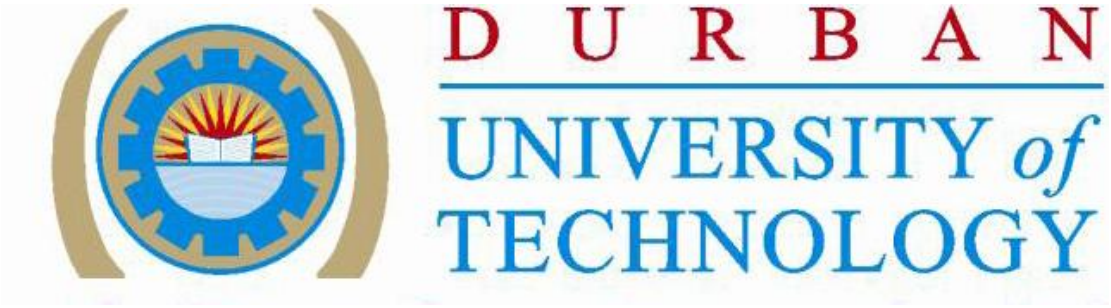

Detection Methods of Organic Acid in Steam/ Water Circuits and Optimisation using HPLC-UV

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Dissertation presented in partial compliance with the requirements for the Masters Degree in Technology: Chemistry, in the Faculty of Applied Sciences at the

Durban University of Technology

2009

Supervisors: Prof K.G Moodley, Mr D.K. Chetty, Prof A. Crouch

Declaration

I, Miss Arthi Ramrung, hereby declare that this dissertation is representative of my own work. It has not been submitted before for any degree or examination in any other institution.

Signature:

Date:

Supervisor (Prof K.G. Moodley)

Co-Supervisor (Mr D.K. Chetty)

Dedication

This work is dedicated to my exceptionally special parents

Mr and Mrs R. Ramrung.

For always being the wind beneath my wings

Love you dearly…

Acknowledgements

I would like to express my gratitude to the following persons who have assisted me in one way or the other towards the completion of this project:

- The supervisors of my project, namely, Professor K G Moodley and Mr D K Chetty of DUT and Professor A M Crouch of the Universities of Stellenbosch and Witwatersrand, for their expert guidance and patience.
- Mr Gerard Gerhard and Mr Theo Jongwana of Eskom Laboratories, Rosherville, for with valuable information on power station processes and collection and transport of field samples, respectively.
- Eskom Tertiary Support Programme and DUT for their financial assistance.
- Professor G G Redhi, HOD of the Chemistry Department for his support.
- My Mom and Dad for their constant love, encouragement and emotional support.
- My family: sister, brother, brother-in-law and nephews, especially Nagesh and Meshar, for all their curiosity, understanding and love.
- My friends: Avy, Shan, John, Naresh, Karen for their words of encouragement.
- My friends for their encouragement, patience and understanding.
- All others who I may have unintentionally omitted from this list.
- Finally to my Special Guides whose help is forever appreciated.

Abstract

This study was mainly a response to a challenge faced by ESKOM in its coal-fired power stations. In spite of using high purity water to drive the turbines, the latter were damaged by 'pitting', possibly related to acids generated at high temperatures. In the light of this a relatively simple method for determination of short chain organic acids was identified by comparing the efficacies of several methods. It was found that high performance liquid chromatography (HPLC) method preceded by derivatization (with *o-nitrophenyl hydrazine)* is suitable for analyzing mixtures of simple acids at ppb levels.

Calibration was effected by using methanoic acid (formic acid), ethanoic acid (acetic acid), propanoic acid (propionic acid) and butanoic acid (butyric acid). The HPLC instrument used was from Thermo Separations with P2000 pump, SN 4000 interface and UV1000 with a column heater. A comparative study between the HPLC methods using ion exclusion and partition chromatography was carried out in order to find a suitable method that can be used with aqueous environmental samples. The two essential columns that were used were ion exclusion Phenomenex Rezex OA column and a Nucleodur C8 column.

The method of partition chromatography using a C8 column showed the most success using a mobile phase consisted of acidified water using HCl (pH4.5) along with a 60:40 Acetonitrile/Methanol mixture. Both isocratic and gradient programs were utilized. Limits of detection were improved from 800ppb (formic acid), 480ppb (acetic), 350ppb (propionic) and 680ppb (butyric acid) to 25ppb (acetic), 60ppb (propionic) and 90ppb (butyric).

Samples used in analysis were collected from the main stream, economiser, condensers, polishing plant and turbines of the Tutuka Power Station in Mpumalanga province and analysed using with final developed method.

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Abbreviation:

CE	capillary electrophoresis
HPLC	high performance liquid chromatography
LC	liquid chromatography
GC	gas chromatography
UV	ultraviolet
ELSD	evaporative light scattering detector
Vis	visible
V	voltage
MS	mass spectrometry
2-NPH	2-nitrophenyl hydrazine
EDC	1-ethyl-3-(dimethylaminopropyl)carbodiimide
GLC	gas liquid chromatography
GSC	gas solid chromatography
FID	flame ionization detector
ECD	electron capture detector
OA	organic acids
ppm	parts per million
ppb	parts per billion
EOF	electroosmotic flow
SCFA	short-chained fatty acid
LCFA	long-chained fatty acid

MeOH	methanol
AcN	acetonitrile
RP-LC	reverse phase liquid chromatography
IC-MS	ion chromatography mass spectroscopy
LOD	limits of detection
pKa	ionisation constant of acid

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Chapter One

Introduction

1.1 Generation of Electricity

ESKOM, the powerhouse in the generation of electricity for South Africa has twenty four power stations that are situated mainly in northern regions of SA. These power stations utilize any one of three main methods of power generation; namely, hydro-electric, nuclear or fossil fuel (coal-fired). The work discussed within this dissertation would focus mainly on the coal-fired power stations.

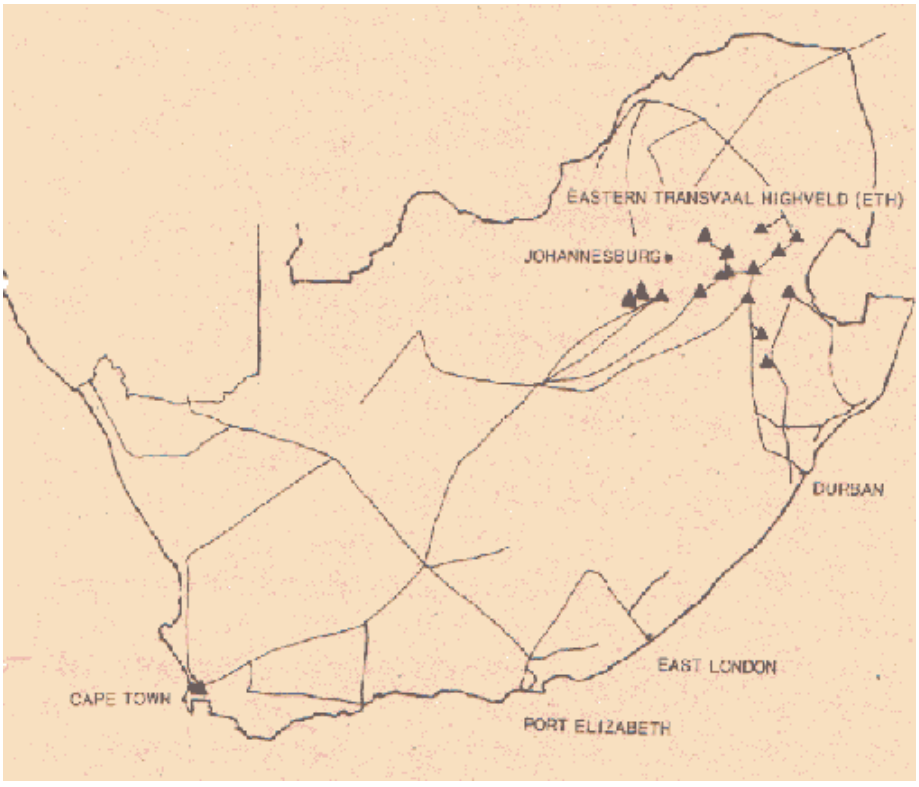

Figure 1.1: Map of South Africa showing the location of power stations

Majority of the coal-fired powers stations are located in the northern region of South Africa as indicated in Figure 1.1. Table 1.1 shows the amount of electricity generated by each of the ten main coal-fired power stations from 2001 to 2006.

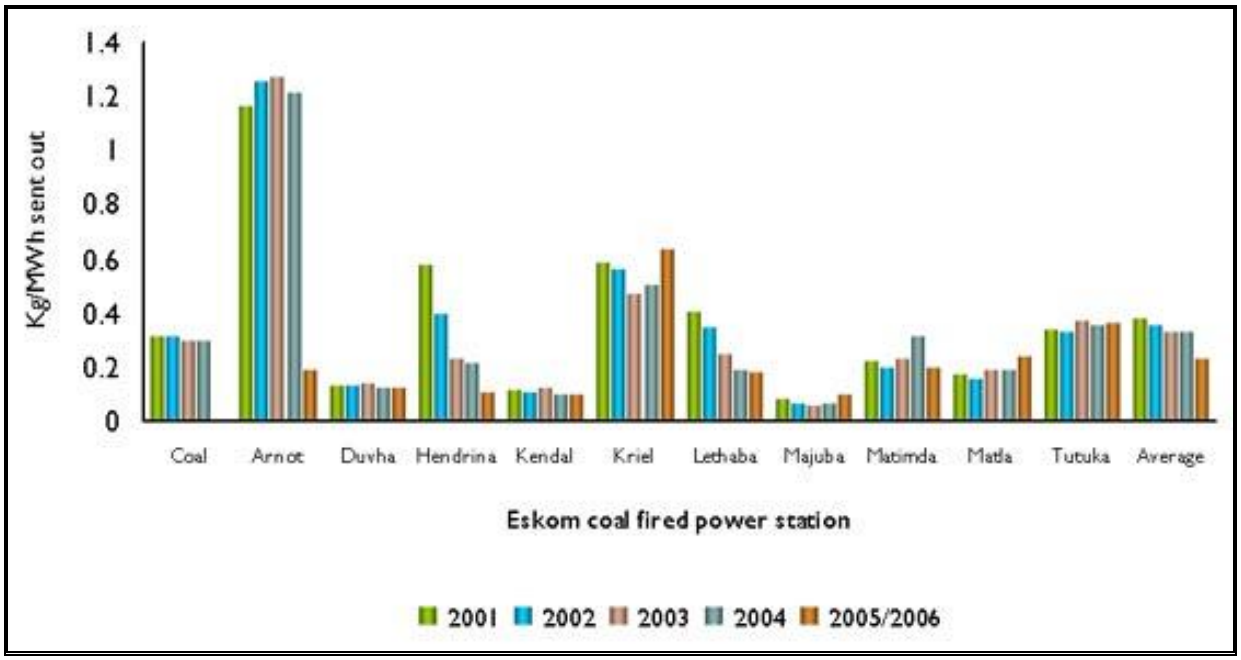

Table 1.1: Bar graph of electricity generated by coal fired power stations

As majority of the nation's electricity comes from these power stations, any problems that would cause failure or damage within the units or unexpected shut downs, would result in a nationwide catastrophe of a shortage of electricity production. This exact situation was unfortunately experienced recently, were the whole of SA suffered dreaded power outages (blackouts) in a periodic fashion in order to reduce the load or strain on the few power station units that were in operation at the time.

This episode, if nothing else, has shown the importance of eliminating any source of damage within the generation units; as well as ensuring that optimum operation

conditions are adhered to at all times. By improving the stringency of the quality of the raw products, the amount of damage experienced within a generation unit could be reduced.

The generation of power in a typical coal-fired power station is discussed below to give the reader an overview of the process. Coal-fired power stations use coal, water and oil as the basic raw material in the generation of electricity. Each power station has a number of generation units which comprise of the following: a boiler unit, turbines, a generator made up of a rotor and stator and a condenser. The numbers of generation units vary from power station to power station.

The larger power stations have 10 to 12 units, with the smaller power stations having approximately 4 units. Not all units are in operation at any given time, due to scheduled periodic maintenance shut downs.

The generation of electricity is achieved by passing ultra-pure water through to a massive boiler, which is heated by burning crushed coal. The water is converted to steam. This steam is then subjected to higher temperatures and pressures within the low and high pressure heaters respectively, converting it to superheated steam.

The high pressured steam is then directed to the turbine blades which drives the generator (Figure 1.2 and Figure 1.3). Electricity is generated when a magnet i.e. rotor spins inside a copper coil i.e. stator. Most power stations generate electricity at a voltage of 22 kV. Transporting of electricity must however be done at higher voltages (220-765 kV) in order to reduce any losses that may occur over long distances.

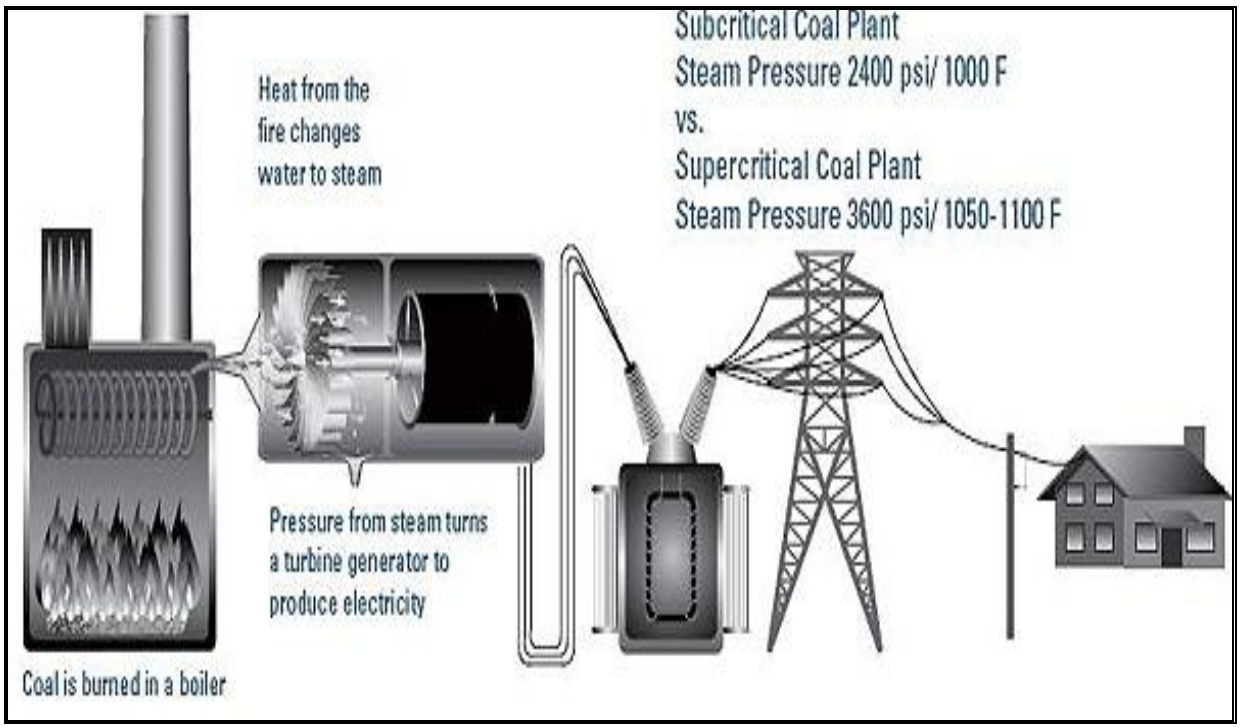

Figure 1.2: Basic diagram of the generation of electricity

It is for this reason that transformers are used. After generation, the voltage is stepped-up to high voltages before being transported to substations. Power lines that feed electricity into ESKOM's national grid transmit at voltages ranging from 22 kV up to 765 kV.

As the work within this dissertation is based on the study of the water/steam used, all discussions henceforth would be centred on an understanding of the role of water and the source of the problems experienced by ESKOM with this regard.

1.2 Steam /Water Circuits

Steam generation incorporates a very simple physical process whereby pressurised water is evaporated with a heat supply. The generated steam can then be used as is (i.e. saturated steam) or after superheating (i.e. superheated steam). Power stations rely greatly on the generation of steam; however the process is also used by many other industries.

Steam that is used to drive both the low and high pressure turbines would be transported to a condenser where it is cooled and condensed by indirect contact with raw water. The raw water acts as a secondary cooler as a result of being passed through a copper/iron piping. Cooling of the steam is achieved by indirect contact with the water. The term steam/water circuits, refers to this conversion of water to steam (within the boiler) and back to water again (in the condenser) (Figure 1.4).

Any water loss during this transfer of states is replenished by topping up with make-up water, i.e. water that has been stored in the demineralization storage tanks (Figure 1.4, E). The water is then passed through a condensate polishing plant first before being recycled to the boiler. Water that is used within these circuits has to be ultra-pure; hence the chemical quality of the water needs to be constantly monitored.

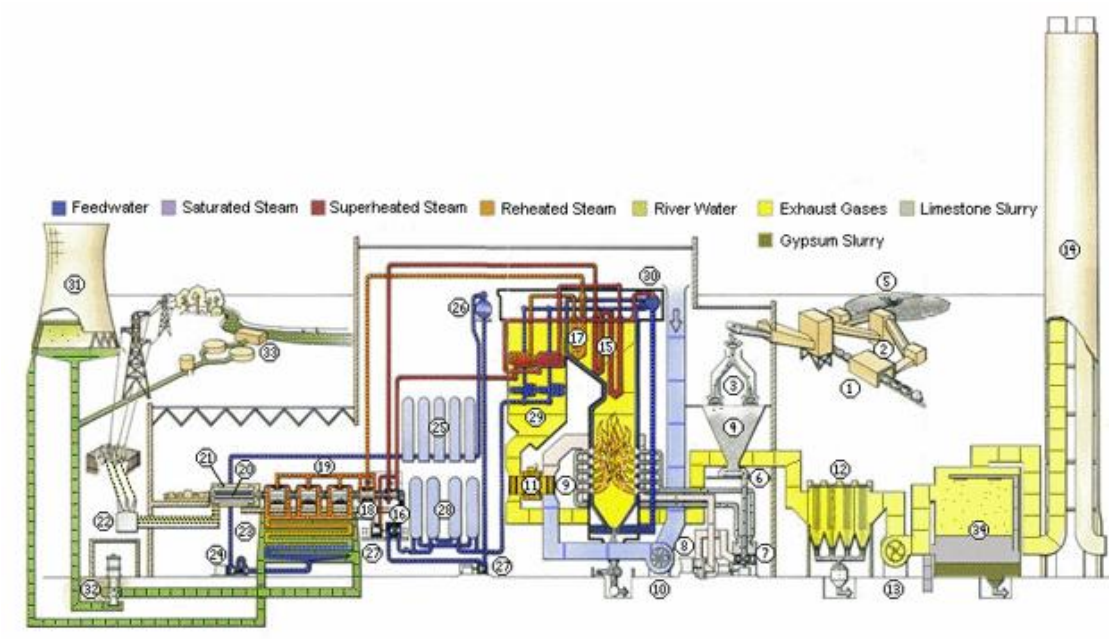

Figure 1.3: Generation unit at a fossil fuel power station showing steam/water circuit

1. Mine	2. Conveyor Belt	3. Silo	4. Bunker	5. Mined Coal	6.Mill
7.Prepared Coal	8. Conveyor B	9. Burners	10. Chain Conveyor	11. Fan	12. Precipitator
13. I.D. Fan	14. Smoke Stake	15. Center Wall	16. Heater	17. Economizer	18.High Pressure
Turbine	19. Low pressure Turbine	20-22 Generator and Transformer		23. Condenser	24. Ex. Pumps
25. Condensate Polishers	26. C.W Pump	27. Make-up water pump		28. Polishers	29. Boiler
30. Air Heaters	31. Cooling Tower	32. Cooling Water	33.Water Treatment Plant		

1.3 Influence of Impurities in Water

The influence of low molecular weight organic acids on industrial processes has been a constant cause for concern [1,2,3]. Aliphatic carboxylic acids found within steam/water circuits could originate from a multitude of proposed sources, the most obvious being the environment. Biodegradation and humification of organic material, herbicides, pesticides etc. release a significant amount of organic acids into natural water resources. Raw water used by power stations is sourced from the surrounding dams.

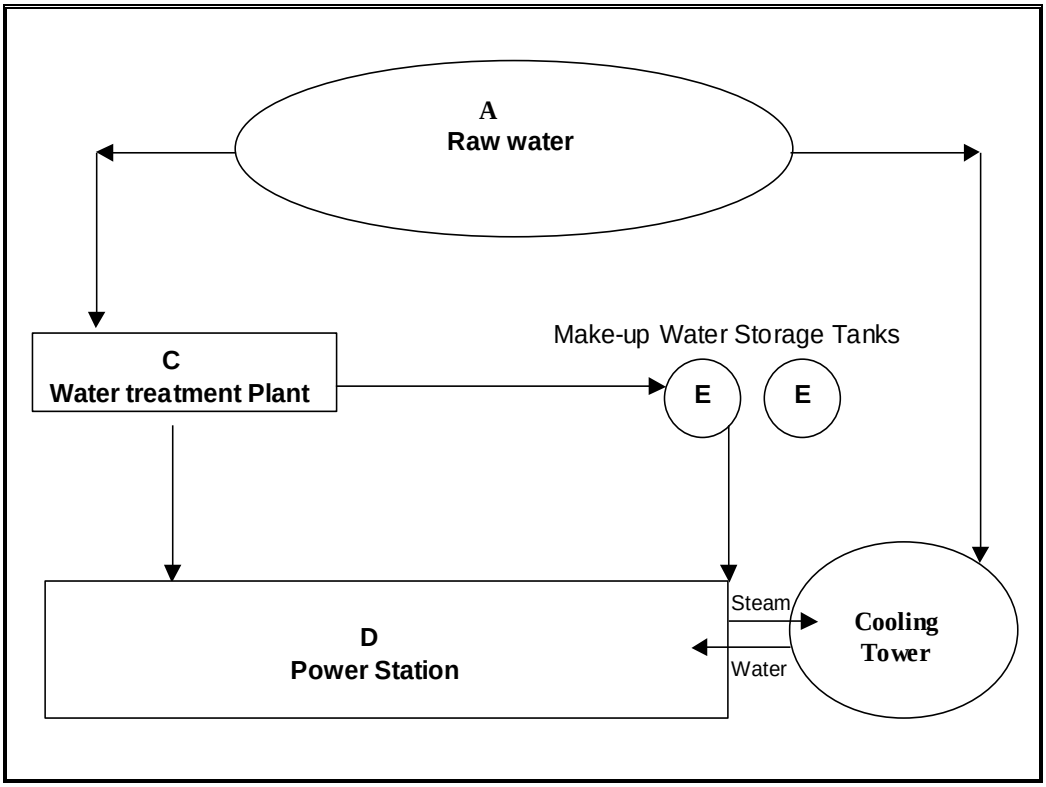

Figure 1.4: Basic Water Circuit

Natural organic matter (NOM) measurements from the relevant dam waters would vary depending on the change in season and environmental conditions. On an average the NOM value would be approximately 5 ppm. An increase in this value would be detrimental to the industrial processes dependent on this water. Measurement of the total organic content along the passage flow of the water from the dams to the power stations show a decrease in the organic compound as it passes through the purification processes. However not all of the organic impurities are removed, and are passed through to the steam/water process at sub µg / L (ppb) levels.

1.4 Monitoring of water quality

Contamination caused by topping up of the boiler with make-up water or in-leakages of cooling water at the condenser increase the amount of impurities. Depending on the type of boiler used, a concentration of the impurities could occur within the boiler itself. Due to the extreme operating conditions utilized within the steam/water circuit, the highest purity water is required in order to ensure the good health of the power plant, hence the levels of contaminates, both inorganic and organic are constantly monitored. However not enough research has been done in order to determine the control parameters under which organic acids can safely exist in the water without causing any extensive damage to the unit.

Tests that are run on a daily basis include the monitoring of inorganic anions such as chlorides, sulphates, phosphates, nitrates, etc, as well as the monitoring of silicates and sodium content in boiler water. Monitoring of total organic carbon (TOC) readings were not given much priority. Results from daily analysis should be within the specifications set by company regulations. If the ion concentrations are found to be out of specification this could cause damage to the components within the units, resulting in unplanned costly shutdowns.

It has been noted that most of the analyses performed at a power station focus on inorganic impurities present within the system. Relatively less attention has been given to those problems associated with unwanted organic impurities [4, 5, 6]. During recent years, however, focus has been redirected to the detection and speciation of organic impurities.

Some of the suspected origins of these organic impurities are listed below:-

- The water supply. The raw water before entering the purification process containing humic and fulvic acids.
- Organic fouling caused by resins used in demineralisation process.
- Organic fouling caused by flocculent and coagulants used in purification process.
- Organic fouling within the sand filters.
- Paints, coating, solvents, cleaning agents and chemicals used in water treatment process.
- Make-up water use for topping up of the boiler.
- In-leakages of water used in cooling system, condenser etc.

The organic acids found within the steam/water circuits are suspected to be the degeneration products of organic compounds [2]. These organic compounds are either partially or totally oxidised to ionic species, if subjected to extreme conditions such as high temperatures and pressures.

Investigations into the relationship between pH, concentration and conductivity with temperature, have shown that there is a considerably large difference between those measured at room temperature and those measured within the circuit at operating temperatures [7]. The pH of the water measured within the steam/water circuit at superheated temperature is shown to decrease significantly [1, 6]. As a result, the water becomes more acidic and behaves more aggressively.

Impurities dissolved in the water at normal temperature would be soluble to a lesser degree in superheated steam, thus leading to the precipitation and deposition of the impurities which is most undesirable.

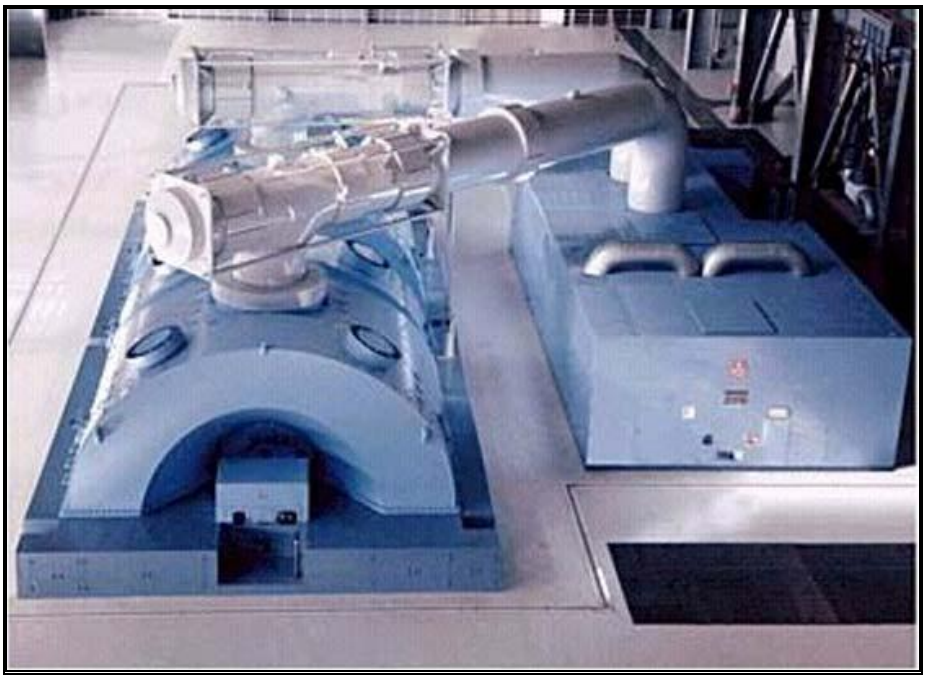

Figure 1.5 Supercritical Fluid Turbine

It has been documented that the most common organic acids that are expected to be found in the steam/water system are: acetic acid, formic acid, propionic acid, butyric acid and oxalic acid [2].

Over the pass few years, ESKOM has been involved in various studies into alternative methods of reducing the amount of organic contamination. Some of the studies that were involved in the improvement of the water chemistry were:

1. Investigations into the effect of certain types of flocculants and coagulants used in the pre-treatment of the water by Wilshire S. [5].
2. Partitioning of acetate and formates by Gruszkiewicz M. et al [7].

3. Investigations involving the resins used in the ion exchange units by Gericke et al [4].

An investigation into the decomposition of larger organic compounds such as 1,2-benzenedicarboxylic acid (phthalic acid), benzenesulphonic acid and chlorobenzenesulphonic acid, has been carried out by Wilshire S [5], in order to understand the fate of undesirable organic matter under high pressure and temperature.

Onsite tests that included varying certain working conditions were also investigated. During these investigations, samples were obtained from various points in the circuit i.e. raw water storage, clarifier outlet, sand filter outlet, cation inlet sump, cation outlet, anion outlet and mixed bed outlet. Figure 1.6 below shows the path through which the raw water follows.

The raw water is subjected to different processes before it is stored in the filtered - water tanks and potable-water tanks respectively. The filtered water is then passed to what is known as a ‘demin’ train as shown in Figure 1.7. This comprises of three ion exchange chambers, the first of which contains a cation resin. The water is then passed through to the anion exchange resin and finally through a mixed bed, which contains a mixture of anion and cation resins.

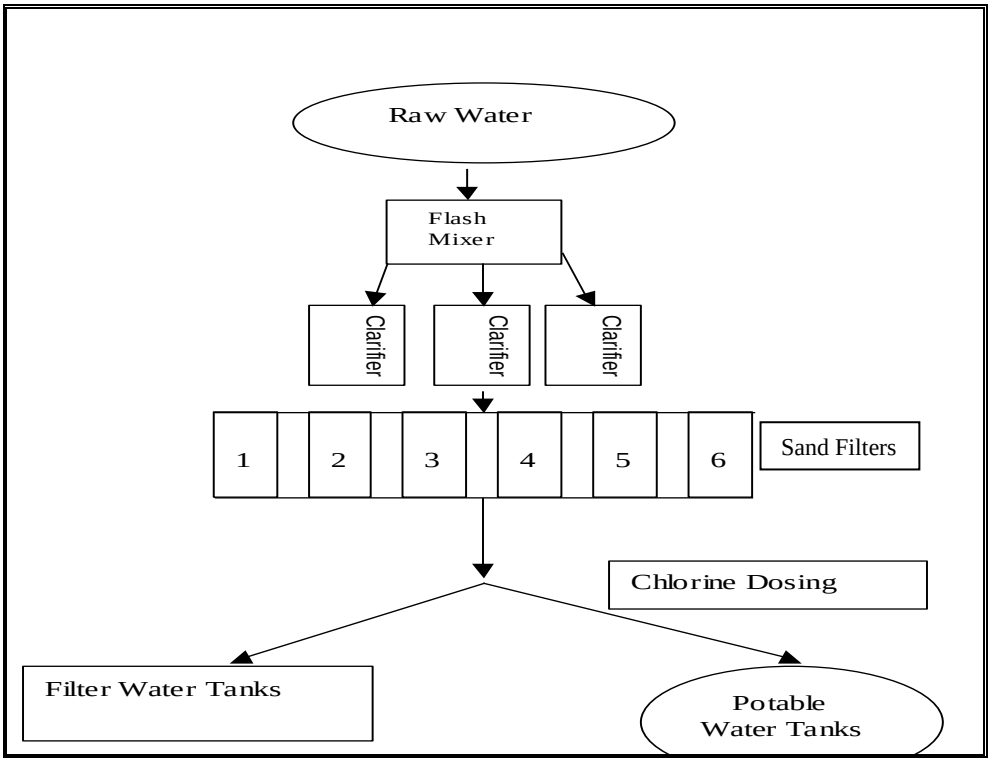

Figure 1.6 Water Treatment Plant

Sampling points in this project would be confined to the steam generation plant.

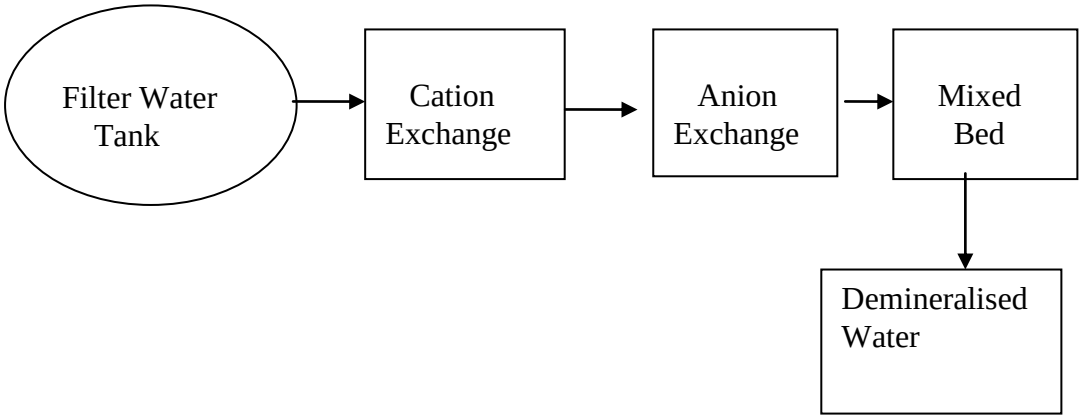

Figure 1.7 Flow of water through Demineralization Train

The concentrations and identification of these acids at various sampling points will help in determining the behaviors these acids have at these targeted points, under various conditions.

1.5 Understanding Total Organic Carbon:

Total Organic Carbon (TOC) analysis is the measure of the total organic carbon present within the water used by the power stations [1, 2, 3]. Monitoring of TOC would help in the control of organic impurities. As mentioned earlier, there are a number of sources for these impurities. As a result of high operating temperatures and pressures (*economizer has a working temperature of 540^{o}C and pressure of 17 MPa*) oxidation of organic impurities gives rise to a range of corrosive organic acids which are undesirable.

There are various types of detection techniques utilized by TOC analyzers. However the detection method used by ESKOM is the membrane/conductivity detector. Traditionally the specification limit for organic impurities determined indirectly by acid conductivity was < 0.2 μS/ cm. This value gives allowance for the carbon dioxide ingression. A significant elevation of cation conductivity by carbon dioxide can be observed on power plants that have neither de-aerators nor condensate polishers.

The impact of organic matter contaminants on power stations has been a problem faced internationally [2, 3]. Researchers abroad have observed trends in the decreasing pH of water sampled from the first condensate at the low turbine stage of the process. This was attributed to the organic compounds that could be found in the boiler make-up water. A strong correlation between the presence of impurities and the corrosion-erosion damage of the low pressure turbines was determined.

Reports from power stations in Russia, Germany and England, claim that organic compounds that enter the steam/water circuit through make-up water break down to

form smaller fraction organic anions such as acetates, formates, oxalates, propionates and butyrates when submitted to the extreme conditions [2].

1.6 Impact on Power Stations

This investigation has been initiated to help reduce the amount of damage caused by simple carboxylic acids to the turbine blade by monitoring the levels of organic acids present. The information obtained could assist in securing prolonged life in the generation units, and reducing unexpected possibilities of power shortages.

The main objective of this research is to test the suitability and feasibility of selected methods in the analysis of organic acids at trace levels in the steam/water circuits found in fossil fuel power stations.

Two methods in high performance liquid chromatography (HPLC) were used in conjunction with a variety of detectors such as ultraviolet-visible detector, evaporative light scattering detector (ELSD), and a conductivity detector. The main objective was to develop a method that could be used by the industries, in the analysis of simple aliphatic acids at traces levels.

Since the organic acids being analysed are expected to be present in trace amounts, a pre-concentration technique would also be investigated. Solid phase extraction has been chosen as the method of pre-concentration in this project.

The method showing most promise was utilised in the analysis of samples obtained from fossil fuel power station/s in Mpumalanga and surrounding provinces.

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Chapter Two

Literature review

2.1 Introduction

Simple aliphatic carboxylic acids are also called short-chained fatty acids (SCFA). Separation and detection of these organic acids has been carried out by using various methods of analysis [1, 12]. Studies that involve the detection of SCFA are carried out in the pharmaceutical, food and beverage industries or in the biological sector [1, 5, 6, 9, 10, 12].

Instrumentation that has been used in reported studies include gas chromatography (GC), high performance liquid chromatography (HPLC), ion chromatography (IC) and recently capillary electrophoresis (CE) [1, 2, 6, 10]. Each of these methods, however, has its limitations. A brief review of the reported applications of each method will be preceded by a short account of the principles on which each rests. Although several databases were searched, it is noted that very few reports on the analysis of short chain fatty acids in aqueous samples were found. The literature review therefore addresses reports covering analyses related to the ones involved in this investigation.

2.2 Gas Chromatography

Gas chromatography, along with methods such as colorimetric and thin layer chromatography was used in the determination of organic acids [1, 2]. As mentioned above, one of the fields in which simple carboxylic organic acids are given the most attention is the food and beverage industry. Quantitative determination of organic acids is of great importance in the

quality control of wines and beverages, as it contributes to the taste of the beverage. Carboxylic acids commonly found in wines are acetic acid, lactic acid, succinic acid, malic acid, citric acid and tartaric acid.

2.2.1 Principles on which GC is based and pertinent applications

In order for one to consider gas chromatography as an option in any analysis, one would have to make sure that the analyte/s in question satisfies certain primary conditions. Firstly the analyte should be volatile to semi-volatile in nature. This would guarantee that the analyte could easily move into the gaseous mobile phase to allow for easy detection of the analyte. The stationary phase used could either be solid or liquid. If the stationary phase was a solid, one would refer to the method as Gas-Solid chromatography (GSC). However if the stationary phase was a non-volatile liquid, the method is known as Gas-Liquid chromatography (GLC).

Long chained fatty acids and SCFA are fairly volatile in nature and hence amenable to analysis by GC. However Ilia Brondz, reported that the volatility of the saturated mono-carboxylic acids decrease with an increase in chain length (Table 2.1). The article also shows a review of the methods that can be used in the separation and detection of a wide range of fatty acids [10].

Formula	Common name	Nomenclature name	Molecular weight	Boiling point
CH_2O_2	Formic	Methanoic	46.03	100.7
$C_2H_4O_2$	Acetic	Ethanoic	60.05	117.9
$C_3H_6O_2$	Propionic	Propanoic	74.08	140.99
$C_4H_8O_2$	Butyric	Butanoic	88.12	163.53
$C_5H_{10}O_2$	Valerianic, propylacetic	Pentanoic	102.13	186.05
$C_6H_{12}O_2$	Caproic	Hexanoic	116.16	205
$C_7H_{14}O_2$	Enanthic	Heptanoic	130.19	223
$C_8H_{16}O_2$	Caprylic	Octanoic	144.22	239.3
$C_9H_{18}O_2$	Pelargonic, nonylic	Nonanoic	158.24	255
$C_{10}H_{20}O_2$	Capric, decylic	Decanoic	172.27	270
$C_{11}H_{22}O_2$	Undecylic, hendecanoic	Undecanoic	186.30	280
$C_{12}H_{24}O_2$	Lauric, laurostearic	Dodecanoic	200.33	225
$C_{13}H_{26}O_2$		Tridecanoic	214.35	236
$C_{14}H_{28}O_2$	Myristic	Tetradecanoic	228.38	250.5
$C_{15}H_{30}O_2$		Pentadecanoic	242.41	257
$C_{16}H_{32}O_2$	Palmitic	Hexadecanoic	256.43	390
$C_{17}H_{34}O_2$	Margaric	Heptadecanoic	270.46	227
$C_{18}H_{36}O_2$	Stearic	Octadecanoic	284.50	360
$C_{19}H_{38}O_2$		Nonadecanoic	298.52	297
$C_{20}H_{40}O_2$	Arachidic, eicosoic	Eicosanoic	312.54	328

Table 2.1 Boiling points of saturated mono-carboxylic acids

Certain fatty acids can be separated and quantified using GC analysis without recourse to derivatization as indicated by James and Martin [11]. However, in order to increase volatility and sensitivity, derivatization is required. It was also noted [11], that tailing of the peaks on the chromatogram increases as volatility of an analyte decreases. Hence when higher sensitivity is demanded, derivatization has to be undertaken.

2.2.2 Detectors used in GC

The choice of an appropriate detector would involve the evaluation of certain characteristics exhibited by the various detectors such as; selectivity, response, noise, linear range, sensitivity and detectability [5]. The selection of a detector depends on the class of compound being analysed.

Electron capture detectors measure the loss of signal in relation to the analyte being determined. As the carrier gas flows through the detector a tritium source ionizes the gas molecules and slow moving electrons are formed. An analyte can only be detected if it contains a molecule that absorbing these electrons. This molecule reduces the current formed, by the electron migration to the anode under a fixed cell voltage. A carrier gas such as nitrogen needs to be used with ECD.

Flame ionization detectors operate on the principle that the electrical current of the gas is directly proportional to the concentration of the charged particles within the carrier gas.

As the analyte enriched gas flows from the column it is mixed with hydrogen and then burned in air. The resulting charged particles decrease the gap resistance within the detector creating a signal. The ionization source within this detector is a flame. The carrier gas used in FID can either be nitrogen, helium or hydrogen. However hydrogen is generally preferred.

An FID detector can be used in the analysis of FA. However the more specialized phosphorous sensitive detector is preferred for detection of trace concentrations of formic acid [10].

Although analysis of carboxylic acids was achievable using gas chromatography, the concentration levels of the various organic acids present in wines and fruit juices are usually very high (i.e.10 to 10 000 ppm); whereas. analysis by GC methods (needing time consuming derivatization) had focused on detection limits of less than 10ppm [1, 2, 3].

2.3 Capillary Electrophoresis

Capillary electrophoresis is a technique that has been used recently in many studies involving environmental and food analysis [6, 7, 9, 12, 13].

2.3.1 Principles on which CE is based and pertinent applications

Capillary electrophoresis makes use of a narrow bore fused silica capillary to execute separation of both large and small molecules. The process is facilitated by the use of a high voltage across the system. The high voltage generates an electroosmotic and electrophoretic flow of the buffered solutions and analyte/s through the capillary.

Chromatography and electrophoresis have a few significant differences; especially in terminology. One of the most fundamental terms, retention time, which refers to the time an analyte is held back or retained on the stationary phase within a column, drops away as nothing is essentially retained in electrophoresis. It is replaced with the term **migration time**. Migration time is the time it takes a solute to move from the beginning of the capillary to the detector [7]. CE however lacks the sensitivity required in organic acid determinations [1].

Within the capillary one would have ionizable silinol groups which are in contact with the buffer solution. The negatively-charged capillary wall would attract the positively charged buffer giving what is known as an electrical double layer. When a high voltage is applied across the capillary, the migration of the cations in the diffused part of the double layer towards the cathode draws the water with it. This results in the net flow of buffer solution to be in the direction of the negatively charged electrode. This flow is called the electroosmotic flow (EOF) [6, 7].

The buffer used in the application would depend on the method of detection being used.

2.3.2 Detectors used in electrophoresis

Detection of analytes by capillary electrophoresis can be attained by using either direct or indirect UV absorption. Due to the lack of strong chromophores in inorganic and organic anions, detection has to done using indirect UV absorption [7, 12, 13]. When using indirect UV absorption, the absorptivity of the electrolyte should be high to allow for lower detection limits. Pyridine-2, 6-dicarboxylic acid (PCDA), can be used as the electrolyte in the analysis of organic acids [6, 9]. Detection can be achieved at an UV absorption wavelength of 254 nm [7]. However in other applications detection was carried out using direct UV absorption at 185 nm. The electrolyte used in this application was sodium phosphate [9, 12, 13].

Although such applications are available it was established that sensitivity within these analysis was not satisfactory [1, 7, 8, 9].

2.4 High Performance Liquid Chromatography: Principles and divisions of HPLC

High performance liquid chromatography primarily uses a liquid as a mobile phase and a solid as a stationary phase. Separation of the analytes is based on the partitioning of the sample between a moving liquid phase and a solid stationary phase. The separation of carboxylic acids more often than not, involves one of these three divisions of HPLC:

- ion exchange
- ion exclusion or
- partition chromatography

It has been found that through the years, one of the abovementioned techniques has been favoured by particular groups of researchers, in an attempt to improve the elected method. Each method carries with it some advantages and disadvantages. All these techniques would be discussed in further detail.

Routine analysis of carboxylic acids is preferably performed using a method of high performance liquid chromatography rather than other techniques [1, 7, 8, 12]. Ion exclusion is usually the method of choice for the analysis of carboxylic acids in wine or fruit juice [17, 18, 19].

From a biological point of view, it can be said that fatty acids form a part of every living organism [10]. Hence most of the metabolic processes would include in some part, fatty acids. Analysis of milk, biotin etc. focused on the analysis of both the content of SCFA as well as LCFA, using partition HPLC methods [15, 16] whereas, analysis of carboxylic acids in environmental samples, by and large, involve the use of ion chromatography [19-23].

2.4.1 Ion exclusion: Principles and pertinent applications

Ion exclusion was one of the first and easiest methods of HPLC that was used in the analysis of carboxylic acids [14, 15]. The method requires the use of a strongly acidic eluent which would ensure that the weaker organic acids remain protonated. An imaginary membrane called the Donnan membrane (i.e. electrostatic repulsion) acts as a barrier (Figure 2.2), allowing only the weak acids towards the stationary phase. The stationary phase is an ion-exchange resin which has an electrical charge of the dissociate functional group of sulfonate.

A weak carboxylic acid remains undissociated, due to its low pKa values, and is allowed to pass over the membrane, whilst the charged, ionic species present are excluded. The carboxylic acids can therefore be separated on the basis of their pKa values.

