Box 6

Analysis of Methyl Tertiary-Butyl Ether (MTBE) and other petroleum oxygenates in water

National Groundwater and Contaminated Land Centre and National Laboratory Service Project NC/99/39

ENVIRONMENT AGENCY

Analysis of Methyl Tertiary-Butyl Ether (MTBE) and other petroleum oxygenates in water

National Groundwater and Contaminated Land Centre and National Laboratory Service

September 2000

James Deuchar and Gill Hardy
National Laboratory Service

Environment Agency
National Groundwater and Contaminated Land Centre
Olton Court
10 Warwick Road
Olton
Solihull
West Midlands
B92 7HX

National Groundwater and Contaminated Land Centre Project
NC/99/39

Publishing Organisation:
Environment Agency
Rio House
Waterside Drive
Aztec West
Almondsbury
Bristol BS12 4UD
Tel: 01454 624400 Fax: 01454 624409

Dissemination status
Internal: **Released to Regions**
External: **Released to Public Domain**

Statement of use
This document provides information on the choice of methodology for the analysis of petroleum oxygenates in water.

Research Contractor
National Laboratory Service
Environment Agency
Meadow Lane
Nottingham NG2 3HN

Tel: 0115 9860325 Fax: 0115 9861739

This document was produced under a National Groundwater & Contaminated Land Centre Project.

Project Manager
The Project Manager for this project was:
Alwyn Hart, National Groundwater and Contaminated Land Centre.

National Groundwater & Contaminated Land Centre Project NC/99/39

CONTENTS

Page Nos.

Executive Summary

Organic ether compounds are added to petrol (gasoline) help to meet octane levels and generally optimise the refinery process management. However, a number of pollution incidents have occurred in the UK and overseas where petrol containing methyl tertiary butyl ether (MTBE) has contaminated groundwater.

Analysis of MTBE and other oxygenates (isopropyl ether, ethyl tertiary butyl ether and tertiary amyl ether) in the UK is mainly on an 'ad hoc' basis and only a small number of laboratories undertake routine analysis for these compounds. The aims of this project were therefore to

- review current analytical techniques
- decide on the best approach for Environment Agency samples.

Although there is currently no drinking water standard for MTBE in Europe, the maximum admissible concentration for dissolved or emulsified hydrocarbons is 10μg l^{-1} (EEC, 1989). A target detection limit for this analysis was therefore set at $1/10^{th}$ of this value. The choice of methodology was primarily concerned with:

- correct identification of MTBE
- removal of known petroleum interferants i.e. 2- and 3- methyl pentanes
- prevention of any possible cross contamination between samples rather than achieving the ultimate detection limit possible.

The final methodology developed comprised:

- Headspace – Gas chromatography/Mass Spectrometry (GC-MS) for quantification.
- Solid phase microextraction – Gas Chromatography/ MS/MS for more definitive confirmation of MTBE identity than normal GC-MS can provide.

Full performance testing to the WRC Water Industry standard NS30 protocol was undertaken for method (1) to provide evidence of method suitability.

For methyl tertiary butyl ether, the method achieved a detection limit of 0.2μg l-1, at10% precision, with a 1% bias at the 10μg l^{-1} level (total error 22%).

The quadrupole GC-MS method can also be used for samples containing levels of petrol higher than 0.5mg l^{-1} but oxygenates other than MTBE are difficult to quantify and confirm due to increased hydrocarbon background. Further work and equipment would be necessary to improve the GC-MS/MS technique to provide more positive confirmation for contaminated samples.

Keywords

MTBE, oxygenates, analysis, petroleum, gas chromatography, headspace, mass spectrometry.

1. Introduction

Aliphatic ethers are currently used in petrol to help manage the refining process and to meet the octane rating. However their higher water solubility, poor soil retention and environmental persistence contribute to a significant possibility of groundwater contamination from leaking storage tanks, pollution incidents and spillages.

To effectively monitor this situation requires an analytical method of known characteristics. The aim of the project is therefore to assess the strengths and weaknesses of current analytical methods and derive a methodology able to produce a definitive result within known parameters.

In the US, the most commonly used methods are EPA 8021B using gas chromatography-photoionisation detection and EPA 8260B using mass spectrometric detection. Both methods use 'Purge and Trap' as a means of sample introduction into the gas chromatograph.

In this country, monitoring of the ethers is usually on an 'ad-hoc' basis often based on BTEX (benzene, toluene, ethyl benzene, xylene) methodology using either 'Purge and Trap' or 'Headspace' sample introduction.

Published analytical methods have used

- multidimensional chromatography (Frysinger and Gaines, 2000),
- membrane –introduction mass spectrometry (Lopez-Avila *et al*, 1999),
- near infra-red spectroscopy (NIR) (Guchardi *et al*, 1998),
- direct water injection (Church *et al*, 1997).

The latter also determined the common degradation products, tertiary butyl alcohol (TBA), tertiary butyl formic acid (TBF) and tertiary amyl alcohol (TAA). Nouri *et al*, 1996 used both Headspace and Purge and Trap techniques with a flame ionisation detector quoting limits of detection for MTBE in water of 50μg l^{-1} for headspace and 2μg l^{-1} for Purge and Trap.

2. Objectives

The purpose of the current work was to better define a method for detection and quantification of MTBE and if possible any other ethers typically used in petrol. Analysis of MTBE and other oxygenates (isopropyl ether, ethyl tertiary butyl ether and tertiary amyl ether) in the UK is mainly on an 'ad hoc' basis and only a small number of laboratories undertake routine analysis for these compounds. The aims of this project were therefore to

- review current analytical techniques
- decide on the best approach for Environment Agency samples.

3. Review and selection of analytical procedures

In order to choose a method for subsequent development a range of options and their implications were considered.

3.1 Sample introduction

The available techniques for this analysis are:

- membrane introduction
- direct water injection
- multiple dimensional chromatography
- purge and trap
- headspace
- solid phase micro-extraction

The choice between these techniques is governed by the interplay between the following factors:

- volatility of the compound
- nature of the sample
- identity of compound
- availability of equipment
- reliability and performance

Briefly, the membrane introduction technique is not commonly employed relying on in-house built equipment and thus was considered not suitable at this time. Direct water injection while offering simplicity and cheapness can result in shifting gas chromatography retention times (See Appendix 1) due to depositing of dissolved salts and involatile organic matter in the gas chromatographic column. As the retention time is a key parameter in the correct identification of compounds and it is necessary here to eliminate the reporting of 'false positives', this technique was considered inadvisable if contaminated waters were to be analysed successfully.

Multidimensional chromatography is a technique, which provides the capability of separating complex mixtures by transferring a defined fraction from one chromatographic column to another. However this requires two gas chromatographs and would only be considered if no column adequately separated MTBE from any interfering petroleum components. See below.

Purge and Trap (dynamic headspace) although the most commonly used technique for MTBE determination and having the highest sensitivity, it is only suitable for clean water samples. With contaminated or dirty samples the system can give rise to cross contamination of samples as it employs glassware common to all samples and with high levels of compound concentration can show 'memory effects' from adsorption on the trap material. This is most evident with compounds containing 'active groups or atoms' such as the oxygen atom in the ethers. Thus the system requires considerable maintenance and numerous blank injections to control these effects when analysing dirty samples.

Headspace (static) although not as sensitive as Purge and Trap, has the advantage of completely separate glassware and no trapping material. With a chemically deactivated introduction path to the gas chromatograph, it rarely shows evidence of any 'memory' effect. Its main drawback is that the sample matrix can influence quantitative results although procedures exist to control this effect.

Solid phase micro-extraction (SPME) is a relatively new technique and requires no solvent or complicated apparatus. Compounds are adsorbed from water using a polymer coated fused silica fibre immersed either in the water or the headspace above it. By varying the type of polymer, it is possible to selectively remove compounds from other compounds present in the water and concentrate only the compounds of interest. The compounds are then desorbed from the fibre by direct exposure to a heated gas chromatographic injector port. The main disadvantage is lower sensitivity when compared to the other techniques as the compounds are only partially extracted (low percentage) by the fibre.

3.2 Quantification and identification

In environmental analysis, determination of concentration is always bound to the requirement that the compound being measured is actually the one of interest. As the concentration falls, confirmation of identity becomes increasingly difficult due to the increased matrix background (natural and man made chemicals already present in the sample). With the known volatility of the ether compounds, there is currently only one proven separation technique, Gas Chromatography, which can fulfil both requirements.

This with the molecular structure of the ethers defines detector choice as one of the following:

- photoionisation
- flame ionisation
- mass spectrometry
- atomic emission

The flame ionisation detector although the cheapest, suffers from poor selectivity (i.e. responds to all compounds containing carbon) and low sensitivity. Photoionisation used in EPA method 8021B cannot distinguish known petroleum interferants (2- and 3- methyl pentanes) from MTBE and would undoubtedly give 'false positives'. Mass spectrometry is now widely used but its suitability can be dependent on how the individual compound molecule fragments. Atomic Emission detection can selectively determine compounds containing oxygen.

3.3 Choice of technique

The final method should provide:

- Positive confirmation of MTBE identity.
- Separation from petroleum constituents which could provide false positives.
- Detection of ethers at or below 1μg l^{-1} of water for clean samples.
- Accurate results of defined precision and accuracy.
- Evidence of satisfactory method performance

In practice, no one analytical technique is entirely suitable for a particular compound and the final choice is usually based on a number of factors. Other factors such as availability, reliability, cost, past experience all inevitably contribute to final technique selection.
For reasons discussed later it was necessary to employ two instrumental systems for this analysis, one for screening and quantification with the other providing positive confirmation of compound identity.

SPME was chosen for its simplicity and ion trap mass spectrometry for its ability to separate the compound from its matrix. For quantification, gas chromatography coupled with mass spectrometer was chosen as the preferred instrumentation since the atomic emission detector was fully occupied with other work.

From past experience and recent improvements in sensitivity, the headspace technique was chosen instead of purge and trap. This enabled a consistent approach to the analysis of all samples irrespective of their nature rather than the purge and trap technique, which probably would be unsuitable for the more dirty samples. Its main advantage, separate glassware, was considered vital if some of the samples proved to be highly contaminated.

4. Method set-up and optimisation

4.1 Identity

Positive identity using GC-MS depends on two parameters, retention time and mass spectrum. The mass spectrum for MTBE and other ethers are shown in Appendix 2. None of the spectra show molecular ions and this fragility of the molecule to electron bombardment results in mass spectra composed mainly of small mass ions, many of which will be common to other compounds. Below a mass of 50, common gases e.g. argon (40) and carbon dioxide (44) are found and many compounds fragment to give ions in this region. Thus the mass spectra of these ethers provide poor fingerprints for identification.

Of the major ions, mass 57 is common to alkanes, the principal constituents of petrol. Only the 73 mass ion is considered suitable as an indicator of the presence of MTBE although reliance on a single ion can cause problems when quantifying in the presence of interference. The other ethers possess at least two ions of mass above 50, useful for confirmatory purposes.

To overcome the single ion confirmation of MTBE, a second analytical system GC-MS/MS based on ion trap technology was employed (Appendix 2: Mass Spectrometry)

In view of the obvious limitations of the mass spectrometry approach, more emphasis was now placed on improving the retention time contribution to the final identity confirmation.

4.2 Interferences

Reference petrols of varying weathering characteristics and octane ratings were obtained and analysed by the selected technique. The retention time of MTBE on the standard environmental column used for solvent analysis and BTEX was found coincident with background alkane peaks.

A specific petroleum column of longer length (100metres) and higher resolution than the standard column was installed in the GC-MS/MS system to enable separation of MTBE from

the petroleum background. Reanalysis on this system now showed clearly those petrols having no detectable MTBE whereas the standard column had indicated its presence in all.

3-methyl pentane, a known interferent when using photoionisation detection, co-eluted with MTBE on the standard column and also contained a minute 73 mass ion in its spectrum. This was confirmed in the instrument spectrum but not by the NIST library mass spectrum. Analysis of blanks gave no detectable peaks for MTBE indicating that carry-over or instrumental memory was not the source.

From this analysis it was possible to estimate that petrol present at 50mg l^{-1} water could indicate a false positive of approximately 10µg l^{-1} MTBE when co-elution with petroleum peaks occurred.

Although the petroleum column separated 3-methyl pentane from MTBE and thus removed the source of the interference, 2-methyl pentane now co-eluted with isopropyl ether. However this compound contains mass ions 59 and 87 which are not present in the alkane (Appendix 3).

Installation of this column in the system used for quantification now showed the absence of MTBE in the petrols in agreement with the GC-MS/MS system. The longer petroleum column (100metres) when compared to the standard column of 30 metres, improved the robustness of the method for identifying a chromatographic peak as MTBE.

4.3 Detection limit

To improve the limit of detection the method was optimised and then calibrated over a low-level range from 0-12.5µg l^{-1} ether designed specifically for clean samples. A higher range method from 10–250µg l^{-1} ether was also set-up for the dirtier samples. Initial work indicated a limit of detection of at least 1µg l^{-1}.

Blank determinations always indicated no MTBE concentration, reflecting the cleanliness of headspace analysis.

When identifying MTBE by GC-MS/MS, the limit of detection was estimated at 25µg l^{-1} for clean samples (i.e. containing no petrol). See Chromatograms in Appendix 8.1

4.4 Accuracy of results

Initial work, checking the ability of the method to accurately quantify the ethers in environmental samples over the concentration range of 10 - 250µg l^{-1}, showed a significant bias. See Appendix 3, Tables A3.1–A3.7 Normal standard addition (i.e. spiking samples with known concentrations) to eliminate the bias gave stepped graphs rather than a straight line necessary for accurate quantification.

Although bias effects are known to occur in headspace analysis, correction is usually by addition of an ionic salt (usually sodium sulphate) to the sample vial. This procedure has been validated for compounds having limited solubility in water (low grams per litre or less) such as BTEX compounds and hydrocarbons. Although the oxygenates have similar boiling points (and thus volatility) to the above compounds, they have higher solubility in water (viz. MTBE 43 grams per litre at 25°C, Environment Agency Report 1999).

In conclusion, the effect could therefore be a result of:

1. The higher solubility of the ethers in water increasing the sample matrix effect.
2. Formation of hydrogen bonds between the ethers and water. As headspace analysis depends on equilibrium achieved by weak molecular forces (Van der Waals), the stronger hydrogen bonds disrupt this process.

Practically, three ways of minimising the sample matrix effect were employed:

1. The internal standard was replaced by deuteurated MTBE matching the behaviour of the MTBE itself.
2. The effect of the water matrix on the headspace was minimised by removing the ionic salt. This reduced the sensitivity of the method but ensured that the headspace was not overloaded by sample components, which were less soluble in water than the ethers.
3. The headspace was standardised (opposite to normal headspace analysis) by adding ether free petrol at a concentration of 0.5mg l^{-1} water.

After undertaking these steps, the spiking exercise was repeated over the lower concentration range, 0-12.5µg l^{-1}, covering the maximum admissible concentration limit for dissolved or emulsified hydrocarbons of 10µg l^{-1}.

The results (Appendix 3 Tables A3.8- A3.10) showed a considerable improvement in bias except when the concentration approached the limit of detection of the method. Overall, the low-level method was now considered suitable for extensive performance testing.

For occasions where the solubility limit of the oxygenates in water is exceeded to form an upper organic layer or where neat oil is present, separate analysis of both layers is necessary to derive the total concentration in the sample.

4.5 Method details

4.5.1 Sample extraction

4.5.1.1 Headspace

10 ml spiked with 0.5mg l^{-1} oxygenate free petrol (QMx Cat.No YA030023)

Zone Temperatures °C	Vial 70	
	Loop 125	
	Transfer Line 125	
Event Times (minutes)	GC Cycle Time 45	
	Vial Equilbrium 20	
	Pressurisation 0	
	Loop Fill 0.1	Loop Volume 1ml
	Loop Equilibrium 0.15	
	Inject 0.5	
Vial Parameters	Shake High	
	Shake High 5 minutes	

4.5.1.2 Solid Phase Micro Extraction (SPME)

Fibre Assembly 75µm Carboxen-Polydimethylsiloxane (PDMS), 24 gauge (Supelco Cat. No 57319)

Headspace Mode

Absorption time (minutes) with vibration	10.0 minutes
Desorption time (minutes) at 220°C	0.4 minutes

4.5.2 Gas Chromatography

4.5.2.1 Quantitative

Gas Chromatograph	Agilent 6890
Column	100metre
	0.25mm id
	0.5µm Petrocol DH Octyl Fused Silica Capillary
(See Mass Spectrometer for oxygenate retention times)	
Carrier Gas	Helium
Column Pressure	25psi
Pressure Mode	Constant Flow
Injector	Volatiles Inlet
Injector Temperature (°C)	200
Injector Split Ratio	10:1
Oven Initial Temperature (°C)	40
Initial Time (minutes)	4
Initial Oven Rate (°C /minute)	6
Oven Final Temperature (°C)	200
Final Time (minutes)	4.33
Run Time (minutes)	35

4.5.2.2 Qualitative

Gas Chromatograph	Varian 3400
Column	100metre
	0.25mm id
	0.5µm Petrocol DH Octyl Fused Silica Capillary
Carrier Gas	Helium
Column Pressure	40psi
Injector	1078 Splitless Mode
Injector Temperature (°C)	220
Oven Initial Temperature (°C)	35
Initial Time (minutes)	1.0
Oven Rate (°C /minute)	10.0
Final Oven Temperature (°C)	200
Final Time (minutes)	17.5
Run Time (minutes)	35.0

4.5.3 Mass Spectrometer

4.5.3.1 Quantitative

Mass Spectrometer	Agilent 5973 *Quadrupole*		
Mode of Operation	Selected Ion Monitoring (SIM)		
Ionisation Mode	Electron Impact (EI)		
SIM Ions	Target	Qualifier	Relative Retention Time
Methyl Tertiary Butyl Ether	73.0	41.1	14.74 minutes = 1.003
Deuterated Methyl Tertiary Butyl Ether	76.1	41.1	14.70 minutes = 1.000
Di-Isopropyl Ether	87.0	45.0	15.52 minutes = 1.056
Ethyl Tertiary Butyl Ether	59.0	87.0	16.16 minutes = 1.099
Tertiary Amyl Methyl Ether	73.0	43.0	18.33 minutes = 1.247

4.5.3.2 Qualitative

Mass Spectrometer	Varian Saturn 2000 Ion Trap
Mode of Operation	MS/MS
Ionisation Mode	Electron Impact (EI) AGC
Parent Ion	73
Waveform Type	Resonance

4.5.4 Method Ranges

Low Level	0.2 – 12.5 micrograms per litre per oxygenate
High Level	10 – 250 micrograms per litre per oxygenate

Both methods operated under the same instrumental conditions but with different concentration standards.

