UNIVERSITY OF JYVÄSKYLÄ

DEVELOPMENT OF ANALYSIS METHOD FOR DETERMINATION OF MINERAL OIL CONTAMINATION IN CARDBOARD

Master's Thesis

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ABSTRACT

Since mineral oil contamination from food packaging to food has become a public health concern, several laboratories are investigating possibilities to develop a simply and affordable analytical method for measuring mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) in cardboard.

The present study investigated on the behalf of Metsä Board Oy (Finland) an efficient analytical method for the determination of mineral oil in cardboard by using cardboard extraction (sample separation), a solid phase extraction (SPE) process (purification) and gas chromatography-flame ionization (GC-FID) (mineral oil quantitation). Also, mass spectrometry (MS) was used for the identification of MOSH and MOAH components. In general a simple test which is easy to carry out on-site is needed to comply with safety regulations in packaging products.

We found out that when used a glass column with a 10 mm inner diameter and filled with activated silver silica gel (containing 0.3 % $AgNO_3$) as a sorbent bed for the SPE process, the MOSH fraction was eluted with 13 mL of *n*-hexane. The MOAH fraction was eluted with 15 mL of solvent mixture of 70 % dichloromethane (DCM), 25 % *n*-hexane. 5 % toluene and gravitational elution to control the elution speed provided the best results.

In this study, it was suggested that the amount of solvent used during the SPE process will gain intensive attention since the handling and evaporation of it is time consuming.

PREFACE

I present my Master's Thesis "DEVELOPMENT OF ANALYSIS METHOD FOR MINERAL OIL IN CARDBOARD" as a part of my compulsory task to achieve a degree in Master of Science in Applied Chemistry at the University of Jyväskylä - Finland. I had previously a BSc. degree in Chemical Process Engineering from the Central Ostrobothnia University of Applied Sciences - Finland.

This thesis was done for Metsä Board Oy (Finland), and the goal was to develop a proper manual method for the determination of mineral oil in cardboard. This was achieved through cardboard extraction, solid phase extraction (SPE), and GC-FID/MS analyses. The cited publications have been found through Academic Journals, books, and internet.

I would like firstly to thank my supervisor Hannu Pakkanen (PhD) for his time, valuable input and support throughout the entire thesis and master period. Furthermore, I would like to thank Metsä Board Oy for the opportunity they gave me to do this research work and the financial support; especially my thanks goes to Pirita Suortamo (Senior R & D Engineer, MSc) for her collaboration. I would like to express my sincere appreciation to laboratory technician Mrs. Maria Salo.

My deepest gratitude goes to my wife, kids, parents and friends for their love, support and patience during my study time. My gratitude goes also to Professor Raimo Alén, the head of the Laboratory of Applied Chemistry.

Finally, my greatest thank to the Great Architect of the Universe who makes everything possible.

Jyväskylä, May 2016 Jovin GREYE

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ABBREVIATIONS

2MN	2-Methylnaphtalene
ACGIH	American Conference of Governmental Industrial Hygienists
BfR	Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment)
C & L Inventory	Classification & Labelling inventory
CEPI	Confederation of European Paper Industries
Cho	5α-Cholestane
CMP	Chemimechanical pulp
CONTAM	The Panel on Contaminants in Food Chain
CONTAM Panel	Members of the Panel on Contaminants in the Food Chain
Cycy	Cyclohexyl cyclohexane
DART-TOF-MS	Direct analysis in real time-of-flight mass spectrometry
DCM	Dichloromethane (methylene chloride)
DIPN	Di-isopropyl naphthalenes
DIBP	Di-isobutyl phthalate
ECHA	European Chemical Agency
EFPRO	European Fibre and Paper Research Organisation
EFSA	European Food Safety Authority
FBB	Folding boxboard
FT-IR	Fourier transform – infrared

GC	Gas chromatography
GC-FID	Gas chromatography- flame ionization detector
GHS	Globally harmonized system of classification and labelling of chemicals
HPLC-GC	High performance liquid chromatography - gas chromatography
IR	Infrared
ISTD	Internal standard
LC-GC	Liquid chromatography – gas chromatography
LPE	Liquid phase extraction
MF	Machine finished
MF resin	Melamine-formaldehyde resin
MG	Machine glazed
MO	Mineral oil
MOAH	Mineral oil aromatic hydrocarbons
MOE	Margin of exposure
MOH	Mineral oil hydrocarbons
MOSH	Mineral oil saturated hydrocarbons
MPPO	Modified polyphenylene oxide
MS	Mass spectrometer
MSPD	Matrix solid-phase dispersion
NMR	Nuclear magnetic resonance

NOAEL	No-observed-adverse-effect level
PAH (PAHs)	Polyaromatic hydrocarbons
PCBs	Polychlorinated biphenyls
PE	Polyethylene
Per	Perylene
PLE	Pressurized liquid extraction
POSH	Polyolefin oligometric saturated hydrocarbons
PP	Polypropylene
PSE	Pressurized solvent extraction
PTFE	Polytetrafluoroethylene (Teflon)
RP	Reference point
SBB	Solid bleached board
SC	Supercalendered
SCF	Scientific committee on food
SGW	Stone groundwood
SPE	Solid phase extraction
SPME	Solid phase microextraction
STD	Standard
SUB	Sublimation paper
TBB	1,3,5-tri-*tert*-butylbenzene
TEF	Toxicity equivalency factor

TLC	Thin-layer chromatography
TMP	Thermomechanical pulp
UF	Urea - formaldehyde
UF resin	Urea-formaldehyde resin
UHQ water	Ultra high quality water
UV	Ultraviolet
WF	Water finish
WLC	White-lined chipboard

1 INTRODUCTION

Mineral oil (MO) consists of a complex mixture of hydrocarbons with two fractions /1/: i) The mineral oil saturated hydrocarbons (MOSH) comprising linear and branched alkanes as well as alkylsubstituted cyclo-alkanes. ii) The mineral oil aromatic hydrocarbons (MOAH) mainly including alkylsubstituted polyaromatic hydrocarbons. Paper and cardboard are known sources of mineral oil, and they are also used for packaging of food product.

According to numerous research, the migration of MO from packaging to food endangers human health because some of these substances especially MOSH and MOAH can have carcinogenic and mutagenic properties /1 - 6/. Lately the estrogenic activity of aromatic compounds present in mineral oil in printing inks has been proven /7/.

Since mineral oil contamination from food packaging to food became a public health concern, several laboratories are investigating possibilities to develop a simple and affordable analytical method for measuring MOSH and MOAH in cardboard.

The method of choice used in many laboratories involves the cardboard extraction with solvent followed by analysis via high performance liquid chromatography-gas chromatography (HPLC-GC) /8 - 14/. However, this method is very complex, requires expensive equipment and highly knowledgeable operators. Thus, a simple test which is easy to carry out on-site is needed to comply with safety regulations in packaging products /1,15,16/. Although many manual analytical methods have been developed /17 - 26/, it is still difficult to control the eluent speed to make it easily replicable, and to avoid the appearing of MOSH in MOAH fraction.

The present study investigated on the behalf of Metsä Board Oy (Finland) an efficient manual analytical method for the determination of mineral oil in cardboard. This was achieved through the following step:

- Optimisation of GC parameters such as the column choice, injection volume, the temperature inlet and column oven, carrier gas flow and solvent choice.

- Use of a commercial cartridge in cardboard analysis: strata tubes.

- Efficiency test for cardboard extraction by using time factor, cardboard sample mass effect and the solvents suitability.

- Output test (standards) to determine the output of the cartridge by using the standards solutions. The output of the cartridge gives information on the retention capacity of the cartridge.

- Optimisation of solid phase extraction by using activated silver silica gel (containing $AgNO_3$) and solvent mixture.

- Analysis of samples with GC-FID to determine the peaks and the “hump” of unsolved components.

- Mass spectrometry (MS) was used to determine the identity of the peaks. Manual integration was used to calculate the amount of MOSH and MOAH in cardboard.

2 PAPERS AND PAPERBOARD

Beside the use in packaging, paper and paperboard have many applications. These include, for example, newsprint, books, tissues, stationery, photography, money, stamps and general printing. In 2012, paper and paperboard produced for packaging applications accounted for 52% of total world paper and paperboard production /27/. Also, about 44% of the fibre used worldwide was virgin fibre and the rest, 56%, was from recovered paper. Non-wood pulp represents 3.4%. Wood is still the main source of raw material for paper and paperboard production.

2.1 Typical wood composition

Wood is essentially composed of cellulose (41-53%), hemicelluloses (25-41%), lignin (16-33%), extractives (2-5%), inorganics (<1%) and nitrogen compounds (traces) (Fig.1) /28/.

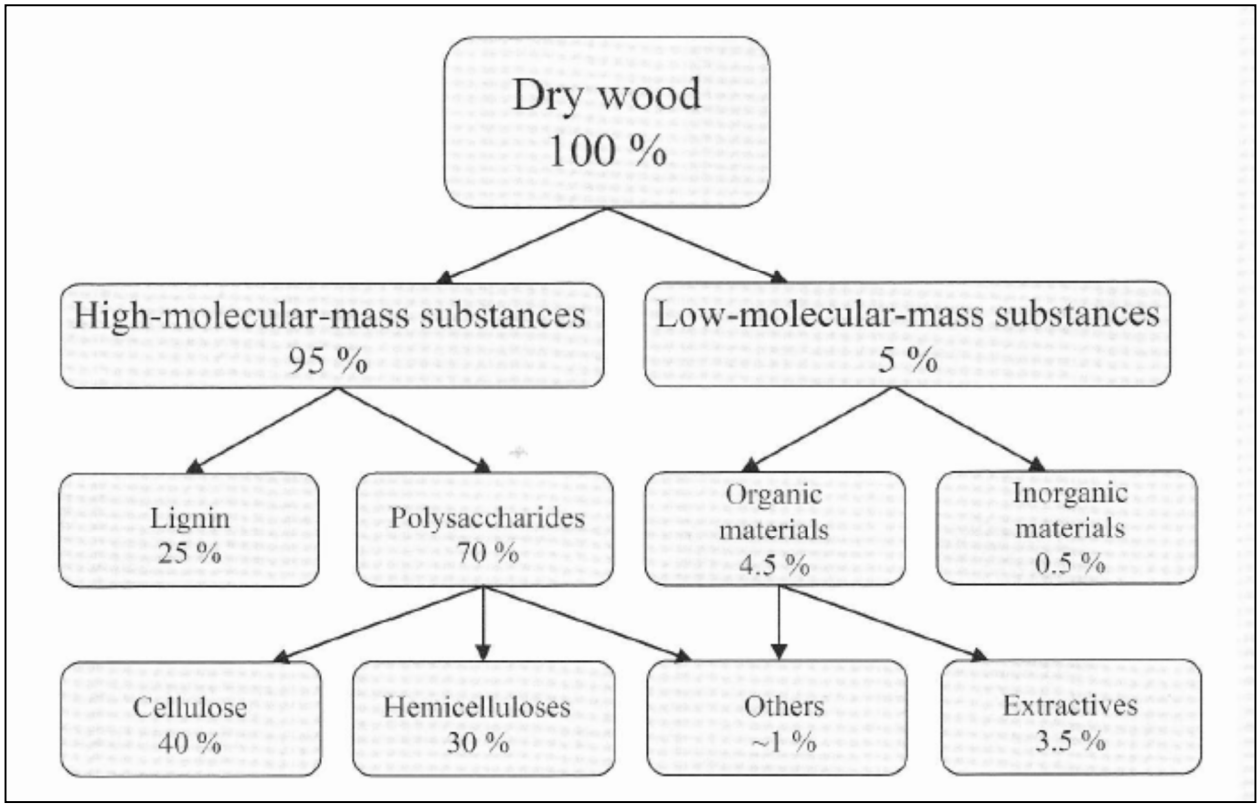


Figure 1. General classification and content of the chemical wood components /28/.

2.1.1 Cellulose

Cellulose, the major (40-45%) chemical component of wood fibre dry weight, is composed of linear chains of D-glucose linked by ß-1,4-glycosidic bonds (Fig. 2) with the degree of polymerisation that range from 10,000 in native wood to 1,000 in bleached kraft pulps /28,29/. Each ß-D glucopyranose unit possesses hydroxyl groups at C2, C3 and C6 positions, capable of undergoing the typical reactions known for primary and secondary alcohols. The molecular structure imparts cellulose with its characteristic properties: hydrophilicity, chirality, degradability and broad chemical variability initiated by the high donor reactivity of hydroxyl groups.

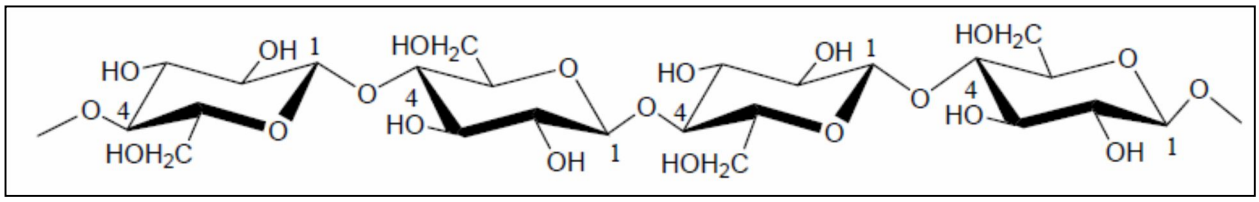


Figure 2. The structure of cellulose /29/.

2.1.2 Hemicelluloses

Hemicelluloses are matrix heteropolysaccharides present in almost all plant cell walls /29/. They are easily hydrolysed by dilute acid or base, and also by hemicellulase enzymes. The main hemicelluloses of softwood are galactoglucomannans and arabinoglucuronoxylan (Fig. 3), while in hardwood glucuronoxylan is the main component.

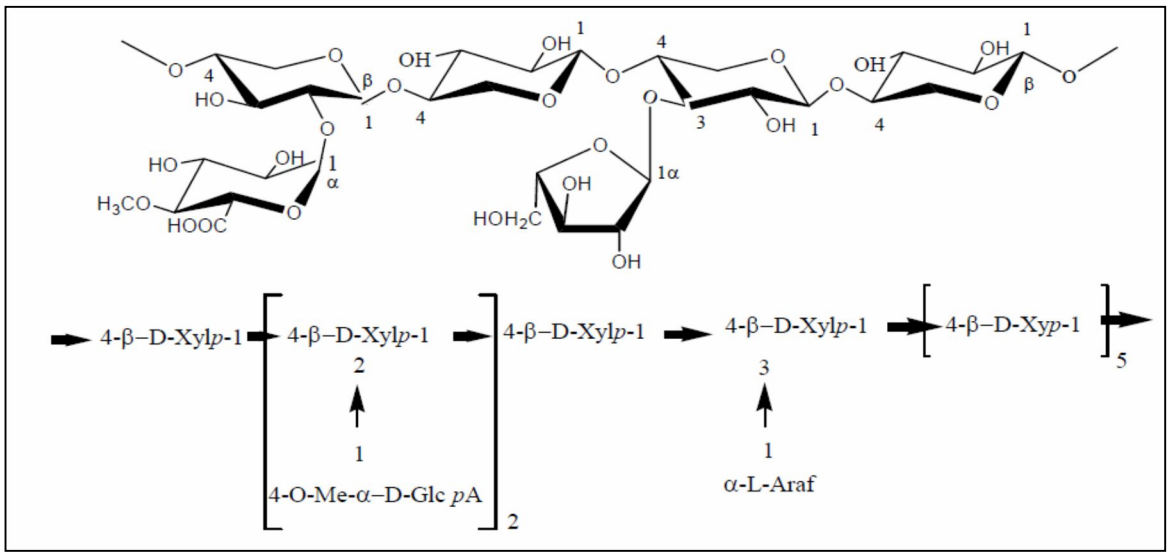


Figure 3. Chemical structure of glucuronoxylan in hardwood /29/.

Hemicellulose building units are hexoses, pentoses and deoxyhexoses. Small amounts of specific uronic acids are also present /28/.

2.1.3 Lignin

Lignin is a complex polymer of aromatic alcohols known as monolignols, binding the cells, fibers and vessels in wood /28/. Lignin can be defined as a polyphenolic material arising primarily from enzymic dehydrogenative polymerisation of three phenylpropanoid unit (Fig. 4): *trans*-coniferyl alcohol, *trans*-sinapyl alcohol and *trans*-*p*-coumaryl alcohol.

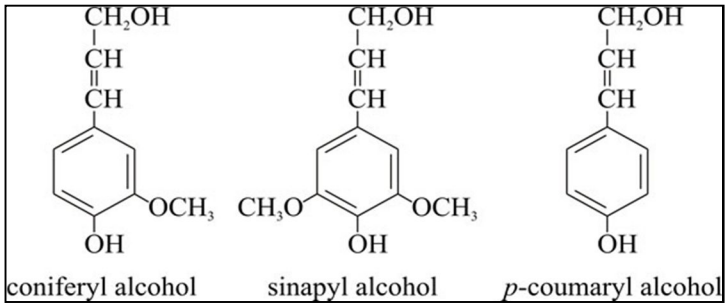


Figure 4. The three phenyl propane monomers in lignin /30/.

2.1.4 Extractives

Extractives are low-molecular-weight compounds present in wood; they can be extracted with neutral organic solvent or water from wood, bark, or foliage /28, 29/. The extractives comprise compounds of both lipophilic and hydrophilic such as: aliphatics, terpenoids, phenolic components, alkanes, proteins, monosaccharides and their derivatives. They play an important role in the pulping and papermaking processes. There are thousands of different extractives present in wood, which are also sources of contaminants in cardboard for food packaging.

2.2 Fibre separation

The processes by which wood or other fibrous feedstocks are converted into a product mass with liberated fibers is called pulping /28/. The pulping process of wood may be based on either mechanical or chemical methods.

2.2.1 Mechanical pulping

Mechanical pulping applies mechanical force to wood in a crushing or grinding action, which generates heat and softens the lignin thereby separating the individual fibres (Fig. 5). As it does not remove lignin, the yield of pulp from wood is very high.

The most basic form of mechanical pulping, which is still in practice in some mills today, involves forcing a debarked tree trunk against a rotating grinding surface /31/. This process uses a large amount of energy and results in a very high-yield product known as stone groundwood (SGW) pulp. Alternatively, lignin can be softened using heat or by the action of certain chemicals; this reduces the mechanical energy needed to separate fibres during pulping and reduces fibre damage, leading to higher quality pulp. Wood in chip form may be heated prior to or during pulping, in which case the pulp is known as thermomechanical pulp (TMP); application of chemicals such as sodium sulphite and sodium hydroxide yields chemimechanical pulp (CMP); and when the two processes are combined, the resulting pulp is called chemi-thermomechanical pulp (CTMP).

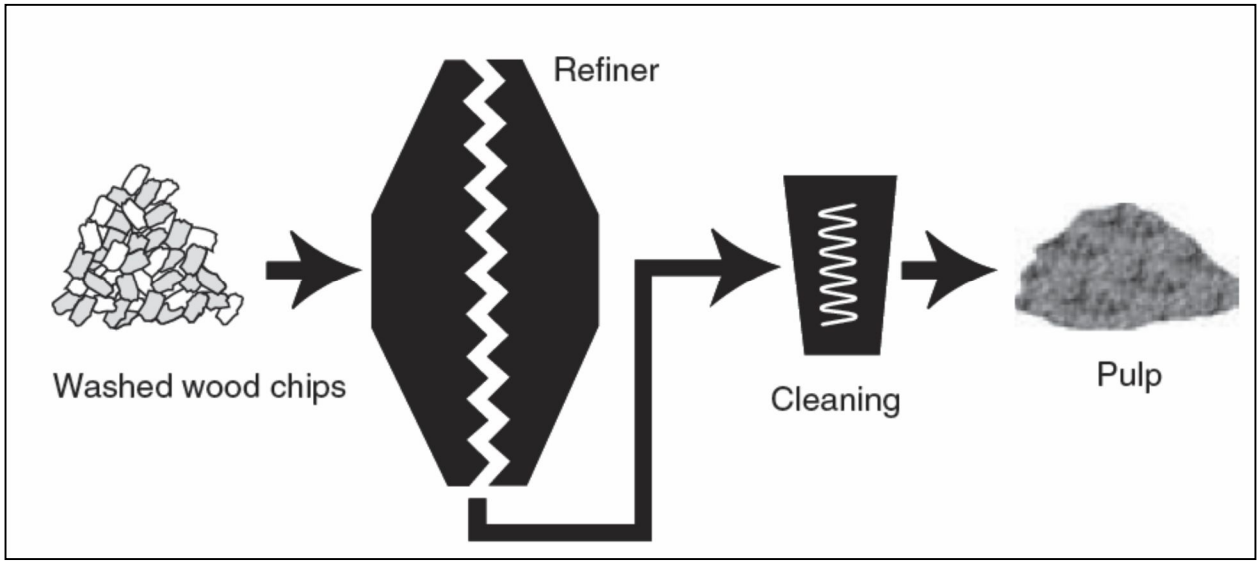


Figure 5. The production of mechanically separated pulp /31/.

2.2.2 Chemical pulping

Chemical pulping uses chemicals to separate the fibres by dissolving the non-cellulose and non-fibrous components of the wood (Fig. 6). There are two main processes characterised by the names of the types of chemicals used:

- The sulphate, or kraft, process uses strong alkali; it is most widely used today because it can operate on all types of wood feedstocks, and the chemicals can be recovered and reused.

- The other main process is known as the acid sulphite process, which uses strong acid.

In both processes, the non-cellulose and non-fibrous material extracted from the wood is used as the main energy source in the pulp mill and in the integrated mills /31/. Chemically separated pulp comprises of 74% of virgin wood fibre production. It has a lower yield than the mechanically separated pulp due to the fact that the non-cellulose constituents of the wood have been removed. This results in pulp which can undergo a high degree of interfibre bonding.

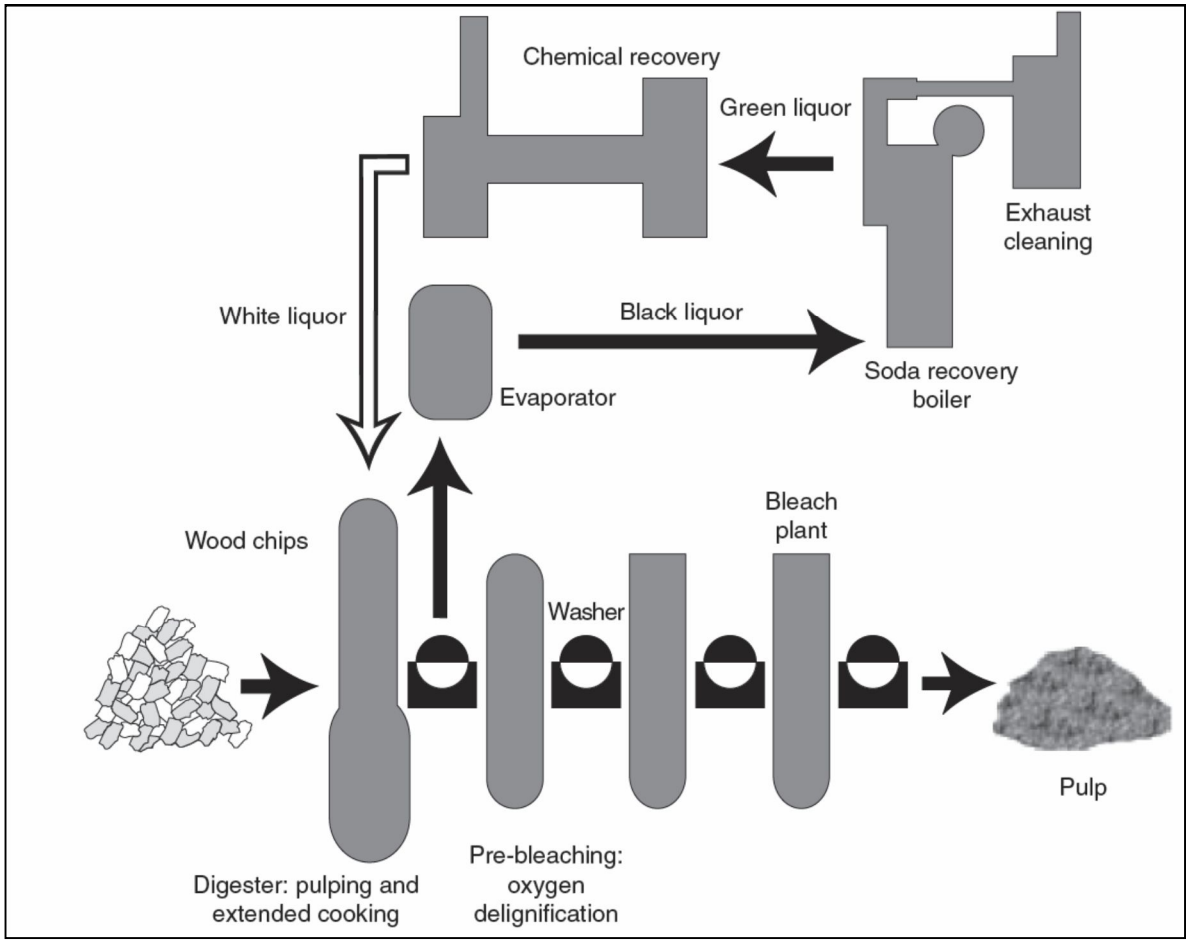


Figure 6. Production of chemically separated bleached pulp /4/.

Kraft pulping

Kraft pulping is the conversion process of wood into pulp by using sodium hydroxide and sodium sulfide in an aqueous solution. In the cooking process the main goal is to facilitate the disintegration of wood into fibrous product such as cellulose, hemicelluloses, lignin and resins. Hemicelluloses can be divided into three major organic group /29/: glucomannan, xylan and other carbohydrate groups. The final product, pulp, is consisting cellulose, hemicellulose with some residue of lignin and resins.

Reaction equation of kraft pulping:

The mixture of sodium hydroxide (NaOH) and sodium sulphide (NaS_2) known as white liquor, is used for the conversion of wood chips into wood pulp.

$$NaOH + NaS_2 + WOOD \longrightarrow Na\text{-org.} + S\text{-org.} + NaHS \qquad \text{(Equation 1)}$$

After cooking, the spent cooking liquor, known as black liquor, is separated by washing from the pulp, is concentrated in an evaporator and then combusted in the recovery furnace for the recovery of cooking chemicals and the generation of energy /28/. The pulp then undergoes different processes such as screening, washing, and bleaching to have the final product.

Sulphite pulping

In sulphite pulping, lignin undergoes two types of reactions /28/: sulphonation and hydrolysis, which are responsible for delignification. Sulphonation generates hydrophilic sulphonic acid ($-SO_3H$) groups, while hydrolysis breaks aryl ether linkage between the phenylpropane units, thus lowering the average molecular mass and creating new free phenolic hydroxyl groups. Both of these reactions increase the hydrophilicity of the lignin and facilitate its water-solubility.

Because of the sensitivity of glycosidic linkages toward acid hydrolysis, depolymerisation of wood polysaccharides cannot be avoided during acid sulphite pulping. Hemicelluloses are attacked more readily than cellulose due to their amorphous state and a relatively low degree of polymerisation.

Kraft pulping vs. sulphite pulping

The production of sulphite pulps is much smaller than the production of kraft pulps. Sulphite pulps are often used in special purposes in papermaking rather than being an alternative market pulp grade for kraft pulps /32/. Very little unbleached sulphite pulp is made and the yield is a little bit higher which can be attributed to the lower pH in the cooking.

The main reasons of more limited applicability of sulphite pulps are as follows:

- It is not possible to use pine as raw material in the acid cooking process which limits the raw material base of sulphite pulping.

- The strength properties of the pulps as measured by the papermaker are generally not as good as those of kraft pulp, although for some specialty pulps these properties may be equally good or even better.

- Environmental problems have in many cases been more expensive to solve and this has decreased the cost-competitively compared to the kraft pulping. The sulphite process is characterised by its high flexibility compared to the kraft process, which is a very uniform method, that can be carried out only with highly alkaline cooking liquor.

2.3 Recycled fibre

Waste paper and paperboard are also collected, sorted and repulped by mechanical agitation in water (Fig. 7) /31/. There are several different qualities of repulped fibre depending on the nature of the original fibre, how it was processed and how the paper or paperboard product was converted and used. Each time paper or paperboard is repulped, the average fibre length and the degree of interfibre bonding is reduced.

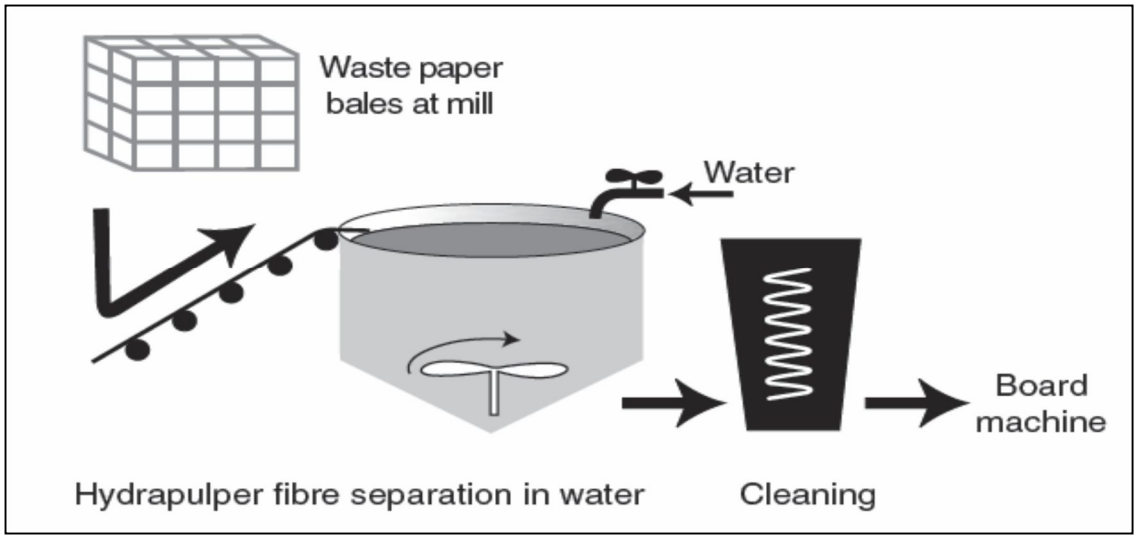


Figure 7. Production of pulp from recovered paper/board (recycling) /31/.

There are several classifications, based on type and source, of recovered paper and paperboard which reflect their value for reuse. Classifications range from 'white shavings' (highly priced), newspapers (medium priced) to 'mixed recovered paper and board' (lowest priced).

2.4 Terminology and classification of paper and paperboard

Preparing fibres for paper manufacture is known as 'stock preparation'. The properties of fibres can be modified by processing and the use of additives at the stock-preparation stage prior to paper or paperboard manufacture /31/. In this way, the papermaker can in theory start with, for example, a suspension of bleached chemically separated fibre in water, and by the use of different treatments produce modified pulps which can be used to make grades as diverse as blotting paper, bag paper or greaseproof paper. The surface structure of the fibre can be modified in a controlled way by mechanical treatment. The classification and terminology of paperboard depends on specific industry, locale and personal choice. The classifications in papers are generally as those described below.

2.4.1 Papers

Tissues

These are lightweight papers with grammages from 12 to 30 g/m^2. The lightest tissues for tea and coffee bags which require a strong porous sheet are based on long fibres such as those derived from manila hemp /31/. To maintain strength during immersion in boiling water, wet strength additives are used. Heat-sealed tea and coffee bags require the inclusion of a heat-sealing fibre, such as PP.

Greaseproof

The fibres are treated (hydrated) so that they become almost gelatinous. Grammage range is 30–70 g/ m^2 /31/.

Glassine

This is a SC greaseproof paper. It is non-porous, greaseproof and can be laminated to paperboard. It may be plasticised with glycerine /31/. It may be embossed, PE coated, aluminium foil laminated, metallised or release-treated with silicone to facilitate product release. Grammage range is 30–80 g/ m^2.

Vegetable parchment

Bleached chemical pulp is made into paper conventionally and then passed through a bath of sulphuric acid, which produces partial hydrolysis of the cellulose surface of the fibres /31/. Some of the surface cellulose is gelatinised and redeposited between the surface fibres forming an impervious layer closing the pores in the paper structure. The process is stopped by chemical neutralisation and the web is thoroughly washed in water. This paper has high grease resistance and wet strength. It can be used in the deep freeze (i.e. −20°C storage environment) and in both conventional and microwave ovens. It can be silicone treated for product release. Grammage range is 30–230 g/m^2.

Label paper

These may be coated, machine glazed (MG), wood-free (FW) or MF (machine finished – calendered) kraft papers (100% sulphate chemical pulp) in the grammage range 70–90 g/m^2 /31/. The paper may be coated on-machine or cast coated for the highest gloss in an off-machine or secondary process. The term 'finish' in the paper industry refers to the surface appearance. This may be:

- MF – smooth but not glazed.
- WF – where one or both sides are dampened and calendered to be smoother and glossier than MF.
- MG – with high gloss on one side only.
- SC – which is dampened and polished off-machine to produce high gloss on both sides.

Depending on the environment in which the label is to be used, various functional chemicals may need to be added, for example for labelling packages containing fatty products, grease-resistant chemicals, such as fluorocarbons, may be included.

Bag papers

It has several uses for wrapping and for bags where it may have an MG and a ribbed finish. Thinner grades may be used for lamination with aluminium foil and PE for use on form, fill, seal machines /31/. For sugar or flour bags, coated or uncoated bleached kraft in the range 90–100 g/ m^2 is used.

Sack kraft

Paper used in wet conditions needs to retain considerable strength, at least 30%, when saturated with water /31/. To achieve this, resins such as UF and MF are added to the stock. These chemicals cross-link

during drying and are deposited on the surface of the cellulose fibres making them more resistant to water absorption.
Microcreping, as achieved for example by the Clupak process, builds an almost invisible crimp into paper during drying, enabling it to stretch up to 7%. When used in paper sacks, this feature improves the ability of the paper to withstand dynamic stresses, such as occur when sacks are dropped.

Impregnated papers

Papers are made for subsequent impregnation off-machine. Such treatment can, for example, be with wax, vapour phase inhibitor for metal packaging and mould inhibitors for soap wrapping /31/.

Laminating papers

Coated and uncoated papers based on both kraft (sulphate) and sulphite pulps can be laminated to aluminium foil and extrusion coated with PE. The grammage range is 40–80 g/ m^2 /31/.

2.4.2 Paperboard

Solid bleached board (SBB)

This board is made exclusively from bleached chemical pulp. It usually has a mineral pigment-coated top surface, and some grades are also coated on the back. This paperboard has excellent surface and printing characteristics /31/. It gives wide scope for innovative structural designs and can be embossed, cut, creased, folded and glued with ease. This is a pure cellulose primary (virgin) paperboard with consistent purity for food product safety, making it the best choice for the packaging of aroma and flavor-sensitive products. SUB is used where there is a high strength requirement in terms of puncture and tear resistance and/or good wet strength is required such as for bottle or can multipacks and as a base for liquid packaging.

Folding boxboard (FBB)

This board comprises middle layers of mechanical pulp sandwiched between layers of bleached chemical pulp /31/. The top layer of bleached chemical pulp is usually coated with a white mineral pigment coating. The back is cream (manila) in colour. This paperboard is a primary (virgin fibre) product with consistent purity for food product safety and suitable for the packing of aroma- and flavour-sensitive products. It is used for packing confectionery, frozen, chilled and dry foods, healthcare products, cigarettes, cosmetics, toys, games and photographic products.

White-lined chipboard

White-lined chipboard (WLC) consists of middle plies of recycled pulp recovered from mixed papers or carton waste. The top layer, or liner, of bleached chemical pulp is usually white mineral pigment coated. The second layer, or under liner, may also comprise bleached chemical pulp or mechanical pulp. This product is also known as newsboard or chipboard. The overall content of WLC varies from about 80 to 100% recovered fibre depending on the choice of fibre used in the various layers. WLC is widely used for dry foods, frozen and chilled foods, toys, games, household products.

2.5 Packaging papers and paperboards advantages

In an age where environmental and waste management issues have a high profile, packaging based on paper and paperboard (Fig. 8) has important advantages /32/: The majority of paper-based packaging grades are now produced using recycled recovered fibre. As such, paper and paperboard packaging forms a very important end product for the recovered paper sector. The main raw material (wood or other suitable vegetation) is based on a naturally renewable resource. In most cases it is sustainably sourced from certified plantations. The growth of these raw materials removes carbon dioxide from the atmosphere, thereby reducing the greenhouse effects. As such they have a smaller carbon footprint than materials made from non-renewable resources, such as petrochemical derivatives. When the use of the package is completed, most types of paper and paperboard packaging can be recovered and recycled. Furthermore, they can all be incinerated with energy recovery, and if none of these options is possible, most are biodegradable in landfill.

Figure 8. Paperboard and cardboard (left) /33/ and paperboard boxes (right) /34/.

3 METSÄ GROUP Oy

Metsä Group can trace its origins back to 1934. Metsä Group focuses on five core businesses: tissue and cooking papers (Metsä Tissue), consumer packaging paperboards (Metsä Board), pulp (Metsä Fibre), wood products (Metsä Wood), and wood supply and forest services (Metsä Forest) /35/. Metsä Group's sales totalled EUR 4.9 billion in 2013, and it employs approximately 11,000 people. The Group operates in some 30 countries.

Metsä Board

Metsä Board is Europe's leading primary folding boxboard and white-top liner producer and a major paper supplier such as high-quality office paper, cast-coated paper and board and wallpaper base /35/. Lightweight and ecological consumer packaging based on fresh forest fibres, and high performance cartonboards for consumer packaging are the core strength of Metsä Board. This company also offers high quality papers for office use, specialty papers for labels, forms and several other end uses.

Metsä Board focuses on renewing product concepts and improving the efficiency of their production units.

4 MINERAL OIL

Mineral oil hydrocarbons (MOH) are hydrocarbons containing 10 - 50 carbon atoms /1/. Crude mineral oils are by far the predominant source of the MOH, but equivalent products can be synthesised from coal, natural gas or biomass.

MOH consist of the three major classes of compounds /1, 14/: paraffin (comprising linear and branched alkanes), naphthenes (comprising alkyl substituted cyclo-alkanes), and aromatics (including polyaromatic hydrocarbons (PAHs), which are generally alkyl-substituted and only contain minor amounts of non-alkylated PAHs). Mineral oil aromatic hydrocarbons (MOAH) may also contain minor amount of nitrogen- and sulphur-containing compounds. Contamination with polyolefin oligomeric saturated hydrocarbons (POSH), example from plastic bags, heat sealable layers or adhesives may interfere with MOSH analysis.

4.1 Mineral oil saturated hydrocarbons

Among MOSH, sub-classes should be distinguished based on molecular mass ranges and structure. Two sub-classes were identified based on molecular mass /1/: MOSH up to n-C_{16} and MOSH from n-C_{16} to n-C_{35}. Based on the MOSH structure, distinction should be made among *n*-alkanes, branched alkanes and cyclic alkanes. Additionally, hydrocarbons with structures similar to MOSH, such as polyalphaolefins and oligomeric polyolefin (POSH), should be distinguished from the MOSH.

MOSH fractions in packaging

The fractions of MOSH in packaging according to the migration phase /17/:

- $<C_{16}$
- C_{16}-C_{24} : Limit for migration through gas phase
- C_{24}-C_{35} : Limit for migration with wetting contact

Structure of MOSH

Mineral oil saturated hydrocarbons are branched or unbranched alkyl groups, the structure of MOSH found in crude oil are presented in Figure. 9.

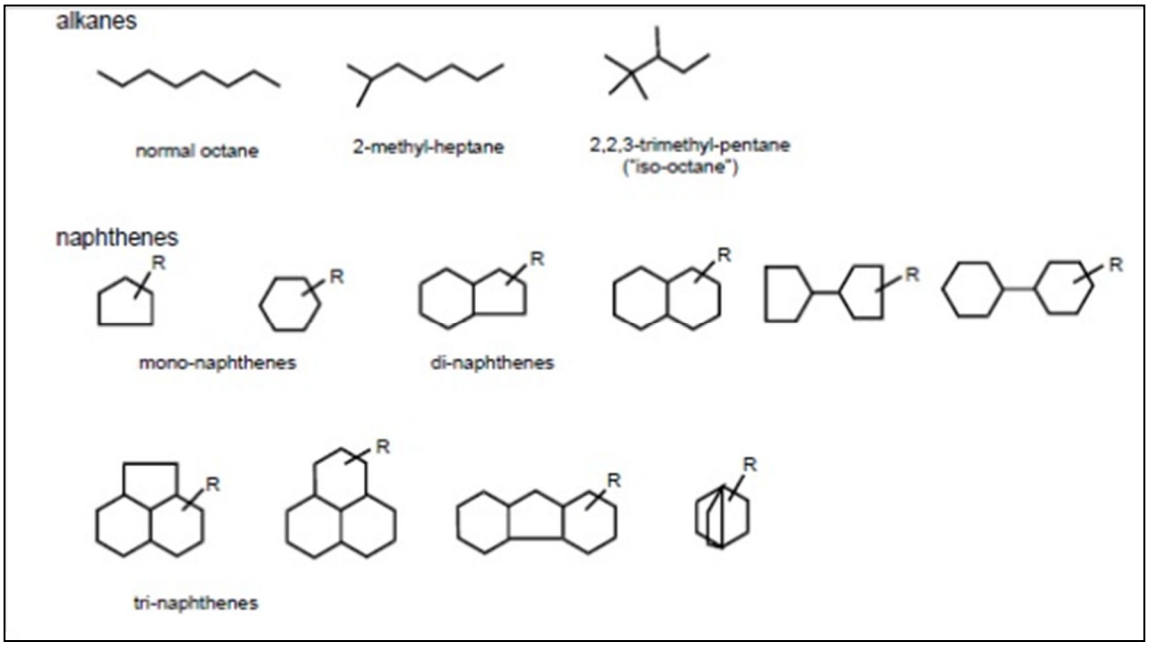


Figure 9. Examples of the different classes of MOSH found in crude oil /1/. R, branched or unbranched alkyl groups with 0 to > 20 C-atoms.

4.2 Mineral oil aromatic hydrocarbons

MOAH are highly alkylated mono- and/or polyaromatic hydrocarbons from mineral oil. In partially hydrogenated mineral oils both, saturated and aromatic rings can be found. Hydrocarbons having at least one aromatic ring are considered as MOAH, even if they predominantly consist of saturated carbons. Technical grades of MOH typically contain 15-35 % MOAH.

MOAH structure and classification in packaging

The MOAH fractions are classified according to the migration phase of the components /1,18,36/:

- $<C_{24}$: Migration through gas phase
- C_{24}-C_{35} : Migration with wetting contact

Structure of MOAH

The different classes of MOAH found in crude oil are described in Figure 10.

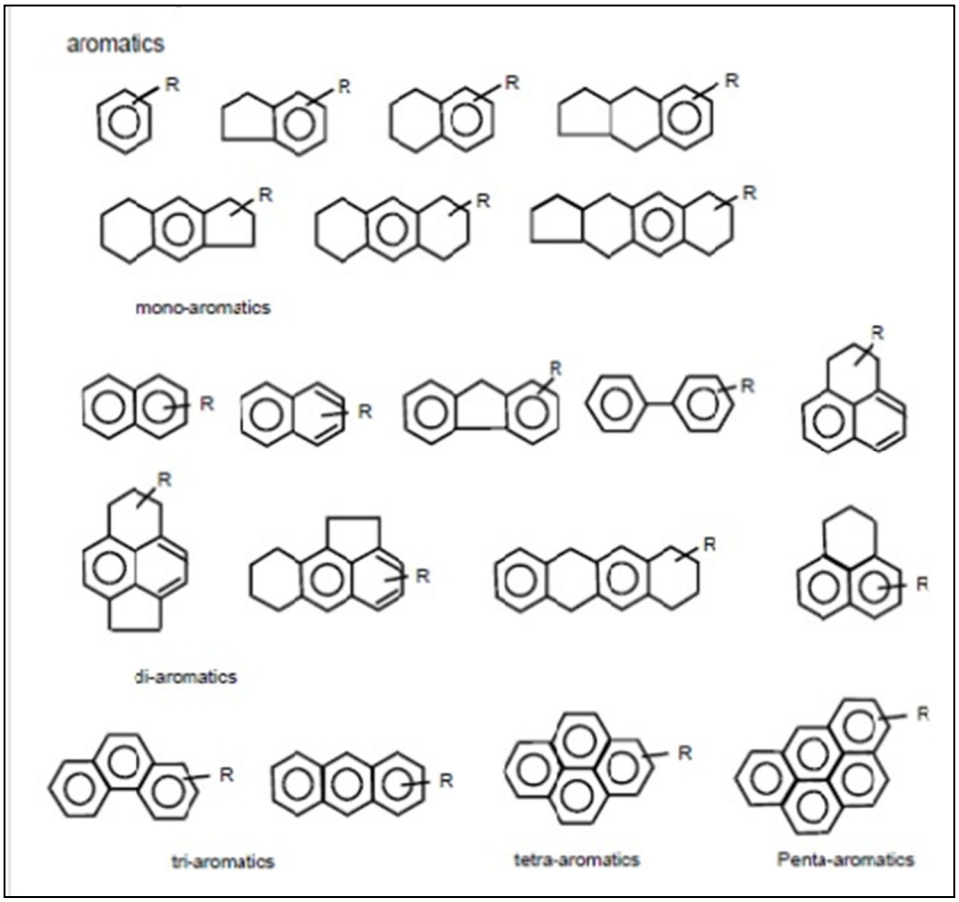


Figure 10. Examples of the different classes of MOAH found in crude oil /1/. R, branched or unbranched alkyl groups with 0 to > 20 C-atoms.

4.3 Structure of mineral oil with sulphur and nitrogen compounds

Mineral oil comprises also sulphur and nitrogen compounds (Fig. 11).

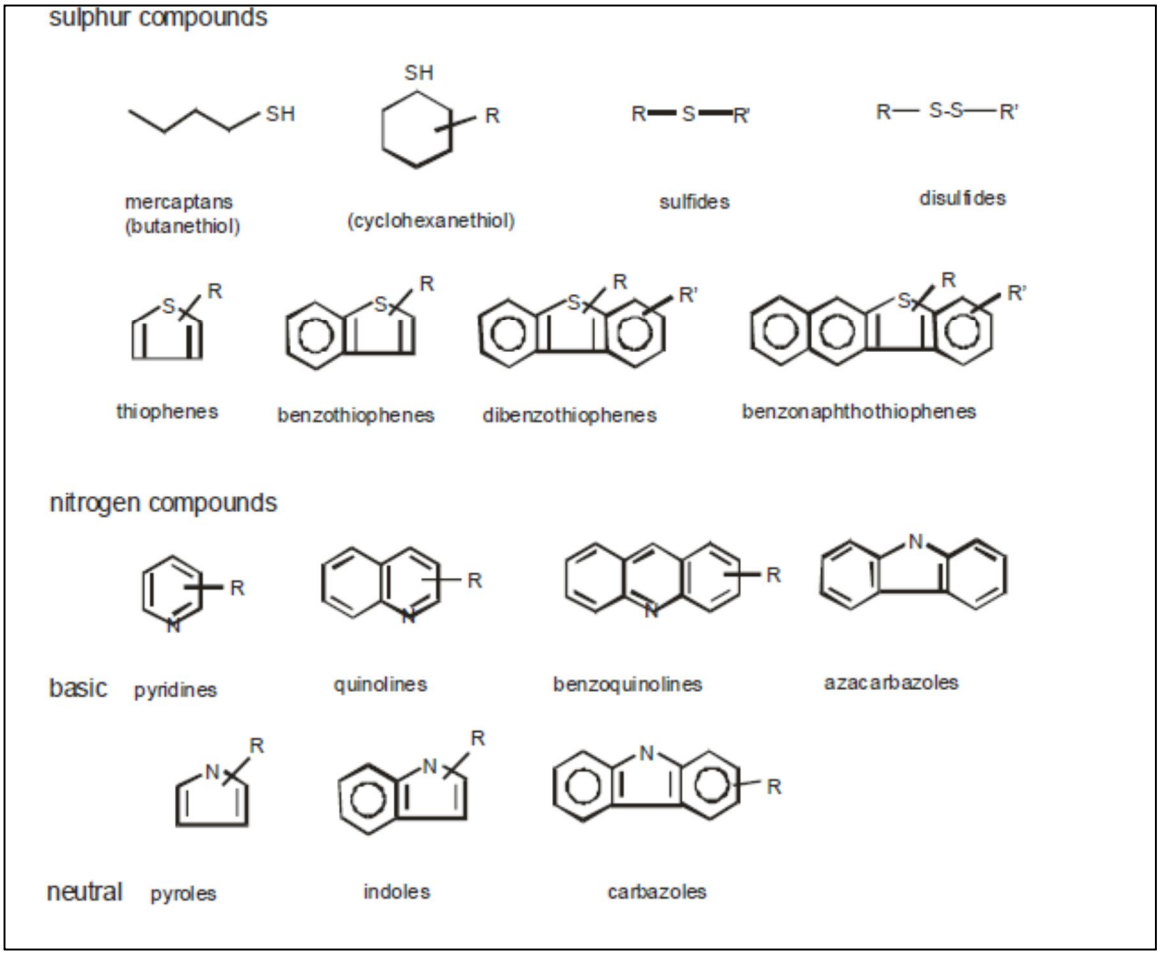


Figure 11. Examples of different classes of sulphur and nitrogen compounds in crude oil /1/.

4.4 Mineral oil sources and migration in cardboard

4.4.1 Mineral oil sources

The main mineral oil sources in cardboard are from recycled paper and board, printing inks applied to paper and board, MOH are used as additives in the manufacture of plastic, adhesives are used in food packaging, wax coating is directly applied to food /1/. Food additives, processing aids and other uses contribute to MOSH level. Further sources are machinery used for harvesting (diesel oil and lubrificating oil) and solvents consisting of individual alkanes or complex MOH mixtures containing cyclic and open

chain alkanes of carbon numbers ranging from C_{10} to C_{14}, used as cleaning agents, may contaminate food products as well.

4.4.2 Migration of mineral oil in food

Migration of mineral oil hydrocarbons into dry foods almost exclusively proceeds through the gas phase by evaporation from paperboard and recondensation in food (Fig. 12), possibly via an intermediate recondensation on an internal bag of paper or plastic /17/. This means that a certain vapour pressure is a prerequisite for a significant mass transfer. In the absence of a functional barrier, the migration of the substances of sufficient volatility tends to be high (70-80% of the content in the paperboard).

There are two types of migrations: Direct migration, and indirect migration. Direct migration proceed through the packaging of the food, while indirect migration proceed through the intermediate packaging of the packed food.

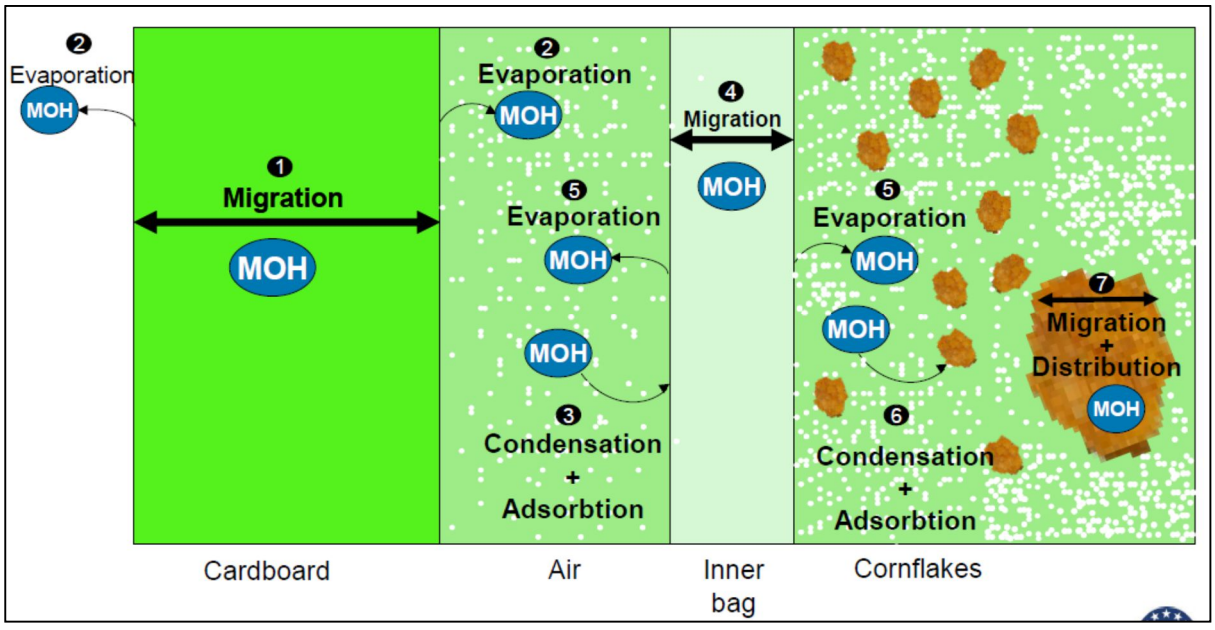


Figure 12. Transfer mechanisms of mineral oil into food /37/.

4.5 Toxicology

All mineral oil hydrocarbons (MOH) are mutagenic unless they are treated specifically to remove MOAH /1,37,38/. The mutagenicity of MOH is caused mainly by 3-7 ring MOAH, including non-alkylated polycyclic aromatic hydrocarbons (PAHs). MOSH are not carcinogenic, though long chain MOSH can act as tumor promoters at high doses.

MOSH from C_{16} to C_{35} may accumulate and cause micro-granulomas in several tissues including lymph nodes, spleen and liver /3/. In the absence of toxicological studies on MOSH mixtures typical of those humans are exposed to, the European Food Safety Authority (EFSA) considered inappropriate to establish a health based guidance value for MOSH.

