THE UNIVERSITY OF HULL

# The Separation and Characterisation of Sorbitan Esters Using Gas and Liquid Chromatographic Techniques

Being a Thesis Submitted for the Degree of
Doctor of Philosophy

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by

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This thesis is dedicated in memory of my wonderful mum Joyce Cooper

12.01.38 to 28.07.08

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## Abstract

The aim of the work carried out, was to develop an analytical method for characterising sorbitan ester surfactants, which could be used to monitor their manufacture and establish structure activity relationships. HPLC was the first analytical technique used, however despite evaluating various stationary and mobile phases a repeatable method could not be found. High temperature GC with splitless injection was then tried. A range of single polyol esters of varying fatty acid chain lengths were prepared and analysed to establish the ester species that would elute from the column. Using optimised conditions for the injector, detector and final oven temperature of 380°C and a DB1-ht column, it was found that all possible ester species elute for the short chain fatty acid esters studied. However as the chain length increases the number of ester species eluting decreases, for the longer fatty acid chain length esters based on oleic and stearic acid the sorbitan and sorbitol tri esters are the largest esters to elute. HT-GC analysis of a range of manufactured sorbitan mono esters based on palmitic, stearic and oleic acid was successful with all the mono ester species separated and identified and these separated from the di and tri esters. Validation of the method with sorbitan mono oleate showed that the method was precise and therefore a suitable method for characterising sorbitan mono esters. HT-GC with on-column injection was also investigated and it was found that for the longer chain fatty acid esters more ester species eluted than when using splitless injection. Validation would be required to establish the precision of the method, however it may also be a suitable method for characterising sorbitan esters.

**Contents:**

**Abbreviations**

| | |
|---|---|
| A | Selectivity Factor |
| AV | Acid Value |
| CSFC | Capillary Supercritical Fluid Chromatography |
| $d_c$ | Column Internal Diameter |
| DCM | Dichloromethane |
| ECD | Electron Capture Detector |
| ELSD | Evaporative Light Scattering Detector |
| F | Flow Rate |
| FID | Flame Ionisation Detector |
| FPD | Flame Photometric Detector |
| GC | Gas Chromatography |
| GC-MS | Gas Chromatography-Mass Spectrometry |
| GLC | Gas Liquid Chromatography |
| GPC | Gel Permeation Chromatography |
| HETP | Height Plate Equivalent |
| HLB | Hydrophilic Lipophilic Balance |
| HPLC | High Performance Liquid Chromatography |
| HPSEC | High Performance Size Exclusion Chromatography |
| HPTLC | High Performance Thin Layer Chromatography |
| $H_2SO_4$ | Sulphuric Acid |
| HT-GC | High Temperature Gas Chromatography |
| IPA | Isopropanol |
| IR | Infra Red |

| | |
|---|---|
| k' | Capacity Factor |
| KOH | Potassium Hydroxide |
| L | Length |
| LC | Liquid Chromatography |
| MeOH | Methanol |
| MM | Molecular Mass |
| MS | Mass Spectrometry |
| MSD | Mass Spectrometer Detector |
| MW | Molecular Weight |
| N | Theoretical Plates |
| NARP | Non-Aqueous Reverse Phase |
| N/D | None Detected |
| NMR | Nuclear Magnetic Resonance |
| NPD | Nitrogen Phosphorus Detector |
| PID | Photo Ionisation Detector |
| PSS | Programmable Split/Splitless |
| PT | Programmed Temperature |
| QC | Quality Control |
| $R^2$ | R-squared Value |
| RID | Refractive Index Detector |
| RSD | Relative Standard Deviation |
| SD | Standard Deviation |
| SEC | Size Exclusion Chromatography |
| SFC | Super Critical Fluid Chromatography |
| SMO | Sorbitan Mono Oleate |

| | |
|---|---|
| SPE | Sucrose Poly Esters |
| TG | Triglyceride |
| TCD | Thermal Conductivity Detector |
| THF | Tetrahydrofuran |
| TLC | Thin Layer Chromatography |
| TMS | Trimethylsilyl |
| $t_o$ | Time taken for mobile phase/unretained solute to pass through a column |
| $t_r$ | Retention time of a component |
| UV | Ultra Violet |
| V | Volume |
| W | Weight |
| $W_{1/2}$ | Peak width at half height |

## 1. Introduction:

The aim of the research was to develop an analytical method for characterising the composition of sorbitan ester surfactants produced by Croda. Croda is a speciality chemicals company, selling a range of naturally derived products. Sorbitan esters are non-ionic surfactants produced by the esterification of sorbitol and fatty acids, very little information is available in the literature regarding compositional analysis of sorbitan esters. This lack of information may be due to the complex nature of the esters, meaning no suitable analytical methods are available, or analytical techniques have been developed for this analysis but have been developed by industry and remain confidential. Due to the lack of information on sorbitan ester analysis, analysis of other polyol esters has also been reviewed, to aid in the development of an analytical method for characterising sorbitan esters.

### 1.1 What are Surfactants?

Surfactant is a shortened form of the term surface active agent and indicates the nature of these compounds, they are a group of compounds that have the ability to modify the interface between two phases. Surfactants consist of a hydrophilic and a hydrophobic group (1), the hydrophobic group is a non-polar hydrocarbon, of not less than 10 carbon atoms, which is bonded to a polar or ionic group, which is the hydrophilic part of the molecule. Surfactants concentrate at the surface of an aqueous solution and alter its surface property. A single surfactant molecule will seek the surface, because the hydrophobic tail will be repelled by the water and the hydrophilic part will be in the water phase. Further molecules will seek the surface or walls of vessels until both are full, then micelles will form, which are clusters of molecules

arranged with the hydrophobic part towards the centre and the hydrophilic part out. The degree of surface activity and application depends on the hydrophilic and hydrophobic groups. Surfactants can be classified according to the nature of their hydrophilic part and include anionic, cationic, non-ionic and amphoteric compounds.

A requirement in surfactancy application is the balance between water and oil solubility. Griffin (2) found it possible to quantify the relationship between the hydrophilic and hydrophobic part on an empirical basis in terms of the hydrophile-lipophile balance (HLB), a value which increases in indirect proportion to the overall polarity of the molecule. The HLB is used to establish the application of a surfactant as shown in table 1.1 (3). Low HLB values indicate relatively oil soluble materials with strong lipophilic characteristics and high values indicate hydrophilic characteristics which are water soluble materials. Surfactants have many applications including textile, metal and leather processing, agriculture, detergents, polymers, cosmetics, pharmaceuticals and food due to the wide range of HLB values.

| HLB RANGE | APPLICATION |
|---|---|
| <3 | Surface Film |
| 3-6 | Water-in-oil emulsifier |
| 7-9 | Wetting Agent |
| 8-15 | Oil-in water emulsifier |
| 13-15 | Detergent |
| 15-18 | Solubiliser |

**Table 1.1: HLB Range and Application of Surfactants**

## 1.2 Polyol Esters:

The sorbitan ester surfactants being studied can be classified as polyol esters. Polyol fatty acid esters are non-ionic and they contain a residue of a polyhydroxy compound as the hydrophilic portion in the ester, in combination with hydrophobic groups derived from fatty acids. Polyol esters are one of the most common surfactant types, partly because esterification is an easy chemical reaction to carry out and polyols such as glycerol, sorbitol and other sugars are readily available. Also a wide variety of fatty acids ensure a range of products can be made for many applications, with both saturated and unsaturated fatty acids of varying chain lengths being used.

The simplest polyol ester is derived from ethylene glycol which has two hydroxyl groups, when reacted with fatty acid it yields a mixture of mono and diesters, plus unreacted acid and diol, the reaction is shown in figure 1.1.

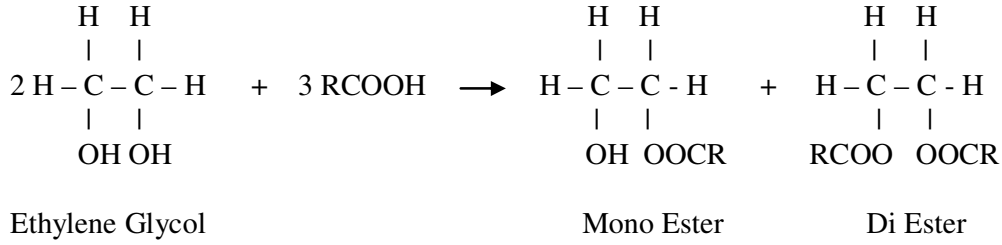



**Figure 1.1: Reaction of Ethylene Glycol and Fatty Acid to Produce Mono and Di Esters**

R = alkyl group of the fatty acid

Polyol esters are usually grouped according to their hydrophilic group (4). The major polyol esters manufactured are derived from glycerol, glycol or polyethylene and sorbitan. The reaction of the residual hydroxyl groups in polyol esters with ethylene

oxide to give products with higher hydrophilic properties, is common in the chemical industry, fats and oils and sorbitan esters are routinely ethoxylated.

Glycerol esters are polyol esters that can consist of mono, di and triglycerides (as shown in figure 1.2) and the volume produced is the most of all the polyol esters, they are of commercial significance because monoglycerides and diglycerides are major emulsifiers used in the food and cosmetic industry. The volume of sorbitan esters manufactured is less than glycerol esters, however they are one of the most common esters produced from the higher members of the polyol series.

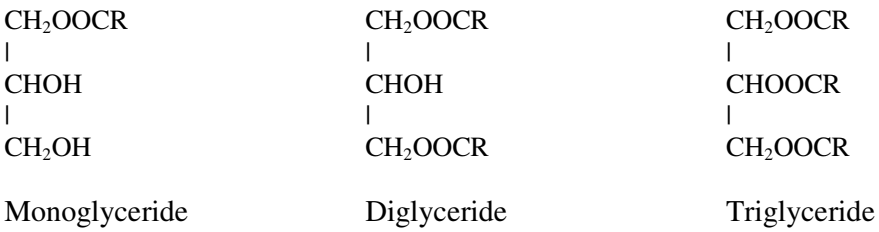



**Figure 1.2 Structures of Mono, Di and Triglycerides**

R = alkyl group

Sucrose esters are polyol esters, produced from the reaction of sucrose and fatty acid, however the volume of sucrose esters sold by industry is relatively small. Sucrose is a disaccharide with eight hydroxyl groups, however it has a high melting point and has a tendency to caramelise rather than melt, making processing difficult. Also due to the eight hydroxyl groups present on sucrose, typical esterification results in mono to penta esters which are very hydrophobic and have limited application potential. Figure 1.3 shows the structure of a sucrose mono ester. However despite production problems and the limited use, the production and analysis of sucrose esters has been

widely studied, this is probably due to the fact that sucrose is produced in million tonne quantities and is very cheap and abundant.

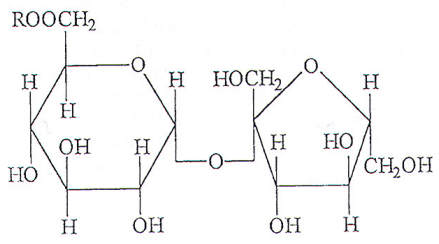



**Figure 1.3: Structure of Sucrose Mono Ester**

Most polyol ester surfactants are complicated mixtures, due to the distribution and degree of esterification and the mixed composition of the starting fatty acids (5).

### 1.3 Chemistry and Manufacture of Sorbitan Esters:

Sorbitan esters are the polyol fatty acid esters derived from the naturally occurring sugar sorbitol reacted with a range of fatty acids. Croda market them under the trade name Crills, other companies sell sorbitan esters under trade names such as Spans (ICI) and Famodan (Grinstead).

Crills are non-ionic surfactants, that are entirely vegetable derived and have long standing food and pharmacopeia approval and a safe history of use in cosmetic products. As non-ionics they offer advantages over ionic surfactants including increased stability, formulating flexibility and wider compatibility. They are also stable to mild acids, alkalis and electrolytes and do not react with ionic ingredients or actives. A range of sorbitan esters are produced by Croda with HLB values typically in the range 1 to 9, depending on the type and amount of fatty acid used. Figure 1.4

shows the chemical structure of a sorbitan mono ester, where R is the alkyl group of the fatty acid.

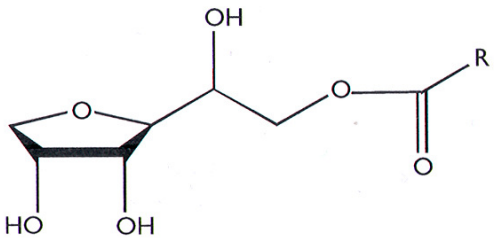



**Figure 1.4: Chemical Structure of Sorbitan Mono Ester (6)**

Table 1.2 shows the standard range of sorbitan esters manufactured by Croda (6). Crill 1 is the most hydrophilic of the Crill range and is soluble in many fatty compositions and solvents and can act as a co emulsifier in oil in water emulsions. In contrast Crill 4, 43, 45 and 6 used alone act as effective water in oil emulsifiers.

| TRADE NAME | DESCRIPTION | FATTY ACID | HLB |
|---|---|---|---|
| Crill 1 | Sorbitan monolaurate | Lauric | 8.6 |
| Crill 2 | Sorbitan monopalmitate | Palmitic | 6.7 |
| Crill 3 | Sorbitan monostearate | Stearic | 4.7 |
| Crill 4 | Sorbitan monooleate | Oleic | 4.3 |
| Crill 41 | Sorbitan tristearate | Stearic | 2.1 |
| Crill 43 | Sorbitan sesquioleate | Oleic | 3.7 |
| Crill 45 | Sorbitan trioleate | Oleic | 1.8 |
| Crill 6 | Sorbitan monoisostearate | Stearic | 4.7 |
| Crill 65 | Sorbitan triisostearate | Stearic | 1.8 |

**Table 1.2: Standard Range of Sorbitan Esters Manufactured by Croda**

Crillets are polyethoxylated partial esters of sorbitol, sorbitan and isosorbide and various HLB values can be obtained by using Crills and Crillets together in various ratios, this then allows the emulsification of many oils and waxes. Figure 1.5 shows a polyethoxylated sorbitan mono ester, where R is the alkyl group of fatty acid and (x + y + z) is the total number of moles of ethylene oxide

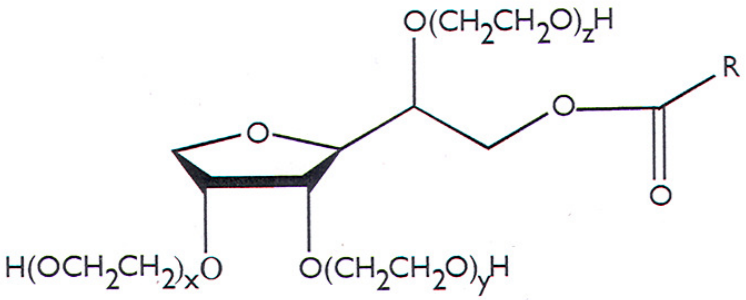



**Figure 1.5: Chemical Structure of Polyethoxylated Sorbitan Mono Ester (6)**

Crills are derived from the sugar sorbitol which dehydrates to other polyols. Sorbitol esters are not commercially available, however sorbitol monoester if it could be manufactured would have a greater water affinity due to the number of free hydroxyl groups and therefore would be a more versatile surfactant compound. The production of sorbitol mono esters would be difficult to obtain by standard chemical esterification due to cyclisation and dehydration of the sorbitol to other polyols and the production of a range of esters. The specificity of the enzyme lipases permits selective esterification of primary alcohols and therefore the use of enzymes in the synthesis of sorbitol mono oleate has been carried out (7). The study showed that a high sorbitol conversion resulted in a low percentage of monoesters in the total esters and to obtain 80% monoester in the final product conversion of the oleic acid and sorbitol was 40 and 60% respectively. However the study showed that a high percentage sorbitol

mono oleate product can be prepared, although it is not used for manufacture of sorbitol esters and commercial implications would have to be considered.

Although Crills are marketed as sorbitan mono or tri esters, a range of esters are present at varying levels in each product. Sorbitan esters are in fact complex mixtures of fatty acid esters of several polyols derived from sorbitol. They are prepared by heating sorbitol and fatty acid, sorbitol dehydrates to its anhydrides sorbitan and dihydride isosorbide as shown in figure 1.6 (4, 8). Sorbitol dehydrates to 1-4, 3-6 and 2-5 sorbitan but 1-4 sorbitan is the preferred cyclisation, due to the one asymmetric hydroxyl group on the '4' carbon atom of sorbitol. The sorbitan further dehydrates to isosorbide, however 2-5 sorbitan is not thought to easily further dehydrate due to stearic reasons, in contrast 3-6 sorbitan is thought to rapidly dehydrate further via the 1,4 ring closure to produce 3-6,1-4 isosorbide.

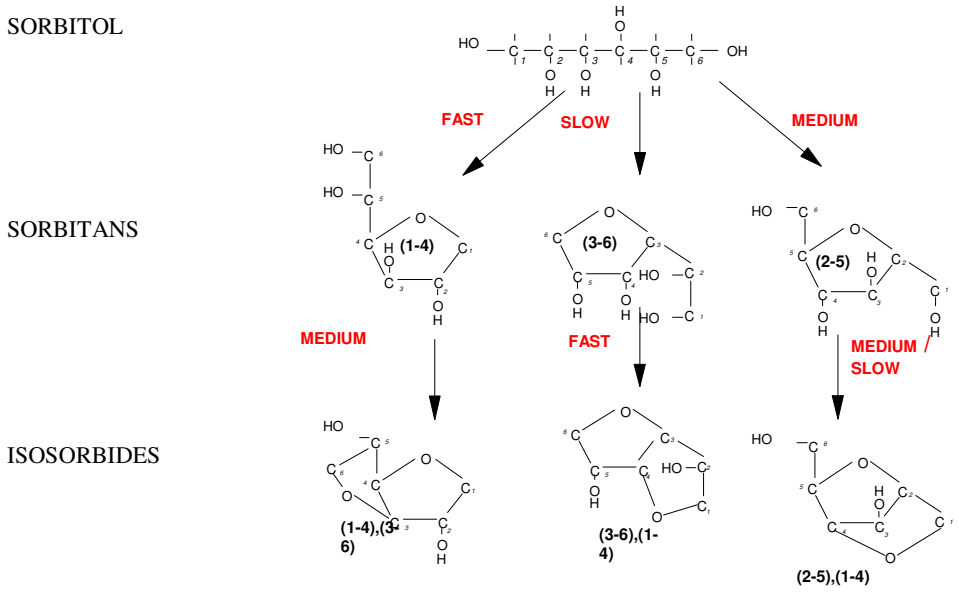



**Figure 1.6: Anhydration Reactions (8)**

The three possible polyols can then be esterified with a range of fatty acids of varying chain lengths, to give a range of esters. The fatty acids used are usually a mixed feedstock and may contain both saturated and unsaturated fatty acids, with chain lengths ranging from C8 to C24. The polyol is esterified at the most accessible hydroxyl group, for sorbitol this is the two end primary hydroxyl groups therefore the mono and di esters will predominate. However sorbitol has six hydroxyl groups, therefore it could be possible to produce a hexa ester. Sorbitan has four hydroxyl groups available for esterification, however due to stearic hindrance and fatty acid ratios hexa esters are unlikely, the presence of 3-6 sorbitan esters are also unlikely due to its rapid dehydration to isosorbide. Isosorbide can produce both mono and di esters, di esters are formed in preference to mono esters when a ratio of 1:1 isosorbide to fatty acid is used.

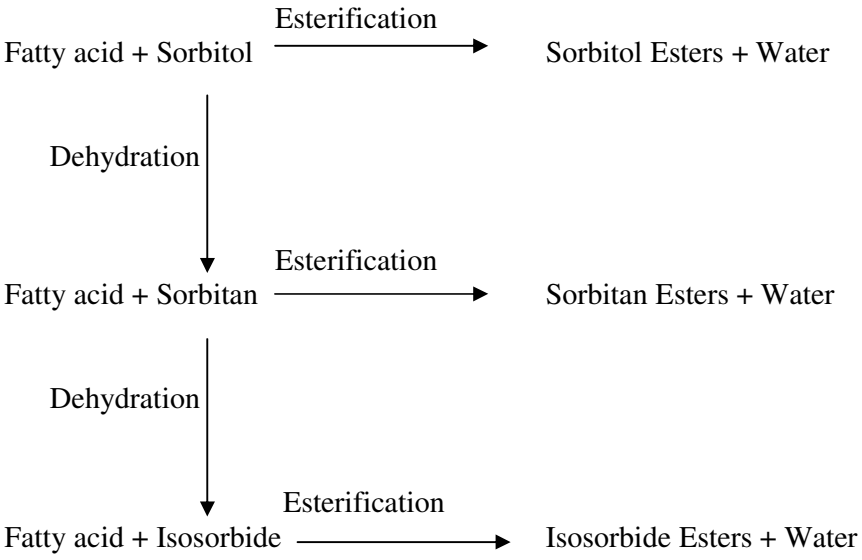



**Figure 1.7: Reaction Scheme**

The complexity of Crills is therefore due to:

- the range of polyols
- degree of esterification of these polyols
- mixed composition of the starting fatty acid
- positional isomers of the starting fatty acids esterified with the hydroxyl groups.

Crills are prepared in either a one or two stage process (9). The one stage process involves reaction of sorbitol and fatty acid to sorbitan ester, with dehydration and esterification occurring simultaneously, using a base catalyst at 230°C. In the two stage process sorbitan esters are prepared by the acid catalysed controlled dehydration of sorbitol at 130°C and then esterification of the resulting polyols with the specified fatty acid under base catalysis at 230°C. Text books (10) indicate that esterification proceeds by acidic catalysis and that alkali esterification is impossible, however esterification occurs because autoesterification occurs at 150 to 250°C, hydrogen ions arise due to dissociation of the carboxylic acid. Therefore the catalyst is not catalysing the esterification but transesterification.

Both sorbitol and fatty acids are natural products therefore can vary depending on the source. The raw material specification and initial selection of molar ratios are important in simplifying the process. The complexity of the mixtures makes determination of the precise composition impossible. Simple analytical techniques are currently used including acid value, hydroxyl value and saponification value and these define the reaction end point but not the product and are used for production control. However an analytical method which will establish the composition of Crills is important for the comparison of emulsifier's performances. When a suitable

analytical method has been developed, a relationship between the composition of sorbitan esters and their application can be established. This relationship can then be used by Croda to design the next generation of emulsifiers.

### 1.4 Chromatography

Chromatography is an analytical technique used to separate, isolate and quantify components in a mixture. It is a very powerful technique available to analysts because it can separate a mixture and quantify components in one step. Samples can be gaseous, liquid or solid and can range from simple mixtures of two or three components to complex mixtures containing very different chemical compounds. Chromatography includes a diverse but related group of methods including thin layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC) and size exclusion chromatography (SEC).

All chromatographic systems consist of a stationary and mobile phase and the components to be separated are distributed between the two phases. The stationary phase is a solid or liquid supported on a solid and the mobile phase a gas, liquid or supercritical fluid. The mobile phase moves across the stationary phase and carries the components with it, different components travel at different rates and this leads to separation, components that partition strongly into the stationary phase spend a greater amount of time in it and are separated from components that stay predominantly in the mobile phase and pass through the system faster. Separation can depend on properties such as boiling point, polarity, electric charge and size of molecules.

The retention factor, also known as the capacity factor is a term used in chromatography to describe the migration rate of an analyte through a column and is defined as

$$k' = t_r - t_o/t_o$$

where $t_r$ is the retention time of a component and $t_o$ is the time taken for the mobile phase to pass through the column. By controlling the capacity factor, separation can be improved. In GC changing the temperature or stationary phase can alter the capacity factor and in LC the composition of the mobile phase or changing the stationary phase can effect the capacity factor.

Another parameter important in chromatography is the selectivity factor and describes the separation of two compounds and is expressed as

$$\alpha = (t_r)b - to/(t_r)a - t_o$$

where $(t_r)a$ is the retention time of component A and $(t_r)b$ is the retention time of component B, with A eluting before B. The selectivity factor can be changed by altering the mobile phase composition, column temperature or changing the stationary phase.

It is also useful in chromatography to measure the column efficiency. The plate model is used which considers the column to contain a number of theoretical plates where the sample equilibrates between the mobile phase and stationary phase in these plates (11). The column efficiency is commonly described by height plate equivalent (HETP), the smaller the better and number of theoretical plates (N), with the more plates the better.

$$HETP = L/N$$

where L is the length of the column

$$N = 5.55(t_r)^2/w_{1/2}^2$$

where $w_{1/2}$ is the peak width at half height.

Both GC and LC are commonly used techniques in the laboratory. GC is a sensitive technique but is limited to the determination of analytes that are readily volatilised without decomposition. LC is suitable for non-volatile compounds or thermally fragile ones, however irreversibly adsorbed compounds are not detected and it can require the use of expensive solvents and solvent waste is generated.

### 1.4.1 Gas Chromatography

Gas Chromatography is a chromatography technique used to analyse complex mixtures of volatile organic and inorganic components. The mobile phase is a gas and the stationary phase is usually a liquid on a solid support or sometimes a solid adsorbent. Separation occurs due to partitioning of the solute between the moving gas and stationary phase. A GC system consists of a carrier gas supply which is typically helium, nitrogen or hydrogen of high purity, injector, column, column oven and a detector.

The injector is a used to introduce a small quantity of sample onto the column. A split/splitless injector uses a syringe to inject the sample into a liner in the injection port which is at a higher temperature than the least volatile component in the mixture, the carrier gas moves all of the sample into the column in the splitless mode or a small proportion in the split mode, with the rest going to the split vent. In contrast on-column injection involves the direct injection of the sample onto the column. Other

methods of introducing the sample onto the column include headspace, purge and trap and pyrolysis.

Columns can be short large diameter packed columns or long narrow capillary columns. The selection of the correct column is important for good separation, a large number of columns are available with a range of stationary phases, internal diameters, film thicknesses and column lengths. Many different interactions occur between the analyte and functional group of the stationary phase, non-polar stationary phases will preferentially retain non-polar compounds. When the non-polar groups of a column are replaced with polar functional groups, the selectivity of the column changes to polar compounds. Polyethylene glycol columns are very polar and therefore are highly selective to polar compounds. The column is contained in a column oven which maintains the temperature of the column because temperature effects retention, the oven also allows the use of temperature programmes.

A range of detectors are available for GC including the flame ionisation detector (FID), thermal conductivity detector (TCD), flame photometric detector (FPD), photo ionisation detector (PID), electron capture detector (ECD), nitrogen phosphorus detector (NPD) and mass spectrometer detector (MSD). The FID is a common universal detector and detects most organic compounds and therefore has many uses, it has been used to analyse a number of esters including sucrose esters (12), galactose and xylose esters (13) and is widely used for triglyceride analysis (14, 15). The ECD is a specific, highly sensitive detector, sensitive to halogens, organometallic compounds, nitriles, or nitro compounds, it is used widely in the analysis of pesticides (16, 17) and polychlorinated biphenyls (18, 19). The NPD is another specific detector

and detects compounds containing nitrogen or phosphorus and therefore is useful for the detection of organophosphate pesticides (20, 21). The MSD is another universal detector and can be used in the full scan mode were the whole molecular weight range is scanned or in the selective ion monitoring mode in which ions that are abundant and specific to the analyte are used which increases the sensitivity of the detector. Due to the amount of carrier gas and fact that the GC operates at high pressure and the mass spectrometer under vacuum, an interface is required to link the GC and MS, a range of interfaces are available including separator interfaces such as the effusion separator, jet separator and membrane separator, as well as splitter interfaces and direct interfaces. The MSD has been used to analyse a range of surfactant compounds including primary and secondary alcohols (22), polyethoxylated surfactants (23), steryl esters (24) and nonylphenol ethoxylates (25).

### 1.4.2 High Performance Liquid Chromatography (HPLC)

HPLC is a chromatography technique which uses liquid as the mobile phase and a solid or liquid on a solid support as the stationary phase. HPLC depends on selective interactions occurring between the mobile and stationary phase, this can lead to the technique having great resolving power. A HPLC system consists of a number of components including the mobile phase, injector, pump, column and detector. The mobile phase is important because it effects separation and the pump is used to pass a constant flow of the mobile phase through the system in either an isocratic or gradient mode. The injector in HPLC is simple in contrast to GC and typically consists of an injection valve and the sample loop. The sample is dissolved in the mobile phase before injection into the sample loop. The sample is drawn into a syringe and injected

into the loop via the injection valve. A rotation of the valve rotor closes the valve and opens the loop in order to inject the sample into the stream of the mobile phase.

There are two types of liquid chromatography depending on the polarity of the stationary and mobile phase. Normal Phase LC has a polar column of silica or a bonded phase, with a non polar solvent. Polar compounds stick longer to the column than non polar compounds which will pass through quickly. Reverse phase chromatography has a non polar column, the silica is typically modified with alkyl chains typically 8 to 18 carbons in length and a polar solvent is used. Elution order is the opposite to normal phase with polar molecules passing through quickly because they are more soluble in the mobile phase and non-polar compounds more attracted to the stationary phase and therefore having longer retention times. The retention can be altered for both by altering the mobile phase polarity or stationary phase packing.

A number of detectors are available for use with HPLC. The UV detector is a commonly used detector for a range of organic compounds including esters such as polyglycerol esters (26), triglycerides (27) and sorbitan esters (28, 29). The sample flows from the column and into the light path of the flow cell and UV radiation is absorbed and a reduced signal is observed by the detector. Different UV detectors are available including single wavelength, variable wavelength and diode array detectors. The major problem with UV detectors is that the sample must contain a UV absorbing component. The refractive index detector is another detector used but can only be used with an isocratic mobile phase. Fluorescence and chemiluminescent detectors are highly sensitive and very specific detectors used with HPLC. The evaporative light scattering detector detects any compound in a sample less volatile than the mobile

phase, regardless of functional group, gradient mobile phases can be used and solvent peaks are eliminated and therefore is considered as a universal detector. HPLC coupled to a mass spectrometer has become a popular method because it is highly sensitive, selective and universal and therefore has been used to analyse a large range of compounds. It has been used to analyse a range of non-ionic surfactants including fatty alcohol ethoxylates (30, 31), alkylphenol ethoxylates (32, 33), ethoxylated sorbitan esters (34, 35), saccharide surfactants (36), polyglycerol fatty acid esters (37) as well as triglycerides in a range of matrices (38, 39 and 40) and simultaneous analysis of different surfactants (41, 42). However an interface is required because the LC solutes are eluted in large amounts of solvent at atmospheric pressure and this must be removed before the eluent enters into the MS which is under vacuum. Interfaces (43) include particle beam, moving belt, thermospray, electrospray and atmospheric pressure chemical ionization.

### 1.5 Analysis of Polyol Esters:

Various chromatographic techniques have been used to analyse a range of polyol esters in an attempt to establish compositional information and have been reviewed to assist in the development of an analytical method for characterising sorbitan esters.

#### 1.5.1 Thin Layer Chromatography (TLC):

TLC is a chromatography technique consisting of a stationary phase which is typically a thin layer of adsorbent material immobilised on a flat sheet and an organic solvent as the mobile phase. The sample is dissolved in solvent and placed at the base of the sheet and the mobile phase is drawn up the sheet by capillary action. The different

components in the mixture move up the plate at different rates due to differences in their partitioning behaviour between the mobile liquid phase and the stationary phase. The advantages of TLC include its simplicity and speed, it is also an inexpensive technique compared to High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC). TLC has been used to analyse polyol esters and in particular sucrose esters.

TLC was used by Gee in 1962 (44) to separate mixtures of sucrose esters, as well as sucrose and raffinose. The technique of qualitative and quantitative silica gel chromatography on glass strips was rapid, with definitive separation of the compounds obtained. A number of different solvents were tried but the best separation for sucrose esters was obtained with toluene/ethyl acetate/95 % ethanol (10:5:5 v/v/v). The plates were developed with dichlorofluorescein indicator solution, esters appeared as yellow spots on an orange background when illuminated with a longwave Ultra Violet (UV) lamp. A spot corresponding to sucrose was obtained, as well as a long spot representing sucrose mono esters and 4-5 spots representing sucrose diesters, faster moving spots were thought to be sucrose esters with a higher degree of esterification than the di ester. The separated components were then eluted from the silica gel and the sucrose content measured using a colorimetric procedure to obtain a quantitative measurement.

Another quantitative TLC method (45) separated the sucrose ester components into mono, di, tri and higher esters. Again individual components were extracted from the plate and quantified colorimetrically using anthrone reagent. The method was also

found to be applicable to the analysis of raffinose esters of fatty acids with some modifications.

However both of those quantitative TLC methods for the analysis of sucrose esters relied on elution of esters from TLC plates, followed by reaction of the esters with a colour-producing reagent. The scraping of each zone of component was the accuracy determining process. Weiss *et al*. (46) attempted to improve the quantitative procedure to better evaluate ester distribution and isomer formation. The sucrose esters were separated on a silica gel plate containing calcium sulphate using toluene/ethyl acetate/95 % ethanol (2:1:1 v/v/v) as the solvent system for mono to tetra esters, further separation of lower esters was achieved using two-dimensional TLC in which the solvent was chloroform/methanol. Petroleum ether/ethyl acetate/glacial acetic acid was found to separate higher esters. Detection of the sucrose esters was obtained using urea-phosphoric acid. The photometrically determined density of each spot on the plate was found to be proportional to the sucrose content, the ester content was then determined using a calculation. Positional isomers were observed at each level of substitution but could not be separated from each other for quantitative evaluation.

The emulsifying properties of different sucrose monoesters have been found to depend on the number and kind of different isomers. Therefore an effective analytical method is essential during synthesis, purification and structural studies. Separation of structural isomers of sucrose monostearate by TLC was carried out by Torres *et al*. (47) with six of the eight possible isomers differentiated by specific colour development with a visualising agent. The TLC plate of sucrose monostearate showed

five well-resolved components, which exhibited different colours. Component 1 appeared as a greyish/blue spot, components 2 and 3 were unresolved and appeared pinkish/brown, although when lower quantities of sample were used, these could be resolved. Component 4 was a blue spot and 5, 6 and 7 different shades of purple. HPLC and GC were also used to analyse the sucrose monostearate sample. HPLC gave baseline separation of three main peaks and the monoesters with different acyl chain lengths were separated. GC also resolved monoesters with different acyl chain lengths and partially separated the isomers. This study showed what separation of isomers is possible, however only concentrated on the mono esters. For sorbitan esters all of the esters would be of importance.

Sucrose polyesters (SPE) are mixtures of esters made up of sucrose esterified with 6, 7 or 8 moles of fatty acid. When sucrose polyesters were growing in importance in food technology, a study was carried out using chromatographic techniques to isolate, characterise and quantify sucrose polyester mixtures, to gain information on the mixtures (48). TLC and TLC flame-ionization detection (FID) as well as reversed phase HPLC were used. Pure fractions of sucrose polyesters of olive oil were obtained by column chromatography, using Infrared and nuclear magnetic resonance spectroscopy to identify them. The lowest polarity fraction contained octa esters, which appeared on the TLC-FID chromatogram as a single peak, two other fractions due to the hepta and hexa esters each gave more than one peak by TLC-FID. Both TLC-FID and HPLC were found to be appropriate techniques for quantification of SPE mixtures of hexa, hepta and octa esters of sucrose and no significant difference in the values by the techniques was noted. Reproducibility was excellent for sucrose octa esters and the maximum coefficient of variation was 7 % for sucrose hexa esters

analysed by TLC-FID. The work showed that analytical techniques are available for quantitative analysis of polyesters. The chromatographic techniques were also thought to be suitable for characterising SPE/Triglyceride mixtures.

The leaf surface chemistry of tobacco is important for plant biology as well as flavour and aromatic characteristics, research has also indicated that sucrose esters also act as antimicrobial agents and could be a potential cancer preventative agent of a new type. Therefore the analysis of sucrose esters found on tobacco leaves has also been important and researched thoroughly. TLC with scanning densitometry was used for the quantification of primary and secondary metabolites of Nicotina tabacum leaf surface components (49), with one of the components of interest being sucrose esters. A single development with isopropanol (IPA)/chloroform/methylene chloride/hexane (7/8/6/79 v/v/v/v) and silica gel was used and quantification with 30 % sulphuric acid followed by densitometry. The results were semi-quantitative but quantitative for all secondary metabolites with the exception of the sucrose esters. Observation of the scanning densitometry trace also showed the sucrose esters as one peak, the possible ester species have not been separated and therefore the method is unsuitable for characterisation of the sorbitan esters. However the work concluded that quantitative TLC is a useful tool in plant breeding and other research programmes interested in rapid screening of chemical traits in a large number of samples.

However more recently sucrose esters from the surface of tobacco plant leaves were analysed using High Performance TLC (HPTLC) silica gel plates (50), with n-hexane/ethyl acetate (1:3 v/v) used as the developing solvent and aniline diphenylamine as a detection reagent. Offline TLC-Mass Spectrometry was used for

detection and identification of the compounds. The chromatograms obtained showed a number of brownish bands and differences are noted in chromatograms of sugar esters from different tobaccos. It was stated that more work is required to identify components and that to date no TLC procedure by which sucrose esters of tobacco leaf surface can be resolved and quantified exists, therefore emphasising the difficulty of the analysis of polyol esters.

The review highlighted that TLC has been widely investigated for sucrose esters, but has been used less for other polyol esters, although in 1969 Sahasrabudhe and Chadha (51) described a procedure for separation, identification and quantitative estimation of mono, di, tri esters of sorbitol and its anhydrides. The lipid classes were separated by liquid partition column chromatography and the fractions analysed by Gas Liquid Chromatography (GLC) as trimethylsilyl ethers, the individual mono and di fatty acid esters and polyols eluted, however higher esters did not and the mono and di esters appeared as broad unresolved peaks. TLC was therefore used to analyse the samples and fractions from the column chromatography used for identification. Absorbosil plates were used and two mobile phases were required. Benzene/methanol (6:4 v/v) was used to separate the polyols and four distinct spots were observed when viewed under a UV light following spraying with 2,6 dichlorofluroscein (1 % in ethanol). Esters however were separated using benzene/ethyl ether/methanol (75:20:5 v/v/v), the largest esters identified were tri esters and they eluted as one spot at the top of the plate. In contrast the di ester of sorbitan was found to give three distinct spots, although no identification of the isomers was possible. In contrast the isosorbide and sorbitan mono esters each appeared as one spot. Further work was required for

identification of the components and no quantitation was possible using this technique.

More recently a quantitative TLC method has been used for sorbitan esters by Smidrkal *et al.* (9). TLC-FID was used to monitor the two stage synthesis of sorbitan esters. A series of sorbitan fatty acid esters with various fatty acid to sorbitol ratios were prepared and the course of the reaction and composition of the final products was determined by TLC-FID. Chromrod SII rods and chloroform-benzene-ethanol developing system were used, the rods were detected by FID. The TLC-FID chromatogram showed four groups of peaks, the highest retardation factor was exhibited by the tri esters, followed by a compact group of four peaks due to the di esters and unreacted fatty acid, two additional not completely separated peaks were present due to the mono esters and two other small peaks closest to the start belonged to isosorbide and sorbitan. The molar ratio of oleic acid and sorbitol was varied at 0.6, 1.2 and 2.4 and the ratio of different esters calculated with time using this method and significant differences were noted. It was therefore concluded that the method is suitable for the preparation of sorbitan esters on an industrial scale.

The review showed that TLC has been used for the analysis of polyol esters with varying success. Quantification has proved difficult, but new advances such as TLC-FID have made it easier. Also due to the complex nature of polyol esters, identification has also been difficult.

### 1.5.2 High Performance Size Exclusion Chromatography (HPSEC):

HPSEC is a technique that separates molecules based on their effective molecular size and shape. The stationary phases are porous particles with closely controlled pore size, molecules of comparable size to the mobile phase diffuse through the entire network. Larger molecules are excluded from narrower parts and very large molecules are excluded completely. HPSEC is a method used by Croda for analysing a range of esters including sorbitan esters (52) and is particularly useful for sorbitan mono oleate, however when used to analyse sorbitan mono laurate many of the components are found to coelute, therefore less information is obtained, although it is still used for comparative purposes (53). The advantages of HPSEC are that very little sample preparation is required and it is very rapid, however only partial separation of various ester species has been possible.

Other polyol esters have been analysed using HPSEC, in 1976, Birch and Crowe (54) used HPSEC, to determine sucrose polyesters in faeces and diet. The samples were freeze-dried, extracted with ethyl ether and analysed by HPSEC. The sucrose polyesters, were found to elute as a single peak before anything else in the sample and were then quantified by refractive index detector. The method was found to be quick and accurate and a linear response was found over a wide concentration range, however separation of the various ester species was not achieved.

HPSEC was used for monitoring the enzymic synthesis of sucrose, glucose, fructose and sorbitol esters (55), using a tetrahydrofuran (THF) mobile phase and refractive index detector (RID). A number of chromatograms were obtained for the various esters produced by enzymic synthesis and these were compared to chromatograms

obtained for esters produced by chemical reaction. The chromatogram obtained for the sucrose-oleic acid ester produced by a chemical reaction showed peaks due to the mono, di, tri and tetra esters although the tri and tetra esters showed coelution. In contrast the sucrose ester produced using the enzyme only showed a mono ester peak. Therefore indicating that HPSEC is useful for comparative purposes but separation of the various ester species is limited.

HPSEC has also been used to characterise sucrose polyester – triglyceride (TG) mixtures (56). A RID was used, with 100 Å and 500 Å PLgel column connected in series and THF as the mobile phase. The SPE and TG were separated, each appearing as a well resolved peak, with minor compounds present not being detected, allowing quantification of the mixture.

HPSEC has some uses for the analysis of polyol esters, including comparative analysis, however due to the lack of separation of all the individual ester species the use is limited.

### 1.5.3 High Performance Liquid Chromatography (HPLC):

HPLC is a chromatography technique which uses liquid as the mobile phase and a solid or liquid on a solid support as the stationary phase. HPLC depends on selective chemical interactions occurring between the mobile and stationary phase, this can lead to the technique having great resolving power and consequently has been used for the analysis of a number of polyol esters.

Aitzetmuller (57) studied the applications of the moving wire detector for Liquid Chromatography (LC) of fats and fatty acid derived oleochemicals in 1975. A range of samples were analysed with this detector, including food emulsifiers, which are a mixture of UV-transparent compounds, varying widely in polarity towards silica gel, making a gradient elution necessary, which meant at the time UV and RID couldn't be used. In carrying out this study he was the first to elute sorbitan esters by LC, with a number of partially resolved peaks observed. The work showed the broad range of applications of the detector.

Garti and Aserin have studied the use of HPLC to analyse a number of polyol esters used as food emulsifiers, including polyglycerol, sucrose and sorbitan esters. Polyglycerol esters are important food emulsifiers and are available commercially as a mixture of various isomers. As with other polyol esters analysis is important during production, to ensure there is no batch to batch variation, to compare emulsifiers from different suppliers and to determine if emulsifiers used in food products are compatible with regulations. Polyglycerol esters are mixtures of hundreds of individual chemical compounds, with differing degrees of esterification of the polyol, chain length of the fatty acid, degree of unsaturation, as well as positional isomers. In 1981 they attempted (26) to analyse a wide range of polyglycerol –poly–fatty acid esters without the need for derivatisation. A 25 cm column packed with 10 μm Lichrosorb Diol was used and the isomers were eluted using a gradient based on a hexane/IPA mixture over 60 minute. The method separated the monoglycerol and polyglycerol monoesters as well as polyglycerol polyesters. However observation of the chromatograms obtained shows that although the first few peaks, identified as the

short chain polyols gave well resolved sharp peaks, later peaks were broad and the analysis ambiguous.

Kaufman and Garti (58) used a reversed phase C18 column and isocratic mobile phase to analyse the composition of sucrose fatty acid esters. The method developed for the quantitative analysis of mono-, di-, tri- and higher isomers, used methanol and isopropanol as the eluent and both UV and RI detection. Both detectors were found to give similar results but the RI detector was less sensitive. The method was simple and rapid but as observed with the polyglycerol esters the mono and di esters resulted in sharper peaks, than the higher esters, which appeared as small broad peaks. An alternative method using methanol and water improved the separation of the mono and di isomers, with elution taking ~40 minutes compared to ~10 minutes using the methanol/IPA mobile phase, however esters greater than diesters were not eluted with the alternative mobile phase.

HPLC analysis of sucrose esters was also attempted by Jaspers *et al.* (59), the work of Kaufman and Garti was tried but similar results could not be obtained, highlighting the complexity of ester analysis. It was found that the method used by Garti resulted in the monoesters eluting with the solvent. Therefore the composition of the eluent was changed to methanol/water (85/15 v/v) and the monoesters determined. Using methanol/ethyl acetate/water (65/25/10 v/v/v) diesters were separated. These methods enabled information about the amount of mono and di esters in the product to be ascertained, as well as the ratio between sucrose monopalmitate and sucrose monostearate and the number of the most important structural isomers. However a

complete separation of all possible di esters was not possible and higher esters were not eluted.

In 1983 Garti *et al.* (28) investigated the use of HPLC with a RP-18 column and no pre-treatment to analyse sorbitan esters. Very little quantitative work had been carried out on sorbitan esters prior to this. Eluents based on IPA/water were used and the best separation was obtained using IPA/water (85/15 v/v) as the mobile phase and UV detection. The chromatogram obtained for Span 80 (sorbitan mono oleate) is shown in figure 1.8. The identification shown in the chromatogram was attempted using fractions thought to be pure esters, collected by column fractionation. The chromatogram shows three peaks with the mono and di esters giving partially resolved peaks followed by a small broad peak thought to be the tri esters.

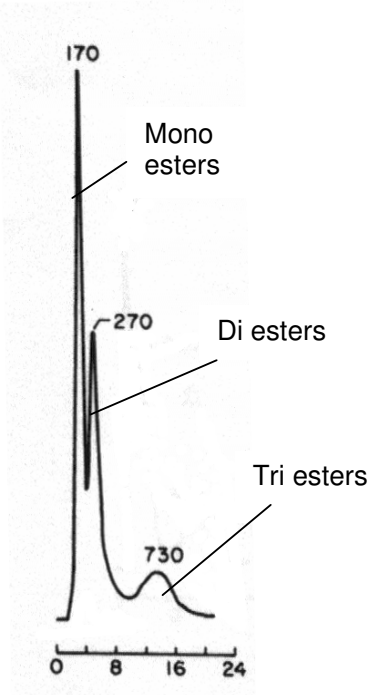



**Figure 1.8: HPLC Chromatogram of Span 80 Obtained by Garti *et al.* (28)**

When the water content of the mobile phase was greater than 15 %, the mono and di esters were found to appear as several peaks due to the different isomers, but the tri esters did not elute. Quantitation was attempted for a range of sorbitan esters but due to the absence of pure standards was carried out by assuming their response factor were close to those obtained for glycerol esters.

In 1994 Wang and Fingas (29) used a very similar method to look at a range of sorbitan esters and again used glycerol esters to determine the response factors. Again a reverse phase C18 column was used, with IPA/water mobile phase and UV detection at 220nm. The chromatogram shown in figure 1.9 was obtained for Span 80.

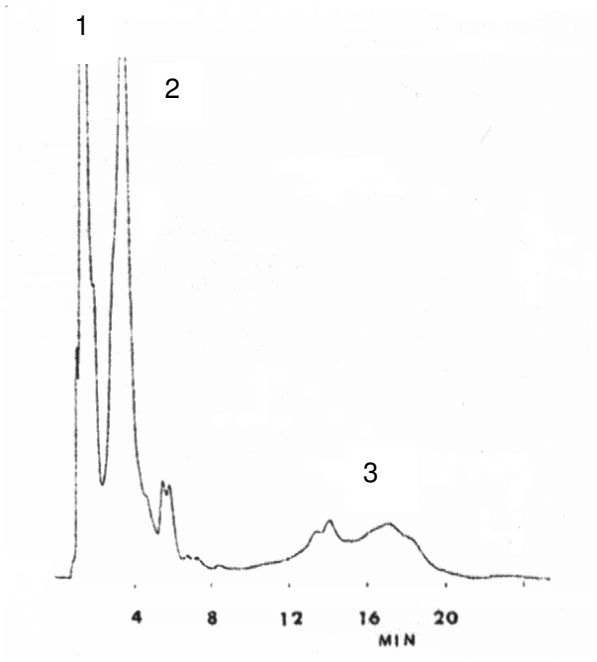



**Figure 1.9: HPLC Chromatogram Obtained for Span 80 by Wang and Fingas (29)**
1 = Mono Esters, 2 = Di Esters, 3 = Tri Esters

Again single components were not separated but separation of mono, di and tri esters were, indicated on the chromatogram by 1, 2 and 3, respectively. Due to the lack of availability of pure standards the identification of the peaks was assumed based on the correlation of retention times with the degree of esterification, molecular weights and comparison with glycerides of fatty acids and similar surfactants. The limitation of the method as with the previous method is that elution of the high poly esters is not complete and these are probably retained on the column, which will result in a short life span of the column. Also as the retention time of the components increases the peaks become broader.

HPLC has also been used for the analysis of glycerol esters (60). Non aqueous reversed phase HPLC was developed using a linear gradient of methanol/IPA and UV detection for separation of molecular species of 45 synthesised tri and diglycerides. The elution order was found to depend on the polarity of the fatty acid constituent, with elution time increasing as polarity decreased. For both tri and diglycerides elution corresponded with chain length, degree of unsaturation and presence of polar groups. It was also found that other structural features affect elution, with a triglyceride containing cis-fatty acids eluting slightly earlier than its isomer containing trans fatty acids. This work emphasises the difficulties of analysing complex mixtures and the various factors that can affect separation.

In 2006 reversed phase HPLC was used for profiling medium chain glycerides which are used to solubilise drugs (61). The ratio of C8 and C10 fatty acids along with the ratio of monoglycerides, diglycerides and triglycerides significantly impact the overall solubilising properties, therefore an analytical method for characterising the

glycerides is necessary. Existing methods did not have the desired selectivity, simplicity or adequate characterisation. A reversed phase HPLC method was used with acetonitrile/water mobile phase and UV detection, it was optimised for selectivity, sensitivity and efficiency and was successfully applied to characterise commercial samples. The method allows adequate quantification of individual components, total monoglycerides, diglycerides, triglycerides, free fatty acids and C8 vs C10 ratios and therefore shows full characterisation of the medium chain glycerides, which is what is required for sorbitan esters. However sorbitan esters typically have fatty acids with a longer chain length.

### 1.5.4 HPLC and Evaporative Light Scattering Detectors:

A versatile detector was needed for HPLC to complement UV detection and to detect solutes that do not absorb UV radiation or do so at an inconvenient wavelength. RI detectors although less sensitive had been considered universal, but cannot be used with gradients and is effected by temperature therefore its use is limited. Both UV and RI detectors also yield a solvent front, which results in fast eluting solutes being obliterated (62). Lipids are vital products of large economic consequence and analysis is difficult due to the complex nature of the mixtures and the number of isomers and homologous. Due to the range of polarity of the various components, gradient elution LC allows separation of many samples but this precludes the use of an RI detector. However most triglycerides have no chromophore active above 210 nm, which makes UV detection difficult. Therefore GC had to be used but high molecular weight triglycerides are difficult by this technique and phospholipids impossible because they cannot withstand high temperatures. Highlighting the need for an alternative detector for HPLC analysis of lipids.

Evaporative light scattering detectors (ELSD) were first considered in 1978, but it was not until 1986 that a version came out using a spray chamber and drift tube and this was commercialised by S.E.D.E.R.E (62). ELSD's cannot detect highly volatile solutes and cannot be used with non-volatile solvents or buffers, therefore are not fully universal. However the advantages of ELSD's (63) include the fact that they detect any compound in a sample less volatile than the mobile phase, regardless of functional group, gradient mobile phases can be used and solvent peaks are eliminated. Also it is a more sensitive detector than both UV and RI and has improved baseline stability due to reduced temperature fluctuations.

Studies have been carried out describing both the theoretical aspects (64, 65) and applications of the ELSD. Stolyhwo *et al.* (66) studied the qualitative and quantitative properties of the ELSD, when in an early stage of development and found it to compare favourably to the RI detector. It was also found that the detector response per unit mass of sample was constant for compounds belonging to a given chemical group, but was not linear, although handling the data for quantitative analysis was easily achieved. Robinson and Macrae (67) compared the ELSD to the RI detector, UV detector, moving wire detector and IR detector for the analysis of complex triglycerides also in the early stages of the ELSD development. It was found to be promising due to its gradient compatibility and insensitivity to eluting solvent composition.

The ELSD has been widely used to analyse polyol esters such as monoglycerides and diglycerides (68), triglycerides (67, 69, 70, 71 and 72), polyglycerol fatty acid esters

(73) and sucrose esters (74) as well as other lipids (75, 76, and 77), steroids (78) and surfactants (79).

Mancini *et al.* (63) studied the performance of three ELSD's, the Alltech Varex K111, Polymer PL-EMD950 and Sedex 45 to analyse triglycerides and phospholipids. The linearity for all the detectors was indicated by regression coefficients and was greater than 0.98 in the range 0-250 μg triglyceride injected and 0-25 ng for the phospholipid. However the three instruments assayed did show differences in behaviour regarding repeatability, linearity, sensitivity and detectability.

Liu *et al.* (68) determined monoglycerides and diglycerides by HPLC with ELSD, using a normal phase column and without derivatization. The 1,3 diglycerol was resolved from the 1,2 positional isomer, although some 1,3 of low molecular weight was found to interfere with the 1,2 diglycerides of high molecular weight. For monoglycerides the separation between 1- (and 3-) acyl and 2-acylglycerols was optimised only between those pairs with identical fatty acyl groups. The response was not found to be linear, so a linear log/log calibration curve was established. However it was concluded that the universal nature of the ELSD makes the method applicable to oils and emulsifiers containing both saturated and unsaturated fatty acyl moieties.

Polyglycerol fatty acid esters are complex mixtures and compounds can appear as diastereoisomers due to stereogenic carbons, therefore the correlation between structure and performance is not easily determined. Polyglycerol fatty acid esters along with fatty ethers were analysed by LC on porous graphitic carbon and octadecyl

silica with ELSD and using Mass Spectrometry (MS) for identification (73). The work showed that with ELSD the response value becomes different when the eluent composition changes, indicating care is required for comparing the response of various compounds in gradient elution. However the work concluded that an octadecyl bonded phase column and ELSD is a suitable method to control the purity of polyglycerol esters, but the porous graphitic carbon column was required for diastereoisomer analysis.

The ELSD was widely used for triglyceride analysis when initially developed and has continued to be used for their analysis. Improvements of the functional properties of vegetable oils have been obtained by altering the fatty acid composition, therefore knowledge of the kind and quantities of individual triglycerides is important. In the past quantitative triglyceride analysis by HPLC was difficult due to the fact they do not have strong chromatophoric groups resulting in absorbance problems for UV detectors and the fact gradient solvent systems are needed for the resolution of triglycerides. However use was made of the FID, for the analysis of triglycerides in vegetable oils and allowed quantitation without the need for detector response, because quantification was based on area percent. However due to the lack of commercial availability of the FID Neff *et al.* (80) used gradient reverse phase HPLC with ELSD for determining the quantitative composition of genetically modified soybean oil, using a newer ELSD with improved linear detection compared to older models. It was demonstrated that ELSD produced quantitative results without the need for response factors for the reverse phase HPLC of triglycerides. The oils showed considerable variation in triglyceride species amount. The analysis showed analysis precision with a standard deviation for triplicate analysis per soybean oil

triglyceride of 0.0 to 0.3 % per triglyceride species. The samples were also analysed by HPLC-FID which is no longer commercially available and the data supported the use of HPLC-ELSD in place of HPLC-FID.

Triglycerides with medium chain fatty acids in the *sn*1- and *sn*3- positions and unsaturated long chain fatty acids in the *sn*2 positions have been reported as alternatives to medium chain triglycerides for treatment of patients with lipid malabsorption. Enzymic methods for synthesis of these triglycerides was appealing. However the positional distribution of acyl groups in the triglyceride must be known as well as the particular number of linoleic acids incorporated at a specific glycerol position. HPLC-ELSD (81) was used for the separation of glyceride positional isomers using silver ion chromatography and a solvent system of n-hexane, 2-propanol, ethyl acetate and acetonitrile. Satisfactory separation of triglyceride positional isomers according to number and the position of linoleic acid was obtained. Good separation of the two positional isomers diglyceride 1,3 dicaprylin and 1,2 dicaprylin was also obtained.

Improved separation of sucrose ester isomers has also been achieved using gradient HPLC and ELSD (74). Sucrose has eight hydroxyl groups therefore 255 different isomers are possible from mono to octa esters, although not all hydroxyl groups are equally as reactive. TLC, GC and HPLC have been used for the separation of the different components of sucrose fatty acid esters. Moh *et al.*(74) felt all previous work was inadequate and used HPLC with a binary gradient to separate sucrose mono and di esters with different acyl chain lengths (C16 and C18), the mono esters resolved in three to six peaks and the di esters in fourteen to eighteen. The method

offered advantages over other analysis in terms of improved resolution and simultaneous separation of mono and di esters. Therefore making it possible to determine the approximate composition of mono and di esters of sucrose esters in one analysis. However quantification of higher esters was based on an estimate and the chromatograms do not show the elution of the higher esters.

