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The Characterisation of Oilfield Chemicals by Electrospray Ionization Multi-Stage Mass Spectrometry (ESI-MS)

McCormack, Paul James

The Characterisation of Oilfield Chemicals by Electrospray Ionization Multi-Stage Mass Spectrometry (ESI-MS^n)

by

Paul James M^{c}Cormack

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ABSTRACT

A diverse range of polar organic chemicals used during the offshore production of crude oils is routinely discharged from oil production platforms in so-called Produced Water (PW). The environmental fate of these chemicals is largely unknown since few methods exist for their detection. In the present study, the use of multistage electrospray ionisation ion trap mass spectrometry (ESI-MS^n) was investigated for the detection, identification, characterisation and quantification of compounds in both speciality oilfield chemicals (corrosion inhibitors, scale inhibitors, biocides and demulsifiers) and in Produced Water.

Both positive and negative ion collision induced dissociation (CID) multi-stage mass spectrometry (MS^n) was shown to allow high specificity detection and characterisation of alkylbenzenesulfonates, di-[alkyldimethylammonium-ethyl]ethers, alkylbenzyl-dimethylammonium and imidazoline compounds. CID MS^n fragmentation pathways were determined for di-[alkyldimethylammonium-ethyl]ethers (up to MS^5) and imidazoline compounds (up to MS^3). CID MS^n fragmentation pathways (up to MS^3) were determined for alkylbenzenesulfonates and alkylbenzyl-dimethylammonium cations and the MS^3 product ions (*m/z* 119 and *m/z* 58 respectively) were identified.

Imidazolines, which are widely used in speciality oilfield corrosion inhibitor products and which were shown to be amenable to ESI-MS detection and MS^n characterisation, were investigated further by high performance liquid chromatography (HPLC) coupled with ESI-MS. A HPLC analytical separation method with ESI-MS detection was devised for synthetic palmitic and oleic acid-derived 2:1-imidazolines (2:1-PI/OI), 1:1-imidazolines (1:1-PI/OI), monoamide and diamide compounds. The HPLC/ESI-MS responses of 2:1-PI and 2:1-OI were investigated and detection limits of 0.01μg mL^{-1} 2:1-PI and 2:1-OI (signal /noise ≥ 3) were determined in the full scan range *m/z* 100 – 1000.

A solid phase extraction (SPE) method for the separation of synthetic 2:1-PI and 2:1-OI compounds from crude oils was developed. When followed by HPLC/ESI-MS detection, this allowed the semi-quantitative but sensitive and specific determination of individual imidazolines at low (<10) parts per million concentrations in crude oils. Whilst non-optimised at present, the method is a significant advance and may prove useful for monitoring downhole and topside oilfield operations.

Although the synthesis of 2-methyl-2-imidazoline was reported over 100 years ago, the existence of reaction intermediates, 1:1-imidazoline products and the reaction pathways for the synthesis of 1:1/2:1-imidazolines by thermal reactions of diethylenetriamine (DETA) with fatty acids has been subject to debate ever since and is still not fully understood. It was shown herein that the thermal synthesis of 2:1-palmitic imidazoline proceeds *via* two different pathways depending on whether the reaction is carried out in the solid phase or in refluxing xylene solvent. Reaction intermediates and final products (1:1- and 2:1-palmitic imidazolines and mono and diamides) were identified and characterised by HPLC/ESI-MS and by MS^n. CID MS^n fragmentation pathways were determined for 1:1- and 2:1-palmitic imidazolines and 1,2- and 1,3-palmitic and oleic diamides.

The SPE and HPLC/ESI-MS techniques developed in this study should allow the environmental effects and fates of some of these polar compounds to be studied even more fully and a better understanding of the consequences of offshore discharges to be reached. In addition, it is clear that HPLC/ESI-MS^n should also allow yet more detailed studies of the reaction mechanisms of the industrial syntheses of some of the commercial products. This too may have consequences for the environment, since improvements in synthesis may lead to higher product purities.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

AA	Activation amplitude and Auto-integrated peak area
AC	Affinity chromatography
ACN	Acetonitrile
AcNA	Sodium acetate
AcOH	Acetic acid
AH	Automatic peak height detection
APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
BDMTDA	Benzyldimethyltetradecylammonium
BP	Base peak
CE	Capillary electrophoresis
CID	Collision induced dissociation
CRM	Charged residue model
Da	Dalton
DBSA	Dodecylbenzenesulfonate
DC	Direct current
DCM	Dichloromethane
DETA	Diethylenetriamine
DLI	Direct liquid introduction
EIC	Extracted ion chromatograph
ESI	Electrospray ionisation
EtOH	Ethanol
FAB	Fast atom bombardment
FT-ICR	Fourier transform ion cyclotron resonance
FT-IR	Fourier transform infrared spectroscopy
GC	Gas chromatography
HFBA	Heptafluorobutyric acid
HPLC	High performance liquid chromatography
HIC	Hydrophobic interaction chromatography
IEM	Ion evaporation model
IEC	Ion-exchange chromatography
IPA	Isopropanol (propan-2-ol)
IPC	Ion-pair chromatography
LAS	Linear alkylbenzenesulfonates

LC	Liquid chromatography
MeCN	Acetonitrile
MeOH	Methanol
MS	Mass spectrometry
MSDS	Material safety data sheet
MS^n	Multistage mass spectrometry (n = ≥2
m/z	Mass to charge ratio
NH_3	Ammonia
NMR	Nuclear magnetic resonance spectroscopy
NPLC	Normal phase liquid chromatography
OCs	Oilfield chemicals
OI	Oleic imidazoline
PB	Particle beam
PI	Palmitic imidazoline
PW	Produced Water
Q	Single quadrupole
QqQ	Triple quadrupole
Quats	Quaternary ammonium compounds
QIT	Quadrupole ion trap
RF	Radio frequency
R_f	Retardation factor
RPLC	Reverse phase liquid chromatography
R_t	Retention time
SEC	Size exclusion chromatography
SFC	Supercritical fluid chromatography
SIM	Selected ion monitoring
S/N	Signal to noise ratio
SPE	Solid-phase extraction
TFA	Trifluoroacetic acid
TIC	Total ion chromatograph
TOF	Time-of-flight
TOFA	Tall oil fatty acid
TSP	Thermospray
TLC	Thin layer chromatography
UV/vis	Ultraviolet/visible light range
ZS	ZoomScan™

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AUTHOR'S DECLARATION

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Publications:

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McCormack, P., Jones, P., Hetheridge, M.J. and Rowland, S.J. (2001) Analysis of oilfield produced waters and production chemicals by electrospray ionisation multi-stage mass spectrometry (ESI-MS^n). *Water Research* **35**, 3567-3578 (Appended).

Presentation and Conferences Attended:

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Signed .....

Date.....

"If we knew what it was we were doing, it would not be called research, would it?"

Albert Einstein

CHAPTER ONE

Introduction

1.1 Introduction

The qualitative and quantitative determination of highly polar or ionisable organic molecules in waters and other matrices is important for a wide range of environmental and industrial applications. An area of great interest is the investigation of products released into industrial and domestic effluents. Here, determination of breakdown products and toxicity and pollution assessments are typically required. Generally, target analytes are present at trace concentrations of $\mu g\ L^{-1}$ or less, often in the presence of large amounts of other organic and inorganic compounds. Analytes of interest may have a wide molecular mass range (<100 to at least 1000 Da) and contain one or more polar or ionisable groups often including hydroxyl, carboxylic, amino, phosphate/phosphonate, sulfate/sulfonates or heterocyclic moieties.

High efficiency extraction, preconcentration and chromatographic separation of such analytes may be difficult due to the hydrophilic nature of the compounds. When examined by traditional chromatographic methods such as high pressure liquid chromatography (HPLC), many polar compounds in complex mixtures elute at or close to the void volume, co-elute with interfering compounds, co-elute with homologous analytes or may have strong surface active properties in reverse phase HPLC systems. This is mainly attributable to the low capacity and selectivity or restricted pH ranges of most established chromatographic supports (bonded silicas). Potential methods of separation may also be limited by the incompatibility with detectors (e.g. liquid chromatography mass spectrometry (LC/MS) is not compatible with high ionic strength or non-volatile buffers, ion-pair reagents or solvent modifiers) or the analytes may not be detectable by the most widely used detectors, such ultraviolet/visible absorption spectrophotometry (UV/vis). A major problem with most detectors is the lack of specificity for the analysis of moderately

complex samples or the ability to obtain structural information for the identification of unknown compounds.

The introduction of commercial atmospheric pressure ionization mass spectrometers (API-MS: electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI)) from at least 1989 onwards[1] and the coupling of these spectrometers with HPLC systems (*viz*: LC/MS) has seen LC/MS evolve into a sensitive, rugged and widely used technique.[2-4] Of the two most commonly applied API techniques, electrospray ionization (ESI), is particularly suitable for highly polar and involatile compounds.[5] ESI as the ionization source for multistage mass spectrometers (MS/MS or MS^n n = >2) allows structural information to be obtained from molecular or pseudomolecular ions. A brief overview of LC/ESI-MS therefore follows. For illustrative purposes only, particular emphasis is given to the Finnigan Matt LCQ™ (Thermo Finnigan, San Jose, CA, USA) quadrupole ion trap mass spectrometer (QIT) which was used for the present study.

1.2 Coupling liquid chromatography to mass spectrometry

The coupling of liquid chromatography to mass spectrometry detectors with various interfaces has been attempted since at least the late 1960s.[6] For successful coupling of LC to a mass spectrometer, several problems needed to be overcome. The main problems were the need for removal of much of the HPLC mobile phase before entry of the eluents into the mass spectrometer and the requirement for formation of volatilized ions from low volatility and high polarity analytes. Amongst the first LC/MS interfaces were direct liquid introduction (DLI), moving belt/wire, thermospray (TSP) and particle beam (PB) devices. These have been described and reviewed.[6,7] More recently, APCI and ESI have proved to

be efficient and versatile methods of coupling LC to MS with various interface designs[1,3,4,7-9] and mass analyser types.[1,4,8,10,11] The APCI and ESI interfaces differ in the method of analyte ion formation, eluent flow rate compatibility and polarity of analyte/eluent optimisation.[2]

APCI was developed for LC-MS in the early 1970s by Horning and co-workers.[12-14] APCI is a gas-phase ionisation process initiated by a corona discharge and sustained by the LC mobile phase vapour which has been rapidly vaporized in a stream of coaxial nitrogen (along with the analytes) in a high temperature (350 – 500 °C) nebulizer.[2,6] APCI is compatible with high flow rates (> 0.5 mL min^{-1} with an optimum at ~1mL min^{-1}), molecular weights generally below 1000 Da with medium to low polarity, reverse and normal phase solvents. ESI has proven to be a successful interface due to its applicability to a wide range of compounds, including small highly polar compounds, low-polarity compounds that can be (de)protonated or which can form cation/anion adduct ions and high molecular weight biomolecules and polymers from which multiply charged ions may be produced.

1.3 Electrospray ionization mass spectrometry (ESI-MS)

In 2002, John B. Fenn received a share of the Nobel Prize in Chemistry for his pioneering work in electrospray ionisation[1].

[1] The prize was awarded jointly to John B. Fenn and Koichi Tanaka "*for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules*"

Electrospray ionization is a process by which gas-phase ions are formed from ions in solution by applying a high electrical potential to a capillary (containing the solution). Malcolm Dole and co-workers in the late 1960s and early 1970s were amongst the first to show that gas-phase ions from polystyrene macromolecules (and later from proteins) could be produced from an atmospheric electrostatic sprayer interfaced with an ion-drift spectrometer.[15] The coupling of electrospray ionization to mass spectrometers was reported in 1984 by Yamishita and Fenn[16-18] and Aleksandrov *et. al.*[8]. However, interest in ESI was greatly increased when Fenn's group in 1988[19,20] showed that proteins may carry multiple charges (2 to <100) and the distribution of multiply charged ions could be used to calculate accurate molecular weights. Since that time, LC/ESI-MS has developed rapidly in applications, interface designs, in MS instrumentation and in computer software for data analysis. The LC/ESI-MS literature is now extensive, with many reviews, special journal issues and books having been published recently.[3,4,11,16,21-24] The content of these excellent works are not repeated here. However, a brief explanation of the ESI process and use with QIT-MS is appropriate.

1.3.1 *Electrospray ionization process*

The formation of gas-phase ions from (de)protonated molecules, ammoniated, alkali metal adducts or anion/cation solution ions can be divided into three stages[25]: *viz*; charged droplet formation; droplet shrinkage and repeated disintegrations and finally gas-phase ion formation.

A high potential is applied to an electrospray capillary (± 3 – 5 kV) whilst the sampling orifice of the mass spectrometer (heated capillary on the LCQ™ instrument) is maintained at low voltage (± 0 – 50 V; LCQ™ instrument) to create a potential gradient (Figure 1.1). Due to the applied electric field, solution ions of the same polarity concentrate at the

capillary tip, which, due to the potential gradient, are drawn out of the capillary tip forming a 'Taylor cone'. Increasing concentration of ions of similar polarity at the liquid surface, increases coulombic forces that eventually overcome the surface tension of the liquid-emitting charged droplets. The charged droplets are drawn down the potential gradient (and vacuum gradient created by the sampling orifice of the high vacuum in the mass spectrometer) with solvent evaporation and shrinkage of the droplet increases the concentration of similar polarity ions at the surface. The coulombic forces again overcome the surface tension, causing droplet disintegration to smaller droplets which with repeated droplet disintegrations lead to very small highly charged droplets, which can produce gas-phase ions. The process is aided at increased flow rates by a coaxial flow of nitrogen (Figure 1.1) and is also known as "Ionspray" and was introduced by Bruins *et. al.*[26] The process of ionization is very soft and results in mostly pseudomolecular ions with very little, or no, fragmentation.

However, the actual mechanisms and processes are subject to much debate. There are two basic theories; the **Dole 'charged residue model' (CRM)**[15] and the **Iribarne and Thompson 'ion evaporation model' (IEM)**.[27,28]

In the **charged residue model,** the charged droplets continue to shrink and disintegrate until a single ion solvated droplet is formed, from which a gas-phase ion is produced on full evaporation. The **ion evaporation model** allows for gas-phase ions to be directly emitted from a very small highly charged droplet surface due to repulsion of charged ions at the droplet surface, although the eventual fate of the droplets is the same as in the charged residue model. As previously stated the mechanisms and processes are subject to much debate. Many reviews and reports detail current thinking in what is an incompletely understood process with many operating variables.[5,8,9,16,22,29-34]

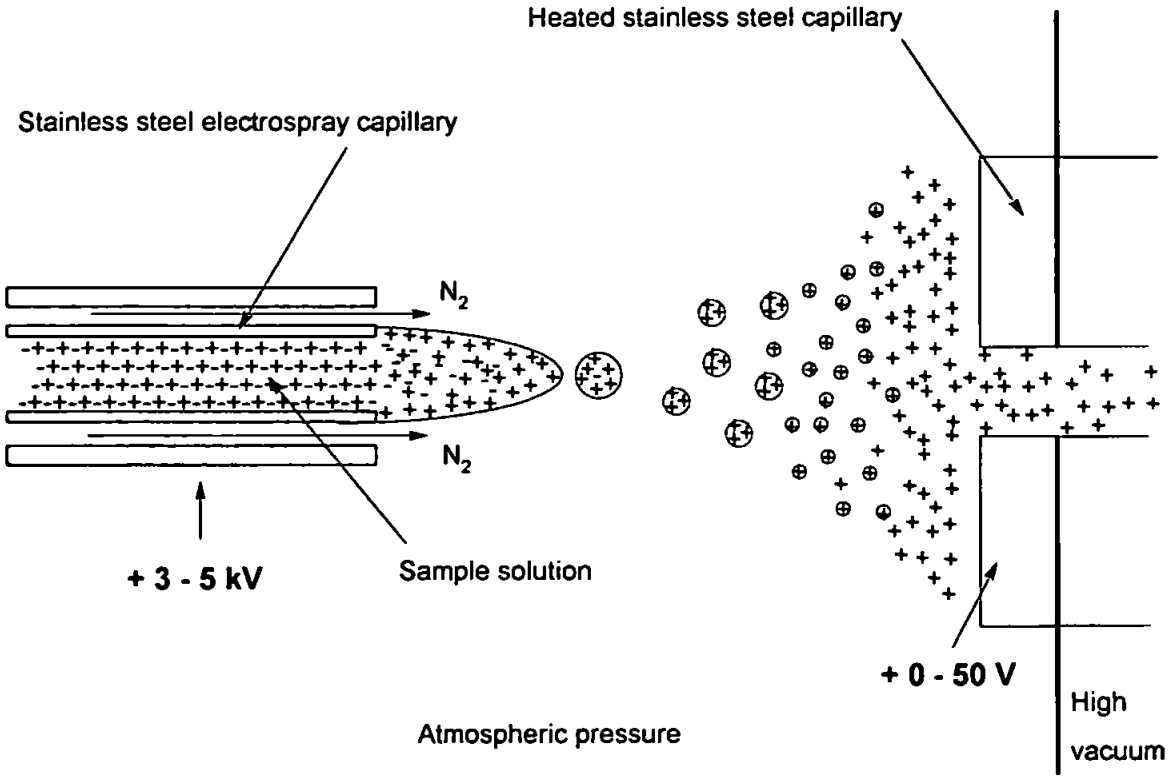


Figure 1.1 Simplified diagram of the Finnigan Matt LCQ™ electrospray ionisation source with droplet and gas phase ion production.

1.3.2 *Advantages and disadvantages of electrospray ionization*

The advantages and disadvantages of ESI interfaces for production of gas-phase ions are subjective and often somewhat dependent on whether an existing, non-MS based chromatographic method is being converted to a method based on LC with ESI-MS detection, or whether a new method is being developed for analytes that were not previously detectable by other methods. The following are a guide to some of the main advantages and disadvantages of ESI:

Advantages:

- Suitable for non-volatile, polar and thermally unstable compounds.
- ESI is sensitive (fmol or lower, dependent on analyte).

- Positive and negative ions can be analysed.
- Soft ionization produces mainly molecular or pseudomolecular ions allowing accurate molecular weights to be determined.
- Optimum chromatographic resolution is not required if analytes that co-elute have different *m/z* and do not cause ESI suppression of one or other analyte.
- Structural information can be determined from molecular or pseudomolecular ions by collision induced dissociation (CID) and multistage mass spectrometry (MS^n).
- Multiply charged ions can be produced, allowing ionised high molecular weight molecules to be within the mass to charge (*m/z*) range of most mass analysers.
- Method is compatible with flow rates from nL min^{-1} to 1-2 mL min^{-1}. (Flow rates over ~ 3 uL min^{-1} require coaxial N_2 nebulization whilst flow rates above ~ 50 uL min^{-1} require auxiliary N_2 nebulization systems (instrument dependent)).
- Method is concentration sensitive. Flow splitting of LC eluents prior to ESI interface therefore does not reduce sensitivity (excluding peak broadening effects), thereby allowing conventional chromatography columns (4.6 mm diameter) with 1 – 2 mL min^{-1} flow rates, to be used. The diverted eluent can be directed to other detectors for complementary data.
- ESI is, or can be made, compatible with many LC techniques such as: reverse phase (RPLC), normal phase (NPLC), supercritical fluid (SFC), size exclusion (SEC), ion-exchange (IEC), affinity (AC), ion-pair (IPC), hydrophobic interaction (HIC), capillary electrophoresis (CE) and other electro-chromatography techniques.

Disadvantages:

- ESI is not compatible with high ionic strength solutions or high concentrations of involatile buffers/mobile phase modifiers used in many traditional chromatographic methods. ESI normally requires modification of such methods to allow use of volatile reagents.

- Many traditional chromatographic phases and methods require the analyte to be non-ionised, whereas ESI requires the analyte to be ionised in solution. Post column addition of acid/base to eluent may be required to adjust solution pH.
- High purity solvents, reagents and chromatographic phases are required in order to reduce background chemical noise.
- Soft ionization produces mainly molecular or pseudomolecular ions. CID methods (in source or tandem instruments (MS/MS or MS^n)) are then required for obtention of structural information.

1.3.3 *Quadrupole ion trap mass spectrometer (QIT-MS)*

ESI has been interfaced with most mass analysers (single (Q) and triple (QqQ) quadrupole, quadrupole ion traps (QIT),[35,36] time-of-flight (TOF), sector and Fourier transform ion cyclotron resonance (FT-ICR) instruments [1,3,4,8,10,11] with Q, QqQ and QIT instruments being the most widespread. The present study was carried out on a Finnigan Matt LCQ™ QIT instrument for which, a detailed report on the fundamentals, operation, capabilities and applications is given by Bier and Schwartz.[37] Both Q/QqQ and QIT instruments use radio frequency (RF) and direct current (DC) voltages for transport, separation and scanning of ions. QqQ and QIT instruments also allow structural information to be obtained from collision induced dissociation (CID) of ions in tandem MS/MS experiments. However, there is a significant difference in the method of performing operations on the ions between QqQ and QIT instruments.

In triple quadrupole instruments, all ions from the ESI source continuously pass into the first quadrupole lens (Q1), and RF and DC voltages are varied to allow ions of a specific *m/z* to pass into the next quadrupole (Q2; or straight through to the detector) where a function on the ions is carried out (*i.e.* CID experiment). The third quadrupole scans the

resultant or selected ions to the detector. Therefore, each operation on the ion beam is separated in space (*i.e.* Q1, Q2 or Q3). In a QIT, the ion beam is allowed to enter the ion trap (ion injection) for a given amount of time, were the ions are stored (ion isolation), then allowed to exit (ion ejection) by increasing *m/z* to the detector. Operations on the ions, such as CID MS^n are carried out on the isolated ions over a period of time, within the same analyser. It is the inherent ability of the QIT to isolate ions for a period of time that allows CID MS^n experiments where $n \geq 2$, and detailed structural information on precursor ions to be obtained.

1.4 The present study

Large quantities of diverse polar organic chemicals are routinely discharged from oil production platforms in so-called Produced Water (PW). The detection and environmental fate of many of these is unknown since few methods exist for their characterisation. At the inception of the present study it was proposed that ESI-(QIT)MS^n had attributes that could be compatible for the detection and characterisation of a least some of these chemicals.

An investigation was therefore carried out with the following aims:

I. To investigate the use of ESI-MS^n and determine its potential for the identification, characterisation of compounds in speciality oilfield chemicals (corrosion inhibitors, scale inhibitors, biocides and demulsifiers).

II. Using appropriate model compounds, to investigate and develop compatible LC methods and chromatographic phases for extraction, separation and quantification of compounds in speciality oilfield chemicals from environmental matrixes.

III. To combine the developed extraction, separation and detection techniques for the qualitative and quantitative analysis of environmental samples.

Chapter 1 has briefly reviewed the development of ESI-MS as a detection method for HPLC.

Chapter 2 describes preliminary investigations into the use of multi-stage electrospray ionisation ion trap mass spectrometry (ESI-(QIT)MS^n for the identification of compounds in speciality oilfield chemicals (corrosion inhibitors, scale inhibitors, biocides and demulsifiers) and oilfield Produced Water. Multiple stage mass spectrometry (MS^n) with both positive and negative ion detection allowed high specificity detection and characterisation of a wide range of polar and charged molecules. Linear alkylbenzenesulfonates (LAS), alkyldimethylbenzylammonium compounds, [2-(2-alkyl-4,5-dihydro-1*H*-imidazol-1-yl)ethyl]amine (1:1-Imidazolines), [2-(2-alkyl-4,5-dihydro-1*H*-imidazol-1-yl)ethyl]alkylamide (2:1-Imidazolines) and a di-[alkyldimethylammonium-ethyl]ether were all identified and characterised in commercial formulations and/or North Sea oilfield Produced Water.

Chapter 3 describes the development of solid phase extraction (SPE) procedures for initial separation of imidazoline/amide oilfield corrosion inhibitors from crude oils prior to examination by HPLC/ESI-MS.

Chapter 4 describes the development of a high performance liquid chromatography (HPLC) separation of commercial and synthesised alkyl-imidazolines and amides with detection by electrospray ionisation mass spectrometry (ESI-MS). Model palmitic and oleic acid derived 2:1-imidazolines and 1:1-imidazolines compounds were synthesised.

The application of the developing HPLC/ESI method was also shown to be a powerful technique for the investigation of imidazoline synthesis reaction intermediates.

Chapter 5 describes the investigation of the reaction intermediates and pathways of solid phase and xylene solvent-based thermal reactions of palmitic acid with diethylenetriamine (DETA). The reaction products were studied by high performance liquid chromatography (HPLC) separation with electrospray ionisation mass spectrometry (ESI-MS) detection and structural characterisation was based on electrospray ionisation multistage mass spectrometry ($ESI\text{-}MS^n$), Fourier transform infra red (FT-IR) and ^1H nuclear magnetic resonance (^1H-NMR) spectroscopy.

Chapter 6 summarises the conclusions from the present study and proposes suggested further work that may be carried out.

CHAPTER TWO

Analysis of Oilfield Produced Water and Production Chemicals by Electrospray Ionization Multi-Stage Ion Trap Mass Spectrometry (ESI-MS^n)

This chapter describes preliminary investigations into the use of electrospray ionisation multi-stage ion trap mass spectrometry (ESI-MS^n) for the detection and characterisation of speciality oilfield chemical components in commercial products and in oil platform Produced Water.

2.1 Introduction

When crude oil is produced from offshore oilfields it is associated with varying proportions of water called Formation Waters.[38] In the early stages of oil production, the water content is usually low, whereas later the proportions can rise to as high as eighty percent.[39] Once the water has been separated from the oil it is known as Produced Water (PW) and this is discharged into the sea.[40,41] PW discharges on the UK continental shelf rose from about 153 million tonnes in 1991 to 244 million tonnes in 2000.[42]

The oil recovery, production and separation processes involve addition of a diverse mixture of oilfield chemicals (OCs) to the oil-water mixtures. For example, these chemical mixtures are formulated to act as scale inhibitors to prevent mineral scale deposition blocking pipework, as corrosion inhibitors to prevent pipe work from attack by the salt water and dissolved gases, as biocides to prevent bacterial degradation of the oil and other products, and as emulsion breakers to facilitate oil-water separation.[41,43-45] Some or all of these OCs may be discharged to the marine environment along with the PW.[46-50] In the North Sea an estimated 5934 tonnes of OCs was discharged in 1989 along with an estimated 84097 tonnes of drilling chemicals[44] whilst in 2003 an estimated 13,790 tonnes of OCs were used and 106,592 tonnes discharged.[51]

Although the syntheses of the individual components of the formulated oilfield chemicals rely substantially on well-known chemical reactions, the products from individual companies may differ due to blending in order to achieve different activities and functions. Many of the organic chemicals in the OCs are polar, hydrophilic compounds, which are not amenable to routine analysis.[43,52-54] Establishment of such methods is, therefore, important to the OC manufacturers, to the oilfield operators and to scientists concerned with the fate and effects, if any, of PW and OCs in the marine environment.[43,50,55,56]

Previously, fast atom bombardment–mass spectrometry (FAB-MS) has been used to good effect to partially characterise OCs in PW[39] and other spectroscopic methods have been used in attempts to characterise oilfield chemicals such as imidazoline corrosion inhibitors.[54] Recently, electrospray ionisation mass spectrometry (ESI-MS) has also proved to be a powerful method for the identification of some constituents of OCs.[43,52,53]

The following study was carried out to determine if active compounds in a selection of commercial speciality oilfield chemicals, known to be used in North Sea operations could be detected, identified and characterised in the products and in extracts of North Sea PW by ESI-MS^n.

2.2 Experimental

2.2.1 *Chemicals*

Ultra pure water was obtained from an Elgastat (Elga, High Wycombe, UK) filtration system. All solvents used were of HPLC grade. Methanol (MeOH) and acetonitrile (ACN) were obtained from BDH (HyPersolv grade, Poole, Dorset, UK), and dichloromethane (DCM) from Rathburn Chemicals Ltd (Walkerburn, UK.). Sodium dodecylbenzenesulfonate (DBSA) was purchased from Aldrich (Poole, Dorset, UK.) and benzyldimethyltetradecylammonium chloride from Fluka (Buchs SG, SW).

PW samples were supplied by various North Sea oilfield operators and speciality OCs by various manufacturers.

2.2.2 *Electrospray ionization mass spectrometry (ESI-MS^n)*

Mass spectrometry analysis was carried out using a Finnigan Mat LCQ™ (ThermoFinnigan San Jose, CA, USA.) bench top mass spectrometer fitted with an electrospray interface. Data were acquired and processed with Navigator 1.1 software for the PW and DBSA samples with a full scan range of *m/z* 50-1850. All other samples were acquired and processed with Xcalibur 1.0 sp1 software in the normal full scan range (*m/z* 50-2000) following an instrument hard/software upgrade. Instrument tuning and mass calibration were carried out and checked using the automatic calibration procedure (tuning and calibration solutions caffeine (Sigma, St. Louis, MO, USA), MRFA (Finnigan Mat, San Jose, CA, USA) and Ultramark 1621 (Lancaster Synthesis Inc., Widham, NH, USA) in methanol:water:acetic acid (50:50:1, v/v/v)). Infusion of PW extracts and OC solutions were carried out using a built in syringe pump with a Hamilton 1725N (250 μL) syringe (Reno CA, USA.). Analytes were infused at 3 μL min^{-1}. Source voltage, (±) 4.5 kV; capillary voltage (±) 0-50 V (auto tune function on ion of interest); capillary temperature, 200 °C; nitrogen sheath gas flow rate, 40 (arbitrary units). Positive and negative ion (±) MS^n analysis of selected ions was performed in the ion trap by collision induced dissociation (CID) with helium damping gas. MS^n ion isolation widths, relative activation amplitudes and activation Qs were optimised to obtain high response and stability of the base peak fragment ion. High-resolution ZoomScan™ (ZS) was recorded for all ions of interest. All spectral data was recorded and averaged over a one-minute acquisition time.

2.2.3 *Produced Water (PW)*

PW samples were extracted with DCM (~50 mL). One extract (PW60) was blown to dryness with nitrogen and the residue re-dissolved in 500 μl methanol and 250 μl Ultra pure water (PW60MW). A DCM extract from a different PW (PW90) was reduced to ~ 1 mL using a Kuderna Danish controlled evaporation and the remaining extract was divided into two portions (~ 0.5 mL) in pre-weighed vials and blown down to dryness with

nitrogen. The dry residues were re-dissolved in methanol (1 mL; PW90M) and acetonitrile (1 mL; PW90A). A further 900 μL ACN was added to 100 μL of PW90A for analysis.

2.2.4 *Stock solutions of standard compounds*

Dodecylbenzenesulfonic acid (DBSA) sodium salt (50 mg) was prepared by dissolution in Ultra pure water and made up to 50 mL. Benzyldimethyltetradecylammonium (BDMTDA) chloride (50 mg) was made up to 50 mL with methanol. Dilutions of the stock were made using the required solvents.

2.2.5 *Oilfield chemicals (OCs)*

Stock solutions of commercial products were prepared as follows: Corrosion inhibitor CI-D2 (50 μL) was diluted to 50 mL with MeOH:H_2O (1:1, v/v); Corrosion inhibitor CI-A3 (25 mg) was made up to 50 mL with MeOH; Corrosion inhibitor CI-B1 (116 mg) was made up to 100 mL with MeOH; Corrosion inhibitor CI-C3 (100 μL) was made up to 10 mL with MeOH; Demulsifier DM-C2 (25 mg) was made up to 50 mL with MeOH. Dilutions of the stock solutions were made using the required solvents.

2.3 Results and discussion

2.3.1 *Produced Water*

Discharged PW is a complex mixture of liquid and particulates and comprises inorganic and organic compounds of natural origin, applied production chemicals (OCs), and residues of other platform effluents and deck washes. For an initial evaluation of ESI-MS^n as an analytical technique for PW analysis, DCM extracts were used to obtain polar organic fractions. After removal of DCM, the extracts were re-dissolved in various

solvents (*e.g.* methanol, acetonitrile or methanol/water) as it is well known that electrospray ionisation of compounds varies for different solvents. Negative and positive ion full scan spectra (± *m/z* 50 - 1850), ZS and CID MS^n spectra of significant ions were acquired in order to provide data that could be used to identify the unknown extracted compounds.

In general, negative ion spectra of PW extracts were relatively simple with low baseline noise. Ions at *m/z* 297, 311, 325 and 339 were the only significant ions in extracts dissolved in methanol/water and methanol (not shown). The spectrum of an extract dissolved in acetonitrile (Figure 2.1A) also contained these ions but in addition a series of ions differing by *m/z* 14 in three clusters were apparent at *m/z* 423, 529 and 635. Compounds that correspond to ions in the clusters were not identified but CID MS^n analysis of the ions at *m/z* 297, 311, 325 and 339 (base peak) indicated a common structure differing only by 14 Da. Thus, CID MS^2 of precursor ions *m/z* 297, 311, 325 and 339 produced neutral fragment losses to give a common product ion *m/z* 183 in each case (*e.g.* Figure 2.2A). CID MS^3 of the *m/z* 183 product ion in each case produced a fragment ion *m/z* 119, representing loss of a neutral 64 Da fragment (Figure 2.2B). Further fragmentation (MS^4) of the *m/z* 119 ions produced no detectable ions.

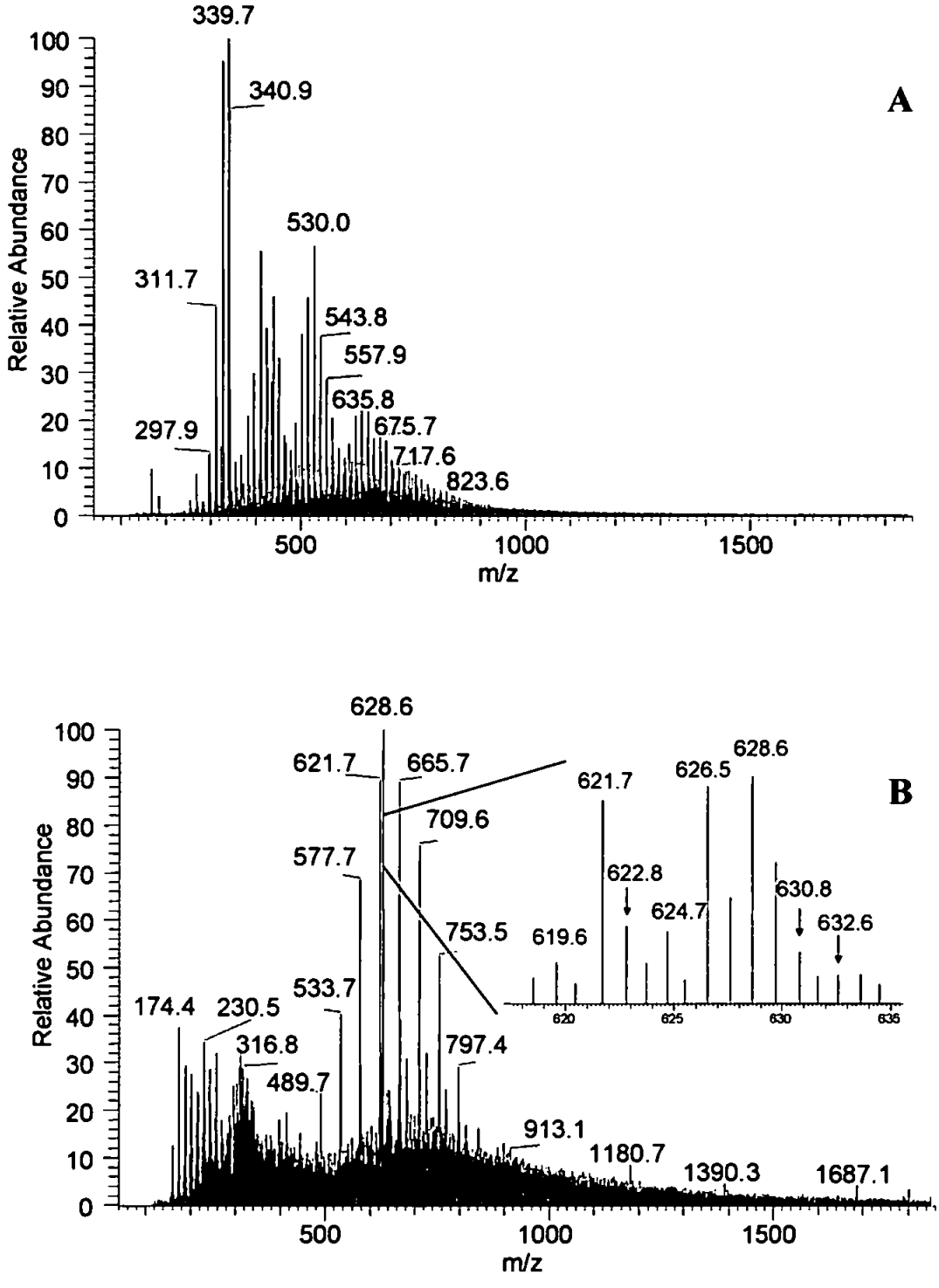


Figure 2.1 Negative (A) and Positive (B) ion ESI-MS mass spectra of Produced Water extracts: A) ACN solvent and B) MeOH solvent.

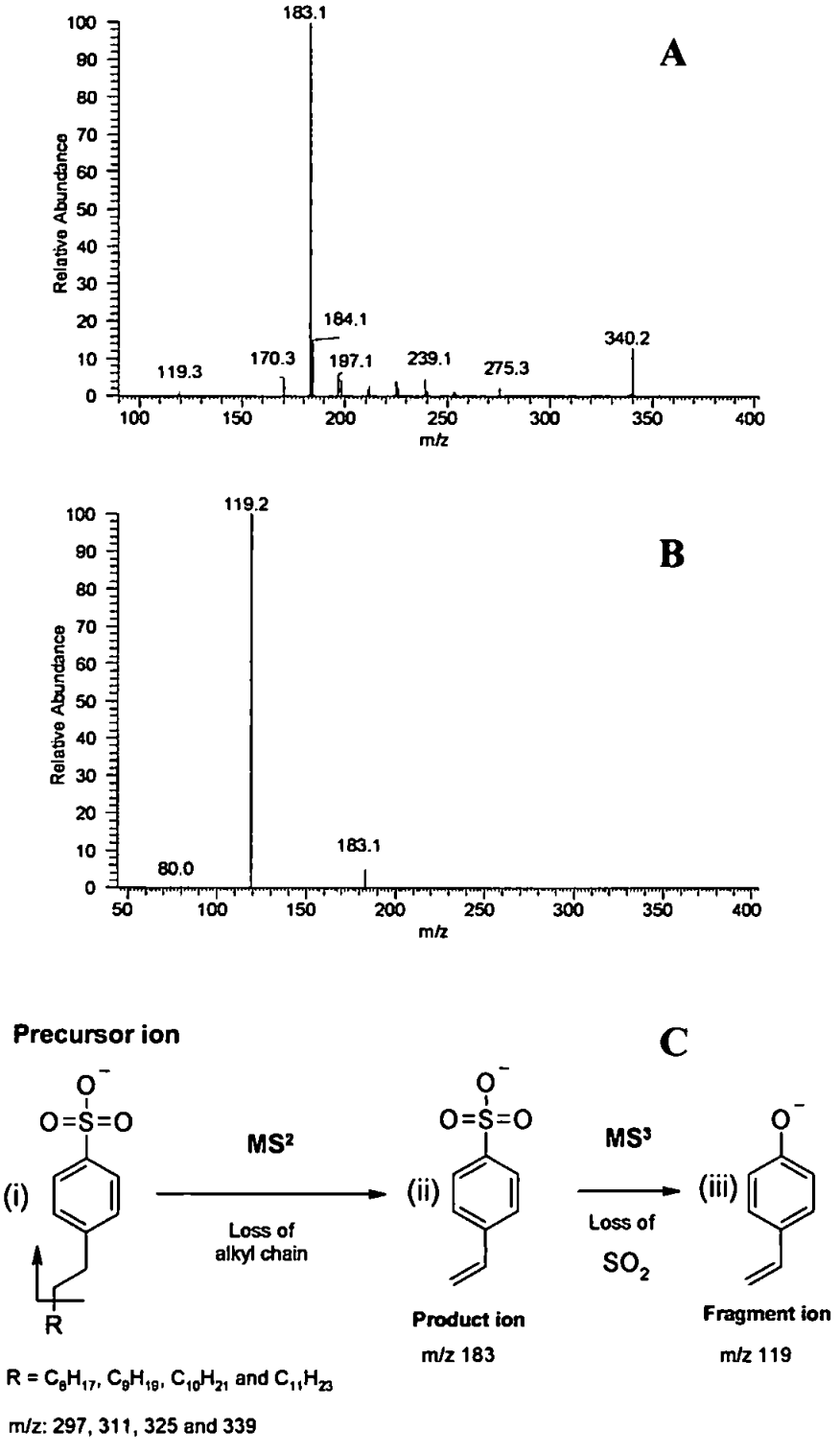


Figure 2.2 Negative ion ESI-MS^n spectra of precursor ion *m/z* 339 shown in Figure 2.1A for Produced Water and CID MS^n fragmentation pathway of alkylbenzenesulfonates: A) MS^2 spectrum of the precursor ion *m/z* 339; B) MS^3 spectrum of the *m/z* 183 product ion and C) CID MS^n fragmentation pathway of alkylbenzenesulfonates. MS^2 cleavage of the alkyl chain is at the same position for all homologues producing identical product ions.

The precursor ions *m/z* 297, 311 325, 339 and the CID MS^2 product ion *m/z* 183 are consistent with those expected of linear alkylbenzenesulfonates (LAS) (Figure 2.2C, (i))[57-63]. The MS^3 product ion *m/z* 119 produced from CID of the *m/z* 183 product ion *via* a neutral loss of 64 Da is consistent with loss of sulfur dioxide (SO_2) from an ethylenebenzenesulfonate ion (Figure 2.2C, (ii)) to yield a ethylenephenoxy ion (Figure 2.2C, (iii)). The fragmentation pathway shown in Figure 2.2C could be deduced for the MS^3 CID of LAS. To the author's knowledge the MS^3 *m/z* 119 ion produced by loss of SO_2 from the CID of the MS^2 *m/z* 183 product ion has not been attributed previously. In order to confirm that the peaks were due to alkylbenzenesulfonates, a commercial sample of sodium dodecylbenzenesulfonate was also examined. The negative ion full scan spectrum (not shown) contained the same four main ions (*m/z* 297, 311, 325 and 339), whilst MS^n produced identical product ions from each precursor ion to those shown in the PW components. The negative ion mass spectrum of an oilfield demulsifier DM-C2 (Figure 2.3A) also contained ions at *m/z* values consistent with the presence of alkylbenzenesulfonates. MS^n analysis shows fragmentations identical to the sodium dodecylbenzenesulfonate. Thus, ESI-MS^n allows unambiguous identification of linear alkylbenzenesulfonates in both PW and OCs.

The positive ion spectra of the PW extracts were more complex than the negative ion spectra and the spectrum of the extract dissolved in methanol was very 'noisy'. This is expected, as the ionisation efficiency of many compounds is greater in methanol/water mixtures compared with pure methanol. The spectra of extracts dissolved in methanol (Figure 2.1B) and acetonitrile were very similar with a base peak ion at *m/z* 628 with a distribution of ions differing by 2 Da. This is consistent with the presence of two homologous series of compounds, one containing a further degree of unsaturation. The spectra are consistent with a diamidoamine synthesised from Tall Oil Fatty Acids (TOFA) and diethylenetriamine (DETA).[52] Such amides are commonly produced during the

manufacture of imidazoline corrosion inhibitors. Also imidazoline compounds readily hydrolyse to their amide precursor compounds on exposure to air[64] and might therefore be produced in PW from hydrolysis of an imidazoline-based product (*vide infra*, below corrosion inhibitor CI-A3). Ions at *m/z* 489.7, 533.7, 577.7, etc, differing by 44 Da are tentatively attributed to polyethoxylated compounds, typical of those found in commercial de-emulsifiers (*vide infra*).

2.3.2 *Oilfield Chemicals (OCs)*

A wide range of OCs are used in offshore oil and gas production. Most are complex mixtures derived from impure raw materials.[65] Furthermore, many commercial products are blends of two or more chemical types. For reasons of commercial confidentiality, the specific chemicals and quantities contained in oilfield products are not generally made public and only the legally required health and safety data are normally specified on Material Safety Data Sheets (MSDS). The information given regarding the active chemicals is generally restricted to the class of compounds (*e.g.* amines, quaternary amines, imidazolines, polycarboxylates, phosphonates) and the solvents (aqueous, methanol or aromatic solvents). Without detailed knowledge of the OC compositions or the availability of suitable analytical methods, it is difficult for the fate of OCs in operational processes or in environmental scenarios to be followed. ESI-MS^n methods begin to allow such knowledge to be assembled, particularly if the mass spectra can be interpreted to identify unknown constituents, as illustrated in the following experiments in which samples of commercial OCs were dissolved and diluted in appropriate solvents and analysed by direct infusion ESI-MS^n.

2.3.2.1 Corrosion inhibitor CI-D2

CI-D2 is a commercial oilfield corrosion inhibitor stated (MSDS) to contain fatty amine quaternary salts (10-30%) in water and methanol (10-30%). A positive ESI full scan mass

spectrum (Figure 2.4A) of CI-D2 dissolved in methanol:water (1:1, v/v) solvent, showed three distinctive odd *m/z* ion series. Ions in the most intense series centred on *m/z* 249 differed from each other by *m/z* 14, whilst those in the series around *m/z* 533 and 1131 differed by *m/z* 28. These differences between the series were elucidated by the use of the so-called 'ZoomScan' (ZS) facility on individual ions in each series (ZS allows resolution of isotope peaks for up to +4 charge-state ions). Thus, pairs of ions in the series at *m/z* 221.4 - 305.4 (*e.g. m/z* 249.3 and 249.7) showed mass differences of 0.4 Da (Figure 2.4B). The *m/z* 249.4/249.7 ion ratio was consistent with that expected for $^{12}C/\ ^{13}C$ isotopes. There is no evidence for the presence of chlorine or bromine. The 0.4 *m/z* differences suggest the *m/z* 221.4-305.4 ions are doubly charged ions (*m/z* = $M^{2+}/2$). By contrast, ZS analysis of the ion series *m/z* 477.5-645.5 shows differences of *m/z* 1.0 between ion clusters (*e.g. m/z* 533.4/ 534.4; Figure 2.4C) consistent with singly charged ions (*m/z* = $M^{+}/1$). However, whilst the *m/z* 533/534 ratio is also consistent with that expected for $^{12}C/\ ^{13}C$ isotopes, the 3:1 ratio of *m/z* 533 and 535 is also indicative of the presence of a chlorine atom (viz: $^{35}Cl/^{37}Cl$). An odd *m/z* value for the ions indicates that an even number of nitrogen atoms are present. As fatty amine quaternary salts are expected from the MSDS description, identification of the compound as a di-quaternary salt is consistent with all the spectral data (viz: ion series at *m/z* 221-305 due to M^{2+}; *m/z* 533 $[M^{2+} + Cl^{-}]^{+}$ and *m/z* 1131 $[2M^{2+} + 3Cl^{-}]^{+}$).

In an attempt to identify the chemical more completely, MS^n analysis of the ions observed in the full scan spectrum was carried out. Highly reproducible product ion spectra were obtained for ions attributed to $M^{2+}$and $[M^{2+} + Cl^{-}]^{+}$ with up to five CID fragmentation steps (MS^5; Figure 2.5). A fragmentation pathway and a molecular structure for the precursor ion at *m/z* 533.5, consistent with the MS^n data (Figure 2.5A-D) are shown in Figure 2.6. The structure is consistent with known synthetic routes to *bis*-quaternary ammonium compounds, involving reaction of β-dichloroethylether with alkyl dimethylamine.[66] Thus,

if the fatty amine was derived from a coco or palm oil source (*cf* Gough and Langley[52]) then all the possible ions resulting from combinations of $C_{8,10,12,14,16,18}$ alkyl groups can be identified in the mass spectrum (Table 2.1).

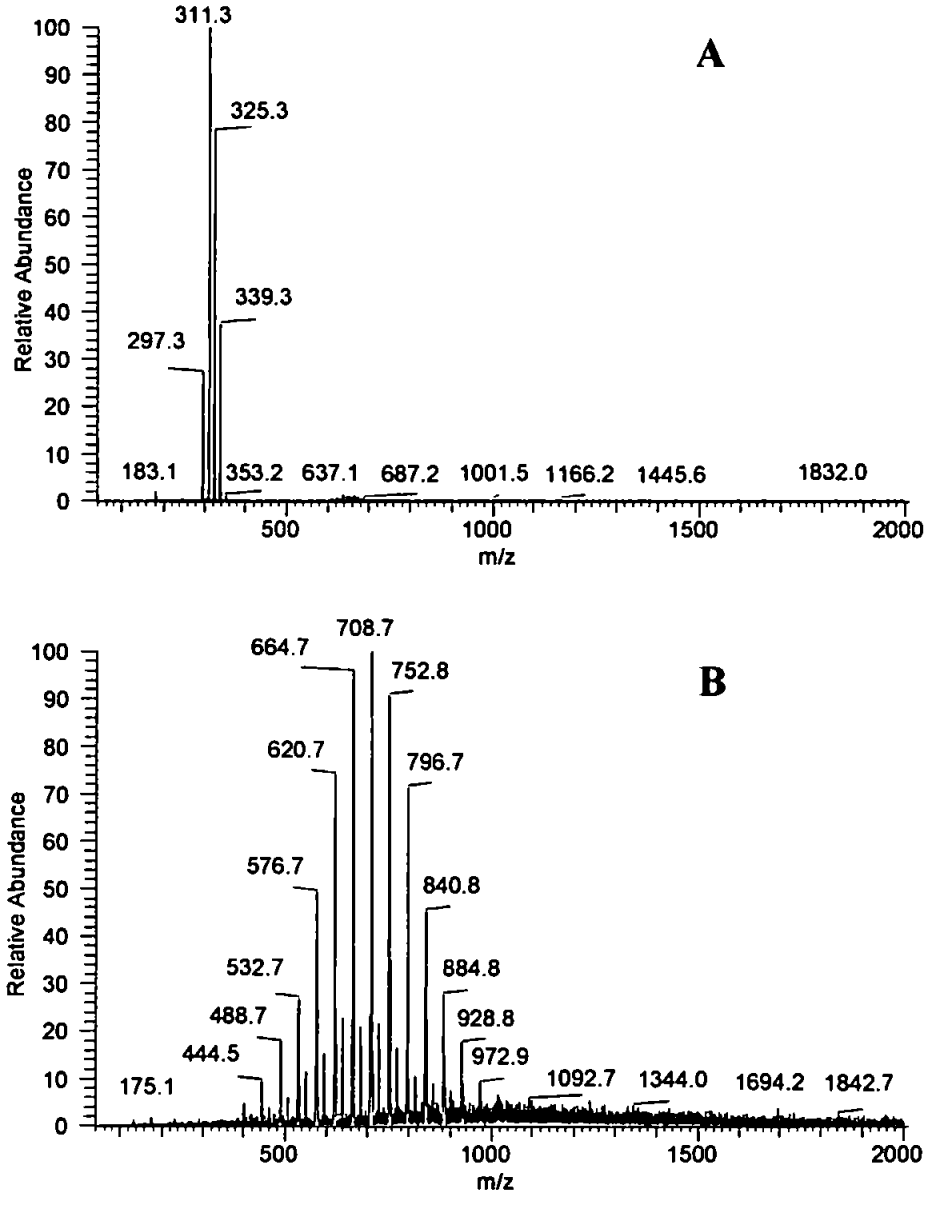


Figure 2.3 Negative (A) and positive (B) ion ESI-MS mass spectra of DM-C2 oilfield demulsifier (7:3 v/v MeOH:H_2O).

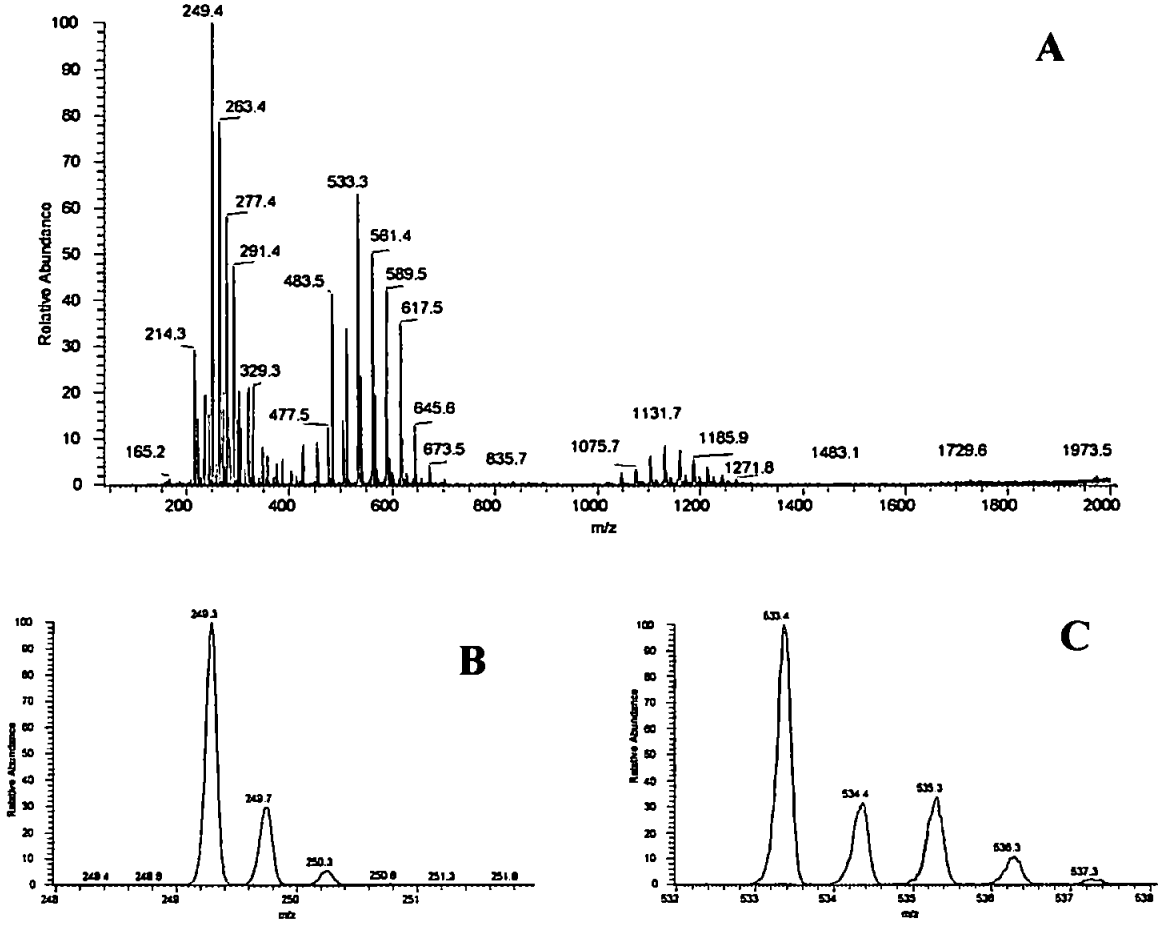


Figure 2.4 Positive ion ESI-MS mass spectra of CI-D2 oilfield corrosion inhibitor (1:1 v/v MeOH:H_2O): A) Full scan *m/z* 50 – 2000; B) ZoomScan *m/z* 249.4 and C) ZoomScan *m/z* 533.4.

