DEVELOPMENT AND IMPLEMENTATION OF GAS AND LIQUID CHROMATOGRAPHIC ORGANIC WATER PROFILES AS A MANAGEMENT TOOL

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DEVELOPMENT AND IMPLEMENTATION OF GAS AND LIQUID CHROMATOGRAPHIC ORGANIC WATER PROFILES AS A MANAGEMENT TOOL

Report to the

WATER RESEARCH COMMISSION

This report consists of 2 sections

Section 1

GAS CHROMATOGRAPHIC PROFILES OF ORGANICS IN WATER
by MI Selala, S. Phirwa and N. Segoe

Section 2

LIQUID CHROMATOGRAPHIC PROFILES OF ORGANICS IN WATER
by *ER Rohwer and C Schoeman*

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EXECUTIVE SUMMARY

In the last century significant advances have been made in our knowledge of the nature of organic compounds present in source and public supply waters. The advances were made possible by developments in analytical instrumentation and the subsequent commercial availability of suitably refined equipment. The motivation for this work was largely an increasing feeling that more needed to be known about the extent to which the population is exposed to environmental chemicals. This stemmed from growing evidence that long-term exposure to low concentrations of certain chemicals is an important factor in some chronic diseases. It is generally assumed, for example, that the great majority of cancer cases are of environmental origin. It is also recognised that organic compounds play an important role in water quality problems not specifically related to health, for example, colour, taste and odour.

It is normal for many drinking water supplies to contain several hundred milligrams per litre of inorganic constituents, but the concentration of organic compounds rarely exceeds 20 mg/l. This small quantity of material is a very complex mixture containing hundreds of different compounds, some of natural and some of synthetic origin. Its analysis is difficult, and even the most advanced analytical techniques cannot yet identify more than 10-20 percent of the organic material present. Progress has been made, however, and in this report the current state of knowledge is summarised. Much more difficult is the question of the significance to public health of these organic substances. Information on this is either totally lacking or highly controversial, and it is clear that considerable more research is required in this field.

The conventional water treatment systems (coagulation, filtration, and disinfection) are primarily designed to remove suspended matter and destroy bacteria, and remove only a small fraction of the total organic carbon. The removal may be somewhat selective, with certain organics being effectively removed while others e.g. phenols and certain pesticides are more or less unaffected.

The analysis of organic substances in water, in relation to potential public health hazards, can be divided into three main approaches:

(i) Quantitative analysis for known determinants
(ii) Comprehensive survey analysis in which the determinants are unknown.
(iii) A third approach is developing profiles as a management tool. Such screening methods usually involve relatively simple analytical techniques. Significant levels would initiate a more detailed investigation using one of the other approaches.

This research report follows the third approach. The objectives of the research program are:

(i) The setting up of Gas and Liquid Chromatographic methods to determine profiles of organic compounds in water and establish fingerprints of volatile and non-volatile fractions of both source and drinking water.
(ii) To compile a database of the profiles from different regions.
(iii) To implement these profiles as a management tool.

- Sample profile differences were monitored on the basis of mass spectra and sensory characteristics.
- A record of the organic profiles of both source and processed water were kept.
- The removal efficiency of a treatment process for a particular profile in a specific region was examined.

Methodology for both Gas and Liquid Chromatography were developed to satisfy these objectives. Mass Spectrometry was used extensively in the development of these methods to

facilitate the identification of unknown compounds in the water samples investigated.
Mass Spectrometry is an expensive technique and is not available in many of the smaller laboratories that analyse water samples. It is thus preferable that cheaper and more accessible technologies are utilised as screening procedures and that Mass Spectrometry is only used as a confirmatory technique. Gas Chromatography coupled to Electron Capture, Nitrogen Phosphorus and Flame Ionisation Detectors can be used to screen samples in this manner. Similarly, Diode Array Detection can be used to screen in Liquid Chromatography.

It was also shown that Dissolved Organic Carbon was used to monitor waters for gross organic contamination. Values for drinking waters were normally in the range of 3 to 5 mg/l while source waters can vary between about 8 and 10 mg/l. Monitoring DOC values can thus indicate if a significant organic pollution incident has occurred.

Odour profiles of waters using an Electronic Nose were also investigated. The technique showed promise but requires further development by the manufacturers before its potential can be fully realised.

Additional studies in this regard should be carried out when the Electronic Nose is fully developed. Also, Total Organic Halogen (TOX) values may also provide a useful Management tool to screen water samples. TOX values from previous studies showed a concentration of between 30 and 50 µg/l in drinking water and would indicate contamination with low levels of halogenated organic pollutants.

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PART 1. GAS CHROMATOGRAPHY

Chapter 1

Introduction

There are two major public concerns in relation to organic drinking water quality, namely the taste and the odour arising from organic micro pollutants in drinking water. The analysis of individual organic micro pollutants is time consuming, labour intensive and generally very expensive. As a result most the organic analyses focus on classes of compounds. The equipment required is beyond reach of most of the smaller water analyses laboratories. [1]

In spite of this of its great significance, organic analyses are still in the early age of development. Since the number of organic compounds is several magnitudes greater than that of inorganic compounds, analytical methodology that would simultaneously determine all organic substances cannot be expected. At best, only group separations can be hoped for. Numerous separation, concentration and extraction techniques have been developed. Although the resin based extraction technique seems to have the largest flexible collecting bandwidth, chromatographic techniques also have also been considered. [1]

Gas Chromatography (GC) used to determine volatile and semi-volatile organic contaminants present in drinking water samples. GC is thus suitable for analysing approximately 15% of the total organics. Liquid Chromatography (LC) is required for the determination non-volatile and thermally labile compounds that make up the other 85% of the organic component of drinking waters. This technology has in recent years advanced greatly and, although expensive, is now widely available. Most of the organics we ingest are harmless [1]. This is important as it costs a great deal of money to treat perceived to-be-hazards in addition to the costs of treating the known and real dangers.

Objectives

i. To develop methodology to determine organic pollutants in water using GC.
ii. Utilise this methodology to monitor organic pollutants.
iii. To establish profiles of these organic pollutants in source and drinking waters.
iv. To utilise these profiles as a management tool.
    - Analyse the chemical data obtained using statistical software to characterise waters (source and drinking).
    - To relate sample sensory characteristics to chemical data.
    - To examine the efficiency of the treatment process for a particular organic pollutants in a specific region.
    - To compare the effects of different regional treatment processes on water quality.

Proposed Methodology

Van Rensburg (1980) developed an Organic Pollution Index (OPI) of types of water using the Liquid/Liquid Extraction (LLE) and Gas Chromatography/Mass Spectrometry (GC/MS). This was not a water profile analysis. He expressed the area of the peaks in a chromatogram in terms of added standards. In order to express these data in a useful form, use was made of an organic pollution index of types of water. The drawback of this was that the extraction step was lengthy and prone to error. Water samples having very different profiles can still give similar OPI's. An OPI is a useful attempt at quantification for a specific area, but may mislead when used to

compare water quality in different regions of the country, or if the nature of the background of some supply changes significantly [1].
The approach to be followed in this section of the Project is as follows,

i. Evaluation of and pre-concentration techniques. These include,
   - Closed Loop Stripping Analysis (CLSA)
   - Headspace
   - Solid Phase Extraction (SPE)
   - Solid Phase Micro Extraction (SPME)
   - Liquid/Liquid Extraction (LLE)

ii. Comparison of Off-Line to automated On-Line extraction techniques discussed above.
iii. *Identify unknown organic pollutants and quantitative target compounds using GC/MS.*
iv. Evaluation of the Electronic Nose to be used for Odour Profile Analysis.

Statistical models able to recognise and characterise patterns in data exist. Profiles are essentially qualitative identifications, but this does not prevent fairly accurate internal and relative comparison from being made by an electronic nose. [1]

Chapter 2

Literature Survey

Organic substances occur in the environment as a result of natural processes or their introduction by human activity [2]. An estimated 70 000 different chemical compounds are currently in common use, a number which grows by about 1000 each year [3]. Many of these chemicals may have direct, indirect or delayed toxic, mutagenic, and carcinogenic or teratogenic effects and indications are that chemicals in the environment may be responsible for 50 to 90 % of human cancer cases [4]. Since water plays an important role in exposing man to chemicals in the environment, research on the incidence of hazardous compounds in water, their health effects and methods for their removal, receives high priority in water research all over the world [5]. In a routine laboratory, the need to accurately analyse a wide range of chemicals in shortest turn around time is essential.

There is a growing need for adequately sensitive analytical procedures that provide confirmation and/or provisional identification. Prior to the actual spectrometric measurement, analytes of interest are commonly separated from each other and/or from interfering sample constituents and if necessary (selectively) concentrated. In many instances, GC will be the method of choice to achieve efficient separation of a mixture components [6].

The application of GC/MS to water analysis since the 1970's has revealed the presence of many hundreds of different organic compounds. These are derived from naturally occurring substances in the environment, as well as from materials produced, used or discarded by industry and agriculture. When present, they usually occur at very low concentrations of less than 1 µg/l. Some are known to be toxic or carcinogenic to animals at concentrations far higher than detected in drinking water. Others are known to be mutagenic (*i.e.* capable of making heritable changes to living cells) under laboratory testing. Assessment of the risk that these compounds might present to human health at the low levels found in water is difficult. However, the potential risk has led to the view that, where possible *the concentration of certain pollutants should be kept as low as* possible in drinking water. [6]

Many compounds are either thermally labile or non-volatilite and are not amenable to GC analysis without prior time-consuming chemical derivitisation procedures [7]. Next to GC, LC is an important and extensively used analytical separation technique. Although the separation efficiency and analyte detectability that can be obtained in LC are generally not as good than those obtainable with GC, the range of substances that can be analysed is much larger as it includes polar, thermally labile, non-volatile and high-molecular weight compounds [8].

1.2.1 Gas Chromatography and Mass Spectrometry

GC has three main advantages,

i. High separation efficiency.
ii. High speed of analysis.
iii. A wide range of sensitive detection devices with low detection limits, many of which are element-specific.

Mass Spectrometry (MS) provides excellent detection and identification possibilities for low amounts of analytes, typically 1ng or less. Since the retention power of GC resides in the stationary phase much attention has always been devoted to the synthesis of novel stationary phases and in the past decade, these activities have resulted in the distinct increase of the number of compound classes that can be analysed routinely by means of GC.

Large Volume Injection (LVI) is the injection of an aliquot of a sample extract greater than the conventional 1-5µl. The injected volume is usually 50µl or more. The sensitivity of existing analytical procedures is thus enhanced [40]. For example, if the detector's detection limit is 100pg/µl in 1µl then the detection limit can be improved down to 1pg/µl in 100µl [38].

Two commonly used techniques for LVI are large volume on-column injection and programmed temperature vaporizer (PTV) injection. If the sample extract is relatively clean on-column injection is preferred because it allows the determination of volatile as well as the less volatile analytes. Hardware modifications, such as cooling with liquid carbon dioxide, are necessary for PTV injection [41]. For this project only large volume on-column injection was investigated.

1.2.2 Sample Pre-treatment

Sample pre-treatment is necessary to isolate and to concentrate analytes from a matrix. Interfering compounds of extreme polarities and/or non-volatile need to be removed during the sample pre-treatment as they can contaminate the GC system leading to rapid deterioration of the performance of the GC system [8]. Derivitisation of the analytes of interest [9,10,] (silylation, acylation, acetylation, alkylation or esterification of polar functional groups) improves the chromatographic behaviour of analytes such as amines, alcohols and acids [9,10].

The proper selection of the pre-treatment procedures depends on several aspects,

- The concentration level of the analytes of interest.
- Characteristics of the analytes of interest such as volatility, thermostatability, reactivity and polarity.
- The difference between the characteristics listed above of the analytes of interest and those of the matrix.

Details of routine sample pre-treatment techniques (SPE, SPME, LLE and CLSA) are included in Appendix 1.1.

1.2.3 On-line systems

Sample pre-treatment is usually the most time consuming, tedious and error prone part of the whole analytical procedure [10]. Traditionally, sample preparation was not considered to be an important operation as the final separation-plus-detection step. Recognition of the poor efficiency with regard to time of analysis, precision as well as long-term performance of sample pre-treatment in general has caused a major upsurge of method development in this area. Today emphasis falls on replacing manual sample pre-treatment by procedures that can, either immediately or in the next stage, be automated. This can be performed by developing on-line sample preparation techniques, or via robotization of the sample preparation [8].

In 1989, on account of its fifteenth anniversary, Hewlett Packard initiated the Rhine Basin Program, a joint co-operation of several Dutch, German and Swiss partners. They decided to devote their attention to an environmental problem of real concern, the detection and identification of 'modern' or 'polar' pesticides. Procedures primarily to be used for early-warning and/or monitoring purposes (1µg/l and 3 µg/l as typical alert and alarm levels respectively) for individual pesticides in surface water needed to de developed. On-line sample handling, full automation and unattended operation were considered critical [36].