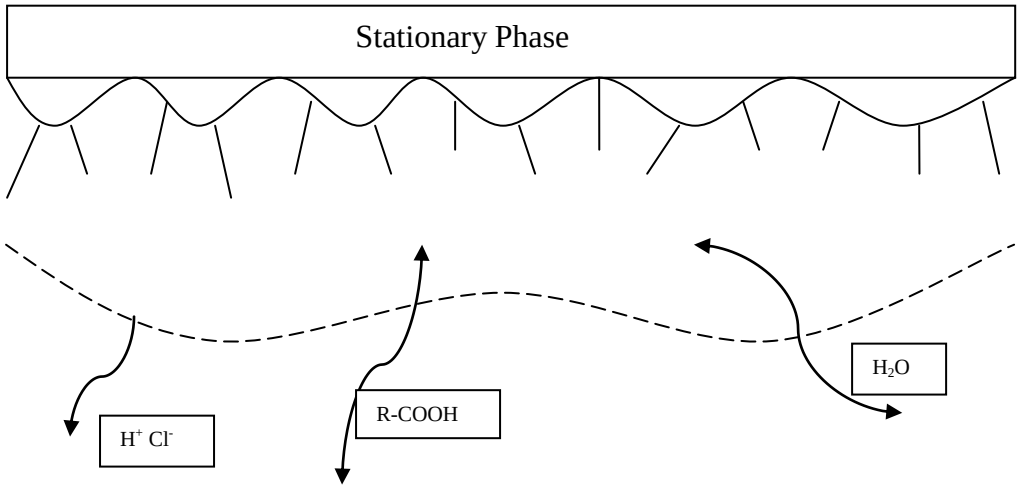

Figure 2.2: Principle for ion exclusion using Donnan membrane

The elution behavior of the aliphatic carboxylic acids was investigated. It was found that the order of elution was dependent on the pKa (Table 2.2) and its hydrophobicity [10].

Name of Organic Acid	Molecular Formula	Boiling Point	pKa Value	IUPAC Name
Formic Acid	CH_2O_2	100 – 101	3.75	Methanoic acid
Acetic Acid	$C_2H_4O_2$	118	4.76	Ethanoic acid
Propionic Acid	$C_3H_6O_2$	141	4.87	Propanoic acid, Ethylformic acid
Butyric Acid	$C_4H_8O_2$	164	4.82	Butanoic acid, n- Butyric acid,
Oxalic Acid	$C_2H_2O_4$	Sublimes 146 - 160	1.27	Ethanedioic acid
Maleic Acid	$C_4H_4O_4$	135	1.5	Cis-butenedioic acid

Table 2.2 pKa values of the simple carboxylic acids

Ion exclusion methods usually use UV/Vis detectors as they were found to be more convenient [1, 10, 14]. However the use of a wavelength of 210nm is not suitable for organic acids in cases of masking of the target analytes caused by a complex matrix [14, 16]. The potential of its use has not been explored to its full extent. Applications have been confined to high concentrations i.e. elevated ppm levels of larger fatty acids and were commonly used in the food and beverage industries [17, 21].

In 1996 Jian Chen, reported on the determination of organic acids in industrial streams using ion chromatography. However it lacks sensitivity giving high detection limits (ppm). Chen also claimed that gradient elution using ion exclusion would not be practical due to the separation mechanism used [25]. The most recently published articles by A. de Villiers et al achieved a LOD of 4.6 ppm for acetic acid [6].

Kazuhiko Tanaka changed the mobile phase from the usual sulphuric acid to 1mM benzoic acid -10mM cyclodextrin solution; to avoid the need for chemical suppression and was able to increase sensitivity and resolution [58]. Detection limit for acetic acid was improved to 590 ppb from the previous ppm levels [58]. Kazutoku Ohta was able to detect the carboxylic acids using a specialized sulfonated – styrene divinylbenzene column with (5-methyl hexanoic acid) as an eluent at pH 4 [23]. Using this method the analyst was able to achieve detection limits of 14 to 40 ppb levels for the aliphatic carboxylic acids.

2.4.2 Ion Chromatography: Principles and pertinent applications

Ion Chromatography has been adopted as the tool of analysis for short chain carboxylic acid and their salts in environmental studies. Methods using ion exclusion coupled to ion chromatography have limited application as only isocratic elution could be used [4]. Ion-exchange chromatography is now commonly being used as an alternative to the ion exclusion technique.

A review by Klaus Fisher showed detection limits of 1-2 ppm levels for methods using ion exclusion with conductivity detection [2]. Further, comparative studies by Jian Chen , and Ming Yu Ding et al, have shown an improvement in detection levels when using ion-

exchange methods instead of ion-exclusion with conductivity detection [2, 25]. A conductivity detector was found to be highly sensitive with respect to the determination of organic acids [21, 23, 24, 29, 33-35, 50-56]. However weak carboxylic acids are difficult to detect using direct conductivity detection. The peak heights of the analytes are relatively small and would therefore not allow detection at lower concentration levels [29]. In order to alleviate the problem, chemical suppression was used to reduce the background conductivity of the eluent.

Suppression is achieved by the use of an ion exchange column which ultimately reduces the conductivity of the commonly used sodium hydroxide-containing eluents [1, 29, 32]. The Dionex Company had previously patented this method but, since then Metrohm (Figure 2.3) has been involved in many investigative studies to improve the detection of these weak organic acids [29-34].

Ion chromatography using an anion exchange column, increases selectivity, thus improving detection limits at trace levels. The use of a suppressor reduces background noise significantly, allowing faster equilibration. The two types of suppressors available are: - Electrolytic (Chemical) Suppressors and Electronic Suppressors [23-26].

Analysis done by Metrohm shows a distinct difference between the peak heights of a 100 ppm acetic acid, 100 ppm propionic, and 0.5 ppm formic acid, detected with and without suppression. [34, 35]. It showed that the peak heights of the organic acids increased with the use of a suppressor column. The eluent was 0.5mM perchloric acid with a 10mM lithium chloride solution as the regeneration solution. The column used was a Metrosep OA column [34, 35].

In reports published by the Dionex Corporation, i.e. Adrian A. Ammann et al, Jian Chen, R. Roehl et al, Mahmood Toofan and Zhongoqing Lu, were the first lot of analysts successful in being able to detect C1 to C8 carboxylic acids in environmental water samples at trace levels [24, 25, 50]. They were equipped to detect some of these acids between 60 to 6 ppb levels. Most of the analyses, however, focused on the detection of the acetate and formate ion. Some analysts were able to separate the acids but found that resolving the acids was difficult. This was attributed to the fact that carboxylic acids absorb weakly [19].

Other analysts [21- 25] have used anion-exchange chromatography with gradient elution were able to obtain a LOD of between 6000 – 600 ppb level.. In 2003, K Tanaka, [58] was able to get a LOD of 590 ppb for acetic acid. Elution time was also a factor to consider when detection of more hydroscopic organic acids is required.

P. Hajós [19] reviewed the retention behavior of different carboxylic acids related to changing pH, stationary phases, etc. It was noted that, by controlling the ion-exchange selectivity, one would be able to manipulate the separation of the carboxylic ions in ion-chromatography [4]. By using the selectivity coefficient, the analyst would be able to determine that increasing the eluent concentration would bring the elution time to a practical operation range, when using a high capacity ion-exchange resin column.

Investigations carried out in the most recent years have used ion-chromatography coupled to a mass spectrophotometer detector (IC-MS) [29, 32, 50, 57]. A group of researchers from Metrohm petrol chemistry branch has carried out a study on the determination of aliphatic mono-carboxylic acids in produced water. They considered the detection of organic acids in crude oil [32].

The mobile phase used was 0.2mM oxalic acid with 50mM sulfuric acid being used as the regeneration solution. In addition to this, 300mM ammonia was used as a post column solution. All the samples were prepared in 100 g/L sodium chloride solution [32].

The results showed conductivity to be much lower in sensitivity using the 100g/L chloride as a matrix. Only standards from 10 to 250 ppm could be analysed for certain acids using conductivity. Chromatograms also showed co-elution of an unknown peak with propionic acid as well as poor detection of acetic acid which manifested as a shoulder on the injection peak. The analysis using MS was said to be more reliable whilst conductivity could only be used for qualitative analysis for this application. Butyric acid exhibited a detection limit of 60ppb whilst acetic and propionic acids had detection limits of 21 ppm and 9 ppm respectively.

Applications using ion chromatography for the analysis of organic acids are well known [1, 17, 29], and although they have not been fully exploited, it is this method of analysis that shows most promise in future analysis.

2.4.3 Partition Methods: Principles and pertinent applications

Partition methods of liquid chromatography refer to either normal phase chromatography or reverse phase chromatography. With regards to normal phase chromatography the stationary phase is always more polar than the mobile phase. Reverse phase chromatography, on the other hand would have a more polar mobile phase and a less polar stationary phase.
Simple short chained carboxylic acids are semi-volatile in nature and should be able to be detected using GC. However, as discussed in 2.2, sensitivity is compromised at lower concentrations.

Detection of organic acids using HPLC is favored by ion exclusion methods, as it is the easiest method to use for this analysis. The carboxylic group has a very poor chromophore that allows a maximum absorption at 210nm [1, 11, 16]. It is for this reason the sensitivity at lower concentration levels diminishes. To increase the sensitivity, the carboxylic group has to be derivatized to a stronger chromophore. The derivatized analyte is then analysed by a method of partition chromatography [16, 26, 27, 36, 37].

However, there has been analysis carried out using direct detection of SCFA using reverse phase liquid chromatography (RPLC) [60, 22].

Guanghou Shui et al [22] and Zohar Kerem et al [60], have both carried out analysis for the determination of carboxylic acids and phenolic compounds in either fruit juices or wine using direct RP chromatography [22, 60]. Acetic acid is the only SCFA of interest that would be found in a beverage. The LOD that was achieved by both analytes were within the ppm levels. Gauanghou Shui had a LOD of 10.9 ppm for acetic acid and Zohar Kerem obtained an

LOD of 8 ppm for acetic acid [22, 60]. Hence sensitivity, selectivity, and speed of analysis are improved by introducing derivatization into an HPLC method [10].

2.4.4 Derivatization in HPLC Analysis

For organic acids HPLC methods commonly use a UV detector set at a wavelength of 210 nm. However the weak chromophoric properties of the carboxyl group result in poor sensitivity.

Derivatization of short chain carboxylic acids in an aqueous solution is difficult due to the low reactivity of the carboxylic acid in water. Pre-column derivatization has been employed as the set method to increase detection of SCFA. Fluorescent derivatization was one of the first methods of derivatizing a carboxylic acid. In a study carried out by Evagelos Gikas [4] 4-bromomethylcoumarin-7-phenyl [6,7-b] (9,6) oxazine, BrMOZPhC, was synthesized and used to in the derivatization of SCFA. However, in spite of achieving a LOD of 18 µg/L for C_5 - C_{10} acids the procedure was long, complicated and time consuming.

Hence a derivatizing procedure less complicated and time consuming derived by Hiroshi Miwa was found to be favored by many analysts [28, 37, 38]. This procedure required the use of 2-nitrophenyl hydrazine (NPH) as the derivatizing agent. The method was reported to be less cumbersome than previous methods [16, 17] which required the more tedious, isolation of the carboxylic acids prior to derivatization [49].

NPH, along with the coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride is used to transform the simple carboxylic acid to a 2-nitrophenyl hydrazide derivative [15, 16]. This method has also been widely used in the analysis of aldehydes and ketones as noted by Peters, R. et al [27]. The resulting hydrazide derivative is non-volatile, thus limiting the application uses to HPLC. Detection of the analytes could be accomplished using a UV detector at 230nm or using a diode array detector (DAD) at 400nm [16, 17, 26, 27].

2.5 Methods of Detection in HPLC

The detection method used in liquid chromatography depends on the chemistry and nature of the analyte to be determined. There are many types of detectors that can be used; but the most common detector is the UV-Vis detector. Absorbance detectors like the UV or fluorescence detectors are excellent for sensitivity on certain compounds; however there are some for which it would lack sensitivity. The reactivity, colour, volatility, electrical charge etc. are all considered when considering the type of detector that can be used.

These are a few types of specialist detectors: Evaporative light scattering detectors and mass spectroscopy detectors require ionization of the analyte. Fluorescence and UV require the analyte to be active at a particular wavelength. Visible detection or colorimetric detectors are suitable for coloured analytes. Each detector will be discussed in detail below.

2.5.1 Evaporative Light Scattering Detector:

An evaporative light scattering detector works in a similar manner to mass spectroscopy. The eluent or sample is drawn into nebulization chamber were it forms a fine mist of droplets.

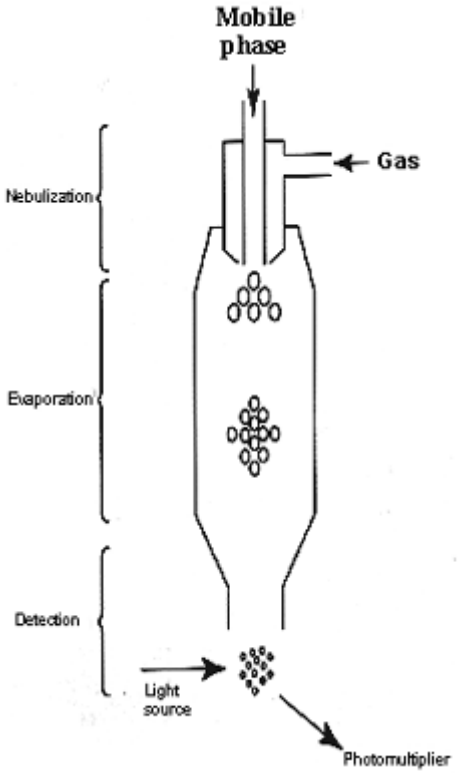

Figure 2.4: Movement of analyte through detector

This is followed by a bombardment of the sample droplets with the carrier gas being air, nitrogen, or helium. The larger droplets are prevented from entering the evaporation tube by gravity and are redirected to waste [42]. The solute molecules are sent into the heated evaporation tube were it is passed through to the detector. ELSD is a sensitive detector and is ideal for use of semi volatile to volatile analytes [38, 42]

The volatility of the analyte must be greater than that of the solvent. This would ensure an easy separation of the analyte from the mobile phase within the nebulization chamber. The solvent droplets are largely responsible for an increase in noise levels. Formic and acetic acids are generally used within the mobile phase as modifiers which makes it difficult to detect as an analyte. However there have been a few applications of organic acids using trifluoroacetic acid as the mobile phase [40-41].

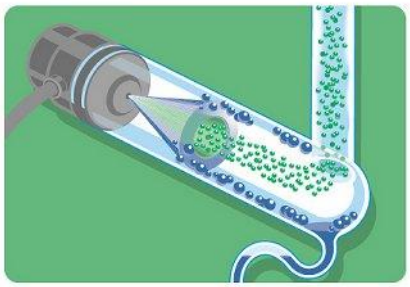

Figure 2.5: Movement of droplets in spray chamber

2.5.2 UV/Visible Detectors:

Analysis of organic acids in wines, fruit juices etc have previously been discussed in detail. Detection is achieved by both direct and indirect UV analysis, depending on the method used. Indirect UV detection is used in capillary electrophoresis [7, 8], whilst direct UV analysis is used with ion exclusion chromatography. Within these applications the concentration levels of the target organic acids are sufficiently high, thus creating a more ideal sample. No methods of pre-concentration are required. However, difficulty would be encountered due to a more complex matrix. Detection of the analyte is thus improved by incorporating a clean-up procedure e.g. SPE [6, 7]. Most detection of organic acids is achieved on a UV-vis detector using ion exclusion as a method of analysis [1, 2, 6, 9, 14, 17].

2.5.3 Conductivity Detection:

Ion chromatography requires the use of a conductivity detector. The principle of operation of the conductivity detector is based on the electrical conductivity of an ionic solution when placed between two oppositely charged electrodes. The presence of ions in the solution allows an electrical current to flow. At low ionic concentration, conductivity is directly proportional to the concentration of the conductive species in the solution. This relationship is dependent on temperature.

2.6 Samples and Sample Handling

Samples and sample handling are the most important parts to any research or project. Prior to any analysis, sampling and sample preparation has to be carried out such that there is minimal interference due to matrices that could result in incorrect deductions [43]. Sample preparation is of utmost importance, it is when one coverts a ‘real matrix’ into a format that would be suitable for the method of analysis being used. Depending on the type of sample that is being used, one would devise appropriate steps of handling the sample. In this investigation the intended sample has no complex matrix that needs to be isolated. However due to the nature of the analysis the low concentration levels of the organic acids expected would probably call for the use of a pre-concentration step.

2.7. Pre-concentration or Enrichment of samples

The analysis of OA or SCFA does not generally require a pre-concentration method [1, 6, 10]. Sample preparation was used to remove the complex matrices in which the acids were usually found [1, 6, 10, 15, 16, 36, 37]. However in this project the most challenging factor was the very low concentration levels of organic acids in the target samples. The development of a non-complicated method of enrichment or pre-concentration is therefore very crucial.

2.7.1 Pre-concentration techniques

There are many pre-concentration methods that are available, which are constantly being improved on. Older methods of sample preparation used liquid-liquid extraction. However recently sample extraction and clean-up methods have improved to such a vast extent that the excessive use of hazardous solvents is obviated.

Recently developed methods of extraction such as solid phase extraction (SPE), solid phase micro-extraction (SPME) and the stir bar sorptive extraction (SBSE) are examples of miniaturization of sample preparation techniques. These methods have improved quantitative recoveries, whilst reducing the amount of laboratory time and solvent used [43-45].

Solid phase extraction requires a sample to be passed through an extraction column made of either polyethylene or glass. The liquid is passed through suitable disks or cartridges. The analytes are adsorbed onto the cartridge and thereafter extracted by eluting with appropriate solvents [44, 45, 49, 50].

In SPME the analytes are absorbed from a liquid onto a PDMS coated fused silica fiber. The analytes are desorbed by means of heat, which is referred to as thermal desorption or by solvent desorption by means of a HPLC interface [46]. This method of desorption is also used in SBSE. SBSE is very similar to SPME in many respects. A thermally stable absorptive extraction phase called polydimethylsiloxane is thinly coated onto a stir bar, which is then placed into an aqueous solution and stirred, thereby extracting the analytes in question. This method is also a means of enrichment [47-48]. The solutes are thereafter desorbed using thermal desorption. The only difference between SPME and SBSE is the higher mass of the adsorption layer of PDMS used in the latter which leads to higher recovery and higher sample capacity.

2.8 Focal Point of Thesis

The determination of OA or SCFA is generally carried out in the food and beverage industries as well as the biological field of study. Quality controls of these acids are paramount, especially in wines and fruit juices. Organic acids in different compartments of the environment have come to attract a lot of attention for various important reasons.

Simple carboxylic acids can be an indicator to the breaking down of larger toxic organic pollutants. However concentration levels of the organic acids in water and the environment are much lower than that which is found in wines and fruit juices. It is mainly for this reason that this investigation on the trace levels of organic acids was carried out. Although focus within this investigation was on power station water, the developed methods can be applied to any industry or environmental study.

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Chapter Three

Experimental

3.1 Chemicals and materials

3.1.1 Chemicals

The following chemicals were used throughout this investigative study. Acid standards were: ethanoic acid (acetic acid) (glacial 100% purity, HiPersolv for HPLC, BDH, Poole, England), methanoic acid (formic acid) (99.5%, Fluka, MS grade, Sigma-Aldrich Chemie, GmbH, Steinheim, Germany), butanoic acid (butyric acid) (99%+, R&D grade, Sigma-Aldrich Chemie, GmbH, Steinheim, Germany), propanoic acid (propionic acid) (99% purity, R&D grade, ACS reagent, Sigma-Aldrich Chemie, GmbH, Steinheim, Germany), and oxalic acid (99% purity, R&D grade, Aldrich-Cheimie, GmbH, Steinheim, Germany). Solvents were sulphuric acid (98% purity Merck, KGaA, Darmstadt, Germany), perchloric acid (Merck, KGaA, Darmstadt, Germany), trifluoroacetic acid (TFA), (Sigma Aldrich, research grade, hygroscopic to be kept in a desiccator), hydrochloric acid (32% Saarchem, Wadeville, Gauteng, South Africa, Merck Chemicals), acetonitrile (200 Romil-SpS, Super Purity, Waterbeach, Cambridge) or (LiChrosolv, Merck, KGaA, Darmstadt, Germany), methanol (LiChrosolv, Merck, Darmstadt, Germany), pyridine (>99.9% Chromasolv plus for HPLC, Sigma Aldrich Aldrich-Cheimie, GmbH, Steinheim, Germany), ethanol absolute (Chromosolv for HPLC, Aldrich-Cheimie, GmbH, Steinheim, Germany) and ammonia (Orion Chemicals, 25% solution).

Other chemicals that were used were lithium chloride (AR grade, Saarchem, Merck, Gauteng, South Africa), 2-nitrophenyl hydrazine (NPH) (Sigma-Aldrich Chemie GmbH, to be refrigerated, light sensitive), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (Bio Chemika, Sigma-Aldrich Chemie GmbH, to be stored -18^{o}C), disodium phosphate heptahydrate, and monosodium phosphate, monohydrate (Saarchem, Merck, Gauteng, South Africa), potassium hydroxide (Saarchem, Wadeville, Gauteng, South Africa, Merck Chemicals) and sodium hydroxide (Saarchem, Wadeville, Gauteng, South Africa, Merck Chemicals).

3.1.2 Other consumables

Syringes (2 mL), syringe filters (0.45 µm nylon) and disposable micropipette tips were obtained from Waters. Cartridges that were used for solid phase extraction were Supelco-Weak Anion Exchange (WAX) cartridges, 3 mL/500 mg silica based tubes and Phenomenex, Strata X-AW mixed mode cartridge, 6 mL/200 mg polymeric sorbent. Disposable 100mL reservoirs (Analyichem International, CA, USA) were used for loading of samples.

The water that was used in preparation of standards and mobile phases was obtained from a Millipore Ultra-pure Water System at 18 MΩ water.

3.2 Preparation of standards and reagent solutions

3.2.1 Acid Standards

Individual stock solutions, 5000 mg/L (ppm), for each of the acid standards were prepared in ultra-pure water and kept refrigerated. Working standards were prepared daily when required, from the stock standards. A mixture of acids was prepared by taking the required volume from the stock solution and diluting with Milli-Q water to obtain a 50 ppm acid mixture standard. This standard was used in establishing the instrumental parameters for each of the methods.

3.2.2 Preparation of Mobile phases:

A 2.5 mM sulphuric acid mobile phase was made-up by adding 140 µL of the concentrated acid to 900 mL of 18 MΩ water then topping-up to mark in a 1 L volumetric flask. A 1.25 mM perchloric acid and 0.5% Trifluoroacetic acid mobile phase was also prepared in 18 MΩ ultra-pure water.

Acidified water with HCl (pH 4.5), used in the partition chromatography was prepared by adding 26 µL of a 1 M HCl solution to 900 mL of 18 MΩ deionised water, then topping up to 1 L in a volumetric flask. The pH was confirmed using a pH meter. A 20 mM (pH 4.5) sodium dihydrogen orthophosphate dihydrate buffer was prepared by dissolving 3.1942 g of the salt in 900 mL of deionised water then transferring to a 1 L volumetric flask before topping-up to mark.

All mobile phases were filtered with a 0.45 µm filter and degassed with a Millipore Vacuum Pump before use.

3.2.3 Preparation of other reagents

The derivatization solutions were prepared in two ways:

1) A fresh NPH *(o-nitrophenyl hydrazine)* solution was prepared daily by dissolving 34 mg of the orange crystals (Fluka, 98% purity) in 5mL of ethanol by ultrasonic agitation, followed by an addition of 2 mL of 1M HCl and 3mL of ultra-pure water. The solution was prepared in a 10 mL volumetric flask.

The stock EDC solution was prepared by dissolving 0.24 g of the white solid (Sigma Aldrich, 99% purity) in 5 mL of ultra-pure water. It can be stored in a refrigerator for a week. Fresh working EDC solution was prepared daily by mixing equal parts of the stock EDC solution with a prepared 3% solution of pyridine in ethanol.

2) A NPH solution was prepared by dissolving 10 mg of NPH in 5 mL ethanol and placing it in an ultrasonic bath for 10-15 minutes. This was followed by 2 mL of 0.1 M HCl and 3 mL of 18 MΩ deionised water. The solution was kept in an amber coloured bottle. The EDC solution was prepared by dissolving 0.24 g of the white solid in a 3% pyridine solution. The 3% pyridine solution was prepared in ethanol.

10% sodium hydroxide and 10% potassium hydroxide solution were prepared in 1:1 methanol: water solution.

A 3% pyridine solution was prepared by adding 750 µL of pyridine to 20 mL of absolute ethanol, then topping-up to 25 mL in a volumetric flask.

The following solutions were prepared for solid phase extraction:
A pH 6 buffer was prepared by combining 12.14 g of monosodium phosphate monohydrate with 3.22 g of disodium phosphate heptahydrate in a 1 L volumetric flask. A sodium dihydrogen phosphate buffer (pH 7), was prepared by dissolving 6 g of the salt in 900 mL water (18 MΩ). The pH was adjusted with a 6 M sodium hydroxide solution and 6 M hydrochloric acid solution and then made up to 1 L.

A 1% HCl solution as well as a 1% NH_4OH was prepared in a 20% methanol/water solution. A range of buffers pH 2, pH 4, pH 6, pH 8 and pH 12 were prepared by adjusting a 50 mM sodium dihydrogen phosphate buffer with either 6 M HCl or 6 M NaOH to the required pH.

3.2.4 Samples

Samples that were obtained from the sample points at the power station were collected in 1 L plastic bottles and transported to the laboratories in Durban, Kwa-Zulu Natal via overnight delivery. Samples were stored in a refrigerator.

3.3 Instrumental

3.3.1 HPLC apparatus

The following instrumentation was used; a SPD-M20A Diode Array Detector with a Waters 600E Pump and LC solutions operating system, a Thermo-Separation UV1000 detector attached to a P2000 pump and SN 4000 interface. Sample injection loop size was 20 µL. The operation program that was used was ChromQuest. A refractive index detector ERC 7515A and an Evaporative Light Scattering Detector Sedex 75 attached to a Waters 590 pump equipped with 20 µL loop and column heater. A Metrohm 753 Suppressor module coupled with a Metrohm 690 Conductivity Detector. A Waters 501 pump was used along with a Waters 740 Data Module. As well as a Metrohm 761 IC Compact with a 758 IC Filteration Sample Processor and IC Net Software from an outside laboratory (SA Bio Products).

3.3.2 List of Columns

A Phenomenex Rezex Organic Acid column with 300 x 7.80 mm dimensions was used for all the analysis carried out on the ELSD, conductivity detectors and UV detectors for ion exclusion analyses. A Metrosep OA, 100 x 7.8 mm, 8 µm column was also used in certain analyses.

The columns used with regards to partition chromatography were, a Hypersil Gold C18 column, 250 x 4.6 mm with a C18 guard column, a C8 Licrospher100, RP8

250mm – 4 mm, 5 µm column and a Nucleodur 100-5 C8ec, 250 mm - 4.6 mm, 5 µm column with a C8 Phenomenex security guard cartridge.

3.4 Ion Exclusion Chromatography

3.4.1 Ion Exclusion using UV Detection

A Phenomenex Rezex OA column with 300 x 7.80 mm dimensions was used for this analysis. The mobile phase that was used was 2.5 mM sulphuric acid or 1.25 mM perchloric acid. Detection was carried out at a wavelength of 210 nm. Acetonitrile was used as an organic modifier in the mobile phase [5]. The flow rate used was 0.5 mL/min.

3.4.2 Ion Exclusion using Evaporative Light Scattering Detector

The mobile phase that was used for this application was 0.5% Trifluoroacetic acid in water. Conditions on the instrument were originally set as follows, *Gain: 9, Detector temperature: 40^{o}C, Column temperature: 40^{o}C, Pressure: 2 bar*. The column that was used in this application was the Phenomenex Rezex Organic Acid 8% H. Flow rate that was used was 0.7 ml/min. Analysis was attempted with both nitrogen and air as a carrier gas.

3.4.3 Ion Chromatography without Chemical Suppression

The column that was used was the Phenomenex Rezex Organic Acid 10µm 8% H. The mobile phase was 1.9mM H_2SO_4 in 2% acetonitrile. A mobile phase of 1.2mM H_2SO_4 was prepared in a 2% acetonitrile/water solution. Column temperature was ambient, with a flow rate of 0.6 ml/min.

4.4.5 Ion Chromatography with Chemical Suppression

The column used was a Phenomenex Rezex Organic Acid 10µm 8% H, at ambient temperature. The mobile phase (eluent) that was used was 0.5 mM perchloric acid along with 10 mM lithium chloride as the regeneration solution. The flow rate was kept at 0.6 ml/min.

3.5 Partition Chromatography

3.5.1 Derivatization of Acids and Detection using UV Detection

The derivatizing agent that was used in this analysis was 2-nitrophenyl hydrazine (NPH) with a coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC). Mobile phase A was either acidified water with HCl (pH 4.5), or phosphate buffer (pH 4.5) and mobile phase B was either 100% acetonitrile, or a 60: 40 acetonitrile: methanol mixture.

3.5.1.1 Derivatization procedure without KOH

Analysis was carried out using a Hypersil Gold C18 column, 250 x 4.6 at a flow rate of 1.8 ml/min, temperature was kept at 40^{o}C. The mobile phase that was used was acidified water with HCl (pH 4.5) and acetonitrile.

The derivatization process used followed the step-by-step procedure listed below:

- 80 µL of NPH solution was injected into a vial.
- 160 µL of EDC was then added to the vial.
- Solution was mixed with a vortex mixer
- 800 µL of sample was then injected into the vial and mixed by the vortex mixer.
- The vial was immediately placed into a water bath at 60^{o}C (Figure 3.1) for 15 minutes.
- After 15 minutes, the sample was analysed.
- This procedure was repeated for each injection.

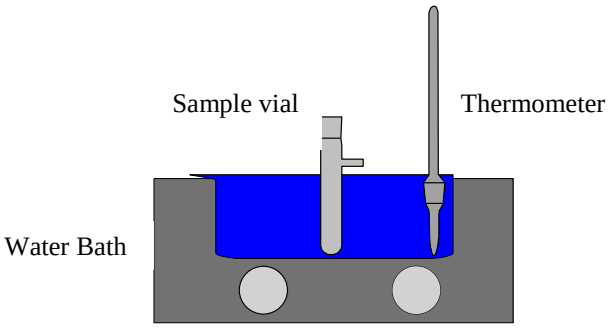

Figure 3.1 Apparatus used for derivatization

3.5.1.2 Derivatization procedure with KOH

A C8 Licrospher100, RP8 250 mm – 4 mm, 5 µm column, and a C8, Nucleodur 100-5 C8ec, 250 mm -4.6 mm, 5 µm column with a C8 security guard column were used along with this derivatization procedure. Column temperature used was 30^{o}C. The mobile phase consisted of acidified water using HCl (pH 4.5) along with a 60:40 Acetonitrile/Methanol mixture.

Table 3.1 below lists the derivatization procedure followed:

STEPS	INSTRUCTION
1	200 µL of EDC was added into a vial
2	200 µL NPH was added to the EDC
3	500 µL of sample was injected into the resulting solution and mixed in a vortex for 5 seconds
4	The solution was then heated in a water-bath set at 60^{o}C for 20 minutes (Figure 3.1)
5	100 µL of 10% KOH was injected into the solution and heated at 60^{o}C for a further 15 minutes
6	The sample was injected into the HPLC

Table 3.1 Step followed for the derivatization of carboxylic acids using method 3.5.1.2

3.6 Sample Collection

Samples used in analysis were collected from the Tutuka Power Station. The main sample points chosen for this analysis had to be contained within the steam/water circuit as shown by arrows in the diagram below:

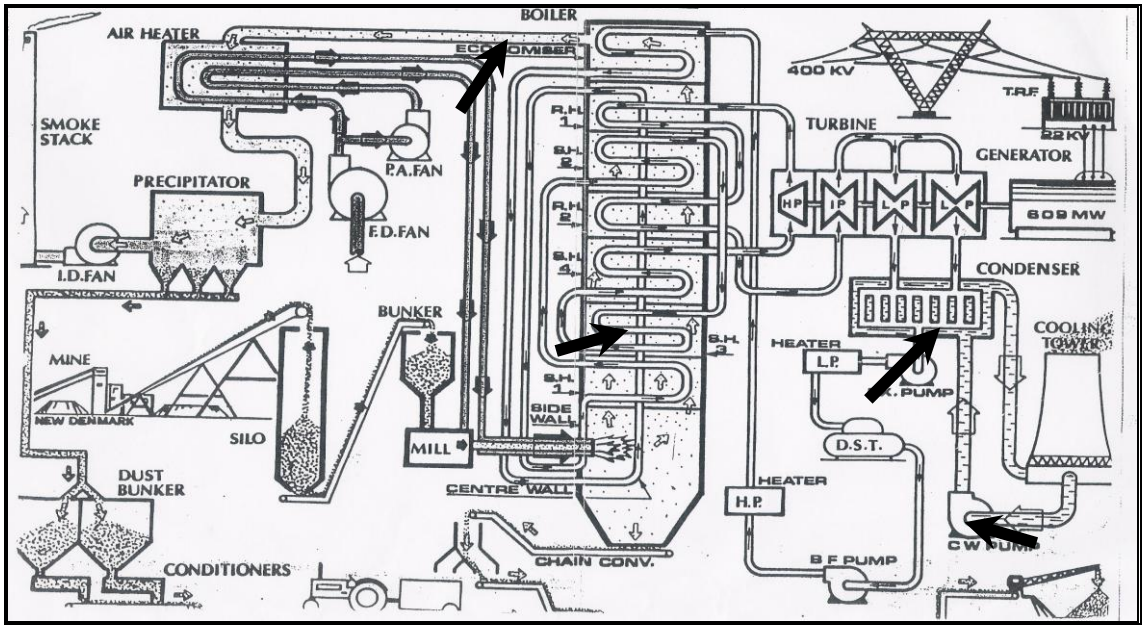

Figure 3.2 Sample points within boiler unit (ESKOM Lethabo PS)

Sampling was carried out at the main steam, economiser, condensers and mix bed outlet.

3.7 Pre-concentration using Solid Phase Extraction

A vacuum manifold was used to carry out the extraction process using either a vacuum pump or the in house vacuum line. The protocol steps of conditioning,

loading, washing and eluting were followed. The flow rate was kept less that 5mL/min during loading of the sample.

3.7.1 Pre-concentration Method One

The solid phase extraction cartridge used in this method was Supelclean-Weak Anion Exchange (WAX) cartridges. The experimental conditions for the pre-concentration techniques are listed below in Table 3.2.

	Trial A	Trial B	Trial C
Conditioning	3 mL Methanol	3 mL Methanol	3 mL Methanol
Equilibration	3 mL Deionised water	3 mL Water	3 mL Water
	3 mL pH6 Buffer		
Sample Loading	100 mL of Sample Flow rate 1 mL/min	100 mL of Sample Flow rate 1 mL/min	100 mL of Sample Flow rate 1 mL/min
Wash	3 mL Buffer pH 6	3 mL pH 6 Buffer	3 mL pH 3 Acid
Elution	pH 3 Acid (H_2SO_4 or HCl) Flow rate 1 mL/min	3 mL pH 3 Acid in Methanol	3 mL pH 6 Buffer Flow rate 1 mL/min

Table 3.2 Table of trials carried out for SPE method development, Supelco-Weak Anion Exchange (WAX) cartridges

3.7.2 Pre-concentration Method Two

The original method of pre-concentration using the Strata X-AW cartridge was obtained by a program recently developed by Strata to find the suitable steps to achieve the desired effect of pre-concentration or clean-up.

The method comprised of the following steps:

Steps	Instructions
Conditioning	5 mL of Methanol using a flow rate up to 12-24mL/min
Equilibration	5 mL of pH 7 buffer (50mM, NaH_2PO_4) at flow rate of 12-24mL/min
Sample Loading	50- 150 mL of sample adjusted to pH 7 using flow rates of 4-8mL/min
Wash 1	5 mL water at flow rates of 4-8mL/min
Wash 2	5 mL methanol at flow rates of 4-8mL/min
	Dry cartridge for 1 minute under full vacuum prior to elution.
Elution	5 mL of a 1% concentrated HCl in 20% methanol at flow rates of 4- 8 mL/min.

Table 3.3 Steps followed for pre-concentration using Strata X-AW

The sample solution was adjusted to a pH 7 using a 0.1 M sodium hydroxide solution.

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Chapter Four

Results and Discussion

4.1 Introduction

Prior to a discussion of the results obtained by the most suitable method set up, an evaluation of the results obtained by the other methods investigated will be given. This will provide an experimental basis for recommending the identified method to ESKOM laboratories where the method will be used routinely for monitoring samples from various power stations for carboxylic acids at trace levels.

The majority of reports in the literature for the determination of simple carboxylic acids occur in the food and biological fields of study, and are not completely focused on within the environmental field of study. Methods used within this dissertation are derived, both from application notes and published journal articles.

4.2 Ion Exclusion Chromatography

4.2.1 Basis for exploring this method

Ion exclusion is the principal method of chromatography used in the separation of organic acids [1]. The stationary phase most commonly used is sulfonated styrene-divinylbenzene (SDVB) co-polymer resin [10]. Stronger acids are excluded from the stationary phase by means of an electrostatic force of the dissociated silanol groups

(Donnan Membrane, Figure 2.2, Chapter 2-8). The order of elution is not prescribed by the size of the ion but rather the pKa value (i.e. the degree of dissociation) of the weak acids. A strong acid would dissociate completely into its ions releasing H^+ into the solution. These acids will not be allowed to adhere to the stationary phase and thus co-elute very quickly out of the column.

The strength of a weak acid is determined by its pKa value. The stronger the acid, the smaller the pKa value, hence a weak organic acid with a lower pKa value would elute earlier then an acid with a higher pKa value. Longer chained aliphatic acids are more hydrophobic in nature, and have a tendency of being retained longer on a SDVB column hence analysis of short chain acids are favoured. The increased retention of the long chain acids on the column, impacts on the broadening of the peaks and longer run time, which are both undesirable in any analysis. Ion exclusion was used in conjunction with various detectors in order to attempt improving the sensitivity of detection of the acids being analysed in this study.

4.2.2 Ion Exclusion Using UV Detection

Analysis of simple carboxylic acids was carried out using an ion exclusion column with detection being achieved on a UV detector at 210 nm. Analysis was carried out on five acids: oxalic acid, acetic acid (ethanoic acid), formic acid (methanoic acid), butyric acid (butanoic acid) and propionic acid (propanoic acid). The mobile phase was chosen from an application note provided with column, which suggested 2.5 mM H_2SO_4. The acids were successfully separated with oxalic acid (pKa 1.27) being the first to be eluted followed by the other four acids from C_1 (pKa 3.75) to C_4 (pKa 4.82)

respectively. Detection of the acids can however be successfully achieved at higher ppm levels (Figure 4.1).

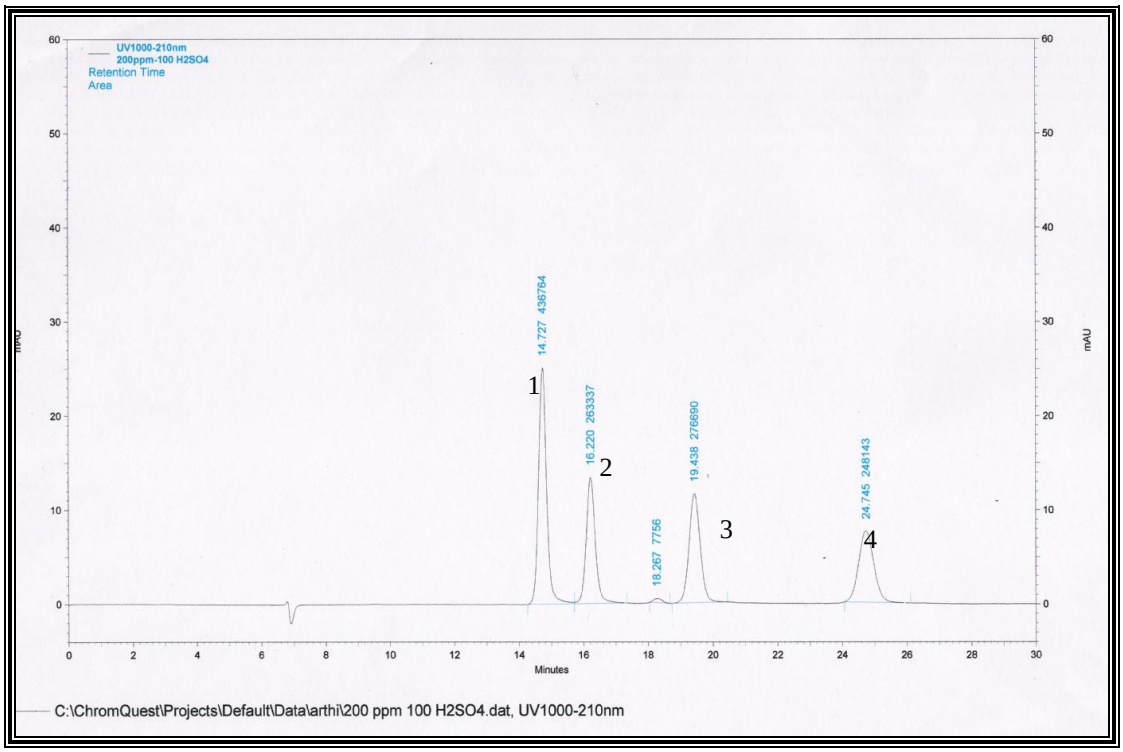


Figure 4.1: Typical chromatogram obtained on the TSP UV detector at 210 nm Using ion exclusion, showing separation of the organic acid mixture (200 ppm of each): 1. formic acid, 2. acetic acid, 3. propionic acid, 4. butyric acid

4.2.2.1 Sensitivity of Detection at chosen Wavelength

A Photodiode Array Detector was used to confirm that the wavelength of 210 nm is the optimum wavelength that can be used for this analysis (Figure A1.1, page A1-2). UV detection can be achieved from a region of 190 nm to 380 nm. The sensitivity of detection is poorer toward the extreme limits of the wavelength range. It is for this

reason that analysis of organic acids at 210 nm was better suited at higher concentration levels.

4.2.2.2 Need for inclusion of a Modifier in Mobile Phase

Analysis was initially carried out on five acids. Subsequently, oxalic acid was omitted from the analysis as it was the only dicarboxylic acid that was used in the analysis and thus reacted differently from the other four acids. The four other acids were simple, single, aliphatic carboxylic acids that ranged from C_1 to C_4. Short chain acids from C_1 to C_3 are more hydrophilic than acids that are greater than C_4. Butyric acid is therefore more hydrophobic in nature hence its increased retention on the column [1]. This interaction resulted in the higher time difference that was observed between propionic (22.6 min) and butyric acids (28.7 min) (Figure 4.2, a). An organic modifier was required to bring the two elution peaks closer together thus improving the total run time.

During method development, parameters such as mobile phases were varied in order to find optimum working conditions. The original mobile phase of 2.5 mM sulphuric acid yielded a run time of approximately 37 minutes (A1.1, page A1-1). The baseline was also very noisy and drifting. A longer time for equilibration was required in order to reduce base line drift.

Alternate mobile phases were also investigated due to baseline drift and noise. According to literature, perchloric acid can be used as an alternative to sulphuric acid as its strength is similar to sulphuric acid. However care must be taken when using

these acids as an increase in strength of the mobile phase could degrade the column and destroy the simple organic acids that are to be determined. This change in mobile phase showed little change to the baseline.