MOAH with three or more, non- or simple-alkylated, aromatic rings may be mutagenic and carcinogenic. Some highly alkylated MOAH can also act as tumour promoters, but they are not carcinogenic themselves. Some simple MOAH, such as naphthalene, are carcinogenic by a non-genotoxic mode of action, involving cytotoxicity and proliferative regeneration.

For MOAH mixtures there are not dose-response data on the carcinogenicity and hence it is not possible to establish a reference point (RP) upon which to base a margin of exposure (MOE) calculation, which would normally be approach for the risk characterization of MOAH mixtures. Some impact of PAHs are shown in Table 1

Table 1. Impact of PAHs on Human health /37/

PAH	CAS Number	SCF	TEF	ECHA C&L inventory GHS
Anthracene	120-12-7		0.01	**not classified**
Benz[a]anthracene	56-55-3	X	0.1	**Carc 1b H350**
Benzo[a]pyrene	**50-32-8**	**X**	**1**	**Muta 1b H340; Carc 1b H350; Repr 1b H360FD**
Benzo[b]fluoranthene	205-99-2	X	0.1	**Carc 1b H350**
Benzo[g,h,i]perylene	191-24-2	X	0.001	**not classified**
Benzo[k]fluoranthene	207-08-9	X	0.1	**Carc 1b H350**
Chrysene	218-01-9	X	0.01	**Muta 2 H341; Carc 1b H350**
Dibenz[a,h]anthracene	53-70-3	X	1	**Carc 1b H350**
Indeno[1,2,3-cd]pyrene	193-39-5	X	0.1	**Carc 2 H351**
Naphthalene	91-20-3		0.001	**Carc 2 H351**
Phenanthrene	85-01-8		0.001	**not classified**
Pyrene	129-00-0		0.001	**not classified**
Benzo[j]fluoranthene	205-82-3	X	0.1	**Carc 1b H350**
Benzo[c]fluorene	205-12-9			**not classified**
Dibenzo[a,l]pyrene	191-30-0	X	10	**Carc 1b H350**
Dibenzo[a,e]pyrene	192-65-4	X	10	**Muta 2 H341; Carc 1b H350**
Dibenzo[a,h]pyrene	189-64-0	X	10	**Muta 2 H341; Carc 1b H350**
Dibenzo[a,i]pyrene	189-55-9	X	10	**Carc 2 H351**
5-Methylchrysene	3697-24-3	X		**not classified**
Cyclopenta[c,d]pyrene	27208-37-7	X	0.1	**not classified**

TEF = Toxicity Equivalency Factor

Mineral Oil from the View of the Adhesives Manufacturer, M. Tönnießen

Typical contaminants in paperboard.

For long, the paper industry has faced a series of challenges to contaminants in recycled fibres such as /39/:

- Polychlorinated biphenyls (PCBs) which came from carbonless copy paper (largely now resolved following phase out of PCBs).
- Di-isopropyl napthalenes (DIPN), which arose from ink jet inks.
- Di-isobutyl phthalate (DIBP) from inks.
- Primary aromatic amines from inks.
- Micheler's ketone and benzophenone related photoinitiators from UV cured inks.
- Mineral oil saturated hydrocarbons and mineral oil aromatic hydrocarbons (MOSH and MOAH) from newspaper inks.
- Other harmful molecules.

The CONTAM Panel considered the toxicological data retrieved on single MOSH and MOAH components of the relevant mixtures as inappropriate for the risk assessment for MOH mixtures /1,17/. However, the CONTAM Panel concluded that it is likely that the no-observed-adverse-effect level (NOAEL) of 19 mg/kg body weight per day for the most potent MOSH used will be sufficiently protective for the range of MOSH to which humans are exposed. The limit of potential toxicological relevant in food was defined as 0.01 mg/kg in food.

5 ANALYSIS OF MINERAL OIL IN FOOD AND CARDBOARD

The migration of mineral oils in food through packaging is harmful for humans. To solve this issue, different methods are being used in mineral oil analysis. These methods have advantages and disadvantages which should be taken into account to evaluate their efficiency. The important steps for method development are the extraction from packing material or foods, aliquot required for analysis, the choice of the analysis devise, and the information obtained from the analysis devise. Prior to the cardboard extraction process, the storage of the sample requires extreme care.

5.1 Sample storage

The sample history is needed in order to establish its reliability, because the paperboard sample should not be taken from the top of the stack to avoid components loss into air /17/. The paperboard should not be in contact with any sources of external contaminants. For best storage condition, the sample should be wrapped in aluminum foil on one hand to avoid evaporation outwards and on the other hand external contamination. The protected sample should be stored at low temperature (refrigerator).

5.2 Cardboard extraction

5.2.1 Solvent extraction

The mineral oil can be extracted from the paperboard by solvent extraction. Lipids extracted with non-polar solvents contained less polar lipids than those extracted with polar solvents /40/. Therefore, the combination of polar and non-polar solvents is recommended. For efficient extraction, the solvent must be able to solubilise the target compounds and minimising co-extraction of interferences /41,42/. The extraction techniques in use are solvent extraction with a solution of ethanol/hexane (1:1, v/v) /36/ or hexane/acetone (1:1, v/v), pressurised solvent extraction (PSE) /25/ and also the use of absorbent material such as modified polyphenylene oxide (MPPO) or molecularly imprinted polymers /43 - 46/. The methods as well as the choice of the extraction solvents are the most important parameters to optimize for the cardboard extraction.

5.2.2 Pressurised liquid extraction

Beside the classic solvent extraction, pressurised liquid extraction (PLE) is a well-established sample preparation technique which uses high temperatures and high pressures for rapid and efficient analyte

extraction from solid samples /25,42/. The solvent is pumped into an extraction vessel containing the sample. The high-pressure allows maintaining the solvent as liquid at temperatures well above its atmospheric boiling point. As the temperature increases, the viscosity of the solvent is reduced thereby increasing its ability to wet the matrix and solubilise the analytes. Higher temperatures also cause a higher diffusion and desorption rate, increasing extraction efficiency.

5.3 Separation process

The separation process or chromatography is a technique that is used in analytical chemistry for the purification, selective extraction and enrichment of analyte in complex samples. The extraction techniques widely employed are liquid-phase extraction (LPE) planar chromatography, solid-phase extraction (SPE), solid-phase microextraction (SPME), and matrix solid-phase dispersion (MSPD) /46/.

5.3.1 Liquid-phase extraction

Liquid phase extraction (LPE) involves the separation of two or more substances in an analyte through a process in which two solvents are employed to separate an analyte from a mixture /47/. The two solvents are such that they do not mix with each other (immiscible). The solvents are also chosen such a way that the analyte is much more soluble in one than the other. This involves analytes being distributed between the two solvents according to certain chemical properties, mainly polarity and pH.

In cardboard extraction, this technique is used to separate the water soluble compounds from the extract by mixing 10 mL of water with 5 mL of the extract /20/.

5.3.2 Planar chromatography (paper chromatography and thin-layer chromatography)

In paper chromatography or thin-layer chromatography (TLC), the mobile phase is a liquid and the stationary phase is a piece of filter paper, or a solid absorbent which is coated onto a solid support as a thin-layer /48/. The sample mixture is applied to a stationary phase, the edge of paper or plate is immersed in a solvent, and the solvent moves up the thin-layer or paper by capillary action. Components of the mixture are carried along with the solvent up the thin-layer to varying degrees, depending on the compound's preference to be adsorb onto the thin-layer versus being carried along with the solvent.

The coupling of planar chromatography with direct analysis in real time time-of-flight mass spectrometry (DART-TOF-MS) is nowadays in use in forensic /49,50/. This consists in cutting the plate within a track led to substance zones positioned on the plate edge which was directly introduced into the DART stream.

Mass signals were obtained instantaneously within seconds. According to forensic science, the coupling was perfectly suited for identification and qualitative purposes, but it was initially critical for quantification of results. Therefore, this method is not suitable for quantification of mineral oil in cardboard.

5.3.3 Column chromatography (solid phase extraction)

SPE is an extraction method that uses a solid phase and a liquid phase to isolate the analyte of interest from a complex solution /50/. The solid phase (usually silica) is the stationary phase in the column which is able to adhere gas or liquid particles on its outer surface. After filling the sorbent, the mobile phase (solvent) is passed through the dry column to make it wet and the column must remain wet throughout the experiment. The sample to be separated is then loaded at the top of the wet column. The solvent used as mobile phase is then eluted through the column, the analytes in the sample interact and retain on the sorbent according to their affinity (example: polarity or pH), the separation occur then. The most used column chromatography techniques in mineral oil analysis are SPE, and HPLC. They are able to separate MOSH and MOAH in cardboard extract. There are many adsorbent materials used for chromatographic separation in solid phase extraction, and the most used are: silica gel, modified or bonded silica, and activated alumina. SPE is robust separation technique; by improving the selectivity of the sorbent bed to pick up the analytes will give a bright future to this separation technique.

5.3.4 Adsorbent materials in solid phase extraction

Sorbents are chemicals or materials that can capture liquids or gases. Adsorbents adhere substances over the surface of the adsorbing material /51/. The mineral oil can be extracted from the paperboard by adsorbent materials such as: activated aluminum oxide, activated carbon, calcium sulfate, calcium oxide, clay, molecular sieves or zeolites, organic polymers and silica gel.

We will focus more on the adsorbents in used: silver silica gel, activated aluminum oxide, and modified polyphenylene oxide.

Activated silver silica gel column

The efficiency of the separation of MOSH and MOAH from the paperboard extract, is the most challenging in method development for mineral oil analysis. This separation can be achieved through activated manual silver silica gel column or commercially manufactured silica gel column. Activated silver silica can also be combined to activated aluminum oxide for better MOSH separation.

Silica

Silica gel has hydroxyl groups termed silanol (Si-OH) groups. Silanol groups are the polar groups through which other functionalities can be attached (see Fig. 13) /52/. The silanol group can absorb compounds onto the silica surface by hydrogen bonding, among others.

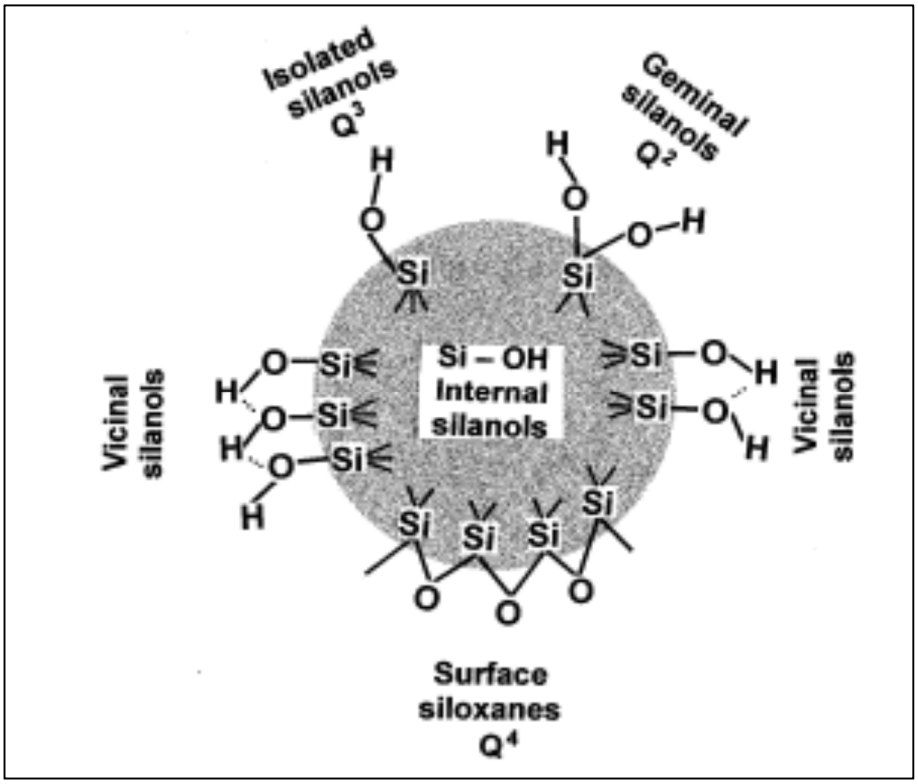
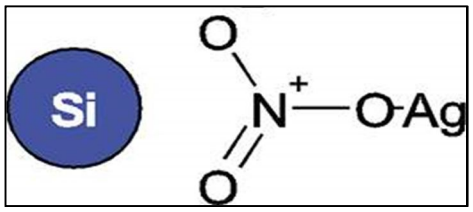


Figure 13. Types of silanol groups and siloxane briges on the surface of amorphous silica /52/.

Silver nitrate and silica

The theory behind the association of silver nitrate and silica (Fig. 14a and Fig. 14b) for the components separation is based on the fact that silver ions can complex with unsaturated compounds (π bond) /53/. Silver ions (Ag+), like the ions of other transition metals, interact specifically with unsaturated compounds to form weak charge transfer complexes with olefinic double bonds. As illustrated in Figure 14b, the unsaturated compound acts as an electron donor and the silver ion as an electron acceptor.



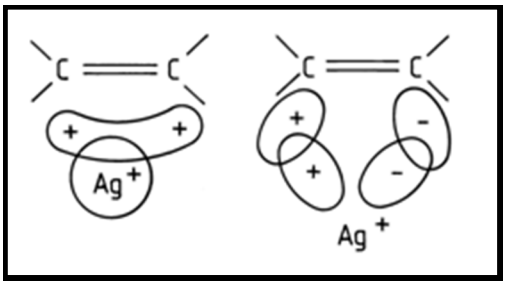


Figure 14a. Silver nitrate and silica /54/.

Figure 14b. The Dewar model of interaction between a silver ion and an olefinic double bond /55/.

The principle of silver silica gel in solid phase separation is described as following:

The properties of macroporous silica gel make it the most important adsorbent used in chromatography column. The polar analytes (white component in Fig. 15) in the sample interact and retain on the polar sorbent while the solvent, and non-polar components (in red) such as MOSH pass through the cartridge /56/.

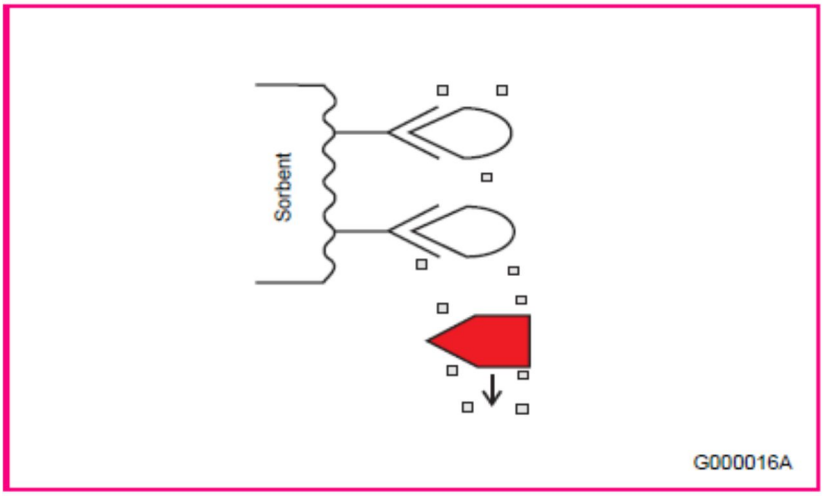


Figure 15. Selective extraction with silica sorbent /56/.

The silver nitrate impregnated silica gel modifies the separation characteristics of the sorbent layer, by increasing discrimination of certain compounds, particularly those containing carbon–carbon double bonds, such as MOAH (see Fig. 16) /56/.

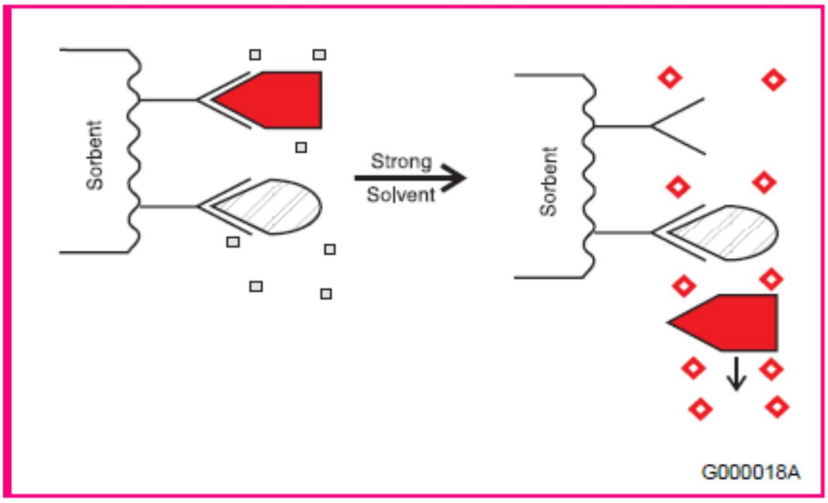


Figure 16. Selective extraction with silver nitrate modified sorbent /56/.

Advantages and disadvantages of manual silver silica cartridge

Before the column is filled with activated silver silica gel for the separation, the preparation of the gel will take almost three days. The amount of silver nitrate used has varied from 0.3% to 10% of the silica weight, according to the degree of separation of MOSH and MOAH wished.

The advantages are mainly the higher retention capacity of mineral oil compared to the use of aluminum oxide. With activated silver silica gel MOSH and MOAH can be separated efficiently prior to gas chromatographic (GC) analysis.

As disadvantages, the preparation of silver silica cartridge is time consuming, it has also a relatively short life time (2 weeks, stored at room temperature in dark) /1,13/.

Commercial silica gel cartridge

The commercial silica gel cartridges are readymade for use.

As advantages, this saves time. They have also long lasting life time since there is no expiraction date for their use.

As disadvantages, the commercial silica gel cartridges deal with contamination. It's extremely difficult to get rid of these contaminants. In consequence more hexane is needed for the washing process, and the analysis result for MO is not reliable if analysis of low concentration is performed.

Activated aluminum oxide

The activated aluminum oxide selectively retains long chain *n*-alkanes /11/. The aluminum oxide (Al_2O_3) activated at 400 °C removes 20 % of the *n*-C_{20}, 80 % of the *n*-C_{21} and virtually all higher mass n-alkanes. Al_2O_3 activated at 600 °C removes 30 % *n*-C_{19} and 90 % *n*-C_{20}. This means that the capacity for retaining *n*-alkanes is achieved by the higher activation temperature. For efficient separation of MOSH and MOAH, the association of silver silica gel first, followed by aluminum oxide in the column has been used prior to GC-FID analysis.

Advantages and disadvantages

As advantages, activated aluminum oxide is good for the separation of aliphatic compounds.

As disadvantages, its preparation is also time consuming, and it selectively retains only long chain alkanes.

Modified polyphenylene oxide

Modified polyphenylene oxide (MPPO) is a porous polymer material with a high molecular weight. It is very stable at high temperature (T_{max} = 350 ºC), it has a high surface area and a low specific mass (0.23 g/cm^3). MPPO has the property to adsorb mineral oils. The adsorption has been tested /43,46/ by covering a sample with MPPO and held at the desired time-temperature test condition, where the maximum temperature applicable was 175 ºC. The exposure was followed by extraction of the adsorbent using an organic solvent depending on the used specific analytical method.

This mineral oil absorbent has a great advantage, only the volatile components are extracted. As disadvantages, the high cost of the MPPO material and a costly special devise are needed for his use.

5.3.5 Matrix solid-phase dispersion

Matrix solid phase dispersion (MSPD) is an analytical process for the preparation, extraction and fractionation of solid, semi-solid and/or highly viscous biological samples /57 - 59/. MSPD is based on several simple principles of chemistry and physics, involving forces applied to the sample by mechanical blending to produce complete sample disruption and the interactions of the sample matrix with a solid support bonded-phase (SPE) or the surface chemistry of other solid support materials. The main difference between MSPD and SPE is that, in MSPD the sample is dispersed throughout the column and retained in not only the first few millimeters. MSPD has some major advantages such as straightforward application, ability to simultaneously perform extraction and cleanup in a single step with good recovery and precision /60,61/. The application of MSDP reduces analysis time, use smaller sample size, increase

sample throughput and shorten turn-around time. This method is providing the reduction in solvent use and the expense of purchase and disposal, as well as providing analytical results that are equal to or better than classical methods, makes MSPD an attractive alternative approach to investigate for cardboard extraction.

5.3.6 Solid phase microextraction

Solid-phase microextraction (SPME) was developed to facilitate rapid sample preparation both in the laboratory and on-site where the investigated system is located. In the technique, a small amount of extracting phase that is dispersed on a solid support is exposed to the coated fiber for a well-defined period of time /62 - 64 /. According to the extraction procedure, the coated fibre is immersed directly in the sample, where the analytes are concentrated. The transport of analytes from the matrix into the coating begins as soon as the coated fiber has been placed in contact with the sample. After equilibrium has been reached (from a few minutes to several hours depending on the properties of the analytes measured) or after a defined time the coated fibre is withdrawn and transferred either to a GC injection port, HPLC valve, or MS for analysis.
SPME sampling can be performed in 3 basic modes (Fig.17): (a) direct extraction, (b) headspace extraction and (c) extraction with membrane protection. Figure 7 illustrates the differences between these modes.

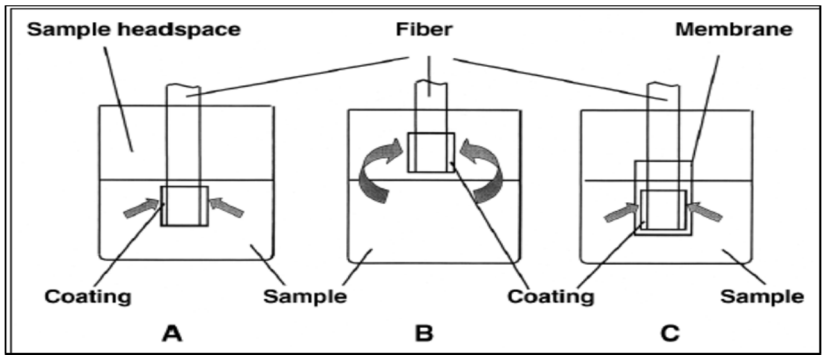


Figure 17 Modes of SPME operation: direct extraction (A), headspace SPME (B), and membrane-protected SPME (C) /62/.

As advantages, SPME saves analysis time, reduces solvent use and apparently a simple techniques. The other advantage is that this technique can be used to studies the distribution of analytes in a complex multiphase system and to specialise in different forms of analytes in a sample. These potential advantages can motivate investigation for the use of SPME in the analysis of mineral oil in cardboard.

5.4 Analysis equipments in mineral oil determination

5.4.1 Gas chromatography

In gas chromatography (Fig. 18), gaseous mobile phase transport the analyte through the column, and separate analytes before the flow through a detector, then the response is displayed on the computer for analysis /65/. Mass spectroscopy (MS) is sometimes used to identify the components prior to determine the amount of analytes.

The standard sample is used to report the different fractions in the mineral oil. The most important in quantitative analysis is proper calibration of the GC /66/. By determining the relationship between the magnitude of a peak for a known amount of analyte in a standard, one can then use that relationship (the calibration curve) to estimate the amount of that analyte in a sample of unknown concentration.

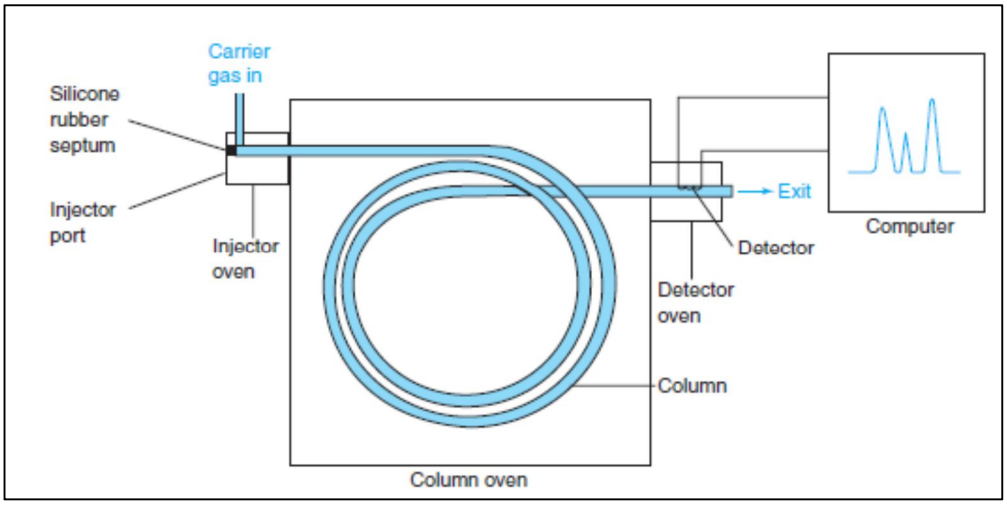


Figure 18. Schematic diagram of gas chromatography /65/.

As advantages, GC allows fast analysis and can be automated. Small samples (µl or µg needed) can be analysed. GC devise is reliable, relatively simple to use and it is also quite cheap. It allows on-line coupling with other devices such as mass spectrometry (MS), GC MS/MS, GCxGC-MS, LP-GC and HPLC-GC. Gas chromatography has some very sensitive detectors (detection limits easily on ppm level, often ppb), and highly accurate quantification (1-5% RSD-relative standard deviation-) /67/.

The disadvantage of GC is the fact that it is limited to volatile samples, and is not suitable for thermally labile samples. Also some samples may require intensive preparation; sample must be soluble and should not react with the column. The use of GC requires usually MS to confirm the peak identity /67/.

5.4.2 HPLC- GC/MS

HPLC is a chromatographic method in which the mobile phase is a liquid which is forced under high pressure through a column containing fine particles that give high resolution separation /65/.

It is possible to combine GC/FID to HPLC (Fig. 19), and MOSH and MOAH determination can be performed using online or off-line liquid chromatography-gas chromatography-flame ionization detector (LC-GC/FID) /68/.

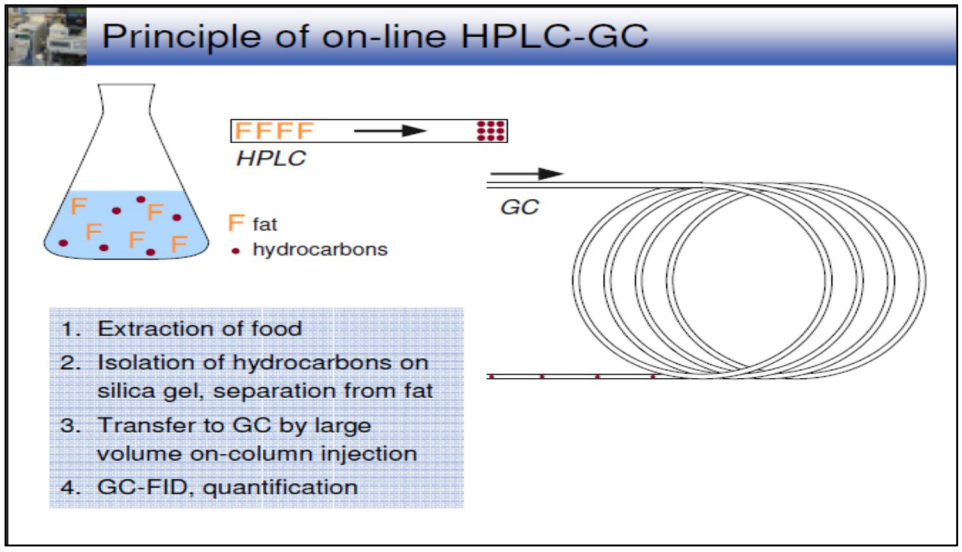


Figure 19. Schematic diagram of HPLC-GC /68/.

The advantages and disadvantages can be compacted as follows /69/:

- High efficiency in pre-separation: efficient sample clean-up.
- Whole fraction of sample material is transferred to GC: Low detection limit.
- Closed system: Rules out sample contamination during preparation.
- Can largely be automated: A minimal amount of manpower.
- Recommended method for routine use (e.g., MOSH/MOAH analysis: 35 injections/day).

- Difficult to detect coelution (two compounds escaping from the tubing at once).
- High cost equipment needed to conduct HPLC.
- Complex operation: Requires a trained technician to operate.
- The equipment has low sensitivity to some compounds because of the speed of the process.

5.4.3 Mass spectroscopy

Mass Spectrometry (MS) is a powerful technique for identification of unknown compounds when studying molecular structure. A mass spectrum is a presentation of the masses of the positively charged fragments versus their relative concentrations /70/. There are several ionisation techniques, the most used ionisation technique is electron impact (EI); where a standardised electron beam energy (70 eV) is generally applied. Mass spectra are then obtained at the electron beam energy of 70 eV. The simplest event that occurs is the removal of a single electron from the molecule in the gas phase by an electron of the electron beam to form the molecular ion, which is a radical cation ($M^{+\bullet}$). The symbol $^{+\bullet}$: indicates that the molecule has lost an electron (Equation 2), the molecular ion has unpaired electron and is positively charged.

$$\underset{\text{Molecule}}{M} \xrightarrow{70\ eV} \underset{\text{Molecular ion}}{M^{+\bullet}} + e^{-} \quad \text{(Equation 2)}$$

The selection of the sample inlet depends on the sample and the sample matrix. If the analyte is sufficiently volatile, with high vapour pressure, and is thermally stable, it is introduced directly into the source region /71/. Liquids and solids are usually heated to increase the vapour pressure for analysis. If the analyte is thermally labile (it decomposes at high temperatures) or if it does not have a sufficient vapour pressure, the sample must be directly ionised from the condensed phase.

For qualitative analysis, a mass spectrometer can identify a chromatographic peak by comparing its spectrum with a library of spectra /64/. The confirmation ion is used for qualitative identification. The confirmation ion or spectra might be expected to be 65% as abundant as the quantitation ion. If the observed abundance is not close to 65%, then we suspect that the compound is misidentified. Another method to identify a peak is to compare its retention time with that of an authentic sample of the suspected compound. Retention time comparison is a common method when FID is used.

Factors affecting MS performance are sample concentration, the matrix, analyte type, buffers and purity, purity of organic solvent, purity of curtain gas and collision cell gas, run time and number of samples run. MS can be coupled to GC (GC-MS), to LC (LC-MS and in tandem (MS-MS). The library of spectra should be updated to make the matching of the unknown molecules easier. Also for better results, high resolution mass spectrometry is recommended since it not only provides a specific molecular mass value, but it may also establish the molecular formula of an unknown compound.

5.4.4 Fourier transform infrared

Most advanced and common IR-spectroscopic method is based on FT-IR (Fourier transformer). The FT-IR principle is the absorption measurement of different IR frequencies by a sample (gas, liquid, or solid) positioned in the path of an IR beam /72,73/. The IR spectrometry use interferometry technique to scan the sample. The interferogram is the superimposed waves obtained after sample scan in order to extract information. Fourier transform which is a mathematical process is then used to convert the interferogram (raw data) into spectrum for easy analysis (Fig. 20).

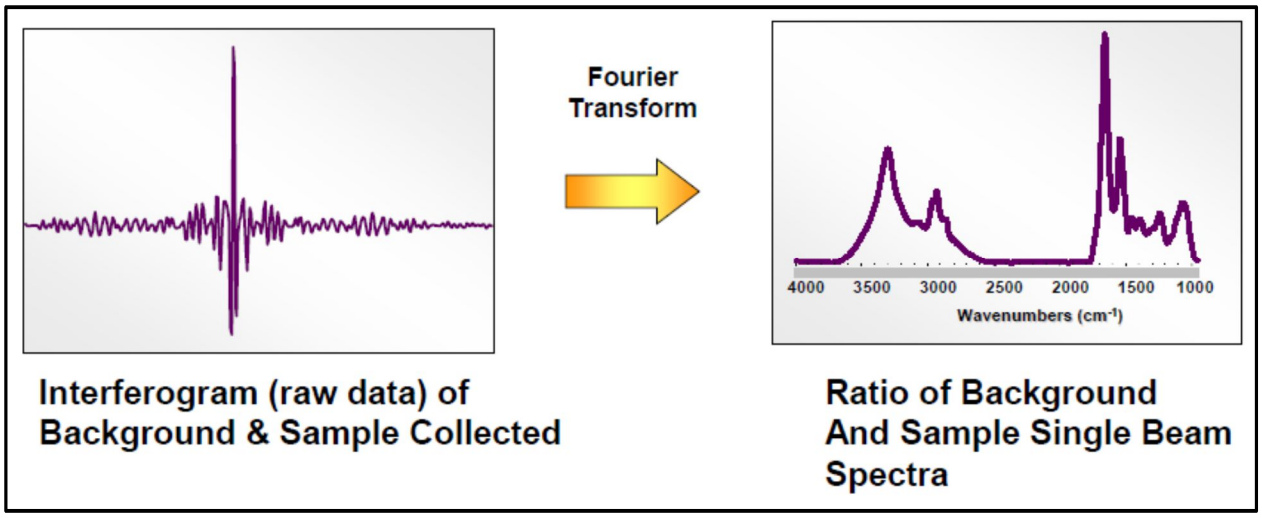


Figure 20. The conversion of the interferogram into spectrum by Fourier transform /74/.

The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. FT-IR can be combined with GC (GC-IR), LC (LC-IR) and HPLC for qualitative and quantitative analysis. By using various sampling accessories, IR spectrometers can analyse gases, liquids and solids samples. Fourier transform Infrared (FT-IR) is the most advanced IR spectrometers.

As advantages, the IR spectrometer is more qualitative rather than quantitative. It is more useful to determine the chemical functional groups in the sample.

The disadvantages are: a lot of compounds are not IR active and therefore, they cannot be detected. The sample preparation is time consuming due to the complexity of IR device. IR is a destructive analysis method due to sample treatment. Minimal elemental information is given for most samples. Background solvent or solid matrix must be relatively transparent in the spectral region of interest.

5.4.5 Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) is a physical property of the nuclei to absorb and re-emit electromagnetic radiation when it is placed in a magnetic field /72/. This magnetic property of nuclei is used to determine qualitatively or quantitatively the component.

Advantages and disadvantages

NMR spectrometer is a non-destructive sample analytical instrument. In addition the technique is quantitative, detects very fine structure and it is good for the identification and proof of structure of chemical compounds. This instrument allows coupling with MS and HPLC as well /72/.

NMR spectrometer is one of the most sophisticated analytical instruments available. Pure compounds are usually required; mixtures are much more difficult to deal with. Background interferences become a limitation with very dilute samples. The method cannot distinguish among magnetically equivalent monomers and dimers. Low sensitivity is the principal limitation of the method. NMR device is also very expensive device, measuring is time consuming and the spectra takes long time to interpret.

5.5 Conclusions

Considering the advantages and limitations of the different methods used in mineral oils analysis above, GC-FID/MS and HPLC-GC/MS will retain positively more attention for their applicability (limited to volatile samples). These analysis methods are fast, cheap, easy to use and also efficient. GC-FID/MS is cheaper compared to HPLC-GC/MS, and also easier to use. Meanwhile, investigations can be done on the new separation processes such as MSPD, SPME and so on to improve the analysis results of mineral oil in cardboard.

Since mineral oil from packaging to food endangers human health, In 2011, an envisioned limit of 0.6 µg/kg for MOSH < *n*-C_{25} and of 0.15 mg/kg for MOAH< *n*-C_{25} have been proposed by the German Federal Ministry of Food, Agriculture and Consumer protection (BMELV) /20,52/. To the European Union level, besides some regulations /15,16/, discussions with the member states on the need for a general maximum level for mineral oil in food are ongoing.

6 GENERAL THEORIES

6.1 Optimization of GC parameters

Laboratories are looking for ways to speed up analysis time and improve peak resolution without compromising results. Sample introduction, column separation and detector performance can be optimised to improve the GC sensitivity, selectivity and baseline stability /75 - 77/. To achieve these goals, different parameters such as column choice, carrier gas linear velocity, temperature ramp rate, injection volume and detector temperature can be tuning up.

Theory

The purpose of developing chromatographic separation is to sort out the mixture of analytes. The optimisation of GC parameters is based on the general resolution equation (Fig 21) /64,75-77/.

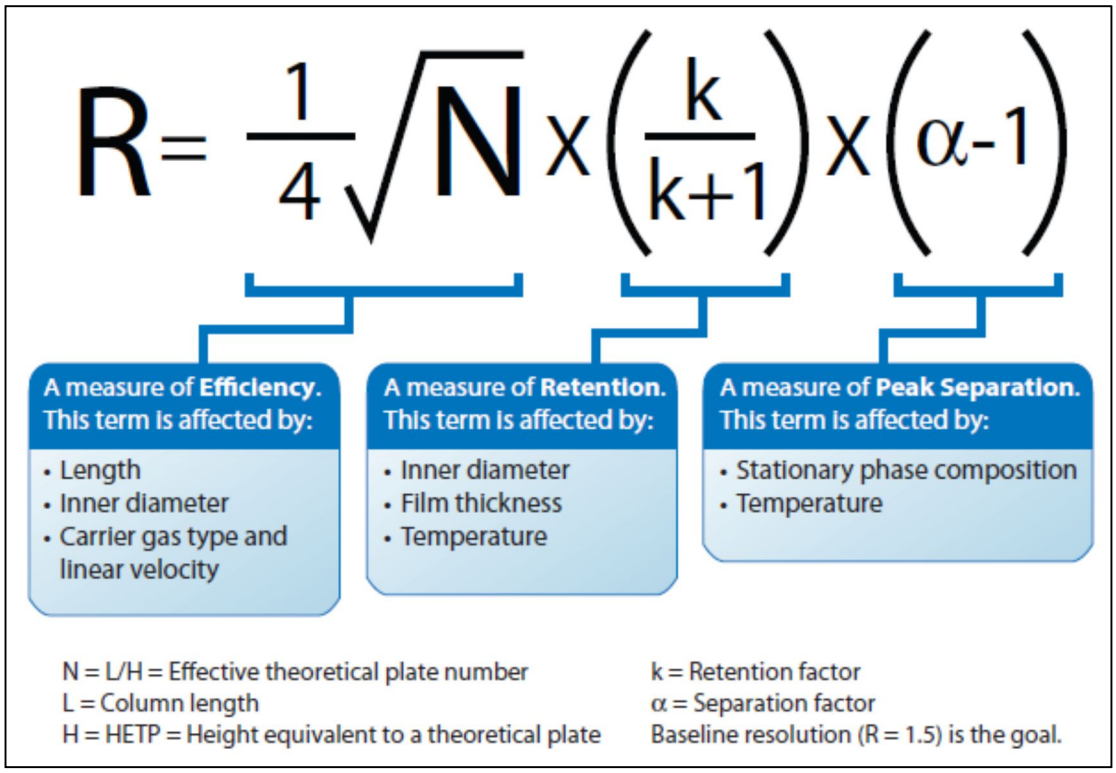


Figure 21. The resolution equation and factors that affect it /77/.

There are three parameters that control resolution, namely **efficiency (N)**, the **retention (k)**, and **selectivity (α)** factor.

Selectivity (α) and stationary phase

Selectivity or separation factor (α) has the greatest impact on resolution, and it is strongly affected by stationary phase polarity and selectivity /77/. Stationary phase selectivity is defined by IUPAC as the extent to which other substances interfere with the determination of a given substance. If the stationary phase and analyte polarities are similar, then the attractive forces (e.g., hydrogen bonding, dispersion, dipole-dipole interactions and shape selectivity) are strong and more retention will result. Greater retention often results in increased resolution. In general, highly polar stationary phases have lower maximum operating temperatures.

Retention factor (k), the choice of film thickness and column inner diameter (ID)

The retention factor (k) of a column is based on the time an analyte spends in the stationary phase relative to the time it spends in the carrier gas /77/. When the temperature increases k decreases, so at higher temperatures analytes stay in the carrier gas longer and are less retained. In practice, if the value of k is too large, the peak will broaden, which can reduce resolution by causing peaks to overlap or coelute. Smaller ID columns produce higher retention factors (k) compared to larger ID columns. This is due to less available mobile phase (carrier gas) volume in the column. When analyzing extremely volatile compounds, a thick film column should be used to increase retention; more separation is achieved because the compounds spend more time in the stationary phase. But when analyzing high molecular weight compounds, a thinner film column should be used, as this reduces the length of time that the analytes stay in the column and minimizes phase bleed at higher elution temperatures.

Efficiency (N)

The efficiency (N) is related to the column length. Longer columns provide more resolving power than shorter columns of the same inner diameter, but they also increase analysis time and should be used only for applications demanding the utmost in separation power /77/. Column length should only be considered once the stationary phase has been determined.

Carrier gas type and linear velocity

Carrier gas choice and linear velocity significantly affect column separation efficiency, which is best illustrated using Van Deemter plots (Fig. 22) /64,77/. The optimum linear velocity for each gas is at the lowest point on the curve, where plate height (H) is minimised, and efficiency is maximised. Nitrogen provides the best efficiency, compared to nitrogen; helium has a wider range for optimal linear velocity,

but offers slightly less efficiency. Helium is the carrier gas of choice, not only for its compatibility with most detectors, but also because it is easier to pump in GC-MS compared to hydrogen. For a flame ionisation detector, nitrogen (N_2) gives a lower detection limit than helium (He).

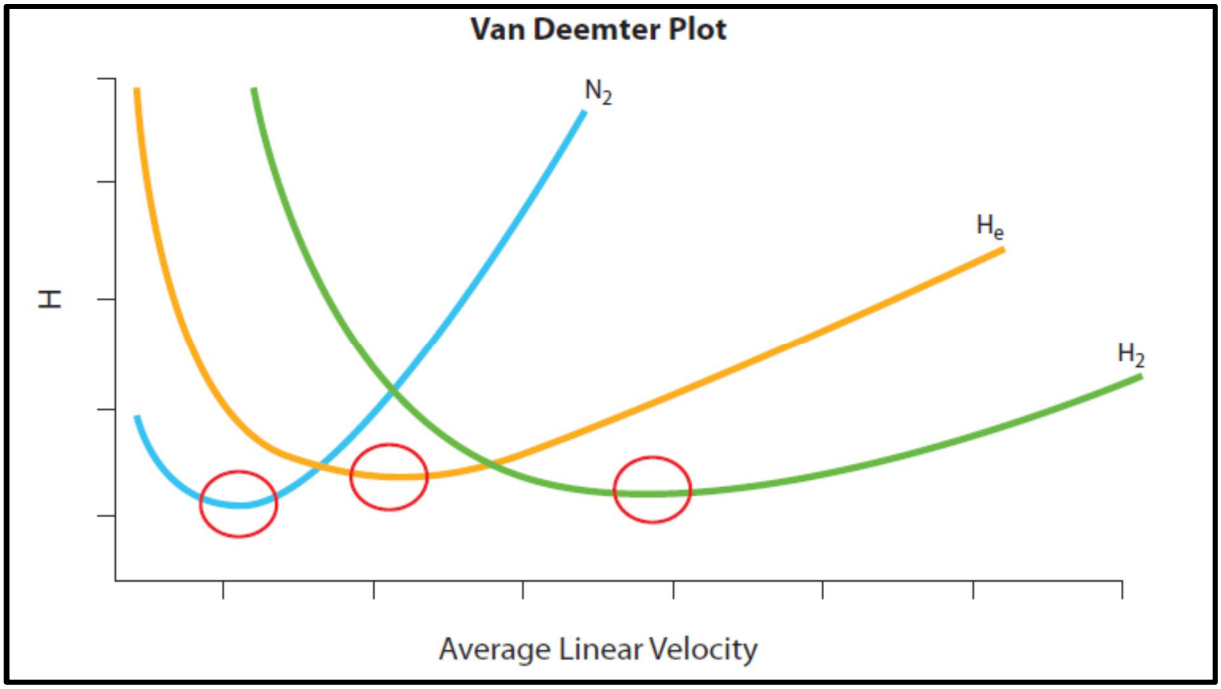


Figure 22. Operating carrier gas at the optimum linear velocity will maximise efficiency at a given temperature. Red circles indicate optimum linear velocities for each carrier gas.

Under the isothermal conditions, if the linear velocity deviates from the optimum linear velocity (U_{opt}), relative peak broadening and loss of resolution is observed /64,76/. At linear velocities above the optimum flow, chromatographic efficiency decreases due to a nonequilibrium of the solute between the stationary and mobile phases. Increasing flow rate results in an increase in the peak capacity, due to the peak widths getting narrower and the temperature gradient effectively being decreased. Selectivity and the retention factor can be improved by changing the column chemistry (stationary phase polarity), carrier gas linear velocity and temperature ramp rate. The thicker the film, the lower the maximum temperature; exceeding the maximum temperature can result in column bleed and should be avoided.

6.2 "Hump" and unresolved components in GC chromatogram

The mineral hydrocarbons primarily or exclusively form a hump of unresolved components, either consisting of branched alkanes, branched paraffins and cyclic naphthenes or of alkylated aromatics /78/.

The peaks integration included also the unsolved humps in MOSH and MOAH chromatograms for reliable results of the determination of mineral oil in cardboard.

The hump of unresolved components that are termed unresolved complex mixture (UCMs) can be identified with comprehensive two-dimensional gas chromatography (GCxGC) /79,80/. GCxGC links two capillary columns, with different stationary phases, via a modulator that creates packets of analytes by temporarily focusing the effluent leaving the first column before entering the second column. The separation of these packets by the second column produces a chromatogram with a high signal-to-noise ratio. Furthermore, the separation power of the first column is conserved into the second column, in a way that compounds not resolved by the first column may be resolved by the second column. However, it is important to know that the overlapping compounds and the mixed spectra have no solution from the GC libraries.

7 EXPERIMENTAL

7.1 Equipment and reagents used for all experimenttals

7.1.1 Glassware and SPE tubes

The glassware equipment was washed and rinsed with UHQ water, acetone and distilled *n*-hexane just before use. The glass column (ID 10 mm, length 13.5 cm) used were emptied and cleaned, they were originally Extrelut NT1 column (Fig. 23), manufacturer Merk KGaA – Germany. The commercial SPE cartridges used were Strata EPH (2.5 g / 6 mL) Teflon tubes (Fig. 24), Company Phenomenex. The vacuum manifold used in fractionation was Phenomenex SPE Teflon (Fig. 25), company Phenomenex.

Figure 23. Extrut NT1 /81/.

Figure 24. Strata EPH 2.5 g/ 6 mL /82/.

Figure 25 . Phenomenex SPE teflon vacuum manifold /83/.

7.1.2 Reagents

The solvents used were *n*-hexane (purity ≥ 97%, manufacturer Sigma-Aldrich USA), ethanol (purity ≥ 99.5%, manufacturer Altia Oy Rajamäki-Finland), acetone (purity ≥ 99.5%, manufacturer: Sigma-Aldrich USA), dichloromethane (DCM) purity ≥ 99.9 (from SIGMA-ALDRICH – USA) and toluene (from RATHBURN Chemical Ltd–Walkerburn, Scotland).

The silica gel (0.063-0.200 mm), was from Merck KGaA-Germany. The silver nitrate ($AgNO_3$) was from J.T.Baker B.V. Deventer, Holland.

7.1.3 Standards and internal standard

- **Standard (STD)**
  - Alkane std (C_7-C_{44}) - 100 ppm (ASTM D2887 Supelco – USA)
  - DIPN – 10 ppm (from Sigma – Aldrich – USA)

Internal standard (ISTD) for solid phase extraction

- **MOSH**
  - C_{13} – 13.66 µg/mL (from Sigma – Aldrich – USA)
  - Cyclohexyl cyclohexane (Cycy) – 13.76 µg/mL (from Sigma-Aldrich – USA)
  - 5α-cholestane (Cho) – 19.80 µg/mL (from Sigma-Aldrich – USA)

- **MOAH**
  - 2-Methylnaphthalene (2MN) – 11.44 µg/mL (from Sigma-Aldrich – USA)
  - 1,3,5-tri-*tert*-butylbenzene (TBB) – 11.25 µg/mL (from Sigma-Aldrich – USA)
  - Perylene (per) – 22.72 µg/mL (from Sigma-Aldrich – USA)

7.2 GC OPTIMISATION

7.2.1 Column information

The column was Phenomenex ZB-5HT Inferno (the length 30 m, ID 0.25 mm and the film thickness 0.25 µm). The minimum temperature limit for the column was 45 °C, and the maximum limit was 400/430 °C (Isothermal program). Column is made from fused silica and outside is outside is polyimide-coated. Stationary phase was 5% phenyl, and 95% dimethylpolysiloxane. The recommended use are: diesel fuels, high boiling petroleum products, high molecular weight waxes, long-chained hydrocarbons, motor oils, polymers/plastics, simulated distillation, surfactants and triglycerides.

7.2.2 GC parameters

Tables 1 through 4 give the different settings used to optimise GC performance. Three methods were used: JGZBO1, JGZBMO1_10, and JGZBMO1_15.

Table 2. Injection parameters

INJECTION PORTSPL 1			
Method name	**JGZBMO1**	**JGZBMO1_10**	**JGZBMO1_15**
Injection volume (µL)	1.0	1.0	2.0

The injection parameters which were same in all methods were the injection mode (splitless), temperature (340 °C) and pressure (133.4 kPa).

Table 3. Column parameters

	Column Oven		
Method name	**JGZBMO1**	**JGZBMO1_10**	**JGZBMO1_15**
Maximum temperature (°C)	350	350	340
Total program time ((min)	28.25	43.50	32.67
Temperature ramp rate (°C/min)	20	10	15

The column parameters which were the same in all methods were the initial temperature (45 °C) and the initial column flow rate (1.92 mL/min). Linear velocity was 40 cm/sec and helium was used as the carrier gas. Thus, pressure and flow rate were changing during temperature program.

Table 4. Detector parameters

	DETECTOR		
Method name	**JGZBMO1**	**JGZBMO1_10**	**JGZBMO1_15**
Temperature (°C)	360	360	340

The detector parameters which were same in all methods were the sampling rate (40 msec) and the makeup gas was helium.

7.3 EXTRACTION

7.3.1 Cardboard extraction efficiency

The efficiency of mineral oil extraction from cardboard is a very important factor for better results in the cardboard mineral oil analysis. This laboratory work aim was to determine the time factor in extraction, the cardboard sample mass effect, and the solvent efficiency in cardboard extraction. The comparative solvents used were *n*-hexane/ethanol (1:1,v:v) and *n*-hexane/acetone (1:1,v:v) with a sample B. Welle as the paperboard grade. The raw cardboard extract was then analyzed with GC-FID, for the comparative study.

Chemicals

The solvents used were *n*-hexane (purity ≥ 97%, manufacturer Sigma-Aldrich USA), ethanol (purity ≥ 99.5%, manufacturer Altia Oy Rajamäki-Finland) and acetone (purity ≥99.5%, manufacturer Sigma-Aldrich USA). Glassware and other equipment were rinsed with distilled acetone and *n*-hexane before use. The properties of the solvents used are given in Table 5.

Table 5. The properties of the solvents used

Solvent	Chemical formula	Boiling point (ºC)	Density (g/mL)	Polarity	Solubility in water (%)
Hexane	C_6H_{14}	68	0.6548	0	0.001
Acetone	CH_3-C(=O)-CH_3	56	0.786	5.1	100
Ethanol	CH_3-CH_2-OH	79	0.798	5.2	100

GC analysis

GC analyses were performed using method JGZBMO1-15. The parameters of this method and the equipment are presented in chapter 7.2.2 GC parameters.

Cardboard extraction

Triplicate samples of B. Welle cardboard 1 g of each were cut into little pieces and were extracted with 10 mL of a mixture of hexane/ethanol (1:1, v/v) for periods of 1 h, 2 h and 3 days with 10 mL of a mixture of hexane/ethanol (1:1, v/v). The same extraction process was performed also with triplicate samples of 2 g each of B. Welle cardboard.

The efficiency evaluation of solvents was performed using two samples of 1 g each of B. Welle extracted for 2 hour with 10 mL of a mixture of hexane/ethanol (1:1, v/v), and 10 mL of hexane/acetone (1:1, v:v). The extracts were then analysed with GC-FID.

7.3.2 Used extraction method

Cardboard samples were extracted according to the method described by Lorenzini *et al.* /84/ and Moret *et al*. /20/, with slight modifications (2 g sample used instead of 1 g). The cardboard B3ARR sample (2 g) was cut into small pieces, and extracted by immersion into hexane/ethanol (1:1) at room temperature for 2 h. The hexane extract phase separation was made by mixing 5 mL of cardboard extract with 10 mL of UHQ water in the tube. The hexane extract on the top was then transferred using a pipette into a narrow closed small bottle (the extraction container).

7.4 FRACTIONATION

7.4.1 Preparation of silver silica gel

Silver nitrate cleaning

A portion (about 3 g) of silver nitrate ($AgNO_3$) was washed with 10 mL of *n*-hexane. The mixture of silica gel and hexane was let settle, and then hexane was pipetted. This washing operation was done 3 times. Silver nitrate was then evaporated to dryness in the oven at around 50 ºC. Washing and heating at 400 °C were used for cleaning. Heating at 400 °C cleaned and activated silica.

Silver silica gel with 10% of silver nitrate preparation

Silver silica gel was prepared according to Moret *et al.* /20,84/. It was prepared by adding drop by drop while agitating, a solution of silver nitrate (3 g in 4 mL UHQ water, previously washed with hexane to remove possible interference) to 30 g of silica gel heated at 400 ºC overnight. The flask was protected from the light, closed and shaked vigorously for about 20 seconds. The silver silica gel was blended for 30 min, using a rotary evaporator without applying the vacuum. It was left to rest for 12 h (protecting from the light). The blended silver silica was dried at 75 ºC overnight to eliminate residual water. Finally, the silver silica gel covered with an aluminum foil was stored in a desiccator.