5. Assessment of method performance

The standard used to evaluate the performance of analytical methods is based on the WRC NS30 Quality Control Manual (Cheeseman and Wilson, 1989). This provides a structured analytical testing procedure for establishing the accuracy of analytical data and the magnitude of errors associated with the method results.

The test comprises the analysis in duplicate of the following samples:

- A blank
- Low Calibration standard
- High Calibration standard
- A real sample
- The sample in (4) spiked with a known concentration of the analytical compounds.

The above comprise one batch. For meaningful data, it is necessary to undertake between 10 and 15 batches to achieve the required 10 degrees of freedom (D.O.F. Appendix 4). The results for the headspace methodology described above are given in Appendix 4. Method details are given again in Appendix 5. A summary of the results is tabulated below.

Ether	%Recovery from Water	Limit of Detection µg l^{-1}	%Bias at 10 µg l^{-1}	%RSD**
Methyl tert. butyl	97.3±4.4	0.2	1.4	10
Di-Isopropyl	115.9±6.3	0.5	9.4	12
Ethyl tert. butyl	110.0±6.7	0.3	6.8	12
Tert.amyl methyl	103.0±5.7	0.3	3.5	10

** Relative Standard Deviation

To further assess method accuracy, external certified standards were purchased, MTBE in petroleum, and a mixture of ethers and alcohols.

Duplicate Analysis gave the following results (micrograms per litre water):

Ether	Supplier: Restek Cat.No 30237		Supplier: Chemservice Cat.No OG4815-1M	
	Cert.Result	Headspace	Cert.Result	Headspace
Methyl tert. butyl	10.6±0.5	10.9	15	13.9
Di-Isopropyl	N/a	N/a	13	11.8
Ethyl tert. butyl	N/a	N/a	15	14.0
Tert.Amyl methyl	N/a	N/a	15	14.3

N/a – not applicable

6. Application to contaminated samples

Samples containing neat petrol represent a considerable analytical challenge as the detector response to the petroleum background is greatly increased.

The GC-MS *quadrupole* ion chromatograms of such a sample are given in Appendix 7.

Assessment of the water layer chromatograms show:

- Although the MTBE is present at approximately 600µg l^{-1} in the water layer, peak size is small when compared to the petroleum hydrocarbon peaks. Separation from these peaks is therefore crucial which the current gas chromatographic column achieves. Appendix 7.1.1
- The MTBE ion chromatogram (Appendix 7.1.2) clearly shows both ions, 73 (target or quantitative) and 41(qualifier) strongly indicating its presence.
- The DIPE ion chromatogram (Appendix 7.1.3) shows both target and qualifier ions indicating its presence in the water layer, but reference to the standard (Appendix 6.2.3) shows the target ion is enhanced by the petroleum background.
- The ETBE ion chromatogram (Appendix 7.1.4) shows only a small target ion and no qualifier within a retention time window of ±0.04 minutes, indicating a limit below which this oxygenate can be measured in the presence of high concentrations of petrol.
- The TAME ion chromatogram (Appendix 7.1.5) shows a large target ion (73) within a retention time window of ±0.03 minutes but no qualifier. The peak at 18.27 minutes can be ignored due to its separation of 0.09 minutes from the target ion (expected ±0.01minutes). As the target ion of 73 is the same as MTBE, this shows the necessity of separating this compound from the petroleum background for accurate measurement.

Assessment of the petrol layer chromatograms show:

- The MTBE is present at approximately 10μg l^{-1} in the petrol layer. If originally present in the oil, this concentration would be expected to be significantly higher and indicates the compound's preference for the water layer. The low value indicates the absence of any significant petroleum interference in its measurement. (Appendix 7.2.2)
- Again the DIPE analysis is inconclusive due to a discrepancy in the target/qualifier ratio. (Appendix 7.2.2)
- The ETBE ion chromatogram (Appendix 7.2.3) indicates it's absence but the peak at 16.02 could be misinterpreted as this compound. This emphasises the importance of retention time stability; a fact confirmed by the maximum shift observed for the deuterated internal standard for this analysis being only 0.01minutes. This is typical of headspace analysis which is unaffected by the type of sample analysed.
- The TAME ion chromatogram (Appendix 7.2.4) is virtually identical with the water layer.

Overall, while the methyl tertiary butyl can be estimated and confirmed with a degree of certainty, the other oxygenates are affected by the petroleum background in contaminated water such that conclusions on their presence or absence is difficult at low concentration levels. This analysis shows the limitation of the GC-MS technique in the presence of petrol.

Confirmation by the GC-MS/MS technique is limited by the excessive petroleum background in the water layer, resulting in the ion trap detector discarding the majority of the 73 ion with the other hydrocarbon ions (See Appendix 1.2 - Mass Spectrometer). This has a detrimental effect on the sensitivity of this technique as shown for a clean sample containing MTBE when petrol is added. These chromatograms and the sample MS/MS spectra in comparison with an MTBE standard are shown in Appendix 8.2.

7. Conclusions

1. A method has been developed to accurately quantify methyl tertiary butyl ether and other oxygenates in water to sub microgram per litre concentrations.

2. The method is suitable for samples containing up to 0.5mg l^{-1} petrol and can provide additional confirmatory evidence on the presence of methyl tertiary butyl ether.

3. The *quadrupole* GC-MS method can also be used for samples containing higher levels of petrol but oxygenates other than MTBE are difficult to quantify and confirm due to increased hydrocarbon background.

4. Further work and equipment would be necessary to improve the GC-MS/MS technique to provide more positive confirmation for contaminated samples.

8. References

Cheeseman, R.V., Wilson, A.L. (1989) *A Manual on Analytical Quality Control for the Water Industry*, Revised by Gardner, M.J. Water Research Centre report no NS 30.

Church, C.D., Isabelle, L.M., Pankow, J.F., Rose, D.L., Tratnyek, P.G. (1997) Method for the determination of methyl tert-butyl ether and its degradation products in water. *Environmental-Science-and-Technology*, **31(12)**: 3723-3726.

Environment Agency, 1999. The fuel additive MTBE – a groundwater protection issue? National Groundwater and Contaminated Land Centre, Solihull.

Frysinger, G.S., Gaines, R.B., (2000) Determination of oxygenates in gasoline by GC x GC, *Journal-of-High-Resolution Chroamtography,* **23(3): 197-201.**

Guchardi, R., da-Costa-Filho, P.A., Poppi, R.J., Pasquini, C., (1998) Determination of ethanol and methyl t-butyl ether (MTBE) in gasoline by NIR-AOTF-based spectroscopy and multiple linear regression with variables selected by genetic algorithm. *Journal-Near-Infrared-Spectroscopy,* **6(1-4)**: 333-339.

Lopez-Avila, V., Benedicto, J., Prest, Bauer, S., (1999) Direct Analysis of MTBE in water using automated membrane-introduction mass spectrometry. *Spectroscopy*, **14(6)**, 36, 38-41.

Nouri, B., Fouillet, B., Toussaint, G., Chambon, R., Chambon, P., (1996) Complementarity of purge-and-trap and headspace capillary gas-chromatography methods for the determination of methyl-t-butyl ether in water. *Journal-of-Chromatography,-A*, **726(1-2)**: 153-159.

9. Appendices

Appendix 1 Chromatography basics

1.1 Retention Time

The heart of the gas chromatograph is the capillary column, which enables separation of compound mixtures. The column is a glass tube of precise narrow diameter, the inner wall upon which is bonded a high boiling liquid usually polymeric in nature. A gas (known as the carrier, usually helium or hydrogen) flows through the column at a known and fixed rate. The column is contained within the gas chromatograph oven, which can be heated precisely at known and variable temperatures rates (temperature programming).
In environmental analysis, the compounds are first removed (extracted) from the sample matrix (usually water) into a solvent (e.g. hexane). A small volume of this solvent extract is placed at the top of the column using a syringe (injection). Headspace introduction replaces the solvent extract with a precise volume of air containing the vaporised compounds.
The data system (computer) starts measuring time elapsed at the moment of injection.
The compound moves through the column depending on its attraction to the gas or the liquid. If more compatible with the liquid, the compound remains longer in the column than a compound, which is predominately in the gas. Transfer between gas and liquid occurs numerous times (equilibrium) such that the compound travels down the column as a band with the highest concentration at the centre and the lowest at the band boundaries.
The temperature program, the nature of the liquid and the flow rate of carrier gas all determine the speed of the compound through the column.
This is illustrated on Page 13.

After leaving the column, the compound passes to the detector (e.g. mass spectrometer, photoionisation, flame ionisation). As the compound band enters the detector, the latter produces an electrical signal, which corresponds to the concentration level of the band at any particular instance.
This is translated by the data system as a change in the background signal of the detector (baseline); being first of increasing magnitude rising to a peak and then decreasing as the band concentration falls. The data system records the time at the top (apex) of the peak and assigns the peak a RETENTION TIME, being the time taken from injection to the detector recording the peak apex (usually expressed in minutes). *This is illustrated on Page 14.*

The area or height of this peak is a direct measure of the amount of compound injected into the column and forms the basis of quantitative measurement in chromatography. The ratio of area or height to amount is known as the 'response factor' and varies according to the compound and the detector employed. As compounds emerge from the column at different times they can be separated by adjustment of the three variables discussed above. If the latter are strictly controlled then the Retention Time is extremely reproducible for that particular compound and can be used as a means of identification. However this time is not entirely unique and certain other compounds may have a very similar or identical Retention Time (false positives). Then additional information is required (e.g. gc-ms analysis) to eliminate other possibilities.

1.2 Mass Spectrometer

As the compound enters the mass spectrometer high-energy electrons bombard it. Fragile objects such as china will break into many small fragments while rigid objects such as granite will remain intact losing only a few fragments.

The mass fragments pass through a filter (*quadrupole*), which determines their actual mass. The mass spectrometer starts at a low mass e.g. 50 then successively increments the filter by unit mass to a high mass e.g. 650 before repeating the cycle (scan). Each time the number of fragments associated with a

particular mass are measured and totalled (abundance). As the compound emerges from the column over a time period due (one second is a long time for a mass spectrometer), several scans from 50 to 650 can be performed before the compound exits the mass spectrometer. One such scan is called a 'mass spectrum'.
The mass spectrums of the ethers are shown on Pages 15 to 21 inc.
A schematic of the above process is on Page 14.

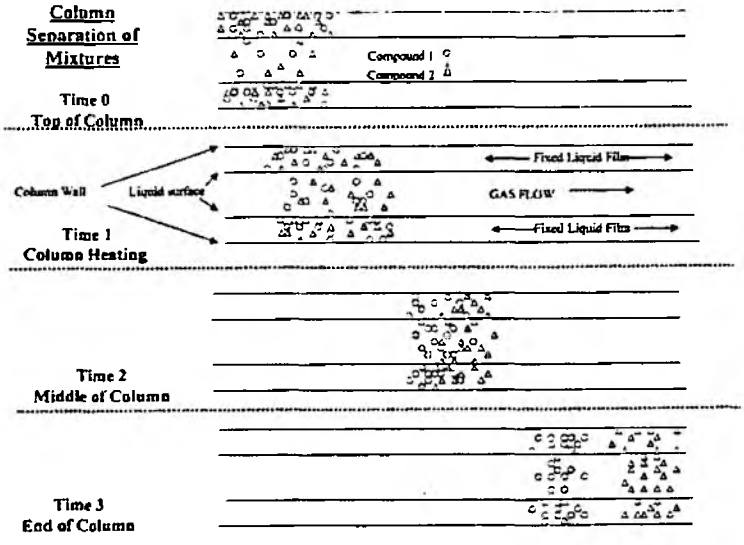
Thus mass chromatographic peaks are in reality summation of individual mass spectrums.
This mode of operation is termed 'mass scan'.

In 'Selected Ion Monitoring' (SIM) mode, the mass spectrometer scans at fixed masses chosen by the user which are relevant to the compounds being measured. Thus a 'scan cycle' is much shorter than when undertaking a full mass scan and results in increased detector signal, enabling higher sensitivity to be achieved for the compound. The ion used for quantification is termed the 'target' while others ions selected to confirm compound identity are termed 'qualifiers'.

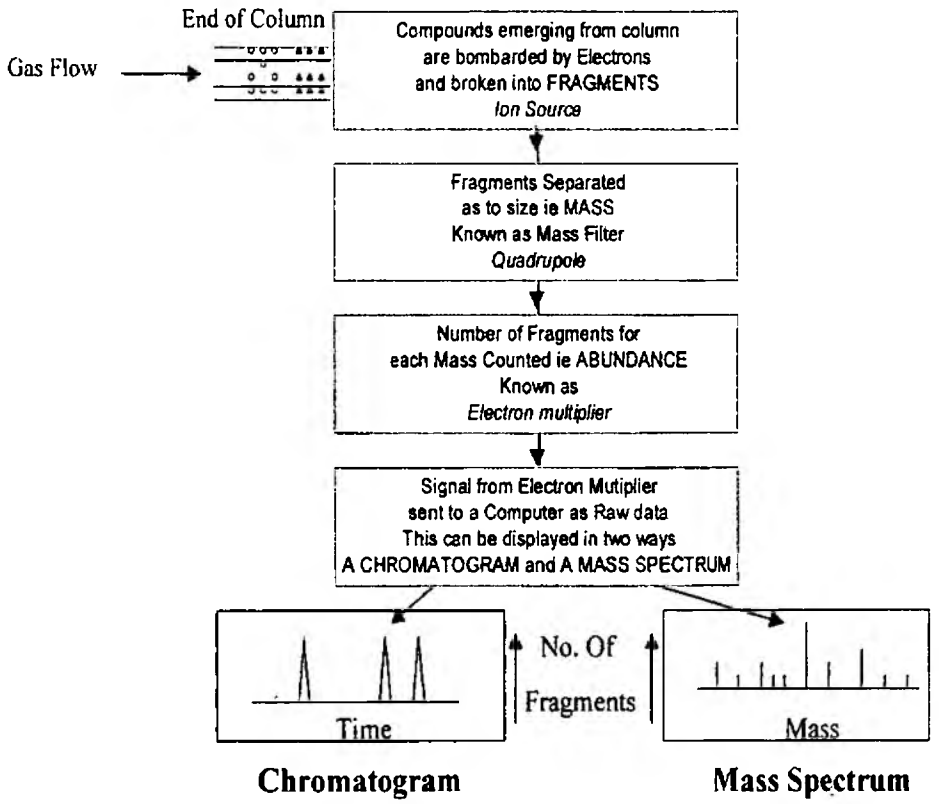
In the real world, compounds such as phenols, which contain a ringed structure, are the 'granite' while compounds such as MTBE represent the 'china'. The molecular weight of MTBE is 88 and the presence of an ion in the spectrum at this mass would be termed its 'molecular ion'.
'Granite' compounds show this ion whereas china compounds often don't. The mass spectrum of MTBE has no ion at mass 88 indicating the fragility of the molecule.

The molecular ion is extremely useful in identifying a compound and its absence in the MTBE spectrum, which also comprises mainly low mass ions, provides limited positive confirmation.

In GC-MS/MS, a suitable mass ion (73 for MTBE) is held in the ion trap while all other ions (e.g. Petroleum) are ejected. The remaining 73 ions are then bombarded by helium molecules (carrier gas) at a predefined voltage which fragments them in a controlled manner to yield a further mass spectrum. The MTBE ion fragments further both by loss of oxygen (16) or water (18) to give 57 and 55 mass ions. The ratios of these three ions and even whether they appear in the spectrum can be tailored by adjustment of the ionisation voltage. See Appendix 2.1.2 for the structural fragmentation of MTBE to give these three ions and Appendix 8.3 for an actual experimental MS/MS spectrum showing these three ions. Thus it is possible to generate a 'fingerprint' type spectrum to confirm the identity of MTBE in a sample by matching with that of a known MTBE standard obtained under identical experimental conditions.