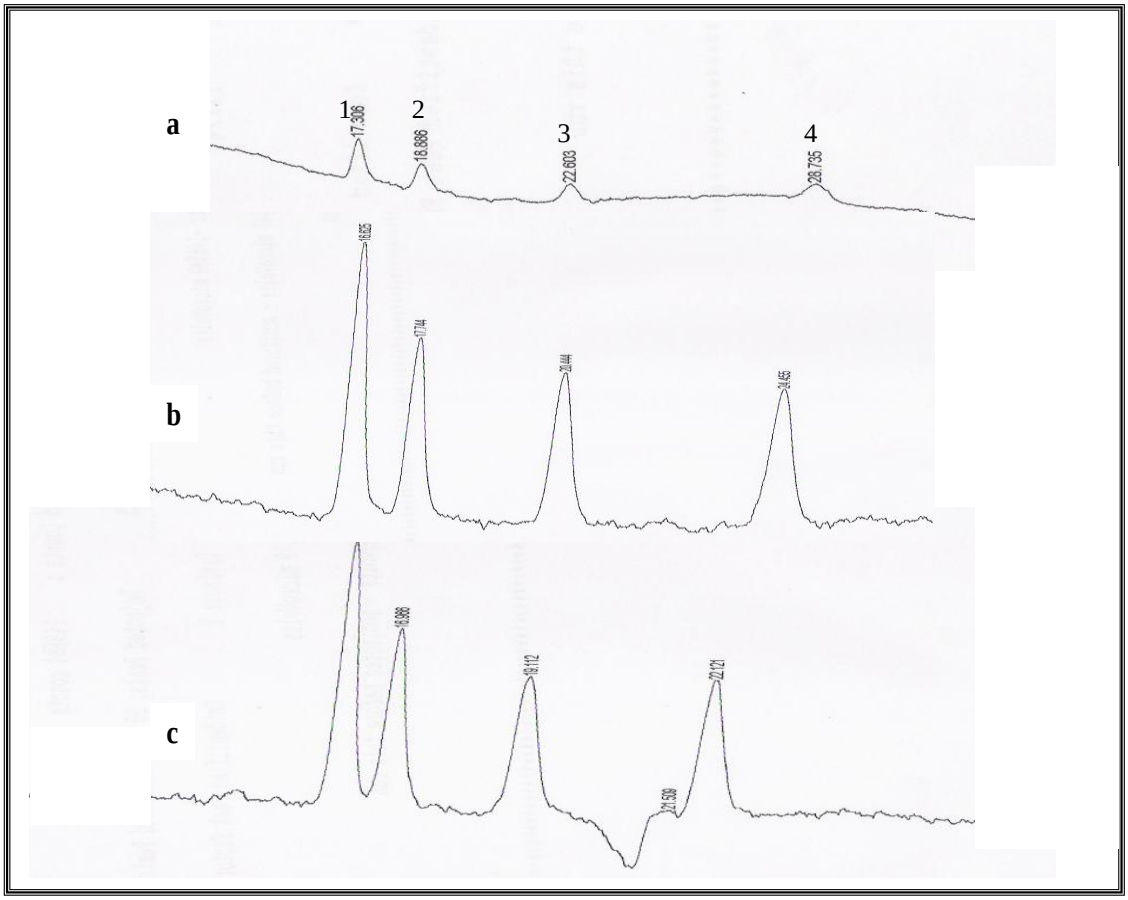


Figure 4.2: Effect of organic modifier on run time. a) 2.5 mM H_2SO_4 only, b) 5% Acetonitrile with 95 % 2.5 mM H_2SO_4, c) 10% Acetonitrile with 90% 2.5 mM H_2SO_4. Acid mixture: 1. formic acid, 2. acetic acid, 3. propionic acid, 4. butyric acid

In order to improve the run time, an organic modifier was introduced. A 5% acetonitrile, mobile phase B was added to the original mobile phase A, (2.5 mM

H_2SO_4). This improved the run time to 30 min. When 10% Acetonitrile was used the run time was reduced to 25 minutes, with the presence of interference between the propionic and butyric peaks (Figure 4.2, c). Hence method 2 was the method chosen to proceed with the rest of the study. Table 4.1 lists the methods investigated.

Method	Mobile Phase	Flow Rate ml/min	Detection λ (nm)	Temperature °C
1	2.5 mM H_2SO_4	0.5	210	Ambient
2	2.5 mM H_2SO_4 and 5% AcN	0.5	210	Ambient
3	2.5 mM H_2SO_4 and 10% AcN	0.5	210	Ambient
4	2.5 mM Perchloric Acid and 5% AcN	0.5	210	Ambient

Table 4.1 List of mobile phases tried on ion exclusion with UV detector, 210 nm

4.2.2.3 Quantitative Analysis of Carboxylic Acid Standards

The limit of detection and limit of quantification was calculated from the signal-to noise, S/N, ratio from the chromatogram of a 4 ppm standard mixture (Figure A1.11). Limit of detection was calculated as 3x the sensitivity ratio and the limit of quantification was calculated from a 10x the sensitivity ratio (Table 4.2). The sensitivity ratio was calculated by dividing the height of the signal peak with the average height of the baseline noise. All statistical data was calculated at a level of 95% confidence.

	Formic Acid	Acetic Acid	Propionic Acid	Butyric Acid
LOD	0.8 ppm	0.48 ppm	0.35 ppm	0.68 ppm
LOQ	2.9 ppm	1.8 ppm	1.19 ppm	2.29 ppm

Table 4.2 List of limit of detection and quantification for each acid calculated From a 4 ppm acid mixture chromatogram (Figure A1.11)

Calibration standards from 2 ppm to 8 ppm were run for each of the acids in order to obtain the linearity of the method used. Below is a typical calibration curve graph that was obtained for one of the acids (Figure 4.3, Table 4.3). Analysis was carried out in duplicate with the average results being used for the calculation.

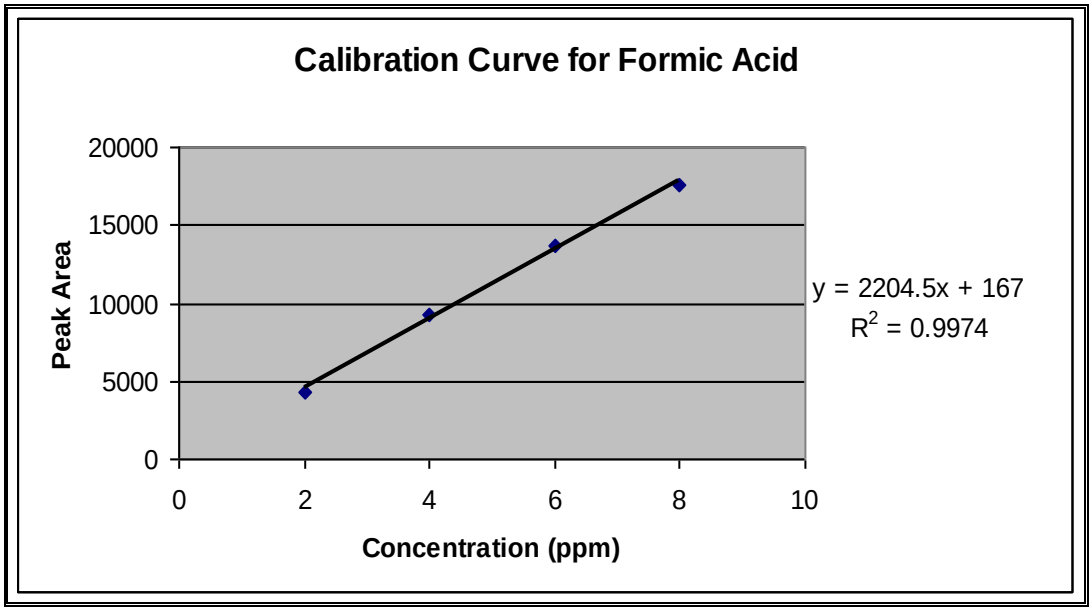


Figure 4.3: Calibration Curve for formic acid obtained from individual standards

Table 4.3 Peak areas and retention time for formic acid (n=2)

Formic Acid	Concentration	Retention Time	Peak Area
	2 ppm	14.22	4337
	4 ppm	14.22	9199
	6 ppm	14.20	13683
	8 ppm	14.20	17539

The linear regression R^2 for each of the four acids calculated using Windows Excel (Table 4.4). The graphs and results can be found in Appendix A1. Using statistical calculation the confidence levels of each concentration used for the calibration curve was calculated using Windows Excel. The confidence levels are as follows: formic acid ±0.23 ppm, acetic acid ±0.03 ppm, propionic acid ± 0.4 ppm and butyric acid ± 0.3 ppm.

Table 4.4 Table of R^2 values for formic, acetic, propionic and butyric acids using ion exclusion on a UV detector, wavelength 210 nm, Phenomenex Rezex OA, flow rate 0,5 mL/min

	Formic Acid	Acetic Acid	Propionic Acid	Butyric Acid
R^2 Value	0.9974	0.9999	0.9860	0.9919

The percent relative standard deviations (RSD) for the repeatability of the acid retention times were calculated to be 0.05% for formic and acetic acid, 0.09% for butyric acid and 2% for propionic acid. The poor % RSD value for propionic acids could be equated to the presence of an impurity peak that is eluted close to the

propionic retention time. The effect of this interference peak was more notable as the concentration was reduced.

Although the calculated detection limits were relatively good the drift and noise of the baseline at lower concentration levels of the acids was amplified, resulting in poor chromatograms (A1.11). Although this method was suitable for the detection of organic acids at concentration levels greater than 3ppm it was not acceptable for this study.

4.2.3 Ion Exclusion using Evaporative Light Scattering Detector

The instrument used in this study consisted of two detectors: a refractive index detector, ERC 7515A, and an Evaporative Light Scattering Detector, Sedex 75 with an ion exclusion column.

No reports were found in literature on the use of ELSD as a detector in the determination of short chain carboxylic acids. An application in the manufacturer's manual for the determination of fatty acids such as malic acid was used as a guide for choosing the conditions listed in 3.4.2 [12].

The mobile phase that was used for this application was 0.5% Trifluoroacetic acid (TFA) due to it being a volatile solvent modifier.

4.2.3.1 Method Development using Organic Acid Standards for ELSD

In addition to the four acids that were selected for this investigation (acetic, formic, propionic and butyric acids) malic acid was included as a reference acid in this application. During method development the column temperature, detector temperature, pressure, gain, and carrier gas was changed systematically in order to find a working method for the detection of the simple short chain carboxylic acids. Either nitrogen or air was used as a carrier gas in the applications.

A 20 ppm standard mixture was used as a trial standard to determine whether these acids could be detected by the ELSD. However the acids were only detected by the refractive index detector. The application was retried with the addition of malic acid. This method was repeated using first nitrogen as the carrier gas then changed to air as a carrier gas. It was found that air was a better carrier gas for the acid than nitrogen. This could possibly be attributed owing to the cooling effect of nitrogen as a gas. Hence the acids could have been removed as waste.

4.2.3.2 Limitations of Method used in ELSD

Formic and acetic acids are usually added to applications using ELSD detection as modifiers to a method in order to improve the detectability of an analyte. As these acids are fairly volatile, there should not have been a problem in their detection. Experiments, using ELSD in our laboratory, have failed to detect the four short chain acids. However oxalic acid and malic acid were detected. This could be attributed to the size of the acid molecule. Further work is needed to investigate this aspect.

4.2.4 Ion Chromatography without Chemical Suppression

Although there were several reports in the literature on applications involving the detection of organic acids using ion chromatography, the instrumentation that was used in all of the applications was supplied by Dionex, a company that was at the cutting edge of analysis involving ion chromatography. The analysis was carried out using a conductivity detector and an anion exchange column called a suppressor. This reduces the noise effect of the mobile phase generally used in this kind of application.

Analysis of ion chromatography was carried out on a Waters 430 Conductivity detector using an ion exclusion column. A mobile phase comprising of 1.9 mM H_2SO_4 in 2% acetonitrile mobile phase was used with the above set up giving a conductivity of 3750μS.

An attempt was made to duplicate the above method using a Metrohm 690 conductivity detector; however the conductivity was found to be over scale. A new mobile phase of 1.2 mM H_2SO_4 in 2% acetonitrile/water solution yielded a conductivity of 760 μS which was more acceptable.

4.2.4.1 Limitation of Method using ion chromatography without suppression

Although this instrumentation utilises electronic suppression it was not sufficient to reduce the noise caused by the mobile phase and thus sensitivity was reduced (Figure 4.4).

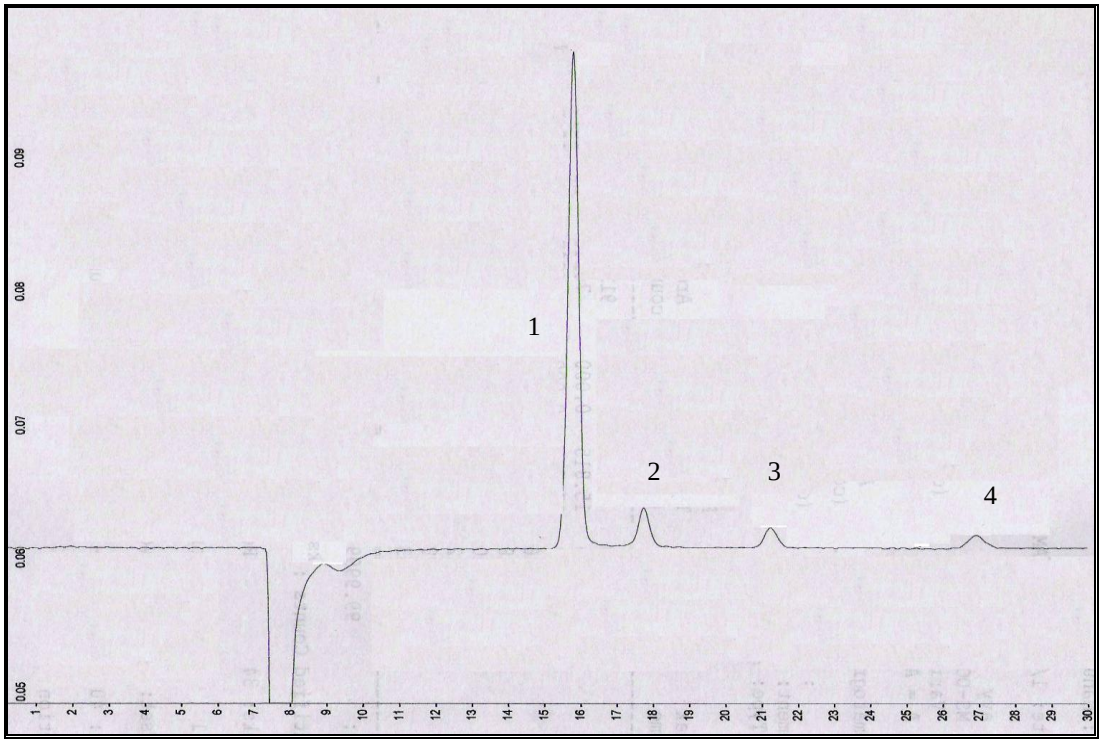


Figure 4.4 A typical chromatogram of 200 ppm acid mixture using Waters 430 conductivity detector: 1. formic acid, 2. acetic acid, 3. propionic acid, 4. butyric acid

The chromatogram above shows the poor sensitivity that was obtained using ion exclusion with a conductivity detector. However formic acid shows a stronger peak than the other 3 acids. Both butyric acid and propionic acid have broad peaks. This method gives less sensitivity than the ion exclusion with UV detector.

4.2.5 Ion Chromatography with Chemical Suppression

In order to improve detection limits that were not achievable with method used in 4.2.4, chemical suppression was investigated. Most literature reports [2-4] involving the determination of organic acids using ion chromatography have resulted from

utilising a system developed by the Dionex Company. This system makes use of an anion exchange process that reduces the background noise of the mobile phase allowing an increase in sensitivity and lowering of detection limits. It uses a column-like component called a suppressor, which contains anion exchange resin that enhances sensitivity by decreasing the noise of the eluent and allowing the compounds of interest to be better detected. Such a system would have been ideal for the investigation being undertaken within this project. However due to the unavailability of such an instrument an alternate method using the principle used by the Dionex Company was attempted.

4.2.5.1 Separation and Detection of Organic Acids

Analysis was carried out at an outside laboratory (SA Bio Products) that was equipped with a conductivity detector and chemical suppression. A range of standards from 200 ppm to 20 ppb was used to determine the limits of the method. The mobile phase (eluent) was 0.5 mM perchloric acid, with 10 mM lithium chloride as the regeneration solution used in the suppressor. Duplicate analysis was run for each concentration of acids. Appendix A2 contains chromatograms of each run. Figure A2.4 shows a slight improvement in the chromatogram with respect to sensitivity of the acids. Formic acid still exhibited the strongest peak, being at least 6x the height of the other peaks. There was no improvement in the peak shape for butyric acid.

4.2.5.2 Limitations of Method using Chemical Suppression

It can be observed that the limits of detection and quantification for propionic, butyric and acetic acids are greater than 2 ppm with only formic acid being detected at 2 ppm (Figure 4.5). The chromatogram at 200 ppb shows only a baseline where none of the acids were detected. Hence it can be concluded that the method of ion exclusion can be improved by using chemical suppression, however the method showed no improvement from ion exclusion with a UV detector. Thus, in order to improve the detection a further step of derivatization needed to be explored.

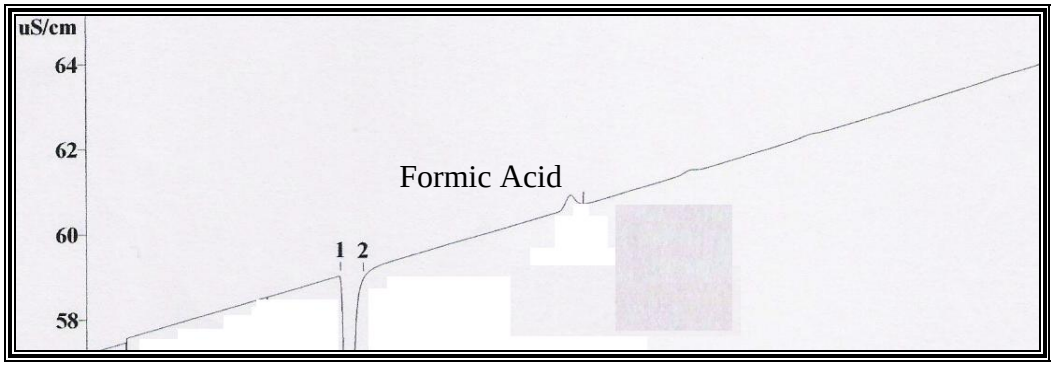


Figure 4.5 Chromatogram of 2 ppm acid mixture on a Phenomenex Rezex column, 0.7 ml/min, using Metrohm 761 IC Compact

4.3 Partition Chromatography using Derivatization

Detection of carboxylic acids is commonly carried out at 210 nm wavelength. However the sensitivity of detection is poor due to the acids weak chromophoric properties. In order to improve the sensitivity of detection using HPLC, derivatization

procedures were investigated. Many derivatization procedures are labour intensive and complicated [listed in 13]. However a simple procedure for the derivatization of carboxylic acids was developed by the work group headed by H. Miwa [6, 7]. The procedure used by the work group was devised to be used in the food and beverage industries.

The derivatizing agent used in the analysis was 2-nitrophenyl hydrazine (NPH) with a coupling reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). This procedure converted the volatile carboxylic acid to a non volatile acid hydrazine derivative. Separation of the hydrazine derivative can be accomplished using a reversed phase column.

With in this study, the work carried out by H. Miwa was adapted for aqueous environmental samples with success in order to develop a less complicated method that could be used on samples collected from the power station.

4.3.1 Detection of the Derivative using UV Detection

Detection of the acid hydrazine derivative can be achieved by using either 230 nm (UV region) or 400 nm (Visible region) wavelengths. A Diode Array Detector was used to determine the optimum wavelength that could be used for this application (A3-23 and A3-24). All analysis was carried out on a UV detector using a reversed phase column.

The two types of columns that were used in this investigation were:

- A C_{18} Hypersil Gold column and
- A C-8 Lichrospher or a Nucleodur C8ec column with a C_8 security guard column.

4.3.1.1 Comparison of the Columns used in partition chromatography

The reversed phase columns used in this study contain a non polar silica based stationary phase. Simple aliphatic carboxylic acids are polar in nature. The polarity of the acid would be reduced with an increase in chain length. Hence the acids that were analysed in this study had to be first converted to a less polar compound before undergoing separation. There were two types of reversed phase columns that were used in this study, C18 and C8. The essential difference between a C18 and a C8 column is the length of the chain attached to the silica based stationary phase.

The C18 solid phase is a longer chain organosilaxane and as a result behaves in a more hydrophobic manner (i.e. more non polar) than a C8 column. Hence, a non polar analyte would be retained much longer on a C18 column than a C8. This was observed by comparing the two methods 4.3.2.1 and 4.3.2.2 used in the partition chromatography study. A drastic reduction of run time from 60 minute on a C18 to 10 minutes on a C8 was observed.

There were two grades of C8 columns used: a Lichrospher C8 and Nucleodur C8ec. As observed in 4.3.2.2. the Nucleodur C8ec column performed much better at separation and had better sensitivity than the Lichrospher column. The main

difference between the two columns is the grade of silica that is used in the stationary phase. Nucleodur contains grade B silica whilst Lichrospher contains grade A silica. Grade A silica is less pure and more acidic silica, which would result in an increased silanol ionisation at extreme pH's. The silica used in the Nucleodur column is more resilient to extreme pH changes. It is for this reason that the Nucleodur column performed better at separation of the acids in this study.

4.3.2 Derivatization Procedures

4.3.2.1. Derivatization procedure without KOH

There were two variations of the derivatization procedures that were used in this study. The first procedure, listed in 3.5.1.1, involved the derivatization of the acid analytes with an acidic solution of 2-NPH and a 1:1 ratio of 3% pyridine: EDC solution. The pyridine solution was used to catalyse the interaction between EDC and 2-NPH as well as raising the pH of the solution as to increase the interaction between the acid coupled EDC and 2-NPH. Separation of the acids mixtures was carried out on a C18 reversed phase column. The mobile phase used was acidified water with HCl (pH 4.5) and acetonitrile. The schematic for the derivatization procedure can be observed in Figure 4.6.

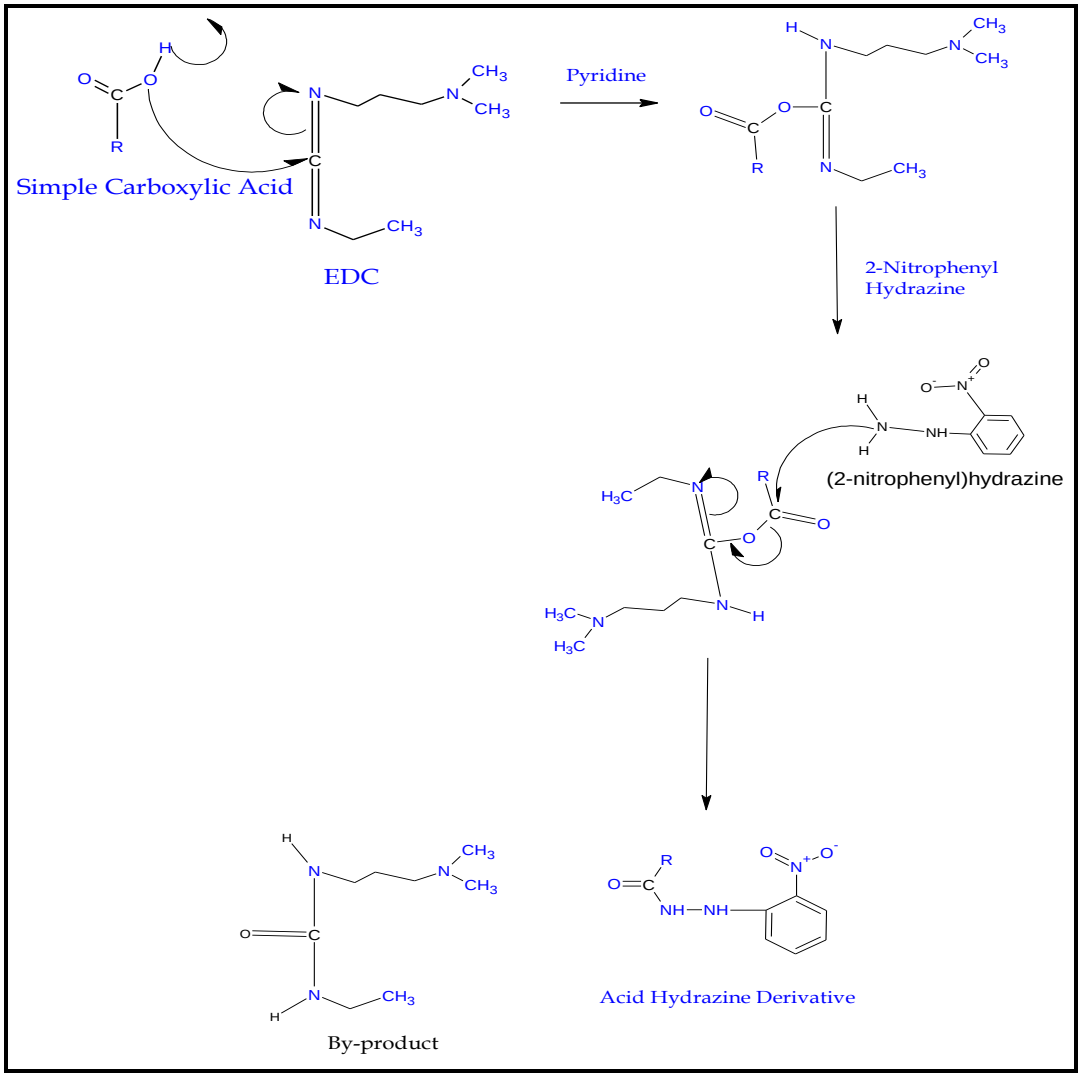


Figure 4.6 Schematic diagram of the reaction of NPH with EDC to form the acid hydrazine derivative and by-product.

After derivatization, the solution containing the acid derivative was bright orange in colour. The orange solution was injected immediately after derivatization. The derivatized standard could not be used for more than one injection, hence a fresh derivative needed to be prepared for every injection due to the degradation caused by light sensitivity. The sensitivity of the derivatization process would be discussed further in detail in 4.3.3.3.

A range of standards from 50 ppm to 10 ppb was used to determine the optimum conditions for the separation of the acids on a C18 column. An isocratic mixture of the mobile phases, comprising of 95% acidified water (pH4.5) and 5% (acetonitrile) was initially used. Run times were found to exceed 60 minutes. Butyric acid was eluted only after 80 minutes, which was unacceptable. Thus gradient elution was used in order to decrease run time without compromising resolution. The first gradient program that was tried is listed in Table 4.5. This program was used to find the optimal gradient that could possible shorten the run time of the analysis.

Time	pH 4.5 acidified water	Acetonitrile
0	95	5
24	95	5
60	80	20

Table 4.5 Gradient elution program 1 used with C18 and UV detection for separation acid mixture, (230nm)

The final gradient program that was used in the analysis is listed in Table 4.6. It shortened the run time to less than 50 minutes with excellent resolution of all four acid peaks. Figure 4.7 shows a typical chromatogram of a standard acid mixture.

Time	Acidified Water pH4.5	Acetonitrile
0	95	5
24	95	5
25	92	8
35	92	8
37	85	15
40	85	15
50	80	20

Table 4.6 Final gradient used with C18 column, 230 nm detection

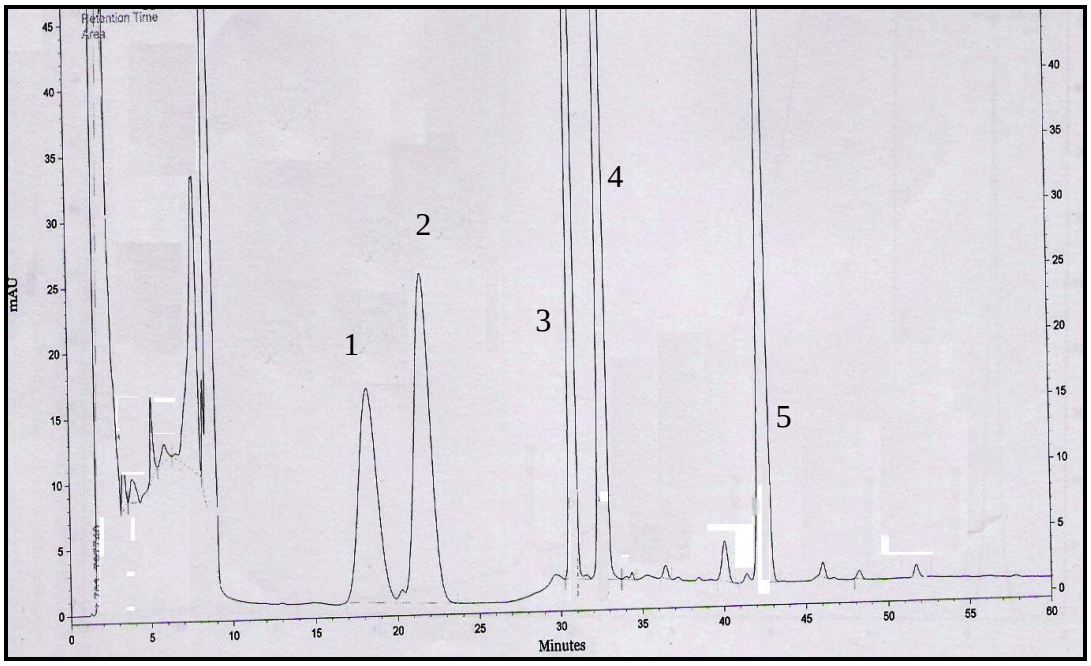


Figure 4.7 Typical chromatogram of an acid mixture after derivatization (50 ppm) on a C18 column, (230 nm), 1. formic acid, 2.aceticacid, 3. un-reacted 2-NPH, 4. propionic acid, 5. butyric acid

4.3.2.2 Derivatization Process using KOH

Separation of acids was achieved on a C8 column successfully. There were two C8 columns used, with the C8 Nucleodur column showing the greater sensitivity. The derivatization procedure in this section of study (3.5.1.2) used a slightly less acidic solution of 2-NPH added to an EDC solution prepared directly in 3% pyridine (ethanol). This variation to the derivatization procedure in 3.5.1.1 increased the pH of the derivatized solution to pH5. A pH range between 3 and 5 should be observed in order to allow optimum derivatization between EDC and 2-NPH [5].

After the first step of the derivatization procedure (3.5.1.2) the derivatized solution was observed to be bright orange in colour (i.e. when the acid was added to the NPH and EDC mixture). However the colour changed from orange to a deep purple after the addition of 10% potassium hydroxide solution. It was observed that the intensity of the purple colour depended on the strength of the acid mixture being analysed. The intense purple colour noticed is due to the conjugation in the anion molecule (Figure 4.8, 2). The purple mixture (was then cooled to room temperature before being injected into the HPLC. The purple colour is a result of the acid hydrazine anion that has been formed by adding potassium hydroxide (Figure 4.8). This reaction converts the hydrazine to its anion which is strongly absorbs at 400 nm. As a result the by products from the coupling reaction and the excess reagent would not absorb at this wavelength giving a relatively cleaner chromatogram.

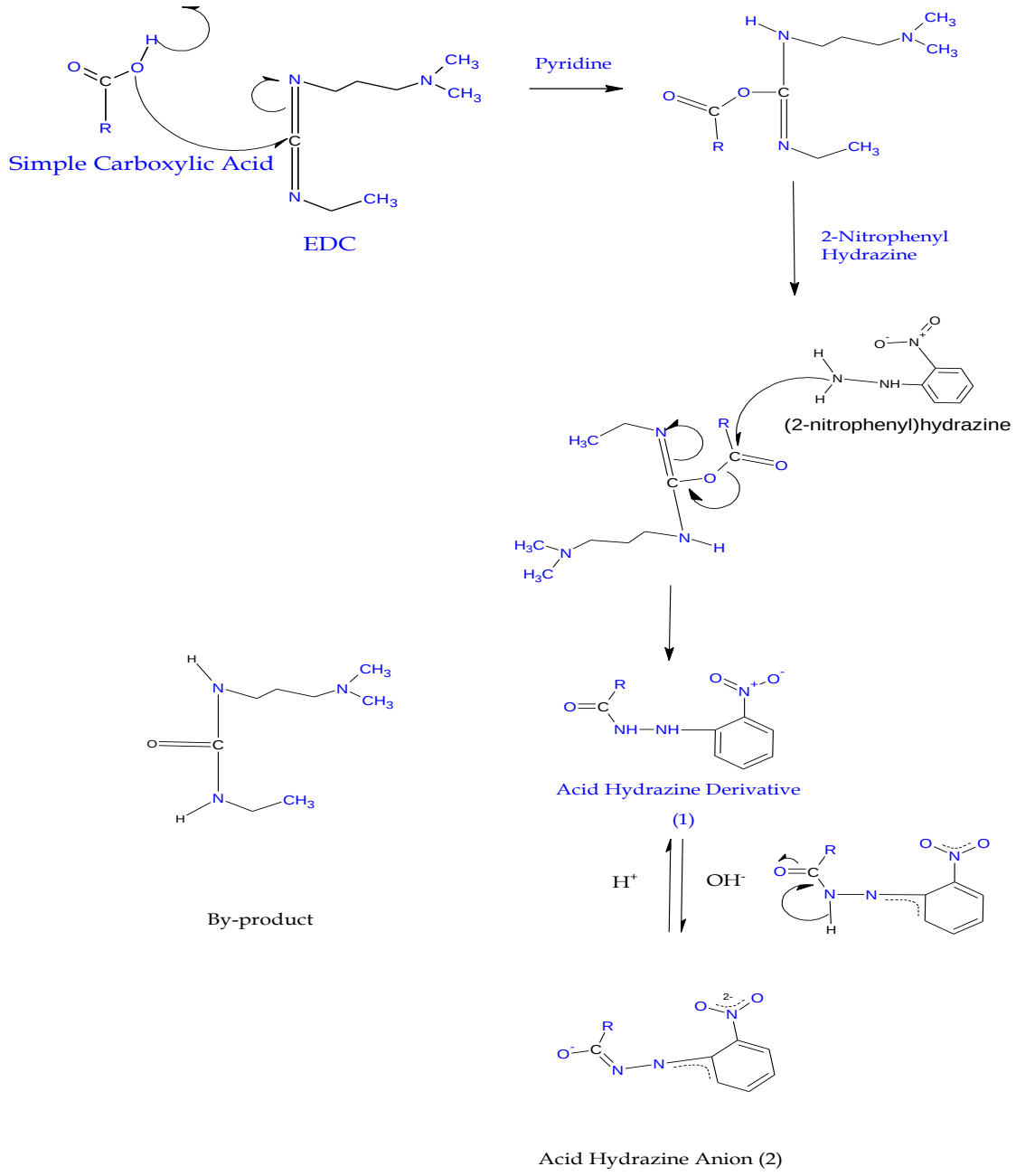


Figure 4.8 Postulated schematic diagram of the reaction of NPH with EDC and KOH to form acid hydrazine anion

In addition, the organic mobile phase B used was a 60%: 40% acetonitrile: methanol mixture in addition to the acidified water with HCl (pH4.5). The use of a mixture of acetonitrile and methanol assisted in the separation of the acid with fewer steps to the gradient program. The elution system combination of acetonitrile, methanol and

acidified water (pH4.5), maintained a pH of 4 to 6. The pH of the eluent was maintained so that the reversed phase column used would not undergo any extensive damage to the stationary phase and the acid hydrazine derivative could be optimally detected within an acidic medium.

Analysis was initially attempted using the gradient from Table 4.6. Figure 4.9 shows the first attempt of running the acid mixture at 400 nm using the gradient program with a flow rate of 1.5 mL/min. An immediate improvement was noticed in the chromatogram obtained. The interference peaks were not detectable at this wavelength as seen in UV spectrum (Figure A3.25). However the peak heights were much lower than that previously observed. Hence a new gradient was applied to the application in order to improve the detection of the acids and its sensitivity ratio.

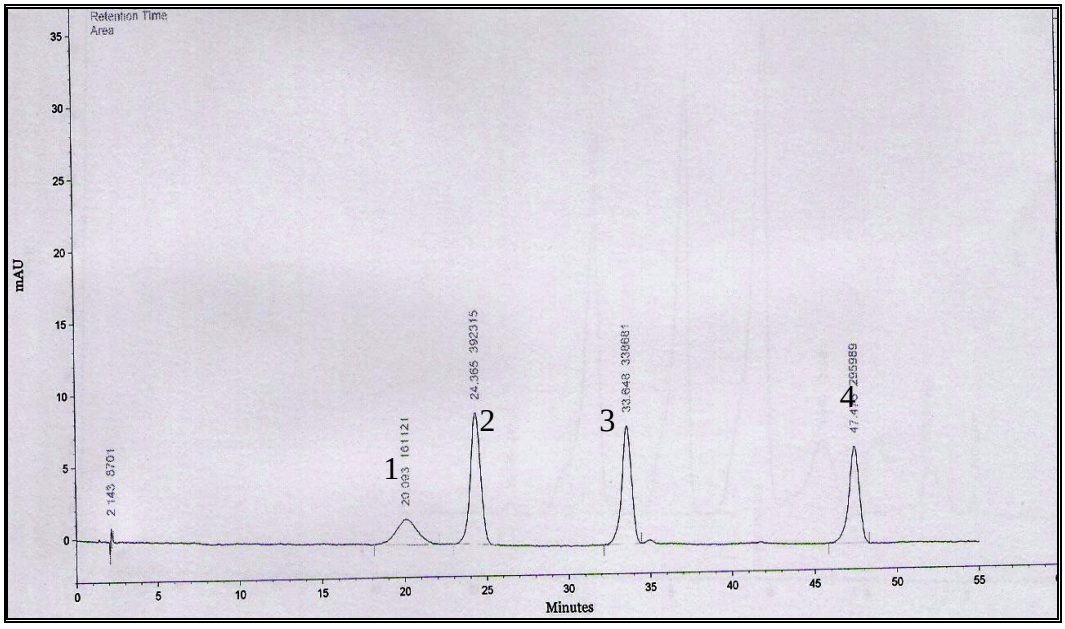


Figure 4.9 Chromatogram of 50 ppm acid mixture using C8 column with a gradient program at 400 nm 1. formic, 2. acetic, 3. propionic, 4. butyric acid

Time	Acidified Water pH4.5	60AcN: 40 MeOH
0	70	30
20	70	30
22	65	35
25	65	35
30	50	50
35	50	50

Table 4.7 Gradient program used for the C8 column, Lichrospher, 400 nm Wavelength

Chromatogram A4.1 in Appendix 4 shows a drastic improvement to both run time and peak height of the acids. The run time was reduced from 45 minutes to less than 10 minutes with peak height 3x that obtained in A4.2. In order to increase sensitivity the Nucleodur C8 column was used to continue the study. The pH of the final derivative was extremely alkaline hence the guard column was used in order to reduce any damage that the column may incur during analysis. A flow rate of 1.2 mL/min was used on this column.

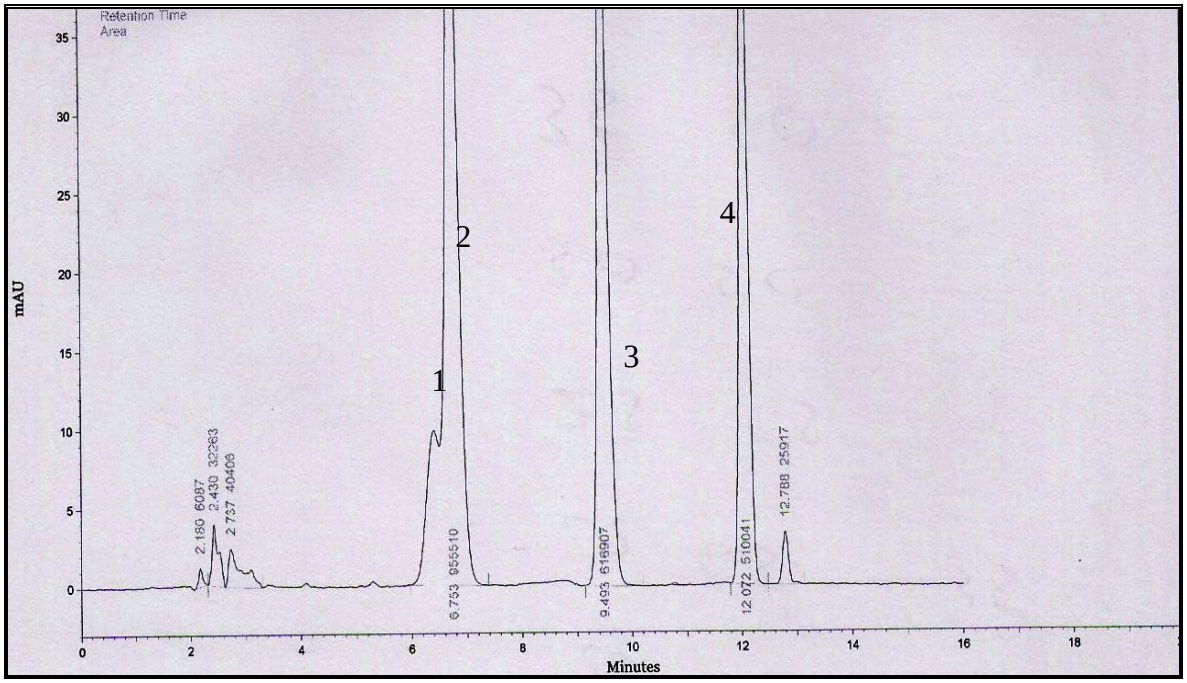


Figure 4.10 Chromatogram using gradient on Nucleodur column showing co-elution of formic and acetic acid peaks at 400 nm using gradient from Table 4.7

A new gradient was used in order to improve the separation between formic acid and acetic acid (Figure 4.10). The gradient program was established by first running an isocratic mobile phase of 80% acidified water and 20% acetonitrile: methanol mixture. This carried out to observe if the two peaks could be resolved. Thereafter a second step was added as to shorten the elution time of the other acids (Figure 4.11, table 4.8).

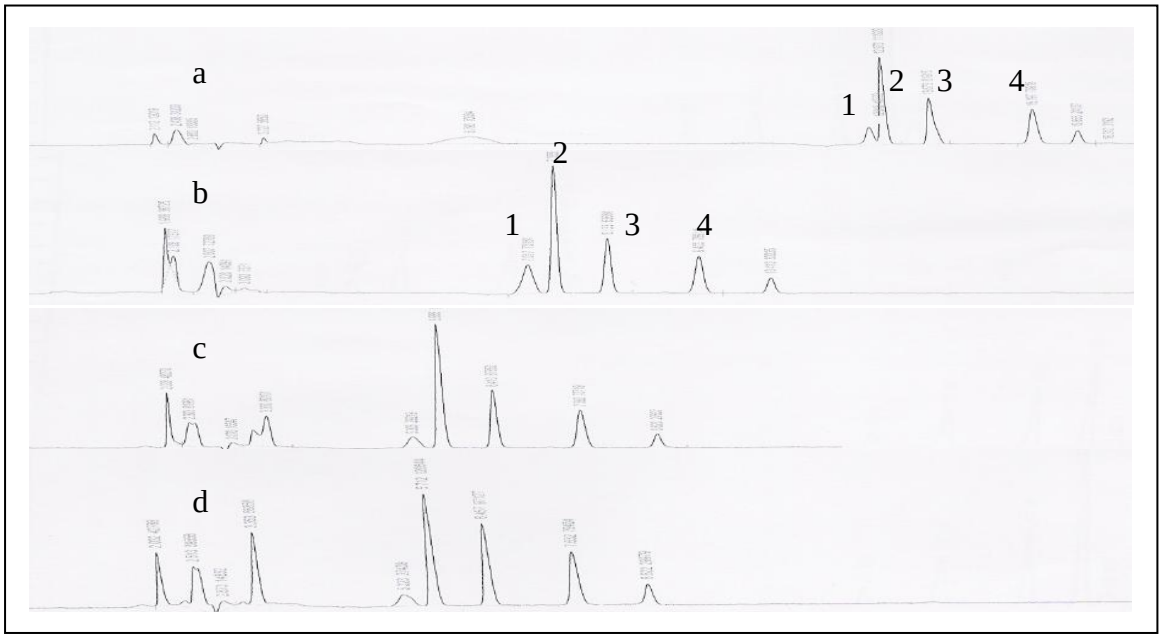


Figure 4.11 Effect of step gradient on the elution times of acid mixture (5 ppm)

1. formic acid, 2. acetic acid, 3. propionic acid, 4. butyric acid.

C8 Nucleodur 400 nm a) gradient starting with 90 Acidified water : 10 AcN/MeOH mix, b) hold 80% acidified water : 20% AcN/MeOH mix for 3 min, c) Hold 80 acidified water : 20 AcN/MeOH mix for 2min, d) hold 80 % acidified water : 20 % AcN/MeOH for 1 min.

Time	Acidified Water pH 4.5	60 AcN: 40 MeOH
0	80	20
1	80	20
1.5	50	50
10	50	50

Table 4.8 Two step gradient used with Nucleodur C 8 column for analysis

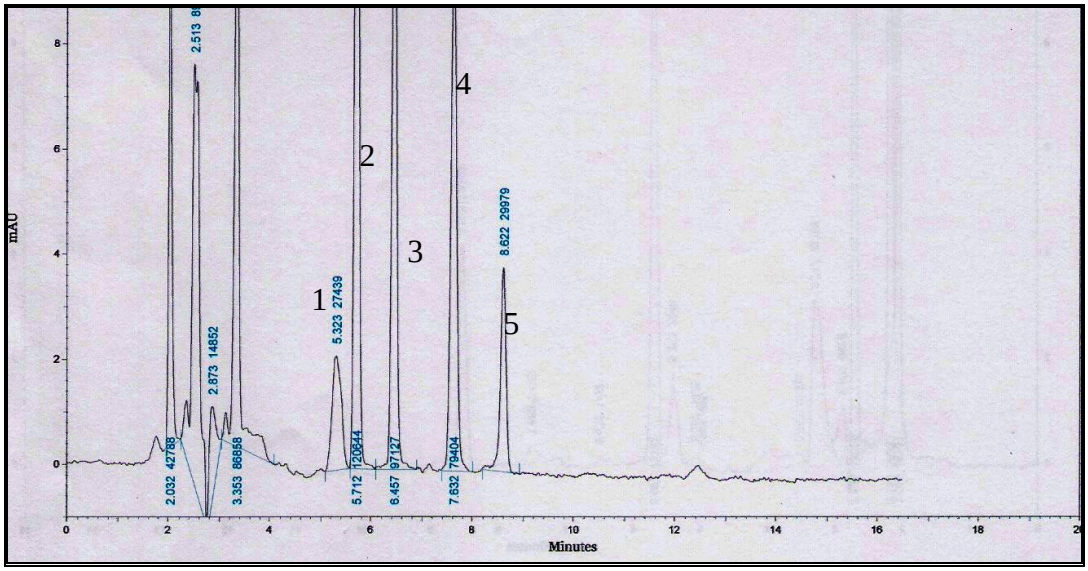


Figure 4.12: Typical Chromatogram of acid mixture (5 ppm) using C 8 Nucleodur, Detection at 400nm, 2 step gradient program. 1. formic acid, 2. acetic acid, 3. propionic acid, 4. butyric acid

The above chromatogram (Figure 4.12) shows the separation of all four acids within less than a 10 minute run time. A range of standards from 10 ppm to 100 ppb was used determine the limitations of the method. The above method has shown an improvement in detection limits for almost all the acids. The detection of formic acid using this method was poor, with a peak height approximately 6x lower than the strongest peak (acetic acid).