Silver silica gel (0.3%)

The silica was activated by heating 33.3 g of silica gel at 400 ºC overnight; it was cooled for 2 hours at least. The washed silver nitrate (100 mg) was transferred into a round flask containing 33.3 g of highly activated silica gel. The flask was protected from the light, closed and shaken vigorously for about 20 seconds. The silver silica gel was blended for 30 min, using a rotary evaporator without applying the vacuum. It was left to rest for 12 h (protecting from the light). The blended silver silica was dried at 75 ºC overnight to eliminate residual water. Finally, the silver silica gel covered with an aluminum foil was stored in a desiccator.

Once ready, the silver silica gel containing 0.3% $AgNO_3$ can be used for two weeks while storing at room temperature in the dark /1,13/.

7.4.2 Fractionation with silver silica (0.3% $AgNO_3$)

The fractionation of MOAH was made with a mixture solvent of 20% DCM, 5% toluene and 75% hexane. The empty glass column was filled with 3 g of silver silica gel (0.3% $AgNO_3$). The filled column was then placed into a vacuum manifold in the close valve position. The cardboard used in this experiment was B3ARR.

Silver silica gel cartridge cleaning up

The dry sorbent was mixed with 5 mL of *n*-hexane. The sorbent bed was soaked with solvent for 3 min and was let to settle by gentle vibration. The solvent was drained by opening the valve and the cartridge conditioned with 10 mL of hexane. The solvent level was lowered to the top of the packed bed avoiding drying of the stationary phase.

Sample loading

Sample: 250 µL of ISTD (see Chapter 7.1.3) was pipetted on the column.

Fractions collection

MOSH fraction: The sample was eluted with 6 mL of *n*-hexane. The eluent was collected into a tube and concentrated to 0.5 mL (weight 0.33 g, density of hexane 0.659 g/mL)

MOAH fraction: Elution was continued with 10 mL of solvent mixture (20% DCM, 5% toluene and 75% hexane), the eluent was collected into a tube and concentrated to 0.5 mL.

The fractions were then analysed by GC.

7.4.3 Fractionation with STRATA EPH SPE tubes

The fractionation of MOAH was made with a mixture solvent of 20% DCM, 5% toluene and 75% hexane. The Strata (2.5 g/6 mL) Teflon tube was placed into a vacuum manifold in the close valve position.

Strata SPE tube cleaning

The dry sorbent was washed with 4 mL of *n*-hexane, 3 mL of solvent mixture, 8 mL of DCM and followed by 20 mL of *n*-hexane. The sorbent bed was soaked with solvent for 3 min and was let to settle by gentle vibration. The solvent was drained by opening the valve and the cartridge conditioned with 20 mL of hexane. The solvent level was lowered to the top of the packed bed avoiding drying.

Sample loading

500 µL of cardboard extract and 250 µL of ISTD (see Chapter 7.1.3) were loaded by pipetting on the column.

Fractions collection

MOSH fraction: The sample was eluted first with 4 mL of *n*-hexane, then with 2 mL of solvent mixture (20% DCM, 5% toluene and 75% hexane). The eluent was collected into a tube and concentrated to 0.5 mL (weight 0.33 g, density of hexane 0.659 g/mL)

MOAH fraction: The elution was continued with 10 mL of solvent mixture, eluent was collected into a tube and concentrated to 0.5 mL.

The fractions were then analysed by GC.

7.4.4 Fractionation with silver silica (0.3% $AgNO_3$) vs. silver silica (1% AgNO3)

Two empty glass columns were filled respectively with 4 g of silver silica gel (0.3% $AgNO_3$) and 4 g of silver silica gel (1% $AgNO_3$). The fractionation procedure was the same for both columns. Elution procedure for each column was performed by gravitation.

Column cleaning

Each dry sorbent was mixed with 7 mL of *n*-hexane. The sorbent bed was soaked with solvent for 3 min and then settled by gentle vibration. The solvent was drained by opening the valve and the cartridge was conditioned with 10 mL of hexane. The solvent level was let down to the top of the packed bed avoiding drying of the stationary phase.

Sample loading

For each column 250 µL of ISTD was loaded by pipetting.

Fractions collection

MOSH fraction: The column loaded with standards sample was eluted with 13 mL of *n*-hexane. The eluent was collected into a tube and concentrated to 0.5 mL (weight 0.33 g, density of hexane 0.659 g/mL)

MOAH fraction: Elution was continued with 15 mL of solvent mixture (70% DCM, 25% *n*-hexane, and 5% toluene), eluent was collected into a tube and concentrated to 0.5 mL.

The fractions were then analysed by GC.

Cardboard extraction

Triplicate samples of B. Welle cardboard 1 g of each were cut into little pieces and were extracted with 10 mL of a mixture of hexane/ethanol (1:1, v/v) for periods of 1 h, 2 h and 3 days with 10 mL of a mixture of hexane/ethanol (1:1, v/v). The same extraction process was performed also with triplicate samples of 2 g each of B. Welle cardboard.

The efficiency evaluation of solvents was performed using two samples of 1 g each of B. Welle extracted for 2 hour with 10 mL of a mixture of hexane/ethanol (1:1, v/v), and 10 mL of hexane/acetone (1:1, v:v). The extracts were then analyzed with GC-FID.

7.4.5 Output test (standards)

Most of the analytical methods used with silver silica gel cartridge for MOSH and MOAH determination in cardboard, do not evaluate the output of the cartridge. Thus, an approach was also to investigate the output of the method (yield) by performing whole procedure for evaluation of the results and method. Through this laboratory work the output of the cartridge can give a clear view of the retention capacity of the sorbent bed.

Elution solvents

For MOSH elution, 13 mL of distilled *n*-hexane was used; while in the case of MOAH elution, 15 mL of the solvent mixture with the composition of 70% DCM, 25% *n*-hexane and 5% toluene was used.

Fractionation procedure

For the packed bed, 4 g of silver silica gel (0.3% $AgNO_3$) was weighed into an empty glass cartridge (ID: 10 mm, length: 13.5 cm) with quartz wool as a bottom frit. The flow rate can affect the retention of certain compounds and therefore, gravity flow was used instead of vacuum manifold which accelerate the flow rate.

Dry sorbent was mixed with 10 mL of *n*-hexane, the sorbent bed was allowed to soak with the sorbent for 3 min and settle by gentle vibration. The solvent was then drained and the cartridge conditioned with 10

mL of *n*-hexane, taking the solvent level to the top of the packed bed and avoiding drying the sorbent bed. The sample was loaded and eluted.

- **Sample loading**

  ISTD (250 µL) solution was loaded onto the silver silica gel.

- **MOSH fraction**

  The MOSH fraction was eluted with 13 mL of *n*-hexane, collected into a tube and concentrated to 0.5 mL. The concentrated weight was 0.33 g according to the density of the *n*-hexane (0.659 g/mL). To determine the accurate volume (0.50 mL) of the concentrated eluent, the tube was weighed two times. First, before collecting a fraction a tare weight for the tube was weighed. After the eluent was collected and concentrated the gross weight (tar weight plus 0.33 g) was weighed.

- **MOAH fraction**

  The MOAH fraction was eluted with 15 mL of solvent mixture (70% DCM, 25% *n*-hexane and 5% toluene), collected into a tube and concentrated to 0.5 mL. The same procedure in MOSH was used to determine the accurate volume of the concentrate (0.50 mL). Nitrogen (N_2) gas was used for the concentration (evaporation) of the samples.

- **GC analysis**

  MOSH and MOAH fraction analysed with GC using method JGZBMO1_15. The STD and ISTD chromatograms were used to identify and classify the MOSH fraction peaks. Also MS was used for identification of the peaks in MOSH and MOAH chromatograms.

7.5 CONTAMINATIONS

7.5.1 Silver silica gel

Cartridge preparation

For the packed bed, 4 g of silver silica gel (0.3% $AgNO_3$) was weighed into an empty glass column (empty EXTRELUT N1) with quartz wool as a bottom frit.

Dry sorbent was mixed with 7 mL of *n*-hexane, and then the sorbent bed was allowed to soak with the sorbent for 3 min and settle by gentle vibration. The solvent was then drained and the cartridge conditioned with 10 mL of *n*-hexane, taking the solvent level to the top of the packed bed while avoiding the drying of the sorbent bed. The sample was loaded and eluted.

- **Sample loading**

  n-hexane of 250 µL was loaded onto the silver silica gel.

- **MOSH fraction**

  The MOSH fraction was eluted with 15 mL of *n*-hexane, collected into a tube and concentrated to 0.5 mL. The empty tube was weighed before using and after the eluent was collected it was concentrated to 0.5 mL.

- **MOAH fraction**

  The MOAH fraction was eluted with 20 mL of solvent mixtures (45% DCM, 50% *n*-hexane and 5% toluene), then collected into a tube and concentrated to 0.5 mL. MOSH and MOAH fractions were analysed with GC. Nitrogen (N_2) gas was used for the concentration (evaporation) of the samples.

7.5.2 Commercial SPE cartridges

Commercial cartridges are useful in cardboard analysis for their time saving. This part of the project work focused on the investigation of possible contaminant presence by using blank test and GC analysis. Commercial SPE – tubes used for these contamination tests were Strata EPH –tubes.

i) First test

Washing Strata tube

The Strata EPH-tube was washed with 20 mL of *n*-hexane.

Fractionation elution solvents

For MOSH elution, 6 mL of *n*-hexane was used; while in the case of MOAH elution, 10 mL of DCM was used. These fractions were collected into tubes and concentrated to 0.5 mL for GC analysis.

ii) Second test

The Strata EPH-tube was washed with 4 mL of n-hexane, followed by 2 mL of solvent mixture 1 (20% DCM, 5% toluene and 75% hexane) and 20 mL of *n*-hexane.

Fractionation elution solvents

For MOSH elution, 4 mL of *n*-hexane was used followed by 2 mL of solvents mixture (20% DCM, 5% toluene and 75% hexane); while in the case of MOAH elution, 10 mL of the same solvent mixture was used. These fractions were collected into tubes and concentrated to 0.5 mL for GC analysis.

iii) Third test

Washing Strata tube

The Strata tube was washed with 5 mL of n-hexane, followed by 6 mL of solvent mixture 2 (45% DCM, 5% toluene, and 50% *n*-hexane) and 15 mL of *n*-hexane.

Fractionation elution solvents

For MOSH elution, 7 mL of distilled *n*-hexane was used; while in the case of MOAH elution, 10 mL of solvent mixture (45% DCM, 5% toluene and 50% *n*-hexane) was used. The different fraction was collected into a tube and concentrated to 0.5 mL for GC analysis

7.5.3 Vacuum manifold valves

To check the possible contaminants coming from the vacuum manifold valves, a blank test was made. Pure solvent such as *n*-hexane, toluene and dichloromethane (DCM) were used in three empty columns connected each to a valve on the vacuum manifold. The eluent of 6 mL of each solvent was collected in three tubes for GC analysis.

7.6 DEVELOPMENT OF THE ANALYSIS METHOD FOR MINERAL OIL IN CARDBOARD

7.6.1 Introduction

The goal of this master thesis was to develop an easy, suitable analytical manual method with high efficiency for MOSH and MOAH measurement, by using a solid phase extraction (SPE) process, gas chromatography-flame ionisation (GC-FID), and mass spectrometry (MS) for the identification of MOSH and MOAH components.

For SPE process, the amount of activated silver silica gel (with 0.3% $AgNO_3$) was increased to 4 g, and the column with 10 mm inner diameter was used. 2% toluene was added in the MOAH elution solvent mixture to deactivate silver nitrate for the efficient elution of MOAH, and also as a keeper preventing loss of volatile components during solvent evaporation.

7.6.2 Equipment and reagents

Glassware material, reagents, standard (STD) and internal standard (ISTD) are presented in Chapters 7.1.2 and 7.1.3.

7.6.3 Preparation of silver silica gel

- **Washing of silver nitrate**

  A portion (about 3 g) of silver nitrate ($AgNO_3$) was washed with 10 mL of *n*-hexane. The mixture of silica gel and hexane was kept to settle and then hexane was pipetted out. This washing operation was done three times. Silver nitrate was then let to evaporate to dryness in the oven at around 50 ºC.

- **Silica gel preparation of is activation heating step**

  The silica was activated by heating 33.3 g of silica gel at 400 ºC overnight; it was let to cool for 2 hours at least. The washed silver nitrate (100 mg) was transferred into a round flask containing 33.3 g of highly activated silica gel. The flask was protected from the light, closed and shaken vigorously for about 20 seconds. The silver silica gel was blended for about 30 min, using a rotary evaporator without applying the vacuum. It was let to rest for about 12 h (protecting from

the light). The blended silver silica was dried at 75 ºC overnight to eliminate residual water. Finally, the silver silica gel covered with an aluminum foil was stored in a desiccator.

Once ready, the silver silica gel containing 0.3% $AgNO_3$ can be used for two weeks while storing at room temperature in the dark /1,13/.

- **The use of silver nitrate impregnated silica gel**

The silver nitrate impregnated silica gel modifies the separation characteristics of the sorbent layer, by increasing the discrimination of certain compounds, particularly those containing carbon–carbon double bonds, such as mineral oil aromatic hydrocarbons (MOAH). The theory behind this separation is based on the fact that silver ions can complex with unsaturated compounds (π bond) /2/.

7.6.4 Extraction of cardboard sample

The cardboard B3ARR sample (2 g) was extracted by immersion hexane/ethanol (1:1) at room temperature for 2 h in a closed tube. The hexane extract phase separation was made by mixing 5 mL of cardboard extract with 10 mL of UHQ water in a tube. The hexane extract on the top was then poured out using a pipette into a narrow closed small bottle.

Pre-separation by column liquid chromatography

For the packed bed, 4 g of silver silica gel (0.3% $AgNO_3$) was weighed into an empty glass cartridge (empty EXTRELUT N1) with quartz wool as a bottom frit. The flow rate can affect the retention of certain compounds, therefore gravity flow was used instead of vacuum manifold which accelerate the flow rate.

Dry sorbent was mixed with 10 mL of *n*-hexane, and then the sorbent bed was allowed to soak with the sorbent for 3 min and settle by gentle vibration. The solvent was then drained and the cartridge conditioned with 10 mL of *n*-hexane, taking the solvent level to the top of the packed bed while avoiding the drying of the sorbent bed. The sample was loaded and eluted.

- **Sample loading**

The phase separated hexane extract of 250 µL and the ISTD of 250 µL were loaded onto the silver silica gel.

- **MOSH fraction**

  The MOSH fraction was eluted with 13 mL of *n*-hexane, collected into a tube and concentrated to 0.5 mL. The empty tube was weighed before using and after the eluent was collected it was concentrated to 0.5 mL. The weight of the concentrated eluent should be 0.33 g; the density (0.659 g/mL) of *n*-hexane was used for the calculation of the volume of the concentrate. This method was used to determine the accurate volume of the concentrate.

- **MOAH fraction**

  The MOAH fraction was eluted with 15 mL of solvent mixtures (70% DCM, 25% *n*-hexane and 5% toluene), then collected into a tube and concentrated to 0.5 mL. The same procedure in MOSH was used for the calculation of the volume of the concentrate, but with the use of toluene density (0.87 g/mL). Nitrogen (N_2) gas was used for the concentration (evaporation) of the samples.

7.6.5 Gas chromatographic analysis

MOSH and MOAH fractions were analysed with GC using method JGZBM01_15 according to Chapter 7.2.2. The ISTD chromatogram was used to identify the MOSH fraction peaks in cardboard B3ARR extract. Also MS was used for identification of the peaks in MOSH and MOAH chromatograms.

8 RESULTS AND DISCUSSIONS

8.1 Optimisation of GC parameters

The same raw extract of cardboard (B3ARR) was used with different analysis method. In the method JGZMO1 (Appendix 1), the initial temperature was 40 °C, a ramp rate of 20 °C/min the maximum column temperature was 360 °C, and the final hold time was 28.25 minutes. With these parameters, baseline drift appeared in the chromatogram (Appendix 1). The reason could be that the rate of the temperature increase was too fast (ramp rate of 20 ºC/min) or the end temperature was too high (column maximum temperature 360 ºC) /77,85/.

The hump of unsolved compounds was very high in this chromatogram. The resolution of the peaks eluting in the middle of the chromatogram were insufficient. By decreasing the ramp rate and increasing the injection volume the resolution can be improved. The solutes elution from the column stopped earlier, which indicates that the program time was short (28.5 minutes).

Some adjustments of the parameters were done in the analysis method JGZMO1_10 (Appendix 2). The ramp rate was reduced to 10 °C/min, and the final hold time for maximum temperature was increased to 43.50 minutes (total run time). As consequences on the chromatogram (Appendix 2), the baseline dropped down a bit, but there was excessive peak resolution.

In the third analysis method JGZMO1_15, the ramp rate was increased to 15°C/min, the final hold time for maximum temperature was reduced to 32.67 minutes, and the injection volume was increased to 2 µL to increase the response of the GC, and increase the sensitivity of the GC method /86/. Chromatogram (Appendix 3) results revealed that the hump of unsolved compounds decreased, the baseline dropped down; the peaks resolution were good, and the hold time (32.67 minutes) was enough to ensure the solution of all the analytes from the column.

Conclusions

The parameters used (injection volume 2.0 µL, ramp rate 15 °C/min, column maximum temperature 340 °C and detector temperature 340 °C) in the third method JGZMo1_15 gave the best results; therefore, this analysis method was the best choice among the three ones. The chromatogram of the raw extract of cardboard showed the hump of unsolved hydrocarbons above the baseline, topped by peaks which are mainly in order of appearance resin acids, alcohols and triglycerides (Appendix 4).

8.2 Commercial cartridge test for contaminants: use of Strata EPH tubes

8.2.1 Strata EPH - first test

In this test, the instructions from the user manual of the Strata tubes were followed. The Strata tubes were washed with 20 mL of *n*-hexane. The chromatogram of the washing hexane (Appendix 5) was compared to the chromatogram of the MOSH eluent (Appendix 6). From retention time fram 10 minutes to 20 minute, a lot of contaminants appeared on MOSH chromatogram as illustrated by the short peaks. When comparing also DCM chromatogram (Appendix 7) with MOAH eluent chromatogram (Appendix 8), the appearance of peaks in MOAH illustrates the presence of contaminants.

8.2.2 Strata EPH- second test

In this second test the volume of washing solvent was increased (5 mL hexane, 6 mL solvent mixture (75%, *n*-hexane, 20% DCM and 5% toluene), and 20 mL of hexane) to clean up the Strata tube. The chromatogram of the washing hexane (Appendix 5) was compared to the chromatogram of the MOSH (Appendix 9). From retention time 10 minutes to 20 minute, a lot of contaminants appeared in MOSH chromatogram. When comparing also solvent mixture chromatogram (Appendix 10) with MOAH chromatogram (Appendix 11), from retention time 5 minutes to 24 minutes a lot of peaks appeared in MOAH region.

8.2.3 Strata EPH - third test

In the third test the washing solvent was also increased (5 mL hexane, 6 mL solvent mixture 2 (45% DCM, 5% toluene and 50% hexane), and 15 mL of hexane) to clean up the Strata tube. The chromatogram of the washing hexane (Appendix 5) was compared to the chromatogram of the MOSH fraction (Appendix 12). There were specially no contaminants in MOSH chromatogram. However, when comparing the solvent mixture chromatogram (Appendix 10) with MOAH solvent mixture eluent (Appendix 13), more contaminants appeared from retention time 6 minutes to 26 minutes. There was a large peak at 12.60 minutes.

8.2.4 Conclusions

Although an important amount of solvent and a solvent mixture were used to clean up the Strata EPH SPE tubes, the contaminants still appeared in the GC analysis of the samples from these three tests. The sources of these contaminants can be the solvents used, the sorbent bed or the valves of the vacuum manifold.

8.3 Silver silica gel cartridge test for contaminants

The silver silica gel cartridge was tested without using the vacuum manifold valves. When the hexane chromatogram (Appendix 5) was comparing with the MOSH chromatogram (Appendix 14), there was no contaminant appeared in MOSH. But when comparing the toluene chromatogram (Appendix 15) with MOAH chromatogram (Appendix 16), the contaminant from toluene appears in MOAH between the retention time 12 minutes and 13 minutes. The contaminants detected in MOAH chromatogram were originated from the toluene used in the solvent mixture. Therefore, this test showed that the silver silica gel itself was clean.

8.4 Contaminant test of the vacuum manifold valve

When comparing the hexane chromatogram (Appendix 5) to the chromatogram of the hexane sample eluted through the valve (Appendix 17); there were several peaks appearing in the chromatogram of the hexane sample flown through the vacuum manifold valve. This clearly indicated that there were contaminants coming from the valves when hexane was used as solvent.

The same thing could be observed when comparing the DCM chromatograph (Appendix 7) with the chromatogram of the DCM sample flown through the valve (Appendix 18). From the retention time 12 minutes to 24 minutes, there were six peaks appearing in the chromatogram (Appendix 18). These peaks were the contaminants coming from the valve when DCM was used as solvent.

The chromatogram of the toluene (Appendix 15) showed clearly contaminants from this solvent with the peaks appearing at retention times of 11 minutes to 14 minutes. The solvent bottle was not opened prior for this laboratory work. The same result appeared after several tests of this solvent. Therefore, these contaminants may come from the manufacturer of the solvent or due to the storage conditions. When comparing the chromatogram of toluene (Appendix 15) with that of the toluene sample eluted through the valve (Appendix 19), beside the contaminants coming from the toluene we could also see the

contaminants coming from the valve (retention time from 14 to 24 minutes) on the chromatogram (Appendix 19).

As conclusion, these valves in the vacuum manifold were not inert to the solvents used (*n*-hexane, dichloromethane (DCM) and toluene). Therefore, there were sources of contaminants in the SPE process. To address this issue, attention should be focused on the choice of the valves of the vacuum manifold. Research should be made using fluorocarbon rubber (FKM) or perfluorocarbon rubber material for the valves /87-89/. The relative inertness of fluorocarbon rubbers is provided by fluorine-carbon bonds on the elastomer backbone. Fluoroelastomers having higher fluorine content have increasing fluids resistance derived from increasing fluorine levels. Therefore, perfluoroelastomers show broad chemical resistance similar to PTFE (Teflon) as well as good heat resistance. Polytetrafluoroethylene (PTFE) would be the best choice for valve material. For this experimental, according to the manufacturer manual, the valves were made from PTFE.

8.5 Cardboard extraction efficiency

8.5.1 Time factor in cardboard extraction

Two samples of 1 g cardboard were extracted for 2 hours and 3 days each. Comparing the chromatographs of 2 hours extraction (Appendix 20) and 3 days extraction (Appendix 21), the total area for 2 h extraction was 7833430 and 8718787 for 3 days extraction time. Thus the total area increased by 11.3% for 3 days extraction time respectively. This indicates that the concentration of the extract has increased by 11.3% when using 3 days extraction time.

The total height of the biggest peaks for 2 h extraction was 1313960 and 1418559 for 3 days. This means that the total height has increased by 7.9% for 3 days extraction.

8.5.2 Mass effect in cardboard extraction

Two samples of 1 g and 2 g cardboard B. Welle were extracted each during 2 hours. The result of the chromatogram (Appendix 20) shows that the total area of 1 g sample was 20417483, and 29489258 for 2 g sample (Appendix 22). Therefore, the total area increase by 30.76% when the cardboard sample used was 2 g. Since the area is proportionally related to the concentration, using 2 g cardboard sample increases the concentration of the extract by 30.76%. The sum of heights (Appendix 20 and 22) increased by 22.76% when using 2 g cardboard. This indicated that the response of the GC increased with the amount of cardboard extracted.

8.5.3 Solvent effect in cardboard extraction

The result of the chromatogram in Appendix 20 shows that the total area of 1 g sample extracted during 2 hours with hexane/ethanol was 7833430. In the Appendix 23, the total area of the sample extracted with hexane/acetone during 2 hours was 7863979. This means that the use of acetone increased the total area by 0.4% (Appendix 23). The sum of heights in Appendix 20 was 1313960 and 1352884 in Appendix 23; which meant that the use of acetone increased the height by 3 %.

Acetone had a positive impact in cardboard extraction; but the increases were not significant (0.4% and 3%). Acetone (boiling point 56 ºC) is extremely volatile compared to ethanol (boiling point 79 ºC); this could easily cause the evaporation of volatile components in the cardboard during SPE process. Therefore, hexane/ethanol is the most adequate solvent for cardboard extraction.

8.5.4 Conclusions

According to the results above, 2 g cardboard sample and 2 hours extraction time were found to be the best setup for cardboard extraction in the laboratory for MOSH and MOAH determination. Since acetone is extremely volatile compared to ethanol, hexane/ethanol is the recommended solvent.

8.6 OUTPUT TEST (standards)

8.6.1 Fractionation with ISTD

The used amount of silver silica gel in the column was 4 g. For the first fractionation ISTD was used as a sample. In the second fractionation ISTD (250 µL) added to STD (250 µL) were used as sample. The results in Table 6 determine the degree of separation of MOSH and MOAH by the cartridge used and also the output of the separation process.

The MOSH (Appendix 24) and MOAH (Appendix 25) were clearly separated, there was no outcome of MOAH in MOSH fraction vice versa. Calculated from the data of Table 6, the average output of MOSH was 69% with R-square value of 0.9683 (close to 1). The average output of MOAH was 67%, with R-square value of 0.9016. The output of the all system was 68%.

Table 6. Output results of MOSH and MOAH by using only ISTD as sample

		ISTD (Area)	MOSH ISTD (Area)	MOSH ISTD (%)	MOSH ISTD (2*%)	MOAH ISTD (Area)	MOAH ISTD (%)	MOAH ISTD (2*%)
	C13	377292	119510	31,68	63,36	0	0	0
MOSH	Cycy	344615	126340	36,66	73,32	0	0	0
	Cholest.	675814	236167	34,95	69,9	0	0	0
					0			0
	2MN	371223	0	0	0	109109	29,39	58,78
MOAH	TBB	315565	0	0	0	111367	35,29	70,58
	Per	714444	0	0	0	210733	29,5	59

Increasing the silver silica gel to 4 g gave more silanol (Si-OL) group to absorb compounds onto the silica surface. By using the solvent mixture of 70% DCM, 25% hexane and 5% toluene, the strength of the MOAH elution solvent was increased.

The retention power of the silver silica gel has an effect on the output of the chromatography columns. The retention of MOAH increases with the number of aromatic rings and decreases with alkylation. Therefore, highly alkylated compounds are eluted first.

Toluene efficiently deactivates the retention power of silver nitrate for unsaturated hydrocarbons. Adding toluene to the eluent for MOAH optimizes the fractionation /18/.

8.6.2 Fractionation with cardboard B3ARR extract

In the fractionation of cardboard extract, MOSH and MOAH in ISTD were the desired components to calculate the output of the cartridge. In the result of Table 7, MOSH and MOAH were clearly separated. The general output of MOSH in ISTD was 100.22%, which clearly showed that there was addition of some similar component in the cardboard extract to the ISTD MOSH components. From Table 7, Cholestane output was 118.26%, which clearly showed that other component from the cardboard sample was added to cholestane peak. The output of MOAH in ISTD was 67% (Table 6), and 63% (Table 7), which meant the increasing of the components in the sample increased the retention capacity of the cartridge.

Table 7. The output of ISTD in the fractionation of cardboard B3ARR extract (Date: 09.04.2014)

		ISTD (Area)	MOSH ISTD (Area)	MOSH ISTD (%)	MOSH ISTD (2*%)	MOAH ISTD (Area)	MOAH ISTD (%)	MOAH ISTD (2*%)
MOSH	C13	371957	161594	43,44	86,88	0	0	0
	Cycy	352671	168421	47,76	95,52	0	0	0
	Cholest.	660587	390588	59,13	118,26	0	0	0
								0
MOAH	2MN	342040	0	0	0	104800	30,64	61,28
	TBB	306308	0	0	0	109920	35,89	71,78
	Per	680586	0	0	0	190465	27,99	55,98

8.6.3 Conclusion

According to the results of Table 6, the average output of the cartridge was 68% with ISTD used as a sample. Beside the retention capacity of the cartridge, the output of the cartridge was also affected by the evaporation of some volatile components of the extract during SPE process and the fractions concentration by nitrogen (N_2). In Table 7, the output of the cholestane was 118.2%, which meant that some other component from the extract was overlapping with cholestane peak or could be even cholestane extracted from the cardboard. Therefore, extreme care had to be taken in the choice of the internal standard (ISTD) to improve the results of MOSH and MOAH calculation. Internal standard should be very similar, but not identical to the chemical species of interest in the samples. The output of MOSH and MOAH components were in the same range, which means that each one of these ISTD components can be used independently or together for the calculation of the concentration of the extract for MOSH and MOAH.

8.7 Analysis methods for mineral oil determination in cardboard

8.7.1 Fractionation with silver silica (0.3% $AgNO_3$) vs. fractionation with Strata EPH

Only the ISTD was used as sample during this experiment. The chromatogram results with silver silica (0.3% $AgNO_3$) showed for MOSH (Appendix 26) a clear separation of C_{13}, cyclohexyl cyclohexane (Cycy)

and 5α-cholestane (Cho). However, in MOAH chromatogram (Appendix 27), C_{13}, Cycy and Cho still appeared besides MOAH compounds such as 2-methylnaphthalene (2MN), 1,3,5-tri-*tert*-butylbenzene (TBB) and perylene (per). Therefore, MOSH and MOAH separation was not good. The reasons could be the insufficiency of MOSH elution, the high concentration of MOSH compounds or the speed control of the elution.

The chromatogram results with Strata tube for MOSH fraction (Appendix 28) showed the appearance of TBB (MOAH component) in addition to C_{13}, Cycy, and Per. There was also a broad peaks of contaminants compared to the silver silica MOSH chromatogram (Appendix 26). MOAH chromatogram (Appendix 29) showed clear separation of Met, TBB and Per. TBB appeared here as very short peak, but higher in MOSH chromatogram. This indicated that TBB is well eluted in MOSH than MOAH fraction.

8.7.2 Fractionation with silver silica (0.3% AgNO3) vs. silver silica (1% AgNO3)

The comparison of the amount of silver nitrate (0.3% and 1%) in silica gel for fractionation process, was done to determine which amount ensure better separation of MOSH and MOAH fractions. The results of MOSH and MOAH for silver silica (0.3%) (Appendices 30 and 31) and silver silica (1%) (Appendices 32 and 33) were used to build the comparison curves for MOSH in Figure 26 and for MOAH in Figure 27.

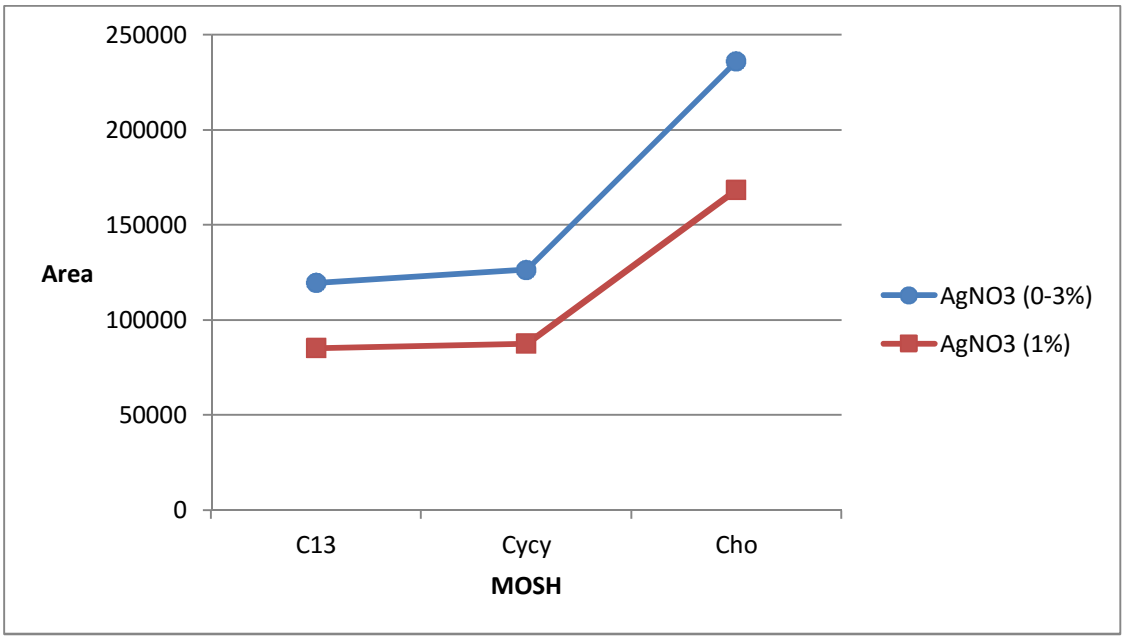


Figure 26. MOSH results with silver silica (0.3%) and silver silica (1%).

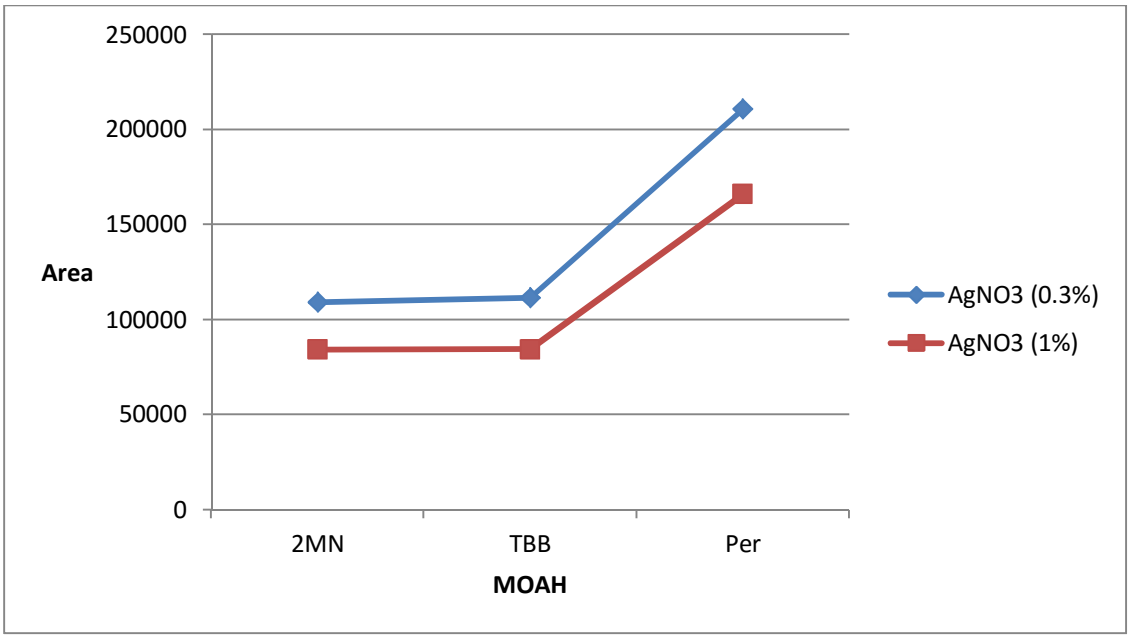


Figure 27. MOAH results with silver silica (0.3%) and silver silica (1%).

In Figure 26 the curve of MOSH fraction for silver silica (0.3%) was above the one for silver silica (1%). The same result occurred also with MOAH curves in Figure 27.The Y-axes unit is the peak area, which was proportionally related to the concentration of the component in MOSH or MOAH. Therefore, silver silica (0.3%) ensure the higher concentration of the solute in MOSH or MOAH fraction. The silver silica gel (0.3%) will be the choice for the development of method for the analysis of mineral oil in cardboard.

8.8 DEVELOPMENT OF THE ANALYSIS METHOD FOR MINERAL OIL IN CARDBOARD

8.8.1 Results and discussion

The internal standard (ISTD) was well separated in MOSH and MOAH fractions according to the chromatogram results in Figures 28 and 29 (see Appendices 34 and 35 for details). TBB did not appear in MOSH, C_{13} and the separation of Cycy was excellent as well as in the case of cholestane (see Appendix 34). In MOAH fraction 2MN, TBB and perylene separation was also excellent, no interfering ISTD MOSH fraction in MOAH were found; see Figure 29 and Appendix 35 for more details.

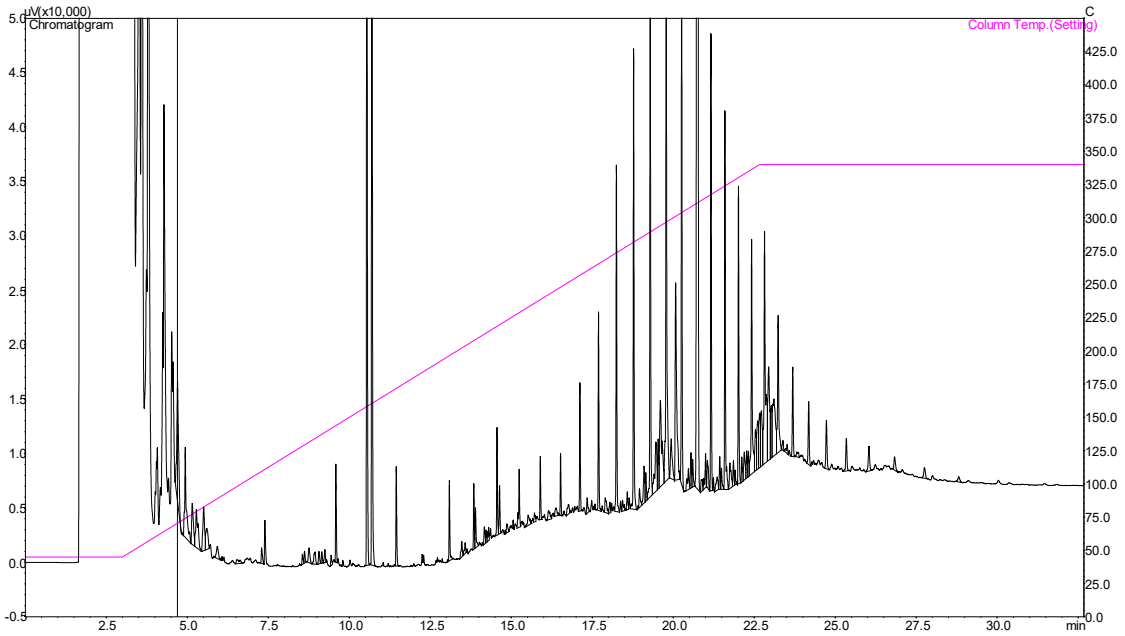


Figure 28. GC-FID of MOSH chromatogram of cardboard B3ARR sample.

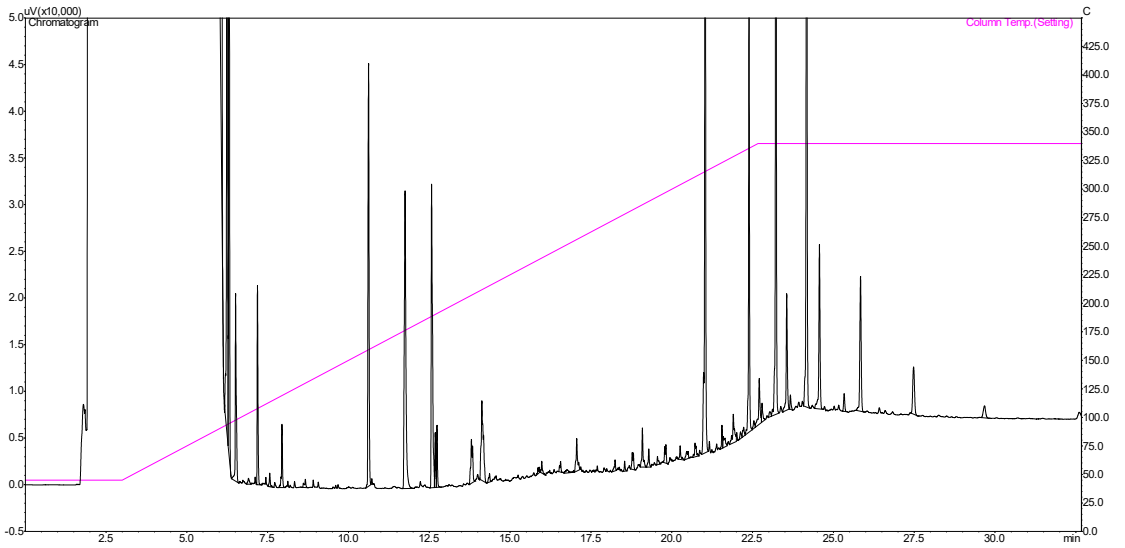


Figure 29. GC-FID of MOAH chromatogram of cardboard B3ARR sample.

MOSH fractions were well eluted due to an increase of the silver silica gel amount to 4 g into the column. This increased the retention surface of the polar sorbent for polar components in the extract. The solvent and non-polar component such as MOSH can pass through the cartridge. Additionally the inner diameter

of the column was relatively small 10 mm; this decreases the longitudinal diffusion effect of the analyte /90/.

The alkanes in MOSH were identified in the chromatogram by using the standard (STD) chromatogram (see Appendix 36) and the retention step separating the peaks. The range of volatile component started from the retention time of 9.59 minutes for C_{12} to the retention time of 23.24 minutes for C_{35}. From the retention time of 23.24 minutes the components migrating with wetting contact started.

The range of alkanes from C_{12} to C_{36} was identified also by the MS (see Appendix 37). The inconvenience in the chromatogram was the increase of the ISTD (Appendix 38) component (cholestane) in MOSH due to the identical or overlapping component present in the cardboard extract.

The MOAH fractions were also well separated as shown by the ISTD in MOAH. The MS result (Appendix 39) shows the aromatic components in the cardboard extract. Only few acid components were present (see Appendix 39).

8.8.2 MOSH Integration in GC chromatogram of B3ARR sample

Integration parameters for MOSH

The chromatograms integration of MOSH and MOAH were complex and time consuming. The manual integration was used to improve the efficiency by monitoring the parameters in Table 8. The integration started from the retention time of 4.75 min to 23.50 min, which was the range of the volatile component of MOSH (C_{10} to C_{35}).

Table 8. Integration parameters of MOSH and MOAH chromatograms

Time (minute)	Command	Value
0.000	Integration Off	******
0.000	HORIZ Baseline On	******
4.750	Width	5.000
4.750	Slope	2000.000
4.750	Drift	3000.000
7.100	Integration On	******
7.100	Min. Area/Height	2000.000
23.500	Integration Off	******
0.000		******

Data comparison of hexane (black line) and MOSH (red line) shows clearly the drift of the baseline and the hump of unresolved components (Fig 30).

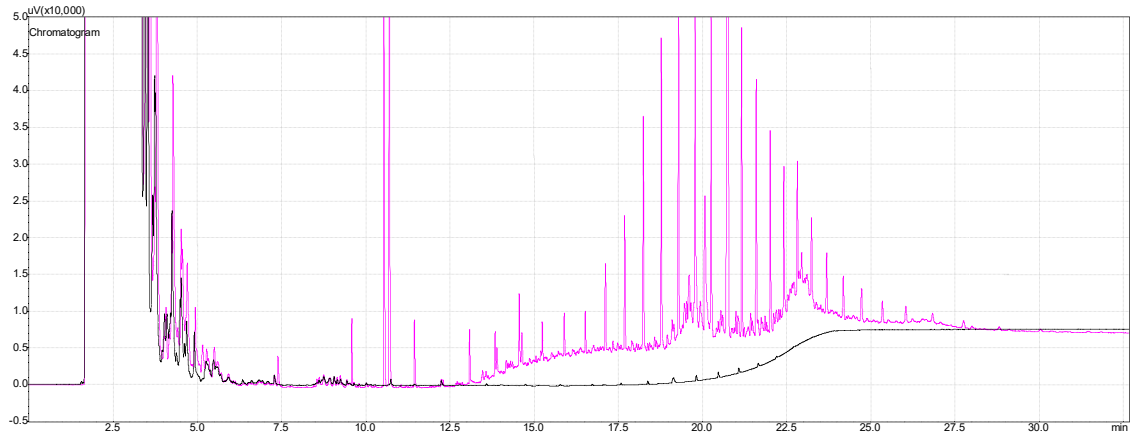


Figure 30. Data comparison of hexane (black) and MOSH (red) for B3ARR cardboard.

Hexane chromatogram was substracted from MOSH and MOAH chromatograms to avoid the drift of the baseline and also to take in account the hump of unsolved components in the integration.

Hexane baseline was used then in the integration parameters of MOSH and MOAH for the determination of mineral oil in cardboard. This helps to draw manually the baseline to unify all the peaks and the hump of unresolved components. The Figure 31 (see Appendix 40 for more details) shows the integration of MOSH. The idea was to subtract hexane baseline with GC-software.

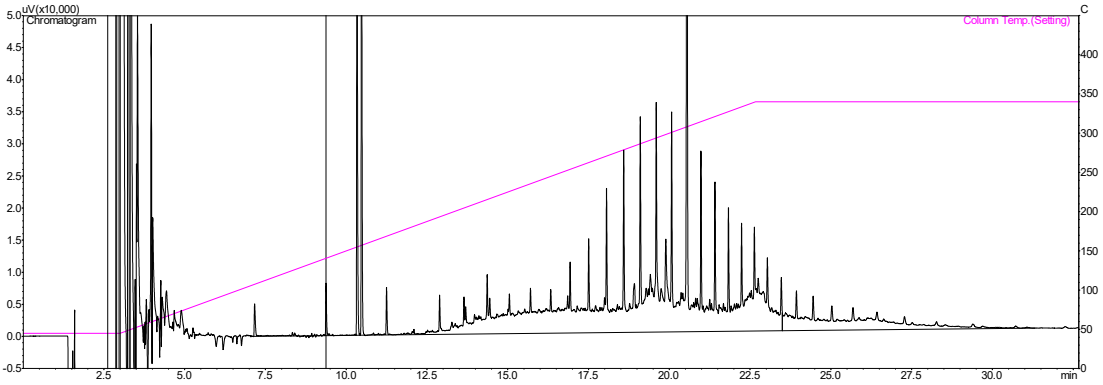


Figure 31. Integration of MOSH (B3ARR).

The determination of MOAH follows the same procedure used for MOSH. Figure 32 (see Appendix 41 for more details) shows the integration of MOAH.

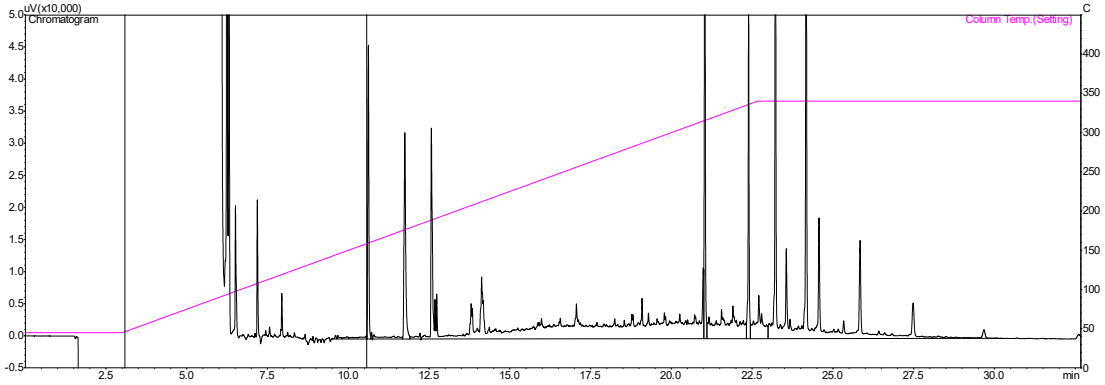


Figure 32. Integration of MOAH (B3ARR)

The integration area of MOSH and MOAH was used to calculate the amount of mineral oil in cardboard (see Fig 32).

8.8.3 Cardboard results

The integration and the calculation results were given as follow: the MOSH fraction was 276.4 mg/kg cardboard (69.8%), and the MOAH fraction 119.6 mg/kg cardboard (30.2%). The total amount of volatile mineral oil in the cardboard B3ARR was 396 mg/kg cardboard. The amount of non-volatile mineral oil in the range over C_{35} is 107.3 mg/kg cardboard. MOSH fraction was 46.8 mg/kg cardboard and MOAH fraction was 60.5 mg/kg cardboard (see Appendix 42 for MOSH and MOAH calculations).

9 GENERAL CONCLUSION

GC-FID is the simplest and affordable analytical equipment for the analysis of mineral oil in cardboard; it allows fast analysis and can be automated. FID is the only method available for a quantitative determination of mixtures of hydrocarbons which are not available as standards. However, FID is of modest sensitivity, which is a particularly severe drawback for MOSH and MOAH analysis as they form broad humps. In fact, 50–100 ng MOSH or MOAH is required to be measurable.

The mineral hydrocarbons primarily or exclusively form a hump of unresolved components, either consisting of branched alkanes, branched paraffins and cyclic naphthenes or of alkylated aromatics. The peaks integration included also the unsolved humps in MOSH (Appendix 40) and MOAH (Appendix 41) chromatograms for reliable results of the determination of mineral oil in cardboard.

According to the experiments, of the cardboard extraction 2 g cardboard samples and 2 hours extraction time were the best practical setup for laboratory work with hexane/ethanol (1:1/v:v) as solvent. For SPE process, the amount of activated silver silica gel (with 0.3% $AgNO_3$) was increased to 4 g, and the column with 10 mm inner diameter was used. 2% toluene was added in the elution solvent mixture to deactivate silver nitrate for the efficient elution of MOAH, and also as a keeper preventing loss of volatile components during solvent evaporation.

The results of the chromatograms (Appendices 34 and 35) show a clear separation of MOSH and MOAH from the ISTD used as samples. The clear sharp peaks on the top of the hump of MOSH mostly represent n-alkanes. The clear sharp peaks on the top hump of MOAH mostly represent the aromatic hydrocarbons. Gravitational elution was used to solve the elution speed control. Conforming to the integration and the calculation results, the MOSH fraction was 276.4 mg/kg cardboard (69.8%), and the MOAH fraction 119.6 mg/kg cardboard (30.2%). The total amount of volatile mineral oil in the cardboard B3ARR was 396 mg/kg cardboard. The amount of non-volatile mineral oil in the range over C_{35} is 107.3 mg/kg cardboard. The MOSH fraction was 46.8 mg/kg cardboard and the MOAH fraction was 60.5 mg/kg cardboard (see Appendix 42 for MOSH and MOAH calculations).

Limitation of this method was the amount of solvent used during the SPE process, whereas less solvent is recommended.

As recommendations, to avoid the overlapping of the chosen ISTD components with cardboard components, the chosen ISTD should not be identical to the cardboard extract components. For the better quality of the peaks, the chromatograph devices (GC and MS) should be cleaned up prior to the sample analysis. Also the GC-MS library should be updated to help the identification of most of the components.

It is suggested that the amount of solvent used during the SPE process will gain intensive attention since it was time consuming. Instead of using gravitational elution speed control one could have performed the experience employing a slower speed elution. Investigation should be done on the new SPE technology such as matrix solid-phase dispersion (MSPD), solid phase microextraction (SPME), nanotechnology in food packaging and food safety and so on.

REFERENCES

1. EFSA, Scientific opinion on mineral oil hydrocarbons in food, *EFSA Journal* 2012, 10(6)(2012)2704. Available at: http://www.efsa.europa.eu/en/efsajournal/doc/2704.pdf

2. Amoruso, M.A., Gamble, J.F., McKee, R.H., Rohde, A.M. and Jacques, A., Review of the toxicology of mineral spirits, *Int. J. Toxicol.* 27(1-2)(2008)97-165.

3. Wright, A., Oral toxicity of mineral oils and related compounds, A review, University of Eastern Finland, Kuopio-Finland.
Available at: http://www.cepi.org/system/files/public/documents/positionpapers/recycling/2011/summary-20111125-00001-01-E.pdf

4. Dalbey, W.E. and Biles, R.W., Recent respiratory toxicology investigations of mineral oils: Post-1990, *ACGIH Symposium On Mineral Oils and Metal Processing Oils*, October 2-4, 2002 (ACGIH: American Conference of Governmental Industrial Hygienists).

5. Nash, J.F., Gettings, S.D., Diembeck, W., Chudowski, M. and Kraus, A.L., A toxicological review of topical exposure to white mineral oils, *Food Chem. Toxicol.*, 34(2)(1996)213-225.

6. Fleming K. A., Zimmermann H. and Shubik P., (1998): Granulomas in the livers of humans and Fischer rats associated with the ingestion of mineral hydrocarbons: A comparison, *Regul. Toxicol. Pharmacol.* 27*(*1998)75–81.

7. Tarnow, P., Hutzler, C., Schön, K., Tralau, T., Luch, A. and Hasse, A., Estrogenic activity of aromatic compounds present in mineral oil used in printing inks, *Toxicol. Lett.*, 229(suppl. 10)(2014)S64–S65.

8. Biedermann, M. and Grob, K., On-line coupled high performance liquid chromatography-gas chromatography for the analysis of contamination by mineral oil. Part 1: Method of analysis, *J. Chromatogr. A*, 1255(2012)56-75.

9. Biedermann, M. and Grob, K., On-line coupled high performance liquidchromatography-gas chromatography for the analysis of contamination by mineral oil. Part 2: Migration from paperboard into dry foods: Interpretation of chromatograms, *J. Chromatogr. A*, 1255(2012)56-75.

10. Vollmer, A., Biedermann, M., Grundböck, F., Ingenhoff, J-E., Biedermann-Brem, S., Altkofer, W. and Grob, K., Migration of mineral oil from printed paperboard into dry foods: survey of the German market, *European Food Research and Technology* 232(2011)175-182.

11. Fiselier, K. and Grob, K., Determination of mineral oil paraffins infoods by on-line HPLC-GC-FID: lowered detection limit; contamination of sunflower seeds and oils, *Eur. Food Res. Technol. 229*(2009)679-688.

12. Grob, K., Biedermann, M., Caramaschi, A. and Pacciarelli, B., LC-GC Analysis of the Aromatics in a Mineral Oil Fraction: Batching Oil for Jute Bags, *J. High Resol. Chromatogr.*, 14(1)(1991).