Monitoring of water samples for the presence of unknown pollutants at the trace level requires fast, sensitive and selective methods. The determination of organic substances in water commonly involves isolation of the compounds of interest and subsequent separation by means of a chromatographic technique [37]. On-line techniques, which combine sample preparation and separation-*plus*-detection in one analytical set-up, are preferred for "early warning/monitoring purposes" [36].

This led to the development and use of the SAMOS system (System for Automated Measurements of Organic micro pollutants in Surface water) for trace enrichment combined on-line with LC or GC separation and subsequent detection (identification and quantification) [36].

Increased hyphenation of techniques has taken place in recent years i.e. SPE with LC (SPE/LC), SPE with GC (SPE/GC), SPME with GC (SPME/GC) and the coupling of Spectrometric/Spectroscopic detectors such as MS-AED and MS-FTIR [48]. The challenges for now and coming years are coupled columns such as two-dimensional GC (GC/GC) and the coupling of such a system to Time Of Flight / MS (TOF/MS).

The advantages of hyphenated systems are a higher separation power (from coupled columns) and yield of better selectivity (from the combination of detectors). Hyphenated systems enhance speed and also increase the possibility to identify new/unknown compounds. Other benefits of hyphenation include total sample transfer during analysis (as in SPE/LC/GC), high sample throughput and automation. The closed system brought by hyphenation minimises the level of contamination, reduces sample manipulation, consumption of sample and organic solvents [38].

The general description and main characteristics of the three approaches, Off-line, On-line and robotisation are given in Table 2.1 [8] detailed explanation of the technique is included in Appendix 1.1.

Table 2.1 Advantages, disadvantages of off-line, on-line sample pre-treatment and robotisation

Advantage	Disadvantages
Off-Line	
Samples can be worked up in parallel	No possibility of automation
Better optimisation of individual steps	Labour-intensive
Simplicity of equipment	Time-consuming
Operational flexibility	Aliquot of sample is analysed
	Risk of solute losses
	Risk of Automation
On-Line	
Automation is possible	Sequential process
Risk of analyte losses is reduced	Procedure has less flexibility
Contamination from external sources is minimised	Optimisation of individual steps is more complicated or impossible
Total sample is analysed, *i.e.* analyte detectability is enhanced	
Precision is improved	
Robotisation	
Automated system	Risk of solute losses
Better optimisation of individual steps	Risk of contamination
Precision is improved	High dependence on good software

Although the composition of the entire organic component of water is of interest, it is not economically feasible to perform comprehensive analyses using the sample pre-treatment techniques discussed on every potable and raw water supply.

It is therefore important to concentrate on parameters that can provide a large amount of significant information without undertaking complicated analytical procedures that cannot be used on a routine basis as a management tool. The two parameters that have been found in this project to meet such criteria are,

i. Odour Profiles Analysis.
ii. Total Organic Carbon (TOC).

1.2.4 Odour Profiles Analysis

Perceptions of the sensations of odour and taste are complementary and it is difficult to distinguish between the two. The sense of smell is generally more sensitive than that of taste and concentrations of µg/l or less of substances may be detected. In contrast to this mg/l are detected by taste. In the assessment of drinking water quality the sense of taste is more useful in detecting inorganic constituents while the sense of smell enables organic constituents more effectively [47].

The detection of odours in water should be viewed as a warning sign indicating the need for

expert assistance. This should not however be interpreted as a direct indicator of the presence of toxic substances in that sample. A range of volatile and semi-volatile organic compounds may cause odours in water and render it unpalatable [48]. Examples of odour-causing compounds are geosmin and 2-methyliscborneol. While very low concentration of these substances result in noticeable odours there is no toxic effect at these levels [48].

Other microbial odorous substances of some importance are organic sulphur compounds. Kadota and Wnorowski [47,61] reported the production of hydrogen sulphide, dim ethyl sulphide and methylmercaptan for a range of bacterial and fungal strains. These are responsible for swampy odours and taste in drinking water [62,63].

Odours in treated water can originate from stagnant water conditions in raw and treated water reservoirs and low flow sections of distribution systems. Water treatment can convert mildly odorous substances, such as phenols and amines, to substances with strong odours, such as chlorophenols and chloroamines. Odours originating in distribution systems may be caused by the use of phenolic pipe-jointing compounds (banned in some municipal areas). Furthermore, certain plastics generate an antiseptic taste and odour as a result of the reaction of residual chlorine in the water with these plastics [47,49,50,59].

The analytical methods available for the determination of odorous compounds in water include sensory evaluation by a human panel and an electronic nose.

1.2.4.1 Human Panel

A description of the taste and odour of a water sample can be obtained by sensory analysis. Since humans make this analysis it is usually considered a subjective measurement [62]. The human panel serves to assess the quality of drinking water following treatment and to confirm consumer's taste/odour complaints.

The practical utilisation of human senses encounters several drawbacks. It is subjective and difficult to standardise. For instance, one panel member may record the smell of hexane in water as fruity while another may identify the odour as rotten. This tends to complicate results [59]. Some panellists are unable to differentiate specific odours in the presence of stronger ones and in addition panellists usually suffer from fatigue rapidly [51].

The most popular method for evaluating odour used for human panel is the Flavour Profile Analysis (FPA). This is based on the rating of odour intensity in an undiluted sample, together with odour description, given by a group of trained panellists.

A library of reference substances and odour descriptors has been established [53]. The method proved to be precise, reproducible and accurate provided the panellists have been properly trained [54]. Literature thoroughly explains the training methods for FPA [55,66].

A larger, untrained taste panel can be used primarily for the assessment of consumer satisfaction with water provided by a given utility [52]. The trained panel's sensitivity is usually more acute than the consumer's [54], but the latter represents more closely the receiver's opinion on drinking water quality [47].

1.2.4.2 The Electronic Nose

An array of sensors simulating the human olfactory organ has become known as an electronic nose [57]. Electronic noses, called eNoses, utilise non or weakly specific arrays of physical sensors to produce an N-dimensional response (where N equals the number of sensors) of specific vapour mixtures (fragrances) and this response can be analysed by principal component analysis [58]. These are a wide variety of sensors *i.e.* Metal Oxide Sensors (MOS), Conducting Polymers and Surface Acoustic Waves (SAW).

The detection is based on a change of resistance when the odour-producing compound is present. Neural networks help to classify new flavours according to odours descriptors. The instrument can be taught to smell and quantify the odour. No extraction is necessary and hence no loss of substance occurs. The instrument can also be used in line with a Headspace/GC or Purge and Trap/GC.

The purpose of the electronic nose is to,

- Confirm a consumer complaint.
- Augment the human panel's findings.
- Identify the compound.
- Quantify the Odour.

The latter implies a huge cost in reduction on MS analyses [58,47]. Extensive studies on various parameters have been conducted at various laboratories including Alpha M.O.S Laboratories [64-69].

1.2.5 Dissolved Organic Carbon

Dissolved Organic Carbon (DOC) can affect the taste of water, particularly on chlorination. Organic carbon arising from soil acids (humic acids) is harmless. Organic carbon arising from industrial or sewage effluents is however undesirable. For South African water a 'recommended' limit for DOC of 5 mg/l (as carbon) and a 'risk' limit of 10 mg/l (as carbon) is suggested [70].

CHAPTER 3

MATERIALS AND METHODS

Protocol

Water samples were collected from the Vaal Dam, Vaal River Barrage and the Vaal River Catchment. Priority was given to those sample points whose DOC content was high. Their concentration ranged from 700 to 100 mg/l. Sampling points chosen for analysis included V9 (Rand Water Vaal River intake 1), V7 (Rand WaterVaal River intake 2), C-K25 (Klip River) and C-E13 (a stream at Boksburg). Refer to the map annexed as Appendix 1.11. Four off-line extraction techniques namely CLSA, SPME, SPE and LLE were utilized. The aim was to optimise methodology to analyse a wide range of organic compounds at trace levels in the water from the Rand Water Catchment area.

1.3.1 *Closed Loop Stripping Analysis*

700 ml of the sample was transferred to a 1000 ml stripping bottle. Approximately, 40 g of sodium chloride was added to increase extraction efficiency. A CLSA (Brecbühler AG, Switzerland) was used for stripping. A carbon filter with 1,5 mg of compressed activated charcoal was used as the adsorbent. Each sample was stripped for 40 minutes at 40°C (condenser temperature 50°C) Analytes were then desorbed from the charcoal using two 10 µl aliquots of carbon disulfide.

GC/MS Parameters :-

Instruments: -	Hewlett Packard 5890 GC, Hewlett Packard 5972 MSD
GC Column: -	J&W Scientific DB-5, 30m x 0.32mm i.d. x 0.25µm
Carrier Gas: -	Helium @ 0.800 ml/min
Injector Temperature: -	250°C
Initial Time: -	4.00 min
Oven: -	70 to 200°C @ 10°C /min, hold for 5.00 min
Interface Temperature: -	280°C
Ionisation Mode: -	Electron Impact
Scan Range: -	50 to 500 m/z
MS Library: -	Wiley 275

1.3.2 Solid Phase Micro Extraction

The coated fused silica fibres 75µm Carboxen/PDMS (Supelco, Bellefonte, USA) were successfully used for extraction and analysis in this study. The fibres were conditioned as prescribed by the manufacturer. Each sample was stirred at 40° C for a minimum of two hours with the fibre placed in the headspace. Approximately 10 g of sodium chloride was added to increase extraction efficiency. The analytes were then thermally desorbed in the GC injection port at 250°C.

GC/MS parameters were as described in section 1.3.2.1.

1.3.3 Solid Phase Extraction

Chromabond®C18 /6ml/500mg solid phase silica material (Düren, Germany) was utilised for extraction. Methanol, dichloromethane and organic free water were used to condition the

extraction cartridge prior to extraction. A 1000 ml filtered water sample was extracted on the sorbent at a flow rate of ± 5 ml/min. The sorbent was allowed to dry for 30 minutes and the adsorbed analytes were eluted using 6 ml of dichloromethane. The eluant was then concentrated to 0.1 ml under a gentle stream of nitrogen at 30°C.

GC/MS Parameters: -

Instruments: -	Hewlett Packard 5890 GC, Hewlett Packard 5972 MSD
GC Column: -	J&W Scientific DB-5, 30m x 0.32mm i.d. x 0.25µm
Carrier Gas: -	Helium @ 0.800 ml/min
Injector Temperature: -	250°C
Initial Time: -	4.00 min
Oven: -	70 to 150°C @ 10°C /min
	150 to 200°C @ 5°C /min, hold for 5.00 min
Interface Temperature: -	280°C
Ionisation Mode: -	Electron Impact
Scan Range: -	50 to 500 m/z
MS Library: -	Wiley 275

1.3.4 Liquid/Liquid Extraction

GC/MS Parameters: -

Instruments: -	Hewlett Packard 5890 GC, Hewlett Packard 5972 MSD
GC Column: -	J&W Scientific DB-5, 30m x 0.32mm i.d. x 0.25µm
Carrier Gas: -	Helium @ 0.800 ml/min
Injector Temperature: -	250°C
Initial Time: -	4.00 min
Oven: -	70 to 150°C @ 10°C /min
	150 to 200°C @ 5°C /min, hold for 5.00 min
Interface Temperature: -	280°C
Ionisation Mode: -	Electron Impact
Scan Range: -	50 to 500 m/z
MS Library: -	Wiley 275

1.3.5 Organic Profiling

Rand Water's raw, drinking and wastewater were sampled in clean 1 litre glass bottles. Samples were analyzed with the Electronic Nose, for organic pollutants using GC/MS and for DOC. Four groups were targeted for profiling.

Table 1.3.1 A list of compounds included in each group

Group1	Group 2	Group 3	Group 4
Volatile Organic Compounds	Phenolic Compounds	Polynuclear Aromatic Hydrocarbons	Sulphur-Containing Compounds
Chlorobenzene	Phenol	Naphthalene	Carbon Disulfide
Xylene	p-Cresol	Acenapthene	Dimethyl sulphoxide
Styrene	2,4-Xylenol	Flourene	
Bromobenzene	2,4-Dichlorophenol	Phenanthrene	
Chlorotoluene	2-Chlorophenol	Pyrene	
1,3-Dichlorobenzene	2,4,6-Trichlorophenol		
1,2-Dichlorobenzene	4-Ethylphenol		
	2,3,4,6-Tetramethylphenol		
	2,3,5,6-Tetrachlorophenol		
	Pentachlorophenol		

Structures and chemical formulae are shown in Appendix 1.2.

1.3.5.1 Group 1 Compounds using CLSA/GC/MS and the Electronic Nose

The CLSA apparatus used for these extractions was supplied by Brecbühler AG, Switzerland. 900ml of the sample and 72g sodium chloride (NaCl) were transferred to a 1000 ml stripping bottle. NaCl was added to increase extraction efficiency. A carbon filter with 1,5 mg of compressed activated charcoal was used as an adsorbent. Each sample was stripped for 90 minutes at 40°C (condenser temperature 50°C). Analytes were then desorbed from the charcoal using three x 10 µl aliquots of carbon disulfide.