4.3.3 Factors Affecting the Derivatization Process

4.3.3.1 Effect of pH

Literature has shown many variations in the derivatization procedures using 2-NPH and EDC in the analysis of carboxylic acids [5-9]. The methods varied in both the manner of preparation and the steps followed for derivatization. Some of the important factors that should be observed when using this type of derivatization are the pH of the sample and concentration of the reagents being used. It was noticed that the pH of the solution being analysed should be between 3 and 2. The pH of the solution is increased with the addition of pyridine in the sample. This is due to the fact that the coupling of EDC with NPH is optimum within this pH range.

The ratio of the solutions volumes used in the derivatization procedure should be 5 (sample): 2(NPH) :2(EDC) :1(KOH). The preparations of the reagent solutions are also important. In Method one the NPH solution was made up with a 1 M HCl solution instead of the 0.1 M used in 3.5.1.2, making the solution acidic. This affected the pH within the vial during derivatization.

4.3.3.2 Effect of potassium hydroxide

The addition of potassium hydroxide in the final step of derivatization improves the detection of the sample peaks. Chromatogram in Figure 4.13 show the interference peaks still observed by the excess 2-NPH when the 10% KOH solution was not added. Whilst the chromatogram from Figure 4.12 shows the elimination of all

interference peaks that where previously observed resulting in a simpler chromatogram after the addition of the 10% KOH solution.

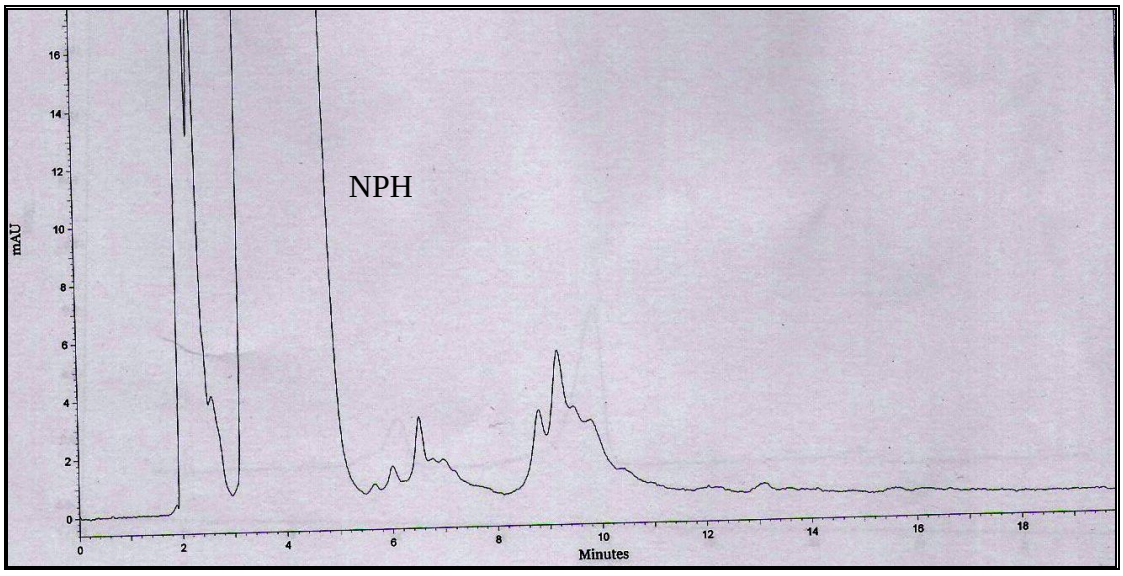


Figure 4.13 Chromatogram of acid mixture without adding KOH on C8 column at 400nm (5 ppm acid mixture)

4.3.3.3 Stability of derivative and derivatizing agents

The derivatizing agent used in this study is very unstable and should be made up fresh everyday before analysis. It is light sensitive and should be stored in the refrigerator when not in use. An intensive study carried out by Adina Musuc, has shown the extremely unstable nature of 2-NPH [11]. The purity of 2-NPH varies with supplier and care should be taken to ensure that the correct assay of the chemical is known. The strength of the derivative is also dependant on the amount of water content, although this is not always stated when purchasing the reagent. A clear way of observing if the water content is high is the colour of the crystals. The darker red crystals have less water content; the lighter the colour the more the water content

(A3.3 and A3.4). It is strongly suggested that the standard be re-crystallized in warm water before being used. Chromatograms in Figure A3.7 and A3.8 show the effect of re-crystallising the 2-NPH.

A fresh derivative needs to be prepared before every injection. As the standard can only be used for one injection (Figure A4.14 and A4.15). It has also been noticed that the run times vary from day to day. This could be a result of the fresh preparation of derivatizing agents daily.

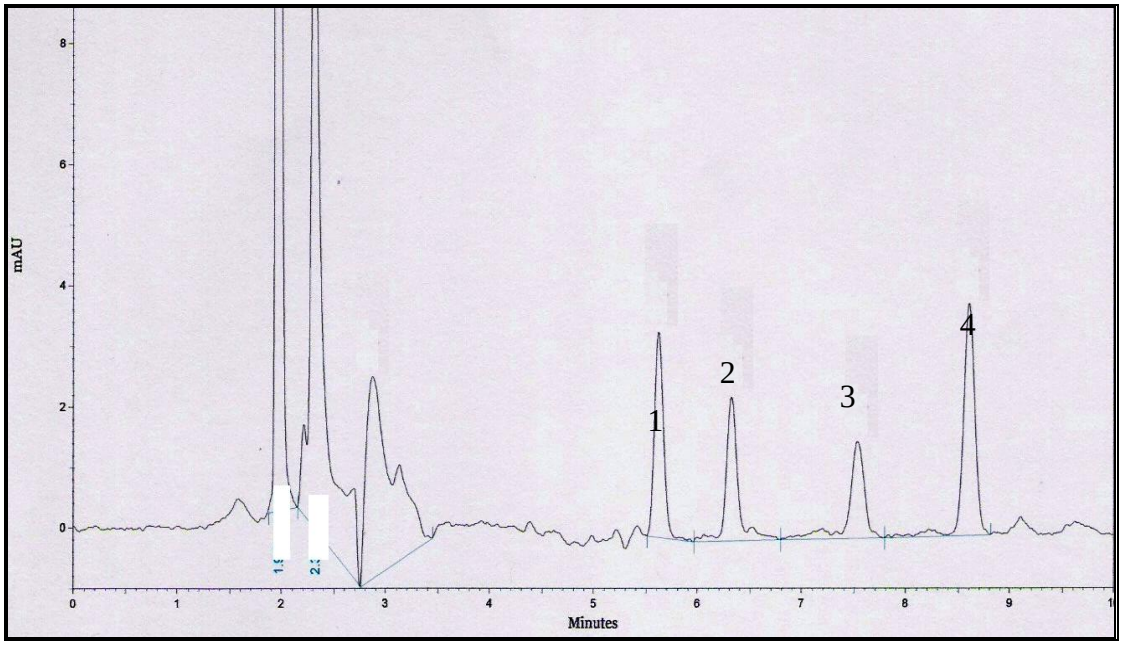


Figure 4.14 Chromatogram of 1 ppm acid standard injection one-C8 column

1. Acetic acid, 2. Propionic acid, 3. butyric acid, 4. unknown peak

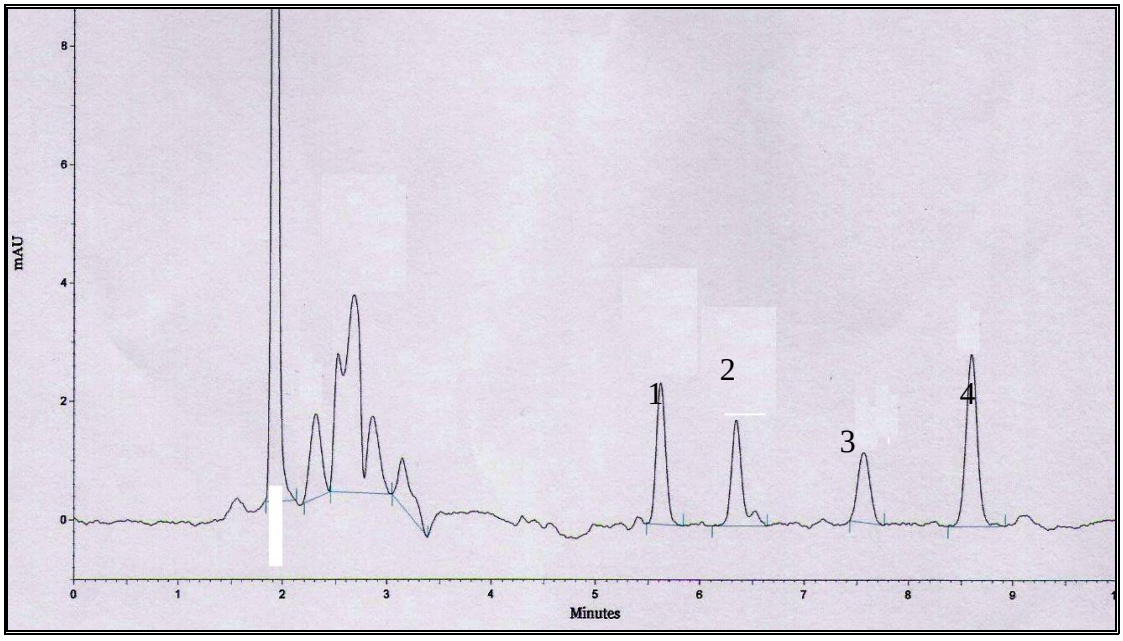


Figure 4.15 Chromatogram of 1 ppm injection 2 showing slight reduction in peak heights using the same derivatized standard. Acid mix 1ppm-C8 Repeat injection 1. acetic acid, 2. propionic acid, 3. butyric acid, 4. unknown peak

4.3.4 Comparison of the Wavelength used in Detection of the Acid Hydrazide

Detection of the derivative can be accomplished at 400 nm and 230 nm [7, 8]. Detection carried out using wavelengths in the UV range is more sensitive than detection at the visible range. However the advantage of using visible detection is that the chromatograms are more selective to the analytes, which are not being masked by the interference peaks. The chromatograms obtained from the analysis at 230 nm showed more interferences, especially with the excess NPH solvent (Figure 4.17). The UV spectrum of NPH indicates that NPH is strongly absorbed at wavelengths 230 nm and 435 nm (Figure 4.16).

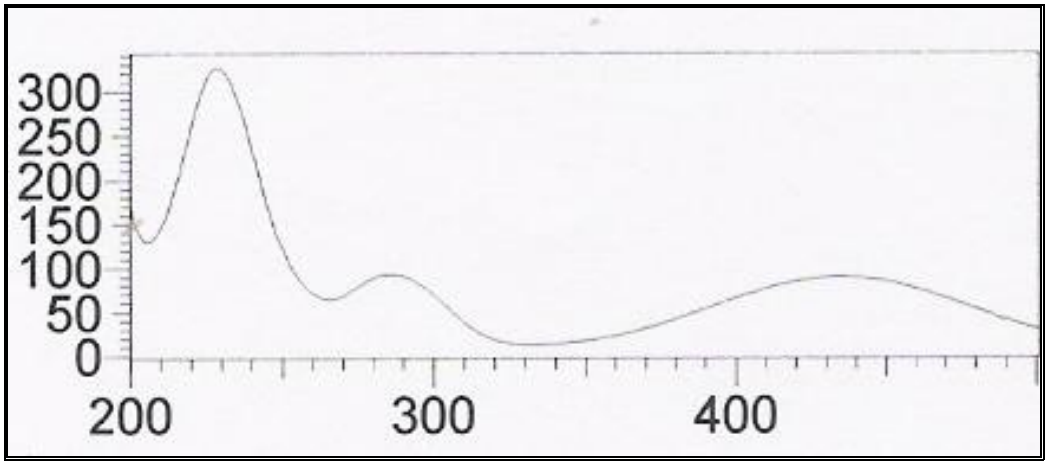


Figure 4.16 UV spectrum of NPH obtained on a PDA

As a result, analysis performed at 400 nm without the use of KOH will still show slight absorbance of the excess NPH (Figure 4.18). However there was a greater difference in the intensity of the peak reducing its ability to interfere with the analyte peaks (Figure 4.17). After the addition of KOH the NPH peak is completely removed from a chromatogram at 400 nm due to change of colour of the solution and its effect on the absorbance of the anions (Figure 4.19).

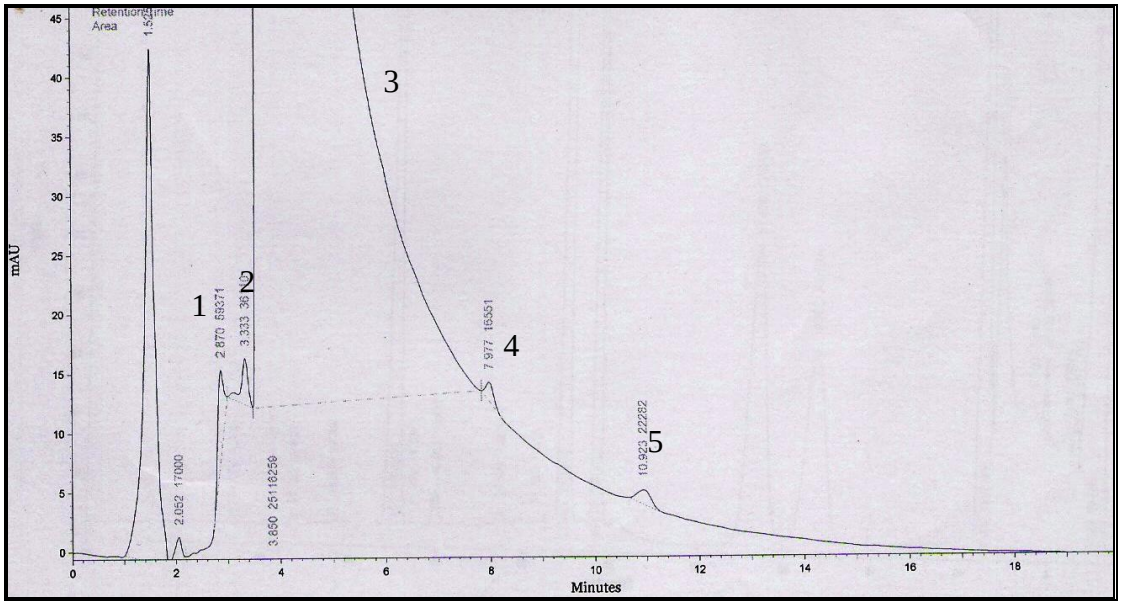


Figure 4.17 Chromatogram using C8 column with detection at 230 nm of 500 ppb acid mixture without KOH 1. formic acid, 2. acetic acid, 3. NPH peak, 4. propionic acid, 5. butyric acid

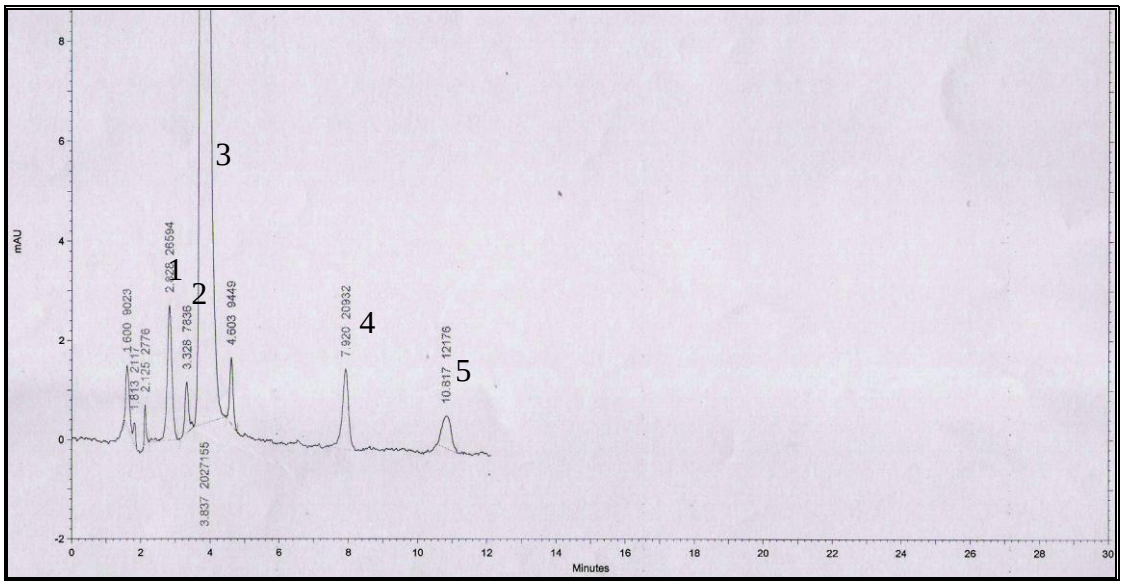


Figure 4.18 Chromatogram using C8 column with detection at 400 nm of 500 ppb acid mixture without KOH 1. formic acid, 2. acetic acid, 3. NPH peak, 4. propionic acid, 5. butyric acid

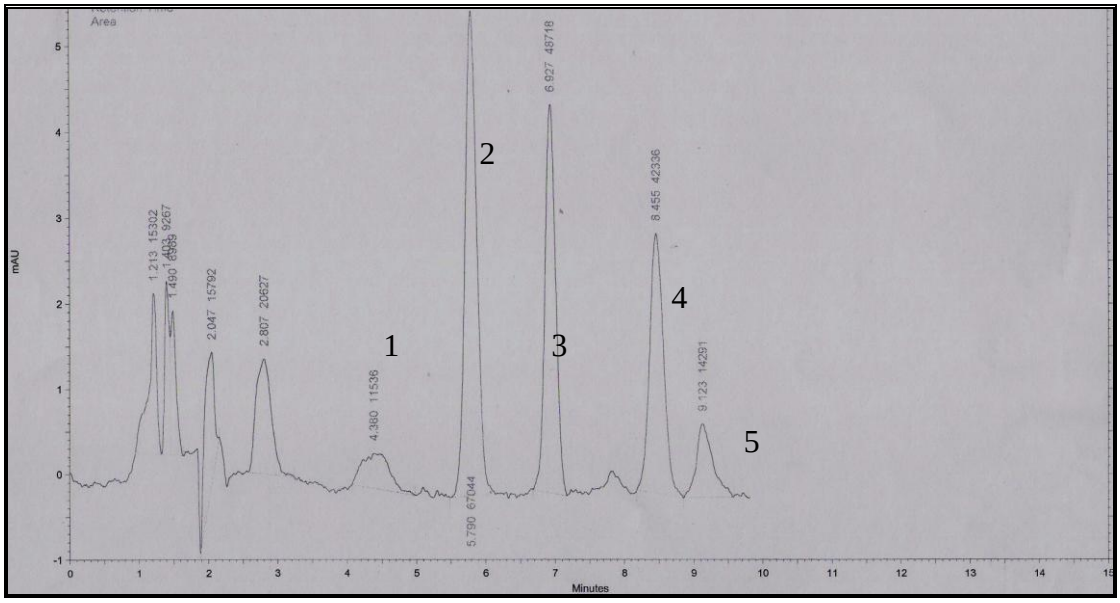


Figure 4.19 Chromatogram using C8 column with detection at 400 nm of 500 ppb acid mixture with KOH 1. formic acid, 2. acetic acid, 3. propionic acid, 4. butyric acid, 5. unknown impurity

4.3.5 Quantification of Methods used to Analyse Organic Acids Derivatives

The limits of detection and quantification for the method using the Nucleodur C8 column were calculated using a 300 ppb acid standard mixture. The method used a two step gradient listed in Table 4.8. Table 4.9 lists the LOD and LOQ calculated from the 300 ppb chromatogram (Figure A4.8). The LOD for formic acid was calculated to be 1.3 ppm with a LOQ of 4 ppm.

	Acetic Acid	Propionic Acid	Butyric Acid
LOD	0.025 ppm	0.064 ppm	0.090 ppm
LOQ	0.083 ppm	0.214 ppm	0.300 ppm

Table 4.9 Limits of detection and quantification for Nucleodur C8 column (n=4)

A range of acid mixtures from concentration 10 ppm to 100 ppb was run in duplicate in order to obtain the calibration curve for each individual acid. The linear regression was calculated for each of the acids from the calibration curves (Table 4.10). The %RSD for the repeatability of the retention times for the acids were calculated to be 1.36% for acetic acid, 1.37% for propionic acid and 1.28% for butyric acid. The calculation for formic acid was carried out on a 5 ppm standard.

	Formic Acid	Acetic Acid	Propionic Acid	Butyric Acid
R^2	0.9969	0.9913	0.9986	0.9988

Table 4.10 Linear regression for each individual acid for derivatization analysis, C8

Acetic Acid	Concentration	Retention Time	Peak Area
	0.1 ppm	5.46	8214
	0.5 ppm	5.46	15365
	5 ppm	5.56	119502
	10 ppm	5.61	198659

Table 4.11 Table of retention time and peak areas for acetic acid at a range of Concentrations (n=3)

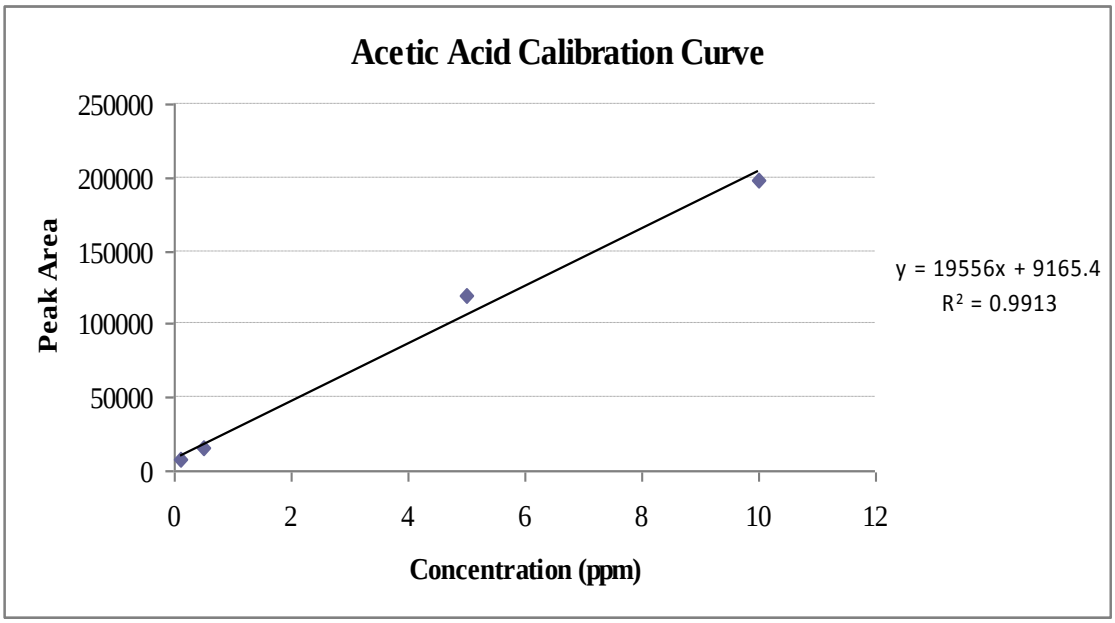


Figure 4.20 Typical calibration curve for Acetic acid on a C8 Nucleodur column, 400 nm, flow rate 1.2 ml/min using a two step gradient

The repeatability of the derivatization process and injections was determined using a 5 ppm acetic acid standard (Table 4.12).

Number of injections	Peak Area	Retention Time
1	104370	5.47
2	101611	5.51
3	106009	5.53
4	104404	5.54
5	106134	5.55
6	103195	5.58
7	101756	5.61

Table 4.12 Repeatability of the injection and derivatization process using a 5 ppm acetic acid standard, on the Nucleodur column

The percent relative standard deviation (%RSD) of the peak areas and retention times for Table 4.12 were calculated to be 1.7% and 0.8% respectively. It was concluded that the method of derivatization used in Chapter 3, 3.5.1.2 was the most favourable, giving good repeatability, with excellent linearity of detection. This method, when used with an appropriate column allowed better sensitivity than the previous methods discussed throughout this chapter.

4.4 Analysis of Power Station Water

From the methods that were used the most favourable method used partition chromatography on a C8 column with the derivatization of the acid analytes using 3.5.1.2.

4.4.1 TOC Results

Power station water sample were obtained from Tutuka. The total organic carbon values that were obtained from the power station were: 0.17 ppm from the output of demineralised water train, 0.21 ppm in the main steam sample, 0.16 ppm in the economiser, and 0.13 ppm in the condenser sample.

4.4.2 Determination of organic acids in power station water

Each samples were first derivatized using the procedure in 3.5.1.2 and then analyses using a C8 column and UV detector. The concentrations of the acids were determined from the slope from the calibration curve obtained from a range of standard. A single

standard mixture was run on the same day of the samples in order to ensure that the concentrations are acceptable and within the calibration. The standard was compared to the calibration curve and was found to be acceptable.

The samples were analysed after a repeated derivatization in order to be certain of the results obtained.

Figures 4.21 to 4.27 are the chromatograms of the samples that were analysed.

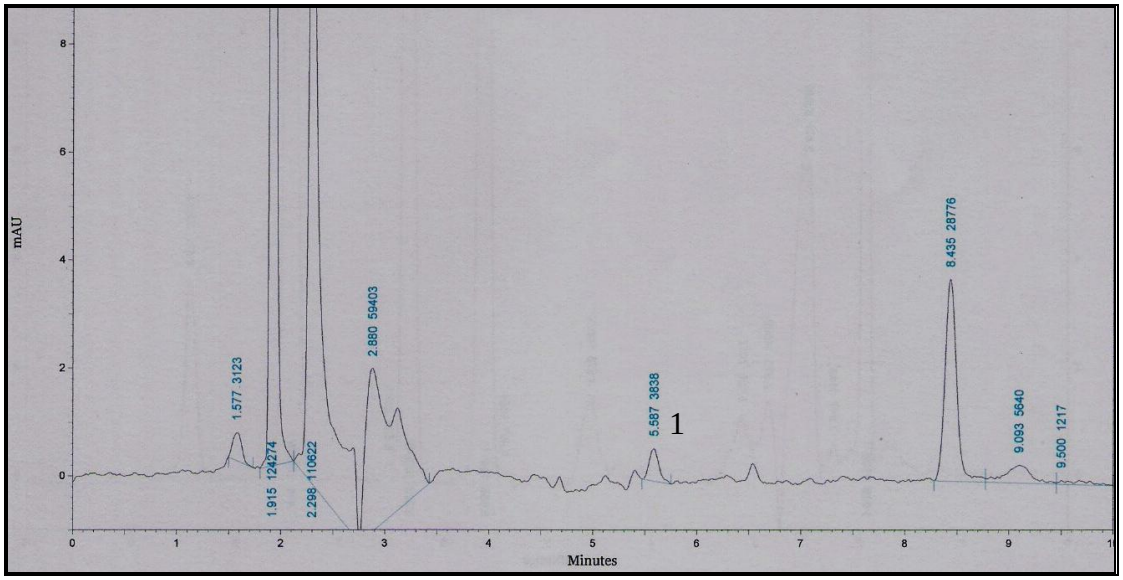


Figure 4.21 Chromatogram of Main steam from first analysis Tutuka PS: 0.276 ppm of acetic acid (n=2) first samples obtained, 1.acetic acid

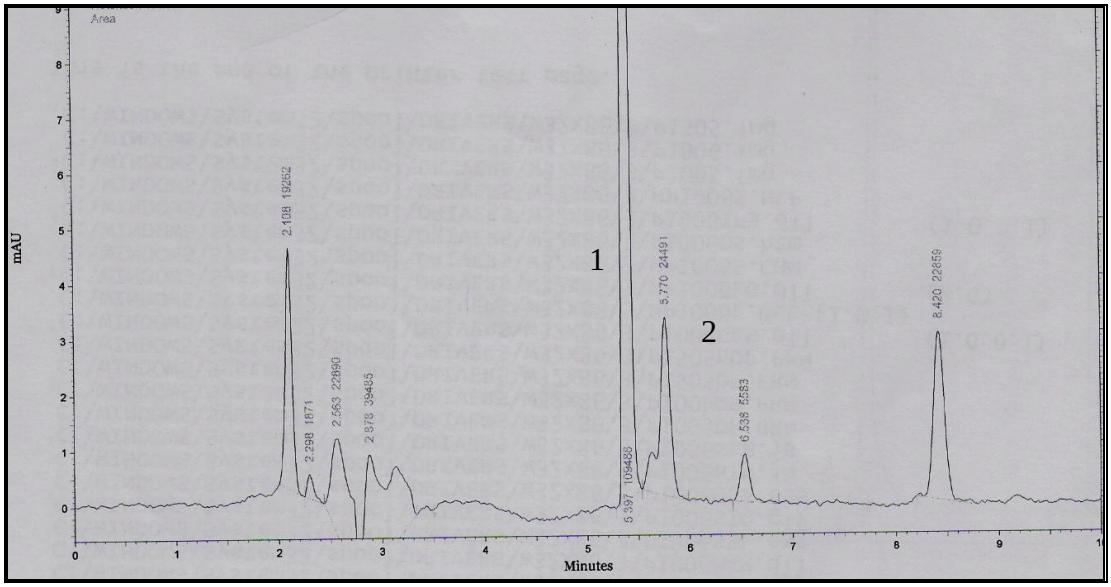


Figure 4.22 Main steam sample run on C8 Nucleodur column with UV detection at 400 nm second samples obtained 1. Formic 2. acetic

The main steam sample (Figure 4.22) above shows a higher concentration in organic acid than the other four samples. This could be attributed to the higher temperatures and pressures that the water is subjected to at that stage in the power station. The bulky organic compound that could have been present would have been reduced to the more simple carboxylic acids like formic acid and acetic acid. The concentration of the acids detected was calculated from the slope a calibration curve (Appendix A4). A 15 ppm standard was run on the same day as the samples, in order to compare run times (Figure 4.26). The peak at 5.3 minutes was identified as formic acid giving an approximate concentration of 14 ppm. The peak at 5.7 minutes was calculated to be 0.27 ppm ± 0.03 ppm acetic acid. When compared to the TOC value, the acid is higher than what is to be expected in the sample. This could have been a result of contamination. Using statistical calculation on Windows Excel the degree of confidence for each acid concentration used in the calibration curve was determined.

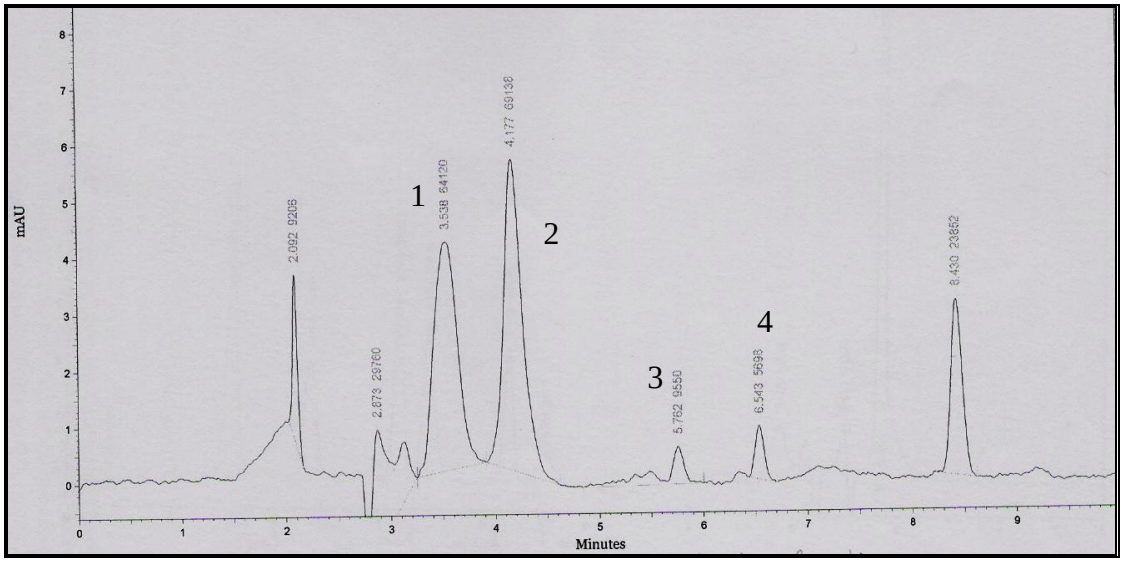


Figure 4.23 Economiser sample run on C8 Nucleodur column with UV detection

1. And 2. Unknown, 3. Acetic, 4. Propionic acid

The economiser (Figure 4.23) and condenser (Figure 4.24) samples both have two large unknown peaks at retention time, 3.5 minute and 4.1 minute in addition to acetic acid and propionic acid. The economiser sample had 0.090 ppm ± 0.04 ppm acetic acid and 0.35 ppm ± 0.04 ppm propionic acid whilst the condenser sample contained 0.055 ppm ± 0.04 ppm of acetic acid and 0.31 ppm ± 0.04 ppm of propionic acid.

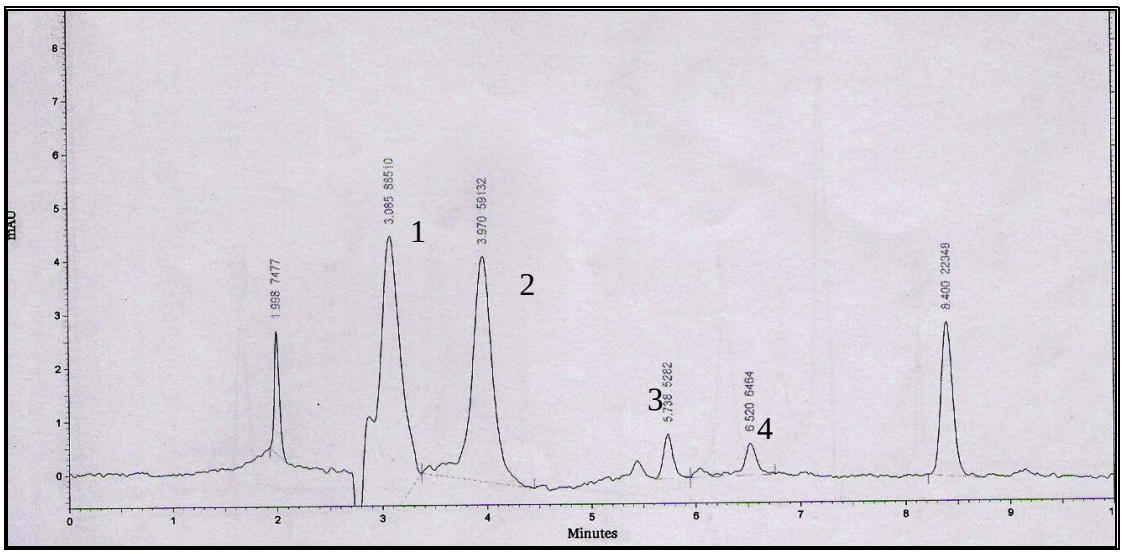


Figure 4.24 Condenser sample from Tutuka power station 1. And 2. Unknown 3. acetic acid, 4. Propionic acid

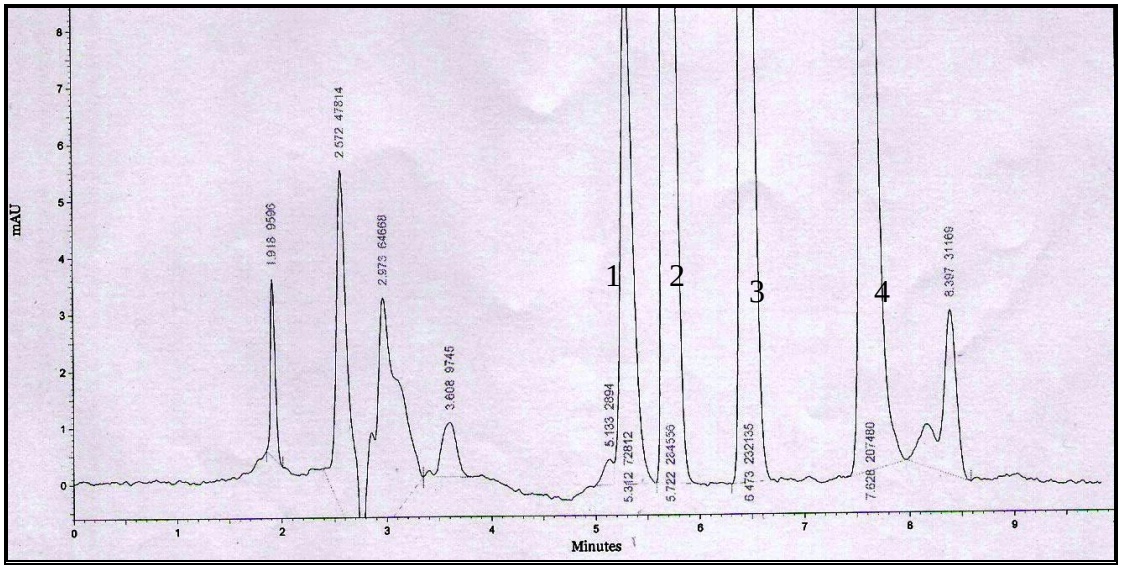


Figure 4.25: 15 ppm acid standard mixture on C8 Nucleodur column using UV Detector 1. Formic, 2. Acetic, 3. Propionic, 4. Butyric

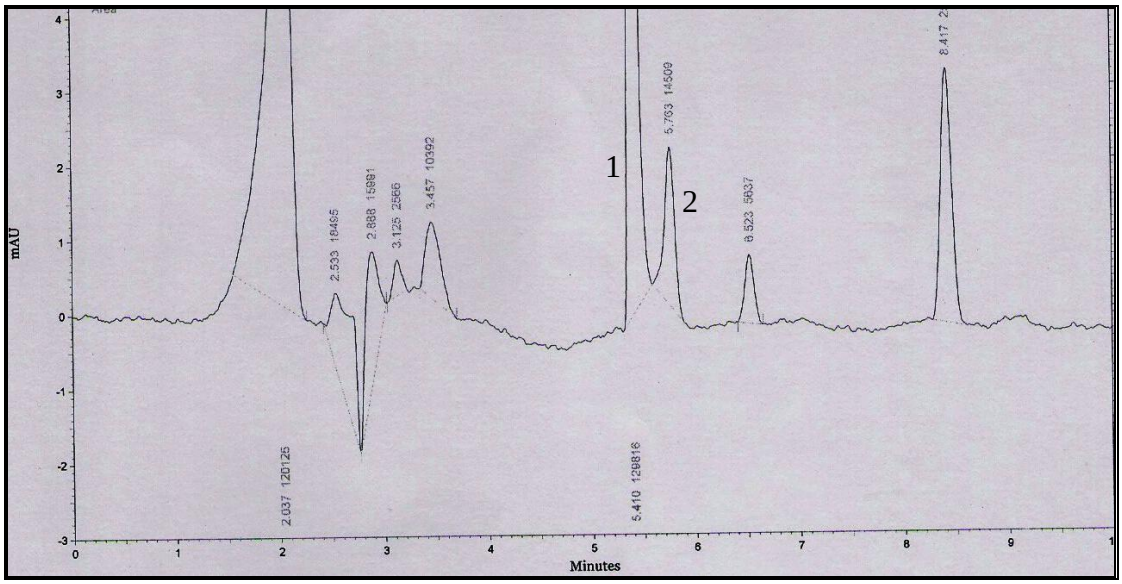


Figure 4.26 Chromatogram of Demin water from mix bed outlet (ESKOM Tutuka) 1, Formic 2. Acetic acid

Table 4.12 Organic acids detected in power station water (Tutuka) (n=2)

Organic Acid				
	Demin water	Condenser	Main Steam	Economiser
Formic Acid	15.27 ppm	N.D	14.06 ppm	N.D
Acetic Acid	0.27 ppm	0.055 ppm	0.28 ppm	0.090 ppm
Propionic Acid	0.36 ppm	0.31 ppm	0.36 ppm	0.34 ppm
Butyric Acid	N.D.	N.D	N.D	N.D

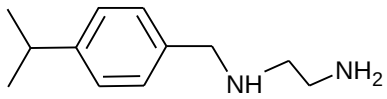
Although the acids were detected in the samples the confidence range between the concentrations as well as the discrepancies between the TOC values and the acid concentrations warrant further investigation. Hence a pre-concentration technique is currently being investigated in order to improve the confidence of the results obtained.

The investigation using a pre-concentration technique before derivatization of the acids is currently being investigated. Some of the method development of the technique has been given within this dissertation below.

4.5 Pre-concentration of Organic Acids using Solid Phase Extraction

4.5.1 Method Development for pre-concentration

The SPE cartridge that was used in the pre-concentration technique is a Strata X-AW. The solid phase i.e. sorbent used in the strata X-AW is polymer based. The structure of the stationary phase on the cartridge can be observed in Figure 4.28.



N-(4-isopropylbenzyl)ethane-1,2-diamine

Figure 4.27 Structure of sorbent for Strata-X-AW

This sorbent works under both, reverse phase and weak anion exchange principles. There were essentially two different methods that were used by the suppliers with regards to the elution of acidic analytes from the cartridge. The same equilibration, conditioning and the wash steps were used, however two different solutions were required for elution. Method development trials are being tested in order to find a suitable method that would be applicable to this study.

A pH6 phosphate buffer was first used as the eluent. It was noticed that the flow rate used during the loading of the sample is extremely important. Although the method suggested by the supplier, recommend a flow rate anywhere between 4 to 8 mL, it was observed that the lower flow rates improved the adhesion of the analytes to the sorbent.

The flow rate that was used in the first elution was 6 mL/min however the chromatogram showed that not all the acids had been pre-concentrated (A5.6). A flow rate below 3 mL/min was used for the rest of the trials. The wash and elution fractions were collected in order to ascertain whether the analytes were being removed in the incorrect solution. The chromatogram for the wash fraction showed that none of the acids had been removed early. The elution fraction i.e. the buffer (Figure 4.29) contained the acids.

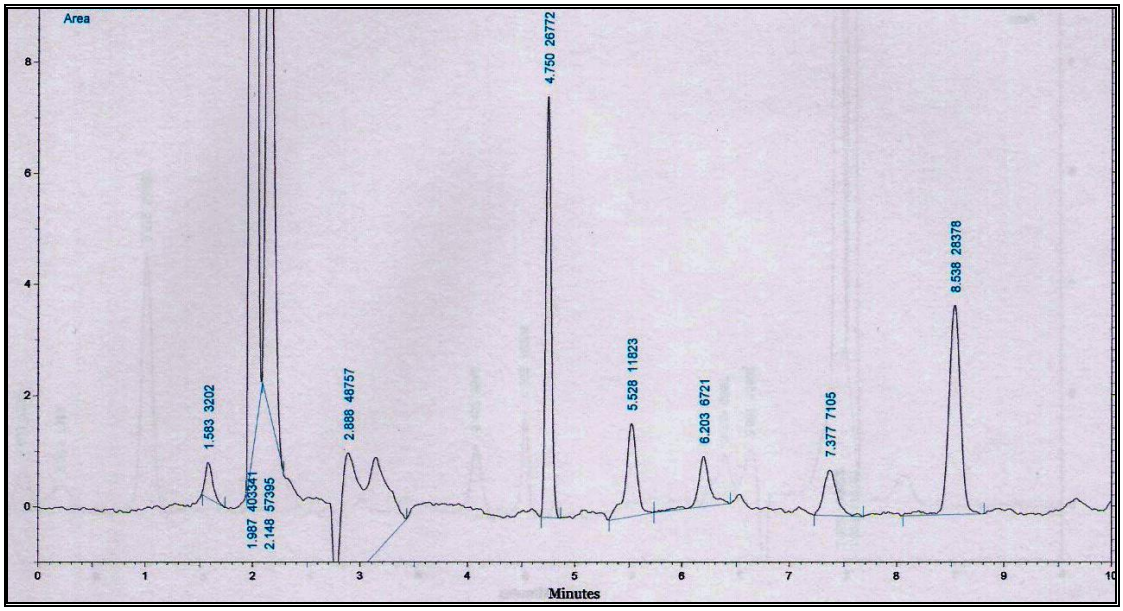


Figure 4.28 Chromatogram of a pre-concentrated 50 ppb acid mixture using Strata X-AW, SPE elution solvent: buffer pH 6

Although the acids were being pre-concentrated and eluted with the pH6 buffer; the percentage pre-concentration was observed to be lower than what was expected. Hence other elution solutions were tried in order to improve the method. Appendix A5 contains all chromatograms for each of the various elution solution tried. Buffer 8 and 12 showed very little or no improvement. A 2% HCl solution in 20% methanol

was also tried. There were many interference peaks that were observed when using this elution solvent. It was also noticed that the guard column cartridge needed to be changed more often when using that solution after derivatization.