13. Hutler, C., Analysis of mineral oil: “Manual” pre-separation, followed by LV-GC/FID off-line HPLC-GC/FID, FbR: Federal Institute for Riskassessment - Germany, *Seminar: Mineral oils in food packaging*, 23.09.2011.

14. Biedermann, M., Fiselier, K. and Grob, K., Aromatic hydrocarbons of mineral oil origin in food: method for determining the total concentration and first results, *J. Agric. Food Chem.*, 57(2009)8711-8721.

15. EU Commission Regulation (EC) N0 1935/2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC, *Off. J. Eur. Communities: Legis.*, L 338/4.

16. EU Commission Regulation (EC) N0 2023/2006 on good manufacturing for material and articles intended to come into contact with food, *Off. J. Eur. Communities: Legis.*, L 384/75.

17. Grob, K., Mineral oils depicted by gas Chromatograms Principals for the analysis in food, *Mineral oils in food packaging – developments and solutions Seminar*, Berlin, 23rd of September 2011.
Available at: http://www.bfr.bund.de/cm/343/mineral-oils-depicted-by-gas-chromatograms-principals-for-the-analysis-in-food.pdf

18. Fiselier, K., Grundbök, F., Schön, K., Kappenstein, O., Pfaff, K., Hutzler, C., Luch, A. and Grob, K., Development of a manual method for the determination of mineral oil in foods and paperboard, *J. Chromatogr. A.*, 1271(2013)192-200.

19. Moret, S., Barp, L., Grob, K. and Conte, L.S. Optimised off-line SPE-GC-FID method for the determination of mineral oil saturated hydrocarbons (MOSH) in vegetable oils, *Food Chemistry*, 129(2011)1898-1903.
Available at:
http://ec.europa.eu/transparency/regexpert/index.cfm?do=groupDetail.groupDetailDoc&id=9275&no=13

20. Moret, S., Barp, L., Purcaro, G. and Conte, L.S., Rapid and sensitive phase extraction-large volume injection-gas chromatography for analysis of mineral oil saturated and aromatic hydrocarbons in cardboard and dried foods, *J. Chromatogr. A*, 1243(2012)1-5.

21. Biedermann, M. and Grob, K., Is comprehensive analysis of potentially relevant migrants from recycled paperboard into foods feasible? *J. Chromatogr. A,* 1272(2013)106-115.

22. Biedermann, M. and Grob, K., Is recycled newspaper suitable for food contact materials? Technical grade mineral oils from printing inks*, Eur. Food Res. Technol. 230*(2010)785-796.

23. Harnsen, J., Hutter, W.J., Win, T., Barnabas, I., Whittle, P., Hansen, N. and Sakai, H., Risk assessment for mineral oil: Development of standardized analytical methods in soil and soil-like materials, Alterra Research Institut (Netherland)-Report 1225/ December/2005/.
Available in pdf at: http://edepot.wur.nl/17143

24. Martteini, P., Rosi, F., Castellano, E., Vestrucci, G., Mostardini, F., Bocci, A., Miniati, A. and Bondi, C., Determination of Mineral Oil Saturated Hydrocarbons (MOSH) and Mineral Oil Aromatic Hydrocarbons (MOAH) in Food and Packaging, *ILSI EUROPE 5th International Symposium on Food Packaging Scientific Developments supporting Safety and Innovation*, 14 – 16 November 2012, Berlin, Germany.

25. Moret, S., Sander, M., Purcaro, G., Scolaro, M., Barp, L. and Conte, L.S., Optimization of pressurized liquid extraction (PLE) for rapid determination of mineral oil saturated (MOSH) and Aromatic Hydrocarbons (MOAH) in cardboard and paper intended for food contact, *Talanta,* 115(2013)246-252.

26. Jamnicki, S., Determination ofmineral oil content in recycled papers, "*EFPRO-CEPI Innovation in Paper"-Early Stage Researcher (ESR) Workshop European PaperWeek*, 13-15 November 2012, Brussels.
Available at: http://www.cepi.org/system/files/public/epw-presentations/2012/innovationinpaper/EFPRO_CEPI_Jamnicki.pdf

27. Magnaghi, G., Recovery paper market in 2012, Bureau International Recycling (BIR) 2012.
Available at: http://www.bir.org/assets/Documents/publications/brochures/BIR-PaperStats-2014-V3.pdf (Read 12.12.2013).

28. Alén, R., *Biorefining of Forest Resources*, Publisher
Paper Engineers' Association/Paperi ja Puu Oy, Helsinki, Finland,2011, pp. 76-89, ISBN 978-952-5216-39-4

29. Sjöström, E., *Wood Chemistry Fundamentals and Applications*, 2nd Edition, Academic Press, San Diego CA, USA, 1993, 293 p. ISBN 0-12-647481-8.

30. Roberts, J.C., *The Chemistry of Paper*, The Royal Society of Chemistry, Cambridge, UK, 1996, ISBN 0-85404-518-X.

31. Kirwan, M.J., *Handbook, Paper and Paperboard Packaging Technology,* 2nd Edition, 2013, pp. 6-25, Wiley Blackwell, Chichester, UK, ISBN: 978-0-470-67066-8.

32. INERIS (French public research body of an industrial and commercial character), visited web page: http://www.ineris.fr/ippc/sites/default/interactive/brefpap/bref_pap/english/bref_gb_sulfite.htm (Read 03.2014).

33. Paperboard and cardboard image source, avaleable at: http://ehs.ucsc.edu/programs/waste-management/recycling-disposal/cardboard-paperboard.html / (Read 02.02.2016)

34. Paperboard boxes image source, avaleable at: http://www.greenwoodms.com/index.aspx?NID=409 (Read 02.02.2016)

35. Metsä Group Oy web page: http://www.metsagroup.com/Metsagroup/Pages/Default.aspx (Read 05.05.2013).

36. Bundesinstitut für Risikkobewertund (BfR)-Germany, *Determination of hydrocarbons from mineral oil (MOSH & MOAH) or plastics (POSH & PAO) in packaging materials and dry foodstuffs by solid phase extraction and GC-FID.*
Available at: http://www.bfr.bund.de/cm/349/determination-of-hydrocarbons-from-mineral-oil-or-plastics.pdf (Read 03.12.2013)

37. Tönnieben, M., Mineral oil from the view of the adhesive manufacturer, European Adhesives and Sealant Industry (FEICA), *Seminar, Safe adhesives for safe food* 20 June 2013.

38. European Adhesives and Sealant Industry (FEICA), Mineral oil hydrocarbons in food: adhesives for food packaging as a source, Brussels, 08/09/2014 source: FEICA FAS-EX-D08-009,http://www.feica.eu
Available at: http://www.feica.com/images/stories/fas-ex-d08-009_mineral-oil-in-foods_v2.pdf (Read 20.10.2014).

39. Irvine, A., Food contamination from recycled paper-fact or fiction? *Corrugated industry Conference*, Pira international September 2011.
Available at: http://www.thepackagingportal.com/assets/Alistair%20Irvine.pdf

40. Finney, K.F., Pomeranz, Y. and Hoseney, R.C., Effects of solvent extraction on lipid composition, mixing time, and bread loaf volume, *Cereal Chem.,* 53(3)383-388.

41. Simpson, N.J.K., *SOLID-PHASE EXTRACTION: Principles, Techniques, and Applications*, Marcel Dekker, 2000, pp. 19-23, ISBN: 0-8247-0021-X.

42. Pawliszyn, J., *Comprehensive Sampling and Sample Preparation: Analytical Techniques for Scientists*, Elsevier, Amsterdam, Netherlands, 2012, pp. 639-640, ISBN: 978-0-12-381373-2.

43. Suomen Standardisoimisliitto SFS-EN 14338 (Finnish Standard Association SFS).

44. Bradley, E.L, Stammati, A., Salkinoja-Salonen, M., Andersson, M., Bertaud, F., Hoornstra, D., Zucco, F., Weber, A., Turco, L., Traussnig, H., Test procedures for obtaining representative extracts suitable for reliable in vitro toxicity assessment of paper and board intended for food contact, *Food Addit. Contam., Part A, 27(2)(*2010)262-71.

45. Chowdhury, G., Kruczek, B., and Matsuura, T., *Polyphenylene oxide and modified polyphenylene oxide membranes*, Kluwer Academic Publishers, Dordrecht, the Netherlands, 2001, pp. 1-127, ISBN 978-0-7923-7511-1.

46. Wang, S., *Food Chemical Hazard Detection: Development and Application of New Technologies*, John Wiley & Sons, Ltd, 2014, 138 p. ISBN: 978-1-118-48859-1.

47. Smith,I., CHROMATOGRAPHY AND ELECTROPHORETIC TECHNIQUES, ISBN 0-8151-7839-5.

48. Morlock, G., and Ueda, Y., New coupling of planar chromatography with direct analysis in real time mass spectrometry*, J. Chromatogr. A.,* 1143(3)(2007)243-251.

49. Houck M.M., and Siegel, J.A., *Fundamentals of Forensic Science*, 2nd Edition, Academic Press, 2010, pp. 125-128, ISBN-13 9780123749895.

50. Zwir-Ferenc, A. and Biziuk, M., Solide Phase Extraction Technique-Trens, Opportunities and Applications, *Pol. J. Environ. Stud.*, 15(5)(2006)677-690.

51. HIS Engineering360, *Absorbents and Adsorbents Information*, Available at: http://www.globalspec.com/learnmore/materials_chemicals_adhesives/chemicals_raw_materials/desiccants_absorbents (read 3.10.2014).

52. Zhuravlev, L.T., The surface chemistry of amorphous silica. Zhuravlev model, *Colloids Surf.,* A, 173(200)1-38.

53. Damyanova, B.N., Retention of lipids in silver ion high-performance liquid chromatography: Facts and assumptions, *J. Chromatogr.* A, 1216(2009)1815–1824.

54. Fisher Scientific – Image gallery
Available at: http://www.fishersci.com/ecomm/servlet/fsproductdetail_10652_5949112__-1_0 (read: 12.02.2014).

55. Boryana Nikolova-Damyanova,Institute Of Organic Chemistry, centre of Phytochemistry, Sofia 1113-Bulgaria. Visited: http://lipidlibrary.aocs.org/Silver/bnd_rev/index1.htm (Read 12.01.2014).

56. Sigma-Aldrich Ltd (SUPELCO) Guide to solid phase extraction - Bulletin 910.

57. Barker, S.A., Matrix solid phase dispersion (MSPD) *J. Biochem. Biophys. Methods.* 70(2)(2007)151-62. Epub 2006 Sep 27.

58. Kristenson, E.M., Ramos, L., and Brinkman, A.Th., Recent advances in matrix solid-phase dispersion, *TrAC, Trends Anal. Chem.,* 25(2)(2006).

59. Bogialli, S., and Di Corcia, A., Matrix solid-phase dispersion as valuable tool for extracting contaminants from foodstuffs, *J. Biochem. Biophys. Methods,* 70(2007)163–179.

60. Monasterio, R.P., Fontana, A.R., and Silva, M.F., Matrix solid-phase dispersion: a simple and fast technique for the determination of phenolic compounds in olive oil by liquid chromatography, *Anal. Methods, 6(*2014)8986–8995.

61. Barker, S.A., Applications of matrix solid-phase dispersion in food analysis, *J. Chromatogr. A,* 880(2000)63-68.

62. Pawliszyn, J., Theory of solid-phase microextraction, *J. Chromatogr. Sci.,* 38(7)(2000).

63. Vas, G., and Vékey, K., Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis, *J. Mass Spectrom.*, *39(*2004)233-254.

64. Jung, D. and Ebeler, S.E., Headphase solid-phase microextraction method for the study of the volatility of selected flavor compounds, *J. Agric. Food Chem., 51(*2003) 200-205.

65. Harris, D.C., *Quantitative Chemistry Analysis*, 8th Edition, Freeman, W.H. & Company,New York,USA, 2010, pp. 565-566, ISBN: 1429218150.

66. Klee, M., Separation Science, web page of Dr. Matthew Klee,
Available at: http://www.sepscience.com/Techniques/GC/Articles/187-/GC-Solutions-19-Calibration-Curves--Part-1 (visited 12.07.2014).

67. http://www.colorado.edu/chemistry/chem5181/Lectures/C3_GC.pdf (read 12.12.2012).

68. Biedermann, M., Mineral oil contaminated food: analysis by on-line HPLC-GC
Available at: http://www.bfr.bund.de/cm/343/mineral-oil-contaminated-food-analysis-by-on-line-hplc-gc.pdf (Read: 12.12.2012).

69. http://www.ehow.com/list_5911530_disadvantages-advantages-hplc.html#ixzz2uvynizFl (Read 20.04.2013).

70. Mistry, B.D., *Handbook of Spectroscopic Data Chemistry (UV, IR, PMR, ^{13}CNMR and Mass Spectroscopy) Edition 2009*, Oxford Book Company, ISBN: 978-81-89473-86-0.

71. Van Bramer, S.E., An Introduction to Mass Spectrometry, Widener University Department of Chemistry-USA,
Available at: http://science.widener.edu/svb/massspec/massspec.pdf (Read 10.07.2014).

72. Settle, F.A., *Handbook of Instrumental Techniques for Analytical Chemistry-*
(chapter 15: Infrared Spectroscopy), Prine Hall PTR, New Jersey, USA, 1997, ISBN 0-13-177338-0.

73. Griffiths, P.R. and De Haseth, J.A., *Fourier Transform Infrared Spectrometry*, 2nd Edition, Wiley & Sons, New Jersey, USA, 2007, pp. 75-93, ISBN 978-0-471-19404-0.

74. Weston, F.S., Gas chromatography-Infrared spectroscopy. Available at: http://www.chem.agilent.com/Library/eseminars/Public/Separate%20and%20Identify%20with%20GC-FTIR.pdf (Read 20.09.2014).

75. Grob, R.L. and Barry, E.F., *Moderne Pratice of Gas Chromatography 4th Edition*, John Wiley & Sons, 2004, chap.2, ISBN 978-0-471-22983-0.

76. Khan, A.I., Optimizing GC Parameters for Faster Separation with Conventional Instrumentation, Thermo Scientific – Technical Note 20743.
Available at:
http://www.bioszeparacio.hu/_user/browser/File/Thermo/Applik%C3%A1ci%C3%B3/TN20743_GC_TraceGOLD_Fast_Analysis.pdf

77. RESTEK, Guide to GC Column Selection and Optimizing Separation. Available at: http://www.restek.com/pdfs/GNBR1724-UNV.pdf

78. Biedermann, M. and Grob, K., How white was the mineral oil in the contaminated Ukrainian sunflower oils? *Eur. J. Lipid Sci. Technol.,* 111(2009)313–31.

79. Phillips, J.B. and Xu, J., Comprehensive multi-dimensional gas chromatography, *J. Chromatogr. A*, 703(1995)327-334.

80. Ventura, G.T., Kenig, F., Reddy, C.M., Frysinger, G.S., Nelson , R.K.N., Ben Van Mooy, B.V.M. and Gaines, R.B., Analysis of unresolved complex mixtures of hydrocarbons extracted from Late Archean

sediments by comprehensive two-dimensional gas chromatography (GGxGC), *Org. Geochem.* 39(2008)846–867.

81. Extrulut NT 1 image source: http://www.merckmillipore.com/FI/en/product/EXtrelut-NT-1,MDA_CHEM-115094 (reade: 18.10.2015).

82. Strata EPH image source: https://www.chromservis.eu/p/strata-eph-200-m-70a-2-5g-6ml-teflon-tubes-30-pk (reade: 18.10.2015).

83. Vacuum manifold image source: http://3.imimg.com/data3/QR/BL/MY-1116047/vacuum-manifold-250x250.jpg (reade: 18.10.2015).

84. Lorenzini, R., Fiselier, K., Biedermann, M., Barbanera, M., Braschi, I. and Grob, K., Saturated and aromatic mineral oil hydrocarbons from paperboard packaging: estimation of long-term migration from contents in the paperboard and data on boxes from the market, *Food Addit. & Contam.* 27(12)(2010)1765-1774.

85. Agilent Technology, GC Method development, 2007. Available at: http://www.chem.agilent.com/library/support/documents/f3964554214.pdf (Read 05.03.2014).

86. Kim, K.H. and Nquyen, H.T., Effects of injection volume change on gas chromatographic sensitivity determined with two contrasting calibration approaches for volatile organic compounds. *J. Sep. Sci.*, 30(3)(2007)367-74.

87. Plastic Design Library, *Chemical resistance, vol. 2, Elastomer, Thermosets, and Rubber*, 2nd Edition, William Andrew Inc., Norwich, NY, USA,1994, 977p. ISBN-13: 978-1884207136.

88. Rubber Technology Info. Available at: http://rubbertechnology.info/en/%20rubber%20compounds/fluorocarbon-rubber-/-fkm/fpm/ (Read 12.06.2014)

89. Trelleborg, Materials Chemical Compatibility Guide.
Available at:
http://www.tss.trelleborg.com/remotemedia/media/globalformastercontent/downloadsautomaticlycreatedbyscript/catalogsbrochures/mat_chem_comp_gb_en.pdf (read 25.09.2014).

90. Eddy Diffusioin. Available at :
http://www.chromacademy.com/lms/sco3/Theory_Of_HPLC_Band_Broadening.pdf (read 25.09.2014).

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32. GC.FID MOSH chromatograms from ISTD loaded in silver silica (1%) column
33. GC.FID MOAH chromatograms from ISTD loaded in silver silica (1%) column

GC-FID chromatograms Integration

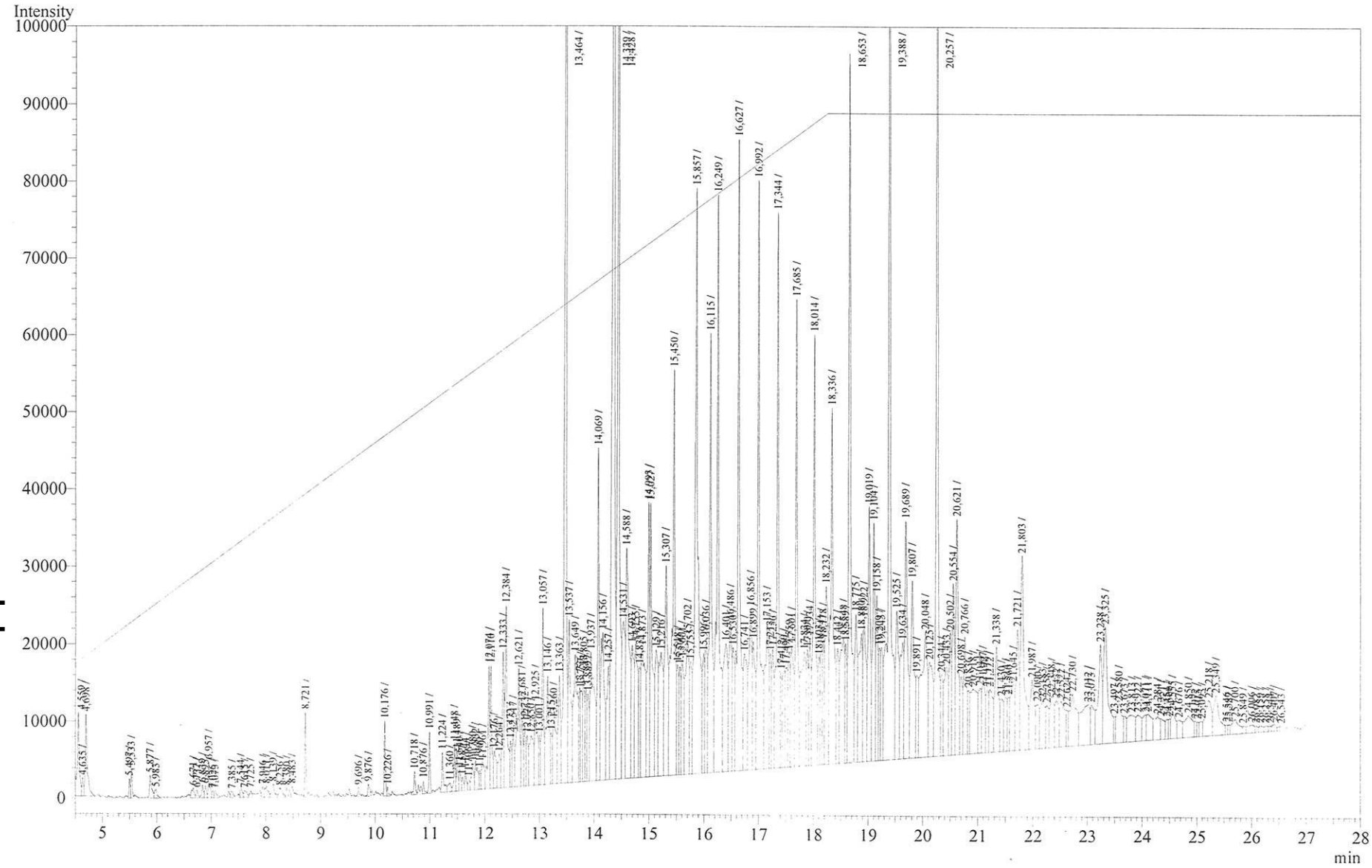
34. GC.FID MOSH chromatograms from cardboard B3ARR
35. GC.FID MOAH chromatograms from cardboard B3ARR

Standards chromatograms and MS chromatograms

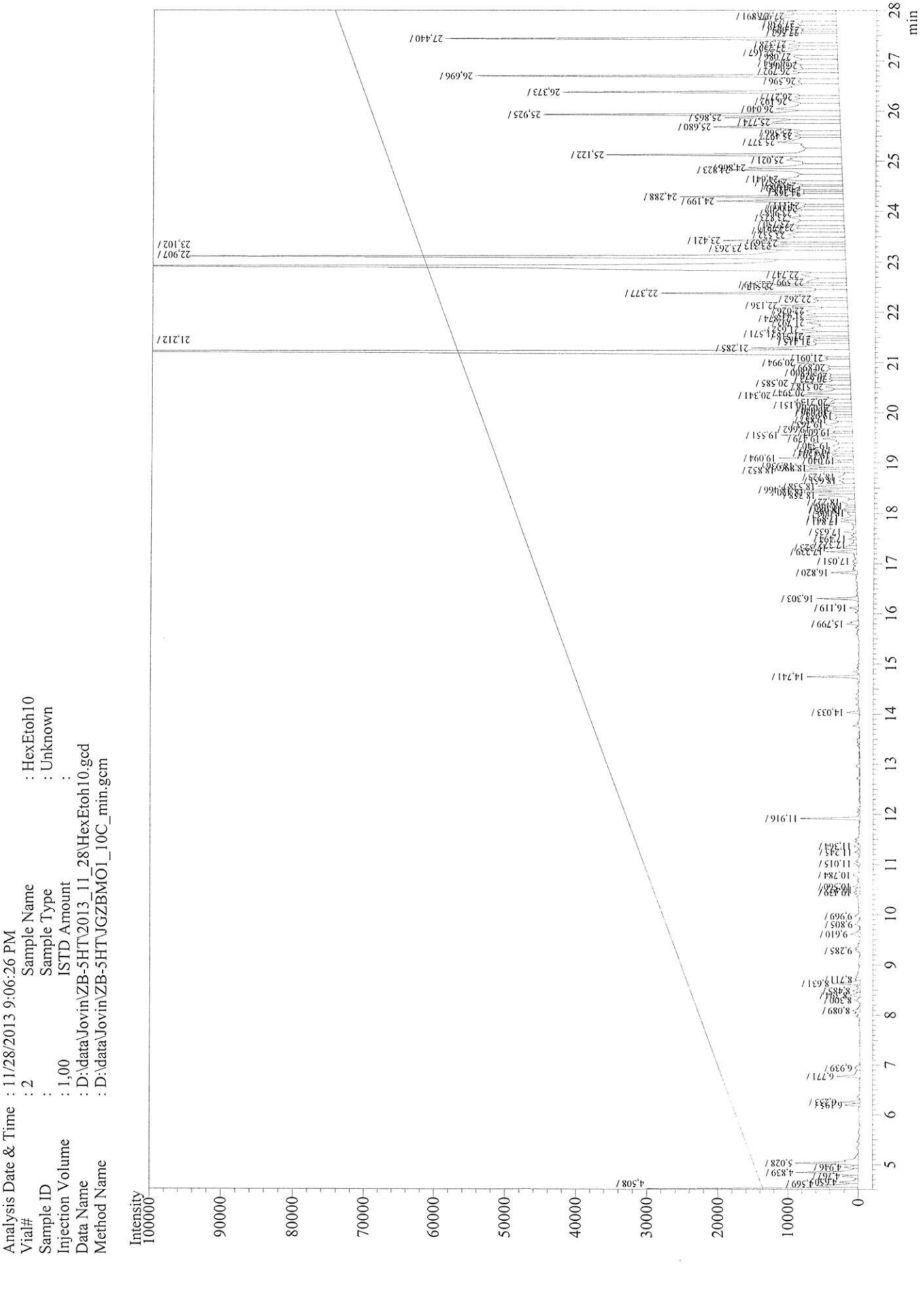
36. GC.FID STD chromatograms
37. GC.FID MS Mosh chromatograms
38. GC.FID ISTD chromatograms
39. MS MOAH chromatograms
40. MOSH integration chromatograms
41. MOAH integration chromatograms
42. MOSH and MOAH calculation

Appendix 1

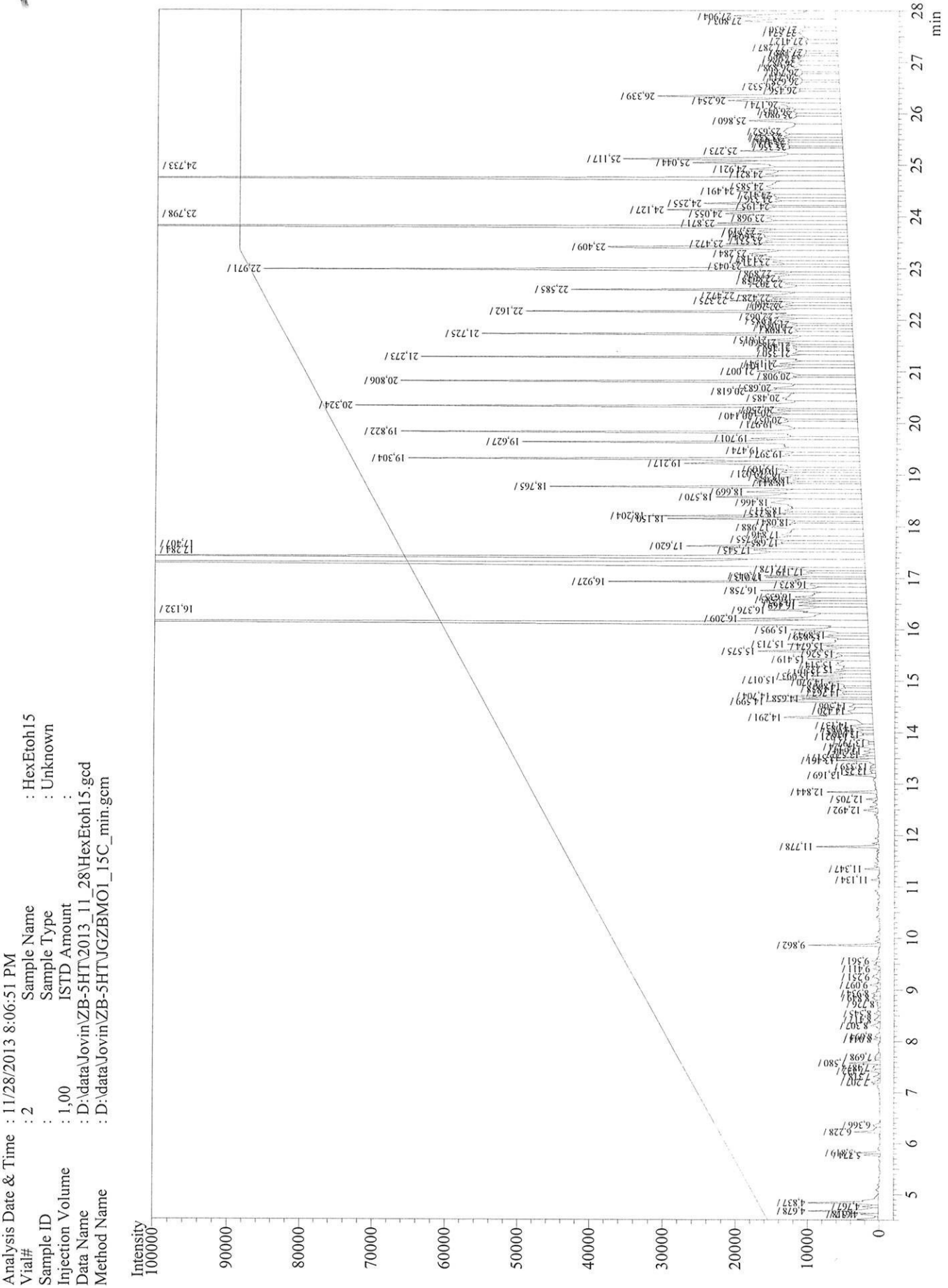
Analysis Date & Time : 11/28/2013 4:27:40 PM
Vial# : 2 Sample Name : HexEtoh1
Sample ID : Sample Type : Unknown
Injection Volume : 1,00 ISTD Amount :
Data Name : D:\data\Jovin\ZB-5HT\2013_11_28\HexEtoh1.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1.gcm



Appendix 2


Analysis Date & Time : 11/28/2013 9:06:26 PM
Vial# : 2 Sample Name : HexEtoh10
Sample ID : Sample Type : Unknown
Injection Volume : 1,00 ISTD Amount :
Data Name : D:\data\Jovin\ZB-5HT\2013_11_28\HexEtoh10.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_10C_min.gcm
Intensity
min
4,508
21,212
22,907
23,102
11,916
14,741
15,799
16,303
16,820
18,852
19,094
20,994
21,285
22,377
24,199
24,288
25,680
25,925
26,373
26,696
27,440

Appendix 3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Sample Name : HexEtoh15
Sample ID : Unknown
Sample Type : Unknown
Injection Volume : 1,00
ISTD Amount :
Data Name : D:\data\Jovin\ZB-5HT\2013_11_28\HexEtoh15.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm
Intensity
min

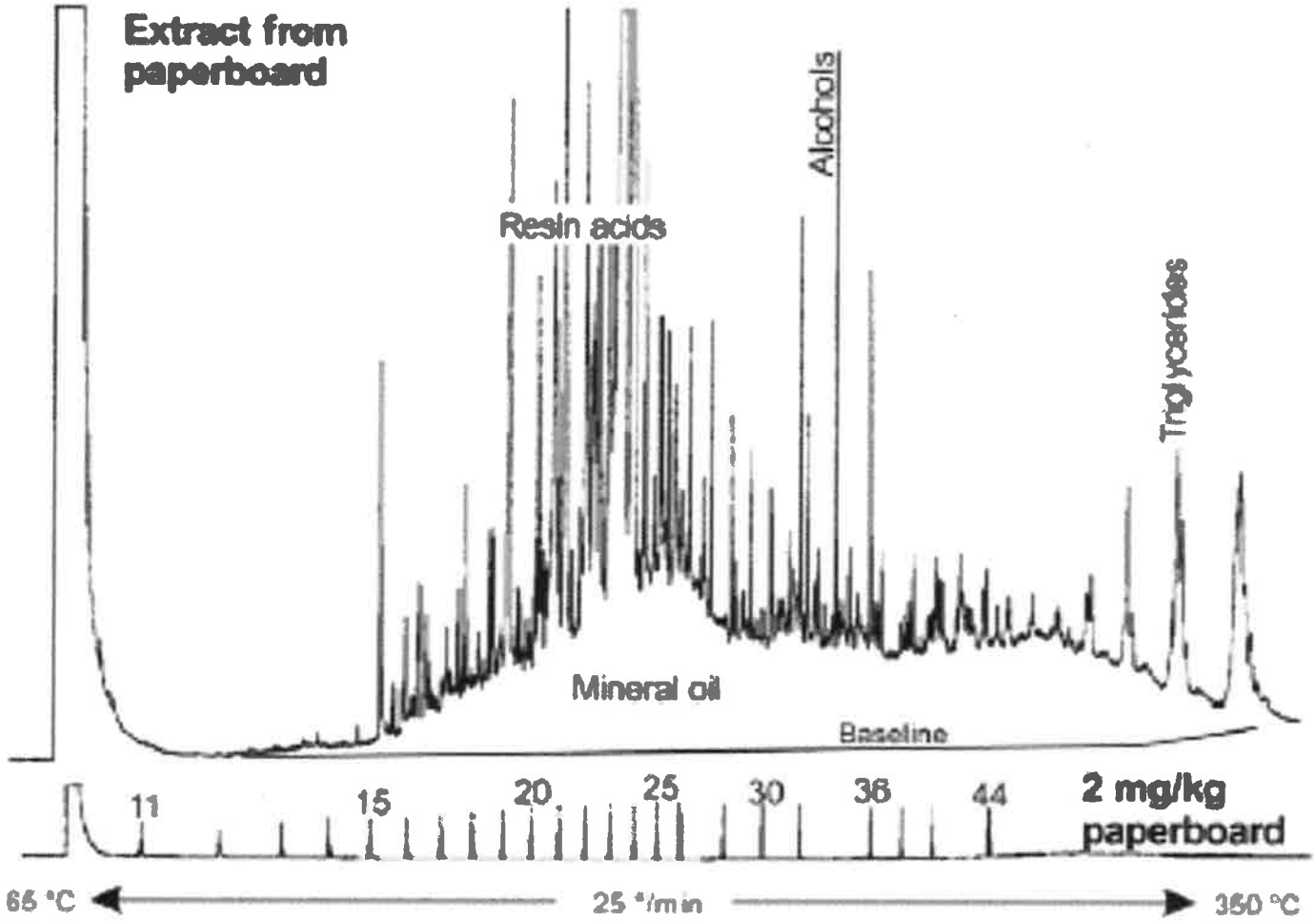
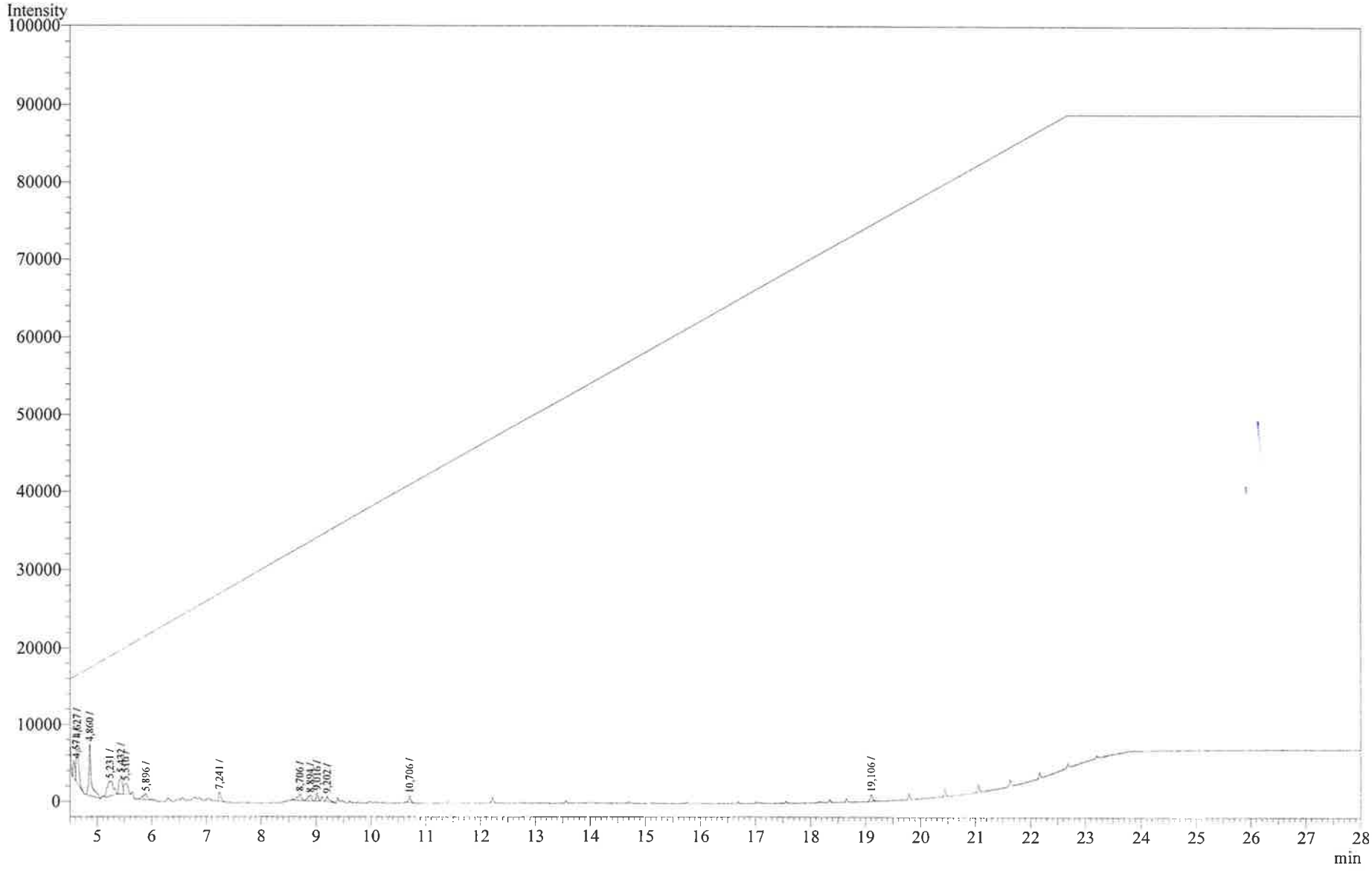


Fig. 1. GC-FID chromatograms obtained from a raw extract of recycled paperboard and a mixture of n-alkanes (with the number of carbon atoms given for some of them) injected in an amount per component corresponding to 2 mg/kg in the paperboard, i.e., 20 times above the required detection limit.

STRATA FIRST TEST

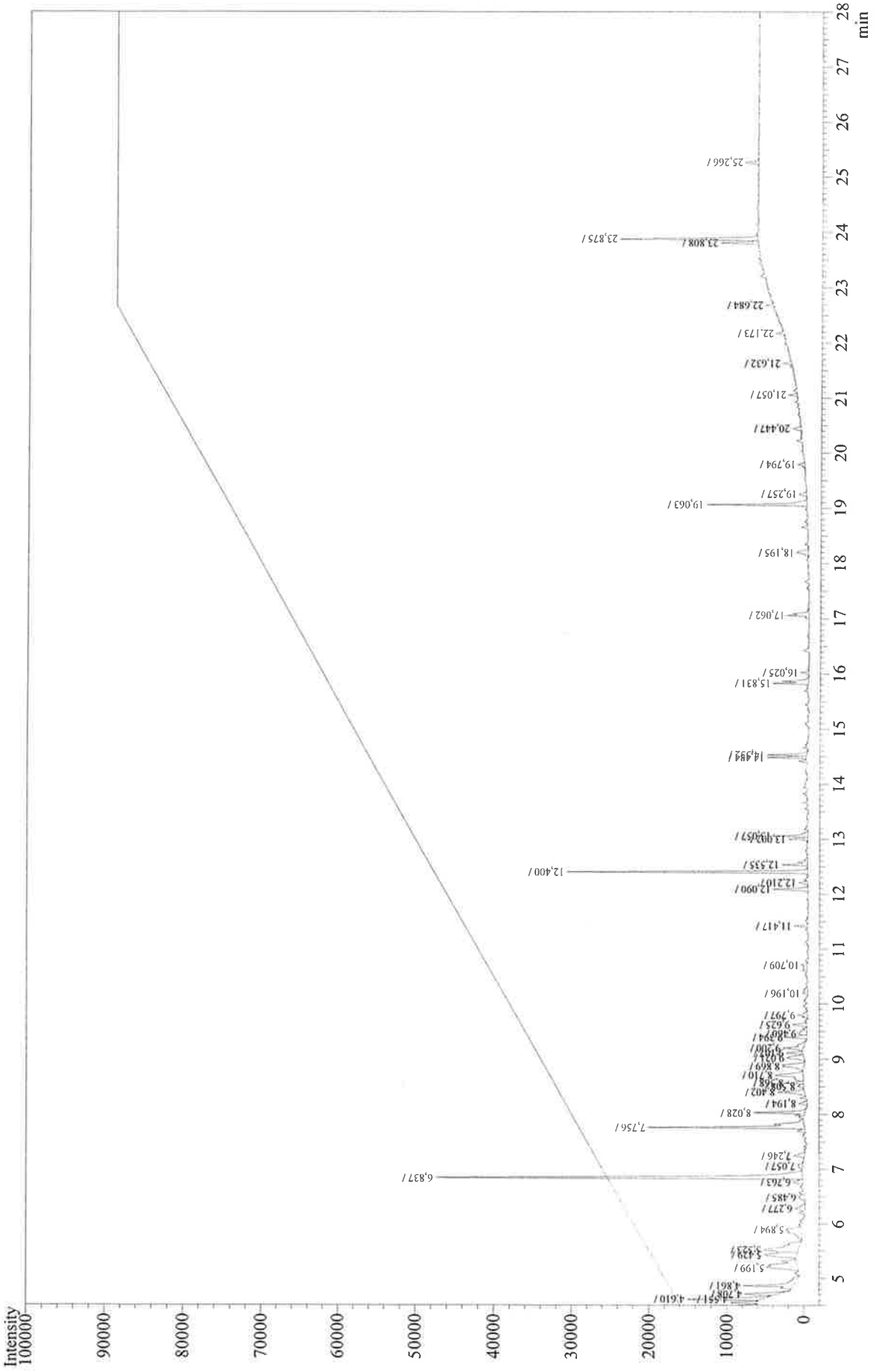
5 (5)

Analysis Date & Time	: 3/28/2014 5:52:43 PM		
Vial#	: 1	Sample Name	: Hexane
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_03_28\HEXANE.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		



Appenix 5

Appendix 6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Vial# : 4
Sample ID : :
Injection Volume : 2.00
Data Name : D:\data\Jovin\ZB-5HT\2014_03_28\EB2-MOAH.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm
Sample Name : EB2-MOAH
Sample Type : Unknown
ISTD Amount :
Intensity
min
6.837
7.756
12.400
23.875
23.808
25.266

Appendix 7

Analysis Date & Time	: 3/27/2014 3:09:24 AM		
Vial#	: 5	Sample Name	: EB2-DCM
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
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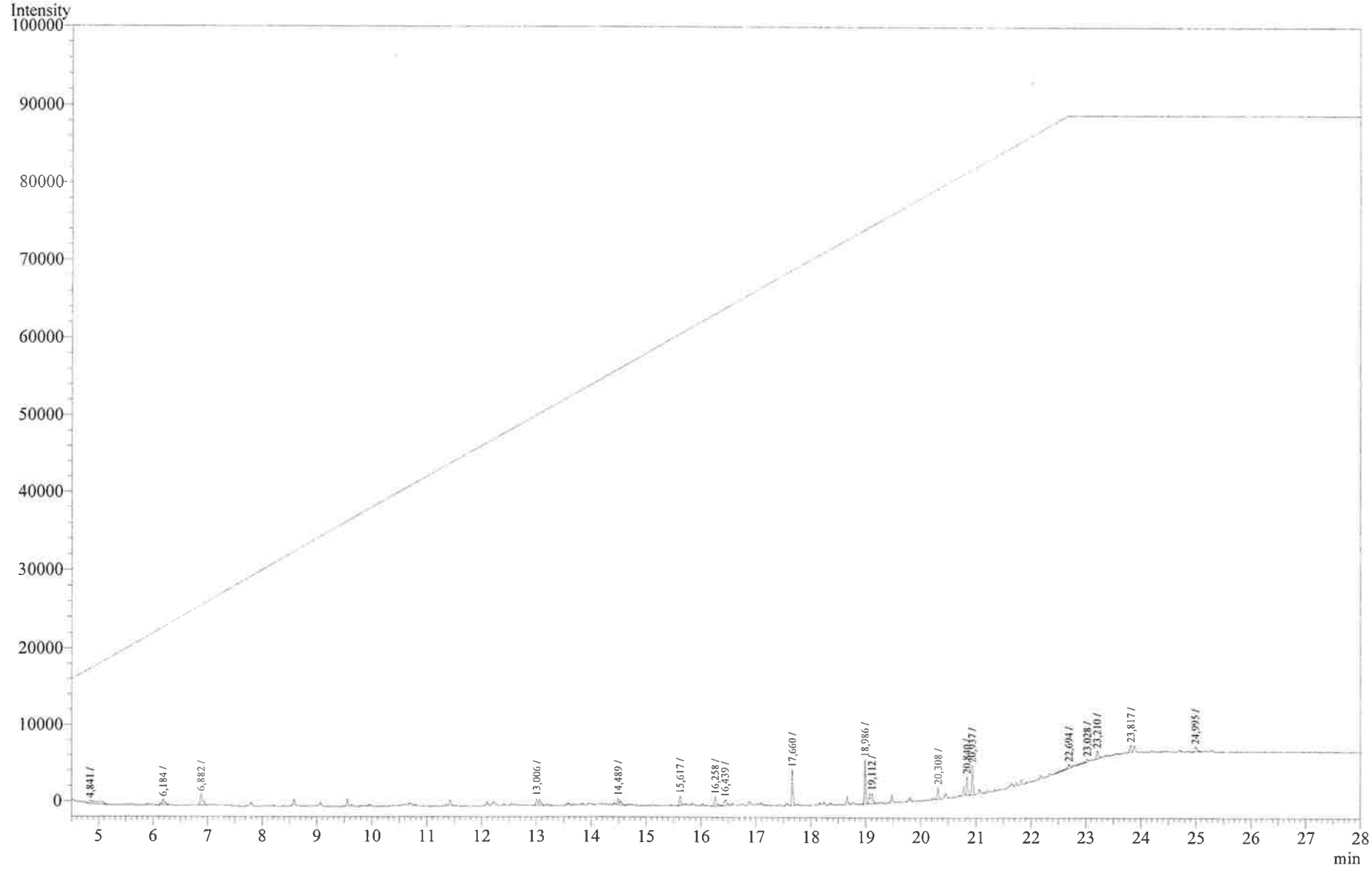
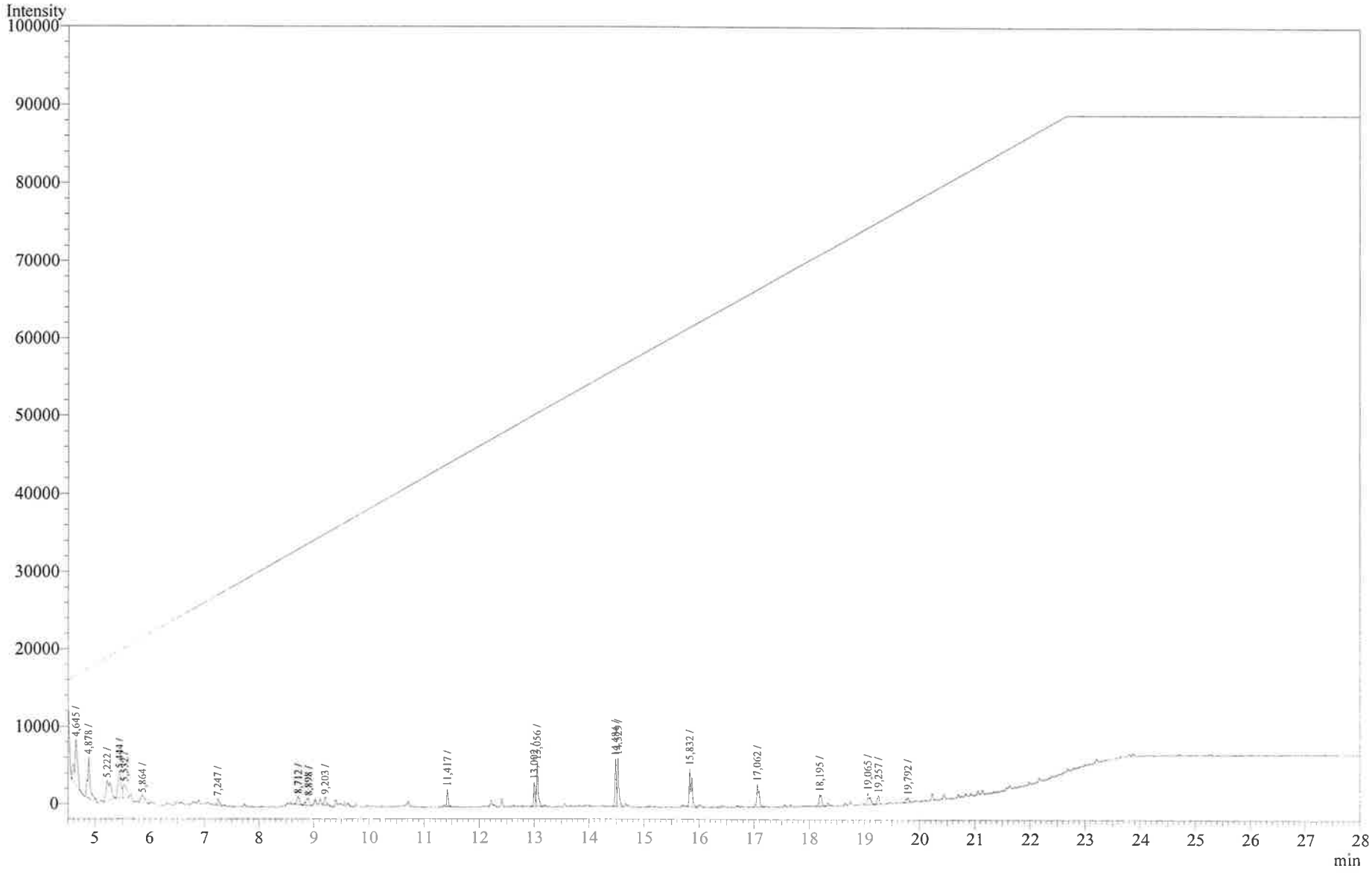
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		



STRATA FIRST TEST

8 (8)

Analysis Date & Time	: 3/28/2014 7:37:42 PM		
Vial#	: 3	Sample Name	: EB2-MOSH
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_03_28\EB2-MOSH.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		



Appendix 8

Appendix 9.1

9

Analysis Date & Time	: 3/28/2014 12:04:47 AM		
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Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_03_027\EB-MOSH.gcd		
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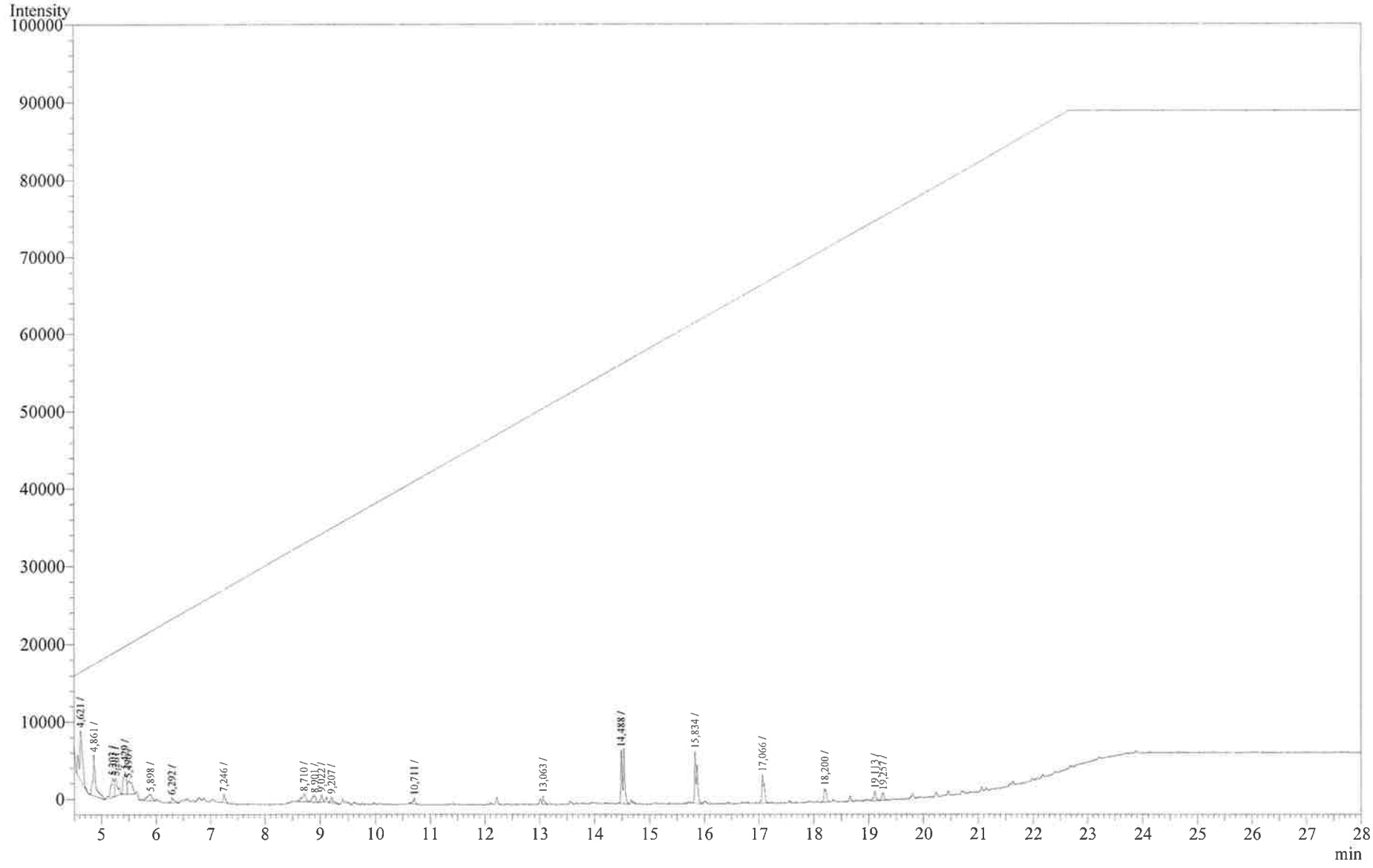
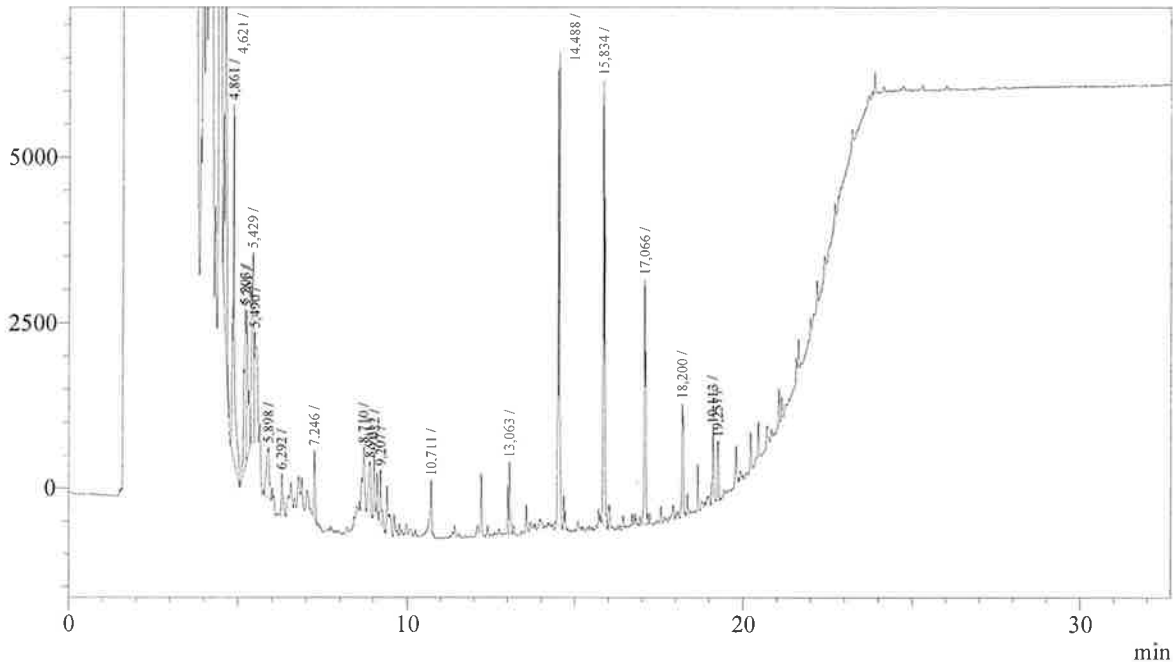