The review highlights that HPLC-ELSD has many uses and that the ELSD is an alternative to the UV detector, particularly for compounds which do not absorb in the UV region. Early problems of non linear detection appears to have been resolved in newer ELSD models. HPLC with ELSD may be suitable for sorbitan ester analysis.

**1.5.5 Capillary Supercritical Fluid Chromatography:**

Capillary Supercritical Fluid Chromatography (CSFC) is a chromatographic technique which uses supercritical fluids as the mobile phase and a narrow bore capillary column with immobilised stationary phase. The high solvating power of the supercritical fluids enables elution of higher molecular weight components at temperatures much lower than those used for high temperature GC. Wang and Fingas (82) investigated the use of capillary supercritical fluid chromatography parallel to HPLC for the analysis of sorbitan esters. The chromatography was performed on a SB-Biphenyl –30 column equipped with an integrated on column frit restrictor and detection was by FID operated at 350°C. SFC grade carbon dioxide was used as the mobile phase and separation was achieved using an isothermal linear pressure programme. The sorbitan esters were separated into five groups, consisting of starting materials, mono-, di-, tri- and tetra esters, with each group consisting of a number of peaks representing different isomers. The chromatogram obtained for Span 80 is

shown in figure 1.10, the numbers 0 to 4 represent the starting material and sorbitan mono, di, tri and tetra esters respectively. Each group consists of a number of partially unresolved peaks, however it was felt to be considerably better than that obtained by HPLC.

As with previous work on sorbitan esters, assumptions were made with identification due to the lack of availability of pure standards and quantitation was carried out using response factors based on those obtained for a range of glycerides. It was concluded that in comparison to HPLC, CSFC broadens the scope of the technique to higher molecular weight sorbitan polyesters while maintaining high separation efficiency.

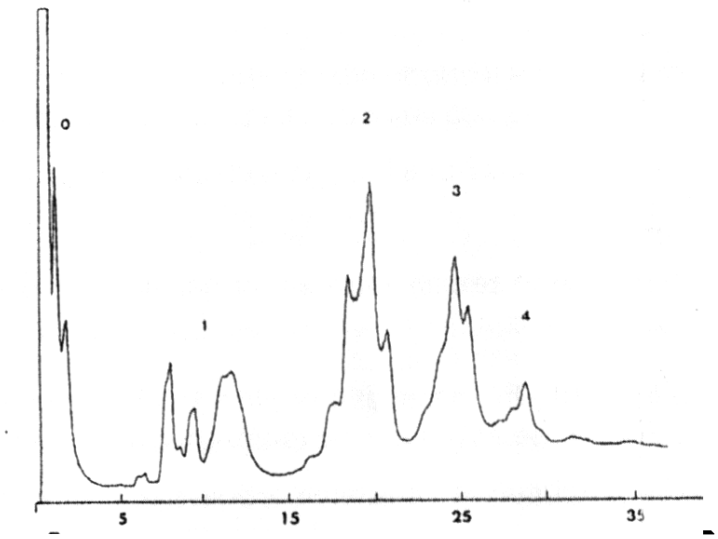



**Figure 1.10: Capillary SFC Chromatogram Obtained for Span 80 by Wang and Fingas (82)**
0 = Starting Material, 1 = Mono Esters, 2 = Di Esters, 3= Tri Esters, 4 = Tetra Esters

CSFC was used by Hunt *et al.* (83) to analyse a sample of sorbitan laurate, which resulted in a chromatogram with a number of partially resolved peaks. Super critical fluid extraction of parts of the sample matrix was used to investigate the composition

of the matrix, by separating it in to constituent fractions that were easier to analyse. The main fractions were analysed by CSFC and this showed that they were simpler than the original sample. The fractions were analysed by $^{13}C$ NMR and this showed that fractionation into four distinct classes occurred, including cyclic monoesters, cyclic di and tri esters, linear esters and a highly polar unidentified fraction. The study demonstrated that supercritical fluid extraction can be used for sample preparation for characterisation of complex mixtures.

### 1.5.6 Gas Chromatography (GC):

Gas Chromatography is a chromatography technique used to analyse complex mixtures of volatile organic and inorganic components, separation occurs due to partitioning of the solute between the moving gas and stationary phase and has been used to analyse polyol esters.

In 1965 Suffis *et al.* (84) presented a GC method for the analysis of some non-ionic surfactants as trimethyl silyl ether derivatives, the hydroxyl groups contained in the surfactant was reacted with hexamethyldisilazone and trimethylchlorosilane to give a more volatile compound suitable for GC analysis using a packed column. Comparison of derivatised surfactants to non derivatised showed the significant improvement derivatisation made. The work concentrated largely on glycerides, although the technique was also tried with several sorbitan esters, resulting in a number of partially resolved peaks being observed. However no attempt was made to assign the peaks, therefore it is uncertain which components eluted and which did not.

GC has also been used to determine sucrose fatty acid esters as TMS derivatives (85). The method involved degradation of the sucrose esters to sucrose and fatty acids, with each then being determined by GC after derivatization. Therefore no separation of the various esters was obtained using this method, just the ratio of the sucrose and the various fatty acids.

Uematsu *et al.* (12) used GC to determine sucrose ester, however only the mono esters were of interest, therefore higher esters were removed by column chromatography. The resulting mono esters were acetylated and then analysed by conventional GC with a wide bore column and splitless injection. The GC work found that sucrose mono esters of $C_{12}$, $C_{14}$, $C_{16}$, $C_{18}$ and $C_{18-1}$ are used as food additives.

A combined LC-GC technique was used (37) for the analysis of polyglycerol fatty acid esters, which are non-ionic surfactants with various applications in the food, cosmetic and the pharmaceutical industry. The samples were fractionated by LC and pure standards of monoester and diesters of diglycerol and triglycerol were obtained, using LC-MS for confirmation. Confirmation of most di esters and cyclic glycerol were proposed. Isolation of the pure esters and subsequent GC analysis indicated that many components coelute and therefore it is necessary to prefractionate by LC to characterise esters correctly and therefore is a lengthy method. However the work concluded that in combination with other chemical analyses a complete profile of the chemical composition of polyglycerol fatty acid esters can be obtained.

GC and GC-MS have been used to analyse a range of crude galactose and xylose monoesters prepared by lipase catalysis (13). The chromatogram obtained for

galactose fatty acid esters showed sharp well resolve peaks for both the fatty acid and galactose, however only three very broad peaks were observed, which were identified as esters. It is possible that other esters were produced but were not volatile enough to be eluted from the GC column. GC analysis of food grade sorbitan esters was undertaken to establish a reference point and again only the starting materials were well resolved. The GC chromatogram obtained for sorbitan mono laurate showed a range of unidentified partially resolved peaks in addition to the fatty acid peaks, however for sorbitan mono oleate, the early eluting components were identified as fatty acids, later eluting components thought to be due to polyol esters were not identified. GC analysis revealed the presence of a large number of individual components (up to 65), many were identified by mass spectrometry as various isomers of sorbitan, isosorbide and their mono and di esters. Ten isomers of sorbitan were identified based on their molecular ions. This analysis was carried out using a maximum oven temperature of 300°C and injection and detector temperature of 285°C.

Giacometti *et al.* (86) monitored the esterification of sorbitol and lauric acid by GC, with and without previous sorbitol cyclisation, although only the concentration of residual fatty acid was determined using GC. The samples were derivatised to produce trimethylsilyl ether and ester derivatives suitable for GC analysis and then analysed using a packed column, injection and detector at 240°C and a maximum oven temperature of 220°C. The chromatogram obtained for sorbitan mono laurate showed characteristic sharp peaks for sorbitan and lauric acid and broader peaks for the mono esters but no higher esters were observed, this is probably because they are insufficiently volatile to elute from the column. However the results confirmed the

possibility of monitoring lauric acid and sorbitol esterification via GC determination of lauric acid concentration.

High temperature-GC (HT-GC) starts at the upper working temperature of conventional GC (330-350°C) it has been made possible by the combination of thermostable bonded stationary phases with a range of columns capable of withstanding the elevated temperatures. HT-GC has been used to characterise high boiling petroleum products, triglycerides, sterol esters and polyglycerols plus other high molecular weight samples including antioxidants and stabilisers (87). Apolar and medium polar gum phases can be operated at temperatures of 400 to 420°C, allowing the analysis of thermally stable organic compounds with molecular weights as high as 1700 amu.

HT-GC and high oven temperature cold on column injection has been used to analyse triglycerides (88) because qualitative and quantitative determination of triglyceride mixtures is important in a variety of fields. An important aspect is quantitative loss of triglycerides in HT-GC by thermal decomposition or polymerisation. The quality of the column and the injection technique are critical for high precision and accuracy. HT-GC was used to analyse tranesterified cocoa butter, cold on-column injection was used with a high oven temperature, however this involved modification of the cold on-column injector with a secondary cooling. The oven temperature was 300°C, increased to 340°C after injection. The analysis showed good repeatability and accuracy and illustrated the potential of the technique.

Cold on-column and programmed temperature (PT) split/splitless injections have been used for a number of HT-GC analysis. Losses above nC60 were observed with PT splitless injection, thought to be due to purging the PT inlet too soon, therefore resulting in incomplete sample vapourisation and this was also found with hot splitless injection. An investigation was carried out to compare cold on-column and PT split/splitless injection for the analysis of polywax 655, n-30 hydrocarbons and triglycerides. PT split injection was preferred over PT splitless for materials above C62 and it has advantages over cold on-column injection for cleaning. It was found (89) that PT split and cold on-column sampling gave equivalent recoveries of materials up to C78, but optimisation of programmed temperature splitless sampling is required. The thermal effects in the column of pure triglycerides were also part of the investigation by varying the temperature programme rate and the gas velocity for a synthetic mixture of triglycerides. Little effect was noted on recoveries by changing the temperature but changing the gas velocity did. A higher linear velocity and thinner stationary phase was found to improve elution of sensitive compounds at temperatures of 400°C, however a reason for the losses is not clear but it is thought it may be due to decomposition.

HT-GC has also been used to analyse sucrose esters (90), the samples were silylated prior to analysis on a number of commercially available high temperature columns, with separation according to boiling point on non-polar phases. A temperature programme was used with a start temperature of 70°C, held for 4 minutes and then increased at 10°C/min to a final temperature of 400°C. The study found the aluminium-clad and HT-polyimide columns to be less suitable compared to the glass and metal columns because of their limited stability at high temperature. The method

was found to be considerably better than conventional GC and the range of compounds amenable to GC widened. However the method was limited to compounds with volatilities less than that of sucrose hexalaurate (MM = 1597.2) and tristearate (MM = 1501.2). However an alternative derivatisation method may extend the range of esters which HT-GC may be applicable to and make it possible to analyse sorbitan esters by HT-GC.

In 1998 Aichholz and Lurbeer (91) investigated the separation of triglycerides by HT-GC using seven different stationary phases. The main limitation of HT-GC is the thermal degradation of triglycerides with a high degree of unsaturation. Three triglycerides were analysed on the seven phases to establish elution order and temperature, three columns were found to offer the best separation and these columns had a dependence on retention on the number of double bonds. It was noted that the elution temperature of individual lipid species could vary significantly depending on the stationary phase. Seed oil of Jatropha multifida was then analysed to illustrate the selectivity of two selected stationary phases (SOP-50 and OV-225-OH). OV-225-OH makes possible both reduced retention time for neutral lipids and increased selectivity for separation of species possessing the same acyl number but different unsaturation, however the drawback was an upper temperature of 380°C, because above this extensive column bleed resulted. The study concluded that the problem of thermal decomposition of highly unsaturated triglycerides cannot be resolved with the stationary phases used in the study.

In 2000 Pereira and Aquino (92) evaluated nine high temperature commercial capillary columns (aluminium clad and polyimide –coated fused silica) for their

thermal stability, activity and dependence on column temperature. A Grob mixture was analysed, along with a mixture containing a range of triglycerides with masses ranging from 218 daltons for triacetin to 1059.8 daltons for tribehenin. The columns were operated using a temperature programme with a maximum temperature of 400°C held for 20 minutes for the triglycerides, however not all columns were able to elute tribehnin. The work showed that the column bleed was higher in columns with a greater quantity of phenyl groups in the stationary phase and when the stability of the capillary external coating was reviewed, it was found that it was poor for the aluminium clad columns. The results also showed that high temperature, high resolution GC capillary columns can be used at high temperatures up to 420°C, but not with long isothermal temperatures. It was felt further development of more stable and inert capillary columns would be required to push GC to its limit of 420°C. However it was concluded that commercial high temperature capillary columns can be used at 390 – 420°C without major problems, except a decrease in column lifetime and that this opens up a new window for molecular masses that can be analysed. It was felt this was very important for various fields of science and the discovery of new compounds.

The review that has been carried out on HT-GC indicates it may be a possible technique for the separation of the various components found in sorbitan esters.

**1.6 Conclusion:**

The literature reviewed highlights the complexity of analysing polyol esters and specifically sorbitan esters, which are to be investigated in this study. However despite the difficulties encountered polyol fatty acid esters have widespread

applications and this leads to the continued research in to the synthesis and analysis of these ester products.

HPLC is to be the first chromatographic technique to be selected for separating the various components of sorbitan esters, because unlike GC it is not limited by sample volatility and thermal stability. As sorbitan esters do not contain a chromaphore it is felt that the use of an evaporative light scattering detector for HPLC rather than a UV detector may be advantageous.

Although TLC was found to be relatively successful for the analysis of sucrose esters, TLC has limitations in that the mobile phase moves by capillary forces and therefore is not as controlled as with GC or HPLC. Also TLC is not one of the better techniques for quantitation and for accurate work expensive scanning equipment is required.

GC will be the second analytical technique investigated, the development of HT-GC has resulted in the characterisation of high molecular weight complex components including triglycerides and sucrose esters and therefore may be suitable for sorbitan esters.

## 2. Initial HPLC Work for the Analysis of Sorbitan Mono Oleate:

HPLC is a popular method of chromatography because it is not limited by volatility or stability of compounds and is used for separation, identification and quantification of compounds. HPLC was therefore the initial analytical technique selected for the separation of sorbitan esters. Sorbitan esters are expected to have a range of components of varying size and polarity and therefore volatility, it was initially thought that due to this GC may be problematic. Sorbitan mono oleate is a major sorbitan ester manufactured by Croda based on oleic acid. It is used in a number of health and personnel care applications as well as the emulsion explosives market. An analytical technique which gives more information on the structure of sorbitan esters is required to follow the manufacturing process of sorbitan esters and it may lead to a better understanding of structure activity relationships.

### 2.1 Samples:

The sample of interest in this experiment sorbitan mono oleate (SMO), is usually manufactured from sorbitol and fatty acids which gives a range of polyol esters, the fatty acid feedstock used also contains a range of fatty acids. The product is therefore a complex material with a large number of components, for this work a sample of SMO was produced from a high purity fatty acid feedstock to give material with a similar polyol profile and ester distribution as the manufactured material but simplified by not having a mixed fatty acid feedstock and therefore making analysis more straight forward.

Before beginning HPLC method development, it is important to know information concerning sample composition and properties, which may include the number of components present, chemical structure of compounds, molecular weight of compounds, pKa values of compounds, concentration range of compounds in the sample of interest and sample solubility.

Sorbitan esters are known to consist of a large range of components of varying polarity and molecular weight, present at varying concentrations. It was therefore felt necessary to gain some information about the single polyol esters of oleic acid prior to HPLC analysis of a sample containing them. Samples of isosorbide oleate, sorbitan oleate, isosorbide dioleate and sorbitol oleate were therefore prepared from high purity oleic acid by Dr C Howarth (Croda). The samples were also to be used as standards to aid identification of components found in the sample of sorbitan mono oleate prepared from high purity oleic acid.

A sample of manufactured sorbitan mono oleate prepared from a mixed fatty acid feedstock was also obtained, so comparison could be made to the high purity fatty acid mixed polyol material.

**2.2 Analysis of Samples for Polyol Profile and Ester Distribution:**

The current methods used by Croda to gain information about the composition of the sorbitan ester samples are polyol profiles and Gel Permeation Chromatography (GPC) and therefore were carried out on the samples detailed in chapter 2.1.

**2.2.1 Polyol Profile:**

The polyol profile determines the levels of sorbitol, sorbitan and isosorbide present in a sample. The sorbitan ester is saponified, the fatty acids removed and the polyols derivatised for subsequent GC analysis. The procedure used is:

3 ± 0.1 g sample weighed into a 250 ml flat bottomed flask and 35 ml methanolic KOH (50 g KOH in 1000 ml anhydrous methanol) reagent added and refluxed for 45 minutes. To the above mixture 35 ml MeOH/$H_2SO_4$ reagent added (100 g $H_2SO_4$ in 1000 ml anhydrous methanol) and refluxed for a further 45 minutes, then 120 ml water added. This is transferred to a 500 ml separating funnel and washed with 3 x 100 ml diethyl ether, discarding the ether layer. The resulting aqueous layer is neutralised to pH 6.5 – 7.5 and evaporated to dryness using a rotary evaporator.

To the above residue 5 ml pyridine and 10 ml acetic anhydride are added and brought to the boil, shaking to dislodge any residue adhering to the sides of the flask and refluxed for 1 hour. 0.3 µl of the resulting mixture is injected on to the GC using the following conditions:

| | |
|---|---|
| Column: | TAP-CB, 0.25 mm id, length 25 m |
| Detector type: | FID |
| Injection temperature: | 350°C |
| Detector temperature: | 380°C |
| Initial oven temperature: | 120°C |

Final oven temperature: 260°C

Ramp rate: 8°C/minute

Carrier gas: Helium

The polyol profiles of the samples detailed in chapter 2.1 were determined and they were found to contain (%):

| | SMO ex Croda | SMO | Sorbitan Oleate | Isosorbide Dioleate | Sorbitol Oleate | Isosorbide Oleate |
|---|---|---|---|---|---|---|
| Isosorbide | ~23 | ~13 | ~5 | >99 | ND | >99 |
| Sorbitan | ~75 | ~78 | ~91 | ND | <1 | <0.5 |
| Sorbitol | ~2 | ~9 | ~4 | <1 | >99 | <0.5 |

ND = None Detected

**Table 2.1: Polyol Profiles of Polyol Ester Samples**

The analysis confirmed that the material prepared from high purity oleic acid with a mixed polyol profile did have a mixture of polyols and was similar to the manufactured sorbitan mono oleate, although it contained slightly different levels of isosorbide and sorbitol. The isosorbide and sorbitol ester samples were found to contain essentially one polyol and the sorbitan oleate prepared to have a high sorbitan content, was found to contain >90 % sorbitan. The material was produced by dehydration of sorbitol, however 100 % conversion to sorbitan did not occur, although the material does still contain a high level of sorbitan.

### 2.2.2 GPC Analysis:

GPC separation is based on the size of the components, it is regularly used by Croda to determine the ester distribution of sorbitan mono esters. From this the sorbitan mono:isosorbide mono ester and higher:mono ester ratios are calculated, because they are believed to give information on the degree of dehydration and esterification.

The samples detailed in chapter 2.1 were analysed using the following conditions:

| | |
|---|---|
| LC System: | Gilson pump |
| | Philips PU4026 refractive index detector |
| Columns: | Two 300 x 7.8 mm Waters Styragel columns (HR1.0 and HR0.5) connected in series |
| Injection volume: | 20 µl |
| Mobile Phase: | Tetrahydrofuran (HPLC grade) |
| Flow rate: | 0.875 ml/min |

The columns used are Waters Styragel columns which are packed with high performance fully porous highly cross linked styrene divinylbenzene copolymer particles. The HR range selected are used for high resolution analysis of low molecular weight polymers, oligomers, additives etc. The HR 1.0 column has an effective molecular weight of 100 to 5000 daltons and the HR0.5 an effective range of 0 to 1000 daltons.

The samples analysed were quantified based on area normalisation, without any response factors being taken into consideration.

Using area normalisation the samples were found to contain (%);

| | SMO ex Croda | SMO | Sorbitan Oleate | Isosorbide Dioleate | Sorbitol Oleate | Isosorbide Oleate |
|---|---|---|---|---|---|---|
| Tri/higher | ~25 | ~35 | ~45 | - | 25 to 30 | - |
| Di esters | ~35 | ~40 | ~35 | ~88 | ~30 | 50 to 55 |
| Sorbitol mono esters | - | - | - | - | 25 to 30 | |
| Sorbitan mono esters | 15 to 20 | ~15 | ~15 | - | - | - |
| Isosorbide mono esters | ~10 | ~6 | ~3 | - | - | ~25 |
| Acid | ~5 | ~1 | ~1 | - | ~15 | ~15 |
| Sorbitan | ~2 | ~2 | ~2 | - | - | - |
| Isosorbide | 2 to 3 | ~1 | <1 | - | - | ~1 |
| Others | - | - | - | ~12 | - | - |

**Table 2.2: Results of GPC Analysis of Polyol Ester Samples**

The mixed polyol high purity fatty acid sorbitan mono oleate was found to have a similar ester distribution to the manufactured material. The high sorbitan SMO was also similar, this is due to a mixture of polyols being present despite sorbitan levels being higher than in manufactured material. The isosorbide dioleate was found to have a high level of di esters and the isosorbide oleate a mixture of mono and di esters as well as starting material. The sorbitol oleate contained a range of esters and free acid.

From the polyol and GPC analysis it is apparent that sorbitan esters will consist of a range of components of varying polarity, present at varying concentrations, as expected.

**2.3 Solubility Study:**

The solubility of the various polyol oleate esters present in sorbitan mono oleate has not been investigated and for HPLC the sample must be soluble in the mobile phase and if a gradient is used, remain soluble throughout the entire eluent composition. Due to the fact solubility of the sample is important, a study was carried out to establish the solubility of various polyol oleate esters in a range of solvents used for HPLC analysis. It was envisaged that the sample to be analysed by HPLC would be prepared at 0.1 % in solvent, therefore a higher concentration was used for the solubility study to ensure solubility at 0.1%.

A solubility study was carried out on all the samples described in chapter 2.1 prepared from high purity oleic acid, as well as isosorbide and sorbitol (ex Aldrich) which are starting materials and therefore could be present in the material and a sorbitan mono oleate manufactured by Croda using a mixed fatty acid feedstock. 0.25 % solutions were prepared of each sample using the following solvents hexane (Fisher – HPLC grade), dichloromethane (Fisher – HPLC grade), tetrahydrofuran (VWR – HPLC grade), ethyl acetate (Fisher – HPLC grade), acetonitrile (Fisher – HPLC grade), isopropanol (Fisher – HPLC grade), methanol (Fisher- HPLC grade) and water (Ronil – HPLC grade). A visual observation was made of each solution to establish if the sample was soluble, dispersable, partially soluble or insoluble in the solvent.

The solubility of each sample in each solvent is shown in table 2.3.

The results show that:

- all the samples in the study, with the exception of sorbitol were soluble in isopropanol and tetrahydrofuran. These are therefore good solvents to select for the initial HPLC analysis.
- a number of the samples were also soluble in hexane, which could be used as the less polar solvent to alter the polarity of the mobile phase.
- the polyol ester samples were found to be dispersible in water, but not fully soluble as expected, this is due to the fact the molecules consist of a hydrophilic portion which will be soluble in the water and a hydrophobic portion which will not.

| | SMO Ex Croda | SMO | Sorbitan Oleate | Isosorbide Dioleate | Sorbitol Oleate | Isosorbide Oleate | Isosorbide | Sorbitol |
|---|---|---|---|---|---|---|---|---|
| Hexane | √ | √ | √ | √ | X | √ | X | X |
| Dichloromethane | √ | √ | √ | √ | X | √ | √ | X |
| Tetrahydrofuran | √ | √ | √ | √ | √ | √ | √ | X |
| Ethyl acetate | √ | √ | X | √ | X | √ | √ | X |
| Acetonitrile | P | P | P | √ | √ | P | X | X |
| Isopropanol | √ | √ | √ | √ | √ | √ | √ | X |
| Methanol | X | X | X | X | X | X | √ | √ |
| Water | D | D | D | D | D | D | √ | √ |

D – dispersible P – partially √- complete solubility X - insoluble

**Table 2.3: Solubility of a Range of Samples in Commonly Used HPLC Solvents**

**2.4. Column Selection:**

The choice of column and mobile phase is important in the development of a HPLC method. A number of columns are available including reversed and normal phases.

Sorbitan mono oleate, prepared from high purity (>99 %) oleic acid was chosen for the initial work because it has a similar polyol and ester distribution to a commercial SMO, but is simplified by not having a mixed composition of fatty acids**.** The separation obtained for the sample using a range of columns and mobile phases was to be compared and from this information the best column and mobile phase selected for further investigation.

The following was used for the initial HPLC analysis:

| | |
|---|---|
| LC System: | HP1050 |
| | HP Interface 35900E |
| | Sedere Sedex 75 ELSD (Gain 6, P 2.2 bar, Temperature 35°C) |
| Injection volume: | 20 µl |
| Flow rate: | 1 ml/min |

Separation of the various components was to be compared using various conditions, with no identification considered.

### 2.4.1 Reverse Phase Chromatography (RPC):

A reversed phased column was the first column selected for LC analysis because it is more rugged than other forms of LC and was thought to be the most likely to give a satisfactory final separation.

From the solubility study, it was shown the samples of interest are not soluble in water, therefore non-aqueous reversed phase (NARP) HPLC was tried. For NARP the mobile phase is a mixture of a more polar (A) and less polar (B) organic solvent, sample retention is controlled by varying the percentage of B, as with RPC. The A solvent selected was IPA and B solvent THF, these were selected because they were the most polar solvents the sample was readily soluble in.

The following were used:

Column: Zorbax C18 5 µm 4.6 x 250 mm, operated at 40°C

Mobile phase: 100 % IPA to 100 % THF in 30 minutes.

The chromatogram shown in figure 2.1, is a typical chromatogram showing poor resolution.

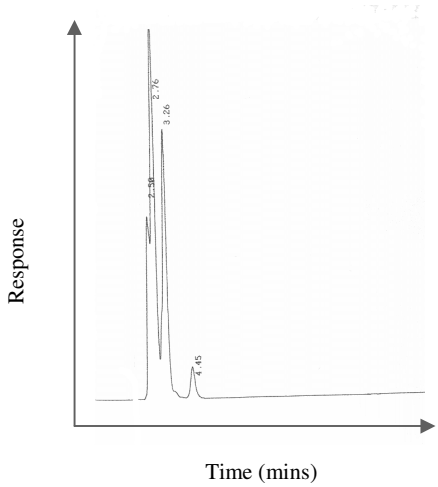



**Figure 2.1: HPLC Chromatogram of SMO Prepared from High Purity Oleic Acid Using NARPC**

Four peaks are observed all of which are eluted in less than 5 minutes. The sample is known to have considerably more components than this, which indicates components are coeluting. Reversed phase separation involves a partitioning of the solutes between the non-polar stationary phase and polar mobile phase. The equilibrium constant for this is called the partition ratio or coefficient and is defined as K = Cs/Cm, where Cs is the concentration of the analyte in the stationary phase and Cm the concentration in the mobile phase. The components in the sample have favoured the mobile phase and passed through the system with little partitioning occurring. To increase retention and therefore resolution of the components present in the sample a more polar mobile phase is required, however this would require the use of another solvent, such as methanol or ideally water, which the sample is not soluble in. Therefore it would appear that if sorbitan mono oleate components are to be separated by HPLC, normal phase will have to be used.

### 2.4.2 Normal Phase Chromatography – Isocratic:

In normal phase the stationary phase is more polar than the mobile phase, a commonly used packing is silica. Separation using bonded stationary phases have started replacing many that were carried out on silica columns, because they show less tailing and respond more rapidly to changes in mobile phase composition. They can also show different selectivities depending on the polar bonded group and gradient elution can be used. Weakly polar bonded phases include diol and cyano, where as more polar types contain amino groups. Cyano is one of the most commonly used bonded phases, due to its rugged packing, followed by diol and amino, however amino columns are less stable than the others (93). Sorbitan mono oleate prepared from high

purity oleic acid was analysed using a range of normal phase columns and mobile phases.

#### 2.4.2.1 Silica Column

Due to the wide range of polarity expected in the sample, it would be ideal to change the polarity of the mobile phase during the separation, however gradient elution is avoided with silica columns, particularly if the two solvents are very different in polarity, due to solvent demixing.

The sorbitan mono oleate prepared from high purity oleic acid under investigation was found to be soluble in hexane and isopropanol, the sample was therefore analysed using a series of mobile phases based on hexane/IPA. In this approach the first mobile phase selected was the most polar, so that long run times were not involved, the amount of hexane was increased sequentially to decrease the polarity of the solvent and therefore increase the retention of the solutes. The effect of polarity on separation could then be observed.

A similar approach was carried out with hexane/THF, because the sample is also readily soluble in tetrahydrofuran.

A 250 x 4.6 mm silica column, operated at room temperature (20°C) was used with the following isocratic mobile phases hexane/IPA 20/80 v/v, 50/50 v/v, 80/20 v/v, 85/15 v/v, 90/10 v/v, 95/5 v/v and hexane/THF 20/80 v/v, 50/50 v/v, 80/20 v/v, 85/15 v/v, 90/10 v/v.

Hexane/IPA series:

The chromatograms obtained for the sample of sorbitan mono oleate prepared from high purity oleic acid and analysed using hexane/IPA as the mobile phase in varying ratios can be seen in figures 2.2 and 2.3. Using the most polar mobile phase hexane/IPA 20/80 v/v, essentially no separation was obtained, one broad peak is obtained with a smaller peak eluting later, however both eluted in less than 5 minutes. The components of the sample have not been well retained by the column. It can be seen that by increasing the hexane concentration and therefore decreasing the polarity of the mobile phase, some adsorption occurs and slightly better separation is obtained up to hexane/IPA 90/10 v/v when a number of partially resolved peaks are obtained. The number of peaks observed is still significantly less than the number of components expected in a sample of SMO, indicating a large number of components are coeluting.

When a mobile phase of hexane/IPA 95/5 v/v was used to elute the sample, the number of peaks decreased, this is probably due to the mobile phase not being polar enough and components being adsorbed strongly by the column and not eluted.

Hexane/THF:

The chromatograms obtained using the series of mobile phases based on hexane/THF are shown in figures 2.4 and 2.5. As observed with the previous mobile phase, essentially no separation of the various components was obtained with the most polar mobile phase, but separation began to increase as the polarity of the mobile phase decreased, up to hexane/THF 85/15 v/v. With a mobile phase of hexane/THF 85/15 v/v distinct groups of partially resolved peaks can be seen, with a broad peak observed

later (~20 minutes). The peaks obtained using hexane/THF 90/10 v/v are well resolved, however the number of peaks is less than with the previous mobile phase, indicating that components are being retained by the column.

Using the two series of mobile phases with a silica column, the best separation for the SMO sample was obtained using a mobile phase of hexane/THF 85/15 v/v, however the peaks were not base line resolved.

Capacity Factor:

The capacity factor (k') describes the migration rate of an analyte through a column, the capacity factor for the first peak observed using the various mobile phases was calculated. The first peak was selected because no identification of the components was attempted, therefore if other peaks were selected there would be uncertainty that they were due to the same component and comparison of the capacity factor for a solute with differing mobile phases was required. The capacity factor requires the retention time of an unretained solute, because an ELSD was used the solvent was not detected, therefore it was necessary to estimate it (94) using the equation

$$t_o \sim 0.5L\, d_c^2/F$$

where L is the length of the column (cm), $d_c$ is the internal diameter of the column (cm) and F is the flow rate (ml/min). The capacity factors for the first peak to elute using the various mobile phases are shown in table 2.4.

The k' values for all the mobile phases are small, indicating retention times are short for the first eluting component. When k' is less than one, elution occurs so rapidly, obtaining an accurate retention time can be difficult, as observed for the first

component. Ideal separation is performed under conditions in which k' for solutes in a mixture lie in the range 2 to 10, values of 20 to 30 results in the elution time being too long (95). The k' values obtained are also all very similar indicating that changing the mobile phase has had no or little effect on the retention of the first eluting components.

| Mobile Phase | Capacity Factor |
|---|---|
| Hexane/IPA 20/80 v/v | 0.3 |
| Hexane/IPA 50/50 v/v | 0.4 |
| Hexane/IPA 80/20 v/v | 0.3 |
| Hexane/IPA 85/15 v/v | 0.3 |
| Hexane/IPA 90/10 v/v | 0.2 |
| Hexane/IPA 95/5 v/v | 0.2 |
| Hexane/THF 20/80 v/v | 0.2 |
| Hexane/THF 50/50 v/v | 0.3 |
| Hexane/THF 80/20 v/v | 0.3 |
| Hexane/THF 85/15 v/v | 0.3 |
| Hexane/THF 90/10 v/v | 0.3 |

**Table 2.4: Capacity Factor of the First Eluting Peak Using A Range of Mobile Phases**

#### 2.4.2.2 Cyano Column:

A cyano column was the first bonded phase column selected to investigate, because it is believed to be the most rugged and it is one of the most extremes of the bonded phases discussed compared to silica. A Supelco 4.6 x 250 mm cyano column was installed to the HPLC system. As a direct comparison to silica was required, the same series of mobile phases were used.

Figures 2.6 and 2.7 show the chromatograms obtained using the various mobile phases based on hexane/IPA and figures 2.8 and 2.9 the chromatograms obtained using the hexane/THF series. It can be seen that the chromatography is poor with essentially no separation achieved with any of the mobile phases.

**2.4.2.3 Diol and Amino Columns:**

As very little was observed with the cyano column it was decided to investigate the use of two other bonded phase columns, diol and amino. From the previous work, it was apparent that using very polar mobile phases of 20/80 v/v and 50/50 v/v hexane/IPA or hexane/THF very little separation was obtained. Therefore the study was started with 80/20 v/v hexane/IPA and hexane/THF as the most polar mobile phases and polarity decreased from here.

The following columns were used:

Diol column: Supelco Diol column 4.6 x 250 mm

Amino column: Nucleosil amino column 4.6 x 250 mm

Both columns were used to analyse the sample of sorbitan mono oleate prepared from high purity oleic acid, with mobile phases hexane/IPA 80/20 v/v, 85/15 v/v, 90/10 v/v, 95/15 v/v and hexane/THF 80/20 v/v, 85/15 v/v, 90/10 v/v.

Diol column:

The chromatograms obtained using hexane/IPA based mobile phases are shown in figure 2.10. The most polar mobile phase selected, resulted in very little separation, consisting of one group of partially resolved peaks, however as the polarity of the mobile phase was decreased the separation began to improve slightly. With a mobile phase of hexane/IPA 95/5 v/v a number of groups of partially resolved peaks were observed, however the peaks are not baseline resolved. The capacity factor for the first eluting peak was calculated for the four hexane/IPA mobile phases used and was 0.4 for all four, indicating decreasing the polarity of the mobile phase did not result in

a longer retention of the early eluting component. Also as seen with the silica column the k' value for the first eluting component was small, indicating little retention.

The chromatograms obtained using hexane/THF based mobile phases are shown in figure 2.11. Using hexane/THF 80/20 v/v two groups of two peaks are detected, as the mobile phase becomes less polar more resolution is obtained and the best separation observed was using hexane/THF 90/10 v/v, nine peaks are observed however the shape of the peaks indicate that they consist of a number of coeluting components. The capacity factor was again determined for the first eluting component and was 0.4 for the 80/20 v/v and 85/15 v/v mobile phases and 0.5 for the 90/10 v/v, indicating that slightly longer retention occurred with the 90/10 v/v mobile phase than with the other two.

Amino Column:

Using the amino column and the hexane/IPA mobile phases very little separation of the various components in the sample is achieved as shown in figure 2.12. When hexane/IPA 95/5 v/v was used it appears that fewer peaks are observed than with the previous mobile phase (hexane/IPA 90/10 v/v) indicating that not everything is eluting from the column. Using hexane/THF essentially no separation is achieved with any of the mobile phase choices, the chromatograms are shown in figure 2.13. One major peak is observed followed by a few smaller peaks for all three mobile phases studied. The k' value for the first eluting major peak was found to be small and similar for all the mobile phases, therefore again the changes in mobile phase had no effect on the retention of this component.

### 2.4.3 Normal Phase Chromatography – Gradient Elution:

Limited separation was obtained for the SMO sample using isocratic normal phase chromatography. Due to the range of polarity expected in the sample, changing the polarity of the solvent during the separation using gradient elution was felt to be advantageous. This was investigated for the bonded phase columns only because silica columns can not be used with a gradient due to solvent demixing.

The amino, cyano and diol columns previously used were operated at room temperature with the following gradients:

i) 100 % Hexane for 5 minutes to 100 % IPA in 30 minutes
   100 % IPA held for 5 minutes

ii) 100 % Hexane for 5minutes to 50/50 v/v hexane/ IPA in 30 minutes
   to 100 % IPA in 5 minutes (held for 5 minutes)

iii) 100 % Hexane for 5 minutes to 100 % THF in 30 minutes
   100 % THF held for 5 minutes

Amino Column:

The chromatograms obtained for the amino column using the initial gradients are shown in figure 2.14. Using the two gradients based on hexane/IPA very little separation was obtained, with only one major peak observed, with a capacity factor of 3.8 with the first gradient and 4.1 with the second gradient tried. The decrease in mobile phase polarity did increase the retention slightly but observation of the chromatogram showed it did not result in an increase in separation of components, because both gradients only showed a few small peaks after the main peak. However using the hexane/THF gradient a number of groups of partially resolved peaks were

observed, with a capacity factor of 0.4 for the first peak observed, showing that retention is very different to the IPA gradients because with the hexane/THF gradient the first component has little retention

Cyano Column:

The chromatograms obtained for the sorbitan mono oleate sample with a cyano column are shown in figure 2.15. The chromatography obtained was found to be poor. The mobile phases using hexane and IPA show a small broad peak followed by a group of peaks thought to consist of a number of partially separated components. The hexane and THF mobile phases show four groups of partially resolved peaks. However the sample is thought to consist of a number of components indicating very little separation is obtained. The baseline for all three chromatograms is also noisy.

Diol Column:

The chromatograms obtained for the sorbitan mono oleate with the diol column are shown in figure 2.16. Using the first hexane/IPA gradient, some separation was observed, with three small broad peaks observed followed by two groups of peaks. Using a slightly less polar gradient the separation is increased slightly with the second group of peaks better resolved than with the first gradient. However the hexane/THF gradient gives better separation, again three peaks are observed before 10 minutes and then a group of peaks, which appear to consist of partially resolved components. The capacity factor for the first eluting component was 0.4 for all three gradients, indicating the differing polarities had no effect on retention of the first eluting components, however observation of the chromatograms shows differences in later eluting components with the different gradients, however due to the lack of

identification of the peaks the capacity factor was not calculated for the other peaks due to uncertainty that the capacity factor for the same component was being determined and compared.

**2.5 Conclusion:**

From the preliminary work carried out using a range of columns to analyse the sample of sorbitan mono oleate, prepared from high purity (>99 %) oleic acid it can be concluded that:

i) A reverse phase column is not suitable. From the solubility study it was established that the expected components are not soluble in water and therefore water could not be used as a solvent in the mobile phase. However using non-aqueous reverse phase (NARP) HPLC, all the components eluted in less then 5 minutes, with very little separation.

ii) The cyano column gave poor chromatography, with both isocratic and gradient mobile phases and therefore is also an unsuitable column.

iii) Using the silica column, minimal separation was achieved, although with hexane/THF 85/15 v/v, two distinct groups of partially resolved peaks, with a few smaller peaks were observed. However when the polarity was decreased further in an attempt to increase absorption and therefore separation, less peaks were observed, indicating components were being retained by the column.

iv) Although little separation was achieved using the amino column with both the isocratic and hexane/IPA gradients, some separation was achieved using the hexane/THF based gradient. Therefore this may merit pursuing further.

v) With the diol column and the isocratic mobile phases 95/5 v/v hexane/IPA and 90/10 v/v hexane/THF, groups of partially resolved peaks were observed. Some separation was also obtained with all three of the screening gradients.

The diol column appears from the initial work to be the most promising, although the amino column using a hexane/THF gradient may also be worth pursuing. The HPLC chromatograms obtained with the amino and diol columns and hexane/THF gradient is already superior to that reported in the literature.

The following pages are chromatograms detailed earlier in the text. No identification of the peaks has been attempted, because the object of the study was to examine separation rather than identify components.

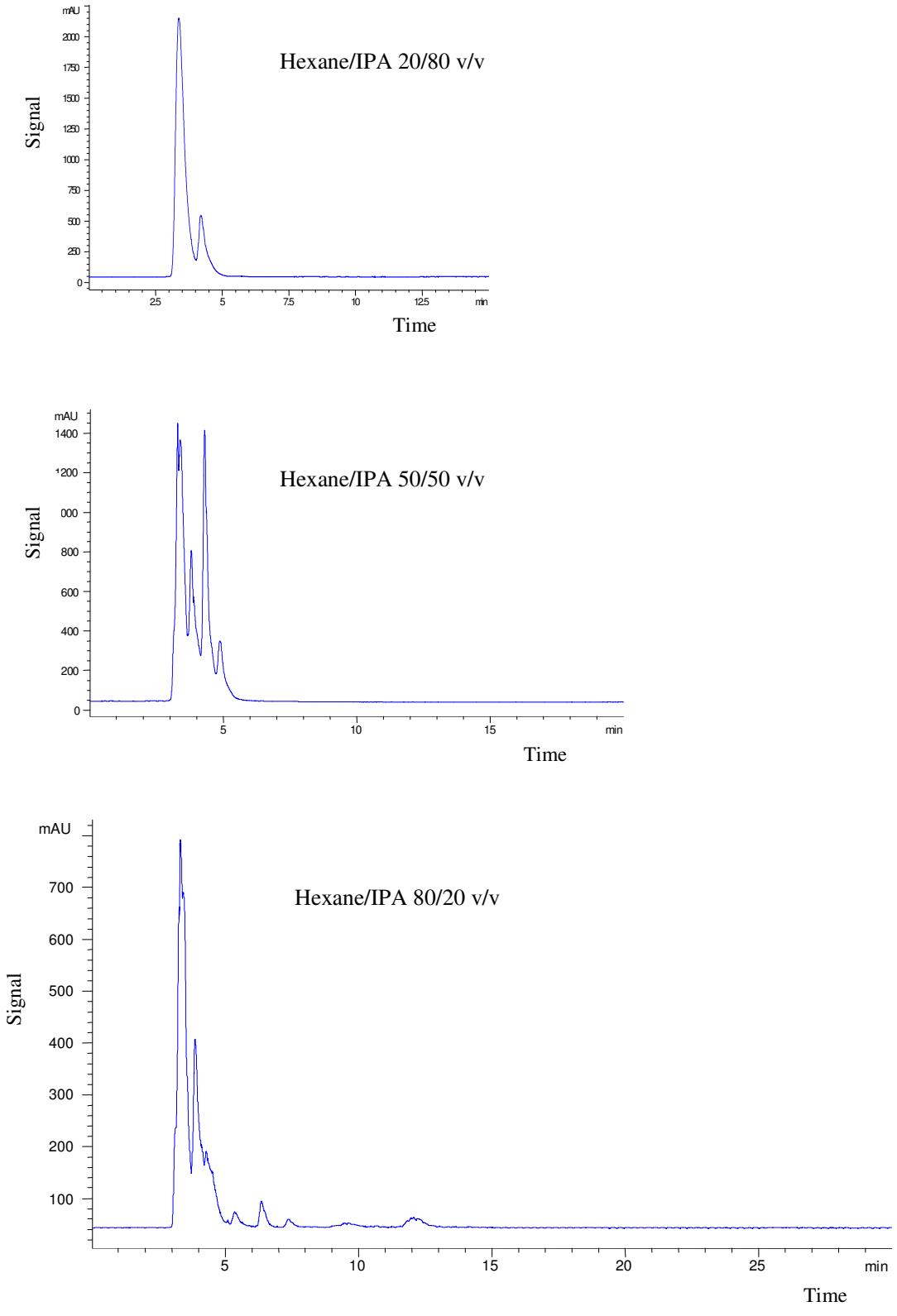



**Figure 2.2: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using a Silica Column and Hexane/IPA Mobile Phases**

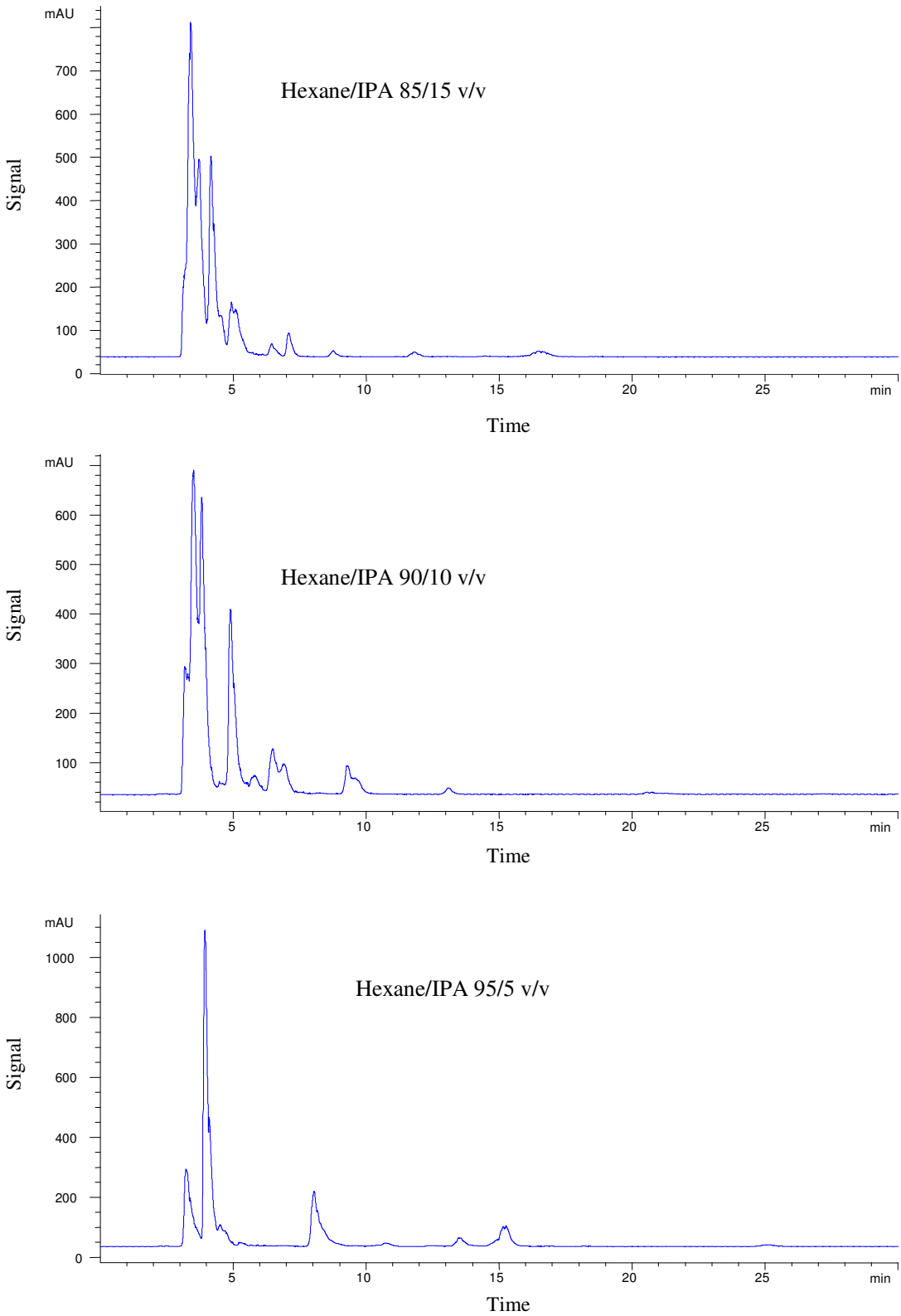



**Figure 2.3: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid, Analysed Using a Silica Column and Hexane/IPA Mobile Phases**