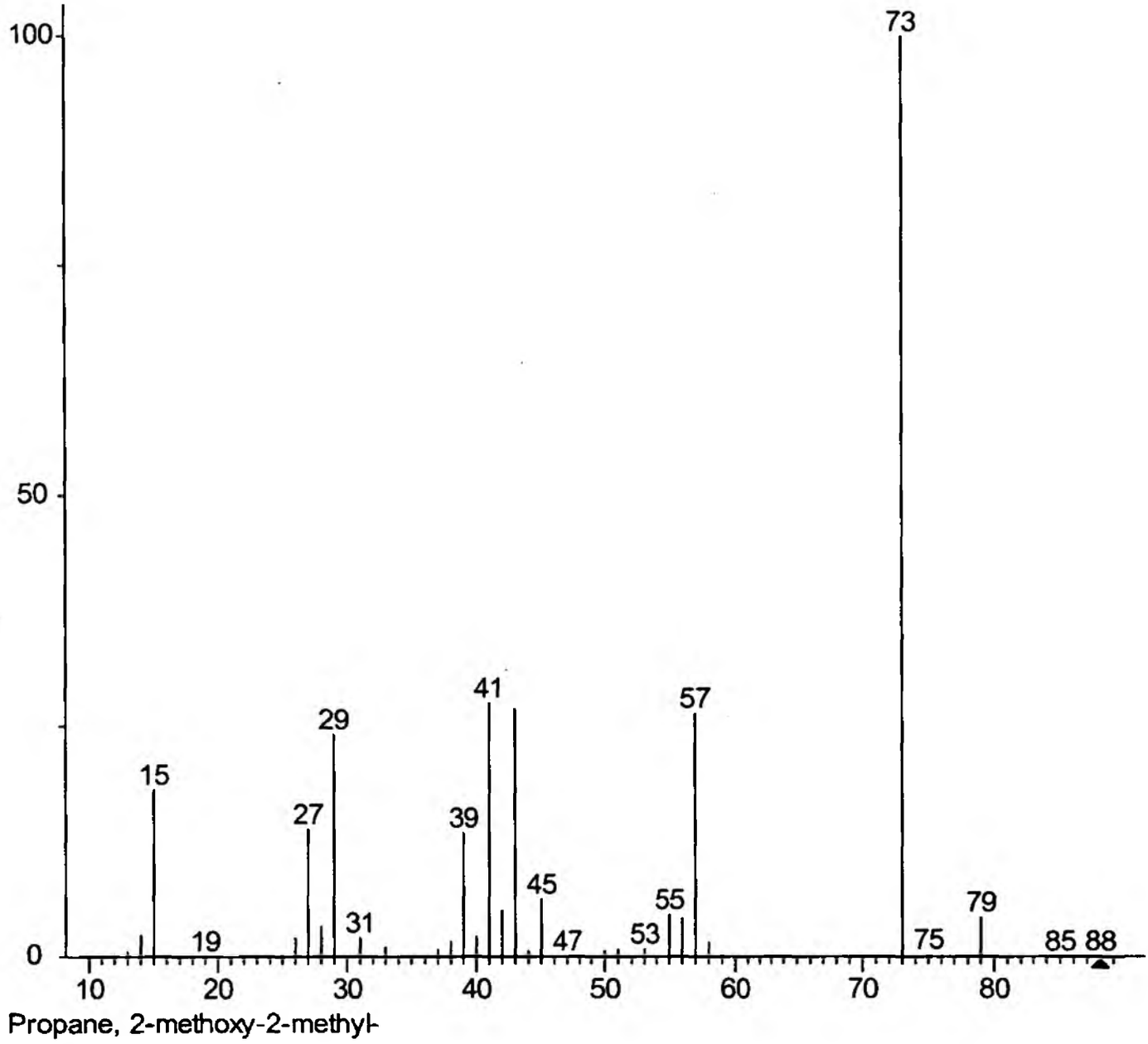
Mass Spectrometer Basics



Appendix 2 Mass spectra

2.1.1 Methyl tert. Butyl Ether CAS No1634-04-04 NIST 98 MS Library

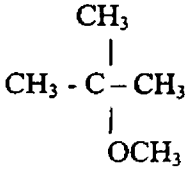
(a) Spectrum



2.1.2 Structure Propane, 2-Methoxy, 2-Methyl

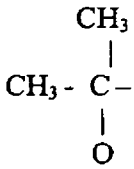
Starting with Propane viz. $CH_3 - CH_2 - CH_3$

Substitute methoxy and methyl groups at middle carbon atom (C) i.e. position 2 gives

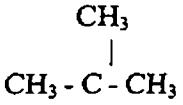


methyl tertiary butyl ether which loses CH_3 (methyl) on breakdown

(*Mass = 5 Carbons at 12 unit mass + 12 Hydrogens at 1 unit mass + 1 Oxygen at 16 unit mass*
*Therefore Molecular Weight is 5*12 + 12*1 + 1*16 =* 88)

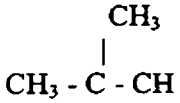
to give  fragment of mass *4*12 + 9*1 +1*16 =* 73

Which when bombarded with helium atoms in the MS/MS technique can give the following: -



By loss of oxygen, mass fragment *4*12 + 9*1* = 57

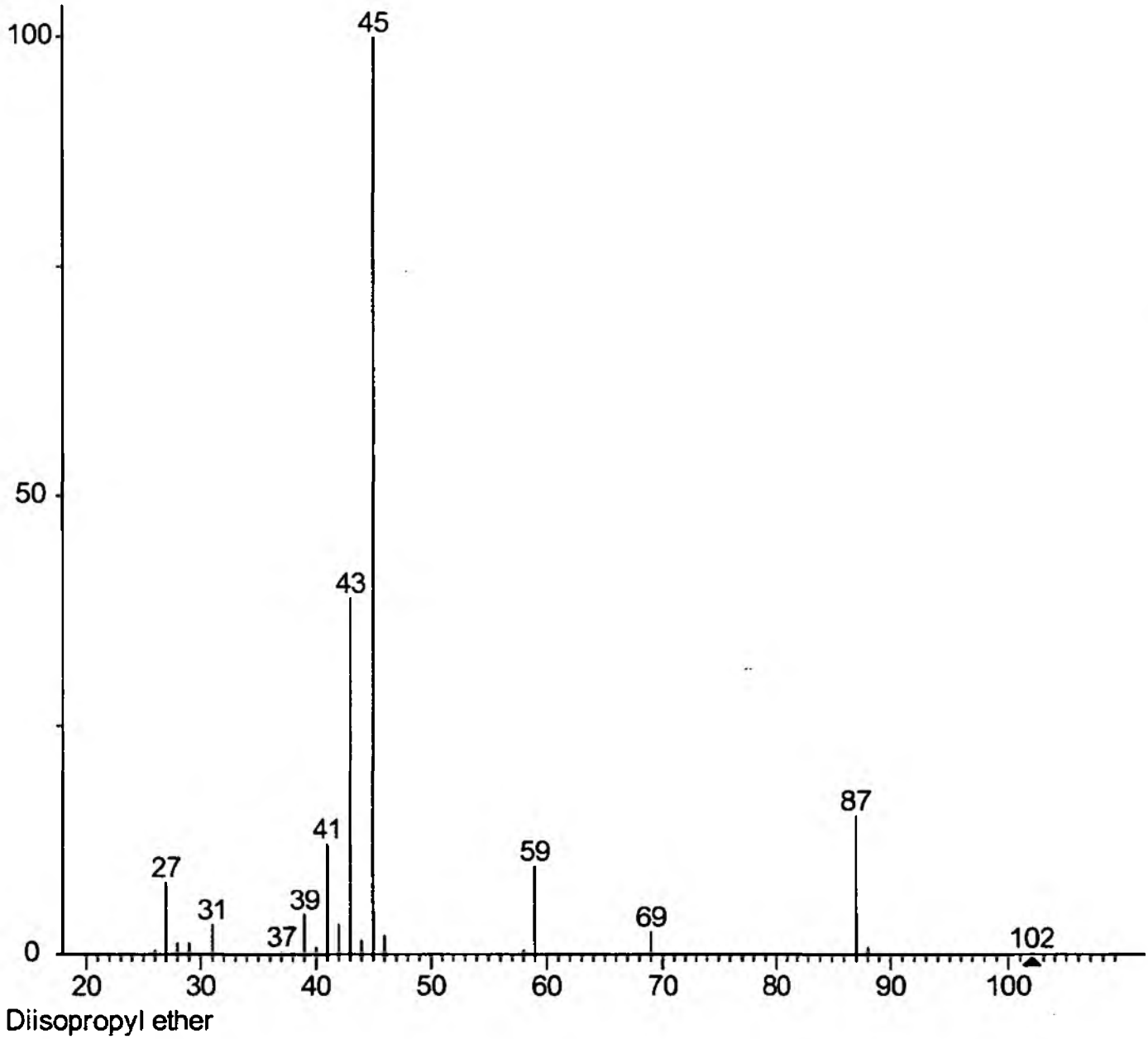
And



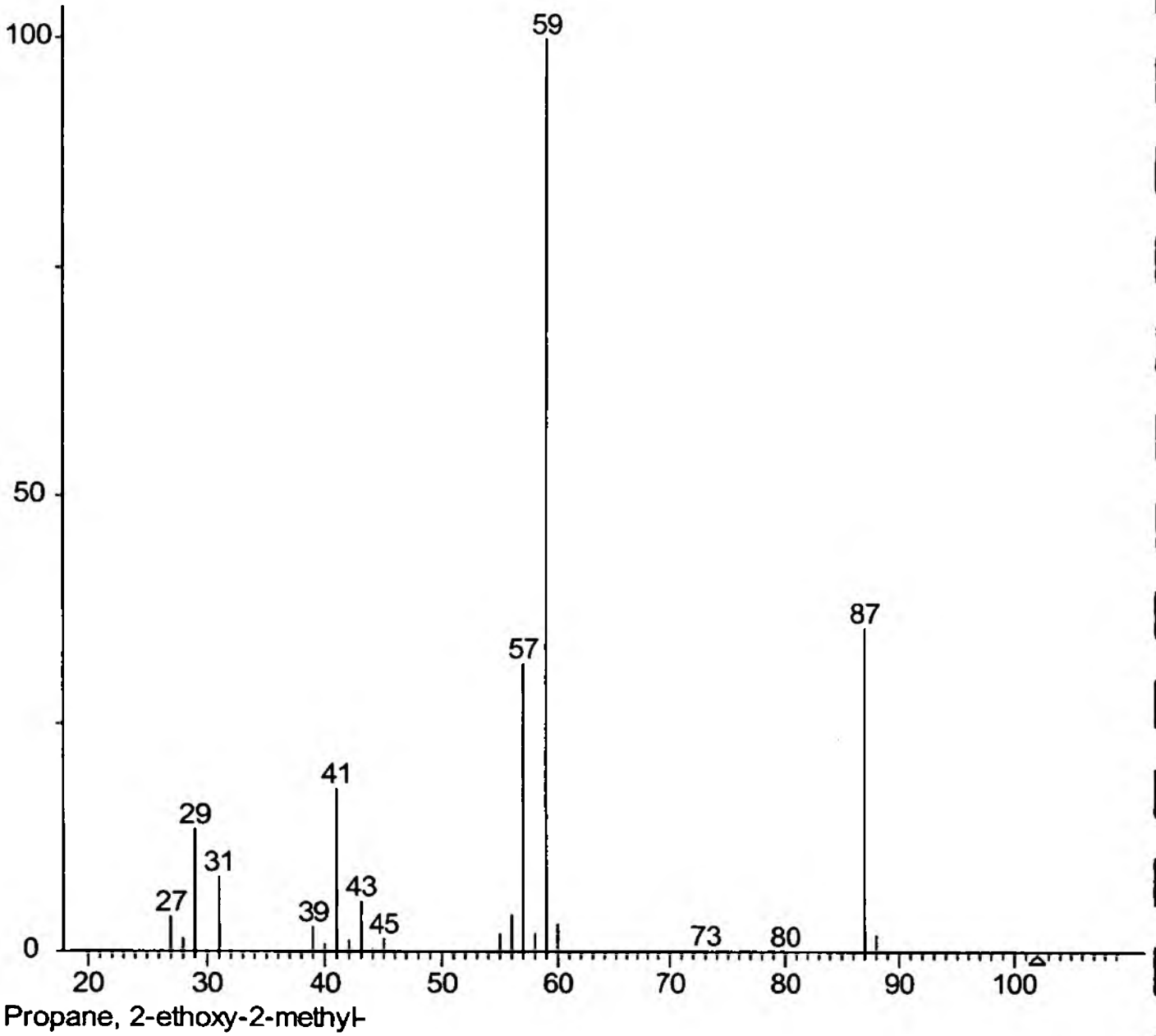
By loss of water H_2O, mass fragment *4*12 + 7*1* = 55

N.B. this is a simplified representation and is not intended to comprehensively show the actual processes.

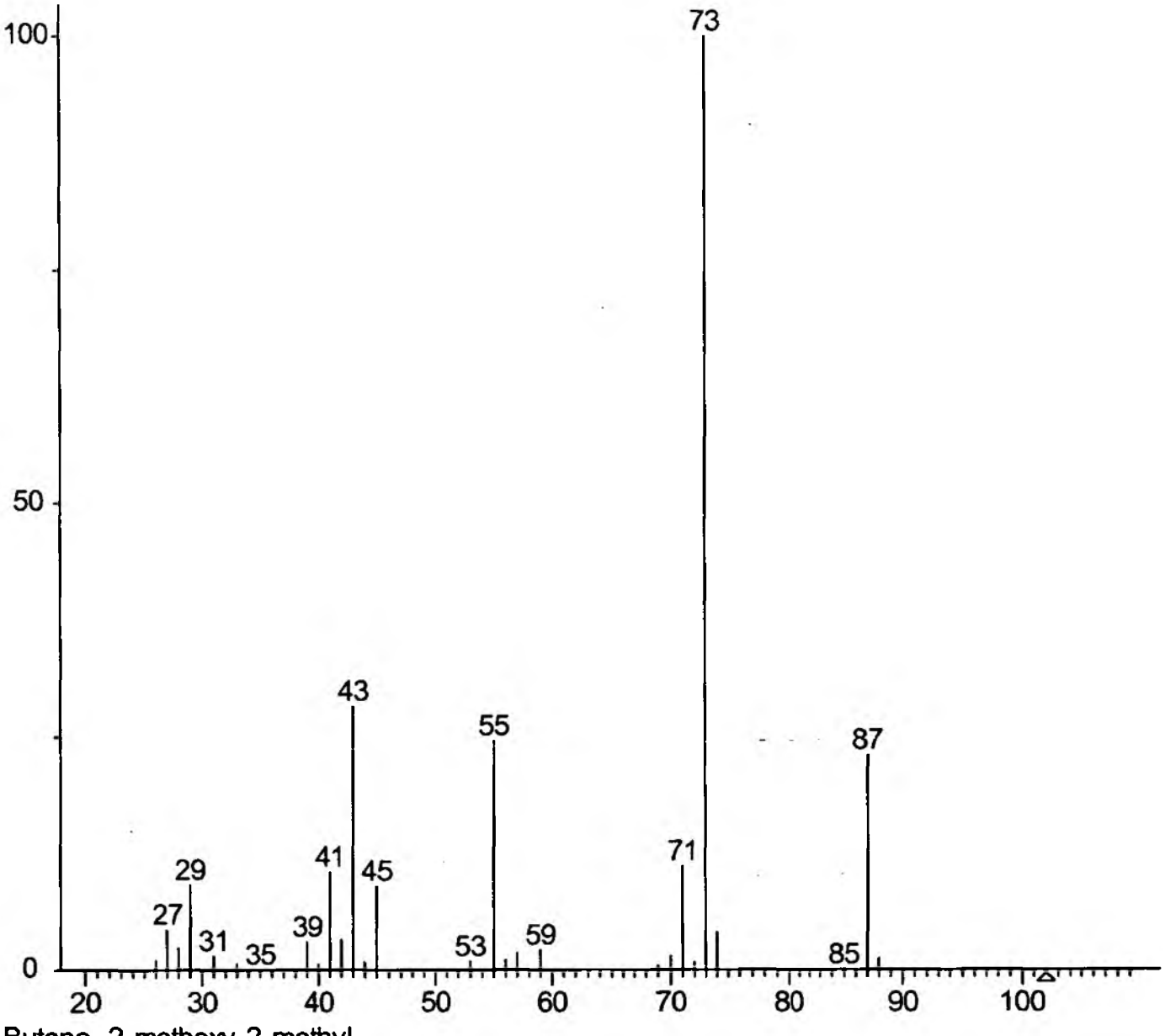
2.2 Di-isopropyl Ether CAS No 108-20-03 NIST 98 MS Library



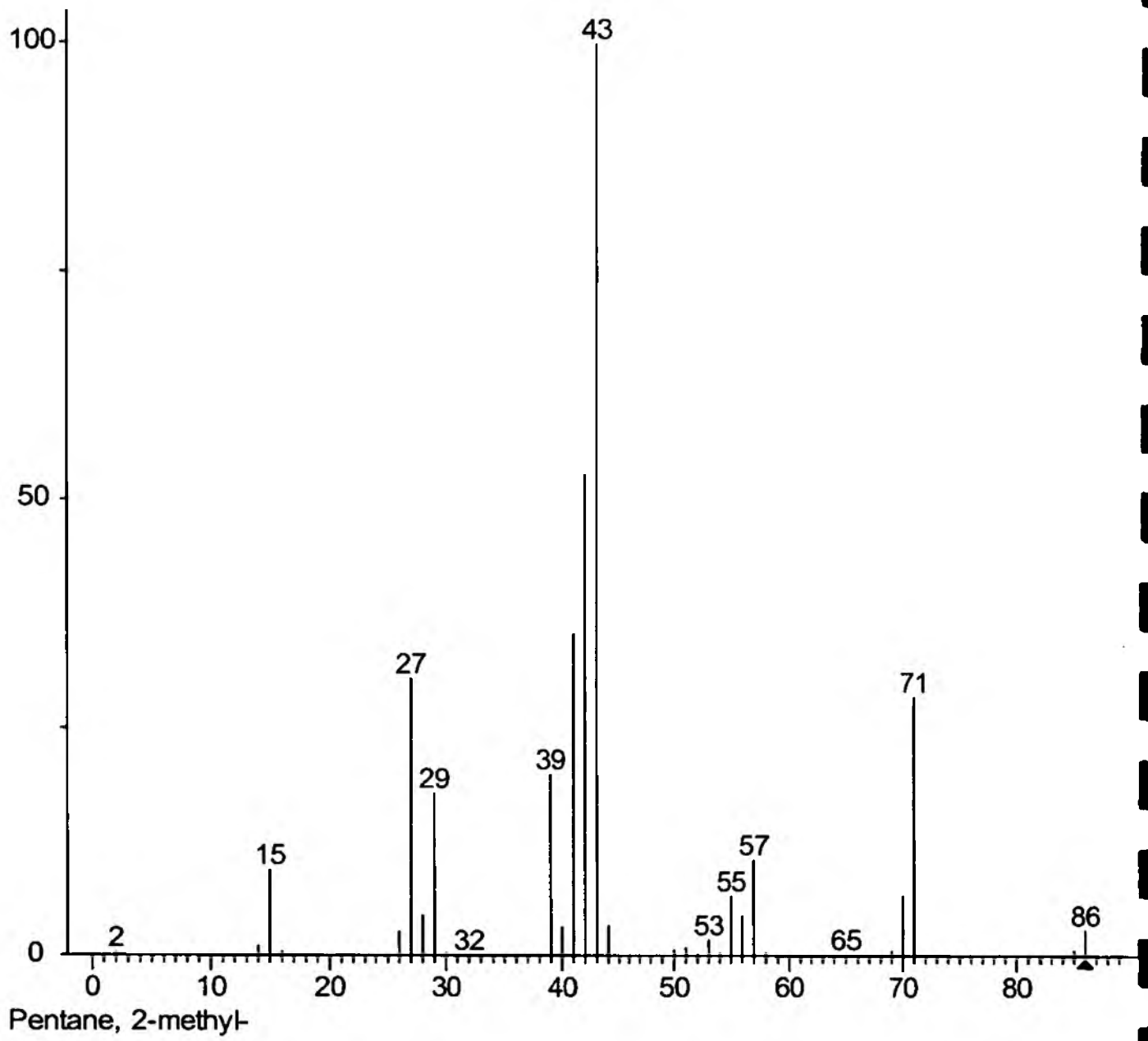
2.3 Ethyl tertiary Butyl Ether CAS No 637-92-3 NIST 98 MS Library