GC/MS Parameters: -

Instruments: -	Hewlett Packard 5890 GC coupled to a Hewlett Packard 5972 MSD
GC Column: -	Zebron-5 MS, 30m x 0.25mm i.d. x 0.25µm
Carrier Gas: -	Helium @ 1.000 ml/min
Injector Temperature: -	200°C
Initial Time: -	2.50 min
Oven: -	35 to 100°C @ 10°C /min
Interface Temperature: -	250°C
Ionisation Mode: -	Electron Impact
Scan Range: -	SIM and Full Scan (50 to 500 m/z)
MS Library: -	Wiley 275

The compounds were identified using the Wiley 275 mass spectra database. Confirmatory match comparisons were performed using the following order of priority: Wiley (>85 %) Pmw_tox2 (>85%) Hppest.

For the Electronic Nose 300 ml of water was transferred to a 500ml Duran bottle. The sample was then equilibrated for at least 30 minutes at 25 °C before the analysis.

For analysis, purging was used. Humidified air (rh=40%) was purged through the water sample at 150 ml/min. The equilibrium time ranged between 185 and 205 seconds. This procedure was used to create the aroma patterns, which were then stored in the database.

1.3.5.2 Group2 Compounds using the Electronic Nose

The electronic nose parameters were the same as in 1.3.5.1

1.3.5.3 Group 3 Compounds using SPE/GC/MS and the Electronic Nose

Chromabond®C18ec /6ml/500mg solid phase silica material (Düren, Germany) was utilised for extraction. Methanol, ethyl acetate and organic free water were used for conditioning the column prior to extraction. Organic pollutants contained in a 1000 ml filtered water sample were extracted onto the sorbent at a flow rate of 3 ml/min. The sorbent was then dried for 30 minutes with nitrogen for 30 minutes. The adsorbed analytes were then desorbed with 6 ml of ethyl acetate. *The extract was concentrated down to 0.1 ml using a Turbovap (35°C) prior to GC/MS analysis.*

GC/MS Parameters: -

Instruments: -	Hewlett Packard 5890 GC coupled to a Hewlett Packard 5972 MSD
GC Column: -	*Zebron-5 MS, 30m x 0.25mm i.d. x 0.25µm*
Carrier Gas: -	Helium @ 1.000 ml/min
Injector Temperature: -	300°C
Initial Time: -	4.00 min
Oven: -	60 to 220°C @ 10°C /min, hold for 1.00 min
Interface Temperature: -	250°C
Ionisation Mode: -	Electron Impact
Scan Range: -	Selected Ion Monitoring

The electronic nose analysis was the same as in 1.3.6.1.

1.3.5.4 Group4 Compounds using the Electronic Nose

The Electronic Nose parameters were the same as in 1.3.5.1.

1.3.6 On-Line Solid Phase Extraction/Liquid Chromatography

The SPE cartridge was conditioned with 2.5 ml Methanol and 2.5 ml HPLC Grade Water at 2.5 ml/min. The system was flushed with 10 ml of sample before passing 50ml of sample through the cartridge. The cartridge was eluted on-line with by the HPLC eluent.

HPLC Parameters: -

Instrument: -	Hewlett Packard 1090 HPLC, Chemstation Software
Detectors: -	Diode Array (DAD), 210 and 254 nm, 0.005 bandwidth. Fluorescence
HPLC Column: -	LC-18 Chromabond, 250mm x 4.6mm, 5µm bonded silica
On-line Pre-treatment: -	PROSPEKT
SPE Pre-column: -	10mm x 2.0mm PTFE 100Å Styrene-Divinyl Benzene copolymer
Mobile Phases: -	A. Water (containing 1% Acetonitrile) B. Acetonitrile (containing 1% Water)
Gradient:-	50 % - 100 % B in 10 min, hold for 5.00 min, return to 50 % B in 2.50 min
Flow Rate: -	0.4 ml/min

25% of methanol modifier to the spiked aqueous sample was also evaluated.

See Appendix 1.3 for PROPEKT parameters and schematics.

1.3.7 In-Vial Liquid/Liquid Extraction and Large Volume Injection (LVI)

Hexane and ethyl acetate were distilled before use. Drinking water was spiked with 0.1 ng/µl of PAH prior to extraction. 1 ml of n-hexane was added to the sample and the sample together with the solvent were hand-shaken for 60 seconds and left to stand for 2 min to allow to layers to separate.

The organic layer was withdrawn from the cylinder using a 1500 µl syringe with a PFTE-coated plunger. After filling and mounting it on the Harvard pump, the sample was transferred to the on-column injector via a stainless steel needle. This extract was injected at the injection rate of 300 µl/min when the evaporation rate was at 200 µl/min. 100µl of the extract was injected into the system. The experiment was repeated using drinking water spiked with 0.1ng/µl.

Details of LVI theory are presented in Appendix 1.4.

The LVI GC system consisted of a Carlo Erba of an HPLC / GC equipped with an on-column injector and the Flame Ionisation Detector (FID). A mass flow meter was installed between the pressure regulator and the on-column injector, flow was determined by means of thermal conductivity and was independent of the pressure.

A 7m diphenyltetramethyldisilazane deactivated retention gap (0.53 mm I.D.) was connected to a 2 m retaining pre-column and a 25m x0.32mm x 1µm GC CP-Sil 80 column via a press fit connector and a T-piece respectively. The Solvent Vapour Exit was connected to a T-piece via an electronically controlled 6-port valve.

Injections were performed with an automated syringe pump using a 1500 µl syringe with a PFTE –coated plunger. After filling the sample was transferred to the on column injector via a stainless-steel needle.

GC Parameters: -

GC: -	Carlo Erba HPLC / HRGC
Retention Gap: -	2m un-coated 0,53mm
Retaining Pre-column: -	7m Diphenyltetramethyldisilazane, deactivated
Analytical Column: -	25m x 0.32mm x 1µm
Detector: -	Flame Ionisation Detector @ 300°C
Initial Time: -	5.00 min
Oven: -	69 to 280°C @10°C/min, hold for 3.00 min

See Appendix 1.5 for details on the optimisation procedure.

CHAPTER 4

RESULTS AND DISCUSSION

Curves and most of the tables have been included in Appendix 1.6, method validations are shown in Appendix 1.10.

1.4.1 Closed Loop Stripping Analysis

The results of the extraction are shown in Figure 1.4.1.1 and show a typical chromatogram of the Vaal River raw water (V9). Table 1.4.1.1 shows compounds detected. Figure 1.4.1.2 shows a chromatogram of the Klip River raw water (C-K25). Table 1.4.1.2 show the compounds identified using the above-mentioned databases.

Raw waters were problematic to analyse. Samples had to be filtered by vacuum suction prior to extraction; this led to the loss of very volatile analytes. In addition, a small glass wool plug had to be inserted at the inlet to the charcoal to prevent algae and other solids from entering the trap. Samples that were soapy could not be extracted using CLSA because during purging the foam that was generated contaminated the charcoal filter.

Compounds having retention times less than that of carbon disulfide compounds (C_2 to C_5 non-substituted alkanes) had to be excluded. Benzene could not be identified due to co-elution with carbon disulphide. Guardilola *et.al.* [34] was able to identify benzene.

Compounds identified in this study as seen in Table 1.4.1.1 and 1.4.1.2 included,

- VOC's such as toluene, ethylbenzene, xylene, styrene, chlorobenzene, and dichlorobenzene.
- Ketones such as heptanone.
- Phenolic compounds such as p-Cresol.
- (PAH's) such as naphthalene, 3-methyl-1H-indole, methylnaphthalene
- Other taste and odour producing compounds like menthol, limonene and beta-cyclocitral [22].

1.4.2 Solid Phase Micro Extraction

The results of the extraction are shown in Appendix 01, Figure 1.4.2.1 shows a typical chromatogram of the Vaal river raw water (V9). Figure 1.4.2.2 shows a chromatogram of the Elsburgspruit raw water (C-E13). Table 1.4.2.1 and table 1.4.2.2 respectively show the compounds identified using the above-mentioned databases.

Fibres were conditioned as specified by the manufacturer (Supelco). Full analysis was done on newly conditioned fibres to verify absence of inferences. Various coated silica fibres such as Polydimethylsiloxane (PDMS), Polyacrylate and Carboxen / PDMS fibre were utilised in this study. Only Carboxen / PDMS fibre produced satisfactory results.

Bao *et.al.* in a study of odour producing compounds also investigated the Carboxen / PDMS fibres. In their study, Carboxen / PDMS fibres were found to be suitable for the extraction of most of the analytes studied (with the exception of citral, beta-ionone and geranylacetone). The major problem encountered during their study was both peak tailing and resolution. PDMS/divinyl benzene was thus chosen for their study [22].

Compounds that were identified in this study as seen in Table 1.4.2.1 and 1.4.2.2,

- VOC's such as toluene,
- Phenolic compounds such as p-cresol,
- PAH's such as 3-methyl-1H-Indole,
- Odour producing compounds such as beta–cyclocitral
- Other compounds such as 3,7-dimethyl-2,6-octadiene, 6-octadiene, 2-ethyl-6-methyl-1,5-heptadiene, calarene, heptadecane, octadecane etc.

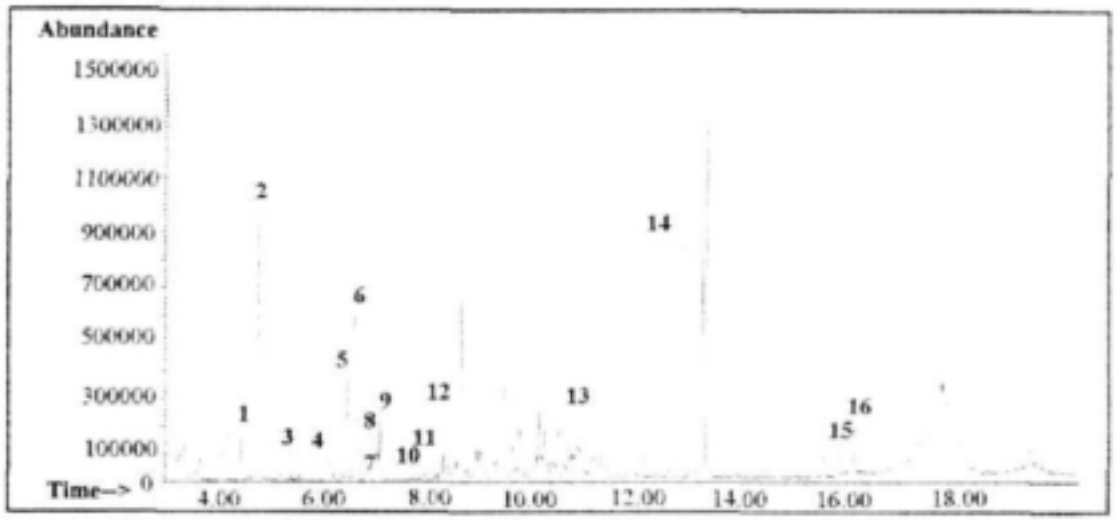

Figure 1.4.1.1 The chromatogram of Vaal River raw water (V9).

Table 1.4.1.1 List of compounds extracted by closed loop stripping.

No.	Compound Name	Retention Time (in min.)	Quality Match (in %)	Molecular weight (in g/mol)	Chemical Formula
1	3-Methylbutanol	4.11	72	88	$C_5H_{12}O$
2	Toluene	4.82	95	92	C_7H_8
3	Tetrachloroethene	5.58	94	164	C_2Cl_4
4	Chlorobenzene	6.22	91	112	C_6H_5Cl
5	Ethylbenzene	6.51	95	106	C_8H_{10}
6	Xylene	6.65	97	106	C_8H_{10}
7	Heptanone	6.87	90	115	$C_7H_{14}O$
8	Styrene	7.09	96	104	C_8H_8
9	Xylene	7.13	96	106	C_8H_{10}
10	1-methyl-4 (1-methylethyl)-cyclohexane, didehydro	8.00	76	136	$C_{10}H_8$
11	Benzaldehyde	8.38	90	106	C_7H_6O
12	Ethyltoluene	8.43	93	120	C_9H_{12}
13	*p-Cresol*	10.29	93	108	C_7H_8O
14	beta-Cyclocitral	13.27	98	152	$C_{10}H_{16}O$
15	Butylbutanoate	15.85	86	144	$C_8H_{16}O_2$
16	3-Methyl-1H-Indole	16.14	94	131	C_9H_9N

1.4.3 Solid Phase Extraction

The results of the extraction are shown in Appendix 01, Figure 1.4.3.1 shows a typical chromatogram of the Vaal river raw water (V9). Table 1.4.3.1 show the compounds identified using the above-mentioned databases. Results from the same sampling point are shown in Figure 1.4.3.2 and Table 1.4.3.2.