Appendix 9.2

Analysis Date & Time : 3/28/2014 12:04:47 AM
User Name : Admin
Vial# : 4
Sample Name : EB-MOSH
Sample ID :
Sample Type : Unknown
Injection Volume : 2,00
ISTD Amount :

Data Name : D:\data\Jovin\ZB-5HT\2014_03_027\EB-MOSH.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm

Intensity



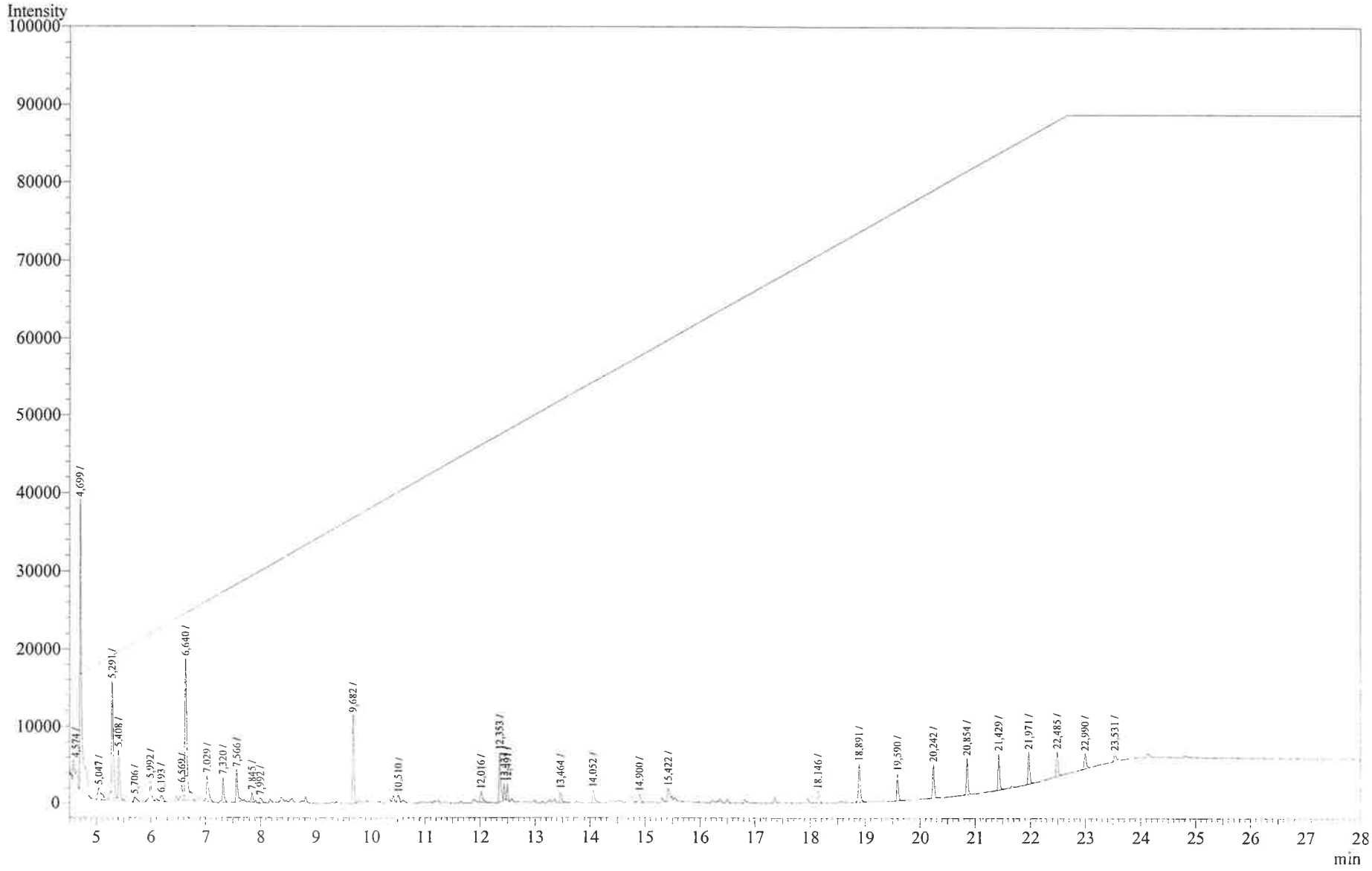
Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	4,621	24918	6316	0,000				
2	4,861	18742	5015	0,000				
3	5,203	10690	2379	0,000				
4	5,261	8458	2232	0,000		V		
5	5,429	11176	2812	0,000				
6	5,490	9310	1667	0,000		V		
7	5,898	4839	770	0,000				
8	6,292	2235	628	0,000				
9	7,246	2628	971	0,000				
10	8,710	4663	938	0,000				
11	8,901	3846	796	0,000				
12	9,022	2345	900	0,000				
13	9,207	2738	772	0,000				
14	10,711	2236	744	0,000				
15	13,063	2504	1034	0,000		V		
16	14,488	24421	6631	0,000				
17	15,834	19240	6514	0,000				
18	17,066	11031	3614	0,000				
19	18,200	5569	1657	0,000				
20	19,113	3193	1134	0,000				
21	19,257	2548	894	0,000				
Total		177330	48418					

Analysis Date & Time	: 6/2/2014 10:14:51 AM		
Vial#	: 52	Sample Name	: HEX-DCM-TOLUEN
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_06_02\HEX-DCM-TOLUEN.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		

02.06.14

10

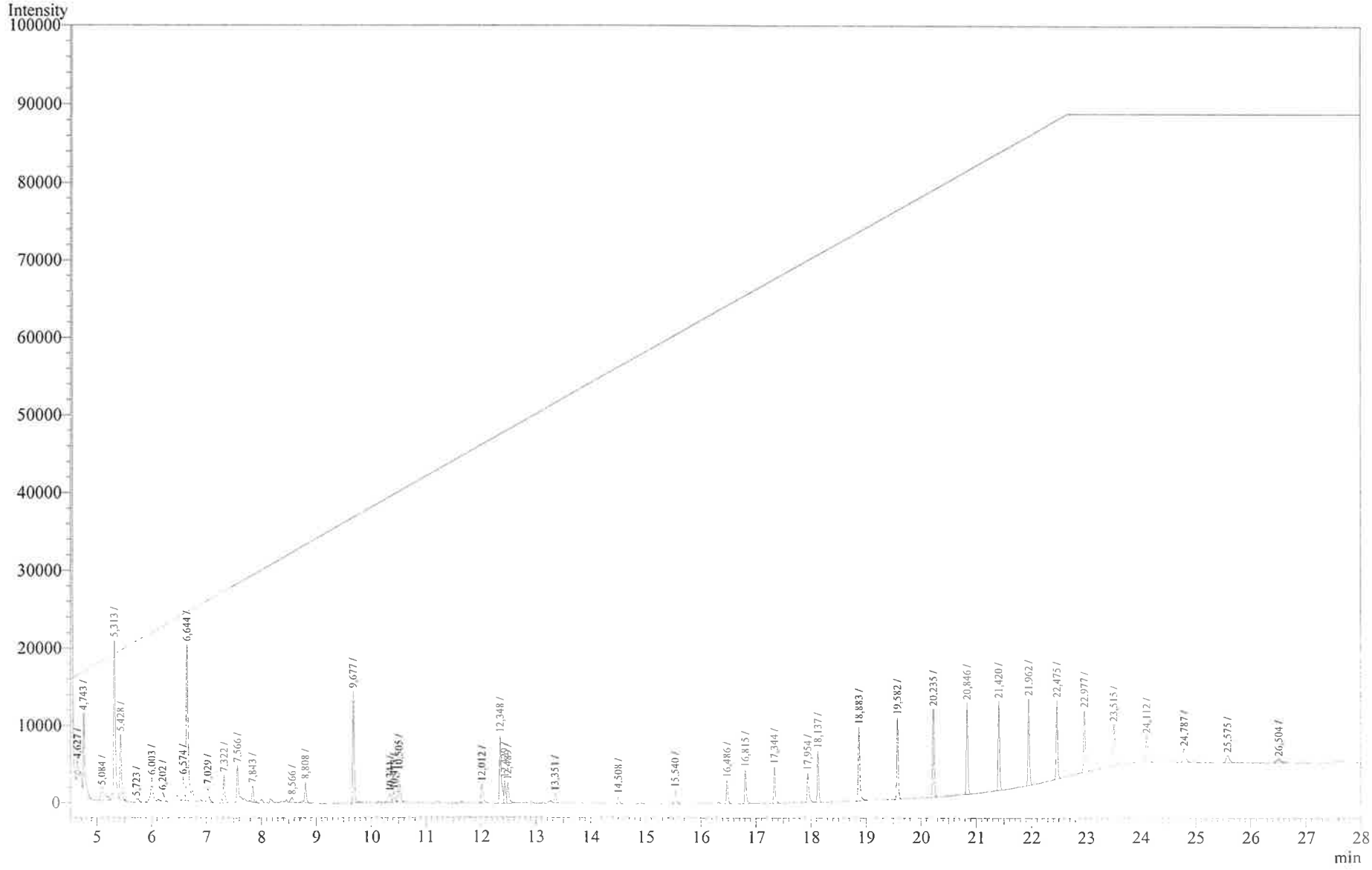
10



Appendix 10.1

10bis

Analysis Date & Time : 6/5/2014 12:21:18 PM
Vial# : 52 Sample Name : HEX-DCM-TOLUEN
Sample ID : Sample Type : Unknown
Injection Volume : 2,00 ISTD Amount :
Data Name : D:\data\Jovin\ZB-5HT\2014_06_05\HEX-DCM-TOLUEN.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm

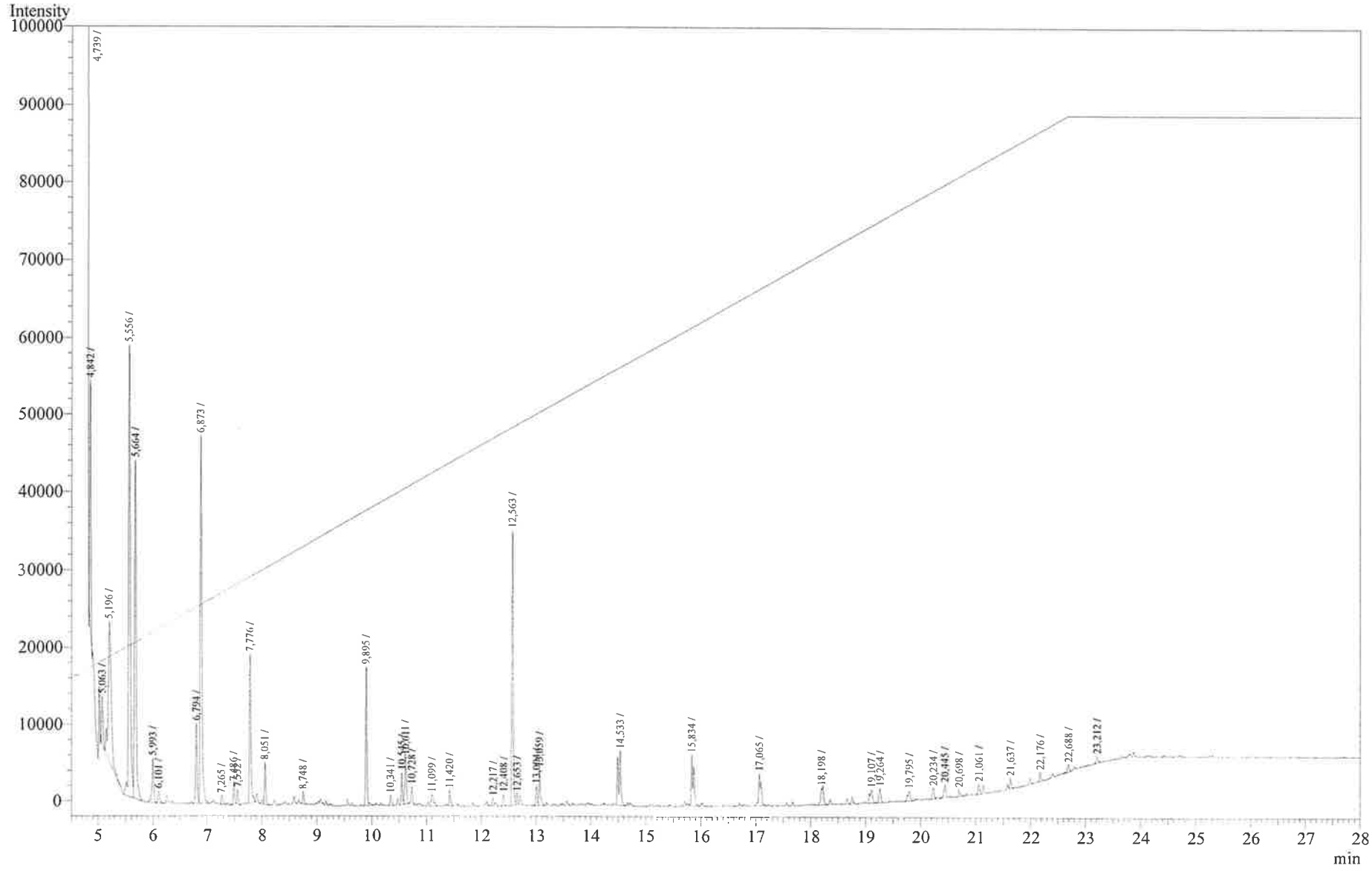


Appendix 10.2

11

Appendix 11

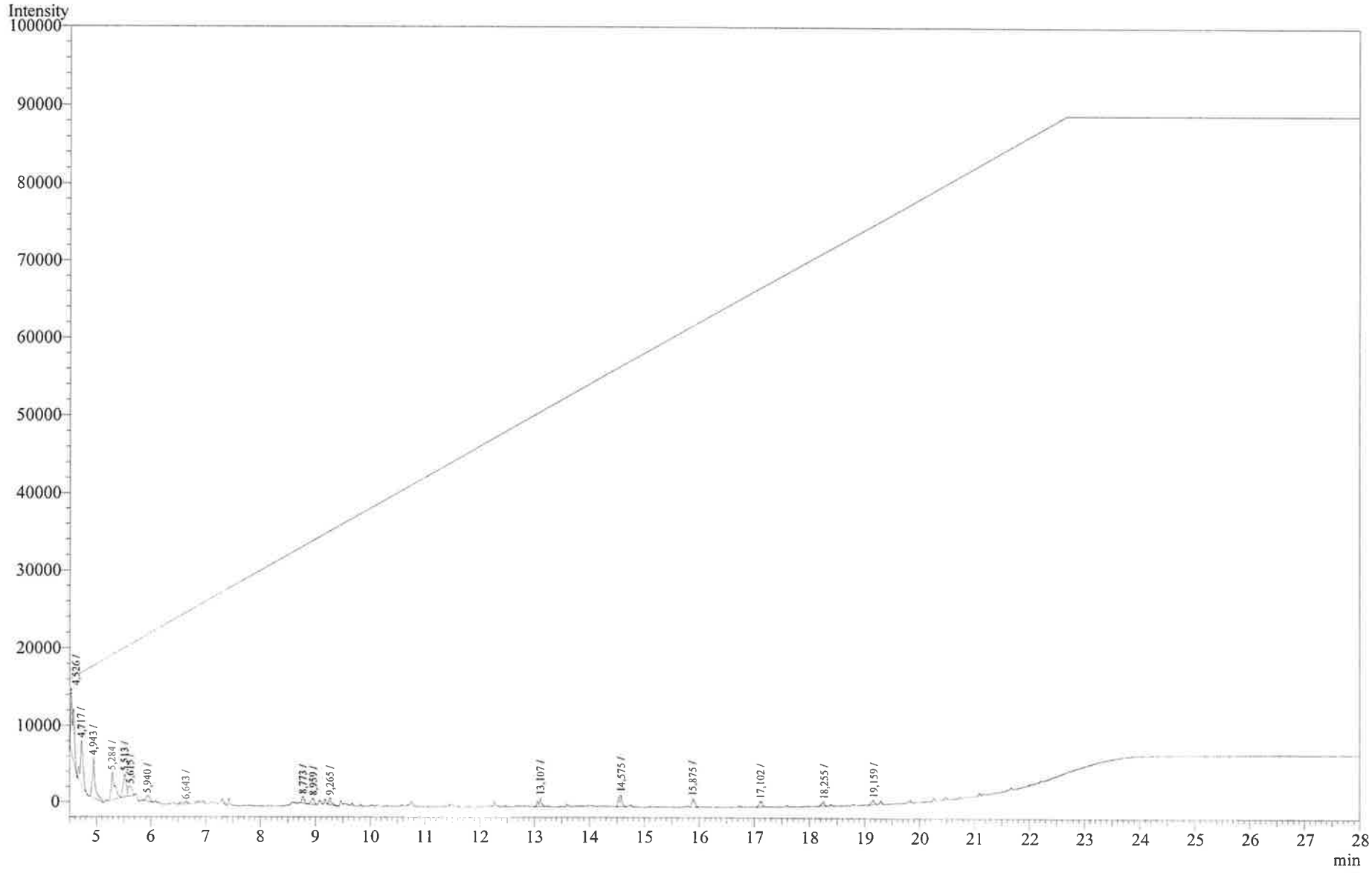
Analysis Date & Time	: 3/28/2014 2:07:36 AM		
Vial#	: 5	Sample Name	: EB-MOAH
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_03_027\EB-MOAH.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		



12

12

Analysis Date & Time	: 4/14/2014 10:36:29 PM		
Vial#	: 6	Sample Name	: EB.MOSH
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_04_14\EB-MOSH.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		

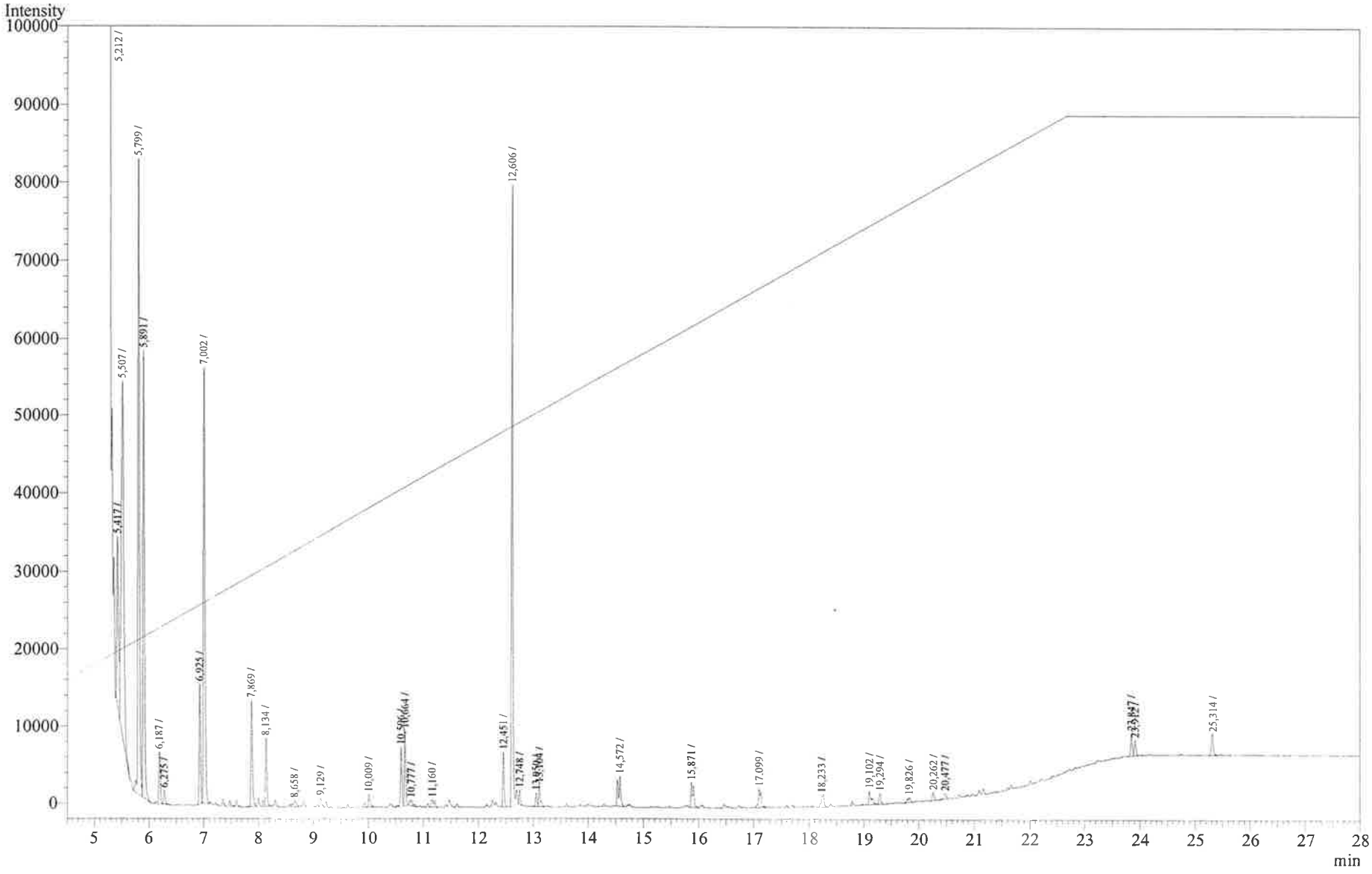


Appendix 12

13

13

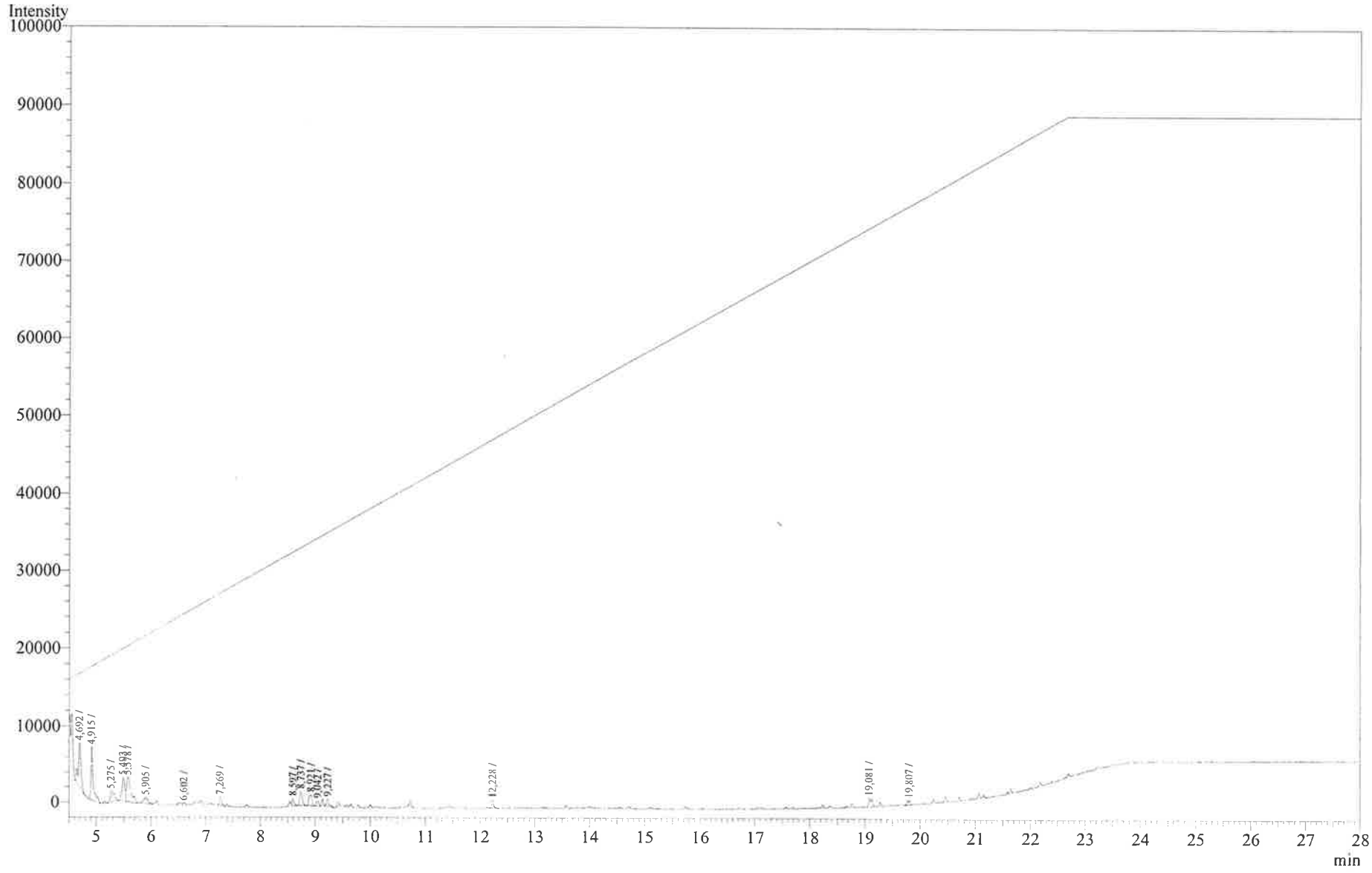
Analysis Date & Time	: 4/14/2014 11:38:49 PM		
Vial#	: 7	Sample Name	: EB.MOAH
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_04_14\EB-MOAH.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		



Appendix 13

14

Analysis Date & Time	: 4/2/2014 9:03:53 PM		
Vial#	: 5	Sample Name	: SB-MOSH
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_04_02\SB-MOSH.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		



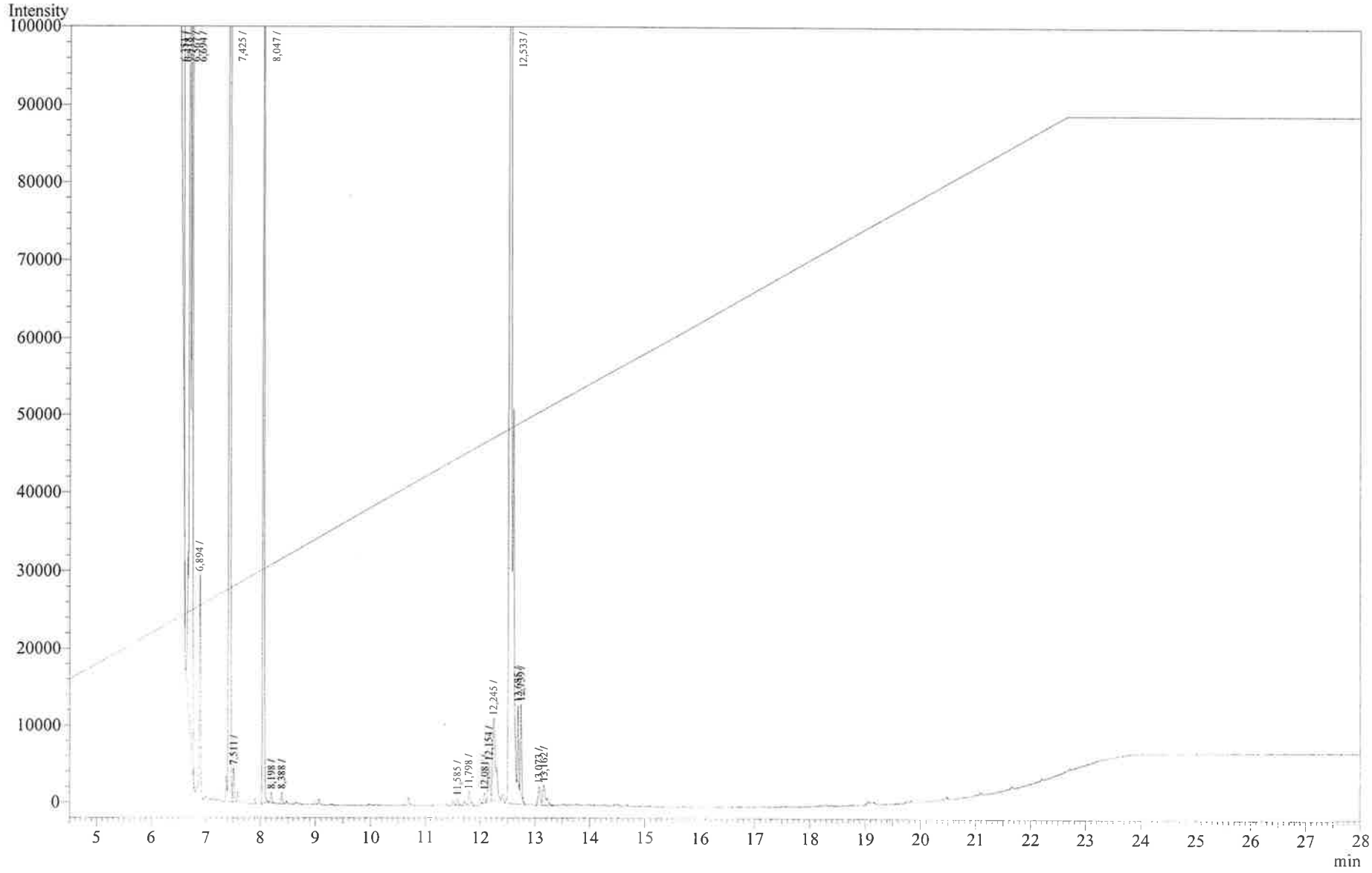
Appendix 14

15

15

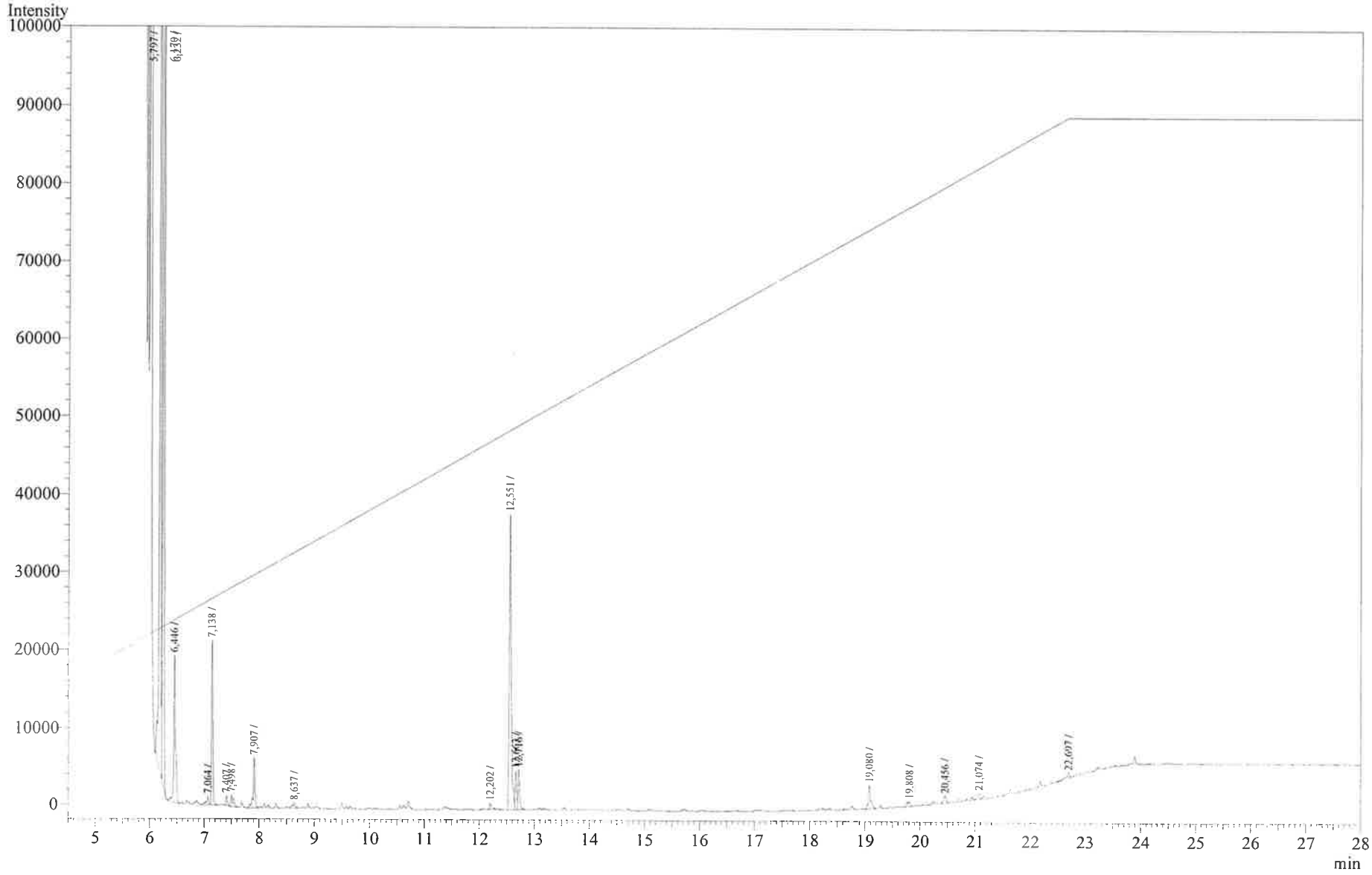
Appendix 15

Analysis Date & Time	: 4/14/2014 8:32:23 PM		
Vial#	: 4	Sample Name	: Toluene
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_04_14\Toluene.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		



16

Analysis Date & Time : 4/2/2014 10:04:45 PM
Vial# : 6 Sample Name : SB-MOAH
Sample ID : Sample Type : Unknown
Injection Volume : 2,00 ISTD Amount :
Data Name : D:\data\Jovin\ZB-5HT\2014_04_02\SB-MOAH.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm

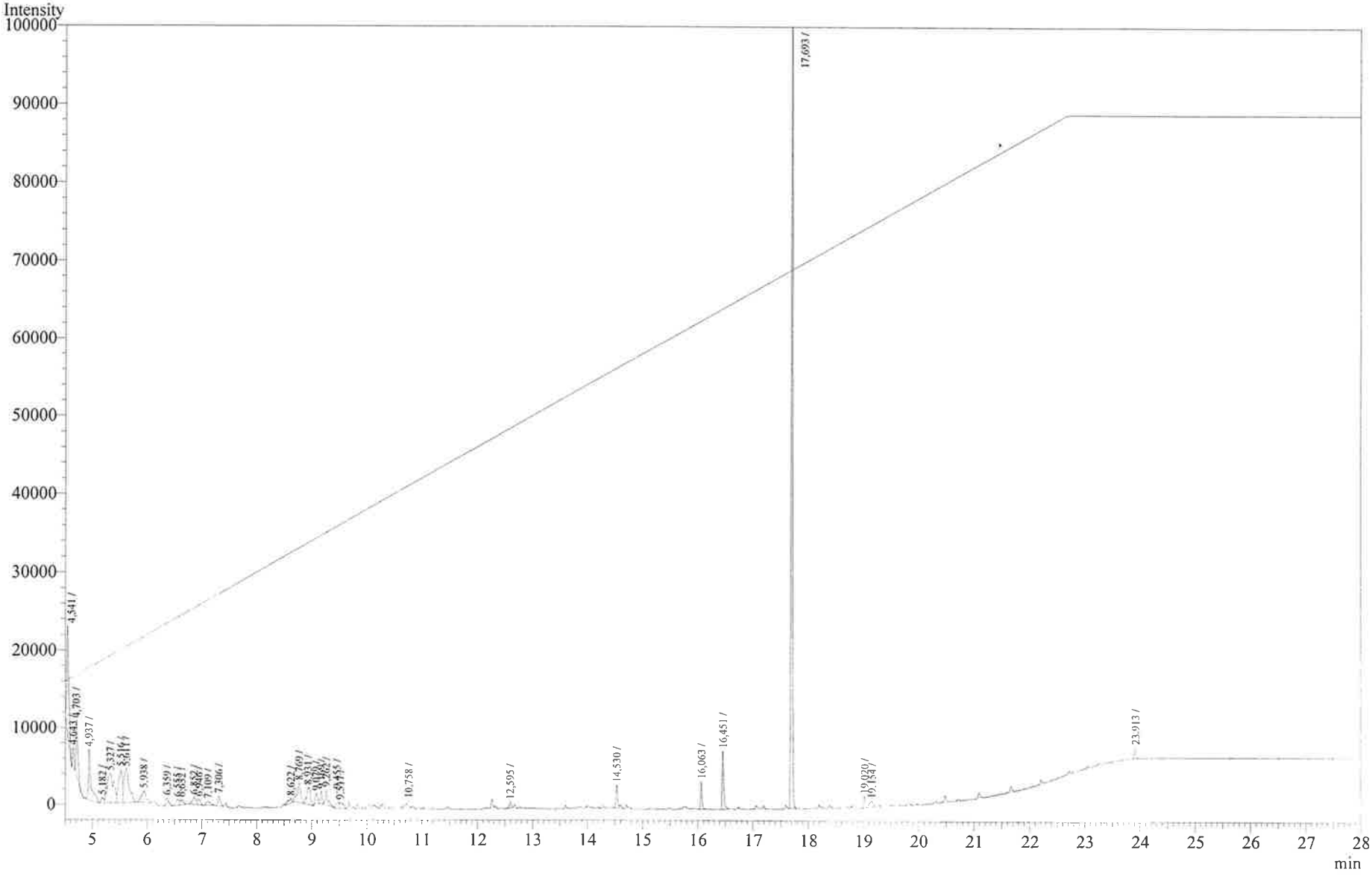


Appendix 16

(17)

17

Analysis Date & Time	: 4/15/2014 12:41:17 AM		
Vial#	: 8	Sample Name	: VM-HEX
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_04_14\VM-HEX.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		

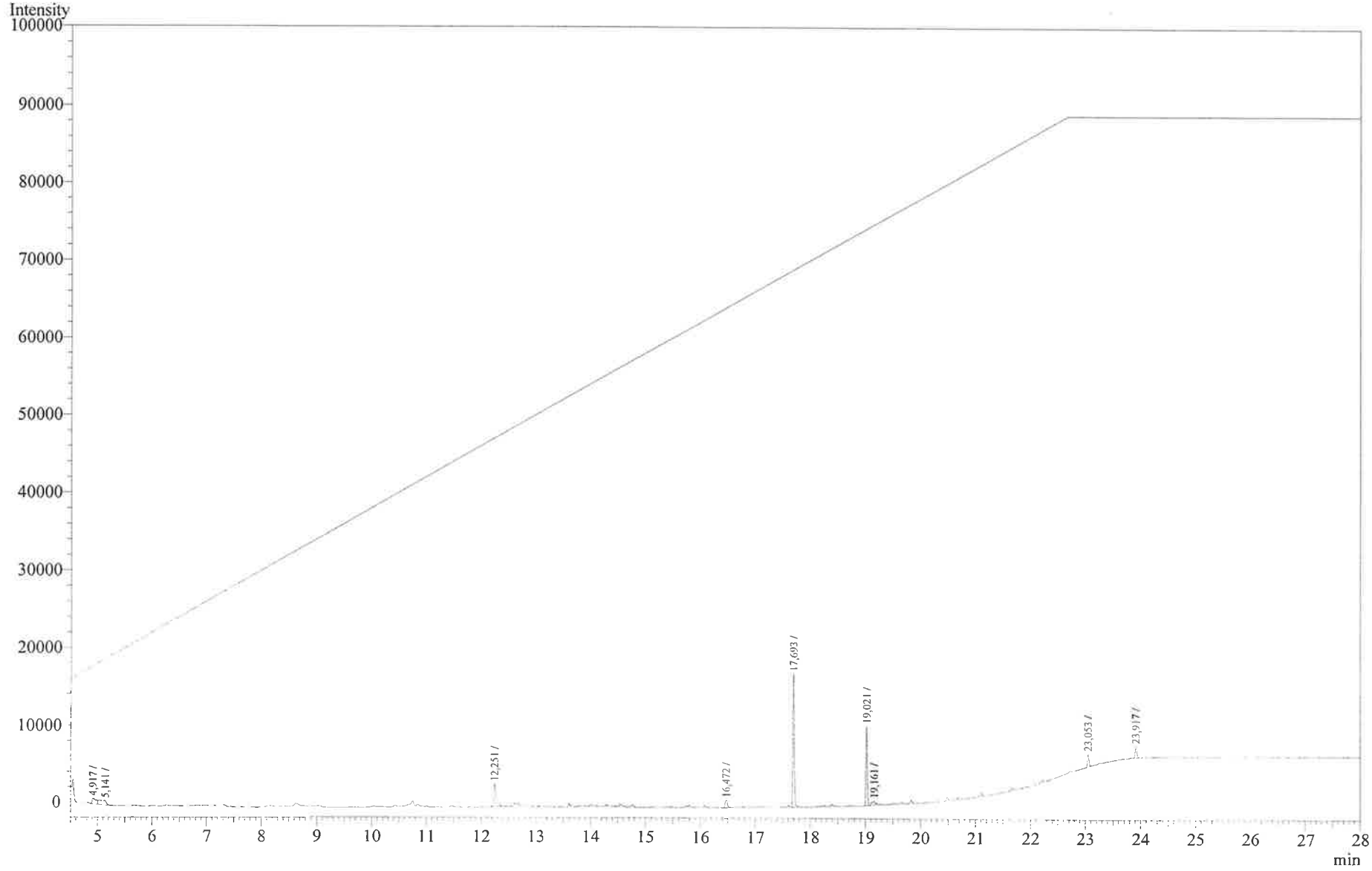


Appendix 17

18

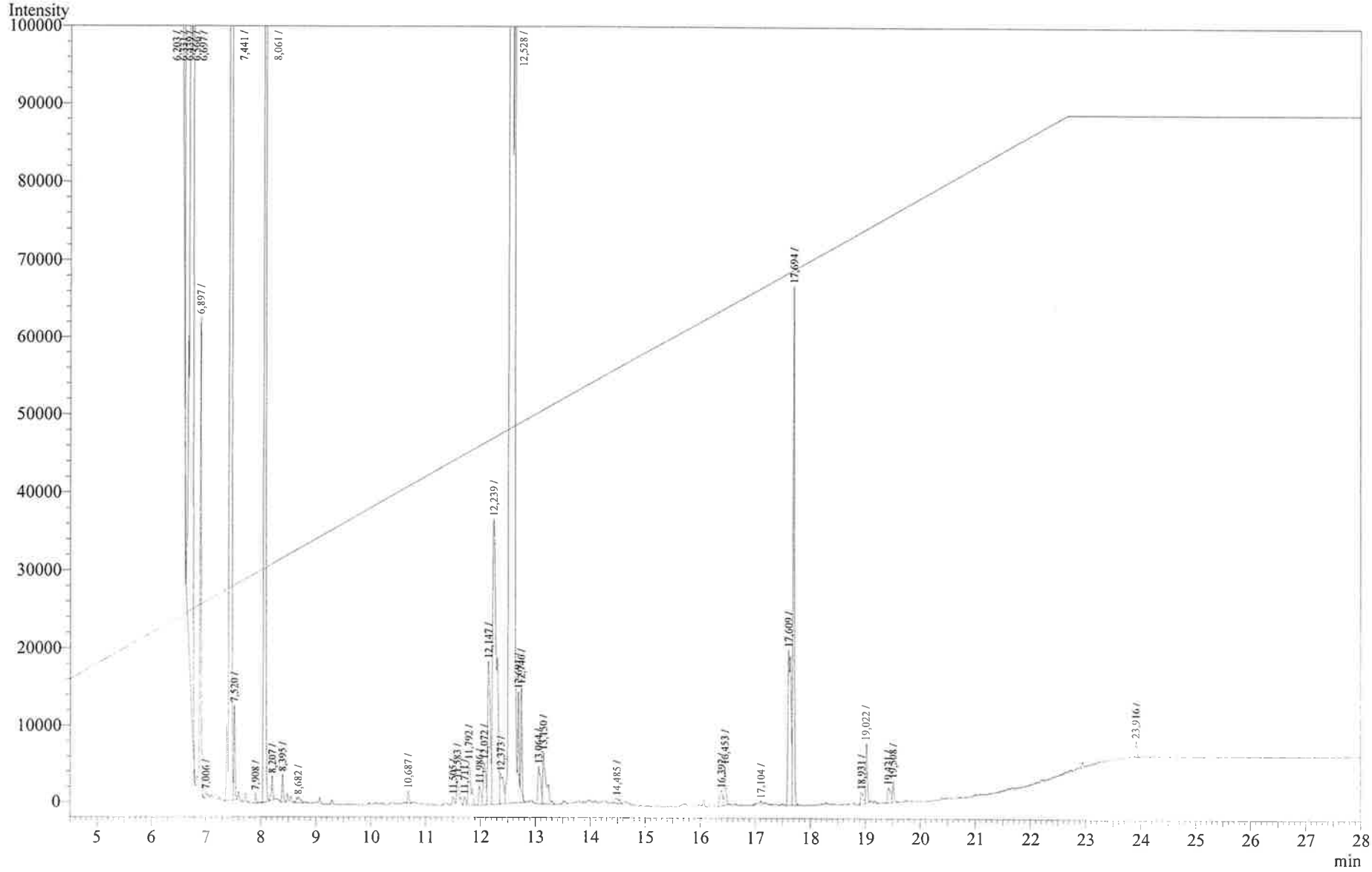
19

Analysis Date & Time	: 4/15/2014 1:43:51 AM		
Vial#	: 9	Sample Name	: VM-DCM
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_04_14\VM-DCM.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		



Appendix 18

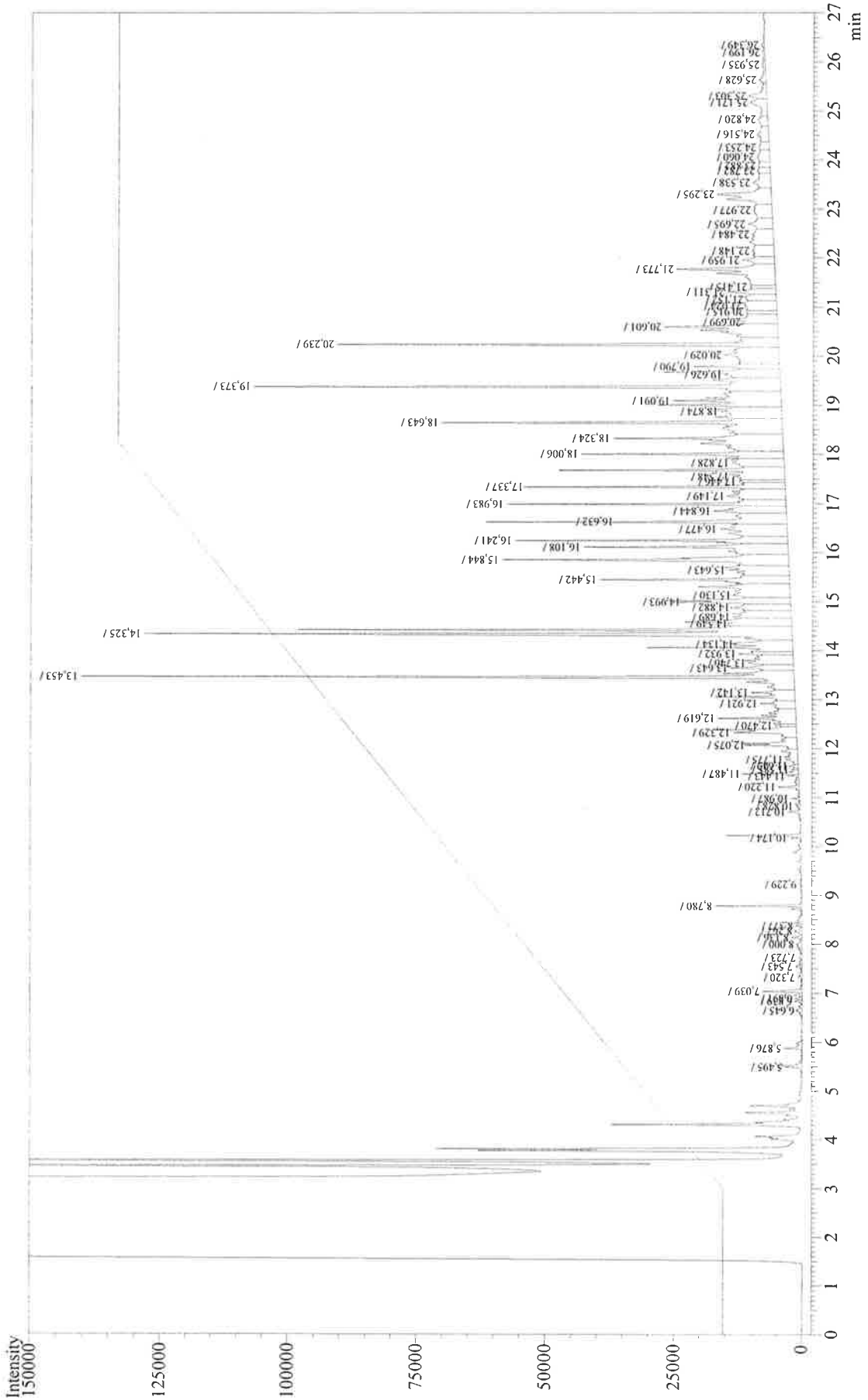
Analysis Date & Time : 4/15/2014 2:46:34 AM
Vial# : 10 Sample Name : VM-TOLUENE
Sample ID : Sample Type : Unknown
Injection Volume : 2,00 ISTD Amount :
Data Name : D:\data\Jovin\ZB-5HT\2014_04_14\VM-TOLUENE.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm