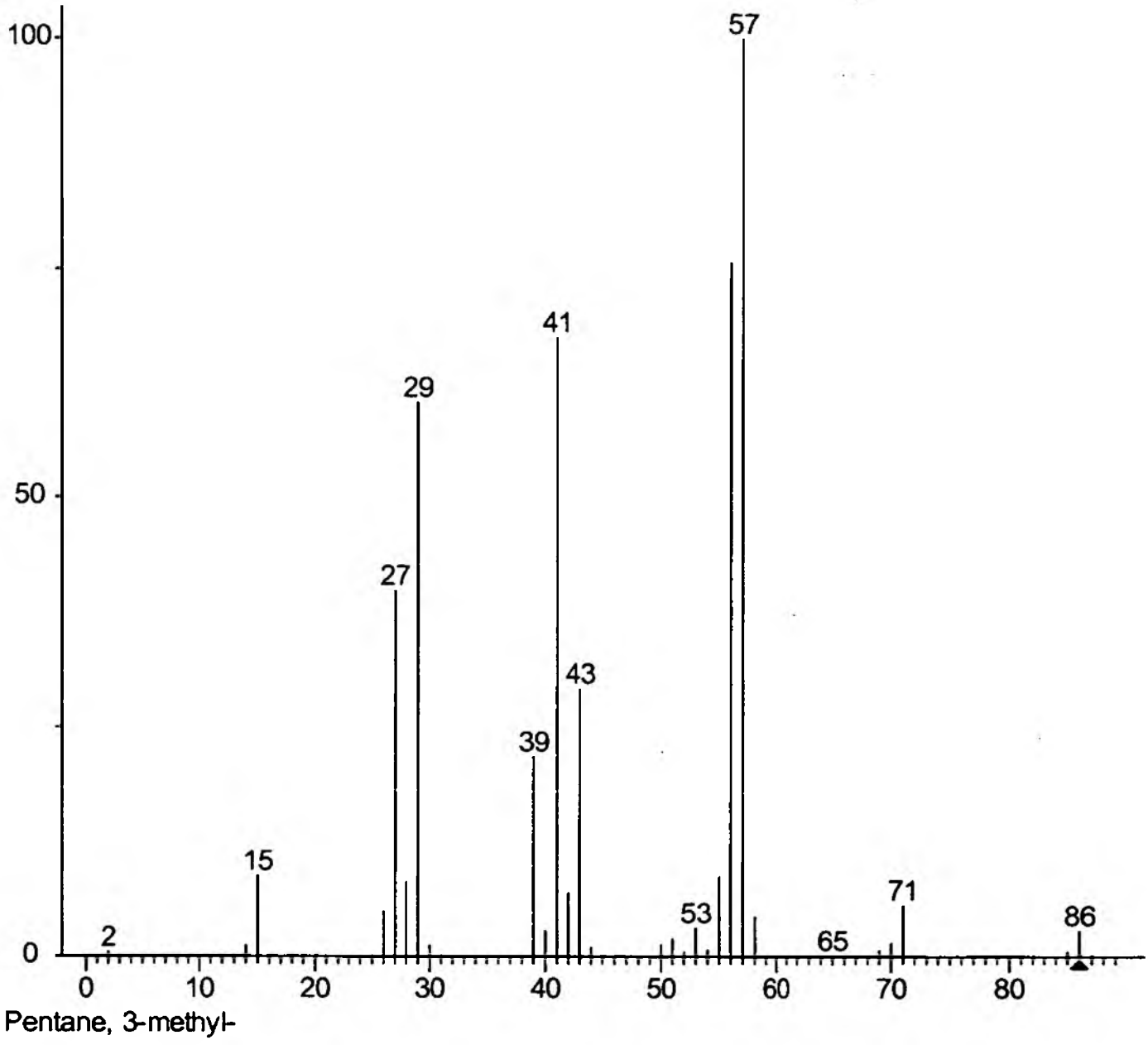
2.4 tertiary Amyl Methyl Ether CAS No 994-05-8 NIST 98 MS Library



2.5 2-methyl pentane CAS No 107-83-5 NIST 98 MS Library



2.6 3- methyl pentane CAS No 96-14-0 NIST 98 MS Library



Appendix 3 Experimental bias

Tables A3.1 to A3.7 Spiking of water sample (river) with oxygenates from 10 to 250µg l-1
Analysis: - 10ml sample with 3 grams sodium sulphate at 70°C
Deuterated 1,4 Dichlorobenzene internal standard

Table A3.1: 10 micrograms per litre

Oxygenate	Experimental Result µg l-1	Bias %
MTBE	6.9	38
DIPE	7.5	50
ETBE	7.4	48
TAME	7.8	56

Table A3.2: 25 micrograms per litre

Oxygenate	Experimental Result µg l-1	Bias %
MTBE	16.8	34
DIPE	18.0	44
ETBE	18.4	47
TAME	18.8	50

Table A3.3: 75 micrograms per litre

Oxygenate	Experimental Result µg l-1	Bias %
MTBE	46.5	21
DIPE	49.2	31
ETBE	50.8	35
TAME	50.5	35

Table A3.4: 125 micrograms per litre

Oxygenate	Experimental Result µg l-1	Bias %
MTBE	74.6	19
DIPE	80.8	29
ETBE	82.2	32
TAME	81.3	30

Table A3.5: 150 micrograms per litre

Oxygenate	Experimental Result µg l-1	Bias %
MTBE	88.3	18
DIPE	94.3	26
ETBE	96.9	29
TAME	96.7	29

Table A3.6: 200 micrograms per litre

Oxygenate	Experimental Result µg l-1	Bias %
MTBE	122.4	22
DIPE	133.2	33
ETBE	133.8	34
TAME	132.7	33

Table A3.7: 250 micrograms per litre

Oxygenate	Experimental Result µg l-1	Bias %
MTBE	153	22
DIPE	165.9	33
ETBE	167.6	34
TAME	166.4	33

Tables A3.8 to A3.10 Spiking of water sample (river) with oxygenates (2.5 to 15µg l-1)
Duplicate Analysis: - 10ml sample, 0.5mg l-1 oxygenate-free petrol at 70°C
Deuterated Methyl tert-butyl Ether internal standard

Table A3.8: 2.5 micrograms per litre

Oxygenate	Experimental Result µg l-1		Bias %	
MTBE	2.9	3.3	18	40
DIPE	3.4	3.7	35	46
ETBE	2.9	3.5	16	42
TAME	3.5	4.2	39	68

Table A3.9: 7.5 micrograms per litre

Oxygenate	Experimental Results µg l-1		Bias %	
MTBE	7.0	7.7	6	3
DIPE	8.3	8.3	11	10
ETBE	7.6	8.2	2	9
TAME	7.9	8.5	5	13

Table A3.10: 15 micrograms per litre

Oxygenate	Experimental Results µg l-1		Bias %	
MTBE	15.6	14.9	4.0	0.6
DIPE	18.6	16.7	24	12
ETBE	17.2	16.5	15	10
TAME	16.7	16.7	11	10

Performance Test (NS30) **Method:- RD3** **Compound:- Methyl tert-butyl Ether**

DataFile ptrd15.xls **Analysis:- MtBE and Oxygenates in Water**
Calc.Date 24/06/00 **ConcnUnits:-** ug/litre
(N.S. = not significant, * = significant at 0.05 level, ** = significant at 0.01 level) **Sample:-** Water:- River, UnFiltered

Results	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Batch 9	Batch 10	Batch 11	Batch 12
BatchDate	01/06/00	02/06/00	05/06/00	06/06/00	07/06/00	08/06/00	09/06/00	13/06/00	14/06/00	20/06/00	21/06/00	
Blank	0.72	0.64	0.65	0.68	0.68	0.5	0.57	0.61	0.65	0.61	0.56	
(Spiked)	0.7	0.71	0.72	0.71	0.68	0.51	0.65	0.59	0.63	0.71	0.48	
Low Calib.	2.48	2.82	2.58	2.89	3.25	2.61	2.78	2.46	2.39	2.57	2.06	
Standard	2.59	2.77	2.62	2.68	3.1	2.63	2.34	2.52	2.32	2.63	2.14	
High Calib.	10.45	10.7	9.46	11.67	10.53	9.18	9.98	10.02	9.4	10.08	9.14	
Standard	10.62	10.53	9.69	11.29	10.2	9.66	9.43	11.71	9.66	10.69	8.84	
Sample	2.78	2.78	2.48	3.04	2.5	2.57	2.48	2.58	2.58	2.49	2.23	
	2.85	2.65	2.58	3.08	3.1	2.52	2.4	2.69	2.72	2.46	2.69	
Spiked	10.54	10.4	9.44	11.45	11.73	9.23	9.5	9.9	9.41	9.46	8.62	
Sample	11.1	11.1	9.52	10.4	10.26	8.92	9.06	9.78	9.52	9.68	9.75	

Statistics	Blank	Low Standard	High Standard	Sample	Spiked Sample
Mean	0.6345	2.6014	10.1432	2.6468	9.9441
Actual	0.5000	2.5000	10.0000	2.5000	10.0000
M1	0.0096	0.1462	1.0826	0.0717	1.1703
M0	0.0016	0.0134	0.2021	0.0297	0.2598
F Value	6.0655	10.8950	5.3574	2.4176	4.5052
Signific.	**	**	**	N.S.	*
sw	0.0398	0.1159	0.4495	0.1722	0.5097
sb		0.2577	0.6635	0.1450	0.6747
st		0.2625	0.8014	0.2251	0.8456

	Low Standard	High Standard	Sample	Spiked Sample	Precision
Target st	0.2601	1.0143	0.2647	0.9944	
F 0.05	1.792	1.723	1.628	1.695	
FCalc	1.180	0.624	0.723	0.723	
D.O.F	11	13	17	14	
st OK	PASS	PASS	PASS	PASS	x%
% RSD	10.9	7.9	8.5	8.5	10
%Bias	4.1	1.4			

Limit of Detection (LOD)

Calculated	0.204	ug/litre
Required	0.2	ug/litre
(Factor 5.12)		

Recovery with 95% Confidence Limits

Mean	7.30	± 0.332	
Expected	7.50		(Spike
Percentage	97.3	± 4.4	Vol. N.S.)

Reference

M1 = between batch mean square
M0 = within batch mean square
sw = within batch standard deviation
sb = between batch standard deviation
st = total standard deviation
Target St = the greater (x% of concentration OR 1/4 LOD)
D.O.F = Degrees of Freedom
RSD = Relative standard deviation (%)

F Value = (M1/M0)
F0.05 = Table value

Performance Test (NS30) **Method:- RD3** **Compound:- Di-isopropyl Ether**

DataFile ptrd15.xls **Analysis:- MtBE and Oxygenates in Water**
Calc.Date 24/06/00 **ConcnUnits:-** ug/litre
(N.S. = not significant, * = significant at 0.05 level, ** = significant at 0.01 level) **Sample:-** Water:- River, UnFiltered

Results	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Batch 9	Batch 10	Batch 11	Batch 12
BatchDate	01/06/00	02/06/00	05/06/00	06/06/00	07/06/00	08/06/00	09/06/00	13/06/00	14/06/00	20/06/00	21/06/00	
Blank	0.62	0.7	0.77	0.81	0.68	0.48	0.52	0.77	0.65	0.55	0.49	
(Spiked)	0.75	0.97	0.84	0.75	0.82	0.63	0.63	0.71	0.69	0.69	0.46	
Low Calib.	2.68	2.87	2.7	3.14	3.81	2.78	2.4	2.73	2.43	2.61	1.94	
Standard	2.47	2.82	2.65	2.75	3.76	2.75	2.47	2.88	2.98	2.55	2.16	
High Calib.	11.02	11.75	9.64	12.45	11.92	10.33	10	11.25	10.07	11.54	9.99	
Standard	11.15	11.29	9.06	12.5	11.7	10.28	10.13	13.03	9.59	12.41	9.55	
Sample	2.79	3.02	2.75	3.54	3.1	2.98	2.79	3.04	2.76	2.62	2.46	
	2.68	2.82	2.68	3.5	3.7	2.88	2.35	3.33	2.93	2.95	2.4	
Spiked	11.85	12.45	10.31	13.31	14.57	10.98	10.4	12.22	11.22	11.44	10.13	
Sample	12.39	12.52	10.64	11.1	13.35	10.76	10.34	12.04	10.84	11.5	11.1	

Statistics	Blank	Low Standard	High Standard	Sample	Spiked Sample
Mean	0.6809	2.7423	10.9386	2.9123	11.6027
Actual	0.5000	2.5000	10.0000	2.5000	10.0000
M1	0.0261	0.3555	2.4559	0.2246	2.3599
M0	0.0081	0.0267	0.2268	0.0385	0.3731
F Value	3.2232	13.3333	10.8397	5.8277	6.3259
Signific.	*	**	**	**	**
sw	0.0900	0.1633	0.4760	0.1963	0.6108
sb		0.4055	1.0558	0.3050	0.9967
st		0.4371	1.1581	0.3627	1.1690

	Low Standard	High Standard	Sample	Spiked Sample	Precision
Target st	0.3291	1.3128	0.3495	1.3923	
F 0.05	1.792	1.792	1.723	1.723	
FCalc	1.764	0.778	1.077	0.705	
D.O.F	11	11	13	13	
st OK	PASS	PASS	PASS	PASS	x%
% RSD	15.9	10.6	12.5	10.1	12
%Bias	9.7	9.4			

Limit of Detection (LOD)
Calculated **0.461** ug/litre
Required **0.5** ug/litre
(Factor 5.12)

Recovery with 95% Confidence Limits
Mean 8.69 ± 0.47
Expected 7.50 (Spike
Percentage **115.9** **± 6.3** Vol. N.S.)

Reference
M1 = between batch mean square
M0 = within batch mean square
sw = within batch standard deviation
sb = between batch standard deviation
st = total standard deviation
Target St = the greater (x% of concentration OR 1/4 LOD)
D.O.F = Degrees of Freedom
RSD = Relative standard deviation (%)
F Value = (M1/M0)
F0.05 = Table value

Performance Test (NS30) **Method:- RD3** **Compound:- Ethyl tert-butyl Ether**

DataFile ptrd15.xls **Analysis:- MtBE and Oxygenates in Water**
Calc.Date 24/06/00 **ConcnUnits:-** ug/litre
(N.S. = not significant, * = significant at 0.05 level, ** = significant at 0.01 level) **Sample:-** Water:- River, UnFiltered

Results	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Batch 9	Batch 10	Batch 11	Batch 12
BatchDate	01/06/00	02/06/00	05/06/00	06/06/00	07/06/00	08/06/00	09/06/00	13/06/00	14/06/00	20/06/00	21/06/00	
Blank	0.64	0.56	0.77	0.6	0.58	0.41	0.48	0.53	0.54	0.52	0.47	
(Spiked)	0.6	0.53	0.57	0.54	0.6	0.53	0.54	0.5	0.51	0.61	0.44	
Low Calib.	2.65	2.72	2.5	2.35	3.48	2.68	2.29	2.47	2.37	2.62	1.93	
Standard	2.57	2.83	2.59	2.9	3.79	2.71	2.77	2.49	2.38	2.54	2.06	
High Calib.	11.11	11.27	9.56	12.35	11.62	10.09	9.67	10.56	9.98	11.01	9.97	
Standard	11.03	11.27	9.15	12.15	11.35	10.21	9.67	12.63	9.19	12.15	8.94	
Sample	2.71	2.99	2.45	3.19	2.7	2.69	2.44	2.64	2.59	2.48	2.25	
	2.86	2.75	2.48	3.15	2.78	2.73	2.33	2.85	2.74	2.63	2.24	
Spiked	11.21	11.87	9.91	12.42	13.87	10.14	10.07	11.17	10.18	10.69	9.45	
Sample	11.9	12.17	9.91	10.92	12.4	10.05	9.92	11	10.06	10.66	10.44	

Statistics	Blank	Low Standard	High Standard	Sample	Spiked Sample
Mean	0.5486	2.6223	10.6786	2.6668	10.9186
Actual	0.5000	2.5000	10.0000	2.5000	10.0000
M1	0.0081	0.3091	2.2033	0.1265	2.2212
M0	0.0034	0.0309	0.3442	0.0087	0.2815
F Value	2.3610	9.9960	6.4022	14.4992	7.8921
Signific.	N.S.	**	**	**	**
sw	0.0585	0.1758	0.5866	0.0934	0.5305
sb		0.3729	0.9642	0.2428	0.9848
st		0.4123	1.1286	0.2600	1.1186

	Low Standard	High Standard	Sample	Spiked Sample	
Target st	0.3147	1.2814	0.3200	1.3102	
F 0.05	1.792	1.723	1.792	1.752	
FCalc	1.717	0.778	0.660	0.729	
D.O.F	11	13	11	12	
st OK	PASS	PASS	PASS	PASS	Precision x%
% RSD	15.7	10.6	9.7	10.2	12
%Bias	4.9	6.8			

