BIOAVAILABILITY STUDIES ON VARIOUS DOSAGE FORMS OF THE ANORECTIC, DIETHYLPROPION HYDROCHLORIDE

by

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SUMMARY

The stereo-chemistry, structure activity relationships and the metabolism of the anorectic drug, diethylpropion hydrochloride, have been reviewed briefly, together with the analytical methods for the determination of this drug and its metabolites in biological fluids. In addition, the physico-chemical properties, mode of action, pharmacology and uses of the metabolites have been presented.

A comprehensive review on general principles of salivary excretion of drugs and their therapeutic drug monitoring in saliva with relevant published data on saliva/plasma drug concentration relationships has been outlined.

Sensitive and specific assay procedures, based on gas-liquid chromatography for the identification, separation and determination of diethylpropion and its two major metabolites i.e. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) in aqueous and biological fluids, have been developed. These methods were used to study the urinary excretion as well as saliva and plasma levels of the two major metabolites and, where possible, the unchanged drug, in man.

Sustained release pellets with diffusion rate-controlled membranes were employed to control the rate of input into the body by oral or rectal route of administration. Urinary excretion data and plasma levels of metabolites II and IV in volunteers, where the urine was controlled at an acidic pH, were used for the evaluation of the bioavailabilities of different dosage forms of diethylpropion hydrochloride. The concentrations of metabolites II and IV were also measured in saliva and in plasma after administration of the drug in different doses and dosage forms; relationships between saliva and plasma concentrations (S/P) and between urinary excretion rates and plasma concentrations (U/P) were developed for each of the two metabolites during plateau levels after oral administration of the sustained release pellets (Lot R 7773). The potential use of salivary excretion of the metabolites as an index to monitor their plasma levels and bioavailabilities, was examined.

The distinct advantage of using a subdivided controlled release system (i.e. sustained release pellets) to a single unit sustained release tablet (erosion-core type) in relation to influence of the physical presence of food on the rate and extent of absorption has been demonstrated. It was found that the route of administration (oral or rectal) did not significantly affect the bioavailability of the sustained release pellets.

The study also involved the investigation of the release of the drug from the pellets. Because the release control step was diffusion, no significant influences on dissolution rates were observed with the use of different dissolution test models and agitation intensities. The influence of the concentration and composition (presence of cations viz. Na^+ and K^+ or anions viz. phosphate and borate) of the dissolution medium on the release of the drug from sustained release pellets, was also studied. Any potential changes in the dissolution pattern on storage of the pellets under different conditions (4°C, room temperature and 37°C) over a period of at least one year, were investigated.

The *in vitro* and *in vivo* correlations of two lots of sustained release pellets, each exhibiting different dissolution profiles, and administered rectally and orally, were developed: the *in vitro* data on the free drug were related to the sum of the urinary excretion data of metabolites II and IV.

An attempt to use an empirical approach to predict urinary excretion rate profiles of metabolite II after oral administration of the sustained release pellets, was promising; the calculated profiles were reasonably comparable with those of *in vivo* studies. However, the complete validity of such equations needs further investigations.

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CHAPTER 1

INTRODUCTION TO ANORECTIC DRUGS, SUSTAINED ACTION DOSAGE FORMS AND TO THE AIMS AND OBJECTIVES OF THE PRESENT STUDY

1.1 Anorectic Drugs

Obesity is the most prevalent nutritional problem in the developed countries. It is associated with a high morbidity and a decreased life expectancy, especially in man under the age of 40 years (Hall, Anderson and Smart, 1974; Bray, 1979) and with an increased incidence of various medical conditions including diabetes mellitus, gall stones, osteoarthritis of the weight bearing joints, angina pectoris and hypertension (Antia, 1966; Gilder, 1969: Obesity and Disease, 1969; Garrow, 1979; de Silva and Turnbridge, 1980: Bray, 1980).

Many attempt to reduce mass either at home or with the help of a slimming organization, while others seek medical attention. Theoretically there are various pharmacological ways to promote mass loss. At present, however, with the exception of thyroid hormones and possibly the biguanides, the available agents primarily work centrally by reducing food intake (Atkinson, 1980). Comprehensive reviews outlining current concepts in obesity have been presented by Gudsoorkar, 1981; Mac Kinnon and Parker, 1983; Lasagna, 1983: Douglas and Munro, 1982; Munro, 1983). In the U.S.A. the majority of clinicians regularly prescribe at least one antiobesity drug (Lasagna, 1973), while more than 3 million prescriptions for anorectics are issued annually in the U.K. In 1975, 1 567 000 prescriptions of diethylpropion were dispensed in the U.K. (Carney and Harris, 1979). A recent survey showed that 58% of their 1000 slimmers had received anorectics (Rudinger, 1978), whereas 81% of the patients referred to a hospital obesity clinic had taken anorectics at some time or other (Douglas, Ford and Munro 1981).

Some anorectics currently available in the Republic of South Africa and in the United Kingdom are listed in Table 1.1. All these anorectics, except mazindol and propylhexedrine, are chemical derivatives of the basic structure, phenylethylamine which is also shown by many antidepressants e.g. phenelzine sulphate, tranylcypromine, pargyline, pheniprazine (Wilson, Gisvold and Doerge, 1977)

Amphetamine was the first drug to be used routinely for the treatment of obesity. However, its anorectic activity is accompanied by undesirable side effects, which are mainly CNS stimulation and potent cardiovascular effects. To overcome this problem, newer antiobesity drugs with more selective activity were developed by introducing structural changes on the phenylethylamine molecule. It was found that:

a) ring hydroxylation (4-hydroxylamphetamine) and shifting the α -methyl group to the β position abolished the CNS and the anorectic activity
b) side chain hydroxylation (norephedrine) decreased the activities
c) the introduction of an oxygen function in the side chain (diethylpropion) or another α-methyl group (phentermine) or a bulky group on the basic centre (benzphetamine) attenuated the CNS activity and had no effect on the anorectic activity
d) the introduction of an electronegative group in the phenyl group (fenfluramine) abolished CNS stimulation and had no effect on the anorectic property (Biel, 1970; Aldous, Brewster, Buxton, Green, Pinder, Rich, Skeels and Tuff, 1974; Antiri-Penrose, 1978).

The recent advances in the design and development of anti-obesity drugs have been reviewed by Sullivan, Baruth and Cheng, 1980.

TABLE 1.1: Anorectics currently available in the Republic of South Africa and the United Kingdom

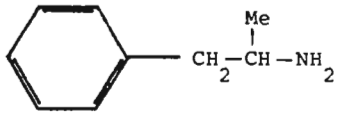
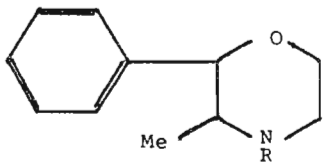
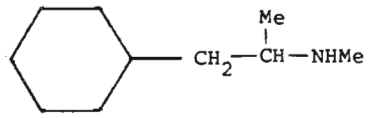
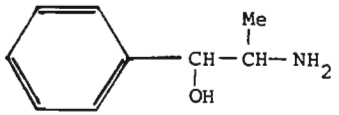
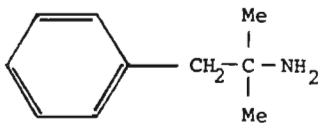
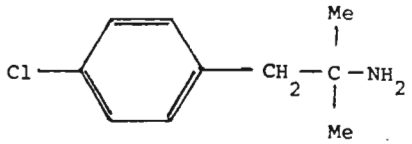
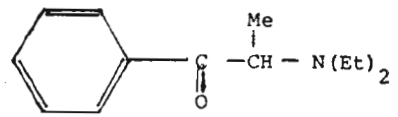
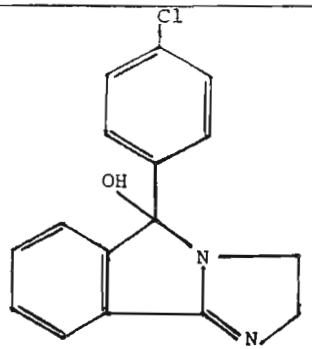
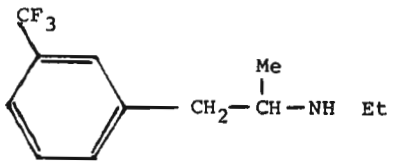
STRUCTURAL FORM		[1]YEAR	DRUG	RECOMMENDED DOSE (PER DAY)	BRANDED PRODUCT	MANUFACTURER
		1944	[2]Amphetamine Resin Complex	12,5 mg	C.D. Durophett S.R. Capsule	Riker
	R=H	1956	[2,3]Phenmetrazine (30 mg) + Phenbutrazate (20 mg)	2 tablets	C.D. Filon Tablet	Berk
	R=Me		Phendimetrazine	105 mg	*Obesan 35mg Tablet *Obex L.A. 105mg Tablet	S.C.S. Rio Ethicals
		1948	Propylhexedrine	100 mg	*Eventin 25mg Dragee *Reducealin 50mg Capsule	Knoll (Holpro) GMP (Protea Pharm)
		1957	[4]D-Norpseudoephedrine	50mg	*Dietene S.R. Capsule *Nobese No. 1 Capsule (Diffucap.) *Thinz Capsule	Bartiss (Restan) Restan Lennon
		1959	Phentermine	30mg	*Duromine 15mg and 30mg S.R. Capsules Ionamin 15mg/30mg Capsules *Minobese 15mg and 30mg S.R. Capsules	*Riker (R.S.A.) and Carnagie Lipha Restan
		1965	[5]Chlorphentermine	65mg	*Pre-sate	Warner-Lambert Technicon (Die-Med) (R.S.A.)

Table 1.1: Anorectics currently available in the Republic of South Africa and the United Kingdom (continued)

STRUCTURAL FORM	[1] YEAR	DRUG	RECOMMENDED DOSE (PER DAY)	BRANDED PRODUCT	MANUFACTURER
	1959	Diethylpropion	75mg	Apisate S.R. Tablet (75mg) *Tenuate 25mg Tablet *Tenuate Dospan 75mg S.R. Tablet	Wyeth Mer-National (R.S.A.) Merrell
	1973	Mazindol	2mg	*Teronac 1mg and 2mg Tablets	Wander
	1973	Fenfluramine	120mg	Ponderax 20mg and 40mg Tablets Pacaps S.R. pellets 60mg	Servier *Servier

*Listed as an Anorectic in "Monthly Index of Medical Specialities" (MIMS) Vol. 23, No. 9, Sept. 1983, R.S.A.

1. Year of introduction as an anorectic.
2. Restricted under Schedule 2 of the Misuse of Drug Act, 1971, U.K.
3. Restricted under Schedule 8 of the Medicines and Related Substances Control Act (Act 101 of 1965) in the R.S.A.
4. Available as an anorectic in the U.S.A., Australia and R.S.A., but not in the U.K. (Greenwood, 1983).
5. Not available in the U.K. but sold in the U.S.A. and in the R.S.A.

1.2 Diethylpropion Hydrochloride

Diethylpropion hydrochloride is one of the several phenylethylamine derivatives (see Table 1) used as an appetite suppressant in the treatment of obesity. Although only introduced on the commercial market in 1959, it was first synthesized by Hyde, Adam and Browing in 1928.

(a) Synthesis*

A mixture of 50% aqueous solution of the diethylamine and α-bromopropiophenone is heated, while being stirred on a waterbath to boiling. The precipitate is filtered off under suction and washed with benzene. The filtrate is shaken up with aqueous hydrochloric acid, and the aqueous solution is made alkaline and etherified. The solution freed of the ether, is then fractionated. The base (B.P. 140°C at 6mm Hg.) is dissolved in acetic ester and then precipitated with ispropanolic hydrochloride. After suction filtration and washing with ether, the yield is found to be 80% and the melting point (m.p.) is 168°C.

(b) Physico-chemical properties**

Chemical name:	1-Propanone, 2 -(diethylamino) -1-phenyl, hydrochloride or 2-diethylaminopropiophenone or 1-benzoyl triethylammonium chloride
Empirical formula:	$C_{13}H_{19}NO.HCl$
Molecular Weight:	241,76
Description:	Creamy-white crystalline powder or crystals.

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* Schutte, J. U.S. Patent 3 001 910 Sept., 1961, assigned to Firma Temmler-Werke, Germany.

** Clarke E.G.G., (1969). Remington XVI (1980)

Stable in dry air with mildly aromatic odour.

Solubility: 1 gram dissolves in about 0,6 ml water, 1,6 ml chloroform, 1 ml absolute methanol and 1 ml of 95% alcohol.
Insoluble in ether.

Some other Names: Prefamone, Regenon, Tenuate, Tepanil, Tylinal, Anorex, Keromin, Dobesin

Melting Point: About 175° with decomposition

pKa: 8,78 (Vree, Musken and van Rossum, 1972)

Partition Coefficient: heptane/water, 525 (Vree et al., 1972).

Ultraviolet absorption spectrum:

diethylpropion in 0,1 N sodium hydroxide, maximum at 246 nm (E1%, 1 cm, 460); in 0,1 N sulphuric acid, maximum at 253 nm (E1%, 1 cm , 580), and in 0,1 N hydrochloric acid, maxima at 245 nm (E1%, 1 cm, 574), 252 nm and 260 nm.

Infra-red spectra: diethylpropion (base)-potassium chloride disc. Principal peaks are A 1682, B 1220, C 689 or 1378 or 1445 cm^{-1}.

Diethylpropion contains one asymmetric carbon atom. The drug itself is a racemate; the (+)- and (-)- isomers are not available commercially.

The stability of diethylpropion was fully investigated by Walters and Walters, 1977, using a HPLC-UV detection method to assess the unchanged compound; GLC/MS was subsequently used to identify the main decomposition product (Walters et al., 1977). Hydrolytic decomposition of the drug in solution (or in dry form) at 45°C occurred at a slow and constant rate at pH 3,5 but increased rapidly as the pH was raised above 3,5 (Figures 1.1 and 1.2 from Walters, 1980). A reaction pathway (Scheme 1.1) for the pH-dependent hydrolytic degradation of diethylpropion was proposed on the basis of the formation of the enamine structure (II) and its hydrolytic reactions (Malhotra, 1969; Weidmann, Wolf and Reisch, 1973).

The enamine (II) formation presumably occurs via pH-dependent rearrangement of diethylpropion (I) to an intermediate ion, followed by dissociation of the latter ion. Rearrangement of the enamine results in a zwitterion (negative charge on the carbon atom and a positive charge on the nitrogen atom) which undergoes protonation to a more stable immonium ion (III). Hydrolysis of III results in a glycerol (IV) which dissociates and rearranges to form diethylamine and the tautomeric species. Various products were anticipated for the product of the latter compounds. The formation of 1-phenyl-1,2-propanedione (VIII) (confirmed previously - Tan, 1978) and its enol tautomer (IX) were identified - these responses coincided with the tautomeric shift for the predominantly diketo form at low pH to the predominantly 1-keto, 2-enol form at high pH (Walters, 1980).

1.2.1 Absorption, metabolism and excretion

The metabolism of diethylpropion and the excretion of the unchanged drug and its basic metabolites have been extensively studied in man (Scheme 1.2 from Beckett and Testa, 1972; Figure 1.3 from Mihailova, Rosen, Testa and Beckett, 1974; Schreiber, Min, Zeiger and Long, 1968; Hossie, 1970; Banci, Cavalli and Monai, 1971; Beckett and Testa, 1973 and 1974a; Lang, Lemieux and Goodfriend, 1975).

Schreiber et al., (1965 and 1968) examined the metabolism and excretion of diethylpropion-1^{14}-C-hydrochloride in humans, using thin-layer chromatography. They reported that diethylpropion-1-^{14}C was completely and quantitatively absorbed (probably by passive diffusion) in the gastrointestinal tract and the radiolabel was excreted exclusively via the renal pathway. Preliminary findings indicated that extensive metabolic alteration of the drug including aromatic hydroxylation, N-de-ethylation, keto-reduction and deamination had taken place.

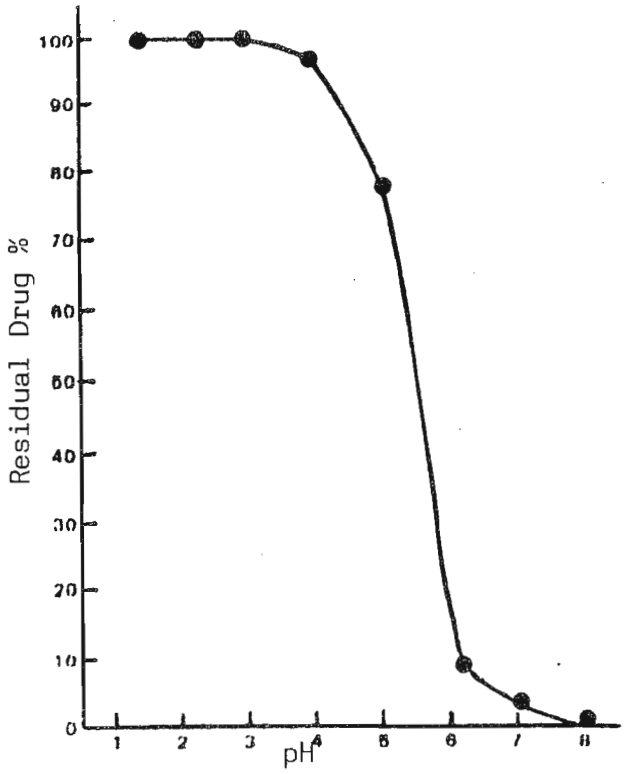


Figure 1.1: Influence of pH, from 1,4 to 8,0 on the hydrolytic decomposition of diethylpropion hydrochloride solutions at 45°C for 4 days - Walters, 1980.

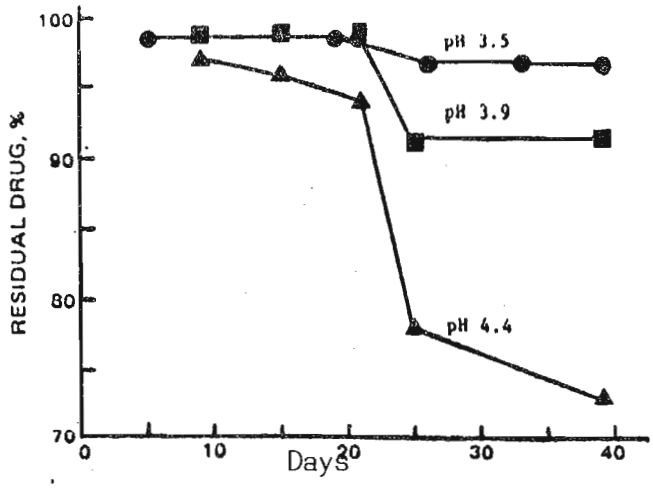
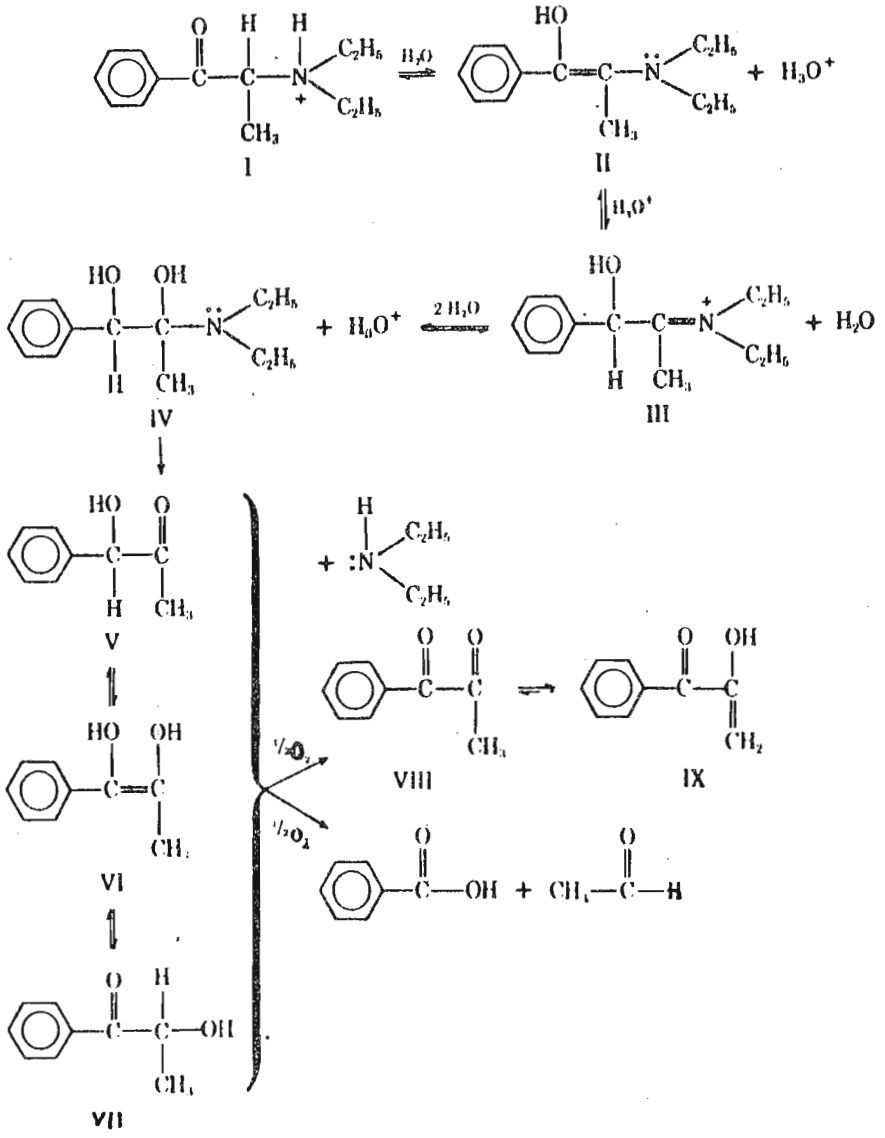
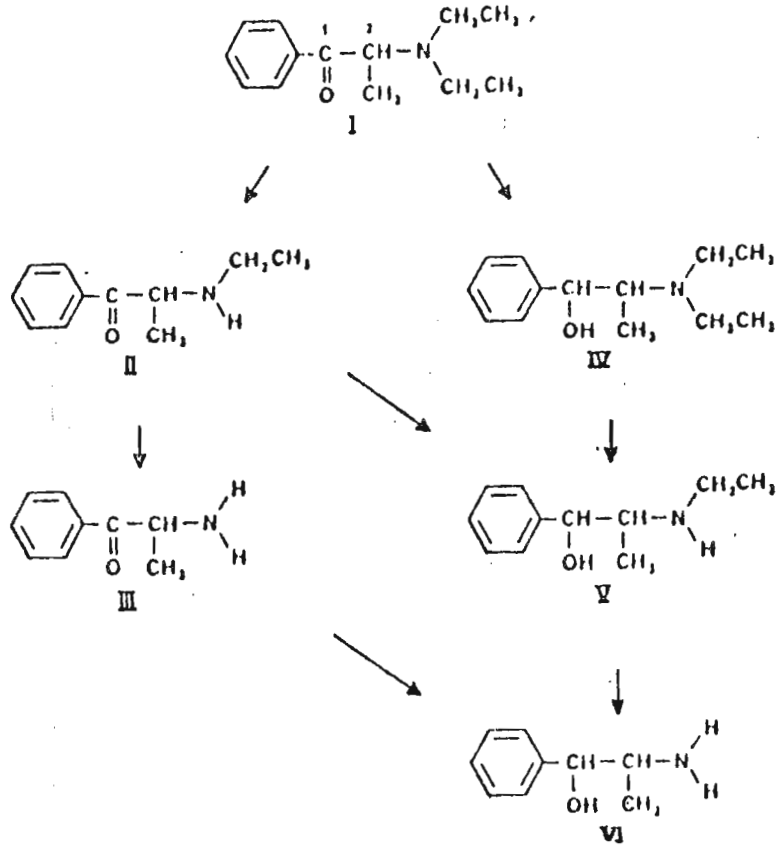


Figure 1.2: Rate plots for the hydrolysis of diethylpropion hydrochloride in weakly acidic solutions at 45°C - Walters, 1980.



Scheme 1.1: Proposed pathway for the hydrolytic decomposition of diethylpropion hydrochloride

Source: Walters, 1980



Scheme 1.2: Pathway for the production of known metabolites of diethylpropion in man-(Beckett et al., 1973).

I	Diethylpropion	IV	N-diethylnorpseudoephedrine
II	N-ethylaminopropiophenone	V	N-ethylnorpseudoephedrine
III	amino-propiophenone	VI	norephedrine

Note: Symbols (I-VI) will be used throughout, to designate any compound.

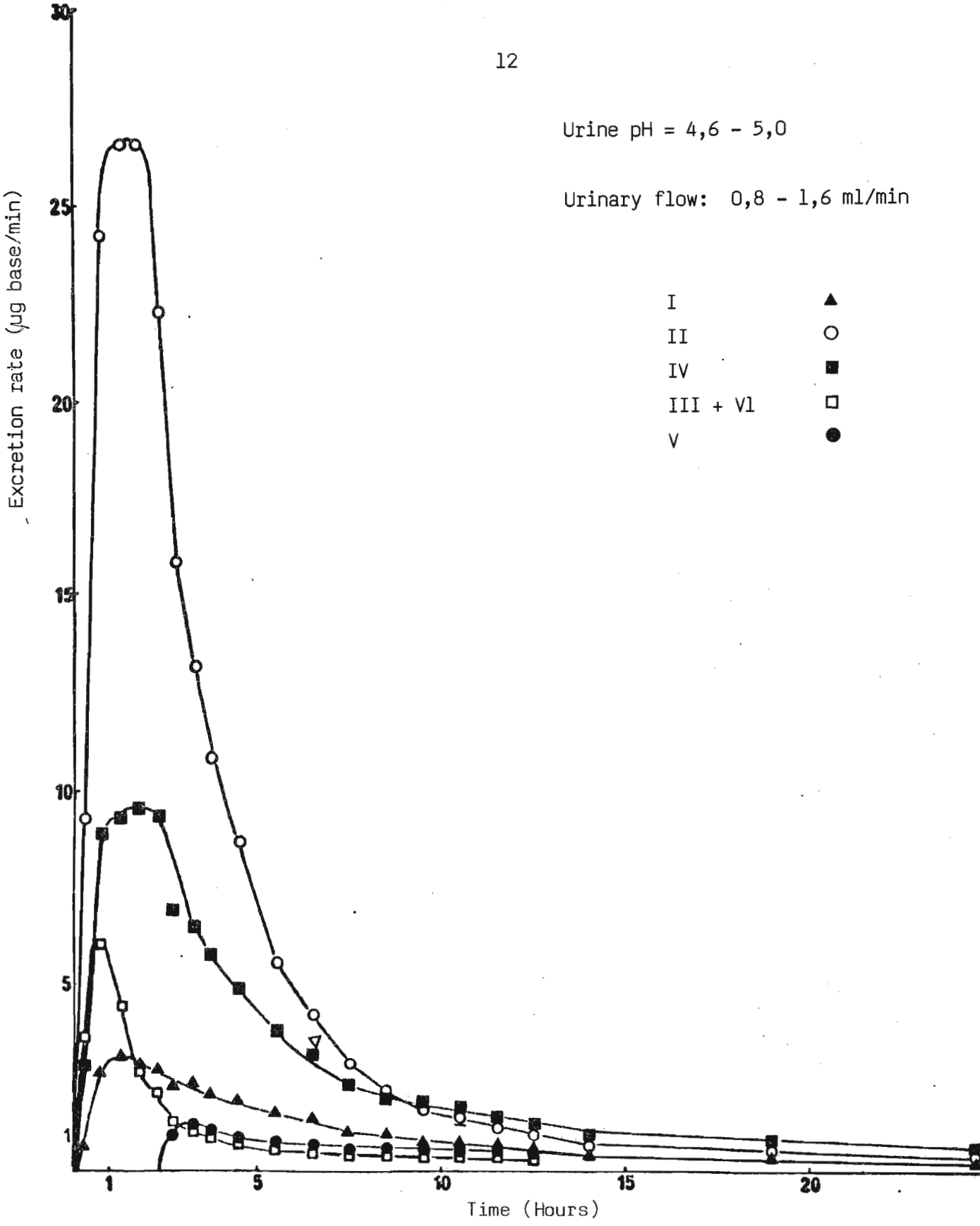


Figure 1.3: Urinary excretion rate profiles of diethylpropion and its basic metabolites in man, under acidic urine conditions, after oral administration of 25 mg of the hydrochloride in aqueous solution.
Source: Beckett and Stanojcic (1979)

Peak plasma values of the radioactivity were observed within 2 hours after oral administration of either 25 mg or 75 mg as a single dose tablet. Twenty-one metabolic products were identified in pooled urine collected over a period of 8-12 hours, adjusted to pH 12 and extracted with diethylether for 20 minutes. Hippuric acid (26,5 - 28,6%) was found to be the major metabolite. Only small amounts of amino-ketones were found because of the strong alkaline conditions in the extraction procedure. Two major basic peaks, which remained unidentified in this work were most probably, artifacts resulting from the instability of amino-ketones in alkaline solutions. The inability to separate the various compounds and the instability of the amino-ketones in alkaline medium have limited the relative importance of these results.

The metabolism and excretion of diethylpropion administered to man or to rabbits as a normal single dose or as a sustained release tablet formulation, were reported by Banci et al. (1968; 1971 and 1972) using a newly-developed chromatographic method. They found the carbonyl reduction of the compound to be stereospecific and established the presence of threo-amino alcohol (see later). In man the main basic metabolites came from N-de-ethylation and reduction of keto groups but in rabbits only the former occurred. However, their conclusion that ethylaminopropiophenone (II) was quantitatively excreted as reduced diethylpropion (IV) is incorrect, as similar amino-ketones were later shown to be unstable in alkaline solutions or decomposed on KOH columns (Beckett, Salmon, Mitchard; 1969; Beckett et al., 1973).

Beckett and Brookes (1970) demonstrated that the introduction of lipophilic or hydrophilic groups at any position in the drug molecule e.g. as in amphetamine and phentermine, may alter the distribution, metabolism and excretion of the drug, in addition to attaining the potential fit at an active site of an enzyme because of the attendant changes in the physico-chemical properties of the molecule. Similar results on studies of the influence of N-alkyl substitution on the metabolism and excretion of aminopropiophenones have been reported. Beckett and Hossie, (1969a, 1969b) examined,

after oral administration in man, the excretion of 4-chloroethyl-aminopropiophenone and its metabolites and found that the total amount of basic compounds excreted was about 50% (Tables 1.2 and 1.3). Vree et al., (1972) examined the metabolism and excretion of several N-alkylsubstituted aminopropiophenones in man. They found that:

a) aminopropiophenone is partly reduced to norephedrine (32%), which is rapidly excreted due to its low lipid solubility

b) N-methylaminopropiophenone is mainly demethylated to amino-propiophenone (60%)

c) Ethylaminopropiophenone is excreted mainly in an unchanged form in the urine (45%) and there is little dealkylation or reduction

d) Isopropylaminopropiophenone is mainly excreted in an unchanged form in the urine but the rapid decrease from the body resembles that of isopropylamphetamine

e) all the compounds N-ethyl, N-propyl, N-isopropyl and N-butylaminopropiophenone have nearly the same pKa values and lipid solubilities (Table 1.4), and this may be the most important factor in the metabolism and renal excretion competition

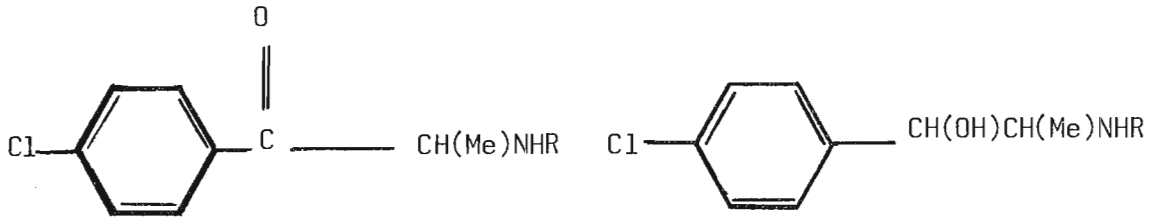
f) Dimethylaminopropiophenone is reduced (40%) and excreted in an unchanged form (25%) and to a small extent demethylated.

g) Dimethylnorephedrine is also slightly demethylated to norephedrine and excreted mainly in an unchanged form

h) Diethylpropion is mainly reduced (20%) and dealkylated (25%). The diethylnorpseudoephedrine formed is mainly excreted in an unchanged form and a small part is dealkylated to ethylnorpseudoephedrine

i) Diethylpropion is excreted in trace amounts as the unchanged drug (2%). This is due to its high lipid solubility and the comparatively low lipid solubility of the metabolites formed, resulting in an increased elimination from the body.

Table 1.2: Structures of the compounds investigated



Amino-ketone Amino-alcohol

Compound	Structure	Chemical Names
I	ketone R = Et	4'-chloro-2-ethylaminopropiophenone
II	alcohol R = Et	1-(4-chlorophenyl)-1-hydroxy-2-ethylaminopropane
III	ketone R = H	4'-chloro-2-aminopropiophenone
IV	alcohol R = H	1-(4-chlorophenyl)-1-hydroxy-2-aminopropane

Table 1.3: The recovery of 4'-chloro-2-ethylaminopropiophenone and its metabolites in two subjects after administration of 150 mg of the salt as a sustained release capsule (S.R.) or as single or divided doses

				% of compound I excreted as compounds				%
Subject	Dose	Urine	Time	I*	II*	III*	IV*	Recovered
1	3x50**	A.D.	30	22,5	15,1	7,1	4,9	49,6
2	3x50**	A.D.	58	17,0	18,4	6,3	6,4	48,1
2	1x150	A.D.	58	25,8	15,5	4,2	4,2	49,7
1	S.R.	A.D.	54.8	16,3	18,4	4,6	9,2	48,5
2	S.R.	A.D.	58	17,8	18,0	6,2	7,3	49,3
1	3x50**	Uncont.	58	5,9	14,6	2,0	2,7	25,2

A.D. - acidic urine with diuresis
S.R. - sustained release pellets placed in a hard gelatin capsule
Uncont. - uncontrolled urinary pH and volume

* Calculated as the equivalent amount of compound I;
** 3 doses of 50 mg of the salt given at 4 hour intervals

Source: Beckett et al., 1969a

Table 1.4 pKa values, apparent and true partition coefficients of some N-alkyl substituted amino-propiophenones

Compound	pKa	TPC_{hept}	TPC_{chl}	APC_{hept}	APC_{chl}
Amino-propiophenone	8,16				
N-methylamino etc ...	7,59			0,22 keto	48 keto
				0,10 enol	20 enol
N-ethyl etc ...	8,40	0,500	214	0,05	19,5
N-propyl etc ...	8,46	27,4	2750	2,25	225
N-isopropyl etc ...	8,45	28,2	3050	2,25	244
N-butyl etc ...	8,47	157	15400	12,2	1200
N-dimethyl etc ...	8,09	4,60	40,5	0,78	6,90
N-diethyl etc ...	8,78	525	15000	21	600

APC_{hept} is the apparent partition coefficient at pH 7,40 in the system heptane/water (Teorell buffer).

TPC_{chl} is the true partition coefficient in the system chloroform/water.

Source: Vree et al., 1972

Comparative in vitro metabolism, using different fractions of liver homogenate of rabbits and guinea-pigs and in vivo metabolism of N-alkylsubstituted aminopropiophenones (i.e. dimethylpropion) in man are presently under extensive investigation in the laboratories at Chelsea College, University of London (Markantonis, personal communication).

For a better understanding of its stereochemistry, and also to overcome inconsistency over reported data i.e. for a better agreement on the percentage of each metabolite formed, Beckett and Testa (1972) developed a GLC/FID, TLC method for the complete analysis in urine, of diethylpropion and its basic metabolites, including the diastereoisomers and enantiomers of the amino-alcohol metabolites. Using this new procedure, the urinary excretion in man of diethylpropion and its metabolites was fully elucidated (Table 1.5; Beckett, 1973; Beckett and Mihailova, 1974b). A pharmacokinetic treatment of the data of excretion (in acidic urine) of compounds I-VI after oral administration of diethylpropion (I) using an analogue and digital programme to evaluate the rate constants for metabolism and excretion of the compounds, was made (Mihailova et al. 1974; Beckett, 1974). Good agreement was obtained between experimental results and computer simulations (Table 1.6).

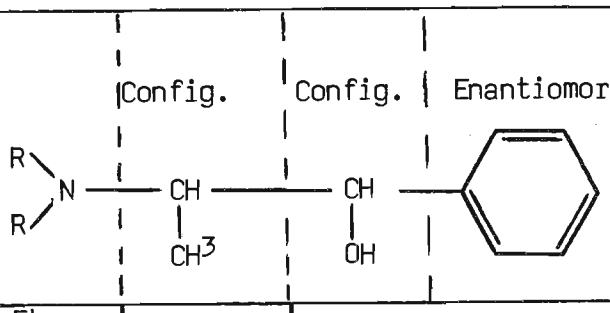
After oral administration, on two separate occasions, of

i) 10 mg of aminopropiophenone (III) as the hydrochloride
ii) 20 mg N-ethylaminopropiophenone (II) as hydrochloride and
ii) 25 mg diethylpropion hydrochloride

the metabolites and unchanged drug in the urine under acidic conditions were analysed and the rate constants computed. The results showed that:

Table 1.5: The urinary recoveries in man after oral administration of 50 mg diethylpropion hydrochloride (racemic).

-- Acidic Urine -- Average of 4 subjects.

Compounds[1] Recovered	 Config.	Config.	Enantiomorph. %	Recoveries in urine[2]
I	Et, N, Et		—C(=O)—	1,8
II	Et, N, Et		—C(=O)—	26,5
III	H, N, H		—C(=O)—	2,6
IV	Et, N, Et	S	S (+)-threo 80	15,8
		R	R (+)-erythro	
		S	S (-)-erythro 2	
		R	R (-)-threo 18	
V	Et, N, H	S	S (+)-threo 58	14,1
		R	R (+)-erythro 15	
		S	S (-)-erythro 7	
		R	R (-)-threo 20	
VI	H, N, H	S	S (+)-threo 12	26,2
		R	R (+)-erythro 39	
		S	S (-)-erythro 3	
		R	R (-)-threo 46	

1. I diethylpropion; II N-ethylpropion; III aminopropiophenone; IV N-diethylnorpseudoephedrine; V N-ethylnorpseudoephedrine; VI norephedrine
   threo is norspeudo-
   erythro is nor-
   Et is ethyl-
   Config. is configuration of aminoalcohols

2. Expressed as % of initial dose

3. The sum of the 4 stereoisomers of each amino-alcohol is considered as 100%

   Total recovery is 87% - while the remaining 13% probably accounts for deamination and hydroxylated metabolites

Table 1.6: Actual and computer-derived constants for one subject for the metabolism and urinary excretion of diethylpropion and its metabolites (Mihailova et al. 1974)

METABOLISM			URINARY EXCRETION OF		
Route	Rate Const. Hours $^{-1}$		Compound	Rate Const. Hours^{-1}	
	Actual	Derived		Actual	Derived
I ⟶ IV	0,77	0,20	I	0,28	0,28
I ⟶ II	1,35	1,00	II	0,25	0,25
IV ⟶ V	0,10	0,10	IV	0,27	0,24
II ⟶ III	0,55	0,66	V	0,24	0,26
II ⟶ V	0,30	0,22	III	0,29	0,29
V ⟶ VI	0,87	0,90	IV	0,22	0,22
III ⟶ others (deamination)	1,00	1,00			

a) N-dealkylation occurs mainly in the liver in a lipid environment. The relative rates of dealkylation of compounds I, IV, II and V are in accord with the lipid/water partition coefficients of these compounds viz. I > II, IV > V, I ≫ V and II ≫ V (Beckett and Mihailova, unpublished information)

b) the rate constant for the reduction of the primary amino-ketone, metabolite III, is greater than those for the reduction of metabolites I and II, which are equal. The reasons for this difference are:

   i) that the stereoselectivity of each metabolite differs (Beckett et al., 1974b) and

   ii) that the ketoreductases in liver favour hydrophilic substrates and III would then be a better substrate than I and II (Culp and McMahon, 1968)

c) metabolite III is involved in an additional metabolic route, which is most likely deamination

d) reduction reactions for amino-ketones are still faster than dealkylation reactions for amino-alcohols; hence amino-alcohols V and VI are produced primarily by a reduction of amino-ketones II and III, rather than by the dealkylation of the amino-alcohols IV and V

e) the percentage reduction and dealkylation of N-alkylsubstituted aminopropiophenones (Testa, 1973) were in reasonable agreement with the values quoted by Vree et al., (1972)

f) the reduction of the primary and secondary amino-ketones, I and II, is substrate specific, as the (2S)-amino-ketones are preferential to the (2R)-amino-ketones

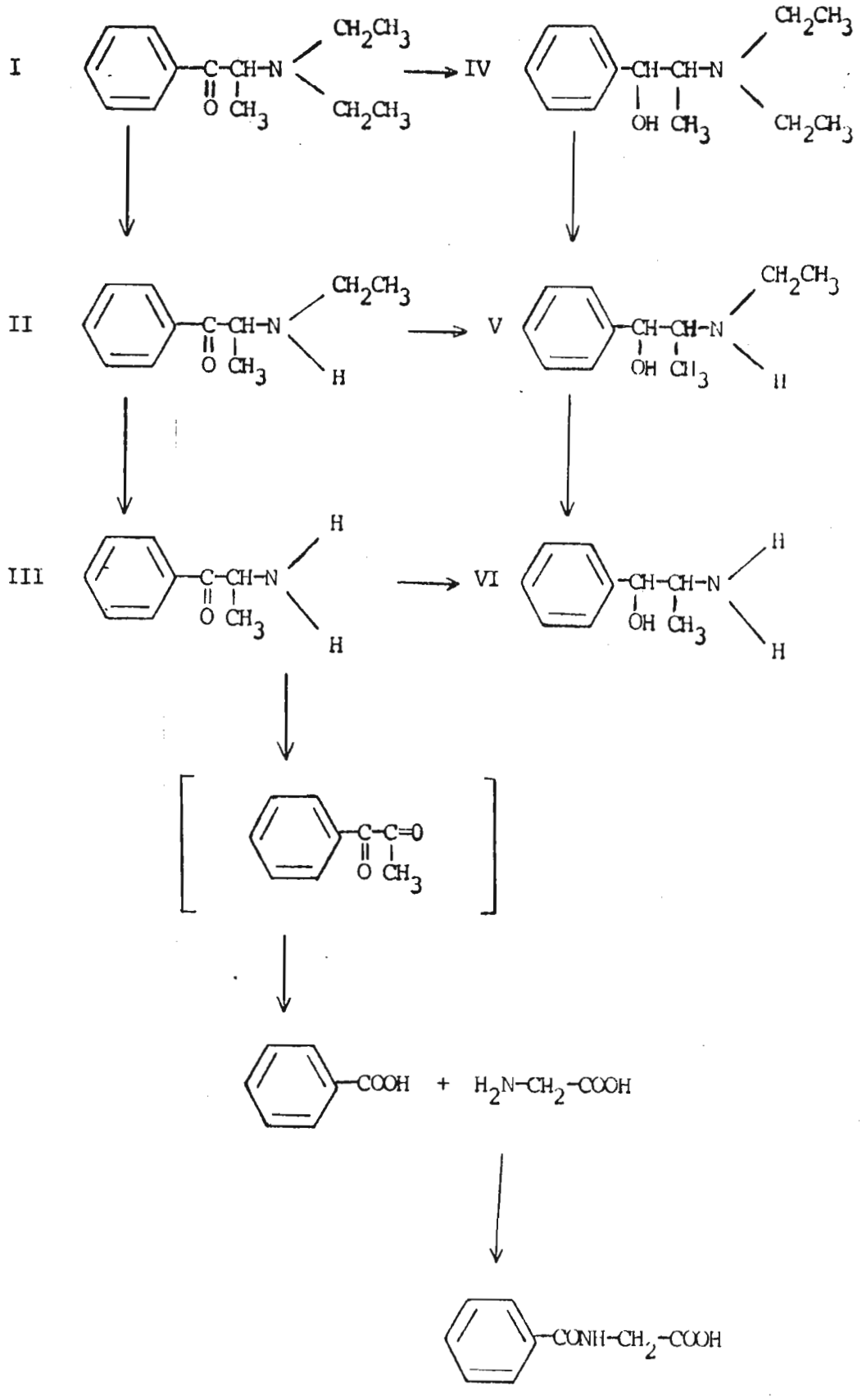
The reduction is also product stereoselective as the newly created centre of the amino-alcohols is of the (1S)-configuration

g) the tertiary amino-ketone, III shows a completely different stereoselectivity for reduction from that of I and II. i.e. here the substrate stereoselectivity is relatively small, ca two-thirds of reduced ketone being the (2R)-enantiomer, but product selectivity is marked for both enantiomers as the newly created centre almost exclusively has the (1R)-configuration (Testa, 1973).

In 1979, Beckett and Stanojcic re-evaluated the metabolism and excretion of diethylpropion in man (Scheme 1.3), by using a newly-developed analytical procedure similar to that of Beckett et al. (1972). They then proposed reasons to account for the observed quantitative differences given in Table 1.7. The total recovery of diethylpropion and its basic metabolites accounted for about 70% of the dose. Mono-deethylation (35% of dose) was more important that the carbonyl reduction (20%) of the unchanged drug. Norephedrine, stated previously (Table 1.5) to be one of the main metabolites, was present only in trace amounts. About 30% of the dose, which was not detected, probably belonged to deaminated product/s - through phenylmethyl diketone to benzoic acid, which is predominantly conjugated with glycine to give hippuric acid (Schreiber et al., 1968). This is also in agreement with findings of Dring, Smith and Williams, (1966) for amphetamine. Less probable is the formation of hydroxylamines and their partial excretion in conjugated forms, since it is considered that, even if hydroxylamines are formed metabolically, they are reduced in vivo to the parent amine (Coutts and Beckett, 1977).

The implications of changes in urinary pH on the metabolism and excretion of sympathomimetic amines like amphetamine, ephedrines, phenmetrazine etc. and their basic metabolites, and the influence

Scheme 1.3 Metabolism of diethylpropion (Beckett and Stanojcic, 1979)



of rate of urine flow on the excretion of these compounds have been clearly illustrated (Beckett, 1966; Beckett et al., 1968a; Raisi, 1977). In an acidic urine, the concentration of unionized drug in the tubules is much lower than in the plasma and this prevents passive tubular reabsorption, thereby avoiding further metabolism, allowing the maximum possible recovery of a basic compound and a good evaluation of the rates of metabolism (Beckett, Boyes and Tucker, 1968b).

Under these conditions, as the rate constant for urinary excretion of the drug and basic metabolites are maximal and proportional to the plasma concentration, meaningful comparisons between the various ways of administration (route, dose and dosage form) of a drug are facilitated - such direct relationships exist for amphetamine, dimethylamphetamine (Beckett, Salmon and Mitchard, 1969c), orphenadrine (Khan, 1972) and phenmetrazine (Raisi, 1977).

As the concentration of some basic compounds in other biological fluids, e.g. saliva, is directly related to that in plasma, monitoring saliva concentrations under acidic urine conditions and developing correlations between urinary excretion rates, salivary concentrations and plasma levels could be useful in assessing the kinetics of absorption and in bioavailability studies - e.g. for digoxin (Huffman, 1975; Kondo et al, 1981) and for phenmetrazine (Raisi, 1977) such studies provided meaningful information.

Investigations on the absorption, metabolism and excretion of diethylpropion administered in different formulations (capsules, sustained release tablets) in man, and the influence of urinary pH on metabolism and excretion of the drug and its metabolites, have been extensively reported (Banci et al., 1968; Beckett et al., 1973 and 1974a). Results of one such study (Table 1.8) highlight the influence of change in urinary pH on the relative importance in metabolic routes and the urinary recovery of the unchanged drug

and its basic metabolites (Beckett et al., 1973 and 1974a), and are similar to those observed for the ephedrines (Beckett and Wilkinson, 1968). The rate of metabolism of diethylpropion showed minimal inter-subject variation when the urinary pH was controlled. Therefore it was proposed that the greatly reduced recovery of metabolites in uncontrolled acidic urine is probably due to increased dealkylation as well as deamination (Table 1.8). Studies on chloroethylaminopropiophenone and subsequently on other compounds (Beckett, 1968a) showed that when excretion of the unchanged drug is difficult to measure (due to extensive and rapid metabolism like "first-pass" effect or where very low doses are given), it is still possible to study the effectiveness of any formulation by minimizing tubular reabsorption (acidic urine with diuresis) and by following the excretion (in a suitable biological fluid) of the metabolite/s which have formed rapidly and directly from the parent compound - comparing the metabolite/s excretion profiles after different dosages (single or multiple) and dosage forms (solution, tablet, sustained release product).

In view of this information, in all the studies undertaken throughout the present project and reported in the thesis, an acidic urine flow was maintained by ingesting sufficient ammonium chloride sustained release pellets with water while monitoring levels in saliva, plasma and urine of the two major metabolites (Chapter 3 and 4).

Recently, the plasma levels of diethylpropion and its metabolites after oral and subcutaneous administration of different doses in man, were studied for the first time (Wright et al., 1975). They used four complementary analytical procedures (e.g. phosphorimetry, GLC/MS, GLC/EC). However, they did not have any specific method to analyse norephedrine in plasma. They reported much higher concentrations of unchanged drug (with delayed metabolism measured by appearance of metabolite II) after subcutaneous administration than when using the oral route. According to their findings,

Table 1.7: The recoveries in man of diethylpropion and its basic metabolites excreted under acidic urine conditions, after oral administration of 25mg of the hydrochloride in an aqueous solution.

Compounds[a]	Recoveries expressed as % of initial dose Subject 1	Mean[b]
I	4,5	1,8
II	36,7	26,5
III	-	2,6
IV	17,3	15,8
V	2,6	14,1
VI	-	26,2
III + VI	9,6	-
Duration (Hours)	26	30
Total of dealkylated compounds (I, II, V, VI)	48,9	69,4
Total of reduced compounds (IV, V, VI)	19,9	56,1
Total Recovery	70,7	87,0

a. I = diethylpropion
II = N-ethylaminopropiophenone
III = aminopropiophenone
IV = N-diethylnorpseudoephedrine
V = ethylnorpseudoephedrine
VI = norephedrine

b. Mean recoveries in 4 subjects, reported by Beckett et al., 1973

Source: Beckett and Stanojcic, 1979

Table 1.8: Recovery in man, under conditions of uncontrolled and controlled urinary pH, of diethylpropion (I) and its basic metabolites after oral administration of the drug

Compounds	Recoveries expressed as % of initial dose		
	Subject 1[a]	Subject2[b]	Mean[c]
I	0,1	0,5	1,8
II	2,3	8,4	26,5
III	4,8	4,3	2,6
IV	3,7	8,8	15,8
V	7,3	5,6	14,1
VI	23,2	20,3	26,2
Total of dealkylated compounds (II, III, V, VI)	34,6	38,6	69,4
Total of reduced compounds (IV, V, VI)	34,2	34,7	56,1
Total Recovery	41,4	47,9	87,0

(a) 96 hour collection, pH fluctuating between 5,9 and 7,1

(b) 30 hour collection, pH fluctuating between 5,3 and 7,0

(c) Mean recoveries (30hr) in 4 subjects under acidic urine pH < 5,0 given a 25 mg dose in aqueous solution

Subjects 1 & 2 received a 50mg dose in a capsule

Source: Beckett et al., 1973.

the systemic availability of diethylpropion is dependent on both size of dose and route of administration and they concluded that the higher dose resulted in an increase in the rate at which diethylpropion enters the intestinal-portal system with concomitant decrease in the percentage of dose metabolized, since high doses saturated the metabolizing enzymes. Because of this fact, blood level studies in one subject after a 600mg subcutaneous dose showed concentrations of unchanged drug in the plasma about three times as high as those found after the equipotent 400mg oral dose; in nine other volunteers, the plasma concentrations of unchanged diethylpropion found after a 75mg oral dose were less than $^{1}/100$ of that observed after a 400mg dose.

Jasinski, Nutt and Griffith (1974) demonstrated conclusively in a crossover trial that orally administered diethylpropion is nearly twice as effective at producing subjective and physiologic effects than when given by the subcutaneous route. They mentioned that plasma concentrations of diethylpropion in one subject were higher after subcutaneous administration than after oral dosing, i.e. the response was not arbitrarily related to the unchanged drug, but rather to the concentration of the compounds reported to be among the major metabolites found in urine (Beckett et al., 1973).

1.2.2 Mode of action, pharmacology and uses

The mode of action of anorectic drugs is not fully understood, although their pharmacological action and clinical use have been much investigated. Comprehensive reviews of diethylpropion, its historical background, assay and pharmacology have been reported (Tan, 1978; Hoekenga, Dillon and Leyland, 1978; Atkinson, 1980; Silverstone, 1982).

The anorectic agents are thought to decrease food intake by interfering with brain monoamines. Amphetamine, phenmetrazine, diethylpropion and mazindol, act on catecholaminergic pathways, although it is unclear whether the noradrenergic or dopaminergic pathway is the more important (Garattini and Samanin, 1978; Munro 1979). A peripheral action on glucose metabolism with increased uptake of glucose in skeletal muscle has been hypothesized in the mass loss associated with fenfluramine (Holmes, Sapeika and Zwarenstein, 1975; Taylor and Goudie, 1975; Sullivan and Comai, 1980).

It appears that brain ascorbic acid levels are unaffected during anorexia caused by diethylpropion and mazindol, but not that caused by fenfluramine in guinea pigs (Odumosu, 1981).

The presence of stereospecific receptor sites in the hypothalamus that mediate the anorectic activity of amphetamine and related drugs (phenylethylamines) have been suggested (Paul, Giblin and Skolnick, 1982). The possible sympathomimetic effect of central stimulating anorectics like diethylpropion upon the thyroid gland, was shown to be only temporary in humans (Stokholm and Hansen, 1983).

Diethylpropion, used as an anorectic (75 mg daily, single long acting or as 3 x 25mg doses) in humans (Sullivan and Comai, 1978) is claimed to have less anorectic activity than d-amphetamine. Like d-amphetamine, it acts preferentially on brain catecholamines, and an intraventricular injection of 6-hydroxydopamine, which markedly depletes brain catecholamines, has been reported to reduce its anorectic effects in rats (Samanin, Bernasconi and Garattini, 1975).

Studies on the locomotor activity of animals showed that most phenylethylamine derivatives probably produce stimulation by a release of catecholamine from neuronal extraglandular pools, while chlorphentermine and diethylpropion are associated with granular pools, and fenfluramine probably has a dual mode of action (Offermeier and Potgieter, 1972). Propylhexedrine and chlorphentermine inhibit the activity of NADH-cytochrome C reductase (in mouse heart homogenates) but to a lesser extent than fenfluramine, while diethylpropion and phendimetrazine have little effect (Holmes, Sapeika and Zwarenstein, 1975).

Borsini, Bendotti, Carli, Pogessi and Samanin, (1979 and references cited therein) reported on the anorectic activity of diethylpropion and d-amphetamine in rats subjected to various treatments known to affect brain monoamines. The effect of both drugs was prevented by a lesion of the ventral noradrenergic bundle, which selectively decreased brain noradrenaline, but was not significantly modified in desimipramine pretreated rats by an intraventricular injection of 6-hydroxydopamine, a condition decreasing only dopamine. Pretreatment with penfluridol significantly reduced the effect of d-amphetamine but not of diethylpropion. A non-significant reduction of drug effect was found with alpha-methyl-p-tyrosine. Lesions of the nucleus medianus raphe which destroys central serotonin neurons, or treatment with methergoline, a central serotonin antagonist, caused no changes in the effects of both compounds. Their findings showed that integrity of central noradrenergic neurons is an important condition for both drugs to exert their anorectic effect. Dopamine

does not seem to play any role in the effect of diethylpropion, but might contribute to that of the action of d-amphetamines. The data indicated lack of involvement of brain serotonin in diethylpropion anorexia (Borsini et al., 1979).

The mechanism of action of anorectic drugs has been clarified in recent years mainly as a result of drug interaction studies employing pharmacological agents that rather selectively affect monoaminergic neurotransmitter processes. Drugs have been classified as either amphetamine-like, acting via a catecholaminergic mechanism, or fenfluramine like, exerting their anorectic effect primarily through endogenous neuronal serotonin or directly on receptors for serotonin. Amphetamine, mazindol, diethylpropion and phentermine fall in the first group, while the second category includes fenfluramine, m-chlorophenylpiperazine, quapazine and MK-212 (Clineschmidt and Bunting, 1980; Thurlby and Samanin, 1981).

The stimulant and anorectic effects of diethylpropion have been studied under a number of conditions using a variety of animal species (Hossie, 1970 and references therein; McQuarrie, 1975; Porikos, Sullivan et al., 1980 and Thurlby et al., 1981). It is generally agreed that diethylpropion has anorectic activity with some stimulation (Hoffman, 1977), although only 4 to 5 cases of dependence have been reported and all these cases had previously abused amphetamine (Silverstone, 1968). Changes in sleeping patterns have been reported (Silverstone, Turner and Humpherson, 1968) and these changes are about equal to those produced by fenfluramine (Oswald, Jones and Mannerheim, 1968; Douglas, Ford and Munro, 1970). The CNS stimulation appears to be much less

than with amphetamine (Sterner and Widbon, 1967) or phenmetrazine (Carpi and Giarolli, 1966) although greater than or equal to, fenfluramine (Oswald et al., 1968; Douglas et al., 1970). Diethylpropion anorexis is less than amphetamine but greater than phenmetrazine or phendimetrazine (Roszkowski and Kelly, 1963; Valle-Jones et al., 1983).

In clinical trials, Spielman (1959) observed that 25 mg diethylpropion given orally 3 to 4 times daily appeared to fulfill the criteria set up by Gadek, Feldman and Lucariello, (1958) for ideal drug treatment of the overeating syndrome. It did not disturb the emotional and psychic balance and appeared to be safe; there was no need for barbiturates to counteract excessive stimulation.

Illig and Illig (1959) studied 48 diabetics who showed no blood glucose changes while taking the recommended dose of 25 mg of diethylpropion orally three times a day. Similar doses showed their high effectiveness in suppressing appetite and reducing mass in obese patients, or in obese patients suffering from cardiac, hypertensive or diabetic problems (Huel, 1959; Ravetz, 1959).

In a series of tests on 15 patients suffering from arterial diseases, intravenous doses up to 10 mg of diethylpropion were given: this is equivalent to four times the recommended single oral dose as determined by LD-50 studies in rats. No acute deleterious effects of the drug upon blood pressure, pulse, respiration or electrocardiograms were noted (Alfaro and Schlueter, 1960).

A double-blind crossover trial of diethylpropion (long acting preparation) in the treatment of obesity with a low caloric diet was described by Hadden and Lucey, (1961).

The drug caused great mass loss and was less effective after a month's treatment with a placebo. Hence it was recommended for clinical use as short intermittent courses. The superiority of diethylpropion over some other anorectics has been well demonstrated by Allen, (1977); Phillips, (1977) and Douglas, Ford and Munro (1981 - Table 1.9).

Williams, (1968); Carney and Twedelli, (1975); Elliot, (1978); Botha, (1979); Abramson, Garg, Cioffari and Rotman, (1980) and Parsons, (1981) demonstrated in obese diabetic patients that a significantly greater loss of mass occurred with long-acting diethylpropion hydrochloride than with placebo. Silverstone, Turner and Humpherson (1968) demonstrated the pharmacological effect of long-acting diethylpropion as an appetite suppressant, with an effect lasting nine to ten hours.

Table 1.9: Results of long term studies on anorectic drugs

References	No. of patients	Drug and method of administration	Mean duration months (Range)	Mean Weight Loss Kg
Smith (1962)	20	Intermittent phentermine/ and or Durophet[1]	16 (2-35)	7,8
Matthews (1975)	28	Continuous diethylpropion	10 (6-15)	18,2
Enzi et al. (1976)	15	Intermittent mazindol	13 (9-16)	14,2
Hudson (1977)	176	Continuous fenfluramine	12	10,4
Craddock (1977)	111	Various drugs Intermittently (usually diethylpropion)	(120-216)	6,9

1. Amphetamine and dexamphetamine in equal parts

Source: Douglas et al., 1981.

Silverman and Okun (1971) showed long-acting diethylpropion to be a safe and effective medication to control mass gain in pregnancy. Botha (1976) confirmed the anorectic efficacy of long-acting diethylpropion in obese patients on a strict diet (Seedat and Reddy, 1974; Van Rooyen and Van Der Merwe, 1971; Allen, 1975 McQuarrie, 1975).

The side effects with diethylpropion , although common, are mild. They include sympathomimetic effects such as increased sweating, palpitations, dry mouth or CNS stimulation as reflected by increased nervousness, irritability or insomnia. The safety, short-term and long-term efficacy of diethylpropion as well as its abuse, have been reviewed by Munro, (1979) and Cohen, (1980). Since 1963, when diethylpropion became available, until December, 1977 there were only 23 cases of drug dependence and 24 of other psychiatric adverse reactions (including 10 of psychosis) which were reported to the Committee of Safety of Medicine, United Kingdom, out of a total of 152 reports of all adverse reactions due to diethylpropion (Carney and Harris, 1979).

Bridgman and Buckler (1974) reported on drug-induced gynaecomastia (reversible) in males taking diethylpropion for 4 weeks - the breasts were enlarged, swollen and tender but there was no loss in libido and the patients produced high urinary levels of luteinizing hormones and oestrogens (17-oxosteroid, oestrone, 17-oxogenic steroids) which returned to normal after drug intake had been stopped. This suggests that diethylpropion exerts a stimulatory effect on the hypothalamus and, via the luteinizing hormone releasing factor, promotes the secretion of luteinizing hormone. Consequently the interstitial cells of the testis produced increased quantities of testosterone and oestrogen.

The low recovery, short half-life and low peaks of unchanged diethylpropion, substantiated by the fact that the oral dose produces greater subjective activity than with subcutaneous administration which produces higher drug plasma levels, are strong evidence that the unchanged drug, because of its rapid and extensive metabolism, contributes very little to the total activity observed. (Jasinski et al., 1974). Its pharmacological properties, and more specifically its anorectic activity are caused by a very complex mixture of metabolites, whose proportions are time dependent and whose activities show large qualitative and quantitative differences.

Thus compounds II, III and (-)-norpseudoephedrine have a central locomotor stimulatory action in mice, whereas (+)- and (-)- norephedrine are inactive (van der Schoot, Ariens, Van Rossum and Hurkmans, 1962; Fairchild and Alles, 1967). The central stimulatory activity is considered to reside in the (+)- norpseudo form (Alles, Fairchild and Jensen, 1961.) The compounds (+)- diethylnorephedrine and (±)-N-ethylnorephedrine have diverse pharmacological activities (Curtis, 1928; Chen, Wu and Henriksen, 1929); the (-)- norephedrine has anorectic activity (Abdallah, 1968). The uses of phenylpropanolamine and ephedrine as effective anorectics, compared to other anti-obesity drugs, have been evaluated in man and reviewed (Malchow-Moller, et al., 1981 and Altschuler et al., 1982). The differences in the pharmacological activities (Patil, La Pidus and Tye, 1970) and in rates of metabolism (Feller, Basu et al., 1973) of the four ephedrine stereo-isomers analogues have been reviewed. Thus the erythro-diastereoisomers ((+)- and (-)- ephedrine) are more potent as CNS stimulants than the threo-diastereoisomers [(i.e. (+)- and (-)- pseudoephedrine] (Lanciault and Wolf, 1965). The (IR)-configuration [(-)-

erythro and (-)- threo] seems to enhance a direct stimulatory activity, whereas the (1S)- configuration [(+)-erythro and (+)- threo] is associated with an indirect action (Patil et al., 1965; 1967). The anorectic activity in mice decreases in the order (-)-ephedrine, (+)-ephedrine, (+)- pseudoephedrine and (-)- pseudo-ephedrine (Abdallah, 1968).

Therefore the five major metabolites (Table 1.10), are likely to account for almost the whole activity of the drug, with probably the major contributions coming from N-ethylaminopropiophenone (II) and (+)- diethyl-norpseudoephedrine [(+)-IV-threo] and (-)-norephedrine [(-)-VI-erythro]. The ((+)-IV-threo form is more likely to have pronounced CNS effects because of its much greater lipid solubility relative to the other amino-alcohols excreted (Taylor, 1972). Presently, pharmacological screening on diethylpropion and the two major metabolites (II and IV) is under investigation (Dr W.A. Behrendt - personal communication - Temmler-Werke, Germany, 1980).

In view of the importance of the basic metabolites viz. (II) and (IV) and their susceptibility in excretion to urinary pH changes, all studies necessitated a well-controlled acid urine, and all evaluations on biological samples analysed (saliva, plasma and urine) were based on metabolites (II) and (IV) and, to a lesser extent, on the unchanged drug (I).

Table 1.10: Mean recoveries, for four subjects under acidic urine conditions, of diethylpropion and its basic metabolites, expressed as percentages of dose (ranges in brackets)

Major compounds excreted		Minor compounds excreted	
II	26,5% (22,4-30,4)	I	1,8% (1,1-2,4)
(+)-IV threo	12,7% (9,6-15,8)	III	2,6% (1,4-6,1)
(+)-V threo	8,2% (6,7- 9,7)	IV erythro	0,3% (0,3-0,4)
(-)-VI erythro	10,2% (7,4-11,8)	(-)-IV threo	2,8% (1,1-4,8)
(-)-VI threo	12,1% (10,4-13,6)	(+)-V erythro	1,0% (0,7-1,2)
		(-)-V erythro	2,1% (1,1-3,1)
		(-)-V threo	2,8% (1,9-3,5)
		(+)-VI erythro	0,8% (0,2-1,7)
		(+)-VI threo	3,1% (2,3-4,2)

Source: Beckett et al., 1973.

I diethylpropion

II N-ethylaminopropiophenone

III aminopropiophenone

IV threo. diethylnorpseudoephedrine

IV erythro. diethylnorephedrine

V threo. N-ethylnorpseudoephedrine

V erythro. N-ethylnorephedrine

VI threo. norpseudoephedrine

VI erythro. norephedrine

1.3 Sustained Action Dosage Forms

The dosage form of any drug is a delivery system which can be modified by both physical (through pharmaceutical technology) or chemical (pro-drug) approaches, to provide some required concentration of drug substance to the desired site of action for a predetermined time interval.

The absorption of drugs through membranes, following oral or rectal administration, depends largely on the drug molecules being in an aqueous solution at the surface of the membrane. The pharmacological and physico-chemical properties of a drug are intrinsic to its molecular structure and cannot be altered except by chemical modifications. However, the rate of delivery, and the concentration and the location of aqueous solutions of drugs at membranes, are controlled by the drug formulation.

The formulation of drugs into tablets, capsules and suppositories has been used to provide a convenient means of administering the drugs in a compact and relatively stable form. At the present time, the importance of formulation is being realized. Increasing emphasis is being placed on formulation to overcome the disadvantages and shortfalls of some drug molecules and to control drug delivery. Once the drug has been delivered at the GIT membrane and has been dissolved in an aqueous medium at the membrane surface, the numerous factors influencing drug absorption clearly come into play. An excellent review on drug absorption, the proceedings of a recent international conference, was published (Prescott and Nimmo, 1981).

Important aims of formulation of drugs to influence absorption are:

a) to increase duration of action and to produce a more convenient dosage regimen and therefore improve patient compliance
b) to reduce side effects

c) to minimise intra-and inter-subject variations in plasma levels without altering the dosage i.e. more reliable absorption and smaller fluctuations of drug levels.

To achieve the above objectives it is necessary to control the rate of delivery of drugs to absorption sites, the location and distribution of the drug delivery systems and the association of the drug with other molecules to alter water/lipid partitioning (Beckett, 1981).

These factors should be considered in relation to anatomical and physiological conditions at the absorption sites. The pH and fluidity of the medium of the GIT, the transit times past different absorption sites or regions should be considered (Florence and Attwood, 1981; Aiache, 1983).

To achieve the therapeutic objectives outlined, a controlled release of the drug from a delivery system is necessary. Drugs formulated for this purpose have been described as sustained action, sustained release, prolonged action, depot, repository, delayed action, retarded release and time release (Notari, 1980; Ballard, 1980). Drug products that have been formulated for the purpose of controlling absorption include dosage forms for oral, injectable and topical use as well as implants and for insertion into body cavities (Manford, 1976, Juliano, 1980, Davis 1981).

Comprehensive reviews on drug delivery systems are available (Ballard and Nelson, 1965: Yates, Benson, Buckles, Urquhart and Zaffaroni, 1975: Gregoriadis, 1977; Heilman, 1978; Juliano, 1978). A survey on controlled-action dosage forms with reported methods of manufacture is available (Colbert, 1974; Robinson, 1978: International Pharm. Congress, 1983; Drug Deliveries Systems, 1983).

1.3.1 The sustained (controlled) action concept

A sustained release product has been defined as "one in which a drug is initially made available to the body in an amount sufficient to cause the desired pharmacological response as rapidly as is consistent with the properties of the drug determining its intrinsic availability for absorption, and one which provides for maintenance of activity at the initial level for a desirable number of hours in excess of the activity resulting from the usual single dose of drug" (Nelson, 1961).

Sustained action formulations provide the means to regulate absorption by formulation. The frequency of administration may thus be reduced, plasma concentrations may be maintained and side effects may possibly be reduced. In addition, it ensures that a minimal quantity of unwanted drug reaches other sites in the body, thereby eliminating adverse reactions viz. anti-cancer, anti-fertility agents and anti-inflammatory steroids. Finally the system is convenient to take so that the patient is easily able to comply with the dosage regimen.

Reducing the frequency of oral administration of drugs is perhaps one of the most important advantages of controlled release delivery systems. One of the main therapeutic problems at present is the lack of patient compliance. Numerous investigators have shown that many patients fail to take their drugs in the prescribed manner (Sackett and Hayes, 1976; Howell, 1977; Mucklow, 1979). Poor communication between doctor or pharmacist and patient and failure by the patient to understand the potential benefits of treatment, can be the cause of poor compliance. Other reasons include psychiatric illness, old age and failing memory. More simply, if a patient has to take a number

of drugs, some twice daily, some four times a day, some before meals and some after meals, then a defined regimen is unlikely to succeed.

It has been shown that even in good hospitals, the proportion of patients who have serum concentrations of drugs within the therapeutic range is remarkably small (Merkus, 1976). There is a correlation between dosage interval and compliance (Gatley, 1968; Porter 1969; Alfredsson and Norell, 1981). Compliance appears to be significantly worse when drugs are prescribed three or four times daily or in patients on long term medication. On a twice daily dosage regimen, doses were generally spread more regularly than on a thrice daily regimen (Alfredsson and Norell, 1981). Also the proportion of missed doses was lower on a twice rather than thrice daily regimen. It is useless to have beneficial drugs with correct formulations if patients fail to take the products regularly. Clearly once or twice daily dosage regimen, with a sustained release formulation, would lead to improved patient compliance.

Drugs undergoing substantial and dose-dependent first-pass metabolism, have also been formulated as controlled release formulations to give predictable and reliable plasma levels (Bogentoft, Carlsson, Ekenved and Magnusson, 1978). It is possible with controlled release formulations to reduce frequency of drug administration irrespective of the different biological half-lives of the compounds to be used.

1.3.2 Rationale for using sustained release pellets

Oral sustained release products can be formulated as single unit or multiple unit doses (Bechgaard and Hegermann-Nelson, 1978). Single unit preparations tend

to follow food, which has a normal transit time through the small intestines of between 3 and 8 hours (Prescott, 1974). Accordingly, 6-10 hours are recommended by many authors as the maximum duration for the in vitro release from depot formulations (Ritschel, 1973; Sjogren, 1975). However, there are instances where it is desirable to detain the drug depot in the upper gut to ensure optimal absorption, or to extend the absorption phase, as with drugs with biological half-lives requiring an absorption period of more than 6-10 hours, in order to facilitate a lower dosage frequency. The large number of sub-units of multiple-unit formulations (distributed freely throughout the GIT) affords wide dispersion of the drug released, thereby offsetting fluctuations in the milieu of the GIT and any variations in the release characteristics of the individual sub-units.

Therefore their transport is less affected than that of single-unit preparations by transit time of food (Ekenved, Bogentoft, Carlsson and Magnusson 1977). Sub-division of dose therefore offers the possibility of achieving a longer lasting and more reliable source of drug - this ensures that the requisite bioavailability and the desired release rate for maintenance of constant blood levels of the drug are more reliably attained (Bechgaard and Ladefoged, 1978).

Some pellets leave the stomach by a zero-order or first-order process, depending on the amount administered (Beckett, 1981). They become widely scattered as they pass down the GIT and do not move downward in the same way as tablets. Their average transit time is much longer than that for tablets (Noormohammadi, 1981; Davis, 1983).

The influence of gastric emptying time and intestinal motility on intra- and inter- subject variations in the rate and extent of availability can be largely avoided by the use of multiple-unit, controlled release dosage forms (Benet, 1973; Bechgaard and Ladefoged, 1978; Bechgaard, 1983). It is desirable therefore that a sustained release product should consist of drug incorporated into small pellets, each with its own rate controlling system to reduce inter-subject variation in absorption and yet obtain complete bioavailability over a 12 or 24 hour period (Beckett, 1981). The gastrointestinal transit of tablets, osmotic pumps and of pellets formulations administered in capsules have been studied using the technique of gamma scintigraphy (Daly, Davis and Frier, 1981). However, in a recent paper Hunter, Fell and Sharma (1982), have commented that in isolated cases the gastric emptying of pellets may occur as a bolus, rather than in a randomized manner, and additionally, when taken with food, the pellets do not necessarily become widely dispersed in the intestines (Wilson, Hardy and Davis, 1983).

In a recent study, the delivery mechanism from sustained release pellets was demonstrated in vivo using radiological studies (Galeone, Nizzola, Cacioli and Moise, 1981). The results confirmed that release and hence the absorption of the active drug material from the pellets take place over a large area of the GIT, thereby avoiding high concentrations of potentially irritating material in any one area. This alone is a major advantage of multiple-unit controlled delivery forms. In the study, pellets releasing drug through non-biodegradable diffusion-controlled membranes were compared to pellets which gradually disintegrated, releasing the drug through erosion. The former were shown to release the drug continuously all the way down the GIT. They demonstrated complete availability, while the

erosion-type pellets provided greater individual variability. A more controlled delivery, with less inter-subject variation, was demonstrated with the diffusion-controlled pellets. Similar studies on the influence of type of controlled release pellets formulations of propoxyphene and norpropoxyphene, demonstrated the superiority of membrane coated pellets which showed minimum intra-subject variance, increased predictability on onset and duration and improved reproducibility of plasma levels (Bechgaard and Baggesen, 1980).

A diffusion rate-limiting membrane-controlling drug release from subdivided dosage units is therefore preferable to one from which the release of drug depends on membrane rupture or unit erosion - the release from the latter type is less predictable and more variable, depending totally on conditions of the GIT (Bechgaard and Baggesen, 1980).

The size and density of pellets have been shown to influence intestinal transit time (Bechgaard and Antonsen, 1977: Noormohammadi, 1981). The permeability of the membrane depends not only on its thickness but also on its composition (polymers, plasticizers, etc) and production factors such as solvents and drying conditions. The core beneath the membrane also influences the release. However, the advantage of this type of formulation is that the release profile can be modified within broad limits, without loss of predictability and repoducibility. In a later study, the density of pellets in ileostomy subjects was found to be an important factor affecting the transit time in the small intestines, while the diameter was of minor significance (Bechgaard and Hegermann-Nelson, 1978; Bechgaard and Ladefoged, 1978).

Localization of high concentrations of some potentially irritant drug in a very small region of the GIT could cause mucosal irritation. Sustained release pellets with diffusion membranes which scatter widely throughout the intestine and so disperse the drug uniformly, are preferable to sustained release tablets which by possibly sticking to the gut or to each other may restrict drug delivery to a limited area (Beckett, 1983). Studies in rabbits showed that sustained release pellets produced considerably less mucosal damage than occurred when using coated or sustained release tablets of potassium chloride (Block and Thomas, 1978). Obstruction in patients with bowel structure, clumping of matrix tablets and local gut damage e.g. from potassium and iron sustained release tablets (Higham and Turnbag, 1980; Whittington and Thompson, 1983) and adverse reactions including gastrointestinal bleeding and perforations e.g. from "Osmosin" (Current Problems Leaflet, 1983) could be avoidable with the use of appropriately designed sustained release pellets (Beckett, 1983).

In all the studies in the present project, sustained release pellets with diffusion rate-limiting membranes, were utilized for both *in vitro* and *in vivo* investigations.

1.3.3 In vitro assessment of availability from sustained action dosage forms

Theoretically, an *in vitro* test for drug availability should indicate the physical factors controlling availability *in vivo*. This is not feasible for orally administered dosage forms because the depot fluids are not constant in composition and the dosage form moves at some unknown rate through a number of depot fluids.

It is not possible to simulate in a single test system such variables in depot as interaction between drug and depot constituents, changes in volume, retention or transit time and various levels of agitation.

In vitro tests can be carried out which will indicate what effects these variables have on the mechanism and kinetics of drug release from a dosage form. Data from such tests may then be used to formulate a product in which drug availability is less sensitive to these variables viz. transit time is of concern when drug release is more rapid in intestinal than in gastric fluid. The kinetics of drug release from the maintenance portion of a formulation can be measured in vitro by placing it in separate fluids which simulate the content, concentration, the volume of gastric and intestinal fluid, heated at 37°C and agitated at a constant low level. The amount of drug remaining in the maintenance portion of the dosage form is determined and availability rate obtained by plotting the percentage remaining versus time (Manford, 1976).

Ideally the rate should be constant (zero-order) in both gastric and intestinal fluids. If there is a significant difference in availability rates, it should be ascertained whether the dosage form would be expected to pass from stomach into intestine in a constant manner. If this is not a reasonable expectation, the dosage form should be reformulated so as to minimize the difference in drug availability rate and or transit time. It is not likely that the overall availability rate will be constant in vivo if it is not constant in both gastric and intestinal fluid in vitro. Conversely it cannot be assumed that availability rate will be constant in vivo if it is constant in vitro. The possibility of correlation must be ascertained by in vivo testing (Manford, 1976; Dakkuri and Shah, 1982). A method for estimating the amount of drug

available for immediate absorption from the dosage form is often needed. This is because dosage forms such as tablets usually do not have a distinctly separate initial dose portion. Such an estimate can be obtained in vitro through tests that are similar to those used to measure the dissolution rate for conventional dosage forms, i.e. the dosage form will release for absorption in vitro a specified quantity of drug in a specified time. For example, a given dosage form should release 30% of its total drug content in less than 30 minutes when the dosage form is placed in 100 ml of simulated gastric fluid maintained at 37°C and stirred at a specific rate.

Components of simulated gastric fluids, such as pancreatin and pepsin, may complicate the analysis of the drug in vitro. Drug availability can be determined through the use of simple buffers and surfactants, which simulate the pH and surface tension of the gastrointestinal fluids. This is acceptable when it has been independently determined that pH and surface tension are the variables that influence availability rate and not some interaction with the other constituents that have been detected.

In vitro tests developed for ensuring lot-lot reproducibility in drug availability for a manufactured product may be distinctly different from those used for arriving at a satisfactory formulation. A large number of procedures have been proposed. The most widely used in vitro test procedure for controlling a manufactured product is based on the method first suggested by Souder and Ellenbogen (1958). A sample of the dosage form is placed in a 90 ml cylindrical screw capped bottle with 60 ml of menstruum and rotated slowly end-over-end in a 37°C controlled temperature waterbath. The released

(or residual) drug is analysed at several intervals to prepare a plot of drug release versus time. Again the initial analysis is usually made after ½ hour and the final one after 8 to 10 hours of rotation. Because one dosage unit is placed in each bottle, one bottle for each data point desired is used.

The common procedure in in vitro testing involves the use of USP simulated gastric fluid for 1 to 2 hours; this fluid is then replaced with USP simulated intestinal fluid and availability measured for as long as 8 hours. It is important that these solutions be held at 37°C during the studies as temperature has a big effect both on diffusion and on the physical parameters producing the sustained release. With the rotating bottle method, separate bottles are used for each time interval at which an availability point is desired; the drug remaining in the sustained action form is usually the information determined, an easier procedure requiring no correction for the background blank of the menstruum.

More precise control of the changes in the menstruum with time have been proposed periodically as better simulations of the biological situation in the gastrointestinal tract. These suggestions culminated in a very complex procedure recommended by the FDA, in which 100 ml of simulated gastric fluid is pump circulated at 37°C through and past the dosage form held in an inert matrix (Wiley, F., 1957). Fifty mls of this menstruum is removed for analysis each hour and replaced with fresh 50 ml of simulated intestinal fluid. The process is continued for the duration of the period over which the dosage form is expected to act.

Although closer simulation of the body systems is afforded by such a system, the manpower and equipment it requires have prevented its general use and simpler methods are still in common use (Manford, 1976).

The compendial *in vitro* test procedures for determining drug release for sustained action products represent a compromise between the method of Souder and Ellenbogen, (1958) and the more closely simulative FDA system. These modifications, or slight modifications thereof, appear to be adequate for controlling the manufacture and examining the stability of most sustained action products. This is true because these tests tend to exaggerate differences in release pattern, i.e. a difference in release that can be demonstrated to occur *in vivo* is always found *in vitro*. The foregoing statement, however, is correct only when the sustained action product being tested is made by a standardised and carefully controlled manufacturing process. For example, a sustained action tablet and a sustained action capsule product can have the same *in vitro* release patterns, but drug availability can be significantly different *in vivo*. When the manufacturing procedure (or dosage form) is changed it is necessary to redetermine drug availability rate *in vivo* even if there is no change *in vitro* (Manford, 1976).

1.4 Monitoring Drugs in Saliva

The salivary excretion of various drugs has attracted much attention in recent years. The observation that drug concentrations in saliva are often proportional to the concentrations in plasma (e.g. anti-epileptics, digoxin, paracetamol) has led to the suggestion that in therapeutic monitoring or in pharmacokinetic studies, saliva might be substituted for plasma (Horning, Brown, Nowlin, Lertratangkoon, Kellaway and Zion, 1977; Dvorchik and Vesell, 1976). Some distinct advantages include:

a) saliva can be collected by non-invasive techniques and many samples can be obtained (collection of a large number of samples increases accuracy of determinations) without exposing patients to discomfort (pain and stress), skin irritation, loss of blood and infection risk, especially in children and elderly patients. Unlike saliva collections which can simply be taken at home, collections of plasma samples need specialised skill or visitations to a centre.

b) There are indications that the concentration of some drugs in saliva is equal to the free or protein-unbound concentration in plasma (assuming passive diffusion transfer), whereas plasma concentrations generally represent both bound and unbound forms of the drugs. Since the extent of the drug protein binding may exhibit substantial inter-individual differences (viz. disease states, displacement from protein sites by concomitant medications), the measurement of drug concentrations in saliva may be of greater therapeutic meaning than of plasma concentrations.

Some limitations connected with salivary monitoring are:

i) concentrations of drug are relatively low, thereby demanding sensitive assays

ii) salivary flow is about 1ml/min, so large volumes are difficult to obtain unless they are collected over an undesirable time or under forced secretion condition (see later) or by supreme conscious effort. Forced conditions cause contamination or changes in both the absorption of the drug (in case of parafilm) and the salivary pH

iii) some find saliva sample handling even more distasteful than urine or plasma handling

iv) the pH-partition hypothesis, with no changes in protein-binding within the clinical range of total concentration in plasma, dictates a constant saliva-to-plasma (S/P) ratio. In practice a number of compounds show wide variations in this ratio, such as lithium (because of active transport), and some organic acids (perhaps because of active transport). Active transport seems a likely method for excretion of penicillins in saliva

v) the S/P ratio may vary with time (diazepam and theophylline) or over the concentration range, or between individuals (e.g., digoxin and procainamide)

vi) of particular concern is the fact that S/P ratios tend to be higher during drug absorption than later in a test. Also, discrepancies between drugs and their metabolites occur. It may be relevant that one unexplored fact concerning salivary secretion is that blood and saliva flow counter-current in the salivary gland, and this could be a crucial influence in diffusion related to lipid-solubility, which requires a finite time to reach equilibrium

vii) salivary monitoring is by definition indirect, and if the drug concerned affects the salivary gland, it may affect its own S/P ratio

viii) a variety of different glands are involved, and the S/P ratio measured may be no more than the mean of a group of ratios (Curry, 1981).

In principle, the analytical procedures which have been developed for the assay of drugs in plasma are equally suitable for drug monitoring in saliva (Horning et al., 1977). However, because of the complex composition of saliva, existing extraction procedures may require modification (as was found in our studies) or development of more sensitive methods.

1.4.1 Composition of saliva

Saliva performs a number of functions. It facilitates swallowing, keeps the mouth moist, serves as a solvent for the molecules that stimulate the taste buds, aids speech by facilitating movements of the lips and tongue, and keeps the mouth and teeth clean (Ganong, 1977).

Saliva is secreted into the mouth by three major paired salivary glands: the parotid, the submaxillary and the sublingual glands (Figure 1.4), and further by numerous small, labial, buccal and palatal glands (Van Dam and Loenen, 1978).

The fluids secreted by these glands differ considerably from each other and the composition is affected by

a) type, intensity and duration of stimulation
b) time of day
c) diet
d) age
e) sex
f) variety of diseases and pharmacological agents (Mandel, 1974; Ferguson and Botchway, 1980).

In general, saliva contains the usual electrolytes and organic consituents which are present in plasma, but often in lower concentrations. The most notable constituents of saliva are mucus and amylase, but the total protein concentration is less than 1% of that of plasma (Table 1.11). In general the osmotic pressure of saliva is about two-thirds that of plasma. As a result of a concomitant increase in bicarbonate concentration, the salivary pH (normal 6,5-7,2) rises from 6,2 to 7,4 with increasing rates of secretion (Knoebel, 1966; Schmidt-Nielsen, 1946). The salivary pH rises on storage in contact with air due to loss of carbon dioxide (Dawes and Jenkins, 1964).

1.4.2 Methods of collection

Several methods for the collection of saliva have been proposed. Human mixed saliva samples are generally collected by asking the subjects to spit into glass vials. Other techniques and devices for collecting specific types of saliva have been reported and it has been postulated that preference should be given to parotid saliva because it has a more constant composition than mixed saliva (Stephen and Speirs, 1976). In many cases, mixed salivary flow is stimulated by having the subjects chew paraffin wax (Hoeprich and Warshauer, 1974; Troupin and Friel, 1975) or parafilm (Koup, Jusko and Goldfarb, 1975; Koysooko, Ellis and Levy, 1974), or by sucking on a small piece of teflon (Boxenbaum, Berkersky, Mattaliano and Kaplin, 1975; Matin, Wan and Karam, 1974) or a glass marble (Inaba and Kalow, 1975; Lampman and Levy, 1975), or chewing on two rubber bands (in our laboratories) or sour chewing gum (Man, 1979). Salivary flow has also been stimulated with citric acid (Reynolds, Ziroyanis, Jones and Smith, 1976; Stephen and Speirs, 1976; Taylor, 1980) or by subcutaneous injection of 8 to 12 mg pilocarpine (Killman and Thaysen, 1955).

The advantages of stimulating salivary flow are:

i) large volumes can be obtained within a short time

ii) the pH of stimulated saliva mostly lies within a narrow range around the value of 7,0 whereas the pH of unstimulated saliva shows a larger variability, which can be of importance for the salivary excretion of weak acidic and basic compounds (Feller, Petit and Marx, 1976; Mandel, 1974; Schmidt-Nielson, 1946)

iii) the inter-subject variability in the saliva to plasma distribution ratio may be diminished when stimulated saliva is used as has been reported for digoxin (Jusko, Gerbracht, Golden and Koup, 1975).

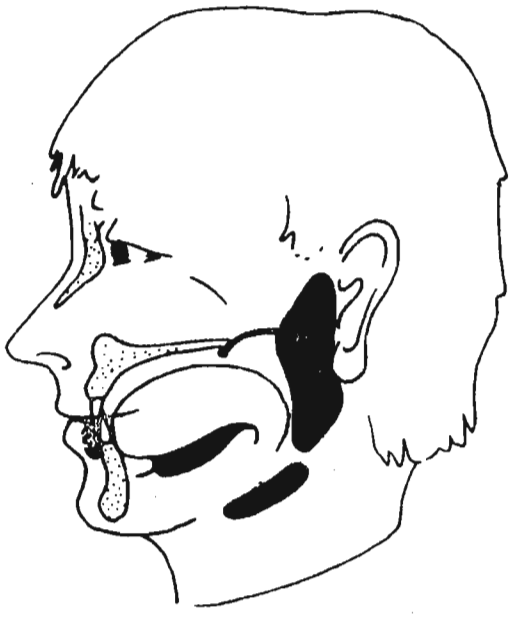


Figure 1.4: Topography of the salivary glands

1. glandula parotis
2. glandula sublingualis
3. glandula labialis
4. glandula submandibularis

Source: Van Dam et al., 1978

Table 1.11.: Salivary composition in normal adults compared with plasma (mean values)

Specimen[1]	Parotid saliva (0,7ml/min)[2]	Submaxillary saliva (0,6 ml/min)[2]	Plasma
	Meq/L		
Potassium	20,0	17,0	4
Sodium	23,0	21,0	140
Chloride	23,0	20,0	105
Bicarbonate	20,0	18,0	27
Calcium	2,0	3,6	5
Magnesium	0,2	0,3	2
Phosphate	6,0	4,5	2
	mg/100ml		
Urea	15,0	7,0	25
Ammonia	0,3	0,2	
Uric Acid	3,0	2,0	4
Glucose	< 1,0	< 1,0	80
Total lipid	2,8	2,0	500
Cholesterol	< 1,0		160
Fatty Acids	1,0		300
Amino Acids	1,5		50
Proteins	250,0	150,0	6 000
pH	6,8-7,2	6,8-7,2	7,35

1. Saliva samples were obtained after stimulation with 2% citric acid.

2. Flow rate in ml/min/gland.

Source: Mandel, (1974)

On the other hand, concentrations of drugs in saliva can be affected by stimulating saliva flow (Taylor, 1980). For instance, the concentration of lipophilic drugs in saliva can be diminished due to adsorption into or onto hydrophobic materials like parafilm.

For chlorpromazine and butaperazine, losses ranging from 15 to 34% and from 8 to 42% respectively at the initial concentration range of 2-20 µg/ml at room temperature (Chang and Chiou, 1976) have been reported. Thus one has to be selective in the method chosen.

It has also been suggested that when salivary excretion of drugs is a passive diffusion process, it would be theoretically possible that an equilibrium between the levels in the plasma and saliva is not reached when salivary flow is stimulated, resulting in too small S/P ratios (Borzelleca and Putney, 1970; Gruneisen and Witzgall, 1974).