A 2% NH_4OH solution in 20% methanol was also used. There were many interference peaks that eluted at similar times of the acids that were first mistaken for the acids but were found to be present in the blank (Figure 4.30).

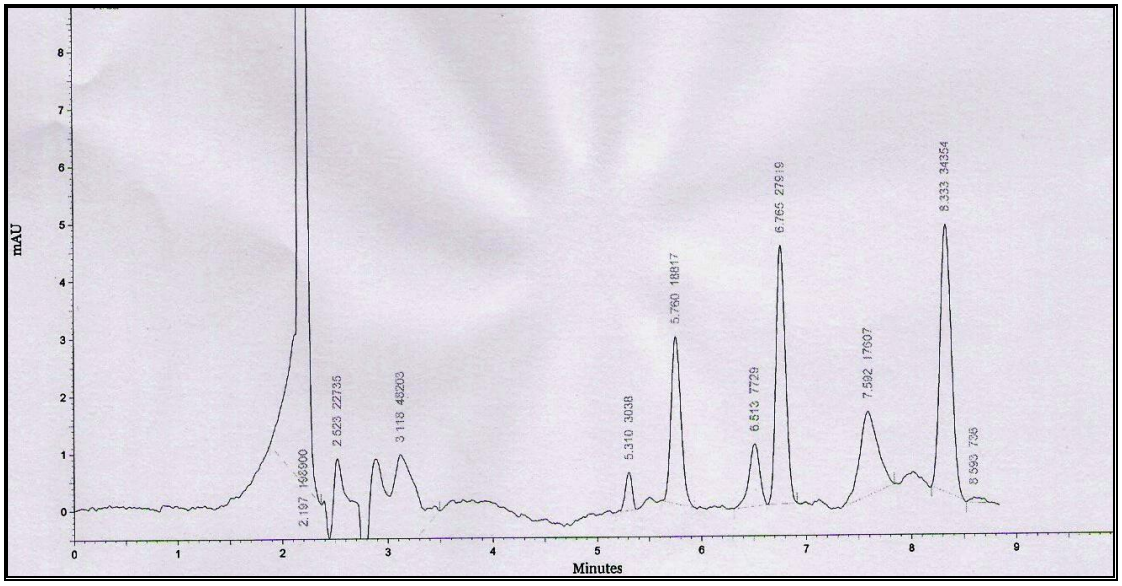


Figure 4.29 Chromatogram of blank using NH_4OH as a SPE eluent

The most crucial factor that needed to be observed was the compatibility of the elution solution to the derivatization process. An attempt was made to reconstitute the acid analytes in a more suitable mobile phase. A solution was spiked in a 1% HCl in 20% methanol solution and evaporated to dryness, then reconstituted in 5mL of water. However none of the acids were able to be detected. It is assumed that due to the volatility of the acids it could have been removed during the evaporation process.

4.52 Quantification of organic acids pre-concentration

A set of calibration standards were run for each method in order to determine the concentration of recoveries of each method. The recoveries were calculated using the gradient of each calibration curve.

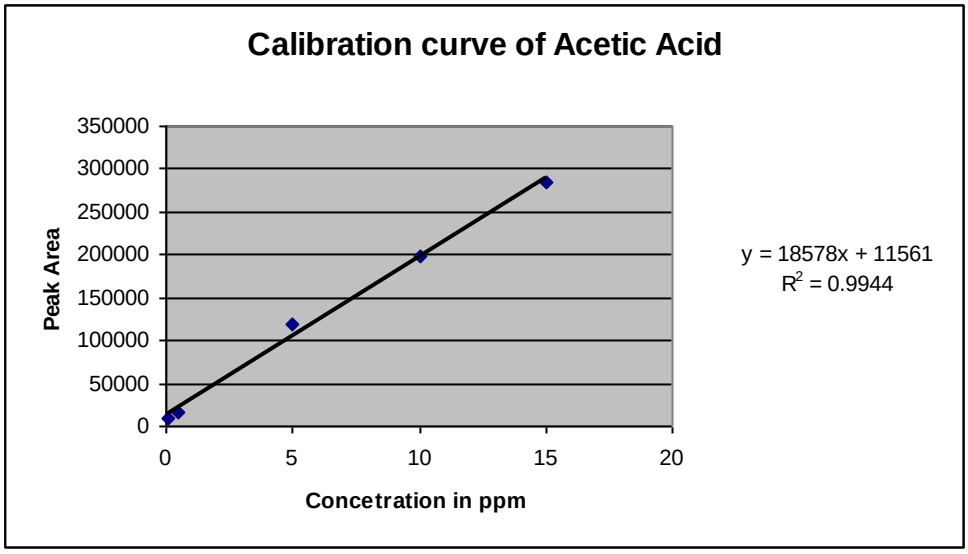


Figure 4.30 Calibration Curve for Acetic Acid used in pre-concentration determination for Strata X-WA

Acetic Acid	Concentration (ppm)	Peak Area
	0.1	8214
	0.5	15365
	5	119502
	10	198659
	15	284556

Table 4.14 Peak Areas for calibration curve of Acetic acid (Figure 4.31)

The calculations of the recoveries on the Strata X-WA cartridge were determined from a 500 ppb acid standard mixture. Since 10 mL of sample was extracted from a 100 mL sample, a 10 times pre-concentration was expected. However the

concentration of acetic acid was calculated to be 1.27 ppm, propionic acid was calculated to be 1.75 ppm and butyric acid was calculated to be 3.6 ppm. Hence the % recovery for acetic acid was 26%, propionic acid was 35% and butyric was calculated to be 72%.

4.5.3 Effects of the SPE eluent on the derivatization process

The poor recoveries on the Strata X-AW could be influenced by the derivatization process. The method was limited by the type of solvents that could be compatible with the derivatization process. The influence of the solvents needs to be explored further. A 10 ppm standard solution was prepared in the pH 6 phosphate buffer (Figures 4.32 and Figure 4.33). The standard solution was then derivatized and the sample analysed on the C8 column. This standard was compared to a standard solution prepared in ultra pure water.

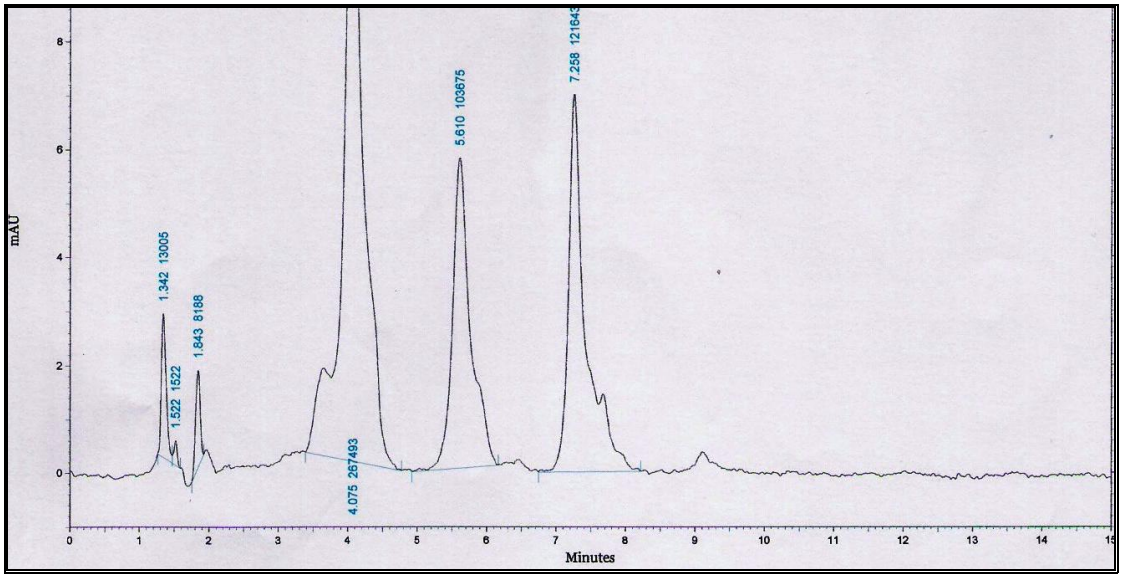


Figure 4.31 Chromatogram of 10 ppm acid mixture prepared in water, 400 nm, C8 column

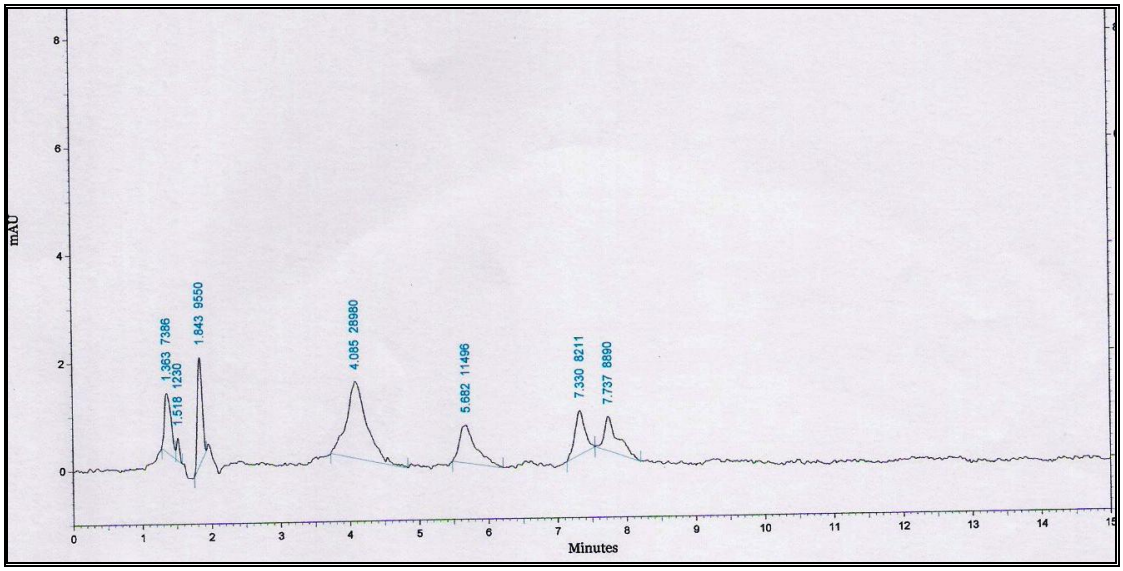


Figure 4.32 Chromatogram of 10 ppm acid mixture prepared in pH 6 phosphate buffer

A decrease in the detectability of the acids was observed which proves that the eluent does effect the detection of the acids from the eluent collected after pre-concentration. Hence further investigation into an appropriate eluent is necessary. In addition it is recommended that an alternate pre-concentration of the acid analytes after derivatization (i.e. acid hydrazine derivative) should be investigated. This could be used to alleviate the effects of the eluent solvents on the analysis.

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Chapter Five

Conclusion and Recommendations

This study involved the analysis of trace level simple carboxylic acids by two methods of HPLC namely, ion exclusion and partition chromatography. Ion exclusion was found to be a simple and easy method, requiring no modification of the acid before separation. Various detectors were used to in order to try to improve the sensitivity of detection. Although ELSD is reported to be a sensitive detector it was found to be unsuitable for the carboxylic acids used in this study. It is recommended that further study should be carried out in order to investigate the improvement of the detection of the acids by coupling to an ion pairing reagent in future investigations.

On the other hand the use of ion exclusion with a UV detector and conductivity detector had yielded results. However the limit of detection for the methods were not low enough for the concentration of the acids expected to be present in field samples. In order to improve the sensitivity of the detection of the acids an additional step of pre-column derivatization was investigated.

A derivatization method used in the determination of carboxylic acids in biological substances was modified for use in this investigation. A comparison between the following was made:

- The two derivatization methods used
- A C18 column and a C8 column used in the analysis
- Detection at wavelengths 230 nm and 400 nm

Each of the above aspects was discussed in detail in Chapter Four. From method development, it was found that the pre-column derivatization for aqueous samples could be successfully carried out using a derivatizing agent *(o-nitrophenyl hydrazine)*, coupling reagent *(1-ethyl-3-(dimethylaminopropyl) carbodiimide)* and 10 % potassium hydroxide solution.

The total run time of the analysis using partition chromatography was improved from 60 minutes on a C 18 column, to 10 minutes on a C 8 column. The latter was therefore used in all subsequent experiments.

By comparing the method used in ion exclusion and partition chromatography with UV detection, the following is noted:

- Both methods showed good R^2 values for all the acids being analysed.
- Formic acid exhibited a weaker response when analysed using partition chromatography.
- The repeatability of the retention times and peak areas for partition chromatography showed good repeatability of the analysis and derivatization process.
- Excellent repeatability was also obtained when using ion exclusion on UV detector.
- The limits of detection was found to be lower when using partition chromatography.

Partition chromatography with a derivatization method was then used in the analysis of the organic acids in the field samples. The detection of the acids was carried out in order to ascertain the level of the acids present in the samples. It was uncertain as to which of the acids would be detected in the field samples. From the analysis formic, acetic and propionic acids were able to be detected within the samples.

By applying statistical calculation, the degree of uncertainty in the concentration value of each acid concentration was determined. In order to improve the certainty of the results, a method of pre-concentration should be used.

A solid phase extraction process using weak anion exchange is being used in a current investigation. The pre-concentration of the acids before derivatization has been attempted. Some difficulties have been experienced in choosing an appropriate eluent solvent for the extraction of the acids. From the analysis carried out it was found that solvents such as a phosphate buffer pH 6, used to extract the acids after pre-concentration, interfere in the derivatization process. Attempts have been made to reconstitute the analytes in a more appropriate solvent but with no success. This could be due to the volatility of the analytes. Poor recoveries were obtained for the acids due to the sensitivity of the derivatization process.

In light of the above the following is recommended:

- A more appropriate solvent for the extraction of the acids during the pre-concentration should be investigated.
- An alternate pre-concentration of the acid after derivatization should be investigated.

- Use of LC-MS in order to identify the impurities that result from the derivatization process.

After carrying out this investigation it has been observed, that these simple organic acids are relatively difficult to detect at trace levels. It is only recently that studies have directed its focus to the effect of these simple carboxylic acids on the environment (including industry). Most analyses found were carried out in the biological and food industries due to the effects these acids have on taste and degradation of the products used in that industry. Detection of the acids in these applications is easier due to the high concentration levels (ppm to %) of the carboxylic acids.

Appendix A

A1: Ion Exclusion Chromatograms and Data:

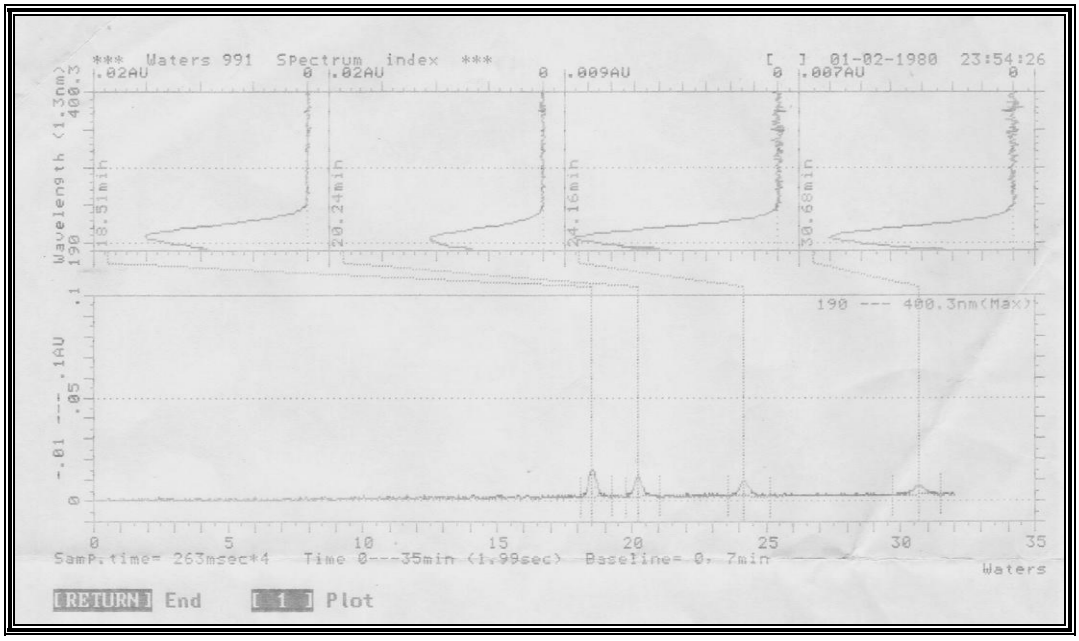


Figure A1.1 Optimum wavelength for each acid investigated

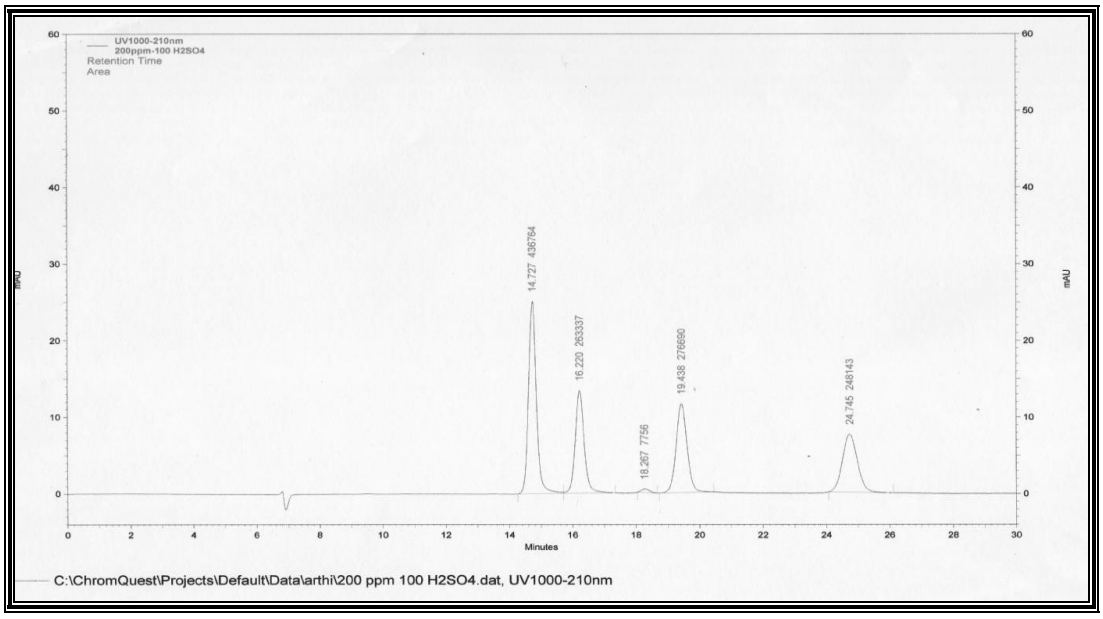


Figure A1.2: Chromatogram of 200ppm acid mixture on ion exclusion column at 210nm, 1. formic acid, 2. acetic acid, 3. propionic, butyric acid

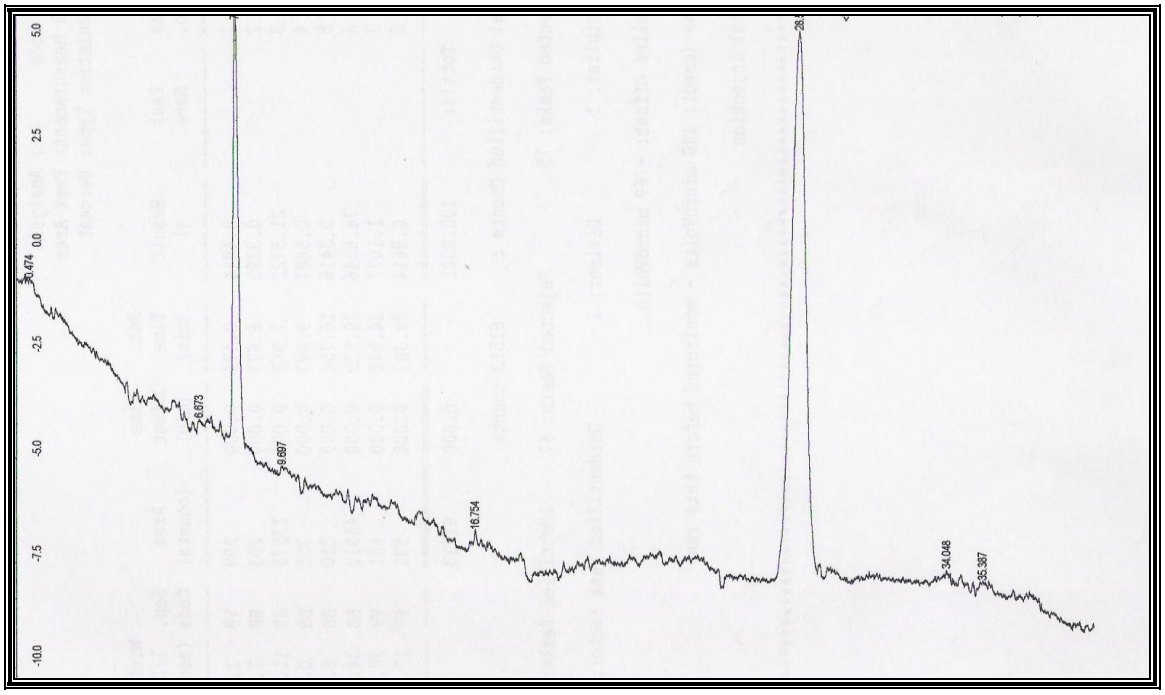


Figure A1.3: Chromatogram showing noisy baseline of acid mobile phase only

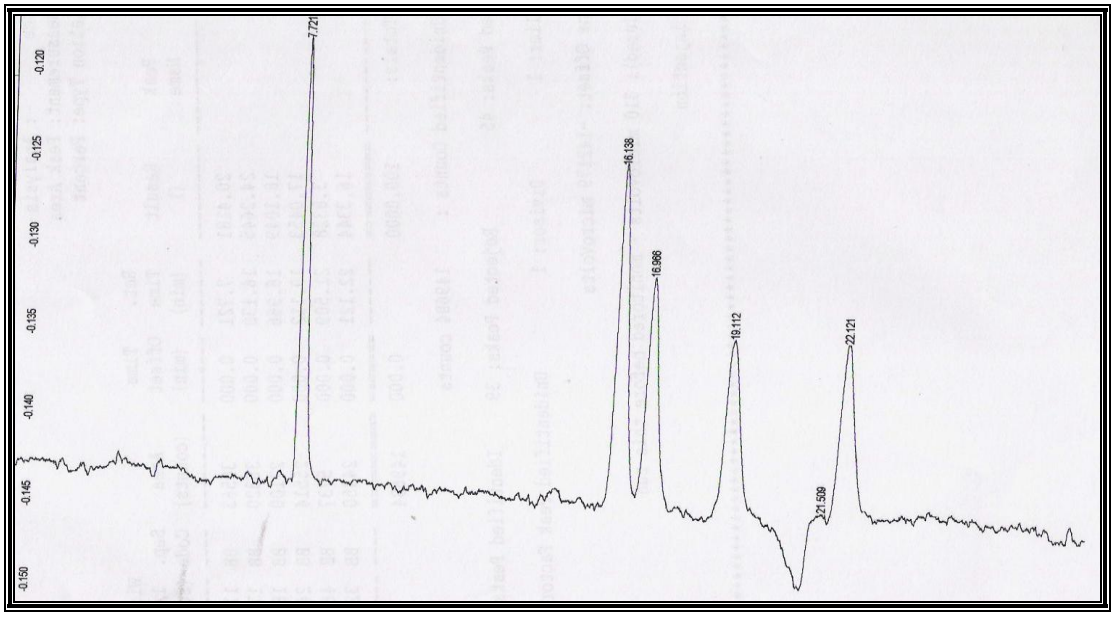


Figure A1.4: Chromatogram of acid mixture with ion exclusion at 210nm, mobile phase A: 90% H_2SO_4 and Mobile Phase B: 10% Acetonitrile

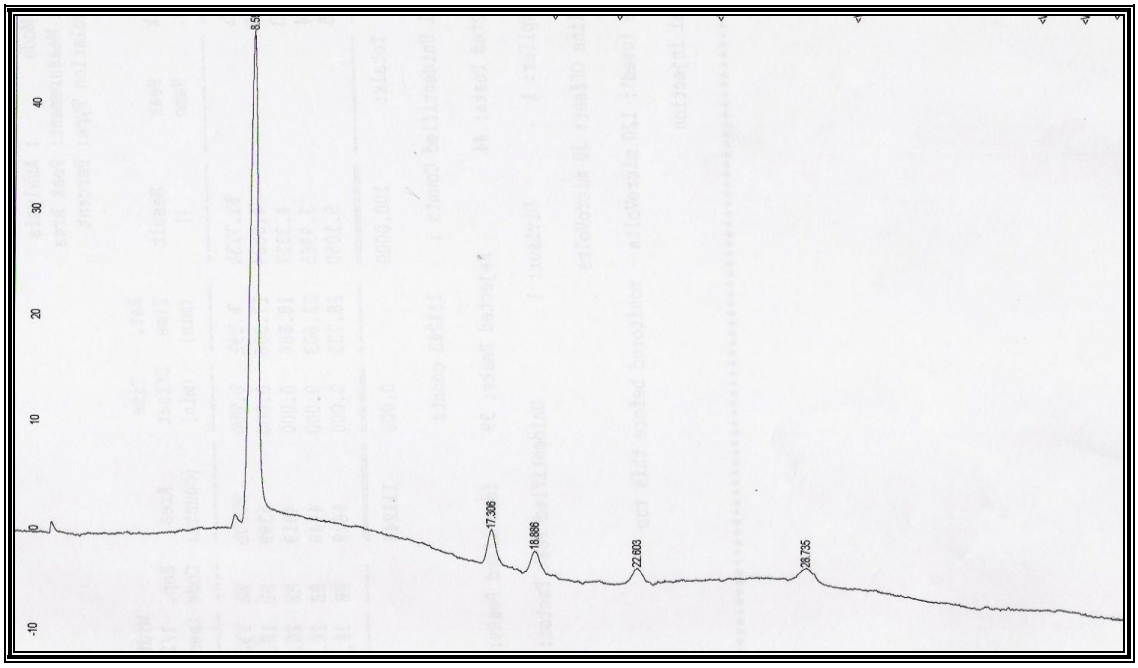


Figure A1.5: Chromatogram of acid mixture with 2.5mM H_2SO_4 acid Mobile Phase only

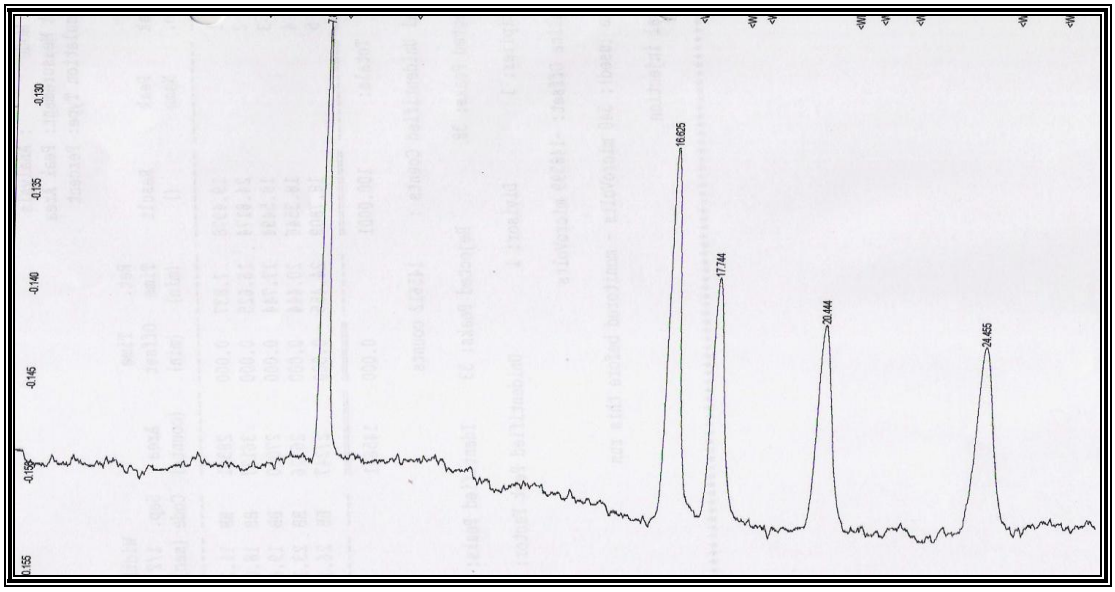


Figure A1.6: Chromatogram of acid mix using ion exclusion column at 210nm with 5% Acetonitrile mobile phase B : 95% H_2SO_4 Mobile phase A.

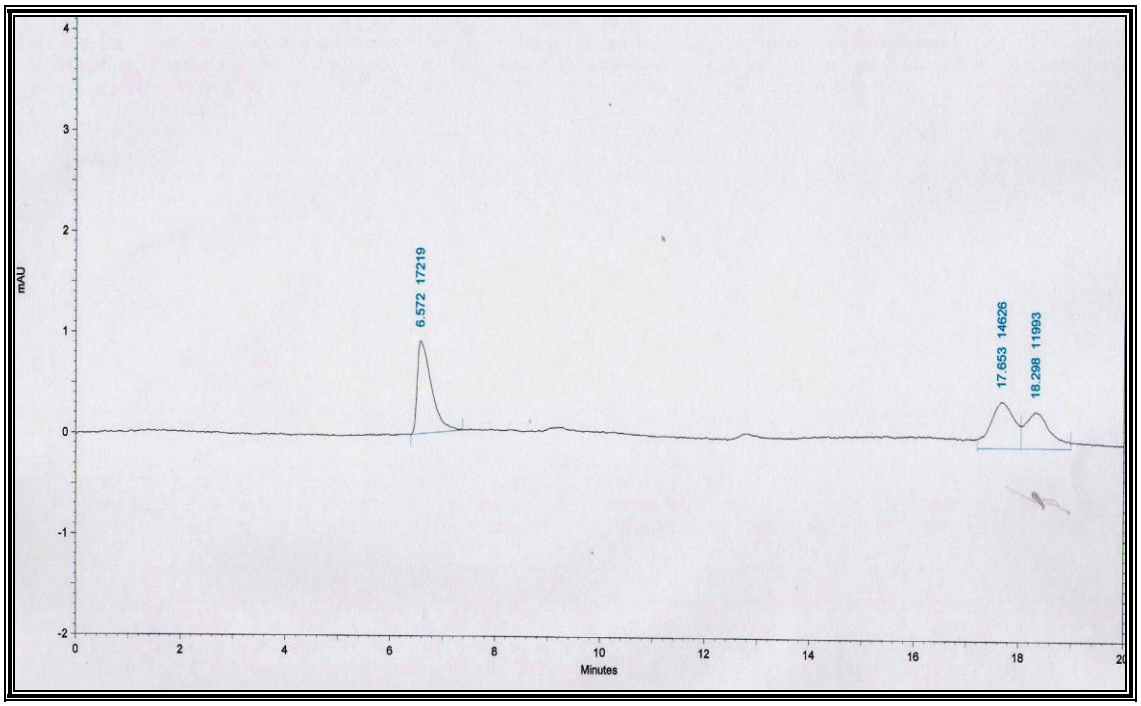


Figure A1.7: Chromatogram of 8ppm propionic acid only, on ion exclusion column

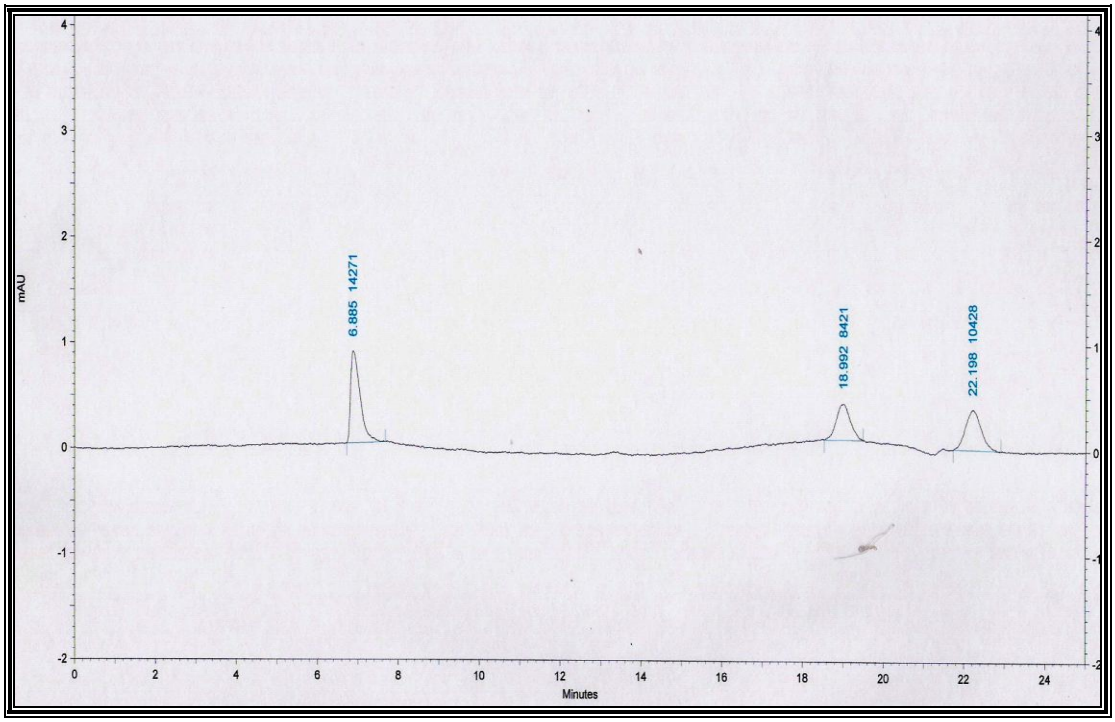


Figure A1.8: Chromatogram of 8ppm butyric acid only, on ion exclusion column

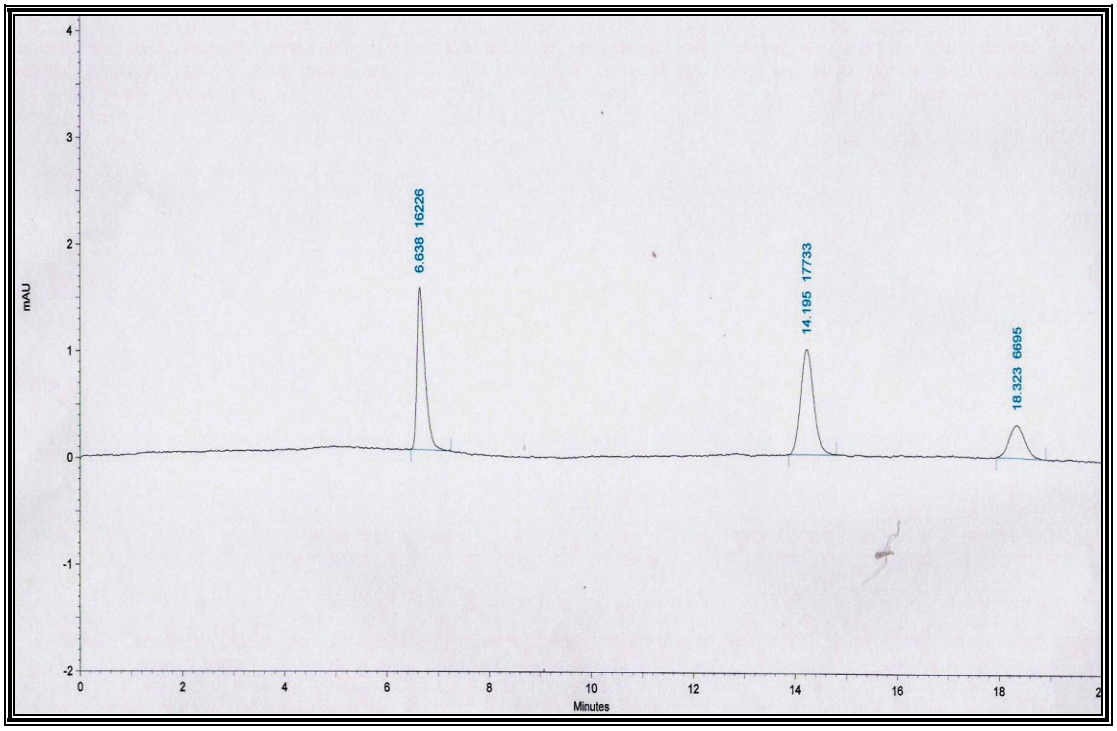


Figure A1.9: Chromatogram of 8ppm Formic acid only, on ion exclusion column

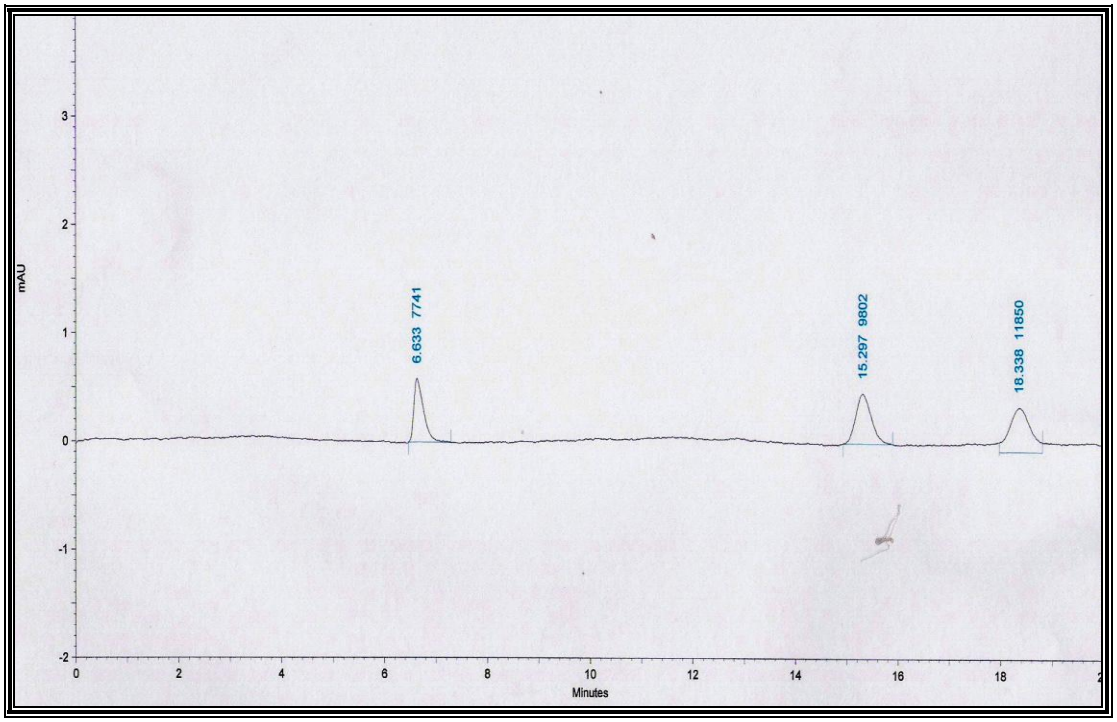


Figure A1.10: Chromatogram of 8ppm acetic acid only on ion exclusion column

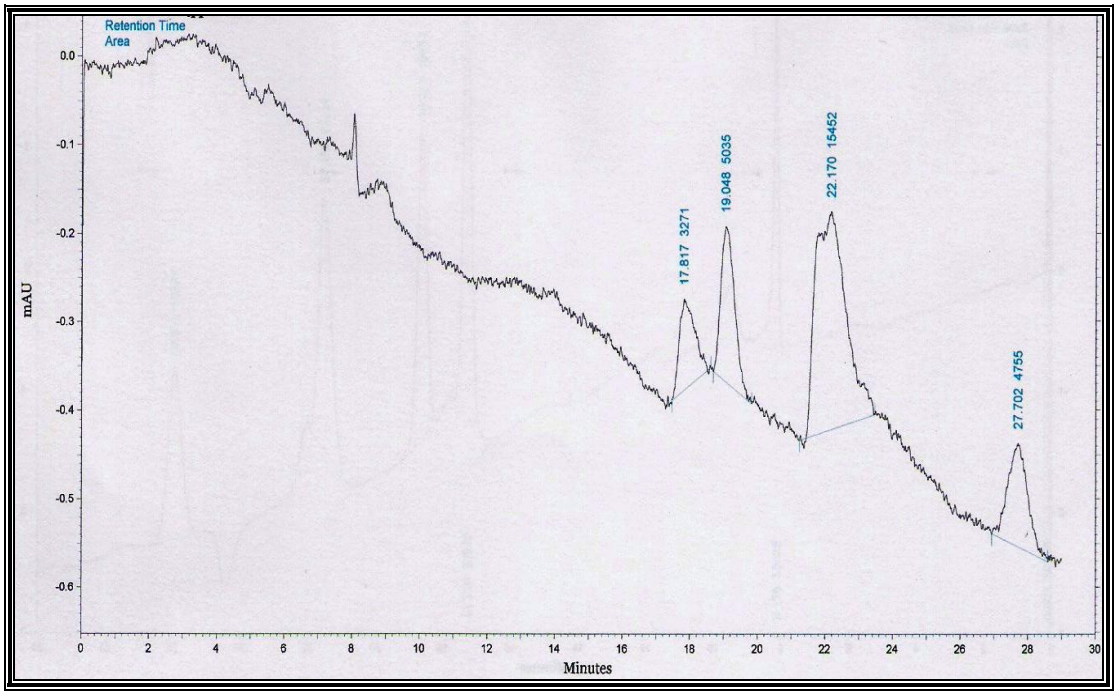


Figure A1.11: Chromatogram of 4ppm acid mixture using ion exclusion at 210nm

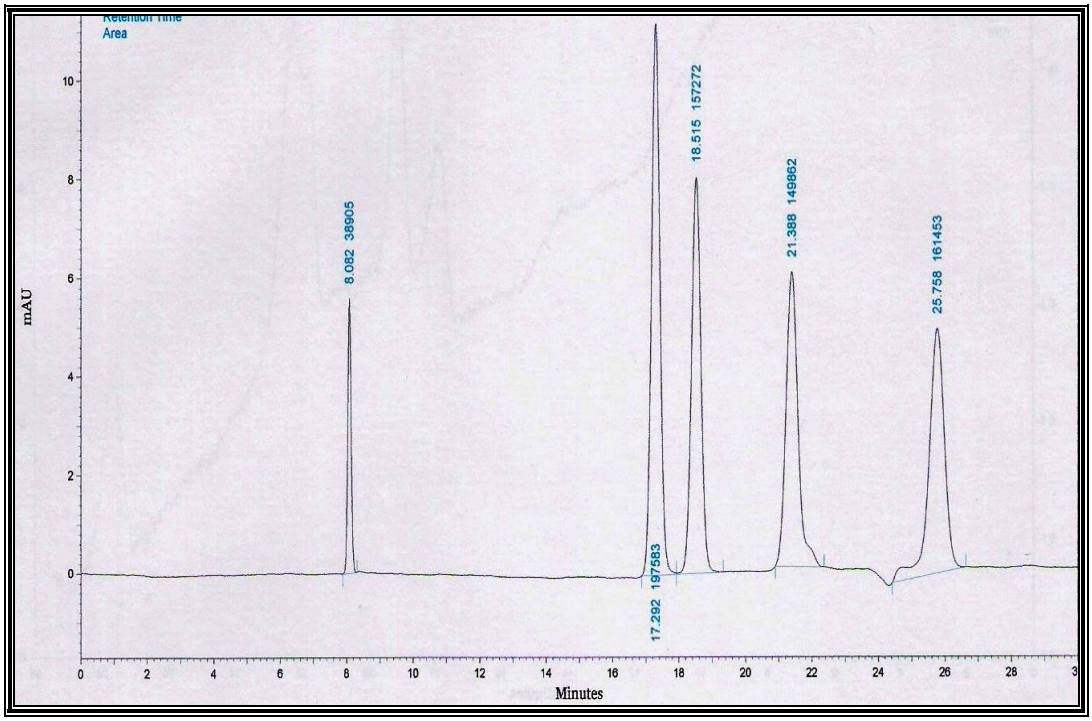


Figure A1.12: Typical chromatogram of organic acid mix 10ppm, ion exclusion, 210nm

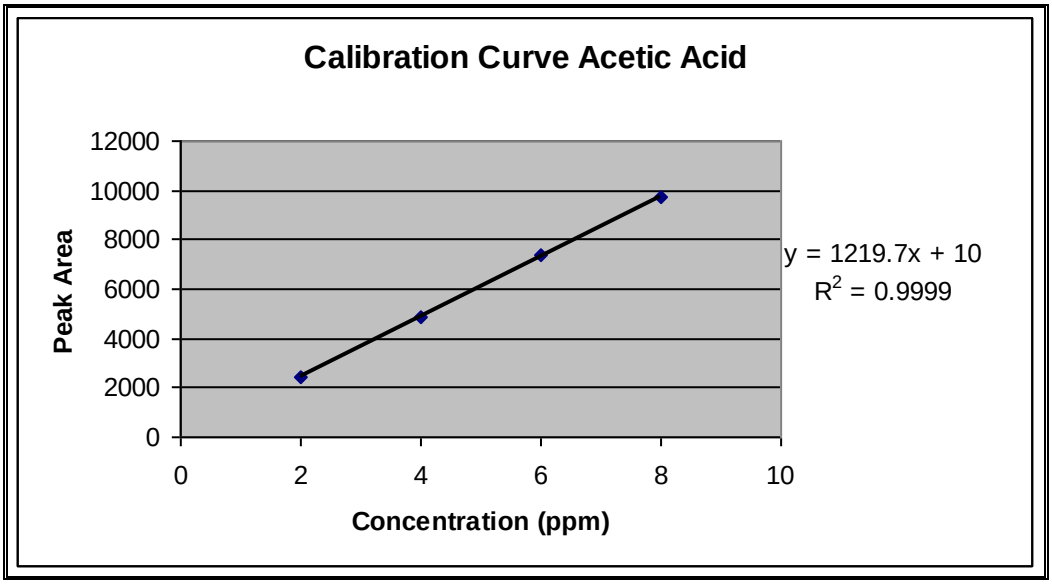


Figure A1.14 Calibration Curve of acetic acid using ion exclusion, 210nm

Table A1.1 Peak areas and retention time for acetic acid

Acetic Acid	Concentration	Retention Time	Peak Area
	2ppm	15.32min	2442
	4ppm	15.30min	4881
	6ppm	15.30min	7366
	8ppm	15.31min	9745