Limit of Detection (LOD)

Calculated **0.300** ug/litre
Required **0.5** ug/litre
(Factor 5.12)

Recovery with 95% Confidence Limits

Mean 8.25 ± 0.499
Expected 7.50 (Spike Vol. N.S.)
Percentage **110.0** **± 6.7**

Reference
M1 = between batch mean square
M0 = within batch mean square
sw = within batch standard deviation
sb = between batch standard deviation
st = total standard deviation
F Value = (M1/M0)
F0.05 = Table value
Target St = the greater (x% of concentration OR 1/4 LOD)
D.O.F = Degrees of Freedom
RSD = Relative standard deviation (%)

Performance Test (NS30) **Method:- RD3** **Compound:- tert-Amyl Methyl Ether**

DataFile ptrd15.xls
Calc.Date 24/06/00
(N.S. = not significant, * = significant at 0.05 level, ** = significant at 0.01 level)

Analysis:- MtBE and Oxygenates in Water
ConcnUnits:- ug/litre
Sample:- Water:- River, UnFiltered

Results	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Batch 9	Batch 10	Batch 11	Batch 12
BatchDate	01/06/00	02/06/00	05/06/00	06/06/00	07/06/00	08/06/00	09/06/00	13/06/00	14/06/00	20/06/00	21/06/00	
Blank	0.88	0.73	0.83	0.85	0.91	0.63	0.66	0.7	0.67	0.79	0.64	
(Spiked)	0.85	0.92	0.83	0.78	0.91	0.77	0.7	0.74	0.62	0.8	0.58	
Low Calib.	2.72	2.87	2.62	3.06	3.67	2.87	2.41	2.6	2.58	2.72	2.08	
Standard	2.71	3.05	2.73	2.85	3.41	2.89	2.41	2.74	2.47	2.54	2.23	
High Calib.	10.81	10.95	8.89	11.87	11.08	10.17	9.38	10.34	8.94	11.09	8.88	
Standard	10.64	11.02	9.28	11.76	11.48	10.23	9.5	12.05	9.13	11.69	8.43	
Sample	2.85	2.96	2.56	3.19	2.86	2.84	2.51	2.75	2.6	2.59	2.22	
	2.67	2.81	2.56	3.2	3.43	2.96	2.41	2.76	2.7	2.76	2.19	
Spiked	10.83	10.96	9.65	11.95	12.91	9.91	9.52	10.43	9.67	10.25	8.68	
Sample	11.44	11.33	9.95	10.45	12.4	9.79	9.2	10.32	9.73	10.74	10.16	

Statistics	Blank	Low Standard	High Standard	Sample	Spiked Sample
Mean	0.7632	2.7377	10.3459	2.7445	10.4668
Actual	0.5000	2.5000	10.0000	2.5000	10.0000
M1	0.0188	0.2547	2.4807	0.1655	1.9951
M0	0.0032	0.0111	0.1772	0.0202	0.2578
F Value	5.8406	23.0284	13.9983	8.1960	7.7381
Signific.	**	**	**	**	**
sw	0.0568	0.1052	0.4210	0.1421	0.5078
sb		0.3490	1.0732	0.2695	0.9320
st		0.3645	1.1528	0.3047	1.0613

	Low Standard	High Standard	Sample	Spiked Sample	Precision
Target st	0.2738	1.0346	0.2745	1.0467	
F 0.05	1.831	1.792	1.752	1.752	
FCalc	1.773	1.242	1.232	1.028	
D.O.F	10	11	12	12	
st OK	PASS	PASS	PASS	PASS	x%
% RSD	13.3	11.1	11.1	10.1	10
%Bias	9.5	3.6			

Limit of Detection (LOD)

Calculated	0.291	ug/litre
Required	0.5	ug/litre
(Factor 5.12)		

Recovery with 95% Confidence Limits

Mean	7.72	± 0.431	
Expected	7.50		(Spike
Percentage	103.0	± 5.7	Vol. N.S.)

Reference
M1 = between batch mean square
M0 = within batch mean square
sw = within batch standard deviation
sb = between batch standard deviation
st = total standard deviation
Target St = the greater (x% of concentration OR 1/4 LOD)
D.O.F = Degrees of Freedom
RSD = Relative standard deviation (%)

F Value = (M1/M0)
F0.05 = Table value

Appendix 5 Methodology

(1) Sample Extraction

(a) Headspace
10 ml spiked with 0.5mg l-1 oxygenate free petrol (QMx Cat.No YA030023)

Zone Temperatures °C	Vial	70	
	Loop	125	
	Transfer Line	125	
Event Times (minutes)	GC Cycle Time	45	
	Vial Equilbrium	20	
	Pressurisation	0	
	Loop Fill	0.1	Loop Volume 1ml
	Loop Equilibrium	0.15	
	Inject	0.5	
Vial Parameters	Shake	High	
	Shake High	5 minutes	

(b) Solid Phase Micro Extraction (SPME)

Fibre Assembly 75µm Carboxen-Polydimethylsiloxane (PDMS), 24 gauge
(Supelco Cat. No 57319)

Headspace Mode
Absorption time (minutes) with vibration 10.0 minutes
Desorption time (minutes) at 220°C 0.4 minutes

(2) Gas Chromatography

(a) Quantitative Gas Chromatograph Agilent 6890
Column 100metre, 0.25mm id, 0.5µm Petrocol DH Octyl Fused Silica Capillary
(See Mass Spectrometer for oxygenate retention times)

Carrier Gas	Helium
Column Pressure	25psi
Pressure Mode	Constant Flow
Injector	Volatiles Inlet
Injector Temperature (°C)	200
Injector Split Ratio	10:1
Oven Initial Temperature (°C)	40
Initial Time (minutes)	4
Initial Oven Rate (°C /minute)	6
Oven Final Temperature (°C)	200
Final Time (minutes)	4.33
Run Time (minutes)	35

Appendix 5 (cont.)

(b) Qualitative

Gas Chromatograph Varian 3400
Column 100metre, 0.25mm id, 0.5μm Petrocol DH Octyl Fused Silica Capillary
Carrier Gas Helium
Column Pressure 40psi
Injector 1078 Splitless Mode
Injector Temperature (°C) 220
Oven Initial Temperature (°C) 35
Initial Time (minutes) 1.0
Oven Rate (°C /minute) 10.0
Final Oven Temperature (°C) 200
Final Time (minutes) 17.5
Run Time (minutes) 35.0

(1) Mass Spectrometer

(a) Quantitative

Mass Spectrometer Agilent 5973 Quadrupole
Mode of Operation Selected Ion Monitoring (SIM)
Ionisation Mode Electron Impact (EI)

SIM Ions	Target	Qualifier	Relative Retention Time
Methyl Tertiary Butyl Ether	73.0	41.1	14.74 minutes = 1.003
Deuterated Methyl Tertiary Butyl Ether	76.1	41.1	14.70 minutes = 1.000
Di-Isopropyl Ether	87.0	45.0	15.52 minutes = 1.056
Ethyl Tertiary Butyl Ether	59.0	87.0	16.16 minutes = 1.099
Tertiary Amyl Methyl Ether	73.0	43.0	18.33 minutes = 1.247

(b) Qualitative

Mass Spectrometer Varian Saturn 2000 Ion Trap
Mode of Operation MS/MS
Ionisation Mode Electron Impact (EI) AGC
Parent Ion 73
Waveform Type Resonance

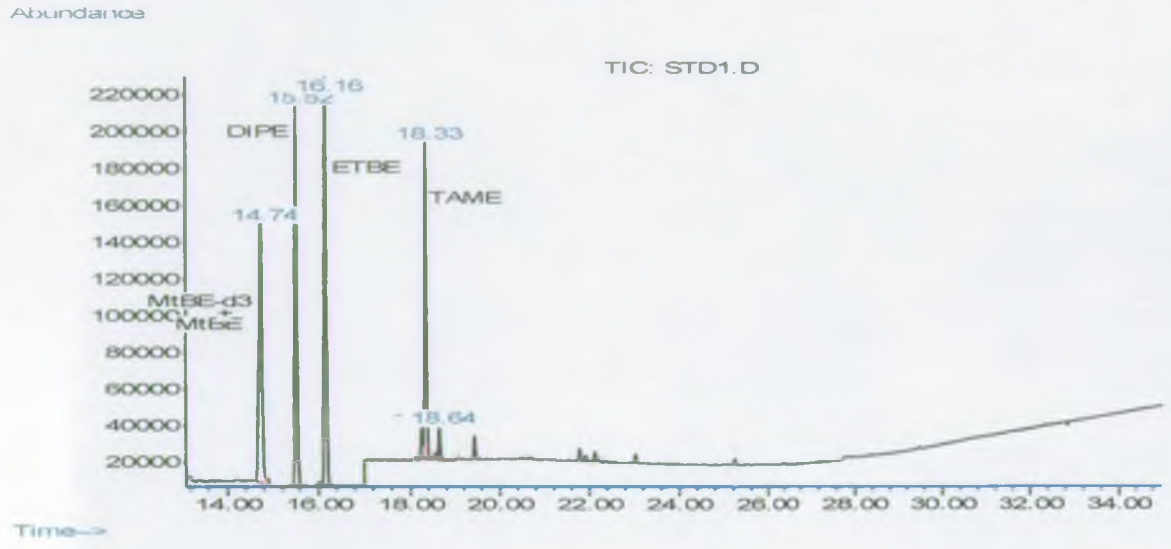
(2) Method Ranges
Low Level 0.2 – 12.5 micrograms per litre per oxygenate
High Level 10 – 250 micrograms per litre per oxygenate

Both methods operated under the same instrumental conditions but with different concentration standards.

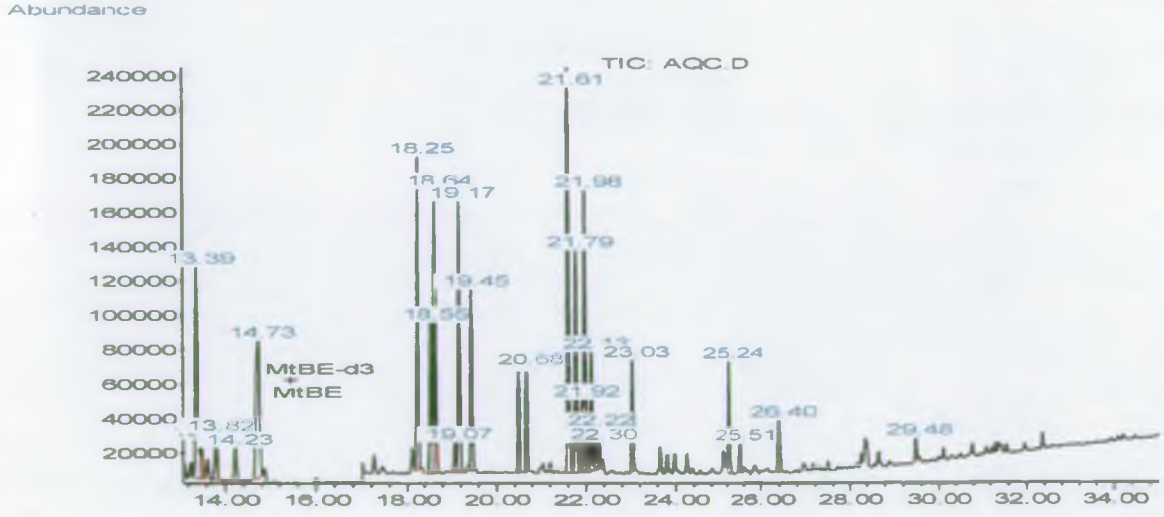
Appendix 6 Chromatograms

6.1 SIM Chromatograms

6.1.1 Calibration Standard (250µg l-1) of Oxygenates



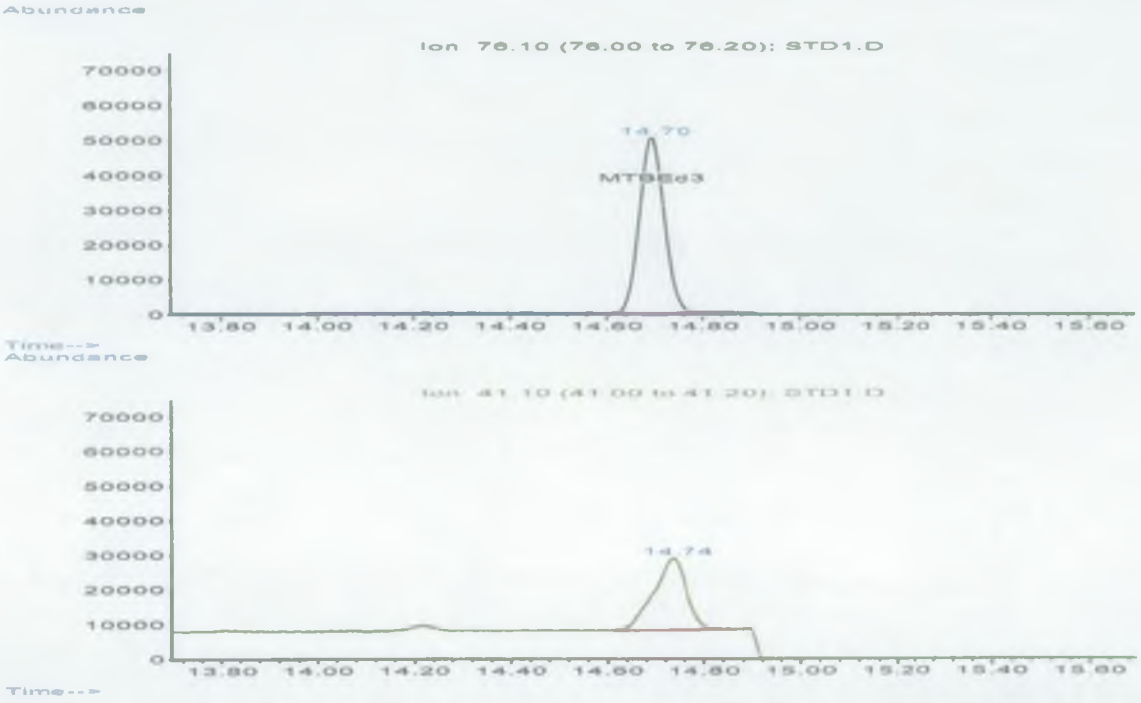
6.1.2 Certified Standard of Methyl tert Butyl Ether 10.6 mg l-1 petrol
Spiked to give Standard Concentration 106µg l-1 water
Petrol Concentration 5,500µg l-1 water



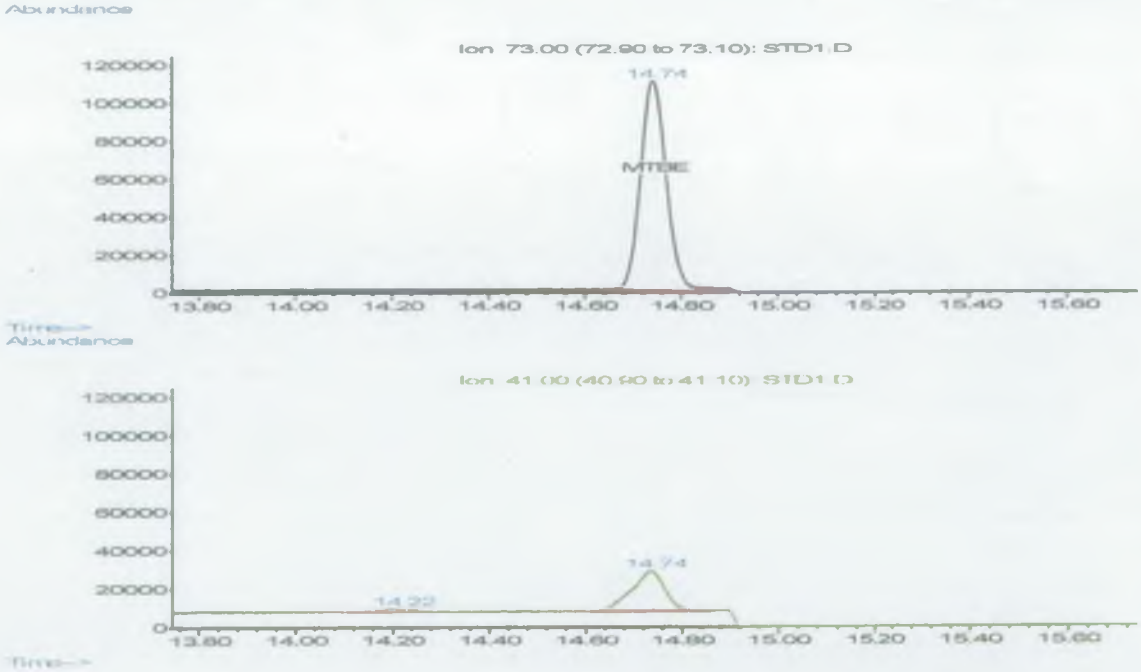
Appendix 6 (cont.)