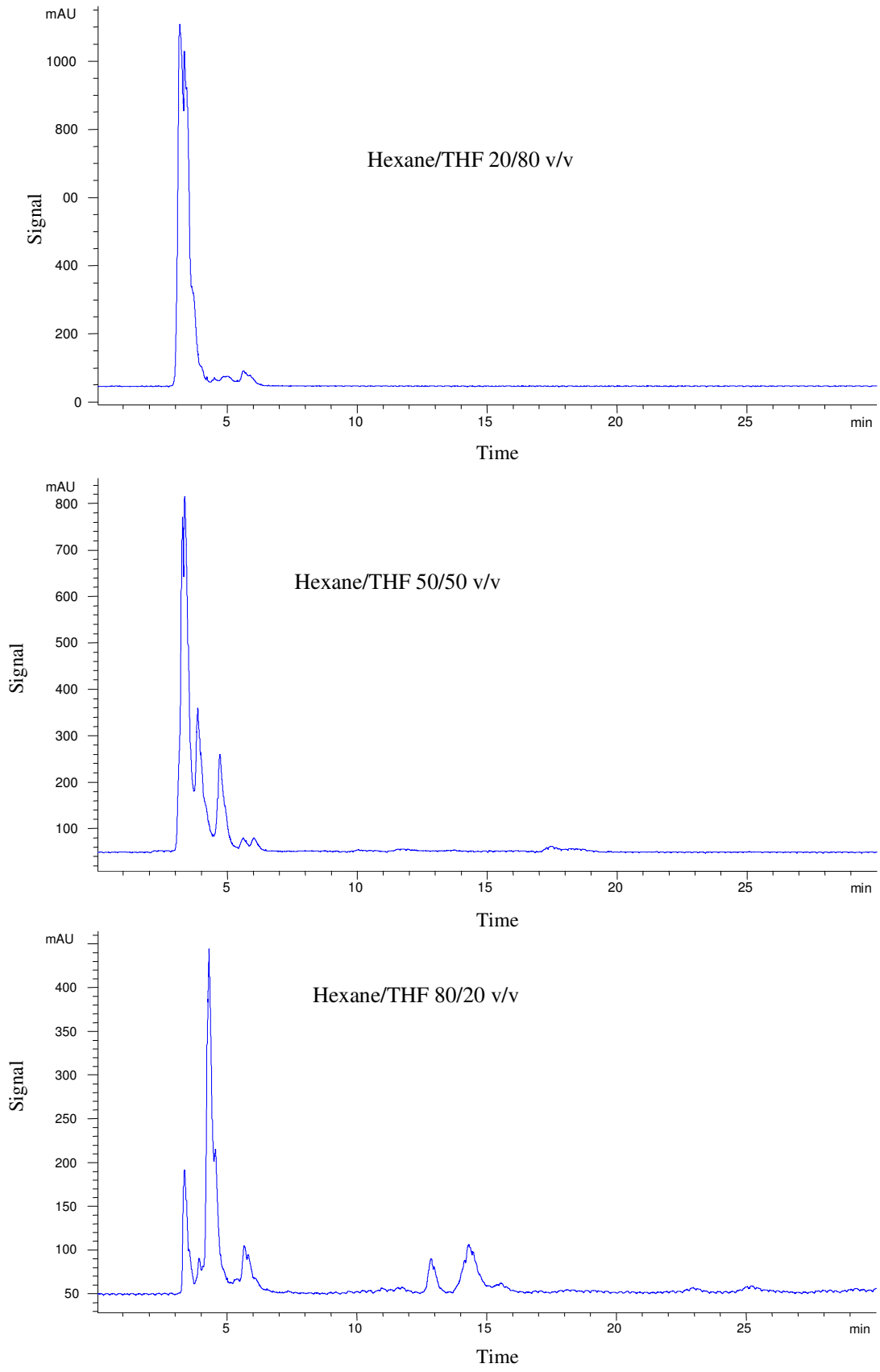



**Figure 2.4: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using a Silica Column and Hexane/THF Mobile Phases**

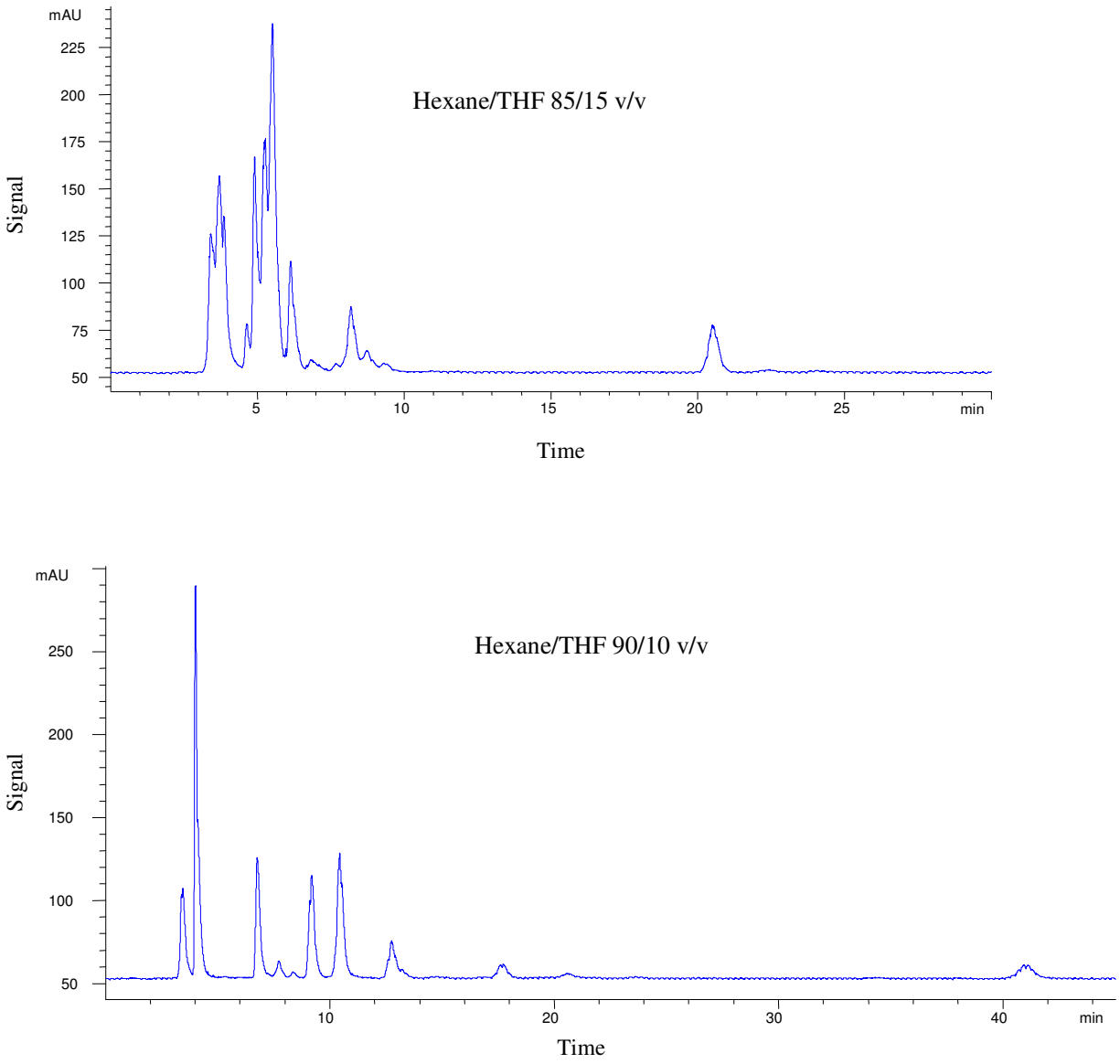



**Figure 2.5: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using a Silica Column and Hexane/THF Mobile Phases**

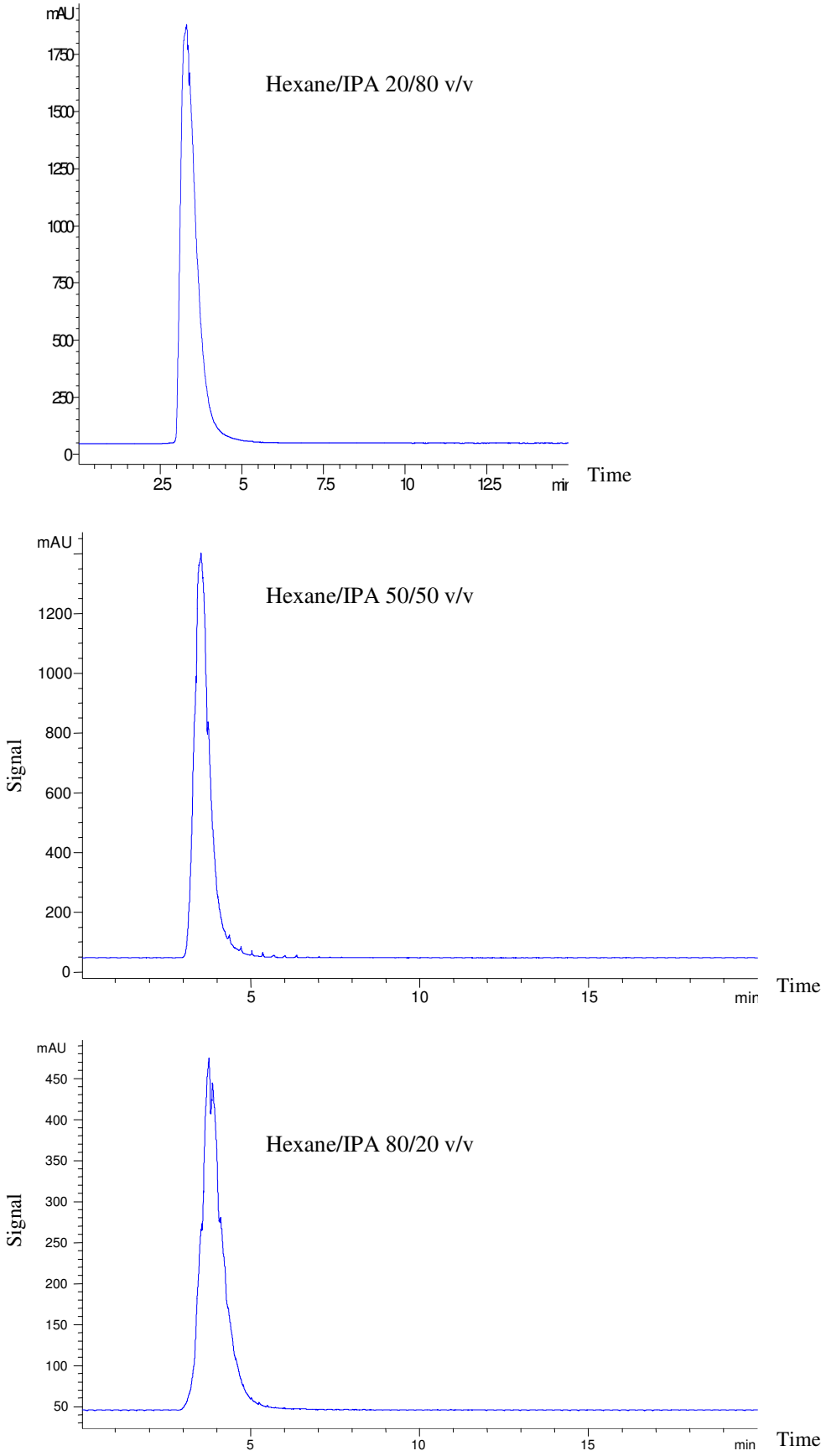



**Figure 2.6: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using a Cyano Column and Hexane/IPA Mobile Phases**

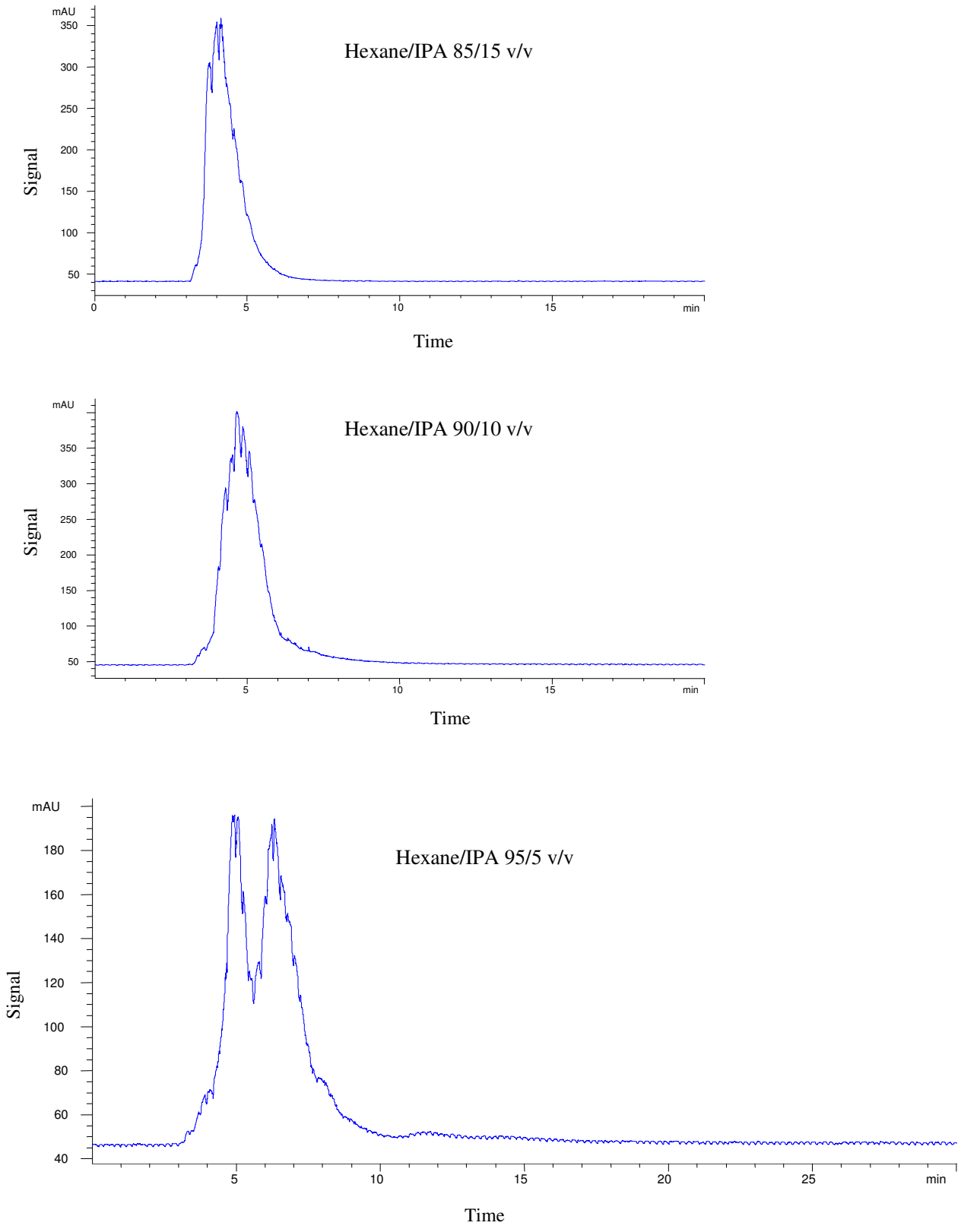



**Figure 2.7: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using a Cyano Column and Hexane/IPA Mobile Phases**

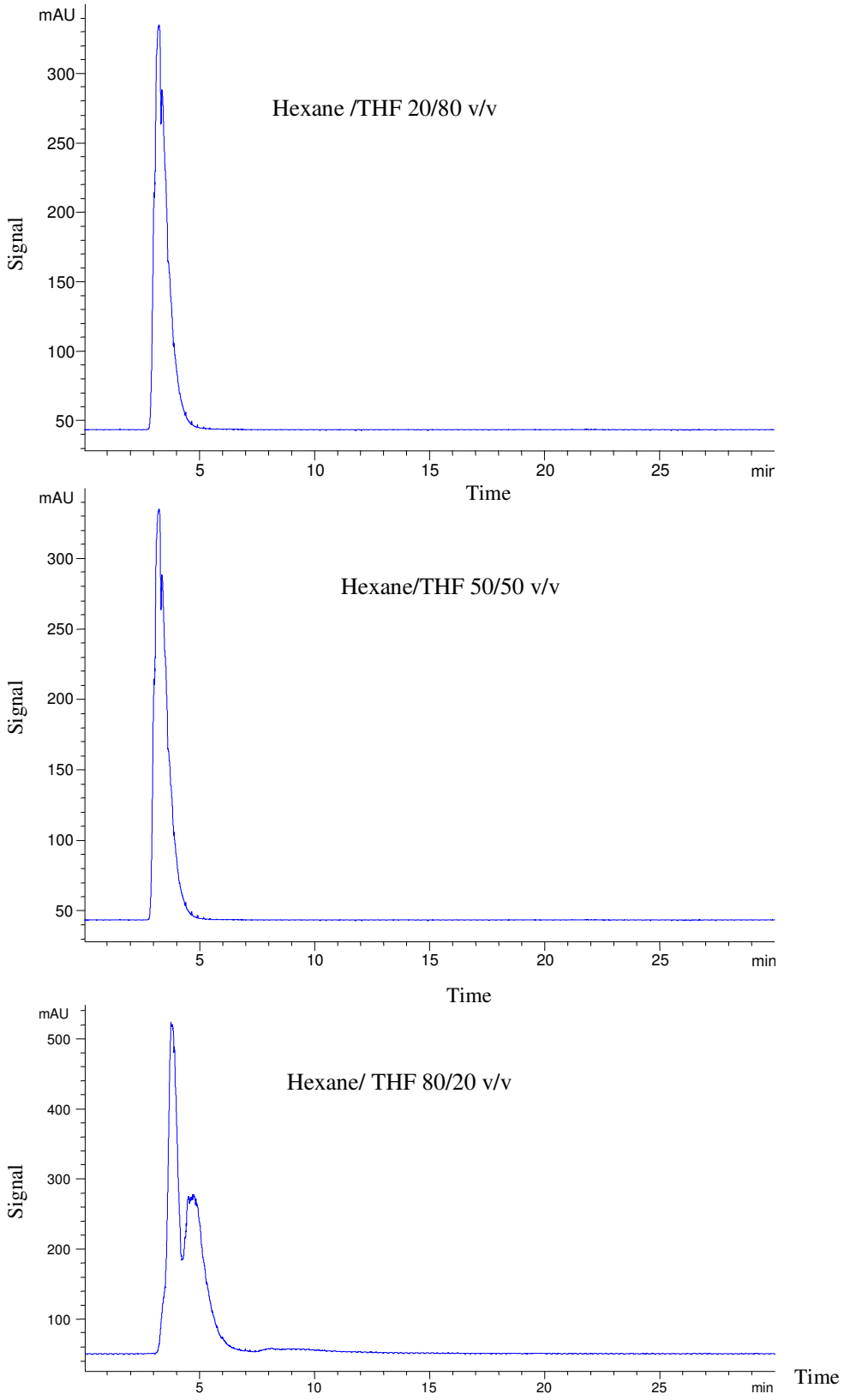
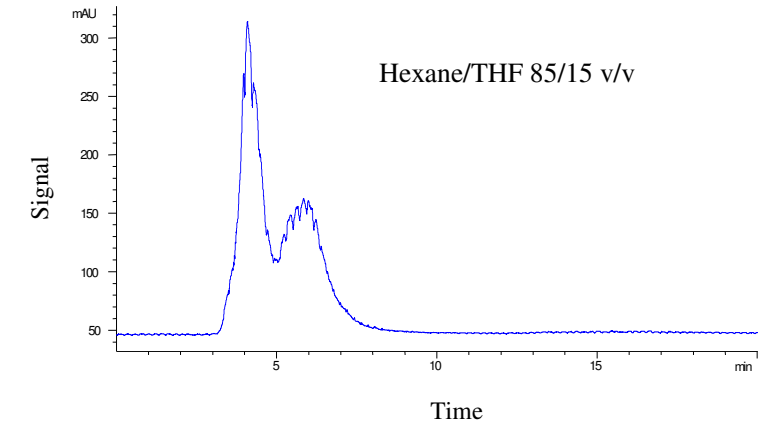



**Figure 2.8: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using a Cyano Column and Hexane/THF Mobile Phases**



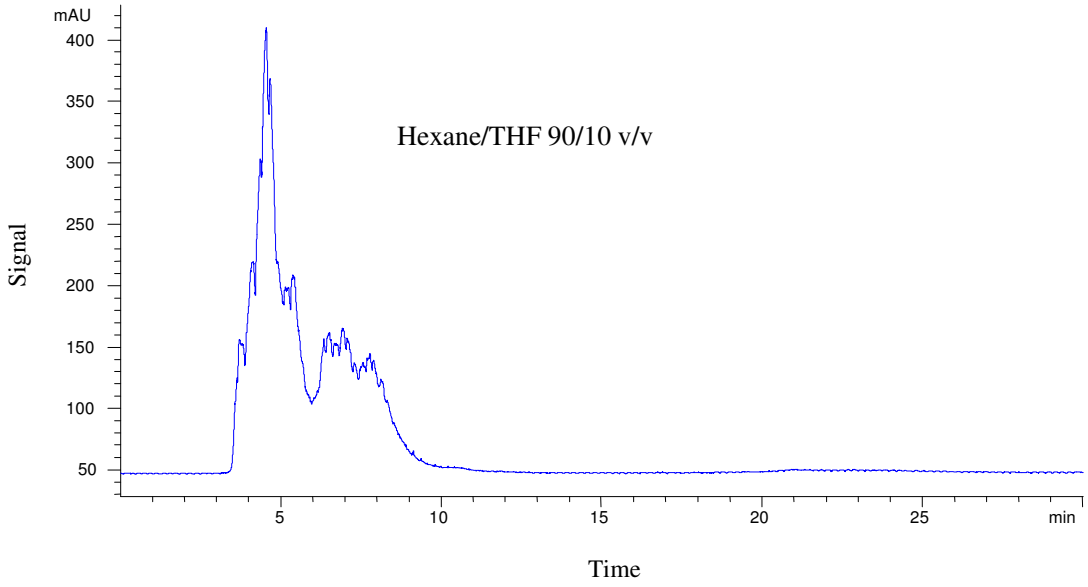




**Figure 2.9: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using a Cyano Column and Hexane/THF Mobile Phases**

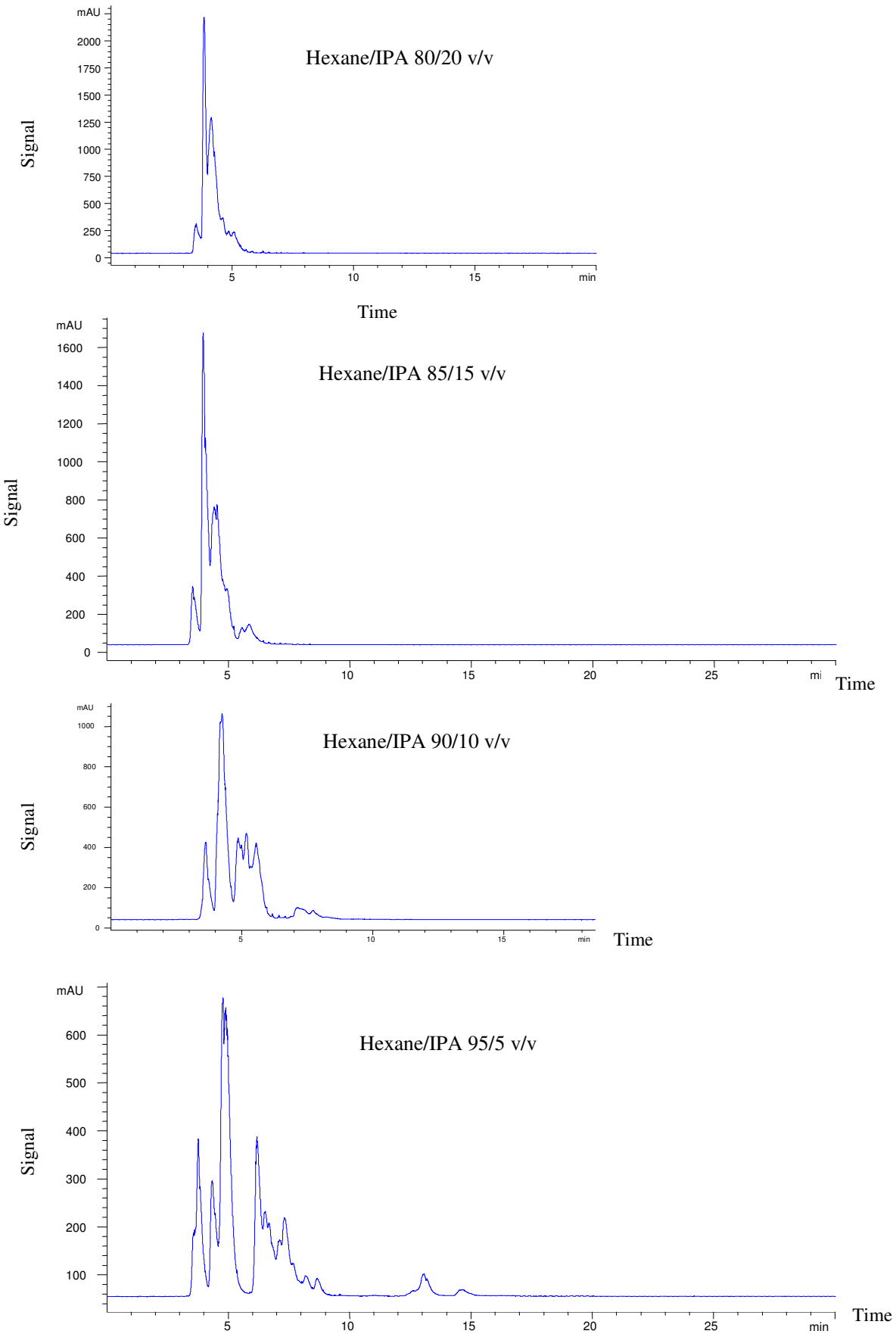



**Figure 2.10: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using a Diol Column and Hexane/IPA Mobile Phases**

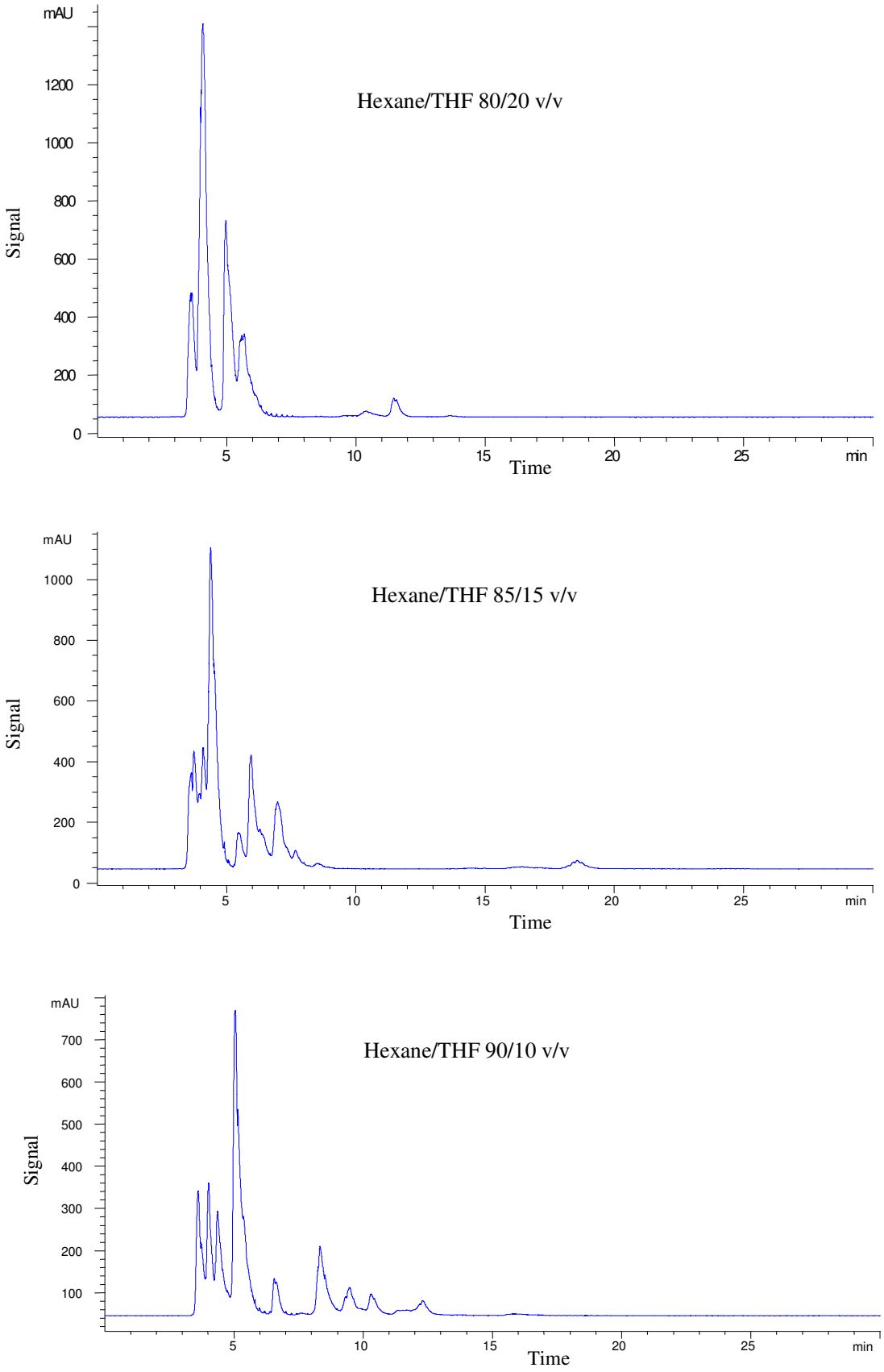



**Figure 2.11: HPLC chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using a Diol Column and Hexane/THF Mobile Phases**

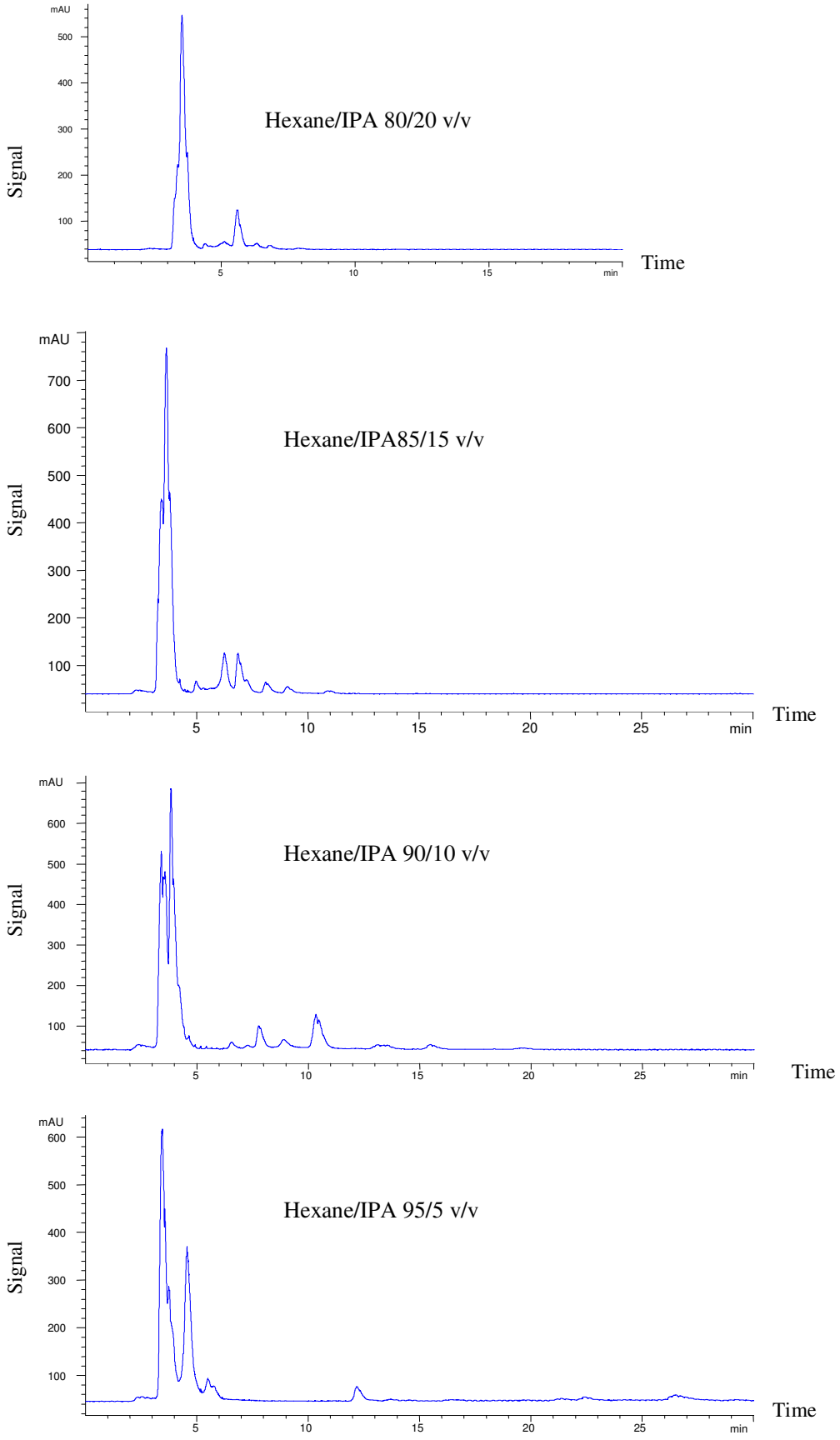



**Figure 2.12: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using an Amino Column and Hexane/IPA Mobile Phases**

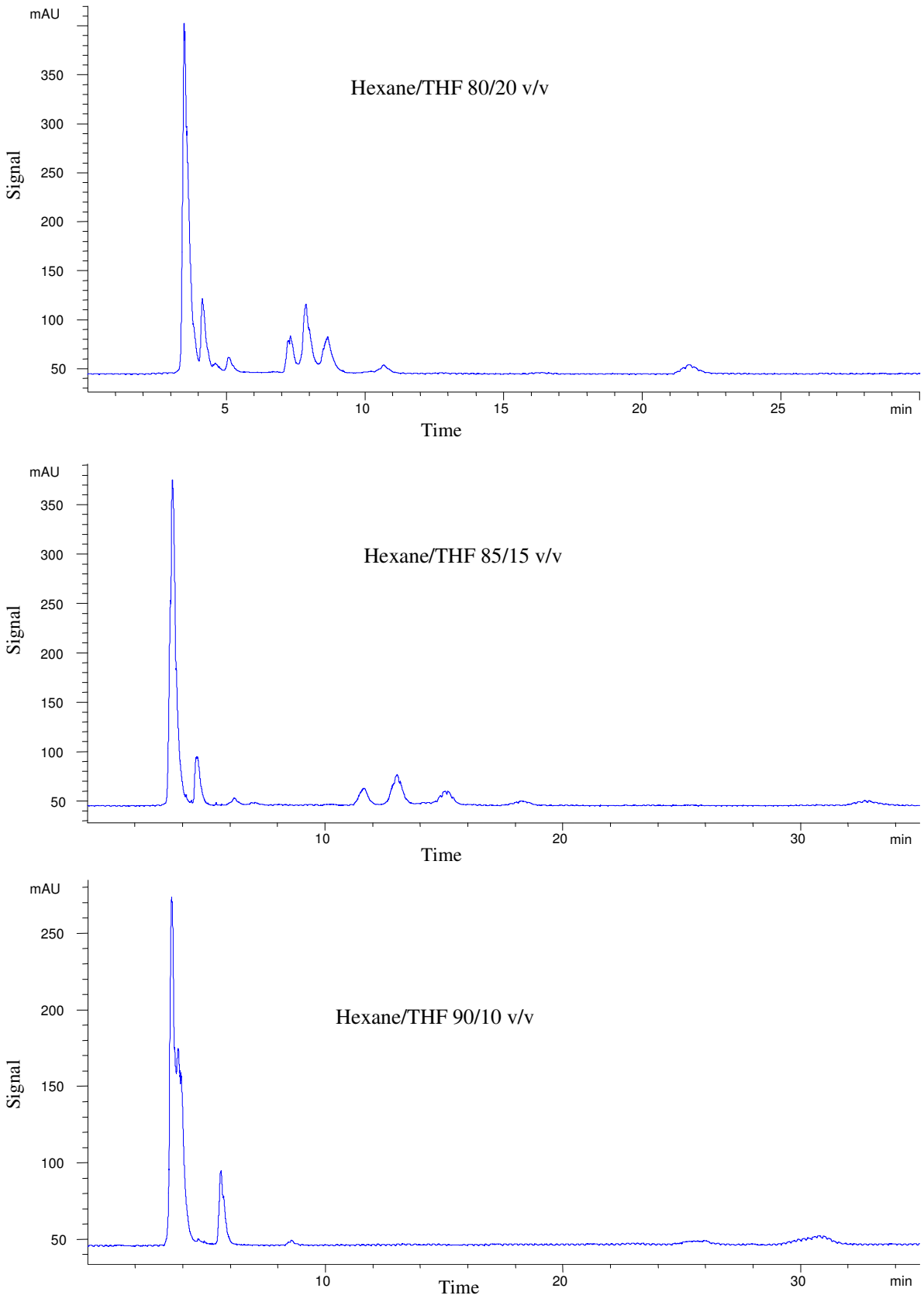



**Figure 2.13: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using an Amino Column and Hexane/THF Mobile Phases**

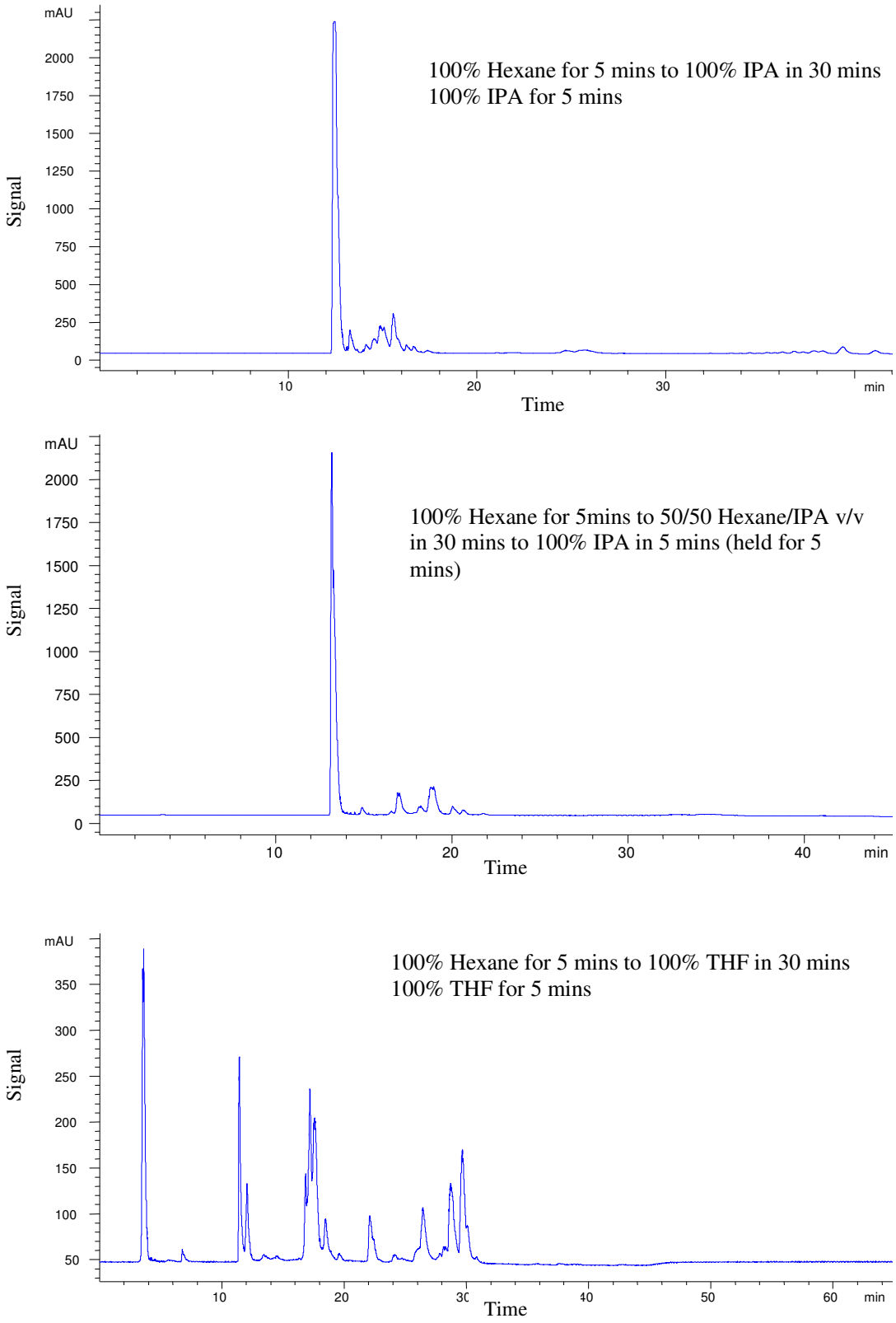



**Figure 2.14: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using an Amino Column and Screening Gradient Mobile Phases**

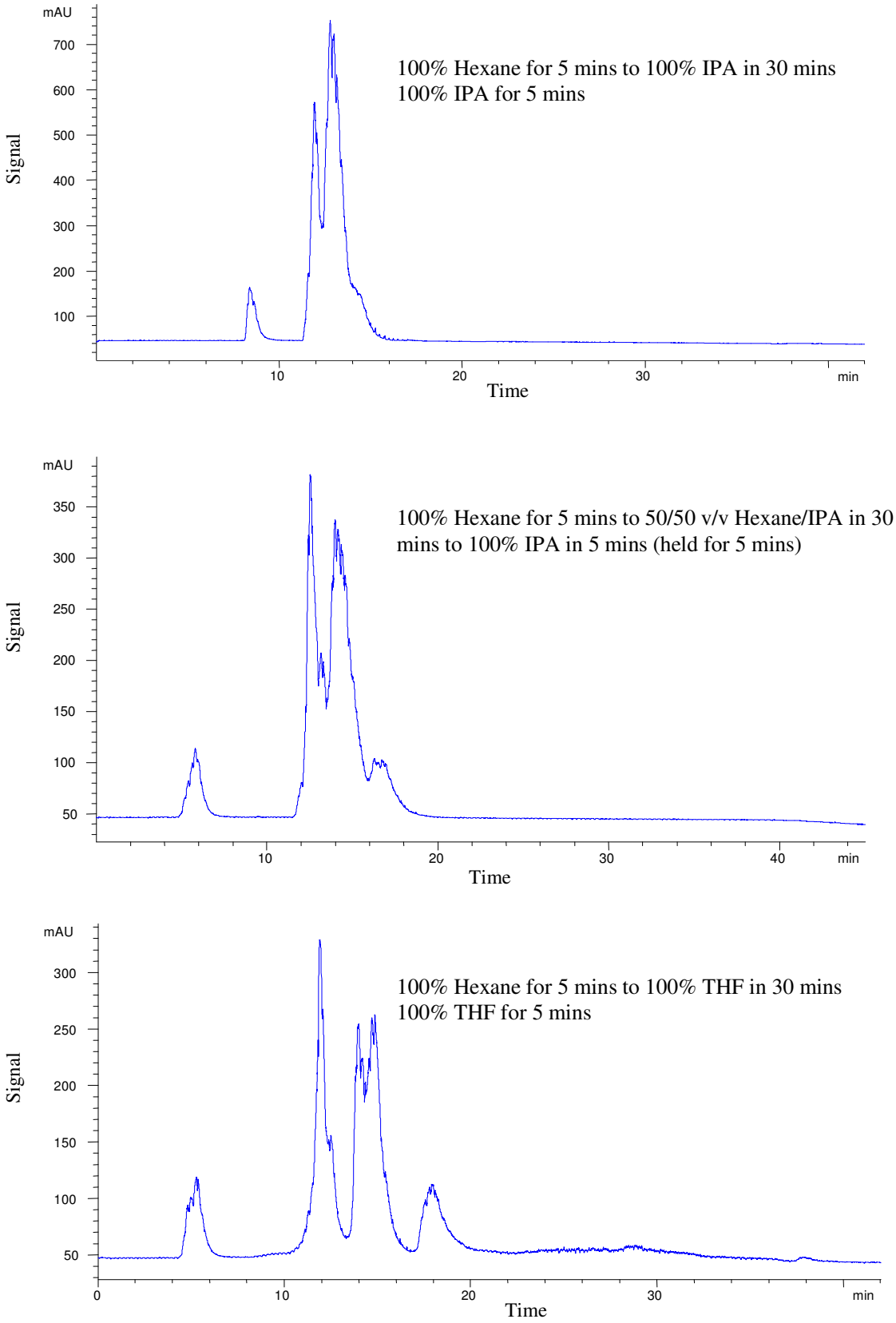



**Figure 2.15: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using a Cyano Column and Screening Gradient Mobile Phases**

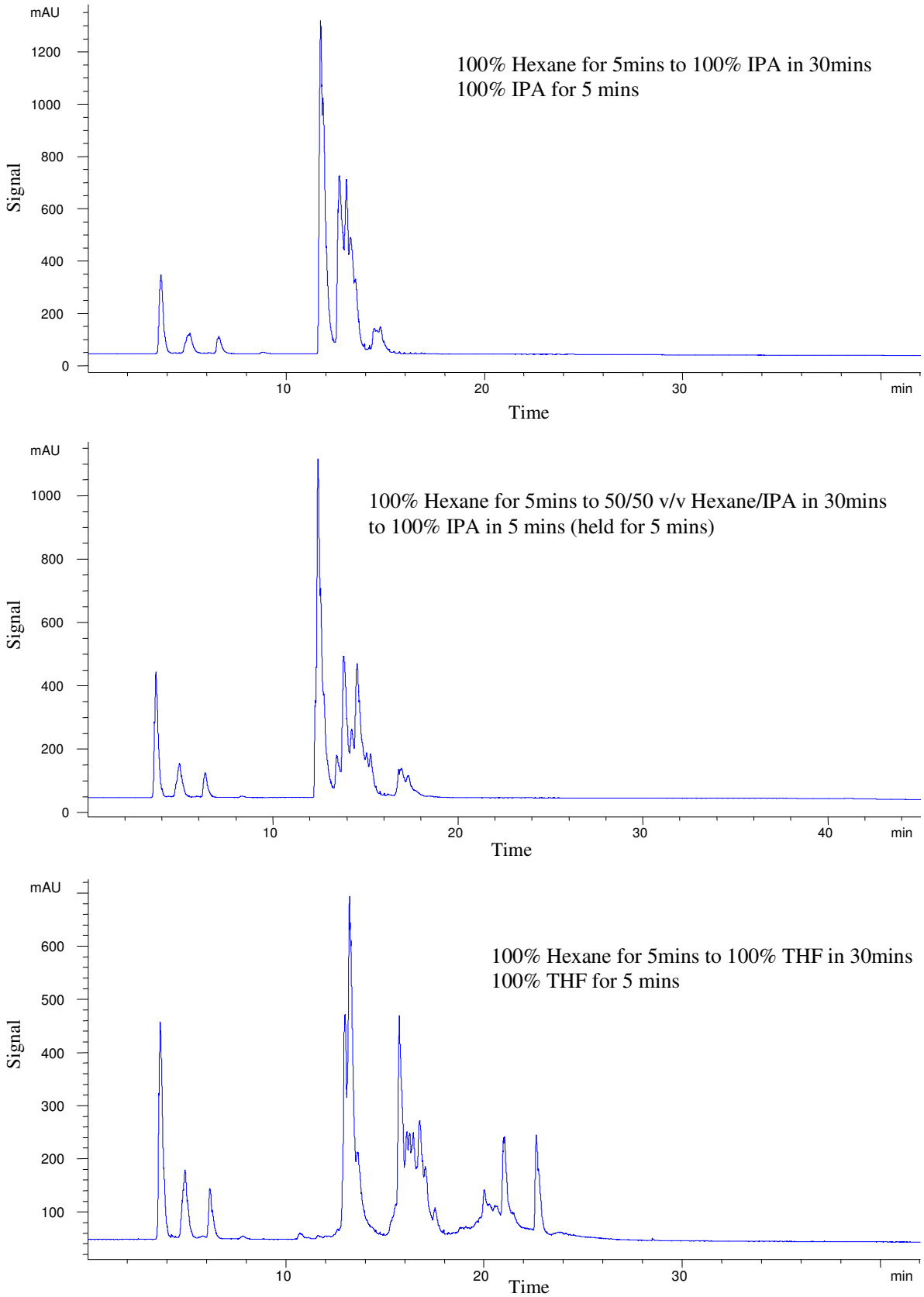



**Figure 2.16: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Analysed Using a Diol Column and Screening Gradient Mobile Phases**

**3. TLC Analysis for the Selection of Mobile Phases for HPLC:**

TLC is a simple, rapid and inexpensive method of separation. TLC can only be used in an isocratic mode and although the process of separation is different to HPLC, it is thought to serve well as a pilot procedure for HPLC. TLC allows the detection of all the components in the sample including the strongly retained ones, therefore it is useful for solvent selection for HPLC. Solvent which will remove strongly absorbed components from the top of a HPLC column can be found and can be used to clean the column between sample injections. Due to the variation in number of peaks observed from the analysis carried out in chapter 2 and therefore the uncertainty of whether all the possible components eluted from the columns, it was felt TLC analysis of the sorbitan esters, using normal phase plates that correlated to the HPLC columns already investigated, would be useful prior to further HPLC method development, to establish if material was being strongly retained.

Normal phase plates are strongly polar and generally the interaction in the mobile phase is complimentary to those in the stationary phase, therefore dispersive solvents are selected. To reduce solute retention, the polar interactions between the solutes and mobile phase must be increased, to compete with the polar interactions between the solute and stationary phase. This is achieved by increasing the polarity of the mobile phase, this will also absorb to the stationary phase deactivating it. For this reason a range of commonly used solvents and blends of varying polarity were investigated.

**3.1 Experimental:**

1 % solutions of the following samples were prepared in IPA and 0.5 µl applied onto the TLC plates (10 x 10 cm).

1 – Manufactured Sorbitan Mono Oleate

2 – Sorbitan Mono Oleate prepared from high purity oleic acid

3 – Sorbitan Oleate, high sorbitan content, prepared from high purity oleic acid

4 – Isosorbide Dioleate, prepared from high purity oleic acid

5 – Sorbitol Oleate, prepared from high purity oleic acid

6 – Isosorbide Oleate, prepared from high purity oleic acid

7 – Oleic Acid

The numbers allocated to the samples above have also been used to identify the samples on the TLC plates in figures 3.1 to 3.8. Esters containing essentially one polyol were also analysed to try and establish the Rf of the individual components expected in manufactured sorbitan esters, if separation was obtained.

The TLC plates used for the screening experiments were TLC Aluminium sheet, silica gel 60 (Merck), HPTLC - Diol $F_{254}$ (Merck), HPTLC – CN (Techware), HPTLC – $NH_2$ (Techware) which correlate to the stationary phases used in the HPLC work (chapter 2).

The solvent systems used were hexane (Fisher – HPLC grade), toluene (Fisher – HPLC grade), diethyl ether (Fisher – HPLC grade), dichloromethane (Fisher – HPLC grade), tetrahydrofuran (VWR – HPLC grade), ethyl acetate (Fisher – HPLC grade), acetonitrile (Fisher – HPLC grade), isopropanol (Fisher – HPLC grade), methanol

(Fisher – HPLC grade), DCM/methanol 80/20 v/v, hexane/THF 50/50, hexane/IPA 50/50 v/v.

The solvent system selected was added to the tank, lined with filter paper to achieve saturation and the plate placed into it. The solvent was allowed to ascend 7 – 8 cm and then the plate was developed by dipping in phosphomolybdic acid solution and then drying with a hot-air drier. Phosphomolybdic acid was selected because it gives blue spots against a green/yellow background for a large range of organic compounds and the colour remains stable for long periods. Visual inspections of the developed plates were made, to establish if separation of a range of components had been achieved.

**3.2 Amino Plates:**

The TLC plates obtained for the samples analysed with the amino plates are shown in figures 3.1 and 3.2. It can be seen that using hexane as the solvent no separation was obtained for any of the samples, all the components were absorbed strongly to the stationary phase and remained at the origin. The other solvents resulted in streaks rather than distinct spots across all or the majority of the plate for most samples under analysis, this may be due to the large number of components in the samples. All of the solvents tried show some components strongly absorbed and remaining at the origin for all samples. The oleic acid (sample 7) showed a spot that remained at the origin using all the solvents, no movement was observed with any solvent indicating it is strongly attracted to the sorbent. If this was repeated with HPLC, material would be retained by the column and would lead to a very short lifetime of the column. Therefore although the amino HPLC column looked worth investigating further, from

the work detailed in chapter 2, TLC shows that it may have severe limitations if material is retained by the column.

**3.3 Cyano Plates:**

The TLC plates obtained for the samples with the cyano plates are shown in figures 3.3 and 3.4.

The non-polar solvent (hexane) resulted in components being strongly retained by the stationary phase and no separation obtained for any sample. With the more polar solvents components showed less affinity for the stationary phase and solubilised in the mobile phase and eluted to the solvent front. However using the mid range polarity solvents, particularly toluene and dichloromethane (DCM), distinct spots were observed showing separation of various components in the samples, although identification of the spots was not possible.

**3.4 Silica and Diol Plates:**

Both the silica and diol plates showed a range of separation with distinct spots observed, the chromatograms obtained are shown in figures 3.5 to 3.8. The chromatographic behaviour of the plates are similar, which will probably be due to the fact the retention mechanism occurring is similar. Both plates have hydroxyl groups, in the diol plate the functional groups are alcoholic residues and with silica the active sites are silanol groups.

Some separation of the polyol esters (samples 1 – 6) was obtained with most solvents, although identification of the spots in the SMO (sample 1) using the single polyol

esters was not possible. With hexane everything remained at the origin and with THF all the components eluted to the solvent front. With toluene and dichloromethane some material remained at the origin and if this occurred on the column would result in a shorter lifetime of the column. If the behaviour seen is reproduced with HPLC, THF will be a useful solvent to ensure all components are eluted from the column, prior to the next injection. And hexane may be useful for varying the polarity of the mobile phase. With the diol plate isopropanol also removes material from the origin and therefore may be another useful solvent.

**3.5 Conclusions from TLC Screening:**

TLC did not result in separation of all of the various components of sorbitan esters with any of the plates and mobile phases tried. When separation of some of the components was obtained, identification of the various spots was not possible.

The TLC analysis showed that:

- with amino plates some components always remain at the origin. If this is repeated with HPLC the component will be retained by the column and a true composition of the sample will not be obtained. The column lifetime will also be shorter.
- with the cyano column most solvents eluted all components to the solvent front, but DCM and toluene gave some separation of the various components in the esters. However initial HPLC work concluded that the cyano column was not a viable stationary phase.
- very similar separation was obtained with the diol and silica plates, this is probably due to similar retention mechanisms occurring. TLC did not result in

separation of all the various components and identification of the various spots was not possible.

Using the information obtained from the solubility study, initial HPLC study and the TLC study it appears that a diol stationary phase HPLC column is the one worth pursuing with THF and IPA as polar solvents and hexane as the non polar solvent. The diol and silica stationary phases on TLC gave similar results but for HPLC the diol stationary phase would be favoured because gradients can be used.