Only Chromabond C18 / 6ml / 500mg was utilized in this study, because it is relatively inexpensive and had a wide variety of applications. All raw samples were filtered prior to SPE extraction. The advantage of this extraction technique is that twelve samples can easily be processed simultaneously. In the study conducted by .Shepard *et.al* for analysis of atrazine, they were able to extract 1000 samples in 18 months using only 10ml of ethyl acetate and 10ml methanol per sample [35].

Compounds identified in this study include,

- Phenolic compounds such as 2,3,6-trichlorophenol and 2,-bis (1,1-dimethyl)-4-methylphenol
- Organic acids such as dodecanoic acid and tetradecanoic acid
- PAH's such as 1H-indole, 3-methyl-1H–indole and 5,6,7,9-tetrahydro-4,4,7-trimethyl(4H)-benzofuranone
- Alkanes such as eicosane, decane, tetradecane and pentadecane
- Other compounds such as 2-(ethoxyethoxy)-ethanol, 2-ethylhexanol and 1–(aminophenyl)-ethanone etc.

1.4.4 Liquid/Liquid Extraction

Please see Appendix 1.6.

The chromatogram of the pentane static LLE is shown in Figure 1.4.4.1 (Vaal River raw water [V9]). Table 1.4.4.1 shows the compounds identified.

Results for Elsburgspruit (C-E13) using a continuous LLE using hexane are shown in Figure 1.4.4.2 and Table 1.4.4.2.

A continuous LLE using dichloromethane was used to extract Vaal River water (V7), the chromatogram and compounds detected are shown in Figure 1.4.4.3 and Table 1.4.4.3.

A continuous pentane LLE was used to extract Vaal River water (V9), the chromatogram and compounds detected are shown in Figure 1.4.4.4 and Table 1.4.4.4.

Two types of LLE techniques were utilised in this study. Static LLE (SLLE) is fast and uses minimal solvent compared to Continuous LLE (CLLE). Three solvents were used namely hexane, pentane and dichloromethane. In SLLE samples were shaken in an ultrasonic bath. Specialised glassware was used for CLLE.

CLLE was time consuming (1litre sample took more than 24 hours to extract) but proved to be the most useful one in preliminary profiling. No filtration was necessary for CLLE. Fortunately, Goosens in her study developed an automated on-line LLE. She further suggested that this method demonstrated practicability in the approach for trace-level detection of unknown organic micropollutants in the screening of ground water samples.

Compounds identified in this study as seen in Table 1.4.4.1 to 1.4.4.4 include,

- VOC's such as methanol-benzene, ethylbenzene, chlorobenzene and ethylxylene.

- Ketones such as 4-hydroxymethylpentanone.
- Phenolic compounds such as phenol and p-cresol.
- PAH's such as 1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene, 3-methyl-1H-indole, methylnaphthalene, 1H-indole, 1H-iso-indole-1,3(2H)-dione
- Other taste and odour producing compounds like beta-cyclocitral [24].
- Alkanes such as tetradecane, pentacosane, tridecane, dodecane etc.

1.4.5 Organic Profiling

1.4.5.1 Group 1 Compounds using CLSA/GC/MS and the Electronic Nose

Ions monitored and their retention times are as shown in table 1.4.5.1

Table 1.4.5.1 Group 1 compounds showing the Selected Ions monitored and their retention times

No	Compound Name	Ions selected	Retention time in min
1	Chlorobenzene	112	6.87
2	Xylene	106	8.15
3	Styrene	104	8.08
4	Bromobenzene	156	9.23
5	Chlorotoluene	126	10.00
6	1,3-Dichlorobenzene	146	11.69
7	1,2-Dichlorobenzene	146	12.38

The compounds were identified using the Wiley 275 mass spectra database. Confirmatory match comparisons were performed using the following order of priority: Wiley (>85 %) Pmw_tox2 (>85%) Hppest.

For Group 1 compounds CLSA was chosen as the final method for extraction. Purge and Trap was not considered as it was not available at the time that the project was initiated and Headspace analysis could not be investigated.

LLE was not suitable because of the concentration step required for analyte enhancement. Modification of the GC inlet systems to facilitate large volume injections is an alternative that should in the future be evaluated. LLE should still be considered for this type of analysis as it is a well-established and reliable extraction procedure that does not require expensive and sophisticated instrumentation. Headspace SPME was suitable for the extraction of VOC's (using polydimethylsiloxane / divinylbenzene fibres). CLSA was chosen because of better automation and reduced extraction times.

Samples were either analysed immediately or stored at 4°C until they were analysed. Prior to analysis carbon disulfide was analysed to ensure that no interfering compounds were present. Catchment and sewage samples required vacuum filtration; this may have led to the loss of very volatile analytes. A small glass wool plug had to be inserted on the trap inlet to prevent contamination of the trap. As discussed previously some sewage samples were excluded because of excessive foaming that was detrimental to the trap.

Each extract was injected twice, in Selected Ion Mode for target analysis and in Full Scan Mode for the determination of unknown compounds. Priority was given to SIM mode, carbon disulfide is

extremely volatile the extract evaporated before it could be injected for the second time using Full Scan mode.

1.4.5.2 Group 2 Compounds using the Electronic Nose

No GC/MS analysis was carried on these compounds.

1.4.5.3 Group 3 Compounds using SPE/GC/MS and the Electronic Nose

Ions monitored and their retention times are as shown in table 1.4.5.2.

Table 1.4.5.2 A list of Group 3 compounds showing the ions monitored and their retention times

No	Compound Name	Ions selected	Retention time (in min)
1	Napthalene	128	11.57
2	Acenapthene	153	15.61
3	Flourene	166	16.91
4	Phenanthrene/Acenapthene	178	19.28
5	Pyrene/Flouranthene	202	22.47

For Group 3 offline SPE was chosen as the most suitable method for extraction. SPME yielded poor recoveries for PAH's. LLE resulted in similar recoveries to those obtained with SPE, disadvantage of LLE is the formation of emulsions and the consumption and subsequent disposal of toxic solvents (relative to SPE).

Samples were only analysed in the SIM. All of the samples analysed displayed Total PAH concentrations of less than 15 ng/l. This is less than the recommended limit of 1 µg/l for drinking water. Odour analysis was not run with this group of compounds.

1.4.5.4 Group 4 Compounds using the Electronic Nose

Dimethyl Sulphoxide (DMSO) was selected as a sulphur-containing compound to monitor for because of its widespread use in industry. It is commonly used as solvent for Orlon, acetylene, sulphur dioxide and other gases. It is also used in paint and varnish [85]. Dimethyl sulphide, dimethyl trisulphide and hydrogen sulphide are naturally occurring microbial odorous substances of importance in the water industry 59]. Their production from a range of bacterial and fungal strains has been reported by Kadota and Hebert [61,72].

No GC/MS analysis was carried out for this group of compounds

Results are shown in Appendix 1.7.

1.4.6 Odour Scanner

The database of each sample is plotted on a 2-dimensional Sammon map where the 32 sensor array is condensed to one point. Sammon mapping is non-linear transformation that reduces the high dimensional space of a pattern X to a 2- or 3- dimensional space of pattern Y. The axes

Sammon mapping represent Euclidean distances. Similar aroma patterns exist in one area. The clusters of different aroma patterns should be separated to such extent that discrimination between the different samples can be identified. The degree of separation is measured by the Quality factor(QF) (see Appendix 1.7). Aromascan recommends that it is a good separation between clusters when the Quality Factor (QF) is equal to or greater than two. In other words, the smaller the QF the more similar the aroma patterns.

The samples were compared with the standard map of the day analysis (see Appendix 1.9). The results were then compiled as in Appendix 1.7. Shades were used to interpret the data in which the QF and the visual position of the Sammon map were taken into consideration. Samples marked with darker shades show a close relation ship between the standard and the sample. The QF is between 0 and 1 and the cluster overlap. Samples marked with less darker shades show a partial relation ship with between a standard and a sample. The QF is between 1 and 2 and the cluster areas are close or partially overlapping. Samples, which are not marked, show no relationship between a sample and a standard. The QF is greater than 2 and the cluster areas are well separated.

Samples marked with a lighter and/or darker for two standards are more likely to contain compounds of both standards. Samples marked with a lighter and/or darker for the blank and a standard are likely to contain compounds of that standard but at a very low concentration. Samples, which are not marked for all standards and show a high QF contain compound not covered within the standards at higher concentrations.

From the results it can be observed that electronic nose technology has the potential to be a successful management tool for the water industry. Odour analysis, including sample preparation of all four groups including blanks, can be completed with seven minutes. In GC analysis, only one group of compounds could be analysed at time. A recommended sensor for an electronic nose is Metal Oxide Sensor (MOS) because they have been found to show best sensor stability. This is important since once the electronic nose system is set-up with the standard groups only one analysis per sample should be sufficient to classify the sample into different standard groups. *Various classification methods are available such as Euclidean distance, mahalanobis distance* and coefficient of correlation; principal component analysis, linear discriminate analysis and neural network (based on kohonen net) are available. If a sample is classified for specific standard group (e.g group1) the analytical techniques required for the analysis in the laboratory can be focused on the compounds of interest.

1.4.7 On-Line SPE/Liquid Chromatography

On-line SPE/LC/DAD/FLD sampling and analysis was performed nine times overnight to determine the reproducibility of this analysis. A typical chromatogram is shown in figure 1.4.6.1. (Appendix 1.6) the statistical data is shown in table 1.4.7.1, fluorene and acenaphthene co-eluted.

Table 1.4.7.1 Mixture of PAH's run at 2μg/l

Analysis No.	Napthalene	Acenaphthene/ Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Chrysene
1st	1.34	1.82	1.85	1.90	1.64	1.58	1.58
2nd	1.30	1.93	2.05	2.17	1.93	1.89	3.00
3rd	1.35	1.93	2.08	2.08	1.92	1.92	3.12
4th	1.27	2.15	2.09	2.16	2.01	1.94	3.57
5th	1.35	1.97	2.15	2.09	1.90	1.98	3.55
6th	1.32	1.88	2.14	2.09	1.93	1.82	3.77
7th	1.35	2.01	2.05	2.11	1.88	1.93	3.72
8th	1.20	1.92	2.10	2.10	1.93	1.89	3.58
9th	1.35	1.76	2.00	2.06	1.88	1.83	3.67
Mean	1.31	1.93	2.05	2.08	1.89	1.86	3.29
STD	0.05	0.11	0.09	0.08	0.10	0.12	0.69
%RSD	3.82	5.78	4.45	3.80	5.37	6.25	21.08

25% of methanol was added to 2 μg/l of tap water as a modifier. The aim was to minimise sorption of PAH's to the "on-line system" (tubing, valves, etc.). The comparison of the chromatograms resulting from 100% water and 25% methanol is shown in Fig 1.4.6.2 (Appendix 1.6).

1.4.8 In-Vial Liquid/Liquid Extraction and Large Volume Injection

Optimised parameters determined in section 1.3.8 were used. The chromatogram is as shown in figure 1.4.6.3 (Appendix 1.6). The bottom chromatogram shows the blank unspiked drinking water. The upper chromatogram shows drinking water spiked with 0.1ng/μl of PAH's. The PAH's used in this case were fluorene, pyrene, phenanthrene, acenaphthene, anthracene and fluoranthene.

CHAPTER 5

Conclusions

Methodology was developed to analyse and to the monitor the GC fraction of organic pollutants in source and drinking waters.

The Volatile Organic Compounds are ideally analysed using CLSA, Purge and Trap or Headspace Analysis. These compounds were designated Group 1 compounds. CLSA was chosen as the final method for extraction.

Semi-Volatile Organic Compounds, such as PAH's, were designated as Group 3 compounds. SPE was chosen as the most suitable method for extracting of these compounds. XAD resins were excluded because they required extensive resin clean up prior to extraction. SPME yielded poor recoveries for PAH's. LLE resulted in similar recoveries to those obtained with SPE. The disadvantage of LLE was the formation of emulsions and the consumption and subsequent disposal of toxic solvents (relative to SPE).

Profiles of Groups 1 and 3 compounds using the GC methodology described above were also determined. Odour profiles were established for group 1 to 4 compounds. The profiles acquired were used to monitor sample profile differences on the basis of mass spectra and sensory characteristics, refer to Appendix 4 and 5. Detection Limits of the Electronic Nose are in the low µg/l levels for the compounds investigated.

A possible alternative to GC/MS would be to couple GC to Flame Ionisation, Eletron Capture and Nitrogen Phosphorus Detectors (FID, ECD, and NPD respectively) instead of MS analysis. MS is expensive relative to FID, ECD and NPD. These detectors will not facilitate the identification of unknown compounds but are useful in Target Analysis i.e. ECD for halogenated compounds (organnochlorine pesticides, THM's, ect.), NPD for compounds containing nitrogen and phosphate (organnophosphate pesticides) and FID (although less sensitive that ECD and NPD is suitable for organic compounds including BTEX's and PAH's). This screening data, in conjunction with the Electonic Nose, could provide valuable data to be used in the Management Tools discussed.