2.Standard Ion Chromatograms

6.2.1 Deuterated Methyl tert Butyl Ether

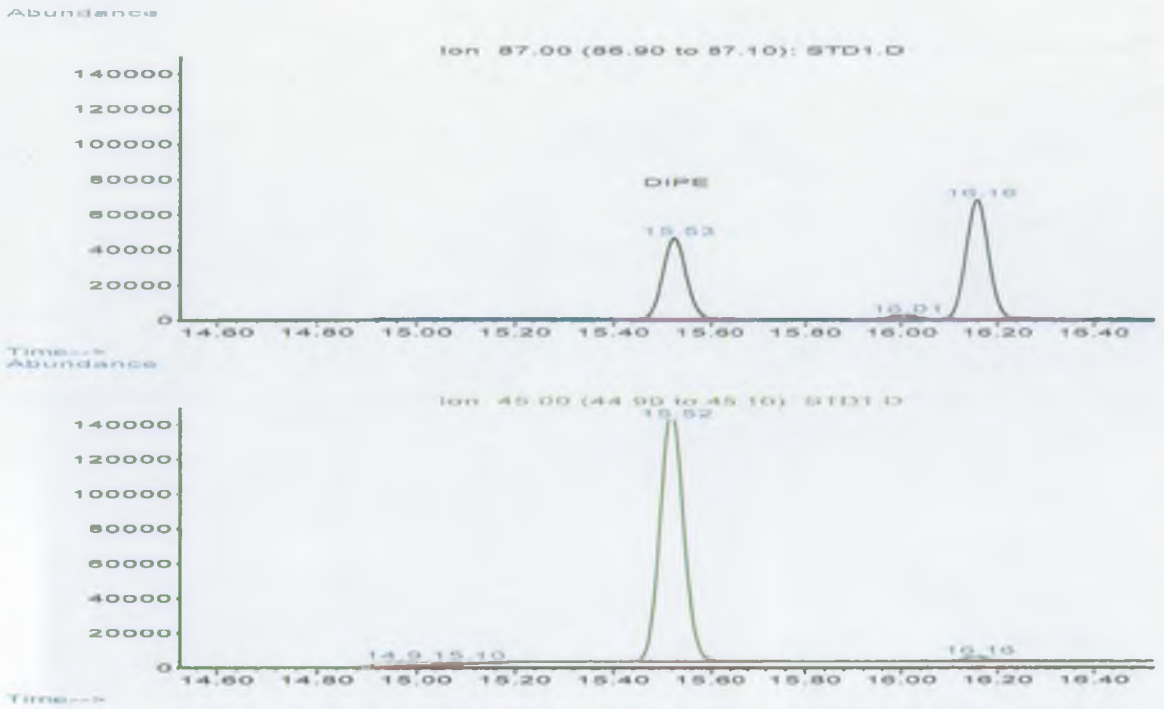


6.2.2 Methyl tert Butyl Ether

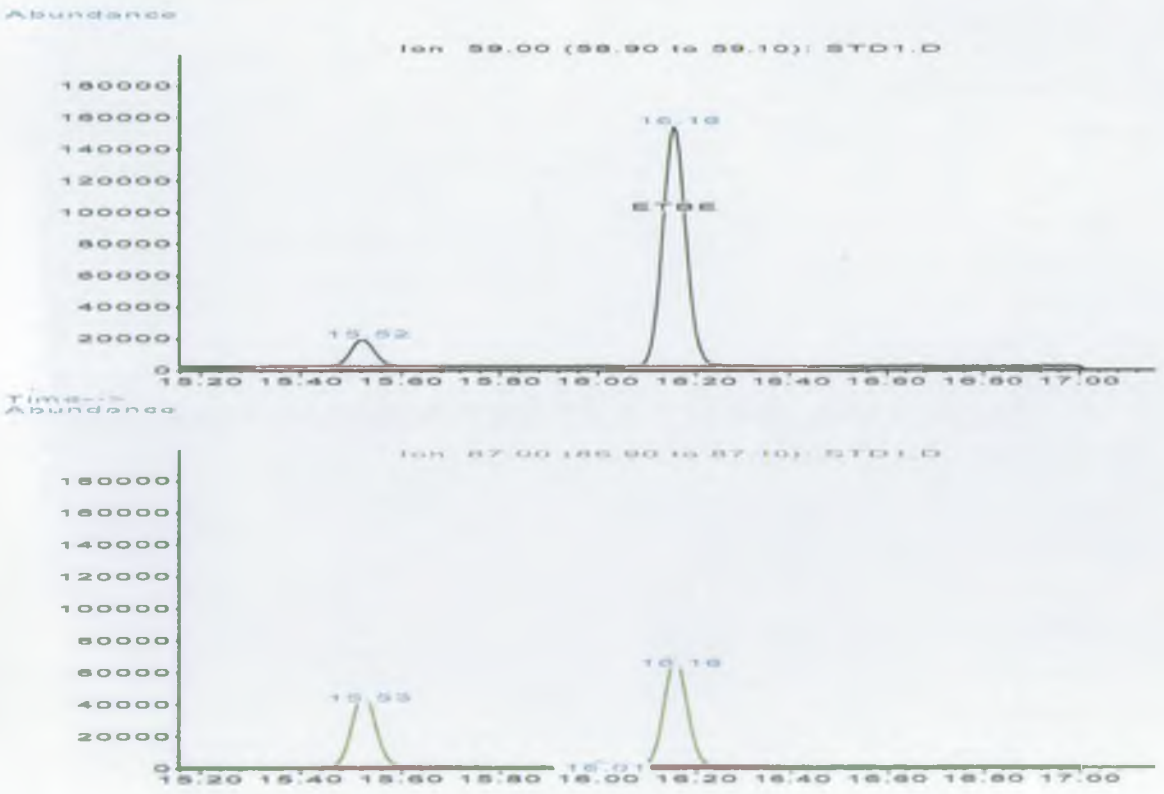


Appendix 6 (cont.)

6.2.3 Di-Ispropyl Ether



6.2.4 Ethyl tertiary Butyl Ether

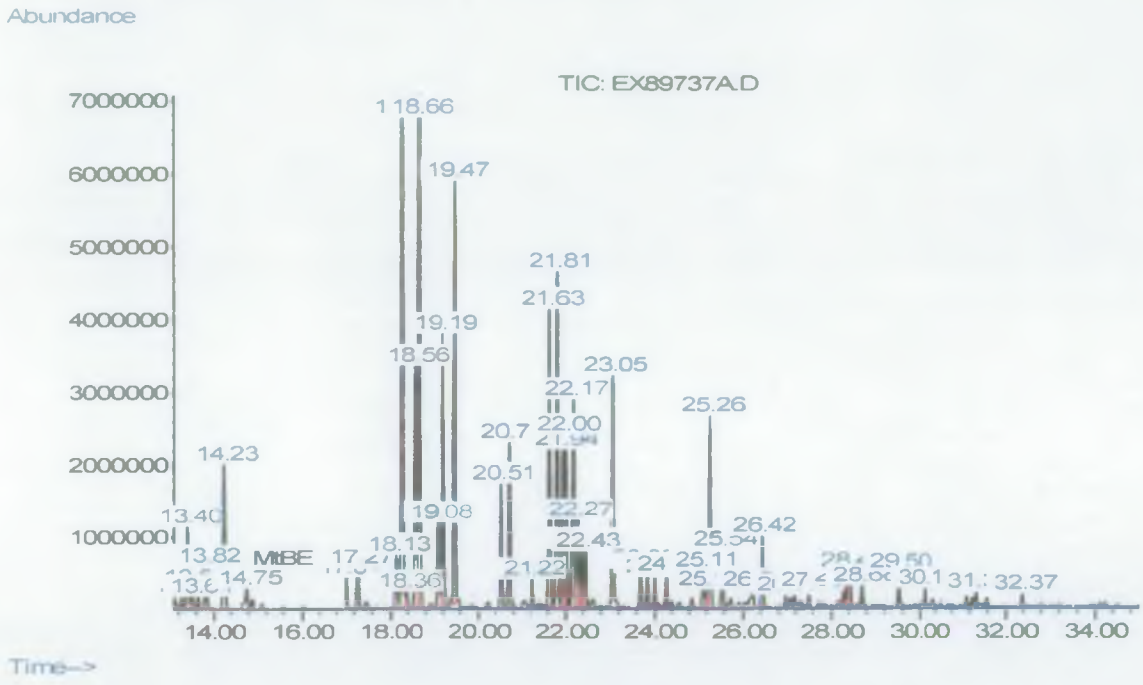


For ion chromatogram of Tertiary Amyl Methyl Ether See Appendix 7.1.6

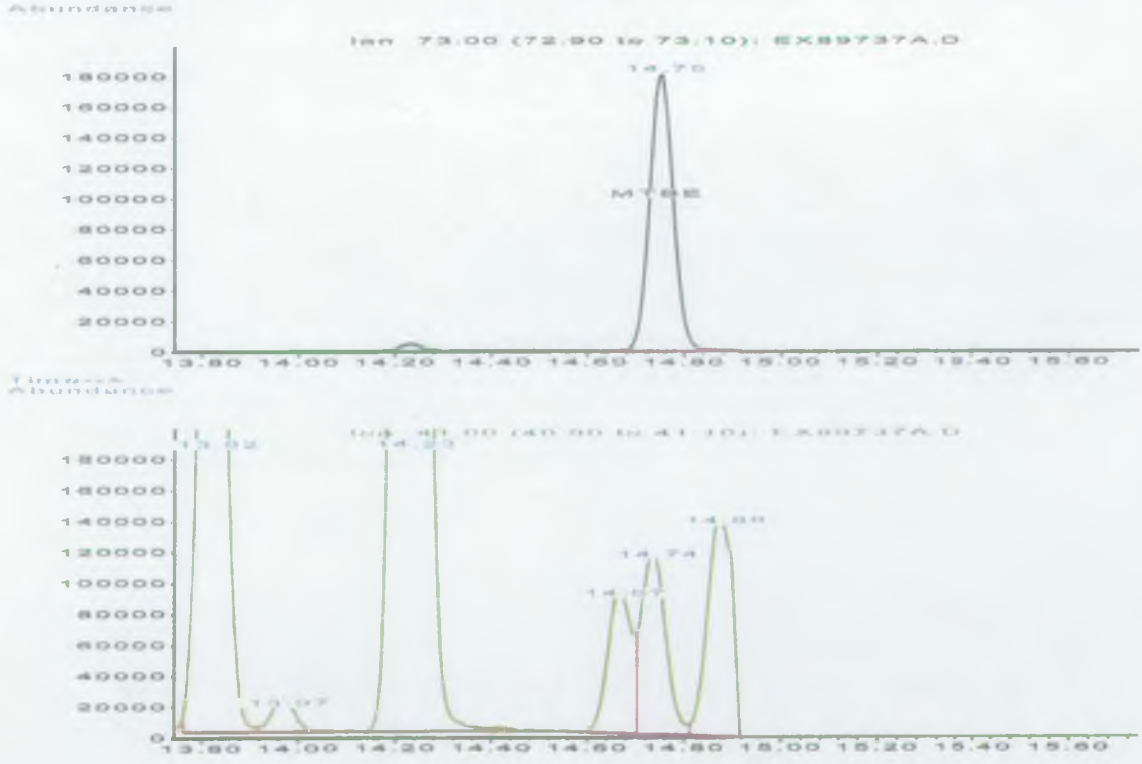
Appendix 7

7.1 Contaminated Sample Chromatograms : Water Layer

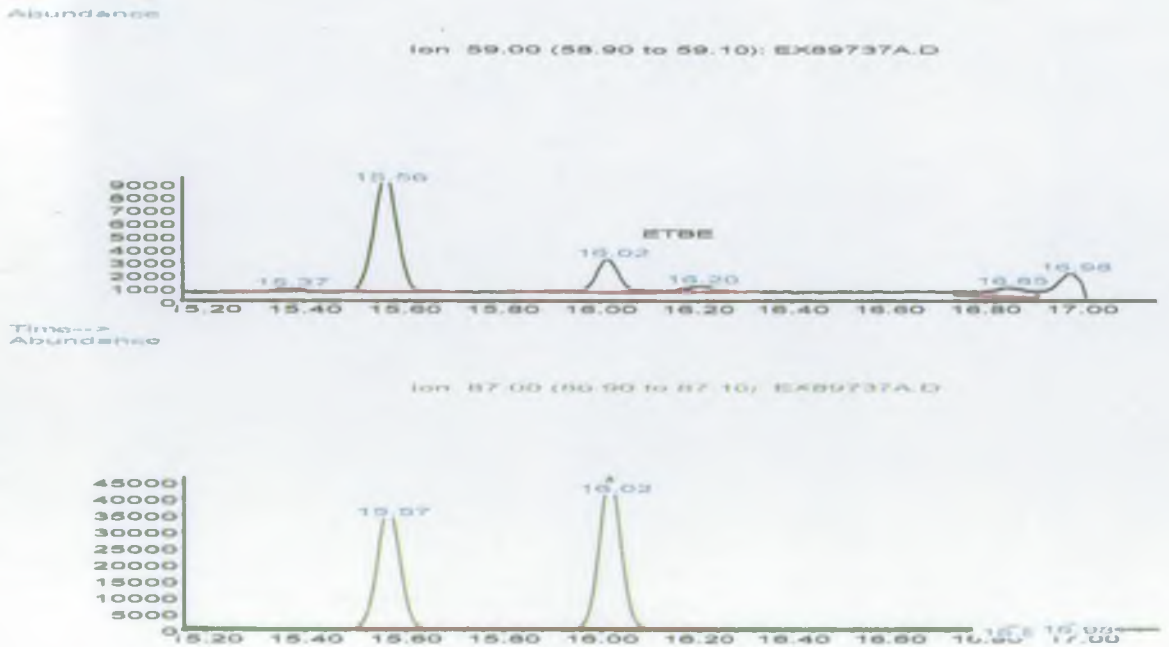
7.1.1 SIM Chromatogram: Water Layer 600µg l-1 MTBE



7.1.2 Ion Chromatogram: Methyl tertiary Butyl Ether/Water Layer

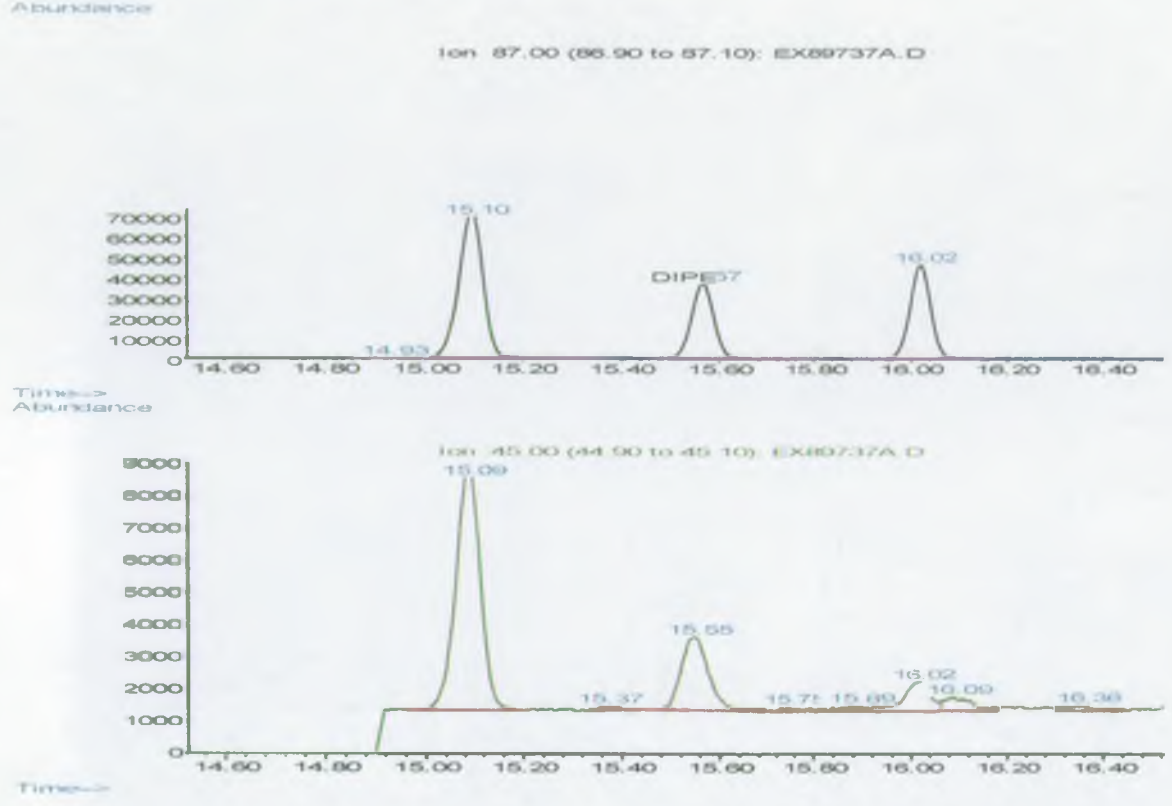


7.1.4 Ion Chromatogram: Ethyl tertiary Butyl Ether/Water Layer



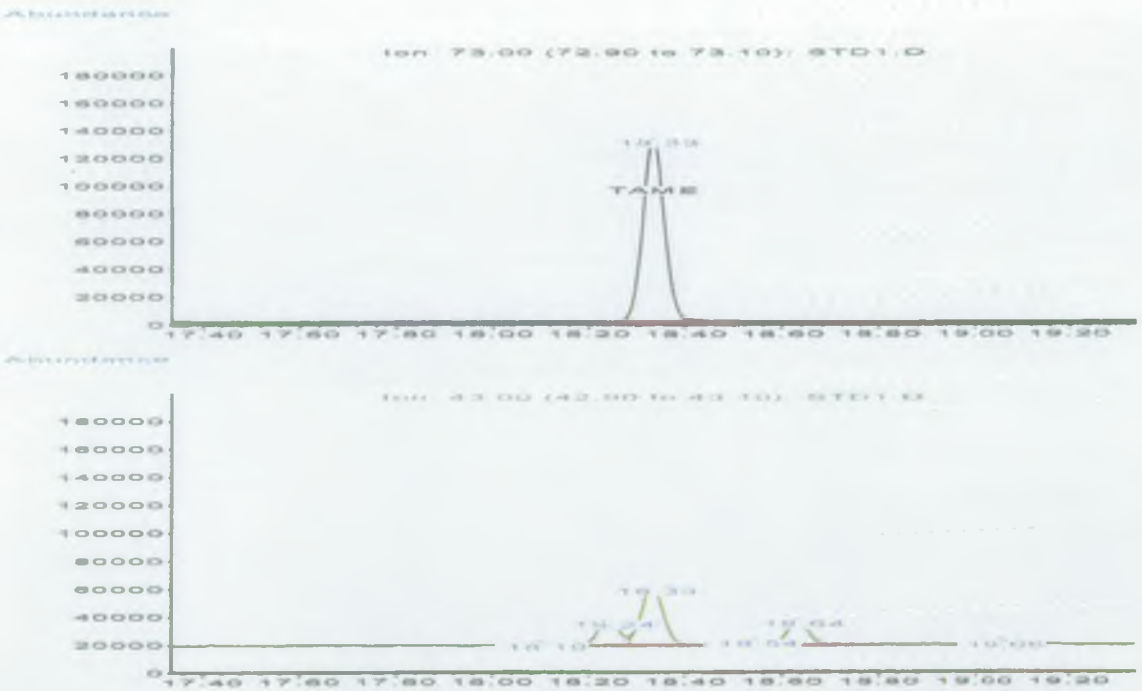
Appendix 7 (cont.)

