CHARACTERISATION AND QUANTIFICATION OF WOOD EXTRACTIVES AND THEIR IMPACT ON PITCH

By

PRINISHA MOODLEY

Submitted in fulfilment of the academic
requirements for the degree of
Master of Science in the
School of Chemistry,
University of KwaZulu-Natal
Durban

January 2011

As the candidate's supervisor I have/have not approved this thesis/dissertation for submission.

Signed: _____ Name: _____ Date: _____

Abstract

The aims of this study were to characterise and quantify wood extractives in *E. grandis* and *E. nitens* and determine the impact of wood extractives on pitch formation. Initially a comparison was made with individual solvent abilities to determine whether the polarity index plays a role in the amount of extracts being removed. After this different methods were used to determine the extractive amounts. These methods included hot water/ethanol-toluene, hot water/acetone and followed by acetone only. Analyses such as UV-Vis, acidolysis and HPLC were carried out to determine the presence of lignin and sugars in the extracts and sawdust respectively. Lastly GC and GC-MS was performed to characterise and quantify extractives present in the extracts from the different methods.

The results showed that acetone is the preferred solvent as it removes higher amounts of extractives than ethanol-toluene. There is also a higher amount of extractives in *E.grandis* sawdust than in the *E. nitens* sawdust and pitch sample. There seems to be more fatty acids and sterols in the *E. nitens* sawdust sample extracted the using acetone (no hot water extraction) method while hydrocarbons are extracted more in *E. grandis* using the same method.

It was found after GC-MS analysis that fatty acids tetradecanoic acid methyl ester and hexadecanoic acid methyl ester and hydrocarbon 1-Octadecene were common to both species.

The common compounds in the pitch and sawdust of *E. nitens* are heptadecanoic, octadecanoic, tetradecanoic and tridecanoic acid methyl esters, gamma and beta sitosterol and Stigmasterol, 1-docosene and lastly 1-nonadecene hence these compounds are more likely to cause pitch.

After analysis using UV analysis and acidolysis there was indication that there were lignin breakdown products present in the wood extracts, in minimal amounts. HPLC indicated no sugars present in the extracts.

It is concluded that GC and GC-MS are the recommended analytical tools in characterising and quantifying wood extractives in *E. grandis* and *E. nitens*. All extractives in both species were quantified and identified using GC and GC-MS respectively.

Preface

The work presented in this thesis was performed at the CSIR and Chemistry, University of KwaZulu-Natal, Durban from August 2005 to September 2007. The work was supervised by Prof. A Kindness.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

P. Moodley (201506912)

DECLARATION 1 - PLAGIARISM

I …***Prinisha Moodley***…… declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
   a. Their words have been re-written but the general information attributed to them has been referenced
   b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed: 

DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

Publication 1

Publication 2

Publication 3

etc.

Signed: 

Acknowledgments

I would like to acknowledge the following people for their contribution to this work:

Firstly, my supervisor, Prof. Andrew Kindness, for his support and ideas during this work. You have no idea how much you have motivated me and I am really appreciative of everything. You are truly a God sent.

I would also like to thank the Eucalyptus Research Cooperative (CSIR, Sappi and Mondi) for funding this project.

To Mr B Parel from UKZN, his assistance for the GC-MS analysis.

On a personal note my wonderful parents, Harry and Sally Moodley, my sister Yershini and last but definitely not least my husband Justin and son Liam, for their support and motivation.

My colleagues, Ajay and Jennings for their friendship, guidance and knowledge.

Table of contents

List of Figures

Chapter 2: Literature Review

List of Tables

Chapter 2: Literature Review

Nomenclature[1]

Symbol / Abbreviation	Meaning
LWE	Lipophilic Wood Extractives
DCM	Dichloromethane
MW	Molecular Weight
TCF	Total Chlorine Free
Py-GC/MS	Pyrolysis-Gas Chromatography/Mass Spectrometry
TMAH	Tetramethylammoniumhydroxide
PEO	Polyethylene oxide
PolyDADMAC	Polydiallyldimethylammoniumchloride
SPE	Solid-Phase Extraction
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
WO	Wet Oxidation
ECF	Elemental Chlorine Free
D	Chlorine dioxide stage
E	Alkaline Extraction stage
HWS	Hot Water Solubility
SEW	Solvent Extractives of Wood
HPLC	High Performance Liquid Chromatography
FID	Flame Ionisation Detector
UV	Ultra-Violet
ASE	Accelerated Solvent Extraction
MTBE	methyl *tert*-butyl ether

[1] Except where otherwise stated, the following symbols and abbreviations have been used in this document.

Chapter 1: Introduction and Aims

1.1. Introduction

Wood extractives are known to have a negative effect on the pulp and paper making processes (Sefara and Birkett, 2004, Speranza *et al*., 2002). Although majority of the extractive compounds dissolve in cooking liquors during pulping, some are carried over to the bleaching processes and accumulate to form sticky deposits called pitch. Pitch is disadvantageous as it impacts on the strength properties of the paper. This is due to resinous extractives blocking reactive groups on the surface of the fibres and hindering inter-fibre bonding (Sefara and Birkett, 2004). Pitch deposits can also impair product quality by causing dirt, holes and scabs (Kostamo and Kukkonen, 2003). Pitch decreases the efficiency of pulp washing, screening, centrifugation, cleaning and refining and can disrupt many paper machine operations (Vercoe *et al*., 2004).

Hence the determination of wood extractives is very important and this is carried out using soxhlet extraction in accordance with standard Tappi T204 om-88 test method (TAPPI Test Methods, 1989). Solvents such as benzene, toluene-ethanol, acetone, hexane, dichloromethane, methanol, and diethyl ether can be used. The amount of extractives and composition depends on the solvent used; this has been shown in numerous publications (Sefara and Birkett, 2004, Sierra, Salvador and Soria, 1991, Demirbas, 1991, Peng and Roberts, 2000, Xiao *et al*., 2001 and Lacorte *et al.,* 2003).

1.2. Aims

The aims of this study were, 1) to characterise and quantify wood extractives in *Eucalyptus grandis* and *Eucalyptus nitens* and; 2) determine the impact of wood extractives on pitch formation.

Chapter 2: Literature Review

2.1. Wood extractives

Extractives are low molecular weight constituents in wood (Vercoe *et al*., 2004) and can be grouped into two main classes i.e. lipophilic (fatty) and hydrophilic extractives (Table 2.1) (Vercoe *et al*., 2004).

Table 2.1: Lipophilic and hydrophilic extractives (Vercoe *et al*., 2004).

Lipophilic	Hydrophilic
Resin acids	Phenols
Free fatty acids	Lignans
Triglycerides	Flavanoids
Steryl esters	Tannins
Free sterols	Sugars
Monoterpenes	Salts

They are also commonly defined as material that can be removed from pulp or paper by a particular solvent or solvent system during soxhlet extraction (Casey, 1952, Tappi Test Methods, 1989). Lipophilic extractives are not soluble in water, acid or alkali making them difficult to remove during the washing stages. They are carried through as fine particles or absorbed in pulp after cooking.

The amount and type of extractives are variable within species (Ucar *et al*., 1995) and is site specific. Megown *et al.,* 2000*,* studied the impact of tree age and site index on extractives in *E. grandis*. It was found that the amount of extractives increased with increasing age and decreasing site index using benzene-alcohol and hot water extractions. Most of the total variation over the range of ages and site indices measured was caused by site index.

Megown and co-workers (Megown *et al.,* 2000) suggested that there is a small inverse effect of SI[2] and a small positive effect of age on hot water (HW) extractives. Hence HW extractives increase significantly with age.

[2] SI in general terms it is a SAPPI index and is a relative measure of forest site quality. This information helps estimate future returns and land productivity for timber and wildlife and varies between 1 and 5, with 1 being good.

The main extractive constituents in softwood include triglycerides, resin acids, free fatty acids, sterols, sterol esters and waxes, while in hardwoods the extractives are composed of sterols, long chain aliphatic acids and alcohols, waxes, triglycerides and sterol esters (Speranza *et al.,* 2002). Gallic and ellagic acid[3], $C_{14}H_6O_8$, Figure 2.1, as well as their glucose derivatives gallo- and ellagitannins are typical extractives in the wood of *Eucalyptus* species (Del Rio *et al*., 1999b).

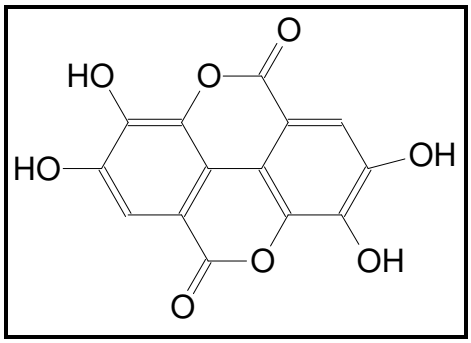


Figure 2.1: Chemical structure of Ellagic acid (Del Rio *et al*., 1999b)

Resin acids appear predominantly in softwood, where sterols such as sitosterol, Figure 2.2, are characteristic of both hardwoods and softwoods (Sithole and Allen, 2003).

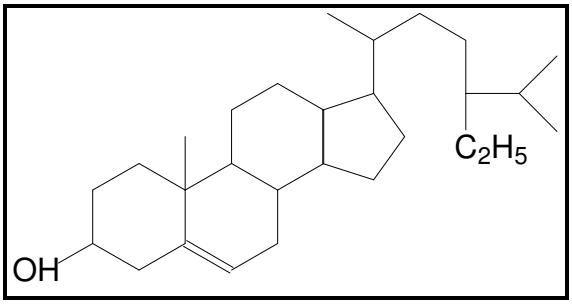


Figure 2.2: Molecular structure of sitosterol (Gutierrez *et al*., 1998a).

Many studies have been carried out to determine the composition of extractives from pine sapwood and eucalyptus commonly used in the pulp and paper industry. Unfortunately, this is not the case for *Eucalyptus* wood which is used primarily in the paper industry in South-Western Europe, South America and South Africa (Gutierrez *et al*., 1999). There are different species of *Eucalyptus* but the most economically important raw material for paper pulp production in Europe is *E. globulus.* Information gathered so far regarding the composition of extractives of this genus showed polar fractions containing phenols and polyphenols (Gutierrez *et al*., 1999b).

[3] A phenolic compound naturally found in plants in the form of ellagitannin known as a potent anti-cancer agent.

Table 2.2 shows the composition of the main lipid classes present in the extractives of eucalypt wood and pine sapwood. The results in Table 2.2 indicate that hardwoods contain a higher mass of extractives than softwoods. Sterol esters and sitosterol are the most abundant of extractives found in eucalyptus *i.e.* 51.7 and 49.4 mg/100g wood respectively, while in pine they only constitute 1.2 and 0.2 mg/100g wood. Resin acids are predominantly found in softwoods.

Table 2.2: Composition of the main lipid classes present in extractives (mean and standard deviation of triplicate analysis) (Gutierrez *et al*., 1998a).

	Eucalypt wood (mg/100g wood)	Pine sapwood (mg/100g wood)
Fatty acids	27.7 ± 1.0	4.0 ± 1.0
Resin acids	0	3.9 ± 0.9
Sitosterol	49.4 ± 1.0	0.2 ± 0.02
Waxes	5.8 ± 0.5	1.6 ± 0.3
Sterol esters	51.7 ± 1.7	1.2 ± 0.2
Triglycerides	13.2 ± 0.4	7.3 ± 2.0

The term resin and sometimes rosin acid is often used as a collective name for those lipophilic wood extractives that are soluble in non-polar organic solvents but insoluble in water (Spark, 2004). There are eight common resin acids found in softwoods, the structures of which can be seen in Figure 2.3**.** They can be divided into three groups: aromatic, conjugated diene class and alkene class and constitute a valuable industrial resource. They are routinely recovered from “tall oil”-acidified Kraft liquor (predominantly softwood) Straight chain saturated and unsaturated fatty acids with 16-24 carbon atoms are the most important fatty acids in wood plants with the C18 mono-, di-, and tri-unsaturated acids dominating (Vercoe *et al.,* 2004).

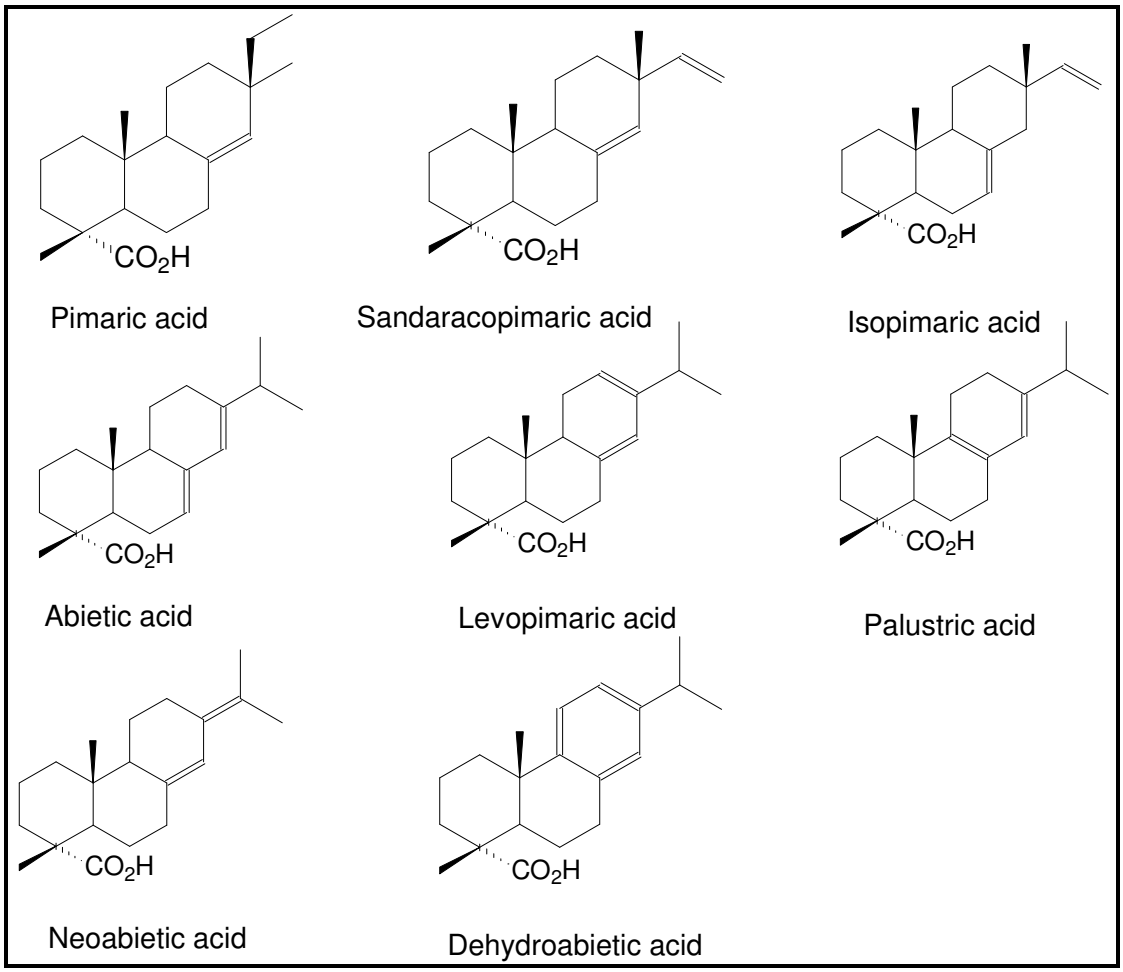
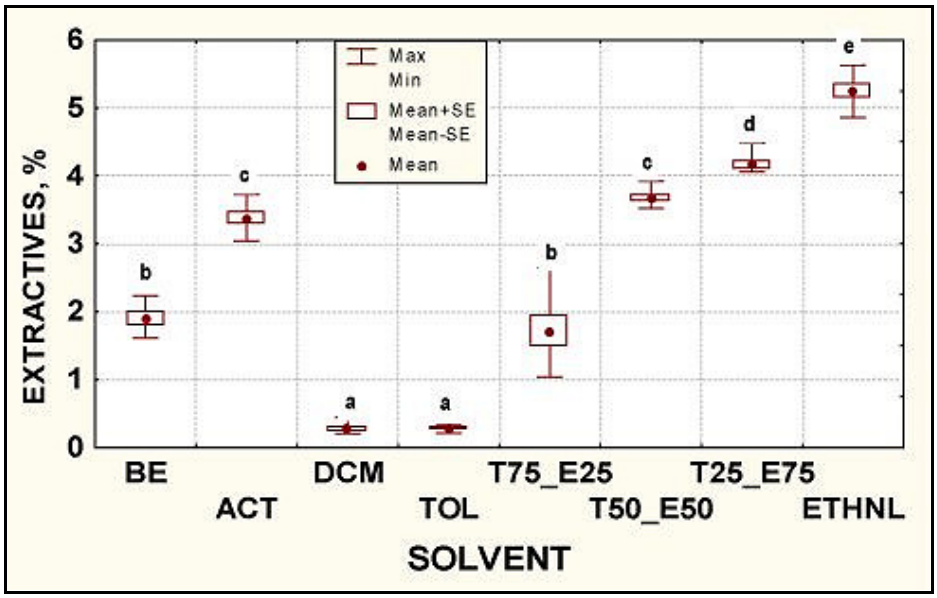


Figure 2.3: Structure of the eight common resin acids found in softwoods (Vercoe *et al*., 2004).

2.2. Quantification of wood extractives

For the quantification of wood extractives wet chemical methods is used. The amount of wood extractives are traditionally measured using hot water and solvent extractions respectively (TAPPI method T207 om-88 and TAPPI method T204 om-88 respectively). However new techniques are being studied as a result of variations in the results due to temperature changes during hot water and solvent extractions. Such techniques include use of the Soxtec apparatus and accelerated solvent extraction (ASE). The advantages of the two methods are they are fast and consume less solvent. Also for ASE the yield is better than the Soxhlet method (Sithole, 1992). Although Soxtec and Soxhlet share a common set of general operating conditions and product composition is very similar, most of the differences can be explained in terms of the increased efficiency of Soxtec, probably due to a better mass transfer.

Different solvents impact on the amount of extractives produced (Lacorte *et al.,* 2003). Different solvents extract different compounds during solvent extraction. Figure 2.4 displays the mean, standard error and range of the extractives removed by the different solvents from *E. grandis*. It shows clearly that toluene and dichloromethane removed the lowest amount of extractives. Extractives removed by toluene amounted to 0.27% in *E. grandis*. The amount of extractives removed by toluene and dichloromethane were on average 40% lower than that removed by benzene-ethanol. This can be attributed to the lower polarity index[4] of toluene (2.4) and dichloromethane (3.1) compared to a polarity index of 3.7 for benzene-ethanol. The extraction with ethanol (4.3) gave a yield of 5.25% which was triple the amount of extractives removed by benzene-ethanol. Further investigation indicated that ethanol also extracts other low molecular weight wood compounds (Sefara and Birkett, 2004).



KEY: BE: Benzene ACT: Acetone DCM: Dichloromethane TOL: Toluene ETHNL: Ethanol
T75_E25: Toluene: Ethanol (2:1) T50_E50: Toluene: Ethanol (1:1) T25_E75: Toluene: Ethanol (1:2)

Figure 2.4: Organic soluble extractives from *E. grandis*. The identical letters on the graph indicate no significant difference was obtained (Sefara and Birkett, 2004).

Sun and co-workers also carried out investigations using different solvents (Sun *et al.*, 2002). They found that a mixture of toluene-ethanol gave high yields i.e. 3.42% while petroleum ether and hexane gave low amounts of extractives, 0.45 and 0.65% respectively. Chloroform and dichloromethane on the other hand gave medium yields of 1.19 and 1.37% respectively. They, therefore, concluded that if lipophilic extractives are of interest then solvents such as

[4] Polarity index is a relative measure of the degree of interaction of the solvent with varies polar test solutes

petroleum ether and hexane should be used. However, if the total extractives and lipophilic extractives are important then solvents such as dichloromethane and chloroform should be considered.

It was found by Sun and Tomkinson, 2003, toluene-ethanol and chloroform-methanol gave high yields of extractives in wheat straw (i.e. 2.38 and 2.32% respectively). They found that methyl *tert*-butyl ether (MTBE) and dichloromethane extracted 1 and 1.17% extractives respectively. According to Sun *et al*, 2002, hexane and petroleum ether extract low amounts of extractives, 0.74 and 0.55% respectively. Other studies also indicated that polarity of the solvent has an effect on the amount of extractives (Sefara and Birkett 2004 and Wallis and Wearne, 1997). The amount of extractives decrease with a decrease in polarity, the order of polarity is as follows:

Most Polar **Least Polar**

Methanol > acetone > dichloromethane > hexane

Most extracted **Least extracted**

According to Wallis and Wearne, 1997, methanol extracted 3.33% extractives followed by acetone which extracted 2.71%, dichloromethane 1.82% and finally hexane extracting 1.55%. It was also mentioned that acetone is fast becoming a widely used solvent. There has been studies documented using acetone as the extracting agent, (Del Rio *et al*., 1999a and 2000).

It was suggested by Demirbas, 1991 that there is no solvent able to affect a complete removal of wood extractives. However acetone is an excellent solvent for extracting fats and fatty acids in wood. It is difficult to conclude from literature which solvent or solvent mixture is the best for extraction Peng and Roberts, 2000.

2.3. Characterisation of wood extractives

2.3.1 Gas chromatography and mass spectroscopy

Gas chromatography (GC) was used to separate the compounds present in extractives while gas chromatography/mass spectroscopy (GC-MS) characterises the individual compounds based on their molecular structure.

Gas chromatography (GC) specifically gas-liquid chromatography is a highly developed technique which involves a sample being vaporised while injected onto the head of the chromatographic column. The sample is transported through the column by the flow of an inert, gaseous mobile

phase. The column itself contains a polymeric phase (stationary phase) which is coated onto the surface of an inert surface.

Separation occurs by partition of the analyte between the stationary phase and the mobile phase (the gas). The lower the vapour pressure, i.e. the higher the boiling point, of any component of the analyte, the more time it spends dissolved in the stationary phase. Thus the components emerge separately at different times (Ambrose, 1971). If the stationary phase is non-polar, then separation is primarily by boiling points and the system operates like an efficient distillation process (Ambrose, 1971). The components of a GC instrument are illustrated diagrammatically in Figure 2.5.

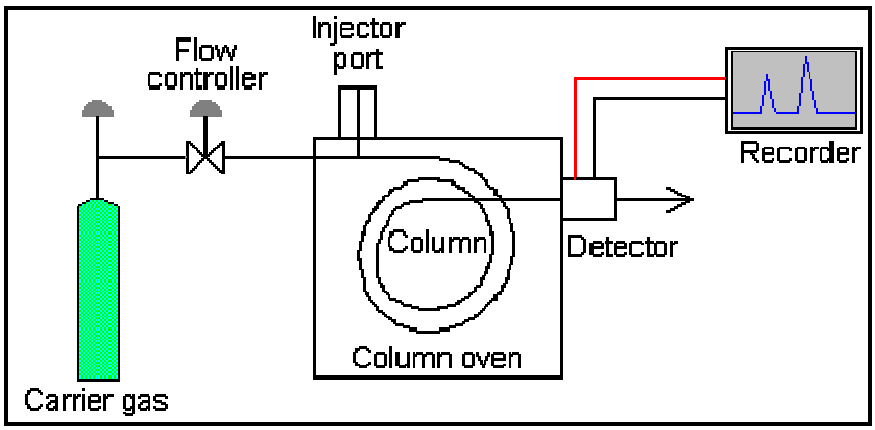


Figure 2.5: Schematic diagram of components of Gas Chromatography (http://www.shu.ac.uk/schools/sci/chem/tutorials/chrom/gaschrm.htm)

The carrier gas must be inert to both the sample and the solid phase. The most often-used carrier gas is nitrogen.

Liquid samples are injected into the column. The GC detector measures the concentration of the column effluent in a differential type analysis i.e. giving a zero signal for the carrier gas and a signal proportional to the concentration or mass of the eluted component. The type of detector used was the Flame Ionisation Detector (FID) (Figure 2.6).

The FID has a minimum detection limit of approximately 10^{-12} g absolute and is sensitive to most organic compounds but is insensitive to water thus is an excellent choice of detector for aqueous extracts. It consists of an H_2/air flame burning at a metal jet through which the column effluent is passed. The organic compounds are burnt in the flame and produce ions and electrons which are detected by the collector electrode. The electrode is normally positive as electron response is faster than the sluggish positive ions (Ambrose, 1971).

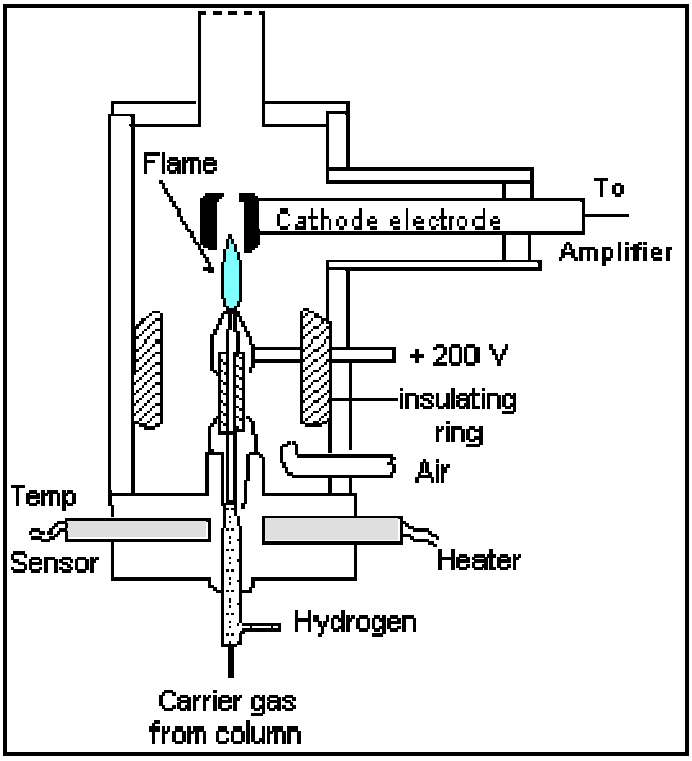


Figure 2.6: Schematic of a FID (Kindness, 2009)

In mass spectroscopy, the sample (liquid, solid, solution, or vapour) enters the vacuum chamber through an inlet and is subjected to bombardment by an electron beam. The resultant ions are sorted in the mass analyzer according to their mass-to-charge ratios and then collected by a detector where the ion flux is converted to a proportional electrical current that is used to produce a mass spectrum (Karasek and Clement, 1988).

The analysis of resin acids in process waters and effluents has been reported by Rigol *et al*., 2003, using gas chromatography, mass spectrometry, liquid chromatography and capillary electrphoresis. Both GC and GC/MS were used to identify 44 compounds and classify a further 26 compounds from quaking aspen (Fernadez *et al*., 2001).

Gutierrez and co-workers used short and medium length high temperature capillary columns respectively for GC and GC-MS (Gutierrez, *et al.,* 1998b). The GC method of separation used different length high temperature capillary columns with rapid temperature programming to separate compounds with a broad range of molecular weights in a short period of time as illustrated in Figure 2.7 (Gutierrez *et al*., 1998b). This paper also showed that fatty acids, sitosterol and sterol esters were found in pitch sample from Pine sapwood and Eucalypt woods (Gutierrez *et al.,* 1998b).

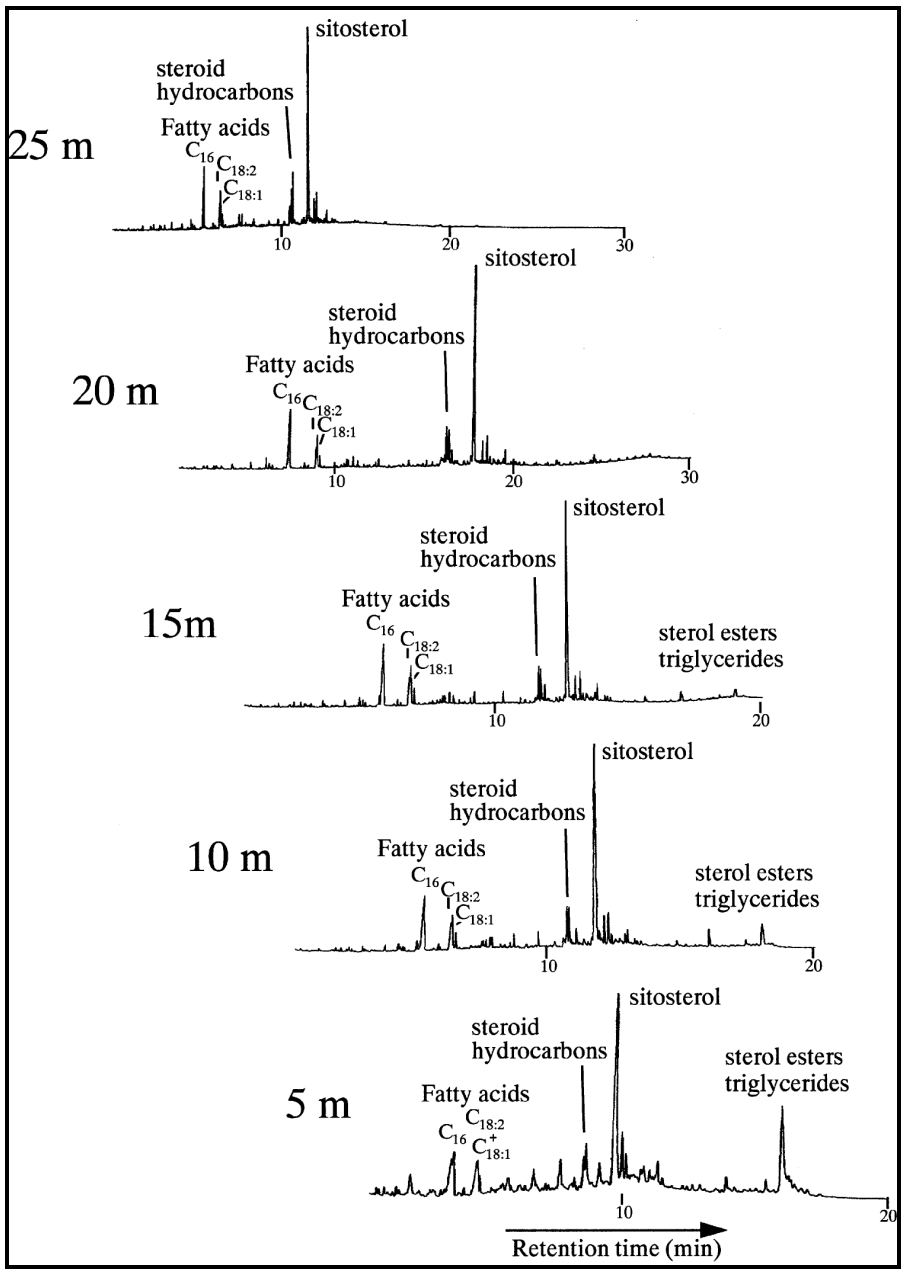
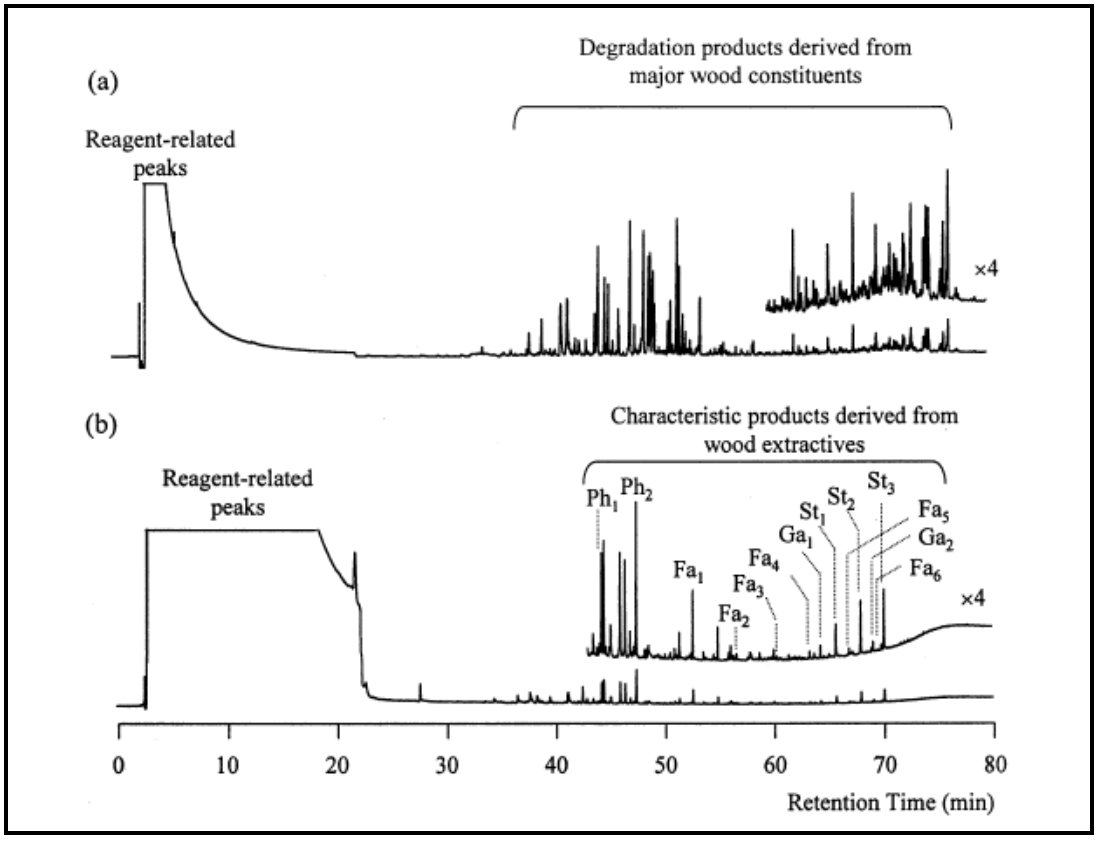


Figure 2.7: Identification of major compounds in lipid extract of *E. globulus* using GC-FID with high-temperature capillary columns of different length (Gutierrez *et al.,* 1998b).

.

Hiroaki *et al.,* (2003) found in their research a rapid and sensitive method to characterise wood extractives in wood samples on the basis of reactive thermal desorption-gas chromatography (RTD-GC). This was carried out in the presence of an organic alkali, tetramethylammonium acetate (TMAAc). This converted the extractives into their volatile methyl esters and allowed for

easier chromatographic analysis. This was done without any pre-treatment such as solvent extraction (Hiroaki *et al*., 2003). Figure 2.8 shows the chromatogram obtained by RTD-GC at 200ºC in the presence of (a) Tetramethylammonium hydroxide (TMAH) and (b) TMAAc respectively.



KEY: Ph: phenolic acid methyl esters, Fa: fatty acid methyl esters, Ga: gallocatechins methyl ethers, and St: stilbenes) and the subscript digit indicates the order of retention time for each RTD product

Figure 2.8: Chromatograms obtained on wood samples by RTD-GC at 200ºC in the presence of (a) TMAH and (b) TMAAc. (Hiroaki *et al*., 2003).

When TMAH was used, reactive pyrolysis of the major wood constituents also proceeded to some extent even at the relatively low temperature of 200ºC through the cleavage of ether bonds in the main constituent chain because of the strong alkalinity of TMAH (Hiroaki *et al.,*2003). This therefore contributed in making the peaks of the wood extractives difficult to distinguish on the chromatogram (Hiroaki *et al*., 2003). This was due to the large number of peaks derived form the dergardation products overlapping with the compounds derived from wood constituents.

However, when using TMAAc the wood constituents did not break down and the peaks were assigned mainly to methyl derivatives of fatty acids, phenolic acids, stilbenes and gallocatechins.

Table 2.3 summarizes the assignment of characteristic peaks derived from wood extractives on the chromatogram in Figure 2.8 (b) by means of RTD-GC/MS in order of peak retention times, together with molecular weight and relative molar sensitivity for flame ionisation detector (FID).

Table 2.3: Peak assignment and relative molar yield in the presence of TMAAc (Hiroaki *et al.*, 2003).

Peak code[5]	**Retention time (min)**	**Compound name**	**MW**	**Rel. molar yield (%)**	**Estimated origin**
Ph_1	44.4	3,4-dimethoxybenzoic acid methyl ester	196	11.8	tannins/lignan/ lignin
Ph_2	47.5	3,4,5-trimethoxybenzoic acid methyl ester	226	20.8	tannins/lignan/ lignin
Fa_1	52.8	hexadecanoic acid methyl ester (C16:0)	270	7.3	triacylglycerol
Fa_2	56.2	octadecanoic acid methyl ester (C18:0)	298	0.9	triacylglyceride
Fa_3	60.2	eicosanoic acid methyl ester (C20:0)	326	0.3	triacylglycerol
Fa_4	61.6	docosanoic acid methyl ester (C22:0)	354	0.4	triacylglycerol
Ga_1	64.5	5,7-dimethyl-2-(3,4,5-trimethoxy-phenyl)-2H-chromen-3-ol	374	9.8	tannins
St_1	65.9	3,3',4,4'-tetramethoxystilbene	300	20.9	Lignan/lignin
Fa_5	67.2	tetracosanoic acid methyl ester (C24:0)	382	0.8	triacylglycerol
St_2	68.1	3,3',4,4',5-pentamethoxystilbene	330	6.4	Lignan/lignin
Ga_2	69.3	isomer of Ga_1	374	14.9	tannins
Fa_6	70.0	hexacosanoic acid methyl ester (C26:0)	410	0.7	triacylglycerol
St_3	71.2	3,3',4,4',5,5'-hexamethoxystilbene	360	6,4	Lignan/lignin

[5] Refer to Figure 2.7 for meaning of abbreviations

From the list of compounds detected by this method; it is apparent that sitosterol was not observed in the chromatogram. This suggests that it is resistant to the formation of methyl esters in the presence of TMAAc (Hiroaki *et al.,* 2003). This is significant as sitosterol is typically a major component of typical wood extractives, see Table 2.2.

A comprehensive review by Challinor of the development and applications of thermally assisted hydrolysis and methylation reactions covers the analysis of wood extractives using this technique (Challinor, 2001).

2.4. The impact of wood extractives in the pulp and paper industry

Wood extractives can form sticky deposits on machinery which also give rise to dark spots in bleached pulp and paper is known as pitch (Spark, 2004). These resin acids are common in softwoods but not in hardwoods.

Sterols and fatty acids, including several α and ω-hydroxyfatty acids and aliphatic alcohols, are the major lipophilic extractives of *E.globulus* unbleached pulp (Freire *et al*., 2005). They can cause operational and quality problems in pulp and paper manufacture due to the formation of spots, specks and other product defects (Speranza *et al*., 2002). This is caused by the accumulation of lipophilic extractives, and can cause production downtime and require extra cleaning of equipment or machinery. Pitch deposits can also impair product quality by causing dirt, holes and scabs (Kostamo and Kukkonen, 2003). Pitch decreases the efficiency of pulp washing, screening, centrifugation, cleaning and refining and can disrupt many paper machine operations (Vercoe *et al*., 2004). The accumulations of lipophilic wood extractives (LWE) also lead to higher consumption of chemicals during bleaching (Gutierrez *et al*., 2002).

In neutral to acidic processing of wood, lipophilic extractives are difficult to remove and resinous woods are more of a problem in pitch control (Gutierrez *et al*, 1999). Extractives and their derivatives play a role in effluent toxicity (European Project, FAIR CT95-0560, 1995).

The group known as the stilbenes are responsible for dark colouring in pine heartwood and can cause major problems in alkaline pulping due to their insolubility (Hills, 1968).

Table 2.4 gives a list of wood extractives known to cause pitch. In conjunction with Table 2.1 it can be seen that most of the wood extractives are lipophilic. Very little of the literature focuses on hydrophilic wood extractive research (Vercoe *et al*., 2004).

Table 2.4: Compounds found in wood extractives known to cause pitch problems.

List of wood extractives	Hardwood or Softwood
Fatty acids	Hardwood and Softwood
Steroid hydrocarbons	Hardwood and Softwood
Sitosterol	Hardwood and Softwood
Sterol esters	Hardwood and Softwood
Stilbenes	Softwood
Resin acids	Softwood

During kraft pulping, glycerol esters are completely saponified and fatty and resin acids dissolved in the liquor (Gutierrez *et al*, 1999). However, some sterols, sterol esters and waxes do not form soluble soaps under alkaline conditions used in kraft pulping and hence have the tendency to deposit and result in pitch formation (Gutierrez *et al*., 1999). Pitch problems are likely to become serious over time with the introduction of more environmentally friendly bleaching processes that have substituted chlorine gas with other reagents such as chlorine dioxide, hydrogen peroxide, or ozone (Gutierrez *et al*., 1999).

It has been reported that some environmentally sound totally chlorine free (TCF) bleaching sequences have increased pitch troubles in eucalypt pulp manufacturing Speranza *et al*., 2002. This is due to deposition of wood extractives that were previously destroyed by chlorine-containing bleaching agents. Presently more focus is placed on *Eucalyptus* wood extractives, since lipophilic compounds in the extractives have been identified as responsible for pitch deposition during manufacturing of kraft pulps from fast growing *E. globulus* (Gutierrez *et al*., 1998b)**.**

Table 2.5 details the main lipophilic compounds of *Eucalyptus globulus*. These are extractives which consist of a complex mixture of compounds such as sterols, long chain aliphatic acids and alcohols, waxes, glycerides and sterol esters.

Colloidal pitch particles can coalesce into larger droplets of pitch which can deposit on the surface of fibres or equipment. They can also remain suspended to be discharged in effluent or waste waters (Gutierrez *et al*.1999a). The means of finding a solution to this problem should

begin with the identification of the extractives present in wood that may lead to pitch formation (Gutierrez *et al*., 1999).

Table 2.5: Main lipophilic compounds produced during manufacturing of TCF-Bleached kraft pulp from *Eucalyptus globulus* wood (Gutierrez *et al*., 1998b).

Compound	**Wood (mg/kg)**	**Brown Pulp (mg/kg)**	**TCF Pulp (mg/kg)**	**Process Water (mg/10L)**	**Pitch Specks (%)**
Fatty acids	277	6	1	0	26
Squalene	38	5	1	3	0
Steroid hydrocarbons	109	49	25	8	16
Sitosterol	494	234	90	221	27
Other sterols	151	92	39	68	9
Steroid ketones	217	34	13	5	11
Sterol esters	517	106	95	80	21
Triglycerides	132	0	0	0	0

Gutierrez *et al*. (1998b) explored the use of different lengths of high capillary columns in GC-FID to make a comparison between the extractives found in softwood and hardwood to that of a pitch deposit in eucalypt kraft pulp. Figure 2.9 shows the chromatogram obtained from the analysis when using a column length of 5m (Gutierrez *et al*., 1998b). Sterol esters have long chain fatty acids which result in the longer retention times.

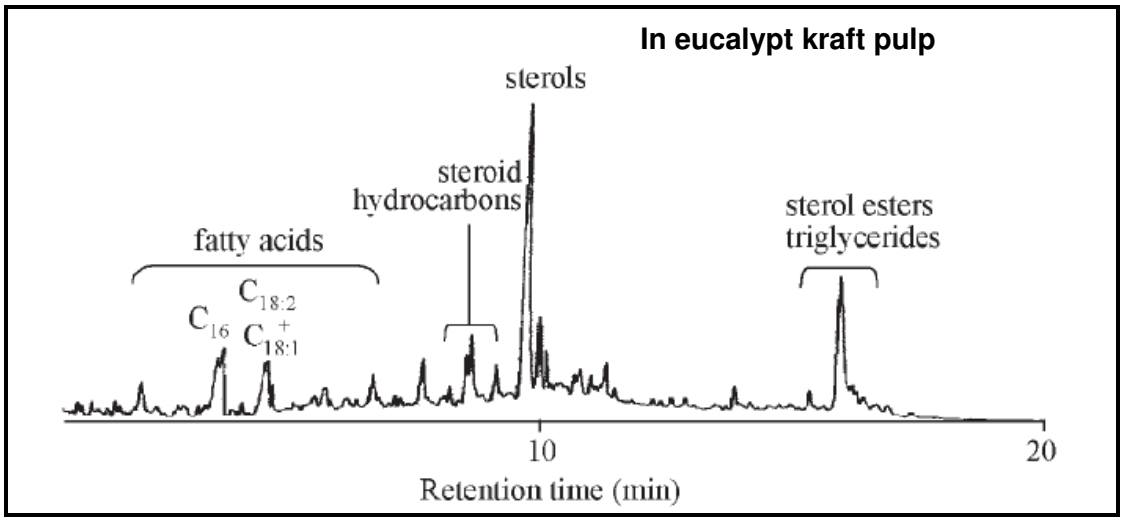


Figure 2.9: GC–FID chromatogram (5 m column) of lipophilic extractives (Gutrierrez *et al*., 1998b).

There are high amounts of fatty acid salts in pitch deposits. It is also well known that pulp mills using eucalypt wood are often plagued with pitch deposition problems caused by the precipitation of ellagic acid soaps of magnesium and sodium ions (Del Rio *et al.,* 1999b). The gallic acid oxidizes rapidly in alkaline solution while ellagic acid is stable in alkaline pulping liquors. This occurs more in the presence of magnesium ions but precipitates on cooling as metal complexes and deposits on the surface of pulp washing equipment and is sometimes found as specks in pulp. Soaps of magnesium and sodium ions (Del Rio *et al*., 1999b) should be considered since they lead to pitch problems when they precipitate (Del Rio *et al*., 1999b).

2.5. The qualitative and quantitative analysis of pitch

Pitch deposits can be extracted using acid/ether or dichloromethane (DCM) (Zheng *et al.,* 2002). The acid/ether mixture extracts fatty acids and esters and salts are converted to their acid form and are then able to be extracted. DCM extracts low molecular weight compounds such as lignans, certain polymers, surfactants and defoamers (Zheng *et al*., 2002).

In pitch deposits acetone insoluble extractives accounted for up to 50% of the whole deposit. These compounds can be analysed by gas chromatography following high temperature pyrolysis (Del Rio *et al*., 1999b) with or without addition of tetramethylammonium hydroxide (Gonzalez-Vila, *et al*., 1997) or tetramethyl ammonium acetate (Yokoi *et al*., 2003).

Del Rio and co-workers 1999b used Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) to analyze different deposits, synthetic polymers and impurities which are found in pulp and paper mills. In their research they found that pitch deposits collected from different parts of the pulp-mill consisted of acetone-soluble and acetone-insoluble fractions. The species used in the pulp-mill were not mentioned in the paper. The pyrograms obtained for the analysis of the insoluble organic fractions which were isolated from pitch deposits are shown in figure 2.10.

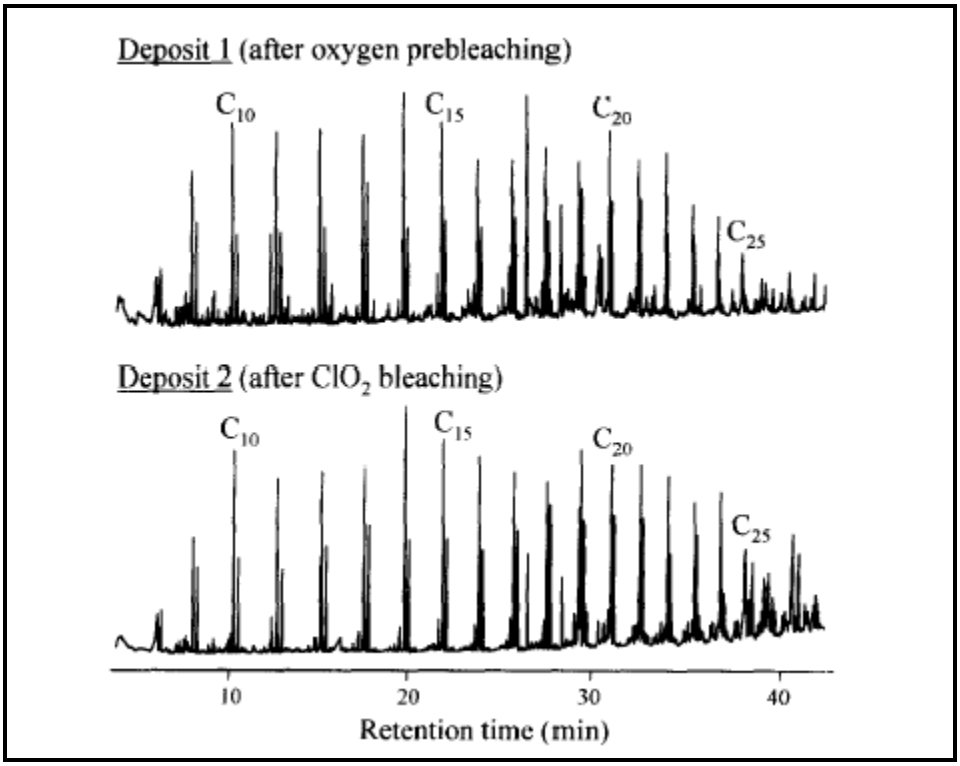
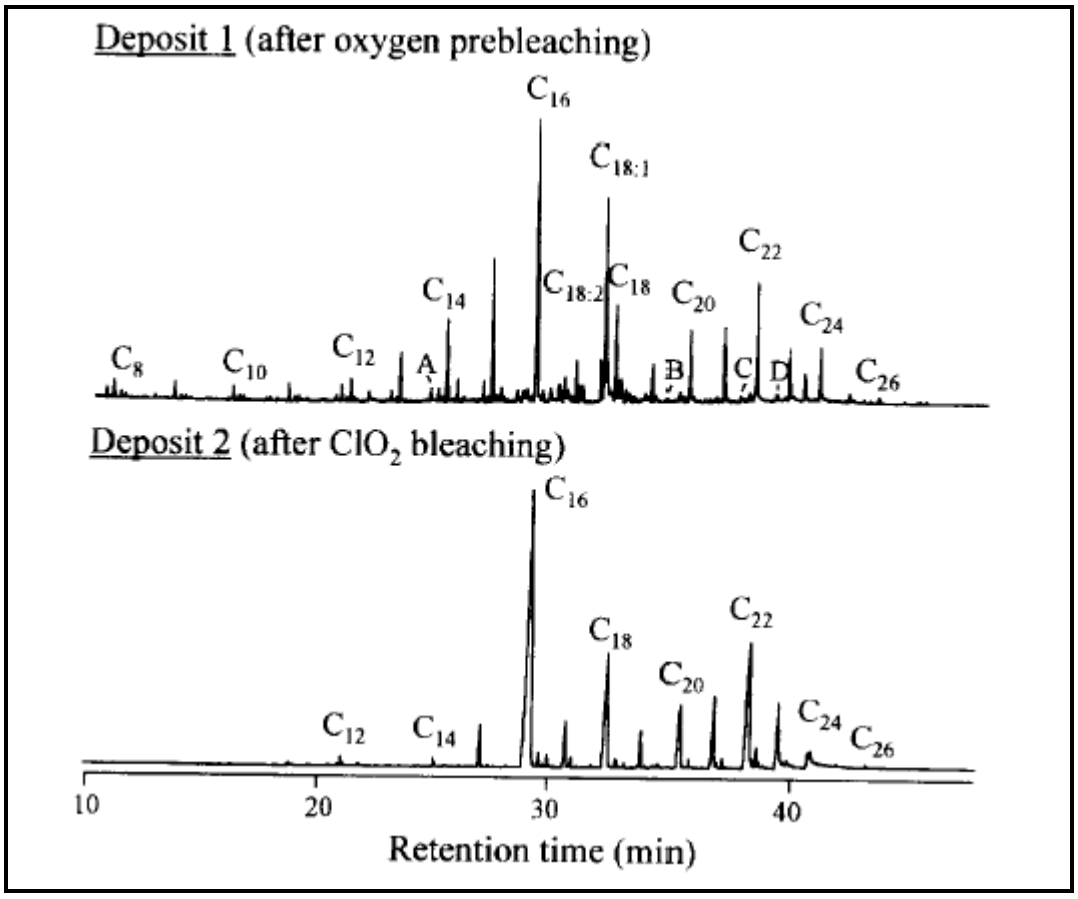


Figure 2.10: Py-GC/MS of pitch deposits showing *n*-alkane/*n*-alkene series (Del Rio *et al*., 1999b).

The pyrograms show a series of C8 to C28 *n*-alkanes/*n*-alkenes, indicating the highly aliphatic character of the insoluble organic matter. No further structural information could be gathered due to the inherent limitations of the technique (Del Rio *et al*., 1999b). The results also indicate that fatty acids exist in large amounts in the pitch deposits collected using Py(TMAH)[6]-GC/MS. This can be seen in the chromatogram in figure 2.11 were the carbon atoms refer to fatty acid methyl ester series. These fatty acids arise from salts of ferric, calcium or magnesium ions which come from additives used for pulping or pitch control.

[6] TMAH, refers to Tetramethylammonium hydroxide



KEY: A: 3,4,5-Trimethoxybenzoic acid methyl ester, B: 2,3,4,2',3',4'-Hexmethoxy-1,1'-diphenyl, C: 2-(2,3,4-Trimethoxyphenyl),2,3,4-trimethoxybenzoic acid methyl ester, D: 2,3,4,2',3',4'-Hexamethoxy-6,6'-dicarbomethoxy-1,1'-diphenyl

Figure 2.11: Py(TMAH)-GC/MS of pitch deposits in pulp-mills (Del Rio *et al*., 1999b).

2.6. Solutions to minimising wood extractives building up or reaching the final product

There are two possible ways to decrease the extractables building up in the machinery or reaching the final product in a form which will reduce the product quality: removing the extractables during the process or stabilising the extractables in a form that can be incorporated in the final product (Gutierrez *et al*., 1998a).

2.6.1. Seasoning

Reduction of pitch has been observed with debarking and seasoning of logs and wood chips (Gutierrez *et al*., 1998a). It is commonly known that storing a felled log will reduce the resin

(collective name for lipophilic wood extractives) content of the wood and change the nature of resin. This however, will depend on the storage conditions. Volatile resin reduction occurs faster when the wood is stored as chips rather than logs. This can be explained since oxidation processes proceed more freely in chip form. This can have negative effects, for instance reduced pulp yield and low pulp quality. Therefore, when seasoning is used it has to be weighed against the loss of pulp quality (Gutierrez *et al.*, 1998a).

Gutierrez and co-workers 1998a also studied the impact of seasoning of *E. globulus* wood (over a three month period) on extractives. Table 2.6 shows the results for *E. globulus* wood and colloidal pitch in black liquors obtained from GC and GC-MS analysis. The experiments were carried out during a three month seasoning period. It shows a reduction of the colloidal pitch by 80% present in black liquors. The colloidal pitch particle concentration was measured using a haemocytometer. The total acetone extract was reduced by 56% while the polar and lipophilic components were reduced by 60% and 38% respectively. Kraft cooking causes nearly all the phenols and polyphenols in the polar fraction of a wood extract to be dissolved, but some lipophilic compounds may remain after kraft pulping and bleaching and will be found in pulp and pulp-mill deposits (Gutierrez *et al.*, 1998a).

Table 2.6: Variation of the total acetone lipophilic and polar fractions (%) from *E. globulus* wood and colloidal pitch in black liquors during a three month seasoning period. The % degradation is represented in brackets (Gutierrez *et al.*, 1998a).

	Control	**1 Month**	**2 Months**	**3 Months**
Acetone extract in wood chips	1.52	1.01 (34)	0.85 (44)	0.67 (56)
Lipophilic in wood chips	0.26	0.26 (0)	0.18 (31)	0.16 (38)
Polar in wood chips	1.26	0.75 (40)	0.67 (47)	0.51 (60)
Colloidal pitch (10^6 particles/cm^3 at 1%) in black liquor	**57.0**	**40.8 (28)**	**14.2 (75)**	**11.5 (80)**

Sterol esters, sterols, fatty acids, triglycerides and steroid ketones are the major lipophilic groups present in fresh *E. globulus* wood accounting for 47, 47, 22, 17 and 5 mg/100g wood respectively. The sterol esters and sterols are mainly constituted from sitosterol.

The variation in the composition of lipophilic extractives from *E. globulus* wood during the three month seasoning experiment is shown below in figure 2.12 (Gutierrez *et al*., 1998a)

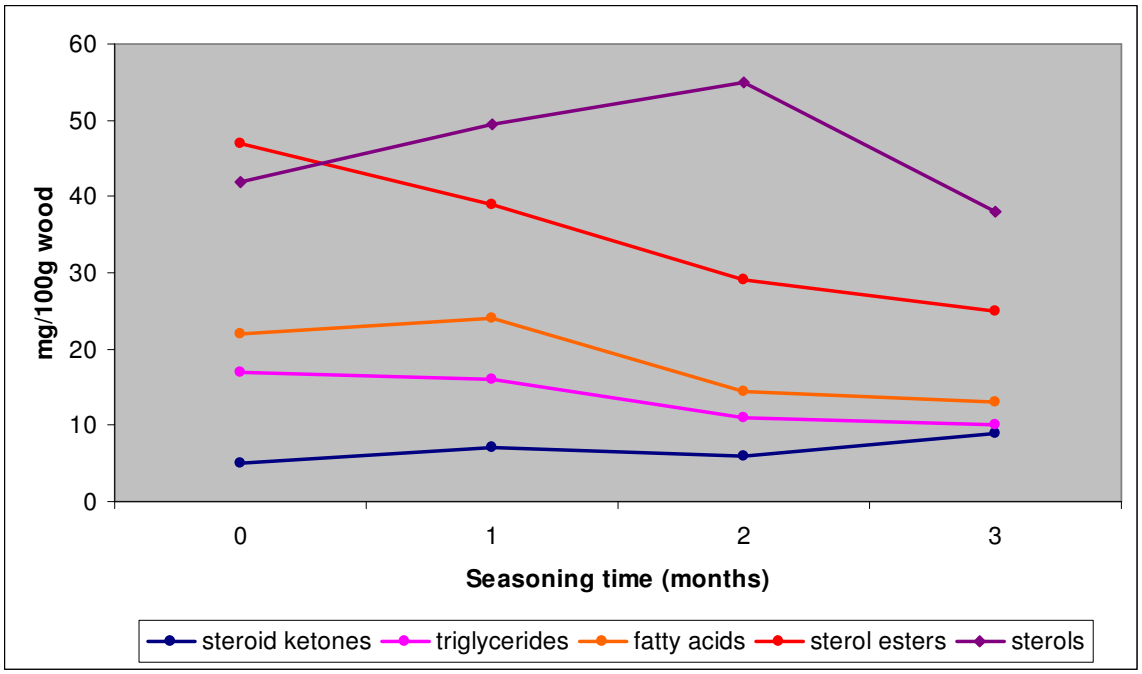


Figure 2.12: Variation in the content of lipophilic extractives from *E. globulus* wood during seasoning (Gutierrez *et al.,* 1998a).

From figure 2.12 it can be seen that during the first month of seasoning there is a decrease in sterol esters. However, the compositions of the different constituents were altered; the most noticeable change was the reduction in the content of sterol esters by 15% (Gutierrez *et al*., 1998a). The second month of seasoning showed a decrease in the sterol esters by 36%, presumably because the sterol esters are being hydrolysed. There was also a parallel increase in the content of free sterols by 31% and steroid ketones by 60%. The result of 31% for the free sterols can be attributed to the hydrolysis of sterol esters taking place faster than the degradation of the sterol moiety. The triglyceride content continued to decrease by 29% during the second month. During the third month, there was a continued decrease in the content of sterol esters, triglycerides and fatty acids, however at a much lower rate than the previous month. Hence from the results it can be seen that by seasoning the *E. globulus* wood chips a decrease in the lipophilic wood extractives responsible for pitch occurs (Gutierrez *et al.,* 1998a).

2.6.2. Chemical Additives

The use of carbon dioxide in brownstock washing in kraft mills without oxygen delignification improves runnability due to increased pulp dewatering capacity, decreased precipitation of calcium soaps, and decreased foaming. Another way to avoid problems caused by extractives is to retain them in the sheet. This can be accomplished by adding retention aids that assist the wood resin attachment to the fibres and increase the first pass retention of fines and fillers (Laleg and Pikulik, 1993). These are additives, such as Chitosan, Polyethylene oxide (PEO), zirconium oxychloride and PolyDADMAC (polydiallyldimethylammonium chloride). The structures for these chemical compounds are shown in figure 2.13.

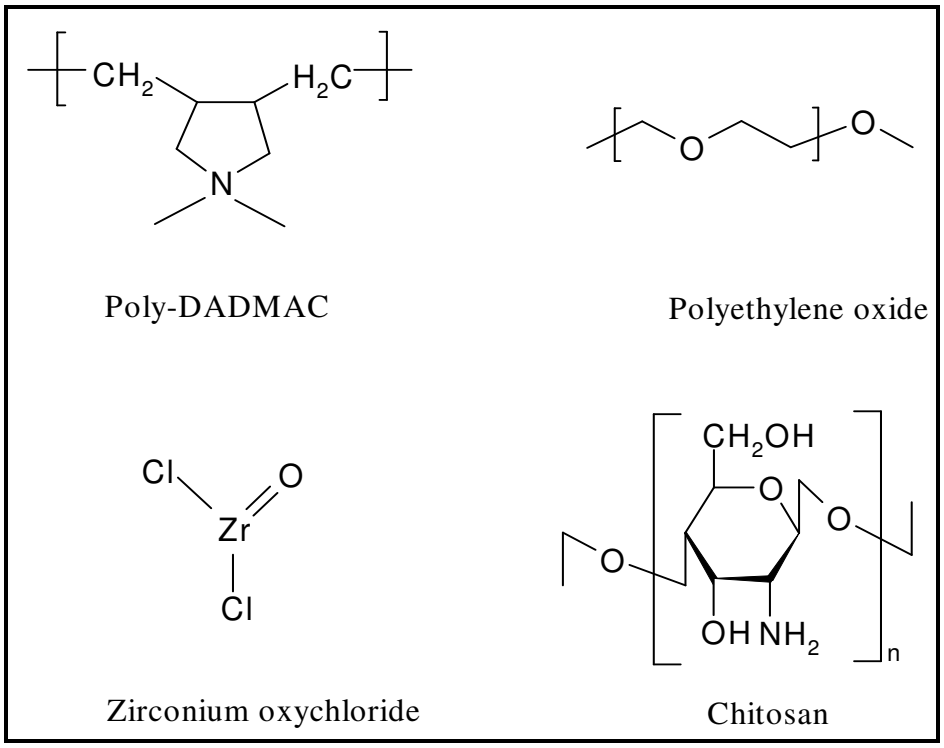


Figure 2.13: Chemical structures of additives used in paper making (Laleg and Pikulik, 1993).

Additives can also be used to change the nature of wood deposits to make them less sticky. Talc ($Mg_3SiO_4O_{10}(OH)_2$) is an example that coats the deposits making them less likely to grow (Douek and Allen, 1980 and Allen *et al.*, 1991). Surfactants are also used to reduce pitch deposits (US Patent, 1995 and Richardson, 1995).

The research of Verenich *et al.,* 2004, concentrated on the treatment of process water, left over after membrane filtration, by wet oxidation (WO). The aim was the complete degradation of lipophilic wood extractives (LWEs) which cause pitch problems. The residue after filtration

contains the LWEs, which are highly toxic. The LWEs concentration was 450 mg/l in the membrane concentrate. Table 2.7 shows the effect of temperature on the removal of LWEs. The table illustrates that the hydrophilic lignans had greater reactivity than the other LWEs, as determined by GC. At higher temperatures the effect of hydrolysis was greater in destroying sterol esters and triglycerides along with oxygen action (Kostamo and Kukkonen, 2003). The fatty and resin acids reacted with oxygen more slowly and therefore remained in the colloidal state.

Table 2.7: The effect of temperature on the removal of LWEs at an oxygen partial pressure of 1MPa after 120 min of the oxidation process (Kostamo and Kukkonen, 2003).

Component	**Removal, %**			
Temperature/ °C	**120**	**130**	**140**	**150**
Fatty Acids	29.5	44.8	65.5	Decomposed during preheating
Resin Acids	Not Analyzed	39.0	68.8	85.3
Lignans	73.6	66.4	90.6	94.0
Sterols	Not Analyzed	36.6	79.0	91.5
Sterol Esters	49.4	41.2	81.3	92.0
Triglycerides	46.1	51.8	92.9	98.5
Total	**57.8**	**51.4**	**85.0**	**92.0**

2.6.3. Bleaching

Many investigations have considered the route of deresination viz. the removal of wood resin. Bergelin and Holmbom, 2003, looked at deresination of birch kraft pulp during bleaching. This can be achieved in two ways, by desorption and dispersion of resin from fibres, followed by removal with washing liquors, or by oxidation of resin components to more hydrophilic/water soluble forms, which then are easily washed out (Bergelin and Holmbom, 2003). In their study, two samples were analysed through the bleaching processes, one using ECF bleaching and the other TCF bleaching. In both processes the birch unbleached pulp showed high levels of residual resin.

Freire *et al*., 2005 investigated the behaviour of extractives during an elemental chlorine free (ECF) bleaching sequence, DEDED[7]. They noted that approximately 80% of the aliphatic extractives were removed from the pulp during bleaching. This constitutes 70% of the sterols, 70% of the fatty acids and 90% of the long-chain aliphatic alcohols. The sterol decrease is mainly due to the degradation of sitosterol by chlorine dioxide, while the decrease in fatty acids and alcohols is essentially due to extraction and elimination with the alkaline filtrates. The

[7] Bleaching sequence were the D refers to the stage with chlorine dioxide and E the stage of alkaline extraction

identification of the high amounts of 24-ethylcholestene-3,5,6-triol in the bleached pulp is evidence of the degradation of β-sitosterol.

High content of long chain aliphatic compounds and sterols are noted in the E_2 stage filtrate (Figure 2.14) (Freire *et al*., 2005). This suggests that the alkaline extraction stages are effective in the removal of lipophilic extractives from pulp. The E_2 stage has a higher lipophilic content than the E_1 stage probably due to their increasing accessibility in the pulp fibre. The accessibility of extractives in pulp fibre can also explain the high content of lipophilic compounds, particularly fatty acids and alcohols which are found in D_2 filtrate, (Figure 2.14). Hence the partial purge of the E_2 and D_2 filtrates may aid in the accumulation of lipophilic extractives in situations of risk pitch deposition (Freire *et al*., 2005).

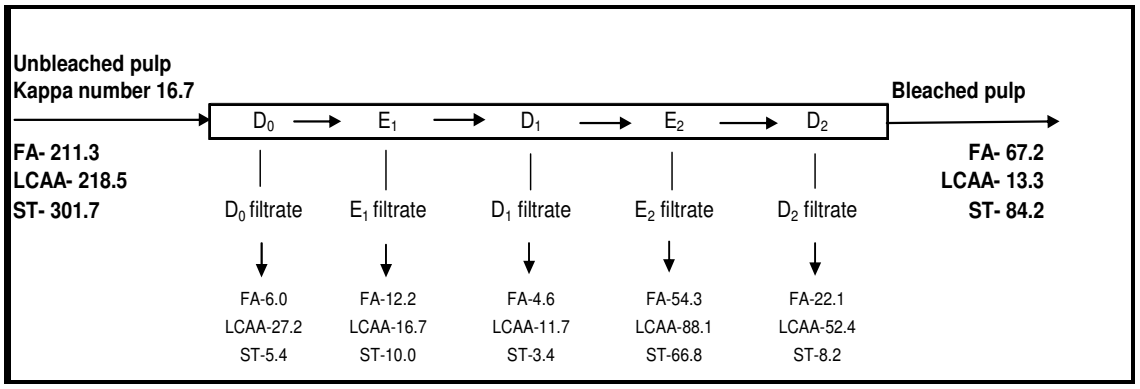


Figure 2.14: Quantities of lipophilic extractives (mg/kg of dry pulp) removed in the different bleaching stages along the ECF bleaching sequence. FA: Fatty acids, ST: Sterols and LCAA: Long Chain Aliphatic Alcohols (Freire *et al*., 2005).

2.7. Conclusion

Measuring extractives in wood involves quantifying and characterising them using wet lab chemistry, GC and GC/MS. Previous studies (Verenich *et al*., 2004 and Gutierrez *et al*., 1998b) have confirmed that lipophilic wood extractives, primarily the sterol esters, sitosterol, fatty acids and resin acids are known to cause pitch. GC and GC/MS has also been used to measure the extractives in pitch. Since accumulation of extractives is a serious problem, mills in South Africa are eager to find an accurate method of measuring wood extractives and understanding which of these extractives are responsible for pitch.

Therefore, the focus of this project was to:-

a) characterise and quantify wood extractives in *E. grandis and E. nitens*

b) determine wood extractives responsible for pitch formation.

Chapter 3: Sampling Methodology

The project design involved the evaluation of wood extractives in *E.grandis* at rotation age from a medium site quality. **Table 3.1** lists the criteria that were identified for compartment selection

Table 3.1: Criteria used to determine the suitability of sites

Property	Criteria
Age Class	9 years
Site Quality	Medium (22.7)
Species	*E. grandis*

3.1. Field Work

Once a suitable site had been identified, a micro site (15m radius plot) of the compartment was selected for relative homogeneity in the area where trees were to be sampled. An enumeration from the micro site area was used to verify suitability according to **Table 3.1** (refer to Appendix A: Enumeration data and Tree Information). After enumeration, site index (at base age 20) was calculated, using the modified Schumacher-difference form. The site index gives an indication of the quality of a site. The greater the site index, the better the performance of the trees.

Site index (HD_2) = *3*HD1*exp[*1(AGE_1 * AGE_2) +*2 {(1/AGE_1) * (1/AGE_2)}]

where: HD_2 = Dominant Height at age 20, in meters

HD_1 (m) = Mean Dominant Height (of 20% tallest trees)

Age_1 = Stand age

Age_2 = 20 years

Sampling was conducted during November 2005 and the stands were located with the assistance of the senior forester at the respective plantation. Site characteristics were recorded prior to sampling (**Table 3.1**). At the site (one site was selected) within the enumerated plot, 5 trees were selected randomly and sufficiently far from the roadside to avoid edge effects. Uncharacteristic or damage trees were avoided. For each selected tree, over bark diameter at breast height (DBH) was measured prior to felling and subsequent to felling total tree height and the merchantable stem height to a top diameter of 7 cm, over bark were measured (Appendix A: Enumeration data and Tree Information).

Table 3.2: Medium site characteristics

Region	Zululand
Plantation	Sappi: Trust A44
Age	9years 1month 16 days
Spacing (m)	2.7×2.4
Area (ha)	12.20
Measured site index	22.7
Mean DBH[1] (cm)	22.00
SD[2] DBH	3.08
Mean Total Height (m)	26.78
SD[2] Height	1.90

[1] DBH = Diameter at breast height of the trees at time of sampling

[2] SD = Standard deviation

Merchantable stem height and DBH were recorded using a 30 m measuring tape and a DBH tape respectively. The total tree height was measured using the Vertex III and Transponder III. The vertex and transponder are manufactured by Haglőf Sweden. The transponder was placed on the bark of the tree to be measured at breast height. The vertex was aimed towards the transponder. Both the distance and angle to the transponder was recorded. The Vertex was then aimed to the top of the tree to measure and record the height that was essentially calculated from the angle and distance between the Vertex and the transponder. Three different height readings were obtained to ensure accuracy. The two units communicate via ultrasonic impulses and this makes measurement in difficult surroundings and conditions possible.

Upon felling destructive sampling locations were marked and the billets were taken at breast height to 7cm in diameter for analytical studies. Samples were labelled using a crayon and identified using alphanumeric code that described the year, which cooperative, which visit and the billet number (**e.g**. 05-PR-001-001).

The billets were de-barked and transported to the Forestry and Forest Products (FFP) Research Centre, Durban, for analysis.

Chapter 4: Experimental Section

4.1. Material

Two eucalyptus species were used for this study, *Eucalyptus grandis* and *Eucalyptus nitens*. Many studies have been carried out on the composition of extractives from softwoods and hardwoods commonly used in the pulp and paper industry. This is not the case for *Eucalyptus* wood which is used primarily in the paper industry in South-Western Europe, South America and South Africa (Gutierrez, *et.al.*, 1999). In South Africa *E. grandis* is the most commonly used specie of wood and studies on wood extractives have previously been done on this specie. However not many studies have been carried out on *E. nitens* and since it is being used by SA mills it would be a good choice for studying.

Five *Eucalyptus grandis* trees, 9 years old were selected, growing at medium site quality (SI 22.7). From each tree, a log was taken from diameter breast height[8], until the trunk diameter of 7cm to be representative of the overall extractive content in a tree. *Eucalyptus nitens* samples were also collected from medium site quality.

4.2. Method

4.2.1. Chipping

Billets were chipped using a 38 inch industrial chipper. The chips were ground using a hammer mill (MACSA) and wiley mill {T.R.U. 267(a)} respectively.

4.2.2. Sample preparation

A bag of chips for each tree was left to dry for three weeks. After being air- dried, five handfuls of chips per tree were taken at random and ground. The ground samples were passed through a 0.40mm screen and placed in plastic bags to avoid loss in extractives for analysis in the laboratory. At this point it is expected that the volatile extractives would have gone during the preparation process.

[8] Diameter Breast Height abbreviated DBH. This has traditionally been the point on a tree where measurements are taken and a multitude of calculations are made to determine things like growth, volume, yield and forest potential

4.3. Investigation of efficiency and accuracy of solvents during extraction process

4.3.1. Extracting abilities of individual solvents

The first phase of the project was the investigation of extracting abilities of different solvents. The solvents chosen for this experiment were acetone, ethanol and toluene. This was done to determine which solvent individually gave the highest yield and whether the polarity index of the solvent impacted on this finding. This was also done to serve as a check for the suitability of these solvents for the project and to confirm the results from the previous studies (Sefara and Birkett, 2004).

Two grams of *E. grandis* sawdust was extracted using a chromatography column (the column was 2 cm in diameter and 30 cm long, packed to the bottom with cotton wool). The solvent was passed trough the column until the solvent eluting was colourless. The collected extract was evaporated on a hotplate and analyzed using GC (For the method refer to section C: Characterisation and quantification of extractives in *E. grandis* and *E. nitens* wood). This was carried out for each of the three solvents used. This was also done on the *E. nitens* pitch sample to determine whether the same tendency was obtained as in the instance of the sawdust.

4.3.2. Determination of extractive amount after extraction using different solvent sytems in *E. grandis* and *E. nitens* wood

The second phase of the chemical analysis was the extraction using, acetone and a combination of ethanol and toluene. Three extractions were carried out, hot water followed by acetone, hot water followed by ethanol - toluene and acetone only. The measurements were carried out in triplicate for *E. grandis* and in duplicate for *E. nitens*. The reason for carrying out the extraction using different methods was to determine the efficiency of the solvent systems.

4.3.2.1. Hot water extraction

Duplicate 5 g samples of dry sawdust were weighed into 400 mL beakers and 300 mL of hot distilled water was added. The beakers were then placed on a hot plate and boiled gently for 3 hours ensuring that the level did not fall below 300mL by topping up with boiling distilled water. The sawdust (now free of water soluble extractives) was quantitatively transferred to a pre-weighed filtering crucible that has been previously dried at 105ºC.

The water soluble extractive free sawdust was washed with 200 mL of hot distilled water and dried at 105ºC overnight. It was then cooled in a desiccator before weighing. After being weighed the eluted water extractives were reacted with 1mL of tetramethylammonium hydroxide, TMAH (5% in methanol) placed in a sealed vial (4 ml) and stored in a refrigerator prior to GC analysis (For the method refer to section C: Characterisation and quantification of extractives in *E. grandis* and *E. nitens* wood)..

4.3.2.2. Solvent Extraction

Duplicate 4 g amounts of the oven dried hot water extracted sawdust samples were weighed out into extraction thimbles and placed into the Soxhlet extraction tubes connected to a pre-weighed flask containing about 300mL of the ethanol-toluene mixture (ratio of 1:2) or acetone The Soxhlet apparatus was placed, with condenser attached into the heating mantle and the temperature of the heating mantle was adjusted to provide a boiling rate that cycled the solvent at least six times per hour. The specimens were extracted for not less than 24 extractions over a 4-5 hour period. After the extraction was completed, most of the solvent in the flask was evaporated leaving behind a few milliliters of solvent containing the extractives. The extractives were dried for 1 hour at 115ºC, then cooled in a desiccator and weighed. After being weighed the eluted extractives were reacted with 1 mL of tetramethylammonium hydroxide, TMAH (5% in methanol) placed in a sealed vial (4 ml) and stored in a refrigerator prior to GC and GC-MS analysis (For the method refer to section C: Characterisation and quantification of extractives in *E. grandis* and *E. nitens* wood).

4.3.3. Characterisation and quantification of extractives in *E. grandis* and *E. nitens* wood

GC and GC-MS were used to characterise and quantify the extractives removed from *E. grandis* and *E. nitens* sawdust. HPLC and UV were used to test for the presence of sugars and lignin respectively. Acidolysis is another technique that was used for the identification of lignin.

4.3.3.1. Gas Chromatography

Fifteen to 20 mg of the dried extractives was reacted with 1 mL of tetramethylammonium hydroxide, TMAH (5% in methanol) in a sealed vial (4 ml). The TMAH treatments convert all fatty acids, sterol esters, triacylglycerides, free acids, etc to methyl esters. The sample was heated to

105˚C until clear and analyzed by GC. Gas Chromatographic analyses was performed using a Perkin Elmer Clarus 500 equipped with a FID detector using nitrogen as the carrier gas (0.8 ml/min), and an Elite 608 column (Perkin Elmer, Massachusetts, USA), coated with 5% phenol methyl silicone. The conditions for the analysis can be seen in Table 4.1.

Table 4.1: Conditions used for GC analysis.

Injector Port Temperature	280ºC
Initial Temperature	120ºC for 1 min
Ramp 1	10.0ºC /min to 290ºC hold for 15.00min
Column dimensions	L=30m I.D=320µm and DF=0.25µm
Detector and temperature	FID, 350ºC

4.3.3.2. Gas Chromatography-Mass spectroscopy

Fifteen to 20 mg of the extractives was dissolved in 1 mL of methanol. GC-MS analysis was performed using a Agilent 6890 GC/5973 MS using helium as carrier gas (30cm/min), equipped with a J & W HPS-MS column (J & W Scientific, Inc., Rancho Cordova, CA), coated with phenyl methyl silicone. The conditions for the analysis can be seen in Table 4.2.

Table 4.2: Conditions used for GC-MS analysis.

Injector Port Temperature	300ºC 1:75 split
Initial Temperature	50ºC for 2 mins
Ramp 1	20.0ºC /min to 300ºC hold for 10.00min
Column	J and W HPS-MS
Column dimensions	L=30m I.D=250µm and DF=0.25µm
Detector Temperature	350ºC

4.3.3.3. Quantification of extracts

The quantification process involved the addition of an internal standard to the extract samples which were then analysed using GC. In this instance the C_{22} fatty acid methyl ester was used. This was chosen since fatty acids are the group of lipids most commonly analyzed by GLC. This method is applicable to biological samples containing compounds with chain length in the range C14 to C24. Also, the logarithm of the relative retention time and the number of carbon atoms is linearly related and may be of some help to identify unknown compounds.

A two step dilution process of the methyl ester had to be done so that the height of the internal standard approximated the peak height of the fatty acids present in the extractives. The internal standard was then added just before the analysis stage. The equation used in the calculation:

$$\text{Amount unknown} = (A_{unknown}/A_{internal\ standard})^* [IS]/R_f$$

The concentration of the C_{22} fatty acid methyl ester stock solution used as an internal standard was 0.0145 mg/ml. The dilution was 2:1 and thus the concentration of the standard was 0.00725 mg/ml.

4.3.4. Chemical analysis of liquid extracts

Figure 4.1 is a summary of the chemical analysis of the acid soluble extracts. The liquid extracts were analysed for two classes of compounds; lignin and sugars. This was done to determine what was responsible for the brownish colour of the extracts.

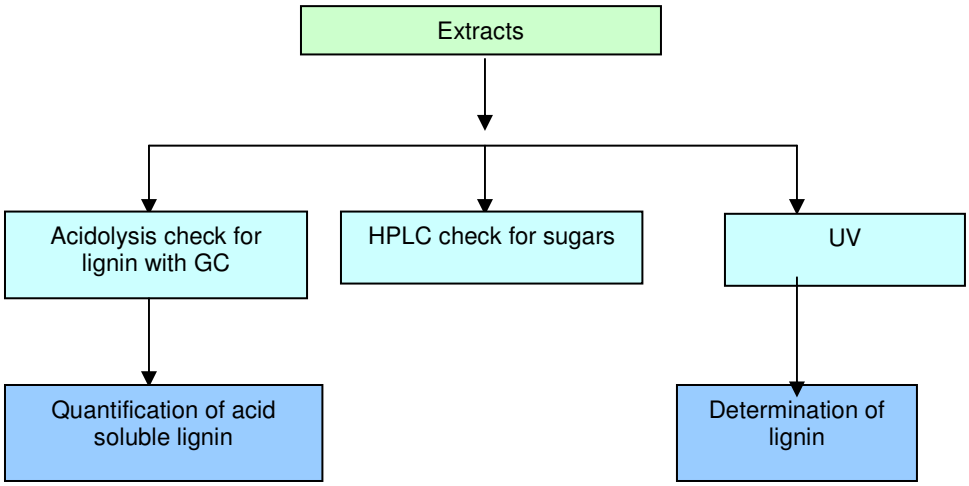


Figure 4.1: Chemical analysis pathway for the determination of acid soluble extracts.

4.3.4.1. High Performance Liquid Chromatography for the detection of sugars

High Performance Liquid Chromatography (HPLC) was used to establish the presence of individual sugars in the extract. The method used was FFP 002 (carbohydrate composition of extractives – free wood and wood pulp by high performance liquid chromatography). The method is attached in Appendix A: Method, Enumeration and Tree Information. The detector used was a Pulsed Amperometric Detection (PAD) to detect sugars.

Three hundred and fifty milligrams of extract was weighed into a test tube and in another test tube 2.0 g of the extract used for moisture determination. Exactly 3 mL of 72% H_2SO_4 was added to the test tube and stirred. The test tube was placed in a 30ºC water bath for 1 hour and stirred regularly while kept in the bath. The contents of the test tube were washed quantitatively into a 250 mL schott bottle with 84 mL deionised water. The closed Schott bottle with the solution was then placed in an autoclave and heated at 10.3kPa (120ºC) for 1 hour.

The hydrolyzate was cooled to room temperature and filtered quantitatively under vacuum through a 0.45µm filter paper, using deionised water to rinse the Schott bottle. The filtrate was transferred into a 200mL volumetric flask and diluted with deionised water. The solution was mixed well. Fifty millilitres was transferred into a plastic bag, sealed and frozen for 24 hours.

Prior to HPLC analysis, the samples were thawed and diluted 1:10 dilution (pipette 50µL of sample and 500µL of deionised water). The samples were then placed into an auto sampler vial. The vial was placed in the auto sampler tray and the position recorded.

Table 4.3: Conditions for HPLC analysis (Flow rate for the steps highlighted)

Filtered deionised water (equilibration)	6.5 mins
Injection	15 µl
Filtered deionised water (separation)	6 min
Ramp to 130 mM or 120 mM sodium acetate in 200 mM NaOH	1 min
Ramp to 130 mM or 120 mM sodium acetate in 200 mM NaOH (acetate loading step)	3 min
Ramp filtered deionised water	1 min
Flow rate	1 ml/min
Post column addition of 350 mM NaOH (flow rate)	0.5 ml/min

4.3.4.2. Ultra-Violet Spectroscopy for the identification of lignin

The extract of *E. grandis* and *E. nitens* were too concentrated and needed to be diluted several times to give an absorbance in the range of 0.0 to 1.0. The samples were diluted using methanol (cut-off wavelength 205 nm), which was also the reference. The samples were then analysed using a Cary 1E UV spectrophotometer Varian Inc. The spectra were recorded between 200 and 700 nm.

4.3.4.3. Acidolysis

This method is used for S/G[9] ratios and was used in this study to determine whether lignin was present. Fifty milligrams of the extractive sample was weighed into a 4 ml sample vial and to it 2 ml of 10:1 dioxane: water with 0.2 M HCl + 1 mg/ml BHA[10] was added. The sample was placed in a suitable rack and placed in the oven set at 105˚C for 4 hours with occasional gentle mixing.

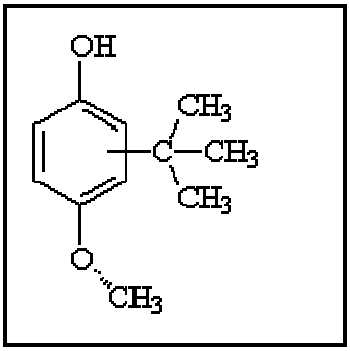


Figure 4.2: Chemical structure of BHA

After the 4 hours the vial was removed from the oven and cooled, and 0.90 ml of 0.40 M sodium bicarbonate solution in water and 1 ml chloroform was added. The vial was then mixed well. After the layers had separated, the organic layer was removed with a glass pasteur pipette and transferred to another 4 ml vial. Using the same pipette 2 ml of distilled water was added to the vial and mixed. The upper layer was removed with a pasteur pipette and a second amount of water was added, the vial was sealed and mixed.

When the sample had settled, the inorganic layer was removed using a pasteur pipette into a GC sampling vial. The analysis was carried out on the GC.

Acidolysis was used to detect for the presence of acid soluble lignin.

4.3.5. Identification and characterisation of wood extractives in pitch and comparison with extractives found in wood

The two pitch samples were obtained from Enstra; this mill uses 100% *E. nitens*. The pitch obtained was blackish lumpy material which stuck together in some instances. The chemical analysis followed the similar pathway to the sawdust i.e. ethanol-toluene after hot water extraction and acetone only extraction. All analyses were carried out in triplicate.

[9] Syringyl (S) and guaiacyl (G) lignin units
[10] Butylated Hydroxyanisole

4.3.6. Summary of Experimental Design

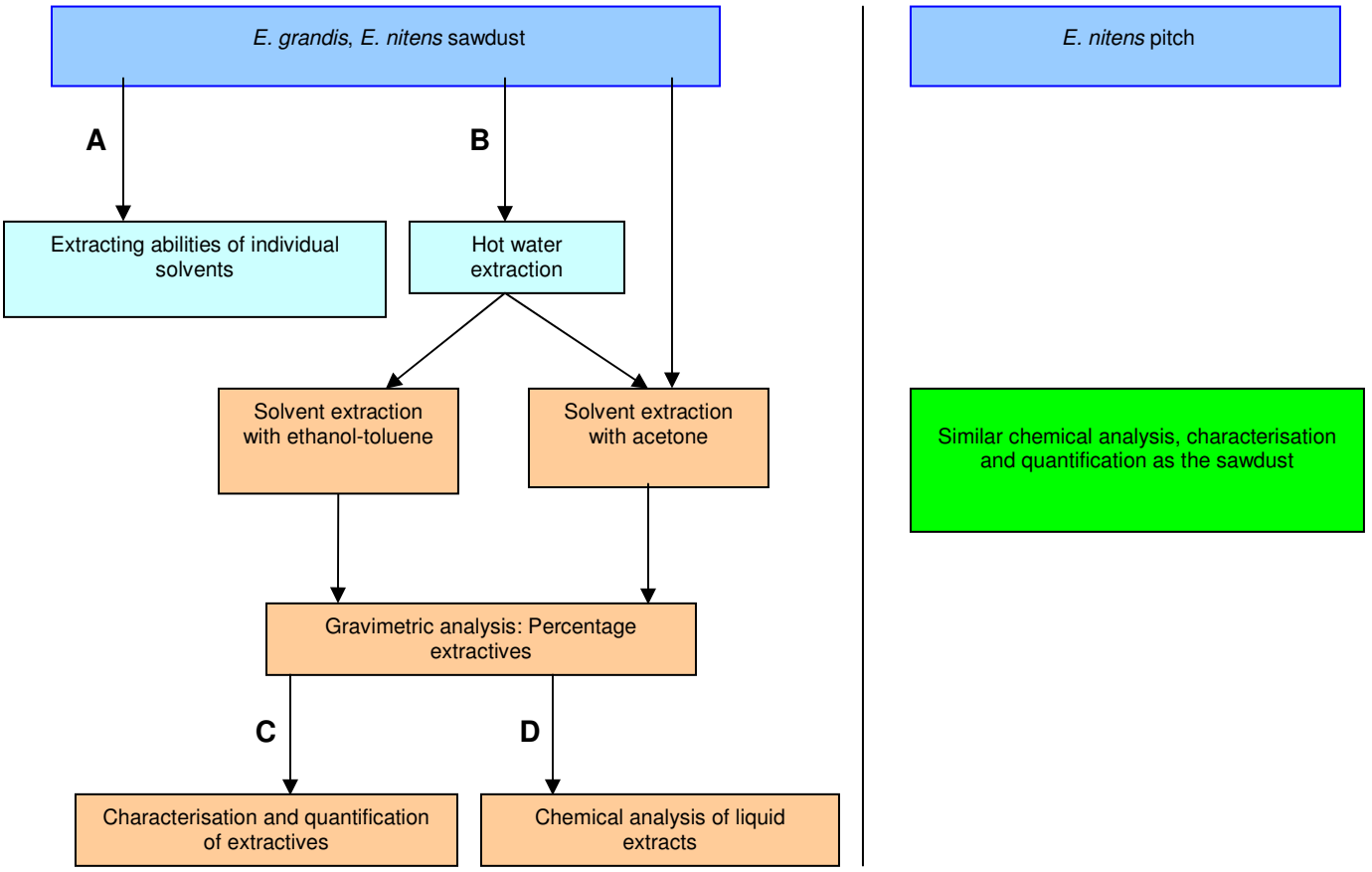


Figure 4.3: Experimental design to test the efficiency of solvents, characterisation and quantification

Chapter 5: Results and Discussion

5.1. Comparison of individual solvent abilities to determine the effect of polarity index

The ability of individual solvents were evaluated to determine which solvent extracts the most and whether the polarity index of the solvent has an impact on the amount extracted. This involved passing the solvent through a column packed with woodmeal. Figure 5.1 shows the amount of extractives removed by individual solvents for *E. grandis* and *E. nitens* woodmeal.

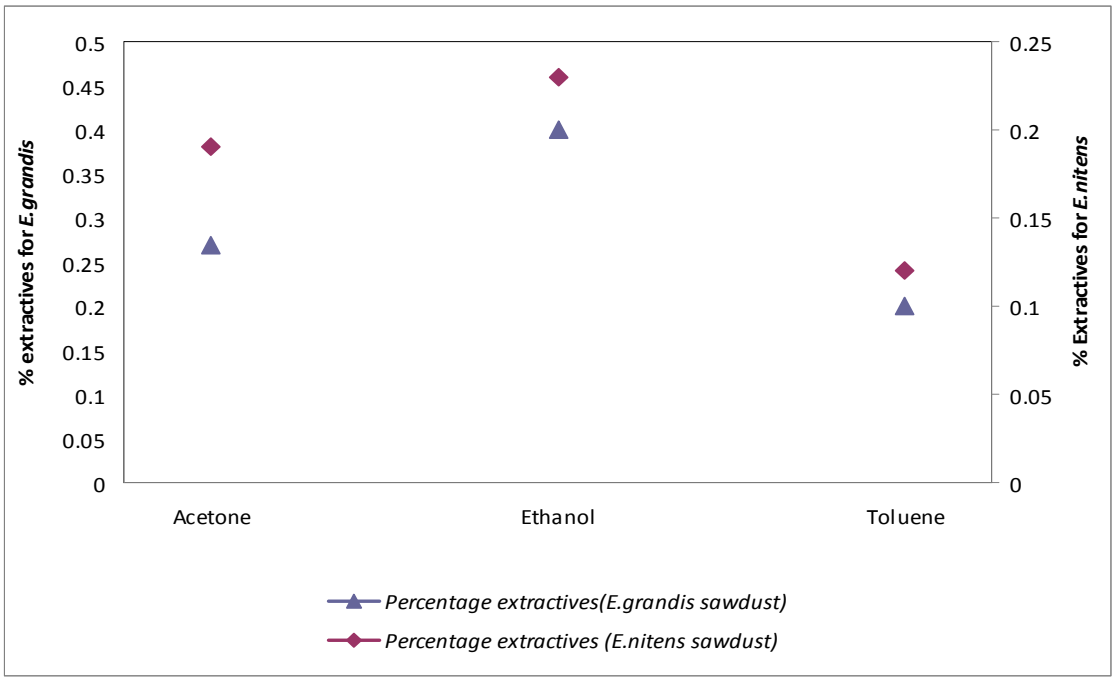


Figure 5.1: Extractives from woodmeal of *E. grandis* and *E. nitens* using individual solvent systems.

The work conducted here showed the same tends as those found by Birkett and Sefara (Sefara and Birkett, 2004); however the amount of extractives found in this study was significantly lower. It was found that toluene removed the lowest amount of extractives as compared to acetone and ethanol. Extractives removed by toluene amounted to 0.27 % in *E. grandis* and 0.12 % for *E. nitens*. The difference of the solvents can be attributed to the different amounts of material extracted. The polar index (measure of the ability of the solvent to interact with various test solutes) of toluene is 2.4, ethanol 4.3, acetone 5.1. Ethanol removed a higher amount of extractives than acetone despite having a lower polar index than acetone, which can be attributed

to the fact that ethanol removes non-resinous compounds as well such as short polymers and lignin. Hence ethanol by itself was not selective to extractives alone but also removed other low molecular weight wood compounds (Sefara and Birkett, 2004).

Figure 5.2 from literature is confirmation of the above results which shows clearly that toluene removed the lowest amount of extractives. Also ethanol removed higher amounts of extractives than acetone. Literature has shown that the extraction time affects the yield of extractives removed from *Pinus pinaster* (Sierra, *et al.*, 1991). Their results indicated that ethanol yields extracts in the range of 0.20-0.83 % over a period of 90 mins. Although the species used were different what is emphasized is that a similar behaviour can be noted for ethanol in both *P.pinaster* and *E. grandis.*

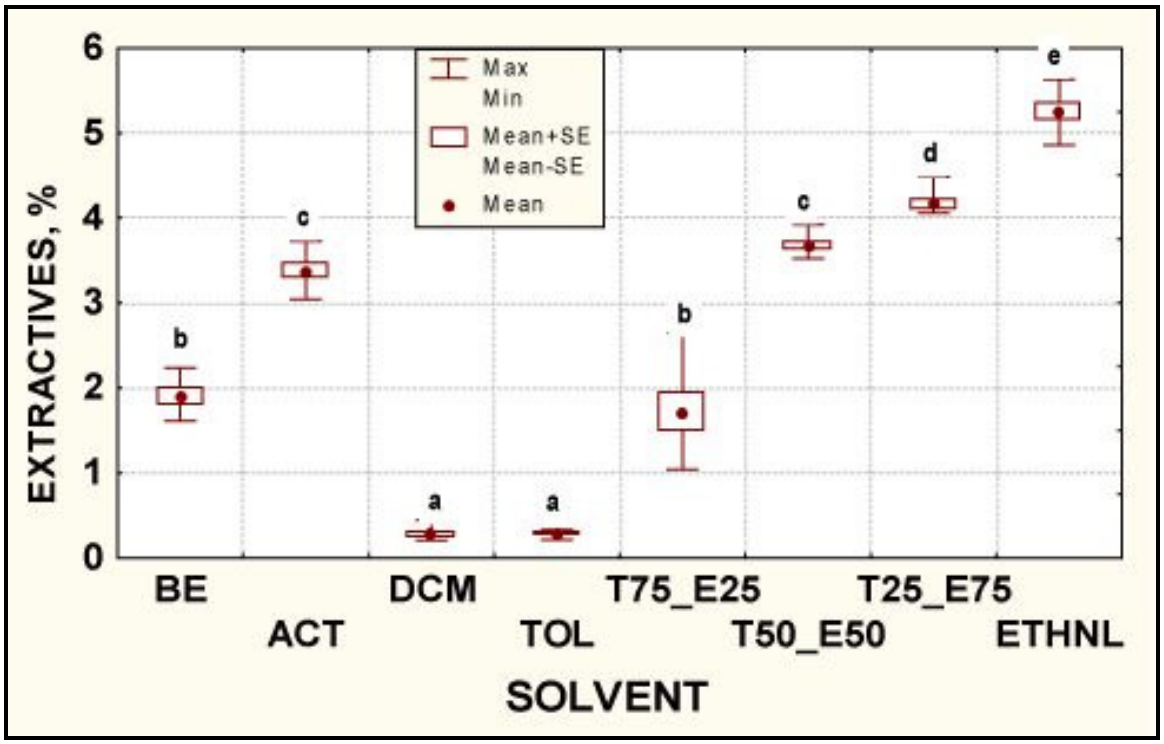


Figure 5.2: Organic soluble extractives from *E. grandis* sawdust. (Sefara and Birkett, 2004).[11]

KEY: BE: Benzene ACT: Acetone DCM: Dichloromethane TOL: Toluene ETHNL: Ethanol T75_E25: Toluene: Ethanol (2:1) T50_E50: Toluene: Ethanol (1:1) T25_E75: Toluene: Ethanol (1:2)

[11] Identical letters represent that no significant difference was noted between that particular solvent.

From the results it can be concluded that the polarity of the solvents impacts on the yield of extractives. The polarity of the solvent determines what type of compounds it is able to dissolve. It was documented that the solvent plays a role in the material removed (Demirbas, 1991). The polarity of the solvent has been suggested as the reason for the difference in the amount of extracts recovered (Wallis and Wearne, 1997). Table 5.1 below shows the total extracts for various solvents on pine wood.

Table 5.1: Results obtained by Wallis and Wearne for different polarity solvents on pine wood (Wallis and Wearne, 1997).

	Hexane	DCM	Acetone	Methanol
Total extracts (%)	1.55	1.82	2.71	3.33

The above results show that different solvents are capable of extracting different amounts of extractives and are in agreement to what has been previously stated by Lacorte *et al*., 2003. Sefara and Birkett, 2004 confirmed that different solvents remove varying amounts of extractive compounds.

5.2. Determination of extractive amount in E. grandis and E. nitens sawdust after extraction using different methods

5.2.1. Comparison of hot water extractives removed from *E. grandis* and *E. nitens*

This section looks at the amount of hot water extractives removed from the both species. The analysis was done using 5 g of dry sawdust, to which 300 mL of hot distilled water was added and boiled for 3 hours. After which the samples were filtered and weighed to determine the percentage of extractives removed.

Figure 5.3 shows the average percentage hot water extractives removed from *E. grandis* and *E. nitens* sawdust (Refer to Appendix B and C respectively, *E.grandis* and *E. nitens* Extractions: Data Collection and Calculations for all raw data). For this experiment duplicate samples of dry sawdust were weighed into beakers and hot distilled water was added. The beakers were then

placed on a hot plate and boiled gently for 3 hours. The water soluble extractive free sawdust was then weighed and calculated based on a percentage.

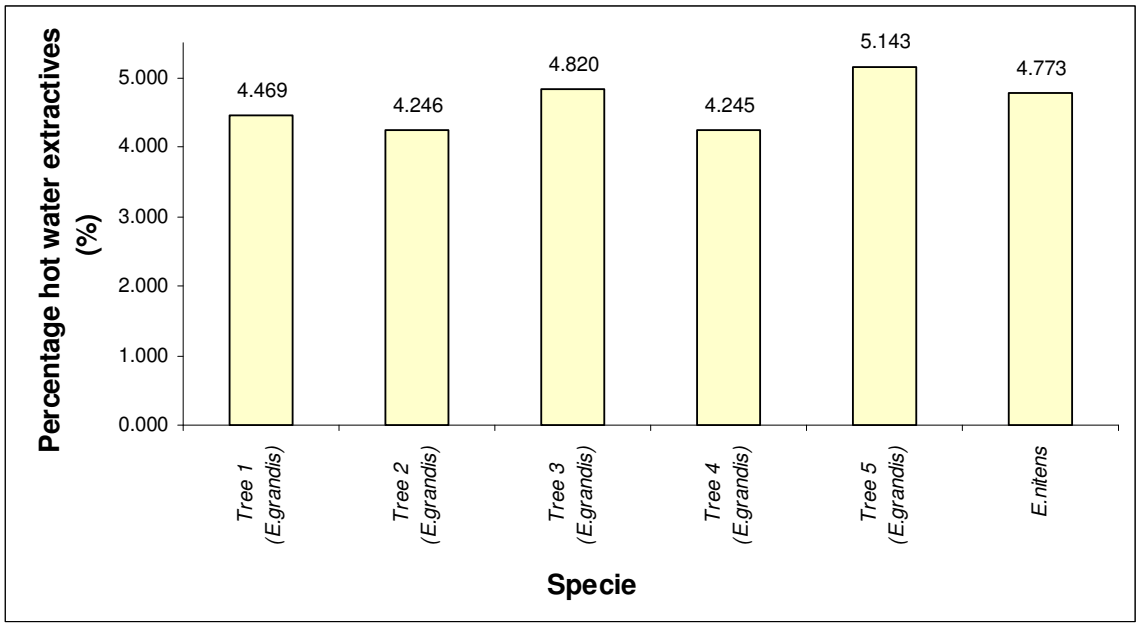


Figure 5.3: Hot water extractives from *E. grandis* and *E. nitens*.

The average amount of hot water extractives in both species varied between 4 and 5 %. However tree 5 (*E.grandis*) showed the tendency to contain slightly higher water extractives than any of the other trees, looking at the data in Appendix B.

5.2.2. Comparison solvent extractives removed from *E. grandis* and *E. nitens*

This part of the analysis involved two parts, firstly weighing 4 g of the water soluble free sawdust (hot water extraction done in triplicate) and refluxing for 240-300 minutes using ethanol-toluene, and then re-weighing and using acetone. Secondly weighing 4 g of sawdust directly and doing a soxhlet extraction using acetone. The solvent was then evaporated, the sample dried and then weighed to determine the percentage extractives removed by soxhlet extraction.

Figure 5.4 shows the average percentage of extractives of *E. grandis* and *E. nitens* obtained after an acetone extraction on sawdusr subjected to hot water extraction, ethanol-toluene extraction on sawdust subjected to hot water extraction and lastly an acetone extraction on sawdust only for *E.*

grandis and for the *E. nitens* sample. The figure also shows results obtained by Sefara and Birkett, 2004, from their study.

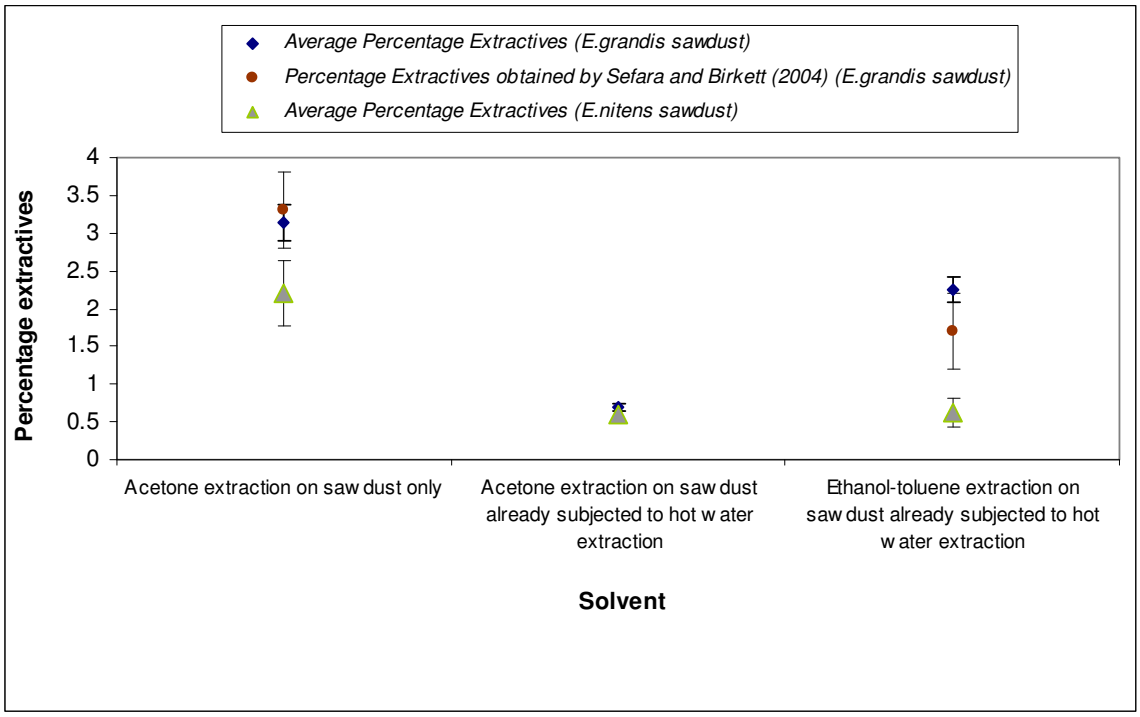


Figure 5.4: Percentage of extractives of *E. grandis* and *E. nitens* woodmeal obtained using different solvents. Results are compared to literature (Sefara and Birkett, 2004).

Table 5.2: Summary of extractives removed for the different methods for *E. grandis* and *E. nitens* sawdust

	(%) extractives acetone extraction on sawdust only	% extractives Ethanol-toluene extraction on sawdust subjected to hot water extraction	% extractives Acetone extraction on sawdust subjected to hot water extraction
E. grandis sawdust	3.13	2.26	0.69
E. nitens sawdust	2.20	0.63	0.61

The graphical representation and table of the results clearly indicate that an acetone extraction on sawdust only removes more extractives then the other technique of a hot water extraction first followed by a soxhlet extraction. Comparing the latter it can be seen that the ethanol-toluene mixture removes far more extractives than the acetone. This would hence indicate that acetone

primarily removes water soluble extracts and therefore the amounts of solvent extracts are minimal after an initial hot water extraction. The data shows that soxhlet extraction using ethanol-toluene extracts minimal amount of extractives from *E. nitens*.

5.2.3. Statistical analysis of extractives using different methods

During the collection and interpretation of the data, it was seen that tree 1 maybe an outlier, therefore to make sure the Dixon's Q test was used:-
For the data:

Tree 1	Tree 2	Tree 3	Tree 4	Tree 5
3.44	2.81	2.09	1.63	2.86

The outlier is 3.44, therefore calculate Q
$Q_{95\%}$: With 5 observations, $Q_{calculated}$ (0.320) < Q_{table} (0.710) so therefore retain it with 95% confidence.

Therefore from the Dixon calculation it was concluded that tree 1 would be used in further calculations.

5.2.4. Repeatability Evaluation

The repeatability evaluation could only be carried out on *E. grandis* as there were insufficient samples to evaluate *E. nitens* (Refer to Appendix B, *E.grandis* Extractions: Data Collection and Calculations for all raw data).

In literature it is explained that a low repeatability indicates good precision (Sefara and Birkett, 2003).This is calculated using the Tappi 1206 method.

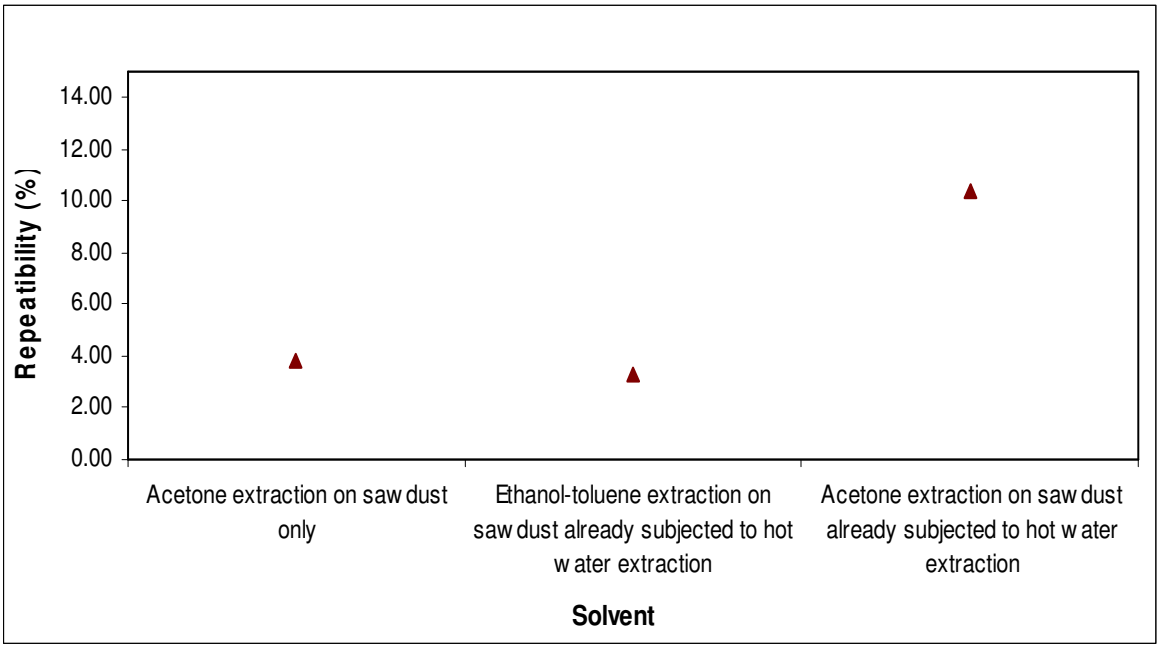


Figure 5.5: Repeatability (%) obtained for the extractions of *E. grandis*.

In figure 5.5, a low repeatability illustrates a good precision in the measurement. It also indicates that the environment and the apparatus for carrying out the experiment were fairly stable and the homogeneity of the material was also good. In this case the two step process using ethanol-toluene after a hot water and soxhlet extraction is preferred in terms of repeatability.

However, acetone was chosen as the solvent for extraction for the following reasons. Acetone has a lower boiling point and can therefore be evaporated easily. It is a polar solvent and can remove water soluble compounds as well, therefore, eliminating the need for doing a hot water extraction hence reducing analysis time significantly. Therefore, from this finding, it was decided not to carry on doing a hot water extraction prior to solvent extraction. Acetone is also much cheaper than ethanol-toluene and is less toxic.

5.3. Analysis to determine the origin of the brownish colour of extracts

After extraction it was noted that the extracts were dark brown in colour. Lignin was suspected as being the source of this brown colour. UV-Vis spectroscopy was used as a quick check to determine if lignin was the source of the colour. However, this proved to be inconclusive.

Therefore, acidolysis was also used which looks at Syringyl/Guaiacyl ratios. This is a more accurate method to identify and quantify lignin using GC.

5.3.1. Ultra-Violet Spectroscopy

UV-Vis spectroscopy was used as a quick technique to determine if lignin was present in the *E. grandis* extracts which could cause the brownish colour of all the extracts. The reference solvent used was methanol (The explanation for the use of methanol is in the experimental section). This was carried out on a soxhlet extracted sample using acetone and soxhlet extracted sample using ethanol-toluene after being subjected to a hot water extraction from *E. grandis*. Figures 5.6 and 5.7 are the UV spectra obtained.

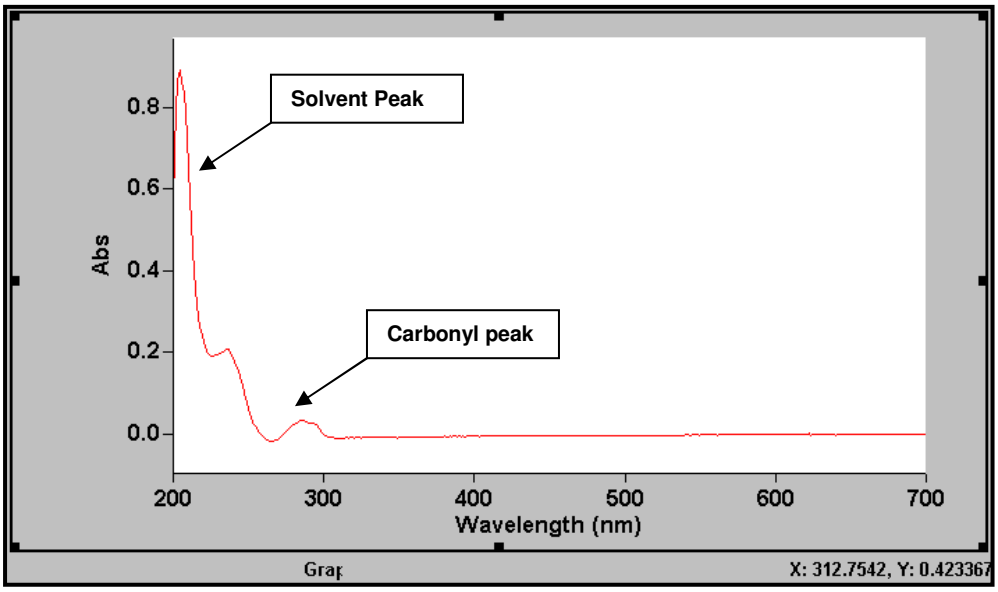


Figure 5.6: UV-Vis spectrum for extractives of *E. grandis* found in acetone (200-700nm)

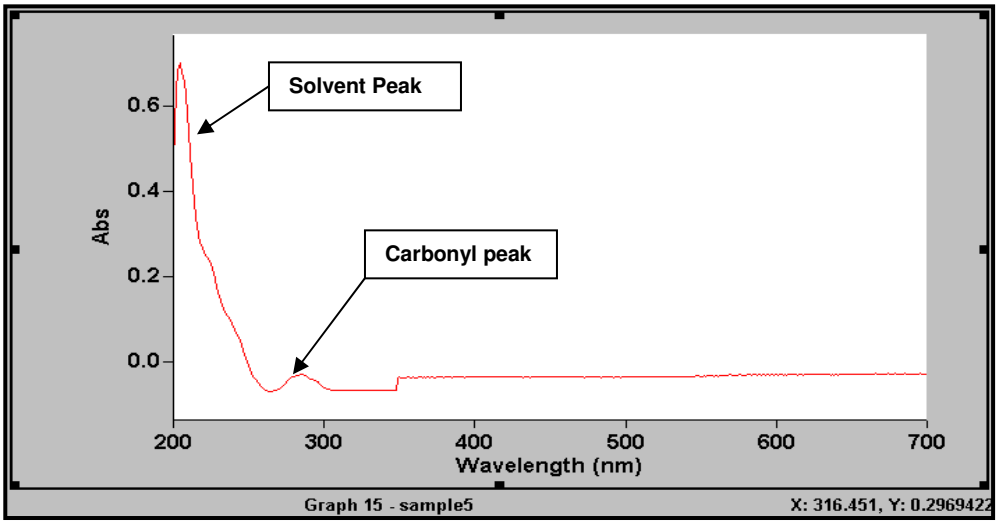


Figure 5.7: UV-Vis spectrum for of extract after an ethanol-toluene extraction on sawdust already subjected to hot water extraction from *E. grandis* (200-700nm).

In both UV spectra there was distinctive peaks at 205, this being the solvent peak and between 280 and 290 nm indicative of a carbonyl (C=O) group, (Figure 5.6 and 5.7). This indicated the possible presence of fatty acids in both samples. The peak at 280 nm can also indicate the presence of lignin. This has been documented in literature (Orsa and Holmbom, 1994) were it is understood that soluble lignin's are the amount of material calculated from the UV absorbance at 280nm.

UV-Vis was not sensitive enough to determine the small amounts of lignin present. Therefore, acidolysis was used to identify and quantify the lignin present.

5.3.2. Acidolysis for quantification of lignin

Acidolysis was carried out to identify if any lignin was present in the extractive sample because as mentioned previously UV was seen as not a conclusive method of determining lignin. Acidolysis involves the break down of lignin into its component parts and these are measured using GC. Figure 5.8 is the chromatogram obtained during GC analysis after acidolysis of the acetone only extract of *E. grandis*.

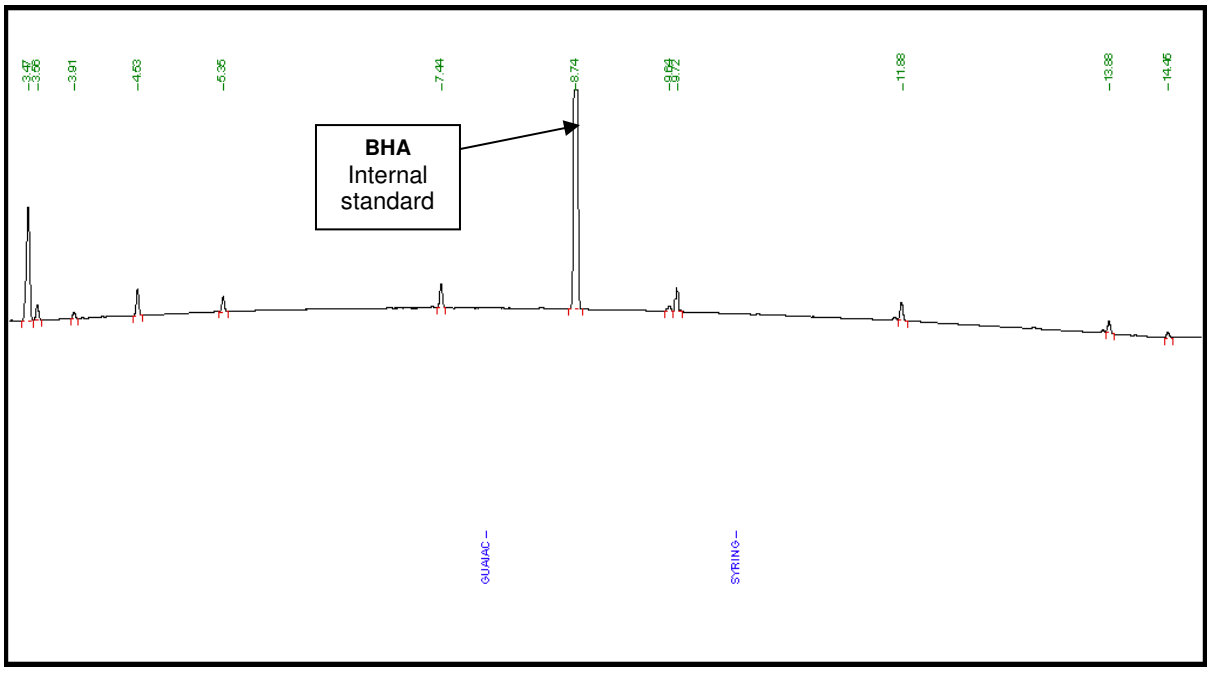


Figure 5.8: GC chromatogram obtained for the acetone extract analyzed after acidolysis

Acidolysis was done on the extracts obtained using the three different methods of extraction, and compared to a lignin chromatogram (Figure 5.9) done at the same conditions. Figure 5.8 shows the peaks at retention times of 7.44 and 9.72 respectively do not align with the lignin breakdown products. These lignin products, guaiacyl and syringyl are formed by the selective cleavage of arylglycerol-β-aryl ethers and other weak ether linkages.

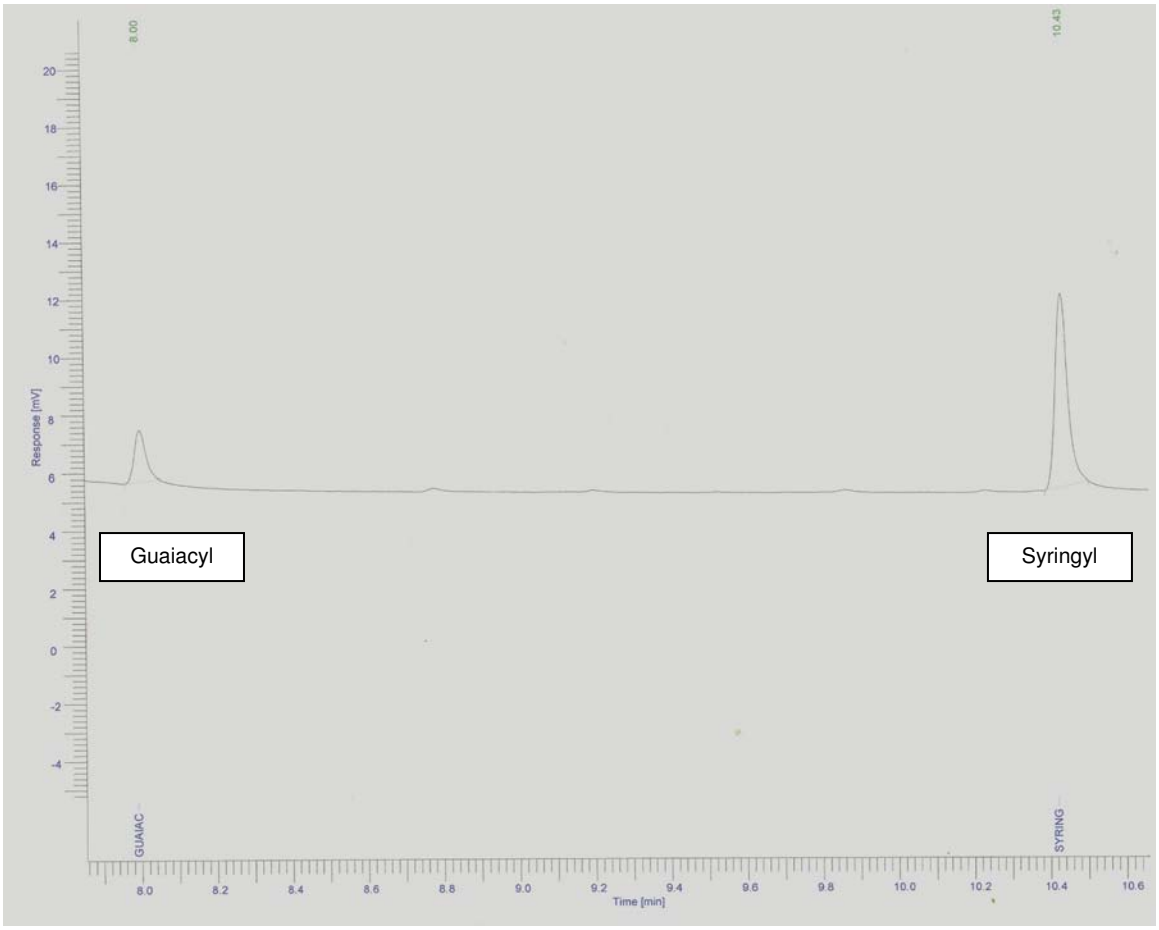


Figure 5.9: GC chromatogram obtained for Klason lignin (Spark, 2006)

It can be concluded that those peaks at the retention times mentioned above are not lignin breakdown products since standards containing such products were run. These results indicate lignin breakdown products occurring at 8.00 and 10.43 mins respectively which do not match with the results obtained. Therefore it can be explained that the discoloration was not due to lignin.

5.4. High Performance Liquid Chromatography to identify sugars retained in the sawdust after the extraction methods

High performance liquid chromatography was used to determine if the six principal monosaccharides that constitute the carbohydrate composition in wood (glucose, mannose, aribinose, xylose, rhamnose and galactose) were present. The procedure is outlined in chapter 3 and is attached in the Appendix D: High Performance Liquid Chromatography. HPLC analysis showed that there was presence of sugars in the sawdust samples of every tree after the

combination of extraction methods. The hot water extracts were also measured to determine if any sugars are extracted during the process.

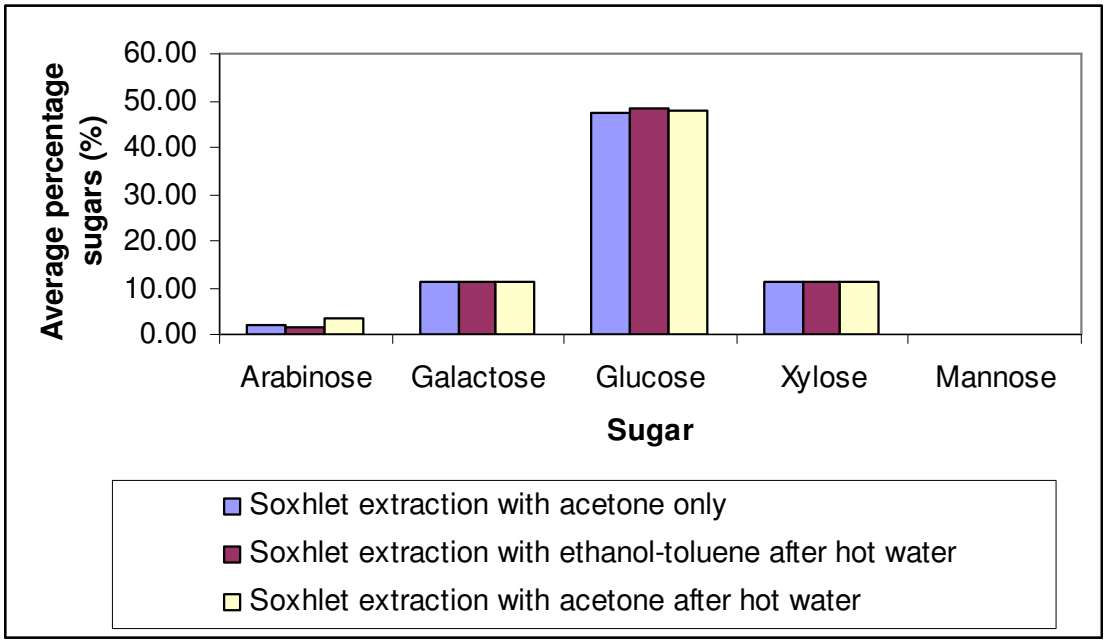


Figure 5.10: Average percentage of sugars of *E. grandis* sawdust obtained after extraction of the different solvents.

A graphical representation of the results indicates that the percentage sugars in the sawdust were approximately 70 %. There is minimal arabinose and no mannose present in the sawdust. Taking note that there is no difference in the two different acetone extraction methods indicating that hot water did not extract any sugars. There are no sugars in the extracts.

5.5. Characterisation of extractives in E. grandis and E. nitens sawdust

5.5.1. Gas Chromatography

Fifteen to 20 mg of the dried extractives was reacted with 1 mL of tetramethylammonium hydroxide, TMAH (5% in methanol) in a sealed vial. The GC analysis using an FID detector was carried out in triplicate on samples of *E. grandis* and *E. nitens* from the three different extraction methods. The chromatograms are shown in Figure 5.11 A (*E. grandis*) and 5.11 B (*E. nitens*) respectively. The complete chromatograms can be located in Appendix E: Gas Chromatography).

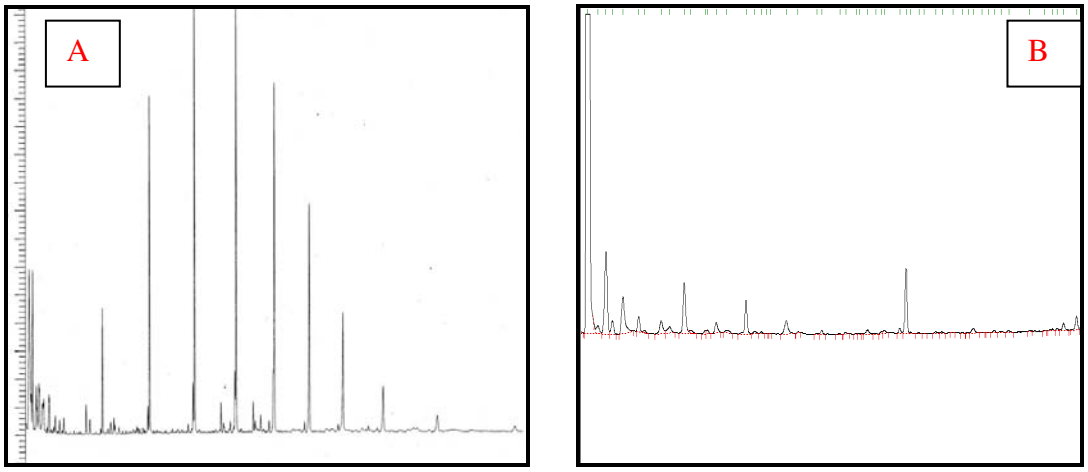


Figure 5.11: Chromatogram A obtained for ethanol-toluene extraction on sawdust already subjected to hot water extraction for *E. grandis* and B for *E. nitens*. The time scales for A and B are equivalent.

The figure 5.12 A is a chromatogram for *E. grandis* and figure 5.12 B for *E. nitens* for an acetone extraction on sawdust only

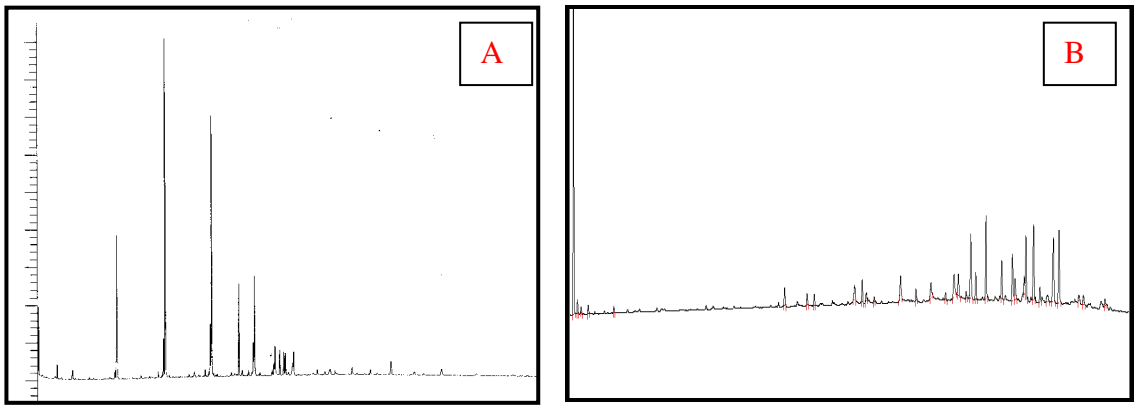


Figure 5.12: Chromatogram A obtained for an acetone extraction on sawdust only for *E. grandis* and B for *E. nitens*. The time scales for A and B are equivalent.

From GC analysis one can deduce the peak patterns between species are different and that within a species different solvent systems extract a different profile of compounds. However looking at the results of a chromatogram, Figure 5.13 from literature, it can be expected that fatty acids should be found at much shorter retention times followed by hydrocarbons, sterols and then sterol esters/triglycerides. Hence it would be interesting to see from GC-MS if the results actually compare with literature.

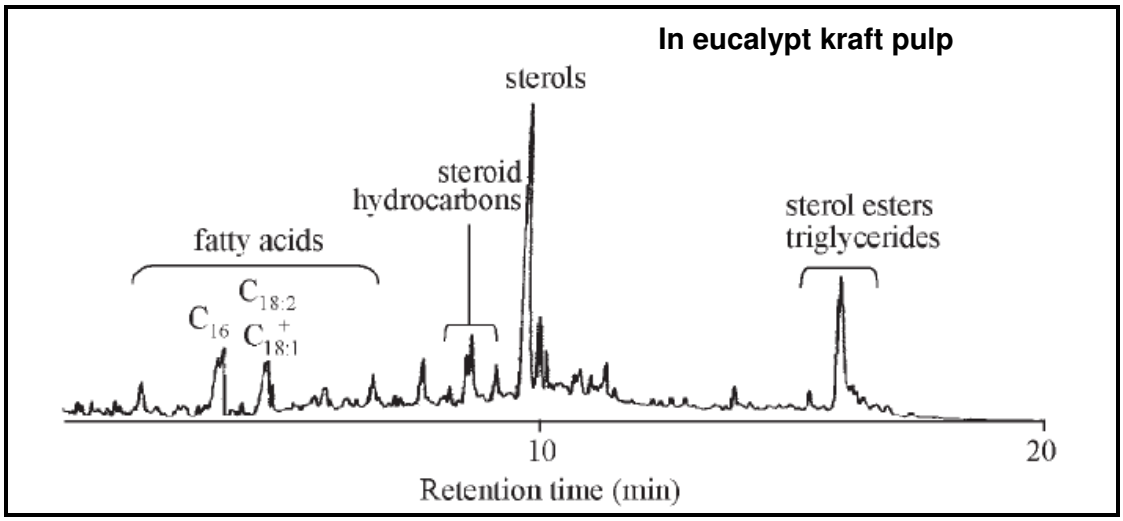


Figure 5.13: GC–FID chromatogram (5 m column) of lipophilic extractives (Gutrierrez *et al*., 1998b).

5.5.2. Quantification of extractive composition in *E. grandis* and *E. nitens* sawdust using Gas Chromatography-Mass spectroscopy

The GC analysis could only give an indication of the profile of compounds and therefore a technique which looked more into identifying the compounds had to be used. Therefore GC-MS was chosen since it looks at both the profile and mass to charge ratio of the compounds. Fifteen to 20 mg of the extractives was dissolved in 1 mL of methanol for GC-MS analysis.

5.5.2.1. Ethanol-toluene extraction on *E. grandis* and *E. nitens* sawdust already subjected to hot water extraction

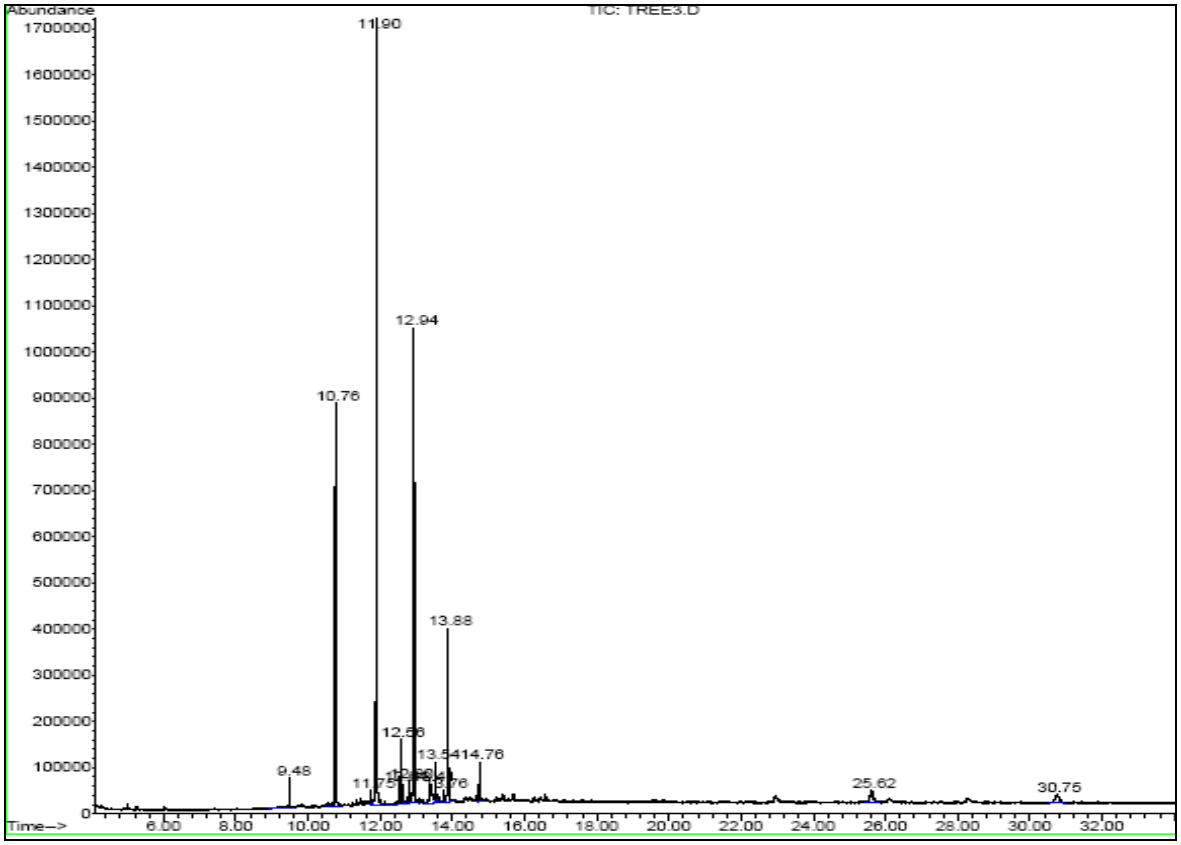
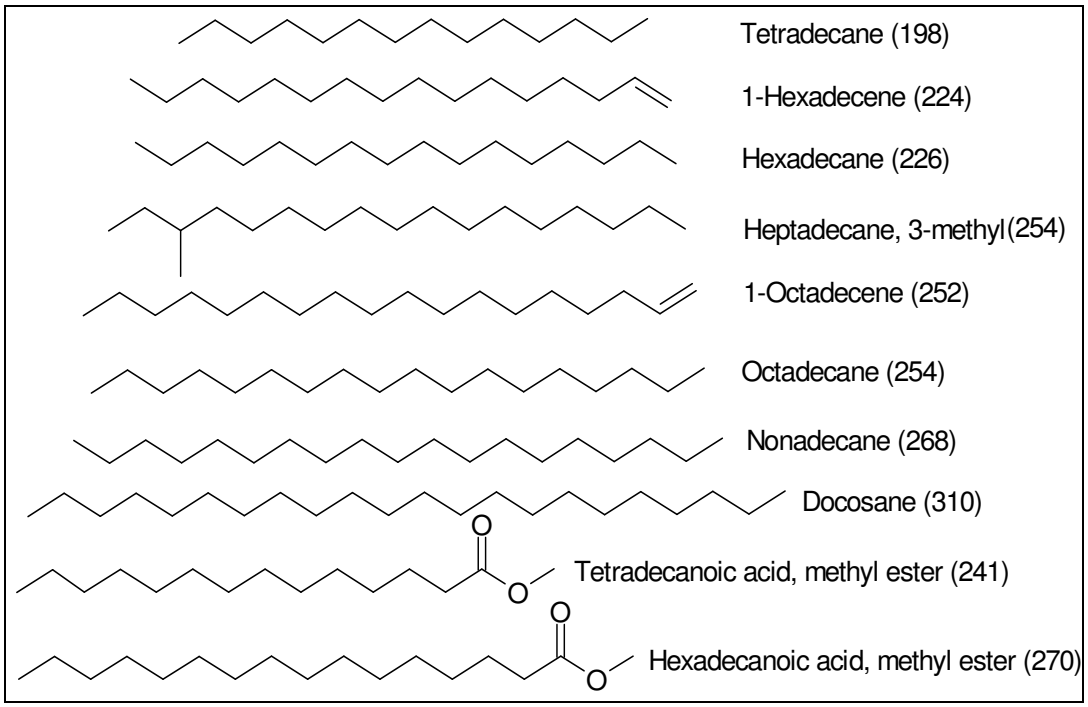


Figure 5.14: GC-MS chromatogram for *E. grandis* extract after an ethanol-toluene extraction on sawdust already subjected to hot water extraction.



Compound	**Retention time (minutes)**
Tetradecane	9.48
1-Hexadecene	10.76
Hexadecane / Nonadecane	12.80
Heptadecane, 3 methyl	11.75
1-Octadecene/ Octadecane	11.90
Docosane	13.88
Tetradecanoic acid and Hexadecanoic acid methyl esters	12.56

Figure 5.15: Possible structures, molecular weight (in brackets) and retention times of compounds extracted from *E. grandis* after an ethanol-toluene extraction on sawdust already subjected to hot water extraction.

Firstly the GC-MS chromatogram obtained for a soxhlet extracted sample using ethanol-toluene after hot water extraction for *E. grandis* looks different from Figure 5.11 due to different columns being used. The GC-MS chromatogram of the extract from the soxhlet using ethanol-toluene after hot water extraction and the suggested structures obtained from a library search confirmed the presence of hydrocarbons and fatty acids in *E. grandis*. The GC-MS chromatogram shows the main peaks to be Tetradecane, 1-Hexadecene Hexadecane / Nonadecane, Heptadecane, 3 methyl, 1-Octadecene/ Octadecane, Docosane and Tetradecanoic acid and Hexadecanoic acid

methyl esters. The methyl esters are conversion of the fatty acids due to the addition of the TMAH during the sample preparation process.

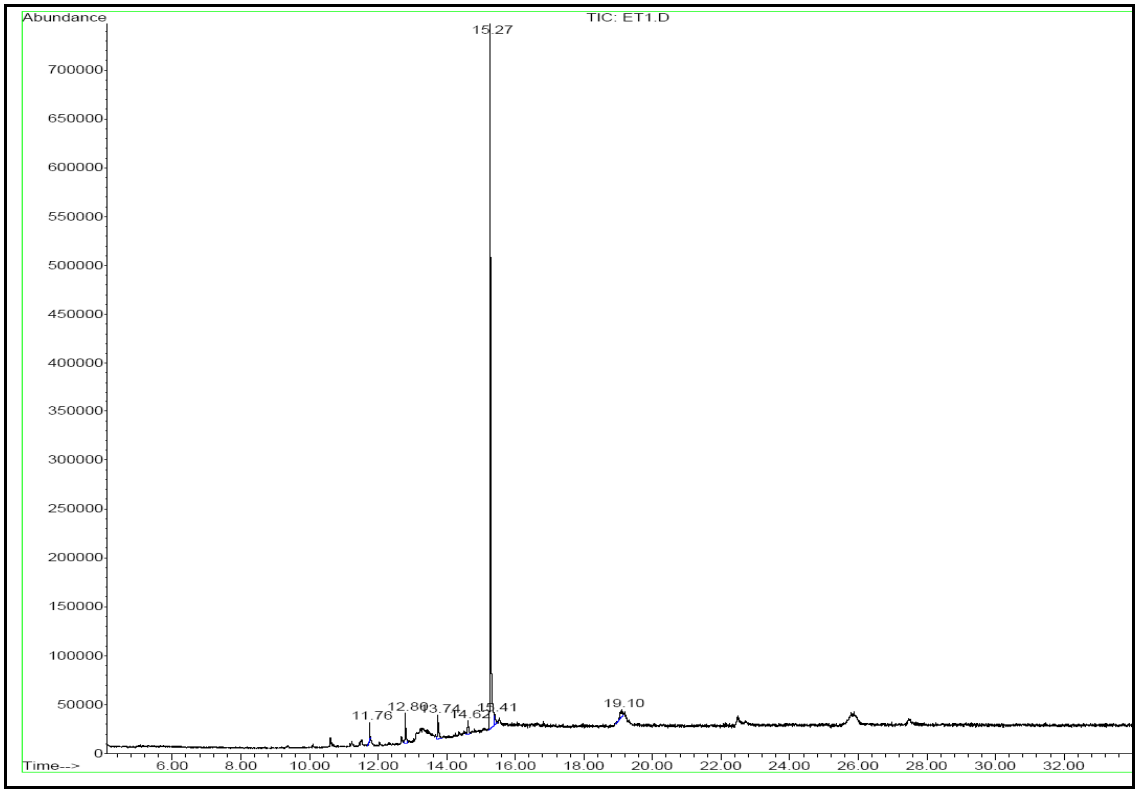
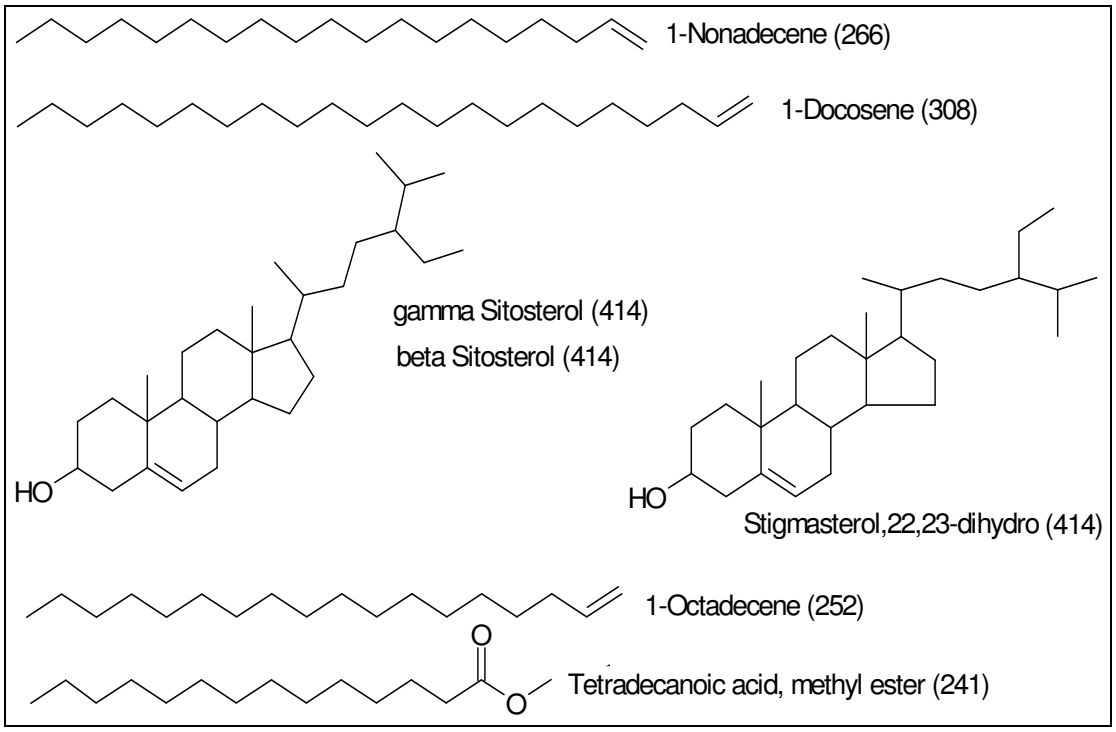


Figure 5.16: GC-MS chromatogram for *E. nitens* extract after an ethanol-toluene extraction on sawdust already subjected to hot water extraction.