19

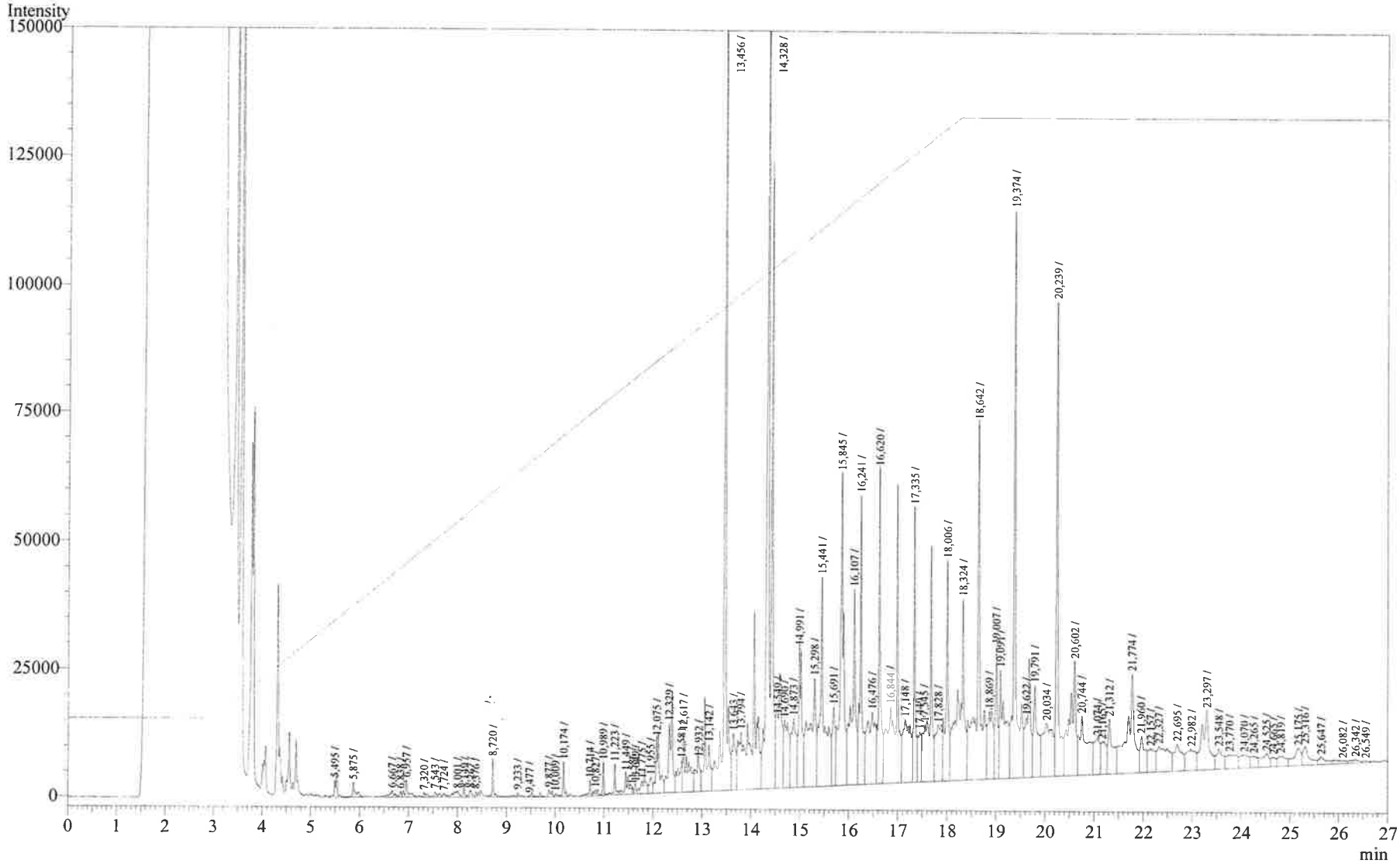
Appendix 19

Appendix 20


Analysis Date & Time : 12/3/2013 7:49:21 PM
Vial# : 6
Sample ID : :
Injection Volume : 1,00
Data Name : D:\data\Jovin\ZB-5HT\2013_12_03\HeEt1G2H.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1.gcm
Sample Name : HeEt1G2H
Sample Type : Unknown
ISTD Amount : :
Intensity
150000
125000
100000
75000
50000
25000
0
min
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
13,453
14,325
20,239
19,373
18,643
21,773
15,844
16,983
17,337
18,006
16,632
15,442
16,241
14,993
12,619
11,487
8,780
7,039

21

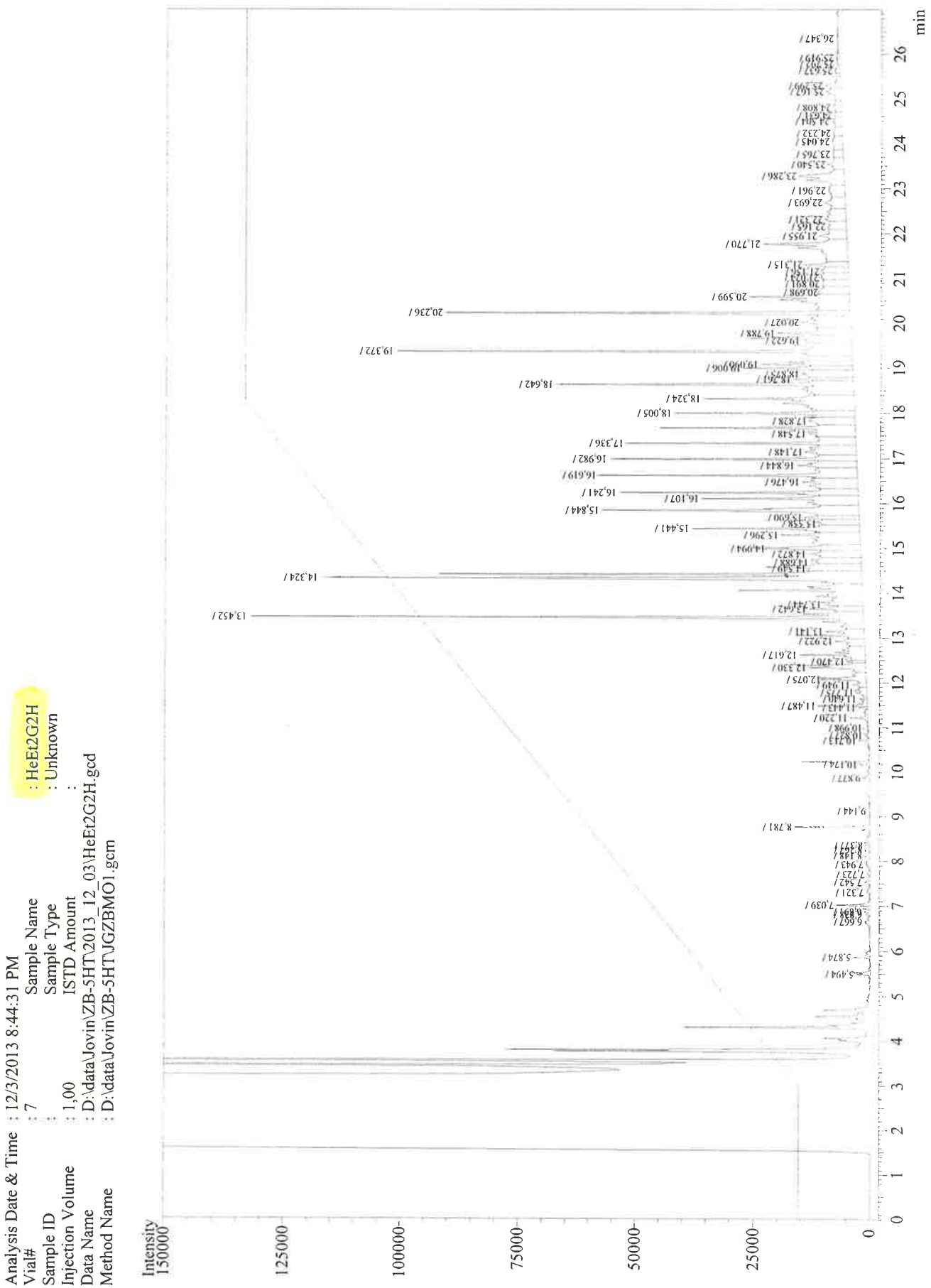
Analysis Date & Time	: 12/3/2013 4:09:06 PM		
Vial#	: 2	Sample Name	: HeEt1G3D
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 1,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2013_12_03\HeEt1G3D.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1.gcm		



1G 3D

Appendix 21

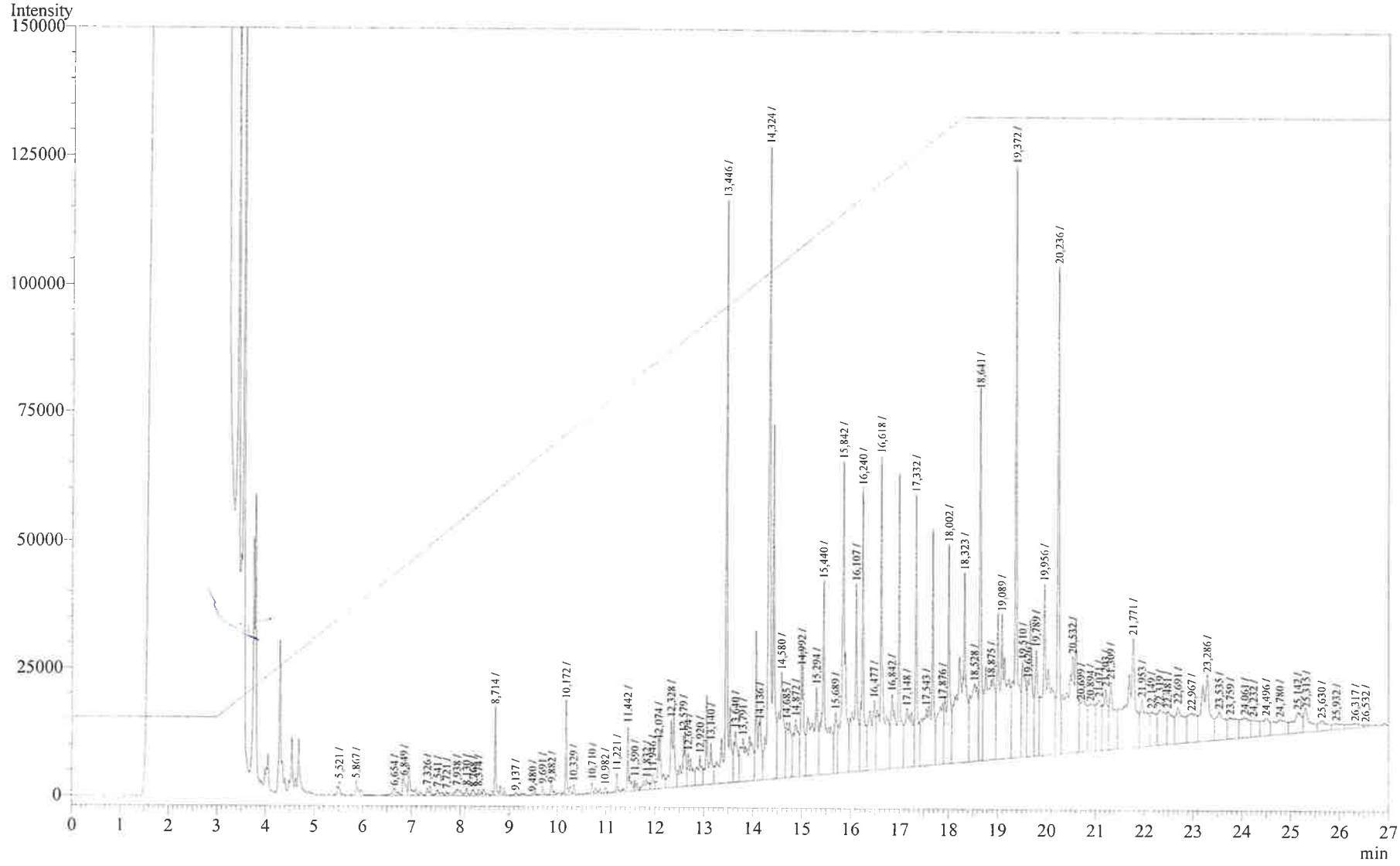
Appendix 22


Analysis Date & Time : 12/3/2013 8:44:31 PM
Vial# : 7
Sample ID :
Injection Volume : 1,00
Data Name : D:\data\Jovin\ZB-5HT\2013_12_03\HeEt2G2H.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1.gcm
Sample Name : HeEt2G2H
Sample Type : Unknown
ISTD Amount :
Intensity
150000
125000
100000
75000
50000
25000
0
min
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
5,494
5,874
6,667
6,898
7,039
7,321
7,542
7,723
7,943
8,148
8,349
8,577
8,781
9,144
9,577
10,174
10,373
10,598
11,220
11,413
11,487
11,649
11,775
11,999
12,075
12,330
12,470
12,617
12,922
13,141
13,442
13,452
14,324
14,539
14,666
14,872
14,994
15,296
15,441
15,558
15,690
15,844
16,107
16,241
16,476
16,619
16,844
16,982
17,148
17,336
17,548
17,838
18,005
18,324
18,642
18,761
18,875
19,099
19,372
19,622
19,788
20,027
20,236
20,599
20,698
20,801
21,155
21,315
21,770
21,955
22,165
22,351
22,693
22,961
23,286
23,540
23,765
24,045
24,232
24,631
24,808
25,162
25,309
25,637
25,919
26,347

HEX/ACE 23

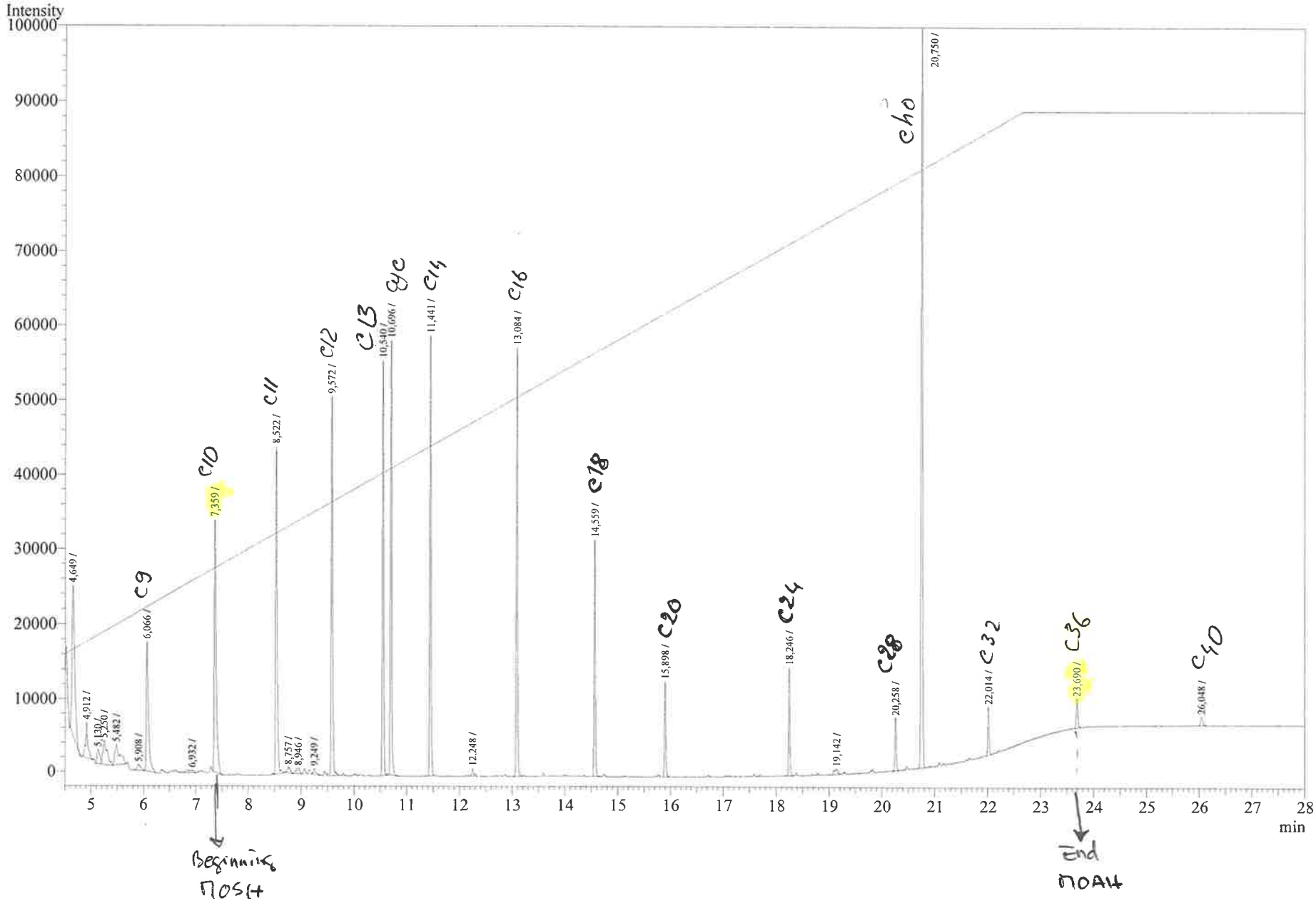
Analysis Date & Time	: 12/4/2013 6:36:08 PM		
Vial#	: 4	Sample Name	: HeAc1G2H
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 1,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2013_12_04\HeAc1G2H.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1.gcm		

(18) → 2H



Appendix 23

Analysis Date & Time : 4/8/2014 1:08:44 AM
Vial# : 5 Sample Name : SS-MOSH
Sample ID : Sample Type : Unknown
Injection Volume : 2,00 ISTD Amount :
Data Name : D:\data\Jovin\ZB-5HT\2014_04_07\SS-MOSH.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm

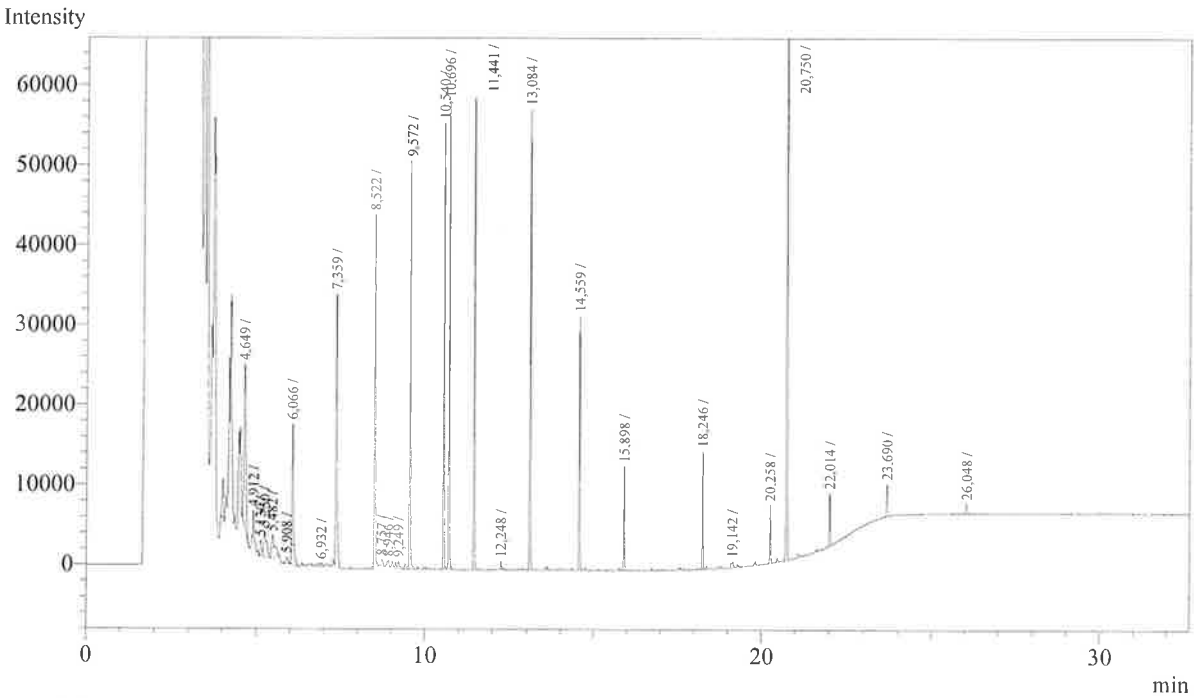


Appendix 24.1

Appendix 24.2

Analysis Date & Time : 4/8/2014 1:08:44 AM
User Name : Admin
Vial# : 5
Sample Name : SS-MOSH
Sample ID :
Sample Type : Unknown
Injection Volume : 2,00
ISTD Amount :

Data Name : D:\data\Jovin\ZB-5HT\2014_04_07\SS-MOSH.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm



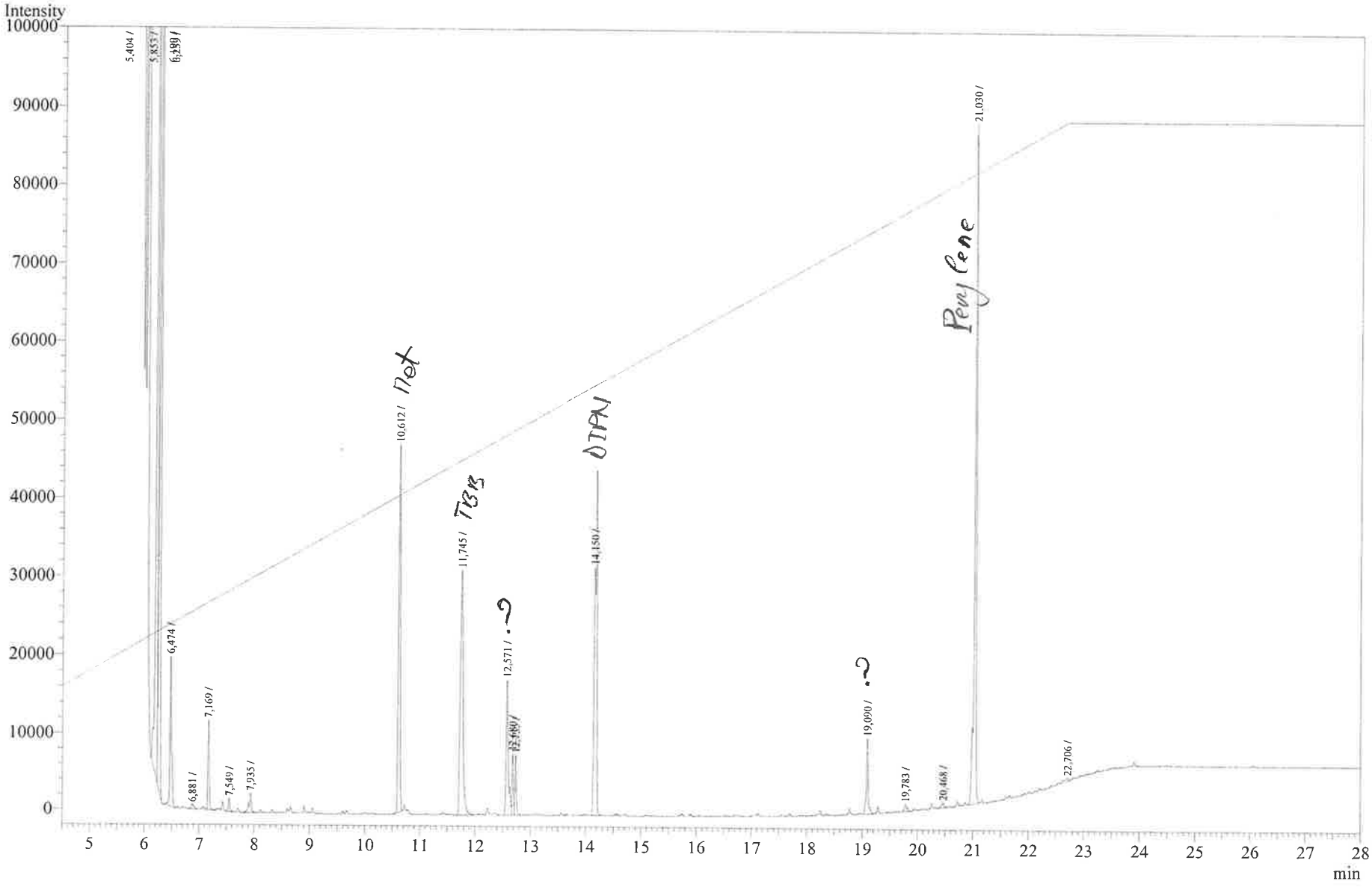
Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	4,649	73824	20044	0,000				
2	4,912	15717	4830	0,000				
3	5,130	7276	1968	0,000				
4	5,250	18890	3261	0,000		V		
5	5,482	18619	2788	0,000				
6	5,908	3568	790	0,000				
7	6,066	51358	17362	0,000				
8	6,932	3098	388	0,000				
C10 9	7,359	86883	33009	0,000				
C11 10	8,522	90865	43334	0,000				
11	8,757	2782	775	0,000				
12	8,946	3273	769	0,000				
13	9,249	2520	822	0,000				
C12 14	9,572	94086	47403	0,000		V		
* C13 15	10,540	92408	51341	0,000				
cyc 16	10,696	97112	56737	0,000				
C14 17	11,441	96786	57646	0,000				
18	12,248	2524	1035	0,000				
C16 19	13,084	91938	53923	0,000				
C18 20	14,559	51051	30236	0,000				
C20 21	15,898	21754	12721	0,000				
C24 22	18,246	23300	13611	0,000				
23	19,142	3419	743	0,000				
C28 24	20,258	12010	7050	0,000				
* cho 25	20,750	213996	121606	0,000				
C32 26	22,014	10591	6130	0,000				
C36 27	23,690	8136	3968	0,000				
28	26,048	3731	1232	0,000				
Total		1201515	595522					

51978

MOAH
- Net
- TBB
- DIPN
- Perylene

25

Analysis Date & Time : 4/8/2014 2:10:05 AM
Vial# : 6 Sample Name : SS-MOAH
Sample ID : Sample Type : Unknown
Injection Volume : 2,00 ISTD Amount :
Data Name : D:\data\Jovin\ZB-5HT\2014_04_07\SS-MOAH.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm

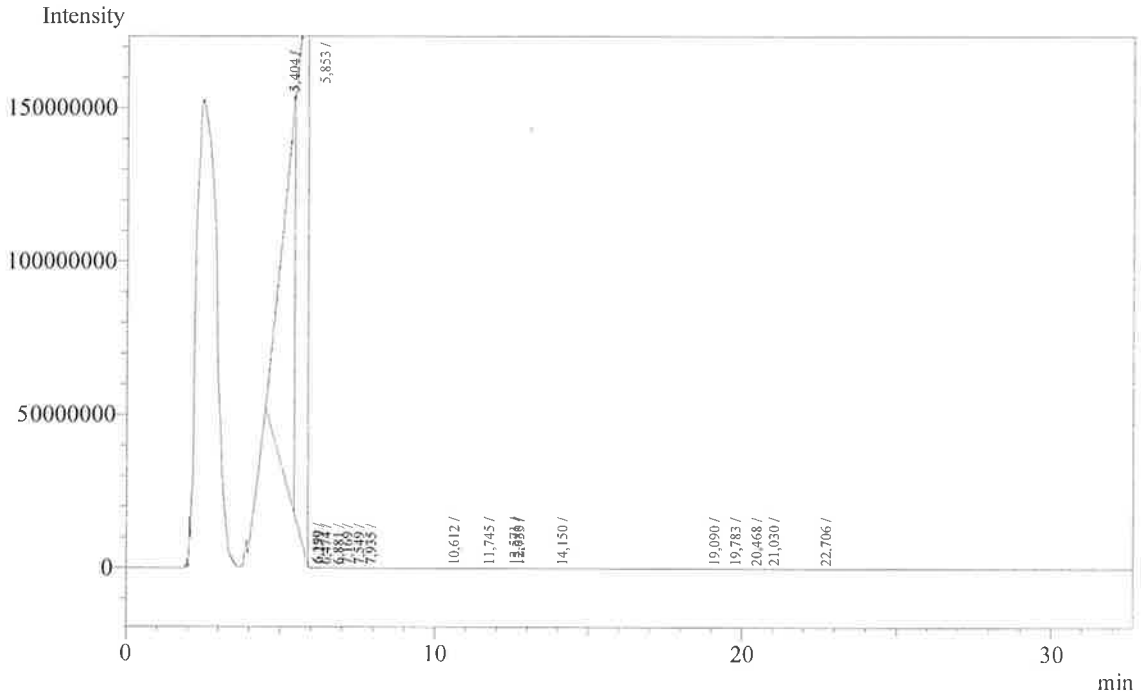


Appendix 25.1

Appendix 25.2

Analysis Date & Time : 4/8/2014 2:10:05 AM
User Name : Admin
Vial# : 6
Sample Name : SS-MOAH
Sample ID :
Sample Type : Unknown
Injection Volume : 2,00
ISTD Amount :

Data Name : D:\data\Jovin\ZB-5HT\2014_04_07\SS-MOAH.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm

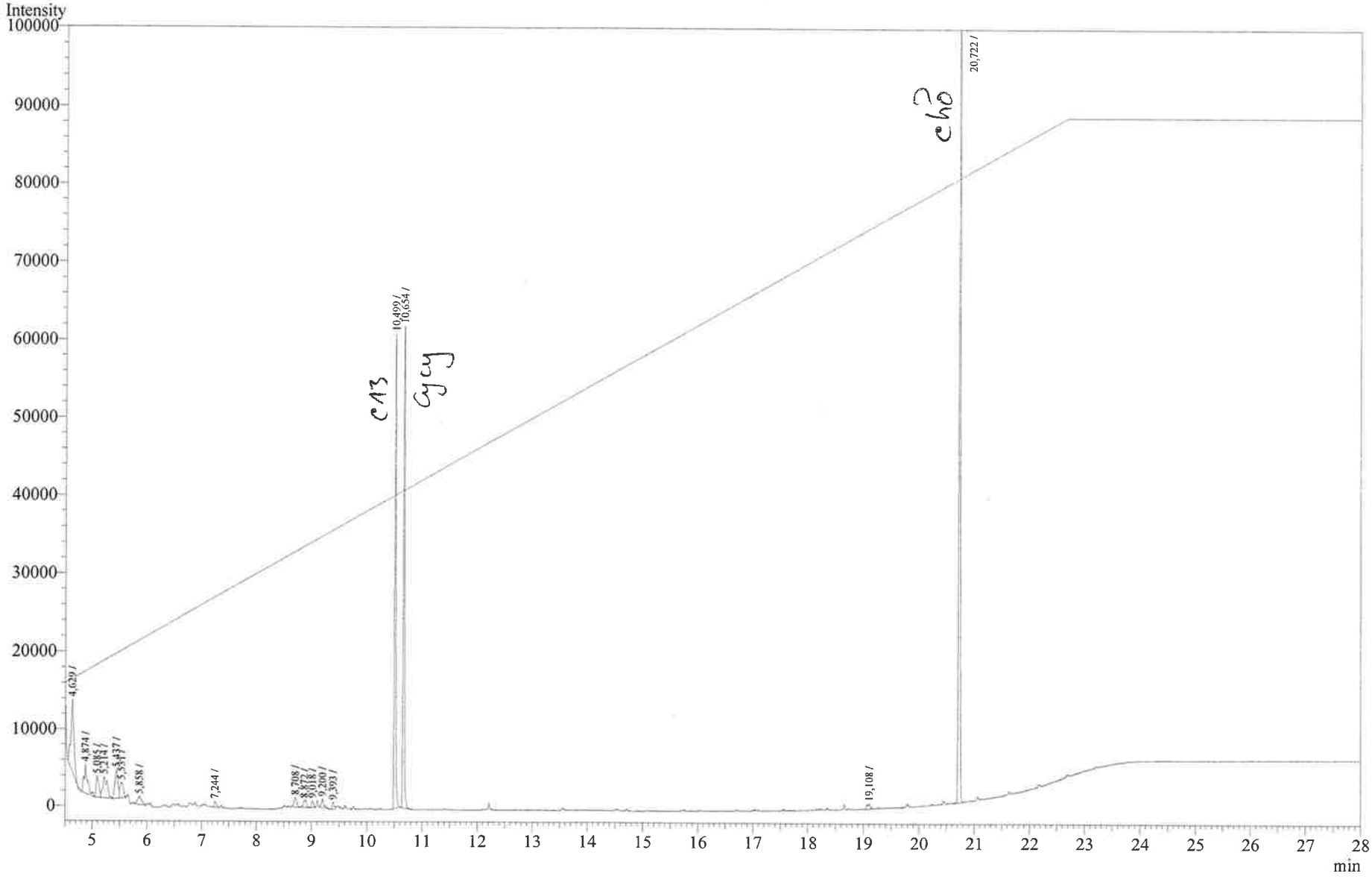


Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	5,404	3674019470	134761050	0,000				
2	5,853	4364759358	190032430	0,000		V		
3	6,199	388279	243508	0,000				
4	6,259	284186	173858	0,000		V		
5	6,474	39944	18789	0,000				
6	6,881	2205	648	0,000				
7	7,169	20427	11471	0,000				
8	7,549	3159	1646	0,000				
9	7,935	7074	2506	0,000				
Net 10	10,612	106020	45621	0,000				
TBB 11	11,745	110761	31197	0,000				
12	12,571	48222	17018	0,000				
13	12,680	13668	7822	0,000		V		
14	12,735	13407	7470	0,000		V		
DIPN 15	14,150	128490	30611	0,000				
16	19,090	21111	9555	0,000				
17	19,783	2889	930	0,000				
18	20,468	2192	594	0,000				
Peryl 19	21,030	177681	83089	0,000				
20	22,706	2366	431	0,000		V		
Total		8040150909	325480244					

Silver silica (0,3g)

26

Analysis Date & Time	: 3/27/2014 11:11:13 AM		
Vial#	: 13	Sample Name	: SB-MOSH
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_03_27\SB-MOSH.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		

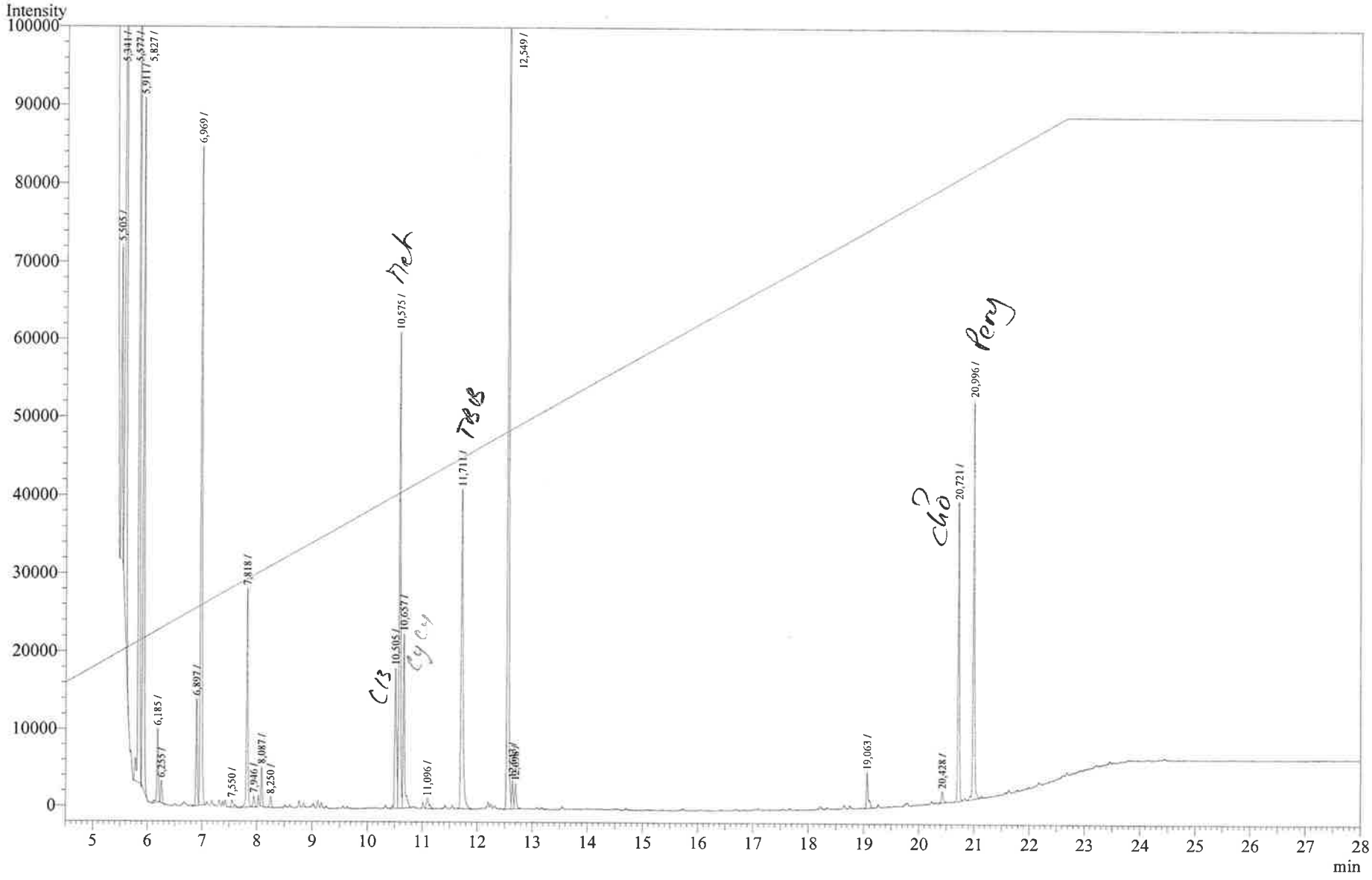


Appendix 26

Silver Silica (0,3g)

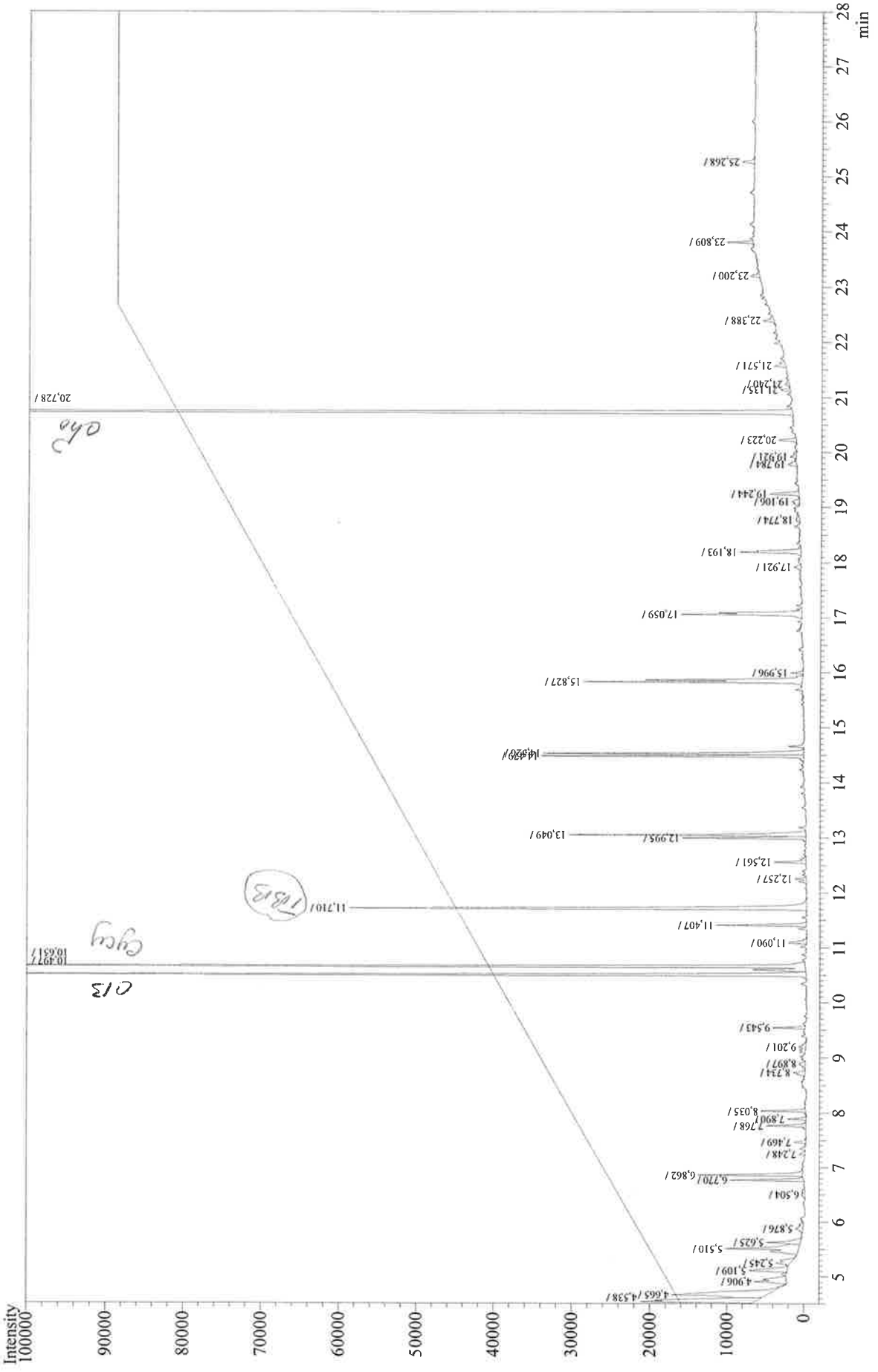
27

Analysis Date & Time	: 3/27/2014 12:10:13 PM		
Vial#	: 14	Sample Name	: SB-MOAH
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_03_27\SB-MOAH.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		



Appendix 27

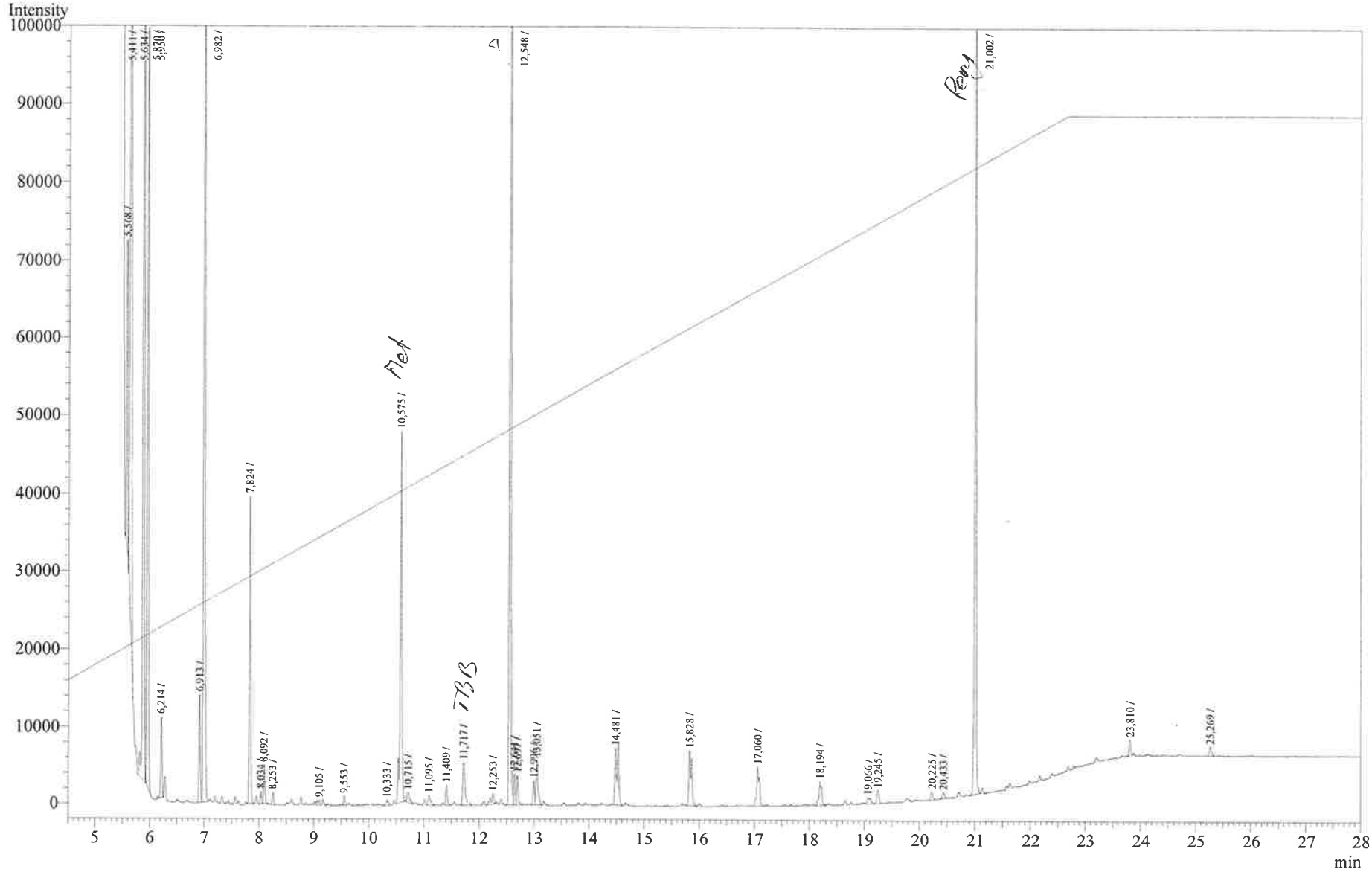
Appendix 28


Analysis Date & Time : 3/27/2014 9:13:01 AM
Vial# : 11
Sample ID : Sample Name : EB2-MOSH
Injection Volume : 2,00 ISTD Amount :
Data Name : D:\data\Jovin\ZB-5HT\2014_03_27\EB2-MOSH.gcd
Method Name : D:\data\Jovin\ZB-5HTJGZBMO1_15C_min.gcm
Sample Type : Unknown
Intensity
min
4,665 / 4,538
4,906
5,109
5,245
5,510
5,625
5,876
6,504
6,770 / 6,862
7,248
7,469
7,768
7,890 / 8,035
8,734
8,897
9,201
9,543
10,631 / 10,897
11,090
11,407
11,710
12,257
12,561
12,995 / 13,049
14,470 / 14,526
15,827
15,996
17,059
17,921
18,193
18,774
19,106 / 19,244
19,784 / 19,921
20,223
20,728
21,135 / 21,240
21,571
22,388
23,200
23,809
25,268

STRATA TUBE

29

Analysis Date & Time	: 3/27/2014 10:12:03 AM		
Vial#	: 12	Sample Name	: EB2-MOAH
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_03_27\EB2-MOAH.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		

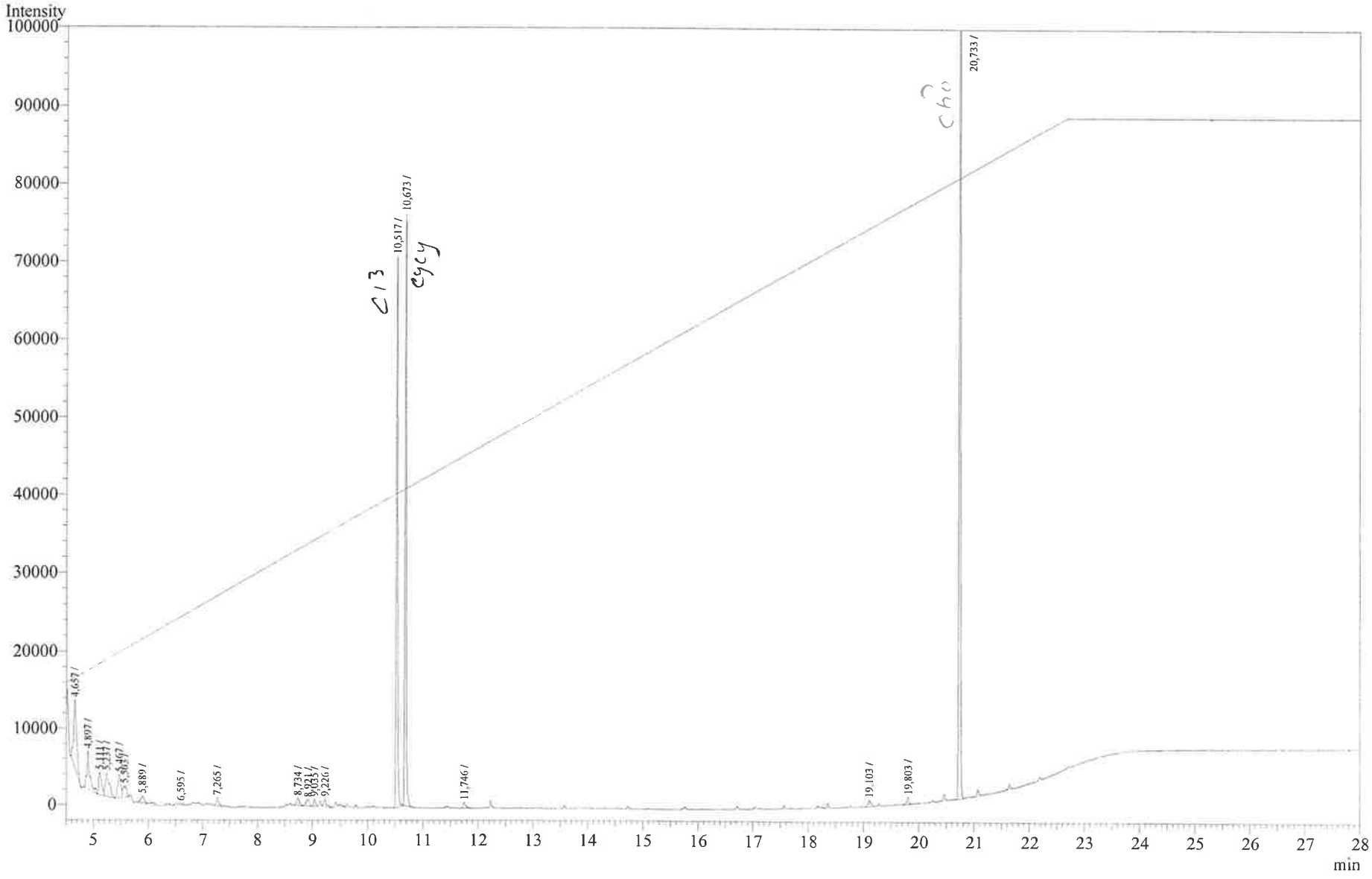


Appendix 29

Silica (0,33 Mg/ml)

30

Analysis Date & Time : 4/3/2014 5:11:50 PM
Vial# : 2 Sample Name : SS-MOSH
Sample ID : Sample Type : Unknown
Injection Volume : 2,00 ISTD Amount :
Data Name : D:\data\Jovin\ZB-5HT\2014_04_03\SS-MOSH.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm



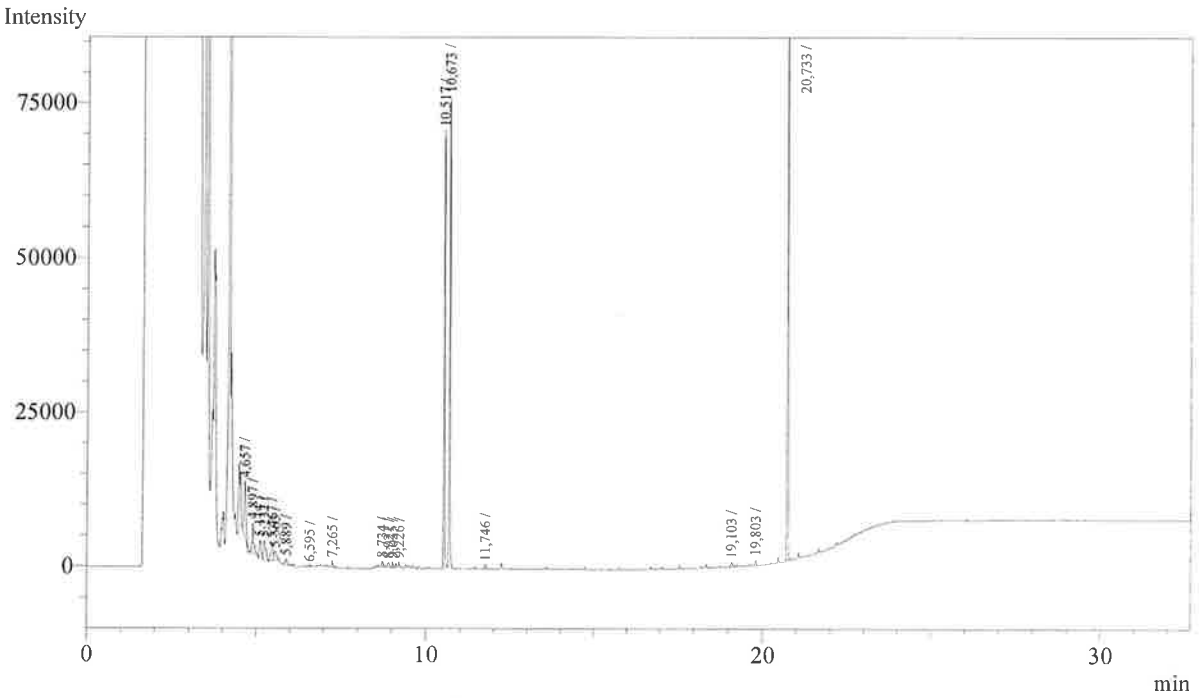
Appendix 30.1

Appendix 30.2

0,3 $AgNO_3$

Analysis Date & Time : 4/3/2014 5:11:50 PM
User Name : Admin
Vial# : 2
Sample Name : SS-MOSH
Sample ID :
Sample Type : Unknown
Injection Volume : 2,00
ISTD Amount :

Data Name : D:\data\Jovin\ZB-5HT\2014_04_03\SS-MOSH.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	4,657	34022	8853	0,000				
2	4,897	18371	4862	0,000				
3	5,111	10785	2818	0,000				
4	5,237	15406	2942	0,000		V		
5	5,467	11541	2967	0,000				
6	5,563	7122	1407	0,000		V		
7	5,889	3842	873	0,000				
8	6,595	2238	283	0,000				
9	7,265	2795	1038	0,000				
10	8,734	3284	932	0,000				
11	8,921	3198	801	0,000				
12	9,035	2198	880	0,000				
13	9,226	2827	954	0,000				
CB - 14	10,517	119510	64590	0,000				
cyty - 15	10,673	126340	74372	0,000				
16	11,746	2399	696	0,000				
17	19,103	2916	815	0,000				
18	19,803	2236	894	0,000				
cho 19	20,733	236167	130382	0,000				
Total		607197	301359					