Dissolved Organic Carbon (DOC) can be utilised as a Management Tool to screen water samples for gross organic contamination. DOC values for drinking waters are normally in the range of 3 to 5 mg/l while source waters can vary between about 8 and 10 mg/l. Monitoring DOC values can thus indicate if a significant organic pollution incident has occurred. Organic contaminants are usually present at much lower concentrations *i.e.* µg/l amounts. Geosmin, for example, can be detected by odour panels at around 30 µg/l and at these levels would not be detected by monitoring DOC values of the waters.

Total Organic Halogen (TOX) values may also be determined, and could also provide a useful Management Tool to screen water samples. TOX values obtained for drinking waters are usually between 30 to 50 µg/l and would thus be more likely to indicate contamination with halogenated organic pollutants at low levels.

Organisations having MS could assist smaller municipalities in developing screening methodologies. For example, MS could be used develop target analyses to run on GC/ECD/NPD/FID systems on behalf of the smaller municipalities that may not have access to MS.

SECTION 2 LIQUID CHROMATOGRAPHY

CHAPTER 1

INTRODUCTION

The aim of this section of the project was to develop High Pressure Liquid Chromatography (HPLC) methodology to analyse source (raw) and drinking water for organic pollutants.

Organic pollutants are generally present at low concentrations (low µg/l) and methodologies to determine these compounds must for this reason incorporate a concentration step.

The traditional method for the extraction and subsequent determination of organic compounds in waters centred mainly on Liquid / Liquid Extractions (LLE) and involve the partitioning of organic compounds into an organic solvent and the subsequent concentration of the organic solvent to facilitate the determination of trace level contaminants. These methods utilise fairly large amounts of organic solvents (usually between 100 and 150 ml) that are both expensive to purchase and expensive to dispose of. Solvents of very high purity must be used because of the concentration step and must generally be redistilled in the laboratory. LLE also tend to be fairly labour-intensive and thus expensive.

The most widely accepted alternative to LLE are Solid Phase Extractions (SPE). SPE utilises a solid adsorbant to selectively remove compounds from a liquid phase, in this case water. Adsorbed compounds are then desorbed from the adsorbant with an organic solvent. Volumes of solvent are typically less than 10 ml. The volume of the solvent is then reduced to facilitate trace analysis.

Although SPE has many advantages over the more traditional LLE it is still neither quick, nor is it a cheap method. An alternative extraction procedure was thus also investigated, Solid Phase MicroExtraction (SPME). SPME utilises a fibre coated with an adsorbant to extract organic compounds from liquid samples. The fibre is then placed into a desorption chamber where the absorbed organic compounds are desorbed onto an HPLC column for subsequent analysis.

Traditional HPLC analyses normally utilise Diode Array Detection (DAD). Other detectors are also used *e.g.* fluorescence detectors are used in the determination of Polynuclear Aromatic Hydrocarbons and offer the advantage of both selectivity and sensitivity. These detectors indicate the possible identity of a compound but will not assist in the identification of completely unknown compounds, for this Mass Spectrometry (MS) is required.

The Particle Beam is an interface between an HPLC and a Mass Spectrometer that facilitates the generation of Electron Impact Mass Spectra that are searchable against Mass Spectral libraries (such as the Wiley library) and enable the identification of unknown compounds.

Because MS is expensive the ideal method would involve a method utilising DAD as a screening procedure and MS to confirm suspected contaminants and to identify unknown compounds.

CHAPTER 2

LITERATURE SURVEY

2.1 Particle Beam Interface

Particle Beam/Mass Spectrometry (PB/MS) has been employed to evaluate its usefulness in the identification of compounds by virtue of the (Electron Impact) EI spectra generated with the Particle Beam Interface (PBI). The technique was originally described as "Monodisperse Aerosol Generating Interface for Chromatography" (MAGIC) and enables the coupling of a wide range of HPLC separations to conventional EI and Chemical Ionisation (CI) MS fragmentation procedures. HPLC/PB/MS is used mainly for the identification of non-target compounds in real-world matrixes because of the library searchable EI spectra generated.

Of all the HPLC/MS interfacing methods, HPLC/PB/MS comes closest to GC/MS. The PB interface is principally a momentum separator and as such is derived from the jet-type GC/MS interface used with packed GC columns (10). Desolvation occurs in steps by leading the HPLC column eluent through several differentially pumped chambers, see Appendix 2.1.

As described earlier two closely related drawbacks of HPLC/PB/MS are the low sensitivity and the non-linearity of the response. The low sensitivity is due to the low analyte transmission efficiency of the interface, which typically lies between 0.5 and 1%. The analyte transmission efficiency and the non-linearity of the response are commonly thought to be related to the efficiency of the formation of solid particles in the evaporation process but no evidence to support this has yet been provided.

Compounds such as maleic acid and ammonium acetate have been added to the HPLC eluent to improve particle formation at low analyte concentrations, the rationalisation behind this approach is that a compound, specific or non specific, carrier effect can be achieved. Such enhancements are not always observed and appear to differ for each analyte.

General acceptance of the PBI in environmental analysis will require considerably improved detection limits, typically in the 10 to 500 ng range for PB, and non-linearity of detection [16, 17]. These limits are often not sufficiently low and measures to pre-concentrate are crucial.

2.2 Extraction Techniques

The extraction techniques investigated and evaluated for use in this project included,

Solid Phase Extraction (SPE)
Solid Phase Micro Extraction (SPME)
Liquid/Liquid Extraction (LLE)

None of these techniques are particularly novel and are discussed in any detail here. Detailed descriptions, and their application to LC are included in Appendix 2.2.

CHAPTER 3

MATERIALS AND METHODS

2.3.1 Optimisation of the Particle Beam Interface

2.3.1.1 Optimisation of Modifier in LC Effluent

The sensitivity of the PBI can be improved by the addition of various modifiers to the HPLC eluent. 0,4mM maleic acid in the eluent [11, 12] was found to result in signal enhancements of up to 30 fold and to improve linearity of the response while 0,01% ammonium acetate added to the eluent was also found to enhance the signal [13]. Other studies using 0,1M ammonium acetate were also reported to enhance response and linearity of PBI [14, 15] but evaluation was difficult as the increased concentration of ammonium acetate was found to lead to deposits on the cones in the interface.

HPLC conditions: -

HPLC	HP1090 Series 2
HPLC Column	Phenomenex Luna 5 micron C18, 250x2,00 mm
Oven Temp	40 °C
Flow Rate	0,3 ml/min
Wavelength	250 nm, bandwidth = 80 nm
Mobile Phase	Water Acetonitrile (50:50 to 0:100 in 10 minutes, hold for 5 minutes)
Injection Volume	25ul (25ul of 50 ng/ul i.e. 1250 ng)
Modifiers Evaluated	0,4 mM Maleic Acid
	1,3 mM Ammonium Acetate
	3,3 mM Ammonium Acetate
	Unmodified eluent

Particle Beam / Mass Spectrometer Conditions:-

Helium Pressure	18 psi
Interface (desolvation) Temperature	65 °C
Ion Source Temperature	250 °C
Analyser Manifold Temperature	100 °C
Mass Range	50 to 400 amu
EMV Offset	509.6V

2.3.1.2 Optimisation of the Particle Beam Temperatures

Because the LC effluent was known to have a high aqueous content the PBI temperature was varied between 55 and 65 °C to determine the optimum temperature for the compounds selected for this study.

Other conditions were as described in 2.3.1.1.

2.3.2 Optimisation of Liquid Chromatography

The effect of varying the gradient from that described in 2.3.1.1 was investigated to determine if improvements in both separation and signal to noise ratio could be achieved.

The LC effluent was passed through both a Diode Array Detector (DAD) and the PBI into the MS to determine the usefulness of the DAD as a cheaper means of analysis for preliminary screening for organic pollutants in water samples.

HPLC Parameters:-

HPLC	Hewlett Packard 1090 Series 2
Column	Phenomenex Luna 5 micron C18, 250x2,00 mm
Flow	0,4 ml/min
Oven Temperature	40 °C
DAD	250 nm, Bandwidth = 80 nm
Mobile Phase	Water:Acetonitrile (70:30 to 40:60 in 30 minutes and 0:100 in a further 10 minutes)
Modifier	3,3 mM Ammonium Acetate
Injection volume	10 µl

PBI and MS parameters were unchanged.

The above MS methodology was slightly modified to run in the Selected Ion Monitoring (SIM) mode to facilitate greater sensitivity. In SIM only selected ions are monitored and this allows for greater sensitivity, typically 100 times more sensitive (this is compound dependant).

Two ions were chosen for each compound, a target ion (specific to that compound) and a qualifying ion. The ions chosen are shown in Table 2.3.1.

Three standards were prepared to determine linearity of the curve, namely 20;50; and 100 ng/ul. A second calibration curve using standards of lower concentrations was also prepared to determine the region in which the curves for the individual compounds was linear i.e. 10, 20, 30, 40 and 50 ng/µl.

Table 2.3.1 Ion selection for Selected Ion Monitoring

Compound	Retention Time (min)	Target Ion (m/z)	Qualifying Ion (m/z)	Class of Compound
1. Carbendazim	3,74	191	159	Carbamate
2. Thiabendazole	4,41	201	174	Fungacide
3. Bromacil	6,82	205	127	Herbicide
4. Monouron	7,20	198	72	Phenylurea Deriv.
5. Simazine	7,60	201	186	Triazine Deriv.
6. Carbofuran	9,64	164	149	Carbamate
7. Pirimicarb	10,67	66	72	Carbamate
8. Carbaryl	10.73	144	115	Carbamate
9. Atrazine	11,51	200	215	Triazine Deriv.
10. Diuron	12,16	232	72	Phenylurea Deriv.
11. Proparil	16,00	217	161	Herbicide
12. Propazine	16,42	229	214	Triazine Deriv.
13. Methiocarb	17,55	168	153	Carbamate
14. Terbuthylazine	17,62	229	214	Triazine Deriv.
15. TMTD	18,97	166	88	Fungacide
16. Prometryne	21,15	241	184	Herbicide
17. Imazalil	21,33	215	173	Fungacide
18. Dodin	24,31	228	128	Fungacide

2.3.3 Examination Of Pyrethroids

A mix of pyrethroids (of unknown concentration) was injected onto HPLC and monitored using PBI/MS to determine the effectiveness of the PBI for these compounds.

The instrumental parameters used were as for Section 2.3.2.

2.3.4 Extraction Techniques

2.3.4.1 Solid Phase Extraction

5 classes of compounds were selected for examination using SPE. These compounds included herbicides, fungicides, carbamates and pyrethroids. 500ng/µl stock solutions of the individual compounds, and mixes of each of the above products were prepared. The mixes were prepared in methanol and diluted 5 times *i.e.* 10ml up to 50ml (except for the pyrethroids which were supplied as a mix and was too dilute for any further studies except for HPLC/PB/MS). Table 2.3.1 shows the compounds that were examined.

2.3.4.1.1 Comparison of Sorbants

Two sorbants (Macherey-Nagel) supplied by Separations(Pty) Ltd., were evaluated with the set of compounds identified in the previous section.

i. Chromabond C18ec / 3 ml / 500 mg. This material is made up of octadecyl modified silica that has been endcapped *i.e.* residual silanol groups have been modified with short-chain alkyl groups. This is a very apolar phase and facilitates hydrophobic interactions with many organic compounds.

Column Conditioning	5 ml methanol followed by 5 ml deionised water.
Sample Application	1000 ml sample passed through the column under vacuum at flow rate of 5,6 ml/min. The sorbant was allowed to dry for ten minutes under vacuum.
Elution	2 x 1 ml methanol/acetone (3:2 v/v) and evaporated down to 250 µl using nitrogen at room temperature.

ii. HR-P is a highly porous adsorbant resin based on polystyrene-divinylbenzene for solid phase extraction of phenols and pesticides from water. The material has a very high binding capacity of about 30% of the adsorbant weight.

Column Conditioning	5 ml methanol followed by 5 ml deionised water.
Sample Application	1000 ml sample passed through the column under vacuum at a flow rate of 11 ml/min. The sorbant was allowed to dry for ten minutes under vacuum.
Elution	2 x1 ml methanol/acetone (3:2 v/v) and evaporated down to 250 µl using nitrogen at room temperature.

Recoveries were determined in triplicate for each of the sorbants. For each sorbent (HR-P and C18ec) a 1000ml deionised water blank was extracted.

Methodologies were obtained from Application Notes supplied by Macherey-Nagel (136,148 and 155).

Instrument parameters used were the same as those in Section 2.3.2 except that the MS was run in the Selected Ion Monitoring mode (EMV Offset = 509.6V)

2.3.4.1.2 Extract Concentration Volume

To reduce the volume of extracts by concentration would be an advantage as the lower the volume, the more concentrated the analyte would be resulting in lower detection limits.

50 µl of the 100 ng/µl mixed stock standard was added to four test tubes (the same as the tubes used for the SPE desorbtions).