1.4.3 Mechanism of drug excretion in saliva

Many drugs enter saliva by a simple diffusion process and thus lipid solubility may be a determining factor in salivary excretion of drugs, although polar substances can also enter into saliva (Hoeprich and Warshauer, 1974; Rasmussen, 1964). Substances can enter into saliva through the membrane lipoid as well as through water-filled pores in the membrane. (Amberson and Hober, 1932; Burgen, 1956).

For lipid soluble acidic or basic compounds, the diffusibility into saliva is dependent on the degree of ionization in plasma and saliva (verified with sulphonamides) (Gruneisen and Witzgall, 1974; Killman and Thaysen, 1955: Rasmussen, 1964). In the case of weakly acidic or basic compounds the S/P ratio can often be predicted based on equations 1.1 and 1.2 (Matin et al., 1974) where;

$$R = \frac{1 + 10^{(pH_s - pK_a)}}{1 + 10^{(pH_p - pK_a)}} \cdot \frac{fp}{fs} \quad \ldots\ldots\ldots \text{Acidic Compounds}$$

(Eq 1.1)

$$R = \frac{1 + 10^{-(pH_s - pK_a)}}{1 + 10^{-(pH_p - pK_a)}} \cdot \frac{fp}{fs} \quad \ldots\ldots\ldots \text{Basic Compounds}$$

(Eq 1.2)

where:

R	=	S/P ratio of drug
pH_s	=	pH of saliva
pH_p	=	pH of plasma
pK_a	=	pK_a value of drug
fs & fp	=	unbound fraction of the total drug concentration in saliva or plasma respectively

Due to the more acidic pH of saliva than of plasma, the concentration of some weak basic compounds e.g. lignocaine in saliva is higher than that in plasma (de Boer, Pronk and Breimer, 1977).

Saliva seems to relate to the free fraction of the drug, whereas data on concentration in plasma represents both the free and protein bound forms of the drug. Since only the free form of the drug is able to produce pharmacological effects, saliva concentrations may be of greater relevance in predicting therapeutic values (pharmacology and toxicity) than plasma levels, especially in the case of altered protein binding e.g. antipyrine. When the saliva concentration of a drug is influenced by the pH of the saliva as well as by the degree of protein binding, the S/P ratio can be predicted when the fp and fs values are considered in equations 1.1 and 1.2.

Drugs are considered as being unbound in saliva (fs=1) and for some substances (e.g. digoxin, sulphonamides, barbiturates and tolbutamide), this has been reliably established (Jusko et al., 1975; Killman and Thaysen, 1955; Matin et al., 1974; Rasmussen, 1964). However, there is evidence that binding of some drugs to saliva constituents cannot be totally excluded as shown with salicylate, propanolol and pentobarbital (Davis, Hartman and Fincher, 1971; Taylor, 1980; Lange, Floridia and Pruyn, 1969). Measurement of drug concentrations in simultaneously obtained samples of saliva and plasma, might be a convenient and simple method of estimating the extent of plasma protein binding of the drug. However, with only a few drugs (eg. theophylline amylobarbitone/amobarbital, tolbutamide, phenytoin and carbamazepine) have comparative studies shown that this is actually a real possibility (Bochner, Hooper, Sutherland, Eadie and Tyre, 1974: Inaba and Kalow, 1975; Koysooko et al., 1974; Matin et al., 1974; Troupin and Friel, 1975).

Active transport mechanisms for some drugs e.g. lithium, penicillin and phenytoin have also been described by Burgen, 1958: Groth, Prellwitz and Jahnchen, 1974; Allen Wrenn, Putney, Ahmad and Rogers, 1980; Borzelleca and Cherrick, 1965. There may be active transport mechanisms for other drugs in the salivary gland and perhaps they may explain the discrepancies in the S/P ratio that are often observed in single dose studies where the value appears to be time-dependent; the S/P ratio in the absorption phase being almost higher than in the elimination phase.

Such a phenomenon was observed in bioavailability studies of theophylline (de Blaey and de Boer , 1976; Knop, Kalafusz, Knols and van der Kleijn, 1975) where the concentrations in saliva sometimes appeared higher than in plasma. Theophylline (an acid with pKa = 8,6 - Martindale, 1972) would be less influenced by salivary pH in the absorptive phase.

1.4.4 Review of some plasma/saliva concentration relationships

If saliva is to be used for drug monitoring there must be a correlation between plasma and saliva drug concentrations over a wide concentration range. Earlier studies (refer 1.4.3) demonstrated that most drugs were transferred rapidly from plasma to saliva, and more recently it has been shown that the concentration of many drugs is proportional to the concentration in plasma (Horning et al., 1977). The view that, for most drugs, salivary concentrations reflects unbound drug concentrations in plasma is now accepted (Dvorchik and Vessell, 1976). Recently, it has been shown that despite no correlation between the two media, saliva concentrations of procainamide, rather than its plasma levels, were better correlated to pharmacological effect and responses (Galeazzi, Benet and Scheiner, 1976). Also, with digoxin, the serial salivary levels might be a better index of pharmacological effects of digitalis than with mean serum levels of the drug in healthy volunteers (Joubert, Muller and Aucamp, 1976a, 1976b).

Extensive studies on salivary excretion of different drugs have been done by many researchers. A comprehensive summary on S/P concentration ratios of drugs has been outlined in Table 1.12 (Danhof and Breimer, 1978). The relevance of such studies has been presented.

The most popular method or determining the relative bioavailability of a drug formulation is to undertake crossover studies to compare the areas under the plasma (or urine) drug concentration time curve ($AUC_{0\rightarrow\infty}$) (Koch-Weser, 1974; Wagner & Nelson, 1963). This method entails collecting enough blood (urine) samples to define the absorption and elimination portion of the concentration-time curve.

Table 1.12 Saliva/plasma concentration ratios of some drugs

Drug	Mean S/P ratio	S/P ratio range	Conditions	No. Subjects
Acetazolamide	0,009±0,001 (SD)	0,008-0,011	Single dose[1]	5
Aminopyrine	0,79±0,04 (SD)	0,73-0,86	Single dose	13
Amylobarbitone (amobartital)	0,35±0,03 (SD)	0,32-0,40	Single dose	5
Antipyrine	0,92±0,02 (SE)		Single dose	10
	0,89±0,14 (SD)	0,77-1,11	Single dose	6
	1,00±0,05 (SD)	0,95-1,09	Single dose	9
	0,92±0,04 (SD)	0,87-1,00	Single dose	12
Carbamazepine	0,37		Steady state[2]	1
	0,26±0,01 (SD)	0,25-0,28	Steady state	7
	0,42±0,05 (SD)	0,34-0,51	Steady state	17
Caffeine	0,55		Single dose	1
Digoxin	1,14±0,48 (SD)		Steady state	34
	0,78±0,07 (SD)		Steady state	18
	1,34±0,44 (SD)	0,77-1,85	Steady state	4
	1,68		Steady state	20
Ethosuximide	1,04±0,07 (SD)	0,92-1,14	Steady State	4
Isoniazid	1,02	0,90-1,17	Single dose	2
Lignocaine (lidocaine)	1,78±0,39 (SD)	1,45-2,21	Single dose	3
Lithium	2,85±0,59 (SD)	2,13-3,47	Single dose	3
	3,43		Single dose	2
	about 2		Steady state	12
		2-5	Steady state[3]	5
Paracetamol (acetaminophen)	1,40		Single dose	8
Penicillin	0,015±0,015 (SD)	0,004-0,050	Single dose	10
Pentobarbitone	0,42±0,18	0,24-0,75	Single dose	6
Phenacetin	0,60±0,19	0,41-1	Single dose	13
Phenobarbitone	0,32±0,02 (SE)	0,22-0,5	Steady state	14
	0,30±0,03 (SE)		Steady state	8
		0,26-0,34	Steady state	
	0,37±0,02		Steady state[3]	
Phenytoin	0,11±0,04 (SE)	0,06-0,19	Steady state	35
	0,101±0,003		Steady state	38
	0,091±0,017 (SE)		Steady state	6
	0,103±0,015 (SD)	0,087-0,129	Single dose[3]	9
	0,015±0,004		Steady State	17
	0,11±0,02 (SD)		[3]	
	0,24±0,24 (SD)	0,06-1,00	Steady state	32
	0,108±0,05 (SD)	0,021-0,316	Steady state	33
Primidone	0,97±0,05 (SE)	0,5-1,4	Steady state	31
	1,08±0,08 (SE)		Steady state	3
	0,85±0,02	0,63-1,09		

Table 1.12 (Continued)

Drug	Mean S/P ratio		S/P ratio range	Conditions	No. Subjects
Procainamide	3,50±2,34	(SD)	0,27-8,93	Steady state	12
	1,62±0,61	(SD)	1,35-2,12	Single dose	4
Quinidine	0,51±0,12	(SD)	0,42-0,65	Single dose	3
Salicylate	0,033±0,005	(SD)	0,029-0,039	Single dose	3
Streptomycin	0,15±0,08	(SD)	0,06-0,27	Single dose	11
Sulphacetamide	0,92			Single dose	1
Sulphadiazine	0,31±0,03	(SD)		Single dose	2
	0,34			Single dose	1
Sulphadimidine	0,72±0,06	(SD)		Single dose	2
Sulphamerazine	0,32±0,02	(SD)		Single dose	2
Sulphanilamide	0,87±0,10	(SD)		Single dose	5
	1,08			Single dose	1
Sulphapyridine	0,81±0,17	(SD)		Single dose	2
	0,49			Single dose	1
Sulphathiazole	0,23±0,04		0,46-0,58	Single dose	2
Theophylline	0,52±0,03	(SD)	0,16-0,58	Single dose	7
	0,58			Steady state	16
	0,77±0,07	(SD)	0,64-0,86	Steady state	6
	0,85		0,77-0,92	Single dose	2
	0,65			Steady state	22
	0,49±0,04	(SD)	0,44-0,52	Single dose	4
	0,75			Single dose	5
Tolbutamide	0,012±0,001		0,012-0,013	Single dose	3

1. Single dose: the S/P ratio was established after intake of a single dose of the compound.

2. Steady state: the S/P ratio was established when the drug was taken regularly.

3. The concentration of the drug was established in parotid saliva.

Source: Danhof et al., 1978

Posti (1979) has presented mathematical expressions to estimate pharmacokinetic parameters and bioavailabilities (absolute and relative) on the basis of drug concentrations in saliva, provided there is correlation of S/P ratios. He has provided rational explanations to justify more elevated S/P ratios of drugs during the invasion (absorption) phase rather than during the elimination phase.

Comparisons of bioavailabilities of two formulations of phenytoin, administered orally, using serum and mixed saliva data have been reported (Paxton and Wilcox, 1980a). There was no significant difference in serum or salivary $AUC_{0\rightarrow\infty}$ values, peak concentrations, time to reach peak concentrations and elimination half-lives after administration of tablets or capsules, indicating bioequivalence for the two products. Previous studies have shown the salivary concentrations of phenytoin to be independent of the degree of stimulation (Paxton, Rowell, Ratcliffe, Lambe, Nanda, Melville and Johnson, 1977b, 1977c) and pH of saliva (Bochner et al., 1974). Salivary concentrations of phenytoin are also representative of the free pharmacologically active fraction of drug in serum (S/P ratio in unity - Cook, Amerson, Poole, Lesser and O'Tuama, 1976). Combined therapy with carbamazepine, sodium valproate, ethosuximide and phenobarbitone does not change the S/P ratio for phenytoin (Cook et al., 1976: Schmidt and Kupferberg, 1975) and S/P concentrations correlated closely in patients with chronic renal failure (Reynolds et al., 1976). These observations have led to the suggestion that bioavailability and pharmacokinetics of phenytoin and management of therapy could be more appropriately monitored with salivary rather than with plasma drug levels (Knott, Hamshaw-Thomas and Reynolds, 1982). It has been reported by several authors that the concentration of phenobarbitone in saliva is proportional to the corresponding plasma level, the mean S/P ratio being

0,29 to 0,33 (Cook et al., 1976; Horning et al., 1977). Calculations based on equation 1.1 (Section 1.4.3) assuming non-binding in saliva, 40% binding to serum protein, a pH for saliva of 6,5 and a pH of 7,4 for plasma, predict a S/P ratio of 0,31 which is less than the actual reported unbound total plasma concentration ratio of 0,4 to 0,5 (Cook et al., 1976, Schmidt and Kupferberg, 1975; Eadie, 1976). This large variability may be due to changes in glandular salivary pH after mixing with contents in mouth, as was suggested by Koup et al., 1975. Therefore it is questionable whether salivary data can substitute plasma data in therapeutic monitoring for all drugs.

In the body, primidone is converted to two active metabolites; phenobarbitone and phenyl-ethyl malonamide, and there is correlation between metabolite levels and anticonvulsant effect. It has been established that there is good correlation between primidone concentrations in plasma and saliva, the mean S/P ratio being close to 1 (Horning et al., 1977). Since primidone is not bound to plasma (Eadie, 1976) this ratio is not influenced by combined therapy with other antiepileptics and the CSF/P ratio appears to be the same as the S/P ratio (Schmidt and Kupferberg, 1975). It seems likely that primidone therapy as well as ethosuximide (acidic drug with negligible plasma protein binding - Eadie, 1976) therapy could be monitored using salivary data instead of plasma levels.

Studies on the kinetics of carbamazepine by enzyme immuno assay, showed that whole salivary and uncontaminated parotid salivary carbamazepine (CBZ) concentrations were not different and were independent of volume of fluid produced, pH of saliva and degree of stimulation. The mean CBZ concentration ratios of whole/total serum and ultrafiltrate/total serum ranged from 19,6 to 34,7% and from 19,0 to 28,8%. Linear

correlations were found between saliva and serum and ultrafiltrate concentrations. These results substantiated the report by Westenberg, Van der Kleijn, Dei and Zeeuw, (1978), that whole saliva would be useful for monitoring carbamazepine therapy, especially in situations in which serum protein binding is altered due to disease, age or interactions with other drugs or endogenous substances (Paxton and Donald, 1980b).

The use of saliva rather than plasma for nortriptyline (Kragh-Sorensen and Larsen, 1980) or with theophylline (de Blaey and de Boer, 1976) cannot be recommended because the S/P ratio varied both intra- and inter-individually by large factors. However, the use of salivary levels of theophylline may be useful for determination of the elimination kinetics and for comparison of the rate and extent of bioavailability of the drug from different theophylline preparations (Nakano, Nakamura, Juni and Tomitsuka, 1980).

The advantages of using saliva rather than plasma in therapeutic drug monitoring or in kinetics have been discussed (Mucklow, Bending, Khan and Dollery, 1978). The existence of good correlations between saliva and plasma levels for amytriptyline (Jeffrey and Turner, 1978), for digoxin (Kondo et al., 1981) and for antipyrine (Chang and Chiou, 1976) has been established. Results on antipyrine indicated that the plasma half-life was predictable from the half-life of the drug in saliva.

In the case of drugs such as amphetamine with excretion rates which are sensitive to urinary pH changes (Beckett and Stenlake, 1967), the use of salivary measurements may have advantages over urinary data in pharmacokinetic studies, provided that only postabsorption data are used (Suk Han Wan, Matin and Azarnhoff, 1978). The measurement of salivary

pyrimethamine concentrations, in addition to allowing further study of the pharmacokinetics of this drug, would allow a check on patient compliance when used for malarial prophylaxis (Allen, et al., 1980). Salivary erythromycin concentrations may be used as an alternate to plasma data when monitoring the course of treatment in conditions where it is imperative to rapidly achieve adequate therapeutic concentrations of the drug in tissues (Henry, Turner, Garland and Esmieu, 1980).

In most cases, simple urine tests are used for phenotyping rapid and slow acetylators. Studies have been done to show that monitoring saliva concentrations could be used for determining the acetylator phenotype in patients treated with sulphasalazine (Bates, Blumenthal and Pieniaszek, 1976) and isoniazid (Boxenbaum et al., 1975).

When S/P ratios in individual subjects have been established by taking a few blood samples, the measurement of saliva concentrations, which are easier and more convenient to collect than to attempt venipuncture of often agitated psychotic patients (Man, 1979), could provide all pharmacokinetic information necessary for rational dosage requirements in lithium therapy (Groth et al., 1974; Neu, Dimascio and Williams, 1975).

Recent reports have suggested that the determination of steroid hormones concentrations in saliva could well become the method of choice for testing endocrine function (Walker, Fahmy and Read, 1978; Walker, Read, Hughes and Fahmy, 1979). Such studies are in progress on prednisolone (Chakraborty, Hayes, English and Marks, 1981) while salivary progesterone assays have been used to monitor menstrual cycles in Bangladeshi women (in Wales) who have superstitious fears about blood sampling (Seaton and Fahmy, 1980), and the circadian

rhythm of testosterone production in man has been demonstrated by using saliva data (Baxendale, 1980).

1.5 Aims and Objectives of Study

Diethylpropion hydrochloride is a popular anorectic agent taken daily in divided doses i.e. 3 x 25 mg or as a sustained release tablet i.e. 1 x 75 mg (1.3).

It is rapidly and extensively metabolised, displaying pronounced "first-pass" effect after oral administration. Thus diethylpropion shows marked inter- and intra-subject variation of metabolism dependent on many factors. The pharmacological activity and mild side-effects like disturbance in sleep patterns, are likely to be accountable to the five metabolites, each having different physico-chemical and pharmacological properties, with probably the greater contribution coming from the two major metabolites viz. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) (Scheme 1- Beckett and Testa, 1973).

To overcome variability in metabolism and also the unpleasant side effects, which may be associated with very high levels of metabolites at various sites, it was proposed to control the rate of input of the drug into the body over a desired duration by the appropriate choice of controlled delivery systems (sustained release pellets) and the possible use of an alternate route of administration (Chapter 3).

It is known that sub-division of an oral dosage form leads to a scatter of the dosage form throughout the GIT, thus releasing the drug at different rates for absorption (see 1.3.2) Hence the chosen dosage form containing the drug is incorporated into several small pellets, each coated with a non-biodegradable diffusion rate-controlled membrane. Such pellets offer more predictable systems than those based upon erosion or membrane rupture (1.3).

The main aim of the present study was to evaluate the bioavailabilities of different formulations of diethylpropion by monitoring the two major metabolites and where possible, the unchanged drug, in plasma, saliva and urine samples. Condition of acidic urine was maintained to minimize kidney tubular reabsorption and to avoid other difficulties (Beckett and Tucker, 1967).

Most drugs which are of interest for sustained release administration have previously been used in conventional products. It is not surprising that one aim of the initial in vivo studies on diethylpropion is generally to establish the advantages of the new dosage form in comparison with the conventional one and to evaluate how the changed absorption pattern affects the in vivo performance of the drug. Examples of relevant questions in such studies are:

- the pattern of drug/metabolite concentration in blood versus time
- reduction in the frequency of drug administration
- frequency of side effects
- physiological availability
- reproducibility of drug/metabolite levels on a dosage regimen.

In pharmacological testing of compounds, it is no longer sufficient to give doses of the compounds through various routes and to note an effect at a stated time. The time course of the response, as well as some indication of the time course of the drug levels (and metabolites in this case) is needed if we are not to be misled by superficial information. In the pre-clinical testing of drug safety, biochemical studies of drug absorption, distribution, metabolism and excretion are important (W.H.O. Report No. 341, 1966). Modern techniques are facilitating these studies. The importance and purpose of measuring the levels of metabolites as well as parent drug in biological fluids i.e. blood and urine have been clearly stressed (Beckett, 1973a).

Salivary excretion of various drugs has attracted much interest in recent years. The observation that drug (or metabolite/s) concentrations in saliva are often proportional to the concentration in plasma has led to the suggestion that in therapeutic monitoring or in obtaining pharmocokinetic parameters or in evaluating bioavailabilities of different formulations (products), saliva may be substituted for plasma (1.4). Therefore it was proposed to compare concentration-time profiles and to establish possible correlations of S/P ratios and urinary excretion rate/plasma concentration (U/P) ratios for each of the two major metabolites under acid urine conditions, after administration of the sustained release pellets formulation Lot R 7773. The use of salivary data, based on the respective correlations established to predict plasma or urinary levels will be considered; alternately, whether salivary measurements of the metabolite/s could be used to substitute plasma in evaluating bioavailability of the different dosage forms.

It was also proposed that the possibilities of avoiding first-pass metabolism by rectal administration should be investigated. Another aim was to establish whether the in vivo behaviour of the sustained release pellets following oral administration could be repeated following rectal administration i.e. would changing the environment (pH 1,5 to 7,5 in the GIT to pH 6,9 in the rectum) alter the release pattern of the drug in the pellets?

Another aim of the study was to determine the minimum influence of food on the bioavailability and metabolism of diethylpropion from sustained release pellets as compared to the conventional sustained release tablet.

The in vitro methods of testing are a necessary part of development of drug preparations. Therefore one aim of this study was to demonstrate the importance of selecting a meaningful dissolution test for correlation with in vivo data.

In vitro dissolution tests were carried out in different ways (Chapter 2), to establish a meaningful dissolution test to measure the constancy of the pellets throughout the present study.

The stability of the sustained release pellets formulations on storage under different conditions had to be confirmed. The release of the drug from suppositories was also investigated for repoducibility.

In vitro/in vivo correlations facilitate the design of suitable sustained release dosage forms. The possibility of such correlations was to be investigated, using urine data of metabolite II and establishing whether the *in vivo* behaviour (i.e. excretion rate profile) of the dosage form administered orally or rectally, could be predicted on the basis of the *in vitro* results.

An attempt was made to predict the urinary excretion rate profile of ethylaminopropiophenone (metabolite II) using the proposed mathematical approach (4.4), and then to compare these results (to check the validity of these equations) with those profiles obtained during different *in vivo* studies using sustained release pellets. Such predictions of the shape of profiles using *in vitro* data could be useful to show, among other things, the relevance of *in vitro* procedures to *in vivo* situations and ultimately to facilitate the design of suitable formulations (with satisfactory *in vitro* release to reflect closely the *in vivo* situation) without the use of many subjects and elaborate trials.

To meet the objectives of this investigation on the conventional and sustained release pellets formulations, the development of specific and sensitive analytical procedures based on gas-liquid chromatography was necessary. Other analytical techniques e.g. thin-layer chromatography and ultra-violet spectrophotometry were also utilized for the study of the compounds.

CHAPTER 2

IN VITRO EXPERIMENTAL

PART A: DISSOLUTION STUDIES

2.A.1 Introduction

The determination of bioequivalence of drug products by means of *in vivo* studies presents innumerable problems. It is time consuming and requires a large number of human subjects; it is affected by many biological variables and it may be economically non-feasable when applied to routine testing. Therefore the development and implementation of *in vitro* standards, that reflect in *in vivo* drug performance, is essential. Likewise, the study of the stability of sustained release preparations demands an *in vitro* approach.

About 30 years ago, disintegration was felt to be the important factor in the testing of the formulation of solid dosage forms. The disintegration data of a tablet were regarded to be directly related to the *in vivo* availability of the drug. However, in the mid 1950's when sustained release formulations were developed, it was felt that there was a need to determine the release patterns of drugs into aqueous solutions from the various dosage forms. The wide variations and anomalies in disintegration and absorption data found between apparently equivalent dosage forms, and the inability of some of the testing methods to distinguish between differences in release rates of the drug from the dosage forms, prompted research into the development of model *in vitro* systems. It was realised that, with few exceptions, disintegration tests did not serve a meaningful index of the availability of poorly water-soluble drugs in the body. There are reports in the literature describing correlations between *in vitro* disintegration times and *in vivo* availability, but there are many where no such correlations have been observed. These studies have been reviewed

(Morrison and Campbell, 1965; Wagner, 1969; Dakkuri and Shah, 1982). In the present study, it was found that the pellets did not disintegrate in in vitro tests (Chapter 2B), and after oral administration were excreted intact in the faeces without rupture of the diffusion membrane coating (A.H. Beckett, personal communication).

Dissolution is the act of dissolving, while rate of dissolution is the rate of dissolving in water or aqueous solution of a chemical or drug from the solid state. There is adequate evidence to conclude that the rate of dissolution often partially or completely controls the rate of absorption (Wagner, 1970).

The development and use of in vitro models to simulate and describe dissolution and in vivo absorption serve several useful purposes. In the present study, in vitro dissolution tests were used to investigate the stability of two batches of pellets, i.e. to investigate any changes in the release profiles of the drugs after storage under various conditions, and to compare these pellets in order to predict their in vivo behaviour. For in vitro models to be of any value they should mimic the in vivo system to such a degree that consistent correlations are obtained.

The inclusion of in vitro dissolution tests in the USP XX/NF XV and the BP 1980 support the realization that there is a significant relationship between in vitro dissolution and in vivo availability.

Edwards (1951), was the first to appreciate that if the absorption process of the drug from the gastrointestinal tract (GIT) was rapid, then the rate of dissolution of drug from the dosage form could be the rate-limiting step to the appearance of the drug in the body. Since then, increasing attention has been paid to the mechanism of dissolution and physico-chemical factors affecting dissolution. The prime objective was, and still is, to establish correlations between in vivo availability and in vitro dissolution data in instances where dissolution is the rate-limiting step.

Mechanism of dissolution

To explain dissolution, various theories have been suggested since 1897 by several different workers. According to Higuchi (1967), there are three processes which either alone or in combination, can be used to describe dissolution rate mechanisms. The simplest is the diffusion layer model, based on the earliest equation expressing rates of solution, Eq. 11.1.

$$\frac{dc}{dt} = K\,(Cs-C) \qquad \text{Eq. 11.1}$$

where $\frac{dc}{dt}$ = the rate of change of concentration with time

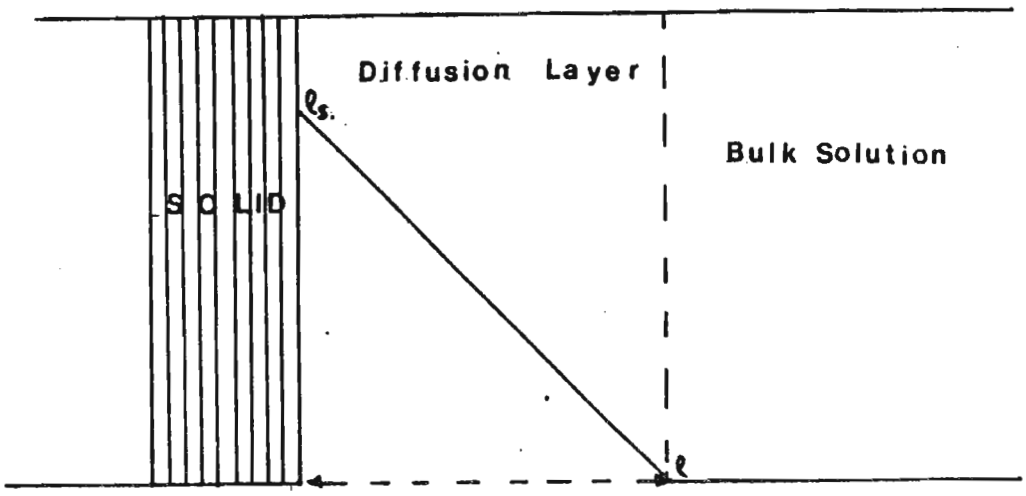
C = concentration of solute at time, t (negligible under sink conditions)

Cs = concentration of a saturated solution of a solute in the dissolution medium and

K = constant with dimension, time $^{-1}$

(Noyes and Whitney, 1897), and extended in studies by Nernst and Brunner (1904), where it is assumed that there is a stationary liquid film attached to the solid surface - Scheme 2.1.

The rate of dissolution is governed entirely by the diffusional transport of solute molecules through the liquid film. Once the solid liquid molecules have passed the liquid film/bulk interface rapid mixing occurs and the concentration gradient is destroyed - Eq. 11.2.



Scheme 2.1: Nernst and Brunner (1904) diffusion layer model of solids - Wagner, (1971).

$$\frac{W}{S} = \left(\frac{D\ Cs}{h}\right).\ t = G.t \quad \text{.....Eq. 11.2}$$

Where

W = mass of solute dissolved in time, t

S = surface area of solute available for dissolution

h = effective diffusion layer (film) thickness

Cs = as explained in Eq. 11.1

D = solute molecule diffusion coefficient

G = intrinsic rate of dissolution = $\frac{1}{S} \cdot \frac{dw}{dt}$

Hence a plot of the amount dissolved, w, divided by the constant surface area, S, against time, will yield the intrinsic rate of dissolution, G.

The interfacial Barrier Model, an extension of the Nernst and Brunner Model, assumes that the reaction at the solid surface is not instantaneous. The process at the solid/liquid interface, due to high free energy of activation, is rate-limiting with respect to the transport process. There is then rapid transport through a static liquid film.

The third model devised by Danckwert (1951), postulates that there is no boundary layer and that turbulence extends to the solid surface. He assumes that transport of solutes away from the solid is achieved by macroscopic packets of solvents reaching the solid liquid surface by eddy diffusion, absorbing solute by diffusion when attached to the surface. The reaction is instantaneous and the rate at which the above occurs is related to solute transport rate and hence dissolution.

The rate laws predicted by different mechanisms of dissolution, both alone and in combination, are discussed (Higuchi, 1967) in an extensive review of drug release rate processes. The different mechanisms of dissolution from solids have also been reviewed and discussed (Swarbrick, 1970; Wagner, 1971; Hanson, 1982).

Factors affecting the dissolution rate

The physico-chemical factors that control the rate of dissolution can in turn affect the onset, duration and intensity of the pharmacological response to the drug by altering the rate of presentation to the active sites. These factors include temperature, agitation, pH, solubility, concentration gradient, composition and viscosity of the dissolution medium and the presence of active or inactive additives. These factors have been discussed (Levy et al., 1960; Wood, 1967). The effects of some of the properties like agitation intensity, drug solubility and surface areas on the dissolution rate have been fully reviewed (Swarbrick, 1970; Barr, 1972; Hanson, 1982; Dakkuri et al, 1982; Roufail, 1983).

The effect of the acid conditions of the stomach on weak acid salts and free bases have been discussed (Wagner, 1971). Morozowitch et al., (1962) reported on the dissolution of benzamphetamine from pellets in 0,12 M hydrochloric acid. In another study, the solubility of tablets containing either warfarin or its sodium salt in aqueous media or different pH values, was studied (O'Reilly et al., 1966). Recently the influence of the composition of dissolution media on the release from sustained release formulations of propoxyphene (Baggesen et al., 1980) and ketoprofen (Hazzanzadeh, 1981) has been reported.

There appears to be relatively little information in the literature concerning the effect of the dissolution medium on the results obtained, although it is a very important factor.

The majority of the monographs calling for dissolution tests in the BP and USP suggest distilled water or dilute hydrochloric acid as a dissolution test medium. Very few attempts are being made to simulate conditions in the GIT. The NF XIV method for the rotating bottle dissolution test, is the only method suggesting the use of buffers to simulate pH conditions following oral administration.

The introduction of *in vitro* dissolution tests in the BP necessitated a considerable programme of laboratory investigations. The importance of minimizing variations, mainly in the rotating basket method, was the objective of the study (Cartwright, 1979). Factors which were identified included sampling position, temperature of dissolution medium, dissolved air, filter adsorption and filter release of interfering substances, and design variables of the apparatus (Underwood and Cadwallader, 1976).

If dissolution is the rate-limiting step in in vivo dissolution and absorption processes, the concentration of the free drug in solution in the gut will be low on account of the relative high absorption rate. Sink conditions are likely to prevail in vivo. For meaningful in vitro data it is therefore necessary to ensure that such sink conditions are used. According to Gibaldi et al., (1967), sink conditions can be assumed if the total amount of drug in solution does not exceed 10-20% of the saturation concentration. In the present study, sink conditions were maintained at a constant temperature of 37°C, by completely renewing the dissolution medium every 1 - 2 hours. The concentrations of drug in any medium did not exceed, 0,5% $^{m}/_{v}$.

In vitro dissolution test methods

A large number of different test methods are adequately described in the literature (Krowczynski, 1978; Swarbrick, 1970). Natural convection methods, where there is no forced agitation, include the hanging pellet method, the static disc method and the sintered filter method. The more popular and common forced convection methods include the three methods employed in the present study; the beaker method (rotating paddle method), the rotating basket method and the rotating bottle method.

The rotating bottle method was first devised (Souder et al., 1958) to follow the release of dextroamphetamine sulphate from sustained release pellets. Samples of pellets were put into bottles containing 60 ml of dissolution medium. One bottle was prepared for each time interval for sampling. The bottles were placed on a rack in a waterbath at 37°C and rotated end over end at 40 r.p.m. The dissolution medium was simulated gastric juice USP XX (pH 1,2) and after 1,5 hours it was replaced by simulated intestinal fluid USP XX (pH 6,9).

Other workers have used the rotating bottle method with different sized containers and at different rates of rotation (Shenoy, Chapman and Campbell, 1959; Krueger and Vliet, 1962).

An inherent disadvantage of the rotating bottle method is said to be the need to stop the apparatus and remove the samples (Hensey, 1969). The method has been criticized by Wagner (1960) on the basis that the intensity of agitation may be too great, therefore effectively obliterating any in vitro differences that one might expect if in vivo differences are seen. His view was supported by Hamlin, Nelson, Ballard and Wagner, (1962).

In March, 1967, the rotating bottle method was included in the NF XII as an in vitro test procedure for time release tablets and capsules. The intent was to set up suitable criteria in order to ensure uniformity of products. Therefore it has not been included in the NF XV as an official specification for any preparation/s. The rotating bottle method, as used today, has been modified by many workers.

The rotating basket method was originally described by Searl and Pernarowski, in 1967, and it is based on the original beaker method (Levy et al., 1960). The NF XIII introduced the rotating basket assembly as one of the two official methods for dissolution studies.

Other "forced convection" dissolution test methods include the stationary basket method, the oscillating tube method, the rotating disc method and dialysis method. Details on these methods have been given (Wagner, 1971; Hanson, 1982).

In a useful critical review with 126 references (Hersey and Marty, 1975) the dissolution method and various modifications, were discussed. In a survey of dissolution test methods (Pernarowski, 1974) 150 apparatus designs were reported. Improvements in equipment for various levels of testing, from one tablet to multitablet simultaneous testing, were also discussed (Miller, 1977; FIP, 1981; Dakkuri et al., 1982).

The hydrodynamics of four dissolution test methods, the USP XIX rotating basket method, the rotating paddle method, the basket spinning filter method and the USP XIX disintegrating apparatus have been characterized and compared (Carstensen, Lai and Prasad, 1978).

Recently an automated dissolution apparatus for non-disintegrating pellets and granules was described (Ramsey, Newton and Shaw, 1980). It was based on the rotating bottle method, but did not suffer from the disadvantages of it, namely the need to interrupt the rotation and hence the dissolution process to withdraw samples. It would not, however, be easy to change the buffer and hence the pH completely every so often as in our study. In another study, an apparatus was described using liquid turbulence to simulate the hydrodynamic conditions generated by gastrointestinal peristalsis (Simmons et al., 1975).

An automated dissolution system that minimizes the shortcomings of previous systems and is suitable for simultaneous testing of five or six samples of either conventional or controlled release dosage forms, has been described (Embil et al., 1983). The system showed excellent correlations with manual dissolution determinations, but the multipoint dissolution profile obtained by the automated procedure more accurately defined the time course of drug release from the products. In addition this system was sensitive enough to discern differences between two controlled release products at the later time periods.

A multicompartment dissolution test system consisting of two or more flow cells was described as a novel method in which the dosage form disintegrated in the gastric part of the model and was continuously pumped into the intestinal part (Slipper, 1981). It was suggested that although the equipment was too complex for routine dissolution testing, it could be used in research and development to obtain <u>in vitro/in vivo</u> correlations.

To attain good *in vitro/in vivo* correlations, endeavours must be made to simulate the physiological conditions as close as possible. In point of fact, however, perfect simulation and consequently a universal standard method is not attainable and, in any case, it could never be adequately assessed since drug levels *in vivo* are not only determined by the dissolution of the drug, but also by absorption, metabolism, distribution and excretion as well as by a possible concentration dependence of all these processes. Therefore it is necessary to develop a predictive testing method for each sustained release formulation. In practice, this is done as follows:

a) Promising test preparations are selected beforehand with the aid of a test method which has been shown to be suitable in our study. Thus two sustained release pellets formulations (Lot R 7773 and R 7574) are selected and the rotating bottle method (2.A.2.2.d) which was found to give good reproducibility (Slipper, 1981), is used.

b) These preparations are tested *in vivo* to ascertain the differences in profiles.

c) The test method is modified until the results achieved with it reflect the differences observed *in vivo* [in this study we used different media (pH 1,5-7,5) for various exposure times]; the speed of rotation of the bottle is kept constant.

The need for a standardized dissolution apparatus as a means of generating meaningful *in vitro* data has been discussed (Skelly, 1977). Recently the FIP (Federation Internationale Pharmaceutique) working group No. 5, published a report on "Guidelines for dissolution testing" FIP, 1981. They reached the following conclusions:

a) It is necessary that uniform *in vitro* testing methods are used for the measurement of dissolution rates. This is important not only for the development of new drugs, but also for the quality assurance of established pharmaceutical formulations. A standardized dissolution test is, for the pharmaceutical manufacturer, an important part of process validation. In the area of drug development, such a test would be equally valuable in providing guidelines which would still allow some flexiblity in selecting testing conditions and would preserve some degree of scientific freedom. In addition to these considerations, there is also a compelling need for world-wide uniformity so as to increase drug safety

b) In test conditions the physicochemical properties of the active ingredient and drug form should be taken into account so that a realistic model of the physiological conditions can be obtained

c) It is very important, not only for the development of new drugs but also for quality control, to attempt to achieve correlations between *in vitro* and *in vivo* results. This is because it is possible, only by the comparative evaluation of *in vitro* and *in vivo* data, to lay down these specifications for the rate of dissolution of a preparation, which are important for process validation and for stability evaluation.

The FIP working group proposed two basic alternatives for testing dissolution rates:-

a) Stirred-tank Method in the form of the paddle apparatus USP XX/NF XV (with a few modifications) and

b) Flow-through Method,

towards obtaining a world-wide standard method, for testing the dissolution rate of different oral dosage forms which will correlate with the *in vivo* absorption rates in man.

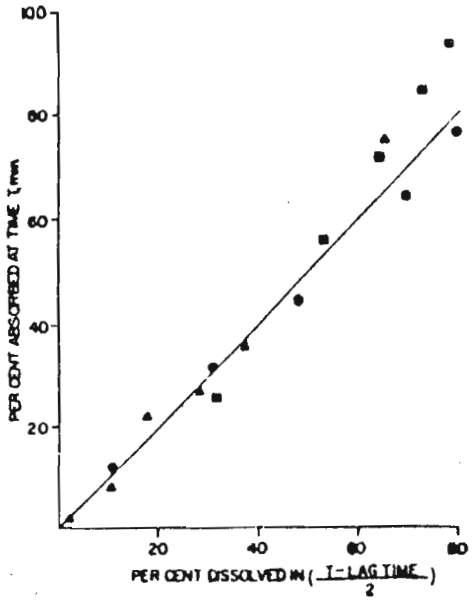
There are many reports in the literature of attempts to establish in vitro/in vivo correlations (Dakkuri et al., 1982; Roufail, 1983). Some examples of different methods and mathematical approaches adopted by investigators to obtain correlatability of the data have been presented in Figure 2.1. A survey, made of the literature (1962-1982) for investigations characterizing both in vitro and in vivo performance of the drug products, unearthed 50 investigations (Banaker and Block, 1983). This review showed that no universal dissolution test method has yet been devised that in every instance gives the same rank order for in vitro dissolution and in vivo availability for different formulations or batches. However, the authors demonstrated that improved correlatability can be achieved if investigators would use the approach of von Hattingberg, Brockmeier and Voegele (1982) which involves:

a) determining dissolution as a function of time from $t = 0$ to $t = \infty$
b) completely characterizing drug uptake or elimination as a function of time
c) transforming the in vitro and in vivo data using the mean time concept or some other scale transformation.

Experimental experiences in the determination of dissolution rates and their correlations with in vivo results have been published (Rothe and Schellhorn, 1977). The authors recommend Pools' Paddle Method (USP - NF 1978, 4th supplement) as a suitable procedure for the determination of dissolution rates for inclusion in the European Pharmacopoeia (EP). They later proposed a formulation for the monograph "Dissolution" for inclusion in the EP (Rothe and Schellhorn, 1978). The method was said to be suitable for worldwide standardization of testing procedures.

The present study is designed to compare and to evaluate the in vitro dissolution profiles of some sustained release pellets formulations of diethylpropion hydrochloride; to investigate the stability of the pellets under different storage conditions and to predict the in vivo behaviour of the pellets. Furthermore, the influence on the dissolution rate when changing the composition of

Figure 21.9. Correlation of percent aspirin absorbed at time T (estimated by method of Wagner and Nelson, 1963) with percent aspirin dissolved in vitro at time (T log time)/1 for absorption from solution, plain tablets and microencapsulated particles. See text for explanation. From Levy et al (1965), reproduced with permission of the copyright owner



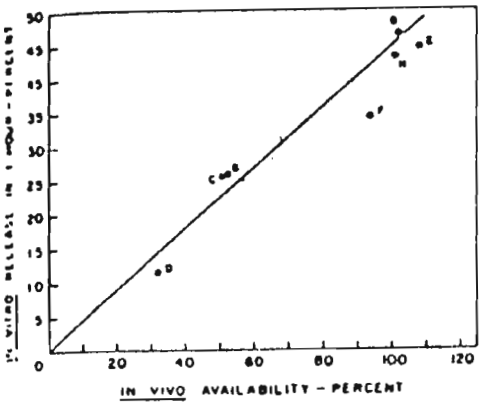


Figure 21.2. Correlation of percent amphetamine released in vitro in one hour with in vivo availability for seven different brands of so-called sustained-release capsules containing coated pellets of amphetamine. From Shenoy et al (1959), reproduced with permission of the copyright owner

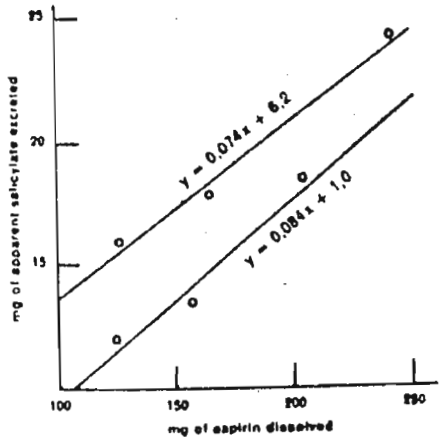
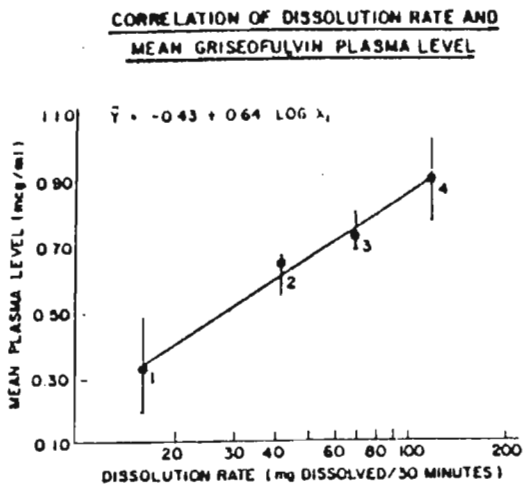


Figure 21.6. Relationship between the mean amount of apparent salicylate excreted in one hour after administration of two 5 grain aspirin tablets and the amount of aspirin dissolved in ten minutes from one tablet in an in vitro test. See text for explanation. From Levy (1961) reproduced with permission of the copyright owner

Figure 2.1: Examples of a few in vivo/in vitro correlations for different drugs (adopted from J.C. Wagner, Biopharmaceutics and relevant pharmacokinetics, 1st Ed., 1971).

Figure 21.14. Correlation of average plasma concentrations of griseofulvin after a single oral dose of 500 mg in 10 healthy subjects with the amount of griseofulvin dissolved in 30 minutes in simulated intestinal fluid for four different griseofulvin preparations. From Kalchen and Symchowicz (1967), reproduced with permission of the copyright owner



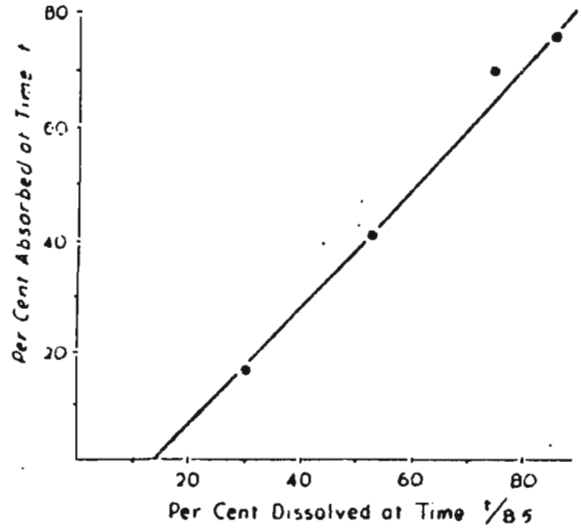


Figure 21.10. Correlation of percent aspirin absorbed at time T (estimated by method of Wagner and Nelson, 1963) with percent aspirin dissolved in vitro at time T/8.5. See text for explanation. Reprinted with permission of Levy (1968) and Pergamon Publishing Company

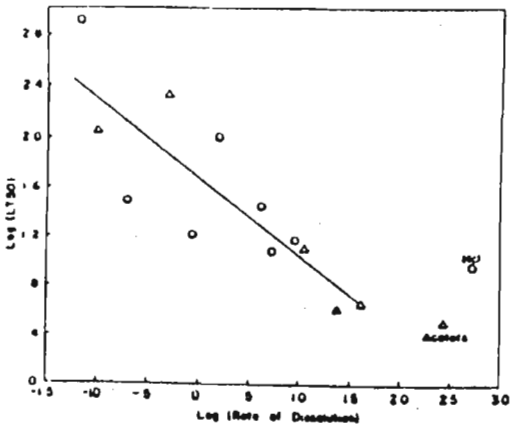
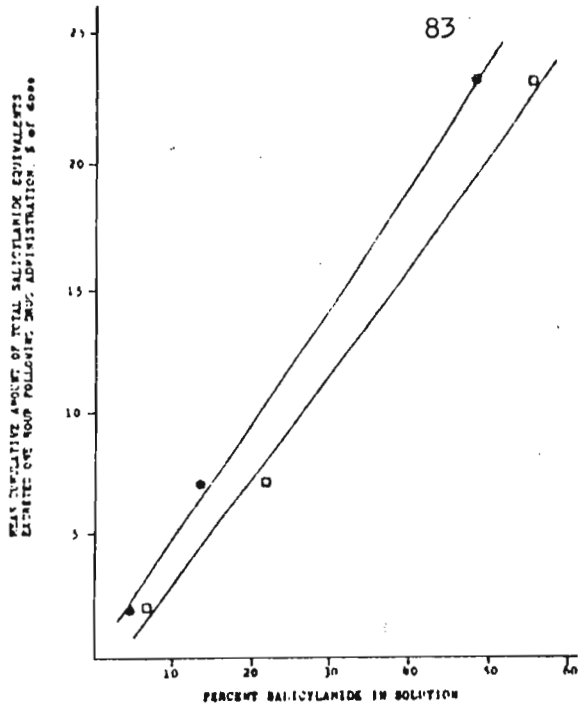


Figure 21.12. Log-log plot of LT in mice against equivalent dissolution rate at pH 7.2 for etryptamine and benzphetamine salts. Key: ○, benzphetamine salts; △, etryptamine free base and four of its salts. See text for explanation. Original data of Moravowich et al. (1962) as re-evaluated by Di Santo and Wagner, *J. Pharm. Sci.* 58, 1077-1085, 1969. Reproduced with permission of the copyright owner

Figure 2.1 (continued): Examples of a few in vivo/in vitro correlations for different drugs (adopted from J.C. Wagner, Biopharmaceutics and relevant pharmacokinetics, 1st Ed., 1971).

Figure 21.16. Correlation between the mean cumulative percent of the dose of salicylamide excreted in the urine of four human volunteers in one hour with the percent salicylamide in solution after 15 mins (●) and 20 mins (○) in vitro. The points from left to right refer to an experimental tablet, a commercial tablet and a commercial suspension of salicylamide. From Bates et al. (1969), reproduced with permission of the copyright owner.



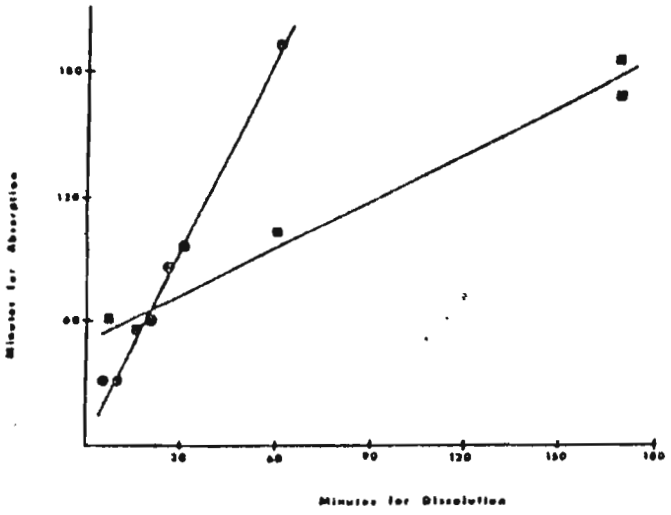
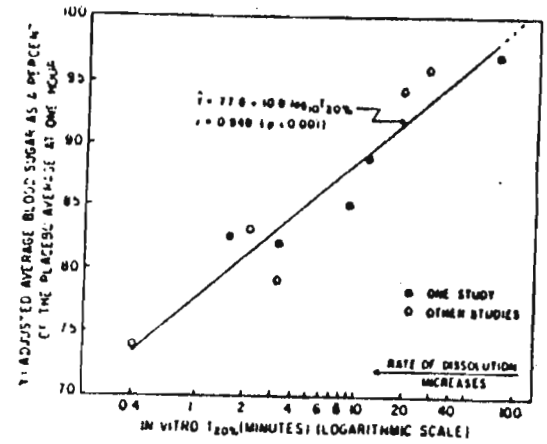


Figure 21.15. Correlation of times required for 25 percent and 50 percent absorption in man (estimated by method of Wagner and Nelson, 1963) with times for 25 percent and 50 percent dissolution in vitro. The absorption times were estimated from measurement of total plasma radioactivity following oral administration of various dosage forms of aminorex Cl⁻. Key: ● time for 25 percent absorption versus time for 25 percent dissolution, ■ time for 50 percent absorption versus time for 50 percent dissolution. From Cressman et al. (1969), reproduced with permission of the copyright owner.



RELATIONSHIP BETWEEN BLOOD SUGAR LEVEL AT ONE HOUR AND TIME FOR 20% OF THE TOLBUTAMIDE TO DISSOLVE FROM THE DOSAGE FORM IN VITRO

Figure 21.13. Correlation between the adjusted average blood sugar level in normals at one hour post administration (expressed as a percent of the placebo group average) with time for 20 percent of the drug to dissolve in an in vitro test (on a logarithmic scale) for ten different types of tolbutamide tablets. From Wagner (1966) with permission of the *Canadian Journal of Pharmaceutical Sciences*.

Figure 2.1 (continued): Examples of a few <u>in vivo/in vitro</u> correlations for different drugs (adopted from J.C. Wagner, Biopharmaceutics and relevant pharmacokinetics, 1st. Ed., 1971).

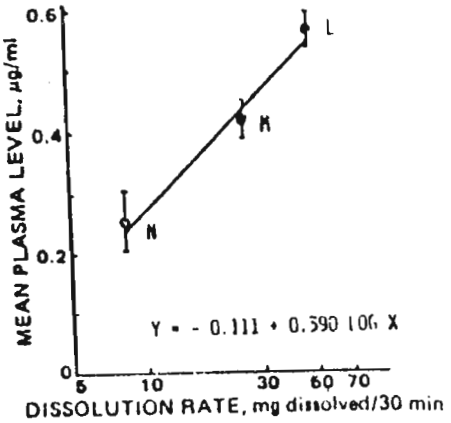


Figure 4—*Correlation of dissolution rates and mean plasma griseofulvin levels in stomach-emptying-controlled rabbits for Formulas L, M, and N. Correlation coefficient = 0.988 (p < 0.10).*

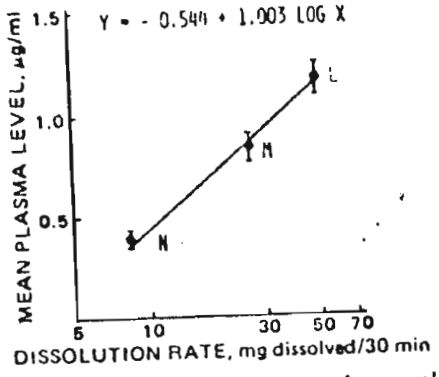


Figure 6—*Correlation of dissolution rates and mean plasma griseofulvin levels in humans for Formulas L, M, and N. Correlation coefficient = 0.995 (p < 0.10).*

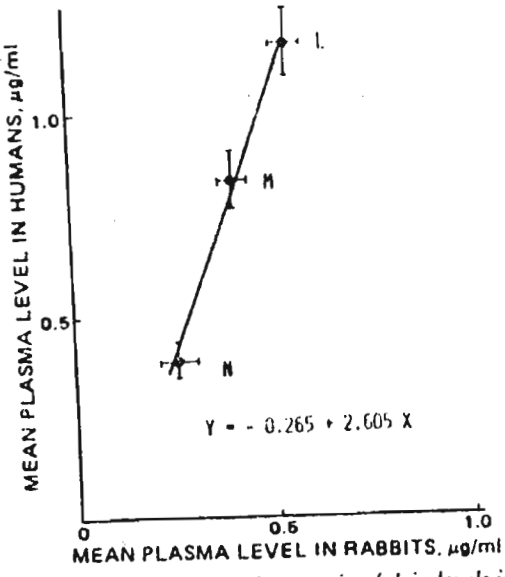


Figure 7—*Correlation of mean plasma griseofulvin levels in humans and in stomach-emptying-controlled rabbits for Formulas L, M, and N. Correlation coefficient = 0.997 (p < 0.05).*

Figure 2.1 (continued): Examples of a few in vivo/in vitro correlations for different drugs (adopted from Maedo et al., 1979).

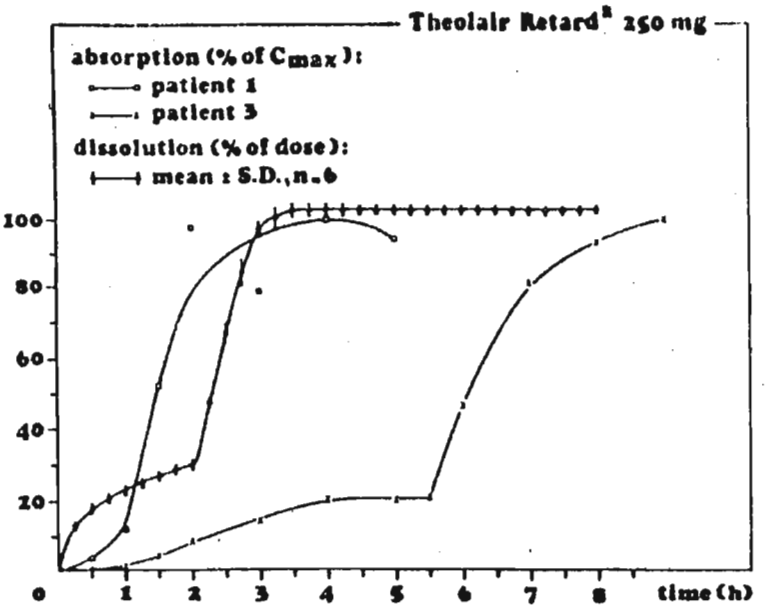


Fig. 1. The in vitro release of theophylline from Theolair Retard 250 mg tablets compared to the in vivo absorption in two typical patients. The in vitro dissolution is expressed as percentage of the dose. The pH of the medium was (arbitrarily) changed after 2 h from 1.0 to 6.8. The in vivo absorption is given by expressing the theophylline serum concentrations at different time points as percentage of the maximum concentration. Patient 1 is typical for a monophasic (intestinal) absorption process. (Note the correlation of the slope of the absorption curve with that of the dissolution curve obtained in a medium of pH = 6.8). Patient 3 is representative for biphasic (gastric and intestinal) absorption phenomenon. (Note the correlation of the two phases of the absorption curve with the slopes of the dissolution curves obtained at pH = 1.0 and pH = 6.8).

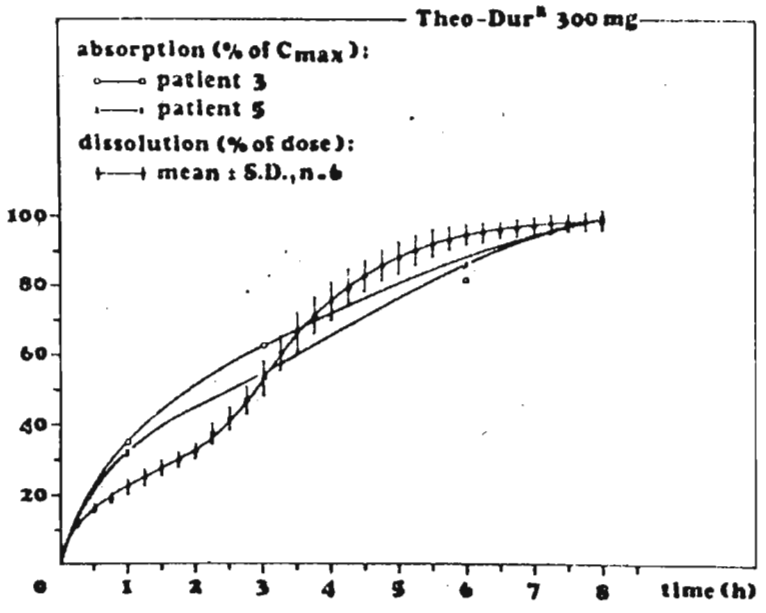


Fig. 2. The in vitro release of theophylline from Theo-Dur 300 mg tablets compared to the in vivo absorption in two typical patients. See also legend of Fig. 1.

Figure 2.1 (continued): Examples of a few in vivo/in vitro correlations for different drugs (adopted from Jonkman et al., 1981).

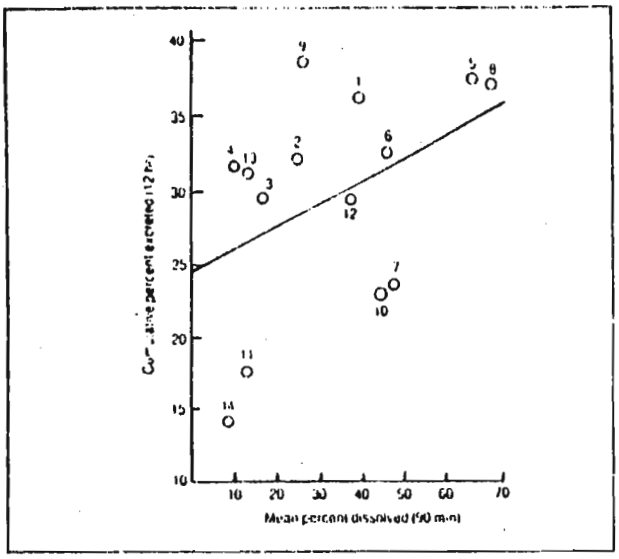


Figure 2: Correlation between cumulative percentage of nitrofurantoin excreted after 12 hr and the mean percentage of nitrofurantoin dissolved after 90 min for the 14 nitrofurantoin products evaluated by Meyer et al. ($r = 0.45$; $p > 0.05$). The numbers adjacent to the points are the product code numbers (from Meyer, M.C., et al., J. Pharm. Sci., Vol. 63, 1974, pp. 1693-1697).

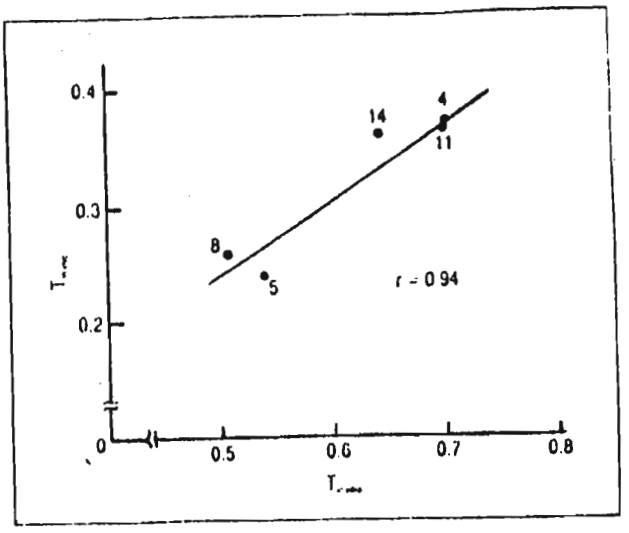


Figure 4: Correlation between $T_{...}$ and $T_{...}$ for the five selected nitrofurantoin products evaluated by Meyer et al. ($r = 0.94$; $p < 0.01$). The numbers adjacent to the points are the product code numbers (from Meyer, M.C., et al., J. Pharm. Sci., Vol. 63, 1974, pp. 1693-1697).

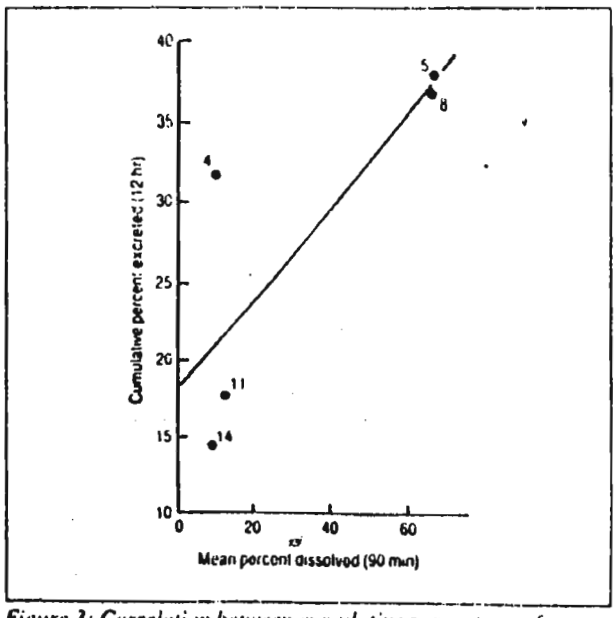


Figure 3: Correlation between cumulative percentage of nitrofurantoin excreted after 12 hr and the mean percentage of nitrofurantoin dissolved after 90 min for five of the nitrofurantoin products evaluated by Meyer et al. ($r = 0.80$; $p > 0.05$). The numbers adjacent to the points are the product code numbers (from Meyer, M.C., et al., J. Pharm. Sci., Vol. 63, 1974, pp. 1693-1697).

Figure 2.1 (continued): Examples of a few in vivo/in vitro correlations for different drugs (adopted from Banaker and Block, 1983).

the dissolution media, the intensity of agitation and the type (design) of dissolution method used (rotating basket, rotating paddle and rotating bottle) have been studied.

The possibility of quantitative correlations between in vivo and in vitro dissolution rates of two lots of diethylpropion sustained release pellets was investigated.

2.A.2 Experimental

2.A.2.1 Apparatus and materials

a. Apparatus

i) A Perkin Elmer 40 Ultraviolet (UV) Spectrophotometer, using 1 cm quartz cells, for measurement of diethylpropion hydrochloride in the dissolution media.

ii) Pye '104' Chromatograph fitted with a flame ionization detector and incorporating a Perkin Elmer Recorder (Model 56) Column - One meter coiled glass (i.e. 4 mm) containing Chromosorb G (A.W.; D.M.C.S. - 100 to 120 mesh) coated with 2% Carbowax 20M and 10% Apiezon L.

Working Conditions:

Column Temperature	120°C
Detector Temperature	200°C
Nitrogen Flow	1,25 cm^3/sec

Internal Standard, I.S.

a) Cinnamyl alcohol (20 ug/ml) - for quantitating the decomposition product, phenylmethyldiketone.

b) Retention times:

	Rt (mins)
1. Internal Standard	3,5
2. Phenylmethyldiketone	9,0

iii) Rotating Bottle Apparatus (Modified NF XIII)

A dissolution cabinet thermostatically controlled (warm air) at 37°C ± 1,0 with three horizontal rotating shafts each fitted with clamps capable of holding up to 12 amber

200 ml capacity bottles. The clamps were designed so that the long shafts rotated the bottles at 30 ± 2 r.p.m. Amber glass bottles (length 10 cm, diameter 6 cm, 200 ml capacity) fitted with bakelite screw caps, (lined with ceresine-coated cardboard). A sintered glass filter stick (Quickfit).

iv) Rotating Basket Dissolution Apparatus - BP 1980

v) Rotating Paddle Dissolution Apparatus (Wagner, 1971)
A one litre capacity, three necked, round bottomed, clearglass flask. The stirring paddle was a 7,5 cm diameter, half-moon shaped teflon paddle that was attached to a 37 cm long shaft.
A motor with adjustable speed, 30 - 120 r.p.m.
A Pye Dynacap pH meter
A waterbath thermostated to 37°C.

b. Materials
Different lots of sustained release pellets used for *in vitro* and *in vivo* studies, as well as all compounds necessary for quantitative analysis were kindly supplied by, or purchased from various suppliers as indicated in Appendix IA. Various substances, different solvents and reagents as well as packing materials for gas-liquid chromatography, were kindly supplied by or purchased from different suppliers (see Appendix IB).

2.A.2.2 Dissolution studies

a (i) Quantitation of diethylpropion hydrochloride in dissolution media
This method was used to measure the amounts of diethylpropion hydrochloride released from the sustained release pellets after dissolution tests and for measuring the potency of the sustained release pellets (2.A.2.2.c).

A stock solution was prepared containing 40 mg of diethylpropion hydrochloride in 100 ml distilled water. The stock solution was diluted in distilled water to produce a standard solution (4 mg/100 ml), which was then diluted to produce standard solutions containing 2,0; 4,0; 8,0; 12,0; 16,0 and 20,0 µg/ml of diethylpropion hydrochloride in 10,0 ml volumes.

The absorption spectra for the standard solutions of the diethylpropion were determined using distilled water as reference solution. The absorbance, measured at 252,5 nm, for each solution was plotted against the concentration of the solution to produce a calibration curve from which the concentration of unknown solutions could be determined.

A satisfactory calibration curve was obtained with a linear regression correlation factor of at least 0,9999 (Figure 2.2).

(ii) Quantitation of the decomposition product, phenylmethyldiketone

The hydrolytic decompositon of diethylpropion hydrochloride, drug substance and tablets, and the effect of it on stability have been reported (Walters et al., 1977; Walters, 1980). Two hydrolysis products, phenylmethyldiketone and diethylamine hydrochloride, isolated and identified earlier, were assayed by an HPLC method, and a pathway for this degradation was proposed (Section 1.2).

In the present study the extent of decomposition of diethylpropion hydrochloride, in the free form and as sustained release pellets, was checked by monitoring the phenylmethyldiketone (1-phenyl-1,2-propanedione) in samples stored under different conditions, using the following procedure:

About 1,0 g of the pellets was crushed and powdered thoroughly in a mortar to obtain a fine powder which was then sieved through a no. 40 mesh.

About 0,25 g of the fine powder (or 40 mg of the free drug powder) was accurately weighed and quantitatively transferred into a 100 ml volumetric flask; 40 ml distilled water was added and the flask stoppered. The contents was then shaken at room temperature for abour 4 hours using a mechanical shaker. The contents was then diluted to volume with water, shaken and then filtered.

The first 20 ml of the filtrate was discarded, then 40 ml was taken and diluted to 100 ml with water.

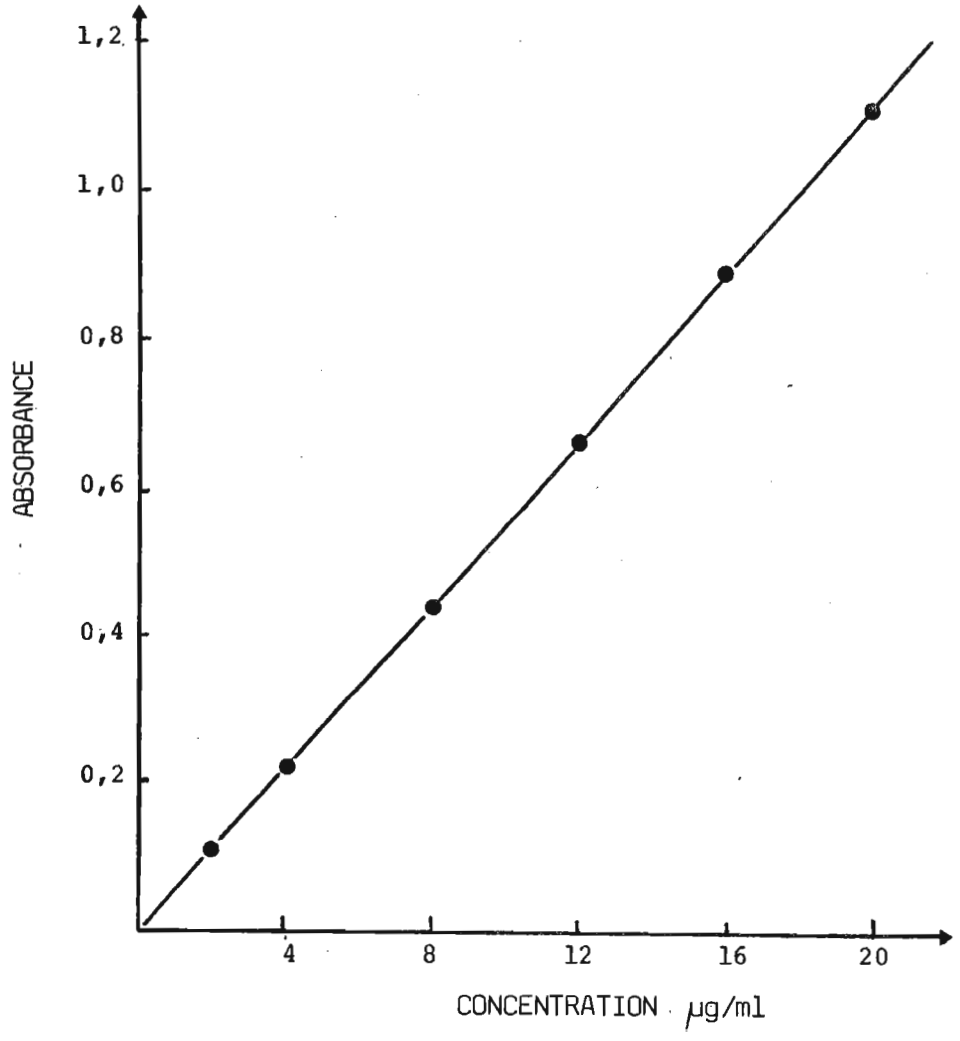
To 1-4 ml of this solution in a tapered tube, 1 ml of internal standard (freshly prepared Cinnamyl alcohol, 20 µg/ml) was added. The mixture was then diluted to 5 ml with water, then acidified with 1 ml 1N H_2SO_4 and mixed: the contents was then extracted with 100 µl freshly distilled chloroform by whirlmixing it for 2 minutes.
After centrifugation at 3 500 r.p.m. on an M.S.E. Speed Bowl for 5 minutes to separate the two immiscible phases, 2-4 µl of the lower chloroform layer was carefully withdrawn into a 5 µl Hamilton syringe and injected onto the gas-liquid chromatography column.
A calibration curve of the compound phenylmethyldiketone was prepared using known amounts: 1,0 - 10,0 µg/assay of the compound, and internal standard 20 µg/ml (Figure 2.3).
The concentration of phenylmethyldiketone in each sample examined was determined by using the peak height ratios (relative to the I.S.) in the straight line equation of the calibration curve (Figure 2.3). All the determinations were done in duplicate. For the determination of the drug content of the free drug substance and sustained release pellets, the solutions were suitably diluted and 1-4 ml of this solution was

Figure 2.2: Typical standard calibration curve for diethylpropion hydrochloride using Ultraviolet Spectrophotometry

$y = 0,0563x - 0,004$

c.c. = 0,9999

λ max = 252,5 nm



extracted and analysed according to the method outlined in 3.5.2(c).

2.A.2.2 b Determination of the densities of sustained release pellets

The densities of the pellets were measured using the methods of Beckett and Stenlake (1966), but distilled water (and/or corn oil) was used in place of benzene. The following pellets were studied: Lot 018010 (Temmler, Marburg), Lot R7574 (Lemmon, U.S.A.) and Lot R 7773 (Lemmon, U.S.A.).

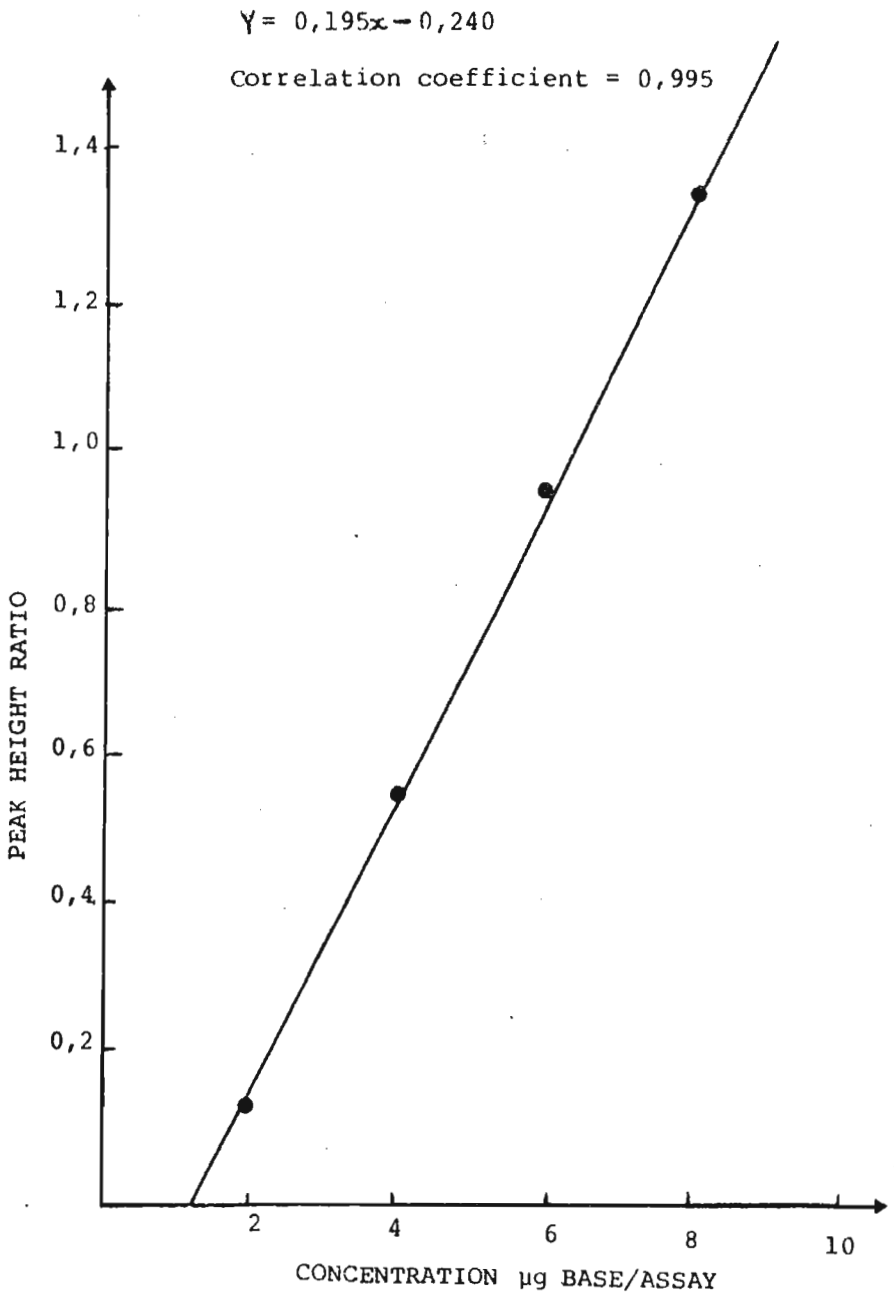
A clean, dry 25 ml specific gravity bottle, fitted with a ground-in stopper pierced by a fine hole was weighed (W_1). About 5 g of pellets was placed into the density bottle which was then reweighed (W_3). Then a small volume of water (and/or oil), sufficient to cover the pellets was added, and the bottle was gently rolled to ensure that individual pellets were completely wetted and air pockets were not trapped amongst the pellets. The bottle was then carefully filled with water (and/or oil), taking care not to allow formation of air bubbles. The stopper was then replaced and any liquid oozing from the stopped hole was wiped off with tissue paper.

The bottle with its contents was then weighed again (W_4). The bottle was emptied, cleaned and the process was repeated with water only (and/or oil only) (W_2). All the determinations were carried out in duplicate.

The density of the pellets (D_p) was calculated using equation 2.3 where D is the density of water at room temperature (25°C) = 0,9971 g/ml.

$$D_p = \frac{W_3 - W_1}{\frac{W_2 - W_1}{D} - \frac{W_4 - W_3}{D}} \quad \text{...Eq. 2.3}$$

Figure 2.3: Typical standard calibration curve for phenylmethyl-diketone using Gas Liquid Chromatography



2.A.2.2 c. Determination of the potencies of sustained release pellets

To measure the potency of sustained release pellets (Lots 018010;R 7574 and R 7773) about 10 g of the pellets was crushed and ground thoroughly in a mortar to obtain a fine powder, which was then sieved through a USP XX 40 mesh screen.

An amount of 0,5 gm of the fine powder, accurately weighed, was quantitatively transferred with washings into a 200 ml volumetric flask; 50 ml distilled water was added, and the flask then stoppered. The contents of the flask was shaken for about 20 hours (overnight) at room temperature using a mechanical shaker. The contents of the flask was then diluted to volume with sufficient water, shaken and then filtered.

The first 50 ml of the filtrate was discarded, then 2 ml was taken and diluted to 100 ml with water and its absorbance measured by Ultraviolet Spectrophotometry (2.A.2.2.a). The determinations were done in triplicate for all three lots of pellets.

In addition, the potencies of the free drug and of sustained release pellets (Lots R 7773 and 018010), stored under different conditions of temperature, were measured, initially and periodically, using the gas-liquid chromatography procedure described under 2.A.2.2.b.
All the determinations were done in duplicate.

2.A.2.2 d Methods of dissolution

(i) Rotating Bottle Method

The thermostated dissolution cabinet was allowed to equilibrate to 37°C prior to use. The sustained release pellets (450 mg equivalent to 75 mg of drug) were accurately weighed, in duplicate, and transferred into clean amber 200 ml bottles containing 150 ml of the required buffer, previously warmed to 37°C $\pm$ 1°C. The bottles were capped tightly to prevent leakage and then placed onto the rotating shaft in the dissolution cabinet

(37°C). The sequence and duration of rotation periods in the various buffers were as follows.

(a) To simulate oral administration

1	One hour (1st hour)	Buffer pH 1,5
2	One hour (2nd hour)	Buffer pH 4,5
3	Two hours (3rd and 4th hours)	Buffer pH 6,9
4	One hour (5th hour)	Buffer pH 6,9
5	Two hours (6th and 7th hours)	Buffer pH 7,2
6	One hour (8th hour)	Buffer pH 7,5

(b) To simulate rectal administration

1	One hour (1st hour)	Buffer pH 6,9
2	One hour (2nd hour)	Buffer pH 6,9
3	Two hours (3rd and 4th hours)	Buffer pH 6,9
4	One hour (5th hour)	Buffer pH 6,9
5	Two hours (6th and 7th hours)	Buffer pH 6,9
6	One hour (8th hour)	Buffer pH 6,9

At the end of each rotation period, two bottles at a time were removed from the cabinet (a maximum of six bottles were used at any one time). An aliquot (about 50 ml) of the buffer was collected from each for analysis. Care was taken not to decant or crush any pellets. A sintered glass filter stick was then attached to a vacuum line and the remaining buffer solution in the bottle was aspirated and discarded, leaving the pellets in the bottle. The pellets were rinsed with about 15 ml distilled water, which was also sucked off. The vacuum line was removed and then 150 ml of the next buffer (pre-warmed to 37°C), was put into the bottle. Positive pressure was then applied through the sintered glass filter stick to free any attached pellets into the bottle. The change of fluid and sampling did not take more than two minutes per bottle.

At the end of the eight-hour period, the dissolution medium was replaced with fresh medium (pH 7,5) and the dissolution process was continued for another 3 to 4 hours to determine that the drug was maximally released from the pellets.

At the end of the eleven or twelve-hour period, having removed the sample and discarded the remaining buffer, the residue of pellets was carefully transferred into a mortar, crushed and the suspension transferred quantitatively to a 100 ml volumetric flask using about 60 ml of acid solution (0,1N HCl). The flask was then shaken for 4 hours at room temperature on a mechanical shaker. Thereafter the volume was adjusted to 100 ml with acidified water, and the contents well mixed, filtered and diluted (1 ml to 50 ml with distilled water) and then analysed by Ultraviolet Spectrophotometry (2.A.2.2.a).

The amount of drug released from the pellets after each hour or two-hour period, and the amount present in the residue, was calculated and then expressed as the percentage of total amount of drug taken. (All the calculations were based on percentages of potency of the pellets).

The buffer solutions (digestive fluids) used in the study were prepared as follows:

pH	Sodium Chloride	Potassium dihydrogen phosphate	Disodium hydrogen phosphate	Adjust* to pH with
	mass in grams per litre			
1,5	2	-	-	1 M HCl**
4,5		6,8	-	1 M HCl or 1 M NaOH
6,9		3,4	3,55	
7,2		6,8		40% NaOH
7,5		6,8		40% NaOH

* Warm to 37°C then adjust pH

** If too much acid added, discard medium - do not adjust back to pH 1,5 with alkali.

The dissolution tests were carried out on sustained release pellets Lots 018010, R 7574 and R 7773 using buffers and time intervals to simulate oral administrations.

A dissolution test (at constant pH) of diethylpropion hydrochloride sustained release pellets Lots R 7574 and R 7773, was also carried out using the dissolution media, pH 1,5 and 4,5 each for 4 hours and pH 6,9 for 6 to 8 hours. The release rates of the pellets were determined. The intention was to define an in vitro dissolution test profile which would then be useful to develop in vitro/in vivo correlations.

To determine the content of free drug (i.e. the non-sustained portion) in the pellets formulations, dissolution tests on diethylpropion sustained release pellets Lots 018010, R 7773 and R 7574 were also done using the dissolution medium (pH 1,5) for exactly 5 minutes in the rotating bottle method. Thereafter the medium was replaced with a fresh dissolution medium (pH 1,5) and the dissolution procedure continued, using the sequence and duration of rotation periods in the various buffers as follows:

1	55 minutes (to complete the 1st hour)	Buffer pH 1,5
2	One hour (2nd hour)	Buffer pH 4,5
3	Two hours (3rd and 4th hours)	Buffer pH 6,9
4	Two hours (5th hour)	Buffer pH 6,9
5	One hour (6th and 7th hours)	Buffer pH 7,2
6	One hour (8th hour)	Buffer pH 7,5

The calculations to determine the free drug in the pellets are based on the following:

$$\% \text{ Free Drug} = \% \text{ Cumulative Release at 5 minutes} - \frac{(\% \text{ drug released in 55 minutes}) \times 5}{55}$$

and

$$\text{Rate of Release (\%/hr)} = \frac{\text{\% drug released in 55 minutes X 60}}{55}$$

The rate of release (%/hr) determined in this way is a good approximation on this study, because with all the batches of sustained release pellets, the rate of release of drug was constant in the first two hours. (Figure 2.B.1).

(ii) Rotating Basket Method

The BP 1980 Rotating Basket Method was used. The rotating basket assembly was immersed in a constant temperature waterbath maintained at 37°C ± 0,5°. About 2,75 g of sustained release pellets was accurately weighed and placed into the stainless steel rotating basket which was then fitted to the stainless steel driving rod. The stirring rod of the rotating basket assembly was placed through the centre hole of the vessel cover (the vessel is described in the BP 1980) and was centred to permit smooth rotation and to prevent wobbling. The assembly was fitted to an electrically-driven motor at right angles and the distance between the bottom of the basket and the bottom of the interior surface of the vessel was adjusted to between 18 and 22 cm. The required buffer solution, (900 ml), previously warmed to 37°C, was introduced into the vessel through one of the four holes on the vessel. All the holes were then stoppered for the duration of the experiment. The stirring rate was maintained at 100 r.p.m.

The sequence and duration of rotation periods in the various buffers were as listed for the rotating bottle method (2.A.2.2.d(i)) to simulate oral administration.

At the end of each rotation period, an aliquot (about 50 ml) of the dissolution medium was taken and the remaining fluid aspirated, before introducing the next appropriate buffer solution. At the end of the eight-hour period, the remaining pellets were dealt with in the same

way as described for the rotating bottle method (2.A.2.2.d.(i)).

The experiment was carried out in duplicate and all the samples were filtered, if necessary, and diluted appropriately for analysis as outlined in 2.A.2.2.a.

(iii) Rotating Paddle Method

The dissolution vessel was a 1 litre three-necked round-bottomed flask, immersed in a waterbath where the temperature was constantly maintained at 37°C. The stirring paddle, a 7,5 cm diameter teflon paddle, was attached to one end of the glass stirring shaft (37 cm long); the other end of the shaft was connected to an electrically-controlled drive. The teflon paddle was centred and positioned 2,0 cm from the bottom of the flask. About 2,75 g of pellets, accurately weighed, was transferred into the round-bottomed flask. 900 ml of the first buffer solution, prewarmed to 37°C, was then poured into the flask. The two side openings of the flask were stoppered, and the buffer stirred at 100 r.p.m.

The sequence and duration of the rotation periods in the various buffers were as listed for the rotating bottle method to simulate oral administration. (2.A.2.2.d.(i)).

At the end of each rotation period, an aliquot (about 50 ml) of the dissolution medium was taken and the remaining fluid aspirated, before the next appropriate buffer solution was introduced. At the end of the eighth-hour period, the remaining pellets were treated as described under 2.A.2.2.d.(i). The experiment was carried out in duplicate and all the samples were filtered, if necessary, and diluted appropriately for analysis as outlined in 2.A.2.2.a.

2.A.2.3 Stability testing

The aim of the present studies (2.A.2.3) was to investigate the stability in relation to the potency and drug release characteristics of the pellets on storage under different conditions. Different presentations of pellets (free pellets, pellets in hard gelatin capsules and in suppositories) were stored for different periods of time at various temperatures: 4°C, room temperature (25°C) and 37°C. To achieve this goal, two lots of diethylpropion hydrochloride sustained release pellets were extensively studied throughout, viz. Lots 018010 and R 7773. All *in vitro* dissolution tests involved the use of the rotating bottle method with buffers to simulate oral administration (2.A.2.2.d.(i)). Analyses of all samples, including potency determinations, were carried out by using the Ultraviolet Spectrophotometric method described earlier (2.A.2.2.a).

(i) Storage of sustained release pellets in hard gelatin capsules at room temperature

In all our *in vivo* studies in humans, the pellets administered were placed in hard gelatin capsules. Therefore it was necessary to check that the release of the drug from the pellets was not affected initially by the presence of the gelatin capsule, or by storage at room temperature (25°C).

About 0,45 g of pellets, (equivalent to one dose of drug ≡ 75 mg), was accurately weighed and placed into a clear, hard gelatin capsule size No. 0. Fifteen capsules were prepared in this manner and then placed in screw-topped clear-glass jars with bakelite lids (as described earlier). The pellets were stored on a shelf at room temperature, tested after overnight storage and then periodically tested for dissolution profiles. (Table 2.A.1). Duplicate determinations were carried out.

(ii) Storage of free drug and sustained release pellets at different temperature conditions

The stability of the free drug and of two batches of diethylpropion hydrochloride sustained release pellets stored for different periods of time at room temperature (25°C), 37°C and 4°C was studied. Each product was stored by placing 15 g of the pellets (or 140 mg of the free drug) in a screw-cap glass bottle, size 2, M 11, and stored as shown in Table 2.A.1. The stability of the pellets was checked initially and periodically by carrying out dissolution tests. Duplicate determinations were carried out.

In addition, the extent of decomposition was checked by monitoring one of the degradation products i.e. phenylmethyldiketone in the free drug and in sustained release pellets, using the gas-liquid chromatography procedure described earlier (2.A.2.2.a.ii). The potencies of all the preparations were also determined by the gas-liquid chromatography procedure outlined later (3.5.2.c).

Table 2.A.1 Storage conditions and duration of storage for diethylpropion hydrochloride sustained release pellets (two batches)

Pellets *1 Lot. No.	Storage Condition	Duration of storage
018010 in gelatin capsules (No. 0)	R.T.	0; 3; 9 and 13,5 months
R 7773 in gelatin capsules	R.T.	0; 1 and 2,5 months
R 7773 (25 mg drug) in suppository	4°C	0 and 2 weeks *2
018010, loose, in closed glass containers	4°C	0;1;3;6;9 and 13,5 months
	R.T.	0;1;3;6;9 and 13,5 months
	37°C	0;1;3;6;9 and 13,5 months
R 7773, loose, in closed glass containers	4°C	0; 1 and 2,5 months
	R.T.	0; 1; 2; 5 and 9,5 months
	37°C	0; 1; 2 and 5 months
Diethylpropion Hydrochloride free drug-powder	R.T.	0; 3; 6 and 9 months
	37°C	0; 3; 6 and 9 months

R.T. Room temperature, ± 25°C.

*1 Pellets Lot No. 018010 was received on 17.3.80 while Lot R 7773 was received on 6.3.81
Storage of pellets commenced within 48 hours after delivery
0 = Initially, or an overnight study
Storage at R.T. was in well-closed, clear glass bottles
Storage at 4°C and 37°C was in well-closed amber glass bottles

*2 The suppositories were administered only after 2 weeks of storage, because it was not intended to be a detailed stability study

(iii) Storage of sustained release pellets as suppositories

In this investigation, the in vitro dissolution release characteristics and short-term (3 weeks) stability of the diethylpropion hydrochloride sustained release pellets (Lot R 7773) incorporated into a suitable lipophilic base (Noormohammadi, 1981) were studied. Six suppositories, each weighing 2 g and containing sustained release pellets equivalent to 75 mg of diethylpropion hydrochloride, were prepared as follows:

A 20 g mixture of Base H and Cream Placebo (2:1 $^{m}/_{m}$) (Appendix IB) was melted and stirred gently at approximately 37,5°C in a beaker. Each single suppository was then made by:

a) Pouring some of the melted base into the bottom of the suppository moulds (6 x 2,0 g) to approximately one third of the mould height
b) Pouring the previously weighed pellets into the mould containing the base
c) Filling each mould with the remainder of base and
d) replacing the mould into the refrigerator (not freezer) to allow the suppositories to set in the normal way.