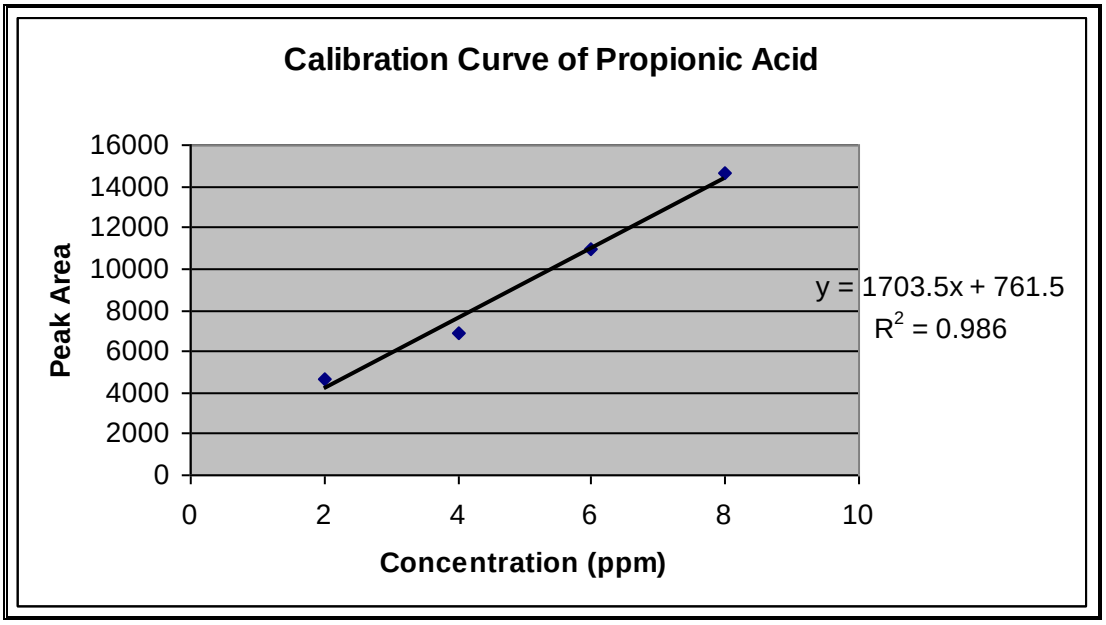


Figure A1.14 Calibration Curve of propionic acid using ion exclusion, 210nm

Propionic	Concentration	Retention time	Peak Area
Acid	2ppm	17.69min	4652
	4ppm	17.69min	6845
	6ppm	17.68min	10992
	8ppm	17.65min	14626

Table A1.2 Peak areas and retention time for propionic acid

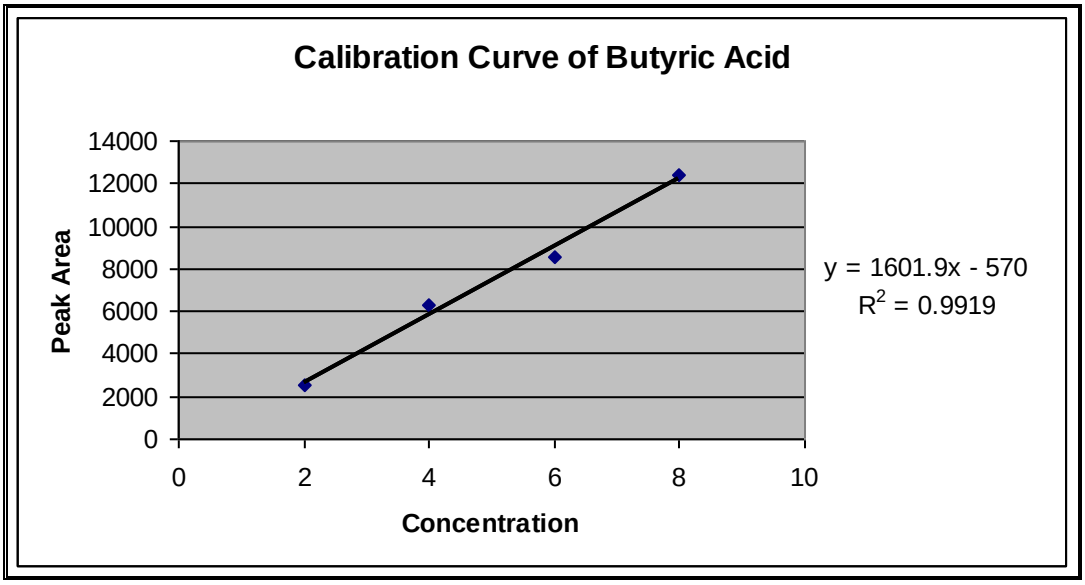


Figure A1.15 Calibration Curve of butyric acid using ion exclusion, 210nm

Butyric	Concentration	Retention time	Peak Area
Acid	2ppm	22.68min	2506
	4ppm	22.80min	6255
	6ppm	19.50min	8590
	8ppm	18.74min	12407

Table A1.3 Peak areas and retention time for butyric acid

A2: Ion Chromatography with Conductivity Detector

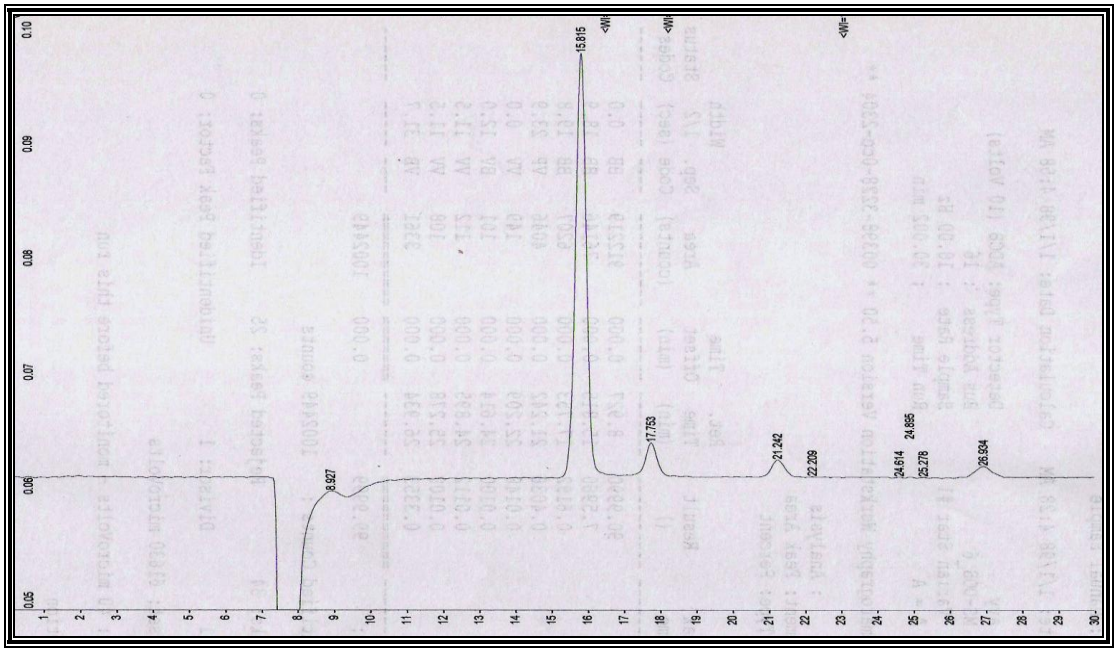


Figure A2.1: Chromatography of acid mixture (200ppm) conductivity detector

Ion exclusion column with electronic suppression

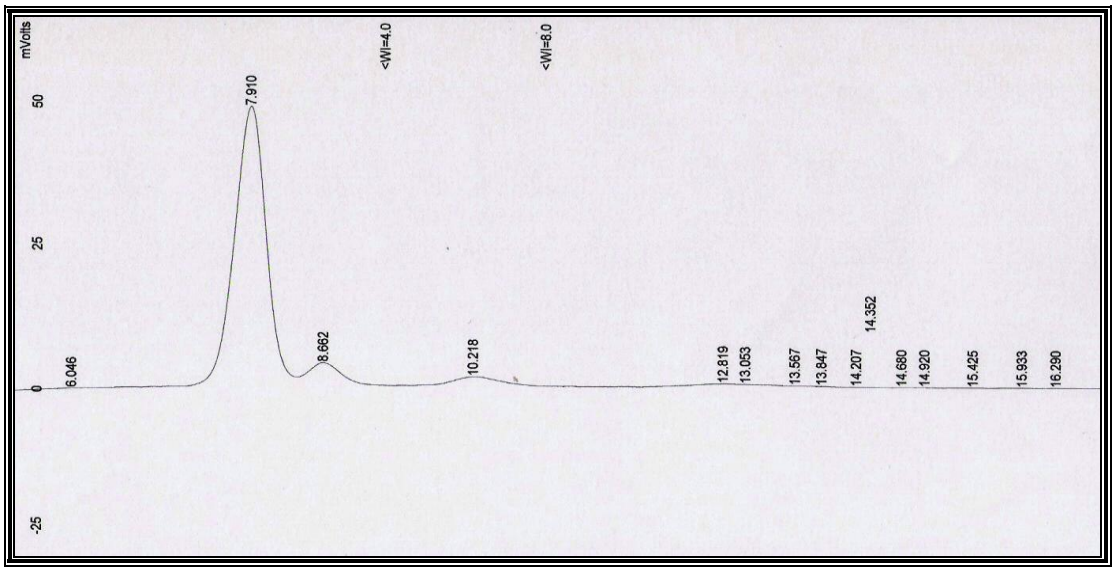


Figure A2.2: Chromatogram of acid mixture 200ppm with 100mm OA Metrosep column, flow rate 0.5mL/min

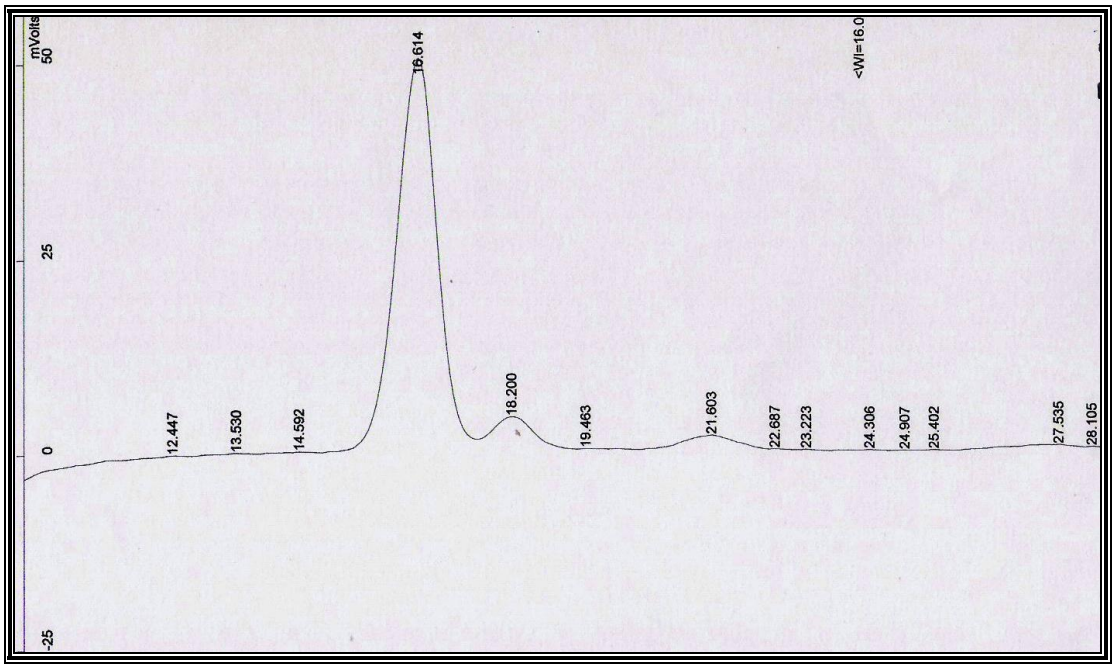


Figure A2.3: Chromatogram of 200ppm acid mixture with 100mm OA Metrosep column, flow rate 0.2mL/min conductivity detector

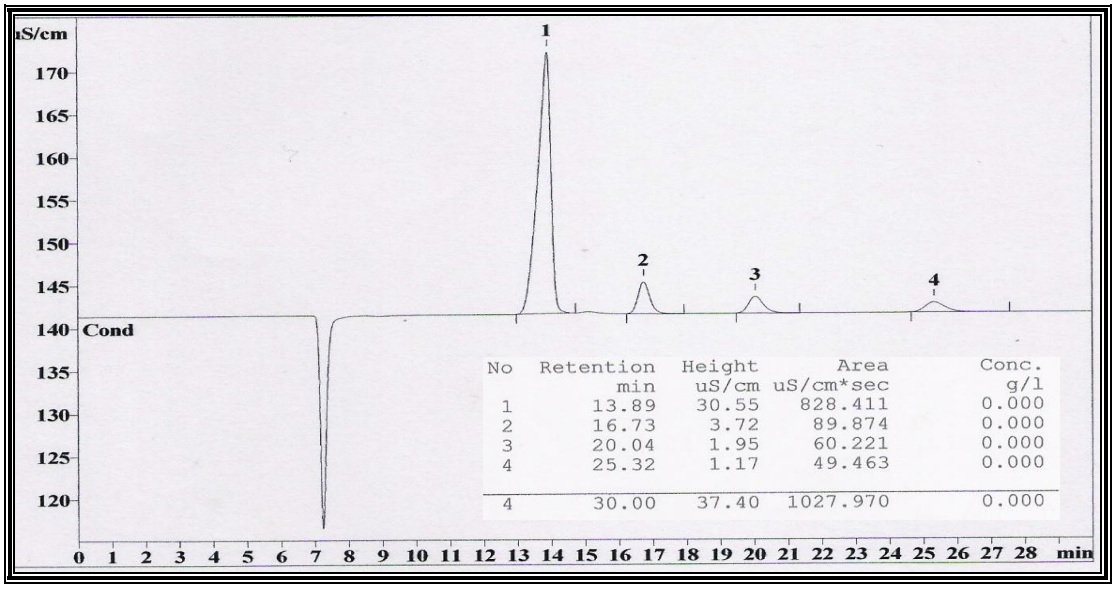


Figure A2.4: Chromatogram of 200ppm aid mixture on 761 IC Compact with Suppressor modular

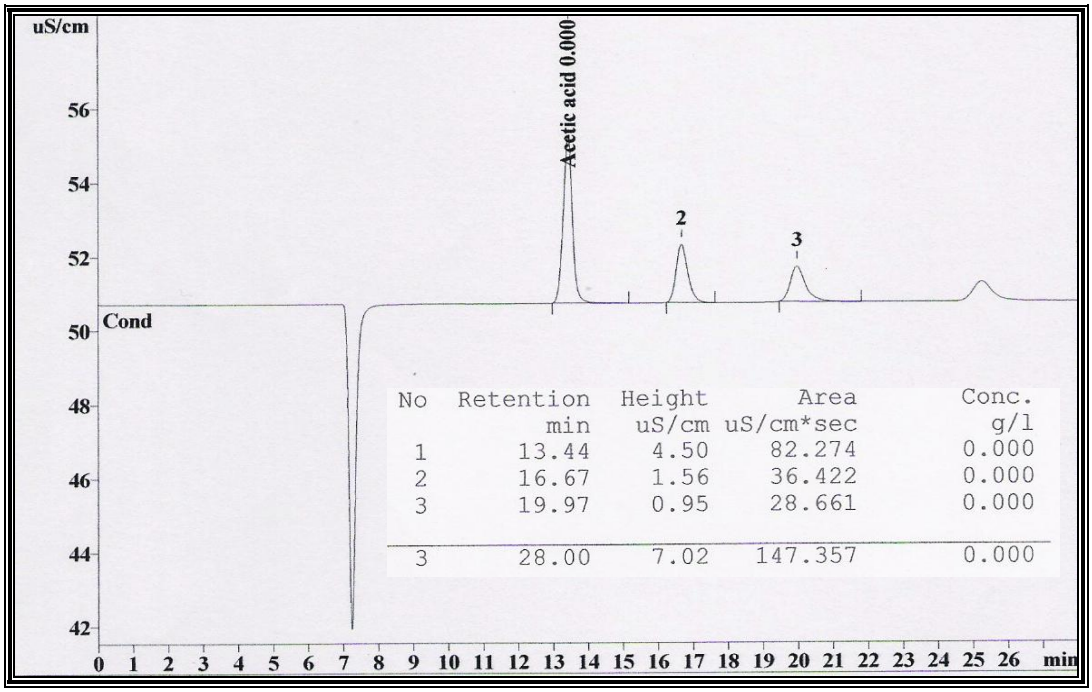


Figure A2.5: Chromatogram of 20ppm aid mixture on 761 IC Compact with suppressor modular

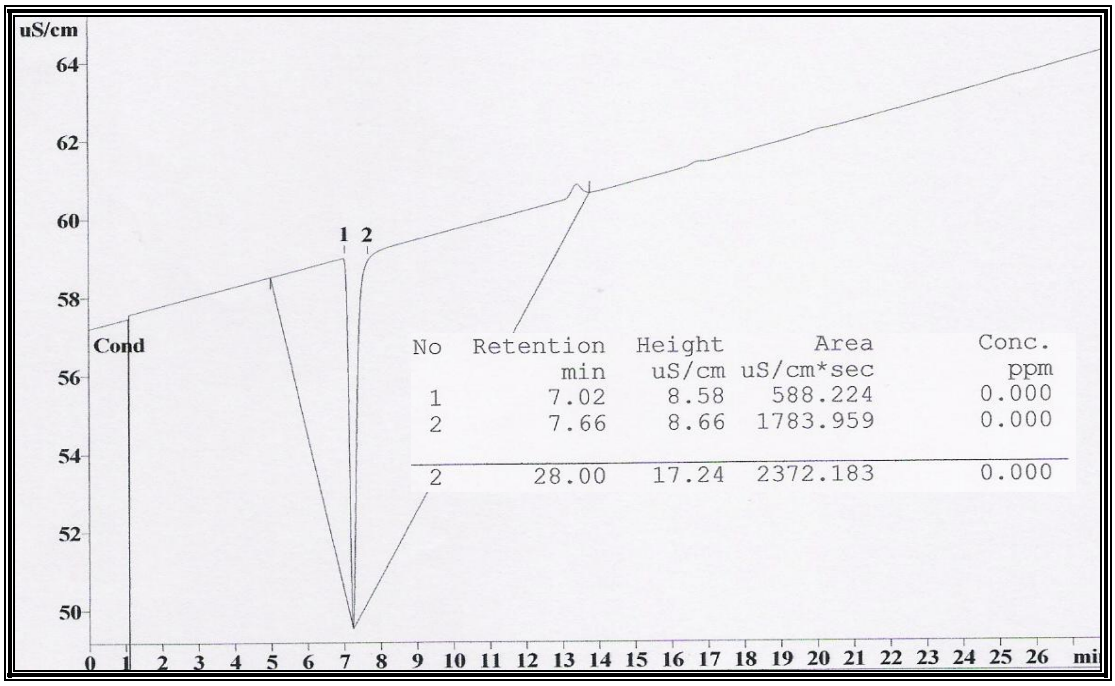


Figure A2.6: 2ppm aid mixture on 761 IC Compact with suppressor

A3: Partition Chromatography-C18

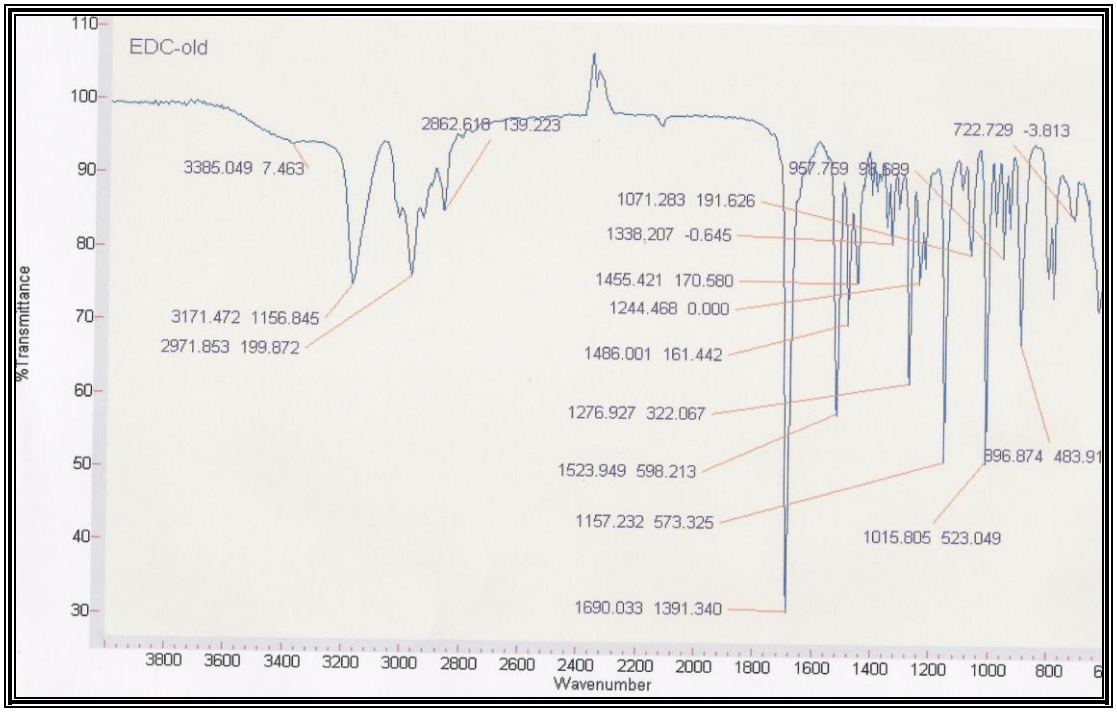


Figure A3.1: IR spectrum for coupling reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) after sublimation

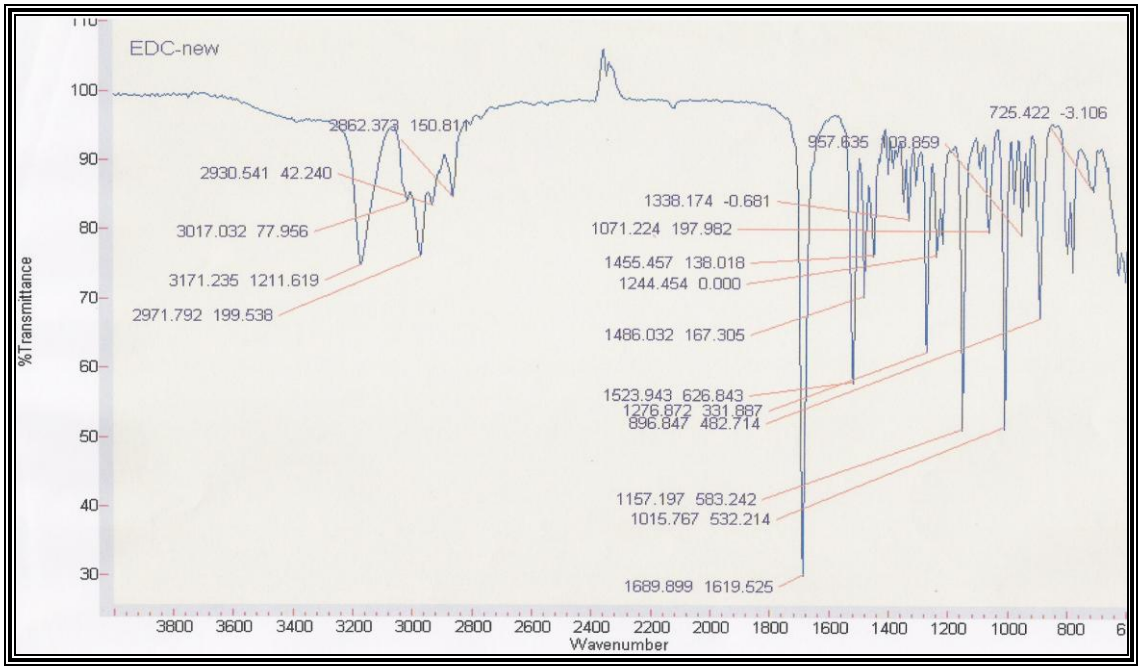


Figure A3.2: IR Spectrum of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) before sublimation

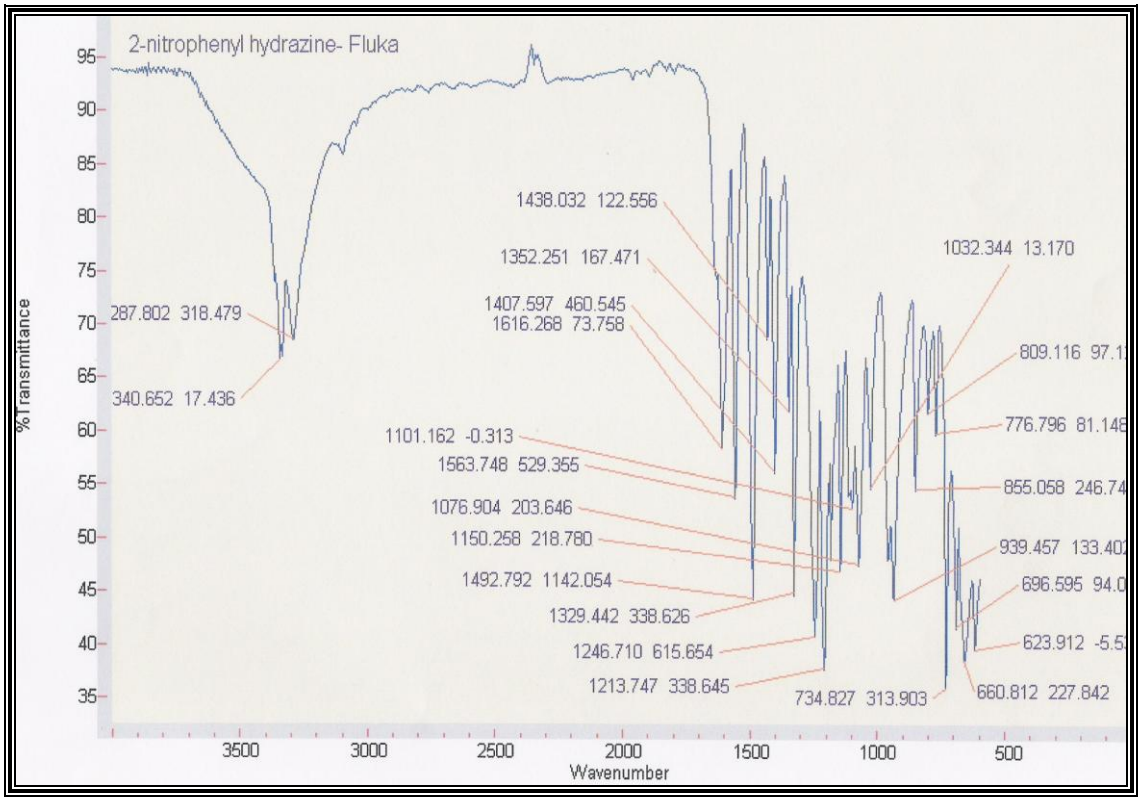


Figure A3.3: IR Spectrum for 2-nitrophenyl hydrazine (orange crystals)

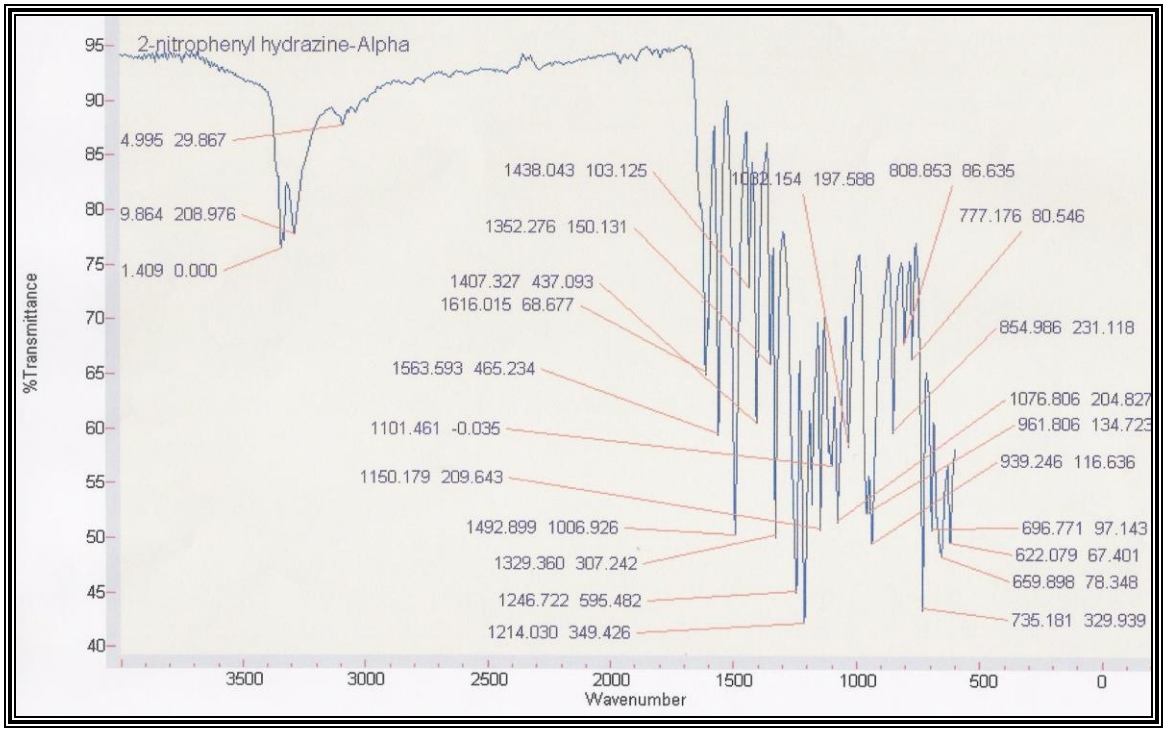


Figure A3.4: IR Spectrum of 2-nitrophenyl hydrazine (red crystals)

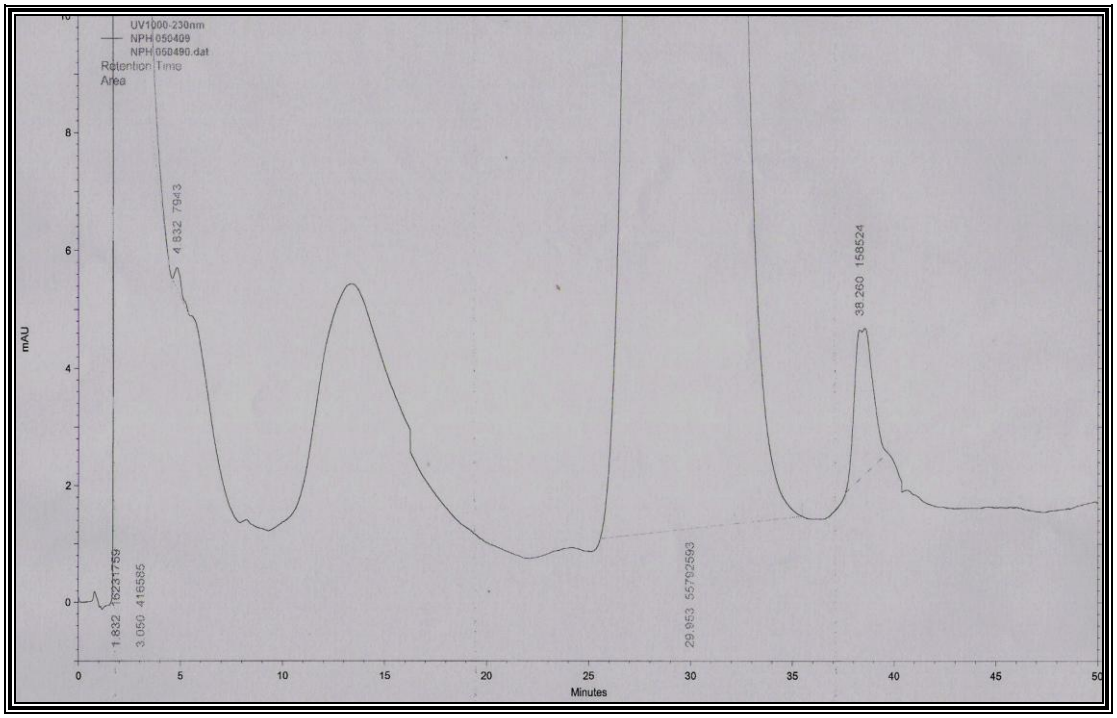


Figure A3.5 Chromatogram of 2-NPH(red crystals) showing more impurities

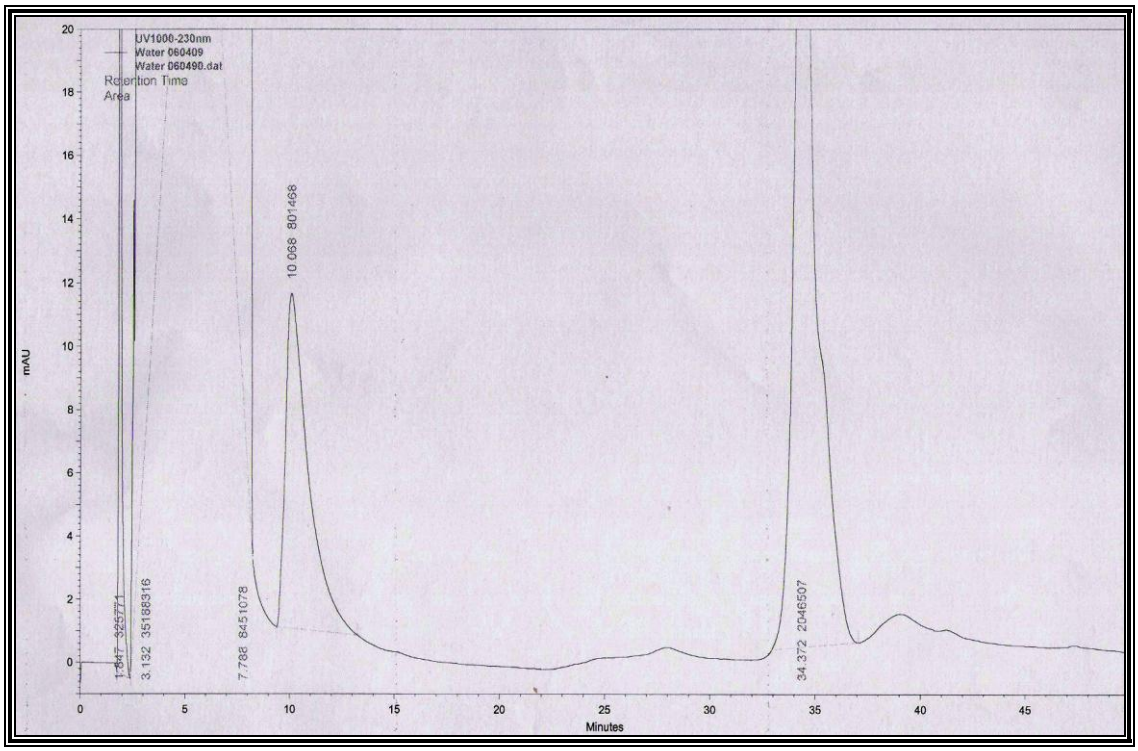


Figure A3-6: Chromatogram of blank with diluted NPH

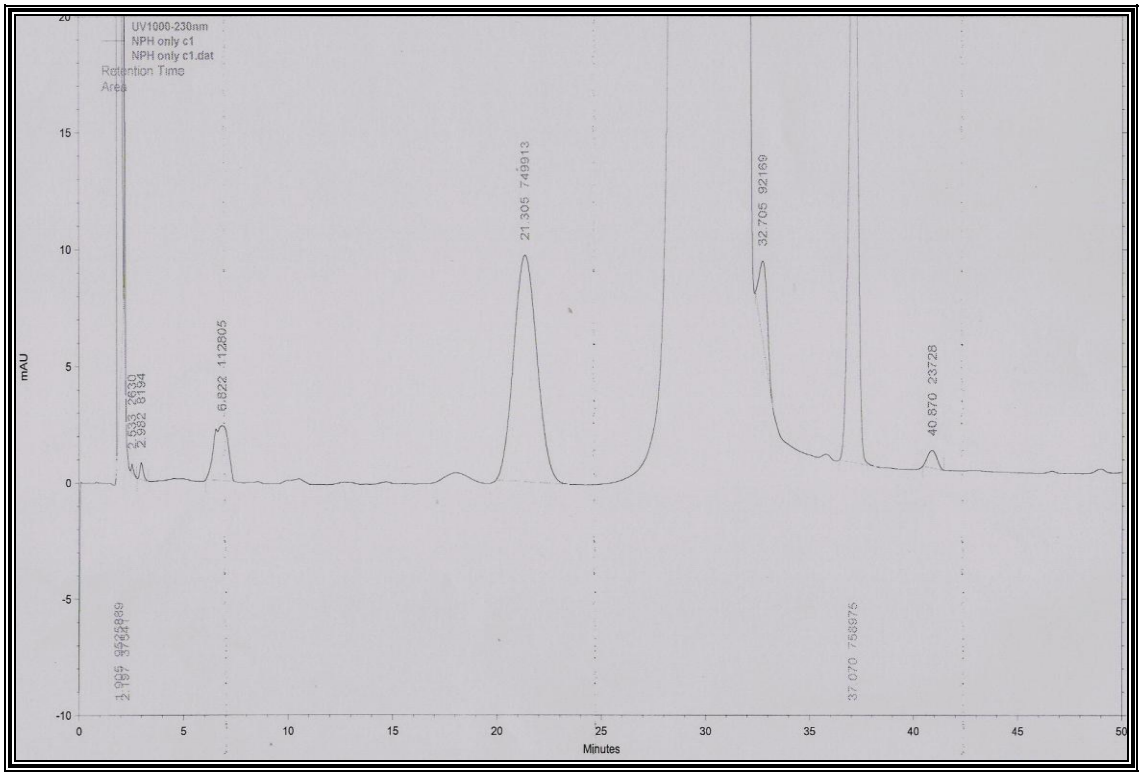


Figure A3-7: Chromatogram of NPH before re-crystallization

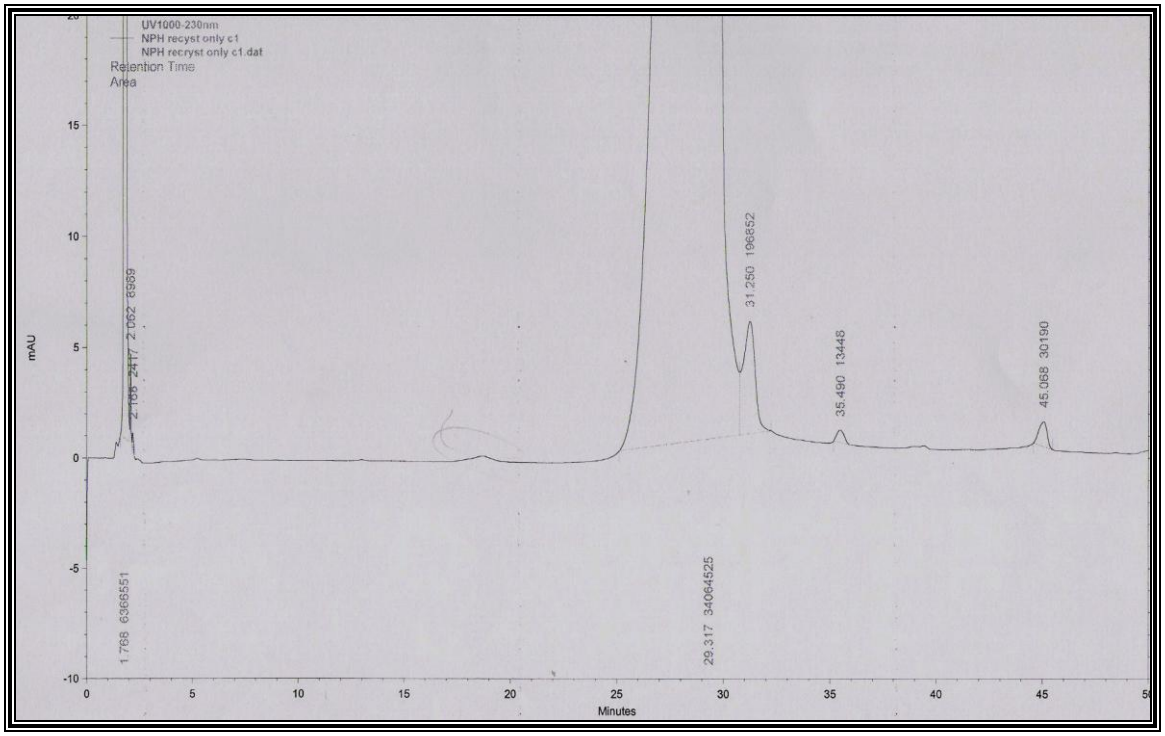


Figure A3-8 Chromatogram of 2-NPH after re-crystallization

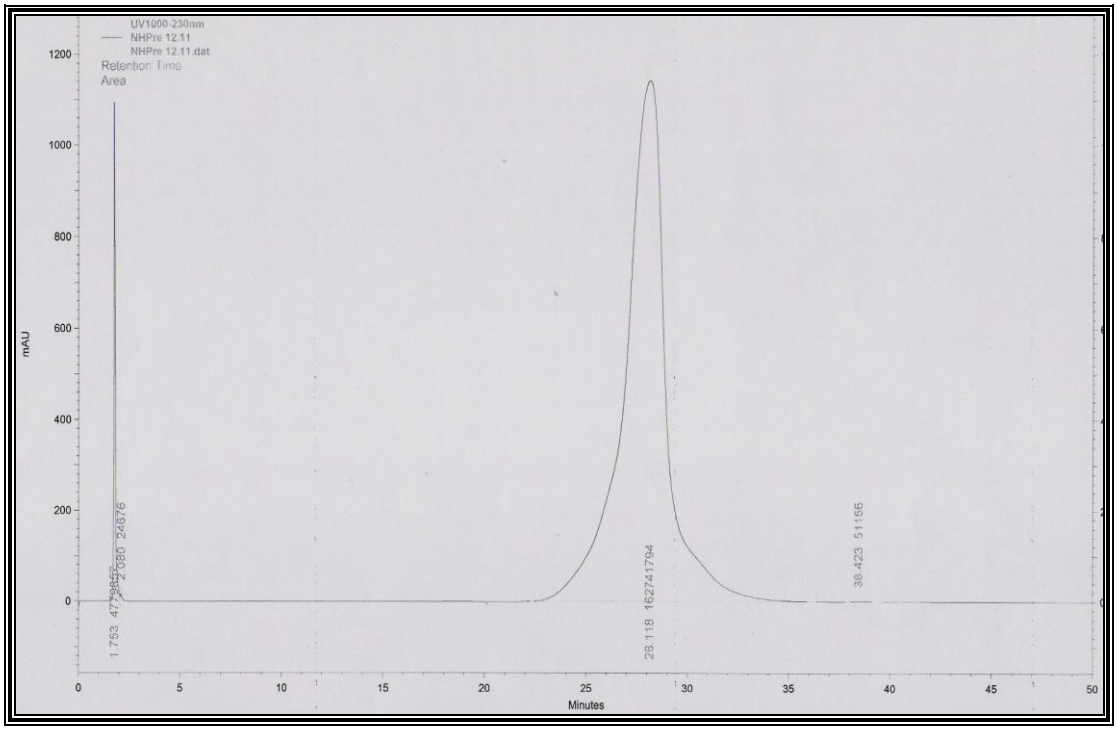


Figure A3.9: Chromatogram of 2-NPH after dilution

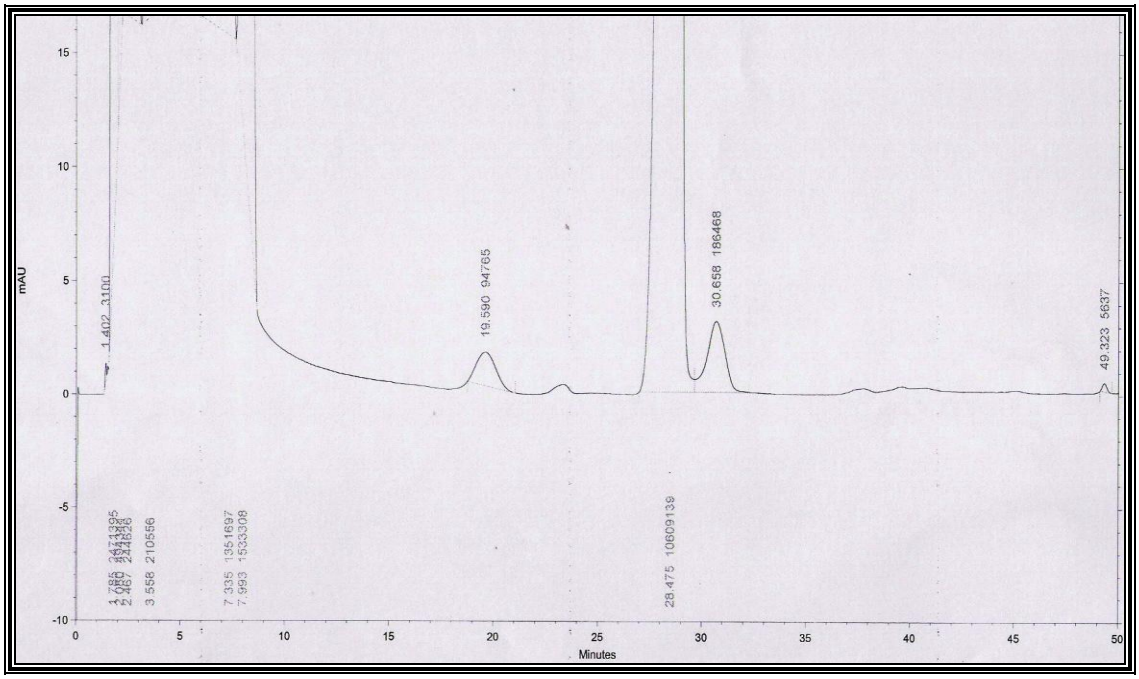


Figure A3.10: Chromatogram of blank using C18 column at 230nm, derivatization without KOH