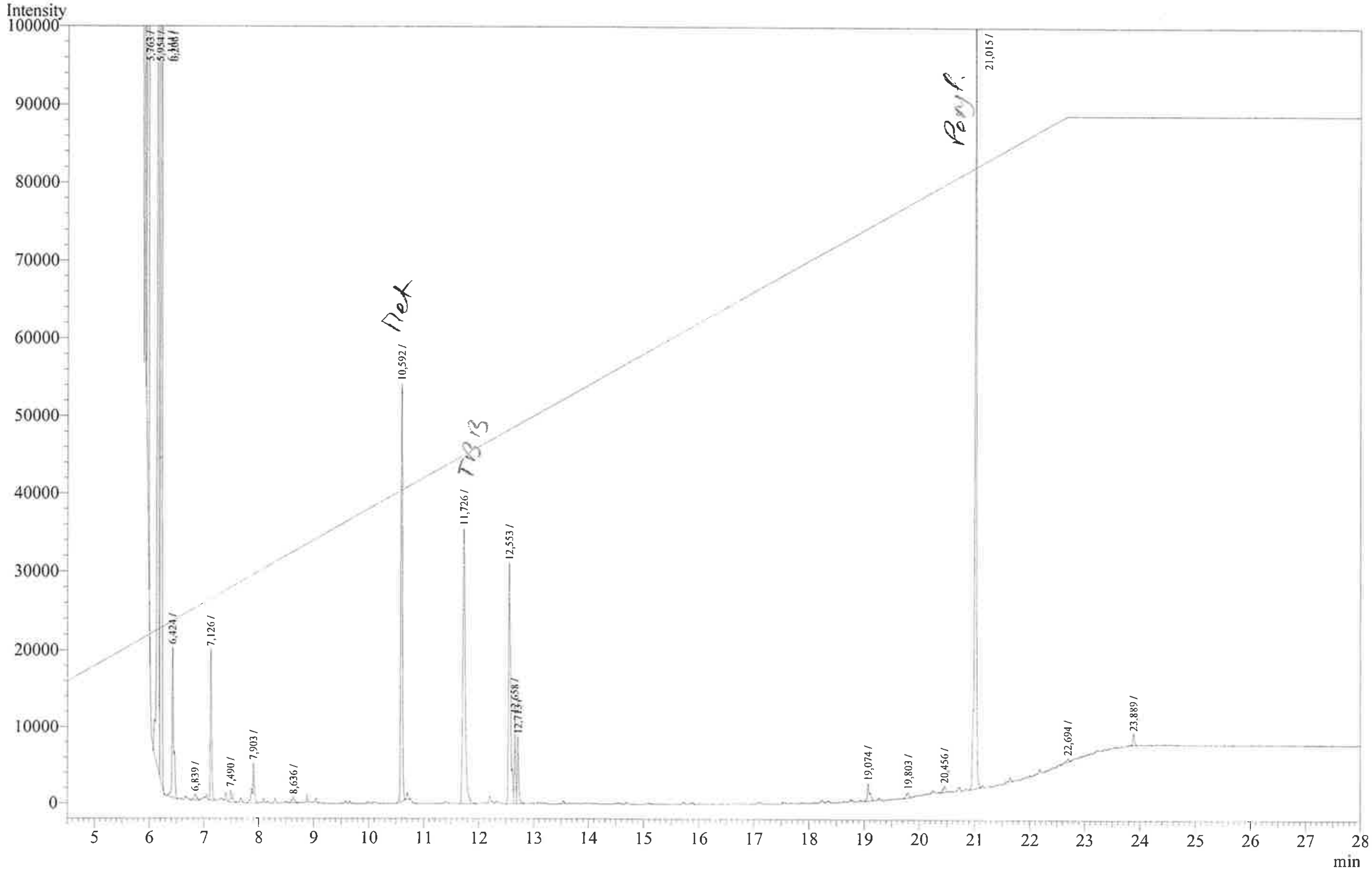
30615

Appendix 31.1

Silu.Silt (0,3% $AgNO_3$)

31

Analysis Date & Time	: 4/3/2014 6:11:10 PM		
Vial#	: 3	Sample Name	: SS-MOAH
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_04_03\SS-MOAH.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		

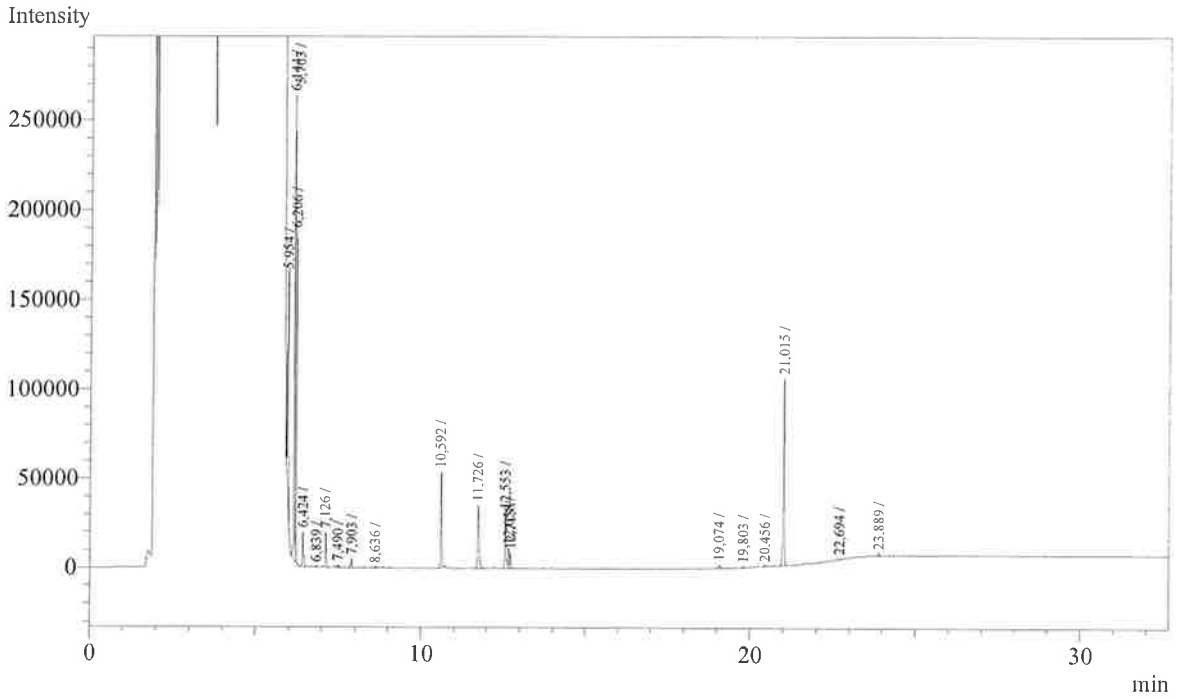


Appendix 31.2

0,3 A_8NIO_3

Analysis Date & Time	: 4/3/2014 6:11:10 PM
User Name	: Admin
Vial#	: 3
Sample Name	: SS-MOAH
Sample ID	:
Sample Type	: Unknown
Injection Volume	: 2,00
ISTD Amount	:

Data Name	: D:\data\Jovin\ZB-5HT\2014_04_03\SS-MOAH.gcd
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	5,763	7780490166	189957842	0,000				
2	5,954	273456	117018	0,000				
3	6,144	398515	249392	0,000				
4	6,206	284868	170674	0,000		V		
5	6,424	41415	19023	0,000				
6	6,839	2604	769	0,000				
7	7,126	34627	18401	0,000				
8	7,490	4225	1538	0,000				
9	7,903	12826	5063	0,000				
10	8,636	2309	691	0,000				
11	10,592	109109	53215	0,000				
12	11,726	111367	34608	0,000				
13	12,553	80006	30544	0,000				
14	12,658	19649	11037	0,000		V		
15	12,713	16119	8540	0,000		V		
16	19,074	6986	2204	0,000				
17	19,803	2318	683	0,000				
18	20,456	2441	801	0,000				
19	21,015	210733	101779	0,000				
20	22,694	2180	518	0,000		V		
21	23,889	3164	1486	0,000				
Total		7782109083	190785826					

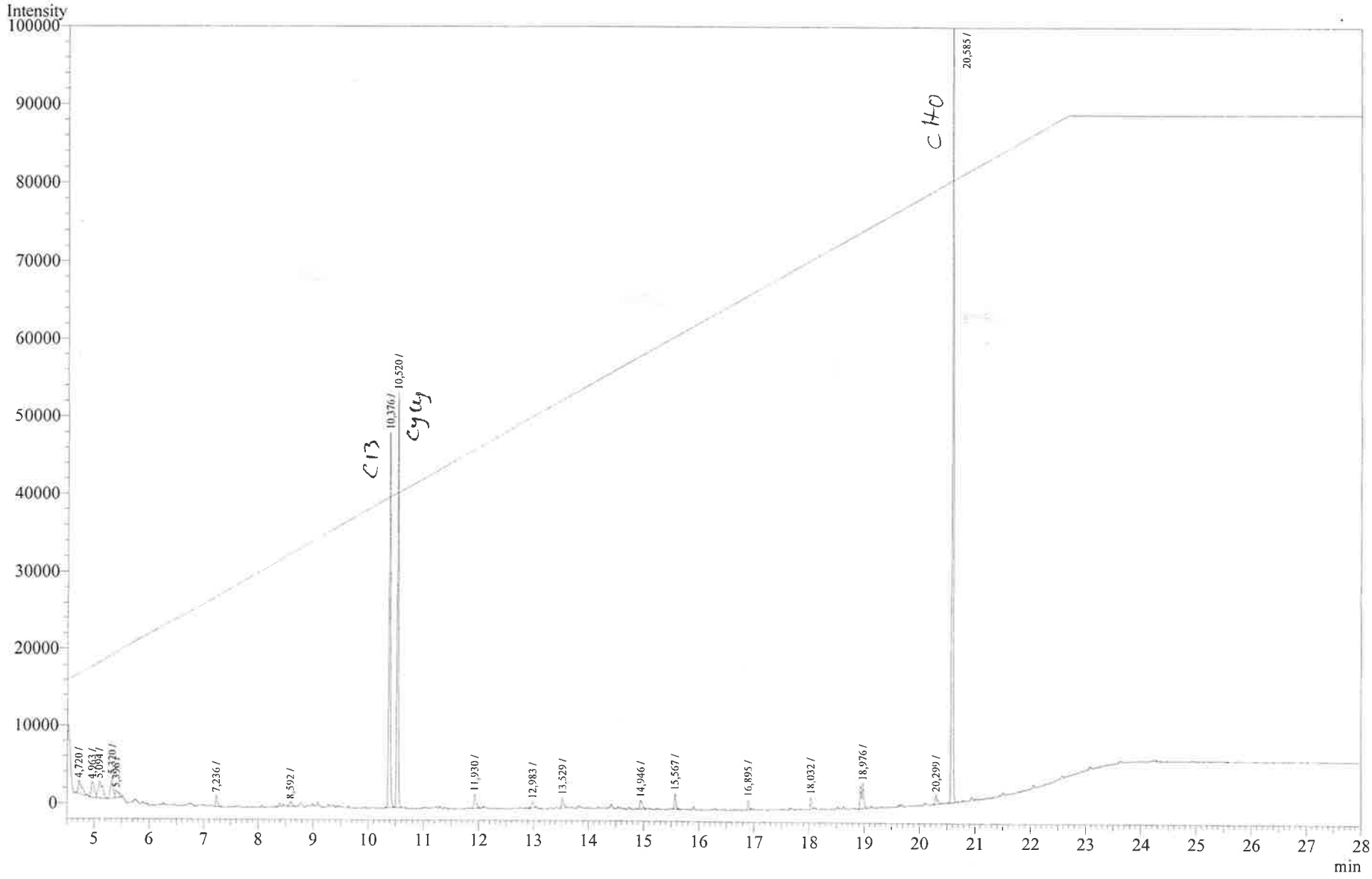
Net

TBB

Peak

silv.sil ($1\%AgNO_3$) 32

Analysis Date & Time	: 4/29/2014 8:00:22 PM		
Vial#	: 4	Sample Name	: SS-MOSH
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_04_28\SS-MOSH.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		



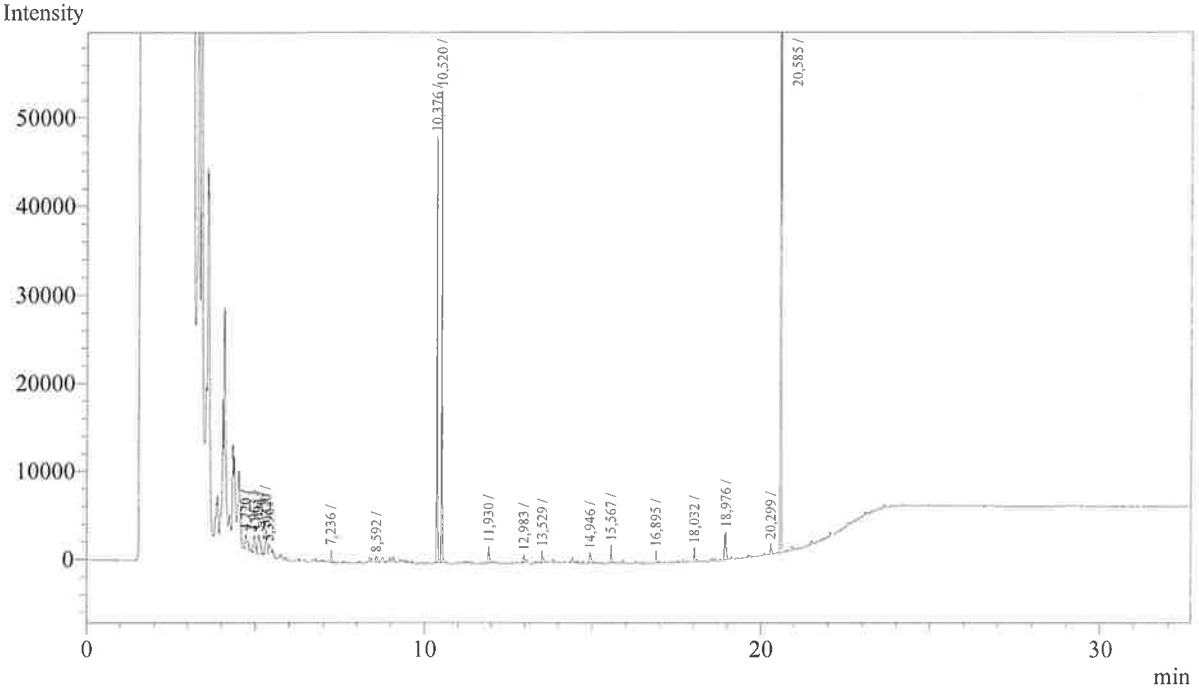
Appendix 32.1

Appendix 32.2

1% $AgNO_3$

Analysis Date & Time : 4/29/2014 8:00:22 PM
User Name : Admin
Vial# : 4
Sample Name : SS-MOSH
Sample ID :
Sample Type : Unknown
Injection Volume : 2,00
ISTD Amount :

Data Name : D:\data\Jovin\ZB-5HT\2014_04_28\SS-MOSH.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	4,720	7617	1601	0,000				
2	4,963	7770	1944	0,000				
3	5,094	11935	2083	0,000		V		
4	5,320	8771	2605	0,000				
5	5,396	4161	908	0,000		V		
6	7,236	3298	1390	0,000				
7	8,592	2095	571	0,000				
8	10,376	85101	46356	0,000				
9	10,520	87560	51815	0,000				
10	11,930	4836	1793	0,000				
11	12,983	2146	803	0,000				
12	13,529	3174	1278	0,000				
13	14,946	3557	1105	0,000				
14	15,567	3699	1918	0,000				
15	16,895	2278	1277	0,000				
16	18,032	2484	1503	0,000				
17	18,976	11398	3128	0,000				
18	20,299	2922	1180	0,000				
19	20,585	168560	101820	0,000				
Total		423362	225078					

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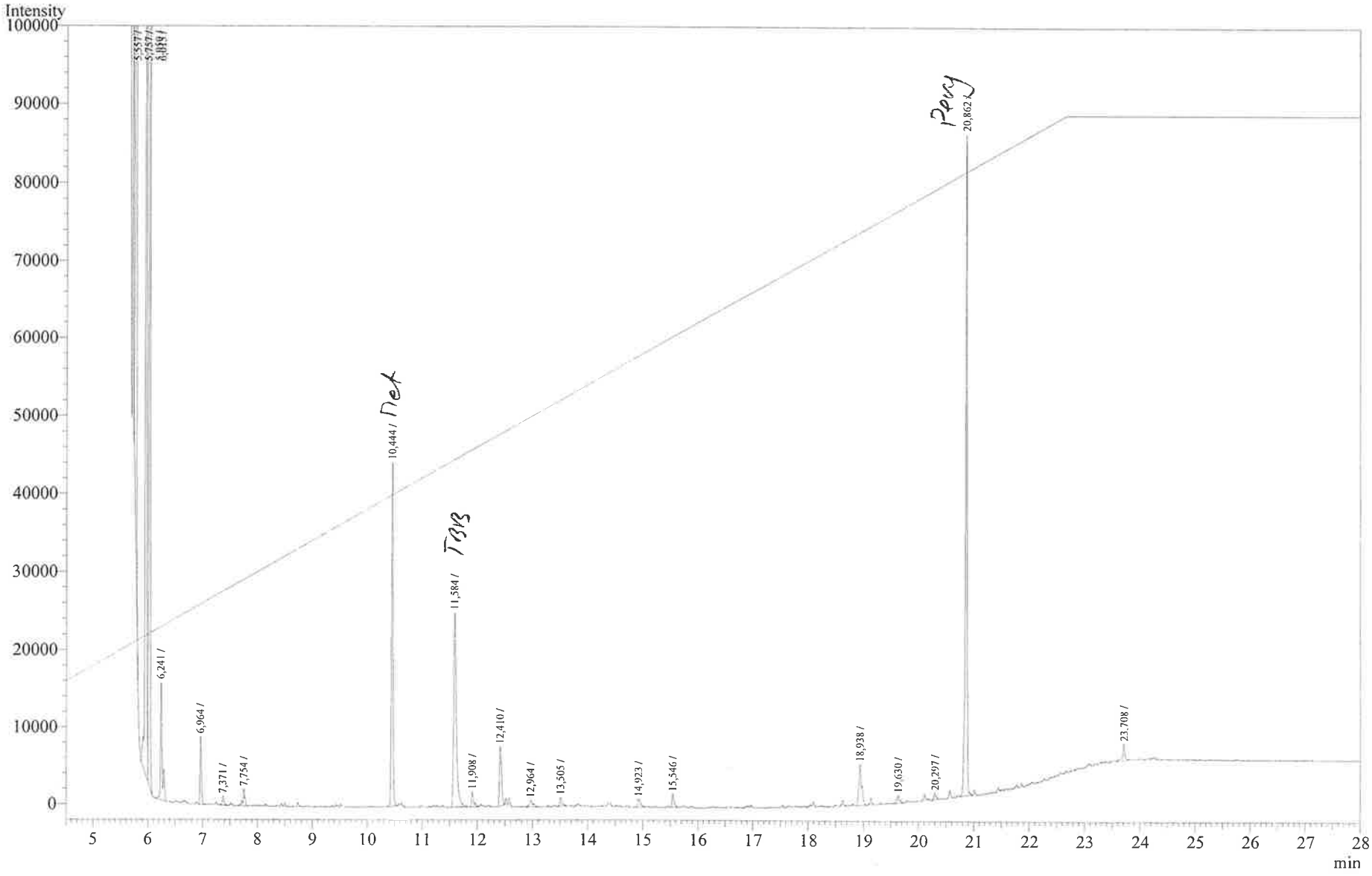
Analysis Date & Time	: 4/29/2014 9:02:13 PM		
Vial#	: 5	Sample Name	: SS-MOAH
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_04_28\SS-MOAH.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		

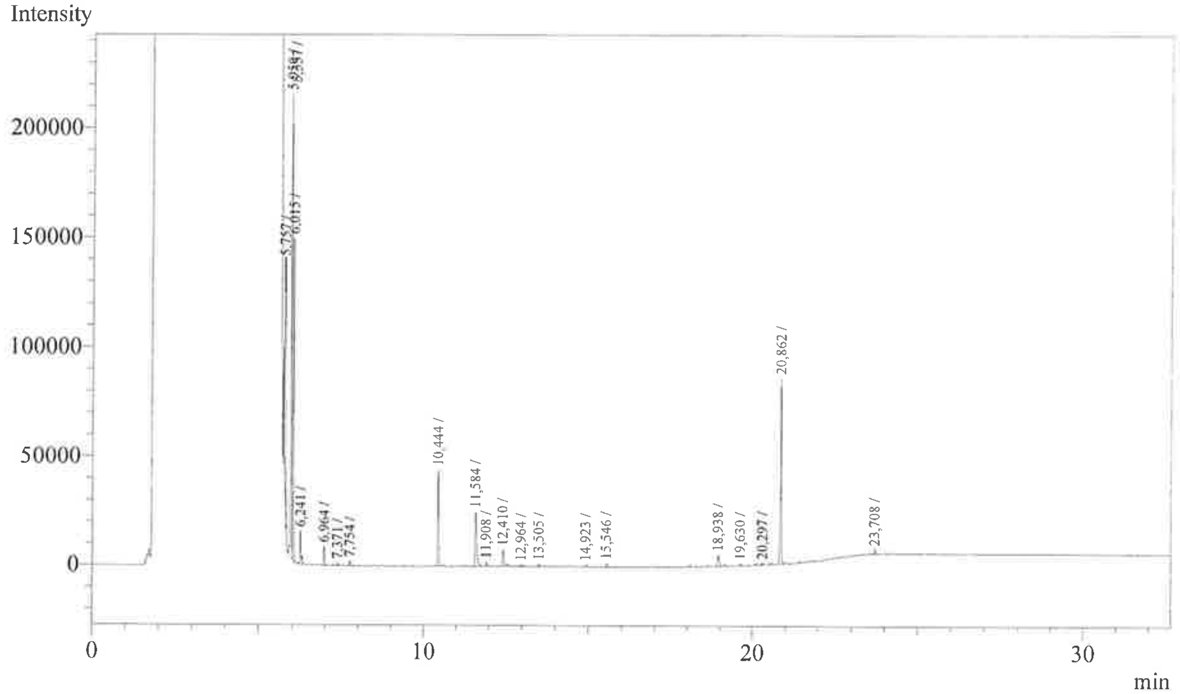


Appendix 33.1

Appendix 33.2

1% $AgNO_3$

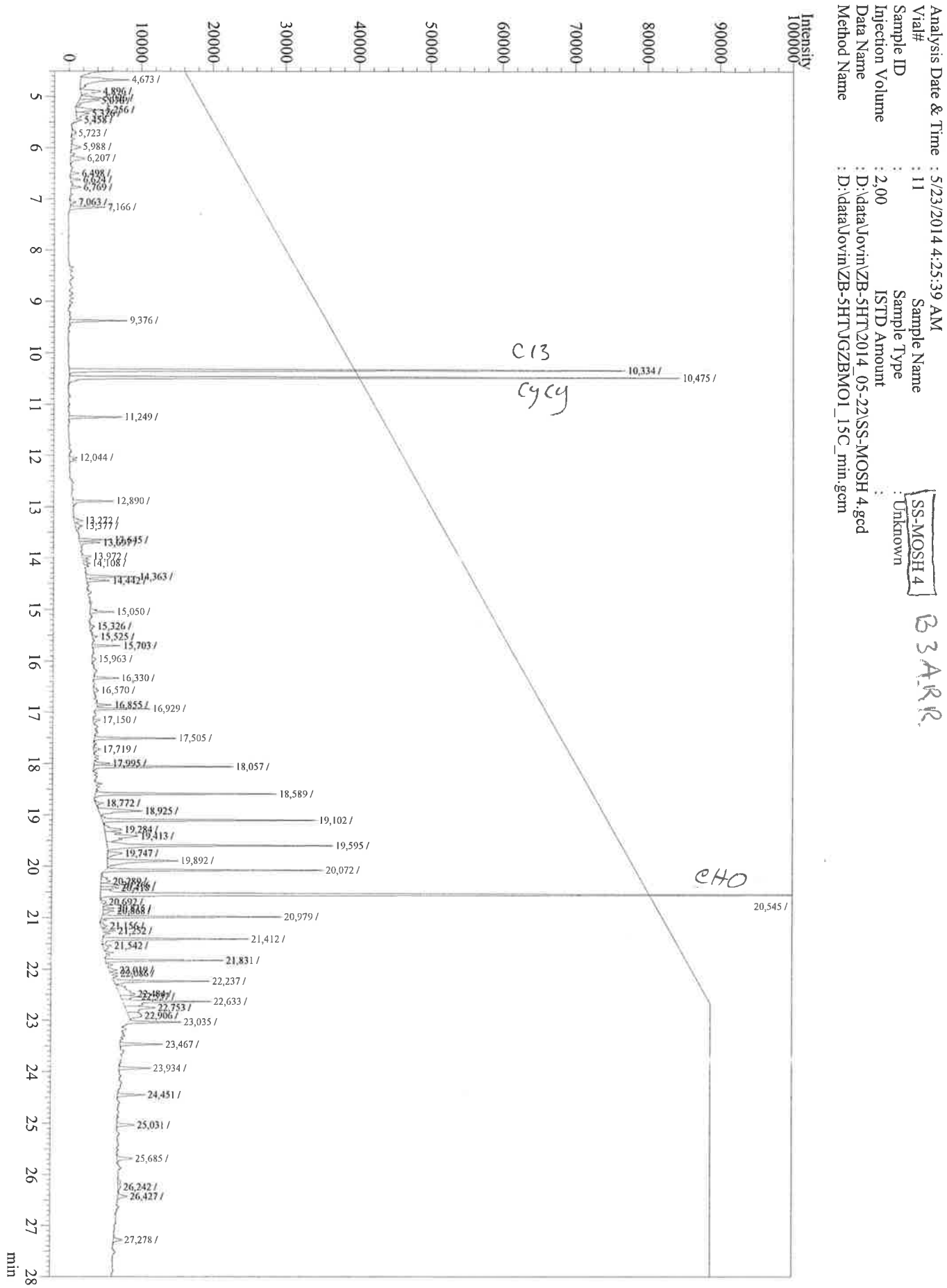
Analysis Date & Time	: 4/29/2014 9:02:13 PM
User Name	: Admin
Vial#	: 5
Sample Name	: SS-MOAH
Sample ID	:
Sample Type	: Unknown
Injection Volume	: 2,00
ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_04_28\SS-MOAH.gcd
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	5,557	6359374038	181748446	0,000				
2	5,757	232918	100779	0,000				
3	5,950	327555	193834	0,000				
4	6,015	234469	138212	0,000		V		
5	6,241	32301	14872	0,000				
6	6,964	15368	8461	0,000				
7	7,371	2259	1143	0,000				
8	7,754	5393	2106	0,000				
Net – 9	10,444	84204	42010	0,000				
TBB – 10	11,584	84239	24734	0,000				
11	11,908	4432	1763	0,000				
12	12,410	20393	7577	0,000				
13	12,964	3702	836	0,000				
14	13,505	2793	1096	0,000				
15	14,923	3392	973	0,000				
16	15,546	4261	1663	0,000				
17	18,938	17640	5213	0,000				
18	19,630	3100	948	0,000				
19	20,297	2500	870	0,000				
Pery – 20	20,862	166028	79172	0,000				
21	23,708	5876	2104	0,000				
Total		6360626861	182376812					

33 515

Appendix 34.1



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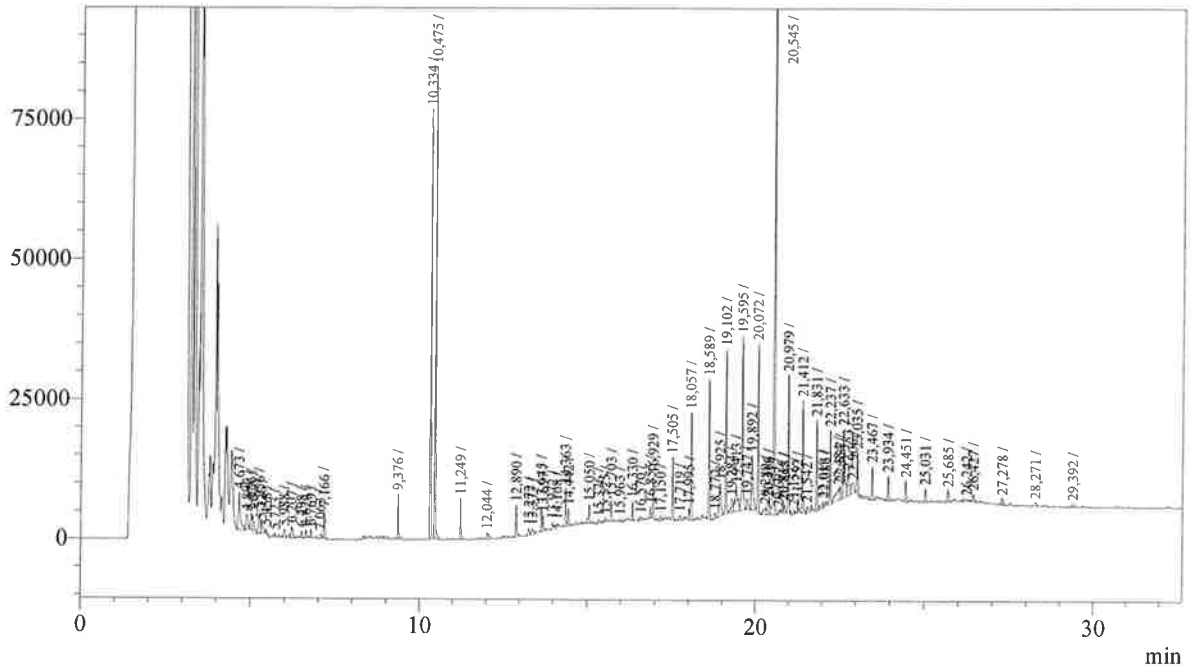
Appendix 34.2

Analysis Date & Time	: 5/23/2014 4:25:39 AM
User Name	: Admin
Vial#	: 11
Sample Name	: SS-MOSH 4
Sample ID	:
Sample Type	: Unknown
Injection Volume	: 2,00
ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_05-22\SS-MOSH 4.gcd
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm

Intensity



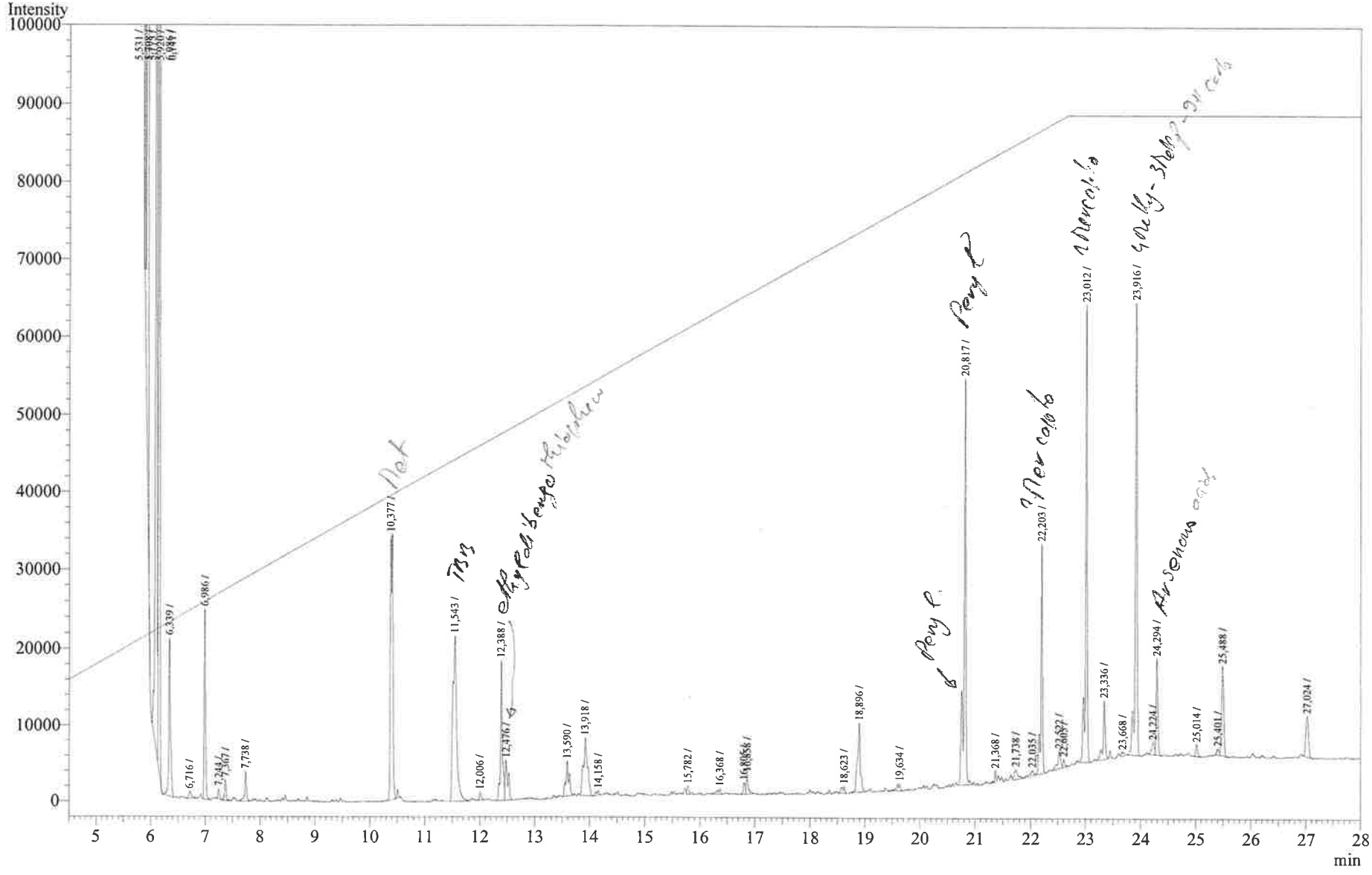
Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	4,673	27036	6594	0,000				
2	4,896	10170	2716	0,000				
3	5,026	8414	2963	0,000				
4	5,076	9459	2779	0,000		V		
5	5,256	10789	3724	0,000				
6	5,326	7113	1787	0,000		V		
7	5,458	2082	803	0,000				
8	5,723	2592	583	0,000				
9	5,988	4823	1307	0,000				
10	6,207	7955	2004	0,000		V		
11	6,498	2480	1180	0,000				
12	6,624	2904	1366	0,000				
13	6,769	2919	1345	0,000				
14	7,063	2288	775	0,000				
15	7,166	10505	4607	0,000				
16	9,376	13462	7728	0,000				
17	10,334	135050	72616	0,000				
18	10,475	144308	81604	0,000				
19	11,249	12967	7171	0,000				
20	12,044	4065	1032	0,000				
21	12,890	10358	5423	0,000				
22	13,272	3764	1174	0,000				
23	13,377	4205	961	0,000		V		
24	13,645	7738	4143	0,000				
25	13,697	6032	2762	0,000		V		
26	13,972	3209	1242	0,000		V		
27	14,108	2635	853	0,000		V		
28	14,363	12658	6553	0,000				
29	14,442	6488	3074	0,000		V		
30	15,050	7126	3186	0,000				
31	15,326	2838	647	0,000				

Appendix 34.3

Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
32	15,525	2168	711	0,000				
33	15,703	6517	3683	0,000				
34	15,963	2303	560	0,000				
35	16,330	5620	3297	0,000				
36	16,570	2518	638	0,000				
37	16,855	3387	1954	0,000		V		
38	16,929	11619	7163	0,000				
39	17,150	2388	876	0,000				
40	17,505	18899	10941	0,000				
41	17,719	2679	892	0,000				
42	17,995	4824	2018	0,000				
43	18,057	32395	18683	0,000		V		
44	18,589	43544	22815	0,000				
45	18,772	2594	1013	0,000				
46	18,925	21439	5751	0,000		V		
47	19,102	51461	27696	0,000		V		
48	19,284	9105	2325	0,000				
49	19,413	25564	4224	0,000		V		
50	19,595	64544	29340	0,000		V		
51	19,747	7261	1920	0,000				
52	19,892	31454	9494	0,000				
53	20,072	49046	29034	0,000				
54	20,289	6391	1225	0,000				
55	20,366	7372	2437	0,000		V		
56	20,418	6010	2377	0,000		V		
57	20,545	237042	119404	0,000		V		
58	20,692	2093	620	0,000				
59	20,815	2753	1464	0,000				
60	20,868	3698	1409	0,000		V		
61	20,979	40459	23254	0,000				
62	21,156	2873	824	0,000				
63	21,252	3628	1597	0,000		V		
64	21,412	35520	18467	0,000				
65	21,542	4204	1135	0,000				
66	21,831	26773	15406	0,000				
67	22,019	2792	1005	0,000				
68	22,086	2054	918	0,000		V		
69	22,237	23677	12164	0,000		V		
70	22,484	16773	2066	0,000		V		
71	22,537	7296	2428	0,000		V		
72	22,633	33684	12013	0,000		V		
73	22,753	17412	4083	0,000		V		
74	22,906	10835	1862	0,000		V		
75	23,035	14569	7089	0,000				
76	23,467	11404	5777	0,000				
77	23,934	8982	4229	0,000				
78	24,451	8220	3675	0,000				
79	25,031	5921	2338	0,000				
80	25,685	6154	2052	0,000				
81	26,242	3428	334	0,000				
82	26,427	4190	1260	0,000				
83	27,278	3742	1132	0,000				
84	28,271	2257	612	0,000				
85	29,392	2264	477	0,000				
Total		1454231	674863					

B3ARR

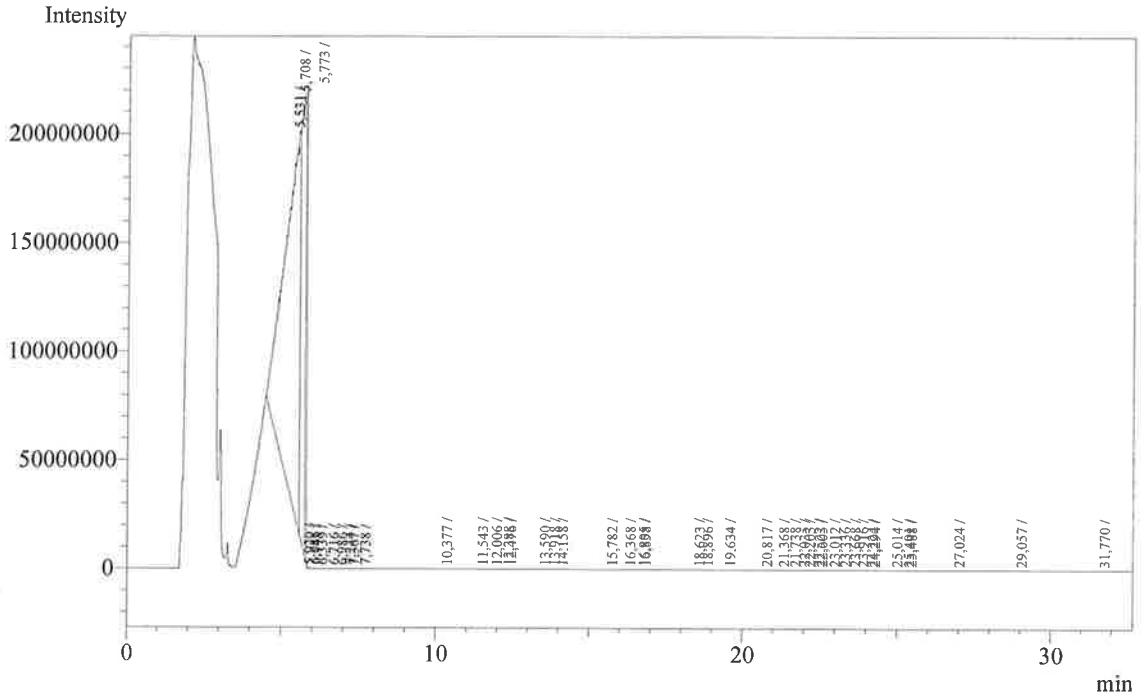
Analysis Date & Time	: 5/23/2014 5:30:05 AM		
Vial#	: 12	Sample Name	: SS-MOAH4
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_05-22\SS-MOAH 4.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		



Appendix 35.2

Analysis Date & Time : 5/23/2014 5:30:05 AM
User Name : Admin
Vial# : 12
Sample Name : SS-MOAH4
Sample ID :
Sample Type : Unknown
Injection Volume : 2,00
ISTD Amount :

Data Name : D:\data\Jovin\ZB-5HT\2014_05-22\SS-MOAH 4.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm

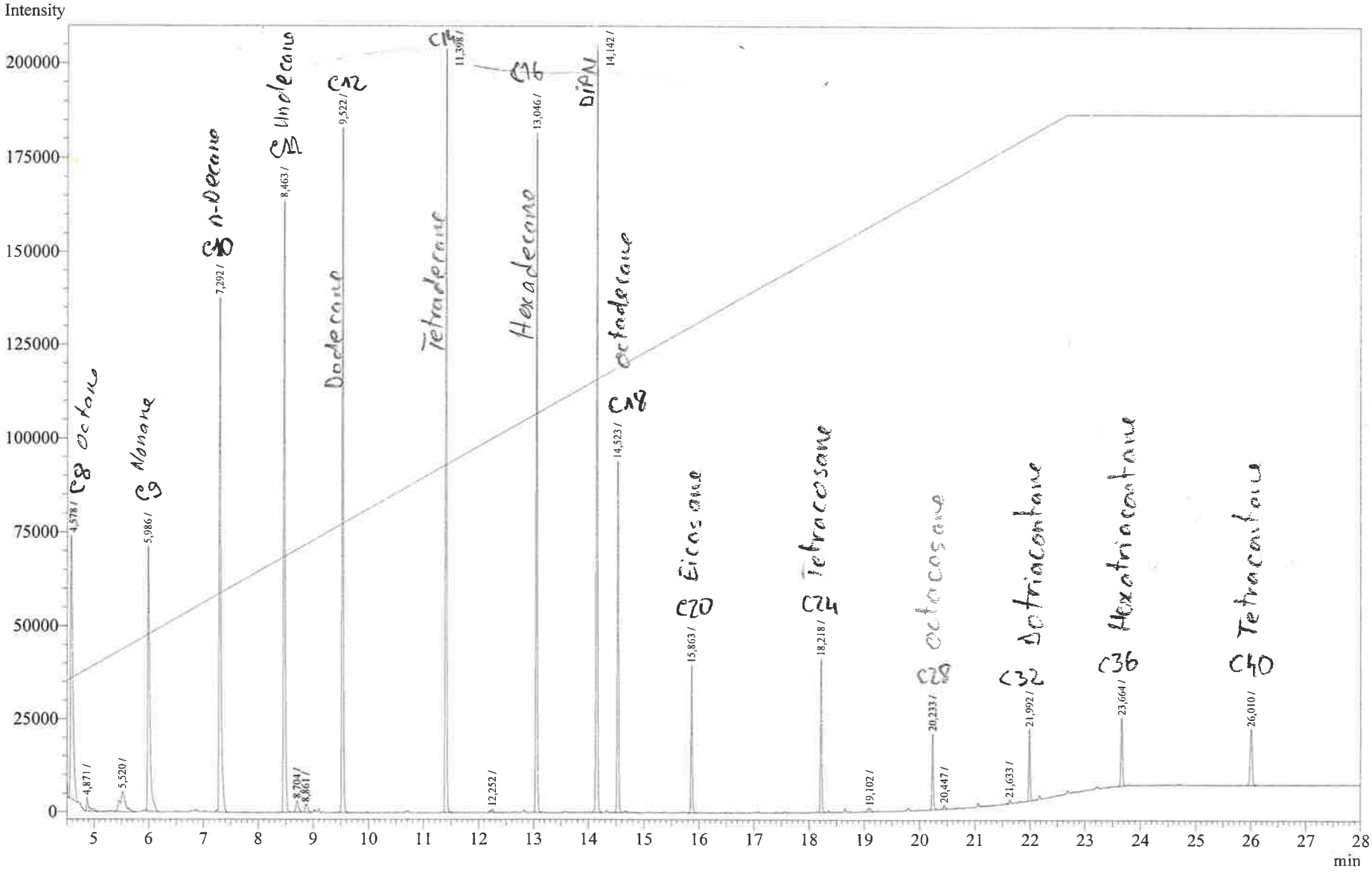


Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	5,531	6010444686	182350579	0,000				
2	5,708	2008848394	208949157	0,000		V		
3	5,773	692762045	217555539	0,000		V		
4	5,920	338689	122515	0,000				
5	6,086	405792	244837	0,000				
6	6,141	287432	173267	0,000		V		
7	6,339	43449	19732	0,000				
8	6,716	3234	916	0,000				
9	6,986	43095	24171	0,000				
10	7,244	2689	1279	0,000				
11	7,367	5585	2636	0,000				
12	7,738	8434	3820	0,000				
13	10,377	109145	33340	0,000				
14	11,543	104537	21255	0,000				
15	12,006	2226	994	0,000				
16	12,388	43858	17280	0,000				
17	12,476	18131	5125	0,000		V		
18	13,590	18139	4383	0,000				
19	13,918	39009	7382	0,000				
20	14,158	2008	546	0,000				
21	15,782	2379	1027	0,000		V		
22	16,368	3027	721	0,000				
23	16,805	3495	1433	0,000				
24	16,858	6140	2530	0,000		V		
25	18,623	3423	859	0,000				
26	18,896	32266	8900	0,000				
27	19,634	3992	915	0,000				
28	20,817	125065	51262	0,000				
29	21,368	2759	1417	0,000				
30	21,738	6208	1297	0,000		V		
31	22,035	3126	674	0,000				

Appendix 35.3

Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
32	22,203	60021	28464	0,000				
33	22,522	8593	2257	0,000				
34	22,605	2059	986	0,000		V		
35	23,012	120019	55657	0,000				
36	23,336	20377	7525	0,000				
37	23,668	2169	530	0,000		V		
38	23,916	132140	55453	0,000				
39	24,224	5471	1578	0,000				
40	24,294	27200	12026	0,000		V		
41	25,014	3592	1497	0,000				
42	25,401	2495	825	0,000				
43	25,488	32352	11565	0,000		V		
44	27,024	18193	5263	0,000				
45	29,057	7332	1590	0,000				
46	31,770	6632	1029	0,000				
Total		87141711	02609796033					

Analysis Date & Time	: 3/26/2014 2:32:49 PM		
Vial#	: 2	Sample Name	: STD
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD_Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_03_26\STD.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		

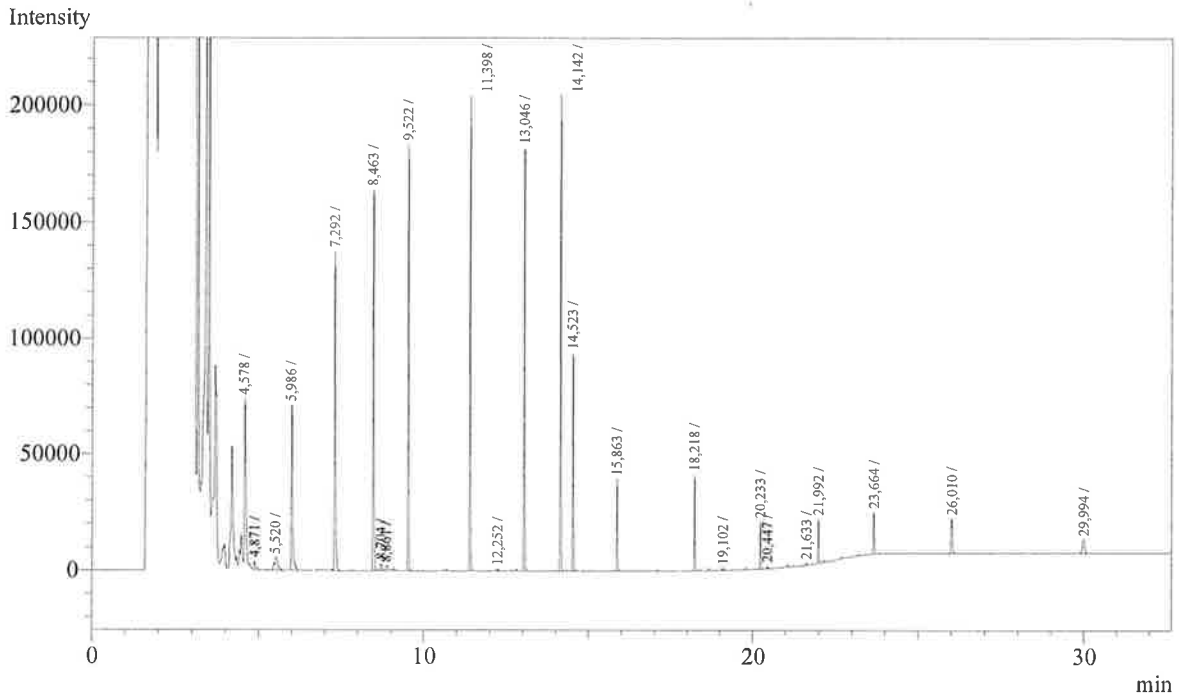


Appendix 36

Appendix 36.2

Analysis Date & Time : 3/26/2014 2:32:49 PM
User Name : Admin
Vial# : 2
Sample Name : STD
Sample ID :
Sample Type : Unknown
Injection Volume : 2,00
ISTD Amount :

Data Name : D:\data\Jovin\ZB-5HT\2014_03_26\STD.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	4,578	198867	69748	0,000				
2	4,871	11596	3564	0,000				
3	5,520	36776	5481	0,000				
4	5,986	185789	70028	0,000				
5	7,292	291975	131156	0,000				
6	8,463	298207	155571	0,000				
7	8,704	9811	3007	0,000				
8	8,861	7982	1930	0,000				
9	9,522	302822	178732	0,000				
10	11,398	313612	187400	0,000				
11	12,252	2505	886	0,000				
12	13,046	279391	170095	0,000				
13	14,142	320086	190379	0,000				
14	14,523	147445	88117	0,000				
15	15,863	62639	37552	0,000				
16	18,218	65260	39949	0,000				
17	19,102	3460	908	0,000				
18	20,233	32805	19817	0,000				
19	20,447	2402	1006	0,000				
20	21,633	2381	990	0,000				
21	21,992	30593	18426	0,000				
22	23,664	34597	17790	0,000				
23	26,010	39760	14742	0,000				
24	29,994	28987	5945	0,000				
Total		2709748	1413219					

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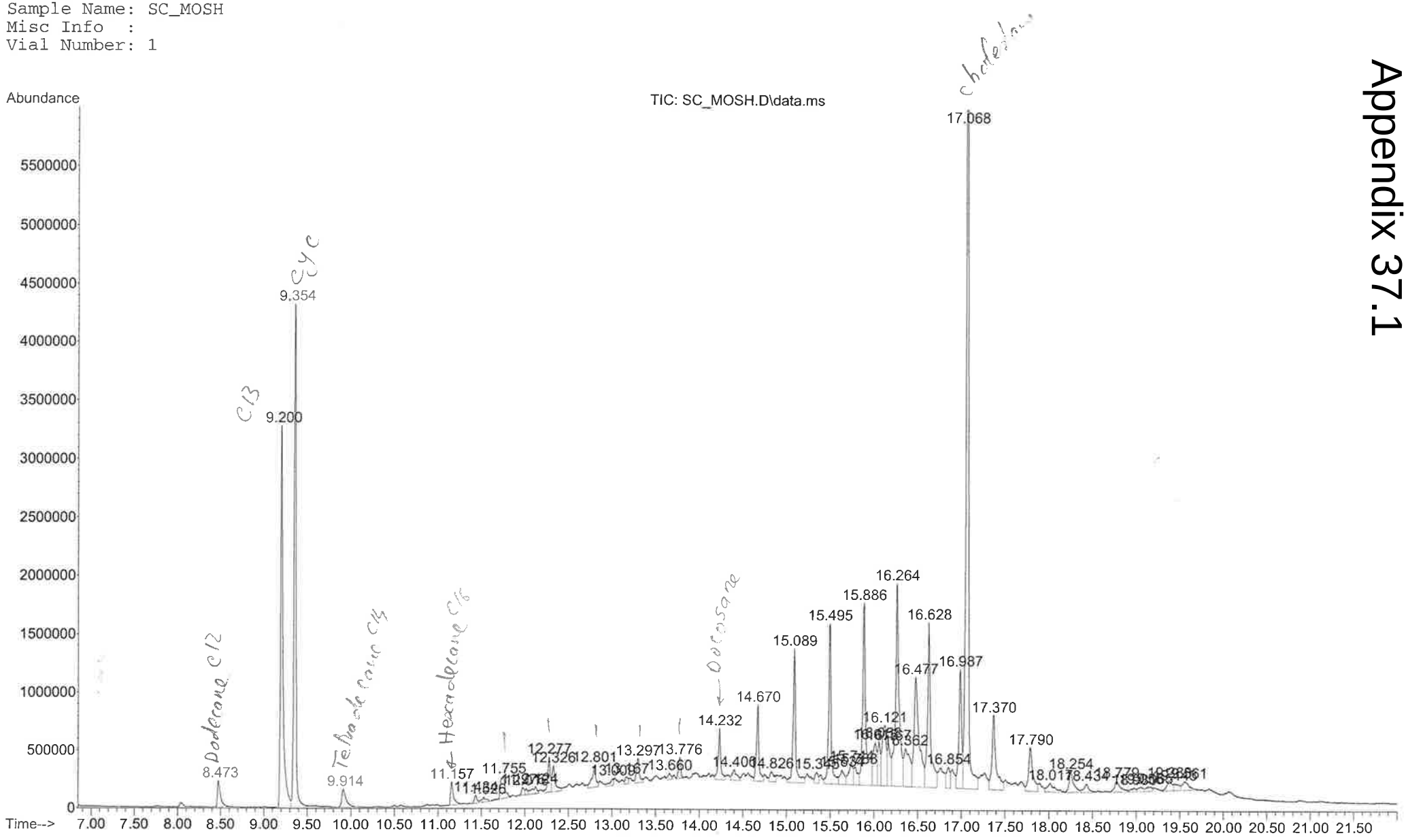
BBARR

File :C:\msdchem\1\data\HannuP\JovinG\20140410\SC_MOSH.D
Operator : Hannu P
Acquired : 10 Apr 14 12:38 pm using AcqMethod MOIL_JG.M
Instrument : msd5973n
Sample Name: SC_MOSH
Misc Info :
Vial Number: 1



Appendix 37.1

Library Search Report

Appendix 37.2

Data Path : C:\msdchem\1\data\HannuP\JovinG\20140410\
Data File : SC_MOSH.D
Acq On : 10 Apr 2014 12:38
Operator : Hannu P
Sample : SC_MOSH
Misc :
ALS Vial : 1 Sample Multiplier: 1