Each suppository was placed in a screw-capped dark bottle, size 2, M 11, and stored as shown in Table 2.A. The dissolution rate of the drug and the potency of the suppositories were determined initially and at the end of the three weeks of storage at 4°C, using the rotating bottle apparatus to simulate rectal administration [2.A.2.2.d(ii)]. Prior to dilution, the dissolution test samples were filtered several times (Whatman No. 5 filter paper), in order to clarify the solutions for Ultraviolet Spectrophotometry.

2.A.2.4 Investigation of factors influencing the release rate of diethylpropion hydrochloride from sustained release pellets

Generally, in the formulation of sustained release pellets, a diffusion rate-limiting membrane is preferred to one from which the drug release depends upon membrane or unit erosion (Beckett, 1981).

One aim of the present investigations was to show that the mechanism of release of the drug from the pellets used was diffusion rate-controlled.

Many factors affect the rate of dissolution of solid dosage forms; the pH, composition and viscosity of medium and temperature are all important (2.A.1). In the following studies, the effects of some of these factors on the *in vitro* dissolution rate of diethylpropion hydrochloride sustained release pellets Lot. 018010 have been investigated. The rotating bottle test method with buffers to simulate oral administration [2.A.2.2.d.(i)] was used throughout the investigation, unless otherwise stated.
Duplicate determinations were carried out routinely in all studies.

a) Type of in vitro dissolution test model

The three *in vitro* dissolution models compared were:

(i) The rotating bottle method, similar to that described in NF XIII

(ii) The rotating basket method, similar to that described in BP 1980 and USP XX

(iii) The rotating paddle method, similar to the one adapted from the beaker method by Poole et al., (1969).

The experimental procedures used have been described [2.A.2.2.d(i), (ii) and (iii)].

The sequence of the buffers used and the duration of time spent in each buffer by the pellets were kept constant for all three methods. All the investigations were done in duplicate. The results of this investigation also determined the choice of dissolution test model used for the bulk of our in vitro work.

b) Speed of agitation

The different speeds of agitation or mixing were compared by using two test models:
35, 45, 65 and 100 r.p.m. with the rotating basket method and with the rotating paddle method. The investigations were done initially and then six months later. Each determination was done in duplicate.

c) Composition of dissolution medium

In the following studies the rotating bottle method was used to establish the in vitro dissolution rates of sustained release pellets Lot 018010 with the following pH gradients which had been established by Hassanzadeh, (1981) to be effective.

pH = 1,5 (1 hr); pH = 6,9 (4 hrs); pH 7,5 (3 hrs)

The sequence of the buffers and the duration of time spent in each buffer were kept constant for all of the following investigations:

(i) Concentration of the buffer solution

The dissolution tests were carried out using buffer solution pH 6,9 with two different concentrations of buffer (0,05 M and 0,10 M) respectively. The purpose was to see if changes in concentration of the buffer had any effect on the dissolution profile of the pellets. The USP XX recommends a buffer concentration of 0,05 M.

(ii) Effect of cations (K^+) and Na^+) on dissolution rates

Dissolution tests were carried out on the pellets, using

a) buffer solutions with only sodium ions (Na^+)

b) buffer solutions with only potassium ions (K^+) and

c) buffer solutions with both potassium and sodium ions (K^+ and Na^+)

to evaluate the effect of different cations on dissolution profiles of the pellets, as both ions are usually present in the buffers.

(iii) Effect of anions on dissolution rates

Buffer solutions (pH 6,9) containing different anions (borate and phosphate) were employed to measure the effect of different anions on dissolution rates of pellets.

PART B: RESULTS AND DISCUSSION

2.B.1 Dissolution Rate Studies on all Sustained Release Pellets used in the Present Study, using the Rotating Bottle Method

These studies were carried out prior to any other *in vitro* or *in vivo* investigations to establish the release patterns and potencies of all the pellets used in the studies. The investigation was necessary to determine which pellets would be used *in vivo*, and to establish the drug release patterns of the pellets prior to storage.

The cumulative release of the drug from the pellets was plotted against the sampling times for each batch of pellets. The mean of the results (m) was plotted where determinations were carried out in duplicate or triplicate.

The mean cumulative percentage release of diethylpropion hydrochloride from the sustained release pellets Lot 018010, established in experiments on six different days, is presented in Table 2.B.1. The good consistency of the results obtained in these experiments by using the rotating bottle dissolution test for sustained release pellets Lot 018010 is clearly shown. The results obtained for the other two batches were equally consistent.

Figure 2.B.1 and Table 2.B.2 show the comparison of the sustained release pellets Lots 018010, R 7574 and R 7773. (Sources of pellets are listed in Table 1, Appendix B). All three batches were used in *in vivo* studies, but because sustained release pellets Lot R 7773 gave excellent constant release profiles and also provided desirable urine profiles in preliminary studies on subject C.D., extensive and well-controlled two-way crossover trials were done on these pellets in 12 subjects from whom saliva, urine and blood samples were collected (Chapter 3).

Table 2.B.1: A comparison of the results *1 obtained for the cumulative percentage release of the drug from sustained release pellets (Lot 018010)

Sampling Time (Hours)	Cumulative Percentage Release of the Drug from sustained release pellets*2, Lot 018010						Mean ± S.D.	Coefficient of Variation
1	25,4	24,4	25,5	27,3	26,3	26,4	25,9 ± 0,9	3,5
2	38,7	38,5	38,7	38,4	39,1	38,3	38,6 ± 0,3	0,80
4	50,5	51,3	50,4	51,0	50,3	50,4	50,7 ± 0,4	0,90
5	55,9	56,8	56,1	53,4	55,7	58,4	56,0 ± 1,6	2,9
7	65,3	65,8	65,4	65,4	65,9	65,2	65,3 ± 0,4	0,6
8	70,6	70,7	71,3	69,9	68,3	72,7	70,6 ± 1,3	1,8
Residue	100,3	99,7	99,5	98,7	97,9	99,9	99,3 ± 0,8	0,8

*1 The in vitro dissolution test was carried out daily for six days.

*2 Each figure is the average of two results.

Sustained release pellets Lot 018010 released only about 70% of their drug content over eight hours, so their use on humans was restricted to the study of the effect of food on the bioavailability of the drug from sustained release pellets comprising a diffusion-controlled membrane that does not disintegrate and remains intact throughout its passage in the GIT (Chapter 1).

The dissolution profiles of diethylpropion sustained release pellets Lots R 7574 and R 7773 at various constant pH levels (i.e. pH 1,5 and pH 4,5 for 4 hours, pH 6,9 for 7 hours and pH 7,2 and pH 7,5 for 4 hours which were used to measure the release rates in each of these dissolution media) are shown in Figures 2.B.2 and 2.B.3 and Tables 2.B.3 and 2.B.4.

Closer examination of the above-mentioned results on cumulative percentage release of the drug from the three lots of sustained release pellets, provided very useful and interesting information regarding release of the drug from the pellets into the dissolution media, viz.:

a) All three formulations contained some free drug (non-sustained portion) which was released instantly in the dissolution medium. The actual content of the "free" unbound drug in each pellet formulation was determined (Table 2.B.2). Such pellets are formulated by coating the outside of the non-biodegradable membrane (associated with release mechanism) with free drug and then coating it with a simple sealant (not involved in the release process) to retain the integrity of the pellet (A.H. Beckett - personal communication).

b) The release of the drug from pellets (Lot R 7574 and R 7773) exposed to constant pH levels for 4 to 8 hours shows that there is minimal change in the release rates at each pH level; i.e. the rate of release is constant and independent of the pH of the environment. Clearly such a formulation, together with

Table 2.B.2: Cumulative percentage release of diethylpropion hydrochloride from three lots of sustained release pellets using the rotating bottle method

pH Value	Time at pH (Hours)	018010 Temmler	R 7773 Lemmon	R 7574 Lemmon
1,5	0,08 (5 mins)	15,82	19,03	17,5
1,5	0,92	24,8	26,1	33,12
4,5	1	36,2	35,8	58,71
6,9	2	50,51	53,6	82,54
6,9	1	56,0	63,4	86,92
7,2	2	66,3	79,9	92,62
7,5	1	71,6	85,2	96,62
7,5	2-4	98,4 (4 hr)	95,4 (3 hr)	100,3 (2 hr)
Residue in Pellets		4,5	4,7	0
Total Recoveries		102,9	100,1	100,3

DEP Diethylpropion hydrochloride

The "free" non-sustained portion present in the pellets is determined according to the procedure outlined in 2.A.2.2.d.

Free DEP in pellets, expressed as % of drug content in pellets, is

15,0 for Lot 018010

18,4 for Lot R 7773

16,1 for Lot R 7574

the benefits of using discrete pellets each containing a diffusion controlled non-biodegradable membrane, would be useful to control the input of drug at any required rate.

c) The release rate of the drug from sustained release pellets Lot R 7773 (used extensively in the in vivo studies, Trials 1 and 2, Table 3.1) definitely maintains zero-order until almost 85% of the drug has been released, while the release rate of Lot 018010 is constant up to about 2 hours. After this period a zero-order release from about 3 hours onwards occurs. (Figure 2.B.1). However, the release of the drug from sustained release pellets Lot R 7574 is very rapid (but not very constant) in the first 2 hours, during which time about 58% of the drug is released, but after the fourth hour, the release is constant. Therefore the release of the drug from sustained release pellets Lot R 7574 appears to be rapid initially and does not seem to follow a general order throughout.

d) The results on the release rates obtained in different media (Tables 2.B.3 and 2.B.4), are referred to in a later chapter (4.3) with regard to the in vivo studies where the intention is to attempt to predict, by using the dissolution test profiles (for the faster releasing pellets R 7574 as well as for the slower ones, R 7773), the in vivo urinary excretion rate profiles of the drug/metabolite/s.

When the solid dosage form is covered with a thin coating that behaves like a dialysis membrane (as in the sustained release pellets used in our studies), the drug is released by a process of diffusion through the membrane. Gastrointestinal fluids (buffer solutions) diffuse through the membrane to form a saturated solution of drug within the pellet. The drug then undergoes passive diffusion when the highly concentrated solution within the pellet diffuses through the membrane to the less concentrated buffer solution. The rate of release is thus governed by the diffusion properties of the drug with respect to the membrane (Notari, 1980).

Figure 2.B.1: Comparison of the cumulative percentage release of diethylpropion hydrochloride from various lots of sustained release pellets using the rotating bottle method:
pH 1,5 (5 min); pH 1,5 (55 min); pH 4,5 (1 hr); pH 6,9 (2 hrs); pH 6,9 (1 hr); pH 7,2 (2 hrs); pH 7,5 (1 hr) and pH 7,5 (2-4 hrs)

x Free Drug (% of pellet content)		Lot	Manufacturer
18,4	- - -□- - -	R 7773	Lemmon
16,1	• • • △ • • •	R 7574	Lemmon
15,0	— O —	018010	Temmler

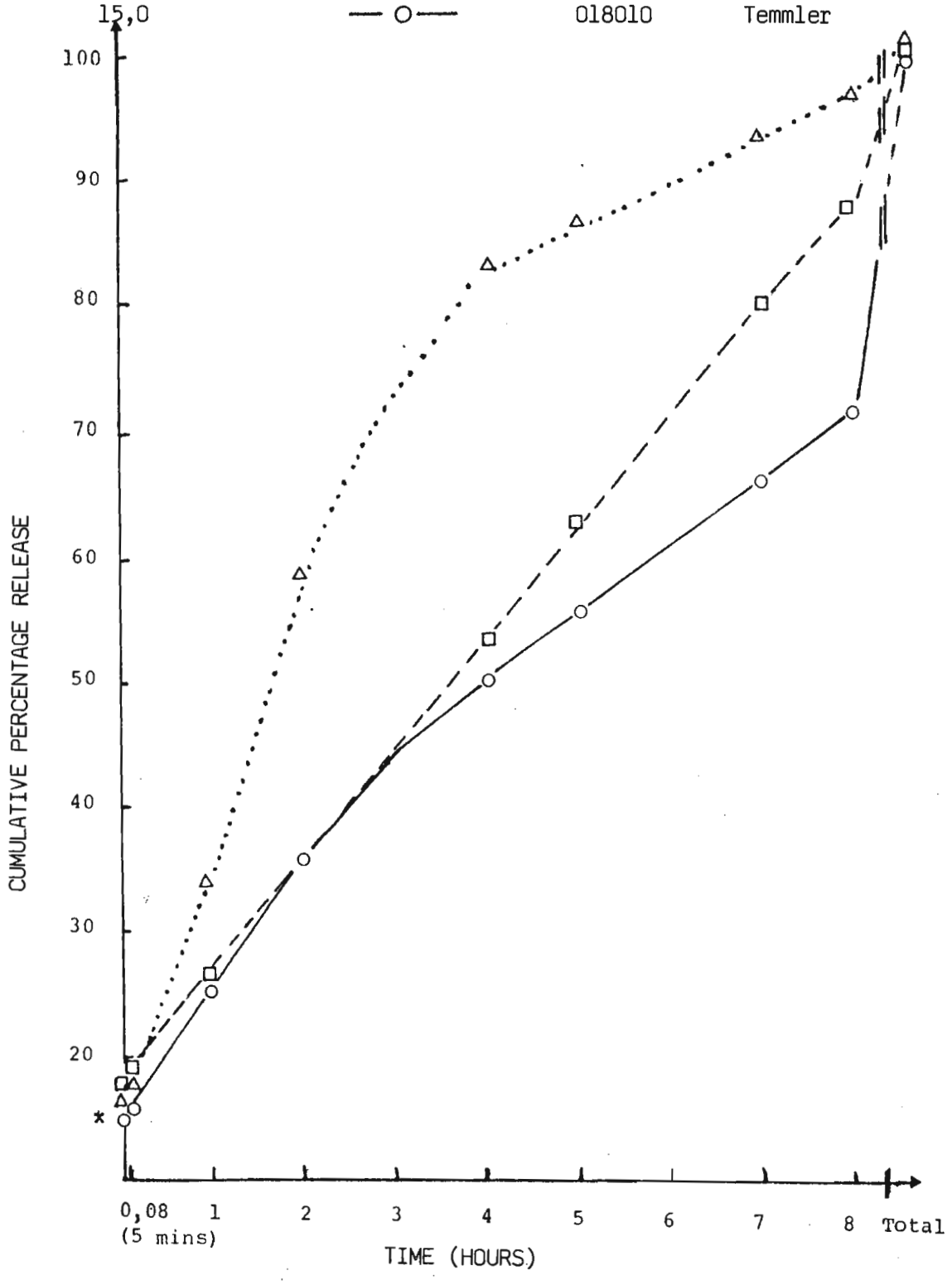


Figure 2.B.2: Comparison of the cumulative percentage release of diethylpropion hydrochloride from sustained release pellets (Lot R 7574 - Lemmon USA) under different conditions:
pH 1,5 (0,08; 1; 2; 3 and 4 hrs); pH 4,5 (0,08; 1; 2; 3 and 4 hrs) pH 6,9 (0,08; 1; 2; 3; 4; 5; 6 and 7 hrs); pH 7,2 (0,8; 1; 2 and 4 hrs) pH 7,5 (0,08; 1; 2 and 4 hrs)
Rotating Bottle Method
0,08 hr = 5 minutes
x Free unbound drug in pellets is 16,1%

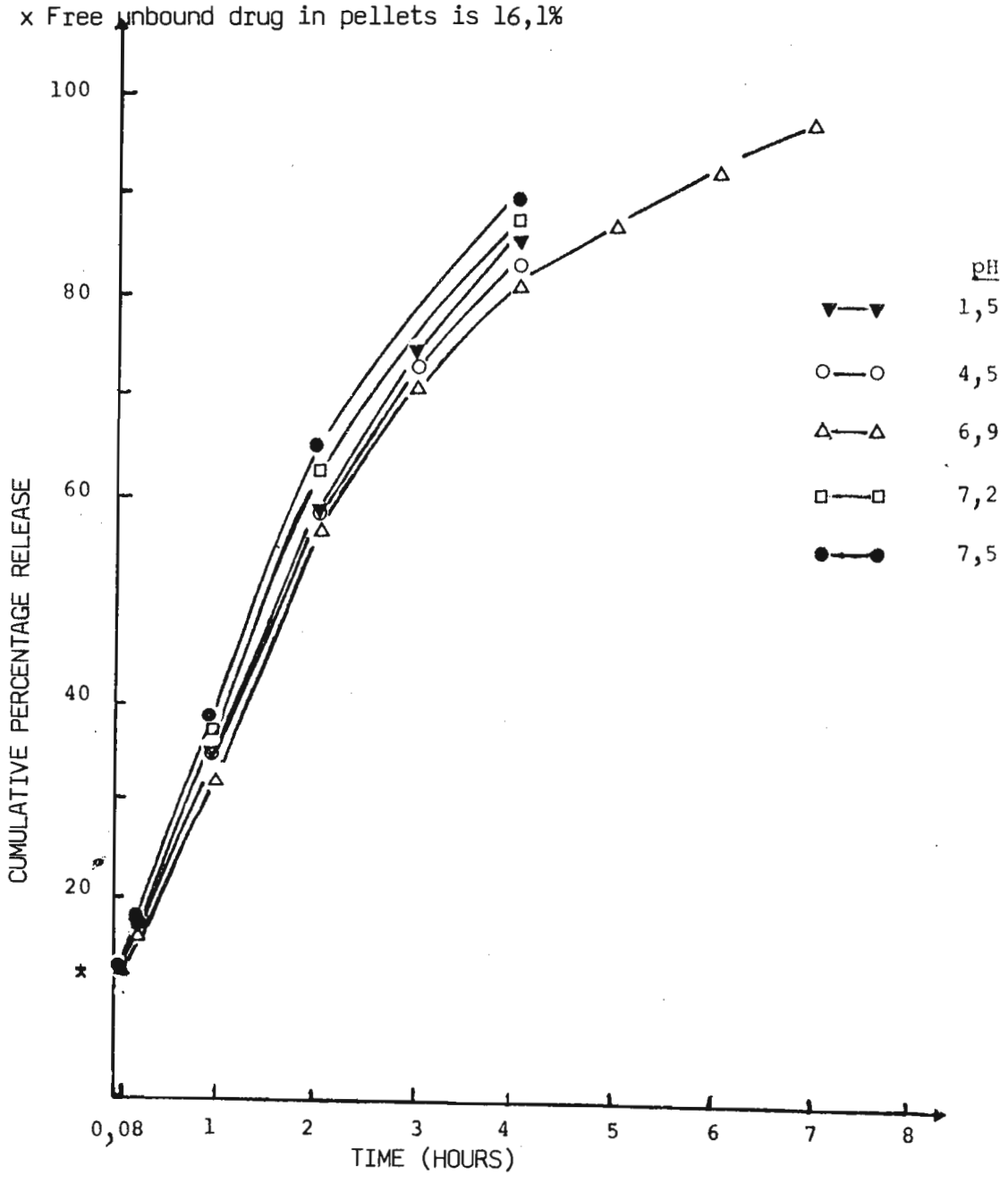


Figure 2.B.3: Comparison of the cumulative percentage release of diethylpropion hydrochloride from sustained release pellets (Lot R 7773 - Lemmon USA) using the rotating bottle method with different dissolution media.

pH 1,5 (0,08; 1; 2; 3 and 4 hrs) ▼——▼
pH 4,5 (0,08; 1; 2; 3 and 4 hrs) O——O
pH 6,9 (0,08; 1; 2; 3; 4; 5; 6; 7 and 8 hrs) △——△
pH 7,2 (0,08; 1; 2 and 4 hrs) □——□
pH 7,5 (0,08; 1; 2 and 4 hrs) ●——●

x Free unbound drug in pellets is 18,4%

0,08 hr = 5 minutes

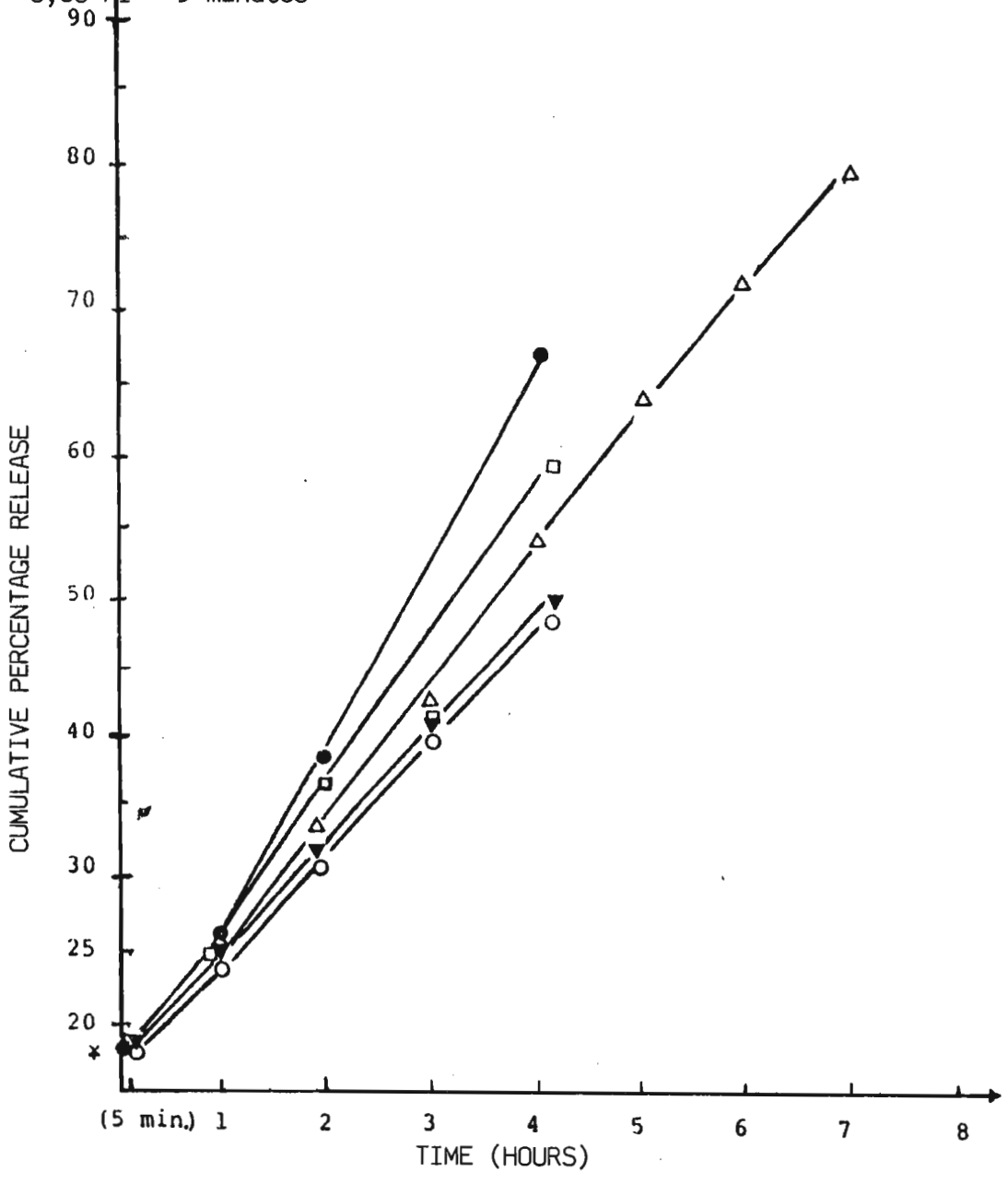


Table 2.B.3: Comparison of the cumulative percentage release of diethylpropion hydrochloride from two lots of sustained release pellets under the following conditions:
pH 1,5 (0,08; 1; 2; 3 and 4 hrs); pH 4,5 (0,08; 1; 2; 3 and 4 hrs); pH 6,9 (0,08; 1; 2; 3; 5; 6; 7 and 8 hrs); pH 7,2 (0,08; 1; 2; 4 and 7 hrs); pH 7,5 (0,08; 1; 2; 4 and 6 hrs):
Rotating bottle method

TIME AT pH (HOURS)	CUMULATIVE PERCENTAGE RELEASE									
	Lot R 7574 (Lemmon)					Lot R 7773 (Lemmon)				
	pH 1,5	pH 4,5	pH 6,9	pH 7,2	pH 7,5	pH 1,5	pH 4,5	pH 6,9	pH 7,2	pH 7,5
0,08 (5 min)	17,5	17,49	17,5	17,8	17,9	19,0	18,9	18,9	18,95	19,0
1	32,9	32,8	32,9	36,1	38,0	25,9	24,4	24,1	24,9	25,7
2	58,5	58,3	57,1	62,9	65,8	31,3	30,3	32,0	36,8	38,5
3	74,4	71,6	70,7			40,9	39,7	42,2		
4	87,2	83,4	82,1	87,4	90,6	50,3	49,5	54,5	59,4	67,3
5			87,1					64,3		
6			92,4					72,8		
7			96,6					79,6		97,0
8								85,6	93,5	

a. Figures are expressed as percentage of potency
b. Potency of R 7574 is 169,18 mg/g pellets
c. Potency of R 7773 is 157,48 mg/g pellets
d. Values are average of duplicates
e. Percentage of free drug (non-sustained) in pellets is
   16,1 for R 7574
   18,4 for R 7773

Table 2.B.4: Comparison of the release rates of diethylpropion hydrochloride from two lots of sustained release pellets under the following conditions:
pH 1,5 (1; 2; 3 and 4 hours); pH 4,5 (1; 2; 3 and 4 hours); pH 6,9 (1; 2; 3; 5; 6 and 7 hours); pH 7,2 and 7,5 (1; 2; 4 and 6 hours each):
Rotating bottle method

TIME AT pH (HOURS)	RELEASE RATES FROM									
	Lot R 7574 (Lemmon USA)					Lot R 7773 (Lemmon USA)				
	pH 1,5	pH 4,5	pH 6,9	pH 7,2	pH 7,5	pH 1,5	pH 4,5	pH 6,9	pH 7,2	pH 7,5
1	16,8	16,7	16,8	20,0	21,9	7,5	6,0	5,7	6,5	7,3
2	25,6	25,5	24,2	26,8	27,8	5,4	5,9	7,9	11,9	12,8
3	15,9	13,3	13,6			9,6	9,4	10,2		
4	12,8	11,8	11,4	12,3	13,9	9,4	9,8	11,3	11,3	14,4
5			5,0					9,8		
6			5,3					8,5		15,83
7			4,2					6,8	11,36	
8								6,0		

a. Figures are expressed as percentage of potency/hour
b. Potency of pellets is 169,2 mg/g and 157,5 mg/g respectively
c. Values are the average of duplicate determinations
d. Both products are obtained from the same source - Lemmon U.S.A.
e. R 7574 - non sustained portion is 16,1% of total content of sustained release pellets.
f. R 7773 - non sustained portion is 18,4% of total content of sustained release pellets.

The solubility of a weak acid or base usually varies considerably as a function of pH. Therefore changes are expected in the solution rate of such drugs in different regions of the GIT. The total solubility (Cs) of a weak acid is given by

$$Cs = [HA] + [A^-] \quad \ldots\ldots\ldots\ldots\ldots\ldots 2.3$$

where

[HA] is the intrinsic solubility of the non-ionized acid (denoted as Co) and $[A^-]$ is the concentration of its anions. The concentration of the anion can be expressed in terms of the dissolution constant, Ka and Co; i.e.:

$$Cs = Co + \frac{Ka \cdot Co}{[H^+]} \quad \ldots\ldots\ldots\ldots\ldots \text{Eq. 2.4}$$

Similarly, for weak bases:

$$Cs = Co + \frac{Co\,[H^+]}{Ka} \quad \ldots\ldots\ldots\ldots\ldots \text{Eq. 2.5}$$

Substituting equations 2.4 and 2.5 into equation 2.6 i.e. the modified Noyes-Whitney relationship,

$$dC/dt = D.S.Cs/h \quad \ldots\ldots\ldots\ldots\ldots \text{Eq. 2.6}$$

where dC/dt is the dissolution rate, S is the surface area of the dissolving solid, Cs is usually approximated as the solubility of the drug or chemical in the solvent, D is the diffusion coefficient of the dissolving material and h is the thickness of the diffusion layer and equation 2.6 describes a diffusion-controlled dissolution process (for details see 2.B.1), the following dissolution rate equations are obtained:

For weak acids

$$\frac{dC}{dt} = K'\left(Co + \frac{KaCo}{[H^+]}\right) \quad \ldots\ldots\ldots\ldots \text{Eq. 2.7}$$

or

$$= K'\,Co\left(1 + \frac{Ka}{[H^+]}\right) \quad \ldots\ldots\ldots\ldots \text{Eq. 2.8}$$

For weak bases

$$\frac{dC}{dt} = K'\,Co\left(1 + \frac{[H^+]}{Ka}\right) \quad \ldots\ldots\ldots\ldots \text{Eq. 2.9}$$

where $K' = DS/h$

Equations 2.8 and 2.9 indicate that the dissolution rate of weak acids increases with increasing pH i.e. decreasing $[H^+]$, whereas the dissolution rate of weak bases decreases with increasing pH. The dissolution rate of weak bases is optimal in gastric fluid, but that of weak acid is minimal. The dissolution rate of the weak acid increases as the undissolved drug particles are transported to the more alkaline regions of the GIT.

According to equation 2.9, a linear relationship should exist between the dissolution rate, dC/dt, of a weak base and the hydrogen ion concentration $[H^+]$, on the assumption that all other conditions, including surface area, remain constant. In practice, however, a plot of dC/dt vs $[H^+]$ may be linear at high $[H^+]$ i.e. low pH. As the $[H^+]$ increases the observed dissolution rate may differ from the predicted value. The reason for this is that the hydrogen ion of the bulk is not equal to the hydrogen ion concentration of the diffusion layer, except at low pH. The hydrogen ion concentration of the diffusion layer is denoted by $[H^+]d$. For a weak acid, $[H^+]d > [H^+]$. Since the diffusion layer is saturated with the drug, it is reasonable to expect that in solutions with a pH greater than the pKa of the drug, the relatively large acidic drug concentration may overcome the buffer capacity of the solution. In this case the buffer capacity of the diffusion layer is lower than the pH of the bulk solution and the dissolution rate is less than predicted. The same principle applies to weak bases (Gibaldi, 1977). The pH of the diffusion layer may be estimated by measuring the pH of the appropriate buffer solution saturated with the drug.

The dissolution rate of a particular salt is usually different from that of the parent compound. Sodium or potassium salts of weak acids dissolve more rapidly than the free acid, regardless of the pH of the dissolution medium (Nelson, 1958). The same is true of the hydrochloric acid or other strong acid salts of a weak base (Gibaldi, 1977).

The potency values determined for each batch of pellets are listed (Table 2.B.5). For calculating the correct dosage for in vivo trials, these determined values were used. The total recovery of the drug from the pellets over eight hours, together with the amount of drug remaining in the residual pellets, served as a check.

The density of the pellets is important in controlling the rate of passage through the GIT (Bechgaard et al., 1978). The average transit times for the light and heavy pellets (density range 1,0 to 1,6 gm/ml) was 7 and 25 hours respectively. The diameter of the pellets is of minor significance in the rate of transit. Noormohammadi (1981) monitored the distribution movement of BaSO4 pellets in the GIT using an X-ray technique. After 24 hours about 80% of the pellets (density = 1,50 g/ml) had passed via the faeces, but with the denser pellets (density = 1,97 g/ml) only 50% of the pellets had passed in the same time. These findings were in good agreement with those of Bechgaard et al., (1978). Therefore, the densities of all the pellets used in these in vivo studies, were determined (Table 2.B.15) and ranged between 1,50 and 1,7 gl/ml. These values, in addition to other data, are of relevance when predicting in vivo profiles from in vitro data on the pellets.

2.B.2 Stability Testing

(i) Storage of sustained release pellets in hard gelatin capsules

The cumulative percentage release of the drug from the sustained release pellets after storage at room temperature (25°C) in hard gelatin capsules, was plotted against the sampling time (Figures 2.B.4 and 2.B.5). The results obtained clearly indicated that storage for at least one year at room temperature in hard gelatin capsules did not affect the release of the drug from the pellets. Sustained release pellets with diffusion rate-limiting membranes similar to the ones used in our studies, but containing pseudoephedrine hydrochloride or disopyramide, were found

Table 2.B.5: The potencies and densities of diethylpropion hydrochloride sustained release pellets formulations

Pellets Lot No.	Density[1] g/ml		Mean Potency[2] mg/g	
	Water	Oil	Found	Given[3]
018010	1,643	1,640	168,8	170,2
R 7574	1,594	1,591	169,18	
R 7773	1,703		157,48	

1. Mean of duplicate readings
2. Mean of triplicate readings. The potency was determined for each dissolution study. Results never varied by more than 5%.
3. Refers to potency stated on the container label i.e. supplied by the manufacturer.

Figures 2.B.4 and 2.B.5: The mean cumulative percentage release of diethylpropion hydrochloride from sustained release pellets Lots 018010 and R 7773 after storage at room temperature in hard gelatin capsules*1. Rotating bottle method: Dissolution medium - pH 1,5 (5 min)*2, pH 1,5 (55 min)*2; pH 4,5 (1 hr); pH 6,9 (2 hrs); pH 6,9 (1 hr); pH 7,2 (2 hr); pH 7,5 (1 hr) and pH 7,5 (2 - 4 hrs)

*1 - Capsules were stored in closed clear glass bottles

*2 - Dissolutions at pH 1,5 for 5 minutes and 55 minutes were carried out initially and at the end of storage period only. At all other storage times, dissolution at pH 1,5 was for 1 hour.

Figure 2.B.4: Lot 018010 (Temmler)

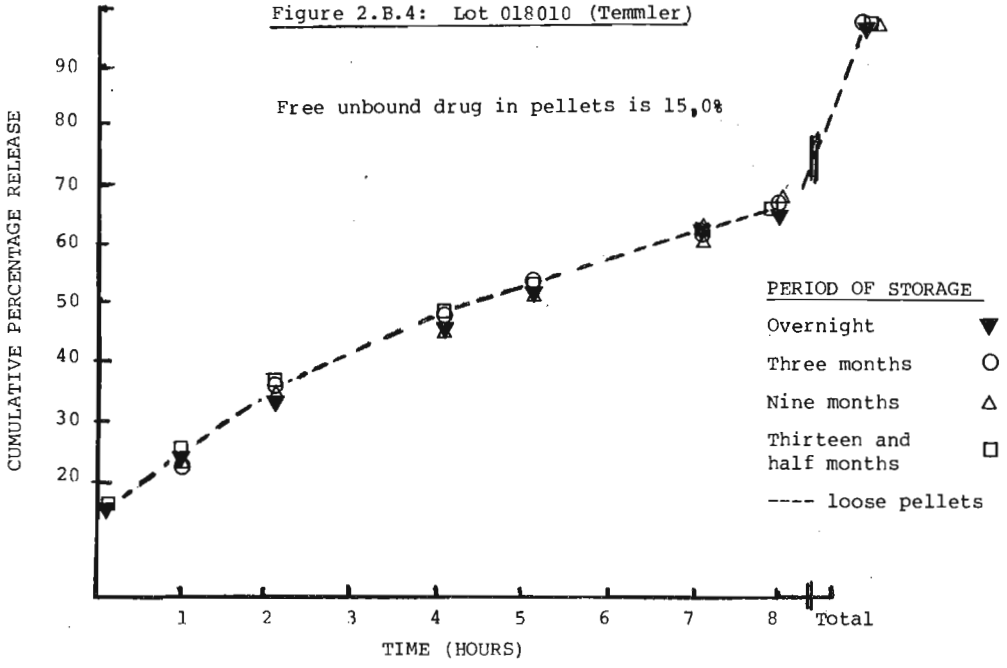
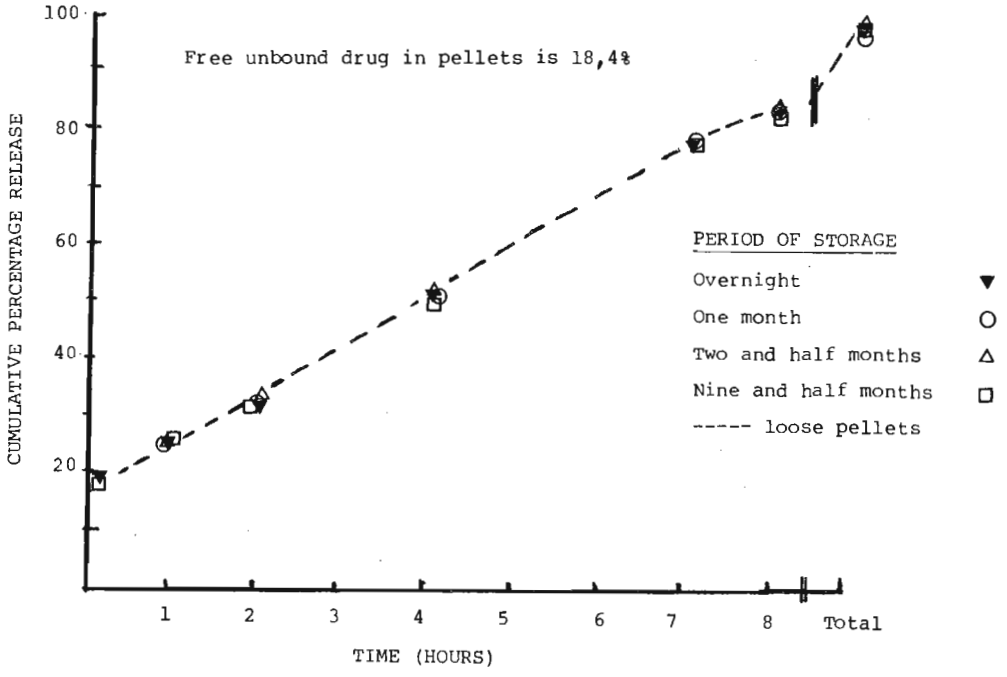


Figure 2.B.5: Lot R 7773 (Lemmon)



to be stable and unaffected when stored in hard gelatin capsules for at least 15 months (Hassanzadeh, 1981; Slipper, 1981). The results were encouraging as they showed that gelatin capsules provided a suitable means of administering the pellets in a premeasured dose and in a convenient, hygienic and compact dosage form.

(ii) Storage of the free drug and of sustained release pellets at different temperatures

The cumulative percentage release and the release rates of diethylpropion hydrochloride from the sustained release pellets (Lots 018010 and R 7773) stored for different periods of time at 4°C, room temperature (25°C) and 37°C, are graphically represented in Figures 2.B.6 to 2.B.10, and in Tables 2.B.6 to 2.B.9 respectively. The results of the potency determinations and the percentage decomposition, expressed in terms of the formation of phenylmethyldiketone (see Scheme 1.1, Section 1.2 for details on its formation), of diethylpropion hydrochloride stored in free form or as sustained release pellets (Lots 018010 and R 7773), are given in Tables 2.B.10 and 2.B.11 respectively.

According to the results obtained, the diethylpropion hydrochloride, free form and the sustained release pellets, were stable at room temperature and at 4°C for the duration of storage (at least one year), as the release characteristics as well as the potencies had not varied significantly. Under these conditions, the shelf-life of the pellets can be predicted to be at least two years which is an acceptable period for a pharmaceutical product.

The long-term stability test was essential to determine accurately the shelf-life of the pellets. Attempts have not been made to use accelerated storage tests to predict stability. Although these tests may be useful in providing information on the possible routes of decomposition which may occur, they are severely limited. Accelerated stability

Figure 2.B.6.: The cumulative percentage release of diethylpropion hydrochloride from sustained release pellets (Lot 018010) after storage at room temperature (using the rotating bottle method) for different periods of time.
pH 1,5 (5 min)*; pH 1,5 (55 min)*; pH 4,5 (1 hr); pH 6,9 (2 hrs); pH 6,9 (1 hr); pH 7 (2 hrs); pH 7,5 (1 hr) and pH 7,5 (2 - 3 hrs).
*Dissolution at pH 1,5 for 5 minutes and 55 minutes was carried out on pellets initially and at the end of storage period only. In all other studies, dissolution at pH 1,5 was for 1 hour.

Free unbound drug in pellets is 15,0%.

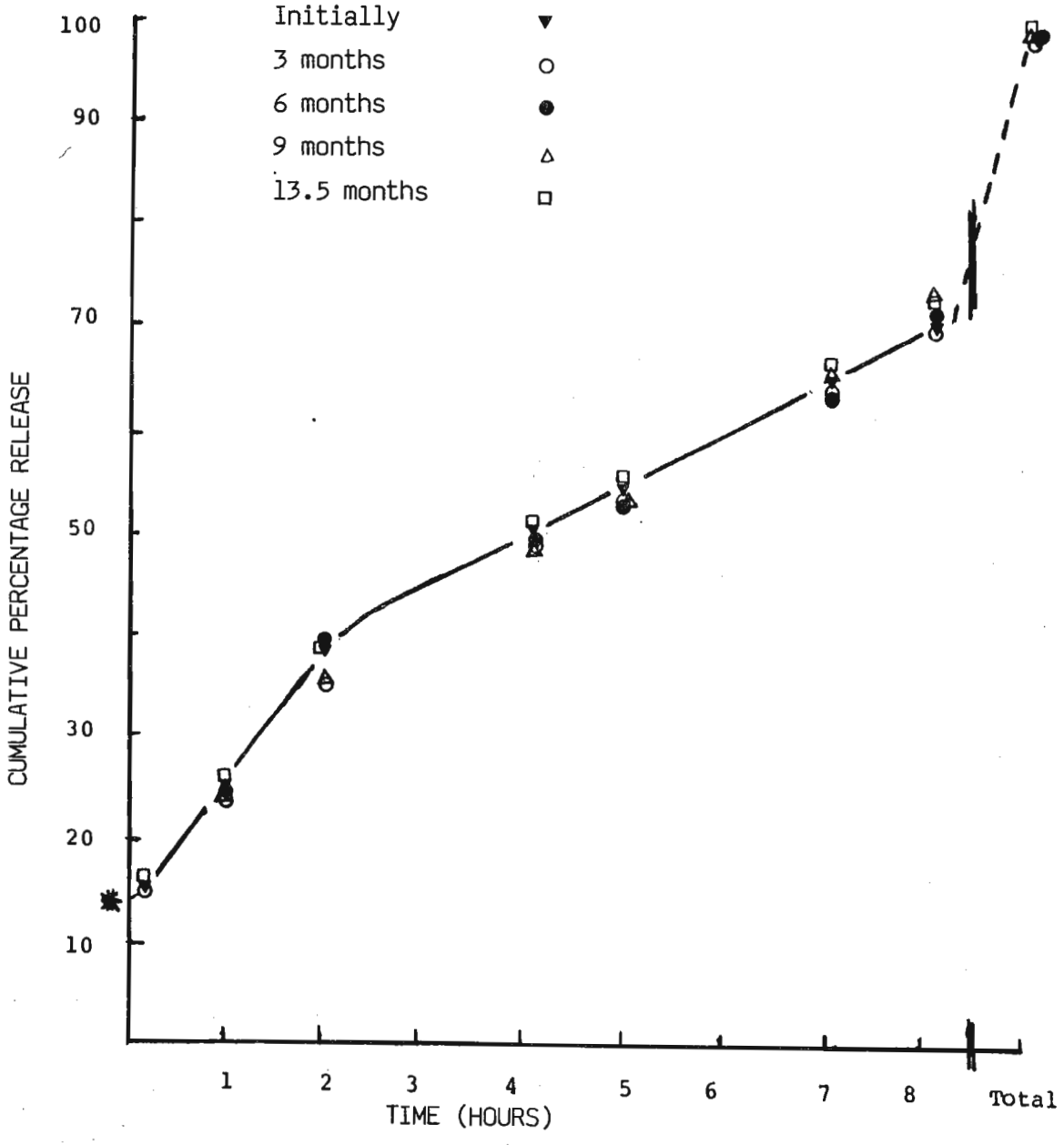


Figure 2.B.7: The cumulative percentage release of diethylpropion hydrochloride from sustained release pellets (Lot 018010) after storage under different conditions.

Method: Rotating Bottle with pH gradients as follows: pH 1,5 (5 min)*; pH 1,5 (55 min)*; pH 4,5 (1 hr); pH 6,9 (2 hrs); pH 6,9 (1 hr); pH 7,2 (2 hrs); pH 7,5 (1 hr) and pH 7,5 (4 hrs).

*Dissolution at pH 1,5 for 5 minutes and 55 minutes was done initially and at the end of 13,5 months of storage - see Table 2.B.6. In all other studies, dissolution at pH 1,5 was for 1 hour.

Free unbound drug in pellets is 15,0%.

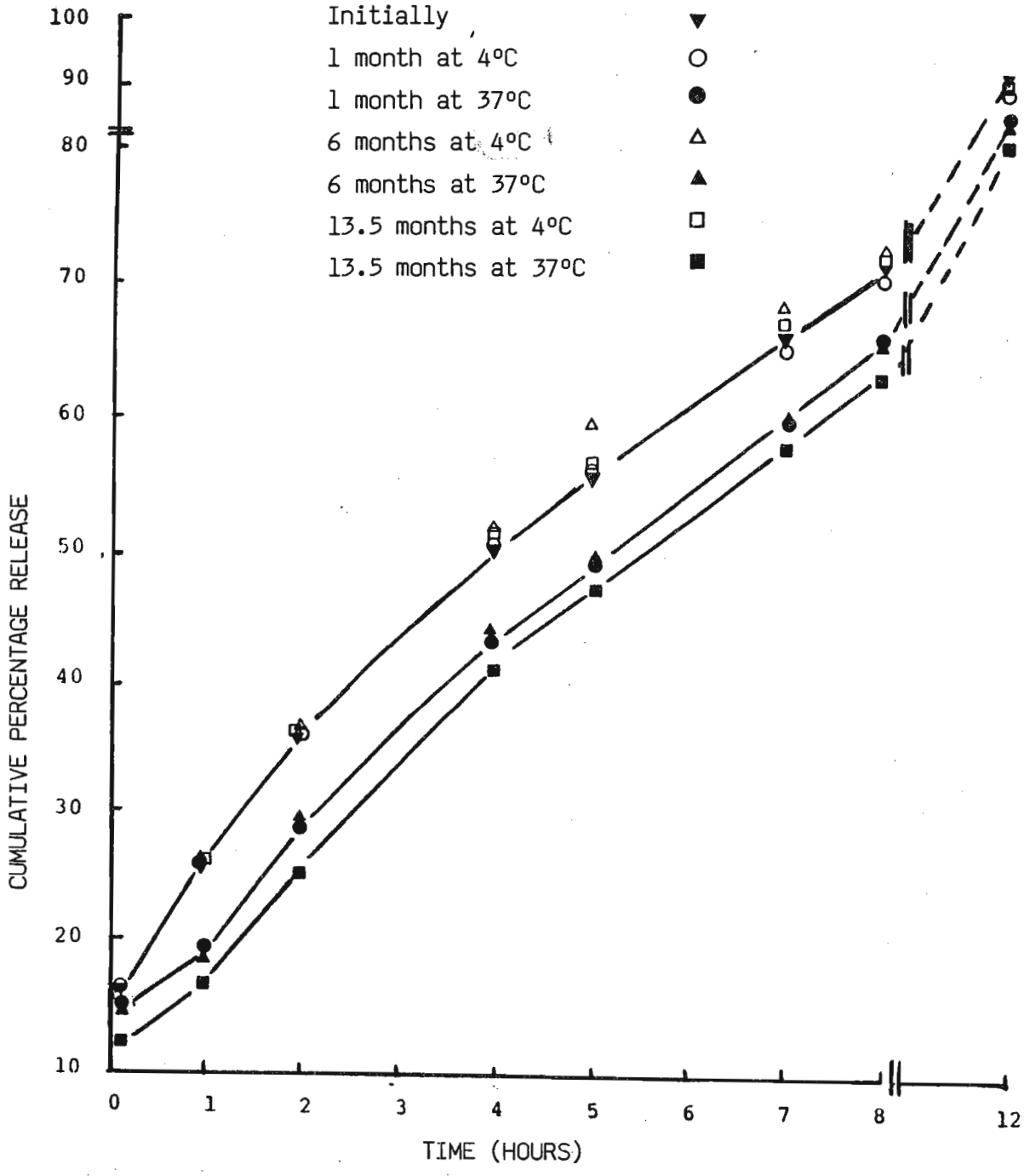


Table 2.B.6: Cumulative percentage release of diethylpropion hydrochloride from sustained release pellets (Lot 018010 - Temmler after storage under different conditions for varying periods of time

pH VALUE	TIME AT pH (Hr/s)	CUMULATIVE PERCENTAGE RELEASE AFTER STORAGE AT														
		START OF STORAGE 21.3.80	4°C for					Room Temperature for					37°C*1 for			
			16.4.80 1 mt	16.7.80 3 mt	28.9.80 6 mt	16.1.81 9 mt	28.5.18 13,5 mt	1 mt	3 mt	6 mt	9 mt	13,5 mt	1 mt	3 mt	6 mt	9 mt	13,5 mt
1,5	0,08	15,82	(15,85)	(15,88)	(15,84)	(15,84)	15,82	(15,78)	(15,81)	(15,81)	(15,71)	15,83	(15,38)	(15,38)	(15,38)	(14,38)	12,38
1,5	1*2	24,8	25,2	25,5	25,1	25,1	24,9	24,4	24,7	24,7	23,5	24,8	19,6	19,5	19,7	18,8	16,6
4,5	1	36,2	36,8	36,7	36,4	36,1	36,2	35,7	35,9	36,6	33,8	36,0	28,9	29,4	29,8	28,5	26,0
6,9	2	50,5	51,3	53,7	52,0	51,9	52,6	48,7	49,6	48,2	49,2	51,8	43,1	43,4	45,2	44,4	41,7
6,9	1	56,0	57,2	60,2	60,8	60,6	57,6	54,0	54,8	53,8	54,4	56,9	49,2	49,8	50,0	50,9	48,1
7,2	2	66,3	65,2	70,3	70,5	66,5	68,1	65,3	65,3	65,5	68,4	68,3	59,8	59,6	60,3	60,7	57,7
7,5	1	71,6	71,0	75,6	74,0	72,0	73,5	70,9	70,9	71,4	73,8	73,6	66,0	65,1	65,6	66,4	63,5
7,5	4	94,8	92,0	92,5	92,5	93,0	93,0	92,0	92,5	92,0	94,0	93,0	87,5	85,6	85,9	87,3	86,7
Drug in Residual Pellets		4,5	8,2	7,3	5,8	6,2	6,8	7,0	7,7	6,9	5,7	7,1	10,5	12,0	12,5	9,3	8,0
Total Recoveries		99,3	100,2	99,8	98,3	99,2	99,8	99,0	100,2	98,9	99,7	100,1	98,0	97,6	98,4	96,6	94,7

(1) Figures are expressed as percentage of the potency i.e. 168,8 mg/g

(2) All results are the average of duplicates

(3) mt/s = month/s Hr/s = hour/s

*1 The pellets darkened to yellow on storage
Free unbound drug in pellets is 15,0%

( ) These values were estimated by back extrapolating (to time, 0) from a plot of Cumulative % Release vs time for each set of data

*2 Determined using the method shown in 2.A.2.2.(d)
For release rates refer to Table 2.B.7

Table 2.B.7: Release rate of diethylpropion hydrochloride from sustained release pellets (Lot 018010 - Temmler) after storage under different conditions.

Rotating Bottle Method: pH 1,5 (1 hr)*, pH 4,5 (1 hr), pH 6,9 (2 hrs), pH 6,9 (1 hr), pH 7,2 (2hrs), pH 7,5 (1 hr)

pH VALUE	TIME OF pH (Hr/s)	RELEASE RATE (% POTENCY/HR) AFTER STORAGE AT											
		START OF STORAGE	4°C for				R.T. for				37°C for			
		21.2.80	1 mt	3 mt	9 mt	13,5 mt	1 mt	3 mt	9 mt	13,5 mt	1 mt	3 mt	9 mt	13,5 mt
		FREE FORM: 15,0	(14,99)	(15,02)	(15,0)	15,0	(15,0)	(15,09)	(14,96)	15,0	(15,0)	(15,0)	(14,2)	12,0
1,5	1	9,8	10,2	10,5	10,1	9,9	9,4	9,7	8,5	9,8	4,6	4,5	4,6	4,6
4,5	1	11,4	11,6	11,2	11,0	11,3	11,3	11,2	10,3	11,2	9,3	9,9	9,7	9,4
6,9	2	7,2	7,8	8,5	7,9	6,2	6,5	6,9	7,7	7,9	7,1	7,0	7,5	7,9
6,9	1	5,5	5,9	6,5	8,7	5,0	5,3	5,2	5,2	5,1	6,1	6,4	6,5	6,4
7,2	2	5,2	4,0	5,1	5,9	5,3	5,7	5,3	7,0	5,7	5,3	4,9	4,9	4,8
7,5	1	5,3	5,8	5,3	5,5	5,6	5,6	5,6	5,4	5,3	6,2	5,7	5,7	5,8

(1) Potency of pellets 168,8 mg/g

(2) Free unbound drug in pellets is 15,0%

(3) For date on cumulative percentage release - refer to Table 2.B.6

mt/s = month/s R.T. = Room Temperature hr/s = hour/s

( ) not determined, but obtained by back extrapolation (to time,0) in a plot of Cumulative Release vs time

* For studies, initially and after storage for 13,5 months, the buffer was substituted: pH 1,5 for 5 mins and pH 1,5 for 55 mins.

(4) All results are the average of duplicates

Table 2.B.8: Cumulative percentage release of diethylpropion hydrochloride from sustained release pellets (Lot R 7773-Lemmon) - after storage under different conditions

pH VALUE	TIME AT pH (Hr/s)	CUMULATIVE % RELEASE AFTER STORAGE AT							
		START OF STORAGE	4°C for		Room Temperature for			37°C*1 for	
			1 Month	2,5 Months	1 Month	2,5 Months	9,7 Months	1 Month	2,5 Months
1,5	0,08	19,03	(19,04)	(19,05)	(19,04)	(19,0)	19,05	(18,73)	(18,73)
1,5	1*2	26,1	26,1	26,3	26,1	25,6	26,2	22,3	22,3
4,5	1	33,8	32,6	32,5	33,4	32,8	33,7	29,8	28,7
6,9	2	53,6	53,1	52,1	53,1	52,9	53,9	49,8	48,6
6,9	1	63,4	63,2	63,0	62,9	63,3	63,5	59,6	58,3
7,2	2	79,9	78,8	79,8	79,0	79,8	80,0	75,8	74,7
7,5	1	85,2	84,0	84,8	84,7	84,9	85,3	80,9	79,7
7,5	3hr	95,4	94,9	95,4	95,0	95,1	95,2	94,3	93,1
Residue remaining in pellets		4,7	4,5	3,8	5,1	4,4	3,6	4,1	5,5
Total recovery		100,1	99,4	99,2	100,1	99,5	98,8	98,4	98,6

(1) Figures are expressed as the percentage of potency i.e. 157,5 mg/g

(2) All results are the average of duplicates

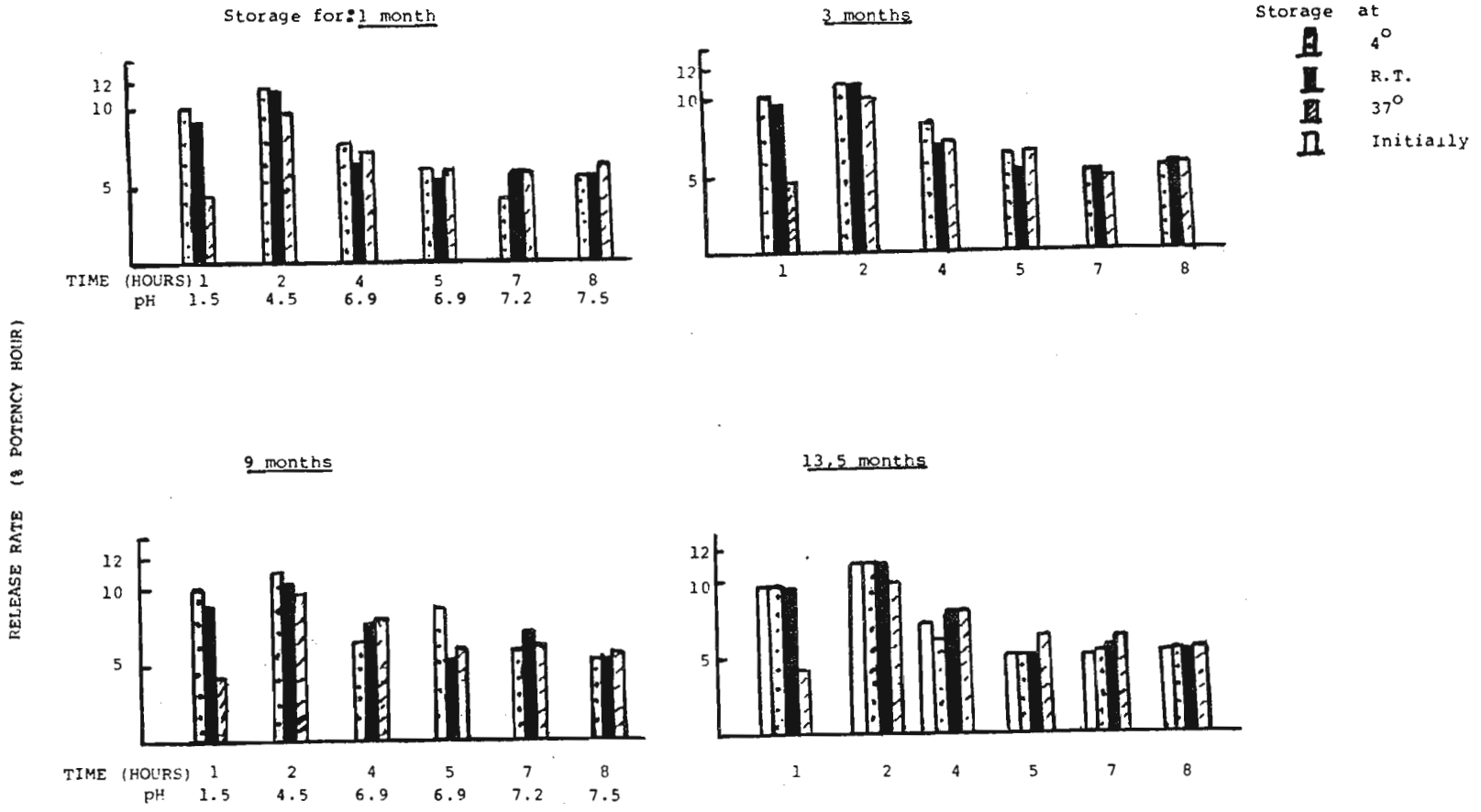
*1 Darkening of pellets on storage hr/s = hour/s

( ) These values were estimated by back extrapolating (to time, 0) a plot of Cumulative % Release vs time

*2 Determined using the method shown in 2.A.2.2.d

For data on release rates, refer to Table 2.B.9

Figure 2.B.8: Release rate (% of potency/hour*) of diethylpropion hydrochloride from sustained release pellets (Lot 018010 - Temmler Werke) after storage under different conditions



Potency of pellets 168,8 mg
Free unbound drug in pellets is 15%
*For data on cumulative percentage release, refer to Figure 2.B.7

Figure 2.B.9: Comparison of the cumulative percentage release of diethylpropion hydrochloride sustained release pellets (Lot R 7773) after storage under different conditions using the rotating bottle method
pH 1,5 (5 min)*; pH 1,5 (55 min)*; pH 4,5 (1 hr); pH 6,9 (2 hrs); pH 6,9 (1 hr); pH 7,2 (2 hrs); pH 7,5 (1 hr) and pH 7,5 (3 hrs).
*Dissolution at pH 1,5 for 5 minutes and 55 minutes was carried out on samples initially and at 9,7 months only. At all other times, the dissolution at pH 1,5 was for 1 hour.

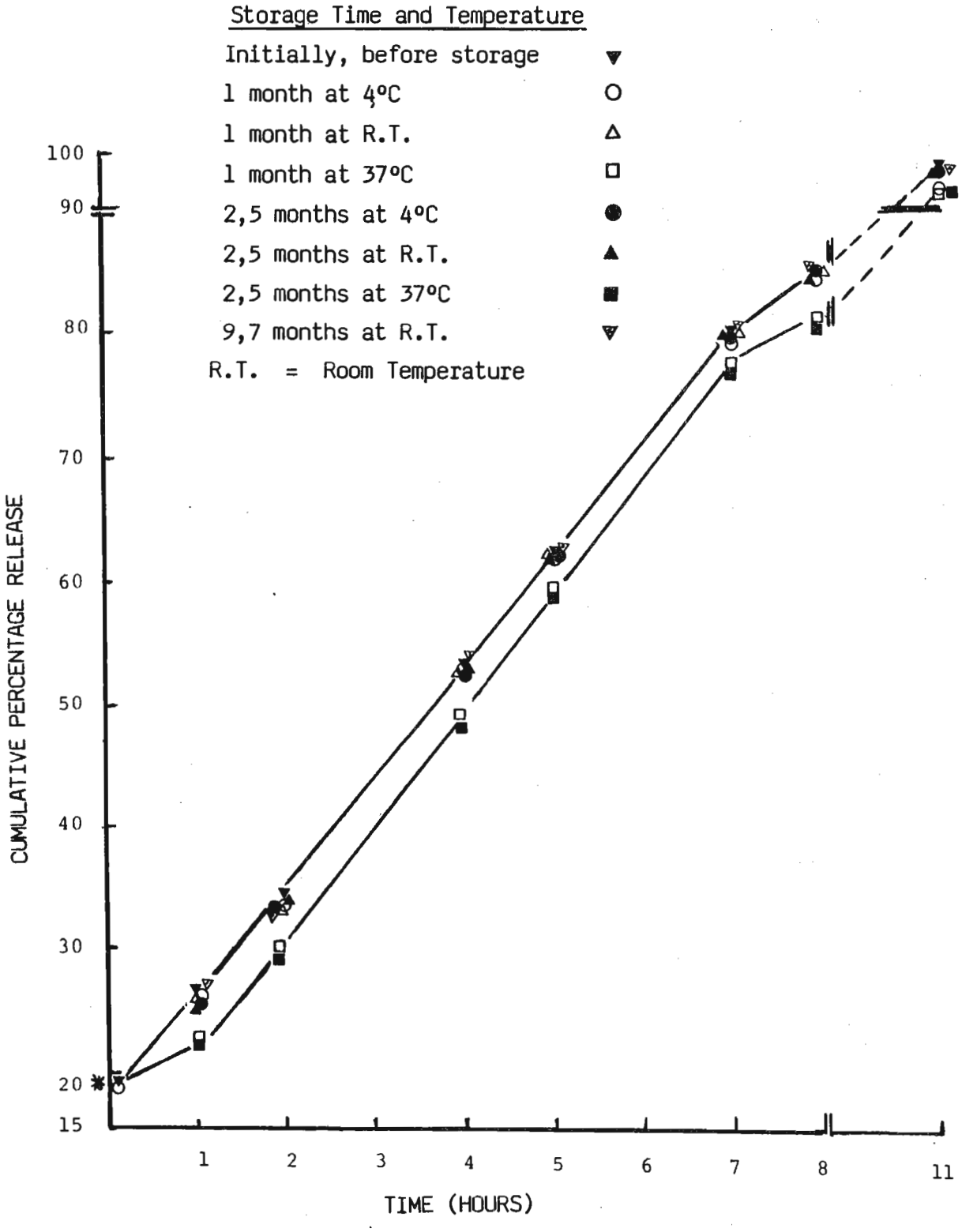


Table 2.B.9: Release rate of diethylpropion hydrochloride from sustained release pellets (Lot R 7773 - Lemmon) after storage under different conditions.

Rotating Bottle Method: pH 1,5*1 (1 hr), pH 4,5 (1 hr), pH 6,9 (2 hrs), pH 6,9 (1 hr), pH 7,2 (2 hrs), pH 7,5 (1 hr)

pH VALUE	TIME AT pH (Hr/s)	RELEASE (% RELEASE/HOUR) AFTER STORAGE							
		START OF STORAGE	4°C for		Room Temperature for			37°C for	
			1 Month	2,5 Months	1 Month	2,5 Months	9,7 Months	1 Month	2,5 Months
		FREE FORM: 19,03	(19,04)	(19,05)	(19,04)	(19,0)	19,05	(18,73)	(18,73)
1,5	1	7,7	7,7	7,9	7,7	7,2	7,8	3,9	3,9
4,5	1	7,7	6,5	6,9	7,3	7,2	7,5	6,5	6,4
6,9	2	10,1	10,25	9,8	9,85	10,1	10,1	10,0	9,9
6,9	1	9,8	10,1	10,9	9,8	10,4	9,6	9,8	9,7
7,2	2	7,8	7,8	8,4	8,1	8,3	8,3	8,1	8,2
7,5	1	5,3	5,2	5,0	5,7	5,1	5,3	5,1	5,0

(1) Figures are expressed as percentage of potency/hour (157,5 mg/g = potency)

(2) All results are the average of duplicates

(3) For data on cumulative percentage release refer to Table 2.B.8

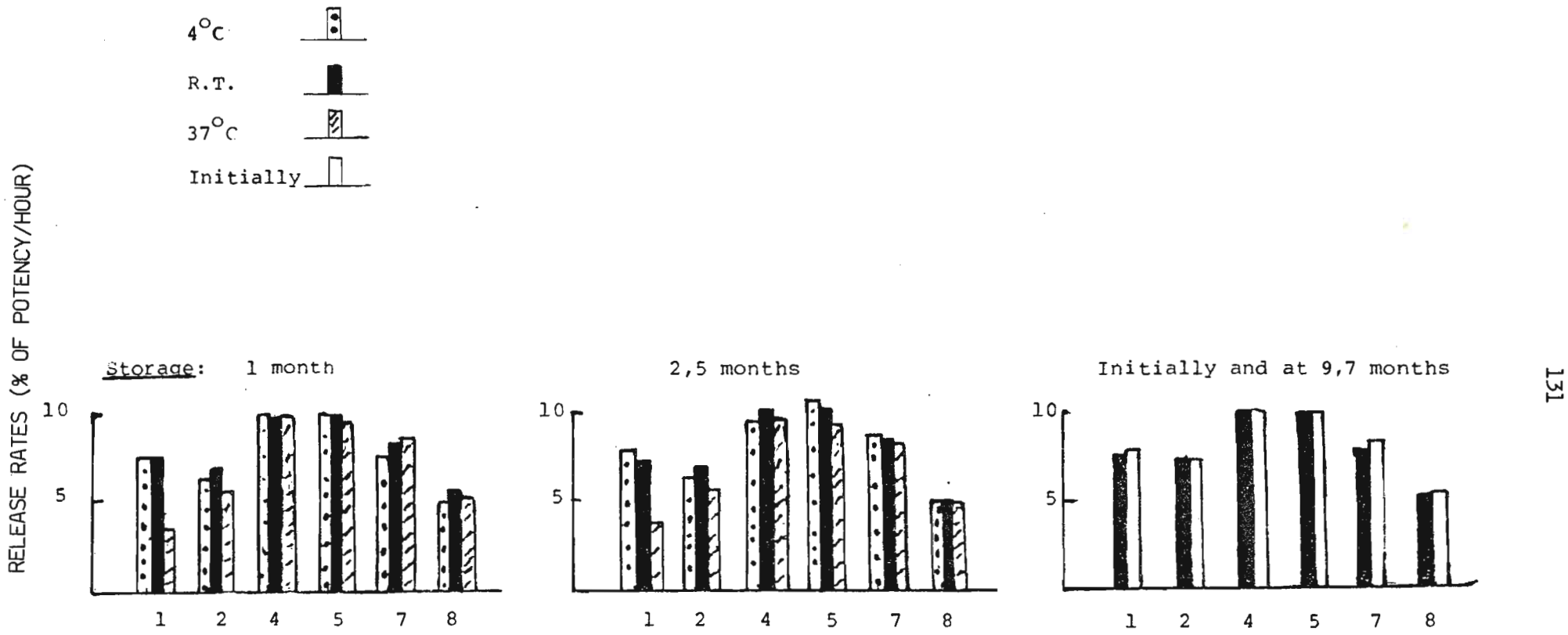
(4) Free non sustained content of drug in pellet is 18,4%

mt/s = month/s hr/s = hour/s

*1 For studies done initially and at 9,7 months of storage, the buffer was substituted pH 1,5 for 5 minutes and pH 1,5 for 55 minutes

( ) Not determined but obtained by back extrapolating (to time, 0) a plot of Cumulative % Release vs time

Figure 2.B.10: Release rate (% potency/hour) of diethylpropion hydrochloride from sustained release pellets (Lot R 7773 - Lemmon) after storage under different conditions



pH 1,5; 4,5; 6,9; 6,9; 7,2; 7,5

Potency of pellets 157,5 mg/g - Table 2.B.10
R.T. Room Temperature
For cumulative release data refer to Figure 2.B.9

tests based on the Arrhenius equation, for example, are only valid when the breakdown is a thermal phenomenon. With diethylpropion hydrochloride several mechanisms, including thermal, pH-dependent and hydrolytic decomposition, seem to be implicated (Walters, 1980; Beckett et al., 1979).

All the pellets formulations (Lots R 7773, R 7574 and Lot 018010) as well as the free powder, showed within 1 month of storage at 37°C, some darkening to a brown-yellow shade, suggestive of possible decomposition. Therefore the "release characteristics", the free drug content, the formation of the decomposition product and the potency of these pellets under stability testing procedure, were routinely checked (Table 2.A.1).

Storage of the pellets (Lots 018010 and R 7773) and the free drug, clearly demonstrated in our studies that formulation into sustained release pellets provided greater stability to the diethylpropion hydrochloride, based on the formation of the smaller percentage (2,13% as against 3,2% in the free form) of the decomposition product, (1-phenyl-1,2, -propanedione), and on the potency of the drug at 13,5 months. (Tables 2.B.10 and 2.B.11). Despite the decrease in the potency of the pellets (Lot 018010) stored for 13,5 months at 37°C, the product still complied with the official limits of content (BP 1980).

Storage of the sustained release pellets (Lot 018010 for 13,5 months and Lot R 7773 for 2,5 months) at room temperature or in a refrigerator (4°C) gave comparable dissolution rate profiles to those of the sustained release pellets stored initially, (Figures 2.B.6 to 2.B.10). There was no significant change in the potencies (Tables 2.B.10 and 2.B.11) over the same periods of time, as the pellets proved to be stable under normal conditions of storage. How-

Table 2.B.10: The effect of storage at different temperatures on the potency of two lots of sustained release pellets

PELLETS LOT NO.	PERIOD OF STORAGE (MONTHS)	POTENCY mg/g AFTER STORAGE AT		
		4°C	R.T.	37°C
018010	0-Initially	168,8	168,8	168,8
	1	169,1	167,1	165,4
	3	168,5	169,1	164,7
	6	165,9	166,9	166,1
	9	167,5	168,3	163,1
	13,5	168,5	170,3	159,9
R 7773	0	157,5	157,5	157,5
	1	156,6	157,6	155,0
	2,5	156,5	156,7	154,0

R.T. = Room Temperature

(1) All results are the average of duplicates

(2) Potencies were determined by gas liquid chromatography - Section 3.5.2(c)

Table 2.B.11: The effect of storage at different temperatures on the potencies and on the degratation of diethylpropion hydrochloride, in free form and as sustained release pellets

Lot 018010 - Temmler - Germany Potency = 168,8 mg/g

R 7773 - Lemmon - U.S.A. 157,5 mg/g

STORAGE (MONTHS)	ROOM TEMPERATURE						37°C					
	% DIKETONE FORMED IN			POTENCY OF			% DIKETONE FORMED IN			POTENCY OF		
	018010	R 7773	F.F.	018010	R 7773	F.F.	018010	R 7773	F.F.	018010	R 7773	F.F.
Initially,0	n.d.	n.d.	n.d.	99,3	101,1	100	n.d.	n.d.	n.d.	99,3	100,1	100
1	n.d.	n.d.	n.d.	99,0	100,1	99,6	n.d.	< 0,2	< 0,2	98,0	98,4	98,4
2,5	n.d.	n.d.	n.d.	a	99,5	101,0	n.d.	0,4	0,4	a	98,0	97,2
3	n.d.	b	n.d.	100,2	b	98,9	0,3	b	0,4	97,6	b	97,6
6	< 0,2	b	n.d.	98,7	b	99,5	0,4	b	0,4	98,4	b	97,6
9	< 0,2	n.d.	n.d.	99,7	98,8	99,7	1,48	b	1,73	96,6	b	95,4
13,5	< 0,2	b	<0,2	100,1	b	98,4	2,13	b	3,2	94,7	b	93,0

1. Diketone refers to the degradation product, phenylmethyldiketone (Walters et al., 1977)
2. Values are the average of duplicate observations
3. F.F. = Diethylpropion hydrochloride (DEP), in the free form
4. All figures are expressed as the percentage of potency

n.d. not detectable

a = Samples were not analysed at 2,5 months

b = Samples were not analysed at 3 and 6 months and beyond 9 months of storage

ever, storage at elevated temperatures did offer some interesting results. The extreme susceptibility of diethylpropion to degradation at elevated temperatures under uncontrolled humidity conditions (Walters, et al., 1977) was also found in our studies. The pellets (Lot 018010) showed signs of decomposition, although less than in the free form, after 3 months of storage at 37°C (Table 2.B.12).

The potency was not altered and the cumulative percentage (%) released over 12 hours for all the pellets stored was comparable. The decomposition of the drug increased progressively thereater for the total period of study (13,5 months).

The rate of release of the drug from both batches of pellets (Lots 018010 and R 7773) stored for 1 month at 37°C showed a marked decrease in the 1st hour of dissolution (pH 1,5) but thereafter (2nd hour to the end of the 11th hour) the drug release profiles were parallel and identical (Figures 2.B.7 and 2.B.9). This characteristic was maintained throughout the study period, and in pellets Lot 018010 (stored for 13,5 months at 37°C) the free drug content showed degradation ($\pm$ 5%), thereby giving a slightly lower profile (Figure 2.B.7). Because the potency and the free drug content of both batches of pellets stored at 37°C for 1 month were the same as in the pellets studied initially, and as the "release characteristics" of the dissolution profiles were constant (i.e. parallel - except for the 1st hour at pH 1,5), the only possible explanation for the lowering of profiles within one month would be the presence of a "lag period" before the drug is released. This "lag period" may be due either to binding of the drug inside the pellet or to some other interaction with additives. Therefore the rate of release may be impeded for a short duration within the first hour period. To be conclusive about the "lag period" one has to do several additional studies following the release rates of the drug

at short time intervals (say every 10 minutes) for the first hour.

Storage at 37°C for over 13,5 months showed no evidence of the total breakdown of the sustained release mechanism and integrity of the pellet membrane which could lead to uncontrolled and non-predictable release of the drug.

The stability of the pellets stored at elevated temperatures is promising as the slight drop in profile and decrease in release rate at one hour (Figures 2.B.7 and 2.B.9) would not be too critical in in vivo studies, for the release rate was found constant in all buffers and was independent of pH of the environment.

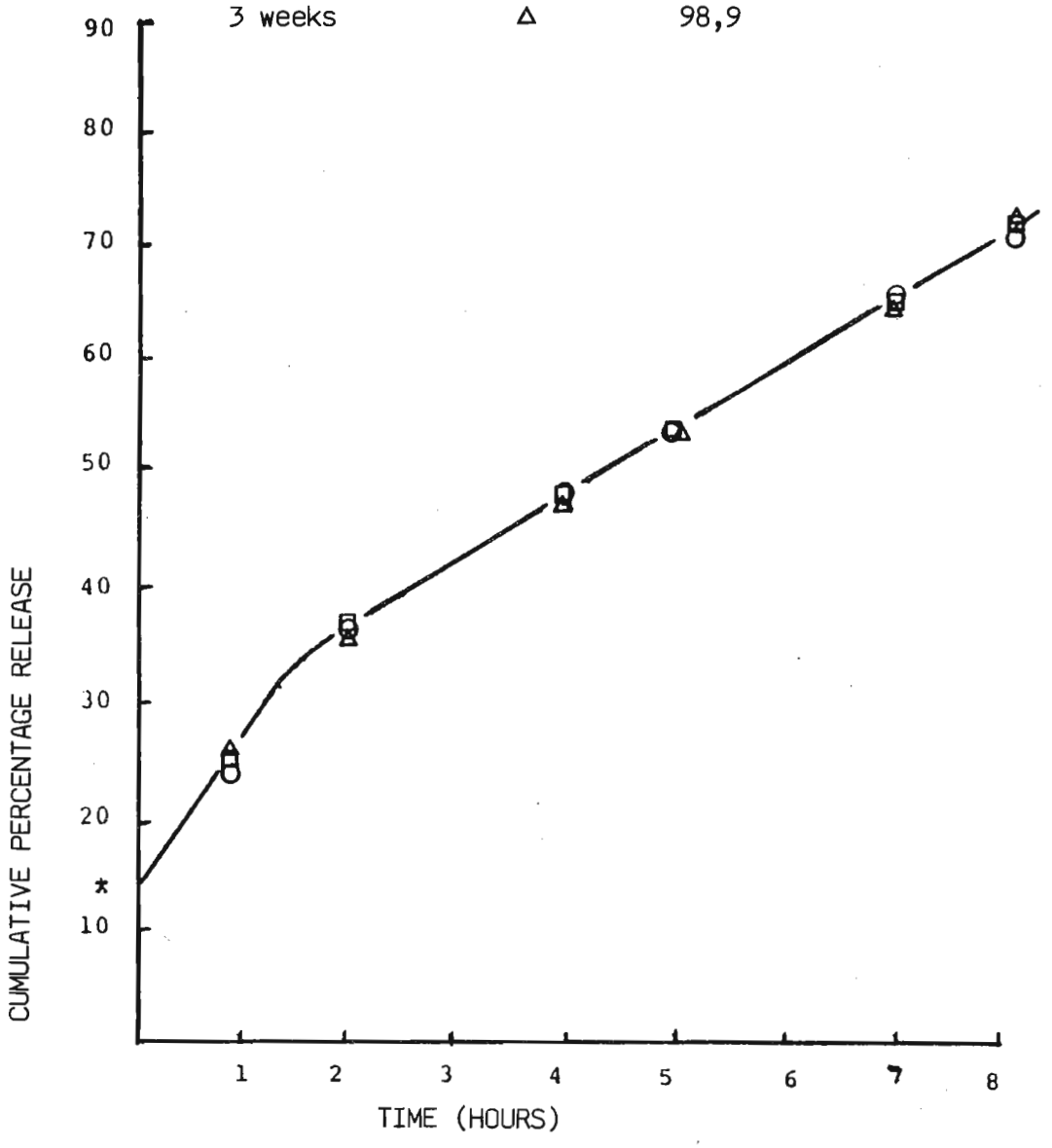
The pellets were not stored at 37°C or higher as part of an accelerated stability test. However, the Food and Drug Administration (FDA) is proposing a satisfactory three months stability testing of the drug product at 37°C to 40°C and 75% or higher relative humidity can be employed to project a tentative expiry date of two years from the date of manufacture. This essentially represents the projection of a tentative expiry date from a one-point accelerated stability test. This proposal has been analysed critically on a theoretical basis and its limitations have been discussed (Yang et al., 1980).

(iii) Storage of sustained release pellets as suppositories

The cumulative percentage release of the drug from the sustained release pellets incorporated into the suppositories was plotted against sampling times (Figure 2.B.11). The release of the drug from the loose pellets at pH 6,9 was also plotted for comparison. The suppository base melted quickly and completely at 37°C, releasing the pellets into the dissolution medium. The release characteristics were similar when stored overnight or for three weeks, and the potency remained unaltered. This suggests that the drug did not diffuse from the pellets into

Figure 2.B.11: The cumulative percentage release*1 of diethylpropion hydrochloride from sustained release pellets (Lot R 7773) as a suppository stored for 3 weeks at 4°C
Rotating bottle method - Constant pH 6,9

		Potency
Loose Pellets*2	□	99,85
Storage at 4°C		
Overnight	○	100,3
3 weeks	△	98,9



* Free unbound drug in pellets is 18,4%

*1 Values are the average of two determinations
*2 Blank suppository was placed in the rotating bottle
*3 Potency values are expressed as percentage of potency, 157,5 mg/g

the suppository on storage. In a separate study, Noormohammadi (1981) showed that ketoprofen sustained release pellets incorporated into the suppository base that was used in this study, were stable over a period of 15 months at room temperature and gave reproducible dissolution test profiles.

Promising results were also shown for lignocaine hydrochloride sustained release pellets formulated into such suppositories (Slipper, 1981).

One of the aims of the present study was to compare the oral and rectal routes of administration in order to consider the possibility of minimizing "first-pass metabolism" of the drug. It is very impractical to administer anorectics rectally, hence the stability studies on suppositories were not meant to be extensive or to include effects of different bases, like lipophilic media, etc. The results confirmed that using such suppositories in our studies would be useful in vivo as it is unlikely to alter the in vivo behaviour of the pellets.

The dissolution rate was determined, using the rotating bottle apparatus with buffers of pH 6,9 to simulate conditions in the rectum. A volume of 150 ml of buffer, replaced every hour or two for eight hours, did not simulate exactly the conditions occuring in the rectum. The rectum is only 15 - 20 cm long and in the resting state it does not show any active mobility. Normally it is empty, containing only 2 - 3 ml of inert mucus fluid (pH 7 to 8) which has no enzymetic activity or buffer capacity (Moolenaar and Schoonen, 1980). Sink conditions do occur, therefore the use of the rotating bottle dissolution test method with 150 ml of pH 6,9 buffer was justifiable.

The rotating bottle dissolution test method was used to simulate both oral and rectal administration (Section 2.A.1)

in order to compare results directly for the same batch of pellets. It was appreciated that the same release characteristics in vitro would not necessarily mean that the in vivo behaviour of the pellets would be the same via the two routes of administration. The in vitro results, however, were useful as such suppositories (stored) would be unlikely to alter the in vivo behaviour of the pellets.

The study on rectal suppositories was also extented to establish any possible in vitro/in vivo correlations (Chapter 4).

Attempts were made to develop in vitro models specifically for the prediction of drug release from lipophilic phases. A typical in vitro model, reported by Muhlemann and Neuenschwander (1956), was later modified for use by Voight and Falk (1968). There have, however, been few reports of in vitro/in vivo correlations concerning suppositories. Recently a new apparatus design was described for dissolution testing of suppositories (Palmeiri, 1981). The method does not appear to have any advantages to the one used in the present study.

The use of natural membranes in the determination of absorption rates of drugs from suppositories was investigated in vitro using the isolated rectum of albino rabbits as natural membranes. Acetaminophen absorption from suppositories prepared from cocoa butter and Witepsol H 15 was also investigated (Günger and Izgü, 1981). The authors concluded that the method could be successfully used in the investigation for the control of formulation parameters of suppositories. The mechanism of drug release from lipophilic media in the GIT has recently been reviewed (Armstrong and James, 1980).