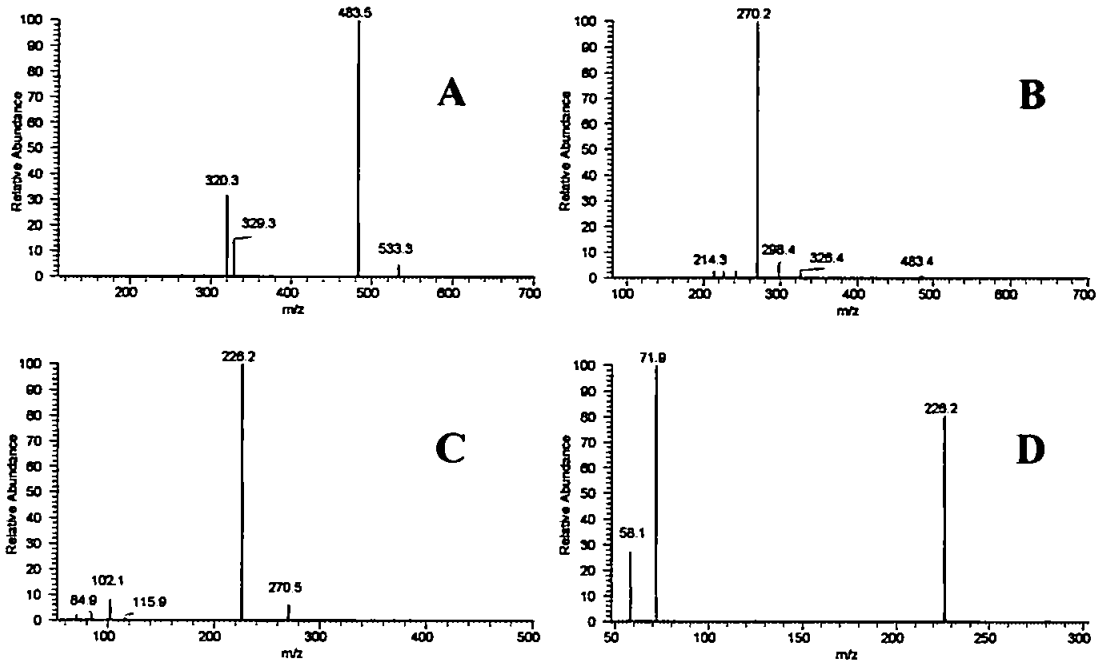


Figure 2.5 Positive ion ESI-MS^n mass spectra of precursor ion *m/z* 533.4 in spectrum Figure 2.4 for CI-D2 oilfield corrosion inhibitor: A) MS^2 on precursor ion *m/z* 533.4; B) MS^3 on product ion *m/z* 483.4; C) MS^4 on product ion *m/z* 270.2 and D) MS^5 on product ion *m/z* 226.2.

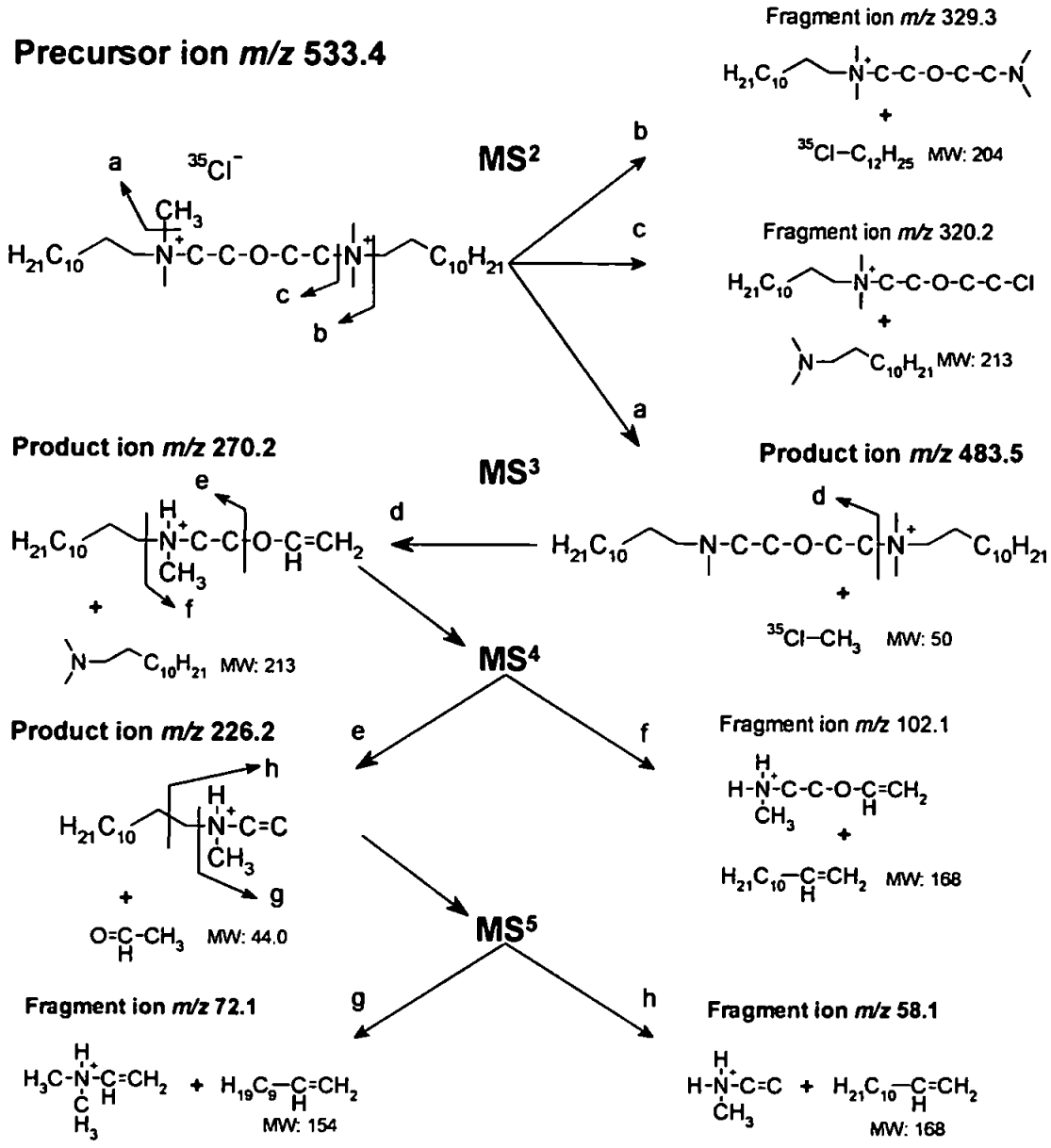
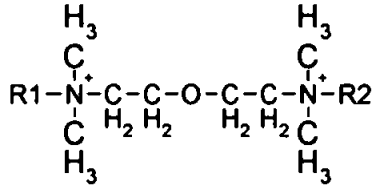


Figure 2.6 Positive ions CID ESI-MS^n fragmentation pathway of precursor ion *m/z* 533.4 in spectrum Figure 2.4A and MS^n spectra Figure 2.5A-D for CI-D2 oilfield corrosion inhibitor.

Table 2.1 Isotopic Masses for $[M^{2+} + {}^{35}Cl^{-}]^{+}$ where R1 and R2 are alkyl moieties derived from coco or palm oils.



R1/R2	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}
C_8	421.4	449.4	477.5	505.5	533.5	561.5
C_{10}	449.4	477.5	505.5	533.5	561.5	589.6
C_{12}	477.5	505.5	533.5	561.5	589.6	617.6
C_{14}	505.5	533.5	561.5	589.6	617.6	645.6
C_{16}	533.5	561.5	589.6	617.6	645.6	673.7
C_{18}	561.5	589.6	617.6	645.6	673.7	701.7

2.3.2.2 Corrosion inhibitors CI-C3 and CI-B1

CI-C3 and CI-B1 are commercial oilfield corrosion inhibitors stated (MSDS) to comprise: ethoxylated amines and quaternary compounds (5-10%), butyl glycol (20-30%) and monoethylene glycol (20-30%) in water (CI-C3) and benzyl chloride quat amine (5-10%) and methanol (1-5%) (CI-B1). Positive ion ESI-MS spectra suggest that both formulations contain quaternary amines corresponding to alkylbenzyldimethylammonium (benzalkonium) compounds (Figures 2.7A and B). The spectra show that the alkyl chain source is different for each product, with CI-C3 having a C_{12} (base peak), C_{14}, C_{16} and C_{18} distribution whilst CI-B1 has only two ions for C_{16} and C_{18} (base peak). This has been shown previously by ESI-MS by Gough and Langley.[52] The more powerful multi-stage MS^n analysis of the benzalkonium ions produced highly reproducible fragmentations with

masses differing only by the alkyl chain lengths. Thus, CID MS^2 of the benzalkonium precursor ion produced two ions (Figure 2.8A) with the base peak *m/z* corresponding to a neutral loss of 92.0 Da, consistent with fragmentation of the benzyl moiety with a proton migration to effect loss of a methylbenzene and yield an iminium ion (Figure 2.8C). The product ion at *m/z* 91.0 is consistent with a benzyl cation (tropylium ion) commonly seen in electron ionisation-MS of aromatic compounds, and a neutral loss of alkyldimethylamine (Figure 2.8C). Both of the MS^2 fragment ions result from cleavage of the same nitrogen-benzyl bond. CID MS^3 of the MS^2 iminium ion (Figure 2.8B) results in loss of an alkene molecule *via* a 1,5 proton shift M^{c}Lafferty rearrangement[67] to yield a *m/z* 58 iminium ion (Figure 2.8C). MS^n analysis of authentic benzyldimethyltetradecylammonium chloride (BDMTDACl) produced identical fragmentation spectra to that for the benzalkonium ions in the corrosion inhibitors. Following the publication of these results[68] a report by Ferrer and Furlong[69] showed similar results for CID MS^2 fragmentation of C_{12} and C_{14} dimethylbenzylammonium ions although MS^3 fragmentations were not carried out. The reproducibility of the CID MS^n spectra allows unambiguous identification of the benzalkonium ions.

Corrosion inhibitor CI-C3 also contained ethoxylated amines (MSDS). Ions due to these are also seen in the positive ESI-MS spectrum (Figure 2.7A) as at least three series of ions differing by 44 Da (*viz*: corresponding to $-C_2H_4O-$) between *m/z* 500 - 1300. Although the MSDS for corrosion inhibitor CI-B1 does not state that it contains ethoxylated compounds there are clearly several series of ions differing by 44 Da in the spectrum and some have similar *m/z* values to those seen in CI-C3 and in the demulsifier DM-C2 (Figure 2.3B). The even *m/z* ions infer an odd number of nitrogen atoms in the compounds and the presence of several series suggests that both CI-B1 and DM-C2 also contain ethoxylated amines. Series differing by *m/z* 44 are also observed in positive ion ESI-MS spectra of the Produced Water (e.g. Figure 2.1B), suggesting these demulsifiers may be discharged into the

environment. However in the PW the ethoxylated compounds differed by 1 Da (reliably measured by ZS) from those in DM-C2 and CI-C3.

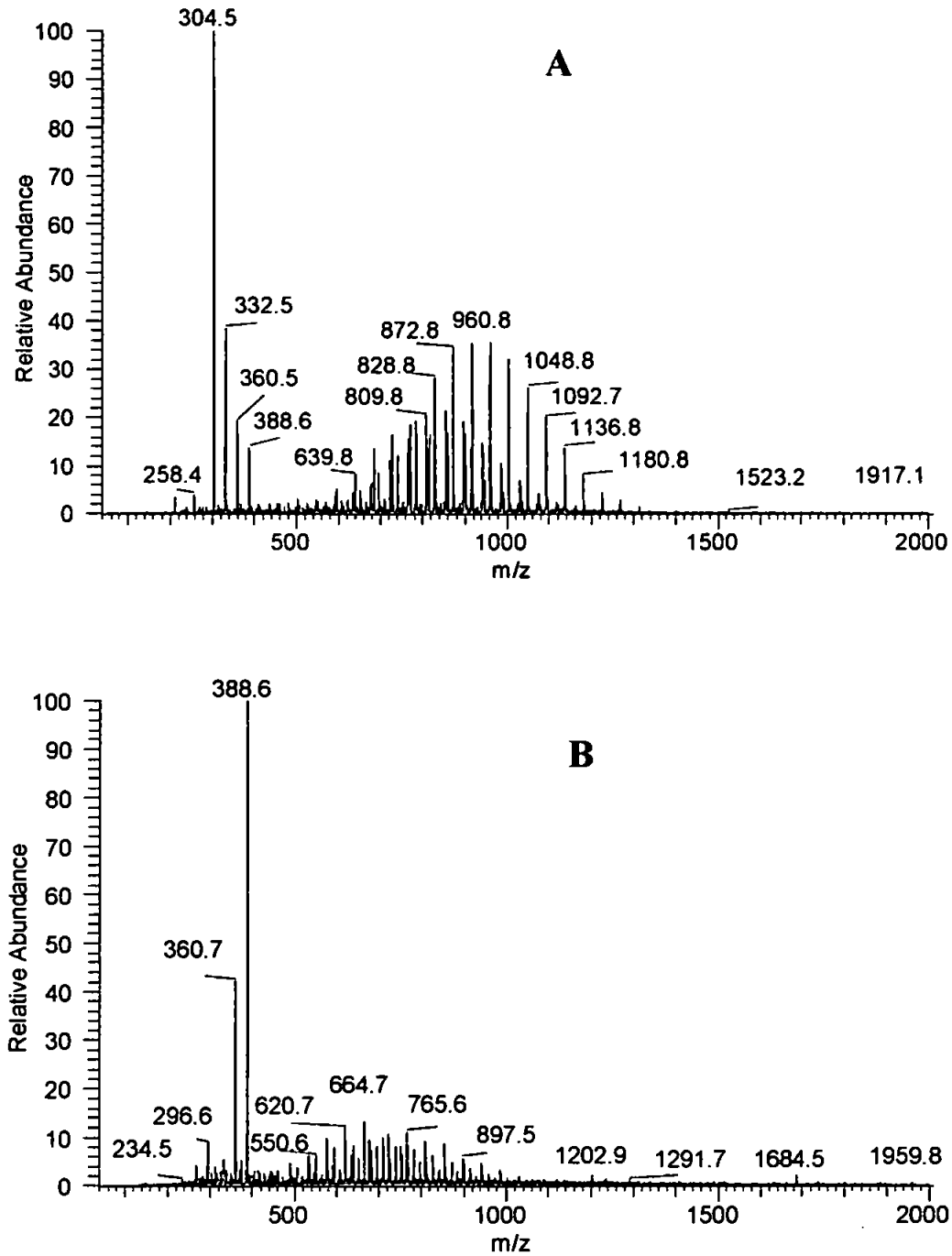


Figure 2.7 Positive ion ESI mass spectra of oilfield corrosion inhibitors A) CI-C3 and B) CI-B1 (1:1 v/v MeOH:H_2O).

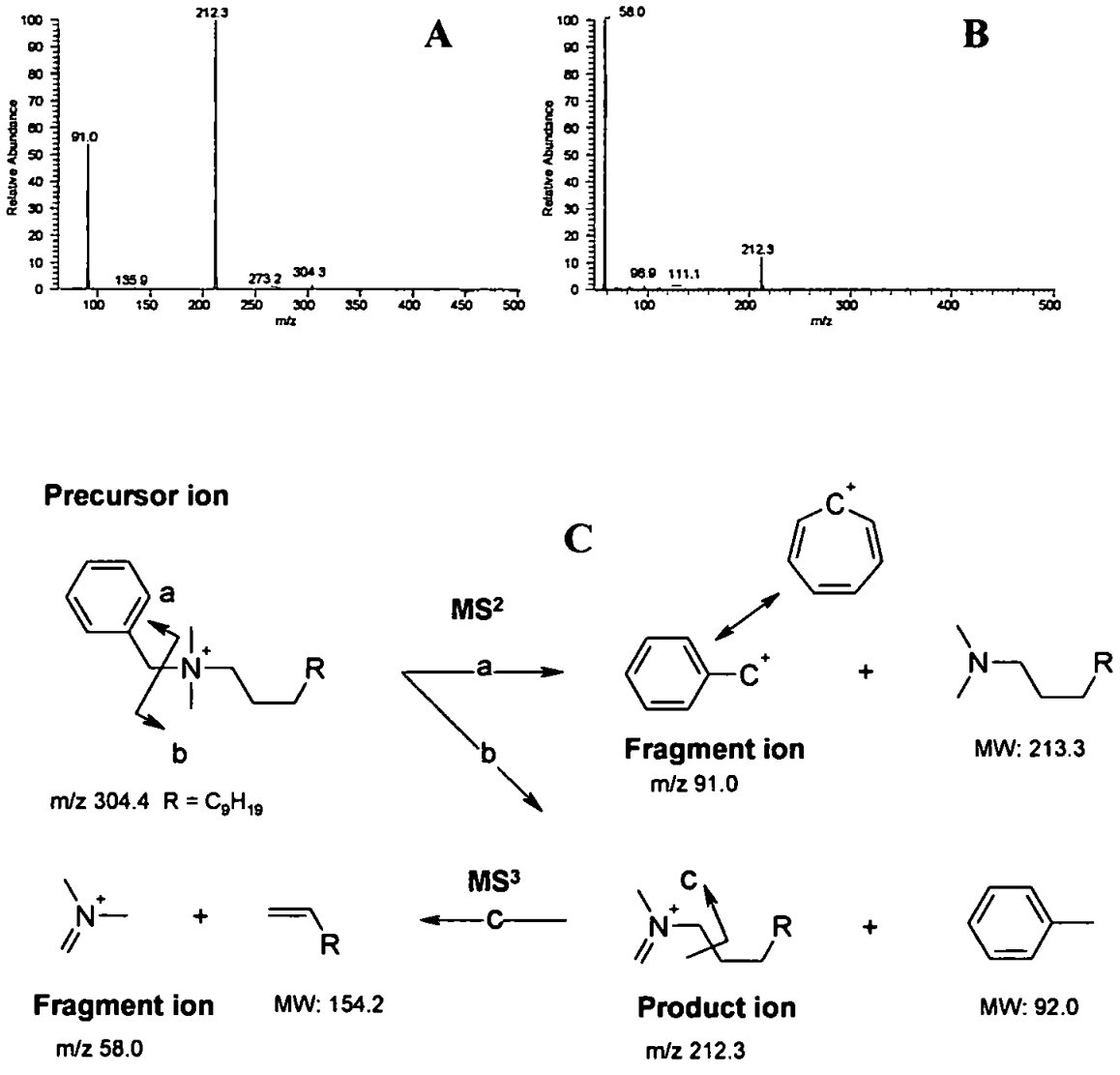


Figure 2.8 Positive ion ESI-MS^n mass spectra of ion *m/z* 304.4 in spectrum Figure 2.7 and CID MS^n fragmentation pathway for CI-C3 oilfield corrosion inhibitor: A) MS^2 on precursor ion *m/z* 304.4; B) MS^3 on product ion *m/z* 212.3; C) CID fragmentation pathway for alkylbenzyldimethylammonium ions.

2.3.2.3 Corrosion inhibitor CI-A3

Corrosion inhibitor CI-A3 is a commercial oilfield corrosion inhibitor with stated composition (MSDS) comprising 10-15% solvent naphtha (petroleum), 1-5% butoxyethanol and 20-40% long chain alkyl imidazoline. A positive ion ESI full scan spectrum (Figure 2.9) of a solution of CI-A3 contained two prominent sets of ions at *m/z* 350 and 612. These are near identical to those reported by Gough and Langley[52] for TOFA/DETA-derived imidazolines. Gough and Langley attribute the spectra to protonated 1:1-imidazolines ([2-(2-alkyl-4,5-dihydro-1*H*-imidazol-1-yl)ethylamine]) and 2:1-imidazolines (2-(2-alkyl-4,5-dihydro-1*H*-imidazol-1-yl)ethyl]alkylamide) (Figure 2.9). The peak distributions differ by 2 Da in each group, which is consistent with a natural distribution of unsaturated acids expected from reactants derived from a natural source such as a Tall Oil. MS^2 analysis of the precursor ions *m/z* 350.7 and 614.7 (Figure 2.10A and C), corresponding to imidazolines with alkyl moieties $R_1=C_{16}H_{31}$ and $R_2=C_{17}H_{33}$, shows the formation of a protonated 2-alkylimidazoline moiety (Figure 2.10E) as the base peak ion for both ions *via* losses of etheneamine and N-ethene-alkylamide respectively. MS^3 fragmentation of the product ion *m/z* 307 (Figure 2.10B and D) produced identical spectra consistent with the loss of the alkyl chain to give a protonated 2-etheneimidazoline (Figure 2.10E). Further fragmentation produced no ions above the *m/z* 50 lower mass limit of the instrument. Spectra were obtained for other ions in the two groups producing product ions that varied by 2 Da, confirming the differences were due to the degree of unsaturation of the alkyl chains. A fragmentation pathway that is consistent with the structures of the imidazoline compounds is given in Figure 2.10E.

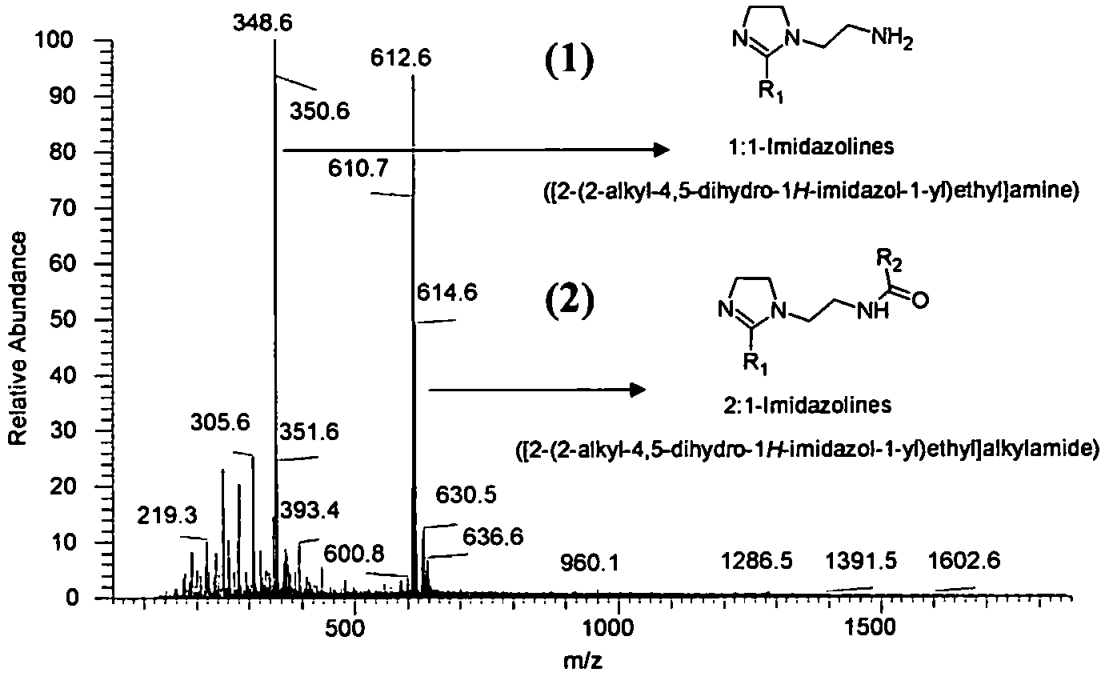


Figure 2.9 Positive ion ESI-MS mass spectrum of CI-A3 oilfield corrosion inhibitor (90:10:0.1 v/v/v MeOH:H_2O:AcOH).

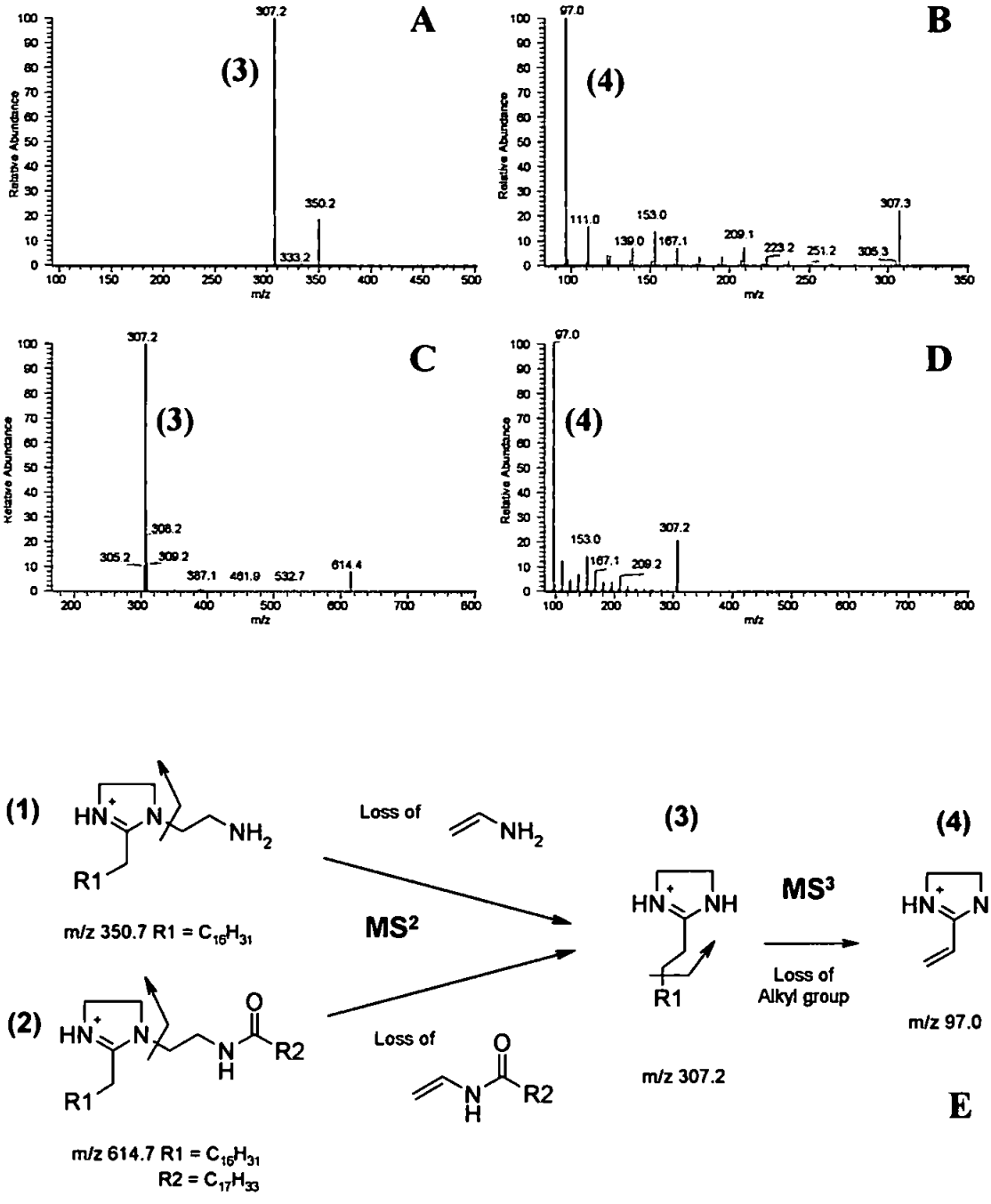


Figure 2.10 Positive ion ESI-MS^n mass spectra of ions *m/z* 350 and 614 shown in Figure 2.9 and CID MS^n fragmentation pathway for CI-A3 oilfield corrosion inhibitor: A) MS2 on precursor ion *m/z* 350.7; B) MS3 on product ion *m/z* 307.2 in A; C) MS2 on product ion *m/z* 614.7; D) MS^3 on product ion *m/z* 307.2 in C and E) proposed CID fragmentation pathway.

2.4 Conclusions

Examination of a range of oilfield chemicals (OCs) and of oilfield platform Produced Water (PW) by electrospray ionization ion trap multi-stage mass spectrometry has demonstrated for the first time that the technique is very powerful for the identification and characterisation of polar chemicals used as demulsifiers, corrosion inhibitors and biocides, for example.

A range of imidazolines, alkylbenzene sulfonates, quaternary ammonium compounds (quats) and ethoxylates have all been identified, some for the first time in OCs and PW.

The particular advantages of the ion trap multi-stage MS method are: the possibility of both positive and negative ion detection (*e.g.* for quats and sulfonates respectively); the ability through the so-called 'ZoomScan™' facility to determine the accurate mass differences between ion pairs, thus allowing differentiation of the multiple charge state of the ions; the collision induced dissociation multistage mass spectrometry allowing fragmentation pathways to be studied in up to five steps, thereby allowing previously unknown compounds to be identified.

CHAPTER THREE

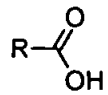
Development of a solid phase extraction (SPE) procedure for the separation of imidazoline and related amide oilfield corrosion inhibitors from crude oils

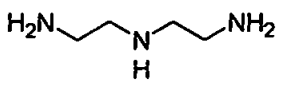
This chapter describes the development of solid phase extraction (SPE) procedures for initial separation of imidazoline/amide oilfield corrosion inhibitors from crude oils prior to examination by HPLC/ESI-MS.

3.1 Introduction

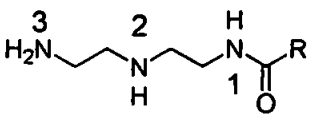
The advent of electrospray ionisation–mass spectrometry (ESI-MS) has seen application of the technique to the identification of a growing number of polar chemicals in environmental and industrial matrices.[3,8,23,24,52,70-72] In Chapter 2, infusion ESI-MS was shown to be applicable to characterisation and determination of oil field chemicals as diverse as positively charged quaternary benzalkonium chlorides ('Quats') and anions of linear alkylbenzene sulfonates ('LAS').[68] Gough and Langley[52] also demonstrated application of LC/ESI-MS techniques to the detection of a variety of polar organics in oilfield chemical formulations and oilfield Produced Water (PW) including Quats (used as biocides) and corrosion inhibitors such as the so-called 'imidazolines' ([2-(2-alkyl-4,5-dihydro-1*H*-imidazol-1-yl)ethyl]amine (1:1-Imidazolines), [2-(2-alkyl-4,5-dihydro-1*H*-imidazol-1-yl)ethyl]alkylamide (2:1-Imidazolines); structures VII and VIII respectively; Figure 3.1) produced by thermal reaction of fatty acids with diethylenetriamine[52,73-78] (Figure 3.1 shows the structures and naming of the main reagents, intermediates and products of the reaction). However, although detection of oilfield chemicals has been reported, few authors have reported ESI-MS methods for the quantitation of polars such as imidazolines[43,52]; and there are no reports of their determination in complex industrial matrices such as crude oil. For imidazolines this is important for the assessment of the efficiency of both downhole inhibitor addition (so-called "squeezing")[79] and to topside operations where control of dosing rates for the protection of metalwork reduces over-dosing and consequent economic and environmental costs. Whilst methods such as thin-layer chromatography (TLC) have been used previously to detect imidazolines in crude oils,[79] such methods at best only allow total imidazolines to be determined. Solid-phase extraction (SPE) methods have been reported[43,52] for extraction of imidazolines from PW[52] and brines,[43] but there appear to be no reports of the separation of imidazolines from crude oils using SPE.

A system is required which allows crude oil (principally hydrocarbon) components to be eluted through a SPE column whilst retaining the imidazoline/amide compounds, followed by elution of the latter in a minimum of solvent whilst asphaltenes and other polar oil compounds are retained. Such an elution solvent system would need to be compatible with ESI-MS.

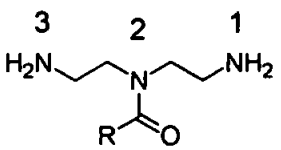
I  Fatty Acid
(Carboxylic acid)

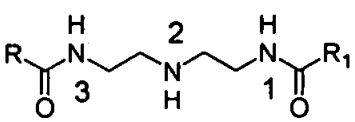
II  Diethylenetriamine (DETA)

(*N*-(2-aminoethyl)ethane-1,2-diamine)

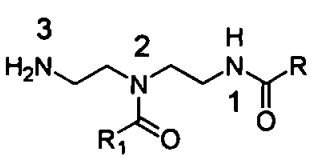
III  1-Monoamide

(*N*-{2-[(2-aminoethyl)amino]ethyl}alkylamide)

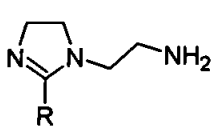
IV  2-Monoamide
(*N*,*N*-bis(2-aminoethyl)alkylamide)

V  1,3-Diamide

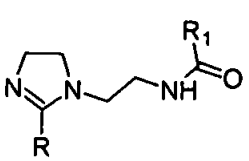
(*N*-[2-(2-alkylamino-ethylamino)ethyl]alkylamide)

VI  1,2-Diamide

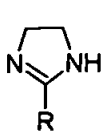
(*N*-(2-amino-ethyl)-*N*-(2-alkylamino-ethyl)alkylamide)

VII  1:1-Imidazolines

([2-(2-alkyl-4,5-dihydro-1*H*-imidazol-1-yl)ethyl]amine)

VIII  2:1-Imidazolines

([2-(2-alkyl-4,5-dihydro-1*H*-imidazol-1-yl)ethyl]alkylamide)

IX  2-alkylimidazolines

(2-alkyl-4,5-dihydro-1*H*-imidazole)

Figure 3.1 The structures and naming of the main reagents and products of the thermal reaction of fatty acids with diethylenetriamine for the synthesis of imidazolines. The name commonly used in the literature and surfactant industry is given first and is used throughout this thesis for brevity; whilst the bracketed name is the most recent systematic name according to guidelines specified by the International Union of Pure and Applied Chemistry (IUPAC) and was generated with ACD/ChemSketch 6 software (Advanced Chemistry Development, Toronto, Canada).

3.2 Experimental

3.2.1 *General chemicals and equipment*

Ultra pure water was obtained from an Elgastat filtration system (Elga, High Wycombe, UK). All solvents used were HPLC grade except glass distilled diethylether (ether) (Rathburn Chemicals Ltd, Walkerburn, UK. and BDH, Poole, UK). Methanol (MeOH), propan-2-ol (IPA), ammonia (0.88) and trifluoroacetic acid (TFA) were HyPersolv grade obtained from BDH (Poole, UK). Dichloromethane (DCM), ethanol (EtOH) and hexane were obtained from Rathburn Chemicals Ltd (Walkerburn, UK). Glass vials (7 and 15 mL) with Teflon lined caps were obtained from Supelco (Poole, UK) whilst auto sampler glass vials (2 mL) with polypropylene screw caps and silicone/PTFE seals were obtained from Chromacol (Welwyn Garden City, UK).

3.2.2 *Thin layer chromatography (TLC)*

3.2.2.1 Corrosion inhibitor and oil samples

A biodegraded North Sea crude oil (Gullfaks crude, PEP Ltd., Plymouth, UK) and an imidazoline corrosion inhibitor intermediate product (our code CI-E1) supplied by a commercial manufacturer, were used. The material safety data sheet (MSDS) for CI-E1 stated that the product comprised: 1,2,4-trimethylbenzene (1-5%), solvent naphtha (petroleum) heavy aromatic (20-30%) and long chain alkyl imidazoline (60-70%).

3.2.2.2 Thin layer chromatography procedure

Thin layer chromatography was carried out using SILGUR-25 (silica gel 60Å with Kieselguhr pre-concentration zone; 250um x 20 x 20 cm glass plates; Machery-Nalgel GmbH & Co. Düren, Germany (Plate 1-5, Table 3.1)) and laboratory-prepared plates of silica gel (60G, 0.5 mm x 20 x 20 cm (Plate 6-9, Table 3.1). Plates were oven-dried (160

°C for 90 minutes) before use. Developing tanks lined with blotting paper were allowed to equilibrate with the solvent for at least 90 minutes before development. Sample application was by 2 μL micro capillaries (micro-caps, CAMAG, Muttenz, Switzerland) to the silica phase (to produce a smaller origin spot) except for plate 1 (Table 3.1) where the sample was applied to the Kieselguhr pre-concentration zone. Samples were diluted in DCM (100 mg oil mL^{-1} and 10 mg CI-E1 mL^{-1}) before use and spots ranging from 1-8 μL were applied. After sample application, plates were air dried (10-15 minutes) before development. After a first development in diethyl ether, hexane or DCM, plates were air-dried (10-15 minutes) before visualisation or a second development stage. After second stage development in propan-2-ol:ammonia (8:2 v/v) plates were air dried (10-15 minutes and 40°C for ~30 minutes). Visualisation was by UV light source at 254 and 366nm followed by development in iodine vapour. Retardation factors (R_f) were calculated for product spots. For large spots or streaks an R_f range was measured for the lower and upper limits of the product.

Table 3.1 Thin layer chromatography of crude oil and imidazoline inhibitor (CI-E1); sample application and development solvents

TLC Plate	Plate development solvents and sample application of crude oil and imidazoline inhibitor (CI-E1).
1	Sample spots applied to the Kieselguhr pre-concentration zone and developed first in diethyl ether and then in propan-2-ol:ammonia (8:2 v/v).
2	Sample spots applied to the silica and developed first in diethyl ether and then in propan-2-ol:ammonia (8:2 v/v).
3	Sample spots applied to the silica and developed in diethyl ether.
4	Sample spots applied to the silica and developed in hexane.
5	Sample spots applied to the silica and developed in dichloromethane.
6	Sample spots applied to the silica and developed first in diethyl ether and then methanol:ammonia (8:2 v/v).
7	Sample spots applied to the silica and developed first in diethyl ether and then methanol.
8	Sample spots applied to the silica and developed first in diethyl ether and then propan-2-ol.
9	Sample spots applied to the silica and developed first in diethyl ether and then methanol:glacial acetic acid (8:1v/v).

3.2.3 *Solid-phase extraction of corrosion inhibitor CI-E1 from North Sea Gullfaks crude oil with analysis by infusion and flow injection ESI-MS (SPE 1-3)*

3.2.3.1 Solid phase extraction procedure for corrosion inhibitor CI-E1 from crude oil

A polyethylene solid-phase extraction reservoir (6 mL; Isolute, International Sorbent Technology, Hengoed, UK) was packed dry with silica gel (60G, 500 mg) and fitted with a defatted cotton wool plug. Solid-phase extraction sample SPE 1 (see below) was eluted under positive pressure using an adapter and Luer-tipped syringe. Samples SPE 2 and 3 were eluted using a vacuum manifold extraction system (Isolute, International Sorbent Technology, Hengoed, UK) with a flow rate of ~ 1mL min^{-1}.

Stock solutions (1 g CI-E1 in 10 mL hexane) were made up daily as required in glass volumetric flasks and transferred to glass vials (15 mL). Samples were: SPE 1, 200 μL of stock CI-E1 solution (20 mg CI-E1); SPE 2, crude oil prepared by diluting 500 mg oil with 5 mL hexane and SPE 3, 2000 ppm (2 mg mL^{-1}). CI-E1 in crude oil was prepared by spiking 10 μL of stock CI-E1 into 500 mg oil and dissolving in 5 mL hexane.

3.2.3.2 Solid-phase extraction procedure for samples SPE 1-3

The SPE column was washed and pre-equilibrated with 6 mL hexane before applying the sample. Sample SPE 1, was transferred onto the column using a glass pipette whilst samples SPE 2 and 3 were syringed onto the column under gentle vacuum (the column was not allowed to run dry except upon final elution). The column was eluted with hexane, diethyl ether and twice with propan-2-ol:ammonia (8:2 v/v) successively and the individual fractions retained. However, because the approach was developmental, the volumes of eluents used varied somewhat (SPE 1: hexane 3 mL, diethyl ether 4 mL, propan-2-ol:ammonia (8:2 v/v) 3 mL and 5 mL; SPE 2: hexane 3 and 5mL, diethyl ether 6 and 2 mL, propan-2-ol:ammonia (8:2 v/v) 6 and 3 mL and SPE 3: hexane 5 and 3 mL, diethyl ether 5

and 2mL, propan-2-ol:ammonia (8:2 v/v) 5 and 2 mL). Glass vials (7 mL) with aluminium-lined caps were used for fraction collection. Selected fractions were blown dry under a nitrogen stream with heating to 60 °C, except fractions from the spiked crude oil (SPE 3) which were blown down to ~ 1 and 0.25 mL.

3.2.3.3 Gas chromatography mass spectrometry (GC/MS) analysis of solid-phase eluate fractions

Gas chromatography mass spectrometry (GC/MS) was carried out using a Hewlett Packard 5890 series II GC with a 5970 mass selective detector (MSD). The column was a HP1 Ultra (12 m x 0.2 mm i.d.) programmed from 40-300 ^{0}C at 5 ^{0}C min^{-1} plus a 10-minute hold. The injection volume was 0.5 µL. Analyte fractions prepared for GC-MS analysis were: SPE 1; 1.8 mg dry residue of 3 mL hexane eluate fraction dissolved in 4 mL dichloromethane (~0.5 mg mL^{-1}) and 2.5 mg dry residue of 4 mL diethyl ether eluate fraction dissolved in 5mL dichloromethane (~0.5 mg mL^{-1}).

3.2.3.4 Infusion and flow injection electrospray ionisation mass spectrometry (ESI-MS) analysis of solid-phase eluate fractions

Mass spectrometry analysis was carried out using a Finnigan Mat LCQ™ (ThermoFinnigan, San Jose, CA, USA.) bench top mass spectrometer. The LCQ was used with an electrospray interface and Xcalibur 1.0 sp1 software (ThermoFinnigan). Infusion of extracts (3 µL min^{-1}) was carried out using the built in syringe pump with a Hamilton 1725N syringe (250 µL; Reno CA, USA.). The instrument was auto-tuned on analyte ion *m/z* 610.7. Flow injection was performed using a 20 µL sample injection into a 150 µL min^{-1} 90:10 propan-2-ol:water eluent.

Analyte fractions prepared for ESI-MS analysis were: SPE 1; 11 mg dry residue of propan-2-ol:ammonia 3 mL eluate fraction dissolved in propan-2-ol (2 mL). 100 µL transferred to 2 mL glass vial and 900 µL propan-2-ol added. SPE 2; 2 mg dry residue of the 6 mL

propan-2-ol:ammonia eluate fraction dissolved in propan-2-ol (2mL). 100 μL transferred to 2 mL glass vial and 900 μl propan-2-ol added. SPE 3; 100 μL of ~1 mL residual propan-2-ol:ammonia eluate (assuming 100% recovery, then 2000 ppm) transferred to 2 mL glass vial and 900 μL propan-2-ol added (200 ppm).

Infusion ESI-MS analysis of corrosion inhibitor CI-E1 (Stock 0.0643g CI-E1 up to 50mL methanol; 1287 μg mL^{-1}, ppm) was carried out at a concentration of ~1 ppm (1000x dilution of stock) in two solvents, *Sample X* (MeOH:water; 7:3 v/v) and *Sample Y* (MeOH:water:formic; 80:20:0.1 v/v/v) in order to characterise the initial product.

3.2.4 *Solid-phase extraction of 2:1-palmitic imidazoline and 2:1-oleic imidazoline (2:1-PI and 2:1- OI) from crude oil with analysis by HPLC/ESI-MS (SPE 4-9)*

3.2.4.1 Stock solutions and Samples

A crude oil sample from a Middle Eastern oilfield which had being treated with imidazoline based corrosion inhibitor was used (supplied by a commercial manufacturer). The oil was supplied in 1 L aluminium flasks. Stock solutions of 2:1-palmitic imidazoline (2:1-PI; R and $R_1 = C_{15}H_{31}$, structure VIII, Figure 3.1; Chapter 4 for synthesis details) and 2:1-oleic imidazoline (2:1-OI; R and $R_1 = C_{17}H_{33}$, structure VIII, Figure 3.1;) were prepared in glass volumetric flasks and transferred to 15 mL glass vials. Standard solutions for spiking crude oil samples for SPE were made up as described in Table 3.2.

Table 3.2 Standard solutions prepared for spiking crude oil

Standard	Analyte/Concentration	Mass/volume standard	Made up to solvent volume
1	2:1-PI (1240 ng μL^{-1})	0.0124g	10 mL MeOH
2	2:1-PI (1012 ng μL^{-1})	0.0253g	25 mL MeOH
3	2:1-PI (112 ng μL^{-1})	0.0028g	25 mL MeOH
4	2:1-OI (1044 ng μL^{-1})	0.0261g	25 mL MeOH
5	2:1-OI (104 ng μL^{-1})	0.0026g	25 mL MeOH
6	2:1-PI (101 ng μL^{-1}) & 2:1-OI (104 ng μL^{-1})	1000 µL of standard 2 + 1000 µL of standard 4	10 mL propan-2-ol

3.2.4.2 Solid Phase extraction

Solid phase extraction was carried out using Isolute Si SPE cartridges (SPE 4-8) (1g silica x 6mL, International Sorbent Technology, Hengoed, UK). A vacuum manifold extraction system (Isolute, International Sorbent Technology, Hengoed, UK) at a flow rate of ~1mL min^{-1} was used for elution. Samples for SPE were made up as described in Table 3.3.

Table 3.3 Samples prepared for solid-phase extraction

SPE Sample	Analyte and matrix concentration	Matrix spiked with standard (std, Table 3.2)
4	5 µg 2:1-PI g oil $^{-1}$	1 g crude oil + 4 µL std. 1
5	1 µg 2:1-PI g oil $^{-1}$	1 g crude oil + 10 µL std. 3
6	1 µg 2:1-PI and 1 µg 2:1-OI g oil $^{-1}$	1 g crude oil + 10 µL std. 3 + 10 µL std. 5
7	1 µg 2:1-PI and 1 µg 2:1-OI mL hexane^{-1}	1 mL Hexane + 10 µL std. 6
8	1 µg 2:1-PI and 10 µg 2:1-OI g oil $^{-1}$	1 g crude oil + 10 µL std. 3 + 10 µL std. 4
9	1 µg 2:1-PI and 10 µg 2:1-OI g oil $^{-1}$	1 g crude oil + 10 µL std. 3 + 10 µL std. 4

3.2.4.3 Extraction procedure for samples SPE 4-8

Samples were diluted with 1mL hexane and transferred with a glass pipette on to SPE columns (pre-washed with 7 ml hexane). Each sample vial was washed 4 times with 0.5mL hexane and the washings transferred to the columns. The SPE columns were then eluted with hexane (7 mL), diethyl ether (30 mL; SPE 6 and 7 were vacuum dried for a few seconds) and propan-2-ol:ammonia (8:2 v/v, 10 mL). Each propan-2-ol:ammonia fraction was blown dry under a nitrogen stream with heating to 60°C.

The SPE 4 residue was reconstituted with 500 µL methanol:0.1% TFA (v/v) whilst SPE 5 - 8 residues were reconstituted with 1000 µL methanol:0.1% TFA (v/v). Each re-dissolved residue was withdrawn from the vial into a 1mL glass syringe and expelled through a 0.2µm PVDF syringe filter (Whatman, Maidstone, UK) into a 2mL glass auto-sampler vial.

3.2.4.4 Extraction procedure samples SPE 9

A solid-phase extraction procedure currently used for commercial analysis of oils was carried out (1g x 6mL SPE cartridge) as a comparison. The procedure is subject to confidentiality agreement and cannot be disclosed. The SPE 9 residue was reconstituted with 1000 µL methanol:0.1% TFA (v/v) and filtered as above (section 3.2.4.3).

3.2.4.5 High Performance Liquid Chromatography (HPLC)

HPLC was carried out using a P580A binary pump (Dionex-Softron GmbH, Germering, Germany) at 1mL min^{-1} flow rate and with a split of around 200 µL (high pressure micro-splitter valve, Upchurch Scientific Ltd., Oak Harbor, WA, USA) to the mass spectrometer. All sample injections were 20 µL except for analyses 1-4 of sample SPE 4 (Table 3.4) were made with an ASI100 autosampler (Dionex-Softron GmbH, Germering, Germany. Elutions were carried out using the gradients shown in Tables 3.4 and 3.5 (solvent A,

MeOH:0.1% TFA v/v; and solvent B, 0.1% TFA in water v/v. A Hamilton PRP-1 reverse phase column was used (5μm, 50 x 4.1mm Reno, CA, USA.).

Table 3.4 Elution gradients used for HPLC analysis of sample SPE 4 (solvent A, methanol: 0.1% trifluoroacetic acid v/v and solvent B, 0.1% trifluoroacetic acid in water v/v)

Analysis 1	**Time (min)**	**0**	**0.1**	**15**	**25**	**30**	**40**
5μL injection	**% A**	70	70	95	95	100	100
	% B	30	30	5	5	0	0
Analysis 2	**Time (min)**	**0**	**0.1**	**15**	**30**	**35**	**40**
5μL injection	**% A**	75	75	95	95	100	100
	% B	25	25	5	5	0	0
Analysis 3	**Time (min)**	**0**	**0.5**	**11**	**20**	**25**	**30**
5μL injection	**% A**	75	75	95	95	100	100
	% B	25	25	5	5	0	0
Analysis 4	**Time (min)**	**0**	**0.5**	**11**	**20**	**25**	**30**
20μL injection	**% A**	75	75	95	95	100	100
	% B	25	25	5	5	0	0

Table 3.5 Elution gradient used for HPLC analysis of 5 μL injections of samples SPE 5-9 (solvent A, methanol: 0.1% trifluoroacetic acid v/v and solvent B, 0.1% trifluoroacetic acid in water v/v)

Time (min)	**0**	**0.1**	**17**	**27**	**28**	**35**
% A	75	75	100	100	75	75
% B	25	25	0	0	25	25

3.2.4.6 Analysis of samples SPE 4-9 using HPLC/ESI-MS

Mass spectrometry analysis was carried out using a Finnigan Mat LCQ™ (ThermoFinnigan, San Jose, CA, USA) ion trap mass spectrometer fitted with an electrospray interface. Data were acquired and processed using Xcalibur 1.0 sp1 software (ThermoFinnigan). Instrument tuning and mass calibration were carried out and checked

using the automatic calibration procedure (tuning and calibration solution; caffeine (Sigma, St Louis, MO, USA), MRFA (Finnigan Mat, San Jose, CA, USA) and Ultramark 1621(Lancaster Synthesis Inc, Widham, NH, USA) in methanol/water/acetic acid (50:50:1, v/v/v)). Instrument method optimisation was carried out by infusing 1M sodium acetate at 1μL min^{-1} into a 200 μL min^{-1} eluent flow from the HPLC system by way of the built in syringe pump, a Hamilton 1725N syringe (250 μL; Reno, CA, USA.) and a PEEK Tee union (Upchurch Scientific Ltd., Oak Harbor, WA, USA). The automatic tune function was used on a suitable sodium trifluoroacetate adduct ion. For the positive ion full scan range *m/z* [100-1000], tuned on adduct ion *m/z* 563 the following instrument parameters were used: source voltage, + 4.5 kV; capillary voltage + 20 V; tube lens offset, +10.00 V; capillary temperature, 220 °C; nitrogen sheath gas flow rate, 60 (arbitrary units); and nitrogen auxiliary gas 20 (arbitrary units). Data was processed and peak areas calculated using the Qualitative programme in the Xcalibur 1.0 sp1 software. Extracted ion mass chromatograms (EIC) were extracted from the full scan; total ion chromatograms (TIC) selected to *m/z* 0.1 and an isolation width of *m/z* 1.

3.3 Results and discussion

3.3.1 *Thin layer chromatography (TLC)*

As a first step in developing a method for the SPE of imidazoline corrosion inhibitor compounds from crude oil, TLC of a Gullfaks crude oil and an imidazoline-based corrosion inhibitor intermediate (CI-E1) was investigated. TLC can provide a visual and relatively simple method of investigating the chromatographic properties of compounds using various solvent systems and stationary phases if the analytes are amenable to a convenient means of visualisation, such as fluorescence under ultra violet (UV) light or

reaction with a chemical reagent producing a coloured or contrasting reaction product. The use of a Kieselguhr pre-concentration zone in the first 28 mm of a plate, allows large volumes of dilute samples to be applied to the chromatographically inactive Kieselguhr layer. On development the applied substances are concentrated in a band at the Kieselguhr/silica interface from which chromatography starts to occur as normal.

TLC using a two-stage development on silica gel plates with a Kieselguhr pre-concentration zone, similar to that described by Buck and Sudbury,[79] was carried out (TLC 1, Table 3.6), although visualisation was achieved using UV and iodine vapour and not the SO_3 charring used by the latter authors. Observation of the plate after development in iodine vapour showed that most of the crude oil components had moved above the mid-point of the plate in a large tear drop spot with a black streak towards the diethyl ether solvent front ($R_{f\text{-ether}}$ 0.54-0.99, also visible to the naked eye and UV at 366 and 254 nm, between developments). These components were moved during the diethyl ether first development as they were clearly above the propan-2-ol (IPA):NH_3 (8:2, v/v) second development solvent front (R_f 0.50 relative to the first development). A faint spot originating from the crude oil at $R_{f\text{-ether}}$ 0.40 was also observed under UV at 366nm between developments. Under UV at 366nm between developments there was faint fluorescence at the origin of the CI-E1 sample and at the Kieselguhr/silica interface indicating that there was some retention of CI-E1 components. Two faint spots at $R_{f\text{-ether}}$ 0.57 and 0.73 originating from the CI-E1 sample were observed above the second development solvent front. In the area of the second development, there were five spots clearly visible originating from the CI-E1 sample at $R_{f\text{-IPA:NH3}}$ 0.04, 0.40, 0.47, 0.70 and 0.80. At the crude oil origin, grey spots remained with faint steaks towards the main spot. When the procedure was repeated (TLC 2, Table 3.7), with the sample applied directly to the silica zone of the plate, the R_f values for the crude oil and CI-E1 components were near identical to those observed in TLC 1, except that an extra spot was present at $R_{f\text{-IPA:NH3}}$

0.40 on TLC 1. The spot at $R_{f\text{-}IPA:NH3}$ 0.40 (TLC 1) may be accounted for if the Kieselguhr had resolved the components in the spot at $R_{f\text{-}IPA:NH3}$ 0.51 on TLC 2. Promisingly and most importantly, these initial results indicated that imidazoline compounds could be separated from crude oil and a more detailed evaluation was undertaken.

Table 3.6 Thin layer chromatography R_f values for resolved crude oil and CI-E1 imidazoline inhibitor components (TLC 1). Conditions and procedure described in experimental section 3.2

Sample	**R_f (First development, diethyl ether)**				
Crude oil	Origin (S)	0.40 (S-vf)	0.54-0.80 (S, d)	0.71-0.99 (SK, d)	
Inhibitor CI-E1	0.57 (S, f)	0.73 (S, f)			
	R_f (Second development, IPA:NH_3, (8:2 v/v))				
Inhibitor CI-E1	0.04 (0.02) (S, d)	0.40 (S, d)	0.47 (S, d)	0.70 (S, d)	0.80 (S, d)

S = spot; SE = spot elongated; SK = streak; f = faint; vf = very faint; d = dark; vd = very dark; TdS = teardrop shaped spot; 366/254 = fluorescence under UV light at 366 or 254nm.