Search Libraries: C:\Database\Extracts.l Minimum Quality: 50
C:\Database\Acid.l Minimum Quality: 50
C:\Database\WILEY138.L

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	8.474	0.75	C:\Database\WILEY138.L			
			Dodecane C12	126000	000112-40-3	97
			Dodecane	126006	000112-40-3	96
			Dodecane	126003	000112-40-3	91
2	9.199	8.32	C:\Database\WILEY138.L			
			Tridecane C13	127254	000629-50-5	94
			Tridecane	127253	000629-50-5	93
			Heptadecane	130827	000629-78-7	91
3	9.354	9.08	C:\Database\WILEY138.L			
			1,1'-Bicyclohexyl cycy	125608	000092-51-3	94
			1,1'-Bicyclohexyl	19751	000092-51-3	93
			1,1'-Bicyclohexyl	125610	000092-51-3	70
4	9.912	0.72	C:\Database\WILEY138.L			
			Tetradecane C14	128277	000629-59-4	93
			Heptadecane	130827	000629-78-7	91
			Hexadecane	130123	000544-76-3	87
5	11.155	0.59	C:\Database\WILEY138.L			
			Hexadecane C16	46975	000544-76-3	95
			Hexadecane	130127	000544-76-3	93
			Hexadecane	130123	000544-76-3	93
6	11.435	0.19	C:\Database\WILEY138.L			
			Heneicosane C21	133323	000629-94-7	80
			C15→PENTADECANE, 2,6,10-TRIMETHYL-	58329	000000-00-0	80
			Tetracosane C24	134673	000646-31-1	72
7	11.526	0.19	C:\Database\WILEY138.L			
			DODECANE, 6-CYCLOHEXYL-	57566	013151-86-5	53
			Cyclohexane, (1-hexyltetradecyl)-	91305	004443-60-1	49
			Undecane 5-cyclohexyl-	51967	013151-80-9	49
8	11.754	0.92	C:\Database\WILEY138.L			
			Tetratetracontane	137346	007098-22-8	90
			Octadecane, 2,6-dimethyl-	67910	075163-97-2	80
			Pentadecane, 2,6,10,14-tetramethyl	132095	001921-70-6	80
9	11.978	0.17	C:\Database\WILEY138.L			
			Tetratetracontane	137346	007098-22-8	52
			1-Nonadecene	132005	018435-45-5	46
			11-Tricosene	80773	052078-56-5	46
10	12.016	0.12	C:\Database\WILEY138.L			
			Nonahexacontanoic acid	115614	040710-32-5	64
			Heneicosane	133323	000629-94-7	52
			Octacosane	135948	000630-02-4	46
11	12.125	0.35	C:\Database\WILEY138.L			
			HAHNFETT	115809	000000-00-0	87
			17-Pentatriacontene	108269	006971-40-0	70
			1-Dotriacontanol	106313	006624-79-9	58
12	12.277	0.83	C:\Database\WILEY138.L			
			Octadecane C18	131491	000593-45-3	95
			Octadecane	131492	000593-45-3	95
			Hexatriacontane	136998	000630-06-8	94

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Appendix 37.3

Data Path : C:\msdchem\1\data\HannuP\JovinG\20140410\
Data File : SC_MOSH.D
Acq On : 10 Apr 2014 12:38
Operator : Hannu P
Sample : SC_MOSH
Misc :
ALS Vial : 1 Sample Multiplier: 1

Search Libraries: C:\Database\Extracts.l Minimum Quality: 50
C:\Database\Acid.l Minimum Quality: 50
C:\Database\WILEY138.L

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
13	12.325	0.77	C:\Database\WILEY138.L			
			Tritetracontane	112525	007098-21-7	87
			Tetratetracontane	137346	007098-22-8	83
			Hexatriacontane	136998	000630-06-8	68
14	12.800	0.75	C:\Database\WILEY138.L			
			Nonadecane	62984	000629-92-5	92
			Tritetracontane	112525	007098-21-7	87
			Octadecane, 1-chloro-	132983	003386-33-2	86
15	13.010	0.22	C:\Database\WILEY138.L			
			HAHNFETT	115809	000000-00-0	91
			Hexadecane, 1-(ethenyloxy)-	132080	000822-28-6	58
			Cyclopentane, 1-methyl-3-(2-methyl propyl)-	9497	029053-04-1	51
16	13.166	0.18	C:\Database\WILEY138.L			
			HAHNFETT	115809	000000-00-0	94
			Cyclopentane, 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediyl]bis-	75420	055255-85-1	62
			(E)-9-((2'S,3'R)-3-HYDROXY-6'-((E)-2''-HYDROXYETHYLIDENE)-2'-METHYL-2'-OXEPANYL)-2,6-DIMETHYL-2-NONEN-5-ONE	85087	071117-51-6	49
17	13.300	0.64	C:\Database\WILEY138.L			
			Eicosane C20	132688	000112-95-8	97
			Eicosane	132690	000112-95-8	93
			Eicosane	67905	000112-95-8	89
18	13.661	0.18	C:\Database\WILEY138.L			
			HAHNFETT	115809	000000-00-0	93
			Cyclopentane, (4-octyldodecyl)-	134961	005638-09-5	53
			1-Hentetracontanol	112195	040710-42-7	49
19	13.775	0.43	C:\Database\WILEY138.L			
			Heptadecane, 2,6,10,15-tetramethyl	72677	054833-48-6	96
			Heneicosane	133324	000629-94-7	92
			Hexatriacontane	136998	000630-06-8	70
(20)	14.232	1.20	C:\Database\WILEY138.L			
			Docosane C22	133783	000629-97-0	96
			Docosane	133781	000629-97-0	95
			Docosane	133786	000629-97-0	93
21	14.398	0.42	C:\Database\WILEY138.L			
			HAHNFETT	115809	000000-00-0	93
			Octadecane, 1-chloro-	132983	003386-33-2	60
			17-Pentatriacontene	108269	006971-40-0	43
22	14.669	1.69	C:\Database\WILEY138.L			
			Tricosane C23	134296	000638-67-5	98
			Tricosane	134294	000638-67-5	93
			Tritetracontane	112525	007098-21-7	91
23	14.826	0.40	C:\Database\WILEY138.L			
			HAHNFETT	115809	000000-00-0	93
			1-Hentetracontanol	112195	040710-42-7	68
			1-Hentetracontanol	137292	040710-42-7	62

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Appendix 37.4

Data Path : C:\msdchem\1\data\HannuP\JovinG\20140410\
Data File : SC_MOSH.D
Acq On : 10 Apr 2014 12:38
Operator : Hannu P
Sample : SC_MOSH
Misc :
ALS Vial : 1 Sample Multiplier: 1

Search Libraries: C:\Database\Extracts.l Minimum Quality: 50
C:\Database\Acid.l Minimum Quality: 50
C:\Database\WILEY138.L

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
24	15.088	3.02	C:\Database\WILEY138.L			
			Tetracosane C24	134673	000646-31-1	97
			Octadecane	131490	000593-45-3	95
			Hexatriacontane	136998	000630-06-8	95
25	15.345	0.33	C:\Database\WILEY138.L			
			HAHNFETT Fatt	115809	000000-00-0	93
			1-Hentetracontanol	137292	040710-42-7	68
			Cyclopentane, 1,1,3-trimethyl-	119102	004516-69-2	30
26	15.496	3.74	C:\Database\WILEY138.L			
			Pentacosane C25	135021	000629-99-2	98
			Eicosane	132691	000112-95-8	95
			Hexatriacontane	136998	000630-06-8	95
27	15.636	0.59	C:\Database\WILEY138.L			
			HAHNFETT	115809	000000-00-0	70
			Tritetracontane	112525	007098-21-7	43
			7-OXABICYCLO[4.1.0]HEPTANE, 1,5-DI METHYL-	5388	000000-00-0	41
28	15.746	0.83	C:\Database\WILEY138.L			
			Hexatriacontane C36	137000	000630-06-8	50
			Hexatriacontane	136998	000630-06-8	50
			HAHNFETT	115809	000000-00-0	50
29	15.781	0.63	C:\Database\WILEY138.L			
			Eicosane, 9-cyclohexyl- C26	91306	004443-61-2	72
			Hexatriacontane	136998	000630-06-8	55
			Hexatriacontane	137000	000630-06-8	55
30	15.886	5.37	C:\Database\WILEY138.L			
			Hexacosane C26	91716	000630-01-3	95
			Hexadecane	130123	000544-76-3	94
			Hexacosane	135387	000630-01-3	93
31	16.014	1.37	C:\Database\WILEY138.L			
			Eicosane C20	132691	000112-95-8	95
			Hexatriacontane	136998	000630-06-8	64
			Nonadecane, 9-methyl-	67908	013287-24-6	60
32	16.061	1.09	C:\Database\WILEY138.L			
			Eicosane C20	67905	000112-95-8	95
			Eicosane	132691	000112-95-8	90
			Eicosane	132690	000112-95-8	64
33	16.119	2.28	C:\Database\WILEY138.L			
			Eicosane	132691	000112-95-8	96
			1-Octadecene	131396	000112-88-9	93
			Hexatriacontane	136998	000630-06-8	87
34	16.165	1.16	C:\Database\WILEY138.L			
			Eicosane	67905	000112-95-8	95
			Eicosane	132691	000112-95-8	92
			Eicosane	132692	000112-95-8	89
35	16.264	6.57	C:\Database\WILEY138.L			
			Heptacosane C27	94436	000593-49-7	96
			Nonadecane	132086	000629-92-5	95
			Heptacosane	135673	000593-49-7	95

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Data Path : C:\msdchem\1\data\HannuP\JovinG\20140410\
Data File : SC_MOSH.D
Acq On : 10 Apr 2014 12:38
Operator : Hannu P
Sample : SC_MOSH
Misc :
ALS Vial : 1 Sample Multiplier: 1

Appendix 37.5

Search Libraries: C:\Database\Extracts.l Minimum Quality: 50
C:\Database\Acid.l Minimum Quality: 50
C:\Database\WILEY138.L

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual	
36	16.363	2.15	C:\Database\WILEY138.L				
			* Eicosane C20	132691	000112-95-8	91	
			Hexatriacontane	137000	000630-06-8	64	
			1-Octadecene	131396	000112-88-9	60	
37	16.474	5.01	C:\Database\WILEY138.L				
			* Eicosane	132688	000112-95-8	91	
			1-Nonadecene	62219	018435-45-5	80	
			Nonadecane	62984	000629-92-5	78	
38	16.631	4.63	C:\Database\WILEY138.L				
			Octacosane C28	135948	000630-02-4	98	
			Nonadecane	132086	000629-92-5	95	
			Tetracosane	134673	000646-31-1	95	
39	16.852	0.66	C:\Database\WILEY138.L				
			HAHNFETT	115809	000000-00-0	89	
			Hexatriacontane	136998	000630-06-8	46	
			Hexatriacontane	137000	000630-06-8	41	
40	16.986	2.92	C:\Database\WILEY138.L				
			Nonacosane C29	99234	000630-03-5	97	
			Eicosane	132691	000112-95-8	97	
			Nonacosane	136145	000630-03-5	96	
41	17.068	19.77	C:\Database\WILEY138.L				
			Ⓜ Cholestane	135542	014982-53-7	99	
			Cholestane	135543	014982-53-7	99	
			Cholestane	93003	014982-53-7	93	
42	17.371	2.74	C:\Database\WILEY138.L				
			Eicosane C20	67905	000112-95-8	97	
			Eicosane	132688	000112-95-8	95	
			Triacontane	136347	000638-68-6	93	
43	17.790	1.70	C:\Database\WILEY138.L				
			Eicosane	67905	000112-95-8	96	
			Eicosane	132691	000112-95-8	95	
			Eicosane	132688	000112-95-8	95	
44	18.017	0.58	C:\Database\WILEY138.L				
			* Cyclotrisiloxane, hexamethyl-	129782	000541-05-9	38	Organosilicone
			Cyclotrisiloxane, hexamethyl-	44380	000541-05-9	38	
			1H-Indole, 5-methyl-2-phenyl-	38053	013228-36-9	27	
45	18.256	0.86	C:\Database\WILEY138.L				
			- Eicosane	67905	000112-95-8	95	
			Octadecane	131492	000593-45-3	95	
			Eicosane	132688	000112-95-8	95	
46	18.436	0.36	C:\Database\WILEY138.L				
			* 1H-Indole, 2-methyl-3-phenyl-	128926	004757-69-1	43	Aromatic $C_{15}H_{13}N$
			1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	129848	001020-31-1	35	
			Cyclotrisiloxane, hexamethyl-	129782	000541-05-9	32	
47	18.780	0.59	C:\Database\WILEY138.L				
			Octadecane C18	131490	000593-45-3	95	
			Octadecane	131492	000593-45-3	89	

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Appendix 37.6

Data Path : C:\msdchem\1\data\HannuP\JovinG\20140410\
Data File : SC_MOSH.D
Acq On : 10 Apr 2014 12:38
Operator : Hannu P
Sample : SC_MOSH
Misc :
ALS Vial : 1 Sample Multiplier: 1

Search Libraries: C:\Database\Extracts.l Minimum Quality: 50
C:\Database\Acid.l Minimum Quality: 50
C:\Database\WILEY138.L

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual	
			Eicosane	132689	000112-95-8	89	
48	18.978	0.15	C:\Database\WILEY138.L				
			Octadecane C20	131491	000593-45-3	91	
			Eicosane	132689	000112-95-8	68	
			Eicosane	67905	000112-95-8	35	
49	19.053	0.20	C:\Database\WILEY138.L				
			Octadecane C18	131491	000593-45-3	91	
			Octadecane	131490	000593-45-3	91	
			Eicosane	132691	000112-95-8	66	
50	19.164	0.23	C:\Database\WILEY138.L				
			Propiophenone, 2'-(trimethylsiloxy)-	44669	033342-87-9	41	organosilicon $C_{15}H_{27}NO_2Si_2$
			1,1,1,3,5,5,5-HEPTAMETHYLTRISILOXANE	44403	001873-88-7	38	
			Cyclotrisiloxane, hexamethyl-	129782	000541-05-9	35	
51	19.385	0.48	C:\Database\WILEY138.L				
			Eicosane C20	67905	000112-95-8	95	
			Eicosane	132688	000112-95-8	90	
			Eicosane	132691	000112-95-8	90	
52	19.443	0.33	C:\Database\WILEY138.L				
			Octadecane C18	131491	000593-45-3	93	
			Octadecane	131490	000593-45-3	93	
			Eicosane	132691	000112-95-8	86	
53	19.560	0.55	C:\Database\WILEY138.L				
			Octadecane C18	131490	000593-45-3	94	
			Hexatriacontane	136998	000630-06-8	50	
			Hexatriacontane	137000	000630-06-8	50	

C12
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13.03.14

±STD

MOSH: C13, cycy, 5α.cho

MOAH: Methyl, TBB, Perylene

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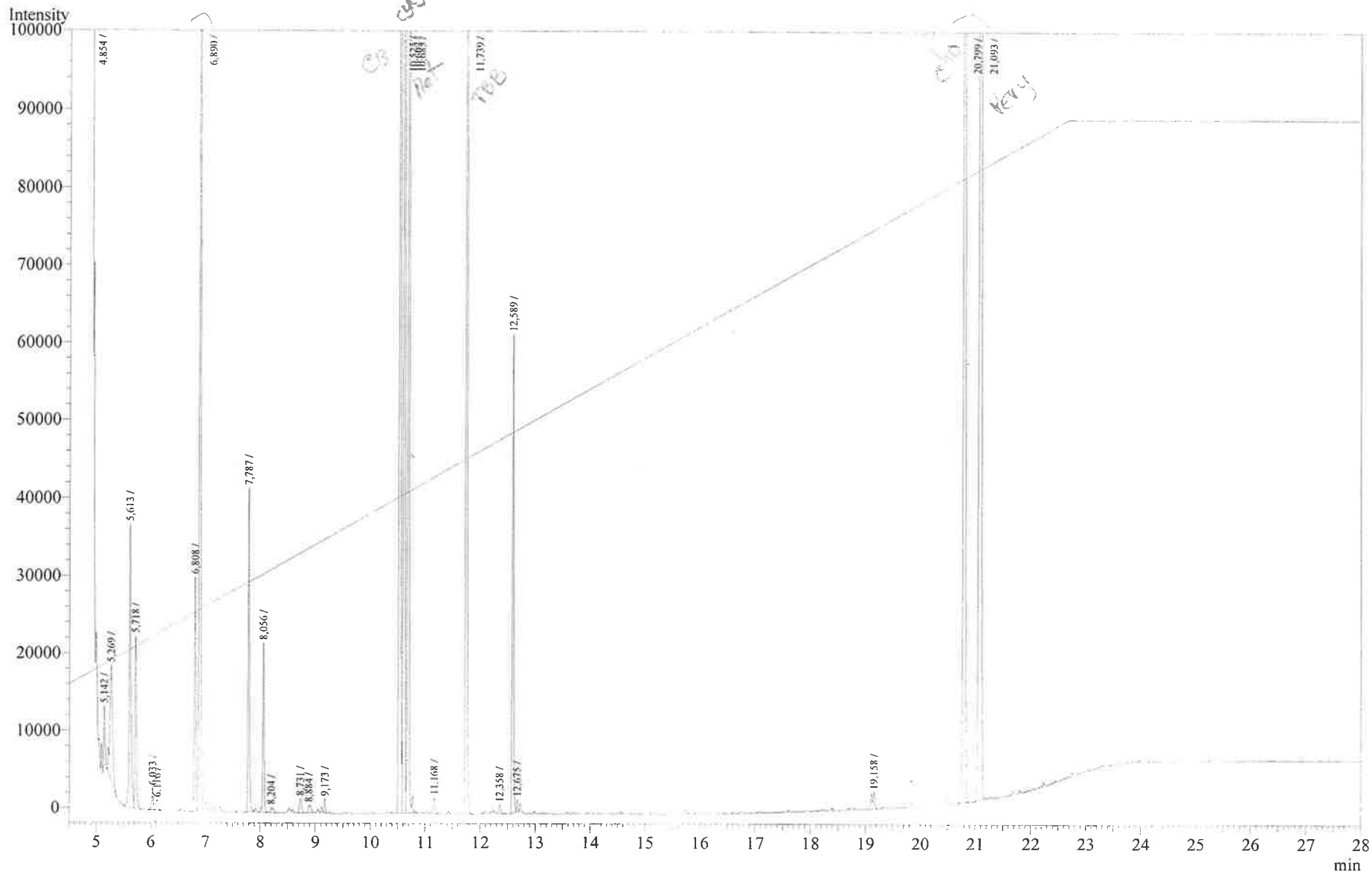
Analysis Date & Time	: 3/14/2014 1:36:33 AM		
Vial#	: 3	Sample Name	: ISTD
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	:
Data Name	: D:\data\Jovin\ZB-5HT\2014_03_13\ISTD.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm		

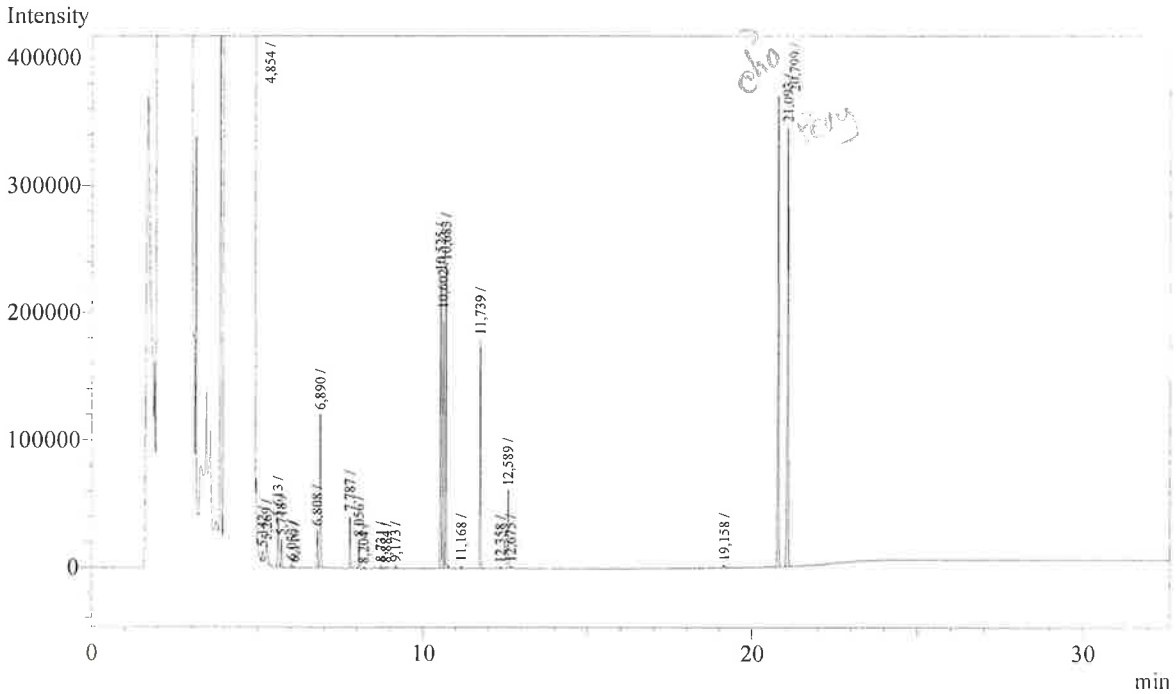


Appendix 38.1

Appendix 38.2

Analysis Date & Time : 3/14/2014 1:36:33 AM
User Name : Admin
Vial# : 3
Sample Name : ISTD
Sample ID :
Sample Type : Unknown
Injection Volume : 2,00
ISTD Amount :

Data Name : D:\data\Jovin\ZB-5HT\2014_03_13\ISTD.gcd
Method Name : D:\data\Jovin\ZB-5HT\JGZBMO1_15C_min.gcm



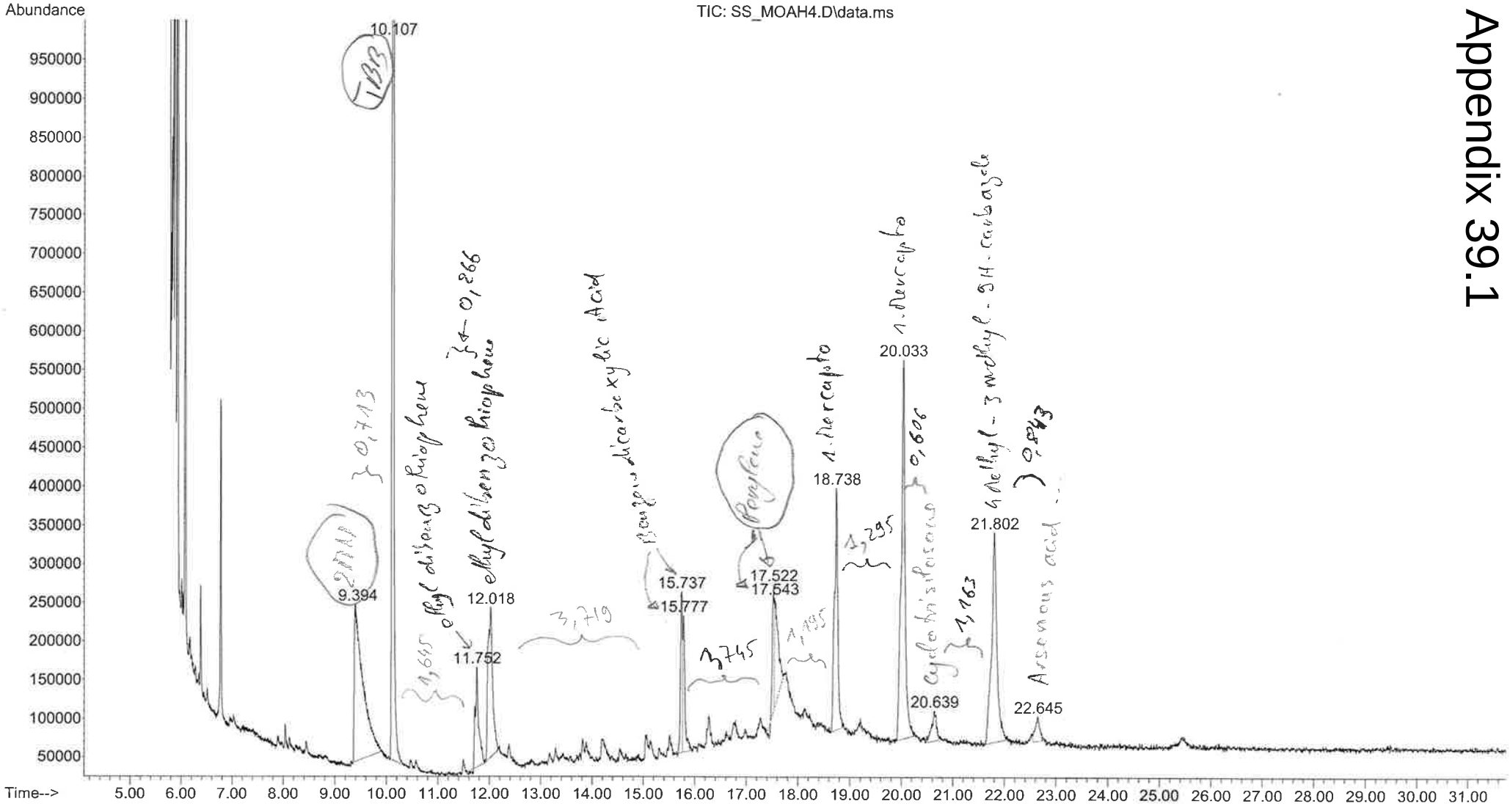
Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	4,854	973119325	82040499	0,000				
2	5,142	14975	7341	0,000				
3	5,269	51868	14467	0,000				
4	5,613	79199	34270	0,000				
5	5,718	43848	20955	0,000		V		
6	6,033	5415	2802	0,000				
7	6,116	2647	1271	0,000				
8	6,808	51768	29259	0,000				
9	6,890	230228	118417	0,000		V		
10	7,787	71055	40525	0,000				
11	8,056	38132	21238	0,000				
12	8,204	2313	667	0,000				
13	8,731	5390	1875	0,000				
14	8,884	3577	986	0,000				
15	9,173	2889	1684	0,000				
16	10,525	384615	208351	0,000				
17	10,602	347747	194967	0,000		V		
18	10,683	373729	228845	0,000		V		
19	11,168	4062	2015	0,000				
20	11,739	317200	173526	0,000				
21	12,358	2465	1110	0,000				
22	12,589	105499	56958	0,000				
23	12,675	2522	1546	0,000		V		
24	19,158	7872	2108	0,000				
25	20,799	698862	358277	0,000				
26	21,093	763423	319445	0,000				
Total		976730625	83883404					

S → C 13
S → Cyw
A → Meth
A → TBE
S → cho
A → Keq

38 bis

B3ARR

File :C:\msdchem\1\data\HannuP\JovinG\20140523\SS_MOAH4.D
Operator : Hannu P
Acquired : 23 May 2014 12:56 using AcqMethod MOIL_JG.M
Instrument : msd5973n
Sample Name: SS_MOAH 4 B3ARR
Misc Info :
Vial Number: 2



Appendix 39.1

Library Search Report

Appendix 39.2

Data Path : C:\msdchem\1\data\HannuP\JovinG\20140523\
Data File : SS_MOAH4.D
Acq On : 23 May 2014 12:56
Operator : Hannu P
Sample : SS_MOAH 4
Misc :
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: C:\Database\Extracts.l Minimum Quality: 50
C:\Database\Acid.l Minimum Quality: 50
C:\Database\WILEY138.L

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	9.395	14.00	C:\Database\WILEY138.L			
			Naphthalene, 1-methyl-	122759	000090-12-0	97
			Naphthalene, 2-methyl-	122764	000091-57-6	95
			Naphthalene, 2-methyl-	122761	000091-57-6	94
2	10.108	26.70	C:\Database\WILEY138.L			
			Benzene, 1,3,5-tris(1,1-dimethylethyl)-	54991	001460-02-2	87
			Benzene, 1,3,5-tris(1,1-dimethylethyl)-	131130	001460-02-2	86
			2,6-Pyridinediol, 3-[(o-hydroxyphenyl)azo]-	48648	021269-89-6	64
3	11.754	3.44	C:\Database\WILEY138.L			
			3-Ethyldibenzothiophene	40520	089817-03-8	83
			2-Ethyldibenzothiophene	40519	089816-98-8	83
			1-Ethyldibenzothiophene	40518	089816-97-7	83
4	12.020	7.10	C:\Database\WILEY138.L			
			2-Ethyldibenzothiophene	40519	089816-98-8	83
			1-Ethyldibenzothiophene	40518	089816-97-7	83
			3-Ethyldibenzothiophene	40520	089817-03-8	83
5	15.735	3.04	C:\Database\WILEY138.L			
			1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	135876	000117-81-7	83
			1,2-Benzenedicarboxylic acid, diisooctyl ester	135878	027554-26-3	74
			1,2-Benzenedicarboxylic acid, 3-nitro-	39769	000603-11-2	74
6	15.775	2.93	C:\Database\WILEY138.L			
			1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	135876	000117-81-7	87
			1,2-Benzenedicarboxylic acid, 3-nitro-	39769	000603-11-2	76
			1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	135866	000117-81-7	59
7	17.522	2.07	C:\Database\WILEY138.L			
			Perylene	131413	000198-55-0	97
			Perylene	57587	000198-55-0	97
			Perylene	131415	000198-55-0	97
8	17.545	3.98	C:\Database\WILEY138.L			
			Perylene	57587	000198-55-0	98
			Perylene	131416	000198-55-0	98
			Perylene	131413	000198-55-0	98
9	18.739	8.11	C:\Database\WILEY138.L			
			1-MERCAPTO-2-HEPTADECANONE	69240	076078-79-0	38
			2,4-Dimethyl-6-(trimethylsilyl)biphenyl	58292	087769-77-5	27
			O-HYDROXYACETOPHENONYLIDENE-4,5-DI	58211	103133-80-8	27

2/95

METHYL-O-PHENYLENEDIAMINE

Appendix 39.3

10	20.031	14.68	C:\Database\WILEY138.L			
			1-MERCAPTO-2-HEPTADECANONE	69240	076078-79-0	43
			Octadecanoic acid, ethenyl ester	77157	000111-63-7	38
			18-Pentatriacontanone	136995	000504-53-0	22
11	20.637	1.51	C:\Database\WILEY138.L			
			Cyclotrisiloxane, hexamethyl-	44380	000541-05-9	49
			Cyclotrisiloxane, hexamethyl-	129782	000541-05-9	49
			Cyclotrisiloxane, hexamethyl-	129783	000541-05-9	47
12	21.801	11.34	C:\Database\WILEY138.L			
			4-Ethyl-3-methyl-9H-carbazole-2-carboxylate	62381	071700-66-8	32
			Benzaldehyde, 2,5-bis[(trimethylsilyl)oxy]-	67552	056114-69-3	25
			Ether, bis(p-tert-butylphenyl)	67892	024085-65-2	25
13	22.646	1.11	C:\Database\WILEY138.L			
			Arsenous acid, tris(trimethylsilyl) ester	85890	055429-29-3	53
			Cyclotrisiloxane, hexamethyl-	129782	000541-05-9	52
			BENZENE, 1,4-BIS(TRIMETHYLSILYL)-	44683	000000-00-0	50

MOil_JG.M Tue May 27 11:54:45 2014

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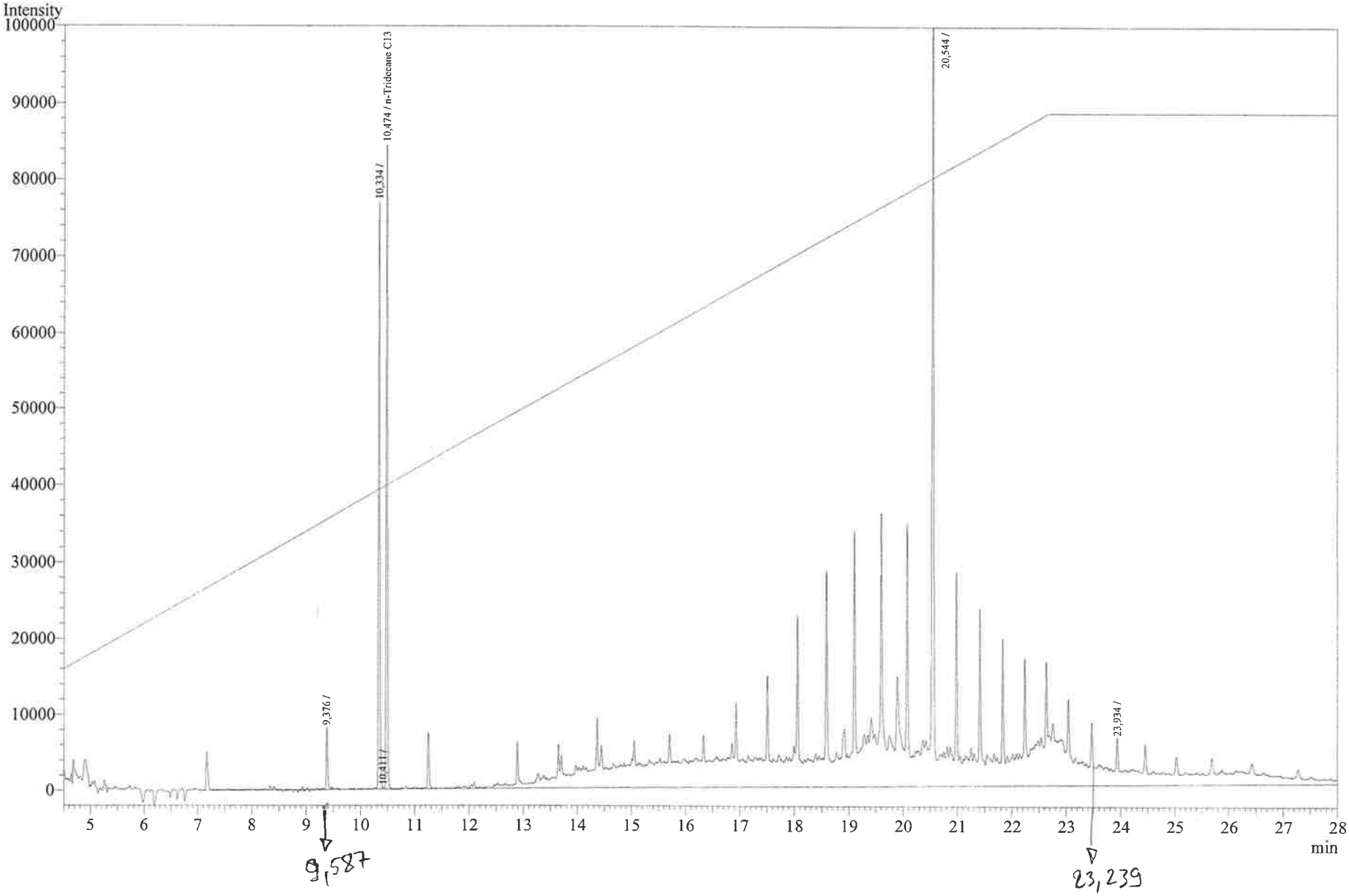
B3ARR

Analysis Date & Time : 5/23/2014 4:25:39 AM
Vial# : 11 Sample Name : SS-MOSH 4
Sample ID : Sample Type : Unknown
Injection Volume : 2,00 ISTD Amount : [1]=1,[2]=1,[3]=1
Data Name : D:\data\Jovin\ZB-5HT\Processed DATA\2014_05-22\SS-MOSH 4_Man Int.gcd
Method Name : D:\data\Jovin\ZB-5HT\Processed DATA\JGZB_MO_HP_Manual Int.gcm

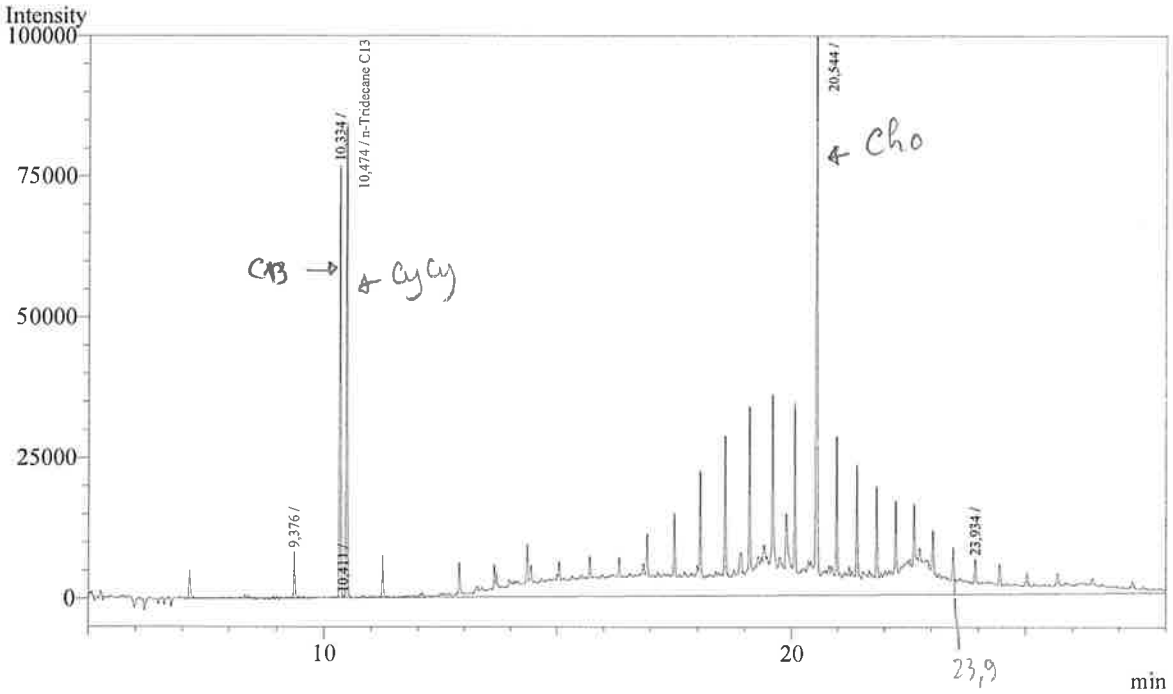


Appendix 40.1

Analysis Date & Time	: 5/23/2014 4:25:39 AM
User Name	: Admin
Vial#	: 11
Sample Name	: SS-MOSH 4
Sample ID	:
Sample Type	: Unknown
Injection Volume	: 2,00
ISTD Amount	: [1]=1,[2]=1,[3]=1

B3ARR

Data Name	: D:\data\Jovin\ZB-5HT\Processed DATA\2014_05-22\SS-MOSH 4_Man Int.gcd
Method Name	: D:\data\Jovin\ZB-5HT\Processed DATA\JGZB_MO_HP_Manual Int.gcm



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	9,376	32816	7979	0,000				
C13 — 2	— 10,334	135697	74939	0,000		V		
3	10,411	471	239	0,000		V		
CyCy — 4	— 10,474	142818	78150	0,000	ppm	V	6	n-Tridecane C13
CHO — 5	20,544	3065906	121037	0,000		V		
6	23,934	⊖484387	6145	0,000		V		
Total		3862095	288489					

3862095 − 484,387 = 3377708

A_{C13} = 163723

A_{CyCy} = 167107 } = 49.137 2,06

A_{CHO} = 160541,06

wrong

≠ 2886336,94

m_{CHO} = 279,2 mg/kg

A_{13} = 135697

A_{CyCy} = 142818

A_{CHO} = 3065906 − (A_{C13} × 1,78) =

− (135697 × 1,78) = 2824365,34

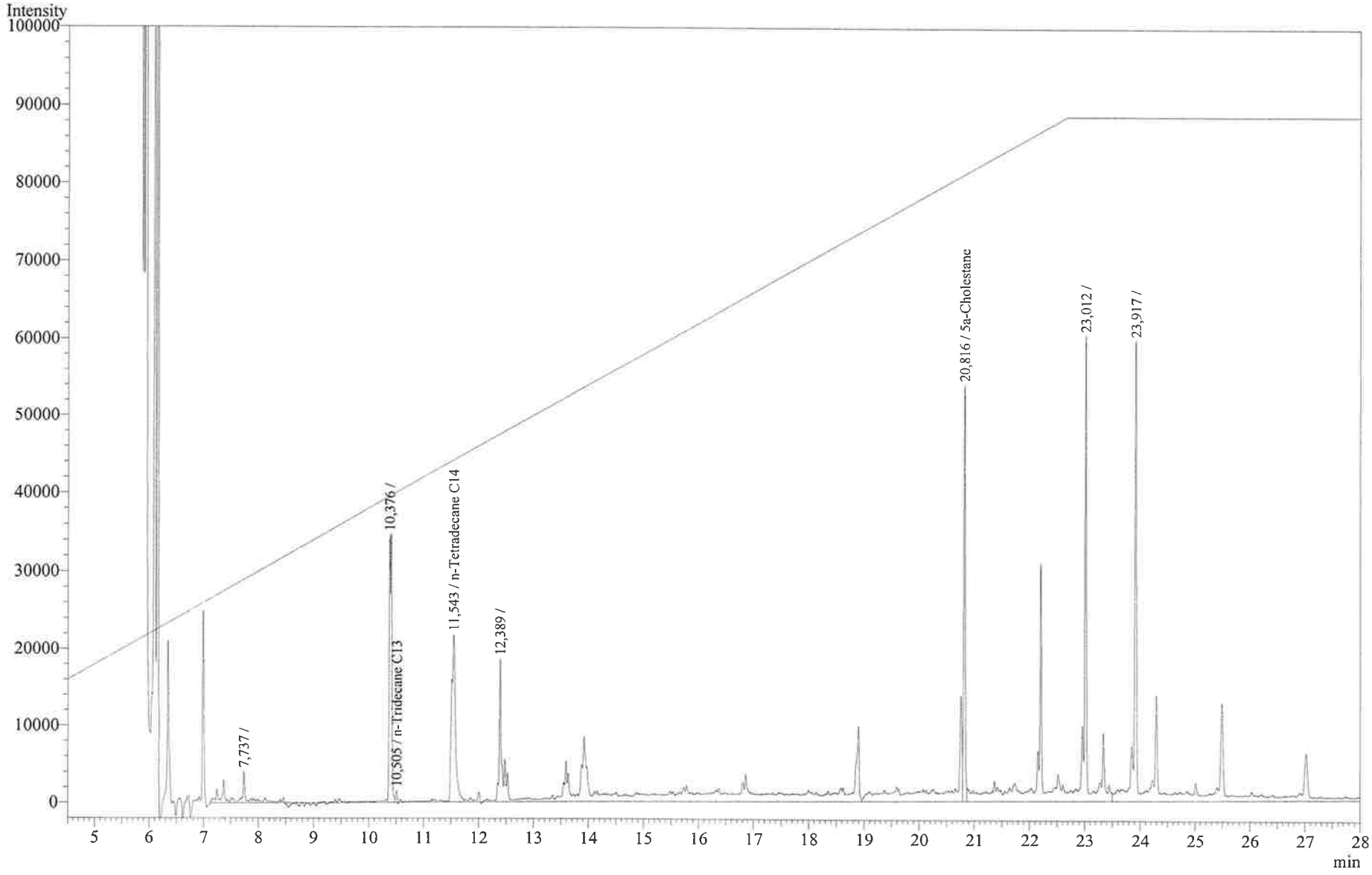
(X)

5/9/14

41

B3ARR

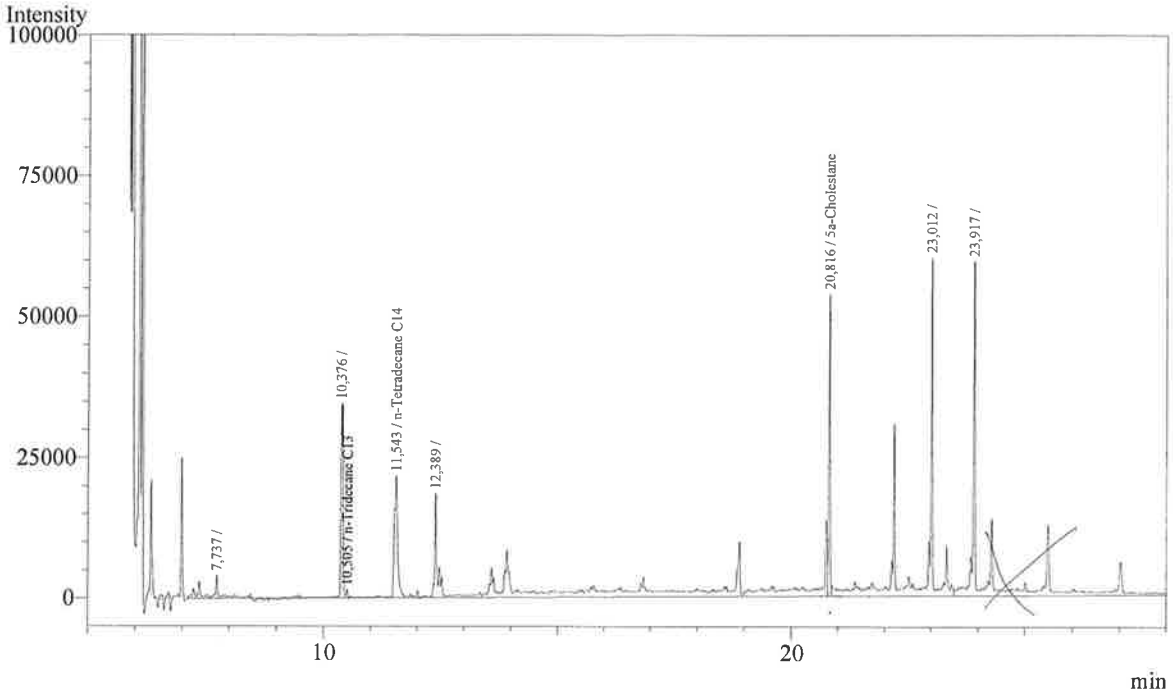
Analysis Date & Time	: 5/23/2014 5:30:05 AM		
Vial#	: 12	Sample Name	: SS-MOAH4
Sample ID	:	Sample Type	: Unknown
Injection Volume	: 2,00	ISTD Amount	: [1]=1,[2]=1,[3]=1
Data Name	: D:\data\Jovin\ZB-5HT\Processed DATA\2014_05-22\SS-MOAH 4_Man Int.gcd		
Method Name	: D:\data\Jovin\ZB-5HT\Processed DATA\JGZB_MO_HP_Manual Int.gcm		



Appendix 40.2

Analysis Date & Time : 5/23/2014 5:30:05 AM
User Name : Admin
Vial# : 12
Sample Name : SS-MOAH4
Sample ID :
Sample Type : Unknown
Injection Volume : 2,00
ISTD Amount : [1]=1,[2]=1,[3]=1

Data Name : D:\data\Jovin\ZB-5HT\Processed DATA\2014_05-22\SS-MOAH 4_Man Int.gcd
Method Name : D:\data\Jovin\ZB-5HT\Processed DATA\JGZB_MO_HP_Manual Int.gcm



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	7,737	39001	3952	0,000				
2	10,376	111476	33843	0,000		V		
3	10,505	10062	1462	0,000	ppm	V	6	n-Tridecane C13
4	11,543	106271	21525	0,000	ppm	V	9	n-Tetradecane C14
5	12,389	665997	17564	0,000		V		
6	20,816	100307	52067	0,000	ppm	V	17	5a-Cholestane
7	23,012	409922	56220	0,000		V		
8	23,917	473242	57290	0,000		V		
Total		1916278	243923					

$A = 1916278 - (A_{2mki} + A_{TBR} + A_{3pcs} + A_{rest})$

$= - (104800 + 109920 + 190465 + 94594 + 57290)$

$= 598773$

MOAH =

MOAH = 51,9 mg/kg candle (15,68 %)

4/6/15

Appendix 42

MOSH AND MOAH MANUAL CALCULATION

1. MOSH calculation

Data

The data are provided by the regents used during the laboratory work and the GC analysis results.

ISTD used: C13
Concentration of the ISTD in MOSH: C_{istd} = 13.66 mg/L = 13.66 µg/mL
Volume of ISTD loaded in the column: V_L= 0.25 mL
Volume of extract from 2 g cardboard: V_{ext} = 5 mL
Mass of C13 in 0.25 mL of istd loaded = 13.66*0.25 = 3.415 µg
Area of C13 A_{istd} = 353087
Total Integrated area of MOSH $\mathbf{A_{TMOSH}}$ = 4820501

Formula used for the calculation of the mass of MOSH and MOAH in cardboard

Equation 1

$$m_{card} = \frac{A_{card}}{A_{istd}} * m_{istd} \qquad (1)$$

Legend:

$\boldsymbol{m_{card=}}$ Mass of the MOSH in cardboard (mg)

$\boldsymbol{m_{istd=}}$ Mass of the ISTD (mg)

$\boldsymbol{A_{card=}}$ Area of the integration of MOSH

$\boldsymbol{A_{istd=}}$ Area of the integrated ISTD

The use of the internal standard components ratio (r) in GC analysis

When several ISTD components are used in the cardboard sample, the ratio (r) of internal standard (ISTD) components is used to determine the area of the overlapping ISTD component with cardboard component.

The Ratio (r) of C13 and cholestane (Cho)

Equation 2

$$r = \frac{A_{cho}}{A_{c13}} = \frac{631454}{353087} = \mathbf{1.78}$$

The determination of the real area of MOSH in the chromatogram

To calculate the real area of MOSH (**A_{RMOSH}**) in cardboard, the area of the internal standard components used such as C13 (**A_{c13}** = 163723), Cycy (A_{cycy} = 167107), Cho (A_{cho} = 451968), and the area of non-mineral oil components must be substrate from the total integrated area of MOSH (**A_{TMOSH}** = 4820501). The real area of MOSH is then used in the equation **3** to calculate the real mass of MOSH.

The real MOSH area calculation

A_{RMOSH} = A_{TMOSH} – (A_{c13} + A_{cycy} + A_{Cho}) *Equation 3*

A_{RMOSH} = Total area MOSH – (A_{c13} + A_{cycy} + (A_{c13}*1.78)) =
= (3862095-484387) – (135697 + 142818 +241540.66) = **2857652.34**

The determination of the real mass of MOSH in GC analysis

Mass of MOSH in 0.25 mL of loaded sample

The real area of MOSH is then used in the equation **1** to calculate the real mass of MOSH.

$$\mathbf{m_{MOSH}} = \frac{A_{card}}{A_{istd}} * m_{istd} = \frac{2857652.34}{353087} * 3.415 = \mathbf{27.64\ \mu g}$$

Mass of MOSH in 0.5 mL extract from 2 g cardboard

m_{MOSH} = 27.64*20 = **552.8 µg = 0.5528 mg**

Mass of MOSH in 1 kg cardboard for the range of C10 to C35

m_{MOSH} = 0.5528*500 = **276.4 mg / kg cardboard**

Non volatile MOSH calculation (range over C35)

Total area of MOSH: A_{MOSH} = 484387

MOSH in 0.25 mL

m_{MOAH} = $\frac{A_{MOAH}}{A_{istd}} * m_{istd}$ = $\frac{484387}{353087} * 3.415 =$ **4.68 µg**

MOSH in 0.5 mL

m_{MOAH} = 4.68*20 = 93.6 µg =**0.0936 mg**

MOSH in 1 kg cardboard

m_{MOSH} = 0.0936*500= **46.8 mg / kg cardboard**

2. MOAH calculation

The procedure used for MOSH calculation is also applied for MOAH.

Data

ISTD used: 2-Methylnaphtalene (2MN)
Concentration of the ISTD used: **C_{2MN}** = 11.44 µg/ mL
Mass of 2MN in 0.25 mL of ISTD loaded: **m_{istd}** = 11.44*0.25 = **2.86 µg**
Area of 2MN: **A_{istd}** = 330205
Total Integrated MOAH area: **A_{MOAH}** = 2703156-817726=**1885430**

Area of MOAH in GC analysis

A_{MOAH} = 1885430- (A_{2MN} +A_{TBB}+A_{Peryl}) -$A_{Mercapto}$
= 1885430-(107492+111984+189209)-95775= **1380970**

Mass of MOSH in 0.25 mL of loaded cardboard sample

m_{MOAH} = $\frac{A_{MOAH}}{A_{istd}} * m_{istd}$ = $\frac{1380970}{330205} * 2.86 =$ **11.96 µg**

Mass of MOAH in 0.5 mL extract from 2 g cardboard

m_{MOAH} = 11.96*20 = **239.2 µg = 0.2392 mg**

Mass of MOAH in 1 kg cardboard for the alkanes range of C10 to C35

m_{MOAH} = 0.2392*500 = **119.6 mg / kg cardboard**

Calculation of MOAH in the range over C35

Area of MOAH: A_{MOAH} = Total area –Non aromatic components
=817726-31004-45667-42132= **698923**

MOAH in 0.25 mL

m_{MOAH} = $\frac{A_{MOAH}}{A_{istd}} * m_{istd}$ = $\frac{698923}{330205} * 2.86 =$ **6.05 µg**

MOAH in 0.5 mL

m_{MOAH} = 6.05*20 = 121 µg =**0.121 mg**

MOAH in 1 kg cardboard

m_{MOAH} = 0.121*500= **60.5 mg / kg cardboard**

3. CALCULATION RESULTS FOR MOSH AND MOAH

Volatile mineral oil in the range of C10 to C35

MOSH = 276.4 mg / kg cardboard (69.80 %)

MOAH = 119.6 mg / kg cardboard (30.2 %)

Total amount of mineral oil in 1 kg cardboard: 396 mg

Non-volatile mineral oil in the range over C35

MOSH= 46.8 mg / kg cardboard
MOAH= 60.5 mg / kg cardboard
Total amount = 107.3 mg / kg cardboard

To make the calculation simple and accurate, **Microsoft Excel** application was used.