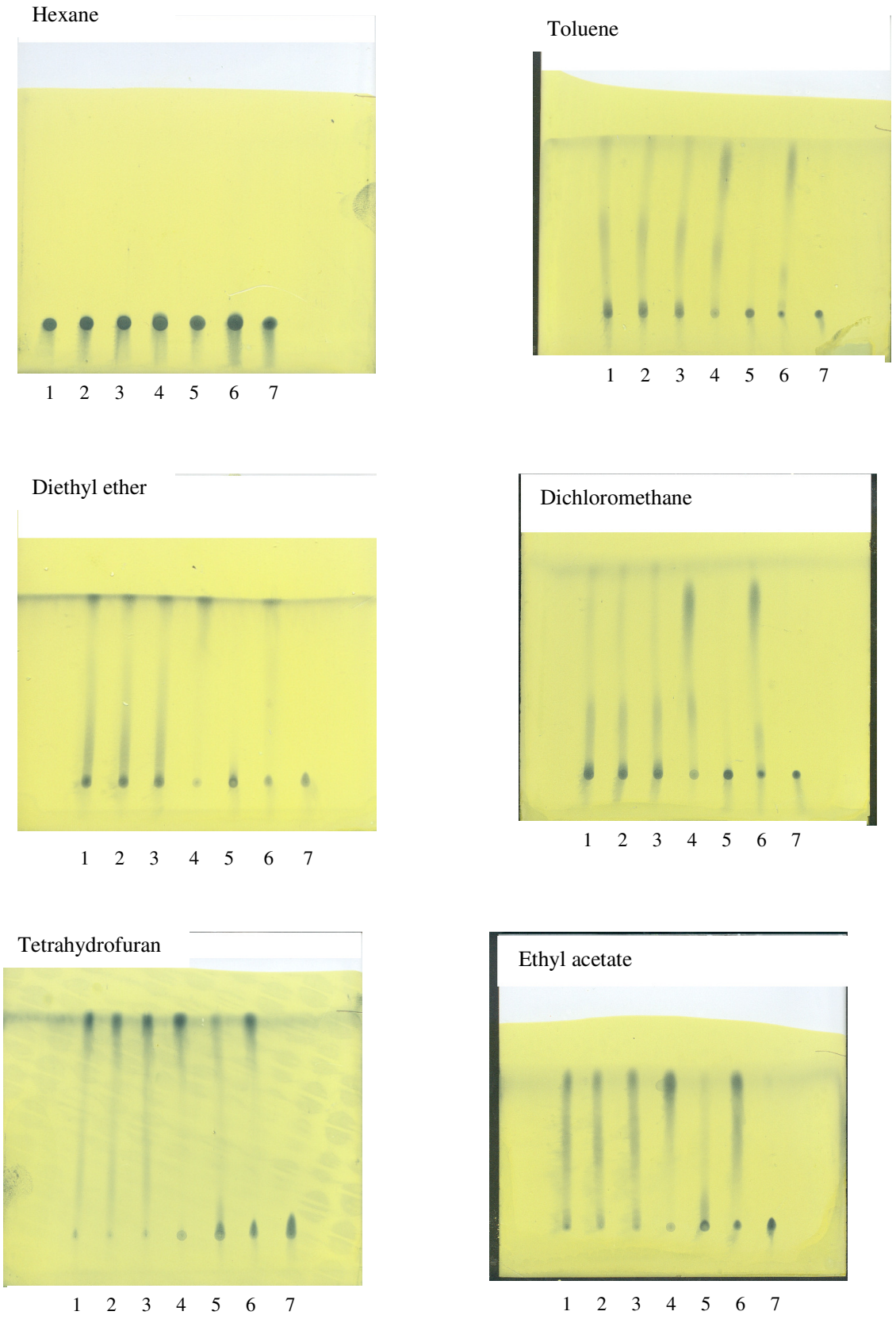



**Figure 3.1: TLC Chromatograms of Polyol Esters of Oleic Acid Using Amino plates and Various Mobile Phases**

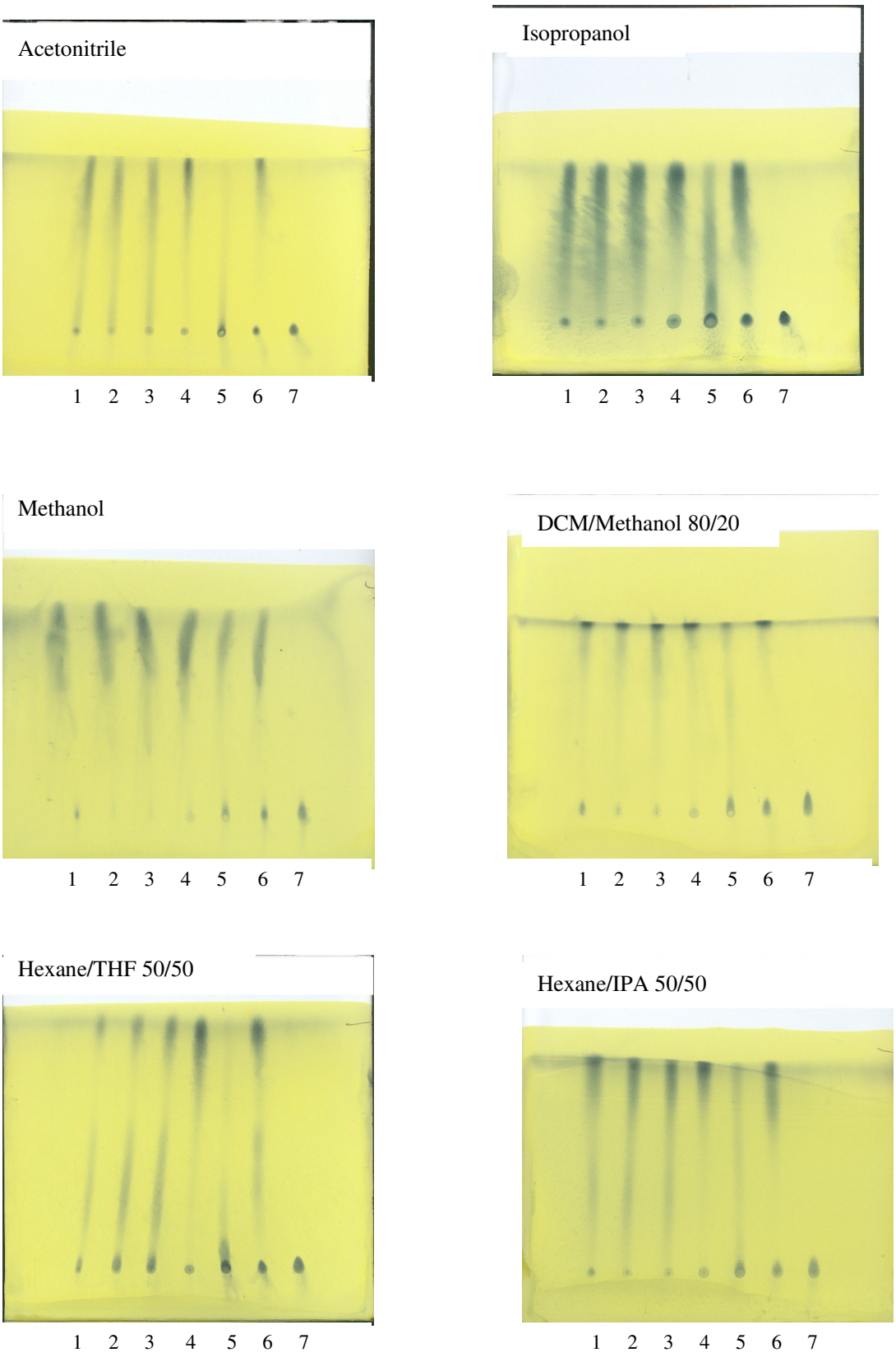



**Figure 3.2: TLC Chromatograms of Polyol esters of Oleic Acid Using Amino plates and Various Mobile Phases**

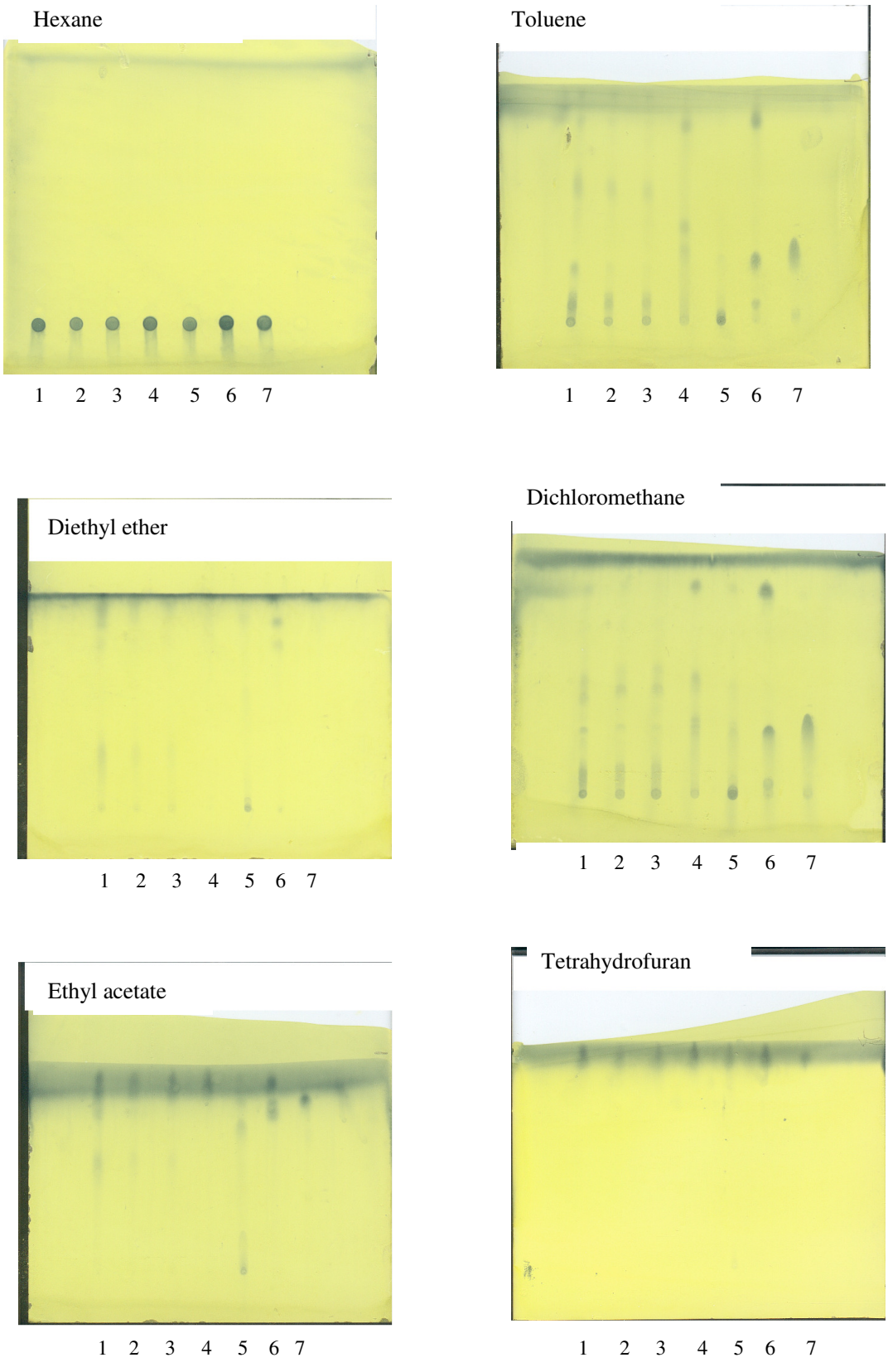



**Figure 3.3 TLC Chromatograms of Polyol Esters of Oleic Acid Using Cyano Plates and Various Mobile Phases**

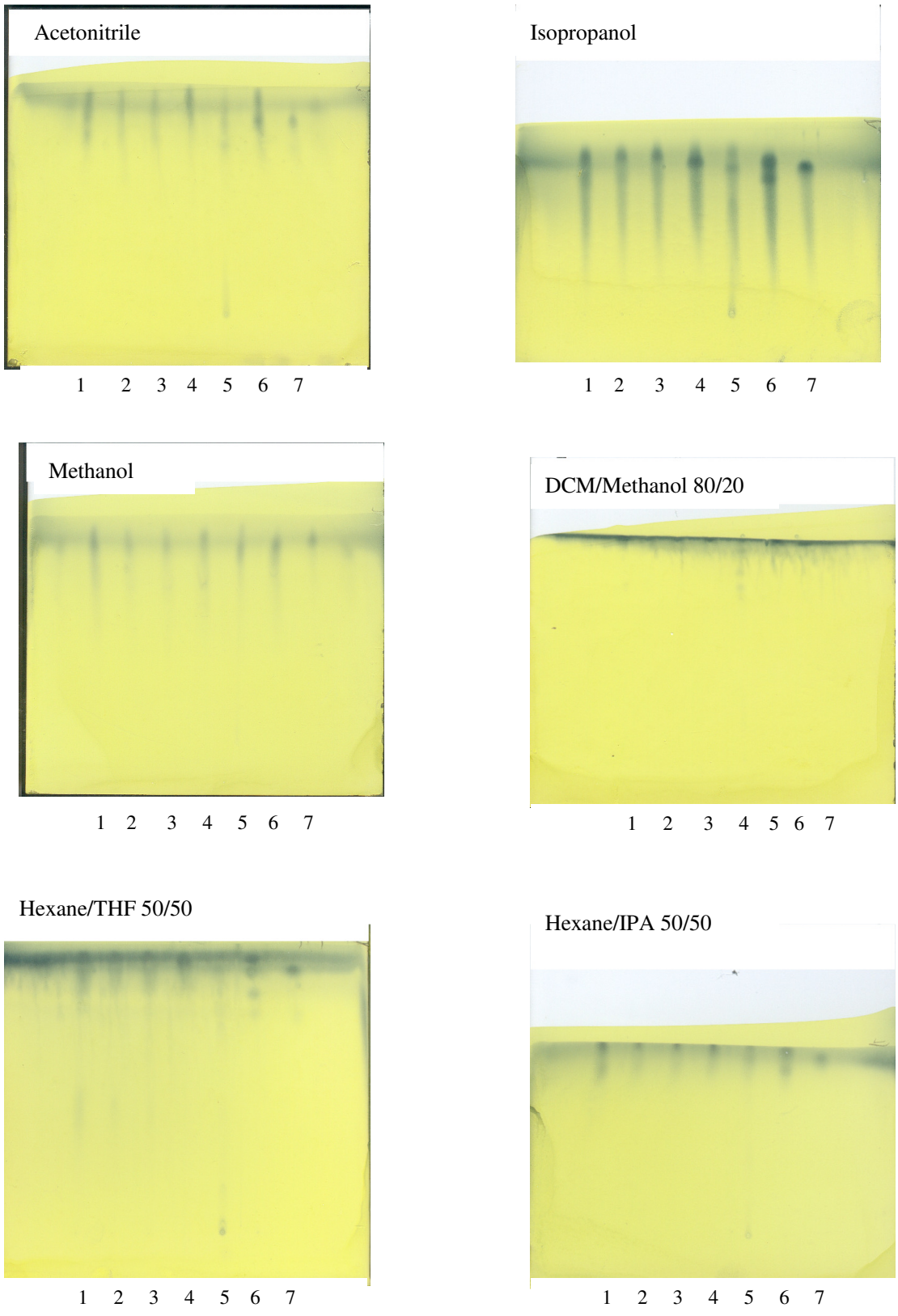



**Figure 3.4: TLC Chromatograms of Polyol Esters of Oleic Acid Using Cyano Plates and Various Mobile Phases**

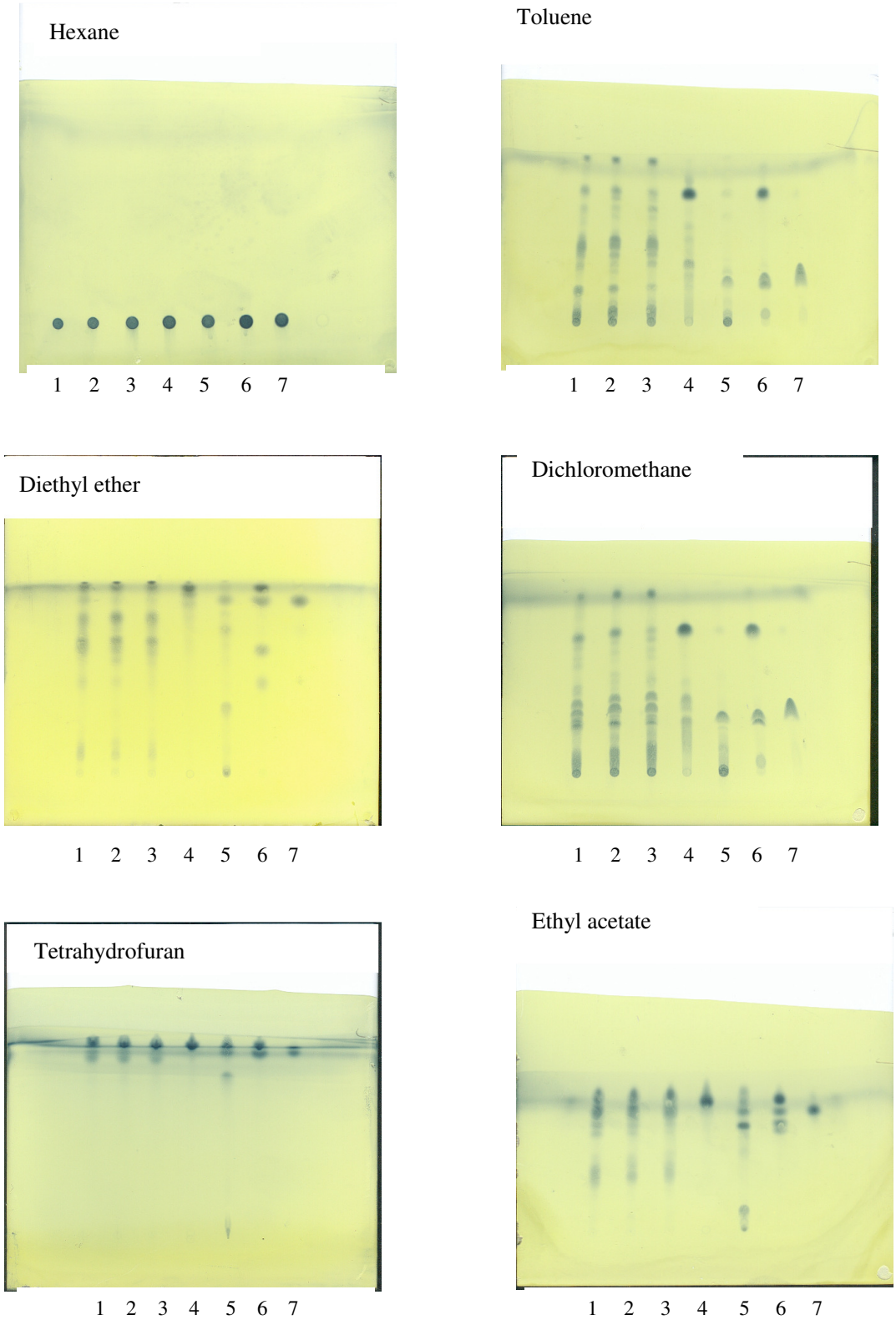



**Figure 3.5: TLC Chromatograms of Polyol Esters of Oleic Acid Using Diol Plates and Various Mobile Phases**

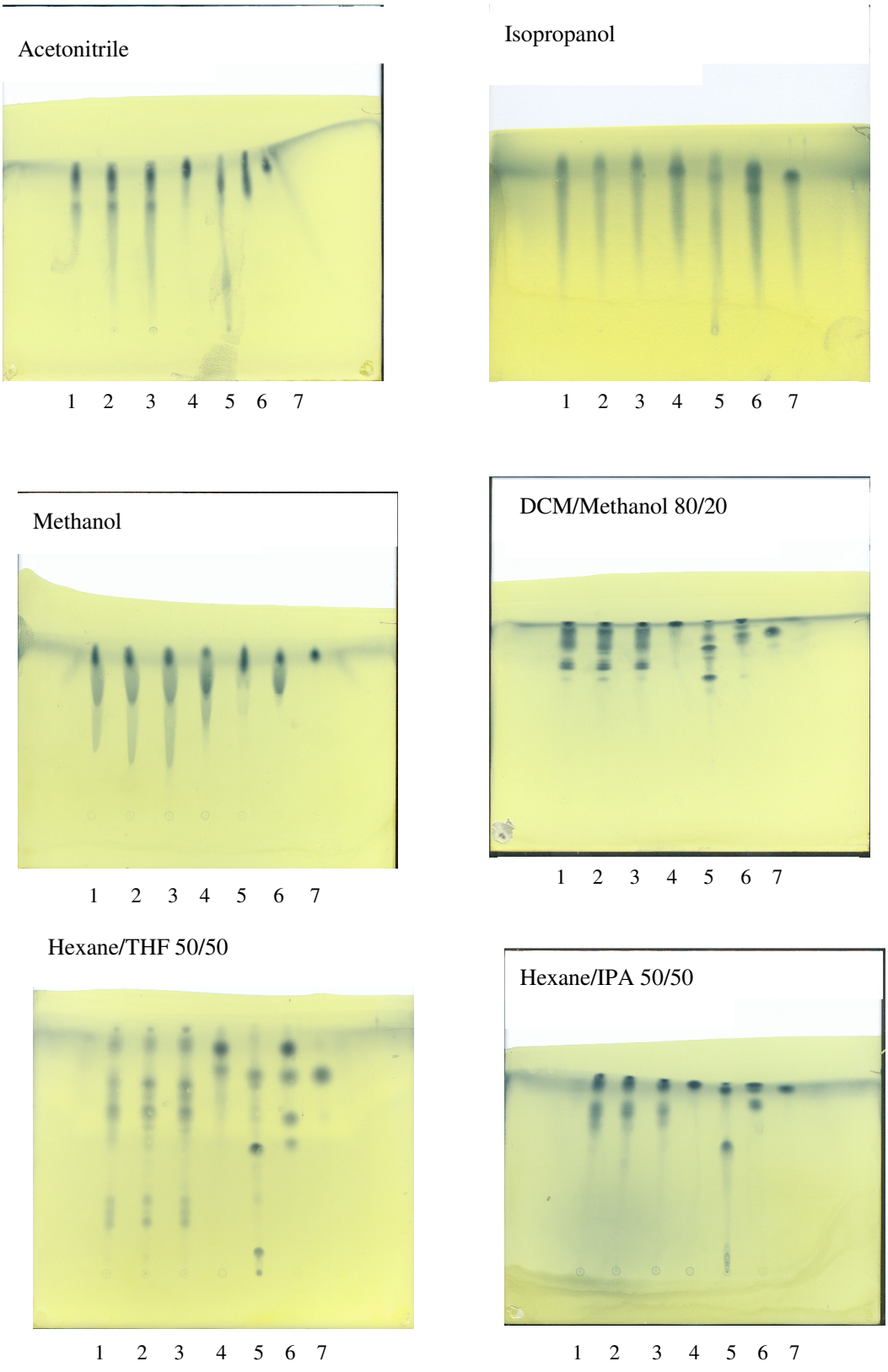



**Figure 3.6: TLC Chromatograms of Polyol Esters of Oleic Acid Using Diol Plates and Various Mobile Phases**

## 4. Further HPLC Analysis of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid:

The initial HPLC work detailed in chapter 2 was used to establish the best column for separation of the various components in sorbitan mono oleate. The work showed that normal phase chromatography was required for any separation to occur and of the various columns tried the best column appeared to be a diol column with a gradient solvent system. TLC was investigated as a screening tool for HPLC in chapter 3 and the diol stationary phase again appeared to be one of the best, the solvents that looked the most promising with the diol stationary phase were THF and IPA. Hexane showed no separation and was therefore felt to be good as the non-polar solvent. From the solubility study carried out in chapter 2 on a range of polyol esters based on oleic acid the esters were also found to be soluble in these solvents.

Sorbitan mono oleate prepared from high purity oleic acid was therefore analysed using the diol column and gradient elutions based on hexane/IPA and hexane/THF.

### 4.1 Hexane/IPA Gradients:

Two hexane/IPA based gradient mobile phases were tried in chapter 2 which started with a low polarity by using 100 % hexane and the polarity increased over 30 minutes to 100 % IPA or 50/50 v/v hexane/IPA. The resulting chromatograms showed very little separation, both showed three small peaks, followed by a large peak and then a group of partially resolved peaks. The mobile phases were therefore too polar and components in the sample were favouring the mobile phase and passing through the column with little separation. It was therefore felt less polar gradients were required to increase retention of the components. The first gradient tried was:

100 % hexane (held for 5 minutes) to 75/25 v/v hexane/IPA over 30 minutes. Decreasing the polarity of the solvent increased the retention and more separation was observed, indicating more interaction of the components with both the mobile phase and stationary phase. Due to a gradient being used the polarity of the solvent altered during the analysis and this is recommended for samples thought to contain a range of components of varying polarity, as with the sorbitan mono oleate under investigation. A number of peaks were observed between 2 and 7 minutes, then very little eluted until ~13 minutes when a large peak thought to consist of a group of partially resolved peaks was observed, followed by a number of smaller peaks from 15 to 21 minutes, although some appeared broad indicating the possibility of coeluting components. The chromatogram obtained is shown in figure 4.1.

It was felt that by decreasing the polarity of the final mobile phase further, retention of the various components could be increased and separation improved, therefore a gradient of 100 % hexane (held for 5 minutes) to 80/20 v/v hexane/IPA over 30 minutes was tried. The chromatogram obtained is also shown in figure 4.1 and the chromatogram was found to be very similar to that obtained using the previous gradient, increased separation was not observed, although elution of the components was slightly longer.

Two other slightly less polar gradients were tried, starting with 100 % hexane (held for 5 minutes) increasing to 85/15 v/v or 87.5/12.5 v/v hexane/IPA over 30 minutes and again a similar chromatogram to the first was obtained, as shown in figures 4.1 and 4.2.

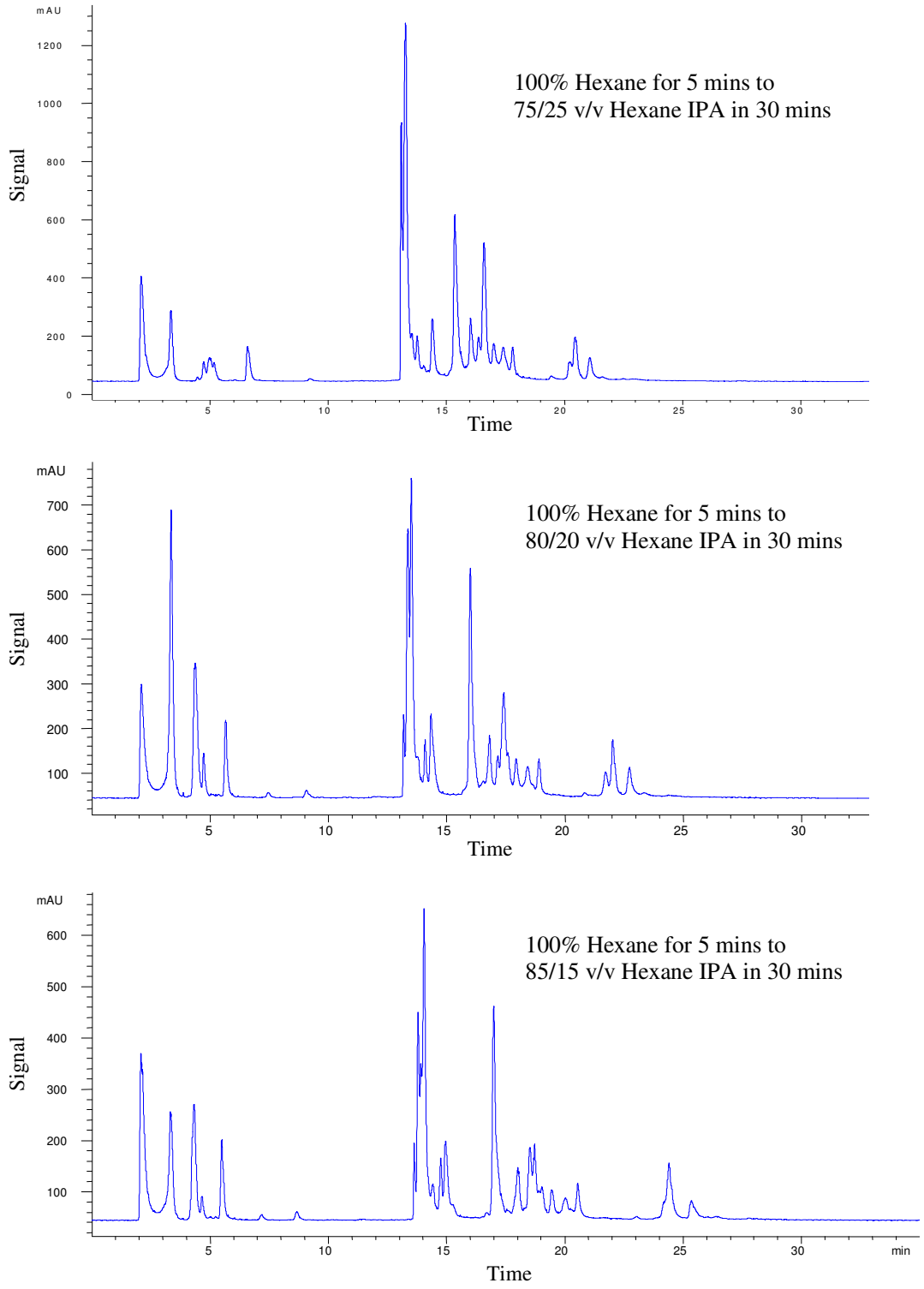



**Figure 4.1: HPLC Chromatograms Obtained for Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Using a Diol Column and Gradient Mobile Phases Based on Hexane/IPA**

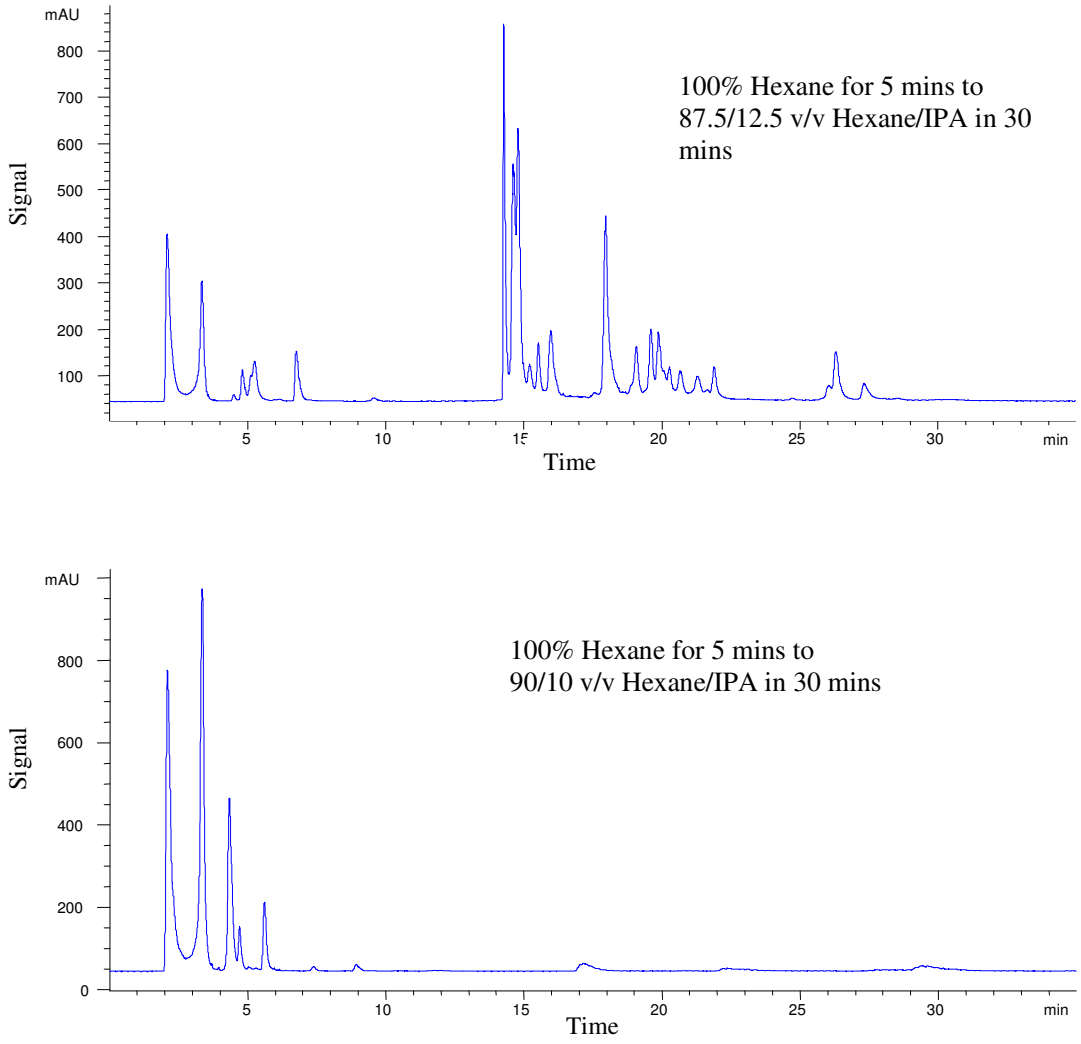



**Figure 4.2: HPLC Chromatograms Obtained for Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Using a Diol Column and Gradient Mobile Phases Based on Hexane/IPA**

The final gradient based on hexane/IPA tried was 100 % hexane (held for 5 minutes) to 90/10 v/v hexane/IPA over 30 minutes and the resulting chromatogram is shown in figure 4.2. The chromatogram shows five peaks which elute in less than 6 minutes, this is very different to the other chromatograms obtained with hexane/IPA gradients which showed more peaks over a longer time. The lack of peaks indicates that the mobile phase is not polar enough and a large number of components have been adsorbed to the column rather than dissolving in the mobile phase and being eluted.

The capacity factor was determined for the first eluting peak with the different gradients, however the retention time of this component was found to be the same as the estimated value for the unretained solute and therefore a k' value of 0 was calculated, indicating no retention of the first component or components. Decreasing the mobile phase polarity can increase retention of a solute in normal phase chromatography, however this was not observed for the early eluting components with the hexane/IPA gradients.

**4.2 Hexane/THF Gradients:**

The other solvent which looked possible as a mobile phase for separation of sorbitan ester species was THF from the initial work detailed in chapters 2 and 3. The initial gradient based on hexane and THF detailed in chapter 2 (chromatogram fig. 2.16) showed a number of peaks, although a number of groups of partially resolved peaks were noted, rather than base line resolved peaks. It was felt that by using a less polar final solvent, retention and therefore separation could be improved. The first gradient tried was 100 % hexane (held for 5 minutes) increased to 50/50 v/v hexane/THF over 30 minutes, the chromatogram obtained is shown in figure 4.3. The chromatogram

shows a number of well resolved peaks over ~26 minutes, improved separation compared to the previous hexane and THF gradient has been achieved, although a number of partially resolved peaks are still observed.

Other less polar gradients based on hexane and THF were therefore tried, in an attempt to improve separation and obtain baseline resolution of all peaks. The gradients tried were:

100 % hexane (held for 5 minutes) increased to hexane/THF (60/40 v/v) over 30 minutes.

100 % hexane (held for 5 minutes) increased to hexane/THF (65/35, v/v) over 30 minutes

100 % hexane (held for 5 minutes) increased to hexane/THF (80/20, v/v) over 30 minutes

The chromatograms obtained are shown in figure 4.3. The chromatograms obtained using 60/40 v/v and 65/35 v/v hexane/THF as the final solvent are similar to that when 50/50 v/v hexane/THF was used as the final solvent, although slightly longer elution times of the components and subtle differences in the peaks are observed. However when the final solvent 80/20 v/v hexane/THF was used the later peaks observed in the previous chromatograms appear to be absent, indicating that the polarity of the mobile phase is too weak to elute these, they are not soluble in the mobile phase and therefore these components have been retained by the column.

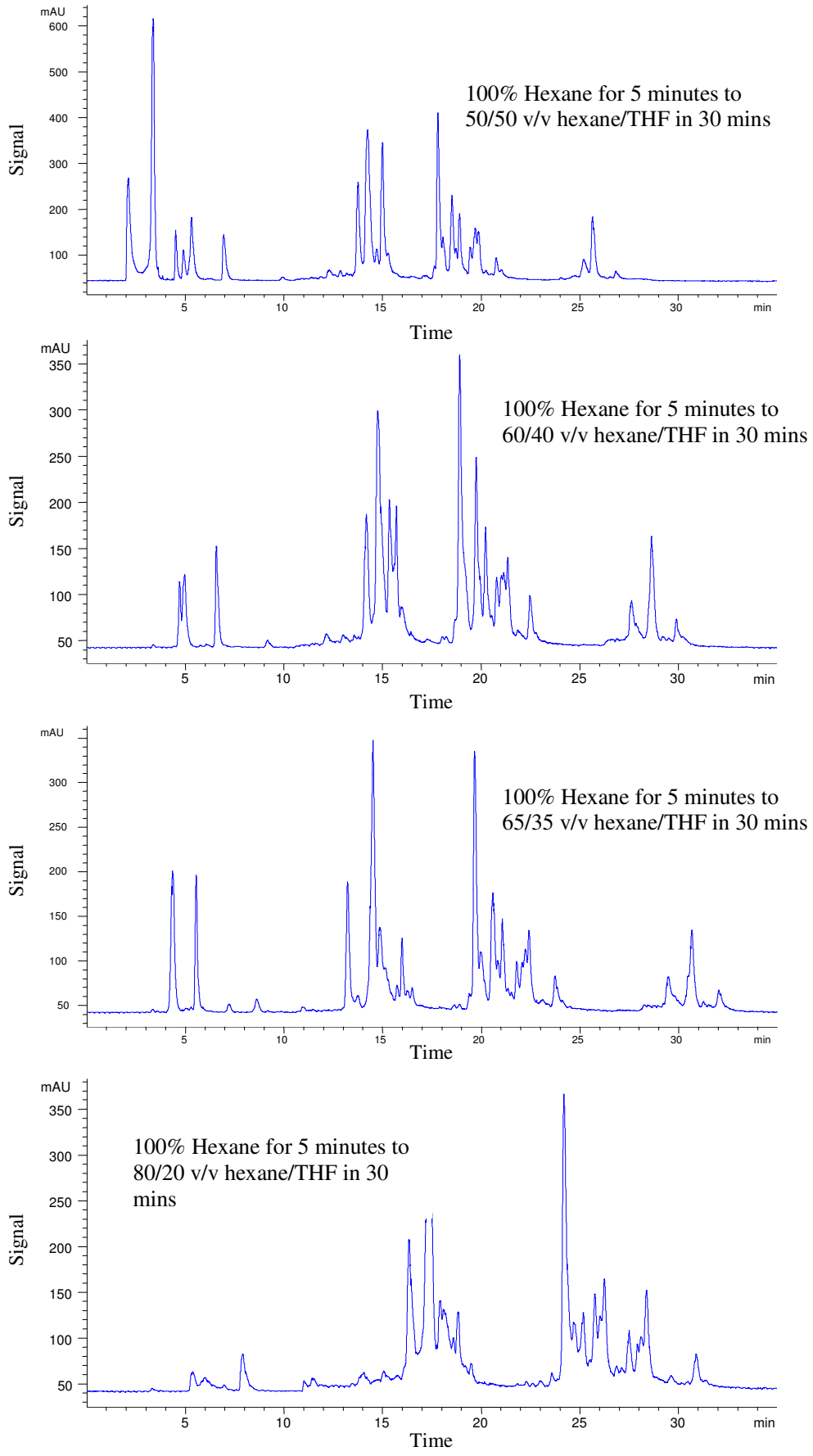



**Figure 4.3: HPLC Chromatograms Obtained for Sorbitan Mono Oleate Prepared from High Oleic Acid Using a Diol Column and Gradient Mobile Phases Based on Hexane/THF**

### 4.3 Hexane/THF Gradient with Various Runtimes:

A review was made of the various gradients tried and the best separation appeared to be obtained with hexane as the starting solvent and 60/40 v/v hexane/THF as the final solvent. A number of peaks are observed indicating separation of many of the components of the sample, however some of the components appear to elute very close together and other peaks appear as two or more partially resolved peaks, it was thought that by increasing the run time of the gradients, the components would have longer to equilibrate between the mobile and stationary phases. The run time was increased to 45 and 60 minutes and the chromatograms obtained are shown in figure 4.4 along with the 30 minute run time for comparison. The extended run times resulted in elution of the components taking longer as expected and the peaks are slightly more spaced out, although significant improvement in separation of the various components was not observed. However it was noted that the ratio of some of the major peaks varies between the gradient runs. It was therefore felt that further work was required to investigate the precision of the gradients.

### 4.4 Precision:

The precision of a method is the closeness of agreement between the results obtained applying the experimental procedure several times. Precision may be considered at various levels and can include instrument precision, intermediate precision and reproducibility.

The instrument precision of the HPLC method was investigated for the 100 % hexane to hexane /THF 60/40 v/v in 30 minutes which resulted in the best separation in the shortest time of all the gradients tried. Instrument precision is a repeatability

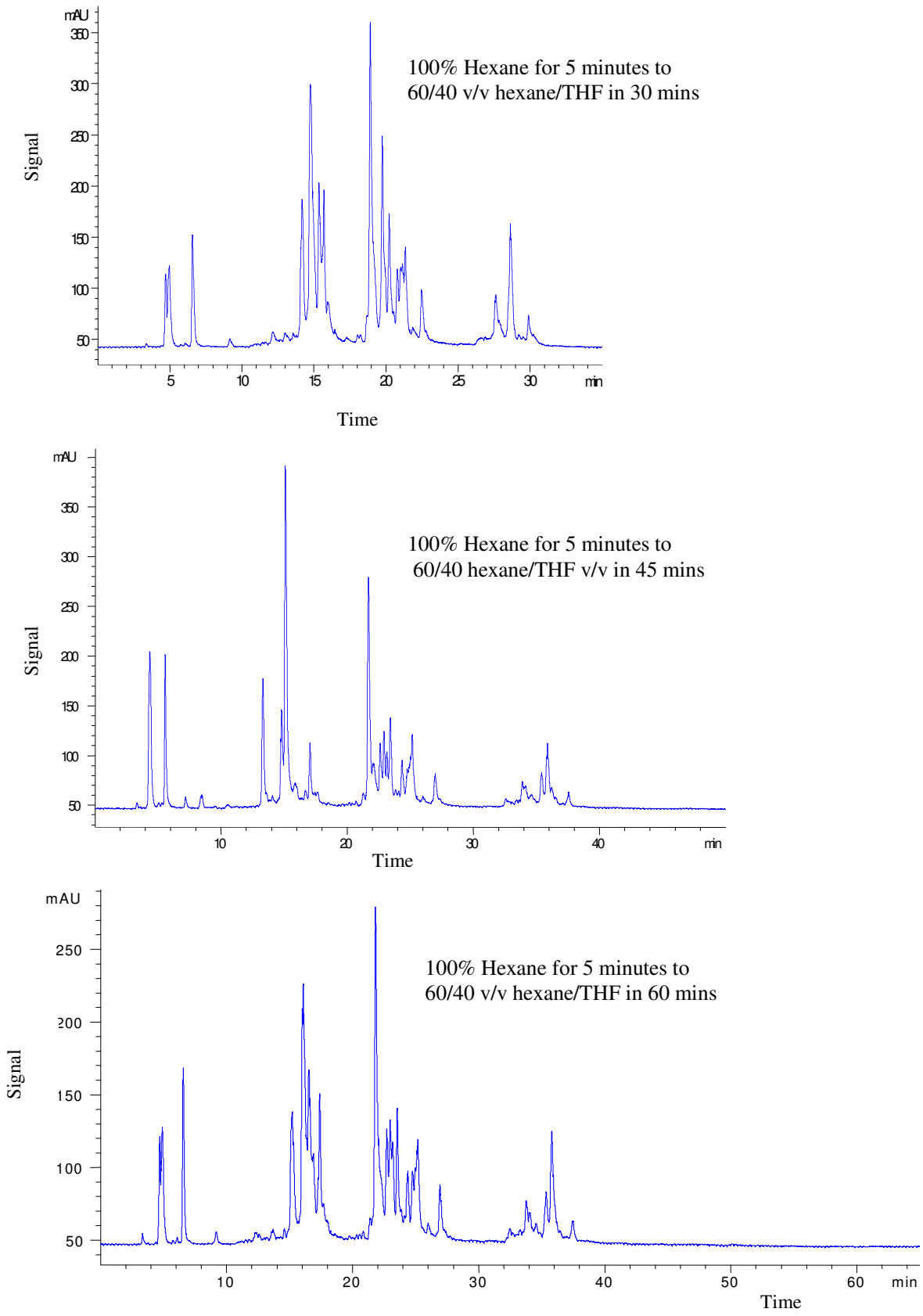



**Figure 4.4: HPLC Chromatograms of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid Using a Diol Column and Hexane to Hexane/THF 60/40 Gradient and Various Run Times**

measurement and indicates the ability of the instrumental method to obtain the same answers for the same sample over a short period of time. A precise method is required to have confidence in the results obtained.

A solution containing sorbitan mono oleate at 0.1 % was prepared and injected onto the column three times from the same vial to establish precision. The chromatograms obtained for the first and last injection obtained are shown in figure 4.5. The third injection shows a group of peaks at ~ 11 minutes, which is absent in the first injection. The first injection shows a group of three peaks around 15 minutes, the third peak is a single peak, however the third injection chromatogram shows this peak as a doublet. The ratio of the peaks are also different in the two chromatograms, for example the ratios of the two major peaks observed at ~15.5 and ~19.5 minutes are 1:1.1 for injection 1 and 1:0.6 for injection 3 and the ratio of two adjacent peaks at ~19.5 and ~20.5 minutes are 1:0.4 for injection 1 and 1:0.6 for injection 3. It can be concluded that the two chromatograms show major differences. The chromatogram obtained for the second injection showed differences to the chromatograms obtained for the first and third injection.

Repeat injections from the same solution were made on a different day and again chromatograms showing significant differences for the same sample were obtained, therefore it was concluded that this gradient method was not precise. Other gradients tried were also checked for precision and were also shown not to be precise

If the gradients do not provide precise results for the same sample on the same day, HPLC is not a suitable QC technique and therefore not worth pursuing further for the

analysis of sorbitan esters outside a research environment and an alternative method of analysis should be tried.

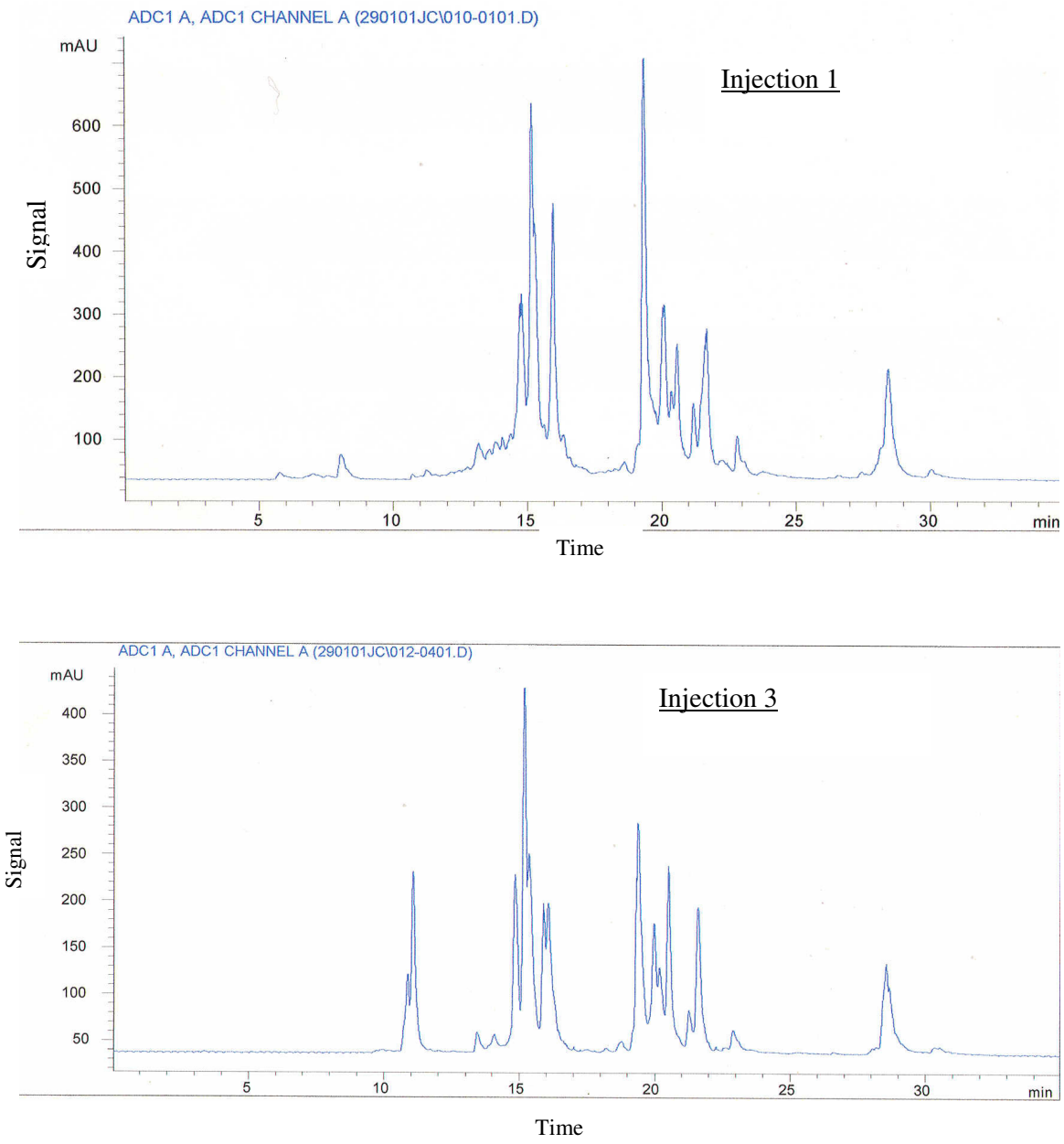



**Figure 4.5: Repeat Injection of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid using Diol Column and Gradient of 100% Hexane to 60/40 Hexane/THF in 30 minutes**

## 5. Initial Gas Chromatography Analysis

### Analysis of Polyol Esters Based on Caprylic Acid Using Split/Splitless Injection:

Gas chromatography is a very useful analytical tool, which is routinely used in a large number of laboratories. It offers rapid and high resolution separation of a wide range of compounds, with the only restriction that the analyte should have sufficient volatility. The analyte needs to be in the gas phase so that it can be transported through the column. For any retention of the solute molecule to take place, the solute molecule must dissolve in the stationary phase for a finite period of time before re-emerging from the liquid surface into the vapour state.

From the work detailed in chapters 2 to 4 it was found that HPLC is unsuccessful for the analysis of sorbitan mono-oleate. GC is another routinely used technique within Croda, therefore would be a good method to use for sorbitan ester analysis particularly sorbitan mono oleate, if suitable. However due to the high molecular size of the polyol esters of oleic acid being investigated it was uncertain whether they would be sufficiently volatile to analyse by GC. It was therefore felt necessary to carry out an investigation using a smaller chain fatty acid ester, which would have a smaller molecular size and therefore be more volatile. This would establish if any polyol esters based on sorbitol can be analysed by GC, prior to the analysis of the less volatile larger esters. The ester selected was sorbitan caprylate, which is the sorbitan ester with the shortest fatty acid chain length manufactured by Croda. It is manufactured from a high purity fatty acid (~98 % caprylic acid) and therefore will have fewer ester species than esters prepared from a mixed fatty acid feed stock.

## 5.1 Initial Analysis:

### 5.1.1 Sample Derivatisation:

The sample chosen was a sorbitan caprylate manufactured by Croda Singapore. Derivatisation is used widely in GC to improve thermal stability, change the separation properties of the compound by adjusting the volatility or introducing a detector orientated tag into the molecule. It was felt necessary to derivatise the sample under study to increase the volatility. Acetylation was chosen because this is the derivatisation used for polyol esters, reducing the polarity of the hydroxyl group. The sample (2 g) was taken and acetylated with pyridine (5 ml) and acetic anhydride (10 ml), refluxed for approximately 1 hour on a sand bath. The resulting sample was then analysed by GC.

### 5.1.2 Instrumentation:

The GC used for this analysis was a Hewlett Packard 5890 GC fitted with a split/splitless injector and flame ionization detector (FID). This is a routinely, readily available GC within Croda. It was felt it would be suitable for sorbitan ester analysis because by using the splitless injection there is total transfer of the sample onto the column and this is commonly used for less volatile analytes in a low boiling point solvent. The detector selected is a universally used detector for organic compounds which is both robust and sensitive.

### 5.1.3 Column Selection:

The majority of analysis can be performed on non polar stationary phases, e.g. DB1, DB5 columns and are good starting points when little information is known about the expected separation. These are preferred stationary phases because they have higher

maximum operating temperatures and are more durable. The separation relies on dispersive interactions so separation is according to the volatility of the components in the sample.

However due to the high molecular weight of sorbitan esters and therefore low volatility it was felt that high temperature GC (HT-GC) would be necessary, this starts at the upper working limit of conventional GC columns. Some non-polar and medium polar gum phases can be operated at temperatures greater than 400°C.

The initial stationary phase selected was therefore a non polar column but one that can be used for HT-GC. The DB1-ht column was selected, this is a 100 % dimethylpolysiloxane column which is specially processed for extended temperatures up to 400°C, compared to a standard DB-1 column which has a maximum operating temperature of 350°C.

Resolution is proportional to the square root of the efficiency and therefore doubling the length of the column results in about 40 % improvement in resolution. However doubling the length of the column doubles the analysis time, increases column bleed and decreases sensitivity. Therefore due to the study being carried out to establish if the ester species of sorbitan caprylate would elute from the column and if any separation of the sorbitan ester could be achieved it was felt that a 15 metre column would be sufficient and decrease the analysis time in comparison to a 30 or 60 metre column. The column diameter selected was a 0.32 mm i.d as these along with 0.25 mm columns are the most commonly used.

The film thickness used was 0.1µm, this is the only commercially available thickness. This is a thin film used for separating low volatile components relatively quickly, which is required for sorbitan ester analysis.

### 5.1.4 GC Conditions:

Temperature markedly influences GC retention because it changes the partition coefficient. Retention decreases with increasing column temperature. The dependence of GC retention on vapour pressure means that mixtures containing a wide range of boiling points can not be analysed using an isothermal run because the volatile components may elute and be well resolved but the higher boiling materials would only be eluted with long retention times and very broad peaks. Due to the number of possible components within the sample under investigation it was felt necessary to use an oven temperature programme. In contrast an injector temperature needed to be selected that was sufficient to volatilise the components but not degrade them.

### 5.1.5 Analysis:

The first analysis carried out was to establish if the ester species would elute from the column. A high starting temperature was selected and a temperature programme with the final temperature of the column being the maximum operating temperature of the column.

The conditions used were:

| | |
|---|---|
| Initial temperature: | 200°C |
| Ramp rate: | 10 °C/minute |
| Final temperature: | 400°C |

| | |
|---|---|
| Injector temperature: | 300°C |
| Detector temperature: | 400°C |
| Injection volume: | 1 µl |
| Carrier Gas: | Helium |
| Flow Rate: | 1 ml/min |

The chromatogram obtained is shown in figure 5.1 and shows that some separation of the various components was obtained however they eluted very close together, with all the components eluting within 12 minutes.

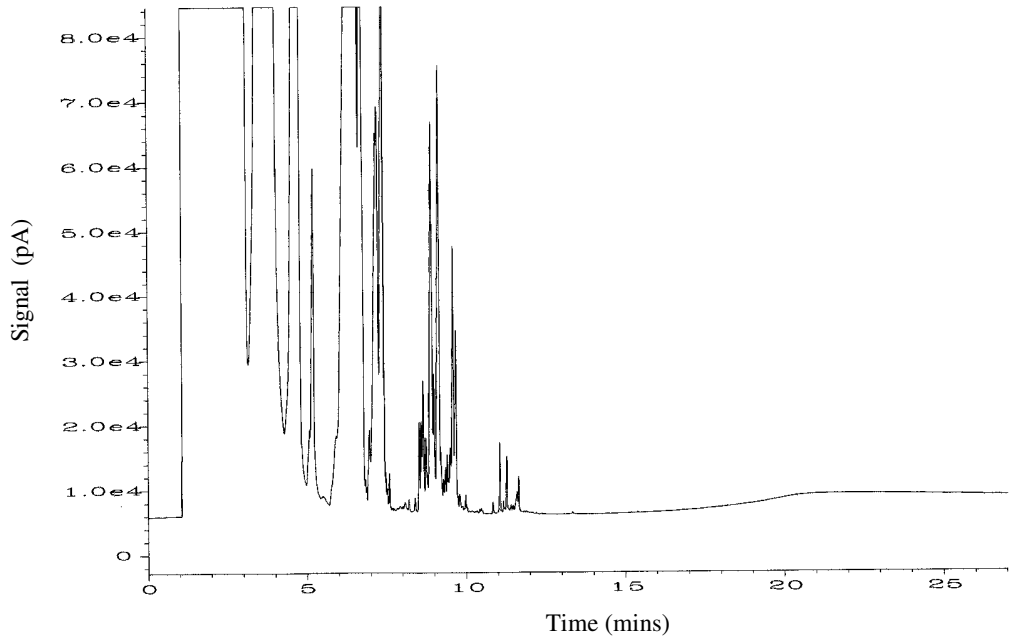



**Figure 5.1: GC Chromatogram of a Manufactured Sample of Sorbitan Caprylate, Analysed Using an Oven Temperature of 200°C to 400°C, With a Ramp Rate of 10°C/min**

## 5.2 Optimisation of GC Conditions:

### 5.2.1 Oven Temperature

From the chromatogram obtained using the initial GC oven conditions it was evident that components of sorbitan esters based on caprylic acid could be eluted from a GC column and that further investigation was worthwhile. However from the chromatogram it was apparent that the column temperature was too high to allow adequate separation of the early eluting components, therefore a lower starting temperature was selected for the next separation. Also because all the components were eluted in the first half of the temperature programme it was felt unnecessary to go to the maximum operating temperature of the column for the next analyses, therefore the final temperature was reduced slightly along with the detector temperature.

The sorbitan caprylate was analysed using the following conditions:

| | |
|---|---|
| Initial temperature: | 150°C |
| Ramp rate: | 10°C/minute |
| Final temperature: | 380°C |
| Injector temperature: | 300°C |
| Detector temperature: | 380°C |

The chromatogram obtained is shown in figure 5.2.

The chromatogram shows better separation of the components compared to the previous conditions, a number of well spaced groups of peaks are seen, with good peak shapes. The peak after the solvent peak appears as partially resolved peaks indicating that the column starting temperature could still be too high to allow adequate separation of the early eluting components in the mixture. It is therefore

also possible that some of the more volatile components are co eluting with the solvent.

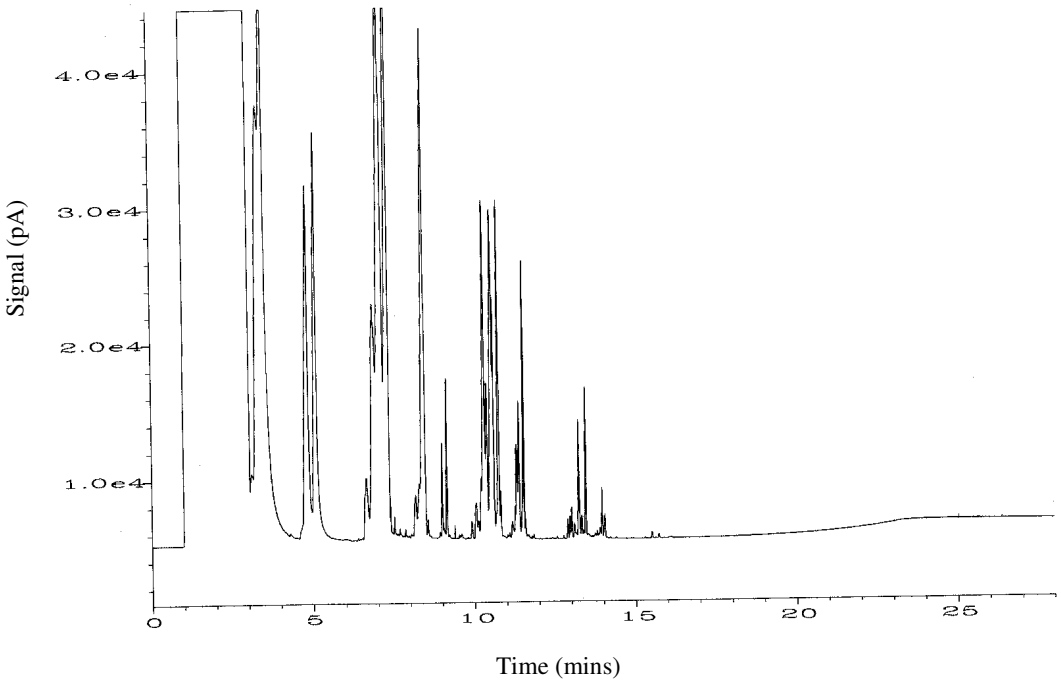



**Figure 5.2: GC Chromatogram of a Manufactured Sample of Sorbitan Caprylate Analysed Using an Oven Temperature of 150°C to 380°C, with a Ramp Rate of 10°C/min**

From the observations made from the chromatogram obtained using the previous conditions, it was felt necessary to reduce the initial oven temperature further. Using the conditions detailed below the chromatogram shown in figure 5.3 was obtained.

Initial temperature: 100°C

Ramp rate: 10°C/minute

Final temperature: 380°C

Injector temperature: 300°C

Detector temperature: 380°C

The chromatogram shows a series of well spaced peaks with good peak shape. The series of peaks observed in figure 5.2 are present but elute later and are more spaced out. Also the partially resolved peak observed after the solvent peak using the previous conditions is absent and has been replaced by a single peak well separated from the solvent peak and a group of peaks at 8-10 minutes. Reducing the initial temperature has allowed sufficient separation of the early components and the use of the temperature programme has not resulted in later eluting components occurring as broad peaks but as sharp resolved ones.

Using an initial oven temperature of 100°C is also beneficial, so that refocusing of the sample at the beginning of the column can occur.

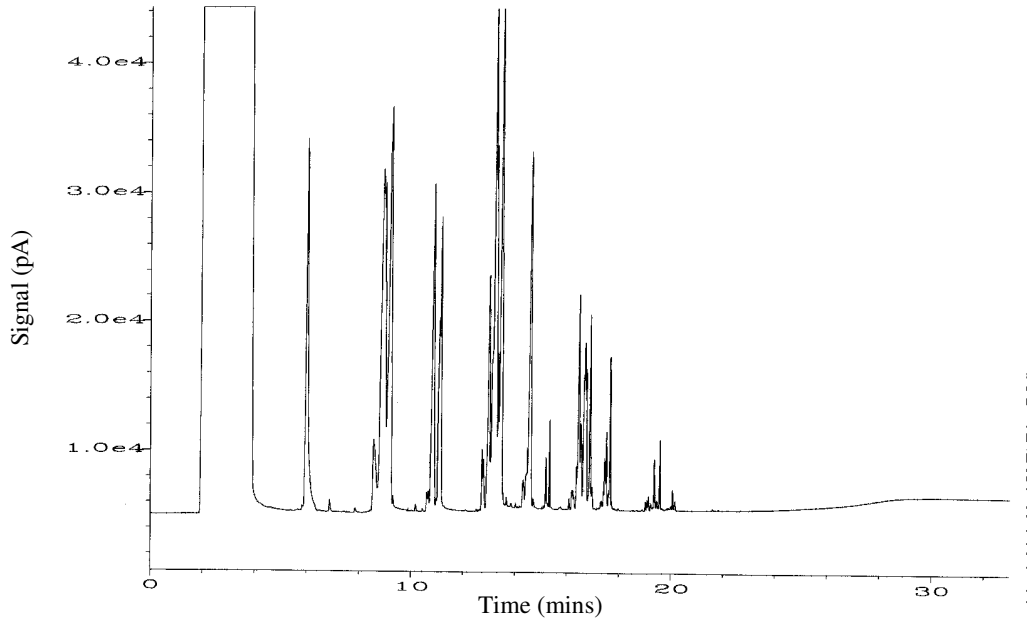



**Figure 5.3: GC Chromatogram of a Manufactured Sample of Sorbitan Caprylate, Analysed Using an Oven Temperature of 100°C to 380°C, With a Ramp Rate of 10°C/min**

### 5.2.2 Ramp Rate:

The previous three GC chromatograms were obtained using a ramp rate of 10°C/minute, the next investigation was to observe the effect altering the ramp rate has on the chromatography. The last oven conditions detailed were used and ramp rates of 8°C and 5°C were used instead of 10°C. The chromatograms obtained are shown in figure 5.4.

The chromatograms are similar to that in figure 5.3, but the peaks are more spaced out. Altering the ramp rate has not had an adverse effect on the peak shapes, however using a ramp rate of 5°C has increased the runtime to almost an hour. Using 8°C has increased the analysis time slightly but the peaks are better separated, therefore this ramp rate was selected for further work. The capacity factor for the first eluting component at each ramp rate was determined and compared, the values obtained are shown in table 5.1 below.

| **Ramp Rate (°C)** | **Capacity Factor** |
|---|---|
| 5 | 2.1 |
| 8 | 1.4 |
| 10 | 1.1 |

**Table 5.1: Capacity Factor of First Eluting Component Using Various Temperature Ramp Rates**

The results show that as the ramp rate increases the capacity factor decreases, indicating less retention as expected. It is likely that the later eluting components will behave similar.

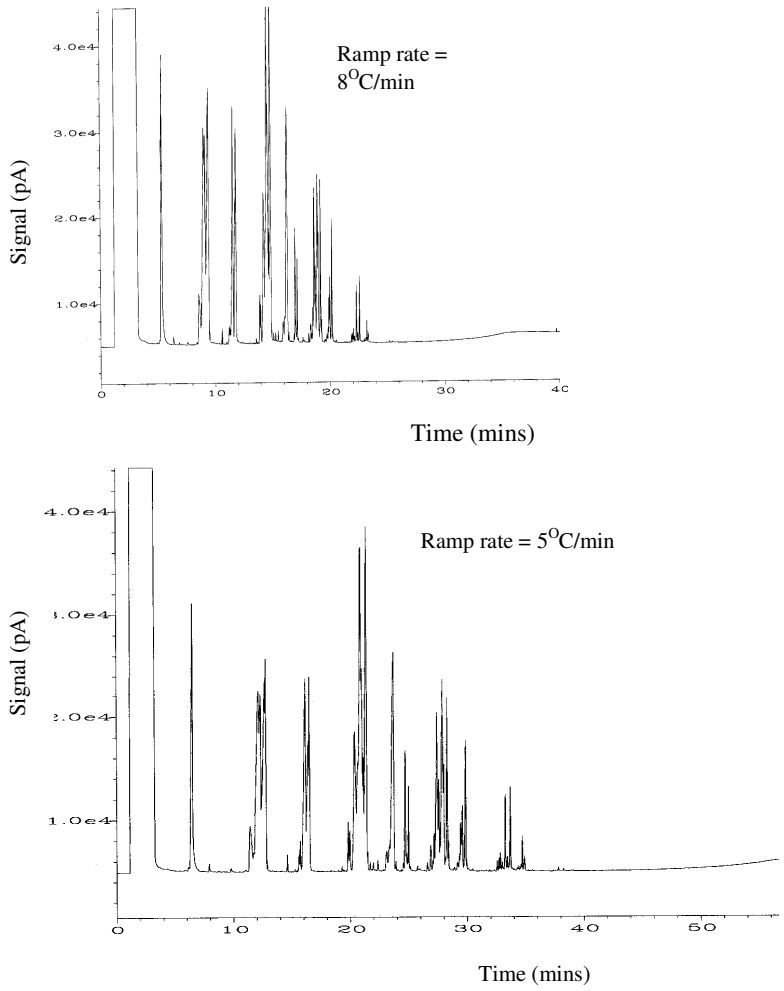



**Figure 5.4: GC Chromatograms of Sorbitan Caprylate Analysed Using Two Different Ramp Rates With an Oven Temperature of 100°C to 380°C**

### 5.2.3 Injector Temperature:

The injector temperature previously used was 300°C, however a higher temperature may be advantageous for volatilising higher molecular weight esters. An investigation was carried out to establish the effect of increasing the injector temperature to 380°C. The chromatograms obtained were comparable to those at 300°C, no other components eluted and components did not appear to degrade with the higher temperature. Therefore the higher temperature was selected for further work, to ensure volatilisation of components.