Figure A3.11 Chromatogram: Acid mixture on C18 column 500ppm on C18 at 230nm, 1. formic acid, 2. acetic acid, 3. excess 2-NPH, 4. propionic, butyric acid

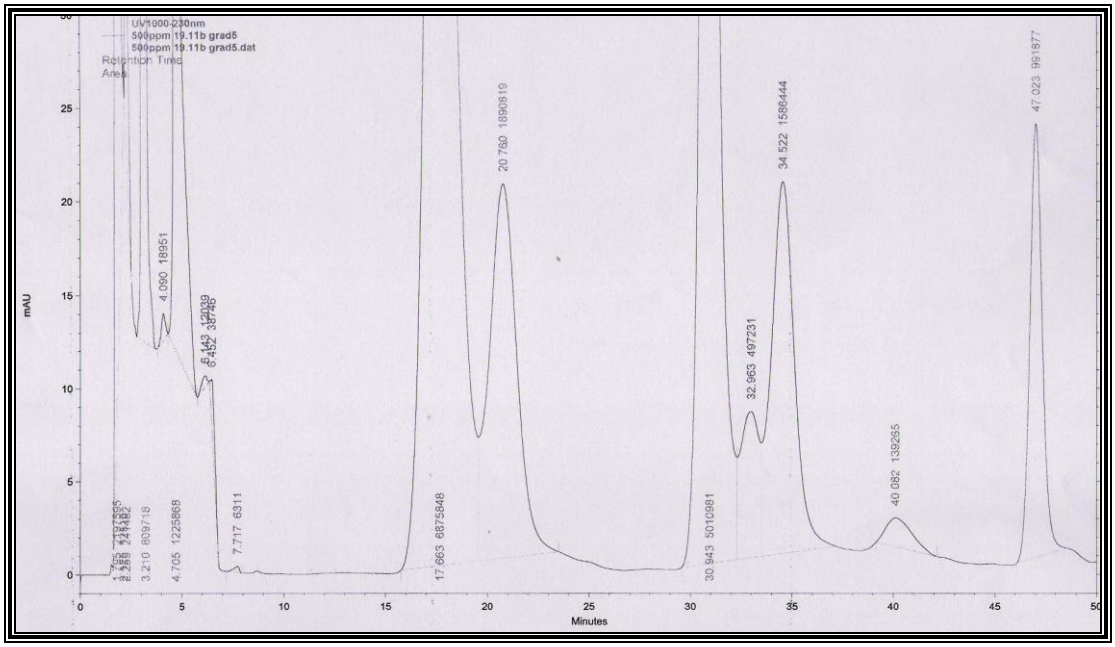


Figure A3.12 Chromatogram of 500ppm with gradient to separate propionic acid and impurity peak

Figure A3.13 Chromatogram of 50ppm acid mixture on C18, 230nm, 1. impurities, 2. formic, 3. acetic, 4. excess NPH, 5. impurity, 6. propionic 7. butyric

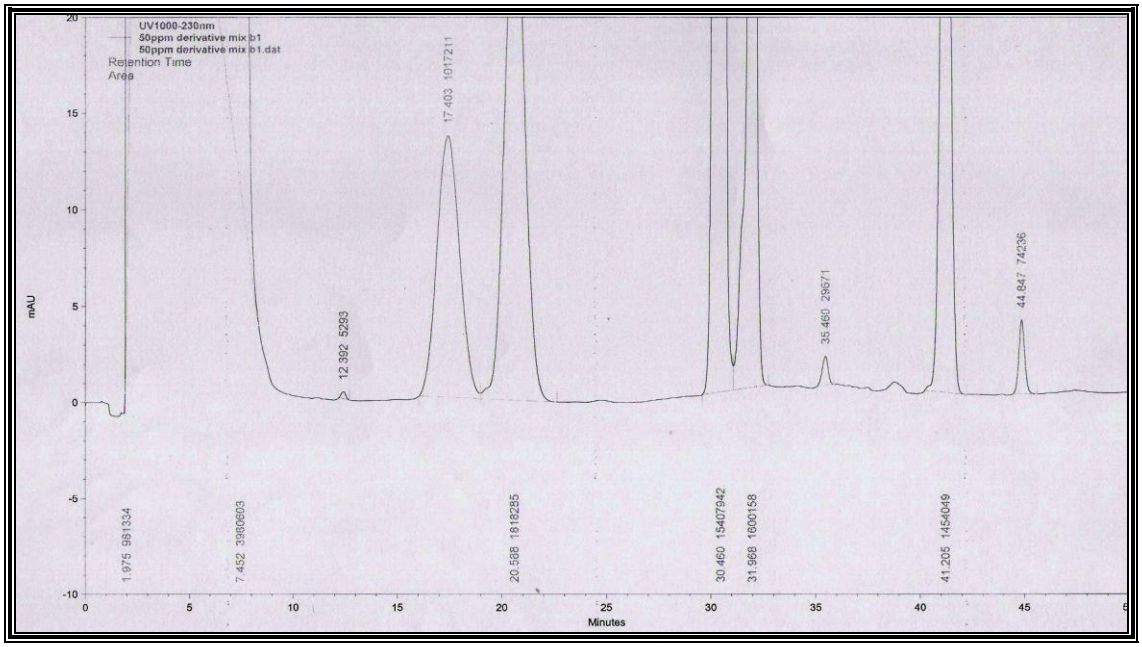


Figure A3.14 Chromatogram of 50ppm acid mixture, improvement of runtime. C18, 1. formic acid, 2. acetic, 3 NPH, propionic, butyric

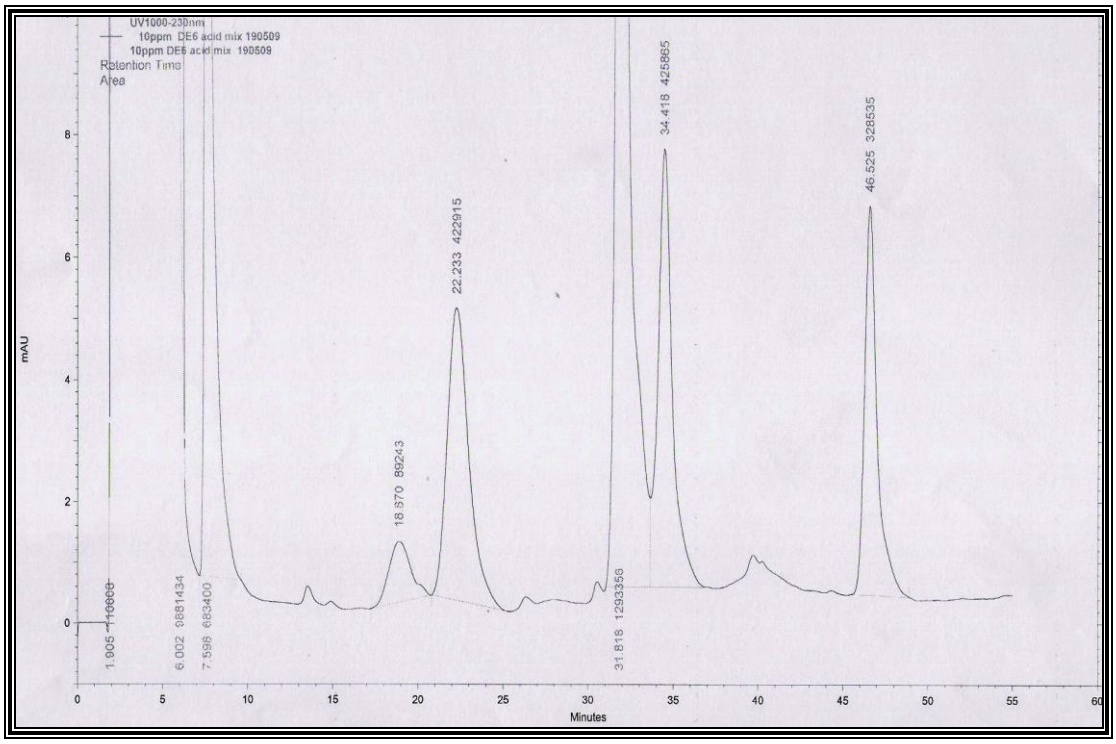


Figure A3.15 Chromatogram of acid mixture 10ppm mixture using higher purity NPH. Masking propionic acid

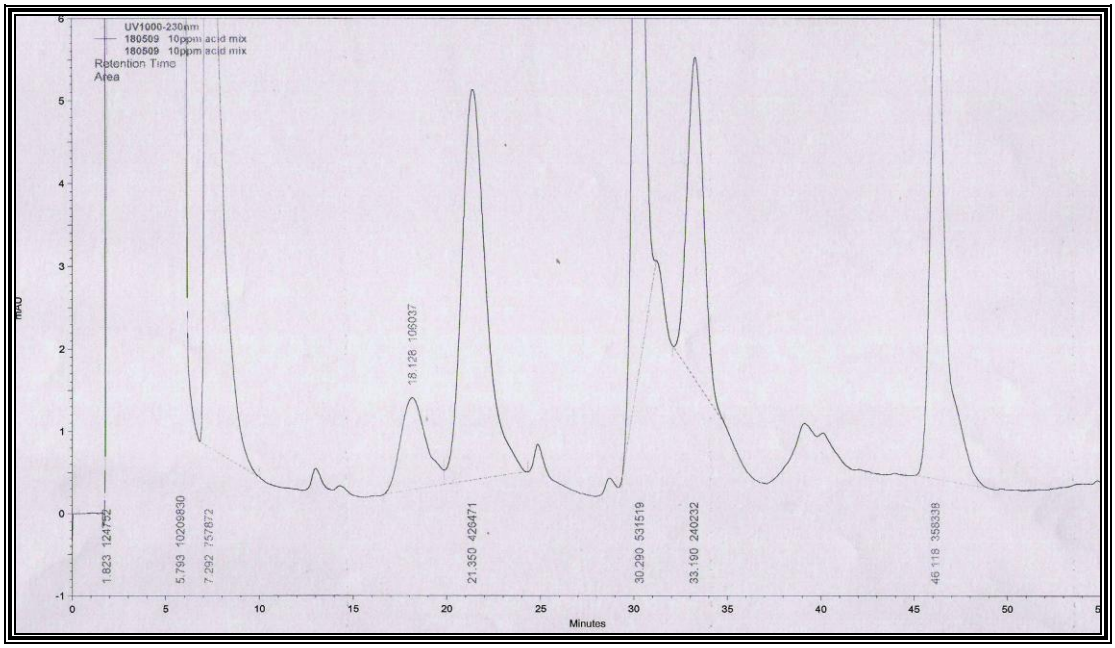


Figure A3.16 Chromatogram of 10ppm acid mixture showing difficulty With separation of 2-NPH peak and propionic acid

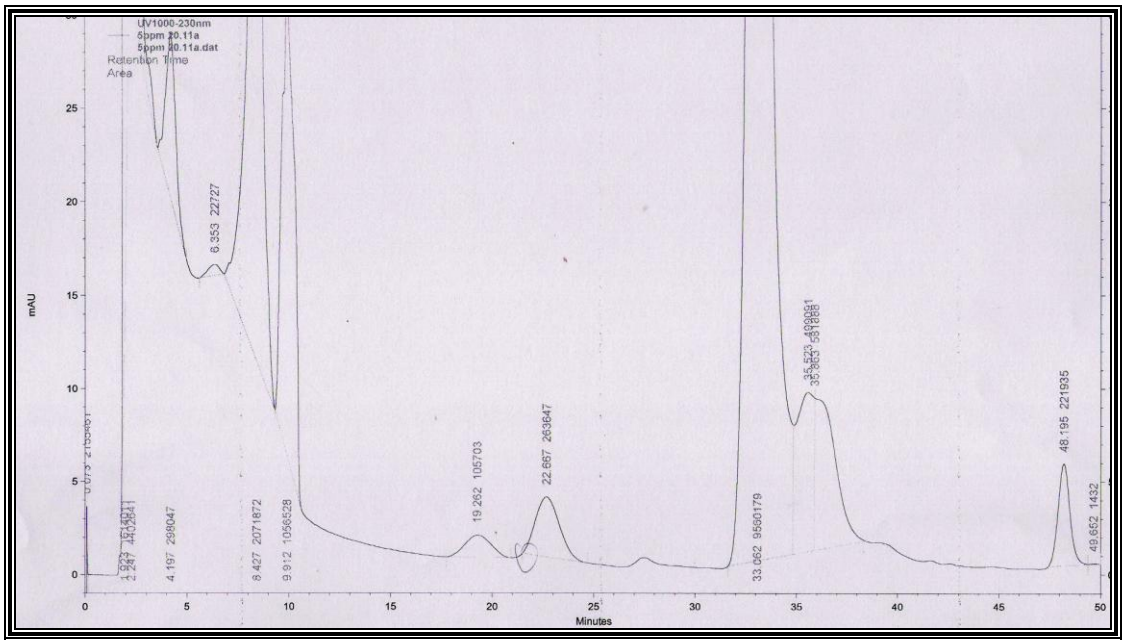


Figure A3.17: Chromatogram of 5ppm acid mixture 230nm, C18 column

1. formic acid, 2. acetic acid, 3. excess 2-NPH, 4. propionic

5. butyric acid

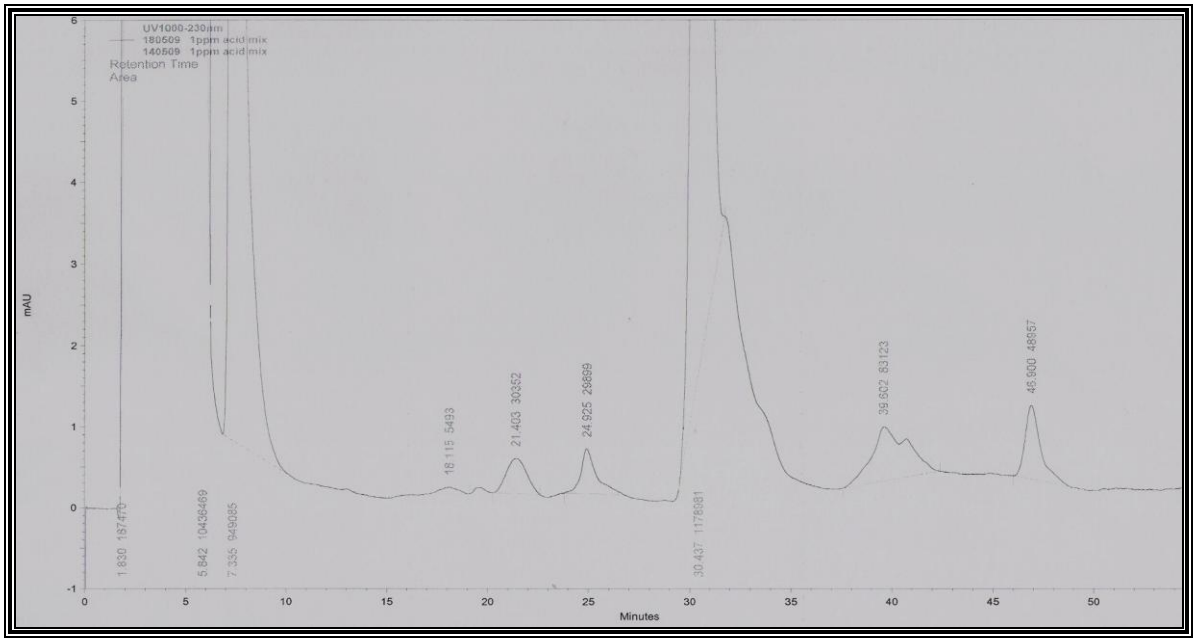


Figure A3.18: Chromatogram of 1ppm acid mixture C18 column, 230nm

1. formic acid, 2. acetic acid, 3. excess 2-NPH, 4. impurity,

5. butyric acid

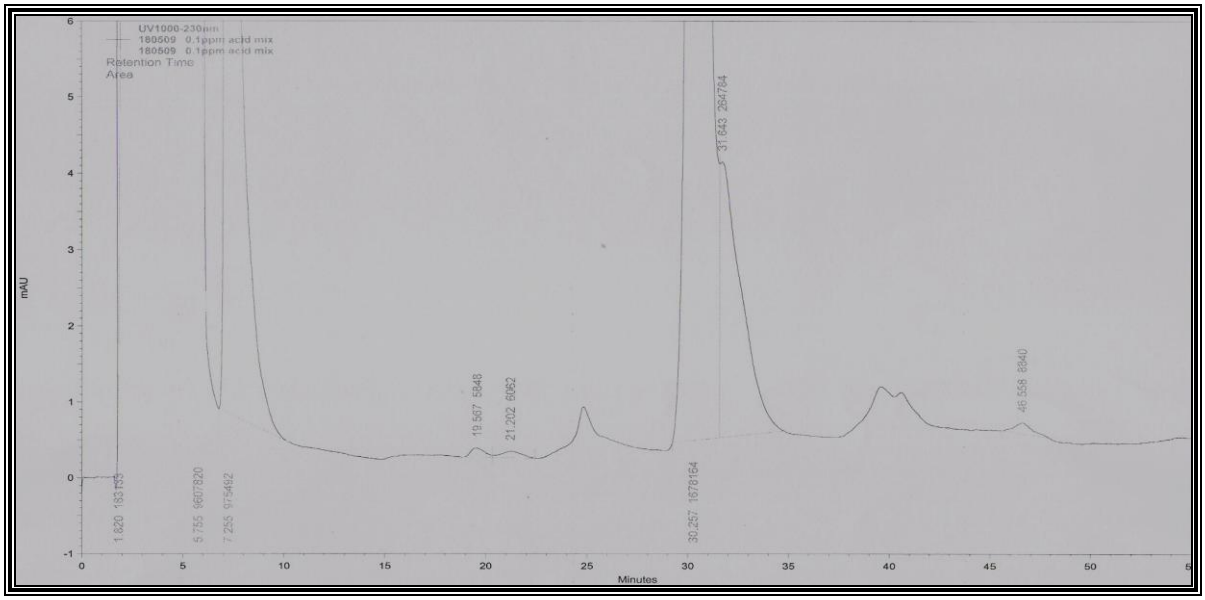


Figure A3-19: Chromatogram of 100ppb of acid mixture, C18, 230nm 1.formic acid,

2. acetic acid, 3. excess NPH, 4. impurity 5. butyric acid

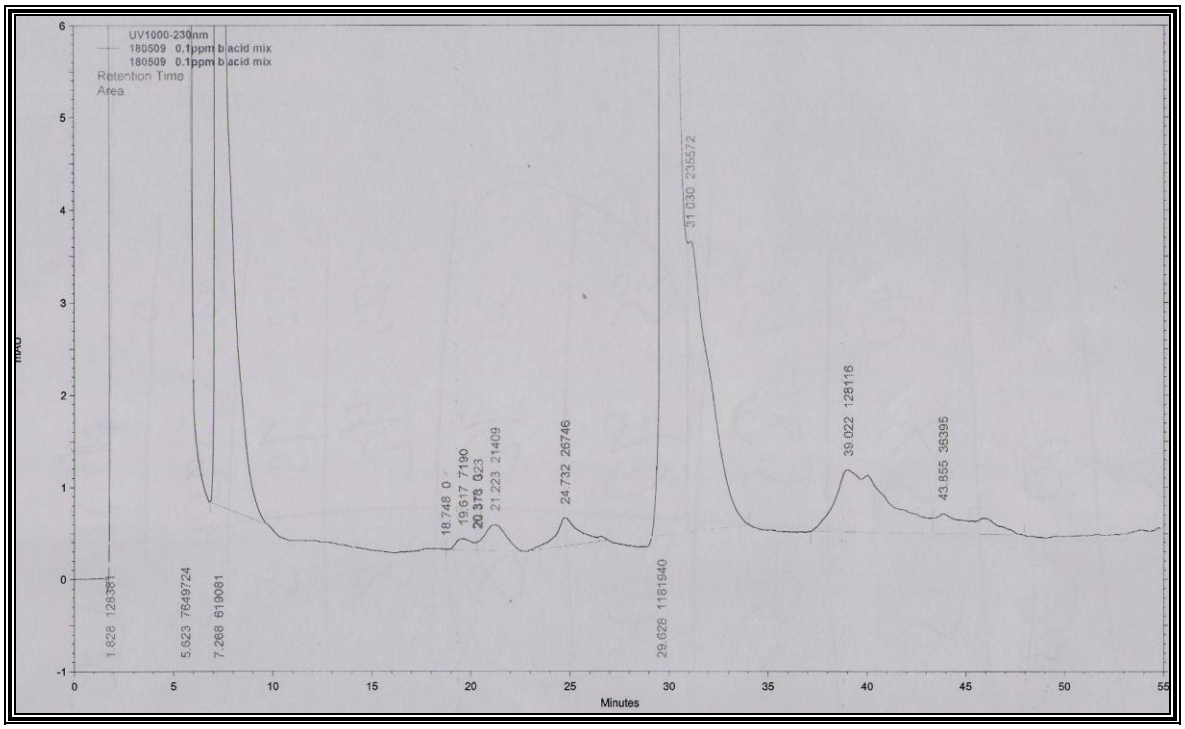


Figure A3.20: Chromatogram of acid mixture 0.1ppm repeated

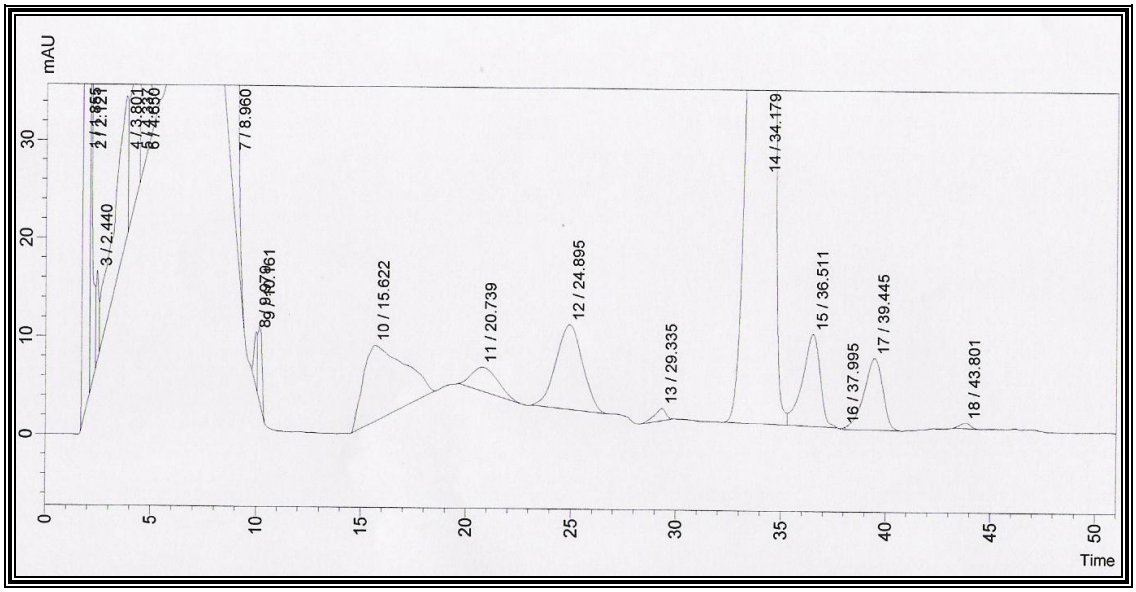


Figure A3.21: Chromatogram of 5ppm acid mixture on PDA, 230nm, for UV spectrum of reagents and derivatives

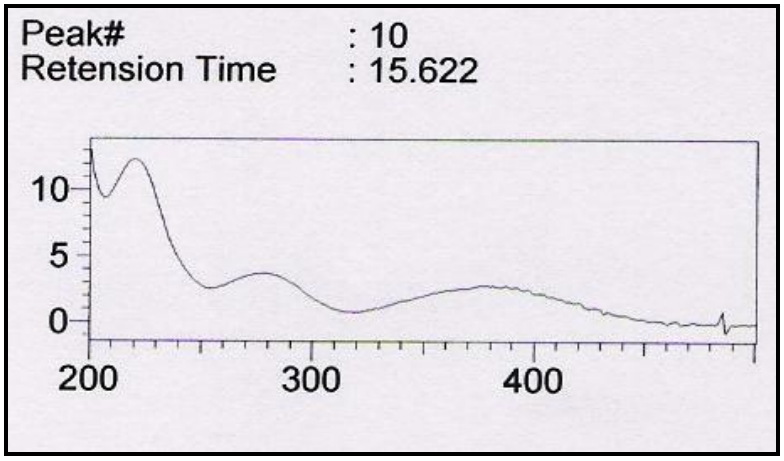


Figure A3.22: UV spectrum of impurity from chromatogram A3.21

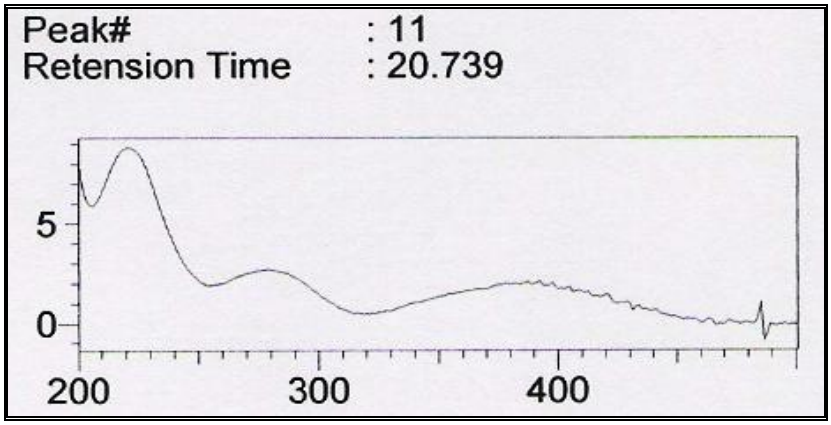


Figure A3.23: UV spectrum of formic acid from chromatogram A3.21

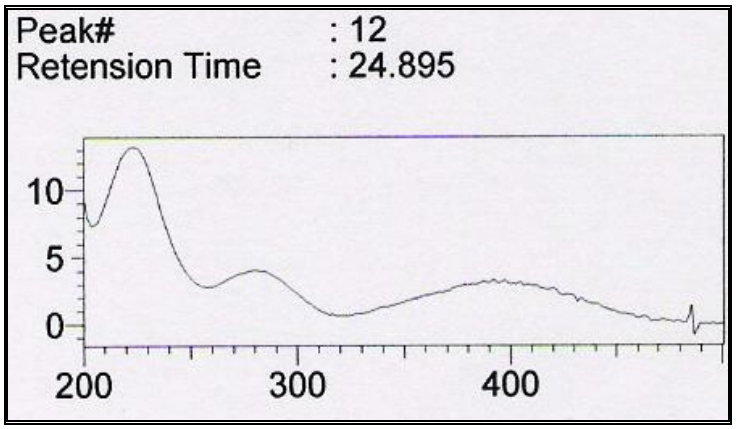


Figure A3.24: UV spectrum for Acetic acid from chromatogram A3.21

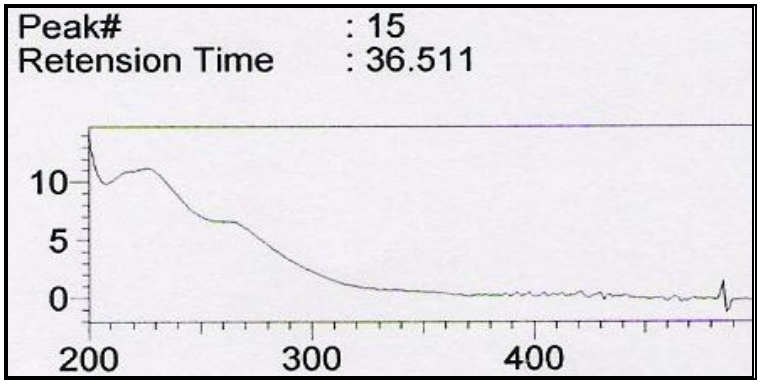


Figure A3.25: UV spectrum of impurity peak 15 on chromatogram A3.2

Appendix 4 - C 8 Columns

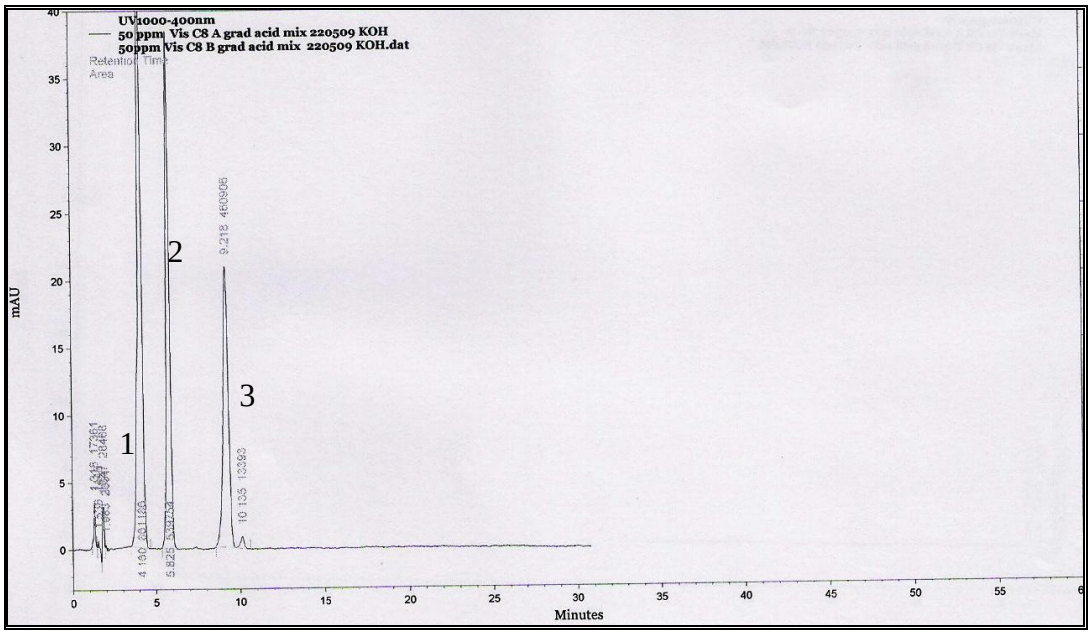


Figure A4.1 Chromatogram of 50ppm acid standard, with gradient using C8 with KOH, 400 nm,

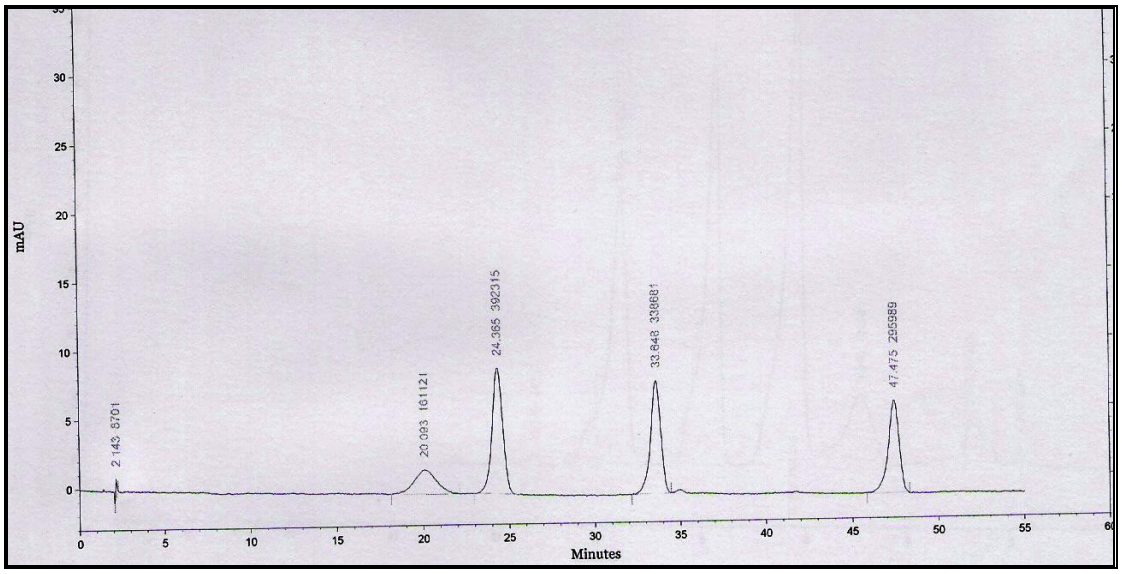


Figure A4.2 Chromatogram of acid mixture with KOH on a C8 column at 400nm

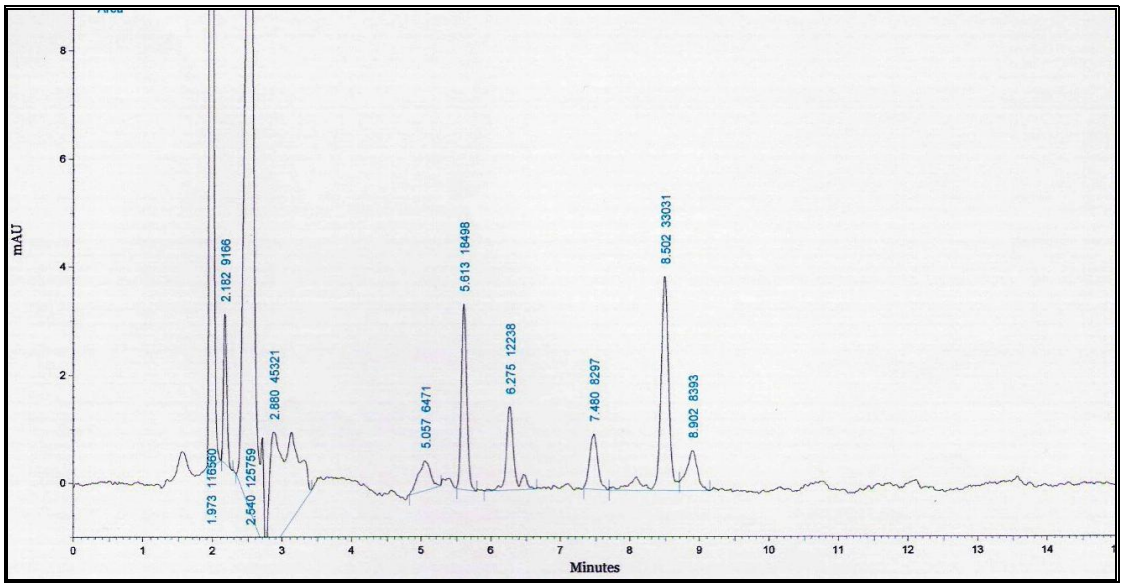
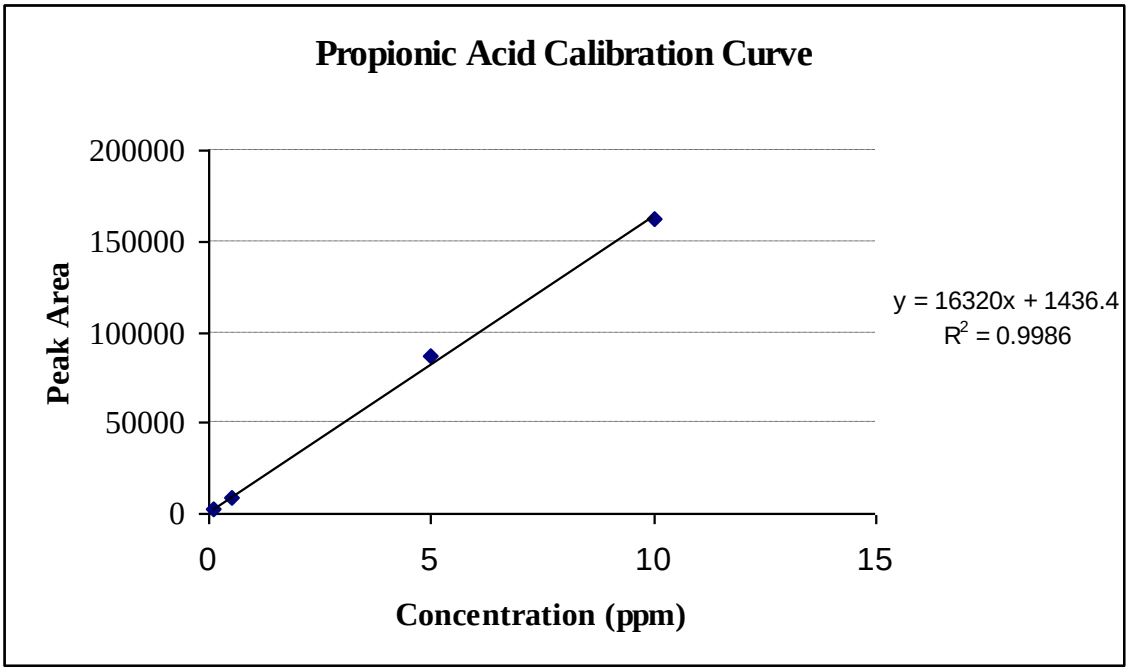


Figure A4.3 Chromatogram of 500ppb using C 8 Nucleodur column, 400nm, 2 step gradient, Flow rate 1.2ml/min



A4.4 Calibration Curve for Propionic acid using C 8 Nucleodur, 400nm

Propionic Acid	Concentration	Retention Time	Peak Area
	0.1	6.15	1956
	0.5	6.25	8572
	5	6.26	87183
	10	6.36	162621

Table A4.1 Table o concentrations, retention time and peak areas for Propionic acid

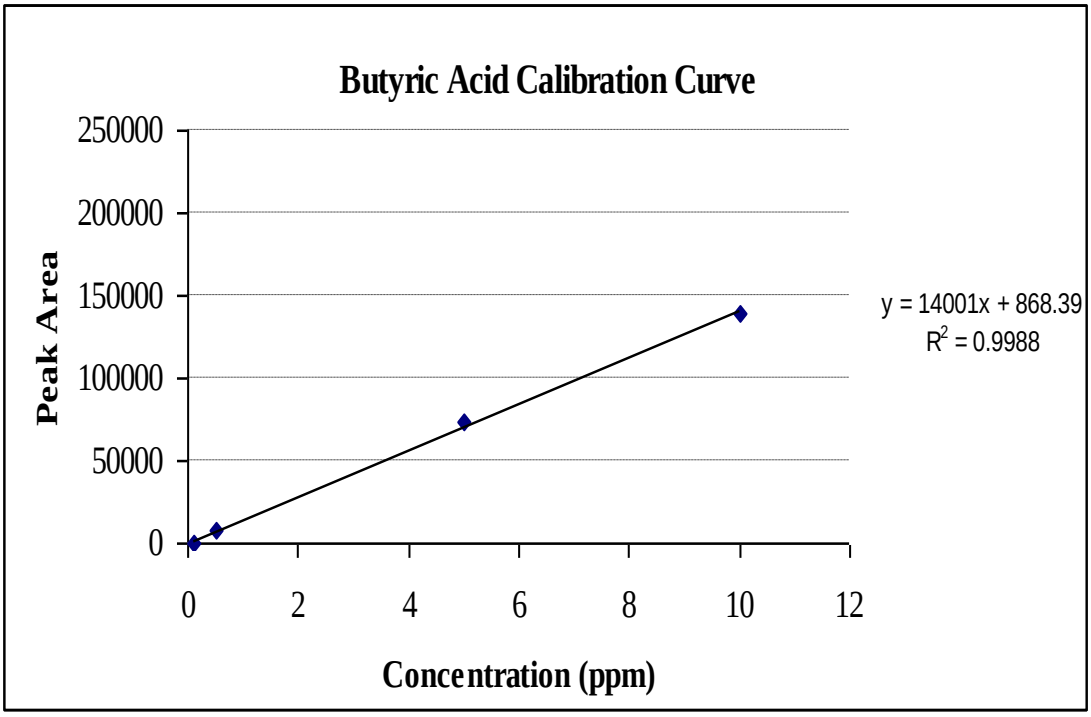


Figure A4.5 Calibration curve for butyric acid using C8 Nucleodur column, 400nm, 1.2ml/min

Butyric Acid	Concentration	Retention Time	Peak Areas
	0.1	7.27	690
	0.5	7.37	7858
	5	7.39	74021
	10	7.50	139324

Table 4.2 Table of concentration, retention time and peak areas for butyric acid

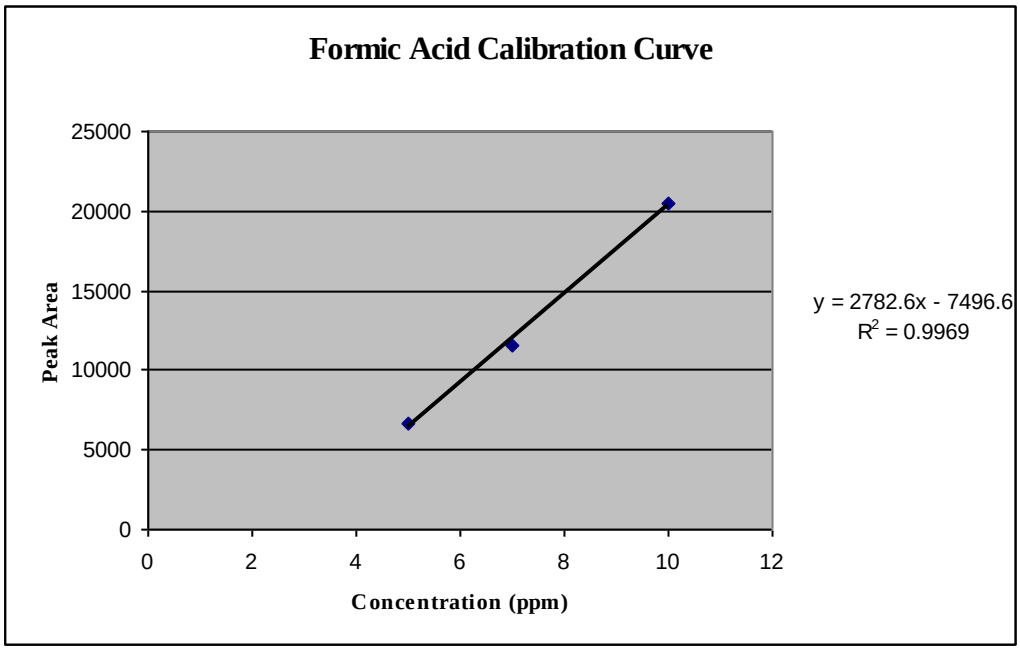


Figure A4.6 Calibration curve for Formic acid using Nucleodur C8, 400nm, Flow 1.2

Formic Acid	Concentration	Retention Time	Peak Areas
	5	4.35	6687
	7	4.40	11531
	10	4.44	20510

Table 4.3 table of concentrations, retention value and peak areas for Formic acid

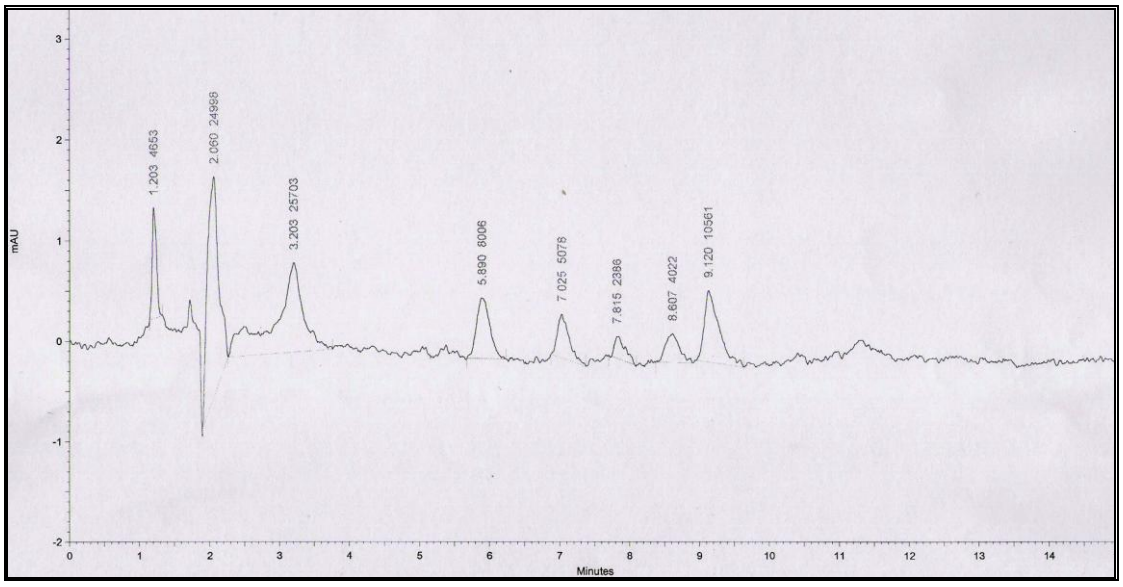


Figure A4.7 500ppb acid mixture on C 8 column used for LOD and LOQ calculations

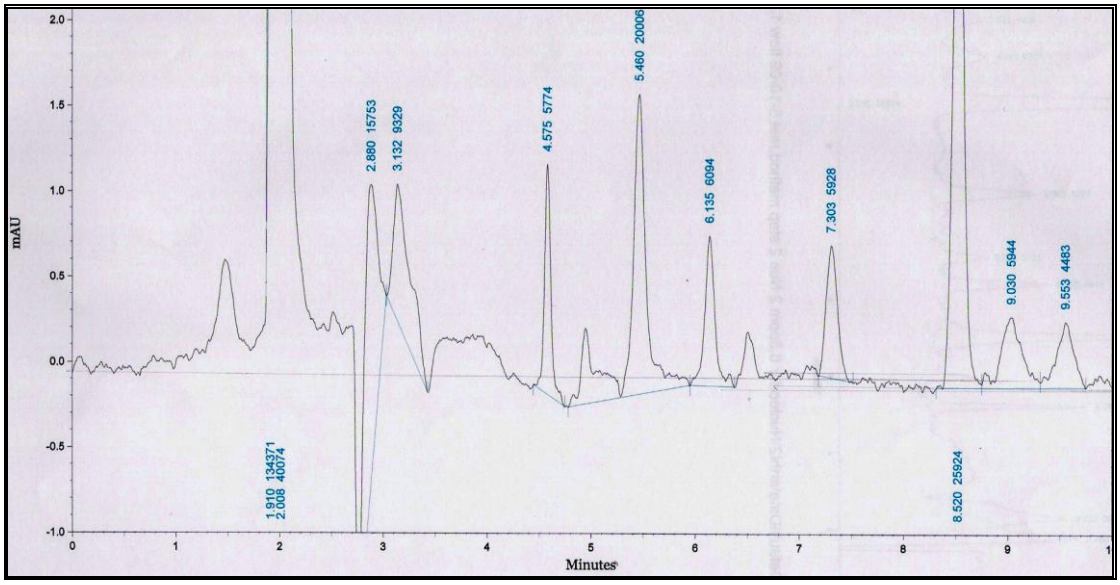


Figure A4.8 Chromatogram of 300ppb acid mixture used in the calculation of LOD and LOQ from method using Nucleodur C 8 column.