7.1.3 Ion Chromatogram: Di-Isopropyl Ethyl Ether/Water Layer



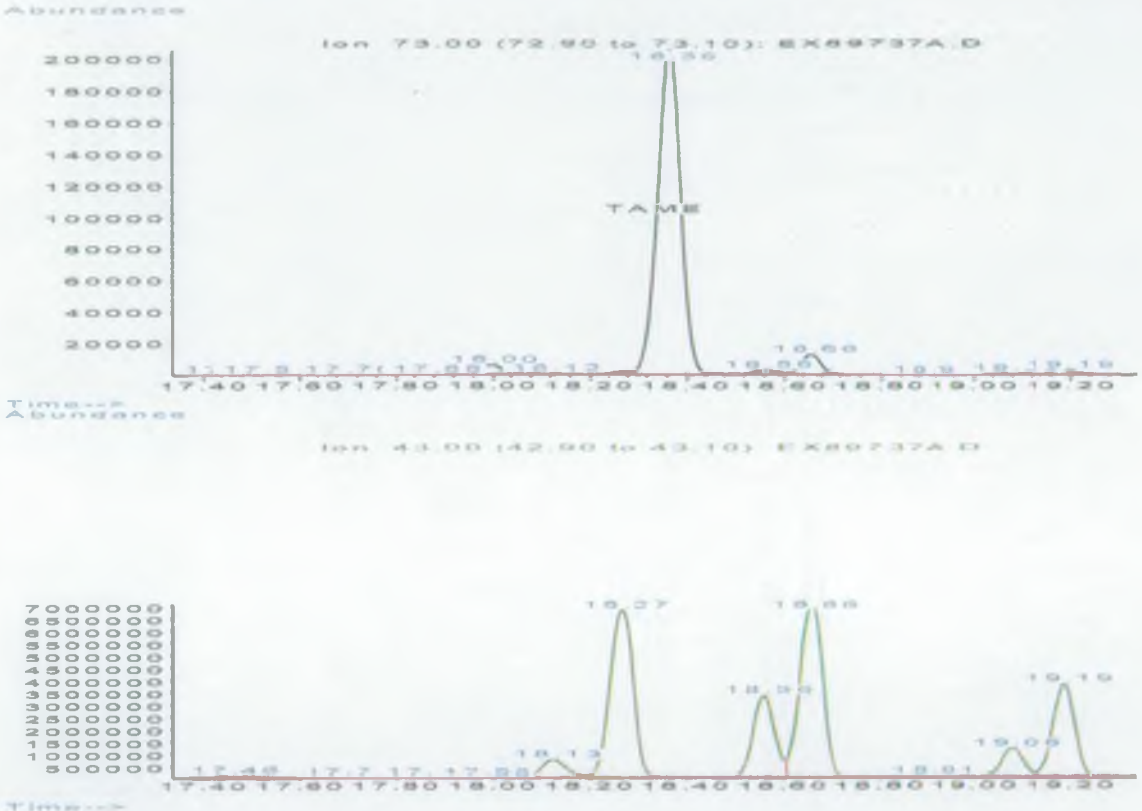
7.1.6 Ion Chromatogram: Tertiary Amyl Methyl Ether Calibration Standard

RetentionTime =18.33mins.



Appendix 7 (cont.)

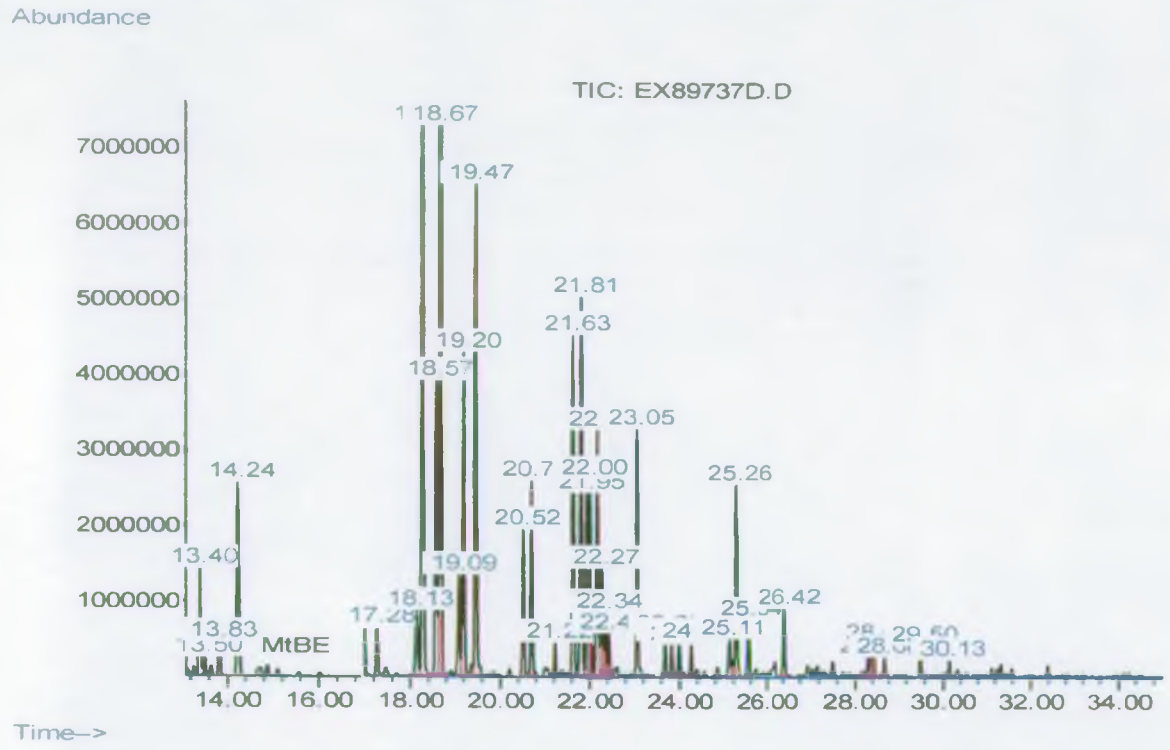
7.1.5 Ion Chromatogram: Tertiary Amyl Methyl Ether/Water Layer



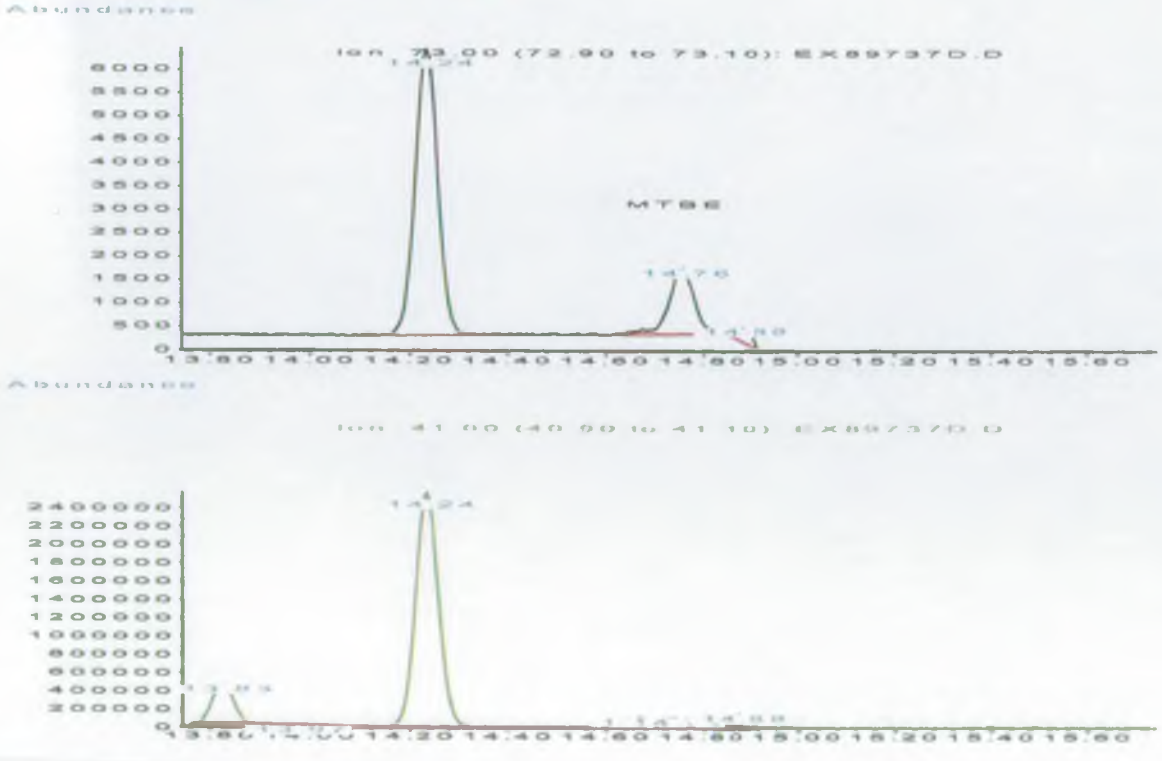
Appendix 7 (cont.)

7.2 Contaminated Sample Chromatograms : Petrol Layer
25µl Petrol in 10mls water

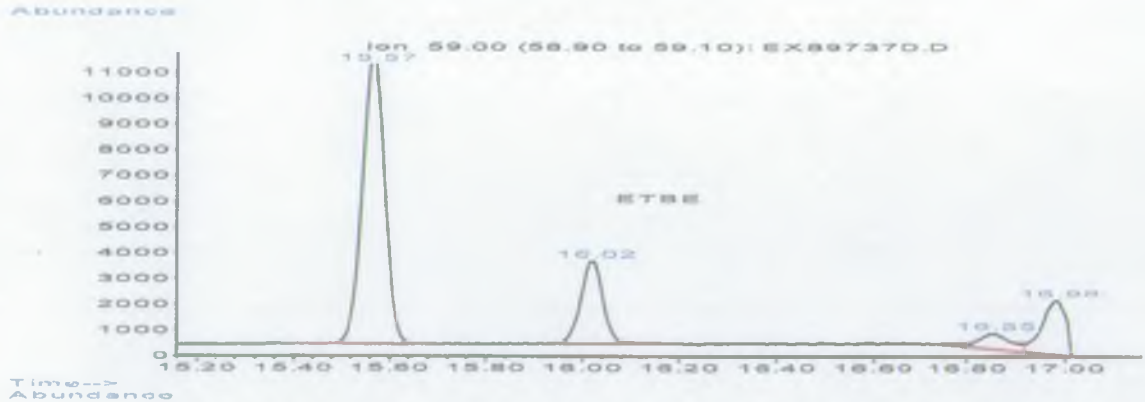
7.2.1 SIM Chromatogram: Petrol Layer 20mg/10mls = 2000mg Petrol l-1 water

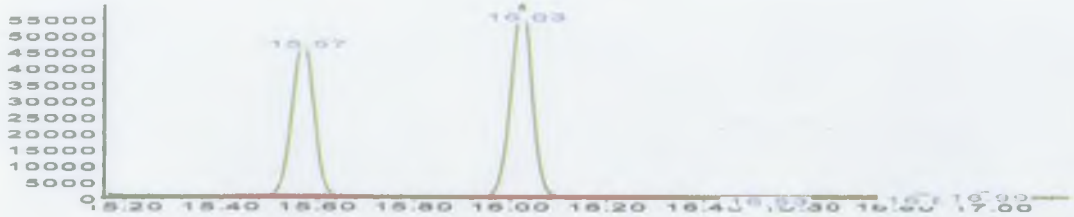


7.2.2 MTBE Ion Chromatogram: Petrol Layer



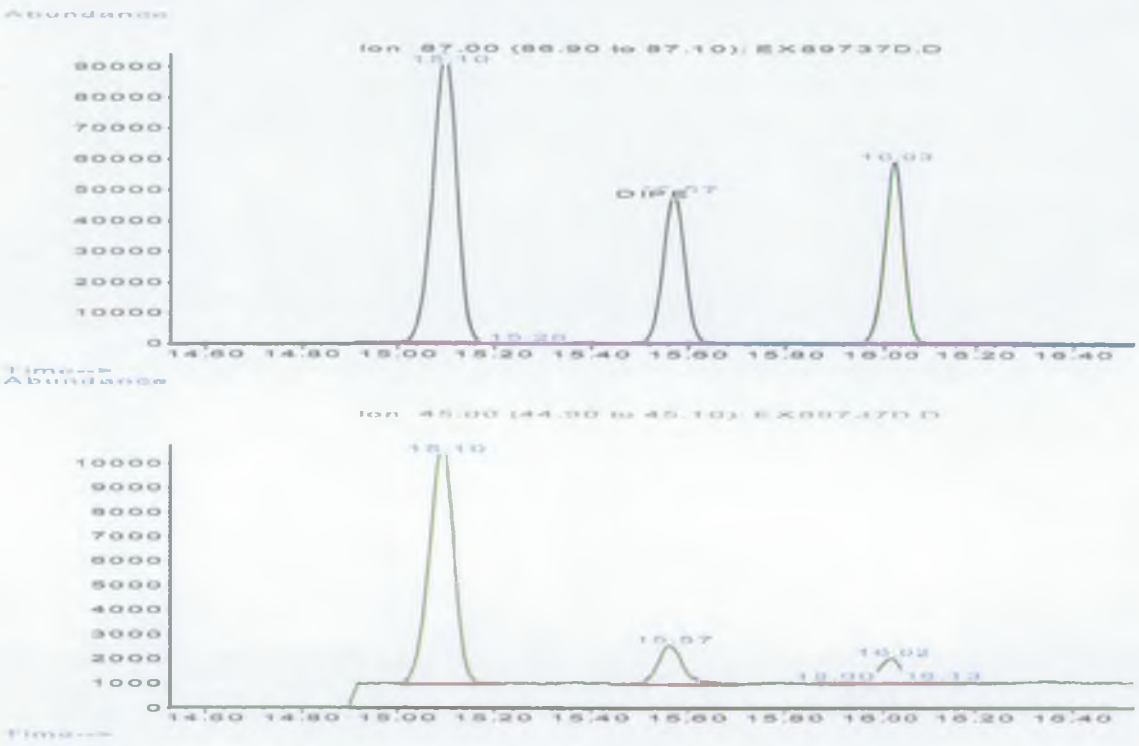
7.2.4 Ion Chromatogram: Ethyl tertiary Butyl Ether/Petrol Layer





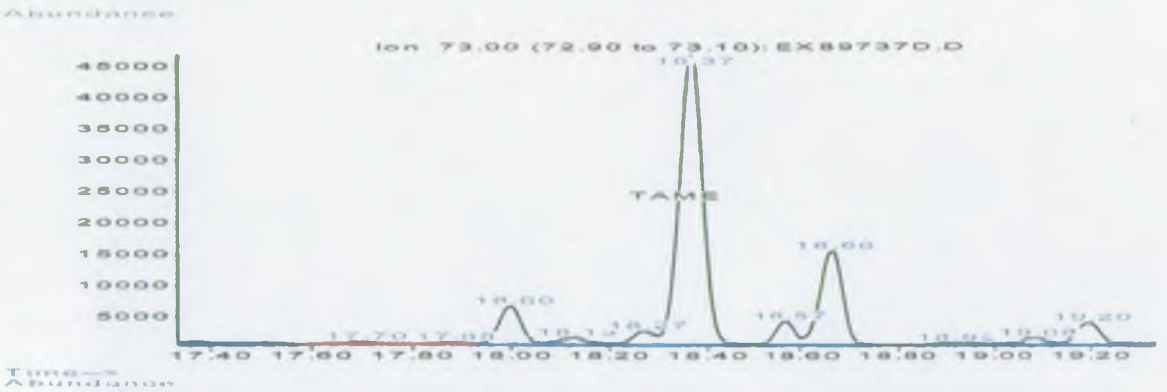
Appendix 7 (cont.)

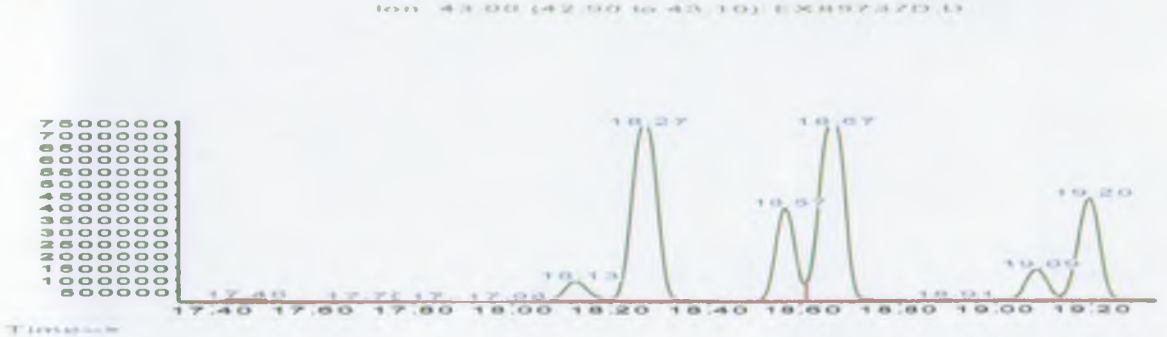
7.2.3 Ion Chromatogram: Di-Isopropyl Ethyl Ether/Petrol Layer



Appendix 7 (cont.)