### 5.2.4 Optimised Conditions:

From this initial study the conditions used for further investigation were:

| | |
|---|---|
| Initial oven temperature: | 100°C |
| Ramp rate: | 8°C/min |
| Final temperature: | 380°C held for 5 minutes |
| Injector temperature: | 380°C |
| Detector temperature: | 380°C |

## 5.3 Peak Identification of Sorbitan Caprylate:

### 5.3.1 Analysis of Starting Materials:

An investigation into the retention time of the starting materials used to produce sorbitan caprylate was carried out. These are the lowest molecular weight components and therefore will be the first to elute, by acetylating them and analysing them using the final GC conditions detailed in chapter 5.2.2, it would be possible to establish their retention time. If no peaks were detected it would indicate that the temperature programme was still too high and they were co eluting with the solvent peak and that more method development was required.

The sorbitan caprylate used for the initial study was prepared from the polyol sorbitol and caprylic acid, during manufacture the sorbitol would have been dehydrated resulting in other polyols, sorbitan and isosorbide. Therefore the starting materials are sorbitol, sorbitan, isosorbide and caprylic acid, high purity isosorbide, sorbitol and caprylic acid were purchased and acetylated (as detailed in chapter 5.1.1). Sorbitan can not be purchased therefore an alternative approach was necessary. By carrying out a polyol profile of the sorbitan caprylate sample as detailed in chapter 2.2, the three

acetylated polyols would result. Using the chromatograms obtained for the pure isosorbide and sorbitol the retention time of sorbitan could also be established. The chromatograms obtained are shown in figure 5.5, peaks are observed for all starting materials indicating that the initial oven temperature is sufficient to separate them from the solvent. Residual polyols may be present in real manufactured sorbitan ester samples and therefore they can now be identified if the same GC conditions are used. If polyols and caprylic acid can be separated from the solvent it is assumed that other components in the sorbitan caprylate can, because they will have a larger molecular weight and therefore will be less volatile and have a longer elution time.

### 5.3.2 Peak Identification of Esters Species:

#### 5.3.2.1 Preparation of Polyol Esters of Caprylic Acid:

The retention time of the starting materials of sorbitan caprylate was possible because pure material is readily available from chemical suppliers except sorbitan which was established from the polyol profile of sorbitan caprylate. However pure samples of the ester species present in the sorbitan caprylate are not available, it was therefore necessary to produce samples. Samples were prepared containing one polyol and using high purity caprylic acids, in an attempt to aid identification of the peaks and establish which esters eluted.

Isosorbide caprylate esters:

The ratio of isosorbide: caprylic acid used was 1:1 w/w. From previous work (Rachael Shaddick MSc. Project (96)) carried out it was found that at this ratio both mono and di esters are produced, with some residual isosorbide and that the isosorbide di ester is produced in preference to the isosorbide mono ester. Therefore

from the material prepared it is the intention that the retention times of both the isosorbide esters species can be established.

182.6 g isosorbide (98 % ex Aldrich) and 180.3 g caprylic acid (98+ % ex. Aldrich) were reacted in the presence of 0.1 % Fascat 4100 as a catalyst at 190°C for 6 hours. The reaction was followed by taking samples at various time intervals and determining the acid value (AV). The reaction was complete when the AV fell to 3, which indicated that the fatty acid had reacted with the isosorbide.

Sorbitol caprylate esters:

The reaction was carried out at low temperature to prevent the dehydration of the sorbitol to the other known polyols. The ratio of sorbitol: acid chloride used was 1:1.5 w/w, this is slightly higher than the ratio used in the preparation of sorbitan esters. This was used in an attempt to prepare some higher esters and establish if they can be eluted from the GC column.

10.9 g sorbitol (99+ % ex Aldrich) was dissolved in 100 ml pyridine and placed in an ice bath, 14.6 g octanoyl chloride (99 % ex Aldrich) added drop wise over ~20 minutes. The mixture was left stirring overnight and then the pyridine was removed using a rotary evaporator.

Sorbitan esters:

Unfortunately sorbitan can not be purchased therefore it was not possible to prepare a sample of sorbitan esters by reacting sorbitan and fatty acid. It was necessary to react sorbitol and fatty acid under conditions that would result in sorbitan being the main

polyol. Previous work carried out by C Newbould at Croda showed that the optimum laboratory condition for cyclisation of sorbitol to sorbitan was 195°C.

The first stage in the preparation of the sorbitan caprylate was therefore to prepare the polyol species required at 195°C. This was then followed by esterification of the resulting polyols with the acids at a higher temperature.

The ratio of sorbitol: acid used was 1:1.3 w/w which is a typical ratio used in the preparation of sorbitan esters.

260 g sorbitol syrup (70 % ex Croda) was cyclised by heating to 195 °C for 2 hours in the presence of 0.9 g hypophosphorus acid and 187.2 g octanoic acid. Then esterified at 215°C for 2 hours using 1.34 g sodium hydroxide as the catalyst. The apparatus used is shown in figure 5.6.

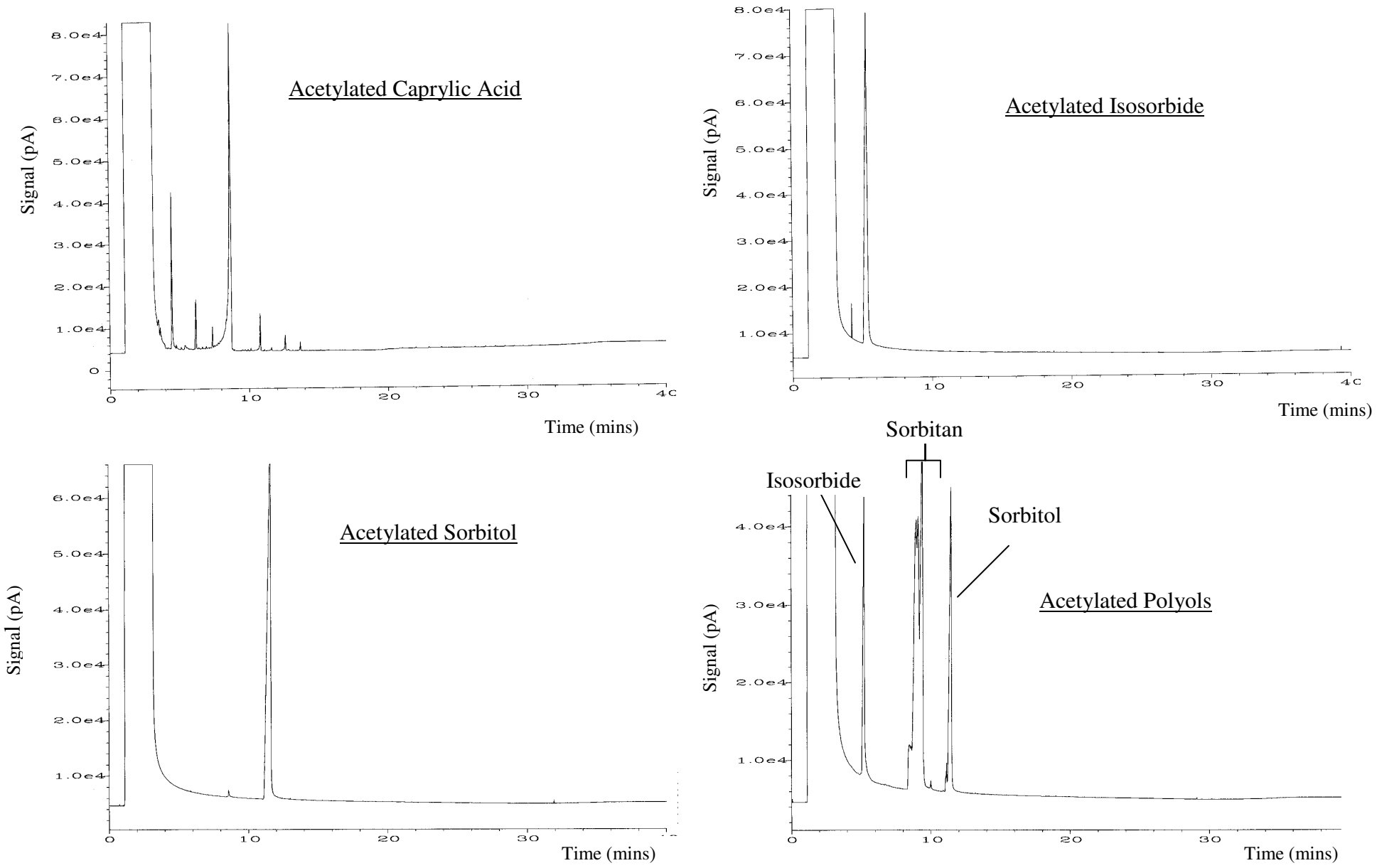



**Figure 5.5: GC Chromatograms of Acetylated Starting Materials used in the Preparation of Sorbitan Caprylate**

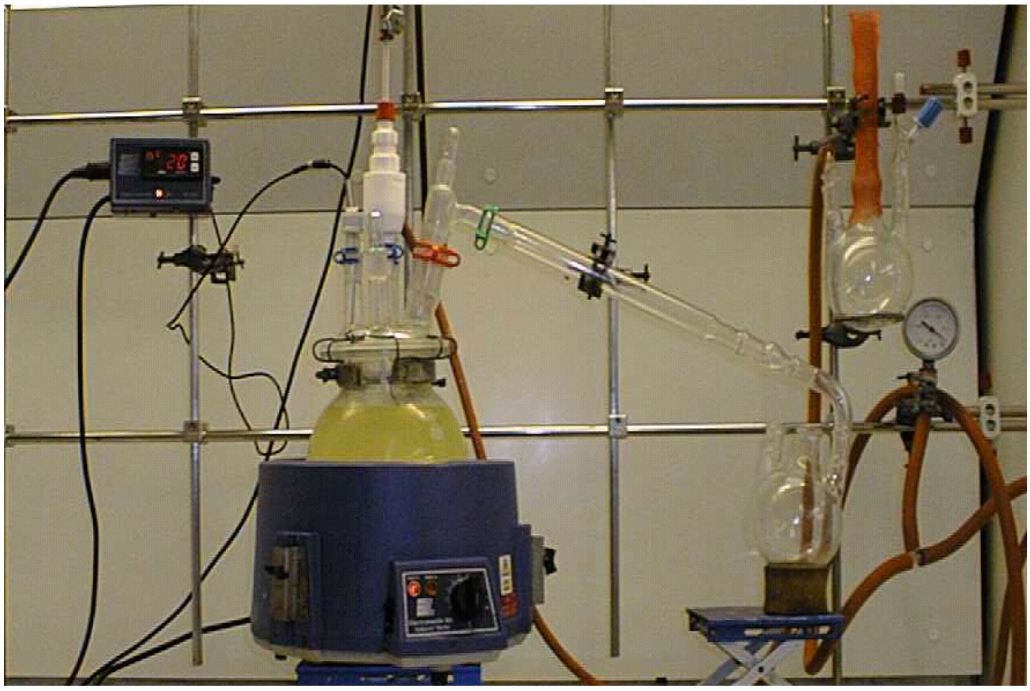


**Figure 5.6: Laboratory Equipment used for the Preparation of Sorbitan Caprylate**

The polyols present in the resulting sample was determined by carrying out a polyol profile and was found to contain:

Isosorbide ~12 %

Sorbitan ~87 %

Sorbitol <1 %

This shows that the sorbitol had been fully dehydrated to sorbitan and isosorbide, with sorbitan being the predominant polyol.

### 5.3.2.2 GC Analysis of Polyol Esters of Caprylic Acid:

The three samples prepared were acetylated and analysed by GC using the details described in 5.1.1 and 5.2.4.

Isosorbide caprylate - the chromatogram obtained (figure 5.7) has three major peaks, from analysis of the starting materials the first peak can be assigned as isosorbide, the two other peaks are assumed to be the mono and di caprylic esters of isosorbide. This indicates that both caprylic esters of isosorbide are sufficiently volatile to elute using the conditions selected.

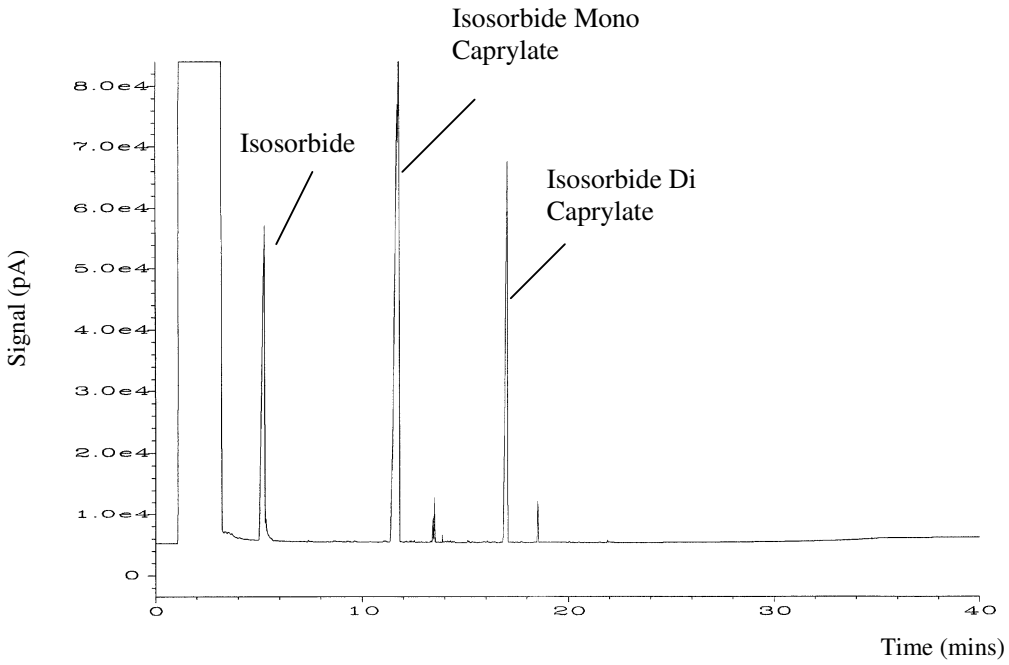



**Figure 5.7: GC Chromatogram of Isosorbide Caprylate**

Sorbitol Caprylate - the chromatogram obtained is shown in figure 5.8, the retention time of sorbitol is 10.5 minutes and was established from the work detailed in chapter 5.3.1. A large peak at 15.7 minutes is thought to be due to sorbitol mono caprylate the

large peak eluting next sorbitol di caprylate and the group of peaks eluting at 22.5 minutes the tri esters. A small peak is also observed at the end of the chromatogram and it is possible this is sorbitol tetracaprylate. The ratio used to prepare the sorbitol caprylate was 1:1.5 w/w sorbitol:acid chloride, therefore it was expected that the main ester species present would be mono and di's. However the chromatogram indicates that sorbitol tri esters of caprylic acid and possibly tetra esters were produced and will elute from the column. Penta and hexa sorbitol esters are not seen, this is possibly due to the fact that they were not produced at the ratio used to prepare the sorbitol caprylate.

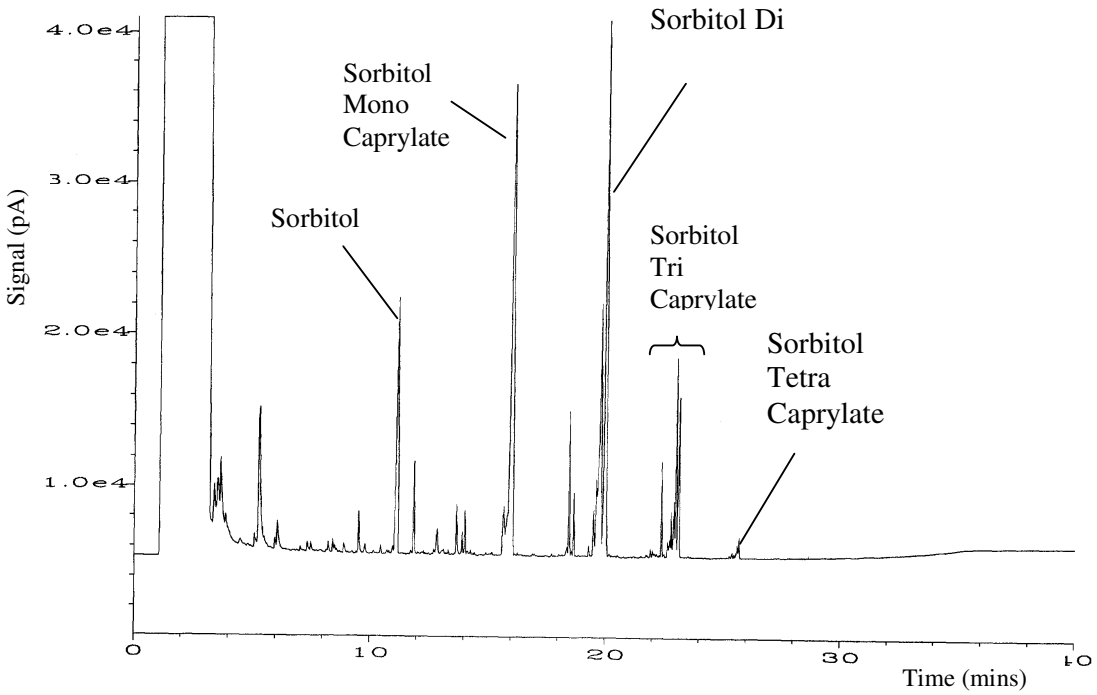



**Figure 5.8: GC Chromatogram of Sorbitol Caprylate**

Sorbitan capylate - the chromatogram obtained is shown in figure 5.9. The polyol profile of the sample showed that the sample was essentially sorbitan but did contain some isosorbide, the retention times obtained for the isosorbide and isosorbide esters was established in figure 5.7. These peaks were identified in the sorbitan caprylate chromatogram, however these were not of interest in this chromatogram, this sample was analysed to establish if sorbitan ester species of caprylic acid will elute from the GC column. The chromatogram shows that the sorbitan ester species appear as groups of peaks, this may be due to a number of different isomers. The peak due to sorbitan was determined from the analysis of the starting materials, three other groups of peaks are observed which are thought to correspond to mono, di and tri esters, as shown in the chromatogram.

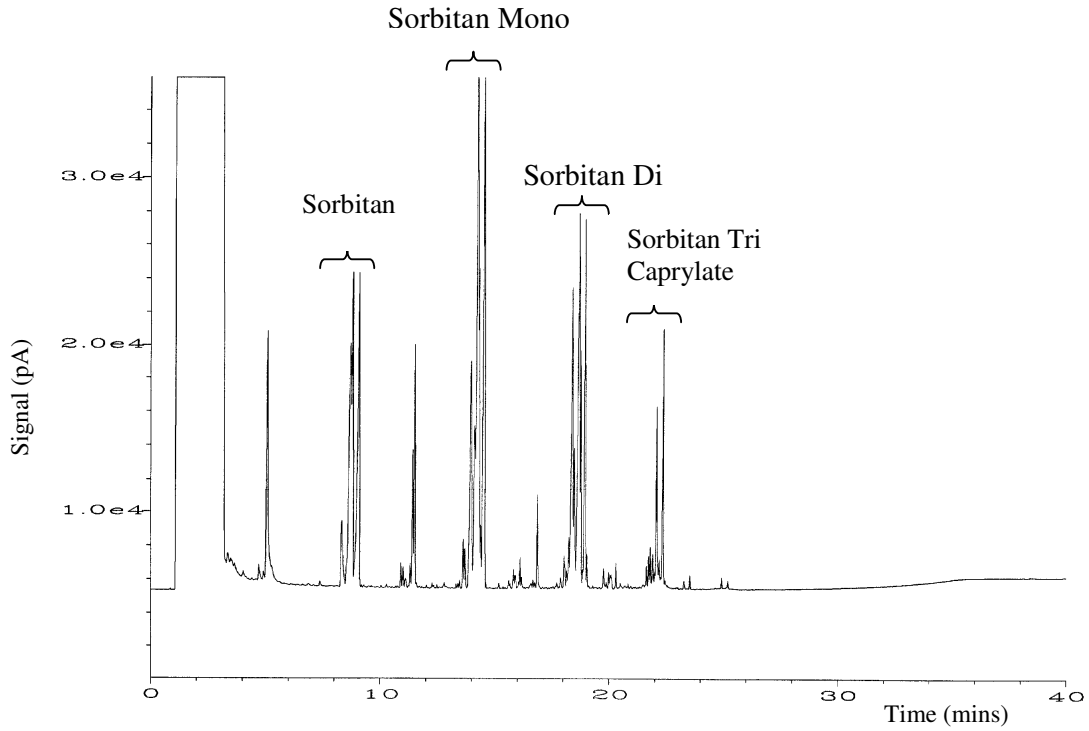



**Figure 5.9: GC Chromatogram of Sorbitan Caprylate**

**5.4 Identification of the Peaks in the Manufactured Sorbitan Caprylate Sample:**

Using the information gained from the analysis of the starting materials and the samples prepared containing one polyol, identification of the peaks in the manufactured sample of sorbitan caprylate has been attempted.The chromatogram obtained with labeled peaks is shown in figure 5.10. This shows that a chromatogram for sorbitan caprylate has been attainable with different species separated and identified. The largest ester species detected were tri esters, although these were small compared to the other ester species, however this is as expected because the material would be manufactured at a ratio of 1:1.3 w/w sorbitol: acid. The chromatogram also shows peaks corresponding to the starting polyols, these will be in excess due to the production of di and tri esters.

The peak identification is based on the species expected to be produced and their size and volatility. GC-MS could be used to confirm these identifications, however this technique was not readily available when the work was carried out.

From this work it can be concluded that GC analysis is a highly successful technique for the analysis of sorbitan caprylate, with information obtained on the various ester species present. It would therefore be advantageous to carry out further investigation on GC analysis of esters prepared from higher molecular weight fatty acids.

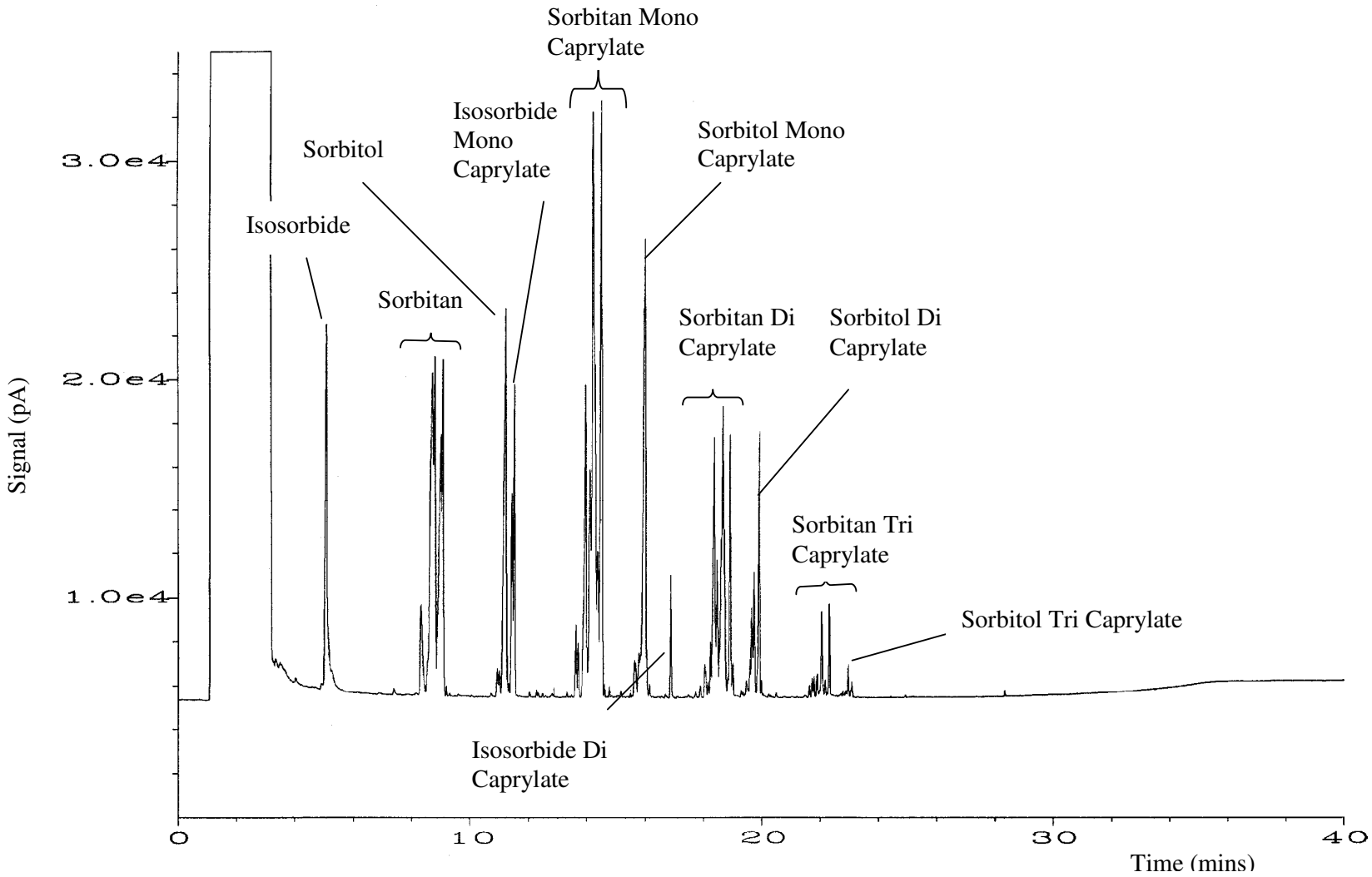



**Figure 5.10: GC Chromatogram of Sorbitan Caprylate Manufactured at Croda Singapore**

## 6. GC Analysis of Sorbitan Esters Using Split/Splitless Injection:

From the initial GC study (chapter 5) it was apparent that GC is a suitable method of analysis for sorbitan caprylate, with a large number of the possible ester species of sorbitan caprylate being eluted from the column. However it was felt that further investigation was required to establish the effect increasing the fatty acid chain lengths of sorbitan esters has on retention, because this will make the molecules larger and less volatile. A study was carried out, by increasing the fatty acid chain length by two carbon atoms at a time for each of the polyol esters. The largest esters investigated were those based on stearic and oleic acid. The samples were analysed using the same GC conditions used for the polyol esters of caprylic acid and the resulting chromatograms obtained observed to establish if the various ester components could be eluted form the GC column or not.

### 6.1 Preparation of Polyol Esters:

The first stage of the study was to prepare each of the polyol esters from high purity fatty acid feed stocks.

#### 6.1.1 Isosorbide Esters:

Isosorbide esters of decanoic acid to stearic acid were prepared as detailed in the chapter 5.3.2.1.

In all cases the isosorbide used was 98 % ex. Aldrich (MW = 146.14) and high purity fatty acids were used. The ratio of isosorbide: acid used was 1:1 w/w and the quantities for each of the isosorbide fatty acid esters prepared are detailed in table 6.1.

| | Isosorbide Decanoate | Isosorbide Laurate | Isosorbide Myristate | Isosorbide Palmitate | Isosorbide Stearate |
|---|---|---|---|---|---|
| Acid purity | 96 % | 98 % | 99.5 % | 95 % | 95 % |
| Acid MW | 172.3 | 200.3 | 228.4 | 256.4 | 284.5 |
| Acid used/(g) | 258.4 | 240.0 | 275.0 | 256.4 | 194.7 |
| Moles of acid | 1.5 | 1.2 | 1.2 | 1.0 | 0.7 |
| Isosorbide used/(g) | 219.2 | 175.0 | 176.0 | 146.1 | 100.0 |
| Catalyst used/(g) | 0.5 | 0.4 | 0.5 | 0.4 | 0.3 |

**Table 6.1: Quantities used for the Preparation of Various Isosorbide Esters**

The C10 – C14 acids were supplied by Aldrich and the C16 and C18 by Avocado.

All of the isosorbide fatty acids prepared were analysed by GPC (as detailed in chapter 2.3) and all showed three major peaks thought to correspond to free isosorbide, isosorbide mono ester and isosorbide di ester as expected.

**6.1.2 Sorbitan Esters**:

Sorbitan esters were prepared using the same acids used to prepare the isosorbide esters and 70 % sorbitol syrup (ex Croda). They were prepared using the method detailed in section 5.3.2.1 for sorbitan esters, the ratio of sorbitol: acid was 1:1.3 w/w, which is a typical ratio used in the preparation of sorbitan esters at Croda. The following chemicals were used as shown in table 6.2.

| | Sorbitan Decanoate | Sorbitan Laurate | Sorbitan Myristate | Sorbitan Palmitate | Sorbitan Stearate |
|---|---|---|---|---|---|
| Sorbitol Syrup/(g) | 260 | 220 | 195 | 175 | 175 |
| Moles of Sorbitol | 1 | 0.9 | 0.8 | 0.7 | 0.7 |
| Fatty Acid /(g) | 224.0 | 220.0 | 222.7 | 224.3 | 248.9 |
| Moles of fatty acid | 1.3 | 1.1 | 1.0 | 0.9 | 0.9 |
| Hypophosphorus acid (50%) /(g) | 0.2 | 0.9 | 0.2 | 0.2 | 0.2 |
| Sodium hydroxide/(g) | 0.7 | 1.3 | 0.7 | 0.6 | 0.6 |

**Table 6.2: Quantities used for the Preparation of Various Sorbitan Esters**

The intention was to dehydrate the sorbitol to sorbitan and esterify this to produce samples containing high levels of sorbitan esters. Polyol profiles for each of the samples prepared were carried out (as detailed in 2.2) to establish if this had been achieved, table 6.3 shows that the samples were found to contain:

| | Sorbitan Decanoate | Sorbitan Laurate | Sorbitan Myristate | Sorbitan Palmitate | Sorbitan Stearate |
|---|---|---|---|---|---|
| Isosorbide | ~5 % | ~22 % | ~7 % | ~3 % | ~5 % |
| Sorbitan | ~90 % | ~76 % | ~93 % | ~80 % | ~76 % |
| Sorbitol | ~5 % | ~2 % | N/D | ~17 % | ~19 % |

**Table 6.3: Polyol Profiles of Sorbitan Decanoate to Stearate**

The polyol profiles show that esters containing only sorbitan as the polyol were not prepared, however all the samples prepared did contain a high level of sorbitan and consequently it is assumed high levels of sorbitan esters.

### 6.1.3 Sorbitol Esters:

Sorbitol esters were produced from sorbitol (99+ % ex. Aldrich, MW = 182) and acid chloride, using a ratio of 1:1.5 w/w sorbitol: acid chloride, all the acid chlorides were purchased from Aldrich. The sorbitol esters were prepared using the method detailed in chapter 5.3.2.1 for sorbitol esters, but using the chemicals detailed in table 6.4.

| | Sorbitol Decanoate | Sorbitol Laurate | Sorbitol Myristate | Sorbitol Palmitate | Sorbitol Stearate |
|---|---|---|---|---|---|
| Acid Chloride purity | 98 % | 98 % | 97 % | 98 % | 99 % |
| Acid chloride MW | 190.7 | 218.8 | 246.8 | 274.9 | 302.9 |
| Acid chloride used/(g) | 18.1 | 19.6 | 22.2 | 24.7 | 13.6 |
| Moles of acid chloride | 0.09 | 0.09 | 0.09 | 0.09 | 0.045 |
| Sorbitol used/(g) | 10.9 | 10.9 | 10.9 | 10.9 | 5.5 |
| Moles of sorbitol | 0.06 | 0.06 | 0.06 | 0.06 | 0.03 |

**Table 6.4: Quantities used to Produce Various Sorbitol Esters Using a Ratio of 1:1.5 w/w Sorbitol:Acid Chloride**

Sorbitol esters were also produced using a ratio of 1:3 w/w sorbitol:acid chloride. Sorbitol mono to hexa esters can be produced, hexa esters with all six hydroxyl groups esterified will be the largest ester produced for each of the acids being investigated and therefore will be the least volatile and the last to elute from the column. At a higher ratio it was thought that the higher esters might be produced and

therefore the likelihood of them eluting could be investigated. The chemicals detailed in table 6.5 were used.

| | Sorbitol Decanoate | Sorbitol Laurate | Sorbitol Myristate | Sorbitol Palmitate | Sorbitol Stearate |
|---|---|---|---|---|---|
| Acid chloride used/(g) | 17.2 | 22.2 | 44.2 | 31.2 | 8.7 |
| Moles of acid chloride | 0.09 | 0.10 | 0.18 | 0.11 | 0.03 |
| Sorbitol used/(g) | 5.5 | 5.5 | 10.9 | 6.9 | 1.8 |
| Moles of sorbitol | 0.03 | 0.03 | 0.06 | 0.04 | 0.01 |

**Table 6.5: Quantities used to Produce Various Sorbitol Esters Using a Ratio of 1:3 w/w Sorbitol:Acid Chloride**

### 6.2 GC Analysis:

It is expected that the same ester species should be produced for each type of polyol ester using the different fatty acids. However the volatility will decrease as the fatty acid chain lengths increase. It is the number of esters species that elute from the column that is being investigated, therefore each of the samples prepared were acetylated as detailed in chapter 5.1.1 and analysed using the GC conditions in chapter 5.2.4 and the resulting chromatograms observed to establish the ester species eluting.

### 6.2.1 Polyol Esters of Decanoic Acid:

The chromatograms obtained for each of the polyol esters of decanoic acid are shown in figure 6.1.

The isosorbide decanoate chromatogram shows three major peaks well resolved from each other, the first peak is due to isosorbide, which was established from previous analysis of the starting materials. The other two peaks will be due to the mono and di esters of isosorbide, this correlates with the information obtained from GPC analysis. Both of the possible ester species of isosorbide decanoate were produced and both eluted from the GC column using the conditions selected.

The sorbitan decanoate gave a more complex chromatogram, this is due to the fact that the sample probably contains some isosorbide and sorbitol esters, because from the polyol profile it was established that some isosorbide and sorbitol were present, 100 % conversion of the sorbitol to sorbitan did not occur. The chromatogram shows that some residual sorbitan is present, again the retention time was established from the polyol profile chromatograms. Three other large groups of peaks are observed that have a similar profile to the sorbitan, it is assumed that these are due to sorbitan mono, di and tri decanoate species, with the tri ester being the last of these groups because it will have three fatty acid groups and therefore will be the largest of these sorbitan ester species and as a consequence will be the least volatile. A small group of peaks is visible with a retention time of ~30 minutes, it is possible that this is due to sorbitan tetra decanoate.

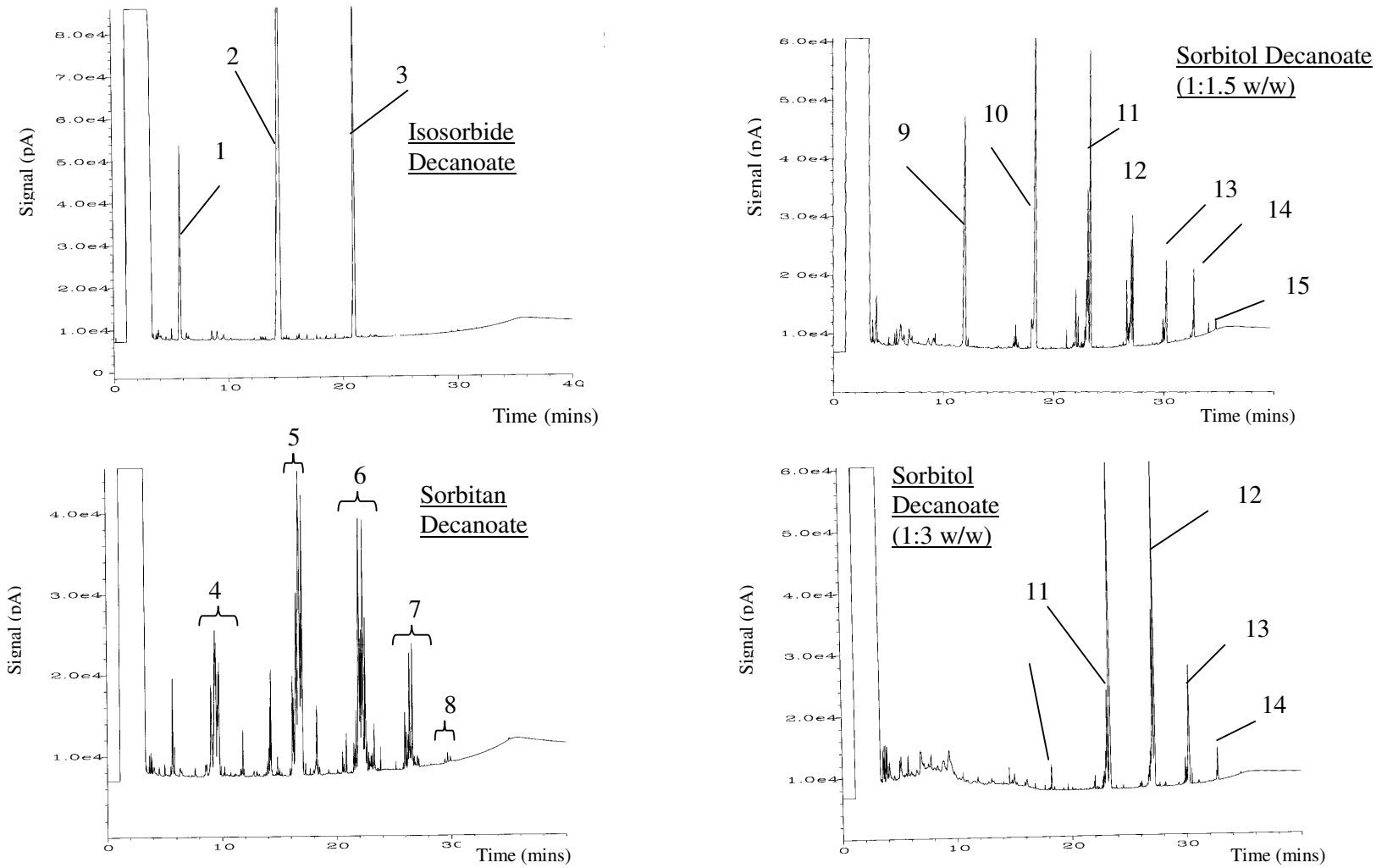



**Figure 6.1: GC Chromatograms of Polyol Esters of Decanoic Acid, Acetylated and Analysed Using a DB1-ht Column, Injector at 380°C, Detector at 380°C and Oven Temperature 100°C to 380°C at 8°C/min**

1 = Isosorbide, 2 = Isosorbide mono decanoate, 3 = Isosorbide di decanoate, 4 = Sorbitan, 5 = Sorbitan mono decanoate, 6 = Sorbitan di decanoate, 7 = Sorbitan tri decanoate, 8 = Sorbitan tetra decanoate, 9 = Sorbitol, 10 = Sorbitol mono decanoate, 11 = Sorbitol di decanoate, 12 = Sorbitol tri decanoate, 13 = Sorbitol tetra decanoate, 14 = Sorbitol penta decanoate, 15 = Sorbitol hexa decanoate

The ratio of sorbitol to acid used for preparation of this sample was 1:1.3 which is a usual stochiometry used in the production of sorbitan esters, at this reaction ratio it is assumed that very little tetra ester will be produced. However there is evidence that a small amount may have been produced and eluted from the column.

Two chromatograms of sorbitol decanoate are shown in figure 6.1 one for the sample produced when a ratio of 1:1.5 w/w sorbitol: decanoyl chloride was used and the other for the sample produced at 1:3 w/w ratio. A ratio of 1:1.5 w/w is a more typical ratio used in manufacture, however a higher ratio was also used, it was thought that at a higher ratio there was a greater possibility of producing higher esters e.g. sorbitol penta and hexa decanoate and establishing if they would elute from the GC column. The chromatogram obtained for the 1:1.5 w/w reaction ratio shows some unreacted sorbitol, followed by the two major peaks of the chromatogram and thought to correspond to sorbitol mono and di decanoate, at the ratio used it was assumed that these would be the major species produced. However three other smaller but well defined later eluting peaks are also observed, thought to be due to the tri, tetra and penta ester species. Due to the ratio of acid to sorbitol used in the preparation these will be at a lower concentration than the other ester species, however their presence does account for the unreacted sorbitol.

In contrast to this chromatogram, the chromatogram obtained for the sorbitol decanoate produced with a ratio of 1:3 w/w starting material shows no unreacted sorbitol and only a very small peak at the retention time expected for sorbitan mono decanoate. Two dominant peaks are observed thought to be sorbitol di and tri decanoate, two smaller peaks elute after these and will probably be due to the tetra

and penta ester species, with a ratio of 1:3 w/w it was expected that the tri ester would be the main ester species produced.

At the 1:1.5 w/w ratio a small peak is detected at the end of the chromatogram that could be due to sorbitol hexa decanaote, however this is not as evident with the higher ratio material. The work does show that it is possible for all sorbitol ester species to be eluted from the column using the conditions selected.

The chromatograms obtained for the polyol esters of decanoic acid show that all of the possible ester species can be eluted to some degree.

**6.2.2 Polyol Esters of Lauric Acid:**

The chromatograms obtained for the polyol esters based on lauric acid are shown in figure 6.2. The chromatogram obtained for isosorbide laurate is very similar to that obtained for isosorbide decanoate, showing three peaks corresponding to unreacted isosorbide, isosorbide mono ester and isosorbide di ester. Although this chromatogram shows less unreacted isosorbide and the two ester species have a slightly longer retention time, this will be due to the larger size of the fatty acid used, making these isosorbide esters less volatile than the isosorbide esters of decanoic acid.

The sorbitan laurate chromatogram shows a peak at the beginning of the chromatogram with the retention time of unreacted isosorbide followed by a group of peaks due to unreacted sorbitan. The polyol profile carried out on the sorbitan laurate sample prepared showed that it has 22 % isosorbide and two peaks with the same retention times of isosorbide mono and di laurate are observed. However three groups

of peaks are also observed which will correspond to sorbitan mono, di and tri esters of lauric acid.

The chromatogram for the sorbitol laurate sample produced with a stochiometry of 1:1.5 w/w shows residual sorbitol, a peak due to sorbitol mono ester and the largest peak due to sorbitol di ester, two later eluting peaks are observed due to the tri and tetra ester. A small peak thought to be due to the penta ester is also seen. In contrast to the chromatogram obtained for sorbitol laurate prepared from a ratio of 1:1.5 w/w starting material, the chromatogram obtained for the material produced from a reaction of 1:3 w/w shows considerably less peaks. No unreacted sorbitol peak is observed and the sorbitol mono ester peak is very small, three peaks are observed after the small mono ester peak, these are thought to be the di, tri and tetra sorbitol ester species, with the di ester being the largest. No penta ester peak is observed at this ratio indicating that it was not produced, at 1:1.5 w/w ratio a penta ester peak is detected indicating that it will elute from the column.

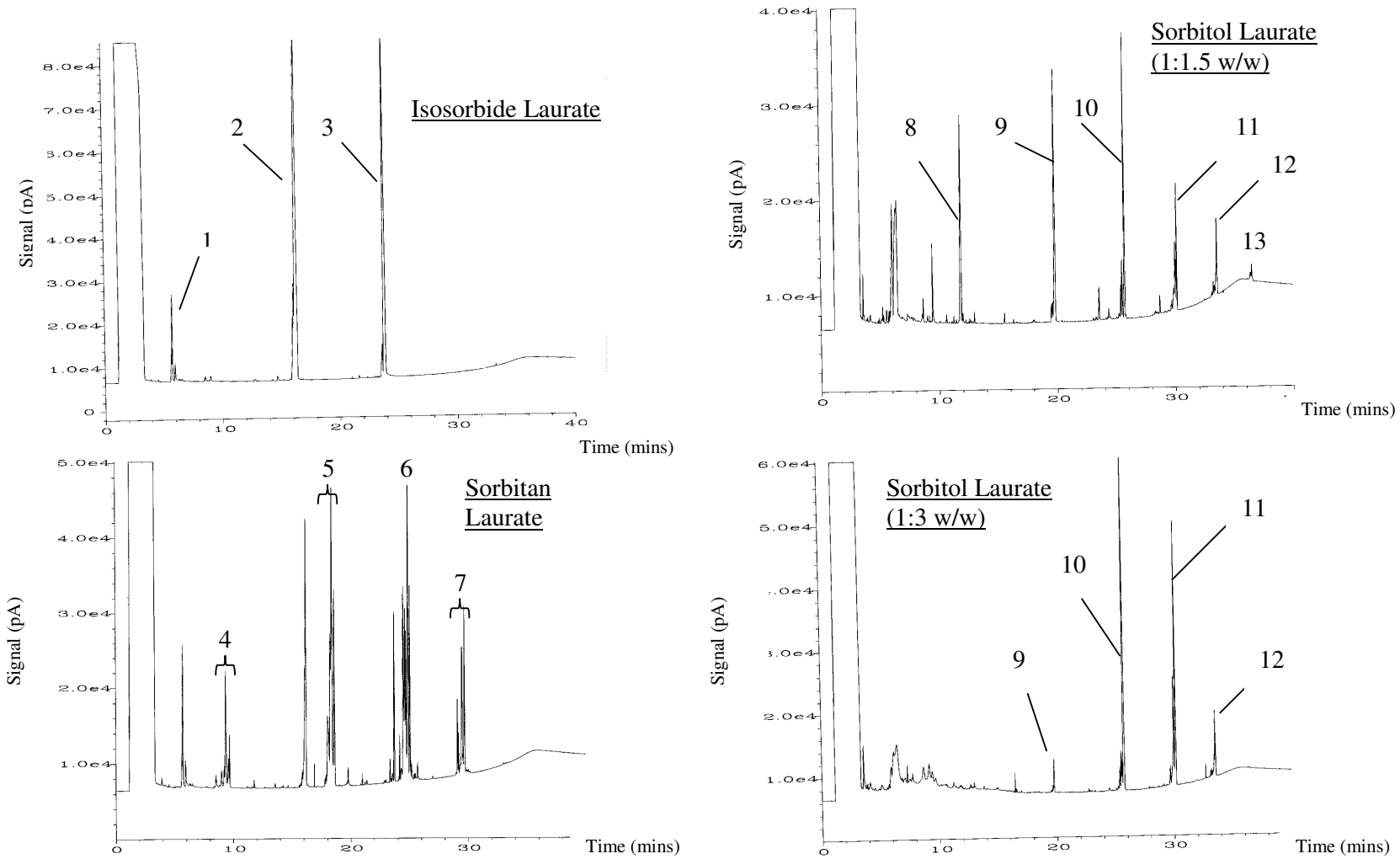



**Figure 6.2: GC Chromatograms of Polyol Esters of Lauric Acid, Acetylated and Analysed Using a DB1-ht Column, Injector at 380°C, Detector at 380°C and oven temperature 100°C to 380°C at 8°C/min**

1 = Isosorbide, 2 = Isosorbide mono laurate, 3 = Isosorbide di laurate, 4 = Sorbitan, 5 = Sorbitan mono laurate, 6 = Sorbitan di laurate, 7 = Sorbitan tri laurate, 8 = Sorbitol, 9 = Sorbitol mono laurate, 10 = Sorbitol di laurate, 11 = Sorbitol tri laurate, 12 = Sorbitol tetra laurate, 13 = Sorbitol penta laurate

### 6.2.3 Polyol Esters of Myristic Acid:

The chromatograms are shown in figure 6.3.

The isosorbide myristate chromatogram shows three peaks due to the unreacted isosorbide and the mono and di ester species, as with the other isosorbide esters investigated. Both of the possible ester species of isosorbide myristate are sufficiently volatile to elute from a DB1-ht column using the conditions selected.

The sorbitan myristate chromatogram shows four groups of peaks due to unreacted sorbitan and sorbitan mono, di and tri myristate. Peaks due to isosorbide and its esters are also seen, this chromatogram is again very similar to that for sorbitan laurate, although the esters for myristic acid have a slightly longer retention time due to the decreased volatility. A very small group of peaks are just evident at the end of the chromatogram which could possibly be sorbitan tetra myristate.

The chromatogram for the sorbitol myristate prepared at a 1:1.5 w/w shows three major peaks after the residual sorbitol thought to be due to the mono, di and tri esters, a very small peak is visible after these and is probably due to the tetra ester species. The chromatogram obtained for sorbitol myristate prepared with a stochiometry of 1:3 w/w shows only two large peaks thought to be the di and tri esters, as in the previous chromatogram a small peak is observed after the tri ester peak thought to be the tetra ester. There is no evidence of any higher sorbitol esters on either chromatogram. The tetra esters elute when the GC is at its final temperature used, therefore the temperature is probably insufficient to elute any higher esters which will be less volatile.

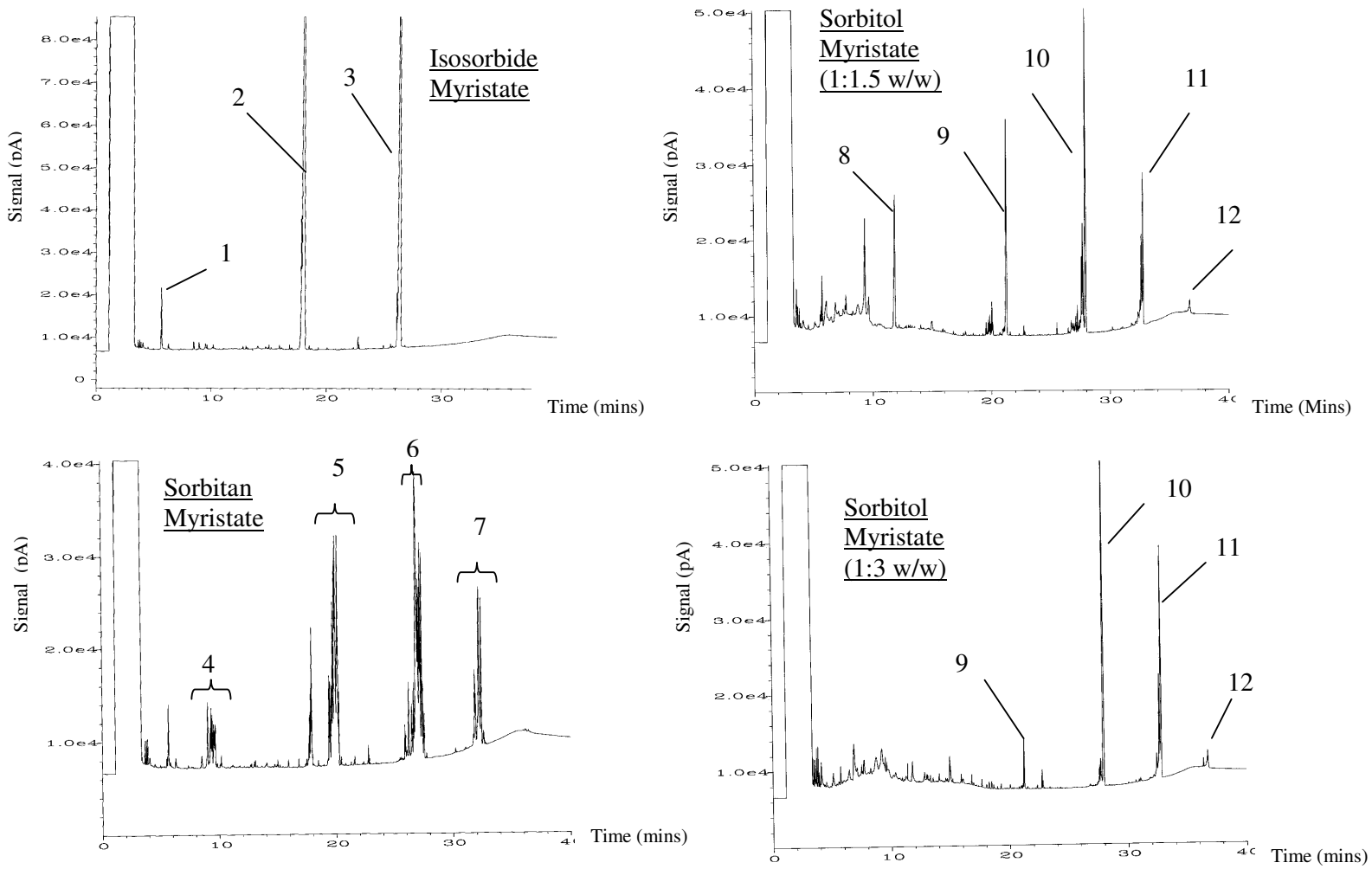



**Figure 6.3: GC Chromatograms of Polyol Esters of Myristic Acid, Acetylated and Analysed Using a DB1-ht Column, Injector at 380°C, Detector at 380°C and Oven Temperature 100°C to 380°C at 8°C/min**

1 = Isosorbide, 2 = Isosorbide mono myristate, 3= Isosorbide di myristate, 4 = Sorbitan 5 = Sorbitan mono myristate, 6 = Sorbitan di myristate, 7= Sorbitan tri myristate, 8 =Sorbitol, 9 = Sorbitol mono myristate, 10 = Sorbitol di myristate, 11 = Sorbitol tri myristate, 12 = Sorbitol tetra myristate

### 6.2.4 Polyol Esters of Palmitic Acid:

The chromatograms of polyol esters based on palmitic acid are shown in figure 6.4. The chromatogram obtained for the isosorbide palmitate sample, as with previous isosorbide samples shows two well defined and separated peaks due to the two possible ester species.

The sorbitan palmitate chromatogram again shows residual sorbitan, as well as groups of peaks that will correspond to the sorbitan mono and di palmitate ester species. The last group of peaks observed will be due to the tri ester species and is smaller than has been seen for the previous sorbitan esters with shorter fatty acid chain length, this may be due to the increase in size and hence decreased volatility. The tri esters elute near the final oven temperature, which may only partially volatilise them and hence the reduction in size compared to previous sorbitan esters studied. No tetra esters are observed.

The sorbitol palmitate chromatograms also show differences to those seen for the previous smaller chain sorbitol esters, for both reaction ratios only three peaks are observed due to the mono, di and tri sorbitol ester species. The last peak to elute is the tri ester, the retention time for this peak is ~35 minutes which corresponds to the time the oven reached it maximum prior to the 5 minute hold time, this indicates that the maximum temperature used was not sufficient to elute any of the higher sorbitol esters, assuming they have been produced.