Figure 2.B.12: Comparison of the cumulative percentage release of diethylpropion from sustained release pellets (Lot 018010) using three <u>in vitro</u> dissolution test methods

Programmed dissolution medium: pH 1,5 (1 hr); pH 4,5 (1 hr); pH 6,9 (2 hrs); pH 6,9 (1 hr); pH 7,2 (2 hrs); pH 7,5 (1 hr) and pH 7,5 (4 hrs)

*Free unbound drug in pellets is 15,0% - Table 2.B.12

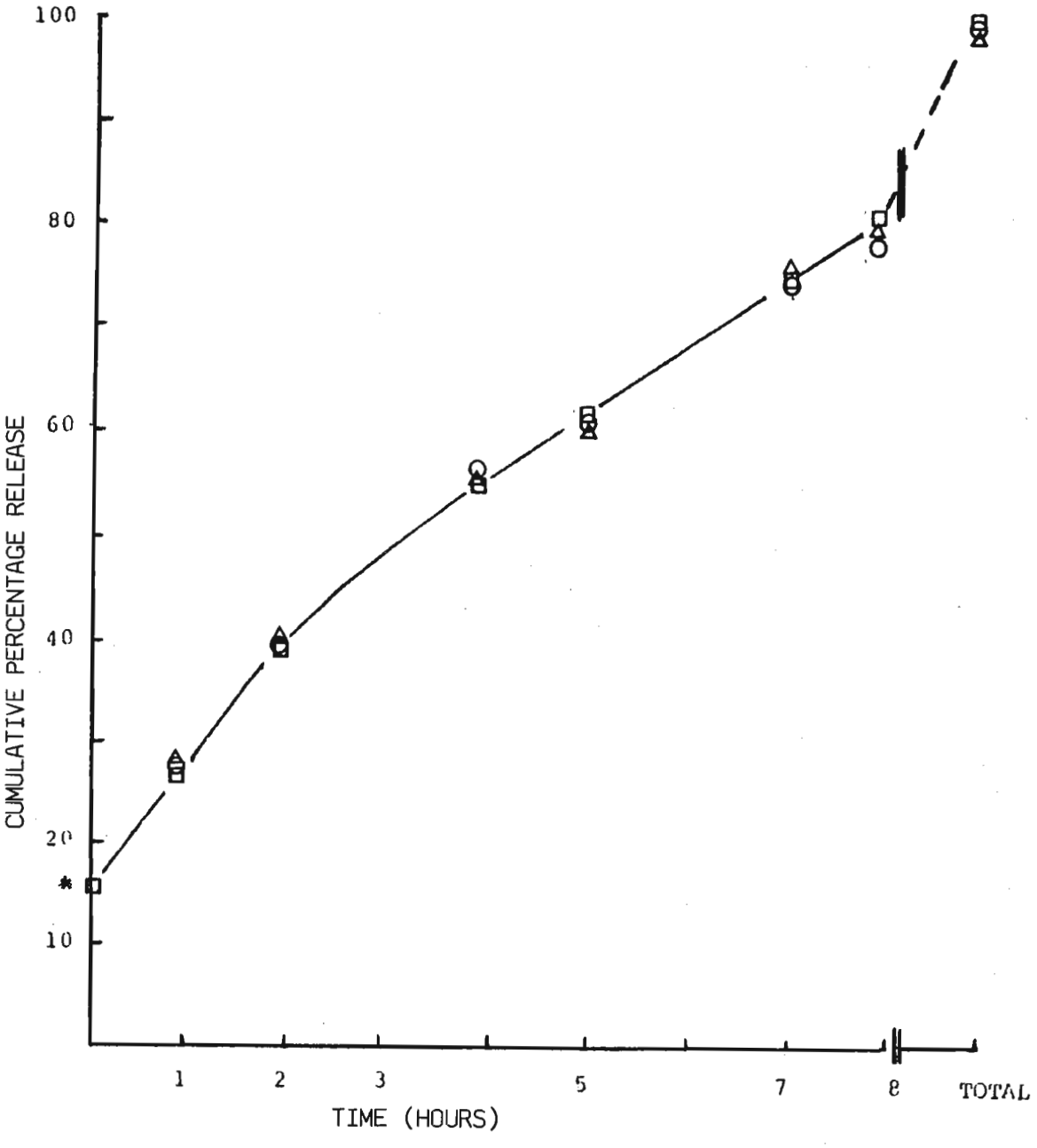


Figure 2.B.13: Comparison of the cumulative percentage release of diethylpropion hydrochloride from sustained release pellets (Lot 018010) using the rotating bottle method
pH 1,5 (1 hr); 4,5 (1 hr); 6,9 (2 hrs); 6,9 (1 hr); 7,2 (2 hrs); 7,5 (1 hr) and 7,5 (4 hrs)
Speed of Agitation (r.p.m.) - Table 2.B.12

35 —▼—
65 --O--
88 --△--
100 --□--

* Free unbound drug in pellets is 15,0%

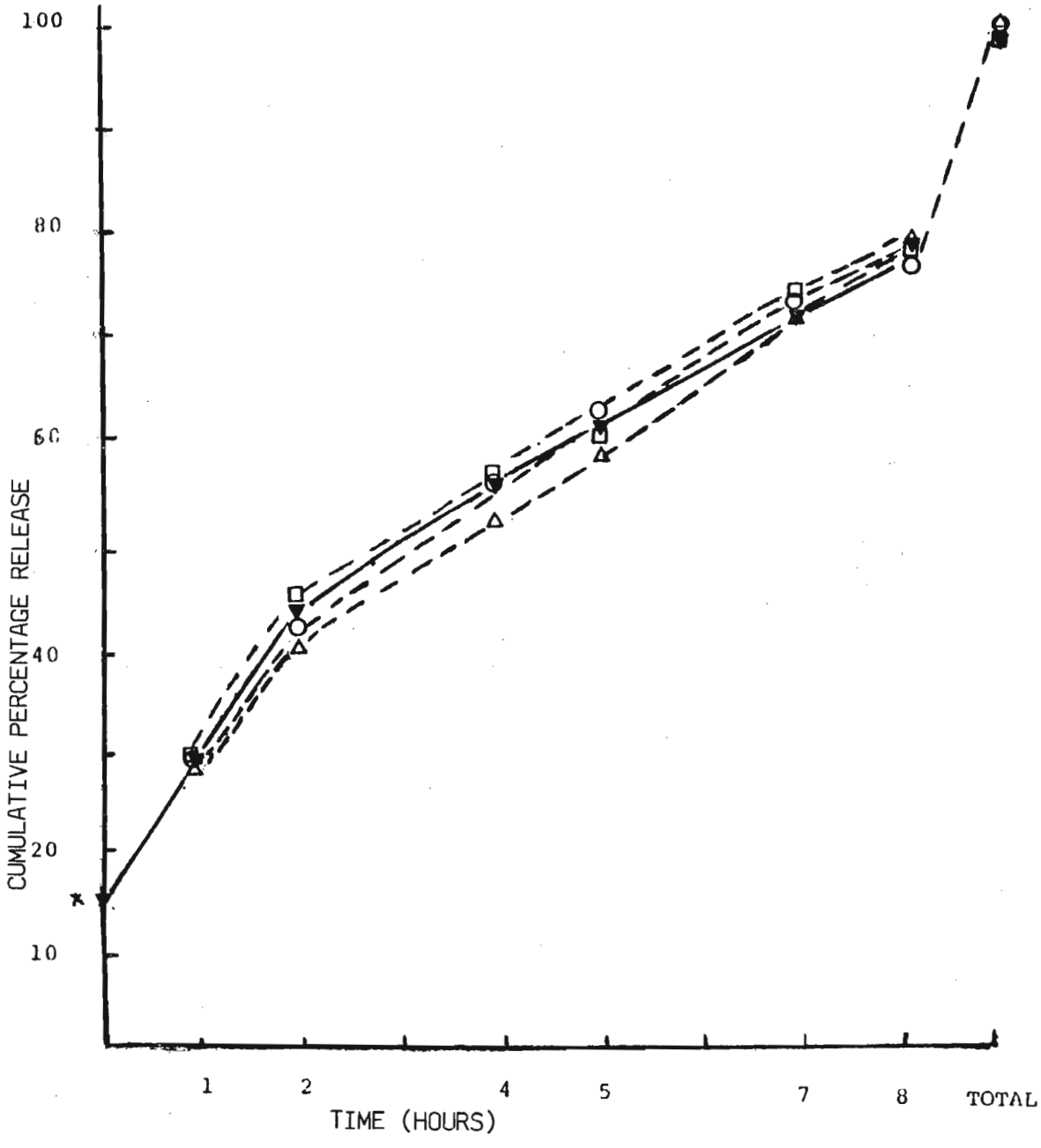


Figure 2.B.14: Comparison of the cumulative percentage release of diethylpropion hydrochloride from sustained release pellets (Lot 018010) using the rotating paddle method
pH 1,5 (1 hr); 4,5 (1 hr); 6,9 (2 hrs); 6,9 (1 hr); 7,2 (2 hrs); 7,5 (1 hr) and 7,5 (4 hrs)
* Free unbound drug in pellets is 15,0%
Speed of Agitation (r.p.m.) - Table 2.B.12

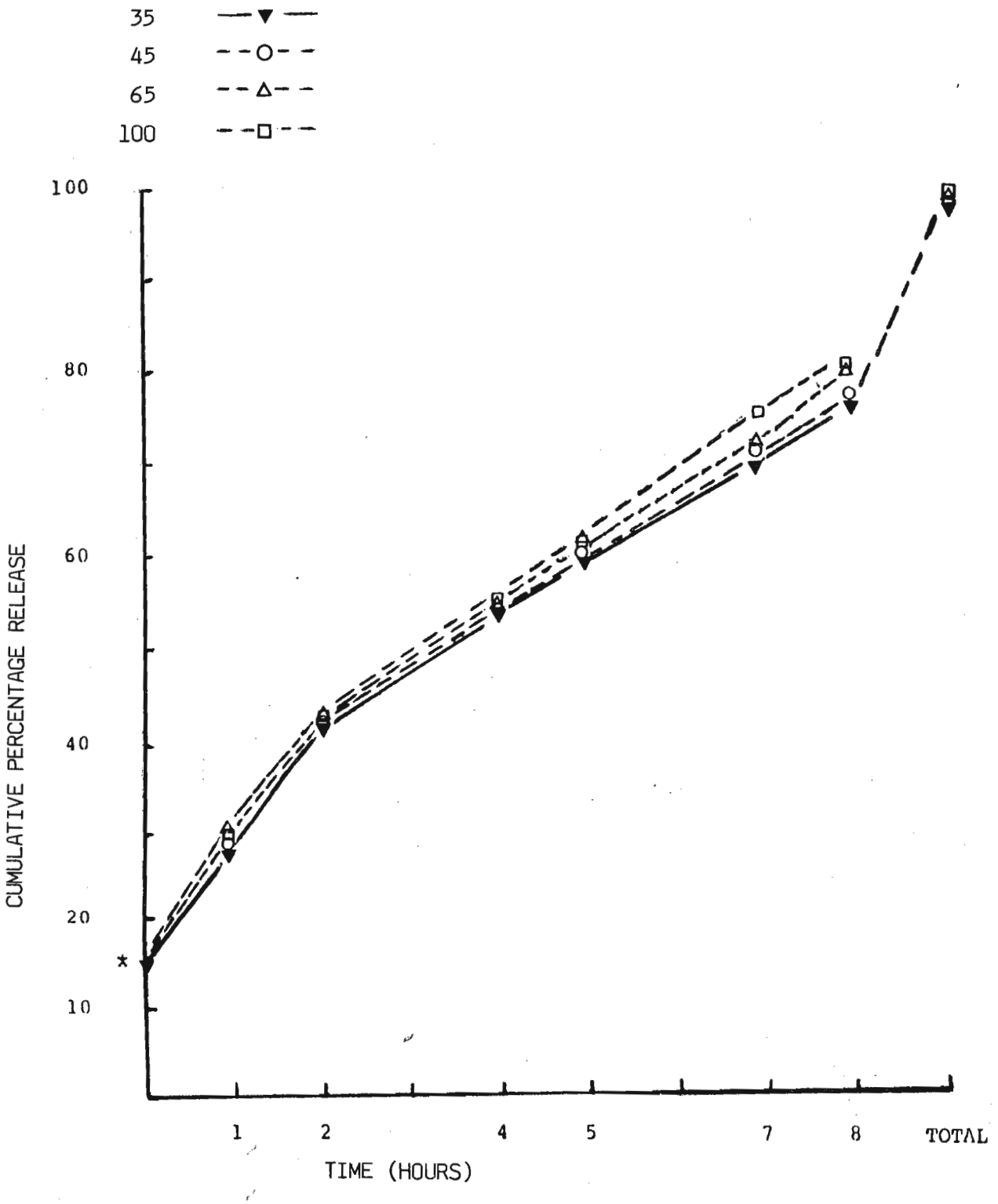


Table 2.B.12: Comparison of the cumulative percentage release of diethylpropion hydrochloride from sustained release pellets (Lot 018010) using different methods of dissolution

pH VALUE	TIME AT pH (HOURS)	CUMULATIVE PERCENTAGE RELEASE USING															
		ROTATING BOTTLE	ROTATING BASKET								ROTATING PADDLE							
			35 rpm		65 rpm		88 rpm		100 rpm		35 rpm		65 rpm		88 rpm		100 rpm	
			A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1,5	1	26,7	29,5	29,4	27,7	27,7	28,3	27,4	29,3	29,8	27,4	27,6	27,8	28,9	30,8	28,4	30,2	31,7
4,5	1	38,8	42,7	42,7	40,8	41,2	40,0	38,7	40,1	40,6	40,0	40,1	39,7	40,5	41,9	38,7	42,1	43,4
6,9	2	54,8	55,0	55,3	54,8	52,1	53,1	52,6	56,0	57,1	52,2	52,3	52,9	53,8	53,9	49,9	54,3	53,6
6,9	1	61,2	60,0	60,4	61,0	61,2	56,7	56,0	58,3	58,4	57,3	57,5	58,6	59,4	59,1	56,0	60,8	61,3
7,2	2	72,8	70,1	70,8	72,2	72,8	70,8	70,4	71,0	73,4	67,6	66,8	68,7	69,7	69,9	66,1	76,3	73,0
7,5	1	78,6	76,4	77,0	77,4	77,6	77,2	76,9	76,0	77,9	72,7	72,9	73,6	74,9	75,8	76,4	78,9	78,5
7,5	4	94,8	95,2	94,7	94,5	94,7	94,7	94,9	95,0	95,6	94,9	95,3	95,2	95,0	94,9	94,6	94,2	95,1
Drug in residual pellets		5,5	4,3	5,4	4,9	5,0	5,3	4,6	4,7	3,9	4,9	5,7	4,4	5,7	6,0	4,4	6,2	4,7
Total Recovery		100,3	99,5	100,1	99,4	99,7	99,0	99,5	99,7	99,5	99,8	101,0	99,6	100,7	100,9	99,0	100,4	99,8

The figures are expressed as percentage of the potency, 168,8 mg/g

All figures are the average of duplicates

A = Analysed on first occasion, June 1980

B = Analysed six months later, January, 1981

Free unbound drug in pellets is 15,0%

rpm = revolutions per minute

2.B.3 Factors Influencing the Release of Diethylpropion Hydrochloride from Sustained Release Pellets

(a) Type of model

The cumulative percentage release of the drug from the pellets has been plotted against sampling times for each dissolution test model, in order to compare the release profiles (Figure 2.B.12). This figure shows conclusively that the different methods of dissolution did not significantly affect the variation in the release rates of the pellets.

Three different modes of agitation were compared. The pellets within the rotating basket were in contact as they swirled within a confined space. In the rotating paddle and rotating bottle methods the pellets moved relatively free in solution and had little contact with one another. If the release of the drug from the pellets had depended on membrane rupture or erosion, different release profiles would have resulted for the three methods.

(b) Degree of agitation

Figures 2.B.13 and 2.B.14 represent graphically the dissolution profiles of diethylpropion hydrochloride sustained release pellets Lot 018010, where the rotating basket and rotating paddle methods (using different rotation speeds) were employed respectively. The results showed that the different speeds of agitation used in the two methods did not significantly affect the release pattern when the intensity of agitation was suitably high to ensure that there was not a semi-stationary film around the pellets. The consistency of the data obtained initially and six months later indicates that perfect sink conditions were maintained in both methods (Table 2.B.12), and a linear profile also suggests that the drug was released from the pellets by a reproducible mechanism closely resembling a zero-order process.

Since the dissolution test model and the intensities of agitation under study did not alter significantly the *in vitro* dissolution profiles of the pellets, it was confirmed that the drug was released from the pellets across a diffusion rate-limiting membrane.

A rotating speed of 100 r.p.m. was selected for both the rotating basket and rotating paddle methods as this is the minimal speed recommended by the NF XX. Several investigators e.g. Wagner, (1971), had shown previously that a rotation speed of 50 r.p.m. would probably have been more realistic but could have presented problems of homogeneity of the vessel fluid.) Because these results showed that the mode and intensity of mixing did not alter significantly the release of the drug from the pellets as the release of the drug was proved to be diffusion controlled, there was no need to compare speed of rotations beyond 100 r.p.m.

Although all three methods provided similar drug release profiles, it was decided that the rotating bottle method would be adopted for all our *in vitro* studies. The merits of this method are highlighted by Slipper, (1981) and Hassanzadeh, (1981). The rotating bottle method, unlike the other two methods, provides a closer representation of the movement of the pellets in the GIT and therefore appears to be a more suitable process for establishing *in vitro/in vivo* correlations and for predictive *in vivo* studies.

(c) Composition of dissolution medium

The effects of using different concentrations of buffer solutions, containing different cations (K^+ or Na^+) and different anions (borate and phosphate) in the dissolution media, are shown in Figures 2.B.15 to 2.B.17. These figures clearly indicate that the dissolution rate of diethylpropion hydrochloride from sustained release pellets

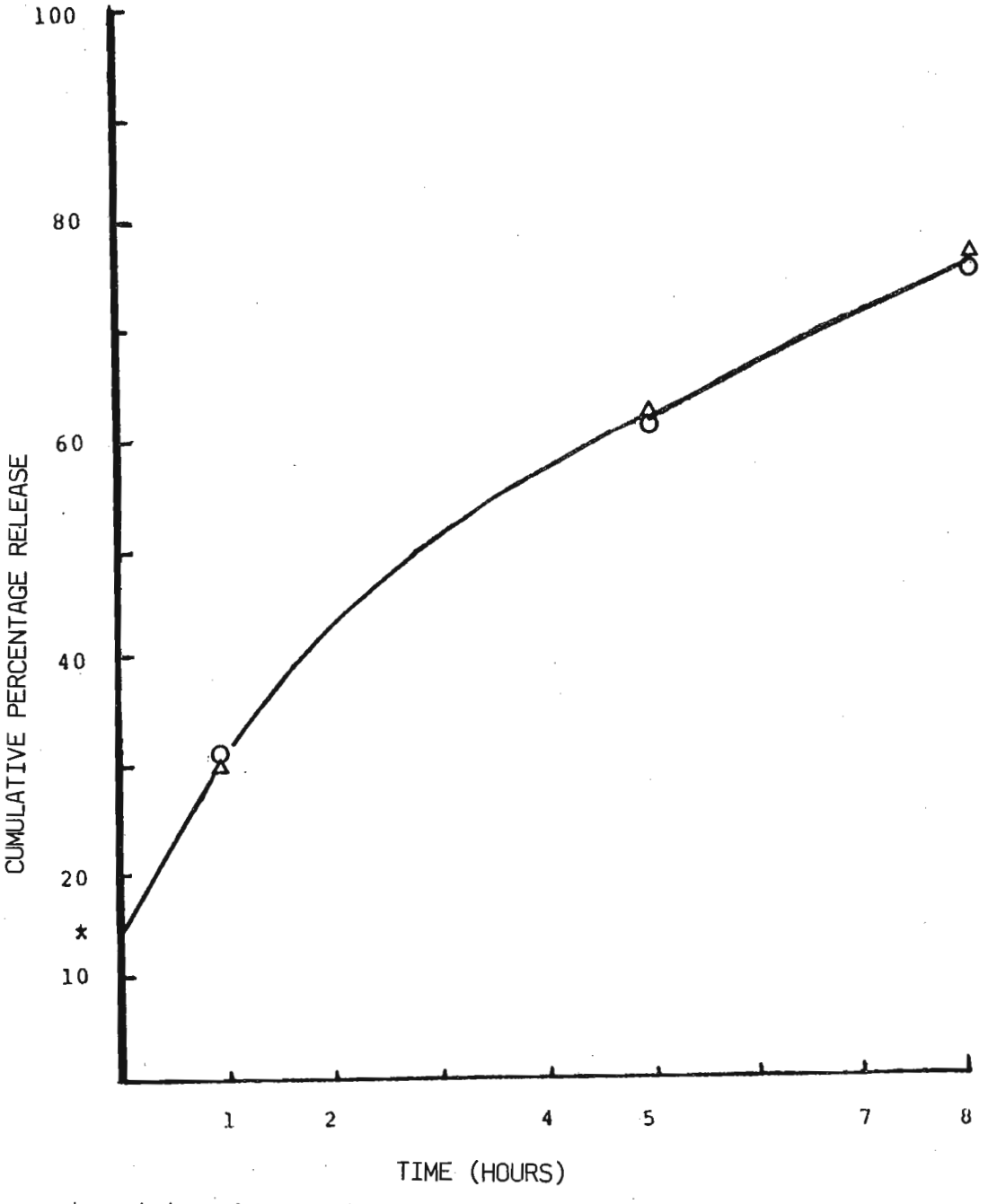
Figure 2.B.15: Comparison of the cumulative percentage release of diethylpropion hydrochloride from sustained release pellets (Lot 018010) using different concentrations of buffer solution (pH 6,9) under the following pH gradients: pH 1,5 (1 hr); 6,9 (4 hrs); 7,5 (3 hrs):

Rotating bottle dissolution test method

Concentrations of Buffer Solutions, USP XX, of pH 6,9 are

0,05M ○

0,10M △



* Free unbound drug in pellets is 15,0%

Figures 2.B.16 and 17: Effect of cations (K^+ and Na^+) and anions (borate, phosphate) on the cumulative percentage release of diethylpropion hydrochloride from sustained release pellets (Lot 018010) using the following pH gradients: pH 1,5 (1 hr); 6,9 (4 hrs); 7,5 (3 hrs): Rotating bottle method

Fig. 2.B.16

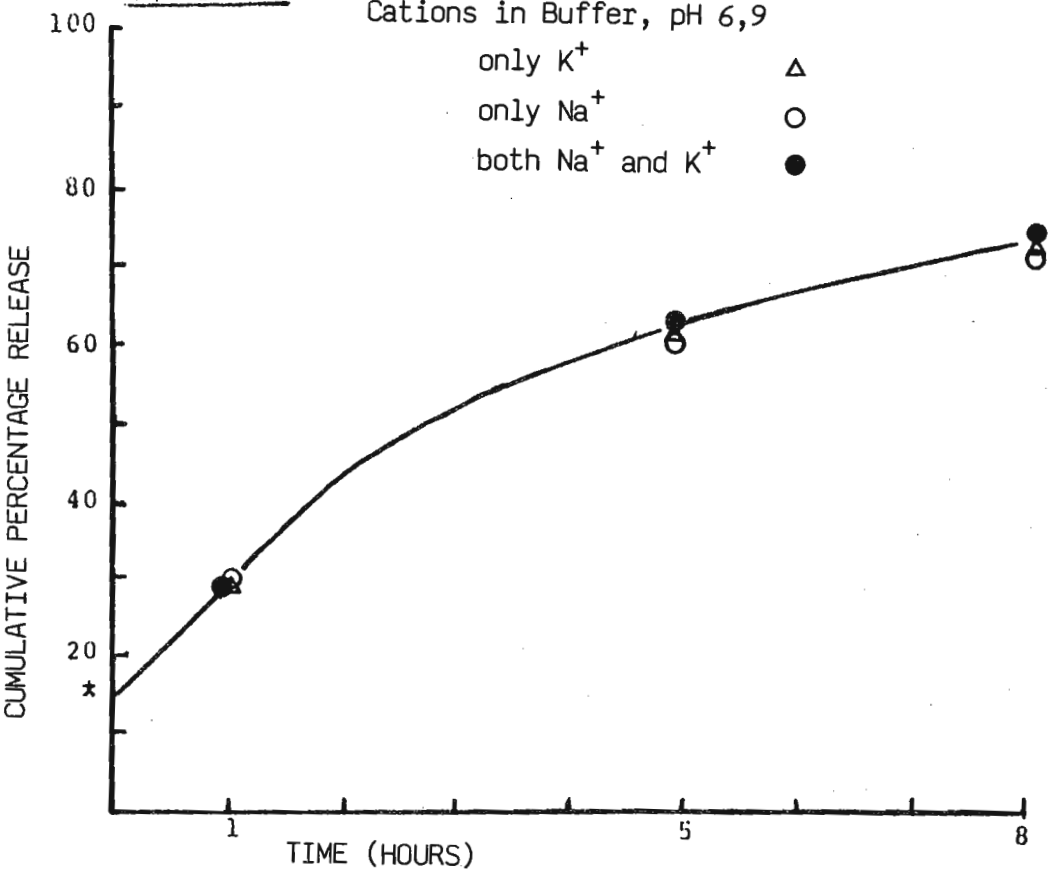
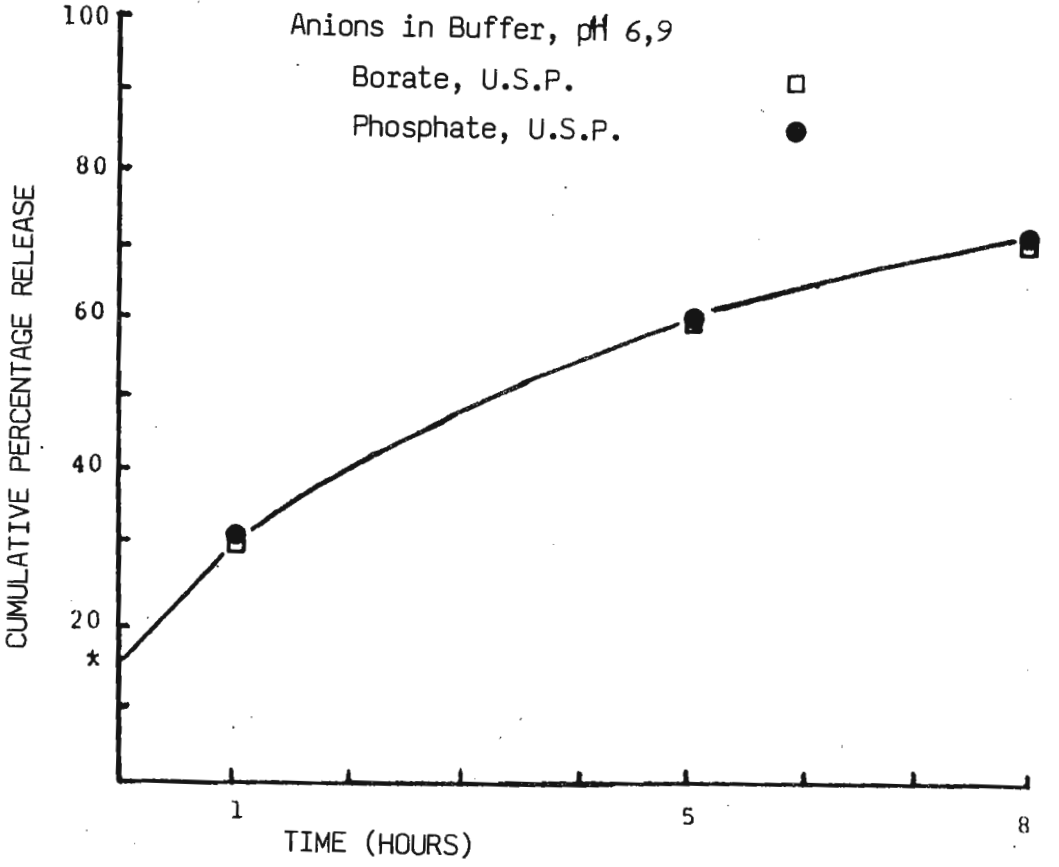


Fig. 2.B.17



* Free unbound drug in pellets is 15,0%

Lot 018010 (Temmler-Werke) was unaffected by any changes in the concentration (0,1 M and 0,05 M) or nature of buffer (Na^+ to K^+ salt or borate to phosphate). However, with ketoprofen an acidic drug, a change in the anion (phosphate, borate and barbital) and/or concentration of buffer solution markedly reduced the release rate of the drug from sustained release pellets - these changes were accountable to solvation and hydrogen-bonding effects (Hassanzadeh, 1981). In another study, the presence of phosphate ions in the buffer solutions (pH 5,5 or 6,9) improved the release rate of the drug from disopyramide base pellets. These studies also demonstrate the preference of phosphate buffers in in vitro dissolution studies.

2.B.4 Conclusion

In vitro dissolution testing was carried out to compare the release of the drug from different batches of sustained release pellets, to investigate the stability of pellets stored under different conditions and to predict the in vivo behaviour of pellets. The conclusions drawn from the various investigations (Chapter 2.B) have been summarized in this section. Reference is made to the relevant discussions and results in the text:

(a) The rotating bottle dissolution test method gave consistent and reproducible results in an investigation extending over six days on the release of the drug from sustained release pellets Lot 018010 (Table 2.B.1). Therefore this test procedure, using buffers pH 1,5 - 7,5 and pH 6,9 to simulate conditions in the GIT and rectum (2.A.2.2.d), has been adopted for the bulk of the in vitro work carried out in the present study. It also enabled me to carry out six dissolution tests simultaneously.

(b) The sustained release pellets (all batches) comprised some unbound "free" drug (the exact contents of each drug is listed in Table 2.B.2) that was released instantly to pro-

provide an initial rapid absorption phase (quick onset). Such pellets were formulated by coating the outside of the non-biodegradable diffusion-controlled membrane associated with the release mechanism, with the "free" drug and then coating the pellets with a sealant (to retain the integrity of the pellets) that would disrupt in contact with body fluids (A.H. Beckett - personal communication).

(c) During the in vitro procedure to simulate oral administration (2.A.2.2.d), the sustained release pellets (Lot R 7773) produced a constant release profile (zero-order for almost 8 hours) until 85% of the drug was released, while Lot 018010 provided constant zero-order release from 3 hours onwards. However, the sustained release pellets Lot R 7574, displayed an initial rapid release (58% within the first 2 hours) followed by a fairly constant release at 4 to 8 hours (Figure 2.B.1).

(d) The release of the drug from pellets Lots R 7574 and R 7773, exposed to media of constant pH levels for 4 to 7 hours, showed minimal changes in the release rates at each pH level. The release rates were relatively constant and independent of the pH of the environment (Figures 2.B.2 and 2.B.3).

(e) The drug release profiles from pellets Lot 018010, were comparative with all three different in vitro dissolution test models, i.e. rotating bottle, rotating paddle and rotating basket methods; (Figure 2.B.12 and Table 2.B.12). The use of different speeds of agitation or change in the concentrations, nature of cation or anion used in the buffer, did not significantly affect the release profile (Figures 2.B.12 to 2.B.17). Therefore, the design of all three pellets formulations to contain a rate-controlled diffusion membrane (explained earlier), together with the advantages of subdivision of dose into discrete units (1.3.2), would be useful to control input of the drug at

any desired rate, and to predict the in vivo release of the drug along the GIT from in vitro data.

(f) Since the sustained release pellets Lot R 7773, were found to provide satisfactory in vitro release profiles (explained in b, c and d above), extensive in vivo trials (Chapter 3) have been carried out on these pellets.

(g) Sustained release pellets Lot 018010, placed as single doses (≡ 75 mg drug) into hard gelatin capsules and stored in clear glass containers at room temperature for just over a year, showed no changes in their in vitro release characteristics (Figures 2.B.4 and 2.B.5). Hence, hard gelatin capsules could provide a suitable and reliable means of delivering pellets in a convenient and well-established dosage form.

(h) Storage of loose pellets (Lots 018010 and R 7773) in closed clear-glass containers at room temperature, or at 4°C in amber glass containers, showed constancies in the potencies (Tables 2.B.10 and 2.B.11) and in the in vitro release characteristics of the drug (Figures 2.B.6 to 2.B.8; Tables 2.B.6 and 2.B.7 for Lot 018010; Figures 2.B.9 and 2.B.10; Tables 2.B.8 and 2.B.9 for R 7773).

(i) Storage of the loose pellets in closed amber glass containers at an elevated temperature of 37°C (uncontrolled humidity) showed only slight reduction (occurring within one month for Lot 018010, and 2,5 months for Lot R 7773) in the release characteristics of the drug in the dissolution medium (pH 1,5 for 1 hour), (Figures 2.B.7 to 2.B.10). The release characteristics in all the other dissolution media of the pellets tested during the storage periods (13,5 months for Lot 018010 and 2,5 months for R 7773) were identical to the initial in vitro data. These results are convincing evidence that the release characteristics were not disrupted and the integrity of the pellets membranes

was not damaged under conditions of study. While there was no decomposition in the drug in pellets Lot R 7773, the decomposition of the drug (detectable only at 3 months of storage) in pellets Lot 018010 was significantly less than that of the free drug stored under similar conditions [Table 2.B.11; Section 2.B.2(ii)].

(j) The release characteristics, potency and stability of pellets (Lot R 7773) incorporated into a lipophilic suppository base [2.A.2.3(iii)], showed good reproducibility with minimum decomposition of drug (Figure 2.B.11). The same rotating bottle test was used to simulate both oral and rectal administrations for direct comparison of results. It is realized that the same in vitro characteristics do not imply similar in vivo behaviour of the pellets via the two routes of administration, but these tests provided suitable information into in vivo behaviour of pellets [detailed discussion in 2.B.2(iii)].

(k) Finally, the densities of two batches were determined (Table 2.B.5). The implications of this parameter in modifying the duration of absorption of the drug from sustained release pellets formulations (programmed dosage forms) to release the drug at specified locations in the GIT have been discussed (2.B.1; c.f. Noormohammadi, 1981).

CHAPTER 3

IN VIVO EXPERIMENTAL

(Drug Administration, Diet, Sampling and the Quantitation of Compounds in Biological Fluids)

3.1 Introduction

Diethylpropion hydrochloride, an effective anorexiant at the recommended dose of 25 mg three times a day (Sullivan et al., 1978), is completely absorbed from the human gut and it is excreted principally via the renal pathway.

Both in free form and as oral sustained release tablets, diethylpropion is rapidly and extensively metabolised by N-de-ethylation and carbonyl reduction (Beckett et al., 1974).

Data show that the major metabolites, especially ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) rather than the parent drug (I) (see Scheme 1.2) are responsible for the observed activity of diethylpropion (Wright et al., 1975; Beckett et al., 1973).

However, Mihailova and co-workers (1974) also showed that diethylpropion does not have a long duration of action because (II) and (IV) have fairly short half-lives (approximately 3 hours). In an investigation where radiolabelled ^{14}C-DEP was used, plasma levels of the individual metabolites were monitored (Wright et al., 1975) because of difficulties in their quantitation. Thus a suitable method to distinguish and to analyse nanogram quantities of diethylpropion (I), ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) in saliva and in plasma has been developed in the present study.

Sustained release preparations can be of advantage with a drug of short half-life, such as diethylpropion, in that they prolong the

action of the drug and increase the dosing interval, whilst minimizing the excessive swing between potential untoward effects and ineffectively low concentration of the active drug in the blood. The advantages of administering an oral-sustained release product in the form of many subunits (i.e. pellets, each individually coated with an insoluble membrane that allows diffusion of the drug in a controlled manner) as compared to a single unit (i.e. sustained release tablet), have been well documented (Beckett, 1981; 1983; Galeone et al., 1981; - Section 1.3).

The objective of the present study was to define and to compare the availability of diethylpropion hydrochloride after oral administration to twelve volunteers (Clinical Trial No. 1) of:

(a) the free drug taken in three divided doses (3 x 25mg) at regular time intervals (0, 4 and 8 hours)

(b) the new sustained release pellets formulation (Lot R7773) - placed in a hard gelatin capsule - in a single 75mg dose.

Comparisons, made of some parameters on bioavailability, were drawn between saliva and/or plasma concentrations and urinary excretion data on the two major metabolites in each subject. The usefulness of salivary data in monitoring plasma data has been discussed (4.1). The pH levels (acidic) and flow rates of the saliva were well controlled to minimize tubular reabsorption of the compounds (1.2.1).

Diethylpropion has a high metabolic clearance and when administered orally it undergoes extensive first-pass metabolism (Beckett et al., 1974). Bypassing the liver by non-portal routes of administration can result in higher bioavailability of some drugs (Gügler et al., 1975). Clearly the intravenous and intramuscular routes can achieve this same result. It has frequently been suggested that following the rectal route of administration, drugs absorbed via the lower haemorrhoidal veins may enter the general circulation without passing through the

liver. The inferior and middle haemorrhoidal veins drain directly into the inferior vena cava whereas the superior haemorrhoidal vein conducts blood via the inferior mesenteric vein into the portal vein. The lower veins therefore offer, in theory, the possibility of bypassing the liver and hence the first-pass metabolism. However, there are extensive anastamoses between the lower and upper haemorrhoidal veins and most of the available evidences indicate that it is not possible to avoid the first-pass metabolism in the liver and gut by rectal administration (Moolenaar and Schoonen, 1980).

In the present study the bioavailabilities were compared using urinary and/or saliva data after the oral and rectal administration of diethylpropion hydrochloride in free form (25 mg) (Trials 3 and 5) or as sustained release pellets (75 mg dose) - Lot R 7773 (Trials 1 and 2 - Table 3.1) in the subject. The pellets were also formulated into suppositories [2.A.2.3(iii)] to determine if the rectal route of administration differed significantly from the oral route for drug absorption and to decide if it offered a viable alternative.

The study of food-drug and fluid-drug interactions, and how these may influence the bioavailability of drugs, has been reviewed by Welling (1980). In the present study, the extent and influence of the physical presence of food on the urinary excretion of metabolites II and IV, were compared to fasted controlled conditions after administration of similar doses (75 mg of drug sustained release pellets formulation Lot 018010) to the same subject (Trails no. 8 - 10 and 11, Table 3.1) From some of the data obtained in the present <u>in vivo</u> studies the following calculations were carried out and parameters determined:

(a) The rate of urinary excretion or concentration in plasma and in saliva of the two major metabolites (and where appropriate the unchanged drug) over each time interval between collections.

(b) The mean time between urine collections.

(c) The cumulative excretions of metabolites II and IV in

urine. The percentage of the dose excreted as metabolites II and IV.

(d) The relative bioavailability of the sustained release preparations relative to free dosage form (3 x 25mg), using urine, saliva and plasma data.

The calculations involved in determining (d) above, and the relevance of these determinations, are discussed below.

Bioavailability studies help to define and control some aspects of the quality of a drug product and medicine. The requirements are that a product:

(a) Contains the quantity of each active ingredient claimed on its label within the applicable levels of its specifications.

(b) Contains the same quantity of active ingredients from one dosage unit to the next.

(c) Maintains its potency, therapeutic availability and appearance until used.

(d) Releases, on administration, the active ingredients for full biological availability; i.e. a product must have the required bioavailability characteristics (Beckett, 1978).

Knowledge of bioavailability is useful for many purposes and some applications are indicated below (Wagner, 1980):

(a) Determination of those formulation factors that alter the bioavailability of an active ingredient in a drug product.

(b) Establishing generic equivalence or inequivalence of two or more drug products or formulations.

(c) Determining the effect of food on the absorption of an active ingredient in a drug product or formulation.

(d) Establishing if one drug interferes with the absorption of another drug and how to avoid the interaction.

(e) Determining if increasing age or specific disease states influence the absorption of an active ingredient in a drug product or formulation.

(f) Assessment of the magnitude and variability of the "first-

pass effect" with specific drugs after oral administration and the degree to which other routes of administration avoid this effect.

(g) As one of the tools in quantitatively assessing drug-drug interactions.

The clinical significance of variations in the bioavailability of a drug from different products has been the subject of much debate. Many examples of bioinequivalence of different generic forms of various drugs have been reviewed (Gibaldi, 1977).

The problems of the definition of bioavailability have been discussed (Beckett, 1978). According to Wagner (1980) bioavailability is a term used to indicate the measurement of both the relative amounts of an administered drug that reaches the general circulation; i.e. the systemic availability and the rate at which it occurs. The absolute systemic availability of a test preparation is expressed as a percentage, which is actually the product of two variables for high clearance drugs which have significant "first-pass effects". One of the variables is the first-pass effect and the other is the efficiency of absorption. Methods of estimating bioavailability have been classified and fully described (Wagner 1971, Ritschel 1980). If the drug is not administered intravenously during the bioavailability trial, then only the relative systemic availability can be estimated, and not the absolute availability.

In the present study the drug was not administered intravenously and plasma levels were not monitored beyond 12 hours after oral administration. The method by which the relative bioavailabilities were determined was similar to that described by Wagner (1980).

The mathematical basis for this evaluation is the relationship between area under curve (AUC) and the delivered dose, which exists in linear systems:-

$$AUC = \frac{f.D}{Cl} \quad \text{..... Eq. 3.1}$$

Where: f = the fraction of the administered dose, (D), which actually reaches the systemic circulation, and is then excreted. Cl = systemic clearance of the drug. Assuming that the change in non-renal clearance is proportional to changing renal clearance, and that the systemic clearance remains constant in the same subject from one treatment to another, then

$$F_R = \frac{F_T}{F_S} = \frac{D_S\ (Ae^{\infty}\quad)T}{D_T\ (A_e^{\infty}\quad)s} \quad \ldots\ldots\ldots \text{Eq. 3.2}$$

Where: D_S and D_T are doses of the standard and test formulations and F_R is the extent of absorption or bioavailability of the drug from the test formulation relative to the standard formulation in a given subject (Jefferey and Gibaldi, 1980).

$(Ae^{\infty}\ )s$ and $(Ae^{\infty}\ )T$ are the amounts of drug (metabolite) excreted in urine in infinite time after single doses of the drug in the form of standard and test forumlations.
N.B. The method used to estimate Ae graphically was that described by Wagner (1980).

$$\frac{(Ae^{\infty}\ )T}{(Ae^{\infty}\ )s} = \frac{f_T\ F_T\ D_T}{fs\ \ Fs\ \ Ds} \quad \ldots\ldots\ldots \text{Eq. 3.3}$$

Where: F = fraction of the dose which is absorbed. If equal doses are given as two treatments T and s to the same panel of subjects then Ds = D_T and fs = f_T.
Hence:

$$\frac{(Ae^{\infty}\ )T}{(Ae^{\infty}\ )s} = \frac{F_T}{F_S} = F_R \quad \ldots\ldots\ldots \text{Eq. 3.4}$$

There is little data in the literature concerning the intra-subject variability of f.

The FDA has now introduced a new requirement referred to as the "75%-75% Rule", regarding the evaluation of bioequivalence of a

drug product to the standard preparation. The regulation simply infers that the test sample is bioequivalent to the standard preparation, provided it shows a bioavailability of 75% or more of that from the standard preparation in at least 75% of all subjects in a well-defined and controlled trial (Beckett 1982 - personal communication).

Elimination half-life of a drug is the time required to reduce to one half that amount of drug which is in the body at the time equilibrium is established (Wagner, 1971). When the term is used it is usually implied that the loss of drug obeys first-order kinetics. The biological half-life of a drug is most probably a function of the magnitude of all volumes and all the rate constants for distribution, metabolism and urinary excretion of unchanged drug. The half-life of elimination K, calculated from a first-order rate constant for overall elimination of unchanged drug from the body (equation 3.5), is a parameter of a particular model elaborated from observed data and should not be confused with the biological half-life (Wagner, 1975).

$$t_{\frac{1}{2}} = \frac{0{,}693}{K} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 3.5}$$

The half-lives of elimination ($t_{\frac{1}{2}}$) of metabolites II and IV investigated in the present study were estimated by plotting the log of the urinary excretion rate against time. The slope of the ß phase (calculated after absorption and distribution have ceased and when the rate of decline of the drug is exponential) multiplied by 2,303 (natural log constant) gives the rate constant of elimination of the drug (K). Using equation 3.5, values for the $t_{\frac{1}{2}}$ were determined. Alternatively the logarithm of ($1 - Ae/Ae^{\infty}$) may be plotted against time to obtain K from the terminal slope of the curve (Ritschel, 1980). The half-life of a drug/metabolite is obviously a very important pharmacokinetic parameter. A knowledge of the half-life is essential in establishing dosage regimens.

Superposition Principle

To predict the drug blood levels during a multiple dosage regimen, pharmacokinetic parameters are obtained from the blood level-time curve generated by a single dose of the drug.

The superposition or overlaying principle provides a method of predicting multiple dose blood levels in linear systems (Wagner, 1975), by assuming that early doses of drug do not affect the pharmacokinetics of subsequent doses. The method is applicable to data obeying any linear pharmacokinetic model and also provides estimates of multiple dose blood levels at any desired sampling times, rather than just the average steady state levels. The principle as described by Shargel and Andrew (1980) was applied to some of the urinary excretion data of ethylaminopropiophenone (II) after oral administration of diethylpropion hydrochloride in different dosage forms. It provided a useful means of roughly predicting the urinary excretion rate of metabolite II (following multiple dosing) and comparing it to the actual in vivo data in multiple dose studies.

3.2 Materials

The different batches of sustained release pellets and the free drug used for the in vivo experimental, as well as other compounds necessary for the quantitative analysis of the metabolites (and unchanged drug), were kindly supplied by, or purchased from various suppliers as indicated (Table A, Appendix I).

All other substances, solvents and packing materials for gas-liquid chromatography and ultraviolet analysis were purchased from suppliers (Table B, Appendix I).

3.3 Drug Administration

3.3.1 Dosage forms and routes

Diethylpropion hydrochloride in different dosage forms was administered orally or rectally to normal, healthy subjects in several separate studies. The various trials carried out on different subjects to investigate the bioavailability of different dosage forms taken orally and rectally, are summarized in Table 3.1 .

Table 3.1: Outline of the different trials involved in the present study

TRIAL REFERENCE NO.	SUBJECT AGE/SEX	DOSAGE FORM	ROUTE OF ADMINISTRATION	BIOLOGICAL FLUID/S ANALYSED
CLINICAL TRIAL*1				
1	Group A (6 subjects) - Table 3.2	F.D.F. vs S.R.P. Lot R 7773	Oral *2	Plasma Saliva Urine
	Group B (6 Subjects) Table 3.2	F.D.F. vs S.R.P. Lot R 7773	Oral *2	Saliva Urine
2	C.D. (35 yr., male)	S.R.P. Lot R 7773	Rectal *3	Saliva Urine
3	C.D.	S	Rectal *4	Urine
4	A.M. (33yr., male)	S	Oral *2	Urine
5	C.D.	S	Oral *2	Urine
6	C.D.	S	Oral	Urine
7	C.D.	S.R.P. Lot 018010	Oral *2	Urine
8	C.D.	S.R.P. Lot 018010	Oral	Urine
9	C.D.	S.R.P. Lot 018010	Oral	Urine
10	C.D.	S.R.P. Lot 018010	Oral *5	Urine
11	C.D.	S.R. Tablets Lot 284 BB	Oral *2	Urine
12	C.D.	S.R. Tablets Lot 284 BB	Oral *5	Urine
13	C.D.	S.R.P. Lot R 7574	Oral *2	Urine
14	A.M.	S.R.P. Lot R 7574	Oral	Urine

Key to Table 3.1 - p.t.o.

KEY TO TABLE 3.1

DEP	Diethylpropion hydrochloride.
S	Single dose, 25 mg DEP (placed in a hard gelatin capsule or dissolved in 25 ml water), taken with 100 ml water at time 0 hours.
F.D.F.	DEP, free form (placed in a hard gelatin capsule) in 3 divided doses i.e. 25 mg at times 0, 4 and 8 hours, with 100 ml water.
S.R.P.	Sustained release pellets (≡ 75 mg DEP placed in a hard gelatin capsule), at time 0 hours, with 100 ml water.
*1	A two-way crossover study. Details have been given in Section 3.3.
*2	The initial dose, in all the trials, except trials no. 10 and 12 (*5), was taken in the morning after overnight fasting. No food was allowed until 6 hours after the first dose.
*3,4	Drug inserted immediately after defaecation, either as a suppository comprising S.R.P. (Trial 2) or as 25 mg DEP dissolved in one ml of water (Trial 3).
*5	Drug taken at 10 minutes after breakfast.

Clinical Trials

The study involved a two-way crossover comparison. Twelve normal, healthy, non-smoking subjects (8 males, 4 females) aged between 19 and 60 years, participated in the study. According to the location of the study (3.3.3) the subjects were arranged into two groups (A and B), each comprising six subjects (4 males, 2 females).

The pre-trial screen on subjects in Group A consisted of a medical history, medical examination and the submission of blood and urine samples for a number of biomedical and haematological tests. On the basis of the results of these tests (Appendix III, Tables A and B), the volunteers were considered to be healthy and suitable for inclusion in the study.

Subjects in Group B were carefully selected from known, healthy post-graduate students in our department who had been participating regularly in earlier clinical trials. All volunteers signed an informed consent statement to confirm their willingness to participate.

Procedure

The subjects were assigned to one of two treatment groups as shown in Table 3.2. After an overnight fast of at least 8 hours, the subjects received the appropriate dose with 50 ml water at 08h00, according to the schedule outline in Table 3.2.

Consumption of water and fruit juices was allowed ad lib. but the subjects continued without food for at least six hours after ingestion of the first dose. All the subjects were recommended to swallow ammonium chloride sustained release pellets (500 mg capsule) according to need, to ensure an acidic urine flow throughout the trial.

After a washout period of one week, the study was repeated, but

with the subjects receiving the second dosage form as described in Table 3.2. Sampling, collection and storage of the biological fluids viz. plasma, saliva and urine, were carried out as outlined in Section 3.5.

All other trials

Two subjects (teetotallers), who had participated in the Clinical Trials, were selected for these trials (Table 3.2). They did not drink any beverages containing caffeine on the day before or during any of the trials.

Several studies, done on separate occasions, where diethylpropion hydrochloride was administered either orally or rectally in different dosage forms under fasting and non-fasting conditions, have been outlined in Table 3.1 (Refer to Trials 2 to 14). Sampling, collecting and storage of the biological fluids viz. saliva and urine were carried out as outlined in Section 3.5.

In rectal studies on subject C.D. who had fasted overnight, it was ensured that the rectum was empty (by defaecating) prior to insertion of the dose in the morning. In Trial 3 (Table 3.1) the free drug, 25mg diethylpropion hydrochloride was dissolved in 1 ml of water and inserted into the empty rectum, using a syringe. The method of preparation of the suppository has been discussed earlier [2.A.2.3(iii)].

All the subjects involved in the in vivo trials were advised to record the time of onset of any unusual signs or symptoms e.g. headaches, dizziness, insomnia etc.

Table 3.2: Details on volunteers and study plan for the clinical trials

SUBJECTS						DOSAGE FORM	
GROUPS	INITIALS	SEX(M/F)	AGE (YEARS)	MASS (KG)	HEIGHT (CM)	FIRST TREATMENT	SECOND TREATMENT
A	A	F	22	58	169	Free Dose	S.R. Pellets
	B	M	29	61	185	Free Dose	S.R. Pellets
	C	M	21	67	180	Free Dose	S.R. Pellets
	D	M	20	74	178	S.R. Pellets	Free Dose
	E	F	19	56	166	S.R. Pellets	Free Dose
	H	M	19	61	178	S.R. Pellets	Free Dose
B	A.M.	M	33	65	170	Free Dose	S.R. Pellets
	A.H.B.	M	60	71	164	Free Dose	S.R. Pellets
	C.D.	M	35	70	167	Free Dose	S.R. Pellets
	D.L.	M	28	62	167	S.R. Pellets	Free Dose
	S.G.	F	28	57	163	S.R. Pellets	Free Dose
	S.M.	F	25	64	174	S.R. Pellets	Free Dose

1. Subjects in group A were kept in a clinic for the first 24 hours of the trial: blood samples as well as saliva and urine samples were collected.
2. Subjects in Group B were engaged in their normal activities and gave saliva and urine samples.
3. Free Dose: 75 mg of free drug in 3 divided doses i.e. 3 x 25 mg diethylpropion hydrochloride at times 0, 4 and 8 hours.
4. S.R. Pellets: 476 mg of sustained release pellets (equivalent to 75 mg diethylpropion hydrochloride) at time 0 hours.

No medication, nor any alcoholic beverages had been consumed during the week preceding the trials, and none was permitted during the study period.

3.3.2 Location of studies

The location for clinical studies on volunteers in Group A, and the times of dosing and the collection of samples were personally supervised by:-
Dr E.L. Harris, Biomedical Services, Station Road, Hook, Basingstoke, Hampshire, England.

All other studies, including those on Group B, and the analysis of the biological fluid samples from all the *in vivo* trials in the present study, were done in the laboratories at Chelsea College, University of London.

3.3.3 Diet

In all the *in vivo* studies, the initial dose was given in the morning after an overnight fast of at least 8 hours. Water was taken *ad lib*. In all the *in vivo* trials, the subjects were allowed a light, low-fat meal six hours after dosing and a more substantial meal four hours later. Individual subjects in Group A followed an identical diet during the second day of dosing (Table C, Appendix III). In the study on the effect of food on the bioavailability of diethylpropion from sustained release pellets Lot 018010 and from a standard sustained release tablet (Merrell Lot 284 BB), subject C.D. took the oral dose immediately after breakfast. The meal consisted of 2 fried eggs, 50g chips and two slices of toast, with butter and jam, and two cups of coffee with milk.

The urinary pH was maintained below pH 5,1 in all the trials by ingesting ammonium chloride sustained release pellets (500mg capsules) before and after each meal starting the day before the trial.

3.4 Collection and Storage of Biological Samples

Control samples (blanks) of urine, plasma and saliva were collected just before administration of the initial dose of each trial. It was ensured that the bladder and, where necessary, the rectum has been completely emptied before the initial dose was administered.

Plasma

Venous blood (20ml) from the forearm, was collected (by vein puncture) initially and at 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hours after the initial dose in all the subjects in Group A. The blood was immediately placed in heparinized tubes containing 1 ml 0,5N sulphuric acid, and the plasma was separated by centrifugation at 3500 r.p.m. for 15 minutes. The plasma samples and remaining pellets were stored at minus 5°C until ready for analysis.

Saliva

Mixed saliva samples were collected at specified times outlined in Table 3.3. The collection starting time and duration of salivary stimulation (± 5 minutes) were recorded. Approximately 7 ml of saliva was provided by each subject.

Resting saliva in the mouth cavity was swallowed and saliva flow was stimulated by chewing on two rubber bands. The volunteers then expectorated into the appropriate preweighed vial every 30 seconds, for a period of 5 minutes. Each stimulated saliva sample was identified, weighed and immediately acidified with 1ml 0,5N sulphuric acid, gently shaken and refrigerated at 4°C until the day of analysis.

Urine

Urine samples were collected as shown in Table 3.3 and the volume and pH of each sample were recorded. A small aliquot (±30ml) of

each sample was then transferred into completely air-tight containers and stored at 4°C until the day of analysis.

Table 3.3: Collection times of the biological fluids after administration of different dosage forms to subjects in the different trials.*

Time of Collection	F.D.F.		S.R.P.		S
Hours	Saliva	Urine	Saliva	Urine	Urine
0	-	-	-	-	-
0 - 2	½ hly	½ hly	½ hly	½ hly	½ hly
2 - 4	1 hly	1 hly	½ hly	½ hly	½ hly
4 - 6	½ hly	½ hly	1 hly	1 hly	1 hly
6 - 8	1 hly	1 hly	1 hly	1hly	1 hly
8 - 12	2 hly	1 hly	2 hly	2 hly	1 hly
12 - 36	3-4 hly	3-4 hly	3-4 hly	3-4 hly	3-4 hly
36 - 48	6-8 hly	6-8 hly	6-8 hly	6-8 hly	6-8 hly

DEP = Diethylpropion hydrochloride

S(oral) = Single dose, 25mg DEP taken orally (50 ml solution) or rectally (1 ml solution)

F.D.F. = DEP free form (placed in a hard gelatin capsule) in 3 divided doses i.e. 25 mg at times 0, 4 and 8 hours, with 100 ml water

S.R.P. = Sustained release pellets (= 75 mg DEP) taken orally (as a capsule) or rectally (as a suppository)

* = Outline of the different trials is given in Table 3.1

hly = Hourly interval

Retention Times (Rt) minutes (min)

	in Urine	in Saliva and Plasma
	Rt (min)	Rt (min)
Unchanged drug 1	6	5,5
Metabolite II	4	3,5
Metabolite IV	8	7,5
Internal Standard	10,8	10,0

3.5.2 Methods for analysis of compounds

In all the studies, the compounds analysed are the two major metabolites and where possible, the unchanged drug, viz.:

I = Diethylpropion
II = Ethylaminopropiophenone
III = Diethylnorpseudoephedrine

3.5.2 (a) Analysis of plasma samples

On the day of analysis, the acidified plasma (pH = 5, measured volume taken) contained in a round-bottomed centrifuge tube, was alkalinized to pH = 11 with 1 ml of diluted ammonia solution and to this was added 1 ml of internal standard solution (2 µg/ml). The compounds present in the alkalinized plasma sample were extracted by adding 5 ml freshly distilled diethyl ether and then shaking the mixture on a mechanical shaker for 15 minutes. The tube was then centrifuged at 3500 r.p.m. for 3 minutes to ensure complete separation of the two layers and the ether layer was then quantitatively transferred (by washing the plasma with an additional 2 ml diethyl ether) to a tapered centrifuge tube (tube 2) that contained 1 ml of 0,1N sulphuric acid. The mixture in tube 2 was whirlmixed for 2 minutes, then centrifuged at 3500 r.p.m. for 5 minutes and the upper ether layer was then discarded by aspiration, using a pasteur pipette.

The entire extracton procedure from the plasma into the aqueous acid solution was repeated using an additional 4 ml of diethyl ether in order to ensure the complete extraction of all compounds.

Any traces of diethyl ether above the acidified aqueous layer were removed by placing tube 2 in a waterbath at 45°C while passing a gentle stream of nitrogen into the tube. The aqueous extract was then alkalinized (pH = 11) by adding 1 ml diluted ammonia solution. It was then extracted with 50-70 µl chloroform in a whirlmixer for 2 minutes and centrifuged at 3500 r.p.m. for 5 minutes. 4 µl of the lower chloroform layer was carefully withdrawn and injected onto the column.

Calibration curves of I, II and IV were obtained by analysing blank plasma samples spiked with known amounts of each compound (100-1000 ng base/Assay) and internal standard (2 µg/Assay) (Figure 3.1).

The amount of I, II and IV in ng base/assay in each plasma sample examined was determined by using the peak height ratio of each (relative to internal standard) in equations of calibration curves (Table 3.4).

Table 3.4: Calibration curves of I, II and IV in plasma

Compound		Equation for Calibration y = mx + c	Correlation Coefficient
Diethylpropion	I	y = 1,03x - 0,012	0,998
Ethylaminopropiophenone	II	= 1,10x - 0,091	0,993
Diethylnorpseudoephedrine	IV	= 0,953x - 0,047	0,998

Figure 3.1: Standard Calibration Curves (prepared on 12.11.80) for

Compounds: I (○—○), II (▲--▲), and IV (■-·-■) in Saliva (S) and Plasma (P)

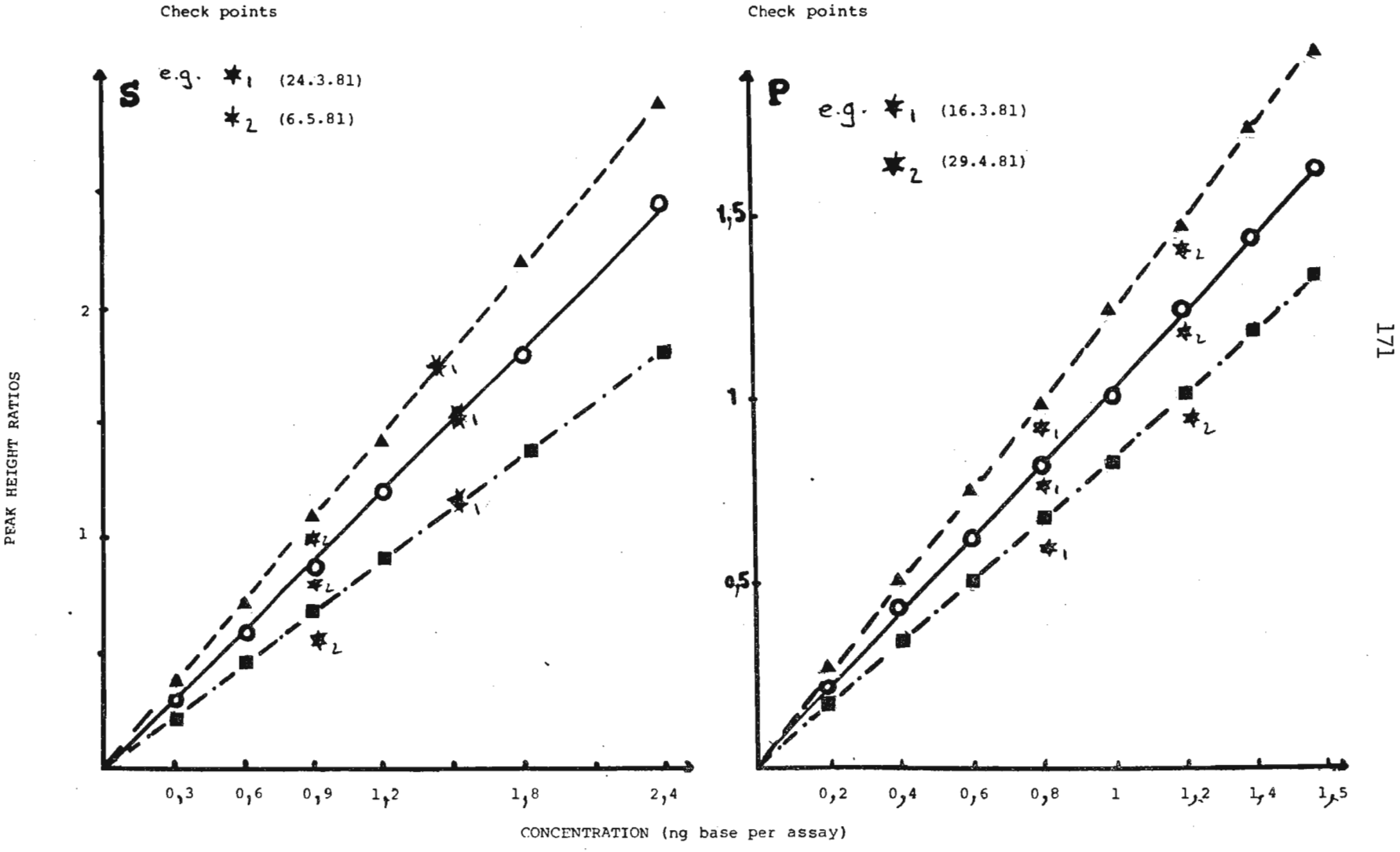
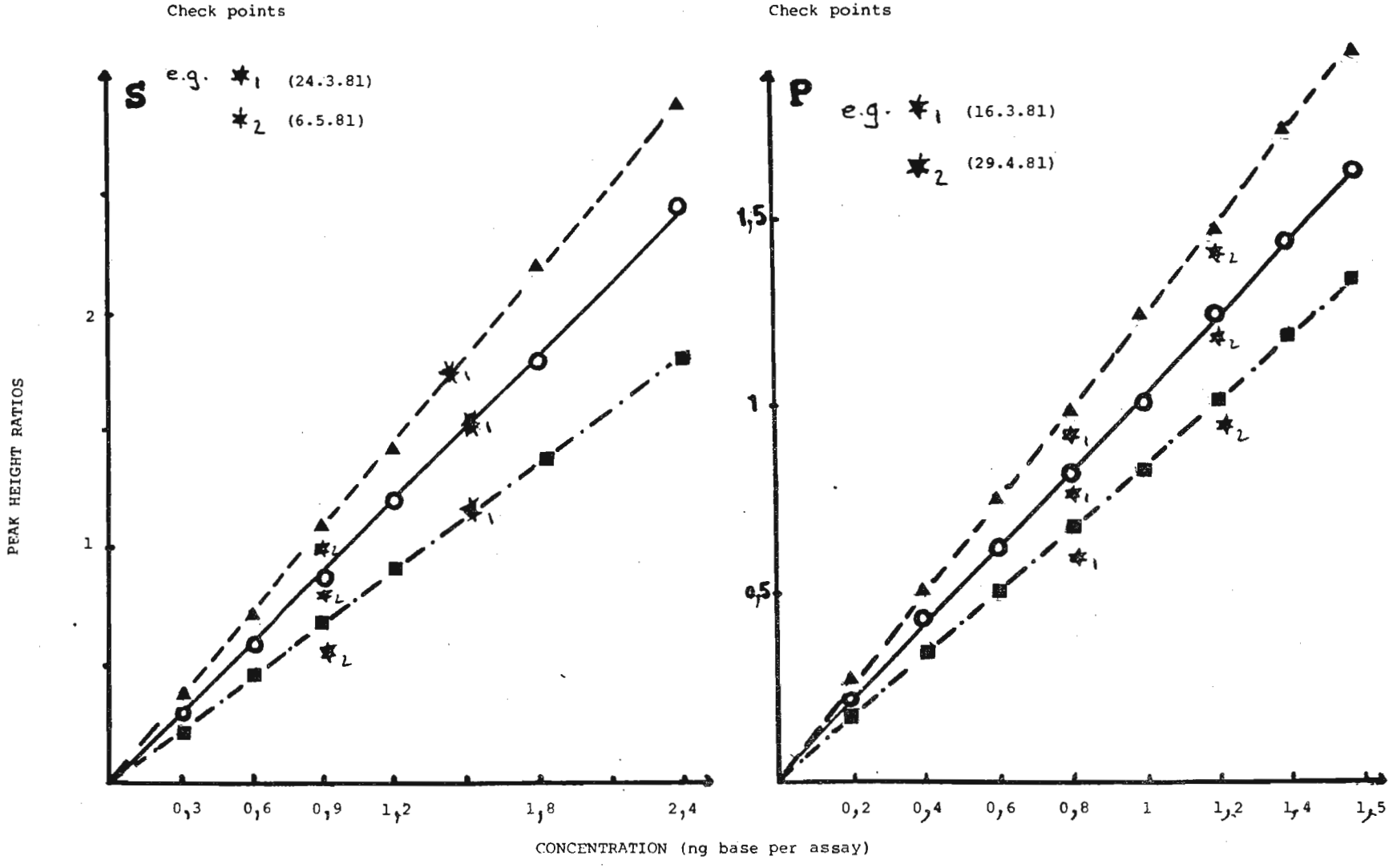


Figure 3.1: Standard Calibration Curves (prepared on 12.11.80) for

Compounds: I (○—○), II (▲--▲), and IV (■··■) in Saliva (S) and Plasma (P)



3.5.2 (b) Analysis of saliva samples

On the day of analysis 5 ml of the acidified saliva was transferred into a tapered centrifuge tube containing 1 ml of internal standard solution (3 µg/ml). Freshly distilled chloroform (100 µl), withdrawn into a Hamilton syringe, was added to the mixture which was then whirlmixed for 1 minute, and then centrifuged at 4000 r.p.m. for 5 minutes. The upper aqueous layer was carefully transferred by means of a Pasteur pipette to a second centrifuge tube. The residue in the first tube was washed with 1 ml of diluted ammonia solution and then with 1 ml distilled water and each washing was transferred into the second tube which contained the previously transferred aqueous solution. 100 µl chloroform was added to the second tube and the solution was then whirlmixed for 2 minutes and then centrifuged at 3500 r.p.m. for 5 minutes. 2-3 µl of the lower chloroform layer was carefully withdrawn and injected onto the column.

Calibration curves of the compounds I, II and IV were obtained by analysing calibrated saliva samples (300-2400 ng base/assay of each) using procedures identical to those mentioned under saliva (Figure 3.1). The amount of I, II and IV in ng/Assay in each saliva sample examined, was determined by using the peak height ratio of each compound (relative to internal standard) in equations of the calibration curves, Table 3.5

Table 3.5: Calibration curves of I, II and IV in saliva

Compound		Equation for Calibration $y = mx + c$	Correlation Coefficient
Diethylpropion	I	$y = 1,03x + 0,015$	0,997
Ethylaminopropiophenone	II	$= 1,207x + 0,010$	0,997
Diethylnorpseudoephedrine	IV	$= 0,79x - 0,075$	0,993

3.5.2 (c) Analysis of urine samples

To 1-4 ml of urine in a tapered centrifuge tube, 1 ml internal standard solution 40 µg/ml, was added. The mixture, after dilution to 5 ml with blank urine, was alkalinized (pH = 10,5) with diluted ammonia solution and then extracted with 100 µl freshly distilled chloroform by shaking the tube for 2,25 minutes on a Fison Wirlmixer. After centrifugation of the tube at 3500 r.p.m. on the M.S.E. Speed Bowl for 5 minutes to separate the two immiscible phases, 1-2 µl of the lower chloroform layer was carefully withdrawn into a 5 µl Hamilton syringe and injected onto the GLC column.

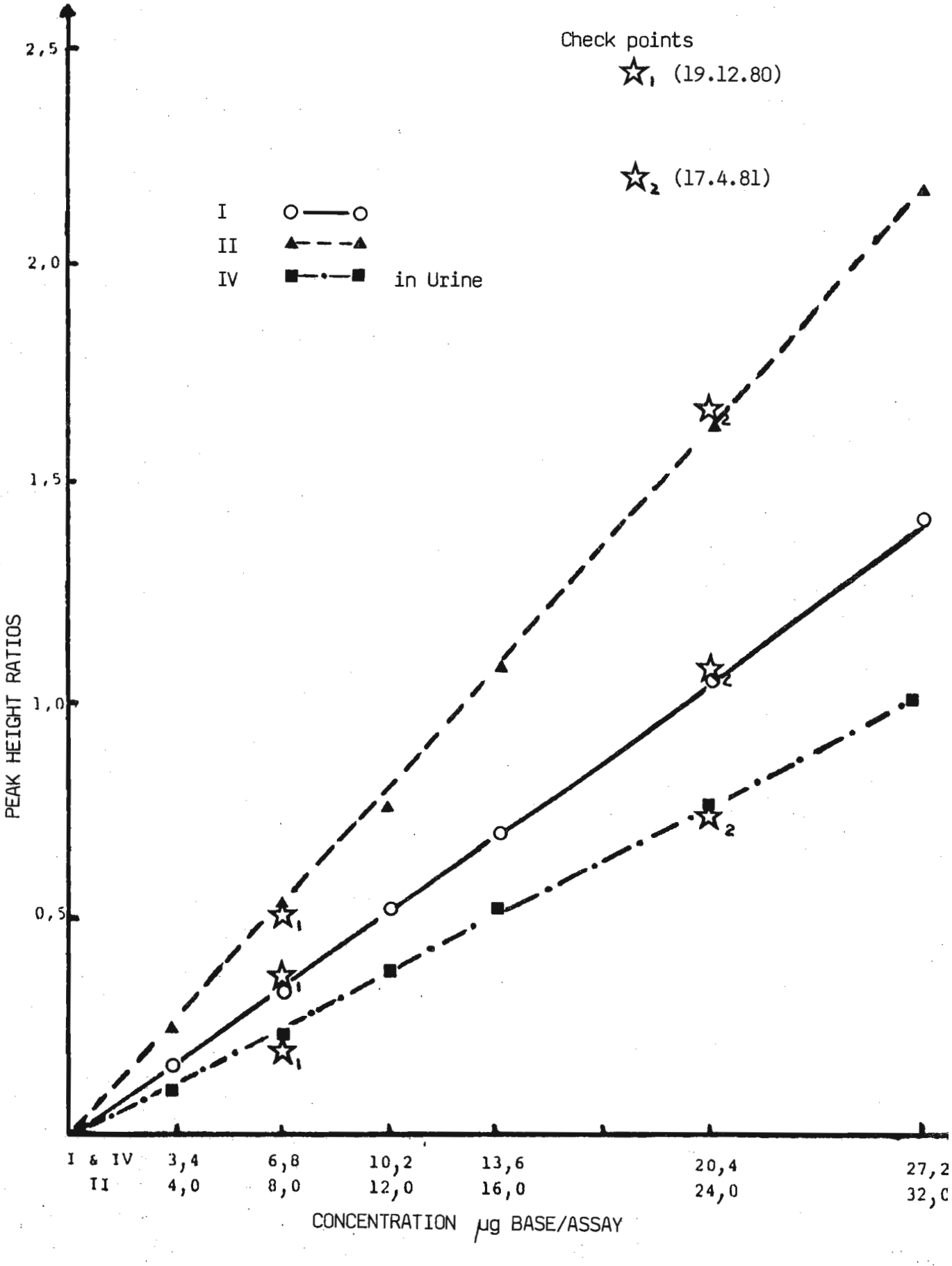
Calibration curves of the compounds, I, II and IV were prepared by analysing blank urine samples spiked with known amounts of the compounds; 3,4 - 34,0 µg base/assay of I and IV; 4,0 - 40,0 µg base/assay of II and internal standard 40 µg/assay (Figure 3.2).

The concentrations of I, II and IV in µg base/ml in each urine sample examined, were determined by using the peak height ratios (relative to internal standard) in equations of calibration curves (Table 3.6).

Table 3.6: Calibration curves of I, II and IV in urine

Compound		Equation for Calibration $y = mx + c$	Correlation Coefficient
Diethylpropion	I	$y = 0,2266x - 0,0035$	0,9990
Ethylaminopropiophenone	II	$= 0,3288x - 0,0262$	0,9990
Diethylnorpseudoephedrine	IV	$= 0,1860x - 0,0114$	0,9987

Figure 3.2: Standard Calibration Curves (pepared on 12.11.80) for Compounds :



Before starting the analysis of biological fluids samples on any day, two check points of the calibration curve of each compound under investigation were made by extracting and analysing any two standard preparations of biological fluid (viz. plasma, saliva and urine). The results were recorded against the corresponding values as shown in Figures 3.1 and 3.2 respectively.

3.6 Extractability, Reproducibility and Stability Studies

3.6.1 Extractability and reproducibility studies

To establish the efficiency of the extraction procedures used throughout the study, the extractabilities of the compounds under investigation from urine, saliva and plasma were compared to that from water while employing identical procedures outlined in Section 3.5.2. The results are given in Table 3.7.

The extraction techniques are essentially based on the ability of compounds to partition into organic solvents from aqueous solutions when in the non-ionic form. The rapid one-step chloroform extraction method for the analysis of saliva samples after a preliminary "clean-up" procedure avoided any deproteinizing process or the evaporation of an extract to dryness, thereby minimizing undue loss of the volatile compounds under study by adsorption, evaporation, or "creeping" along the inner walls of the tube. The use of small volumes of chloroform (50 - 100 µl) in biological fluids extraction allowed the solvent peaks to be low even when operating at high sensitivities ($< 5 \times 10^{-11}$ A) because of negligible traces of impurities in the extracts. Initial acidification of the saliva (Section 3.4), as compared to direct extraction (i.e. without acidification) had the advantage of giving clean chromatograms and sharpness of peaks with minimal interfering peaks (Figure 3.3).

Table 3.7: Extractability test of diethylpropion (I) and its two major metabolites (II and IV) from biological fluids

COMPOUND	CONCENTRATION (ng/µl of $CHCl_3$)	$CHCl_3$ *1	EXTRACTION FROM WATER *2, *3						EXTRACTION FROM BIOLOGICAL UNITS					
			URINE		SALIVA		PLASMA		URINE		SALIVA		PLASMA	
		Mean (±S.E.) P.H. Values	Mean (±S.E) P.H. Values	*3 %	Mean (±S.E) P.H. Values	*3 %	Mean (±S.E) P.H. Values	*3 %	Mean (±S.E) P.H. Values	*4 %	Mean (±S.E) P.H. Values	*4 %	Mean (±S.E) P.H. Values	*4 %
I A	400	140 ± 2	173 ± 3	95					139 ± 5	80				
I B	40	121 ± 1	138 ± 0	88					122 ± 1	88				
I C	20	167 ± 1			155 ± 1	71	208 ± 5	96			120 ± 5	77	159 ± 8	100
I D	8	135 ± 1			130 ± 5	74	166 ± 2	95			112 ± 8	86	128 ± 3	100
II A	400	206 ± 2	225 ± 5	84					199 ± 1	88				
II B	40	168 ± 1	190 ± 0	87					177 ± 1	93				
II C	20	207 ± 1			200 ± 5	74	225 ± 7	84			190 ± 2	95	173 ± 5	77
II D	8	166 ± 1			160 ± 5	74	172 ± 2	80			155 ± 10	97	145 ± 2	84
IV A	400	123 ± 1	152 ± 2	95					126 2	83				
IV B	40	114 ± 1	128 ± 0	87					112 1	87				
IV C	20	135 ± 1			140 ± 2	80	166 ± 8	95			92 ± 2	66	119 ± 5	72
IV D	8	106 ± 0			110 ± 5	80	135 ± 2	98			80 ± 5	73	100 ± 2	74

*1 Solution A (i.e. containing 400 ng of each comp. per µl of $CHCl_3$) was obtained after extracting 2 ml (i.e. 4 mg/ml) of aqueous solution with 4 x 5 ml of $CHCl_3$. Solutions B, C and D were prepared by diluting solution A with $CHCl_3$. Identical volumes of each solution were injected at a constant attenuation repeatedly; i.e. A: 4 µl at 1000, B: 3 µl at 100, C: 5 µl at 50, and D: 4 µl at 20.

*2 5 ml of water containing various amounts of compounds (i.e. identical to solution A, B, C and D) was extracted by employing the procedures outlined for different biological fluids i.e. Urine (U), Saliva (S), and Plasma (P).

*3 Using 100 µl of $CHCl_3$, for extraction from water (as well as from biological fluids), it was observed that only 77 µl of $CHCl_3$ was recovered after centrifugation. Therefore, extractability of each compound was calculated after considering a 23% reduction in $CHCl_3$ volume.

*4 Calculated after comparing the mean peak height values of each compound extracted from biological fluid with those obtained after extraction from water (i.e. see *2).

P.H. Values - peak height values

The minimal detectable amount of each compound was 10 ng and this is certainly good to measure the amount present in plasma (e.g. Figure 3.4).

With saliva samples, and at times with plasma, the final chloroform layer into which the compounds were extracted, became turbid due to the spread of mucilagenous salivary material from the saliva/chloroform interface - this was easily overcome (when additional injections of the chloroform extract were necessary) by scratching the chloroform layer at the interface with a needle and recentrifuging the mixture.

While establishing the extraction procedures from the three biological fluids, all the intermediate phases of the solvents, fluids or residues that were to be discarded were first checked for the absence of trace peaks so as to confirm the complete or maximal extraction of the compounds into the desired extracting layer. Therefore "in-step" controls were introduced to avoid undue loss by incomplete extraction of the compounds under investigation.

To check for the interference from endogenous constituents of blood, urine and saliva, samples were collected from six different normal subjects, who had not ingested any of the compounds under study and these samples were analysed using the developed method. All the samples gave no extraneous peaks except for the appearance of a nicotine peak (in those of some smokers) and some artifacts of diet in some urine samples (Figure 3.5). The acidification step in the extraction from plasma and saliva did not appear to affect the recoveries of the compounds, whilst ensuring the removal of many interfering peaks and providing stability to compounds in the fluid samples (Walters, 1980).

Figure 3.3: Chromatogram of a 2 µl Injection of an extract of saliva prepared by:*1 A) Extraction without addition of acid or
B) Acidification and final extraction

*1 Procedure outlined under Section 3.4.

I Diethylpropion
II Ethylaminopropiophenone
IV Diethylnorpseudoephedrine
I.S. Internal Standard

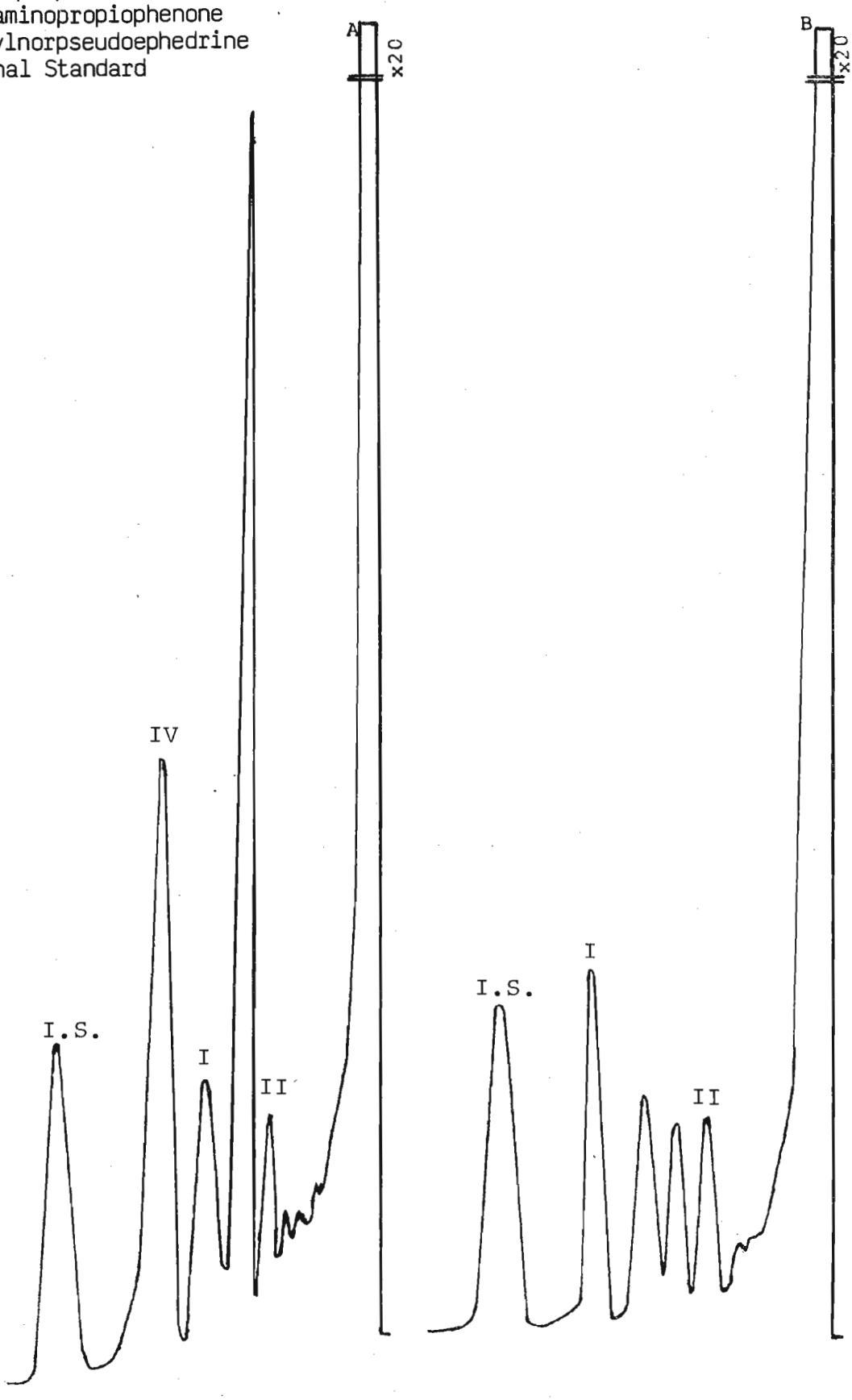


Figure 3.4: Chromatogram of a 2 µl Injection of an extract of plasma collected at 2 hours after administration of sustained release pellets (75 mg DEP HCl)

I.S. = Internal Standard, Furfurylamphetamine 2 µg/ml

II = Ethylaminopropiophenone 38,5 ng/ml

IV = Diethylnorpseudoephedrine 28,5 ng/ml

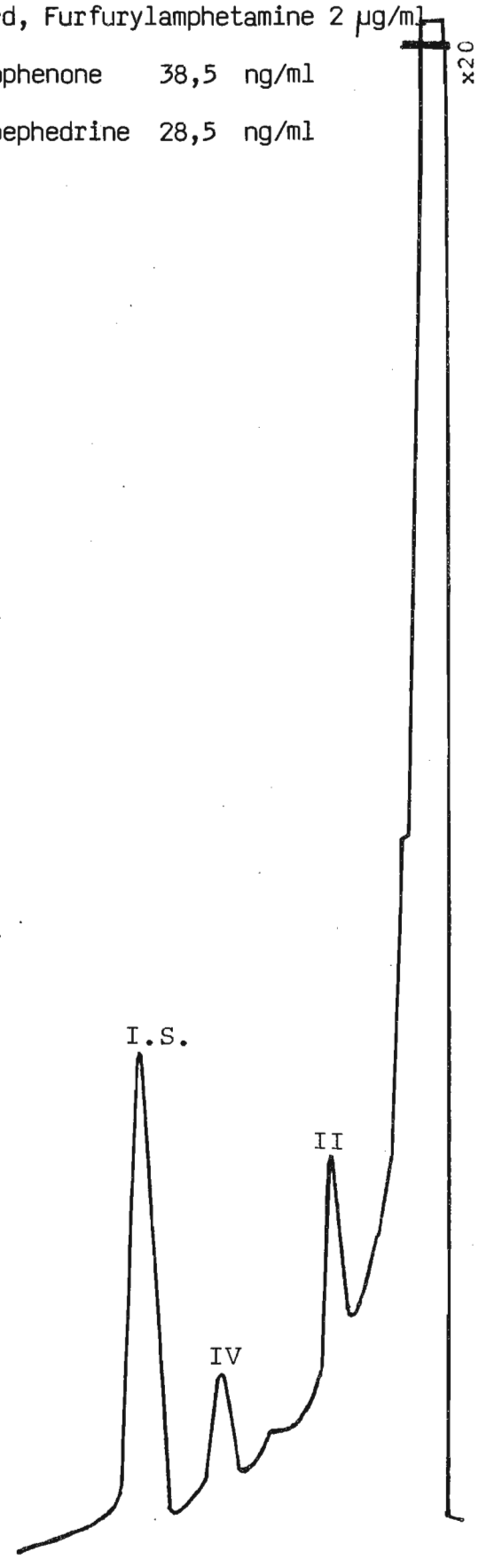


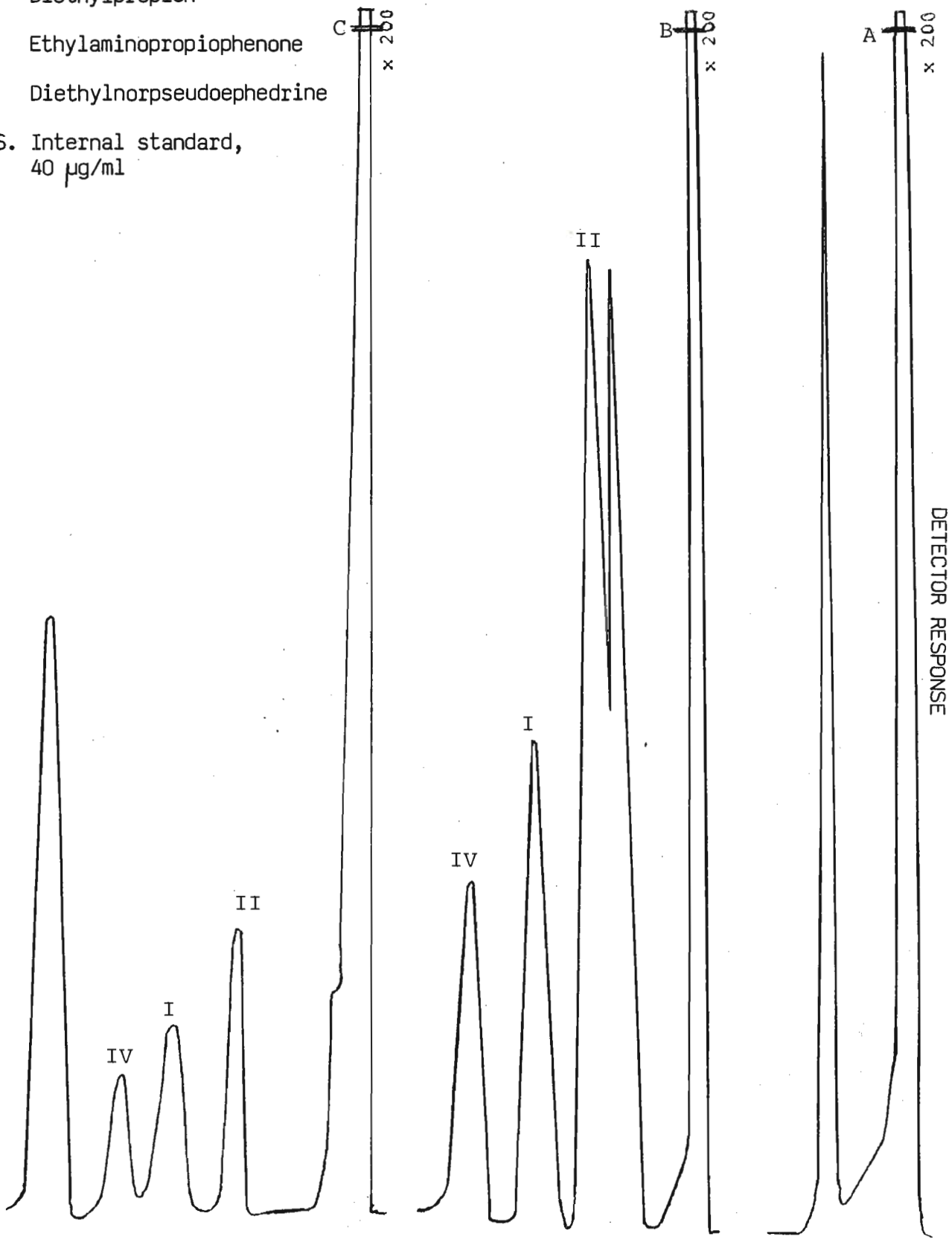
Figure 3.5: Chromatograms of a 1,0 µl Injection of an extract of A) Blank urine from a smoker; B) Smoker's urine spiked with compounds I, II and IV; C) Urine of subject, M.A. (Non-Smoking) at 15 hours after a 75 mg dose of sustained release pellets

I Diethylpropion

II Ethylaminopropiophenone

IV Diethylnorpseudoephedrine

I.S. Internal standard, 40 µg/ml



An attempt to use plasma extraction procedures to analyse compounds in saliva showed minimal improvement in the recovery of any compounds, although the chromatograms gave a "cleaner" scan.

3.6.2 Storage and stability

The nature of the project undertaken, necessitated the examination of a multitude of samples. Since some of the extraction procedures were elaborate and slow, and due to unforeseen delays in the delivery of some of these samples, many of the biological fluids had to be stored for some time (1 to 3 weeks) prior to analysis. A stability study was therefore undertaken whereby duplicate samples of urine containing 20 µg base/ml of each compound, and saliva and plasma samples comprising 1 µg base/ml of each compound, were prepared. Finally three preparations (each in duplicate) of each biological fluid, were adjusted to pH 5, 7 and 11 respectively using suitable volumes of 0,5 N sulphuric acid or diluted ammonia solution.

All the samples were stored for up to 3 weeks in a refrigerator (4°C), and analysed initially and periodically, using the method of extraction and analysis outlined earlier (3.5.2). Results on these tests, shown in Table 3.8, suggest improved stability of compounds under acidic conditions (pH = 5) of storage. It was therefore a standard procedure in the present studies to acidify all the plasma and saliva samples with 1 ml of 0,5 N sulphuric acid, immediately after collection (i.e. prior to storage), until ready for analysis. Urine samples were relatively acidic due to the ingestion of ammonium chloride sustained release pellets and therefore they were not acidified.

Table 3.8: Stability studies of diethylpropion (I), ethylamino-propiophenone (II) and diethylnorpseudoephedrine (IV) on storage at 4°C in acidic, neutral and alkaline conditions of biological fluids

BIOLOGICAL FLUID	Duration of Storage	Concentrations of compounds after storage at								
		Acidic pH (=5)			Neutral pH (≅7)			Alkaline pH (=11)		
		I	II	IV	I	II	IV	I	II	IV
URINE *1	20 hours	20	20	20	20,00	20,00	20,00	19,40	19,50	20,00
	1 week	20	20	20	18,40	17,00	18,10	-	-	-
	3 weeks	20	20	20	17,70	15,60	15,80	-	-	-
SALIVA *2	20 hours	1	1	1	0,81	0,72	0,75	0,70	0,90	0,60
	1 week	1	1	1	0,75	0,67	0,75	-	-	-
	3 weeks	1	1	1	0,70	0,57	0,75	-	-	-
PLASMA *3	20 hours	1	1	1	1,00	0,95	1,00	0,80	0,90	0,60
	1 week	1	1	1	1,00	0,90	1,00	-	-	-
	3 weeks	1	1	1	1,00	0,82	1,00	-	-	-

* Each value is the mean of 3 duplicate sample injections

*1 Urine contained 20 µg base of each compound

*2 Saliva and plasma contained 1 µg base of each compound

*3 The neutral pH of plasma was taken as 7,4 (normal value)

CHAPTER 4

IN VIVO EXPERIMENTAL (RESULTS AND DISCUSSION)

4.1 Two-way Crossover Trial

In this study (Trial No. 1, Table 3.1) twelve subjects were divided into two groups; Group A (six subjects), where plasma, saliva and urine samples were available, after oral administration of diethylpropion hydrochloride in sustained release pellets (Lot R 7773) and the free form; and Group B (six subjects), where only saliva and urine samples were available.

In addition to Tables 1 to 10 in Appendix IV, which provide detailed information of results, some data obtained in individual subjects and the mean values on plasma, saliva and urine data of twelve subjects (Table 3.2) are graphically represented in Figures 4.1-4.28.

4.1.1 Plasma Data

In the six subjects (Group A) on whom plasma determinations were made, the peak plasma levels of metabolites I, II and IV were reached at 45 to 90 minutes after administration of each portion of the drug; the mean of results is shown in Figure 4.1. A rapid decline in the levels of each metabolite occurred 2,5 to 4 hours after each dose of the free drug, thereby producing typical "peaks and troughs" profiles. On the other hand, after administration of the sustained release pellets, the plasma concentration profiles for compounds I, II and IV gave broad plateau levels which were intermediate between the "peaks and troughs" using free drugs and extended over 8 to 10 hours after drug administrations (Figures 4.1 to 4.7).

The mean plasma concentrations during steady plateau levels (4-10 hours) for metabolite II, was 34,88 ng/ml, ranging from 29,1 - 37,9 ng/ml, while for metabolite IV, it was 23,22 ng/ml, with a range of 23,2-25,5 ng/ml (Table 4.1); the mean plasma concentrations of metabolites II and IV at their peaks and troughs are given in Table 4.2.

Although the peak plasma levels for the unchanged drug 1, and its two metabolites II and IV after administration of the free drug, were higher than after administration of sustained release pellets, the relative areas under the curves, calculated over 12 hours, for the sum of the two metabolites II and IV, showed that the subjects gave a relative bioavailability result between 81,8% and 98,3% (Table 4.3). When the plasma data of the unchanged drug itself was considered, the sustained absorption of drug after a dose of sustained release pellets is evident; plasma levels were measurable for about 8 hours after the dose whereas, after each dose of free drug, levels could only be measured up to 4 hours (Figure 4.1).

Figure 4.1: Mean (±S.E.) plasma concentration profiles of diethylpropion (I;△) and its two major metabolites i.e. ethylaminopropiophenone (II, ○) and diethylnorpseudoephedrine (IV;●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to six subjects under acidic (pH = 4,8 ± 0,2) urinary conditions.

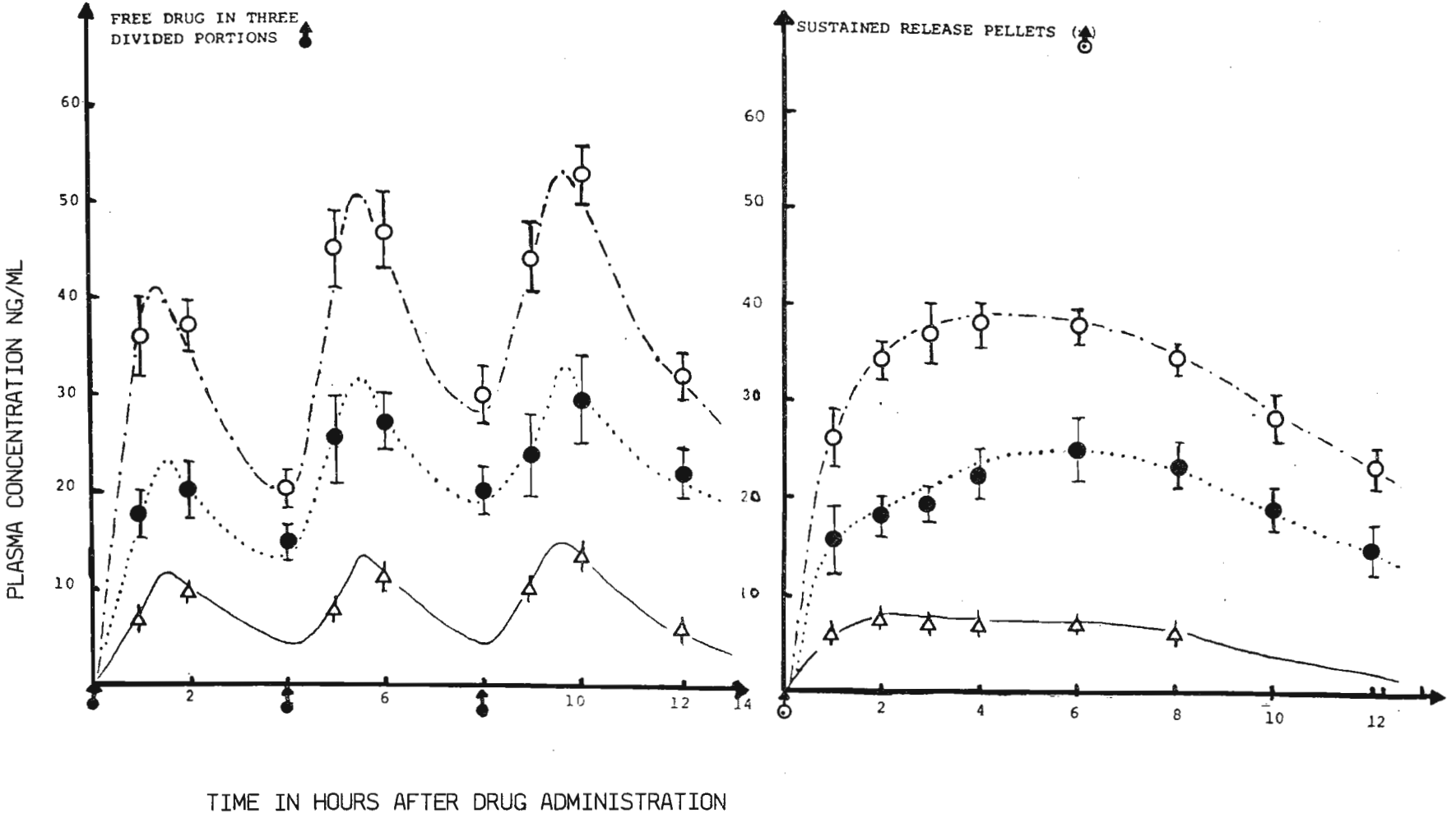


Figure 4.2: Plasma concentration profiles of diethylpropion (I; △) and its two major metabolites i.e. ethylaminopropiophenone (II, ○) and diethylnorpseudoephedrine (IV; ●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to subject A (female, 22 years) under acidic (pH = 4,8 ± 0,2) urinary conditions.