7.2.5 Ion Chromatogram: Tertiary Amyl Methyl Ether/Petrol Layer





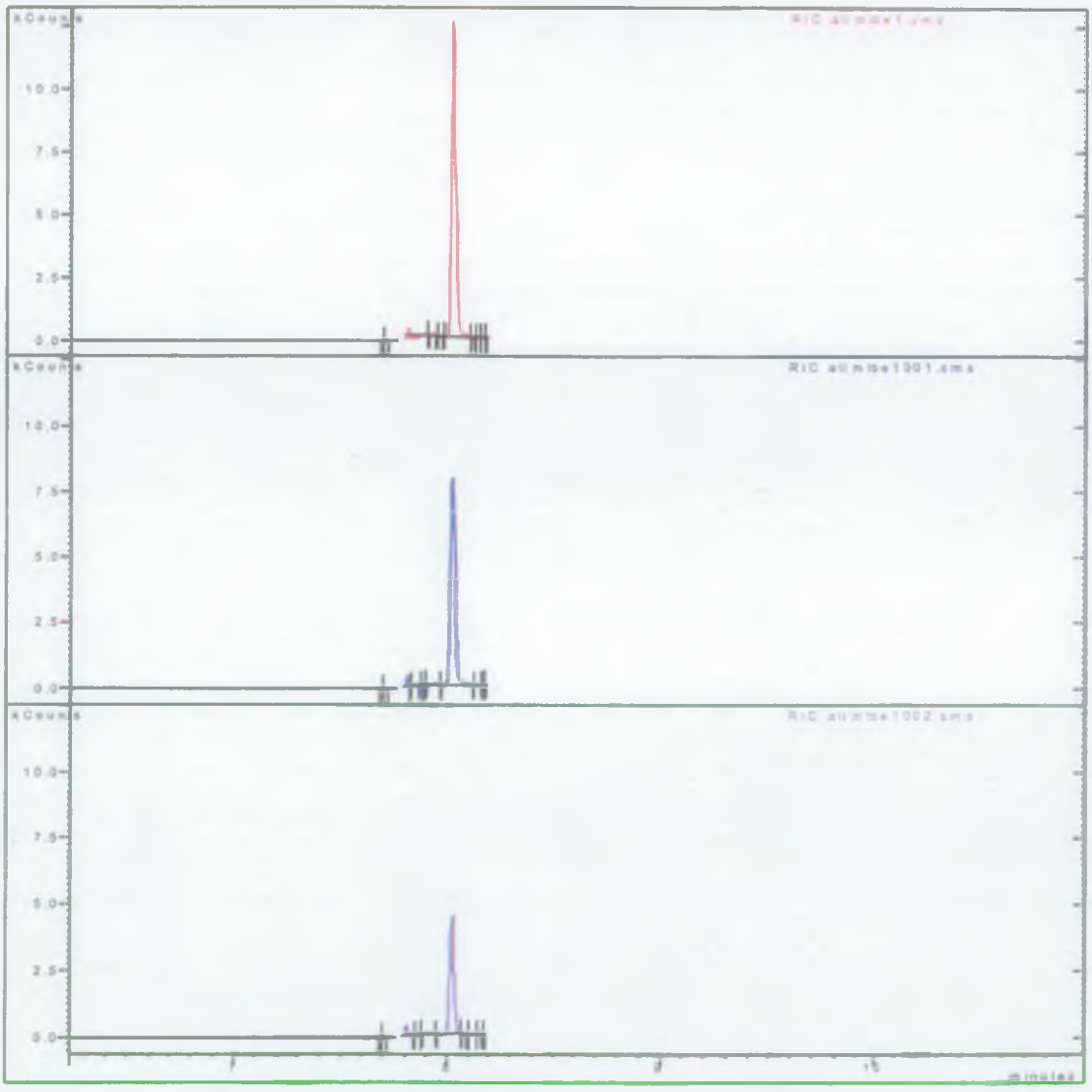
Appendix 8 GC –MS/MS Chromatograms

8.1.1 Calibration standards

Top Chromatogram	125µg l-1 water MTBE
Middle Chromatogram	50µg l-1 water MTBE
Bottom Chromatogram	25µg l-1 water MTBE

Chromatogram Plots

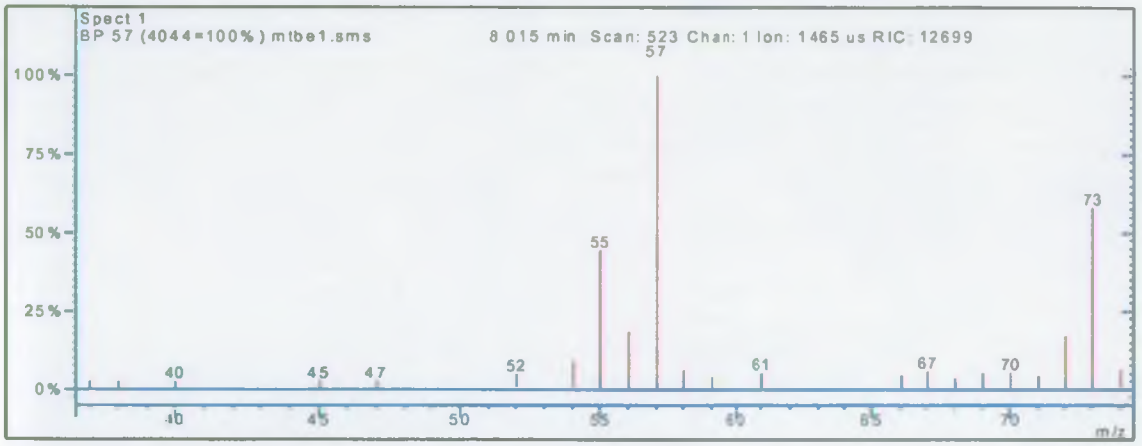
Plot 1: c:\saturnws\data\11aprmtb\mtbe1.sms RIC all
Plot 2: c:\saturnws\data\11aprmtb\mtbe1001.sms RIC all
Plot 3: c:\saturnws\data\11aprmtb\mtbe1002.sms RIC all



Appendix 8 (cont.)

8.1.2 GC-MS/MS Mass Spectrum of Methyl tertiary Butyl Ether

Scan 523 from c:\saturnws\data\11aprmtb\mtbe1.sms



Spectrum from c:\saturnws\data\11aprmtb\mtbe1.sms
Scan No: 523, Time: 8.015 minutes
No averaging. Not background corrected.
Comment: 8.015 min, Scan: 523 Chan: 1 Ion: 1465 us RIC: 12699
Pair Count: 22 MW: 0 Formula: None CAS No: None Acquired Range: 37 - 74

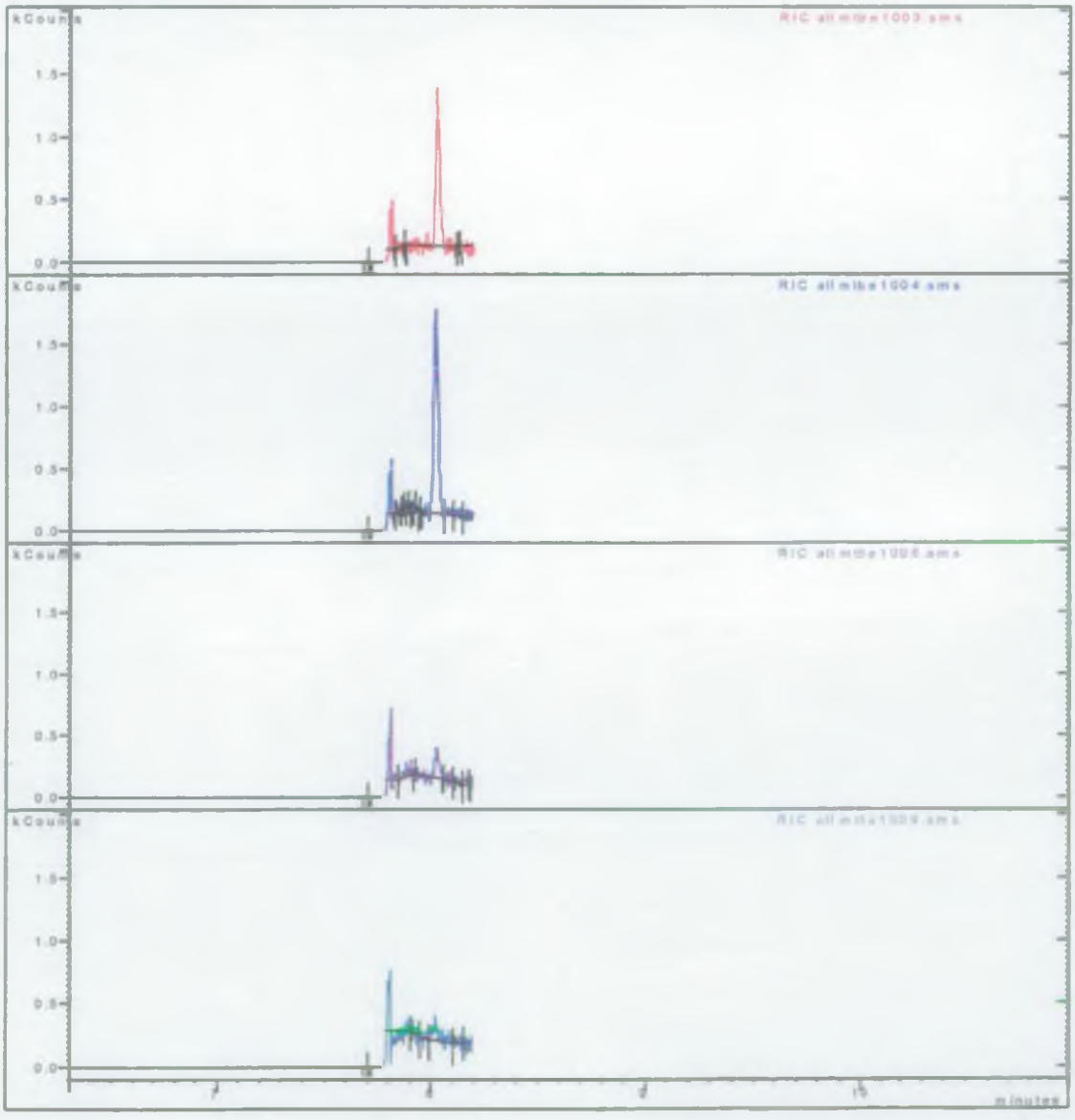
Appendix 8 (cont.)

8.2.1 Effect of petrol addition on sensitivity

Chromatogram 1 (from top) Sample containing MTBE
Chromatogram 2 Sample containing MTBE + 50µg l-1 MTBE
Chromatogram 3 Sample containing MTBE + 50µg l-1 MTBE + 100µl Petrol
Chromatogram 4 Sample containing MTBE + 100µl Petrol

Chromatogram Plots

Plot 1: c:\saturnws\data\11aprmtb\mtbe1003.sms RIC all
Plot 2: c:\saturnws\data\11aprmtb\mtbe1004.sms RIC all
Plot 3: c:\saturnws\data\11aprmtb\mtbe1005.sms RIC all
Plot 4: c:\saturnws\data\11aprmtb\mtbe1009.sms RIC all

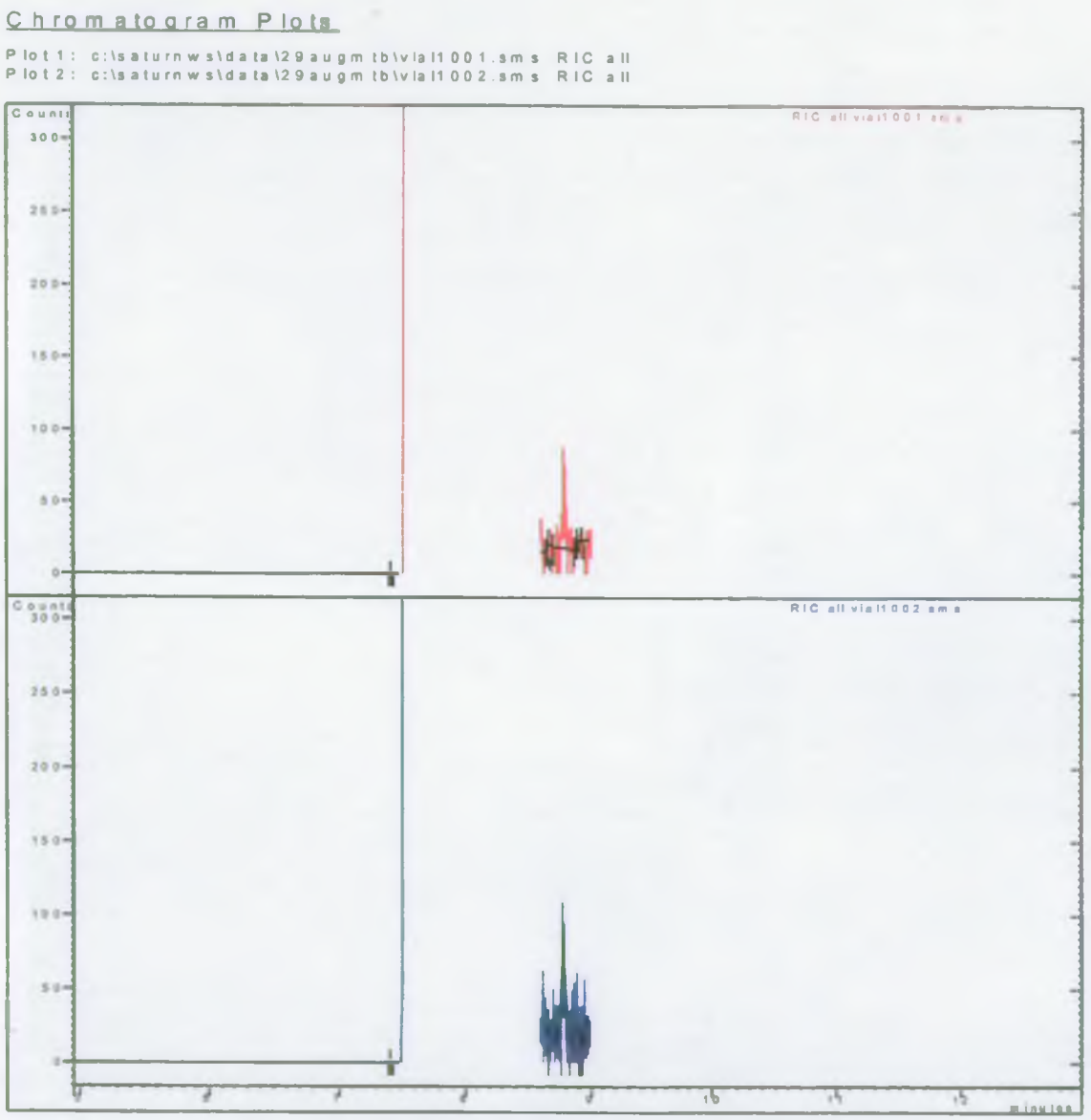


Appendix 8 (cont.)

8.2.2 Contaminated Sample

Chromatogram 1 (top) Contaminated Sample

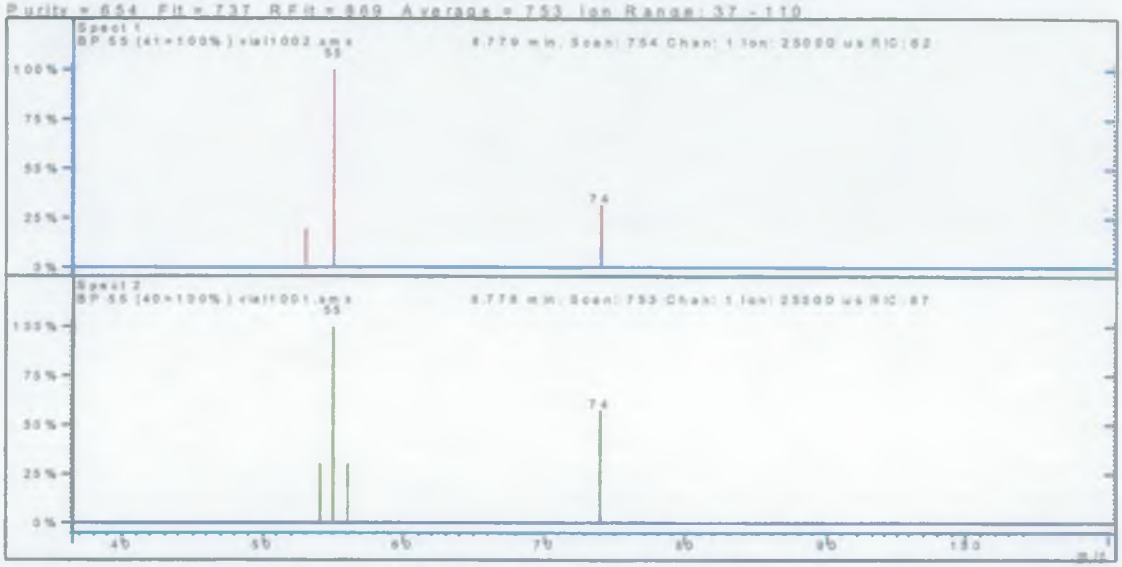
Chromatogram 2 MTBE Standard 106µg l-1 MTBE



Appendix 8 (cont.)

8.2.3 MS/MS Spectra of MTBE Standard (top) and Contaminated Sample (bottom)

Scan 754 from c:\saturnws\data\29augmtb\vial1002.sms
Scan 753 from c:\saturnws\data\29augmtb\vial1001.sms
Purity = 654 Fit = 737 RFit = 869 Average = 753 Ion Range: 37 - 110



Target Spectrum from c:\saturnws\data\29augmtb\vial1002.sms
Scan No: 754, Time: 8.779 minutes
No averaging. Not background corrected
Comment: 8.779 min. Scan: 754 Chan: 1 Ion: 25000 us RIC: 62
Pair Count: 3 MW: 0 Formula: None CAS No: None Acquired Range: 37 - 74

Match Spectrum from c:\saturnws\data\29augmtb\vial1001.sms
Scan No: 753, Time: 8.778 minutes
No averaging. Not background corrected
Comment: 8.778 min. Scan: 753 Chan: 1 Ion: 25000 us RIC: 87
Pair Count: 4 MW: 0 Formula: None CAS No: None Acquired Range: 37 - 74