Compound	Retention time (minutes)
1-Nonadecene	15.27
1-Docosene	13.83
Gamma, beta Sitosterol / Stigmasterol, 22,23-dihydro	22.85
1-Octadecene	11.83
Tetradecanoic acid methyl esters	12.56

Figure 5.17: Possible structures, molecular weight (in brackets) and retention times of compounds extracted from *E. nitens* after an ethanol-toluene extraction on sawdust already subjected to hot water extraction.

The suggested structures obtained from a library search after GC-MS analysis obtained for *E.nitens* after hot water followed by a solvent extraction with ethanol-toluene confirmed the presence of hydrocarbons, a fatty acid methyl ester and sterols, Figure 5.16. The GC-MS chromatogram shows the main peaks to be 1-Nonadecene, 1-Docosene, 1-Octadecene, Gamma, beta Sitosterol / Stigmasterol, 22, 23-dihydro and Tetradecanoic acid methyl esters.

5.5.2.2. An acetone extraction on *E. grandis* and *E. nitens* sawdust

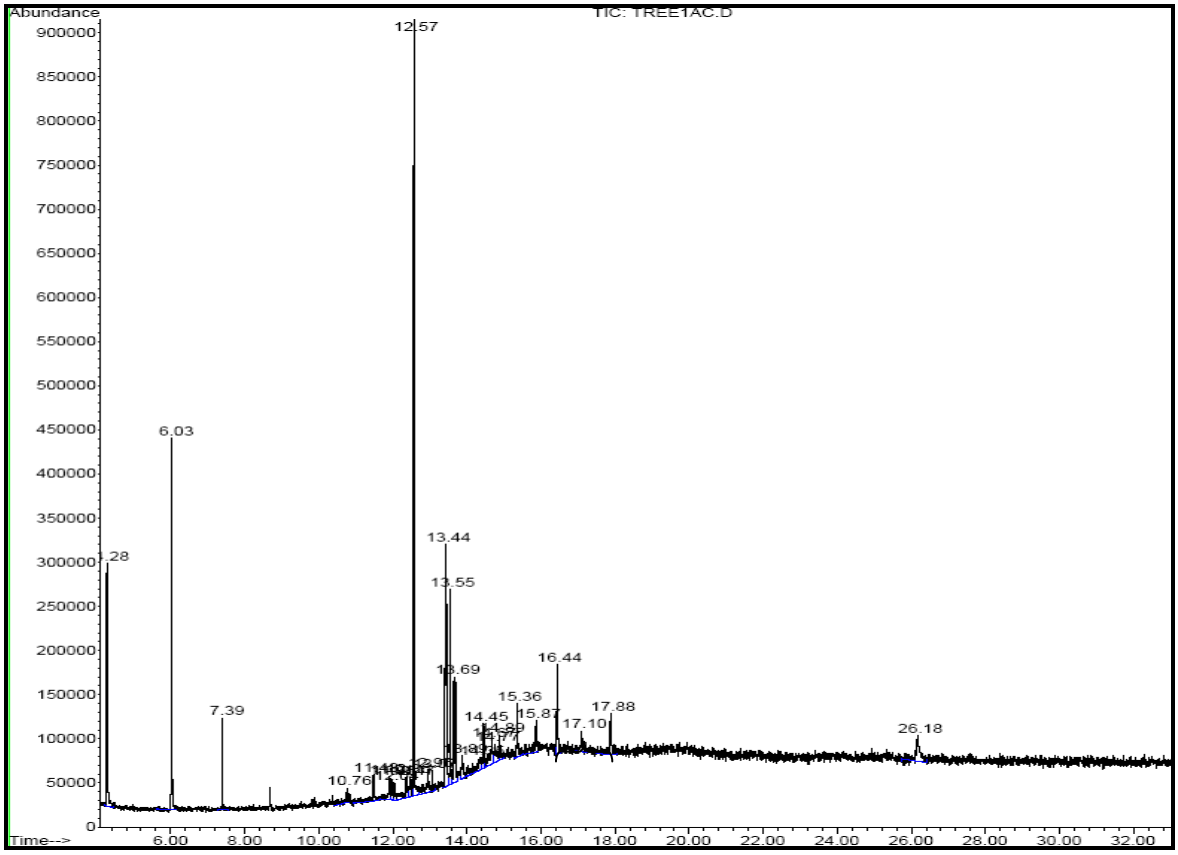
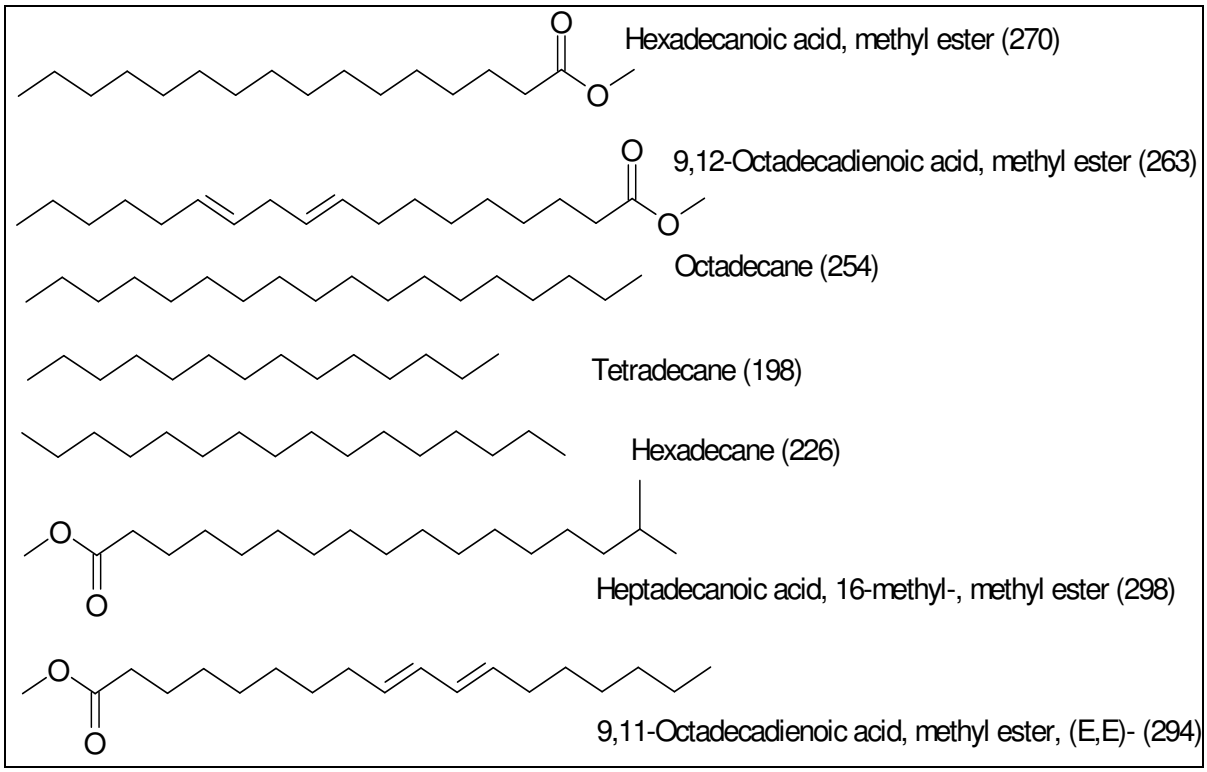


Figure 5.18: GC-MS chromatogram for *E. grandis* extract after an acetone extraction on sawdust only.



Compound	Retention time (minutes)
Hexadecanoic acid methyl esters	12.57
9,12-Octadecadienoic acid, methyl ester / 9,11-Octadecadienoic acid, methyl ester	13.44
Octadecane	11.91
Tetradecane	9.48
Hexadecane	12.80
Heptadecanoic acid, 16-methyl-, methyl ester	13.55

Figure 5.19: Possible structures, molecular weight (in brackets) and retention times of compounds extracted from *E. grandis* after an acetone extraction on sawdust only.

The chromatogram for *E. grandis* (Figure 5.16) differs from Figure 5.18, in that there are more fatty acids than hydrocarbons. These being Hexadecanoic acid methyl esters, 9,12-Octadecadienoic acid, methyl ester / 9,11-Octadecadienoic acid, methyl ester and Heptadecanoic acid, 16-methyl-, methyl.

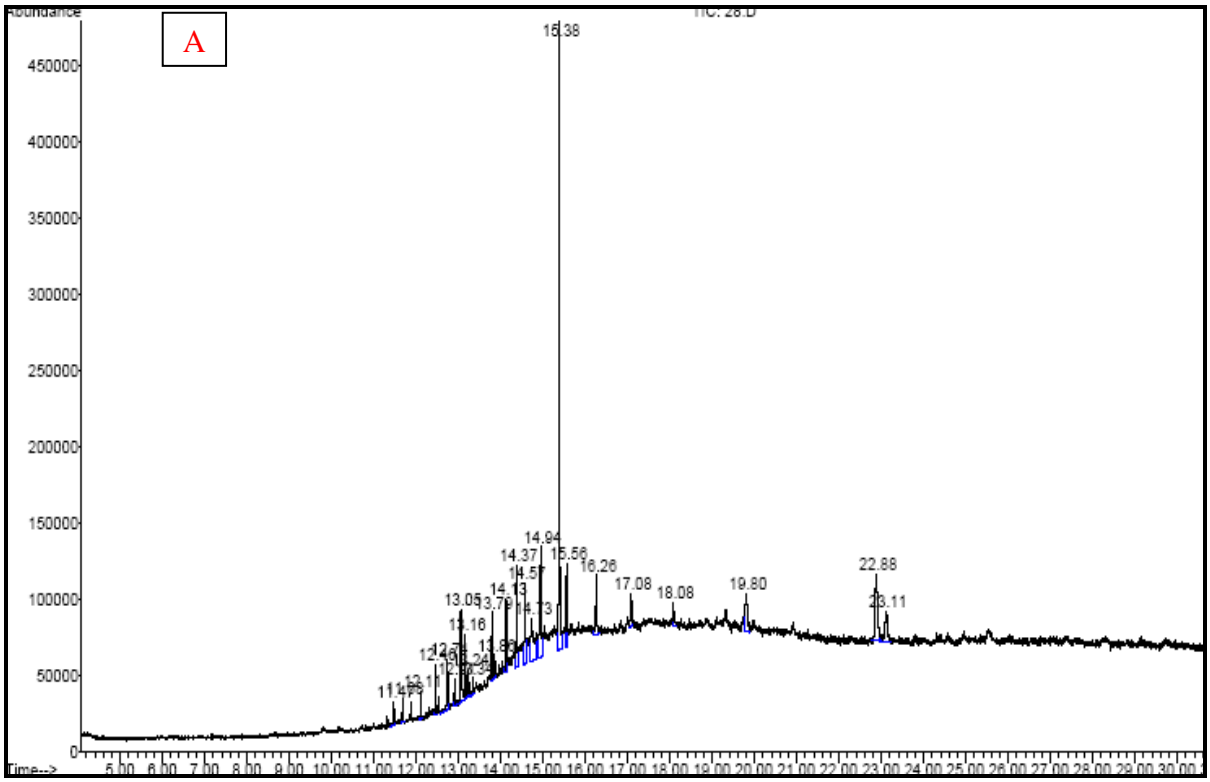
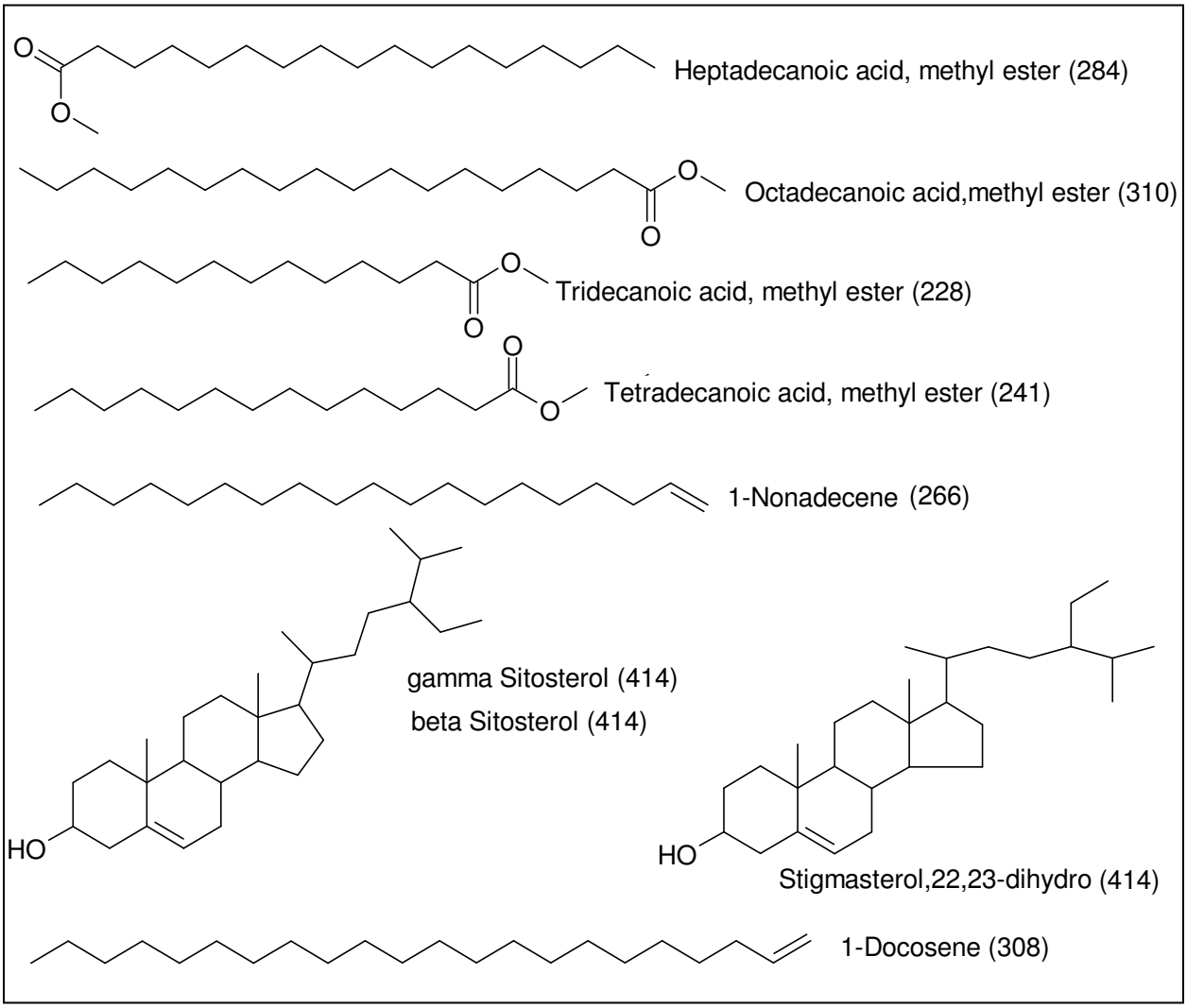


Figure 5.20: GC-MS chromatogram for *E.nitens* extract after an acetone extraction on sawdust only.



Compound	Retention time (minutes)
Heptadecanoic acid, methyl ester	13.55
Octadecanoic acid, methyl ester	13.86
Tridecanoic acid / Tetradecanoic acid, methyl ester	13.55
1-Nonadecene	15.38
Gamma, beta Sitosterol / Stigmasterol, 22,23-dihydro	22.88
1-Docosene	13.88

Figure 5.21: Possible structures, molecular weight (in brackets) and retention times of compounds extracted from *E. nitens* after an acetone extraction on sawdust only.

The suggested structures obtained from a library search after GC-MS analysis obtained for *E.nitens* after a solvent extraction with acetone were Heptadecanoic acid, methyl ester,

Octadecanoic acid, methyl ester, Tridecanoic acid / Tetradecanoic acid, methyl ester, 1-Nonadecene, Gamma, beta Sitosterol / Stigmasterol, 22,23-dihydro and 1-Docosene

It was found in the GC-MS analysis that no sterols were found in *E. grandis* for both methods of extraction. Therefore it can be shown that different solvents extracted different profile of components.

5.4.2.3. Quantification of extracts

In order to quantify the extracts, the process involved the addition of an internal standard to the 15-20 mg of sample extracts and then analysis using GC. The chromatogram and raw data are in Appendix F, Quantification of *E. grandis*, *E. nitens* and *E. nitens* pitch extracts

The equation used in the calculation was defined as:

$$\text{Amount unknown} = (A_{unknown}/A_{internal\ standard})^* [IS]/R_f$$ [12]

The resultant figures from the calculation are given in Table 5.3, the total amount of extractives removed from the *E. grandis* sawdust; (average of four trees) and *E. nitens*.

Table 5.3: Average mass of extractives for *E. grandis* and *E nitens* sawdust after an ethanol-toluene extraction on sawdust already subjected to hot water extraction.

	Total extractives (mg)	Fatty acids (mg)	Hydrocarbons (mg)
E. grandis	1.0	0.13	0.87
E. nitens	0.015	0.011	0.004

To summarise the above, comparing the two species, the total amount of extractives is higher in *E. grandis* than in *E. nitens*. There are higher amounts of hydrocarbons and fatty acids found in *E. grandis* than in *E. nitens*. It was also noted there was no sterols identified in *E. grandis* (therefore mass of 0.13 mg is only fatty acids) however it was identified in *E. nitens*. It is also noted that *E. grandis* consists of more hydrocarbons, 0.87 mg then fatty acids, while *E. nitens* consists of higher fatty acids and sterols then hydrocarbons.

[12] For Rf value refer to Appendix D, Quantification of *E. grandis*, *E. nitens* and *E. nitens* pitch extracts

The results given in Table 5.4 show the total amount of extractives removed from the *E. grandis* sawdust; (average of four trees) and *E. nitens*.

Table 5.4: Composition of extractives identified after an after an acetone extraction on sawdust only of *E. grandis* and *E. nitens*.

	Total extractives (mg)	Fatty acids (mg)	Hydrocarbons (mg)
E. grandis	0.991	0.021	0.97
E. nitens	0.064	0.038	0.026

To summarise, comparing the two species and referring to Table 5.4, the total amount of extractives is higher in *E. grandis* than in *E. nitens*. There were no sterols identified in *E. grandis* however sterols were identified in *E. nitens*. It is also noted that *E. grandis* consists of more hydrocarbons 0.97 mg than fatty acids, while *E. nitens* has higher amounts of fatty acids and sterols than hydrocarbons.

For the different extraction methods *E. grandis* yields a higher percentage of extractives than *E. nitens*. In percentage, acetone extracts more hydrocarbons and less fatty acids; this is the opposite for hot water followed by ethanol-toluene. Therefore it can be shown that different solvents extracted not only different amounts of extractives but also different components.

5.5. Comparison of extractives component in sawdust and pitch of E. nitens

5.5.1. Identification of extractives in pitch using gas chromatography

The chromatograms (Figure 5.22 and 5.23), represents the peak pattern of the extracts from the pitch and sawdust sample of *E. nitens* for the extraction with acetone and an ethanol-toluene extraction after being subjected to a hot water extraction.

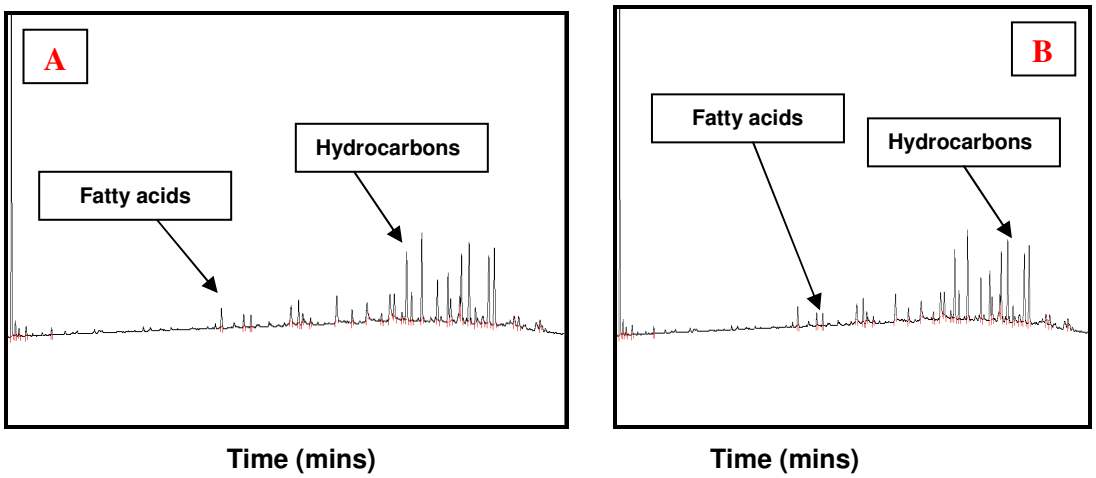


Figure 5.22: Chromatogram 5.19 A for the acetone extracted *E. nitens* sawdust and 5.19 B for the acetone extracted *E. nitens* pitch obtained from Enstra mill. The time scales for A and B are equivalent.

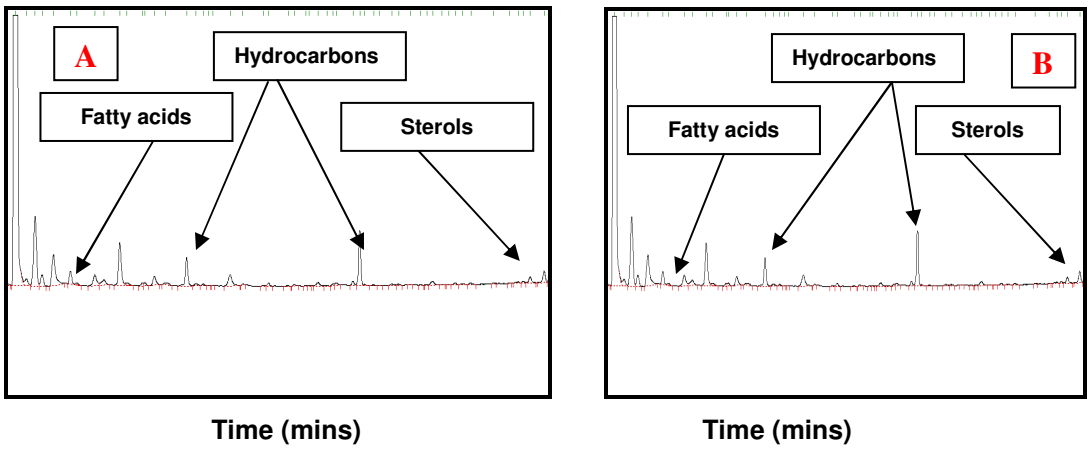


Figure 5.23: Chromatogram 5.20 A represents the hot water followed by ethanol-toluene extracted *E. nitens* sawdust and 5.20 B for *E. nitens* pitch. The time scales for A and B are equivalent.

From figure 5.22 and 5.23 A and B, the peak patterns for the *E. nitens* pitch and sawdust are very similar. They both show the presence of hydrocarbons and fatty acids in the extractives.

5.5.2. Gas Chromatography-Mass spectroscopy for extractives from pitch of *E. nitens*

The sample preparation and analysis followed the same path as that of the sawdust. In order to compare the extracts from *E. nitens* sawdust and the pitch, two methods of extraction were carried out, acetone only and hot water followed by ethanol-toluene.

The following chromatogram (Figure 5.24) represents an extractive sample obtained from a pitch sample after extraction using acetone. The analysis was carried out in triplicate. The chromatogram shows the presence of fatty acids, sterols and hydrocarbons. The compounds suggested by the GC-MS library are shown in Figure 5.25.

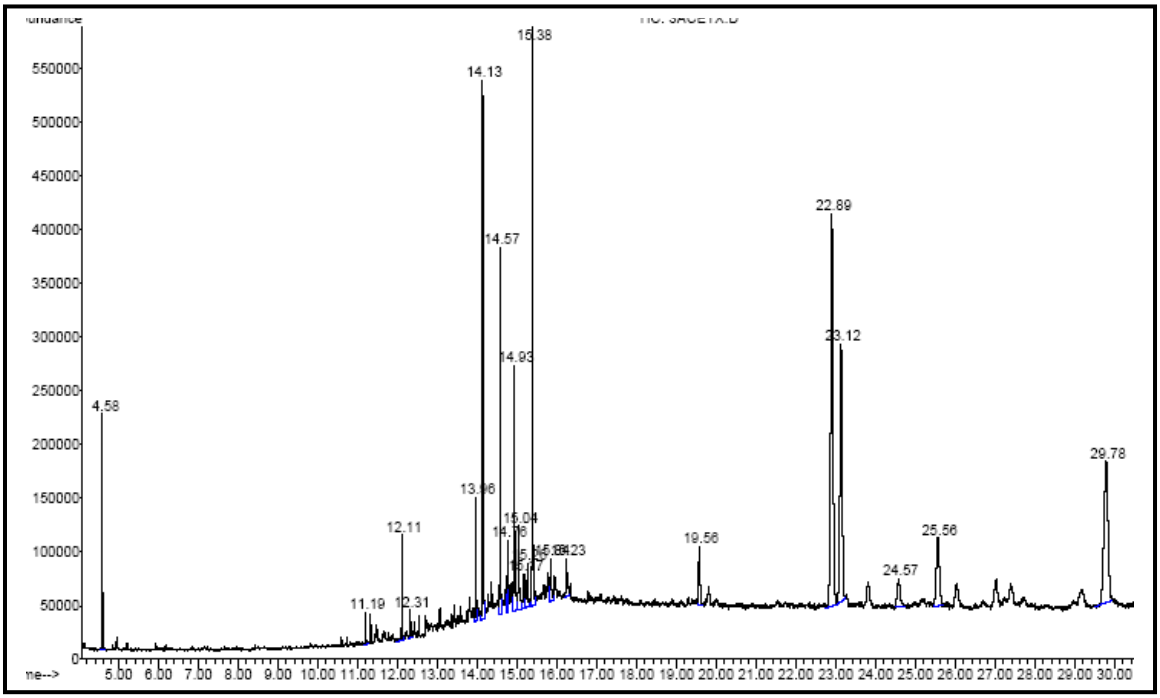
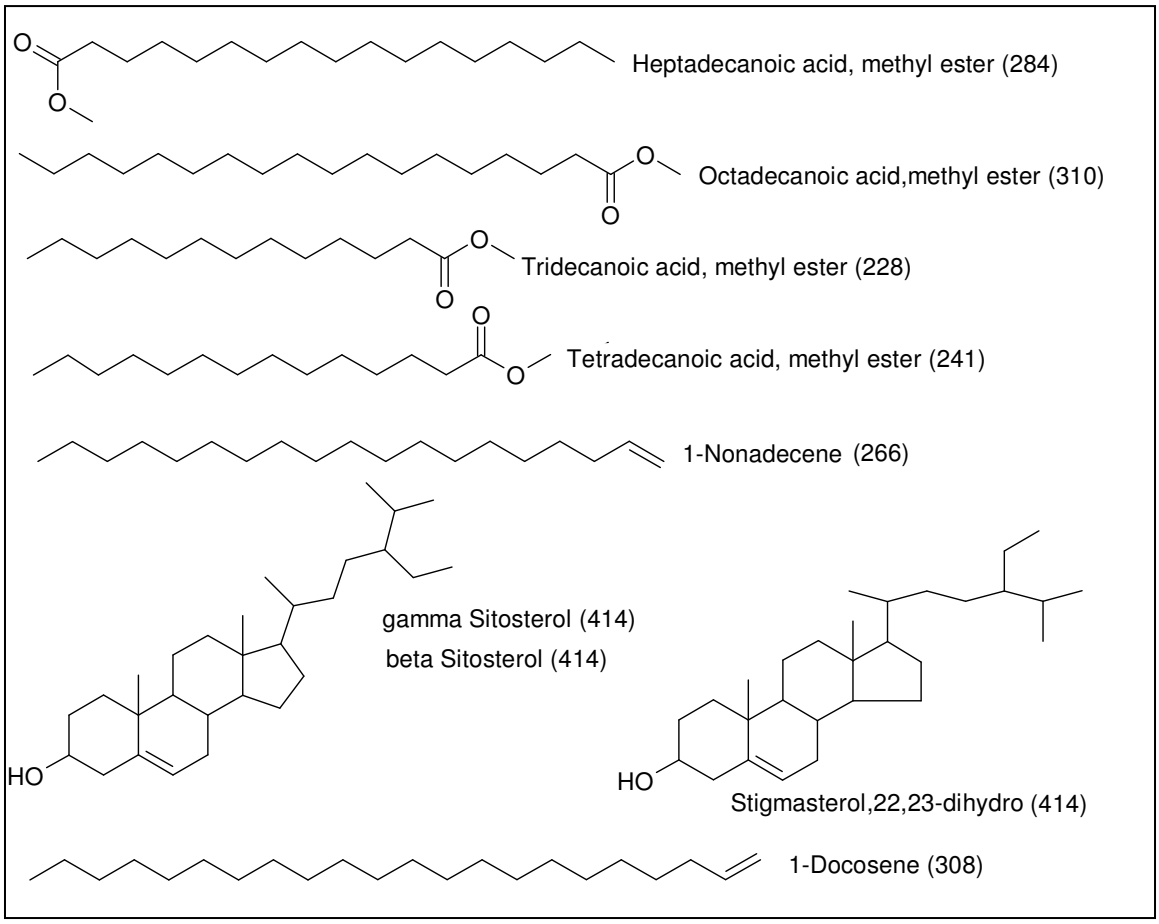


Figure 5.24: GC-MS chromatogram for *E.nitens* pitch extract after an acetone extraction only.



Compound	Retention time (minutes)
Heptadecanoic acid, methyl ester	13.07
Octadecanoic acid, methyl ester	13.52
Tridecanoic acid / Tetradecanoic acid, methyl ester	13.55
1-Nonadecene	15.38
Gamma, beta Sitosterol / Stigmasterol, 22,23-dihydro	22.89
1-Docosene	13.88

Figure 5.25: Possible structures, molecular weight (in brackets) and retention times of compounds of *E. nitens* pitch extract after an acetone extraction only.

The GC-MS analysis (Figure 5.26) of the ethanol-toluene extract of a pitch sample indicates the presence of hydrocarbons, fatty acids and sterols.

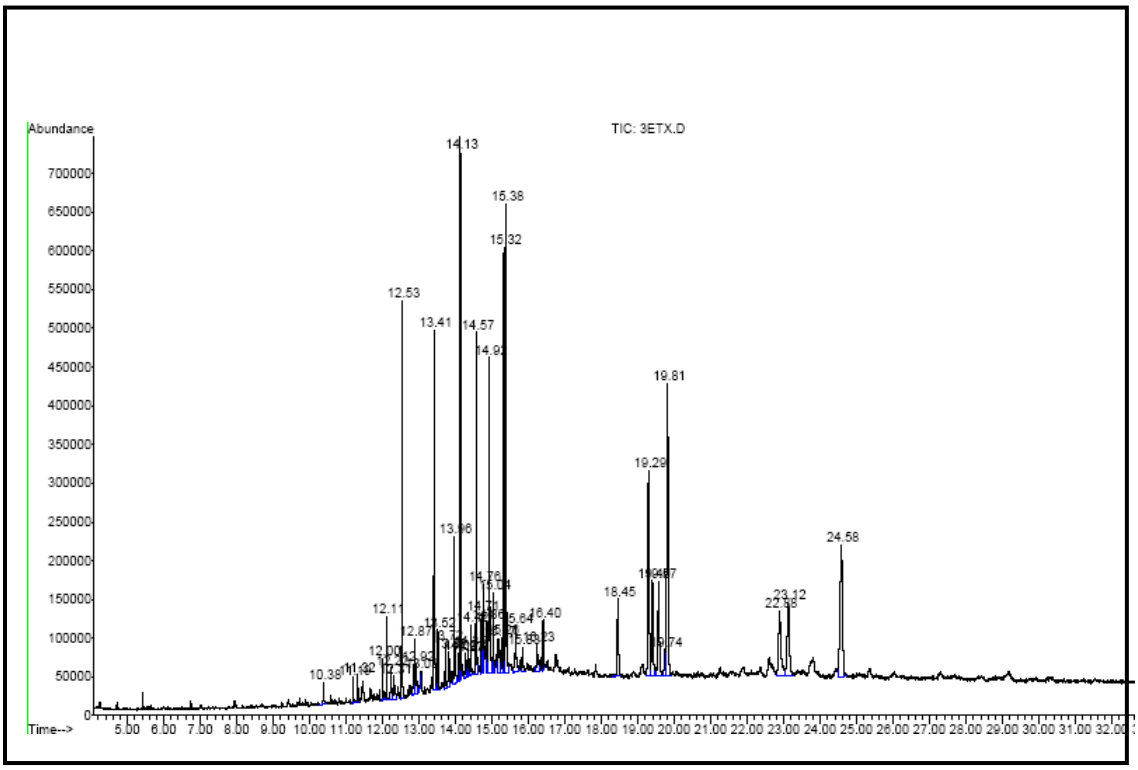
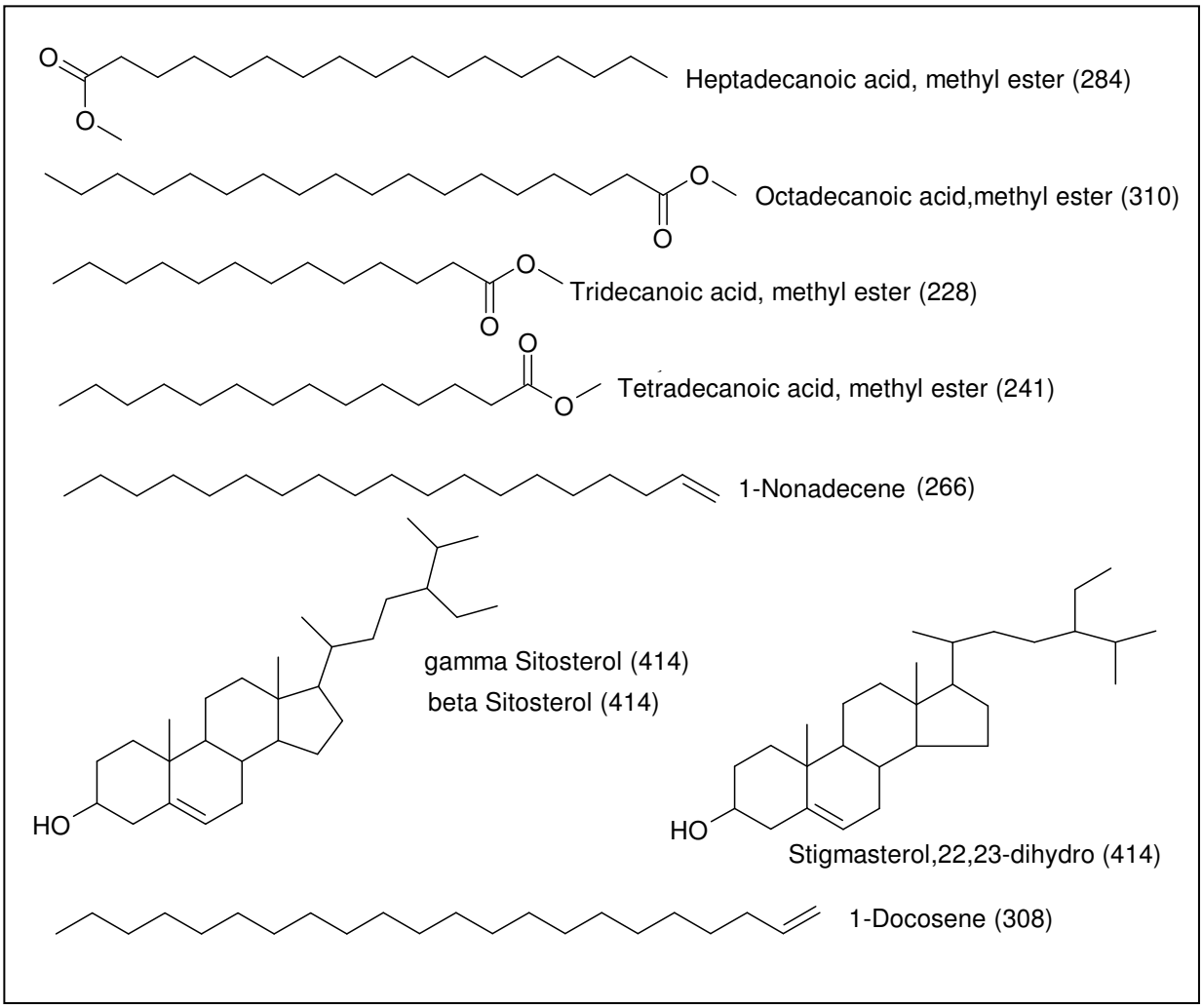


Figure 5.26: GC-MS chromatogram for *E.nitens* pitch extract after an ethanol-toluene extraction on sawdust already subjected to hot water extraction.



Compound	Retention time (minutes)
Heptadecanoic acid, methyl ester	13.41
Octadecanoic acid, methyl ester	13.52
Tridecanoic acid / Tetradecanoic acid, methyl ester	14.57
1-Nonadecene	15.38
Gamma, beta Sitosterol / Stigmasterol, 22,23-dihydro	22.88
1-Docosene	13.89

Figure 5.27: Possible structures, molecular weight (in brackets) and retention times of compounds of *E. nitens* pitch extract for an ethanol-toluene extraction on already subjected to hot water extraction.

The suggested structures obtained from a library search for *E.nitens* after a solvent extraction with acetone and ethanol-toluene after hot water extraction were Heptadecanoic acid, methyl

ester, Octadecanoic acid, methyl ester, Tridecanoic acid / Tetradecanoic acid, methyl ester, 1-Nonadecene, Gamma, beta Sitosterol / Stigmasterol, 22,23-dihydro and 1-Docosene

Comparing the chromatograms of *E. nitens* sawdust (Figures 5.11 and 5.12) and *E. nitens* pitch (Figures 5.22 and 5.23), they share some similarities but are not identical. Sterols were identified in GC-MS however not in GC this could be due to the sample was left to be analysed for a longer time and they possibly decomposed. It could have also been due to sensitivity; since MS is more sensitive. The common compounds in the pitch and sawdust of *E. nitens* are heptadecanoic, octadecanoic, tetradecanoic and tridecanoic acid methyl esters (fatty acids were converted to methyl esters during sample preparation), gamma and beta sitosterol and Stigmasterol, 1-docosene and lastly 1-nonadecene.

The quantification results for an acetone extraction only are given in Figure 5.28, for the *E. nitens* pitch and sawdust respectively.

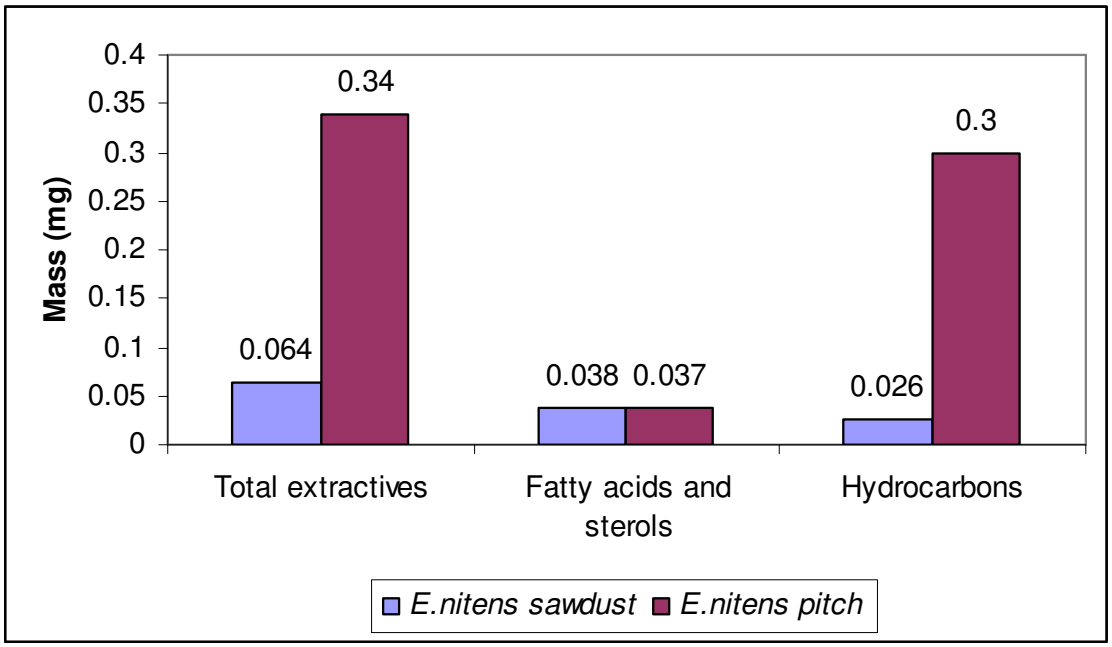


Table 5.28: Composition of extractives identified for *E. nitens* pitch and sawdust after an acetone extraction only.

The quantification results for hot water followed by a soxhlet extraction using ethanol-toluene are given in Figure 5.29, for both pitch and sawdust.

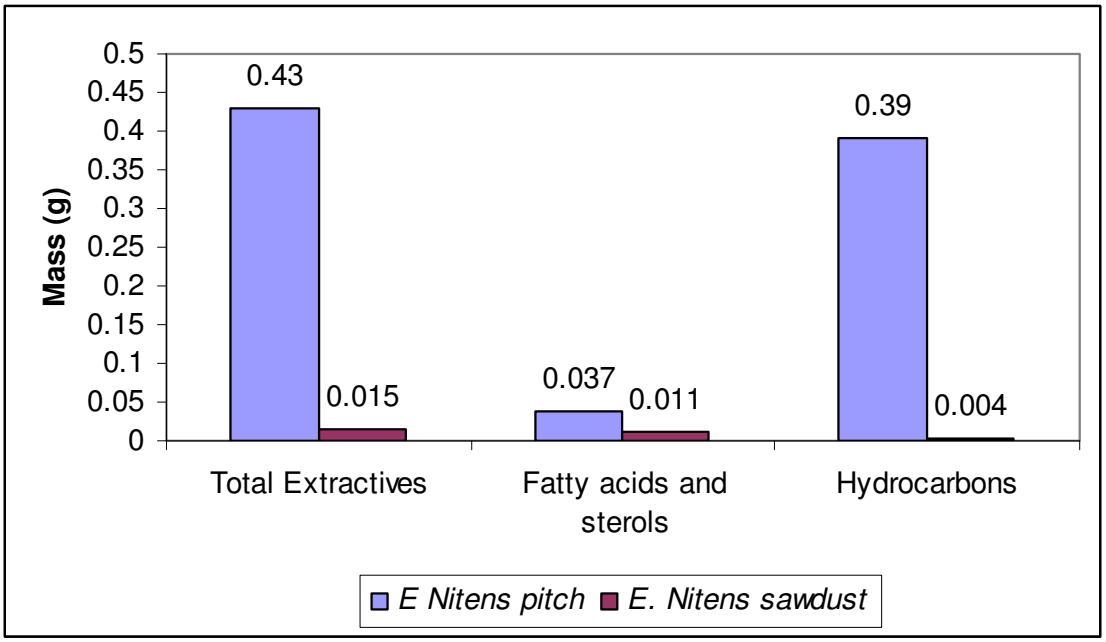


Table 5.29: Composition of extractives identified for *E. nitens* pitch and sawdust after an ethanol-toluene extraction already subjected to hot water extraction.

E. nitens pitch sample has higher amounts of hydrocarbons compared to fatty acids and sterols. Expressed as a percentage, ethanol-toluene extracts more hydrocarbons and less fatty acid. Acetone on the other hand extracts both compounds moderately.

Chapter 6: Conclusion and Recommendations

Initially when this study was initiated certain aims had to be set in order to determine the correct approach. As mentioned earlier in chapter one the following aims was outlined: - 1) to characterise and quantify wood extractives in *Eucalyptus grandis* and *Eucalyptus nitens* and; 2) determine the impact of wood extractives on pitch formation. Hence the start was to determine different extraction methods. Two methods were employed i.e. soxhlet extraction using ethanol-toluene (after hot water extraction) and soxhlet extraction using acetone (no hot water extraction).

GC and GC-MS have been found to be the recommended analytical tools in characterising and quantifying wood extractives in *E. grandis* and *E. nitens*. The reason is due to GC basically separating components based on the rates at which they are carried through the stationary phase and quantifying by the use of internal standards. While GC-MS, identifies the molecular weight and formula of a molecule and the various structural units within it. Hence once you identify the extractives by GC-MS then you can identify the peaks in GC-FID. Table 6.1 is the summary of the results obtained.

Table 6.1: Is a summary of amounts of extractives for the different methods of. extraction.

	***E. grandis* sawdust**	***E. nitens* sawdust**	***E. nitens* pitch**	***E. grandis* sawdust**	***E. nitens* sawdust**	***E. nitens* pitch**
	Soxhlet extraction using ethanol-toluene (after hot water extraction)			**Soxhlet extraction using acetone (no hot water extraction)**		
Total extractives (g)	1	0.015	0.43	0.99	0.25	0.34
Fatty acids and sterols (g)	0.13	0.011	0.037	0.021	0.18	0.037
Hydrocarbons (g)	0.87	0.004	0.39	0.97	0.068	0.30

The table clearly shows that there is a higher amount of extractives in *E.grandis* sawdust than in the *E. nitens* sawdust and pitch sample. There seems to be more fatty acids and sterols in the *E. nitens* sawdust sample extracted the using acetone (no hot water extraction) method while hydrocarbons are extracted more in *E. grandis* using the same method. During GC-MS analysis the following compounds were identified in *E. grandis*, *E. nitens* sawdust and pitch (Table 6.2). In

order to ensure that all extractives removed from both methods were characterised and quantified other techniques such as HPLC, UV-Vis and acidolysis was also used to ensure, that all extractives were characterised. .

UV-Vis and acidolysis was used to determine if any lignin was in the extractives. Both methods indicated that there were no traces of lignin present. HPLC was used to determine if there were any sugars in the extracts. This technique also indicated that the sugars had remained in the sawdust after extraction.

Table 6.2: Summary of compounds identified during GC-MS in both species for the different methods of extraction.[13]

E. grandis sawdust	*E. nitens* sawdust	*E. grandis* sawdust	*E. nitens* sawdust	*E. nitens* pitch	*E. nitens* pitch
soxhlet extraction using ethanol-toluene (after hot water extraction)		soxhlet extraction using acetone (no hot water extraction)		soxhlet extraction using ethanol-toluene (after hot water extraction	soxhlet extraction using acetone (no hot water extraction)
Tetradecane	1-Nonadecene	Hexadecanoic acid methyl ester	Heptadecanoic acid, methyl ester	Heptadecanoic acid, methyl ester	Heptadecanoic acid, methyl ester
1-Hexadecene	1-Docosene	9, 12-Octadecadienoic acid, methyl ester / 9, 11-Octadecadienoic acid, methyl ester	Octadecanoic acid, methyl ester	Octadecanoic acid, methyl ester	Octadecanoic acid, methyl ester
Hexadecane / Nonadecane	Gamma, beta Sitosterol / Stigmasterol, 22, 23-dihydro	Octadecane	Tridecanoic acid / Tetradecanoic acid, methyl ester	Tridecanoic acid / Tetradecanoic acid, methyl ester	Tridecanoic acid / Tetradecanoic acid, methyl ester
Heptadecane, 3-methyl	1-Octadecene	Tetradecane	1-Nonadecene	1-Nonadecene	1-Nonadecene
1-Octadecene / Octadecane	Tetradecanoic acid methyl esters	Hexadecane	Gamma, beta Sitosterol / Stigmasterol, 22, 23-dihydro	Gamma, beta Sitosterol / Stigmasterol, 22, 23-dihydro	Gamma, beta Sitosterol / Stigmasterol, 22, 23-dihydro

[13] The similar colours represent compounds which are found to be common in the different extraction methods.

Docosane		Heptadecanoic acid, 16-methyl-, methyl ester	1-Docosene	1-Docosene	1-Docosene
Tetradecanoic acid and Hexadecanoic acid methyl esters					

Referring to table 6.2 above, it is quite evident that acetone and ethanol-toluene both in some instances extract similar and different compounds. However, it is interesting to see that the *E. nitens* sawdust and pitch have similar compounds extracted. This observation would tie in closely with the second aim of the study which referred to the impact on wood extractives on pitch formation. Since most of the compounds identified in the sawdust were also found to be in the pitch it is safe to conclude that wood extractives play a vital role in the formation of pitch. This has become a huge problem in the paper industry and therefore employing extraction methods to remove extractives prior to papermaking would be solution to this problem.

Also making reference to table 6.1, it seems that a acetone extraction on wood only with acetone removes higher amounts of extractive i.e. fatty acids / sterols and hydrocarbons. Also on a health side acetone is less toxic than toluene and much cheaper (Appendix H: MSDS). Table 6.2 is a cost summary of the chemicals and although toluene is the cheapest of the three it has to be used in conjunction with ethanol. Hence acetone is still the cheaper option.

Table 6.2: Cost summary of chemicals.

Chemical (AR grade)	Cost
Acetone (2.5 L)	R56.00
Ethanol (2.5 L)	R445.00
Toluene (2.5 L)	R45.00

Doing a quick calculation to determine which would work out cheaper in the long term, referring to table 6.3.

Table 6.3: Calculation indication the cost of usage for each chemical.

Acetone @ R56 per 2.5 L	***Usage***
	300 ml
Assume	10 extractions / day
	3000 ml / day
Assume extractions done 5 times a week	60 000 ml / month
	60 L / month
Cost of using acetone	60 L * R56 /2.5 L = R1344 per month
Ethanol @ R445 per 2.5 L	
Assume	200 ml
	10 extractions / day
Assume extractions done 5 times a week	2000 ml / day
	40 000 ml / month
	40 L / month
Cost of using ethanol	40 L * R445 /2.5 L = R7120 per month
Toluene @ R45 per 2.5 L	
Assume	100 ml
	10 extractions / day
Assume extractions done 5 times a week	1000 ml / day
	20 000 ml / month
	20 L / month
Cost of using toluene	20 L * R45 /2.5 L = R360 per month

It is evident from the calculations that acetone works out to be more cost effective than using ethanol-toluene as a mixture.

Hence as a final conclusion of this study it is recommended that extractives be removed as a prevention method to the formation of pitch on the paper and the method to be implemented is a acetone extraction on wood only using acetone. The method saves time, as the step of a hot water extraction would be eliminated.

References

Allen, L.H., Sithole, B.B., Macleod, J.M., Lapointe, C.L. and Mcphee, F.J., 1991, *Journal of Pulp and Paper Science*, Vol. 17, No. 3, J85-J91.

Ambrose, D., 1971, 2nd edn, Butterworth and Company Ltd, London, UK, pp. 75-77.

Back, E.L. and Allen, L.H., 2000, *Tappi Press*, Atlanta, USA, p. 38.

Bergelin, E., von Schoultz, J. and Holmbom, B., 2003, *Nordic Pulp and Paper Research Journal*, Vol. 18, No. 2, p. 133.

Bergelin, E. and Holmbom, B., 2003, *Journal of Pulp and Paper Science*, Vol. 29, No. 1, pp. 29-33.

Burton, G. W. and Ingold, K. U., 1981, *Journal of the American Chemical Society*, Vol 103, pp 6472 - 6477.

Casey, J.P., 1952, *Pulp and Paper Chemistry and Chemical Technology*, Vol. 1, Interscience Publishers, New York, pp. 544-547.

Centrifugation cleaning of pulp and paper process liquids, 1995, *US Patent* **5**, **468**, **396**.

Challinor, J.M, 2001, *Journal of Analytical and Applied Pyrolysis*, Vol. 61, Issue 1-2, pp. 3-34

Christie, W.W., 1992, *The Oily Press*, Dundee, Scotland, pp 1-18.

Del Rio, J.C., Gutierrez, A. and Gonzalez-Vila, F.J., 1999a, *Journal of Chromatography* A, p. 227-232.

Del Rio, J.C., Gutierrez, A., Gonzalez-Vila, F.J. and Martin, F., 1999b, *Journal of Analytical and Applied Pyrolysis*, Vol. 49, pp. 165-177.

Del Rio, J.C., Ramero, J. and Gutierrez, A., 2000, *Journal of Chromatography A*, Vol. 2, p. 239.

Demirbas, A., 1991, *Wood Science and Technology*, Vol. 25, pp. 366-370.

Douek, M and Allen, L.H., 1980, *Pulp Paper. Can.,* Vol. 81, No. 11, T317-T322.

Ekman, R. and Holmbom, B., 1989, *Nordic pulp and paper research journal*, No. 1, p.19.

European Project, *FAIR CT95-0560*, 1995,

Fast GC Columns, Technical article, 2000.

Fernandez, M.P., Watson, P.A., and Breuil, C., 2001, *Journal of Chromatography*, Vol. 922, No. 1-2, pp 225-233.

Freire, C.S.R., Silvestre, A.J.D. and Neto Pascoal, C., 2005, *Journal of Wood Chemistry and Technology*, Vol. 25, pp. 67-80.

Gonzalez-Vila, F.J., Gutierrez, A., Martin, F. and Verdejo, T., 1997, *Journal of Analytical and Applied Pyrolysis*, Vols. 40-41, pp. 501-501.

Gutierrez, A., del Rio, J.C. and Gonzalez-Vila, F.J., 1998a, *Journal of Wood Chemistry and Technology*, Vol.18, No. 4, pp. 439-446.

Gutierrez, A., Del Rio, J.C., Gonzalez-Vila, F.J. and Martin F., 1998b, *Journal of Chromatography A*, Vol. 823, pp. 449-455.

Gutierrez, A., Del Rio, J.C. and Gonzalez-Vila, F.J. and Martin, F., 1999, *Holzforschung*, Vol. 53, No. 5, pp. 481-485.

Gutierrez, A., Romero J. and del Rio, J.C., 2002, *Holzforschung*, vol. 55, pp. 260-264.

Hiroaki, Y., Takahito, N., Kuniyoshi, G., Yasuyuki, I., Hajime, O., Shin, T., Tetsuya, S. and Toshihiro, O., 2003, *Journal of Analytical and Applied Pyrolysis*, Vol. 67, pp. 191-200.

Howard, E.T., 1975, *US Forest Research Note*, p.2.

Jennings, W., 1987, *Academic Press*, Orlando Florida.

Karasek, F.W. and Clement, R.E., 1988, Elsevier Science Publishers B.V., Netherlands, pp. 41-42.

Kindness, A., Chemical Manual, UKZN, 2009

Kolaczkowski, S.T., Plucinski, P., Beltran, F.J., Rivas F.J. and McLurgh, D.B., *Journal of Chemical Engineer*, Vol. 73, pp 143-160.

Kostamo, A. and Kukkonen, J.V.K., 2003, *Water Research*, Vol. 37, No. 12, pp. 2813-2820.

Lacorte, S., Latorre, A., Barcelo, D., Rigol, A., Malmqvist, A. and Welander, T., 2003, *Tr Ac Trends in Analytical Chemistry,* Vol. 22, pp. 725-737.

Laleg, M. and Pikulik, I., 1993, *Nordic Pulp and Paper Research Journal*, Vol. 1 No. 8, pp 41-47.

Matinez-Inigo M.J., Immerzeel P., Gutierrez A., del Rio J.C. and Sierra-Alvarez R., *Holzforschung*, Vol. 53, 1999, pp. 247-252.

Megown, K.A., Philip, T., Male, J.R. and Retief, R.J., 2000, Forestry and Forest Products Research Centre, CSIR Durban, Report Number: ENV-S-R-2000-36.

Mehes, D., 1989, Buckman Laboratories of Canada, Ltd.

Örså, F. and Holmbom, B., 1994, *Journal of Pulp and Paper Science*, Vol.20, No.12, p J364.

Peng, G. and Roberts, J.C., 2000*, Tappi Journal*, Vol. 82, No. 12, pp 1-2.

Richardson, P.E., 1995, TAPPI Papermakers Conference, TAPPI PRESS, pp 205-214.

Rigol, A., Lacorte, S. and Barcelo, D., 2003, Tr *Ac Trends in Analytical Chemistry*, Vol. 22, No. 10, pp. 738-749.

Sandstrőm, M., Norborg, M.A. and Ericsson, A., 1996, *Journal of Chromatography*, Vol. 730, pp 373-379, Abstracts Refs only.

Sefara, N.L. and Birkett, M., 2004, African Pulp and Paper Week, pp. 1-7.

Sierra, A.C., Salvador, A.R. and Soria, F.G.O, 1991, *Tappi Journal*, p. 193.

Sithole, B.B., 1992, *Appita*, Vol. 45, No. 4, p. 264.

Sithole, B.B. and Allen, L., 2003, TAPPSA Technical Article.

Skoog, D.A., West, D.M., Holler, F.J. and Crouch, S.R., 2000, Analytical Chemistry: An Introduction, 7th ed., Saunders College Publishing, New York, USA, pp 683-690.

Spark, A., 2004, Literature Review: Wood Extractives and Pitch, Forestry and Forest Products research Centre, CSIR Durban, Report Number: ENV-D-C 2004-025.

Speranza, M., Martinez M.J., Gutierrez A., del Rio , J.C. and Martinez A.T., 2002, *Journal of Pulp and Paper Science*, Vol. 28, No. 9, pp. 292-297.

Sun, R., Sun, X.F. and Xiao, B., 2002, *Journal of Wood Chemistry and Technology,* Vol. 22, No. 1, pp. 1-9.

Sun, R. and Tomkinson, J., 2003, *Journal of Wood Science*, Vol. 49, p. 49.

TAPPI Tests Methods. TAPPI Press. 1989, 1996-1997.

Ucar, G. and Fengal, D., 1995, *Phytochemistry*, Vol. 38, pp. 877-880 Abstract Refs only.

US Patent 5468396, 1995

Vercoe, D., Stack, K., Blackman, A., Yates, B. and Richardson, D., 2004, *Journal of Wood Chemistry and Technology*, Vol. 24, No.2, pp. 115-137.

Verenich, S., Molina, V.G. and Kallas, J., 2004, *Advances in Environmental Research*, Vol. 8, pp. 293-301.

Wallis, A.F.A. and Wearne, R.H., 1997, *Appita Journal*, Vol. 50, No. 5, pp. 411-414.

Wise, L.E. and Ratliff, E.K., 1947, Mannose Content in Hardwoods, Institute of Paper Science and Technology, Report Number: 22, p. 122.

“Wood Extractives Biocontrol” Wood Extracts in Pulp and Paper Manufacture, Technical and Environmental Implications and Biological Removal FAIR CT95-0560 1995 R&D project of Fisheries and Agro-Industry Programme of the European Commission.

Xiao, Q.H, Qin M.H., Shao, Z.Y. and Gao, Y., 2009, *Bioresource Technology*, Vol 100, Issue 12, pp 3082-3087

Yokoi, H., Nakase, T., Goto, K., Ishida, Y., Ohtani, H., Tsuga, S., Sonoda, T., and Ona, T., 2003, *Journal of Analytical and Applied Pyrolysis*, Vol. 67, pp. 191-200.

Zheng, H., Uhing, M.C. and Cosper, D.R., 2002*, Journal of Pulp and Paper Science*, Vol.28, pp. 204-210.

Web References

Sheffield Hallam University, 2009, Gas Chromatography. Available from http://www.shu.ac.uk/schools/sci/chem/tutorials/chrom/gaschrm.htm (Accessed 17 April 2009)

Sci-Tech Dictionary: Acidolysis. Available from http://www.answers.com/topics/acidolysis (Accessed 17 April 2009)

UV-Spectrometry. Available from: http://www.cem.msu.edu/~reusch/VirtualText/Spectrpy/UV-Vis/uvspec.htm (Accessed 17 April 2009)

Helmenstine. A. M. (2010). BHA or Butylated Hydroxyanisole Available from http://chemistry.about.com/od/factsstructures/ig/Chemical-Structures---B/BHA-or-Butylated-Hydroxyanisol.htm (24 June 2010)

Appendix A: Method, Enumeration data and Tree information

Table A1: Enumeration data

DATE OF SAMPLING:	2005/11/17	**TIME OF SAMPLING:**	12:30
DATE OF ENUMERATION:	2005/09/27	**TIME OF ENUMERATION:**	11:00
	Equipment	**Measured by:**	
DBH - Sampled trees	DBH Class Tape #2	Wesley	
Height - Sampled trees	30 m Yellow tape	Prinisha	
Climatic conditions	cloudy, slight breeze and humid		

Table A2: GPS points of enumerated plot.

		Lat	**Long**
Centroid		-28.56152	32.13772
Corner points of plot	**Corner 1**	-28.56164	32.1378
	Corner 2	-28.56149	32.13778
	Corner 3	-28.5615	32.13780
	Corner 4	-28.56162	32.13777

COMMENTS
Understorey consisted of mostly weeds close to ground - 'medium' coverage
Thick leaf litter
Few double stems - compartment not coppiced (confirmed with Geoff Gallaway)
Seedling material - variable DBH's
Large stumps within compartment

Table A3: Information for the sampled trees.

SAMPLE NUMBER	TREE NO.	DBH (cm)	TOTAL HEIGHT (m)	SAMPLE Core/Disk/billet	SAMPLE HEIGHT UP TREE (m)	Tree HT to 7 cm DBH (m)
05-PR-001-001	5	21.0	25.6	B	1.5	20
05-PR-001-002	5			B	3	
05-PR-001-003	5			B	4.5	
05-PR-001-004	5			B	6	
05-PR-001-005	5			B	7.5	
05-PR-001-006	5			B	9	
05-PR-001-007	5			B	10.5	
05-PR-001-008	5			B	12	
05-PR-001-009	5			B	13.5	
05-PR-001-010	5			B	15	
05-PR-001-011	5			B	16.5	
05-PR-001-012	5			B	18	
05-PR-001-013	5			B		
05-PR-001-014	4			B	1.5	
05-PR-001-015	4	24.0	29.4	B	3	23
05-PR-001-016	4			B	4.5	
05-PR-001-017	4			B	6	
05-PR-001-018	4			B	7.5	
05-PR-001-019	4			B	9	
05-PR-001-020	4			B	10.5	
05-PR-001-021	4			B	12	
05-PR-001-022	4			B	13.5	
05-PR-001-023	4			B	15	
05-PR-001-024	4			B	16.5	
05-PR-001-025	4			B	18	
05-PR-001-026	4			B	19.5	
05-PR-001-027	4			B	21	
05-PR-001-028	4			B	22.5	
05-PR-001-029	3	26.0	27.3	B	1.5	22
05-PR-001-030	3			B	3	
05-PR-001-031	3			B	4.5	
05-PR-001-032	3			B	6	
05-PR-001-033	3			B	7.5	
05-PR-001-034	3			B	9	
05-PR-001-035	3			B	10.5	
05-PR-001-036	3			B	12	
05-PR-001-037	3			B	13.5	
05-PR-001-038	3			B	15	
05-PR-001-039	3			B	16.5	
05-PR-001-040	3			B	18	
05-PR-001-041	3			B	19.5	
05-PR-001-042	3			B	21	

05-PR-001-043	3	21.0	24.4	B	22.5	
05-PR-001-044	2			B	1.5	20
05-PR-001-045	2			B	3	
05-PR-001-046	2			B	4.5	
05-PR-001-047	2			B	6	
05-PR-001-048	2			B	7.5	
05-PR-001-049	2			B	9	
05-PR-001-050	2			B	10.5	
05-PR-001-051	2			B	12	
05-PR-001-052	2			B	13.5	
05-PR-001-053	2			B	15	
05-PR-001-054	2			B	16.5	
05-PR-001-055	2			B	18	
05-PR-001-056	2			B	19.5	
05-PR-001-057	1	18.0	27.2	B	1.5	19.5
05-PR-001-058	1			B	3	
05-PR-001-059	1			B	4.5	
05-PR-001-060	1			B	6	
05-PR-001-061	1			B	7.5	
05-PR-001-062	1			B	9	
05-PR-001-063	1			B	10.5	
05-PR-001-064	1			B	12	
05-PR-001-065	1			B	13.5	
05-PR-001-066	1			B	15	
05-PR-001-067	1			B	16.5	
05-PR-001-068	1			B	18	
05-PR-001-069	1			B	19.5	

METHODS MANUAL	Section: FFP
	Page No.: Page 1 of 15
Natural Resources and the Environment, Forestry Competence Area, Forestry and Forest Products Research Centre, CSIR	Date of Issue: March 2007
	Issue No.: 04
	Compiled by: A. Spark / N. Gounden
CARBOHYDRATE COMPOSITION OF EXTRACTIVE-FREE WOOD AND WOOD PULP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	Approved by: T. Bush

1. SCOPE AND DEFINITION

This method is used to determine the six principal monosaccharides that constitute the carbohydrate composition in wood, wood pulp and final sheet. The constituents determined quantitatively are glucose, mannose, arabinose, xylose, rhamnose and galactose. Concentrations of the individual components as low as 0.1% can be determined. This method is applicable to extractive-free wood as well as to wood pulp and final sheet.

2. APPARATUS

2.1 High Performance Liquid Chromatograph comprising an autosampler, quaternary LC pump, column oven, pulsed amperometric detector, isocratic LC post column pump, Dionex CarboPac PA1 analytical column (with Dionex borate trap and Phenomenex SAX guard cartridge).

2.2 Constant temperature water bath, regulated to 30 ± 3 °C

2.3 Autoclave, capable of operation at 103 ± 7 kPa (120 °C)

METHODS MANUAL	Section: FFP
	Page No.: Page 2 of 15
Natural Resources and the Environment, Forestry Competence Area, Forestry and Forest Products Research Centre, CSIR	Date of Issue: March 2007
	Issue No.: 04
	Compiled by: A. Spark / N. Gounden
CARBOHYDRATE COMPOSITION OF EXTRACTIVE-FREE WOOD AND WOOD PULP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	Approved by: T. Bush

2.4 Other apparatus: conical glass test tubes, 15 ml; glass stirring rods, beakers, 50 mL; graduated cylinders, 100 mL; bulb pipettes, 3 mL, 10 mL, 25 mL; volumetric flasks, 100 mL, 200 mL, 250 mL, 1000 mL; Schott bottles, 250 mL.

3. REAGENTS

Glucose, mannose, arabinose, xylose, rhamnose and galactose - monosaccharide standards used for calibration.

Fucose - internal standard, (1µg/1µL) 0.1g into 100 mL deionised water.

Hydrochloric acid (standardised 1N)

Sodium hydroxide 50% (m/v)

To 400 mL of deionised water contained in a 1000 mL beaker, slowly add 500 g of NaOH. Keep the beaker submerged in cold water while continuously stirring the solution until the NaOH is completely dissolved. Allow the solution to cool to room temperature and transfer it to a 1000 mL volumetric flask. Make up to the mark with deionised water. Allow the solution to stand until the suspended carbonates have settled. Pipette 10 mL of the solution and make up to 100 mL in a volumetric flask. Use 3 x 10ml from the 100 mL volumetric flask for the standardision. Add a few

METHODS MANUAL	Section: FFP
	Page No.: Page 3 of 15
Natural Resources and the Environment, Forestry Competence Area, Forestry and Forest Products Research Centre, CSIR	Date of Issue: March 2007
	Issue No.: 04
	Compiled by: A. Spark / N. Gounden
CARBOHYDRATE COMPOSITION OF EXTRACTIVE-FREE WOOD AND WOOD PULP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	Approved by: T. Bush

drops of phenolpthalein indicator and standardise using standardised 1N HCl. Degas solution for 30 min in ultra-sonic bath.

Sodium hydroxide (0.350 M and 0.200 M)

Calculate the volume of the 50% (m/v) NaOH needed to prepare 1000 mL of the 0.350 M and 0.200 M solutions, using the equation below:

$$V_1 = \frac{1000ml \times C_2}{C_1}$$

Where V_1 = Volume of the 50% (m/v) NaOH solution required, mL

C_1 = Exact concentration (M) of the 50% (m/v) NaOH solution (from standardisation)

C_2 = Concentration of the NaOH solution required (ie. 0.500, 0.350 or 0.200 M)

130 mM Sodium Acetate in 200 mM NaOH

Weigh out 17.69 g ± 0.01 g AR Grade sodium acetate trihydrate and make up to 1000 mL in a volumetric flask with 200 mM NaOH. Degas solution for 30 min in ultrasonic bath.

120 mM Sodium Acetate in 200 mM NaOH

Weigh out 16.33 g ± 0.01 g AR Grade sodium acetate trihydrate and make up to 1000 mL in a volumetric flask with 200 mM NaOH. Degas solution for 30 min in ultrasonic bath.

Sulphuric acid (72% (m/m) or 24N)

To 250 mL of deionised water in a 1000 mL volumetric flask, slowly add 665 mL of concentrated sulphuric acid 98% (m/m) (sp gr 1.84). When temperature has equilibrated to ambient, make up to the mark with deionised water and mix. Standardise the solution using the standardised sodium hydroxide – the concentration of the sulphuric acid should be 24 N ± 0,1 N.

Sulphuric acid (3% (m/m))

Add 15ml of 72% Sulphuric Acid into 1000ml Volumetric flask using a bottle top dispenser and make up to the mark with deionised water.

4. ANALYTICAL PROCEDURE

4.1 Preparation of wood pulp sample

Wet pulp should be dried overnight at 105 ± 3°C.

4.2 Preparation of wood sample

4.2.1 Preparation of sawdust is carried out in accordance with 'The Preparation of Wood for Chemical Analysis'.

4.2.2 Prepare 6 - 8g of extractive free wood.

4.3 Preparation of final sheet sample

Final sheet should be scraped with a sharp knife to separate fibres.

4.4 Analysis of wood, pulp and final sheet samples

4.4.1 Allow sawdust, pulp or final sheet sample to reach moisture equilibrium in the atmosphere near the balance.

4.4.2 In order to achieve a constant mass of cellulose in the sample for HPLC analysis the following masses should be weighed:
Extractive-free wood: 0.35 g (50% cellulose, 8% moisture)
Pulp: 0.2 g oven-dry (90% cellulose, 60% moisture)
Final Sheet: 0.16 g (98% cellulose, 7% moisture)

Masses should be ± 0.01 g to the nearest 0.1 mg and transferred into 15 mL glass test tubes.

4.4.3 At the same time weigh another 2.0 g specimen for moisture determination.

4.4.4 To the specimen in the test tube, add exactly 3 mL of 72% (m/m) H_2SO_4 with a bottle top dispenser. Stir the contents of the tube with a stirring rod to dissolve the sample.

4.4.5 Place tube in a 30 ± 3 °C water bath for 1 hour to 1 and half hours. Stir occasionally (every 15 minutes). Wash the contents of the tube quantitatively into a 250 mL Schott bottle with 84 mL of deionised water. If any specimen solution is lost during the hydrolysis and transfer steps, terminate the analysis, weigh out a new specimen, and repeat the analysis.

4.4.6 Place the Schott bottle with specimen solution in an autoclave set at 103 ± 7 kPa and 120 °C for 1 hour. Ensure that the cap of the Schott bottle is partially tightened.

METHODS MANUAL	Section: FFP
	Page No.: Page 7 of 15
Natural Resources and the Environment, Forestry Competence Area, Forestry and Forest Products Research Centre, CSIR	Date of Issue: March 2007
	Issue No.: 04
	Compiled by: A. Spark / N. Gounden
CARBOHYDRATE COMPOSITION OF EXTRACTIVE-FREE WOOD AND WOOD PULP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	Approved by: T. Bush

4.4.7 Cool the hydrolyzate to room temperature and allow the lignin to settle. Filter quantitatively under vacuum through a 0.45 μm filter paper, finally using deionised water to transfer and wash the precipitate and to rinse the Schott bottle.

4.4.8 Transfer the filtrate quantitatively into a 200 mL volumetric flask and dilute to the mark with deionised water. Mix well.

4.4.9 Transfer about 50 mL into a plastic bag, seal and place in the freezer for a minimum of 24 hours.

4.4.10 Thaw sample and pipette exactly 50 μL of sample and 500 μL deionised water using a fixed volume pipette into the autosampler vial and place in the autosampler tray, recording the position and identity.

4.4.11 When all the samples for the batch are in the tray, place the vial containing the internal standard in a convenient position in the tray.

METHODS MANUAL	Section: FFP
	Page No.: Page 8 of 15
Natural Resources and the Environment, Forestry Competence Area, Forestry and Forest Products Research Centre, CSIR	Date of Issue: March 2007
	Issue No.: 04
	Compiled by: A. Spark / N. Gounden
CARBOHYDRATE COMPOSITION OF EXTRACTIVE-FREE WOOD AND WOOD PULP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	Approved by: T. Bush

4.4.12 Before running the samples and standards, program the autosampler offline to add 20 µl of internal standard solution (1 mg/ml fucose) from the designated internal standard vial (suggested: vial 100) and mix for 3 cycles. (See Appendix A, page 13, for details of how to program the 200 Autosampler).

4.4.13 Program the HPLC for analysis – Use Totalchrom software.

4.4.14 Conditions for HPLC analysis are set as follows:

(a) Filtered deionised water for 6.5 min (equilibration)

(b) Injection (15 µl)

(c) Filtered deionised water for 6 min (separation)

(d) Ramp to 130 mM or 120 mM sodium acetate in 200 mM NaOH for 1 min.

(e) 130 mM or 120 mM sodium acetate in 200 mM NaOH for 3 min (acetate loading step)

(f) Ramp to filtered deionised water for 1 min
Flow rate for steps (a), (c) – (f) is 1 mL/min.

(g) Post-column addition of 350 mM NaOH at a flow rate of 0.5 mL/min.

METHODS MANUAL	Section: FFP
	Page No.: Page 9 of 15
Natural Resources and the Environment, Forestry Competence Area, Forestry and Forest Products Research Centre, CSIR	Date of Issue: March 2007
	Issue No.: 04
	Compiled by: A. Spark / N. Gounden
CARBOHYDRATE COMPOSITION OF EXTRACTIVE-FREE WOOD AND WOOD PULP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	Approved by: T. Bush

4.4.15 Peak areas from the sample chromatograms are converted into percent composition for each carbohydrate by comparison of peak area ratio of each carbohydrate / internal standard to a calibration of peak area ratio of each carbohydrate / internal standard vs. concentration for each carbohydrate standard.

4.4.16 Report the percentages of each carbohydrate to the nearest 0.1%.

4.4.17 The maximum concentration expected for 150 mg cellulose/ hemicellulose in the samples are as follows:

	Hardwoods		Softwoods	
	g/ 100 mL	mg/ mL	g/ 100 mL	mg/ mL
Glucose	0.0850	0.850	0.085	0.850
Xylose	0.0220	0.220	0.010	0.100
Galactose	0.0010	0.010	0.0025	0.025
Arabinose	0.0005	0.005	0.0015	0.015
Rhamnose	0.0005	0.005	0.0015	0.015
Mannose	0.0030	0.030	0.0100	0.100

4.5 Preparation of Standards for HPLC Analysis

4.5.1 Dry all sugars to constant mass. (This converts glucose to the anhydrous form). DO NOT OVERDRY – if sugar discolours discard.

METHODS MANUAL	Section: FFP
	Page No.: Page 10 of 15
Natural Resources and the Environment, Forestry Competence Area, Forestry and Forest Products Research Centre, CSIR	Date of Issue: March 2007
	Issue No.: 04
	Compiled by: A. Spark / N. Gounden
CARBOHYDRATE COMPOSITION OF EXTRACTIVE-FREE WOOD AND WOOD PULP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	Approved by: T. Bush

The stock solutions must be concentrated enough for convenient storage but not so concentrated that excessive dilution is required (leading to dilution errors). Because of the very low concentrations of some of the sugars (0.005 g/ 100 mL), two dilutions are presently carried out so that the primary solution is not so dilute that weighing errors occur. To ensure accurate dilutions (evident from the calibration line), standard volume pipettes and volumetric flasks of large enough size (min 10 mL pipette) must be used.

4.5.2 Prepare stock solutions of each monosaccharide standard by weighing the following dry masses ± 0.01 g to the nearest 0.1 mg and transferring each mass quantitatively into a separate 100 mL volumetric flask and making up to the mark with deionised water:

Glucose: 1.00 g
Xylose: 0.25 g
Mannose: 0.30 g
Galactose: 0.20 g
Arabinose: 0.10 g
(Rhamnose: 0.15 g) (not generally included)

METHODS MANUAL	Section: FFP
	Page No.: Page 11 of 15
Natural Resources and the Environment, Forestry Competence Area, Forestry and Forest Products Research Centre, CSIR	Date of Issue: March 2007
	Issue No.: 04
	Compiled by: A. Spark / N. Gounden
CARBOHYDRATE COMPOSITION OF EXTRACTIVE-FREE WOOD AND WOOD PULP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	Approved by: T. Bush

4.5.3 **Primary stock mix:** Pipette 10 mL each of arabinose and galactose stocks into a 100 mL volumetric flask A. (Pipette 10 mL rhamnose standard into flask A if required). Mix and make up to volume with deionised water. Pipette 20 mL each of the glucose, xylose, and mannose stocks and 20 mL from volumetric flask A into another 200 mL volumetric flask B. Mix and make up to volume with 3 % Sulphuric acid.(This is the 100% standard).

4.5.4 The working standards are prepared from the 100% standard as follows using bulb pipettes:

10% standard: pipette 10 mL of the 100% standard (4.5.4) into a 100 mL volumetric flask, and make up to the mark with 3% Sulphuric acid.

25% standard: pipette 25 mL of the 100% standard into a 100 mL volumetric flask, and make up to the mark with with 3% Sulphuric acid.

50% standard: pipette 50 mL* of the 100% standard into a 100 mL volumetric flask, and make up to the mark with with 3% Sulphuric acid.

* 2 x 25 mL - using the same 25 mL pipette.

75% standard: pipette 75 mL* of the 100% standard into a

METHODS MANUAL	Section: FFP
	Page No.: Page 12 of 15
Natural Resources and the Environment, Forestry Competence Area, Forestry and Forest Products Research Centre, CSIR	Date of Issue: March 2007
	Issue No.: 04
	Compiled by: A. Spark / N. Gounden
CARBOHYDRATE COMPOSITION OF EXTRACTIVE-FREE WOOD AND WOOD PULP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	Approved by: T. Bush

100 mL volumetric flask, and make up to the mark with with 3% Sulphuric acid.

* 3 x 25 mL - using the same 25 mL pipette.

This will give 100 mL each of the diluted standards and 40 mL of the 100 % standard.

4.5.6 The exact concentration of each of the components in each standard is calculated using the exact masses weighed.

4.5.7 The standards are pipetted (50 µl per vial) into autosampler vials and are then refridgerated for a minimum of 24 hours before analysis.

4.5.8 After thawing the standards, pipette exactly 50 µL of standard and 500 µL deionised water using a fixed volume pipette into the autosampler vial, and place in the autosampler tray, recording the position and identity.

4.5.9 The procedure for analysing the standards using HPLC is the same as for the samples ie steps 4.4.13 – 4.4.16.

METHODS MANUAL	Section: FFP
	Page No.: Page 13 of 15
Natural Resources and the Environment, Forestry Competence Area, Forestry and Forest Products Research Centre, CSIR	Date of Issue: March 2007
	Issue No.: 04
	Compiled by: A. Spark / N. Gounden
CARBOHYDRATE COMPOSITION OF EXTRACTIVE-FREE WOOD AND WOOD PULP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	Approved by: T. Bush

4.5.10 Full set of standards should be run after every 10 –12 samples.

5. REFERENCES

5.1 TAPPI Test Methods (1996-1997), test method no. T249 cm-85.

5.2 Wright, P.J., and Wallis, A.F.A. 1996. *Rapid determination of carbohydrates in Hardwoods.* Holzforschung. Walter de Gruyter, Berlin. **50**: 518-524.

5.3 Wallis, A.F.A., Wearne, R.H., Wright, P.J. 1996. *Chemical analysis of polysaccharides in plantation eucalypt woods and pulps.* Appita Journal. **49(4)**: 258-262.

Appendix A

Addition of Internal Standard (Fucose):

10 µL fucose solution (1% m/v) is added to both standards and samples once samples and standards are loaded in vials in the autosampler tray.

Programming the 200 Autosampler to add and mix Fucose:

TotalChrom must be on manual operation (Release control)

Follow the instructions below:

METHODS MANUAL	Section: FFP
	Page No.: Page 14 of 15
Natural Resources and the Environment, Forestry Competence Area, Forestry and Forest Products Research Centre, CSIR	Date of Issue: March 2007
	Issue No.: 04
	Compiled by: A. Spark / N. Gounden
CARBOHYDRATE COMPOSITION OF EXTRACTIVE-FREE WOOD AND WOOD PULP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	Approved by: T. Bush

F7 - CNFG

F1 - INST

F1 - OPT

Deriv/Dilute **F4** (**D.O.**) – Enter

Press **RETURN** (3x)

(Back to Method 18 on screen)

F2 - METHOD

(use arrow)

First	Last	Vol	Replicates	Time
Add number	Add number	15	1	0
eg. 2	eg. 30			

F4 - DRV

Add number of reagents (0-5): **1**

Enter

Fill in:

Sample vol	**550 µL**
Vial (no. of IS)	**100**
Vol. (IS)	**20 µL**

METHODS MANUAL	Section: FFP
	Page No.: Page 15 of 15
Natural Resources and the Environment, Forestry Competence Area, Forestry and Forest Products Research Centre, CSIR	Date of Issue: March 2007
	Issue No.: 04
	Compiled by: A. Spark / N. Gounden
CARBOHYDRATE COMPOSITION OF EXTRACTIVE-FREE WOOD AND WOOD PULP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	Approved by: T. Bush

Mix cycles **3**

Reaction time **0**

Enter

Return

F6 - Store

STORE METHOD: Change to **18**

Enter

Overwrite existing method: **Y**

Name method: **N**

Press "**Start**" on Autosampler

Appendix B: *E. grandis* Extractions: Data Collection and Calculations

Table B1 illustrates the data collected during column chromatography using individual solvents acetone, ethanol and toluene for *E. grandis* sawdust. Its purpose is to show the different extracting abilities of the solvents. The method and graphical representation is found in chapter 4 and 5 respectively.

Table B1: Data collected during column chromatography using individual solvents acetone, ethanol and toluene.

	Sample	**Mass of oven dried sample, g**	**Mass of flask, g**	**Mass of flask + sample, g**	**% Solvent Extractives**
Acetone	Tree 3	2.0257	93.1571	93.1625	0.27
Ethanol	Tree 3	2.0233	95.3397	95.3478	0.40
Toluene	Tree 3	2.0303	161.8845	161.8885	0.20

Table B2 below is the data collected during the hot water extraction process with the necessary calculations. After every hot water extraction a soxhlet extraction was carried out using different combinations of solvent i.e. ethanol-toluene and acetone. Table B3 and Table B4 is the data collected during the soxhlet extraction can be found directly after the results for the hot water extraction.

Table B2: Data collected during the hot water extraction process.

Sample	Mass of dish, g	Mass of dish plus sample before drying, g	Mass of dish plus sample after drying, g	% Moisture	Mass of sample, g	Mass of oven dried sample, g	Mass of crucible, g	Mass of crucible + sample after drying, g	% Hot Water Extractives
Tree 1	43.702	45.8965	45.6971	9.08	5.0943	4.6315	32.0549	36.487	4.31
Tree 1	43.7	45.701	45.5265	8.72	5.0709	4.6288	32.0559	36.4651	4.74
Tree 1	45.192	47.2928	47.1097	8.72	5.0022	4.5662	19.5803	23.9406	4.51
Tree 1	45.192	47.2928	47.1097	8.72	5.0011	4.5652	18.5854	22.964	4.09
Tree 1	46.699	48.6972	48.4832	10.71	5.0068	4.4706	51.8801	56.1479	4.54
Tree 1	46.699	48.6972	48.4832	10.71	5.0089	4.4724	51.287	55.5522	4.63
							Mean % extractives ± s		4.47 ± 0.24
Tree 2	52.981	55.1704	54.9575	9.72	5.0489	4.5580	34.3992	38.8018	3.41
Tree 2	50.827	52.6288	52.46079	9.32	5.0443	4.5739	34.4092	38.7633	4.81
Tree 2	46.713	48.8625	48.6725	8.84	5.0055	4.5631	18.8861	23.2956	3.37
Tree 2	46.713	48.8625	48.6725	8.84	5.0015	4.5595	19.5928	24.0068	3.19
Tree 2	43.704	45.7041	45.4887	10.77	5.0006	4.4620	51.8973	56.2082	3.39
Tree 2	43.704	45.7041	45.4887	10.77	5.0072	4.4679	51.8115	56.1193	3.58
							Mean % extractives ± s		3.69 ± 0.59
Tree 3	45.19	47.3504	47.1443	9.54	5.0529	4.5709	34.6822	39.0464	4.52
Tree 3	56.737	58.7992	58.5915	10.07	5.0141	4.5091	51.8783	56.1665	4.90
Tree 3	56.737	58.7992	58.5915	10.07	5.0166	4.5113	52.263	56.5533	4.90
							Mean % extractives ± s		4.77 ± 0.22
Tree 4	46.71	48.7100	48.5359	8.70	5.0479	4.6085	32.853	37.2822	3.89
Tree 4	46.671	48.6709	48.4726	9.91	5.0051	4.5089	51.6344	55.9419	4.47
Tree 4	46.671	48.6709	48.4726	9.91	5.006	4.5098	50.8373	55.1497	4.38
							Mean % extractives ± s		4.24 ± 0.31
Tree 5	44.047	46.0416	46.0091	1.63	5.0003	4.9188	51.8983	56.7058	2.26
Tree 5	44.047	46.0416	46.0091	1.63	5.0004	4.9189	52.3503	57.1527	2.37
Tree 5	55.7	57.699	57.4905	10.43	5.0015	4.4800	49.6634	53.9513	4.29
Tree 5	55.7	57.699	57.4905	10.43	5.0068	4.4847	50.6339	54.9225	4.37
							Mean % extractives ± s		3.32 ± 1.16

Table B3: Data collected for a soxhlet extraction using ethanol-toluene after a hot water extraction.

Sample (Soxhlet extraction using ethanol-toluene)	Mass of oven dried sample, g	Mass of flask, g	Mass of flask + sample, g	% Solvent Extractives
Tree 1	4.001	153.5048	153.5900	2.13
Tree 1	4.0049	155.0191	155.1100	2.27
Tree 2	4.0042	161.8876	161.9592	1.79
Tree 2	4.0089	134.8833	134.9282	1.12
Tree 3	4.0009	153.5233	153.6071	2.09
Tree3	4.0076	155.041	155.1223	2.03
		Mean % extractives ± s		1.91 ± 0.42

Table B4: Data collected for a soxhlet extraction using acetone after a hot water extraction.

Sample (Soxhlet extraction using acetone	Mass of oven dried sample, g	Mass of flask, g	Mass of flask + sample, g	% Solvent Extractives
Tree 1	4.0005	161.9375	161.9545	0.42
Tree 1	4	153.5562	153.5758	0.49
Tree 2	4.0008	153.548	153.5761	0.70
Tree 2	4.0004	161.9273	161.9558	0.71
Tree 3	4.0118	155.0546	155.0877	0.83
Tree 3	4.0012	134.9317	134.9449	0.33
Tree 4	4.0005	165.0359	165.1003	1.61
Tree 4	4.0051	153.5351	153.6052	1.75
Tree 5	4.0109	153.5399	153.5732	0.83
Tree 5	4.0066	165.1066	165.1229	0.41
		Mean % extractives ± s		0.81 ± 0.49

Table B5: Data collected for a soxhlet extraction using acetone eliminating the hot water extraction.

Sample	Mass of oven dried sample, g	Mass of flask, g	Mass of flask + sample, g	% Solvent Extractives
Tree 1	4.0097	162.1508	162.2514	2.51
Tree 1	4.0073	153.5445	153.7014	3.92
Tree 2	4.0045	161.9054	162.0018	2.41
Tree 2	4.0019	155.0627	155.2143	3.79
Tree 3	4.0077	153.5294	153.6100	2.01
Tree 3	4.0032	161.9268	155.2143	3.73
Tree 4	4.0025	155.0611	155.1221	1.52
Tree 4	4.0049	134.9177	134.9978	2
Tree 5	4.0023	153.5118	153.6444	3.31
Tree 5	4.0011	153.5027	153.6384	3.39
		Mean % extractives ± s		2.86 ± 0.87

Table B6: The statistical repeatability of the data obtained for each tree. [14]

Hot water extraction					
Replicate	**Tree 1**	**Tree 2**	**Tree 3**	**Tree 4**	**Tree 5**
1	4.09	3.19	1.63	0.97	2.26
2	4.51	3.37	1.89	0.59	2.37
3	4.54	3.39	4.90	4.47	4.29
4	4.63	3.58	4.90	4.39	4.37
Average, x	4.56	3.45	4.90	4.43	4.33
Standard deviation, S_e	0.06	0.12	0.00	0.06	0.06
Std. dev of the test result, $S_r = S_e/(5)^{1/2}$	0.03	0.05	0.00	0.03	0.03
Repeatability, r = 2.77 S_r	0.08	0.14	0.00	0.07	0.07
Repeatability in % = 100 r/x	1.70	4.17	0.00	1.58	1.62
Average repeatability in %					**1.63%**
Soxhlet Extraction					
Ethanol-Toluene after hot water extraction					
Replicate	**Tree 1**	**Tree 2**	**Tree 3**	**Tree 4**	**Tree 5**
1	2.13	1.12	1.97	3.12	3.14
2	2.27	1.79	2.03	2.99	3.31
3	2.35	1.68	2.50	4.12	1.05
Average, x	2.31	1.74	2.00	3.06	3.23
Standard deviation, S_e	0.06	0.08	0.04	0.09	0.12
Std. dev of the test result, $S_r = S_e/(5)^{1/2}$	0.03	0.03	0.02	0.04	0.05
Repeatability, r = 2.77 S_r	0.07	0.10	0.05	0.11	0.15
Repeatability in % = 100 r/x	3.03	5.55	2.63	3.73	4.62
Average repeatability in %					**3.13%**

Acetone without hot water extraction					
Replicate	**Tree 1**	**Tree 2**	**Tree 3**	**Tree 4**	**Tree 5**
1	2.31	1.79	2.09	3.12	3.14
2	2.27	2.41	2.03	4.12	3.31
3	2.51	1.12	2.01	1.52	3.39
Average, x	2.36	2.10	2.02	3.62	3.35
Standard deviation, S_e	0.10	0.44	0.01	0.71	0.06
Std. dev of the test result, $S_r = S_e/(5)^{1/2}$	0.05	0.20	0.01	0.32	0.03
Repeatability, r = 2.77 S_r	0.13	0.54	0.02	0.88	0.07
Repeatability in % = 100 r/x	5.50	25.86	0.87	24.20	2.09
Average repeatability in %					**3.80%**

Acetone only no hot water extraction					
Replicate	**Tree 1**	**Tree 2**	**Tree 3**	**Tree 4**	**Tree 5**
1	0.42	0.70	0.83	1.61	0.83
2	0.49	0.71	0.33	1.75	0.41
Average, x	0.46	0.71	0.58	1.68	0.62

[14] The numbers highlighted indicate outliers.

Standard deviation, S_e	0.05	0.01	0.35	0.10	0.30
Std. dev of the test result, $S_r = S_e/(5)^{1/2}$	0.02	0.00	0.16	0.04	0.13
Repeatibility, $r = 2.77\ S_r$	0.06	0.01	0.44	0.12	0.37
Repeatibility in % = 100 r/x	13.48	1.24	75.51	7.30	59.34
Average repeatibility in %					**10.39%**

Appendix C: *E. nitens* Extractions: Data Collection and Calculations

Table C1: Data collected during column chromatography using individual solvents acetone, ethanol and toluene.

Sample	Mass of oven dried sawdust, g	Mass of flask, g	Mass of flask + sample, g	% Solvent Extractives
Acetone	2.5074	93.1423	93.1625	0.81
Ethanol	2.5025	95.1589	95.1818	0.92
Toluene	2.504	161.4251	161.4404	0.61

Table C2: Summary of results obtained for soxhlet extraction of *E. nitens* sawdust using acetone with no hot water extraction, acetone and ethanol-toluene after a hot water extraction.

Sample	Mass of oven dried sample, g	Mass of flask, g	Mass of flask + sample, g	% Solvent Extractives
Acetone	4.0027	153.5432	153.6329	2.24
AHW	4.003	155.3285	155.3528	0.61
Ethanol-toluene	4.0015	161.1134	161.1385	0.63

Table C3: Summary of results obtained for soxhlet extraction of *E. nitens* pitch using acetone with no hot water extraction and ethanol-toluene after a hot water extraction.

Sample	Mass of oven dried sample, g	Mass of flask, g	Mass of flask + sample, g	% Solvent Extractives
Acetone	2.5074	94.2156	94.4817	10.61
Ethanol-toluene	2.5049	94.159	94.518	14.33

Appendix D: High Performance Liquid Chromatography

Below is the result for each tree after the extraction processes, analysed for sugar content.

Table D1: Summary of results obtained for HPLC analysis.

		Arab %	Galac %	Gluc %	Xyl %	Mann %
Soxhlet extraction with acetone only	Tree 1	2.12	11.53	48.14	11.29	0.00
Soxhlet extraction with ethanol-toluene after hot water	Tree 1	1.65	10.00	48.37	10.58	0.00
Soxhlet extraction with acetone after hot water	Tree 1	1.89	10.77	48.26	11.94	0.00
Soxhlet extraction with acetone only	Tree 2	2.27	15.67	48.77	10.39	0.00
Soxhlet extraction with ethanol-toluene after hot water	Tree 2	1.72	15.28	48.69	10.81	0.00
Soxhlet extraction with acetone after hot water	Tree 2	1.73	10.72	46.12	11.29	0.00
Soxhlet extraction with acetone only	Tree 3	1.36	10.07	46.21	11.42	0.00
Soxhlet extraction with ethanol-toluene after hot water	Tree 3	2.09	11.36	46.03	11.16	0.00
Soxhlet extraction with acetone after hot water	Tree 3	1.79	10.37	47.26	11.01	0.00
Soxhlet extraction with acetone only	Tree 4	2.02	10.67	46.67	10.81	0.00
Soxhlet extraction with ethanol-toluene after hot water	Tree 4	1.55	10.07	47.85	11.20	0.00
Soxhlet extraction with acetone after hot water	Tree 4	2.00	15.48	48.73	10.60	0.00
Soxhlet extraction with acetone only	Tree 5	1.69	9.14	46.66	11.21	0.00
Soxhlet extraction with ethanol-toluene after hot water	Tree 5	1.38	9.34	50.16	11.16	0.00
Soxhlet extraction with acetone after hot water	Tree 5	1.54	9.24	48.41	11.19	0.00

Appendix E: Gas Chromatography

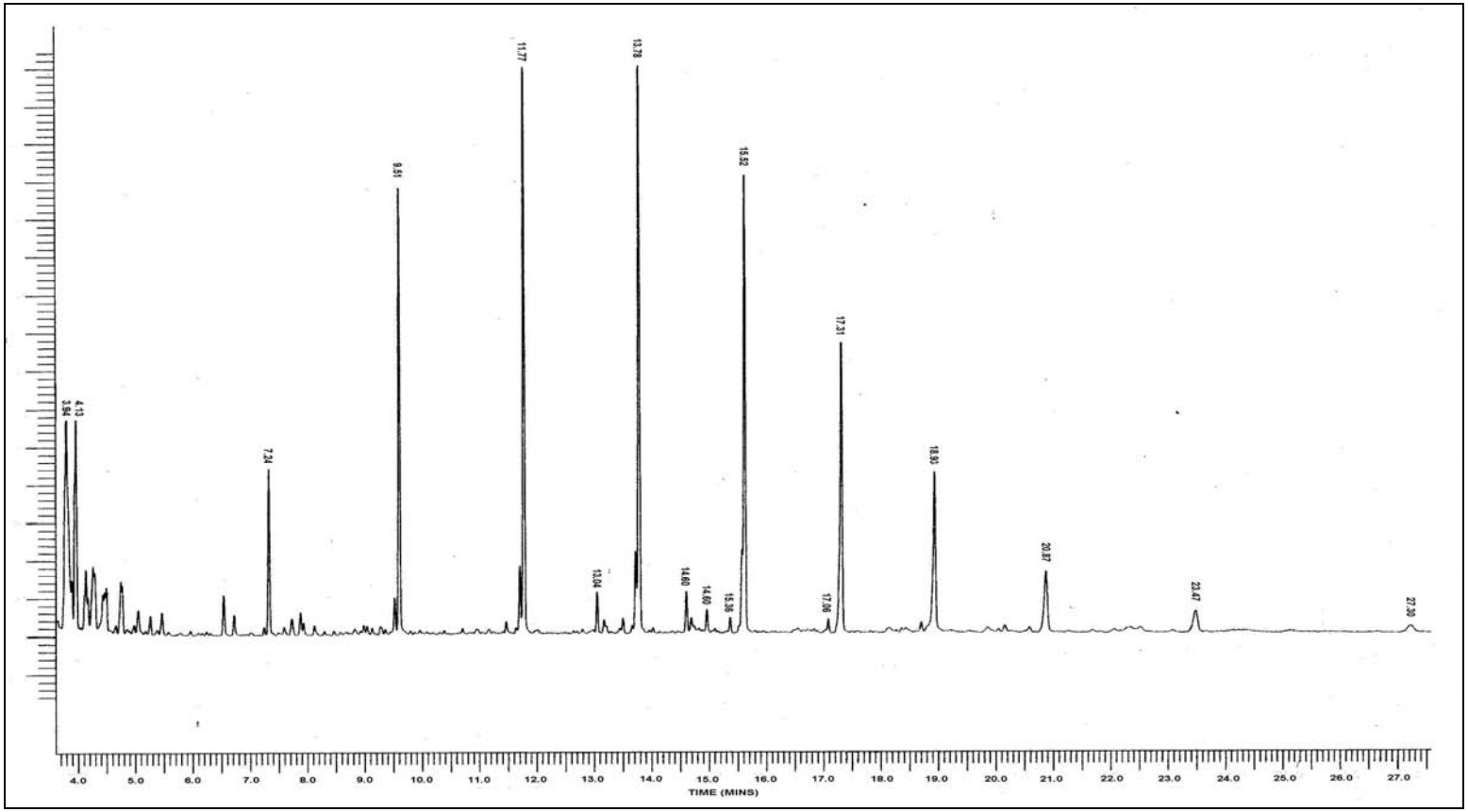


Figure E.1: GC Chromatogram for the extraction with ethanol-toluene after hot water (*E.grandis* extracts)

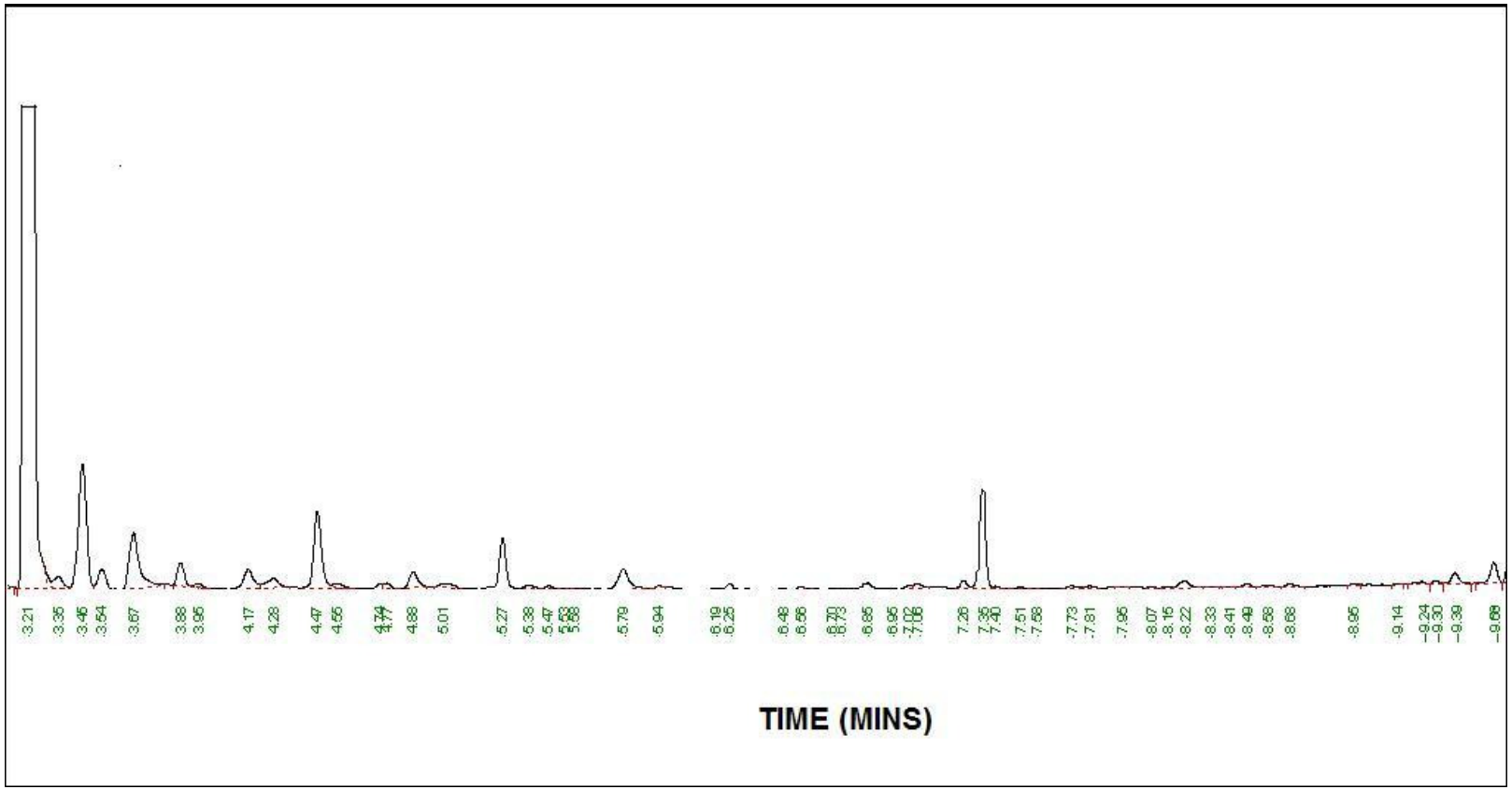


Figure E.2: GC Chromatogram for the extraction with ethanol-toluene after hot water (*E.nitens* extracts)

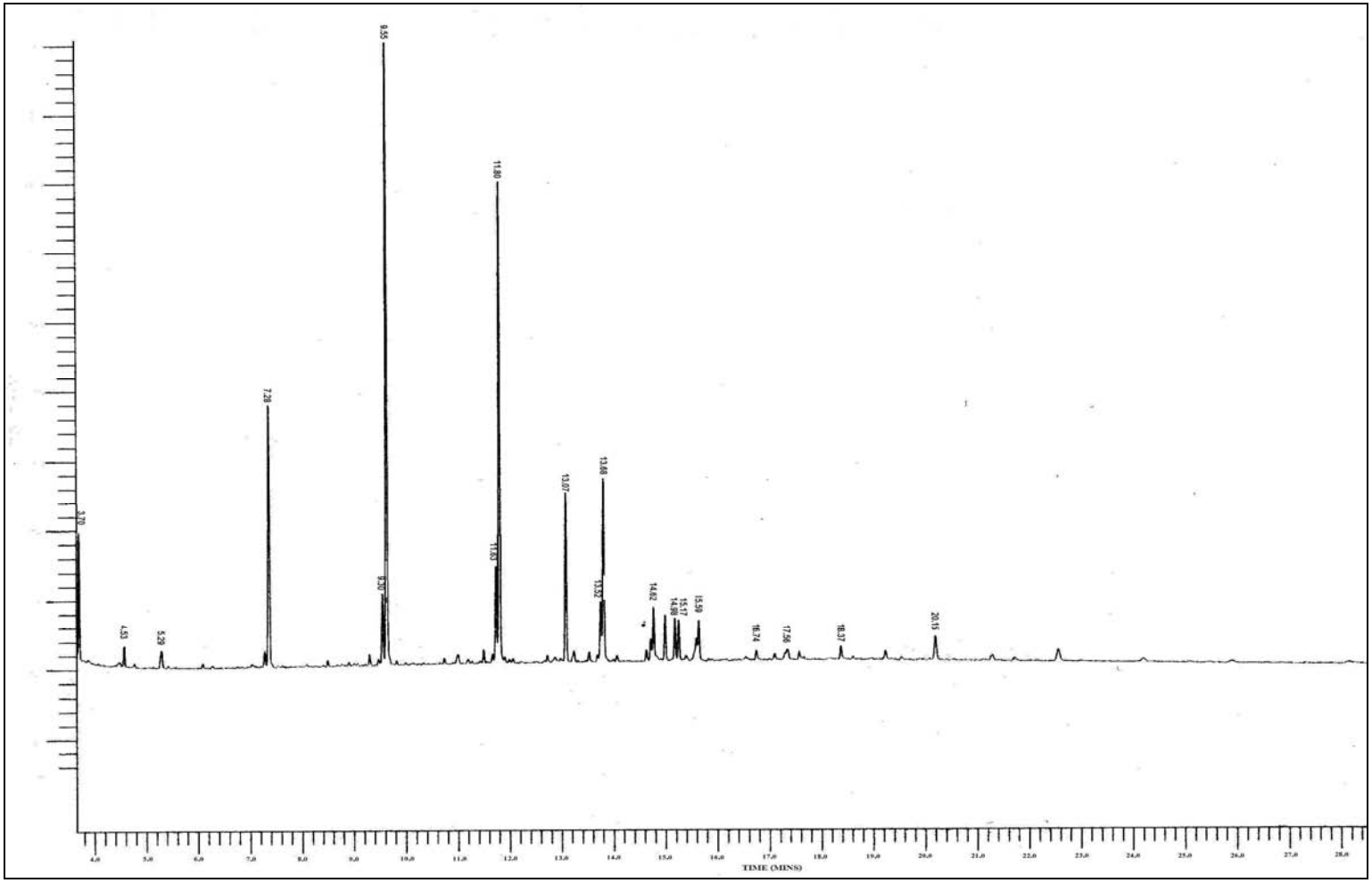


Figure E.3: GC Chromatogram for the extraction with acetone only. (*E.grandis* extracts)

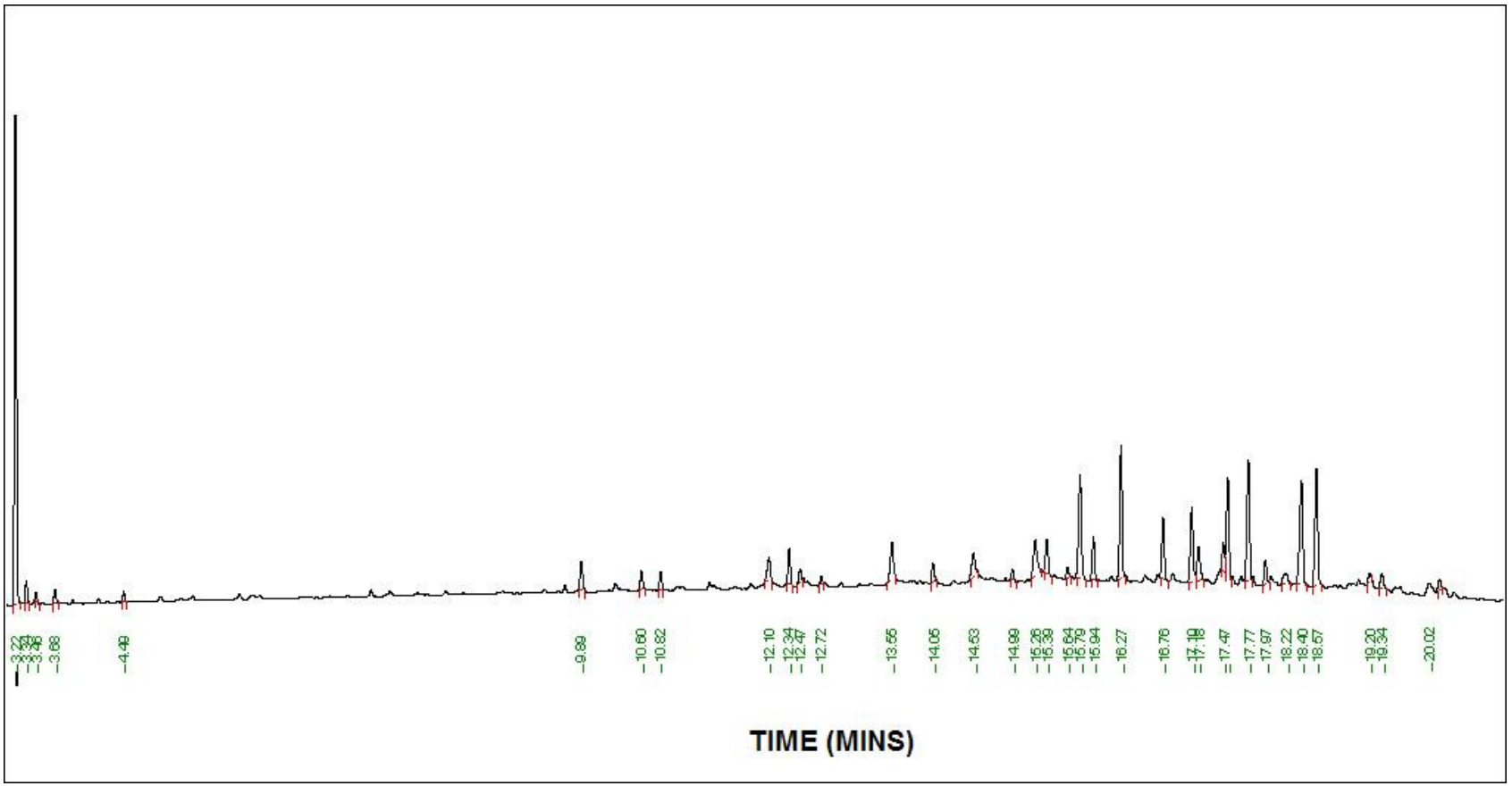


Figure E.4: GC Chromatogram for the extraction with acetone only. (*E.nitens* extracts)

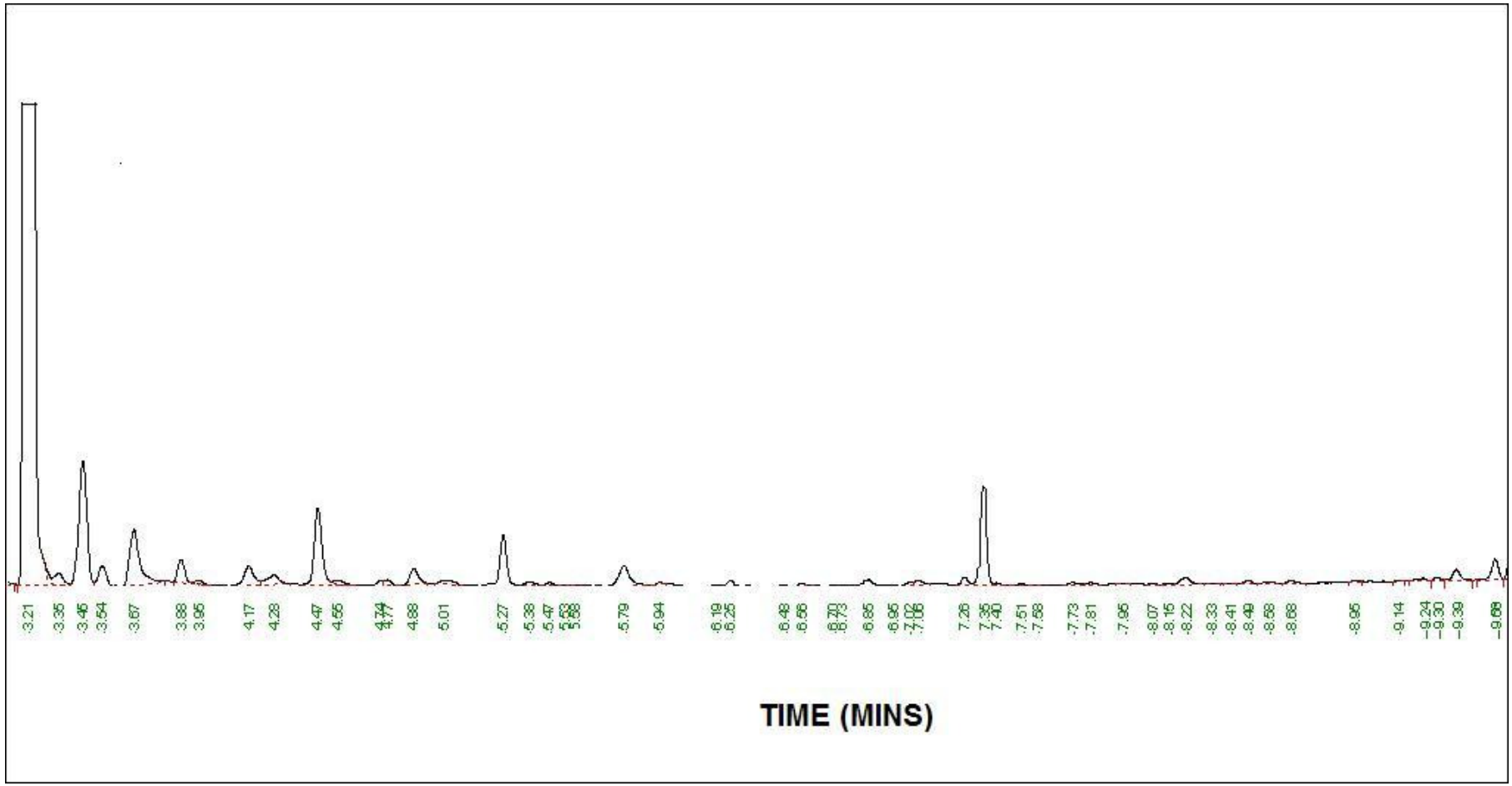


Figure E.5: GC Chromatogram for an ethanol-toluene extraction on sawdust already subjected to hot water extraction. (*E.nitens* pitch extracts)

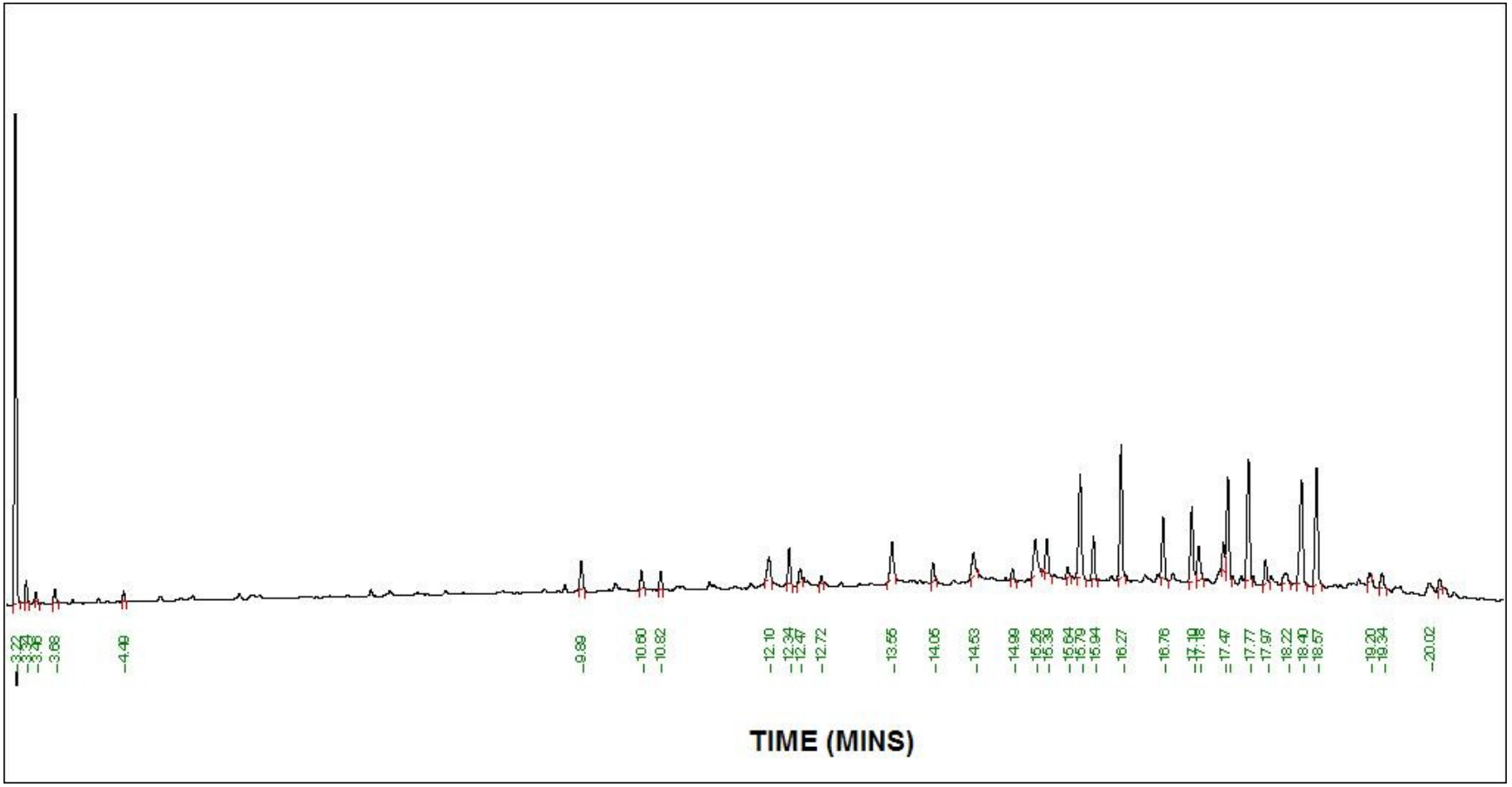


Figure E.6: GC Chromatogram for an ethanol-toluene extraction on sawdust already subjected to hot water extraction. (*E.nitens* pitch extracts)

Appendix F: Quantification of *E. grandis*, *E. nitens* and *E. nitens* pitch extracts

The table and graphs below represents the peak areas and total areas obtained during GC analysis using internal standards C_{22} fatty acid methyl esters. The calculation for the quantification is also shown. In analysis purposes 15-20 mg sub-samples were used.

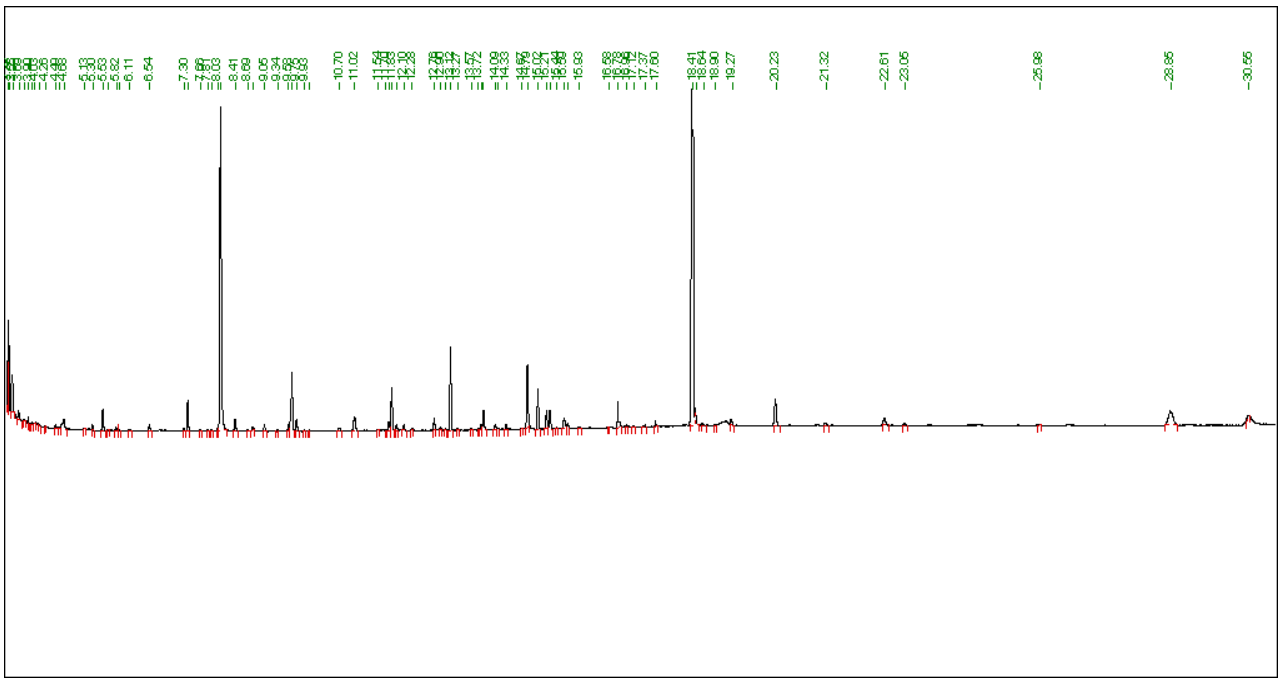


Figure F1: Chromatogram obtained with the internal standard added to the acetone

Table F1: Peak area and total areas from GC analysis for *E. grandis* extracts.

	E. grandis **(acetone) Tree 1**	***E. grandis*** **(acetone) Tree 4**	***E. grandis*** **(acetone) Tree 5**
Total area	98855.75	100052.79	102311.58
Peak area of internal standard	22226.61	13794.00	15152.03

Calculation of mass of Fatty acids and sterols present in sample:

Masses of fatty acids and sterols were calculated using the peak area of the internal standard and the total area of the sample excluding the solvent. Since the mass of the internal standard is known the mass of the fatty acids and the sterols present in the sample can be calculated. Assuming that the IS has the same response factor as the other components

Sample calculation:

Total area of the internal standard: 51172.64

Total area of all the peaks including the internal standard: 301220.12

Note that all the values were obtained from the report obtained from GC analysis

Therefore mass fatty acids and sterols: (301220.12/51172.64)×0.00725 mg/ml
: 0.0427 mg/ml in 0.5 ml (volume of IS)
: 0.0213 mg in 15-20 mg sub-samples

Table F2: Peak area and total areas from GC analysis for *E. nitens* extracts and pitch extracts.

	***E. nitens* (acetone)**	***E. nitens* (ethanol-toluene)**	**Pitch (acetone)**	**Pitch (ethanol-toluene)**
Peak area of internal standard	313045.27	300051.21	300282.29	309358.21
Total area	30032.09	103292.00	29153.01	30279.73

Calculation of mass of Fatty acids and sterols present in sample:

Masses of fatty acids and sterols were calculated using the peak area of the internal standard and the total area of the sample excluding the solvent. Since the mass of the internal standard is known the mass of the fatty acids and the sterols present in the sample can be calculated.

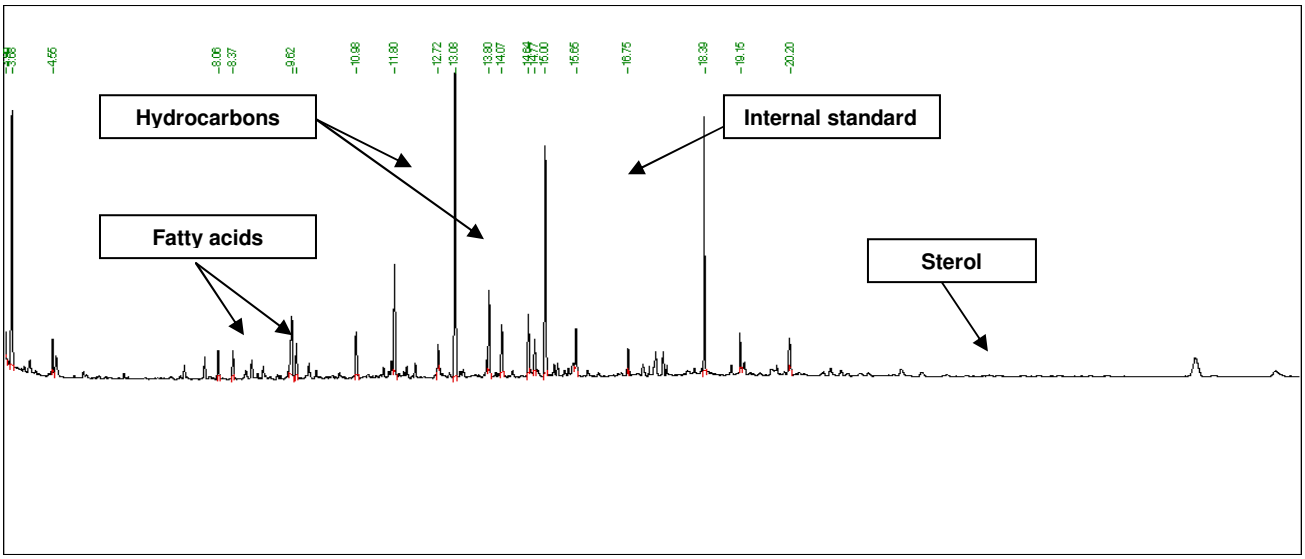


Figure F2: Chromatogram obtained with the internal standard added to the acetone and ethanol-toluene samples for *E. nitens* and pitch.

Sample calculation using *E. nitens* extract:

Area of the internal standard: 313045.27

Total area of all the peaks including the internal standard: 30032.09

Note that all the values were obtained from the report obtained from GC analysis.

Therefore mass of fatty acids and sterols: (313045.27/30032.09)×0.00725 mg/ml
: 0.0756 mg/ml in 0.5 ml (volume of IS)
: 0.0378 mg in 15-20 mg sub-samples

Table F3: Summary of the mass results obtained for *E. nitens* sawdust and *E. nitens* pitch.

Extraction Method	Sample	Total extractives, mg	Total Fatty acids, mg	Total Hydrocarbons, mg
Ethanol-toluene extraction on sawdust already subjected to hot water extraction.	Wood meal	0.0145	0.0105	0.004
Acetone extraction only		0.064	0.0378	0.026
Acetone extraction only	Pitch	0.337	0.0373	0.300
Ethanol-toluene extraction on sawdust already subjected to hot water extraction.		0.428	0.0370	0.391

Table F4: Data collected during the quantification of E. *grandis.* 15-20 mg sub-samples were used for the determination of the mass of extractives.