Table 3.7 Thin layer chromatography R_f values for resolved crude oil and CI-E1 imidazoline inhibitor components (TLC 2). Conditions and procedure described in experimental section 3.2

Sample	**R_f (First development, diethyl ether)**			
Crude oil	Origin (S,)	0.43 (S, vf)	0.52-0.71 (S)	0.66-0.92 (SK)
Inhibitor CI-E1	0.52 (S, f)	0.67 (S, f)		
	R_f (Second development, IPA:NH_3, (8:2 v/v))			
Inhibitor CI-E1	0.06 (S, d)	0.51 (S, d)	0.72 (S, d)	0.79 (S, d)

The TLC elution of crude oil and CI-E1 components by development in diethyl ether, hexane and DCM solvents (TLC 3-5; Tables 3.8-3.10, respectively) was compared. Developed in diethyl ether (TLC 3), the oil components eluted as a tear drop spot at R_f values near identical to those in TLC 1. For CI-E1 the origin was a very dark spot indicating that most of the applied sample was not eluted. Two components at $R_{f\text{-ether}}$ 0.54 and 0.67 were eluted, corresponding to spots in TLC 1, which was consistent with the trimethyl benzene and naphtha components reported in the MSDS data provided. There was a faint spot at $R_{f\text{-ether}}$ 0.03 which indicated the spot at $R_{f\text{-IPA:NH3}}$ 0.04 ($R_{f\text{-ether}}$ 0.02) on TLC 1 was eluted by diethyl ether but not by IPA:NH_3. Development in hexane (TLC 4) showed no movement from the origin for CI-E1 except for a very faint spot at $R_{f\text{-hexane}}$ 0.35, whilst the oil components were eluted from the origin to $R_{f\text{-hexane}}$ 0.67 as a streak comprising four distinct areas. Development of a plate with DCM (TLC 5) eluted crude oil in a teardrop spot ($R_{f\text{-DCM}}$ 0.62-0.87) similar to that observed in diethyl ether. The origin of the crude oil spot was darker, indicating perhaps that not as many components were eluted. There was also a streak from the origin to $R_{f\text{-DCM}}$ 0.12. The CI-E1 origin was again very dark, but there was also a light streak to $R_{f\text{-DCM}}$ 0.67. A faint spot was observed at $R_{f\text{-DCM}}$ 0.73.

From the results of development of CI-E1 in diethyl ether, hexane and DCM it was found that the imidazoline components were not moved from the origin whilst the trimethyl benzene and naphtha components were. The crude oil components were eluted to varying degrees by the three solvents, with hexane producing the widest spread of components and diethyl ether the greatest elution with the least separation of components. To evaluate solvent systems for eluting the imidazoline compounds and the asphaltene crude oil compounds, diethyl ether was used as a first development stage to move the crude oil components and trimethyl benzene and naphtha compounds from CI-E1 away from the second development zone.

Table 3.8 Thin layer chromatography R_f values for resolved crude oil and CI-E1 imidazoline inhibitor components (TLC 3). Conditions and procedure described in Experimental section 3.2

Sample	R_f (Development, diethyl ether)			
Crude oil	Origin (S)	0.54-0.72 (S, d)	0.71-0.97 (SK, d)	
Inhibitor CI-E1	Origin (S, vd)	0.03 (S, f)	0.54 (S, f)	0.67 (S, f)

Table 3.9 Thin layer chromatography R_f values for resolved crude oil and CI-E1 imidazoline inhibitor components (TLC 4). Conditions and procedure described in Experimental section 3.2

Sample	R_f (Development, hexane)			
Crude oil	Origin-0.14 (SK, d)	Origin-0.35 (SK,366/254)	0.35-0.56 (SK)	0.57-0.67 (S)
Inhibitor CI-E1	Origin (S, vd)	0.35 (S, vf)		

Table 3.10 Thin layer chromatography R_f values for resolved crude oil and CI-E1 imidazoline inhibitor components (TLC 5). Conditions and procedure described in Experimental section 3.2

Sample	R_f (Development, DCM)			
Crude oil	Origin (S, d)	Origin-0.12 (SK)	0.62-0.76 (S)	0.73-0.87 (SK)
Inhibitor CI-E1	Origin (S, vd)	Origin-0.67 (SK,)	0.73 (S, f)	

Second development eluents (MeOH:NH_3, 8:2 v/v; MeOH; IPA; MeOH:AcOH, 8:1 v/v; TLC 6-9; Table 3.11 – 3.14 respectively) were evaluated to find a system that would allow rapid elution of the imidazoline compounds. None of the above systems moved the crude oil components remaining from the origin after the first development. MeOH:NH_3 (8:2, v/v) (TLC 6) elution produced greater resolution of CI-E1 components and an extra spot was detectable compared with that obtained using IPA:NH_3 (8:2, v/v) (TLC 2). However, some of the latter eluting components were not moved far from the origin. MeOH, IPA and MeOH:AcOH (8:1, v/v) proved unsuitable as elution solvents as CI-E1 components remained at, or close to, the origin.

Table 3.11 Thin layer chromatography R_f values for resolved crude oil and CI-E1 imidazoline inhibitor components (TLC 6). Conditions and procedure described in Experimental section 3.2

Sample	**R_f (First development, diethyl ether)**				
Crude oil	Origin (S)	0.66-097 (TdS)			
Inhibitor CI-E1	Origin (S)	0.51 (S, vf)	0.79 (S, vf)		
	R_f (Second development, MeOH:NH_3, (8:2 v/v))				
Inhibitor CI-E1	0.06 (S)	0.11 (S)	0.30 (SE)	0.50 (SE)	0.69 (S)

Table 3.12 Thin layer chromatography R_f values for resolved crude oil and CI-E1 imidazoline inhibitor components (TLC 7). Conditions and procedure described in Experimental section 3.2

Sample	**R_f (First development, diethyl ether)**				
Crude oil	Origin (S)	0.71-098 (TdS)			
Inhibitor CI-E1	Origin (S)	0.57 (S, vf)	0.86 (S, vf)		
	R_f (Second development, MeOH)				
Inhibitor CI-E1	Origin-0.13 (SK, vd)	0.34 (SK, d)	0.51 (S)	0.68 (S, f)	0.73 (S, f)

Table 3.13 Thin layer chromatography R_f values for resolved crude oil and CI-E1 imidazoline inhibitor components (TLC 8). Conditions and procedure described in Experimental section 3.2

Sample	R_f (First development, diethyl ether)			
Crude oil	Origin (S)	0.77-0.98 (TdS)		
Inhibitor CI-E1	Origin (S)	0.77 (S, vf)	0.93 (S, vf)	
	R_f (Second development, IPA)			
Inhibitor CI-E1	Origin-0.15 (SK, vd)	0.15-0.17 (SK, d)	0.69 (S, vf)	0.95 (S, vf)

Table 3.14 Thin layer chromatography R_f values for resolved crude oil and CI-E1 imidazoline inhibitor components (TLC 9). Conditions and procedure described in Experimental section 3.2

Sample	R_f (First development, diethyl ether)		
Crude oil	Origin (S)	0.66-0.98 (TdS)	
Inhibitor CI-E1	Origin (S)	0.61 (S, vf)	0.80 (S, vf)
	R_f (Second development, MeOH:AcOH (8:1 v/v))		
Inhibitor CI-E1	Origin-0.11 (SK, vd)	0.11-0.19 (SK, vd)	0.78 (S, f)

3.3.2 *Infusion ESI-MS analysis of CI-E1 imidazoline corrosion inhibitor intermediate*

Active compounds in the CI-E1 corrosion inhibitor product were determined by infusion ESI-MS. Positive ESI spectra of CI-E1 in two solvent systems (1 ppm CI-E1 in methanol:water, 7:3 v/v; and methanol:water:formic, 8:2:0.1 v/v/v) are shown in Figures 3.2 and 3.3. It is apparent from the spectra that the relative abundance maximum in the ion

current for the base peak (*m/z* 610.7) is higher in the methanol:water:formic solvent system than the methanol:water solvent (1.42E6 > 4.08E5). This is consistent with higher proton concentration, owing to the presence of formic acid, leading to an increase in the number of protonated molecular ions formed during ESI. It is also seen that the relative abundance of *m/z* 348.5 is increased from ~5% to ~ 25% in the presence of formic acid. The spectral ion masses are similar to those shown by Gough and Langley,[52] for a TOFA/DETA imidazoline product. The ion masses are consistent with the presence of oleic and linoleic acid-derived imidazolines with so-called 2:1-imidazolines as the predominant compounds. Further examination of the *m/z* values and relative responses of spectral peaks for the methanol:water:formic acid system (Figure 3.3) showed that linoleic acid ($C_{18:2}$) was the main acid starting component (*i.e. m/z* 348.5 and 610.7) with oleic ($C_{18:1}$) and linolenic ($C_{18:3}$) acids also present (expanded regions of the spectra showing ions due to 2:1-imidazoline and 1:1-imidazoline are shown in Figures 3.4 & 3.5). Ions at *m/z* 374.5 and 636.7 indicated the presence of a $C_{20:3}$ acid in the reactant fatty acids mixture. Tables 3.15 and 3.16 show *m/z* values for ions expected to arise from mass spectrometry of combinations of various fatty acids used in the synthesis of 2:1-imidazoline and 1:1-imidazolines. Evidence for monoamide and diamide precursors of imidazolines (or hydrolysis products of imidazolines) could also be seen in the spectra (*i.e. m/z* 628.7 and 366.5) at ~ 50% abundance of the corresponding imidazoline-derived ions. Negative ion ESI analysis of CI-E1 showed no peaks distinguishable from background.

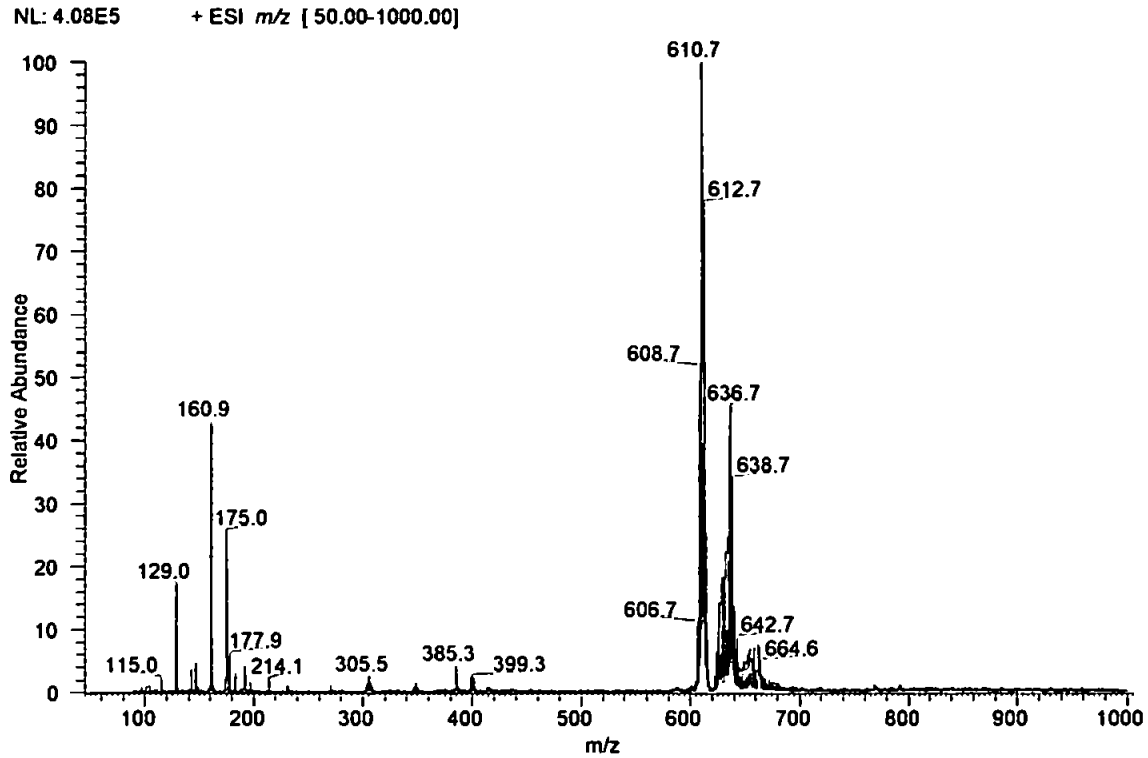


Figure 3.2 Infusion positive ion ESI-MS mass spectrum of 1ppm CI-E1 imidazoline corrosion inhibitor in methanol:water (7:3 v/v).

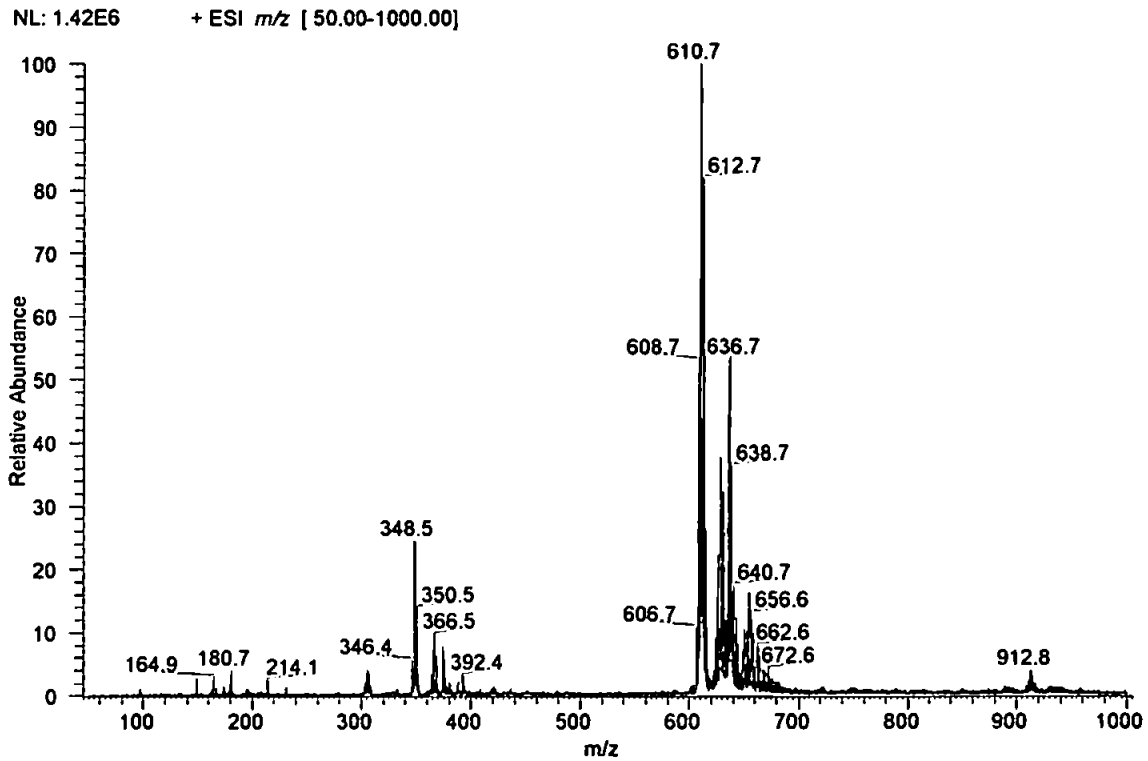


Figure 3.3 Infusion positive ion ESI-MS mass spectrum of 1ppm CI-E1 imidazoline corrosion inhibitor in methanol:water:formic acid (8:2:0.1 v/v).

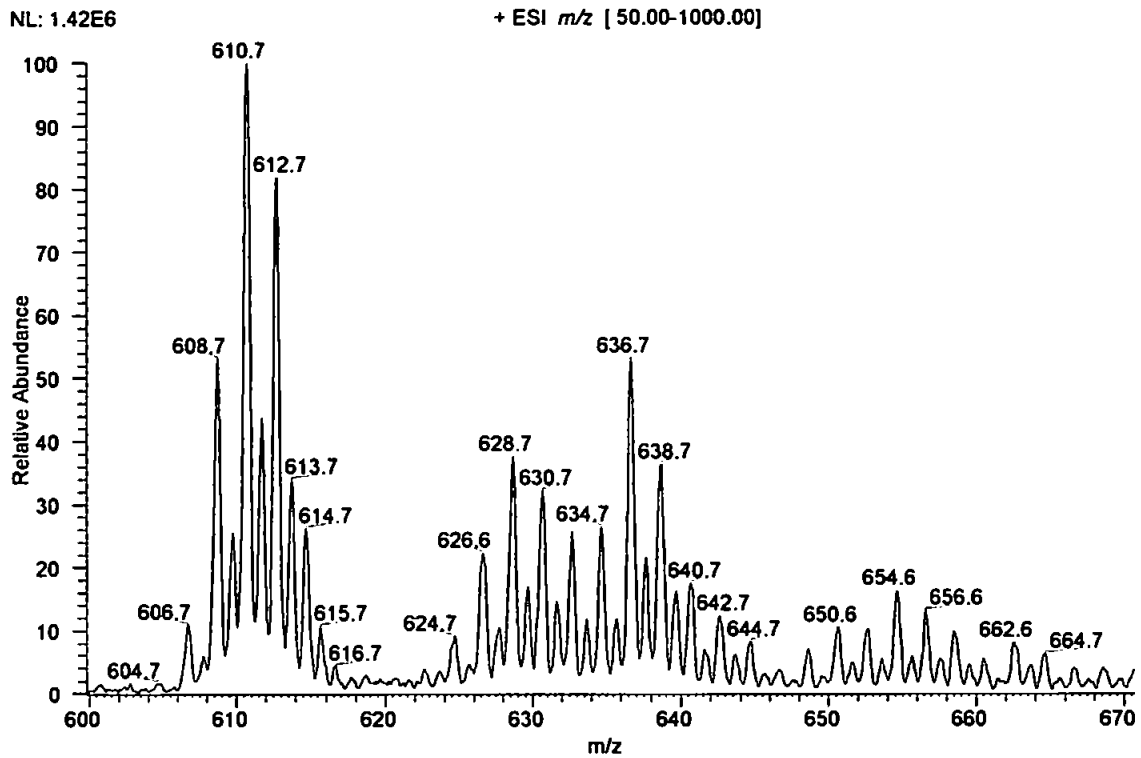


Figure 3.4 Expanded spectrum of *m/z* 600-670 region of Figure 3.2.

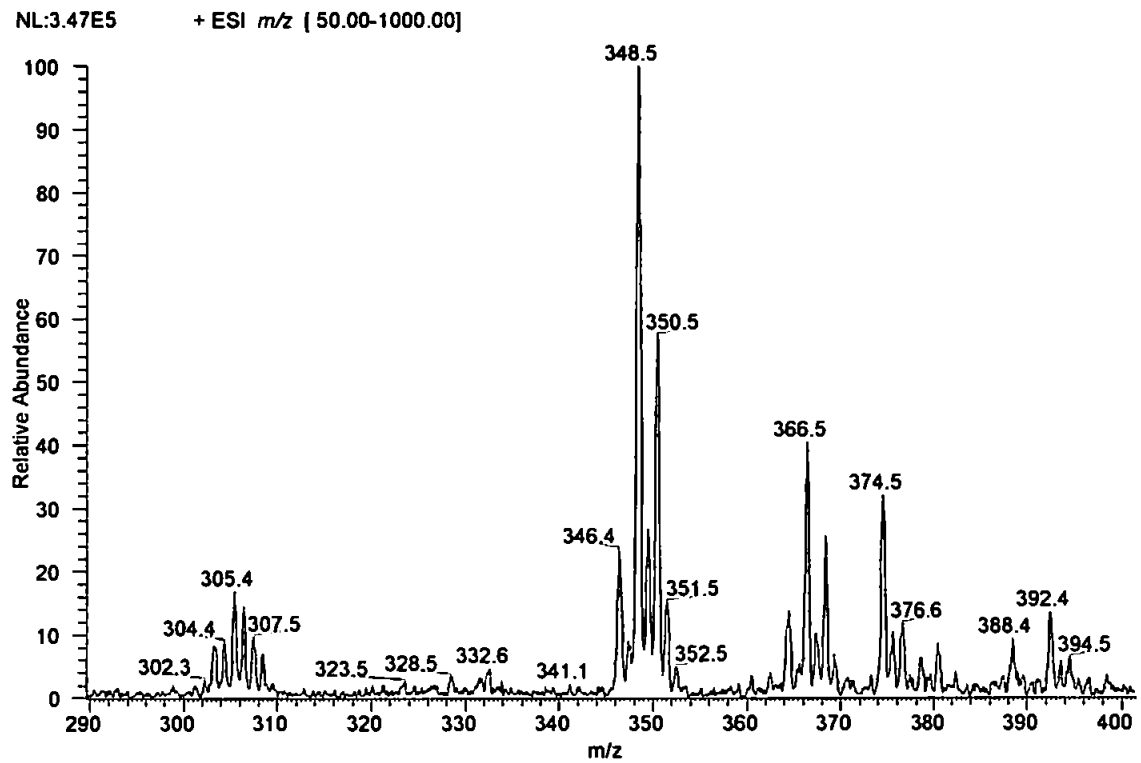
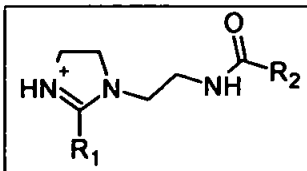


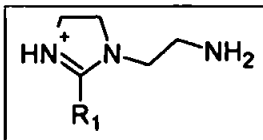
Figure 3.5 Expanded spectrum of *m/z* 290-400 region of Figure 3.3.

Table 3.15 Calculated mono-isotopic masses for some C_{18} fatty acid-derived 2:1-imidazolines



Mono-isotopic masses for protonated 2:1-imidazolines				
R1/R2	**$C_{17:1}$**	**$C_{17:2}$**	**$C_{17:3}$**	**$C_{20:3}$**
$C_{17:1}$	614.6	612.6	610.6	638.6
$C_{17:2}$	612.6	610.6	608.6	636.6
$C_{17:3}$	610.6	608.6	606.6	634.6
$C_{20:3}$	638.6	636.6	634.6	662.7

Table 3.16 Calculated mono-isotopic masses for some C_{18} fatty acid derived 1:1-imidazolines



Mono-isotopic masses for protonated 1:1-imidazolines				
R1	**$C_{17:1}$**	**$C_{17:2}$**	**$C_{17:3}$**	**$C_{20:3}$**
	350.5	348.5	346.5	374.5

3.3.3 *Solid-phase extraction (SPE)*

Following the results of the TLC analyses; (Section 3.3.1), a SPE system using columns packed with silica gel was investigated to determine whether active corrosion inhibitor components in CI-E1could be retained and subsequently eluted without decomposition and free from crude oil-derived interferents. The sequence of development of the SPE procedures is outlined below.

3.3.3.1 Solid-phase extraction of CI-E1 (SPE 1)

A sample of CI-E1 was extracted as described previously (Section 3.2.3; SPE1). Hexane and diethyl ether fractions were analysed by GC/MS whilst the IPA:NH_4 eluent fraction was analysed by ESI-MS. The GC/MS total ion chromatogram (TIC) of the hexane eluate comprised early eluting volatile components (Figure 3.6).The components of the main

chromatographic peaks (*e.g.* Figure 3.7) were tentatively identified by comparison with the online library (Chemstation software, Hewlett Packard) as a series of alkylbenzenes. The GC/MS chromatogram (TIC) of the diethyl ether eluate indicated later eluting, higher molecular weight components (Figure 3.8), which were tentatively identified from mass spectra as originating from naphtha (e.g. Figure 3.9). Thus the components eluted by hexane and diethyl ether were consistent with the designation of 'trimethylbenzenes' and 'heavy aromatic naphtha' made in MSDS sheets accompanying the CI-E1 product.

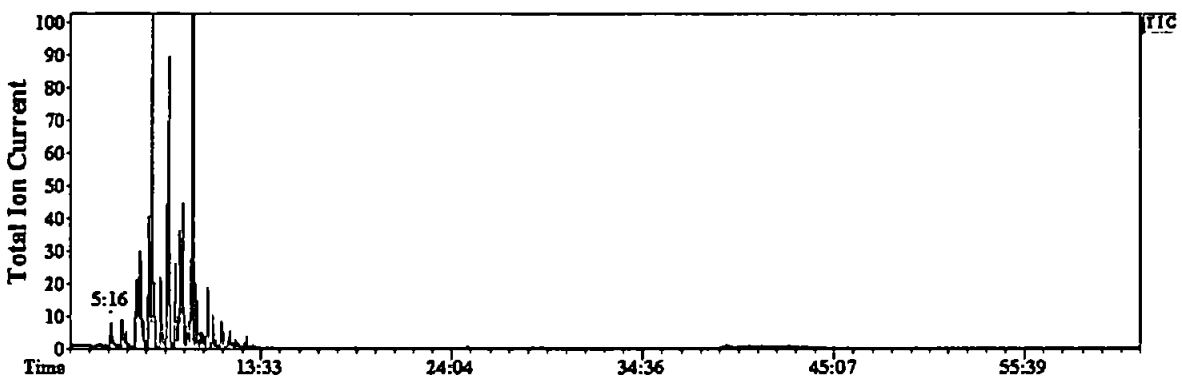


Figure 3.6 GC/MS total ion chromatogram of the SPE hexane eluate from CI-E1 corrosion inhibitor.

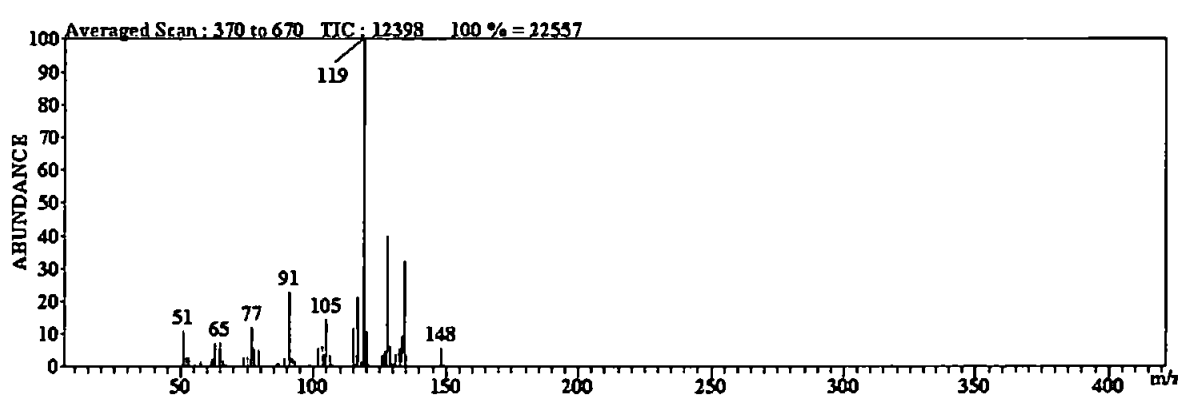


Figure 3.7 Averaged mass spectrum of the major chromatographic peaks shown in Figure 3.6.

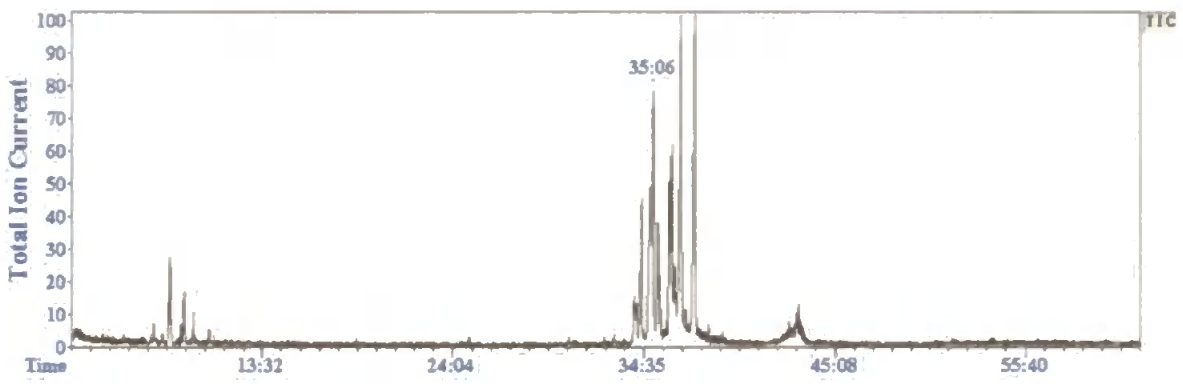


Figure 3.8 GC/MS total ion chromatogram of the diethyl ether SPE eluate from corrosion inhibitor CI-E1.

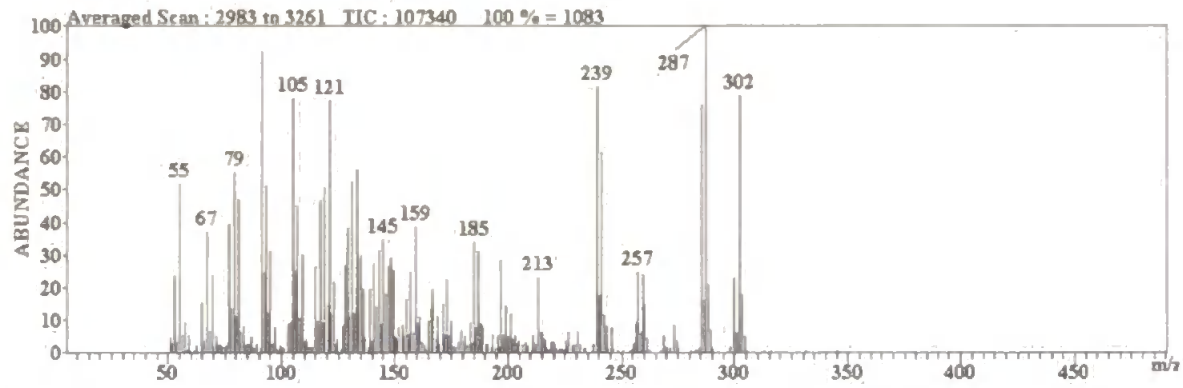


Figure 3.9 Averaged mass spectrum of the major chromatographic peaks shown in Figure 3.8.

The ESI-MS positive ion mass spectrum of the first 3 mL of IPA:NH_4 SPE eluate of CI-E1 (Figure 3.10), was similar to that of unfractionated product (Figures 3.2 and 3.3). The only significant difference was in the relative abundances of the 1:1- and 2:1-imidazoline ions. In the unfractionated product (Figure 3.3) *m/z* 610.7 (2:1-imidazoline) is the base ion with *m/z* 348.5 ion (1:1-imidazoline) at *ca* 30 % relative abundance, whereas the *m/z* 348.5 is the base ion whilst the *m/z* 610.7 is *ca* 70 % relative abundance. This may have been due to

partial fractionation of the 2:1- and 1:1-imidazolines during elution. However, this could not be confirmed as the second 5 mL IPA:NH_4 eluent fraction was not analysed, as the residue proved difficult to re-dissolve in IPA. The ion at *m/z* 388.5 was not observed in the spectra of unfractionated CI-E1 (Figures 3.2 and 3.3). The spectrum does not indicate any significant changes in the imidazoline/amide ratios (*viz* no alterations in the ratios of ions *m/z* 610.7 and 628.7) and therefore hydrolysis of the imidazoline components was considered to be insignificant.

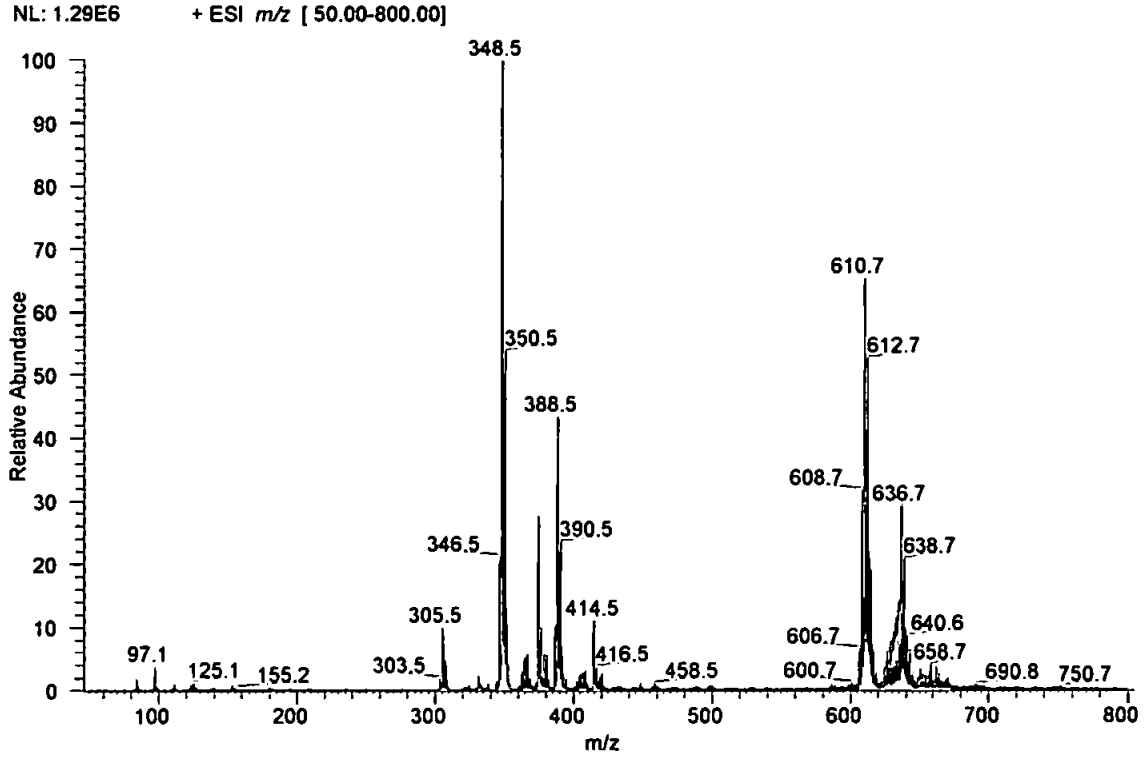


Figure 3.10 Infusion positive ion ESI-MS full scan mass spectrum *m/z* [50-800] of the propan-2-ol:ammonia eluent fraction (0-3mL) from CI-E1 corrosion inhibitor extraction SPE 1.

3.3.3.2 Crude oil and CI-E1 spiked crude oil solid-phase extraction (SPE 2 and 3)

A biodegraded North Sea crude oil from the Gullfaks Field[80,81] was extracted (Section 3.2.3; SPE 2) and the IPA:NH_4 eluent fraction (0-6 mL) analysed by positive ion ESI-MS. The mass spectrum of the crude oil eluate (Figure 3.11) contained a complex series of ions in the range *m/z* 200-1400 with a base peak ion at *m/z* 607.7. No peaks at *m/z* values corresponding to expected imidazoline or amides were detected.

The positive ion ESI-MS mass spectrum (Figure 3.12) of the IPA:NH_4 eluate (0-5 mL) extracted from the same oil spiked with 2000 ppm CI-E1 (Section 3.2.3; SPE 3) contained only ions due to the CI-E1 product components (*cf* Figures 3.2 and 3.3) and no discernible interference ions from the constituents of the crude oil. Although the spectrum was similar to that of unfractionated CI-E1 (Figures 3.2 and 3.3), the 2:1-imidazoline/1:1-imidazoline relative ion abundances were approximately equal, whilst in the spectrum of the same sample, analysed by flow injection ESI-MS the 2:1-imidazoline/1:1-imidazoline relative ion abundances are approximately 2:1 (Figure 3.13).

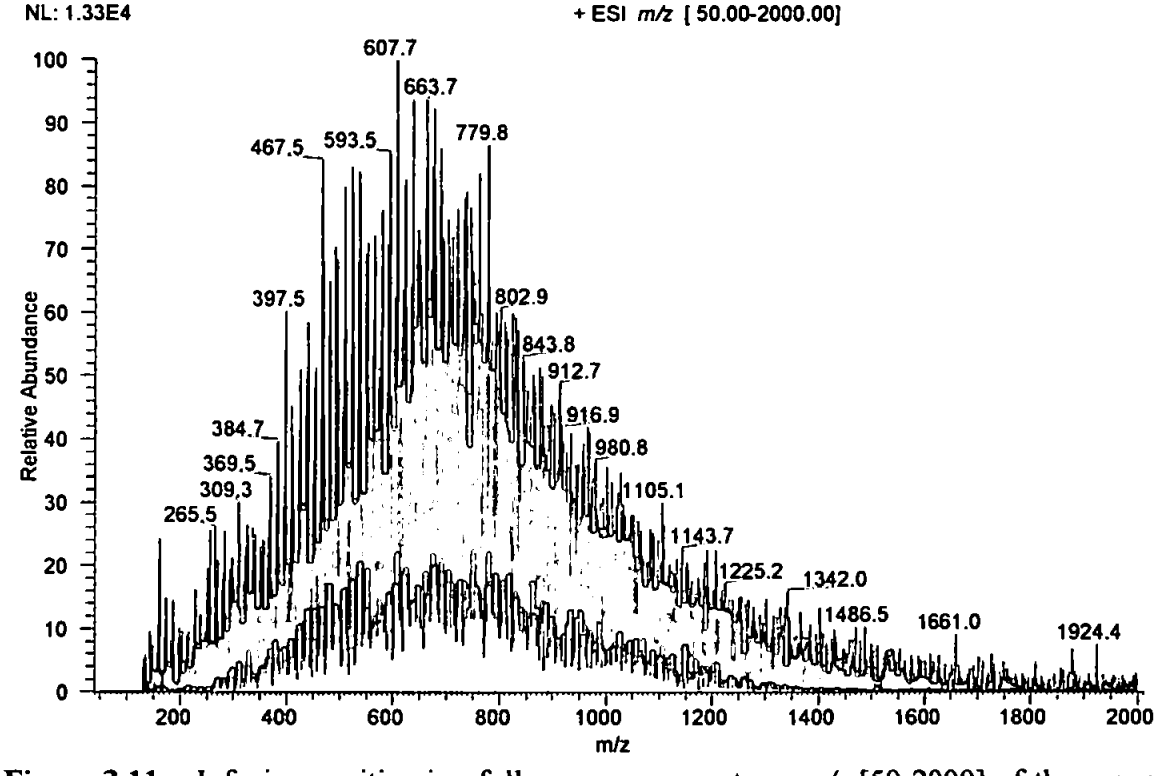


Figure 3.11 Infusion positive ion full scan mass spectrum *m/z* [50-2000] of the propan-2-ol:ammonia SPE eluate (0-5mL) from Gullfaks crude oil (SPE 2).

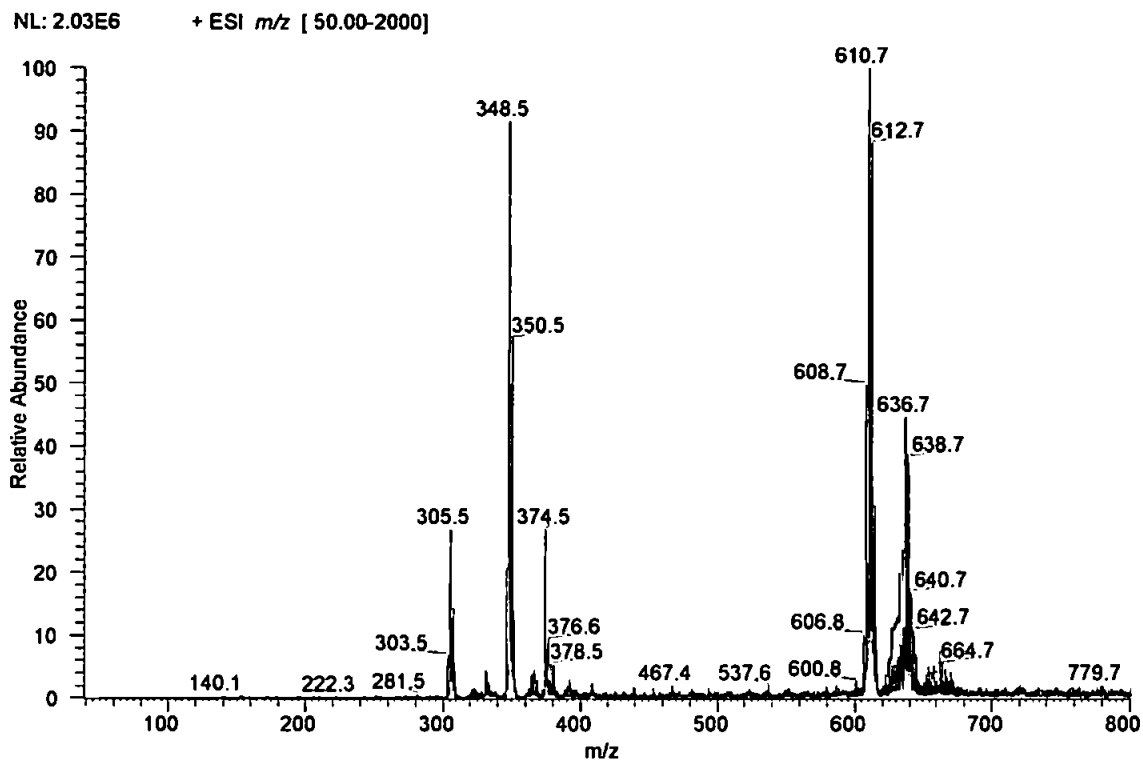


Figure 3.12 Infusion positive ion full scan mass spectrum [*m/z* 50-800] of the propan-2 ol:ammonia eluate (0-6mL) extracted by SPE from Gullfaks crude oil spiked with 2000 ppm CI-E1 (SPE 3).

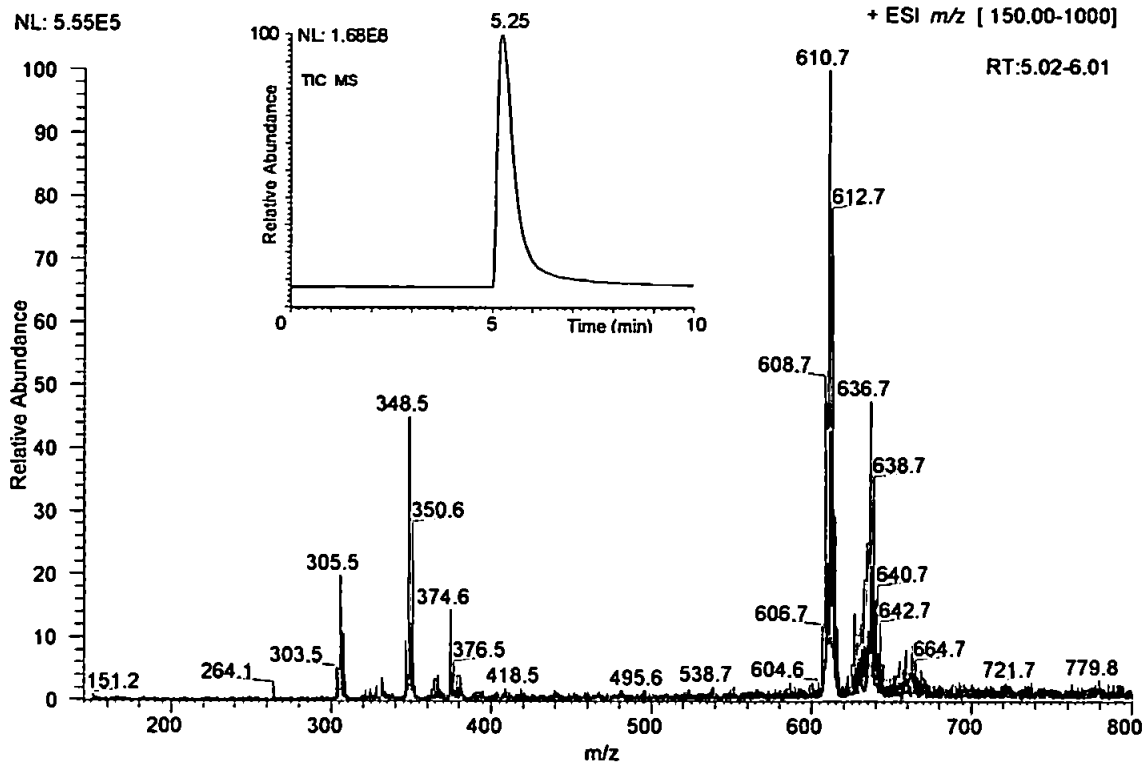


Figure 3.13 Flow injection positive ion full scan mass spectrum [*m/z* 50-800] (averaged across peak) of the propan-2-ol:ammonia eluate (0-6mL) extracted by SPE from a 2000 ppm CI-E1 spiked crude oil (SPE 3). Insert shows the total ion chromatogram of the 20 μL sample injection into a 150 μL min^{-1} 90:10 propan-2-ol:water eluent.

3.3.3.3 Conclusions from the initial solid phase extractions

From the mass spectral analyses of the eluates of initial solid phase extractions of CI-E1 and of crude oil spiked with CI-E1 at 2000 ppm, it can be concluded that the imidazoline/amide components are initially retained on the SPE column and may be readily separated from the 'trimethylbenzene', 'naphtha' formulation components and from crude oil constituents, by sequential elution. Hydrocarbons are first eluted with hexane and diethyl ether. The imidazoline/amide components could then be eluted with IPA:NH_4, blown down to dryness and re-dissolved in IPA without apparent decomposition or

interference from crude oil constituents at the nominal concentration of 2000ppm CI-E1 in the crude oil. This is a significant advance on any method published hitherto.

However, the ratios of 2:1-imidazoline/1:1-imidazoline ion groups in the unfractionated CI-E1 and the fraction obtained by SPE of spiked crude oil were different. For an imidazoline produced by reaction of two parts of fatty acid to one part diethylenetriamine,[52] the 2:1-imidazoline ions (*e.g. m/z* 610.7) would be expected to be at least twice as abundant as those of the 1:1-imidazoline (*e.g. m/z* 348.5). Indeed, this was seen in the mass spectra of unfractionated CI-E1 (Figures 3.2 and 3.3). However, in the infusion ESI-MS spectra of IPA:NH_4 eluates of SPE 1 & 3 (Figures 3.10 and 3.12) the ions derived from 1:1-imidazolines were as, or more abundant, than those arising from the 2:1-imidazolines . Such differences in ratios were also observed between the infusion and flow injection spectra of the same IPA:NH_4 eluate of SPE 3 (Figure 3.12 & 3.13) and may be attributable to instrument tuning and/or operating conditions. Differences in the MS conditions and solvents were also shown to produce different ion ratios when unfractionated CI-E1 was examined. The ESI-MS responses observed for the 2:1- and 1:1-imidazolines were at least an order of magnitude greater when an infusion of acidified methanol/water CI-E1 (Figure 3.3) was made, relative to an infusion in methanol/water (Figure 3.2). A number of other factors may have contributed to the apparent differences in 2:1-/1:1-imidazoline ion ratios. First, the volume of the IPA:NH_4 eluates in each fraction were not identical (SPE 1, 3 mL; SPE 3, 5 mL). Thus, different proportions of the 2:1-/1:1-imidazoline may have been eluted from each sample in these developmental extractions. This was also consistent with the partial separations observed in the TLC experiments. Second, the eluate from SPE 3 was not blown down to dryness and therefore did not require resolvation, unlike that from SPE 1. Differential solubility may have altered the ratios of the 2:1-/1:1-imidazolines in SPE 1.

3.3.3.4 Solid Phase Extraction of palmitic and oleic imidazolines from crude oil and analysis using HPLC/ESI-MS

To allow further development and optimisation of the SPE methods and to enable quantitation, an HPLC/ESI-MS system utilising pure, single, authentic compounds was needed. The use of HPLC should enable at least some separation of components, and standardisation of the solvent system would allow the ESI-MS responses to be calibrated for quantitative analyses. No literature detailing a HPLC/ESI-MS procedure for the separation of imidazoline and their amide precursor/hydrolysis compounds could be found. Although a reverse phase HPLC/ESI-MS procedure was used by Gough and Langley[52] for the analysis of oilfield chemicals, no details of the stationary or liquid phases or operating conditions were published. Development of a suitable HPLC system would also require pure authentic compounds of known quality.

The complexity and the potential of developing an HPLC/ESI-MS system was demonstrated by a preliminary investigation of CI-E1 components using a HyPurity Advance column (Hypersil, Thermo-Hypersil, Runcorn, UK). Figure 3.14 shows the total ion chromatogram of ~200 ng CI-E1 injected on column, with isocratic elution (methanol:water:formic, 70:30:0.1 v/v/v). A clear peak is seen at the solvent front (peak 1, Figure 3.14) followed by numerous broad unresolved peaks. Ions at *m/z* values typical of 1:1-imidazolines and monoamides (III and IV; Figure 3.1) were observed in the mass spectra of scans across peak 1 (*e.g.* Figure 3.15). The co-elution of 1:1-imidazolines and monoamide components at, or close to, the solvent front was shown by the corresponding extracted ion chromatograms (*e.g.* Figure 3.15). Mass spectra of peaks 2-5 (Figure 3.16) showed *m/z* values corresponding to the main 2:1-imidazoline components. Extracted ion chromatograms of the 2:1-imidazolines (*m/z* 608.7, 610.7, 612.7 and 614.7; Figure 3.17) clearly corresponded to peaks 2-5 respectively and demonstrated separation of the compounds. It was also apparent there was some separation of isomers as secondary peaks could be distinguished, particularly for ions *m/z* 610.7, 612.7 and 614.7. The total ion and

extracted ion chromatogram peaks are very broad suggesting that there were several competing interactions between the compounds and the solid phase and/or the solvent. As a first trial the results were promising, showing that separation was possible and compatible with ESI-MS detection. However, the complex nature of CI-E1 was evident and due to the large number and unknown quantities of each component the product would be unsuitable for further development of the system.

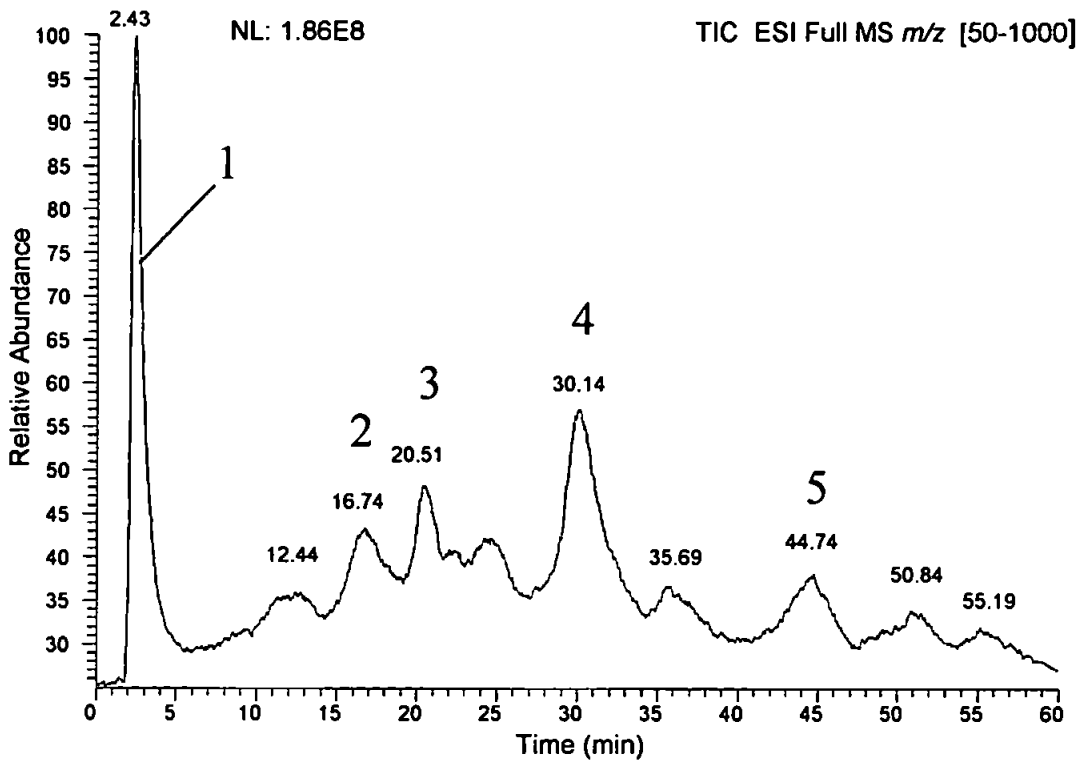


Figure 3.14 HPLC/ESI-MS Total ion chromatogram of CI-E1 corrosion inhibitor. HyPurity Advance column, 200 ng CI-E1 on column and isocratic elution with methanol:water:formic 70:30:0.1 v/v/v (See text for details of peaks 1-5).

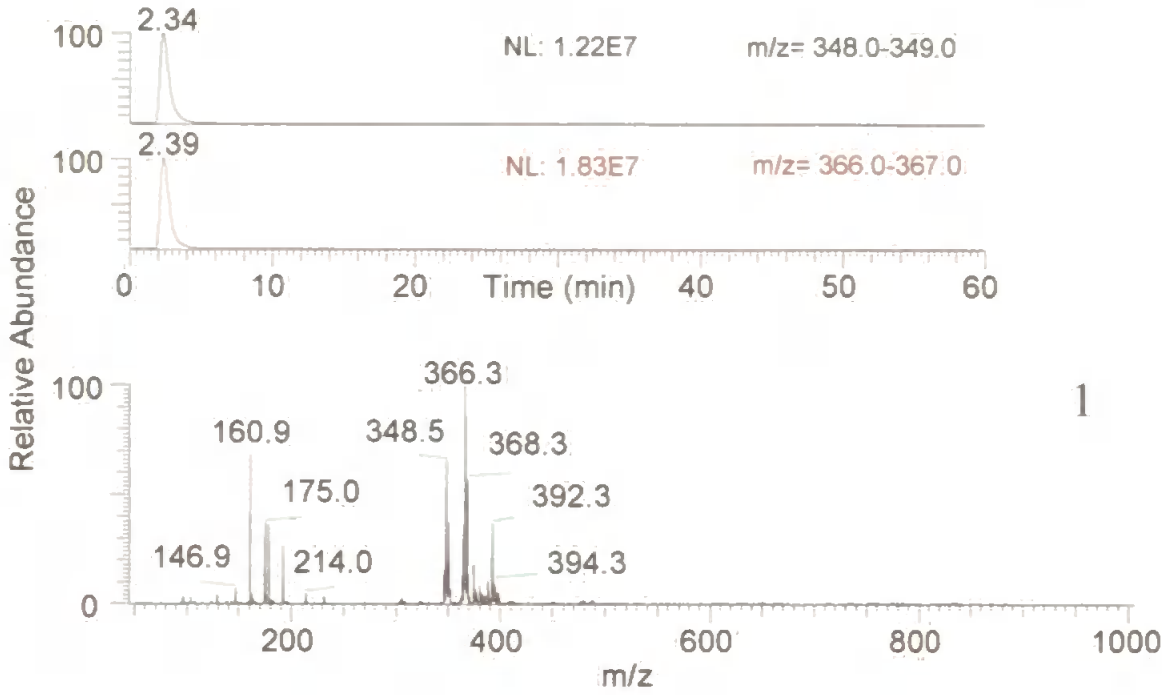


Figure 3.15 Extracted ion mass chromatograms of the main 1:1-imidazoline (*m/z* 348.5) and monoamide (*m/z* 366.5) observed in CI-E1 and the mass spectrum of peak 1 shown in Figure 3.14.