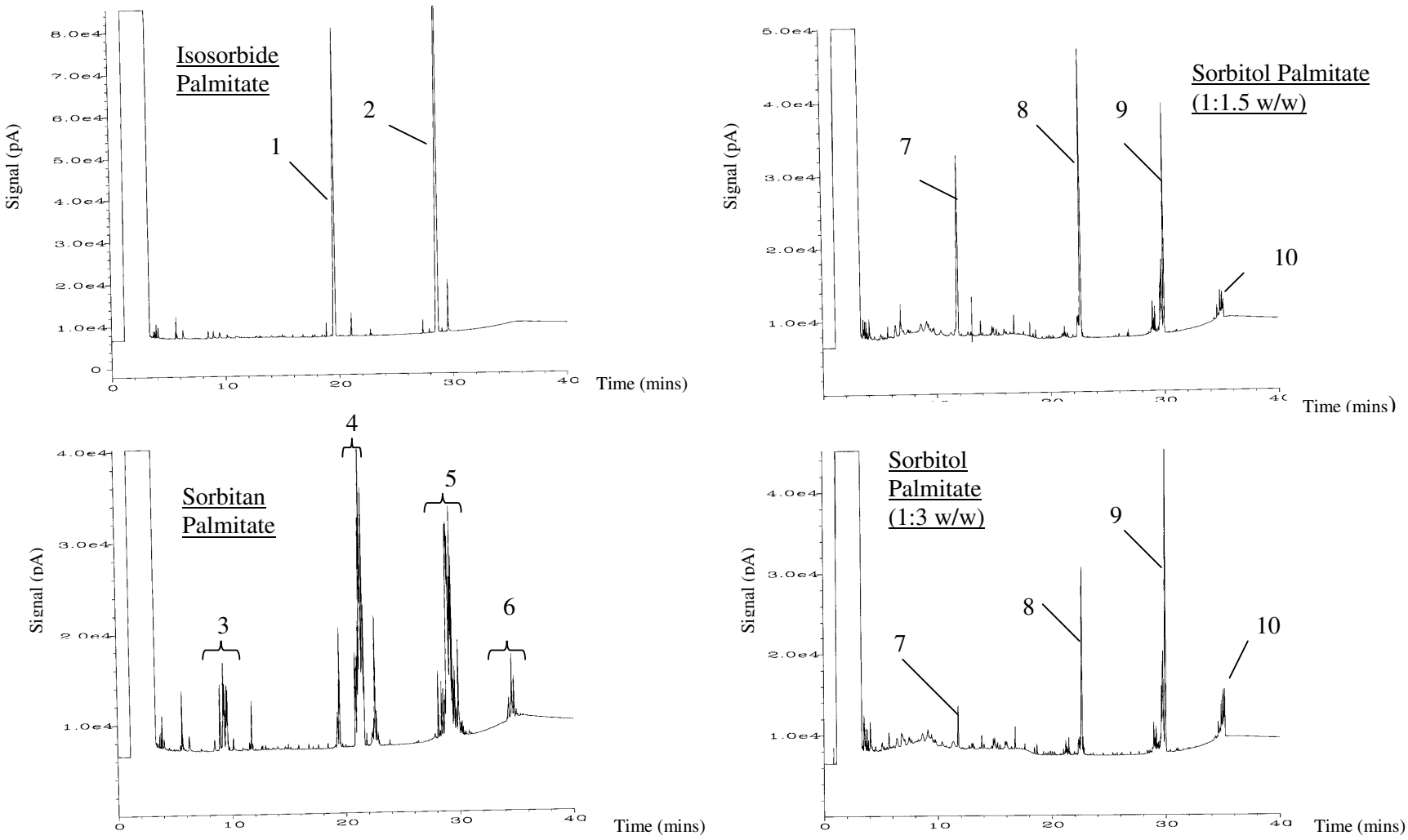



**Figure 6.4: GC Chromatograms of Polyol Esters of Palmitic Acid, Acetylated and Analysed using a DB1-ht Column, Injector at 380°C, Detector at 380°C and Oven Temperature 100°C to 380°C at 8°C/min**

1 = Isosorbide mono palmitate, 2 = Isosorbide di palmitate, 3 = Sorbitan, 4 = Sorbitan mono palmitate, 5= Sorbitan di palmitate, 6 = Sorbitan tri palmitate, 7= Sorbitol, 8 = Sorbitol mono palmitate, 9 = Sorbitol di palmitate, 10 = Sorbitol tri palmitate

### 6.2.5 Polyol Esters of Stearic Acid:

The chromatograms are shown in figure 6.5, as expected the two isosorbide ester species of the isosorbide stearate elute. The sorbitan stearate chromatogram shows peaks thought to be due to sorbitan mono and di ester species. The tri ester peak is very small, sorbitan tri esters have been observed for all the other shorter fatty acid sorbitan esters, indicating that at the ratio of starting materials used and the specified conditions tri esters would be produced. It is probable that the GC conditions are not sufficient to fully elute the larger sorbitan tri stearate. No tetra ester peaks are observed, unlike sorbitan decanoate to myristate.

The chromatogram of the sorbitol esters of stearic acid also show less peaks than the previous sorbitol esters prepared and analysed. At a ratio of 1:1.5 w/w, peaks are observed for the mono and di ester and at 1:3 w/w only the di ester peak is observed. At 1:3 w/w starting material ratio the tri ester is expected to be the major component and this is not observed, this is probably due to the fact it is insufficiently volatile to elute from the column using the GC temperature used.

### 6.2.6 Polyol Esters of Oleic Acid:

The previous GC work on polyol esters (chapters 5 and 6.1 to 6.2.5) was carried out on saturated fatty acid esters, this is because saturated fatty acids are readily available at high purity commercially in contrast to unsaturated fatty acids. However the initial HPLC work was carried out on sorbitan mono oleate and this is one of Croda's major Crills produced and therefore one that would be regularly analysed, if a method was developed.

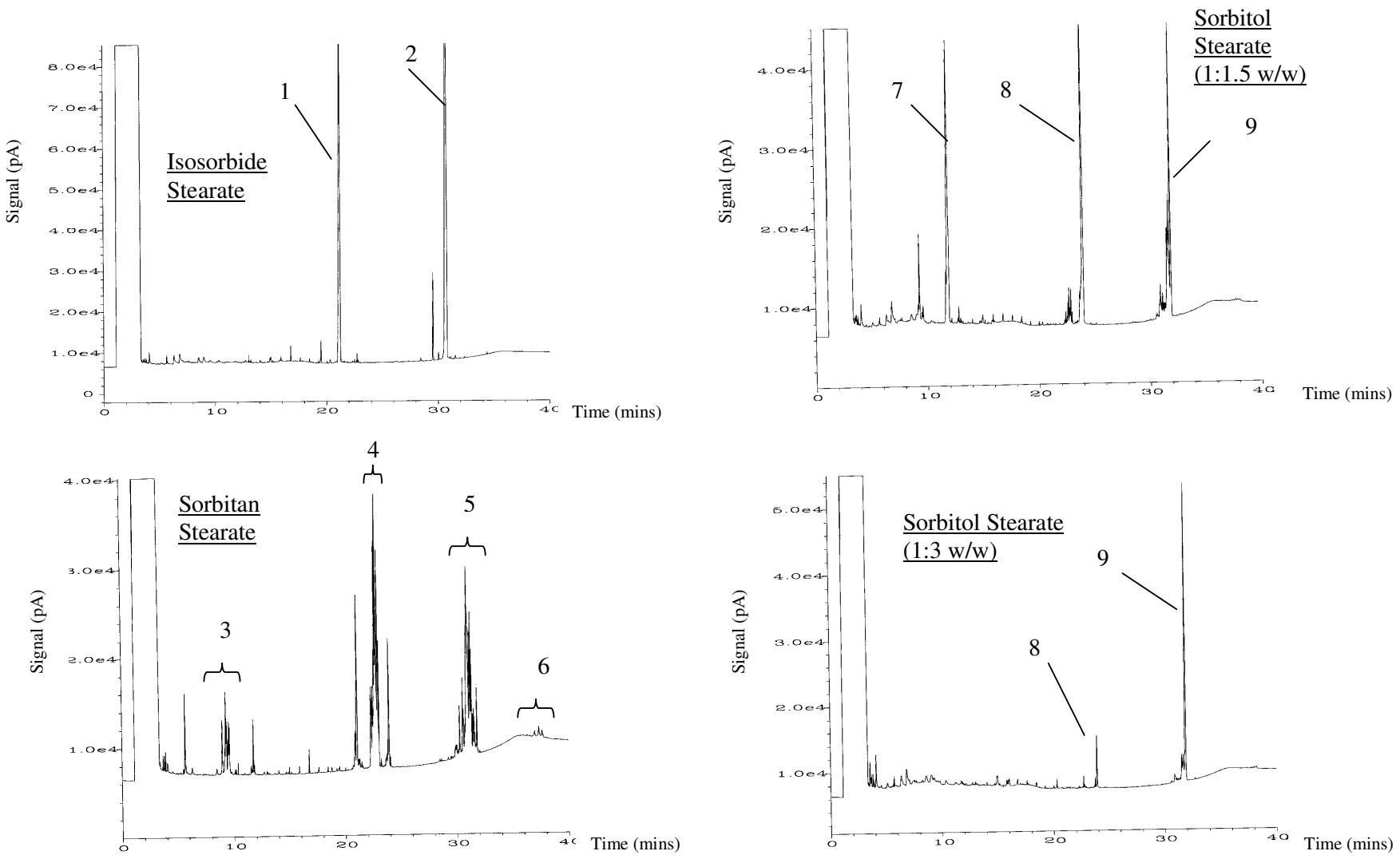



**Figure 6.5: GC Chromatograms of Polyol Esters of Stearic Acid, Acetylated and Analysed using a DB1-ht Column, Injector at 380°C, Detector at 380°C and Oven Temperature 100°C to 380°C at 8°C/min**

1 = Isosorbide mono stearate, 2 = Isosorbide di stearate, 3 = Sorbitan, 4 = Sorbitan mono stearate, 5 = Sorbitan di stearate, 6 = Sorbitan tri stearate, 7 = Sorbitol, 8 = Sorbitol mono stearate, 9 = Sorbitol di stearate

Isosorbide oleate, sorbitan oleate (~90% sorbitan) and sorbitol oleate prepared from high purity oleic acid were therefore also acetylated and analysed. These samples are those detailed in chapter 2, prepared for the initial HPLC work on sorbitan mono oleate. The comparison of saturated and unsaturated C18 fatty acid esters can be established, by comparing the chromatograms obtained for the polyol esters based on oleic and stearic acid.

The chromatograms obtained for polyol esters of oleic acid are shown in figure 6.6.

The chromatogram obtained for isosorbide oleate shows two major peaks, the first will correspond to isosorbide mono oleate and the later isosorbide di oleate. Around these major peaks are a number of smaller peaks, these will be isosorbide esters of different acids, because the purity of the acid was not as high as that purchased from Aldrich and Avocado for the other esters. Oleic acid is an unsaturated acid and is more difficult to obtain at high purity than the other fatty acids used in the investigation which are all saturated.

The chromatogram for the sorbitan esters shows a small group of peaks due to unreacted sorbitan and two later eluting groups of peaks thought to be due to sorbitan mono and di oleate. A single peak before the sorbitan mono oleate peak is isosorbide mono oleate. In the preparation of the sample the conversion from sorbitol to sorbitan also resulted in some isosorbide, this was determined in the polyol profile of the sample carried out and detailed in chapter 2.2.

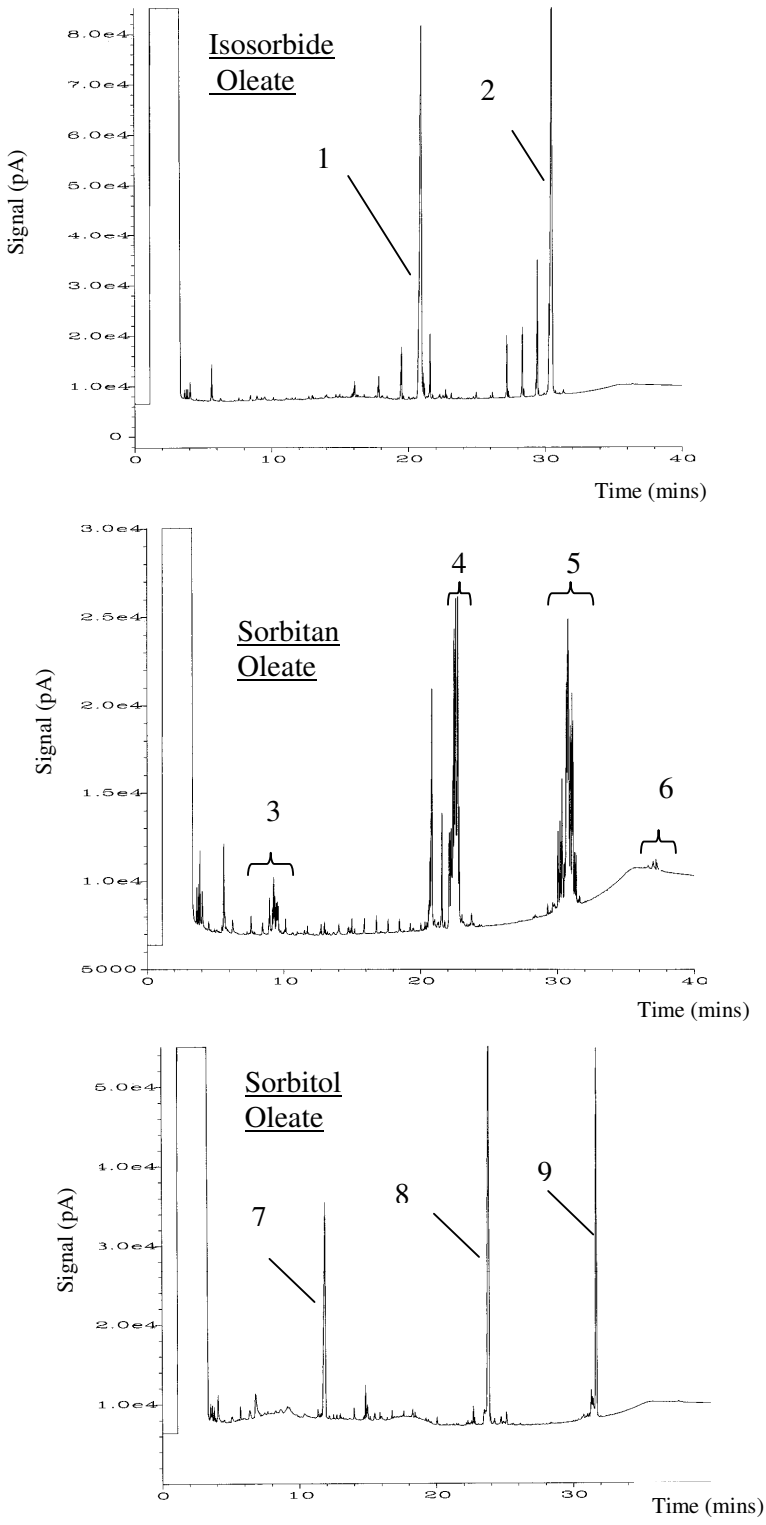



**Figure 6.6: GC Chromatograms of Polyol Esters of Oleic Acid, Acetylated and Analysed Using a DB1-ht Column, Injector at 380°C, Detector at 380°C and Oven Temperature 100°C to 380°C at 8°C/min**

1 = Isosorbide mono oleate, 2 = Isosorbide di oleate, 3 = Sorbitan,
4 = Sorbitan mono oleate, 5 = Sorbitan di oleate, 6 = Sorbitan tri oleate,
7 = Sorbitol, 8 = Sorbitol mono oleate, 9 = Sorbitol di oleate

The sorbitol oleate chromatogram shows peaks thought to be due to mono and di esters, no higher esters are observed, probably because the higher esters are not sufficiently volatile. Higher esters were expected to be produced because they were produced for the smaller chain sorbitol esters.

Comparison of chromatograms in figures 6.5 and 6.6, show that the same number of ester species elute for the esters based on a C18 saturated and unsaturated fatty acid. Although the retention time for the esters based on oleic acid are slightly shorter than those based on stearic acid.

### 6.2.7 Capacity Factor

**Effect of Chain Length**

The capacity factor was determined for isosorbide mono ester and sorbitol mono ester with the varying saturated fatty acids and the values are listed in table 6.6 below.

| | **Capacity Factor** | |
|---|---|---|
| **Fatty Acid Chain Length** | **Isosorbide Mono Ester** | **Sorbitol Mono Ester** |
| C8 | 5.0 | 7.0 |
| C10 | 6.0 | 7.8 |
| C12 | 6.7 | 8.4 |
| C14 | 7.6 | 9.1 |
| C16 | 8.3 | 9.8 |
| C18 | 9.1 | 10.5 |

**Table 6.6: Capacity Factor of Isosorbide and Sorbitol Mono Esters With Varying Fatty Acid Chain Lengths**

The capacity factor for these two esters was determined because they appear as single peaks, sorbitan monoesters appear as groups of peaks due to the varying isomers and

obtaining a retention time would be difficult. The value for the isosorbide mono ester is smaller than that for the sorbitol mono ester of the same fatty acid as expected, due to the different volatilities and therefore the retention on the column. For both esters studied the capacity factor increases with increasing fatty acid chain length.

The plot of the data is shown in figure 6.7. For both esters studied the capacity factor increases with increasing fatty acid chain length, this was as expected because as the fatty acid chain length of the ester increases the volatility will decrease and the ester will be retained longer. The $R^2$ value of the line for the isosorbide mono esters is 0.9976 and for the sorbitol mono esters 0.9992, indicating a linear relationship between the capacity factor and fatty acid chain length of the esters. It is assumed a similar effect would be observed for the higher esters.

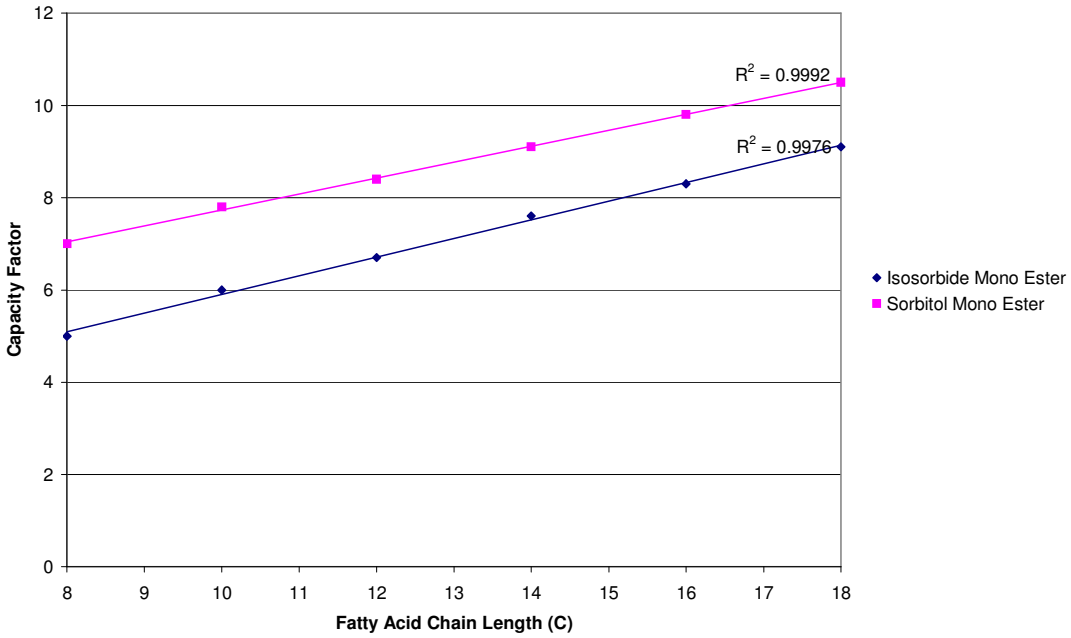



**Figure 6.7: Graph Showing Capacity Factor of Isosorbide and Sorbitol Mono Esters With Varying Fatty Acid Chain Lengths**

**Effect of Number of Ester Groups:**

The GC analysis of the high purity esters showed that for sorbitol decanoate all possible ester species elute from the GC column. The capacity factor for sorbitol and each of the possible sorbitol decanoate esters was determined. All the possible sorbitol ester species did not elute for esters prepared with longer fatty acids, however the capacity factor was determined for the esters that did elute. The values obtained are shown in table 6.7 below.

| | **Capacity Factor** | | | | |
|---|---|---|---|---|---|
| **Ester Groups** | **C10** | **C12** | **C14** | **C16** | **C18** |
| **0** | 4.7 | 4.7 | 4.7 | 4.7 | 4.7 |
| **1** | 7.8 | 8.4 | 9.1 | 9.7 | 10.4 |
| **2** | 10.1 | 11.2 | 12.3 | 13.2 | 14.4 |
| **3** | 11.9 | 13.3 | 14.6 | 15.5 | N/D |
| **4** | 13.4 | 15.0 | 16.5 | N/D | N/D |
| **5** | 14.6 | 16.4 | N/D | N/D | N/D |
| **6** | 15.5 | N/D | N/D | N/D | N/D |

N/D – Not detected

**Table 6.7: Capacity Factor of Sorbitol Ester Species Analysed Using Splitless Injection**

The results show that for a specific fatty acid, as the number of ester groups on the sorbitol increases, the capacity factor increases. This was expected because as the number of ester groups increases, the volatility will decrease and therefore the retention on the column will increase

Plotting the data graphically shows that it is curved for each of the sorbitol fatty acid esters studied and is shown in figure 6.8.

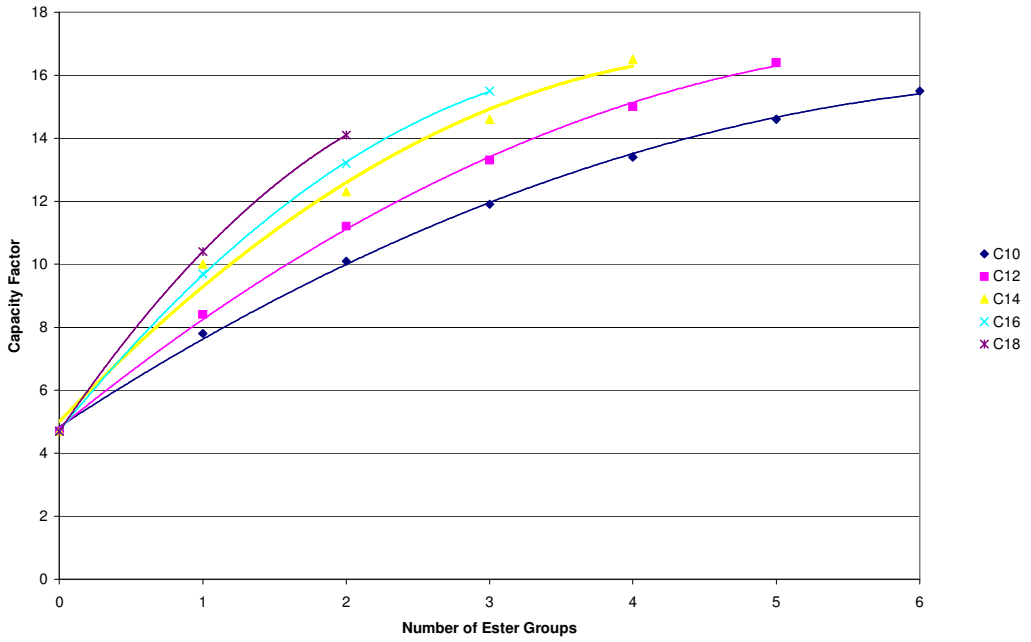



**Figure 6.8: Graph Showing Capacity Factor of Sorbitol Esters Analysed Using Splitless Injection**

### 6.2.8 Summary of GC Analysis of Polyol Esters using a DB1-ht Column with a Final Oven Temperature of 380°C:

From the investigation carried out it was found that all of the isosorbide ester chromatograms showed peaks corresponding to the mono and di esters. Both isosorbide esters for all fatty acids investigated eluted from the GC column. The sorbitan ester chromatograms showed that sorbitan mono and di esters for all the fatty acids eluted from the GC column using the selected GC conditions. Peaks thought to correspond to tri esters are observed for sorbitan decanoate to sorbitan palmitate but are smaller for the larger fatty acids of stearic and oleic acid. The tri ester was observed for the sorbitan esters of decanoate to palmitate and because all the esters were prepared using the same conditions it is assumed that the tri ester would be

produced for the larger fatty acid esters. However due to the larger sizes may not be sufficiently volatile to fully elute from the column.

As with the isosorbide and sorbitan esters, peaks for the sorbitol mono and di esters of all the fatty acids were observed on the chromatograms. However as the fatty acid chain length increased the number of esters eluting decreased. For decanoic acid a small peak corresponding to sorbitol hexa decanoate was observed, but a hexa ester was not observed for any of the larger fatty acids. The largest esters observed for lauric acid was the penta esters and for myristic acid the tetra esters, although they were small. When a ratio of 1:3 w/w sorbitol: acid chloride was used it was expected that the tri ester would be the major peak observed, however for the larger fatty acids no tri ester peaks are observed, this indicates that the esters are not sufficiently volatile to elute from the column under the conditions detailed. Table 6.8 shows the esters detected using the conditions detailed in chapter 5.2.4.

The work indicates that GC with splitless injection may be a suitable method of analysis for characterising the sorbitan mono ester range of Crills produced by Croda. At the ratio of 1 sorbitol:1.3 fatty acid used in the manufacture of the mono ester range, it is likely that the mono and di esters will be produced and these can be eluted from the GC column and identified for all the fatty acids investigated. However the method will not be suitable for the tri ester range of Crills, which are produced with a sorbitol to fatty acid ratio to give the higher esters, which can not be eluted from the GC column using the conditions used in the study.

| | C10 | C12 | C14 | C16 | C18 | C18:1 |
|---|---|---|---|---|---|---|
| Isosorbide mono | √ | √ | √ | √ | √ | √ |
| Isosorbide di | √ | √ | √ | √ | √ | √ |
| Sorbitan mono | √ | √ | √ | √ | √ | √ |
| Sorbitan di | √ | √ | √ | √ | √ | √ |
| Sorbitan tri | √ | √ | √ | √ | * | * |
| Sorbitan tetra | * | X | * | X | X | X |
| Sorbitol mono | √ | √ | √ | √ | √ | √ |
| Sorbitol di | √ | √ | √ | √ | √ | √ |
| Sorbitol tri | √ | √ | √ | √ | X | X |
| Sorbitol tetra | √ | √ | * | X | X | X |
| Sorbitol penta | √ | * | X | X | X | X |
| Sorbitol hexa | * | X | X | X | X | X |

**Table 6.8: Summary of Esters Detected For the Various Fatty Acids Studied**

√ = detected

X = not detected

* = trace detected

### 6.3 Optimising GC Conditions:

The work carried out on the various polyol esters showed that a large number of the polyol esters would elute from the GC column. However as the fatty acid chain length increased the number of ester species that eluted decreased. Therefore it was decided to investigate other conditions including the use of higher temperatures and higher temperatures with an alternative column in an attempt to elute more ester species.

#### 6.3.1 DB1-ht Column, Injector and Final Oven Temperature of 400°C:

The work detailed in chapter 6.1 and 6.2 used an injection and final oven temperature of 380°C, increasing the injector and oven temperature to 400°C may be sufficient to volatilise some of the polyol esters which did not volatilise at 380°C, therefore increasing the number of polyol esters that elute from the GC column and are detected. The 400°C final oven temperature selected is the upper limit of the DB1-ht column. The following GC conditions were used to analyse all of the high purity polyol esters prepared:

| | |
|---|---|
| Injection temperature: | 400°C |
| Detector temperature: | 400°C |
| Initial oven temperature: | 100°C |
| Ramp rate: | 8 °C/min |
| Final oven temperature: | 400°C, held for 5 minutes |
| Total analysis time: | 42.5 minutes |

The chromatograms obtained for each of the polyol esters were reviewed and compared to those obtained in chapter 6.2 when an injector temperature and final

oven temperature of 380°C was used. The increased temperature had no effect on the number of esters detected, the chromatograms obtained were comparable to those in figures 6.1 – 6.6, but with a slightly longer run time.

### 6.3.2 HT5 Column:

The use of the increased injector and final oven temperature of 400°C with a DB1-ht column had no significant effect on the esters eluted from the column, therefore the use of an alternative GC column with a higher operating temperature was investigated. The column selected was an aluminium clad HT5 column, which is a unique high temperature phase, suited for simulated distillation and petroleum applications. This column has the highest maximum operating temperature available with a maximum operating temperature up to 480°C. An extensive programme of work was undertaken using this column. The dimensions of the column used were 12 m x 0.32 mm x 0.1 μm.

#### 6.3.2.1 GC Conditions 1:

The polyol esters of caprylic, stearic and oleic acid were analysed using the GC conditions detailed in 6.3.1. These are the fatty acid esters with the shortest and longest chain lengths, the extremes were studied to establish if any differences in chromatograms were seen using the two different columns with the same operating conditions. The chromatograms obtained for the various esters using the DB1-ht and HT-5 column were visually compared and found to be comparable, the number of esters eluted and resolution obtained were similar for each sample on the two different columns. The columns are both non-polar and the same operating conditions were used, therefore the chromatograms were expected to be similar.

The capacity factor was determined for isosorbide mono caprylate and stearate and sorbitol mono caprylate and stearate using the two different columns with the same operating conditions and the values obtained are shown in table 6.9.

| | Capacity Factor | |
|---|---|---|
| | DB1-ht Column | HT5 Column |
| Isosorbide Mono Caprylate | 5.0 | 6.2 |
| Sorbitol Mono Caprylate | 7.0 | 8.5 |
| Isosorbide Mono Stearate | 10.1 | 11.2 |
| Sorbitol Mono Stearate | 11.5 | 12.5 |

**Table 6.9: Capacity Factors for Isosorbide and Sorbitol Esters of Caprylic and Stearic Acid Using the Same GC Conditions on DB1-ht and HT5 Columns**

The k' values obtained are slightly higher with the HT5 column compared to the DB1-ht column, indicating longer retention times, however the HT5 column offers the possibility of using higher temperatures and therefore is worth pursuing further, to establish if more ester species can be eluted with higher temperatures.

#### 6.3.2.2 GC Conditions 2:

A higher final oven temperature and detector temperature were used with the HT5 column in an attempt to elute more esters. An injector temperature of 400°C was used as before because this is the maximum injection temperature possible for the HP5890 GC used.

The following GC conditions were used to analyse all the polyol ester samples

Injector temperature: 400°C

Detector temperature: 430°C

Initial oven temperature: 100°C

Ramp Rate: 8°C/minute

Final oven temperature 430°C

The chromatograms obtained were compared to those obtained in chapter 6.2 and it was found that the increase in the final oven temperature had no effect on the number of esters eluted.

#### 6.3.2.3 GC Conditions 3:

A final oven temperature and detector temperature of 450°C were tried, which are the maximum for the GC.

Injector temperature: 400°C

Detector temperature: 450°C

Initial oven temperature: 100°C

Ramp Rate: 8 °C/minute

Final oven temperature 450°C

The above conditions were used to analyse all the polyol esters, however the chromatograms obtained were still found to be similar to those obtained with the DB1-ht column and maximum temperatures of 380°C. The increased oven and detector temperature had no effect on the number of ester species eluted.

### 6.3.3 Conclusions from Optimisation Investigation:

The investigations carried out using the DB1-ht column and higher temperatures and the HT5 column found that no more ester species could be eluted then those eluted

using the DB1-ht column and injection and final oven temperature of 380°C. The injection temperature of the GC is limited to 400°C and may be part of the reason. The HT5 column is stated to be suitable for use up to 480°C, however it was found to go very brittle and had a tendency to break, the column could not withstand the high temperatures used for any length of time and therefore would be of limited use for regular analysis of polyol esters. Due to the HT5 column also offering no advantage for the separation of polyol esters of various fatty acids compared to the DB1-ht column further work reverted to the DB1-ht column which is more stable and the conditions detailed in chapter 6.2.

### 6.4 GC Analysis of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid and Manufactured Sorbitan Mono Ester Samples:

The results obtained in chapters 5 and 6.2 have shown that GC has the potential as an analytical method for comparison of a range of sorbitan mono esters manufactured by Croda, although as the chain length of the fatty acid used increases it is not possible to elute all of the higher ester species. Only samples containing essentially one polyol and prepared from high purity fatty acid feed stocks have been analysed, with the exception of sorbitan caprylate (chapter 5) when a sample of material manufactured on the plant was analysed, although this is produced from a high purity fatty acid feedstock. Therefore more complex samples were analysed including SMO prepared from high purity oleic acid and manufactured sorbitan mono ester samples that have varying polyols and fatty acids.

### 6.4.1 GC Analysis of Sorbitan Mono Oleate Prepared from High Purity Oleic Acid:

The first sample analysed was a sample of sorbitan mono oleate, prepared from high purity (>99 %) oleic acid, which has a similar polyol and ester distribution to a commercial SMO, but is simplified by not having a mixed composition of fatty acids. This sample was used in the HPLC investigations. The sample was acetylated and analysed on the GC using the conditions detailed in chapter 5.2.4.

The single polyol esters prepared using high purity oleic acid (detailed in chapter 6.2.6) were also analysed at the same time to aid identification of the various peaks in the chromatogram of sorbitan mono oleate prepared from high purity oleic acid. The chromatogram is shown in figure 6.9 and shows that it contains very little unreacted material, which would be evident at the beginning of the chromatogram after the solvent peak. The sample is found to contain isosorbide mono and di esters, as well as mono and di esters of sorbitan. The polyol profile carried out on the sample (chapter 2.2) indicated that sorbitol was present, peaks corresponding to the sorbitol mono and di esters are also observed, although are small compared to the peaks for the other mono and di polyol esters. The chromatogram shows that the largest esters to elute are the tri esters, it is probably because the higher esters are insufficiently volatile to elute from the column, the tri esters elute close to the maximum oven temperature, a higher temperature would therefore be required to elute the less volatile higher esters. Alternatively higher esters were not produced. The results obtained are in contrast to those detailed in chapter 6.2.6 were only di esters were eluted using the same GC conditions and the tri ester peak was very small. The column used for the work detailed in chapters 5 and 6.1 – 6.2 was new at the start of the experimental work but had begun to show poor resolution prior to this analysis and therefore was

replaced with another DB1-ht column with the same dimensions but from a different batch. The increase in ester species detected using columns from the same manufacturer but different batches shows that if the method was used routinely, a system suitability would be required prior to analysis of samples, to ensure the column eluted the maximum number of ester species possible.

### 6.4.2 Analysis of Real Samples:

The analysis of the sorbitan mono oleate sample prepared from high purity oleic acid was successful, therefore a number of real sorbitan ester samples produced by Croda were also analysed by GC, they were:

Sorbitan mono laurate

Sorbitan mono palmitate

Sorbitan mono stearate

Sorbitan mono oleate

These are part of the range of sorbitan mono esters produced by Croda and vary in fatty acid feedstock used.

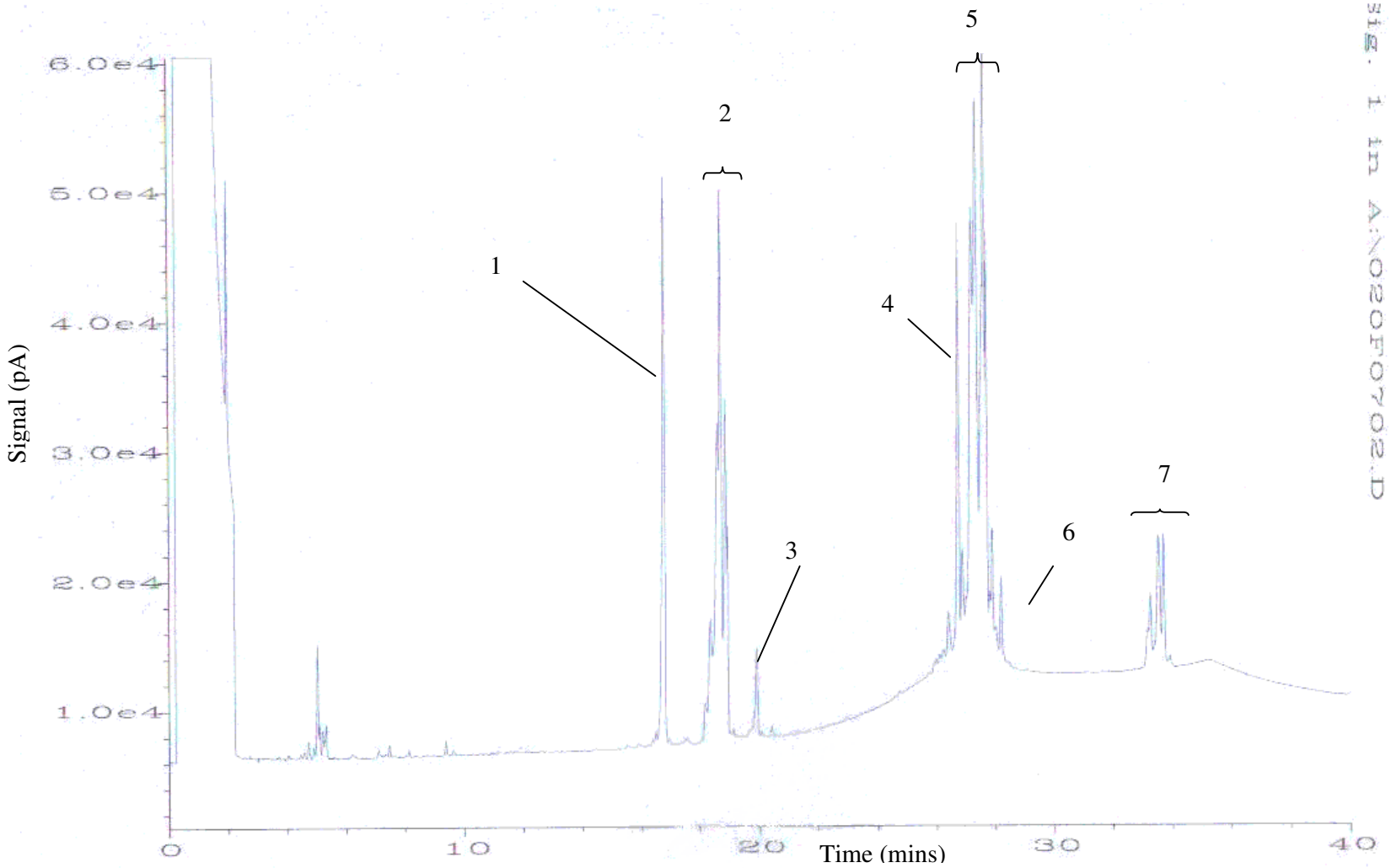



**Figure 6.9: GC Chromatogram of Sorbitan Mono Oleate Made Using High Purity Oleic Acid**

1 = Isosorbide mono oleate, 2 = Sorbitan mono oleate, 3 = Sorbitol mono oleate, 4 = Isosorbide di oleate
5 = Sorbitan di oleate, 6 = Sorbitol di oleate, 7 = Sorbitan tri oleate

#### 6.4.2.1 Standard Croda Analysis of Sorbitan Esters:

The samples were analysed using Croda's standard methods to establish the usual information on them including AV, hydroxyl value, ester distribution (GPC), polyol profile and lipid profile.

Acid value is defined as the number of milligrams of potassium hydroxide required to neutralise the free acids in 1 gram of the sample. Therefore gives an indication of any free acid, this is usually low because the acid reacts to form esters, by following the acid value during production it is possible to establish when the acid has reacted and the reaction can be stopped.

Hydroxyl value is the number of milligrams of potassium hydroxide required to neutralize the amount of acetic acid capable of combining, by acetylation, with 1 g of the test sample. It gives an indication of the free hydroxyl groups. Each sorbitan ester has a specification for the hydroxyl value which must be met.

Ester Distribution is determined by GPC as detailed in chapter 2.2.2. This is an LC separation based on molecular size and gives an indication of the ester species present. It is routinely used to give an indication of the ester species present, although is only possible for some sorbitan esters and resolution is limited with tri and higher esters eluting as one peak.

Polyol profiles determine the level of each polyol present in a sorbitan ester and is carried out as detailed in chapter 2.2.

Fatty acid profiles give information on the range of fatty acid used to prepare the esters. The sorbitan esters are saponified and the resulting fatty acids are converted to methyl esters and subsequently analysed by GC.

The results obtained for the sorbitan mono ester samples can be found in table 6.10 and the GPC chromatograms are shown in figure 6.10.

**6.4.2.2 GC Analysis of Manufactured Sorbitan Mono Esters Samples:**

The samples were analysed by GC using the method detailed in chapter 5.2.2. The single high purity polyol esters were also analysed to aid identification of the peaks in the manufactured samples.

Sorbitan Mono Laurate:

The chromatogram obtained for sorbitan mono laurate is shown in figure 6.11. The chromatogram shows a large number of peaks which indicates a large number of components present and shows the complex nature of the material. The fatty acid profile for sorbitan mono laurate shows that the fatty acid feedstock is mixed and despite the material being called sorbitan monolaurate, lauric acid constitutes less than 50 % of the fatty acids, the fatty acids present range from caprylic acid (C8:0) to linoleic acid (C18:2). The fatty acid range used for the production of sorbitan mono laurate is more varied than for the other sorbitan esters and is the reason for the complex nature of the material. The polyol profile showed that the material contained only isosorbide and sorbitan, the sorbitol had been fully dehydrated and therefore the chromatogram could have been more complex if sorbitol esters were also present.

The standard results are typical of sorbitan mono laurate. Analysis of manufactured sorbitan mono laurate by GPC has not been possible due to the large number of components resulting in poor resolution and no identification of components being possible. The GC chromatogram shows that manufactured sorbitan mono laurate is too complex to analyse and characterise by GC, there are too many peaks and insufficient resolution to make identification of components possible. The large number of components present in the sorbitan mono laurate is due to the large range of fatty acids used. However it may be possible to use the GC chromatogram as a 'finger print' type analysis for comparison of different samples.

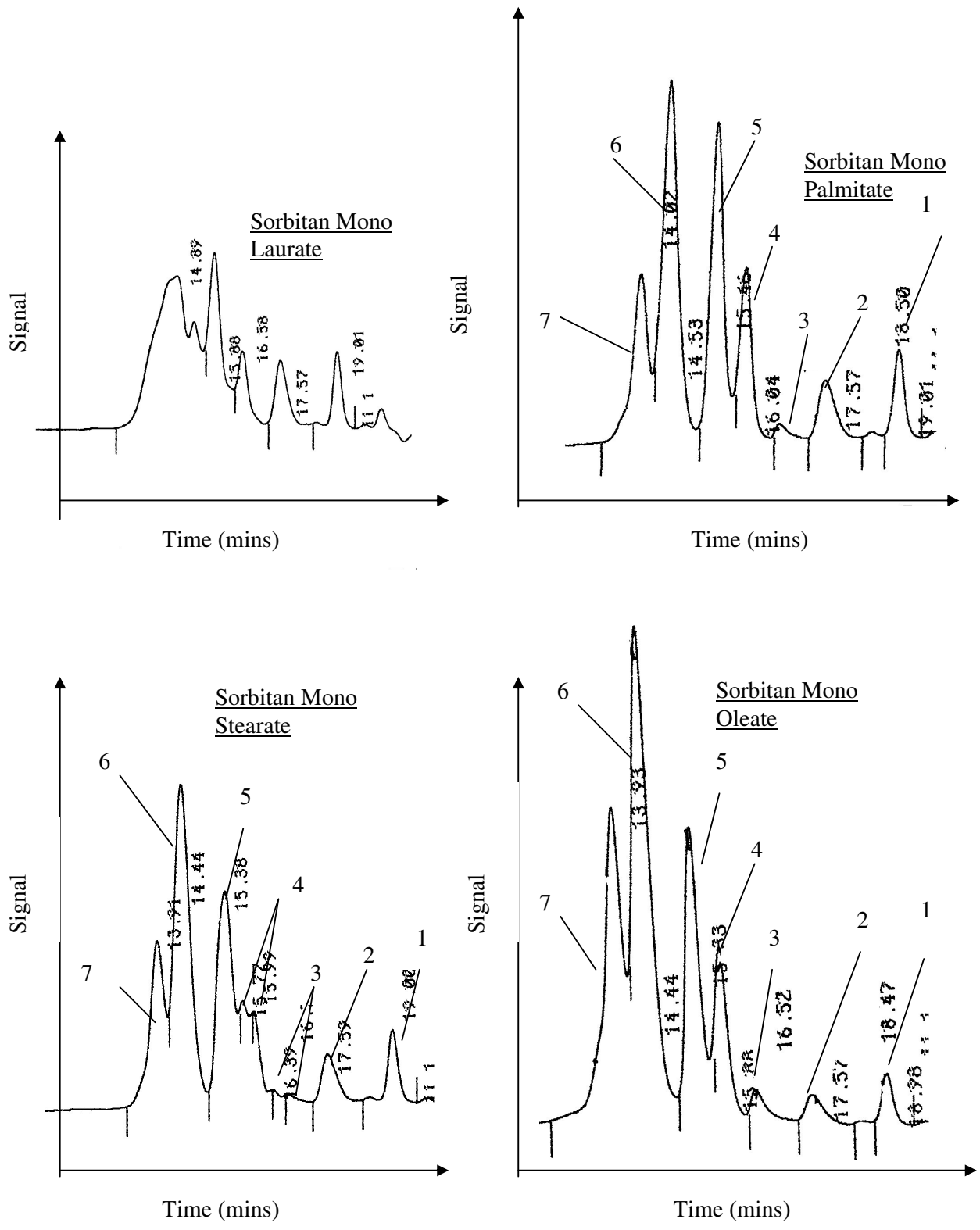



**Figure 6.10: GPC Chromatograms of Manufactured Sorbitan Mono Ester Samples**
1 = Isosorbide, 2 = Sorbitan, 3 = Acid, 4 = Isosorbide mono esters, 5 = Sorbitan mono esters
6 = Di esters, 7 = Tri/higher esters

| | Sorbitan Mono Laurate | Sorbitan Mono Palmitate | Sorbitan Mono Stearate | Sorbitan Mono Oleate |
|---|---|---|---|---|
| Acid Value | 5.4 | 5.6 | 9.4 | 6.4 |
| Hydroxyl value | 336 | 284 | 246 | 191 |
| Polyol Profile (%) | | | | |
| Isosorbide | 27 | 24 | 19 | 20 |
| Sorbitan | 73 | 76 | 77 | 79 |
| Sorbitol | N/D | N/D | 4 | N/D |
| Ester Distribution (%) | * | | | |
| Tri/higher esters | | 15 | 20 | 30 |
| Di esters | | 30 | 35 | 35 |
| Sorbitan mono esters | | 25 | 20 | 17 |
| Isosorbide mono esters | | 15 | 8 | 10 |
| Acid | | 3 | 3 | 4 |
| Sorbitan | | 6 | 3.5 | 2 |
| Isosorbide | | 5 | 2.5 | 2 |
| Fatty acid Profile (%): | | | | |
| C8:0 | 2 | N/D | N/D | N/D |
| C10:0 | 4.3 | N/D | N/D | N/D |
| C12:0 | 47.5 | 0.8 | N/D | 0.2 |
| C14:0 | 21.6 | 1.6 | 2.6 | 2.2 |
| C16:0 | 11.6 | 93.7 | 42.9 | 4.8 |
| C16:1 | N/D | N/D | N/D | 4.3 |
| C18:0 | 3.5 | 3.2 | 49 | 1.7 |
| C18:1 | 8.3 | N/D | 0.4 | 77.3 |
| C18:2 | 1.2 | N/D | N/D | 2.6 |
| C20:0 | N/D | N/D | 0.6 | N/D |
| C20:1 | N/D | N/D | N/D | 0.6 |
| C22:0 | N/D | N/D | 0.1 | N/D |
| Others | N/D | 0.7 | 4.4 | 6.3 |

**Table 6.10: Standard Analysis of Manufactured Sorbitan Mono Ester Samples**

* Not possible due to poor resolution and peak identification not possible

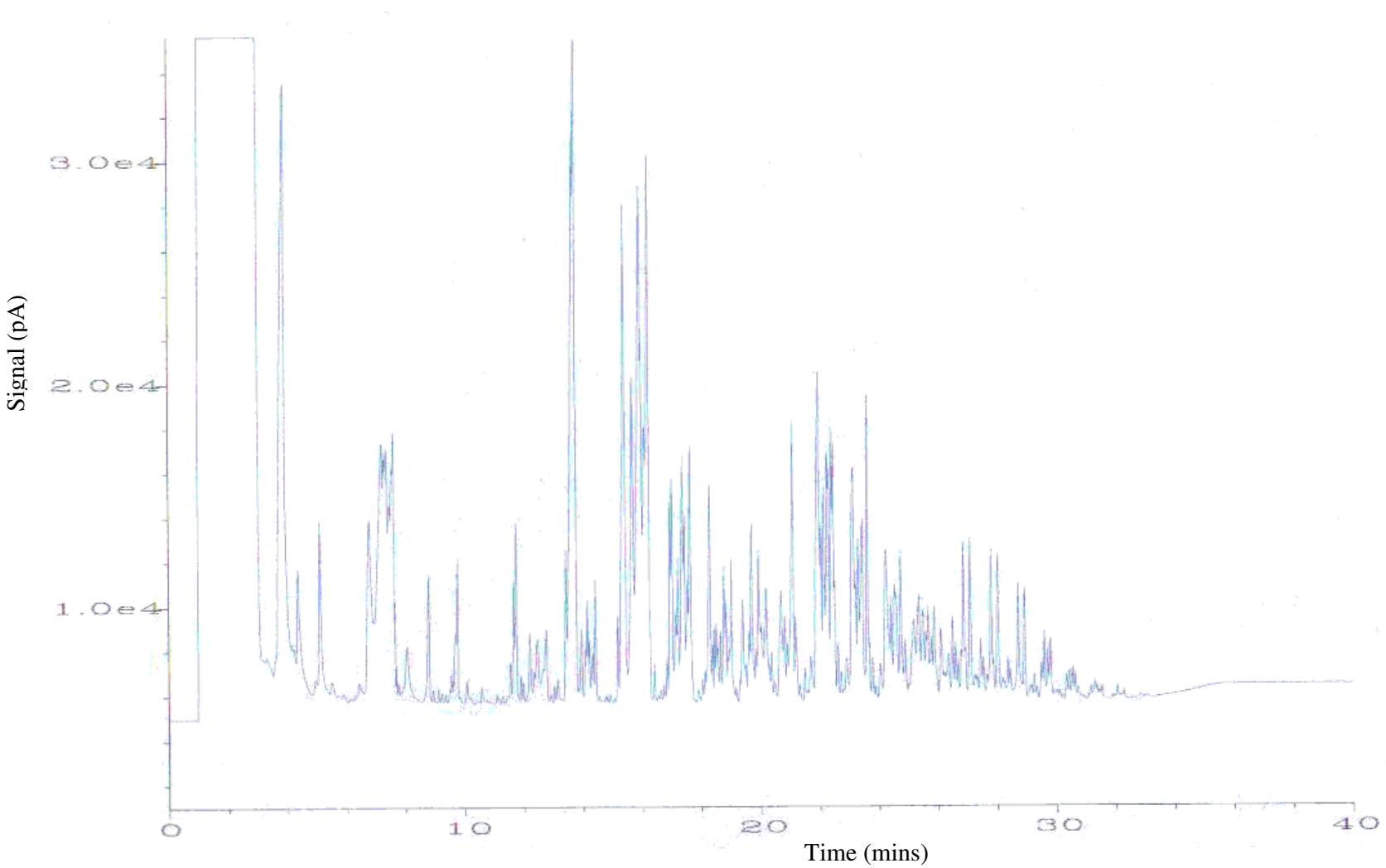



**Figure 6.11: GC Chromatogram of Manufactured Sorbitan Mono Laurate**

Sorbitan Mono Palmitate:

The standard analysis carried out showed that the sorbitan mono palmitate sample contained only isosorbide and sorbitan as with the sorbitan mono laurate, however the fatty acid feed stock was found to be less varied with palmitic acid being the major constituent (94 %). The chromatogram was therefore expected to have less peaks than the sorbitan mono laurate and this was found to be the case, the chromatogram obtained is shown in figure 6.12. It is similar to that obtained for sorbitan mono oleate prepared from high purity oleic acid but the esters have a slightly shorter retention time, this will be because they are more volatile due to being based on a shorter fatty acid, also there is evidence of unreacted polyols at the beginning of the chromatogram. The chromatogram shows that the sample contains isosorbide and sorbitan mono and di esters. The largest esters to elute were the tri esters, the highest esters that could be present in the sample would be sorbitan tetra esters due to no sorbitol being present, however it is uncertain if tetra esters would be produced at the ratio of 1:1.3 w/w sorbitol:acid used in the production of this material and it is probable they would not elute from the column even if they were produced.

The GC analysis shows that it may offer advantages over GPC analysis because the mono esters are baseline resolved unlike with GPC. Also the di and tri esters are well separated by GC but not by GPC.

Sorbitan Mono Stearate:

The routine analysis indicated that all three polyols are present in the sample, although sorbitol is only present at a low level. The fatty acid profile showed that the esters were prepared from a mixed feedstock containing largely palmitic and stearic

acid in almost equal quantities. Due to two major fatty acids being used the chromatogram was expected to be more complex than that of the sorbitan mono palmitate, the esters present in sorbitan mono palmitate were expected along with esters based on stearic acid. The chromatogram obtained is shown in figure 6.13, the mono ester region has doublets due to esters of the two main acids. Two isosorbide mono ester peaks are identified, with the shorter chain length, more volatile, isosorbide mono palmitate eluting prior to isosorbide mono stearate. Two sorbitan mono ester group of peaks are also seen.

The di and tri ester regions do not show distinct doublets due to the different fatty acids but the peak area is broader than when the esters are prepared from essentially one fatty acid, as in sorbitan mono palmitate. Due to the fatty acid feedstock, esters based on palmitic and stearic acid could be produced as well as esters with mixed fatty acids. As observed with sorbitan mono oleate prepared from high purity oleic acid and manufactured sorbitan mono palmitate the highest esters eluted are thought to be tri esters, it is possible that this consists of sorbitan and sorbitol tri esters of both acids and mixed acid esters.

Again it appears that GC analysis may offer advantages over GPC, largely due to the baseline separation of the various mono ester species which is not possible with GPC.