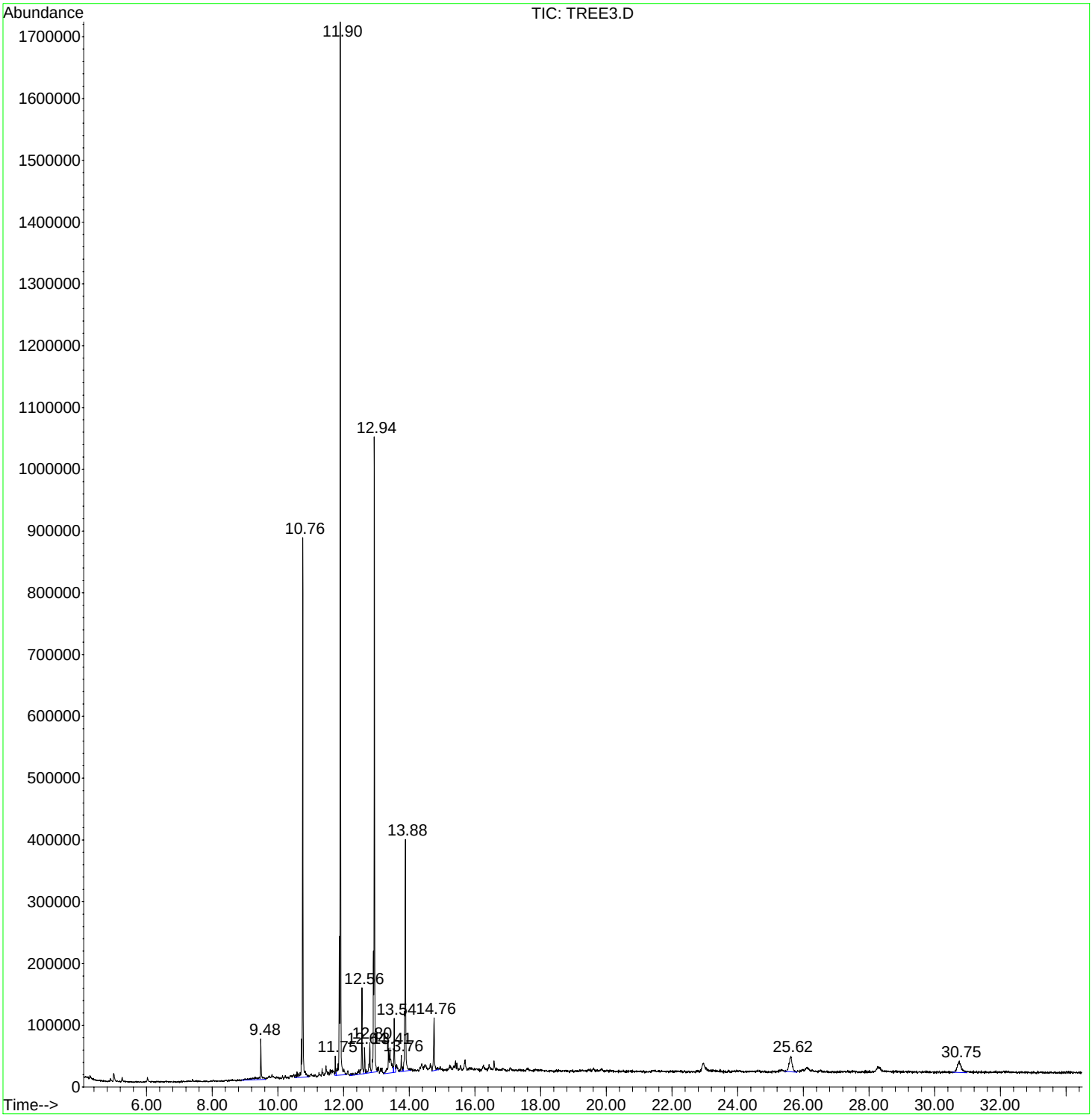
Extraction Method	Total extractives, mg	Total Fatty acids, mg	Total Hydrocarbons, mg
Ethanol-toluene extraction on sawdust already subjected to hot water extraction.	1.0007	0.1277	0.8730
Acetone extraction on sawdust already subjected to hot water extraction.	0.3236	0.1159	0.2077
Acetone extraction only	0.9955	0.0213	0.9742

Appendix G: Gas Chromatography-Mass Spectroscopy

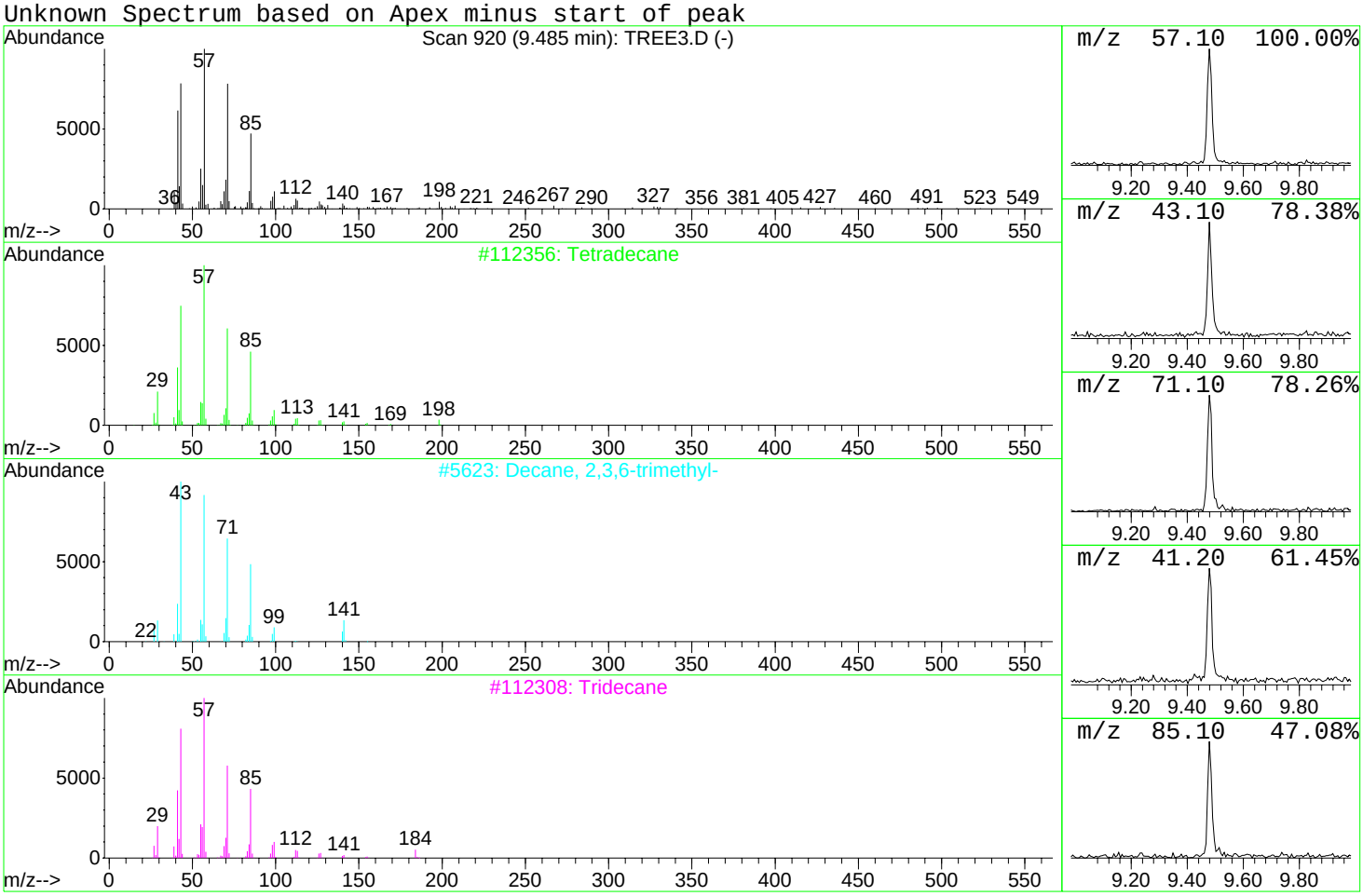
Library Search Report

Data File : D:\PRENISHA\TREE3.D Vial: 26
Acq On : 16 May 2006 8:39 Operator: Bret
Sample : Tree 3 Alcona Inst : Instrumen
Misc : 1µl inject, split 1:75, methanol Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e
Method : C:\MSDCHEM\1\METHODS\NEW.M (Chemstation Integrator)
Title :



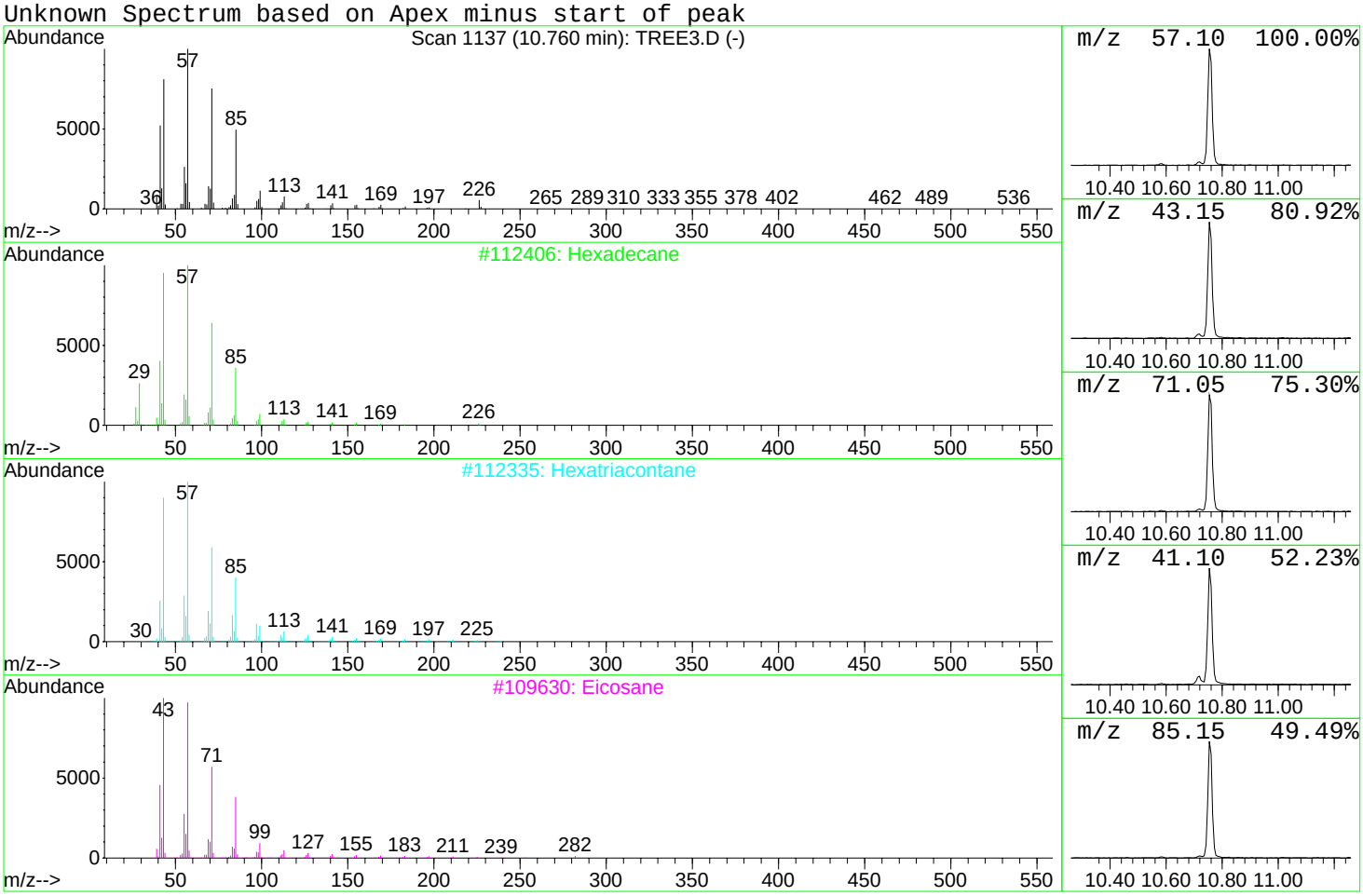
Unknown Spectrum based on Apex minus start of peak



Peak Number: 1 at 9.48 min Area: 1456893 Area % 2.18

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Tetradecane	112356	000629-59-4	96
2 Decane, 2,3,6-trimethyl-	5623	062238-12-4	86
3 Tridecane	112308	000629-50-5	86

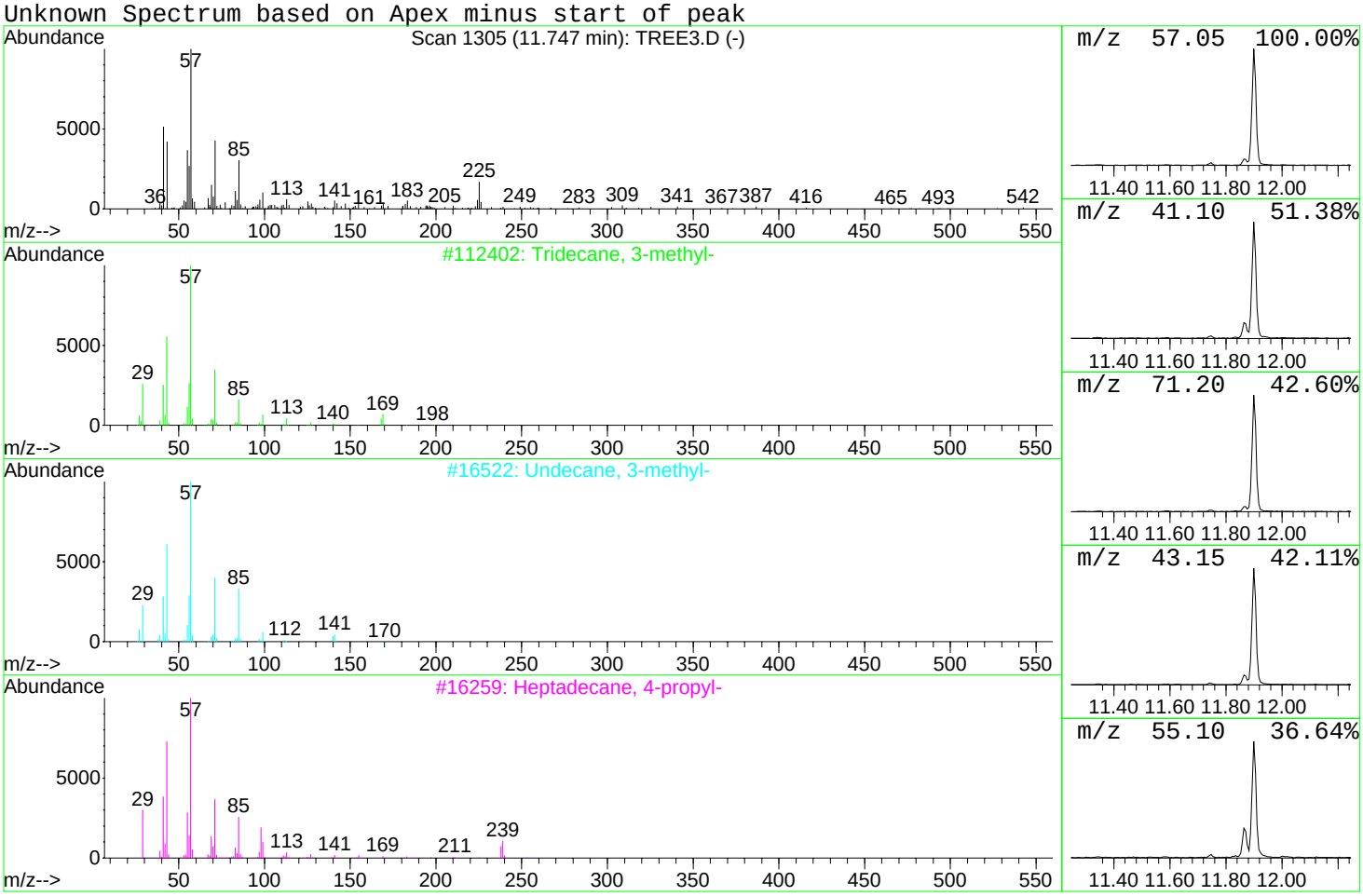
Unknown Spectrum based on Apex minus start of peak



Peak Number: 2 at 10.76 min Area: 11233839 Area % 16.78

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Hexadecane	112406	000544-76-3	97
2 Hexatriacontane	112335	000630-06-8	91
3 Eicosane	109630	000112-95-8	91

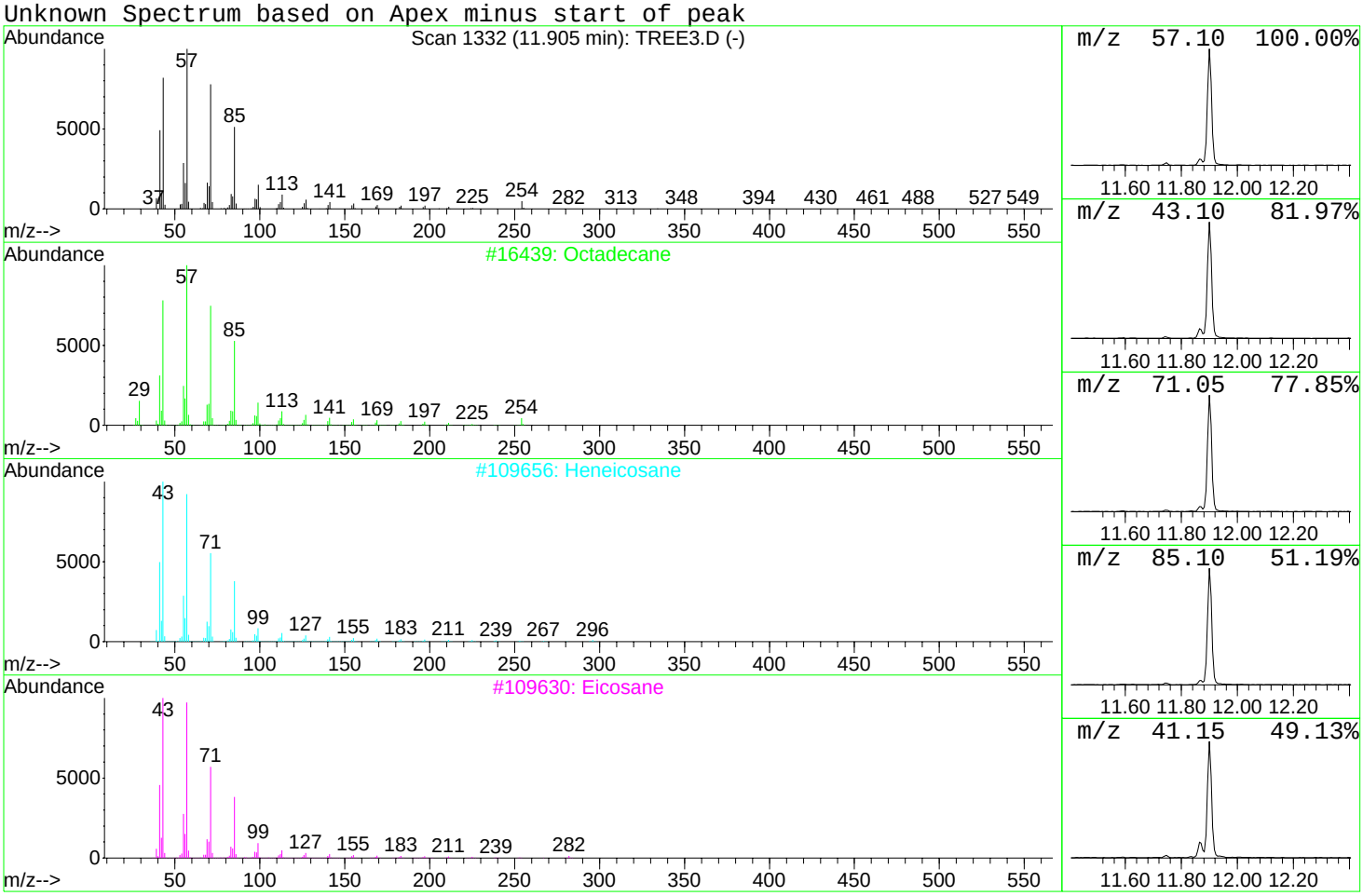
Unknown Spectrum based on Apex minus start of peak



Peak Number: 3 at 11.75 min Area: 360358 Area % 0.54

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Tridecane, 3-methyl-	112402	006418-41-3	64
2 Undecane, 3-methyl-	16522	001002-43-3	64
3 Heptadecane, 4-propyl-	16259	055044-10-5	64

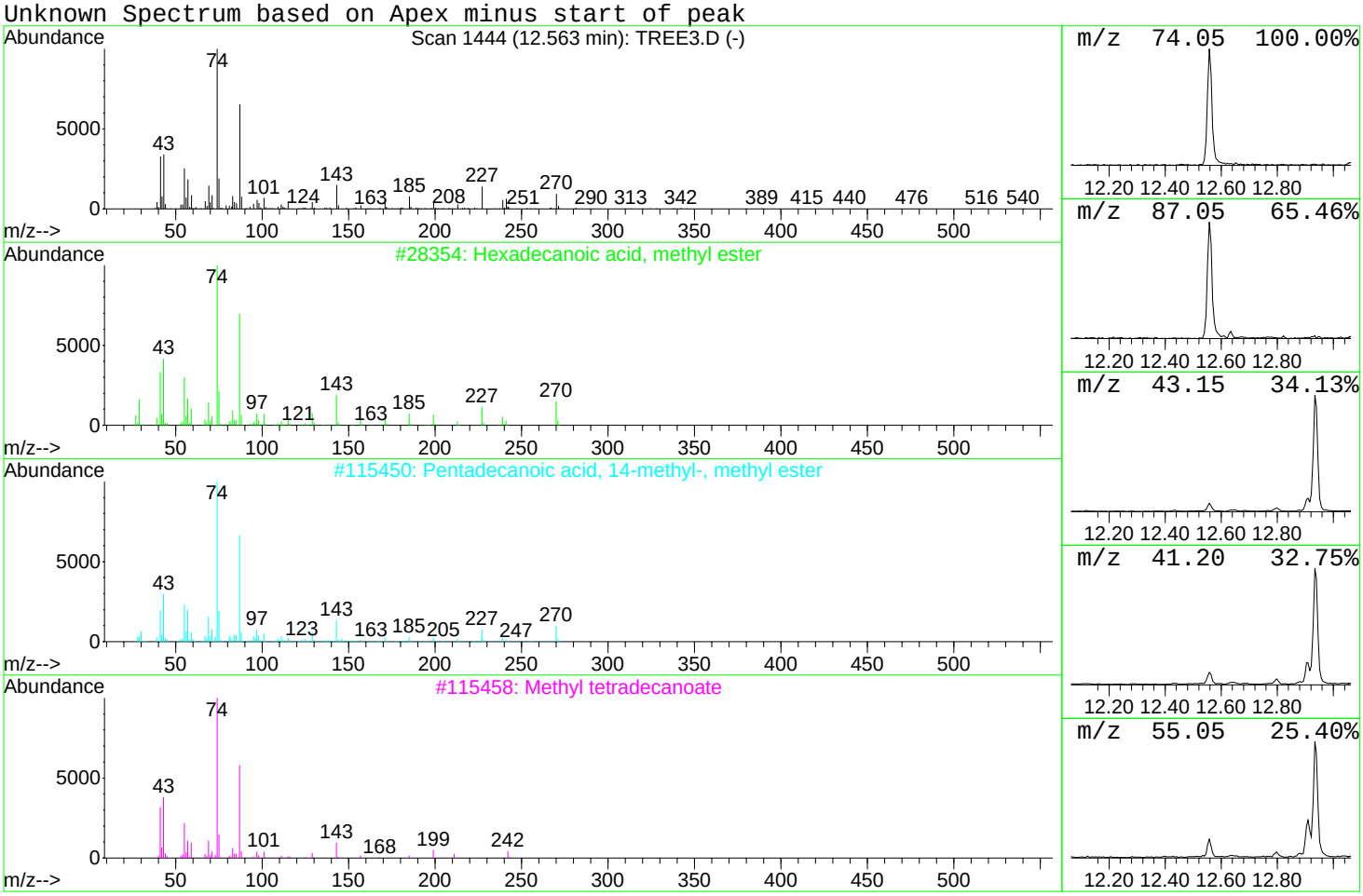
Unknown Spectrum based on Apex minus start of peak



Peak Number: 4 at 11.91 min Area: 21450082 Area % 32.05

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Octadecane	16439	000593-45-3	99
2 Heneicosane	109656	000629-94-7	97
3 Eicosane	109630	000112-95-8	93

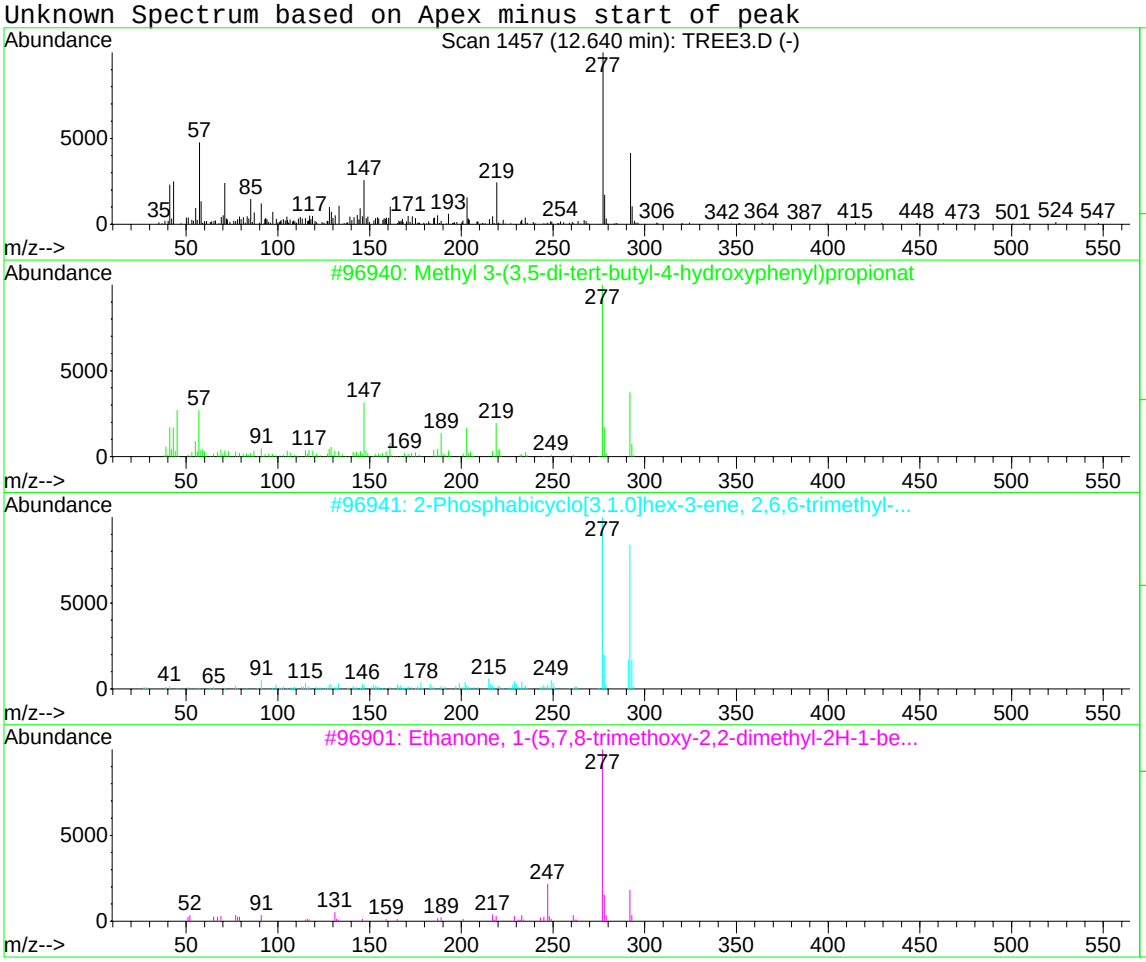
Unknown Spectrum based on Apex minus start of peak

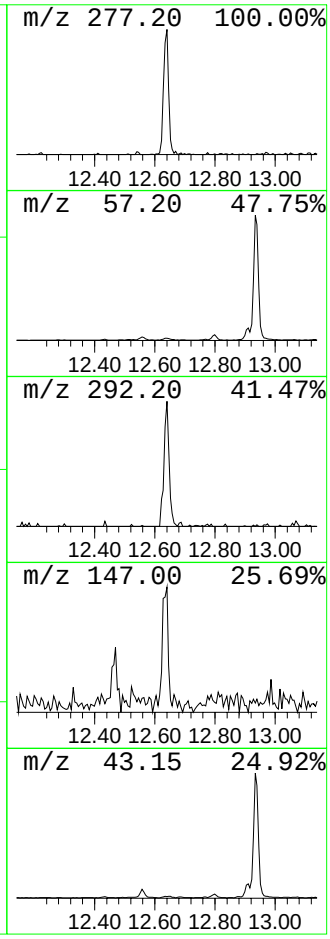


Peak Number: 5 at 12.56 min Area: 2246581 Area % 3.36

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Hexadecanoic acid, methyl ester	28354	000112-39-0	97
2 Pentadecanoic acid, 14-methyl-, ...	115450	005129-60-2	96
3 Methyl tetradecanoate	115458	000124-10-7	94

Unknown Spectrum based on Apex minus start of peak

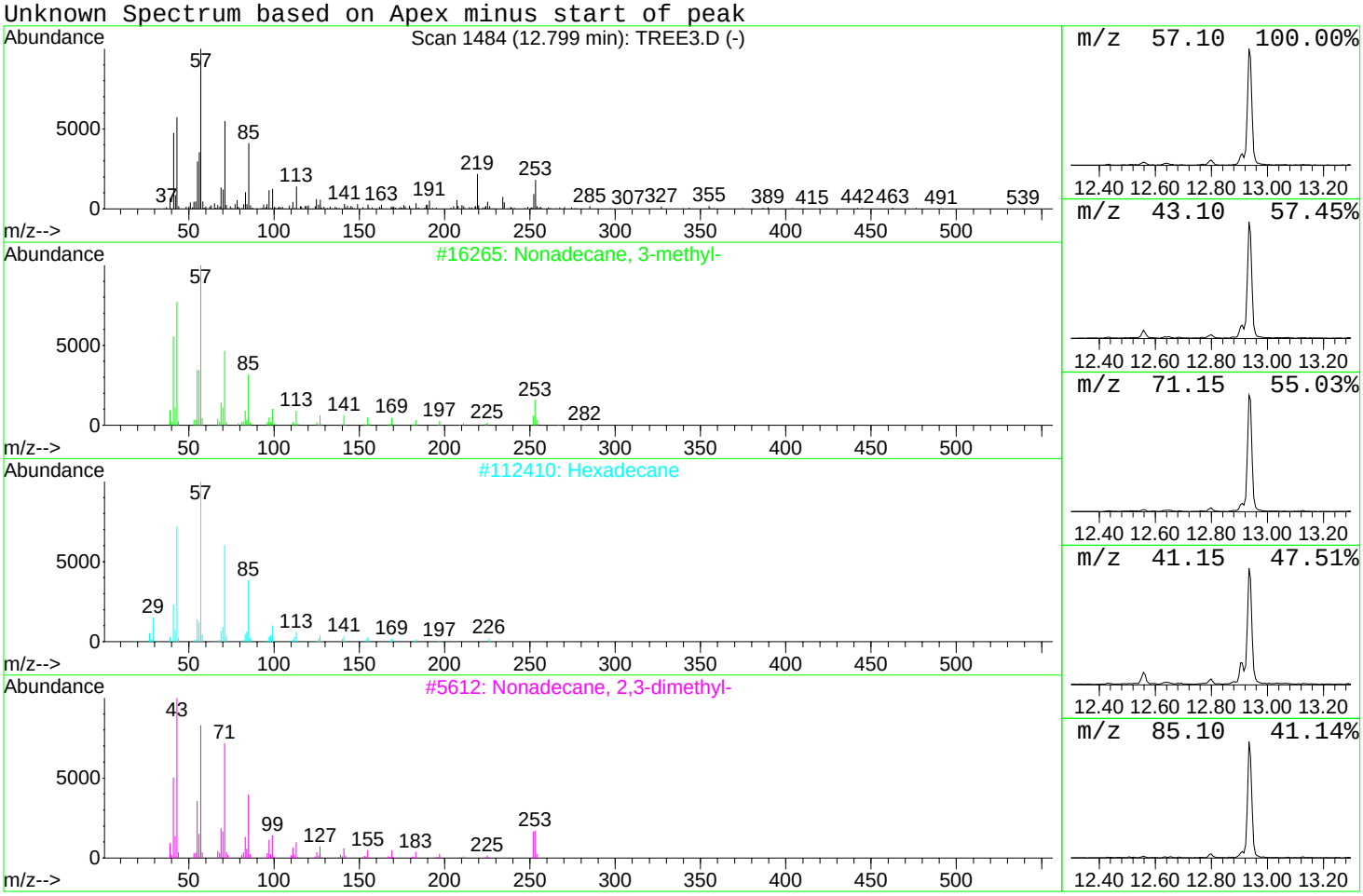




Peak Number: 6 at 12.64 min Area: 698143 Area % 1.04

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Methyl 3-(3,5-di-tert-butyl-4-hy...	96940	1000245-76-2	93
2 2-Phosphabicyclo[3.1.0]hex-3-ene...	96941	1000161-75-2	53
3 Ethanone, 1-(5,7,8-trimethoxy-2,...	96901	000482-07-5	37

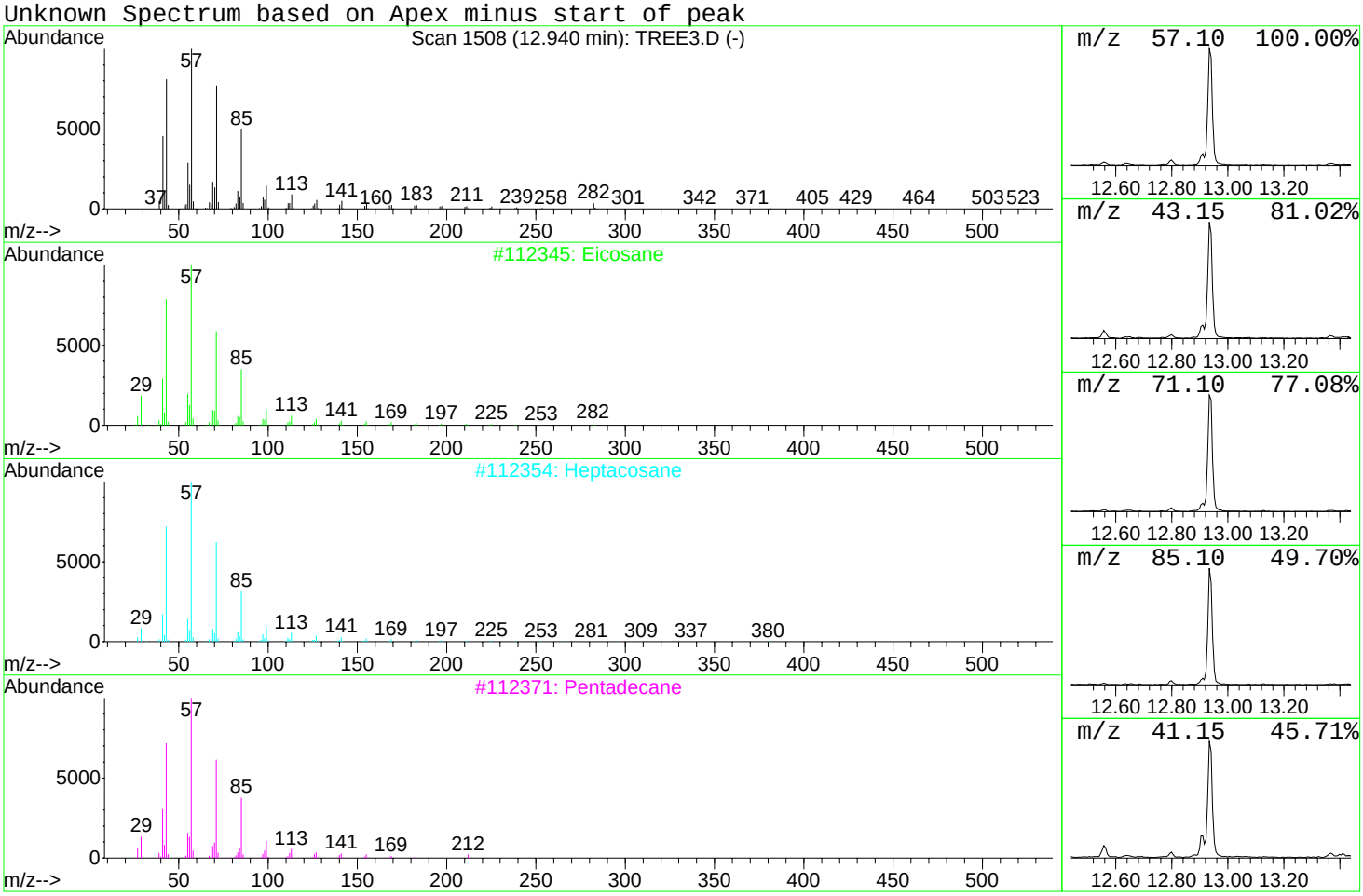
Unknown Spectrum based on Apex minus start of peak



Peak Number: 7 at 12.80 min Area: 875056 Area % 1.31

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Nonadecane, 3-methyl-	16265	006418-45-7	64
2 Hexadecane	112410	000544-76-3	60
3 Nonadecane, 2,3-dimethyl-	5612	075163-99-4	60

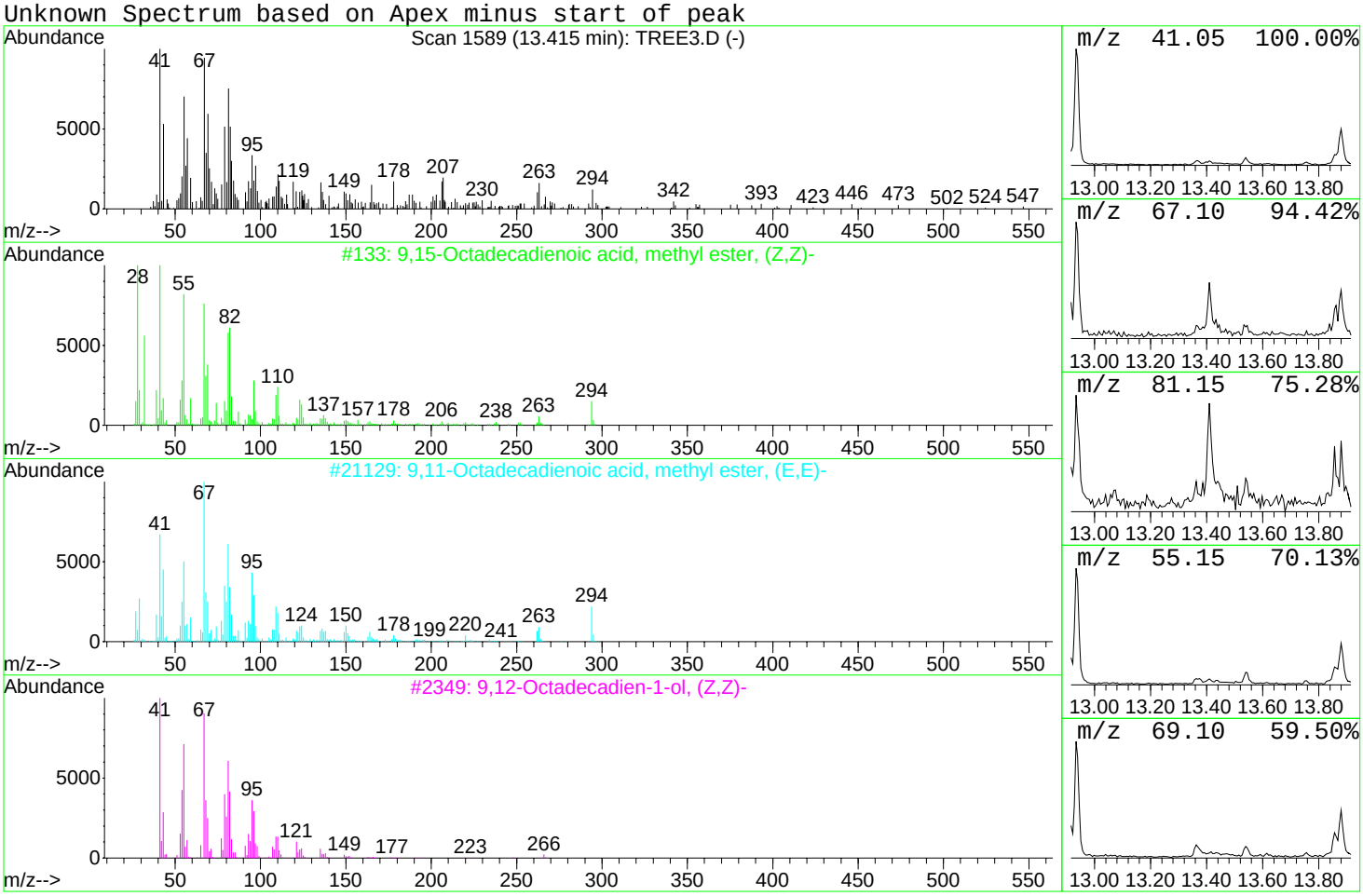
Unknown Spectrum based on Apex minus start of peak



Peak Number: 8 at 12.94 min Area: 14178344 Area % 21.18

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Eicosane	112345	000112-95-8	98
2 Heptacosane	112354	000593-49-7	98
3 Pentadecane	112371	000629-62-9	94

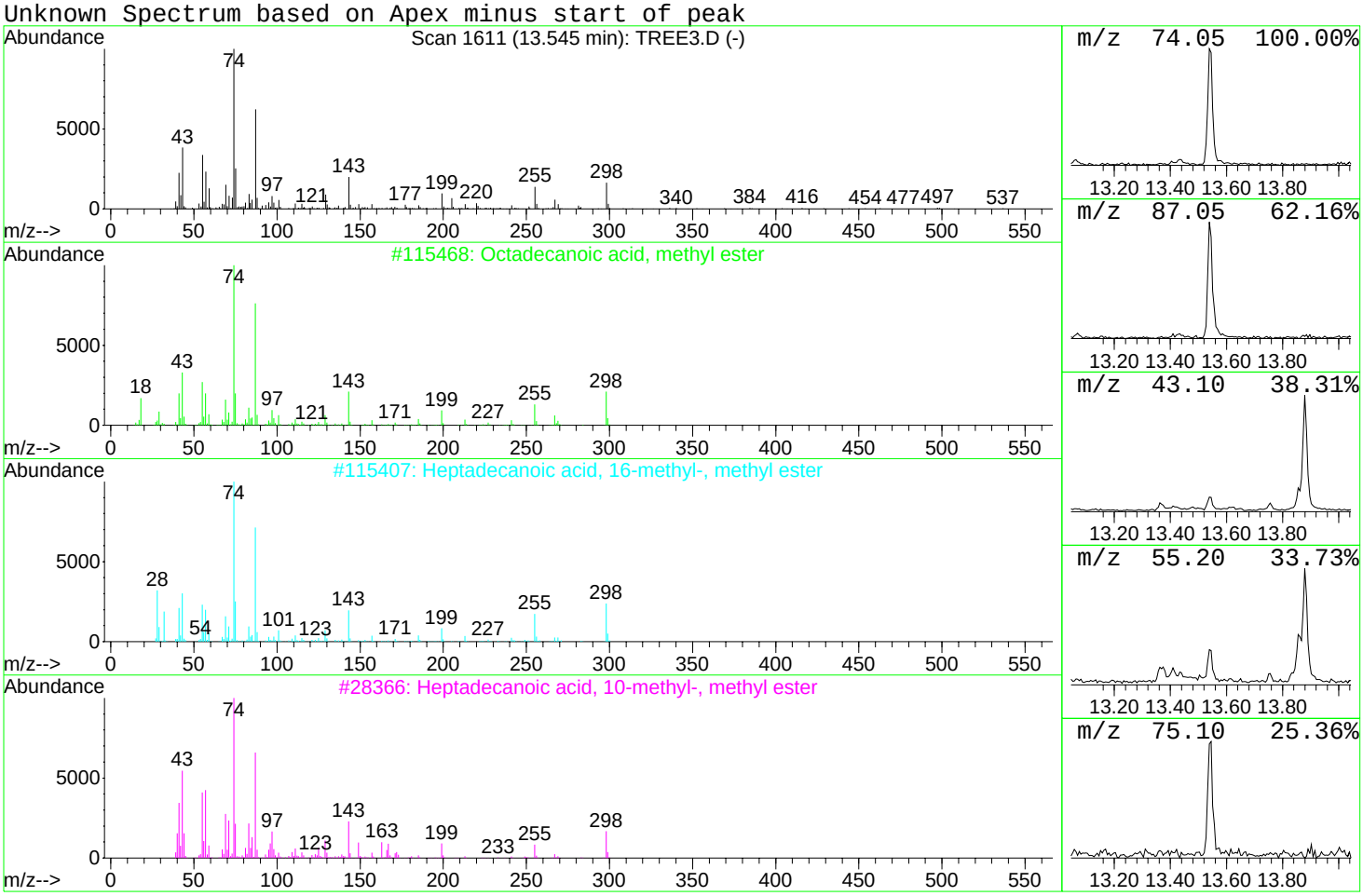
Unknown Spectrum based on Apex minus start of peak



Peak Number: 9 at 13.42 min Area: 2286530 Area % 3.42

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 9,15-Octadecadienoic acid, methy...	133	017309-05-6	60
2 9,11-Octadecadienoic acid, methy...	21129	013038-47-6	56
3 9,12-Octadecadien-1-ol, (Z,Z)-	2349	000506-43-4	50

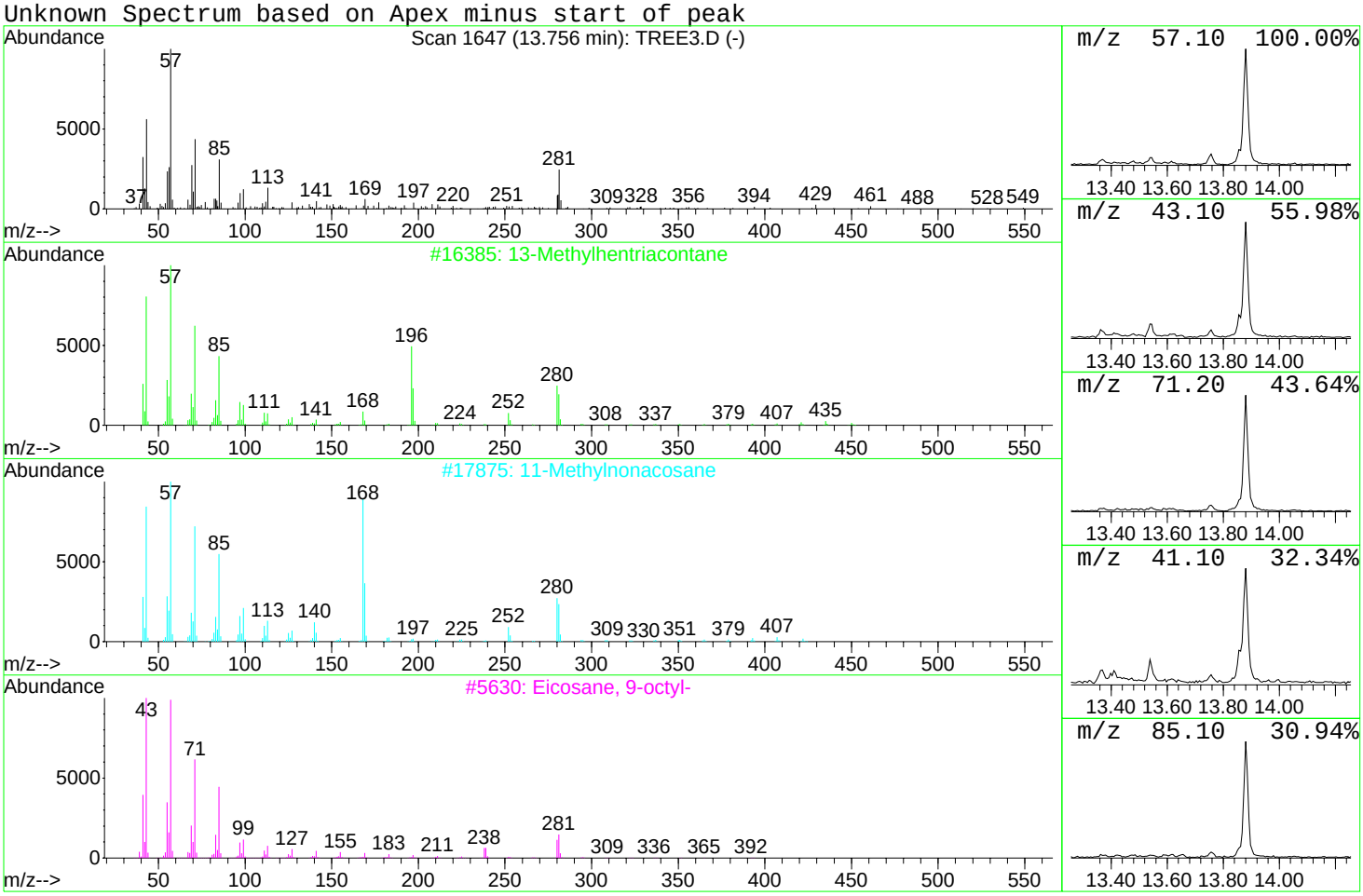
Unknown Spectrum based on Apex minus start of peak



Peak Number: 10 at 13.54 min Area: 1267793 Area % 1.89

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Octadecanoic acid, methyl ester	115468	000112-61-8	95
2 Heptadecanoic acid, 16-methyl-, ...	115407	005129-61-3	94
3 Heptadecanoic acid, 10-methyl-, ...	28366	002490-25-7	90

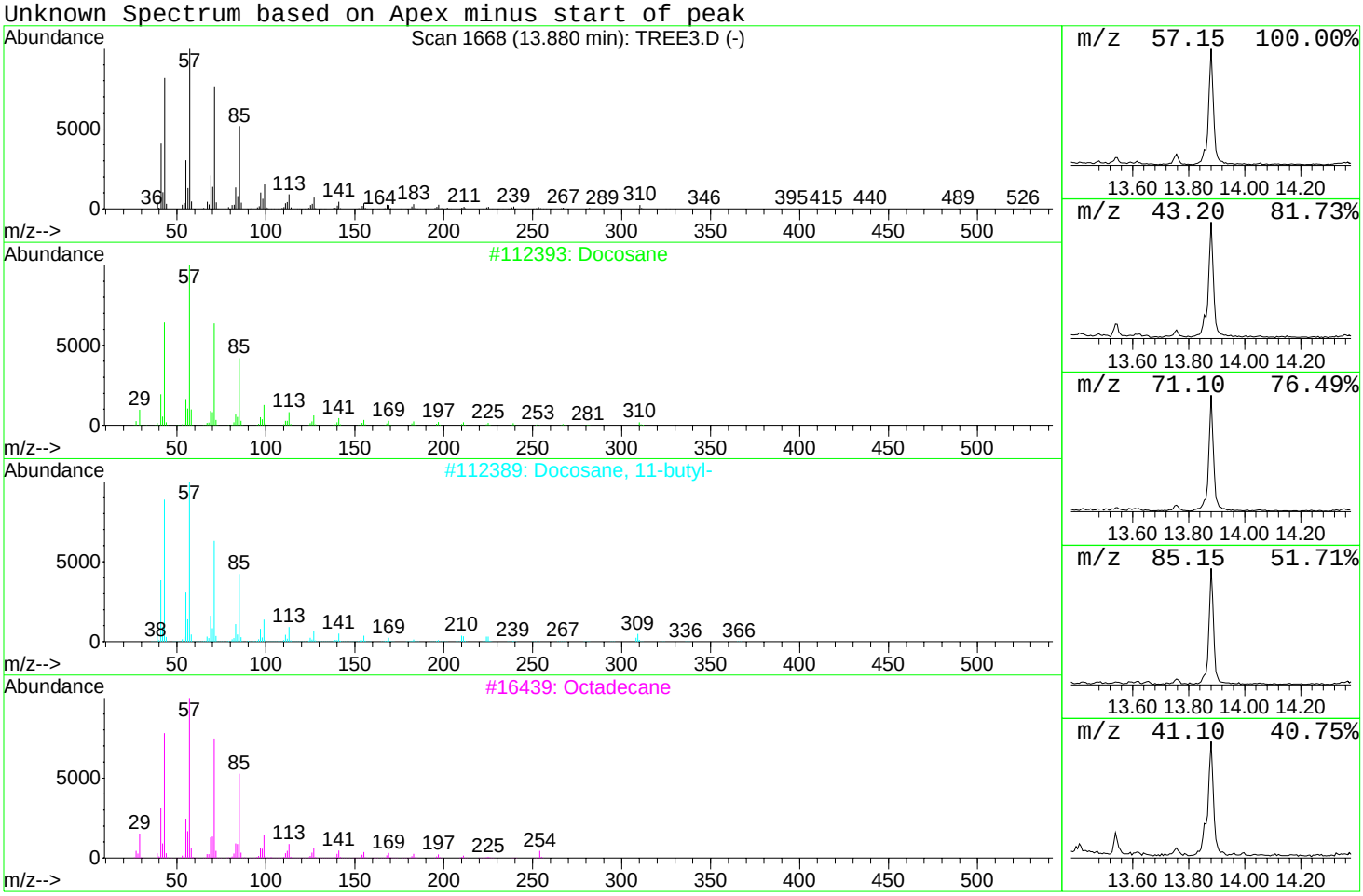
Unknown Spectrum based on Apex minus start of peak



Peak Number: 11 at 13.76 min Area: 468444 Area % 0.70

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 13-Methylhentriacontane	16385	1000131-19-4	59
2 11-Methylnonacosane	17875	1000131-18-7	50
3 Eicosane, 9-octyl-	5630	013475-77-9	50

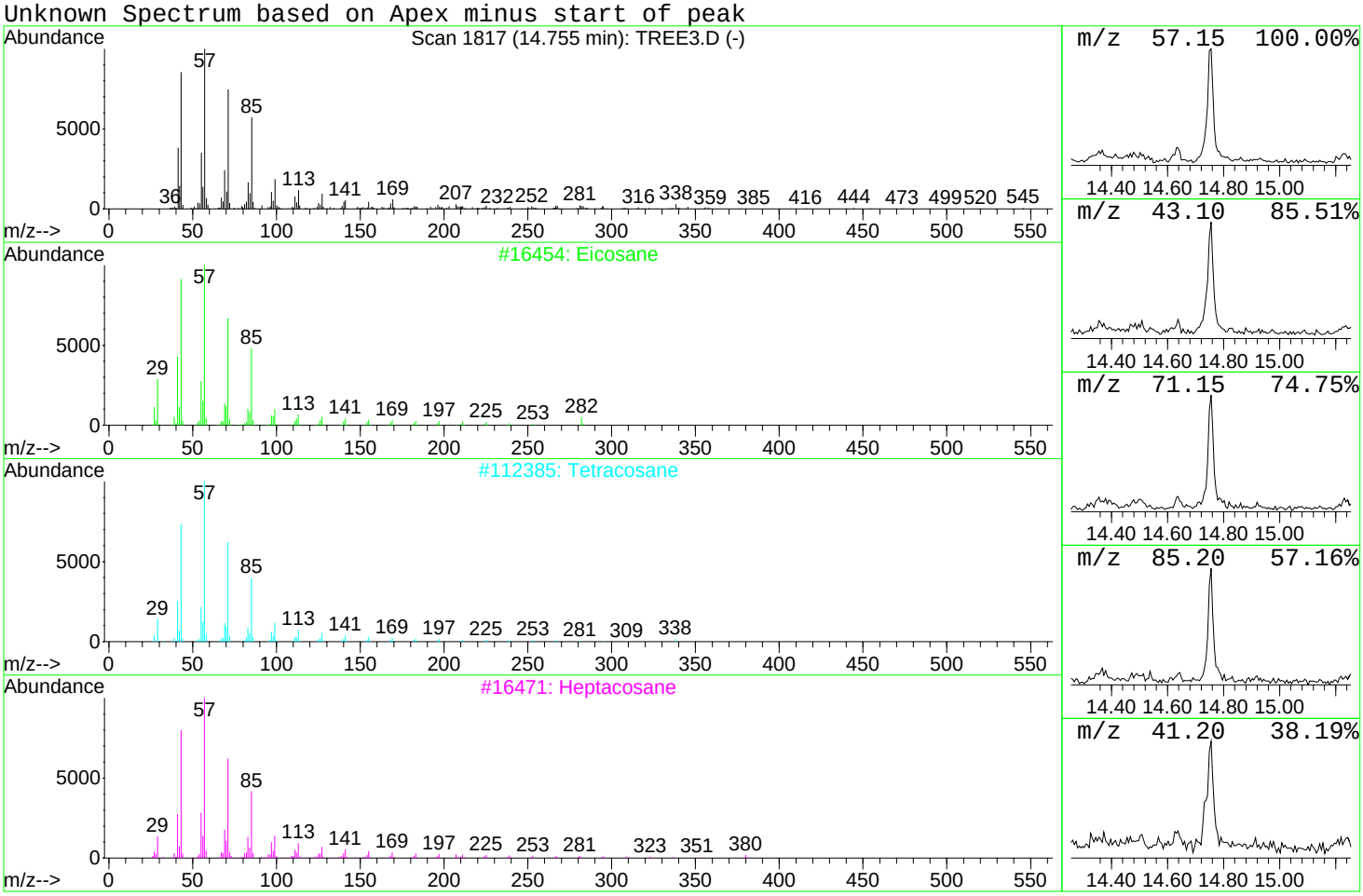
Unknown Spectrum based on Apex minus start of peak



Peak Number: 12 at 13.88 min Area: 5679917 Area % 8.49

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Docosane	112393	000629-97-0	94
2 Docosane, 11-butyl-	112389	013475-76-8	91
3 Octadecane	16439	000593-45-3	91

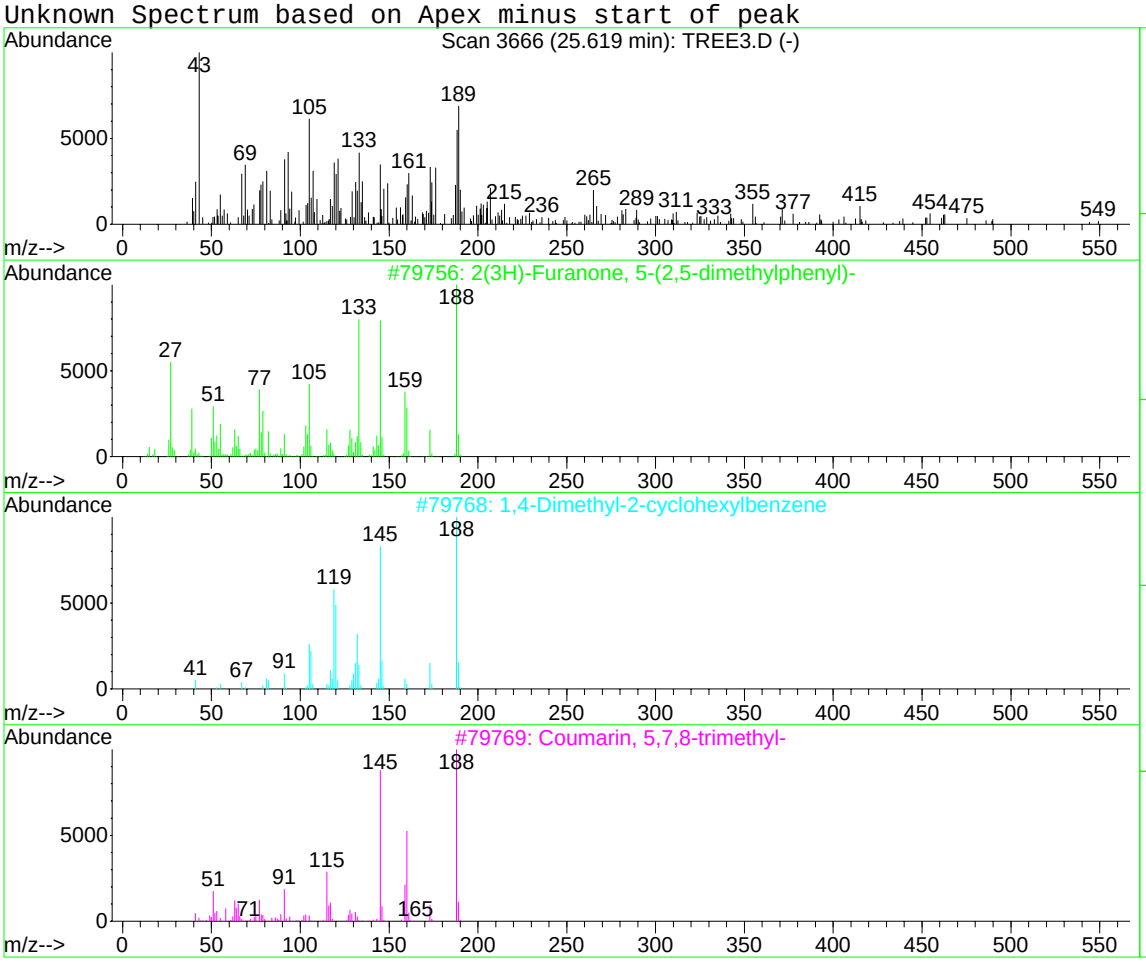
Unknown Spectrum based on Apex minus start of peak

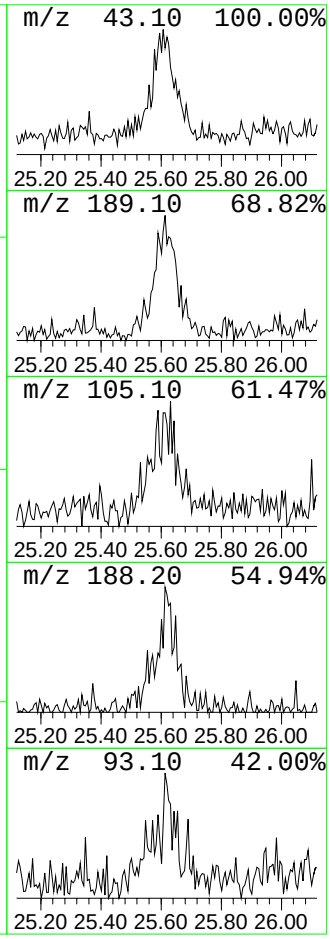


Peak Number: 13 at 14.76 min Area: 1648783 Area % 2.46

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Eicosane	16454	000112-95-8	97
2 Tetracosane	112385	000646-31-1	96
3 Heptacosane	16471	000593-49-7	94

Unknown Spectrum based on Apex minus start of peak

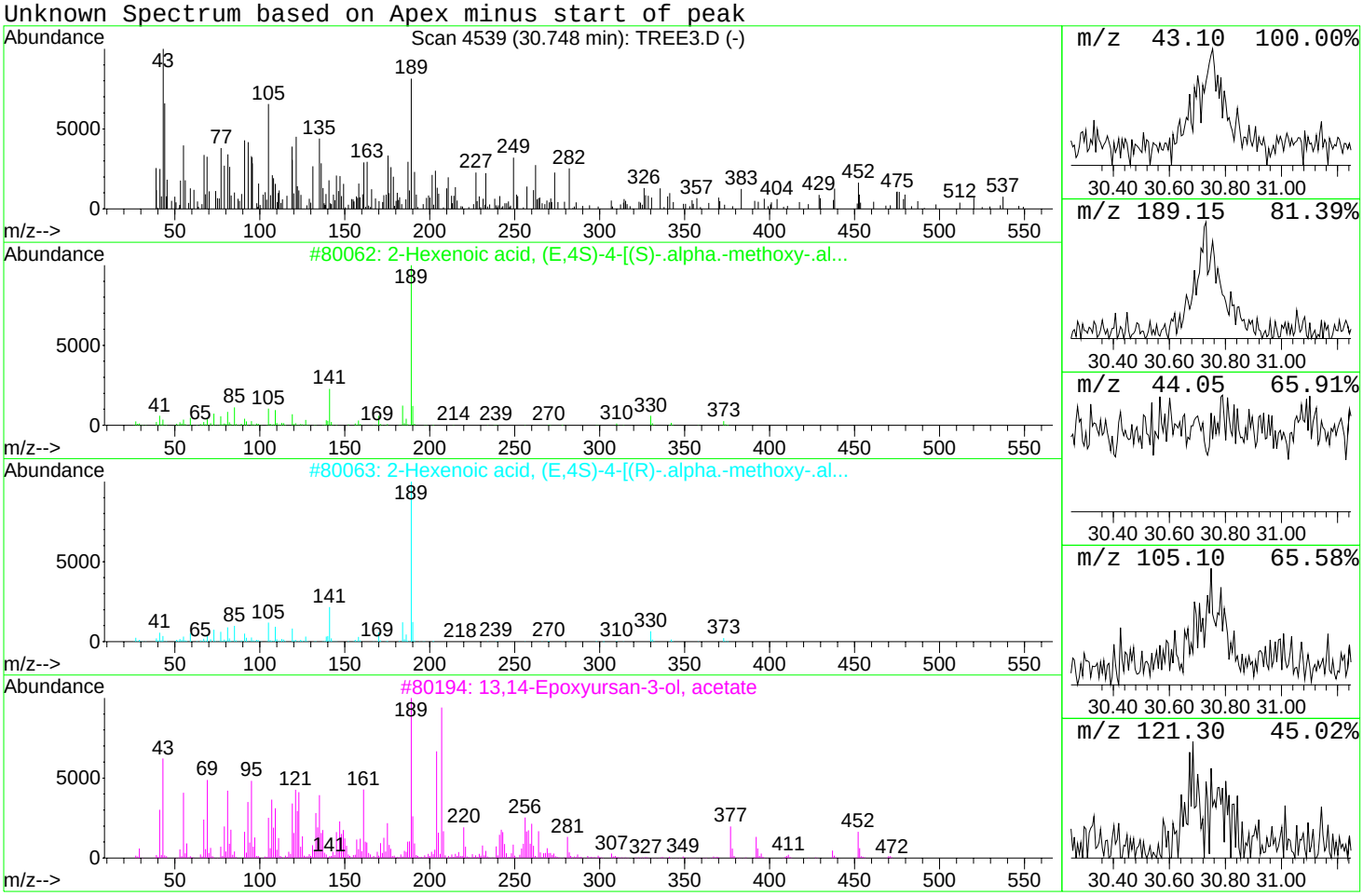




Peak Number: 14 at 25.62 min Area: 1587973 Area % 2.37

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2(3H)-Furanone, 5-(2,5-dimethylp...	79756	055669-87-9	49
2 1,4-Dimethyl-2-cyclohexylbenzene	79768	004501-52-4	30
3 Coumarin, 5,7,8-trimethyl-	79769	1000132-62-2	30

Unknown Spectrum based on Apex minus start of peak



Peak Number: 15 at 30.75 min Area: 1492755 Area % 2.23

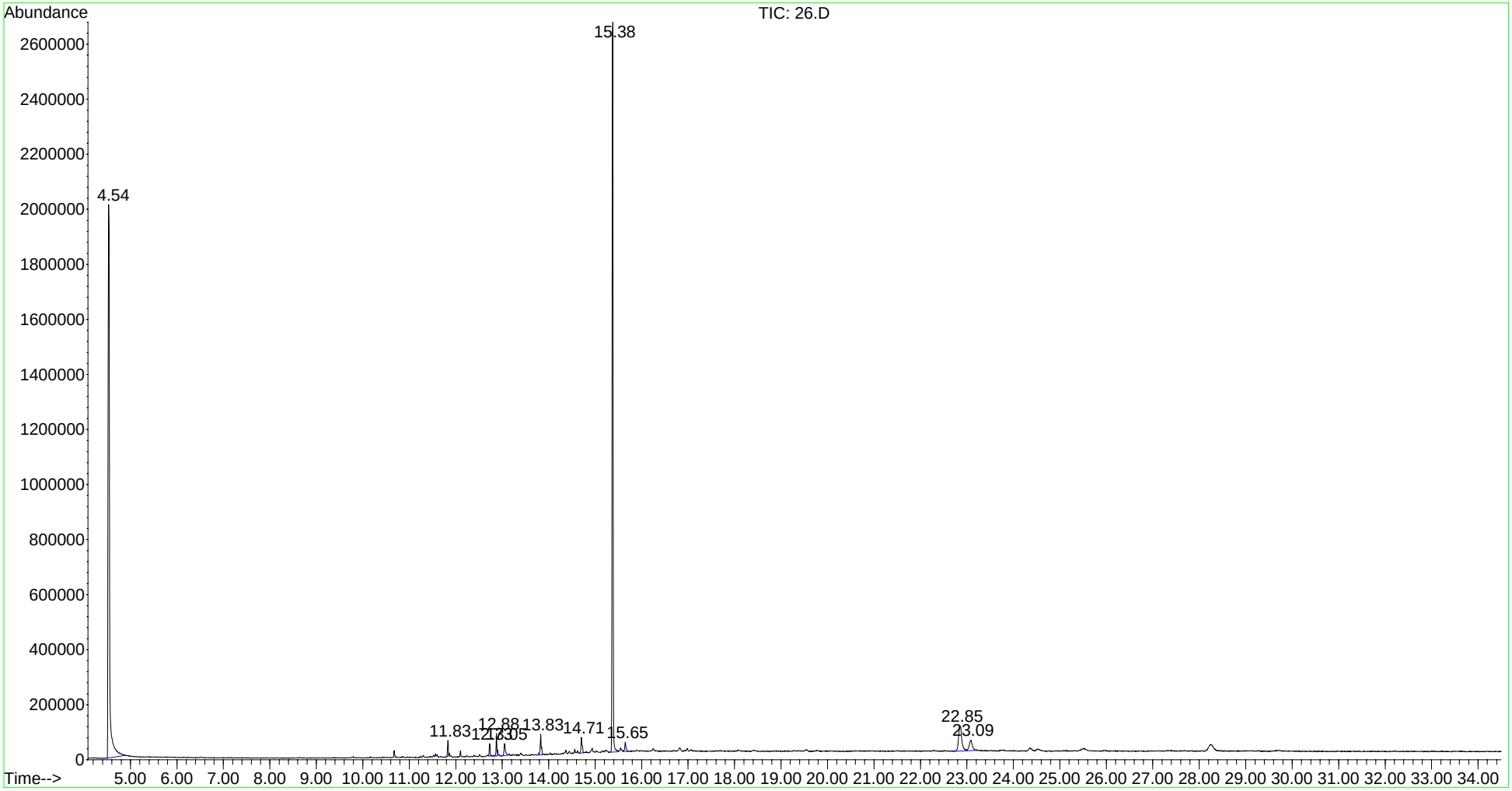
The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Hexenoic acid, (E,4S)-4-[(S)-....	80062	1000163-90-2	38
2 2-Hexenoic acid, (E,4S)-4-[(R)-....	80063	1000163-90-1	38
3 13,14-Epoxyursan-3-ol, acetate	80194	1000188-40-5	37

Library Search Report

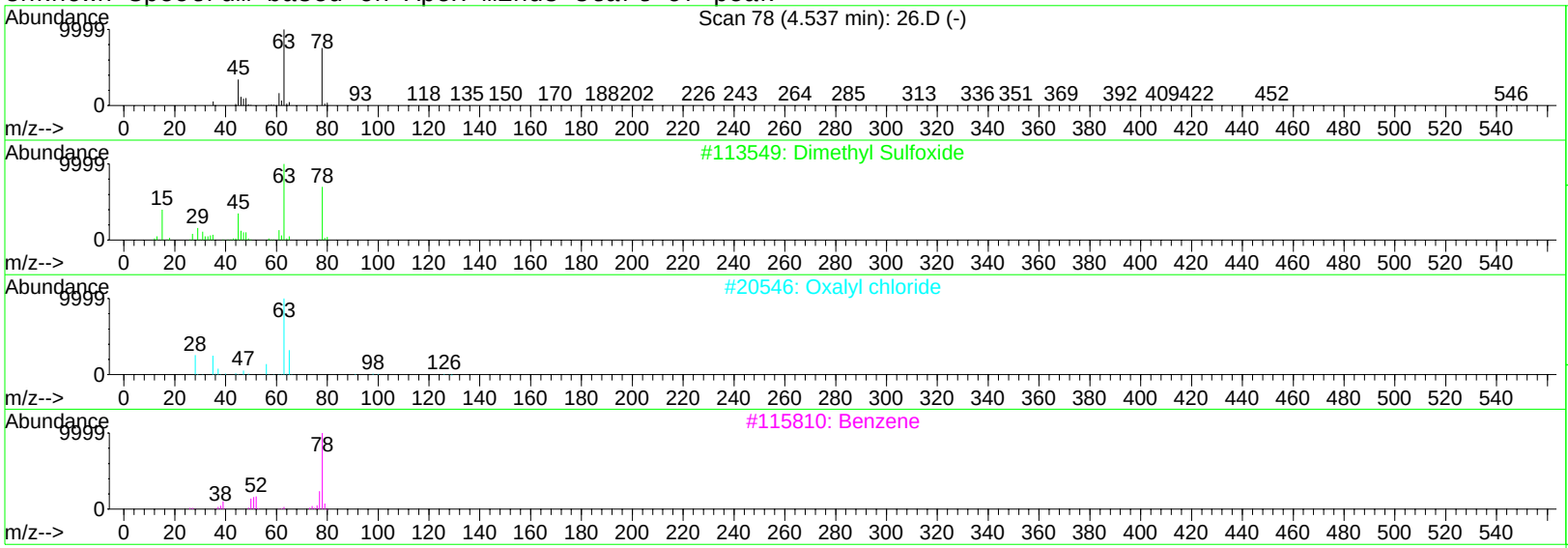
Data File : D:\PRENISHA\300\26.D
Acq On : 23 Aug 2007 9:54
Sample : Clear Extract in alcohol
Misc : 1µl inject, ethanol, splitless

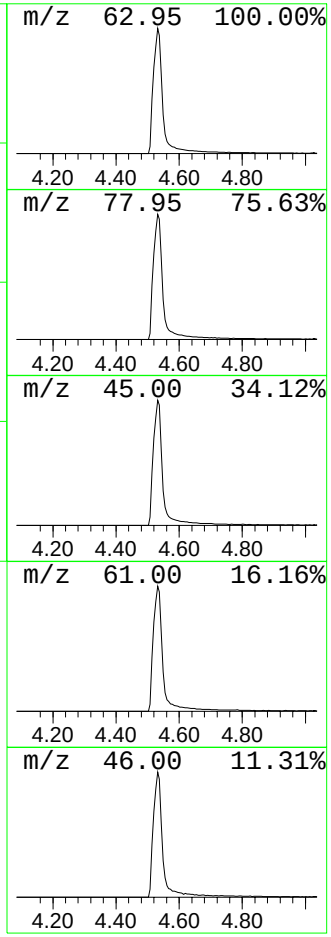
Vial: 26
Operator: Prenisha
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e
Method : C:\MSDCHEM\1\METHODS\NEW.M (Chemstation Integrator)
Title :



Unknown Spectrum based on Apex minus start of peak

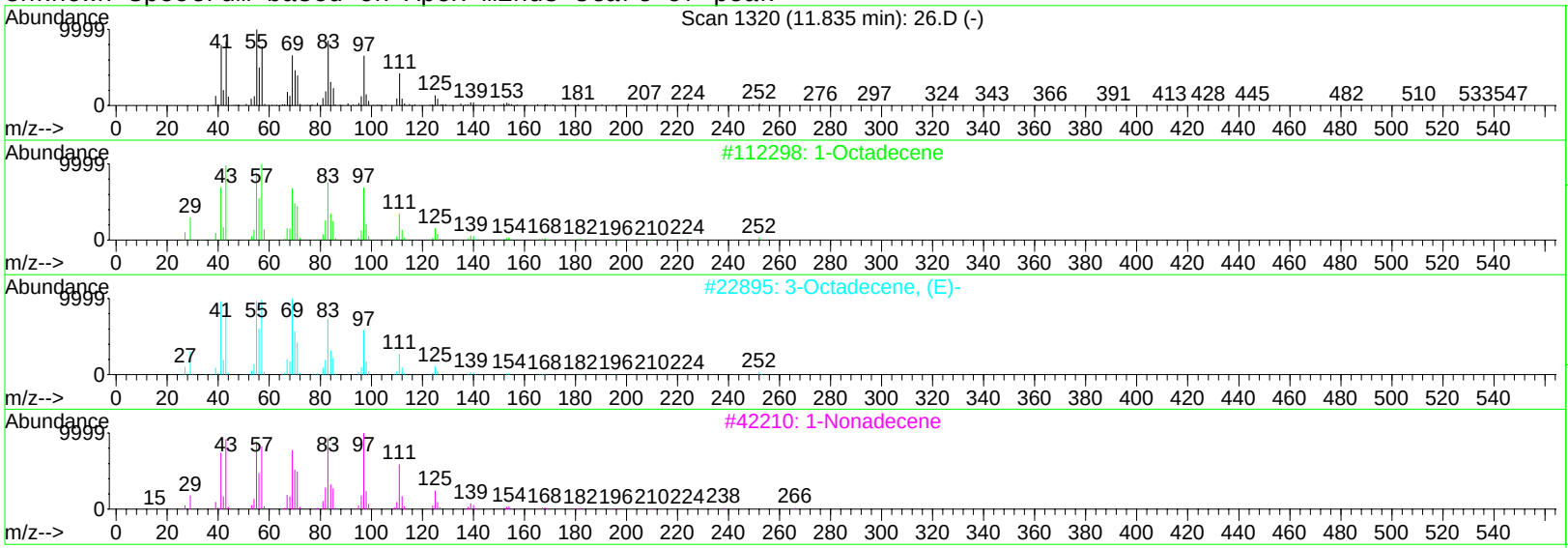


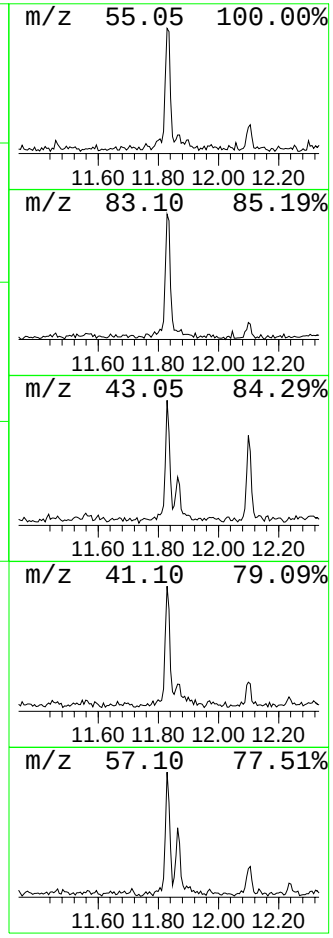


Peak Number: 1 at 4.54 min Area: 42410126 Area % 47.46

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Dimethyl Sulfoxide	113549	000067-68-5	94
2 Oxalyl chloride	20546	000079-37-8	9
3 Benzene	115810	000071-43-2	4

Unknown Spectrum based on Apex minus start of peak

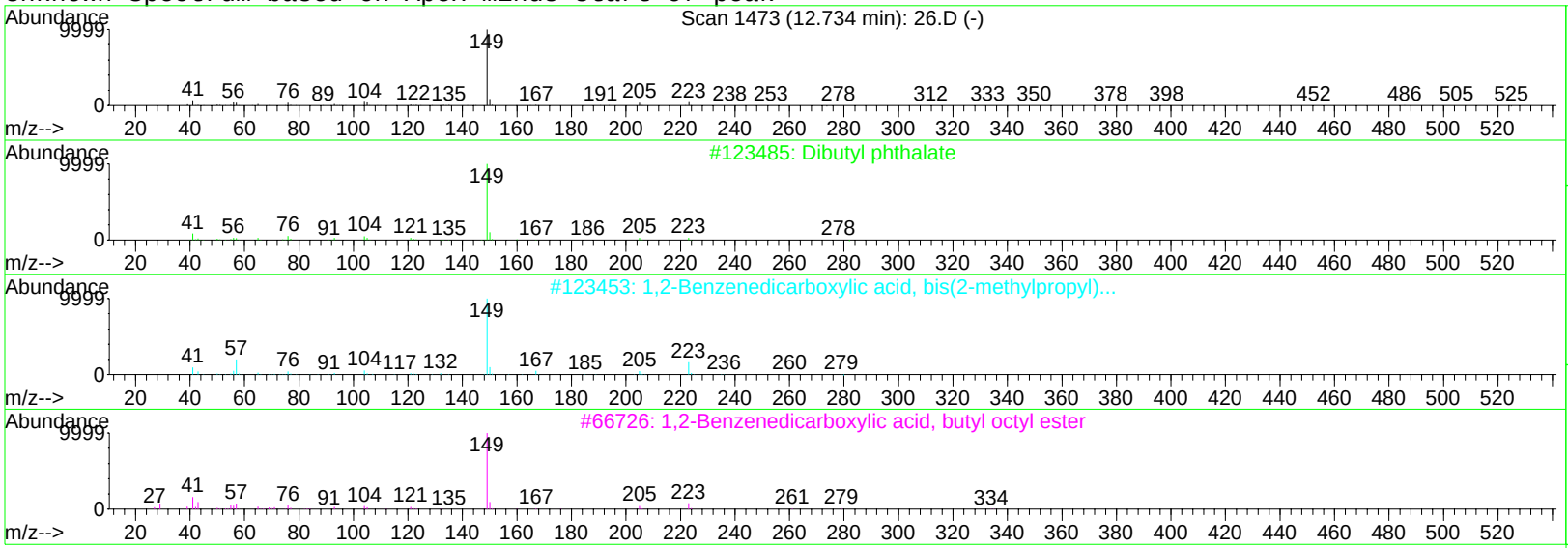


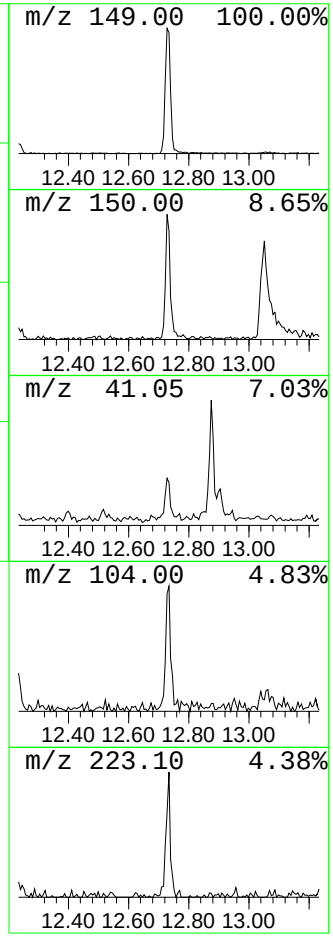


Peak Number: 2 at 11.83 min Area: 592637 Area % 0.66

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1-Octadecene	112298	000112-88-9	99
2 3-Octadecene, (E)-	22895	007206-19-1	96
3 1-Nonadecene	42210	018435-45-5	94

Unknown Spectrum based on Apex minus start of peak

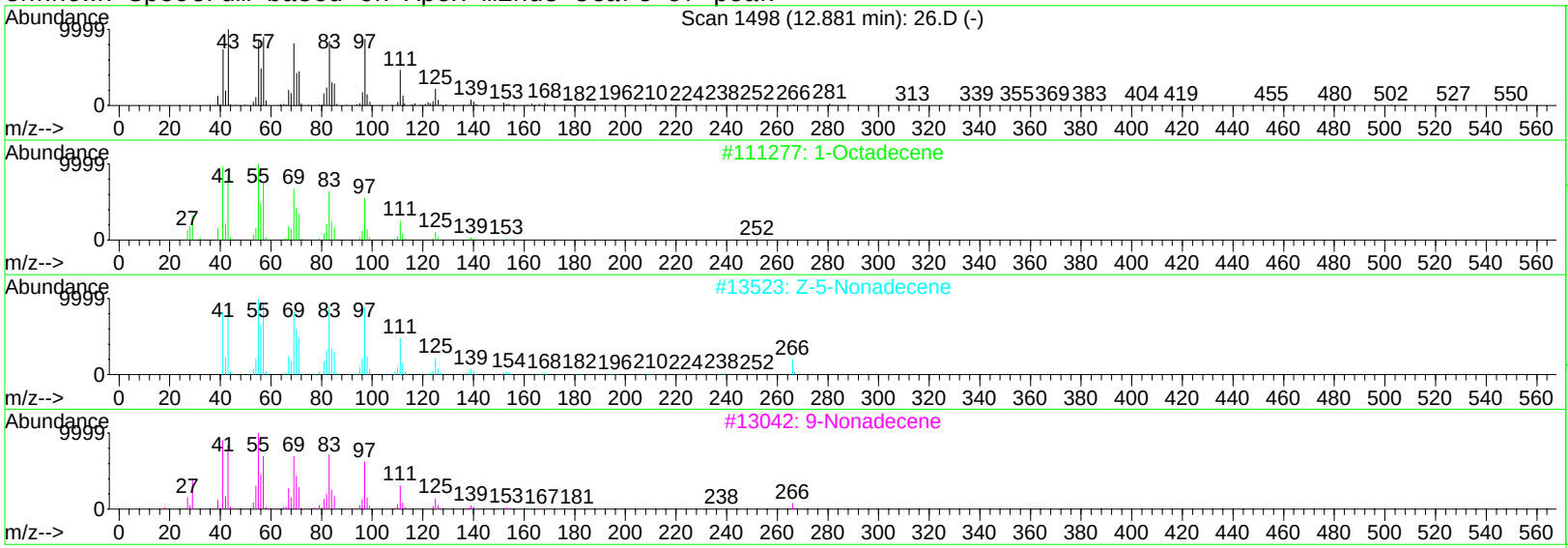


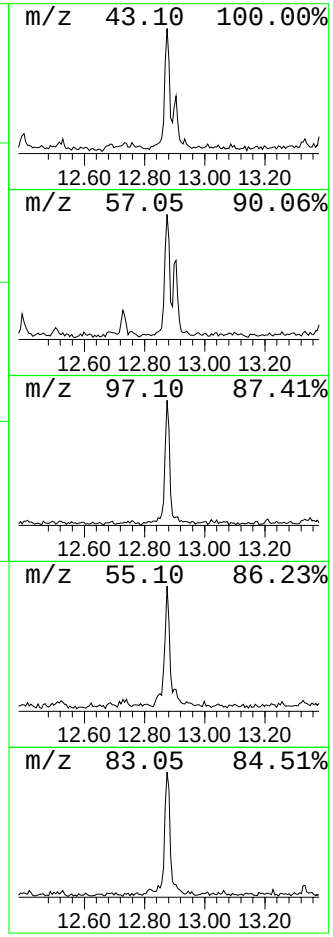


Peak Number: 3 at 12.73 min Area: 572744 Area % 0.64

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Dibutyl phthalate	123485	000084-74-2	93
2 1,2-Benzenedicarboxylic acid, bi...	123453	000084-69-5	80
3 1,2-Benzenedicarboxylic acid, bu...	66726	000084-78-6	64

Unknown Spectrum based on Apex minus start of peak

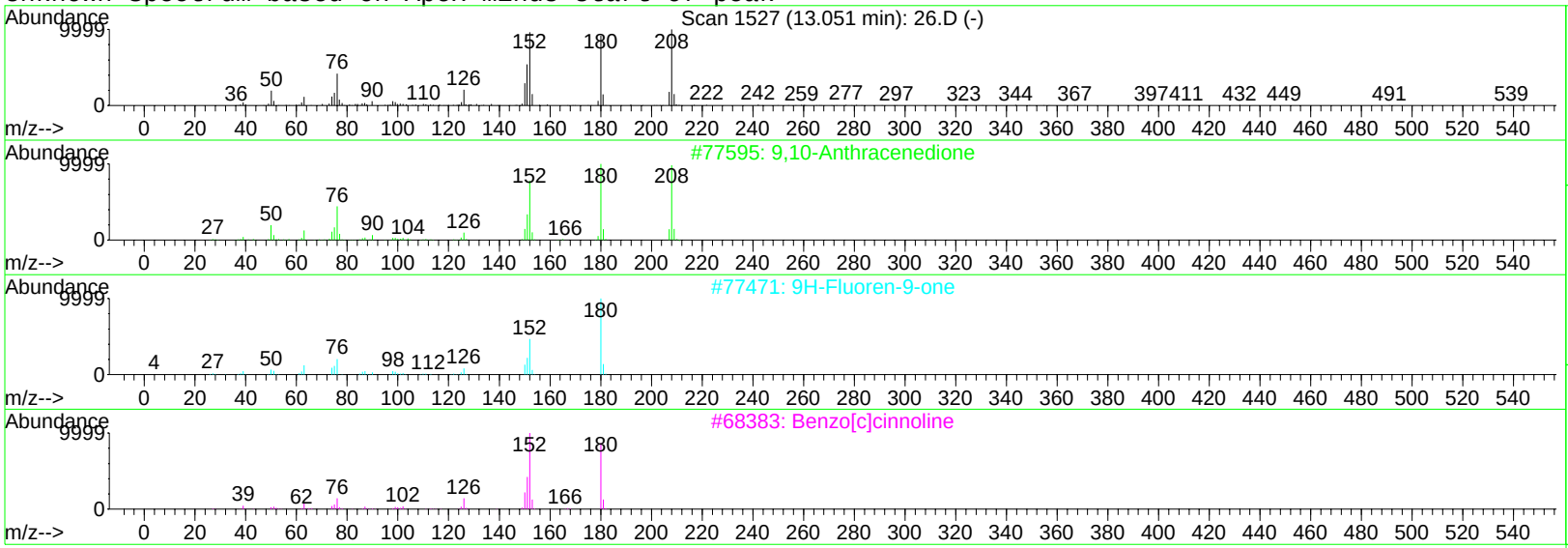


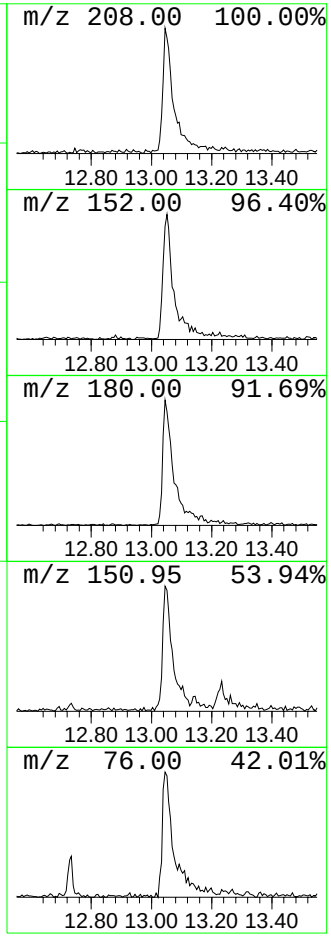


Peak Number: 4 at 12.88 min Area: 925732 Area % 1.04

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1-Octadecene	111277	000112-88-9	95
2 Z-5-Nonadecene	13523	1000131-11-8	94
3 9-Nonadecene	13042	031035-07-1	93

Unknown Spectrum based on Apex minus start of peak

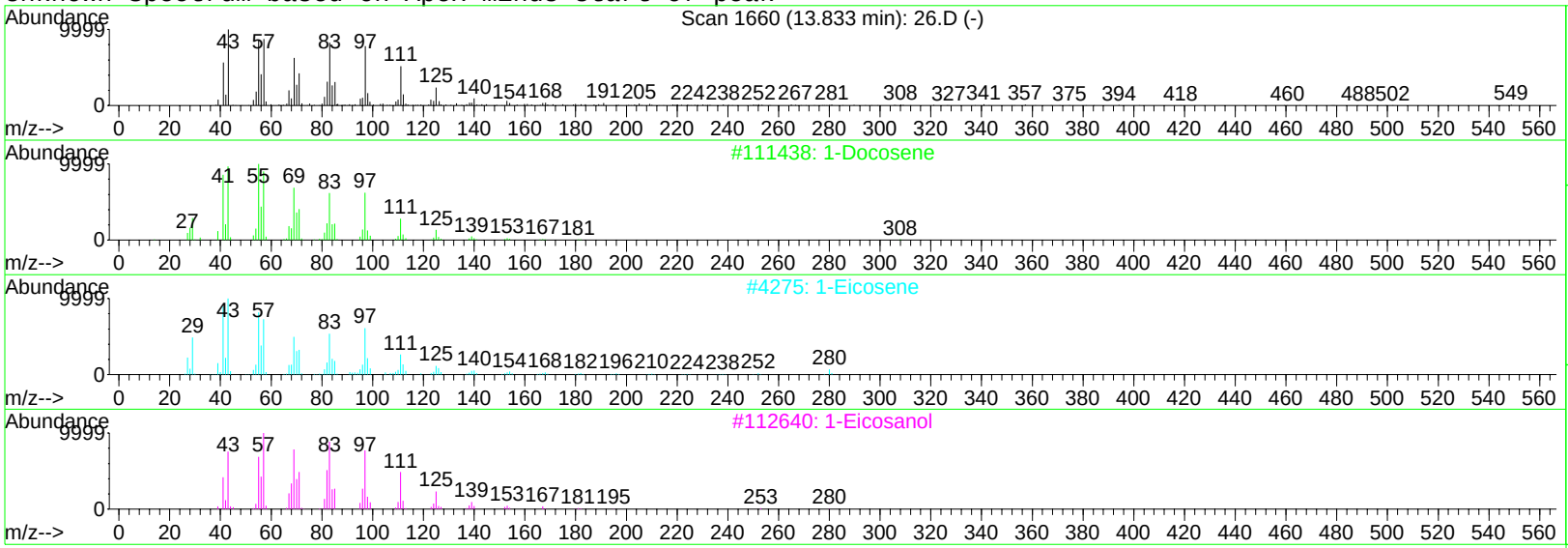


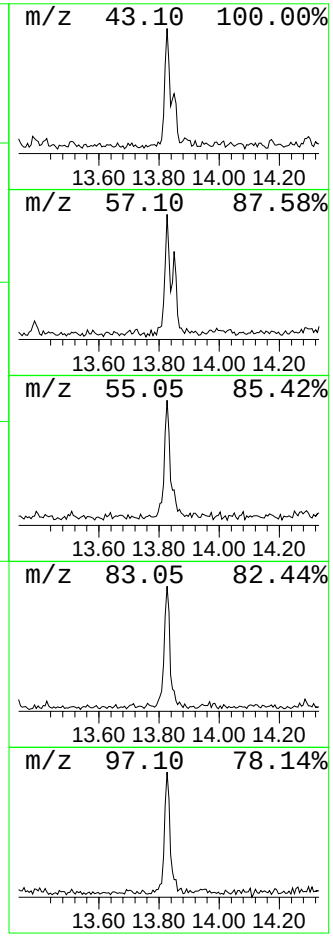


Peak Number: 5 at 13.05 min Area: 971537 Area % 1.09

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 9,10-Anthracenedione	77595	000084-65-1	98
2 9H-Fluoren-9-one	77471	000486-25-9	89
3 Benzo[c]cinnoline	68383	000230-17-1	76

Unknown Spectrum based on Apex minus start of peak

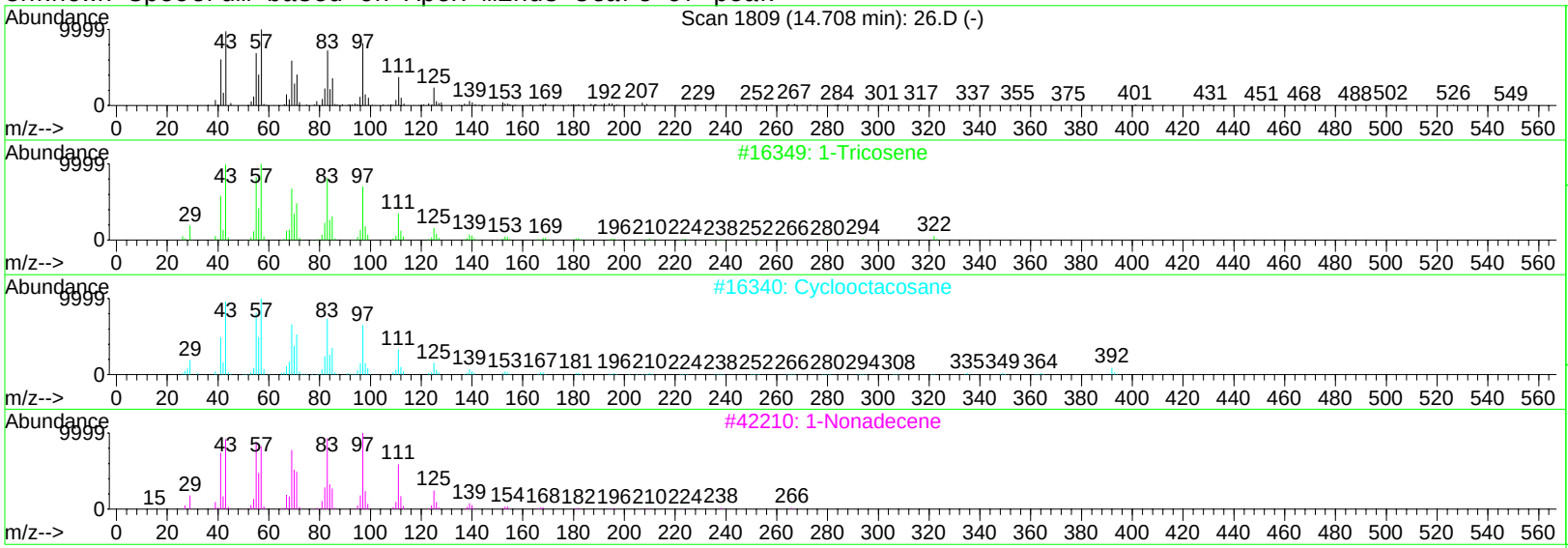


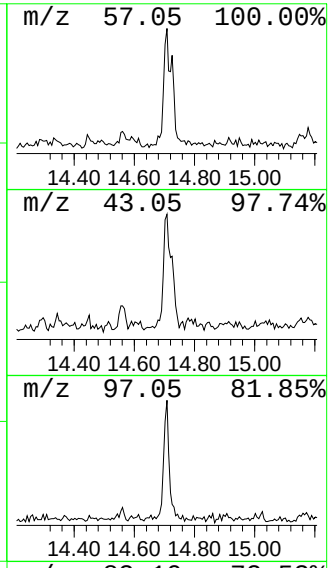


Peak Number: 6 at 13.83 min Area: 1162605 Area % 1.30

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1-Docosene	111438	001599-67-3	92
2 1-Eicosene	4275	003452-07-1	91
3 1-Eicosanol	112640	000629-96-9	90

Unknown Spectrum based on Apex minus start of peak

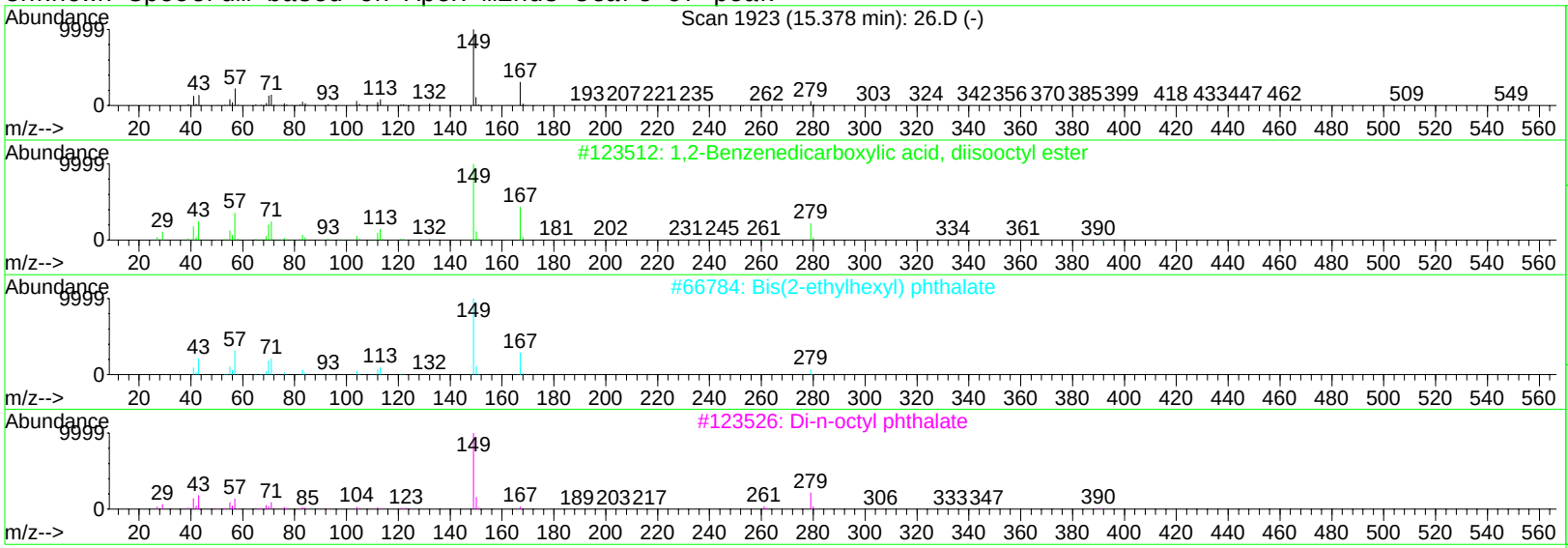


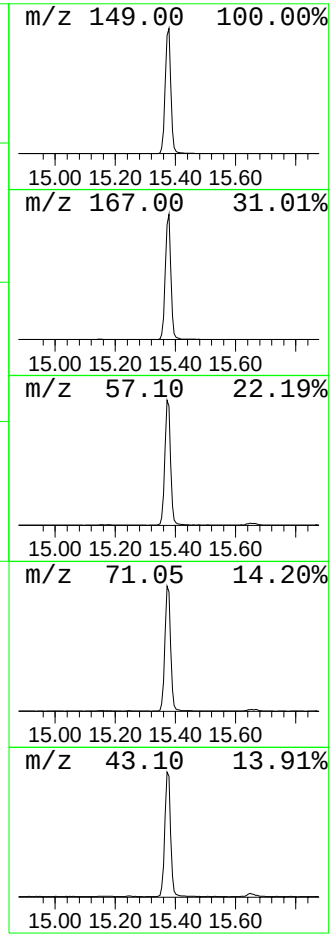


Peak Number: 7 at 14.71 min Area: 966629 Area % 1.08

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1-Tricosene	16349	018835-32-0	91
2 Cyclooctacosane	16340	000297-24-5	90
3 1-Nonadecene	42210	018435-45-5	87

Unknown Spectrum based on Apex minus start of peak

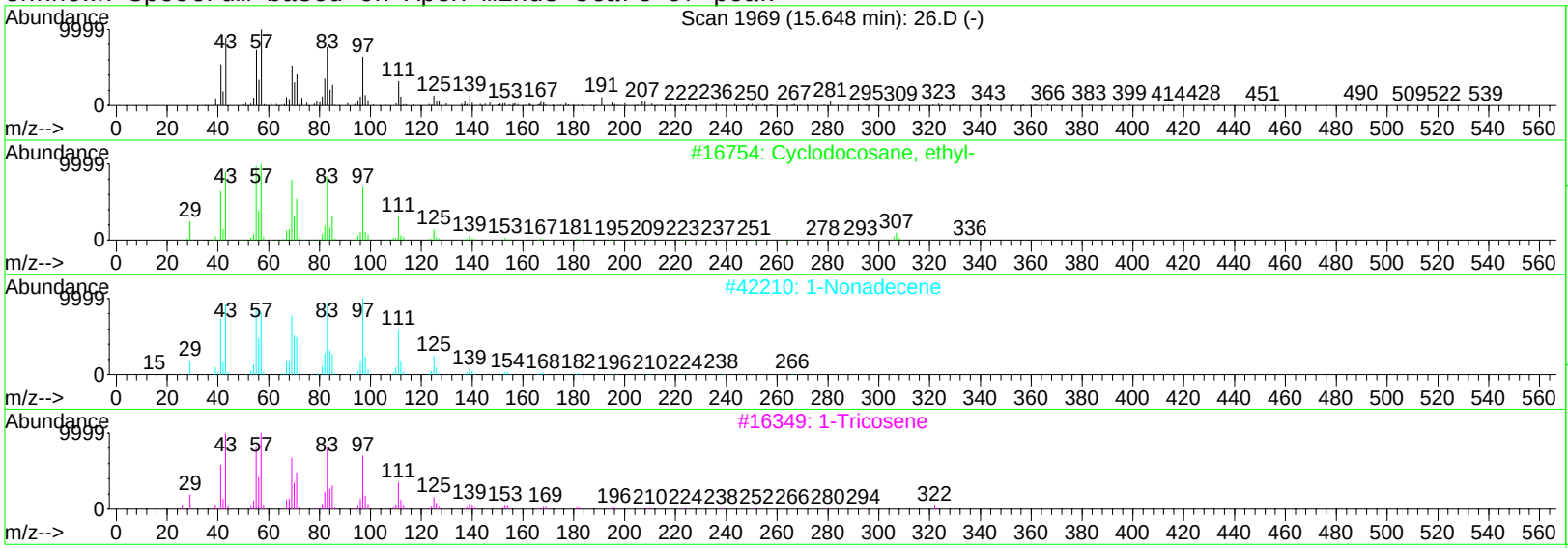


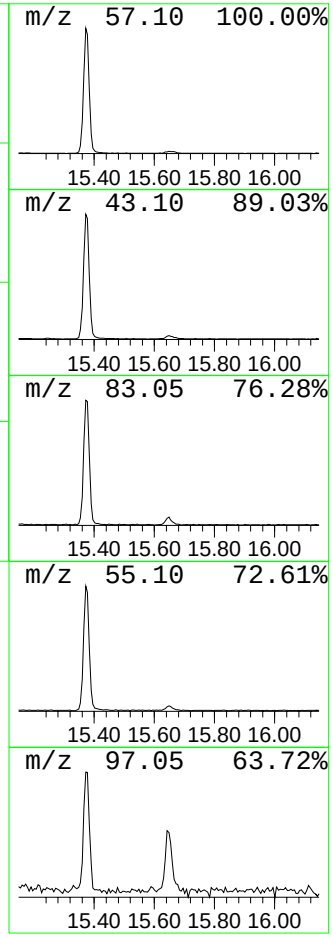


Peak Number: 8 at 15.38 min Area: 34888345 Area % 39.04

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1,2-Benzenedicarboxylic acid, di...	123512	027554-26-3	93
2 Bis(2-ethylhexyl) phthalate	66784	000117-81-7	91
3 Di-n-octyl phthalate	123526	000117-84-0	81

Unknown Spectrum based on Apex minus start of peak

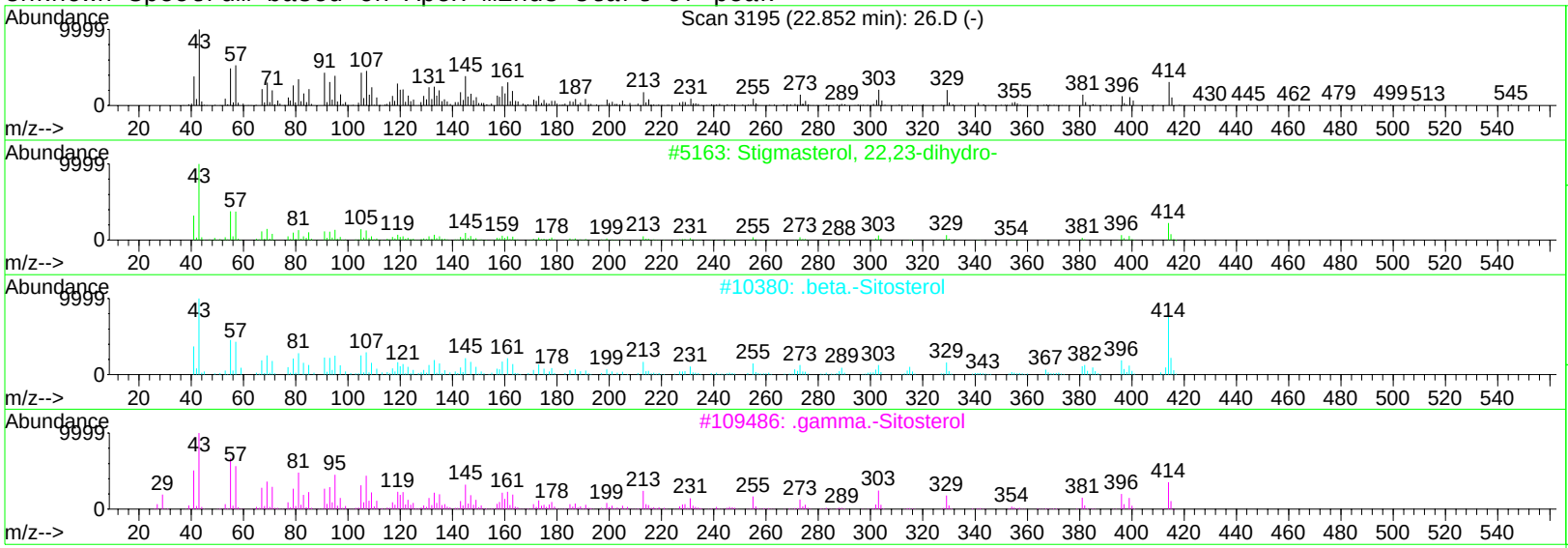


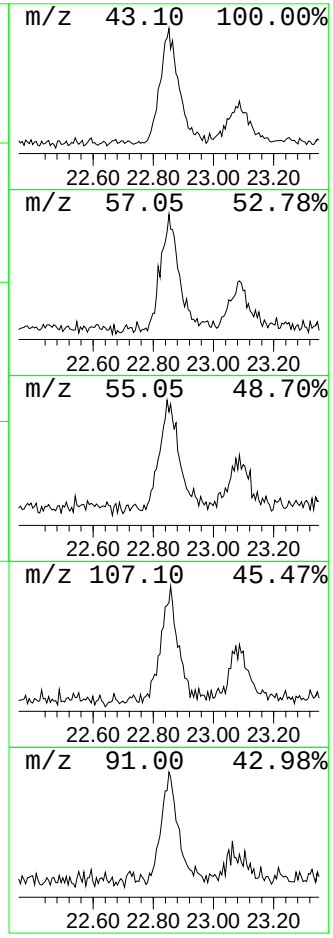


Peak Number: 9 at 15.65 min Area: 690469 Area % 0.77

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclodocosane, ethyl-	16754	1000151-22-6	93
2 1-Nonadecene	42210	018435-45-5	92
3 1-Tricosene	16349	018835-32-0	91

Unknown Spectrum based on Apex minus start of peak

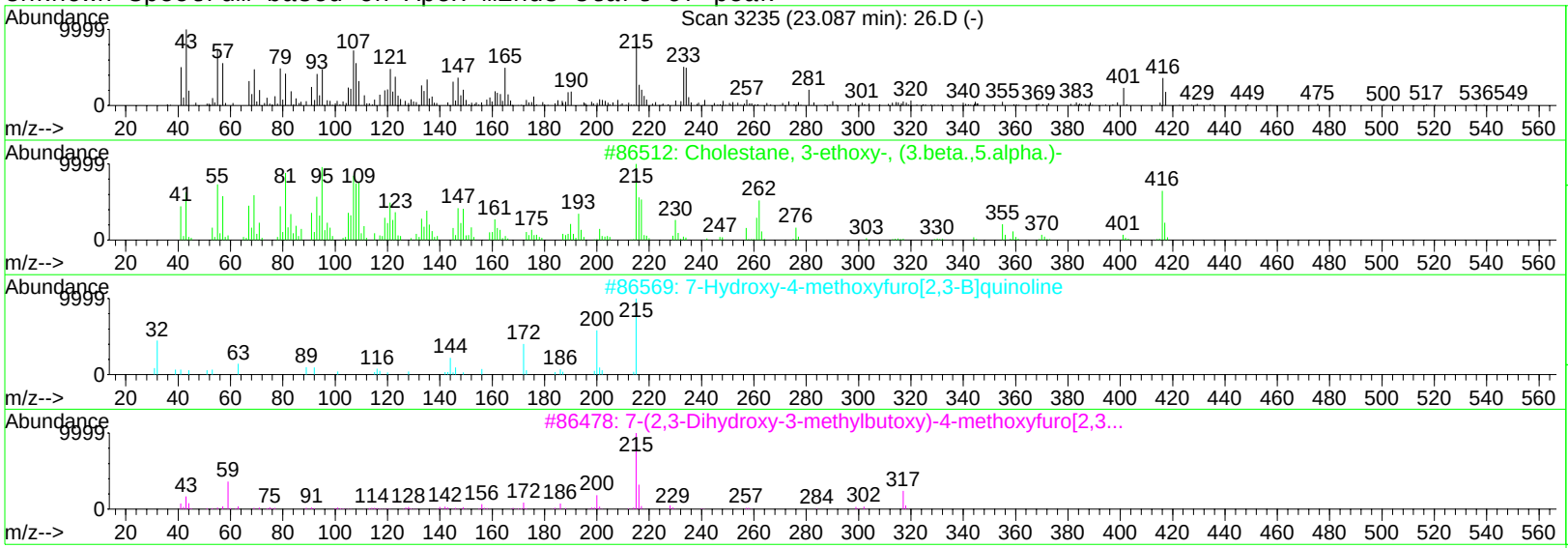


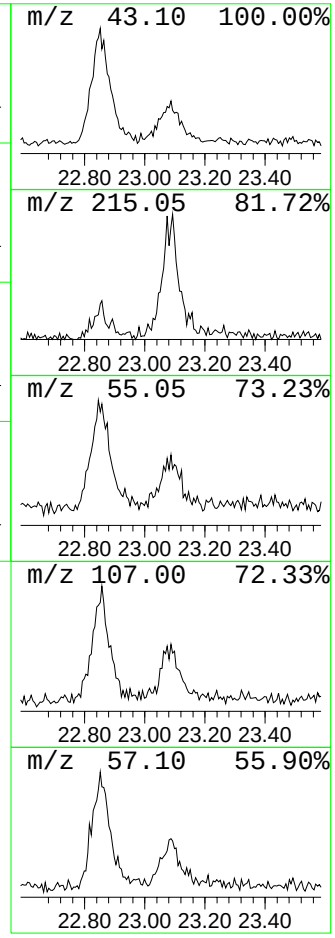


Peak Number: 10 at 22.85 min Area: 4421246 Area % 4.95

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Stigmasterol, 22,23-dihydro-	5163	1000214-20-7	94
2 .beta.-Sitosterol	10380	000083-46-5	94
3 .gamma.-Sitosterol	109486	000083-47-6	90

Unknown Spectrum based on Apex minus start of peak





Peak Number: 11 at 23.09 min Area: 1762752 Area % 1.97

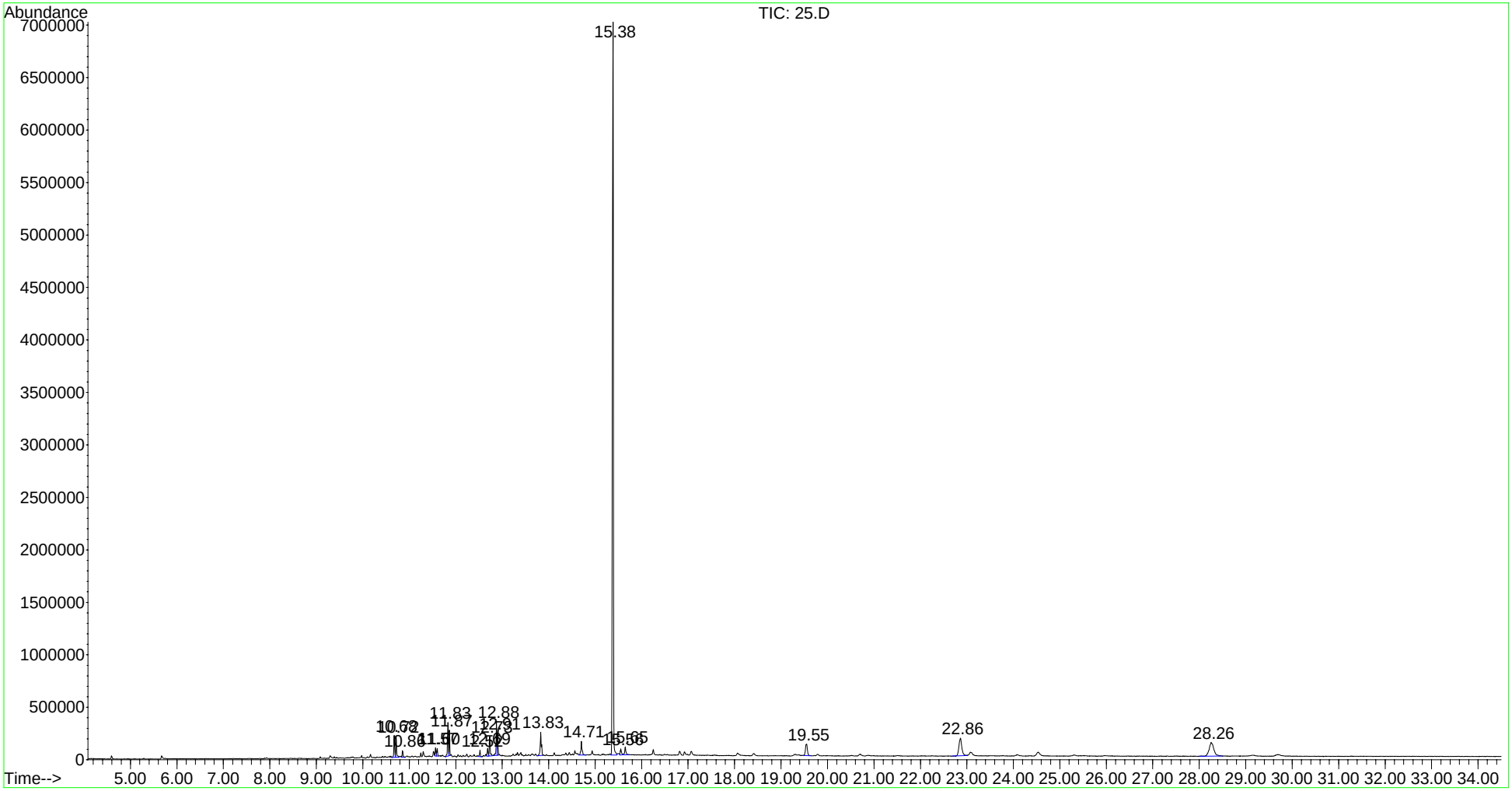
The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cholestane, 3-ethoxy-, (3.beta.,...	86512	002089-02-3	38
2 7-Hydroxy-4-methoxyfuro[2,3-B]qu...	86569	020643-71-4	11
3 7-(2,3-Dihydroxy-3-methylbutoxy)...	86478	085849-53-2	10

Library Search Report

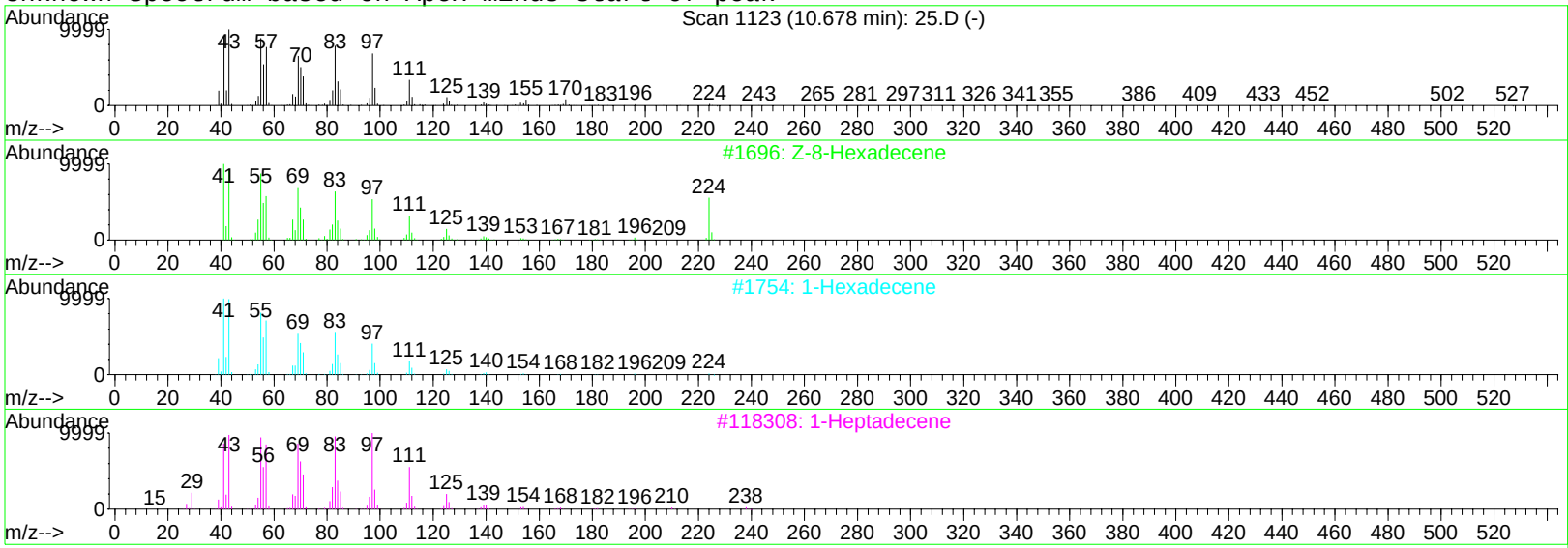
Data File : D:\PRENISHA\300\25.D
Acq On : 23 Aug 2007 10:43
Sample : 25
Misc : 1µl inject, neat, splitless

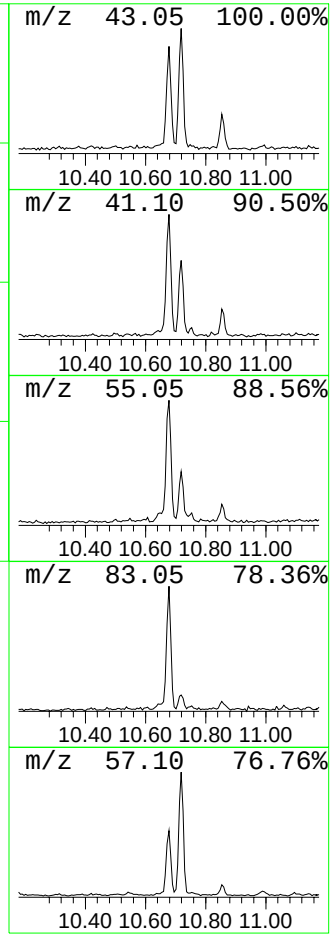
Vial: 25
Operator: Prenisha
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e
Method : C:\MSDCHEM\1\METHODS\NEW.M (Chemstation Integrator)
Title :



Unknown Spectrum based on Apex minus start of peak

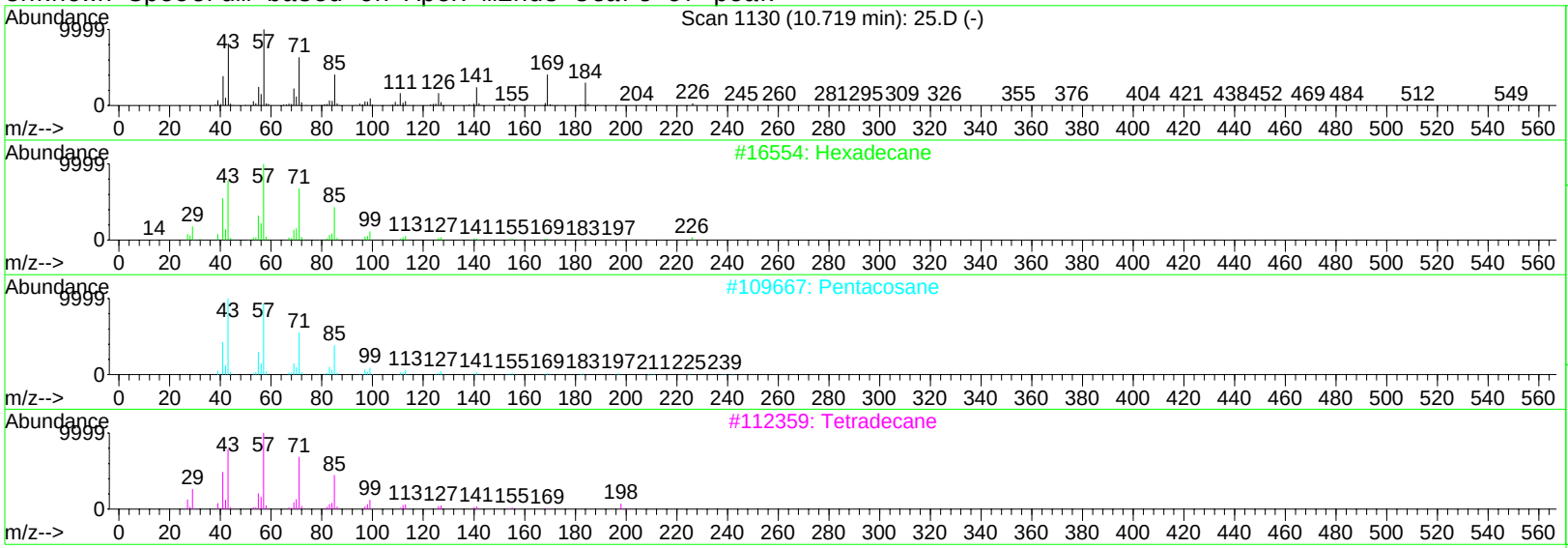


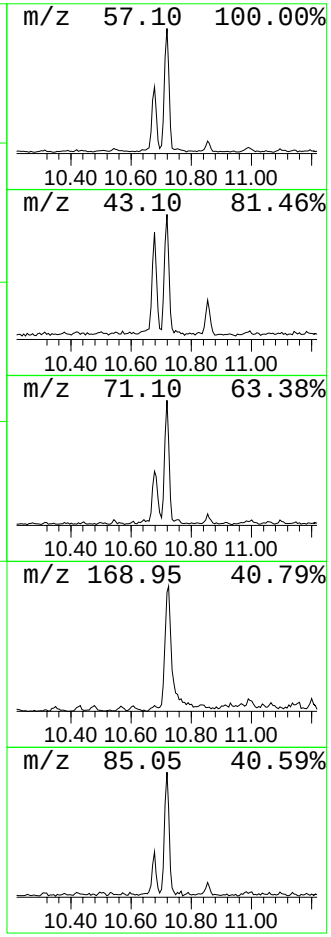


Peak Number: 1 at 10.68 min Area: 2397890 Area % 1.60

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Z-8-Hexadecene	1696	1000130-87-5	94
2 1-Hexadecene	1754	000629-73-2	92
3 1-Heptadecene	118308	006765-39-5	91

Unknown Spectrum based on Apex minus start of peak

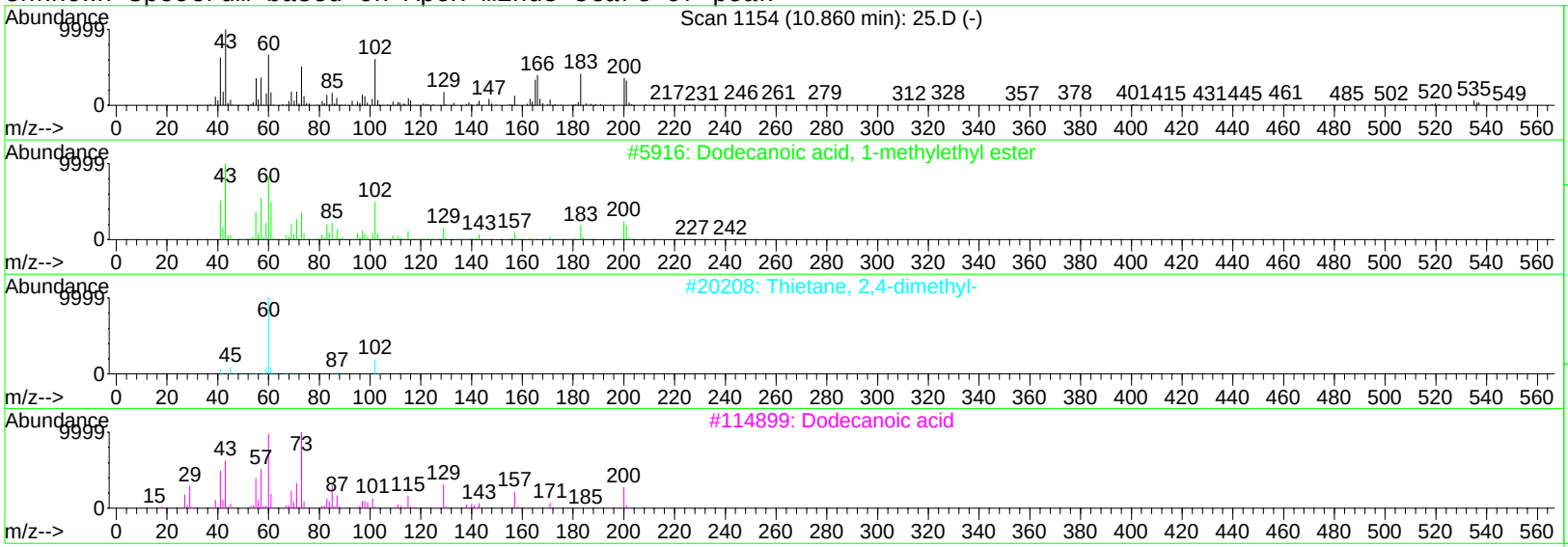


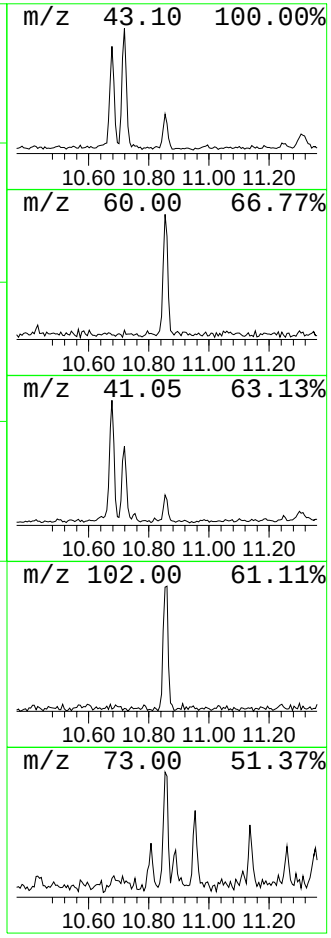


Peak Number: 2 at 10.72 min Area: 2611353 Area % 1.74

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Hexadecane	16554	000544-76-3	95
2 Pentacosane	109667	000629-99-2	49
3 Tetradecane	112359	000629-59-4	49

Unknown Spectrum based on Apex minus start of peak

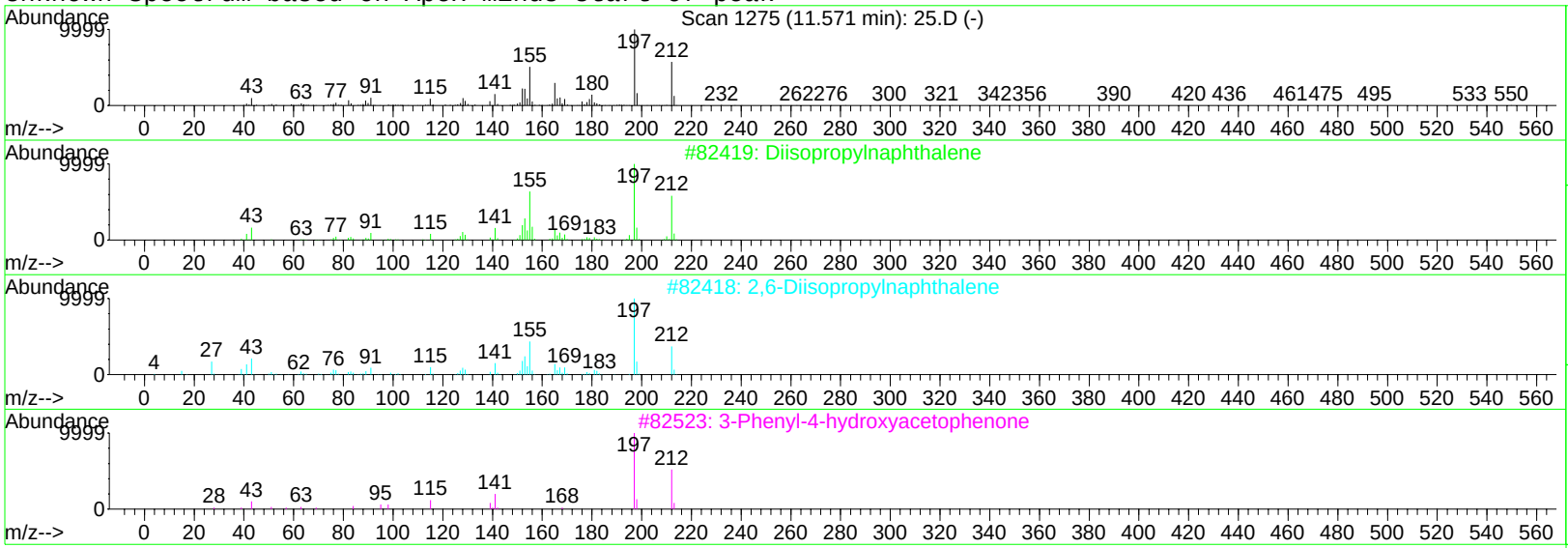


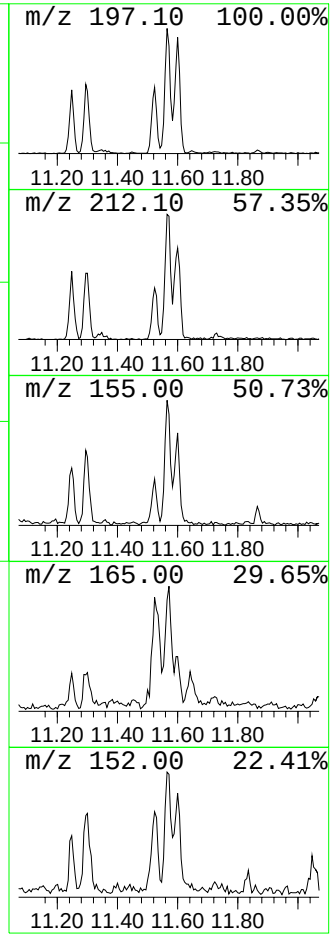


Peak Number: 3 at 10.86 min Area: 838057 Area % 0.56

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Dodecanoic acid, 1-methylethyl e...	5916	010233-13-3	38
2 Thietane, 2,4-dimethyl-	20208	043044-24-2	27
3 Dodecanoic acid	114899	000143-07-7	25

Unknown Spectrum based on Apex minus start of peak

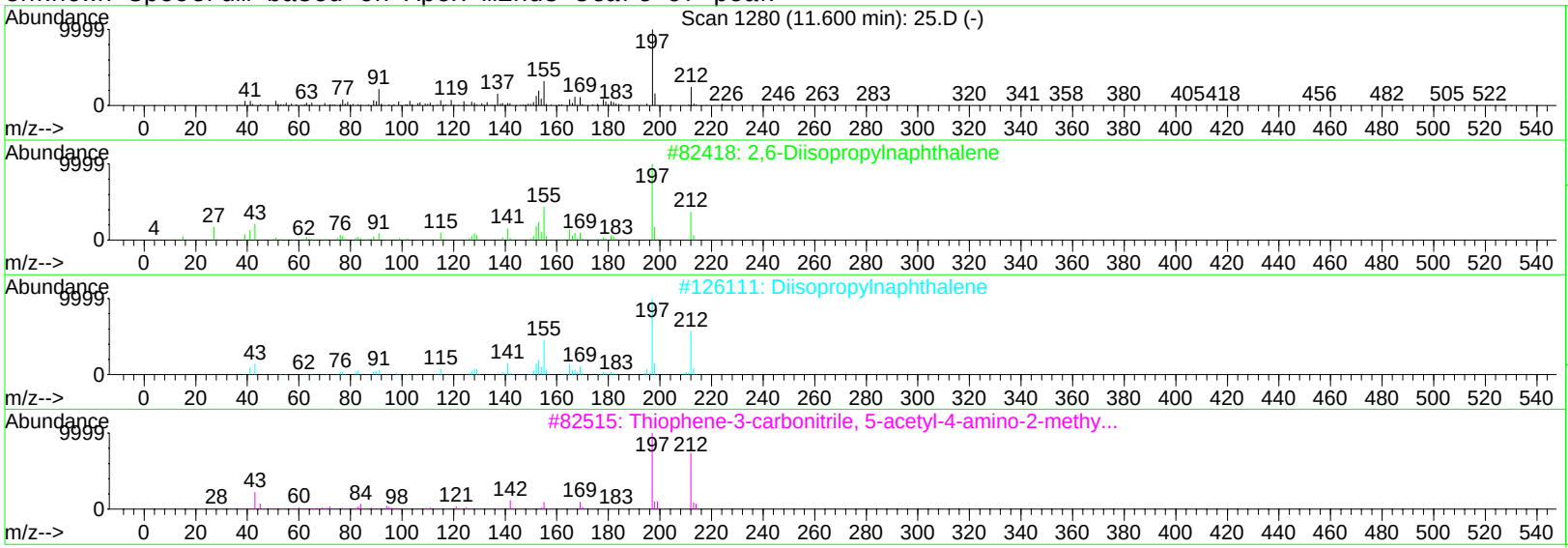


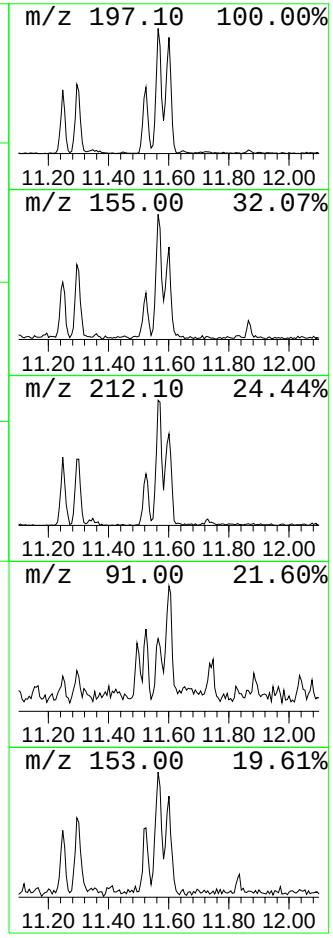


Peak Number: 4 at 11.57 min Area: 978111 Area % 0.65

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Diisopropylnaphthalene	82419	038640-62-9	93
2 2,6-Diisopropylnaphthalene	82418	024157-81-1	87
3 3-Phenyl-4-hydroxyacetophenone	82523	021424-82-8	49

Unknown Spectrum based on Apex minus start of peak

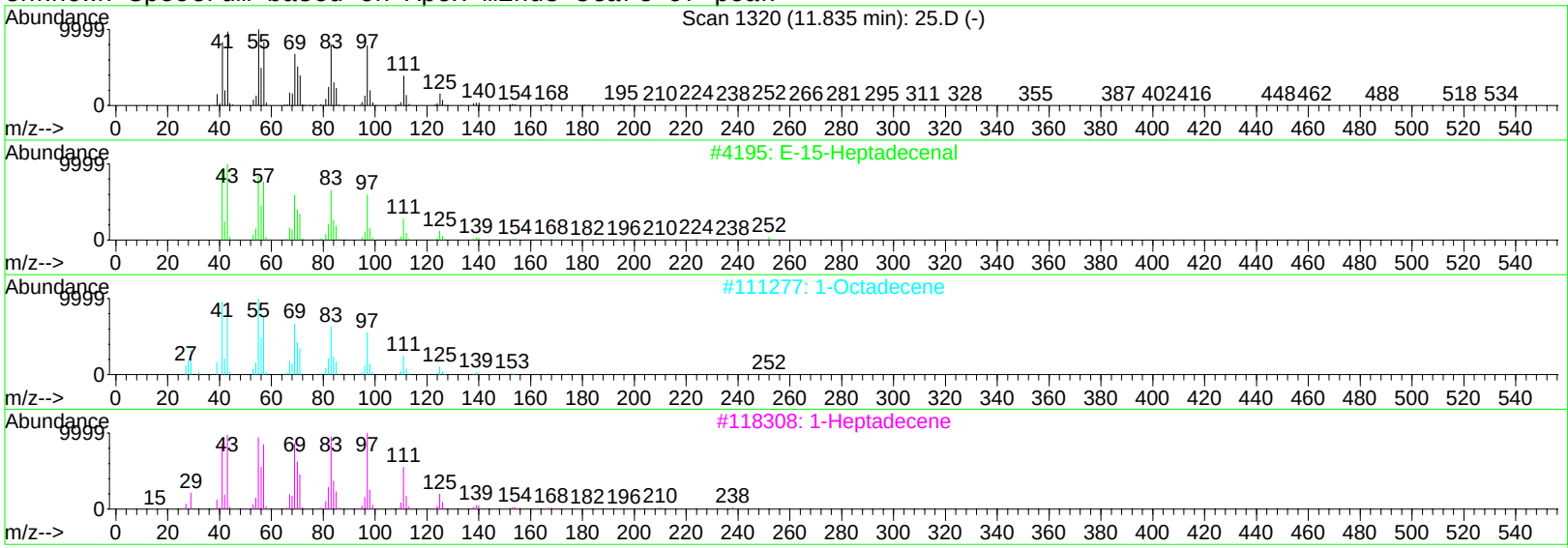


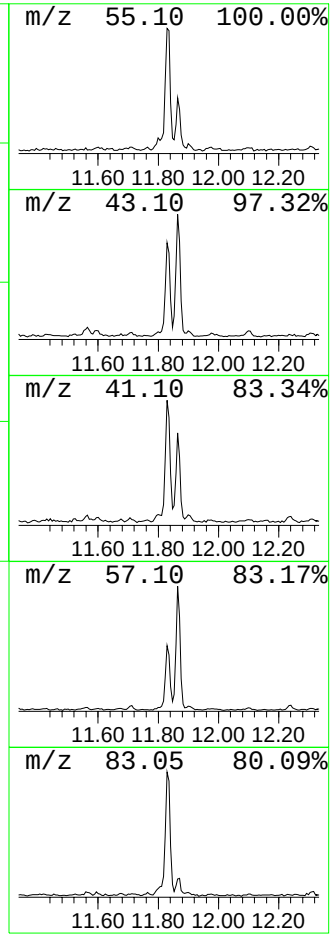


Peak Number: 5 at 11.60 min Area: 905641 Area % 0.60

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2,6-Diisopropylnaphthalene	82418	024157-81-1	89
2 Diisopropylnaphthalene	126111	038640-62-9	83
3 Thiophene-3-carbonitrile, 5-acet...	82515	116171-01-8	50

Unknown Spectrum based on Apex minus start of peak

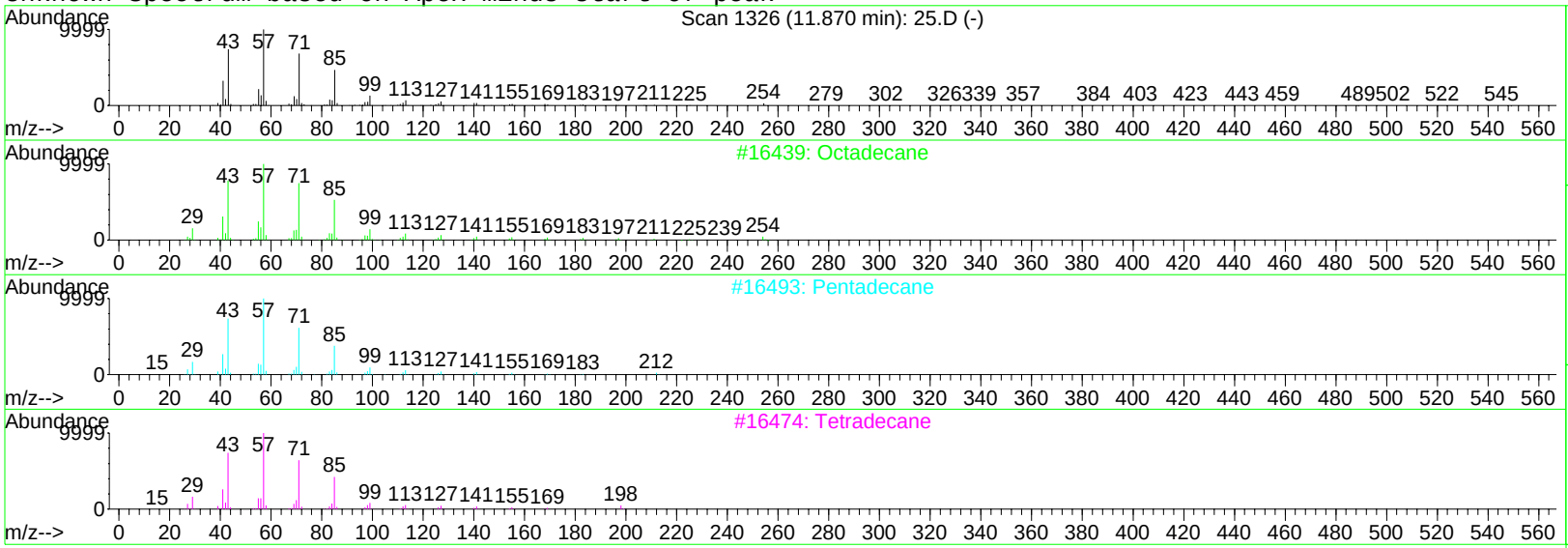


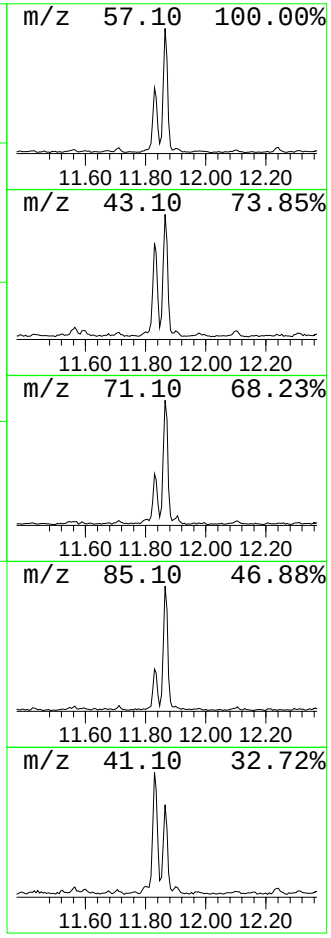


Peak Number: 6 at 11.84 min Area: 3632081 Area % 2.43

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 E-15-Heptadecenal	4195	1000130-97-9	99
2 1-Octadecene	111277	000112-88-9	98
3 1-Heptadecene	118308	006765-39-5	94

Unknown Spectrum based on Apex minus start of peak

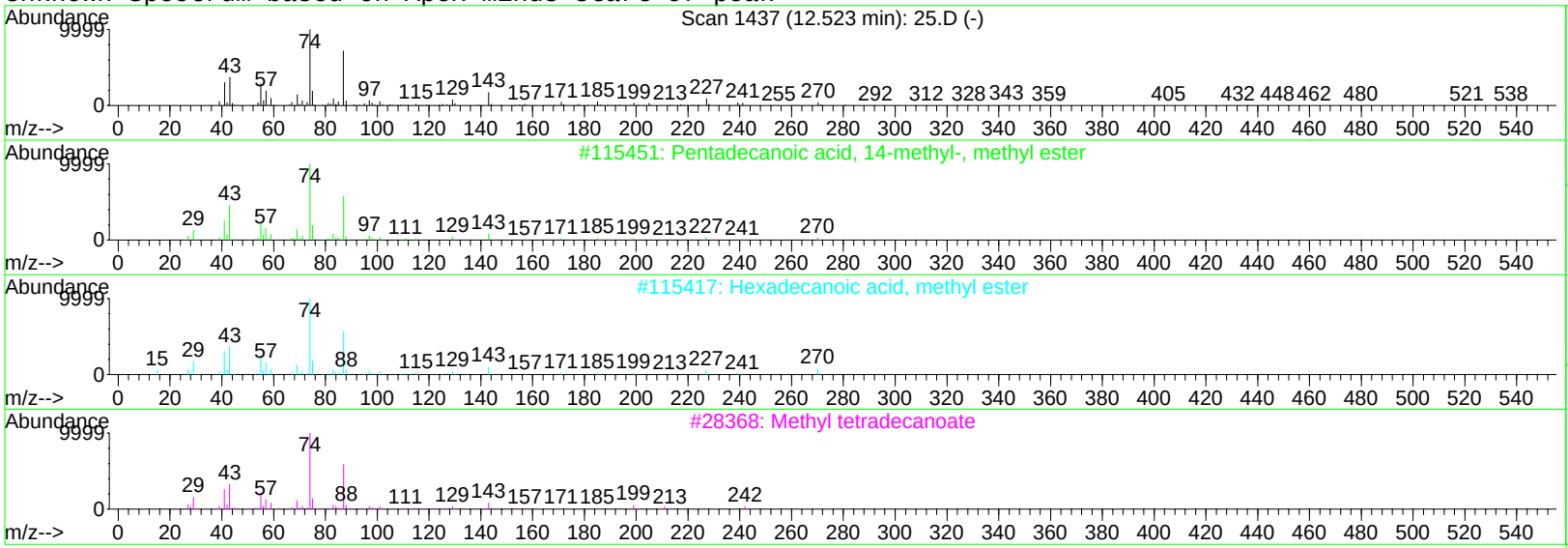


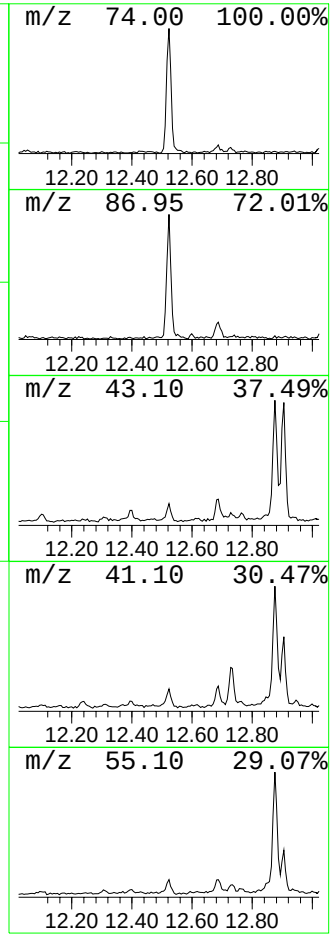


Peak Number: 7 at 11.87 min Area: 2356372 Area % 1.57

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Octadecane	16439	000593-45-3	97
2 Pentadecane	16493	000629-62-9	91
3 Tetradecane	16474	000629-59-4	91

Unknown Spectrum based on Apex minus start of peak

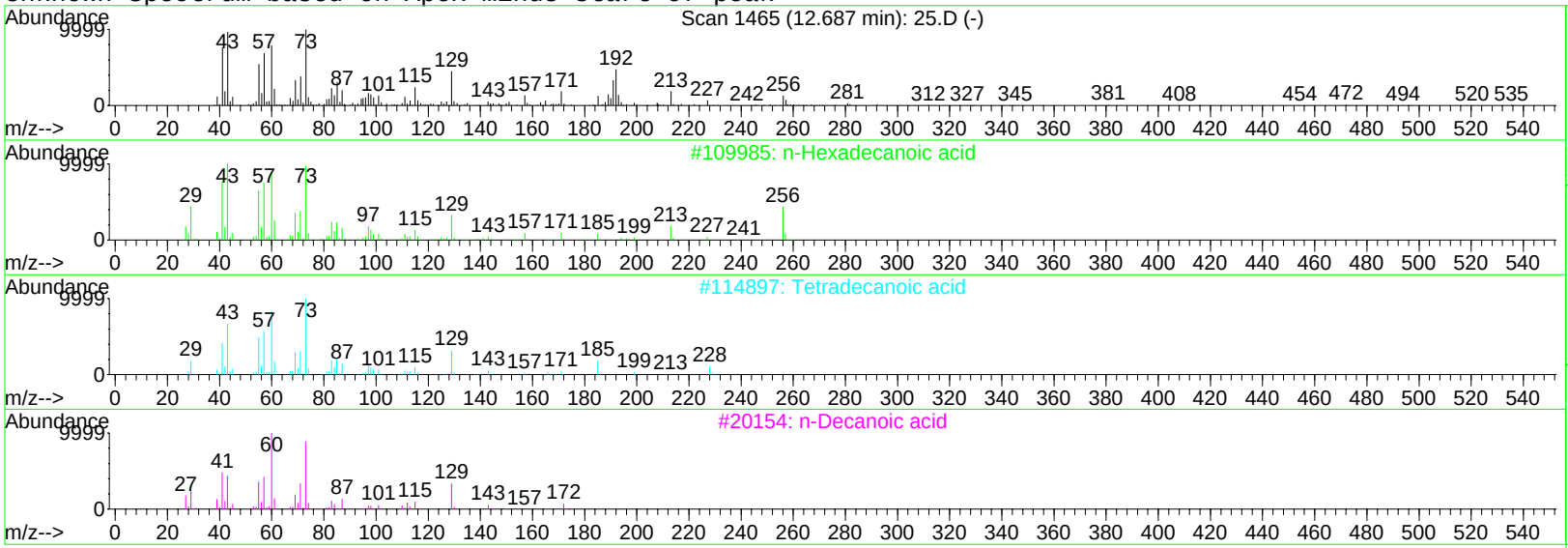


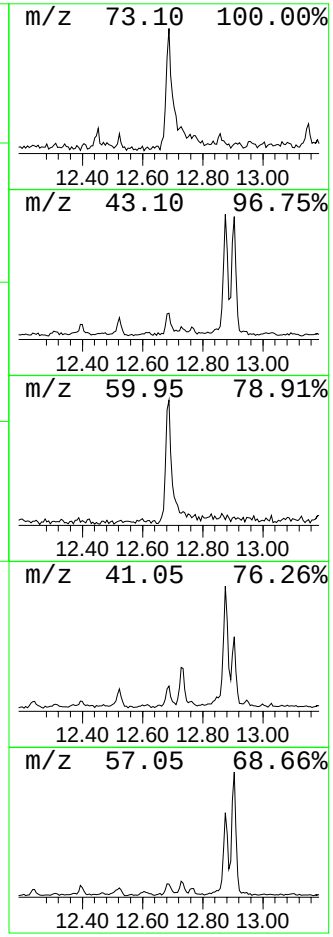


Peak Number: 8 at 12.52 min Area: 744274 Area % 0.50

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Pentadecanoic acid, 14-methyl-, ...	115451	005129-60-2	97
2 Hexadecanoic acid, methyl ester	115417	000112-39-0	95
3 Methyl tetradecanoate	28368	000124-10-7	87

Unknown Spectrum based on Apex minus start of peak

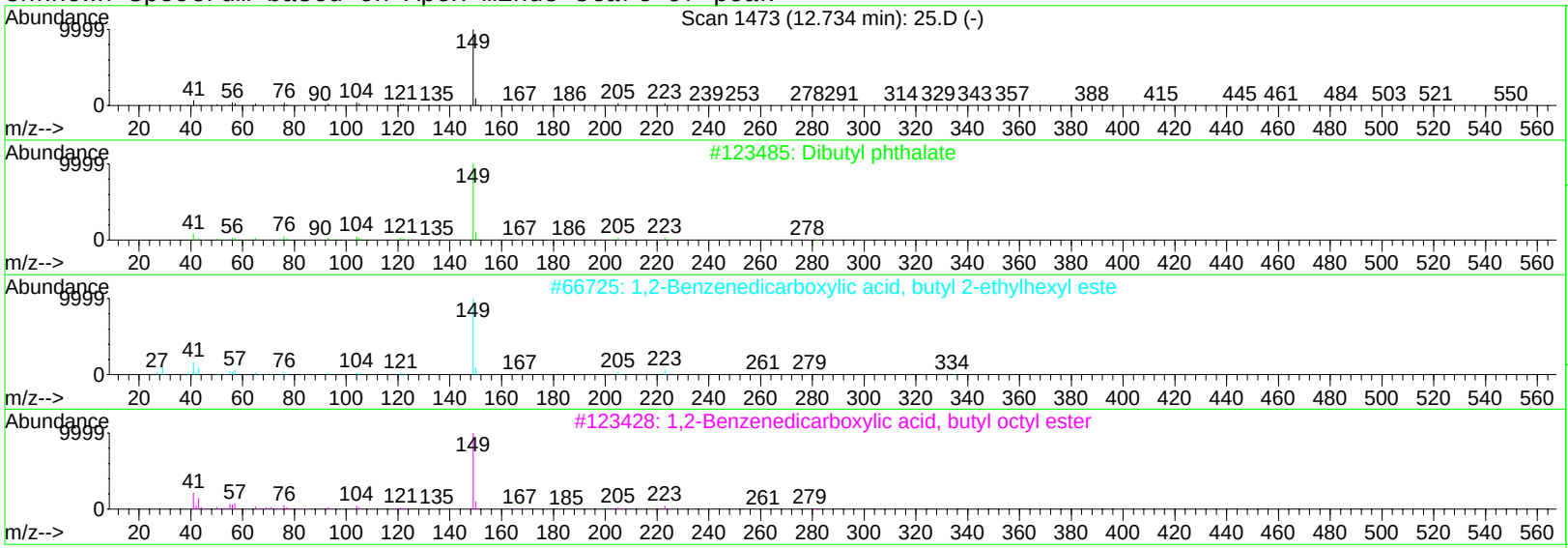


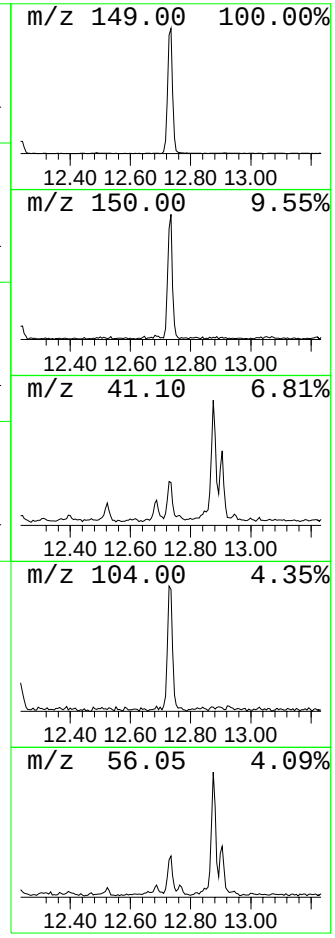


Peak Number: 9 at 12.69 min Area: 1008833 Area % 0.67

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 n-Hexadecanoic acid	109985	000057-10-3	92
2 Tetradecanoic acid	114897	000544-63-8	72
3 n-Decanoic acid	20154	000334-48-5	70