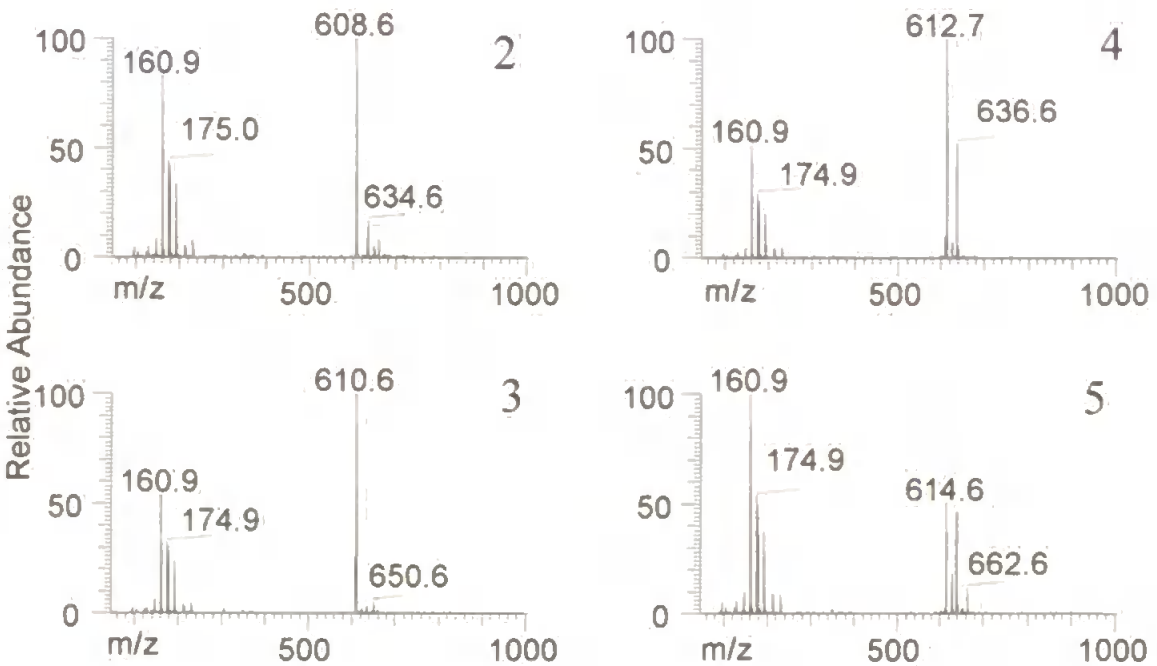


Figure 3.16 Positive ESI-MS mass spectra of peaks 2-5 shown in Figure 3.14.

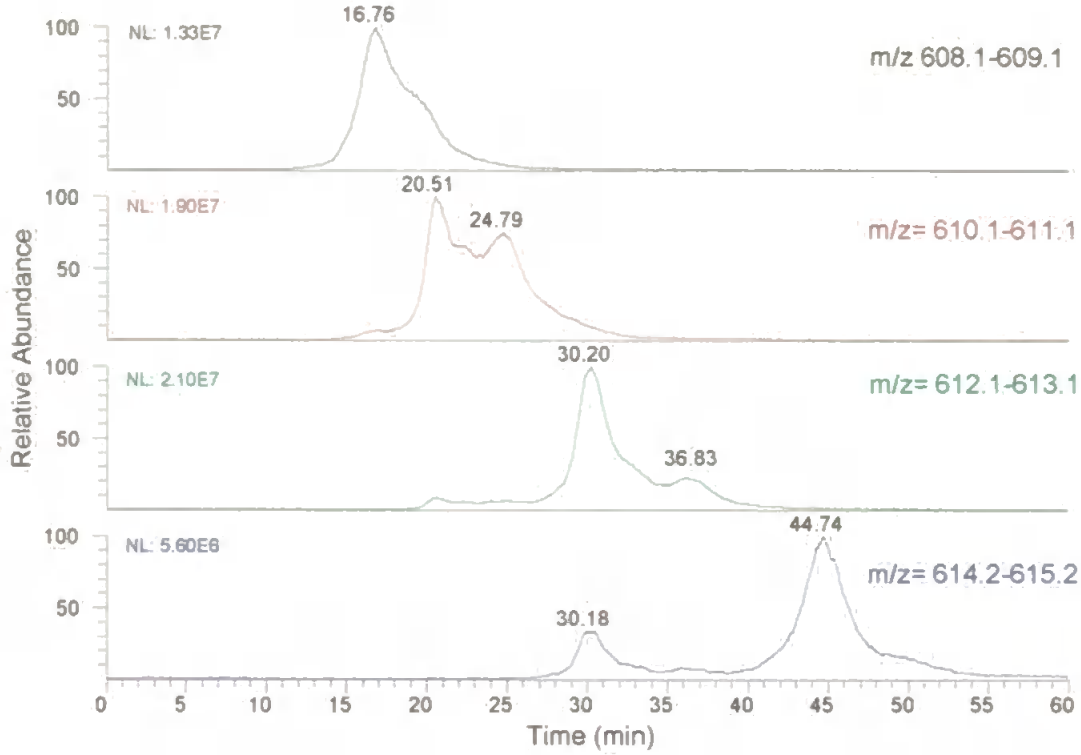


Figure 3.17 Extracted ion mass chromatograms (from TIC Figure 3.14) of the main 2:1-imidazolines observed in CI-E1: Top to bottom *m/z* 608.7, 610.7, 612.7 and 614.7.

3.3.4 *Authentic compounds*

Oleic and palmitic acid-derived imidazolines compounds were synthesised and a HPLC/ESI-MS system developed, simultaneously (Chapter 4). Indeed, an investigation of the synthesis of imidazolines by thermal reaction of fatty acids with diethylenetriamine was carried out as described in Chapter 4 and 5. 2:1-Oleic imidazoline (2:1-OI) was used for further systematic investigation of the SPE extractions because it is known to be one of the major components in imidazoline corrosion inhibitor products and indeed was observed by ESI-MS of the CI-E1 product (Section 3.3.2). 2:1-Palmitic imidazoline (2:1-PI) was chosen as an internal standard spike for quantitation because it is not present in the CI-E1 product; the protonated molecular ion at *m/z* 562.6 falls between that of the protonated C_{18} 1:1-imidazoline/monamide and 2:1-imidazoline/diamide compounds, because it was

expected to behave in a somewhat physically and chemically similar manner to the major C_{18} imidazoline compounds, and because palmitic acid is readily available at low cost. Although deuterated analogues of analytes are conventionally used as internal standards for mass spectral quantitation,[82] deuterated $C_{18:1}$ imidazoline was not chosen in the present study as the molecular mass of a mono-deuterated OI would be isobaric with some of the isotopic masses of imidazolines expected to be present in the commercial products. An efficient synthesis of imidazolines was still to be determined and therefore the cost of multi-deuterated analogues was prohibited.

3.3.5 *HPLC/ESI-MS*

A reverse phase HPLC separation was developed using a gradient elution profile (Table 3.5) which allowed separation of the synthetic 2-alkylimidazolines, 1:1-imidazolines, 2:1-imidazolines, monoamides and diamides. For convenience the development of the HPLC/ESI-MS method is discussed in conjunction with the imidazoline syntheses in Chapter 4.

3.3.6 *Solid-phase extraction of crude oil spiked with 2:1-palmitic imidazoline (2:1-PI; SPE 4 and SPE 5)*

3.3.6.1 Solid-phase extraction of 2:1-palmitic imidazoline spiked crude oil (5 μg 2:1-PI g oil^{-1}; SPE 4)

A Middle East crude oil was supplied from an oilfield in which the drill well had been treated ('squeezed')[79] with imidazoline-based corrosion inhibitors. A 1g sample of the oil was spiked with 5μg (5ppm in oil) 2:1-PI as an internal standard, and extracted (Section 3.2.4; SPE 4). As this was a first extraction using commercial Isolute silica cartridges, the

volumes of eluate were determined by applying aliquots until hexane eluted with a light yellow (9 mL) and diethyl ether with a very slight yellow colouration (30 mL). To ensure elution of analytes the volume of eluent used was about five times the sorbent volume (10 mL). The dried IPA:NH_3 eluate was re-solvated in 500 µL 0.1% TFA in methanol solvent, equivalent to a two times pre-concentration. Since at this stage, the HPLC/ESI-MS method was under development, the re-solvated extract was examined four times with small changes in the gradient for the first three analyses and an increase in the injection volume from 5 to 20 µL for the fourth analysis (Table 3.2; Section 3.2.4.1). Positive ion ESI-MS was carried out in full scan mode (*m/z* 100-1000) and selected ion monitoring mode *(m/z* 562.6 and 324.4).

The *m/z* 562.6 and *m/z* 324.4 selected ion monitoring (SIM) chromatograms showed peaks at retention times expected for the 2:1-PI standard (R and R_1 = $C_{15}H_{31}$, structure VIII, Figure 3.1; Chapter 4 contains details of analysis and *m/z* values of 2:1-PI and 2:1-OI). Single ion monitoring of *m/z* 324.4 for 1:1-PI (R = $C_{15}H_{31}$, structure VII, Figure 3.1) a minor impurity in the 2:1-PI internal standard, showed small peaks with signal to noise ratios of 2-3 and was therefore at the limit of detection. Extracted ion chromatograms (EIC) for *m/z* 324.4 showed no peaks. Peak retention time, area, height and signal to noise ratio for SIM and EIC of *m/z* 562.6 are presented in Tables 3.17 and 3.18. It was clear from the peak area data that the change from 5 to 20 µL injections (analyses 3 and 4, respectively; Table 3.17 and 3.18) resulted in a 2.5 times increase in response for both peak area and height. It was also observed that SIM was twice as sensitive as the EIC for *m/z* 562.6.

Table 3.17 Peak data determined from HPLC/ESI-MS chromatograms of single ion monitoring of *m/z* 562.6 (positive ion) for internal standard 2:1-palmitic imidazoline spiked into crude oil (extraction SPE 4)

Single ion monitoring chromatograms, *m/z* 562.6				
Analysis	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
1	16.87	277607527	7643447	347
2	16.75	333322537	8734094	221
3	13.04	238168435	6695585	252
4	13.07	589072070	16916800	244

Table 3.18 Peak data determined from HPLC/ESI-MS extracted ion chromatograms of *m/z* 562.6 (positive ion) for internal standard 2:1-palmitic imidazoline spiked into crude oil (extraction SPE 4)

Extracted ion chromatograms, *m/z* 562.6				
Analysis	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
1	16.92	141134837	4088380	61
2	16.73	169583445	4660826	87
3	13.05	130226114	3800347	107
4	13.06	313464454	9307471	63

Having confirmed that there was some recovery of the internal standard, *m/z* values corresponding to the main C_{18} 2:1-imidazoline, 1:1-imidazoline, diamides and monoamides compounds were extracted from the full scan data. The only *m/z* values to show evidence of a peak were at *m/z* 610.7, 612.7 and 614.7 (Figure 3.18). However, the peaks were at the limit of detection with signal to noise ratios (S/N) of 8, 3 and 3, respectively (Table 3.19). Analysing the mass spectra from these peaks (Figure 3.19), indicated that the extracted ion *m/z* 614.7 (actual *m/z* extracted is ± *m/z* 0.4; Figure 3.18c; for a $C_{18:1}$/ $C_{18:1}$ 2:1-imidazoline), corresponded to the observed mass *m/z* 614.7 (Figure 3.19c). The observed m/z for the extracted ions *m/z* 612.7 and 610.7 peaks were *m/z* 612.0 and 610.0 respectively (Figure 3.19d and e), a significant *m/z* 0.7 difference. The generally

observed variation in *m/z* values is normally ± *m/z* 0.2, therefore, although the *m/z* 612 and 610 peaks are at retention times estimated for $C_{18:1}$/ $C_{18:2}$, $C_{18:2}$ / $C_{18:2}$ 2:1-imidazoline, there is considerable doubt over their identities. Indeed, when extracted ion chromatograms of *m/z* 612.0 and 610.0 were examined (Figure 3.20), identical retention times were observed with an increase in peak area, height and signal to noise values (Table 3.19). Extraction of ion chromatogram *m/z* 614.0 (Figure 3.20) resulted in a small rise in the base line noise at the same retention time (12.75 minutes) as *m/z* 612.0 and 610.0, but no peak at the retention time observed for the extracted ion *m/z* 614.7 (R_t =14.68 min). This may suggest that the peak at *m/z* 614.7 with a signal to noise ratio of 8, and a 2x pre-concentration is evidence for a very low concentration of $C_{18:1}$/ $C_{18:1}$ 2:1-imidazoline being present in the sample.

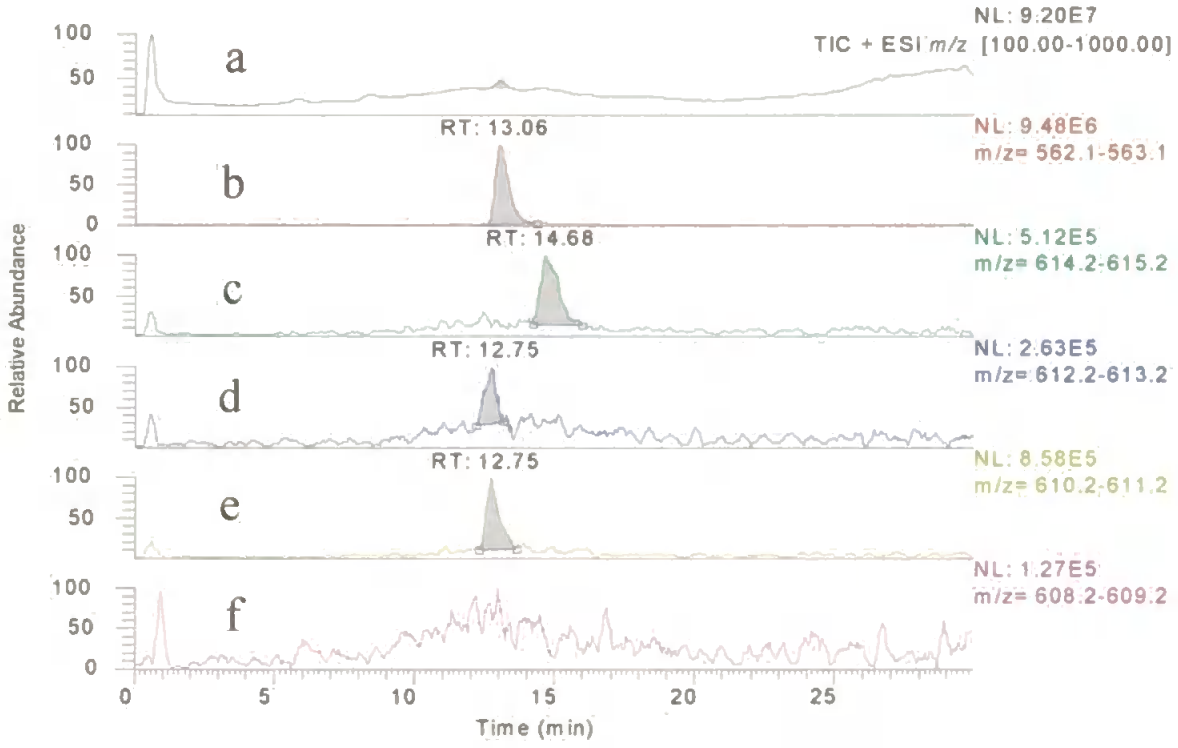


Figure 3.18 HPLC/ESI-MS chromatograms of internal standard 2:1-palmitic imidazoline (5 µg g oil^{-1}) spiked crude oil extraction SPE 4. a. Total ion chromatogram (*m/z* 100-1000), b. extracted ion chromatogram of internal standard (*m/z* 562.6), c-f. Main C_{18} 2:1-imidazoline corrosion inhibitor components (*m/z* 614.7, 612.7, 610.7 and 608.7, respectively).

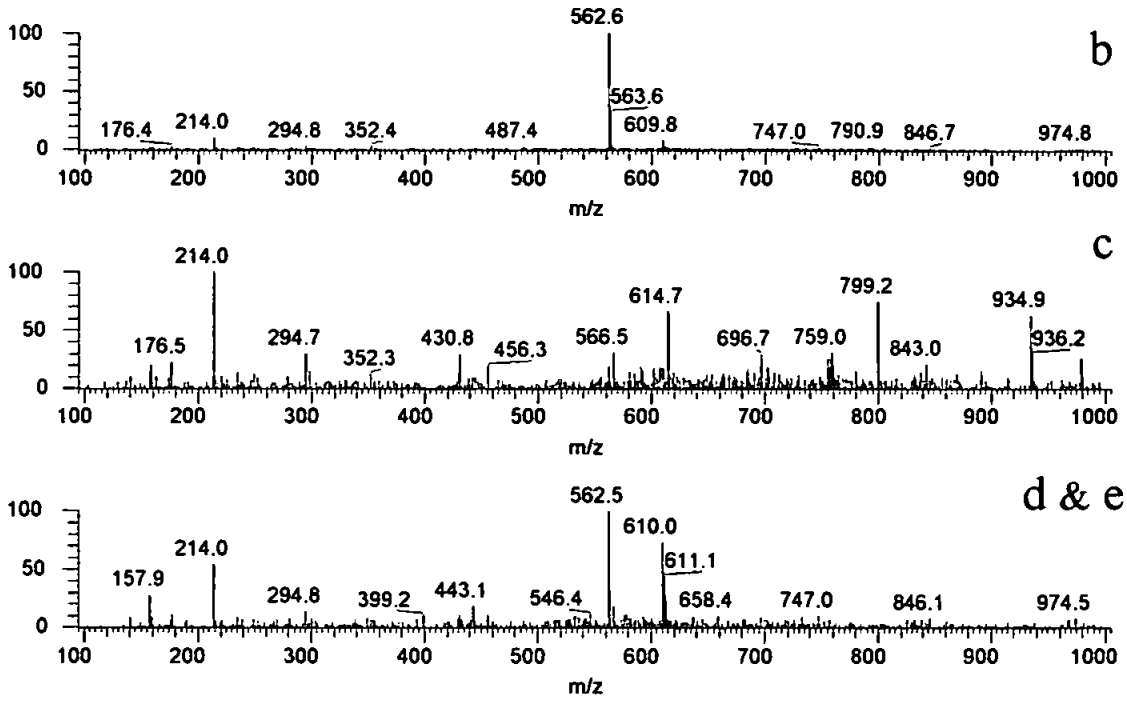


Figure 3.19 Mass spectra at the peak retention time, for peaks b, c and d/e shown in Figure 3.18.

Table 3.19 Peak data determined from HPLC/ESI-MS extracted ion chromatograms of analysis 4 for internal standard 2:1-palmitic imidazoline spiked into crude oil (extraction SPE 4)

Extracted ion chromatograms of SPE 4, analysis 4				
EIC *m/z*	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
614.7	14.68	19725010	439970	8
612.7	12.75	5162535	183293	3
610.7	12.75	23807243	765256	8
612.0	12.75	17061536	392760	10
610.0	12.75	48277662	1376148	24

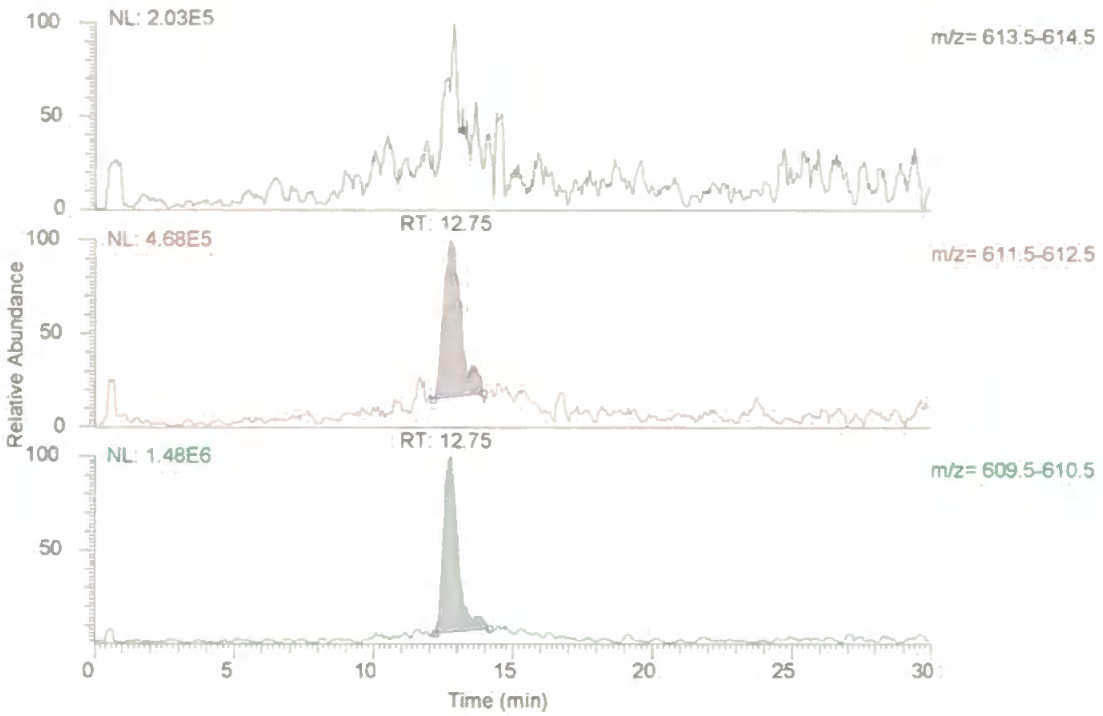


Figure 3.20 HPLC/ESI-MS extracted ion chromatograms of crude oil extraction SPE 4. Top to bottom, *m/z* 614.0, 612.0 and 610.0.

3.3.6.2 Solid-phase extraction of 2:1-palmitic imidazoline spiked crude oil (1 μg 2:1-PI g oil $^{-1}$; SPE 5)

Following further developments in synthesising palmitic and oleic imidazoline standards, a chromatographic gradient elution profile (Table 3.5) was developed that provided retention and separation of the main compounds without excessive peak broadening for the diamides observed in the synthesis of the standard compounds. This gradient was used for all subsequent studies and allowed direct comparison between analyses. A separation of the commercial inhibitor product CI-E1 was carried out (section 4.3.10; Chapter 4) and provided retention data for the compounds, other than the standards, expected to be observed in extractions of crude oil, if present. This information was not available at the time of the previous extraction (*SPE 4*) and for the gradient used. A repeat extraction of the crude oil spiked with the internal standard at 1μg g oil^{-1} was carried out (Section 3.2.4.2; SPE 5). Again, recovery of the internal standard was observed, and peak retention

time, area, height and signal to noise ratios could be determined from the extracted ion chromatograms of duplicate analyses of the extract (Table 3.20). Extracted ion chromatograms of the main C_{18} compounds *m/z* values did not exhibit any peaks. However a broad hump was observed in the base line for the main 2:1-imidazolines (Figure 3.21) that was not seen in the equivalent solvent blank extracted ion chromatogram (Figure 3.22). The hump maximum responses were similar to the expected retention times for the compounds. As the reconstituted extract was equivalent to the original sample (residue made up to 1 mL), then compared to the previous extract (*SPE 4(1)*) in which the response for *m/z* 614.7 was close to the limit of detection, then no peak would be expected. The oil for further spiking experiments was essentially C_{18} 2:1-imidazoline, 1:1-imidazoline, diamide and monoamide free.

Table 3.20 Peak data determined from HPLC/ESI-MS extracted ion chromatograms of *m/z* 562.6 (positive ion) for 1 μg g^{-1} internal standard 2:1-PI spiked into crude oil (SPE 5)

Extracted ion chromatograms, *m/z* 562.6				
Analysis	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
1	14.44	28885000	1273951	33
2	14.47	42378559	1868293	29

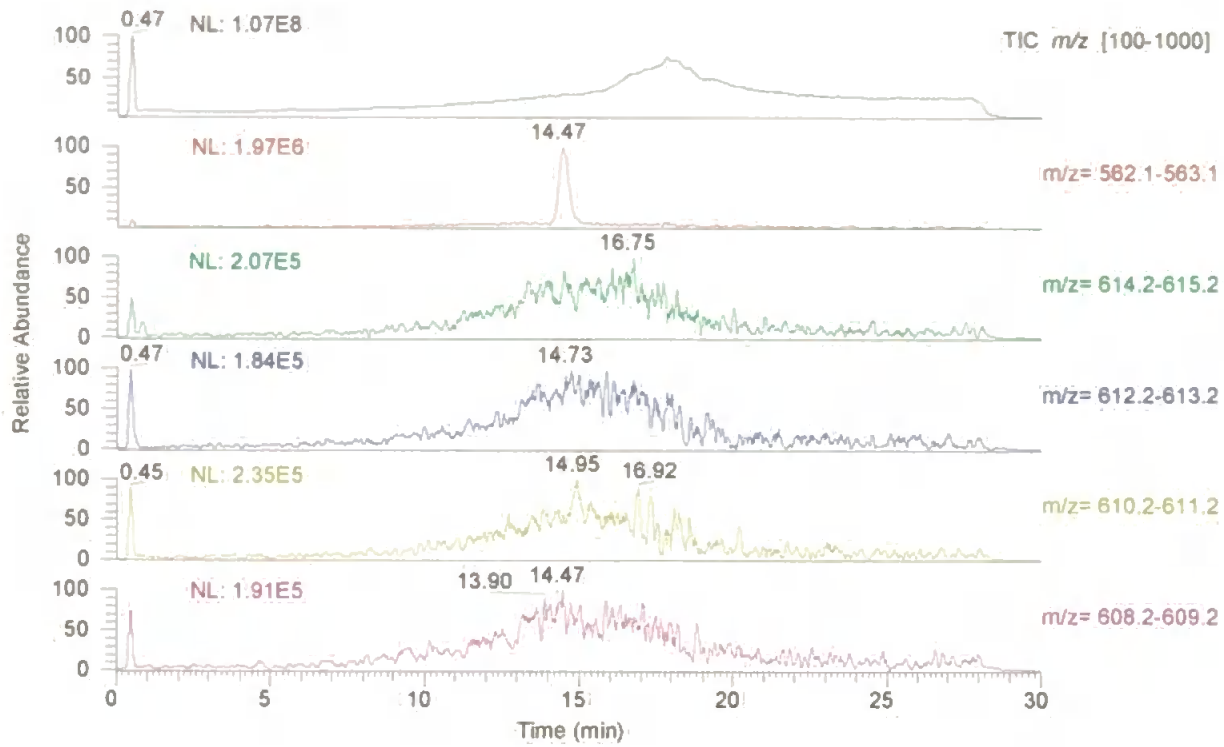


Figure 3.21 HPLC/ESI-MS chromatograms of internal standard 2:1-PI (1μg g^{-1}) spiked crude oil extraction SPE 5. a. Total ion chromatogram (*m/z* 100-1000), b. Extracted ion chromatogram of 2:1-palmitic imidazoline internal standard (m/z 562.6), c-f. Main C_{18} 2:1-imidazoline corrosion inhibitor components (*m/z* 614.7, 612.7, 610.7 and 608.7, respectively).

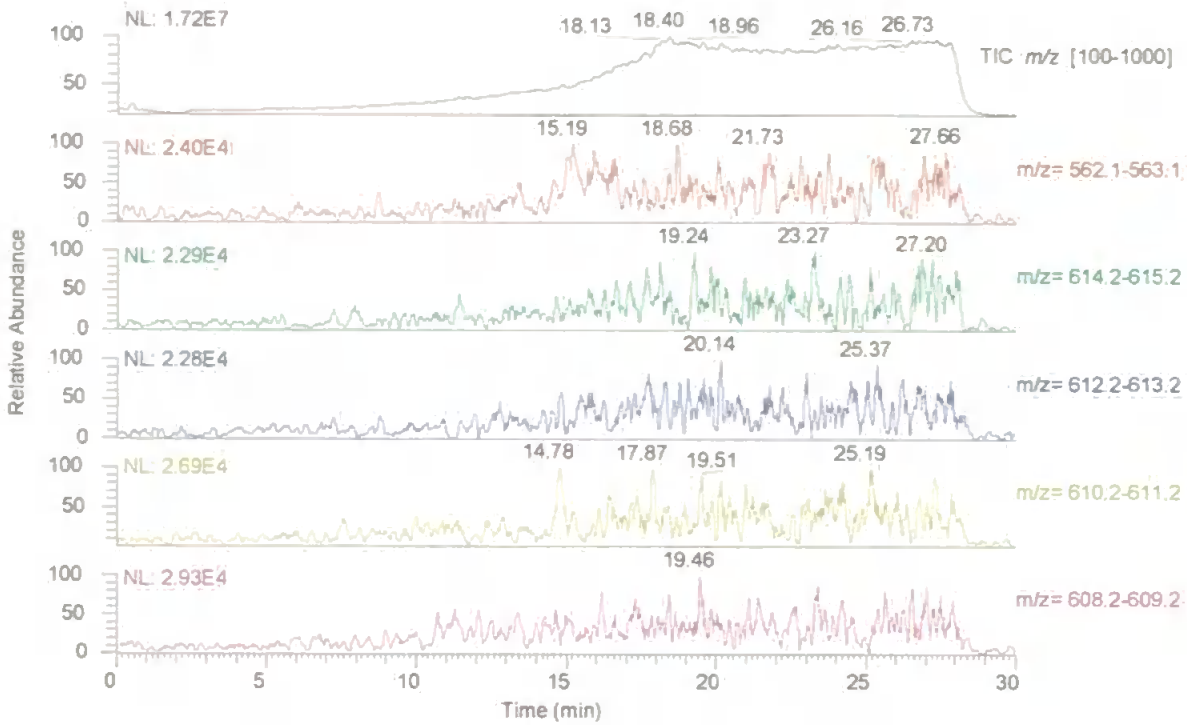


Figure 3.22 HPLC/ESI-MS chromatograms of solvent blank injection for crude oil extraction SPE 5. a. Total ion chromatogram (*m/z* 100-1000), b. Extracted ion chromatogram of 2:1-palmitic imidazoline internal standard (*m/z* 562.6), c-f. Main C_{18} 2:1-imidazoline corrosion inhibitor components (*m/z* 614.7, 612.7, 610.7 and 608.7, respectively).

3.3.6.3 Solid-phase extraction of crude oil spiked with 2:1-palmitic and 2:1-oleic imidazolines (1 μg 2:1-PI and 1 μg 2:1-OI g^{-1}; SPE 6)

To further investigate the recovery, a 1g sample of the oil was spiked with 1 μg 2:1-palmitic and 1 μg 2:1-oleic imidazolines. The 1 μg g^{-1} of each component was used initially because recovery of 2:1-palmitic imidazoline at the 1 μg g oil^{-1} was detected in the previous extraction (SPE 5) and was detected at concentrations lower than established methods. As no external calibration had been developed, a 1 μg mL^{-1} solution made up from the same standards used to spike the oil was analysed in order to provide a reference response. The 1 mL re-solvated extracted residue was divided into two samples (S1, first

500μL and S2, remaining solution). The extracted ion chromatogram data acquired from the extract samples S1, S2 and the standard are given in Table 3.21. Recovery of both 2:1-palmitic and 2:1-oleic imidazoline was evident as observed by peaks at expected retention times with expected *m/z* values. However, it was obvious that the response for 2:1-palmitic imidazoline was greater than 2:1-oleic imidazoline. Relative to the standard, the peak area for 2:1-palmitic imidazoline was 37% and 25% for sample S1 and S2 respectively compared to 4% and 2% for 2:1-oleic imidazoline from the same samples. A decrease in response was observed for sample S2 relative to sample S1, for 2:1-palmitic and 2:1-oleic imidazoline (66% and 47 %, respectively). The extraction of both compounds was poor compared to the standard response but recovery of 2:1-oleic imidazoline was extremely low.

Table 3.21 Peak data determined from HPLC/ESI-MS extracted ion chromatograms of *m/z* 562.6 and 614.7 (positive ion) for 2:1-PI and 2:1-OI spiked into crude oil (1.12/1.04 μg g^{-1} oil)

Extracted ion chromatograms, *m/z* 562.6				
Analysis	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
Standard	14.96	178625276	5909611	225
S1	15.31	65863295	2708316	78
S2	14.72	43782861	1665033	69
Extracted ion chromatograms, *m/z* 614.7				
Analysis	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
Standard	15.84	141509611	53533052	375
S1	16.16	6043503	228639	6
S2	15.64	2834681	123589	5

3.3.6.4 Solid-phase extraction of hexane spiked with 2:1-palmitic and 2:1-oleic imidazolines (1 μg 2:1-PI and 1 μg 2:1-OI mL^{-1} hexane; SPE 7)

To investigate the reasons for the poor recoveries and the difference between recovery of 2:1-palmitic and 2:1-oleic imidazolines, an extraction of spiked hexane was carried out using the same procedure (Section 3.2.4.2; SPE 7). From the extracted ion chromatogram data of a 1ug mL^{-1} standard and the extract (Table 3.22), a 42% and 24% area response of the 2:1-palmitc and 2:1-oleic imidazolines relative to the standard was observed respectively. At 42% the increase in response for 2:1-palmitic imidazoline was small (5-15%) compared to the extracted oil (*SPE 6*) and could easily have been attributable to variations in instrumental or experimental conditions. The 24% response for 2:1-oleic imidazoline was up to 20% greater than observed in the oil extraction. This would suggest the oil matrix was affecting recovery of 2:1-oleic imidazoline to a greater extent than the 2:1-palmitic imidazoline compound. Although the recoveries were increased when a refined solvent (hexane) was used compared to the crude oil, they were still less than 42%. As one of the important physical properties of 2:1-imidazoline is strong sorption to surfaces, particularly metallic surfaces, sorption to exposed metal surfaces may have caused the loss of analytes. A stainless steel needle was used in the transfer of sample from the vial containing the reconstituted extract residues to a glass syringe for expulsion through a syringe filter (PVDF, polypropylene housing) and was considered likely to be responsible for the loss of analytes. A simple test was carried out in which an auto sampler vial containing 1 μg mL^{-1} each of 2:1-palmitic and 2:1-oleic imidazolines standards was analysed and then transferred to another vial using the filtration method above. The result (Table 3.23) shows a 39 % reduction in peak area for 2:1-palmitic imidazoline and 51 % reduction for 2:1-oleic imidazoline. These were significant losses for a 1 μg mL^{-1} standard. A more detailed investigation would be required not only for the filtration procedure at different concentrations but also for the sample vials used for preparation and collection, and glass capillaries used for transfer of sample to the extraction cartridge. Teflon, plastic

or silanized glassware and equipment needs to be compared for sorption of analytes in order to provide an optimum system for analyses.

Table 3.22 Peak data determined from HPLC/ESI-MS extracted ion chromatograms of *m/z* 562.6 and 614.7 for 2:1-PI and 2:1-OI spiked into hexane (1.01/1.04 μg mL^{-1} hexane)

Extracted ion chromatograms, *m/z* 562.6				
Analysis	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
Standard	14.71	188930407	5978190	76
S1	14.76	79161052	2791403	205
Extracted ion chromatograms, *m/z* 614.7				
Analysis	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
Standard	15.68	211266279	6787619	186
S1	15.66	51297101	1852033	105

Table 3.23 Peak data determined from HPLC/ESI-MS extracted ion chromatograms of *m/z* 562.6 and 614.7 for 2:1-PI and 2:1-OI standards before and after filtration (1.01/1.04 μg mL^{-1})

Extracted ion chromatograms, *m/z* 562.6				
Analysis 1.01 μg mL^{-1}	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
Unfiltered	14.95	262277819	8590937	89
Filtered	14.98	160170362	5828561	97
Extracted ion chromatograms, *m/z* 614.7				
Analysis 1.04 μg mL^{-1}	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
Unfiltered	15.88	314545566	9884390	1653
Filtered	15.92	152762911	5171078	125

3.3.6.5 Comparison of solid-phase extraction methods for crude oil spiked with 2:1-palmitic and 2:1-oleic imidazolines (1 μg 2:1-PI and 10 μg 2:1-OI g oil^{-1}; SPE 8 and 9)

To compare the extraction method being developed with a confidential SPE procedure used for analyses of oils, extractions of oil samples spiked with 2:1-palmitic and 2:1-oleic (1 μg & 10 μg g oil $^{-1}$) were carried out using both methods. Comparison of the peak area responses (Tables 3.24 and 3.25) indicates that the method being developed was 50% and 60% more efficient at extracting 2:1-palmitic and 2:1-oleic (average of duplicate analyses of each extract).

Table 3.24 Peak data determined from HPLC/ESI-MS extracted ion chromatograms of *m/z* 562.6 and 614.7 (positive ion) for 2:1-PI and 2:1-OI spiked into crude oil (1.12/10.4 μg g^{-1})

Extracted ion chromatograms, *m/z* 562.6				
Analysis	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
S1	15.17	45322942	1928019	30
S2	15.10	43934279	1938563	53
Extracted ion chromatograms, *m/z* 614.7				
Analysis	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
S1	16.04	343452238	12767957	280
S2	16.02	355988605	12747084	306

Table 3.25 Peak data determined from HPLC/ESI-MS extracted ion chromatograms of *m/z* 562.6 and 614.7 (positive ion) for 2:1-PI and 2:1-OI spiked into crude oil (1.12/10.4 μg g^{-1}) and extracted by a confidential SPE method

Extracted ion chromatograms, *m/z* 562.6				
Analysis	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
S1	14.79	20809552	860821	41
S2	14.75	24158678	1001944	44
Extracted ion chromatograms, *m/z* 614.7				
Analysis	**Peak Retention Time (min)**	**Peak Area**	**Peak Height**	**Signal to Noise Ratio**
S1	15.69	148121788	5433384	196
S2	15.66	157185149	5539308	197

3.4 Conclusion

A semi-quantitative but sensitive and specific method, involving SPE followed by HPLC/ESI-MS, has been developed for the determination of individual imidazolines at low (<10) parts per million concentrations in crude oils. Whilst the method requires further optimisation due to the high experimental losses of imidazolines during work-up, it nonetheless represents a considerable improvement on previous techniques and should prove valuable for operational and environmental studies of corrosion inhibitor and surfactant behaviour.

CHAPTER FOUR

Development of high performance liquid chromatography (HPLC) separation of alkyl-imidazolines and amides with detection by electrospray ionisation mass spectrometry (ESI-MS)

This chapter describes the development of high performance liquid chromatography (HPLC) separation of alkyl-imidazolines and amides with detection by electrospray ionisation mass spectrometry (ESI-MS). The synthesis of authentic palmitic and oleic 2:1 and 1:1-imidazoline is also described.

4.1 Introduction

Liquid chromatography (LC) with atmospheric pressure ionisation mass spectrometry (API-MS) detection has been used for determination of many anionic, nonionic and cationic surfactants in industrial waste waters, aquatic and marine waters.[45,61,63,69,83-87] However, there are relatively few reports of LC separation methods for alkyl-imidazolines and related amides.[52,88,89] Neither are such compounds amenable to gas chromatographic separation due to their high polarities and low volatilities. The lack of separation and sensitive detection methods has made attempts to investigate the partitioning behaviour of these compounds difficult.[43]

ESI-MS has been shown to be a powerful detection method for these compound types[43,52] (Chapter 2 and 3). However, flow injection ESI-MS does not allow for separation of the individual components of products or of environmental extracts. A liquid chromatography technique coupled to the ESI-MS detector would enable separation of the individual components of imidazoline corrosion inhibitor products and interferences from co-extracted compounds. The liquid chromatographic properties determined from HPLC experiments might also be indicative of conditions needed for solid-phase extraction absorbents and elution solvents for extraction of analytes from environmental waters such as oilfield Produced Water and their discharges in the marine environment.

The aim of this investigation was therefore to develop a LC/ESI-MS method for the separation and quantitation of alkyl-imidazoline and amide components of corrosion inhibitors used in oilfield applications.

4.2 Experimental

4.2.1 *Chemicals*

Ultra pure water was obtained from an Elgastat filtration system (Elga, High Wycombe, UK). All solvents used were HPLC grade except glass distilled diethylether (ether; Rathburn Chemicals Ltd, Walkerburn, UK and BDH, Poole, UK). HyPersolv grade methanol (MeOH), propan-2-ol (IPA), ammonia (0.88), trifluoroacetic acid (TFA), formic acid and xylene (sodium-dried) were from BDH. Dichloromethane (DCM), ethanol (EtOH), hexane, acetone and toluene were from Rathburn. Sodium acetate (AcNa, 99%) was Aristar grade from BDH. Diethylenetriamine (DETA, 98.5%) and oleic acid (97%) were supplied by ACROS Organics Ltd (Geel, Belgium). Heptafluorobutyric acid (99%), palmitic and oleic acids (90%) were from Aldrich Chemical Company Inc. (Milwaukee, WI, USA). The corrosion inhibitor intermediate product CI-E1 was from a commercial supplier.

4.2.2 *High performance liquid chromatography (HPLC)*

HPLC was carried out using a P580A binary pump (Dionex-Softron GmbH, Germering, Germany). 1mL min^{-1} flow rate was split post column (high pressure micro-splitter valve; Upchurch Scientific Ltd, Oak Harbor, WA, USA), with ~200 µL min^{-1} to the mass spectrometer and the residue to a UV6000LP diode array detector (Thermoquest, San Jose, CA,USA). 200 and 150 µL min^{-1} flow rates used with 3mm and 2.1 mm diameter columns were direct to the mass spectrometer. Sample injections (5 and 20 µL) were made with an ASI 100 autosampler (Dionex-Softron). HPLC columns investigated were; a stainless steel column (3 x 100 mm; Alltech Associates Applied Science Ltd, Carnforth, UK.) packed with Absorbosphere C_{18} (5µm; Alltech), amino-bonded C_8 (Advance; 150 x 2.1, 3 µm, ThermoHypersil, Runcorn, UK), porous graphitic carbon (PGC; Hypersil Hypercarb, 100 x

2.1, 5 µm, ThermoHypersil), a stainless steel column (2.1 x 100 mm; Alltech) packed with a hypercrosslinked polystyrene resin (MN200, 5µm; Purolite Int., Moscow, Russia) and poly(styrene-divinylbenzene) (PRP-1; 50 x 4.1 mm, 5 µ, Hamilton, Reno, NA, USA). Mobile phases, eluent compositions and gradient elution times are given in the text or with the respective figures.

4.2.3 *Electrospray ionisation mass spectrometry (ESI-MS^n)*

Mass spectrometry was carried out using a Finnigan Mat LCQ™ (ThermoFinnigan, San Jose, CA, USA) ion trap mass spectrometer fitted with an electrospray interface. Data were acquired and processed with Xcalibur 1.0 sp1 software. Instrument tuning, mass calibration and data processing was carried out as described in Chapter 3 (Section 3.3.4.6). Selected ion monitoring (SIM) was carried out using the SIM mode with the mass(es) selected to *m/z* 0.1 and an isolation width of *m/z* 1. Extracted ion mass chromatograms (EIC) were extracted from the full scan; total ion chromatograms (TIC) selected to *m/z* 0.1 and an isolation width of *m/z* 1. Positive ion ESI-MS^n was carried out as described in Chapter 2 (Section 2.2.2).

4.2.4 *Fourier transform infrared (FT-IR) and ^{1}H nuclear magnetic resonance (NMR) spectroscopy*

Fourier transform infrared (FT-IR) spectroscopy was carried out on a Bruker FT-IR IFS66 spectrometer (Bruker Inc, USA) and data acquired with OPUS 2.0 Optics software (Bruker, Inc). Samples were prepared as KBr disk. ^{1}H nuclear magnetic resonance (NMR) spectroscopy was carried out on a JEOL EX-270 FT-NMR spectrometer. Samples (10-20 mg mL^{-1}) were dissolved in $CDCl_3$.

4.2.5 *Imidazoline synthesis procedures*

4.2.5.1 2:1 Thermal reaction of oleic acid with diethylenetriamine

Oleic acid (2.48g, 8.8 m mole, 97 % Acros) was heated in a three neck, round bottom flask (50 mL). A thermometer was fitted through a side neck and positioned in the reaction mixture above the Teflon coated magnetic stirring flea. The top neck was open to the atmosphere, whilst the second side neck was stoppered after addition of DETA. The mixture was heated to 45°C whilst stirring. DETA (0.53g, 5.1 m mole) was added drop wise (*ca* 10 mins). The temperature rose to 60°C as a clear, gel like soap was formed. This became liquid on heating to 105°C and steam was evolved. The solution was heated to 155°C over 35 minutes and continued for 240 minutes. The reaction mixture was allowed to cool. A side condenser was fitted to the top neck, and a vacuum was then applied (the reaction mixture erupted around the flask on initial application of vacuum). The reaction mixture was heated to 160°C at ~ 0.5 Torr and heating continued for 120 minutes. On stopping the reaction, condensation and dark brown oily spots were visible on the side of the reaction flask above the surface of a dark yellow oil product. After cooling overnight the reaction flask was stoppered. A yellow waxy material and dark brown spots were visible on the flask wall above a viscous dark yellow oil product. After four days storage in the flask at room temperature a waxy yellow solid was visible around the flask and into the viscous oil. An aliquot of the oil was taken up in a glass Pasteur pipette and 0.0158 g transferred to a glass volumetric flask and made up to 10 mL with methanol (Sample OI/1, 1.58 mg mL^{-1}). The reaction flask was heated to185°C. The yellow oil became darker on heating and over *ca* 2h became dark brown in colour. A temperature of 185 °C was maintained for 24 hours. On cooling a black, tar-like substance was produced. Product (0.0051 g) was transferred to a 7 mL glass vial and IPA (5 mL) added by auto-pipette (sample OI/2, 1.02 mg mL^{-1}).

4.2.5.2 HPLC/UV analysis of 2:1-oleic imidazoline synthesis product (sample OI/1)

An isocratic elution was carried out on C_{18} reverse phase column (Absorbosphere C_{18}) with an 80:20 % A:B eluent at a flow rate of 200 μL min^{-1}. Eluent A, 1% acetic acid in methanol (v/v) and B, water:methanol:acetic acid (90:10:1 %, v/v/v). UV detection was carried out in the range 190-360 nm and at a discrete 230 nm wave length. No signal was observed for 5 μL injections of 10 and 100 x dilutions (in 2:1 A:B) of the sample OI/1.

4.2.5.3 2:1 Thermal reaction of palmitic acid with diethylenetriamine reflux in xylene

A 2:1-palmitic imidazoline was synthesised by Mr. Andrew Tonkin (laboratory technician, University of Plymouth, UK). Palmitic acid (5.12g, 20 m mole) was reacted with DETA (10 m moles, 1.08 cm^3) by refluxing in xylene (160mL) 144 -145°C, and water (0.36 cm^3, 66%) removed azeotropically by way of a Dean and Stark apparatus. The reaction was stopped after 144 hours when no further water could be collected. Sub samples were removed at intervals (7, 23, 30, 47 and 71 hours) using a syringe and PTFE tubeing. After stopping the reaction, a white precipitate started to form at room temperature and settled out over 24 hours. Xylene was removed by rotary evaporation. The crude product was re-crystallised in acetone to yield 4.28g (76%) white powder, m.p. 81°C (lit. m.p. 78-79 °C,[76] m.p. 85-87 °C xylene solvent synthesis[89] and m.p. 80-82 °C solid-phase synthesis[89]). FT IR spectrum (υ/cm^{-1}): 1643, 1550 (-NH-C=O), 1608 (C=N) (Appended; Figure A.1; FTIR discussed in Chapter 5, Section 5.3 4). The sub samples where blown dry with nitrogen.

Stock solutions of each sub sample were prepared by dissolving (1.4 mg 7 hr, 1.6 mg 23 hr, 1.2 mg 30 hr, 1.4 mg 47 hr and 1.2 mg 71 hr) in 1000 μL methanol (samples PI/1-7hr/23hr/30hr/47hr and 71hr respectively). The final product (11.2 mg) was made up to 10 mL with methanol (Sample PI/1-fp). A 1000 μL methanol blank was prepared. Stock solutions where diluted as required (*e.g.* for a 10 x dilution; 100 μL of each stock solution was diluted 10 times by adding to 900 μL methanol:water:TFA (80:20:0.1% v/v/v)).

Serially diluted standards (0.0001 to 100 μg mL^{-1}; 0.0124 to 124 μg mL^{-1} and 0.062 to 62.0 μg mL^{-1}) for HPLC/ESI-MS response were prepared.

4.2.5.4 1:1 Thermal reaction of oleic acid with diethylenetriamine in xylene

A 1:1 molar ratio synthesis of oleic acid with diethylenetriamine in xylene was carried out as described for 2:1-PI (Section 4.2.4.3), by reacting oleic acid (8.47 g, 30 m mole, 90 % Aldrich) with DETA (3.2 g, 31 m mole) for 91 hours. Aliquots where taken at 1, 3, 19 and 91 hours and blown down with N_2 with heating to 50 °C.

Stock solutions of each sample were prepared by weighing out (0.0259 g 1 hr, 0.0277 g 3 hr, 0.0261 g 19 hr and 0.0252 g 91 hr) and making up to 25 mL with ethanol in a glass volumetric flask (Samples OI/2-1hr/3hr/19hr and 91hr respectively). 100 x dilutions for HPLC/ESI-MS analysis were prepared by transferring 10 μL to 990 μL of required solvent.

4.2.5.5 2:1 Thermal reaction of oleic acid with diethylenetriamine in xylene

A 2:1 molar ratio synthesis of oleic acid with diethylenetriamine in xylene was carried out as described above, by reacting oleic acid (100 g, 350 m mole, 90 % Aldrich) in 200 mL in xylene with DETA (18 g, 175 m mole) for 95 hours. After 2 hours, there were two layers visible in the trap, a cloudy white lower and clear upper layer. The trap contents (9 mL), containing both layers was transferred to a 10 mL measuring cylinder. This on cooling separated to two clear layers (*ca.* 4 mL lower water layer). At 5 hours a further 4 mL lower water layer was visible in the trap. After 95 hours excess xylene was removed by opening the drain tap on the Dean and Stark trap, preventing condensed xylene from returning to the reaction flask. Heating was stopped after 20 minutes when the temperature rose from 149 to 160 °C. 9 mL of water was collected *via* the Dean and Stark trap (95 %). Aliquots of the reaction solution (~2 mL) where taken at 5, 23 and 95 hours. The golden brown, viscous oil product (sample OI/3) was stoppered and sealed on cooling to room

temperature and stored at 4°C. Xylene was removed from the sub-samples by rotary evaporation with heating to 100°C in a water bath. The 23hr sub-sample on cooling contained white precipitate on the flask wall and was the result of hydrolysis to the product amide, due to steam from the water bath entering the flask under vacuum due to a poor seal.

Stock solutions of each sample were prepared by weighing out (0.0270 g 5 hr, 0.0264 g 23 hr and 0.0271 g 95 hr) and making up to 25 mL with methanol in a glass volumetric flask (Samples OI/3-5hr/23hr and 95hr respectively). 100 x dilutions for HPLC/ESI-MS analysis were prepared by transferring 10 μL to 990 μL of required solvent. Calibration standards (0.005 – 10 μg mL^{-1}) were prepared by sequential dilution in 0.1% TFA in methanol of a 1008 μg mL^{-1} 2:1-OI stock solution (0.0252 g OI/3 up to 25 mL methanol).

4.2.5.6 Vacuum distillation of 95 hour reaction product

The 95 hour 2:1 product (63.9 g) was vacuum distilled by way of a condenser and a three way trap. A clear fraction (xylene) was collected (27°C at 2 mm Hg). The temperature rose to 140°C, (0.8 mm Hg) as a white vapour was observed. Clear yellow oil started condensing out (fraction 2). This continued as the temperature rose slowly to 160°C. It became apparent that air was being drawn in around the top of the condenser and could not be sealed. The temperature rose to 212°C and the oil started solidifying (white) as it reached the collection flask. Changed to the third collection flask, temperature dropped and rose as air leak sealed and unsealed as darker oil was condensing out and solidifying. Heating and vacuum was stopped. On cooling the remaining oil was a dark black, very sticky semi-solid.

4.3 Results and discussion

In order to develop an efficient HPLC/ESI-MS separation and detection method for imidazoline compounds, authentic compounds of known composition were required. As no commercial authentic compounds were available, synthesis of representative compounds was required. Since it is one of the major components of commercial corrosion inhibitor products, 2:1-Oleic imidazoline (2:1-OI; structure VIII, R and $R_1 = C_{17}H_{33}$, Figure 3.1) was chosen as an initial synthetic target. An internal standard was also required to enable quantitation of extraction methods of real samples. 2:1-Palmitic imidazoline (2:1-PI; structure VIII, R and $R_1 = C_{15}H_{31}$, Figure 3.1) was not observed in any of the commercial products previously analysed (Chapters 2 and 3), and was thought to be a reasonable surrogate. The mass to charge ratio of the protonated 2:1-palmitic imidazoline ion (m/z 562.6) falls between those of the major 1:1 and 2:1-imidazoline components (1:1-OI *m/z* 350.4 and 2:1-OI *m/z* 614.6) and thus would not be expected to cause interferences in mass chromatography. A di-deuterated 2:1-oleic imidazoline would not be as suitable as it would be isobaric with 2:1-stearic imidazoline (structure VIII, R and $R_1 = C_{17}H_{35}$, Figure 3.1, *m/z* 618.7) whilst a mono-deuterated analogue would be isobaric with the ^{13}C masses. A review of published methods of imidazoline synthesis[74-79,90-100] revealed that rather surprisingly, although the synthesis of imidazolines has been known since at least 1888,[101] there is no well-accepted synthetic method and much confusion remains over the exact mechanisms operating. Both solid-phase and solvent/reflux methods combining selected amines with fatty acids, have been employed in the literature. Figure 4.1 shows the various intermediates and pathways proposed for thermal syntheses of 2:1-imidazolines and are discussed in detail in Chapter 5. Therefore it was decided that both solid-phase and solvent/reflux methods would be investigated for the production of pure imidazolines.