1ml of desorption solvent (methanol:acetone, 3:2 v/v) was added to each of the tubes and the solvents blown gently with a stream of nitrogen at room temperature to final volumes of 50, 100, 200 and 500 µl. resulting in concentrations of 100, 50, 25 and 10 ng/µl respectively.

10 µl of each solution was injected (conditions as in Section 2.3.2).

2.3.4.2 Solid Phase Micro Extraction

Three different fibres were compared to determine which would be most effective for the extraction of organic compounds for subsequent HPLC analysis. The fibres compared were the Polydimetholsiloxane (PDMS), the Carbowax/Templated Resin (CW/TPR) and Polydimethylsiloxane/Divinylbenzene (PDMS/DVB).

Additional parameters investigated included analyte concentration, extraction volume, extraction time and extraction temperature.

All results are expressed as percentages for comparative purposes as direct quantitation with SPME is not possible.

General Extraction Procedures

De-ionised water was spiked to a final concentration of 50 µg/l with a mixture of fungicides, herbicides and carbamates. The solvent was allowed to evaporate before the de-ionised water was added to minimise the effect of the solvent on the extraction procedure. Sodium chloride was added to the water (10%, m/v) to increase the ionic strength. A de-ionised water blank was used to establish the cleanliness of fibres and was prepared by addition of sodium chloride (10%, m/v) to de-ionised water.

Static,static/dynamic and dynamic desorptions were compared and found to result in similar desorption efficiencies. For this reason a ten-minute static desorption was chosen as it allows for the removal of the fibre from the SPME/HPLC interface after the ten-minute desorption period is complete. The fibre can then be used for further extractions while the previous extract is analysed on the HPLC (conditions as in Section 2.3.2).

2.3.4.2.1 Comparison of PDMS, PDMS/DVB and CW/TRP fibres

Samples were extracted for 45 minutes (vigorous stirring with a glass covered magnetic stirrer bar) at room temperature, 23 °C to 26 °C, HPLC conditions were as described in Section 2.3.2.

2.3.4.2.2 Analyte Concentration

Samples of varying concentration of analyte were extracted using the adsorption/desorption parameters and HPLC conditions described in Sections 2.3.2. Concentrations of the analyte examined were 5; 10; 20 and 50 µg/l.

2.3.4.2.3 Extraction Volume

A spiked solution containing 20 µg/l was prepared and various volumes of this solution were extracted in order to determine optimal extraction volumes. The adsorption/desorption parameters and HPLC conditions described in Sections 2.3.2 were again used. Volumes examined were 10; 30 and 80ml.

2.3.4.2.4 Extraction Time

Samples containing the same concentration (20 µg/l) of analyte were extracted for different periods using the same adsorption/desorption parameters and HPLC conditions described in Sections 2.3.2.

Samples were extracted for 30; 45 and 75 minutes respectively.

2.3.4.2.5 Extraction Temperature and Time

Samples of the same concentration (20 µg/l) of analyte were extracted for different times at 6°C using the same adsorption/desorption and HPLC parameters described in Sections 2.3.2. For comparative purposes the results at 6°C (45, 90 and120 minutes) were compared to those extractions carried out at 24°C (45 minutes extraction time).

2.3.4.3 Liquid/Liquid Extraction

The extraction was carried out using ethyl acetate as the organic extractant. Five litres of a spiked solution were prepared. (500 µl of the 100 ng/µl mixture (described in 3.1) was added to 5 litres of de-ionised water). This solution was used to determine the recoveries for liquid/liquid extractions.

One litre samples were extracted according to the following procedure:-

i) A 1 litre aliquot of spiked solution was placed in 2 litre separating funnels.
ii) 50 g sodium chloride was added to the sample and dissolved.
iii) The pH of spiked solution was lowered to 2 by the addition of 2 ml concentrated sulphuric acid.
iv) 100 ml ethyl acetate was added to the solution and shaken vigorously by hand for two minutes.
v) The organic and aqueous phases were allowed to stand for 5 minutes until they had separated and the lower aqueous phase removed to a 1 litre glass beaker.
vi) The upper organic layer was then filtered through Whatman 541 filter paper (containing ± 2 g anhydrous sodium sulphate to remove residual water).
vii) The aqueous phase was returned to the separating funnel and extracted with two further 50 ml aliquots of ethyl acetate.
viii) The ethyl acetate extracts were pooled and concentrated down to approximately 1 ml using a Turbovap (0,8 bar nitrogen at 40 °C).
ix) Extracts were reduced to 100 ul with a gentle stream of nitrogen.
x) Extracts were then analysed using SIM on the HPLC/PB/MS (as in Section 2.3.2).

2.3.5 Analysis of Source and Finished Waters

Raw and final waters from the Vereeniging, Molopo and Cape Metro were subjected to target analysis using the methodology described in 2.3.4.1.1 (SPE using C18ec Chromabond cartridges). As described in the method, Selected Ion Monitoring was used to optimise the

sensitivity of the method for target analysis. A deviation from the method was that the injection volume was increased from 10 to 25 µl to lower detection limits.

Raw and final waters from the Vereeniging and Zuikerbosch Pump Stations were analysed during October and November in 2001 using the methodology described section 2.3.4.1.1, *i.e.* SPE using the C18ec Chromobond cartridges that resulted in the greatest recoveries of the spiked compounds. The only instrument parameters that were changed from the methodology were that the volume injected onto the HPLC was again increased from10 to 25 µl (to improve sensitivity) and the scan mode of the MS was changed from Selected Ion Monitoring to Full Scan. Full Scan facilitates the detection and identification of unknown compounds while Selected Ion Monitoring is more suited to Target Analysis.

The extracts were analysed in series using a Diode Array Detector (DAD) and the Particle Beam Mass Spectrometer (PBMS). A major shortcoming was that the DAD available for the project was not computerized and was only connected to an integrator to monitor the signal.

Method blanks were analysed to determine influences of the solvents and the C18ec SPE cartridges. Spiking and recoveries of the method were also checked during the survey to ensure that was performing satisfactorily. Also, the mixed standard was also analysed with each batch of extracts to monitor performance of the particle beam and the MS.

CHAPTER 4

RESULTS AND DISCUSSION

2.4.1 In-Vial Liquid/Liquid Extraction and Large Volume Injection

Hewlett Packard recommends that spectral averaging and background subtractions should be utilised in PBI analyses. Use was made of the EI Sensitivity Tune, which incorporates the High Energy Diode tuning parameters that select against low masses that are associated with Liquid Chromatography mobile phases. No further manipulations of tuning parameters to boost sensitivity were carried out.

2.4.1.1 Optimisation of Modifier in LC Effluent

For the comparison of the effect of modifiers on chromatography and signal enhancement raw chromatograms are utilised, *i.e.* no spectral averaging or background subtraction (as described in Section 2.4.1).

Chromatograms are shown in Figures A 2.3.1 to A2.3.8 in Appendix 2.3.

As can be seen from the chromatograms 0,4 mM maleic acid resulted in better responses than those observed with 3,3 mM ammonium acetate for many of the compounds evaluated. It also however resulted in a higher background signal than that observed with the ammonium acetate. As discussed previously 0,1 M ammonium acetate was also evaluated but found to block the holes in the cones of the PBI and was thus unsuitable. Blockages in the momentum separator cones was characterised (ironically) by a marked improvement in vacuums of the PBI and no signal.

2.4.1.2 Optimisation of the Particle Beam Temperatures

Temperatures in excess of 65°C caused compound degradation, see Figure A 2.3.9 and Figure A2.3.10 in Appendix 2.3.

2.4.2 Optimisation of Liquid Chromatography

The chromatography (as well as the signal to noise ratio) was improved by the addition of 3,3 mM ammonium acetate to the mobile phase and slowing down the gradient as follows,
Water (plus modifier) Acetonitrile (60:40 to 40:60 in 15 minutes and 0:100 in a further 5 minutes).

Note that the ammonium acetate also resulted in increased sensitivity without increasing the baseline as the 0,4mm maleic acid did. Results are shown in Figures 2.4.2.1 and 2.4.2.2.

UV traces are included and show retention times of 0,2 to 0,3 minutes quicker than those observed with the PBI due to the additional time taken to reach the MS through the PBI. UV spectra of peaks detected are included to facilitate rapid identification (probable) of peaks identity in the absence of MS data. This would facilitate screening of extracts using only DAD detection and subsequent MS analysis, which is expensive, only if it is necessary.

Mass spectra of the compounds selected for the study and library searches (Wiley Library, 275) are included in Appendix 2.4 (A2.4.1 to A2.4.17) to show the agreement of the PBI generated mass spectra with traditional EI mass spectra. UV specta of the compounds are shown in Appendix 2.6 (A2.6.1 to A2.6.5).

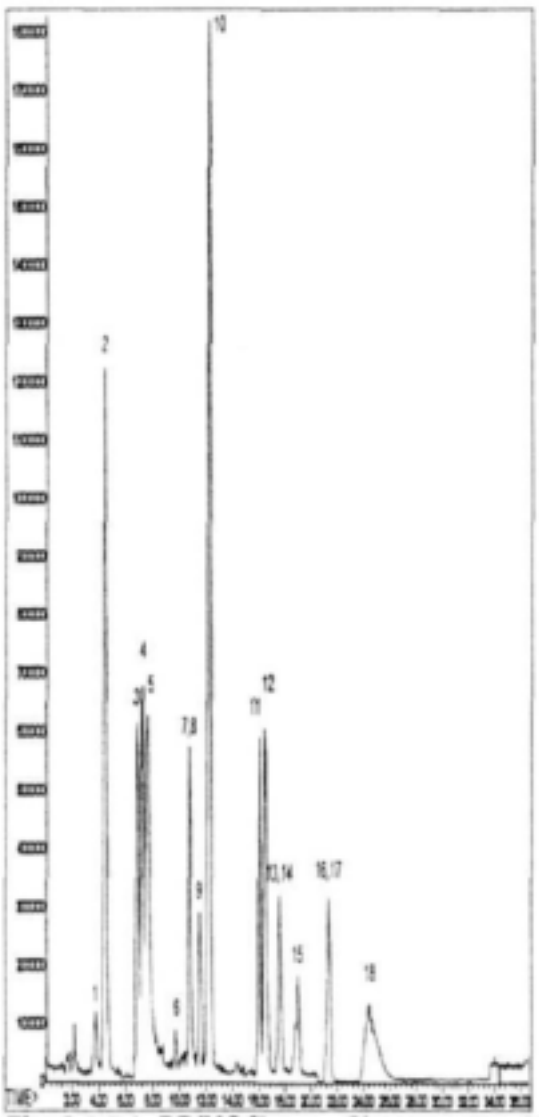

Fig. 2.4.2.1 PB/MS Trace - Chromatography Optimised.

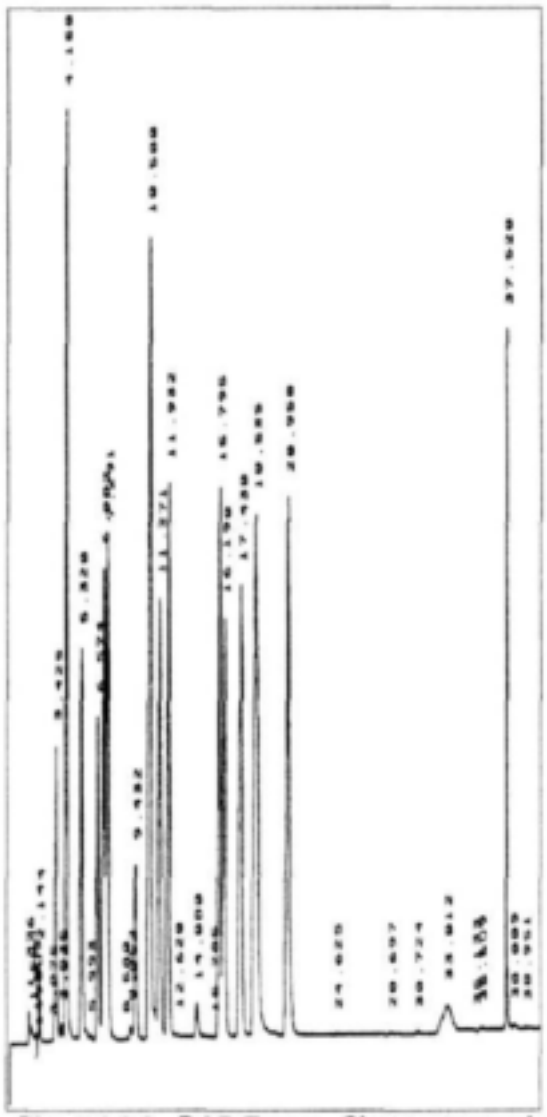

Fig. 2.4.2.2 DAD Trace - Chromatography Optimised

Key to Chromatograms:-

1.	Carbendazim	2.	Thiabendazole
3.	Bromacil	4.	Monouron
5.	Simazine	6.	Carbofuran
7.	Pirimicarb	8.	Carbaryl
9.	Atrazine	10.	Diuron
11.	Propanil	12.	Propazine
13.	Methiocarb	14.	Terbuthylazine
15.	TMTD	16.	Prometryne
17 .	Imazalil	18.	Dodin

The calibration curves, shown in Appendix 2.5 (A2.5.1 to A2.5.18), indicate that, even at the low concentrations used in the second attempt, the curves are not linear. The curves have been plotted using the standard Hewlett Packard Chemstation Quadratic Best Fit (forcing the curve to pass through the origin).