Unknown Spectrum based on Apex minus start of peak

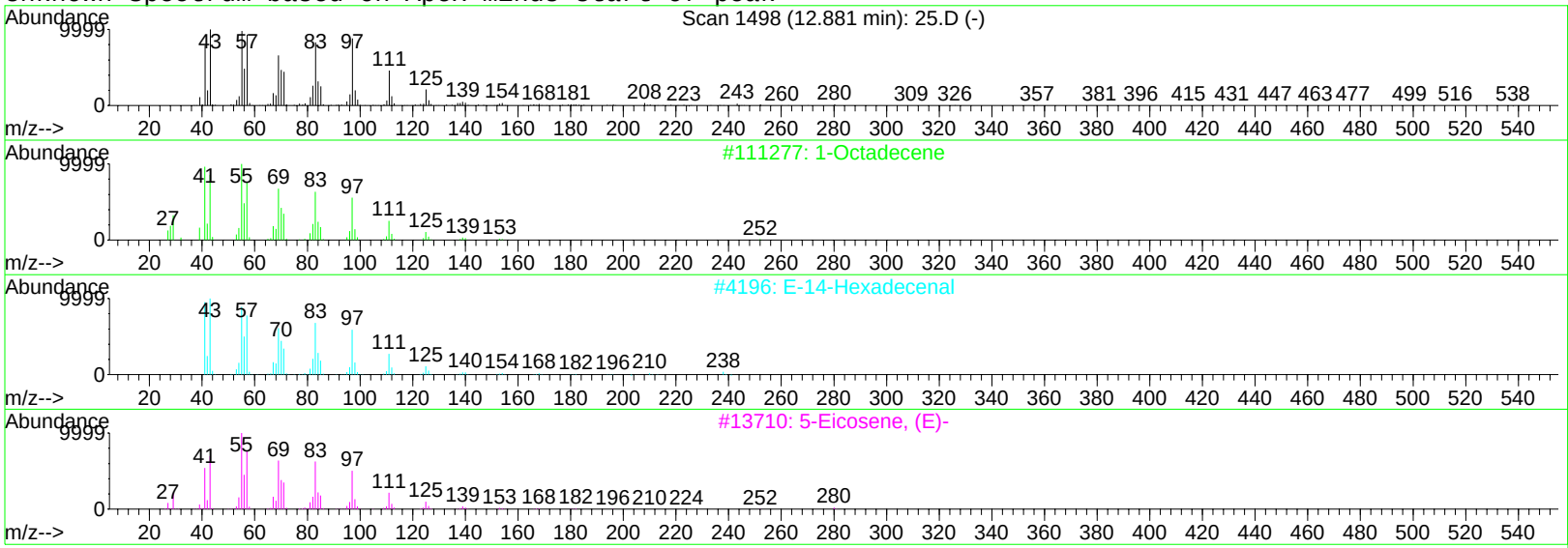


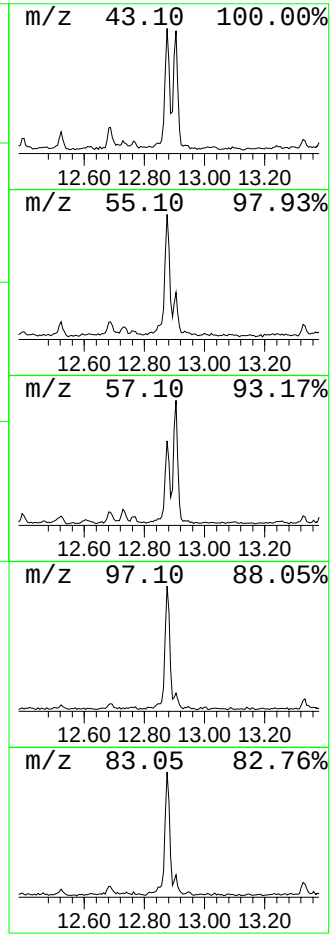


Peak Number: 10 at 12.73 min Area: 2436602 Area % 1.63

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Dibutyl phthalate	123485	000084-74-2	95
2 1,2-Benzenedicarboxylic acid, bu...	66725	000085-69-8	83
3 1,2-Benzenedicarboxylic acid, bu...	123428	000084-78-6	83

Unknown Spectrum based on Apex minus start of peak

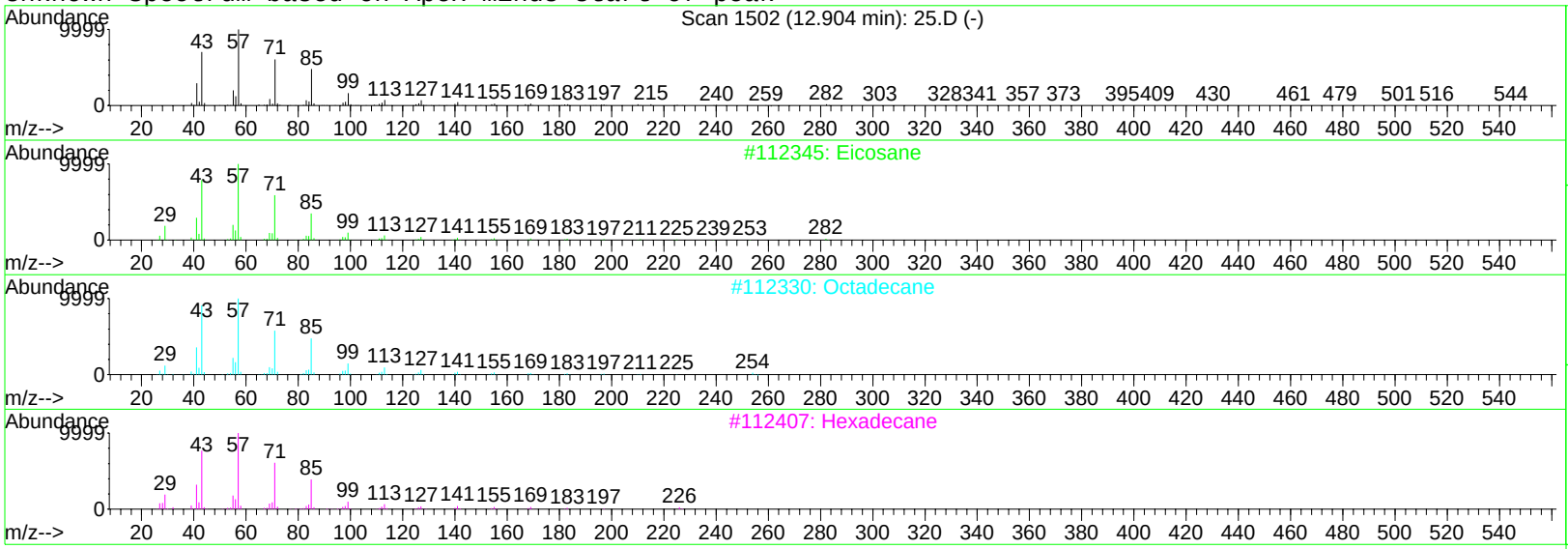


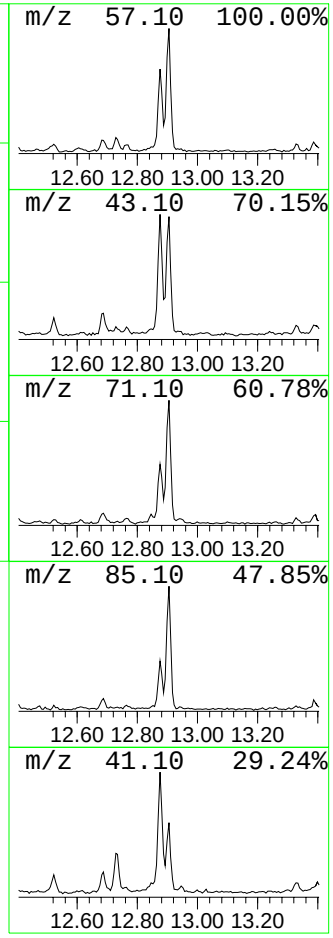


Peak Number: 11 at 12.88 min Area: 3561658 Area % 2.38

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1-Octadecene	111277	000112-88-9	98
2 E-14-Hexadecenal	4196	1000130-98-0	98
3 5-Eicosene, (E)-	13710	074685-30-6	95

Unknown Spectrum based on Apex minus start of peak

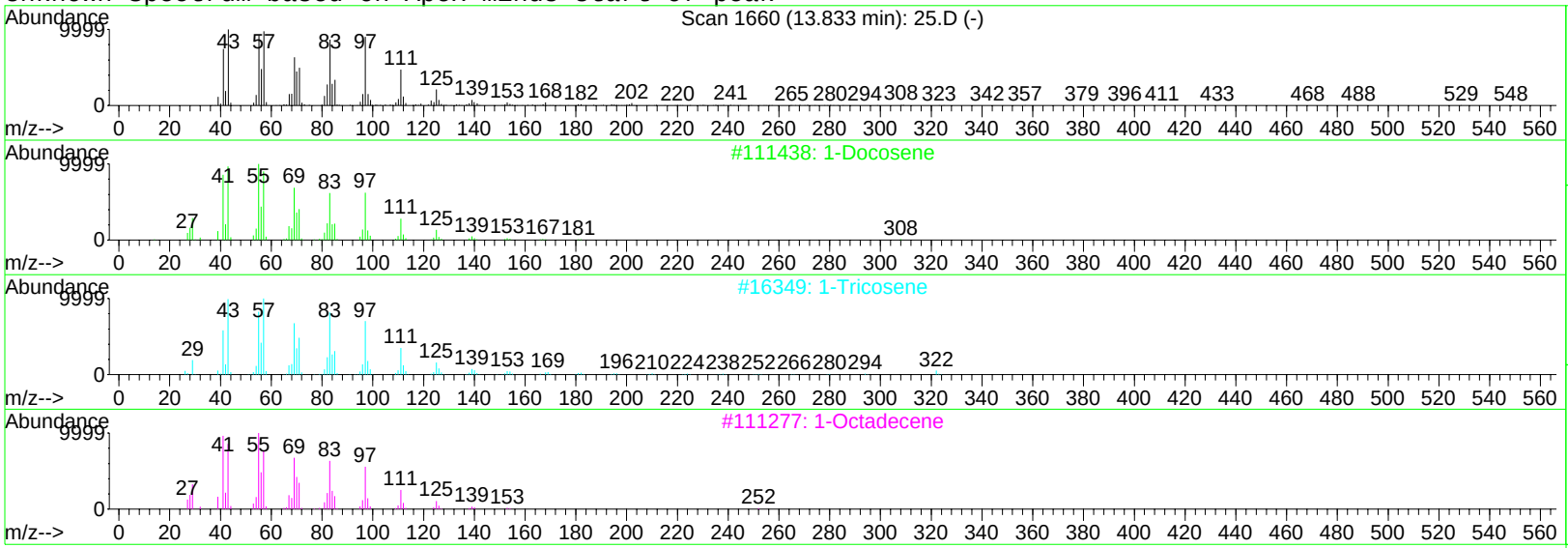


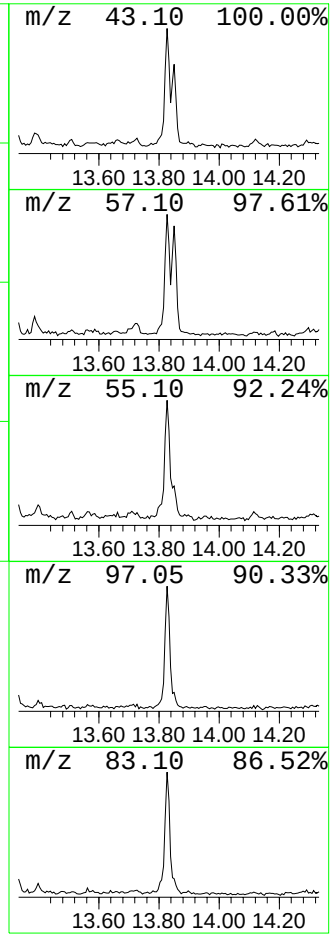


Peak Number: 12 at 12.90 min Area: 2000645 Area % 1.34

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Eicosane	112345	000112-95-8	93
2 Octadecane	112330	000593-45-3	91
3 Hexadecane	112407	000544-76-3	91

Unknown Spectrum based on Apex minus start of peak

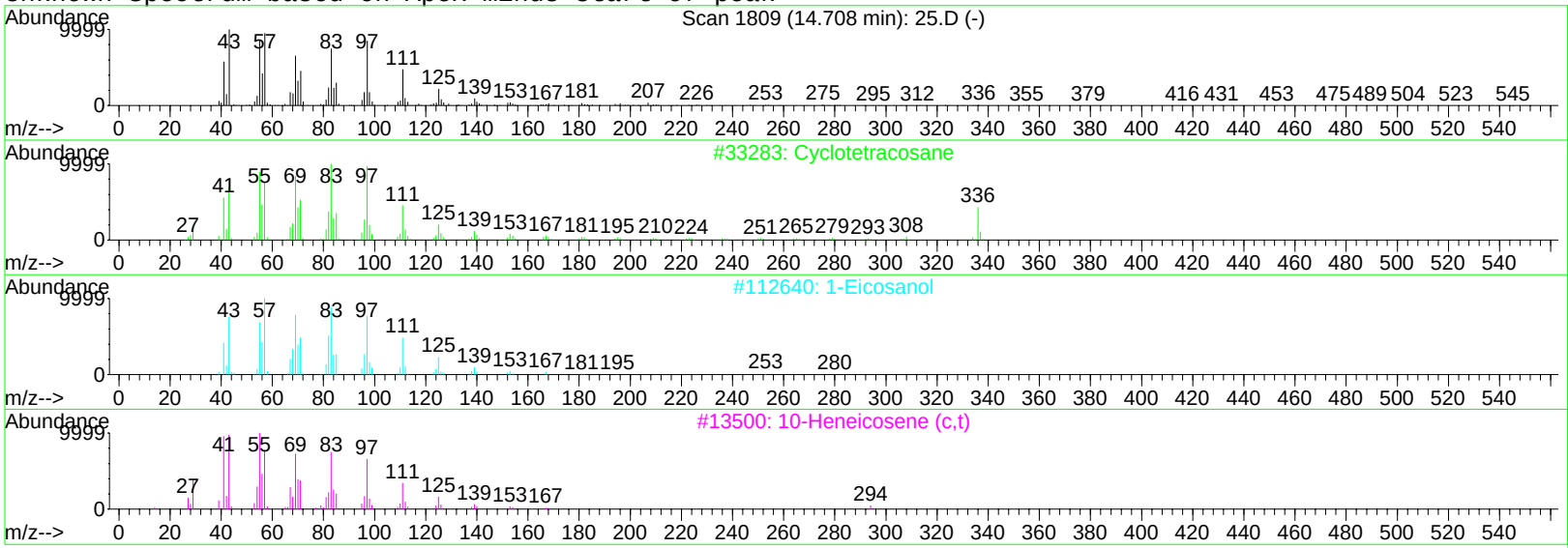


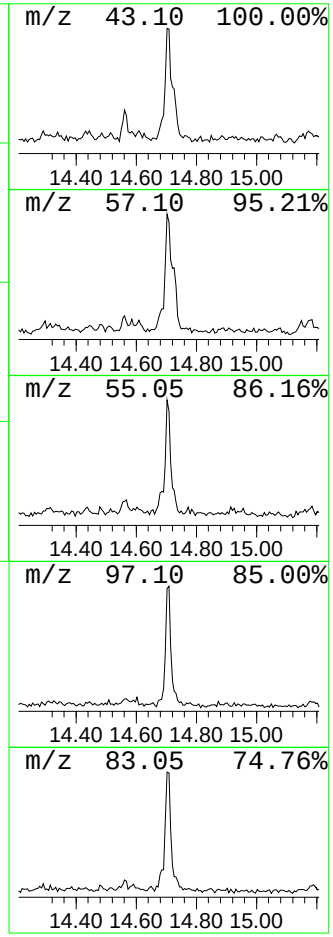


Peak Number: 13 at 13.83 min Area: 3777590 Area % 2.52

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1-Docosene	111438	001599-67-3	98
2 1-Tricosene	16349	018835-32-0	91
3 1-Octadecene	111277	000112-88-9	91

Unknown Spectrum based on Apex minus start of peak

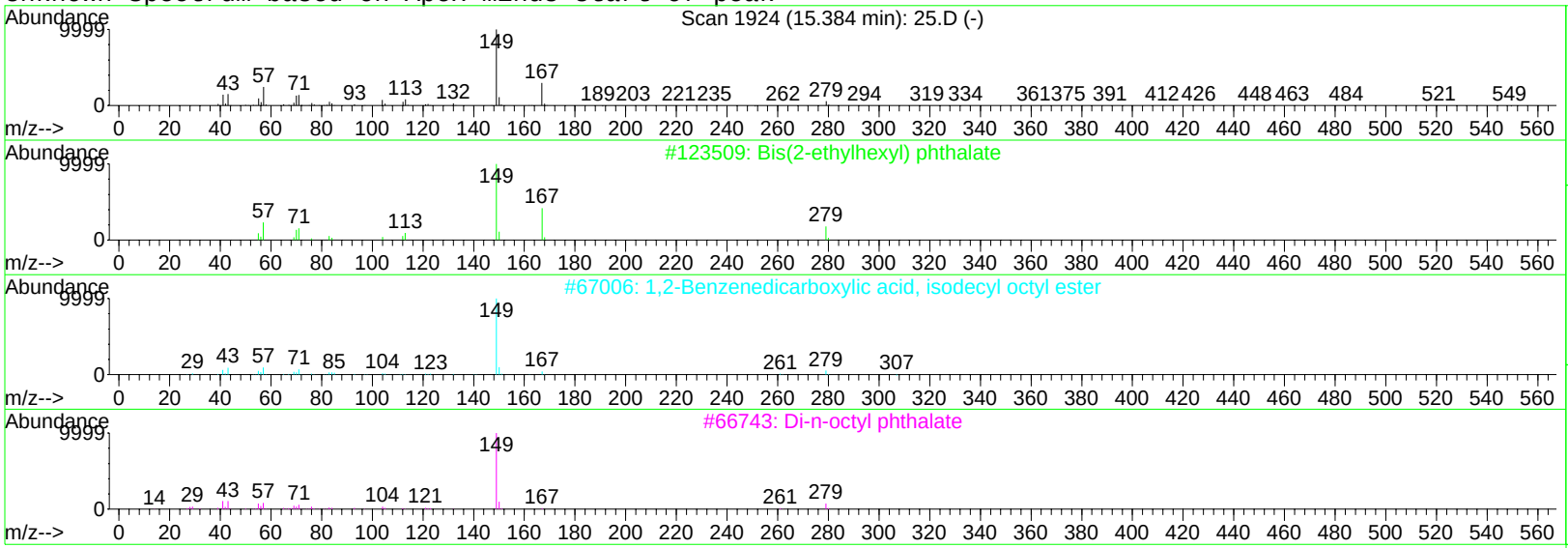


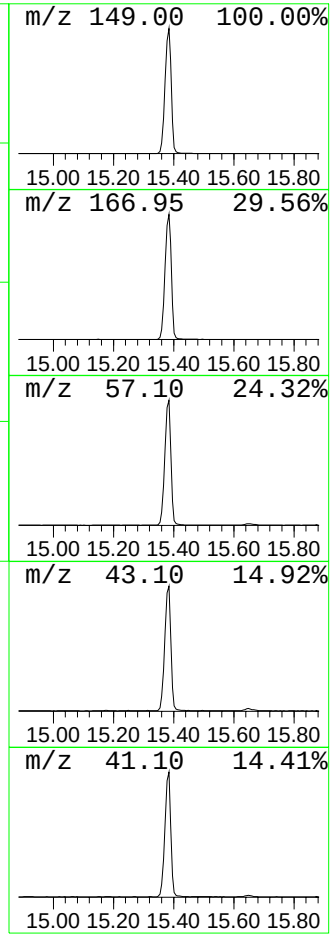


Peak Number: 14 at 14.71 min Area: 2382818 Area % 1.59

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclotetracosane	33283	000297-03-0	97
2 1-Eicosanol	112640	000629-96-9	91
3 10-Heneicosene (c,t)	13500	095008-11-0	91

Unknown Spectrum based on Apex minus start of peak

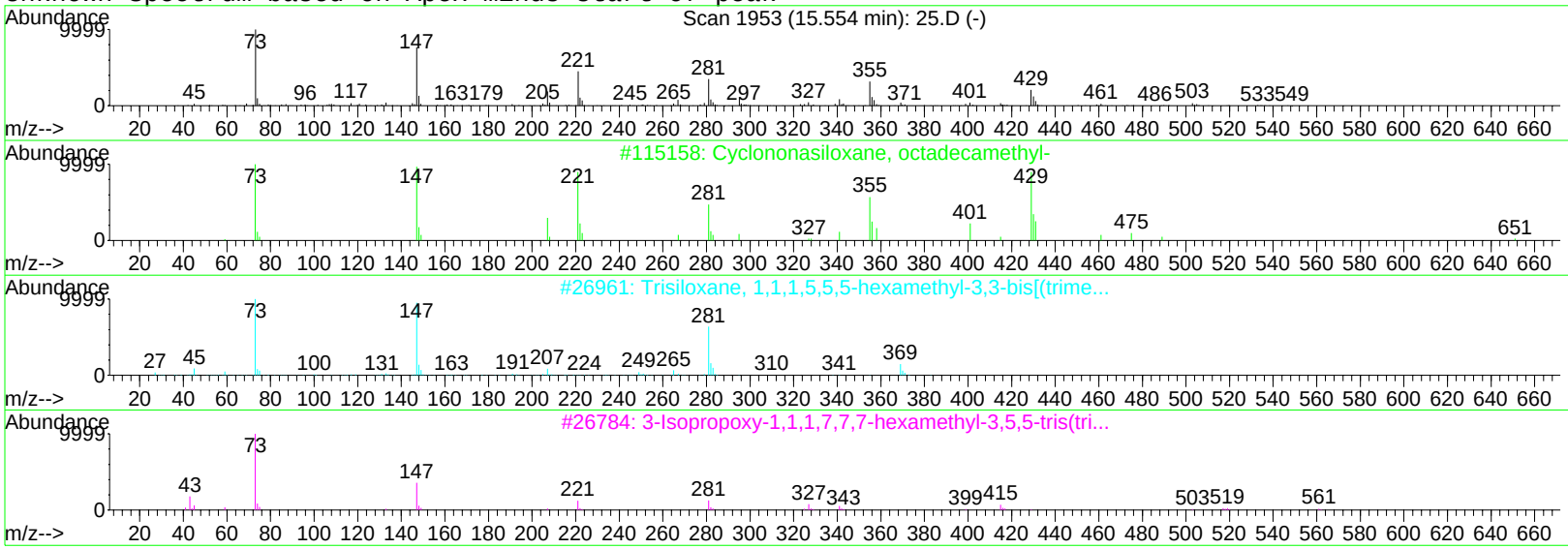


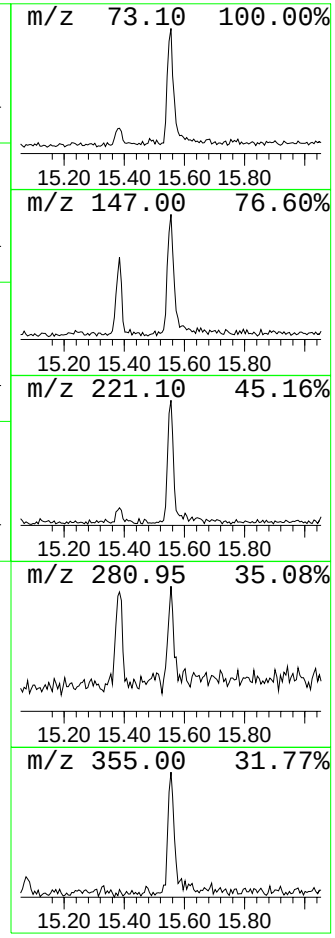


Peak Number: 15 at 15.38 min Area: 97893885 Area % 65.37

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Bis(2-ethylhexyl) phthalate	123509	000117-81-7	86
2 1,2-Benzenedicarboxylic acid, is...	67006	001330-96-7	64
3 Di-n-octyl phthalate	66743	000117-84-0	59

Unknown Spectrum based on Apex minus start of peak

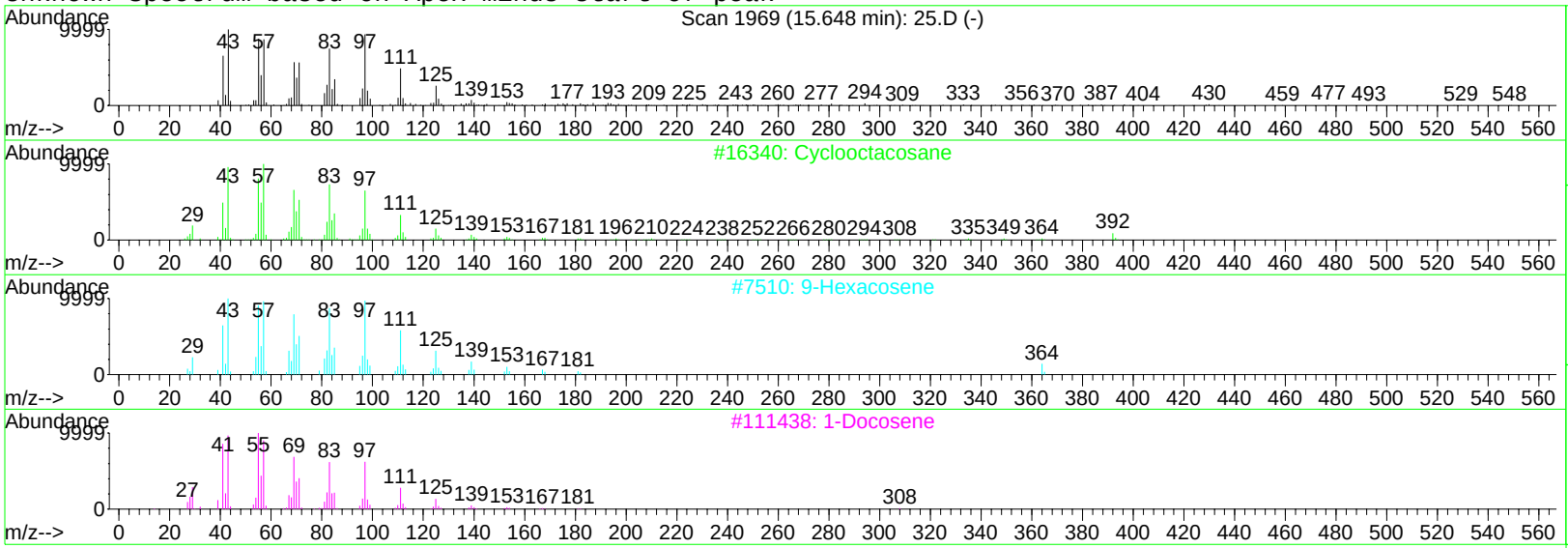


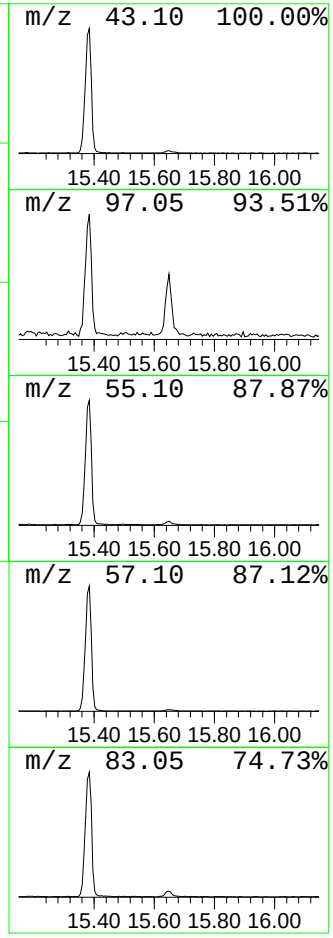


Peak Number: 16 at 15.55 min Area: 1044428 Area % 0.70

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclononasiloxane, octadecamethyl-	115158	000556-71-8	80
2 Trisiloxane, 1,1,1,5,5,5-hexamet...	26961	003555-47-3	32
3 3-Isopropoxy-1,1,1,7,7,7-hexamet...	26784	071579-69-6	28

Unknown Spectrum based on Apex minus start of peak

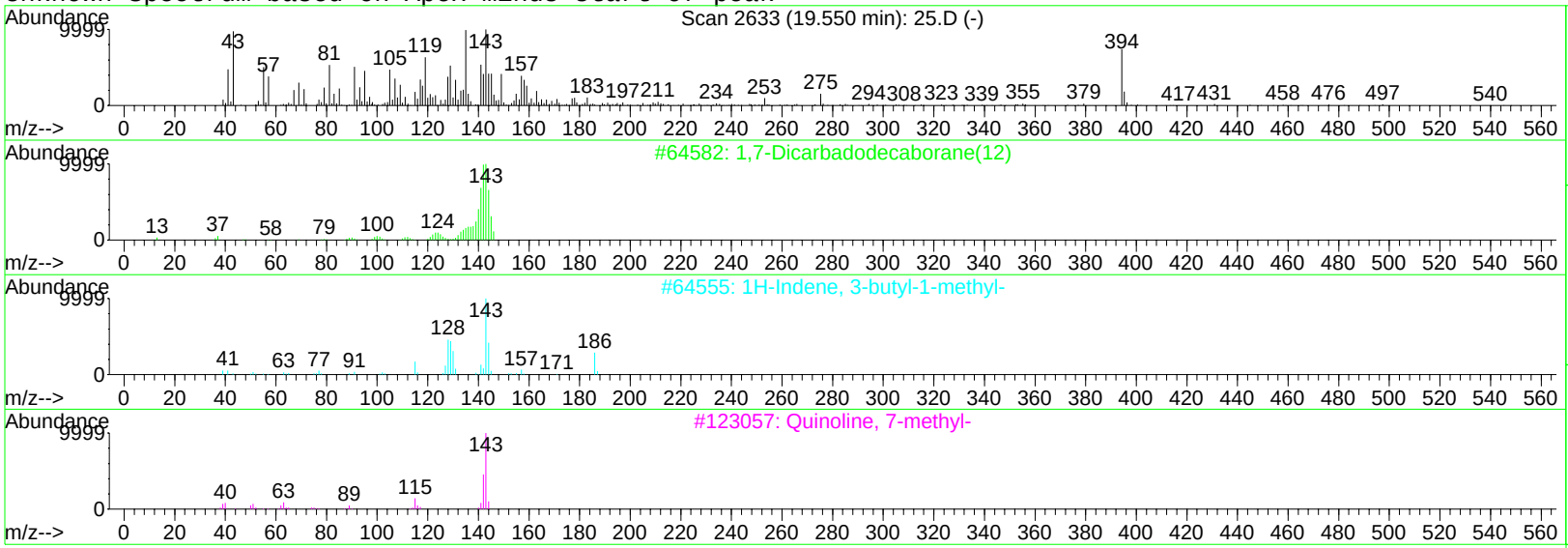


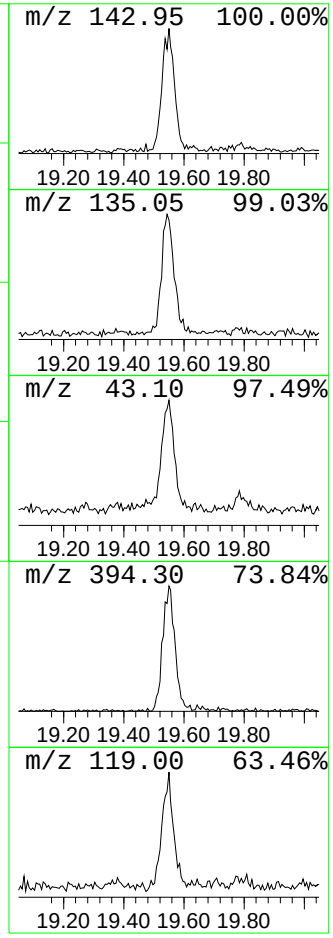


Peak Number: 17 at 15.65 min Area: 1486114 Area % 0.99

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclooctacosane	16340	000297-24-5	94
2 9-Hexacosene	7510	071502-22-2	91
3 1-Docosene	111438	001599-67-3	91

Unknown Spectrum based on Apex minus start of peak

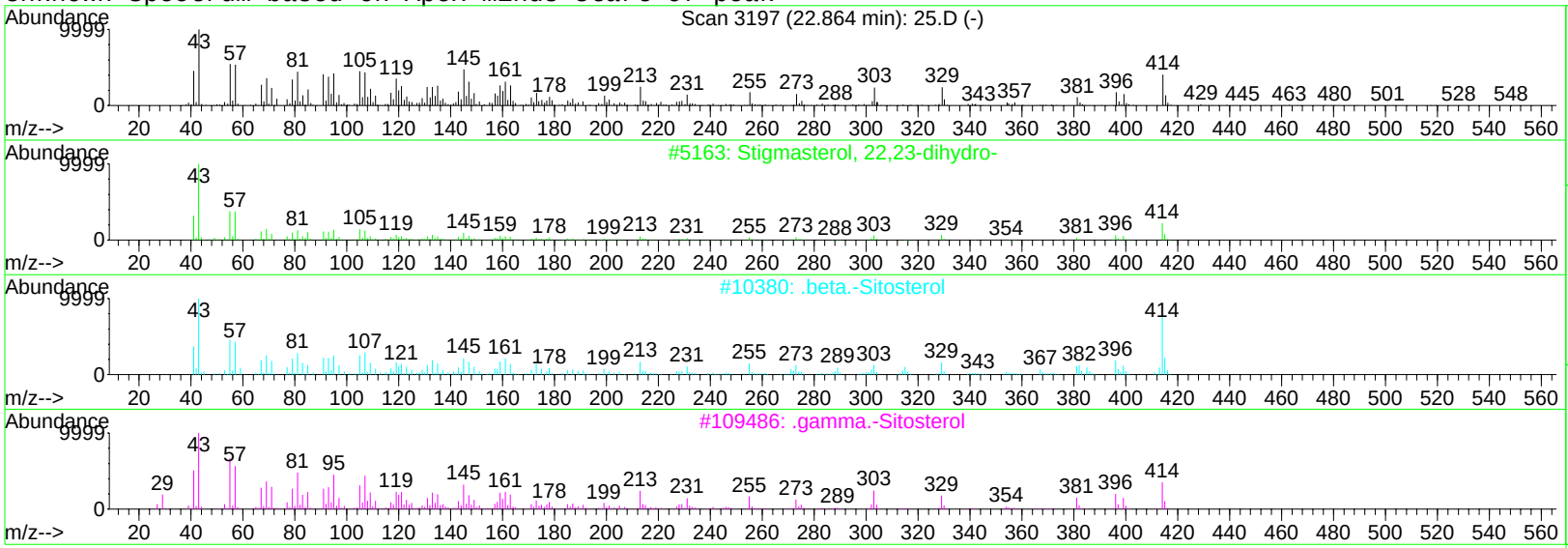


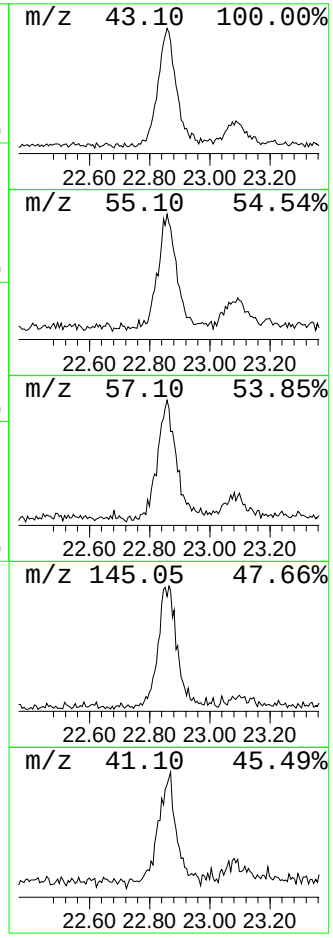


Peak Number: 18 at 19.55 min Area: 3251921 Area % 2.17

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1,7-Dicarbadodecaborane(12)	64582	016986-24-6	18
2 1H-Indene, 3-butyl-1-methyl-	64555	111400-84-1	12
3 Quinoline, 7-methyl-	123057	000612-60-2	10

Unknown Spectrum based on Apex minus start of peak

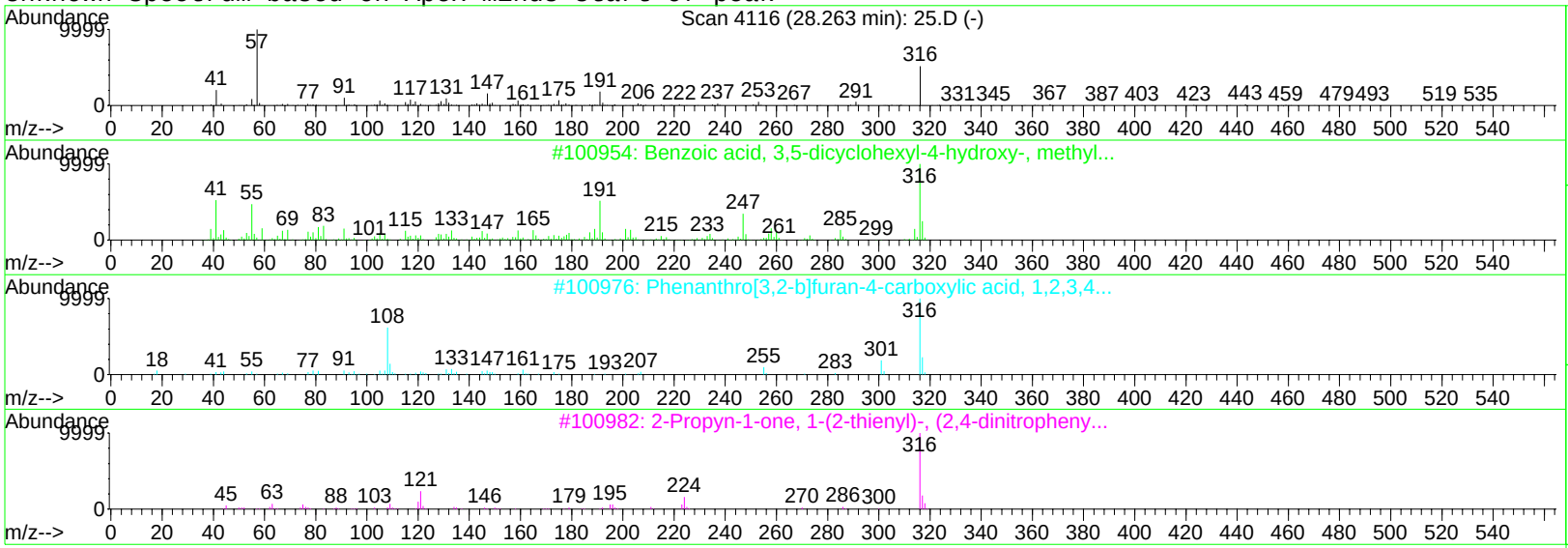


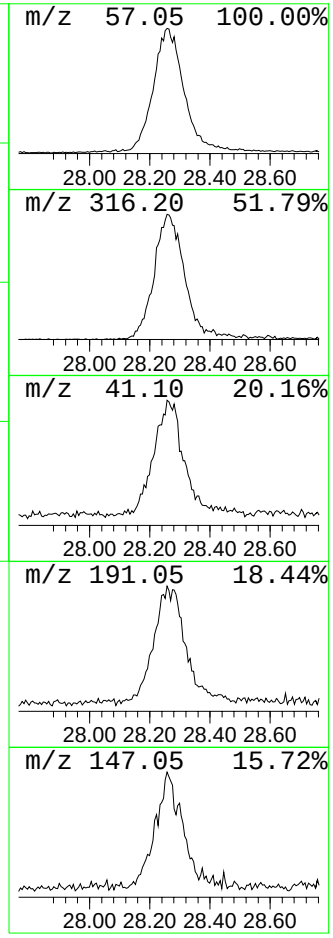


Peak Number: 19 at 22.86 min Area: 7106714 Area % 4.75

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Stigmasterol, 22,23-dihydro-	5163	1000214-20-7	96
2 .beta.-Sitosterol	10380	000083-46-5	89
3 .gamma.-Sitosterol	109486	000083-47-6	76

Unknown Spectrum based on Apex minus start of peak





Peak Number: 20 at 28.26 min Area: 9337475 Area % 6.24

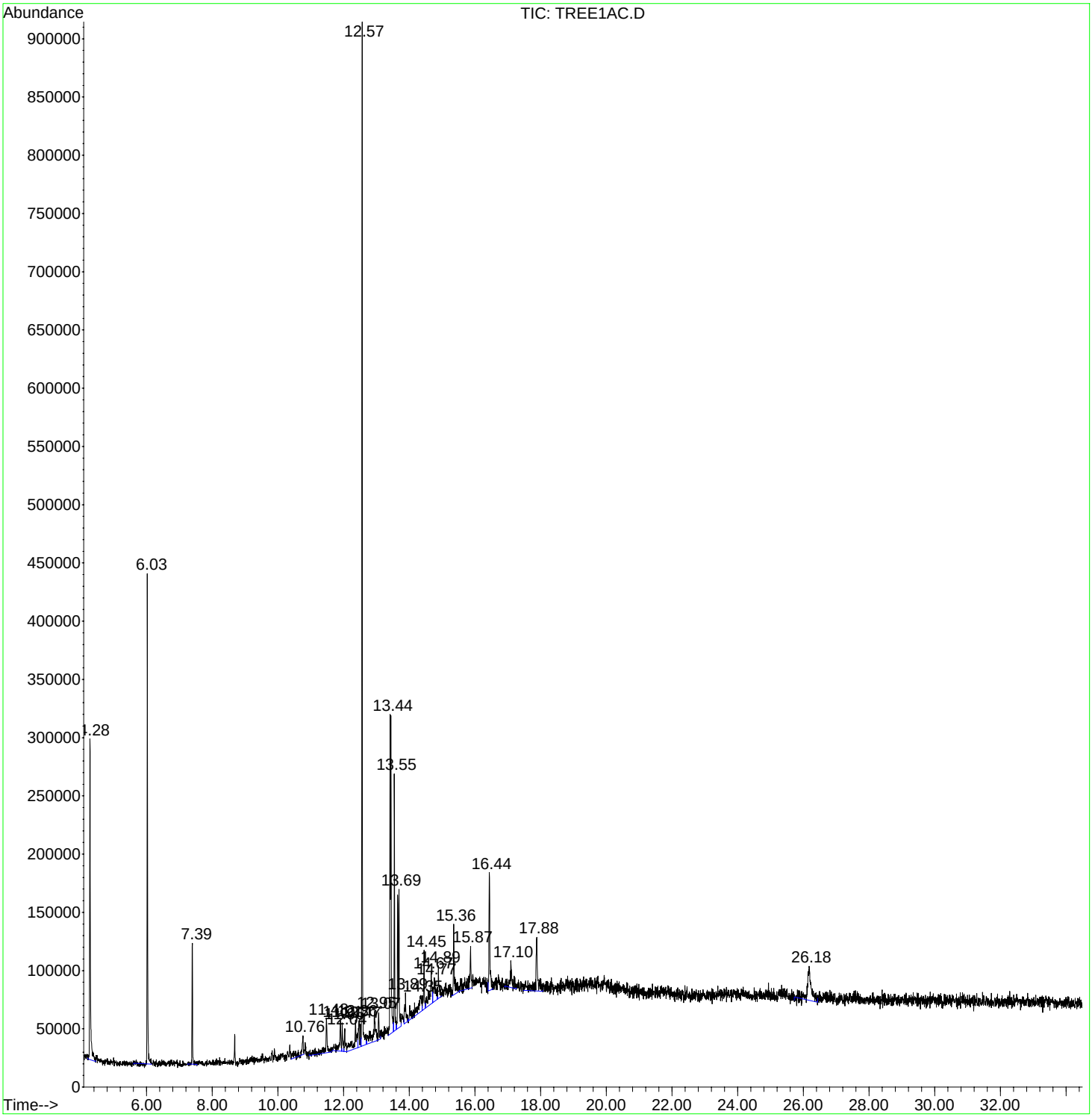
The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Benzoic acid, 3,5-dicyclohexyl-4...	100954	055125-23-0	23
2 Phenanthro[3,2-b]furan-4-carboxy...	100976	019941-61-8	12
3 2-Propyn-1-one, 1-(2-thienyl)-, ...	100982	056588-20-6	9

Library Search Report

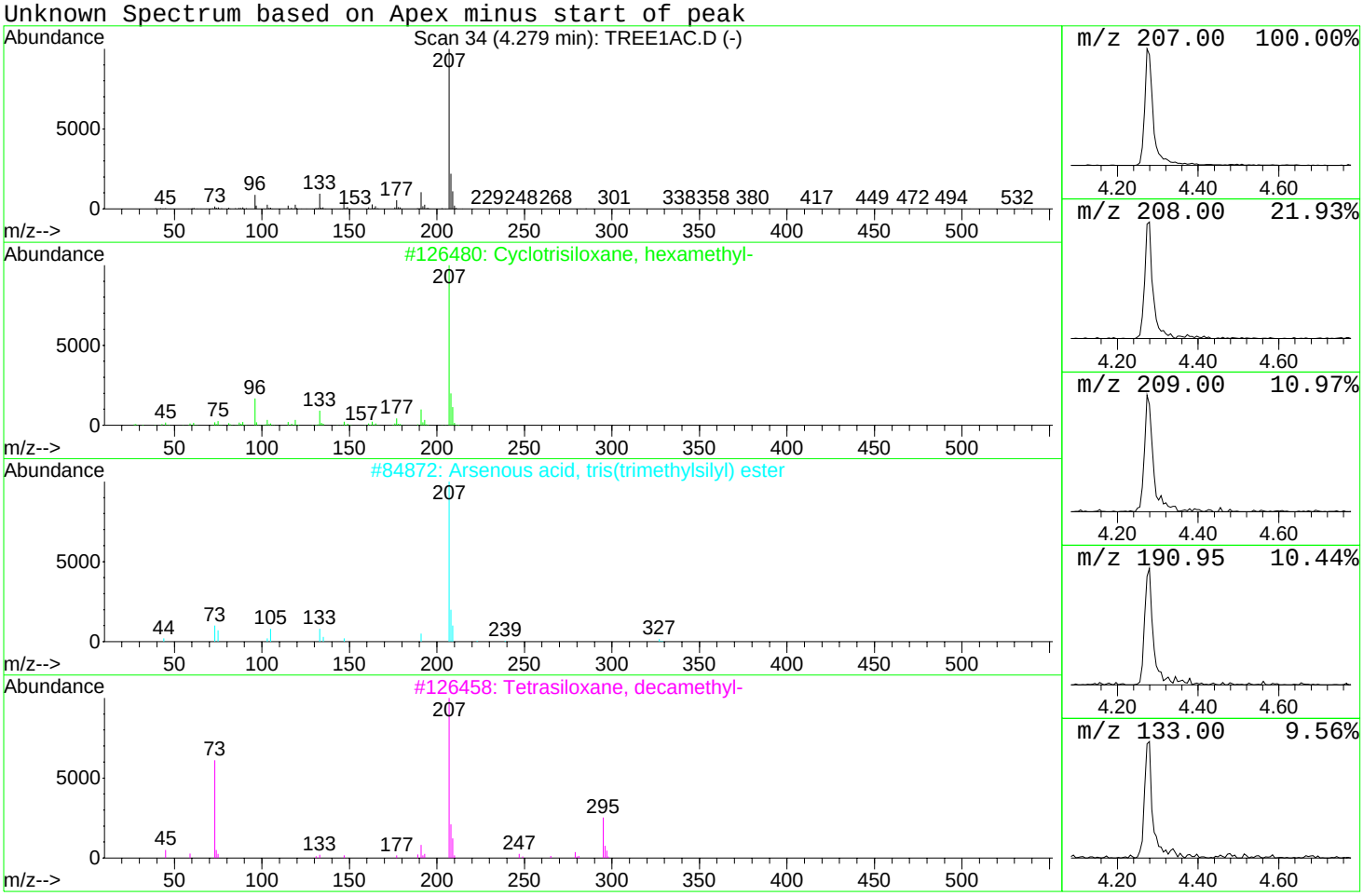
Data File : D:\PRENISHA\TREE1AC.D
Acq On : 28 Jun 2006 11:06
Sample : Tree 1 acetone
Misc : 1µl inject, 1:75 split, neat

Vial: 1
Operator: Prenisha
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e
Method : C:\MSDCHEM\1\METHODS\NEW.M (Chemstation Integrator)
Title :



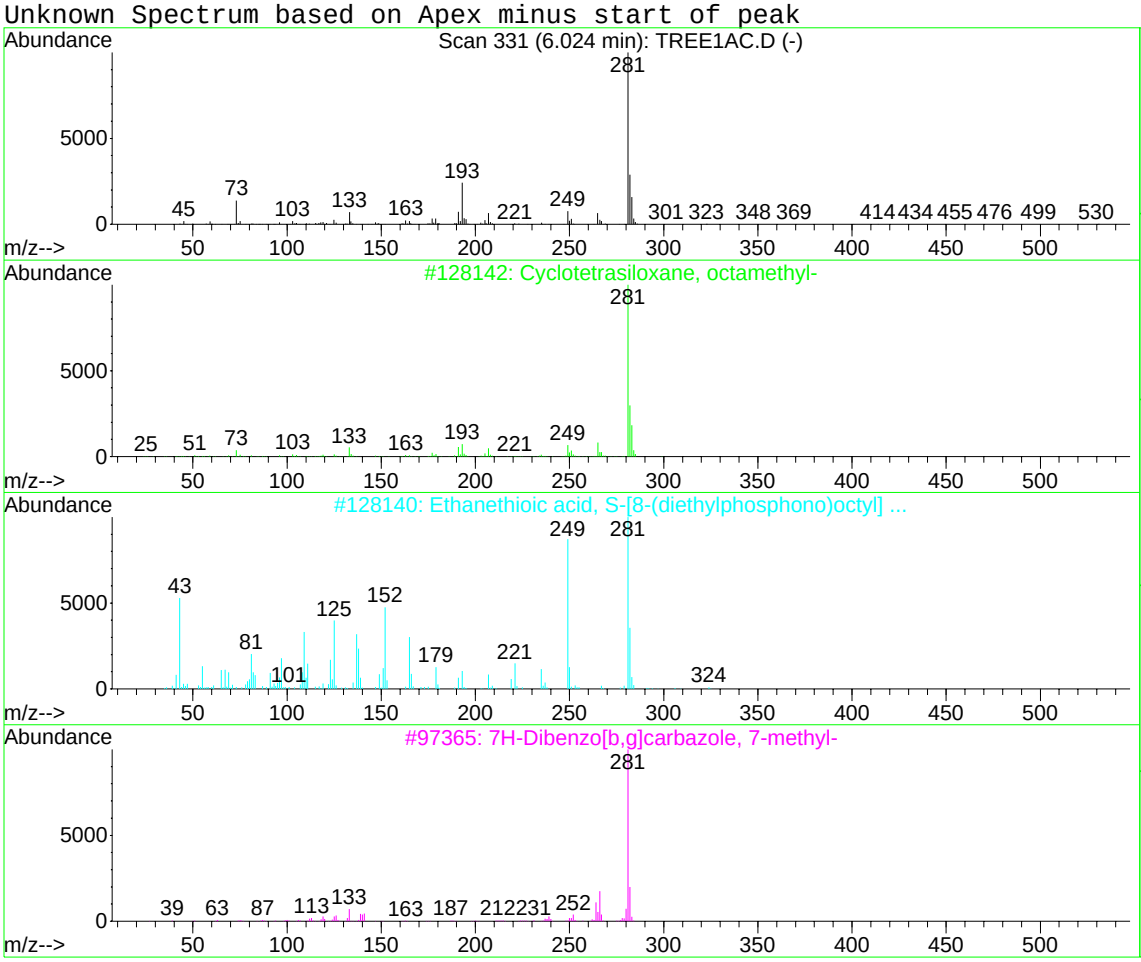
Unknown Spectrum based on Apex minus start of peak

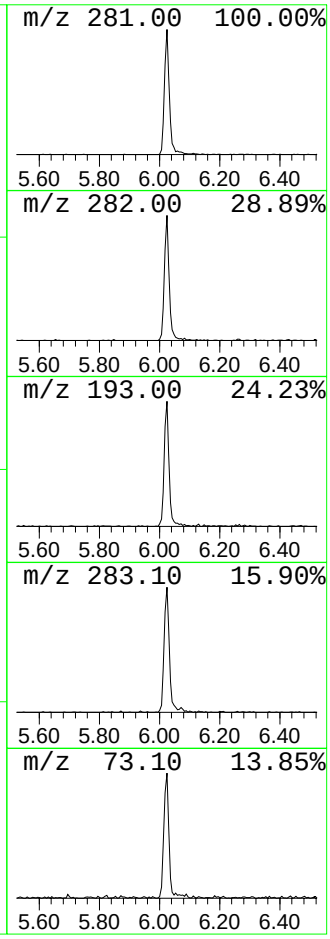


Peak Number: 1 at 4.28 min Area: 4390318 Area % 7.87

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclotrisiloxane, hexamethyl-	126480	000541-05-9	87
2 Arsenous acid, tris(trimethylsil...	84872	055429-29-3	72
3 Tetrasiloxane, decamethyl-	126458	000141-62-8	56

Unknown Spectrum based on Apex minus start of peak

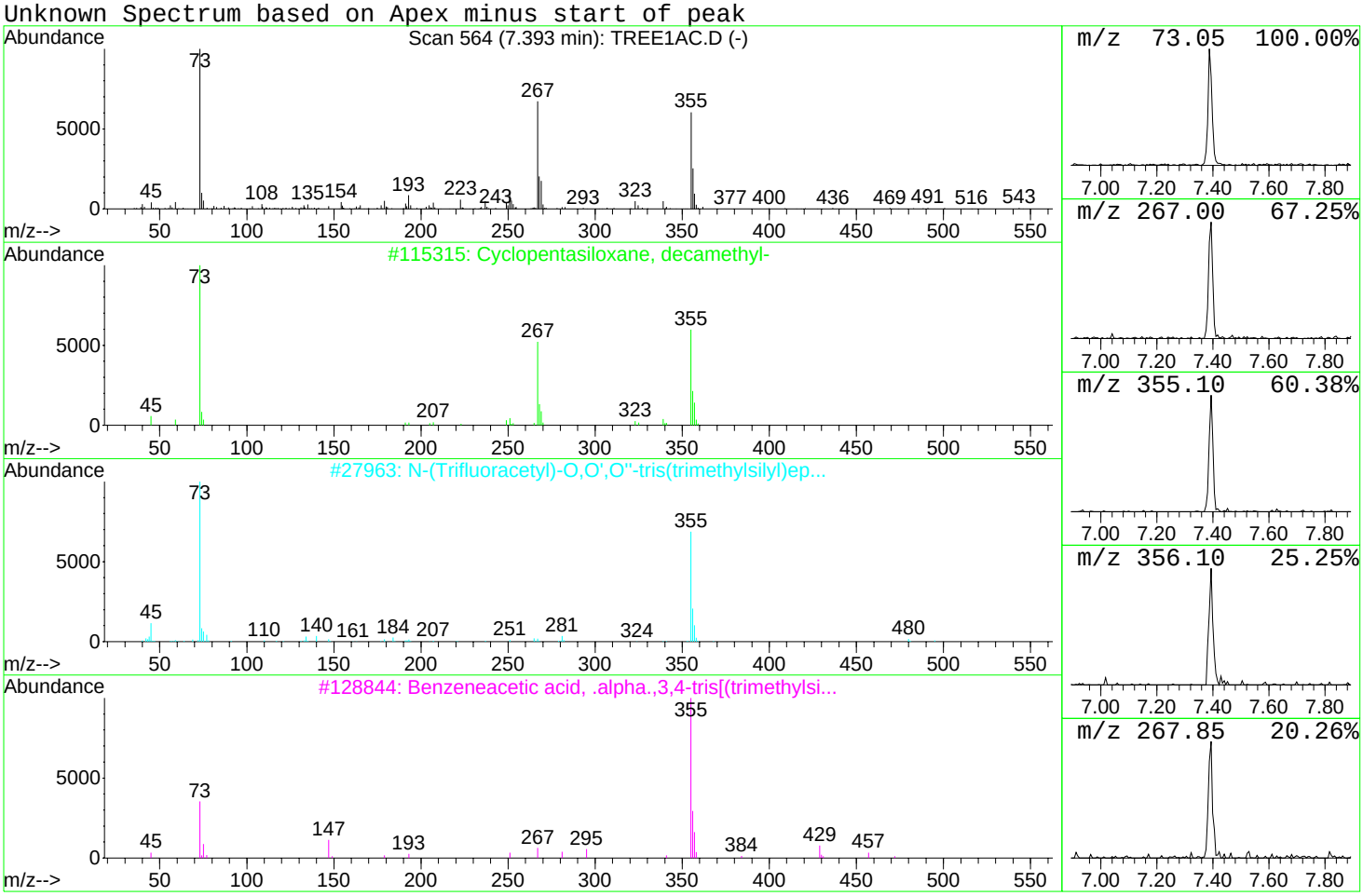




Peak Number: 2 at 6.02 min Area: 4822890 Area % 8.65

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclotetrasiloxane, octamethyl-	128142	000556-67-2	74
2 Ethanethioic acid, S-[8-(diethyl...	128140	129065-11-8	62
3 7H-Dibenzo[b,g]carbazole, 7-methyl-	97365	003557-49-1	50

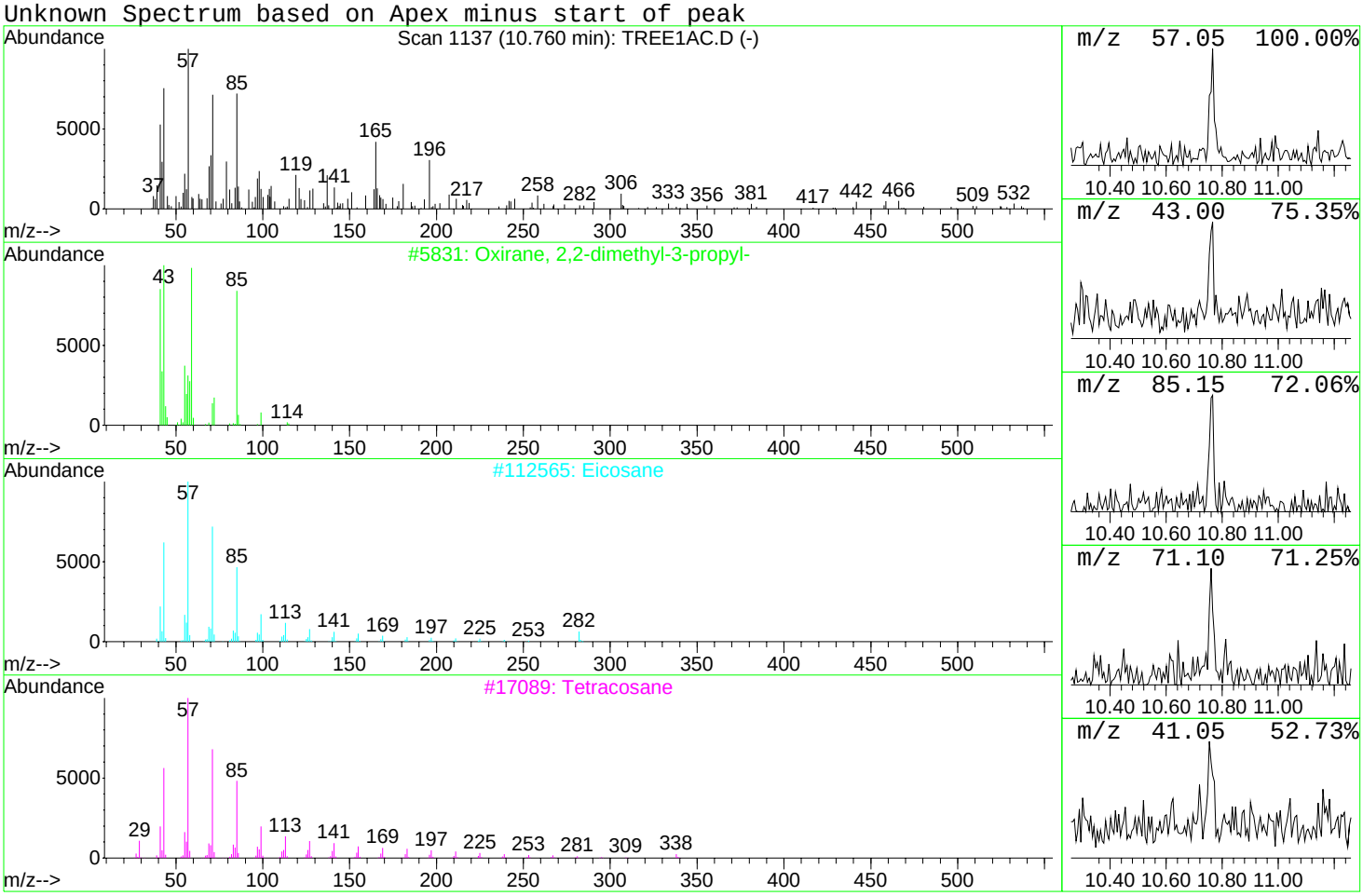
Unknown Spectrum based on Apex minus start of peak



Peak Number: 3 at 7.39 min Area: 1305067 Area % 2.34

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclopentasiloxane, decamethyl-	115315	000541-02-6	86
2 N-(Trifluoracetyl)-O,O',O''-tris...	27963	054135-51-2	43
3 Benzeneacetic acid, .alpha.,3,4-...	128844	037148-65-5	40

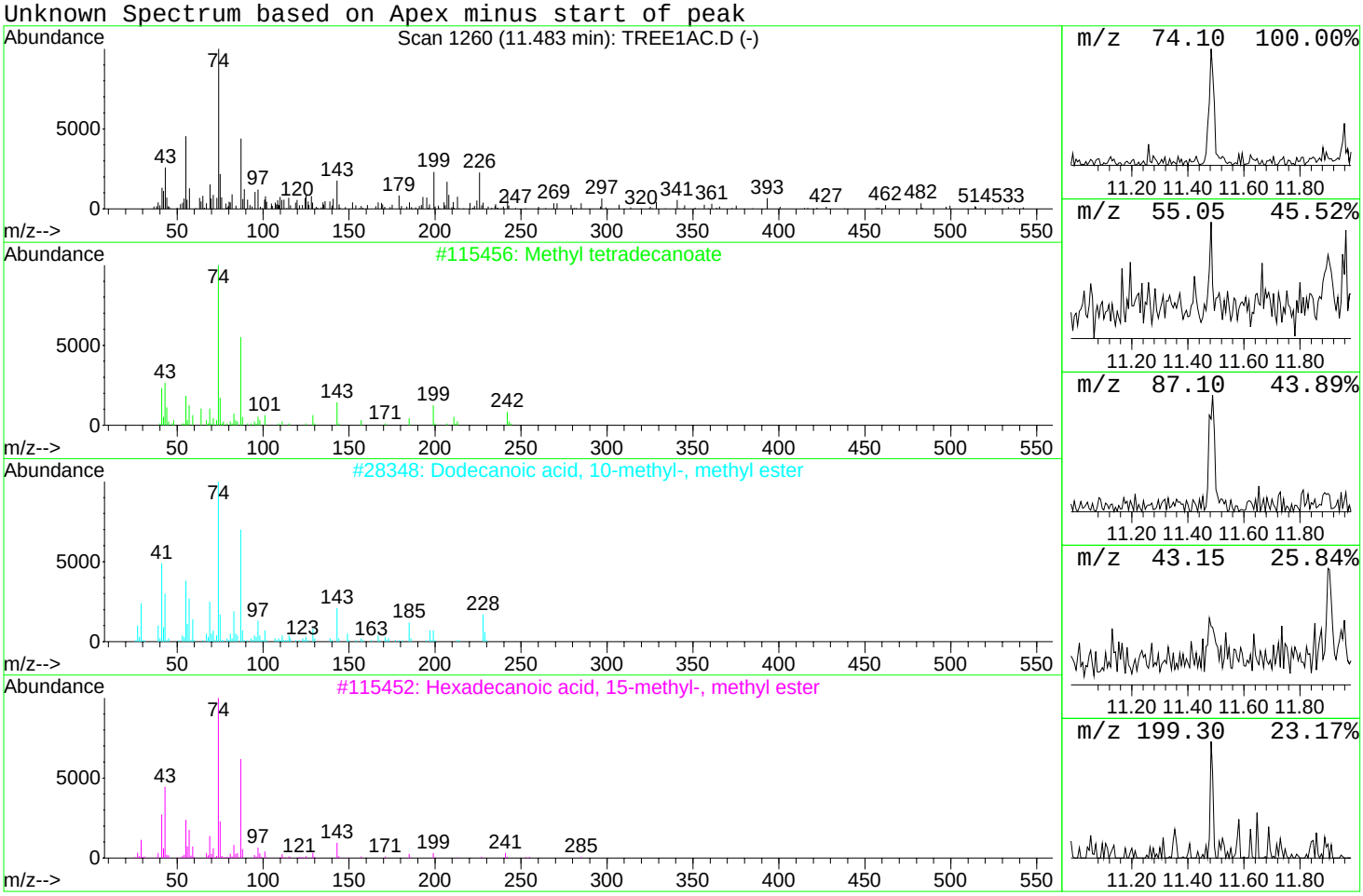
Unknown Spectrum based on Apex minus start of peak



Peak Number: 4 at 10.76 min Area: 695882 Area % 1.25

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Oxirane, 2,2-dimethyl-3-propyl-	5831	017612-35-0	25
2 Eicosane	112565	000112-95-8	25
3 Tetracosane	17089	000646-31-1	22

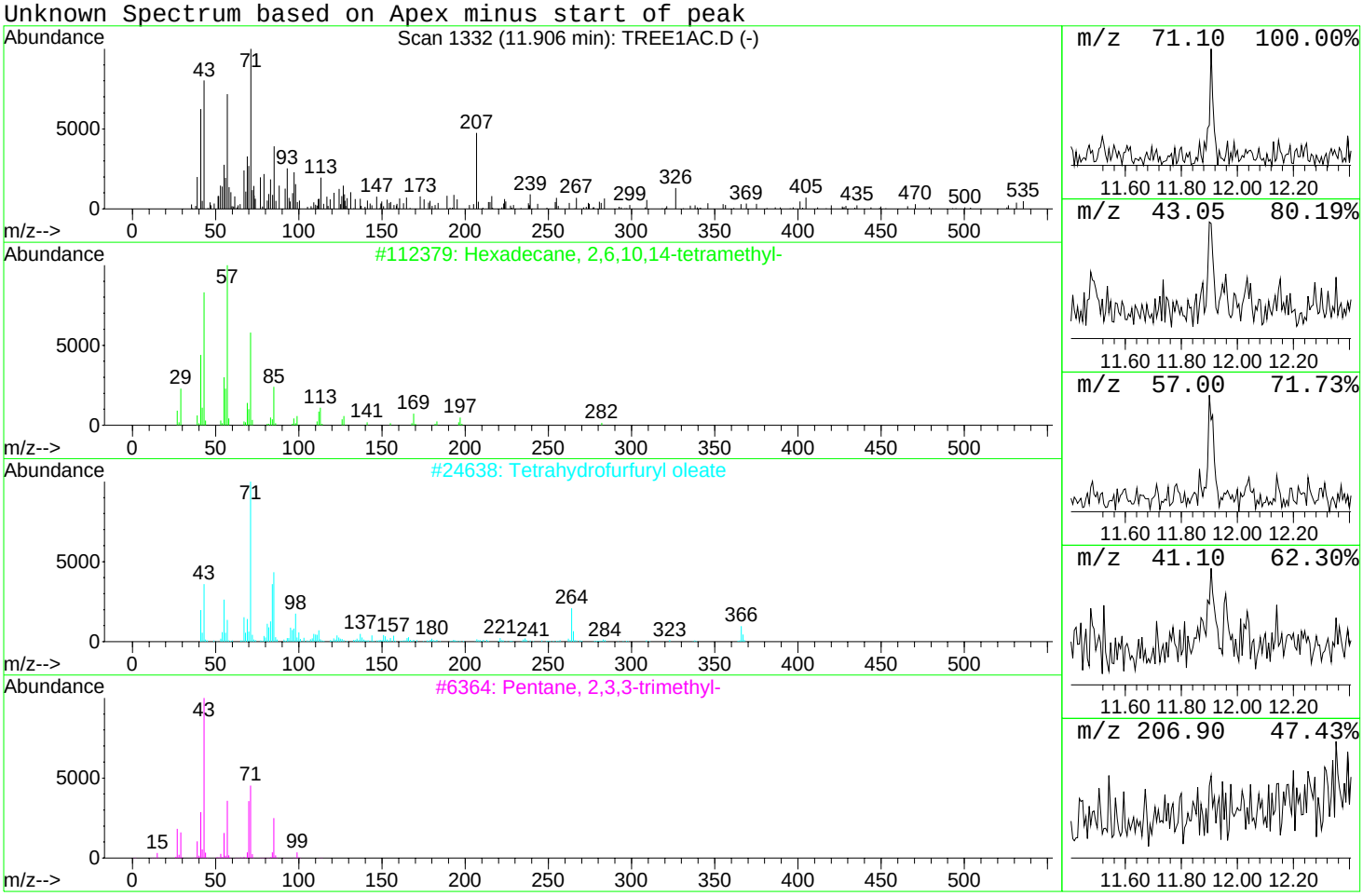
Unknown Spectrum based on Apex minus start of peak



Peak Number: 5 at 11.48 min Area: 1144851 Area % 2.05

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Methyl tetradecanoate	115456	000124-10-7	58
2 Dodecanoic acid, 10-methyl-, met...	28348	005129-65-7	58
3 Hexadecanoic acid, 15-methyl-, m...	115452	006929-04-0	53

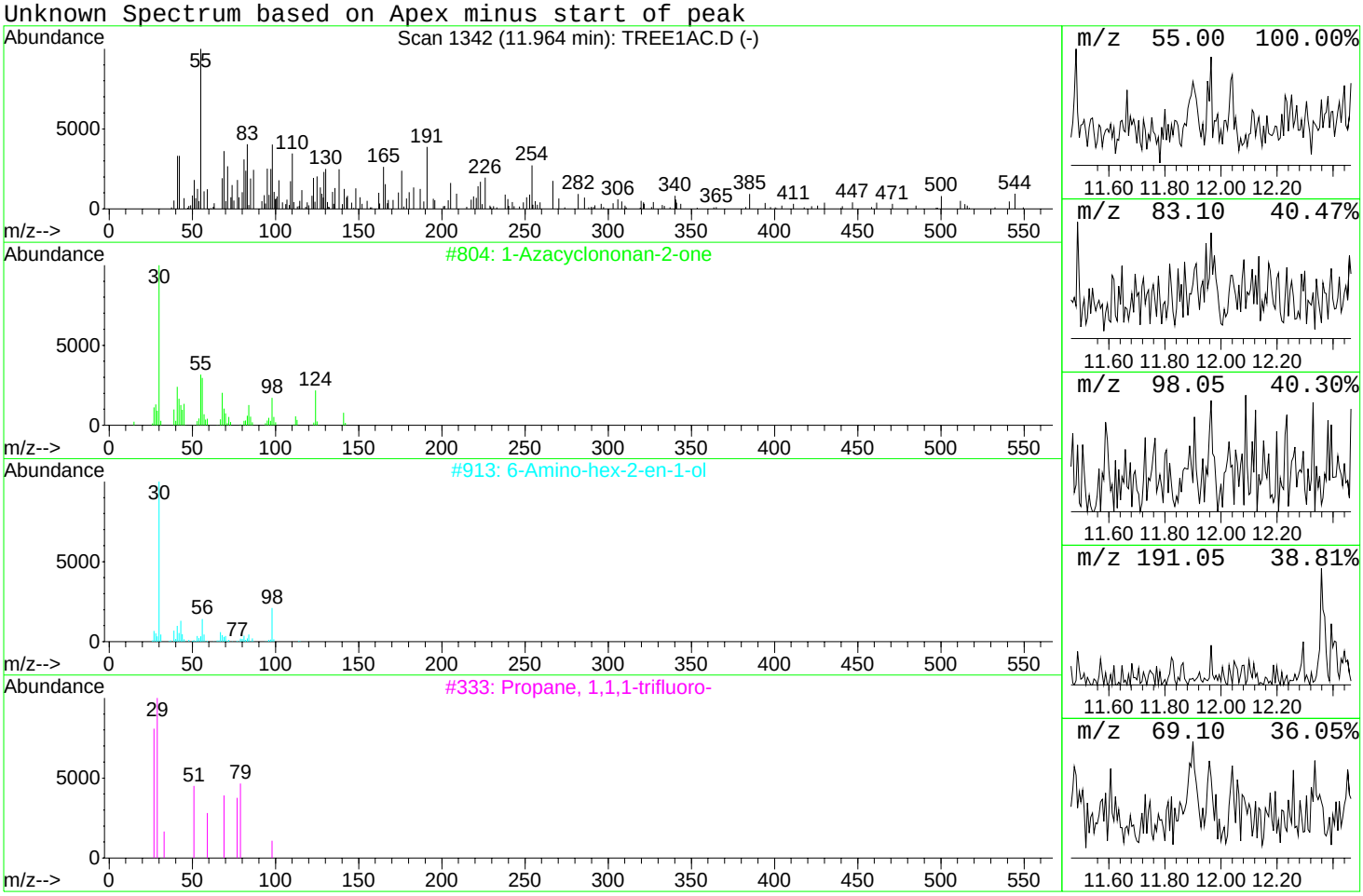
Unknown Spectrum based on Apex minus start of peak



Peak Number: 6 at 11.91 min Area: 879835 Area % 1.58

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Hexadecane, 2,6,10,14-tetramethyl-	112379	000638-36-8	49
2 Tetrahydrofurfuryl oleate	24638	1000131-42-2	47
3 Pentane, 2,3,3-trimethyl-	6364	000560-21-4	47

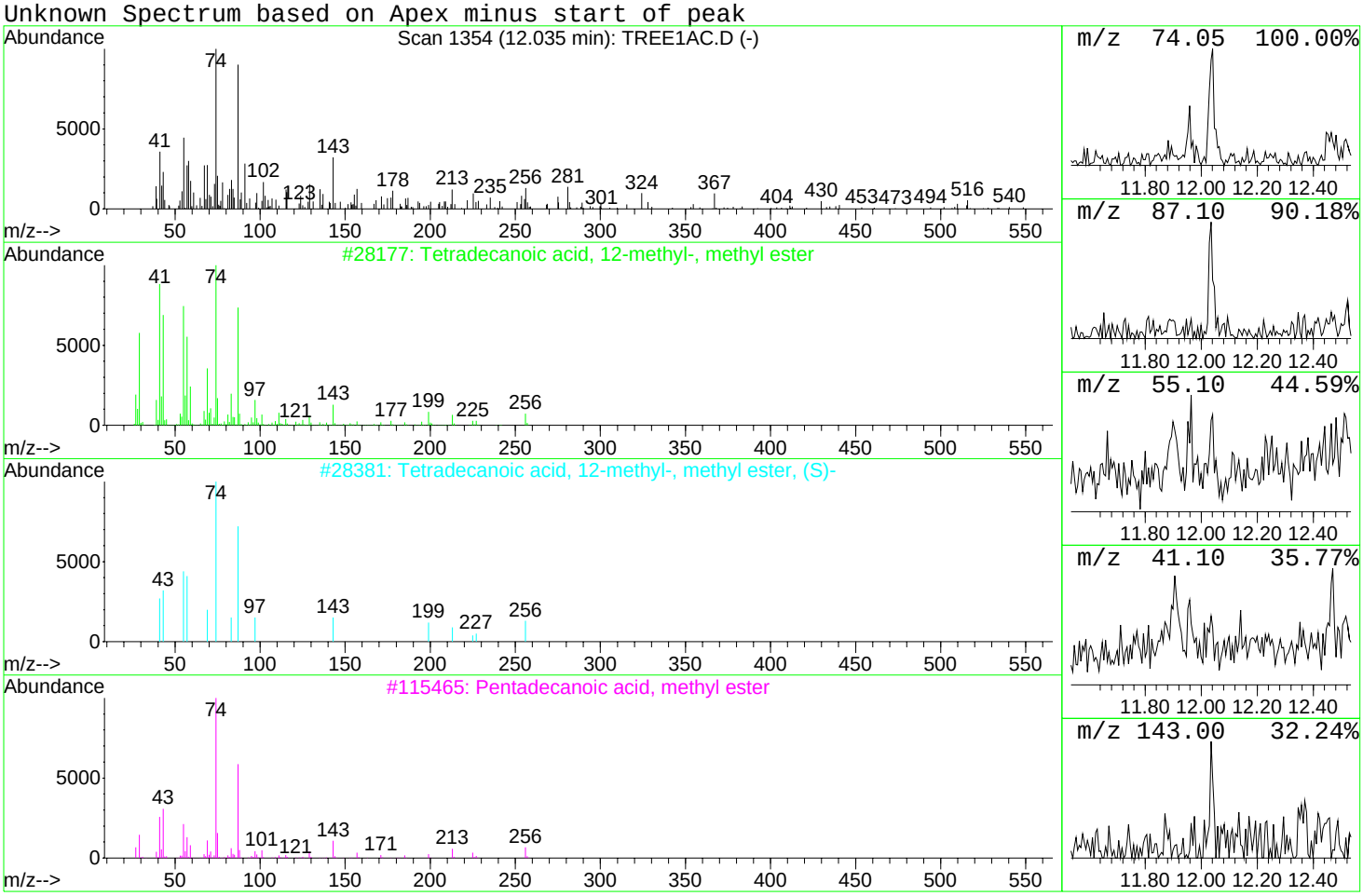
Unknown Spectrum based on Apex minus start of peak



Peak Number: 7 at 11.96 min Area: 427998 Area % 0.77

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1-Azacyclononan-2-one	804	000935-30-8	8
2 6-Amino-hex-2-en-1-ol	913	1000190-02-5	2
3 Propane, 1,1,1-trifluoro-	333	000421-07-8	1

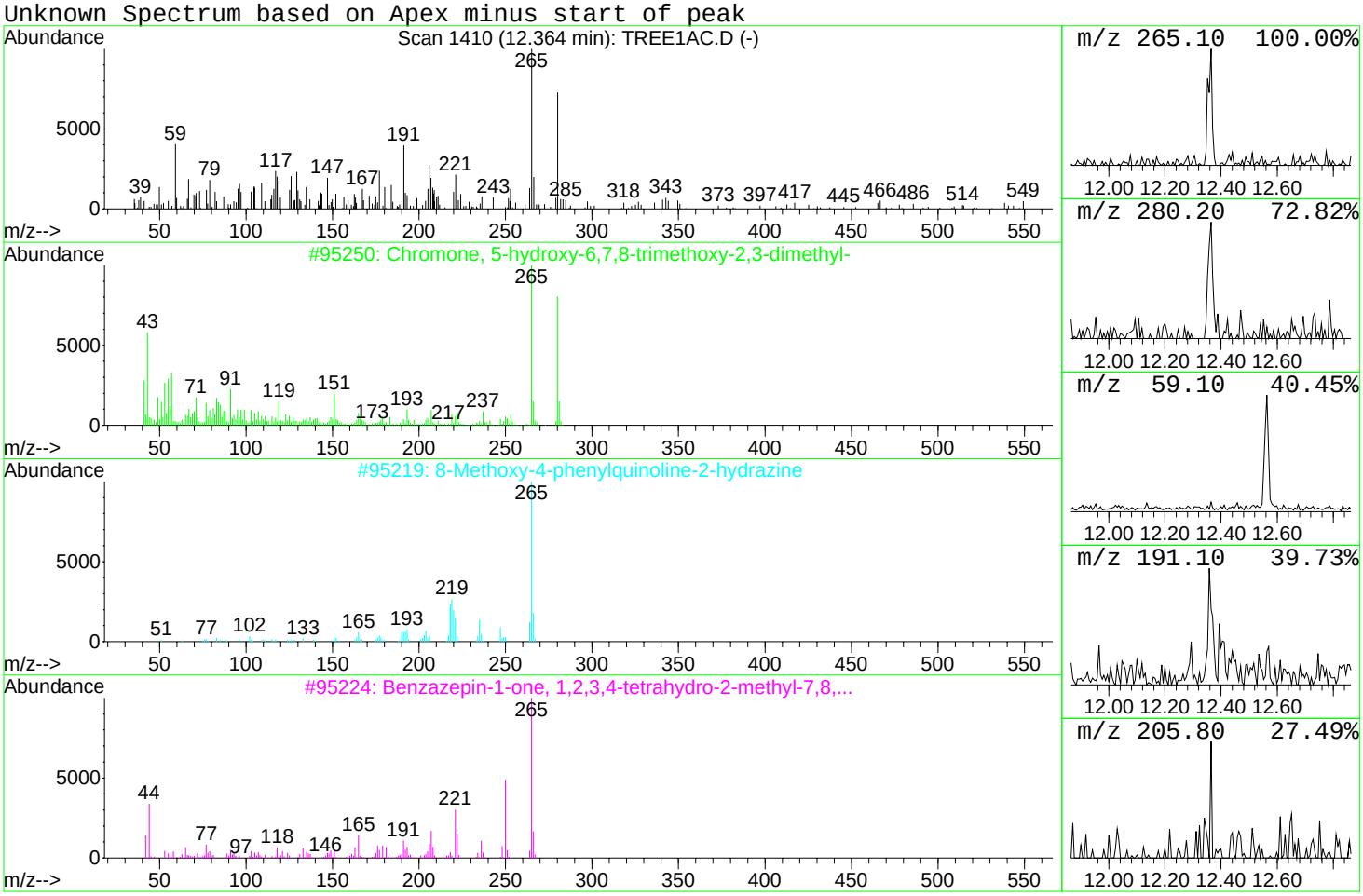
Unknown Spectrum based on Apex minus start of peak



Peak Number: 8 at 12.03 min Area: 417361 Area % 0.75

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Tetradecanoic acid, 12-methyl-, ...	28177	005129-66-8	81
2 Tetradecanoic acid, 12-methyl-, ...	28381	062691-05-8	72
3 Pentadecanoic acid, methyl ester	115465	007132-64-1	72

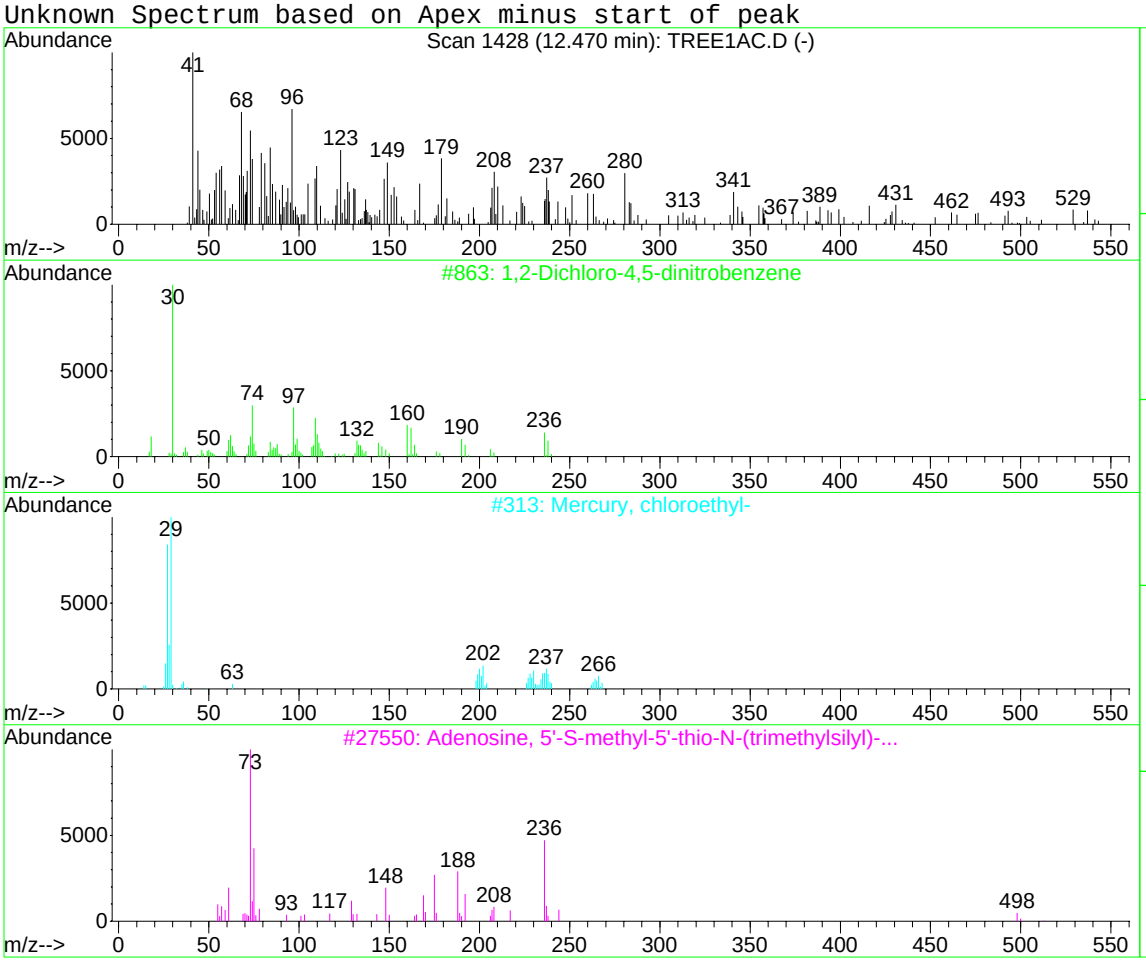
Unknown Spectrum based on Apex minus start of peak

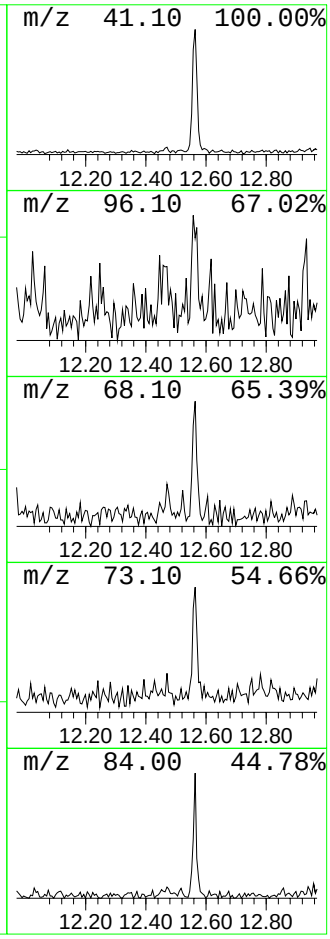


Peak Number: 9 at 12.36 min Area: 1289718 Area % 2.31

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Chromone, 5-hydroxy-6,7,8-trimet...	95250	1000124-95-9	41
2 8-Methoxy-4-phenylquinoline-2-hy...	95219	1000110-62-8	30
3 Benzazepin-1-one, 1,2,3,4-tetrah...	95224	1000129-96-5	27

Unknown Spectrum based on Apex minus start of peak

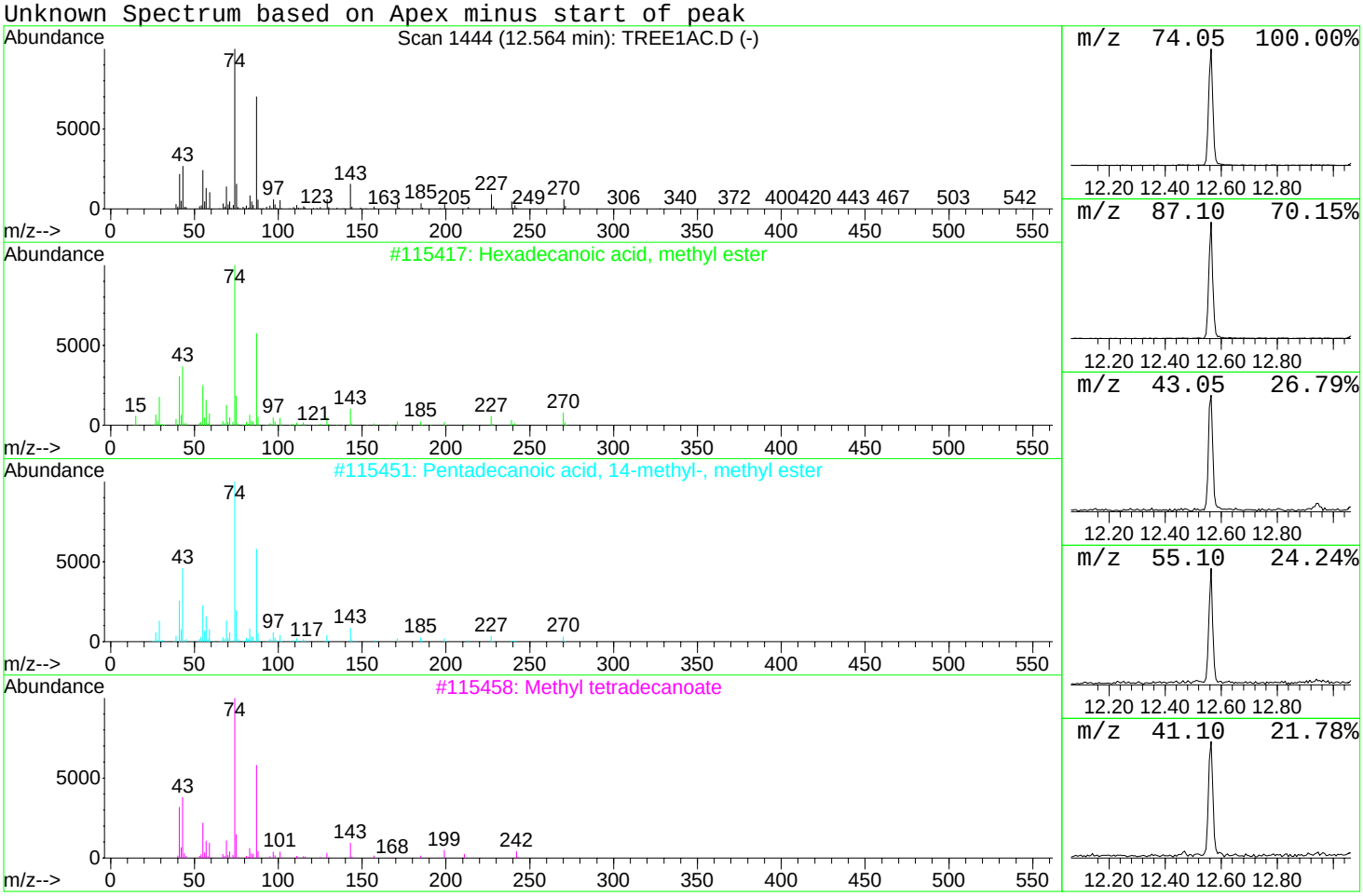




Peak Number: 10 at 12.47 min Area: 673925 Area % 1.21

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1,2-Dichloro-4,5-dinitrobenzene	863	006306-39-4	10
2 Mercury, chloroethyl-	313	000107-27-7	9
3 Adenosine, 5'-S-methyl-5'-thio-N...	27550	054623-28-8	9

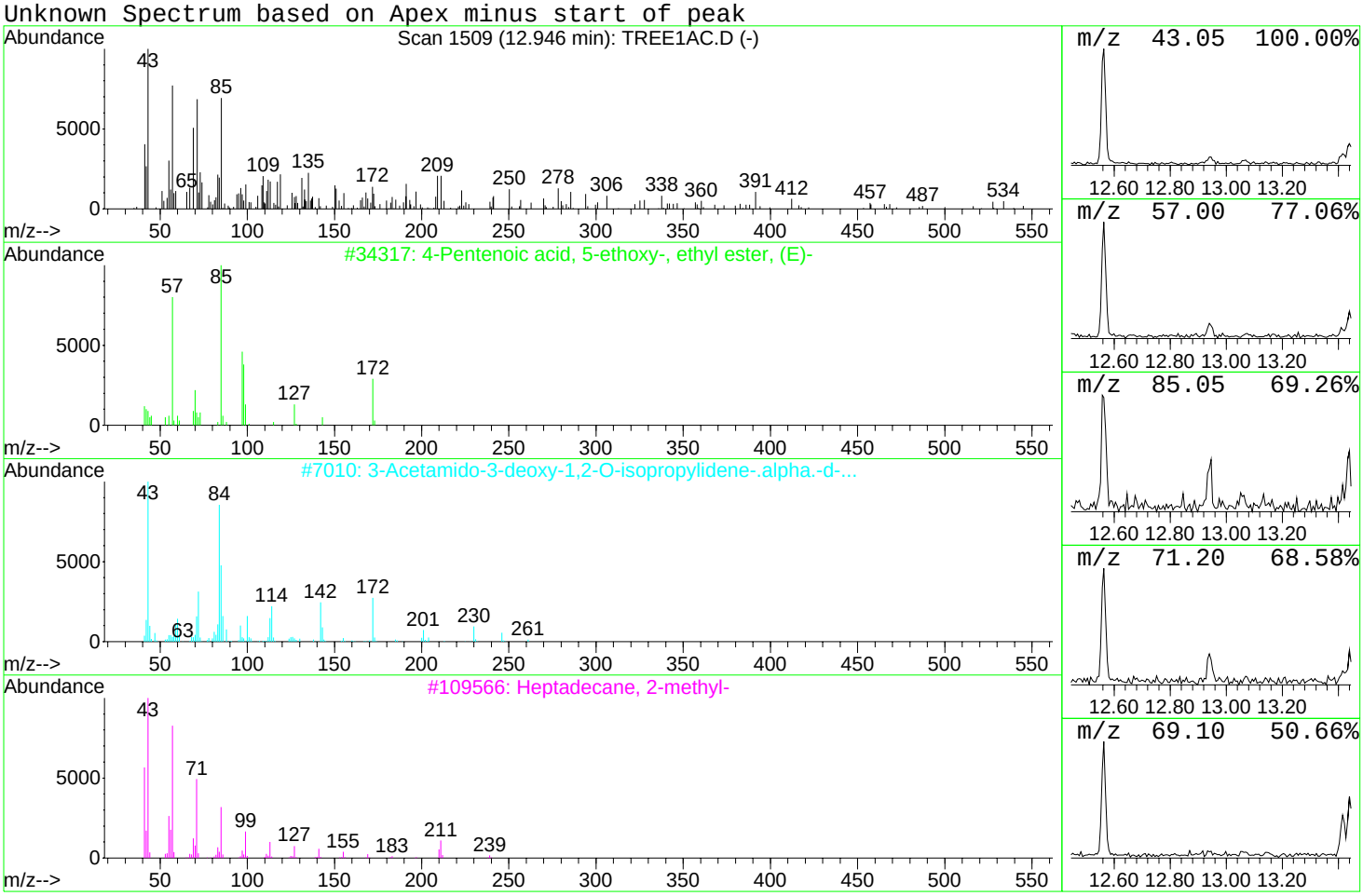
Unknown Spectrum based on Apex minus start of peak



Peak Number: 11 at 12.56 min Area: 9554857 Area % 17.13

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Hexadecanoic acid, methyl ester	115417	000112-39-0	98
2 Pentadecanoic acid, 14-methyl-, ...	115451	005129-60-2	94
3 Methyl tetradecanoate	115458	000124-10-7	91

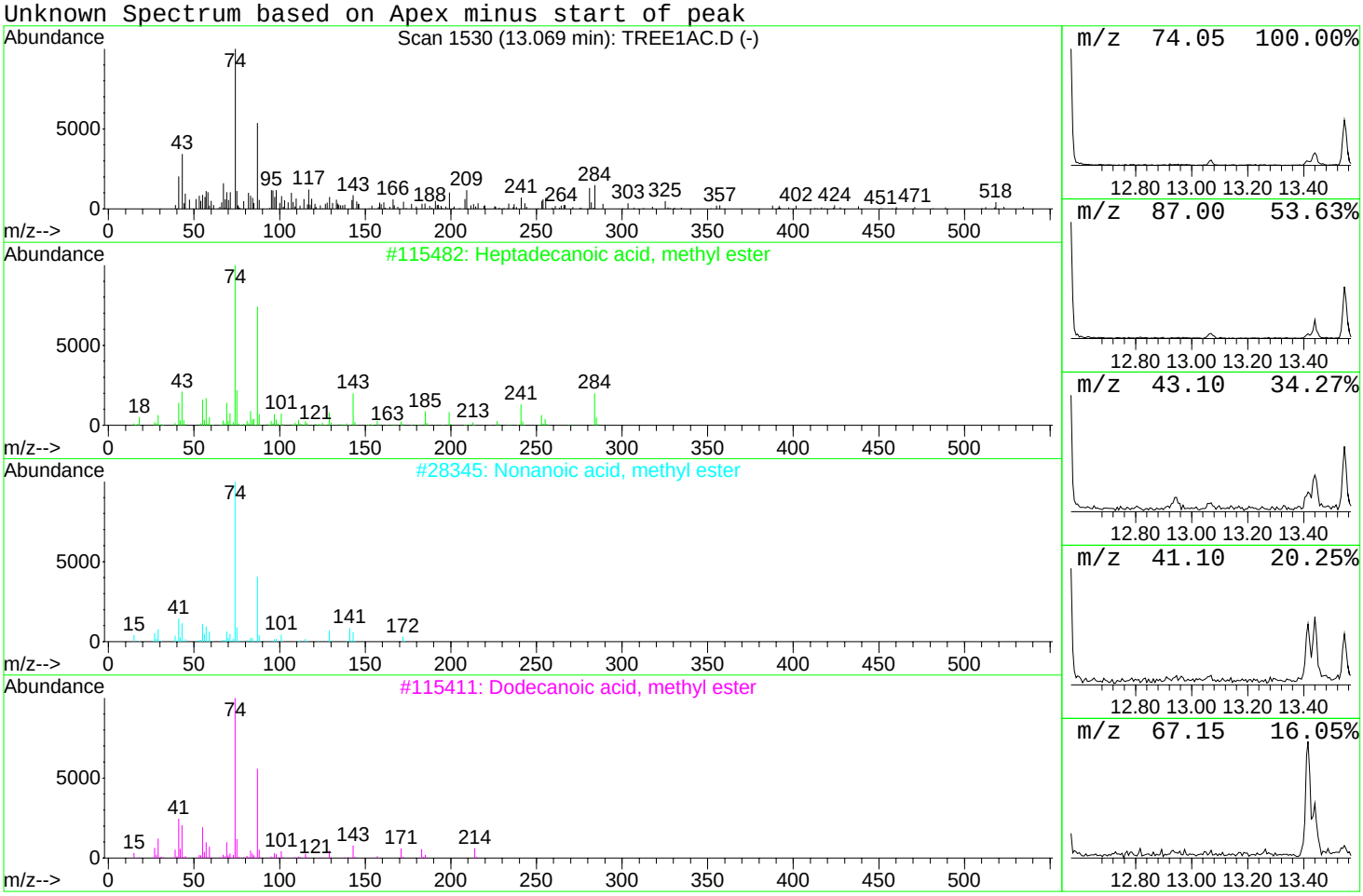
Unknown Spectrum based on Apex minus start of peak



Peak Number: 12 at 12.95 min Area: 1432400 Area % 2.57

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 4-Pentenoic acid, 5-ethoxy-, eth...	34317	055162-84-0	37
2 3-Acetamido-3-deoxy-1,2-O-isopro...	7010	1000214-30-6	25
3 Heptadecane, 2-methyl-	109566	001560-89-0	22

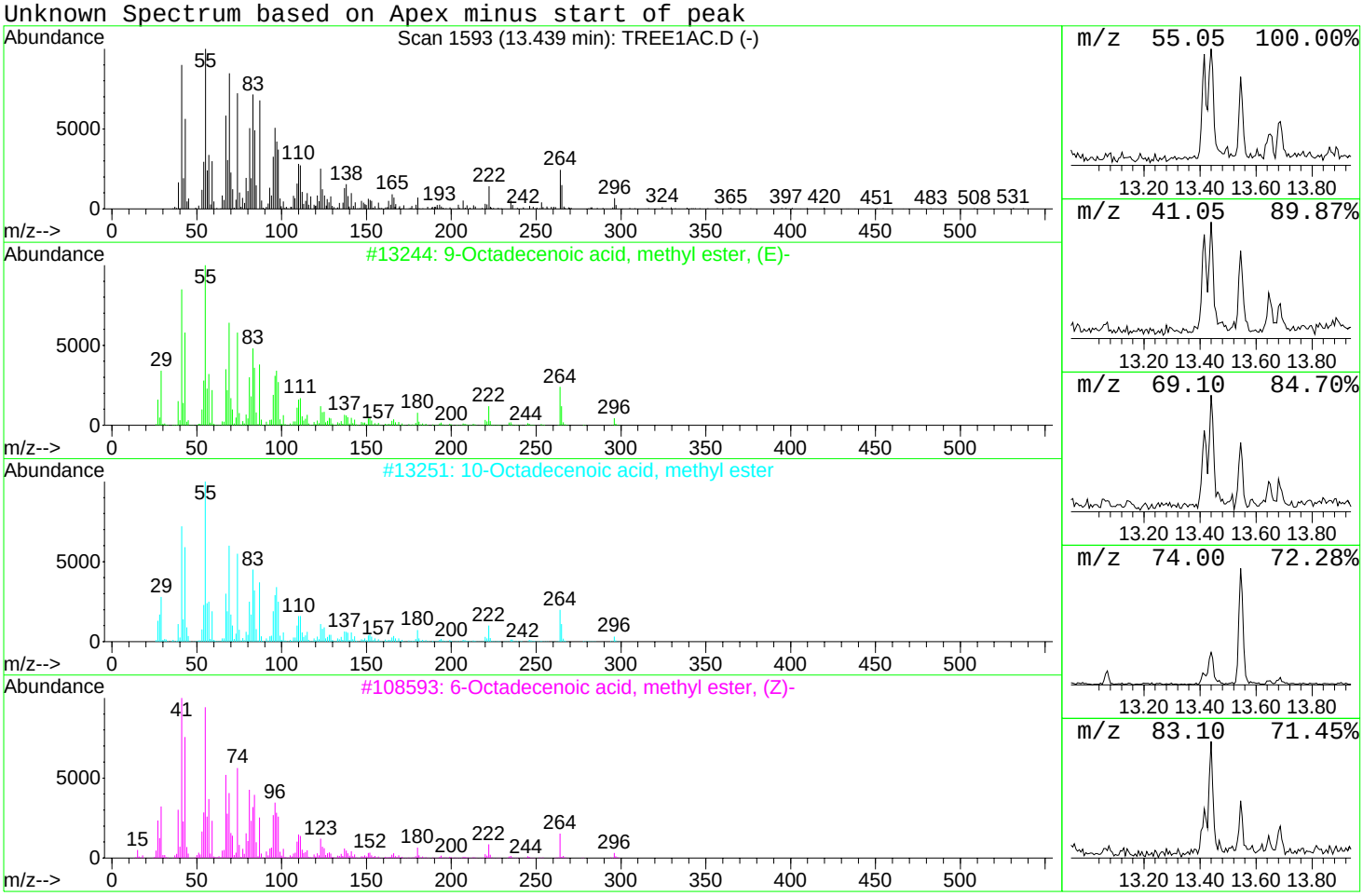
Unknown Spectrum based on Apex minus start of peak



Peak Number: 13 at 13.07 min Area: 445789 Area % 0.80

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Heptadecanoic acid, methyl ester	115482	001731-92-6	81
2 Nonanoic acid, methyl ester	28345	001731-84-6	68
3 Dodecanoic acid, methyl ester	115411	000111-82-0	68

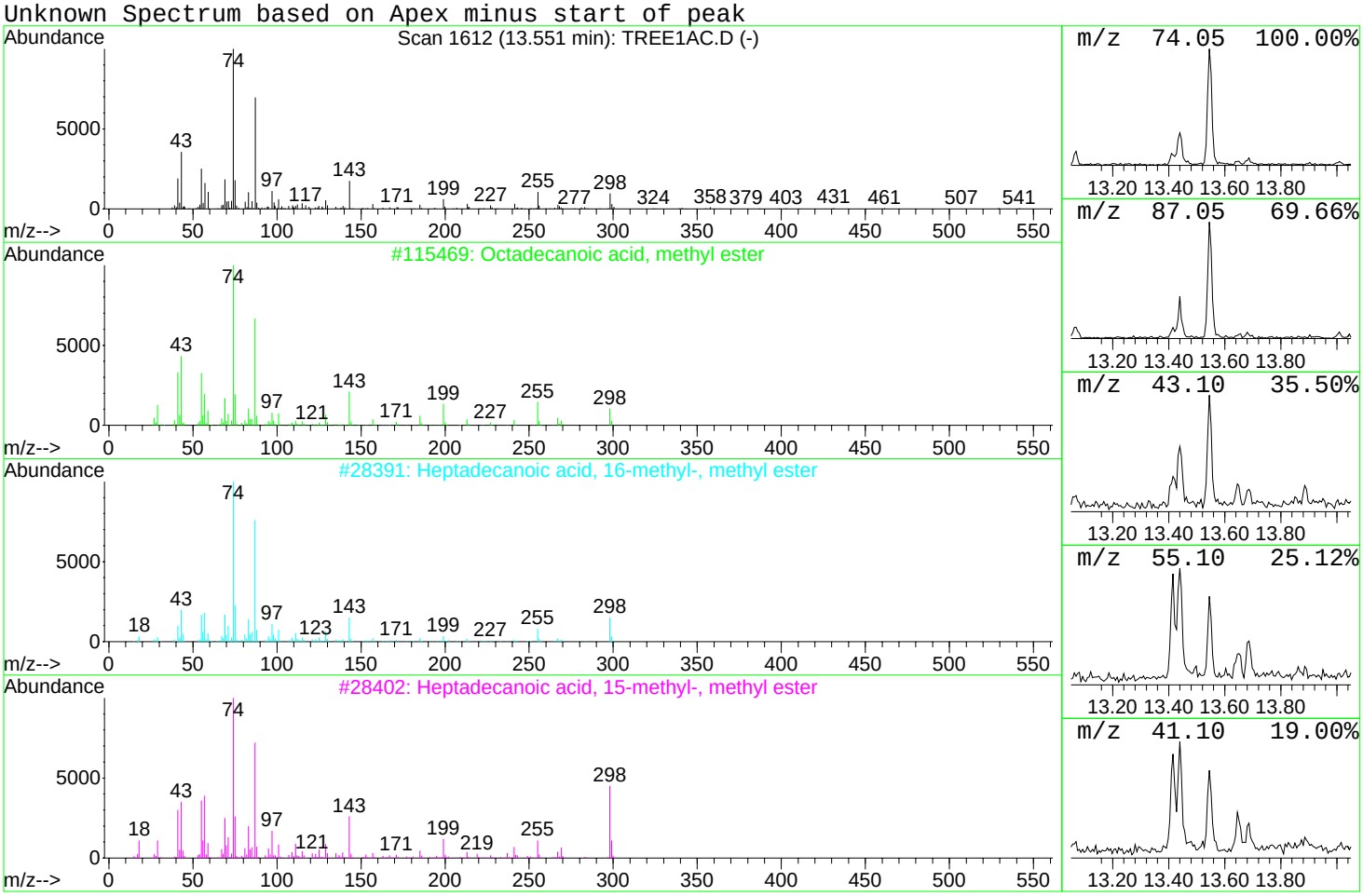
Unknown Spectrum based on Apex minus start of peak



Peak Number: 14 at 13.44 min Area: 6520305 Area % 11.69

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 9-Octadecenoic acid, methyl este...	13244	001937-62-8	96
2 10-Octadecenoic acid, methyl ester	13251	013481-95-3	94
3 6-Octadecenoic acid, methyl este...	108593	002777-58-4	91

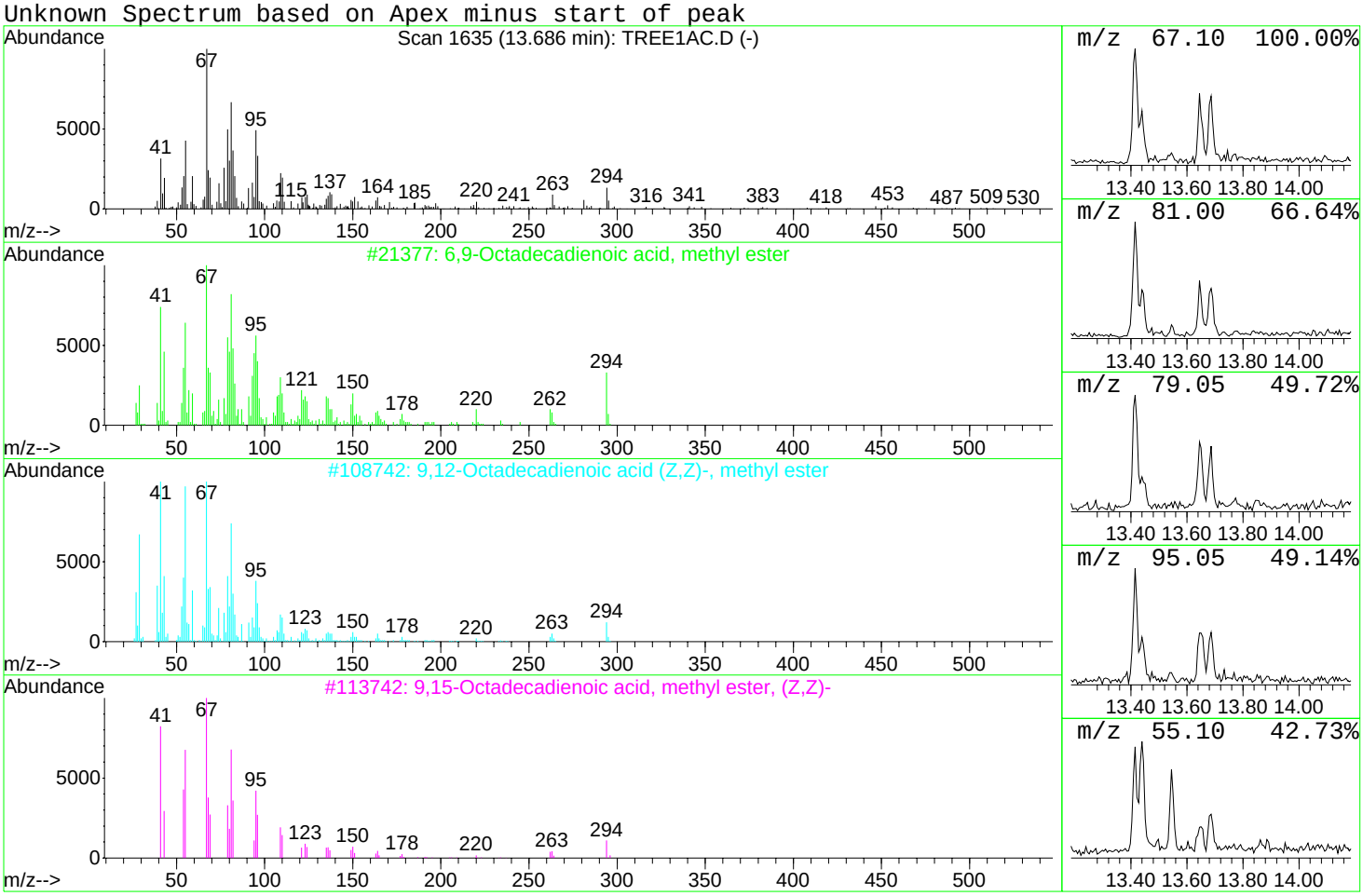
Unknown Spectrum based on Apex minus start of peak



Peak Number: 15 at 13.55 min Area: 2572865 Area % 4.61

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Octadecanoic acid, methyl ester	115469	000112-61-8	94
2 Heptadecanoic acid, 16-methyl-, ...	28391	005129-61-3	93
3 Heptadecanoic acid, 15-methyl-, ...	28402	054833-55-5	93

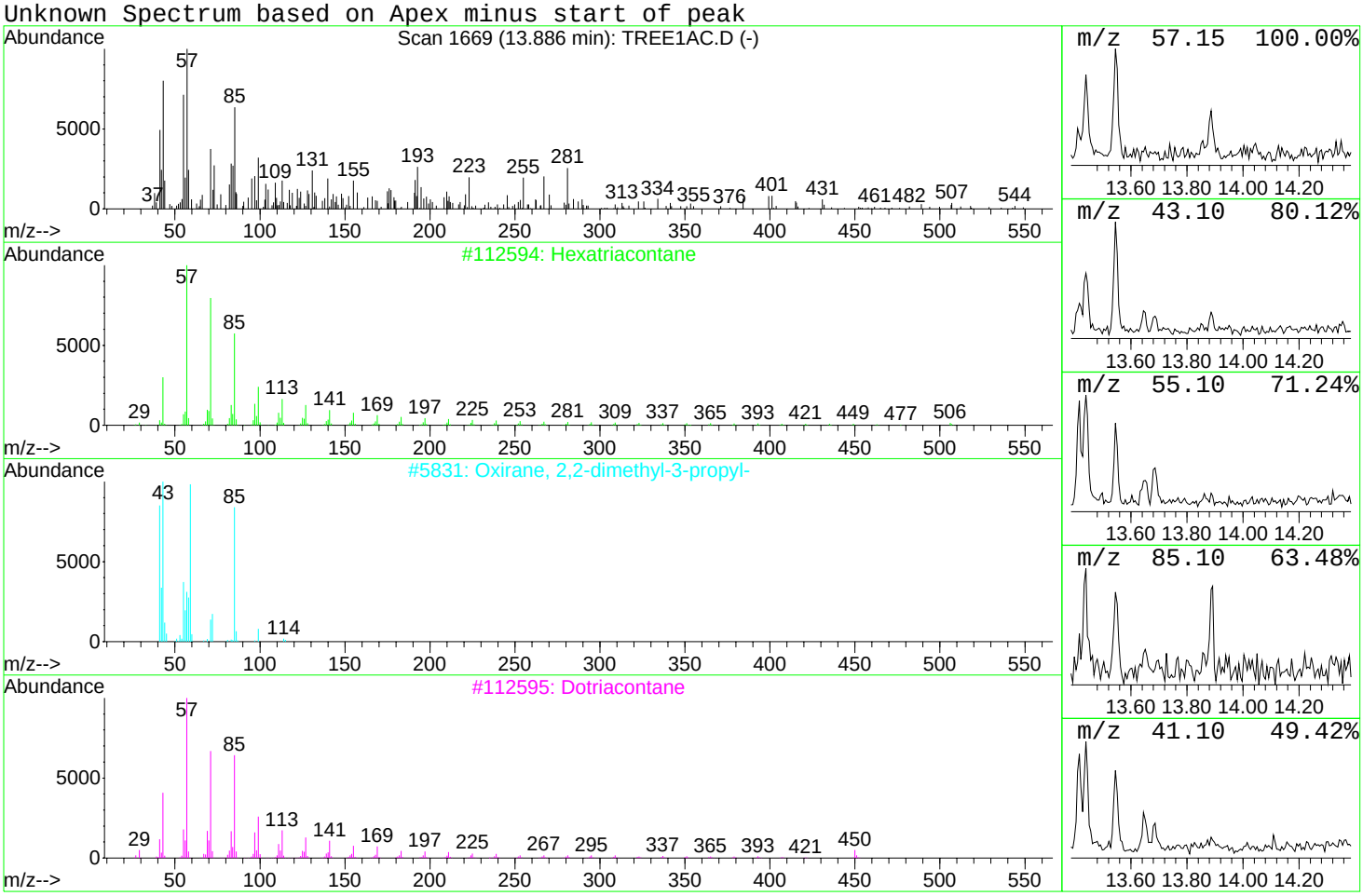
Unknown Spectrum based on Apex minus start of peak



Peak Number: 16 at 13.69 min Area: 3099892 Area % 5.56

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 6,9-Octadecadienoic acid, methyl...	21377	056599-55-4	95
2 9,12-Octadecadienoic acid (Z,Z)-...	108742	000112-63-0	93
3 9,15-Octadecadienoic acid, methy...	113742	017309-05-6	90

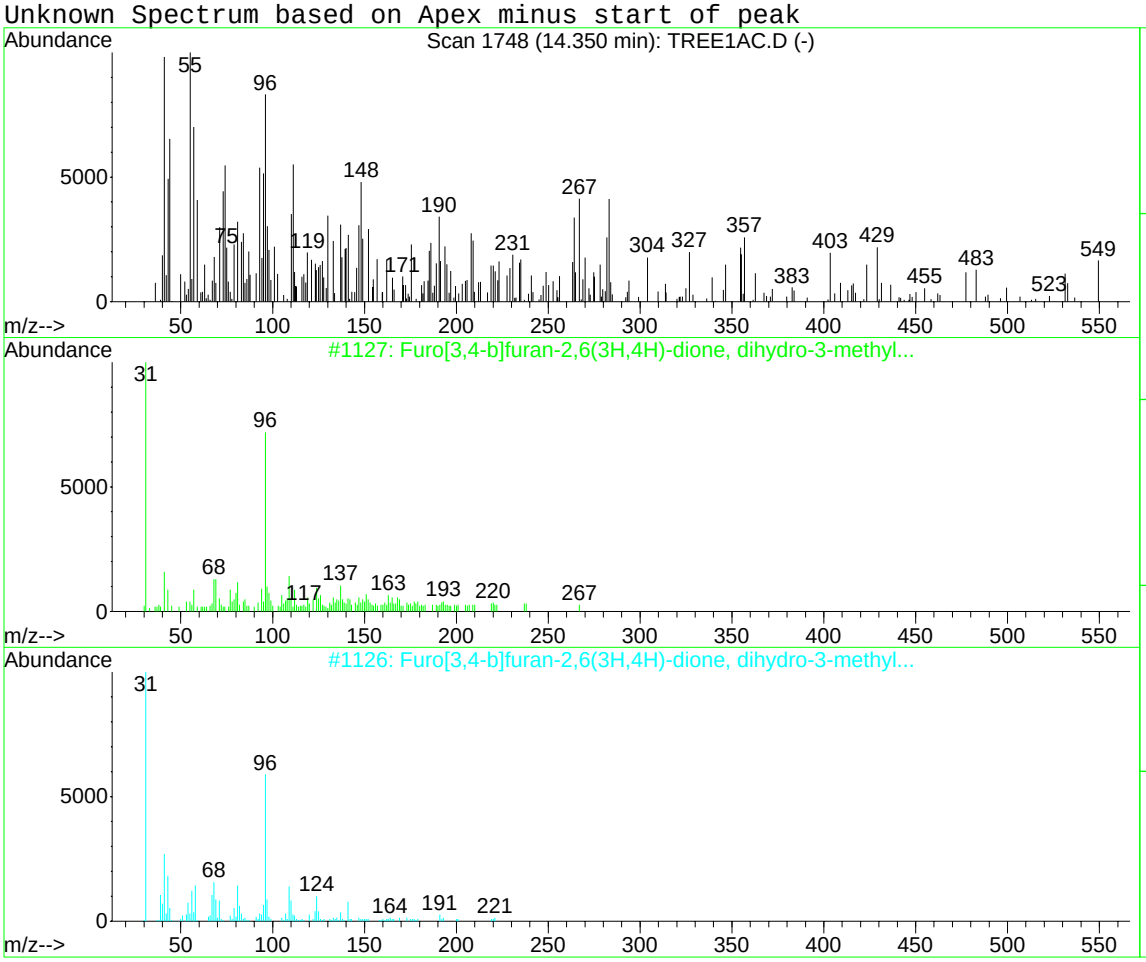
Unknown Spectrum based on Apex minus start of peak

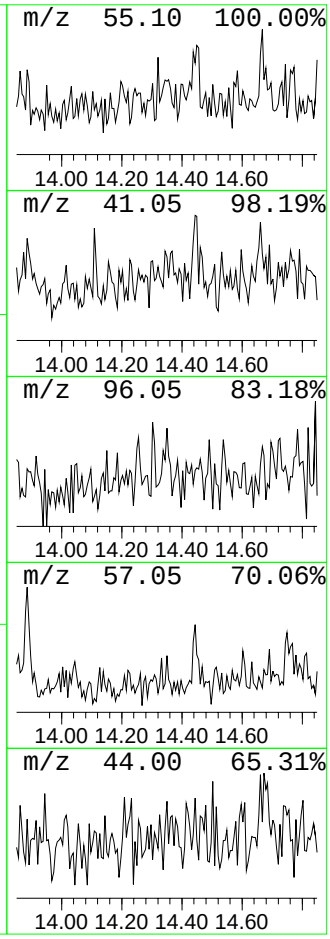


Peak Number: 17 at 13.89 min Area: 691598 Area % 1.24

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Hexatriacontane	112594	000630-06-8	50
2 Oxirane, 2,2-dimethyl-3-propyl-	5831	017612-35-0	38
3 Dotriacontane	112595	000544-85-4	35

Unknown Spectrum based on Apex minus start of peak

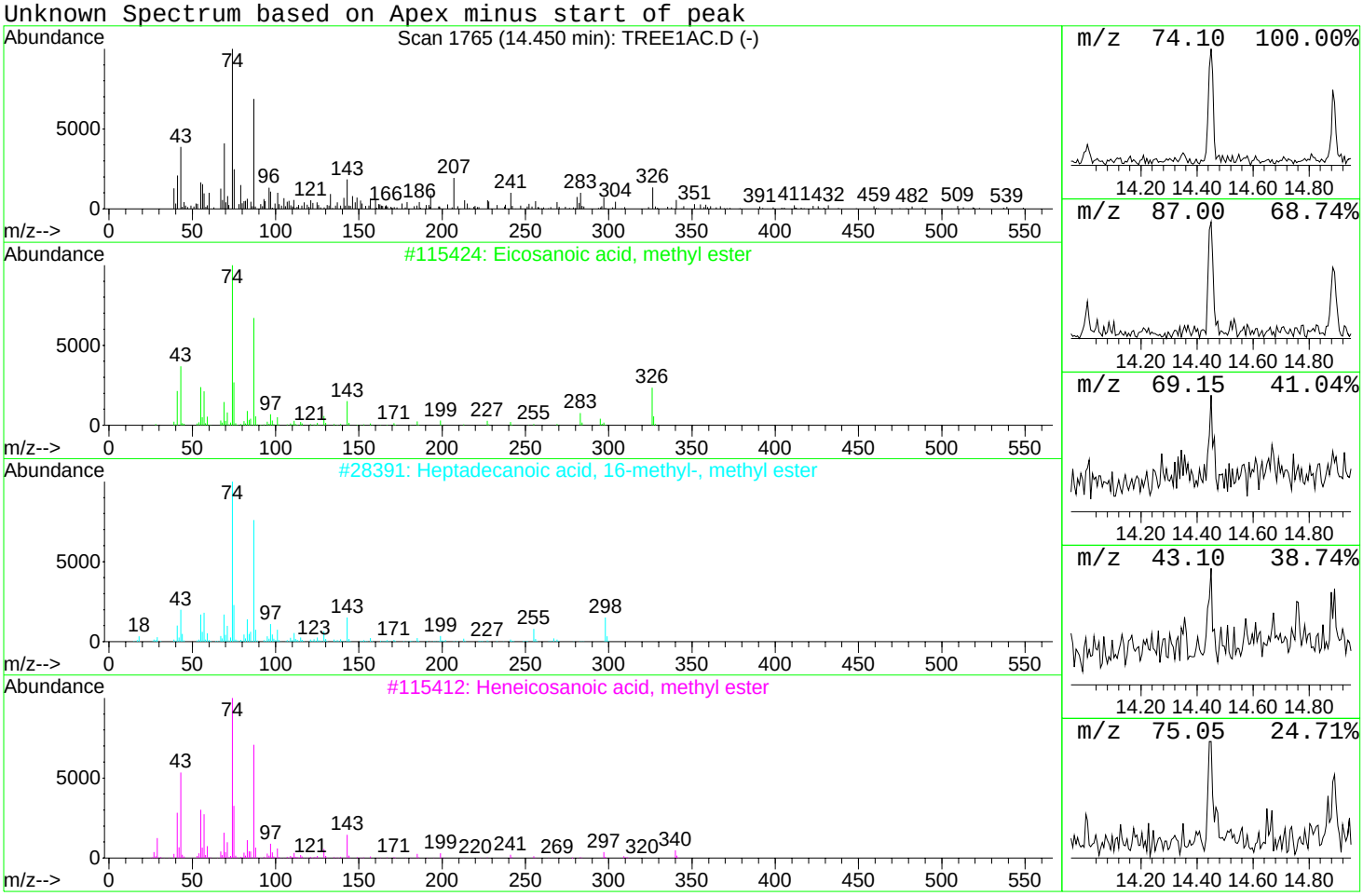




Peak Number: 18 at 14.35 min Area: 1372038 Area % 2.46

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Furo[3,4-b]furan-2,6(3H,4H)-dion...	1127	033644-09-6	4
2 Furo[3,4-b]furan-2,6(3H,4H)-dion...	1126	020223-76-1	2

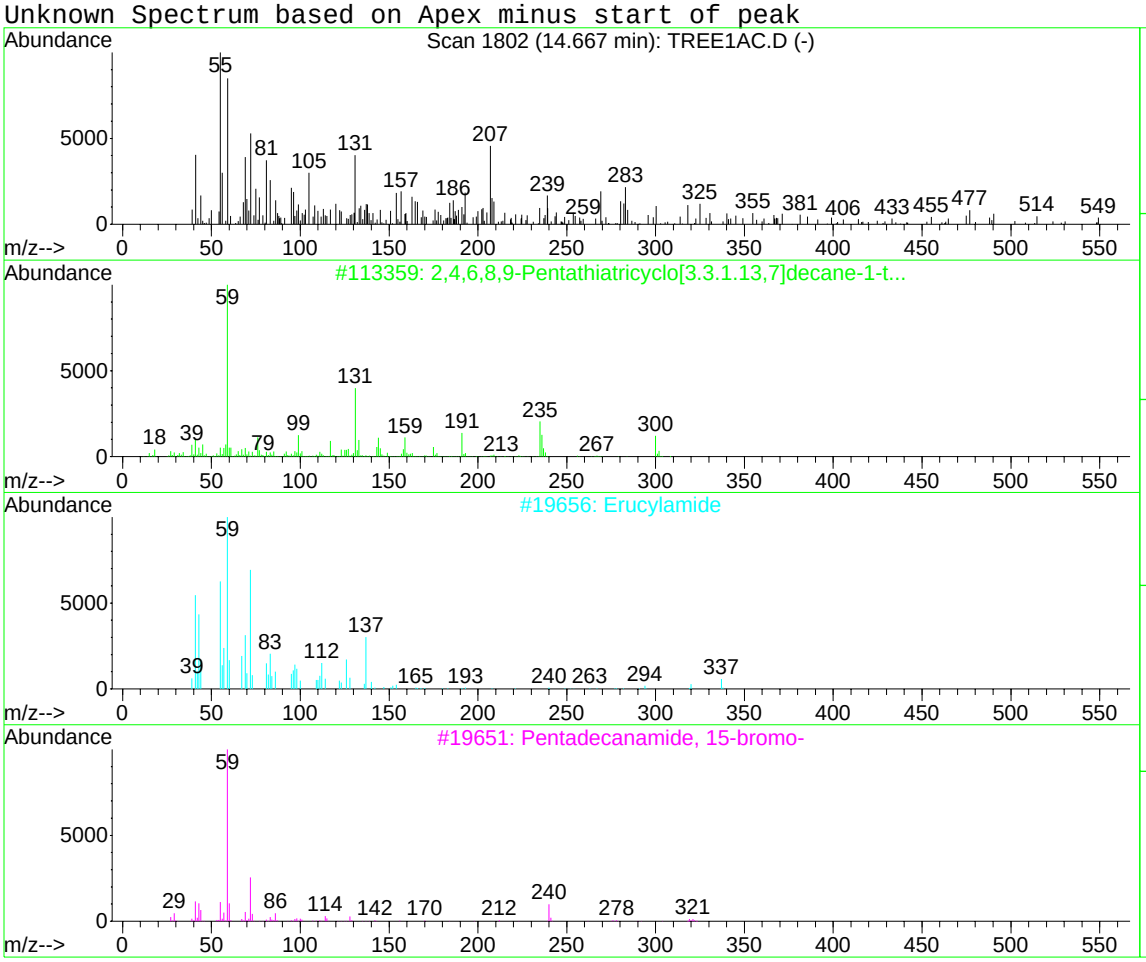
Unknown Spectrum based on Apex minus start of peak

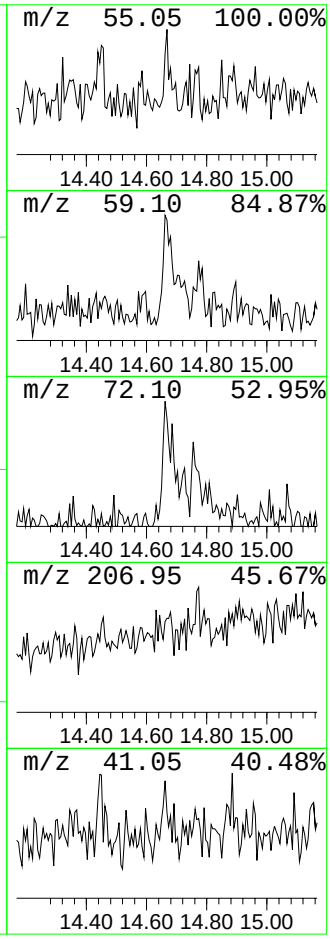


Peak Number: 19 at 14.45 min Area: 837317 Area % 1.50

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Eicosanoic acid, methyl ester	115424	001120-28-1	76
2 Heptadecanoic acid, 16-methyl-, ...	28391	005129-61-3	62
3 Heneicosanoic acid, methyl ester	115412	006064-90-0	58

Unknown Spectrum based on Apex minus start of peak

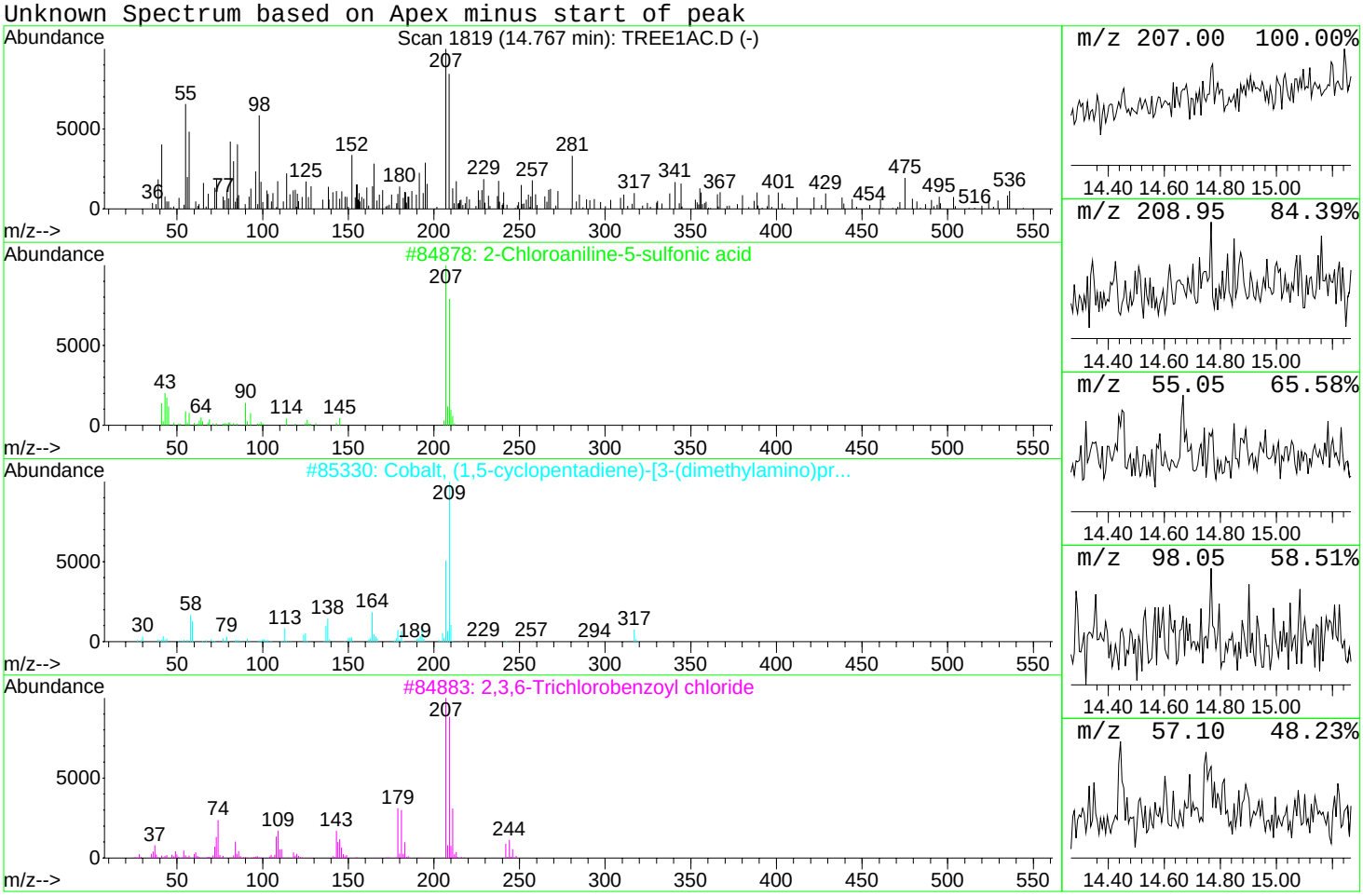




Peak Number: 20 at 14.67 min Area: 1295642 Area % 2.32

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2,4,6,8,9-Pentathiatricyclo[3.3....	113359	057274-31-4	49
2 Erucylamide	19656	000112-84-5	37
3 Pentadecanamide, 15-bromo-	19651	1000163-86-1	37

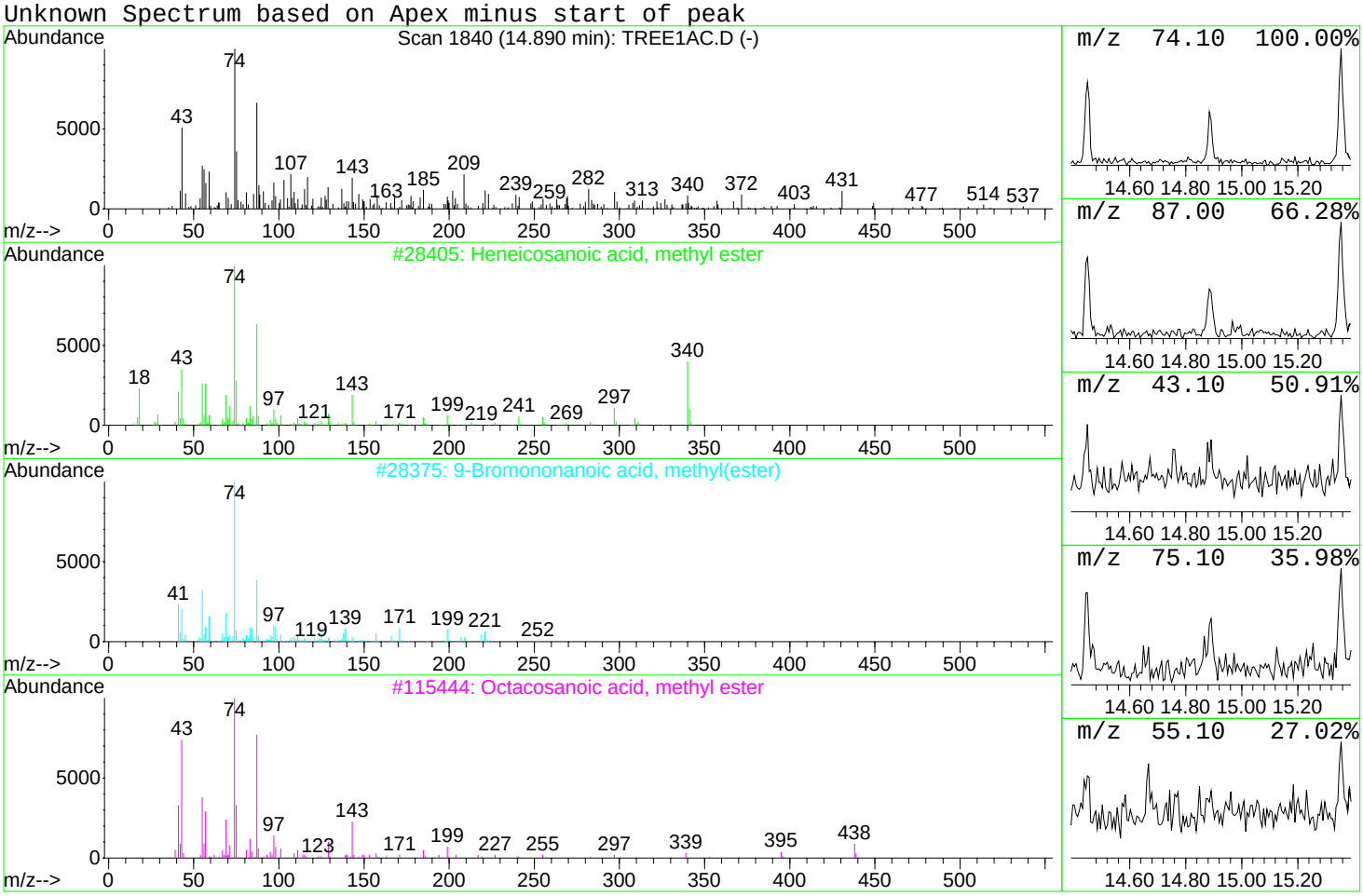
Unknown Spectrum based on Apex minus start of peak



Peak Number: 21 at 14.77 min Area: 792686 Area % 1.42

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Chloroaniline-5-sulfonic acid	84878	000098-36-2	42
2 Cobalt, (1,5-cyclopentadiene)-[3...	85330	1000158-65-1	38
3 2,3,6-Trichlorobenzoyl chloride	84883	004093-17-8	36

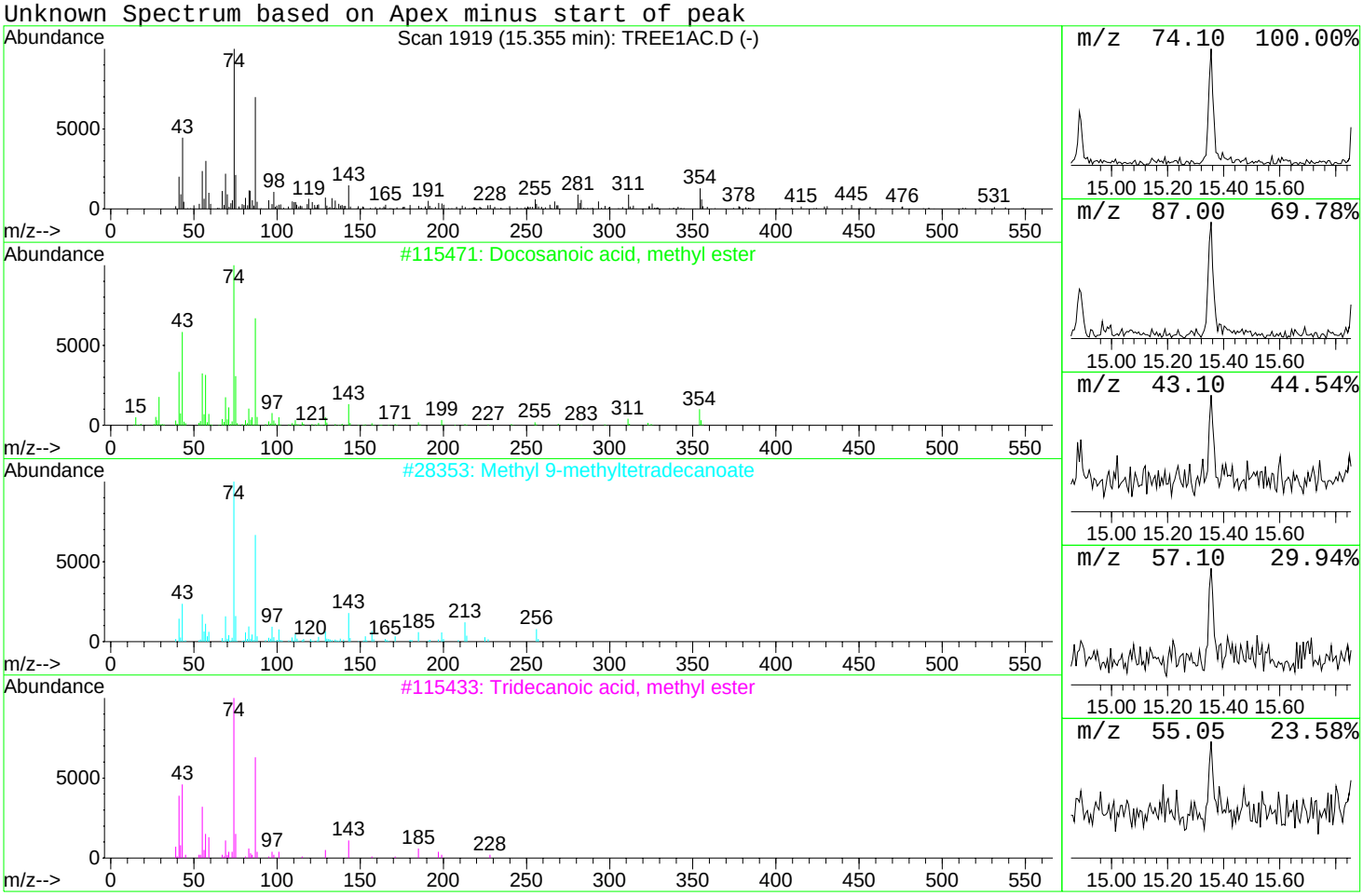
Unknown Spectrum based on Apex minus start of peak