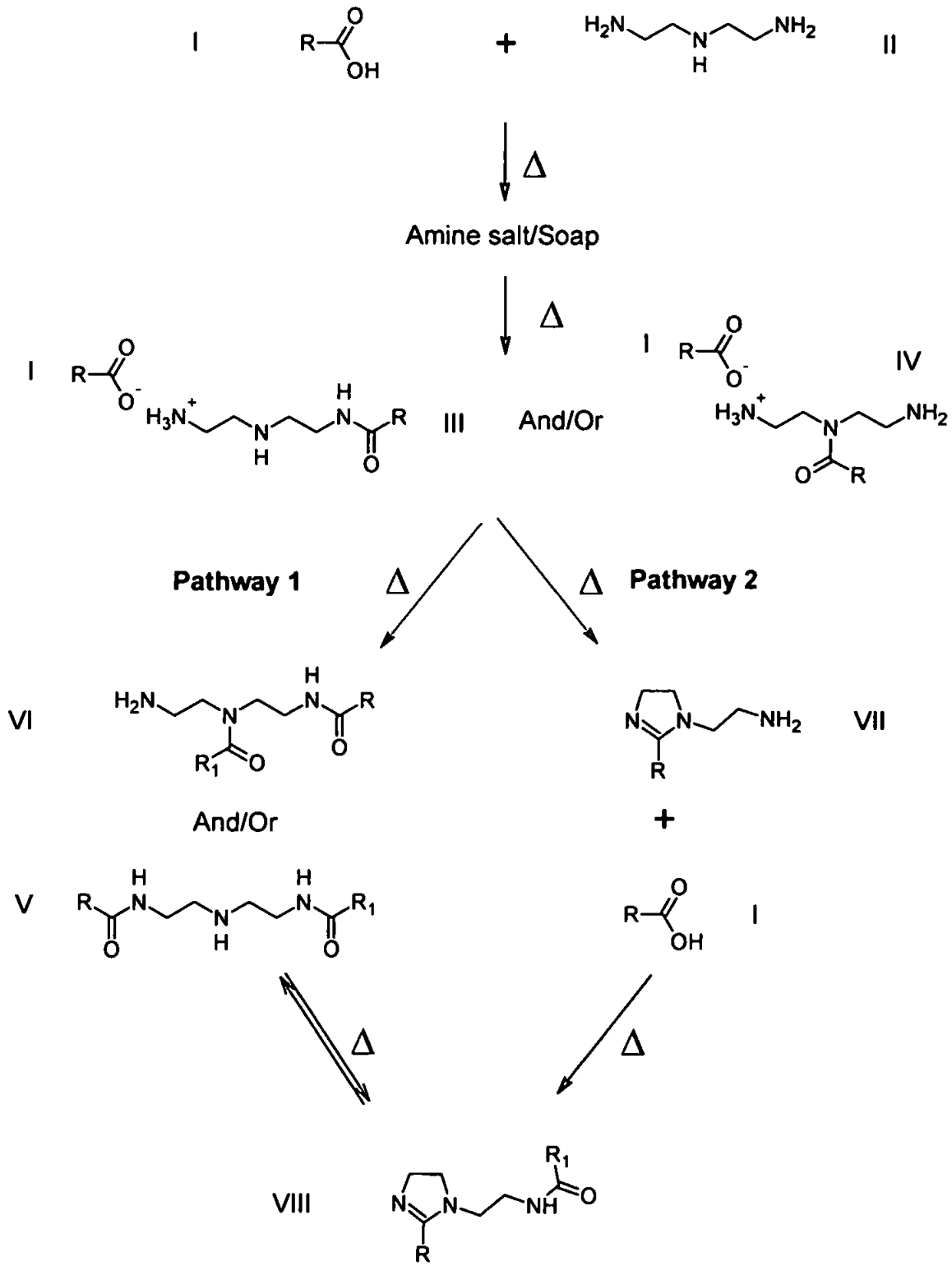


Figure 4.1 Proposed reaction pathways for the thermal condensation reaction of fatty acids with diethylenetriamine. Naming as Figure 3.1.

4.3.1 *Solid-phase synthesis of 2:1-oleic imidazoline*

A solid-phase thermal condensation synthesis of 2:1-oleic imidazoline based on the method described by Bistline *et. al.*[76] was carried out. The reaction proceeded as described (Section 4.2.5.1) without problem. However, after four days the yellow waxy substance observed on the flask wall of the product had visibly increased in amount at the oil/flask surface. It was thought this was the diamide (either structure V or VII, Figure 3.1) hydrolysis product.[54,77] After removal of an aliquot of the oil product (Sample OI/1; Section 4.2.5.1), the reaction mixture was further heated (185°C) for 24 hrs to observe if the waxy material re-condensed. On cooling, after 24 hours the product was a black tar-like substance.

Infusion positive ESI-MS analysis of the original yellow oil (Sample OI/1: Figure 4.2), shows a base peak ion at *m/z* 614.7 consistent with the presence of a protonated 2:1-oleic imidazoline. The only other ion of significance in the spectrum was *m/z* 307.5 (5%) which was attributed to a protonated 2-oleic imidazoline (structure IX, Figure 3.1). It was unclear whether this was due to in-source fragmentation of 2:1-oleic imidazoline (fragmentation during the electrospray process) or to the presence of protonated 2-oleic imidazoline. The latter is a known fragmentation ion of the 1:1- and 2:1-imidazolines (Chapter 2; Section 2.). There was a very small ion at *m/z* 350.6 (~1%) attributed to the protonated 1:1-oleic imidazoline. The mass spectrum suggested that the product was reasonably pure (ca 95%).

The reheated final product was also analysed by flow injection positive ion ESI-MS (sample OI/2; Figure 4.3). The base peak *m/z* 612.6 showed a loss of 2 Daltons (Da) from the molecular mass of the protonated 2:1-Oleic imidazoline ion attributed to loss of H_2. Further ESI-MS analysis was not carried out on this product to prevent instrument contamination as it was difficult to remove residues from the ESI source and the priority at this stage of the research was to develop a HPLC/ESI-MS method. It was noted however

that *m/z* 612 is a common mass observed in imidazoline products and has previously been assigned to 2:1-oleic/linoleic imidazolines by multistage mass spectrometry (MS^n) (see Chapter 2 and 3) and would imply loss of H_2 from an alkyl group. However, MS^n was not carried out and the *m/z* 2 loss may be loss of H_2 from the imidazoline moiety to give an aromatic imidazole.

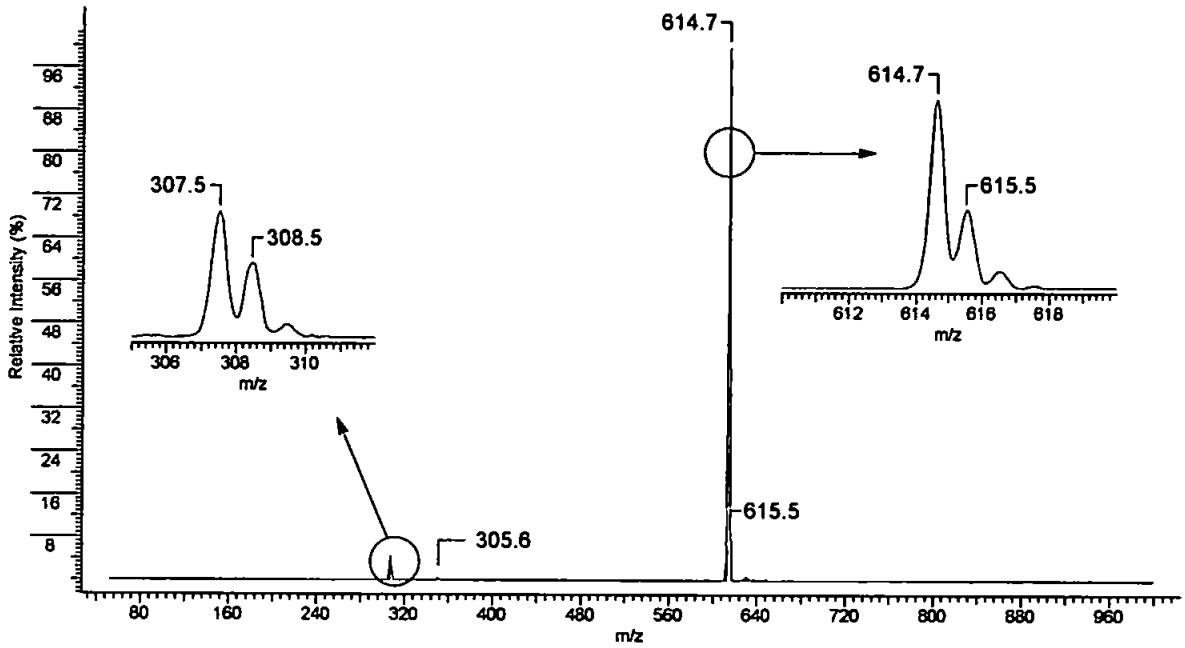


Figure 4.2 Positive ion ESI mass spectrum of 2:1-oleic imidazoline synthesis product sample OI/1. Infusion at 3 μL min^{-1}, Full scan *m/z* [50-2000].

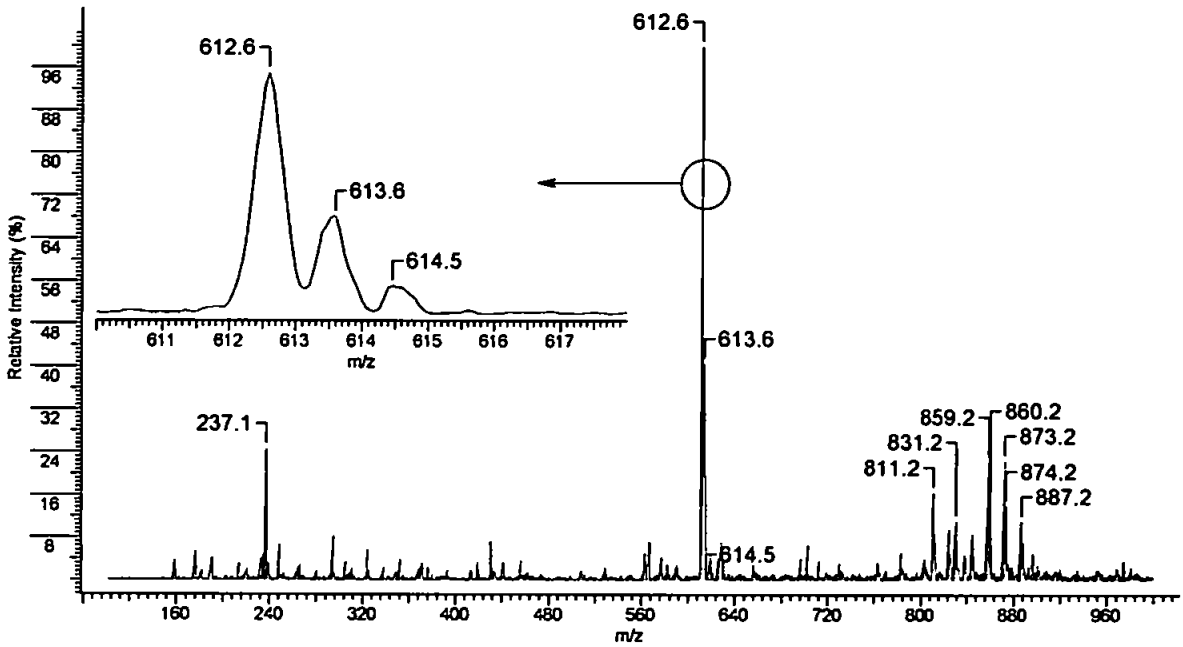


Figure 4.3 Flow injection positive ion ESI mass spectrum of 2:1- oleic imidazoline synthesis product sample OI/2. 5 µL Injection via "T" connection into 150 µL min^{-1} 0.1% TFA in MeOH, full scan *m/z* [100-1000].

4.3.2 *Synthesis of 2:1-palmitic imidazoline by Dean and Stark reflux in xylene*

Apart from the solid state reaction method outlined above, imidazolines have also been prepared by heating the reagents in solvents (e.g. Butler and O'Regan,[78] Maddox[97]). Often the latter methods have employed azeotropic removal of water produced in the reaction with toluene.[97] Azeotropic removal of water is a simple method of removing water of reaction whilst maintaining a constant temperature. The reagents are refluxed in a suitable solvent which forms an azeotrope with water in a flask fitted with a Dean and Stark trap and condenser (Figure 4.4). The azeotrope vapour condenses and runs back into the trap were the heavier water separates out and remains in the trap whist excess solvent returns to the reaction flask. The trap should ideally be fitted with a drain tap to allow collected water to be removed if the volume exceeds that of the trap. It was thought that if a Dean and Stark method worked, it would provide a simple one-pot synthesis without the requirement

of high temperatures and reduced pressure. 2:1-Palmitic imidazoline was prepared by way of a Dean and stark reflux in xylene (mixed isomers) which boils at 144-145°C and forms an azeotrope with water (Section 4.2.4.4). The reaction was monitored by analysis of sub-samples of the reaction solution taken at 7 and 47 hours for flow injection ESI-MS. The mass spectrum for the 7 hour sample (Figure 4.5) showed two main ions; *m/z* 324.4 as the base ion and *m/z* 562.6 (28%). The masses are consistent with 1:1-PI (*m/z* 324.4; structure VII, R = $C_{15}H_{31}$, Figure 3.1) and 2:1-PI (*m/z* 562.6; structure VIII, R and R_1 = $C_{15}H_{31}$, Figure 3.1). For the 47 hour sample (Figure 4.6) the major ions were *m/z* 562.6 (100%) and *m/z* 324.4 (49%). No ions due to DETA, palmitic acid, monoamide or diamide starting products or possible intermediates were detected. Between 7 and 47 hours the 2:1-PI increased relative to 1:1-PI and the postulated intermediate amides were not observed. Of the theoretical yield of water, 66% was collected in the trap. This low yield of water was probably mostly due to loss of vapour during collection of the sub-samples.

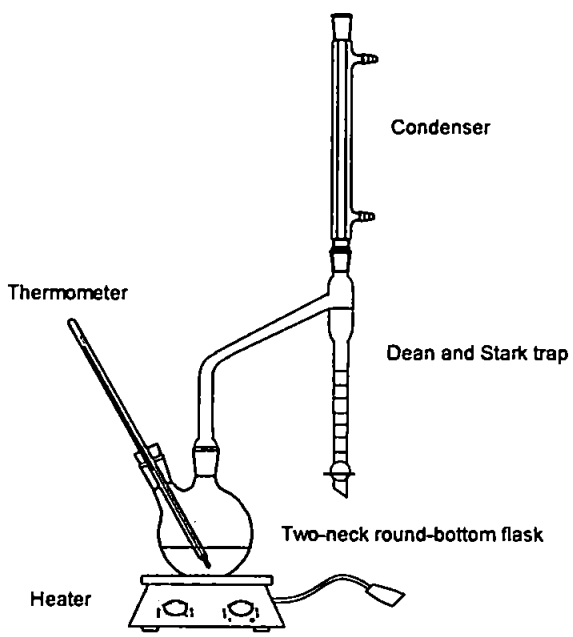


Figure 4.4 Apparatus and setup used for xylene reflux synthesis of imidazolines using a Dean and stark trap.

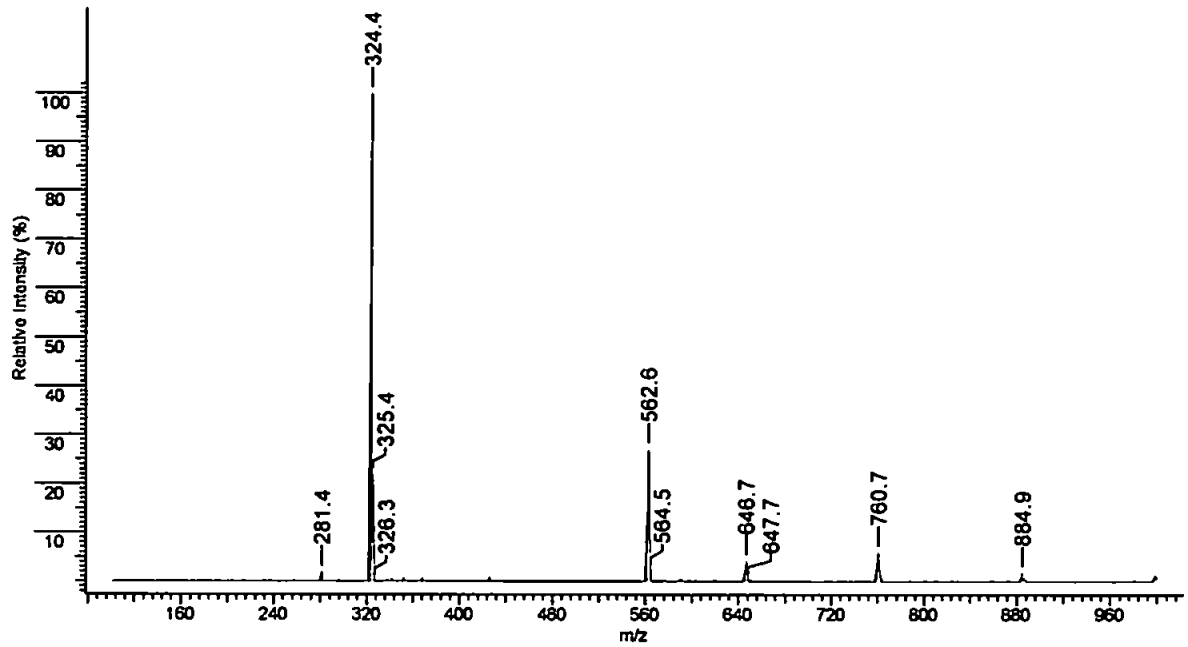


Figure 4.5 Flow injection positive ion ESI mass spectrum of 2:1-palmitic imidazoline synthesis product sample PI/1-7hr. 5 µL Injection via "T" connection into 150 µL min^{-1} 0.1% TFA in MeOH, full scan *m/z* [100-1000].

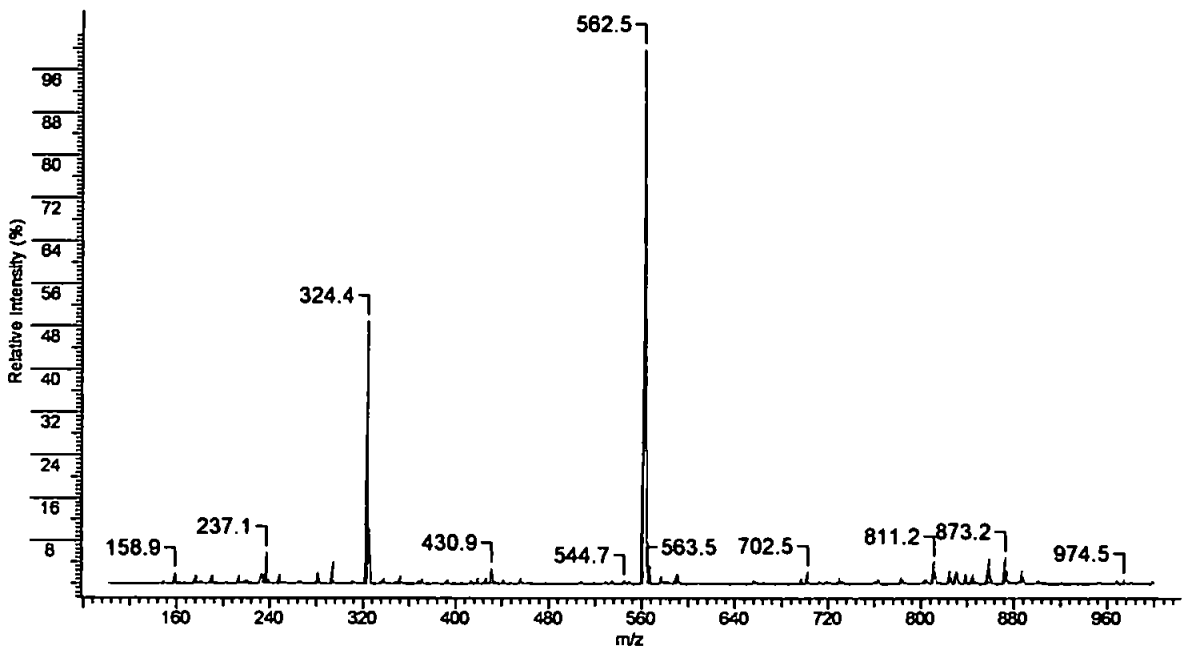


Figure 4.6 Flow injection positive ion ESI mass spectrum of 2:1-palmitic imidazoline synthesis product sample PI/1-47hr. 5 µL Injection via "T" connection into 150 µL min^{-1} 0.1% TFA in MeOH, full scan *m/z* [100-1000].

4.3.3 *Investigation of the HPLC separation, UV and ESI-MS detection of 2:1-OI*

There are few HPLC methods for the separation of imidazolines and related amides.[52,53,88] Gough and Langley[52] reported the use of reverse-phase HPLC/UV and ESI-MS for commercial imidazolines added to production fluids. However, no HPLC column phase or eluent details were given. They reported HPLC/UV (230 nm) calibrations of an imidazoline/quat corrosion inhibitor standard in the range 50-250 ppm. A reverse-phase HPLC/refractive index (RI) method for the separation of imidazolines from their diamides, was reported by Hampson *et. al.*[88]. The method described was for short chain fatty acid (C_9-C_{12}) derived imidazoline and diamides using an octadecyl bonded silica phase and isocratic methanol:0.1 N acetic acid eluents (70:30-90:10 v/v). The refractive index detection was not very sensitive with "barely detectable peak heights" for 40 μg 200 μL^{-1} (200 ppm) injections of pelagonic acid (nonanoic acid) derived 2:1-imidazoline and diamide. A HPLC/UV (192-360 nm, photo-diode array detector (PDA)) analysis of the 2:1-OI based on that described by Hampson *et. al.*,[88] was carried out on a C_{18} reverse-phase with isocratic methanol:water:acetic acid (82:18:1 v/v/v) elution (Section 4.2.4.3). No significant UV signal was observed for 158 μg mL^{-1}, 5 μL injections even when the eluent was switched to 100% methanol:1% acetic acid after 30 minutes. As an imidazoline based product (CI-E1) had previously been analysed by HPLC/ESI-MS herein (Chapter 3; Section 3.3.4) and separation demonstrated on an amino-bonded C_8 phase (HyPurity Advance, Hypersil) no further analysis were carried out on the C_{18} phase herein .

The HPLC/ESI-MS separation of the CI-E1 imidazoline product on Hypersil Advance® column, (Discussed in Chapter 3; Section 3.3.4) produced very broad peaks for the imidazoline components (Figures 3.14 – 3.17; Chapter 3), suggesting several competing interactions between the compounds and the solid-phase, stainless-steel column components and/or eluents. The imidazolines and amides are basic and readily protonate in solution to give a hydrophilic cationic species which with the hydrophobic non-polar alkyl

moieties, give competing interactions and a surface-active nature. The alkyl moieties will have strong interactions with alkyl groups on chromatographic phases, such as C_{18} and C_8 bonded silica, whilst the protonated sites will interact with the un-bonded silanol sites. The surface active nature can have interactions with the stainless-steel column and fittings. It was thought that an ion-pair modified eluent might alleviate the ionic and surface active interactions. Polymeric resin chromatographic phases such as polystyrene-divinylbenzene are generally more tolerant of wide pH ranges (pH 1-14) than bonded silica (pH 2-7), and are more tolerant of ion-pair reagents and have strong reverse-phase interactions for many polar compounds. A Hamilton PRP-1 (5μ poly(styrene-divinylbenzene)) column (50 x 4.1 mm) was therefore investigated with trifluoroacetic acid (TFA) as an ion-pair reagent for the retention of 2:1-OI.

Extracted ion chromatograms (EIC) from an initial isocratic elution of 2:1-OI on PRP-1 with 0.1% TFA in methanol (Figure 4.7), showed that 2:1-OI (*m/z* 614.7; t = 0.66 min) eluted close to the solvent front (t_0 = 0.43 min) with a capacity factor (k) of 0.5, and very little peak tailing. It was also observed that an *m/z* 307.5 ion elutes on the solvent front 0.43 min, k = 0) with a smaller peak at k 0.5 (0.66 min), directly under the 2:1-OI peak. This shows that 2-oleic imidazoline (OI, structure IX, R = $C_{17}H_{33}$; Figure 3.1) is present in the sample as the peak at k = 0 and is the result of ESI source fragmentation of the 2:1-OI precursor ion. To increase retention of OI and resolution from 2:1-OI, an isocratic elution with 0.1% TFA in methanol:water (90:10 v/v) was carried out (Figure 4.8). This resulted in a sharp peak for OI at k = 0.4 and a very broad peak for 2:1-OI between k = 13-64. In order to improve the 2:1-OI peak, gradient elutions of 0.1% TFA in methanol (eluent A) 0.1% TFA (eluent B) were investigated. For the gradients [(time/%A) 0/70-1/70-15/100 and 0/80-1/80-15/100] (Figures 4.9 and 4.10 respectively), the 2:1-OI peaks are sharp with little tailing and well resolved from 1:1-OI and OI. Changing the eluent starting composition from 70 to 80% A, had a larger effect on the retention of both 1:1-OI (5.64 to

1.75 min) and OI (9.50 to 5.05 min) compared to 2:1-OI (16.03 to 14.68 min). Both gradients produced good baseline resolution and good peak shapes for the three components of the 2:1-OI synthesis products and showed that PRP-1 was a suitable chromatographic phase for separation of 2:1-OI, 1:1-OI and OI from each other.

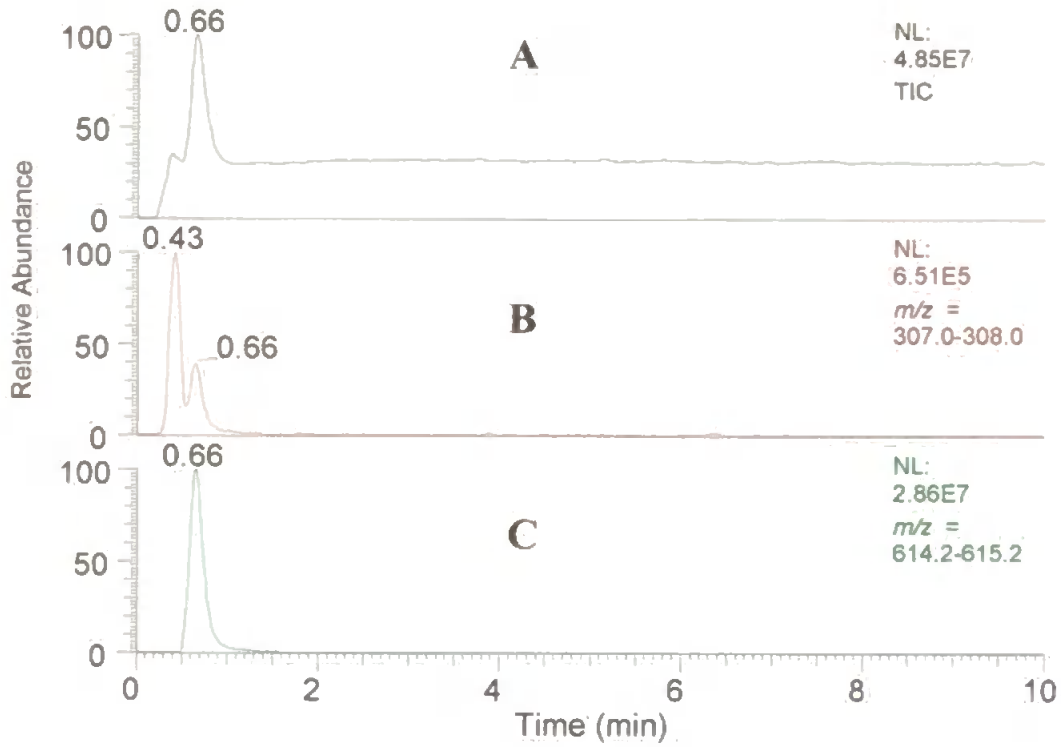


Figure 4.7 HPLC/ESI-MS mass chromatograms of 2:1-oleic imidazoline product by isocratic elution with 0.1% TFA in methanol on PRP-1 column: (A) Total ion current of positive ion full scan *m/z* [100-1000]; (B) EIC for 2-oleic imidazoline *m/z* 307.5 and (C) 2:1-oleic imidazoline *m/z* 614.7. 5 µL injections (50 µL sample OI/1 in 450 µL methanol:water:TFA (70:30:0.1 v/v/v)).

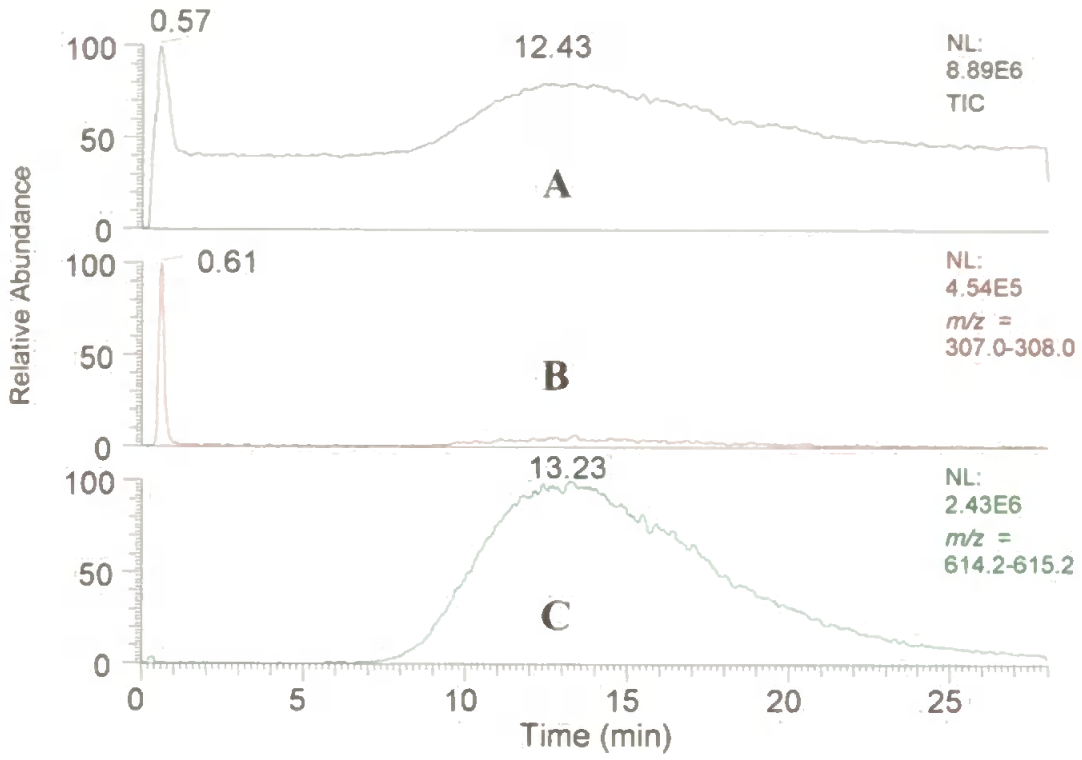


Figure 4.8 HPLC/ESI-MS mass chromatograms of 2:1-oleic imidazoline product by isocratic elution with 0.1% TFA in methanol:water (90:10 v/v) on PRP-1 column: (A) Total ion current of positive ion full scan *m/z* [100-1000]; (B) EIC for 2-oleic imidazoline *m/z* 307.5 and (C) 2:1-oleic imidazoline *m/z* 614.7. 5 µL injections (50 µL sample OI/1 in 450 µL methanol:water:TFA (70:30:0.1 v/v/v)).

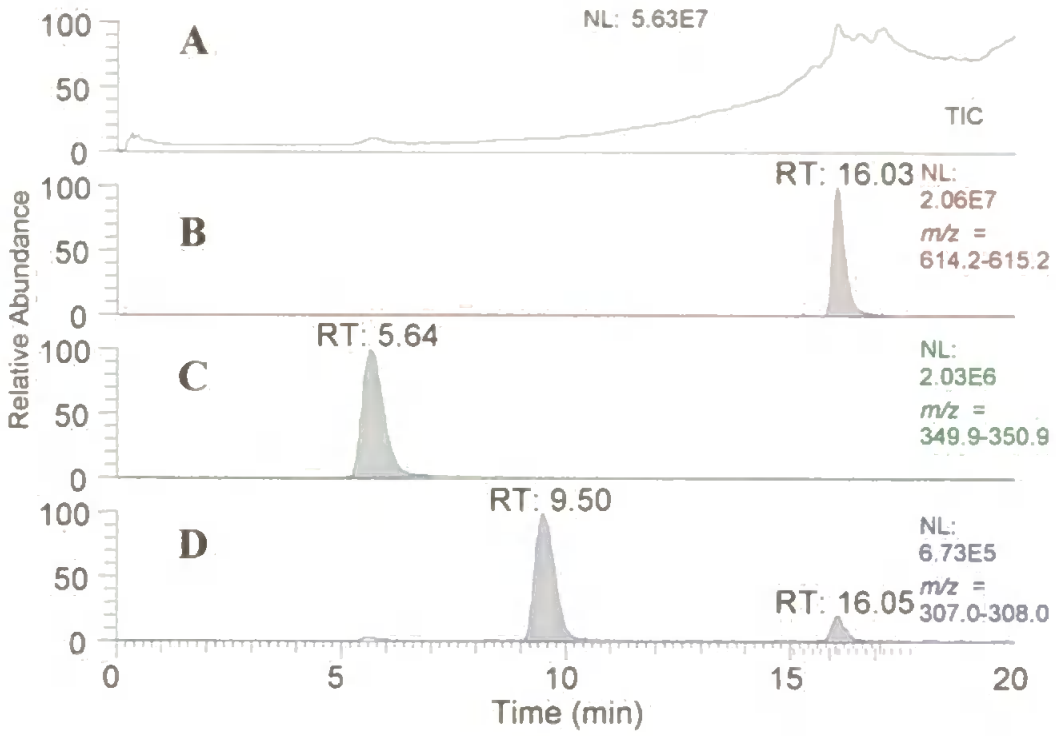


Figure 4.9 HPLC/ESI-MS mass chromatograms of 2:1-oleic imidazoline product by gradient elution [(time/%A) 0/70-1/70-15/100; A) 0.1% TFA in methanol and B) 0.1% TFA] on PRP-1 column: (A) Total ion current of positive ion full scan *m/z* [100-1000]; (B) EIC for 2:1-oleic imidazoline *m/z* 614.7, (C) 1:1-oleic imidazoline and (D) 2-oleic imidazoline *m/z* 307.5. 5 µL injections (50 µL sample OI/1 in 450 µL methanol:water:TFA (70:30:0.1 v/v/v)).

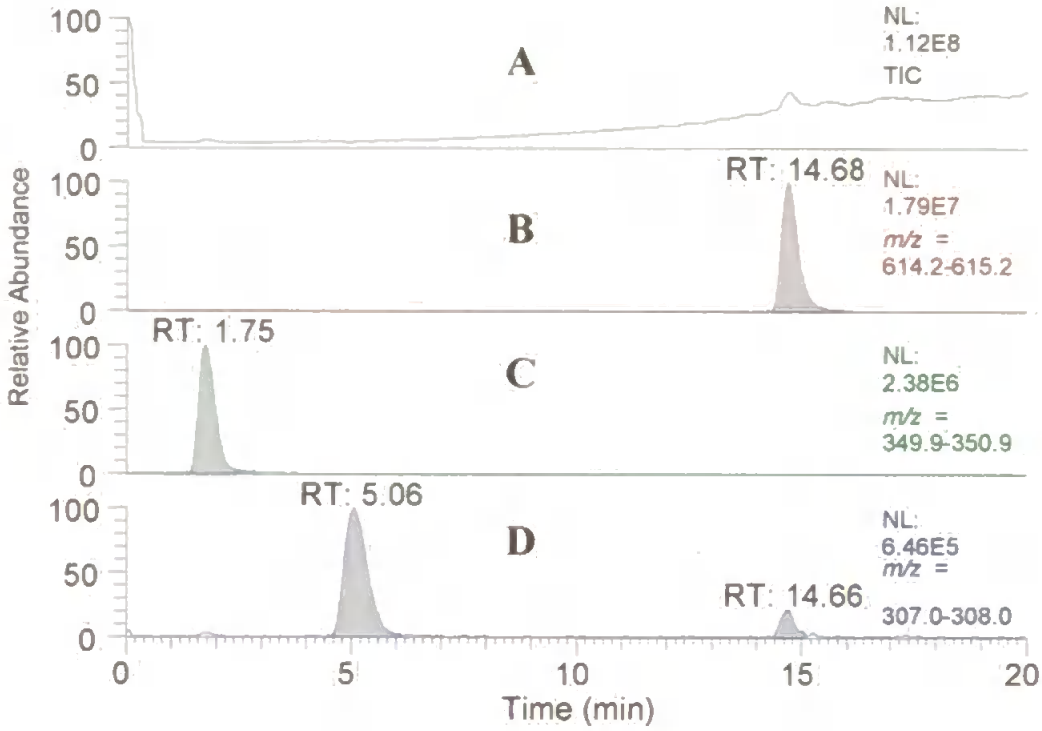


Figure 4.10 HPLC/ESI-MS mass chromatograms of 2:1-oleic imidazoline product by gradient elution [(time/%A) 0/80-1/80-15/100; A) 0.1% TFA in methanol and B) 0.1% TFA] on PRP-1 column: (A) Total ion current of positive ion full scan *m/z* [100-1000]; (B) EIC for 2:1-oleic imidazoline *m/z* 614.7, (C) 1:1-oleic imidazoline and (D) 2-oleic imidazoline *m/z* 307.5. 5 µL injections (50 µL sample OI/1 in 450 µL methanol:water:TFA (70:30:0.1 v/v/v)).

Two other chromatographic phases were investigated. MN200 is a novel hyper-crosslinked polystyrene-divinylbenzene phase that shows strong retention for hydroxybenzenes, polyphenols (Appended is a report[102] (Analysis of Highly Polar Low Molecular Weight Organic Molecules: Part 2 –New Methods for the Identification of Polar Chemicals in Complex Mixtures) of an investigation carried out on this phase by the present author) and other ionic or polar compounds.[103-111] Elution of 2:1/1:1-OI was observed with 0.1% TFA in methanol. However, 2:1-OI eluted as a very broad peak (k = 4.5-11). A porous graphitic carbon phase (Hypersil Hypercarb)[112-119] was also investigated, but no elution of 2:1/1:1-OI or PI was observed with 0.1% TFA in methanol. No further analysis was carried out with either phase.

4.3.4 *HPLC/ESI-MS analysis of 2:1-PI synthesised by Dean and Stark reflux*

Having shown that the PRP-1 stationary phase gave good chromatographic separations of oleic acid based imidazolines, the 2:1-PI synthetic product and the reaction sub-samples (Section 4.2.5.3) were analysed by HPLC/ESI-MS using gradient elution determined for 2:1-OI. Protonated ions for the main products and intermediates: 2:1-PI (*m/z* 562.6; Structure VIII, R and R_1 = $C_{15}H_{31}$, Figure 3.1), 1:1-PI (*m/z* 324.4; Structure VII, R = $C_{15}H_{31}$, Figure 3.1), PI (*m/z* 281.3; Structure IX, R = $C_{15}H_{31}$, Figure 3.1), monoamide (*m/z* 342.4; Structure III and/or IV, R = $C_{15}H_{31}$, Figure 3.1) and diamide (*m/z* 581.6; Structure V and/or VI, R and R_1 = $C_{15}H_{31}$, Figure 3.1) could be observed in the TICs obtained. The retention times (R_t, Table 4.1), and auto-integrated peak areas (AA, Table 4.2), were determined for each extracted ion mass. The most striking feature comparing TIC and EIC peak heights, was that the ratio of 1:1-PI decreased relative to 2:1-PI from ~ 1:1 for the 7 hour sample to 1:3 at 71 hours and is clearly seen in the *m/z* 562.6 and *m/z* 324.4 EIC (Figure 4.11) and as a plot of the percentage relative ratios of the auto-integrated peak areas (AA) (Figure 4.12). This suggests that the reaction proceeds *via* reaction pathway 2 shown in Figure 4.1, in which a monoamine is initially produced from the fatty acid/DETA

salt, followed by cyclisation to 1:1-PI with subsequent condensation at the amine with fatty acid to 2:1-PI. The mono and diamide precursors are present in only small quantities relative to there imidazoline products (<10% and <1% respectively; Table 4.3) and therefore cyclisation must be rapid. The final recrystallised (from acetone) 2:1-PI product contained ~ 6% 1:1-PI relative to 2:1-PI by peak area and even lower quantities of the mono/diamides (Table 4.3). Comparison of the retention times for the palmitic and oleic compounds indicates that they would be resolved by the HPLC method.

Table 4.1 HPLC/ESI-MS retention times for extracted mass chromatograms for protonated ions of: PI (*m/z* 281.3), 1:1-PI (*m/z* 324.4), monoamide (*m/z* 342.4), 2:1-PI (*m/z* 562.6), and diamide (*m/z* 580.6). Conditions as given in Figure 4.11

2:1-PI Sample	Retention Time (R_t)					
	t_0 #	*m/z* 281.3	*m/z* 324.4	*m/z* 342.4	*m/z* 562.6	*m/z* 580.6
Blank	0.51	nd*	nd	nd	nd	nd
7 hr	0.46	3.37	1.05	2.23	13.36	14.33
23 hr	0.45	3.47	1.07	2.27	13.38	14.43
30 hr	0.46	3.42	1.05	2.25	13.3	14.33
47 hr	0.45	3.38	1.05	2.27	13.37	14.49
71 hr	0.45	3.48	1.08	2.37	13.27	14.32
FP (144 hr)	0.43	nd	1.08	2.32	13.36	14.36

Unretained compound (t_0) * Not detected (nd)

Table 4.2 Peak areas for detected peaks given in Table 4.1

2:1-PI Sample	Peak Area (Auto Integrated)				
	m/z 281.3	*m/z* 324.4	*m/z* 342.4	*m/z* 562.6	*m/z* 580.6
Blank	nd	nd	nd	nd	nd
7 hr	1227078	91438661	7092118	113142354	729504
23 hr	1645295	89359329	8096950	162514597	1193184
30 hr	1120770	61585189	4436593	131399597	1137245
47 hr	1202958	55279426	1245089	135720318	646090
71 hr	688960	55560829	868047	156507653	347757
FP (144 hr)	nd	9885036	709099	216430843	3472371

Table 4.3 Relative percentage peak area ratios of peaks given in Table 4.1

2:1-PI Sample	% Relative ratios of EIC *m/z* Peak Areas		
	324.4/562.6	580.6/562.6	324.4/342.4
7 hr	80.8	0.6	7.8
23 hr	55.0	0.7	9.1
30 hr	46.9	0.9	7.2
47 hr	40.7	0.5	2.3
71 hr	35.5	0.2	1.6
FP (144 hr)	4.6	1.6	7.2

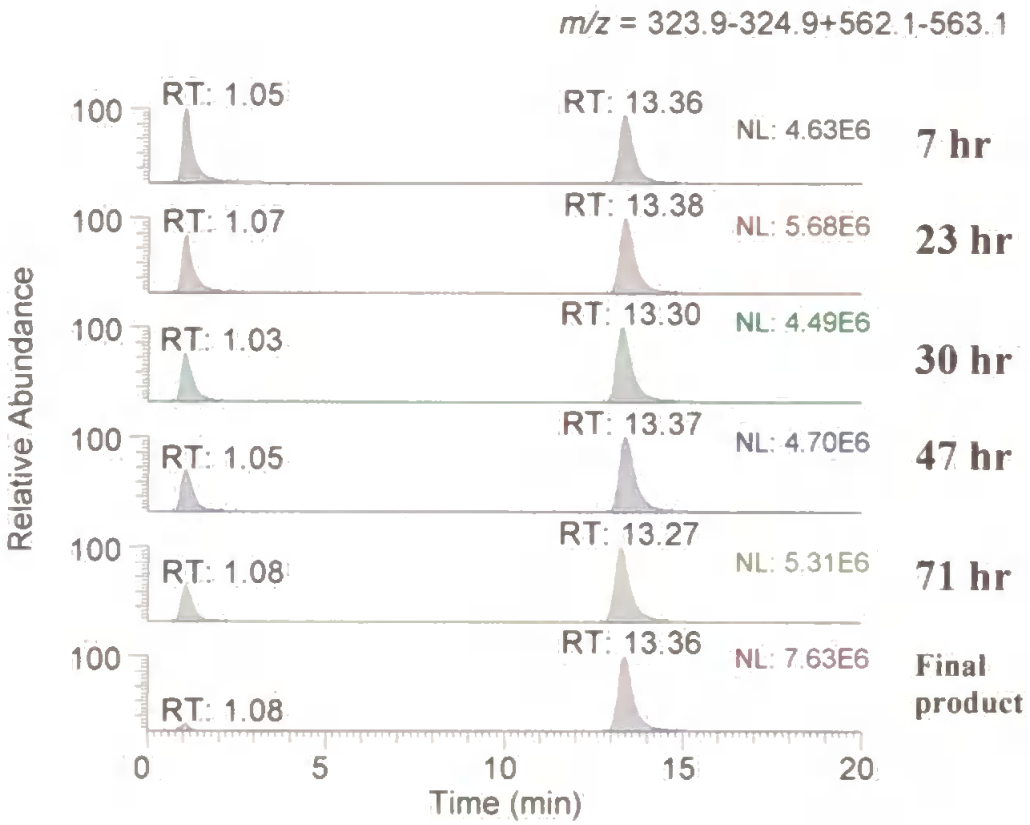


Figure 4.11 HPLC/ESI-MS mass chromatograms of 2:1-palmitic imidazoline synthesis sub samples by gradient elution [(time/%A) 0/80-1/80-16/100; A) 0.1% TFA in methanol and B) 0.1% TFA] on PRP-1 column: EIC's for 1:1-PI and 2:1-PI (*m/z* 325.4 and 562.6) at 7/23/30/47/71 hours and final product.

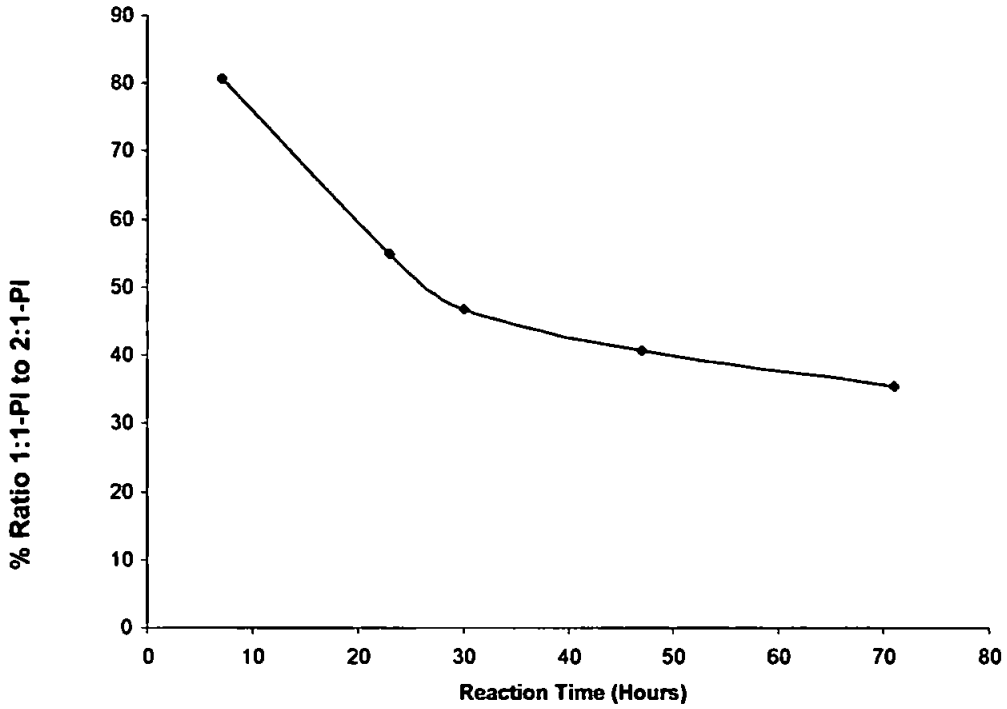


Figure 4.12 Plot of the percentage relative peak area ratios of 1:1-PI/2:1-PI against reaction time for solvent reflux reaction of palmitic acid with diethylenetriamine.

4.3.5 *Investigation of the HPLC/ESI-MS limits of detection for 2:1-PI in full scan and selected ion monitoring mode*

A series of standards (0.0001 to 100 μg mL^{-1}) of 2:1-PI (Sample PI/1-fp; Section 4.2.5.3) were analysed by HPLC/ESI-MS using positive ion, full scan mode (*m/z* 100-1000) and selected ion monitoring (SIM) for 1:1-/2:1-PI (*m/z* 324.4, 0-6 min and *m/z* 562.6, 6-30 min; isolation width *m/z* 1.5) in order to determine appropriate concentrations for further evaluations, to obtain an idea of the potential limits of detection and to establish external calibration responses. Peak retention time (Rt; Automatic peak detection), auto-integrated peak areas (AA), peak height (AH; Automatic peak height detection) and signal to noise ratios (S/N; Xcalibur software calculated) from extracted ion and selected ion chromatograms of the 2:1-PI (*m/z* 562.6) of each standard are given in Table 4.4. For 2:1-PI (*m/z* 562.6) the lowest detectable peak, was obtained for the 0.1 μg mL^{-1} standard in

both SIM and EIC mode. However, the peak areas for the SIM mode were twice that of the EIC peaks. The signal to noise ratio of four at the 0.1 μg mL^{-1} level for the EIC (*m/z* 562.6) is about the limit of detection (S/N = 3) whilst for the SIM at S/N= 11, is considered to be about the limit of quantitation (S/N = 10). Plotting the peak areas against concentration (Figure 4.12), a good linear trend was shown for the SIM and EIC chromatograms (R^2 = 0.9991(1) and 0.9990(6) respectively) in the concentration range 0.1 to 100 μg mL^{-1}. Only one data set was obtained because the chromatographic and mass spectrometer conditions were still being evaluated at this point and were subject to constant change; therefore repeatability tests and statistical analyses could not be conducted. The lowest detected standard at 0.1 μg mL^{-1} equates to 0.5 ng analyte on column (for the 5 μL injection), which with the eluent split ($1/5^{th}$ to the mass spectrometer) means only 0.1 ng sample is reaching the mass spectrometer. However, even with this amount the detection limits determined were lower than previously published for imidazolines (50μg mL^{-1} and 150 μg mL^{-1}).[52,88] It was determined that 1 – 10 μg mL^{-1} would be optimum for further evaluation both for HPLC/ESI-MS and for solid-phase extraction procedural development. As the objective was the determination of unknown samples and not achievement of the lowest possible detection limit, selected ion monitoring was not considered necessary for development and all further analyses were carried out in the full scan mode with a wide mass range (*m/z* 100-1000 or greater) in order to observe ions from a greater range of compounds.

Table 4.4 Selected ion and extracted ion chromatogram peak data for a series of 2:1-PI (*m/z* 562.6) standards; 5μL injections on PRP-1 with gradient [(time/%A) 0/75-0.5/75-15/95; A) 0.1% TFA in methanol and B) 0.1% TFA]

Standard	Selected ion *m/z* 562.6				Extracted ion *m/z* 562.6			
μg mL^{-1}	R_t	AA	AH	S/N	R_t	AA	AH	S/N
100	12.93	2040366555	76589953	314	12.94	1.11E+09	40741369	411
10	13.17	140138626	3829811	126	13.19	75588309	2081006	110
1	13.25	15460647	442662	182	13.28	8330346	236135	103
0.1	13.14	1223257	40123	11	13.17	564874	16937	4
0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

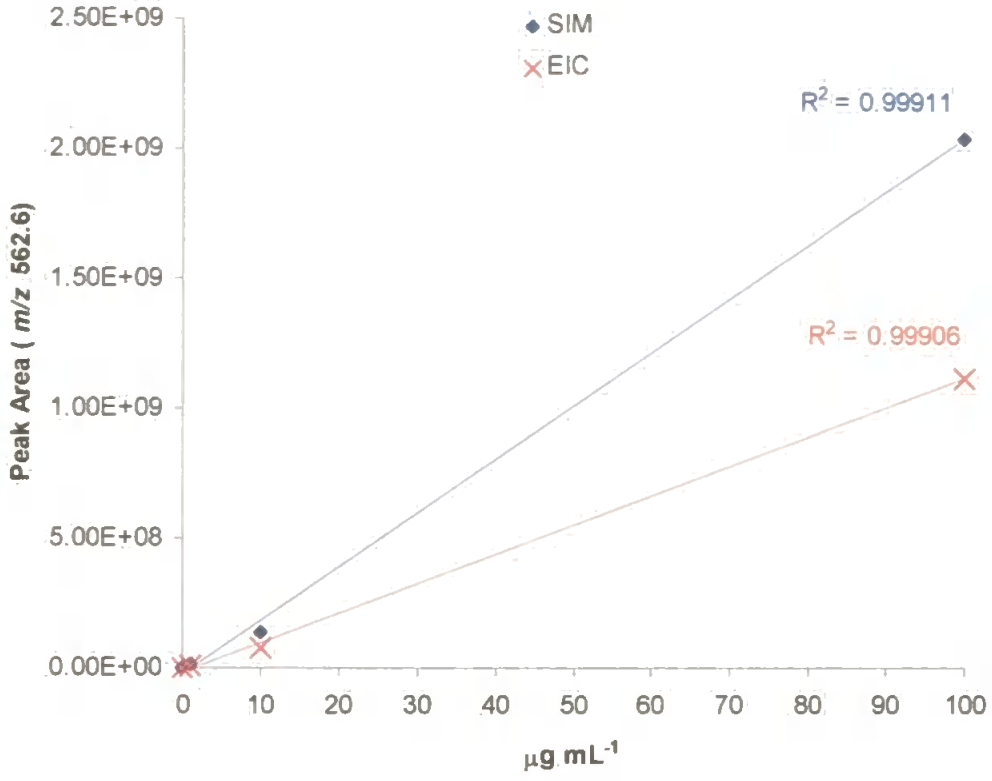


Figure 4.13 Plot of the selected (SIM) and extracted (EIC) ion peak area for 2:1-PI (*m/z* 562.6) standards against concentration. Linear trend line and R^2 value shown. Conditions given in Table 4.4.

Following the promising results of the above calibration, a second calibration series of standards (0.0124 to 12.4 µg mL^{-1}) of 2:1-PI (Sample PI/1-fp; Section 4.2.5.3) was carried out in full scan mode (*m/z* 100 – 1000) with an increase in injection volume (5 to 20 µL), a small change in gradient (to 95% A in 11 minutes compared to 15minutes previously) and half magnitude dilutions at the 0.0620 and 0.620 µg mL^{-1} level. Extracted ion chromatogram (*m/z* 562.60) peak data for 2:1-PI, are given in Table 4.5. An order of magnitude improvement in detection was observed, with a peak at a signal to noise ratio of three, for the 0.0124 µg mL^{-1} standard. A plot of the peak areas against concentration (Figure 4.14) show a very good order two polynomial trend (R^2 = 1.0000) compared to the previous linear calibration (Figure 4.13). However, when the previous calibration was re-examined and an order two polynomial trend line fitted (Figure 4.15), an R^2 = 1.0000 was

also observed, except with an opposite slope (increasing slope with increasing concentration). The reason for these differences was not clear and required further investigation once an optimised system had been determined.

Table 4.5 Extracted ion chromatogram peak data for a series of 2:1-PI (*m/z* 562.6) standards; 20µL injections on PRP-1 with gradient [(time/%A) 0/75-0.5/75-11/95; A) 0.1% TFA in methanol and B) 0.1% TFA]

Standard	Extracted ion *m/z* 562.6			
µg mL^{-1}	R_t	AA	AH	SN
12.4	12.65	366931542	11493193	124
1.24	12.68	59875892	1946530	96
0.620	12.74	32735977	1113637	44
0.124	12.78	5693611	248173	14
0.0620	12.74	3777264	140890	9
0.0124	12.81	792544	48515	3
Blank	n.d.	n.d.	n.d.	n.d.