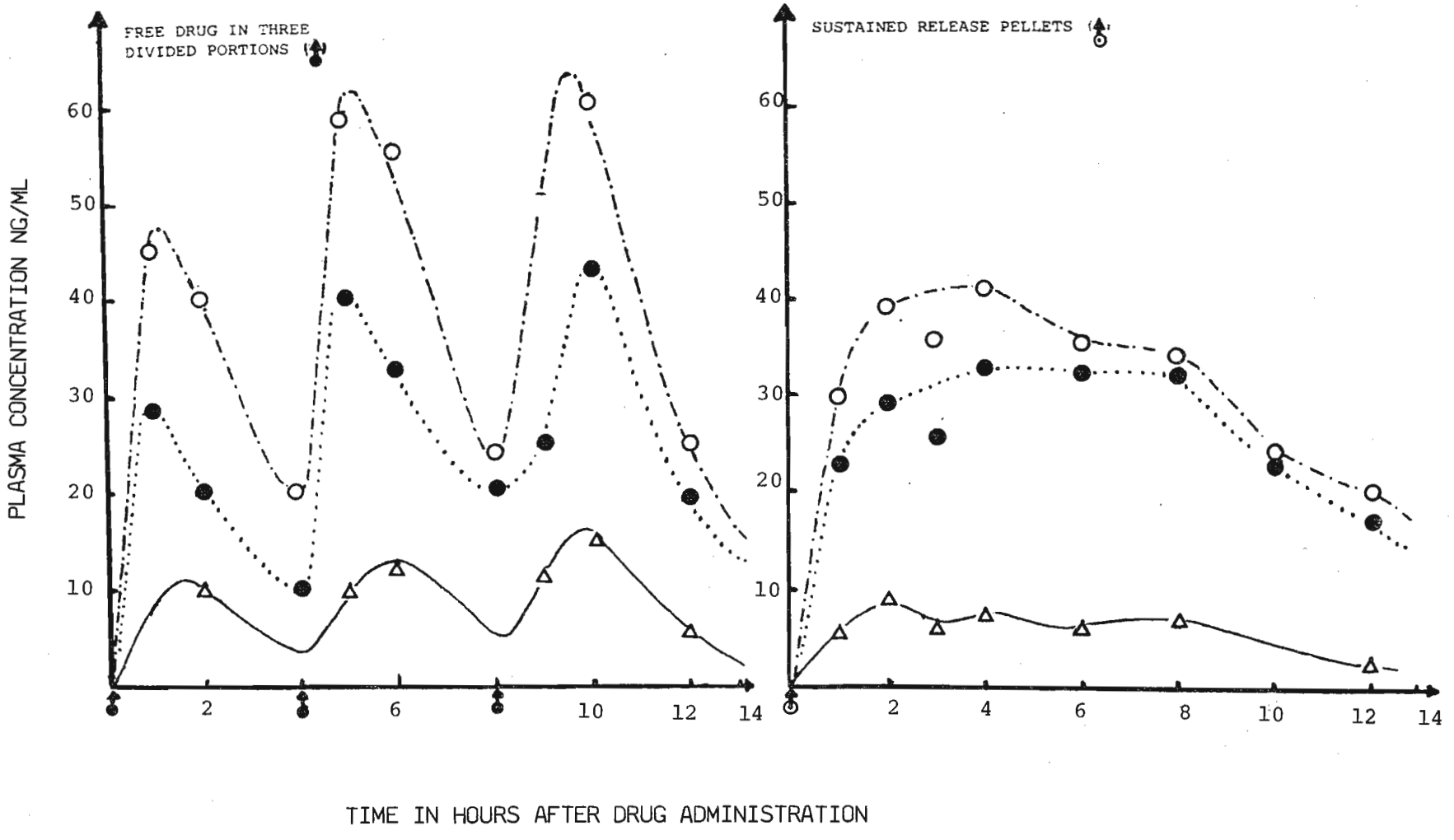


Figure 4.3: Plasma concentration profiles of diethylpropion (I;△) and its two major metabolites i.e. ethylaminopropiophenone (II,○) and diethylnorpseudoephedrine (IV;●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to subject B (male, 19 years) under acidic (pH = 4,8 ± 0,2) urinary conditions.

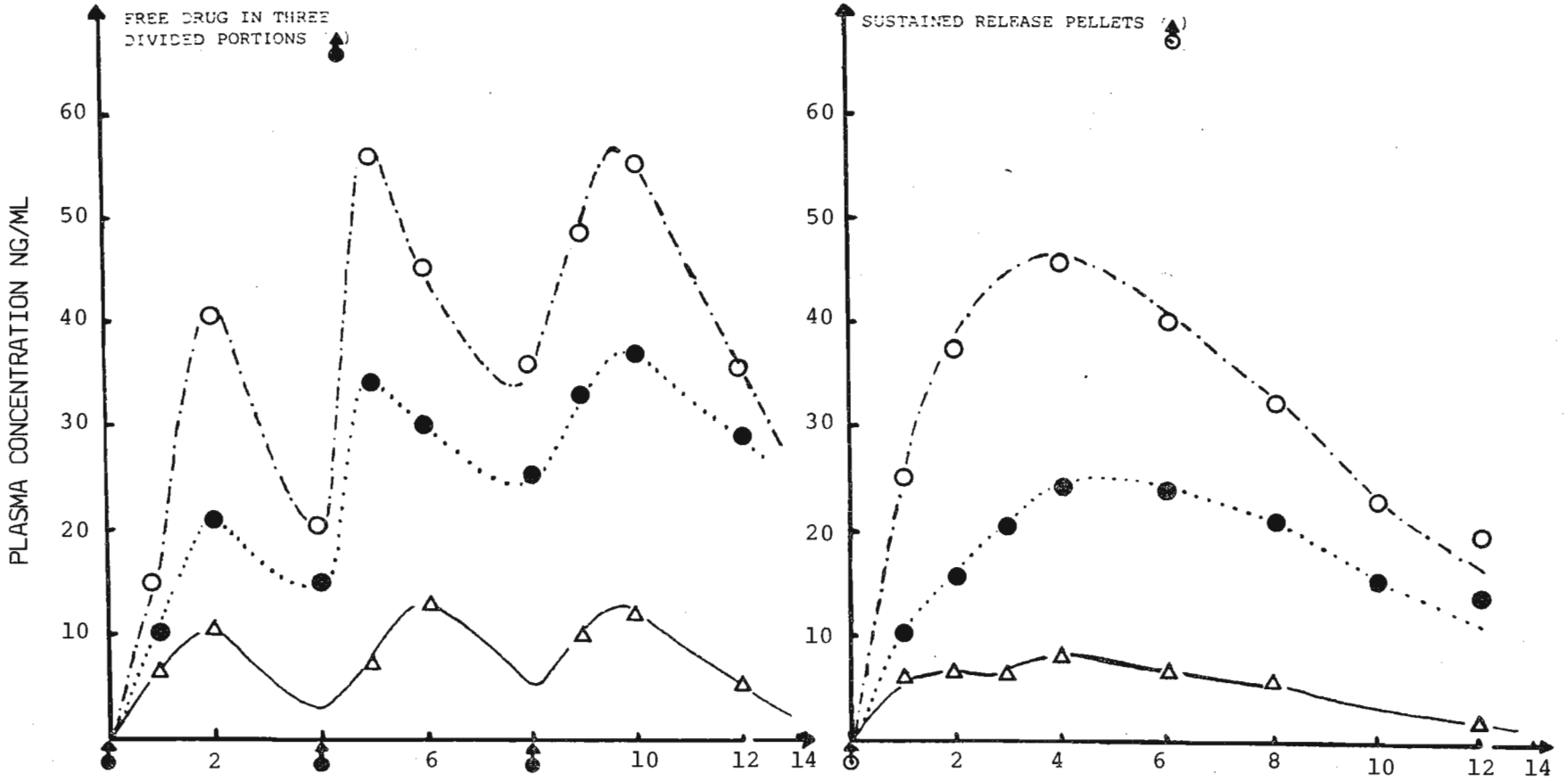


Figure 4.4: Plasma concentration profiles of diethylpropion (I;△) and its two major metabolites i.e. ethylaminopropiophenone (II,○) and diethylnorpseudoephedrine (IV;●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to subject C (male, 21 years) under acidic (pH = 4,8 ± 0,2) urinary conditions.

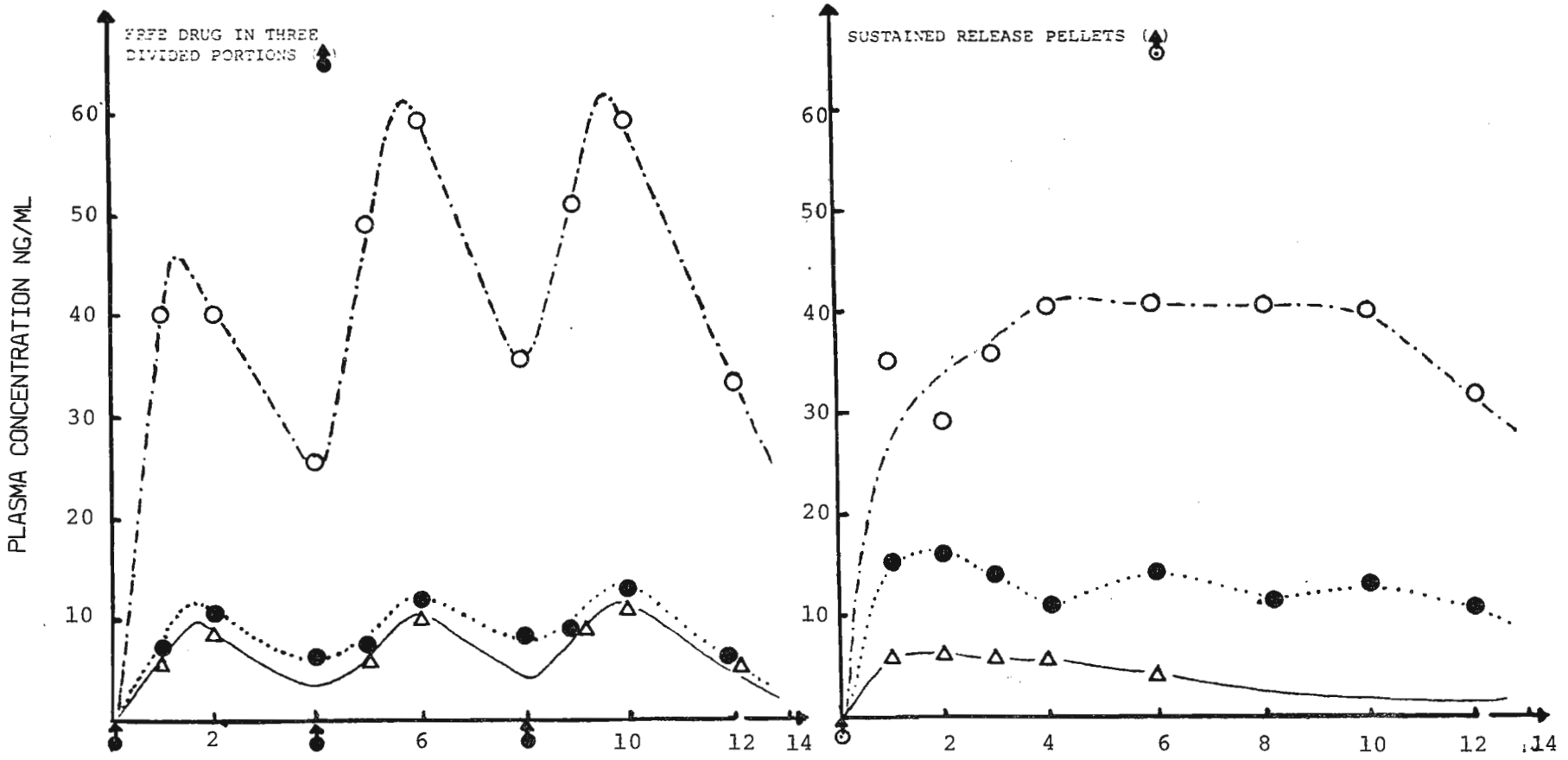


Figure 4.5: Plasma concentration profiles of diethylpropion (I;△) and its two major metabolites i.e. ethylaminopropiophenone (II,○) and diethylnorpseudoephedrine (IV;●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to subject D (male, 20 years) under acidic (pH = 4,8 ± 0,2) urinary conditions.

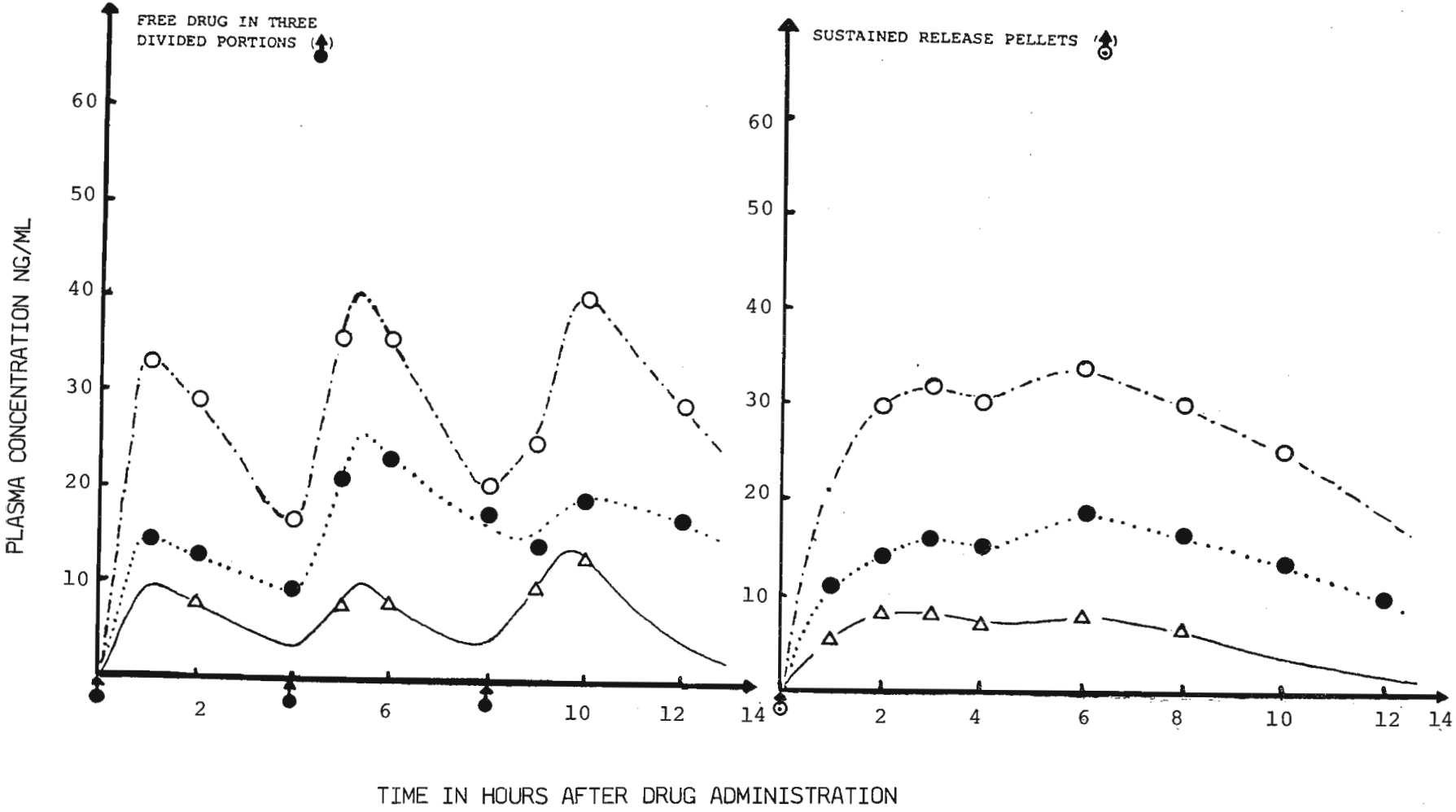


Figure 4.6: Plasma concentration profiles of diethylpropion (I; △) and its two major metabolites i.e. ethylaminopropiophenone (II,○) and diethylnorpseudoephedrine (IV;●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to subject E (female, 19 years) under acidic (pH = 4,8 ± 0,2) urinary conditions.

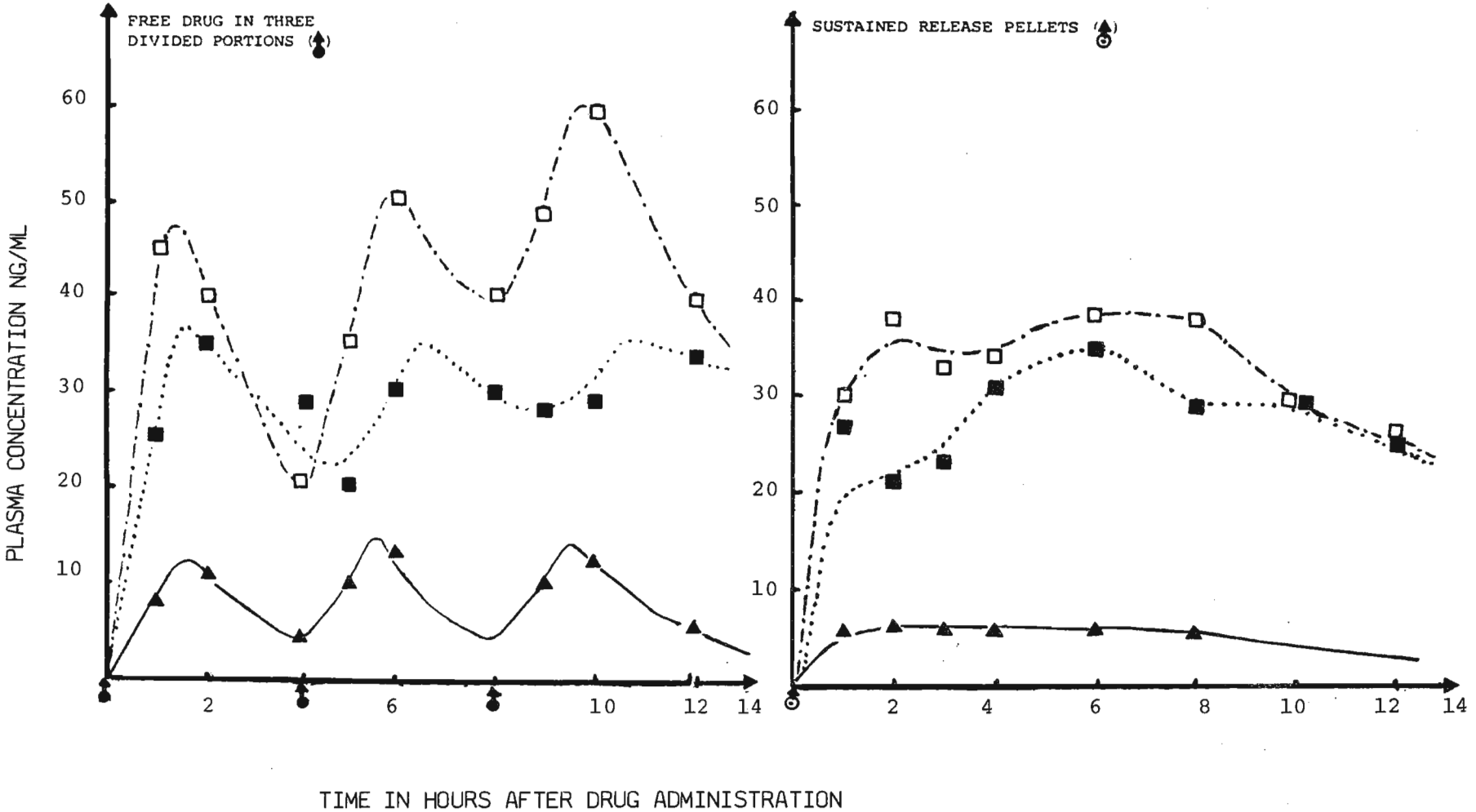


Figure 4.7: Plasma concentration profiles of diethylpropion (I; △) and its two major metabolites i.e. ethylaminopropiophenone (II,○) and diethylnorpseudoephedrine (IV; ●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to subject H (male, 19 years) under acidic (pH = 4,8 ± 0,2) urinary conditions.

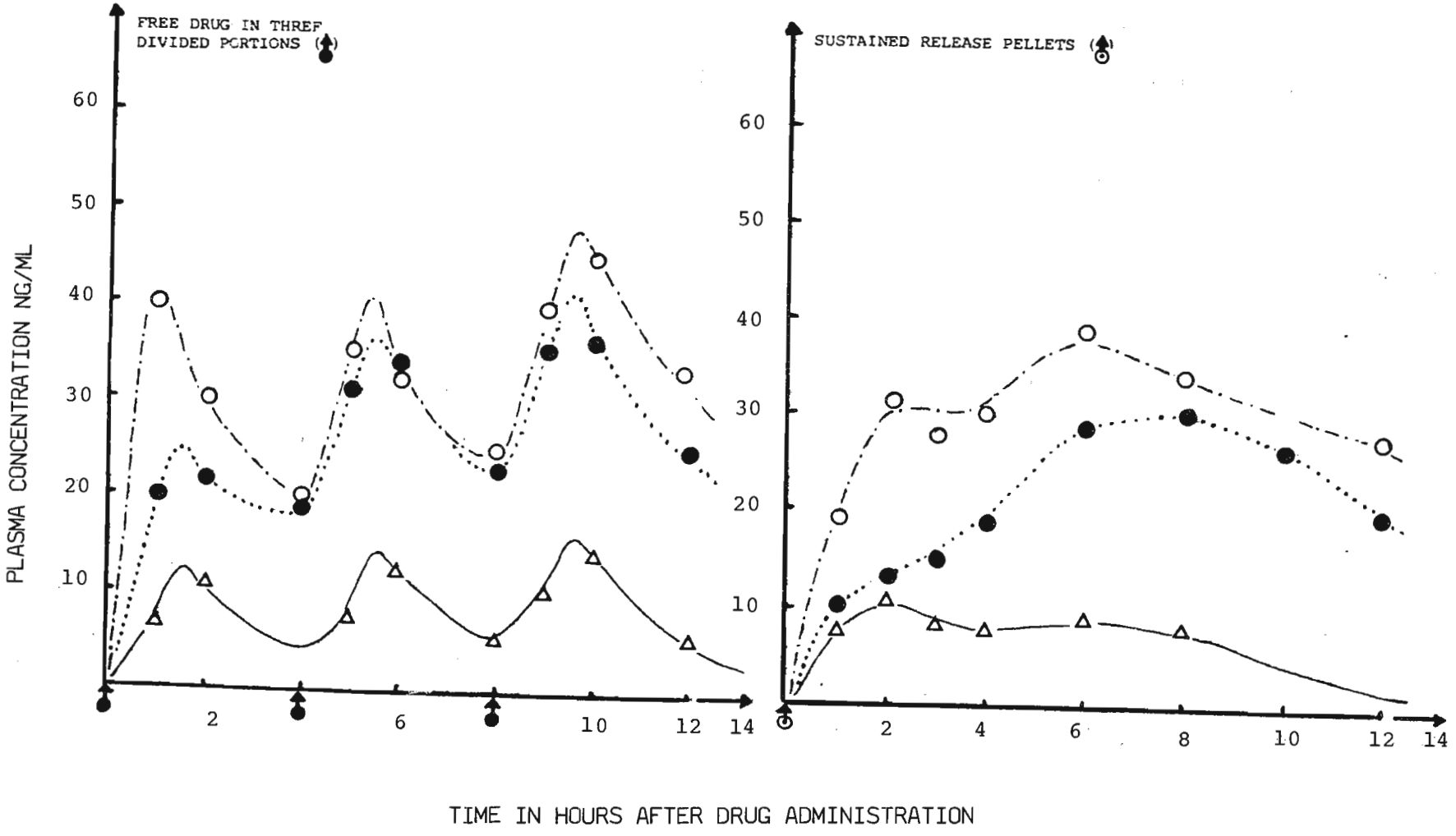


Table 4.1: The plasma and saliva concentrations and urinary excretion data on metabolites *1 II and IV at plateau levels after oral administration of sustained release pellets *2 to six subjects (Group A)

	Metabolite II	Metabolite IV
Plateau Levels - Duration ± 6 hours i.e. 4 to 10 hours after dose		
*3 Plasma ng/ml		
Mean ± S.D.	34,88 ± 3,5	23,22 ± 1,79
Range	(29,1 - 37,9)	(23,2 - 25,5)
*4 Saliva ng/ml		
Mean ± S.D.	209,8 ± 22,52	163,5 ± 27,19
Range	(180 - 212)	(132 - 207)
*5 Urine µg/min		
Mean ± S.D.	20,25 ± 0,73	12,99 ± 0,35
Range	(19,49 - 21,17)	(12,64 - 13,36)

*1 Metabolite II - Ethylaminopropiophenone

Metabolite IV - Diethylnorpseudoephedrine

*2 Sustained release pellets Lot R 7773 (≡ 75 mg diethylpropion hydrochloride) at 0 hours

*3 Data from Tables 2, Appendix IV

*4 Data from Tables 4, Appendix IV

*5 Data from Tables 3, Appendix IV

Table 4.2: The Plasma and Saliva concentrations and urinary rate datas of metabolites*1 II and IV at their "Peaks and Troughs" times after oral administrations of F.D.F.*2 to six subjects (Group A).

	PLASMA ng/ml		SALIVA ng/ml		URINE µg/min	
	II	IV	II	IV	II	IV
FIRST DOSE - 0 hrs						
Peak						
Mean ± S.D.	40,8±1,2	20,3±3,1	290,8±42	138±28	25,6±3,0	12,7±2,3
	*3(33-46)	(11-35)	(130-435)	(32-130)	(17,5-34,3)	(9,3-19,9)
Time after dose (hrs)	1,3	1,3	1,1	1,1	1,33	1,3
	(1-2)	(1-2)	(1-1,5)	(1-1,5)	(1,25-1,75)	(0,75-1,25)
Trough						
Mean ± S.D.	20,8±1	14,5±3	83±12	63±17	8,2±1,2	5,6±1
Time after dose (hrs)	(17-26)	(6-27)	(40-120)	(35-123)	(4,6-11,8)	(1,1-8,0)
	4	4	4,1	4,0	4,1	4,0
			(4-4,5)		(3,8-4,8)	(3,75-4,25)
SECOND DOSE - 4 hrs						
Peak						
Mean ± S.D.	49,5±4,0	28,8±4,0	386,3±44	203,3±3,1	33,3±3,1	19,0±3,3
	(36,2-59)	(11-40)	(219-506)	(90-356)	(25,4-4,6)	(5,6-28,2)
Time after first dose (hrs)	5,1	5,1	5,1	5,1	5,2	5,2
	(5-5,5)	5-5,5)	(5-5,5)	(5-5,5)	(4,75-5,5)	(4,75-5,25)
Trough						
Mean ± S.D.	30,6±3,1	20,5±2,7	110±18	74±20	13,8±2	9,7±1,0
	(20-37)	(8-29)	(86-182)	(49-150)	(8,1-16)	(4,1-15,9)
Time after first dose (hrs)	8,0	8,0	8,0	8,1	8,2	8,0
			(7,75-8,25)	(8,0-8,5)	(8,0-8,4)	(7,75-8,25)
THIRD DOSE - 8 hours						
Peak						
Mean ± S.D.	53,2±2,3	29,4±4,4	337,8±37	188±17	43,2±3,0	18,9±3,4
	(40-61)	(123-43)		(22-132)	(33,2-50,2)	(8,9-33,2)
Time after first dose (hrs)	9,5	9,5	9,8	9,85	9,85	10
	(9-9,5)	(9-9,8)	(9,5-10)	(9,5-10)	(9,25-10,75)	(9,75-10,25)

*1 II Ethylaminopropiophenone
IV Diethylnorpseudoephedrine

*2 F.D.F. Free Dosage Form, ≡ 25 mg diethylpropion hydrochloride in a hard gelatin capsule at times 0 ,4 and 8 hours

*3 Value within brackets gives the range

hrs = hours

Table 4.3: Relative Areas Under the Curves (cm^2)*1 calculated for 12 hours Plasma concentrations of ethylaminopropiophenone (Metabolite II) and diethylnorpseudoephedrine (Metabolite IV) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to six subjects (Group A)

Subject	F.D.F.*2			S.R.P.*3			Relative Percentage*4		
	Met. II	Met. IV	Total	Met. II	Met. IV	Total	Met. II	Met. IV	Total
A	99,1	60,8	159,9	75,6	67,0	142,6	76,3	110,2	89,2
B	90,6	59,3	149,9	79,7	43,0	122,7	88,0	72,5	81,8
C	100,3	18,0	118,3	87,1	29,2	116,3	87,0	162,0	98,3
D	67,2	37,2	104,4	67,2	34,7	101,9	100,0	93,0	97,6
E	97,4	66,6	164,0	79,6	67,1	146,7	81,7	100,7	89,4
H	76,5	64,2	140,3	73,0	48,3	121,3	95,4	75,2	86,2
Mean	88,5	51,0	139,5	77,0	48,2	125,2	87,0	94,5	90,0

*1 Area under curve (cm^2) for 12 hour period, was estimated by counting the squares on the graph.

*2 F.D.F.: Free Dosage Form in 3 divided doses, i.e. 25 mg at times 0, 4 and 8 hours.

*3 S.R.P.: Sustained Release Pellets (≡75 mg) Lot 7773 at time 0 hours.

*4 Results obtained after administration of S.R.P. against results obtained after adminisration of F.D.F.

Table 4.4: Range* of the relative bioavailability percentages using the sum of the two metabolites II and IV in plasma, saliva and urine of subjects in Groups A and B

Group	Plasma	Saliva	Urine
A	81,8% - 98,3%	87,4% - 136,1%	88,4% - 127,0%
B	-	64,5% - 122,3%	94,7% - 112,6%

* From Table 4.3 for Plasma
Table 4.5 for Saliva
Table 4.6 for Urine

4.1.2 Saliva Data

The results on the determinations of the salivary concentrations of diethylpropion (I), and its two metabolites, II and IV, after oral administration of sustained release pellets and the free drug to twelve subjects, are summarized in Tables 3 to 6, Appendix IV. The graphical representations of these results in individual subjects and the mean of the salivary concentrations, have been given in Figures 4.8 to 4.16. The salivary concentration profile of compounds I, II and IV after oral administration of the sustained release pellets, (supported by the plasma concentration profiles in each of the six subjects), gave broad plateau levels which were intermediate between the "peaks and troughs" using free drugs, and extended over 8 to 10 hours after drug administration. The mean $\pm$ S.D. saliva concentrations during plateau levels (4 to 10 hours) for metabolite II was 209,8 ng/ml ranging from 180 - 212 ng/ml, while for metabolite IV, it was 163,5 $\pm$ 27,19 ng/ml, ranging from 132,5 - 207,0 ng/ml (Table 4.1); the mean salivary concentrations and the times when the "peaks and troughs" appeared, have been outlined in Table 4.2. The "peaks and troughs", for each metabolite, appeared at the same time in plasma and saliva samples.

The relative bioavailability results, based on salivary data on all twelve subjects are shown in Table 4.5.

Group A

All the areas under curve (AUC) values were above 75% when the results for either metabolite II or metabolite IV were considered. The range was 89,3% to 148,6% for metabolite II and 83,7% to 120,0% for metabolite IV.

Group B

The relative bioavailability when considering metabolite II data, gives a range of 60,5% to 122,0% with two of the subjects (A.M. and S.M.) below 75,0%. For metabolites IV, the results range from a relative availability of 41,3% to 123,4%.

In general, the saliva data on the six subjects of Group A who were kept at the clinic on the day of dosing (Table 3.1), were more reliable than the data of the results on saliva samples collected from subjects of Group B, who were involved in their normal activities throughout the day. It must be appreciated, though, that saliva data, due to many difficulties of sample stimulation and collections (discussed in 1.4) are less valuable than the plasma or urine data for consideration of relative bioavailabilities.

Figure 4.8: Mean (± S.E.) saliva concentration profiles of diethylpropion (I;△) and its two major metabolites i.e. ethylaminopropiophenone (II, ○) and diethylnorpseudoephedrine (IV;●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to six subjects (i.e. group A, Table 1) under acidic (pH = 4,8 ± 0,2) urinary conditions.

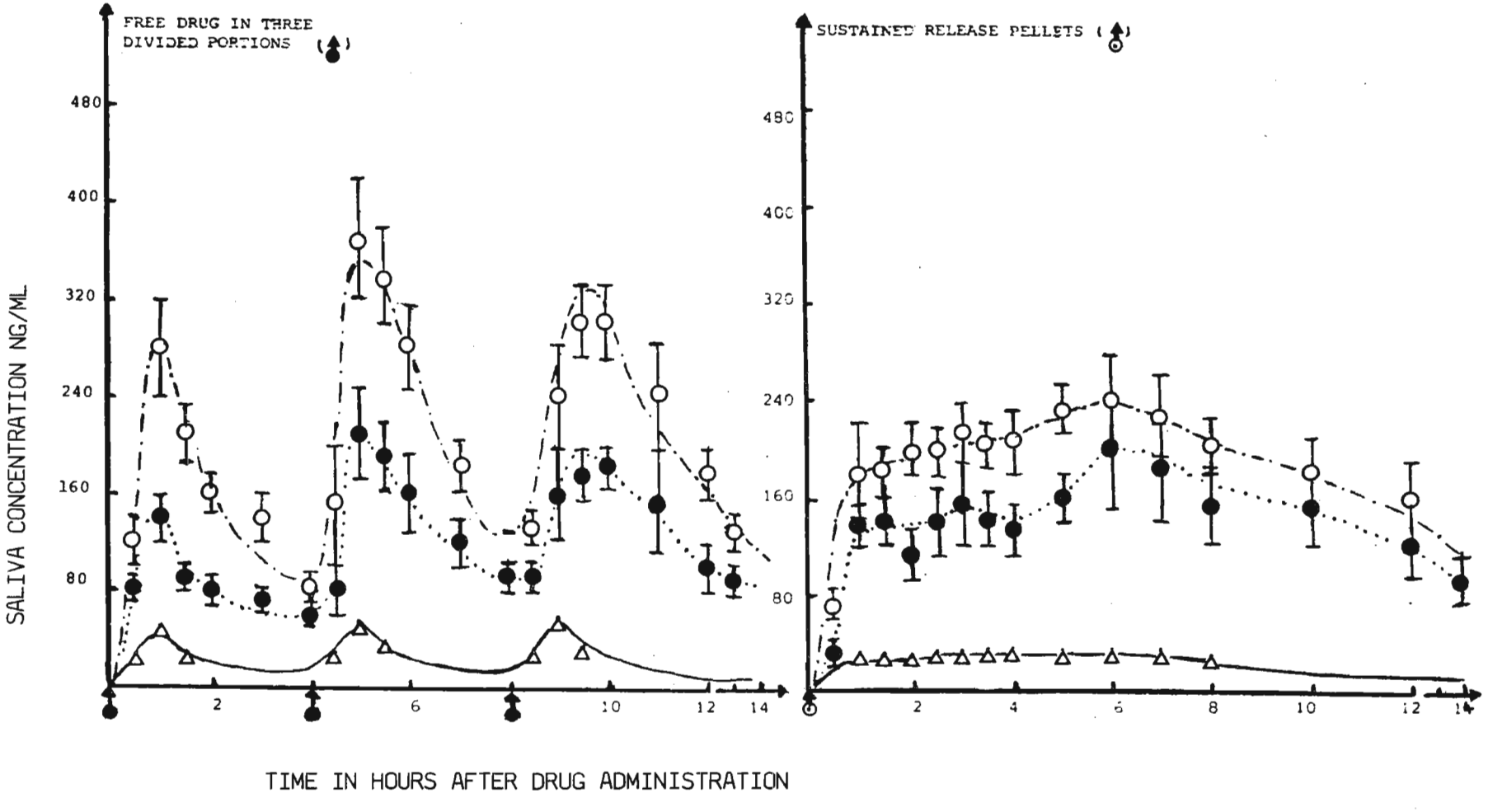


Figure 4.9: Saliva concentration profiles of ethylaminopropiophenone (Metabolite II,●■) and diethylnorpseudoephedrine (Metabolite IV;○□) after oral administration of two different dosage forms (F.D.F.: solid lines. S.R.P.: broken lines)* of diethylpropion (75 mg hydrochloride salt) to two subjects.

* F.D.F.: Free dosage form in three divided doses i.e. 25 mg at times 0, 4 and 8 hours

S.R.P.: Sustained release pellets (75 mg) at time 0 hours

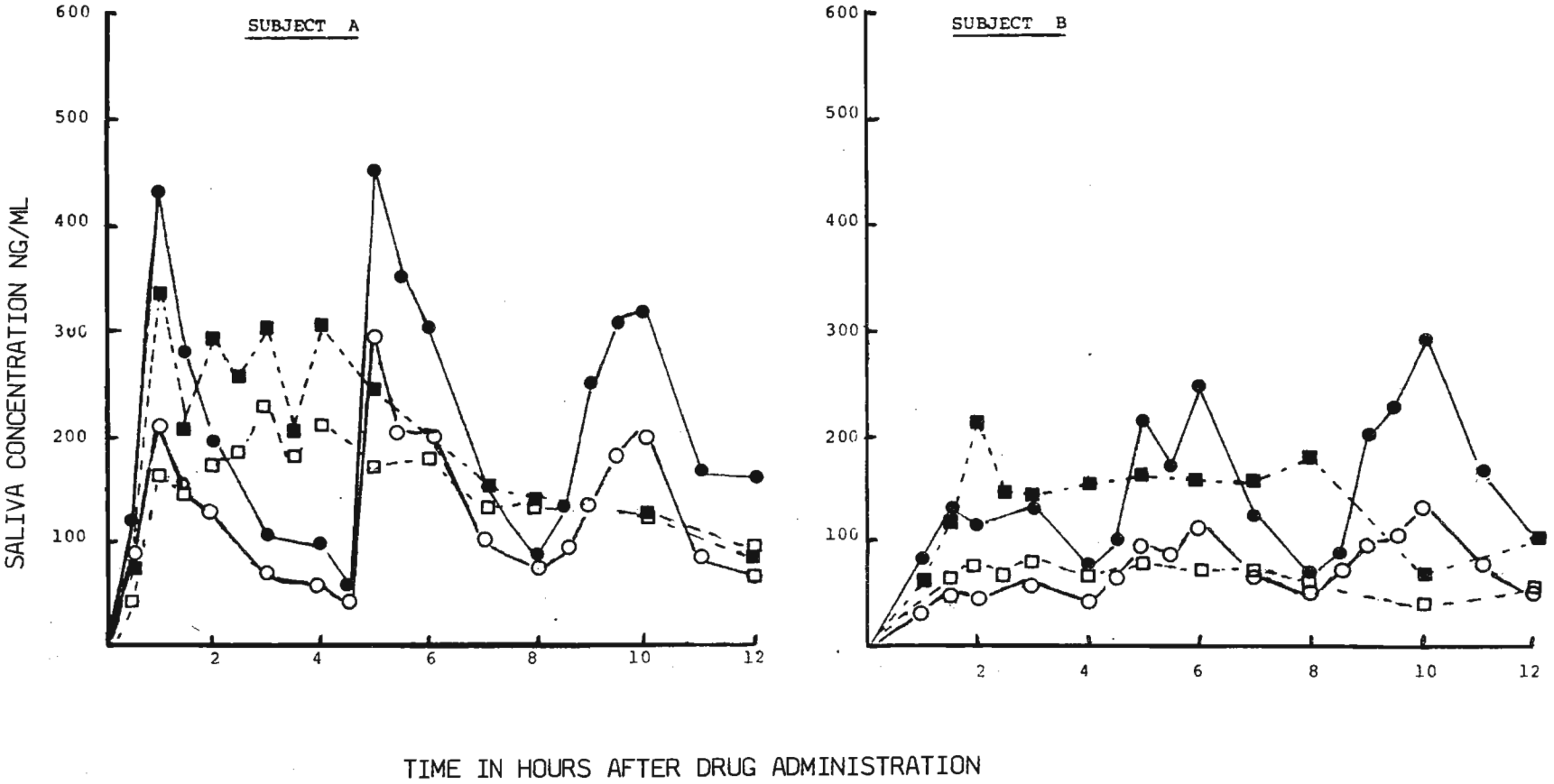


Figure 4.10: Saliva concentration profiles of ethylaminopropiophenone (Metabolite II,●■) and diethylnorpseudoephedrine (Metabolite IV;○□) after oral administration of two different dosage forms (F.D.F.: solid lines. S.R.P.: broken lines)* of diethylpropion (75 mg hydrochloride salt) to two subjects.

* F.D.F.: Free dosage form in three divided doses i.e. 25 mg at times 0,4 and 8 hours

S.R.P.: Sustained release pellets (75 mg) at time 0 hours

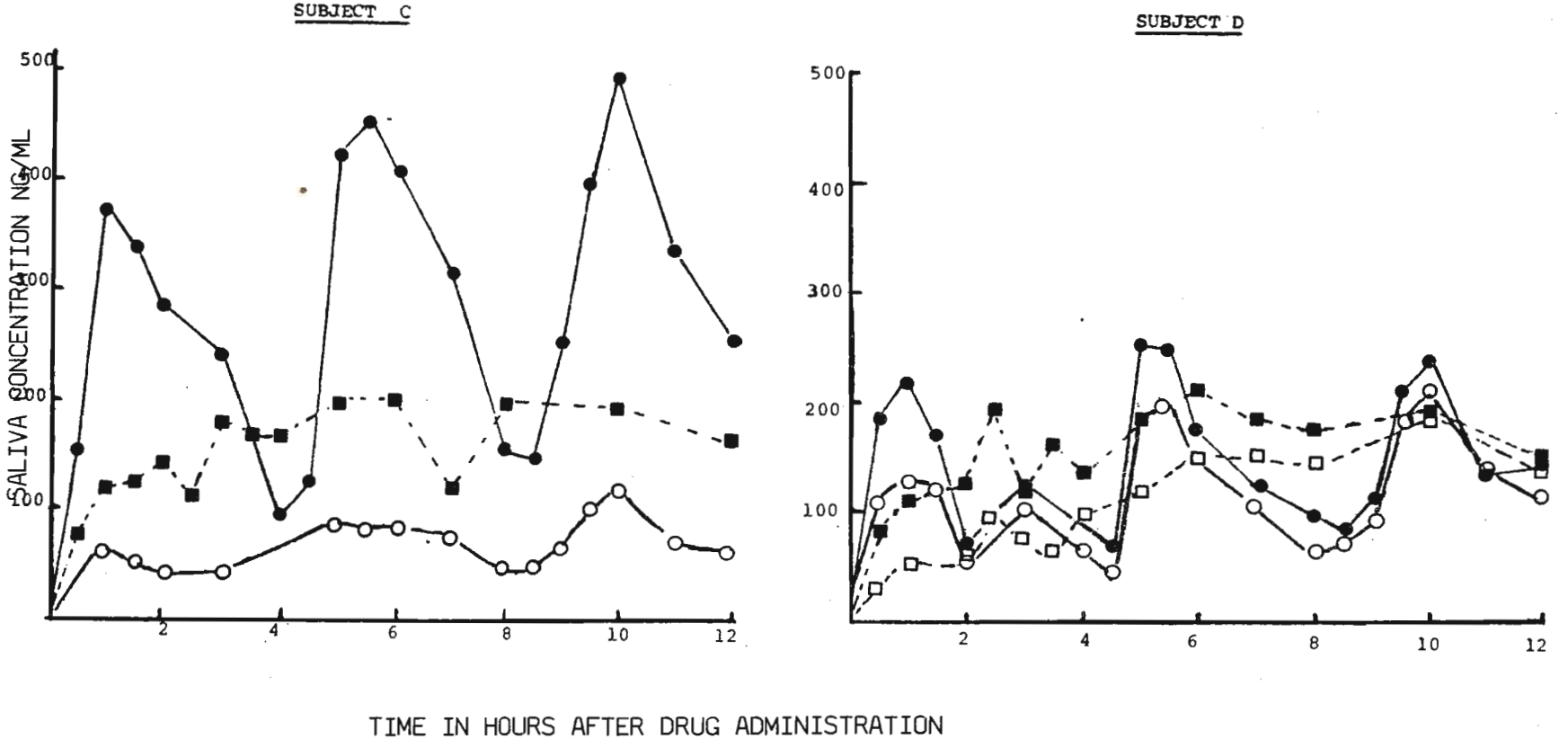


Figure 4.11 Saliva concentration profiles of diethylpropion (I; △) and its two major metabolites i.e. ethylaminopropiophenone (II, ○) and diethylnorpseudoephedrine (IV; ●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to subject E (female, 19 years) under acidic (pH = 4,8 ± 0,2) urinary conditions.

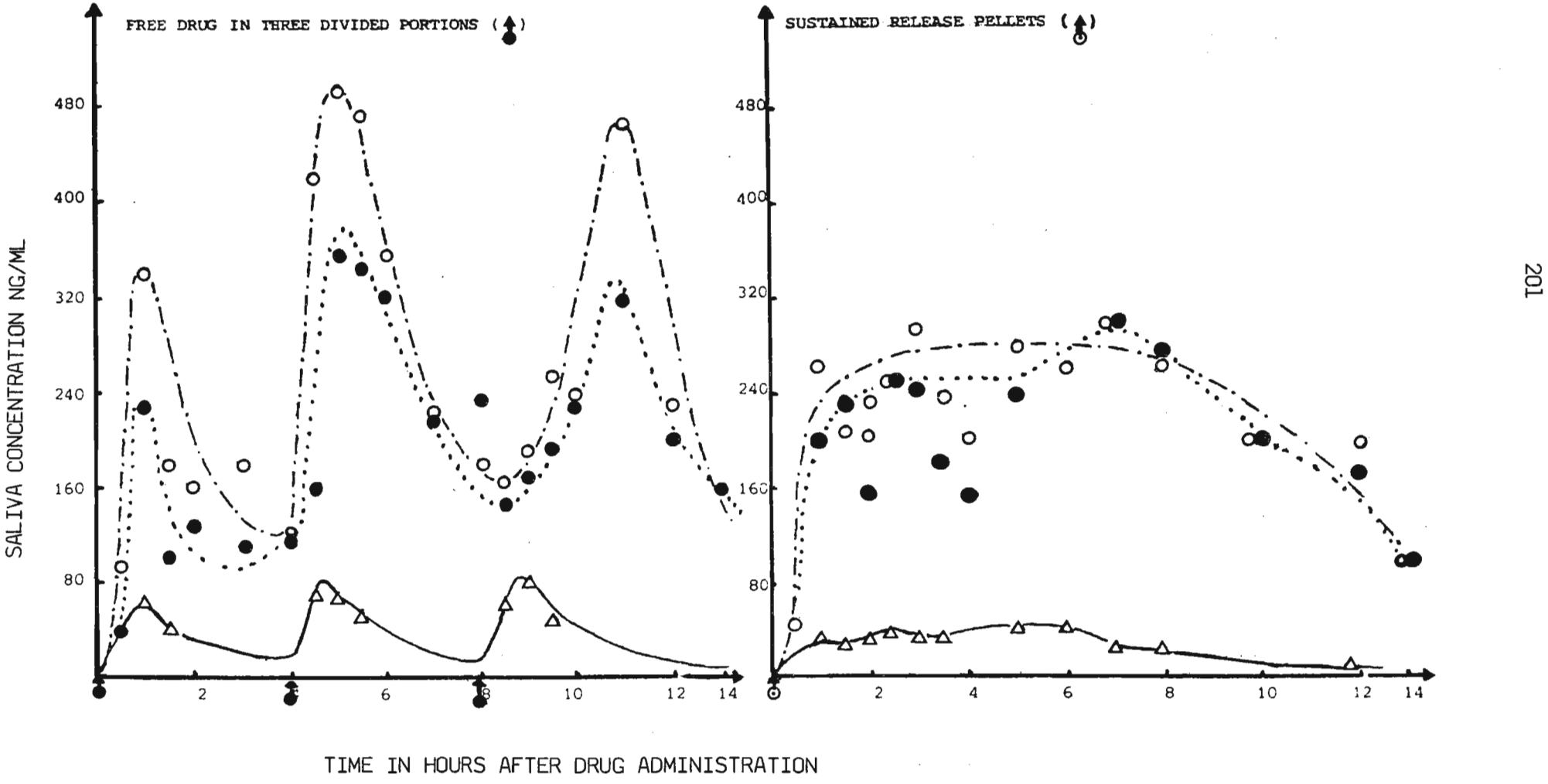


Figure 4.12 Saliva concentration profiles of diethylpropion (I; △) and its two major metabolites i.e. ethylaminopropiophenone (II, ○) and diethylnorpseudoephedrine (IV; ●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to subject H (male, 19 years) under acidic (pH = 4,8 ± 0,2) urinary conditions.

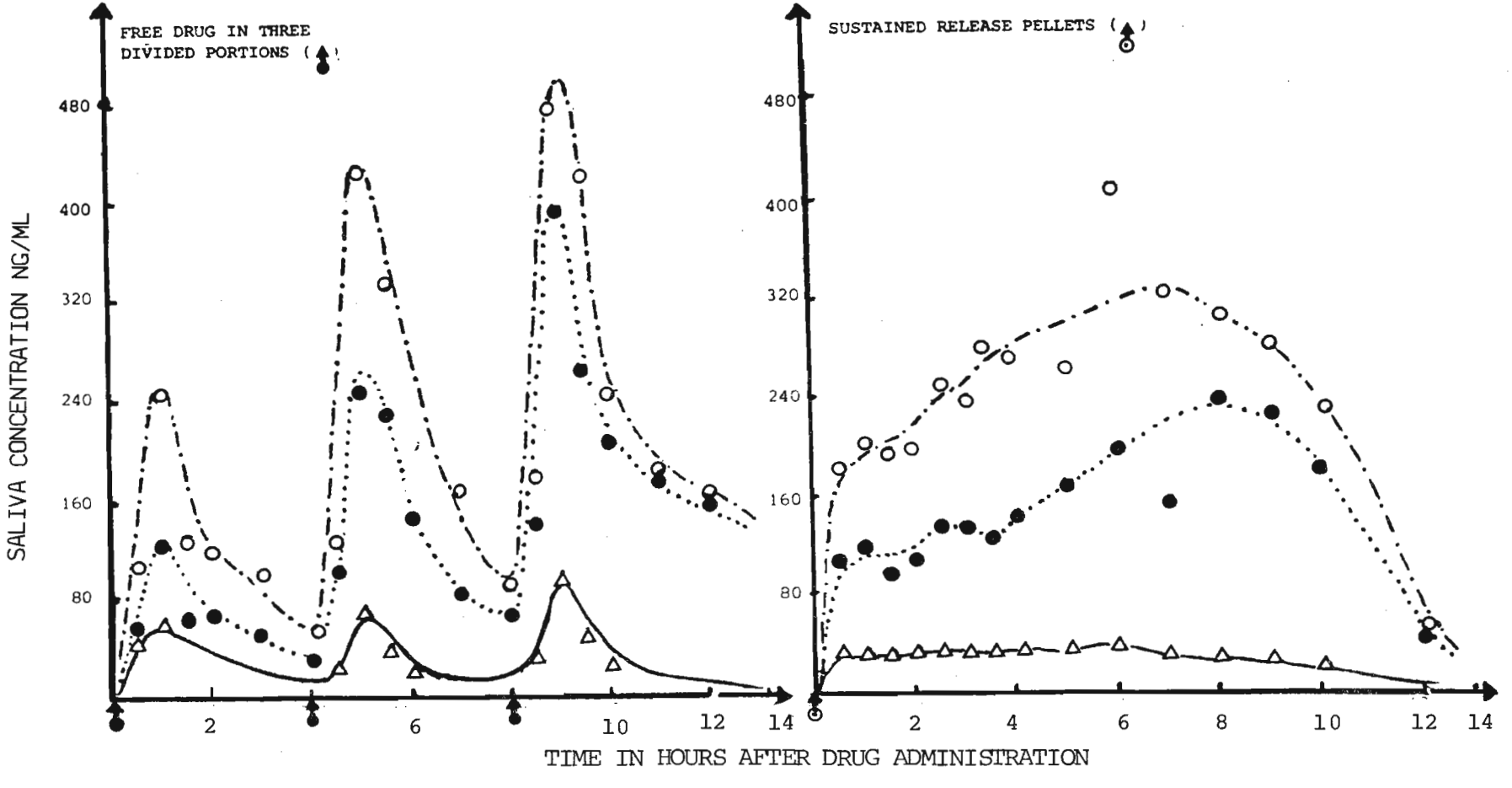


Figure 4.13: Mean (± S.E.) saliva concentration profiles of diethylpropion (I;△) and its two major metabolites i.e. ethylaminopropiophenone (II, ○) and diethylnorpseudoephedrine (IV;●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to six subjects (i.e. group B, Table 1) under acidic (pH = 4,8 ± 0,2) urinary conditions.

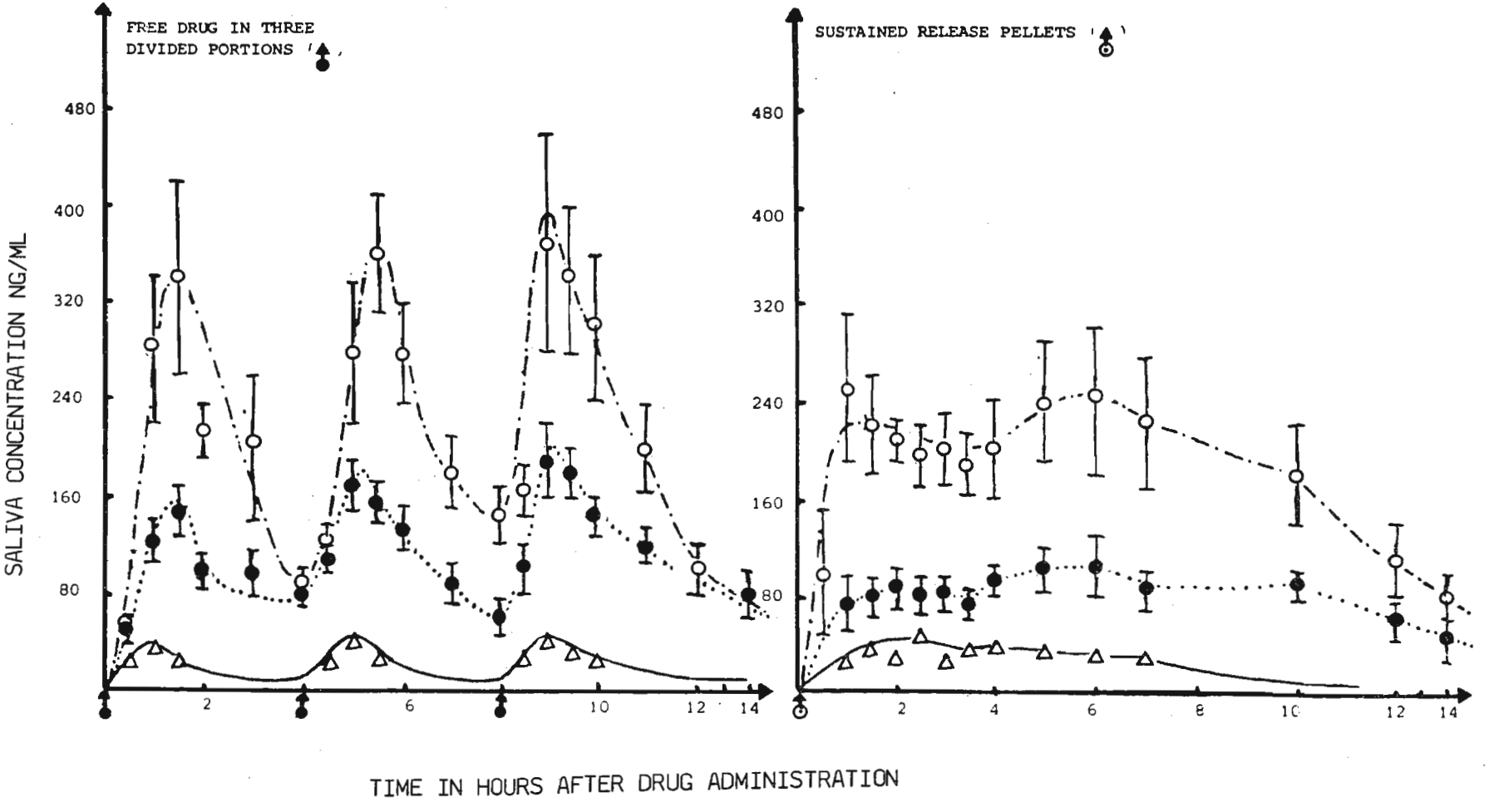


Figure 4.14: Saliva concentration profiles of ethylaminopropiophenone (Metabolite II,●■) and diethylnorpseudoephedrine (Metabolite IV;○□) after oral administration of two different dosage forms (F.D.F.: solid lines. S.R.P.: broken lines)* of diethylpropion (75 mg hydrochloride salt) to two subjects.

* F.D.F.: Free dosage form in three divided doses i.e. 25 mg at times 0, 4 and 8 hours

S.R.P.: Sustained release pellets (75 mg) at time 0 hours

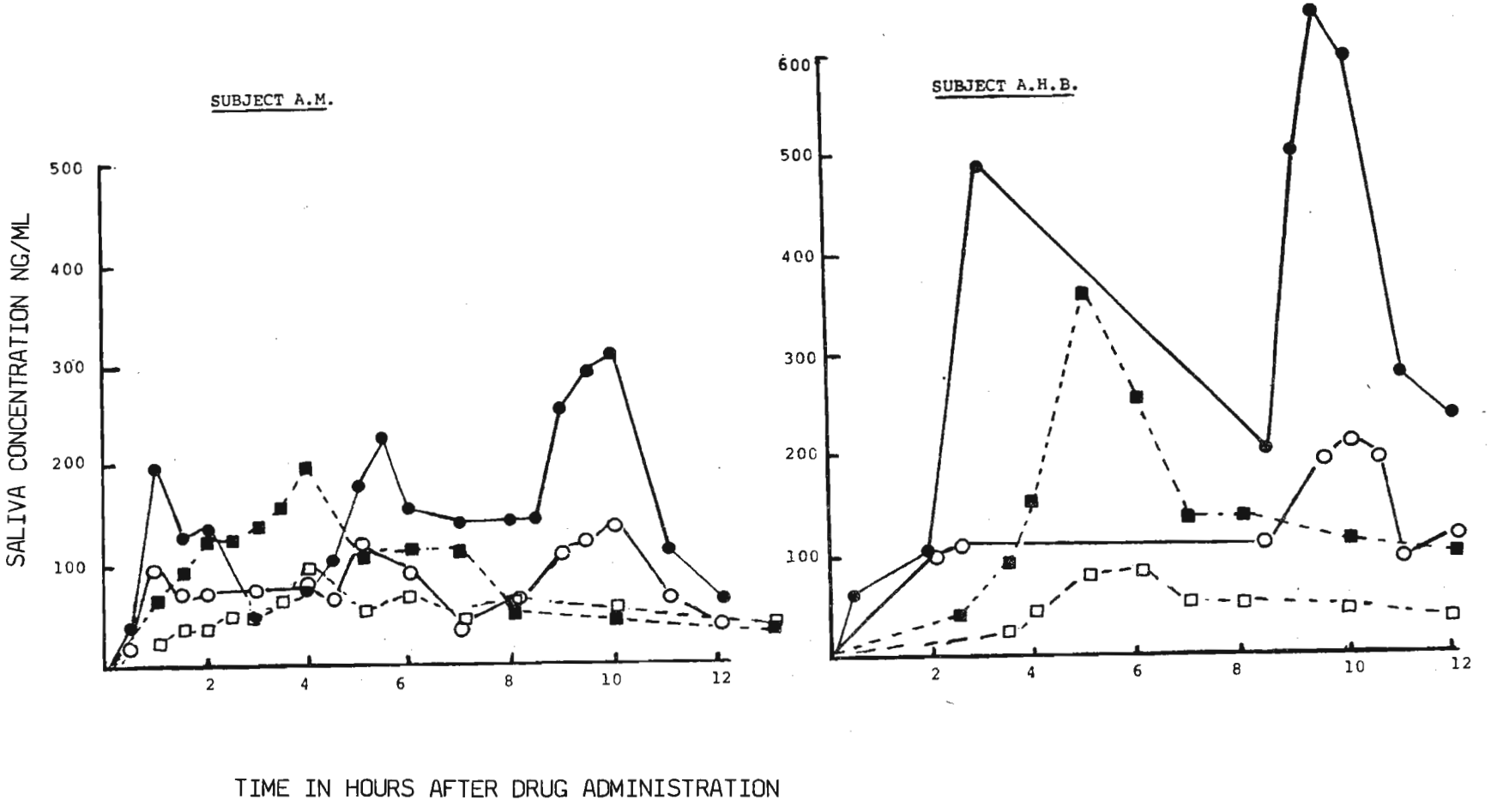
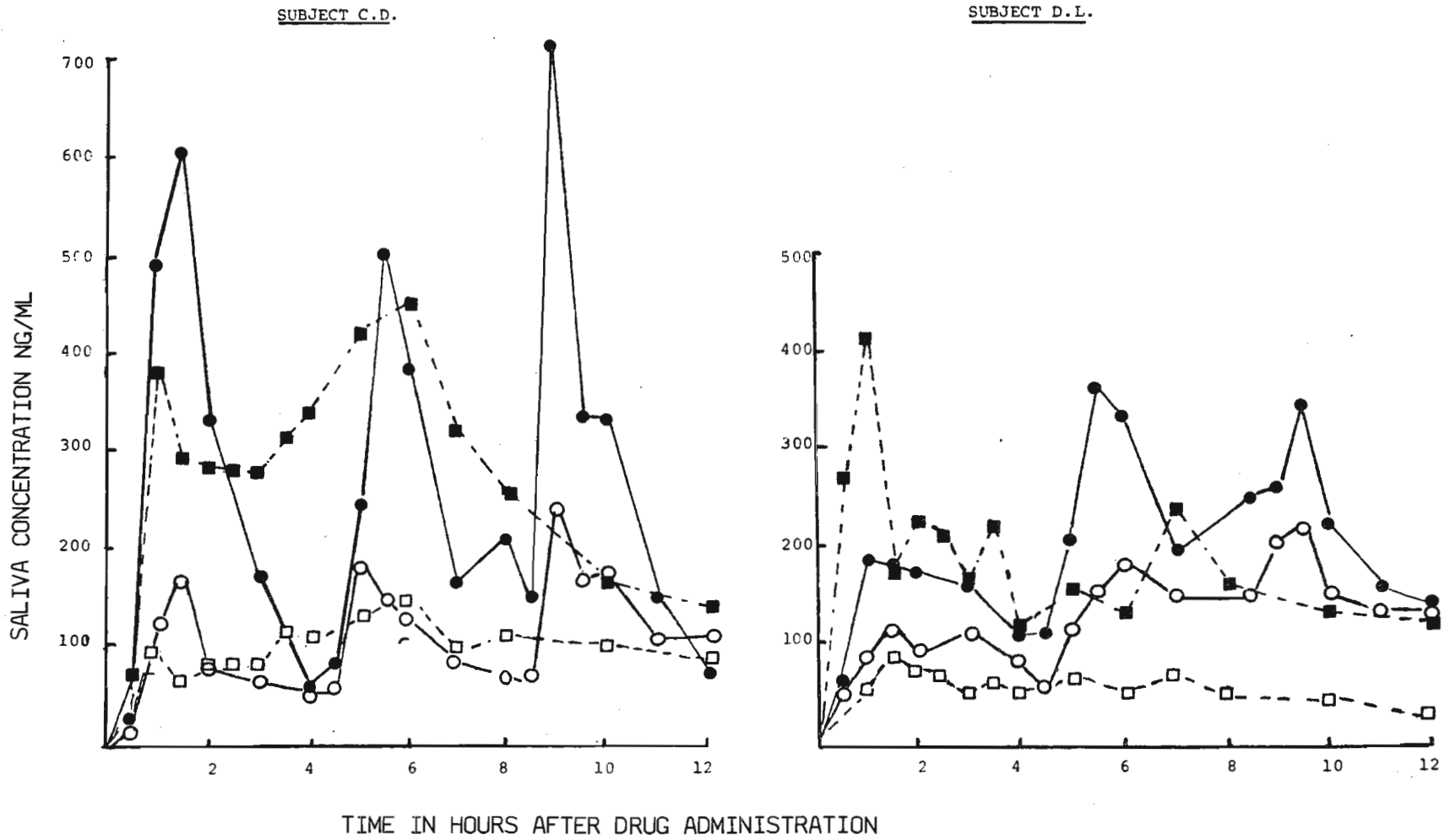


Figure 4.15: Saliva concentration profiles of ethylaminopropiophenone (Metabolite II,●■) and diethylnorpseudoephedrine (Metabolite IV;○□) after oral administration of two different dosage forms (F.D.F.: solid lines. S.R.P.: broken lines)* of diethylpropion (75 mg hydrochloride salt) to two subjects.



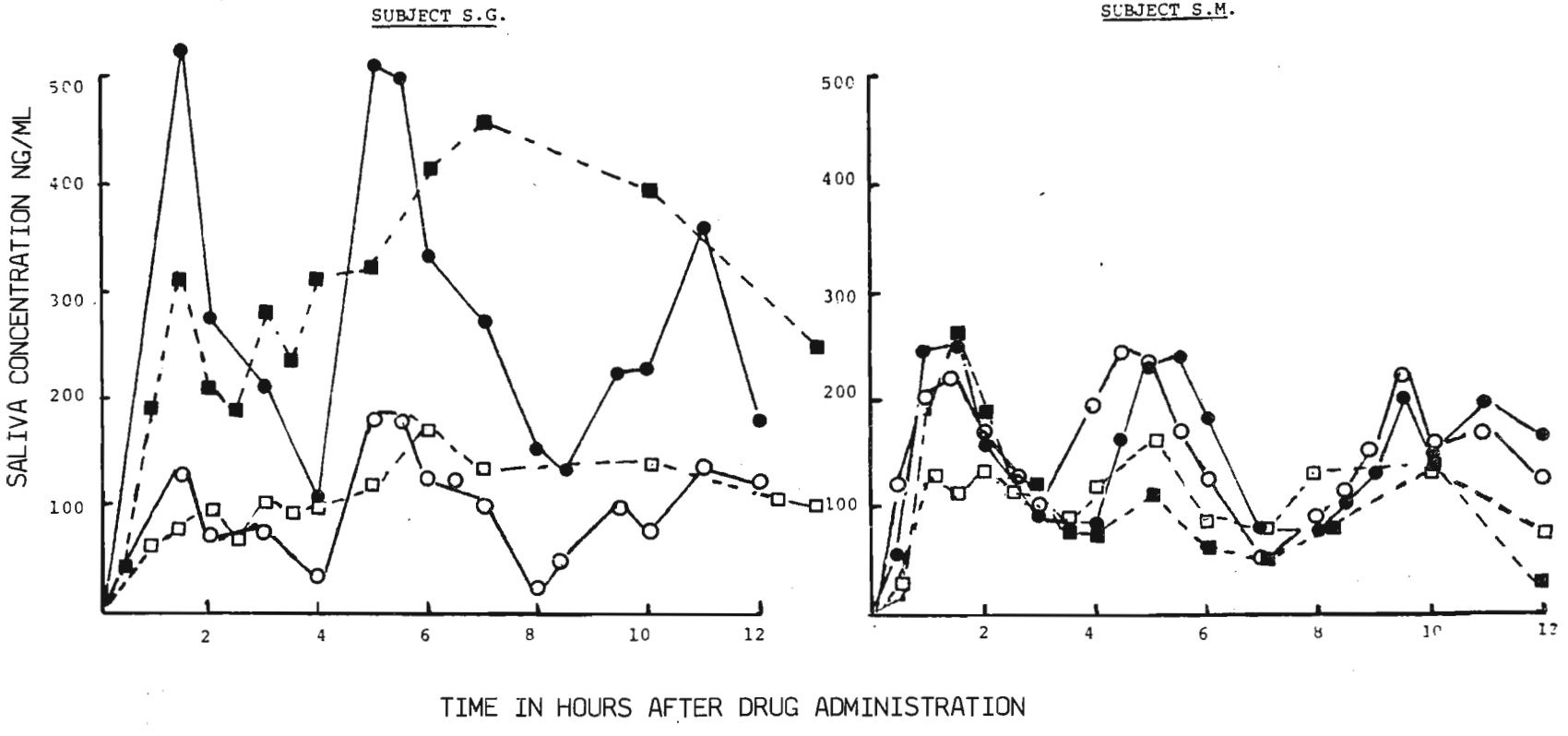
* F.D.F.: Free dosage form in three divided doses i.e. 25 mg at times 0, 4 and 8 hours

S.R.P.: Sustained release pellets (75 mg) at time 0 hours

Figure 4.16: Saliva concentration profiles of ethylaminopropiophenone (Metabolite II,●■) and diethylnorpseudoephedrine (Metabolite IV;○□) after oral administration of two different dosage form (F.D.F.: solid lines. S.R.P.: broken lines)* of diethylpropion (75 mg hydrochloride salt) to two subjects.

* F.D.F.: Free dosage forms in three divided doses i.e. 25 mg at times 0, 4 and 8 hours

S.R.P.: Sustained release pellets (75 mg) at time 0 hours



4.1.3 Urine Data

The results on the urinary excretion rates of compounds I, II and IV, after oral administration of the sustained release pellets and the free drug to twelve subjects, are summarized in Tables 7 to 10, Appendix IV; the graphical representations of these results in some individuals (A, E and H of Group A) and the mean of the urinary excretion rates of twelve subjects have been shown in Figures 4.17 to 4.21. In the six subjects of Group A, the peak urinary excretion rates of compounds I, II, IV were reached at 45 to 90 minutes after administration of each of the first two portions of the dose, with a slight delay at 50-150 minutes after the third portion; the mean of the results is shown in Figure 4.17 and Table 4.2. Each successive peak was slightly higher than the previous one, c.f. for amphetamine (Beckett et al., 1967), fenfluramine (Shenoy, 1971), phendimetrazine (Raisi. 1977) , and a rapid decline in the levels of each compound occurred at 3-4 hours after each dose, thereby giving typical "peaks and troughs" profiles; these corresponding characteristic patterns were also seen in saliva and plasma concentration vs time profiles (Figure 4.22). The "peaks" in the urinary data profiles appeared slightly delayed compared to those in plasma and in saliva (which appeared almost simultaneously) (Figures 4.22 and 4.23). The reason for the small time delay in appearance of peaks is that the excretion of compounds in saliva occurs via the arterial system, while that in urine is related to the venous system (Posti, 1979).

The slight delay in the appearance of the peak times of all the compounds (I, II and IV) after administration of the final portion (3rd dose) of the free drug (Table 4.2) can be accountable to delay in absorption of drug from the GIT due to the presence of food, taken at 6 hours after the first dose (Table C, Appendix III), which may have altered the transit time of the pellets.

The mean profiles of compounds I, II and IV seen in all the biological fluids of subjects taking the free drug or sustained release pellets, reflected similar patterns (Figures 4.22, 4.23).

On the other hand, after oral administration of the sustained release pellets, the mean urinary excretion rate profiles, supported by the appropriate mean saliva and plasma concentration profiles in all subjects, presented, after rapid initial absorption, broad plateau levels which were intermediate between the "peaks and troughs" using the free drug, and extended over 8 to 10 hours after drug administration; Figures 4.1, 4.8 and 4.17 refer to plasma, saliva and urine data in six subjects of group A; Figures 4.13 and 4.21 refer to saliva and urine data in six subjects of group B. Table 4.1 outlines the means of plasma, saliva and urine data at plateau levels after administration of sustained release pellets to six subjects of Group A.

The cumulative urinary excretion profiles of compounds II and IV after oral administration of the free drug and sustained release pellets in three subjects (A, C and A.M.) are given in Figures 4.25, 4.27 and 4.28 respectively; the mean results on the twelve subjects were also plotted (Figures 4.24 and 4.26). These profiles clearly demonstrate the "staircase" effect (corresponding to "peaks and troughs" shown in Figures 4.17 and 4.21) of metabolites II and IV after oral administration of the free drug.

Figure 4.17: Mean (± S.E.) urinary excretion rate profiles of diethylpropion (I;△) and its two metabolites i.e. ethylaminopropiophenone (II, ○) and diethylnorpseudoephedrine (IV;●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to six subjects (i.e. group A, Table 1) under acidic (pH = 4,8 ± 0,2) urinary conditions.

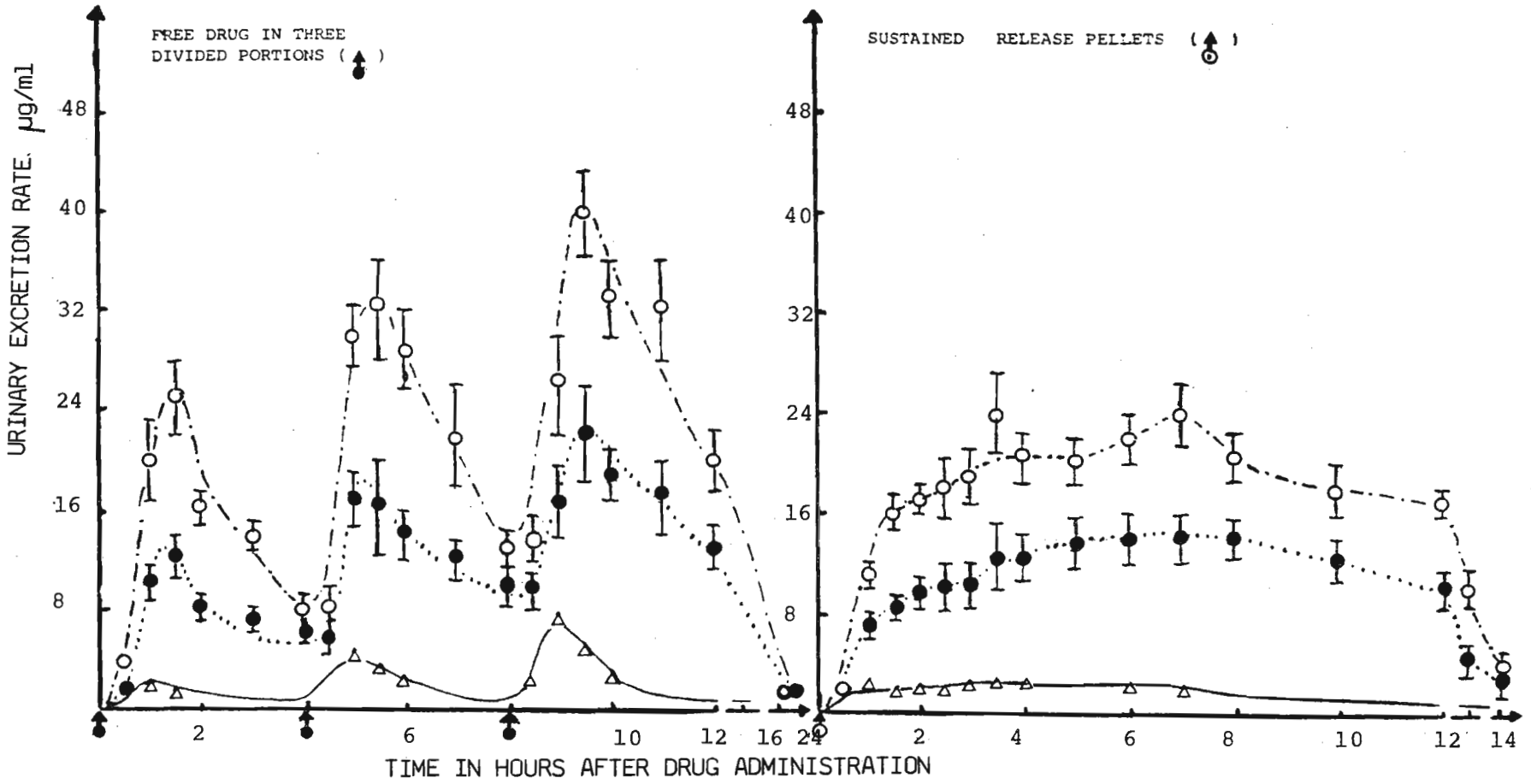


Figure 4.18: Urinary excretion rate profiles of diethylpropion (I;△) and its two major metabolites i.e. ethylaminopropiophenone (II,○) and diethylnorpseudoephedrine (IV;●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to subject A (female, 22 years) under acidic (pH = 4,8 ± 0,2) urinary conditions.

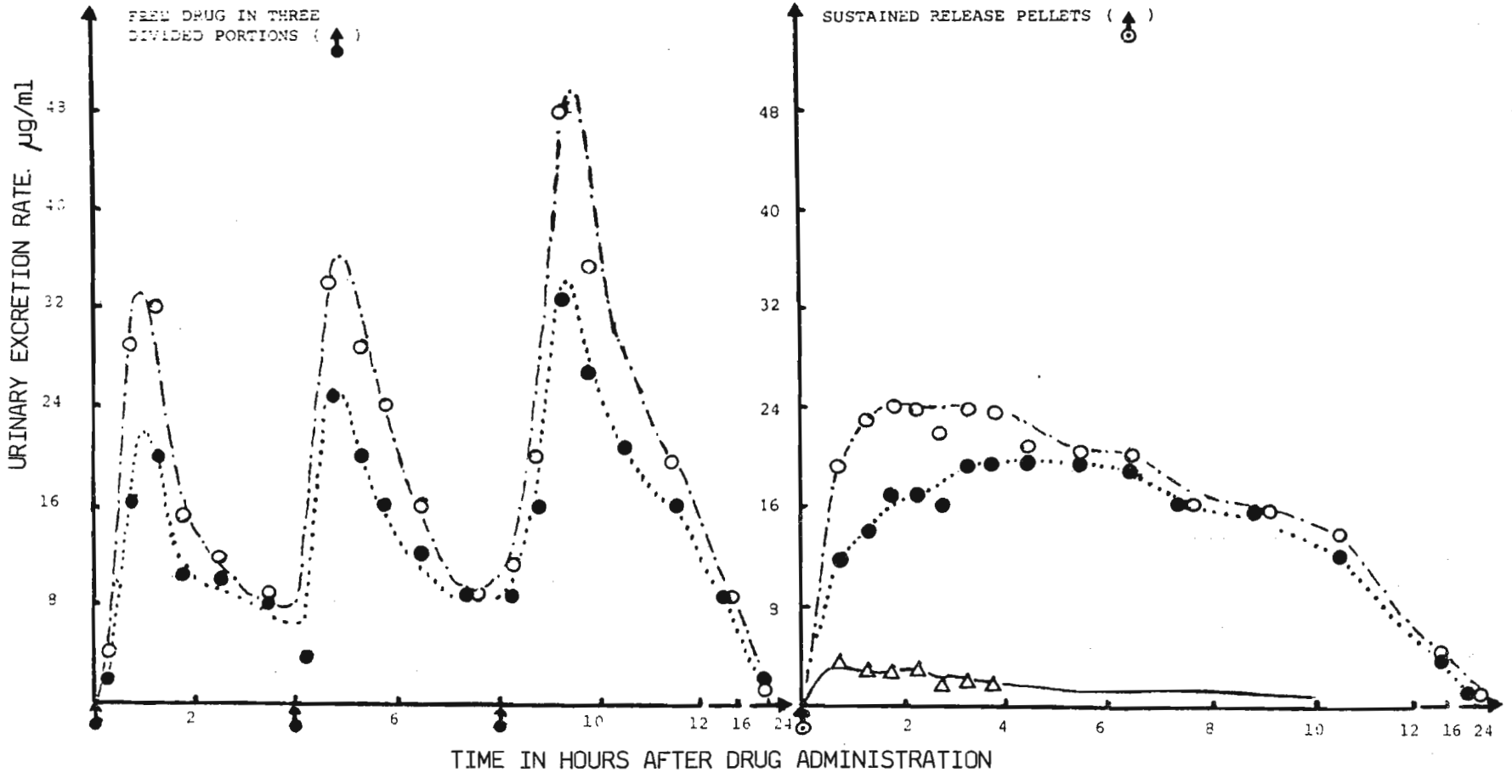


Figure 4.19: Urinary excretion rate profiles of diethylpropion (I;△) and its two major metabolites i.e. ethylaminopropiophenone (II,○) and diethylnorpseudoephedrine (IV;●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to subject E (female, 19 years) under acidic (pH = 4,8 ± 0,2) urinary conditions.

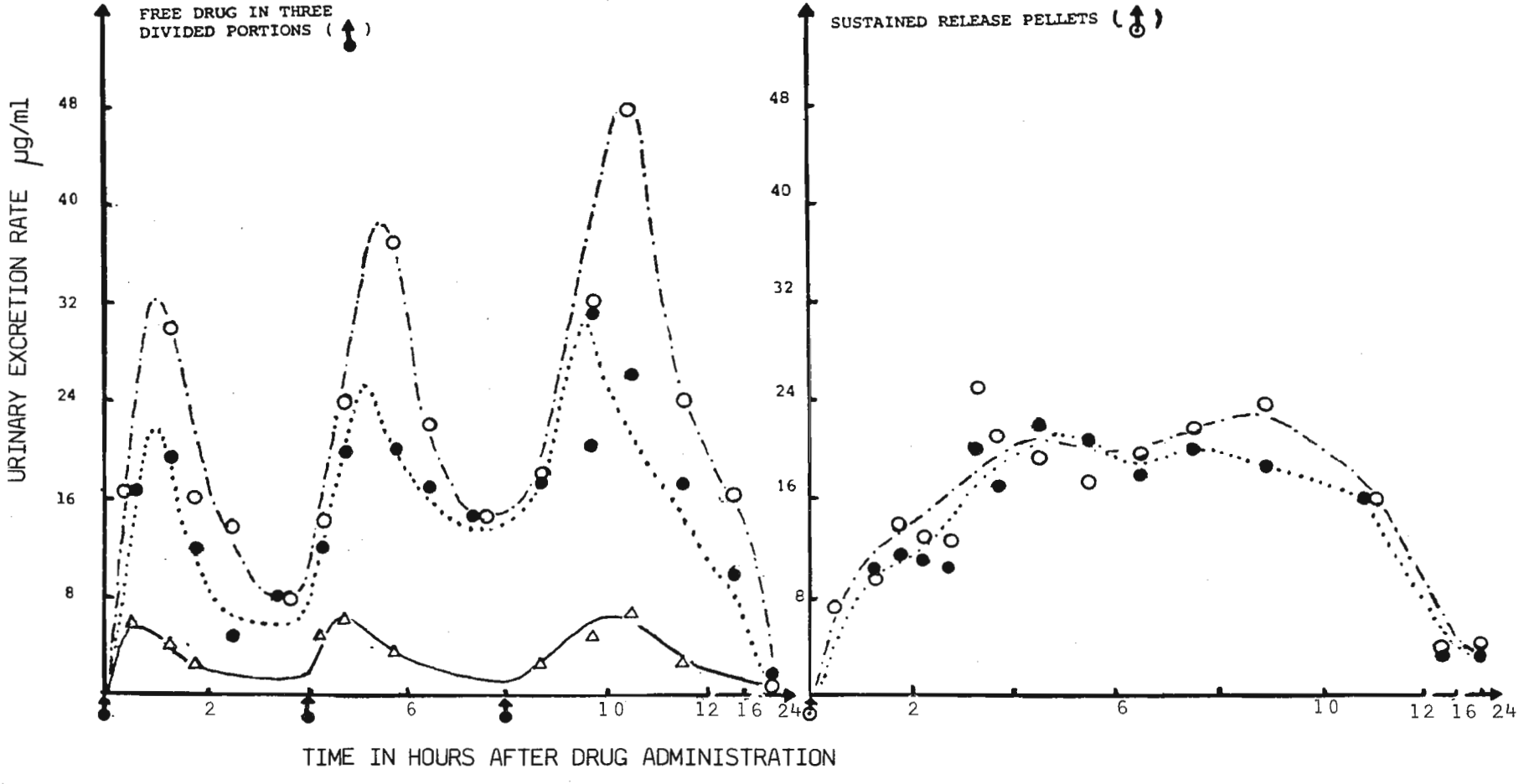


Figure 4.20: Urinary excretion rate profiles of diethylpropion (I;△) and its two major metabolites i.e. ethylaminopropiophenone (II,○) and diethylnorpseudoephedrine (IV;●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to subject H (male, 19 years) under acidic (pH = 4,8 ± 0,2) urinary conditions.

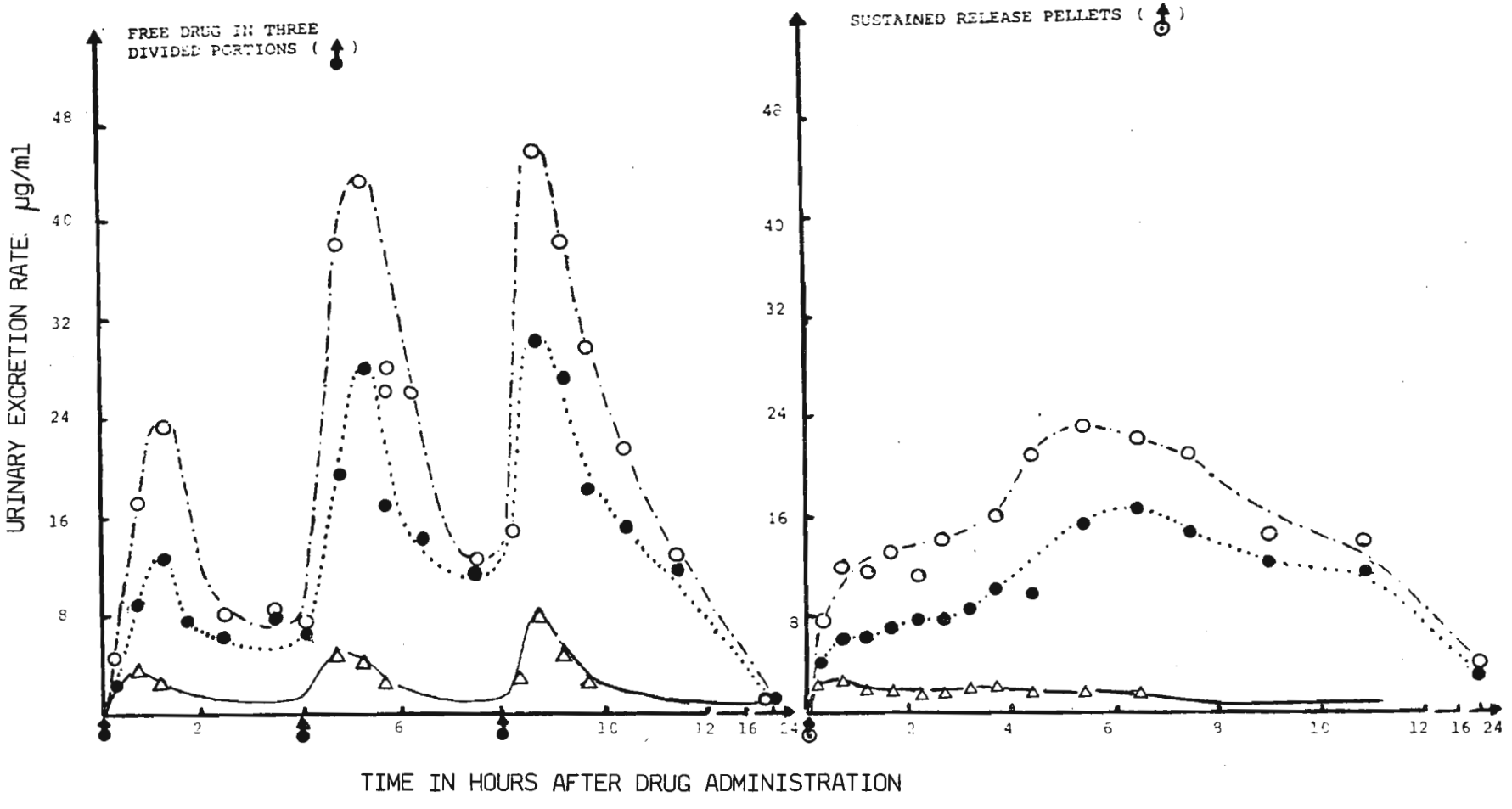


Figure 4.21: Mean (± S.E.) urinary excretion rate profiles of diethylpropion (I;△) and its two metabolites i.e. ethylaminopropiophenone (II, ○) and diethylnorpseudoephedrine (IV;●) after oral administration of two different dosage forms of diethylpropion (≡ 75 mg hydrochloride salt) to six subjects (i.e. group B, Table 1) under acidic (pH = 4,8 ± 0,2) urinary conditions.