Peak Number: 22 at 14.89 min Area: 593164 Area % 1.06

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Heneicosanoic acid, methyl ester	28405	006064-90-0	90
2 9-Bromononanoic acid, methyl(ester)	28375	1000131-06-7	58
3 Octacosanoic acid, methyl ester	115444	055682-92-3	58

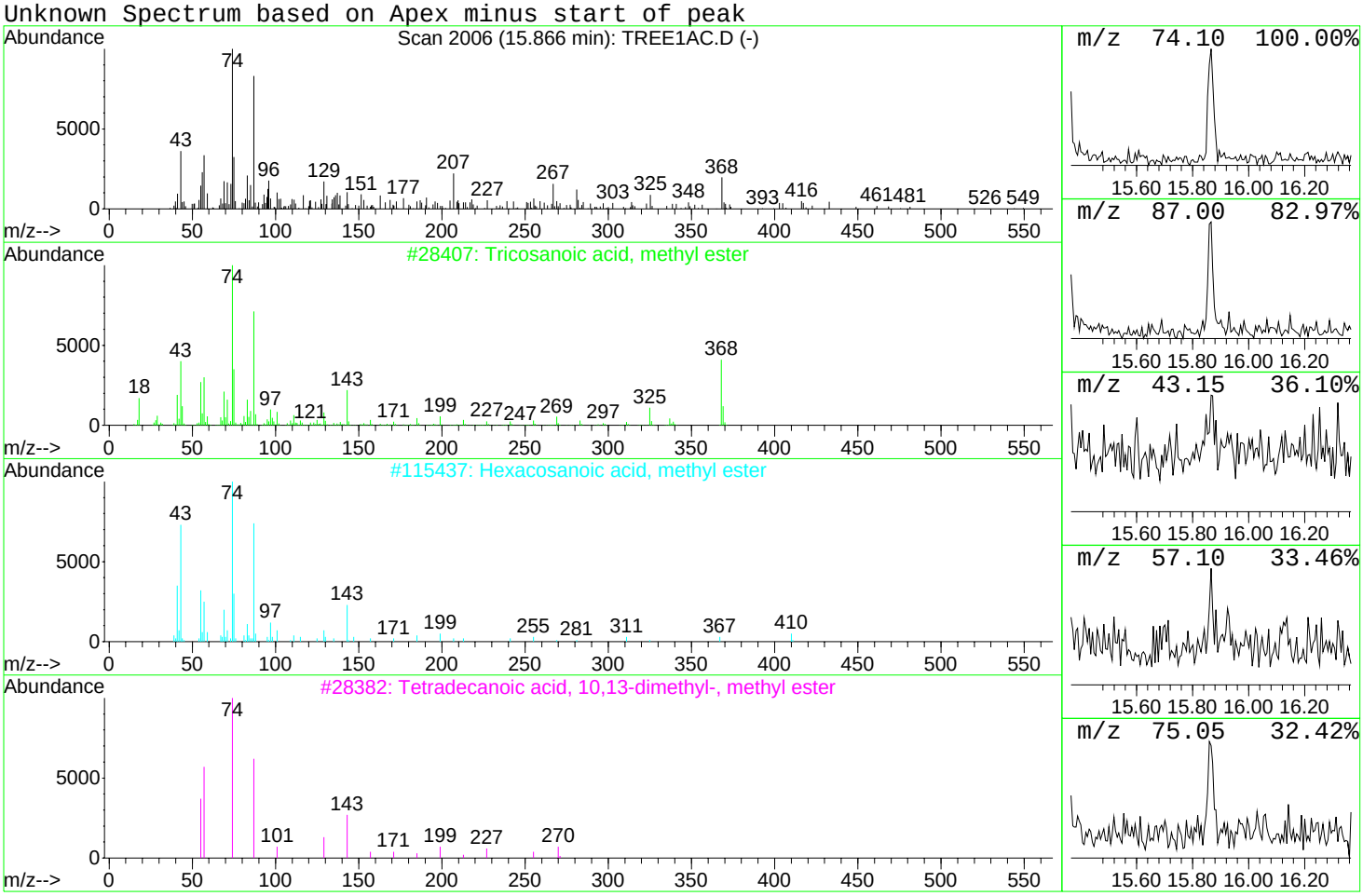
Unknown Spectrum based on Apex minus start of peak



Peak Number: 23 at 15.35 min Area: 1398146 Area % 2.51

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Docosanoic acid, methyl ester	115471	000929-77-1	93
2 Methyl 9-methyltetradecanoate	28353	1000110-16-1	76
3 Tridecanoic acid, methyl ester	115433	001731-88-0	68

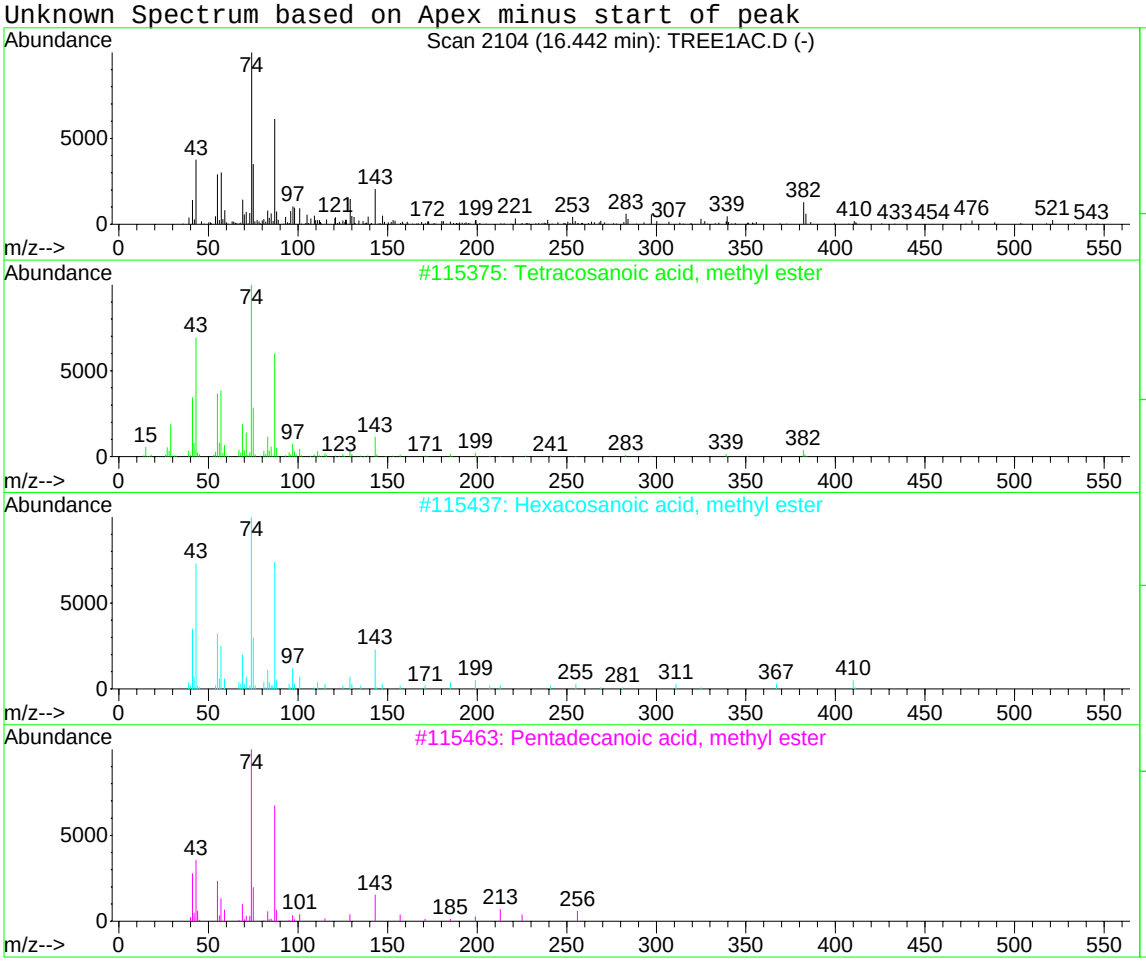
Unknown Spectrum based on Apex minus start of peak

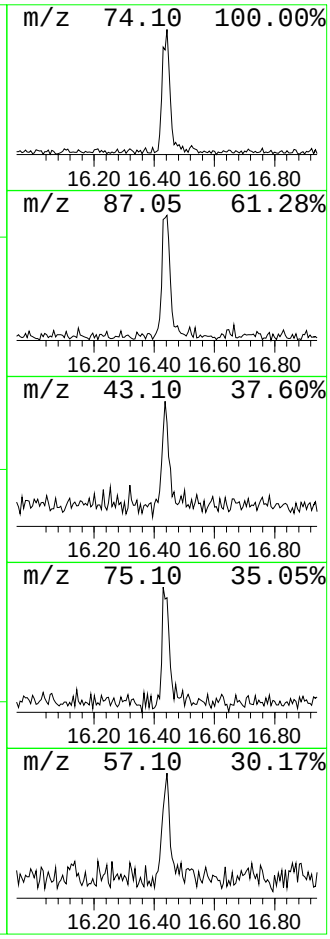


Peak Number: 24 at 15.87 min Area: 1423453 Area % 2.55

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Tricosanoic acid, methyl ester	28407	002433-97-8	76
2 Hexacosanoic acid, methyl ester	115437	005802-82-4	53
3 Tetradecanoic acid, 10,13-dimeth...	28382	1000112-13-7	50

Unknown Spectrum based on Apex minus start of peak

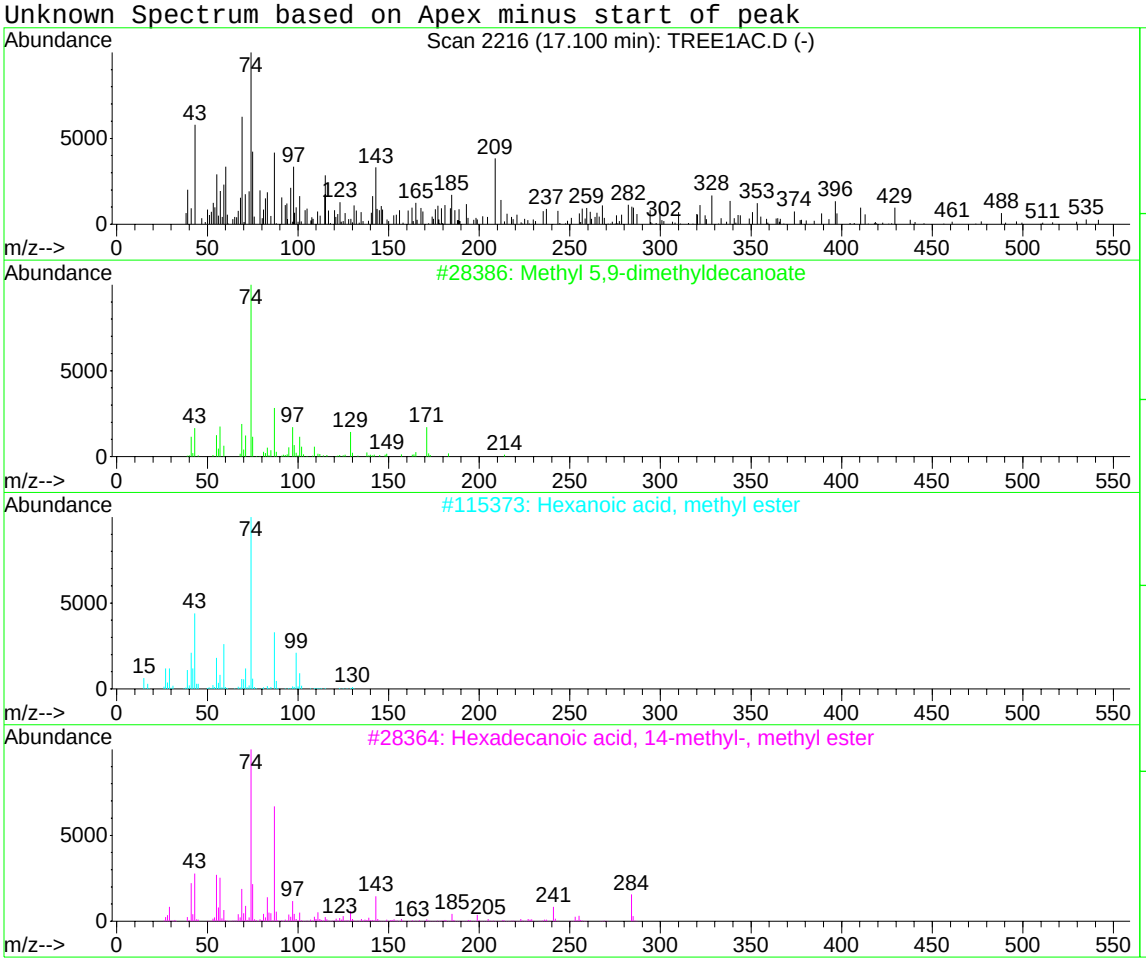


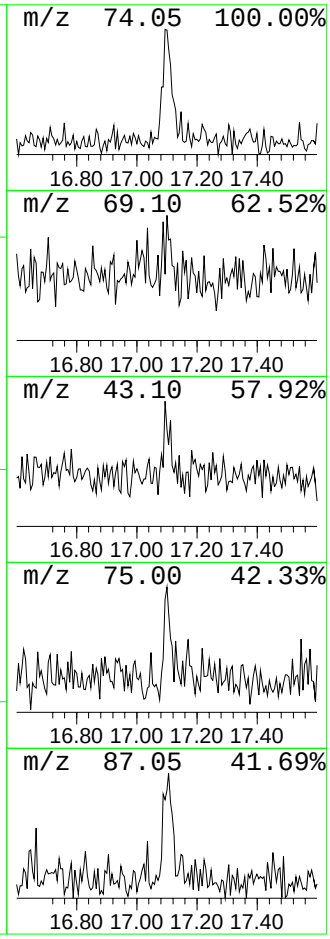


Peak Number: 25 at 16.44 min Area: 2122955 Area % 3.81

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Tetracosanoic acid, methyl ester	115375	002442-49-1	93
2 Hexacosanoic acid, methyl ester	115437	005802-82-4	87
3 Pentadecanoic acid, methyl ester	115463	007132-64-1	72

Unknown Spectrum based on Apex minus start of peak

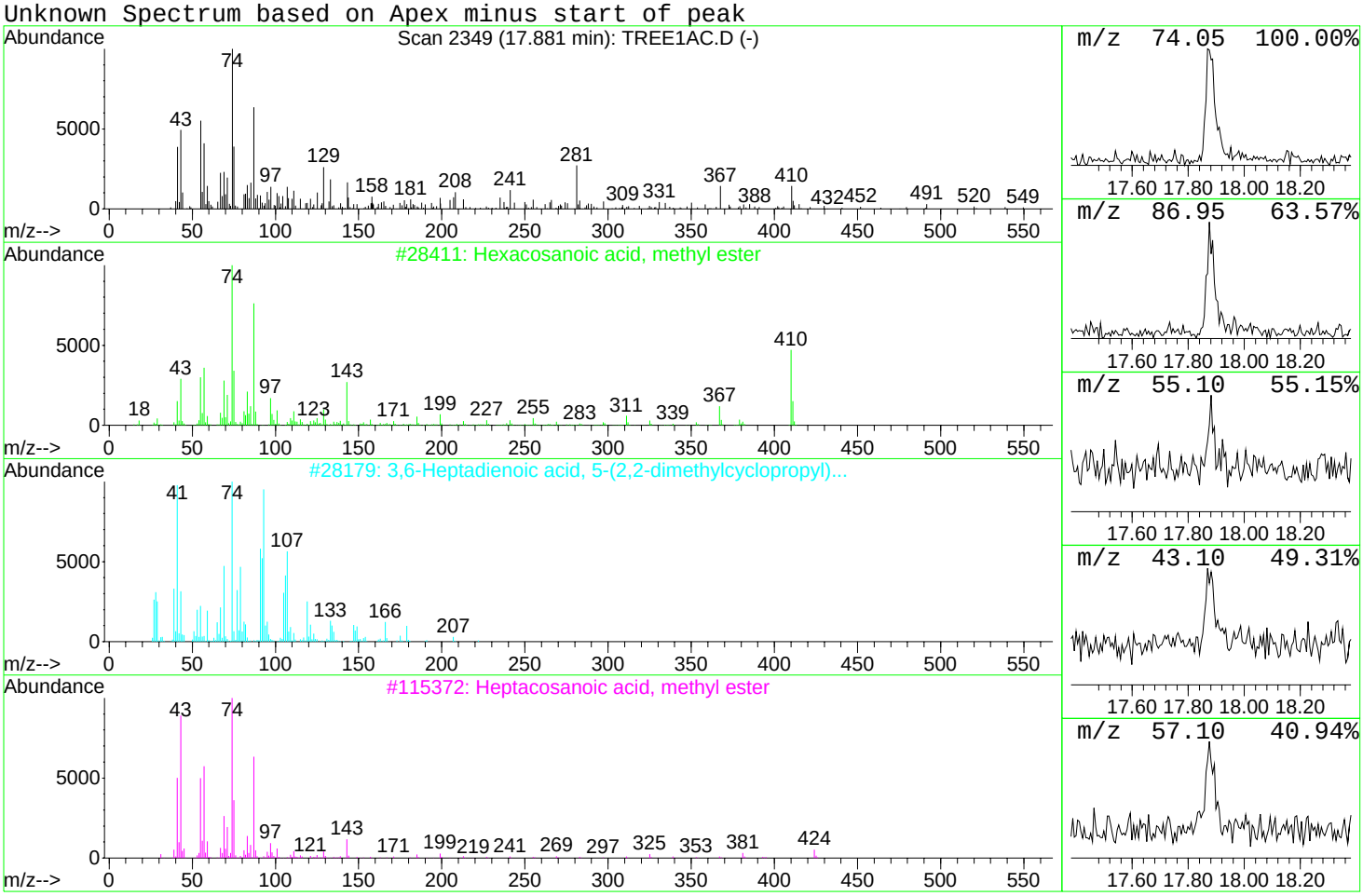




Peak Number: 26 at 17.10 min Area: 906673 Area % 1.63

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Methyl 5,9-dimethyldecanoate	28386	068043-22-1	49
2 Hexanoic acid, methyl ester	115373	000106-70-7	47
3 Hexadecanoic acid, 14-methyl-, m...	28364	002490-49-5	47

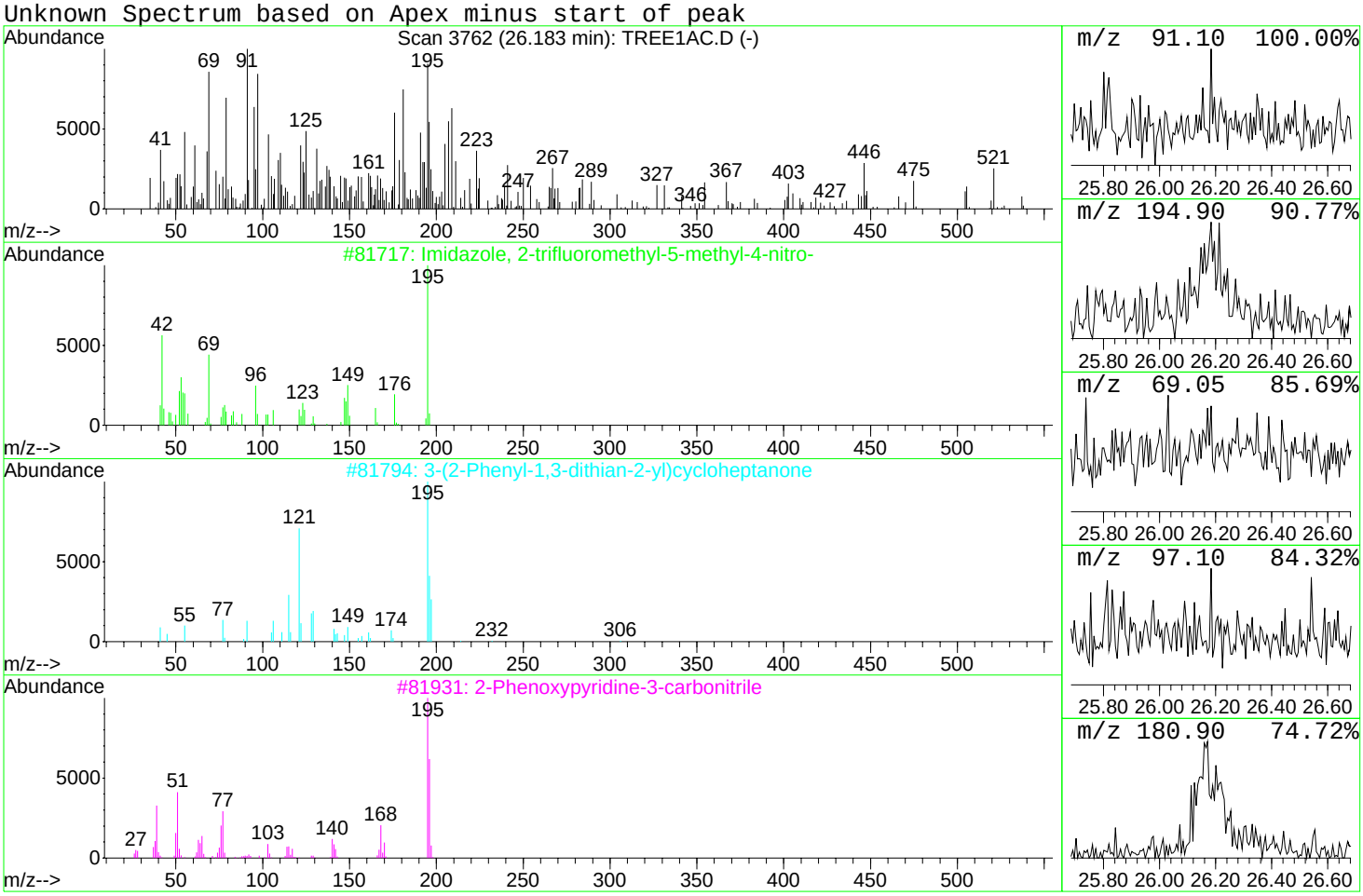
Unknown Spectrum based on Apex minus start of peak



Peak Number: 27 at 17.88 min Area: 2286364 Area % 4.10

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Hexacosanoic acid, methyl ester	28411	005802-82-4	93
2 3,6-Heptadienoic acid, 5-(2,2-di...	28179	103620-10-6	83
3 Heptacosanoic acid, methyl ester	115372	055682-91-2	52

Unknown Spectrum based on Apex minus start of peak



Peak Number: 28 at 26.18 min Area: 2382611 Area % 4.27

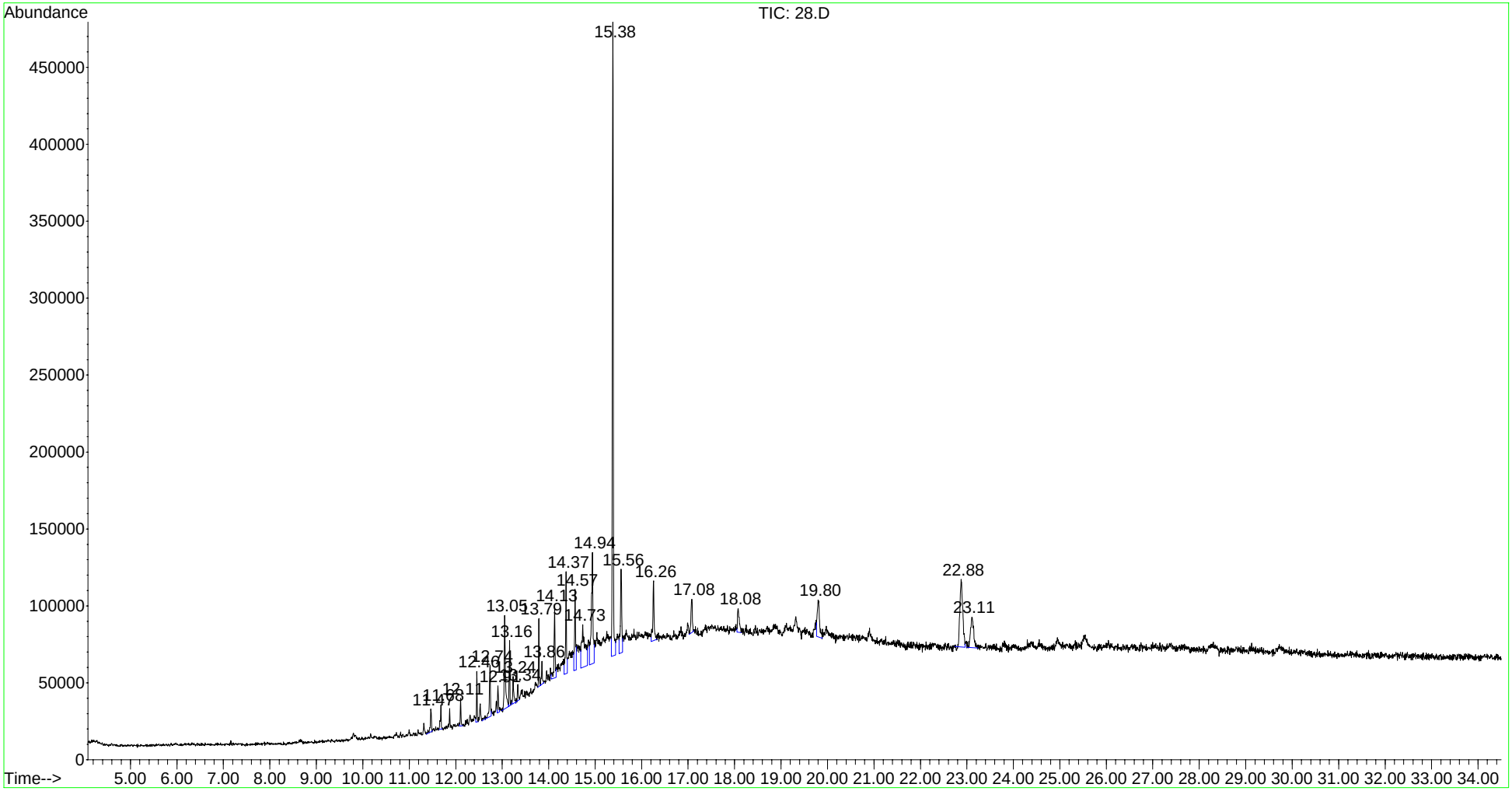
The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Imidazole, 2-trifluoromethyl-5-m...	81717	104752-69-4	15
2 3-(2-Phenyl-1,3-dithian-2-yl)cyc...	81794	1000147-46-7	12
3 2-Phenoxypyridine-3-carbonitrile	81931	014178-15-5	11

Library Search Report

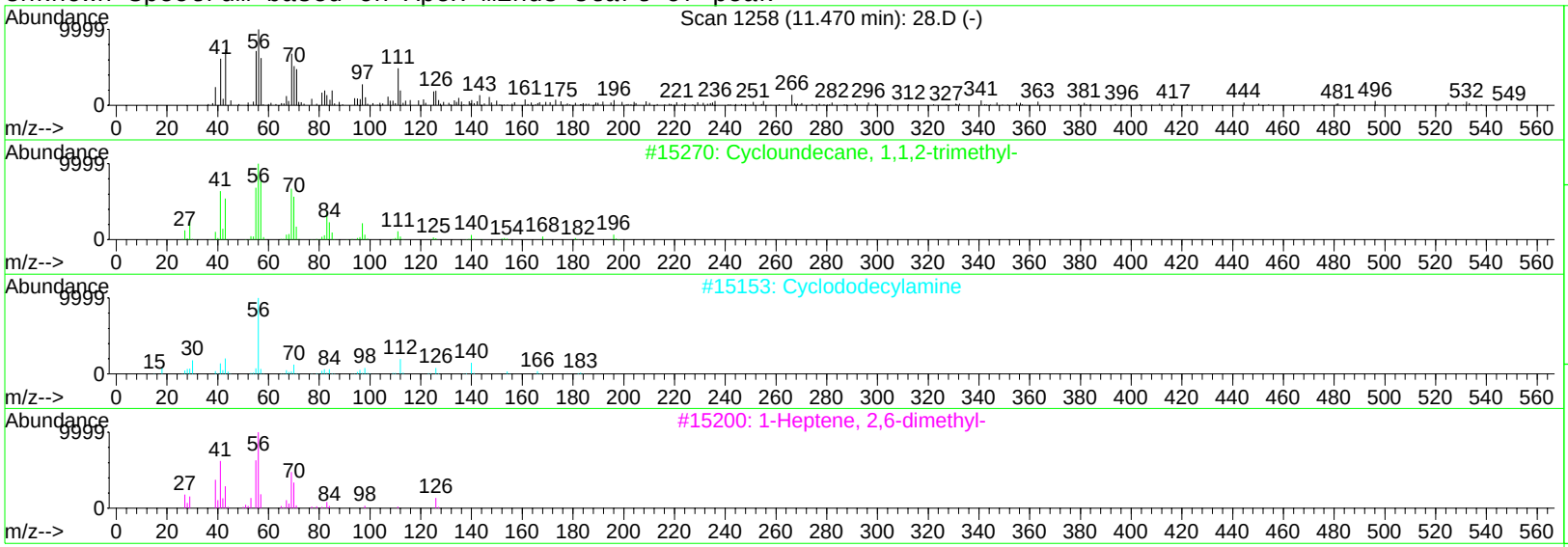
Data File : D:\PRENISHA\300\28.D
Acq On : 2 Aug 2007 13:19
Sample : 28
Misc : 1µl inject, acetone, splitless

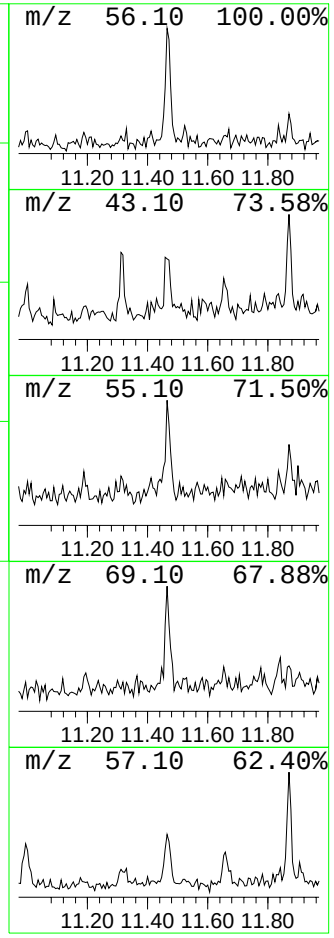
Vial: 28
Operator: Bret
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e
Method : C:\MSDCHEM\1\METHODS\NEW.M (Chemstation Integrator)
Title :



Unknown Spectrum based on Apex minus start of peak

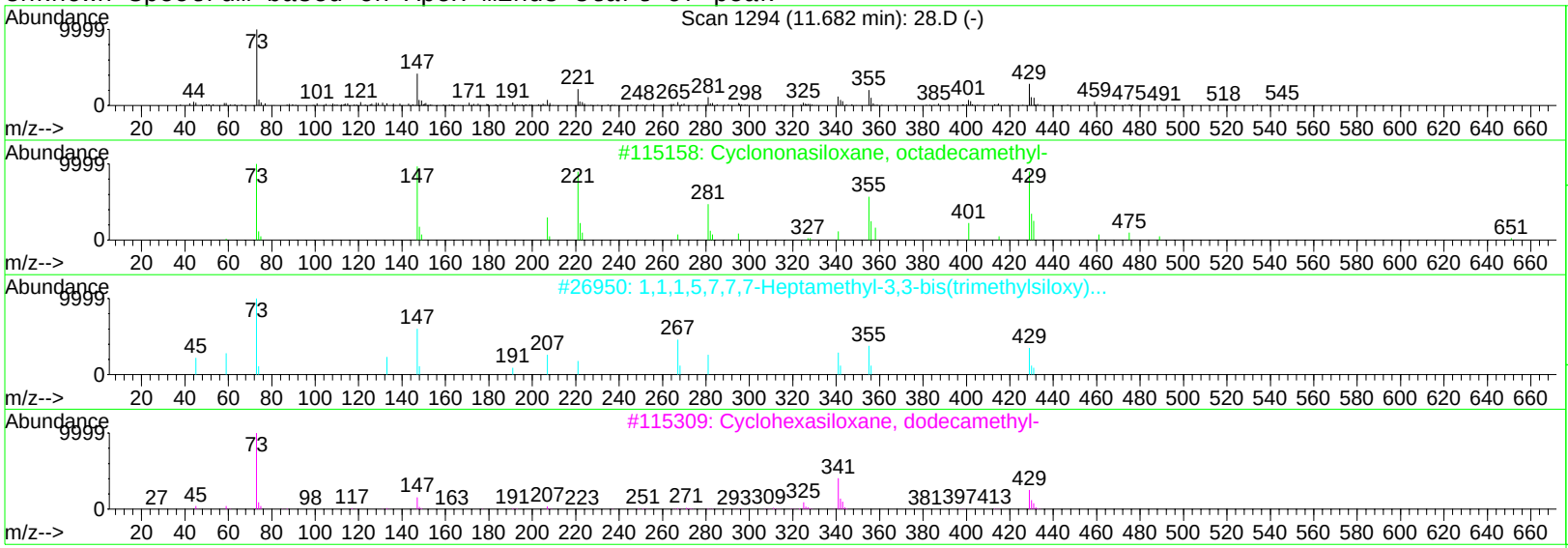


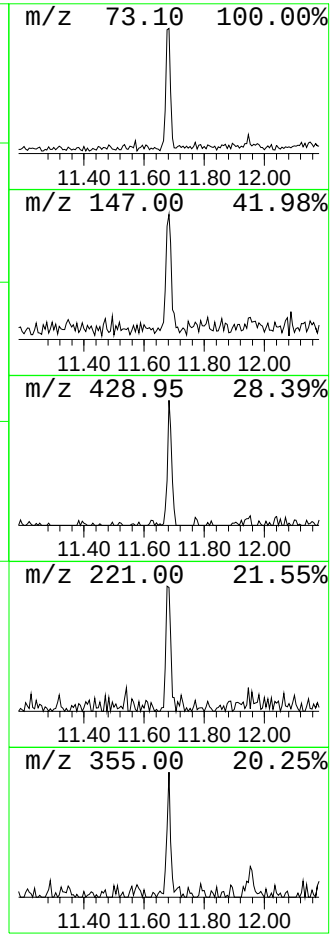


Peak Number: 1 at 11.47 min Area: 237695 Area % 1.09

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cycloundecane, 1,1,2-trimethyl-	15270	062376-15-2	49
2 Cyclododecylamine	15153	001502-03-0	45
3 1-Heptene, 2,6-dimethyl-	15200	003074-78-0	45

Unknown Spectrum based on Apex minus start of peak

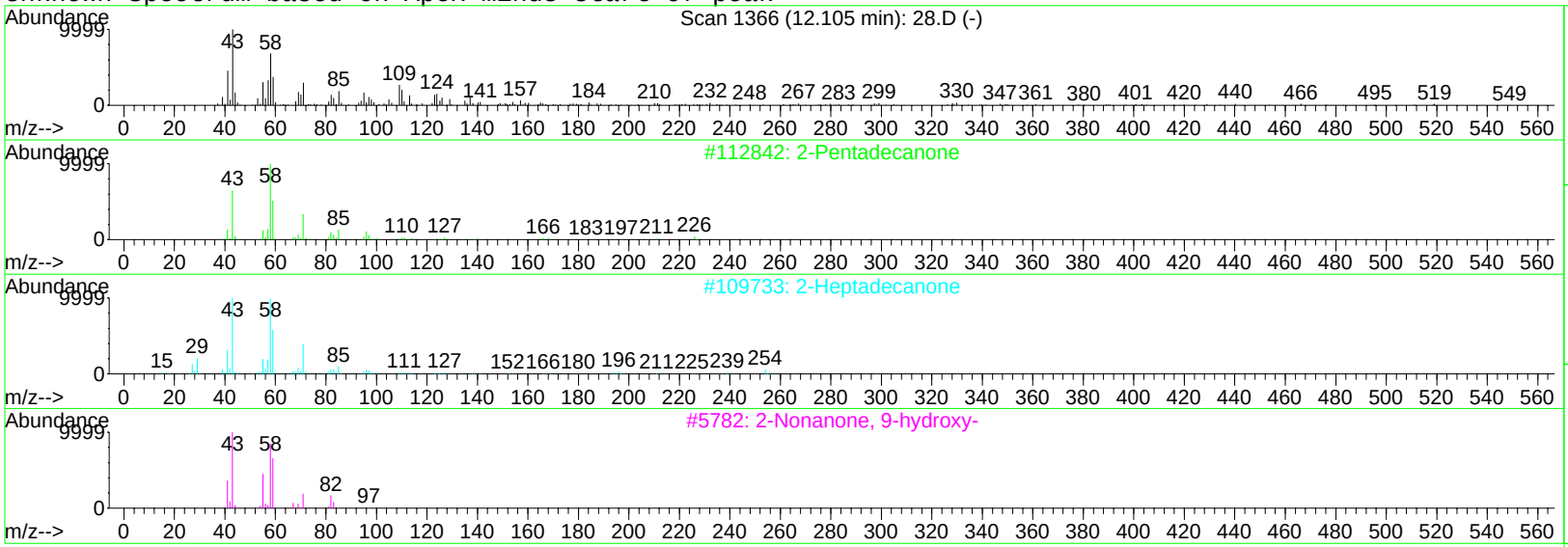


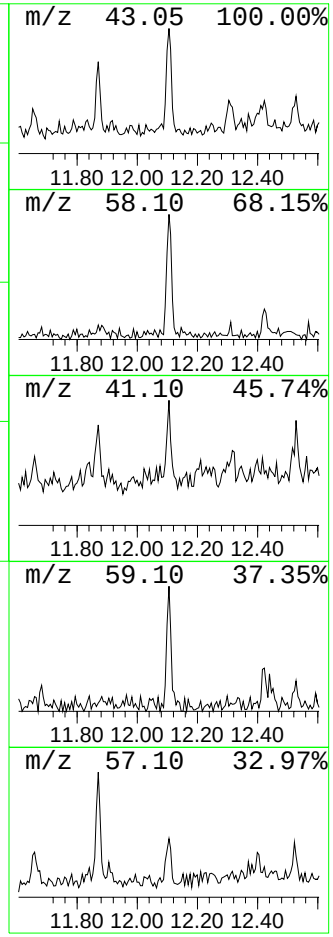


Peak Number: 2 at 11.68 min Area: 217180 Area % 1.00

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclononasiloxane, octadecamethyl-	115158	000556-71-8	47
2 1,1,1,5,7,7,7-Heptamethyl-3,3-bi...	26950	038147-00-1	32
3 Cyclohexasiloxane, dodecamethyl-	115309	000540-97-6	10

Unknown Spectrum based on Apex minus start of peak

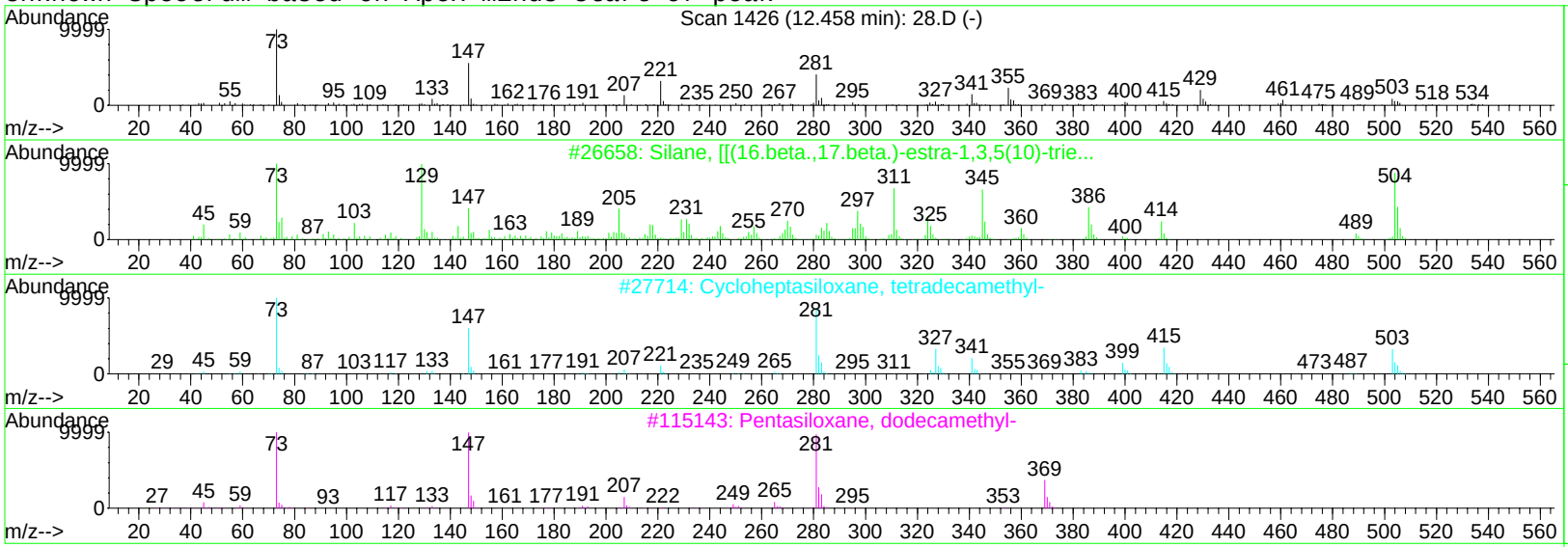


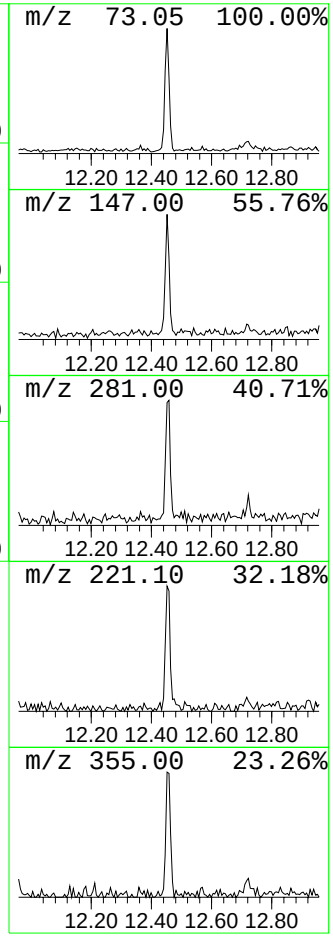


Peak Number: 3 at 12.11 min Area: 204951 Area % 0.94

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Pentadecanone	112842	002345-28-0	45
2 2-Heptadecanone	109733	002922-51-2	43
3 2-Nonanone, 9-hydroxy-	5782	025368-56-3	38

Unknown Spectrum based on Apex minus start of peak

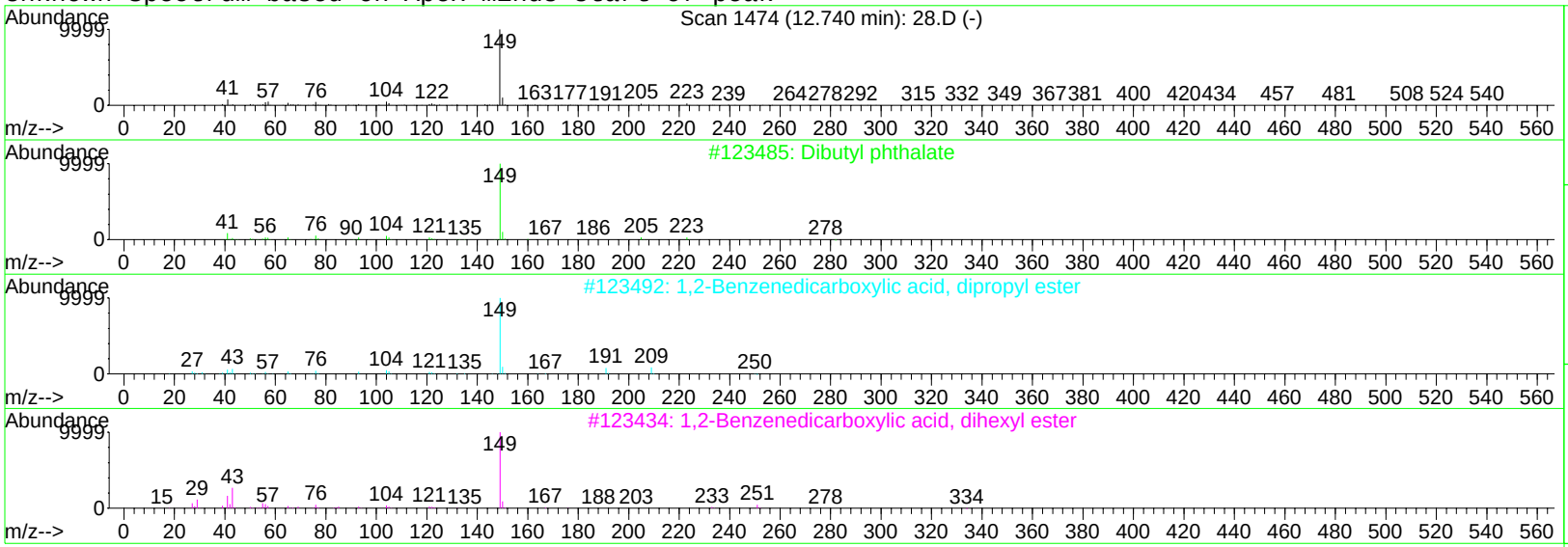


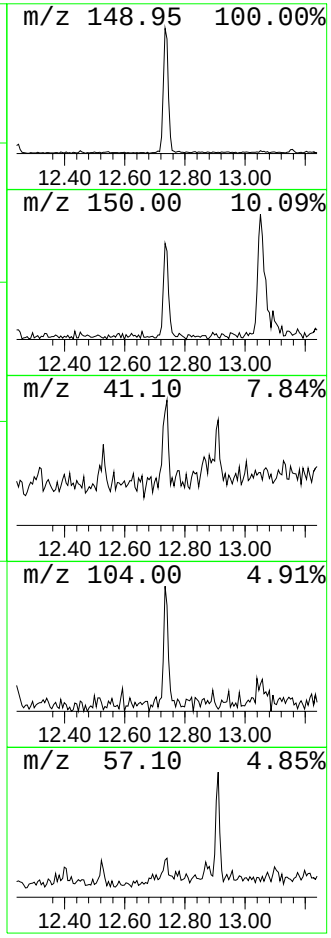


Peak Number: 4 at 12.46 min Area: 340227 Area % 1.56

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Silane, [[(16.beta.,17.beta.)-es...	26658	057305-21-2	78
2 Cycloheptasiloxane, tetradecamet...	27714	000107-50-6	33
3 Pentasiloxane, dodecamethyl-	115143	000141-63-9	23

Unknown Spectrum based on Apex minus start of peak

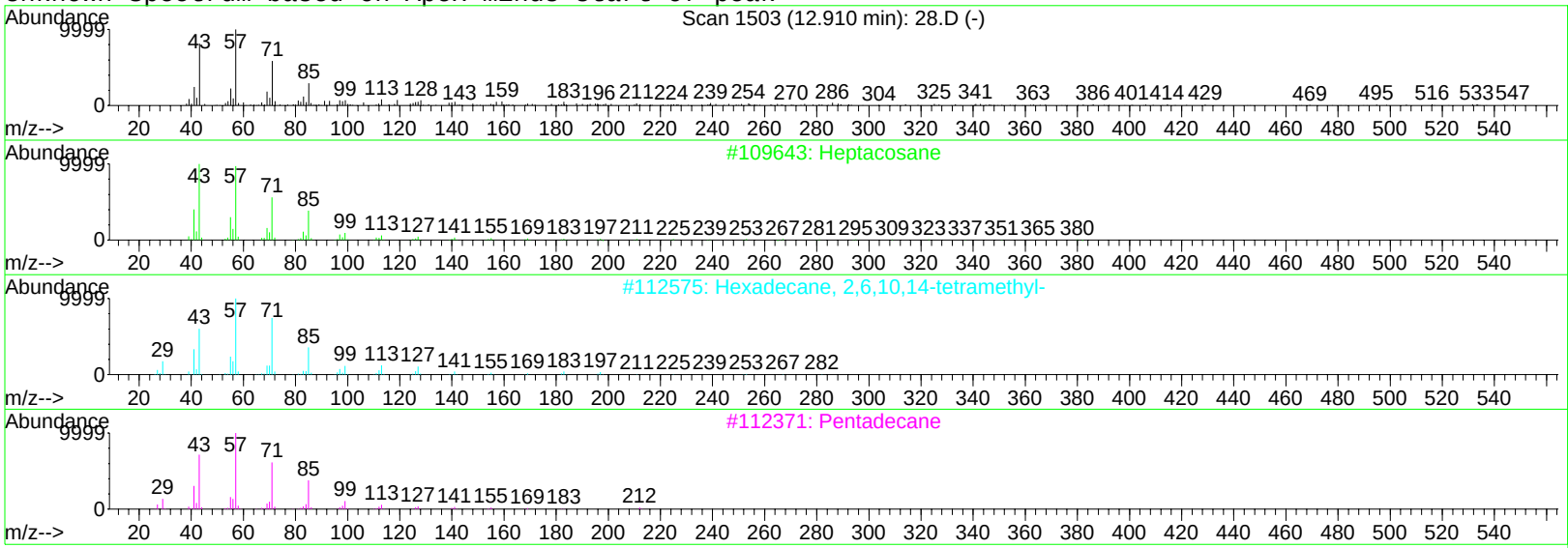


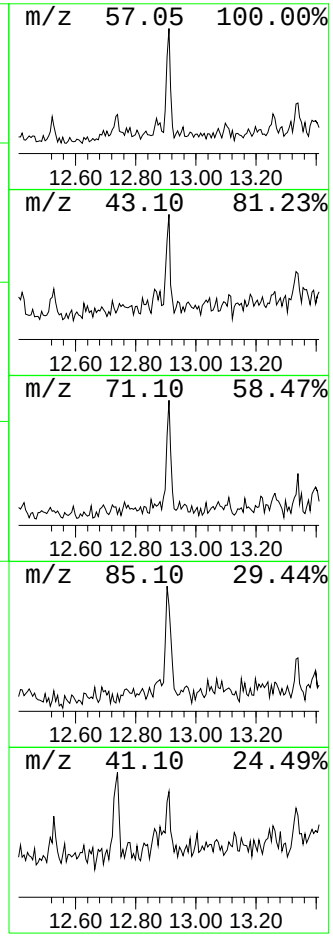


Peak Number: 5 at 12.74 min Area: 550555 Area % 2.53

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Dibutyl phthalate	123485	000084-74-2	91
2 1,2-Benzenedicarboxylic acid, di...	123492	000131-16-8	83
3 1,2-Benzenedicarboxylic acid, di...	123434	000084-75-3	80

Unknown Spectrum based on Apex minus start of peak

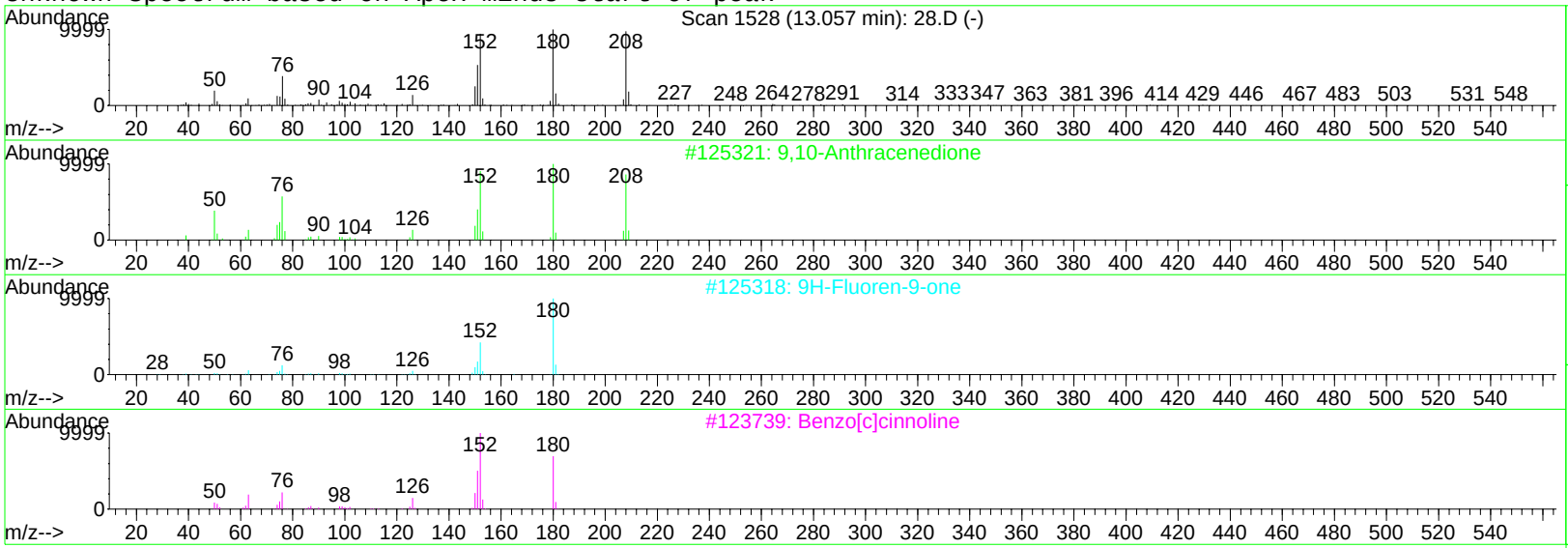


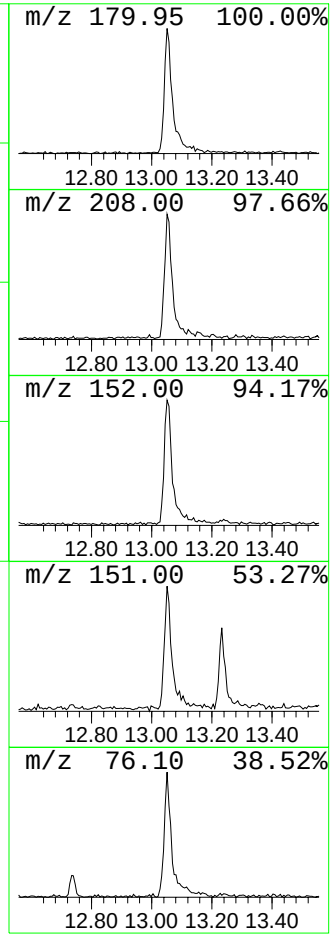


Peak Number: 6 at 12.91 min Area: 198069 Area % 0.91

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Heptacosane	109643	000593-49-7	93
2 Hexadecane, 2,6,10,14-tetramethyl-	112575	000638-36-8	89
3 Pentadecane	112371	000629-62-9	76

Unknown Spectrum based on Apex minus start of peak

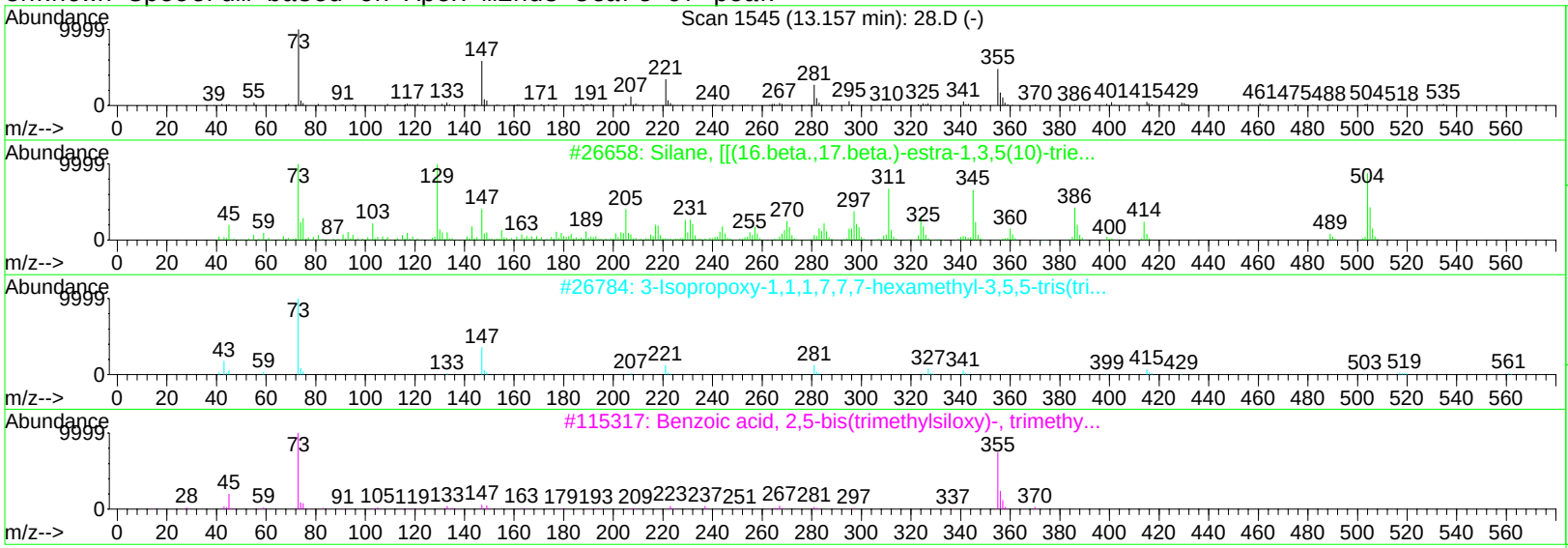


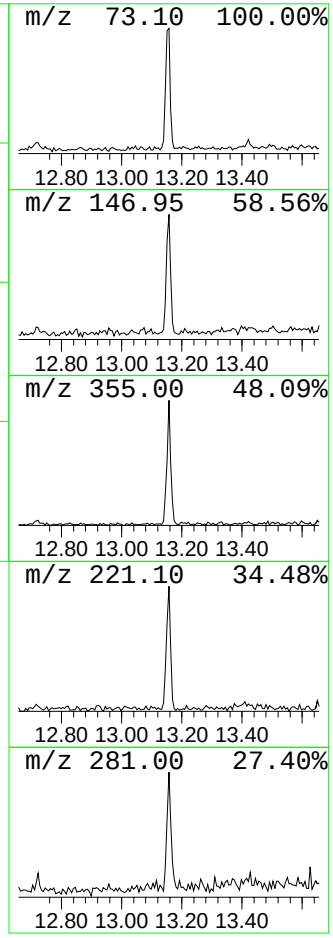


Peak Number: 7 at 13.06 min Area: 1160658 Area % 5.33

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 9,10-Anthracenedione	125321	000084-65-1	94
2 9H-Fluoren-9-one	125318	000486-25-9	89
3 Benzo[c]cinnoline	123739	000230-17-1	64

Unknown Spectrum based on Apex minus start of peak

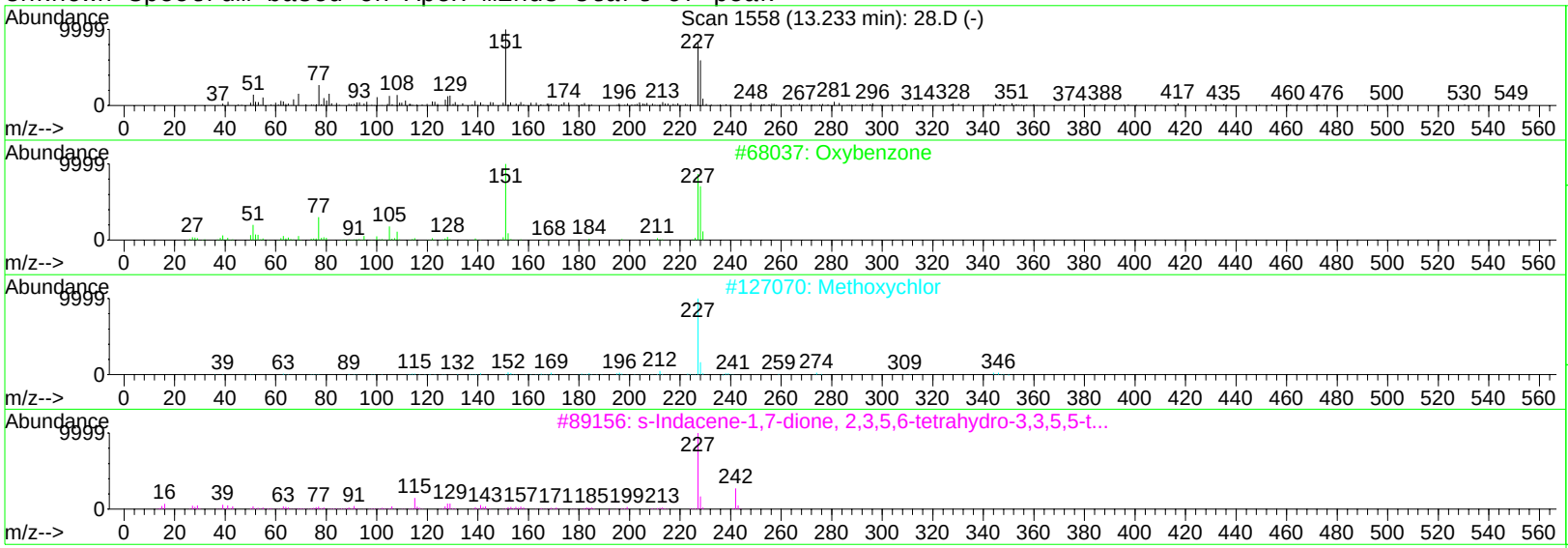


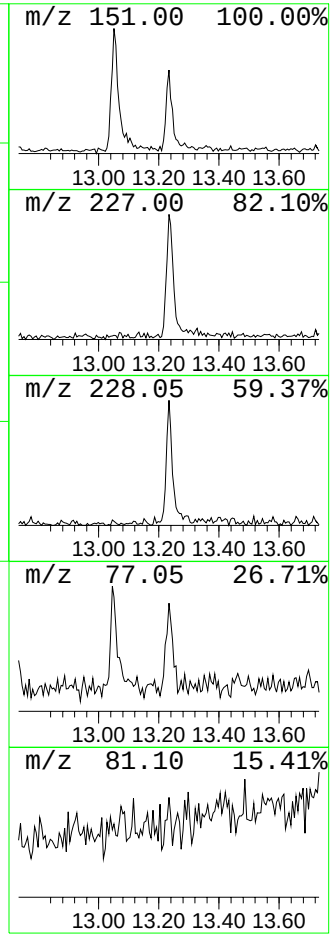


Peak Number: 8 at 13.16 min Area: 419046 Area % 1.92

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Silane, [[(16.beta.,17.beta.)-es...	26658	057305-21-2	93
2 3-Isopropoxy-1,1,1,7,7,7-hexamet...	26784	071579-69-6	35
3 Benzoic acid, 2,5-bis(trimethyls...	115317	003618-20-0	30

Unknown Spectrum based on Apex minus start of peak

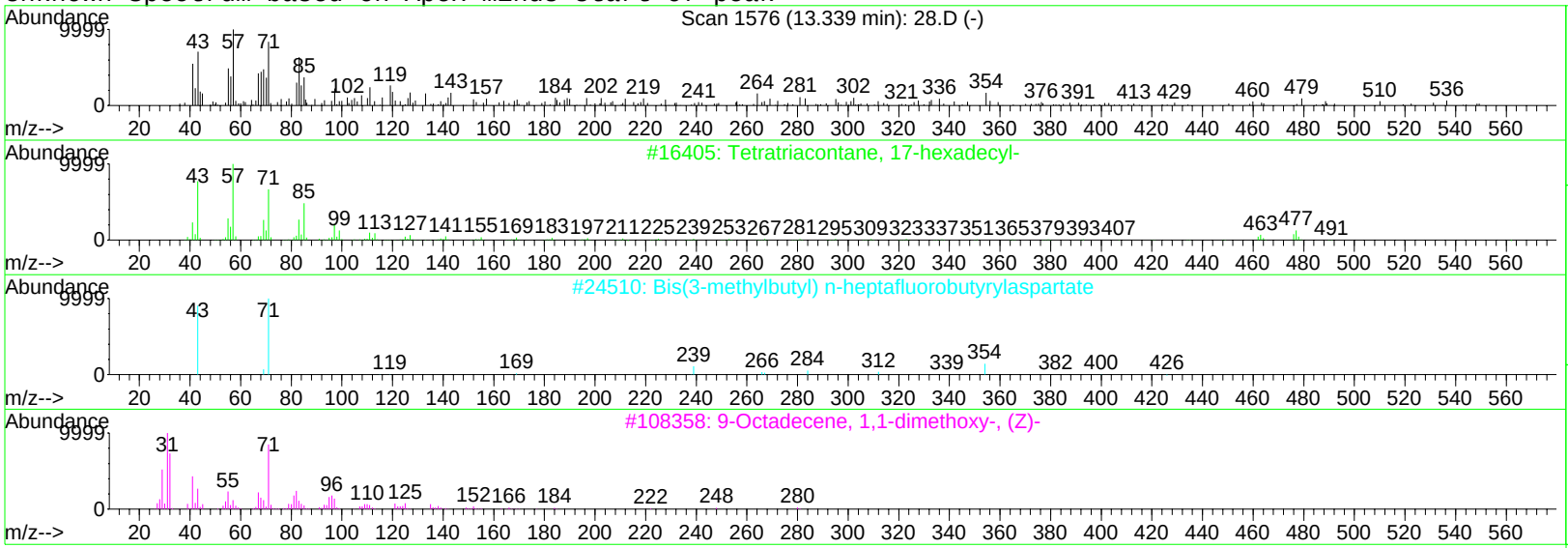


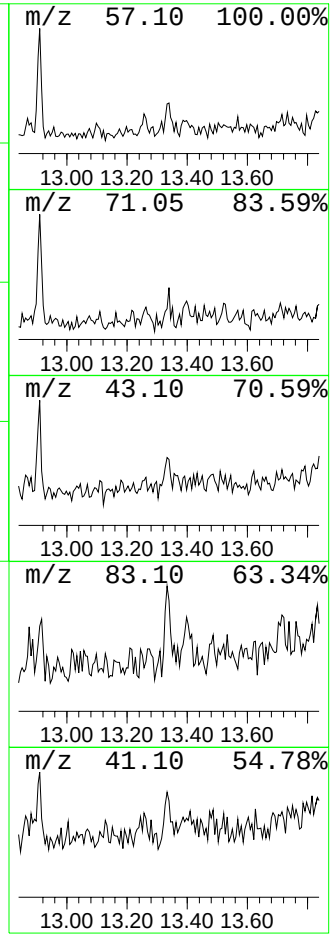


Peak Number: 9 at 13.23 min Area: 264888 Area % 1.22

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Oxybenzone	68037	000131-57-7	93
2 Methoxychlor	127070	000072-43-5	38
3 s-Indacene-1,7-dione, 2,3,5,6-te...	89156	055591-17-8	27

Unknown Spectrum based on Apex minus start of peak

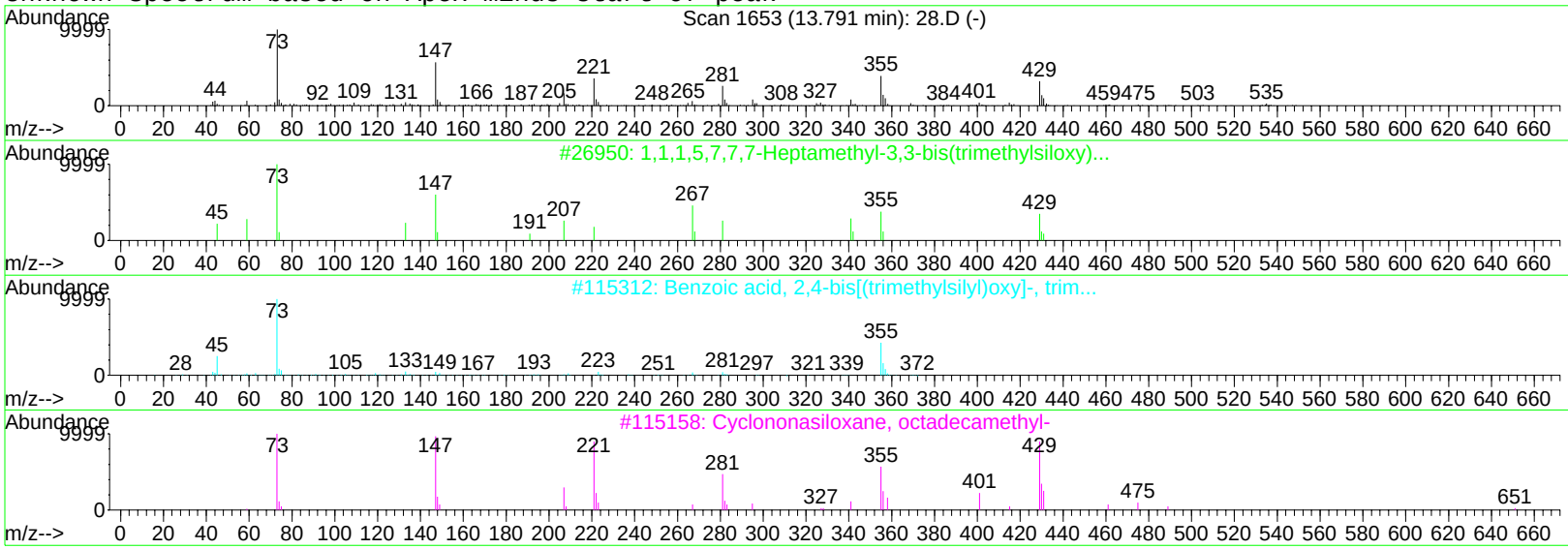


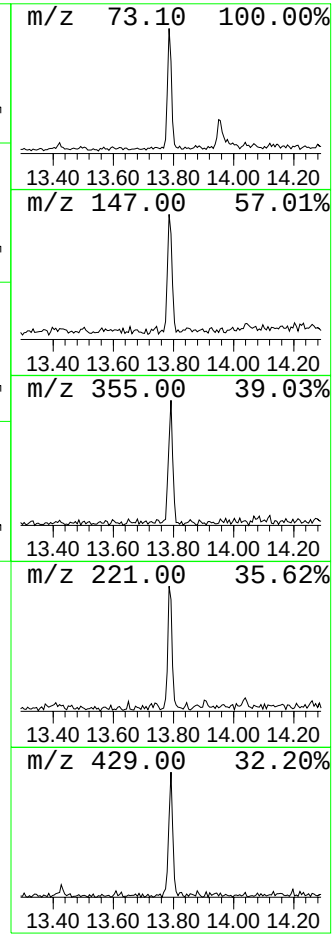


Peak Number: 10 at 13.34 min Area: 128598 Area % 0.59

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Tetratriacontane, 17-hexadecyl-	16405	055256-07-0	33
2 Bis(3-methylbutyl) n-heptafluoro...	24510	075743-06-5	33
3 9-Octadecene, 1,1-dimethoxy-, (Z)-	108358	015677-71-1	33

Unknown Spectrum based on Apex minus start of peak

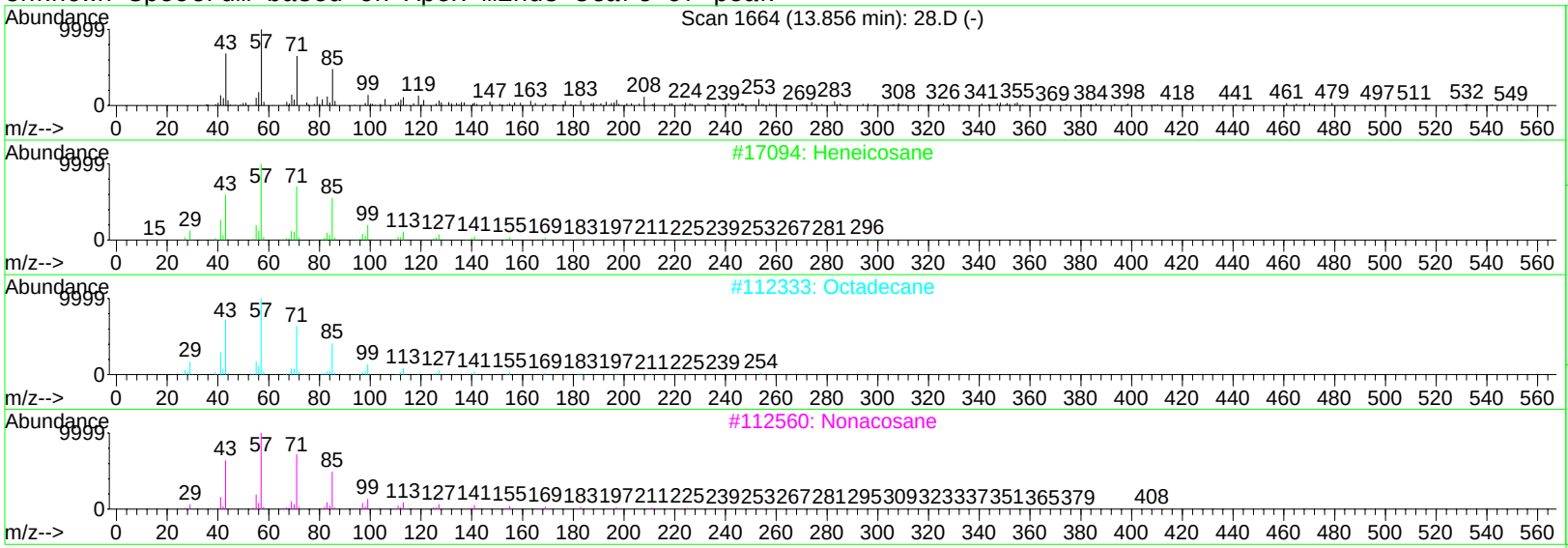


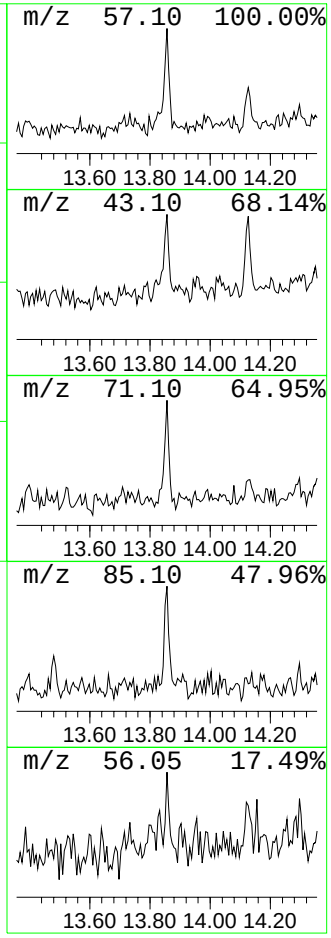


Peak Number: 11 at 13.79 min Area: 472383 Area % 2.17

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1,1,1,5,7,7,7-Heptamethyl-3,3-bi...	26950	038147-00-1	56
2 Benzoic acid, 2,4-bis[(trimethyl...	115312	010586-16-0	35
3 Cyclononasiloxane, octadecamethyl-	115158	000556-71-8	28

Unknown Spectrum based on Apex minus start of peak

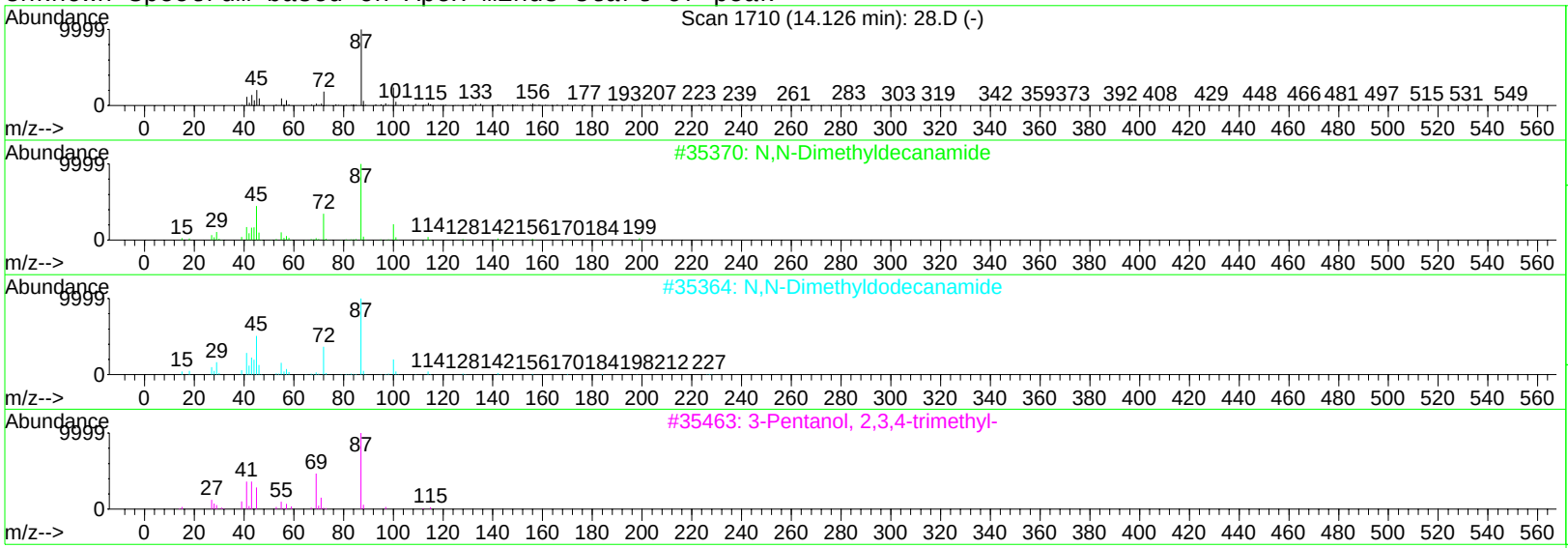


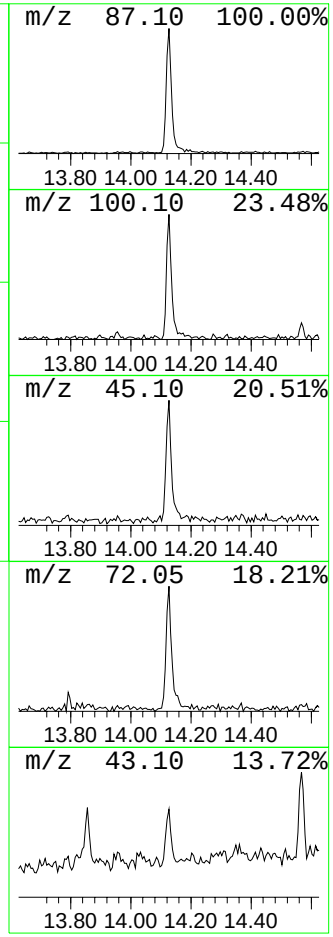


Peak Number: 12 at 13.86 min Area: 213118 Area % 0.98

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Heneicosane	17094	000629-94-7	94
2 Octadecane	112333	000593-45-3	91
3 Nonacosane	112560	000630-03-5	89

Unknown Spectrum based on Apex minus start of peak

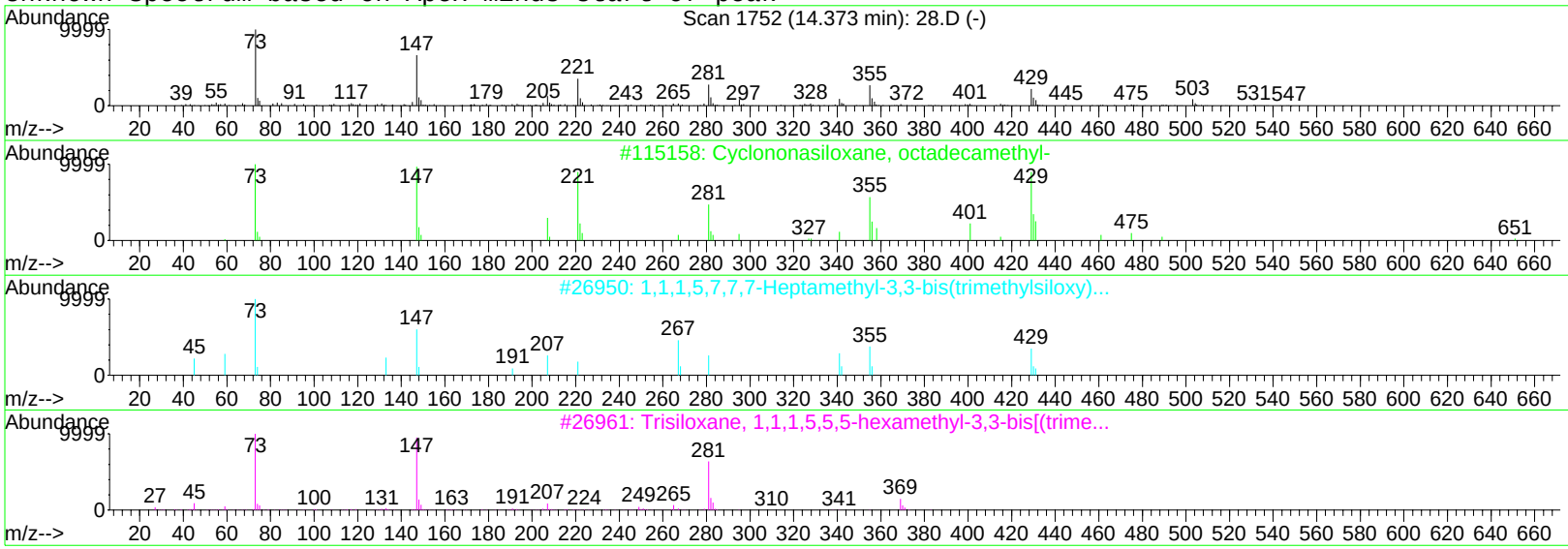


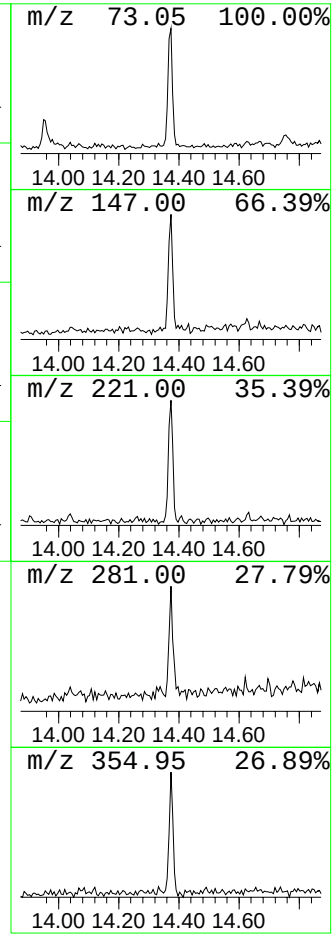


Peak Number: 13 at 14.13 min Area: 711429 Area % 3.27

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 N,N-Dimethyldecanamide	35370	014433-76-2	56
2 N,N-Dimethyldodecanamide	35364	003007-53-2	52
3 3-Pentanol, 2,3,4-trimethyl-	35463	003054-92-0	50

Unknown Spectrum based on Apex minus start of peak

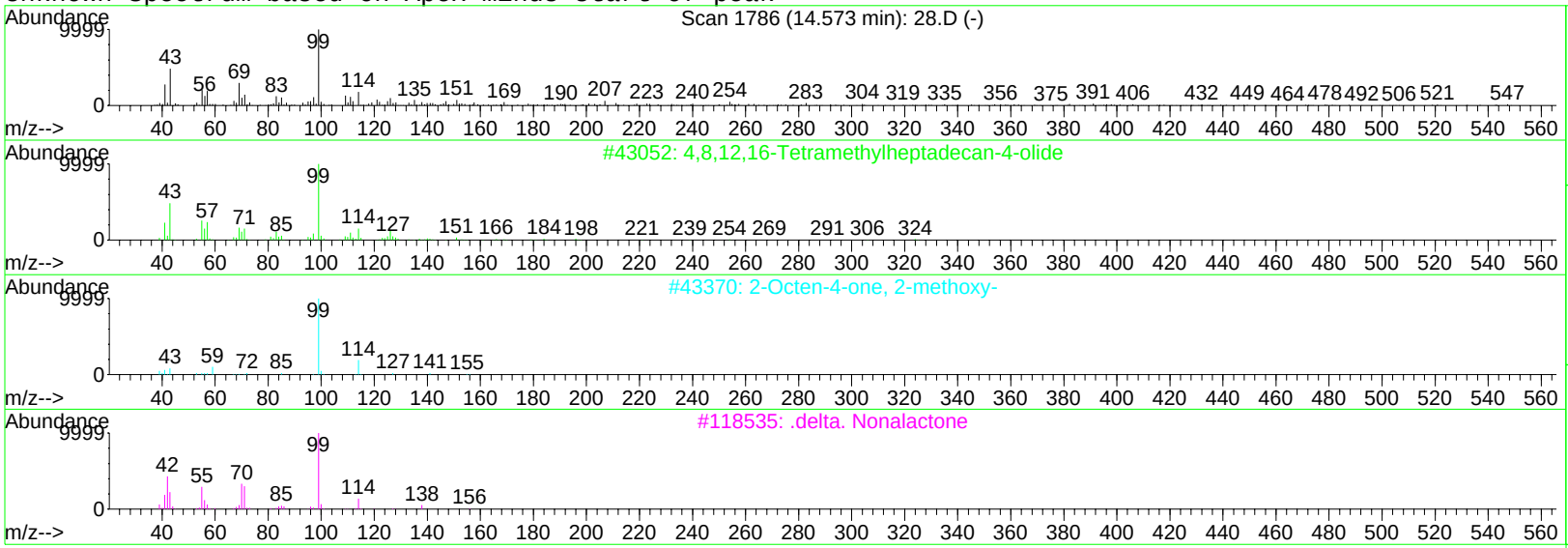


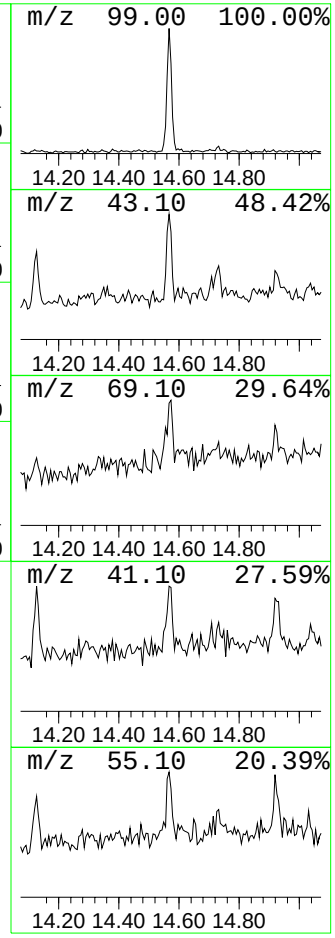


Peak Number: 14 at 14.37 min Area: 908276 Area % 4.17

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclononasiloxane, octadecamethyl-	115158	000556-71-8	45
2 1,1,1,5,7,7,7-Heptamethyl-3,3-bi...	26950	038147-00-1	33
3 Trisiloxane, 1,1,1,5,5,5-hexamet...	26961	003555-47-3	25

Unknown Spectrum based on Apex minus start of peak

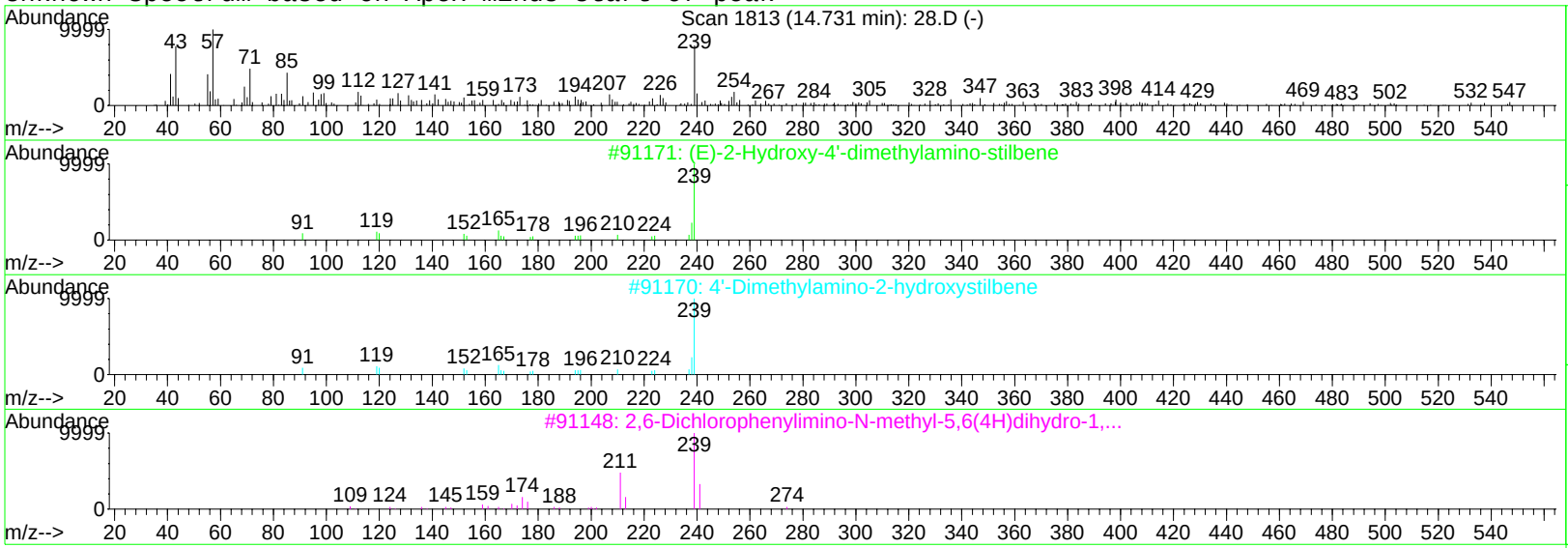


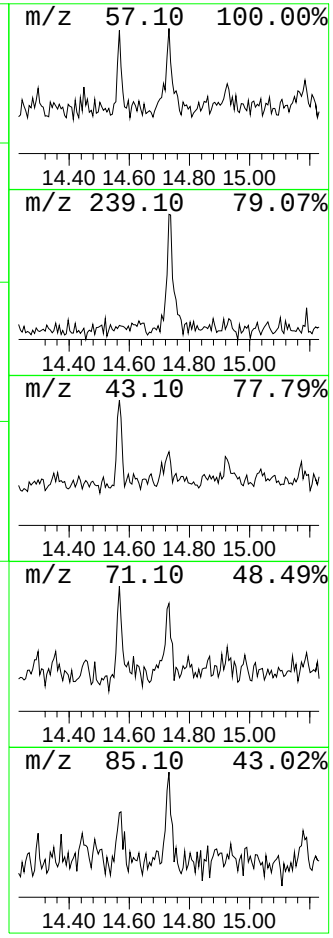


Peak Number: 15 at 14.57 min Area: 826780 Area % 3.80

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 4,8,12,16-Tetramethylheptadecan-...	43052	096168-15-9	86
2 2-Octen-4-one, 2-methoxy-	43370	024985-48-6	64
3 .delta. Nonalactone	118535	003301-94-8	64

Unknown Spectrum based on Apex minus start of peak

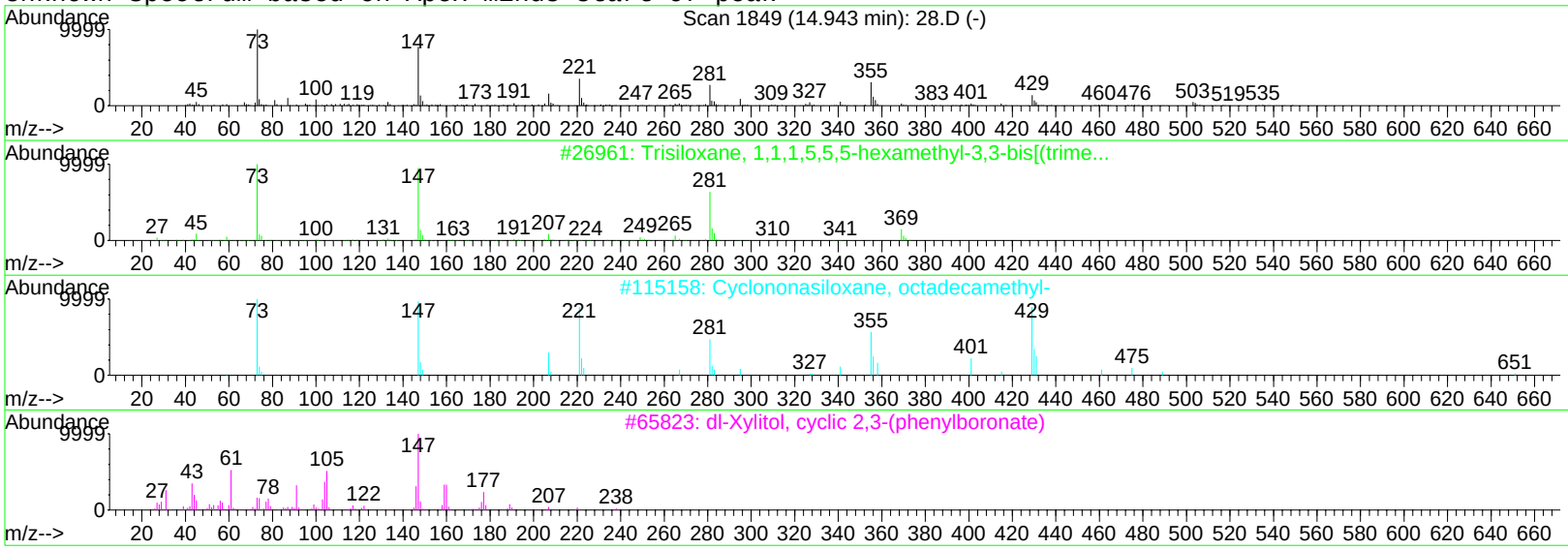


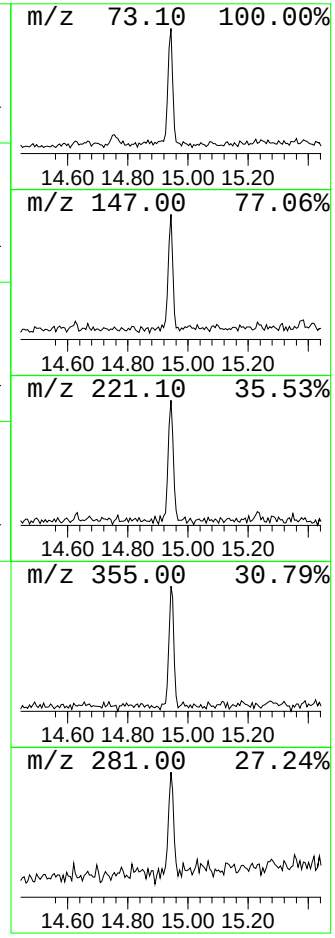


Peak Number: 16 at 14.73 min Area: 1349395 Area % 6.19

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 (E)-2-Hydroxy-4'-dimethylamino-s...	91171	1000148-08-3	64
2 4'-Dimethylamino-2-hydroxystilbene	91170	1000148-08-2	64
3 2,6-Dichlorophenylimino-N-methyl...	91148	1000148-09-4	64

Unknown Spectrum based on Apex minus start of peak

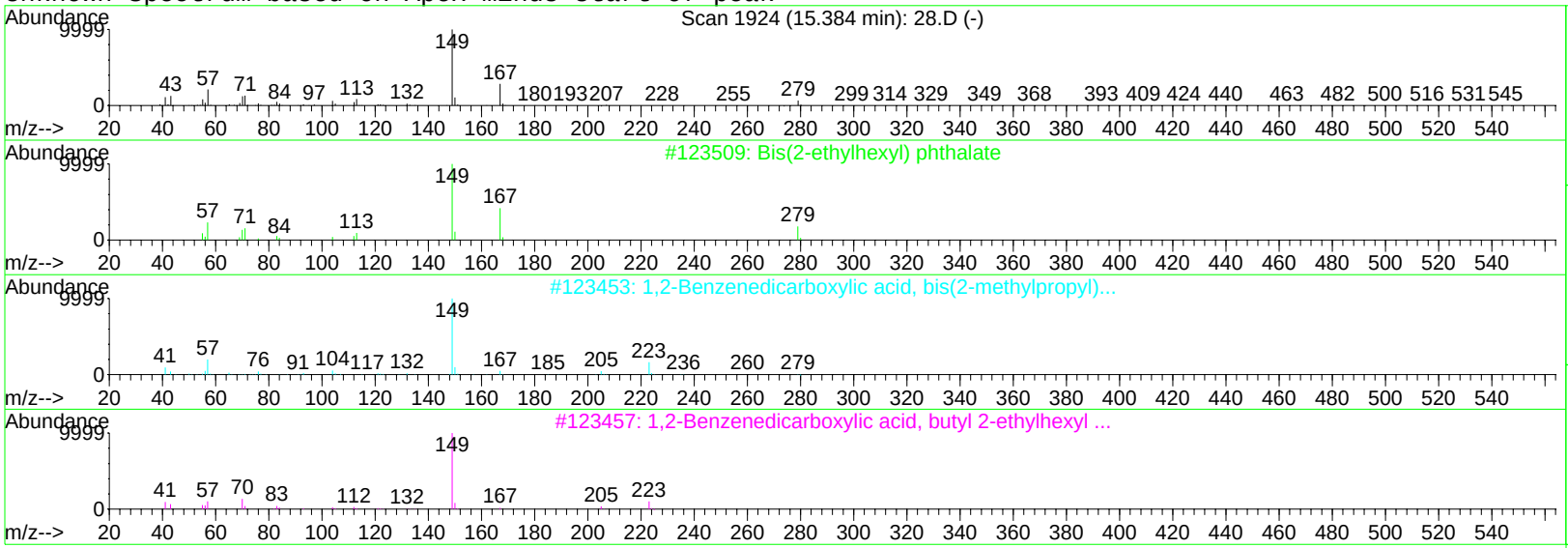


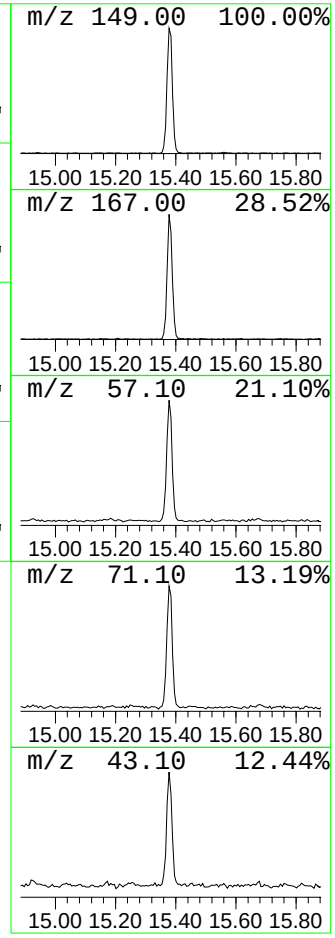


Peak Number: 17 at 14.94 min Area: 1834251 Area % 8.42

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Trisiloxane, 1,1,1,5,5,5-hexamet...	26961	003555-47-3	50
2 Cyclononasiloxane, octadecamethyl-	115158	000556-71-8	43
3 dl-Xylitol, cyclic 2,3-(phenylbo...	65823	074793-46-7	38

Unknown Spectrum based on Apex minus start of peak

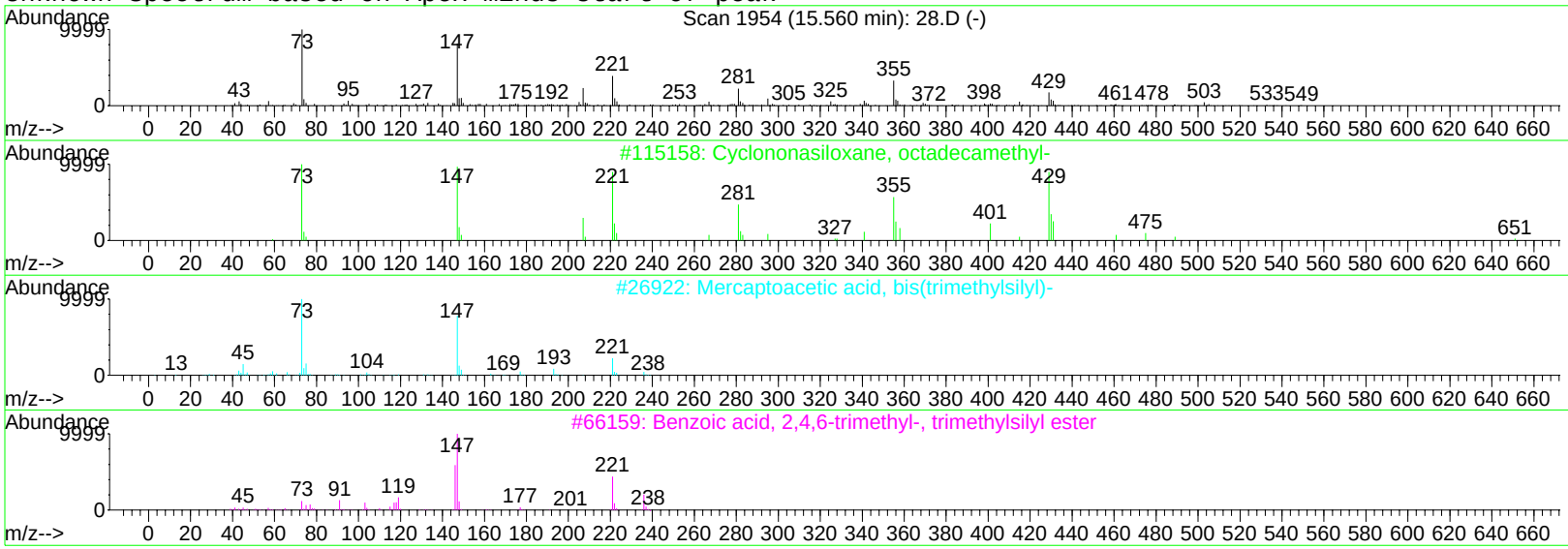


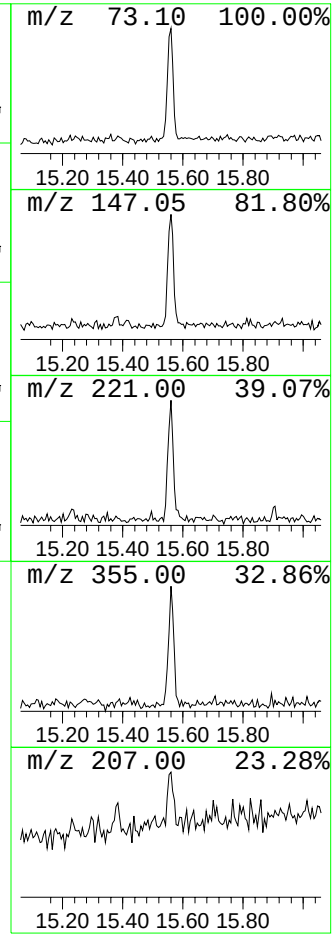


Peak Number: 18 at 15.38 min Area: 5410820 Area % 24.84

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Bis(2-ethylhexyl) phthalate	123509	000117-81-7	86
2 1,2-Benzenedicarboxylic acid, bi...	123453	000084-69-5	60
3 1,2-Benzenedicarboxylic acid, bu...	123457	000085-69-8	59

Unknown Spectrum based on Apex minus start of peak

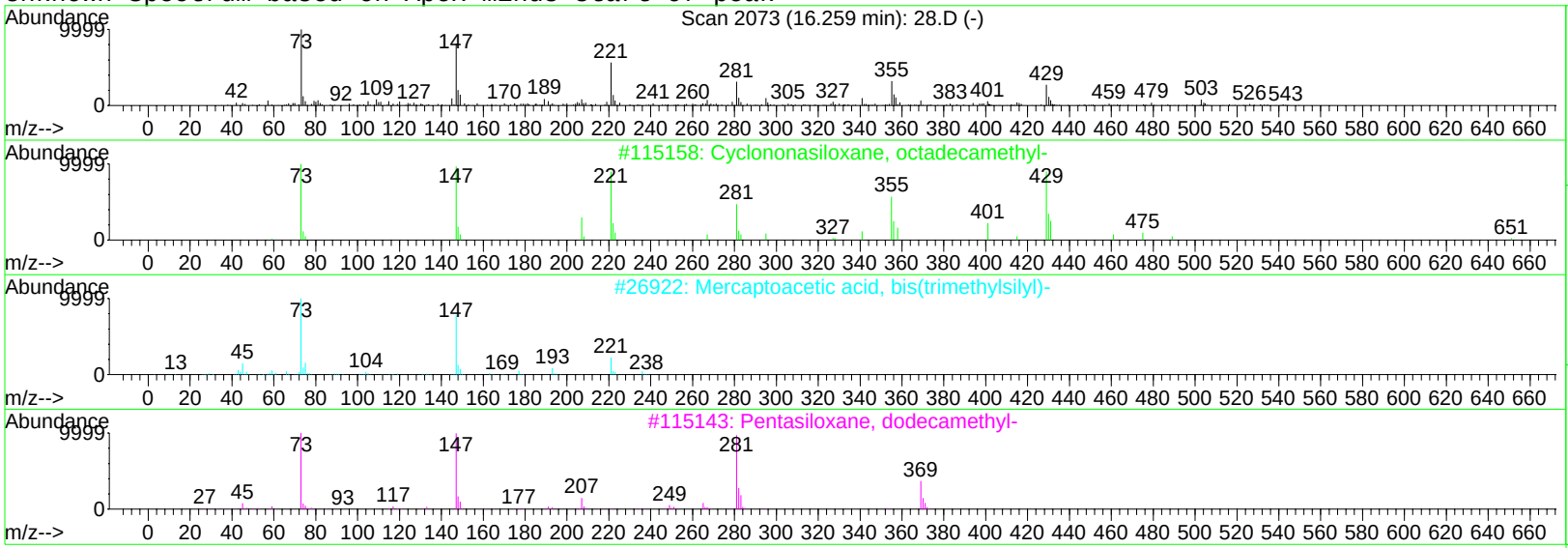


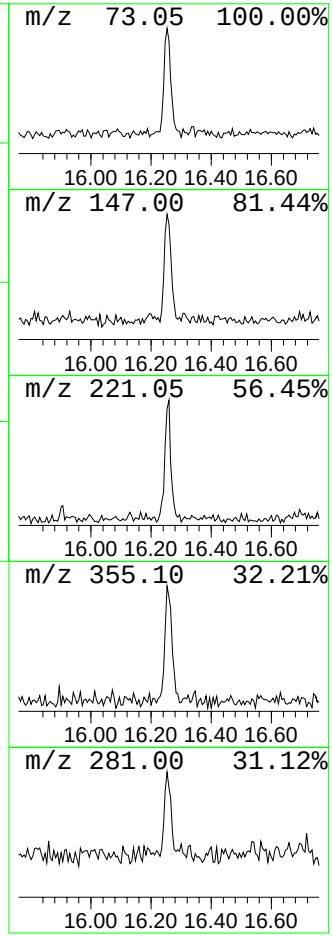


Peak Number: 19 at 15.56 min Area: 1034390 Area % 4.75

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclononasiloxane, octadecamethyl-	115158	000556-71-8	50
2 Mercaptoacetic acid, bis(trimeth...	26922	006398-62-5	47
3 Benzoic acid, 2,4,6-trimethyl-, ...	66159	070079-88-8	38

Unknown Spectrum based on Apex minus start of peak

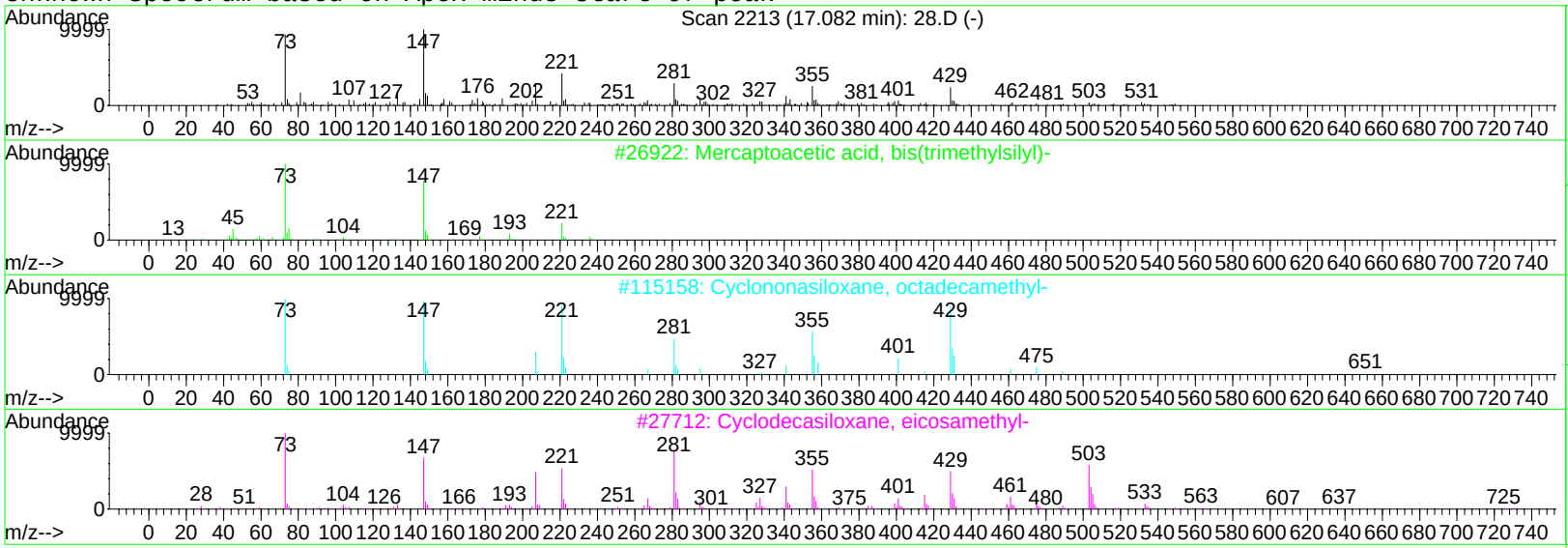


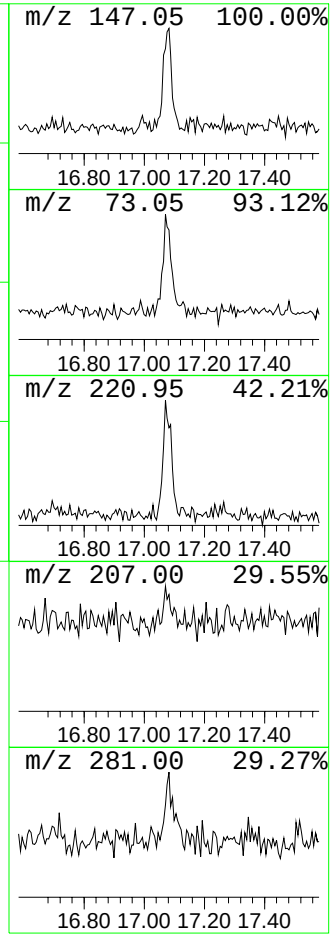


Peak Number: 20 at 16.26 min Area: 795090 Area % 3.65

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclononasiloxane, octadecamethyl-	115158	000556-71-8	87
2 Mercaptoacetic acid, bis(trimeth...	26922	006398-62-5	37
3 Pentasiloxane, dodecamethyl-	115143	000141-63-9	35

Unknown Spectrum based on Apex minus start of peak

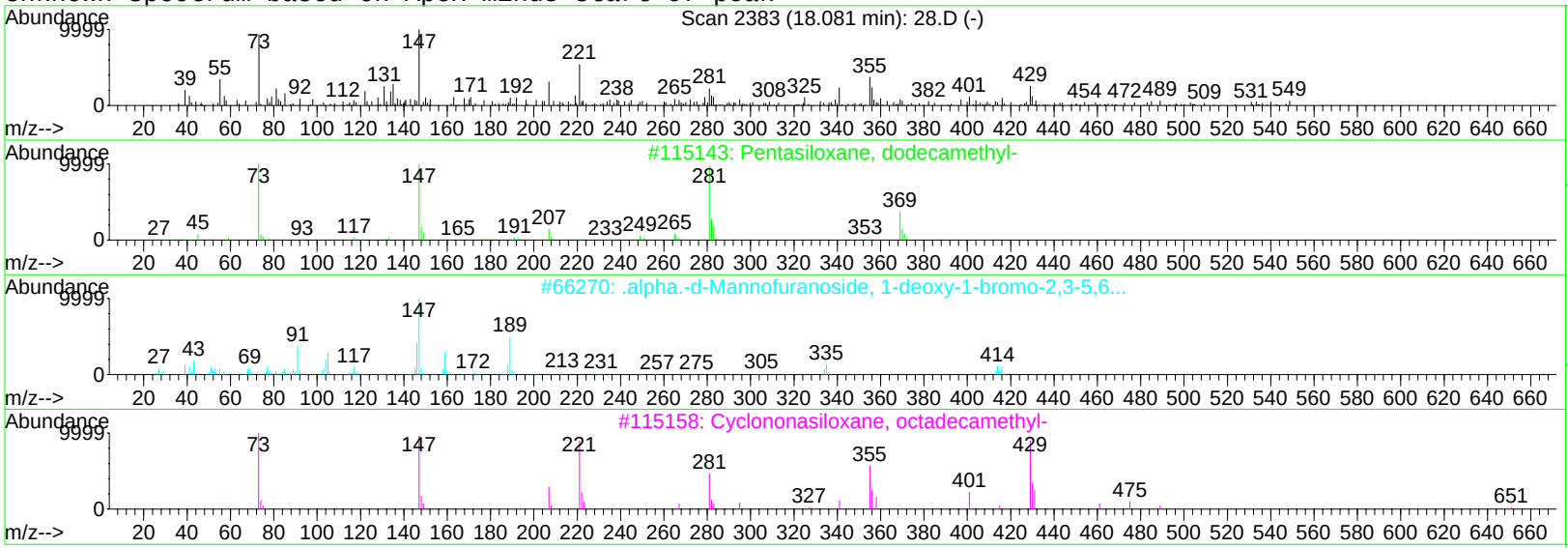


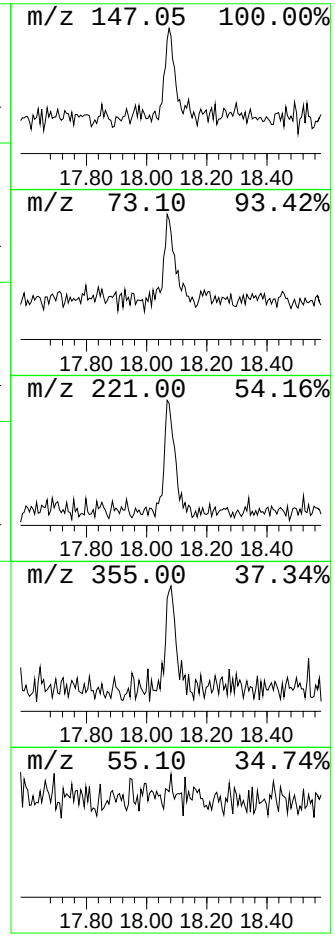


Peak Number: 21 at 17.08 min Area: 450168 Area % 2.07

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Mercaptoacetic acid, bis(trimeth...	26922	006398-62-5	38
2 Cyclononasiloxane, octadecamethyl-	115158	000556-71-8	14
3 Cyclodecasiloxane, eicosamethyl-	27712	018772-36-6	12

Unknown Spectrum based on Apex minus start of peak

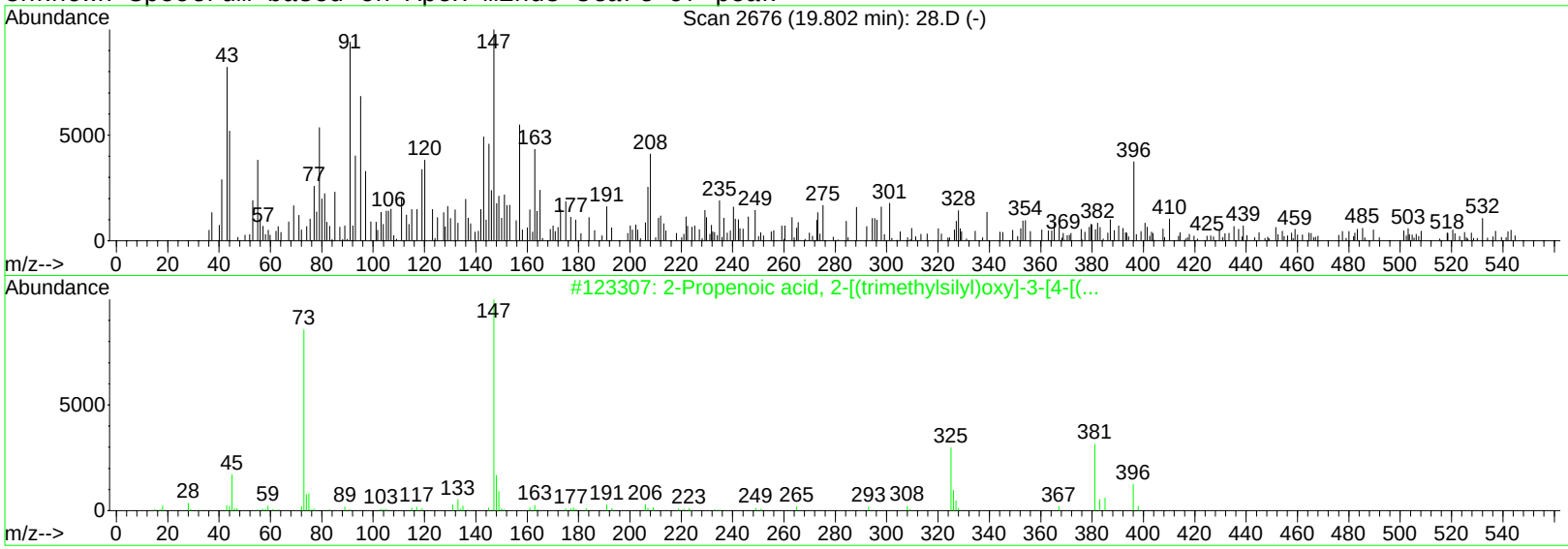


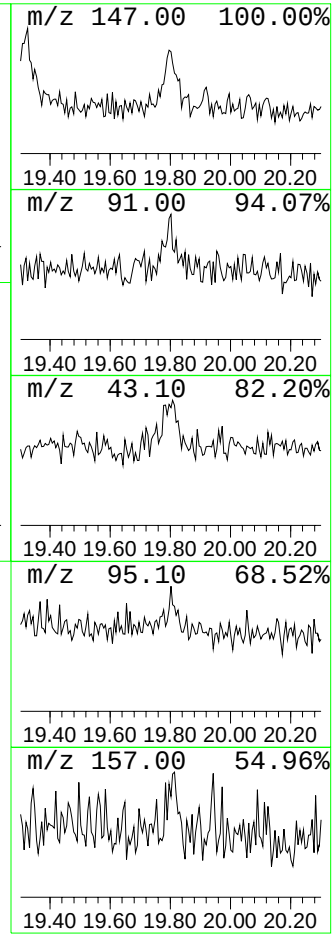


Peak Number: 22 at 18.08 min Area: 410183 Area % 1.88

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Pentasiloxane, dodecamethyl-	115143	000141-63-9	32
2 .alpha.-d-Mannofuranoside, 1-deo...	66270	1000150-77-4	28
3 Cyclononasiloxane, octadecamethyl-	115158	000556-71-8	27

Unknown Spectrum based on Apex minus start of peak

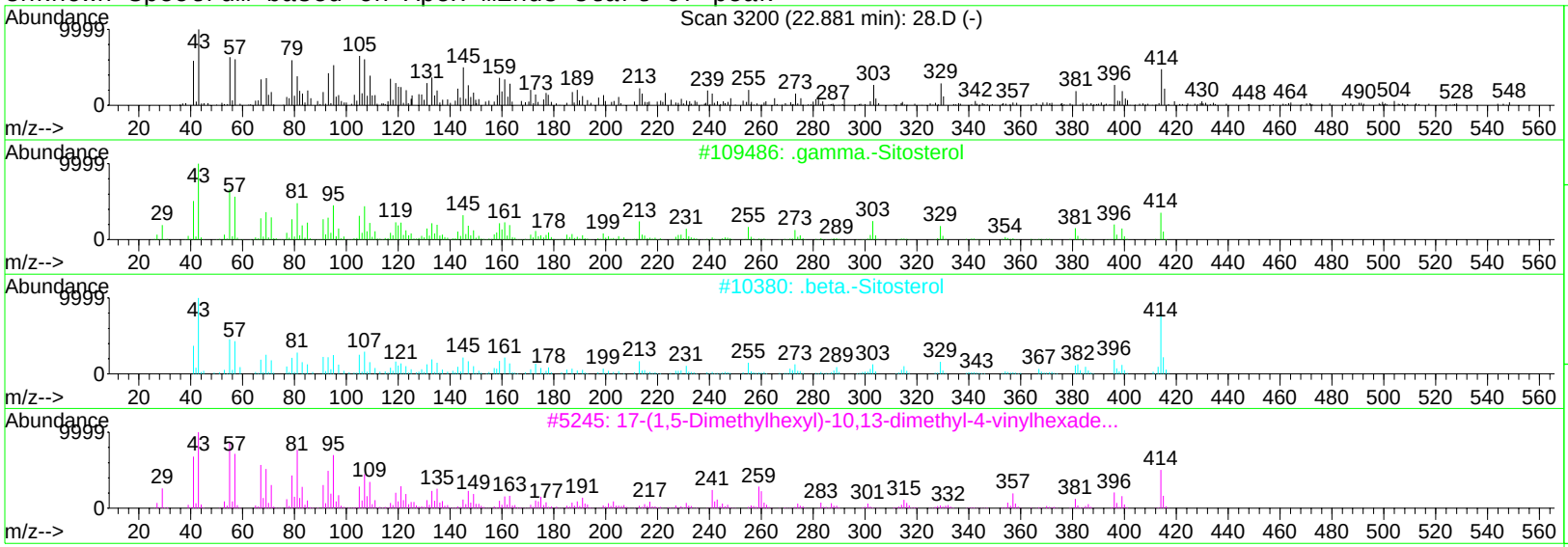


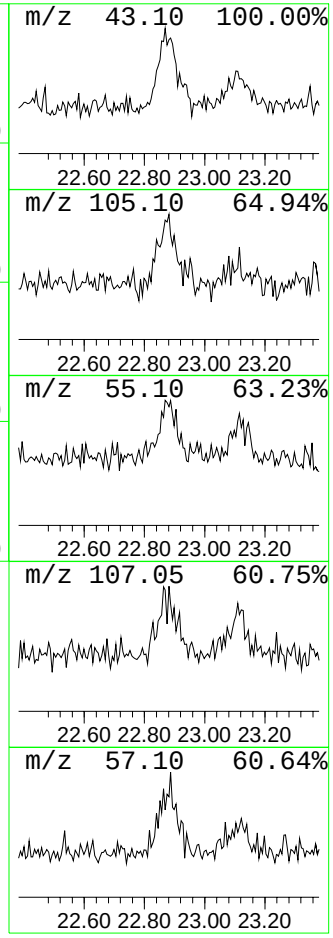


Peak Number: 23 at 19.80 min Area: 861079 Area % 3.95

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Propenoic acid, 2-[(trimethyls...	123307	027750-74-9	2

Unknown Spectrum based on Apex minus start of peak

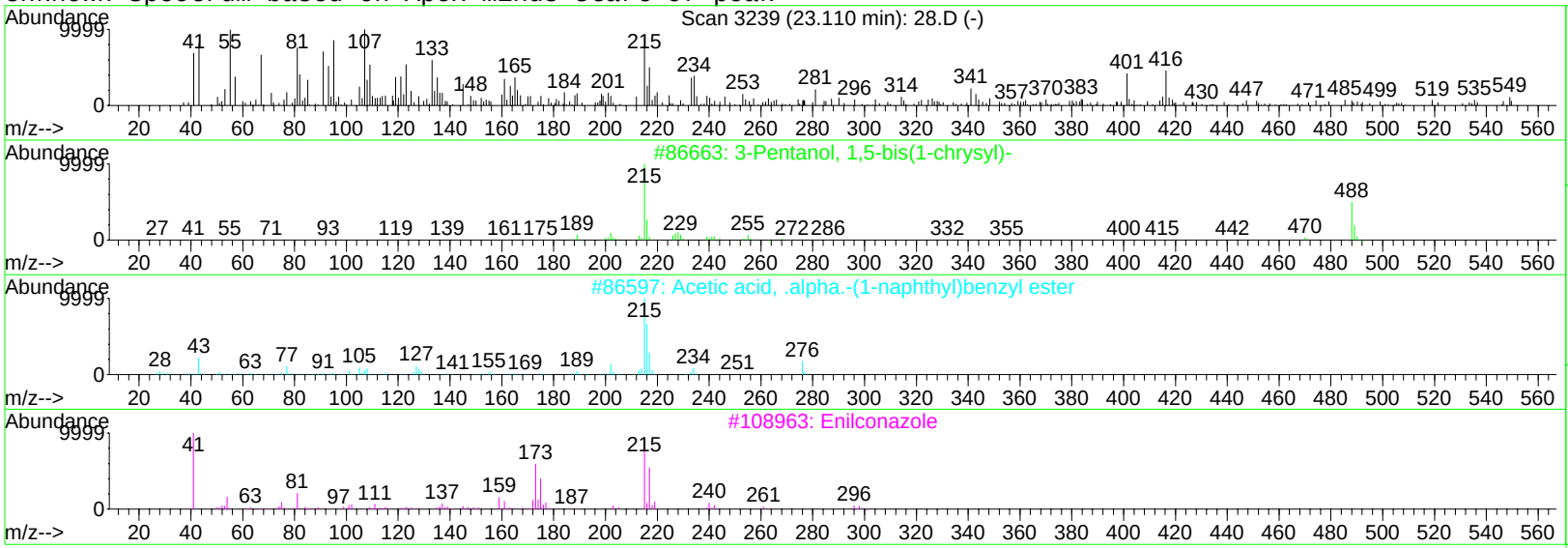


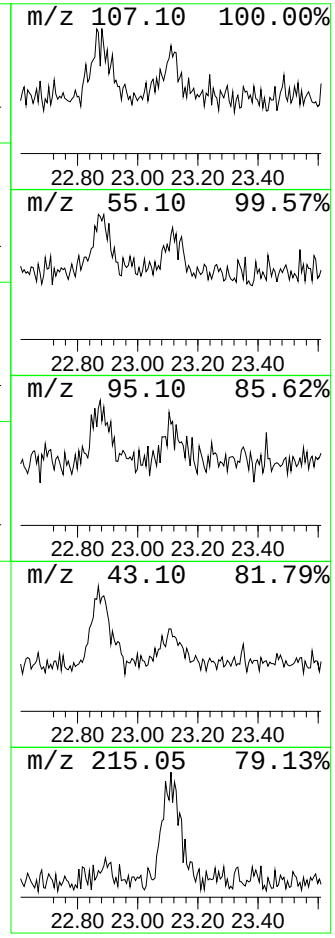


Peak Number: 24 at 22.88 min Area: 1843502 Area % 8.46

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 .gamma.-Sitosterol	109486	000083-47-6	81
2 .beta.-Sitosterol	10380	000083-46-5	58
3 17-(1,5-Dimethylhexyl)-10,13-dim...	5245	1000210-86-9	53

Unknown Spectrum based on Apex minus start of peak





Peak Number: 25 at 23.11 min Area: 942174 Area % 4.32

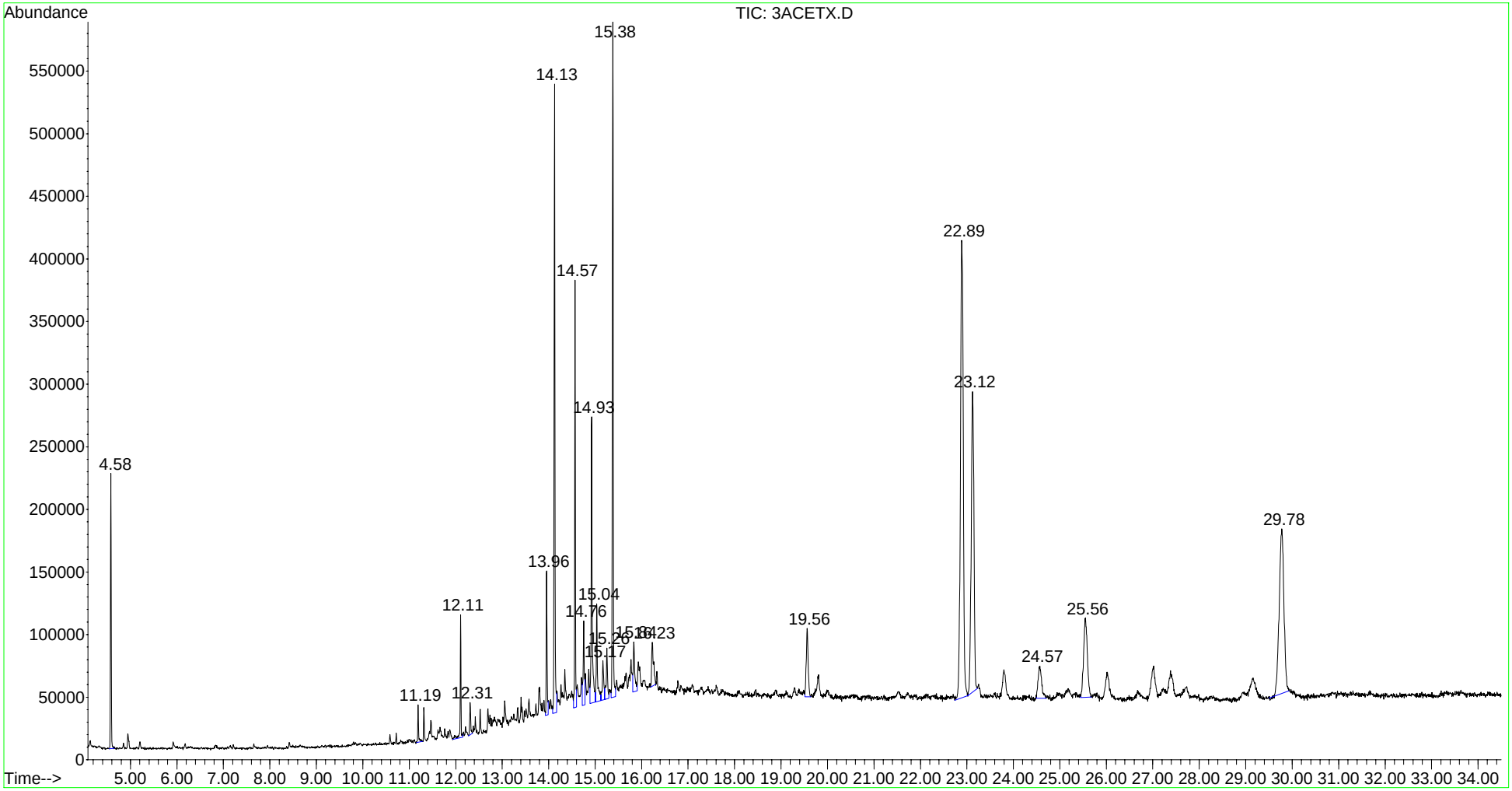
The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 3-Pentanol, 1,5-bis(1-chrysyl)-	86663	1000152-49-6	30
2 Acetic acid, .alpha.-(1-naphthyl...	86597	1000217-27-2	11
3 Enilconazole	108963	035554-44-0	11

Library Search Report

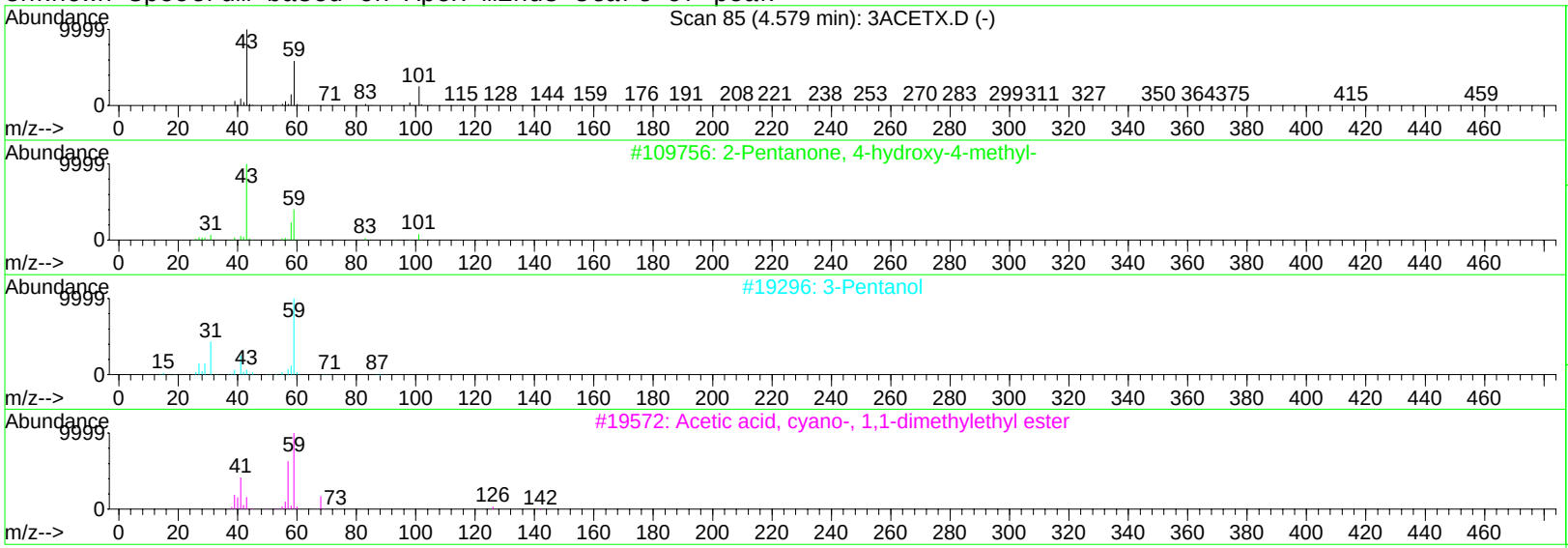
Data File : D:\PRENISHA\300\3ACETX.D
Acq On : 2 Aug 2007 15:37
Sample : Pitch Sample acetone extract
Misc : 1µl inject, acetone, splitless

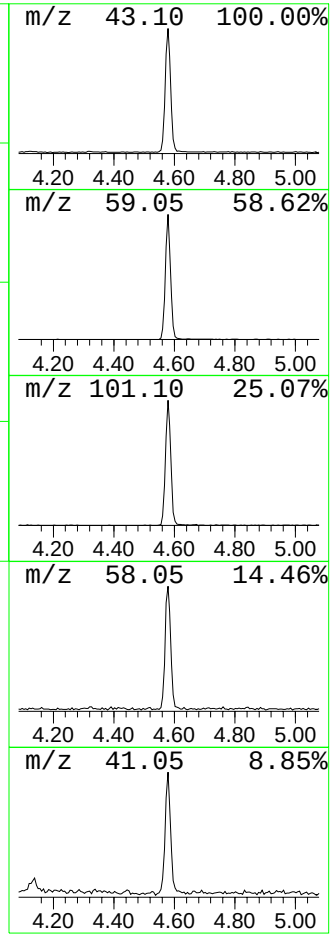
Vial: 92
Operator: Bret
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e
Method : C:\MSDCHEM\1\METHODS\NEW.M (Chemstation Integrator)
Title :



Unknown Spectrum based on Apex minus start of peak

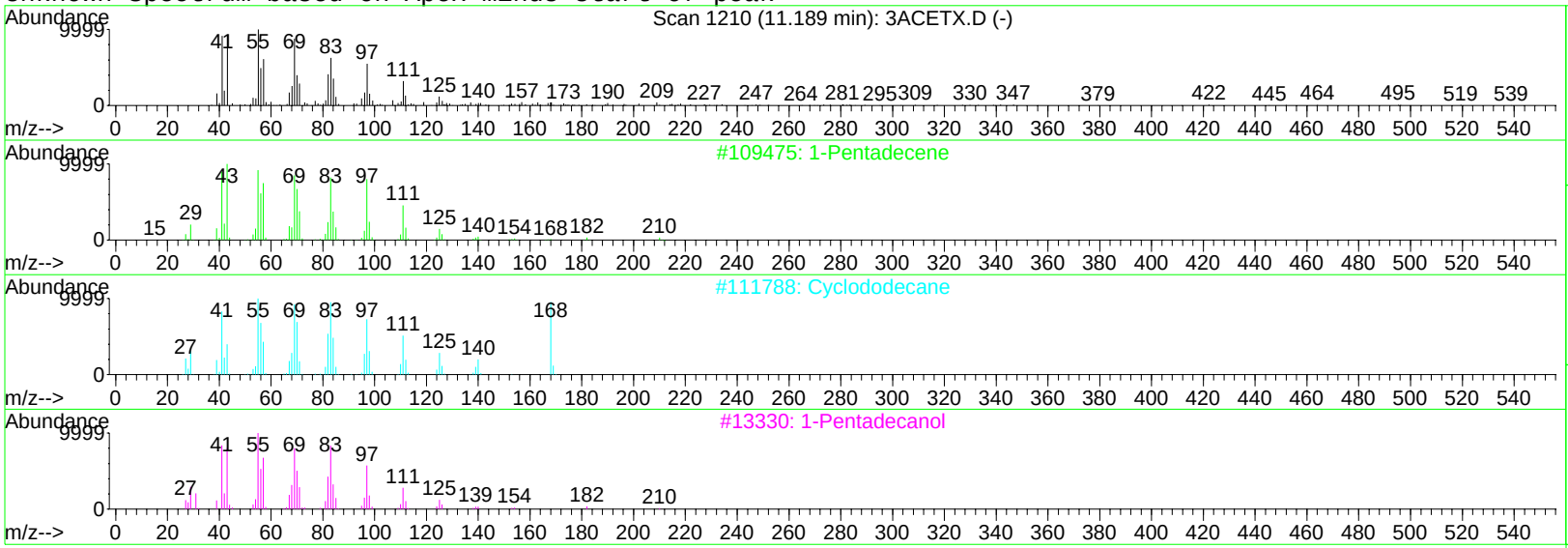


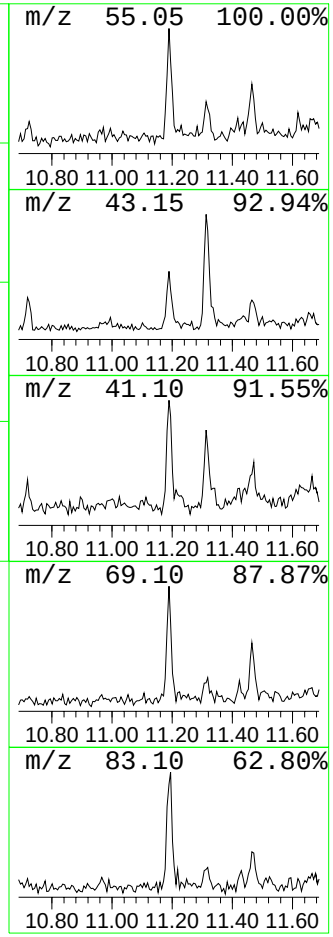


Peak Number: 1 at 4.58 min Area: 2720288 Area % 3.70

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Pentanone, 4-hydroxy-4-methyl-	109756	000123-42-2	50
2 3-Pentanol	19296	000584-02-1	25
3 Acetic acid, cyano-, 1,1-dimethy...	19572	001116-98-9	17

Unknown Spectrum based on Apex minus start of peak

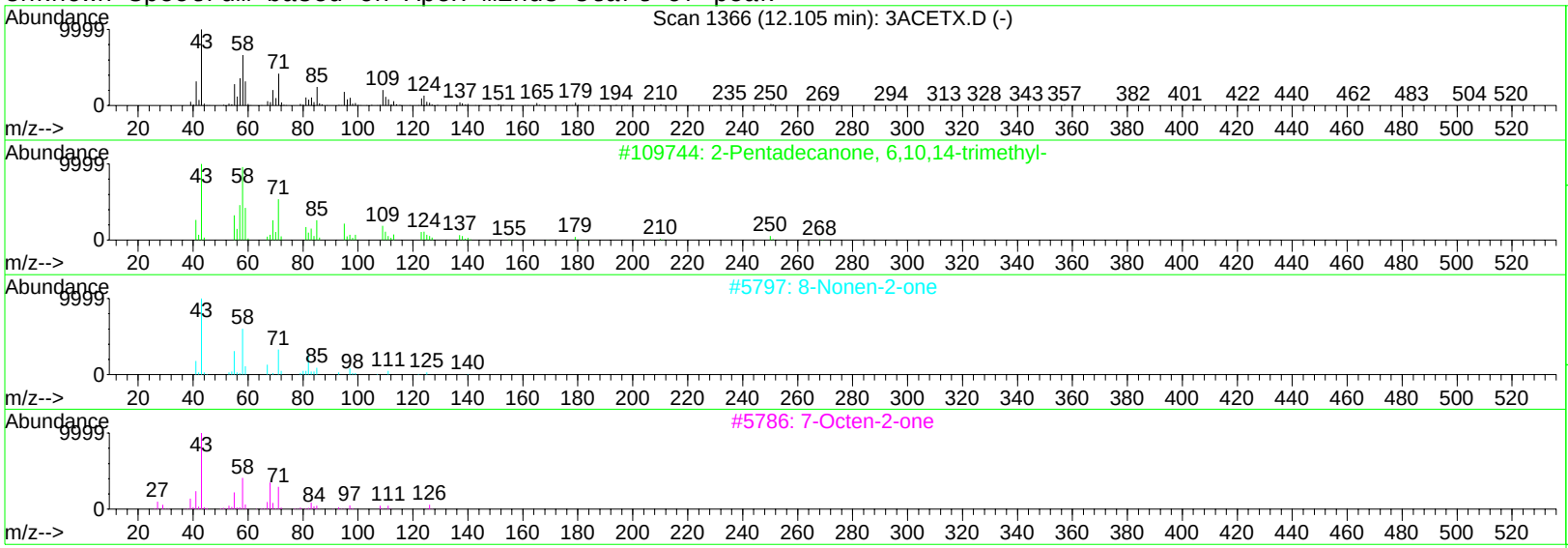


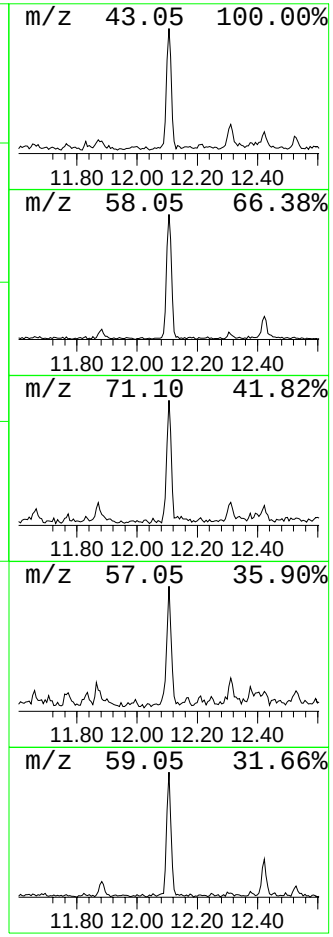


Peak Number: 2 at 11.19 min Area: 389878 Area % 0.53

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1-Pentadecene	109475	013360-61-7	96
2 Cyclododecane	111788	000294-62-2	93
3 1-Pentadecanol	13330	000629-76-5	91