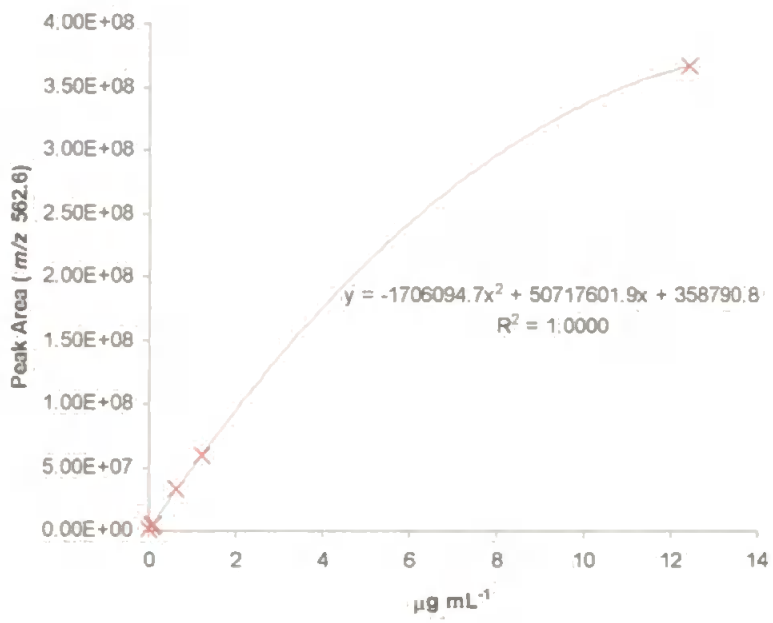


Figure 4.14 Plot of the extracted (EIC) ion peak area for 2:1-PI standards (*m/z* 562.6) against concentration. Order two polynomial trend line, equation and R^2 value shown. Conditions given in Table 4.5.

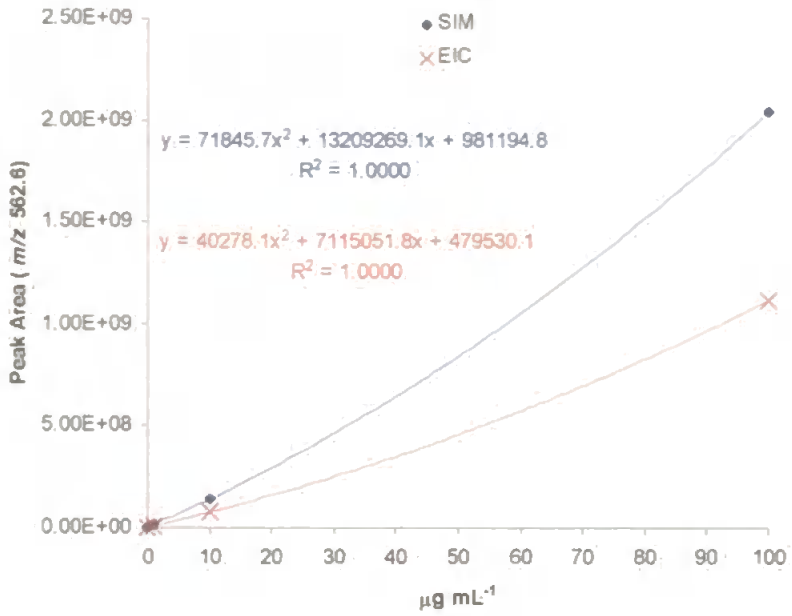


Figure 4.15 Plot of the selected (SIM) and extracted (EIC) ion peak area for 2:1-PI standards (*m/z* 562.6) against concentration. Order two polynomial trend line, equation and R^2 value shown (*cf* Figure 4.13). Conditions given in Table 4.4.

4.3.6 *Investigation of ion-pair and solvent systems for the HPLC/ESI-MS detection 1:1-PI and 2:1-PI*

It is widely known that TFA can cause signal suppression of analytes during electrospray ionisation.[120,121] Therefore, heptafluorobutyric acid (HFBA) was investigated as a possible alternative ion pairing agent. HFBA generally provides greater retention due to the longer per-fluoro chain, but can have less ESI suppression.[112,121] 0.1% TFA was replaced with 0.1% HFBA in both mobile phases (A; methanol and B; water) and the 23 hour 2:1-PI synthesis sub-sample analysed (Sample PI/1-23hr; Section 4.2.4.3). The PI/1-23hr sample was ideal for investigating the chromatographic conditions as it contained approximately equal quantities of both 1:1-PI and 2:1-PI. HFBA had a detrimental effect on the elution of 1:1-PI and caused 'fronting' of the peak (Figure 4.15). It was also observed that the

background was generally noisier than spectra obtained with TFA as the agent (spectra not shown) and therefore HFBA was not further evaluated.

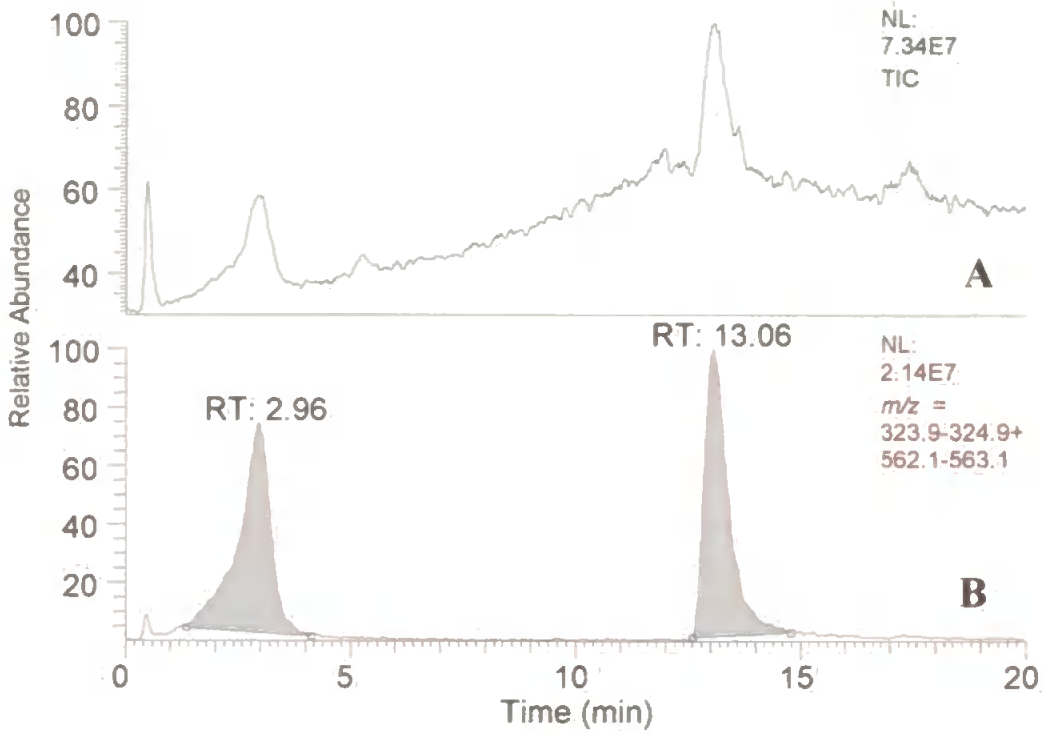


Figure 4.16 HPLC/ESI-MS mass chromatograms of 2:1-palmitic imidazoline synthesis 23hr sub sample by gradient elution with heptafluorobutyric acid ion-pair reagent [(time/%A) 0/80-1/80-16/100; A) 0.1% HFBA in methanol and B) 0.1% HFBA] on PRP-1 column: (A) total ion current chromatogram and (B) EIC's for 1:1-PI and 2:1-PI (*m/z* 325.4 and 562.6).

The organic/water content of eluent is also known to affect electrospray ionisation efficiency. Both 100% water and 100% organic are detrimental. Generally 50% to 80% organic phase is optimal for LC/ESI-MS of many compounds. For elutions carried out so

far, the gradients have started at 75% organic content (methanol) and finished with 95 or 100% organic. The 2:1-imidazoline compounds do not elute until the organic content is about 95%. Ethanol as the organic eluent was evaluated to see if the 2:1-imidazoline compounds could be eluted with a higher aqueous content. A gradient elution (60 – 100% in 15 minutes of 0.1% TFA in ethanol) of PI/1-23hr sample (Figure 4.17) showed that 2:1-PI eluted in 10.49 minutes and ~ 80%, 0.1% TFA in ethanol with a good peak shape. 1:1-PI eluted in 1.71 minutes with a symmetrical peak and sharper than observed in previous methanol elutions. The improvement in tailing of the 2:1-PI peak was due to a 0.1 minute delay in the start of the gradient rather than the 0.5 minute delay used previously. The initially 0.5 minute delay was set in order to allow increased retention (relative to the solvent front) of the 1:1-PI and also the requirement of the P580 binary pump to have a time set after t_0 for the start of a gradient profile. 0.1% TFA in both methanol and ethanol are suitable for elution of 1:1-PI and 2:1-PI and required further evaluation with other imidazoline compounds. Acetonitrile (MeCN), which is also a common reverse-phase eluent, was not evaluated as neither the synthesised compounds nor the commercial imidazoline products were soluble in MeCN.

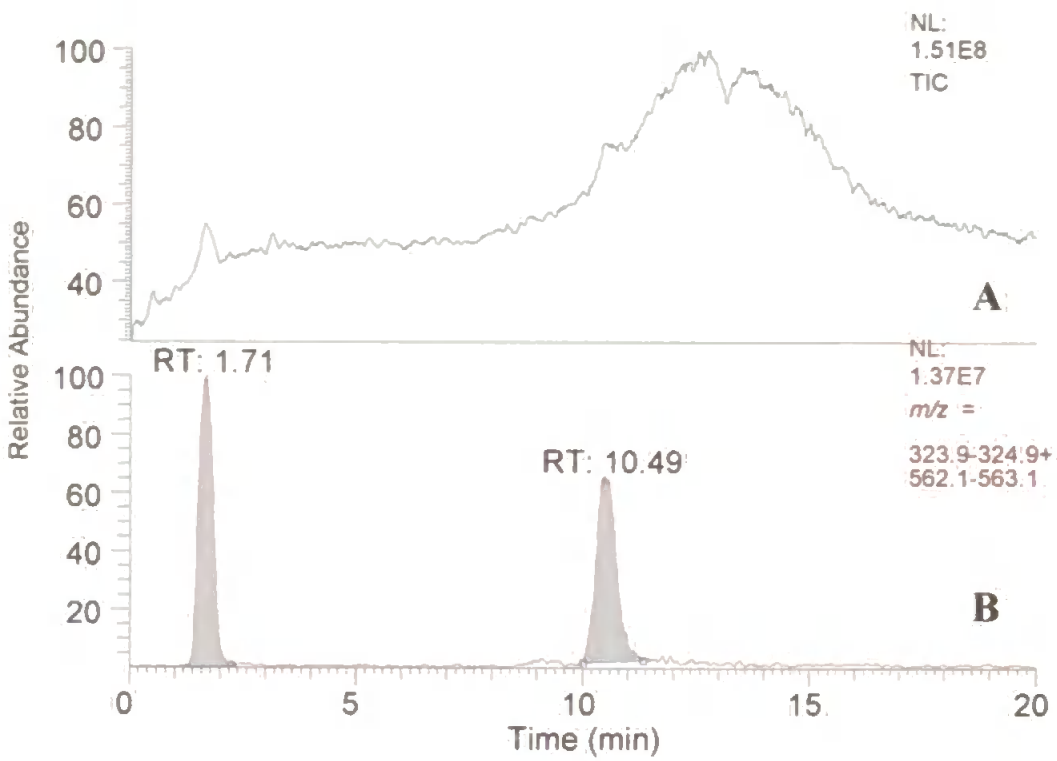


Figure 4.17 HPLC/ESI-MS mass chromatograms of 2:1-palmitic imidazoline synthesis 23hr sub sample by gradient elution with ethanol [(time/%A) 0/60-0.1/60-15/100; A) 0.1% TFA in ethanol and B) 0.1% TFA] on PRP-1 column: (A) total ion current chromatogram and (B) EIC's for 1:1-PI and 2:1-PI (*m/z* 325.4 and 562.6).

4.3.7 *Synthesis of 1:1-oleic imidazoline by Dean and Stark reflux in xylene*

The 23 hour sub-sample from the 2:1-PI synthesis was useful for developing the HPLC/ESI-MS due to the presence of both the 1:1 and 2:1-PI compounds. However, in order to quantitate the relative responses, pure individual compounds were required. The 1:1-imidazoline is predicted to be the major product if DETA and fatty acid are allowed to react in a 1:1 molar ratio[75,76,78,90,98,99,122] although only Bondareva *et. al.*[75] isolated and characterised the monoamine intermediate and 1:1-imidazoline product. A 1:1 molar ratio of DETA and oleic acid were reacted by Dean and Stark reflux in xylene for 91 hours (Section 4.2.5.4). Sub samples of the reaction solution, were taken at 1, 3, 19 and 91 hours. HPLC/ESI-MS analysis of each sample was carried out. In the 1 hour sample, ions could be identified in the mass spectra and extracted for the 1:1-OI (*m/z* 350.4; peak 1; 100%), monoamide (*m/z* 368.3; peak 2; 30%), 2:1-OI (*m/z* 614.7; peak 3; 80%) and the diamide (*m/z* 632.6; peak 4; 10%) (Figures 4.18 and 4.19). 1:1-OI is the main product (100% relative abundance in extracted ion chromatogram; Figure 4.18) whilst the 2:1-OI product is present at 60% relative abundance. This was not expected for such a short reaction time, as an excess of fatty acid, higher temperature or longer reaction times are generally required for production of 2:1-imidazolines.[75,76,78,90] Over the course of the reaction (Figure 4.20), it can be seen that the monoamide (*m/z* 368.3; peak 2) decreased from 30% to 3%, whilst the 2:1-OI (*m/z* 614.7; peak 3) decreased from 80% to 46%. This suggests that there was little change in the amount of 2:1-OI produced after 1 hour and that the relative abundance change was due to condensation of the monoamide to the 1:1-OI product. It could also be seen that the diamide product was present at 1 hour and decreased from 10% to <1% throughout the reaction (*m/z* 632.6; peak 4; (Figures 4.18 and 4.20). This means that both the monoamide and diamide were formed before cyclisation to the respective imidazoline and that both reaction pathways 1 and 2 (Figure 4.1) are possible. The reactions carried out so far, thus highlight why the literature has reported conflicting results and disagreements on the reaction mechanisms and pathways. It was also becoming

apparent from the above studies that there were several potential remaining problems for the method. For example, the presence of fatty acid, fatty acid amine or amide salts could not be detected by the HPLC/ESI-MS system; hydrolysis of the imidazolines during sample preparation might occur (during or before HPLC/ESI-MS); the oleic imidazolines were oils and recrystallisation was not possible thus a method of purification was required.

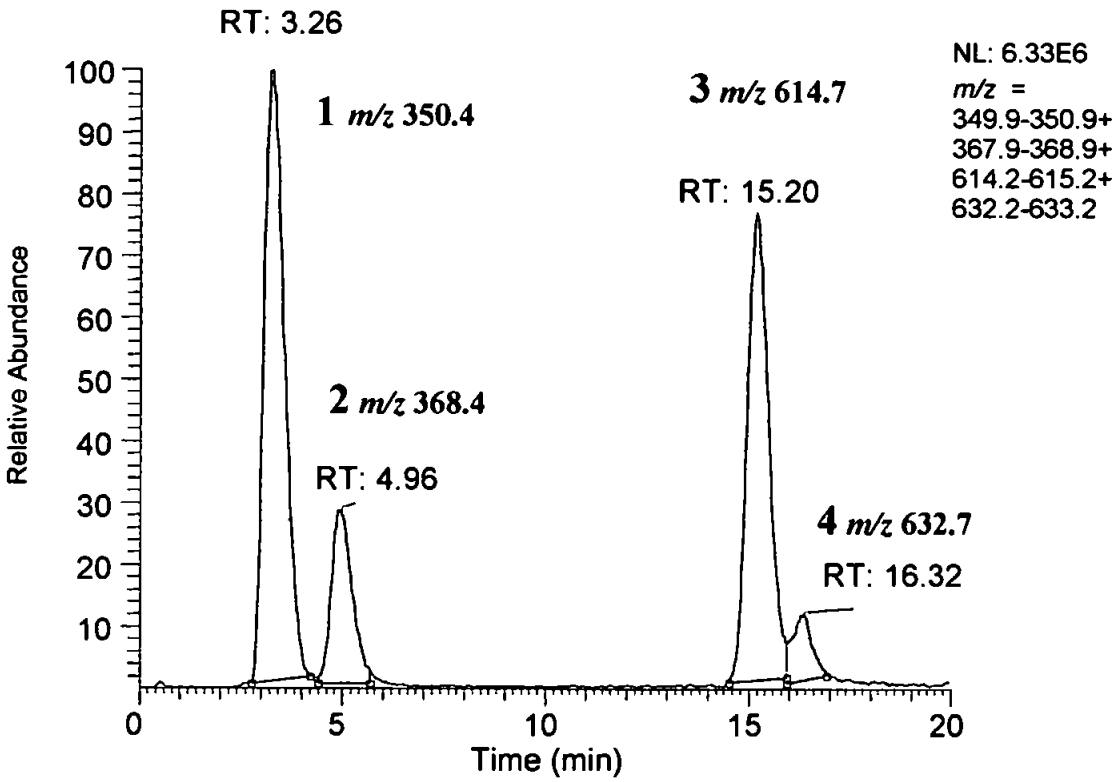


Figure 4.18 HPLC/ESI-MS extracted mass chromatogram of; 1:1-OI (*m/z* 350.4; peak 1), monoamide (*m/z* 368.3; peak 2), 2:1-OI (*m/z* 614.7; peak 3) and the diamide (*m/z* 632.6; peak 4) for 1:1-oleic imidazoline synthesis 1hr sub sample, by gradient elution with ethanol [(time/%A) 0/55-0.1/55-20/100; A) 0.1% TFA in ethanol and B) 0.1% TFA] on PRP-1 column.

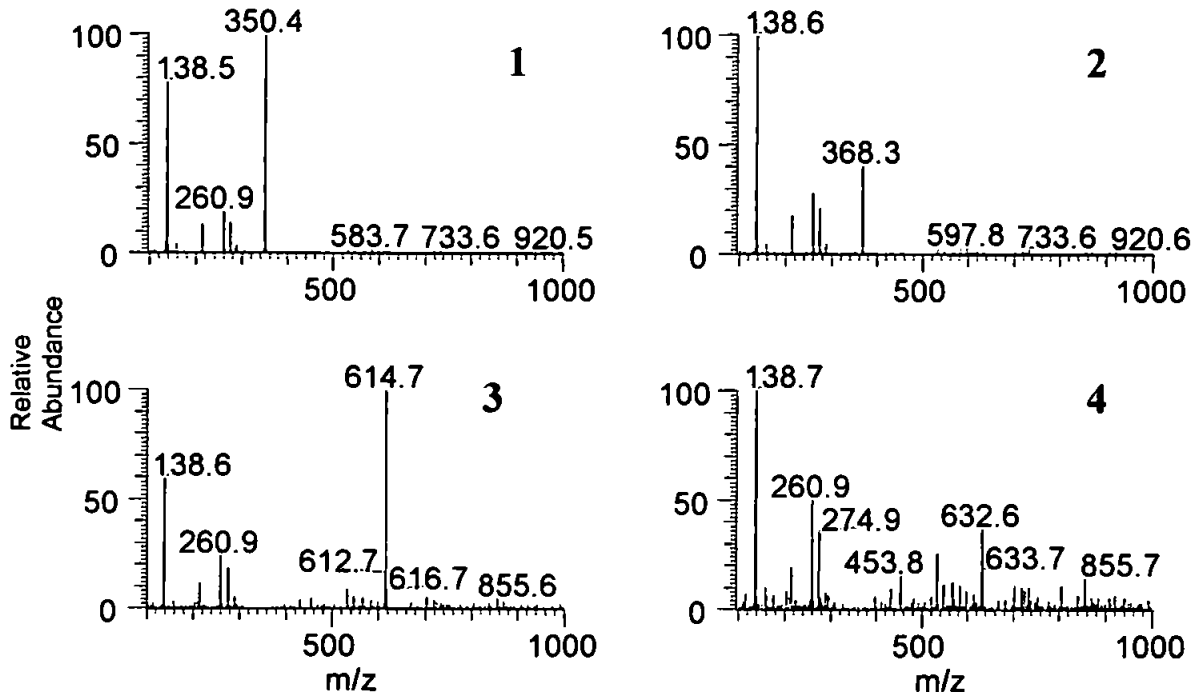


Figure 4.19 Mass spectra of peaks 1-4 shown in Figure 4.18.

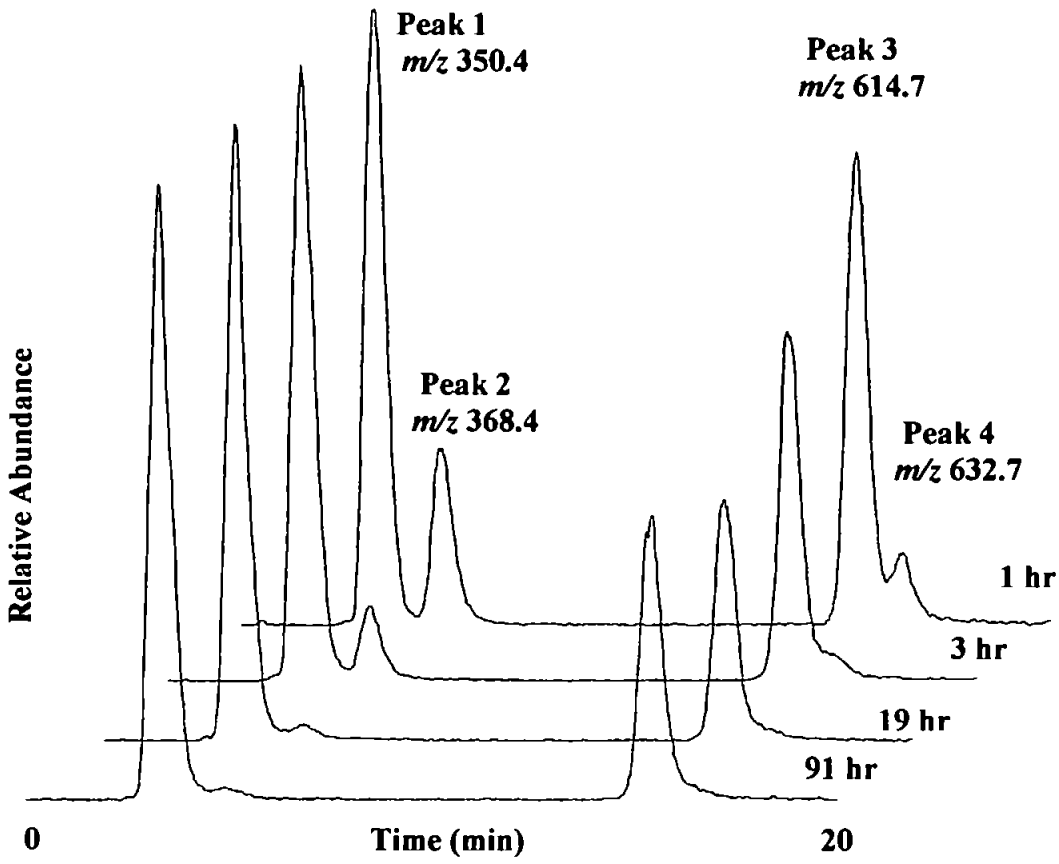


Figure 4.20 HPLC/ESI-MS extracted mass chromatograms of 1, 3, 19 and 91hr sub samples for 1:1-oleic imidazoline synthesis. Peaks 1-4 and conditions given in Figure 4.18.

4.3.8 *Synthesis of 2:1-oleic imidazoline by Dean and Stark reflux in xylene and purification by vacuum distillation*

Bondareva *et. al.*[75] used vacuum distillation to isolate monoamine, 1:1-imidazoline and 2:1-imidazoline produced by sequential time/temperature stages by solid-phase reaction of DETA with 2-ethylhexanoic acid. In the present study, in an attempt to determine a distillation procedure, a larger scale (100 g oleic acid) Dean and Stark reflux synthesis of 2:1-OI was carried out over 95 hours with sub samples taken at 5, 23 and 95 hours (Section 4.2.5.5). HPLC/ESI-MS analysis of each sample was carried out and for each sample, ions could be identified and extracted for the 1:1-OI (*m/z* 350.4; peak 1, monoamide (*m/z* 368.3; peak 2), 2:1-OI (*m/z* 614.7; peak 3) and the diamide (*m/z* 632.6; peak 4) (Figure 4.21) products. 2:1-OI was the main product (100% relative abundance in extracted ion chromatograms; Figure 4.21) whilst the 1:1-OI product decreased as the reaction progressed and was present at 55, 38 and 28% relative abundance at 5, 23 and 95 hours respectively. An increase in the diamide is observed in the 23 hour EIC (Peak 4; Figure 4.21); however, this is due to hydrolysis of the 2:1-OI during the rotary evaporation of xylene. This highlights the potential and ease of hydrolysis of the 2:1-OI. This was further confirmed when an attempt to vacuum distil a quantity (64 g) of the 2:1-OI final product (Section 4.2.5.6) resulted in the formation of a white solid from what was initially a yellow vapour which condensed to a yellow oil. An air leak could clearly be seen but proved difficult to seal. A further vacuum distillation was not carried out as the remaining 2:1-OI product was used for solid-phase extraction experiments (Chapter 3). Over 12 weeks storage at 4 ^{O}C in a stoppered and sealed round bottom flask (except for removal of aliquots), a crystalline white precipitate appeared and gradually increased in amount over time. The contents of the flask were filtered under vacuum with a washing of hexane. The collected precipitate was washed with hexane and dried at 40^{O}C for 24 hours. HPLC/ESI-MS analysis of the collected solids, showed that the major peak in the extracted chromatogram (Peak 2, *m/z* 632.7; Figure 4.22 and 4.23) corresponded to the expected

mass for protonated oleic-diamides. Indeed, MS^n analysis (Appended; Figures A2 – A6) was consistent with identification of the product as oleic-diamides and ^1H nuclear magnetic resonance spectroscopy (Appended; Figure A7) was consistent with the product being 1,3-oleic diamide (Structure V, Figure 3.1) as discussed in Chapter 5 (section 5.3.3.3 and 5.3.3.4). A small amount of 2:1-OI was also present (Peak 1, *m/z* 614.7; Figure 4.22 and 4.23).

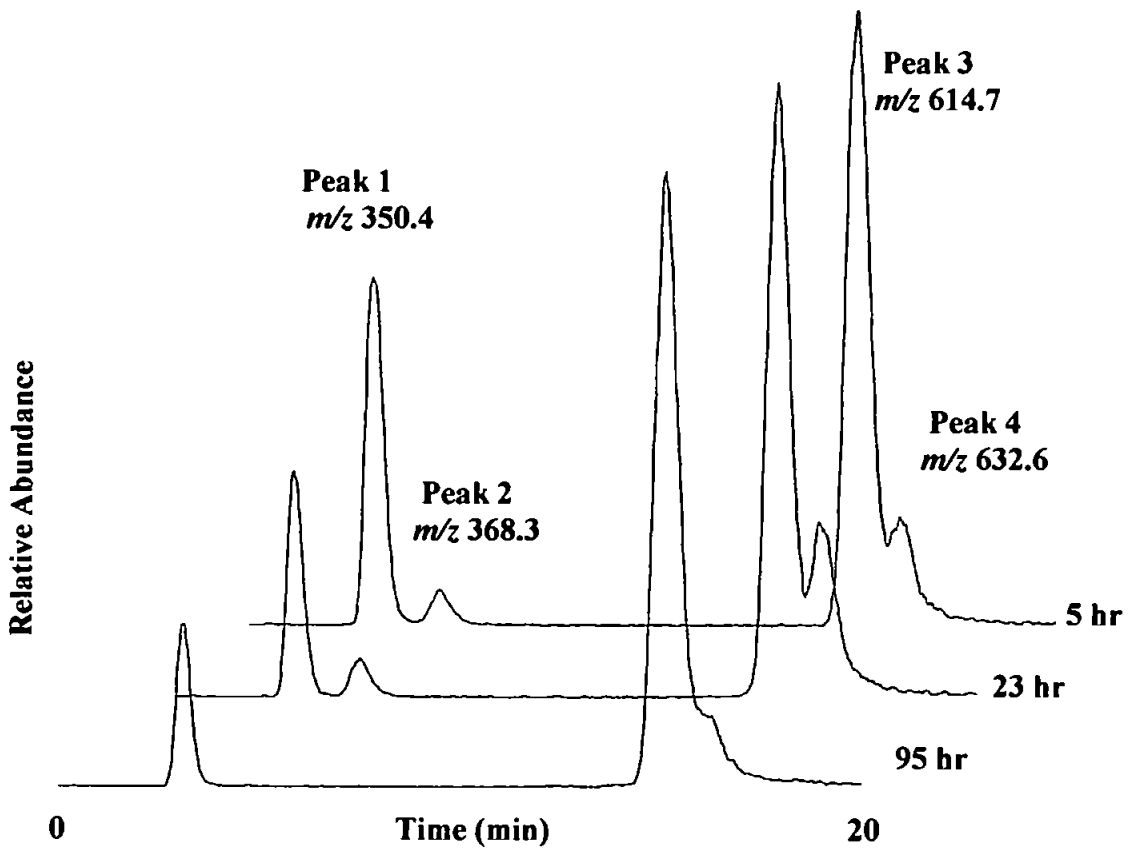


Figure 4.21 HPLC/ESI-MS extracted mass chromatograms of 1:1-OI (*m/z* 350.4; peak 1), monoamide (*m/z* 368.3; peak 2), 2:1-OI (*m/z* 614.7; peak 3) and the diamide (*m/z* 632.6; peak 4) for 5, 23 and 95hr sub samples of 2:1-oleic imidazoline synthesis. Gradient elution with ethanol [(time/%A) 0/55-0.1/55-20/100; A) 0.1% TFA in ethanol and B) 0.1% TFA] on PRP-1 column.

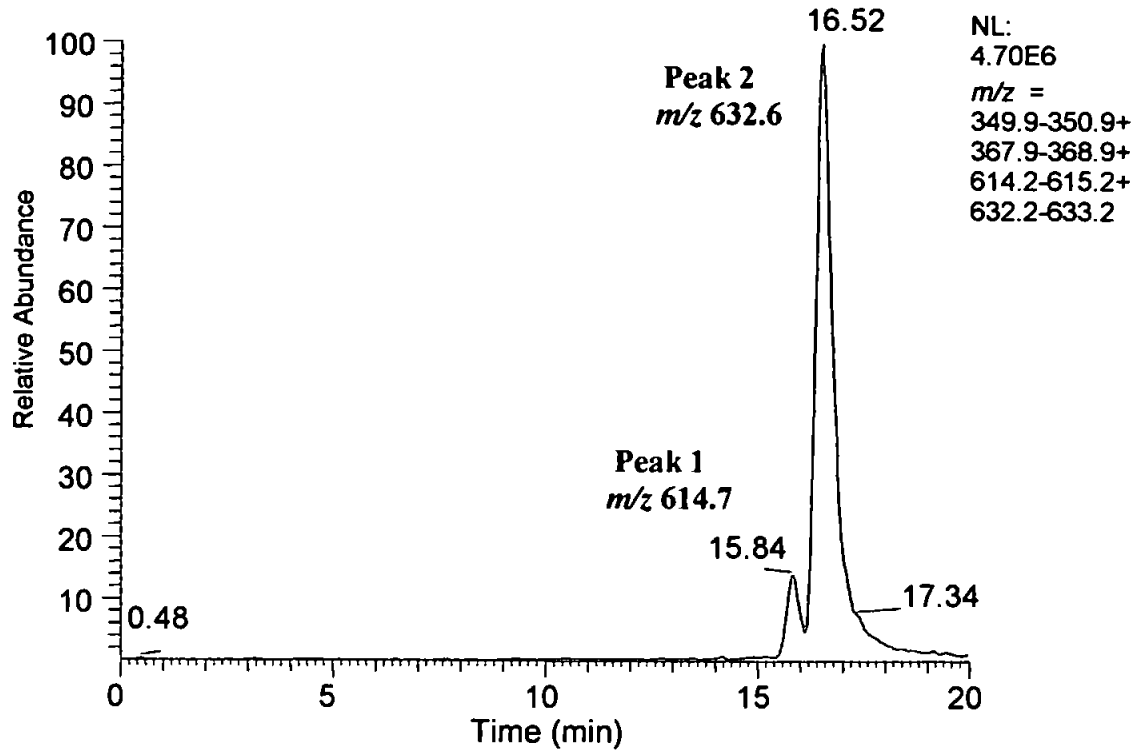


Figure 4.22 HPLC/ESI-MS extracted mass chromatogram of; 1:1-OI (*m/z* 350.4; no peak), monoamide (*m/z* 368.3; no peak), 2:1-OI (*m/z* 614.7; peak 1) and the diamide (*m/z* 632.6; peak 2) for oleic diamide. Gradient elution with methanol [(time/%A) 0/75-0.1/75-17/100; A) 0.1% TFA in methanol and B) 0.1% TFA] on PRP-1 column.

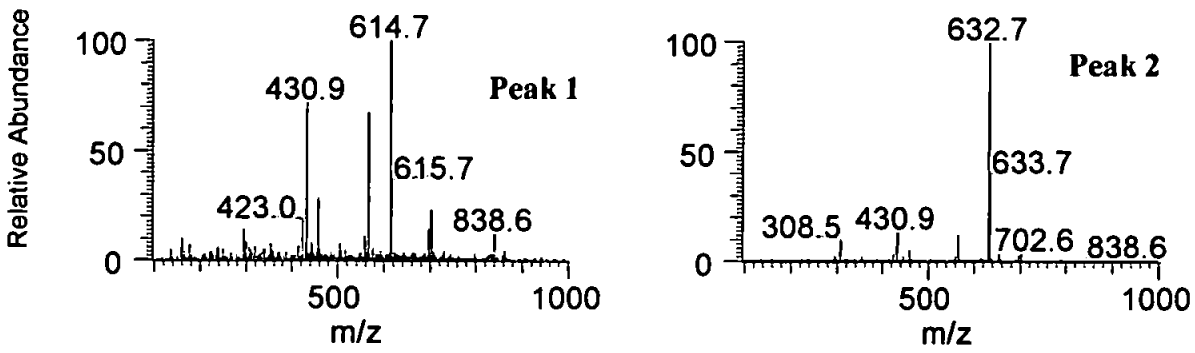


Figure 4.23 Mass spectra of peaks 1 and 2 Figure 4.22.

4.3.9 *Investigation of the HPLC/ESI-MS response for 2:1-OI*

A series of 2:1-OI standards (0.005 – 10 μg mL^{-1}; Section 4.2.5.5) was analysed to determine the response by HPLC/ESI-MS in order to determine the best method for evaluating and providing an external calibration method for both 2:1-OI and 2:1-PI. Peak retention time, integrated area and signal to noise ratio for the protonated 2:1-OI ion (*m/z* 614.7) are given in Table 4.6. The lowest detectable peak was at the 0.005 μg mL^{-1} concentration (S/N = 3) with the 0.01 μg mL^{-1} standard giving a signal to noise ratio of four. The 0.01 – 10 μg mL^{-1} standards were re-analysed the following day (data in Table 4.6). The Xcalibur software-determined signal to noise levels show that the background signal response was highly variable for the eluent system with the signal to noise ratios increasing to maximum values for the 1μg mL^{-1} concentrations (S/N = 480 and 218, day 1 and 2 respectively; Table 4.6) and decreasing at the higher concentrations. Plotting the *m/z* 614.7 peak area response against concentration for the day 1 and 2 standards (0.01 – 10 μg mL^{-1}; Figure 4.24) a curvilinear response was observed. The relative response decreased above 1 μg mL^{-1}. This decrease in response at higher concentrations is normal for the electrospray ionisation process and is dependent on the ease of ionisation of the compound and the total concentration of ions in the eluent system. The response is linear for both day one and day two analyses in the range (0.01 – 1 μg mL^{-1}; Figure 4.25 and 4.26 respectively) with R^2 values of 0.9900 and 0.9777 respectively. There was greater variation observed in the day to day response of the 0.5 and 1 μg mL^{-1} samples (Figure 4.25 and 4.26 respectively). However, when the day one and day two responses were averaged (Figure 4.27) a very good linear trend (R^2 = 0.9997) was observed. Solvent blanks were examined by LC/ESI-MS between each standard and show that sample carryover was present only in the inter-sample blanks from the 1 μg mL^{-1} concentration and higher (Table 4.6). This indicates that sample carryover due to the system should not be a problem in the linear response concentration range. The calibrations carried out showed that an external

calibration method for 2:1-OI could be developed, although a more detailed study would be needed to be carried out on sample repeatability and day to day variability of response.

Table 4.6 Extracted ion chromatogram peak data for a series of 2:1-OI (*m/z* 614.7) standards; 20μL injections on PRP-1 with gradient elution [(time/%A) 0/75-0.1/75-17/100; A) 0.1% TFA in methanol and B) 0.1% TFA]

Standard	Day 1				Day 2		
(μg mL^{-1})	R_t	AA	SN		R_t	AA	SN
Blank	*n.d*						
0.001	n.d				Not run		
0.005	15.77	274417	3		Not run		
Blank	*n.d*				*n.d*		
0.01	15.88	483151	4		15.91	607910	6
Blank	*n.d*				*n.d*		
0.05	15.69	3644524	32		16.17	3944367	23
Blank	*n.d*				*n.d*		
0.1	15.66	8136643	79		16.08	8099689	37
Blank	*n.d*				*n.d*		
0.5	15.61	32884677	208		16.13	40737031	163
Blank	*n.d*				*16.05*	*309155*	*3*
1.0	15.61	81621473	480		16.11	62187936	218
Blank	*15.67*	*534463*	*5*		*15.92*	*679758*	*5*
5.0	15.64	202699140	168		15.95	193559699	107
Blank	*15.74*	*2760026*	*20*		*16.02*	*3105846*	*23*
10	15.63	352526470	90		15.95	354972761	98

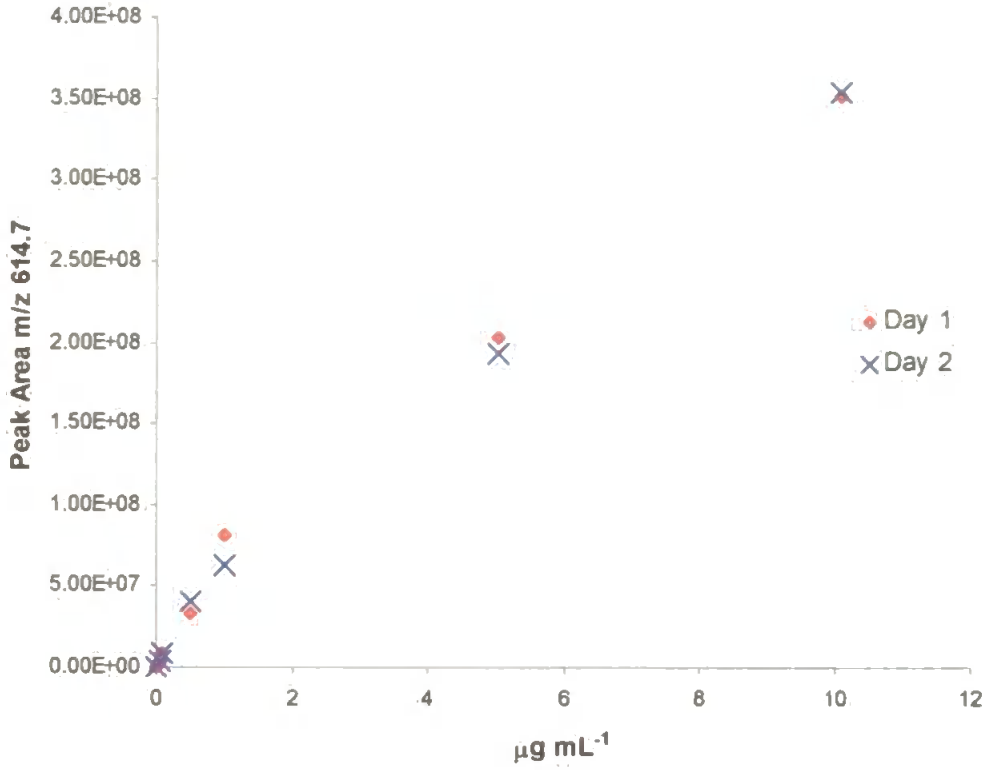


Figure 4.24 Mass spectral ion current response (*m/z* 614.7) of 2:1-OI standard solutions (0.01 - 10 µg mL^{-1}) on consecutive days.

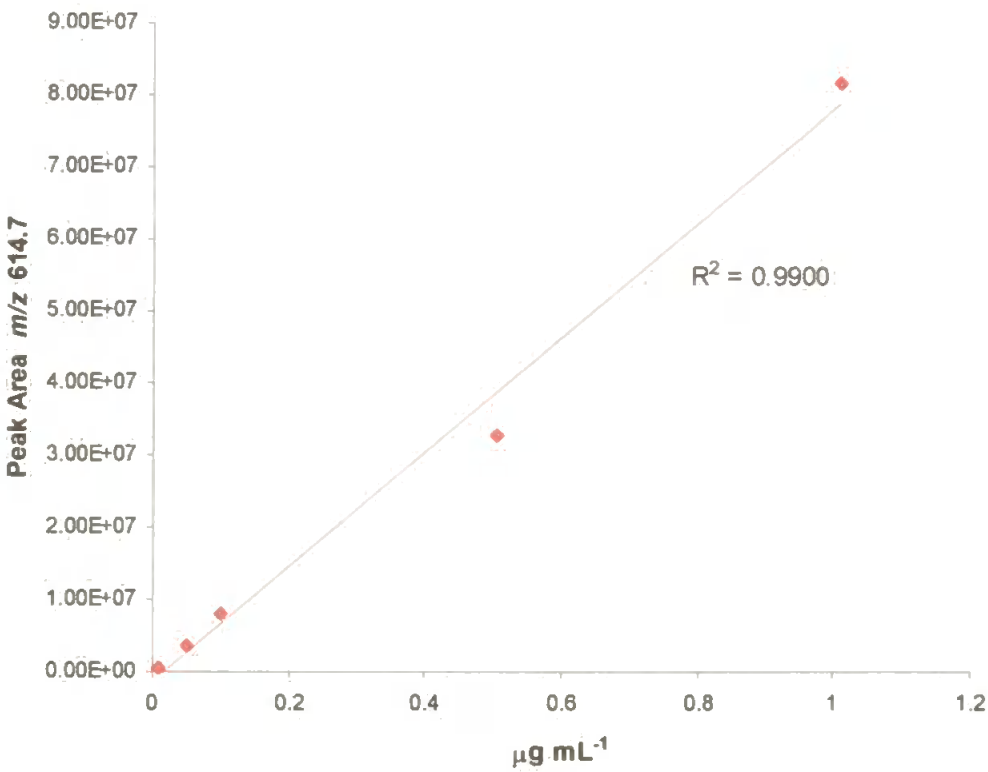


Figure 4.25 Mass spectral ion current response (*m/z* 614.7) of 2:1-OI standard solutions (0.01 - 1 µg mL^{-1}) on day 1.

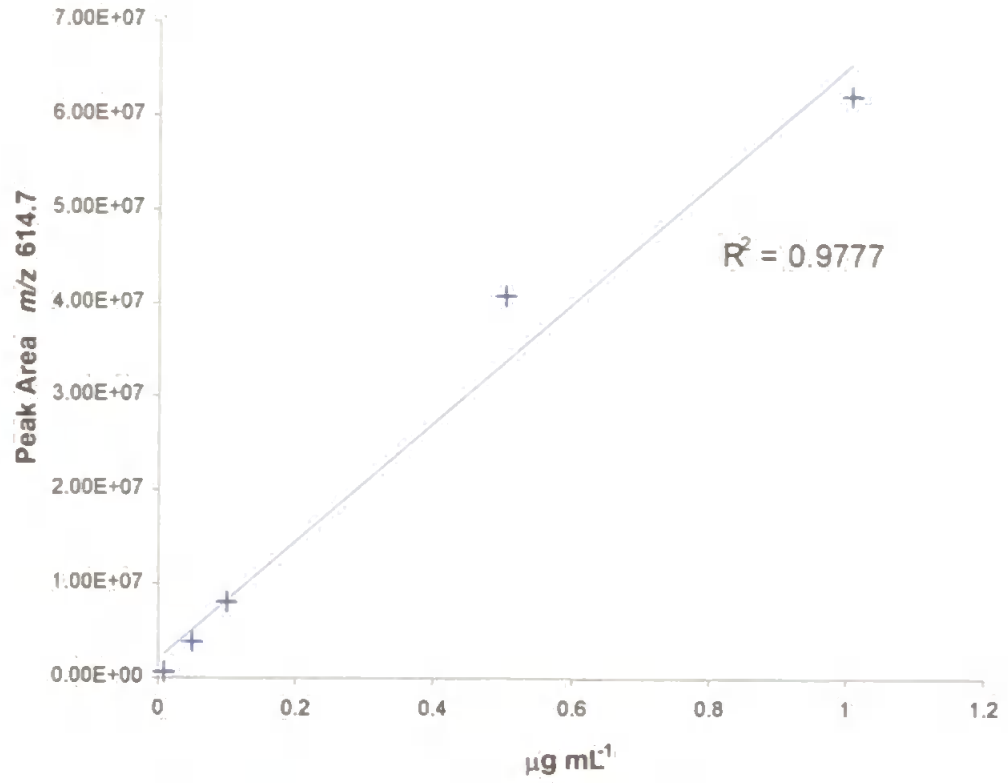


Figure 4.26 Mass spectral ion current response (*m/z* 614.7) of 2:1-OI standard solutions (0.01 - 1 µg mL^{-1}) on day 2.

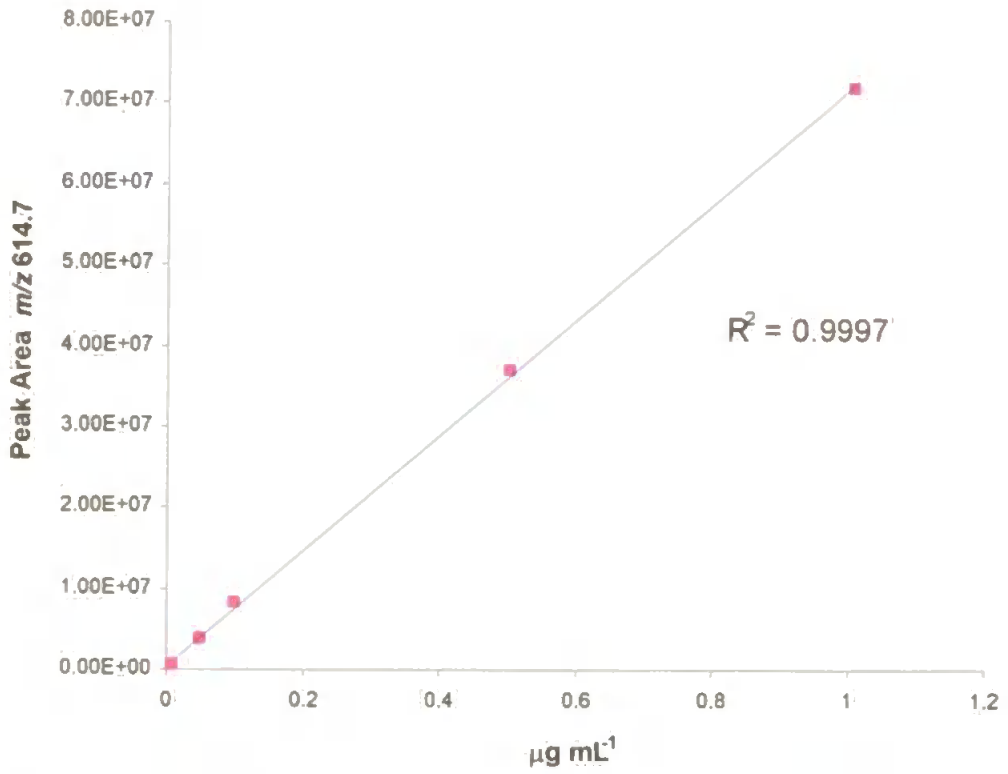


Figure 4.27 Averaged (day 1 and 2) mass spectral ion current response (*m/z* 614.7) of 2:1-OI standard solutions (0.01 - 1 µg mL^{-1}).

4.3.10 *HPLC/ESI-MS analysis of a commercial imidazoline-based corrosion inhibitor*

Having shown that synthetic palmitic and oleic acid-derived imidazolines and amides could be separated, the HPLC/ESI-MS method was applied to a commercial imidazoline corrosion inhibitor product (CI-E1). The total ion chromatogram (Figure 4.28A) revealed a series of small broad peaks in the first five minutes and a large broad peak at about sixteen minutes, which showed enhanced resolution and intensity in the extracted base peak chromatogram (Figure 4.28B). Extracting selected ion chromatograms of *m/z* values observed in the mass spectra under the peaks and observed in a previous infusion ESI-MS analysis (See Chapter 3 section 3.3.2) of the product, the presence of a range 1:1- and 2:1-imidazolines (Figure 4.29) and the corresponding monoamides and diamides (Figure 4.30) derived from C_{18} acids with up to three degrees of unsaturation ($C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$) and 1:1- and 2:1-Imidazolines (Figure 4.31) derived from C_{20} acids with up to three degrees of unsaturation ($C_{20:0}$, $C_{20:1}$, $C_{20:2}$ and $C_{20:3}$) was observed. The extracted masses correlated with those observed in the infusion ESI-MS spectra of the product and some separation of the various structural isomers (Chapter 3, section 3.3.2) was observed (Figure 4.29, 4.30 and 4.31). The resolution was poor for most of the isomers and needed to be enhanced to near baseline resolution to enable MS^n analysis. The optimised method may allow 'fingerprinting' of commercial products and their detection in environmental and other samples.

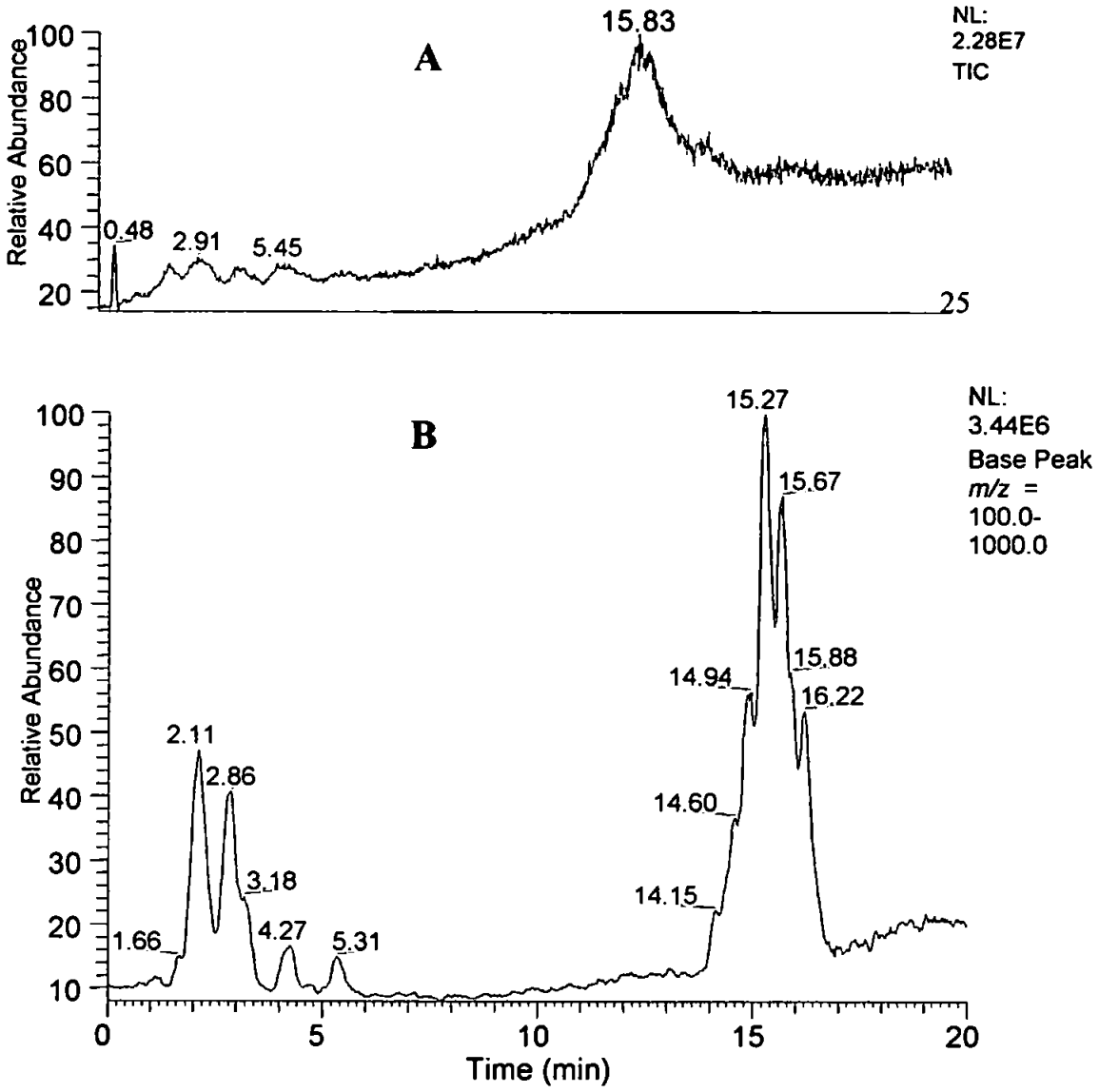


Figure 4.28 HPLC/ESI-MS mass chromatograms of imidazoline corrosion inhibitor product (CI-E1); (A) total ion current chromatogram and (B) base peak chromatogram. Gradient elution with methanol [(time/%A) 0/75-0.1/75-17/100; A) 0.1% TFA in methanol and B) 0.1% TFA] on PRP-1 column.

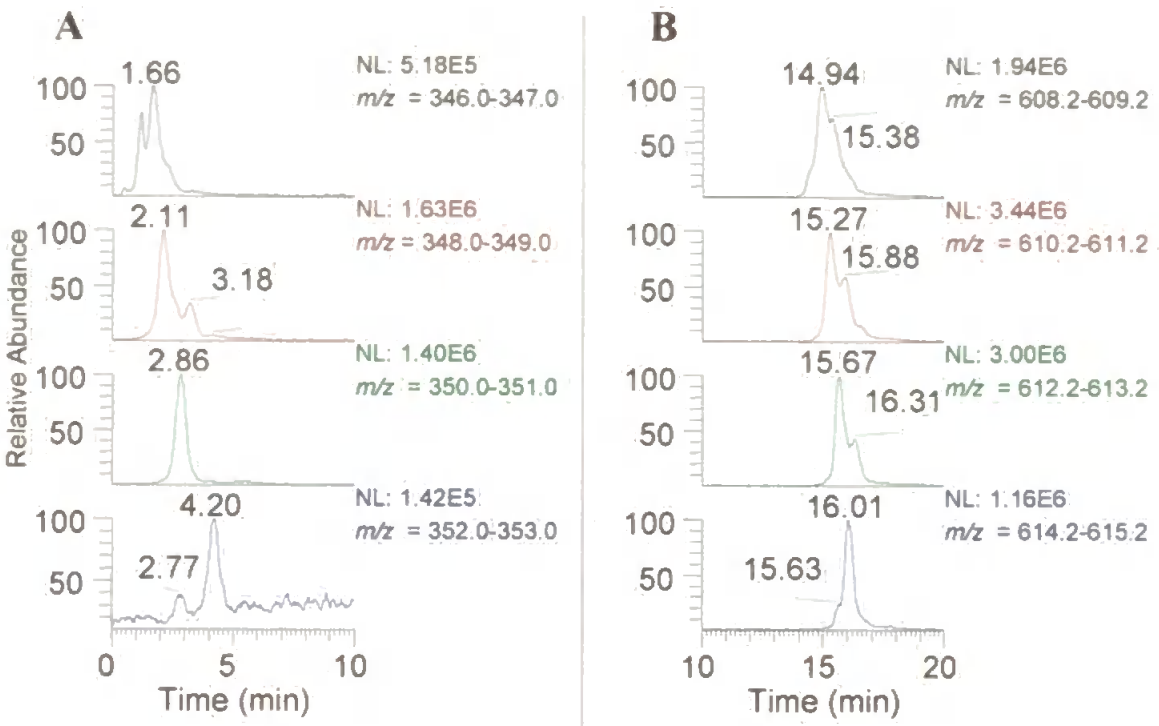


Figure 4.29 HPLC/ESI-MS extracted mass chromatograms of imidazoline corrosion inhibitor product (CI-E1); (A) $C_{18:n}$ 1:1-imidazolines and (B) $C_{18:n}/C_{18:n}$ 2:1-imidazolines. Conditions as Figure 4.28.