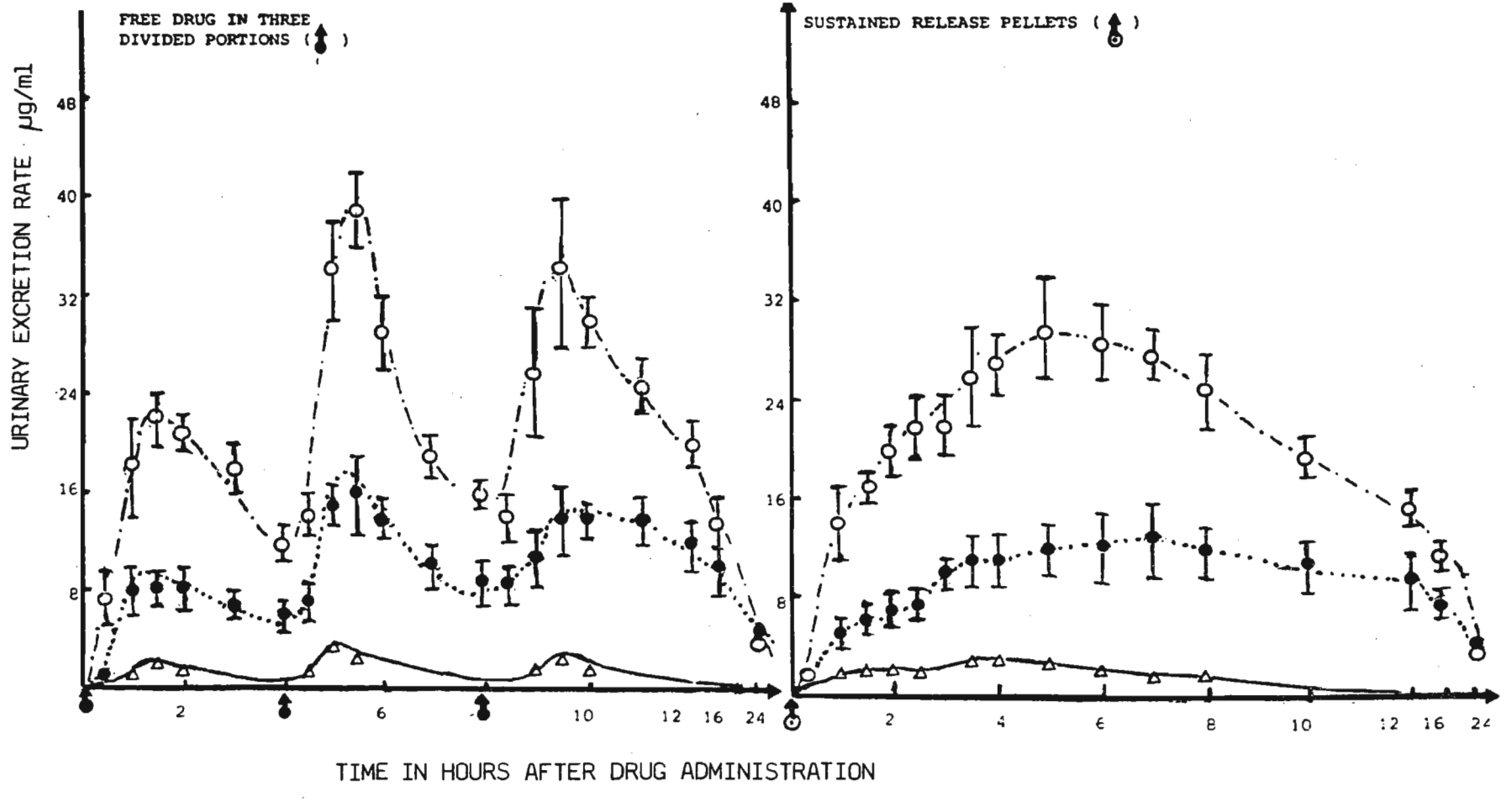


Table 4.5: Relative Areas Under the Curves (cm^2) calculated for 12 hours SALIVA concentrations of ethylaminopropiophenone (Metabolite II) and diethylnorpseudoephedrine (Metabolite IV) after oral administration of two different dosage forms of DIETHYLPROPION (≡ 75 mg hydrochloride salt) to twelve subjects

GROUP A

Subject	F.D.F.*1			S.R.P.*2			Relative Percentage*3		
	Met. II	Met. IV	Total	Met. II	Met. IV	Total	Met. II	Met. IV	Total
A	48,5	28,9	77,4	43,7	34,7	78,4	90,1	120,0	101,3
B	33,7	16,6	50,3	30,1	13,9	44,0	89,3	83,7	87,4
C	69,0	16,4	85,4	?	?	?	?	?	*4
D	34,0	26,8	60,8	38,3	28,4	66,3	112,6	105,9	109,7
E	62,5	49,0	111,5	57,0	55,6	112,6	91,2	113,4	100,9
H	43,0	31,5	74,5	63,9	37,5	101,4	148,6	119,0	136,1
Mean	48,4	28,2	76,6	46,6	34,0	80,6	96,3	120,5	105,2

GROUP B

Subject	F.D.F.*1			S.R.P.*2			Relative Percentage*3		
	Met. II	Met. IV	Total	Met. II	Met. IV	Total	Met. II	Met. IV	Total
AM	34,0	16,5	50,5	20,6	12,0	32,6	60,5	72,7	64,5
AHB	-	-	-	-	-	-	-	-	*4
CD	62,2	24,8	87,0	62,4	23,2	85,6	100,3	93,5	98,4
DL	46,9	30,5	77,4	40,9	12,6	53,5	87,2	41,3	69,1
SG	62,7	22,2	84,9	76,5	27,4	103,9	122,0	123,4	122,3
SM	34,5	35,7	70,2	23,1	27,8	50,9	67,0	77,8	72,5
Mean	48,0	25,9	74,0	44,7	20,6	65,3	93,1	79,5	88,2

Met.: = Metabolite

*1F.D.F.: Free Dosage Form in 3 divided doses, i.e. 25 mg at times 0, 4 and 8 hours.

*2S.R.P.: Sustained Release Pellets (75 mg) at times 0 hours.

*3Results obtained after administration of S.R.P. against results obtained after administration of F.D.F.

*4Data omitted due to the decomposition of compounds in chloroform extract (which was left overnight because of breakdown of g.l.c.) and due to irregular collection times of samples in the cases of subjects C and A.H.B. respectively.

Table 4.6: Cumulative urinary recoveries (mg in 36 hours) of ethylaminopropiophenone (Metabolite II) and diethylnorpseudoephedrine (Metabolite IV) after oral administration of two different dosage forms of DIETHYLPROPION (75 mg hydrochloride salt) to twelve subjects

GROUP A

Subject	F.D.F.*1			S.R.P.*2			Relative Percentage*3		
	Met. II	Met. IV	Total	Met. II	Met. IV	Total	Met. II	Met. IV	Total
A	17,4	14,2	31,6	16,6	15,3	31,9	95,4	107,7	100,9
B	12,9	10,0	22,9	18,9	10,2	29,1	146,5	102,0	127,0
C	25,1	4,8	29,9	24,3	6,7	31,0	96,8	139,6	103,7
D	13,6	11,1	24,7	16,7	11,5	28,2	112,8	103,6	114,2
E	19,6	14,1	33,7	15,1	14,7	29,8	77,0	104,2	88,4
H	16,4	13,8	30,2	18,2	14,0	32,2	110,0	101,4	106,6
Mean	17,5	11,3	28,8	18,3	12,1	30,4	108,1	109,8	106,8

GROUP B

Subject	F.D.F.*1			S.R.P.*2			Relative Percentage*3		
	Met. II	Met. IV	Total	Met. II	Met. IV	Total	Met. II	Met. IV	Total
AM	19,2	10,2	29,4	21,9	10,6	32,5	114,0	104,0	110,5
AHB	18,4	8,2	26,6	20,2	6,0	26,2	109,8	73,2	98,5
CD	21,5	7,9	29,4	20,3	9,9	30,2	94,4	125,3	102,7
DL	18,5	12,9	31,4	23,9	8,9	32,8	129,1	69,0	104,4
SG	20,0	8,1	28,1	18,4	8,5	26,9	92,0	104,9	94,7
SM	18,0	22,6	40,6	16,9	28,8	45,7	93,9	127,4	112,6
Mean	19,3	11,7	30,9	20,3	12,1	32,4	105,5	100,6	103,9

Met.: = Metabolite

*1F.D.F.: Free Dosage Form in 3 divided doses, i.e. 25 mg at times 0, 4 and 8 hours.

*2S.R.P.: Sustained Release Pellets (75 mg) at times 0 hours.

*3Results obtained after administration of S.R.P. against results obtained after administration of F.D.F.

Table prepared by Dr. A. Noormohammadi

The use of sustained release pellets totally eliminated this effect in both metabolites, by providing a steady constant (release of drug) increase in the cumulative excretion of each compound (Figures 4.24 and 4.26).

The metabolism and excretion of diethylpropion followed by monitoring metabolites II and IV after oral administration of the sustained release pellets were compared with those of the free drug in three divided doses. Although the amount of each metabolite excreted varied slightly between subjects using any dosage form, the change in formulation did not markedly influence the overall pattern of metabolism of the drug (Table 4.6). For instance, those subjects with pronounced monodealkylation relative to carbonyl reduction (e.g. subject C of group A) showed the same emphasis using both formulations (see Figure 4.4 relative to Figure 4.6 in plasma; Figure 4.10 relative to Figure 4.11 in saliva; and Table 4.3 in urine). Beckett et al., (1967, 1974) reported similar findings using urine data after oral administration of diethylpropion or p-chloroethylaminopropiophenone to man in different dosage forms.

The relative bioavailabilities, by comparison, of the cumulative urinary recoveries of the two metabolites, II and IV, after oral administration of the sustained release pellets and the free drug to twelve subjects are shown in Table 4.6. All the cumulative results for the six subjects in Group A were above 75% for metabolites II and for metabolites IV (range 77,0% to 146,5% for metabolite II and 101,4% to 139,6% for metabolite IV). In Group B, the results when considering metabolite II gave a relative bioavailability above 75% (range 92,0% to 129,1% with a mean of 105,5%) but with metabolite IV, two of the six subjects gave apparent bioavailabilities under 75% i.e. A.H.B. 73,2% and D.L. 69,0% (range in six subjects is from 69,0% to 127,4% with a mean of 106,6%).

When the total of metabolites II and IV are considered, all relative bioavailabilities are above 75% (range 94,7% to 112,6% with a mean of 103,9% - Table 4.6).

The concentration vs time profiles of metabolites II and IV in saliva and in plasma, as well as the urinary excretion data after oral administration of the sustained release pellets indicated an efficient sustained release that extended over 8 to 10 hours. The presence of various slight fluctuations during the duration of the plateau levels would be unlikely to alter the efficacy of the pellet formulation as an anorectic agent - the profile in plasma is "bowed-type" for both metabolite II and metabolite IV and would seem to be suitable, when compared to the free drug in three divided doses, as an appetite suppressant which is required to give "protection" during the normal times for lunch and dinner and not produce any stimulation (i.e. insomnia) in the late evening. No side effects were reported after the use of sustained release pellets (Lot R 7773), while nausea, headache irritability and excitability occurred after the third dose in four subjects receiving the free drug (Table 4.8).

Figure 4.22: Comparison of the mean (±S.E.) plasma (--o--), saliva (—▲—) concentrations and mean urinary excretion rates (··□··) of ethylaminopropiophenone (Metabolite II) after oral administration of diethylpropion hydrochloride (free dosage form) in divided doses (3 x 25 mg) at 0, 4 and 8 hours (↑) to six subjects in Group A, under acidic urine conditions

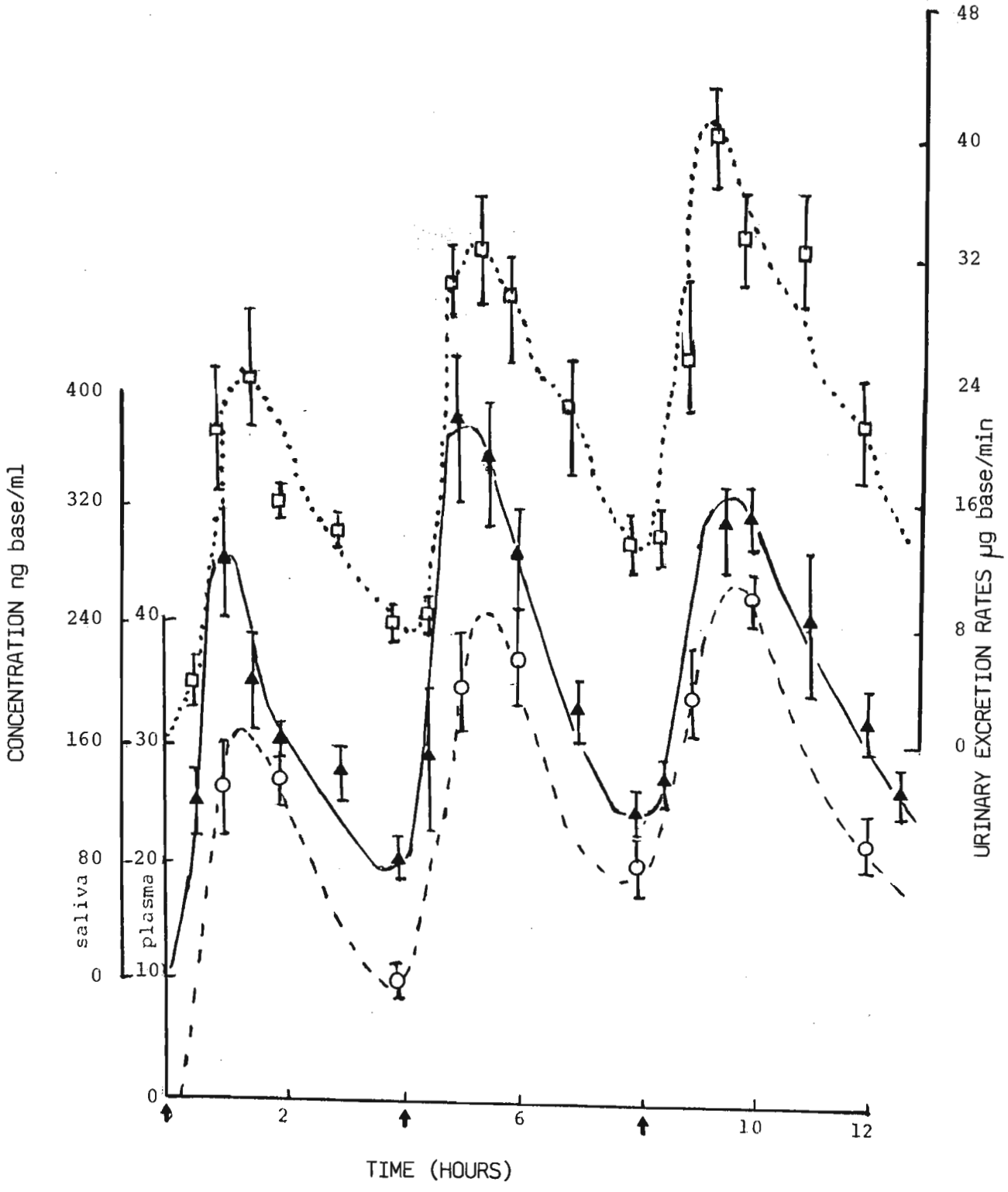


Figure 4.23: Comparison of the mean (±S.E.) plasma (◇ ◆), saliva (□■) concentrations and urinary excretion rates (△ ▲) of the two metabolites, ethylaminopropiophenone (·—·—) and diethylnorpseudoephedrine (· · · ·) after oral administration of 75 mg of diethylpropion hydrochloride (Sustained Release Pellets, R 7773) to six subjects (Group A) under acidic urine conditions

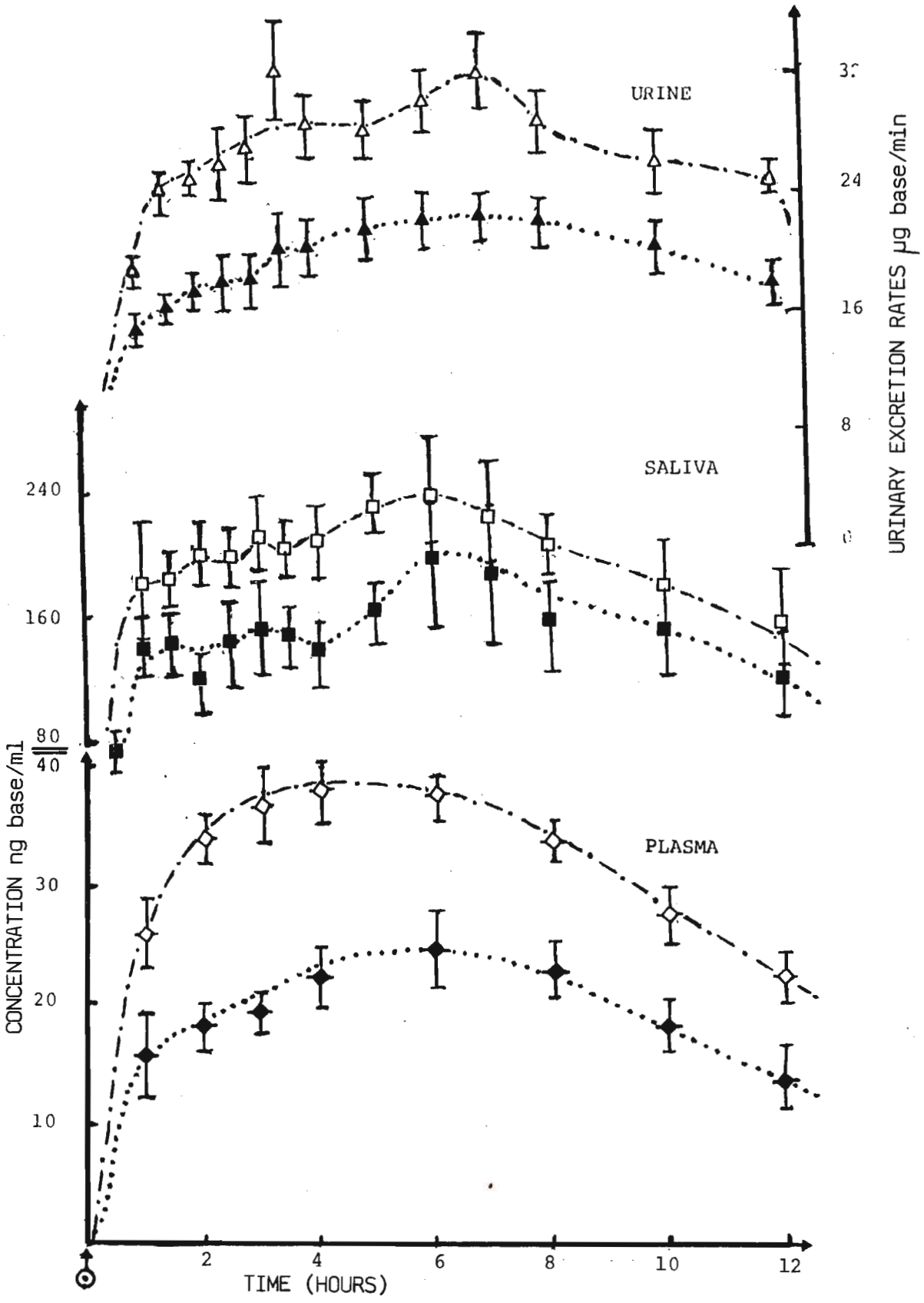


Figure 4.24: Mean (±S.E.) cumulative urinary excretion*1 of ethylaminopropiophenone (Metabolite II;●■) and diethylnorpseudoephedrine (Metabolite IV;○□) after oral administration of two different dosage forms (F.D.F. solid lines; S.R.P. broken lines)* of diethylpropion (75 mg hydrochloride salt) to six subjects (Group A).

*1 Expressed as percentage of total dose administered

*2 F.D.F. Free dosage form in three divided doses i.e. 25 mg at times 0, 4 and 8 hours (↑)

*3 S.R.P. Sustained release pellets Lot R 7773 (75 mg) at time 0 hours

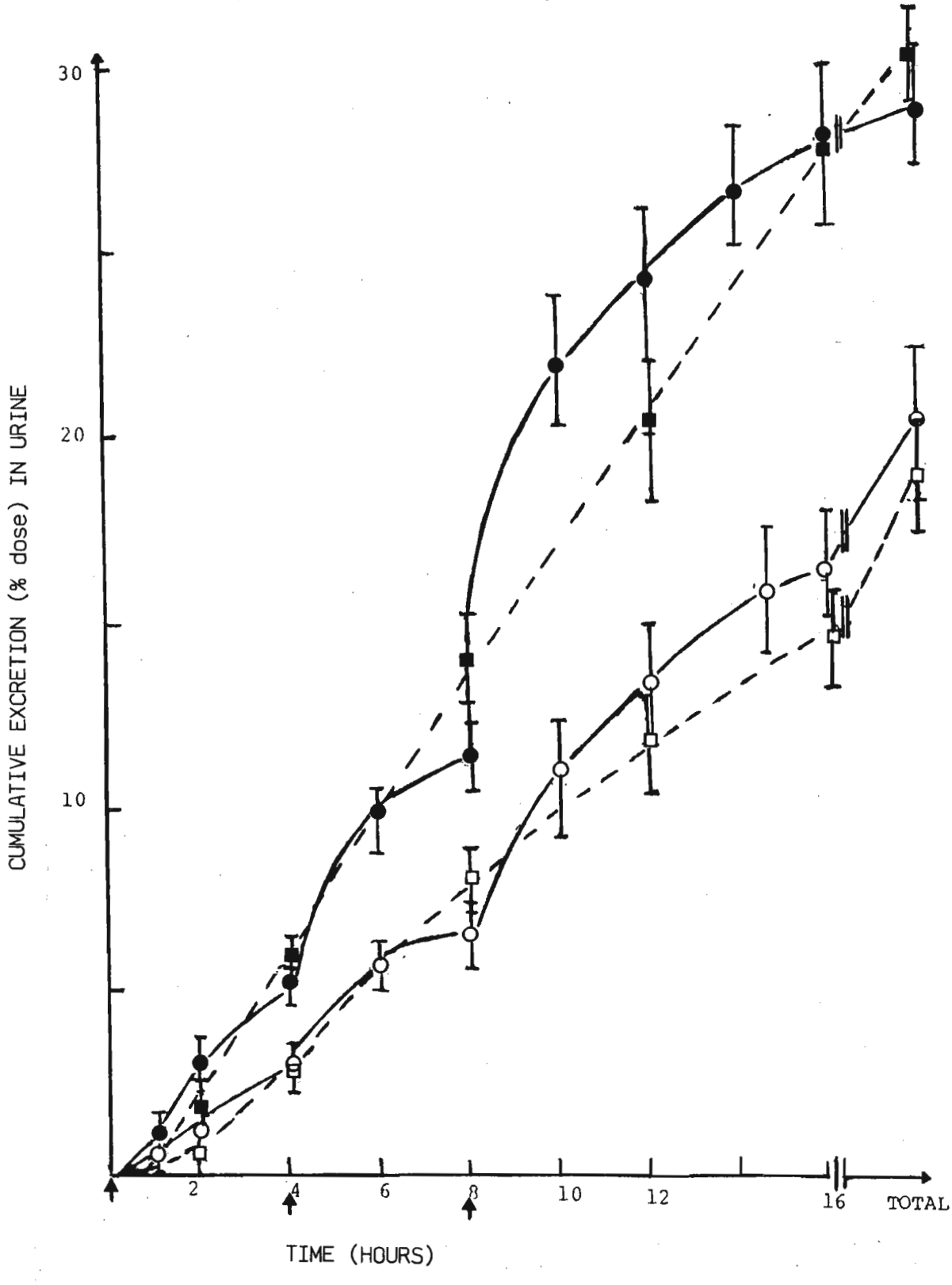


Figure 4.25: The cumulative urinary excretion (% dose)*1 of ethylaminopropiophenone (Metabolite II; ● ■) and diethylnorpseudoephedrine (Metabolite IV; ○ □) after oral administration of two different dosage forms (F.D.F. ——; S.R.P.– – –)*2 of diethylpropion (75 mg of hydrochloride salt) to subject A of Group A.

*1 Expressed as percentage of total unchanged drug administered

*2 F.D.F. Free dosage form in three divided doses i.e. 25 mg at times 0, 4 and 8 hours (↑)

*3 S.R.P. Sustained release pellets Lot R 7773 (≡75 mg) at time 0 hours

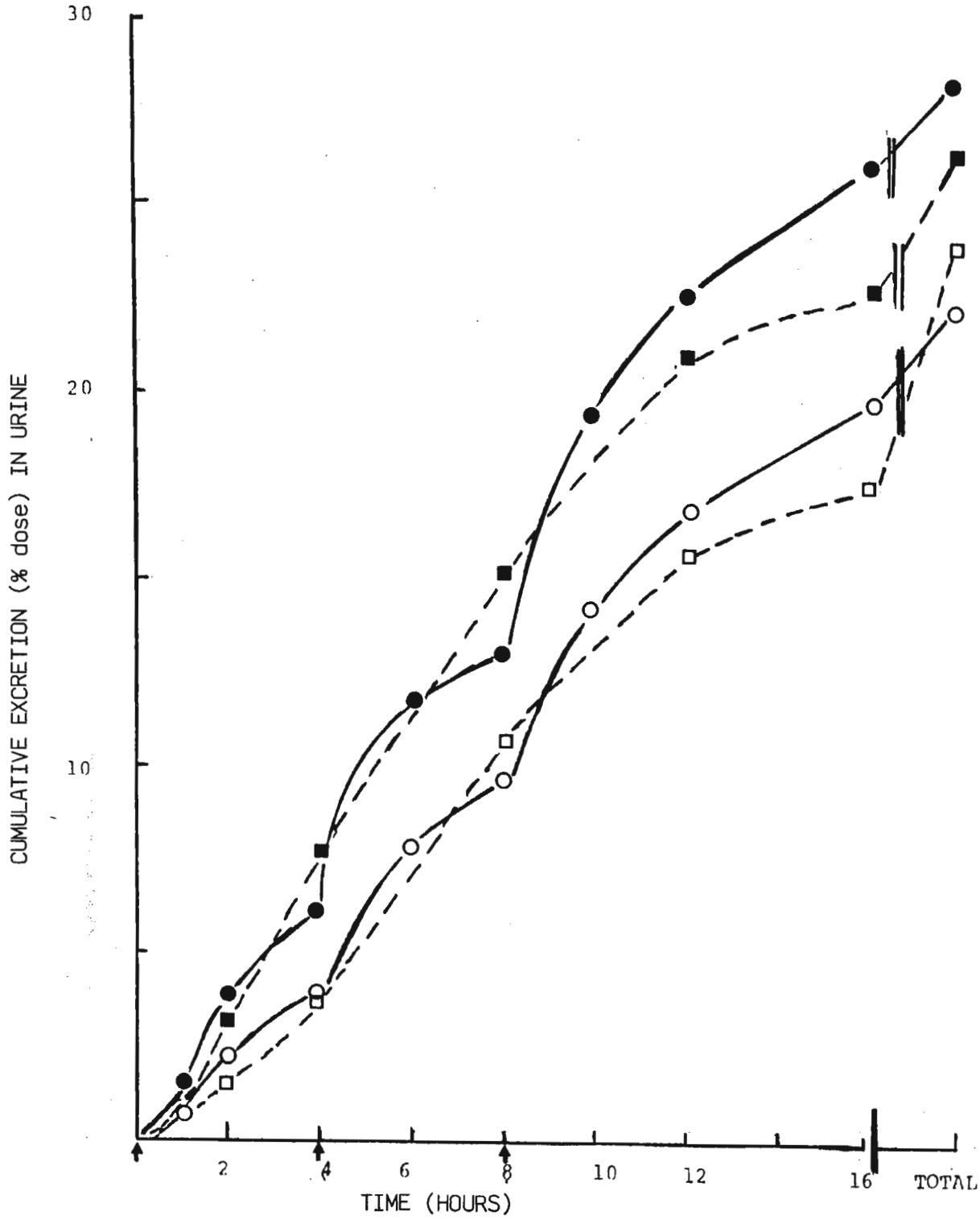


Figure 4.26: The mean (+S.E.) cumulative urinary recovery (% dose)*1 of ethylaminopropiophenone (Metabolite II;●■) and diethylnorpseudoephedrine (Metabolite IV; ○□) after oral administration of two different dosage forms (F.D.F. —— ; S.R.P. — — —)*2 of diethylpropion (75 mg hydrochloride salt) to six subjects (Group B).

*1 Expressed as percentage of total unchanged drug administered

*2 F.D.F. Free dosage form in three divided doses i.e. 25 mg at times 0, 4 and 8 hours (↑)

*3 S.R.P. Sustained release pellets Lot R 7773 (≡75 mg) at time 0 hours

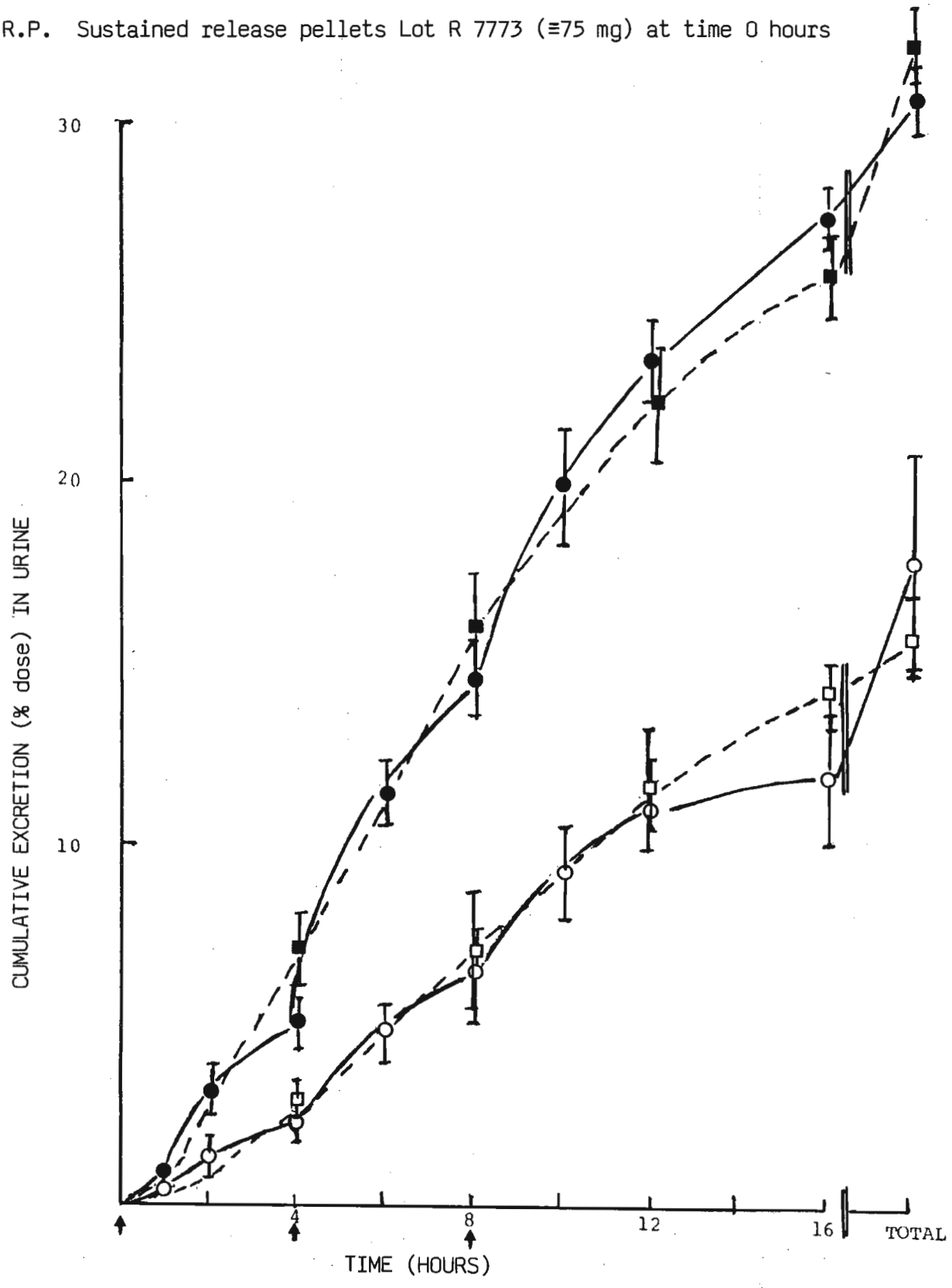


Figure 4.27: The cumulative urinary excretion (% dose)*1 of ethylaminopropiophenone (Metabolite II;●■) and diethylnorpseudoephedrine (Metabolite IV;○□) after oral administration of two different dosage forms (F.D.F.——; S.R.P.- - -)*2 of diethylpropion (75 mg hydrochloride salt) to subject C (Group A).

*1 Expressed as percentage of total drug administered
*2 F.D.F. Free dosage form in three divided doses i.e. 25 mg at times 0, 4 and 8 hours (⬆)
*3 S.R.P. Sustained release pellets Lot R 7773 (75 mg) at time 0 hours

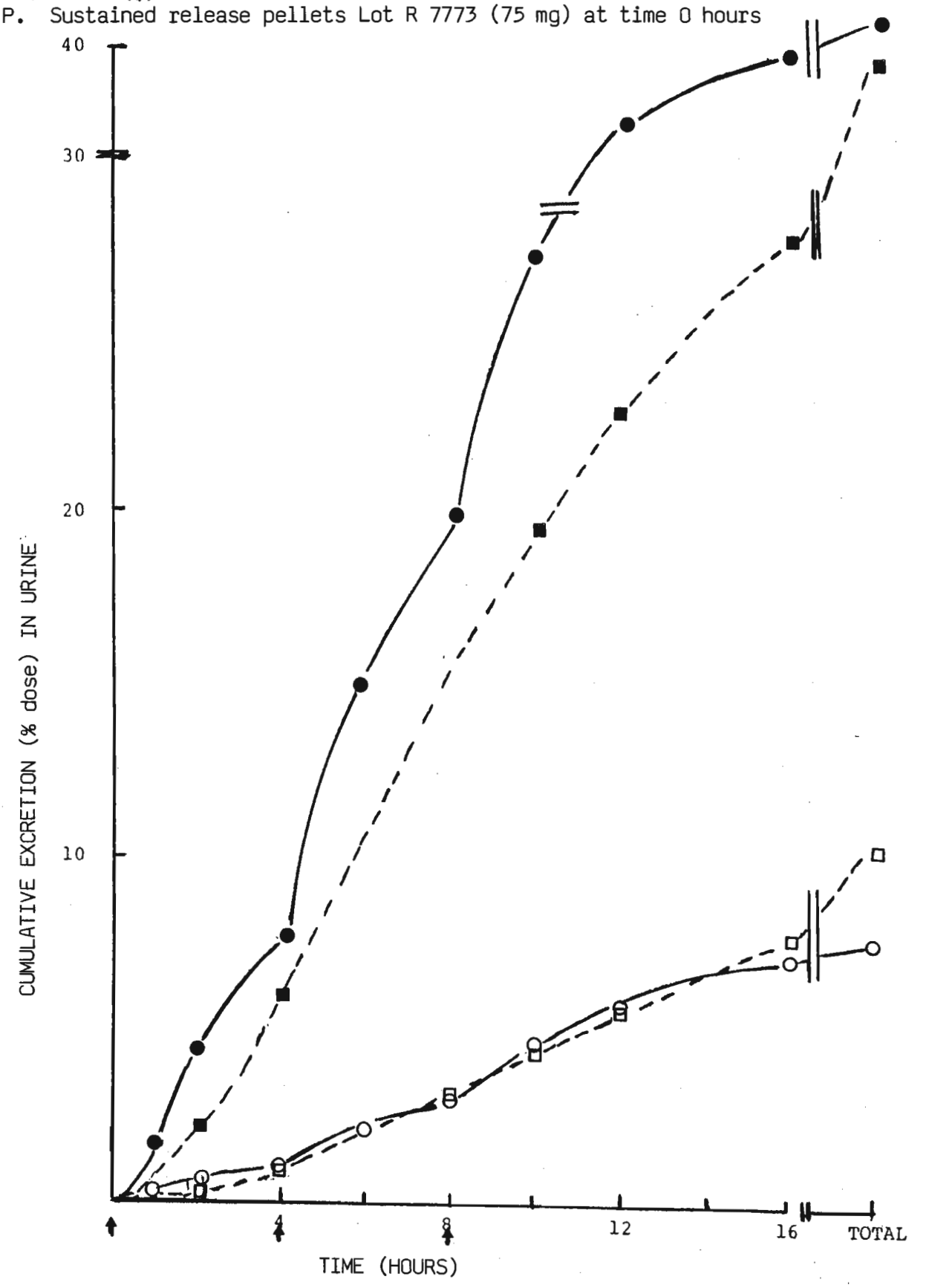


Figure 4.28: The cumulative urinary excretion (% dose)*1 of ethylaminopropiophenone (Metabolite II; ● ■) and diethylnorpseudoephedrine (Metabolite IV; ○ □) after oral administration of two different dosage forms (F.D.F. —— ; S.R.P. — —)*2 of diethylpropion (75 mg hydrochloride salt) to subject A.M. (Group B).

*1 Expressed as percentage of total drug administered

*2 F.D.F. Free dosage form in three divided doses i.e. 25 mg at times 0, 4 and 8 hours (⬆)

S.R.P. Sustained release pellets Lot R 7773 (75 mg) at time 0 hours

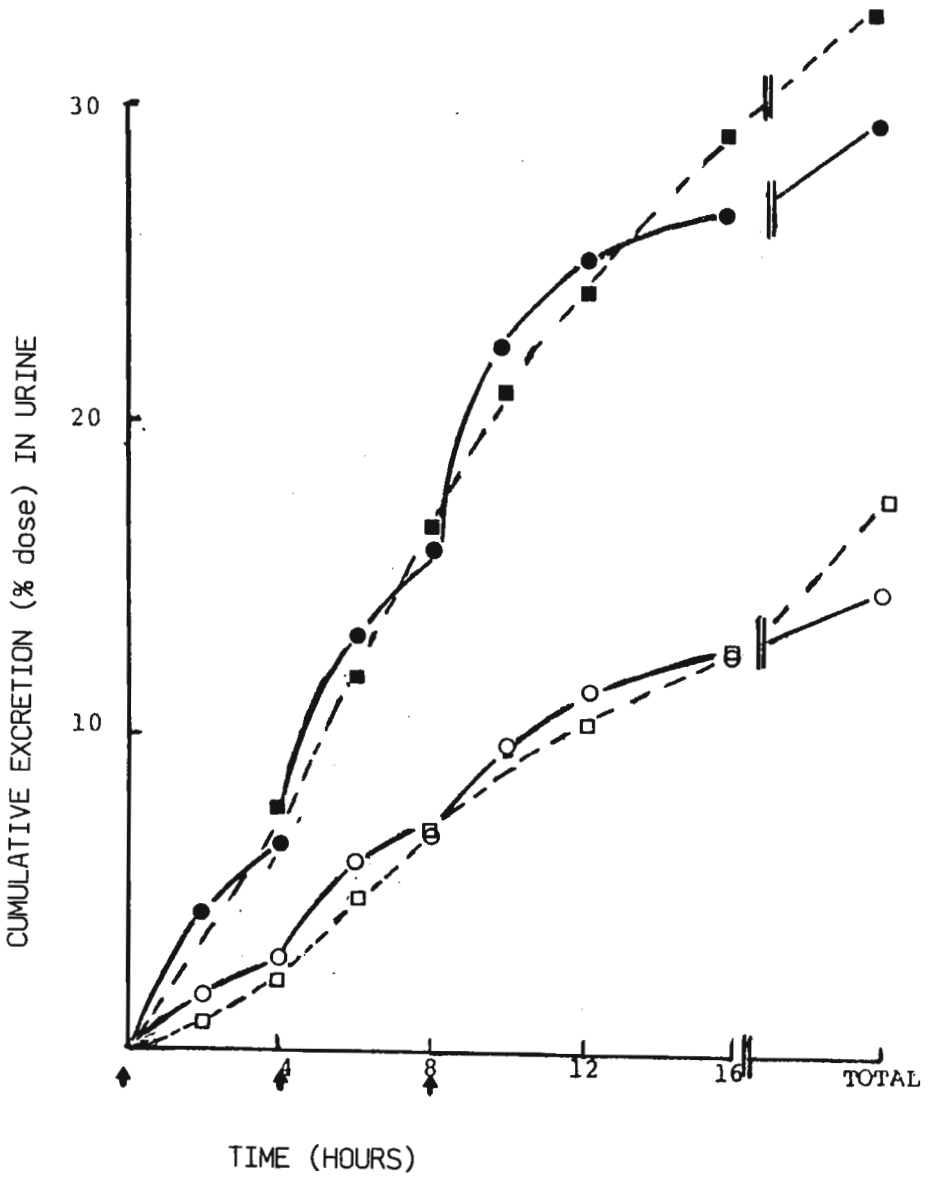


Table 4.7 Bioavailability of diethylpropion from sustained release pellets*1 relative to that of free dosage form*2 as measured by the determination of the two major metabolites of diethylpropion in biological fluids

Subjects	Compounds	Percentage of Subjects with						Mean Values Calculated For all Subjects		
		Plasma		Saliva		Urine				
		<75%	>100%	<75%	>100%	<75%	>100%	Plasma	Saliva	Urine
GROUP A (six subjects)	Met. II	0	17	0	40	0	50	87%	96%	108%
	Met IV.	17	50	0	80	0	100	94%	120%	110
	II + IV	0	0	0	80	0	83	90%	105%	107%
GROUP B (six subjects)	Met. II	-	-	40	40	0	50	-	93%	105%
	Met. IV	-	-	40	20	33	67	-	79%	101%
	II + IV	-	-	60	20	0	67	-	88%	104%

*1 Equivalent to 75 mg of hydrochloride salt administered at time 0 hours.

*2 75 mg of hydrochloride salt (powder in solution) administered in three divided doses, i.e. 25 mg at times 0, 4 and 8 hours.

Met. = Metabolite

(For details see Tables 4.3, 4.5 and 4.6 respectively)

Table 4.8: Summary of reported side effects by different subjects

Side Effect	Subject	Free Drug	Sustained Release Pellets (R 7773)
Nausea	E	1,5 hours after 3rd dose	Nil
	S.G.	1,0 hours after 2nd dose	Nil
Headache	S.G.	1,5 hours after 3rd dose	Nil
	A	At 2 hours	Nil
	C.D.	1,5 hours after 3rd dose	Nil
Irritability	A.M.	1,5 hours after 3rd dose	Nil
	D.L.	1,5 hours after 2nd dose	Nil
	S.G.	1,25 hours after 3rd dose	Nil
Insomnia	S.G.		Nil

All other subjects reported no side effects

4.1.4 Consideration of saliva and plasma concentrations of metabolites II and IV and the ratio of their concentrations

The mean concentration time profiles of ethylaminopropiophenone (metabolite II) and diethylnorpseudoephedrine (metabolite IV) seen in plasma and saliva of the six subjects in Group A (Trial 1), after oral administration of the free drug (three equal doses at 0, 4 and 8 hours), or sustained release pellets (R 7773, 75 mg drug salt) under acidic urine conditions, reflected similar patterns (Figures 4.22 and 4.23) - especially in the post-absorptive phase i.e. between 4 to 10 hours after dose of sustained release pellets had been administered.

The applications of drug/metabolite measurement in saliva (as a substitute for plasma) in therapeutic monitoring or in biopharmaceutical and pharmacokinetic studies (1.4), are also extensively reviewed by Borzelleca et al. (1970) and Danhof et al., (1978). For a number of drugs, e.g. antiepileptics and digoxin, salivary drug/metabolite concentrations seem to be satisfactory under steady state conditions to predict plasma concentrations, while for single dose studies many discrepancies have been described (Danhof, 1978). Often satisfactory results have been obtained with a constant S/P ratio during the elimination phase in certain drugs e.g. phendimetrazine (Raisi, 1979) and amphetamine (Wan, Matin and Azarnoff, 1978). In these studies the saliva concentration decay curve was a reliable reflection of the concentration decay in plasma, and hence a reliable estimation of the elimination half-life was obtained. Unfortunately, in our studies plasma samples were not collected beyond 12 to 13 hours, and the saliva data beyond 13 hours was not reliable due to extremely low levels of the compounds which precluded accurate determinations.

Therefore, to obtain meaningful information, our discussions on the relationships of S/P ratios and also U/P ratios have been confined to studies on sustained release pellets taken orally by six subjects (Group A), where a reasonable degree of steady plateau levels of each metabolite, especially between 4 to 10 hours after dose, has been obtained (Figures 4.1, 4.8 and 4.17).

The S/P ratios of ethylaminopropiophenone (metabolite II) and diethylnorpseudoephedrine (metabolite IV) at different times after oral administration of sustained release pellets to six subjects (Group A) are given in Table 4.9. The mean S/P ratio of each metabolite at each time of collection, was calculated by averaging all the ratios obtained in the six subjects.

The concentrations of metabolites II and IV in saliva were higher than in plasma in samples taken at the same time, (Figure 4.1 in relation to Figure 4.8) as was expected according to the pH-partition theory where: the degree of ionization of a weak base (pK_a = 8,4 for metabolite II and pK_a = 8,8 for metabolite IV) is higher in saliva than in plasma as a result of pH differences between the two fluids. However, during the absorptive phase (i.e. 1 to 2 hours after dose), the S/P ratios were markedly elevated in each subject (Table 4.19). At 1 hour, the S/P ratio (mean $\pm$ S.D.) in subjects (A-H) was 8,71 $\pm$ 2,18 (range 5,33 - 11,3) for metabolite II: and 7,62 $\pm$ 1,94 (range 5,09 - 10,55) for metabolite IV - such high saliva concentrations relative to plasma concentrations in the absorptive phase compared to later times have been reported for many drugs c.f. acetaminophen (Glynn and Bastain, 1973), theophylline in oral studies (Koysooko et al., 1974) and rectal studies (de Boer, Pronk and Breimer, 1977), pentobarbital (de Boer et al., 1980) and amphetamine (Wan et al., 1978). A rational explanation for this occurrence has been presented by Posti, (1979) and is based on the following hypothesis:

Table 4.9: The Saliva/Plasma (S/P) Ratios of ethylaminopropiophenone (Metabolite II) and diethylnorpseudoephedrine (Metabolite IV) at different times after oral administration of S.R.P. to six subjects (Group A) under acidic urine conditions.

SUBJECT \ HRS.	SALIVA CONC. (ng/ml)/ PLASMA CONC. (ng/ml) RATIO OF METABOLITES II AND IV AT .... HOURS AFTER DOSE																	
	1		2		3		4		6		8		10		12		MEAN ± S.D.	
	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV
A	11,3	7,30	7,61	6,21	8,42	9,05	7,38	5,59	5,75	5,46	4,05	4,16	5,35	5,39	4,2	5,11	5,63±1,18	5,15±0,58
B	a		5,82	5,13	2,59	4,20	3,27	2,8	3,93	3,17	4,05	2,79	2,86	2,38	5,2	4,15	3,34±0,57	3,07±0,62
C	b		b		b		b		b		b		b		b		b	
D	5,33	5,09	4,29	3,71	3,74	4,56	4,43	6,87	5,24	8,26	5,74	8,52	a		8,29	12,8	4,79±0,76	7,05±1,57
E	8,56	7,56	6,04	7,52	8,88	10,75	5,65	4,74	11,07	12,8	6,74	9,93	6,53	6,39	7,34	6,84	6,95±1,19	7,02±2,17
H	9,63	10,55	5,84	6,88	8,93	10,0	10,3	6,68	8,72	9,58	9,5	5,03	9,7	8,81	10,11	11,65	9,43±0,56	8,02±1,88
MEAN ± S.D. II	8,7 ± 2,18		5,95 ± 1,18		6,04 ± 2,89		6,21±2,46		5,91±1,75		6,02±2,02		6,11±2,46		6,71±2,27		6,03±2,07	
MEAN ± S.D. IV	7,62±1,94		5,89±1,35		6,25±2,65		5,33±1,48		6,62±2,49		6,08±2,70		5,74±2,31		6,94±2,89		6,06±1,76	

II = Ethylaminopropiophenone

IV = Diethylnorpseudoephedrine

a = Saliva sample not collected

b = Data omitted due to decomposition of compounds in chloroform extract which was left overnight due to the breakdown of the G.L.C.

S.R.P. = Sustained Release Pellets (R 7773), ≡75mg diethylpropion hydrochloride (DEP) at time 0 hours

a) Drug concentrations in the arterial blood during absorption (invasion phase) are higher than in peripheral venous blood (collected in forearm). This concentration difference is at any moment directly proportional to the actual invasion rate.

b) The drug concentrations in saliva are in equilibrium with concentrations in arterial blood. Consequently, relative drug concentrations in saliva during invasion are higher than after invasion (i.e. during the elimination phase). Hence, in the determination of the mean S/P ratios in each individual (Table 4.9), only the ratios between 3 hours to 10 hours after the dose were considered. The S/P ratios in each subject at 12 hours after dose were disregarded due to the extremely low levels of plasma concentrations of metabolites II and IV which might be inaccurate, thereby giving incorrect S/P ratio values. Some S/P ratios (those circled) of individual samples collected between 3 to 10 hours have been deliberately omitted viz. (a) the S/P ratios at 3 hours in subject A for metabolites II and IV, and in subject E for metabolite IV, which could perhaps have been due to a change in the pH of saliva; (b) the S/P ratios at 6 hours in subject E for metabolites II and IV which could have been accountable to a high saliva concentration, due to a very small saliva flow rate - 0,4 ml/min (mean $\pm$ S.D. is 0,75, $\pm$ 0,13 ml/min).

The overall mean $\pm$ S.D. S/P ratios of the saliva and plasma concentrations in samples collected between 3 to 10 hours in the six subjects are: 6,03 $\pm$ 2,07 (range 3,31 to 9,61) for metabolite II and 6,06 $\pm$ 1,76 (range 3,07 to 9,02) for metabolite IV (Table 4.9).

The differences in the S/P ratios between subjects (inter-subject variation) and also the scatter of S/P ratios at different collection times within each subject could be due to fluctuations in the salivary excretion related to changes in salivary pH. The saliva flow was fairly constant in all subjects (mean ± S.D. 1,07 ml/min ± 0,33 - range 0,57 to 1,57 ml/min). Unfortunately we were unable to determine the pH of saliva samples (at time of collection) due to the distant location of study (3.3.2), but the pH value of mixed saliva is known to range between 6,5 to 7,1 (Danhof et al., 1978). Although the urine pH was well-controlled by the ingestion of ammonium chloride sustained release pellets, there is no significant relationship between pH of saliva and the pH of urine (Lampman et al., 1975). The S/P ratios for metabolites II and IV in subject H compared to those in the others (subjects A to E) were abnormally high throughout the study. It is suspected that this could be due to altered or further metabolism of the drug in the salivary glands in subject H, which is very unusual but possible.

The mean ± S.D. S/P ratios for each metabolite at each collection time between 3 to 10 hours after dose are relatively constant; the overall mean ± S.D. for metabolite II is 6,06 ± 0,10 (range 5,91 - 6,21) and for metabolite IV it is 6,00 ± 0,44 (range 5,74 - 6,62). These results (mean ± S.D.) are in close agreement with the predicted S/P ratios (Table 4.10) at a salivary pH of 6,60 to 6,63. As anticipated according to the lipid solubilities of the compounds, the S/P ratios for metabolite II, are on the average generally higher than those of metabolite IV.

A method to predict the S/P ratio

Assuming the pH of plasma is 7,4 and pH of saliva is between 6,6 and 7,1, the amount of ionized and unionized ethylaminopropiophenone, (metabolite II, pK_a = 8,40) and diethylnorpseudoephedrine (metabolite IV, pK_a = 8,80) in plasma and saliva can be calculated using the equation:

$$\% \text{ ionized (weak bases)} = \frac{100}{1 + \text{antilog } (pH - pK_a)}$$

Then the saliva/plasma concentration ratio can be obtained by: dividing the amount of ionized plus unionized compound present in saliva, by the corresponding value in plasma (Table 4.10).

When the pH value of saliva is between 6,5 - 7,0 the calculated ratios are close to most of those obtained experimentally, but more variation of salivary pH (e.g. 6,3) gave values with greater differences than those obtained experimentally (Table 4.9). Provided that the excretion of the compound into the saliva is only dependent upon salivary pH and there is no binding of the compound in plasma or saliva and also no active secretion into the saliva, the above method can be valuable to predict S/P ratios for the compound.

Table 4.10: Calculation of saliva/plasma concentration ratio (S/P) of ethylaminopropiophenone (Metabolite II) and diethylnorpseudoephedrine (Metabolite IV) at different pH values of saliva using the pKa* values of the compounds

		%I	%U	$^{I}/_{U}$	$I+^{I}/_{U}$	S/P
PLASMA	II	90,9	9,09	10,00	11,0	
pH = 7,4	IV	96,17	3,83	25,1	26,1	
SALIVA	II	96,17	3,83	25,1	26,1	2,4
pH = 7,0	IV	98,43	1,57	62,7	63,7	2,45
SALIVA	II	96,9	3,1	31,3	32,3	2,94
pH = 6,9	IV	98,76	1,24	79,64	80,64	3,09
SALIVA	II	97,6	2,4	40,7	41,7	3,79
pH = 6,8	IV	99,0	1,0	99,0	100	3,83
SALIVA	II	98,0	2,0	49,0	50	4,55
pH= 6,7	IV	99,21	0,79	125,58	126,58	4,84
SALIVA	II	98,25	1,75	56,14	57,14	5,20
pH = 6,65	IV	99,30	0,70	139,85	140,85	5,40
SALIVA	II	98,33	1,67	58,88	59,88	5,44
pH = 6,63	IV	99,33	0,67	147,81	148,81	5,70
SALIVA	II	98,43	1,57	62,70	63,70	5,79
pH = 6,60	IV	99,37	0,63	158,7	159,7	6,12
SALIVA	II	98,61	1,39	71,30	72,30	6,57
pH = 6,55	IV	99,44	0,56	177,57	178,57	6,84
SALIVA	II	98,76	1,24	79,64	80,64	7,33
pH = 6,5	IV	99,50	0,50	199,0	200,0	7,66
SALIVA	II	99,21	0,79	125,6	126,6	11,42
pH = 6,3	IV	99,68	0,32	311,5	312,5	11,97

I = Ionized

U = Unionized

S/P = Saliva/Plasma ratio

*pK_a of IV = taken as 8,8 (equal to diethylpropion)

II = 8,40 (Vree et al., 1972)

4.1.5 Relationship of plasma concentration and urinary excretion rate

The concentrations of many drugs in plasma are frequently so low that it is difficult to preclude accurate determinations: urinary excretion data are thus often used to study the fate of drugs and to compare biopharmaceutical data and bioavailabilities from different dosage forms. The basis for utilization of urinary excretion studies is that the rate of excretion of the drug is proportional to plasma concentration. Only when renal tubular reabsorption is minimized (made negligible) by using acidic urine conditions with diuresis, do good drug relationships exist between the urinary excretion rate and the plasma levels for some basic drugs eg. amphetamine (Beckett et al., 1969), orphenadrine (Khan, 1972) and phendimetrazine (Raisi, 1978). In the present study, we have attempted to determine the relationship between plasma concentrations (ng/ml) at the mean time of collection and urinary excretion rates (µg/min) at these times for the two metabolites ethylaminopropiophenone (II) and diethylnor-pseudoephedrine (IV) in six subjects (A-H), after oral administration of sustained release pellets Lot R 7773 (Trial 1) under acidic urine conditions. The results (U/P ratios) in this study have been outlined in Table 4.11.

In the evaluation and assessment of the U/P ratio in all six subjects, only the urinary excretion rates and plasma concentration data between 2 to 10 hours after dose were considered. The reason is that the 1st hour samples did not give a true average urine excretion rate due to a lag time associated with absorption, distribution, metabolism and finally the excretion of the metabolites; in the 12th-hour samples the plasma concentrations were too low to preclude accurate determinations.

Table 4.11: The Urine/Plasma Ratio (U/P) of ethylaminopropiophenone (Metabolite II) and diethylnorpseudoephedrine (Metabolite IV) at different times after oral administration of S.R.P.* to six subjects

HRS. / SUBJECT	URINE EXCRETION RATE (µg/min) / PLASMA CONCENTRATION (ng/ml) RATIO. (U/P) OF METABOLITES II AND IV AT .... HOURS AFTER DOSE																MEAN ±S.D.	
	1		2		3		4		6		8		10		12			
	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV
A	0,64	0,55	0,64	0,60	0,59	0,60	0,59	0,50	0,61	0,60	0,48	0,51	0,69	0,64	0,71	0,71	0,60±0,06	0,58±0,05
B	0,81	0,76	0,57	0,58	0,53	0,70	0,57	0,57	0,71	0,54	0,77	0,46	0,74	0,66	0,58	0,45	0,64±0,09	0,59±0,08
C	0,29	0,16	0,47	0,25	0,36	0,28	0,48	0,48	0,67	0,59	0,56	0,66	0,55	0,59	0,53	0,45	0,52±0,10	0,48±0,16
D	0,83	0,73	0,62	0,66	0,68	0,45	0,49	0,63	0,39	0,46	0,54	0,85	0,54	0,87	1,27	1,16	0,54±0,09	0,55±0,10
E	0,28	0,43	0,25	0,52	0,42	0,47	0,59	0,54	0,38	0,43	0,48	0,57	0,79	0,6	0,58	0,64	0,53±0,15	0,52±0,06
H	0,58	0,62	0,41	0,59	0,41	0,51	0,54	0,56	0,60	0,53	0,51	0,47	0,45	0,45	0,56	0,62	0,49±0,07	0,51±0,05
MEAN ±S.D. II	0,57±0,22		0,54±0,09		0,50±0,11		0,54±0,05		0,56±0,13		0,56±0,10		0,59±0,11		0,59±0,06		0,55 ± 0,03	
MEAN ±S.D. IV	0,62±0,12		0,53±0,13		0,50±0,13		0,55±0,05		0,53±0,01		0,53±0,07		0,59±0,07		0,57±0,11		0,53 ±0,04	

II = Ethylaminopropiophenone

IV = Diethylnorpseudoephedrine

S.R.P. = Sustained Release Pellets (R 7773), ≡75mg diethylpropion hydrochloride (DEP) at time 0 hours

The large urine flow rates (8,8 ml/min and 5 ml/min) at 8 hours and 10 hours after dose in subject D, gave very high excretion rates for the more polar metabolite (diethylnorpseudoephedrine, IV); thus the respective U/P ratios for this subject were not included in the calculation of the mean values.

The mean U/P ratios of each metabolite (II and IV) in each individual subject (i.e. across Table 4.11 between 2 and 10 hours after dose) or at each time of sample collection (down Table 4.11 - at 2, 3, 4, 6, 8 and 10 hours after dose) were calculated by averaging all the ratios, except those specifically excluded for reasons given earlier. The overall mean $\pm$ S.D. U/P ratio of the six subjects was 0,55 $\pm$ 0,03 (range 0,49 to 0,64) for metabolite II, while for metabolite IV it was 0,53 $\pm$ 0,04 (range 0,48 to 0,59); these results demonstrate minimal inter-subject variation. Such results are possible only because of good control of urinary pH and these results differ from the corresponding S/P ratios where fluctuations in salivary pH (which is difficult to control) promote large variations (Table 4.9).

There was an excellent relationship between mean urinary excretion rate (µg/min) at various mean times of urinary collection and mean plasma concentrations (ng/ml) at these times for both metabolites in the period between 2 to 10 hours after the dose (r^* = 0,87 for II and r = 0,85 for IV); the mean $\pm$ S.D. ratios were 0,54 $\pm$ 0,03 (range 0,50 to 0,59) for metabolite II and 0,53 $\pm$ 0,03 (range 0,50 to 0,59) for metabolite IV. Therefore under acidic urine conditions, it would be possible to predict plasma levels of these compounds from urinary excretion data (in the post-absorptive phase) even after single dose studies.

r^* = correlation coefficient

4.2 Other Studies

In addition to the major study involving the two-way crossover trial (Trial 1), the results on several small studies, on separate occasions and on two subjects, where diethylpropion hydrochloride was administered either orally or rectally in different dosage forms under fasting and non-fasting conditions (Table 3.1), are presented in this section.

4.2.1 Rectal administration

The urinary excretion rate profiles of diethylpropion and its metabolites, ethylaminopropiophenone (II) and diethylnorpseudoephedrine after oral and rectal administration of 25 mg diethylpropion hydrochloride (Trials 5 and 3 respectively) in the same subject are shown in Figure 4.29. It is clear that the urinary excretion rates of all three compounds did not differ in any significant manner following oral and rectal administration of the drug. The peak urinary excretion rates for metabolites II and IV were 28,46 µg/ml and 5,41 µg/ml after rectal and 26,61 µg/ml and 9,52 µg/ml after oral administration, and occurred at 90-150 minutes and 45-90 minutes after administration in each case (Figure 4.29). The cumulative urinary recoveries of the compounds I, II and IV after rectal administration were comparable to those after oral administration (Table 4.12).

In another study (Trial 2, Table 3.1), the urinary excretion rates and salivary concentrations as well as the total drug recovered (as metabolites I, II and IV) after rectal administration of 75 mg diethylpropion hydrochloride as a special suppository (2.A.2.3 (iii)), were determined. These results did not differ significantly from the results obtained when the pellets were used orally in the same subject (Figure 4.30 and Table 4.12).

However the salivary concentration profiles, supported by urinary excretion data, (Figures 4.31 and 4.30) of all three compounds showed that when the pellets were administered rectally, there was a delay in attaining the plateau levels which were slightly lower, but sustained longer (12 to 14 hours) than those obtained when the pellets were used orally. Although about 50% more of the unchanged drug was recovered after the rectal than after oral administration, the results do not seem to be conclusive regarding reduction of first-pass metabolism via the rectal route. However, there is a reduction in the urinary recovery of metabolite IV after rectal administration of the solution or the suppository, as compared to the recovery after oral administration (Table 4.12). There is some evidence, in the case of lignocaine that rectal administration can lead to avoidance of first-pass metabolism in the rat (de Boer, Breimer and Pronk, 1980). The systemic availability of a slow-release rectal preparation of lignocaine was also studied (Beckett et al., 1978b); the results indicated that first-pass metabolism was avoided to some extent (Beckett, 1981).

After rectal administration of diethylpropion, either as free drug in solution, or as a suppository which contains sustained release pellets, the extent of metabolism was similar to that of a dose given orally. This possibly suggests that the pellets must have moved upwards in the rectum into a region from where the veins drain predominantly into the portal vein and there released most of their drug content. Furthermore, the pellets must have been in a correct environment for complete drug release (Banker and Rhodes, 1979; Noormohammadi, 1981).

Table 4.12: Cumulative urinary recoveries of diethylpropion and its two major metabolites, after administration of single doses of two different dosage forms (Free drug, and S.R.P. R 7773) of its hydrochloride salt to the same subject, C.D. under acidic urine conditions

TRIAL NO.*1	5	3	1	2
Route / Metabolite	Oral 25 mg DEP in a capsule	Rectal 25 mg DEP in 1 ml water	Oral S.R.P. 75 mg DEP in a capsule	Rectal S.R.P. as a special suppository *2 = 75 mg DEP
I	2,98	2,27	1,00	1,51
II	35,04	34,49	30,58	29,56
IV	17,10	16,5	15,79	13,48
Total I+II+IV	55,12	53,26	47,37	44,55
Duration of collection (Hours)	26	24	24	24

Figures indicate the amount excreted, expressed as the percentage of the unchanged drug dose.

I Diethylpropion

II Ethylaminopropiophenone

IV Diethylnorpseudoephedrine

DEP Diethylpropion hydrochloride

S.R.P. = Sustained release pellets Lot R 7773

*1 = refer to Table 3.1

*2 = the preparation of suppositories is outlined in Section 2.A.2.3(iii).

Figure 4.29: Urinary excretion rates of diethylpropion (△ ▲) and its two major metabolites i.e. ethylaminopropiophenone (○ ●) and diethylnorpseudoephedrine (□ ■) after oral and rectal administration of its hydrochloride salt (25 mg) to subject C.D. (male, 35 years) under acidic urine conditions.

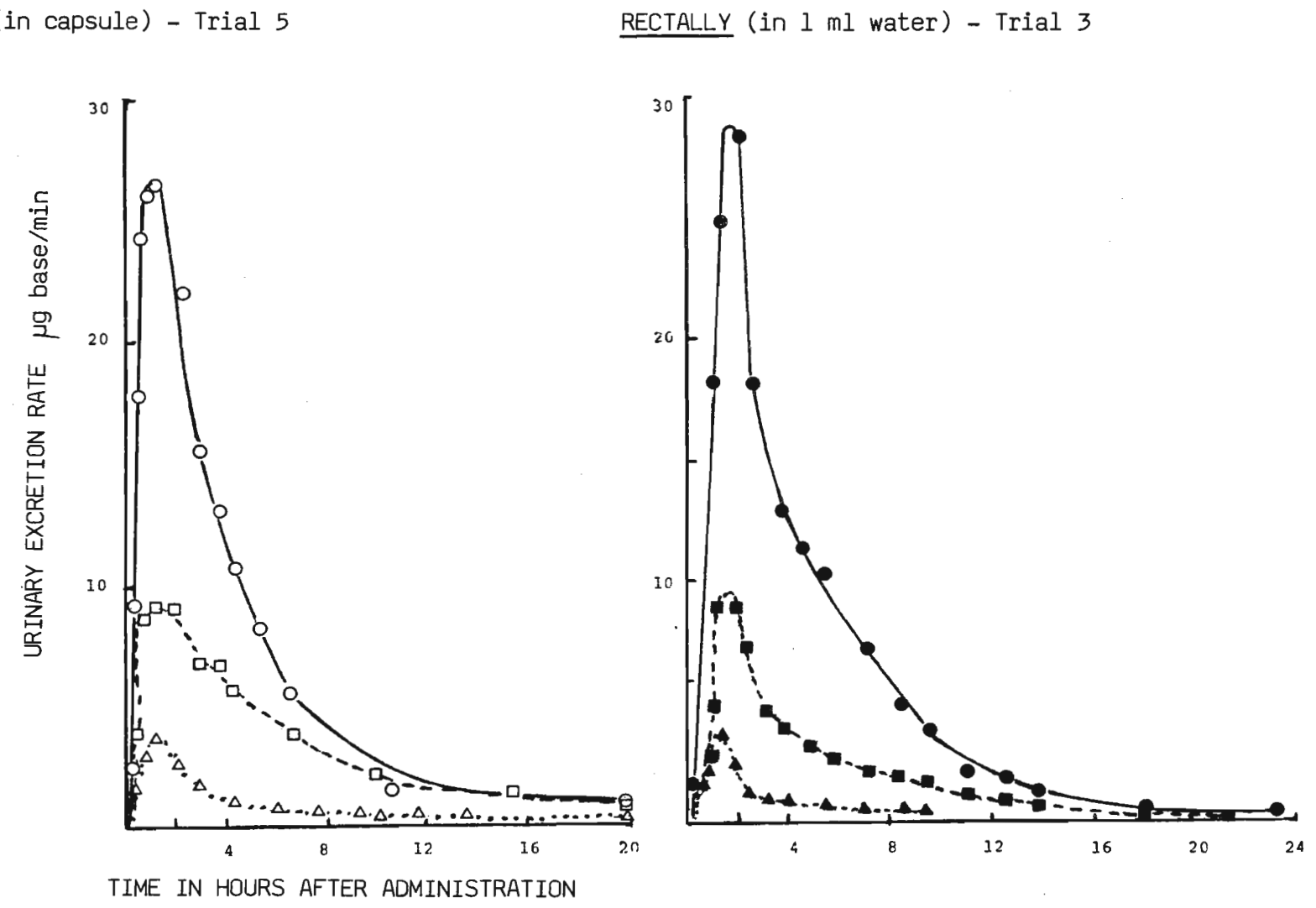


Figure 4.30: Urinary excretion rates of diethylpropion (I, △) and its major metabolites i.e. ethylaminopropiophenone (II, ○) and diethylnorpseudoephedrine (IV;□) after oral and rectal administration of sustained release pellets, R 7773 (≡ 75 mg diethylpropion hydrochloride) to subject C.D. (male, 35 years) under acidic urine conditions.

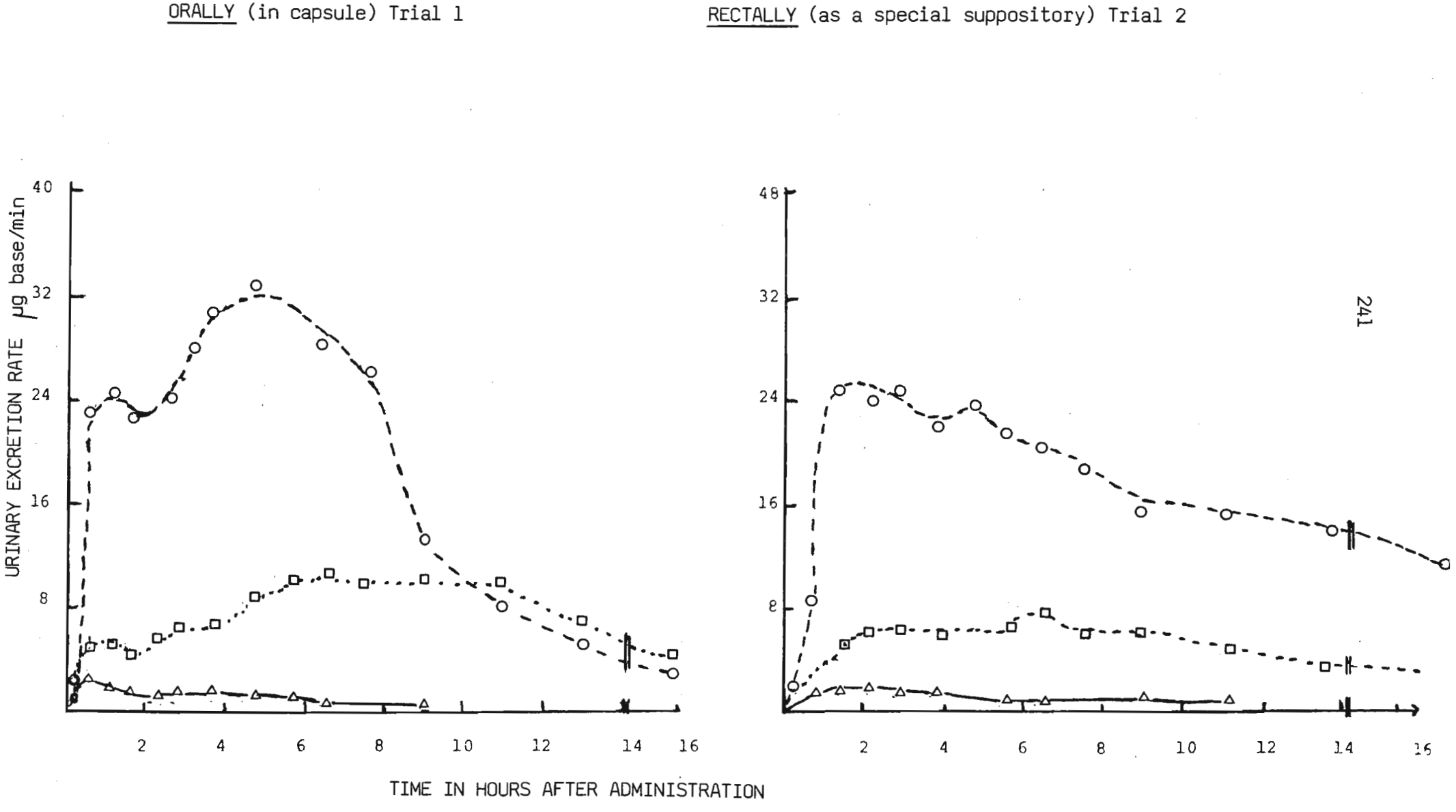
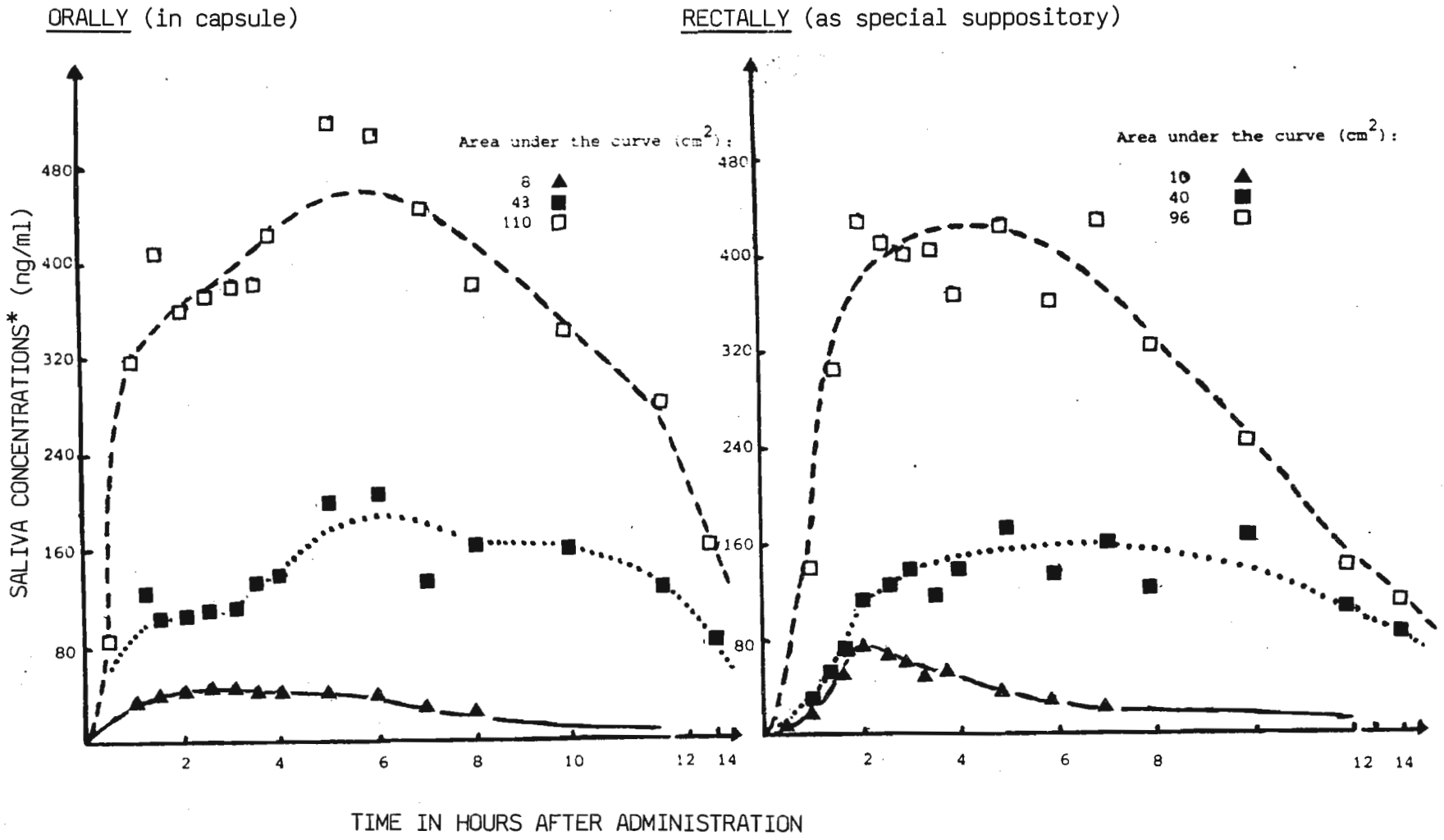


Figure 4.31: Saliva concentration profiles of diethylpropion (I, ▲) and its major metabolites i.e. ethylaminopropiophenone (II, □) and diethylnorpseudoephedrine (IV;■) after oral and rectal administration of sustained release pellets, R 7773 (≡ 75 mg diethylpropion hydrochloride) to subject C.D. (male, 35 years) under acidic (pH = 4,8 ± 0,2) urinary conditions.

* Calculated for a constant flow rate; i.e. 1 ml saliva/minute



4.2.2 Effect of food

The urinary excretion rate profiles of metabolites II and IV after oral administration of diethylpropion hydrochloride (≡ 75 mg) sustained release pellets Lot 018010 to the same subject under fasting and non-fasting conditions (Trials 7, 8, 9 and 10 - Table 3.1) are given and compared in Table 4.13 and Figure 4.32. In both studies, acidic urine conditions were maintained in order to make meaningful comparisons. The physical presence of food (details on meal content are given in Section 3.3.3) delayed the onset of plateau levels marginally from 2,42 hours (mean of 3 separate trials) to 3,75 hours after the dose; the plateau levels and their durations for both metabolites II and IV in the two studies were comparable. Therefore, from a practical viewpoint, the slight shift in the peak would not alter the efficacy of the sustained release pellets formulations, if the pellets were taken after a meal because the dose would still provide appetite suppressant activity for the rest of the day. The urinary recoveries of the two metabolites (separately and in total) in Trials 7 to 9 showed minimal intra-subject variations and compared to Trial 10, they were nearly the same; thus minimal influence of food from the pellets on the total bioavailability of diethylpropion is indicated - Table 4.13 and Figures 4.32 and 4.33. Therefore it would be reasonable to infer (although much more work is necessary to prove this point) that the release of the drug from the pellets which dispersed widely in the GIT and were able to leave the stomach almost independently of food content, was not influenced by the presence of food. The study of food-drug and fluid-drug interactions and how these may influence the bioavailability of drugs has been extensively examined and reviewed by Welling et al., (1977), D'Arcy and Merkus, (1980) and Welling, (1980).

Drug formulations (e.g. enteric-coated tablets), may also affect drug-food interaction and therefore the availability of the drug, and are thus principally designed to release drugs in the intestine, so that delayed stomach emptying may considerably delay the absorption of the drug. More disperse systems like suspensions and solutions (Welling, 1980) or sustained release pellets would be far less vulnerable to the effect of food because of their diffuse nature, greater mobility within the GIT and minimal dissolution problems. In a separate study, the effect of the presence of food on the bioavailability from a single unit sustained release tablet* (Tenuate Dospan - Merrell Lot 284BB) was therefore determined in subject C.D. (Trials 11 and 12; Table 3.1) under conditions identical to those described with the use of sustained release pellets Lot 018010 (Trials 7 to 9; Table 3.1). The urinary excretion rate profiles, and the cumulative urinary recoveries of the two metabolites II and IV (Figures 4.34 and 4.35) showed that food taken 10 minutes before the dose not only reduced the bioavailability to 80% (relative to fasting state - Table 4.14), but onset (time to reach peak) of plateau levels for each metabolite was delayed from 4 to 6 hours (Figure 4.34). The peak levels for metabolite II did not change significantly in the presence of food. It was interesting that with the use of sustained release tablets Lot 284BB but not with the use of the sustained release pellets Lot 018010 the subject reported a severe headache, which was more pronounced when the dose had been taken after breakfast, at a time period corresponding to more or less the peak plasma level of metabolite II.

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* Tenuate Dospan - erosion type, single unit, sustained release tablet - available commercially in South Africa - equivalent to 75 mg diethylpropion hydrochloride.

Clearly the results on Trials 8 to 13 demonstrate the advantage of using sustained release pellets rather than a single unit tablet in relation to the influence of food. As single unit preparations tend to follow food, which has a normal transit time through the small intestine of between 3 and 8 hours (Prescott, 1974), drug availability from such formulations would be dependent on the location of the tablet in the GIT. Therefore drug release from single unit preparations would be influenced more by variables like gastric emptying than release from sustained release pellets, which disperse and distribute freely throughout the GIT and are thus less affected by transit time of food and give more uniform drug release profiles (Ekenved et al., 1977). In contrast to the high reproducibility of transit times of pellets throughout the small intestine observed both within and between subjects (Bechgaard and Ladefoged, 1978), transit times of single unit tablets show great variations between and within subjects (Bechgaard and Ladefoged, 1981).

Figure 4.32: Effect of food on the urinary excretion rate of the two major metabolites of diethylpropion after oral administration to the same subject, C.D., of 75 mg of the hydrochloride in sustained release pellets formulation (Lot 018010)

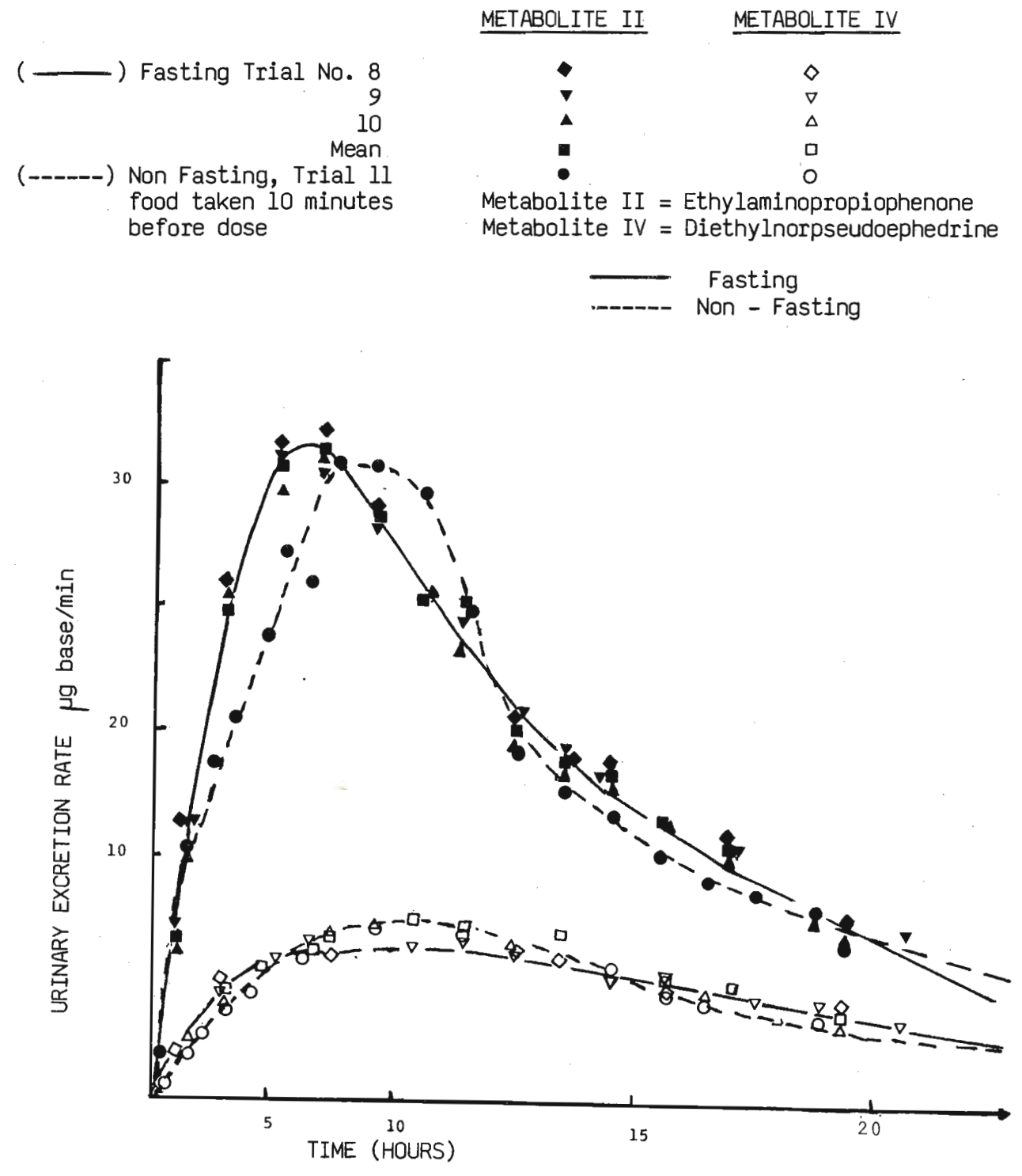


Figure 4.33: Effect of food on the cumulative urinary recovery*1 of the two major metabolites of diethylpropion after oral administration to the same subject, C.D. of 75 mg of the hydrochloride in sustained release pellets formulation (Lot 018010)

		METABOLITE II	METABOLITE IV
Fasting: Mean		—— ■	□
Trial No.	7	◆	◇
	8	▼	▽
	9	▲	△
Non fasting: Trial No.	10	---- ●	○

Metabolite II = Ethylaminopropiophenone
Metabolite IV = Diethylnorpseudoephedrine

*1 Expressed as percentage of dose administered

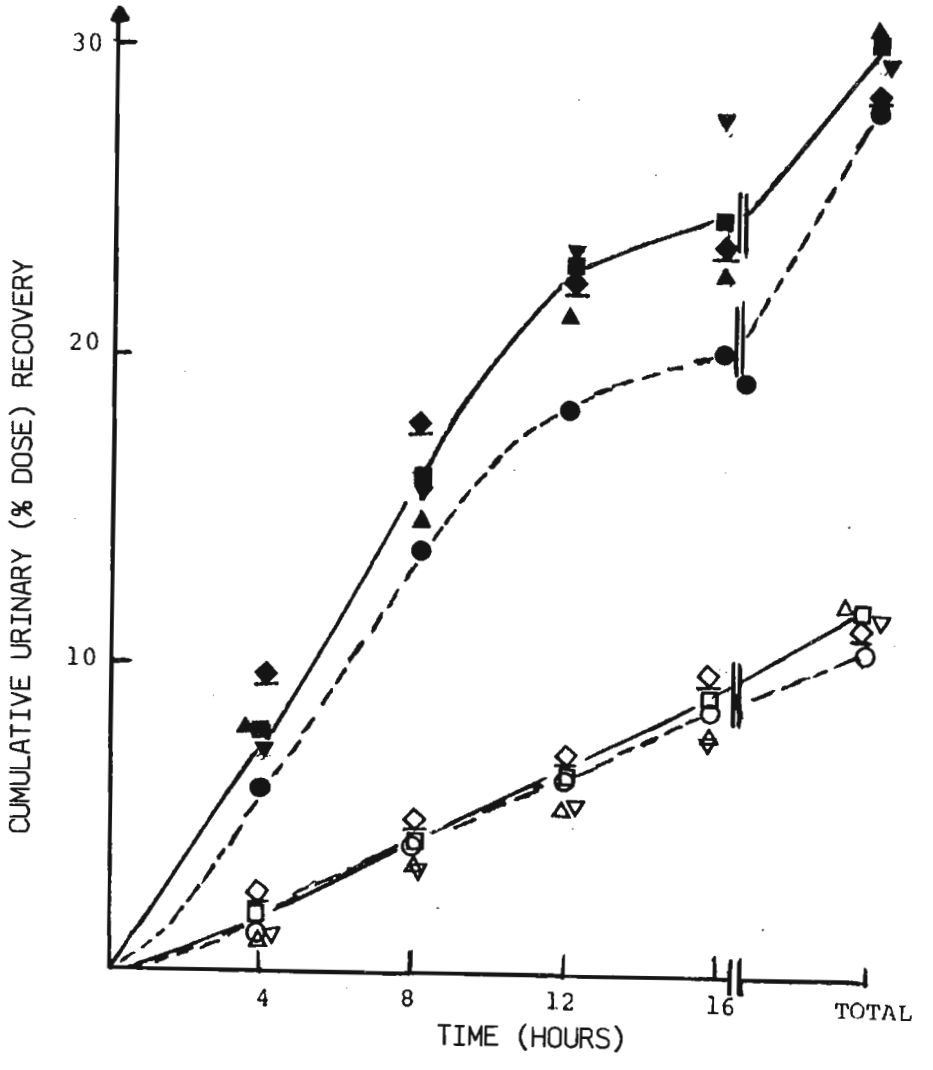


Table 4.13: Effect of food on the metabolism and bioavailability*1 of diethylpropion after oral administration to the same subject, C.D. of 75 mg of the hydrochloride in sustained release pellets formulation (Lot 018010)

	Fasting				Non Fasting*2
Trial No. *3	7	8	9	Mean	10
II Ethylaminopropiophenone	29,20	30,60	29,64	29,8	27,07
IV Diethylnorpseudoephedrine	11,60	12,49	11,97	12,0	10,40
Total Recovery II + IV	40,80	43,09	41,61	41,8	37,47
Plateau Levels					
Range µg/min					
II	25-26	26-28	23-25	23-28	23-25
IV	5,7-6,3	5,9-7,0	4,9-5,8	4,9-7,0	6,0-7,0
Onset (Hours)					
II	2,75	2,25	2,25	2,42	3,75
IV	2,72	2,25	2,25	2,40	3,75
Duration (Hours)					
II	2,75	3,25	3,25	3,08	2,75
IV	5,25	4,75	5,25	5,08	5,00

*1 Total urinary recovery in 36 hours, of the sum of the two metabolites II and IV, at time, t_{∞} , expressed as percentage of unchanged drug.

*2 Dose taken at 10 minutes after breakfast (2 eggs, toast, butter and coffee)

*3 Details have been outlined in Table 3.1

Figure 4.34: Effect of food*1 on the urinary excretion rate of the two major metabolites*2 of diethylpropion after oral administration to the same subject C.D. of 75 mg of the hydrochloride in sustained release tablets (Merrell Lot 284 BB)
Urine: Acidic

*1 Trial 11. Fasting ———
Trial 12. Non Fasting — — — . (2 eggs, 2 slices bread, butter, tea - taken at breakfast, 15 minutes before dose. Supper was taken at 12 hours after dose.)