Unknown Spectrum based on Apex minus start of peak

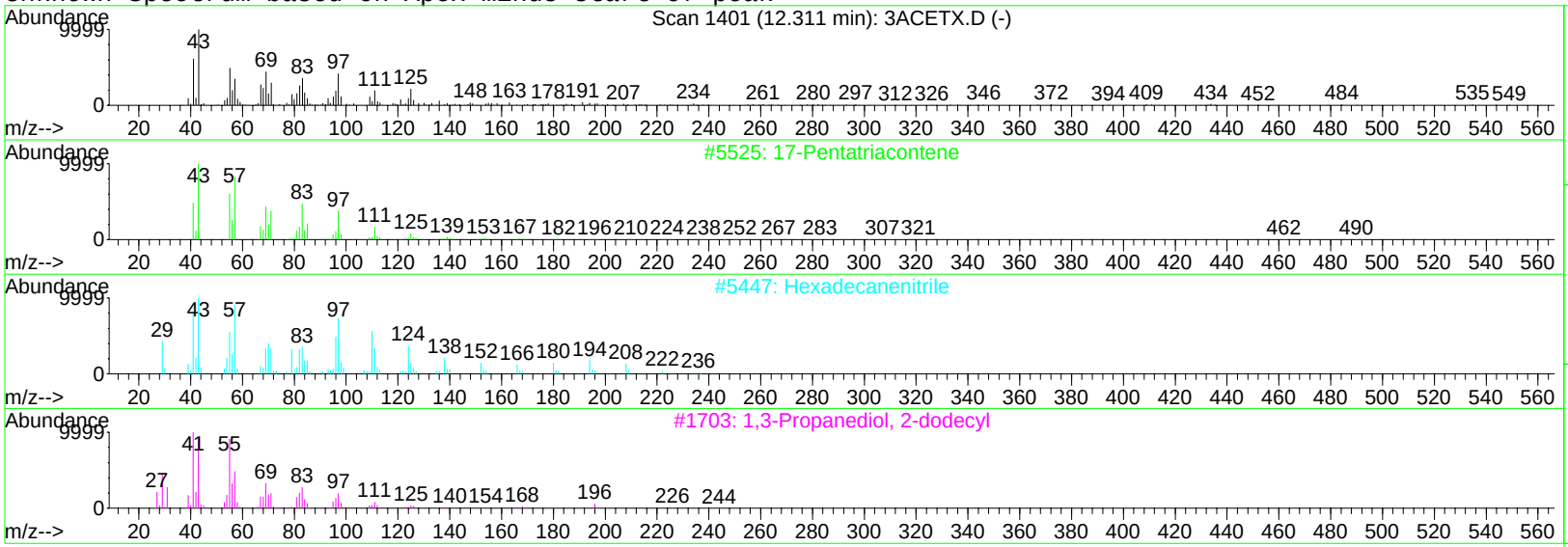


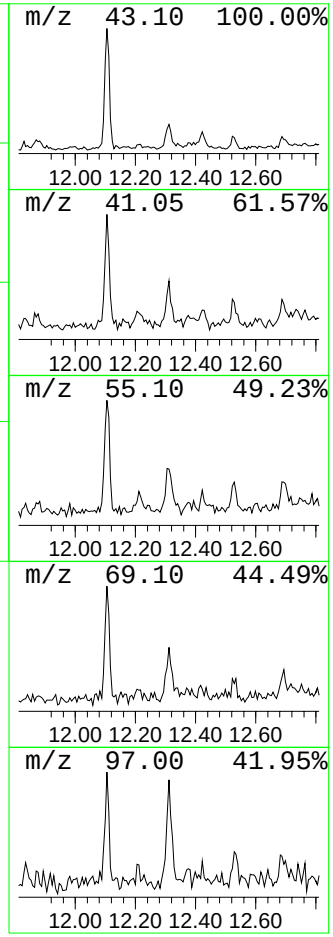


Peak Number: 3 at 12.11 min Area: 1248322 Area % 1.70

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Pentadecanone, 6,10,14-trimethyl-	109744	000502-69-2	87
2 8-Nonen-2-one	5797	005009-32-5	46
3 7-Octen-2-one	5786	003664-60-6	38

Unknown Spectrum based on Apex minus start of peak

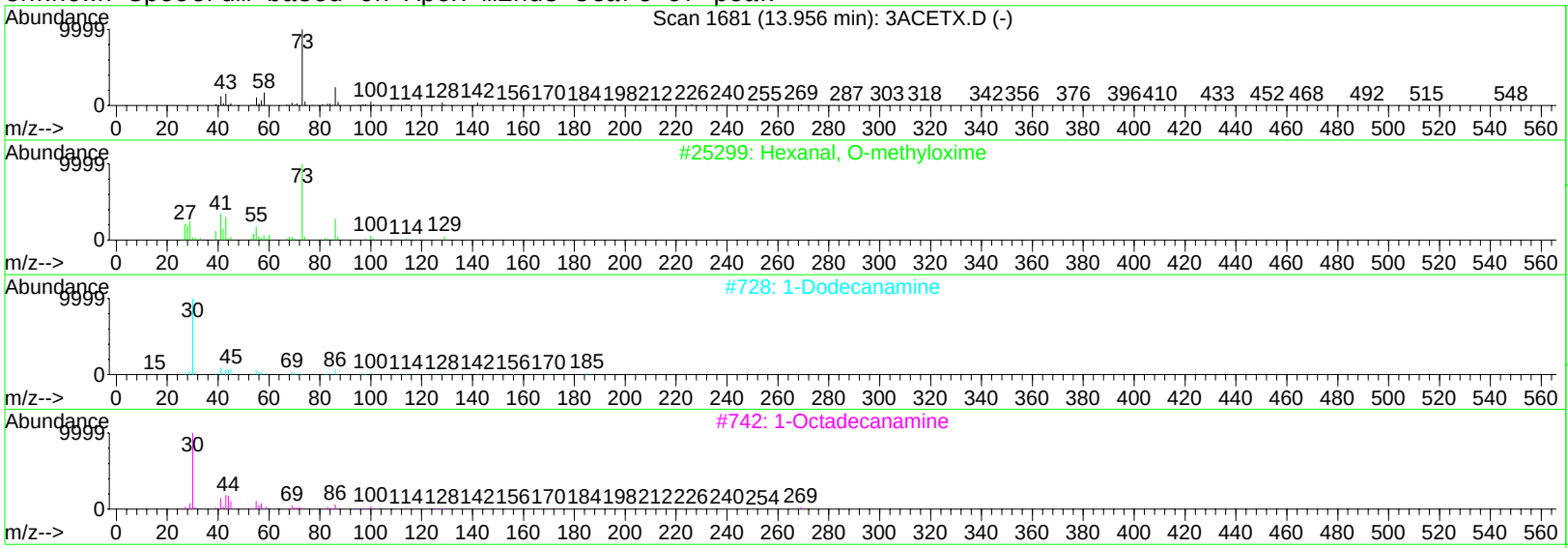


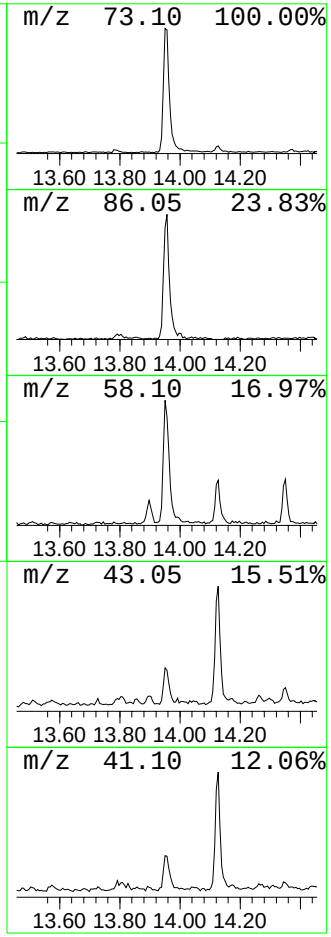


Peak Number: 4 at 12.31 min Area: 361359 Area % 0.49

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 17-Pentatriacontene	5525	006971-40-0	64
2 Hexadecanenitrile	5447	000629-79-8	58
3 1,3-Propanediol, 2-dodecyl	1703	1000197-46-7	41

Unknown Spectrum based on Apex minus start of peak

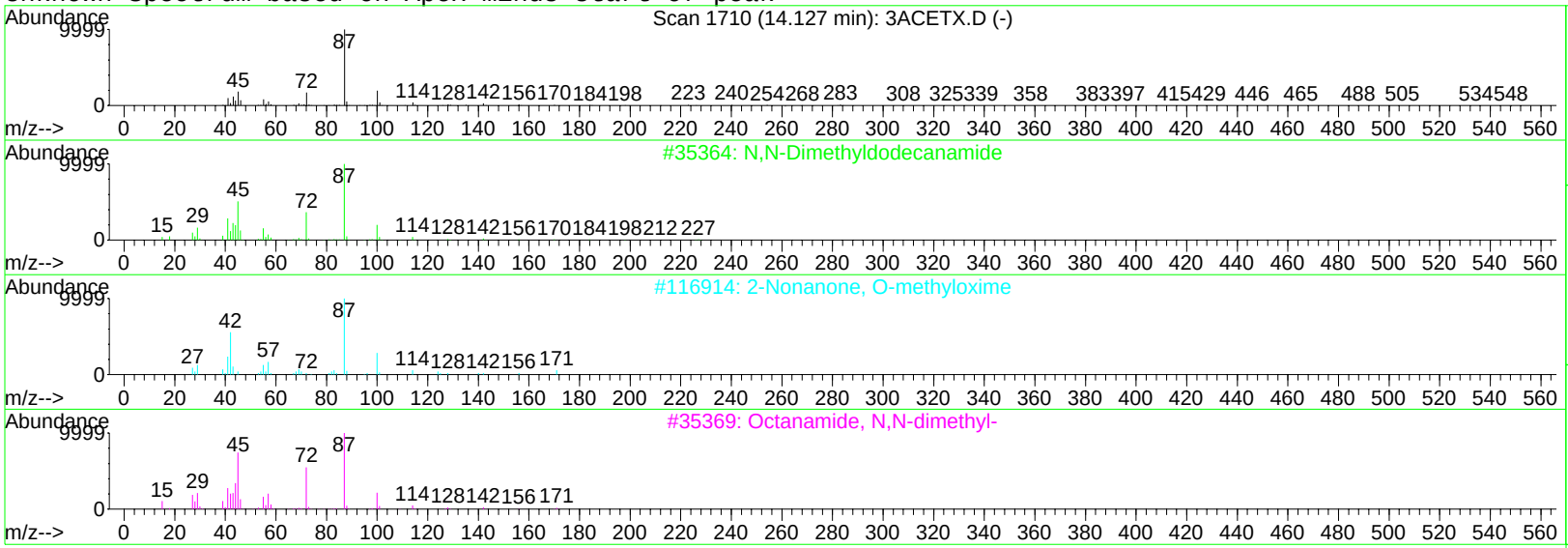


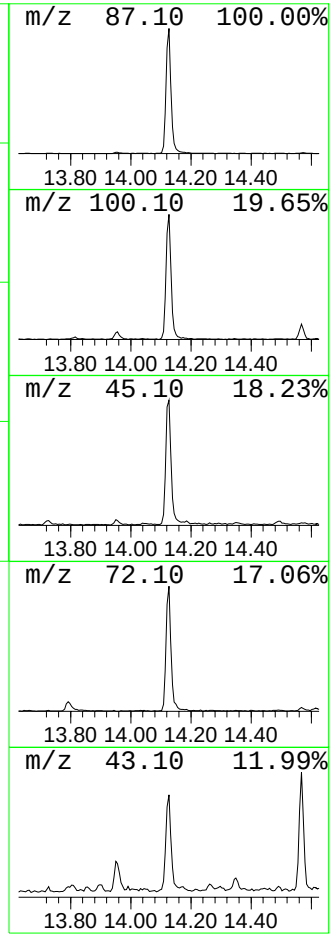


Peak Number: 5 at 13.96 min Area: 1628216 Area % 2.22

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Hexanal, O-methyloxime	25299	056292-95-6	50
2 1-Dodecanamine	728	000124-22-1	27
3 1-Octadecanamine	742	000124-30-1	25

Unknown Spectrum based on Apex minus start of peak

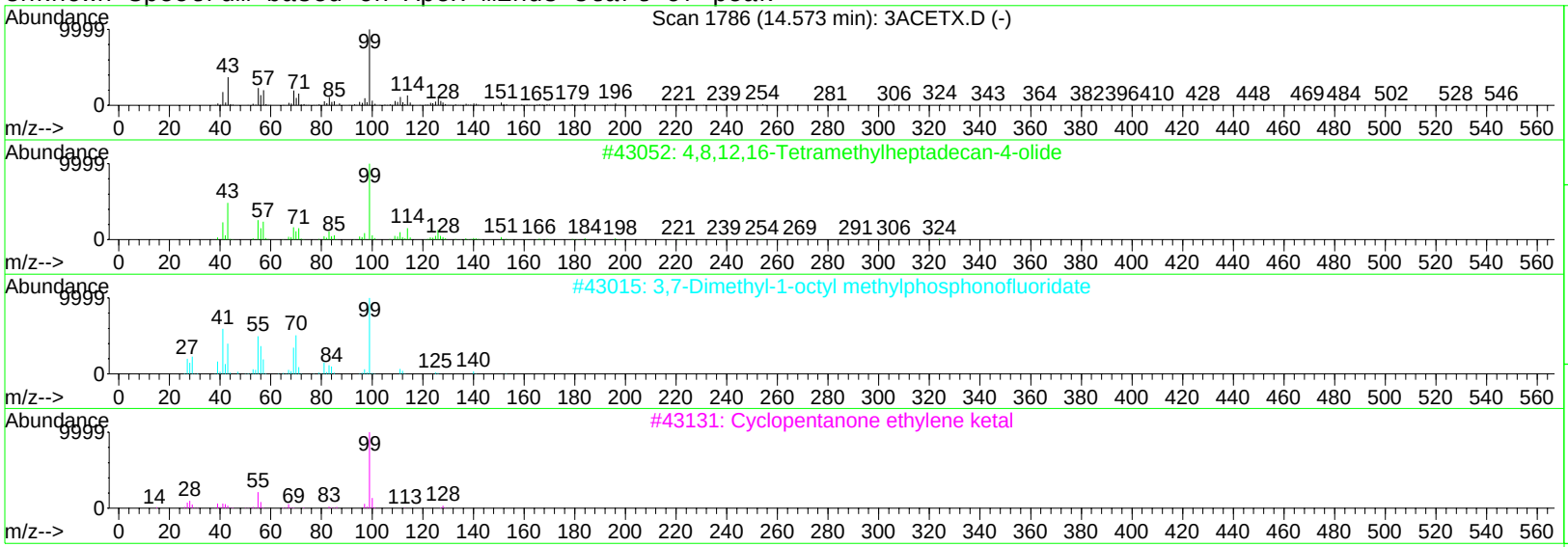


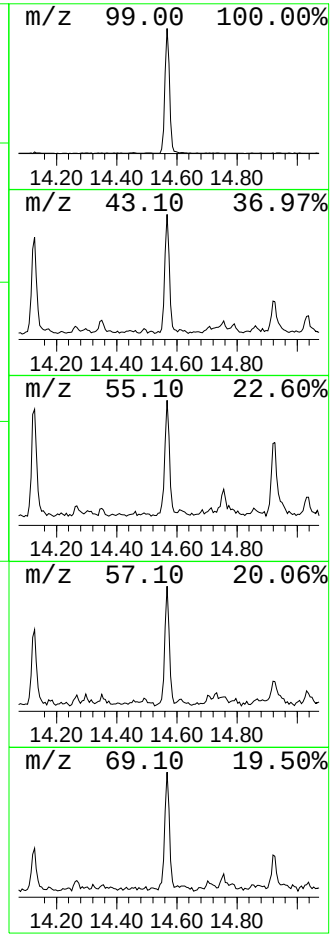


Peak Number: 6 at 14.13 min Area: 5960257 Area % 8.11

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 N,N-Dimethyldodecanamide	35364	003007-53-2	50
2 2-Nonanone, O-methyloxime	116914	056292-72-9	50
3 Octanamide, N,N-dimethyl-	35369	001118-92-9	50

Unknown Spectrum based on Apex minus start of peak

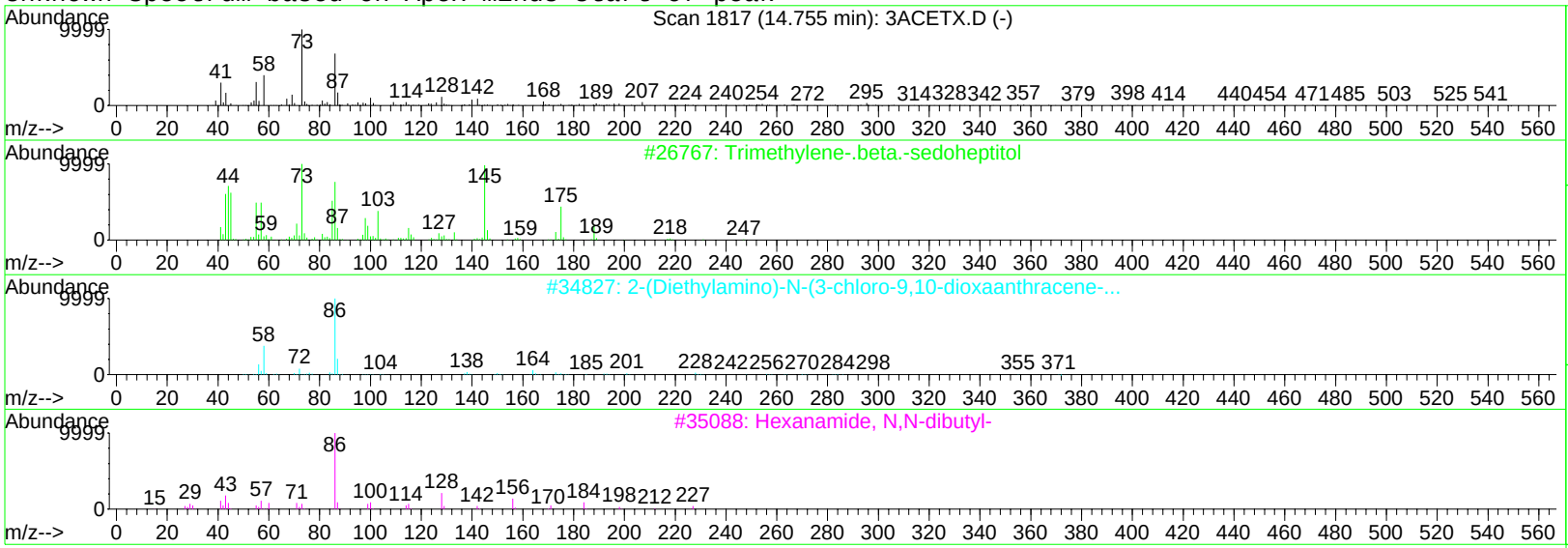


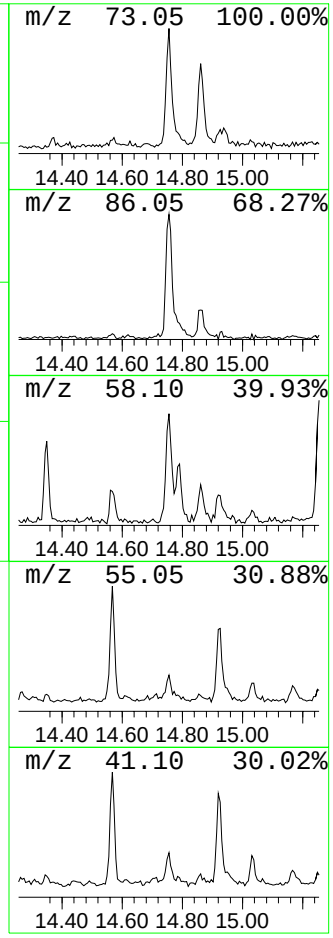


Peak Number: 7 at 14.57 min Area: 3760608 Area % 5.12

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 4,8,12,16-Tetramethylheptadecan-...	43052	096168-15-9	99
2 3,7-Dimethyl-1-octyl methylphosp...	43015	1000216-71-6	59
3 Cyclopentanone ethylene ketal	43131	000176-32-9	58

Unknown Spectrum based on Apex minus start of peak

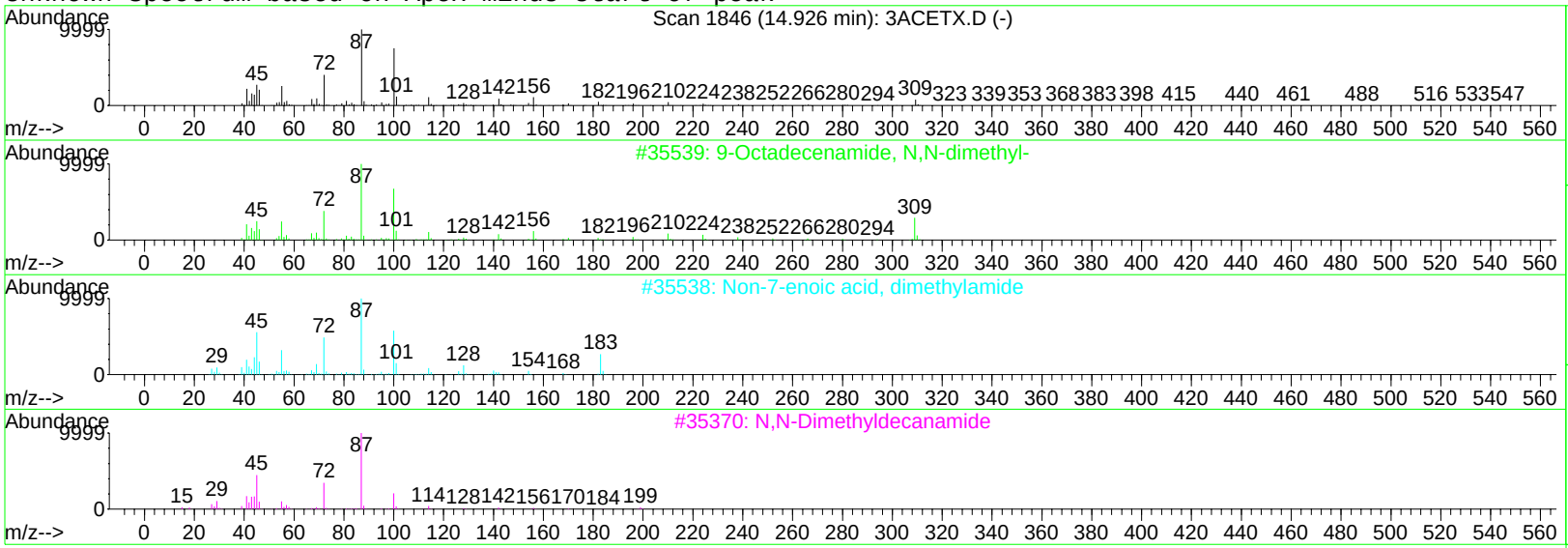


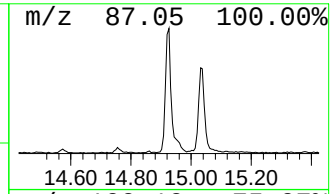


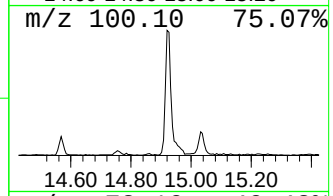
Peak Number: 8 at 14.76 min Area: 1144637 Area % 1.56

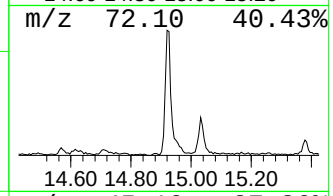
The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Trimethylene-.beta.-sedoheptitol	26767	094625-22-6	32
2 2-(Diethylamino)-N-(3-chloro-9,1...	34827	1000111-05-1	23
3 Hexanamide, N,N-dibutyl-	35088	052868-51-6	23

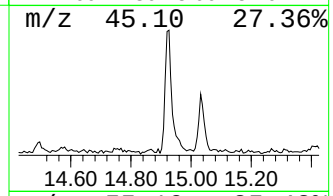
Unknown Spectrum based on Apex minus start of peak

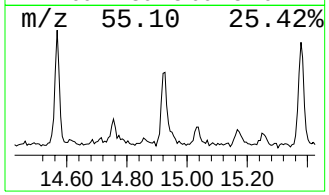








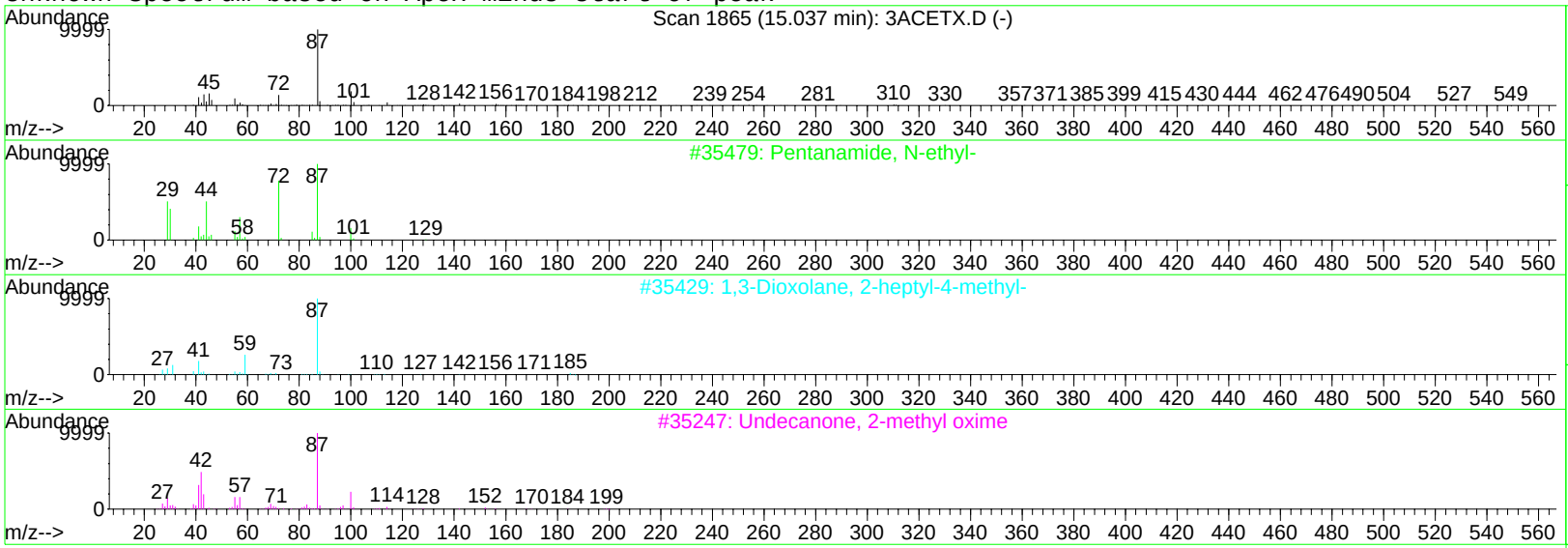


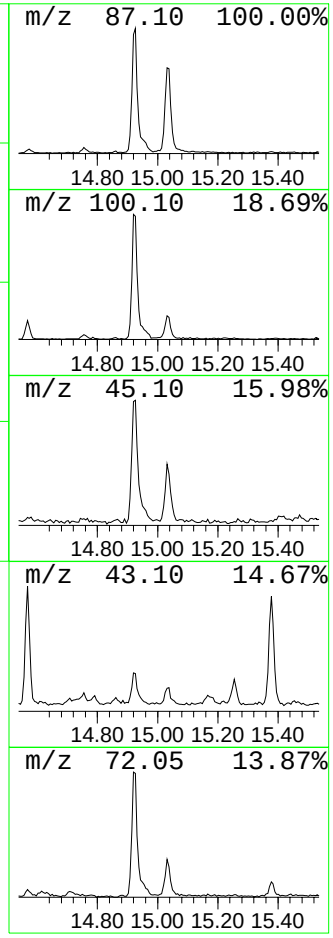


Peak Number: 9 at 14.93 min Area: 3763760 Area % 5.12

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 9-Octadecenamide, N,N-dimethyl-	35539	003906-30-7	90
2 Non-7-enoic acid, dimethylamide	35538	1000187-26-5	50
3 N,N-Dimethyldecanamide	35370	014433-76-2	43

Unknown Spectrum based on Apex minus start of peak

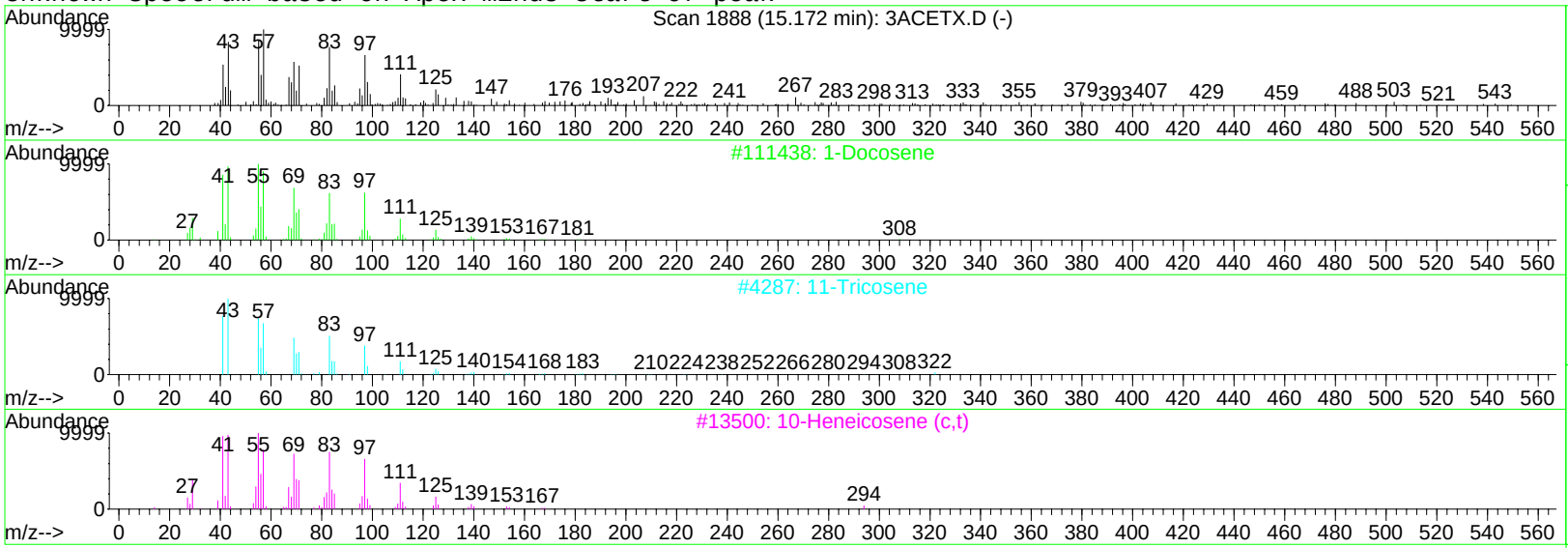


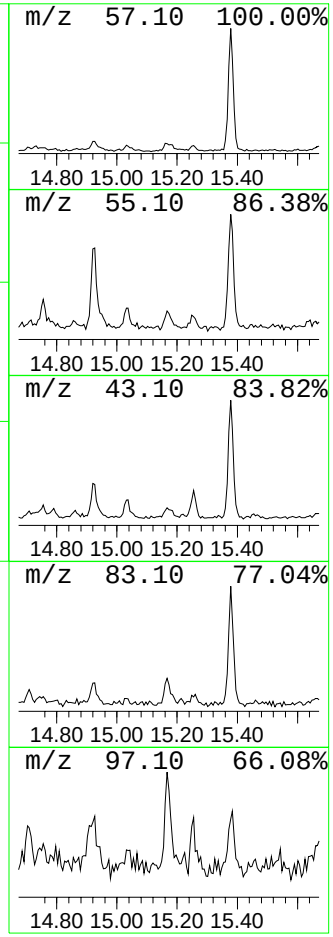


Peak Number: 10 at 15.04 min Area: 1471797 Area % 2.00

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Pentanamide, N-ethyl-	35479	054007-33-9	59
2 1,3-Dioxolane, 2-heptyl-4-methyl-	35429	074094-61-4	50
3 Undecanone, 2-methyl oxime	35247	1000062-84-8	45

Unknown Spectrum based on Apex minus start of peak

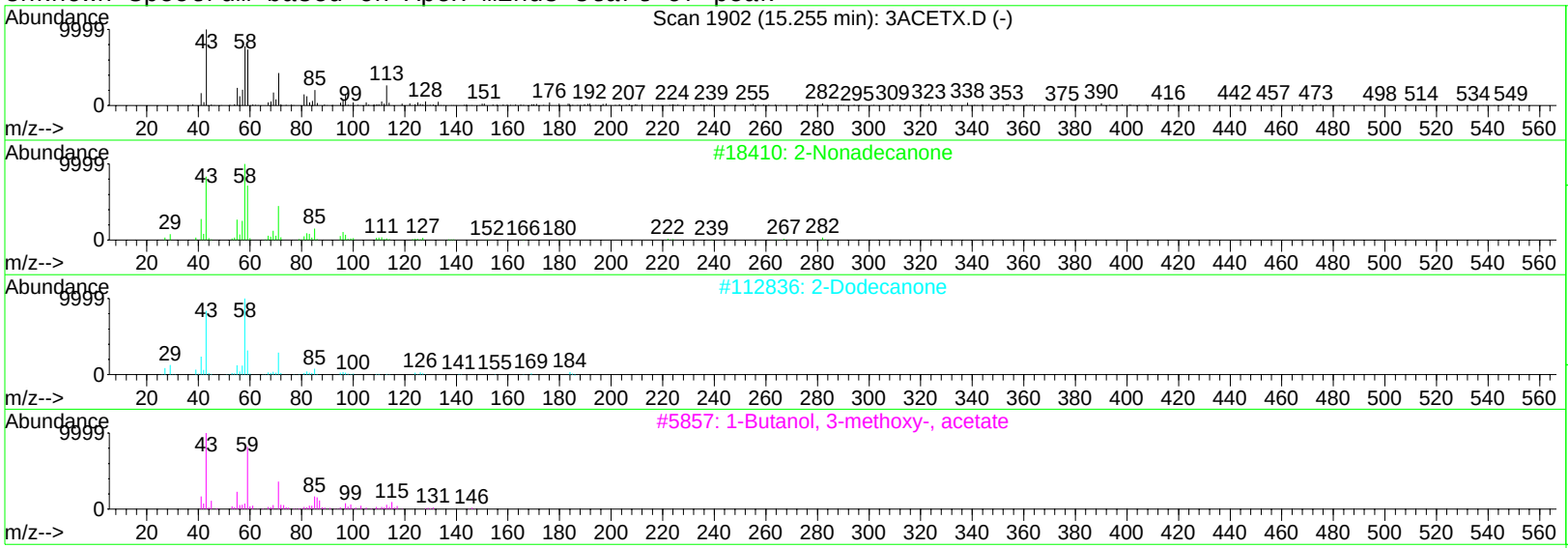


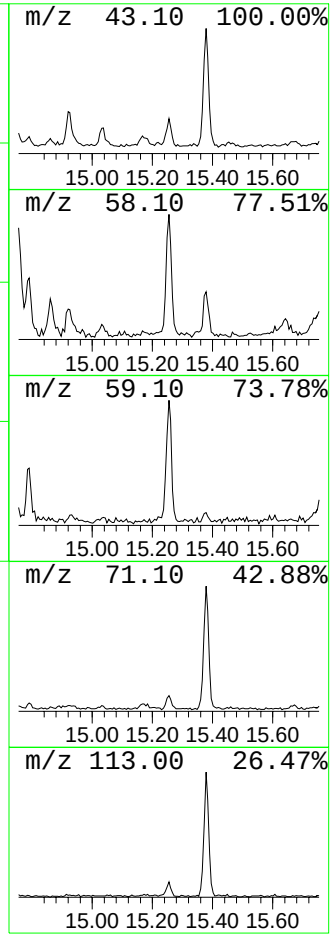


Peak Number: 11 at 15.17 min Area: 696620 Area % 0.95

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1-Docosene	111438	001599-67-3	94
2 11-Tricosene	4287	052078-56-5	87
3 10-Heneicosene (c,t)	13500	095008-11-0	87

Unknown Spectrum based on Apex minus start of peak

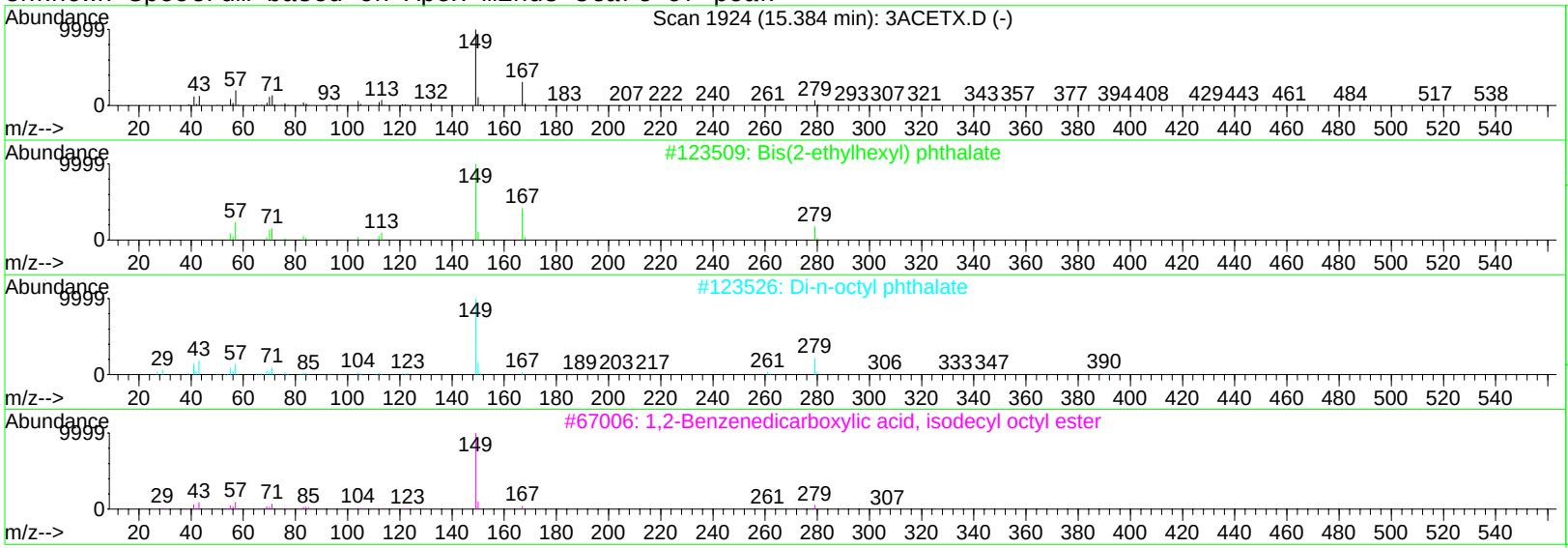


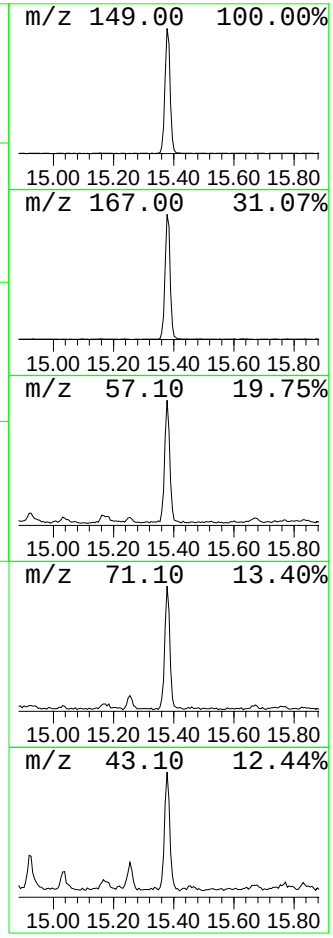


Peak Number: 12 at 15.25 min Area: 810981 Area % 1.10

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Nonadecanone	18410	000629-66-3	81
2 2-Dodecanone	112836	006175-49-1	43
3 1-Butanol, 3-methoxy-, acetate	5857	004435-53-4	43

Unknown Spectrum based on Apex minus start of peak

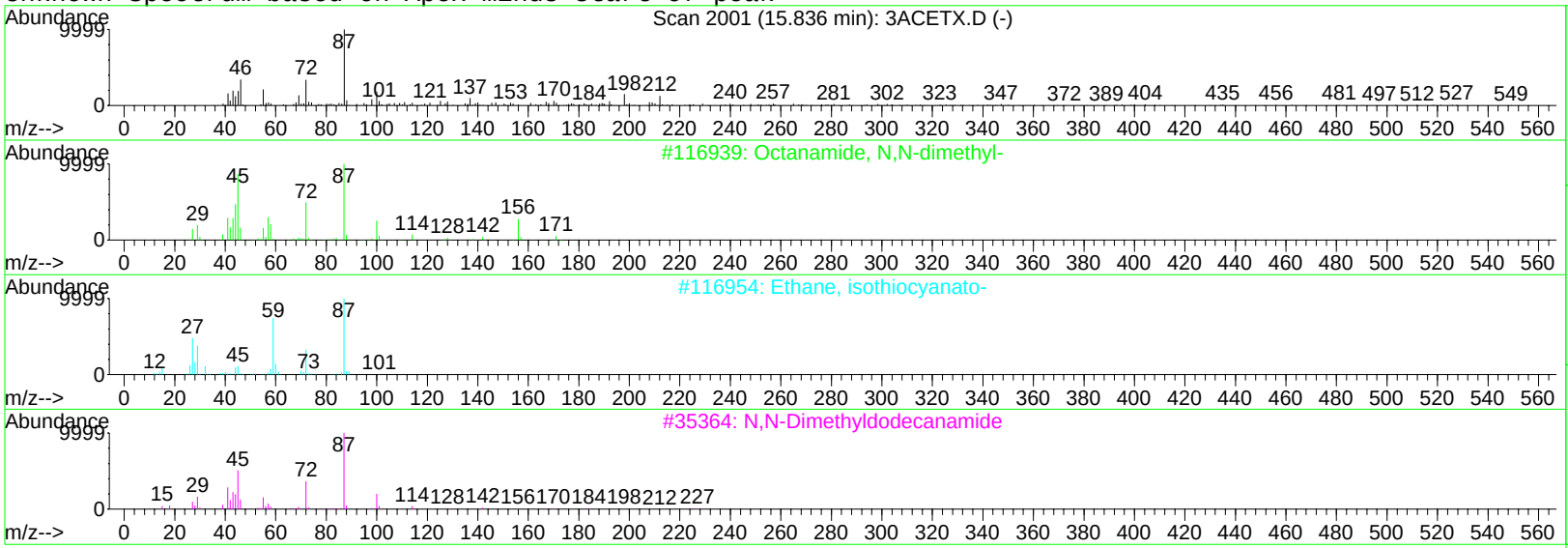


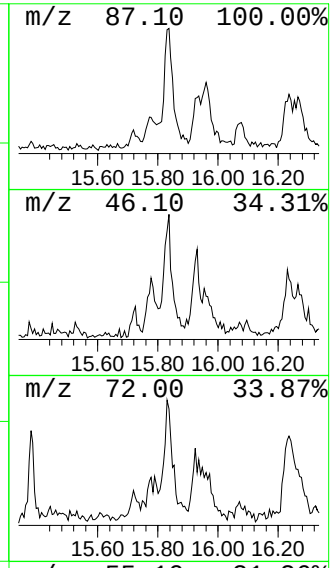


Peak Number: 13 at 15.38 min Area: 6619640 Area % 9.01

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Bis(2-ethylhexyl) phthalate	123509	000117-81-7	91
2 Di-n-octyl phthalate	123526	000117-84-0	64
3 1,2-Benzenedicarboxylic acid, is...	67006	001330-96-7	64

Unknown Spectrum based on Apex minus start of peak

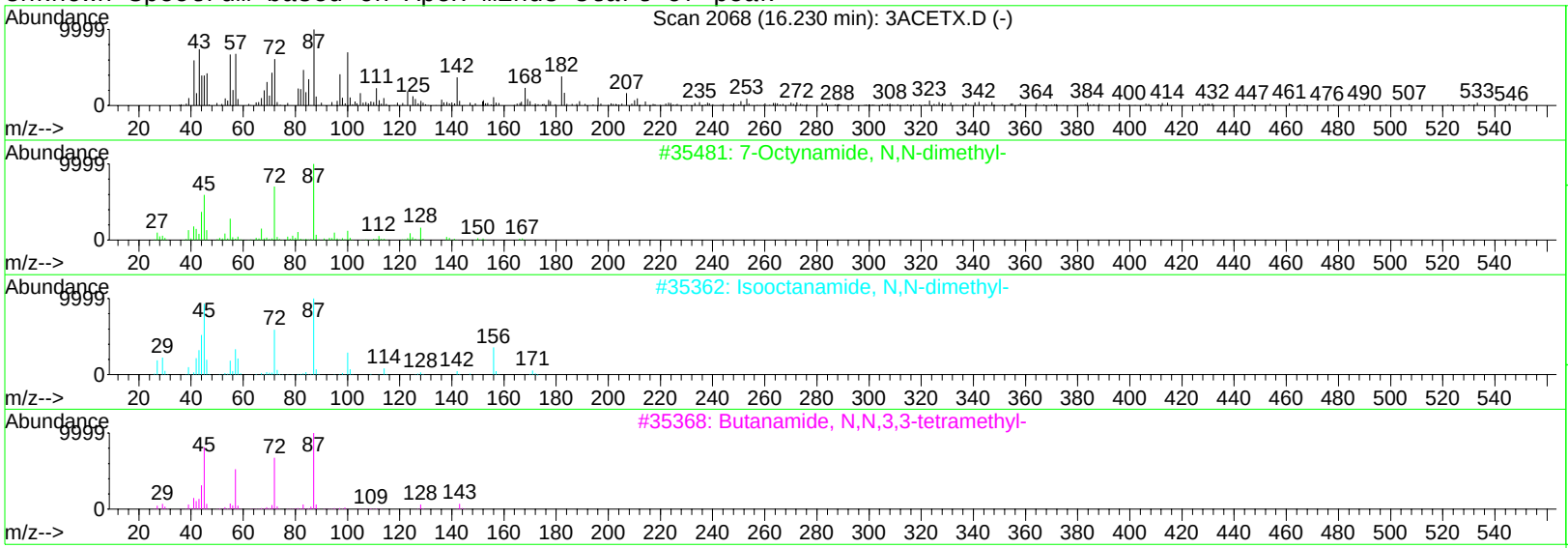


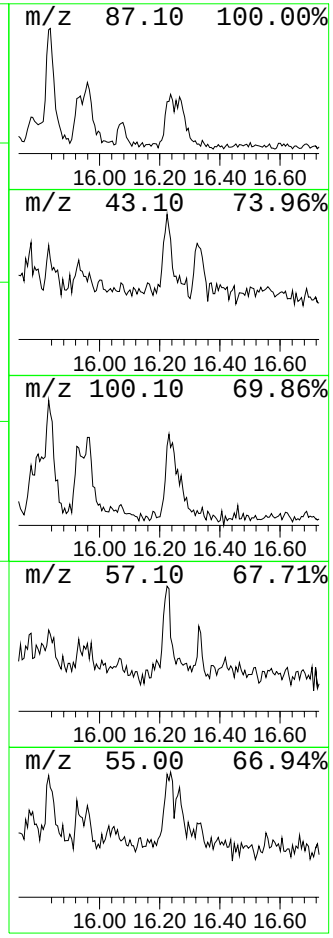


Peak Number: 14 at 15.84 min Area: 959338 Area % 1.31

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Octanamide, N,N-dimethyl-	116939	001118-92-9	53
2 Ethane, isothiocyanato-	116954	000542-85-8	50
3 N,N-Dimethyldodecanamide	35364	003007-53-2	50

Unknown Spectrum based on Apex minus start of peak

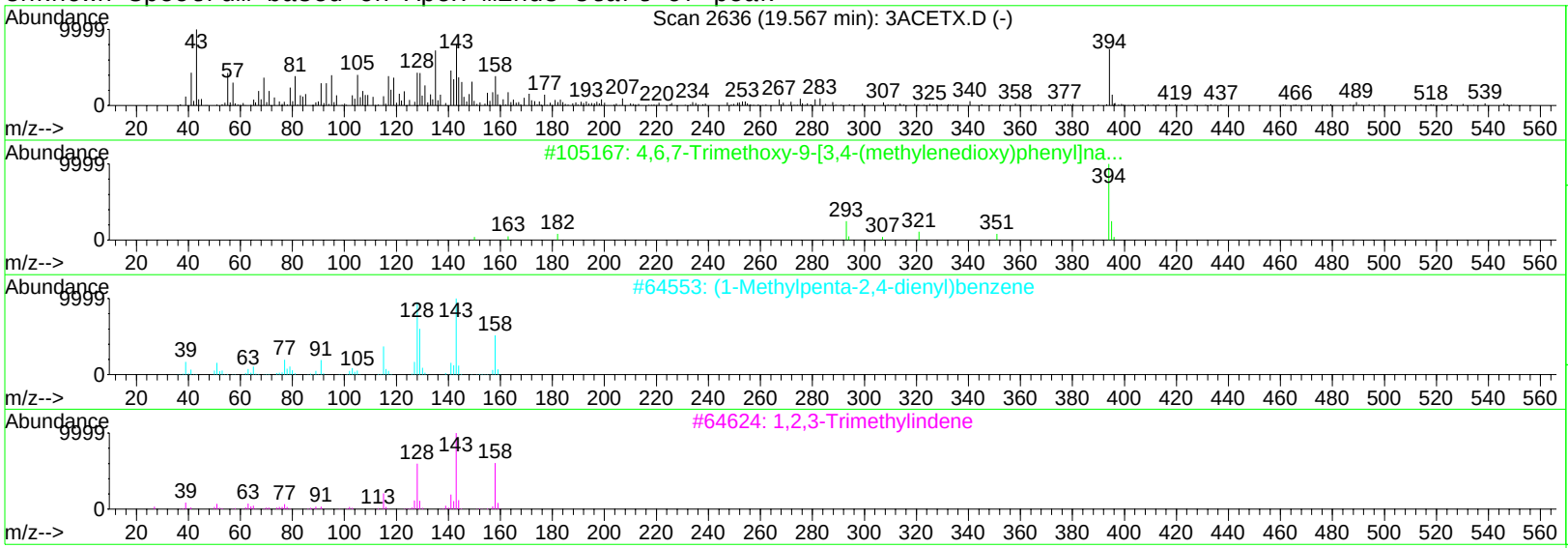


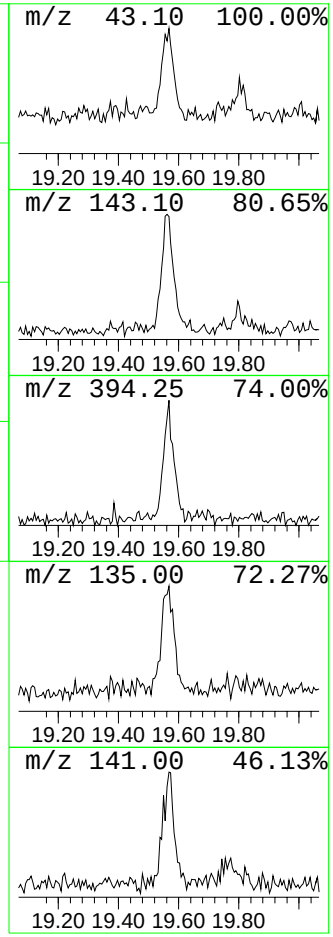


Peak Number: 15 at 16.23 min Area: 959313 Area % 1.31

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 7-Octynamide, N,N-dimethyl-	35481	035066-53-6	35
2 Isooctanamide, N,N-dimethyl-	35362	1000139-76-9	32
3 Butanamide, N,N,3,3-tetramethyl-	35368	026153-90-2	30

Unknown Spectrum based on Apex minus start of peak

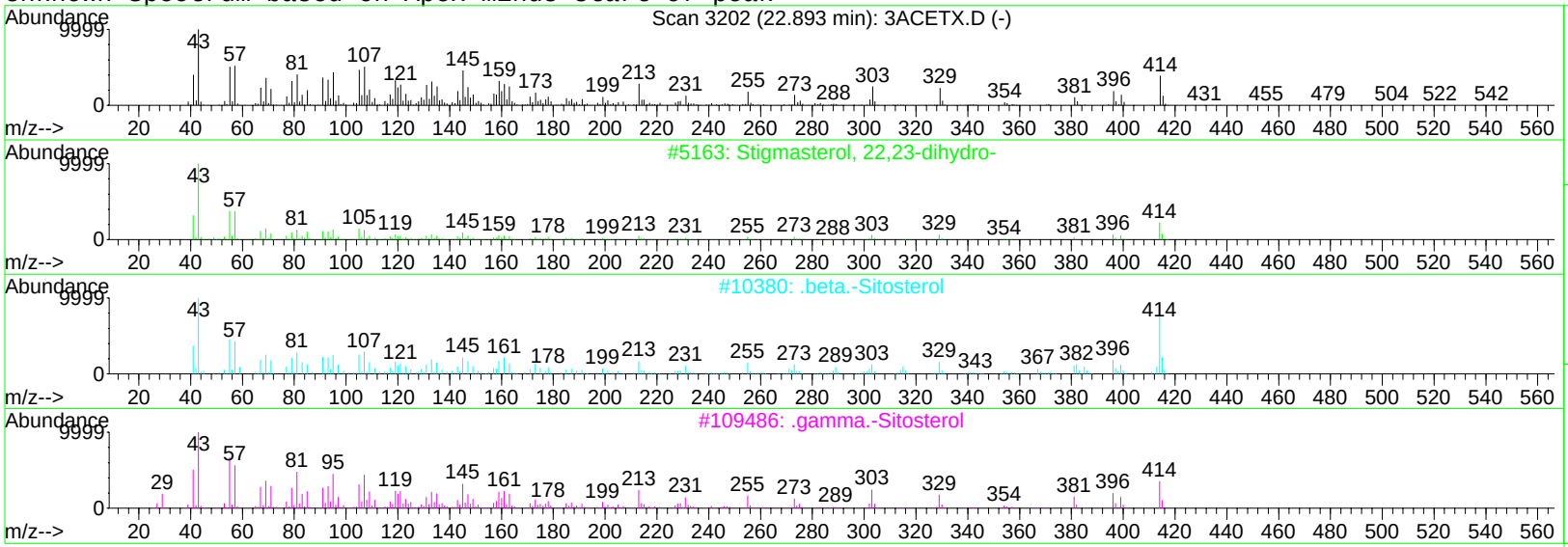


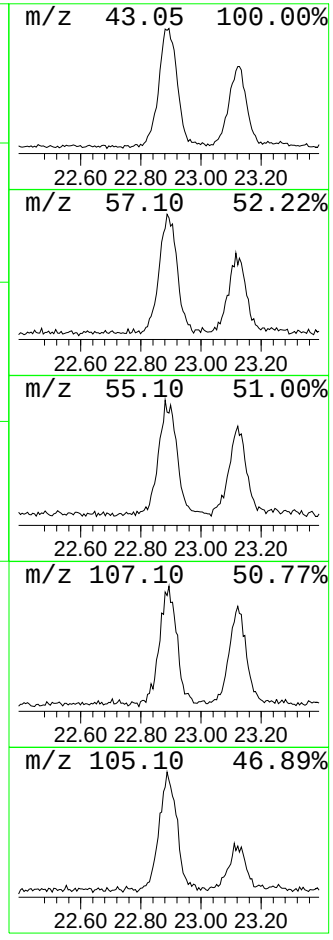


Peak Number: 16 at 19.57 min Area: 1555171 Area % 2.12

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 4,6,7-Trimethoxy-9-[3,4-(methyle...	105167	025001-57-4	33
2 (1-Methylpenta-2,4-dienyl)benzene	64553	1000210-01-1	16
3 1,2,3-Trimethylindene	64624	004773-83-5	16

Unknown Spectrum based on Apex minus start of peak

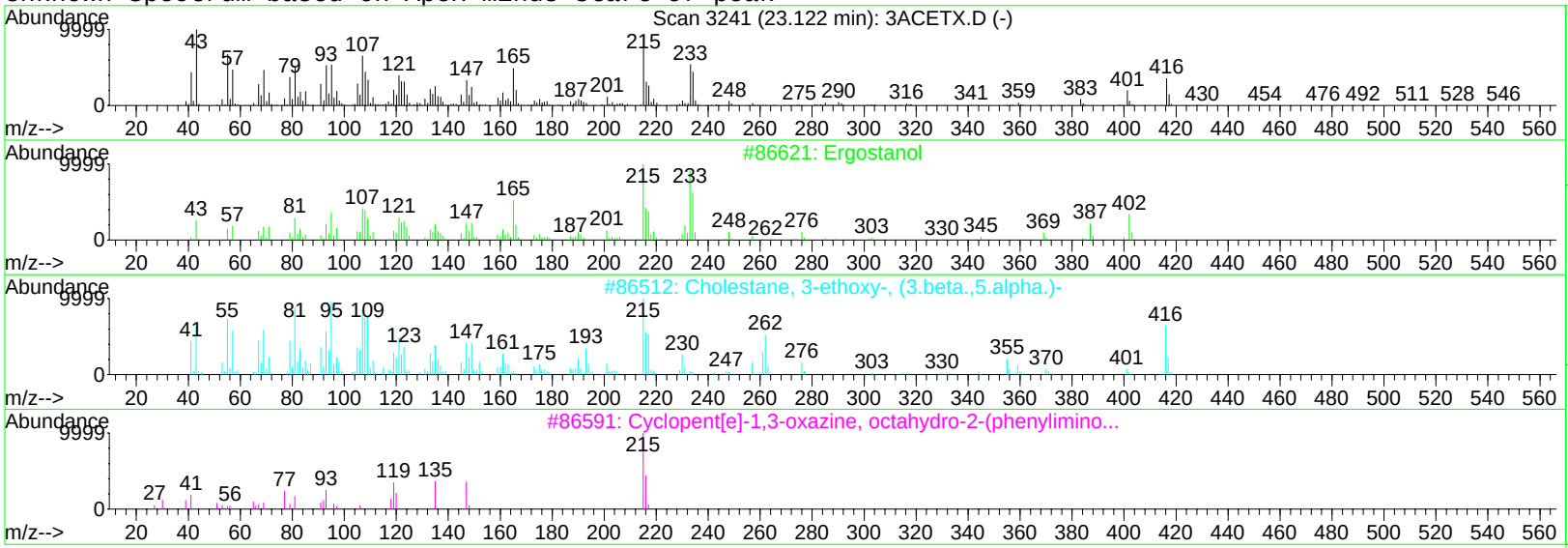


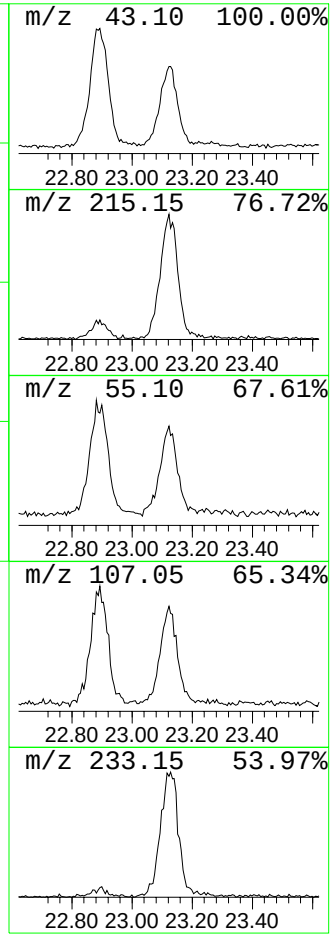


Peak Number: 17 at 22.89 min Area: 15530725 Area % 21.13

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Stigmasterol, 22,23-dihydro-	5163	1000214-20-7	99
2 .beta.-Sitosterol	10380	000083-46-5	94
3 .gamma.-Sitosterol	109486	000083-47-6	91

Unknown Spectrum based on Apex minus start of peak

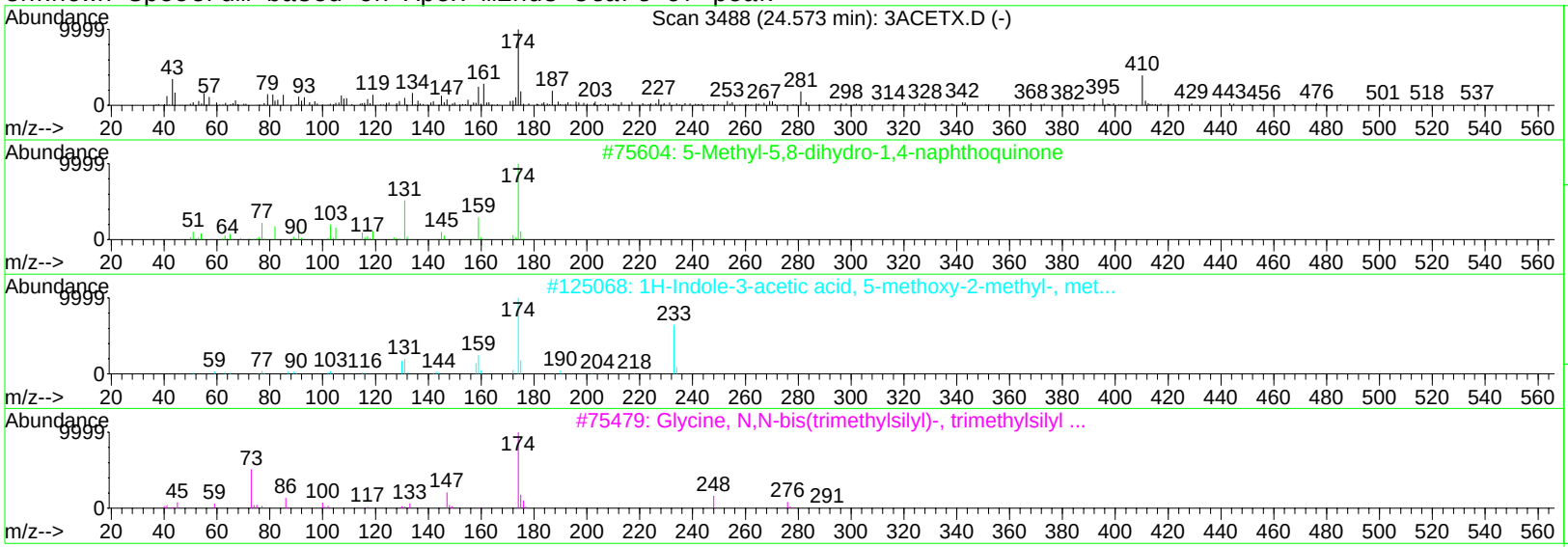


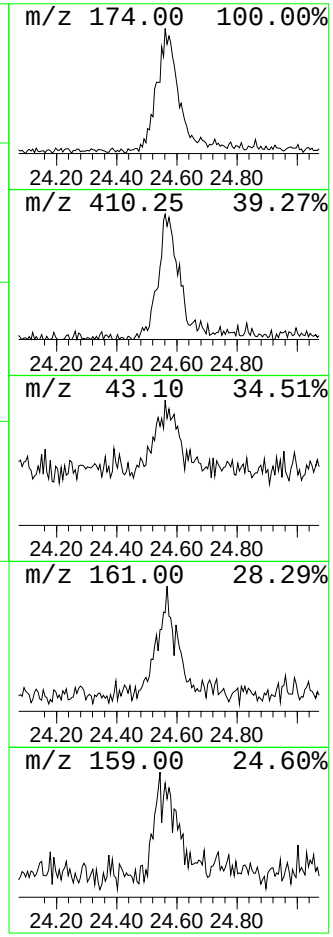


Peak Number: 18 at 23.12 min Area: 9696712 Area % 13.19

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Ergostanol	86621	006538-02-9	95
2 Cholestane, 3-ethoxy-, (3.beta.,...	86512	002089-02-3	55
3 Cyclopent[e]-1,3-oxazine, octahy...	86591	1000138-92-2	11

Unknown Spectrum based on Apex minus start of peak

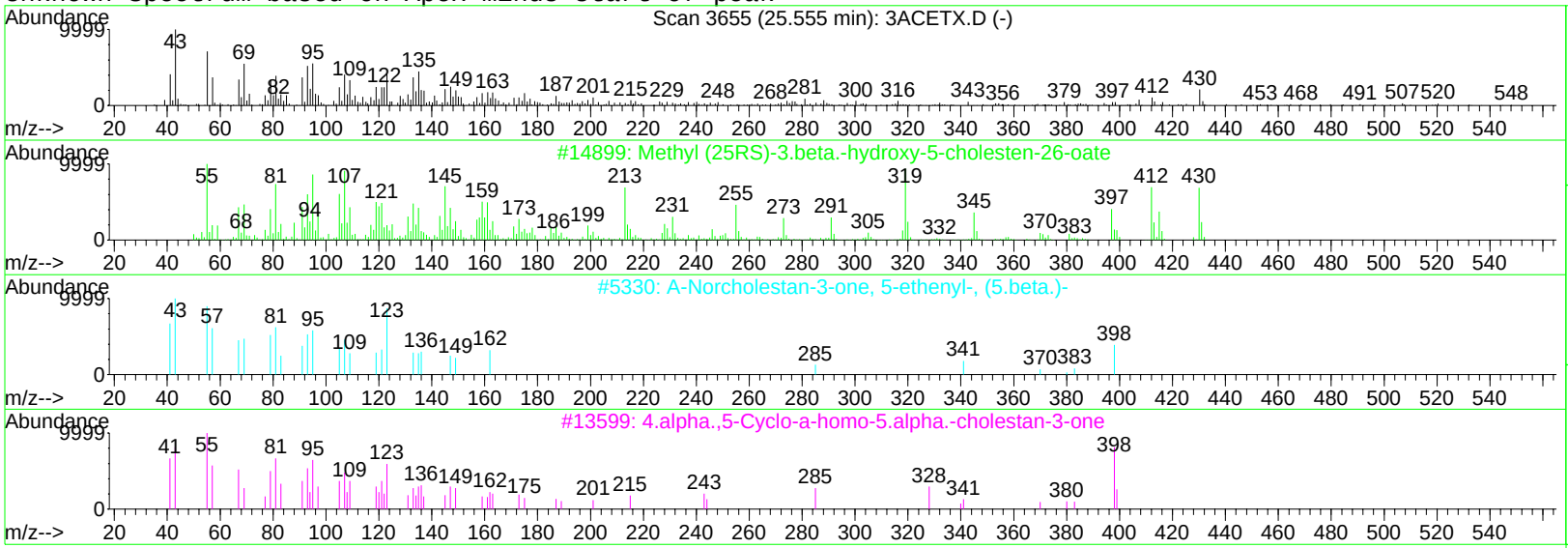


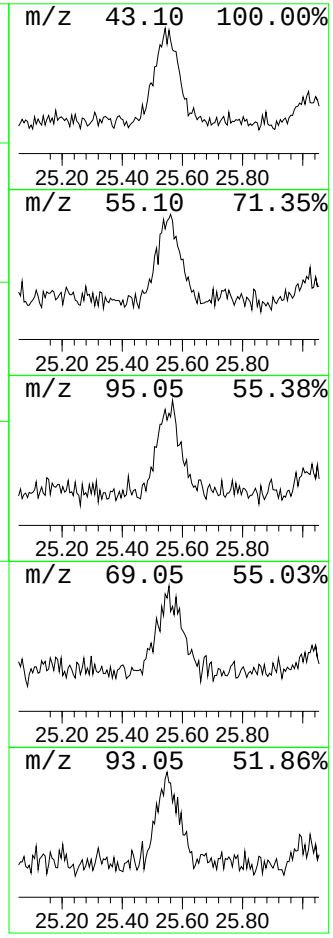


Peak Number: 19 at 24.57 min Area: 1361518 Area % 1.85

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 5-Methyl-5,8-dihydro-1,4-naphtho...	75604	086759-40-2	49
2 1H-Indole-3-acetic acid, 5-metho...	125068	007588-36-5	37
3 Glycine, N,N-bis(trimethylsilyl)...	75479	005630-82-0	35

Unknown Spectrum based on Apex minus start of peak

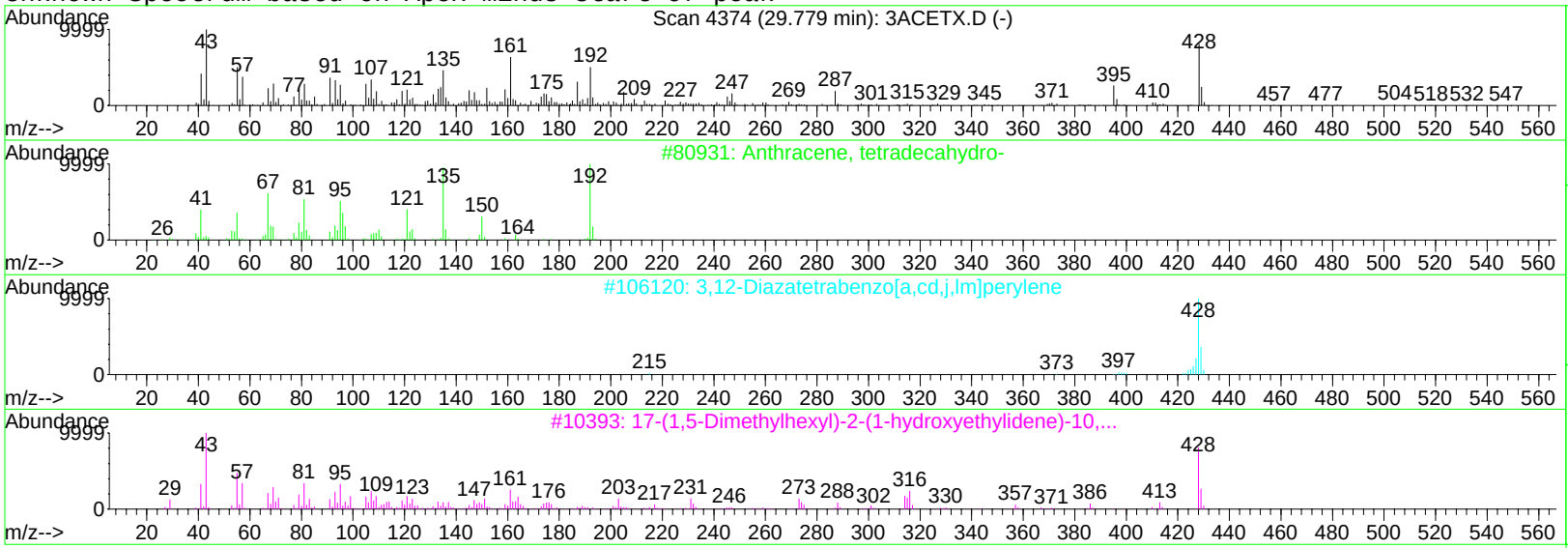


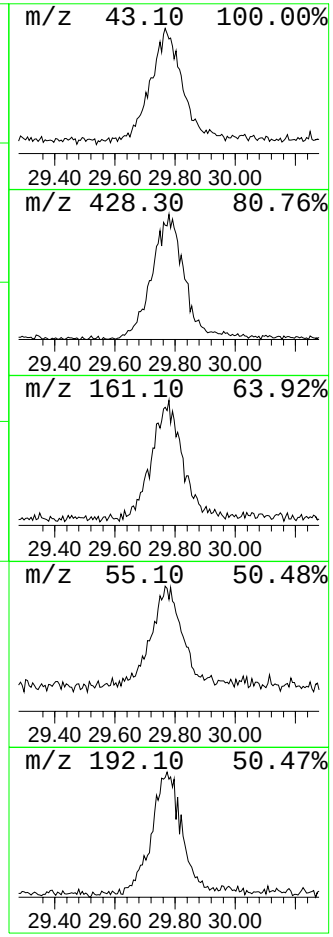


Peak Number: 20 at 25.55 min Area: 3532670 Area % 4.81

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Methyl (25RS)-3.beta.-hydroxy-5-...	14899	056845-86-4	46
2 A-Norcholestan-3-one, 5-ethenyl-...	5330	019594-90-2	45
3 4.alpha.,5-Cyclo-a-homo-5.alpha....	13599	1000100-12-7	36

Unknown Spectrum based on Apex minus start of peak





Peak Number: 21 at 29.78 min Area: 9331532 Area % 12.70

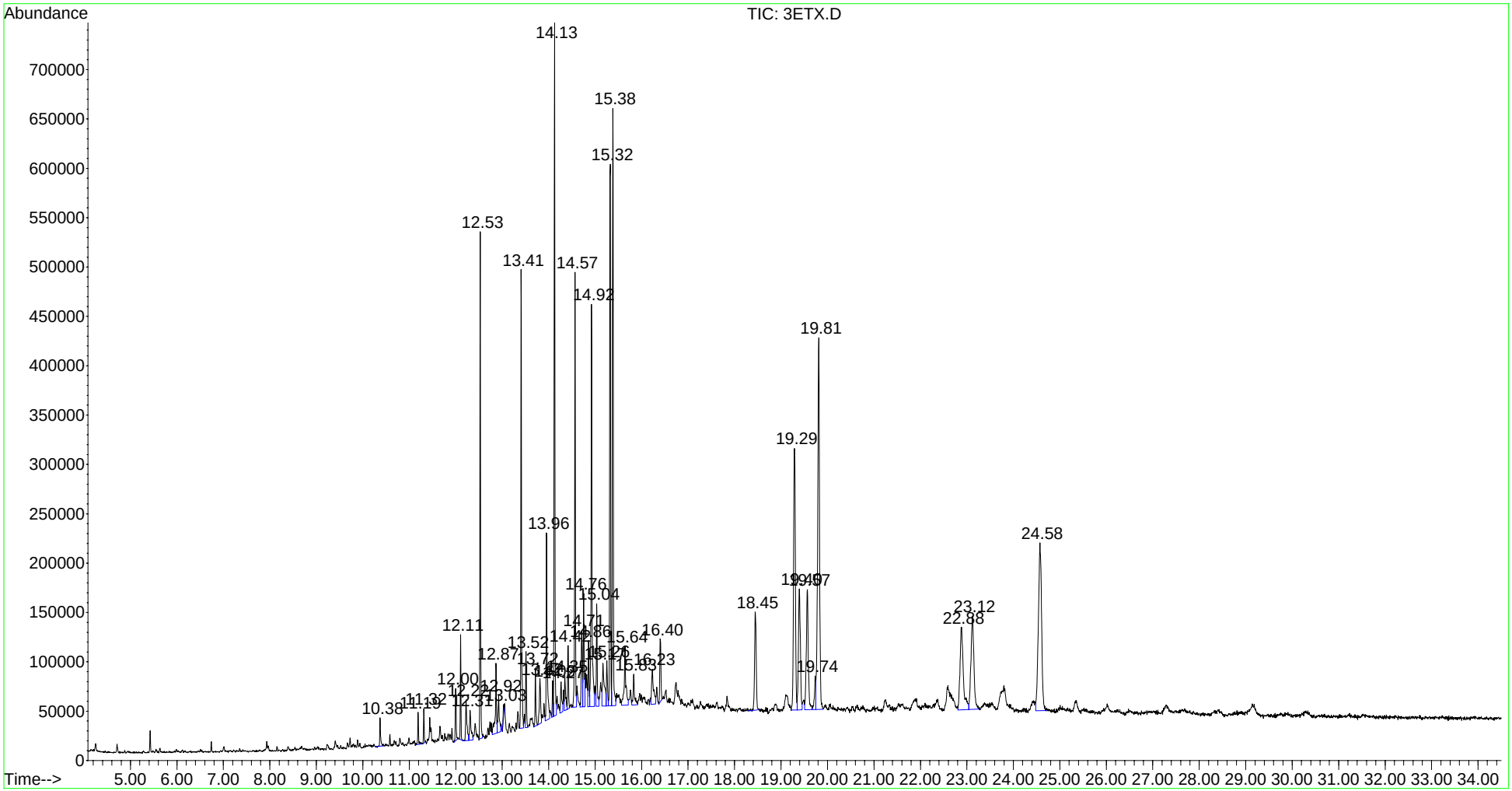
The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Anthracene, tetradecahydro-	80931	006596-35-6	47
2 3,12-Diazatetrabenzo[a,cd,j,lm]p...	106120	095264-24-7	45
3 17-(1,5-Dimethylhexyl)-2-(1-hydr...	10393	1000210-79-8	32

Library Search Report

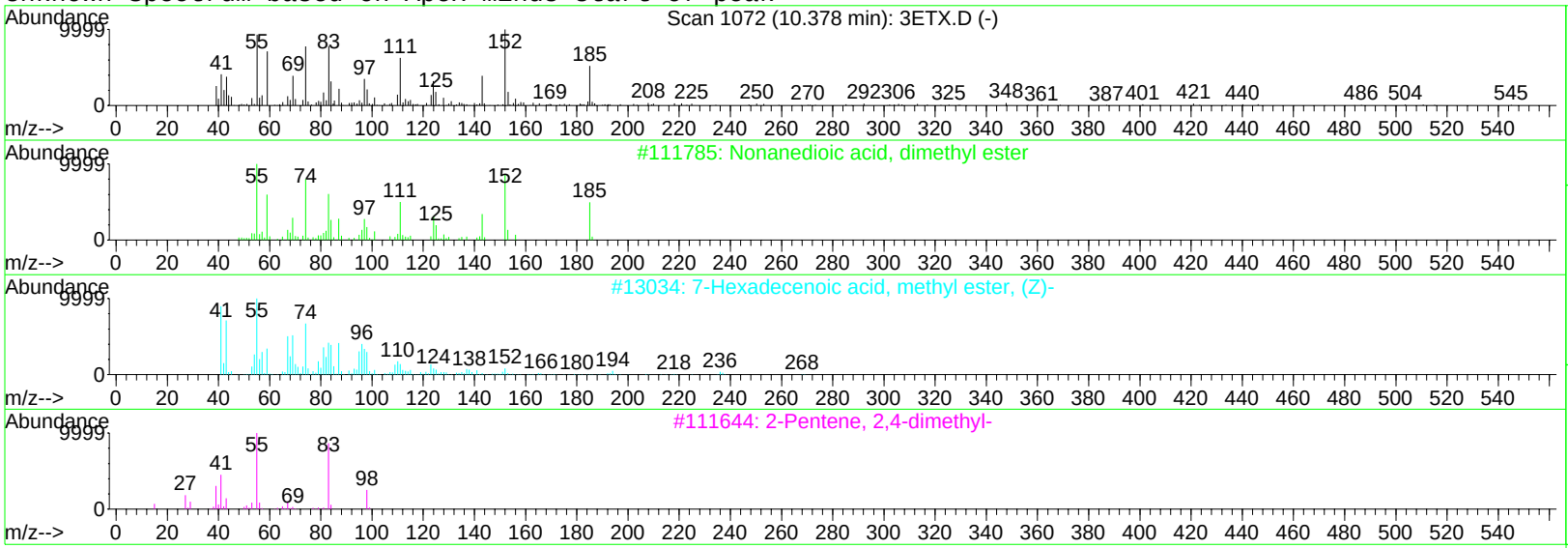
Data File : D:\PRENISHA\300\3ETX.D
Acq On : 2 Aug 2007 16:23
Sample : Pitch Sample ethanol extract
Misc : 1µl inject, acetone, splitless

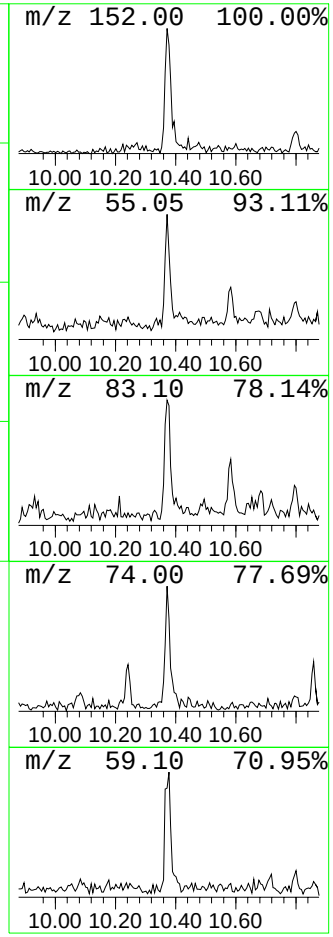
Vial: 93
Operator: Bret
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e
Method : C:\MSDCHEM\1\METHODS\NEW.M (Chemstation Integrator)
Title :



Unknown Spectrum based on Apex minus start of peak

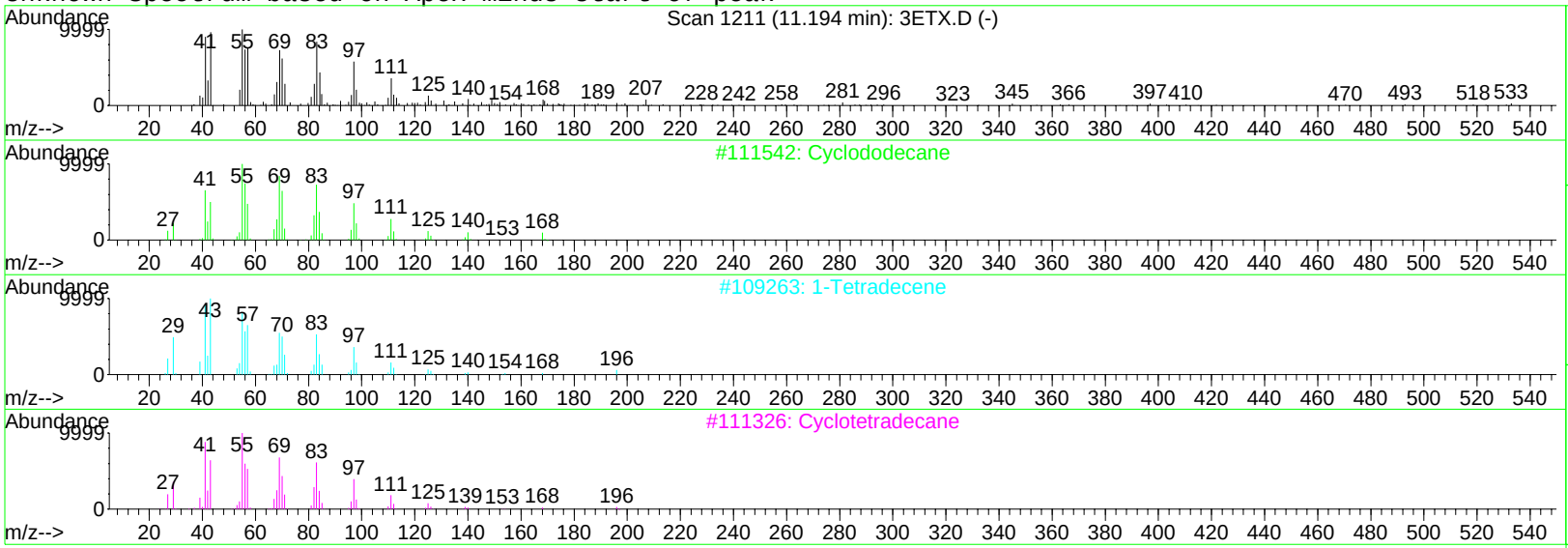


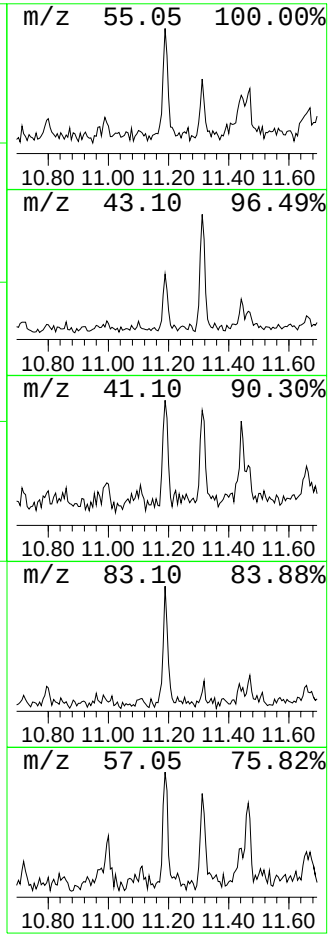


Peak Number: 1 at 10.38 min Area: 372683 Area % 0.33

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Nonanedioic acid, dimethyl ester	111785	001732-10-1	87
2 7-Hexadecenoic acid, methyl este...	13034	056875-67-3	20
3 2-Pentene, 2,4-dimethyl-	111644	000625-65-0	11

Unknown Spectrum based on Apex minus start of peak

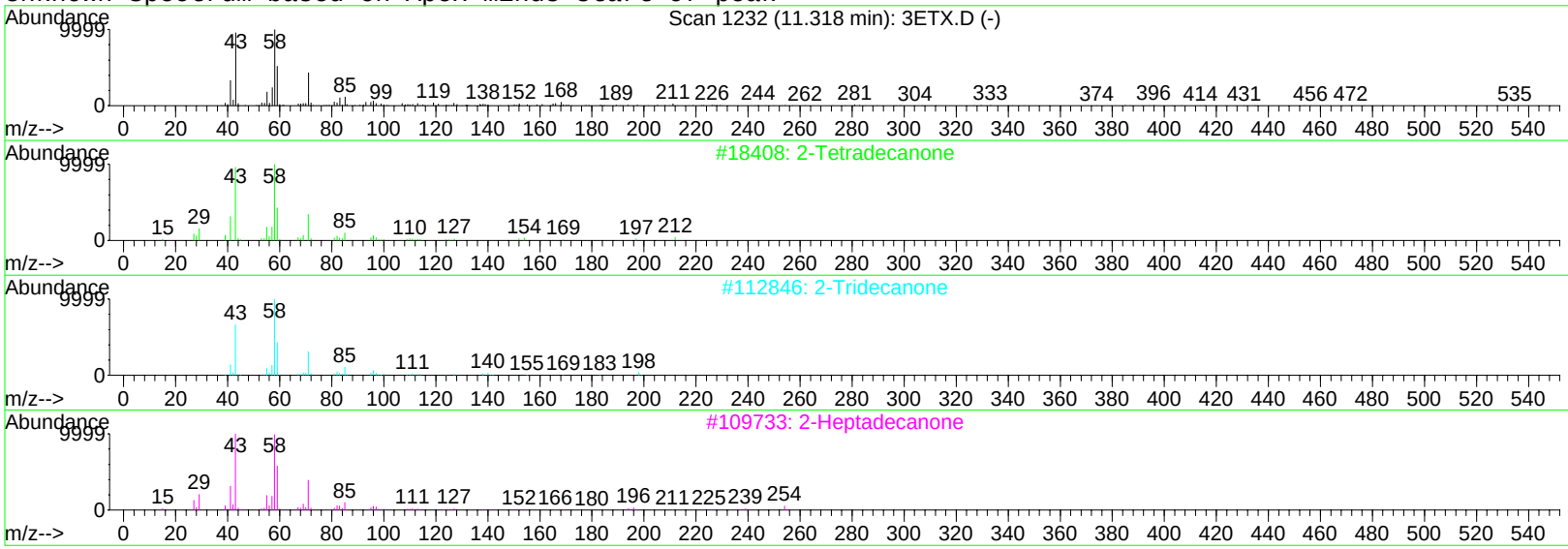


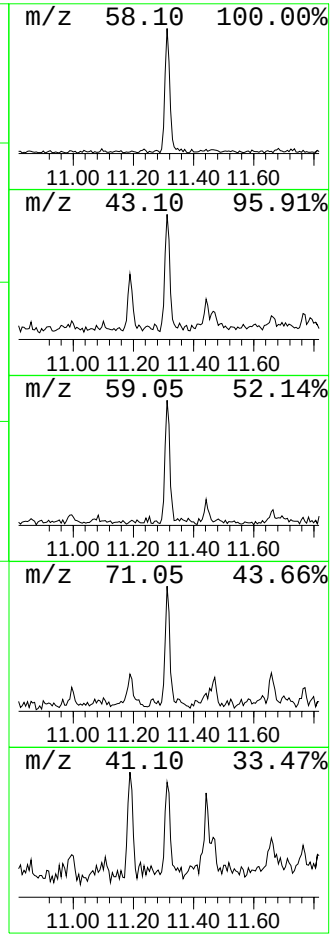


Peak Number: 2 at 11.19 min Area: 395053 Area % 0.35

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclododecane	111542	000294-62-2	98
2 1-Tetradecene	109263	001120-36-1	96
3 Cyclotetradecane	111326	000295-17-0	95

Unknown Spectrum based on Apex minus start of peak

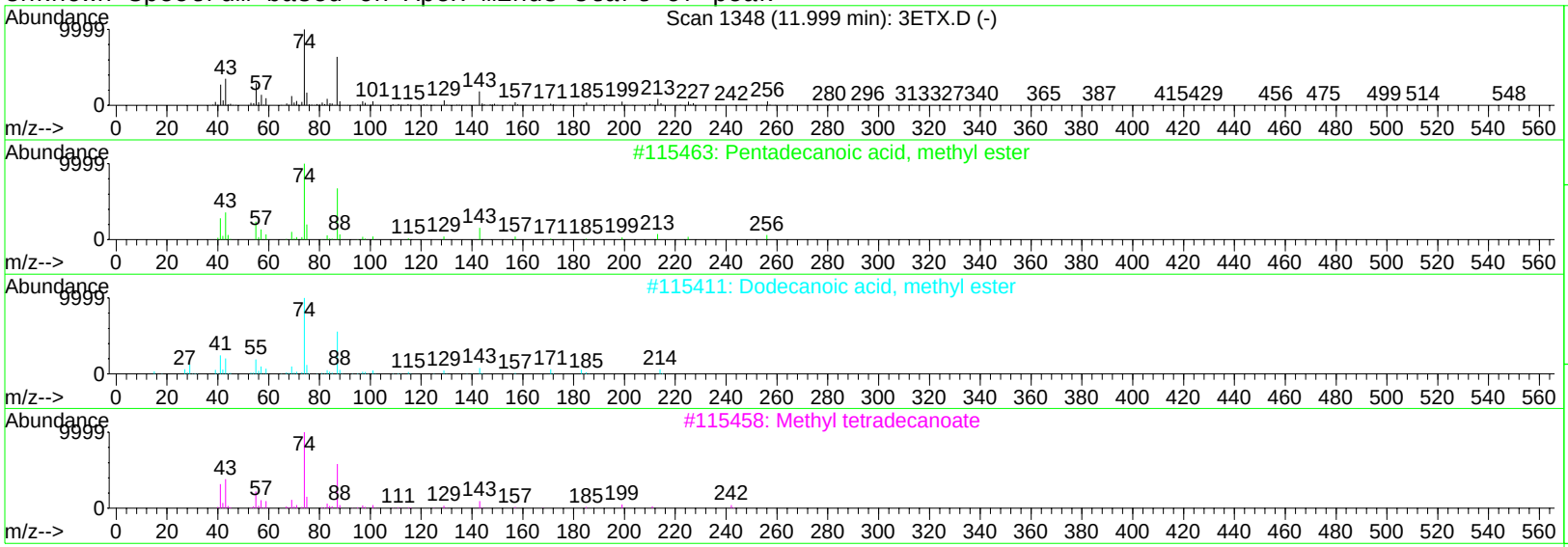


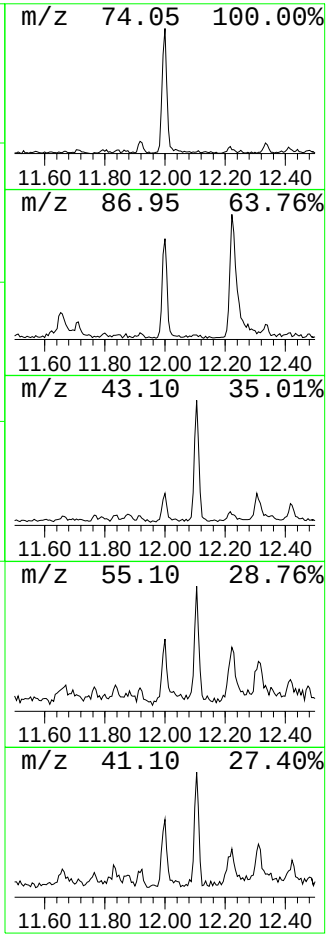


Peak Number: 3 at 11.32 min Area: 375937 Area % 0.33

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Tetradecanone	18408	002345-27-9	86
2 2-Tridecanone	112846	000593-08-8	78
3 2-Heptadecanone	109733	002922-51-2	64

Unknown Spectrum based on Apex minus start of peak

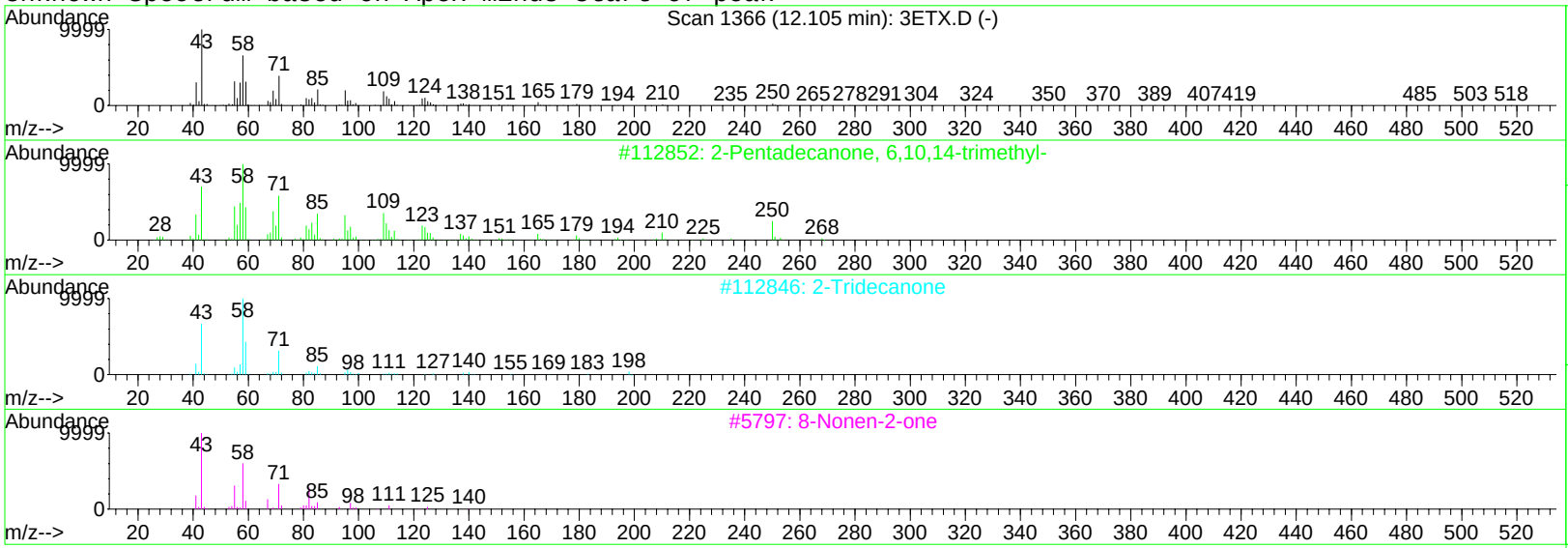


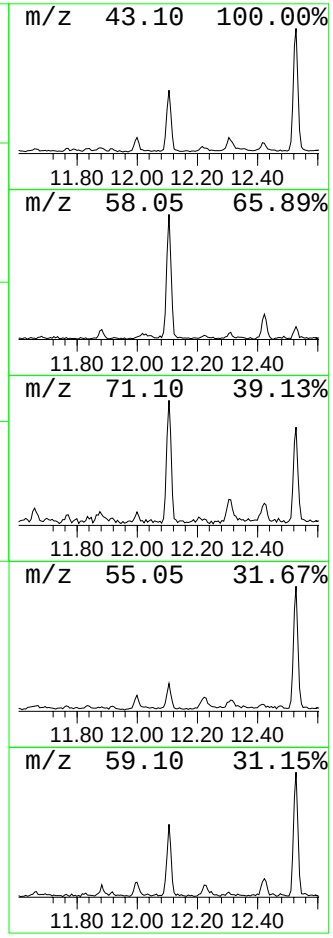


Peak Number: 4 at 12.00 min Area: 608291 Area % 0.53

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Pentadecanoic acid, methyl ester	115463	007132-64-1	97
2 Dodecanoic acid, methyl ester	115411	000111-82-0	90
3 Methyl tetradecanoate	115458	000124-10-7	83

Unknown Spectrum based on Apex minus start of peak

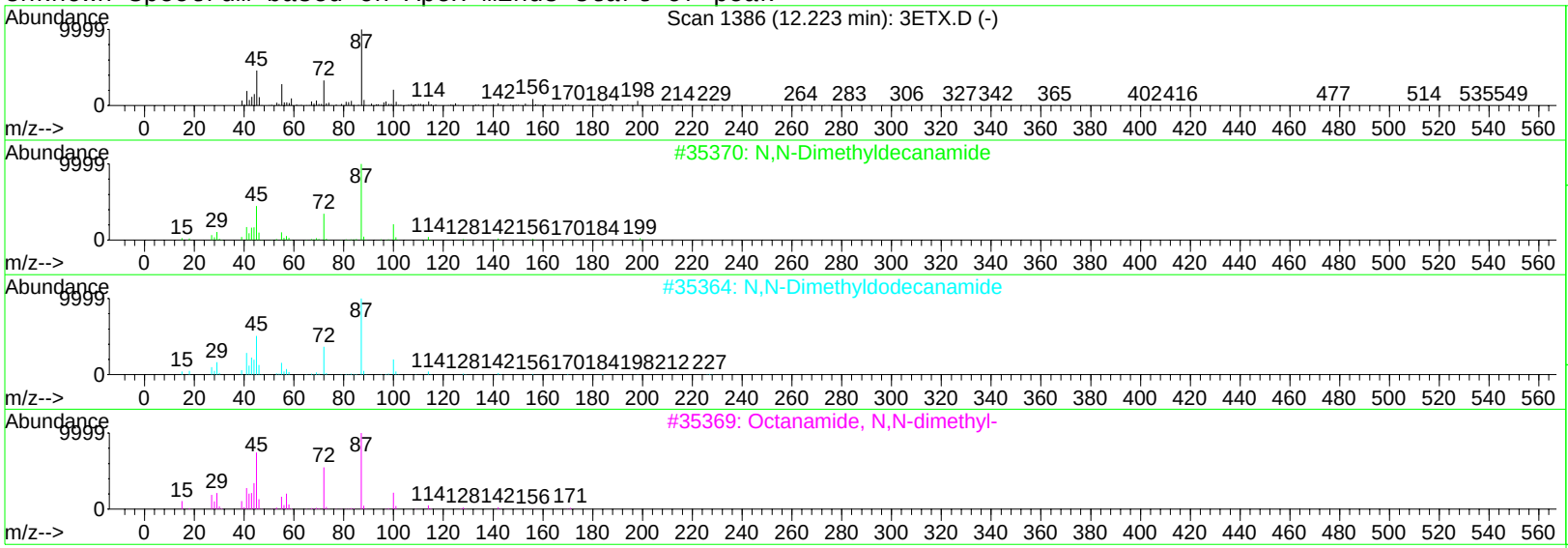


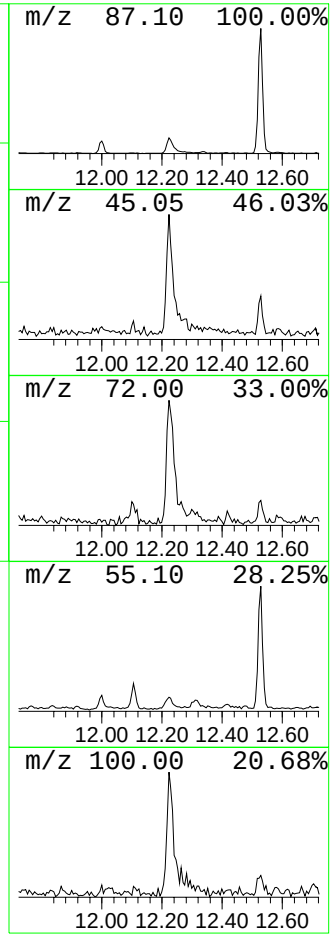


Peak Number: 5 at 12.11 min Area: 1093741 Area % 0.96

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Pentadecanone, 6,10,14-trimethyl-	112852	000502-69-2	53
2 2-Tridecanone	112846	000593-08-8	50
3 8-Nonen-2-one	5797	005009-32-5	43

Unknown Spectrum based on Apex minus start of peak

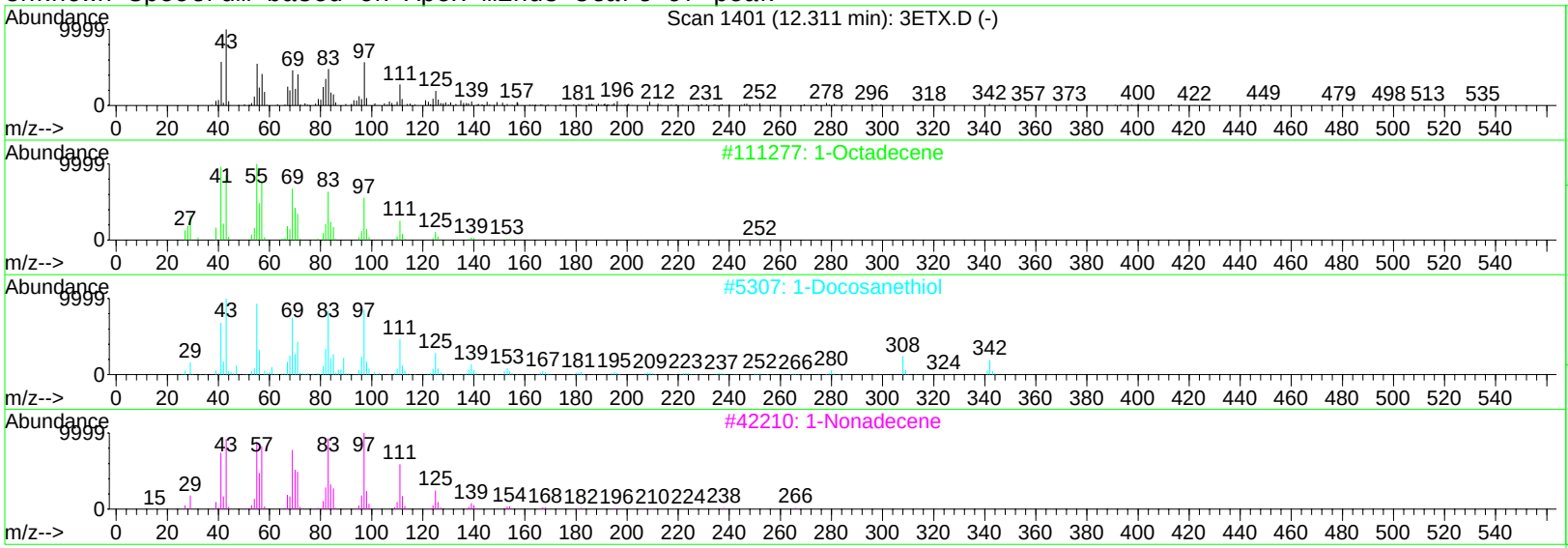


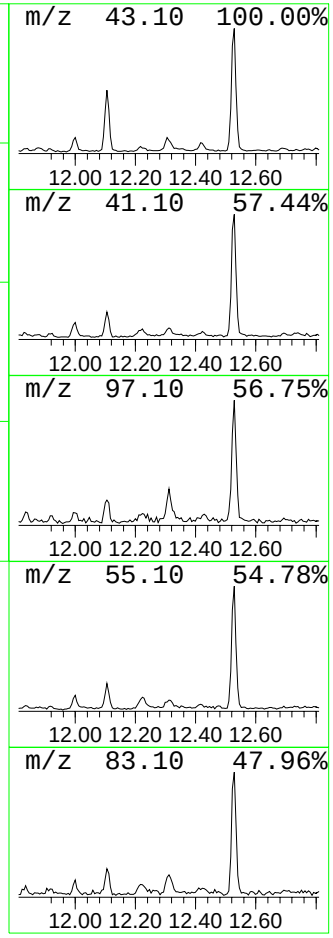


Peak Number: 6 at 12.22 min Area: 835594 Area % 0.73

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 N,N-Dimethyldecanamide	35370	014433-76-2	86
2 N,N-Dimethyldodecanamide	35364	003007-53-2	64
3 Octanamide, N,N-dimethyl-	35369	001118-92-9	64

Unknown Spectrum based on Apex minus start of peak

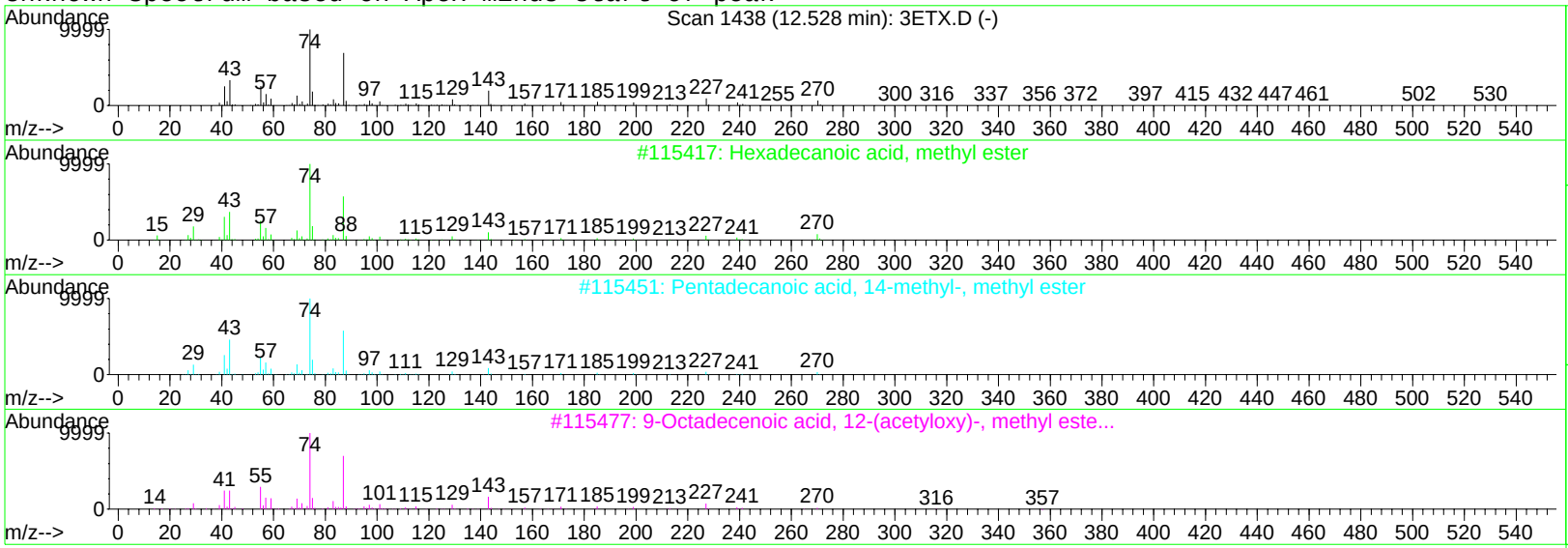


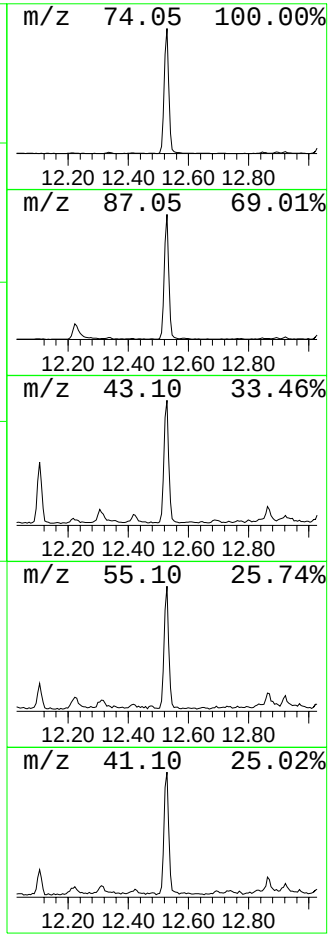


Peak Number: 7 at 12.31 min Area: 654689 Area % 0.57

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1-Octadecene	111277	000112-88-9	92
2 1-Docosanethiol	5307	007773-83-3	76
3 1-Nonadecene	42210	018435-45-5	76

Unknown Spectrum based on Apex minus start of peak

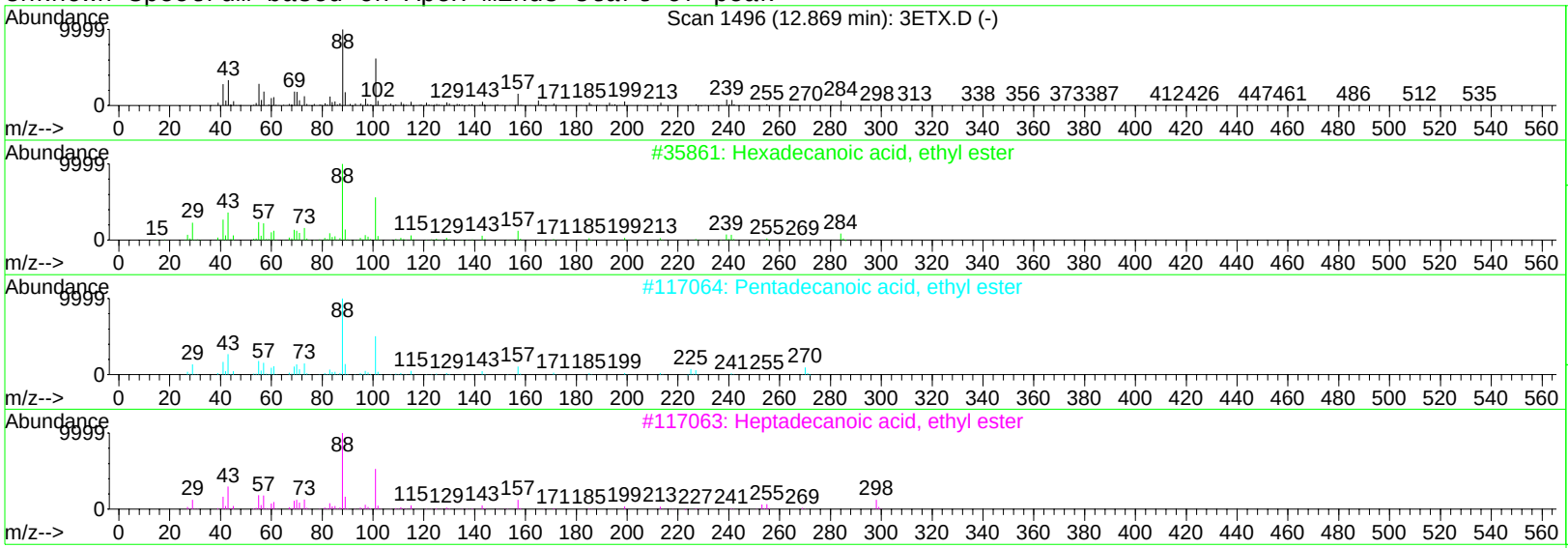


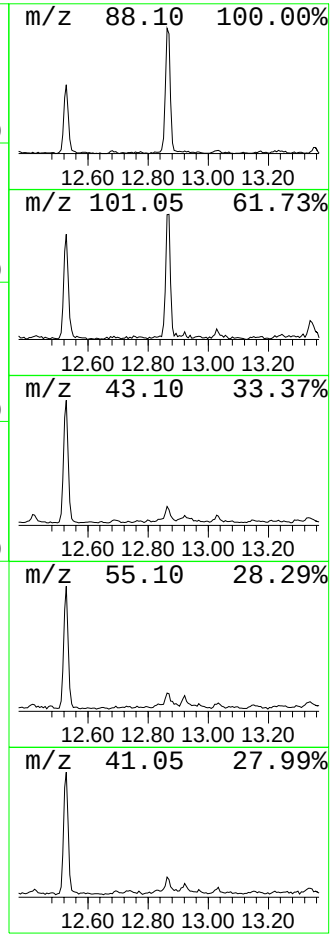


Peak Number: 8 at 12.53 min Area: 5360307 Area % 4.69

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Hexadecanoic acid, methyl ester	115417	000112-39-0	97
2 Pentadecanoic acid, 14-methyl-, ...	115451	005129-60-2	96
3 9-Octadecenoic acid, 12-(acetylo...	115477	000140-03-4	86

Unknown Spectrum based on Apex minus start of peak

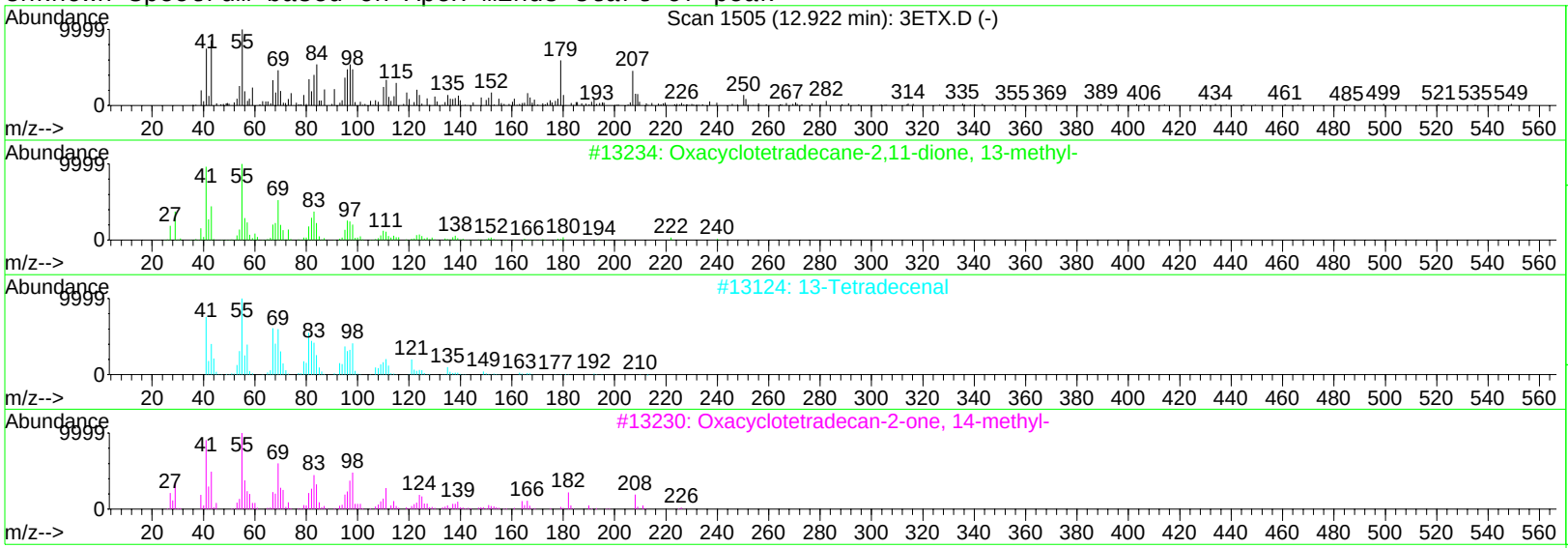


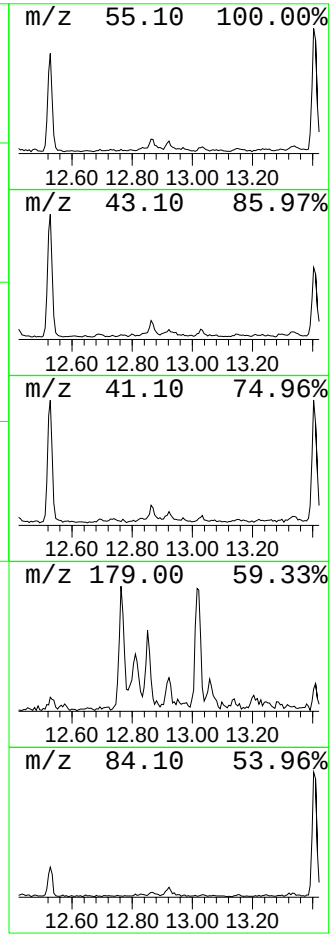


Peak Number: 9 at 12.87 min Area: 1223451 Area % 1.07

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Hexadecanoic acid, ethyl ester	35861	000628-97-7	95
2 Pentadecanoic acid, ethyl ester	117064	041114-00-5	91
3 Heptadecanoic acid, ethyl ester	117063	014010-23-2	91

Unknown Spectrum based on Apex minus start of peak

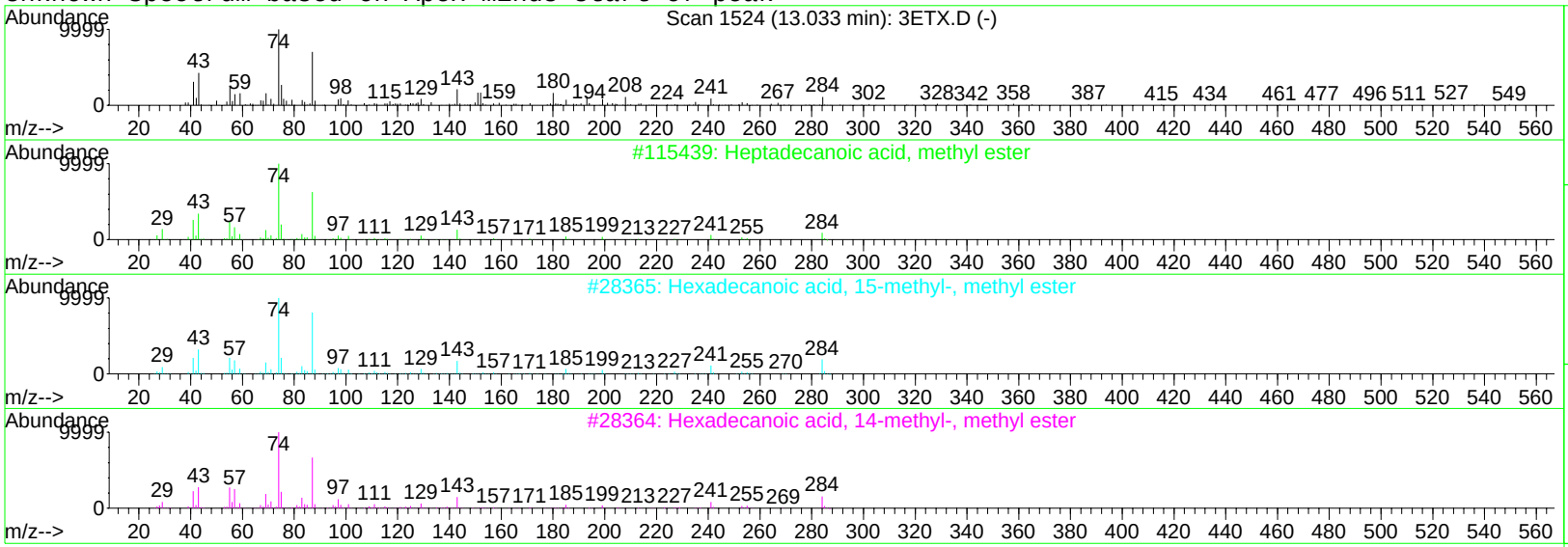


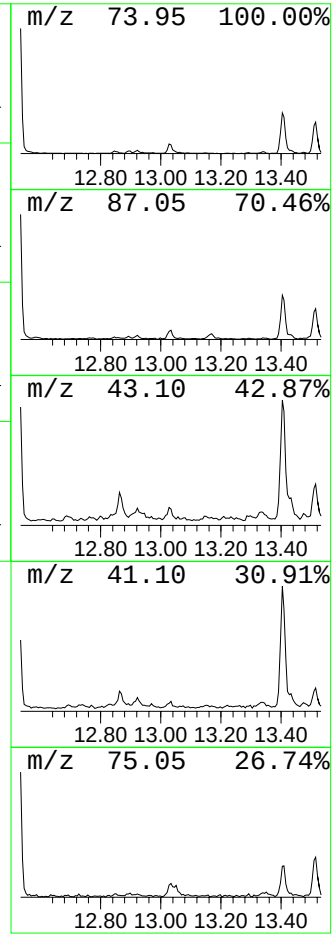


Peak Number: 10 at 12.92 min Area: 741281 Area % 0.65

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Oxacyclotetradecane-2,11-dione, ...	13234	074685-36-2	49
2 13-Tetradecenal	13124	085896-31-7	43
3 Oxacyclotetradecan-2-one, 14-met...	13230	027198-63-6	38

Unknown Spectrum based on Apex minus start of peak

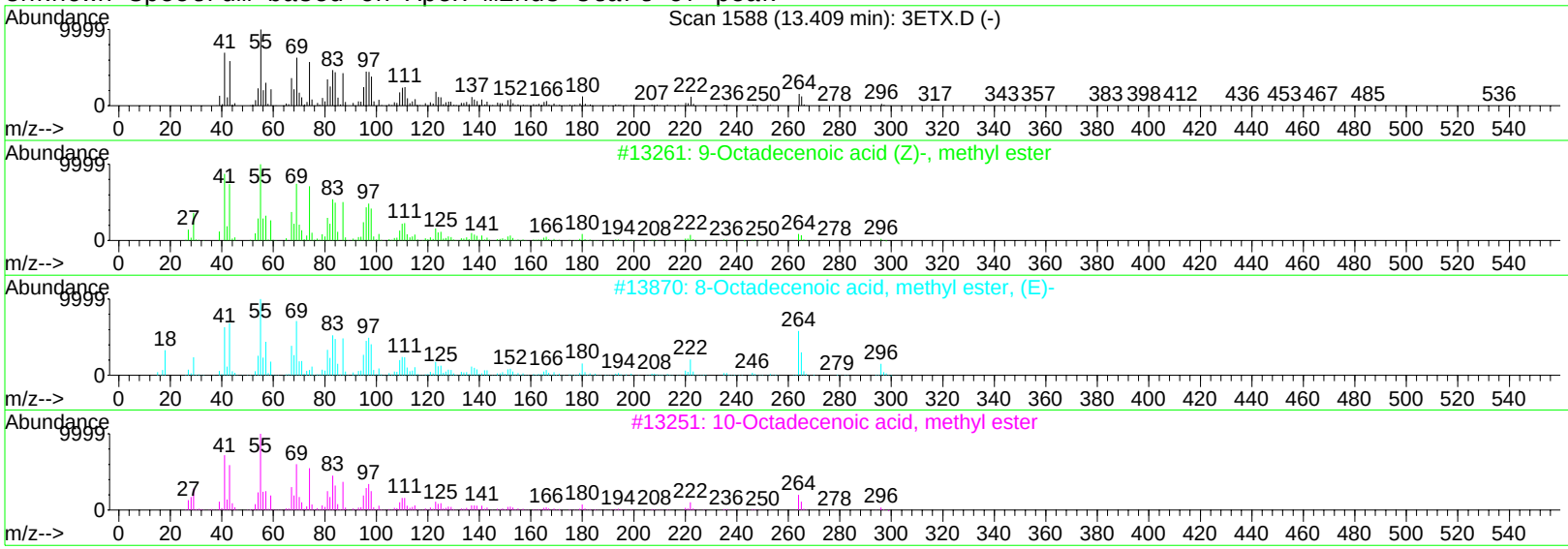


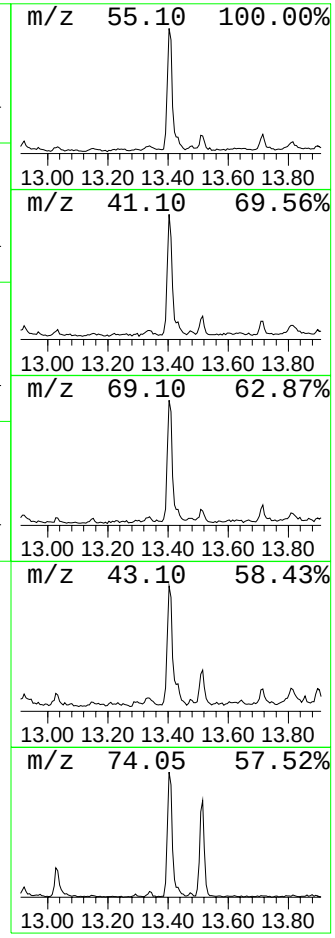


Peak Number: 11 at 13.03 min Area: 81201 Area % 0.07

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Heptadecanoic acid, methyl ester	115439	001731-92-6	96
2 Hexadecanoic acid, 15-methyl-, m...	28365	006929-04-0	93
3 Hexadecanoic acid, 14-methyl-, m...	28364	002490-49-5	83

Unknown Spectrum based on Apex minus start of peak

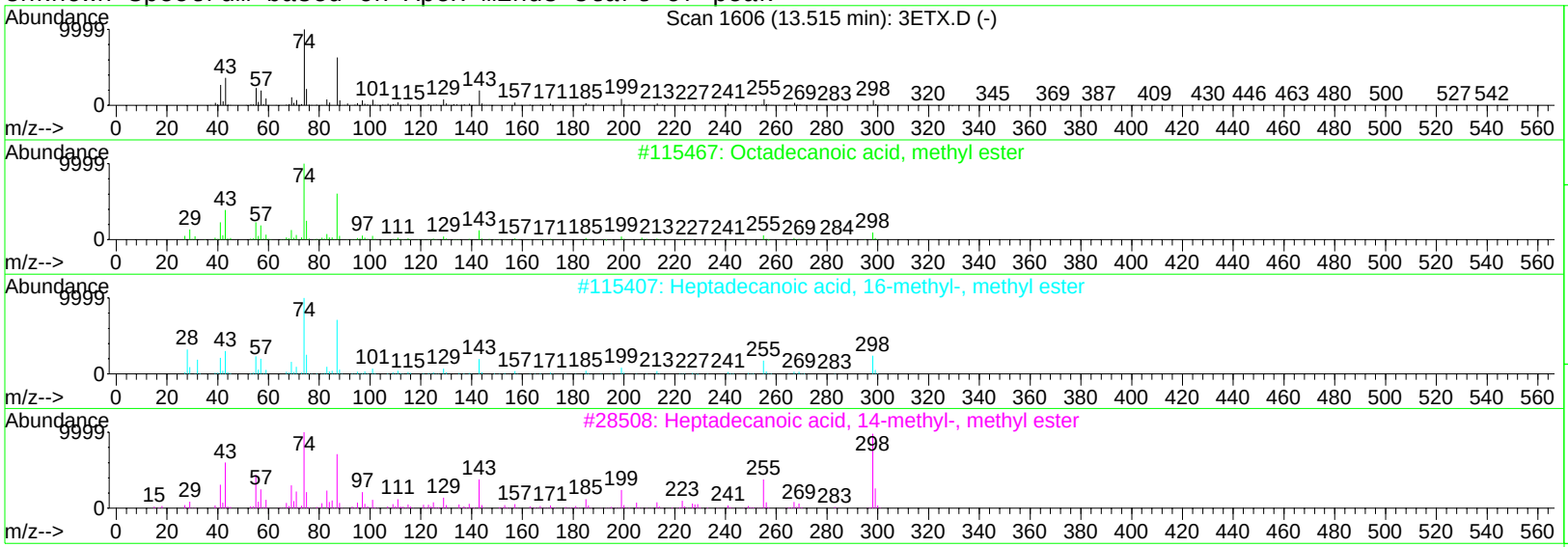


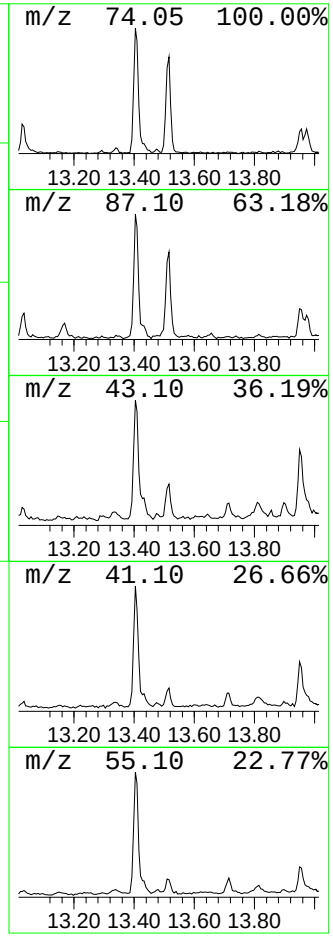


Peak Number: 12 at 13.41 min Area: 5500604 Area % 4.82

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 9-Octadecenoic acid (Z)-, methyl...	13261	000112-62-9	99
2 8-Octadecenoic acid, methyl este...	13870	026528-50-7	99
3 10-Octadecenoic acid, methyl ester	13251	013481-95-3	99

Unknown Spectrum based on Apex minus start of peak

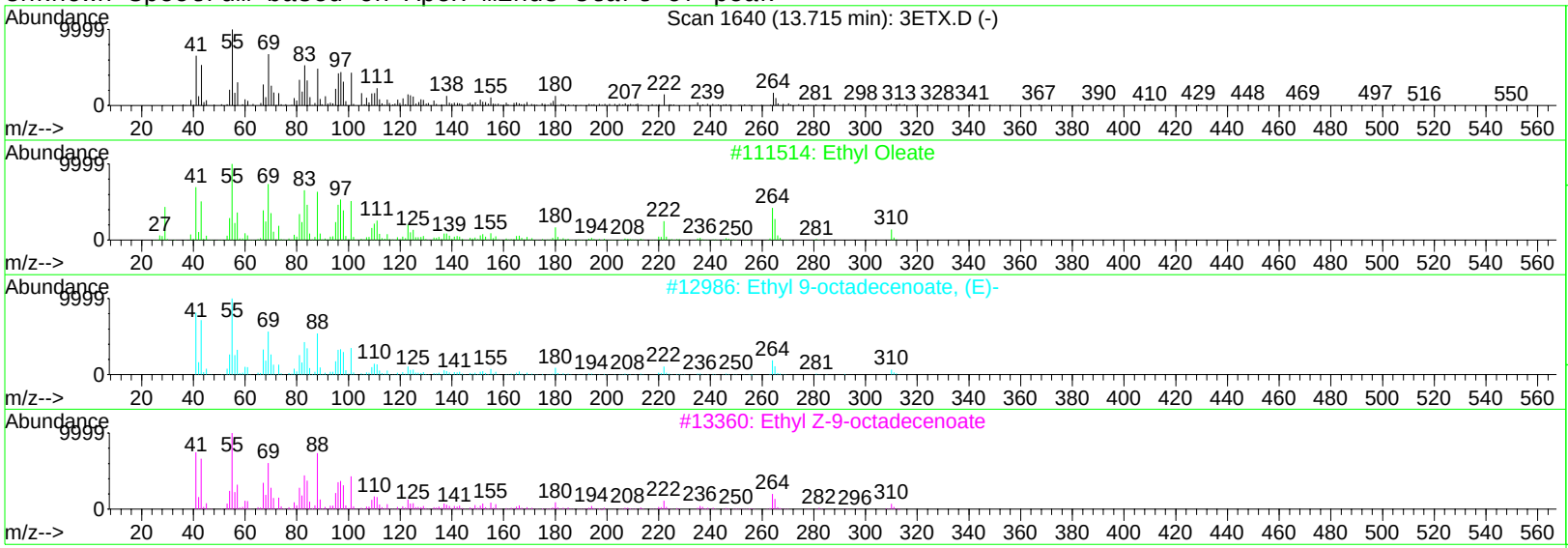


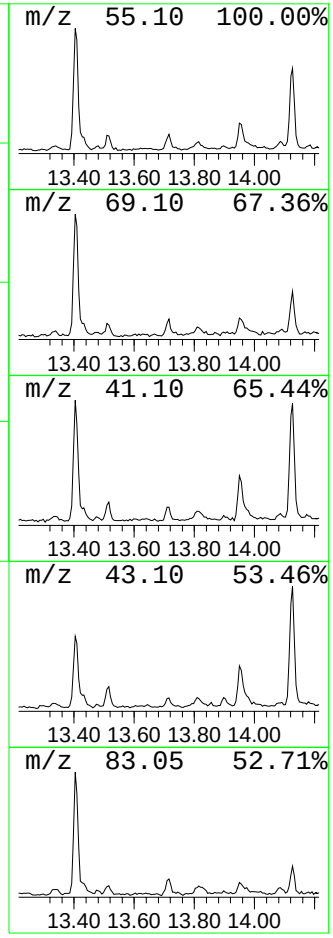


Peak Number: 13 at 13.52 min Area: 891781 Area % 0.78

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Octadecanoic acid, methyl ester	115467	000112-61-8	97
2 Heptadecanoic acid, 16-methyl-, ...	115407	005129-61-3	96
3 Heptadecanoic acid, 14-methyl-, ...	28508	002490-23-5	91

Unknown Spectrum based on Apex minus start of peak

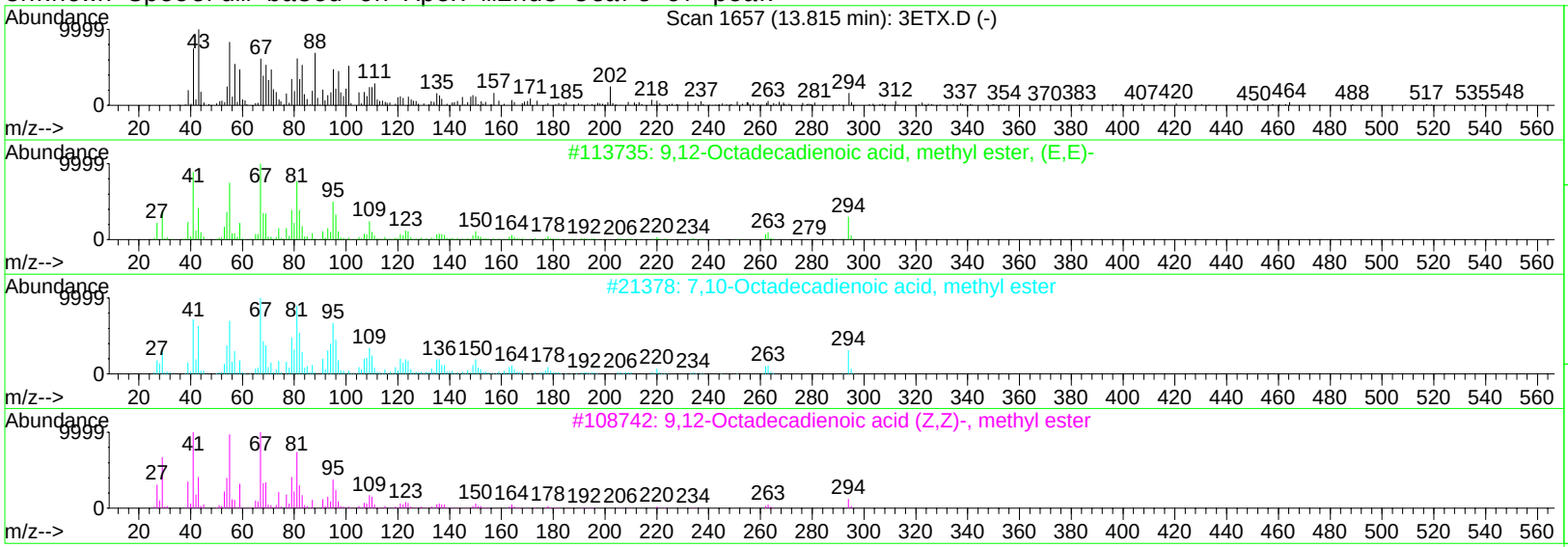


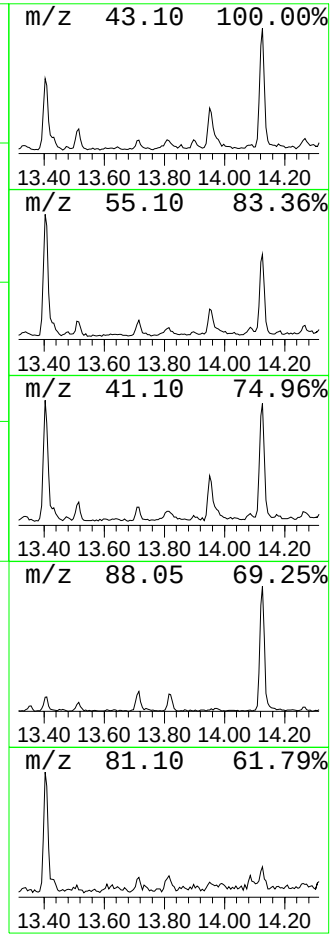


Peak Number: 14 at 13.71 min Area: 734425 Area % 0.64

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Ethyl Oleate	111514	000111-62-6	98
2 Ethyl 9-octadecenoate, (E)-	12986	1000130-91-6	91
3 Ethyl Z-9-octadecenoate	13360	1000130-91-7	62

Unknown Spectrum based on Apex minus start of peak

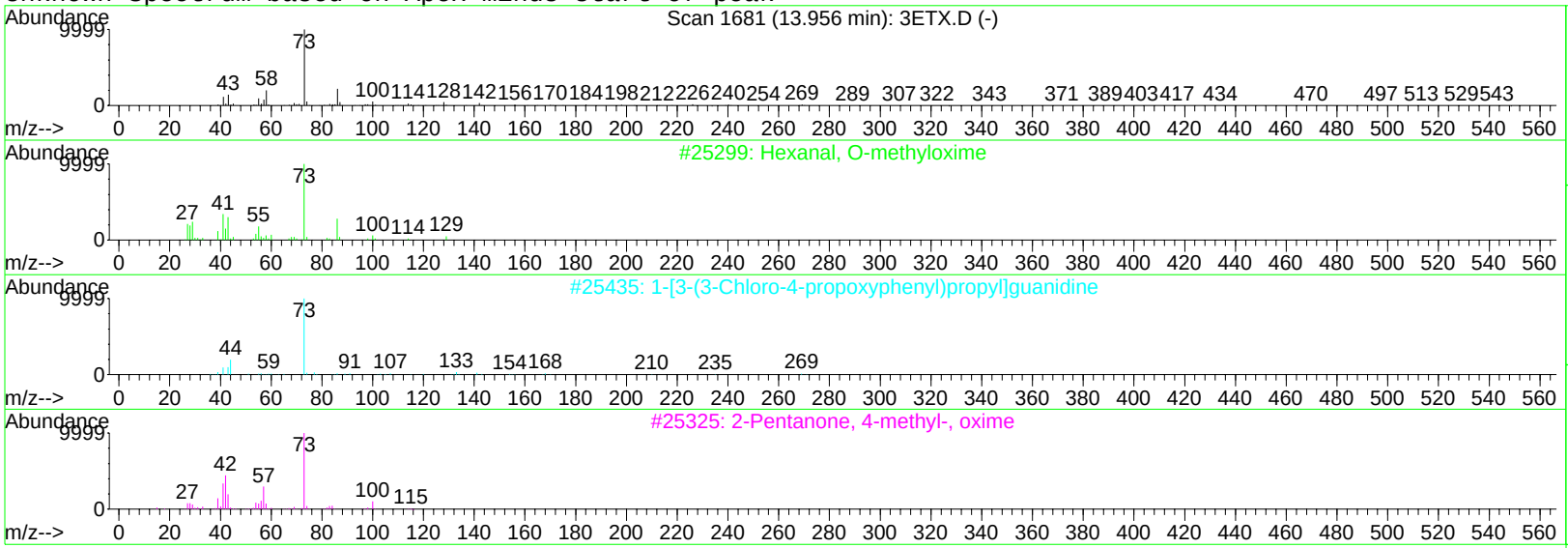


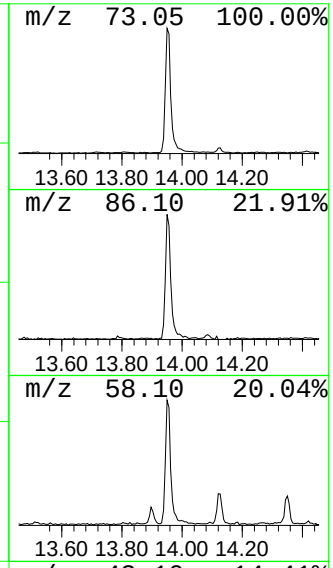


Peak Number: 15 at 13.81 min Area: 821454 Area % 0.72

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 9,12-Octadecadienoic acid, methy...	113735	002566-97-4	55
2 7,10-Octadecadienoic acid, methy...	21378	056554-24-6	45
3 9,12-Octadecadienoic acid (Z,Z)-...	108742	000112-63-0	44