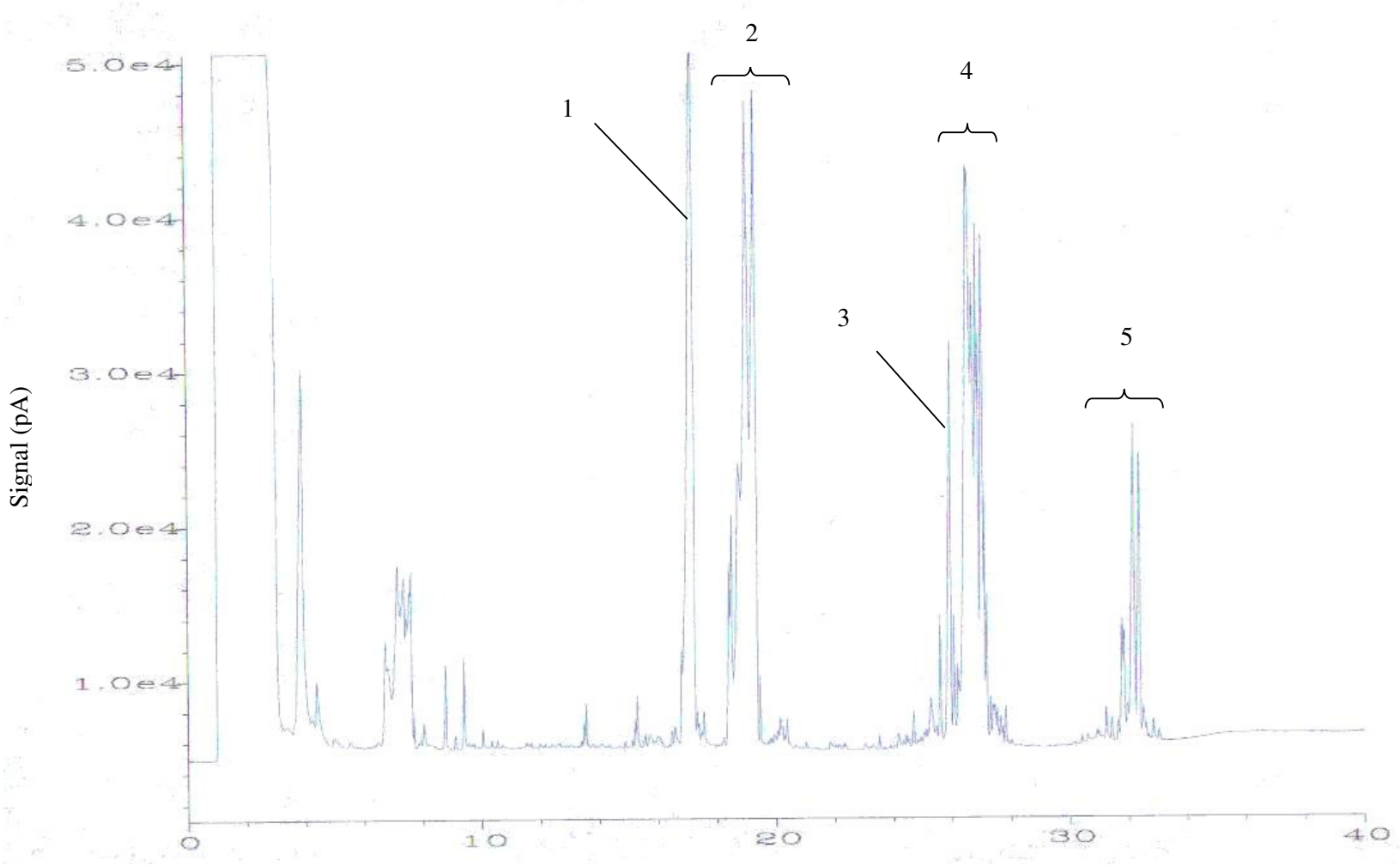



**Figure 6.12: GC Chromatogram of Manufactured Sorbitan Mono Palmitate**

1 = Isosorbide mono plamitate, 2 = Sorbitan mono palmitate, 3 = Isosorbide di palmitate, 4 = Sorbitan di palmitate, 5 = Sorbitan tri palmitate

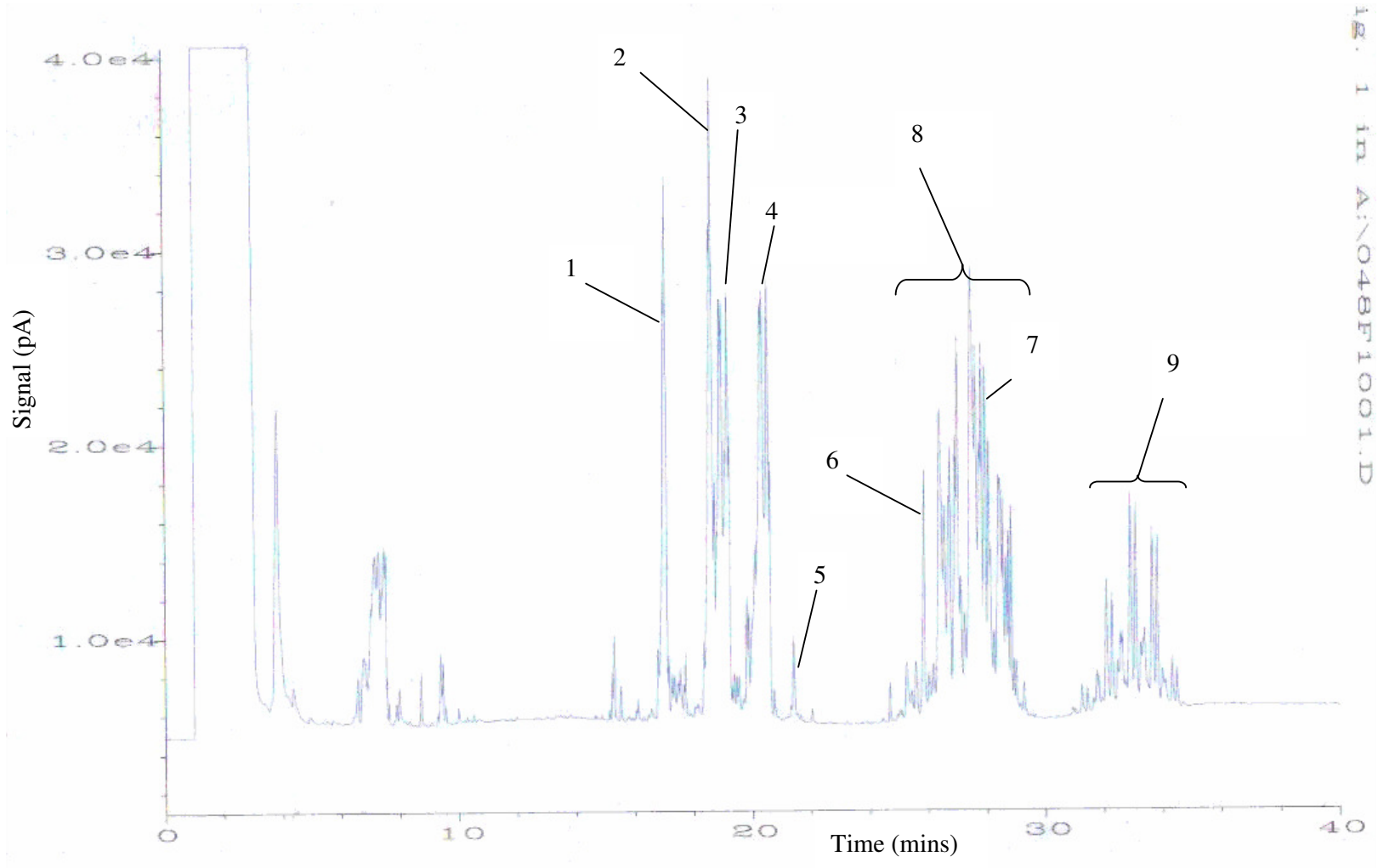



**Figure 6.13: GC Chromatogram of Manufactured Sorbitan Mono Stearate**

1 = Isosorbide mono palmitate, 2 = Isosorbide mono stearate, 3 = Sorbitan mono palmitate, 4 = Sorbitan mono stearate, 5 = Sorbitol mono stearate, 6 = Isosorbide di palmitate, 7 = Isosorbide di stearate, 8 = Sorbitan di esters, 9 = Sorbitan tri esters

Sorbitan Mono Oleate:

The chromatogram obtained for sorbitan mono oleate is shown if figure 6.14, it is similar to that obtained for sorbitan mono oleate prepared from high purity oleic acid although the di and tri ester peaks are broader due to the mixed fatty acid feedstock, esters based on oleic acid could be present as well as mixed fatty acid esters. A single peak due to isosorbide mono oleate is evident followed by a group of peaks thought to be sorbitan mono oleate. The di esters of isosorbide and sorbitan are also observed followed by a group of peaks thought to be the sorbitan tri oleate esters. The polyol profile indicated that no sorbitol was present, that it had been fully dehydrated, therefore sorbitol esters were not expected and were not seen. The largest esters possible would therefore be sorbitan tetra esters, however these were not seen with the other sorbitan mono ester samples, the largest esters to elute from the column were again sorbitan tri esters.

As seen with sorbitan mono palmitate and sorbitan mono stearate GC may be a possible alternative to GPC analysis for determining ester distributions for sorbitan mono oleate.

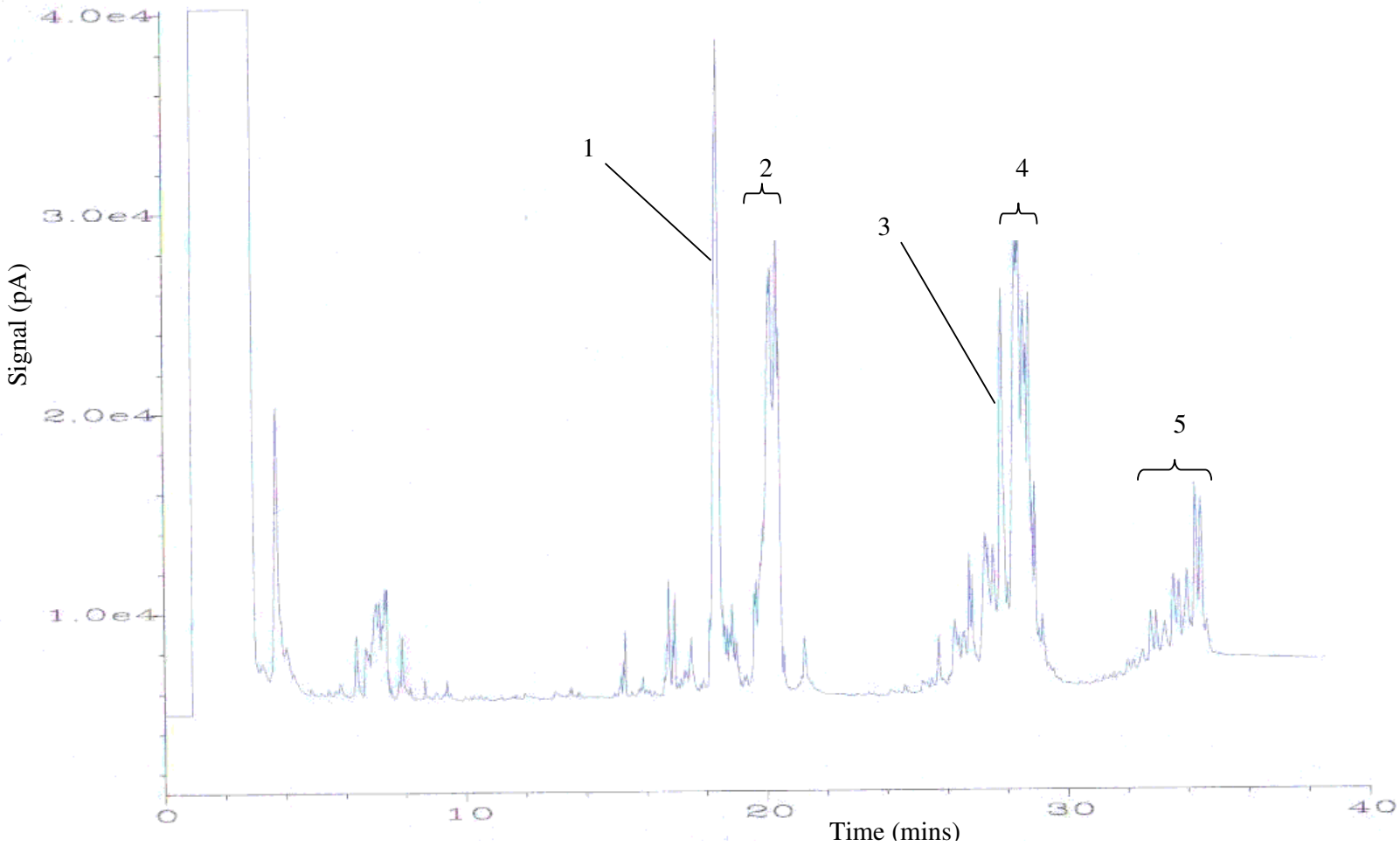



**Figure 6.14: GC Chromatogram of Manufactured Sorbitan Mono Oleate**

1 = Isosorbide mono oleate, 2 = Sorbitan mono oleate, 3 = Isosorbide di oleate, 4 = Sorbitan di oleate, 5 = Sorbitan tri esters

### 6.4.3 Summary:

The work carried out shows that GC with splitless injection can successfully be used for the analysis of a number of sorbitan mono ester samples, although due to the complex nature of sorbitan mono laurate characterisation is not possible. It was found that it is not possible to elute all the ester species from the GC column for the higher fatty acid chain length esters, however a number of esters do elute with the tri esters being the largest to elute for the real samples analysed. It was found that the various mono ester species are well resolved and these are well separated from the di and tri esters which are well separated from each other, therefore comparison of these ester species between samples would be possible. Currently the best method available for comparison of differences in ester species in manufactured sorbitan mono ester samples is GPC, however it does appear that GC may offer advantages over it due to better resolution of the various esters, although further work including work on repeatability is required.

## 6.5 Limited Validation:

Validation is the assessment of a method to ensure that it is fit for purpose. Validation ensures that results obtained are reliable, consistent and there is confidence in the results, this is carried out prior to routine use of the analytical procedure. A number of characteristics can be assessed including specificity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness. The characteristics used are dependent on the method and its proposed application. The GC work carried out on the high purity esters and real samples showed that GC was successful in the analysis of sorbitan mono esters and could be used routinely to give information about material made. Therefore a limited validation programme was performed.

The accuracy of an analytical procedure expresses the closeness of the determined value with the true value. The samples to be analysed by this GC method are complex mixtures and currently it is not possible to establish the true value of each component and therefore determination of accuracy would not be possible. Also the method under investigation will involve the comparison of peak areas of different samples and therefore this characteristic is not relevant. The linearity verifies that the sample for analysis is in the concentration range were the analyte response is linearly proportional to concentration. It is not possible to investigate this parameter in the timescale given due to the complex nature of the samples and it is not envisaged that there will be major differences between the concentration of components in the samples. The method is for comparison of samples, the samples will be analysed at concentrations where the limit of detection and quantification will also be unnecessary. Therefore the two important parameters to be investigated were specificity and precision.

Specificity demonstrates that the analytical procedure is specific for an analyte, in the case of the method under study, it is important no peaks will coelute with those of the sample. The GC method investigates all of the various components of the sample, the only other things present which may give a response that cannot be distinguished from the analytes will be from the acetylating reagents. Analysis of these showed no peaks that would interfere with those of the sample. Therefore the method is specific to the analytes in the sample.

Precision is a measure of how close the data values are to each other under stipulated conditions and is usually stated in terms of standard deviation (SD) and relative standard deviation (RSD). This validation characteristic can be considered at various levels. Instrument precision is obtained by making repeat injections of a sample solution and tests the performance of the chromatographic instrument. Analysis precision is obtained by analysis of a sample which has been independently prepared according to the procedure over several days, using one laboratory and one piece of equipment. Intermediate precision is obtained when analysis is performed by multiple analysts, using multiple instruments on multiple days in one laboratory. Only one instrument was available for the study therefore intermediate precision was not investigated, along with reproducibility which involves multiple laboratories.
The precision study was performed on sorbitan mono oleate prepared from high purity oleic acid, this samples has the mixed polyol composition of a real sample but will be simpler than a real sorbitan mono oleate sample manufactured on a plant at Croda because of the putiy of the acid. A real SMO sample manufactured at Croda was also analysed for precision.

Instrument precision – the sample was acetylated as detailed in chapter 5.1.1 and the resulting solution injected six times on to the GC using the GC conditions detailed in chapter 5.2.4.

The chromatograms obtained were integrated and due to the complex nature of the samples not all peaks were investigated for precision, the first major eluting peak (isosorbide mono oleate) and the last eluting peaks (tri esters) were used for the precision investigation. An assumption was made that if the first and last peaks were precise the others also would be. The peak areas obtained were investigated, this gives an indication that the same amount of sample is being injected each time to give a similar peak area. The area percentages were also considered because this will relate the two esters under investigation to the other components in the mixture.

The results obtained using peak area were:

| | **SMO (High purity acid)** | | **Manufactured SMO** | |
|---|---|---|---|---|
| Injection | Isosorbide Mono-Peak Area | Tri Ester Peak Area | Isosorbide Mono-Peak Area | Tri Ester Peak Area |
| 1 | 274471 | 214226 | 419459 | 335907 |
| 2 | 277478 | 203191 | 395930 | 360259 |
| 3 | 281423 | 167758* | 420532 | 365864 |
| 4 | 273085 | 232028 | 399558 | 374845 |
| 5 | 270108 | 256893 | 421265 | 376553 |
| 6 | 275145 | 250475 | 422128 | 390735 |
| Average | 275285 | 231363 | 413145 | 367361 |
| SD | 3868 | 22939 | 12017 | 18595 |
| RSD (%) | 1.4 | 9.9 | 2.9 | 5.1 |

**Table 6.11: Peak Areas Obtained for the Two Esters Investigated for Instrument Precision, SMO Prepared From High Purity Oleic Acid and Manufactured SMO**

*This value was found to be an outlier using the Dixon Q test and therefore was rejected.

The acceptance criteria for precision, depends very much on the type of analysis. While for compound analysis in pharmaceutical quality control precision of better than 1 % RSD is easily achieved, for more complex samples the precision RSD is much higher because the precision is dependent on the sample matrix, the concentration of the analyte and on the analytical technique and therefore it can vary between 2 % and more than 20 %. Due to the complex nature of the samples under investigation the RSD values obtained for instrument precision are thought to be acceptable, all were <20 %. This indicated that there was little variation in the peak areas between injections, this was particularly evident for the isosorbide mono oleate peak which had RSD of 1 % for the SMO prepared from high purity oleic acid and 3 % for the manufactured SMO. The RSD was greater for the tri ester peak, indicating more variation, however this may be due to the fact that this is a group of peaks and integration was more difficult.

The results obtained when studying the area percent were:

| | SMO (high purity acid) | | Manufactured SMO | |
|---|---|---|---|---|
| Injection | Isosorbide Mono- Area % | Tri Ester Area % | Isosorbide Mono- Area % | Tri Ester Area % |
| 1 | 9.2 | 7.2 | 13.3 | 10.7 |
| 2 | 9.3 | 6.8 | 12.4 | 11.3 |
| 3 | 9.4 | 5.6* | 13.0 | 11.3 |
| 4 | 8.9 | 7.6 | 12.0 | 11.2 |
| 5 | 8.6 | 8.2 | 12.5 | 11.2 |
| 6 | 8.5 | 7.8 | 12.4 | 11.5 |
| Average | 9.0 | 7.5 | 12.6 | 11.2 |
| SD | 0.4 | 0.5 | 0.5 | 0.3 |
| RSD (%) | 4.2 | 7.2 | 3.7 | 2.4 |

**Table 6.12: Area Percentages Obtained for the Two Esters Investigated for Instrument Precision, SMO Prepared From High Purity Oleic Acid and Manufactured SMO**

* This value was found to be an outlier using the Dixon Q test and therefore was rejected.

Using the area percentages for the peaks the RSD are comparable to when the peak areas were used, showing little variation between injections, this indicated that the other components also showed little variation. The area percent results show that the two esters considered are only a small proportion of the components in the sample, both being <20 % of total components in SMO prepared from high purity oleic acid and <25 % in manufactured SMO.

The results show that when a sample is prepared and injected on the GC several times there is little variation, the chromatograms obtained are similar.

The chromatograms obtained for each injection were overlaid for both samples under investigation and visual inspection showed that the chromatograms for each injection are very similar. All of the peaks looked comparable not just those for the isosorbide mono ester and tri ester peaks used in the precision investigation. The chromatograms obtained for the sorbitan mono oleate prepared from high purity oleic acid are shown in figure 6.15.

Analysis Precision:

To determine the analysis precision of the method each sample was independently prepared five times as detailed in chapter 5.1.1 and then analysed as detailed in 5.2.4. The analysis was carried out over several days.

As for instrument precision, the chromatograms obtained were integrated and the peak area and area percent considered for the isosorbide mono oleate and tri ester peaks to establish the analysis precision.

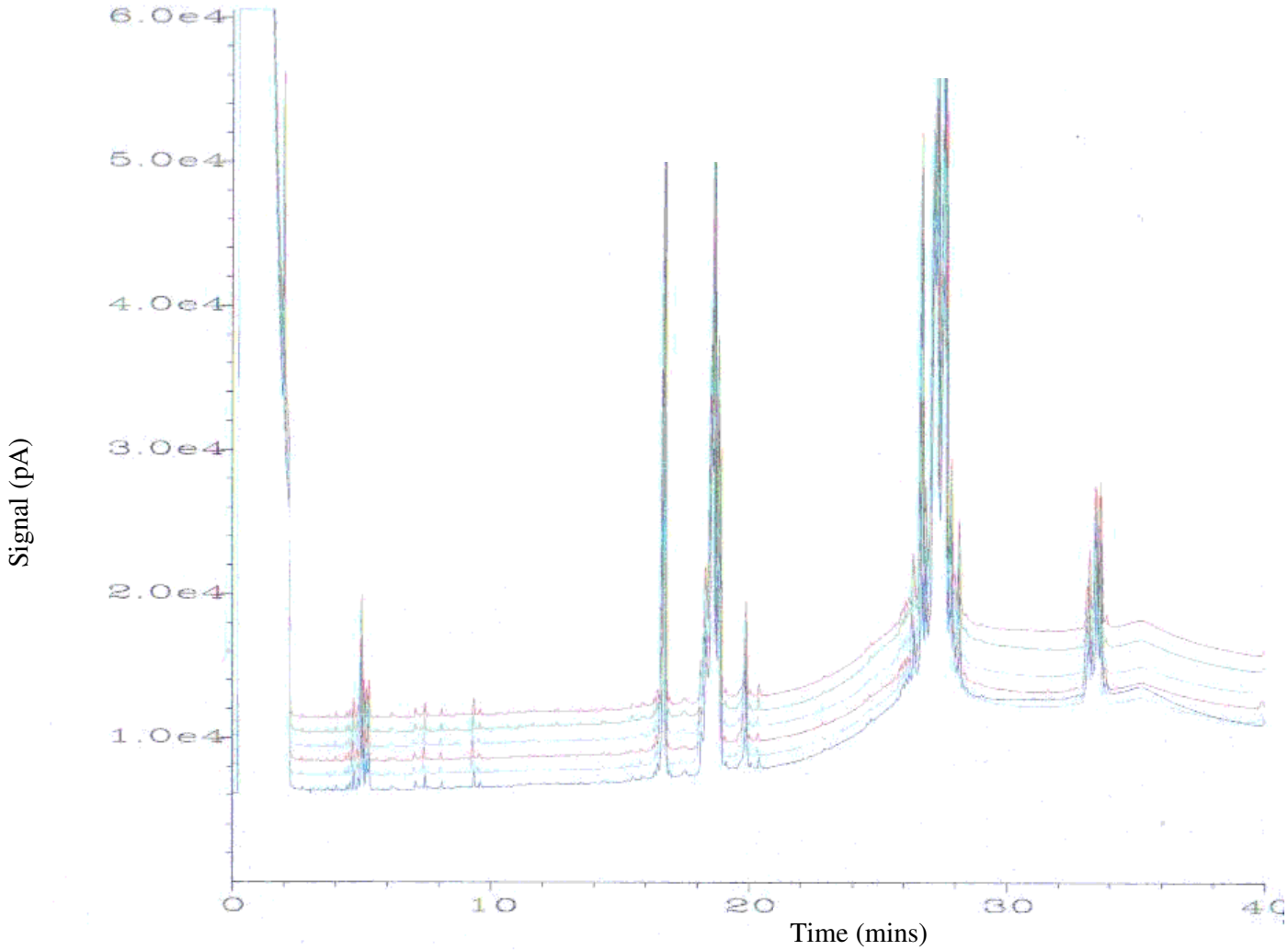



**Figure 6.15: Overlaid GC Chromatograms of SMO Prepared from High Purity Oleic Acid for Instrument Precision Study**

Using peak area the following results were obtained:

| | SMO (high purity acid) | | Manufactured SMO | |
|---|---|---|---|---|
| Injection | Isosorbide Mono-Peak Area | Tri Ester Peak Area | Isosorbide Mono-Peak Area | Tri Ester Peak Area |
| 1 | 285523 | 299243 | 398679 | 293419 |
| 2 | 278455 | 313224 | 371552 | 303462 |
| 3 | 330451 | 342777 | 388544 | 256314 |
| 4 | 289290 | 307539 | 409224 | 310339 |
| 5 | 276876 | 252963 | 419459 | 313645 |
| Average | 292119 | 303149 | 397492 | 295436 |
| SD | 22022 | 32500 | 18537 | 23199 |
| RSD (%) | 7.5 | 10.7 | 4.7 | 7.9 |

**Table 6.13: Peak Areas Obtained for the Two Esters Investigated for Analysis Precision**

The RSD values are <20 % and therefore are thought to be acceptable considering the complex nature of the samples. The samples were acetylated using the same weight of sample and volume of acetylating reagents and this has resulted in the peak areas investigated being very similar despite the sample being independently prepared for each injection. There is little variation in the peak areas, which infers that the esters are being fully acetylated each time.

Using Area percent the results in table 6.14 were obtained.

| | SMO (high purity acid | | Manufactured SMO | |
|---|---|---|---|---|
| Injection | Isosorbide Mono-Area % | Tri Ester Area % | Isosorbide Mono-Area % | Tri Ester Area % |
| 1 | 10.6 | 11.1 | 13.6 | 10 |
| 2 | 10 | 11.3 | 13.8 | 11.3 |
| 3 | 9.7 | 10.1 | 13.3 | 8.8 |
| 4 | 9.7 | 10.3 | 13.5 | 10.2 |
| 5 | 9.6 | 8.8 | 13.1 | 9.8 |
| Average | 9.9 | 10.3 | 13 | 10 |
| SD | 0.4 | 1.0 | 0.3 | 0.9 |
| RSD (%) | 4.1 | 9.6 | 2.0 | 8.9 |

**Table 6.14: Area Percentages obtained for the Two Esters Investigated for Analysis Precision**

The area percent result gave acceptable RSDs, with all being ≤10 %, indicating little variation in the results obtained from the independently prepared sample as when peak area was used. The area percent would be the parameter used for the analysis, because the object of the method is comparison between peaks of different samples and using this parameter removes any discrepancies that would occur due to varying weights/volumes used and/or losses.

The chromatograms obtained for each of the independently prepared sample were overlaid and the chromatograms showed that each of the injections for the independently prepared sample are very similar for all the components not just the isosorbide mono oleate and tri esters, as with instrument precision. The procedure can therefore be considered to be precise.

Conclusion of limited validation:

The results obtained from this limited validation show that the method is specific and precise for sorbitan mono oleate. If a sample is prepared and injected several times on the GC there will be very little difference in the results because the GC analysis is repeatable. Also if the same weights and volumes are used to independently prepare a sample there is very little variation in the peak area results because the acetylation method is also repeatable. Both parts of the method are precise indicating that the method is suitable for the analysis of samples, with repeatable analysis being possible.

## 7. GC Analysis of Sorbitan Esters Using On-Column Injection:

The work detailed in chapter 6 showed that GC analysis was a success for the analysis of sorbitan mono esters including sorbitan mono oleate, which could not be analysed successfully by HPLC due to lack of repeatability, in contrast the GC analysis of SMO was found to be repeatable. However it was found that as the fatty acid chain length of the polyol esters increased the number of ester species eluting decreased and as a consequence a number of ester species of SMO are not detected by GC. It was felt that the limitation of the method may be a consequence of using a split/splitless injector which may not have resulted in complete transfer of material onto the column despite using the maximum operating temperature for the injector.

Therefore an investigation into the use of an alternative injection technique was felt to be worthwhile, due to the purchase of GC's capable of on-column injection by Croda. This technique applies the liquid sample directly to the column and is therefore thought to eliminate the errors associated with flash vaporisation techniques. The high boiling compounds with lower volatility are placed on the front of the column, therefore the problem of them not reaching the column due to incomplete vaporisation by other injection methods is eliminated. A study was therefore to be carried out similar to that detailed in chapter 6 but using on-column injection.

### 7.1 Analysis using On-Column Injection:

#### 7.1.1 Gas Chromatograph:

The GC used is a Perkin Elmer Autosystem XL fitted with a FID and uses a programmable split/splitless (PSS) on-column mode. An hourglass liner and the oven programme mode are used, a 0.53 mm id column is used and is inserted into the hour

glass liner, a special syringe is required that has a needle outside diameter of 0.47 mm, this enables the syringe to enter the column and deposit the sample directly onto the column.

### 7.1.2 GC Column:

The GC columns used for the GC work detailed in chapters 5 and 6 were a DB1-ht column and a HT5 column. The DB1-ht is not commercially available with an internal diameter of 0.53 mm which is required for on-column work and previous work found the HT5 column to become very brittle with high temperatures. It was therefore necessary to find a column that was suitable for on-column analysis. The column selected was a WCOT ultimetal 10 m x 0.53 mm with coating HT SIMDIST CB, which is an unbreakable inert ultimetal capillary column guaranteed to a maximum temperature of 450°C. It is only available with 0.53 mm id and therefore is suitable for on-column analysis.

### 7.1.3 Operating Temperature:

The maximum operating temperature using the DB1-ht column was 400°C and this upper temperature was the starting point for the on-column work, with an initial temperature of 100°C.

| | |
|---|---|
| Initial temperature: | 100°C |
| Ramp rate: | 8°C/min |
| Final temperature: | 400°C (held for 5 mins) |
| Detector temperature: | 400°C |
| Carrier gas: | Helium |
| Flow rate: | 1 ml/min |

Injection volume: 1 µl

### 7.2 GC Analysis of Polyol Esters

#### 7.2.1 Samples:

The previous work using the DB1-ht and HT5 columns was a study to find out the ester species which would elute from the column as the fatty acid chain length of the polyol esters increased and therefore became less volatile. A similar approach was therefore carried out with the on-column analysis, starting with the polyol esters of caprylic acid and finishing with the polyol esters of stearic and oleic acid. The samples used were those prepared and detailed in chapters 2, 5.3, 6.1 and 6.2.6, with each being acetylated as detailed in chapter 5.1.1 and analysed using the GC conditions detailed in 7.1.

Note: It was only possible to analyse sorbitol laurate prepared at 1:1.5 w/w sorbitol:acid chloride due to insufficient sample of the 1:3 w/w ratio material. Due to lack of availability of the lauryl chloride starting material preparation of more was not possible.

#### 7.2.2 Polyol Esters of Caprylic Acid:

The chromatograms obtained for each of the polyol esters of caprylic acid are shown in figure 7.1.

The isosorbide caprylate chromatogram shows three major peaks well resolved from each other. The first peak to elute just after the solvent peak is due to isosorbide. The two other peaks will be due to isosorbide mono caprylate and isosorbide di caprylate, with the di ester being the last to elute because it is larger and therefore will be less

volatile. Two smaller peaks are also observed and will probably be isosorbide mono and di esters of a larger fatty acid produced due to the starting material only being 98 % pure. The chromatogram shows that both possible isosorbide esters of caprylic acid will elute from the column and be detected using on-column injection.

The sorbitan caprylate chromatogram shows that unreacted isosorbide and sorbitan are present, this was expected because the polyol profile of the sample showed that it was essentially sorbitan but did contain some isosorbide (chapter 5.3.2). A peak elutes after the unreacted polyols and will be due to isosorbide mono caprylate, the retention time of this peak is the same as that of isosorbide mono caprylate seen in the previous chromatogram. The next large peaks to elute are groups of peaks and are thought to correspond to sorbitan mono, di and tri caprylate. A small group of peaks are also observed between 22 and 23 minutes and could be due to sorbitan tetra caprylate. The work has therefore shown that it is also possible to elute all the possible sorbitan caprylate ester species using on-column injection.

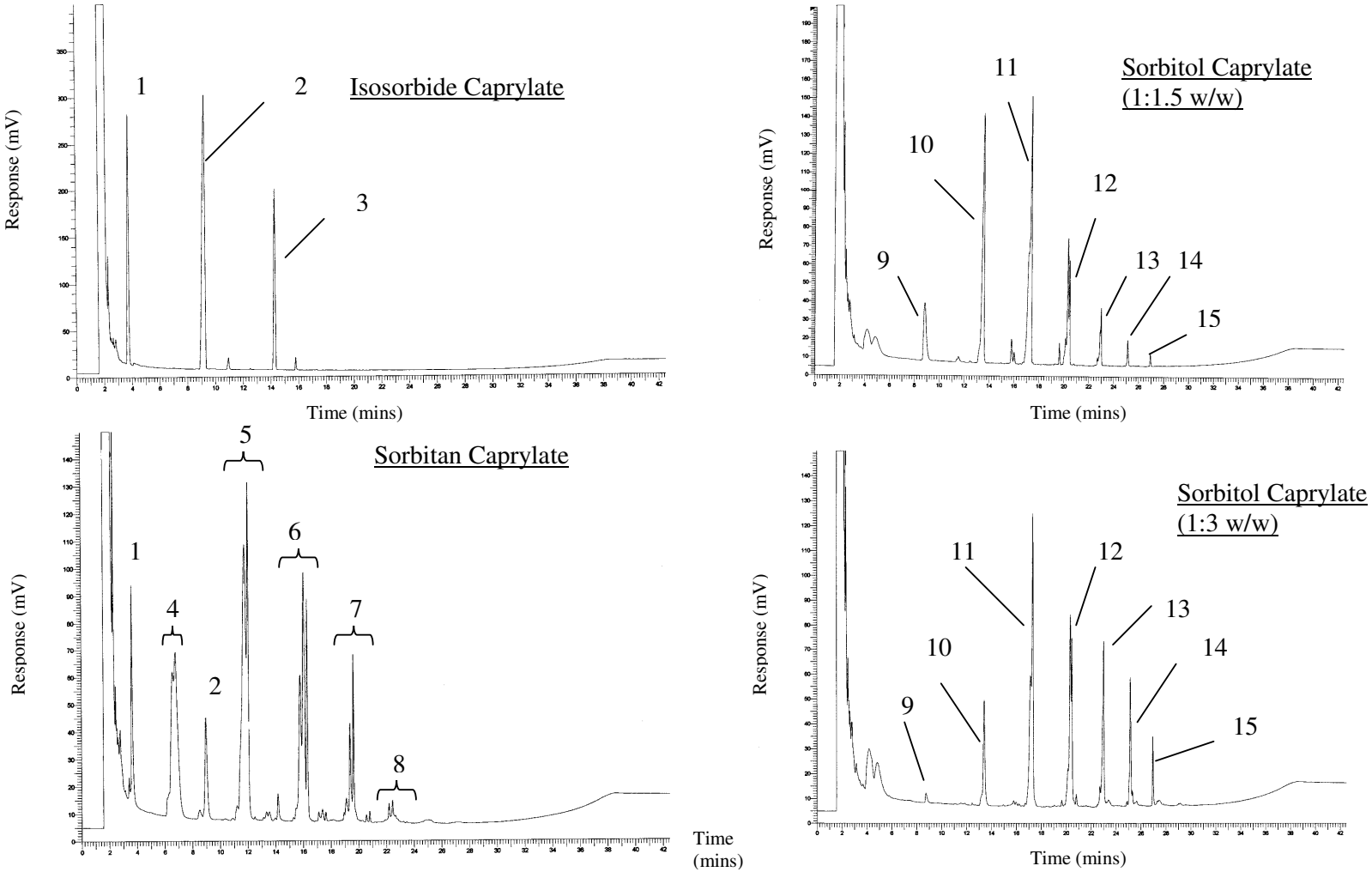



**Figure 7.1: GC Chromatograms of Polyol Esters of Caprylic Acid, Acetylated and Analysed Using an On-Column Injector, Detector at 400°C and Oven Temperature 100°C to 400°C at 8°C/min**

1 = Isosorbide, 2 = Isosorbide mono caprylate, 3 = Isosorbide di caprylate, 4 = Sorbitan,
5 = Sorbitan mono caprylate, 6 = Sorbitan di caprylate, 7 = Sorbitan tri caprylate, 8 = Sorbitan tetra caprylate,
9 = Sorbitol, 10 = Sorbitol mono caprylate, 11 = Sorbitol di caprylate, 12 = Sorbitol tri caprylate,
13 = Sorbitol tetra caprylate, 14 = Sorbitol penta caprylate, 15 = Sorbitol hexa caprylate

Two chromatograms are shown for sorbitol caprylate, one for the sample prepared with a ratio of 1:1.5 w/w sorbitol:octanoyl chloride and the other 1:3 w/w. The chromatogram obtained for the material prepared at a 1:1.5 w/w ratio shows a broad peak thought to be due to unreacted sorbitol followed by six peaks thought to be sorbitol mono, di, tri, tetra, penta and hexa caprylate and therefore showing that all possible sorbitol caprylate esters were prepared and eluted from the column. The largest peaks are due to the mono and di ester as expected at a preparation ratio of 1 sorbitol:1.5 octanoyl chloride, the tri ester peak is considerably smaller and the last three peaks smaller still. The chromatogram obtained for sorbitol caprylate prepared using 1:3 w/w ratio shows a very small peak due to free sorbitol and then again peaks corresponding to all six possible sorbitol esters of caprylic acid. The largest peaks are due to the di and tri esters and will be due to the increase in the octanoyl chloride compared to the previous sample. The tetra, penta and hexa peaks are also large compared to the previous chromatogram.

The study showed that all the possible polyol ester species of caprylic acid will elute from the column when using on-column injection.

### 7.2.3 Polyol Esters of Decanoic Acid:

The chromatograms obtained for each of the polyol esters of decanoic acid are shown in figure 7.2.

The chromatogram obtained for the isosorbide decanoate shows peaks that correspond to free isosorbide and isosorbide mono and di ester.

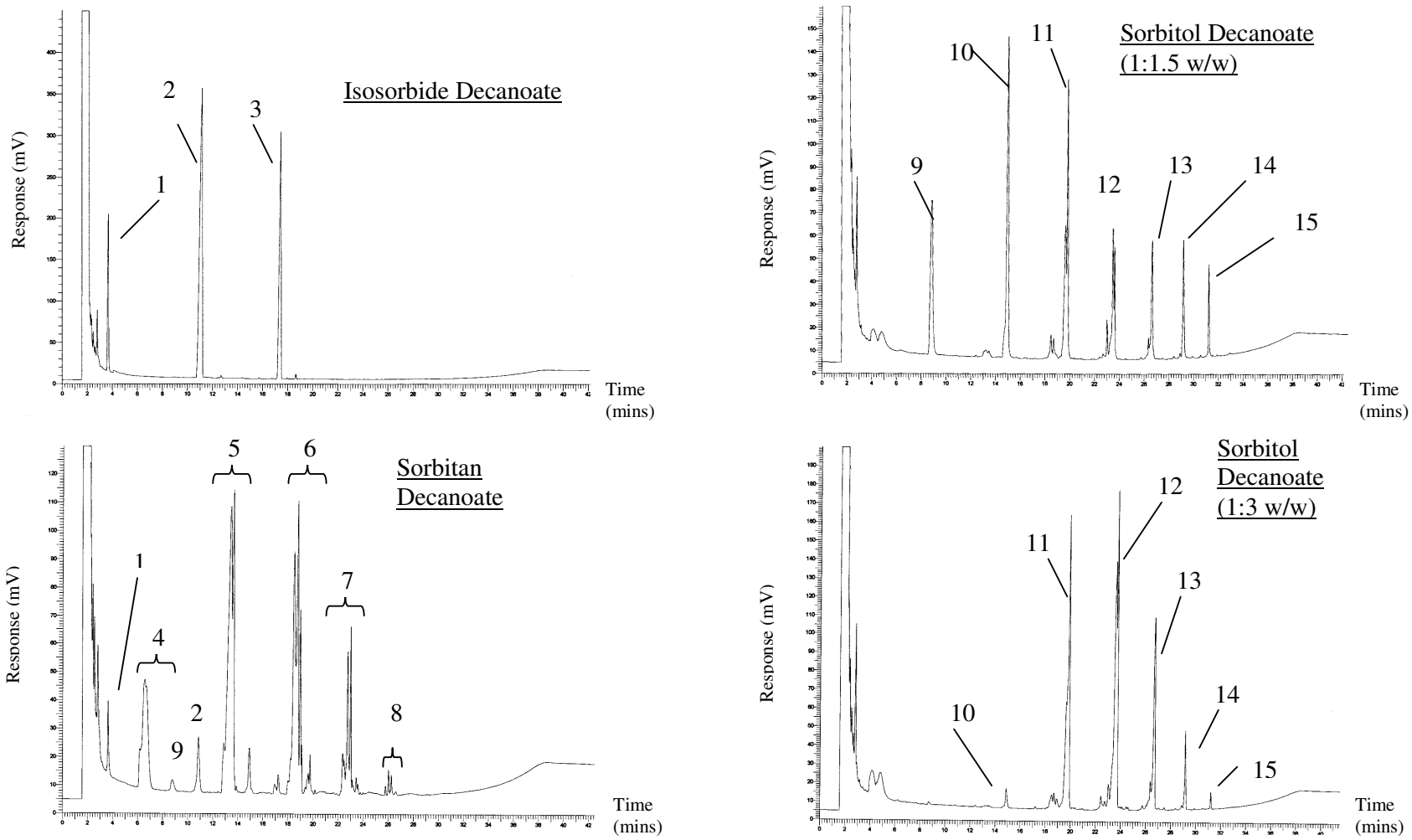



**Figure 7.2: GC Chromatograms of Polyol Esters of Decanoic Acid, Acetylated and Analysed Using an On-Column Injector, Detector at 400°C and Oven Temperature 100°C to 400°C at 8°C/min**

1 = Isosorbide, 2 = Isosorbide mono decanoate, 3 = Isosorbide di decanoate, 4 = Sorbitan, 5 = Sorbitan mono decanoate, 6 = Sorbitan di decanoate, 7 = Sorbitan tri decanoate, 8 = Sorbitan tetra decanoate, 9 = Sorbitol, 10 = Sorbitol mono decanoate, 11 = Sorbitol di decanoate, 12 = Sorbitol tri decanoate, 13 = Sorbitol tetra decanaote, 14 = Sorbitol penta decanoate, 15 = Sorbitol hexa decanoate

The chromatogram obtained for the sorbitan decanoate shows peaks due to free isosorbide, sorbitan and sorbitol and their esters, from the polyol profile it was found that isosorbide and sorbitol were present in the sorbitan decanoate sample, complete dehydration of the sorbitol to sorbitan did not occur. From the chromatogram it can be seen that the first eluting peaks are due to the unreacted polyols, these are followed by the mono esters of each of the polyols with the sorbitan mono decanaote being in the middle of the other two and considerably larger in height. The di and tri sorbitan decanoate esters are the next large groups of peaks seen, the final small group of peaks observed on the chromatogram are thought to be sorbitan tetra decanoate.

The two chromatograms obtained for the sorbitol decanoate show that all the possible sorbitol decanoate ester species were eluted. The chromatogram obtained for the sorbitol decanoate produced at 1:1.5 w/w stoichiometry shows unreacted sorbitol followed by the two largest peaks present due to sorbitol mono and di decanoate, four later eluting peaks are also noted and are all of a similar height and are thought to correspond to the other four possible sorbitol esters (tri – hexa esters). The chromatogram obtained for the sorbitol decanoate produced with 1:3 w/w sorbitol: decanoyl chloride shows no unreacted sorbitol and only a very small peak corresponding to sorbitol mono decanoate. Two large peaks are then observed due to the di and tri esters followed by a reasonable sized peak due to the tetra ester, the two later eluting components are thought to correspond to the penta and hexa esters. Due to the larger ratio of acid chloride used it was expected that the most abundant peaks would be larger esters compared to the previous chromatogram.

The study shows that all the possible polyol esters of decanoic acid were detected, as with the polyol esters of caprylic acid.

### 7.2.4 Polyol Esters of Lauric Acid:

The chromatograms obtained for each of the polyol esters of lauric acid are shown in figure 7.3.

The chromatogram obtained for isosorbide laurate shows three peaks corresponding to free isosorbide, isosorbide mono laurate and isosorbide di laurate. The chromatogram is similar to those obtained for isosorbide caprylate and decanoate, although the retention times of the ester species are slightly longer for the laurate esters due to the longer fatty acid used and the esters therefore being less volatile than the corresponding esters of the other acids.

The chromatogram obtained for sorbitan laurate is also similar to those for the sorbitan esters of the previous acids studied with four groups of peaks being observed for the four possible sorbitan ester species. As with the previous samples unreacted polyols are noted, however this chromatogram shows a larger peak due to the isosorbide mono ester, than seen in the other samples and a reasonable sized peak corresponding to the isosorbide di laurate is also seen.

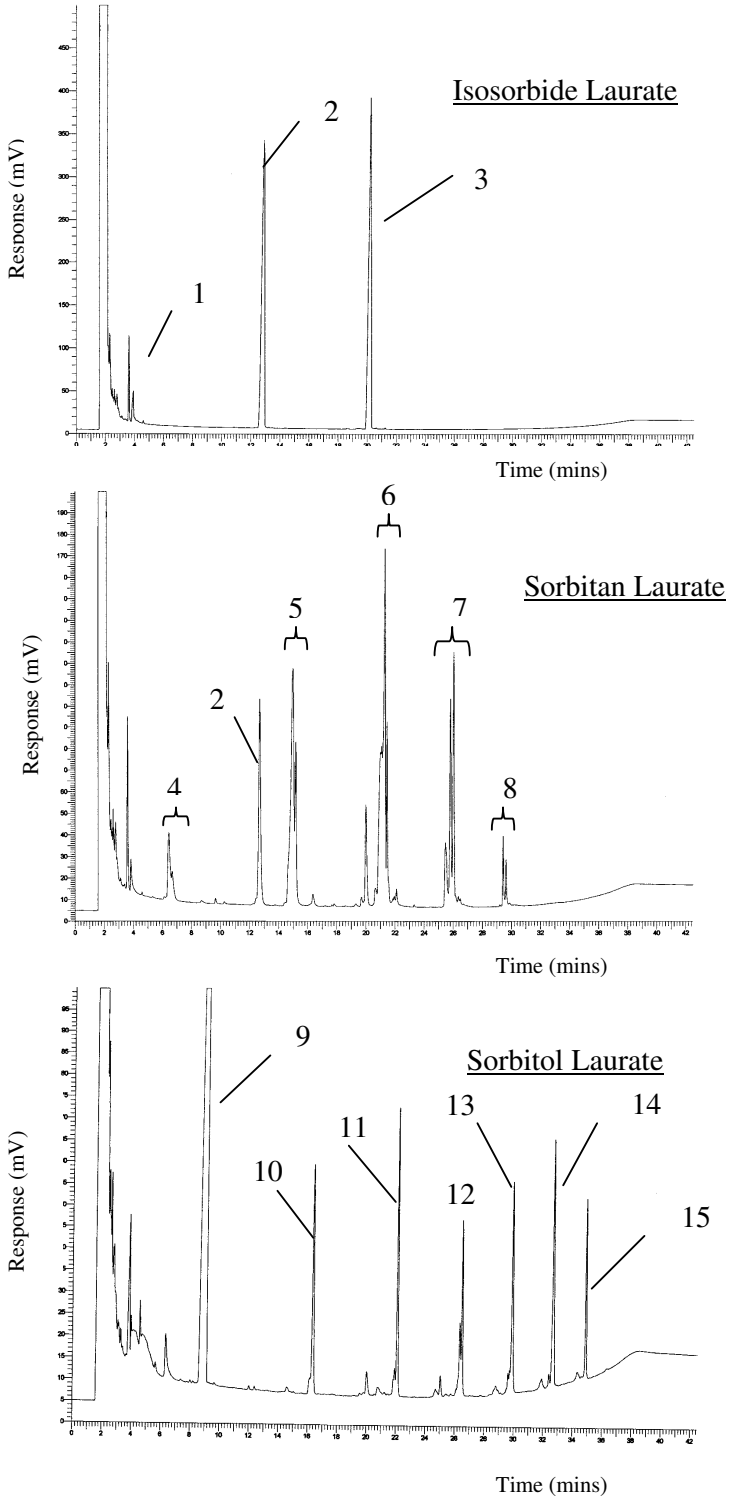



**Figure 7.3: GC Chromatograms of Polyol Esters of Lauric Acid, Acetylated and Analysed Using an On-Column Injector, Detector at 400°C and Oven Temperature 100°C to 400°C at 8°C/min**

1 = Isosorbide, 2 = Isosorbide mono laurate, 3 = Isosorbide di laurate, 4 = Sorbitan, 5 = Sorbitan mono laurate, 6 = Sorbitan di laurate, 7 = Sorbitan tri laurate, 8 = Sorbitan tetra laurate, 9 = Sorbitol, 10 = Sorbitol mono laurate, 11 = Sorbitol di laurate, 12 = Sorbitol tri laurate, 13 = Sorbitol tetra laurate, 14 = Sorbitol penta laurate, 15 = Sorbitol hexalaurate

The sorbitol laurate chromatogram shows a very large peak corresponding to unreacted sorbitol, followed by six peaks of varying size that are thought to be due to the six possible sorbitol esters of lauric acid.

Again this study shows that all the possible polyol esters under investigation eluted from the column and were detected.

**7.2.5 Polyol Esters of Myristic Acid**:

The chromatograms obtained for the polyol esters of myristic acid are shown in figure 7.4. The isosorbide myristate chromatogram shows three peaks due to the unreacted isosorbide and the mono and di ester species, as with the other isosorbide esters investigated. The ester species are therefore sufficiently volatile to elute form the column.

The sorbitan myristate chromatogram shows a small peak due to unreacted sorbitan followed by an isosorbide mono myristate peak due to the sample containing isosorbide and sorbitan, not just sorbitan, this was established from the polyol profile. Four groups of peaks can then be seen due to sorbitan mono, di, tri and tetra myristate. The tetra esters are a smaller group of peaks with a retention time of 32 to 33 minutes, it is expected that there would be less tetra esters from the stochiometry of the starting material, however the chromatogram indicates that sorbitan tetra esters were produced and can be eluted.

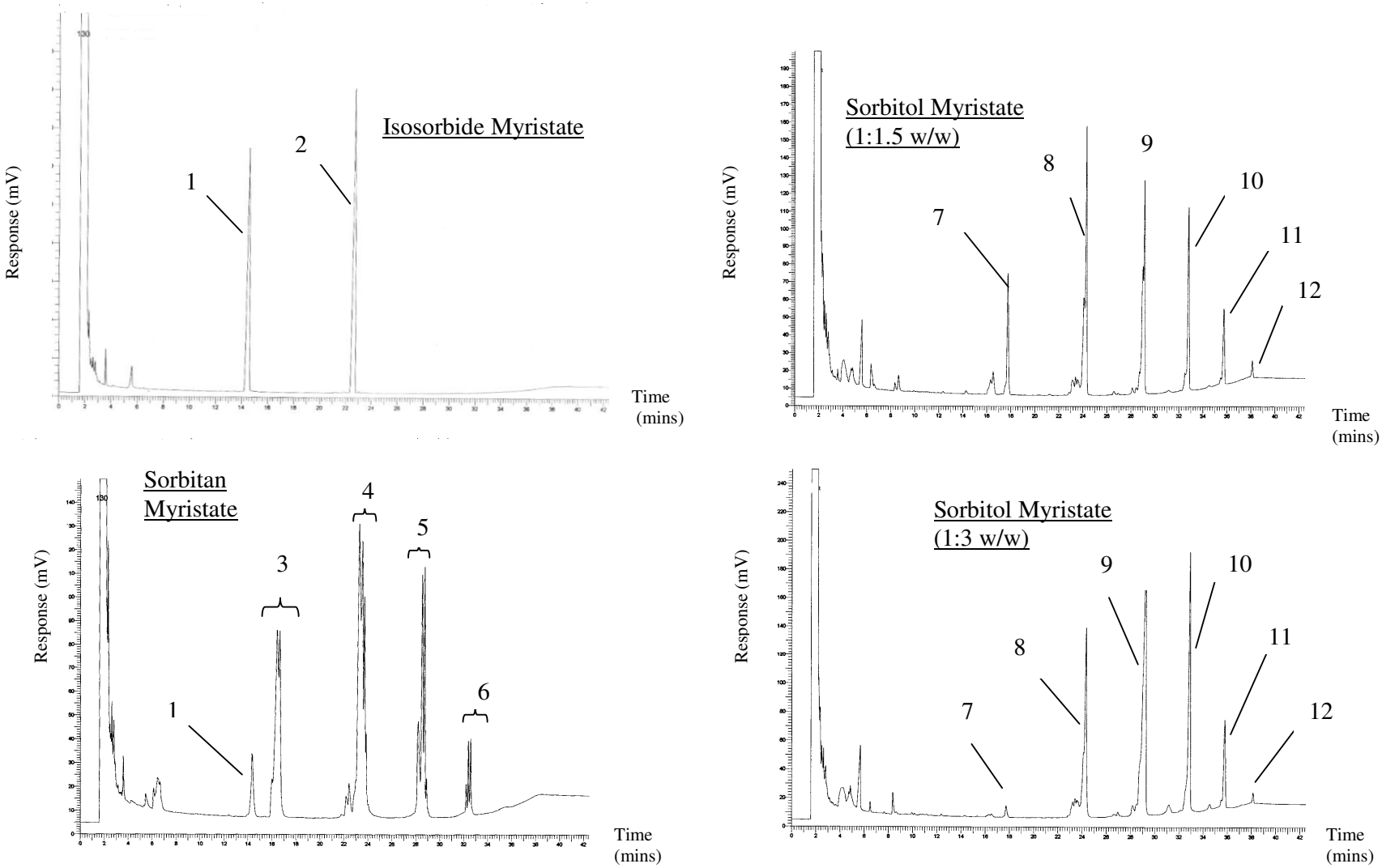



**Figure 7.4: GC Chromatograms of Polyol Esters of Myristic Acid, Acetylated and Analysed Using an On-Column Injector, Detector at 400°C and Oven Temperature 100°C to 400°C at 8°C/min**

1 = Isosorbide mono myristate, 2 = Isosorbide di myristate, 3 = Sorbitan mono myristate, 4 = Sorbitan di myristate, 5 = Sorbitan tri myristate, 6 = Sorbitan tetra myristate, 7 = Sorbitol mono myristate, 8 = Sorbitol di myristate, 9 = Sorbitol tri myristate, 10 = Sorbitol tetra myristate, 11 = Sorbitol penta myristate, 12 = Sorbitol hexa myristate

The chromatogram for the sorbitol myristate prepared at a 1:1.5 w/w ratio shows very little residual sorbitol, a small peak due to sorbitol mono myristate is then seen followed by larger peaks thought to be due to the di, tri and tetra esters, a smaller peak follows these and is probably due to the penta ester species. A small peak at the end of the chromatogram is thought to be due to the hexa esters, which again indicates that all the possible ester species were produced and all eluted. The chromatogram obtained for sorbitol myristate prepared with a stochiometry of 1:3 w/w shows small peaks due to unreacted sorbitol and the mono ester. Peaks due to the di, tri, tetra and penta esters of sorbitol are seen followed by a small peak at the end again thought to be due to the hexa ester species. The largest peak is due to the tri ester, which at a stochiometry of 1 sorbitol:3 acid chloride was expected.

**7.2.6 Polyol Esters of Palmitic Acid**:

The chromatograms for the polyol esters of palmitic acid are shown in figure 7.5. The chromatogram obtained for isosorbide palmitate shows no residual isosorbide and two major peaks due to isosorbide mono palmitate and isosorbide di palmitate. Smaller peaks are evident to the right of the main peaks, these are probably isosorbide mono and di esters of a larger fatty acid produced due to the palmitic acid feedstock not being 100% pure.

The sorbitan palmitate chromatogram shows groups of peaks due to free sorbitan and all four possible esters of sorbitan palmitate. Two smaller peaks are visible on either side of the sorbitan mono palmitate and have retention times that correspond to isosorbide and sorbitol mono esters of palmitic acid.

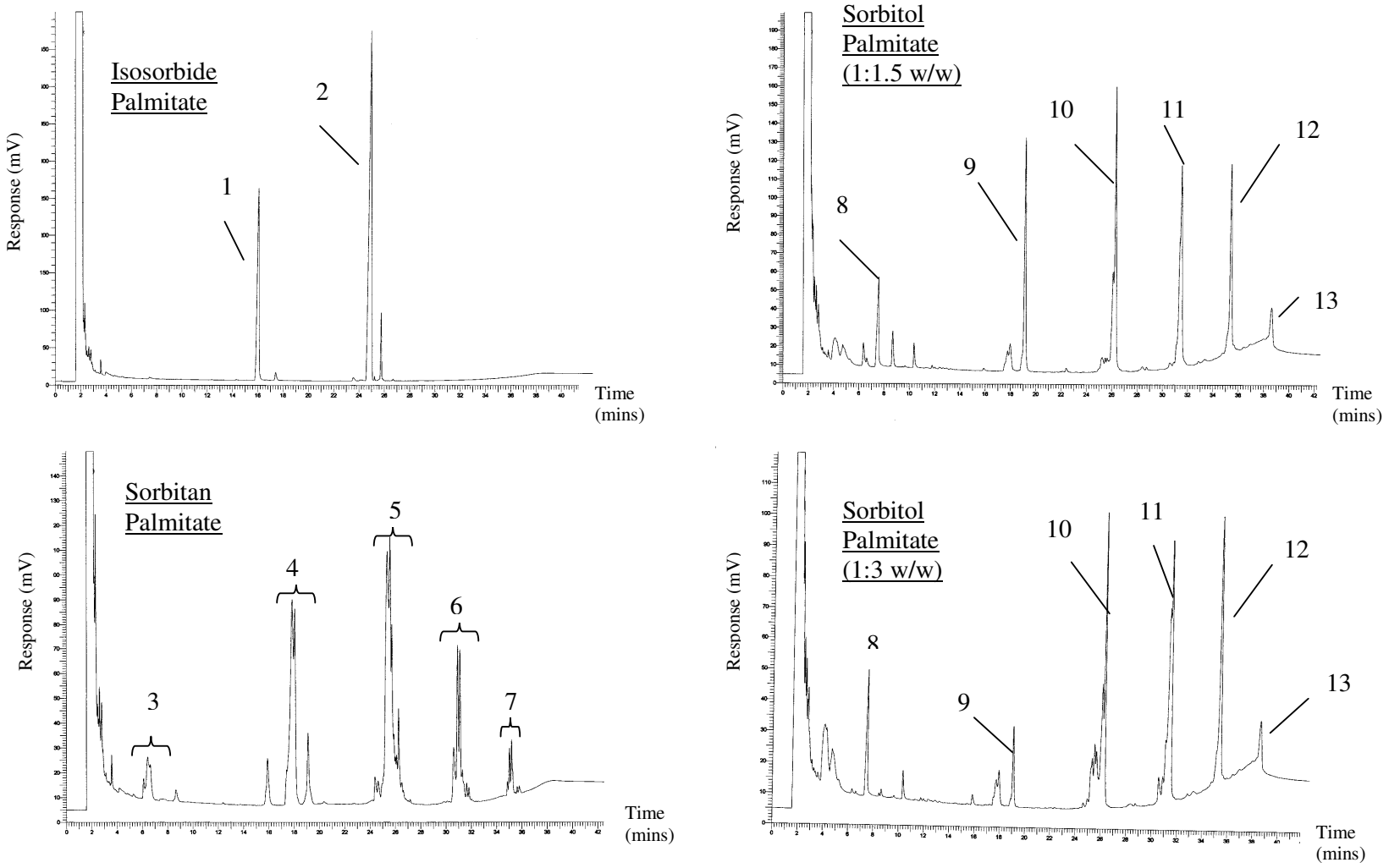



**Figure 7.5: GC Chromatograms of Polyol Esters of Palmitic Acid, Acetylated and Analysed Using an On-Column Injector, Detector at 400°C and Oven Temperature 100°C to 400°C at 8°C/min**

1 = Isosorbide mono palmitate, 2 = Isosorbide di palmitate, 3 = Sorbitan, 4 = Sorbitan mono palmitate, 5 = Sorbitan di palmitate, 6 = Sorbitan tri palmitate, 7 = Sorbitan tetra palmitate, 8 = Sorbitol, 9 = Sorbitol mono palmitate, 10 = Sorbitol di palmitate, 11 = Sorbitol tri palmitate, 12 = Sorbitol tetra palmitate, 13 = Sorbitol penta palmitate

These would be produced as a consequence of the sorbitol used to produce the material not converting to 100 % sorbitan, the polyol profile indicated that only 80 % sorbitan was produced, with both isosorbide and sorbitol also being present and therefore the possibility of the esters being produced.