As can be seen from the calibration curves some compounds were found to be particularly poor with respect to linearity. Some of the compounds could not even be fitted with the quadratic best fit *i.e.* simazine, pirimicarb, diuron and dodin.

2.4.3 Examination Of Pyrethroids

The MS trace and EI spectra (and Wiley Library searches) are shown in Figures A2.7.1 to A2.7.3 (Appendix 2.7) and show that HPLC and the PBI are suitable for the analysis of pyrethroids. The MS chromatogram shows good HPLC peak shapes and both pyrethrin I and pyrethrin II displayed EI spectra quite consistent with those displayed in the Wiley Library when searched against that library.

2.4.4. Extraction Techniques

2.4.4.1 Solid Phase Extraction

2.4.4.1.1 Comparison of Sorbants

Two sorbants, supplied by (Macherey-Nagel) supplied by Separations (Pty) Ltd., were evaluated.

Recoveries were determined in triplicate for each of the sorbants. For each sorbent (HR-P and C18ec) a 1000ml deionised water blank was extracted. Recoveries are shown in Table 2.4.1, chromatographs in Figures 2.4.4.1 and 2.4.4.2

Table 2.4.1 Comparison of Recoveries using different SPE cartridges

Compound	HR-P (%)	C18 ec (%)
1. Carbendazim	89,4	64,1
2. Thiabendazole	82,0	84,8
3. Bromacil	99,6	89,7
4. Monouron	104,0	95,5
5. Simazine	78,9	93,9
6. Carbofuran	83,4	71,3
7. Pirimicarb	105,7	104,7
8. Carbaryl	101,7	88,0
9. Atrazine	79,1	93,7
10. Diuron	91,4	91,2
11. Proparil	79,2	84,1
12. Propazine	81,1	91,6
13. Methiocarb	58,6	57,4
14. Terbuthylazine	15,9	15,6
15. TMTD	3,5	8,5
16. Prometryne	67,5	73,2
17. Imazalil	58,2	23,6
18. Dodin	-	-

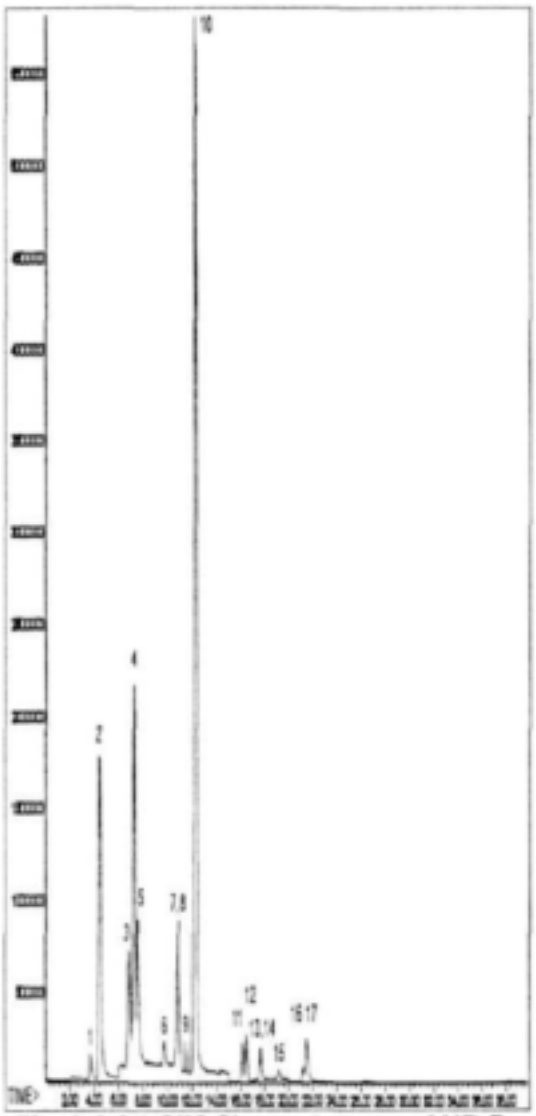

Fig. 2.4.4.1 SIM Chromatogram of HR-P Recoveries.

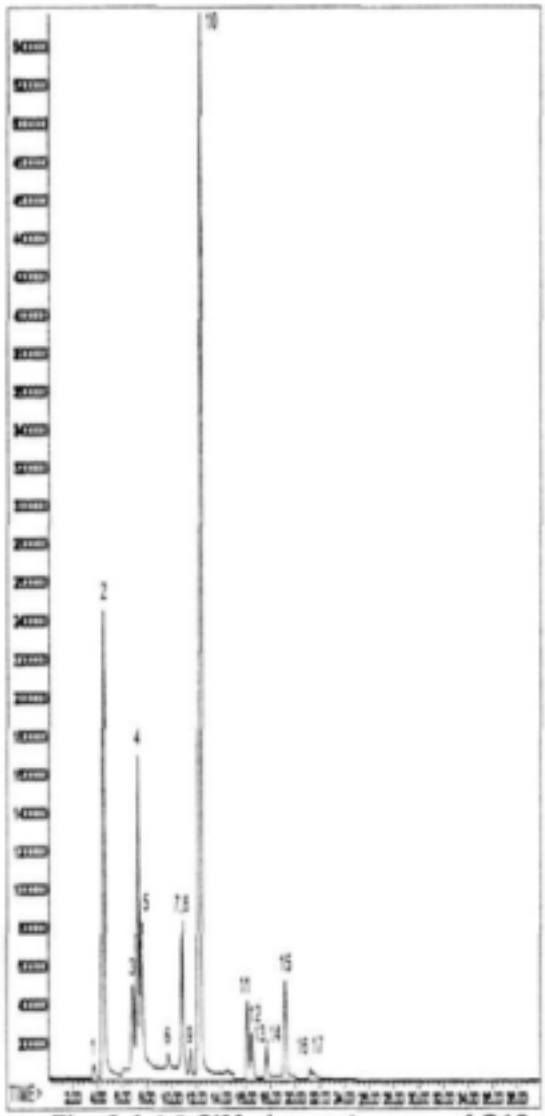

Fig. 2.4.4.2 SIM chromatogram of C18-ec Recoveries.

Key to Chromatograms:-

1.	Carbendazim	2.	Thiabendazole
3.	Bromacil	4.	Monouron
5.	Simazine	6.	Carbofuran
7.	Pirimicarb	8.	Carbaryl
9.	Atrazine	10.	Diuron
11.	Propanil	12.	Propazine
13.	Methiocarb	14.	Terbuthylazine
15.	TMTD	16.	Prometryne
17 .	Imazalil	18.	Dodin

2.4.4.1.2 Extract Concentration Volume

Peak areas are shown in Table 2.4.2 and indicate that 100 µl is the optimum volume to concentrate the SPE extracts down for quantitative work. When concentrating down to 50 µl, losses of analytes were observed *i.e.* peak areas should have been 100% greater than those observed for the 100 µl extractions volume but were instead observed to be only about 50% greater.

For qualitative analyses one could concentrate down to 50 µl as the absolute amount of analyte would be greater (50% greater) and would aid in elucidation of unidentified compounds.

Table 2.4.2 Peak Areas obtained with different extract volumes

Compound	50 µl Extract Vol.	100 µl Extract Vol.	200 µl Extract Vol.	500 µl Extract Vol.
Carbendazim	3 467	2 252	1 126	578
Thiabendazole	13 566	8 246	3 757	1 875
Bromacil	5 161	3 095	1 414	699
Monouron	8 723	5 383	2 600	1 315
Simazine	10 680	7 138	3 428	1 759
Carbofuran	3 137	1 920	899	451
Pirimicarb	18 442	12 245	6 404	3 405
Atrazine	9 917	6 369	3 096	1 594
Diuron	10 943	6 942	3 253	1 646
Proparil	10 359	6 408	2 979	1 507
Propazine	9 234	6 088	3 000	1 545
Methiocarb	12 433	8 101	3 984	2 041
TMTD	3 373	1 998	1 338	1 100
Prometryne	2 365	1 506	676	340
Imazalil	12 361	7 952	3 874	1 971

2.4.4.2 Solid Phase Micro Extraction

All results are expressed as percentages for comparative purposes as direct quantitation with SPME is not possible.

2.4.4.2.1 Comparison of PDMS, PDMS/DVB and CW/TRP Fibres

Chromatograms of the comparison (and of blank runs in which the three fibres were placed in the de-ionised water) are shown in Appendix 2.8, Figures A2.8.1 to A2.8.3 and recoveries in Table 2.4.3.

Table 2.4.3 Comparison of CW/TPR, PDMS and PDMS/DVB fibres

Compound	PDMS (%)	CW/TRP (%)	PDMS/DVB (%)
1. Bromacil	-	19	100
2. Monouron	-	18	100
3. Simazine	-	16	100
4. Carbofuran	-	33	100
5. Atrazine	-	16	100
6. Diuron	-	33	100
7. Propanil	2,3	50	100
8. Propazine	21	22	100
9. Methiocarb	-	40	100
10. Terbuthylazine	32	26	100
11. TMTD	-	36	100
12. Prometryne	12	21	100
13. Imazalil	58	38	100

As can be seen from the chromatograms and the results (in Table 2.4.3 and Appendix 2.8, Figures A2.8.1 to A2.8.3 PDMS/DVB is the fibre of choice for the compounds chosen. Greater amounts were absorbed and subsequently desorbed using this fibre.

Although this fibre appears ideal it suffers from a major drawback in that it is fragile. The coatings on two fibres disintegrated with very limited use. Extra care was taken with the second PDMS/DVB fibre, which only lasted for five extractions. The PDMS and CW/TPR fibres were both far more robust and tended to fail in the area of attachment (to the coloured plastic hub). These failures were easily repaired by drilling the hubs, using a needle to wedge the fibre into the hub and trimming the excess with wire cutters.

Fibres also need to to be rinsed in de-ionised water after use to prevent a sodium chloride build-up that may lead to fibres becoming jammed in the outer barrel of the needle assembly.

Communications with the supplier have indicated that the fibres could be swelling excessively in the organic solvent (acetonitrile in this case) and the coating on the fibres is being stripped from the fibre when it is passed through the sealing ferrule. It was recommended that the desorption chamber be filled with water and that the fibre be allowed to stand in the water for one minute to allow the organic solvent to be replaced and for the swelling to reduce.

2.4.4.2.2 Analyte Concentration

Results are shown in Tables 2.4.4 to 2.4.6.

Detection limits were generally around 10 µg/l for most of the compounds selected, if bromacil and carbofuran are excluded, then the remainder of the compounds could be detected at 5 µg/l with both the CW/TPR and PDMS/DVB fibres.

Table 2.4.4 Behaviour of CW/TPR with different concentrations of analyte (constant volume)

Compound	5 µg/l (%)	10 µg/l (%)	20 µg/l (%)	50 µg/l (%)
1. Bromacil	-	-	75	100
2. Monouron	10	20	70	100
3. Simazine	16	26	63	100
4. Carbofuran	-	25	50	100
5. Atrazine	11	22	39	100
6. Diuron	9	24	39	100
7. Propanil	9	26	38	100
8. Propazine	7	25	29	100
9. Methiocarb	3	27	39	100
10. Terbuthylazine	8	33	38	100
11. TMTD	8	29	33	100
12. Prometryne	29	39	43	100
13. Imazalil	12	35	39	100

Table 2.4.5 **Behaviour of PDMS/DVB with different concentrations of analyte (constant volume)**

Compound	5 µg/l (%)	10 µg/l (%)	20 µg/l (%)	50 µg/l (%)
1. Bromacil	-	-	64	100
2. Monouron	21	85	100	100
3. Simazine	23	48	100	100
4. Carbofuran	22	33	66	100
5. Atrazine	11	18	49	100
6. Diuron	5	6	25	100
7. Propanil	6	14	48	100
8. Propazine	5	41	48	100
9. Methiocarb	6	25	42	100
10. Terbuthylazine	3	25	45	100
11. TMTD	7	12	50	100
12. Prometryne	11	27	51	100
13. Imazalil	11	28	48	100

Table 2.4.6 **Behaviour of PDMS with different concentrations of analyte (constant volume)**

Compound	5 µg/l (%)	10 µg/l (%)	20 µg/l (%)	50 µg/l (%)
1. Bromacil	-	-	-	-
2. Monouron	-	-	-	-
3. Simazine	-	-	-	-
4. Carbofuran	-	-	-	-
5. Atrazine	-	-	-	-
6. Diuron	-	-	-	-
7. Propanil	14	63	65	100
8. Propazine	23	33	35	100
9. Methiocarb	-	-	-	-
10. Terbuthylazine	20	29	49	100
11. TMTD	-	-	-	-
12. Prometryne	26	53	58	100
13. Imazalil	19	24	38	100

2.4.4.2.3 Extraction Volume

Results are shown in Table 2.4.7.