Unknown Spectrum based on Apex minus start of peak

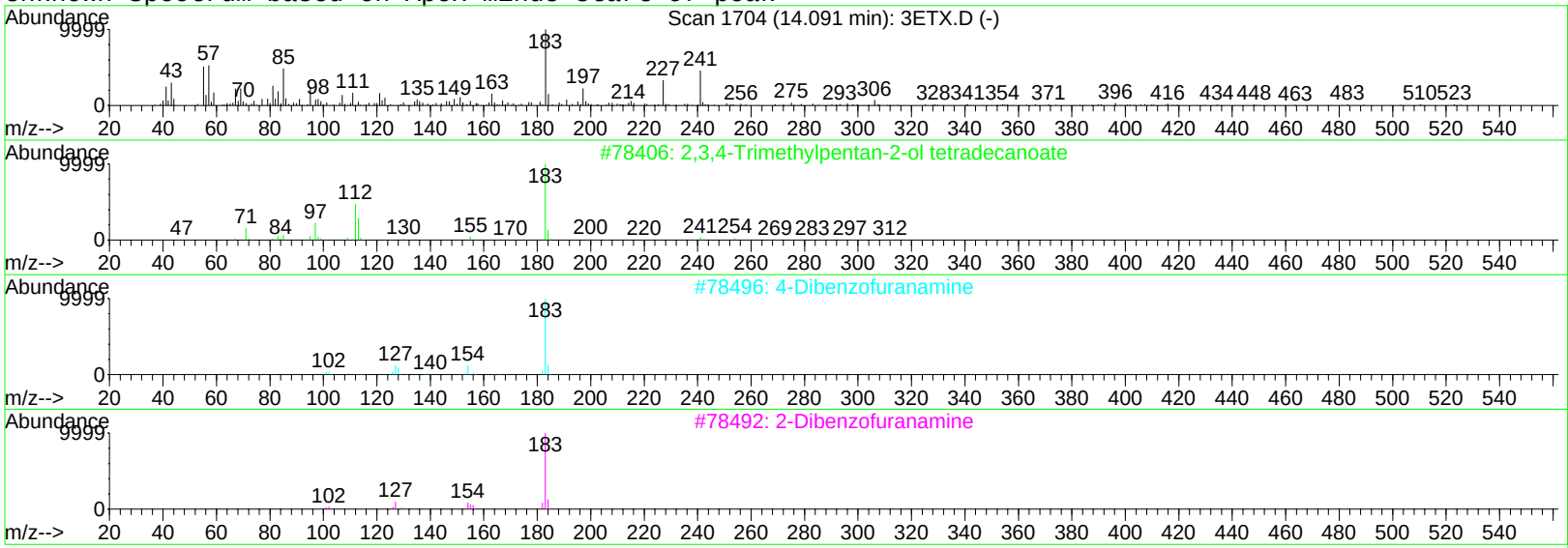


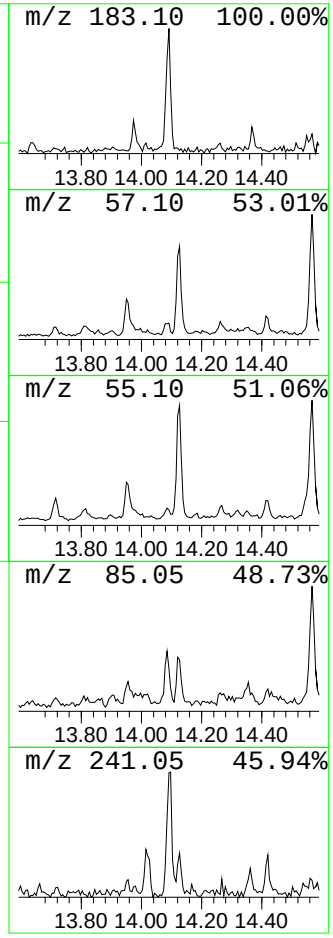


Peak Number: 16 at 13.96 min Area: 2919254 Area % 2.56

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Hexanal, O-methyloxime	25299	056292-95-6	38
2 1-[3-(3-Chloro-4-propoxyphenyl)p...	25435	1000223-85-0	9
3 2-Pentanone, 4-methyl-, oxime	25325	000105-44-2	9

Unknown Spectrum based on Apex minus start of peak

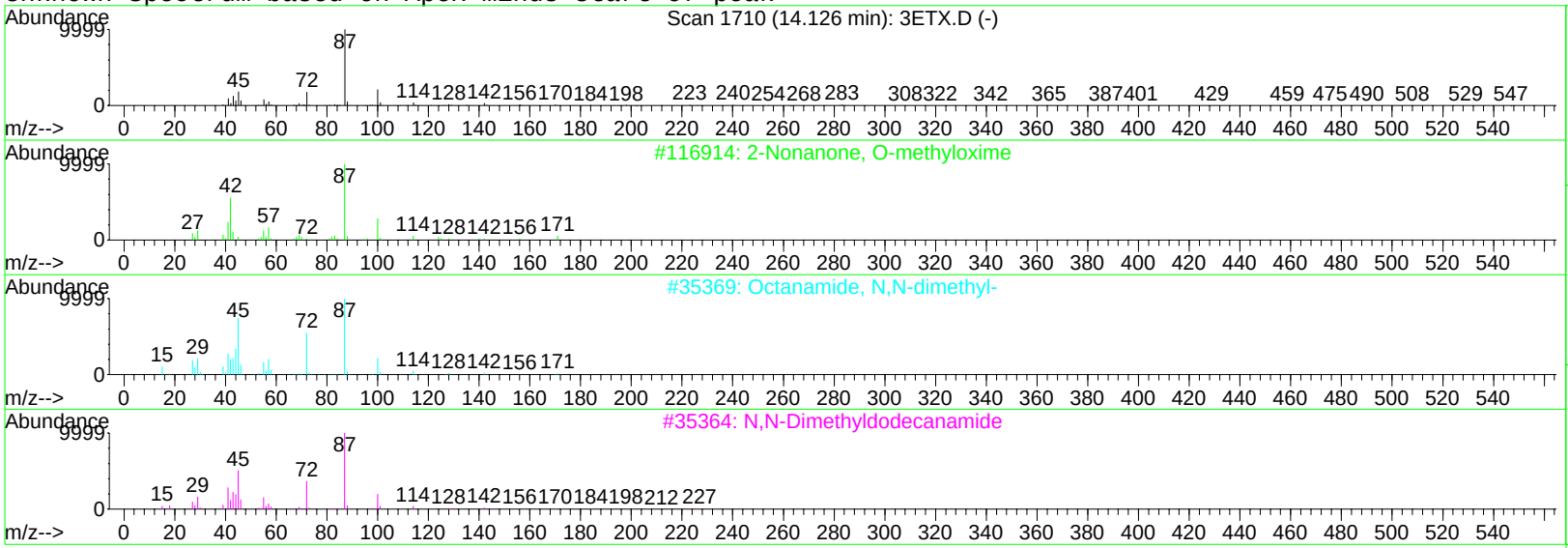


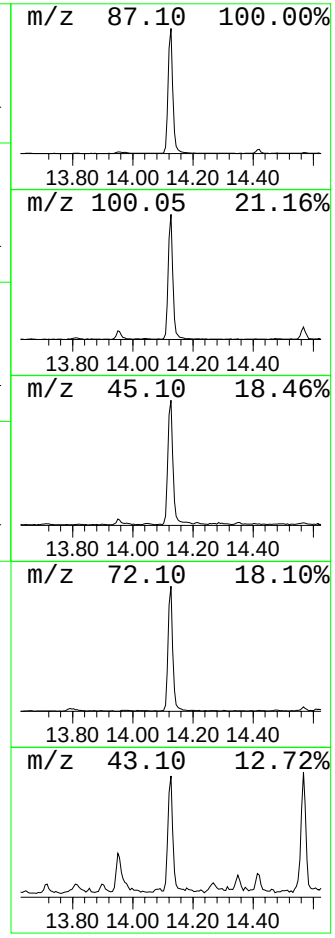


Peak Number: 17 at 14.09 min Area: 457209 Area % 0.40

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2,3,4-Trimethylpentan-2-ol tetra...	78406	1000130-74-1	35
2 4-Dibenzofuranamine	78496	050548-43-1	32
3 2-Dibenzofuranamine	78492	003693-22-9	32

Unknown Spectrum based on Apex minus start of peak

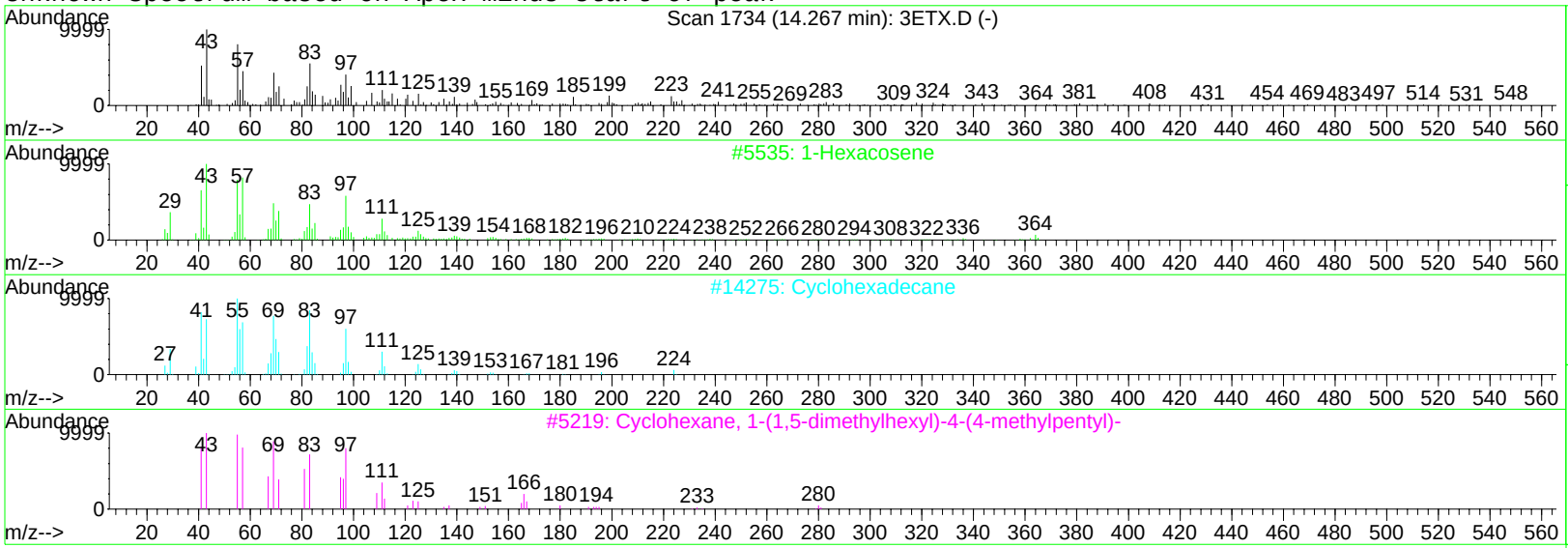


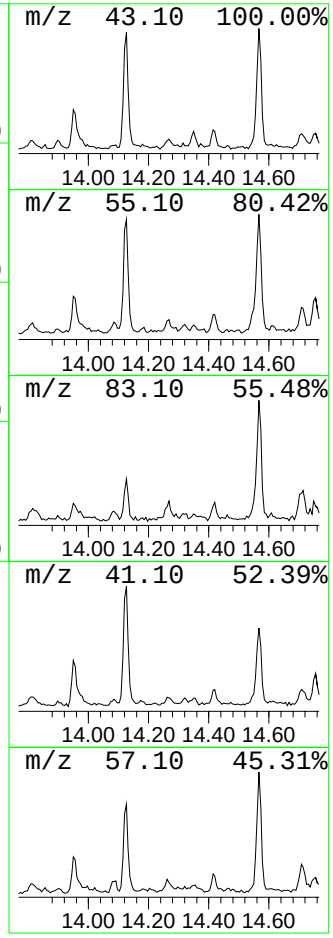


Peak Number: 18 at 14.13 min Area: 7708891 Area % 6.75

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Nonanone, O-methyloxime	116914	056292-72-9	50
2 Octanamide, N,N-dimethyl-	35369	001118-92-9	50
3 N,N-Dimethyldodecanamide	35364	003007-53-2	47

Unknown Spectrum based on Apex minus start of peak

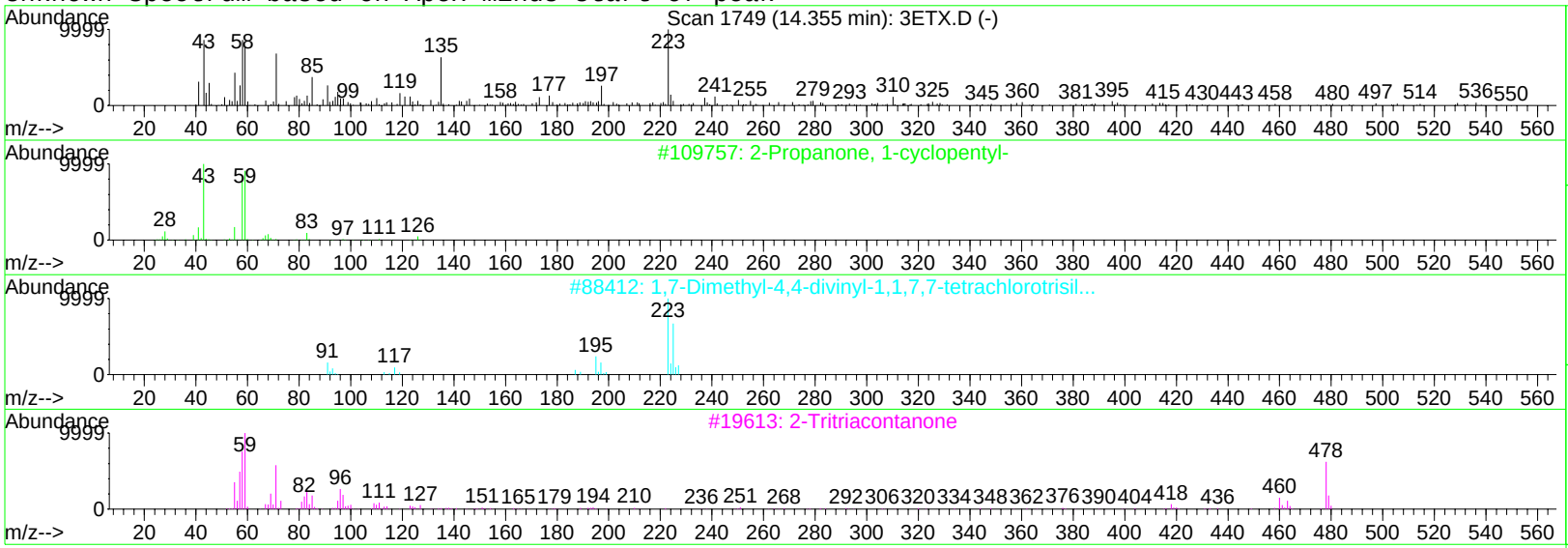


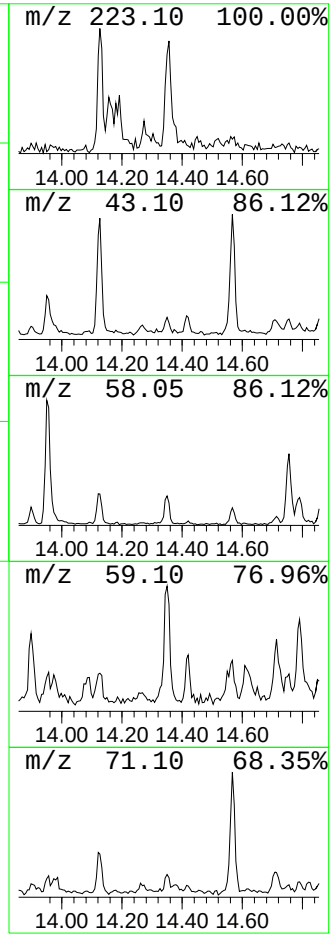


Peak Number: 19 at 14.27 min Area: 585369 Area % 0.51

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1-Hexacosene	5535	018835-33-1	91
2 Cyclohexadecane	14275	000295-65-8	83
3 Cyclohexane, 1-(1,5-dimethylhexy...	5219	056009-20-2	83

Unknown Spectrum based on Apex minus start of peak

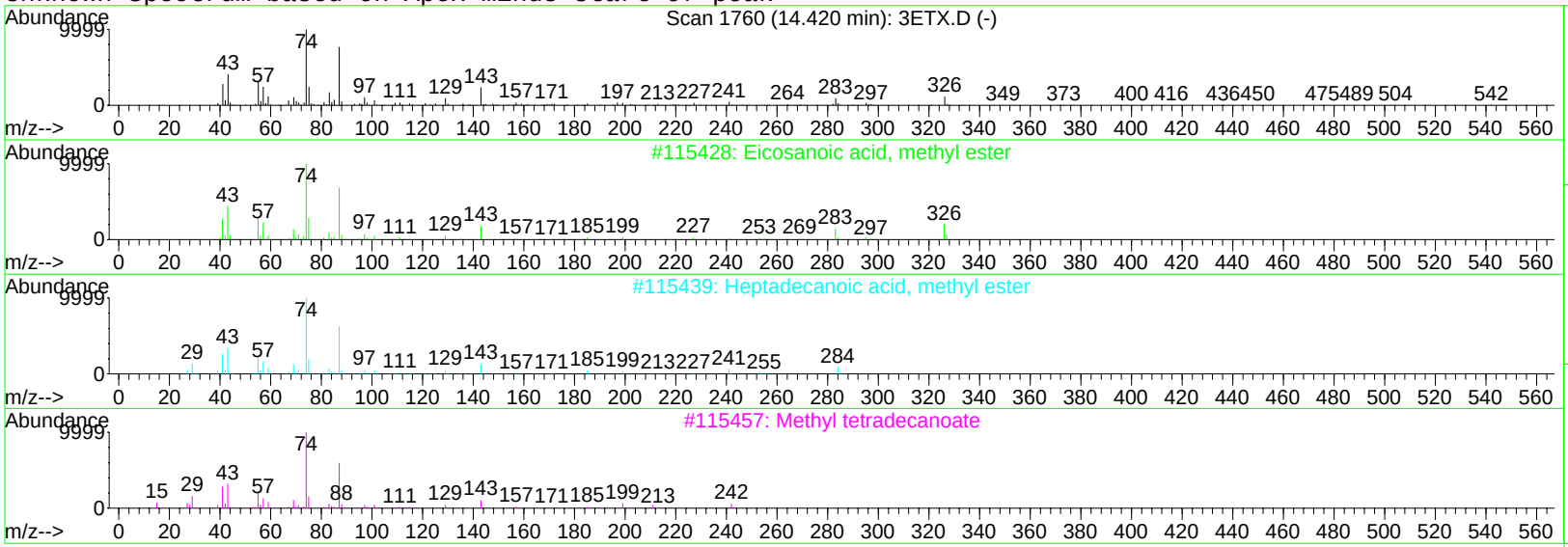


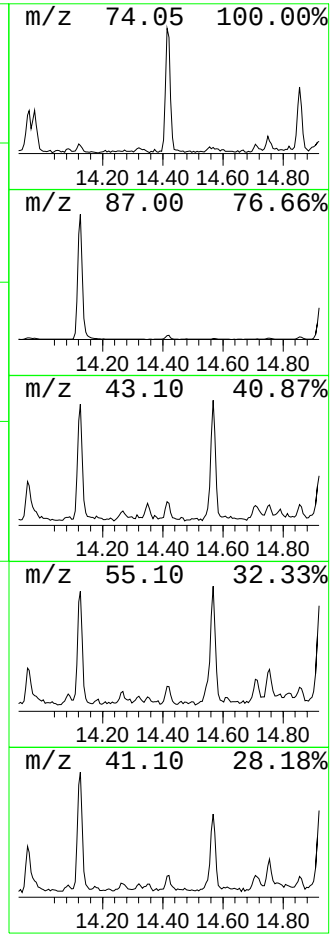


Peak Number: 20 at 14.36 min Area: 558619 Area % 0.49

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Propanone, 1-cyclopentyl-	109757	001122-98-1	43
2 1,7-Dimethyl-4,4-divinyl-1,1,7,7...	88412	080153-60-2	43
3 2-Tritriacontanone	19613	075207-55-5	38

Unknown Spectrum based on Apex minus start of peak

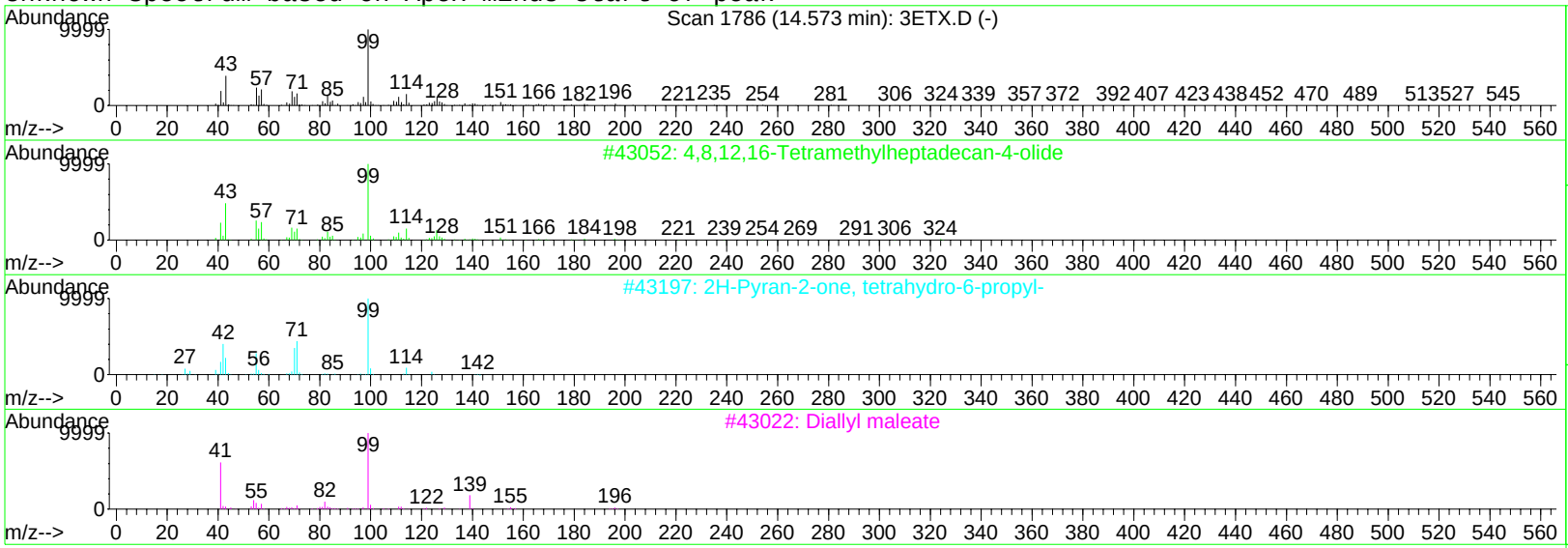


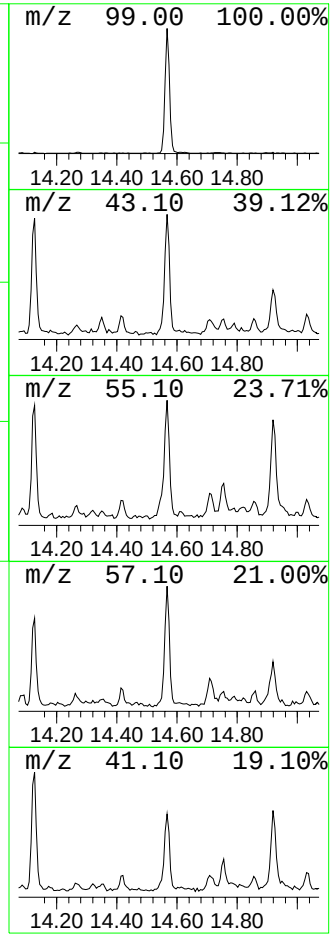


Peak Number: 21 at 14.42 min Area: 766771 Area % 0.67

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Eicosanoic acid, methyl ester	115428	001120-28-1	94
2 Heptadecanoic acid, methyl ester	115439	001731-92-6	91
3 Methyl tetradecanoate	115457	000124-10-7	87

Unknown Spectrum based on Apex minus start of peak

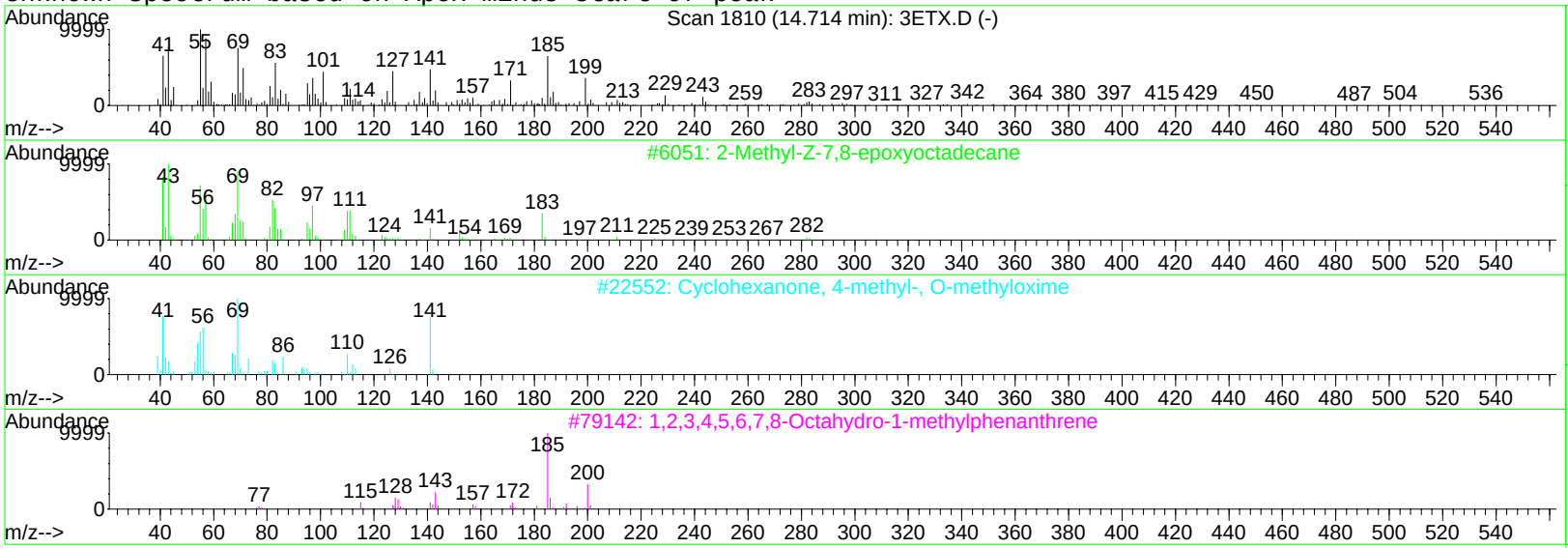


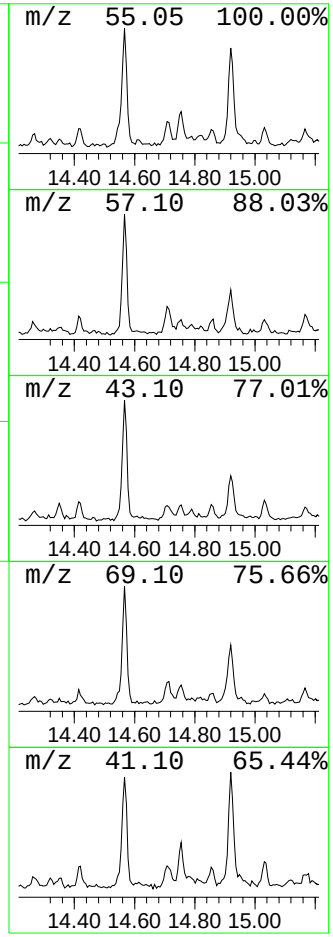


Peak Number: 22 at 14.57 min Area: 5193359 Area % 4.55

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 4,8,12,16-Tetramethylheptadecan-...	43052	096168-15-9	99
2 2H-Pyran-2-one, tetrahydro-6-pro...	43197	000698-76-0	50
3 Diallyl maleate	43022	000999-21-3	47

Unknown Spectrum based on Apex minus start of peak

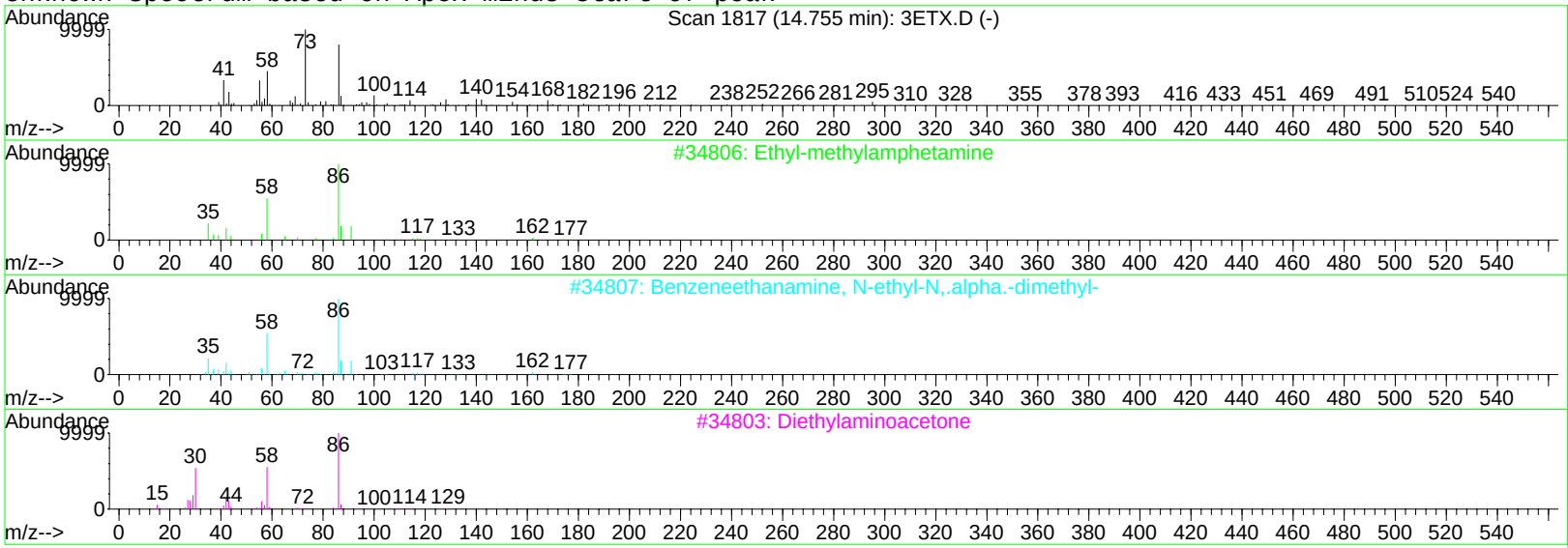


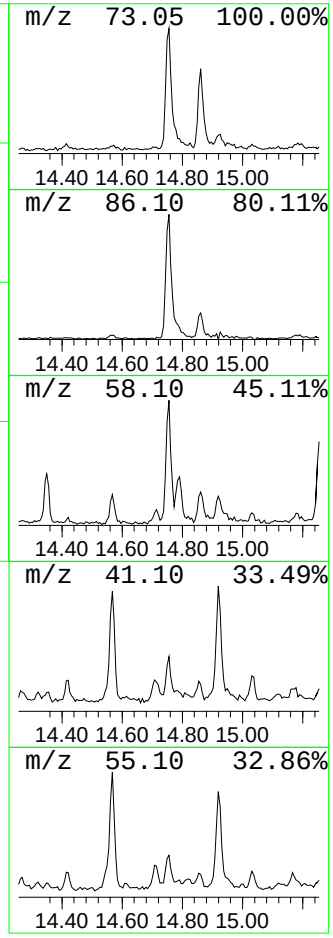


Peak Number: 23 at 14.71 min Area: 1196757 Area % 1.05

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Methyl-Z-7,8-epoxyoctadecane	6051	1000130-91-4	45
2 Cyclohexanone, 4-methyl-, O-meth...	22552	039477-43-5	22
3 1,2,3,4,5,6,7,8-Octahydro-1-meth...	79142	1000080-19-4	22

Unknown Spectrum based on Apex minus start of peak

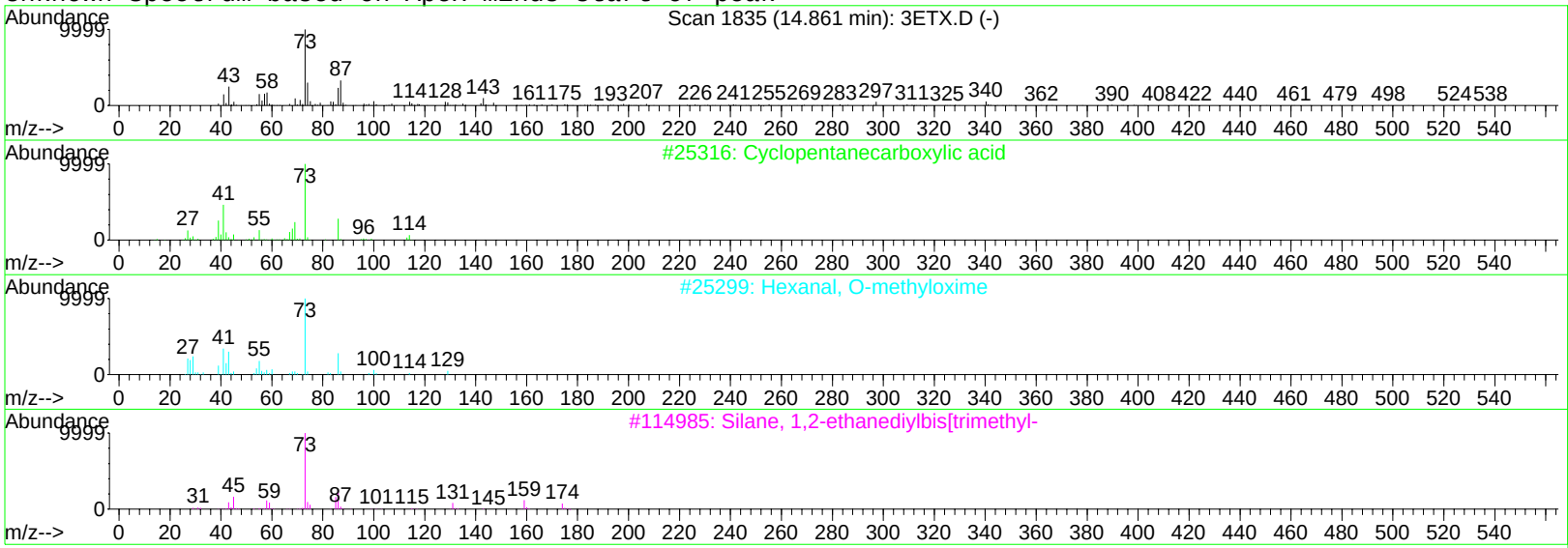


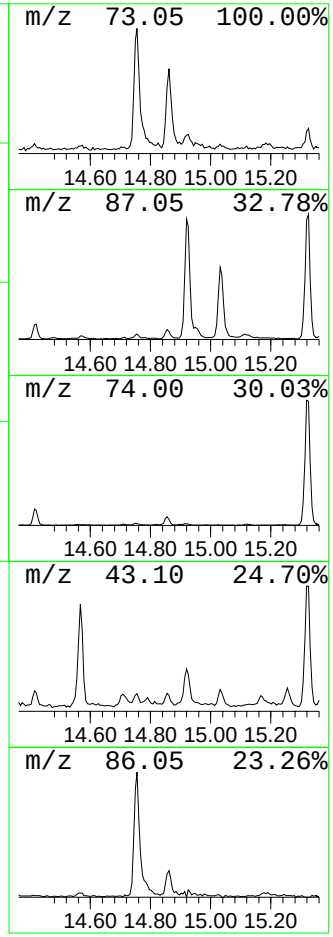


Peak Number: 24 at 14.75 min Area: 1654696 Area % 1.45

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Ethyl-methylamphetamine	34806	1000120-30-0	53
2 Benzeneethanamine, N-ethyl-N,.al...	34807	119290-77-6	42
3 Diethylaminoacetone	34803	001620-14-0	42

Unknown Spectrum based on Apex minus start of peak

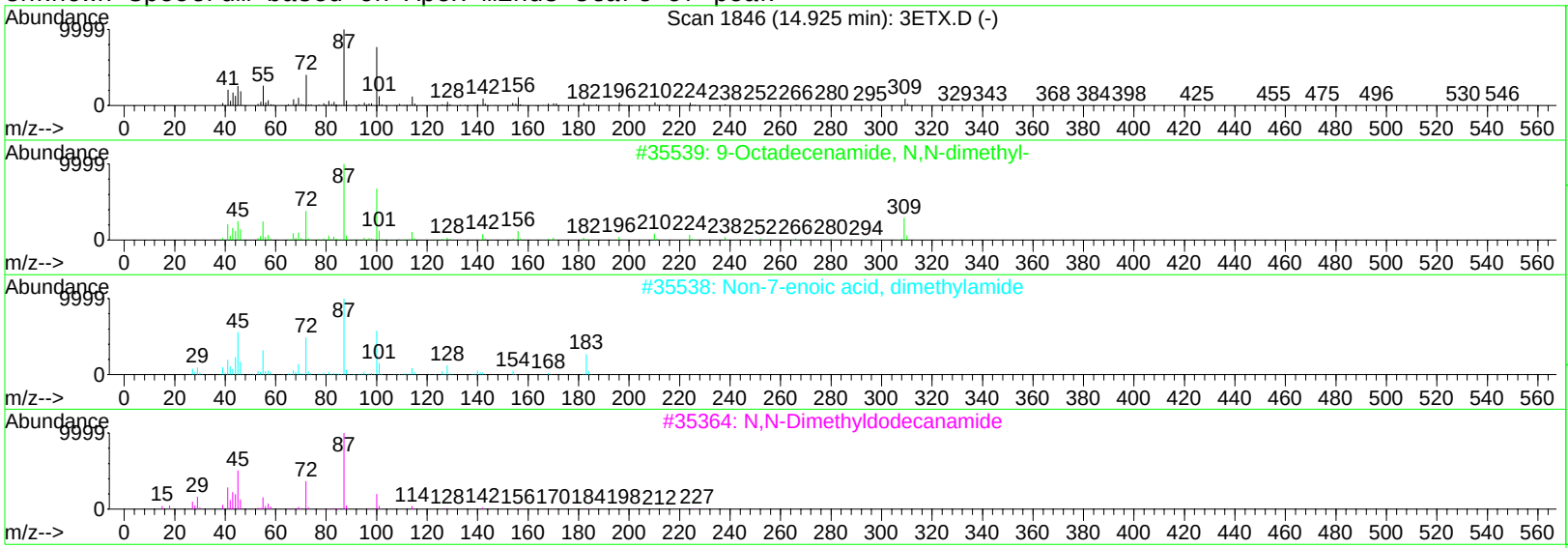


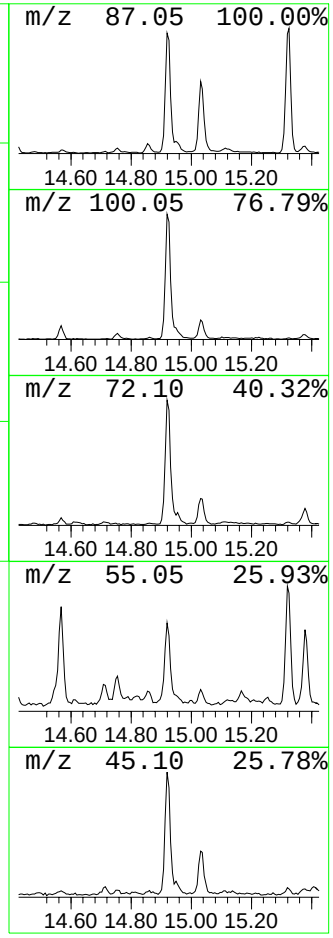


Peak Number: 25 at 14.86 min Area: 950582 Area % 0.83

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cyclopentanecarboxylic acid	25316	003400-45-1	38
2 Hexanal, O-methyloxime	25299	056292-95-6	22
3 Silane, 1,2-ethanediylbis[trimet...	114985	006231-76-1	17

Unknown Spectrum based on Apex minus start of peak

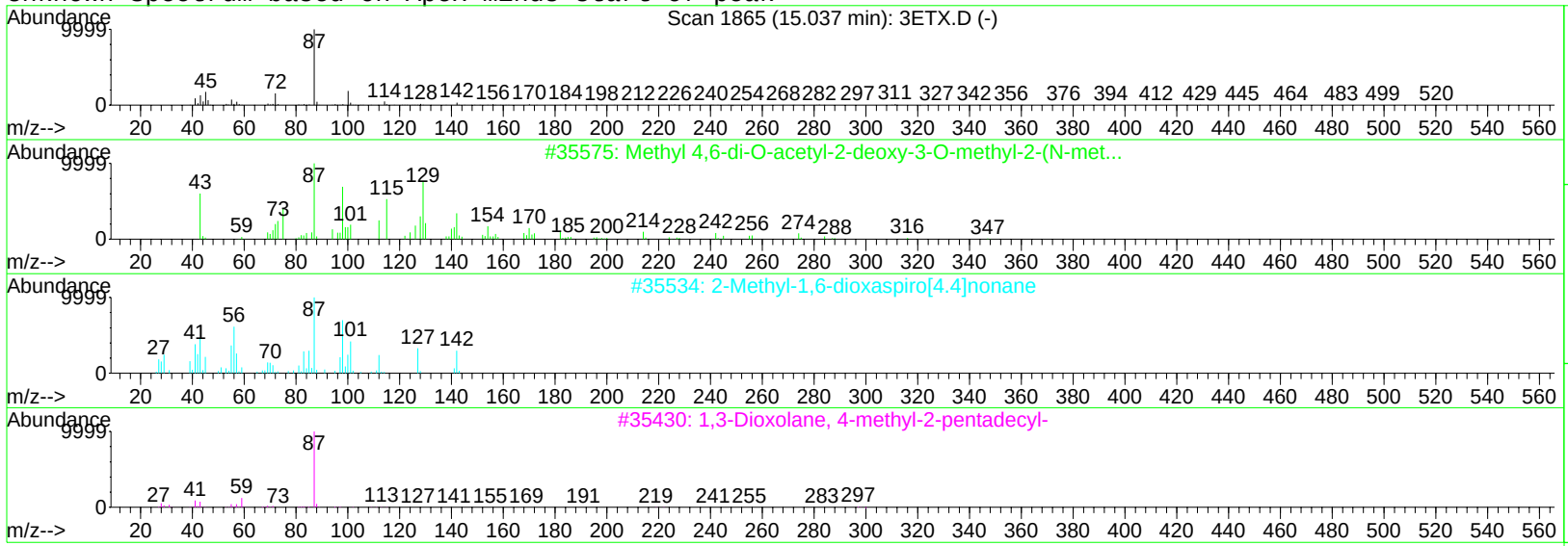


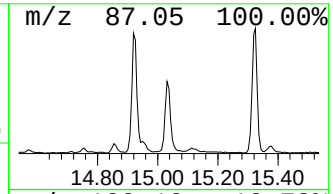


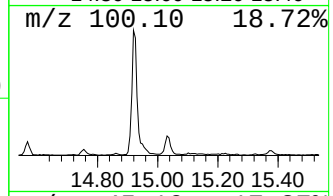
Peak Number: 26 at 14.93 min Area: 6053885 Area % 5.30

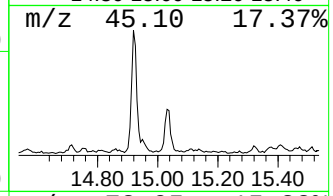
The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 9-Octadecenamide, N,N-dimethyl-	35539	003906-30-7	98
2 Non-7-enoic acid, dimethylamide	35538	1000187-26-5	56
3 N,N-Dimethyldodecanamide	35364	003007-53-2	43

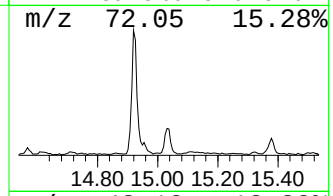
Unknown Spectrum based on Apex minus start of peak

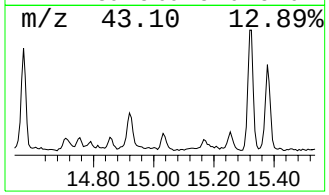








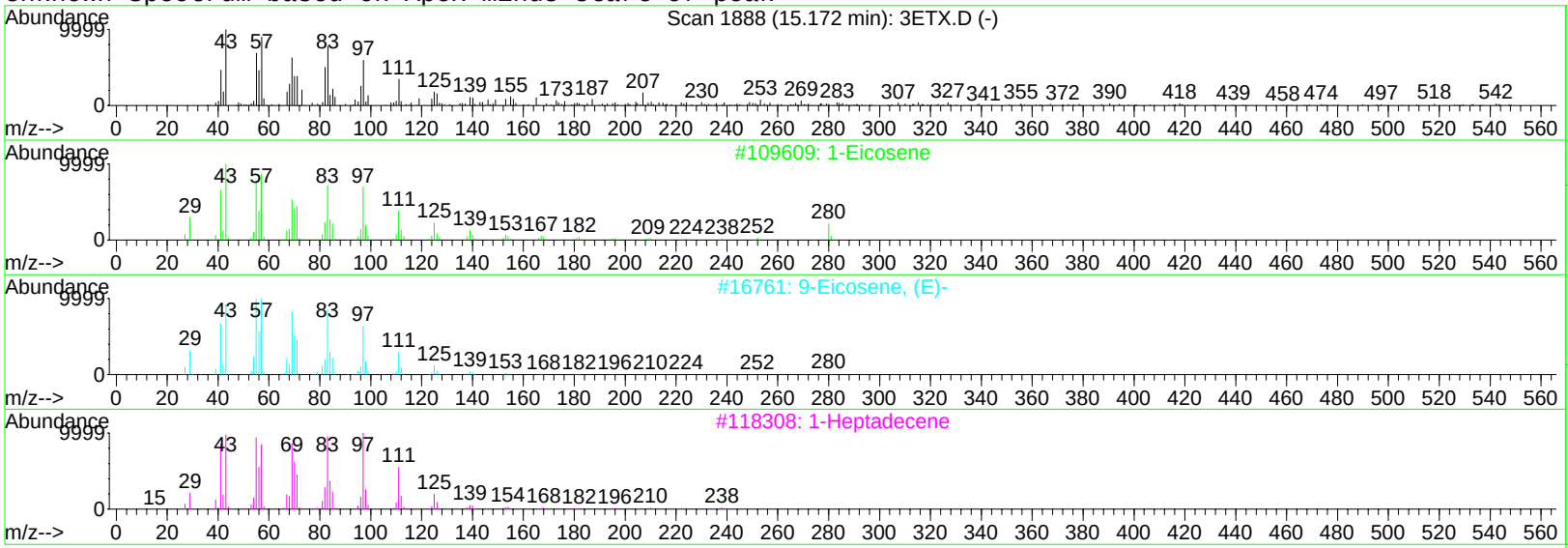


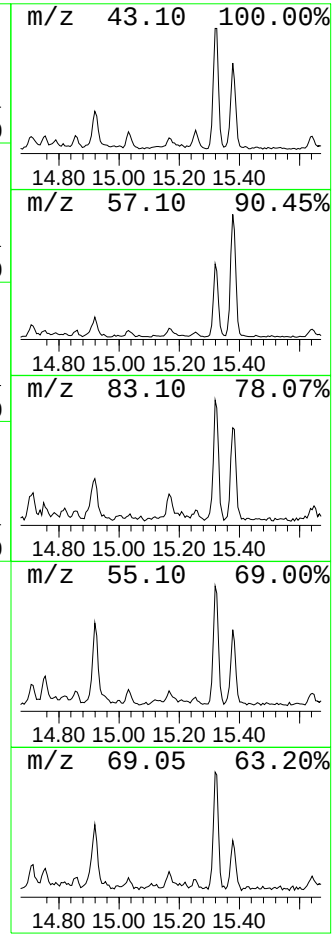


Peak Number: 27 at 15.04 min Area: 1468704 Area % 1.29

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Methyl 4,6-di-O-acetyl-2-deoxy-3...	35575	056341-51-6	59
2 2-Methyl-1,6-dioxaspiro[4.4]nonane	35534	005451-15-0	53
3 1,3-Dioxolane, 4-methyl-2-pentad...	35430	054950-56-0	50

Unknown Spectrum based on Apex minus start of peak

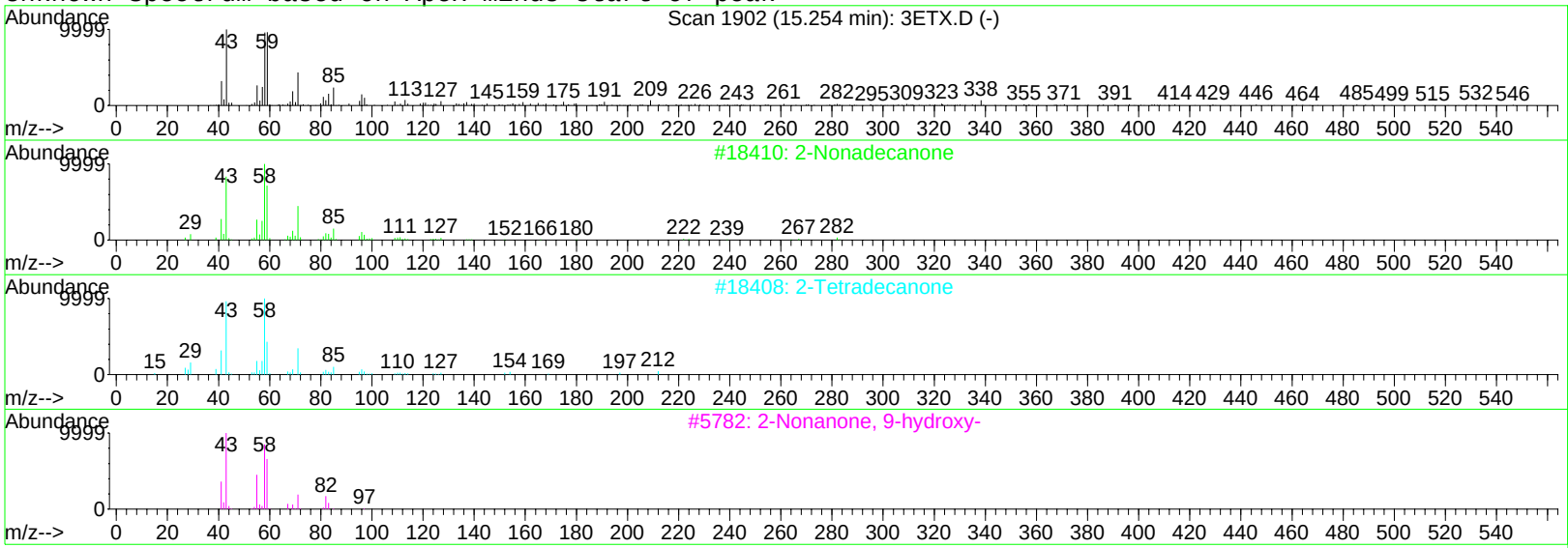


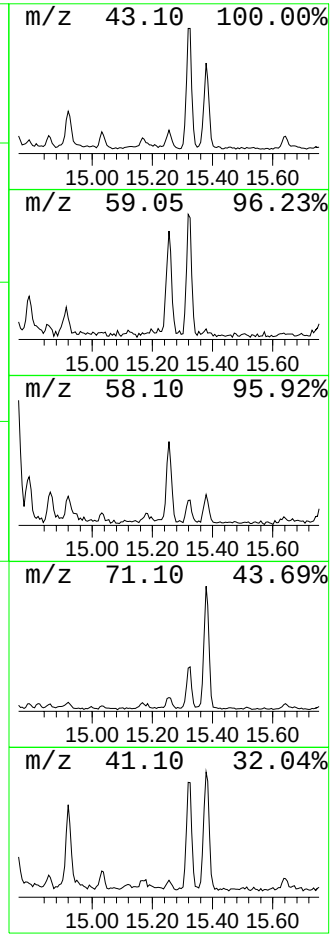


Peak Number: 28 at 15.17 min Area: 1321266 Area % 1.16

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1-Eicosene	109609	003452-07-1	91
2 9-Eicosene, (E)-	16761	074685-29-3	91
3 1-Heptadecene	118308	006765-39-5	91

Unknown Spectrum based on Apex minus start of peak

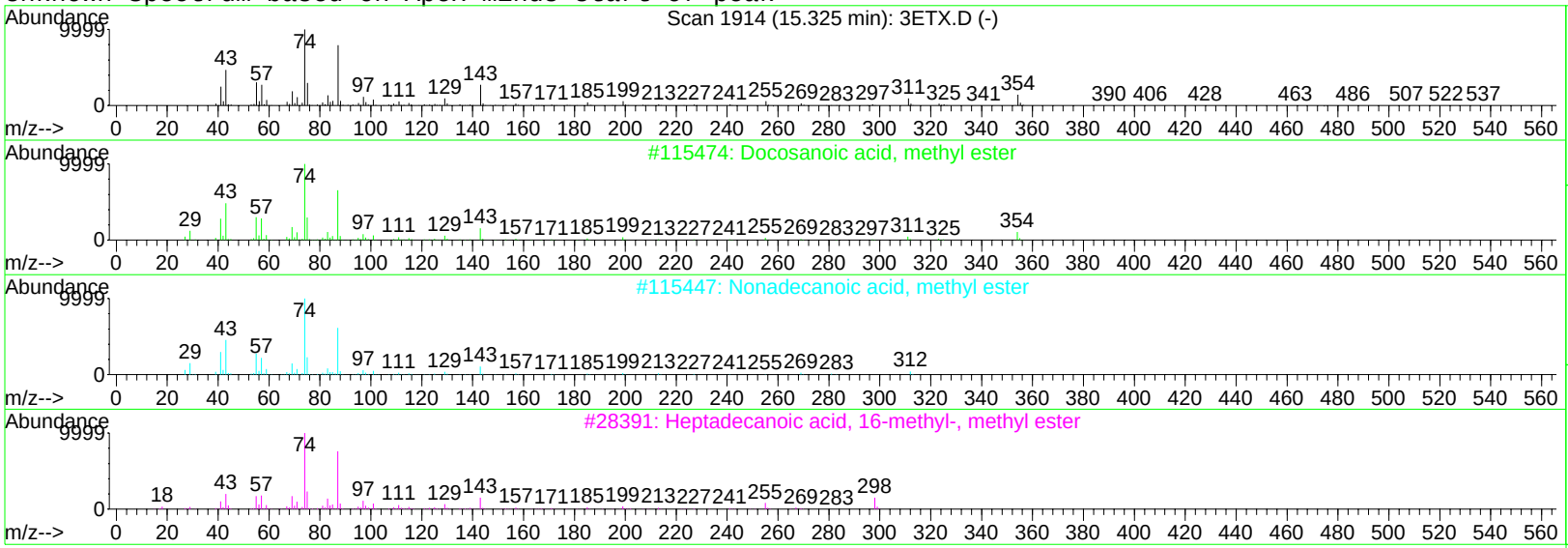


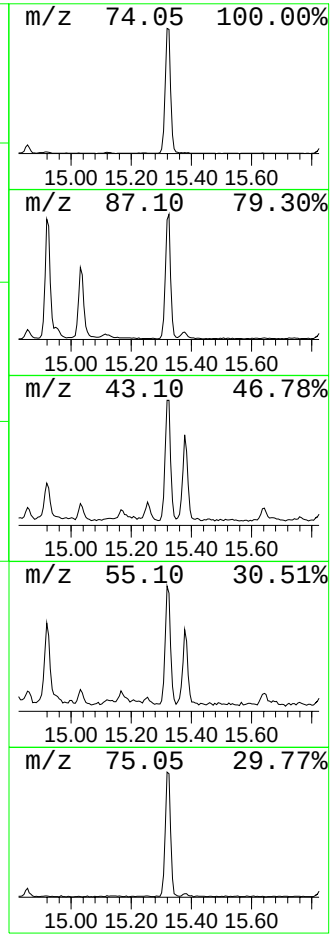


Peak Number: 29 at 15.25 min Area: 667912 Area % 0.58

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 2-Nonadecanone	18410	000629-66-3	70
2 2-Tetradecanone	18408	002345-27-9	62
3 2-Nonanone, 9-hydroxy-	5782	025368-56-3	59

Unknown Spectrum based on Apex minus start of peak

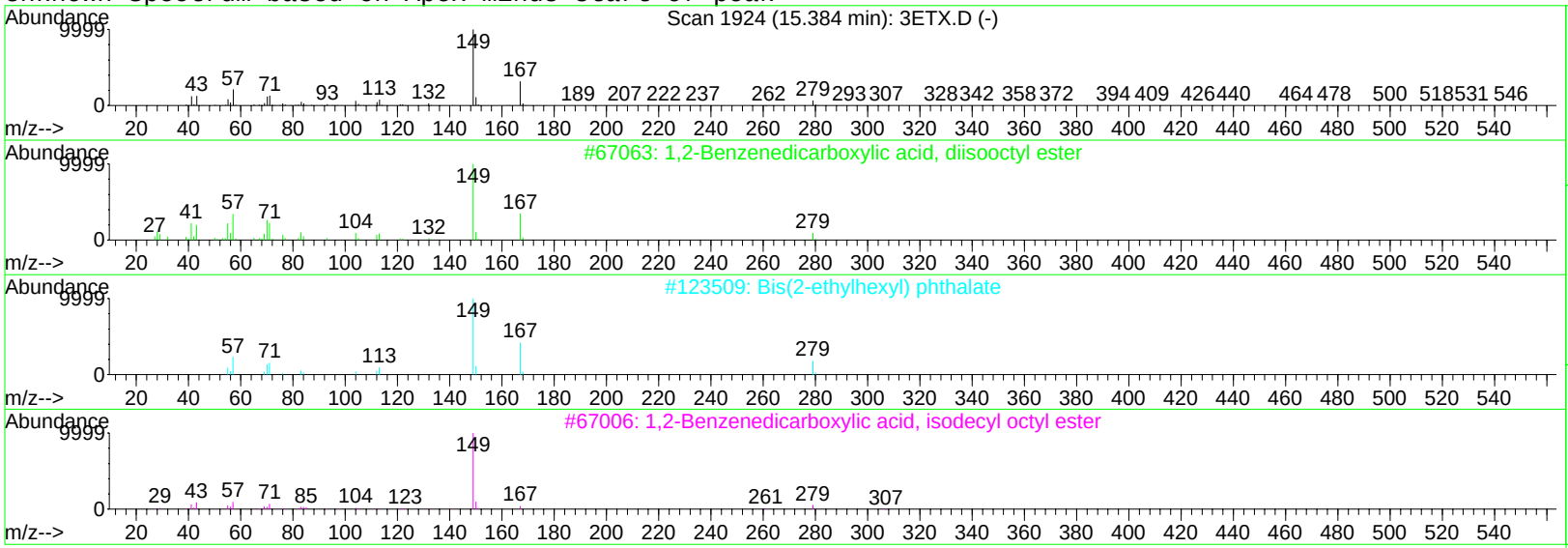


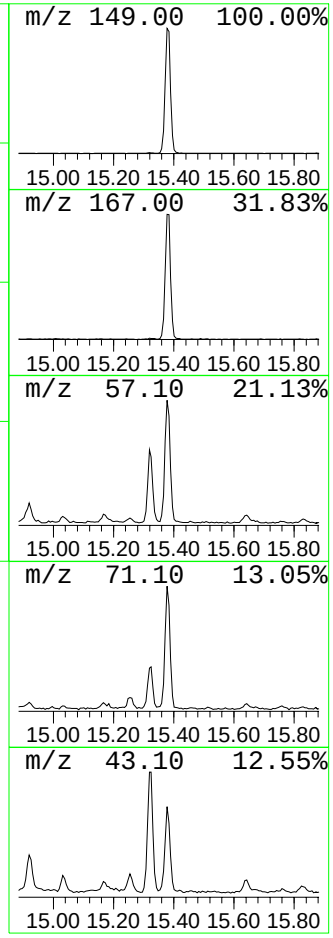


Peak Number: 30 at 15.32 min Area: 6719895 Area % 5.89

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Docosanoic acid, methyl ester	115474	000929-77-1	99
2 Nonadecanoic acid, methyl ester	115447	001731-94-8	93
3 Heptadecanoic acid, 16-methyl-, ...	28391	005129-61-3	83

Unknown Spectrum based on Apex minus start of peak

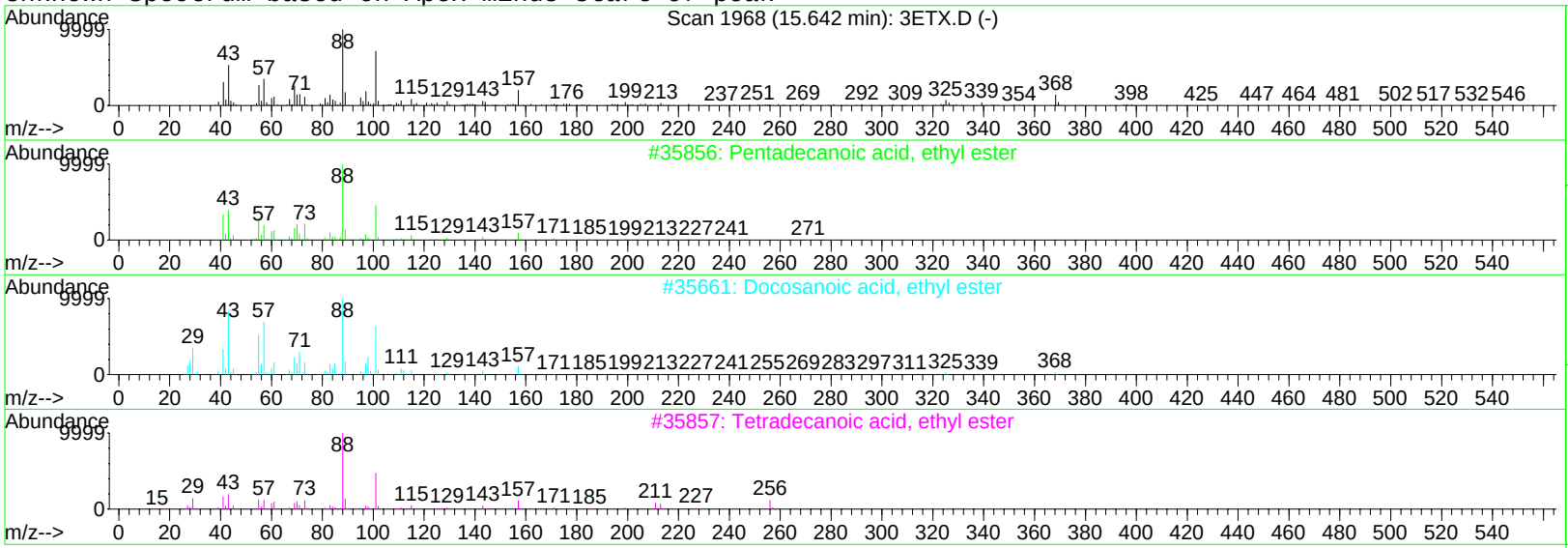


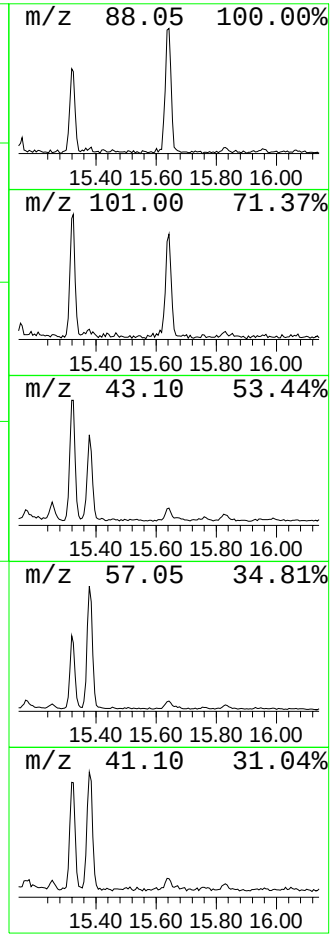


Peak Number: 31 at 15.38 min Area: 7690583 Area % 6.74

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 1,2-Benzenedicarboxylic acid, di...	67063	027554-26-3	91
2 Bis(2-ethylhexyl) phthalate	123509	000117-81-7	91
3 1,2-Benzenedicarboxylic acid, is...	67006	001330-96-7	64

Unknown Spectrum based on Apex minus start of peak

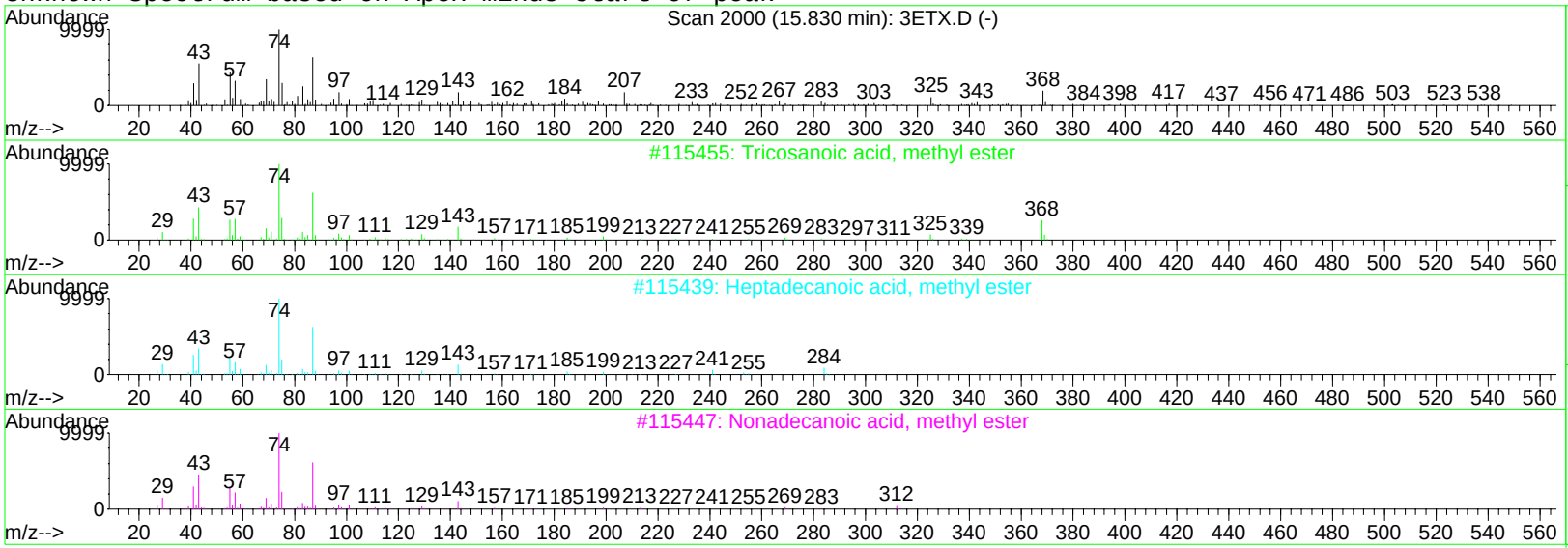


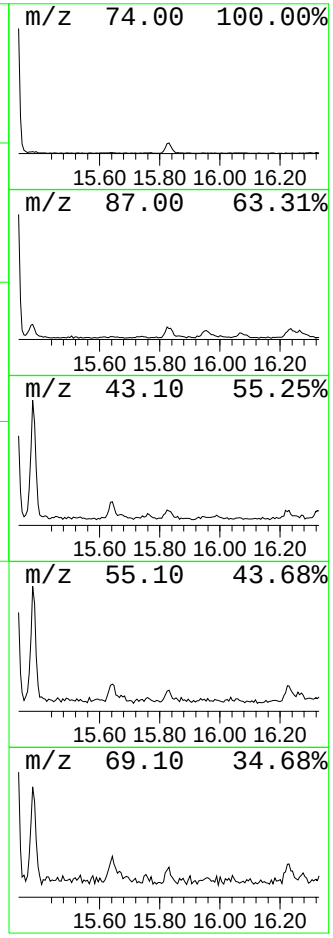


Peak Number: 32 at 15.64 min Area: 1488775 Area % 1.30

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Pentadecanoic acid, ethyl ester	35856	041114-00-5	87
2 Docosanoic acid, ethyl ester	35661	005908-87-2	78
3 Tetradecanoic acid, ethyl ester	35857	000124-06-1	76

Unknown Spectrum based on Apex minus start of peak

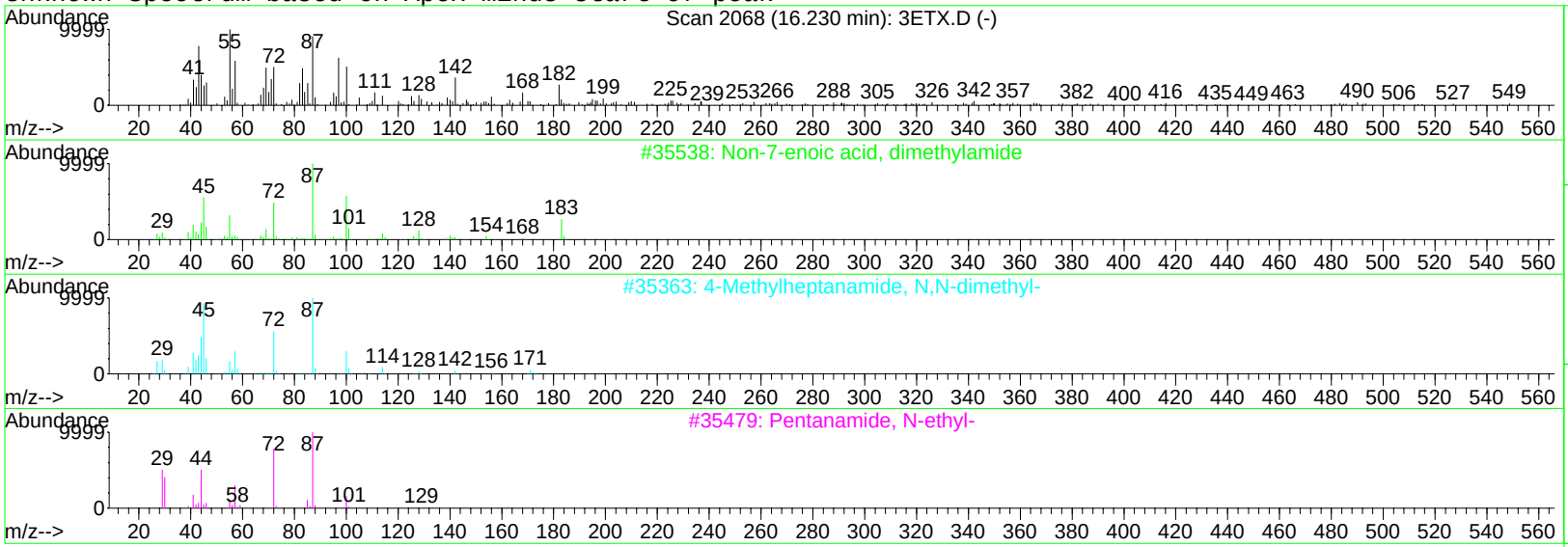


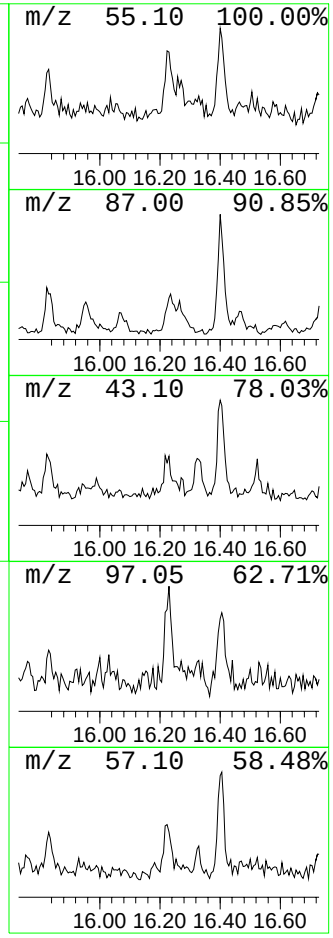


Peak Number: 33 at 15.83 min Area: 668006 Area % 0.59

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Tricosanoic acid, methyl ester	115455	002433-97-8	93
2 Heptadecanoic acid, methyl ester	115439	001731-92-6	70
3 Nonadecanoic acid, methyl ester	115447	001731-94-8	58

Unknown Spectrum based on Apex minus start of peak

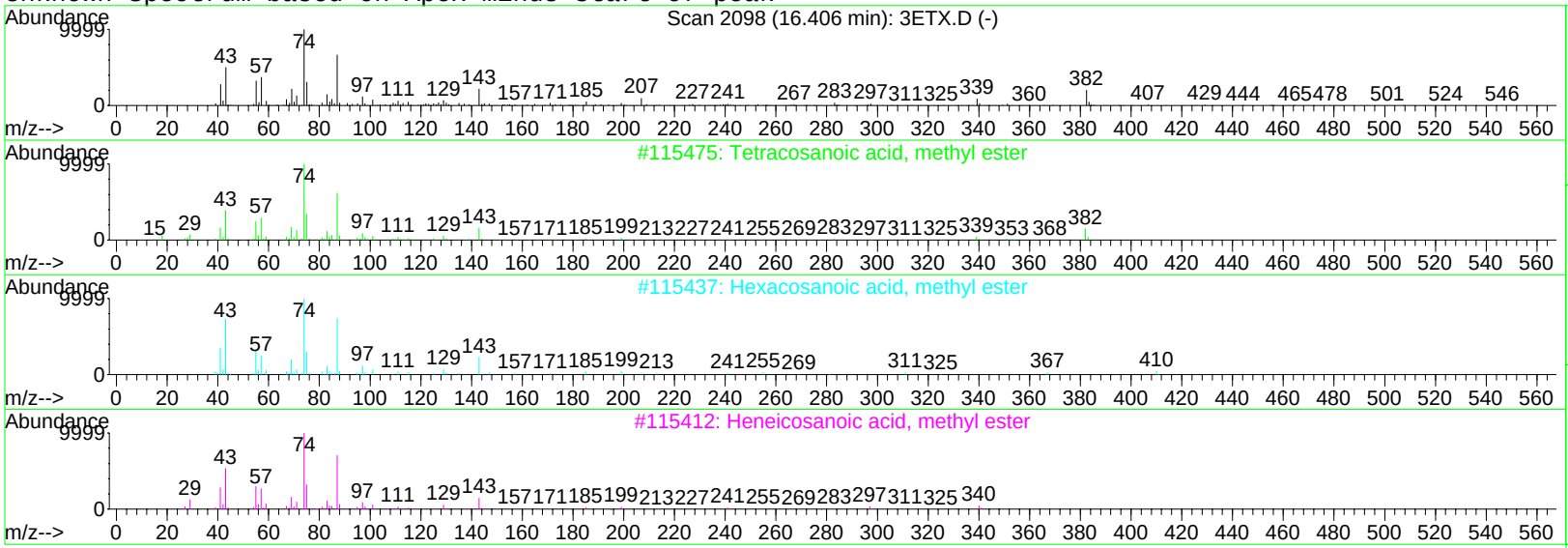


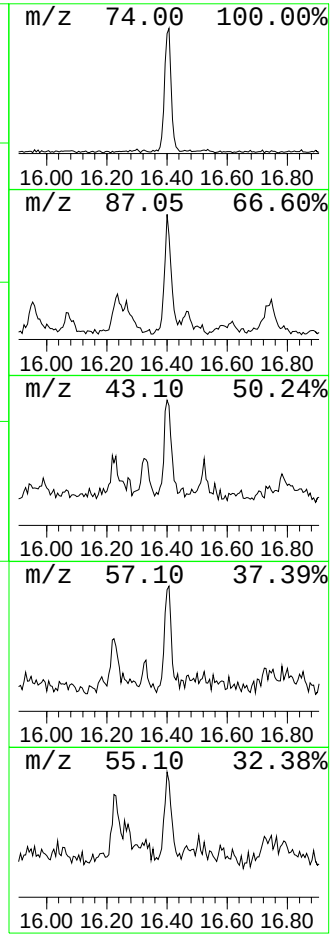


Peak Number: 34 at 16.23 min Area: 978381 Area % 0.86

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Non-7-enoic acid, dimethylamide	35538	1000187-26-5	70
2 4-Methylheptanamide, N,N-dimethyl-	35363	1000139-77-1	50
3 Pentanamide, N-ethyl-	35479	054007-33-9	43

Unknown Spectrum based on Apex minus start of peak

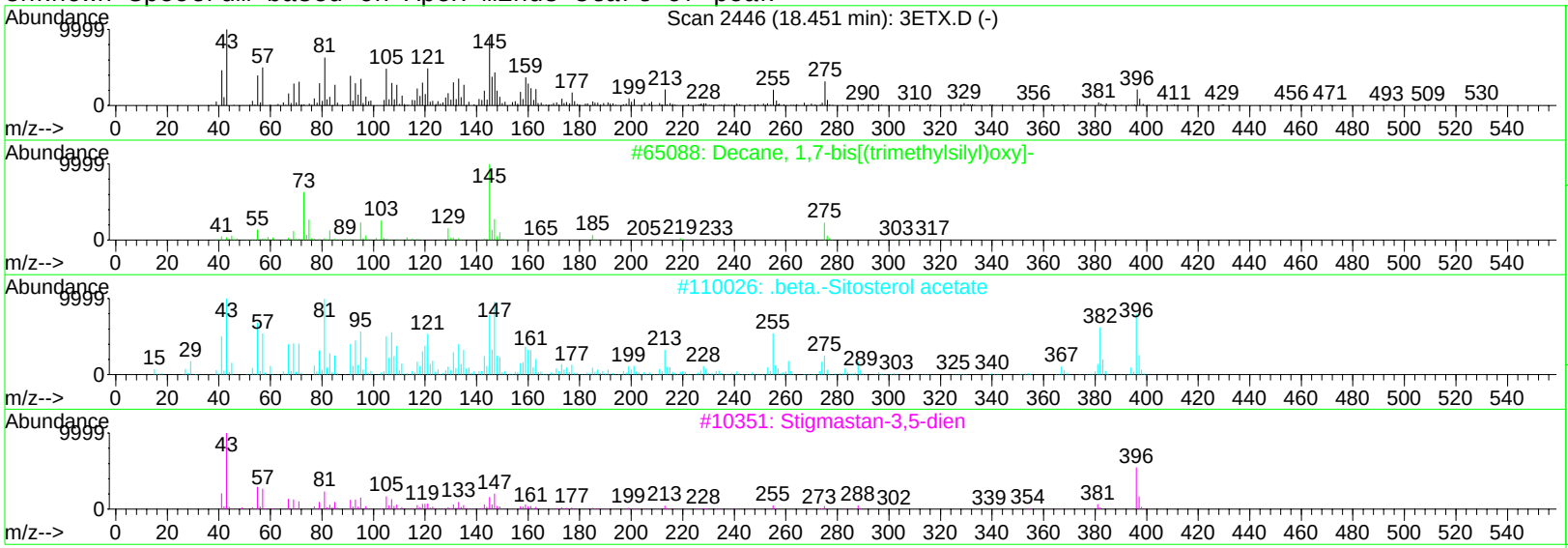


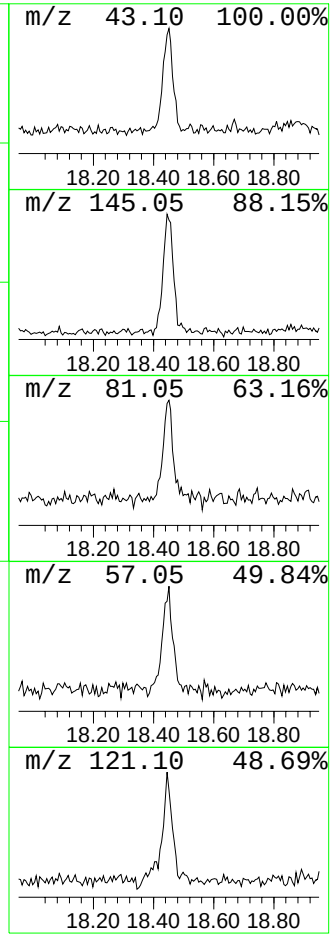


Peak Number: 35 at 16.41 min Area: 993140 Area % 0.87

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Tetracosanoic acid, methyl ester	115475	002442-49-1	93
2 Hexacosanoic acid, methyl ester	115437	005802-82-4	90
3 Heneicosanoic acid, methyl ester	115412	006064-90-0	76

Unknown Spectrum based on Apex minus start of peak

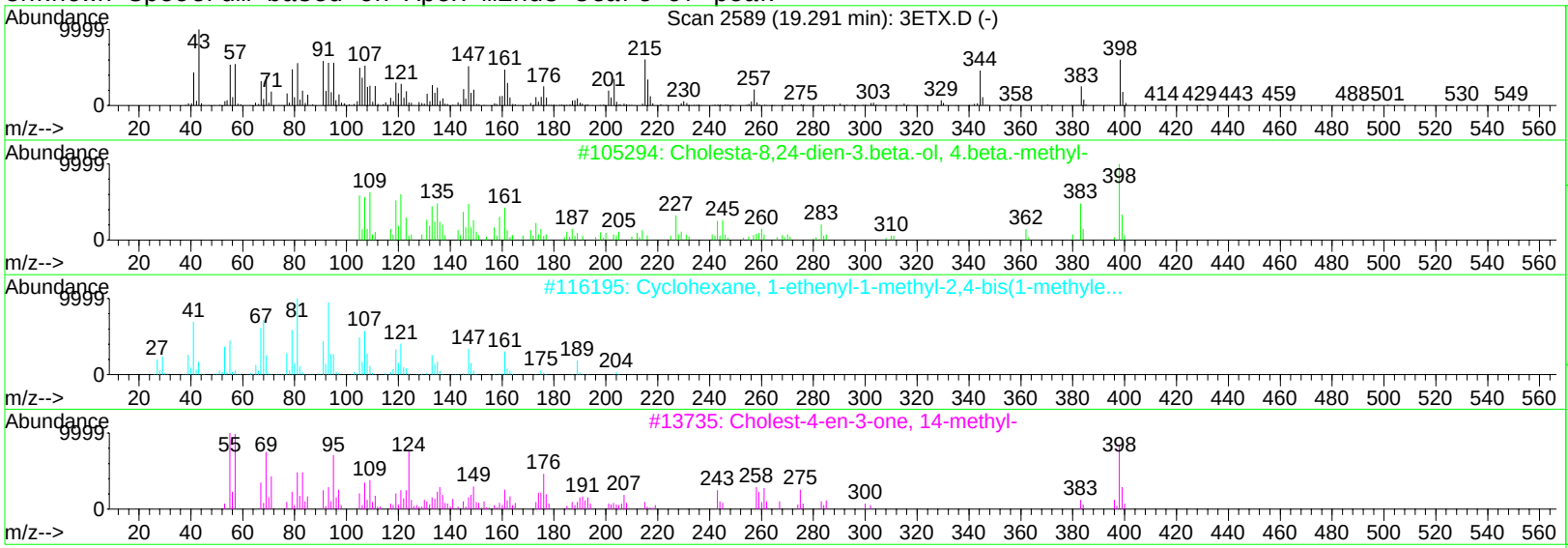


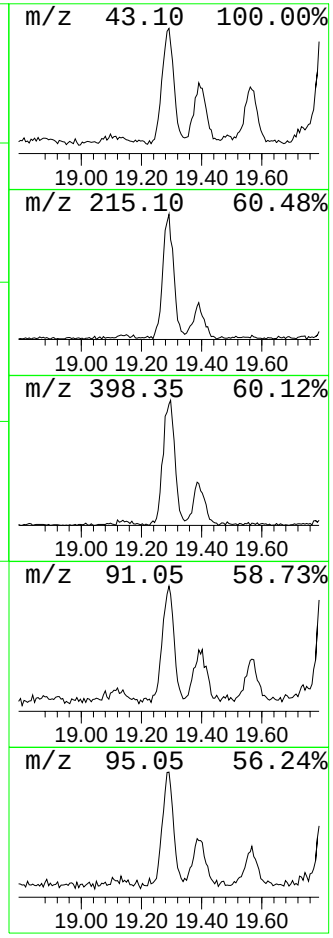


Peak Number: 36 at 18.45 min Area: 2356296 Area % 2.06

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Decane, 1,7-bis[(trimethylsilyl)...	65088	1000142-15-9	25
2 .beta.-Sitosterol acetate	110026	000915-05-9	4
3 Stigmastan-3,5-dien	10351	1000214-16-4	2

Unknown Spectrum based on Apex minus start of peak

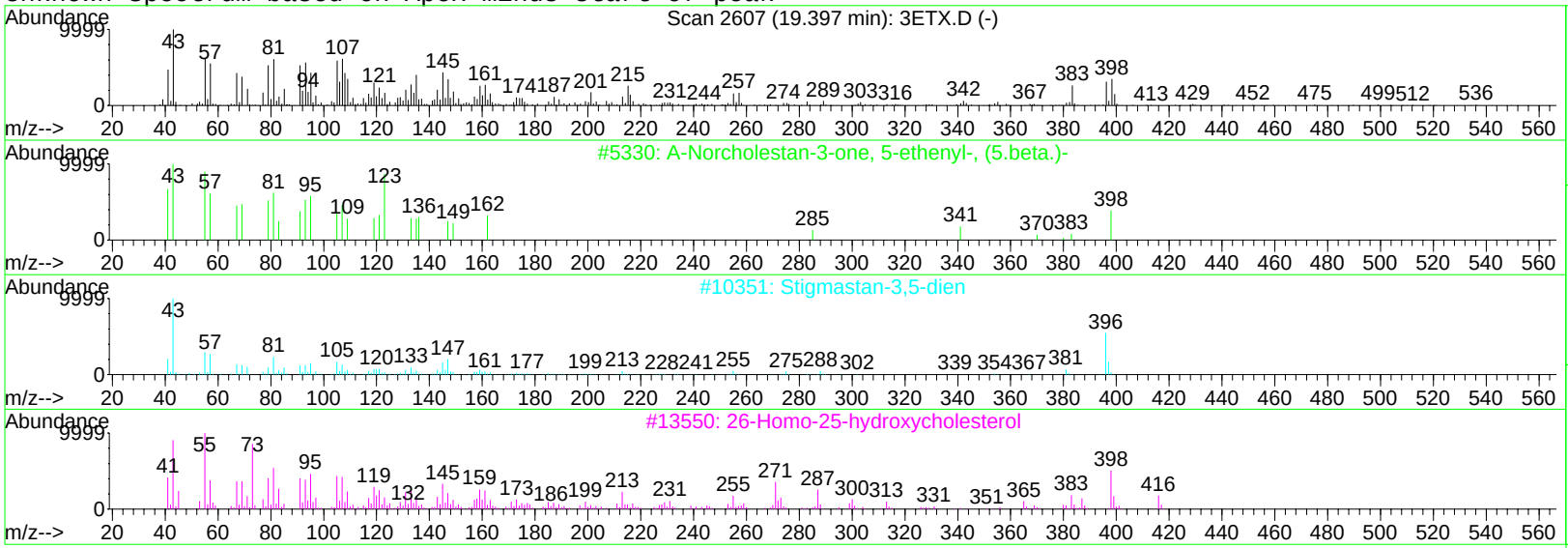


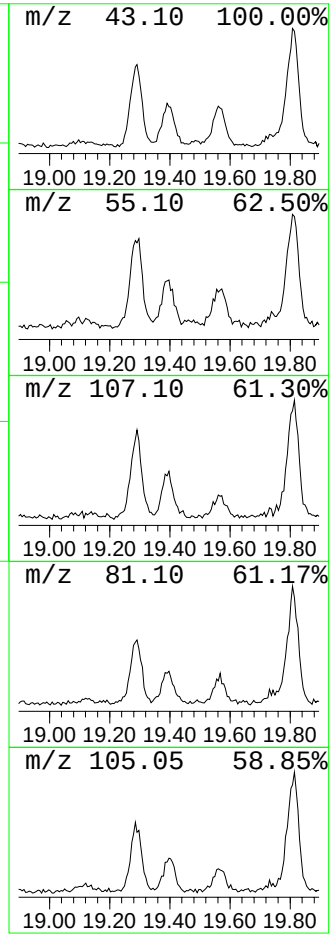


Peak Number: 37 at 19.29 min Area: 7065343 Area % 6.19

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Cholesta-8,24-dien-3.beta.-ol, 4...	105294	015737-15-2	56
2 Cyclohexane, 1-ethenyl-1-methyl-...	116195	000515-13-9	46
3 Cholest-4-en-3-one, 14-methyl-	13735	021857-92-1	45

Unknown Spectrum based on Apex minus start of peak

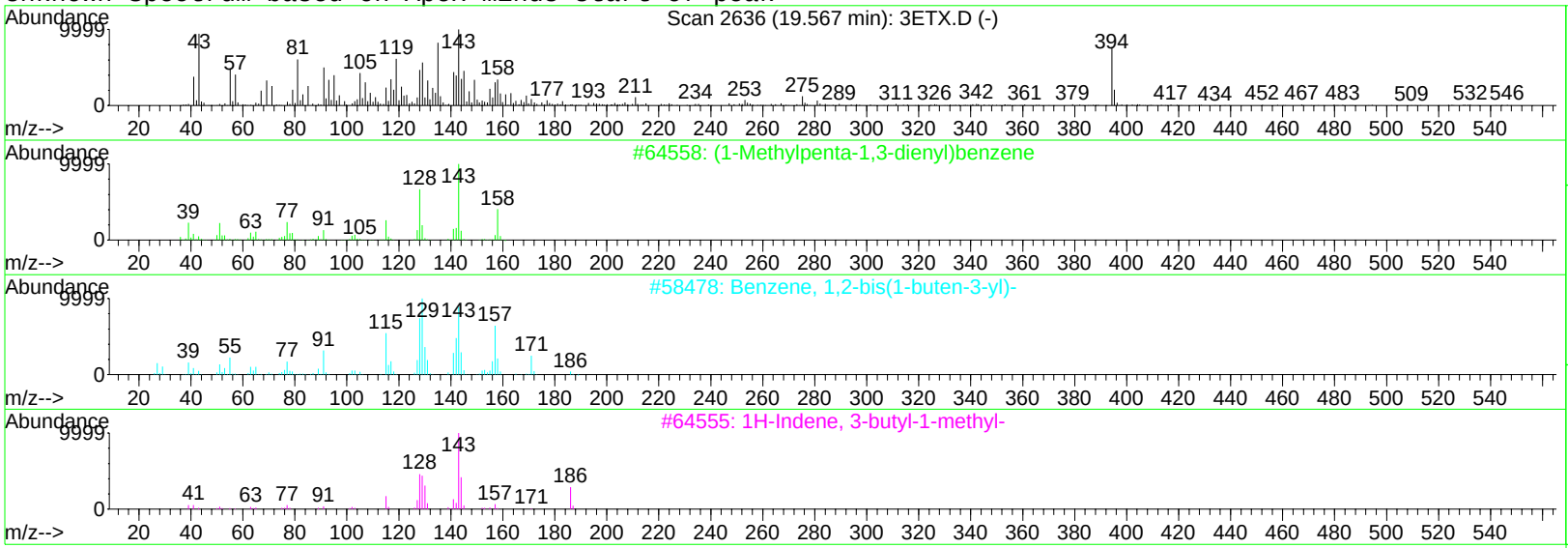


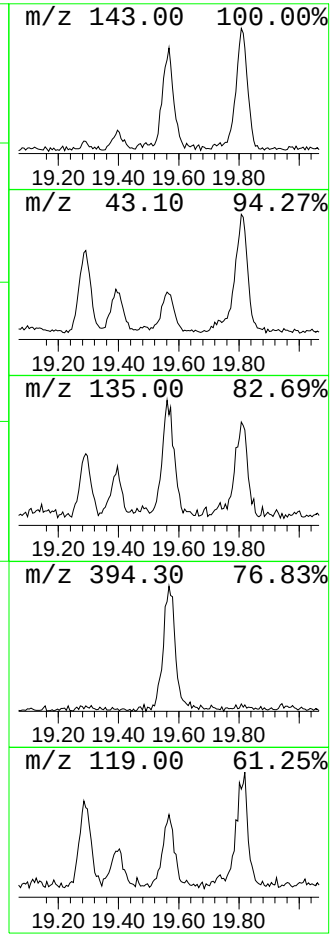


Peak Number: 38 at 19.40 min Area: 3580083 Area % 3.14

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 A-Norcholestan-3-one, 5-ethenyl-...	5330	019594-90-2	51
2 Stigmastan-3,5-dien	10351	1000214-16-4	41
3 26-Homo-25-hydroxycholesterol	13550	1000124-49-0	37

Unknown Spectrum based on Apex minus start of peak

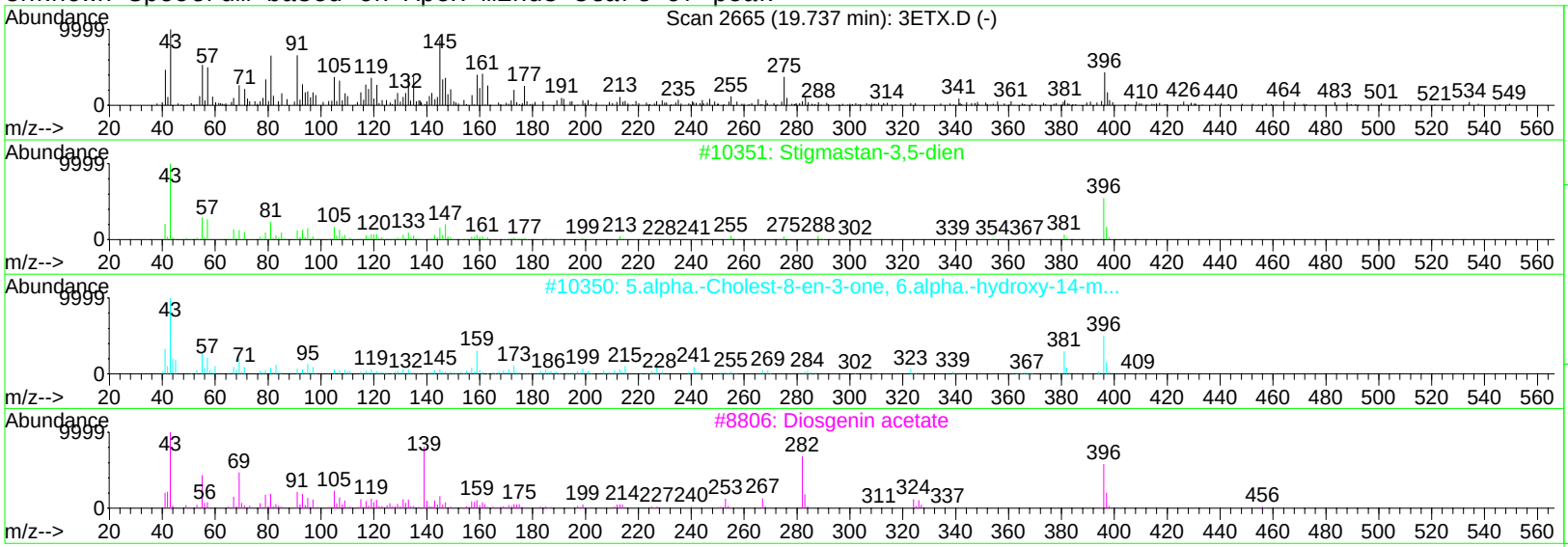


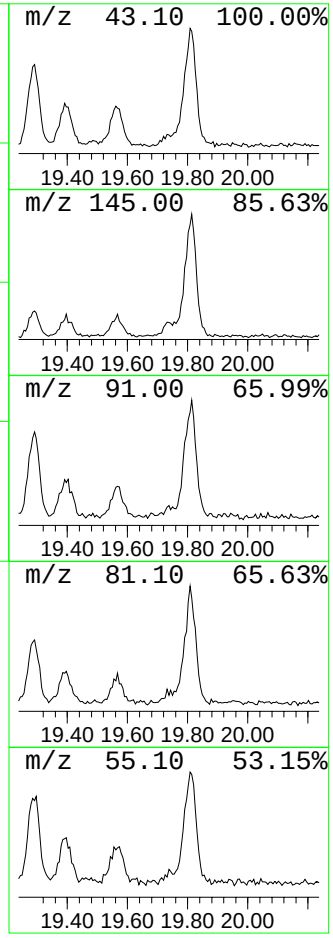


Peak Number: 39 at 19.57 min Area: 3600528 Area % 3.15

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 (1-Methylpenta-1,3-dienyl)benzene	64558	116669-49-9	18
2 Benzene, 1,2-bis(1-buten-3-yl)-	58478	1000162-73-9	14
3 1H-Indene, 3-butyl-1-methyl-	64555	111400-84-1	12

Unknown Spectrum based on Apex minus start of peak

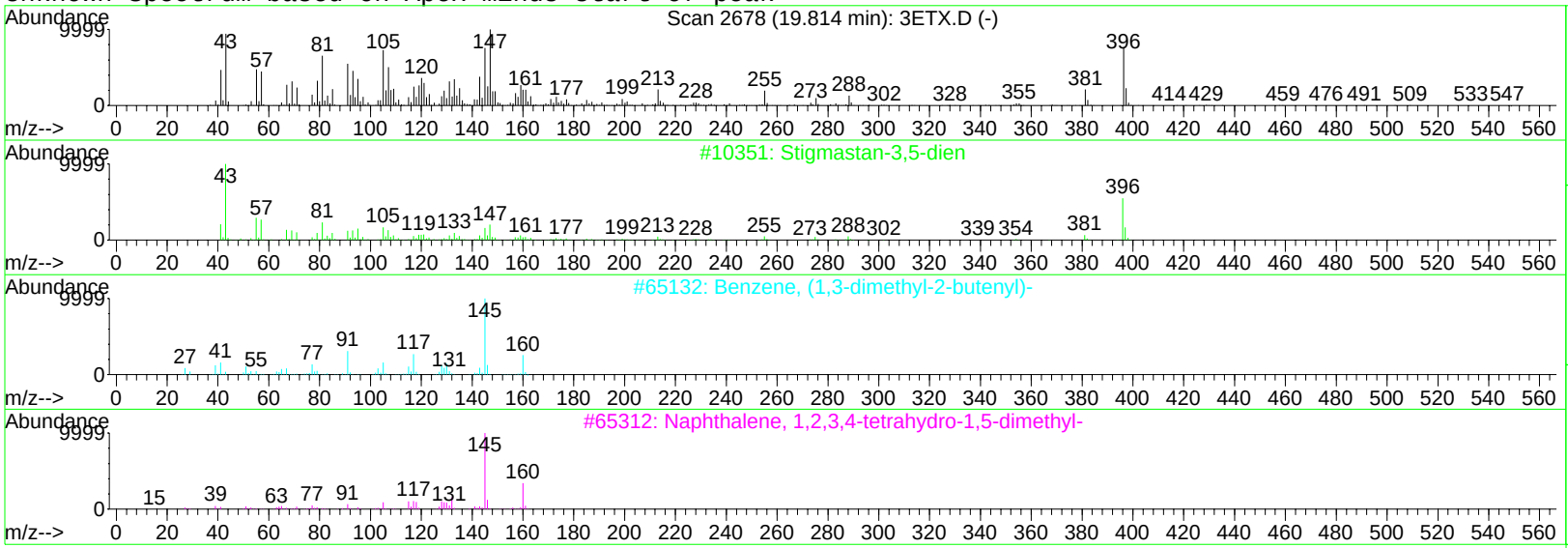


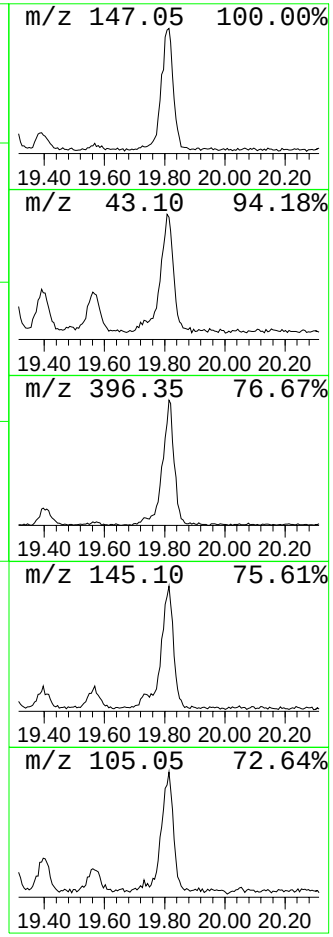


Peak Number: 40 at 19.74 min Area: 644476 Area % 0.56

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Stigmastan-3,5-dien	10351	1000214-16-4	10
2 5.alpha.-Cholest-8-en-3-one, 6.a...	10350	005259-17-6	10
3 Diosgenin acetate	8806	1000127-06-9	10

Unknown Spectrum based on Apex minus start of peak

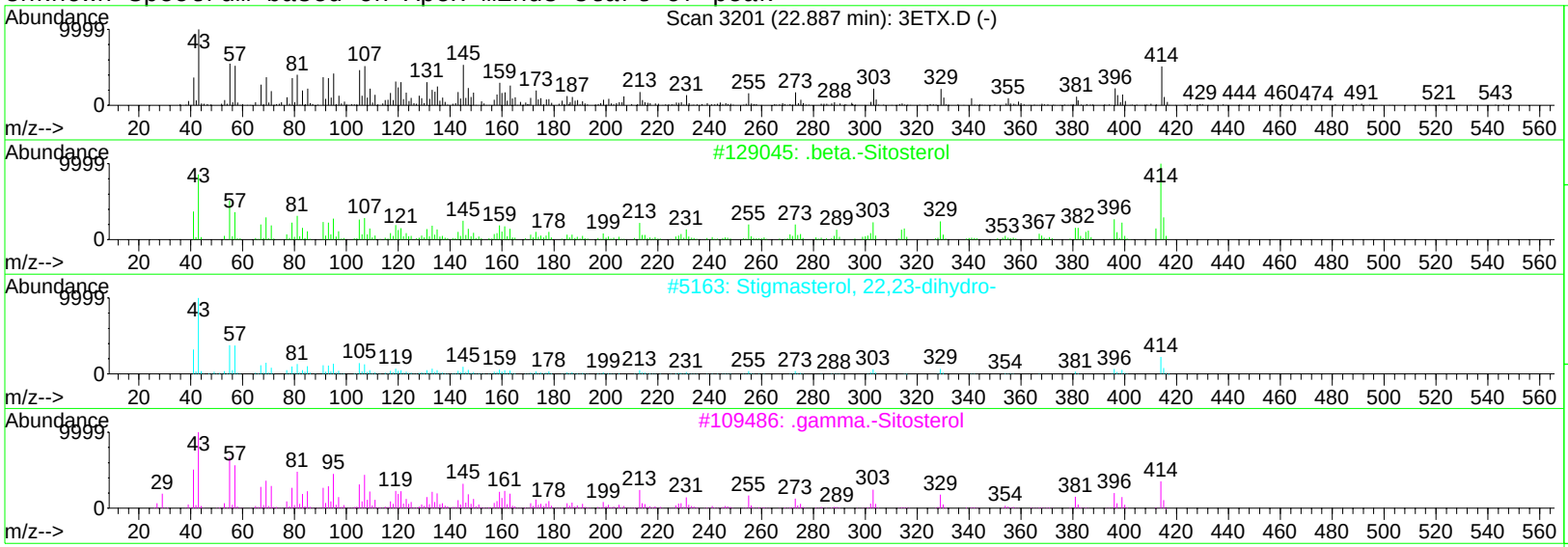


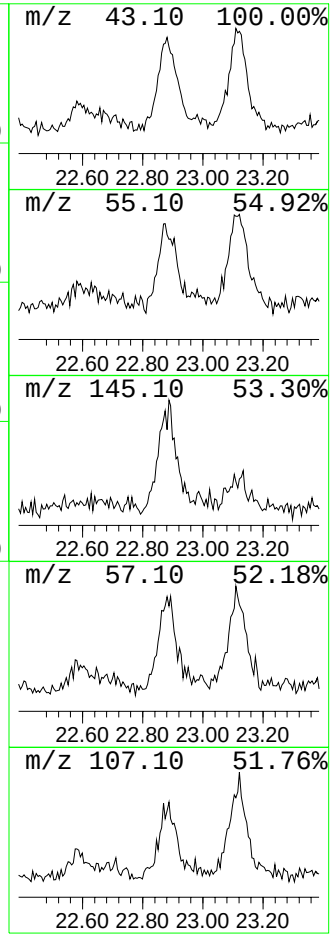


Peak Number: 41 at 19.81 min Area: 10686845 Area % 9.36

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Stigmastan-3,5-dien	10351	1000214-16-4	93
2 Benzene, (1,3-dimethyl-2-butenyl)-	65132	050704-01-3	15
3 Naphthalene, 1,2,3,4-tetrahydro-...	65312	021564-91-0	11

Unknown Spectrum based on Apex minus start of peak

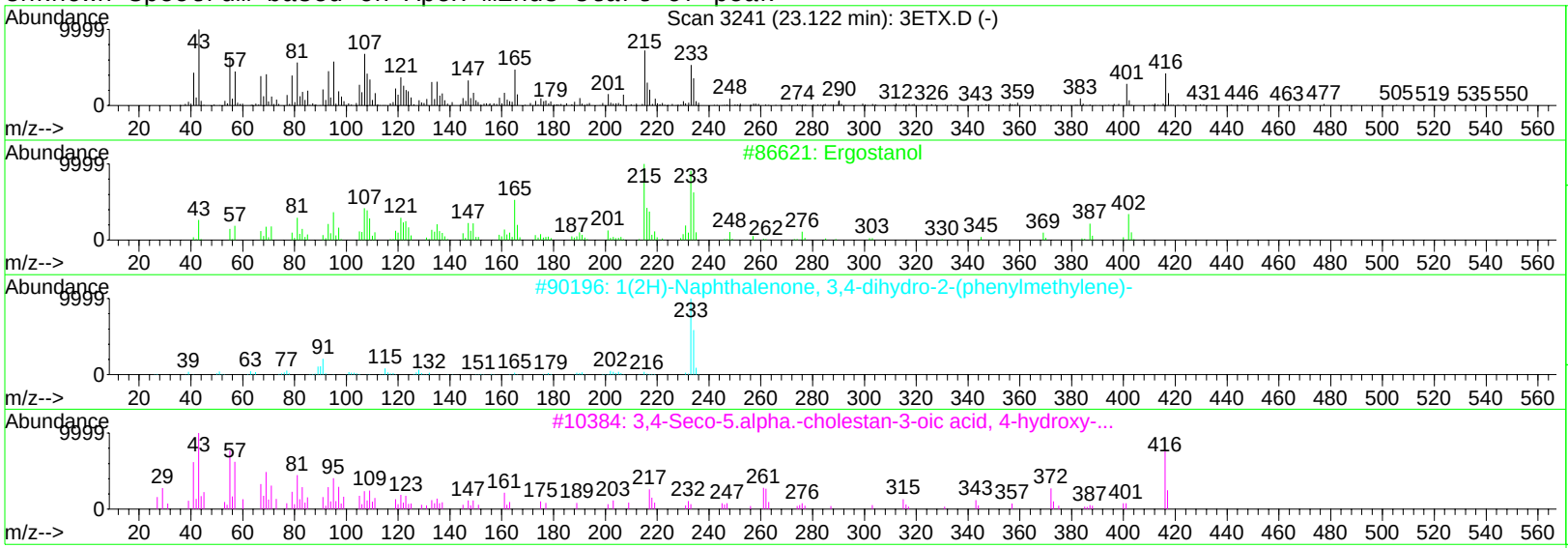


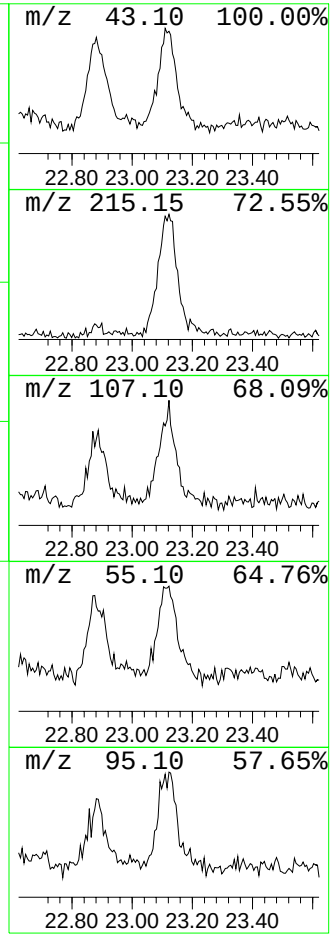


Peak Number: 42 at 22.89 min Area: 4152960 Area % 3.64

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 .beta.-Sitosterol	129045	000083-46-5	89
2 Stigmasterol, 22,23-dihydro-	5163	1000214-20-7	86
3 .gamma.-Sitosterol	109486	000083-47-6	64

Unknown Spectrum based on Apex minus start of peak

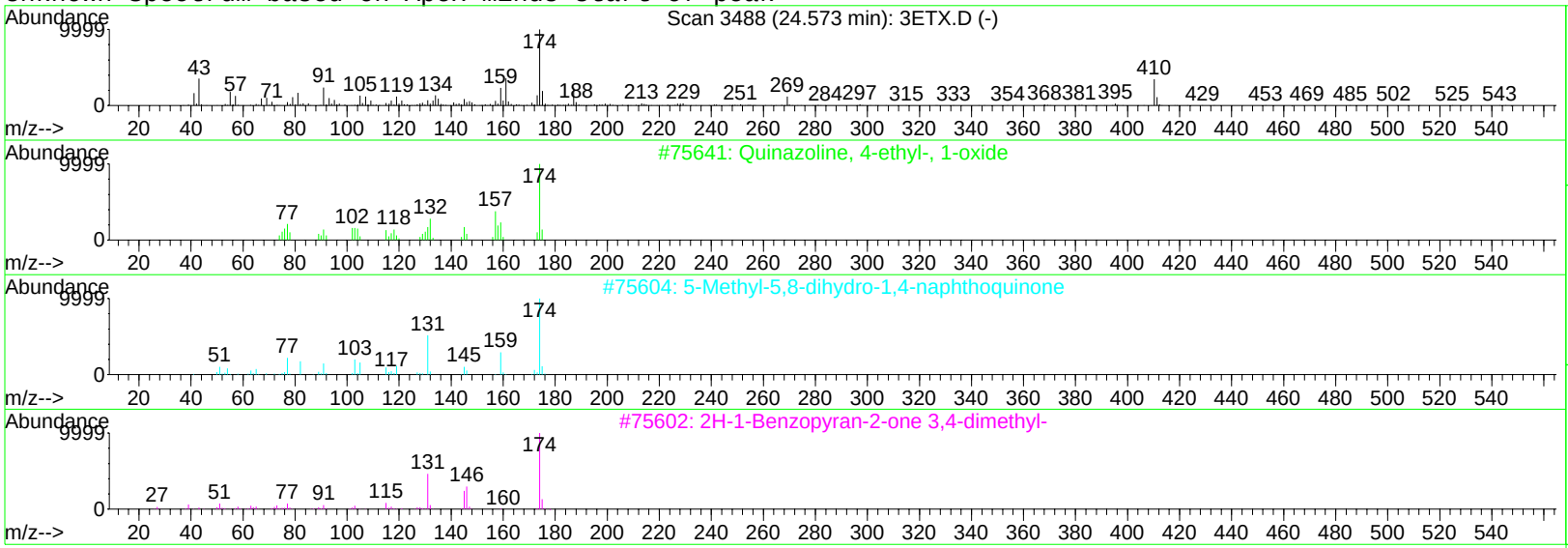


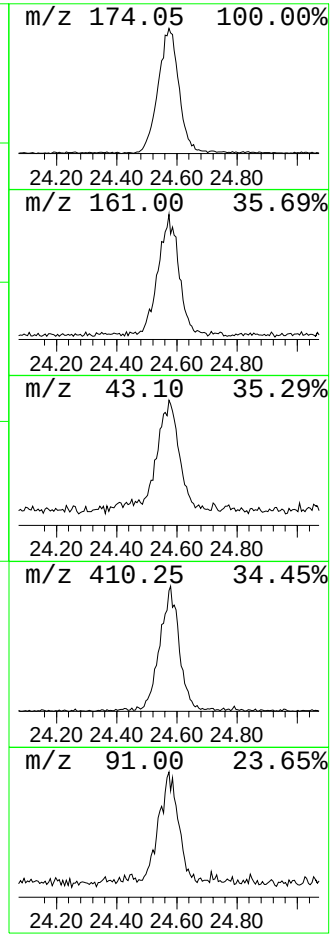


Peak Number: 43 at 23.12 min Area: 4203149 Area % 3.68

The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Ergostanol	86621	006538-02-9	62
2 1(2H)-Naphthalenone, 3,4-dihydro...	90196	006261-32-1	15
3 3,4-Seco-5.alpha.-cholestan-3-oi...	10384	005061-93-8	11

Unknown Spectrum based on Apex minus start of peak





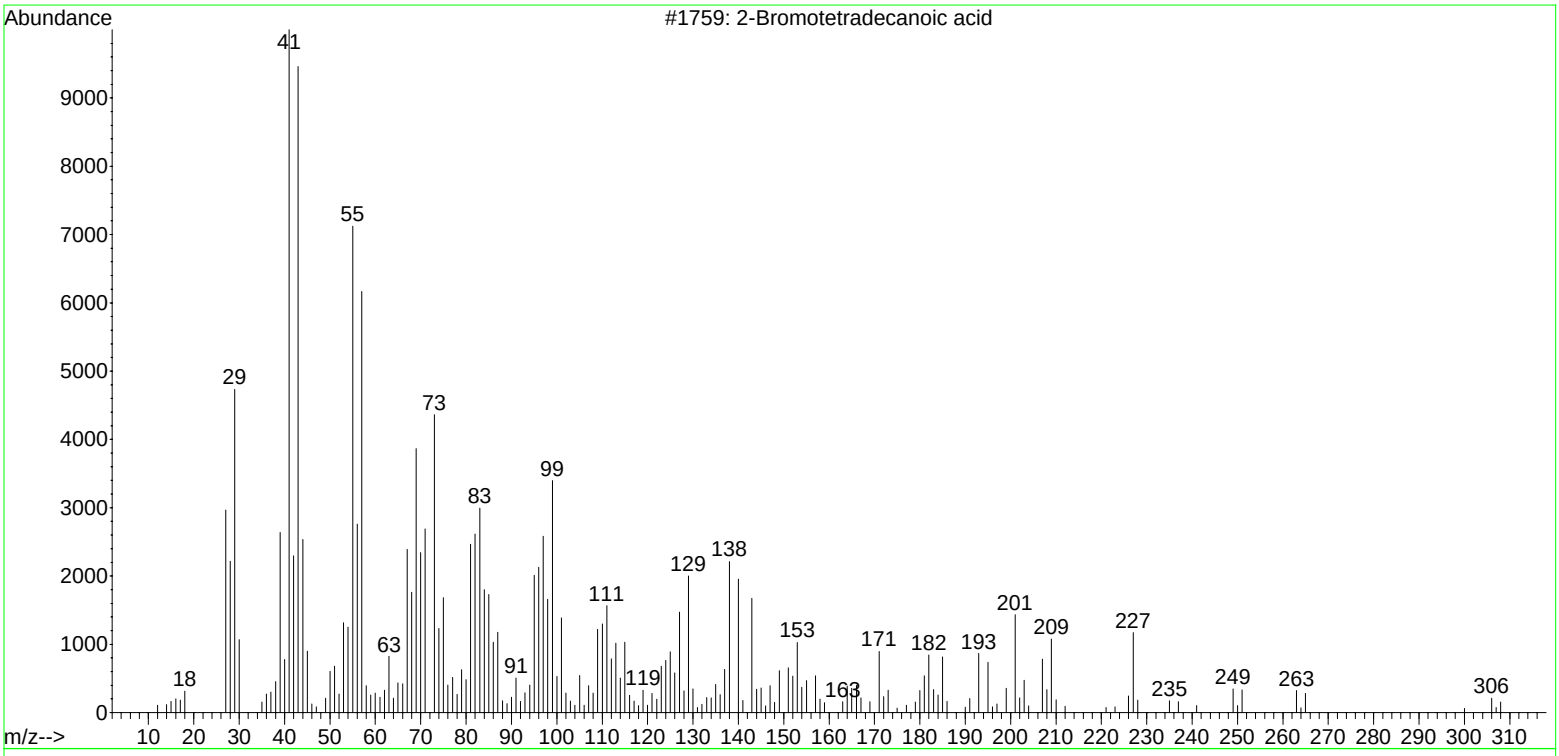
Peak Number: 44 at 24.57 min Area: 8161719 Area % 7.15

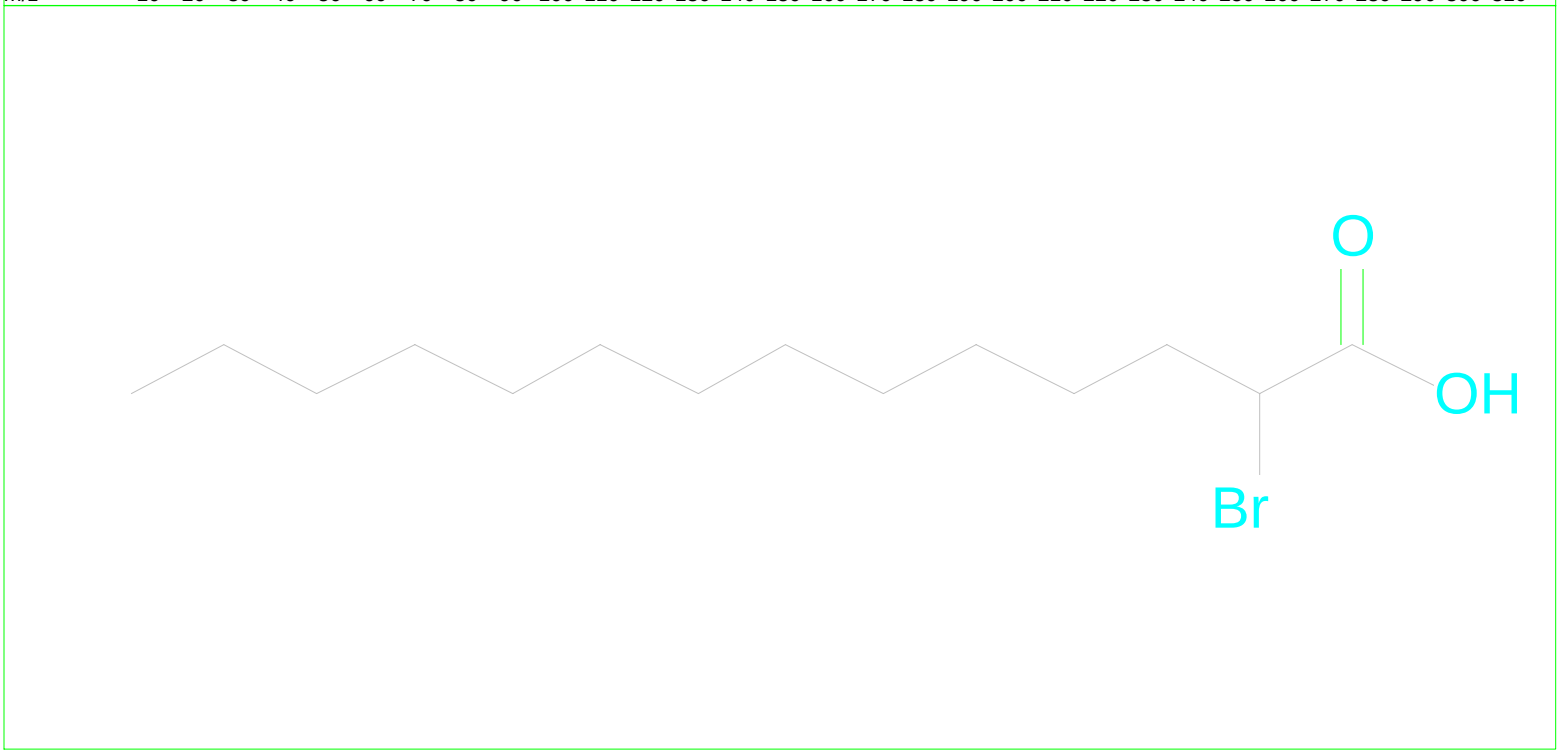
The 3 best hits from each library.	Ref#	CAS#	Qual
C:\Database\Nist98.L			
1 Quinazoline, 4-ethyl-, 1-oxide	75641	037920-75-5	46
2 5-Methyl-5,8-dihydro-1,4-naphtho...	75604	086759-40-2	46
3 2H-1-Benzopyran-2-one 3,4-dimethyl-	75602	004281-39-4	38

2-Bromotetradecanoic acid

Entry Number	1759	from	C:\Database\Nist98.L
CAS	010520-81-7		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C14H27BrO2		
Mol Weight	306.119		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 125223, Seq# M1759

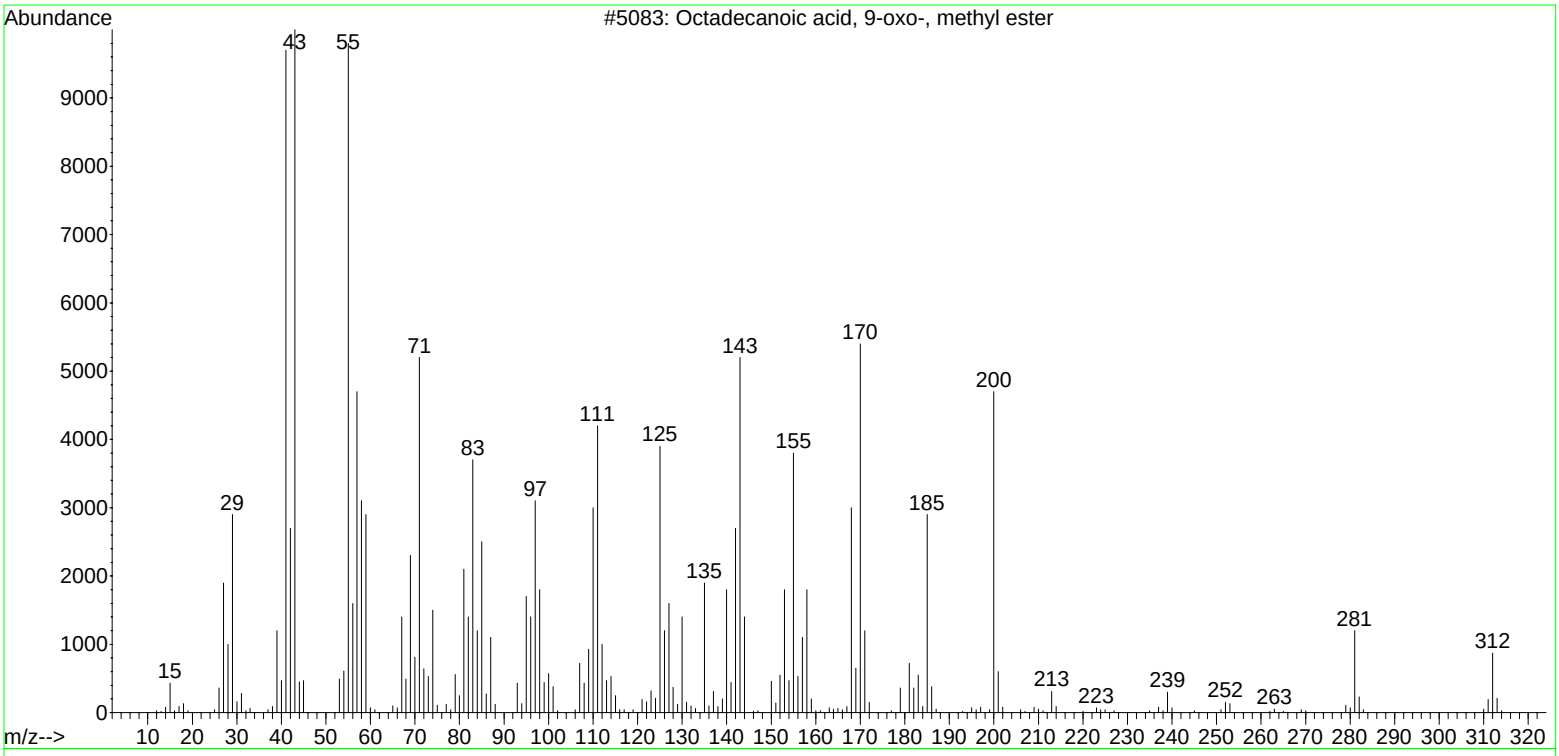


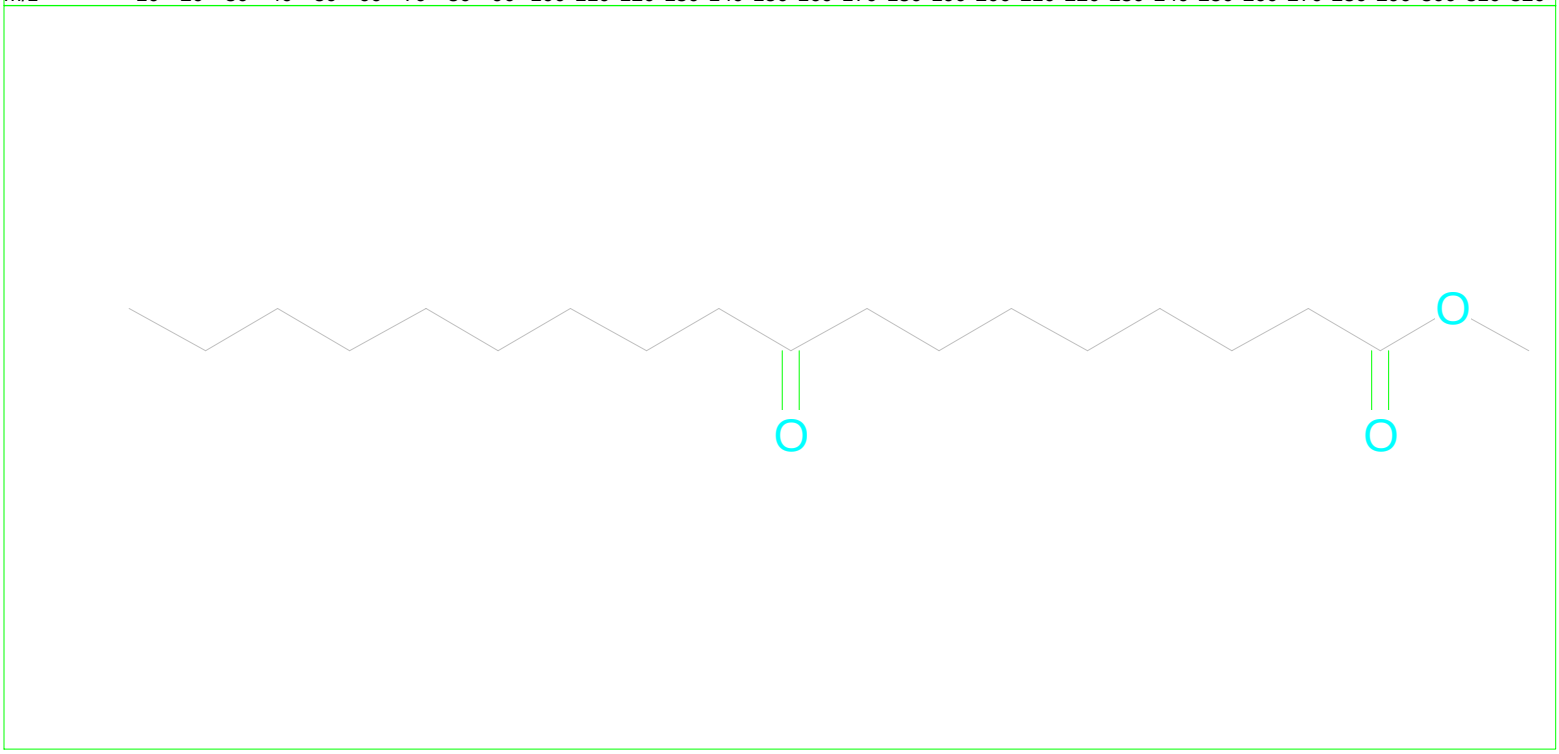


Octadecanoic acid, 9-oxo-, methyl ester

Entry Number	5083	from	C:\Database\Nist98.L
CAS	001842-70-2		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C19H36O3		
Mol Weight	312.266		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 14761, Seq# M5083

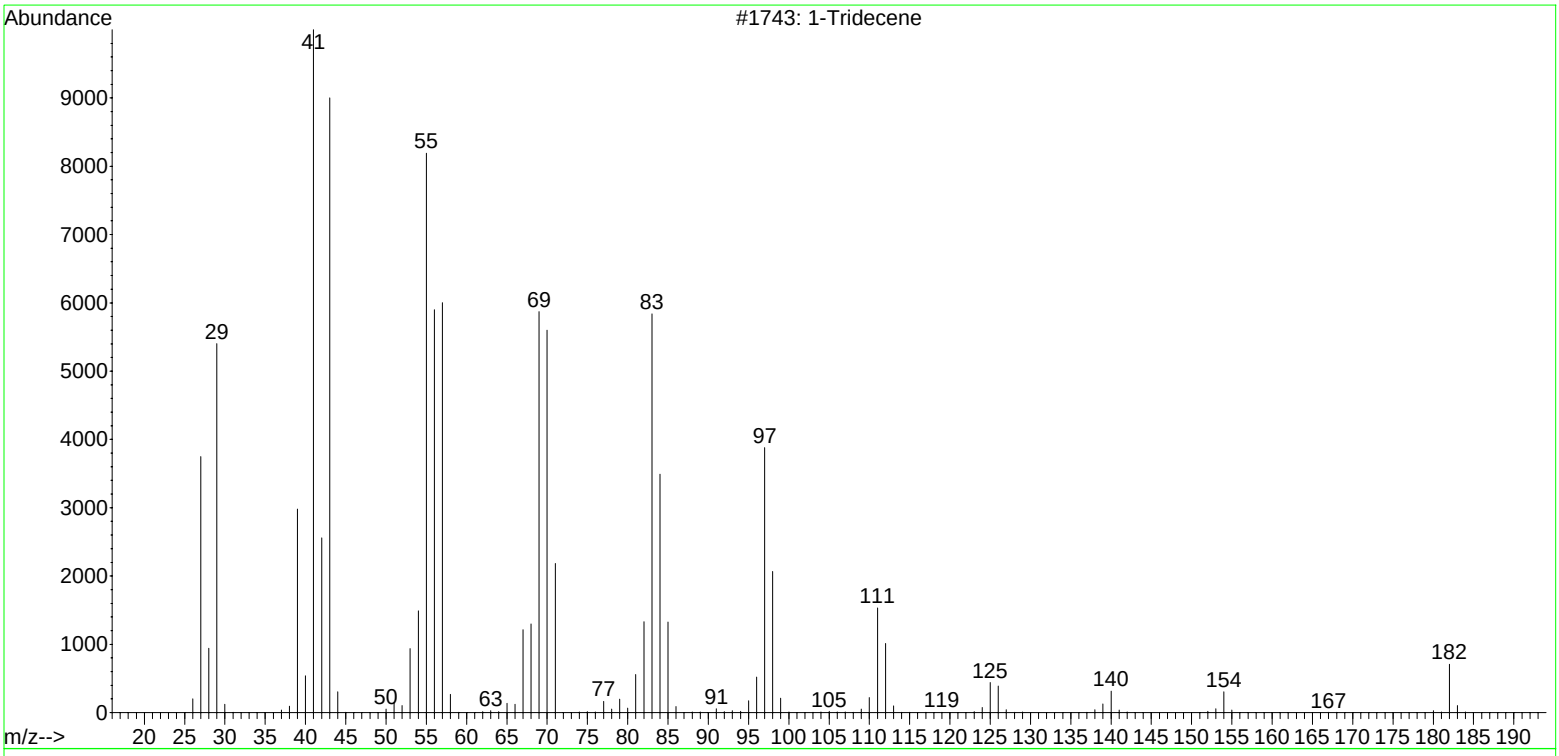




1-Tridecene

Entry Number	1743	from	C:\Database\Nist98.L
CAS	002437-56-1		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C13H26		
Mol Weight	182.203		
Company ID	NIST 1998		

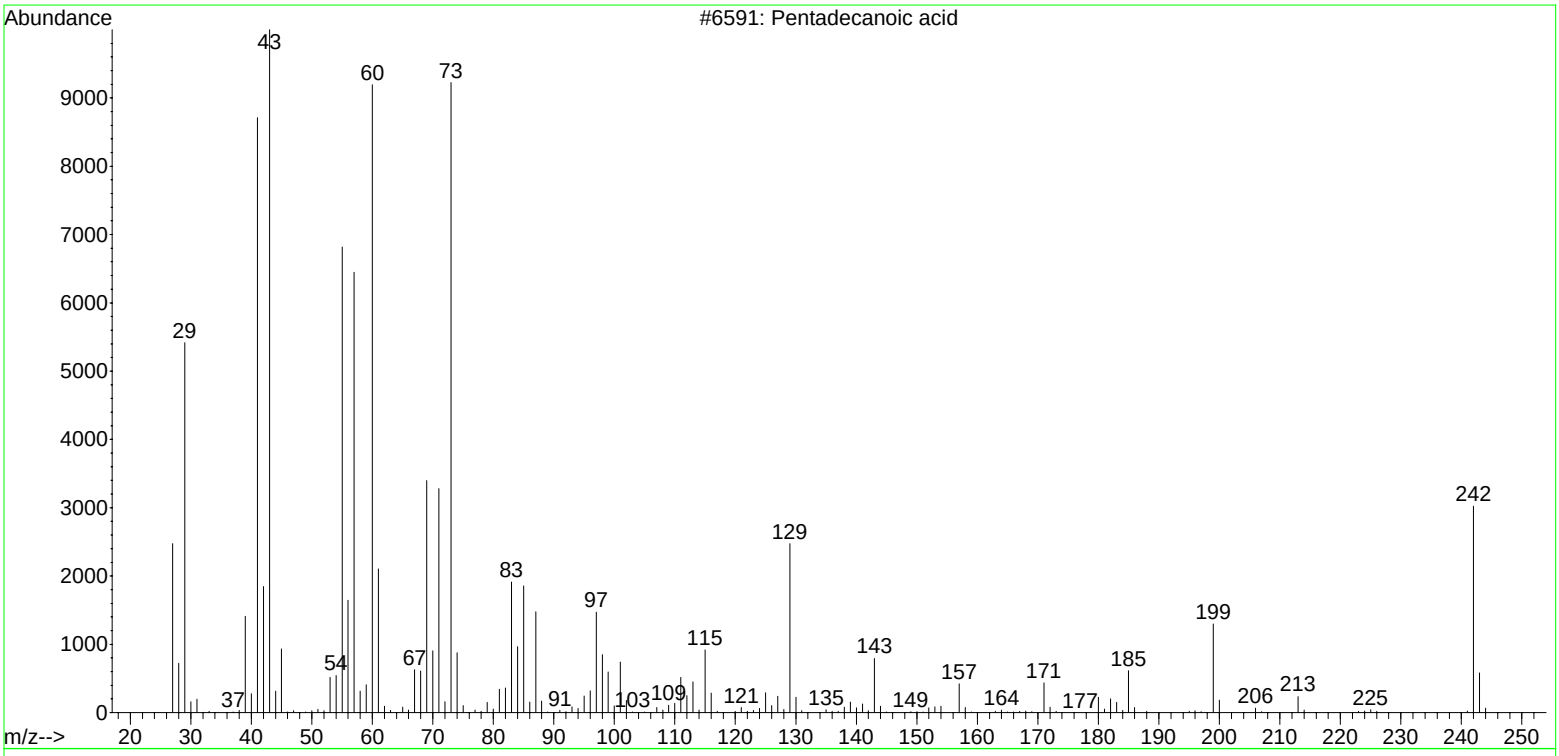
Miscellaneous Information
NIST MS# 34733, Seq# M1743

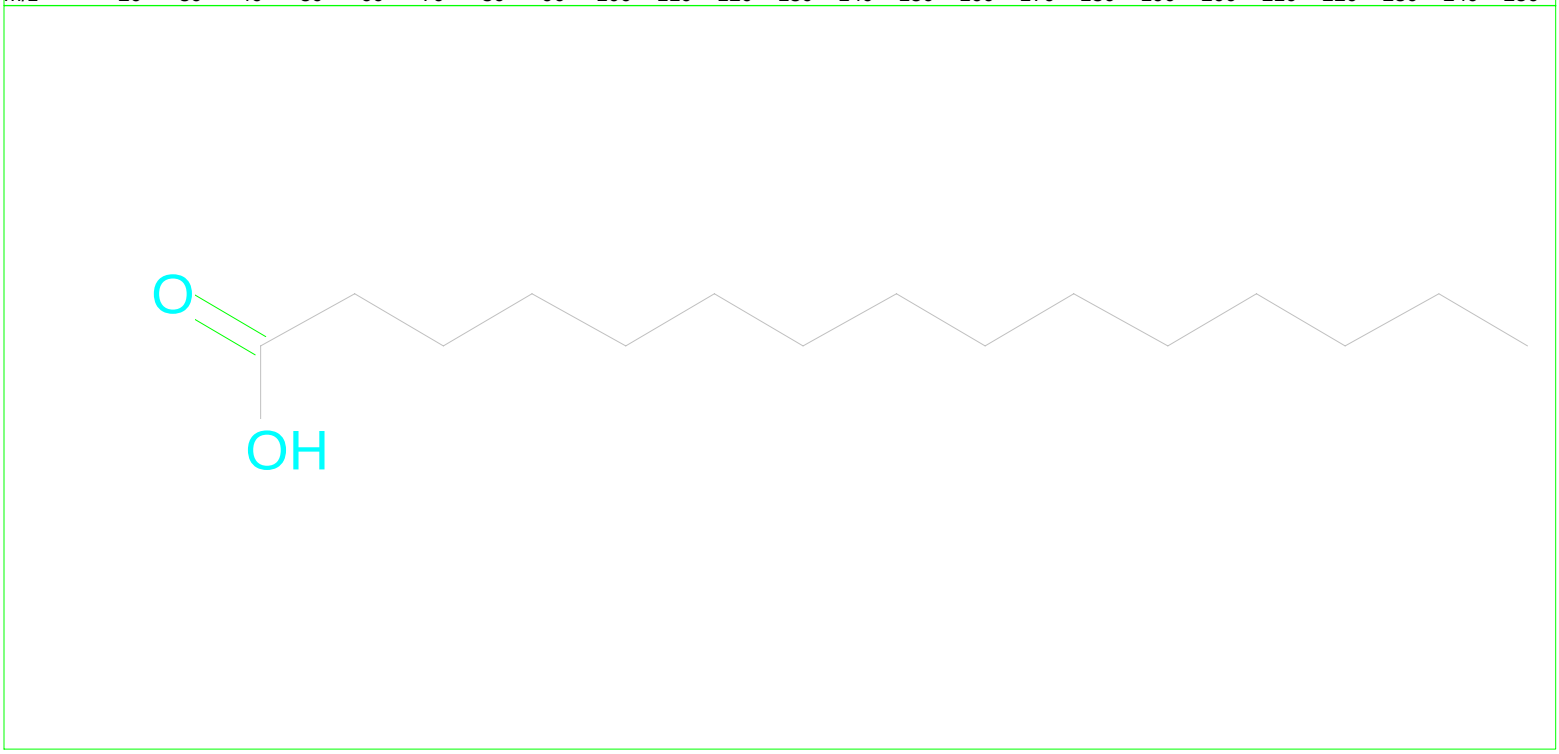


Pentadecanoic acid

Entry Number	6591	from	C:\Database\Nist98.L
CAS	001002-84-2		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C15H30O2		
Mol Weight	242.225		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 63741, Seq# M6591

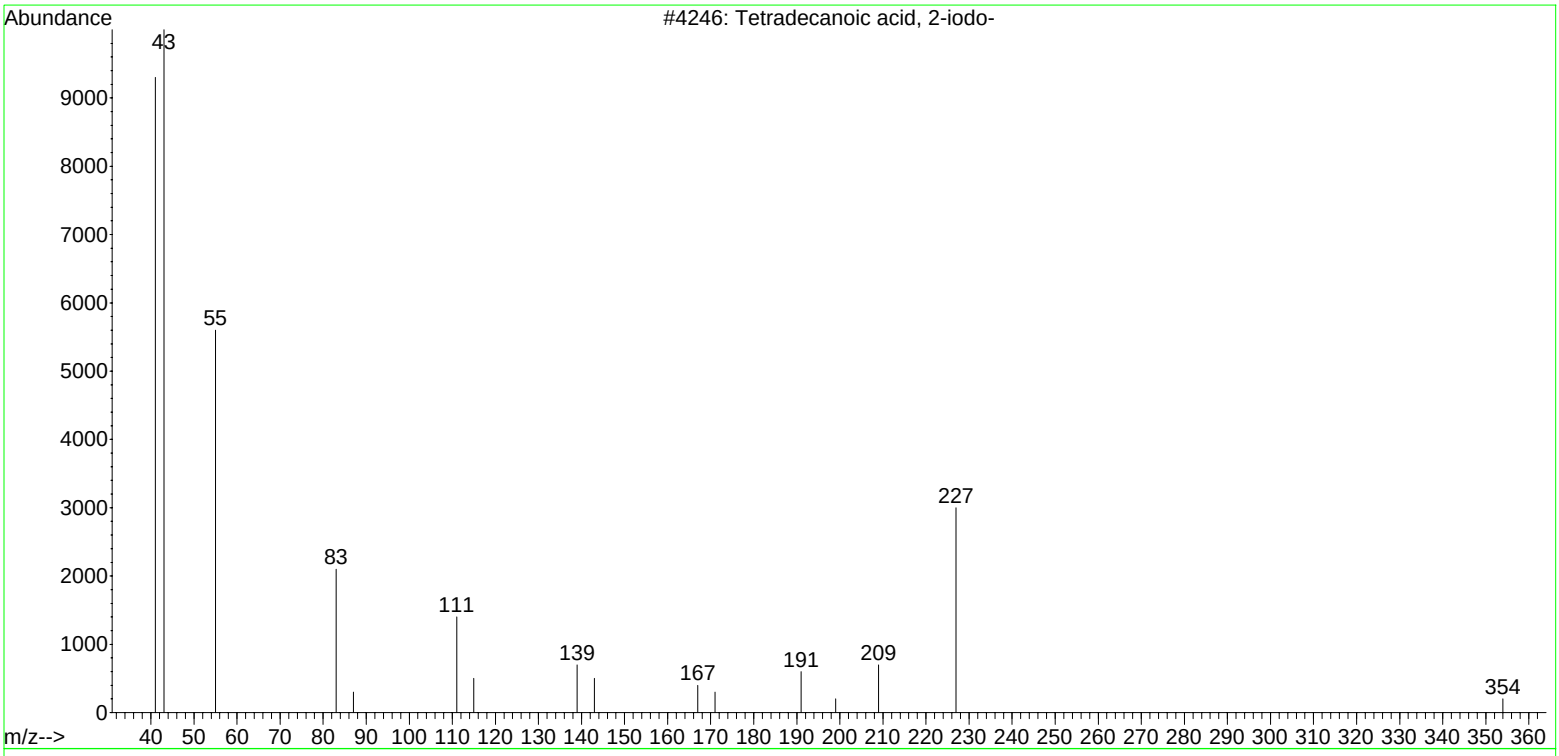


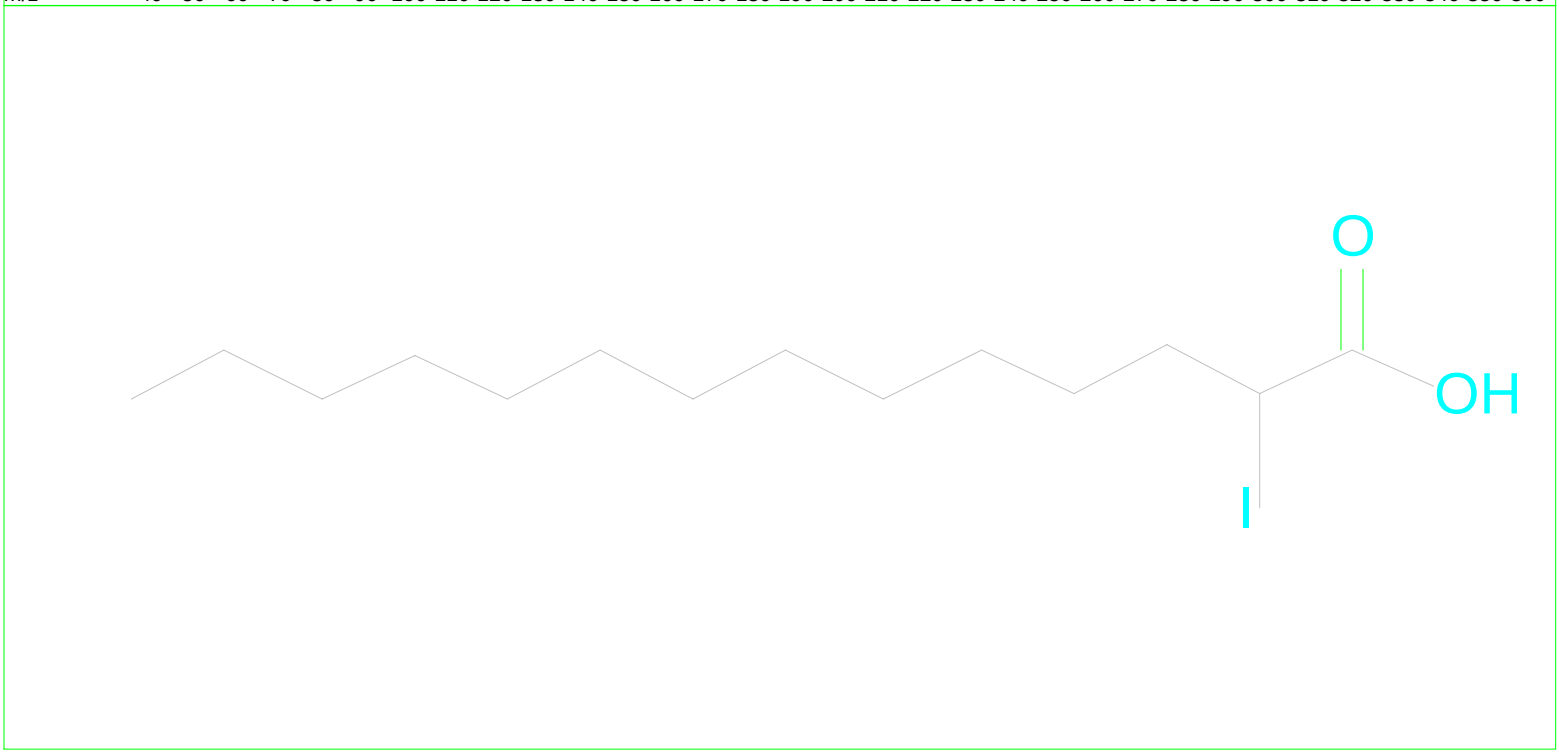


Tetradecanoic acid, 2-iodo-

Entry Number	4246	from	C:\Database\Nist98.L
CAS	109105-98-8		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C14H27IO2		
Mol Weight	354.106		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 115247, Seq# M4246