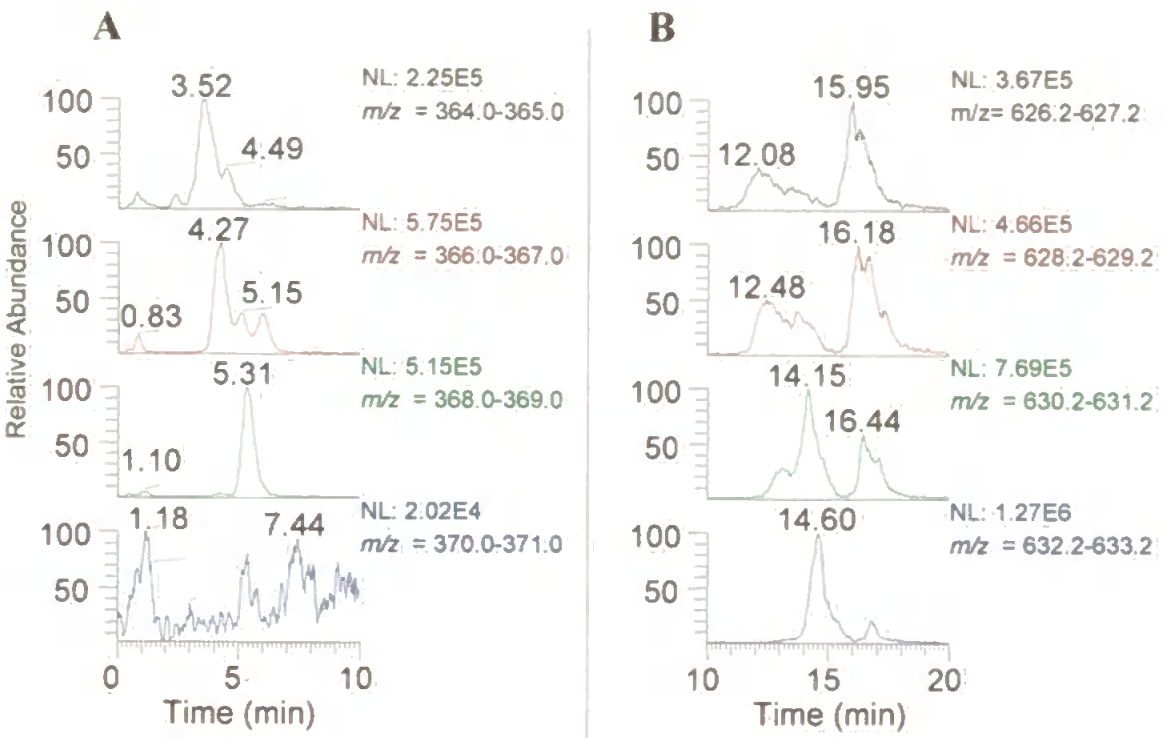


Figure 4.30 HPLC/ESI-MS extracted mass chromatograms of imidazoline corrosion inhibitor product (CI-E1); (A) $C_{18:n}$ monoamides and (B) $C_{18:n}/C_{18:n}$ diamides. Conditions as Figure 4.28.

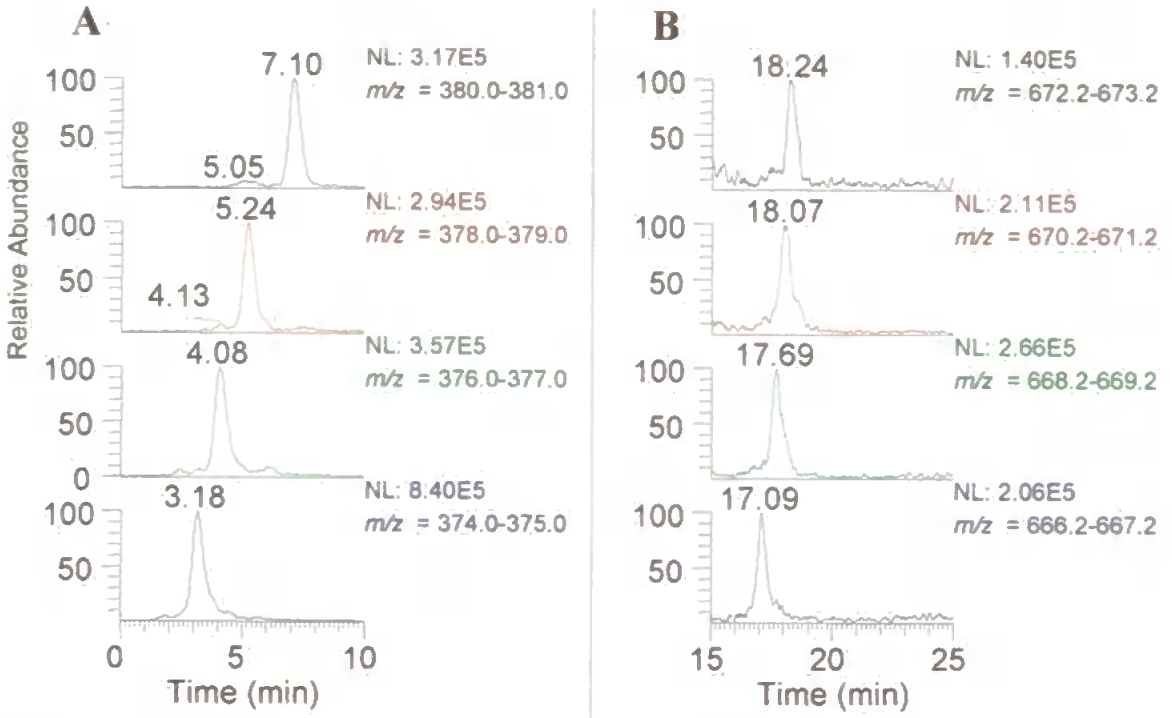


Figure 4.31 HPLC/ESI-MS extracted mass chromatograms of imidazoline corrosion inhibitor product (CI-E1); (A) $C_{20:n}$ 1:1-imidazolines and (B) $C_{20:n}/C_{20:n}$ 2:1-imidazolines. Conditions as Figure 4.28.

4.4 Conclusion

A HPLC/ESI-MS method for the separation and detection of palmitic and oleic acid-derived 2:1-imidazolines, 1:1-imidazolines, monoamide and diamide compounds was developed and the ESI-MS responses investigated. The method was used to separate the imidazoline and amide components of a commercial imidazoline product and separation of structural isomers was shown. A simple synthesis and purification method of the compounds was not identified, consistent with confusing literature reports,[74-79,90-100] and therefore pure compounds could not be obtained for investigation of the relative responses for the individual compounds. However, the HPLC/ESI-MS method proved to be a powerful method for monitoring the progress of synthesis and the intermediate compounds and highlighted some reasons for the contradictory results in the published literature.

CHAPTER FIVE

Investigation of the thermal synthesis of palmitic acid with diethylenetriamine (DETA)

This chapter describes the investigation of the reaction intermediates and pathways of solid phase and xylene solvent-based thermal reactions of fatty acids with diethylenetriamine (DETA). The reaction products were studied by high performance liquid chromatography (HPLC) with electrospray ionisation mass spectrometry (ESI-MS) detection. Structural characterisation was based on electrospray ionisation multistage mass spectrometry ($ESI-MS^n$), Fourier transform infra red (FT-IR) and ^1H nuclear magnetic resonance (NMR) spectroscopy.

5.1 Introduction

Ever since the initial synthesis of 'imidazolines' described by Hofmann[101] in 1888, there has been debate concerning the mechanism of the reaction between fatty acids and triamines.[76,88-90] Indeed, even the existence of the imidazoline compounds in corrosion inhibitor products has been questioned.[54] Yet the subject is of major importance for synthesis of pure amides and imidazolines and for the efficient deployment of anti-corrosion measures since the composition of the synthetic mixtures controls their self assembly as monolayers on metal surfaces and hence their efficency.[123]

Much of the confusion may have arisen from the existence of different mechanisms in the solid-phase and solvent-based reactions.[89] However, attempts to elucidate the mechanisms have also been hampered by the lack of sensitive, specific methods for identification of the reactants, intermediates and products in the complex mixtures resulting from such reactions. Thus, even recent studies[89] have required isolation and recrystallisation of the chemicals at each stage, followed by spectroscopic examination by nuclear magnetic resonance spectroscopy (NMR) and other techniques. Powerful though these methods are, they are not sensitive to (perhaps important) minor products and intermediates, most of which are in any case, removed by recrystallisation procedures. This may have led to some of the misleading conclusions concerning the mechanisms.

The following study was carried out to determine an efficient method for the synthesis of 2:1-palmitic imidazoline. The presence of palmitic-monoamides, palmitic-diamides and 1:1-palmitic imidazoline in reaction sub samples and crude products also allowed an investigation of the reaction mechanisms to be undertaken.

5.2 Experimental

5.2.1 *Chemicals*

The chemicals used were as described in Chapter 4 (Section 4.2.1).

5.2.2 *High performance liquid chromatography with electrospray ionisation mass spectrometry (HPLC/ESI-MS) and electrospray ionisation multistage mass spectrometry (ESI-MS^n)*

HPLC/ESI-MS analyses were carried out with the equipment described in Chapter 4 (Section 4.2.2 and 4.2.3) on a poly(styrene-divinylbenzene) reverse phase column (PRP-1; 50 x 4.1 mm, 5 μ, Hamilton, Reno, NA, USA). Sample injections (20 μL) were made by an ASI 100 auto-sampler. Gradient elution (Table 5.1) was made with the following eluents: A) 0.1% TFA in water and B) 0.1% TFA methanol. ESI-MS^n analyses were carried out with the equipment and as described in Chapter 4 (Section 4.2.2).

Table 5.1 Gradient elution profile for HPLC/ESI-MS analysis of imidazoline syntheses. Eluents; A) 0.1% TFA in water and B) 0.1% TFA methanol

Time (min)	0	0.1	17	27	28	35
% A	25	25	0	0	25	25
% B	75	75	100	100	75	75

5.2.3 *Fourier transform infrared (FT-IR) and ^{1}H nuclear magnetic resonance (NMR) spectroscopy*

FT-IR and ^{1}H NMR spectroscopy were carried out as described in Chapter 4 (Section 4.2.4)

5.2.4 *Thermal reaction of palmitic acid with diethylenetriamine in xylene solvent*

A thermal reaction of palmitic acid with DETA was carried out in xylene solvent with removal of water by way of a Dean and Stark apparatus, using the procedure described by Wu and Herrington[89], the only change being that the reaction was conducted at half scale. Palmitic acid (2.5 g, 9.7 m mole) dissolved in xylene (25 mL) was added, dropwise, over 1 hour, to a solution of DETA (0.98 mL, 9.1 m mole) in xylene (12.5 mL) under reflux. The reaction mixture was refluxed (4 hr). After cooling a white precipitate was collected by filtration. The collected solids were recrystallised from ethyl acetate to give a 2:1-palmitic imidazoline product (1.80 g), m.p. 95 °C with a wax like softening below the liquid point at 95 °C (*lit.* 85 – 87 °C for the palmitic acid complex[89]). A sub sample of the crude reaction mixture was collected before filtration, and xylene removed by N_2 blow down. The crude and recrystallised 2:1-PI products were prepared for HPLC/ESI-MS from a 1 mg mL^{-1} standard (1000 μL methanol added to 0.0010 g) and diluted to 5 μg mL^{-1} (5 μL of 1 mg mL^{-1} standard added to 995 μL methanol).

5.2.5 *Thermal reaction of palmitic acid with diethylenetriamine without solvent*

A thermal reaction of palmitic acid with DETA without solvent was carried out using the procedure described by Wu and Herrington.[89] DETA (10.32 g, 100 m mole) was heated to 150°C and palmitic acid (51.29 g, 200 m mole) was added in small portions over 2 hours. The reaction was continued for 5 hours with stirring and maintaining the temperature at 150°C. A sample of the crude cooled, hard waxy product was retained, whilst the remainder was recrystallised from acetone:toluene (1:1) to give 1,3- palmitic-diamide, m.p. 118 °C (*lit.* m.p. 117 – 119 °C[89]; 116 – 118 °C[76]).

1,3-Palmitic-diamide (5.00 g) was heated to 235°C under reduced pressure (30 mm Hg) for 2 hours (*ca* 0.5 hr ; Wu and Herrington). The residue was recrystallised from hexane (100 mL) to give a 2:1 palmitic imidazoline product (4.32 g), m.p. 80 °C (*lit.* 80 – 82 °C[89]; 78 -

79 °C[76]). The crude and recrystallised diamide products were prepared for HPLC/ESI-MS from a 1 mg mL^{-1} standard (0.0100 g made up to 10 mL cyclohexane:methanol:trichloromethane (4:3:3 v/v/v)) and diluted to 5 μg mL^{-1} (5 μL of 1 mg mL^{-1} standard added to 995 μL methanol). The 2:1-PI product was prepared similarly except the 1 mg mL^{-1} standard was made up with methanol (1000 μL methanol added to 1.0 mg).

5.3 Results and discussion

Having carried out a synthesis of 1:1-oleic imidazoline (Chapter 4; Section 4.3.7) in which the 1:1-OI was in greater abundance (by HPLC/ESI-MS analysis) relative to the 2:1-OI in the final crude product and 1:1-PI was observed in sub samples and products of 2:1-PI syntheses (Chapter 4), it was intriguing to discover, that Wu and Herrington[89] did not encounter the 1:1-imidazolines or monoamides in their study of the thermal reactions of fatty acids (palmitic and decanoic acids) with diethylenetriamine, even when reacted in 1:1 molar ratios. Although the present author was not aware of the work of Wu and Herrington[89] at the time the syntheses carried out in Chapter 4 were conducted, the methods employed by Wu and Herrington were nonetheless similar to those employed herein, in that reactions were carried out in xylene solvent and by solid phase reaction. However, there were also some significant differences between them:

- In the xylene solvent method (Wu and Herrington[89]), a dilute solution (0.02 mol) of fatty acid in xylene was added drop wise by syringe over 1 hour to a refluxing solution of 0.2 mol DETA in xylene with reflux continued for a further four hours to complete the reaction. A 2:1-imidazoline-fatty acid complex was isolated by recrystallisation. The method used herein,

employed addition of DETA (undiluted) over *ca* 10 minutes, to a non boiling solution of fatty acid in xylene and then refluxed for up to 95 hours.

- For the solid phase synthesis (Wu and Herrington [89]), fatty acid (0.2mol) was added in small portions over 2 hours to DETA (0.1 mol) heated to 150 °C. The reaction mixture was maintained at 150 °C with stirring for a further 5 hours after which the palmitic-1,3-diamide intermediate was recovered by recrystallisation. The purified 1,3-diamide was then heated under reduced pressure (240 °C; 30 mm Hg) for 0.5 hour and 2:1-imidazoline recovered by recrystallisation. The methods used by the present author employed addition of DETA over *ca* 10 minutes to heated (melted) fatty acid. The diamide intermediate was not isolated.

Wu and Herrington[89] characterised the recrystallised products by Fourier transform infrared (FTIR) spectroscopy, ^{1}H nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), normal phase high performance chromatography (NP-HPLC) and Schiff base-forming reactions monitored at 410 nm by ultraviolet/visible (UV/vis) diode-array spectrophotometer.

The methods described by Wu and Herrington[89] appeared to offer a simple and efficient route to high yields of a 2:1-palmitic imidazoline-palmitic acid complex (76 %) with xylene solvent and 1,3-diamide (62 %) and 2:1-palmitic imidazoline (94 %) by solid phase syntheses. The xylene solvent and solid phase thermal reactions of palmitic acid with DETA were therefore carried out as described by Wu and Herrington,[89] to prepare palmitic-diamide and 2:1-PI for subsequent HPLC/ESI-MS monitoring of oilfield corrosion products and environmental samples (*cf* McCormack *et. al.*[68]).

5.3.1 *Thermal reaction of palmitic acid with diethylenetriamine in xylene solvent*

A thermal reaction of palmitic acid with diethylenetriamine in xylene solvent was carried out by the method described by Wu and Herrington[89] (Section 5.2.4). A sample of the crude reaction mixture was collected and the xylene removed with a stream of nitrogen in order that both the crude reaction products as well as the recrystallised final product could be studied. HPLC/ESI-MS analyses of both crude and recrystallised products were carried out and two peaks were clearly seen in each of the total ion (TIC) and base peak chromatograms (BP) (Figures 5.1 and 5.2). However, the first peak in the crude product (Figure 5.1; 1.8 min) eluted *ca* 2.5 minutes before the first peak in the recrystallised product (Figure 5.2; 4.4 min). Examination of the mass spectra of each component and mass chromatography (Figures 5.3 and 5.4) of ions typical of 1:1-PI (*m/z* 324.4), monoamide (*m/z* 342.4), 2:1-PI (*m/z* 562.6) and the diamide (*m/z* 580.6) allowed the separate products to be identified. Calculation of signal to noise (S/N) ratios and peak areas allowed estimation of the relative proportions of each component in the crude and recrystallised products (Table 5.2).

Table 5.1 HPLC/ESI-MS peak retention times (R_t), signal to noise ratios (S/N) and peak area data acquired from the extracted mass chromatograms for protonated ions of: 1:1-PI (*m/z* 324.4), monoamide (*m/z* 342.4), 2:1-PI (*m/z* 562.6), and diamide (*m/z* 580.6) for the crude and recrystallised products of xylene solvent synthesis (Figures 5.3 and 5.4)

	***m/z* 324.4**		***m/z* 342.4**		***m/z* 562.6**		***m/z* 580.6**	
	R_t (min)	**S/N**	**R_t (min)**	**S/N**	**R_t (min)**	**SN**	**R_t (min)**	**S/N**
Crude	1.84	901		23	14.89	266	15.82	32
Recrystallised	2.17	115	4.4	300	15.14	256	16.06	70

	Peak Area (% of total area)							
	***m/z* 324.4**		***m/z* 342.4**		***m/z* 562.6**		***m/z* 580.6**	
Crude	149393804	(46.4)	5254287	(1.6)	162367249	(50.4)	5062358	(1.6)
Recrystallised	5190259	(1.7)	73734232	(23.8)	216442600	(69.9)	14157290	(4.6)

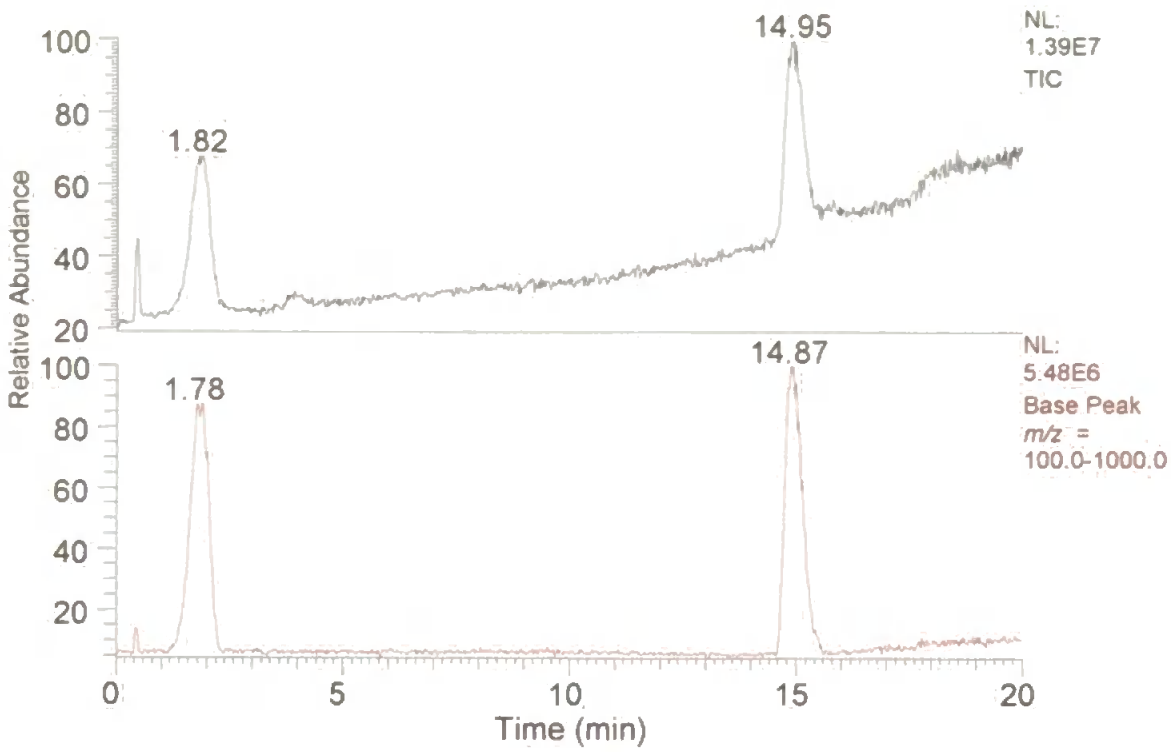


Figure 5.1 Total ion (TIC; Top) and base peak (BP; Bottom) HPLC/ESI-MS chromatograms of the crude product of the thermal reaction of palmitic acid with DETA in xylene solvent.

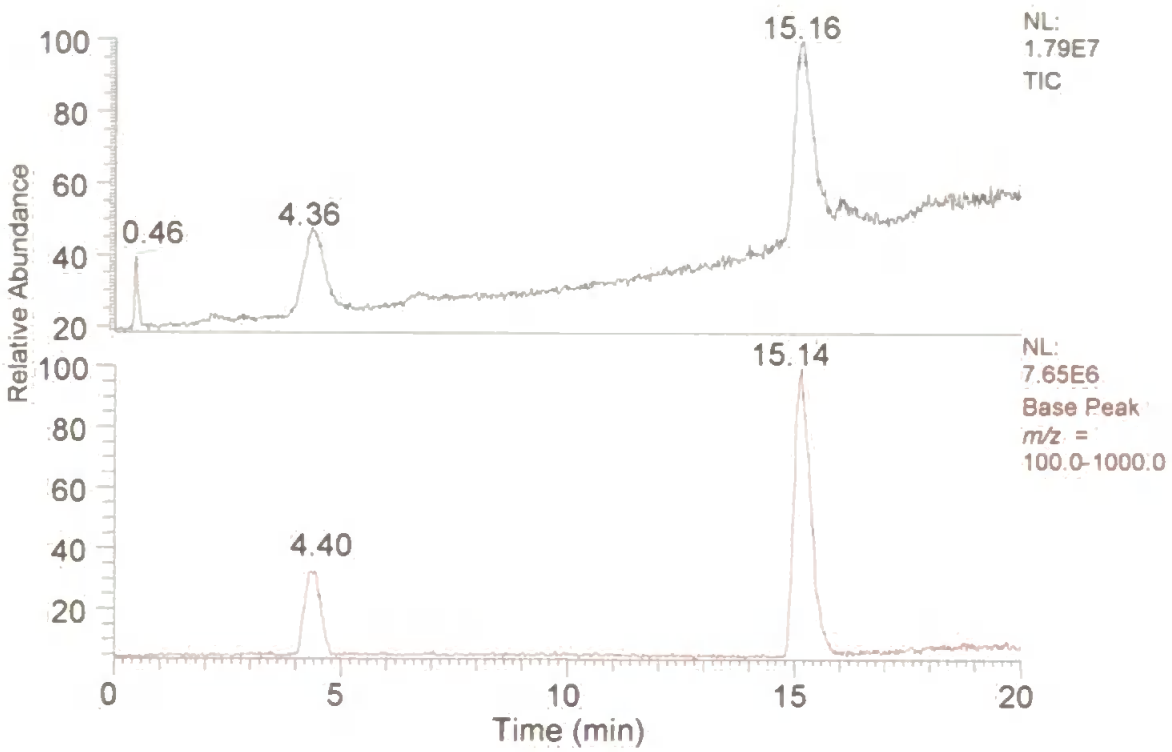


Figure 5.2 Total ion (TIC; Top) and base peak (BP; Bottom) HPLC/ESI-MS chromatograms of the recrystallised product of the thermal reaction of palmitic acid with DETA in xylene solvent.

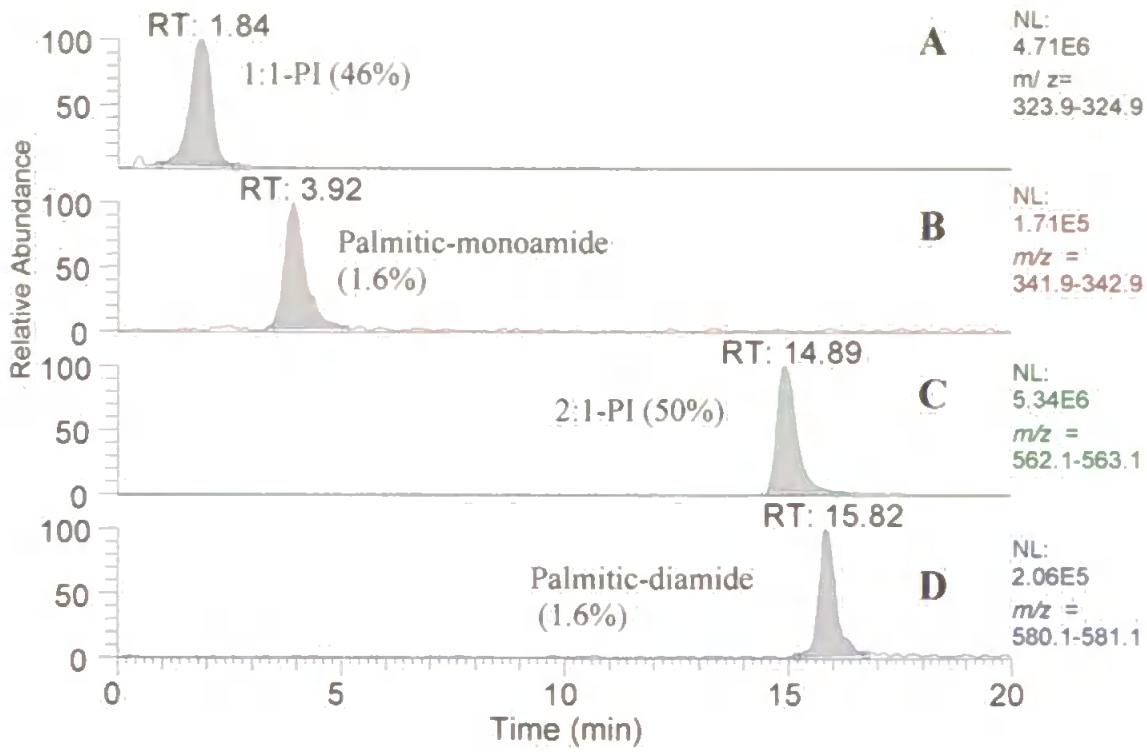


Figure 5.3 HPLC/ESI-MS extracted mass chromatograms from the crude product of the thermal reaction of palmitic acid with DETA in xylene solvent: (A) 1:1-PI (*m/z* 324.4), (B) palmitic-monoamide (*m/z* 342.4), (C) 2:1-PI (*m/z* 562.6) and (D) palmitic-diamide (*m/z* 580.6).

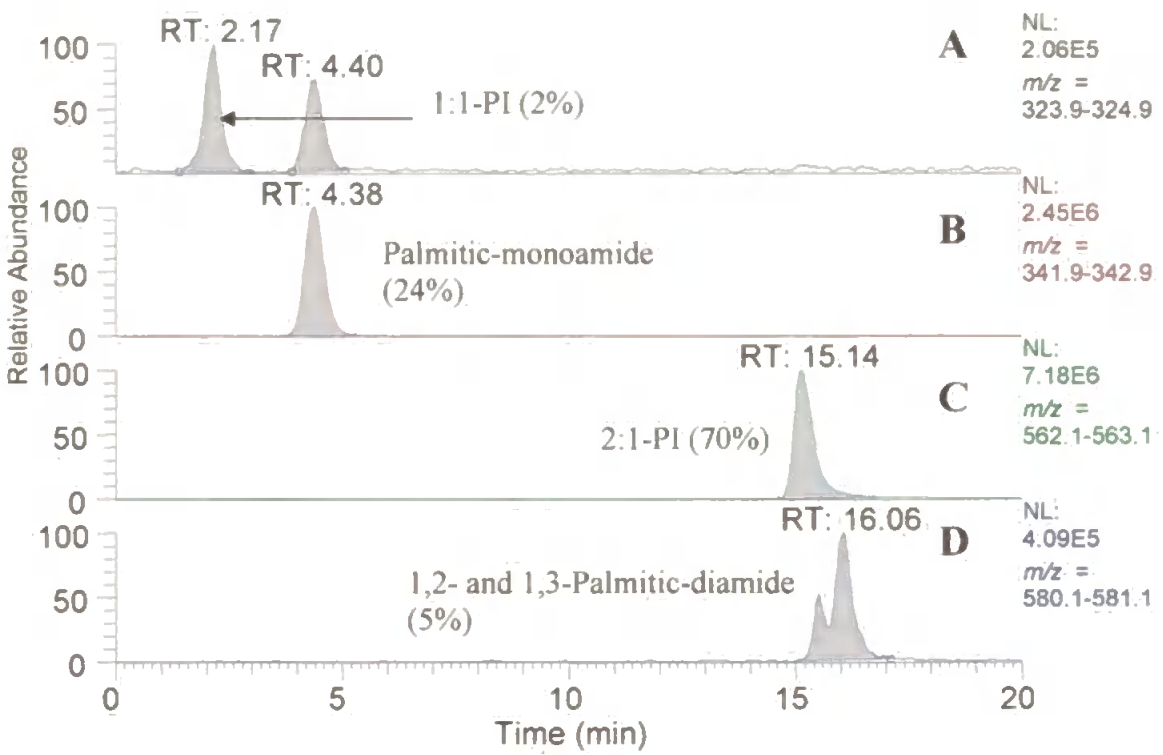


Figure 5.4 HPLC/ESI-MS extracted mass chromatograms from the recrystallised product of the thermal reaction of palmitic acid with DETA in xylene solvent: (A) 1:1-PI (*m/z* 324.4), (B) palmitic-monoamide (*m/z* 342.4), (C) 2:1-PI (*m/z* 562.6) and (D) palmitic-diamide (*m/z* 580.6).

In the crude product, 1:1-PI (*m/z* 324.4) was present at 46 % of the total peak area of the four main ions and the 2:1-PI (*m/z* 562.6) at 50 % (Figure 5.3A and C). This was not expected as Wu and Herrington did not report the formation of 1:1-PI or monoamide. However, it was consistent with other syntheses of imidazolines conducted herein (Chapter 4). No ions could be observed or attributed to 2:1-PI-palmitic acid complex which was the product reported by Wu and Herrington, although this may have been due to disassociation of the complex to the protonated 2:1-PI (*m/z* 562.6) which was observed (Figure 5.3C and 5.4C). The monoamide (*m/z* 342.4) and diamide (*m/z* 580.6) intermediates were present (each 1.6 %). Only one peak was observed for the diamide (*m/z* 580.6) at 15.8 minutes (Figure 5.3D). No distinction could be made as to whether the peak was due to the 1,2-diamide or the 1,3-diamide (or both) as the masses are isobaric.

The product recrystallised (from ethyl acetate) contained 70 % 2:1-PI (*m/z* 562.6) by total peak area of the four main compounds (Table 5.2), whilst the 1:1-PI was *ca* 2 %. The monoamide (*m/z* 342.4) and diamide (*m/z* 580.6) both showed an increase in relative amounts to *ca* 24 % and 5 % respectively (*cf* 1.6 % in the crude product). This was probably due to hydrolysis of the 1:1/2:1-PI during the recrystallisation (a boiling water bath was used to heat the solution). A valley indicating two peaks was observed in the extracted diamide mass chromatogram (*m/z* 580.6; Figure 5.5) of the recrystallised product probably reflects some separation of the 1,2- and 1,3-diamides.

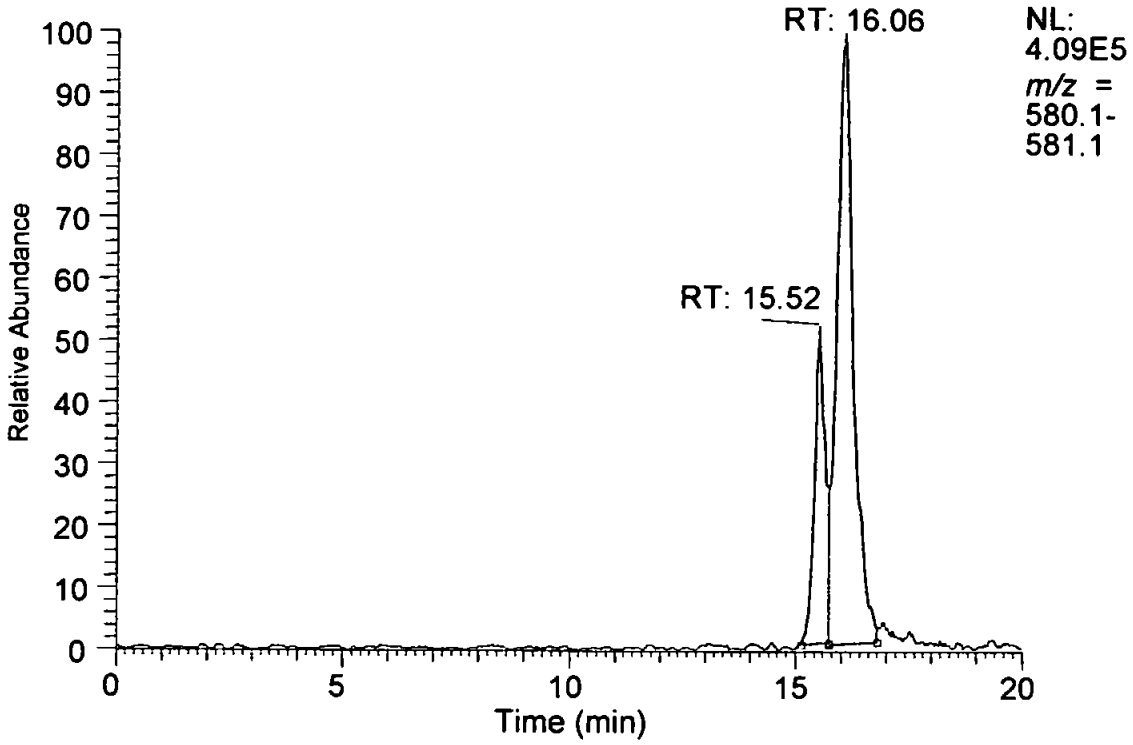


Figure 5.5 Enlarged HPLC/ESI-MS extracted mass chromatogram of 1,3- and 1,2-palmitic-diamide (*m/z* 580.6) shown in Figure 5.4(D).

5.3.2 *Solid phase thermal reaction of palmitic acid with diethylenetriamine*

A solid phase thermal reaction of palmitic acid with diethylenetriamine was also carried out by the method of Wu and Herrington[89] (Section 5.2.5). Compared with the solvent-based reaction described in the preceding section (5.3.1) the reaction was carried out in two steps and at a 2:1 molar ratio of fatty acid to DETA (*cf* one stage and a 1:1 molar ratio). In the first step, 1,3-palmitic diamide (Wu and Herrington[89]) is reported to be synthesised by heating the reagents at 150 °C for 7 hr and is then isolatable by recrystallisation. In the second step the 1,3-palmitic diamide is cyclized to 2:1-PI at 235 °C under reduced pressure (30 mm Hg) for 2 hr (*cf* 0.5 hr Wu and Herrington[89]). Samples of the crude 1,3-palmitic

diamide, recrystallised 1,3-palmitic diamide and the recrystallised 2:1-PI products were analysed by HPLC/ESI-MS and the total ion and base peak ion chromatograms are shown in Figures 5.6, 5.7 and 5.8 respectively. Two major peaks are clearly seen in the total ion (TIC) and base peak (BP) chromatograms of both the crude and recrystallised 1,3-diamide products (Figures 5.6 and 5.7).The two peaks (3.9 and 15.8 min) are present in approximately equal proportions in the crude product (Figure 5.6), whist the peak eluting after 3.8 min was relatively much smaller in the recrystallised product (Figure 5.7). A single peak with a small shoulder is observed in the recrystallised 2:1-PI product with a retention time of 15.1 min. Examination of the mass spectra of each component and mass chromatography of ions indicative of 1:1-PI (*m/z* 324.4), monoamide (*m/z* 342.4), 2:1-PI (*m/z* 562.6) and the diamides (*m/z* 580.6) (Figures 5.9, 5.10 and 5.11) allowed identification of the components. Calculation of signal to noise ratios and peak areas for both the crude and recrystallised products allowed the relative amounts to be estimated (Table 5.3).

In the first step of the reaction, reported to be the formation of the diamide (Wu and Herrington[89]), the crude product actually contains both monoamide (*m/z* 342.4) and a diamide (*m/z* 580.6) (Figure 5.9B and D) with the monoamide at 51 % and the diamide at 44 % (Table 5.3). This was not expected as Wu and Herrington had not encountered the monoamide. The 1:1-PI and 2:1-PI compounds were present in the crude product at 3.5 % and 1.5 % respectively and therefore are not readily formed under the reaction conditions employed (Figure 5.9A and C; Table 5.3). A single recrystallisation produced a mixture that comprised 86.5 % diamide (*m/z* 580.6), 11.5 % monoamide (*m/z* 342.4), 1 % 1:1-PI (*m/z* 324.4) and 1 % 2:1-PI (*m/z* 562.6) (Figure 5.10; Table 5.3). There does not appear to be any detrimental effects from the crystallisation procedure and therefore repeated recrystallisations might allow the product to be purified further. The second step of the reaction to convert the diamide to the 2:1-PI produced a 2:1-PI with *ca* 95 % purity (by

HPLC/ESI-MS) with 0.2 % 1:1-PI (*m/z* 324.4), 0.2 % monoamide (*m/z* 342.4) and 4.3 % diamide (*m/z* 580.6) (Figure 5.11; Table 5.3) from a single recrystallisation. Conversion of the diamide to imidazoline does not appear to be the problematic step of the reaction; rather formation of the initial mono or diamide is more complicated than stated by Wu and Herrington.[89]

Table 5.3 HPLC/ESI-MS peak retention times (R_t), signal to noise ratios (S/N) and peak area data acquired from the extracted mass chromatograms for protonated ions of: 1:1-PI (*m/z* 324.4), monoamide (*m/z* 342.4), 2:1-PI (*m/z* 562.6), and diamide (*m/z* 580.6) for the crude and recrystallised palmitic-diamide and recrystallised 2:1-PI products of solid phase synthesis (Figures 5.9, 5.10 and 5.11)

	***m/z* 324.4**		***m/z* 342.4**		***m/z* 562.6**		***m/z* 580.6**	
	R_t(min)	**S/N**	**R_t(min)**	**S/N**	**R_t(min)**	**S/N**	**R_t(min)**	**S/N**
Crude diamide	3.82	43	3.75	531	14.99	50	15.77	248
Diamide	3.81	27	3.81	263	14.88	32	15.69	198
2:1-PI	2.18	12	4.63	11	15.1	4561	16	94

	Peak Area (% of total area)							
	***m/z* 324.4**		***m/z* 342.4**		***m/z* 562.6**		***m/z* 580.6**	
Crude diamide	6911107	(3.3)	109204991	(51.4)	2959222	(1.4)	93438432	(44.0)
Diamide	1524770	(0.7)	26865167	(11.6)	2823968	(1.2)	199668674	(86.5)
2:1-PI	707887	(0.2)	528287	(0.2)	312749178	(95.3)	14056288	(4.3)

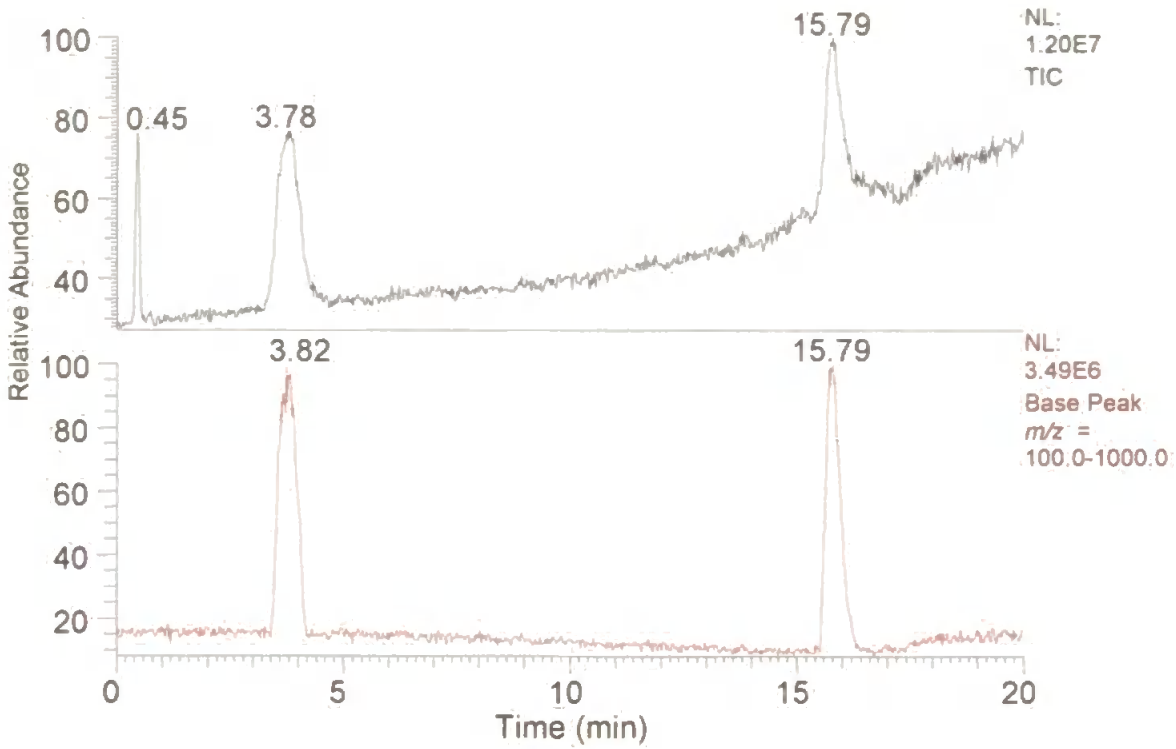


Figure 5.6 Total ion (TIC; Top) and base peak (BP; Bottom) HPLC/ESI-MS chromatograms of the crude palmitic-diamide product of the solid phase thermal reaction of palmitic acid with DETA.

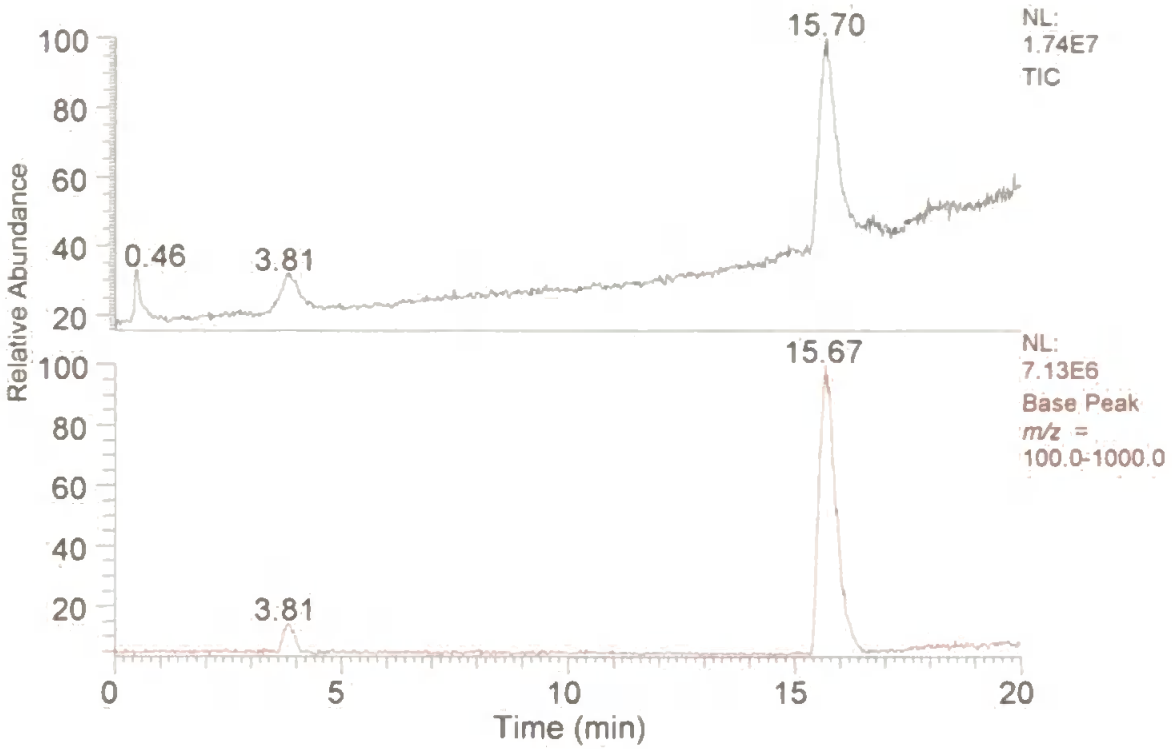


Figure 5.7 Total ion (TIC; Top) and base peak (BP; Bottom) HPLC/ESI-MS chromatograms of the recrystallised palmitic-diamide product of the solid phase thermal reaction of palmitic acid with DETA.

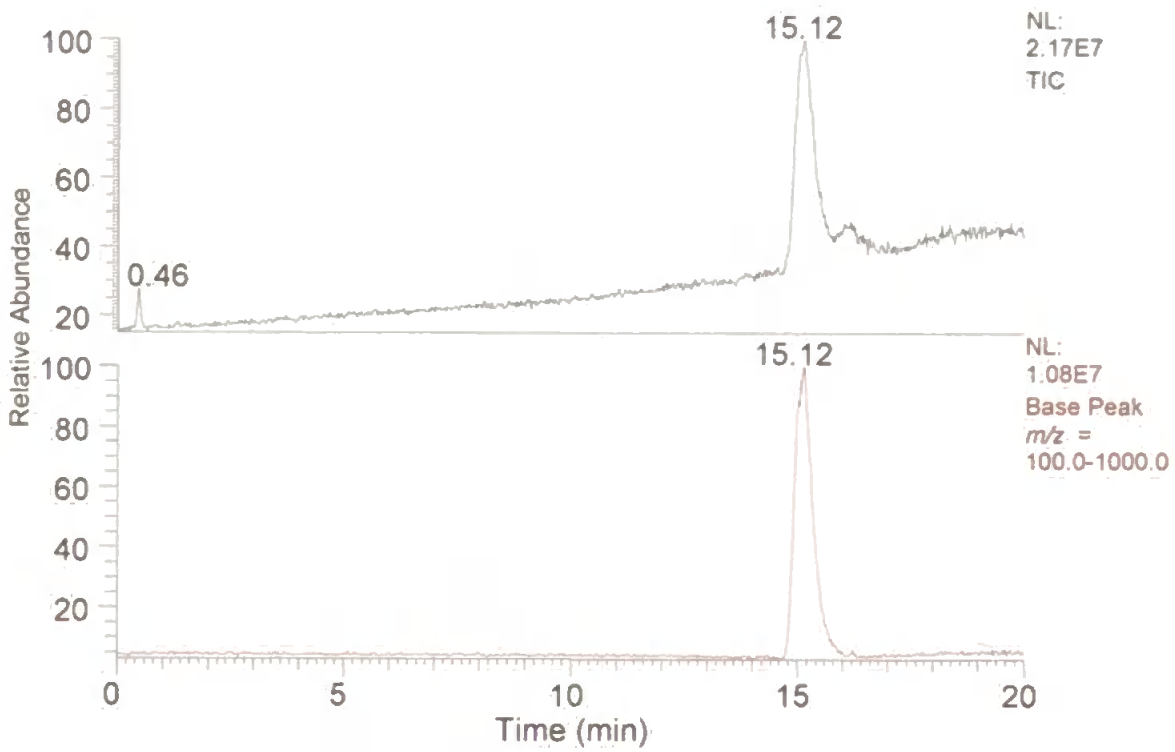


Figure 5.8 Total ion (TIC; Top) and base peak (BP; Bottom) HPLC/ESI-MS chromatograms of the recrystallised 2:1-PI product of the solid phase thermal reaction of palmitic acid with DETA.

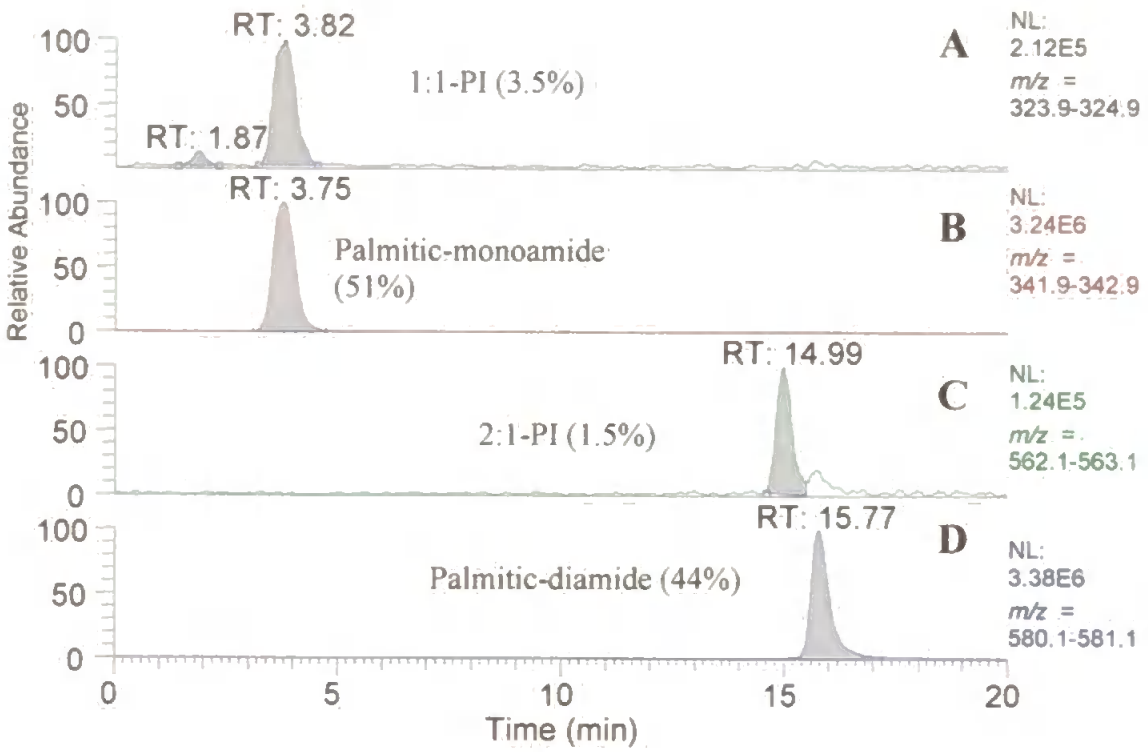


Figure 5.9 HPLC/ESI-MS extracted mass chromatograms from the crude palmitic-diamide product of the solid phase thermal reaction of palmitic acid with DETA: (A) 1:1-PI (*m/z* 324.4), (B) palmitic-monoamide (*m/z* 342.4), (C) 2:1-PI (*m/z* 562.6) and (D) palmitic-diamide (*m/z* 580.6).

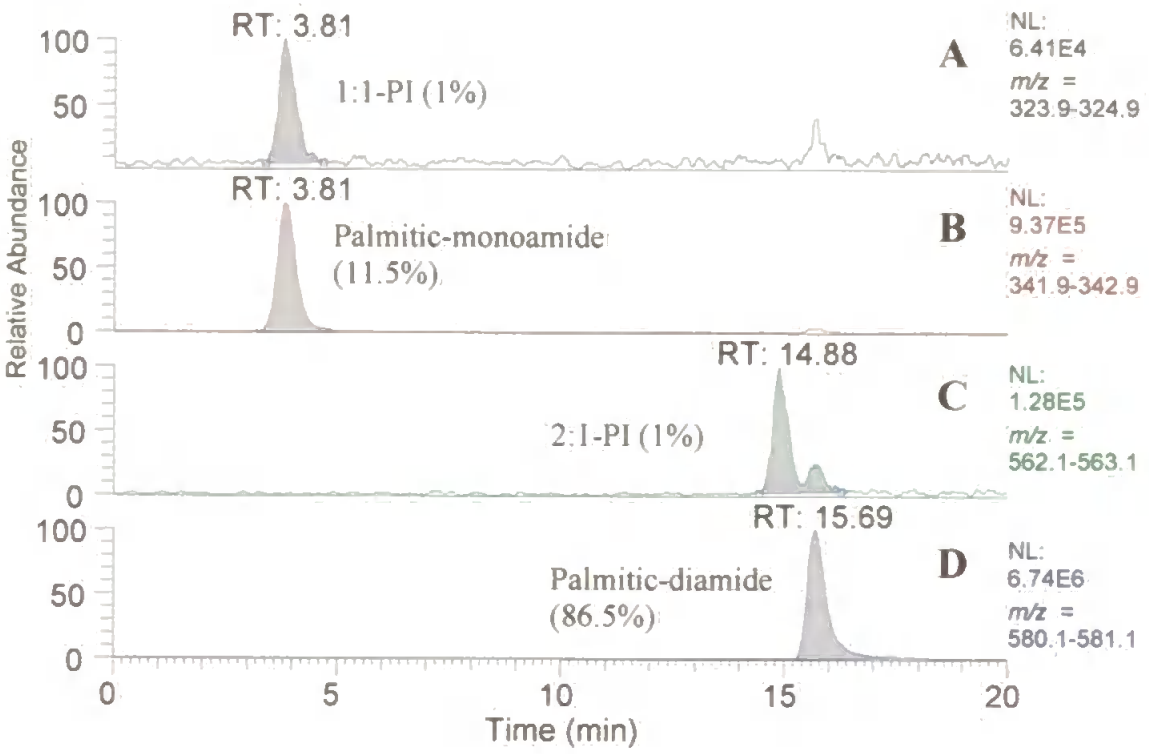


Figure 5.10 HPLC/ESI-MS extracted mass chromatograms from the recrystallised palmitic-diamide product of the solid phase thermal reaction of palmitic acid with DETA: (A) 1:1-PI (*m/z* 324.4), (B) palmitic-monoamide (*m/z* 342.4), (C) 2:1-PI (*m/z* 562.6) and (D) palmitic-diamide (*m/z* 580.6).