*2 Metabolite II = Ethylaminopropiophenone
Metabolite IV = Diethylnorpseudoephedrine

• • • • • Single dose data (Trial 5, Table 3.1) multiplied by X3 for comparison

METABOLITE	TRIAL 5	11 (FASTING)	12 (NON FASTING)
■ II ▲ ●	35,0	29,45	25,50
□ IV △ ○	17,0	10,78	6,75
TOTAL RECOVERY*3	52,0	40,23	32,25

*3 Expressed as percentage of unchanged dose in urine over 36 hours

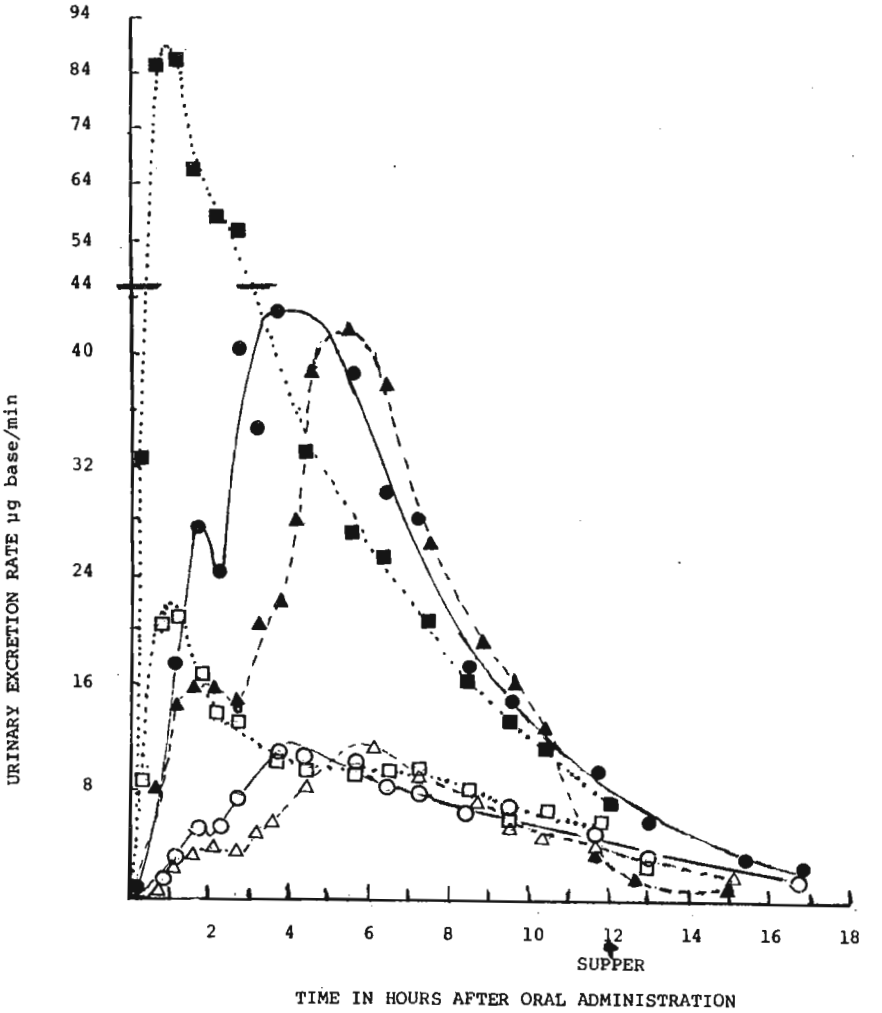


Figure 4.35: Effect of food on the cumulative urinary recovery*1 of the two major metabolites i.e. ethylaminopropiophenone (Metabolite II;●○) and diethylnorpseudoephedrine (Metabolite IV; ■ □) after oral administration of diethylpropion hydrochloride (≡ 75 mg S.R. tablets - Tenuate Dospan - Merrell Lot 284 BB) to subject C.D. under acidic urine conditions

		Metabolite II	Metabolite IV
Fasting	———	●	■
Non-Fasting	- - - - -	○	□

*1 Expressed as percentage of dose excreted in urine over 48 hours

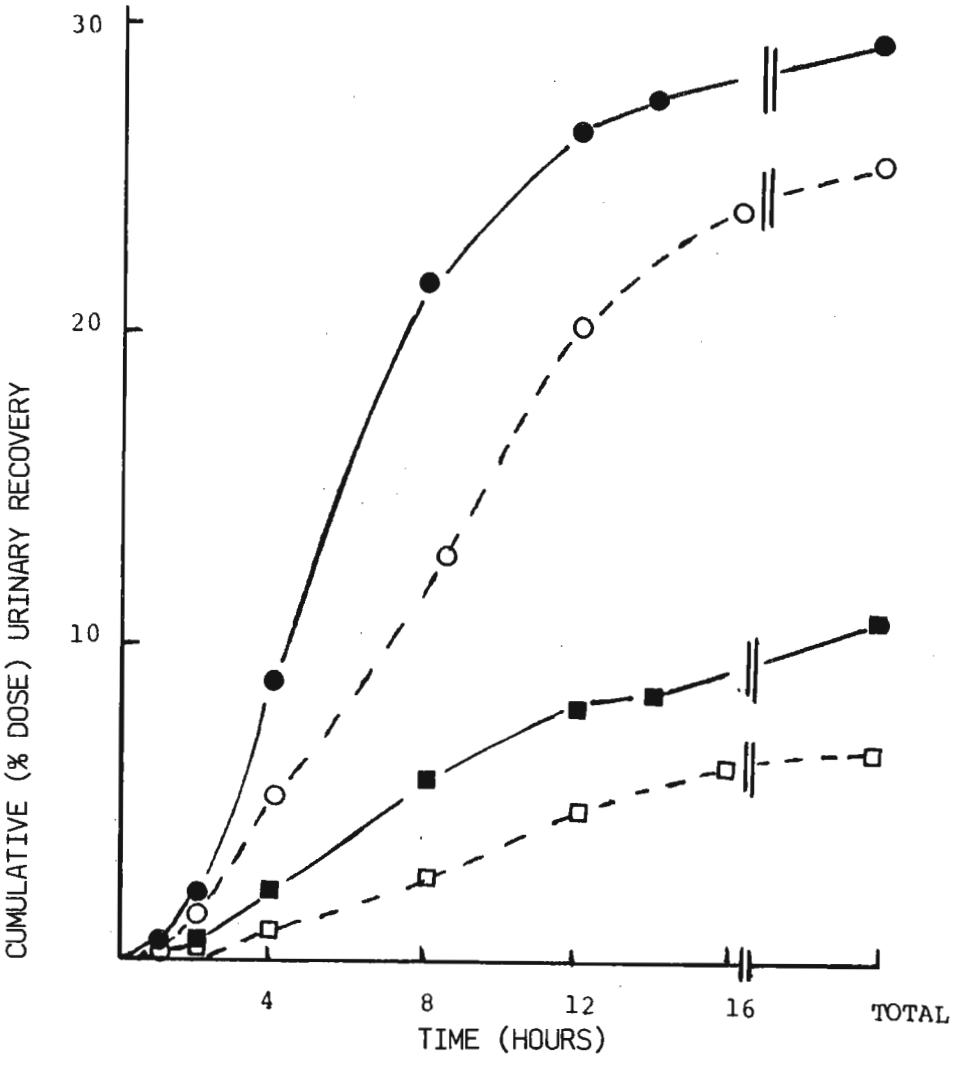


Table 4.14: Comparison of the effect of food on the urinary recoveries*1 (over 48 hours) of ethylaminopropiophenone (Metabolite II) and diethylnorpseudoephedrine (Metabolite IV) after oral administration of 75 mg diethylpropion hydrochloride in different dosage forms i.e. sustained release pellets (Lot 018010) versus sustained release tablet (Lot 284 BB) to the same subject C.D. under acidic urine conditions

Metabolite	Fasting		Non Fasting	
	S.R. Pellets	S.R. Pellets	S.R. Pellets	S.R. Pellets
II	29,8	29,5	27,1	25,5
IV	12,0	10,8	10,2	6,8
Total Recovery	41,8	40,3	37,3	32,3
Relative Percentage Availability*2			89,2%	80,2%

*1 Expressed as the percentage of unchanged dose excreted in urine over 48 hours

*2 The percentage of the ratio of total recovery in non fasting state and fasting state

S.R. = Sustained Release

4.2.3 Single dose studies using 25 mg diethylpropion hydrochloride

The urinary excretion rate profiles of ethylamino-propiophenone (Metabolite II) and diethylnorpseudoephedrine (Metabolite IV) after oral administration of 25 mg diethylpropion hydrochloride (DEP HCI), on separate occasions (Trials 4, 5 and 6 - Table 3.1) to different subjects with acidic urine conditions are shown in Figure 4.36. The absorption of the drug was rapid, giving an average peak urinary excretion rate of 26,9 µg/min and 8,22 µg/min for metabolites II and IV respectively at about 1,25 hours after administration. Thereafter the decline in urinary excretion rate was rapid and it appears that 70-80% of the drug was absorbed in 4-5 hours. The pharmacokinetic parameters of the two metabolites in individual trials and their average values are summarised in Table 4.15. The cumulative urinary recoveries (total) as well as the pharmacokinetic data suggest that under acidic urine conditions, there are minimal inter-subject and intra-subject variations. Therefore it is possible to use a small number of subjects in studies on diethylpropion to obtain meaningful results, provided acidic urine conditions are maintained. The average elimination half-lives ($t_{\frac{1}{2}}$ β) for metabolites II and IV were 3,10 hours and 3,74 hours respectively, while those reported by Mihailova et al., (1974) were 2,77 hours and 2,48 hours - the differences may be due to the inclusion of the early distribution phase (α phase) in the determination of the β phase. The semi-logarithmic plot of the average urinary excretion rate of ethylaminopropiophenone (Metabolite II) after oral administration of 25 mg diethylpropion hydrochloride in subject C.D. (Trials 5 and 6; Tables 3.1) under acidic urine conditions showed that, after a short distribution phase (α phase), a biexponential decline was obtained (Figure 4.37); the slow phase (β phase) generally started at about 3,5 hours after administration of the drug; the third phase (γ phase) was attributed to the

presence of a deep tissue compartment which released the drug slowly. Therefore, it is important when determining elimination half-lives to establish that absorption of drug has ceased, especially in the case of slow release pellets (Trials 1, 2, 7 to 14) and that the extreme tail end (γ phase) should not be considered. This terminal γ phase is probably associated with the release of the bound compound from deep tissues and fat and comprises a minor percentage of the total amount of drug and metabolites excreted in urine.

Table 4.15: Pharmacokinetic parameters of ethylaminopropiophenone (Metabolite II) and diethylnorpseudoephedrine (Metabolite IV), calculated from urinary excretion data, after oral administration of 25 mg diethylpropion hydrochloride (in a capsule) to two subjects under acidic urine conditions

	TRIALS			
	4	5	6	AVERAGE
SUBJECT	A.M.	C.D.	C.D.	
Peak Excretion Rate µg/min				
II	29,17	26,61	24,93	26,9
IV	6,82	9,52	7,32	8,22
Time of Peak Excretion Rate, Hours.				
II	1,25	1,25	1,25	1,25
IV	1,25	1,50	1,25	1,35
β Slope *1(hr^{-1})				
II	0,214	0,226	0,231	0,224
IV	0,182	0,191	0,184	0,186
Half Life ($t_{½}$ β) hours				
II	3,24	3,07	3,0	3,10
IV	3,81	3,63	3,77	3,74
% Urinary Recovery*2 (24 hrs)				
II	36,7	35,06	34,49	35,42
IV	14,15	17,10	16,50	15,92
TOTAL	50,85	52,16	50,99	51,34

*1 Obtained from a semilog plot of data between 4 to 10 hours for II and 3-8 hours for IV, after administration - Figures 4.41 and 4.42 respectively.

*2 Expressed as the percent of unchanged dose excreted over 24 hours.

hr=hour

Figure 4.36: Urinary excretion rate of metabolites II and IV after oral administration of diethylpropion hydrochloride (25 mg - placed in a capsule) under acidic conditions to two subjects.

Metabolite II — Ethylaminopropiophenone ● ■ ▲
Metabolite IV — Diethylnorpseudoephedrine ○ □ △

TRIAL NO	SUBJECT	URINE FLOW RATE ml/min	pH
4 ———	A.M.	2,5 ± 0,8	4,95 ± 0,2
5 ------	C.D.	1,8 ± 1,0	4,85 ± 0,2
6	C.D.	2,5 ± 1,2	4,9 ± 0,3

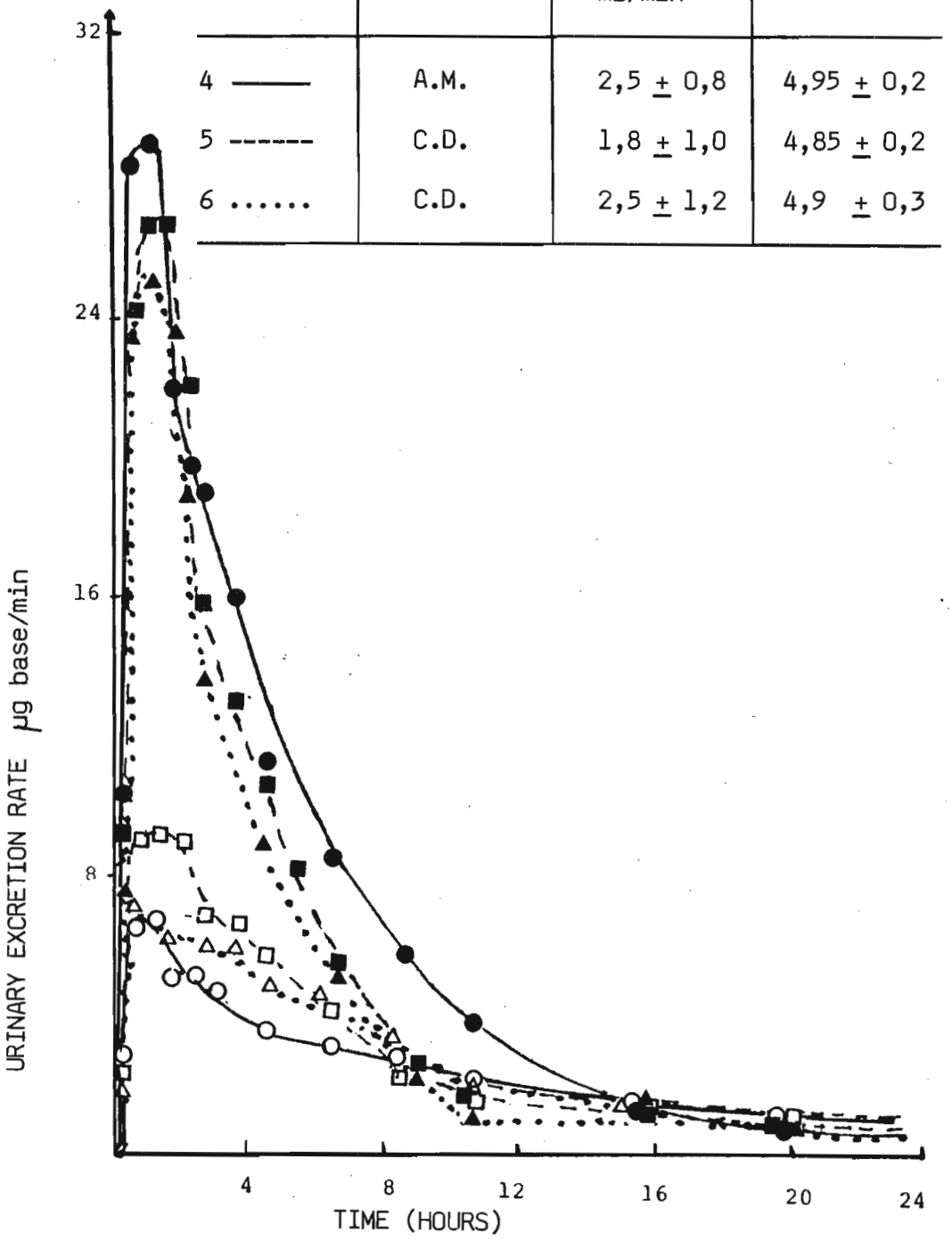
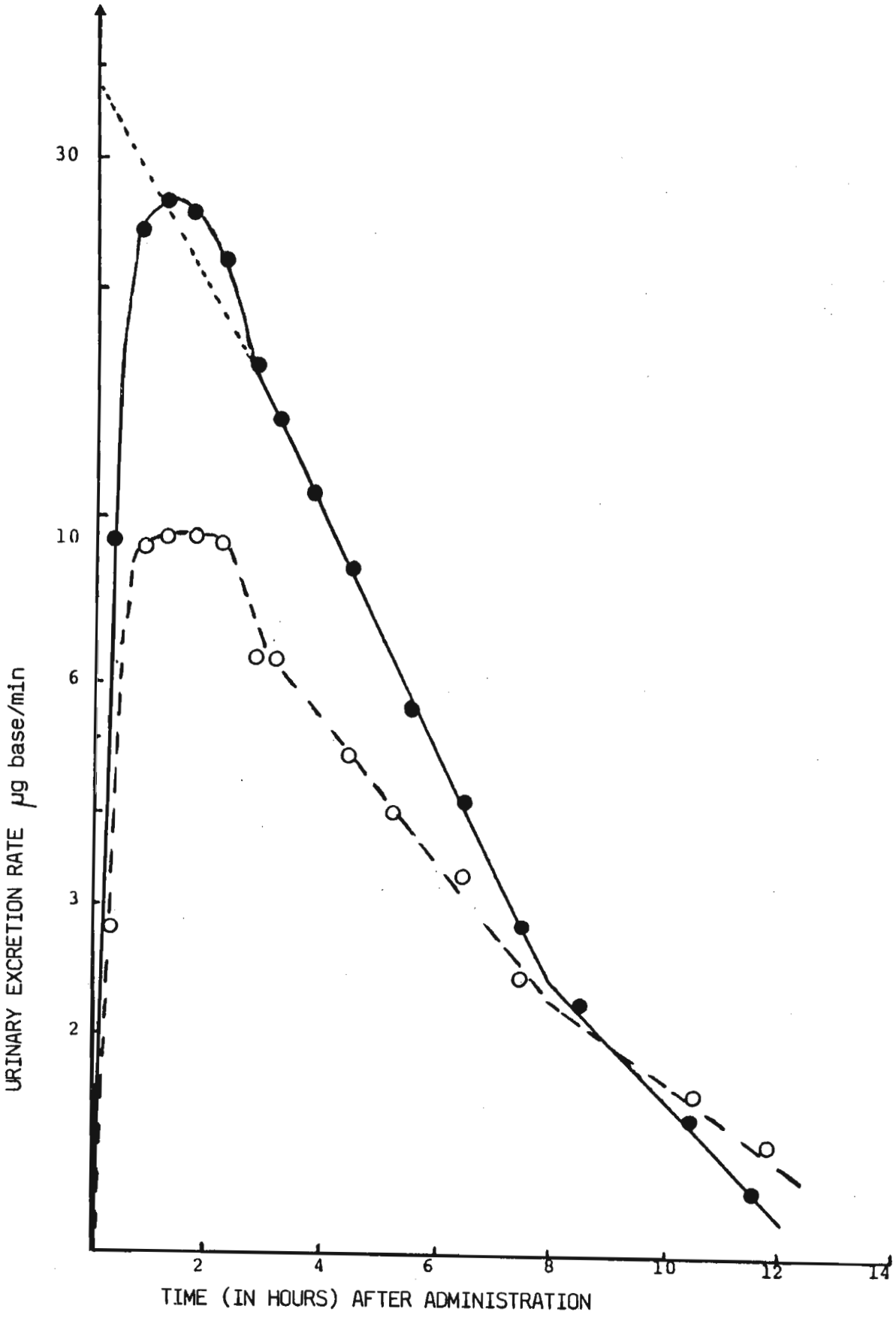


Figure 4.37: Mean urinary excretion rate of ethylaminopropiophenone (Metabolite II; ●) and diethylnorpseudoephedrine (Metabolite IV; O) (Trials 5 & 6) after oral administration of 25 mg diethylpropion hydrochloride in a capsule, to the same subject C.D., under acidic urine conditions



4.2.4 Study on sustained release pellets Lot R 7574

To establish the *in vivo* release profile of sustained release pellets Lot R 7574 which showed faster *in vitro* release characteristics than the sustained release pellets Lot R 7773, the urinary excretion rate profiles of ethylaminopropiophenone (Metabolite II) and diethylnorpseudoephedrine (metabolite IV) were determined after oral administration of these pellets (Lot R 7574) to two subjects, C.D. and A.M. (Trials 13 and 14 - Table 3.1). The results of these two preliminary trials on sustained release pellets Lot R 7574 were compared with the *in vivo* data on Lot R 7773 in the same subjects (Trial 1 - Table 3.1) to enable us to select the most suitable lot of pellets formulation with desirable urinary excretion profiles (of the two metabolites) for possible replacement of the conventional drug dosage forms. The mean urinary excretion rate profiles and the cumulative urinary recoveries of ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of sustained release pellets Lot R 7574 (≡75 mg diethylpropion hydrochloride), on separate occasions, in subjects C.D. and A.M. (Figures 4.38 and 4.39) respectively, demonstrate the excellent reproducibility and minimal intra-subject variations.

Comparisons of the urinary excretion rates of metabolites II and IV after oral administration of three different dosage forms in subject C.D. (Figure 4.40) show that the two sustained release pellets formulations gave profiles which were intermediate between the "peaks and troughs" seen in three divided doses administrations; the apparent initial rates of absorption from all three dosage forms were similar because of the presence of a portion of the drug in the sustained release pellets formulations (Lots R 7574 and R 7773) in the "free" form (Table 2.B.2).

However, sustained release pellets Lot R 7574 gave plateau levels for II and IV which were higher and of shorter duration than those seen with the use of sustained release pellets Lot R 7773 - the latter formulation also provided satisfactory plateau levels of 6 to 8 hour duration.

The total cumulative urinary recoveries (sum of metabolites II and IV) after oral administration of the different dosage forms, suggest that despite the lower profiles with the use of the sustained release pellets formulations, all the dosage forms gave relatively good bioavailabilities.

The results (Figure 4.40 and Table 4.16) suggest clearly that sustained release pellets Lot R 7773 provided suitable urinary excretion profiles of the two major (active) metabolites II and IV; hence this formulation was selected for further investigations, involving two-way crossover studies on twelve subjects (Trial 1 - Table 3.1).

Figure 4.38: Mean urinary excretion rate of ethylaminopropiophenone (Metabolite II; ○ ●) and diethylnorpseudoephedrine (Metabolite IV; △ ▲) after oral administration of diethylpropion hydrochloride sustained release pellets (Lot R7574) to two subjects.

SUBJECT	II	IV	TRIAL NO
A.M.	○	△	14
C.D.	●	▲	13
MEAN	■	□	

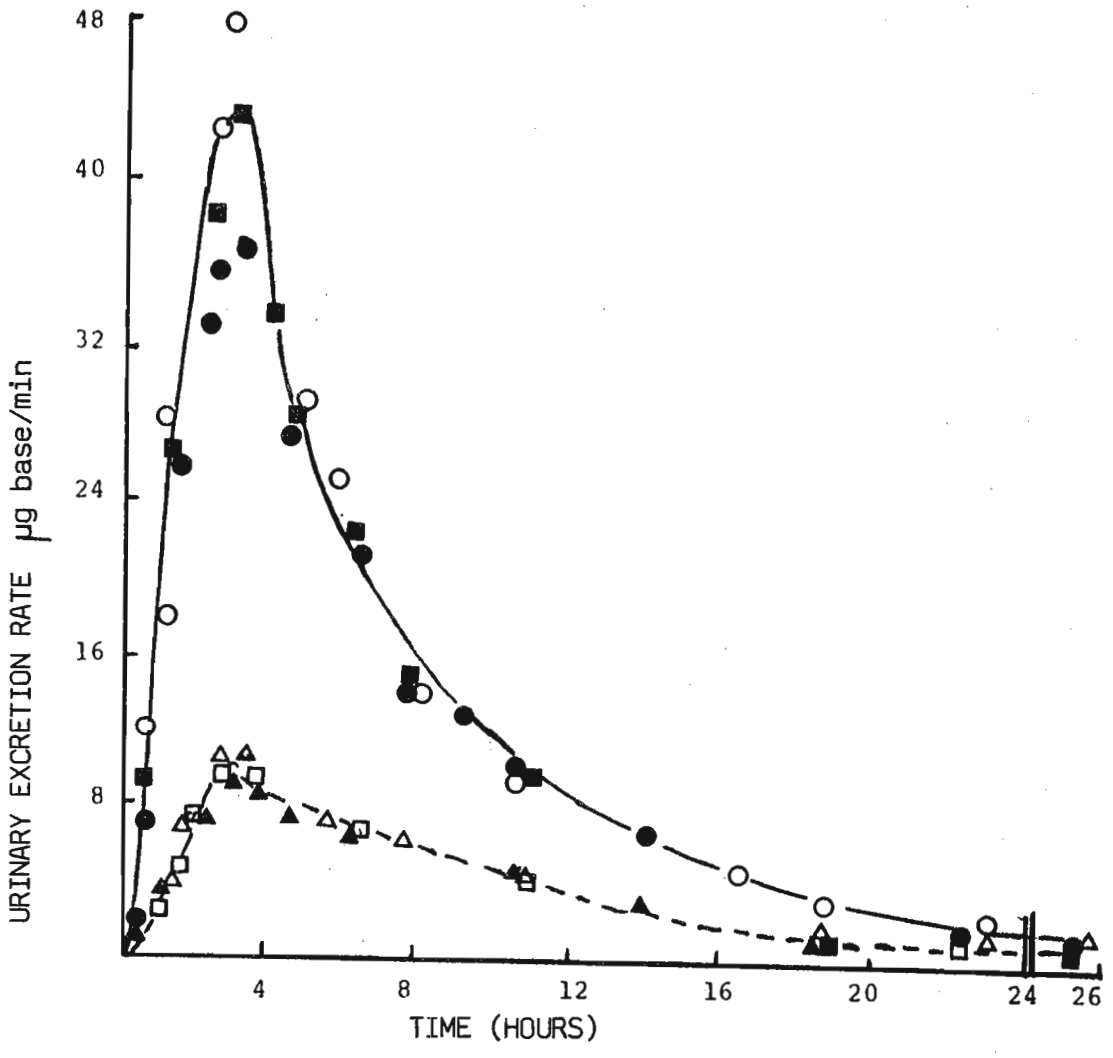


Figure 4.39: Cumulative urinary excretion of ethylaminopropiophenone (Metabolite II; ● ■) and diethylnorpseudoephedrine (Metabolite IV; ○ □) after oral administration of diethylpropion sustained release pellets Lot R7574 (75 mg hydrochloride salt) to two subjects.

TRIAL	SUBJECT	% RECOVERY (48 HOURS)		
		II	IV	TOTAL
13	C.D. ——	27,25	10,9	38,15
14	A.M. -----	28,69	11,97	40,66

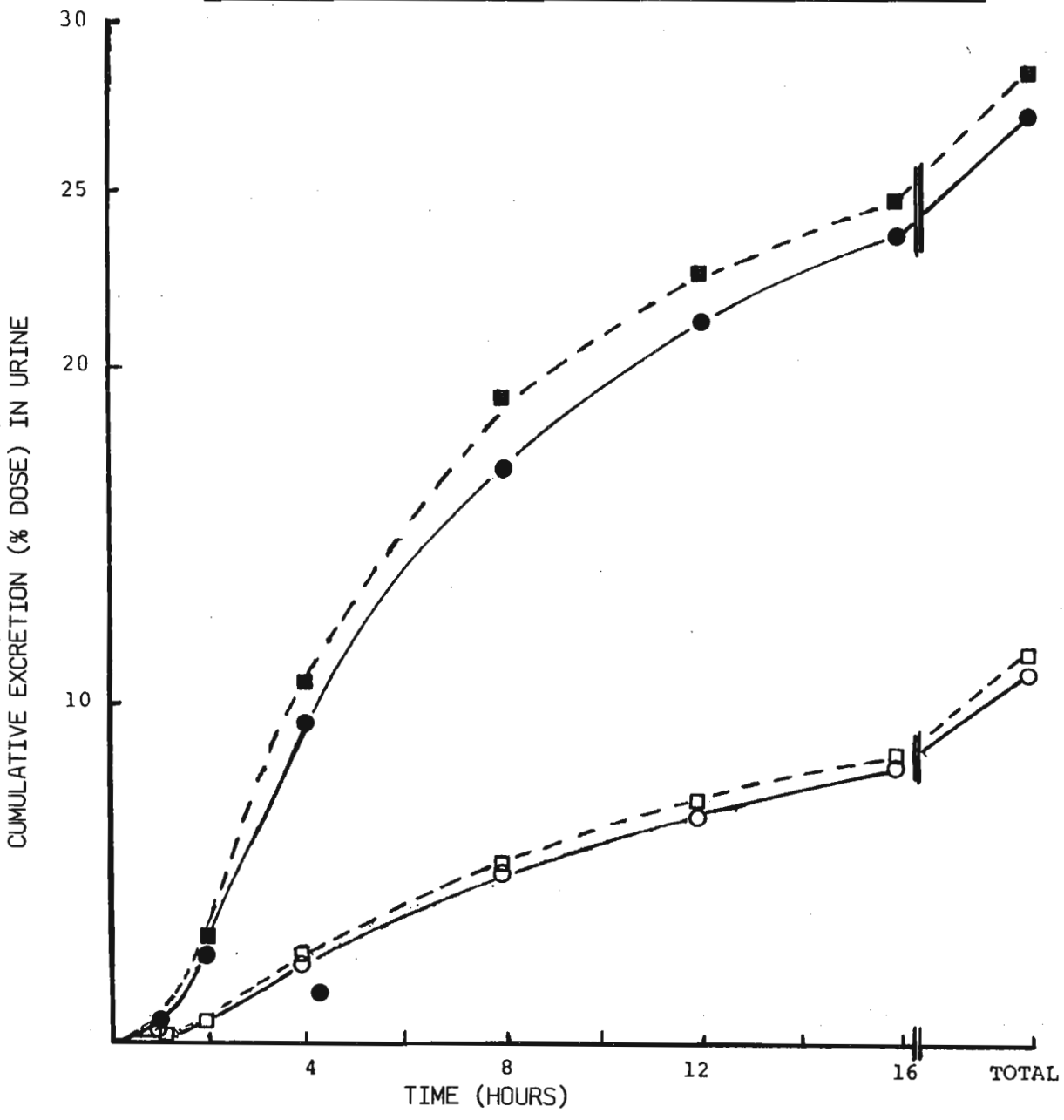


Figure 4.40: Comparison of the urinary excretion rate profiles of Metabolite II(○△□) and IV (● ▲ ■) after oral administration of 75 mg of diethylpropion hydrochloride (DEP) in the form of F.D.F.*1 (-------), S.R. Pellets Lot R 7773 (– – –) and S.R. Pellets Lot. R 7574 (———) in the same subject, C.D., under acidic urine conditions.

F.D.F. Free drug, DEP 25 mg at 0, 4 and 8 hours

S.R.P. Sustained release pellets at 0 hours

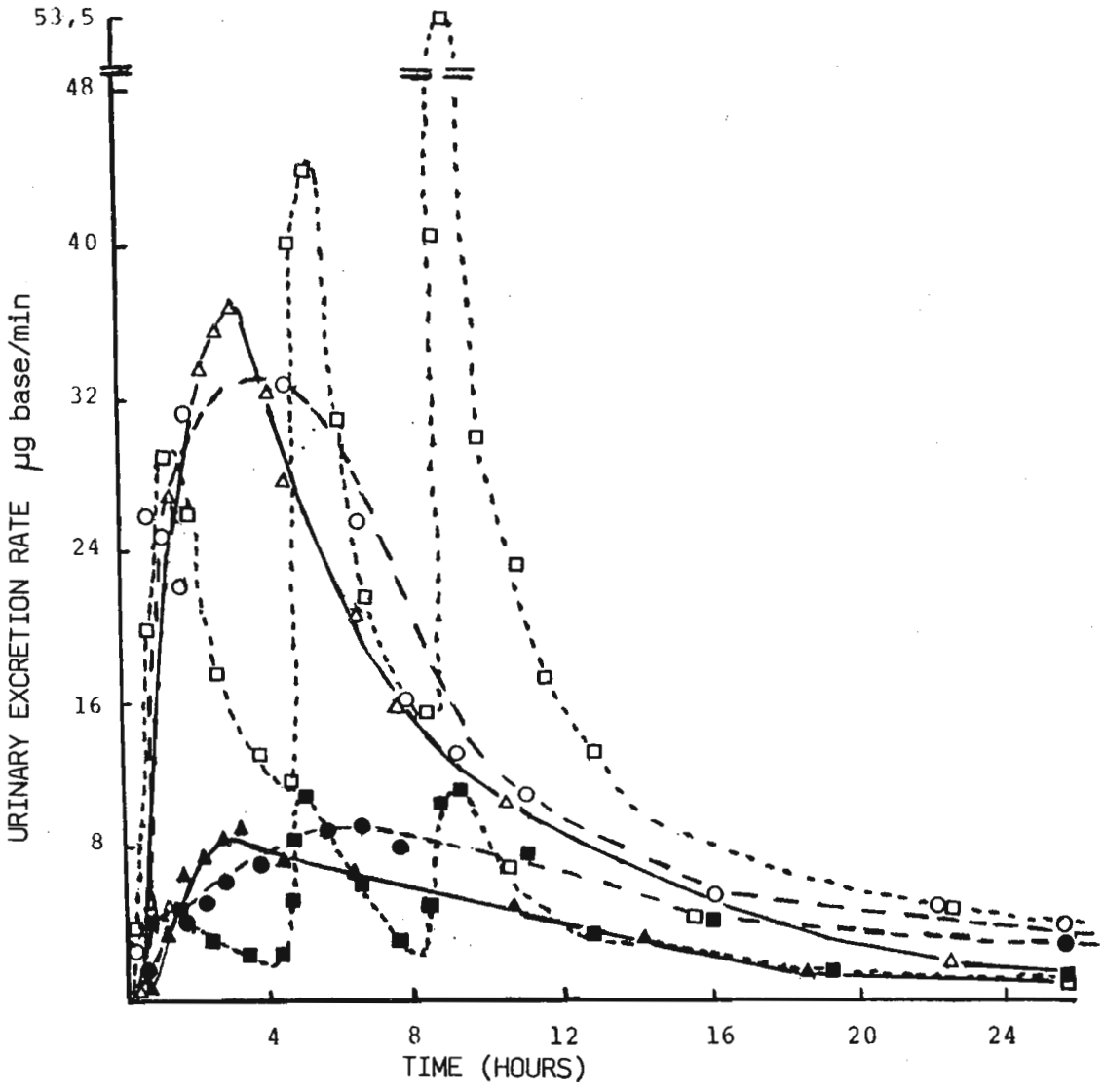


Table 4.16 Cumulative urinary recoveries (% dose)*1 of ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of 75 mg diethylpropion hydrochloride (DEP) in different dosage forms, on separate occasions, to the same subject, C.D., under acidic urine conditions.

TRIAL NUMBER	1	13	1
Metabolite	F.D.F.*2	S.R.P.*3 Lot R 7574	S.R.P.*3 Lot R 7773
II	33,74	27,25	30,59
IV	12,5	10,9	15,0
Total (II + IV)	46,24	38,15	45,59

*1 Figures are expressed as the percentage (of dose taken) excreted over 48 hours.

*2 F.D.F. Free dosage form, 25 mg DEP at 0, 4 and 8 hours.

*3 S.R.P. Sustained release pellets (≡ 75 mg DEP), at 0 hours.

4.2.5 Elimination half-life of ethylaminopropiophenone (II)

The elimination half-life ($t_{\frac{1}{2}}$ ß) and the elimination rate constant (ß) of the major metabolite (metabolite II) were determined as described (Section 3.1) for each trial in subjects C.D. and A.M. The full details on the trials are given in Table 3.1.

A semilog plot of excretion rate of metabolite II after oral administration of the different dosage forms in subject C.D. or in subject A.M., shows that the decline (ß slope) of the metabolite decreases exponentially after the initial rapid distribution phase. (Figures 4.41 for C.D. and 4.42 for A.M.). The slow ß phase generally starts at about 4 hours post-administration of the last dose of the free drug and at least 7-10 hours after dosing in the case of the slow-releasing formulations. Therefore, to avoid obtaining large ß values, it is important when determining the $t_{\frac{1}{2}}$ ß values to establish that absorption and distribution of the drug into the body have ceased.

The ß values and the corresponding half-lives in each trial are given in Table 4.17. The remarkably close values in all the studies suggest that changing the dosage form or route of administration does not significantly alter the elimination kinetics of this metabolite (and possibly of any of the others) in different subjects under controlled acid urine conditions. Similar findings on dose and dosage form independent kinetics on the metabolism of diethylpropion and its chloro-derivative (chloro-ethylamino-propiophenone), have been reported (Beckett et al., 1969a, 1973, 1974a).

Figure 4.41: Semilog plot of the urinary excretion rate of the major metabolite, ethylaminopropiophenone (II) after oral administration of different dosage forms* of 75 mg diethylpropion hydrochloride (DEP) to subject C.D. under acidic urine conditions.

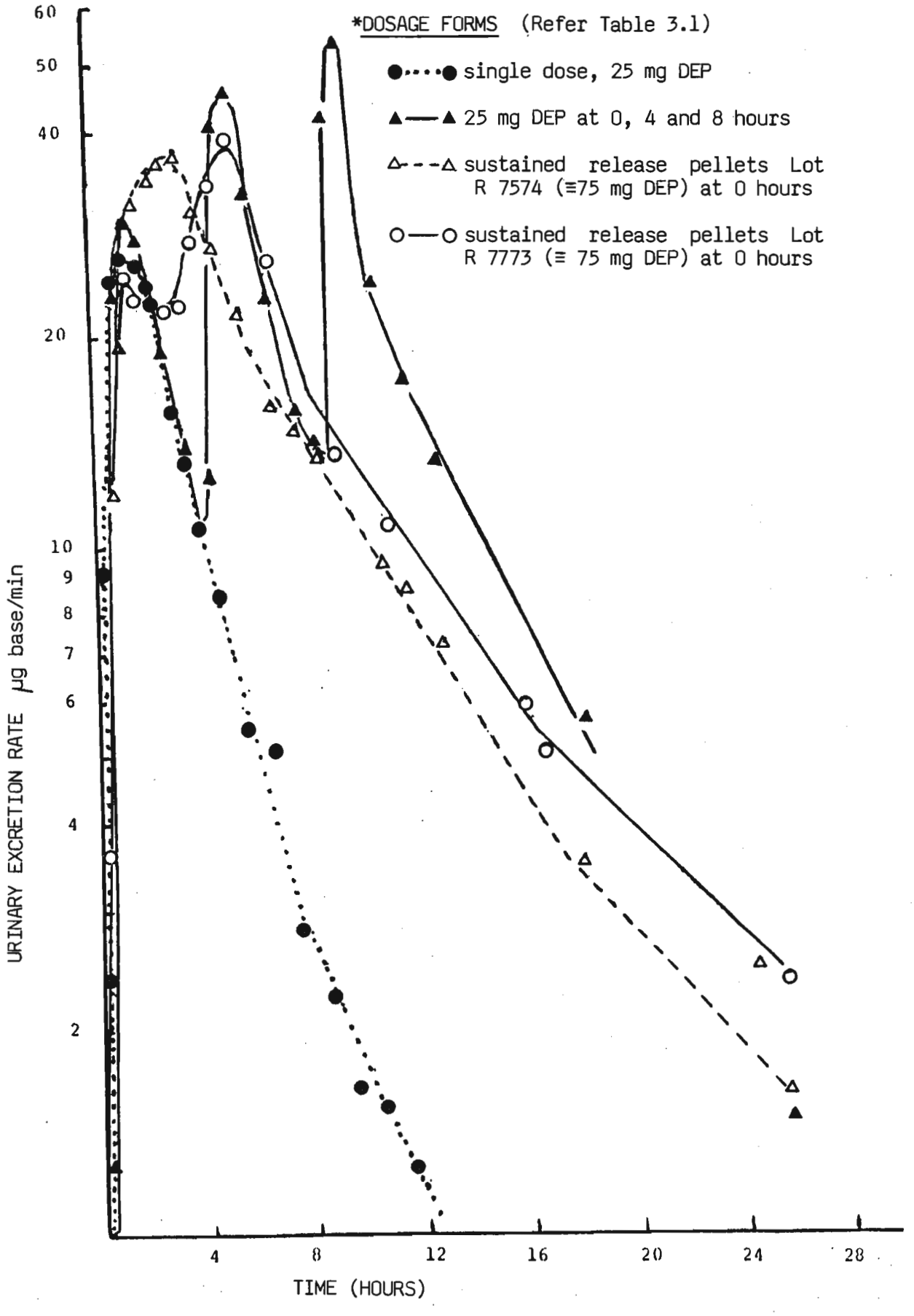


Figure 4.42: Semilog plot of the urinary excretion rate of the major metabolite, ethylaminopropiophenone (II) after oral administration of different dosage forms* of 75 mg diethylpropion hydrochloride (DEP) to subject A.M. under acidic urine conditions.

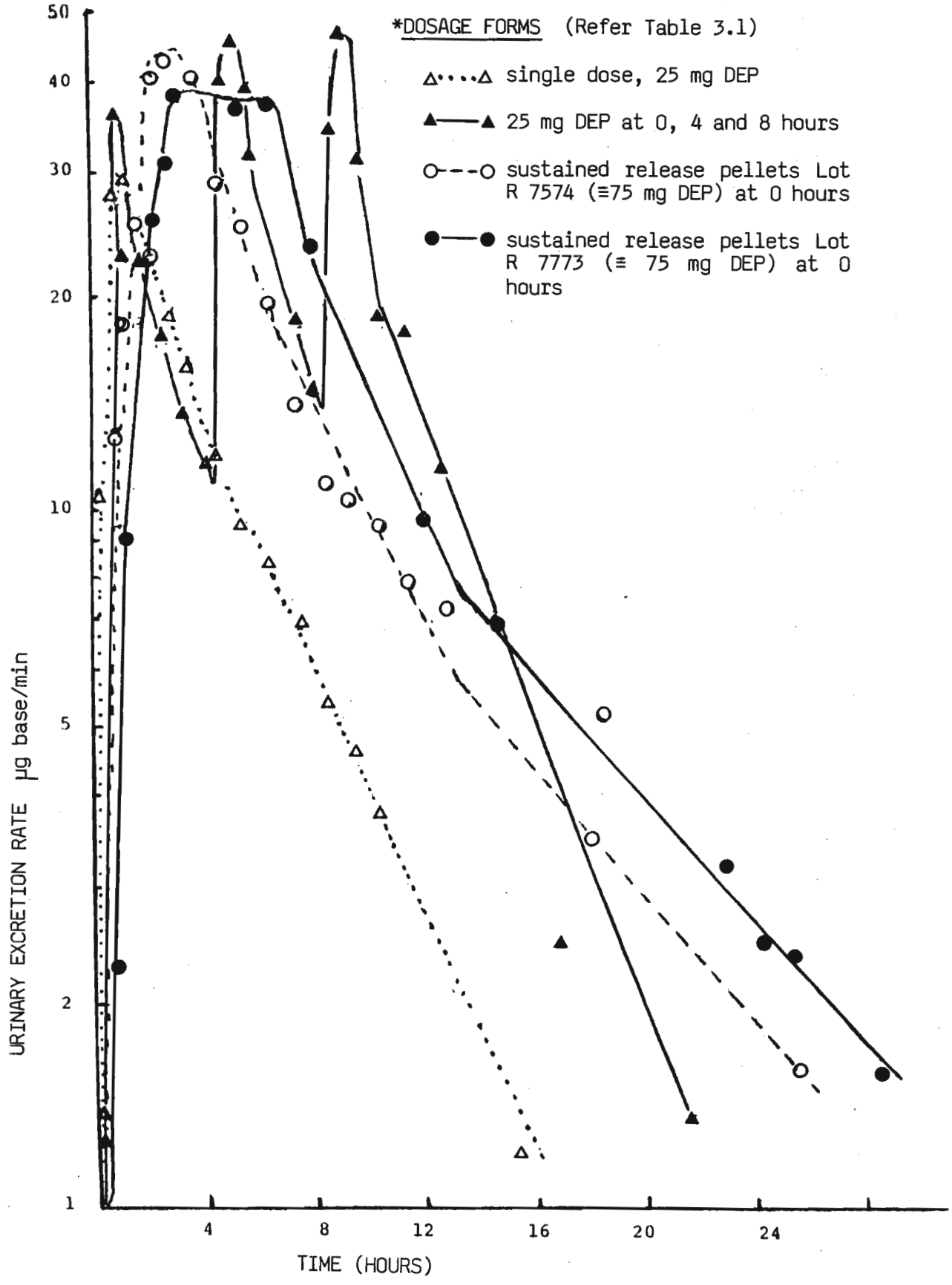


Table 4.17 The elimination half life ($t_{\frac{1}{2}}\ \beta$) of the major metabolite ethylaminopropiophenone (Metabolite II) as determined for each trial to two subjects, C.D. and A.M., using urinary data

Trial No: see Table 3.1	β (Hours)$^{-1}$		$t_{\frac{1}{2}}\ \beta$ (hours)		Dosage form & route*1
	A.M.	C.D.	A.M.	C.D.	
1	0,2190 0,2210	0,2033 0,1790	3,2 3,1	3,4 3,9	F.D.F. Oral S.R.P. Lot R 7773 Oral
2		0,167		4,20	S.R.P. Lot R 7773*2 Rectal
3		0,247 (9-13)		2,81	S*3 Rectal
4 5 6	0,2269	 0,2040 0,189	3,05	 3,40 3,67	S Oral
7 8 9		0,198 0,203 0,178		3,5 3,4 3,9	S.R.P. Lot 08010 Oral
10		0,180 (12-20)		3,90	S.R.P. Lot 08010*4 Oral
11		0,248 (12-18)		2,8	S.R. Tablets Oral Lot 284 BB
12		0,197 (12-19)		3,5	S.R. Tablets*4 Oral Lot 284 BB
13	0,2330	0,1732	2,97	4,00	S.R.P. Lot R 7574 Oral

DEP = Diethylpropion Hydrochloride
S = Single dose, 24 mg DEP (placed in a hard gelatin capsule or dissolved in 25 ml water), taken orally with 100 ml at time, 0 hours.
F.D.F = 25 mg DEP, free form, (placed in a capsule) at times 0, 4 and 8 hours, with 100 ml water
S.R.P.= S.R. Tablets= Sustained Release Pellets (≡ 75 mg DEP placed in a hard gelatin capsule)
Sustained Release Tablets (≡ 75 mg DEP) at time 0 hours, with 100 ml water
*1 = Details in Table 3.1
*2.3 = Drug inserted immediately after defaecation, either as a suppository comprising S.R.P. or a 1 ml solution containing 25 mg DEP
*4 = Drug taken at 10 minutes after breakfast

4.3 In Vitro/In Vivo Correlations

The subject of *in vitro/in vivo* correlations has been reviewed by Wagner (1971) while Robinson (1978) cites a number of articles with regard to pharmacokinetics after controlled release product administration, but few articles dealing with *in vitro* and/or *in vivo* test methodologies. The Ritschel (1973) review discusses the theoretical release of drugs from various types of controlled release dosage forms. Sjogren (1971) has discussed the difficulties of using *in vitro* methods to evaluate the performance of this type of product. A number of *in vitro* methodologies have been used as a means of evaluating controlled release preparations; most of these methods have ignored the necessity of correlation with *in vivo* parameters (Wagner, 1971).

Few discussions of solely bioequivalency or methods to assess bioequivalency, between different formulations of a drug in controlled release products exists in literature. Recently a general protocol has been proposed (Vallner, Honigberg, Kotzan and Stewart, 1983; Roufail, 1983) to evaluate controlled release drug products from both *in vitro* and *in vivo* investigations. The release patterns can be effectively evaluated in practice, by calculation of a controlled release effectiveness parameter and an absorption rate effectiveness parameter.

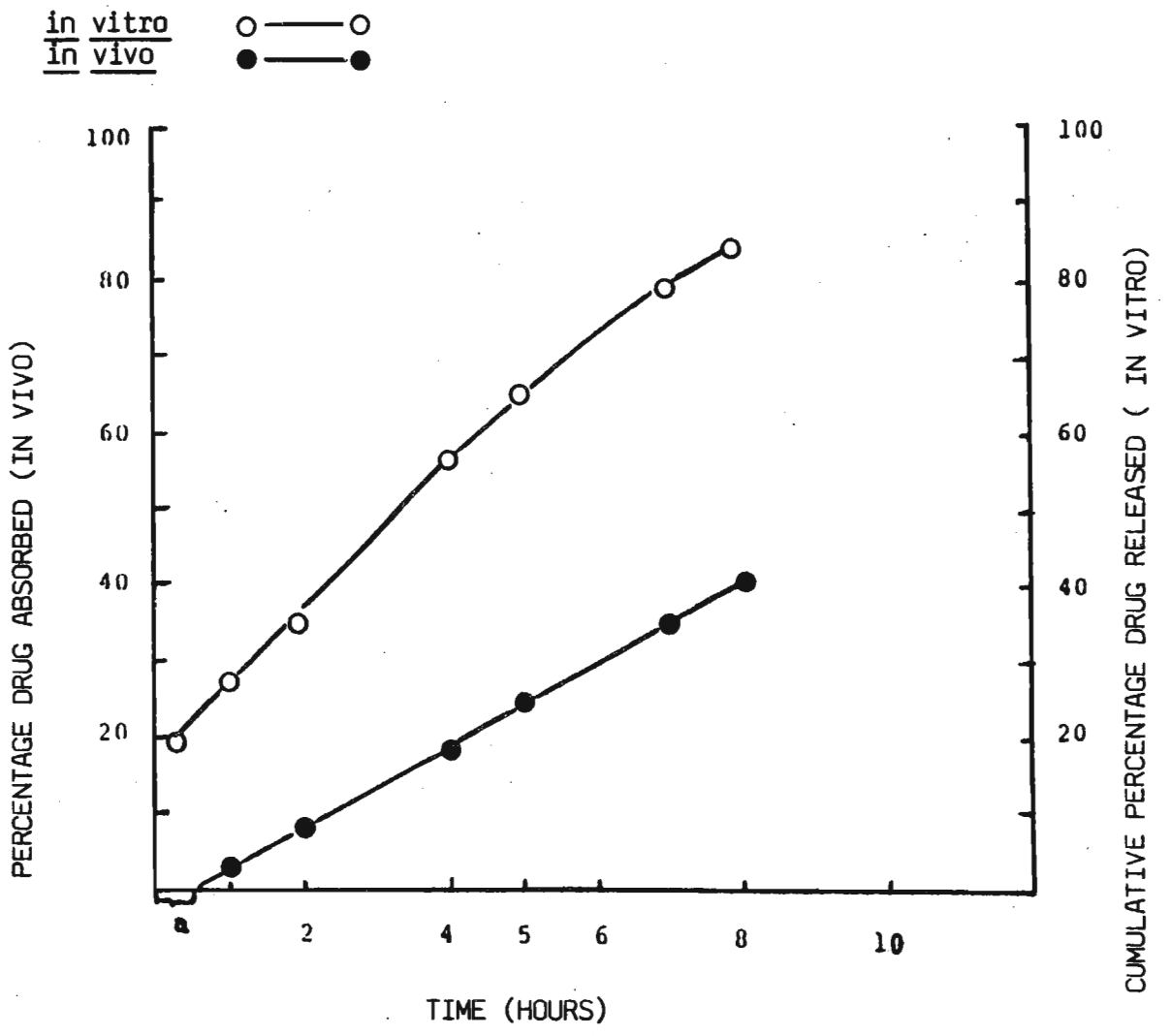
In the present study extensive *in vitro* investigations were carried out on diethylpropion sustained release pellets dosage forms; the results, given in Section 2.B.1, were obtained using the rotating bottle method with a range of buffers (pH 1,5 to 7,5) to simulate pH conditions following oral administration, or buffer pH 6,9 to simulate pH conditions following rectal administration (2.A.2.2.d). Since the release of diethylpropion hydrochloride from each

lot of sustained release pellets (Lots R 7574 and R 7773) was seen to be relatively constant in each of the different dissolution media (Figures 2.B.2 and 2.B.3) the in vitro results on the standard pH gradients were found to be most suitable i.e. pH 1,5 (1 hour); pH 4,5 (1 hour); pH 6,9 (2 hours); pH 6,9 (1 hour); pH 7,2 (2 hours) and pH 7,5 (1 hour).

Several in vivo studies were also carried out on the different lots of sustained release pellets, viz. Lot R 7773 which was administered orally in a two-way crossover trial to 12 subjects; Lot R 7773 which was administered rectally as a special suppository to one subject (C.D.); and Lot 7574 which was administered orally to two subjects (C.D. and A.M.). For details, refer to Table 3.1. The results on these studies are discussed below:

(a) Figure 4.43 shows the comparison of the mean percentage of dose absorbed (calculated by considering the total amount of the sum of the two metabolites (II and IV) at time, t_∞ as 100%) at different periods of time over 8 hours, after a single dose of diethylpropion hydrochloride sustained release pellets (Lot R 7773) in six subjects (Group A), using urine data, with the in vitro cumulative release data. Scatter of the in vivo results from the mean percentage in six subjects, represented in Figure 4.44, indicates that the spread of data is reasonably good. The in vitro/in vivo correlation of DEP sustained release pellets after single dose in six subjects (A-H), is excellent (c.c. = 0,996 - Figure 4.45). There is a lag time of absorption which is related to the delay in appearance (excretion) of the metabolites in the urine after the release of the drug in the GIT which is then followed by absorption and metabolism.

Figure 4.43: Comparison of mean percentage of diethylpropion absorbed* (in vivo) at different periods of time after a single oral dose of diethylpropion hydrochloride sustained release pellets (Lot R 7773) to six subjects (A, B, C, D, E, and H) and of the cumulative in vitro release data using the following pH gradients:
pH 1,5(5 min); 1,5 (55 mins); 4,5 (1 hr); 6,9 (2 hrs); 6,9 (1 hr); 7,2 (2 hrs) and 7,5 (1 hr)



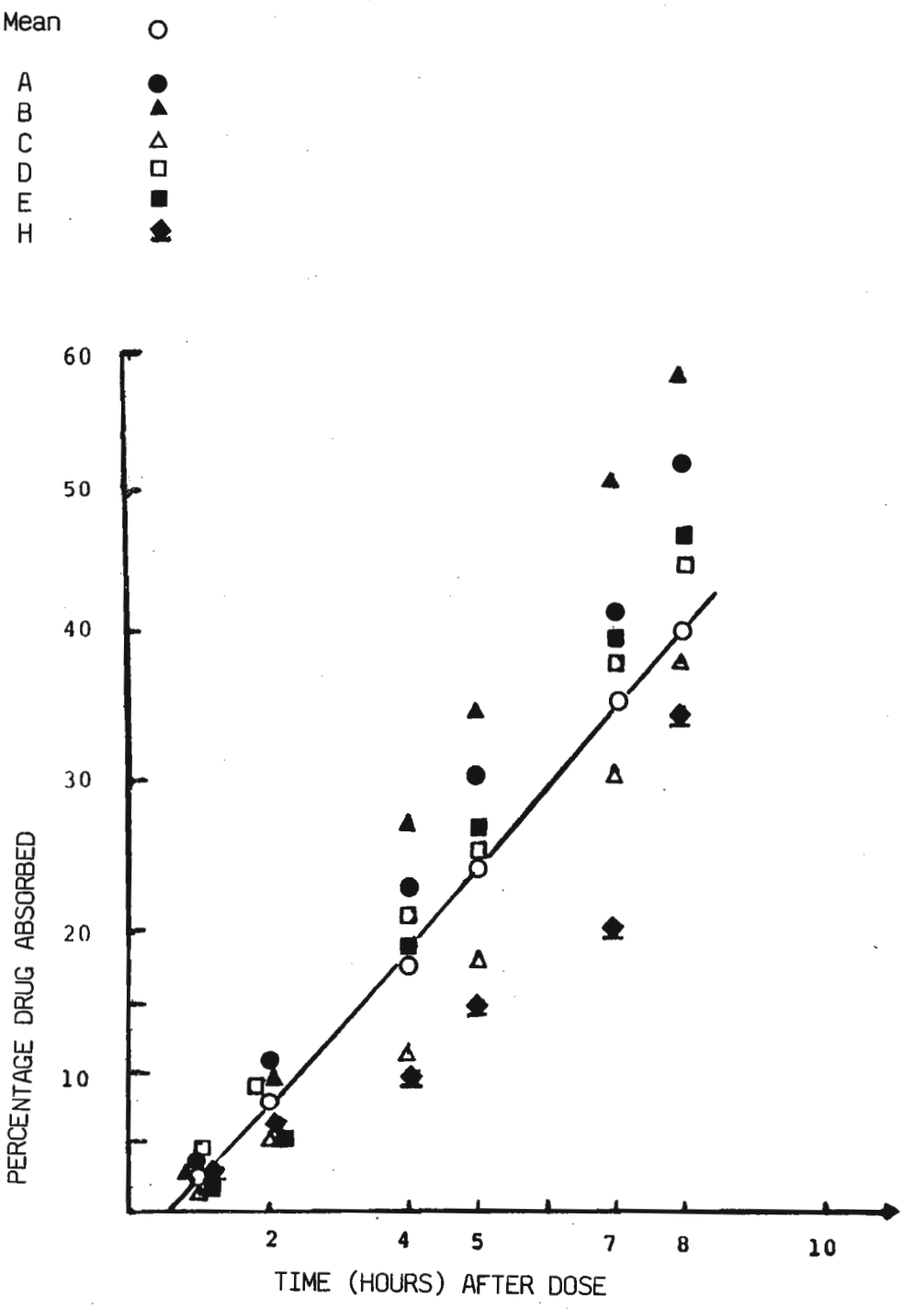
* Total amount (sum of the two metabolites) excreted at time, t_∞ is taken as 100%.

Drug Absorbed =

$$\frac{\text{Sum of cumulative amount in urine (Metabolite II and IV) at time, t}}{\text{Sum of the cumulative recovery (Metabolite II and IV) at time, } t_\infty} \times 100$$

a = lag time (for absorption) i.e. time taken for the metabolites to be excreted after the release of drug in the GIT

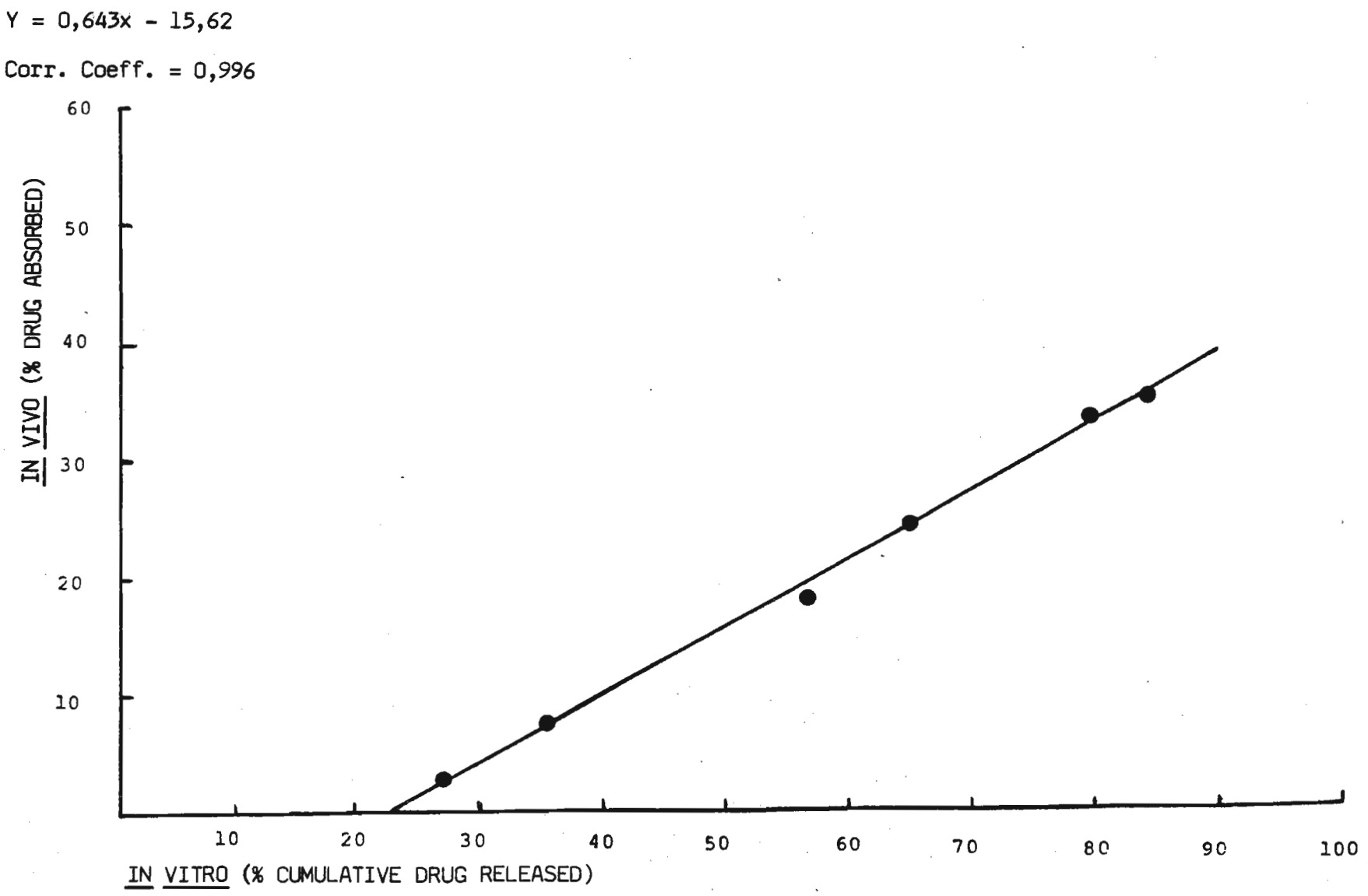
Figure 4.44: Percentage of diethylpropion absorbed* at different periods of time after a single dose of diethylpropion sustained release pellets Lot R 7773 (= 75 mg hydrochloride) to six different subjects (A, B, C, D, E, and H) as indicated using urinary data.



* % Absorbed is calculated as follows:

$$\frac{\text{Sum of cumulative amount of two metabolites (II and IV) at time, } t}{\text{Sum of the cumulative recovery of both (II and IV) at time, } t_{\infty}} \times 100$$

Figure 4.45: In vitro/in vivo correlation of the release of diethylpropion hydrochloride from sustained release pellets Lot R7773 after oral administration to six subjects (A, B, C, D, E and H) using the mean values for urine data (in vivo) and the following pH gradients -pH 1,5 (5 minutes); 1,5 (55 minutes); 4,5 (1 hour); 6,9 (2 hours); 6,9 (1 hour); 7,2 (2 hours); and 7,5 (1 hour) for the in vitro release data



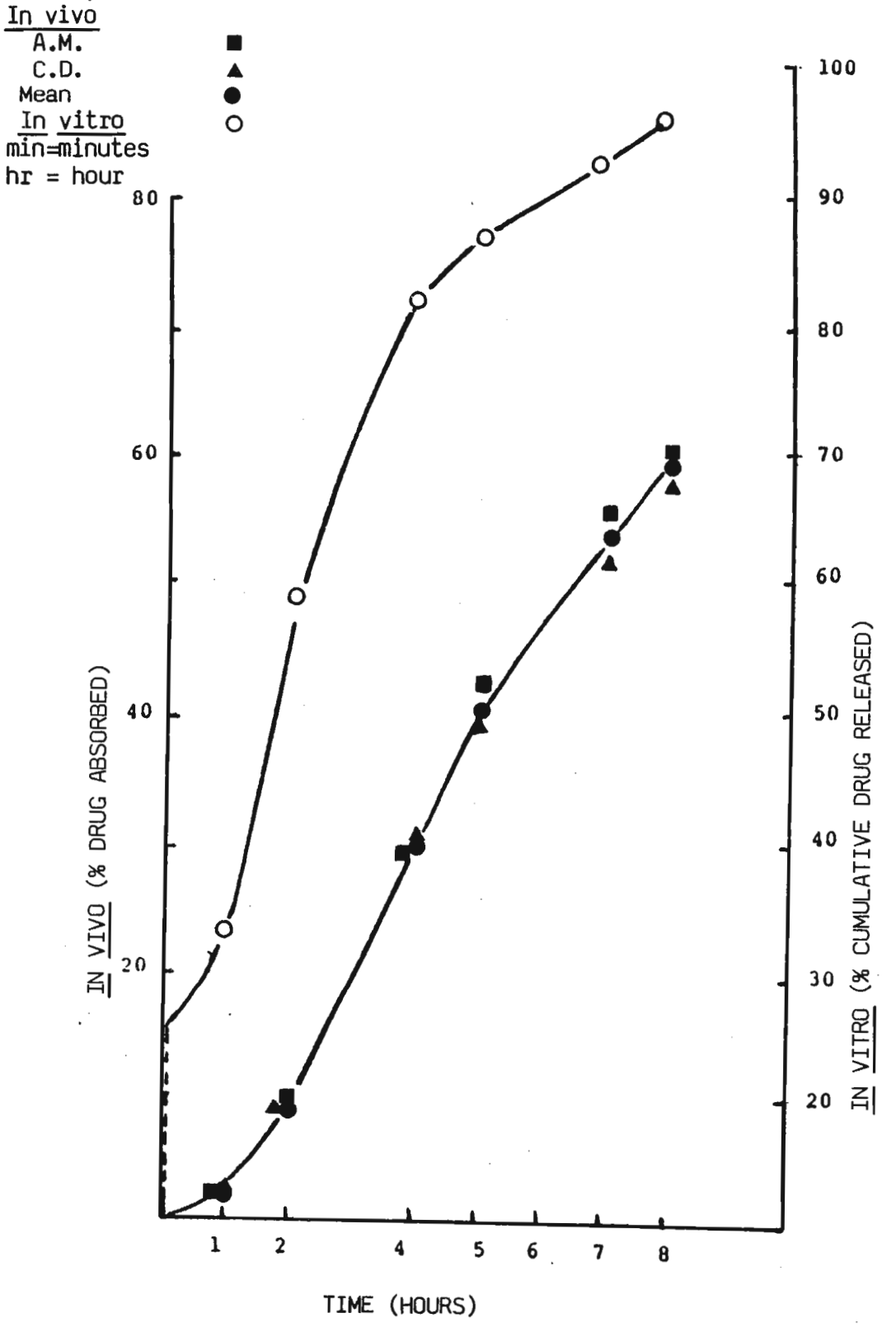
(b) To emphasize the validity of the good in vitro/in vivo correlation obtained with Lot R 7773 the results were compared with the in vitro/in vivo correlations of sustained release pellets Lot R 7574 which showed a faster in vitro release rate (Figure 2.B.1).

Figure 4.46 shows the comparison of the percentage of drug absorbed after a single oral dose of sustained release pellets Lot 7574 using urinary data (subjects C.D. and A.M.) with the in vitro cumulative release using the standard pH gradients (mentioned earlier). For these pellet formulations, the in vitro/in vivo correlation is reasonably good (c.c. = 0,996 - Figure 4.47). When 33,1% and 58,7% of the drug was released from the pellets, only about 2% and 9% of the drug (sum of metabolites II and IV) at 1 and 2 hours respectively after the dose, was excreted in the urine. Therefore the lag time appears to be rather long. However, a better in vitro/in vivo correlation exists if the data point at the 1st hour is not considered in the calculations i.e. the quantitive in vitro/in vivo correlation for the time period from 2 hours to 8 hours after the dose is expressed by: $y = 0,726\ x + 55,66$ where c.c. = 0,97.

From the in vitro study on Lot R 7773, it is possible to predict the in vivo behaviour of sustained release pellets. It was therefore suitable to use the rotating bottle dissolution test method (2.A.2.2.d).

(c) The in vitro dissolution data, using sustained release pellets Lot R 7773 at a constant pH of 6,9, is presented in Figure 2.B.2; the pellets were formulated into a special suppository-2.A.2.3(iii).

Figure 4.46: Comparison of average percentage of diethylpropion absorbed* (in vivo) at different times after a single oral dose of diethylpropion hydrochloride sustained release pellets (Lot R 7574) to two subjects (A.M. and C.D.) and of the cumulative in vitro release data using the following pH gradients:
pH 1,5 (5 mins); 1,5 (55 mins); 4,5 (1 hr); 6,9 (2 hrs); 6,9 (1 hr); 7,2 (2 hrs) and 7,5 (1 hr)



* % Drug absorbed =

$$\frac{\text{Sum of cumulative amount in urine (Metabolites II and IV) at time, t}}{\text{Sum of the cumulative recovery in urine (Metabolites II and IV) at time, t} \infty} \times 100$$

Figure 4.47: In vitro/in vivo correlation of the release of diethylpropion hydrochloride from sustained release pellets (Lot R 7574) after single oral administration to two subjects (A.M. and C.D.) using the average values for urine data (in vivo) and the following pH gradients -pH 1,5 (5 minutes); 1,5 (55 minutes); 4,5 (1 hour); 6,9 (2 hours); 6,9 (1 hour); 7,2 (2 hours); and 7,5 (1 hour) for the in vitro release data

$Y = 0,996x - 42,92$

Corr. Coeff. = 0,943
mins = minutes
hr = hour

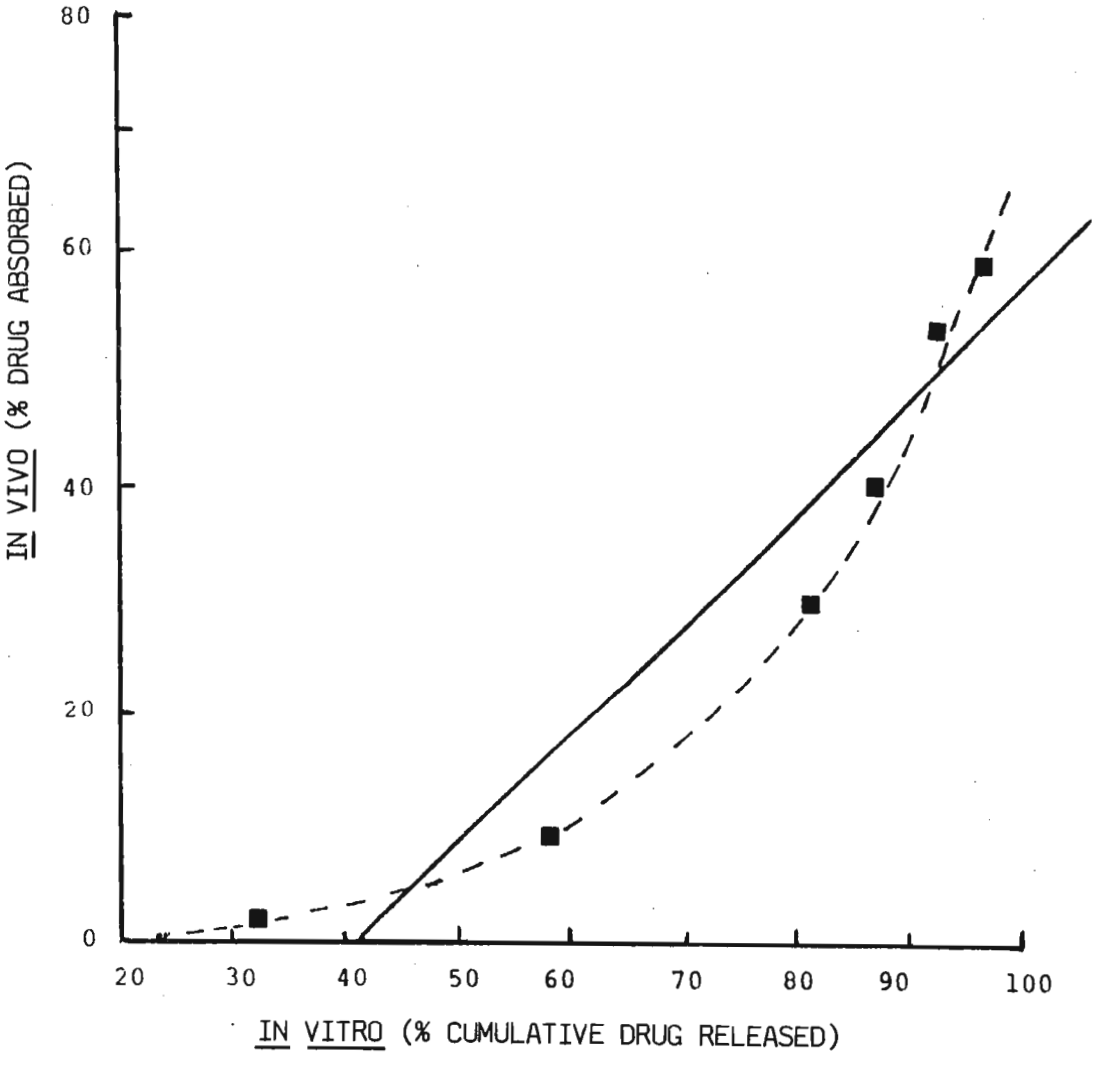
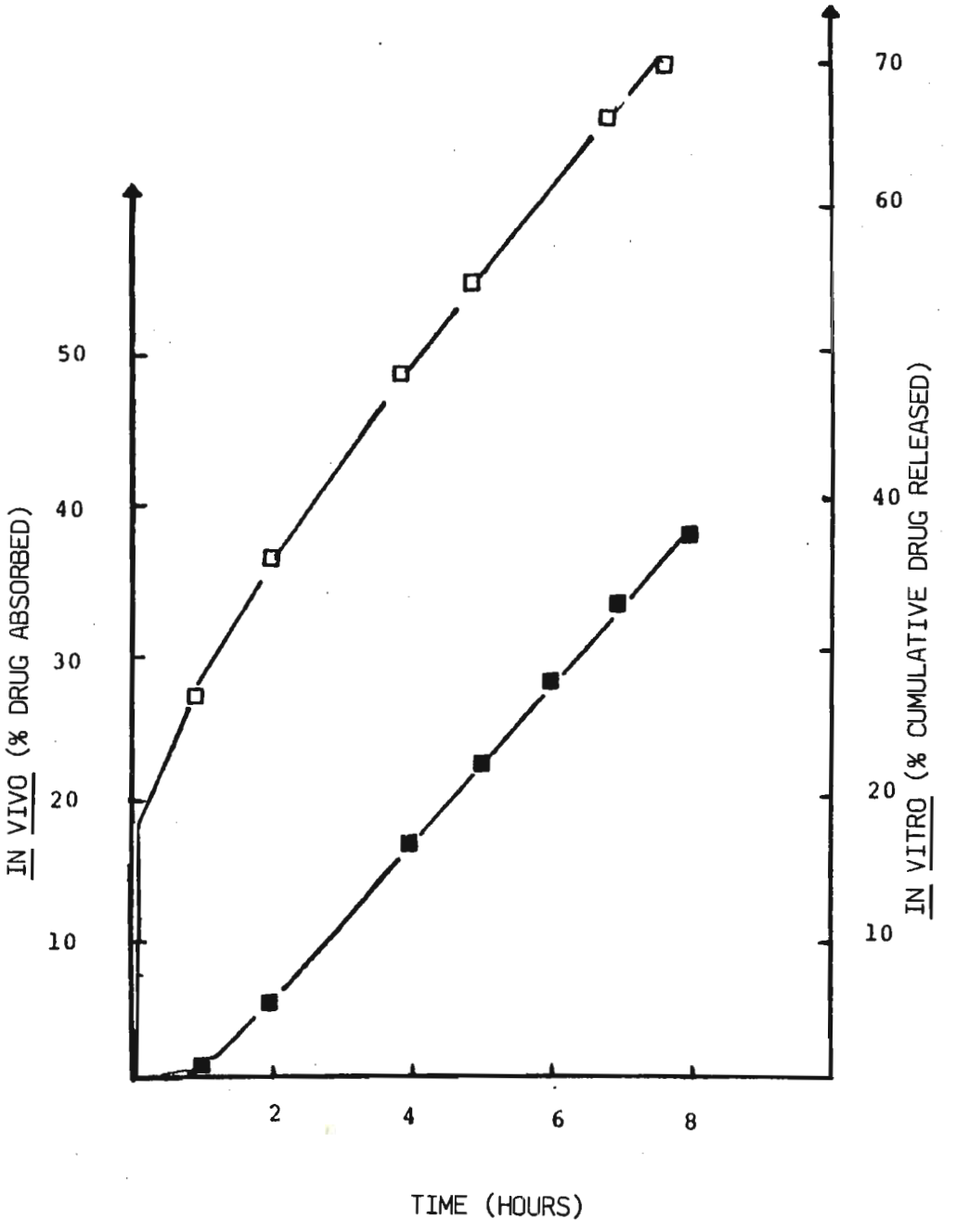


Figure 4.48: Comparison of the percentage of diethylpropion absorbed* (in vivo) at different times after a single rectal dose of diethylpropion hydrochloride sustained release pellets (Lot R 7773 - in a special suppository) to subject C.D. and of the cumulative in vitro release data at a constant pH of 6,9

in vivo ■——■

in vitro □——□



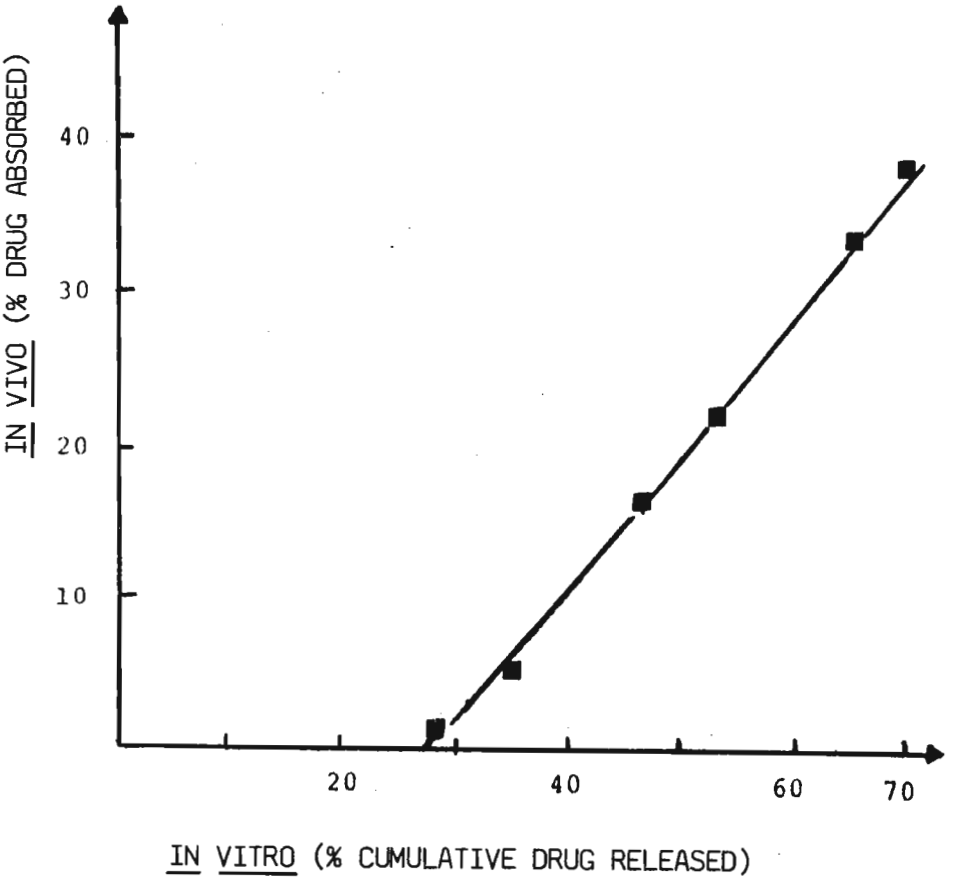
* % Drug absorbed =

$$\frac{\text{Sum of cumulative amount of Metabolite II and IV excreted at time, t} \times 100}{\text{Sum of cumulative recovery of Metabolite II and IV excreted at time, t}\infty}$$

Figure 4.49: In vitro/in vivo correlation of the release of diethylpropion hydrochloride from sustained release pellets (Lot 7773) administered in a special suppository, after single rectal administration to subject C.D. using urinary data (in vivo) and the cumulative in vitro release data at a constant pH of 6,9

$Y = 0,8925\ x - 25,37$

Corr. Coeff. = 0,997



The in vitro cumulative release and the percentage of drug absorbed after rectal administration of sustained release pellets (Lot R 7773) as a suppository, (Figure 4.48) gave very good in vitro/in vivo correlations (Figure 4.49). The resulting linear relationship ($y = 0,89 x - 25,37$; c.c. = 0,99) did not pass through the origin, due to the lag time for absorption (explained earlier). The different rates of agitation (in vitro) as compared to gut mobility in vivo during the trials, may also explain the differences of the slopes obtained for the three studies (Figures 4.45, 4.47 and 4.49).

A comparison of the urinary excretion rate data obtained after the sustained release pellets Lot R 7773 were administered orally or rectally (Figure 4.30) also confirms that the in vitro release of the drug corresponds to the in vivo data. This correlation facilitates the design of suitable sustained-release dosage forms using the principle involved in the release of drug from pellets described herein i.e. pellets with non-biodegradable membranes through which the drug diffuses.

There are no official dissolution test methods for monitoring drug release from suppositories. In vitro/in vivo correlations concerning rectal dosage forms are uncommon. The rotating bottle method clearly provided a useful dissolution test method for monitoring the drug release from the suppositories investigated in the present study i.e. suppositories into which sustained release pellets have been incorporated.

4.4 A Method to Predict the In Vivo Urinary Excretion Rate Profiles of Ethylaminopropiophenone (Metabolite II)

The in vitro/in vivo correlations on different lots of sustained release pellets taken rectally or orally, have been discussed (Section 4.3).

In the present investigations, we attempted to develop and to predict the urinary excretion rate profiles of ethylaminopropiophenone (metabolite II) by using a mathematical technique (proposed by a fellow researcher, A. Kyroutis, at Chelsea College, University of London) and then to compare these results with those profiles obtained during the different in vivo studies using sustained release pellets. This mathematical approach had been tested and found to give profiles that simulated very closely the experimental data in several bioavailability studies done by other research students in the laboratories at Chelsea College, University of London, viz. plasma levels of aminophylline, urinary excretion rate profiles of lithium and dimethylpropion after oral administration of the appropriate sustained release pellets, etc., (A. Kyroutis - personal communication, 1982). Therefore we have attempted to investigate the applicability of this method to the present studies.

Various methods for predicting the release of drugs in vivo from preparations, particularly those where the release of the drug is delayed or sustained, have been investigated by various researchers. Steinbach et al., (1980) described a mathematical technique to evaluate the bioavailability of drug preparations, and to predict blood-level profiles using single and multicompartment systems; the approach assumed that the release of the drug was zero-order.

The prediction of the shape of in vivo profiles (on blood or urine data) from in vitro release data would be very useful. Theoretical curves given by such mathematical expressions could be compared with those obtained from actual measurements of drug levels in vivo, and this comparison would in turn show how relevant the in vitro technique was to the in vivo situation.

Application of this process in reverse would enable the calculation of ideal in vitro release profiles from the in vivo drug levels; - this would facilitate the design of suitable formulations with desirable in vitro release profiles to reflect the in vivo situation as closely as possible without the use of many subjects and elaborate trials.

The general mathematical expressions and relevant calculations involved in the approach to develop the predictive urinary excretion rate profiles, have been outlined in Appendix V. The model which has been developed uses pharmacokinetic parameters (obtained after administration of the free drug, i.e. 25 mg diethylpropion hydrochloride in a single dose), in particular the rate constants of apparent absorption (s phase), distribution (α phase) and elimination (β phase), as well as in vitro dissolution data, in order to simulate (or predict) both the shape and maxima of the urinary excretion rate profiles of metabolite II.

Results on the in vitro release of the drug from different lots of sustained release pellets under investigation, which are to be substituted in the equations (outlined in Appendix V), are given in Table 4.18.

The pharmacokinetic parameters and related data on the apparent absorption (s phase, S value), distribution (α phase, A value) and elimination (β phase, B value) of ethylaminopropiophenone (metabolite II) obtained by using the Residual Method (Ritschel, 1980) in the different in vivo studies, are tabulated in Table 4.19 and illustrated, as a typical example, in Figure 4.50.

Using the superposition principle (Wagner, 1975) it has been shown that for two subjects viz. C.D. and A.M. the extrapolated urinary excretion rate profiles of ethylaminopropiophenone (metabolite II) are superimposable onto the actual urinary excretion data (Figure 4.52); in each case the extrapolated profiles were

Table 4.18 In vitro dissolution data[*1] on different sustained release pellets formulations, (for substitution into equation[*2]) used for predicting urinary excretion rate profiles

Time (Hours)	Sustained Release Pellets - Lot:		
	R 7773[*3]	R 7574[*3]	R 7773[*4]
1[*5]	24,8	32,9	27,7
2	5,7	25,1	7,4
3	10,1	14,2	6,0
4	13,5	11,7	6,0
5	10,2	3,2	7,2
6	8,5	5,3	5,6
7	6,8	4,7	5,6
8	6,0		4,5
9	6,0		

*1 Expressed as the percentage release rate (%/hr) of diethylpropion hydrochloride

*2 Outlined in Appendix V

*3 Craded pH profiles - procedure outlined in section 2.A.2.2.d(i)(a)

*4 Constant pH 6,9 profiles - procedure outlined in section 2.A.2.2.d(i)(b)

*5 Data at 1 hour includes the free unbound drug present in pellets

Table 4.19 Pharmacokinetic parameters on ethylaminopropiophenone (metabolite II), obtained by using the Residual Method[*1] in different trials

TRIAL[*2]			PHASE (SLOPE) $(hours)^{-1}$			INTERCEPT VALUE (µg/min)		
No.	Subject/s	Route	s	α	ß	S	A	B
1	A to H[*3]	Oral	2,18	1,75	0,235	63,43	46,99	16,44
3	C.D.	Rectal	1,79	1,365	0,246	61,39	34,81	26,58
5 & 6	C.D.[*4]	Oral	2,066	1,257	0,1964	60,89	33,78	27,11

*1 Demonstrated using urine data, in example, Figure 4.50:

*2 Details in Table 3.1

*3 Mean of six subjects, calculated from urine data after the third dose (at 8 hours) - Figure 4.51

*4 Mean of two trials in the same subject, C.D. - Figure 4.41 and Table 4.17

Figure 4.50: Determination of the mean pharmacokinetic parameters by the residual method, using the urinary excretion rate profile of metabolite II (ethylaminopropiophenone) after oral administration of 25 mg diethylpropion hydrochloride to the same subject, C.D., on two separate occasions*1, under acidic urine conditions.