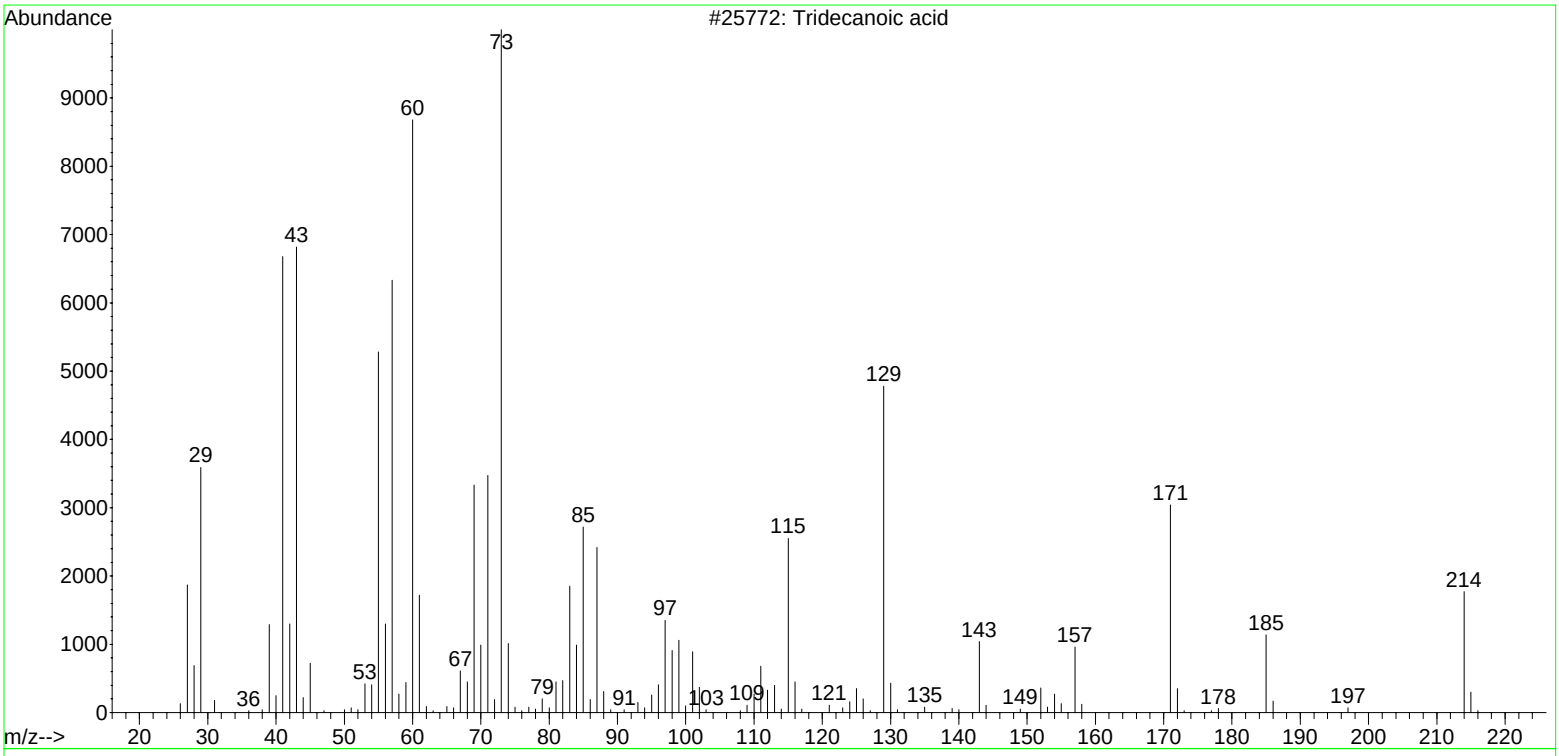


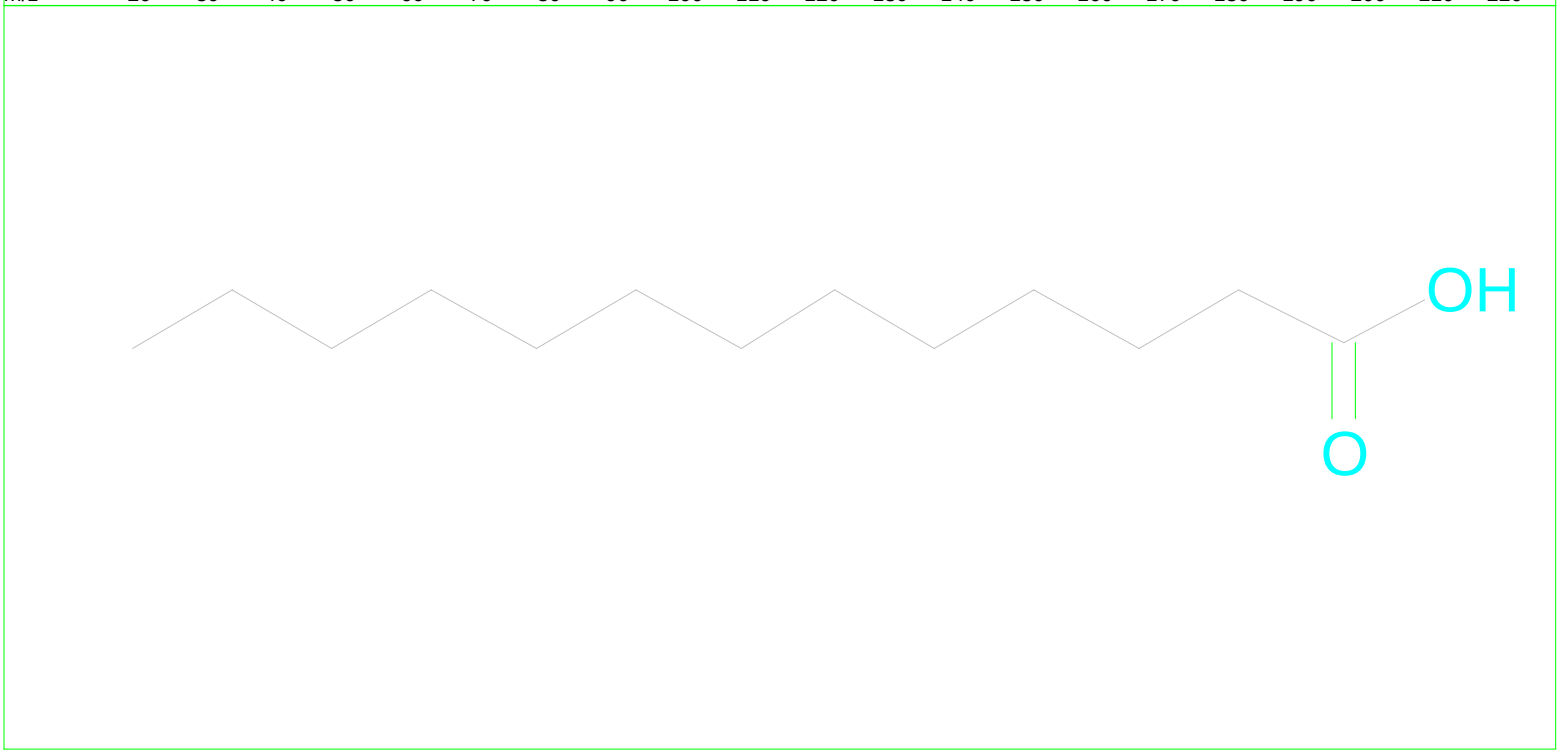


Tridecanoic acid

Entry Number	25772	from	C:\Database\Nist98.L
CAS	000638-53-9		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C13H26O2		
Mol Weight	214.193		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 221175, Seq# M25772

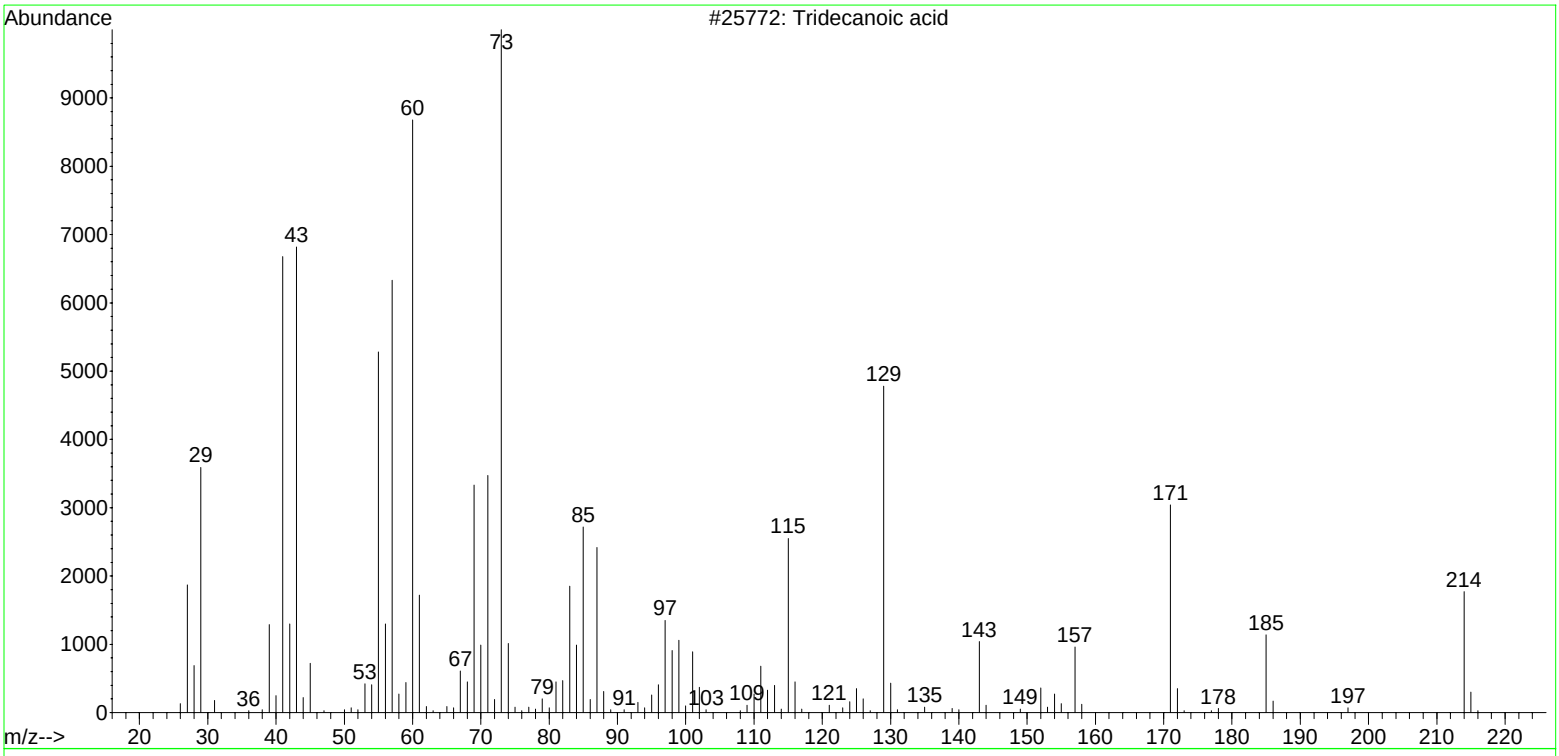


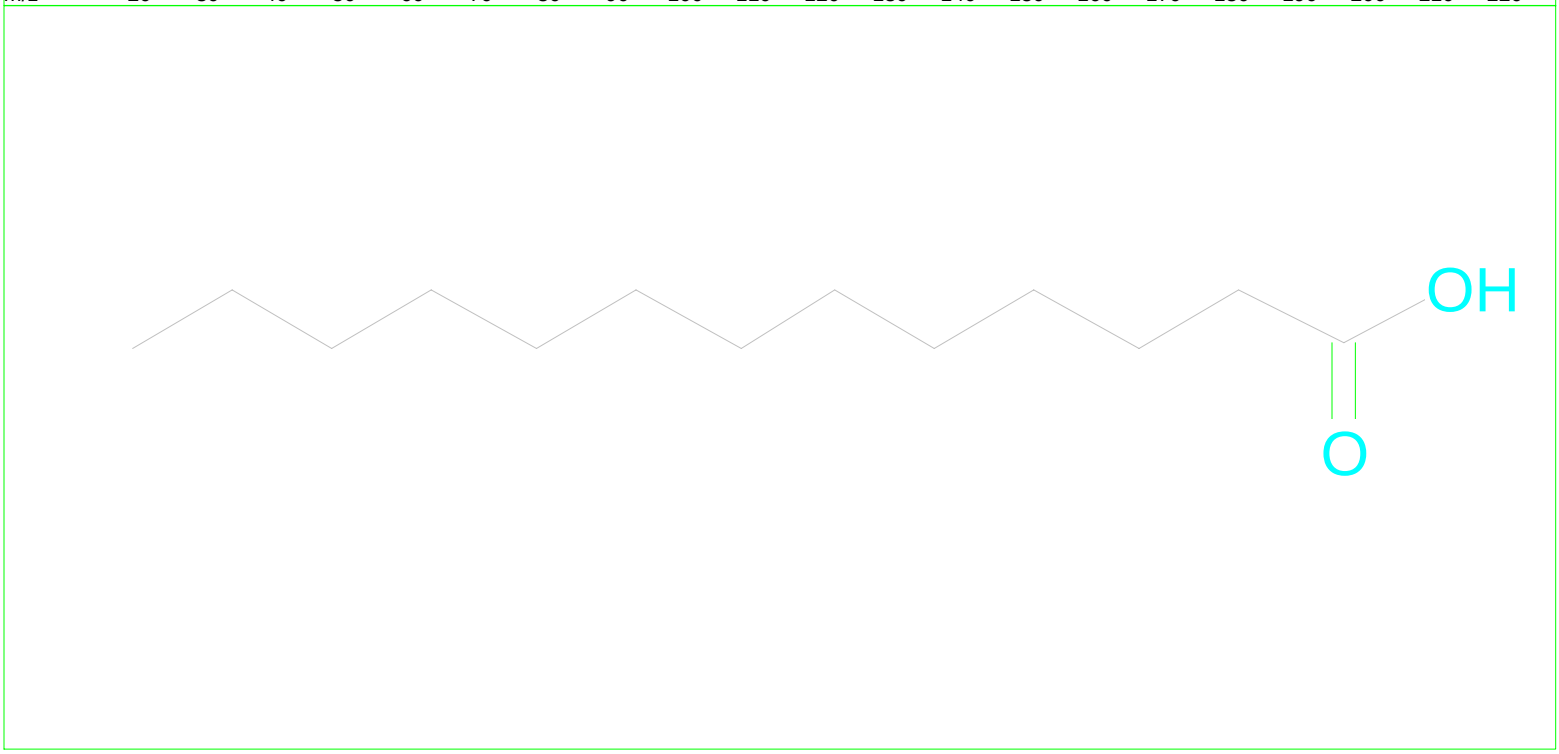


Tridecanoic acid

Entry Number	25772	from	C:\Database\Nist98.L
CAS	000638-53-9		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C13H26O2		
Mol Weight	214.193		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 221175, Seq# M25772

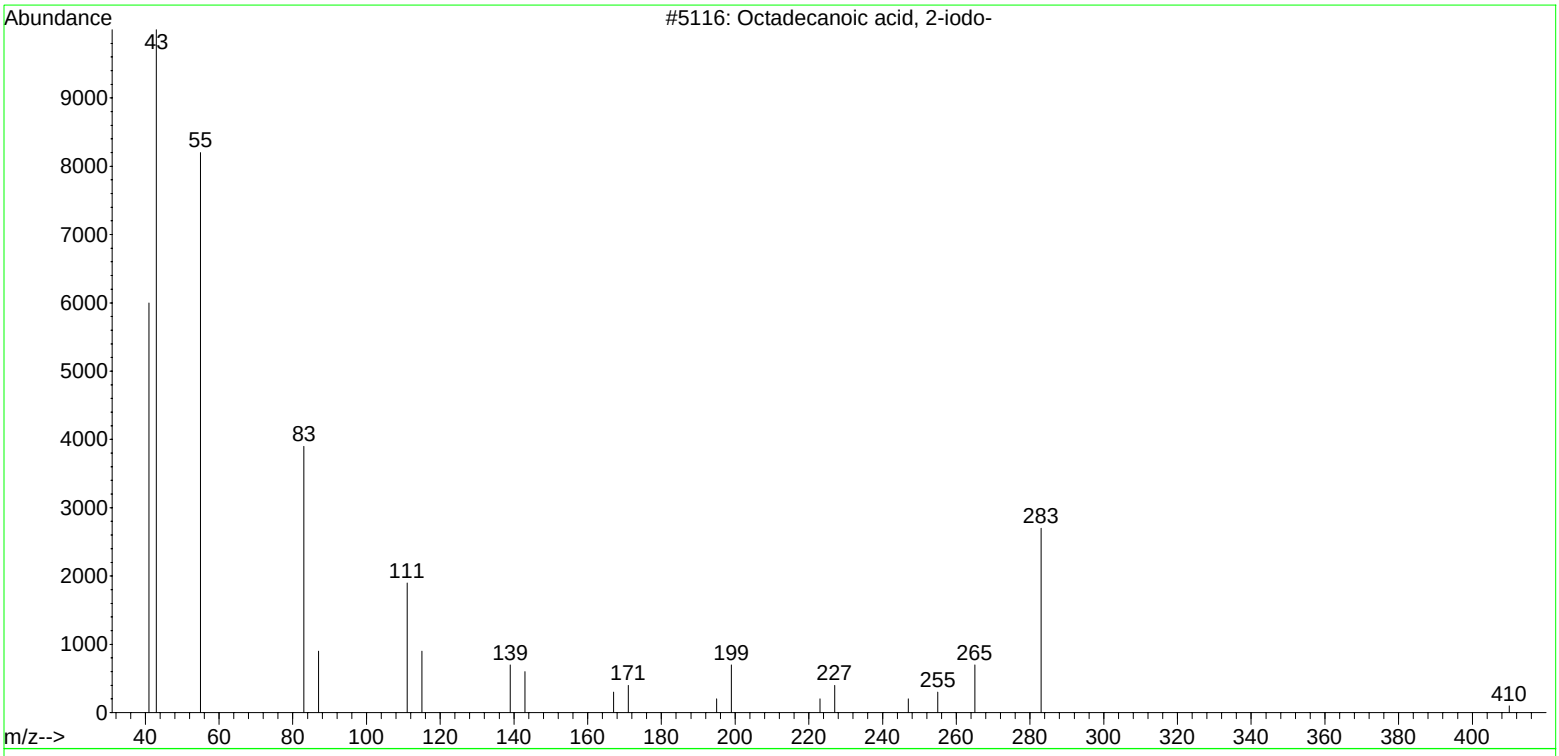


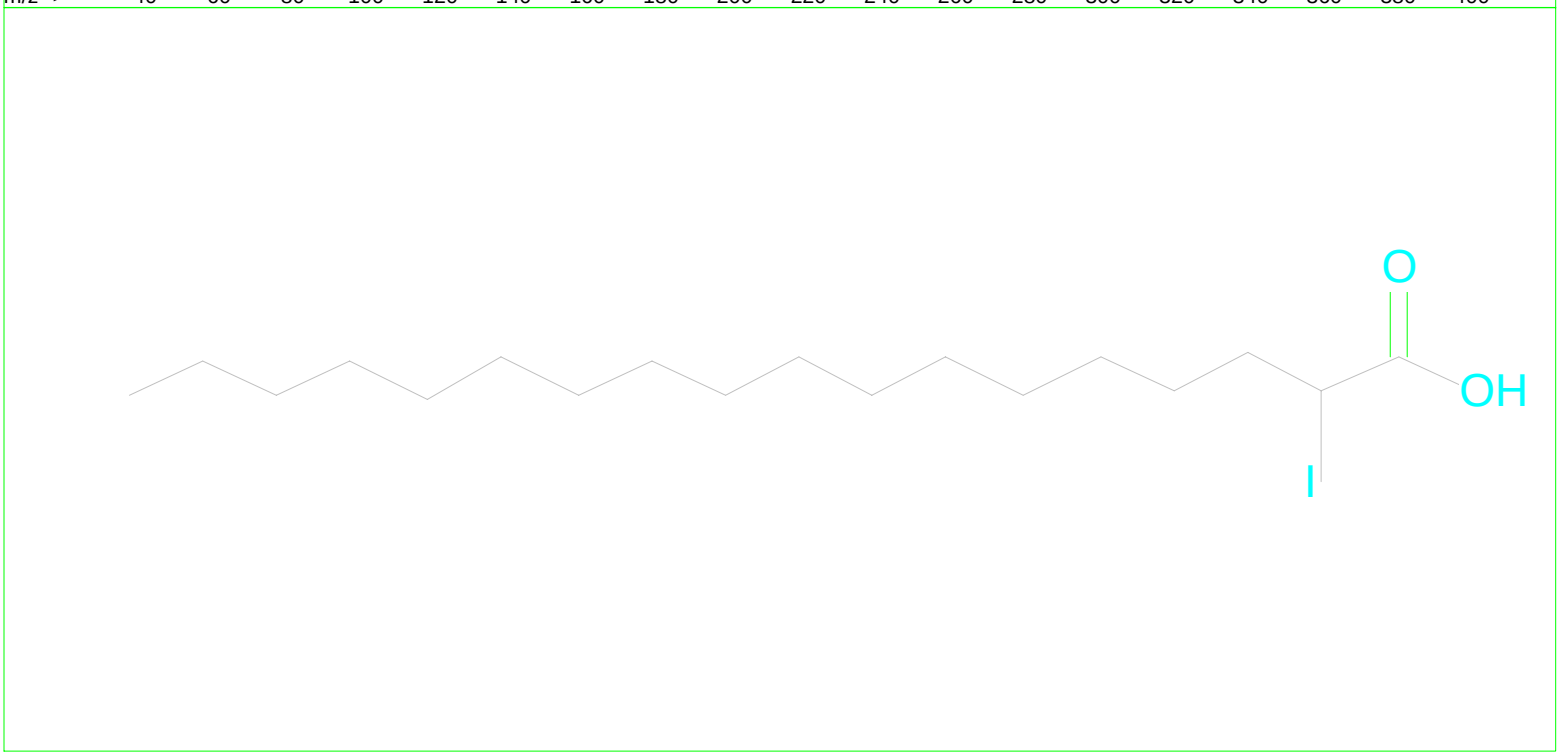


Octadecanoic acid, 2-iodo-

Entry Number	5116	from	C:\Database\Nist98.L
CAS	065164-70-7		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C18H35IO2		
Mol Weight	410.168		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 115245, Seq# M5116

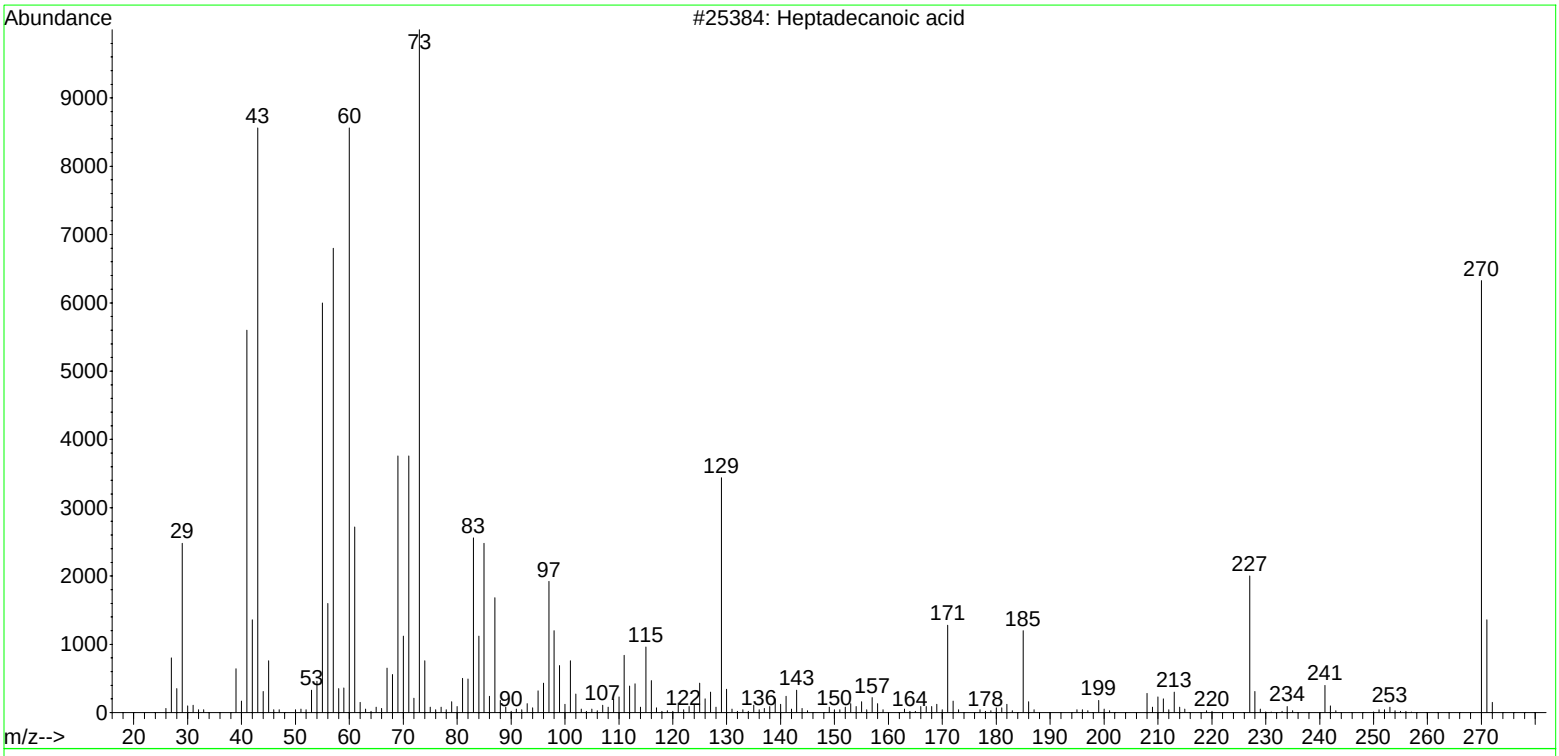


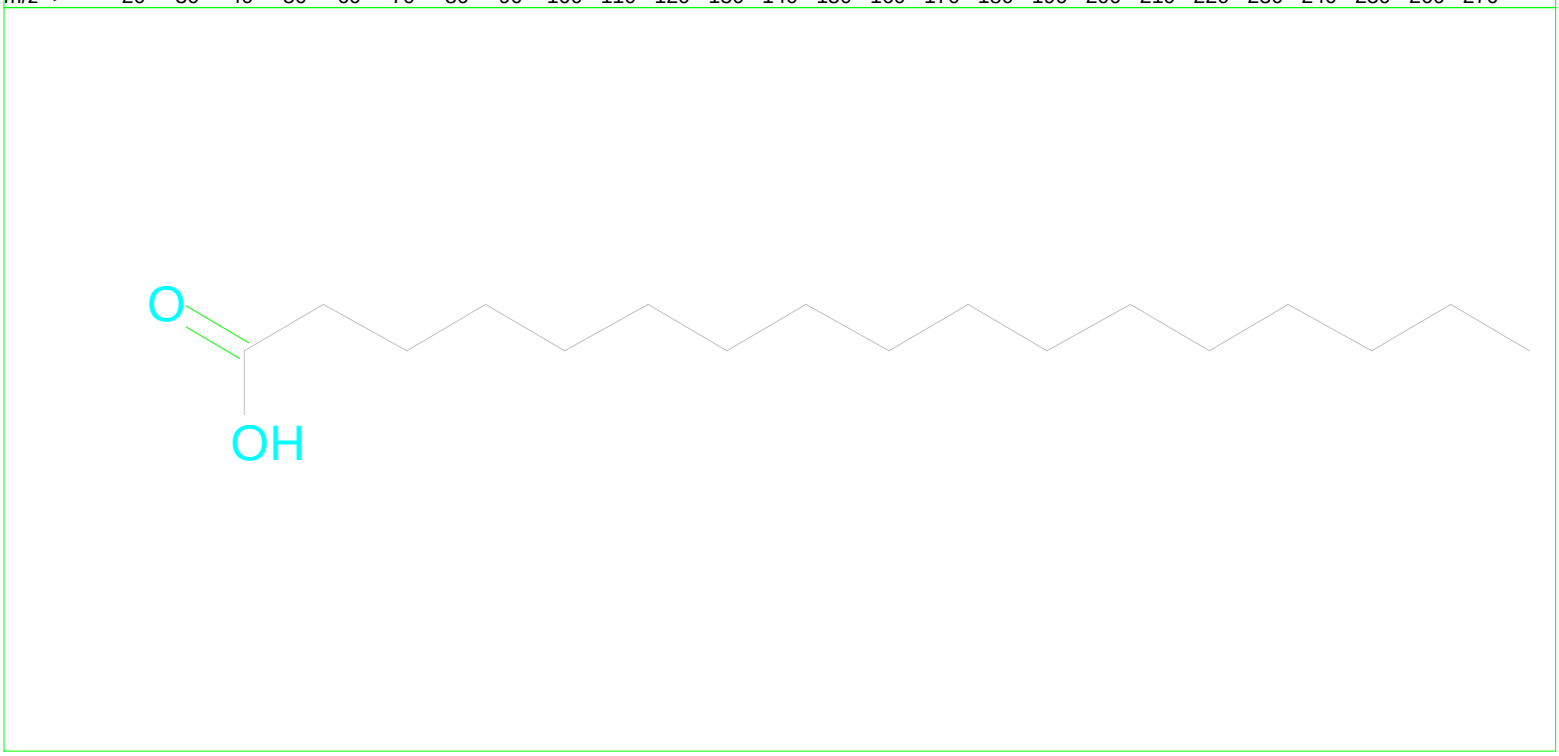


Heptadecanoic acid

Entry Number	25384	from	C:\Database\Nist98.L
CAS	000506-12-7		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C17H34O2		
Mol Weight	270.256		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 36447, Seq# M25384

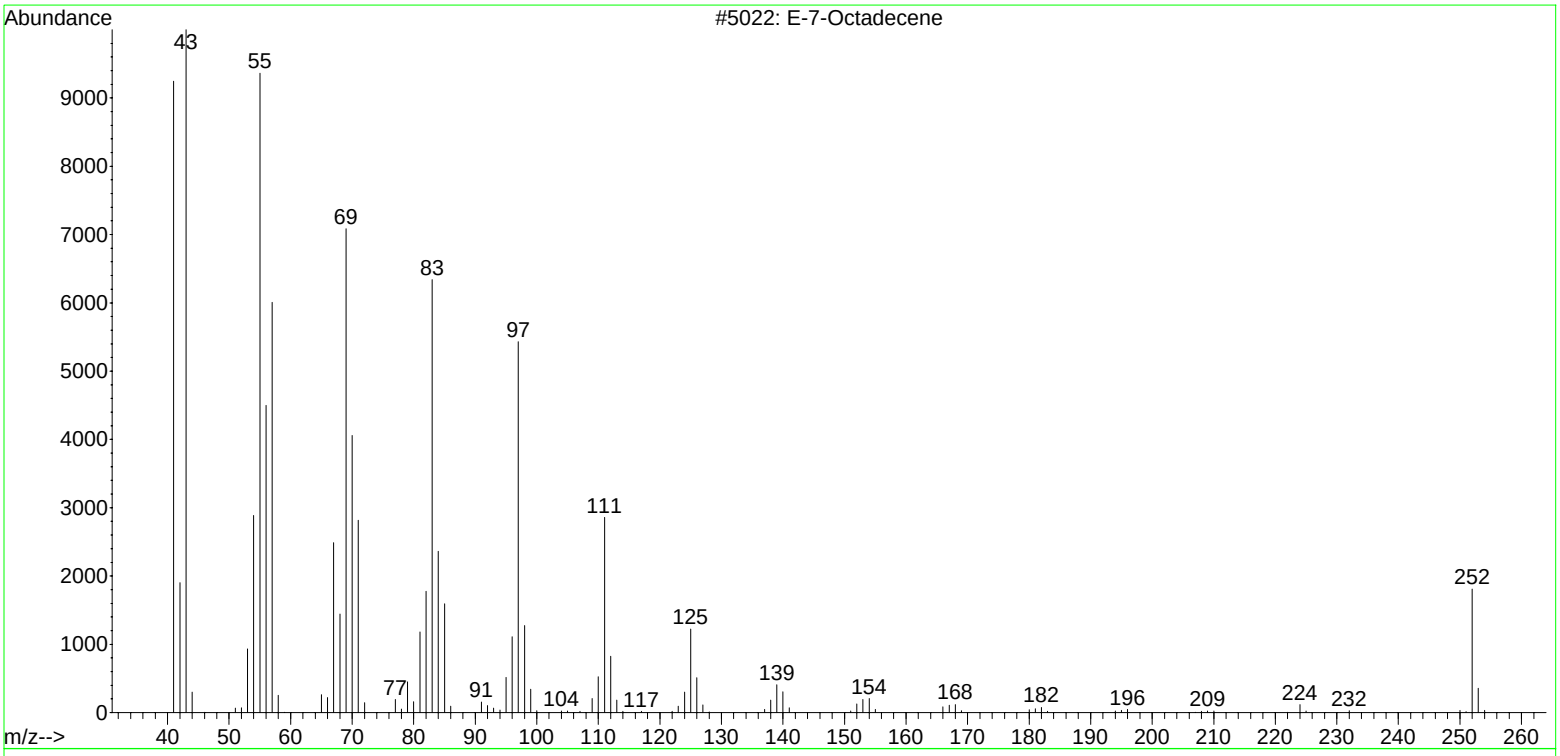


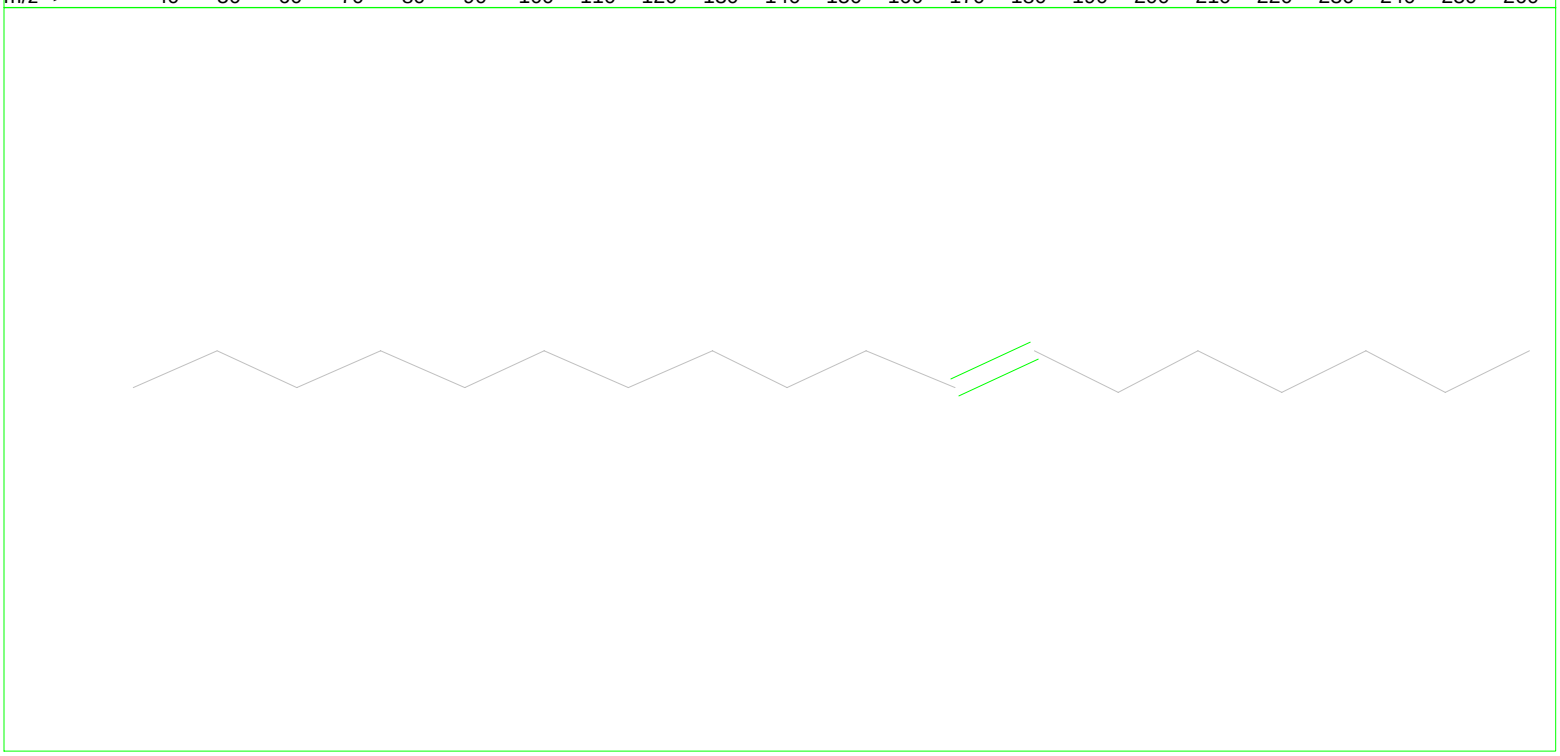


E-7-Octadecene

Entry Number	5022	from	C:\Database\Nist98.L
CAS	1000130-92-0		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C18H36		
Mol Weight	252.282		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 130920, Seq# M5022, CAS number = 10^9 + NIST MS#

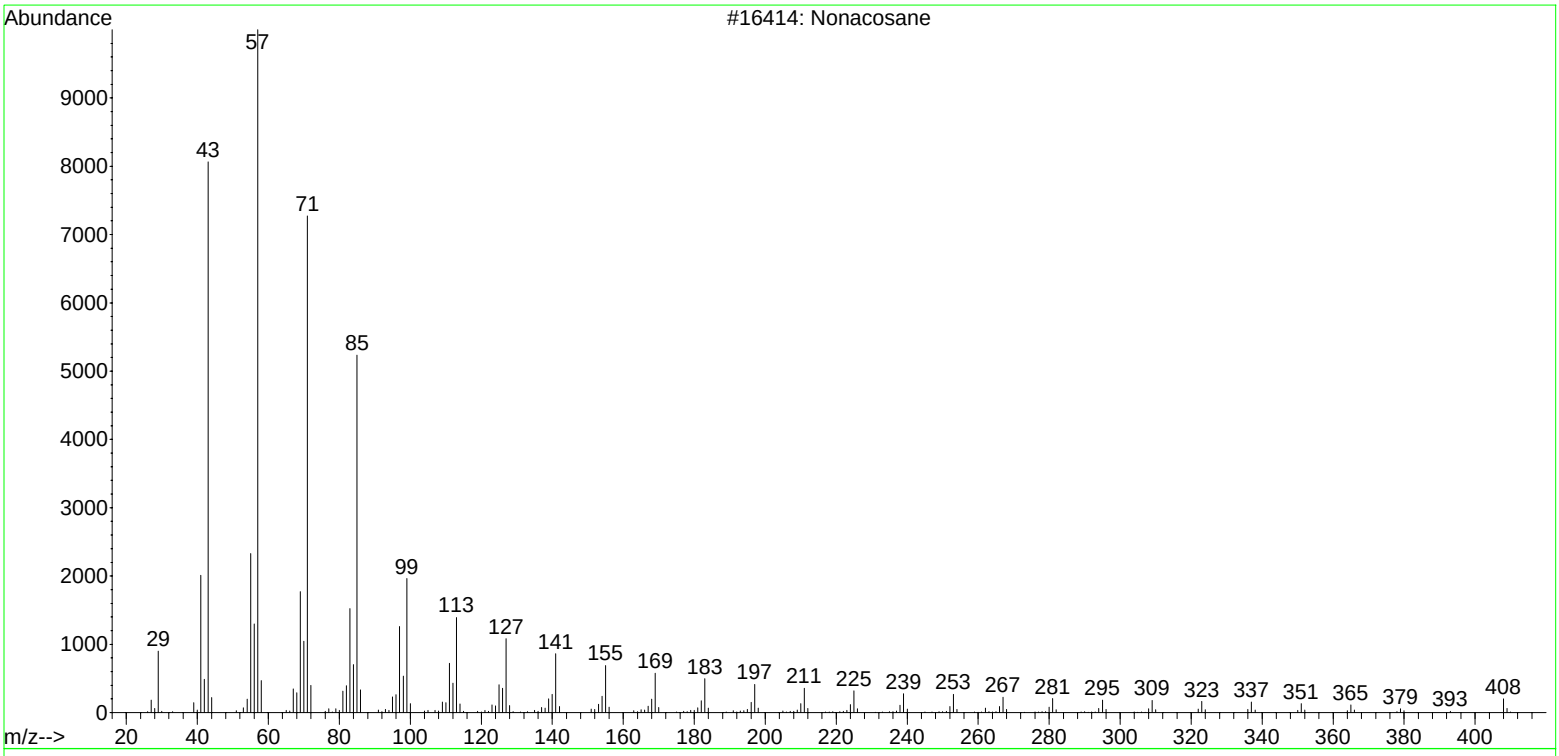




Nonacosane

Entry Number	16414	from	C:\Database\Nist98.L
CAS	000630-03-5		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C29H60		
Mol Weight	408.47		
Company ID	NIST 1998		

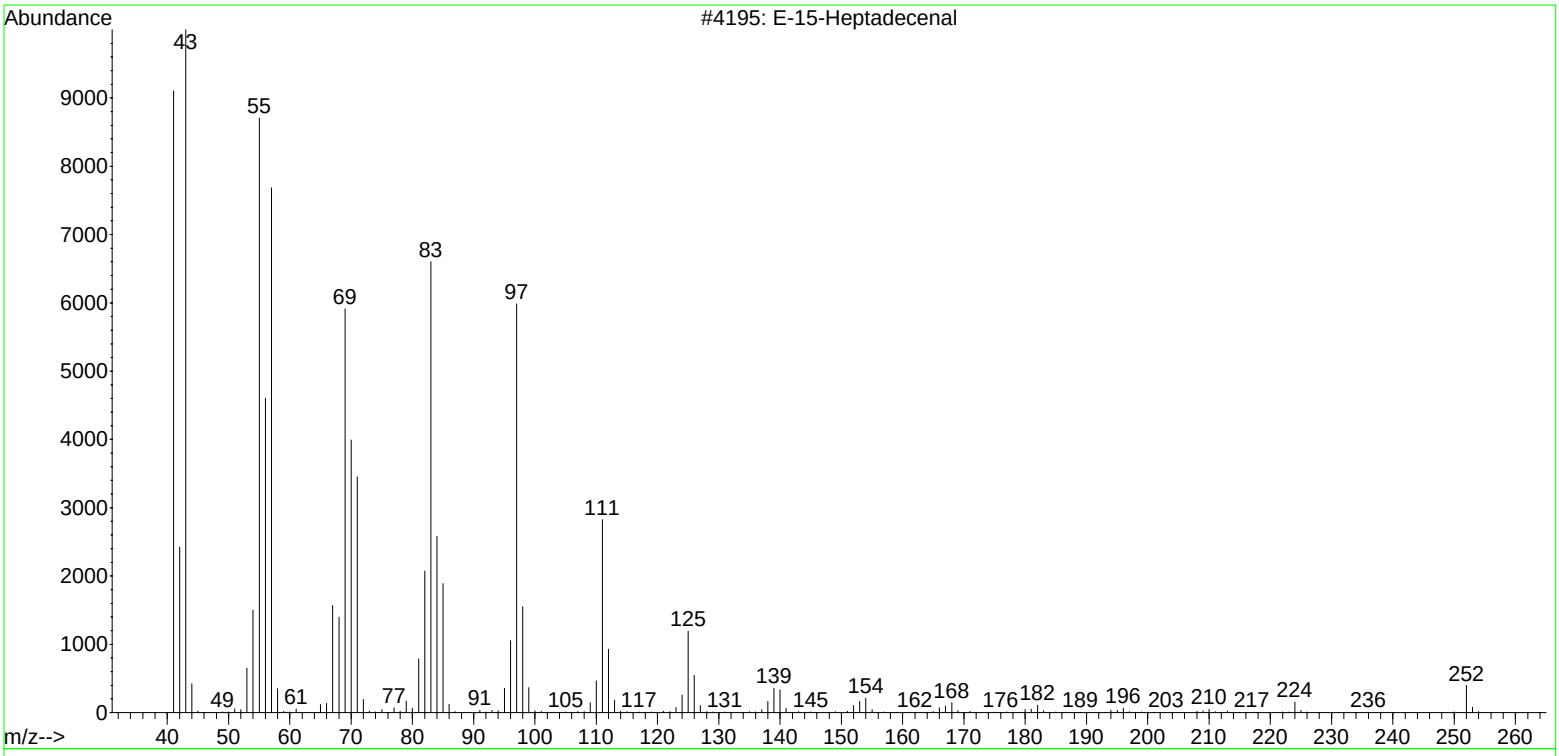
Miscellaneous Information
NIST MS# 197624, Seq# M16414

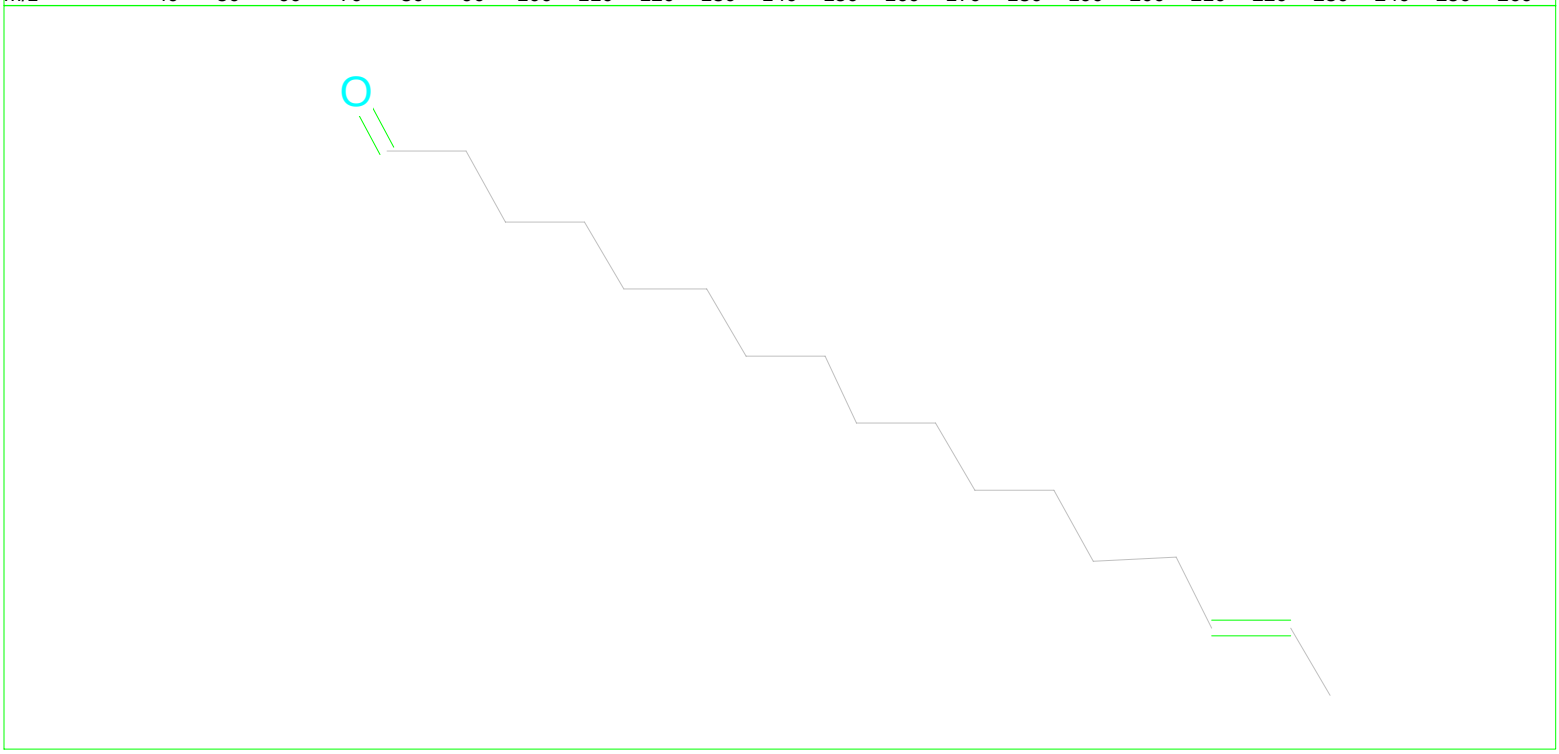


E-15-Heptadecenal

Entry Number	4195	from	C:\Database\Nist98.L
CAS	1000130-97-9		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C17H32O		
Mol Weight	252.245		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 130979, Seq# M4195, CAS number = 10^9 + NIST MS#

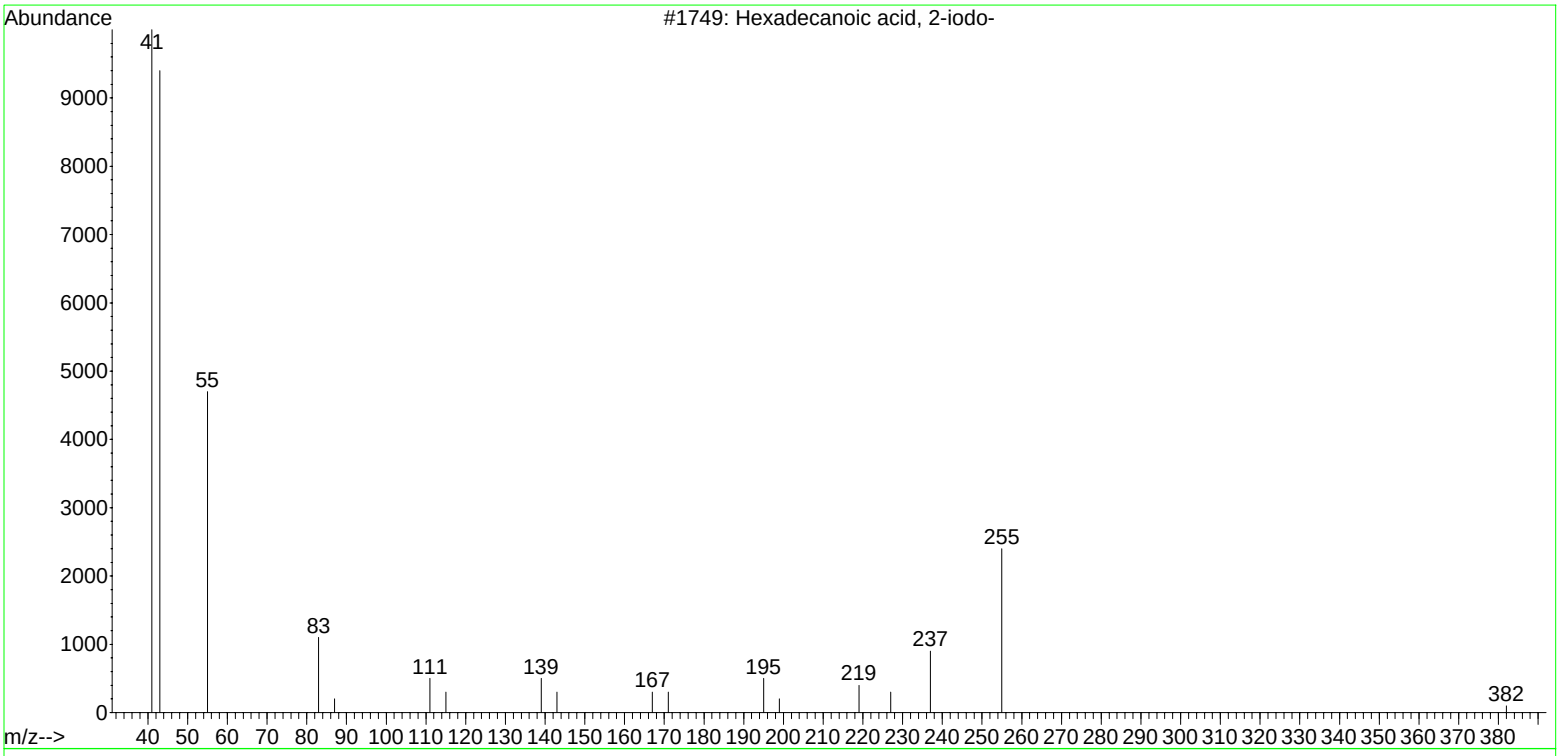


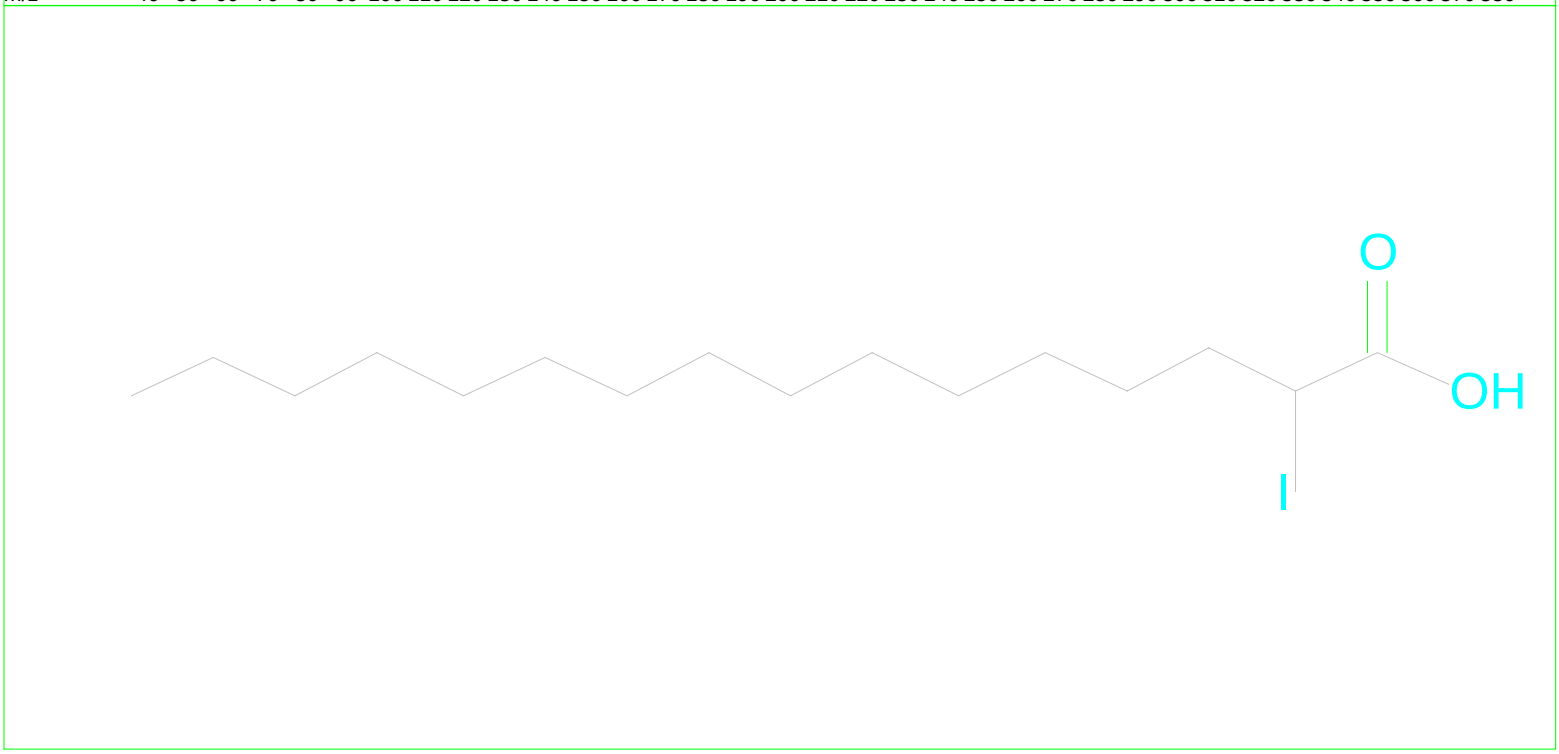


Hexadecanoic acid, 2-iodo-

Entry Number	1749	from	C:\Database\Nist98.L
CAS	101434-56-4		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C16H31IO2		
Mol Weight	382.137		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 115246, Seq# M1749



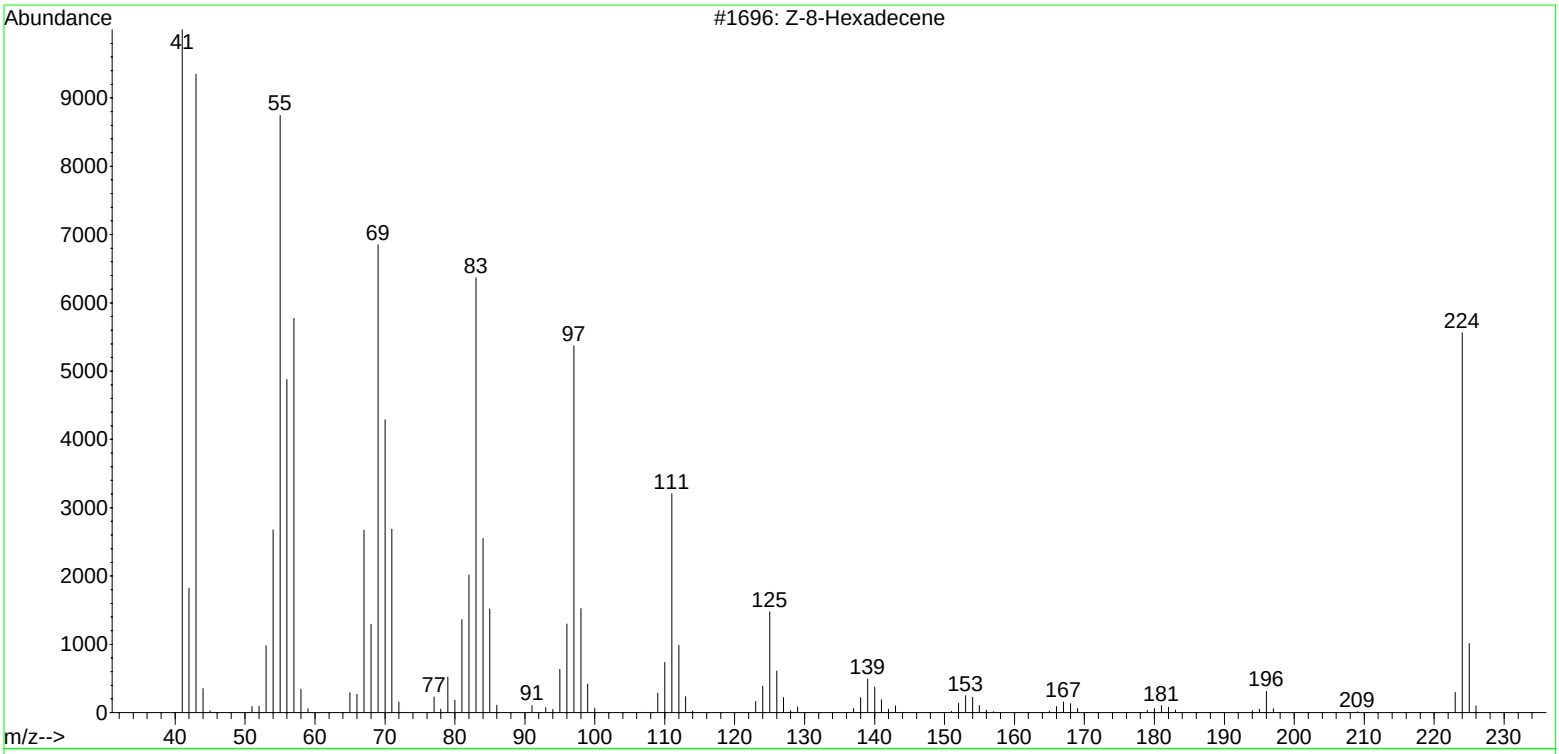


Z-8-Hexadecene

Entry Number	1696	from	C:\Database\Nist98.L
CAS	1000130-87-5		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C16H32		
Mol Weight	224.25		
Company ID	NIST 1998		

Miscellaneous Information

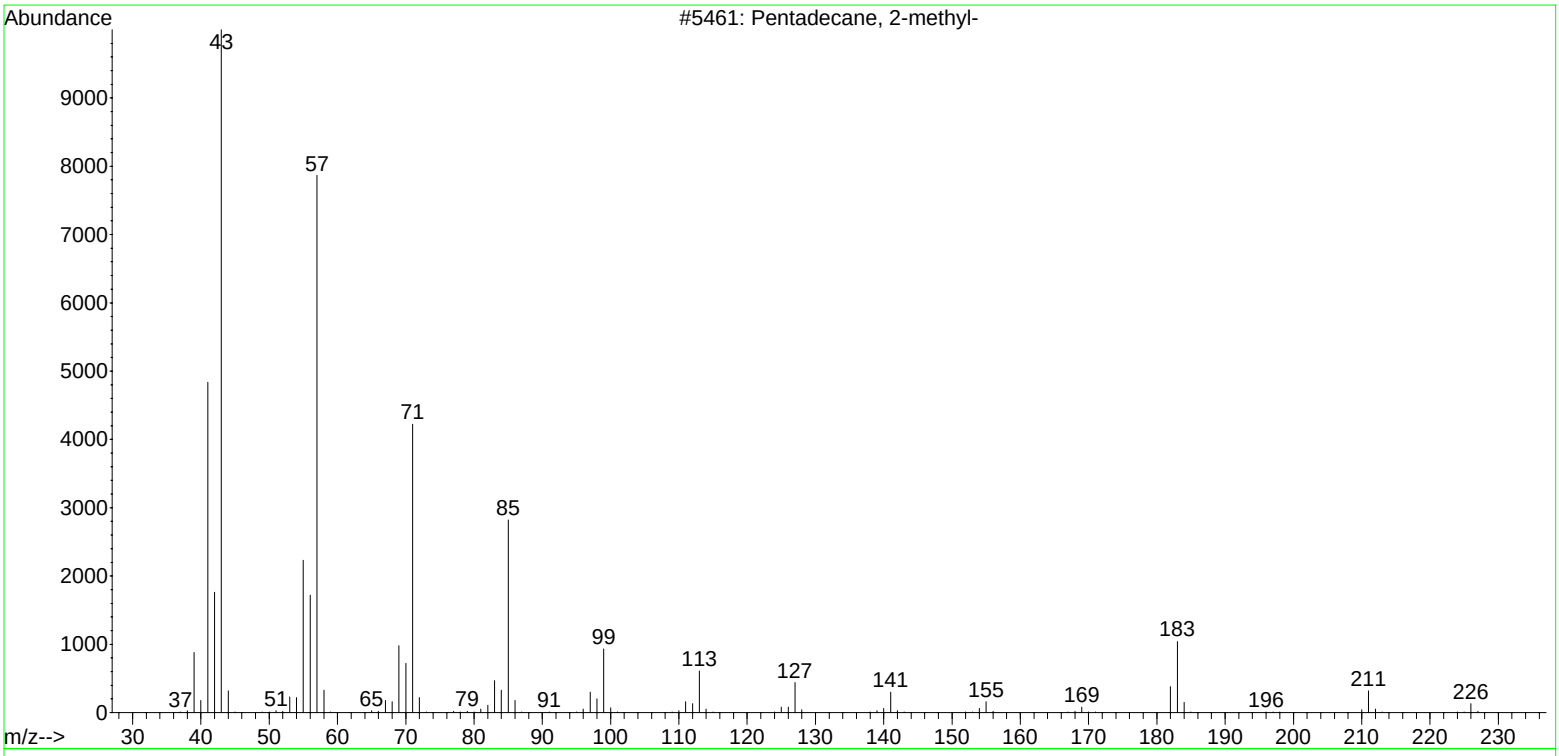
NIST MS# 130875, Seq# M1696, CAS number = 10^9 + NIST MS#



Pentadecane, 2-methyl-

Entry Number	5461	from	C:\Database\Nist98.L
CAS	001560-93-6		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C16H34		
Mol Weight	226.266		
Company ID	NIST 1998		

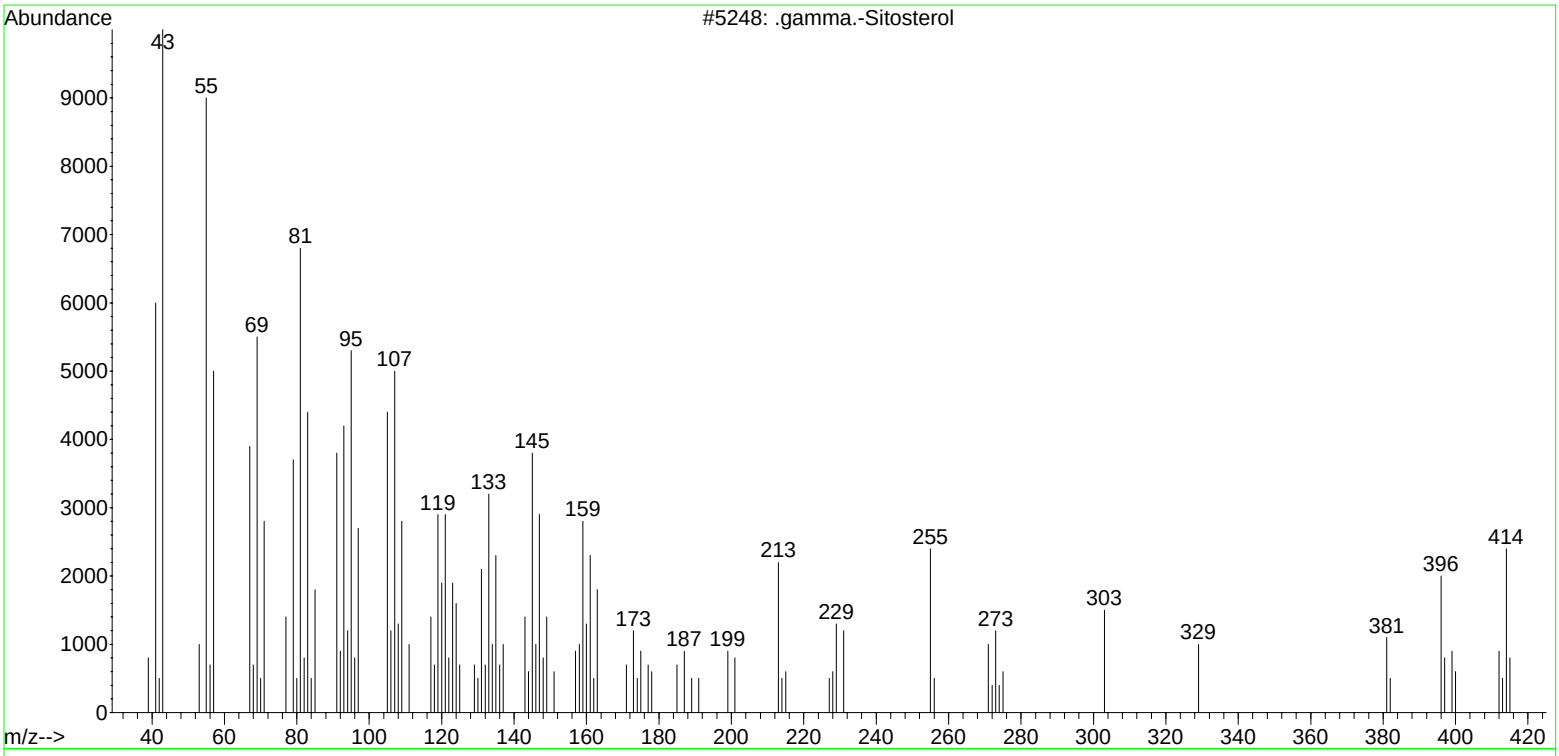
Miscellaneous Information
NIST MS# 22753, Seq# M5461

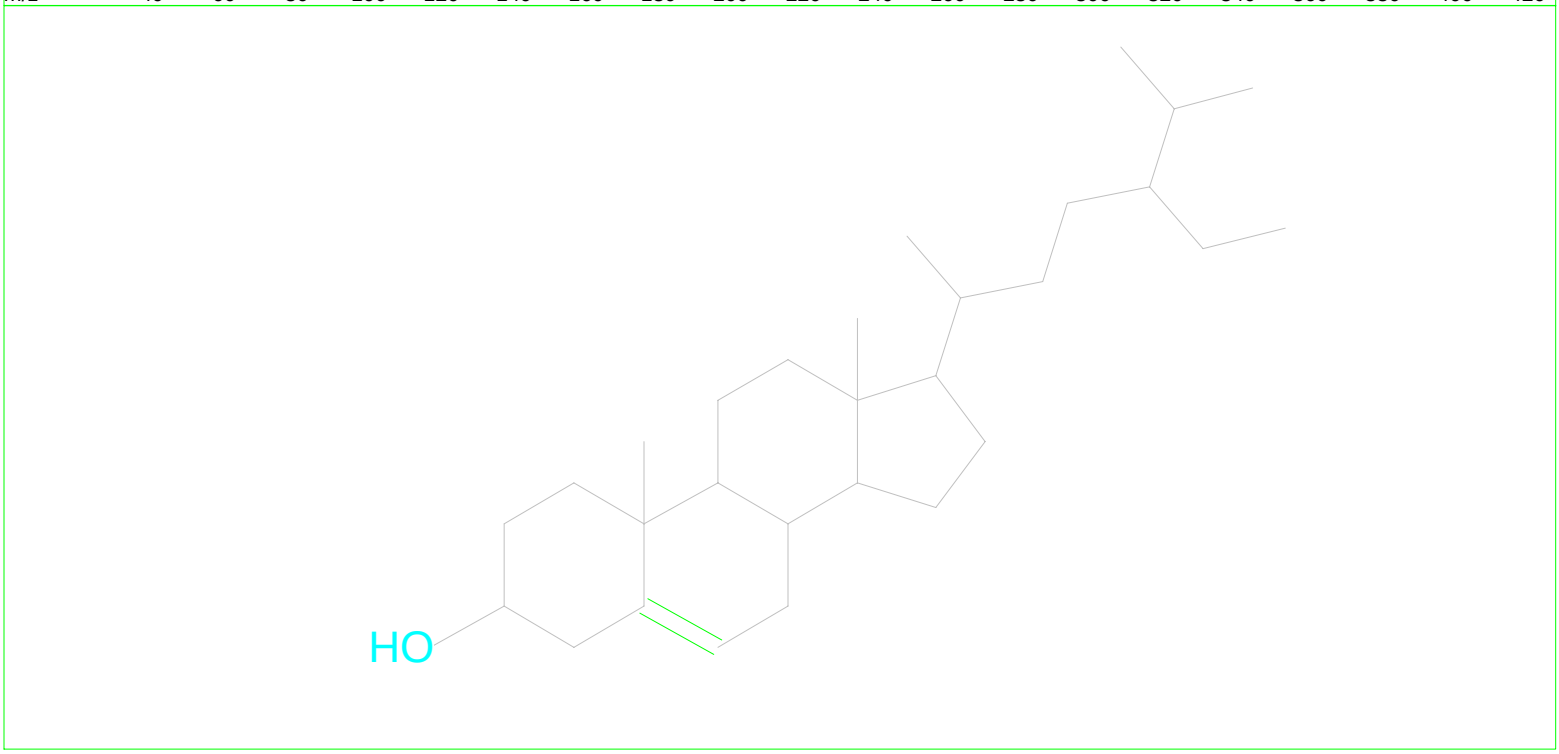


.gamma.-Sitosterol

Entry Number	5248	from	C:\Database\Nist98.L
CAS	000083-47-6		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C29H50O		
Mol Weight	414.386		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 36773, Seq# M5248

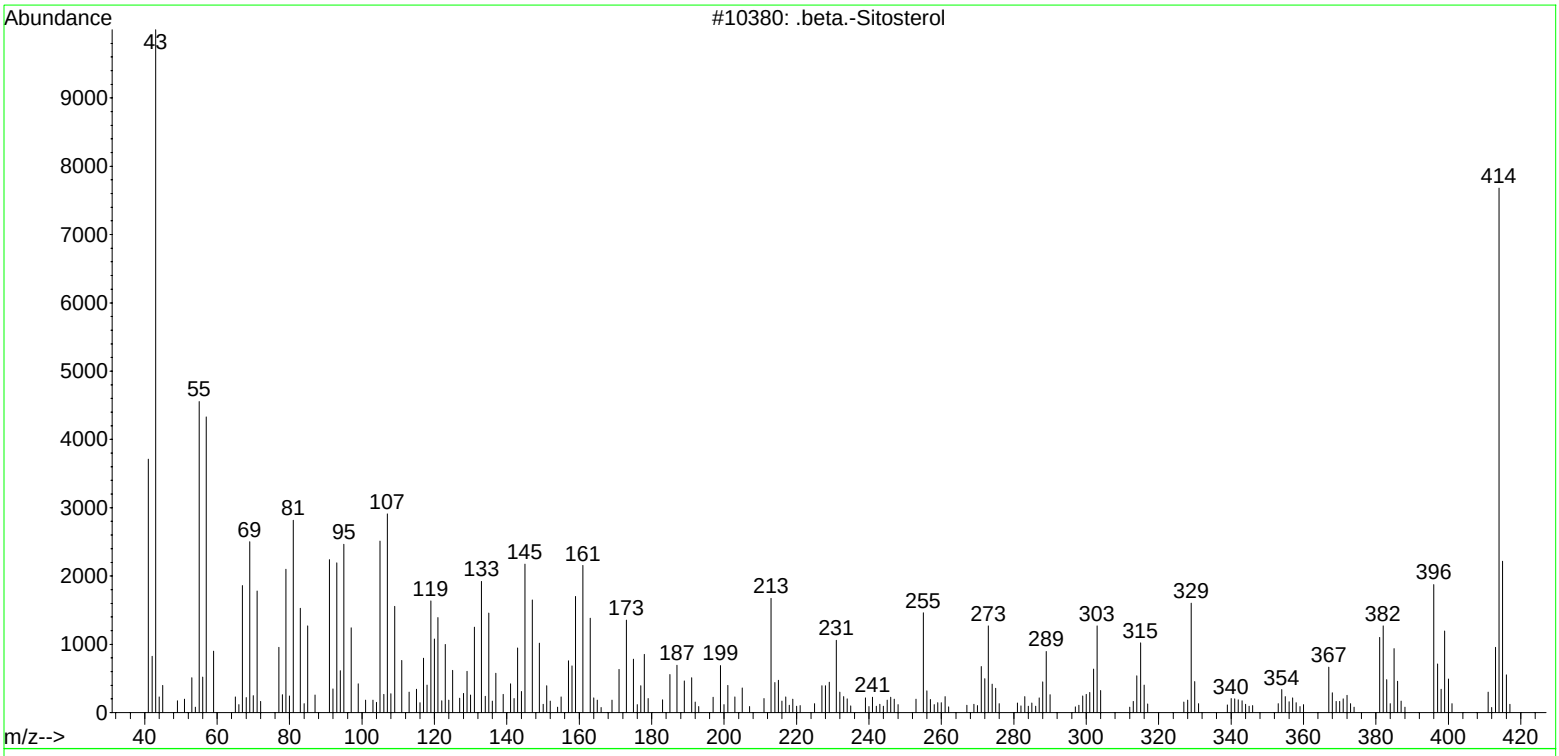


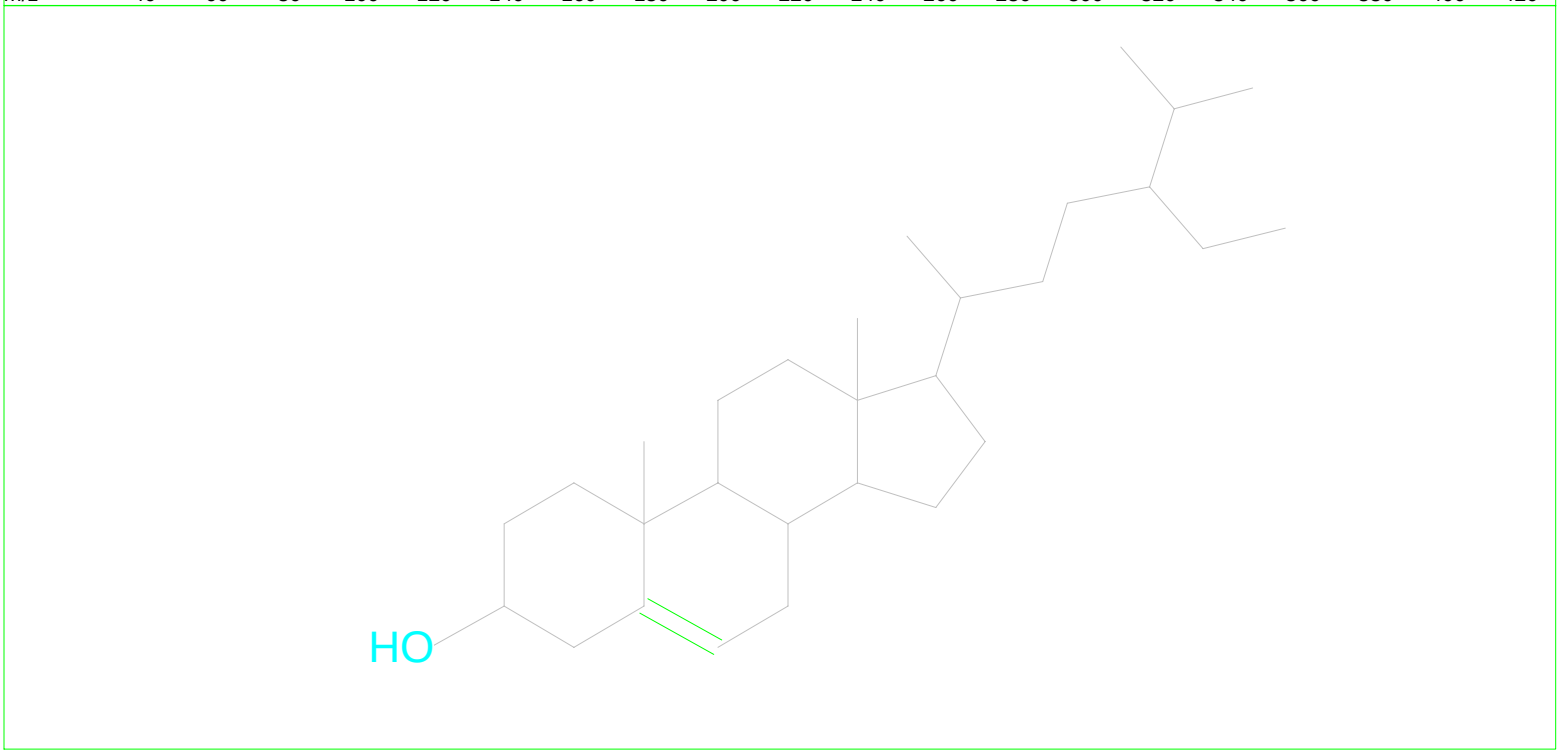


.beta.-Sitosterol

Entry Number	10380	from	C:\Database\Nist98.L
CAS	000083-46-5		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C29H50O		
Mol Weight	414.386		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 129178, Seq# M10380



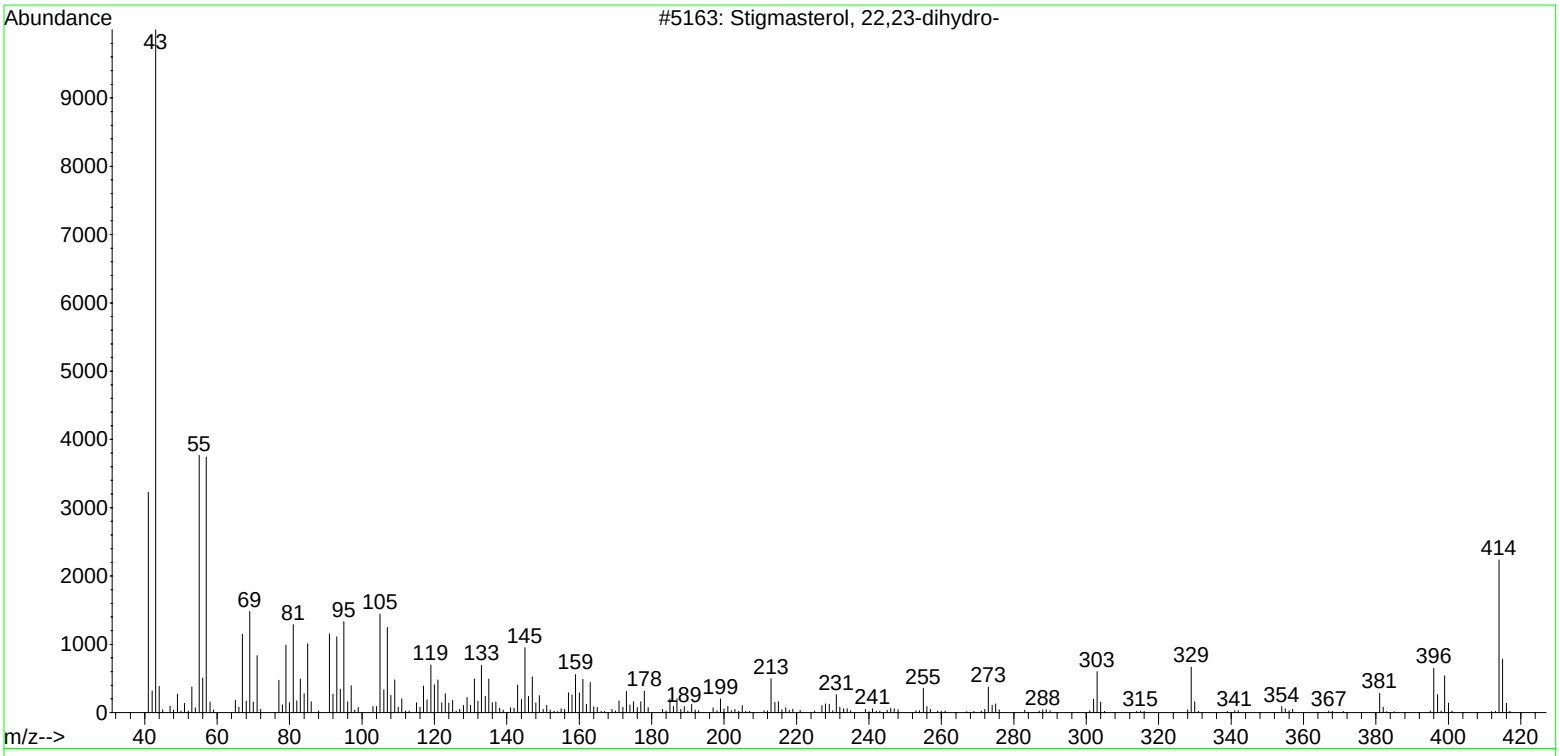


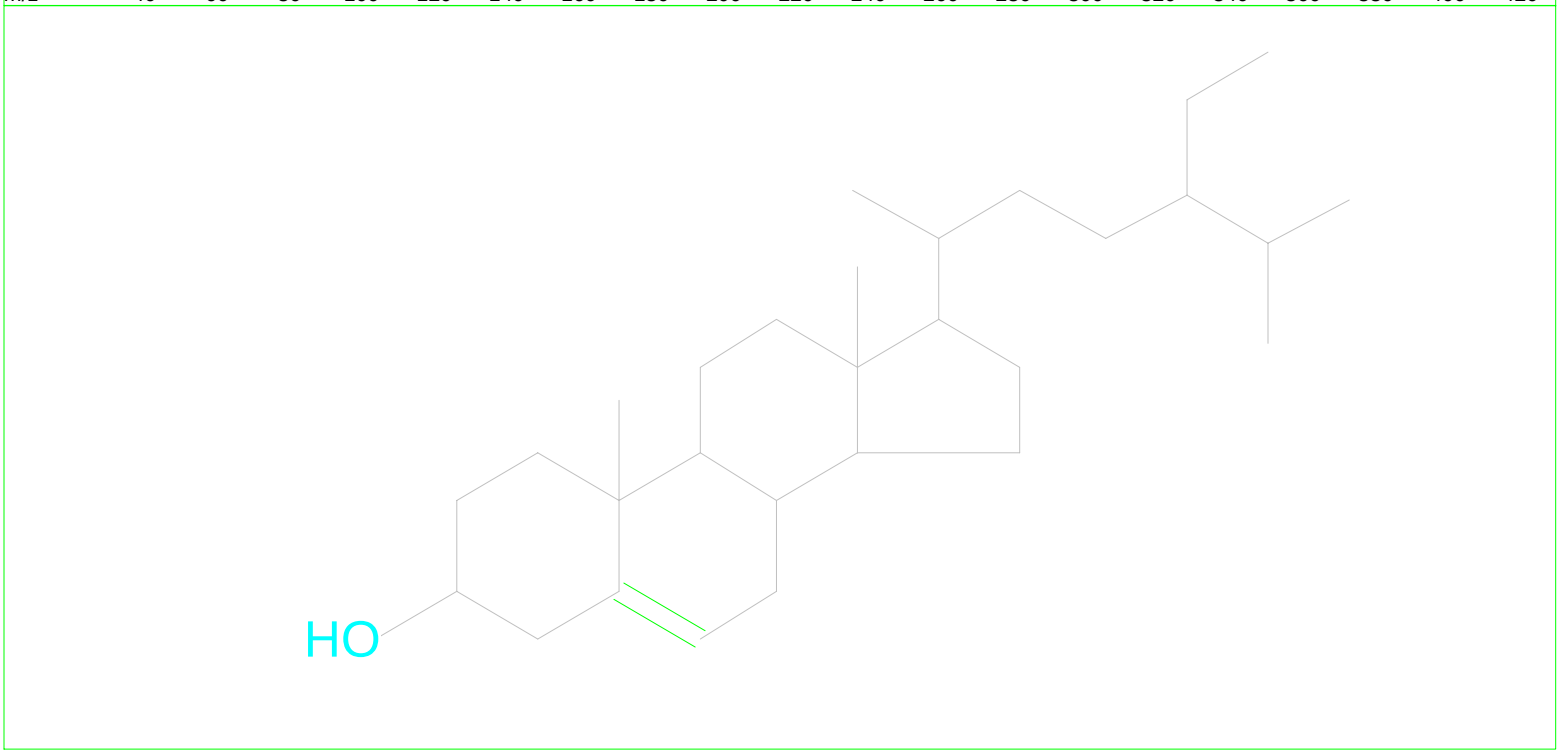
Stigmasterol, 22,23-dihydro-

Entry Number	5163	from	C:\Database\Nist98.L
CAS	1000214-20-7		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C29H50O		
Mol Weight	414.386		
Company ID	NIST 1998		

Miscellaneous Information

NIST MS# 214207, Seq# M5163, CAS number = 10^9 + NIST MS#

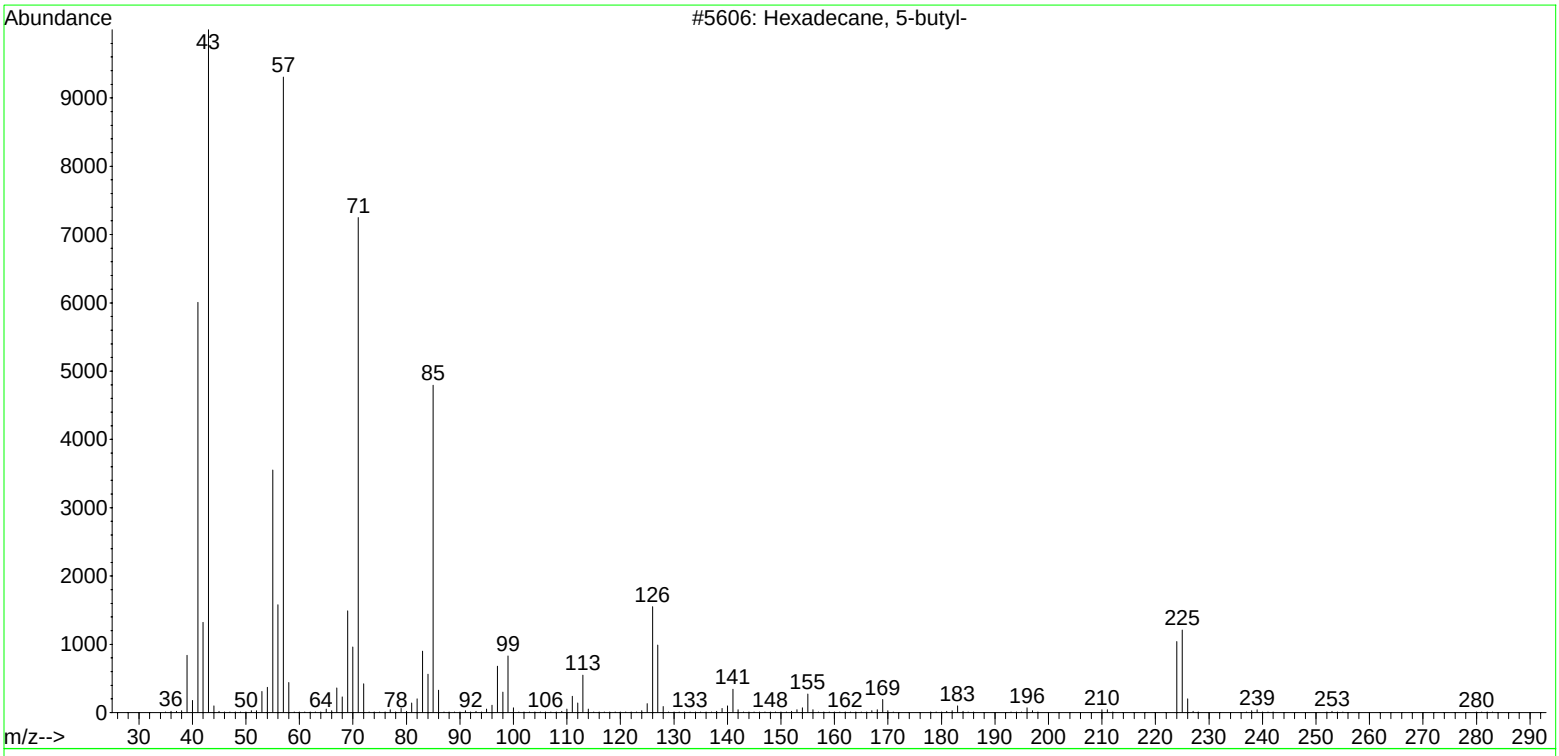


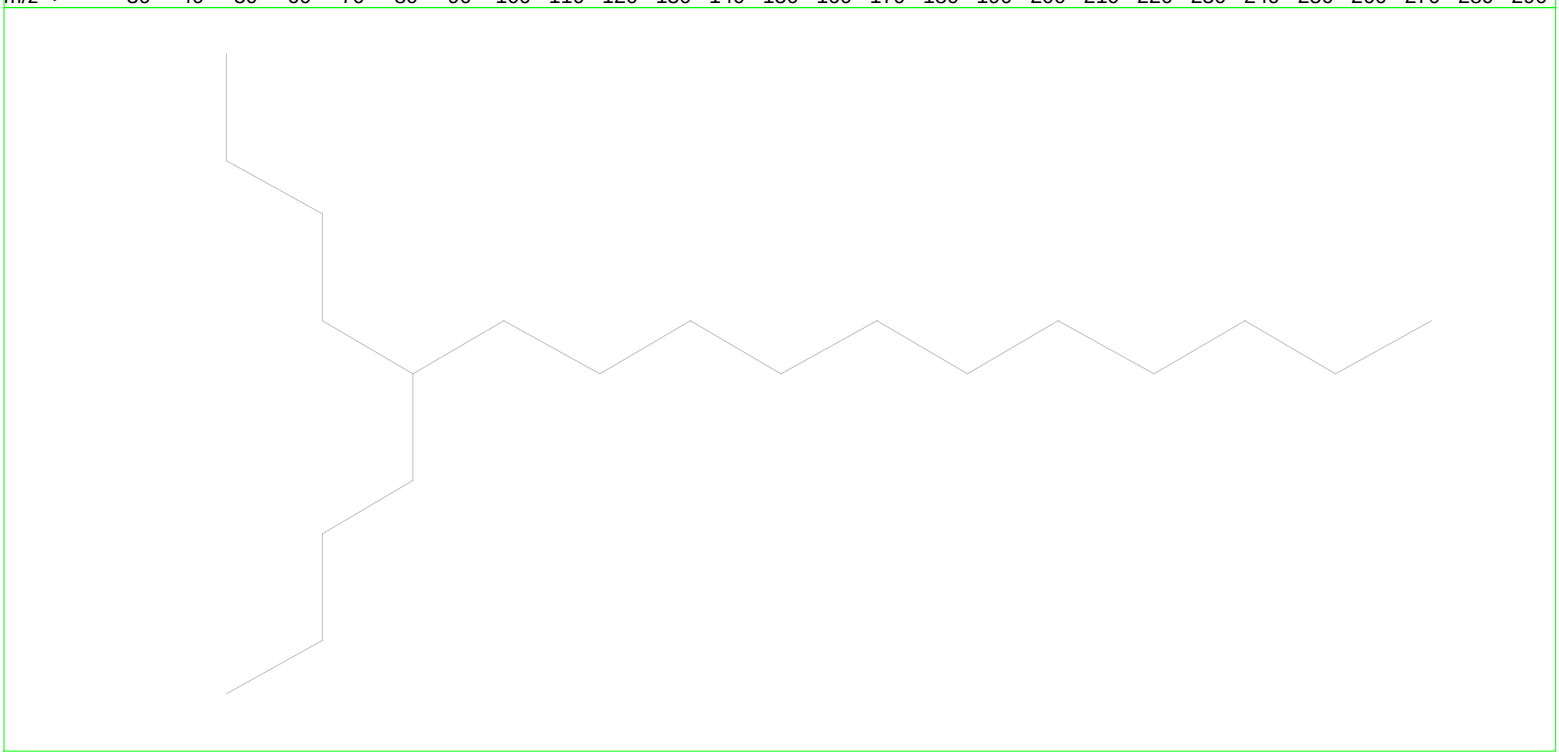


Hexadecane, 5-butyl-

Entry Number	5606	from	C:\Database\Nist98.L
CAS	006912-07-8		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C20H42		
Mol Weight	282.329		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 13572, Seq# M5606

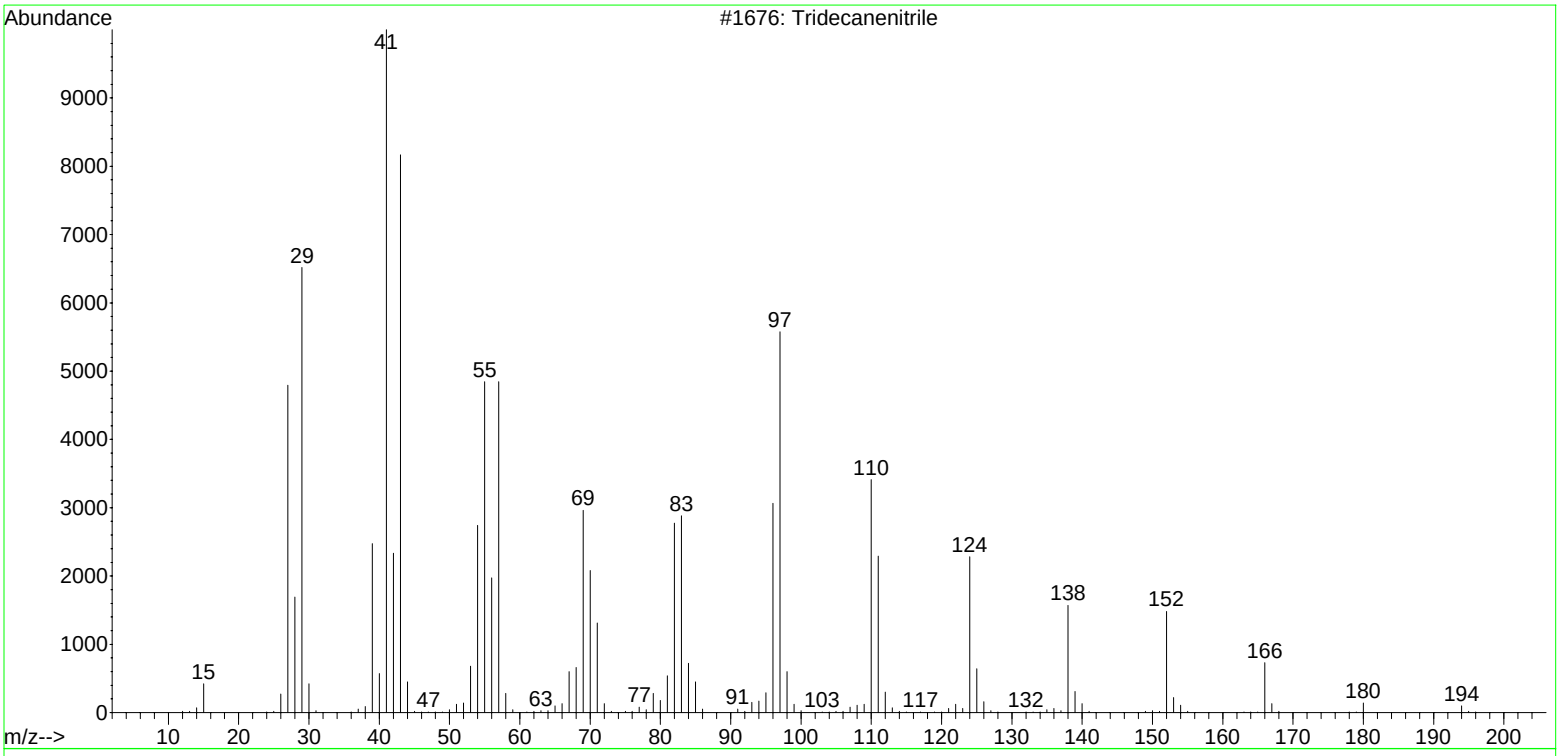


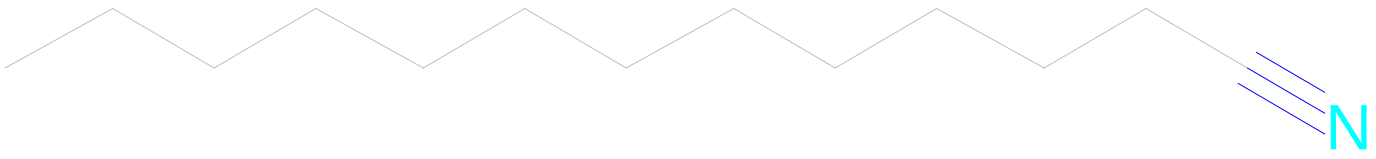


Tridecanenitrile

Entry Number	1676	from	C:\Database\Nist98.L
CAS	000629-60-7		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C13H25N		
Mol Weight	195.199		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 8573, Seq# M1676

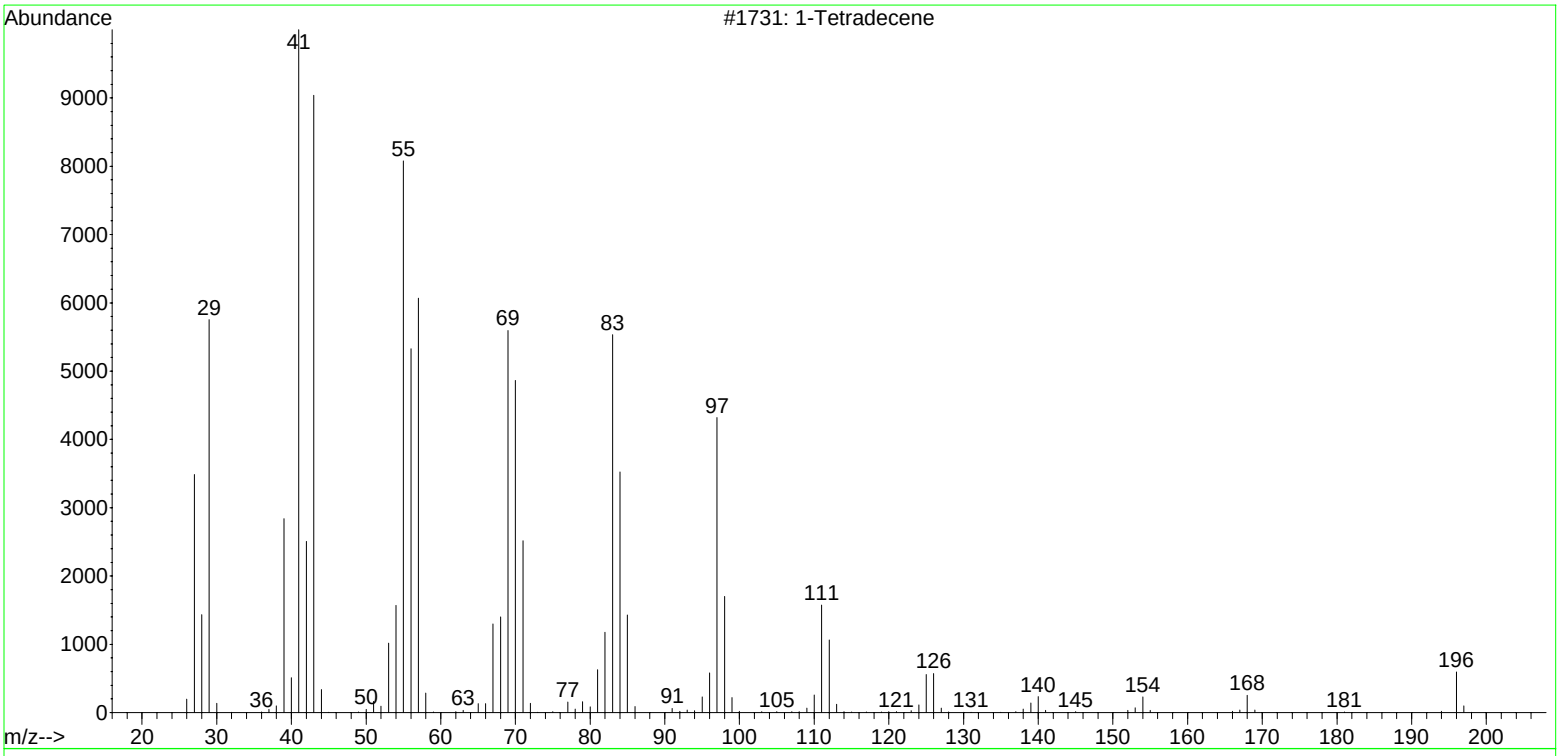




1-Tetradecene

Entry Number	1731	from	C:\Database\Nist98.L
CAS	001120-36-1		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C14H28		
Mol Weight	196.219		
Company ID	NIST 1998		

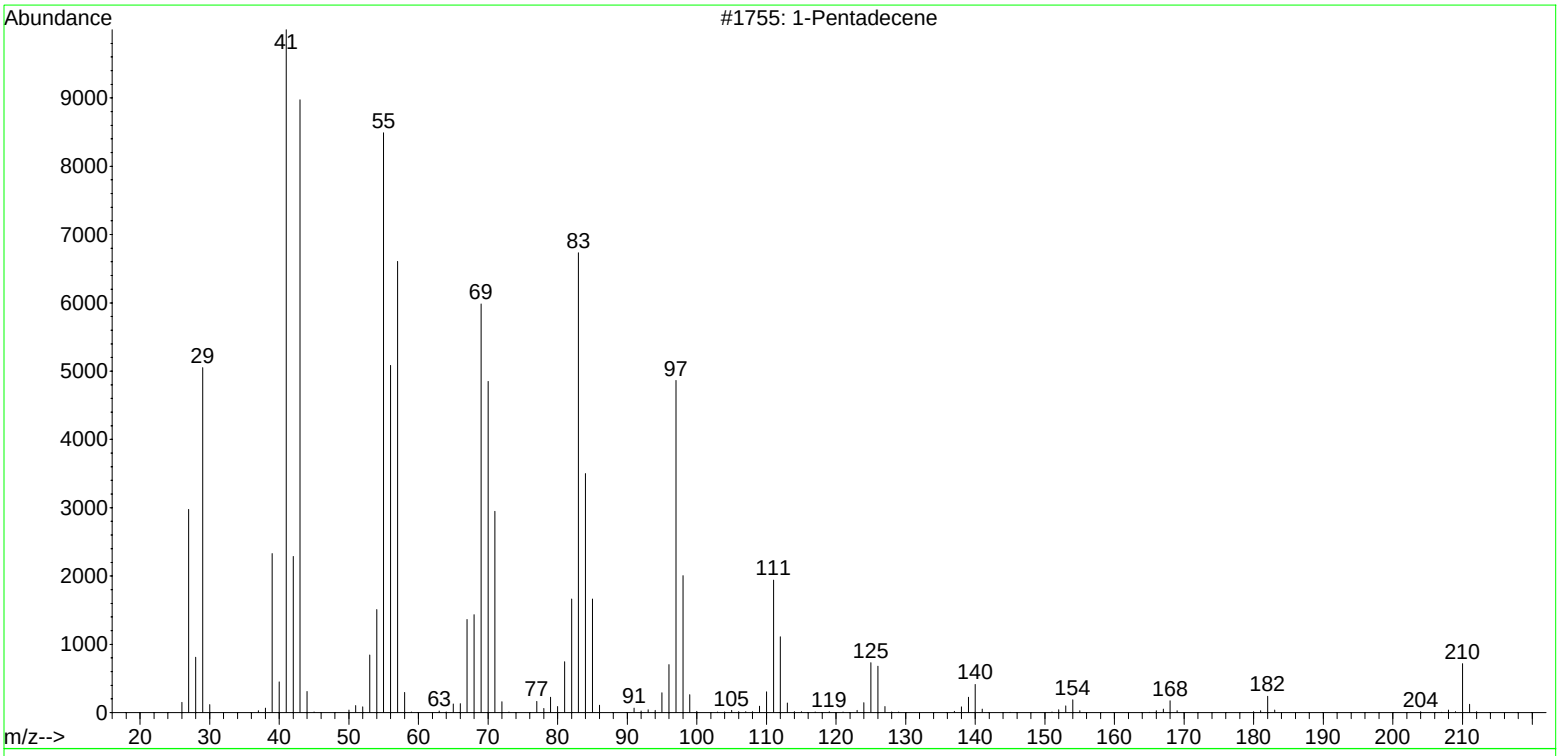
Miscellaneous Information
NIST MS# 34720, Seq# M1731



1-Pentadecene

Entry Number	1755	from	C:\Database\Nist98.L
CAS	013360-61-7		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C15H30		
Mol Weight	210.235		
Company ID	NIST 1998		

Miscellaneous Information
NIST MS# 34735, Seq# M1755



Appendix H: MSDS

Material Safety Data Sheet
Acetone

Section 1 - Chemical Product and Company Identification

MSDS Name: Acetone

Synonyms: Dimethylformaldehyde; Dimethyl ketone; 2-Propanone; Pyroacetic acid; Pyroacetic ether.

Company Identification:

ROCHELLE CHEMICALS & LAB EQUIPMENT cc
54 Meson Road, Electron, Johannesburg, South Africa

For information, call: +27 11 613 5638
Emergency Number: +27 83 269 7693

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
67-64-1	Acetone	100.0	200-662-2

Hazard Symbols: XI F
Risk Phrases: 11 36 66 67

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: colourless. Flash Point: -4 deg F. Causes respiratory tract irritation. Causes eye irritation. Breathing vapors may cause drowsiness and dizziness. Prolonged or repeated contact may dry the skin and cause irritation. **Danger!** Extremely flammable liquid and vapor. Vapor may cause flash fire.
Target Organs: Central nervous system, respiratory system, eyes, skin.

Potential Health Effects

Eye: Produces irritation, characterized by a burning sensation, redness, tearing, inflammation, and possible corneal injury.
Skin: May be absorbed through the skin. Repeated or prolonged exposure may cause drying and cracking of the skin.
Ingestion: May cause irritation of the digestive tract. May cause central nervous system depression, characterized by excitement, followed by headache, dizziness, drowsiness, and nausea. Advanced stages may cause collapse, unconsciousness, coma and possible death due to respiratory failure.

Inhalation: Inhalation of high concentrations may cause central nervous system effects characterized by nausea, headache, dizziness, unconsciousness and coma. Causes respiratory tract irritation. May cause motor incoordination and speech abnormalities.
Chronic: Prolonged or repeated skin contact may cause dermatitis. Chronic inhalation may cause effects similar to those of acute inhalation.

Section 4 - First Aid Measures

Eyes: Flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid immediately.
Skin: Flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or persists. Wash clothing before reuse.
Ingestion: Do NOT induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid immediately.
Inhalation: Get medical aid immediately. Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid. Do NOT use mouth-to-mouth resuscitation. If breathing has ceased apply artificial respiration using oxygen and a suitable mechanical device such as a bag and a mask.
Notes to Physician: Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information: Containers can build up pressure if exposed to heat and/or fire. As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion. Use water spray to keep fire-exposed containers cool. May be ignited by heat, sparks, and flame. Vapors are heavier than air and may travel to a source of ignition and flash back. Vapors can spread along the ground and collect in low or confined areas.
Extinguishing Media: For small fires, use dry chemical, carbon dioxide, water spray or alcohol-resistant foam. For large fires, use water spray, fog, or alcohol-resistant foam. Use water spray to cool fire-exposed containers. Water may be ineffective. Do NOT use straight streams of water. Cool containers with flooding quantities of water until well after fire is out.
Flash Point: -4e deg F (-20.00 deg C)
Autoignition Temperature: 869 deg F (465.00 deg C)
Explosion Limits, Lower:2.5%
Upper: 12.8%
NFPA Rating: (estimated) Health: 1; Flammability: 3; Instability: 0

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Avoid runoff into storm sewers and ditches which lead to waterways. Wear appropriate protective clothing to minimize contact with skin. Remove all sources of ignition. Provide ventilation. A vapor suppressing foam may be used to reduce vapors. Water spray may reduce vapor but may not prevent ignition in closed spaces. Clean up residual material by washing area with a 2-5% solution of soda ash.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Ground and bond containers when transferring material. Avoid contact with eyes, skin, and clothing. Empty containers retain product residue, (liquid and/or vapor), and can be dangerous. Keep container tightly closed. Avoid ingestion and inhalation. Do not pressurize, cut, weld, braze, solder, drill, grind, or expose empty containers to heat, sparks or open flames. Use only with adequate ventilation. Keep away from heat, sparks and flame.
Storage: Keep away from heat, sparks, and flame. Keep away from sources of ignition. Store in a tightly closed container. Store in a cool, dry, well-ventilated area away from incompatible substances. Flammables-area.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits.
Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Acetone	500 ppm TWA; 750 ppm STEL	250 ppm TWA; 590 mg/m3 TWA 2500 ppm IDLH	1000 ppm TWA; 2400 mg/m3 TWA

OSHA Vacated PELs: Acetone: 750 ppm TWA; 1800 mg/m3 TWA
Personal Protective Equipment
Eyes: Wear chemical goggles.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin exposure.
Respirators: Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Always use a NIOSH or European Standard EN 149 approved respirator when necessary.

Section 9 - Physical and Chemical Properties

Physical State: Liquid
Appearance: colourless
Odor: acetone-like
pH: 7
Vapor Pressure: 180 mm Hg
Vapor Density: 2.0 (Air=1)
Evaporation Rate:7.7 (n-Butyl acetate=1)
Viscosity: Not available
Boiling Point: 133.2 deg F
Freezing/Melting Point:-139.6 deg F
Decomposition Temperature: Not available.
Solubility: Soluble.
Specific Gravity/Density:0.79 (Water=1)
Molecular Formula:C3H6O
Molecular Weight:58.08

Section 10 - Stability and Reactivity

Chemical Stability: Stable at room temperature in closed containers under normal storage and handling conditions.
Conditions to Avoid: High temperatures, ignition sources, temperatures above 220°C.
Incompatibilities with Other Materials: Strong acids, strong oxidizing agents.
Hazardous Decomposition Products: Carbon monoxide, irritating and toxic fumes and gases, carbon dioxide.
Hazardous Polymerization: Has not been reported.

Section 11 - Toxicological Information

RTECS#:
CAS# 67-64-1: AL3150000
LD50/LC50:
CAS# 67-64-1:
Dermal, guinea pig: LD50 = >9400 uL/kg;
Draize test, rabbit, eye: 10 uL Mild;
Draize test, rabbit, eye: 20 mg Severe;
Draize test, rabbit, eye: 20 mg/24H Moderate;
Draize test, rabbit, skin: 500 mg/24H Mild;
Inhalation, mouse: LC50 = 44 gm/m3/4H;
Inhalation, rat: LC50 = 50100 mg/m3/8H;
Oral, mouse: LD50 = 3 gm/kg;
Oral, rabbit: LD50 = 5340 mg/kg;
Oral, rat: LD50 = 5800 mg/kg;<br.

Carcinogenicity:
CAS# 67-64-1:
ACGIH: A4 - Not Classifiable as a Human Carcinogen
Epidemiology: No information available.

Teratogenicity: No information available.
Reproductive Effects: TDLo(Oral, rat) = 273 gm/kg;Reproductive - Paternal Effects - spermatogenesis (incl. genetic material, sperm morphology, motility, and count).
Neurotoxicity: No information available.
Mutagenicity: Sex chromosome loss and nondisjunction(Yeast - Saccharomyces cerevisiae) = 47600 ppm; Cytogenetic analysis(Rodent - hamster Fibroblast)= 40 gm/L.
Other Studies: Standard Draize Test: Administration onto the skin (human) = 500 mg/7days (Mild). Standard Draize Test: Administrat ion onto the skin (rabbit) = 500 mg/24H (Mild). Standard D raize Test(Eye, Rabbit) = 20 mg; Severe.

Section 12 - Ecological Information

Ecotoxicity: Material Safety Data Sheet Brown trout: ; ; Rainbow trout LC50=5540 mg/L/96H Sunfish (tap water), death at 14250 ppm/24H Mosquito fish (turbid water) TLm=13000 ppm/48HCas# 67-64-1:LC50 (96Hr.) rainbow trout = 5540 mg/L; Static conditions, 11-13 degrees CLC50 (96Hr) Fathead Minnow = 7280-8120 mg/L; Flow-through ConditionsLC50 (96Hr) Bluegill = 8300 mg/L
Environmental: Volatilizes, leeches, and biodegrades when released to soil. TERRESTRIAL FATE: If released on soil, acetone will both volatilize and leach into the ground. Acetone readily biodegrades and there is evidence suggesting that it biodegrades fairly rapidly in soils. AQUATIC FATE: If released into water, acetone will probably biodegrade. It is readily biodegradable in screening tests, although data from natural water are lacking. It will also be lost due to volatilization (estimated half-life 20 hr from a model river). Adsorption to sediment should not be significant.
Physical: ATMOSPHERIC FATE: In the atmosphere, acetone will be lost by photolysis and reaction with photochemically produced hydroxyl radicals. Half-life estimates from these combined processes are 79 and 13 days in January and June, respectively, for an overall annual average of 22 days. Therefore considerable dispersion should occur. Being miscible in water, wash out by rain should be an important removal process. This process has been confirmed around Lake Shinsei-ko in Japan. There acetone was found in the air and rain as well as the lake.
Other: Not expected to bioconcentrate in fish. he recommended log octanol/water partition coefficient for acetone is -0.24 and therefore its potential for bioconcentration in fish is negligible. One experimental study of bioconcentration in adult haddock at 7-9 deg C (static test), resulted in a BCF of 0.69.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
RCRA P-Series: None listed.
RCRA U-Series: CAS# 67-64-1: waste number U002 (Ignitable waste).

Section 14 - Transport Information

	US DOT	IATA	RID/ADR	IMO	Canada TDG
Shipping Name:	No information available.				ACETONE
Hazard Class:					3
UN Number:					UN1090
Packing Group:					II
Additional Info:					FLASHPOINT -20 C

Section 15 - Regulatory Information

US FEDERAL

TSCA
CAS# 67-64-1 is listed on the TSCA inventory.
Health & Safety Reporting List
None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules
None of the chemicals in this product are under a Chemical Test Rule.
Section 12b
CAS# 67-64-1: 4/12b
TSCA Significant New Use Rule
None of the chemicals in this material have a SNUR under TSCA.
SARA

CERCLA Hazardous Substances and corresponding RQs
CAS# 67-64-1: 5000 lb final RQ; 2270 kg final RQ
SARA Section 302 Extremely Hazardous Substances
None of the chemicals in this product have a TPQ.
SARA Codes
CAS # 67-64-1: acute, chronic, flammable.
Section 313
No chemicals are reportable under Section 313.
Clean Air Act:
This material does not contain any hazardous air pollutants. This material does not contain any Class 1 Ozone depletors. This material does not contain any Class 2 Ozone depletors.
Clean Water Act:
None of the chemicals in this product are listed as Hazardous Substances under the CWA. None of the chemicals in this product are listed as Priority Pollutants under the CWA. None of the chemicals in this product are listed as Toxic Pollutants under the CWA.
OSHA:
None of the chemicals in this product are considered highly hazardous by OSHA.
STATE
CAS# 67-64-1 can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Minnesota, Massachusetts.
California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations

European Labeling in Accordance with EC Directives

Hazard Symbols:

XI F

Risk Phrases:

R 11 Highly flammable.
R 36 Irritating to eyes.
R 66 Repeated exposure may cause skin dryness or cracking.
R 67 Vapors may cause drowsiness and dizziness.

Safety Phrases:

S 16 Keep away from sources of ignition - No smoking.
S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S 9 Keep container in a well-ventilated place.

WGK (Water Danger/Protection)

CAS# 67-64-1: 0

Canada - DSL/NDSL

CAS# 67-64-1 is listed on Canada's DSL List.

Canada - WHMIS

This product has a WHMIS classification of B2, D2B.

Canadian Ingredient Disclosure List

CAS# 67-64-1 is listed on the Canadian Ingredient Disclosure List.

Exposure Limits

CAS# 67-64-1: OEL-AUSTRALIA:TWA 500 ppm (1185 mg/m3);STEL 1000 ppm OEL-AUSTRIA:TWA 750 ppm (1780 mg/m3) OEL-BELGIUM:TWA 750 ppm (1780 mg /m3);STEL 1000 pp OEL-CZECHOSLOVAKIA:TWA 800 mg/m3;STEL 4000 mg/m3 O EL-DENMARK:TWA 250 ppm (600 mg/m3) OEL-FINLAND:TWA 500 ppm (1200 mg/m 3);STEL 625 ppm (1500 mg/m3) OEL-FRANCE:TWA 750 ppm (1800 mg/m3) OEL -GERMANY:TWA 1000 ppm (2400 mg/m3) OEL-HUNGARY:TWA 600 mg/m3;STEL 120 0 mg/m3 OEL-INDIA:TWA 750 ppm (1780 mg/m3);STEL 1000 ppm (2375 mg/m3) OEL-JAPAN:TWA 200 ppm (470 mg/m3) OEL-THE NETHERLANDS:TWA 750 ppm (1780 mg/m3) JAN9 OEL-THE PHILIPPINES:TWA 1000 ppm (2400 mg/m3) OEL-P OLAND:TWA 200 mg/m3 OEL-RUSSIA:TWA 200 ppm;STEL 200 mg/m3 OEL-SWEDEN :TWA 250 ppm (600 mg/m3);STEL 500 ppm (1200 mg/m3) OEL-SWITZERLAND:TW A 750 ppm (1780 mg/m3) OEL-TURKEY:TWA 1000 ppm (2400 mg/m3) OEL-UNIT ED KINGDOM:TWA 750 ppm (1810 mg/m3);STEL 1250 ppm OEL IN BULGARIA, CO LOMBIA, JORDAN, KOREA check ACGIH TLV OEL IN NEW ZEALAND, SINGAPORE, VIETNAM check ACGI TLV

Section 16 - Additional Information

Date of Issue : 06/09/2010

Material Safety Data Sheet
ETHANOL

Section 1 - Chemical Product and Company Identification

MSDS Name: ETHANOL
Synonyms: Ethyl alcohol; Ethyl hydroxide; Fermentation alcohol; Grain alcohol; Methylcarbinol, Ethanol absolute 99.9%, Ethanol rectified 96%.
Company Identification:
ROCHELLE CHEMICALS & LAB EQUIPMENT cc
54 Meson Road, Electron, Johannesburg, South Africa
For information, call: +27 11 613 5638
Emergency Number: +27 83 269 7693

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
64-17-5	Ethyl alcohol	95-99.9	200-578-6
7732-18-5	Water	1-5	231-791-2

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: colorless clear liquid. Flash Point: 16.6 deg C.
Warning! Causes severe eye irritation. **Flammable liquid and vapor.** Causes respiratory tract irritation. This substance has caused adverse reproductive and fetal effects in humans. May cause central nervous system depression. May cause liver, kidney and heart damage. Causes moderate skin irritation.
Target Organs: Kidneys, heart, central nervous system, liver.

Potential Health Effects

Eye: Causes severe eye irritation. May cause painful sensitization to light. May cause chemical conjunctivitis and corneal damage.
Skin: Causes moderate skin irritation. May cause cyanosis of the extremities.
Ingestion: May cause gastrointestinal irritation with nausea, vomiting and diarrhea. May cause systemic toxicity with acidosis. May cause central nervous system depression, characterized by excitement, followed by headache, dizziness, drowsiness, and nausea. Advanced stages may cause collapse, unconsciousness, coma and possible death due to respiratory failure.

Inhalation: Inhalation of high concentrations may cause central nervous system effects characterized by nausea, headache, dizziness, unconsciousness and coma. Causes respiratory tract irritation. May cause narcotic effects in high concentration. Vapors may cause dizziness or suffocation.
Chronic: May cause reproductive and fetal effects. Laboratory experiments have resulted in mutagenic effects. Animal studies have reported the development of tumors. Prolonged exposure may cause liver, kidney, and heart damage.

Section 4 - First Aid Measures

Eyes: Get medical aid. Gently lift eyelids and flush continuously with wate r.
Skin: Get medical aid. Wash clothing before reuse. Flush skin with plenty of soap and water.
Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid.
Inhalation: Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid. Do NOT use mouth-to-mouth resuscitation.
Notes to Physician: Treat symptomatically and supportively. Persons with skin or eye disorders or liver, kidney, chronic respiratory diseases, or central and peripheral nervous sytem diseases may be at increased risk from exposure to this substance.
Antidote: Replace fluid and electrolytes.

Section 5 - Fire Fighting Measures

General Information: Replace fluid and electrolytes. As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. Vapors may form an explosive mixture with air. Vapors can travel to a source of ignition and flash back. Will burn if involved in a fire. Flammable Liquid. Can release vapors that form explosive mixtures at temperatures above the flashpoint. Use water spray to keep fire-exposed containers cool. Containers may explode in the heat of a fire.
Extinguishing Media: For small fires, use dry chemical, carbon dioxide, water spray or alcohol-resistant foam. For large fires, use water spray, fog, or alcohol-resistant foam. Use water spray to cool fire-exposed containers. Water may be ineffective. Do NOT use straight streams of water.
Flash Point: 16.6 deg C (61.88 deg F)
Autoignition Temperature: 363 deg C (685.40 deg F)
Explosion Limits, Lower:3.3 vol %
Upper: 19.0 vol %
NFPA Rating: (estimated) Health: 2; Flammability: 3; Instability: 0

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section

8.
Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Remove all sources of ignition. Use a spark-proof tool. Provide ventilation. A vapor suppressing foam may be used to reduce vapors.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Use only in a well-ventilated area. Ground and bond containers when transferring material. Use spark-proof tools and explosion proof equipment. Avoid contact with eyes, skin, and clothing. Empty containers retain product residue, (liquid and/or vapor), and can be dangerous. Keep container tightly closed. Keep away from heat, sparks and flame. Avoid ingestion and inhalation. Do not pressurize, cut, weld, braze, solder, drill, grind, or expose empty containers to heat, sparks or open flames.
Storage: Keep away from heat, sparks, and flame. Keep away from sources of ignition. Store in a tightly closed container. Keep from contact with oxidizing materials. Store in a cool, dry, well-ventilated area away from incompatible substances. Flammables-area. Do not store near perchlorates, peroxides, chromic acid or nitric acid.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Use explosion-proof ventilation equipment. Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits.
Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Ethyl alcohol	1000 ppm TWA	1000 ppm TWA; 1900 mg/m3 TWA 3300 ppm IDLH	1000 ppm TWA; 1900 mg/m3 TWA
Water	none listed	none listed	none listed

OSHA Vacated PELs: Ethyl alcohol: 1000 ppm TWA; 1900 mg/m3 TWA Water: No OSHA Vacated PELs are listed for this chemical.
Personal Protective Equipment
Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin exposure.
Respirators: A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant respirator use.

Section 9 - Physical and Chemical Properties

Physical State: Clear liquid
Appearance: colorless
Odor: Mild, rather pleasant, like wine or whis
pH: Not available.
Vapor Pressure: 59.3 mm Hg @ 20 deg C
Vapor Density: 1.59
Evaporation Rate: Not available.
Viscosity: 1.200 cP @ 20 deg C
Boiling Point: 78 deg C
Freezing/Melting Point:-114.1 deg C
Decomposition Temperature: Not available.
Solubility: Miscible.
Specific Gravity/Density:0.790 @ 20°C
Molecular Formula:C2H5OH
Molecular Weight:46.0414

Section 10 - Stability and Reactivity

Chemical Stability: Stable under normal temperatures and pressures.
Conditions to Avoid: Incompatible materials, ignition sources, excess heat, oxidizers.
Incompatibilities with Other Materials: Strong oxidizing agents, acids, alkali metals, ammonia, hydrazine, peroxides, sodium, acid anhydrides, calcium hypochlorite, chromyl chloride, nitrosyl perchlorate, bromine pentafluoride, perchloric acid, silver nitrate, mercuric nitrate, potassium-tert-butoxide, magnesium perchlorate, acid chlorides, platinum, uranium hexafluoride, silver oxide, iodine heptafluoride, acetyl bromide, disulfuryl difluoride, tetrachlorosilane + water, acetyl chloride, permanganic acid, ruthenium (VIII) oxide, uranyl perchlorate, potassium dioxide.
Hazardous Decomposition Products: Carbon monoxide, irritating and toxic fumes and gases, carbon dioxide.
Hazardous Polymerization: Will not occur.

Section 11 - Toxicological Information

RTECS#:
CAS# 64-17-5: KQ6300000
CAS# 7732-18-5: ZC0110000
LD50/LC50:
CAS# 64-17-5:
Draize test, rabbit, eye: 500 mg Severe;
Draize test, rabbit, eye: 500 mg/24H Mild;
Draize test, rabbit, skin: 20 mg/24H Moderate;
Inhalation, mouse: LC50 = 39 gm/m3/4H;
Inhalation, rat: LC50 = 20000 ppm/10H;
Oral, mouse: LD50 = 3450 mg/kg;
Oral, rabbit: LD50 = 6300 mg/kg;
Oral, rat: LD50 = 7060 mg/kg;
Oral, rat: LD50 = 9000 mg/kg;

.

CAS# 7732-18-5:
Oral, rat: LD50 = >90 mL/kg;
.

Carcinogenicity:
CAS# 64-17-5: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
CAS# 7732-18-5: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: Ethanol has been shown to produce fetotoxicity in the embryo or fetus of laboratory animals.Prenatal exposure to ethanol is associated with a distinct pattern of congenital malformations that have collectively been termed the "fetal alcohol syndrome".
Teratogenicity: Oral, Human - woman: TDLo = 41 gm/kg (female 41 week(s) after conception) Effects on Newborn - Apgar score (human only) and Effects on Newborn - other neonatal measures or effects and Effects on Newborn - drug dependence.
Reproductive Effects: Intrauterine, Human - woman: TDLo = 200 mg/kg (female 5 day(s) pre-mating) Fertility - female fertility index (e.g. # females pregnant per # sperm positive females; # females pregnant per # females mated).
Mutagenicity: DNA Inhibition: Human, Lymphocyte = 220 mmol/L.; Cytogenetic Analysis: Human, Lymphocyte = 1160 gm/L.; Cytogenetic Analysis: Human, Fibroblast = 12000 ppm.; Cytogenetic Analysis: Human, Leukocyte = 1 pph/72H (Continuous).; Sister Chromatid Exchange: Human, Lymphocyte = 500 ppm/72H (Continuous).
Neurotoxicity: No information found
Other Studies:

Section 12 - Ecological Information

Ecotoxicity: Fish: Rainbow trout: LC50 = 12900-15300 mg/L; 96 Hr; Flow-through @ 24-24.3°CFish: Rainbow trout: LC50 = 11200 mg/L; 24 Hr; Fingerling (Unspecified)Bacteria: Phytobacterium phosphoreum: EC50 = 34900 mg/L; 5-30 min; Microtox test When spilled on land it is apt to volatilize, biodegrade, and leach into the ground water, but no data on the rates of these processes could be found. Its fate in ground water is unknown. When released into water it will volatilize and probably biodegrade. It would not be expected to adsorb to sediment or bioconcentrate in fish.
Environmental: When released to the atmosphere it will photodegrade in hours (polluted urban atmosphere) to an estimated range of 4 to 6 days in less polluted areas. Rainout should be significant.
Physical: No information available.
Other: No information available.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
RCRA P-Series: None listed.
RCRA U-Series: None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	ETHANOL	No information available.
Hazard Class:	3	
UN Number:	UN1170	
Packing Group:	II	

Section 15 - Regulatory Information

US FEDERAL

TSCA

CAS# 64-17-5 is listed on the TSCA inventory.
CAS# 7732-18-5 is listed on the TSCA inventory.

Health & Safety Reporting List

None of the chemicals are on the Health & Safety Reporting List.

Chemical Test Rules

None of the chemicals in this product are under a Chemical Test Rule.

Section 12b

None of the chemicals are listed under TSCA Section 12b.

TSCA Significant New Use Rule

None of the chemicals in this material have a SNUR under TSCA.

CERCLA Hazardous Substances and corresponding RQs

None of the chemicals in this material have an RQ.

SARA Section 302 Extremely Hazardous Substances

None of the chemicals in this product have a TPQ.

SARA Codes

CAS # 64-17-5: immediate, delayed, fire.

Section 313 No chemicals are reportable under Section 313.

Clean Air Act:

This material does not contain any hazardous air pollutants.
This material does not contain any Class 1 Ozone depletors.
This material does not contain any Class 2 Ozone depletors.

Clean Water Act:

None of the chemicals in this product are listed as Hazardous Substances under the CWA.
None of the chemicals in this product are listed as Priority Pollutants under the CWA.
None of the chemicals in this product are listed as Toxic Pollutants under the CWA.

OSHA:

None of the chemicals in this product are considered highly hazardous by OSHA.

STATE

CAS# 64-17-5 can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Minnesota, Massachusetts.
CAS# 7732-18-5 is not present on state lists from CA, PA, MN, MA, FL, or NJ.

California Prop 65

WARNING: This product contains Ethyl alcohol, a chemical known to the state of California to cause developmental reproductive toxicity.
California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations

European Labeling in Accordance with EC Directives

Hazard Symbols:

F

Risk Phrases:

R 11 Highly flammable.

Safety Phrases:

S 16 Keep away from sources of ignition - No smoking.
S 33 Take precautionary measures against static discharges.
S 7 Keep container tightly closed.
S 9 Keep container in a well-ventilated place.

WGK (Water Danger/Protection)

CAS# 64-17-5: 0
CAS# 7732-18-5: No information available.

Canada - DSL/NDSL

CAS# 64-17-5 is listed on Canada's DSL List.
CAS# 7732-18-5 is listed on Canada's DSL List.

Canada - WHMIS

This product has a WHMIS classification of D2B.
This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.

Canadian Ingredient Disclosure List

CAS# 64-17-5 is listed on the Canadian Ingredient Disclosure List.

Section 16 - Additional Information

Date of Issue : 10/09/2010

1. Identification of the substance/preparation and of the company/undertaking

Identification of the product:
Product number: TO0077
Name of material: Toluene, HPLC grade

Use of the substance/preparation:
synthesis of organic products, solvents, as gasoline additive.
Manufacturer/supplier identification:
Company:

Scharlau Chemie S.A.
Ctra. Polinyà-Sentmenat Km. 8.2
08081 Sentmenat (Barcelona) SPAIN
Tel. 34-93 715 18 11- FAX 34-93 715 31 75

Regional representation:

Scharlau Chemie S.A.
Ctra. Polinyà-Sentmenat Km. 8.2
08081 Sentmenat (Barcelona) SPAIN
Tel. 34-93 715 18 11 - FAX 34-93 715 31 75
E-mail: export@scharlau.com
Internet Web Site:
http://www.scharlau.com

Emergency telephone:
Scharlau Chemie, S.A. 34 - 93 715 18 11

2. Composition/information on ingredients

Identification and amount of the components:
Synonyms:Methylbenzene. Phenylmethane
CAS: 108-88-3
Molecula weight:92.14
EC index no,:601-021-00-3
EC number:203-625-9
Formula: C7H8

3. Hazards identification

Hazards classification for the substance according to the european directives:
Highly flammable. Harmful by inhalation.

4. First aid measures

After inhalation: Fresh air. If necessary, apply mechanical ventilation or mouth-to-mouth resuscitation . If necessary oxygen mask. Summon doctor.
After skin contact: wash off with plenty of water. Immediately change contaminated clothing.
After ingestion: Avoid vomiting (risk of aspiration). Keep airways free. Immediately summon doctor.
After eye contact: wash off with plenty of water. Summon eye specialist.

5. Fire-fighting measures

Suitable extinguishing media: Powder, foam.
Special risks: combustible. Vapours heavier than air. Formation of explosive mixtures possible with air at normal temperatures. Formation of combustion gases or dangerous vapours possible in event of fire.
Special protective equipment for fire fighting: Do not stay in dangerous zone without suitable chemical protection clothing and self-contained breathing apparatus.
Further information: Take measures to prevent electrostatic charging. Cool container with spray water from a save distance. Prevent fire-fighting water from entering surface water or groundwater.

6. Accidental release measures

Person-related precautionary measures: Do not inhale vapours/aerosols. Ensure supply of fresh air in enclosed rooms. Avoid substance contact.
Environmental precautions: Do not allow to enter sewerage system (risk of explosion!).
Procedures for cleaning / absorption: Take up with liquid-absorbent material. Forward for disposal. Clean up.

7. Handling and storage

Handling: Keep away from sources of ignition. Take measures to prevent electrostatic charging.
Storage: Tightly closed in a well-ventilated place, away from sources of ignition and heat. Storage temperature: no restrictions.

8. Exposure controls/personal protection

Exposure limit values: (MAK, Germany): 50 ml/m3 , 190 mg/m3
Exposure controls:
Occupational exposure controls: The personal protective equipment must be selected according to the working place, based on the concentration and amount of the dangerous substance. The supplier should indicate the stability of the personal protective equipment to chemical reagents.
Respiratory protection: required when vapours/aerosols are generated.
Hand protection: required
Eye protection: required
Skin protection: Application of skin-protective barrier cream recommended.
Industrial hygiene: Change contaminated clothing. Wash hands and face after working with substance.

9. Physical and chemical properties

General information:
Form: liquid
Colour: colourless
Odour: characteristic
Important health, safety and environmental information:
pH value: ---
Boiling temperature: 111 ºC
Flash point: 4 ºC
Explosion limits (low): 1,2 Vol%
Explosion limits (high): 8 Vol%
Vapour pressure: (20 ºC) 29 hPa
Density (20 °C): 0,87 g/cm3
Solubility in water: (20 °C): 0,52 g/l
Partition coefficient n-octanol/water: log P(o/w): 2,65 (experimentally)
Viscosity: (20 ºC) 0,58 mPas
Relative vapour density: 3,18
Refractive index: ---
Melting temperature: -95 ºC
Ignition temperature: 535 ºC

10. Stability and reactivity

Conditions to be avoided: Strong heating.
Substances to be avoided: nitric acid, halogen-halogen compounds, nitrogen oxides, organic nitro compounds, oxidizing agents, sulfur.
Hazardous decomposition products: No information available.
Further information:
Highly flammable.
Unsuitable working materials: rubber, various plastics.
Explosible with air in a vaporous/gaseous state.

11. Toxicological information

Acute toxicity:
The literature data available to us do not conform with the labelling prescribed by the EC. The EU has dossiers which have not been published.
LD_{50}(oral, rat): 636 mg/kg
LC_{50} (inhalation, rat): 49 mg/l /4h.
LD_{50} (dermal, rabbit): 12124mg/kg
Subacute to chronic toxicity:
Animal experiments suggest that the substance may lead to an impairment of reproductive performance also in man.
Mutagenicity: Bacterian mutagenicity: Ames-Test: negative
Mutagenicity (test of mammal cells): negative
Teratogenicity: The possibility of an embryotoxic effect has not yet been fully assessed.
Further toxicological information:
After inhalation: Irritating to respiratory system, headhache, drowsiness, dizziness, absorption.
After skin contact: Slight irritations; by prolonged exposure: dermatitis, degreasing effect on the skin, possibly followed by secondary inflammation. Danger of skin absorption.
After eye contact: Slight irritations, mucosal irritations.
After ingestion: nausea, vomiting, absorption. Risk of aspiration upon vomiting.
After ingestion (large amounts): pneumonia, respiratory paralysis.
After absorption of large quantities: Systemic effect: CNS disorders, inebriation, spasms, unconsciousness, respiratory paralysis, cardiovascular failure, death.
Further information:
The product should be handled with the care usual when dealing with chemicals.

12. Ecological information

Ecotoxic effects: Damage of aquatic organisms. Toxic effect on fish and plankton. Change in the flavour characteristics of fish protein.
Fish toxicity:
C. auratus CL_{50} : 13 mg/l /96h.
P. promelas CL_{50} : 36,2 mg/l /96h. (in soft water).
Daphnia toxicity: Daphnia magna EC_{50}: 11,5 mg/l /48h.
Algeal toxicity: Selenastrum capricornutum IC_{50} : 12 mg/l /72h.
Bacterial toxicity: Photobacterium phosphoreum CE_{50} : 20 mg/l /30min.
Mobility: log P(o/w): 2,65 (experimentally)
Bioaccumulative potential:
Bioconcentration factor: 90
Persistence and degradability: Water-dissolved constituents biodegradable.
Further ecologic data:
BOD_5 : 0,86 g/g; COD: 0,7 g/g.

Do not allow to enter waters, waste water, or soil!

13. Disposal considerations

Product: There are no uniform EU Regulations for the disposal of chemicals or residues. Chemical residues generally count as special waste. The disposal of the latter is regulated in the EU member countries through corresponding laws and regulations. We recommend that you contact either the authorities in charge or approved waste disposal companies which will advise you on how to dispose of special waste.
Packaging: Disposal in compliance with official regulations. Handle contaminated packaging in the same way as the substance itself. If not officially specified differently, non-contaminated packaging may be treated like household waste or recycled.

14. Transport information

Road transport:
UN-No: 1294
ADR class: 3 F1 II
Correct technical name: TOLUENE
Sea transport:
UN-No: 1294
IMDG class: 3 II
Correct technical name: TOLUENE
Air transport:
UN-No: 1294
IATA/ICAO class: 3 II
Correct technical name: TOLUENE

15. Regulatory information

EC-classification: This product has been included in the dangerous substances index with its corresponding EC index number, so it has been classified according to the 67/548/EEC directive and its later adaptations.
Symbol: F (Highly flammable), Xn (Harmful)
R-phrases: 11-20 Highly flammable. Harmful by inhalation.
S-phrases: 16-25-29-33 Keep away from sources of ignition - No Smoking. Avoid contact with eyes. Do not empty into drains. Take precautionary measures against static discharges.
EC-Index-No: 601-021-00-3

16. Other information

Reason for the revision: General update.
Date: 11/2/2003

The information supplied in this data sheet, is based on the present state of our knowledge. The purpose of this information, is only to describe the security measures to follow in the handling of the product. Therefore it does not represent a guarantee about the properties of the product.