The chromatogram for the sorbitol palmitate prepared at a 1:1.5 w/w shows some residual sorbitol and peaks due to mono, di, tri, tetra and penta palmitate, with the largest peak due to the di ester. The chromatogram obtained for sorbitol palmitate prepared with a stochiometry of 1:3 w/w also shows peaks due to unreacted sorbitol and the mono to penta ester. The largest sorbitol ester to elute from the column was therefore the penta ester, the elution time is ~39 minutes and therefore is at the final temperature of the GC programme and if the hexa ester was produced the temperature would not be sufficient to volatilise it. This is the first of the polyol ester series were all the esters did not elute from the column when using on-column injection.

**7.2.7 Polyol Esters of Stearic Acid**:

The chromatograms obtained for the polyol esters based on stearic acid are shown in figure 7.6.

The chromatogram obtained for isosorbide stearate shows that both the possible ester species are present and elute from the column, as seen with the isosorbide esters of shorter fatty acid chain lengths.

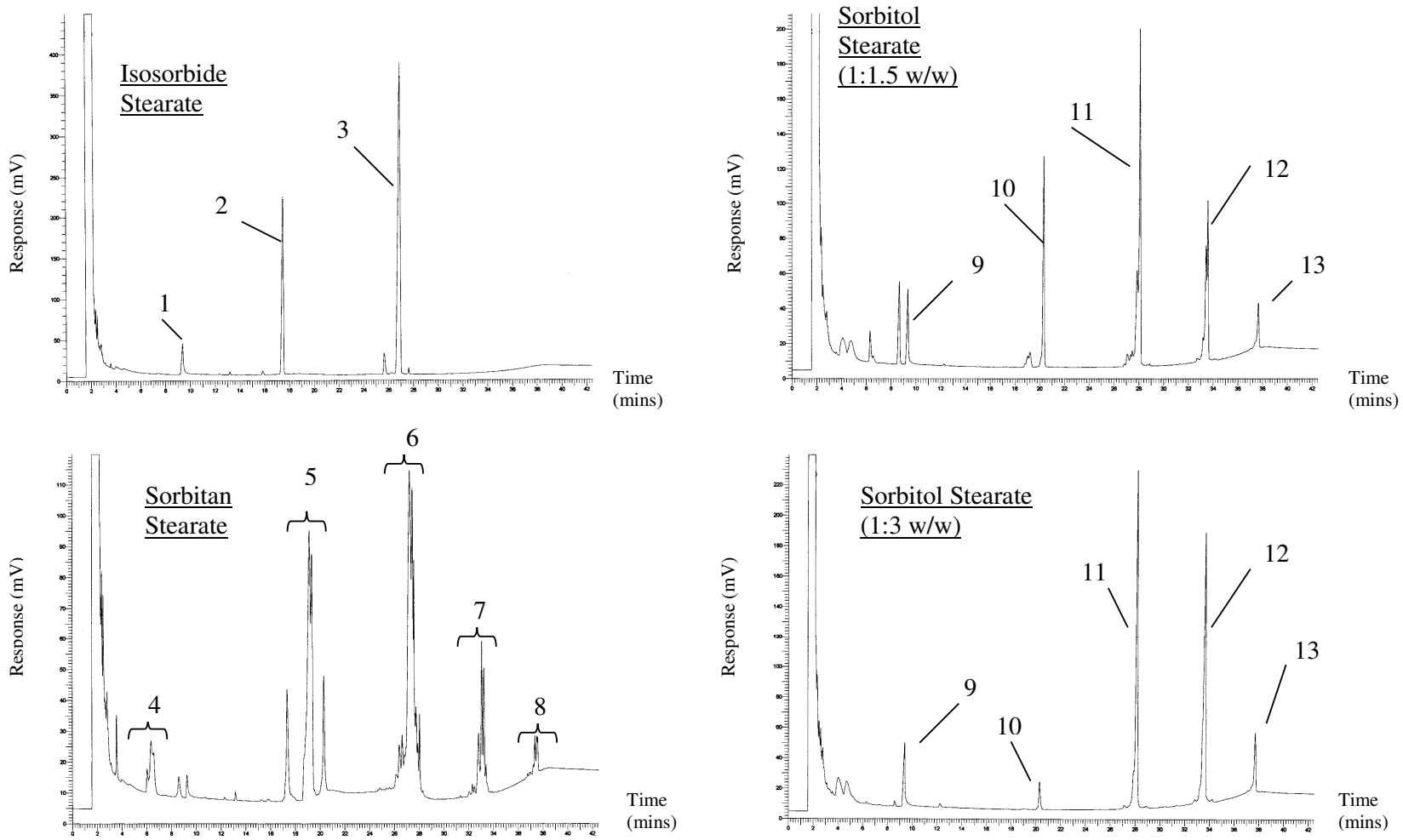



**Figure 7.6: GC Chromatograms of Polyol Esters of Stearic Acid, Acetylated and Analysed Using an On-Column Injector, Detector at 400°C and Oven Temperature 100°C to 400°C at 8°C/min**

1 = Isosorbide, 2 = Isosorbide mono stearate, 3 = Isosorbide di stearate, 4 = Sorbitan, 5 = Sorbitan mono stearate, 6 = Sorbitan di stearate, 7 = Sorbitan tri stearate, 8 = Sorbitan tetra stearate, 9 = Sorbitol, 10 = Sorbitol mono stearate, 11 = Sorbitol di stearate, 12 = Sorbitol tri stearate, 13 = Sorbitol tetra stearate

The chromatogram of sorbitan stearate shows groups of peaks due to residual sorbitan and the four possible esters. At either side of the sorbitan mono stearate peak are smaller peaks, with retention times that indicate they are isosorbide mono stearate and sorbitol mono steartate. The tetra esters elute at ~38 minutes which indicates they elute close to the final oven temperature used, due to a 5 minute hold time at the end of the run.

The chromatogram obtained for sorbitol stearate produced at 1:1.5 w/w stochiometry shows residual sorbitol, followed by peaks due to the mono, di and tri esters of sorbitol. A smaller peak is observed at the final oven temperature which is assumed to be sorbitol tetra stearate, this is the largest ester to elute, higher esters of sorbitol were detected for the shorter chain fatty acid, sorbitol esters indicating it is possible to produce them, therefore the higher esters of stearic acid were probably produced but not eluted. The chromatogram obtained for sorbitol stearate produced at a stochiometry of 1:3 w/w also shows residual sorbitol and peaks due to sorbitol mono, di, tri and tetra stearate. In contrast to the 1:1.5 w/w material the mono ester is very small, however as with the 1:1.5 w/w material the tetra ester peak is small, this may indicate that it is only partially volatile under the conditions used.

The study shows that a number of the polyol ester species based on stearic acid will elute from the column, however the largest to elute are the tetra esters. Other lower chain polyol esters resulted in all possible esters eluting and because the samples were prepared in the same way it infers that the higher esters of stearic acid were produced but the GC conditions were not adequate for them to elute from the column.

### 7.2.8 Polyol Esters of Oleic Acid

The chromatograms obtained for the polyol esters of oleic acid are shown in figure 7.7.

As with the previous isosorbide esters both the mono and di esters elute. However no residual isosorbide is detected and smaller peaks are detected prior to the large peaks of the isosorbide mono oleate and isosorbide di oleate, this is due to the purity of the fatty acid used to prepare the material, the smaller peaks are isosorbide esters of shorter fatty acid chain length, this can be assumed because the retention times are shorter than for the oleic acid based esters.

The chromatogram obtained for the sorbitan oleate shows groups of peaks due to the sorbitan mono, di, tri and tetra oleate, indicating that all the possible sorbitan oleate esters elute from the column.

The chromatogram obtained for sorbitol oleate shows two major peaks due to sorbitol mono oleate and sorbitol di oleate, a small peak is detected after this thought to be the tri ester and an even smaller peak after this due to the tetra ester. Previous sorbitol esters of shorter fatty acid chain lengths produced in the same way, have shown a larger range of esters, however the oleic acid is larger and therefore the esters produced are less volatile, it is probable that the GC conditions are not sufficient to elute these larger components.

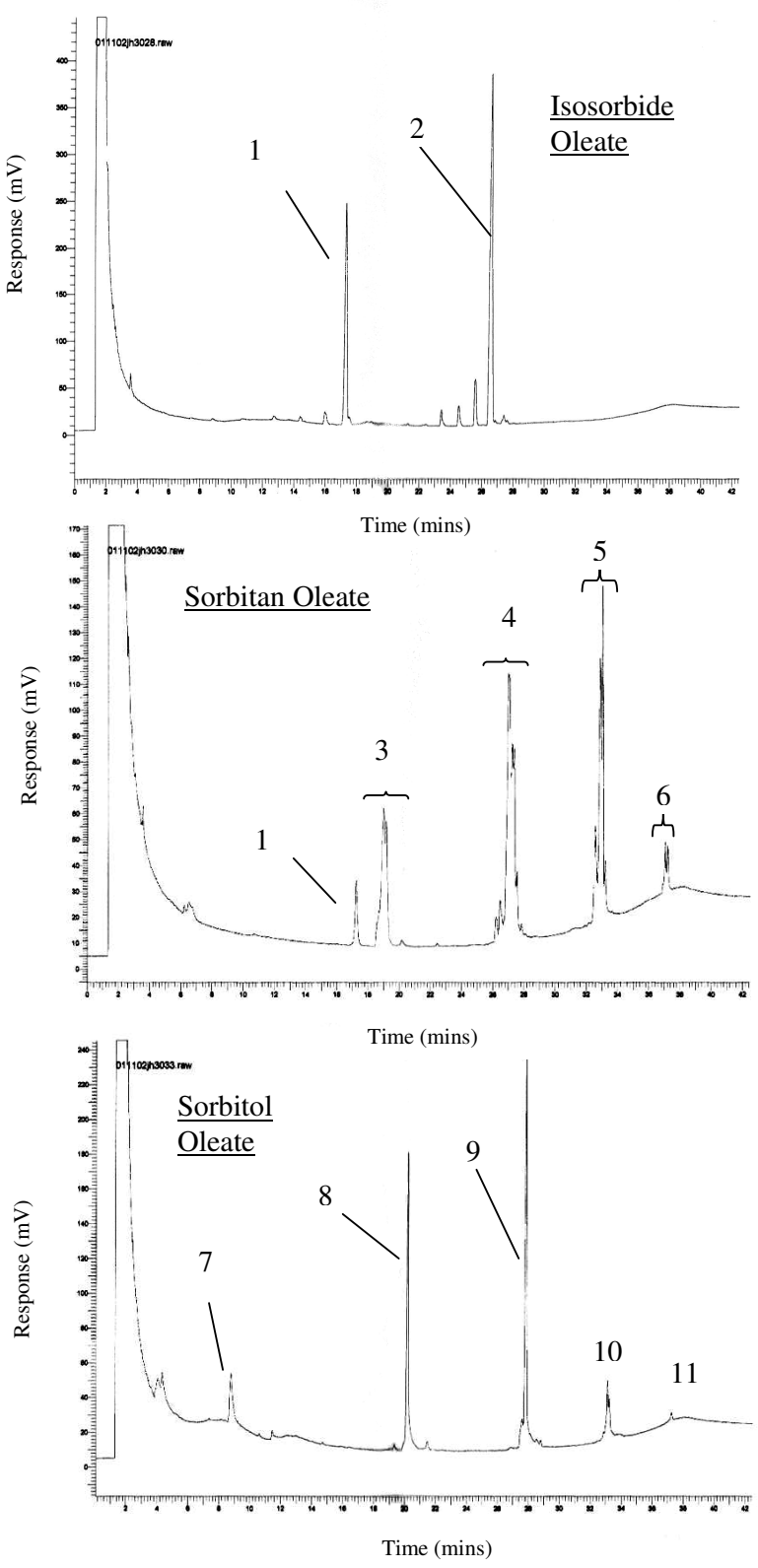



**Figure 7.7: GC Chromatogram of Polyol Esters of Oleic Acid**

1 = Isosorbide mono oleate, 2 = Isosorbide di oleate, 3 = Sorbitan mono oleate, 4 = Sorbitan di oleate, 5 = Sorbitan tri oleate, 6 = Sorbitan tetra oleate, 7= Sorbitol, 8 = Sorbitol mono oleate, 9 = Sorbitol di oleate, 10 = Sorbitol tri oleate, 11 = Sorbitol tetra oleate

### 7.2.9 Capacity Factor:

The effect of varying the fatty acid chain length and the number of ester groups on capacity factor was studied with the on-column injection, as with splitless injection in chapter 6.

**Effect of Chain Length**

The capacity factor was determined for the isosorbide mono and sorbitol mono esters with varying fatty acid chain lengths, as with the splitless injection analysis in chapter 6. The values obtained are shown in table 7.1 below.

| | Capacity Factor | |
|---|---|---|
| **Fatty Acid Chain Length** | **Isosorbide Mono Ester** | **Sorbitol Mono Ester** |
| C8 | 4.2 | 6.7 |
| C10 | 5.3 | 7.5 |
| C12 | 6.3 | 8.2 |
| C14 | 7.3 | 9.1 |
| C16 | 8.1 | 9.9 |
| C18 | 8.9 | 10.5 |

**Table 7.1: Capacity Factor of Isosorbide and Sorbitol Mono Esters With Varying Fatty Acid Chain Lengths Using On-column Injection**

The plot of the data is shown in figure 7.8. As seen with the splitless injection and Db1-ht column a linear relationship exists between capacity factor and fatty acid chain length of the ester, with the $R^2 = 0.9958$ for the isosorbide mono esters and 0.9978 for the sorbitol mono ester. Again the capacity factor for the sorbitan mono esters was not determined due to the difficulty obtaining a precise retention time due to the sorbitan mono esters appearing as group of peaks rather than a single peak.

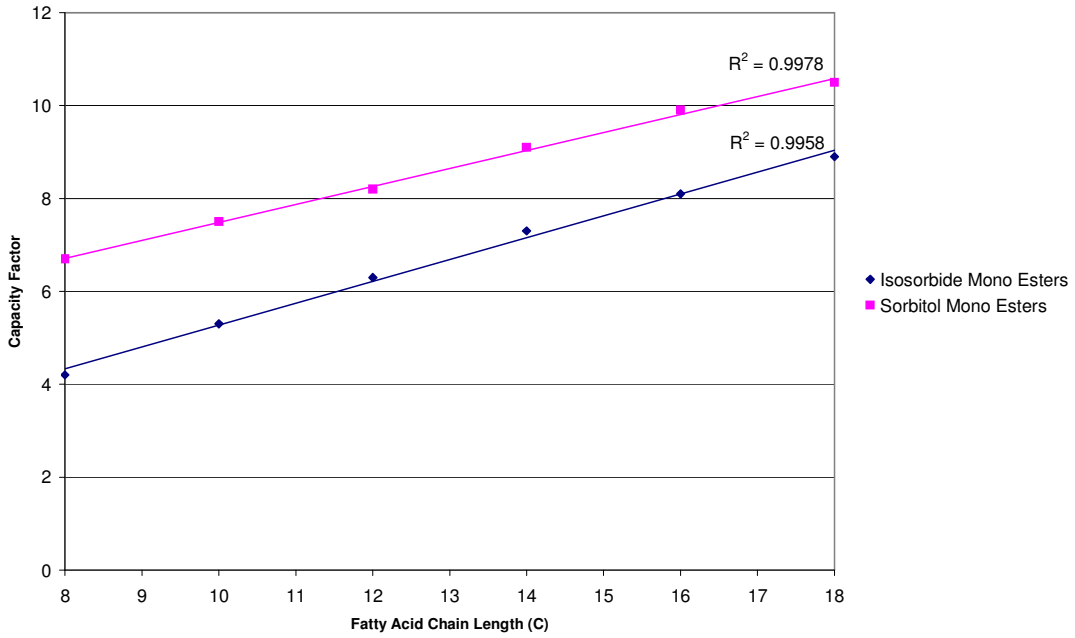



**Figure 7.8: Graph Showing Capacity Factor of Isosorbide and Sorbitol Mono Esters With Varying Fatty Acid Chain Lengths Analysed Using On-Column Injection**

**Effect of Number of Ester Groups:**

The capacity factors for each of the sorbitol ester species that eluted from the GC column were determined and the values obtained are shown in table 7.2.

For a specific fatty acid, as the number of ester groups on the sorbitol increases, it is expected that the volatility will decrease and therefore the retention on the column will increase. This was confirmed by the fact that for each of the fatty acids studied, as the number of ester groups on the sorbitol increased the capacity factor increased.

A plot of all the data is shown in figure 7.9 and as observed with the splitless injection, for each sorbitol fatty acid ester studied the data is curved.

| | Capacity Factor | | | | | |
|---|---|---|---|---|---|---|
| **Ester Groups** | **C8** | **C10** | **C12** | **C14** | **C16** | **C18** |
| **0** | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| **1** | 6.6 | 7.5 | 8.3 | 9.1 | 9.9 | 10.6 |
| **2** | 8.8 | 10.2 | 11.5 | 12.8 | 14.0 | 15.0 |
| **3** | 10.6 | 12.4 | 14.0 | 15.5 | 16.9 | 18.0 |
| **4** | 12.0 | 14.1 | 16.0 | 17.6 | 19.1 | 20.4 |
| **5** | 13.3 | 15.5 | 17.6 | 19.3 | 20.9 | N/D |
| **6** | 14.3 | 16.7 | 18.8 | 20.7 | N/D | N/D |

N/D – Not detected

**Table 7.2: Capacity Factor of Sorbitol Ester Species Analysed Using On-Column Injection**

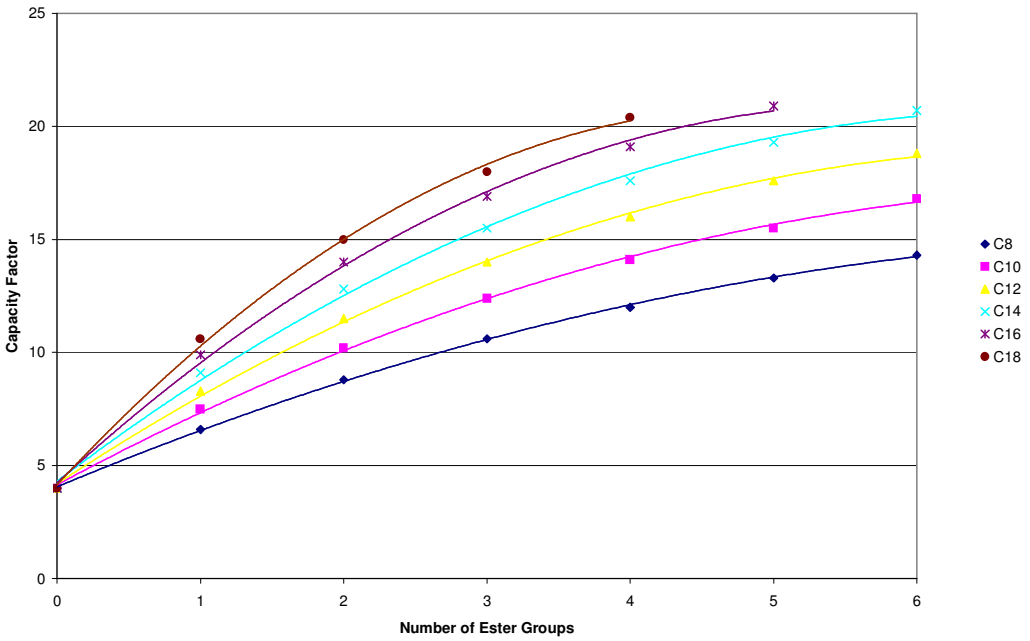



**Figure 7.9: Graph Showing Capacity Factor of Sorbitol Esters Analysed Using On-Column Injection**

### 7.2.10 Summary of the Use of On-Column Injection for Analysis of High Purity Polyol Esters:

From the investigation it was found that:

- all the isosorbide ester chromatograms showed peaks corresponding to the mono and di esters
- all the sorbitan ester chromatograms showed peaks for the mono, di, tri and tetra esters
- all the possible sorbitol ester species eluted from the column for caprylic, decanoic, lauric and myristic acid based esters. For palmitic esters five of the six possible esters eluted, with only the hexa esters not detected. However for sorbitol esters based on oleic and stearic acid the penta and hexa esters did not elute from the column.

The investigation has shown that the majority of ester species do elute from the column when on-column injection is used. Table 7.3 shows the esters detected for each polyol ester of each fatty acid studied.

| | C8 | C10 | C12 | C14 | C16 | C18 | C18:1 |
|---|---|---|---|---|---|---|---|
| Isosorbide mono | √ | √ | √ | √ | √ | √ | √ |
| Isosorbide di | √ | √ | √ | √ | √ | √ | √ |
| Sorbitan mono | √ | √ | √ | √ | √ | √ | √ |
| Sorbitan di | √ | √ | √ | √ | √ | √ | √ |
| Sorbitan tri | √ | √ | √ | √ | √ | √ | √ |
| Sorbitan tetra | √ | √ | √ | √ | √ | √ | √ |
| Sorbitol mono | √ | √ | √ | √ | √ | √ | √ |
| Sorbitol di | √ | √ | √ | √ | √ | √ | √ |
| Sorbitol tri | √ | √ | √ | √ | √ | √ | √ |
| Sorbitol tetra | √ | √ | √ | √ | √ | √ | * |
| Sorbitol penta | √ | √ | √ | √ | √ | X | X |
| Sorbitol hexa | √ | √ | √ | √ | X | X | X |

**Table 7.3 : Summary of Esters Detected Using On-column Injection For the Various Fatty Acids Studied**

√ Present X Absent * Trace

### 7.2.11 Comparison of Results using On-Column and Splitless Injection:

Chapter 5 and 6 detailed the GC analysis of the same high purity polyol esters detailed in this chapter but using splitless injection rather than on-column injection. Table 6.8 shows the ester species which eluted from the column with splitless injection and table 7.3 the ester species eluted with on-column injection. Comparison of table 6.8 and 7.3 shows that more ester species eluted from the GC column using on-column injection than splitless injection.

It was found that:

- using splitless injection the technique is not capable of eluting all possible esters for any of the fatty acid esters.

- using on-column injection all possible esters can be eluted for caprylic to myristic acid.

- using on-column injection all isosorbide and sorbitan esters will elute for all fatty acid esters under study.

- using splitless injection sorbitan tetra esters are a problem for all fatty acids esters and for oleic and stearic acid only trace amounts of the tri esters were detected. Although it was noted that when real samples were analysed with splitless injection using another column sorbitan tri esters of stearic and oleic acid were detected, so the column could have been partially responsible for the reduction in ester species detected compared to on-column injection.

### 7.3 Optimising GC Conditions:

The work carried out in chapter 7.2 analysing the various polyol esters using on-column injection showed that the majority of the polyol esters species would elute from the GC column. Considerably more ester species eluted using on-column injection than splitless injection. However again as the fatty acid chain length increased the number of ester species that eluted decreased, with some of the higher sorbitol ester species of palmitic, stearic and oleic acid not eluting. In an attempt to elute all the esters it was felt that an investigation using a higher final oven temperature should be tried, the higher temperature may be required to volatilise and elute the higher sorbitol esters from the column.

#### 7.3.1 Final Oven Temperature of 430 °C:

Increasing the final oven temperature to 430°C may be sufficient to volatilise some of the sorbitol esters which did not volatilise at 400°C and therefore did not elute from the column. The temperature selected was 430°C which is higher than the initial upper temperature tried with the on-column injection but not the upper limit of the column.

The following GC conditions were therefore used to analyse all of the high purity polyol esters prepared:

| | |
|---|---|
| Detector temperature: | 430°C |
| Initial oven temperature: | 100°C |
| Ramp rate: | 8°C/min |
| Final oven temperature: | 430°C, held for 5 minutes |

The chromatograms obtained for each of the polyol esters were reviewed and the ester species eluting noted, however it was found that the increased temperature had no effect on the number of esters detected. The chromatograms obtained were comparable to those in figures 7.1 – 7.6.

### 7.3.2: Final Oven Temperature of 450 °C

The use of a final oven temperature of 430°C had no significant effect on the esters eluting from the column, therefore the use of an even higher final oven temperature was studied. The temperature selected was 450°C which is the maximum operating temperature for the column. The following GC conditions were used to analyse all the high purity polyol esters:

| | |
|---|---|
| Detector temperature: | 450°C |
| Initial oven temperature: | 100°C |
| Ramp rate: | 8°C/min |
| Final oven temperature: | 450°C, held for 5 minutes |

Again the chromatograms obtained were studied and the ester species eluting for each sample noted. All of the ester species for the polyol esters of caprylic and decanoic acid eluted, for the polyol esters of lauric, myristic and palmitic acid all the isosorbide and sorbitan ester species eluted and the mono to penta esters of sorbitol. The chromatograms for the polyol esters of stearic acid showed that both the possible isosorbide esters eluted from the column, however only the mono to tri esters of sorbitan and sorbitol stearate eluted. The results were compared to the previous on-column results obtained for polyol esters of stearic acid when a final oven temperature of 400 and 430°C was used and it was found that with a final oven temperature of

450°C fewer ester species eluted. The various chromatograms obtained for the polyol esters of stearic acid were also compared and it was found that at 450°C the peaks were broader, this indicates that the column could be deteriorating, hence the reason for fewer ester species eluting. Finally the polyol esters of oleic acid were analysed with a final oven temperature of 450°C and again the ester species eluting were established and compared to the previous on-column work for polyol esters of oleic acid. It was found that with a final oven temperature of 450°C both isosorbide esters eluted, the mono to tri sorbitan esters and the mono and di sorbitol esters of oleic acid. The previous temperatures tried did not elute all of the possible esters of oleic acid but more were eluted than this investigation showed and when reviewed the chromatograms showed broad peaks. It was therefore concluded that the column had deteriorated. The column was new at the start of the on-column work and therefore a limited number of samples had been analysed, it is possible that although it is stated to be suitable for use up to 450°C it can not withstand use at this temperature for any length of time, as was found with the HT5 column used for the optimisation study using splitless injection.

The optimisation of the GC conditions, in an attempt to elute all the possible ester species from the GC column was a lengthy study, however it was found that the use of a higher upper temperature had no effect on the number of ester species that can be eluted from the column.

### 7.4 GC Analysis of Real Sorbitan Mono Ester Samples:

The work detailed in chapter 6 proved that GC is a useful analytical tool for the analysis of real sorbitan mono ester samples. The results obtained in chapters 7.2 and 7.3 have shown that using GC with on-column rather than splitless injection a wider range of esters can be eluted from the GC column, with only the largest sorbitol esters not eluting. However these results were obtained with samples containing essentially one polyol and prepared from high purity fatty acid feedstocks. Therefore real sorbitan mono ester samples that have varying polyols and fatty acids were analysed as in chapter 6.4.

#### 7.4.1 Analysis of Manufactured Sorbitan Mono Ester Samples:

The following real sorbitan ester samples produced by Croda were therefore analysed by GC using on-column injection:

Sorbitan mono palmitate

Sorbitan mono stearate

Sorbitan mono oleate – sample 1 and 2

These are part of the range of sorbitan mono esters produced by Croda and vary in fatty acid feedstock used. The samples are the same as the real samples analysed in chapter 6, however the analysis of real samples with splitless injection also included sorbitan mono laurate, however this was found to have a large number of components and be a very complex material therefore was eliminated from this study.

The samples were analysed by GC using the method detailed in chapter 7.1.3. The single high purity polyol esters were also analysed to aid identification of the peaks in the manufactured sorbitan mono ester samples.

Sorbitan mono palmitate:

The standard analysis (chapter 6.4.2.1) carried out showed that the manufactured sorbitan mono palmitate sample under analysis contained only isosorbide and sorbitan and that palmitic acid was the major fatty acid in the feedstock (94 %). The chromatogram obtained for the manufactured sorbitan mono palmitate sample analysed by GC using on-column injection is shown if figure 7.10. The chromatogram shows that the sample contains both of the possible isosorbide esters of palmitic acid. All of the possible sorbitan esters of palmitic acid were also found to be present, the sorbitan tetra palmitate peaks were the last peaks observed on the chromatogram and were small, however at the ratio of sorbitol to fatty acid used in the production of sorbitan mono palmitate, the amount of tetra esters produced would be limited and therefore the peaks were expected to be small. The polyol profile indicated that sorbitol was not present, therefore the tetra esters would be the largest esters expected.

The chromatogram obtained was compared to that obtained for the manufactured sorbitan mono palmitate using splitless injection (figure 6.12) and were found to be comparable for the mono, di and tri esters. However the on-column injection resulted in peaks thought to be due to sorbitan tetra palmitate eluting which were not seen with the splitless injection. In chapter 6 it was assumed that at the production ratios used the tetra esters would not be produced. However the on-column analysis of sorbitan mono palmitate indicates that the tetra esters were produced and do elute from the column.

The GC analysis using splitless injection offered advantages over GPC analysis because the mono esters were baseline resolved and the di and tri esters were well separated by GC but not by GPC. On-column injection offers the same advantages as GC with splitless injection has over GPC, however the tetra esters elute from the column. Therefore the potential for more information on ester species is possible using GC with on-column injection.

Sorbitan mono stearate:

It was established in chapter 6 that all three polyols are present in the manufactured sorbitan mono stearate sample under analysis, although sorbitol is only present at a low level. The fatty acid profile showed that the esters were prepared from a mixed feedstock containing largely palmitic and stearic acid in almost equal quantities.

The GC chromatogram obtained for sorbitan mono stearate using on-column injection is shown in figure 7.11, the mono ester region has doublets due to esters of the two main acids. Two isosorbide mono ester peaks are identified and two sorbitan mono ester groups of peaks are also seen. The palmitic acid esters will be more volatile and therefore will elute prior to the corresponding stearic esters.

The di and tri ester regions show a range of peaks, however the di and tri esters are well separated from each other. Due to the fatty acid feedstock, esters based on palmitic and stearic acid could be produced as well as esters with mixed fatty acids. The isosorbide di palmitate is the first peak of the di ester group to elute, this is followed by a number of peaks which could consist of isosorbide di stearate and sorbitan and sorbitol di esters of both acids and mixed acid esters.

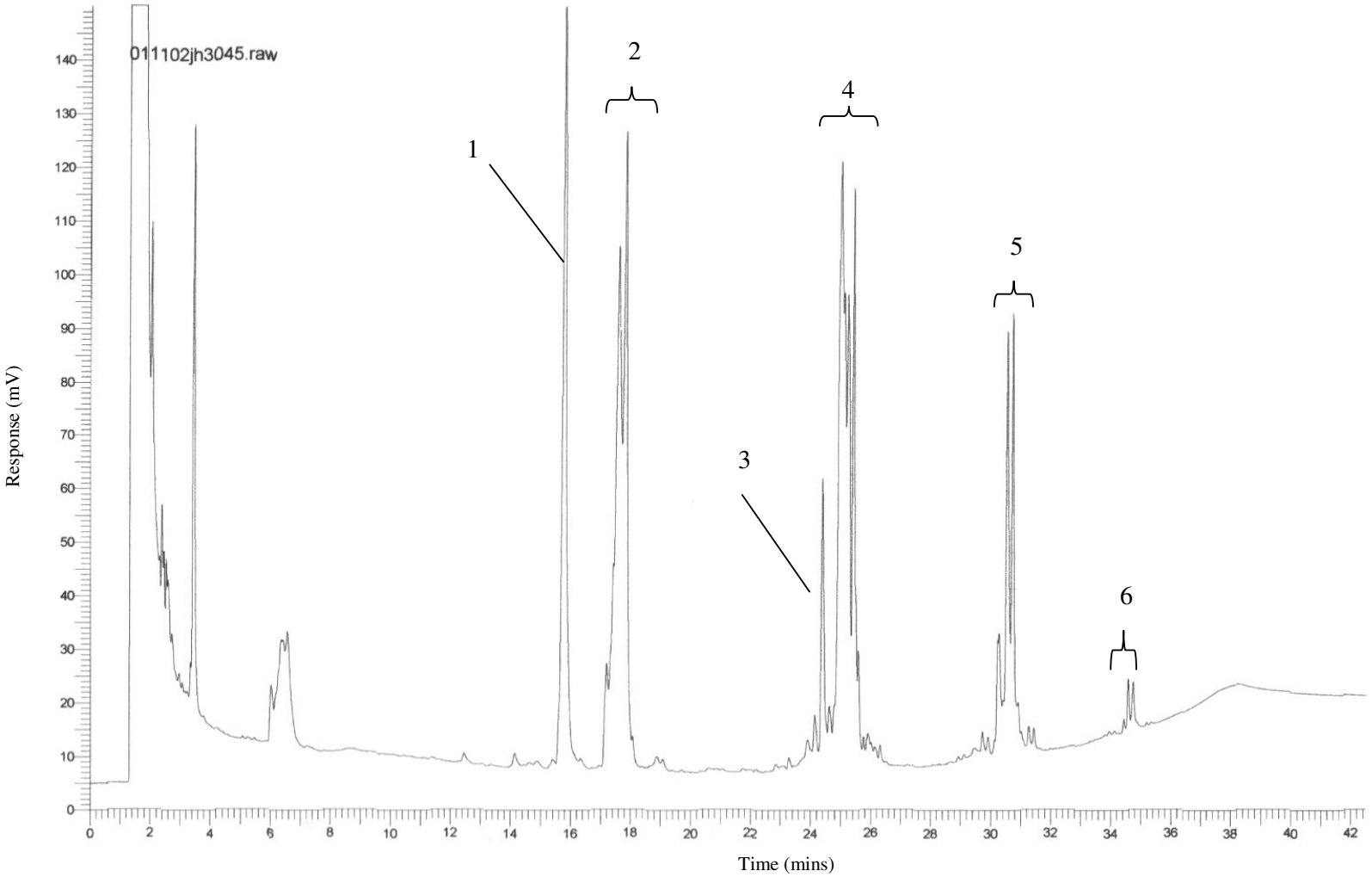



**Figure 7.10: GC Chromatogram of Manufactured Sorbitan Mono Palmitate Using On-Column Injection**

1 = Isosorbide mono palmitate, 2 = Sorbitan mono palmitate, 3 = Isosorbide di palmitate,
4 = Sorbitan di palmitate, 5 = Sorbitan tri palmitate, 6 = Sorbitan tetra palmitate

The group of tri esters elute next and are thought to be due to sorbitan and sorbitol tri esters. A small group of peaks elute after 34 minutes and are thought to be the tetra esters, however no larger ester groups are detected, these would be due to sorbitol penta and hexa esters however at the manufacturing ratio used and the polyol profile only showing 4 % sorbitol to be present it is unlikely these esters would be produced. The analysis using high purity esters also showed that only the sorbitol penta palmitate could elute.

The chromatogram obtained was compared to that obtained for the sorbitan mono stearate using splitless injection and as with the sorbitan mono palmitate were found to be comparable for the mono, di and tri esters but using on-column injection the tetra esters are also detected. Therefore GC analysis with on-column injection may offer advantages over both GPC and GC with splitless injection, as was found with sorbitan mono palmitate.

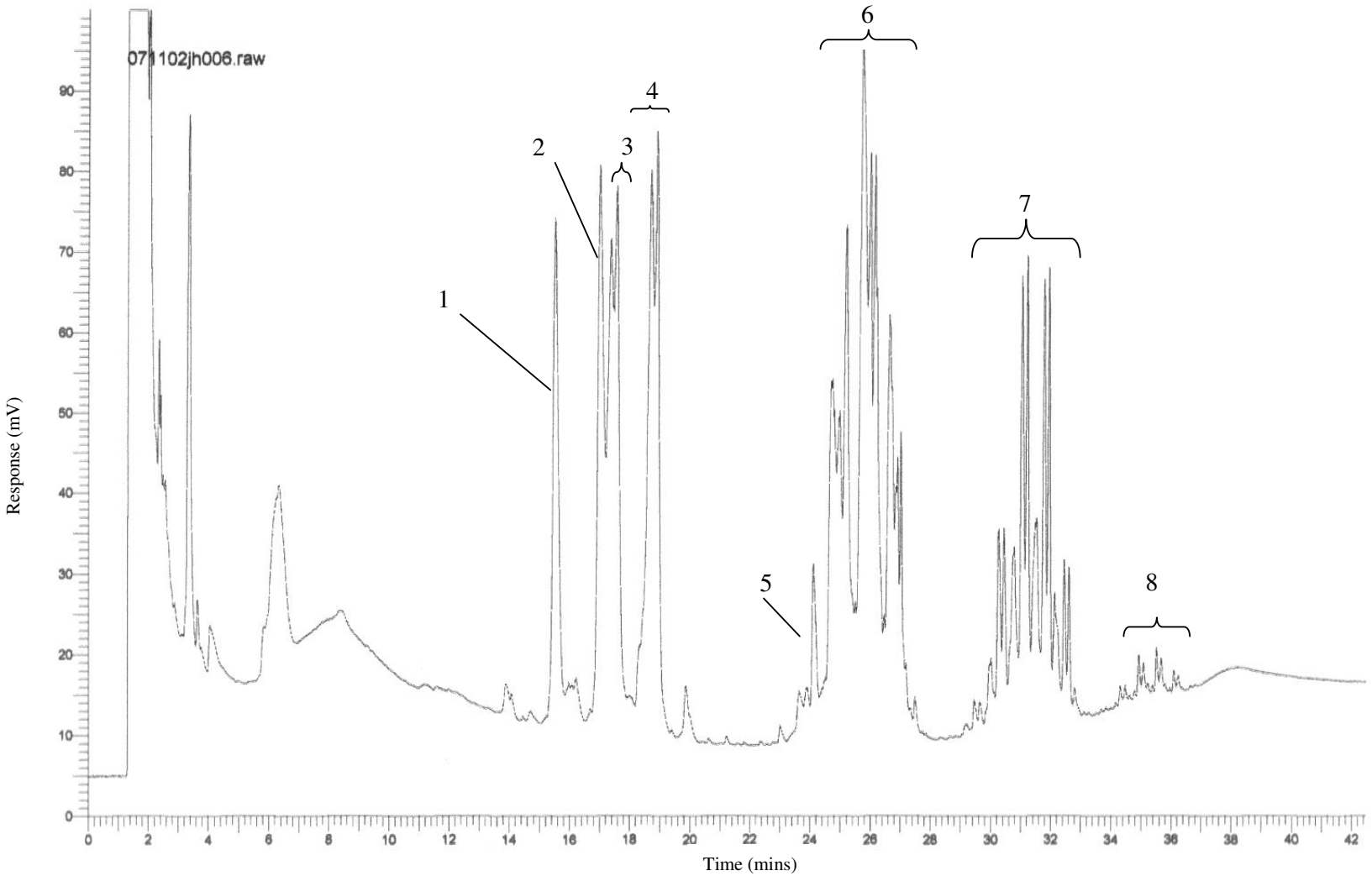



**Figure 7.11: GC Chromatogram of Manufactured Sorbitan Mono Stearate Using On-Column Injection**
1 = Isosorbide mono palmitate, 2 = Isosorbide mono stearate, 3 = Sorbitan mono palmitate, 4 = Sorbitan mono stearate, 5 = Isosorbide dipalmitate, 6 = Di esters, 7 = Tri esters, 8 = Tetra esters

Sorbitan Mono Oleate:

The GC chromatogram obtained for manufactured sorbitan mono oleate is shown in figure 7.12. A single peak due to isosorbide mono oleate is evident followed by a group of peaks thought to be sorbitan mono oleate. The di esters elute next with isosorbide di oleate evident followed by sorbitan di oleate. A group of peaks are then detected thought to be the sorbitan tri oleate esters, which is followed by a small group of peaks which elute after 35 minutes and are thought to be sorbitan tetra esters. The polyol profile indicated that no sorbitol was present, that it had been fully dehydrated, therefore sorbitol esters were not expected and were not seen. The largest esters possible would therefore be sorbitan tetra esters which were detected at the end of the chromatogram, indicating that all possible esters of the sorbitan mono oleate sample under analysis were detected.

As seen with sorbitan mono palmitate and stearate GC with on-column injection may be a possible alternative to GPC analysis for determining ester distributions for sorbitan mono oleate.

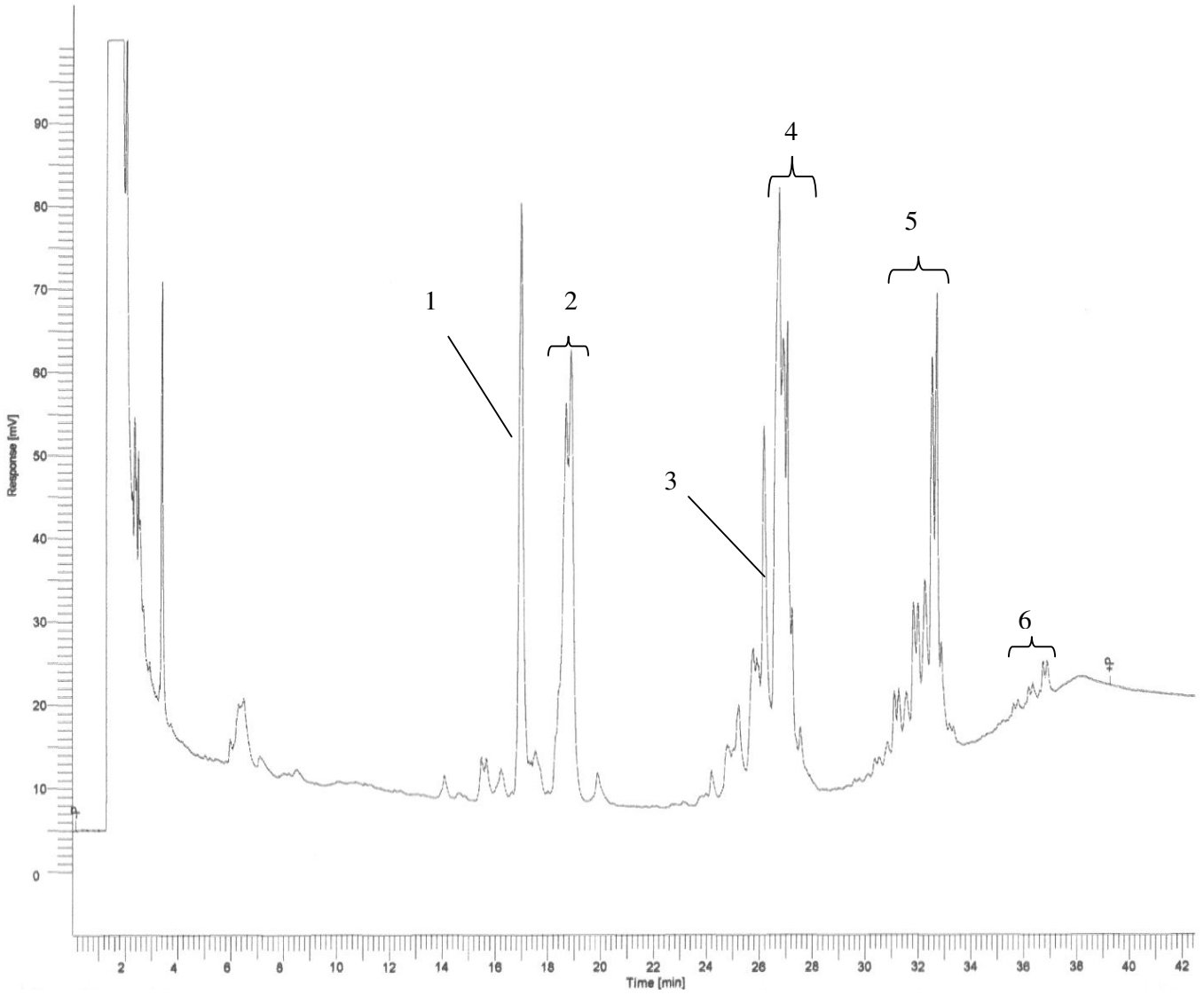


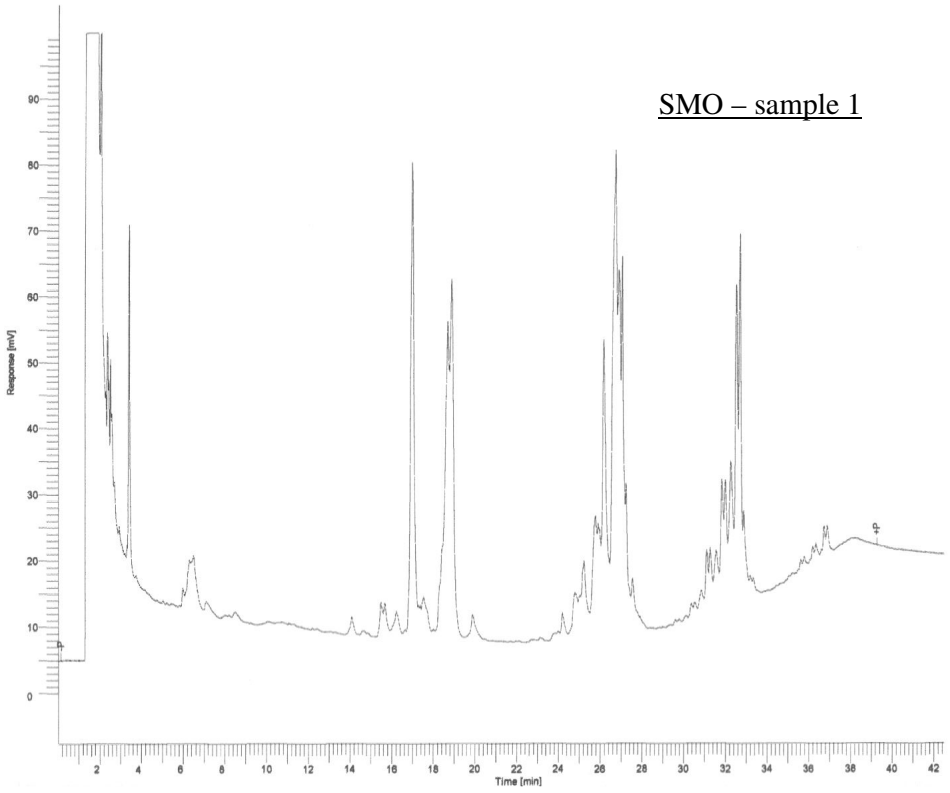

**Figure 7.12: GC Chromatogram of Manufactured Sorbitan Mono Oleate Using On-Column Injection**
1 = Isosorbide mono oleate, 2 = Sorbitan mono oleate, 3 = Isosorbide di oleate, 4 = Sorbitan di oleate, 5 = Sorbitan tri oleate, 6 = Sorbitan tetra oleate

Comparison of two manufactured sorbitan mono oleate samples:

The analysis of a real manufactured sorbitan mono oleate sample showed good separation of a number of ester species in the sample. The intention of the analytical method is to compare samples, therefore two manufactured sorbitan mono oleate samples were selected and analysed by GC using on-column injection and the chromatograms obtained compared to indicate if comparisons will be possible. The two chromatograms obtained are shown in figure 7.13. Studying the chromatograms obtained shows a number of differences, sample 2 has a distinct sorbitol mono-oleate peak, however this peak is very small in sample 1. Also in sample 1 the peaks due to the mono, di and tri esters are all similar sizes, but in sample 2 this is not found, with the tri ester group of peaks in particular being considerably smaller. Also on close inspection it can be seen that the profile of the groups of peaks for the two samples are different. This initial work indicates that differences in sorbitan mono oleate samples can be observed from a visual assessment, therefore with further work it may be possible to use GC for comparison of sorbitan ester samples.

**7.4.2 Summary:**

The work carried out shows that GC analysis with an on-column injector has proved to be of success in the analysis of manufactured sorbitan mono palmitate, stearate and oleate samples. A large number of esters were found to elute with the highest esters to elute being the tetra esters for the sorbitan mono palmitate and oleate, this was the largest expected ester, it was possible that sorbitol penta and hexa ester could have been produced, however it was felt unlikely, indicating that all expected esters eluted. For each manufactured sorbitan mono ester sample the various mono



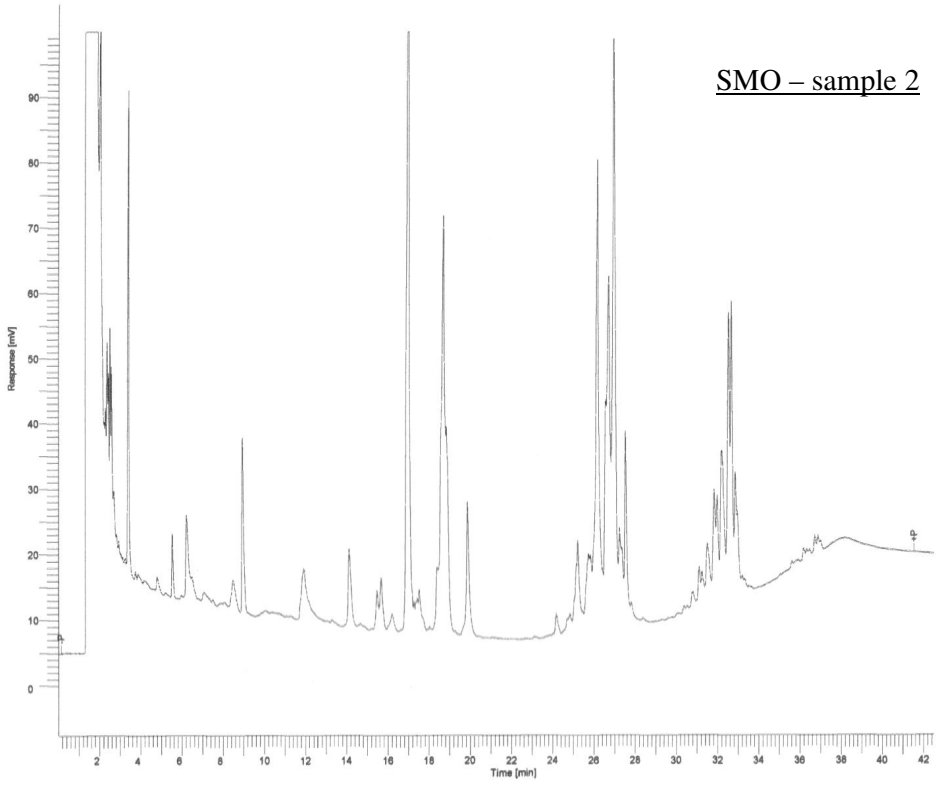




**Figure 7.13: GC Chromatograms of Two Manufactured Sorbitan Mono Oleate Samples**

ester species expected were well resolved and these were well separated from the di, tri and tetra esters which were also well separated from each other.

The work has shown that GC with on-column injection could be used for the comparison of ester species between samples. As detailed in chapter 6 currently the best method available for comparison of differences in ester species in manufactured sorbitan mono ester samples is GPC, GC with splitless injection appeared to offer advantages over it due to better resolution of the various esters, however the use of GC with on-column injection allows the tetra esters to be considered which did not elute with splitless injection. However further work using on-column analysis is required, including a repeatability study.

## 8. Conclusion and Further Work:

### 8.1 Conclusion

The objective of the work carried out was to develop a suitable method of analysis for characterising sorbitan esters, which can then be used to monitor sorbitan esters during manufacture, as well as to compare samples and establish structure activity relationships.

Sorbitan esters have a range of components of varying size and polarity and therefore volatility. HPLC was the first technique tried because it does not have the volatility and thermal stability issues associated with GC. However despite the use of various mobile and stationary phases for the analysis of a sample of sorbitan mono oleate, HPLC was not found to be a suitable method of analysis. The best resolution was obtained with a diol column and gradient elution with hexane and THF, a number of partially resolved peaks were obtained indicating separation of some of the components of the sample, however further work showed that the method was not repeatable. This method would therefore be of little use in a QC department and therefore was not felt to be worth pursuing further.

The next technique tried, GC with splitless injection, was found to be a suitable method for the analysis of sorbitan mono esters. A DB1-ht column was required, which is a high temperature column which operates at temperatures above normal columns and allows elution of higher molecular weight, less volatile compounds. Initial GC analysis confirmed that some sorbitan ester species would elute from a HT-GC column. Components expected in Sorbitan Caprylate were prepared and analysed, they have a shorter fatty acid chain length and therefore are more volatile and likely to elute from the GC column than the large ester species in the sorbitan mono oleate

sample analysed by HPLC. Both isosorbide esters of caprylic acid, sorbitan mono to tri caprylate and sorbitol mono to tetracaprylate eluted from the column. Analysis of a manufactured sorbitan caprylate sample resulted in a chromatogram with different ester species separated and identified, indicating that GC is a suitable method for characterising it.

Elution from the HT-GC column of larger less volatile ester species present in sorbitan esters with larger fatty acid chain lengths was investigated, by the preparation of a series of polyol esters, of varying chain lengths, with the largest esters based on oleic and stearic acid. GC analysis of the samples using an injector, detector and final oven temperature of 380°C showed that both isosorbide esters, sorbitan mono, di and tri esters and sorbitol mono and di esters for all the fatty acids investigated eluted. Larger esters eluted from the column for esters with smaller fatty acid chain lengths and for decanoic acid a small peak corresponding to sorbitol hexadecanoate was observed, indicating all possible ester species of decanoic acid will elute from the column. However the larger ester species for the longer chain fatty acids did not elute. The use of a higher final oven temperature or alternative column did not increase the number of ester species that could be eluted.

The work showed that HT-GC with splitless injection would be a suitable method of analysis for the sorbitan mono ester range manufactured by Croda, which are manufactured with a low fatty acid to sorbitol ratio and are expected to produce largely mono and di esters, which can be detected by GC. In contrast the sorbitan tri ester range, produced with a higher fatty acid to sorbitol ratio, to produce the higher

esters would not be suitable for analysis by GC because many of the higher esters produced would not be detected.

GC analysis of samples of manufactured sorbitan mono palmitate, sorbitan mono stearate and sorbitan mono oleate gave chromatograms that showed although not all the ester species could be eluted, a large number could and that the various mono esters are well resolved and are separated from the di and tri esters, which are also well separated from each other. Validation of the method with sorbitan mono oleate showed that it is specific and precise, demonstrating that the method is suitable for characterising real sorbitan mono ester samples.

Analysis of a sample of manufactured sorbitan mono laurate resulted in a chromatogram with a large number of peaks, indicating the complex nature of the material, which is probably due to the mixed fatty acid starting material. Baseline resolution was not obtained for the components and the large number of peaks means identification of the peaks is not possible, however it may be possible to use the chromatograms as 'finger print' type analysis for comparative purposes.

HT-GC with splitless injection offers advantages over the current GPC method used for the analysis of sorbitan mono esters due to better resolution of the various ester species.

The work showed that different columns from the same manufacturer, with the same stationary phase gave different results, therefore it would be necessary to set up a

system suitability using a sample of known composition to verify that the resolution of the chromatographic system is adequate prior to the analysis of samples.

Work carried out with HT-GC with on-column injection using a WCOT ultimetal column with HT SIMDIST CB coating, showed it may also be a suitable method of analysis for characterising the sorbitan mono ester range. Analysis of the range of single high purity polyol esters by GC with on-column injection showed that more ester species could be eluted for the larger fatty acid esters with on-column injection than with splitless injection. This is probably due to the fact that all of the sample is placed on the column, unlike splitless injection where the sample has to be transferred from the injection port and complete transfer onto the column may not occur. Analysis of real manufactured sorbitan mono ester samples using on-column injection was also successful, with a number of well resolved, identified peaks observed from mono to tetra esters. However validation of the method would be required to prove that it is precise and acceptable for its intended purpose. Also the column used became brittle when used at high temperatures for a length of time and this would need to be considered if the method was to be used as a routine QC test.

The work carried out shows that HT-GC with splitless injection is a suitable repeatable method of analysis for characterising a range of sorbitan mono esters. This analysis could be used to allow a relationship between the composition of sorbitan mono esters and their application to be established. This relationship could then be used to design the next generation of emulsifiers. HT-GC with on-column injection may also be a suitable method of analysis following further work.

**8.2 Further Work:**

GC with on-column injection showed potential for the analysis of sorbitan mono ester samples. However validation of the method is required to indicate the repeatability of the method and show that it is fit for purpose.

When the final analytical method has been determined, a range of sorbitan mono esters could be produced using conditions that will result in varying polyol profiles and therefore varying ester species. The samples could then be analysed using the selected method to establish that differences in the composition of the sorbitan mono esters can be determined using the analytical method developed.

The samples could then be used in one of the product application tests used within Croda, to determine the effect sorbitan ester composition has on performance. Sorbitan mono oleate is used as an emulsifier in emulsion explosives, a screening test is used to establish a product's applicability as an emulsifier. The test involves emulsifying ammonium nitrate, water, paraffin and an emulsifier using equipment designed at Croda, the resulting emulsion stability is then evaluated. The samples of varying composition could be used as the emulsifier and a study carried out to establish if a relationship between the composition of sorbitan mono esters and the application exists.

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