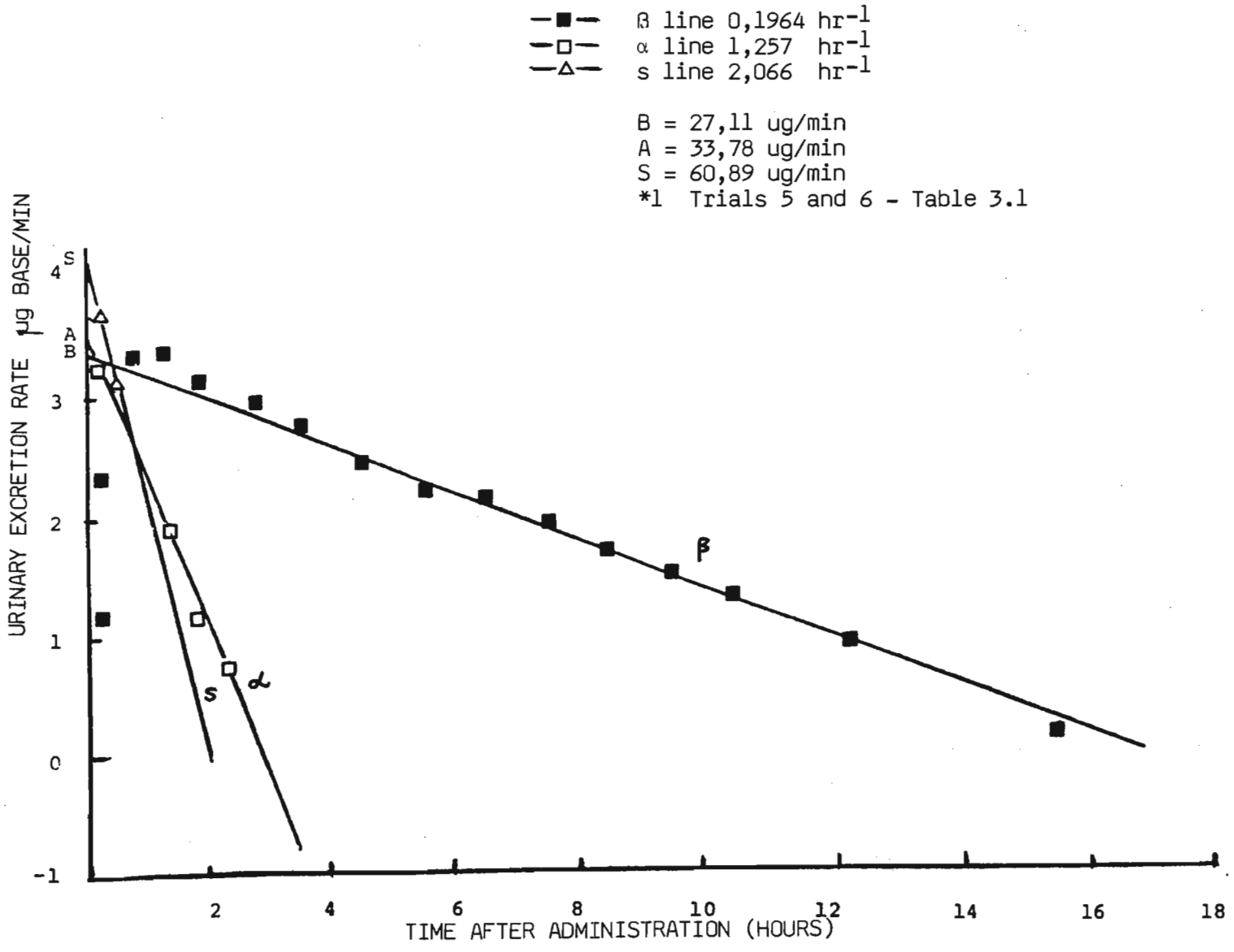


Figure 4.51 Semilog plot of the urinary excretion rate of ethylaminopropiophenone (metabolite II) after oral administration of 75 mg diethylpropion hydrochloride (DEP) as F.D.F.*1 to six subjects (A-H, group A*2) under acidic urine conditions

*1F.D.F. = Free dosage form, 25 mg DEP at 0, 4 and 8 hours (⬆ dose)

*2Group A, Trial 1, Table 3.1

Subject	β (10-17 hrs) hrs^{-1}
A	0,2261
B	0,2607
C	0,2123
D	0,240
E	0,234
H	0,236
Mean	0,235 hr^{-1}
± S.D.	± 0,03

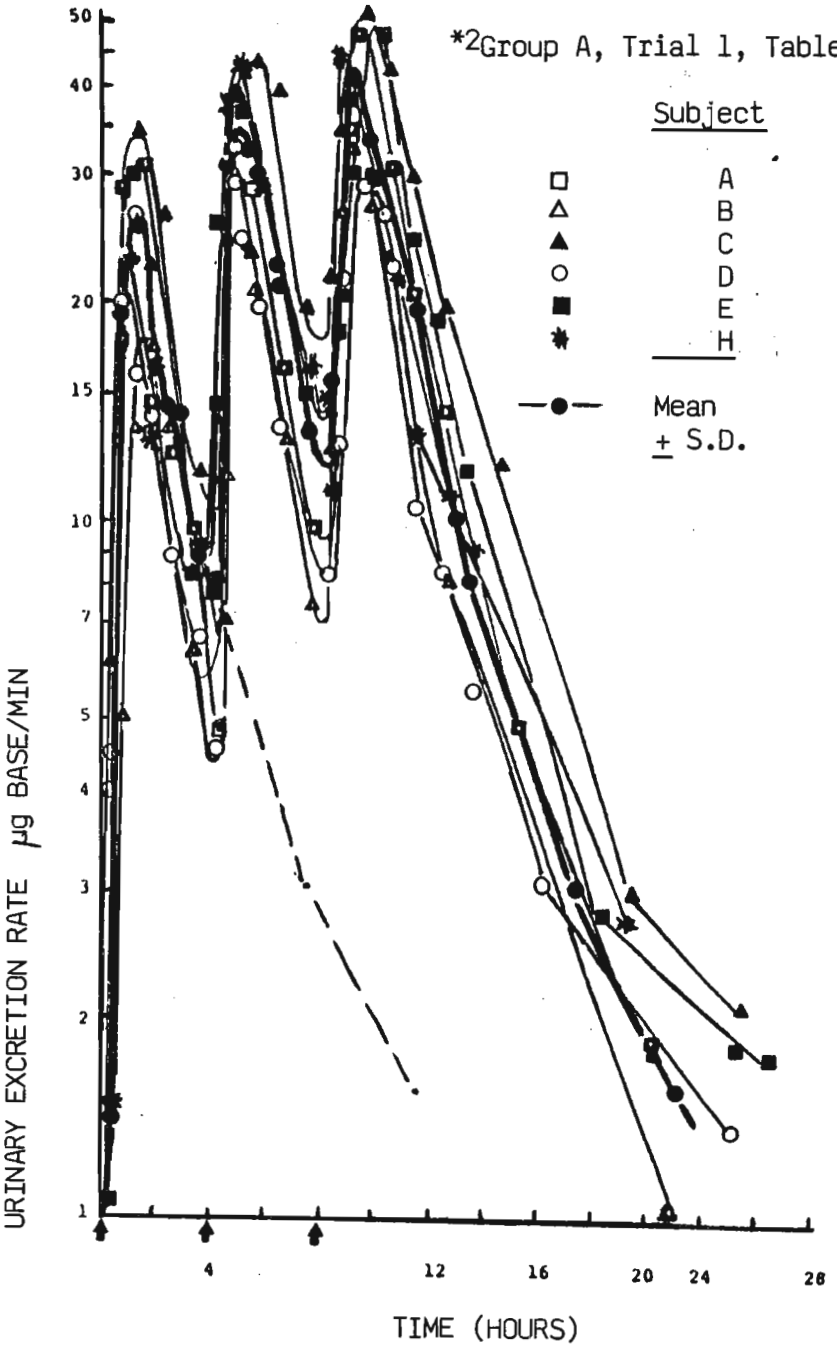


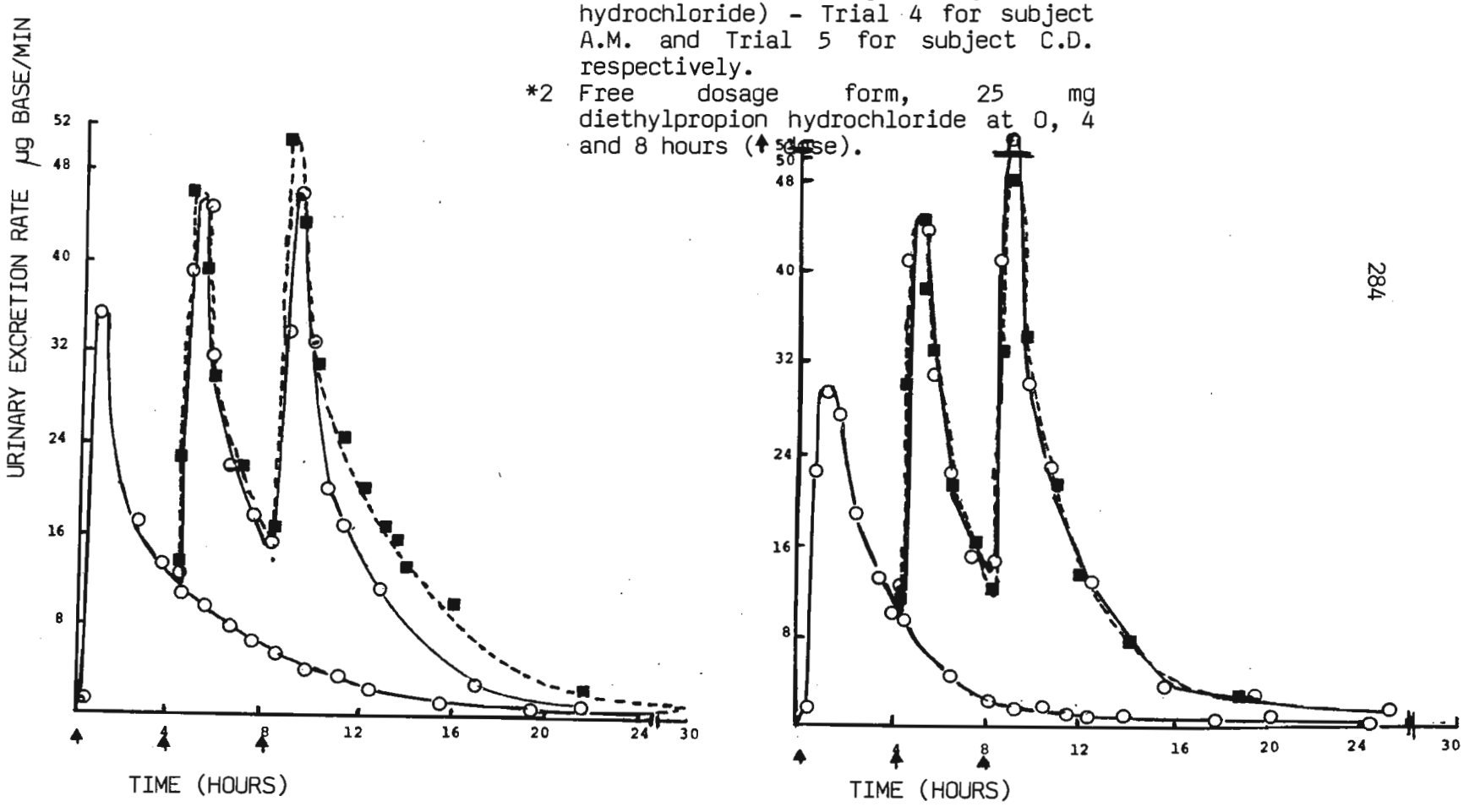
Figure 4.52 Comparison of the actual urinary excretion data to the extrapolated urinary excretion data (using superposition principle)*1 of ethylaminopropiophenone (metabolite II) after oral administration of F.D.F.*2 to two subjects under acidic urine conditions

o——o Experimental data - Trial 1 - Table 3.1

■·····■ Extrapolated data

*1 Data points of terminal slope (4 to 30 hours, o——o) after the first dose at 0 hours was taken from single dose studies (25 mg diethylpropion hydrochloride) - Trial 4 for subject A.M. and Trial 5 for subject C.D. respectively.

*2 Free dosage form, 25 mg diethylpropion hydrochloride at 0, 4 and 8 hours (▲dose).



obtained using data (0-4 hours) after oral administration of the first dose of Trial 1, Table 3.1, while the data from 4 to 30 hours were taken from the results of the single dose studies (Trial 4 for subject A.M. and Trial 5 for subject C.D. respectively - outlined in Table 3.1). Since the terminal slope (ß phase - Figure 4.52) after the third dose at 8 hours for the extrapolated and actual data were parallel to each other and comparable (identical) to the ß phase after the first dose, it could be considered proper to transpose the terminal slope starting at 4 hours after the third dose to the terminal slope starting at 4 hours after the first dose in each case. Since the elimination (ß) phases of metabolite II in each of the six subjects (Trial 1 - Table 3.1) were similar (Figure 4.51), the mean ß value was used in calculating the predictive profiles. In order to obtain the mean pharmacokinetic parameters associated with absorption, (S value and s phase) and distribution (A value and αphase) given in Table 4.19, and since single dose studies were not done on all six subjects (A-H), the mean ß slope (starting at 4 hours after the third dose) was transposed and linked at the 4th-hour point after the first dose and this completed profile was then utilized (Figure 4.51) - such a transposition is logical for reasons explained earlier.

4.4.1 Comparison of the in vivo and calculated profiles

(a) Sustained release pellets Lot R 7773 - oral dose

Figure 4.53 shows that the calculated profiles resemble closely the in vivo profiles. The differences in the two profiles may be due to the fact that the pharmacokinetic parameters were calculated from data following the third dose (Figure 4.51) instead of from single dose studies (which were not carried out). However, the terminal end of the profiles are parallel and therefore indicative of similar elimination rate constants.

(b) Sustained release pellets Lot R 7574 - oral dose

Figure 4.54 shows that the calculated profiles and the in vivo urinary excretion profiles are in close agreement especially in the first three hours and after 10 hours following administration of the drug. The use of mathematical expressions in calculating (predicting) profiles presupposes minimal variations in the biological system, but such limitations are not always possible with urinary data where urine flow and its pH are so variable. Hence, during the distributive phase (α phase) significant differences in the in vivo and the calculated profiles are observed.

(c) Sustained release pellets R 7773 - rectal dose

Figure 4.55 shows that the calculated profile does not resemble closely to the in vivo profile. The differences, particularly the higher peak and slower decline of the in vivo profile, may probably be due to the free drug present in the pellet, and the influence of its rapid availability in the small rectal environment of limited fluid contents.

It must be appreciated that different mathematical expressions (which are based on a fixed rate constant and order of process and on a model system) have limited applicabilities in predicting plasma levels and urinary excretion profiles. However, the expressions used in our studies (outlined in Appendix V) which relate to a single metabolite only, seem to be fairly useful in providing meaningful profiles that are reasonably superimposable on profiles obtained in studies following oral administration of the sustained release pellets (Lots R 7773 and R 7574).

Figure 4.53: Comparison of the mean urinary excretion rate profile of ethylaminopropiophenone i.e. metabolite II (■- - -■) with the calculated profile*1 (□——□), after oral administration of a single dose of sustained release pellets Lot R 7773 (≡75 mg diethylpropion hydrochloride) to six subjects*2 (Group A)

*1 obtained by substituting the relevant pharmacokinetic data (Table 4.19) and dissolution test data (Table 4.18) into expressions in Appendix V

*2 subjects (A-H) of Group A, Trial 1, Table 3.1

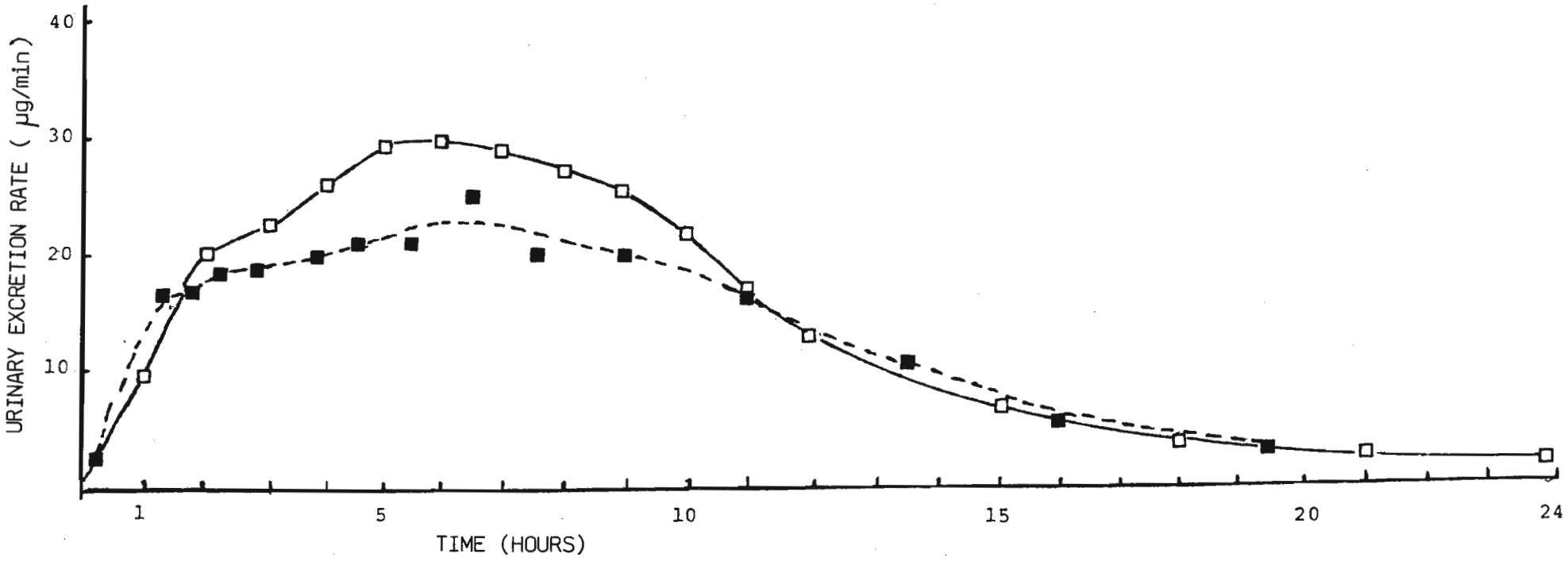


Figure 4.54: Comparison of the mean urinary excretion rate profile of ethylaminopropiophenone i.e. metabolite II (■- - -■) with the calculated profile*1 (□———□), after oral administration of a single dose of sustained release pellets Lot R 7574 (≡75 mg diethylpropion hydrochloride) to two subjects*2

*1 obtained by substituting the relevant pharmacokinetic data (Table 4.19) and dissolution test data (Table 4.18) into expressions in Appendix V

*2 Trials 13 and 14, subjects C.D. and A.M. respectively, Table 3.1

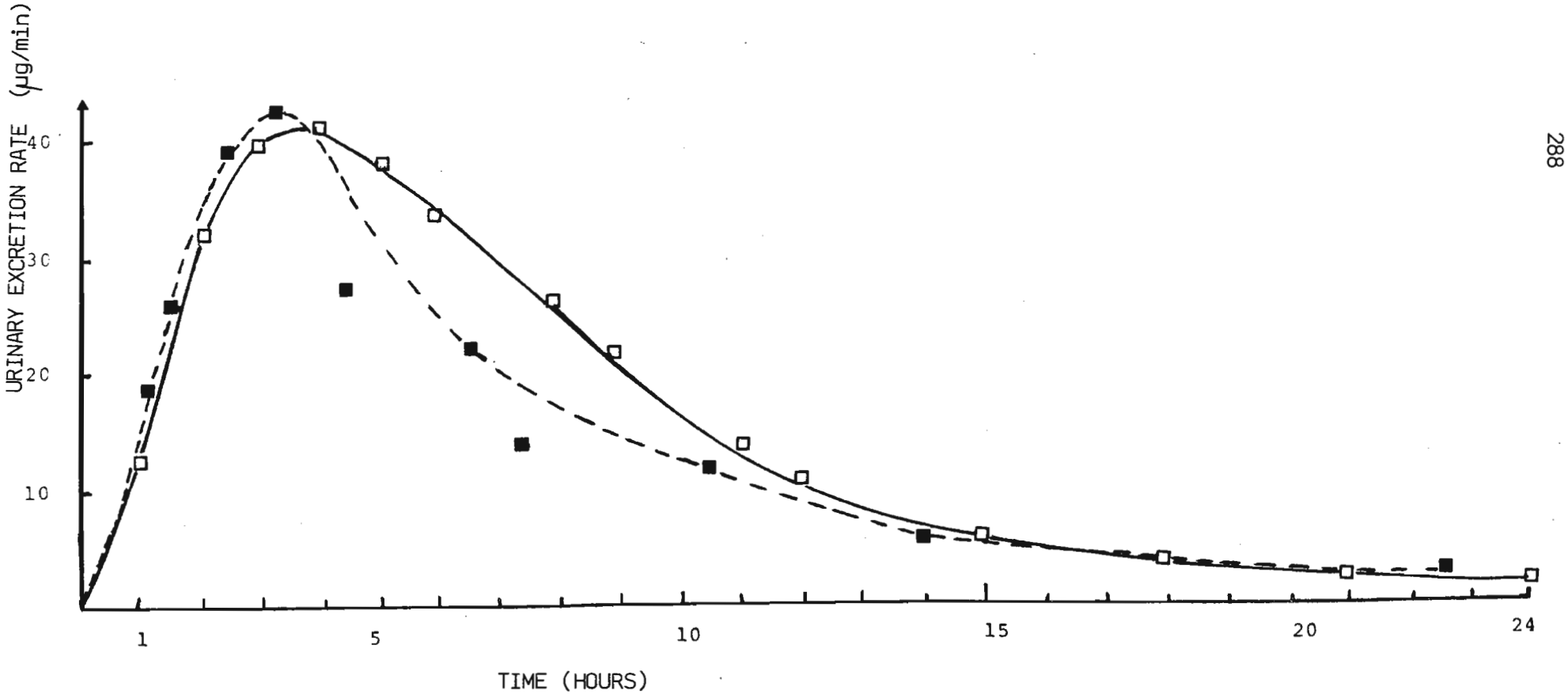
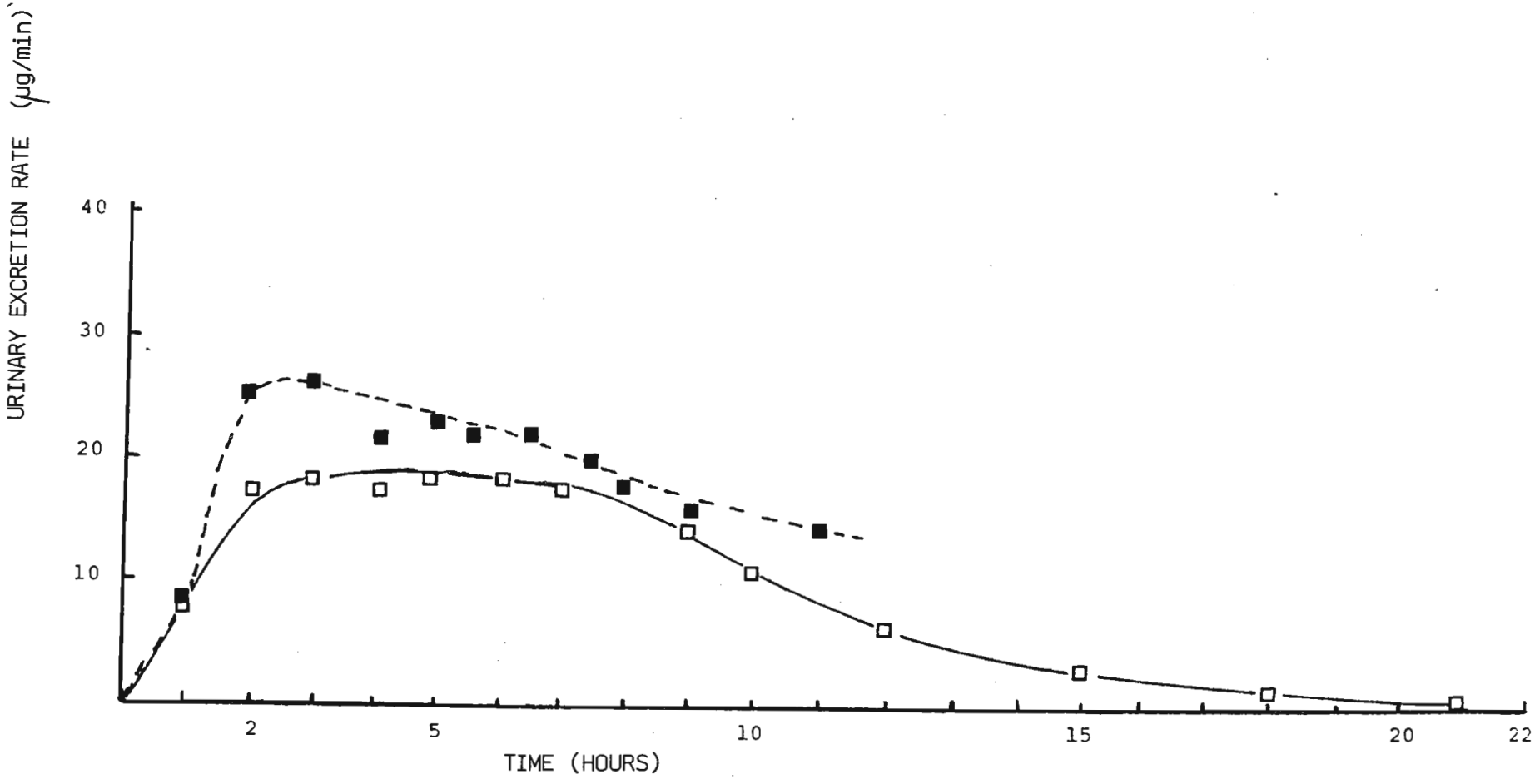


Figure 4.55: Comparison of the urinary excretion rate profile of ethylaminopropiophenone i.e. metabolite II (■- -■) with the calculated profile*1 (□——□), after rectal administration of a single suppository*2 containing sustained release pellets, Lot R 7773 (≡75 mg diethylpropion hydrochloride) to subject C.D.*3

*1 obtained by substituting the relevant pharmacokinetic data (Table 4.19) and dissolution test data (Table 4.18) into expressions in Appendix V
*2 prepared as outlined in 2.A.2.3 (iii)
*3 Trial 2, Table 3.1



4.5 Conclusions

The use of sustained release preparations could be of advantage in the administration of a drug of short half-life, such as diethylpropion hydrochloride, in that they prolong the average activity of the "active" compound/s and increase the dosing interval, whilst minimizing the excessive swing between potential unpleasant side effects related to central nervous system stimulation and ineffectively low concentrations of the drug in the blood. The advantage of administering sustained release pellets, each with a diffusion rate-controlled membrane, as a subdivided controlled delivery system has been discussed (1.3.2). Therefore, in the present studies, several investigations of such sustained release pellets formulations were undertaken (Chapter 3). Some conclusions drawn from the results of these studies (4.1 to 4.4) are summarised in this section: reference is made to the relevant results and discussions in the text.

1. In the two-way crossover study on twelve subjects (Trial 1, Table 3.1) the following results were obtained:

   (a) Diethylpropion hydrochloride, given in free from as three divided doses (3 x 25 mg) at 4-hourly intervals gave typical "peaks and troughs" in mean plasma concentration data of both unchanged drug and metabolites II and IV (Figure 4.1).

   (b) Diethylpropion hydrochloride (75 mg) taken as a single dose of sustained release pellets gave a broad plateau in plasma levels of both drug and metabolites II and IV, which extended for 8 to 10 hours. The mean plateau concentration was intermediate between the "peaks and troughs" after the free drug (Figure 4.1) was administered.

   (c) The free form gave profiles with a "staircase effect" so that the last dose in this dosage form gave a higher level than the first dose (Figure 4.1). Six of the twelve subjects experienced side effects after the last

dose. On the other hand, side effects were not observed when the sustained release pellets had been administered (Table 4.8).

(d) The change in formulation from free drug to sustained release pellets in any individual did not markedly influence the overall pattern of metabolism of the drug (Table 4.6); for instance, those subjects with pronounced monodealkylation relative to carbonyl reduction (e.g. subjects C and E of group A) showed the same emphasis when both formulations had been administered (Figure 4.4 relative to Figure 4.6).

(e) Salivary concentrations as well as urinary excretion data support the above conclusions (1 a-d) in Figure 4.8, Table 4.5 and Figure 4.17, Table 4.6 respectively.

(f) Comparison of mean plasma, saliva and urinary data profiles of either metabolite II or IV, (Figures 4.22 and 4.23) after administration of both dosage forms, shows the appearance of saliva peaks in advance of those in the urine; also saliva levels relative to plasma levels are higher in the absorptive phase compared to the same data at later times. An explanation for this latter result has been discussed (4.1.4; c.f. Posti, 1979).

(g) The usefulness of drug/metabolite concentrations in saliva during steady state levels in therapeutic monitoring or in biopharmaceutical and pharmacokinetic studies, in general, instead of in plasma, has been outlined (1.4). The mean S/P ratios for each metabolite (II and IV) at each collection time between 3 - 10 hours post-dose of sustained release pellets, are relatively constant (Table 4.9) and are in close agreement with the predicted S/P ratios (Table 4.10) for a salivary pH of 6,6 to 6,63. The S/P ratios of metabolite II are generally slightly higher than those of metabolite IV, and this difference is as a result of their differing pKa

and lipid solubilities (4.1.4). Therefore, with the use of diethylpropion sustained release pellets the plasma levels of the major metabolites (II and IV) could be predicted from salivary data during the period of plateau concentration, provided the salivary and plasma pH values are known.

(h) There was an excellent linear relationship between the mean urinary excretion rate (U) at various mean times of urine collection and mean plasma concentration (P) at these times for both metabolites, in the period between 2,5 $\pm$ 0,5 to 9,0 $\pm$ 1,0 hours after the oral administration of the sustained release pellets to six subjects in Group A (Table 4.11). The U/P ratios between individuals (inter-subject) during plateau levels, showed consistency and minimal fluctuations due to the good control of urinary pH. This was contrary to S/P ratios where more fluctuations (especially with metabolite IV) occurred because of the difficulty to control the pH and flow rate of saliva. The results support predictions of plasma levels from urinary excretion data during the post-absorptive phase even with single dose studies with provisio of acidic urine conditions (4.1.5).

(i) The results of the bioavailability of the sustained release pellets relative to a dose of the free drug of diethylpropion are shown in Table 4.7.

For subjects in Group A, the bioavailabilities calculated from the results of metabolite II, using either plasma, saliva or urine data, indicate that all subjects gave a bioavailability result above 75%. Results calculated from metabolite IV indicate that in all the subjects the bioavailability was above 75% when the saliva and urine data were considered, whilst 72,5% was obtained when the plasma data were considered. When the sum of metabolites II and IV was considered, all the subjects gave results

indicating bioavailability above 75% in plasma, saliva or urine data calculations. The bioavailability of the drug from the pellets is 81,8% to 93,8% when plasma data is considered, 87,4% to 136,1% when saliva and 88,4% to 127% when urine data is used (Table 4.4).

For subjects in Group B, the bioavailabilities calculated from urine results of metabolite II indicate that all subjects gave bioavailability results above 75% and the sum of metabolites II and IV also indicates that all the subjects produced results of more than 75% bioavailability. However, when metabolite IV is considered, 33% of the subjects gave a result indicating less than 75% bioavailability, but the two results under 75% are close to this figure i.e. 73,2% and 69,0%.

The results on salivary data indicate that it is less reliable than urine and plasma data as a measure of relative bioavailability. When the sum of two metabolites, II and IV are considered, 60% of the subjects give bioavailability results below 75%. The mean values, however, indicate 88% bioavailability for the sum of metabolites II and IV in salivary data. The reason why the salivary data in Group B are less reliable than in Group A, is that the flow rate was not well controlled in Group B, and hence the concentration fluctuated with the flow rate of the saliva.

(j) The broad plateau levels starting from about 2 hours to about 10 hours after administration and intermediate between the "peaks and troughs" seen after administration of the free drug, the lack of any side effects and the excellent bioavailability relative to a dose of the free drug, are conclusive evidences that sustained release pellets of diethylpropion hydrochloride (R 7773) can be used advantageously to replace the drug in free form administered at four-hour intervals.

(k) Statistical evaluations of the plasma data of six subjects in Group A, involving the Analysis of Variance (Tables 1 and 2 - Appendix VI) at each collection time for metabolite II and IV respectively (Tables 3 - 9, Appendix VI), the "areas under the curve" for metabolites II and IV, and also the sum of both metabolites plus unchanged drug (Tables 10 and 11, Appendix VI), showed, in most instances, no significant differences amongst subjects and within subjects. However, some of the lower values ($\measuredangle 0,5$) obtained for the significance could have been due to one of the following facts:

(i) the lower levels of both metabolites after administration of pellets than after using the free form, and

(ii) inter-subject variations in the rate and type of metabolism, i.e. subjects C and E produced significantly lower and higher amounts of metabolite IV (respectively) than the others.

2. The relative bioavailability and metabolism of diethylpropion hydrochloride (based on urine and saliva data of metabolites II and IV) after rectal administration of the free drug (25 mg, single dose) or of sustained release pellets (Lot R 7773; $\equiv$ 75 mg drug) formulated into a special suppository, did not significantly differ from those when equivalent doses of the free drug of the same sustained release pellets (in capsule), were given orally to the same subject, C.D. (Table 4.12). However, after rectal administration of either dosage form there was a delay in absorption with the plateau levels lower and of longer duration (12 to 14 hours) relative to the oral route (Figures 4.29 and 4.30). There was no convincing evidence of avoidance of first-pass metabolism with rectal use. This suggests that the formulation must have moved upward and remained in the rectum in a region from where veins drain predominantly into the portal system.

3. The distinct advantage of using a subdivided controlled delivery system (i.e. sustained release pellets each with a diffusion rate-controlled membrane - Lot 018010), to a single unit sustained release tablet (erosion-core type - Lot 284 BB) in relation with the influence of the physical presence of food, has been vividly demonstrated in studies on subject C.D. under controlled acid urine conditions. (Figure 4.32, Vs Figure 4.34; Table 4.14); identical food (details in 3.3.3) taken 10 minutes before the oral dose, markedly delayed the release and significantly decreased the bioavailability of the drug (relative to fasting conditions) from the single unit dosage form, while with the sustained release pellets a minimal change was seen in release and bioavailability of the drug (4.2.2).

4. The metabolism of diethylpropion, and the urine excretion rate of its metabolites after oral administration were reported to be dose-independent and unaffected by the type of dosage form used (Beckett et al., 1974a; Mihailova et al., 1974). This was confirmed in different studies on two subjects (C.D. and A.M.) who were given different dosage forms via oral and rectal routes (4.2.5). The urinary excretion kinetics (i.e. β values and the corresponding half-lives) of the major metabolite, ethylaminopropiophenone, were similar in all the studies (Table 4.17) and showed minimal inter- and intra-subject variations with different formulations and routes of administration.

5. The bioavailabilities of two batches of pellets of similar design but with different <u>in vitro</u> release profiles (Table 2.B.1: Lot R 7574 faster releasing than Lot R 7773), based on the total amount of metabolites (II and IV) excreted over 48 hours, were comparable to each other and to the free drug (25 mg at 0, 4 and 8 hours) after oral administration in the same subject, C.D. (4.2.4). However, the faster releasing pellets (R 7574) gave urinary plateau levels for metabolites II and IV that were higher and of shorter duration than those seen after

oral administration of the slower releasing pellets Lot R 7773 (Figure 4.40, Table 4.16). The latter formulation gave satisfactory profiles and was selected (reasons outlined in 2.B.4) for detailed investigations in twelve subjects (Trial 1, Table 3.1).

6. The need for quantitative correlations between *in vitro* and *in vivo* data from a quality control point of view and in the design of different dosage forms of the drug, as well as in the predictions of the behaviour of dosage forms in the body, have been discussed (2.A.1). Excellent *in vitro/in vivo* correlations (4.3) have been observed in studies on different lots of sustained release pellets viz. Lot R 7773 (Figure 4.45) and Lot 7574 (Figure 4.47) given orally and Lot R 7773 given rectally (Figure 4.49). The possible reasons for the occurrence of a lag time have been outlined (4.3).

7. Attempts to develop and to predict the urinary excretion rate profiles of ethylaminopropiophenone, i.e. metabolite II, by using a mathematical technique (detailed in Appendix V) showed promise as the calculated profiles were comparable with those profiles obtained during *in vivo* studies (Table 3.1) using single oral doses of two different formulations of sustained release pellets Lots R 7773 and R 7574 (Figures 4.53 and 4.54 respectively). The possible explanations for differences in *in vivo* profiles and calculated profiles have been discussed (4.4).

APPENDIX IA

The name and form of different pharmaceutical compounds and the names of suppliers

No.	Name*	Form	Supplier
1	Diethylpropion Hydrochloride	Free	Temmler-Werke (Germany)
2	Diethylpropion Hydrochloride Lot 018010	S.R.P.	Temmler-Werke
3	Diethylpropion Hydrochloride Lot R 7574	S.R.P.	Lemmon S.A. (U.S.A.)
4	Diethylpropion Hydrochloride Lot R 7773	S.R.P.	Lemmon S.A.
5	Diethylpropion Hydrochloride Lot Merrell 284 BB	S.R. Tabs.	Zenith Labs. U.S.A.
6	Diethylnorpseudoephedrine Hydrochloride	Free	Temmler-Werke
7	Ethylaminopropiophenone Hydrochloride	Free	Temmler-Werke
8	Furfurylamphetamine Acetate	Free	Diamant Lab. S.A. (France)
9	Cinnamyl Alcohol		B.D.H.

S.R.P. Sustained Release Pellets

S.R. Tabs. Sustained Release Tablets

* All compounds were kindly donated by suppliers, except item 9

APPENDIX IB

Different substances (1), packing materials (2), solvents and solutions (3) employed during research.

		Supplier
1:	Base H (Witepsol H-15)	Brome and Schimmer, Ltd.
	Boric Acid	B.D.H.
	Cream Placebo	Hoechst. A.G.
	Corn Oil (Mazola - pure)	Safeway Food Store Ltd.
	di-Potassium hydrogen phosphate Analar	Fisons
	di-Sodium hydrogen phosphate Analar	Fisons
	Gelatin Capsules (hard)	Lilly Laboratories
	Potassium chloride Analar	Fisons
	Potassium dihydrogen phosphate Analar	Fisons
	Sodium chloride, Analar	Fisons
	Sodium dihydrogen phosphate, Analar	Fisons
	Sodium hydroxide pellets, Analar	Fisons
2:	Apiezon L	Perkin Elmer
	Carbowax 20M	Perkin Elmer
	Chromosorb G (A.W., D.M.C.S., 100-120)	Perkin Elmer
3:	Ammonia Solution (Strong) BP	Fisons
	Chloroform, Analar	Fisons
	Diethyl ether, Analar	Fisons
	Hydrochloric acid conc; IN; 0,IN;	Fisons
	Methanol, Analar	B.D.H.
	Sulphuric acid 0,5N; 1,0N; 0,1N;	Fisons
	Toluene, Analar	Fisons

APPENDIX II

Official requirements for dissolution tests

The BP 1980 requires dissolution tests to be carried out on only 14 drug substances. These drugs are selected as "materials that might give rise to clinical problems if the required dosages were not made available".

The drugs are also selected if "they have been the subject of allegations on bioinequivalence". The dissolution test (rotating basket method) is "simply a physical requirement of the dosage form in question and it has not been considered essential to attempt to correlate results obtained with those obtained in vivo".

The BP 1980 does not specify any dissolution tests for sustained release preparations.
"Preparations purporting to exhibit delayed or sustained release characteristics are also being studied but certain of these materials are giving rise to particular problems."

The current edition of USP/NF requires dissolution testing for 52 drugs and describes three types of apparatus for dissolution testing: rotating basket, rotating paddle and a third one similar to the disintegration test apparatus (Banaker and Block, 1980).

The USP and NF both introduced dissolution tests in 6 monographs in 1970. In the subsequent 5 years period leading to the publication of USP XIX and NF XIV, requirements were introduced into several more monographs. The USP executive committee of revision favoured the inclusion, during the 1975 - 1980 review period, of a dissolution test in the monographs of all official oral solid dosage forms except where such inclusions were judged inappropriate.

Implementation of the policy was not achieved. However, dissolution tests are required in many monographs.

The dissolution media specified are all distilled water or buffers in which a constant pH is maintained throughout the dissolution test.

Controlled or sustained release preparations are not mentioned with respect to dissolution testing. There is therefore no Official BP of USP method for the dissolution testing of controlled/sustained release preparations.

The important points to be considered in the evaluation of controlled release formulations, particularly in regard to *in vivo* and *in vitro* release behaviour, have now been well documented by Roufail (1983).

Recommendations

On the basis of consistent *in vitro* results (see Chapter 2 and Slipper, 1981), and good *in vitro*/*in vivo* correlations reported by many workers in the laboratories of Chelsea College, University of London (Slipper, 1981; Hassanzadeh, 1981) and together with the present study, it is suggested that the rotating bottle method as used in these studies may be considered for inclusion in the BP as a means of carrying out meaningful *in vitro* dissolution tests on controlled/sustained release preparations. Also distilled water should not be taken as the only suitable dissolution medium for the *in vitro* dissolution testing of all controlled/sustained release preparations.

APPENDIX III

Diet and screening tests data on six subjects (Group A) involved in the clinical trials (Trial 1 - Chapter 3).

APPENDIX IIIA

Clinical chemistry screening test results

Test	Subject					
	A	B	C	D	E	H
sodium (mmol/l)	139	138	142	141	140	140
potassium (mmol/l)	4,1	4,0	3,8	4,5	3,9	3,6
urea (mmol/l)	3,1	6,9	4,0	4,9	3,6	3,5
creatinine (umol/l)	82	92	64	82	82	72
phosphate (mmol/l)	1,01	1,12	0,99	1,14	1,20	1,03
alkaline phosphatase (iu/l)	49	72	68	67	81	44
bilirubin (umol/l)	13	11	39	9	10	9
∂ GT (iu/l)	8	13	15	11	10	10
total protein (g/l)	68	68	81	78	70	68
albumin (g/l)	42	44	50	49	46	40
Calcium (mmol/l)	2,20	2,32	2,47	2,45	2,38	2,14

APPENDIX IIIB

Haematology screening test results

Test	Subject					
	A	B	C	D	E	H
WBC x 10^9/l	5,9	9,0	3,7	6,4	4,9	7,1
RBC x 10^{12}/l	4,76	4,81	4,82	5,21	5,11	4,47
Haemoglobin (g/dl)	13,9	15,6	14,7	15,6	15,5	12,8
PCV	0,41	0,45	0,44	0,42	0,46	0,38
MCV (fl)	84	94	91	90	90	86
MCH (pg)	28,9	32,3	30,4	29,9	30,4	28,7
MCHC (g/dl)	34,1	34,7	33,7	33,3	34,0	33,6
Neutrophils. (%)	40	52	33	43	45	52
Lymphocytes (%)	45	40	56	46	41	45
Monocytes (%)	9	4	10	7	9	3
Eosinophils (%)	6	4	1	4	5	0
Basophils (%)	0	0	0	0	0	0
Platelets x 10^9/l	204	220	221	265	242	304

APPENDIX IIIC

Diet

Subject	6 hours after dose	10 hours after dose
A	3 slices toast and jam	½pint vegetable soup, ½lb hamburger in bun.
B	3 slices toast and jam	½ pint vegetable soup, ½ lb hamburger in bun, 1 banana
C	3 slices toast and jam	½ pint vegetable soup, 1 slice bread, ½ lb hamburger in bun, 1 banana
D	4 slices toast and jam	½ pint vegetable soup, 2 slices bread, ½ lb hamburger in bun, 1 banana
E	2 slices toast and jam	½ pint vegetable soup, 1 slice bread, 1 small pizza
H	3 slices toast and jam	½ pint vegetable soup, ½ lb hamburger in bun, 1 banana

NOTE : ½lb = ± 227,5 g

½ pint = ± 225 ml

APPENDIX IV

Detailed results on plasma, saliva and urine for diethylpropion (I) and its two metabolites, i.e. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of sustained release pellets and the free drug, to twelve subjects, (Trial 1 in Table 3.1 - Chapter 3).

Table 1: Plasma concentrations of diethylpropion (I) and its two metabolites, i.e. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of the free drug in three divided doses of diethylpropion hydrochloride (25 mg at 0, 4 and 8 hours) to 6 subjects (Group A)

Mean Time (Hours)	Unchanged Drug (I) ng/ml							Ethylaminopropiophenone (II) ng/ml							Diethylnorpseudoephedrine (IV) ng/ml						
	A	B	C	D	E	H	Mean ± SE	A	B	C	D	E	H	Mean ± SE	A	B	C	D	E	H	Mean ± SE
1	8	7	6,5	6	8	7,7	7,2 ± 0,31	46	15	40	33	45	40	36,5 ± 4,3	28	10	7	14	26	20	17,5 ± 3,2
2	10	11	8	8	11	11	9,8 ± 0,55	42	41	40	29	40	30	37,0 ± 2,2	20	21	11	13	35	22	20,3 ± 3,1
4	b.m.	b.m.	b.m.	b.m.	b.m.	b.m.		21	21	26	17	20	20	20,8 ± 1,1	10	15	6	9	29	20	14,8 ± 3.2
5	10	8	6,5	8,2	10	8	8,4 ± 0,5	58	56	49	36	35	36,2	45,0 ± 4,0	40	34	6,5	21	20	31,8	25,5 ± 4,5
6	12	13	10	8	13	12,5	11,4 ± 0,7	55	45	59	36,5	51	33,6	46,7 ± 3,8	33	30	11	23	30	34,6	27,0 ± 3,2
8	b.m.	b.m.	b.m.	b.m.	b.m.	b.m.		24	37	36	21	41	25	30,6 ± 3,1	20	25	8	17,5	29	23,5	20,5 ± 2,7
9	12	10	9	10	10	11	10,3 ± 0,4	50	48	52	25	49	40	44,0 ± 3,8	25	33	8	14,1	28	36,5	24,1 ± 4,1
10	15	12	11	13	13	14	13,0 ± 0,5	61	55	59	40	60	44,6	53,2 ± 3,3	43	37	12,3	19	28	37,2	29,4 ± 4,4
12	6	6	7	6	7	6	6,3 ± 0,2	25	36	33	29	40	33	32,6 ± 2,0	19	26	6	17,5	34	25,7	21,8 ± 3,7
24	b.m.	b.m.	b.m.	b.m.	b.m.	b.m.		b.m.	b.m.	b.m.	b.m.	b.m.	b.m.		b.m.	b.m.	b.m.	b.m.	b.m.	b.m.	

b.m. below limit of detection

Table 2: Plasma concentrations of diethylpropion (I) and its two metabolites, i.e. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of a single dose of sustained release pellets Lot R 7773 (≙ 75 mg diethylpropion hydrochloride salt) to 6 subjects (Group A)

Mean Time (Hours)	Unchanged Drug (I) ng/ml							Ethylaminopropiophenone (II) ng/ml							Diethylnorpseudoephedrine (IV) ng/ml						
	A	B	C	D	E	H	Mean ± SE	A	B	C	D	E	H	Mean ± SE	A	B	C	D	E	H	Mean ± SE
1	6	6,5	6,5	6	6	7,2	6,3 ± 0,17	30	25	34,8	21,2	30	19	26,6 ± 2,2	23	10	15	11	27	10	16,0 ± 2,7
2	8	7	7	8	6,8	11	7,9 ± 0,6	38,5	37,6	29	30,1	38,4	32	34,2 ± 1,6	28,5	16	16	14	21	12,5	18,0 ± 2,2
3	6	6,5	6,5	8	5,9	6,7	6,6 ± 0,3	36,3	54,8	36	32,6	33	28	36,8 ± 34	25,5	20,5	14	16	22,6	15	18.9 ± 1,7
4	7	8	6	7	6	7	6,8 ± 0,3	41,6	46,7	41	30,7	34	30,5	37,4 ± 2,5	38,1	24	11	15	32,1	19	23,2 ± 3,8
6	6	7	4,5	8	6	9	6,8 ± 0,6	35,3	40	41	34	38,2	39	37,9 ± 1,0	32,6	23	14	19	35,2	29	25,5 ± 3,0
8	7	6	11,5	7	4	6,6	7,0 ± 0,9	34,3	32,6	41	31	38	34	35,1 ± 1,4	32,4	21,5	11,5	17	29	31	23,7 ± 3,1
10	b.m.	b.m.	b.m.	b.m.	b.m.	b.m.		24,1	23	40	26,4	30	31	29,1 ± 2,3	24,1	15,1	12,2	14	31	27	20,5 ± 2,9
12	b.m.	b.m.	b.m.	b.m.	b.m.	b.m.		20	20	32	18,1	27,8	28	24,3 ± 2,1	18	13,5	11	11	26	20	16,6 ± 2,2
24	b.m.	b.m.	b.m.	b.m.	b.m.	b.m.		b.m.	b.m.	10	b.m.	b.m.	b.m.		b.m.	b.m.	5,3	b.m.	b.m.	b.m.	

b.m. below limit of detection

Table 3: Saliva concentrations of diethylpropion (I) and its two metabolites, i.e. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of the free drug in three divided doses of diethylpropion hydrochloride (25 mg at 0, 4 and 8 hours) to 6 subjects (Group A)

Mean Time (Hours)	Unchanged Drug (I) ng/ml							Ethylaminopropiophenone (II) ng/ml							Diethylnorpseudoephedrine (IV) ng/ml						
	A	B	C	D	E	H	Mean ± SE	A	B	C	D	E	H	Mean ± SE	A	B	C	D	E	H	Mean ± SE
0,5	23		21	23	11	40	23,5 ± 4	121		154	189	36	106	121 ± 23	81			113	99	48	85 ± 12
1,0	52	26	37	41	72	55	47 ± 6	435	84	375	222	343	240	283 ± 47	216	33	71	130	231	127	135 ± 29
1,5	24		24	22	40	31	28 ± 4	282	130	344	170	175	123	204 ± 33	151	52	52	125	105	59	91 ± 16
2,0								195	118	286	70	160	124	159 ± 28	127	46	46	46	142	77	80 ± 16
3,0								105	133	244	128	181	66	143 ± 23	69	63	47	107	116	51	75 ± 11
4,0								97	76	93		120	40	85 ± 12	55	41			123	35	63 ± 17
4,5	30		22	25	79	29	37 ± 9	55	101	128	70	425	128	151 ± 51	38	65		43	160	106	82 ± 20
5,0	51	32	41	42	78	63	51 ± 6	456	219	428	257	506	423	373 ± 51	300	96	90	200	356	247	215 ± 40
5,5	28	25	27	21	57	38	32,6 ± 5	353	173	455	252	477	336	341 ± 43	207	86	82	208	337	225	191 ± 35
6,0					43	22		306	249	410	175	359	213	285 ± 33	202	119	90	148	320	87	161 ± 33
7,0								152	126	319	126	225	174	187 ± 28	102	67	77	122	229	151	124 ± 22
8,0								86	69	158	99	182	88	114 ± 17	78	49	47	63	238	68	95 ± 32
8,5	31	21	21	22	70	33	33 7	130	88	152	84	166	176	132 ± 14	88	70	50	73	150	140	95 ± 15
9,0	42	36	40	40	81	89	55 9	251	203	255	116	189	462	246 ± 44	136	99	70	94	170	383	158 ± 43
9,5	31	26		22	45	44	34 4	311	230	400	214	252	425	305 ± 33	183	103	107	182	190	262	171 ± 22
10,0						28		322	294	494	240	234	231	302 ± 37	212	135	122	213	232	217	188 ± 17
11,0								168	162	339	136	465	181	242 ± 49	83	75	76	146	329	179	148 ± 37
12,0								163	100	258	146	230	170	178 ± 21	70	45	65	110	207	140	106 ± 22
13,0								100	57	232	83	198	119	131 ± 25	58	44	60	90	162	113	90 ± 16

Table 4: Saliva concentrations of diethylpropion (I) and its two metabolites, i.e. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of a single dose of sustained release pellets of Lot R 7773 (≡ 75 mg diethylpropion hydrochloride salt) to 6 subjects (Group A)

Mean Time(Hours)	Unchanged Drug (I) ng/ml							Ethylaminopropiophenone (II) ng/ml							Diethylnorpseudoephedrine (IV) ng/ml						
	A	B	C	D	E	H	Mean ± SE	A	B	C	D	E	H	Mean ± SE	A	B	C	D	E	H	Mean ± SE
0,5								74		80	85	48		72 ± 7	41			32			36,5 ± 3
1,0	27			24	36	41	32 ± 3	340	63	123	113	257	183	180 ± 38	168			56	204	116	136 ± 28
1,5	29				23	32	27 ± 2	208	120	126		217	215	177 ± 20	144	64			231	119	140 ± 30
2,0	26			20		23	23 ± 1,5	293	219	147	129	232	187	201 ± 22	177	82		52	158	86	111 ± 21
2,5	28			31	40	27	31 ± 2,5	258	148	113	195	250	216	197 ± 21	182	71		99	254	95	140 ± 30
3,0	30			27	30	37	31 ± 1,8	306	142	182	122	293	250	215 ± 29	231	86		73	243	150	156 ± 31
3,5	28			30	30	38	31,5 ± 2	205		170	165	236	235	202 ± 14	173			56	182	146	139 ± 25
4,0	31			30		32	31	307	153	169	136	192	314	212 ± 29	213	68		103	152	127	132 ± 22
5,0	27			29		36	31 ± 2	246	163	264	189	275	274	235 ± 18	174	83		120	249	148	155 ± 25
6,0	27			32	43	38	35 ± 3	203	157	203	212	423	262	243 ± 35	178	73		157	450	178	207 ± 57
7,0				43	23	40	31 ± 5	152	158	120	184	300	402	219 ± 40	134	72		150	310	207	174 ± 36
8,0				30	23	32	28 ± 2	139	132	200	178	256	323	204 ± 27	135	60		145	288	156	157 ± 33
10,0						30		129	66	196	196	196	301	180 ± 29	130	36		188	198	238	158 ± 31
12,0						23		84	104	164	150	204	283	165 ± 27	92	56		141	178	233	140 ± 28
14,0						27		61	64	75	71	110	231	102 ± 25	54			184	105	184	107 ± 24

Table 5: Saliva concentrations of diethylpropion (I) and its two metabolites, i.e. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of the free drug in three divided doses of diethylpropion hydrochloride (25 mg at 0, 4 and 8 hours) to 6 subjects (Group B)

Mean Time (Hours)	Unchanged Drug (I) ng/ml							Ethylaminopropiophenone (II) ng/ml							Diethylnorpseudoephedrine (IV) ng/ml						
	AM	AHB	CD	DL	SG	SM	Mean ± SE	AM	AHB	CD	DL	SG	SM	Mean ± SE	AM	AHB	CD	DL	SG	SM	Mean SE
0,5						46		37	61	29	62		62	50 ±6	20		18	50		123	52 ± 21
1,0						72		198		489	187		249	280 ± 61	104		125	85		201	128 ± 22
1,5						54		129		608	180	527	251	339 ± 86	64		178	122	145	228	147 ± 24
2,0						20		137	104	331	176	274	160	197 ± 32	71	102	81	87	65	165	95 ± 13
3,0								49	490	171	160	214	92	196 ± 58	76	112	63	115	84	104	92 ± 8
4,0								72		60	110	104	83	86 ± 8	75		58	80	31	204	90 ± 27
4,5						33		102		131	113		163	127 ± 11	66		56	53		248	105 ± 41
5,0						23		177		243	205	513	233	274 ± 54	118		190	118	188	236	170 ± 20
5,5						51		224		501	364	496	240	365 ± 53	108		145	154	184	167	152 ± 11
6,0								155		382	333	333	183	277 ± 40	94		127	183	124	128	131 ± 13
7,0								140		164	195	275	79	170 ± 29	41		87	150	105	56	58 ± 17
8,0								140		208		155	80	146 ± 23	62		71		18	91	60 ± 13
8,5								141	202	147	251	133	106	163 ± 20	60	114	60	148	48	116	91 ± 15
9,0						18		255	502	712	260		132	372 ± 93	112	198	246	202		155	190 ± 24
9,5						20		291	638	334	344	266	206	340 ± 58	121	215	167	224	104	235	177 ± 21
10,0						21		307	595	333	225	229	146	306 ± 58	141	197	170	152	75	153	148 ± 15
11,0								110	276	148	162	360	197	209 ± 34	63	101	107	138	139	175	120 ± 14
12,0						18		62	238	71	144	181	166	108 ± 24	36	123	112	135	123	121	108 ± 13
14,0									118		97	88	97	75 ± 17		72		87	56	113	82

Table 6: Saliva concentrations of diethylpropion (I) and its two metabolites, i.e. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of a single dose of sustained release pellets Lot R 7773 (≙ 75 mg diethylpropion hydrochloride salt) to 6 subjects (Group B)

Mean Time (Hours)	Unchanged Drug (I) ng/ml							Ethylaminopropiophenone (II) ng/ml							Diethylnorpseudoephedrine (IV) ng/ml						
	AM	AHB	CD	DL	SG	SM	Mean ± SE	AM	AHB	CD	DL	SG	SM	Mean ± SE	AM	AHB	CD	DL	SG	SM	Mean ± SE
0,5										72	274	46	17	102 ± 50						25	
1,0					24	56		69		382	416	191	190	250 ± 58	25		96	57	62	132	74 ± 16
1,5					37	44		92		294	172	312	263	226 ± 37	34		65	93	78	117	77 ± 12
2,0					25	50		126		283	227	210	182	205 ± 23	36		76	75	104	131	84 ± 14
2,5					51			127		281	211	185	125	186 ± 26	51		77	70	65	119	76 ± 10
3,0					22			141		278	166	283	121	198 ± 31	56		80	50	110	108	81 ± 11
3,5					43			156	94	314	222	234	77	183 ± 34	57	22	112	68	95	82	73 ± 12
4,0					41			201	152	338	118	316	77	200 ± 40	109	39	105	57	103	116	87 ± 12
5,0					38			107	362	422	156	324	116	248 ± 51	57	79	132	70	118	160	102 ± 15
6,0					36			115	258	452	133	415	62	240 ± 61	69	86	150	52	176	94	104 ± 18
7,0					35			117	137	322	242	458	57	222 ± 55	51	53	94	68	132	77	80 ± 11
8,0								54	140	259	161		80	140 ± 32	60	54	112	49		136	82 ± 16
10,0								42	112	214	132	400	166	178 ± 46	56	49	102	46	145	142	90 ± 17
12,0									100	189	122		24	109 ± 30		36	89	29		77	57 ± 12
13,0								29			57	251			35			15	103		
14,0										155							73				
15,0										114		113					55				

Table 7: Urinary excretion rates of diethylpropion (I) and its two metabolites, i.e. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of the free drug in three divided doses of diethylpropion hydrochloride (≡ 25 mg at 0, 4 and 8 hours) to 6 subjects (Group A)

Time (Hours)	Unchanged Drug (I) µg/min							Ethylaminopropiophenone (II) µg/min							Diethylnorpseudoephedrine (IV) µg/min						
	A	B	C	D	E	H	Mean ± SE	A	B	C	D	E	H	Mean ± SE	A	B	C	D	E	H	Mean ± SE
0,5	0,97		1,60	0,68		1,54		4,01		6,19	4,53	1,06	4,51	4,06 ± 0,74	2,0			2,11	0,78	2,76	1,91±0,36
1,0	3,97	0,74	1,40	1,09	6,10	3,79		29,08	5,06	28,64	20,13	17,61	17,55	19,68±3,29	16,48	3,26	3,93	9,25	17,0	9,28	9,87±2,13
1,5	2,10	1,32		0,77	4,63	2,64		32,28	13,88	34,32	15,88	30,44	23,44	25,03±3,24	19,68	9,65	4,32	8,43	19,86	12,79	12,46±2,34
2,0		0,28		0,81	2,54	1,15		14,80	17,45	22,38	13,56	16,53	13,19	16,32±1,268	10,95	10,46	3,44	8,35	12,18	7,24	8,77±1,18
3,0					1,01	0,875		12,58	13,77	26,03	8,98	14,40	8,58	14,06±2,37	10,58	8,94	3,78	6,34	11,30	6,78	7,95±1,06
4,0						0,80		9,64	6,36	11,96	6,74	8,39	9,29	8,73±0,769	7,49	5,24	3,11	5,08	7,99	8,43	6,22±0,77
4,5					5,69	0,56		5,14	7,11	11,82	4,53	14,55	7,21	8,39±1,474	4,63	4,41	2,75	3,80	12,81	6,48	5,82±1,35
5,0	4,88	1,00		6,04	6,60	5,74		34,00	24,54	38,65	29,61	25,43	38,17	31,73±2,30	25,24	14,10	5,32	20,23	20,44	18,68	17,34±2,57
5,5	2,08	1,87		2,23		4,80		28,40	23,48	42,48	23,78		43,59	32,36±3,98	20,51	12,85	5,56	16,56		28,18	16,75±3,37
6,0	1,98	2,54		1,64	3,10	2,36		24,42	20,99	43,5	19,35	37,61	28,18	29,01±3,59	16,18	17,07	6,95	15,60	20,53	17,76	15,68±1,72
7,0	0,524			0,69	1,29	0,93		16,17	13,69	39,50	13,38	22,40	17,69	20,81±3,654	12,89	9,76	5,79	12,64	16,98	14,24	12,05±1,44
8,0	0,422			0,54	0,89	0,55		9,73	7,4	19,8	12,3	15,29	16,74	13,54±1,718	9,00	4,68	4,15	12,36	15,86	12,76	9,80±1,75
8,5	0,422					1,58		11,02	12,79	21,48	8,14		15,41	13,77±2,023	9,71	11,72	4,11	7,16		15,16	9,57 ±1,69
9,0	2,56	2,75		1,20	2,25	8,70		20,52	26,47	34,97	12,81	18,05	45,23	21,18±3,104	16,58	17,26	6,32	13,58	17,29	29,60	16,77±2,81
9,5	5,26	2,88		2,99		5,31		48,26	32,98	48,15	34,95		38,07	40,48±2,91	33,65	23,14	8,91	20,63		26,99	22,66±3,65
10,0	2,84	1,73		3,26	5,74	2,32		27,27	27,09	50,16	28,52	29,88	29,45	33,73±3,83	27,35	19,75	8,49	19,35	21,95	18,30	19,20±2,29
11,0	1,49	1,23		2,10	6,79	1,94		30,95	23,58	42,26	26,18	47,11	21,76	31,91±3,85	21,22	18,48	7,64	17,71	25,48	15,26	17,63±2,24
12,0	0,426	0,69		0,56	2,56	0,65		20,17	20,08	29,33	10,33	24,08	13,35	19,55±2,58	16,76	16,78	6,10	9,23	17,04	11,78	12,95±1,73
13,0	0,454	0,32			1,94	0,85		14,33	8,21	19,39	8,36	18,96	10,70		13,05	6,69	4,84	7,78	14,9	9,91	9,53±1,44
14,0					1,22			9,22				11,73			9,95				10,44		
16,0								4,90		11,76					6,15		2,61				
18,0											3,04							3,35			
24,0					0,094			1,82	1,42	2,05	1,34	1,79	0,77	1,53 0,17	2,23	1,88		1,77	2,60	1,86	2,07 0,3
30,0									0,32		0,24					0,27		0,46	0,30		
34,0													0,183						0,07	0,44	

Table 8: Urinary excretion rates of diethylpropion (I) and its two metabolites, i.e. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of a single dose of sustained release pellets Lot R 7773 (≙ 75 mg diethylpropion hydrochloride salt) to 6 subjects (Group A)

Time (Hours)	Unchanged Drug (I) µg/min							Ethylaminopropiophenone (II) µg/min							Diethylnorpseudoephedrine (IV) µg/min						
	A	B	C	D	E	H	Mean ± SE	A	B	C	D	E	H	Mean ± SE	A	B	C	D	E	H	Mean ± SE
0,5	0,25					2,01		1,64		2,83	4,01		7,61	2,82 ± 0,55	1,02		0,54	1,29		2,69	1,39±0,40
1,0	3,71	3,74	0,44	0,79		2,87		19,06	20,18	10,20	17,56	8,53	11,93	14,44 ± 1,89	12,83	7,63	2,39	8,06	7,26	6,16	7,38±1,25
1,5	2,05	3,92		0,65		1,26		23,13	21,81	15,7	17,18	10,84	11,83	16,74 ± 1,87	14,62	7,91	3,92	8,40	11,55	6,71	8,85±1,39
2,0	2,43	1,60				1,85		24,72	21,75	13,71	18,63	9,76	13,09	16,94 ± 2,13	16,99	9,24	3,92	9,29	10,91	7,33	9,61±1,61
2,5	2,67	1,84				1,43		23,69	27,36	11,93	19,68	14,70	11,01	18,06 ± 2,45	17,01	11,09	3,5	6,54	11,73	3,86	8,96±1,96
3,0	1,11	2,71				1,50		21,4	28,88	12,73	22,21	13,94	11,34	18,42 ± 2,56	15,38	14,39	3,85	7,19	10,41	7,60	9,80±1,66
3,5	1,93	1,53		1,53		1,48		24,50	37,34	19,79	25,37	26,43	12,48	24,32 ± 3,06	18,40	14,91	6,05	7,53	20,01	5,55	12,74±2,3
4,0	1,74	1,55				2,26		24,46	26,82	19,88	14,85	22,02	16,48	20,75 ± 1,72	19,1	13,89	5,30	9,53	17,46	10,58	12,64±1,03
5,0	1,43	0,83				1,01		21,69	25,31	25,44	13,29	18,83	21,0	20,93 ±1,69	19,48	13,78	5,55	10,2	22,75	9,62	13,56±2,01
6,0	1,34	1,54		0,50		2,44		21,96	28,5	27,53	13,38	14,48	23,65	21,17 ± 2,58	19,73	12,47	8,34	8,79	15,09	15,55	13,33±1,63
7,0	1,36	0,74		1,30		2,38		20,30	27,74	35,94	23,48	17,46	25,55	25,30 ±2,23	15,88	12,13	5,55	14,28	17,59	16,45	14,64±1,42
8,0	1,16	0,49				1,55		16,33	25,24	22,76	16,72	18,44	17,43	19,49 ± 1,36	16,62	10,08	7,57	14,53	16,67	14,70	13,36±1,38
10,0	0,793	0,50				0,95		16,65	17,14	21,85	14,20	23,83	14,05	19,62 ± 2,03	15,59	10,00	7,33	12,23	18,66	12,04	12,64±1,49
12,0	0,73	0,11				1,25		14,22	11,65	16,93	23,05	16,12	15,58	16,26 ± 1,42	12,83	6,01	4,98	12,76	16,58	12,36	10,92±1,67
14,0	0,179							3,99				3,56		3,77 ± 0,15	3,46				3,31		
15,0		0,28							7,95	15,02	10,03			11,0 ±1,713		6,46	3,90	7,39			5,91±0,85
20,0	0,156							3,64							4,81						
24,0	0,145							1,30	3,35	8,06	1,63	2,49	4,33	3,52 ± 0,92	1,66	2,8	2,04	1,8	4,33	4,09	2,79±0,44
30,0								0,963	0,383			0,613	1,03		1,241	0,386			0,79	1,31	
36,0								0,498		1,76		0,716	0,554		0,424		0,498		0,840	0,79	
38,0								0,431		1,15					0,086		0,373				
46,0												0,346									
48,0								0,234	0,10	0,85					0,196	0,123			0.496		

Table 9: Urinary excretion rates of diethylpropion (I) and its two metabolites, i.e. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of the free drug in three divided doses of diethylpropion hydrochloride (≙ 25 mg at 0, 4 and 8 hours) to six subjects (Group B)

Time (Hours)	Unchanged Drug (I) µg/min							Ethylaminopropiophenone (II) µg/min							Diethylnorpseudoephedrine (IV) µg/min						
	AM	AHB	CD	DL	SG	SM	Mean ± SE	AM	AHB	CD	DL	SG	SM	Mean ± SE	AM	AHB	CD	DL	SG	SM	Mean ± SE
0,5						0,32		1,3	0,07	2,4	14,7		1,13	3,92 ± 2,43	0,5		0,7	5,9		1,53	0,9 ± 0,2
1,0						0,83		35,9	14,5	22,5	24,8	2,9	8,05	18,11±4,49	12,2	4,2	2,3	11,8	0,6	8,5	7,8 ± 1,8
1,5						1,65		20,3	27,9	30,3	17,0	18,6	18,63	22,1 ± 2,1	7,8	6,90	5,0	8,50	7,8	11,8	6,0 ± 0,8
2,0						1,48		20,4		27,7	18,7	22,7	18,41	21,6 ± 1,5	10,5		4,5	7,9	6,9	12,2	8,4 ± 1,2
3,0						0,52		17,6	19,0	18,9	17,6	28,8	11,20	18,9 ± 2,1	8,0		4,7	5,2	4,7	11,7	6,9 ± 1,2
4,0								13,4	13,8	13,7	11,4	11,8	8,44	12,1 ± 0,8	6,1	4,7	3,5	6,5	5,3	9,3	5,9 ± 0,7
4,5						1,14		12,5		12,7		19,3	12,8	14,3 ± 1,4	7,0		3,6		6,2	11,3	7,0 ± 1,4
5,0						3,95		39,2		41,1	19,3	44,5	28,10	34,4 ± 4,2	14,0		10,9	9,6	26,4	24,4	17,06±3,1
5,5						2,72		45,3		44,4	39,5	32,8	31,9	38,8 ± 2,5	15,7		10,2	20,4	9,10	25,4	16,16±2,75
6,0						1,21		31,6	14,5	31,7	33,0	31,8	18,4	29,3 ± 2,5	14,4	5,6	8,9	16,6	10,3	18,8	13,9 ± 1,6
7,0						0,95		12,9	20,9	22,1	22,9	19,0	18,1	19,3 ± 1,4	8,3	7,9	6,9	13,5	7,00	19,6	10,53±1,89
8,0						0,71		18,2	16,0	15,3	16,3	12,9	16,3	15,83±0,65	9,9	6,6	3,5	10,1	5,3	19,1	9,1 ± 2,1
8,5								14,8	19,4	14,1			10,6	14,7 ± 1,3	8,6	7,9	5,0			15,0	9,13 ± 1,83
9,0						0,99		34,0	34,3	41,2	10,8	14,6	14,9	24,97±4,84	14,9	12,7	11,1	7,0	5,4	16,4	11,25±1,62
9,5						2,05		46,5	31,3	53,5	7,3	13,2	15,7	27,92±7,08	19,0	11,3	11,2	5,9	5,4	20,0	12,13±2,3
10,0						2,54		30,8	26,5	30,8	16,2	17,1	24,7	24,35±2,37	14,8	10,9	10,3	12,4	12,6	20,26	13,54 ± 1,3
11,0						2,05		19,8	33,6	23,2		23,5	26,2	25,3 ± 2,1	11,6	12,6	9,1		8,6	23,9	13,2 ± 2,5
12,0						1,95		17,0		17,1	25,6	22,6	25,6	21,6 ± 1,7	8,8		8,0	14,2	9,3	23,8	12,8 ± 2,6
14,0						1,04			13,0	13,2	13,8	16,1	14,10	14,0 ± 0,5		8,0	6,4	10,3	6,4	23,2	10,9 ± 2,8
16,0								11,2	13,3		15,8	13,0			6,2	7,9		10,6	2,5		
24,0						0,57		2,4	7,4	3,9	3,8	4,1	5,2	4,4 ± 0,64	2,4	7,7	2,8	4,7	2,5	16,8	6,2 ± 2,1
26,0								0,9	2,3		1,7	2,4			1,2	3,4		2,9	1,9		
28,0								1,0	1,1						0,7	2,4	0,7				
30,0								0,84	0,7	0,6	0,8	0,8	0,9			1,9		1,5	1,0	2,9	
32,0								0,74	0,5	1,3	0,6	0,4			0,3	1,23	1,7	1,3	0,6		

Table 10: Urinary excretion rates of diethylpropion (I) and its two metabolites, i.e. ethylaminopropiophenone (II) and diethylnorpseudoephedrine (IV) after oral administration of a single dose of sustained release pellets Lot R 7773 (≙75 mg diethylpropion hydrochloride salt) to 6 subjects (Group B)

Time (Hours)	Unchanged Drug (I) µg/min							Ethylaminopropiophenone (II) µg/min							Diethylnorpseudoephedrine (IV) µg/min						
	AM	AHB	CD	DL	SG	SM	Mean ± SE	AM	AHB	CD	DL	SG	SM	Mean ± SE	AM	AHB	CD	DL	SG	SM	Mean ± SE
0,5		0,59	0,40			1,65		0,08	3,8	2,61	3,4	4,1	0,8	2,465± 0,62	0,13	0,9	0,6	0,7	1,5		0,8±0,2
1,0		1,14	2,29			2,23		1,3	17,8	26,2	26,7	5,9	9,4	14,55±3,4	0,8	3,1	5,6	7,1	2,8	10,4	5,0±1,3
1,5		0,55	1,04			1,88		9,2	21,5	24,5	25,1	12,2	12,3	17,47±2,61	3,5	2,7	5,1	7,2	5,7	11,3	5,9±1,2
2,0		0,77	1,11			1,34		19,0	18,9	22,2	27,6	20,8	12,0	20,1±1,9	5,7	3,0	4,8	7,6	7,5	9,5	6,4±0,9
2,5		0,73	1,28			1,25		25,9	25,2	20,6	30,2	19,1	11,7	22,1±2,4	9,2	4,1	5,21	8,1	8,6	10,5	7,62±0,9
3,0		0,85	0,60			1,15		30,8	18,6	21,9	30,9	19,5	11,8	22,25±2,8	11,8		6,2	9,6	8,4	12,8	9,36±1,13
3,5			0,36			3,18		38,9		21,6	34,1	20,3	11,0	25,2 ± 4,5	11,7	7,1	6,6	10,5	8,3	20,3	11,1±1,8
4,0		0,96	0,80			3,40		38,9	22,4	28,0	25,8	20,7	18,0	25,63±3,76	12,9	4,5	7,3	5,7	8,3	23,2	10,8±2,5
5,0		0,77	0,88			3,09		45,3	27,0	33,8	36,2	20,7	18,8	30,3±3,8	14,6	5,8	8,4	10,7	8,9	23,9	12,05±2,42
6,0		0,71	0,79			2,98		36,5	33,3	39,67	27,3	26,4	17,7	30,15±2,97	13,2	6,7	9,07	6,39	9,2	28,4	12,16±3,1
7,0			0,59			2,67		37,3	31,8	25,4	27,5	24,4	20,4	26,72±2,55	15,5	7,8	9,5	10,0	8,5	27,2	13,1±2,0
8,0						2,95		20,5	37,9	25,3	29,0	21,0	18,3	25,3±2,7	10,0	9,5	8,0	10,6	6,1	21,2	10,9±1,94
10,0			0,39			1,86		17,8	22,6	13,34	23,8	15,0	17,0	18,26±1,55	9,7	8,6	8,4	9,7	5,0	24,4	11,0±2,5
12,0		0,91	0,331			1,11		10,7	16,2	10,81	20,9	12,0	16,2	14,47±1,50	7,0	6,5	8,0	7,5	4,9	24,2	9,7±2,7
14,0		0,5				0,86		8,6	9,7		12,19		15,6	11,52±1,34	6,0	4,5	7,2	7,5		18,0	8,8±2,3
18,0								6,2		5,7	4,5				5,3		4,16	4,6			
20,0		0,3							5,4							2,9					
24,0			0,098			0,64		3,7		5,1	4,0		4,5	4,33 0,27	3,2		4,3	2,0		12,4	5,5 2,0
27,0								2,3		2,4	2,8				2,4		2,8	1,1			
30,0								1,6		2,2	1,6				1,6		1,7	1,0			
33,0								1,3			0,8				1,1			0,6			
36,0								0,6				0,3			0,77				0,35		
40,0								0,3			0,5				0,6						
48,0								0,17			0,28				0,42						

APPENDIX V

Expressions used to predict urinary excretion rate profiles of ethylamino-propiophenone i.e. metabolite II, after oral administration of sustained release pellets.

Method used for calculating the urinary excretion rate profile by using the in vitro dissolution data on the sustained release pellets and in vivo data on the free drug.

Step 1: Calculation of the following parameters from the urinary excretion rate profile of metabolite II after administration of the free drug (25 mg diethylpropion hydrochloride) orally or rectally.

A = Intercept of line α corresponding to the phase of distribution.

B = Intercept of line β corresponding to the phase of elimination.

S = Intercept of line s corresponding to the phase of absorption

α = Slope of the line α

β = Slope of the line β. See Figure 4.50 as an example.

s = Slope of the line s.

Step 2: Calculation of R_1, R_2, R_3 .... R_t, from equation (1).

$$R_t = \frac{D_t + D_{t-1}}{2 \times 100} \qquad \text{.....Eq.(1)}$$

where D_t = % amount of drug released in dissolution test at time from t to t + 1 hours. D_{t-1} = % amount of drug released in dissolution test at time from t-1 to t hours.

Step 3: Calculation of the urinary excretion rate ($U_t\beta$) at time t due to the β line, using equation (2)

$$U_t\beta = BR_1e^{-t\beta} + BR_2e^{-(t-1)\beta} + BR_3e^{-(t-2)\beta} \ldots BR_te^{-\beta}$$

.....Eq.(2)

where $U_{t\beta}$ = expected urinary excretion rate at time, t, due to the β line.

B, β, R_1, R_2, R_3 ..... R_t as defined previously.

Step 4: Calculation of the urinary excretion rate ($U_{t\alpha}$)at time t, due to the α line, using equation (3).

$$U_{t\alpha} = AR_1e^{-t\alpha} + AR_2e^{-(t-1)\alpha} + AR_3e^{-(t-2)\alpha} \ldots AR_te^{-\alpha} \quad \ldots\ldots\ldots\ldots\ldots\text{Eq.(3)}$$

where $U_{t\alpha}$ = urinary excretion rate at time t due to the α line.

A, α, R_1, R_2, R_3 .....R_t as defined previously.

Step 5: Calculation of the urinary excretion rate (U_{ts}) at time t due to the s line, using equation (4).

$$U_{ts} = SR_1e^{-ts} + SR_2e^{-(t-1)s} + SR_3e^{-(t-2)s} .. + SR_te^{-s}. \quad \ldots\ldots\ldots\ldots\ldots\text{Eq.(4)}$$

where U_{ts} = urinary excretion rate at time t due to the s line.

S, s, R_1, R_2, R_3 ..... R_t as defined previously.

Step 6: Calculation of the overall urinary excretion rate at time t (U_t), using equation (5)

$$U_t = U_{t\beta} + U_{t\alpha} - U_{ts} \quad \ldots\ldots\ldots\ldots\ldots\ldots\text{Eq. (5)}$$

APPENDIX VI

Statistical evaluations of the plasma data after oral administration of two different dosage forms of diethylpropion hydrochloride (≡ 75 mg) to six subjects (Group A, Trial 1 - Table 3).

Table 1: Crossover comparison studies of diethylpropion hydrochloride (1 x 75 mg sustained release pellets at time 0 hour VS 3 x 25 mg free dosage form at times 0, 4 and 8 hours

RAW DATA

DOSE	SUBJECT	PLASMA ETHYLAMINOPROPIOPHENONE (METABOLITE II) LEVELS (ng/ml) Time (hours)												PLASMA DIETHYLNORPSEUDOEPHEDRINE (METABOLITE IV) LEVELS (ng/ml) Time (hours)													
		0	1	2	3	4	5	6	8	9	10	12	*1 A.U.C.	0	1	2	3	4	5	6	8	9	10	12	*1 A.U.C.	*1,2 A.U.C
3 x 25 mg	A	0	46	42		21	58	55	24	50	61	25	47,5	0	28	20		10	40	33	20	25	43	19	30,5	83
	B	0	15	41		21	56	45	37	48	55	36	45,0	0	10	21		15	34	30	25	33	37	29	29,0	80
	C	0	40	40		26	49	59	36	52	59	33	51,0	0	7	11		6	6,5	11	8	8	12,3	6	9,5	66
	D	0	33	29		17	36	36,5	21	25	40	29	33,5	0	14	13		9	21	23	17,5	14,1	19	17,5	19,0	58
	E	0	45	40		20	35	51	41	49	60	40	48,0	0	26	35		29	20	30	29	28	28	34	34,0	86
	H	0	40	30		20	36,2	33,6	25	40	44,6	33	37,5	0	20	22		20	31,8	34,6	23,5	36,5	37,2	25,7	32,0	65
MEAN		0	36,5	37		20,8	45	46,7	30,6	44	53,2	32,6	43,5	0	17,5	20,3		14,8	25,5	27	20.5	24,1	29,4	21,8	26,0	73
1 x 75 mg	A	0	30	38,5	36,3	41,6		35,3	34,3		24,1	20	42,0	0	23	28,5	25,5	38,1		32,6	32,4		24,1	18	33,5	82,0
	B	0	25	37,6	54,8	46,7		40	32,6		23	20	40,0	0	10	16	20,5	24		23	21,5		15,1	13,5	22,0	71
	C	0	34,3	29	36	41		41	41		40	32	43,5	0	15	16	14	11		14	11,5		12,2	11	14,0	65
	D	0	21,2	30,1	32,6	30,7		34	31		26,4	18,1	34,0	0	11	14	16	15		19	17		14	11	17,6	58
	E	0	30	38,4	33	34		38,2	38		30	27,8	40,0	0	27	21	22,6	32,1		35,2	29		31	26	33,5	81
	H	0	19	32	28	30,5		39	34		31	28	36,5	0	10	12,5	15	19		29	31		27	20	27,0	71
MEAN		0	26,6	34,2	36,8	37,4		37,9	35,1		29,1	24,3	39,0	0	16	18	18,9	23,2		25,5	23,7		20,5	16,6	23,0	71,3

*1 A.U.C.: Area Under the Curve (CM^2) calculated by counting the number of squares

*1, 2 A.U.C.: Total Area Under the Curve for the three compounds i.e. metabolites II and IV and the unchanged drug

Table 2: Method employed for calculation of analysis of variance (Metabolite II: 1st hour)

Subject	3 x 25 mg X_1	$(X_1)^2$	1 x 75 mg X_2	$(X_2)^2$	(X_1+X_2)	$(X_1+X_2)^2$
A	46	2116	30	900	76	5776
B	15	225	25	625	40	1600
C	40	1600	34,8	1211	74,8	5595
	101		89,8			
D	33	1089	21,2	449	54,2	2938
E	45	2025	30	900	75	5625
H	40	1600	19	361	59	3481
	118		70,2			
	219	8655	160	4446		25015

$X^2 = 8655 + 4446 = 13101$

C.F. (Correcting Factor) $= \frac{(\Sigma X_1 + \Sigma X_2)^2}{N} = \frac{(219 + 160)^2}{12} = 11970$

T.S.S. (Total sum of squares) $= \Sigma X^2$ - C.F. $= 13101 - 11970 = 1131$

.. C.F. = 11970; T.S.S. = 1131

Among subjects (sum of squares) : $\frac{(X_1+X_2)_A^2 + (X_1+X_{2B})^2 + \ldots}{2}$ - C.F.

$= \frac{25105}{2} - 11970 = 537,5$

Within subjects (S.S.): T.S.S. - Among Subjects (S.S.)

$= 1131 - 537,5 = 539,5$

.. Sum of squares for: Among subjects = 537,5

Within subjects = 593,5

Treatment (3 x 25 mg Vs. 1 x 75 mg): $\frac{(\Sigma X_1)^2 + (\Sigma X_2)^2}{6}$ - C.F.

$= \frac{(219)^2 + (160)^2}{6} - 11970 = 290$

Table 2: continued

Time (subjects A, B and C on 3 x 25 mg plus subjects D, E and H on 1 x 75 mg Vs. subjects A, B and C on 1 x 75 mg plus subjects D, E and H on 3 x 25 mg):

Subjects		X		X
A,B,C	on 3 x 25 mg	101	on 1 x 75 mg	89,8
D,E,H	on 1 x 75 mg	70,2	on 3 x 25 mg	118
		ΣX = 171,2		ΣX = 207,8

$$\text{Time (S.S.)} = \frac{(171,2)^2 + (207,8)^2}{6} - \text{C.F.} = 111,7$$

Residual = Within Subjects - (Time + Treatment)

$\therefore$ 593,5 - (111,7 + 290) = 191,8

S.S. for: Treatment = 290

Time = 111,3

Residual = 191,8

Final Table:

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance* p
Among Subjects	537,5	5	$\frac{537,5}{5}$=107,5	$\frac{107,5}{48}$=2,24	>0,2 ; NS
Within Subjects	593,5				
Treatment	(290)	(1)	$\frac{290}{1}$ =290	$\frac{290}{48}$ =6,04	>0,05 ; NS
Time	(111,7)	(1)	$\frac{111,7}{1}$=111,7	$\frac{111,7}{48}$=2,33	>0,2 ; NS
Residual	(191,8)	(4)	$\frac{191,8}{4}$= 48		
Total	1131	11			

* Calculated from H.W. Norton Variance Ratios; P values greater than 0,05 will be shown as NS

* Statistical evaluations were conducted by Dr A. Noormohammadi

Table 3 : Analysis of variance at different time intervals for crossover comparison studies of various dosage forms of diethylpropion hydrochloride, i.e. free dosage form (F.D.F.: 3 x 25 mg at times 0, 4 and 8 hours) Vs sustained release pellets (S.R.P.: 1 x 75 mg at time 0 hours).

Time: 1 Hour

Compound*: Metabolite II

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance* p
Among Subjects	537,5	5	107,5	2,24	NS
Within Subjects	593,5	6			
Treatment	(290,0)	(1)	290	6,04	NS
Time	(111,7)	(1)	111,7	2,33	NS
Residual	(191,8)	(4)	48		
Total	1131	11			

Compound*: Metabolite IV

Among Subjects	542,75	5	108,5	5,86	NS
Within Subjects	99,5	6			
Treatment	(6,75)	(1)	6,75	0,36	NS
Time	(18,75)	(1)	18,75	1,01	NS
Residual	(74,0)	(4)	18,50		
Total	642,25	11			

* Using S.R.P., lesser amounts of both metabolites (i.e. II: ethylaminopropiophenone, IV: diethylnorpseudoephedrine) but larger amounts of unchanged drug were recovered when compared with the results obtained after using F.D.F. (see Table 11 for Total Area Under the Curve).

Table 4 : Analysis of variance at different time intervals for crossover comparison studies of various dosage forms of diethylpropion hydrochloride, i.e. free dosage form (F.D.F.: 3 x 25 mg at times 0, 4 and 8 hours) Vs sustained release pellets (S.R.P.: 1 x 75 mg at time 0 hours).

Time: 2 Hours

Compound*: Metabolite II

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance* p
Among Subjects	214,48	5	42,89	7,62	< 0,05
Within Subjects	76,29	6			
Treatment	(22,41)	(1)	22,41	3,98	NS
Time	(31,36)	(1)	31,36	5,57	NS
Residual	(22,52)	(4)	5,63		
Total	290,77	11			

Compound*: Metabolite IV

Among Subjects	344,5	5	68,9	2,54	NS
Within Subjects	204,75	6			
Treatment	(16,36)	(1)	16,38	0,6	NS
Time	(80,11)	(1)	80,11	2,96	NS
Residual	(108,28)	(4)	7,07		
Total	549,27	11			

* Using S.R.P., lesser amounts of both metabolites (i.e. II: ethylaminopropiophenone, IV: diethylnorpseudoephedrine) but larger amounts of unchanged drug were recovered when compared with the results obtained after using F.D.F. (see Table 11 for Total Area Under the Curve).

Table 5 : Analysis of variance at different time intervals for crossover comparison studies of various dosage forms of diethylpropion hydrochloride, i.e. free dosage form (F.D.F.: 3 x 25 mg at times 0, 4 and 8 hours) Vs sustained release pellets (S.R.P.: 1 x 75 mg at time 0 hours).

Time: 4 Hours

Compound*: Metabolite II

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance* p
Among Subjects	187	5	37,4	4,6	NS
Within Subjects	902	6			
Treatment	(825)	(1)	825	101,6	< 0,01
Time	(44,5)	(1)	44,5	5,5	NS
Residual	(32,5)	(4)	8,12		
Total	1089	11			

Compound*: Metabolite IV

Among Subjects	637,3	5	127,46	3,09	NS
Within Subjects	471	6			
Treatment	(210)	(1)	210	5,09	NS
Time	(96,3)	(1)	96,3	2,33	NS
Residual	(164,8)	(4)	41,2		
Total	1108,4	11			

* Using S.R.P., lesser amounts of both metabolites (i.e. II: ethylaminopropiophenone, IV: diethylnorpseudoephedrine) but larger amounts of unchanged drug were recovered when compared with the results obtained after using F.D.F. (see Table 11 for Total Area Under the Curve).

Table 6 : Analysis of variance at different time intervals for crossover comparison studies of various dosage forms of diethylpropion hydrochloride, i.e. free dosage form (F.D.F.: 3 x 25 mg at times 0, 4 and 8 hours) Vs sustained release pellets (S.R.P.: 1 x 75 mg at time 0 hours).

Time: 6 Hours

Compound*: Metabolite II

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance* p
Among Subjects	317	5	63,4	1,71	NS
Within Subjects	468	6			
Treatment	(230,5)	(1)	230,5	6,23	NS
Time	(89,5)	(1)	89,5	2,42	NS
Residual	(148)	(4)	37		
Total	785	11			

Compound*: Metabolite IV

Among Subjects	661,4	5	132,28	8,84	0,05
Within Subjects	66,28	6			
Treatment	(6,45)	(1)	6,45	0,43	NS
Time	(0)	(1)	0	0	NS
Residual	(59,83)	(4)	14,95		
Total	727,68	11			

* Using S.R.P., lesser amounts of both metabolites (i.e. II: ethylaminopropiophenone, IV: diethylnorpseudoephedrine) but larger amounts of unchanged drug were recovered when compared with the results obtained after using F.D.F. (see Table 11 for Total Area Under the Curve).

Table 7 : Analysis of variance at different time intervals for crossover comparison studies of various dosage forms of diethylpropion hydrochloride, i.e. free dosage form (F.D.F.: 3 x 25 mg at times 0, 4 and 8 hours) Vs sustained release pellets (S.R.P.: 1 x 75 mg at time 0 hours).

Time: 8 Hours

Compound*: Metabolite II

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance* p
Among Subjects	303,5	5	60,7	2,25	NS
Within Subjects	170,25	6			
Treatment	(60,3)	(1)	60,3	2,23	NS
Time	(2,1)	(1)	2,1	0,08	NS
Residual	(107,85)	(4)	26,96		
Total	473,75	11			

Compound*: Metabolite IV

Among Subjects	536,62	5	107,32	5,13	NS
Within Subjects	117,38	6			
Treatment	(31,36)	(1)	31,36	1,50	NS
Time	(2,43)	(1)	2,43	0,11	NS
Residual	(83,59)	(4)	20,90		
Total	654	11			

* Using S.R.P., lesser amounts of both metabolites (i.e. II: ethylaminopropiophenone, IV: diethylnorpseudoephedrine) but larger amounts of unchanged drug were recovered when compared with the results obtained after using F.D.F. (see Table 11 for Total Area Under the Curve).

Table 8 : Analysis of variance at different time intervals for crossover comparison studies of various dosage forms of diethylpropion hydrochloride, i.e. free dosage form (F.D.F.: 3 x 25 mg at times 0, 4 and 8 hours) Vs sustained release pellets (S.R.P.: 1 x 75 mg at time 0 hours).

Time: 10 Hours

Compound*: Metabolite II

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance* p
Among Subjects	331,10	5	66,22	0,58	NS
Within Subjects	2281,27	6			
Treatment	(1345,5)	(1)	1745,5	15,27	0,05
Time	(78,54)	(1)	78,54	0,69	NS
Residual	(457,23)	(4)	114,3		
Total	2612,37	11			

Compound*: Metabolite IV

Among Subjects	759,3	5	151,86	3,3	NS
Within Subjects	487,5	6			
Treatment	(235)	(1)	235	5,1	NS
Time	(68,5)	(1)	68,5	1,49	NS
Residual	(184)	(4)	46		
Total	1246,8	11			

* Using S.R.P., lesser amounts of both metabolites (i.e. II: ethylaminopropiophenone, IV: diethylnorpseudoephedrine) but larger amounts of unchanged drug were recovered when compared with the results obtained after using F.D.F. (see Table 11 for Total Area Under the Curve).

Table 9 : Analysis of variance at different time intervals for crossover comparison studies of various dosage forms of diethylpropion hydrochloride, i.e. free dosage form (F.D.F.: 3 x 25 mg at times 0, 4 and 8 hours) Vs sustained release pellets (S.R.P.: 1 x 75 mg at time 0 hours).

Time: 12 Hours

Compound*: Metabolite II

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance* p
Among Subjects	220	5	44	2,35	NS
Within Subjects	287	6			
Treatment	(209)	(1)	209	11,16	< 0,05
Time	(3,1)	(1)	3,1	0,16	NS
Residual	(74,9)	(4)	18,7		
Total	507	11			

Compound*: Metabolite IV

Among Subjects	547	5	109,5	3,91	NS
Within Subjects	202,5	6			
Treatment	(84)	(1)	84	3	NS
Time	(6,5)	(1)	6,5	0,23	NS
Residual	(112)	(4)	28		
Total	750	11			

* Using S.R.P., lesser amounts of both metabolites (i.e. II: ethylaminopropiophenone, IV: diethylnorpseudoephedrine) but larger amounts of unchanged drug were recovered when compared with the results obtained after using F.D.F. (see Table 11 for Total Area Under the Curve).

Table 10 : Analysis of variance at different time intervals for crossover comparison studies of various dosage forms of diethylpropion hydrochloride, i.e. free dosage form (F.D.F.: 3 x 25 mg at times 0, 4 and 8 hours) Vs sustained release pellets (S.R.P.: 1 x 75 mg at time 0 hours).

Area Under the Curve

Compound*: Metabolite II

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance* p
Among Subjects	262,35	5	54,47	9,75	$< 0,05$
Within Subjects	88,38	6			
Treatment	(58,52)	(1)	58,52	10,48	$< 0,05$
Time	(7,52)	(1)	7,52	1,34	NS
Residual	(22,34)	(4)	5,58		
Total	350,73	11			

Compound*: Metabolite IV

Among Subjects	733	5	146,6	13	$< 0,05$
Within Subjects	53	6			
Treatment	(3,4)	(1)	3,4	0,3	NS
Time	(4,5)	(1)	4,5	0,4	NS
Residual	(45,1)	(4)	11,27		
Total	786	11			

* Using S.R.P., lesser amounts of both metabolites (i.e. II: ethylaminopropiophenone, IV: diethylnorpseudoephedrine) but larger amounts of unchanged drug were recovered when compared with the results obtained after using F.D.F. (see Table 11 for Total Area Under the Curve).

Table 11 : Analysis of variance at different time intervals for crossover comparison studies of various dosage forms of diethylpropion hydrochloride, i.e. free dosage form (F.D.F.: 3 x 25 mg at times 0, 4 and 8 hours) Vs sustained release pellets (S.R.P.: 1 x 75 mg at time 0 hours).

Total "Area Under the Curve" for both Metabolites* plus Unchanged Drug

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance* p
Among Subjects	1018	5	203,6	15,97	$< 0,01$
Within Subjects	72	6			
Treatment	(8,6)	(1)	8,6	0,67	NS
Time	(12,4)	(1)	12,4	0,97	NS
Residual	(51)	(4)	12,75		
Total	1090	11			

* Using S.R.P., lesser amounts of both metabolites (i.e. II: ethylaminopropiophenone, IV: diethylnorpseudoephedrine) but larger amounts of unchanged drug were recovered when compared with the results obtained after using F.D.F.

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