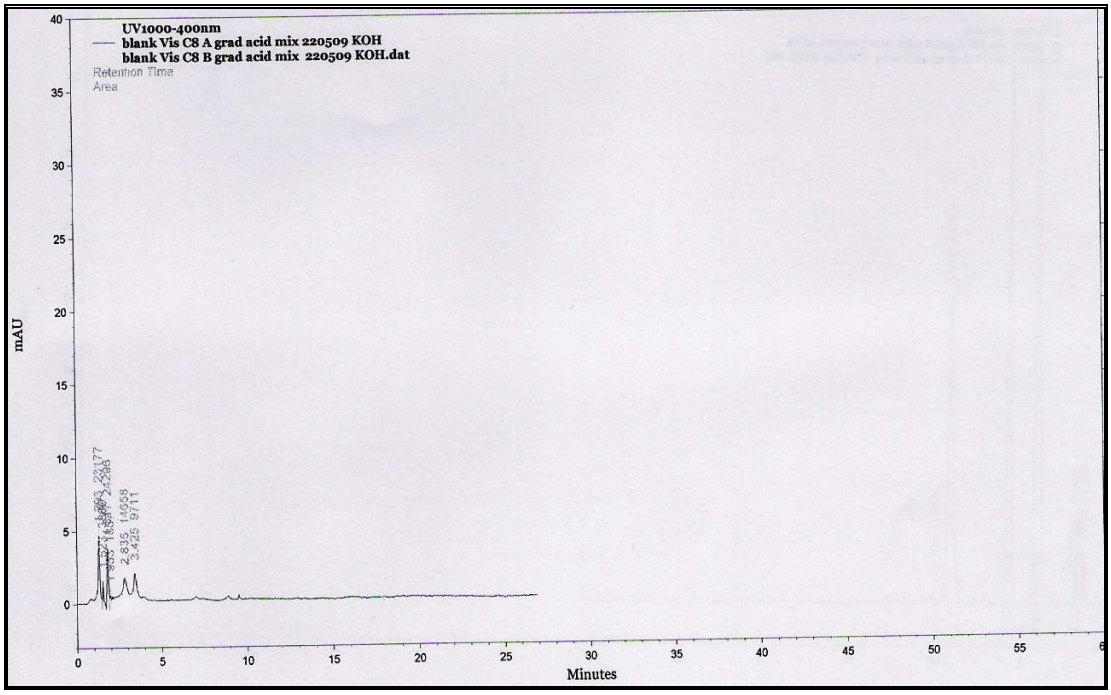


Figure A4.9 Chromatogram of blank on C 8 column at 400nm

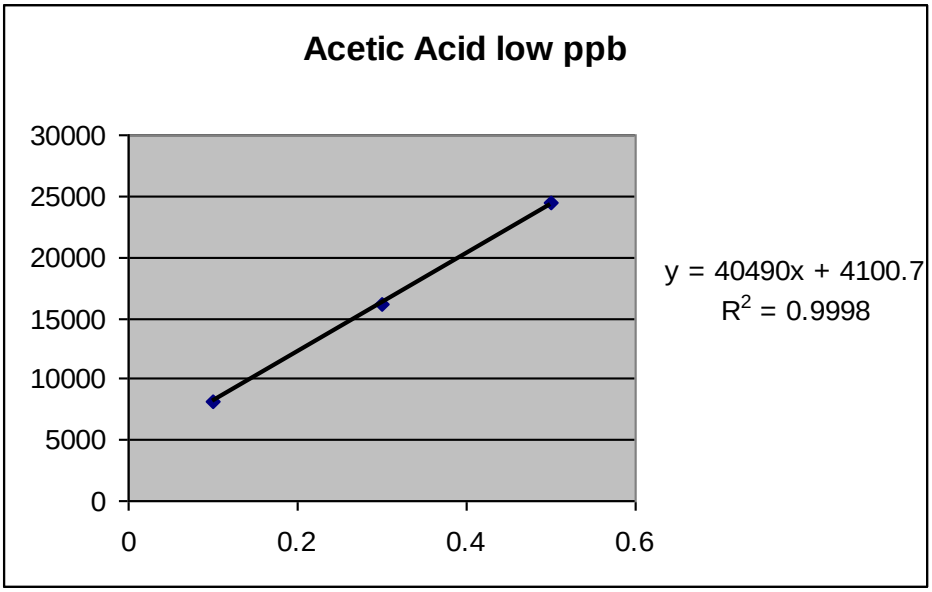


Figure A4.10 Calibration of low concentration level acids (acetic acid)

Concentration	Peak areas		
0.1ppm	8214		
0.3ppm	14513		
0.5ppm	24410		
40490	Slope		
3565.333	intercept		
1468.877	STEYX		
3	count		
0.08	DEVSQ		
15712.33	Average		
2	No of samples		
0.033239	Std dev in C	for acetic acid	

Table A4.4 Concentration of acetic acid used fro calibration and calculation of statistical data

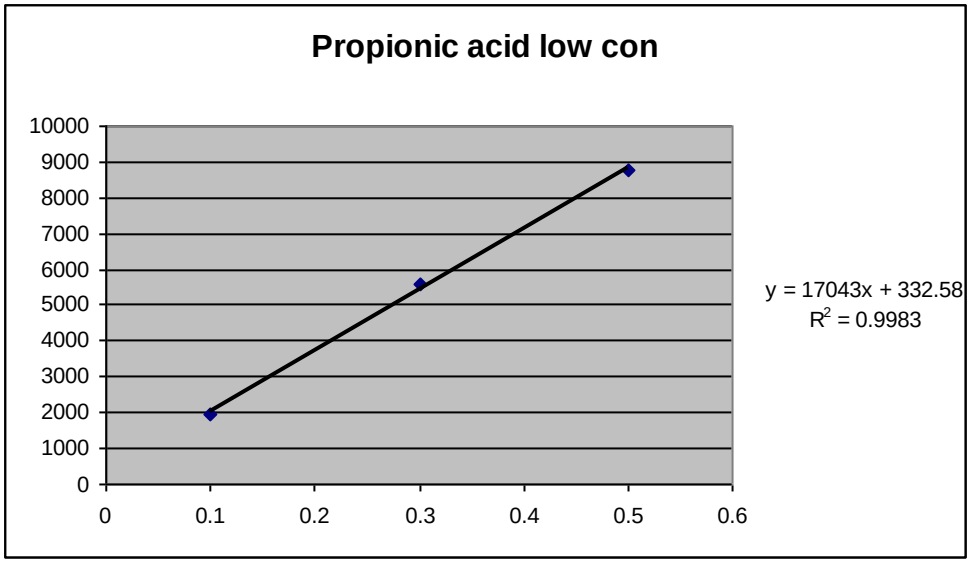


Figure A4.11 Calibration of low concentration level acids (propionic acid)

Concentration	Peak areas		
0.1ppm	1956		
0.3ppm	5607		
0.5ppm	8773		
14326.26	Slope		
1002.589	intercept		
663.805	STEYX		
4	count		
0.2675	DEVSQ		
7091.25	Average		
2	No of samples		
0.040523	Std dev in C	for propionic acid	

Table A4.5 Concentration of propionic acid used for calibration and calculation of statistical data

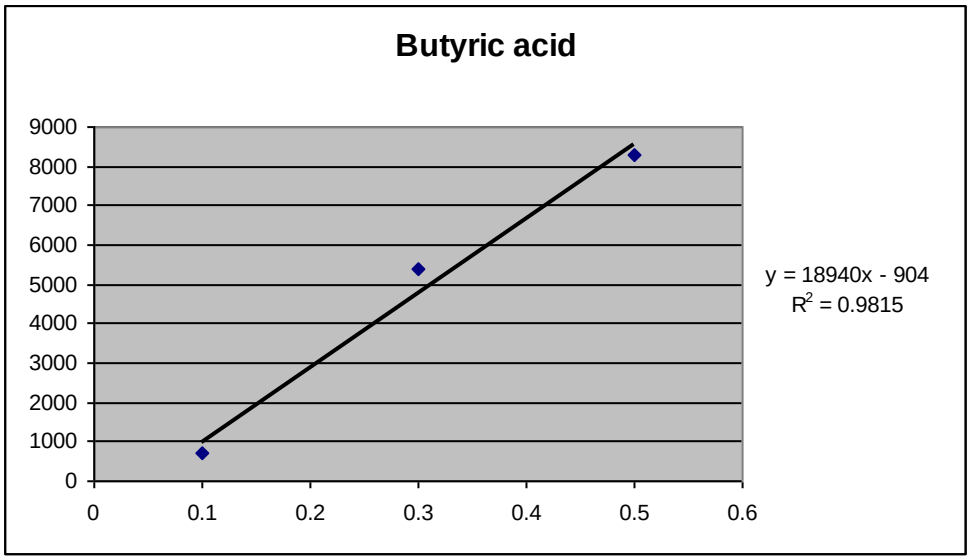


Figure A4.12 Calibration of low concentration level acids (butyric acid)

Concentration	Peak areas		
0.1ppm	690		
0.3ppm	5378		
0.5ppm	8266		
18940	Slope		
-904	intercept		
734.8469	STEYX		
3	count		
0.08	DEVSQ		
4778	Average		
2	No of samples		
0.049517	Std dev in C	For butyric acid	

Table A4.6 Concentration of butyric acid used fro calibration and calculation of statistical data

Appendix 5 – Pre-concentration

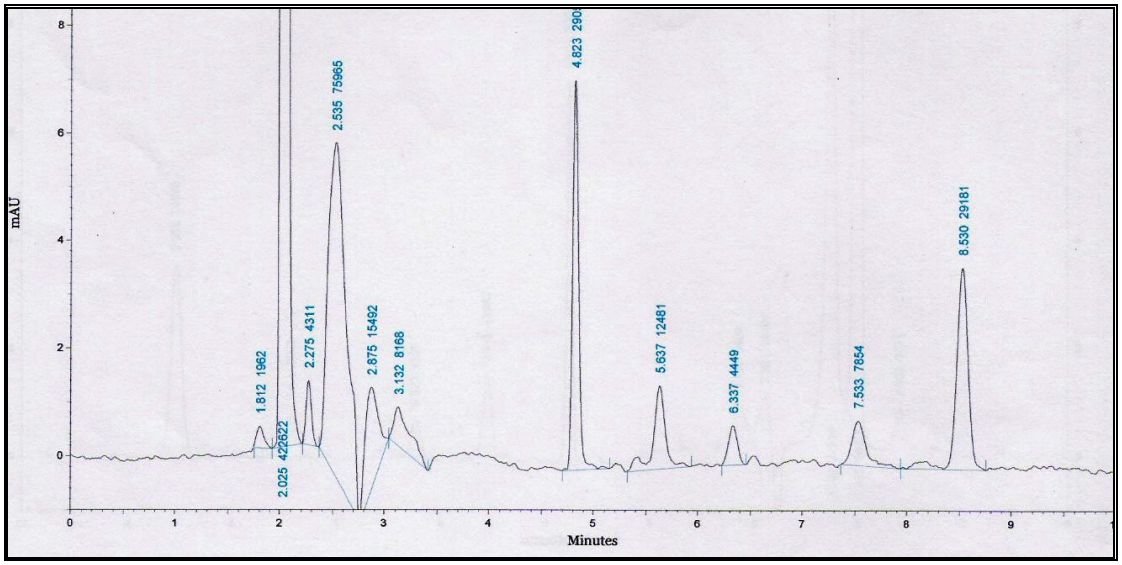


Figure A5.1 Chromatogram of pre-concentration of 50ppb on Strata X-AW, elution with pH6 Buffer repeated

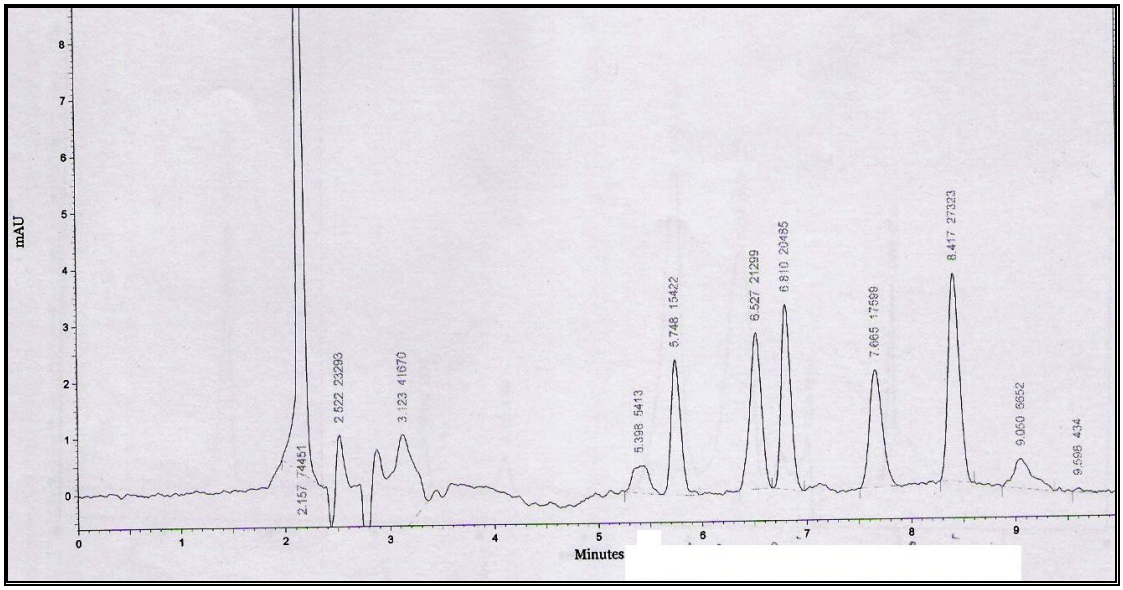


Figure A5.2 Chromatogram of pre-concentration of 500ppb using NH_4OH as eluent on Strata X-AW,

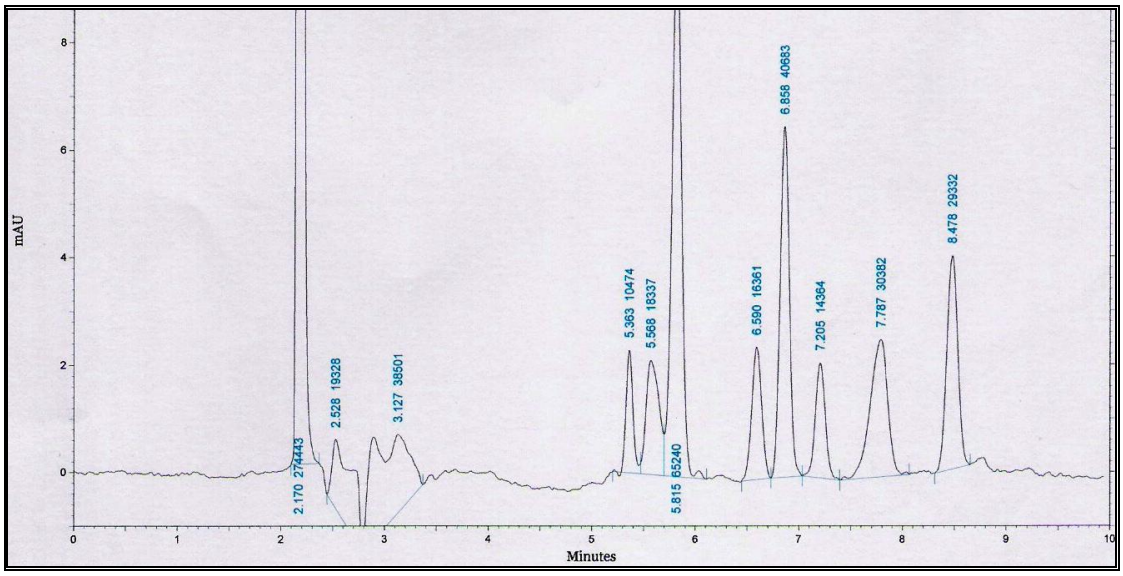


Figure A5.3 Chromatogram of 500ppb pre-concentration using Strata X-AW, Elution with NH_4OH, solution pH 1,19

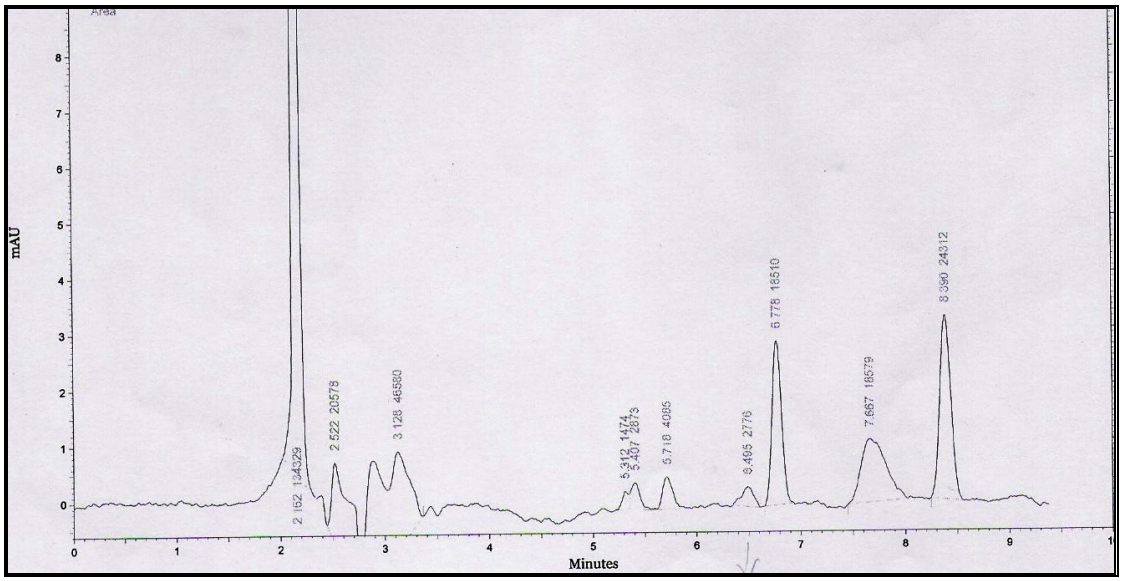


Figure A5.4 Chromatogram of 500ppm extraction with a further 5mL of eluent

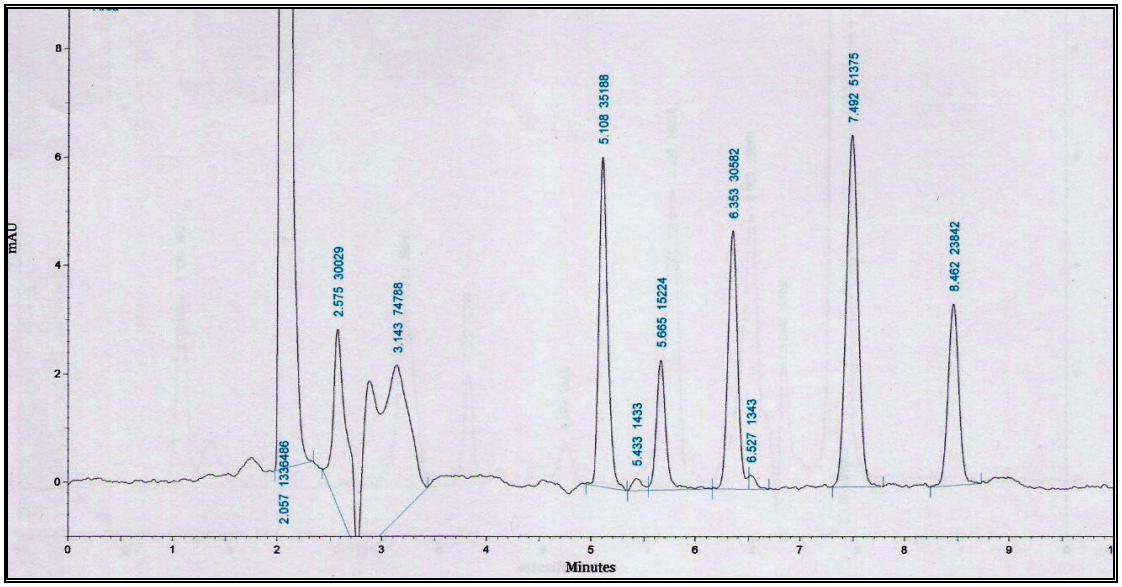


Figure 5.5 Chromatogram of pre-concentration of 500ppb acid mixture spiked on Main steam sample on Strata X-AW using pH6 buffer

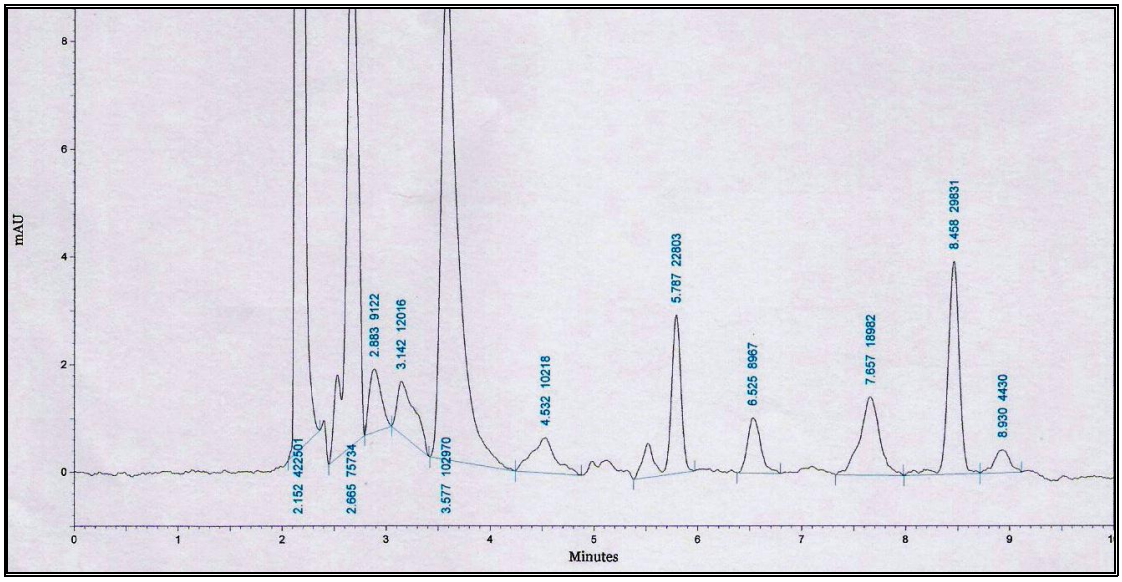


Figure 5.6 Chromatogram of SPE trial L using Strata X-AW of 50ppb acid mixture using 1% HCl as eluent

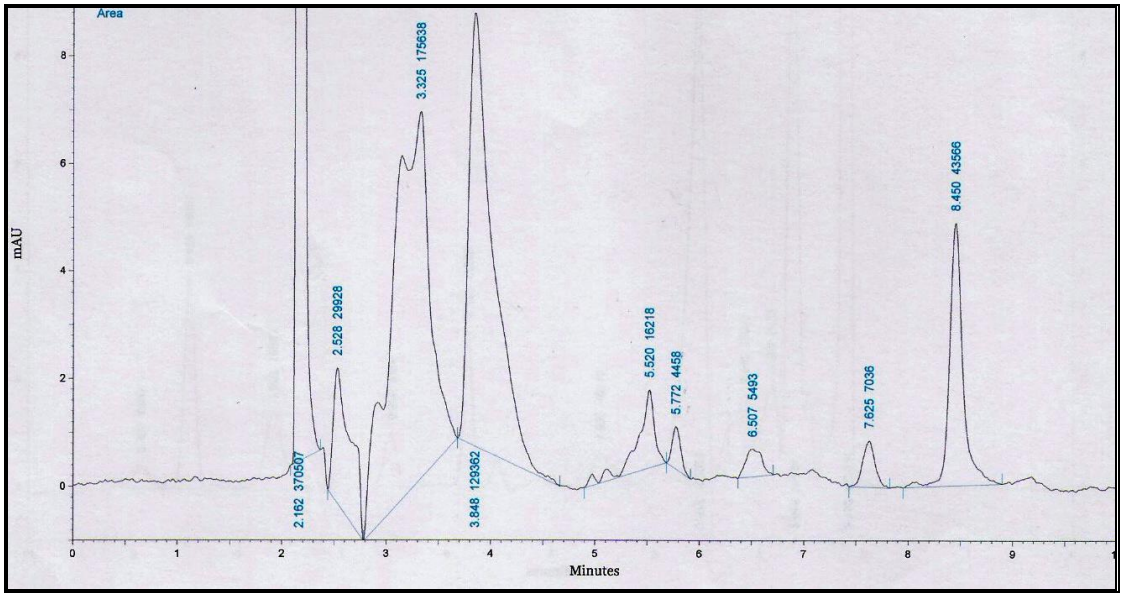


Figure 5.7 Chromatogram using Strata X-AW, pre-concentration of 50ppb acid standard, elution with 1% HCl solution, trial L

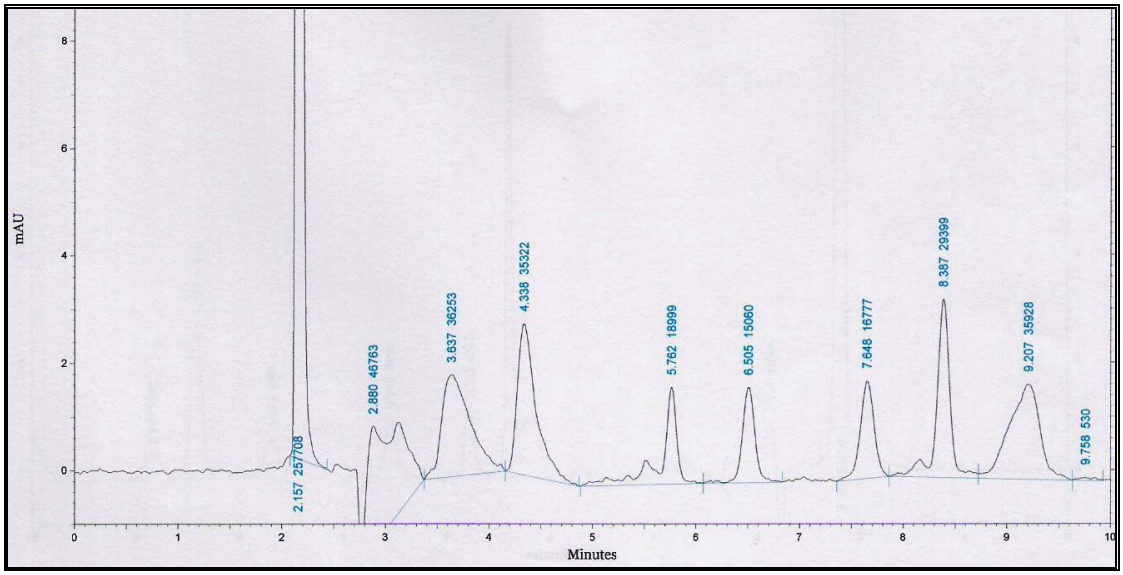


Figure A5.8 Chromatogram of pre-concentration of 1ppm spiked on main steam sample, elution with pH 12 buffer

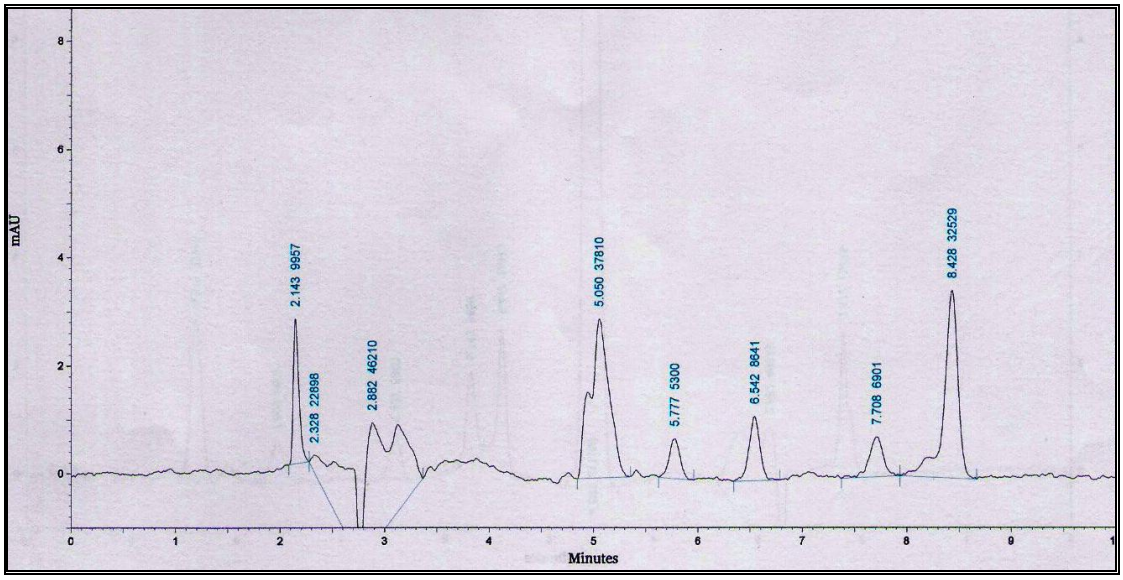


Figure A5.9 Chromatogram of 50ppb spiked in main stream sample, Strata X-AW, Elution with pH 8 buffer

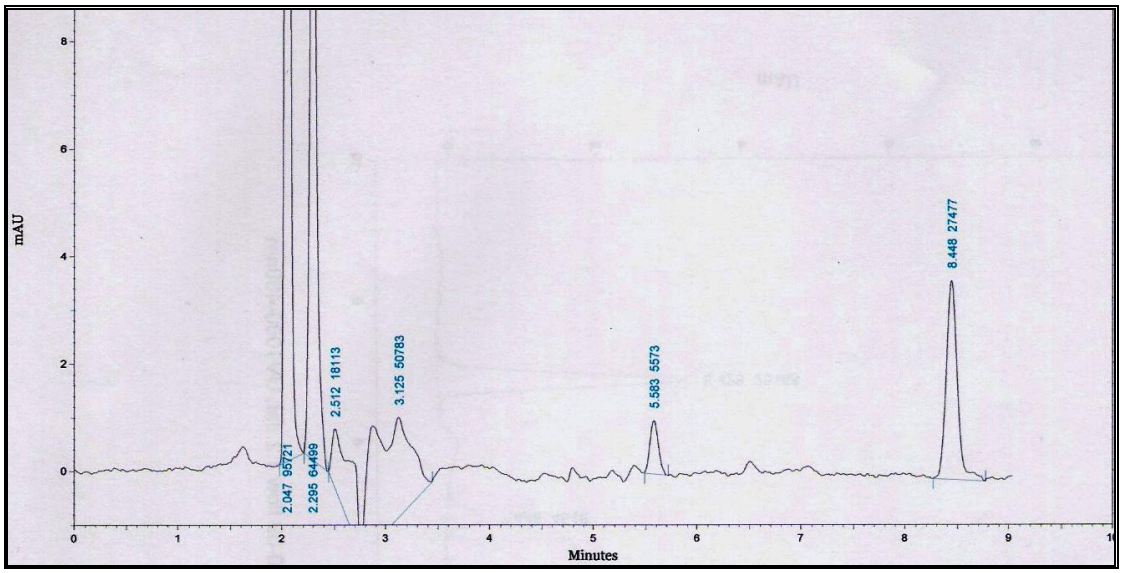


Figure A5.10 Chromatogram of blank using Strata X-AW, elution with pH 6 buffer

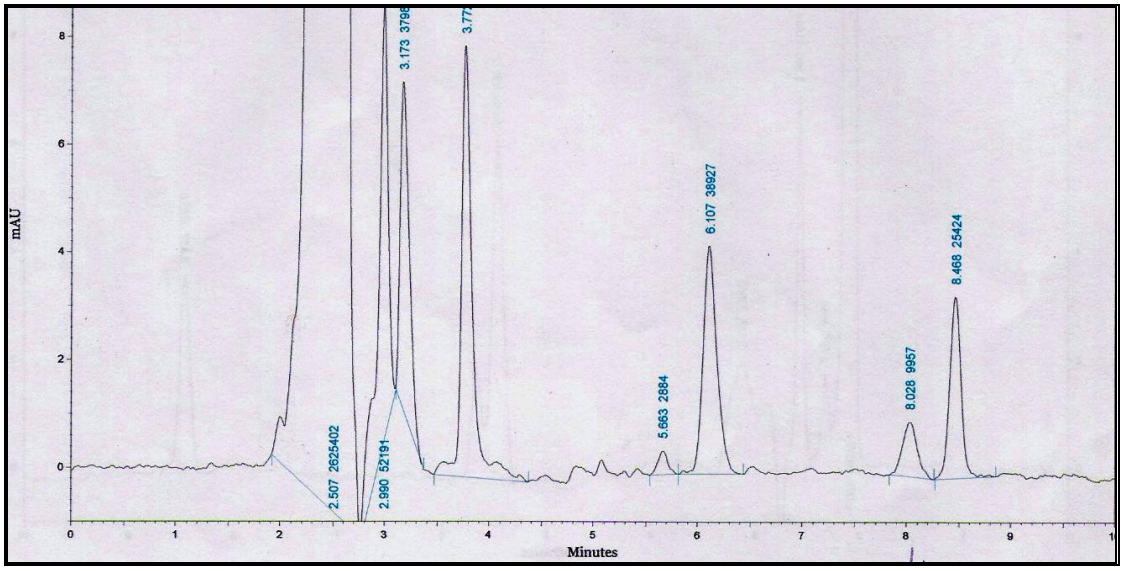


Figure A5.11 Chromatogram of Trial B using Strata X-AW, elution with pH6 buffer

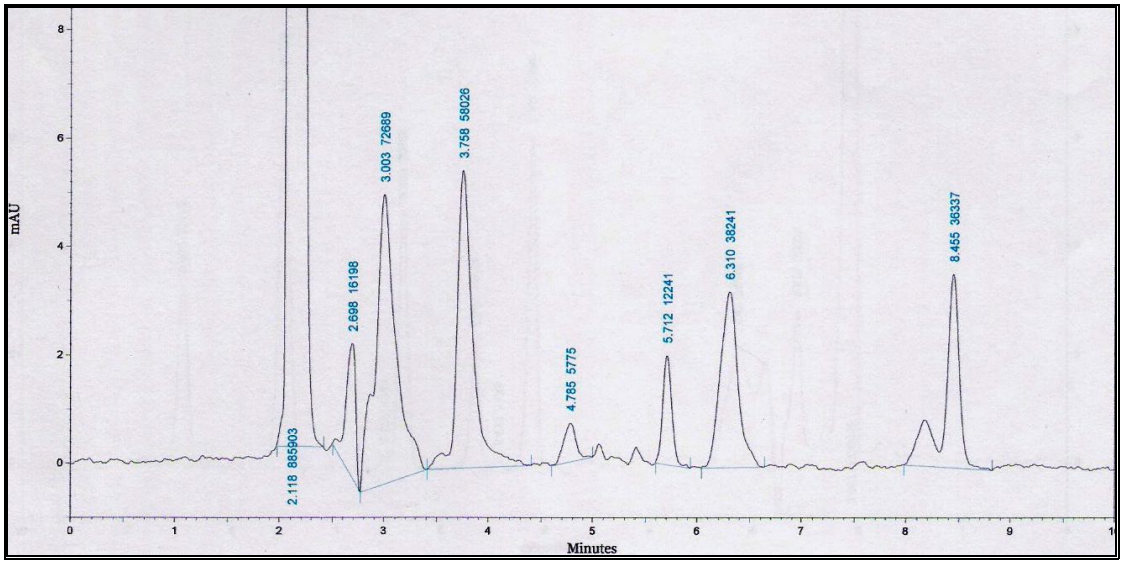


Figure A5.12 Chromatogram of pre-concentration of main steam, using trial C, elution with buffer pH6

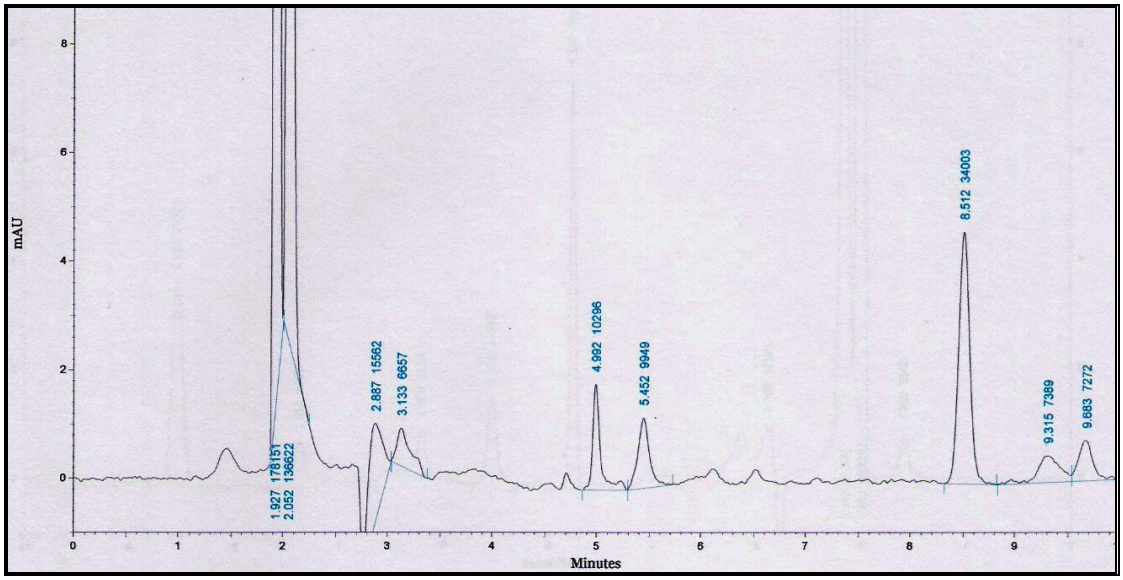


Figure A5.13 Chromatogram of wash before elution with pH 6 buffer, Strata X-AW, pre-concentration of 50ppb acid

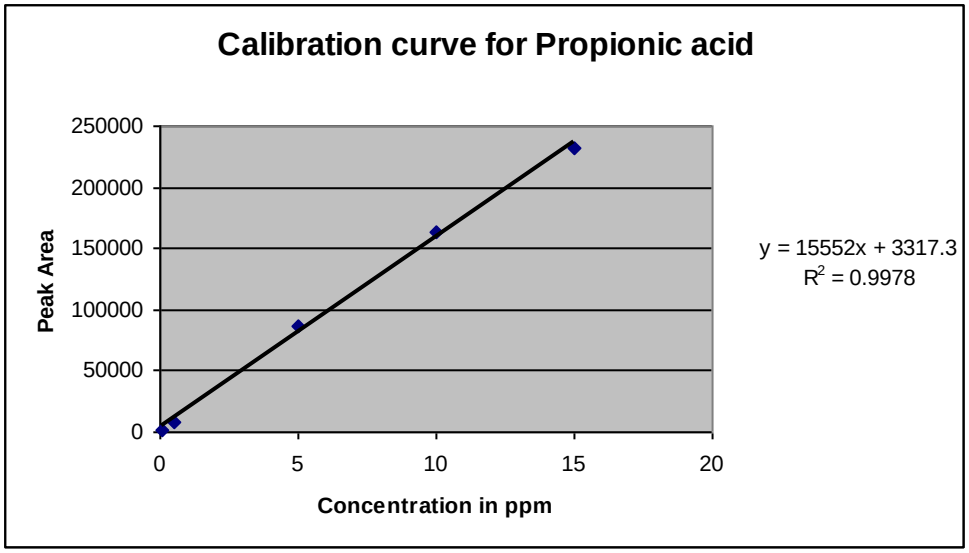


Figure A5.14 Calibration Curve for propionic acid used for pre-concentration determination, on Strata X-WA

Propionic Acid	Concentration (ppm)	Peak Area
	0.1	1956
	0.5	8572
	5	87183
	10	162621
	15	232135

Table A5.1 Peak areas for propionic acid from calibration curve A5.27

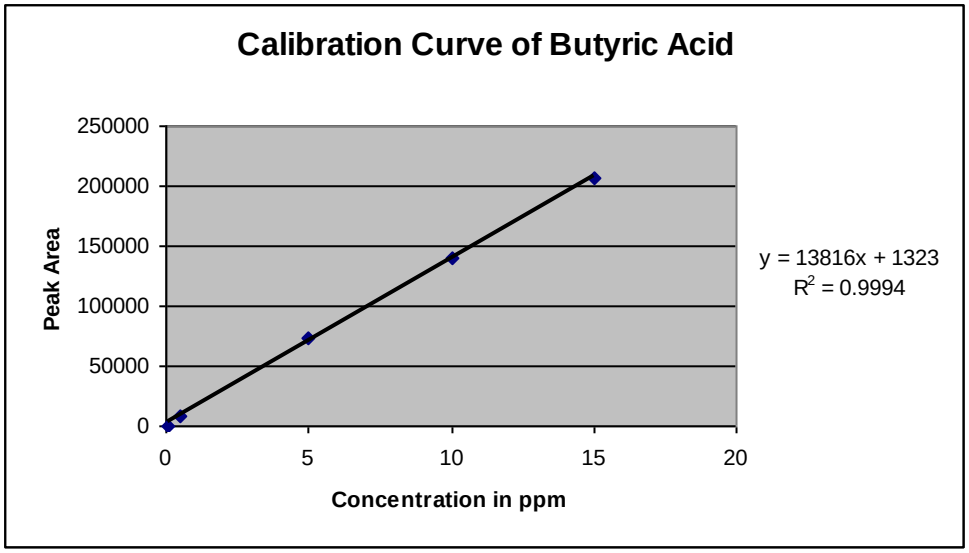


Figure A5.15 Calibration Curve for butyric acid for pre-concentration determination on Strata X-AW

Butyric Acid	Concentration (ppm)	Peak Area
	0.1	690
	0.5	7858
	5	74021
	10	139324
	15	207480

Table A5.2 Peak areas for butyric acid used in calibration curve A5.28