Table 2.4.7 Varying Extraction Volume (CW/TPR Fibre)

Compound	10ml (%)	30ml (%)	80ml (%)
1. Bromacil	100	100	100
2. Monouron	80	100	93
3. Simazine	83	100	92
4. Carbofuran	50	100	50
5. Atrazine	98	100	97
6. Diuron	96	100	111
7. Propanil	78	100	91
8. Propazine	80	100	87
9. Methiocarb	93	100	96
10. Terbuthylazine	93	100	97
11. TMTD	84	100	98
12. Prometryne	87	100	100
13. Imazalil	86	100	100

As can be seen from the results in Table 2.4.7 the optimal extraction volume is 30ml. The fibre appears saturated with the 30 ml sample volume and any further increases that would be gained with the 80 ml sample volume are offset by losses due to factors such as irreversible binding to a larger glass surface area.

2.4.4.2.4 Extraction Time

Samples were extracted for 30; 45 and 75 minutes. Results are shown in Table 2.4.8.

Table 2.4.8 Comparison of Extraction Time

Compound	30min (%)	45min (%)	75min (%)
1. Bromacil	100	100	100
2. Monouron	93	100	93
3. Simazine	90	100	90
4. Carbofuran	80	100	80
5. Atrazine	113	100	69
6. Diuron	101	100	102
7. Propanil	83	100	112
8. Propazine	85	100	105
9. Methiocarb	80	100	96
10. Terbuthylazine	82	100	96
11. TMTD	35	100	127
12. Prometryne	80	100	96
13. Imazalil	75	100	93

An extraction time of 45 minutes resulted in the greatest overall recoveries. An extraction time of 30 minutes was found to yield reduced recoveries for most compounds, an exception was atrazine. Extraction time of 75 minutes resulted in better recoveries for diuron, propanil, propazine and TMTD (102, 112, 105 and 127 % respectively).

For this reason 45 minutes was chosen as the extraction time of choice as the small advantage gained by extracting for 75 minutes was outweighed by better recoveries for most of the other compounds and the obvious time saving is also an important factor to consider.

2.4.4.2.5 Extraction Temperature and Time

Samples of the same concentration (20 µg/l) of analyte were extracted for different times at 6°C using the same adsorption/desorption and HPLC parameters described in sections 2.4.2.3.1.3. For comparative purposes the results at 6°C (45, 90 and120 minutes) were compared to those extractions carried out at 24°C (45 minutes extraction time).

Results are shown in Table 2.4.9.

Table 2.4.9. Comparison of Extraction Time at 6° Compared to Extraction at 24°C

Compound	45min at 6°C (%)	90min at 6°C (%)	120min at 6°C (%)	45min at 24°C (%)
1. Bromacil	66	100	100	100
2. Monouron	80	107	107	100
3. Simazine	83	104	108	100
4. Carbofuran	66	66	33	100
5. Atrazine	68	88	103	100
6. Diuron	84	124	145	100
7. Propanil	67	108	131	100
8. Propazine	61	83	96	100
9. Methiocarb	70	93	112	100
10. Terbuthylazine	68	99	116	100
11. TMTD	62	105	140	100
12. Prometryne	61	85	110	100
13. Imazalil	52	76	107	100

An increased extraction time at 6°C generally resulted in better recoveries of analytes and recoveries at 120 minutes in particular resulted in the best recoveries. Comparison to recoveries at 24°C (45 minutes extraction time) was generally better at 6°C (120 minutes extraction time). However, the gains achieved by extraction at 6°C for 120 minutes were not sufficiently great to make this viable and an extraction at 24°C for 45 minutes is preferable.

2.4.4.3 Liquid/Liquid Extraction

Recoveries are shown in Table 2.4.10.

Table 2.4.10 Percent Recoveries obtained with Liquid/Liquid Extraction

Compound	Extraction 1 % Recovery	Extraction 2 % Recovery	Extraction 3 % Recovery	Average % Recovery
Carbendazim	0,2	0,0	0,2	0,1
Thiabendazole	0,2	0,1	0,0	0,1
Bromacil	0,0	0,0	0,0	0,0
Monouron	57,3	45,7	52,4	51,8
Simazine	34,3	30,5	33,6	32,8
Carbofuran	25,6	22,8	24,0	24,1
Pirimicarb	62,2	54,0	64,1	60,1
Carbaryl	32,7	32,0	32,8	32,5
Atrazine	7,6	8,0	8,2	7,9
Diuron	36,0	26,6	32,9	31,8
Propanil	49,0	41,0	46,0	45,3
Propazine	2,0	2,2	2,0	2,1
Methiocarb	38,0	34,0	37,1	36,4
Terbuthylazine	55,2	57,3	54,4	55,6
TMTD	5,6	4,2	4,8	4,9
Prometryne	52,9	51,9	52,3	52,4
Imazalil	1,0	1,3	0,8	1,0
Dodin	0,0	0,0	0,0	0,0

2.4.5 Analysis of Water from the Vereeniging and Zuikerbosch Pumping stations

The target analysis carried out yielded on the raw and final waters from Vereeniging, Molopo and Cape Metro did not detect any of the target compounds analysed for (as shown in Table 2.4.1). For this reason remaining analyses of the waters from the Vereeniging and Zuiferbosch Pumping Stations were carried out using the Full Scan mode on the MS. Although this mode is less sensitive than Selected Ion Monitoring mode it has the advantage of identifying unknown compounds.

The DAD and PBMS traces are shown in Appendix 2.9.

It is immediately obvious that the DAD and PBMS traces do not always correlate exactly. This is because the two methods of detection have different responses to different compounds and the PBMS is vastly less sensitive than the DAD (typically only between 0,5 and 1,0% transmission efficiency of analytes through the PM interface into the MS). As described earlier, the MS was run in the Full Scan mode to facilitate the identification of unknown compounds. If it can be established which compounds need to be monitored for, one could use Selected Ion Monitoring which will improve sensitivity greatly (this will depend on the compound and on the number of ions scanned).

Compounds detected and identified were,

Tri(butoxyethyl)phosphate : Raw water Vereeniging 24 October 2001.
: Final water Vereeniging 31 October 2001.
: Raw water Vereeniging 28 November 2001.
: Final water Vereeniging 28 November 2001.

Carbamazepine : Final water Vereeniging 24 October 2001.

Library search data is included in Appendixes 2.9, Figures A2.9.35 and A2.9.036.

All extracts were scanned for tri(butoxyethyl)phosphate post run using Reconstructed Selected Ion Monitoring once its characteristic ions were identified. It was fortunate that both the tri(butoxyethyl) phosphate were detected on the DAD trace and on the PBMS trace.

Several other compounds were also detected but no suitable search matches were obtained. This is usually due to co-eluting peaks, excessive background or the compounds were just not present in the Wiley 275 Library used to search.

Tri(butoxyethyl) phosphate is a compound that is used as a plasticiser, flame retardant and floor waxes (18) and exhibits an LD_{50} of 3000mg/kg (rat). Tri(butoxyethyl) phosphate was detected on four occasions at the Vereeniging Treatment Plant. Results indicate that tri(butyoxyethyl) phosphate both enters and leaves the treatment plant *i.e.* is unaffected by the process within the treatment plant. On the first occasion the compound was detected (24 October) it was only detected in the raw water. A week later (31 October) it was detected in the final water indicating that the compound had passed through the treatment plant. Tri(butoxyethyl) phosphate was again detected in both the raw and final waters (28 November) i.e. the timing was such that sampling occurred as the tri(butoxyethyl) phosphate had already entered the treatment plant and was already in the process of being pumped away.

Carbamazepine is a drug used for the treatment of epileptics (simple and complex seizures and neuralgia) and for manic depressive disorders[19]. Carbamazepine is metabolized primarily in the liver by oxidative enzymes. It is then conjugated with glucuronic acid and excreted in the urine. The metabolite, carbamazepine-10,11-epoxide is active and may achieve up to 50% of the activity of the parent compound. This would indicate that the carbamazepine was unlikely to have entered the water system via an excretory pathway. The possibility that the compound entered the water via illegal dumping does exist. Carbamazepine was not found at any other time during the survey. Follow up to determine if this result was an artifact should be carried out.

CHAPTER 5

Conclusions

From the results presented it is obvious that SPE is the method of choice for the determination of HPLC compounds in water samples.

The SPE cartridges that yielded the best recoveries were the C18-ec, these cartridges cost approximately R30 each. Minimal solvents were required to desorb the organic pollutants (5 ml of methanol and 2 ml of methanol:acetone,3:2) and once the methodology has been established requires very little analyst time. HPLC analyses, in general, examine the less volatile organic fraction in comparison to Gas Chromatography. For this reason extracts destined for HPLC analysis are more easily concentrated *i.e.* blown almost to dryness with nitrogen and made up to volume.

Use was made of the DAD to screen samples. No compounds were detected on the DAD trace (or on the SIM MS traces). Ordinarily the samples would not be analysed on MS unless suspected positive samples required confirmation or if an unknown contaminant required identification. Samples from Rand Water, Molopo Water and Cape Metro Water (source and treated water) were analysed.

Use was also made of UV spectral data to assist in the partial confirmation of compounds eluted from the HPLC. A standard solution was injected onto the HPLC and the UV spectra of peaks were plotted, these spectra are shown in Appendix C and can be compared (manually) to those obtained for unknown samples. Software that can automatically compare UV spectra of compounds detected in samples to UV Spectral Libraries is available. While such matches are generally not accepted as being definitive identifications, they are important as a screening method.

SPME as a screening technique was promising but the expense of the fibres in relation to their fragile nature was disappointing. The fibre that yielded the best recoveries (PDMS/DVB) was the most fragile of all the fibres and two such fibres only lasted for about 5 extractions each. Discussions with the supplier indicated that the coatings on the fibres were stripped off as the fibres were swollen with absorbed organic solvent and that a soak period of about 1 minute in water should be sufficient to reduce the swelling. The validity of this claim must still be tested. The fibres are expensive at approximately R850 each, thus if a fibre only lasts for a limited number of extractions *i.e.* 5 extractions each extraction would cost in the vicinity of R160, more than five times the cost of a SPE analysis.

Use was made of the DAD to screen samples. No compounds were detected on the DAD trace (or on the SIM MS traces). Ordinarily the samples would not be analysed on MS unless suspected positive samples required confirmation or if an unknown contaminant required identification. Samples from Rand Water, Molopo Water and Cape Metro Water (source and treated water) were analysed.

The analysis of raw and final waters from the Vereeniging and Zuikerbosch Treatment Plant was carried out over a period of eight weeks at the end of 2001 using the methodology developed in section 4.1 of the project *i.e.* Solid Phase Extraction using the C18ec Chromobond SPE cartridges.

The DAD and PBMS were used in tandem to evaluate the effectiveness of utilising the DAD as a screening tool for monitoring organic compounds with HPLC. The technique is relatively inexpensive and easy to use (compared to PBMS) and is in use in most water laboratories. The DAD and PBMS traces, although different, were found to correlate relatively well. Different

compounds respond differently to the two detectors and the PBMS is much less sensitive than the DAD.

Of the compounds detected two were identified, tri(butoxyethyl) phosphate and carbamazepine. These compounds were identified using Electron Impact Spectral Libraries (Wiley 275) as the PBMS generates true Electron Impact spectra. The identification of carbamazepine was not as definitive as that of tri(bytoxyethyl) phosphate but the major ions in the unknown and carbamazepine corresponded very well. It is not unlikely that tri(butoxyethyl) phosphate could be found in the water, its uses include as plasticiser, a flame retardant and in floor wax and it was detected on four occasions at the Vereeniging Treatment Plant. Also, it appears to pass through the treatment process unaffected. Carbamazepine was only detected once and its presence is very difficult to explain. Carbamazepine is a drug used in the treatment of epileptics and manic-depressives. It was only found in the final water on 24 October and must already have entered the Vereeniging Treatment Plant prior to sampling. The compound is metabolised in the body and excreted in the urine as a metabolite and would thus be unlikely to be detected as the parent compound if it entered the water system as an excretion product (unless it naturally reverts to the parent compound once excreted). An alternative could be the illegal dumping of the compound. Follow up studies should be carried out to determine if the source of this compound could be established.

The survey made use of the PBMS in the Full Scan mode, which facilitates the generation of true Electron Impact specra that allows unknown compounds to be identified. The alternative-scanning mode, Selected Ion Monitoring, allows far greater sensitivity but is only really useful for Target Analyses where the identity of the compounds to be monitored is already known. The use of alternative LC/MS interfaces should also be investigated.

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