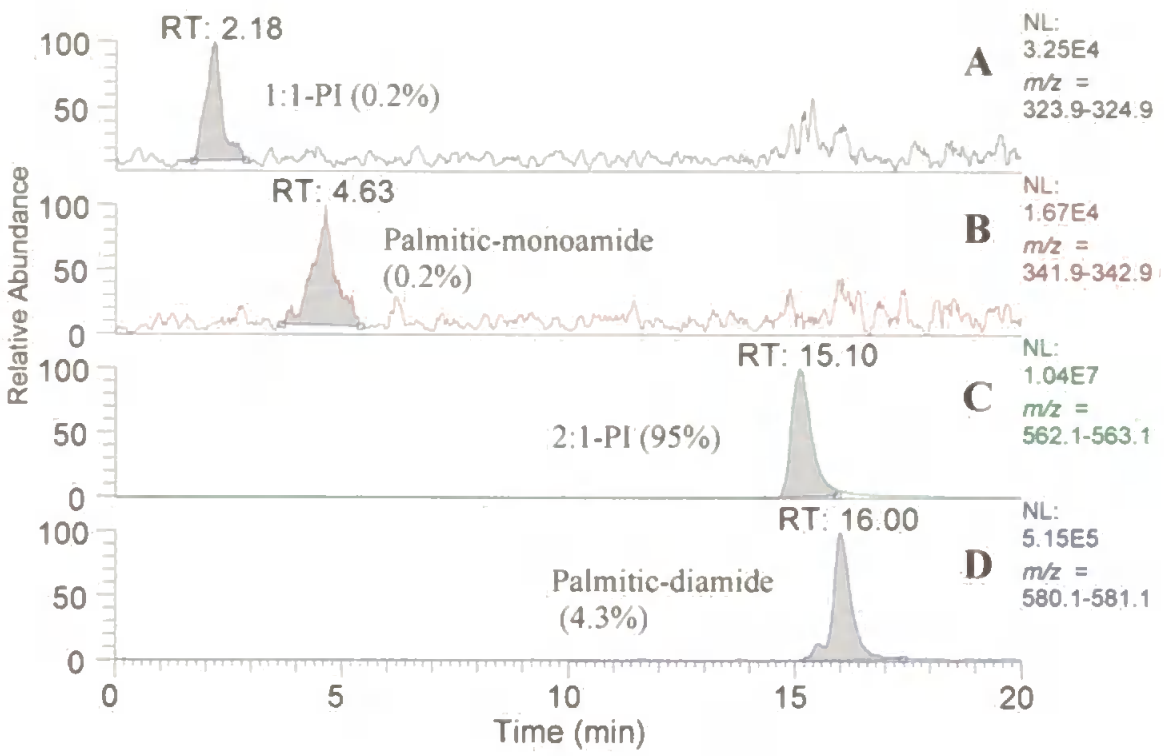


Figure 5.11 HPLC/ESI-MS extracted mass chromatograms from the recrystallised 2:1-PI product of the solid phase thermal reaction of palmitic acid with DETA: (A) 1:1-PI (*m/z* 324.4), (B) palmitic-monoamide (*m/z* 342.4), (C) 2:1-PI (*m/z* 562.6) and (D) palmitic-diamide (*m/z* 580.6).

5.3.3 *Infusion electrospray ionisation multistage mass spectrometry (ESI-MS^n) analysis of the solvent and solid phase synthesis products*

The *m/z* values identified in the HPLC/ESI-MS analyses of the crude and recrystallised products were consistent with protonated ions for 1:1-PI (*m/z* 324.4), monoamide (*m/z* 342.4), 2:1-PI (*m/z* 562.6) and the diamides (*m/z* 580.6) although no structural information was obtained to confirm the assignments. Multistage mass spectrometry allows characteristic collision induced dissociation (CID) fragmentation of a precursor ion in an MS^1 full scan spectrum. As previously shown in Chapter 2, CID MS^n fragmentation pathways can allow identification of unknown compounds as well as unambiguous identification of known compounds previously characterised from pure standards. The crude and recrystallised products from the solvent and solid phase syntheses were therefore also analysed by infusion ESI-MS^n in order to obtain structural information which would allow identification of the products and by-products. Infusion of the sample into the mass spectrometer was used in preference to either HPLC or flow injection as a continuous flow of sample allows a wider range and optimisation of CID experiments to be carried out without interruption of the sample flow.

5.3.3.1 Infusion electrospray ionisation multistage mass spectrometry (ESI-MS^n) analysis of the crude reaction products of the thermal reaction of palmitic acid with diethylenetriamine in xylene solvent

Figure 5.12A shows the MS^1 full scan mass spectrum of the crude product over the mass range *m/z* 50 – 1000, with the instrument optimised (auto-tuned) on the ion at *m/z* 324.4. Ions with relative abundances consistent with protonated ions for 1:1-PI (*m/z* 324.4), monoamide (*m/z* 342.4), 2:1-PI (*m/z* 562.6) and the diamides (*m/z* 580.6) which were also observed in the HPLC/ESI-MS mass chromatograms (Section 5.3.1) were identified. Changing the full scan mass range to *m/z* 50 – 700 and again auto-tuning on *m/z* 324.4 (Figure 5.12B), the relative abundance of *m/z* 324.4 increases relative to *m/z* 562.6. This is

due to instrument bias towards higher masses, particularly when wide mass ranges are used. For all MS^n experiments the instrument was auto-tuned on the ion of interest prior to analysis. High resolution ZoomScan mass spectra were acquired of the two prominent ions (*m/z* 342.4 and *m/z* 562.6; Figure 5.13) shown in the full scan spectra. For both ions, base-line resolution with a peak width of *m/z* 1, which is indicative of singularly charged ions. Multistage mass spectrometry on the MS^1 *m/z* 324.4 and *m/z* 562.6 precursor ions (Figure 5.12B) was carried out.

Figure 5.14A shows the MS^2 CID mass spectrum of *m/z* 324.4, in which a *m/z* 281.4 ion is the only major ion product of decomposition. A *m/z* 307.4 ion at *ca* 1 % relative abundance was also observed. MS^3 CID of *m/z* 281.4 (Figure 5.14B) produces a series of ions differing by 14 Da (CH_2 group) with *m/z* 97.1 as the most abundant. The MS^2 and MS^3 product ions are consistent with the previously proposed ion trap CID fragmentation pathway for 1:1-imidazolines (Chapter 2; Section 2.3.2.3) and is shown in Figure 5.14C for the 2:1-PI. Identical MS^2 and MS^3 spectra to those obtained for the *m/z* 324.4 ion (Figure 5.14A and B) were obtained (Figure 5.15A and B) from the CID of the *m/z* 562.6 precursor in Figure 5.12B. Again this fragmentation pathway (Figure 5.15C) is consistent for 2:1-imidazolines as previously proposed (Chapter 2; Section 2.3.2.3) and shows that MS^2 CID fragmentation is at the same position (N-1) on the imidazoline moiety regardless of whether the parent ion is the 1:1- or 2:1-imidazoline. The optimum activation amplitude (AA; the percentage Normalized Collision Energy™) to induce fragmentation in the ion trap, is the same for both precursor ions (MS^2; 42 % AA and MS^3; 53 % AA).

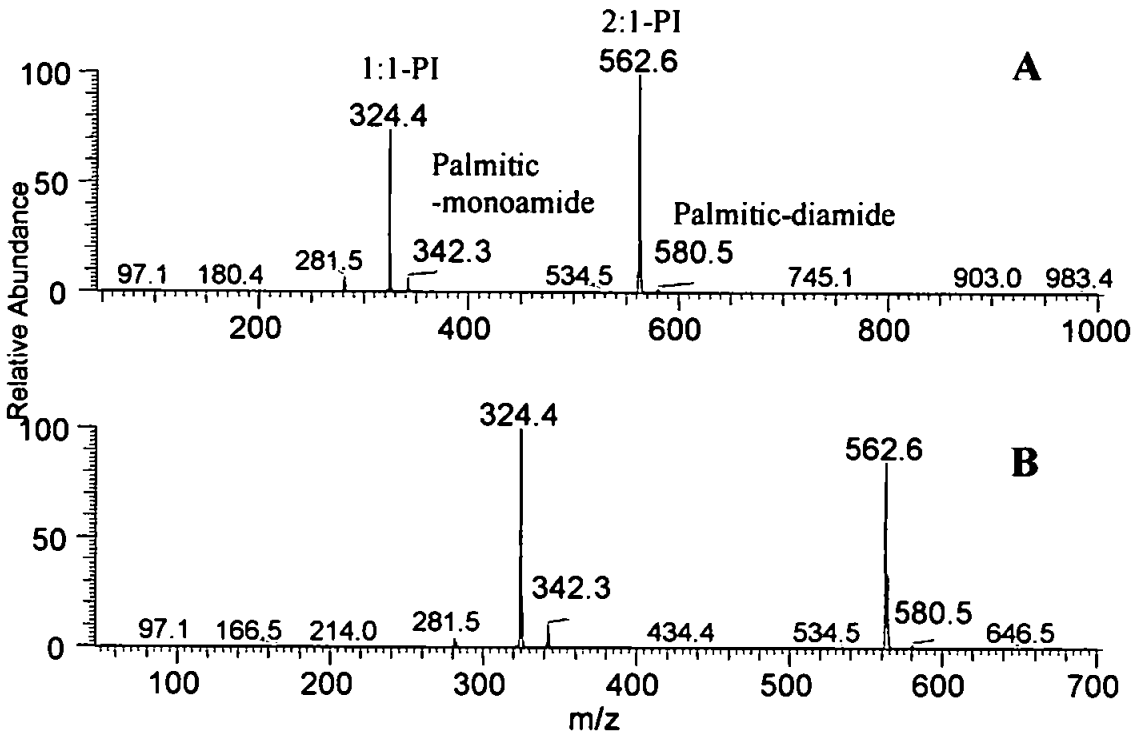


Figure 5.12 Infusion ESI-MS mass spectra of the crude products of the thermal reaction of palmitic acid with DETA in xylene solvent. Positive ion full scan spectra: **(A)**, *m/z* 50 - 1000 (capillary voltage (+3.88 V), 1st octapole (-1.39 V), 2nd octapole (-1.39 V) and tube lens (-39.24 V) and **(B)**, *m/z* 50 – 700 (capillary voltage (+2.85 V), 1st octapole (-1.47 V), 2nd octapole (-6.96 V) and tube lens (-33.22 V). Both spectra acquired after auto-tuning on ion m/z 324.4 at each *m/z* range. The differences illustrate changes in response dependent on *m/z* range and tune parameters.

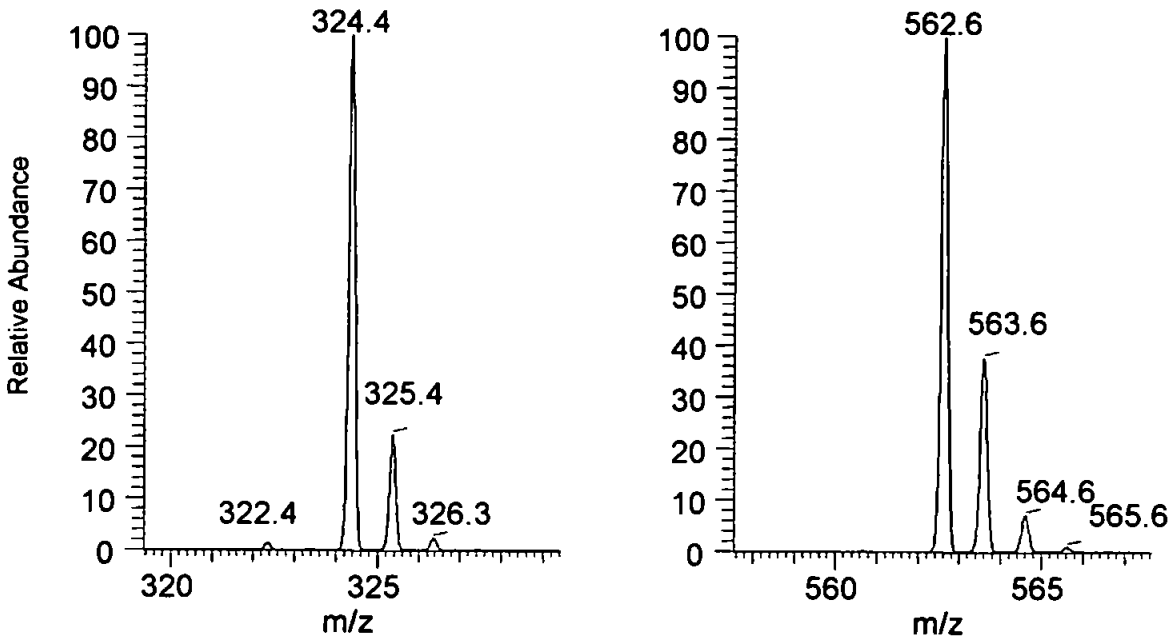
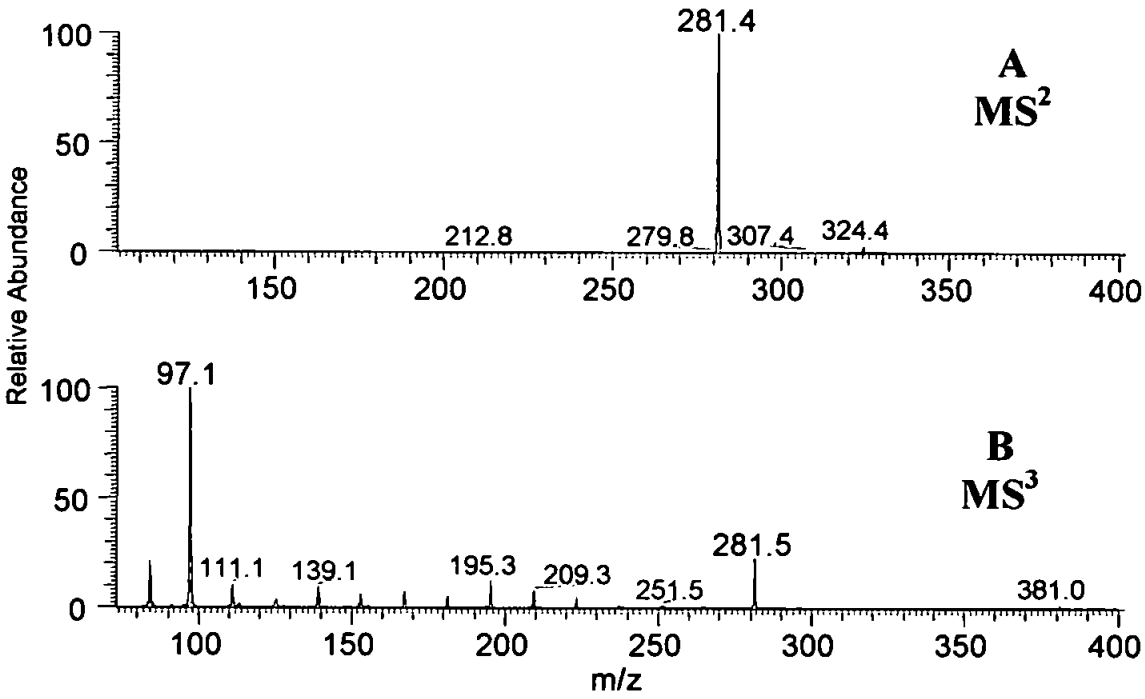
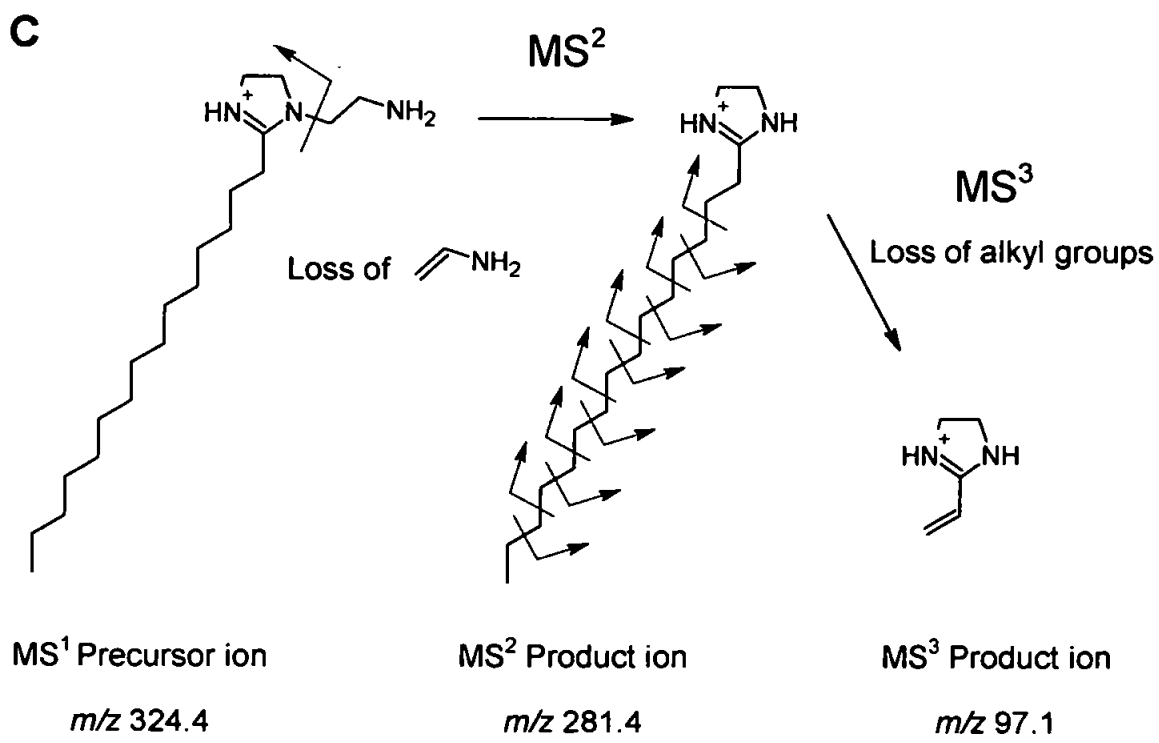


Figure 5.13 ESI-MS ZoomScan mass spectra of the 1:1-PI ion (Left) and 2:1PI ion (Right) shown in the full-scan spectrum (Figure 5.12B).



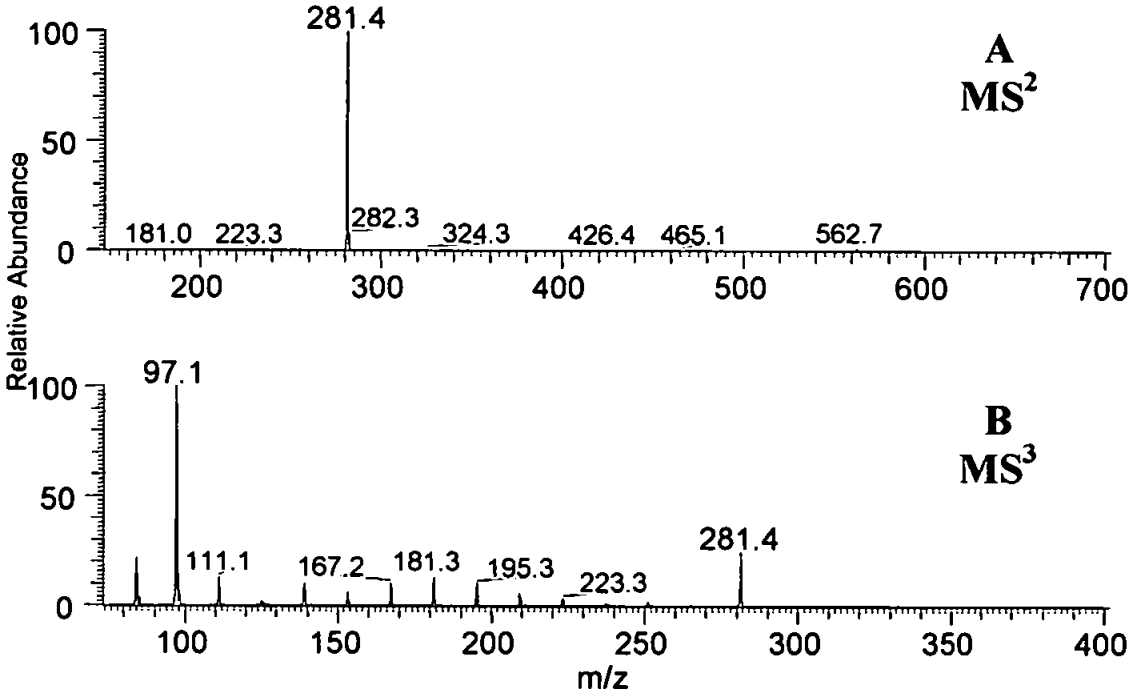


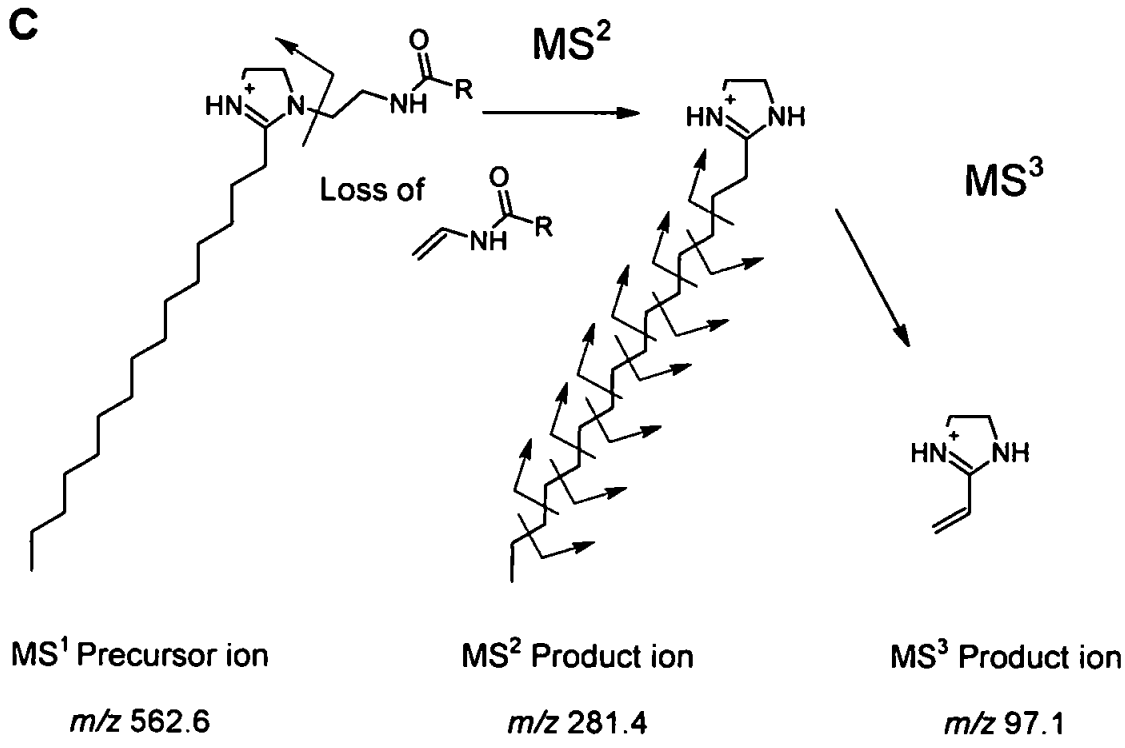
MS[1] Precursor ion *m/z* 324.4

MS[2] Product ion *m/z* 281.4

MS[3] Product ion *m/z* 97.1

Figure 5.14 ESI-MS[n] mass spectra and proposed fragmentation pathways of the 1:1-PI precursor ion (*m/z* 324.4) in the full-scan spectrum (Figure 5.12B). **(A)** MS[2] of precursor ion *m/z* 324.4 (42 % AA), **(B)** MS[3] of product ion *m/z* 281.4 (53 % AA) and (**C**) proposed CID fragmentation pathway.





MS[1] Precursor ion *m/z* 562.6

MS[2] Product ion *m/z* 281.4

MS[3] Product ion *m/z* 97.1

Figure 5.15 ESI-MS[n] mass spectra of the 2:1-PI precursor ion (*m/z* 562.6) in the full-scan spectrum (Figure 5.12B). **(A)** MS[2] of precursor ion *m/z* 562.6 (42 % AA), **(B)** MS[3] of product ion *m/z* 281.4 (53 % AA) and (C) proposed CID fragmentation pathway.

5.3.3.2 Infusion electrospray ionisation multistage mass spectrometry (ESI-MS^n) analysis of the recrystallised product of the thermal reaction of palmitic acid with diethylenetriamine in xylene solvent

Figure 5.16 A and B show the MS^1 full scan mass spectra of the recrystallised products of the solvent synthesis, over the mass range *m/z* 50 – 700, with the instrument optimised (auto-tuned) on the ion at *m/z* 562.6 (Figure 5.16A) and *m/z* 342.4 (Figure 5.16B). There is only a small change in response (*c.a.* 25 % to 35 % relative abundance) for the lower mass ion due to a smaller difference between tuned masses (Δ *m/z* 220) and no change in full scan mass range (*c.a.* Figure 5.12A and B). Two prominent ions were observed with *m/z* values and relative abundances consistent with protonated ions of palmitic-monoamide (*m/z* 342.4), and 2:1-PI (*m/z* 562.6) observed in the HPLC/ESI-MS mass chromatograms (Section 5.3.1) of the recrystallised products. The ions for 1:1-PI (*m/z* 324.4) and the diamide (*m/z* 580.6) were also observed in the spectra at < 5 % relative abundance.

ZoomScan, MS^2 and MS^3 mass spectra (not shown) of the MS^1 precursor ion *m/z* 562.6, were identical to those obtained for the same *m/z* value in the crude products discussed in Section 5.3.3.1 and confirmed the ion to be due to 2:1-PI. The *m/z* 342.4 ion which had increased in abundance during and after recrystallisation was at a relative abundance which allowed multistage experiments to be carried out, which was not the case in the spectra of the crude product (Figure 5.12A and B). The ZoomScan spectrum of *m/z* 342.4 (Figure 5.17) showed base-line resolution with a peak width of 1 Da indicative of $^{12}C/^{13}C$ isotopes of a singularly charged ion. MS^2 CID of the precursor ion *m/z* 342.4 (Figure 5.18A) resulted in an *m/z* 324.4 product ion, which on MS^3 and MS^4 (Figure 5.18C and D) fragmented identically to that of the 1:1-PI (*m/z* 324.4) precursor ion in the crude product (Figure 5.14 and section 5.3.3.1). This is consistent with the loss of water (18 Da) from protonated palmitic-monoamide with cyclisation to protonated 1:1-PI. The MS^2 fragmentation does not allow distinction of the 1-monoamide (Structure III, Figure 3.1) or the 2-monoamide (structure IV, Figure 3.1), to the precursor palmitic-monoamide ion (*m/z*

342.4) to either the precursor palmitic-monoamide ion (*m/z* 342.4), therefore, a proposed fragmentation pathway is shown (Figure 5.19) assuming that ion trap MS^2 CID is the same for both 1- and 2-monoamides.

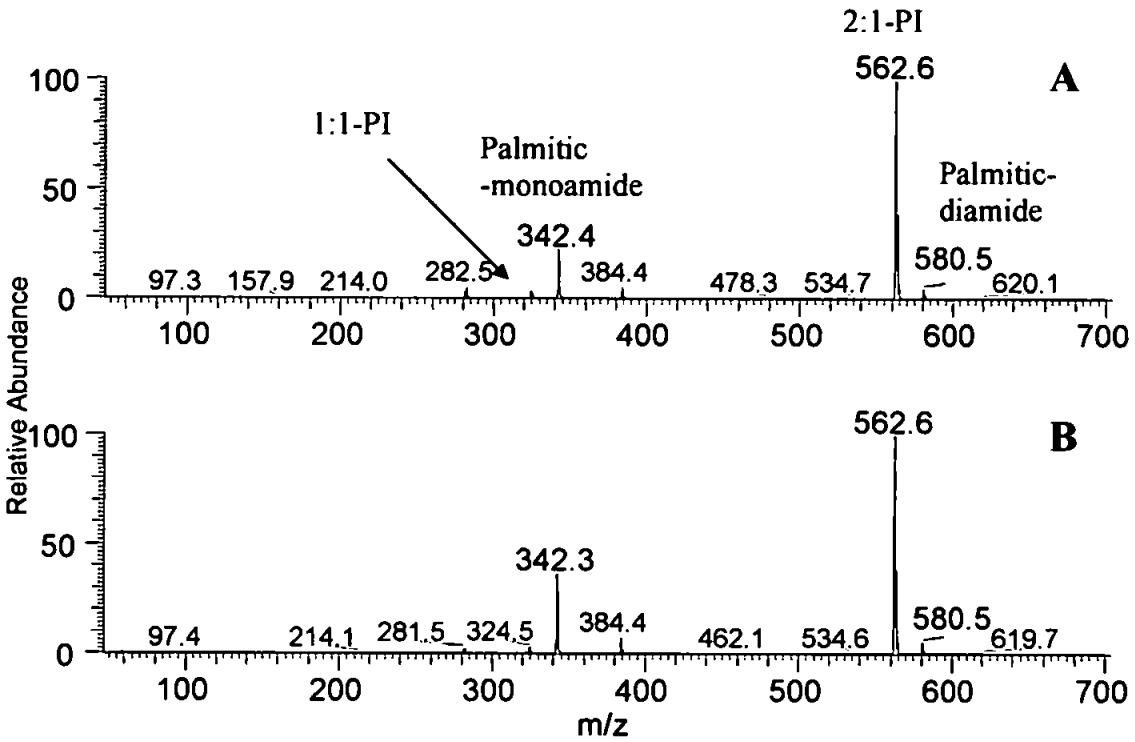


Figure 5.16 Infusion ESI-MS mass spectra of the recrystallised product of the thermal reaction of palmitic acid with DETA in xylene solvent. Positive ion full scan spectra: **(A)**, auto- tuned on ion m/*z* 562.6 (*m/z* 50 – 700, capillary voltage (+30.15 V), 1st octapole (-2.42 V), 2nd octapole (-5.93 V) and tube lens (-23.12 V) and **(B)**, auto-tuned on ion m/*z* 342.4 (*m/z* 50 – 700, capillary voltage (+30.15 V), 1st octapole (-1.17 V), 2nd octapole (-6.88 V) and tube lens (-27.19 V). Spectra illustrate changes in response dependent on m/*z* auto-tuned values.

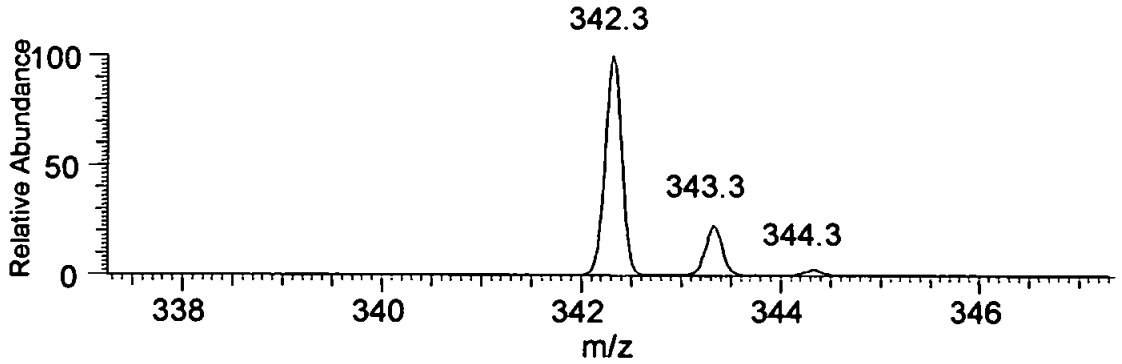


Figure 5.17 ESI-MS ZoomScan mass spectrum of the palmitic-monoamide ion shown in the full-scan spectrum (Figure 5.16B).

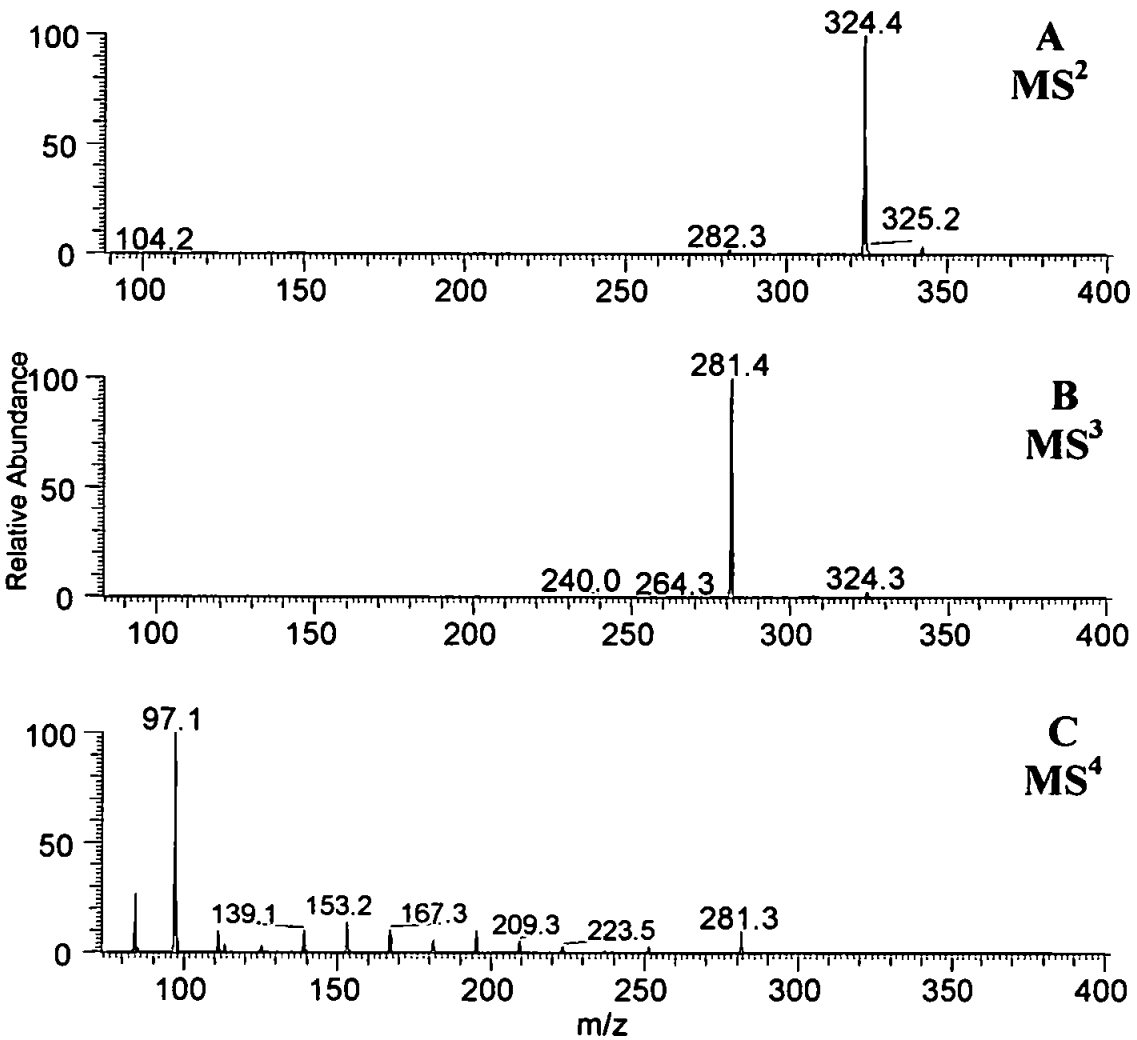


Figure 5.18 ESI-MS^n mass spectra of the palmitic-monoamide precursor ion (*m/z* 342.4) in the full-scan spectrum Figure 5.16B. **(A)** MS^2 on precursor ion *m/z* 342.4 (30 5 AA), **(B)** MS^3 on product ion *m/z* 324.4 (42 % AA) and **(C)** MS^4 product ion m/z 281.4 (54 % AA).

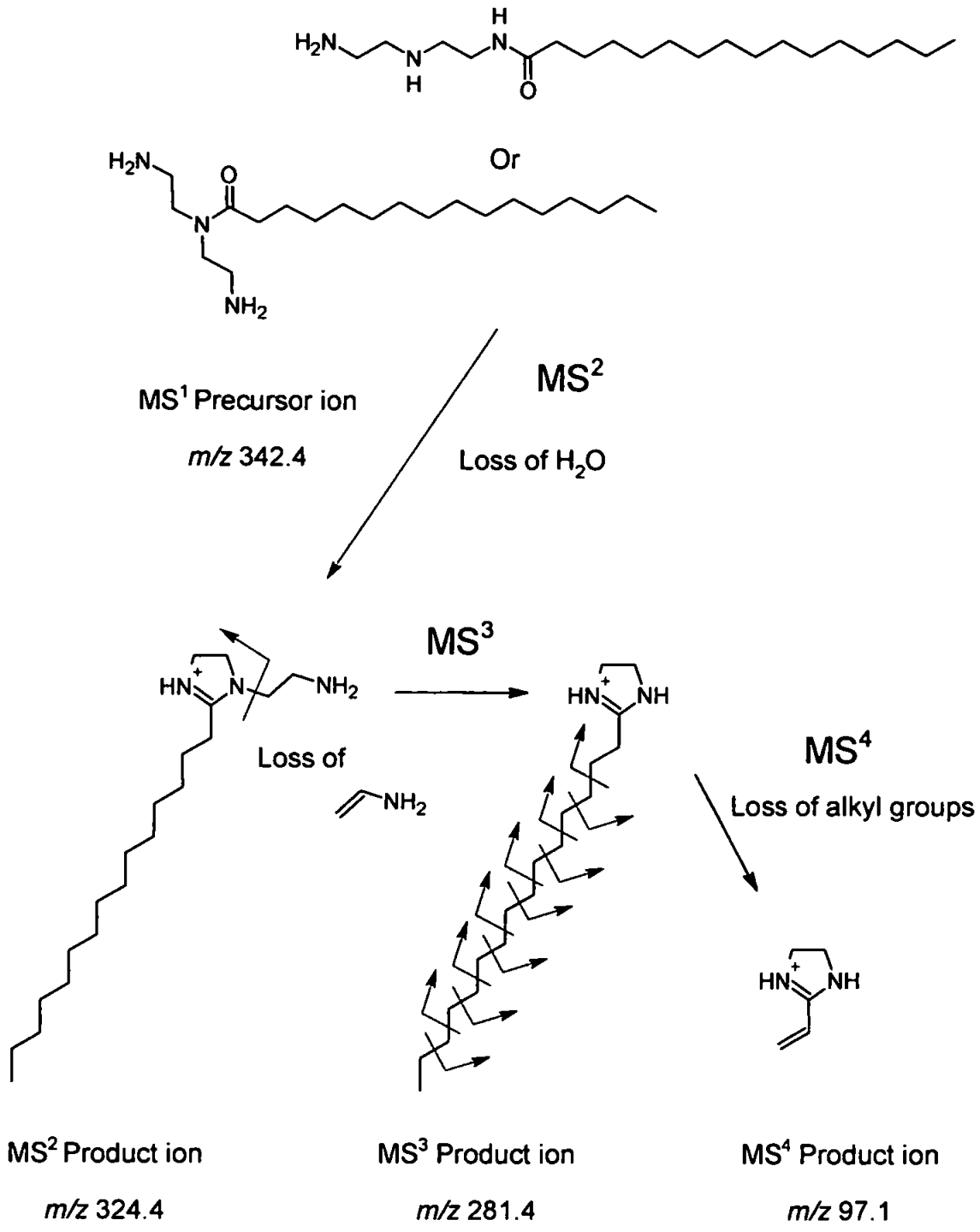


Figure 5.19 Proposed CID fragmentation pathway of compounds assigned as 1- and 2-monoamides.

5.3.3.3 Infusion electrospray ionisation multistage mass spectrometry (ESI-MS^n) analysis of the crude and recrystallised palmitic-diamide products and the recrystallised 2:1-PI product of the solid phase thermal reaction of palmitic acid with diethylenetriamine

The MS^1 full scan mass spectra (over the mass range *m/z* 50 – 700) of the crude and recrystallised palmitic-diamide intermediate products (Figure 5.20A and B) and the recrystallised 2:1-PI final product (Figure 5.21), showed ions with relative abundances consistent with the protonated ions for 1:1-PI (*m/z* 324.4), monoamide (*m/z* 342.4), 2:1-PI (*m/z* 562.6) and the diamide (*m/z* 580.6) observed in the respective HPLC/ESI-MS mass chromatograms (Section 5.3.1). Multistage MS^n analysis of the base peak ion, *m/z* 342.4 in the crude palmitic-diamide (Figure 5.20A) and the second most abundant ion in the recrystallised palmitic-diamide product (Figure 5.20B), produced MS^2, MS^3 and MS^4 spectra (not shown), identical to those of the corresponding ions in the recrystallised 2:1-PI product of the solvent based synthesis (Figure 5.18; Section 5.3.3.2). They can therefore be assigned as arising from decomposition of the protonated ion, of the palmitic-monoamide (either 1- or 2-monoamides). MS^n analysis of the almost exclusive ion, *m/z* 562.6 (Figure 5.21), produced MS^2 and MS^3 mass spectra (not shown) identical to those of the corresponding ions in the recrystallised 2:1-PI product of the solvent based synthesis (Figure 5.18; Section 5.3.3.2) and can be assigned as due to the protonated 2:1-PI ion. The ion *m/z* 580.6 is the base peak ion in the spectrum of the recrystallised palmitic-diamide product (Figure 5.20B) and second most abundant ion in that of the crude palmitic-diamide product (Figure 5.20A). MS^2, MS^3 and MS^4 CID spectra of the *m/z* 580.6 ion were identical in both the crude and recrystallised palmitic-diamide products and are discussed below for the recrystallised palmitic-diamide product (Figure 5.20B). The ZoomScan spectrum of the ion *m/z* 580.6 (Figure 5.22) shows base-line resolution with a peak width of *m/z* 1 indicative of a singularly charged ion. MS^2 CID of the precursor ion *m/z* 580.6 (Figure 5.23A) resulted in four significant product ions (*m/z* 282.3 (95 %), *m/z* 299.2 (40 %), *m/z* 562.5 (55 %) and *m/z* 563.5 (100 %)). The ions *m/z* 562.5 and *m/z* 563.5 are shown to be distinct separate ions in the enlargement of the spectrum (Figure 5.23B) and since the

higher mass ion is *ca* 40 % more abundant, this cannot be due to a ^{13}C isotope. Ion *m/z* 563.5 is due to loss of 17 Da from the precursor ion *m/z* 580.6 (Figure 5.23A), which is the mass expected for the 1,3- and 1,2-palmitic diamides (structures V and IV respectively, R and $R_1 = C_{15}H_{31}$, Figure 3.1) . Although both amides are isobaric, only the 1,2-diamide can lose ammonia (17 Da) by MS^2 CID to give the *m/z* 563.5 product ion observed in Figure 5.23. MS^3 CID of the *m/z* 563.5 involves an approximately symmetrical cleavage to *m/z* 282.3 (Figure 5.24), which on MS^4 CID produces a *m/z* 95.2 ion (Figure 5.25). The *m/z* 562.5 MS^2 CID product ion observed in Figure 5.23A and B, can be accounted for by loss of water from a diamide and cyclisation to form the protonated 2:1-PI ion. Indeed, this is confirmed by MS^3 and MS^4 CID spectra (Figures 5.26 and 5.27 respectively) in which product ions corresponding to a ion trap CID fragmentation pathway previously proposed for 2:1-PI (Figure 5.15C and Chapter 2; 2.3.2.3) are observed. An *m/z* 562.5 ion is also observed as the base peak in the MS^3 CID spectrum (Figure 5.24), which might be construed as evidence of loss of hydrogen radical from *m/z* 563.5 (and therefore loss of a hydroxyl radical rather than ammonia from the diamide precursor). However, loss of a hydrogen radical following loss of hydroxyl was ruled out on the basis that MS^4 and MS^5 CID (not shown) of the *m/z* 562.5 ion produced spectra identical to that consistently obtained for protonated 2:1-PI proposed by CID fragmentation (Figure 5.15C and Chapter 2; Section 2.3.2.3). It is suspected that the *m/z* 562 ion is in fact not a CID product ion at all since it was also present at 0% activation amplitude (AA) and the actual ion count remained constant at 0, 31, 33 and (the optimum) 35% AA. Rather, it is suggested that the *m/z* 562.5 ion was coincidentally isolated with the *m/z* 563.5 ion. Unfortunately attempts to prove this by reducing the isolation width from the normal *m/z* 1 to *m/z* 0.9 resulted in lower relative abundances for all ions and even lower isolation width values resulted in trapping of too few ions and signal instability. However, in a similar experiment with a oleic acid derived diamide (*m/z* 632.6; Chapter 4, section 4.3.8) the corresponding M-18 ion (*m/z* 614.6 *cf* 562.5) was not present in the MS^3 spectrum (Appended; Figure A.4)

which supports the suggestion that 562.5 was not a CID product ion. All other equivalent MS^n spectra (Appended; Figures A.4 – A.7) of the MS^2 product ions of oleic-diamide (*m/z* 632.6; Appended; Figure A.3) are identical except for the difference in mass (52 Da) and support the fragmentation pathway for 1,2-/1,3-diamides.

The MS^3 CID spectrum (Figure 5.28) of the second most abundant product ion (*m/z* 282.3, 95 %) of MS^2 CID on the *m/z* 580.6 precursor (Figure 5.23A) was identical to that of the corresponding MS^4 product ion from *m/z* 563.5 ((Figure 5.25). Also the MS^3 CID spectrum (Figure 5.29) of the fourth most abundant product ion (*m/z* 299.2, 40 %) produced an *m/z* 282.3 ion by loss of ammonia (17 Da), which on MS^4 CID was identical to that described in the previous sentence.

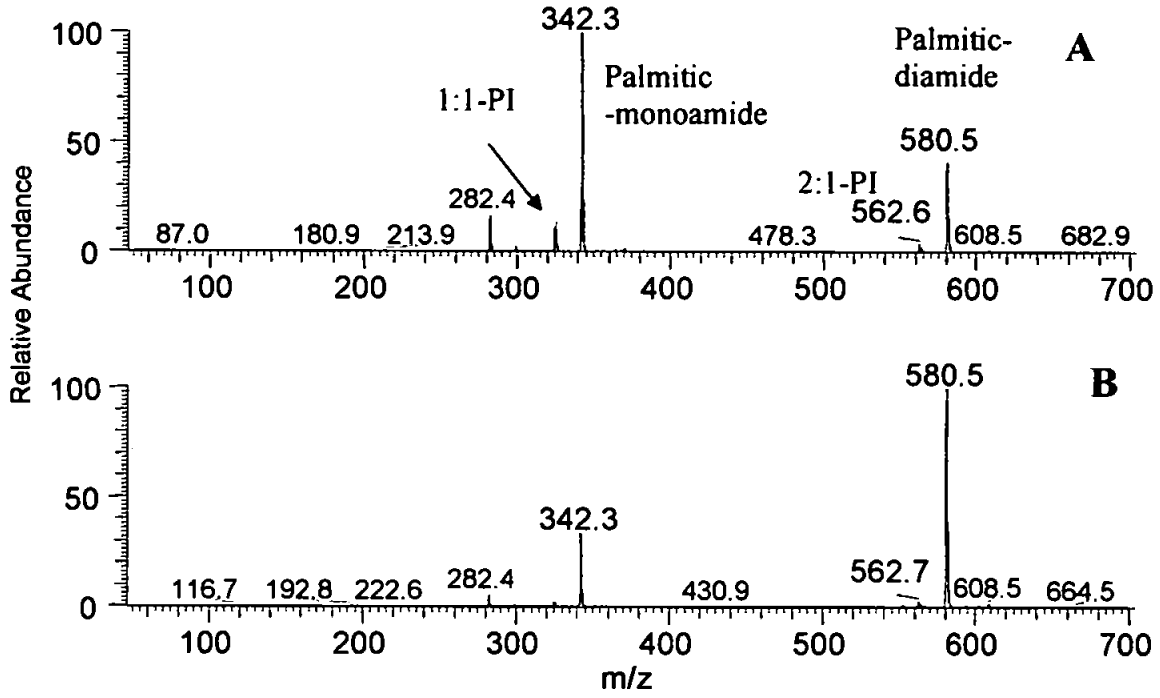


Figure 5.20 Infusion ESI-MS mass spectra of the crude and recrystallised palmitic-diamide products of the solid phase thermal reaction of palmitic acid with DETA. Positive ion full scan spectra: **(A)**, crude product (auto- tuned on ion m/z 580.6, *m/z* 50 – 700, capillary voltage (+42.30 V), 1st octapole (-2.64 V), 2nd octapole (-6.44 V) and tube lens (-19.05 V) and **(B)**, recrystallised product, (auto-tuned on ion m/z 580.6, *m/z* 50 – 700, capillary voltage (+22.10 V), 1st octapole (-2.86 V), 2nd octapole (-5.93 V) and tube lens (-23.12 V).

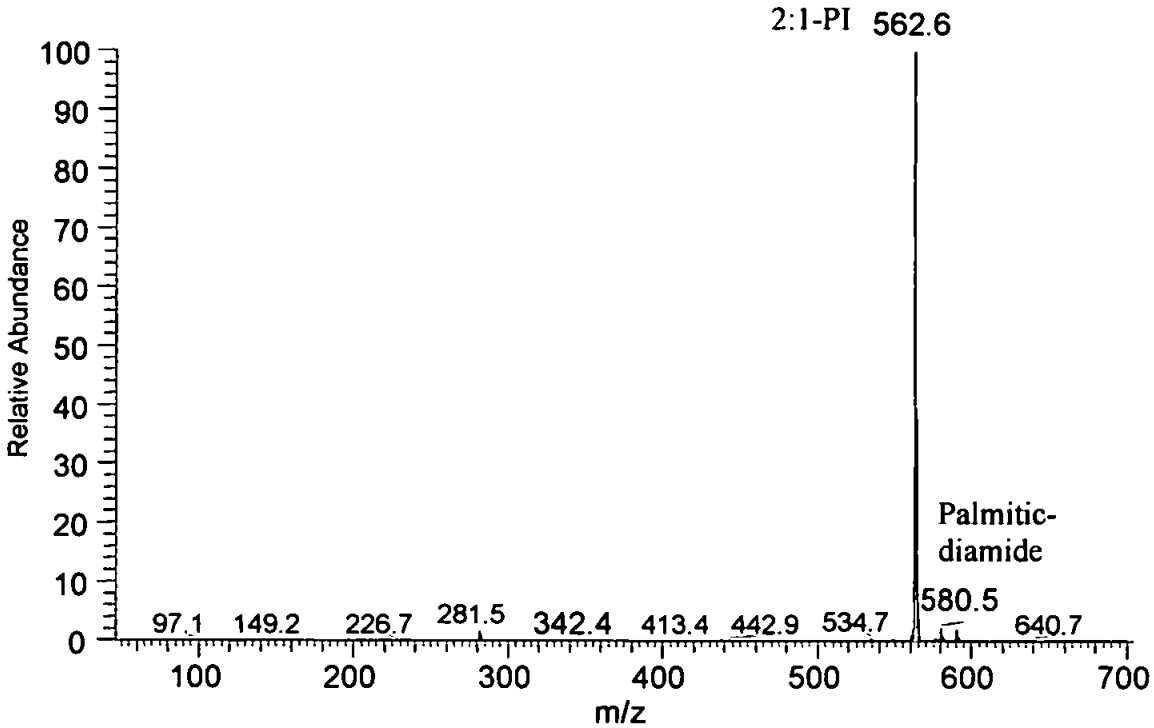


Figure 5.21 Infusion ESI-MS mass spectrum of the recrystallised 2:1-PI product of the solid phase thermal reaction of palmitic acid with DETA. Positive ion full scan spectra: (auto- tuned on ion m/z 562.6, *m/z* 50 – 700, capillary voltage (+24.08 V), 1st octapole (-2.93 V), 2nd octapole (-6.37 V) and tube lens (-27.05 V).

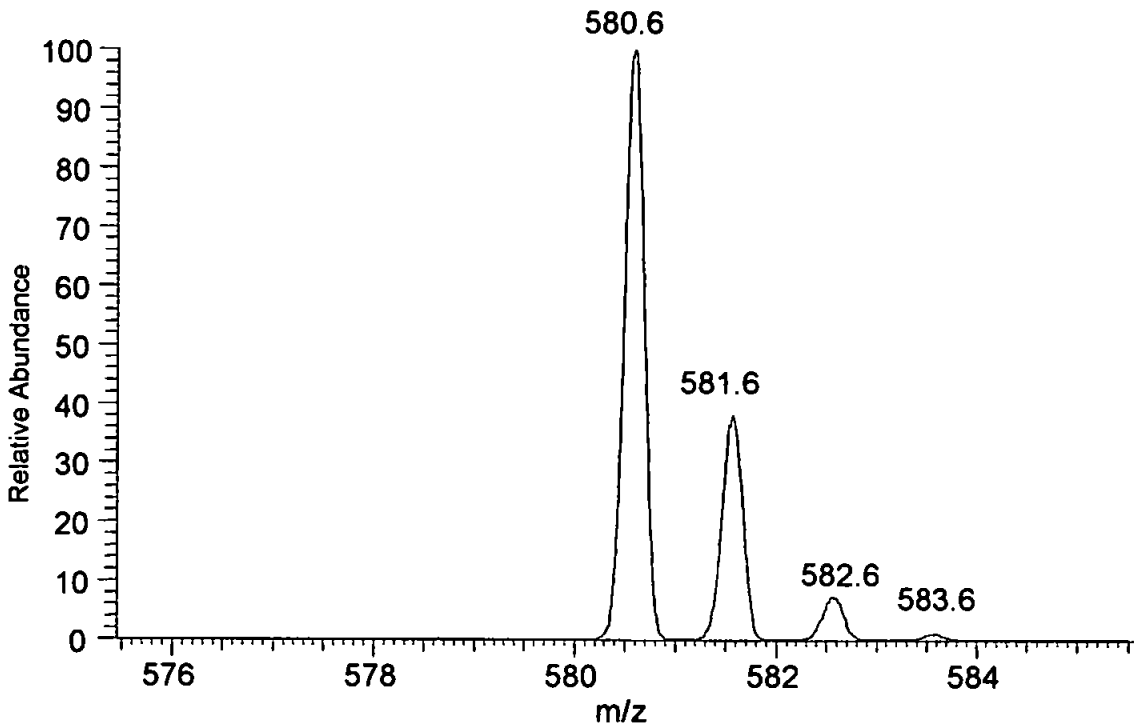


Figure 5.22 ESI-MS ZoomScan mass spectrum of the palmitic-monoamide ion shown in the full-scan spectrum (Figure 5.21).

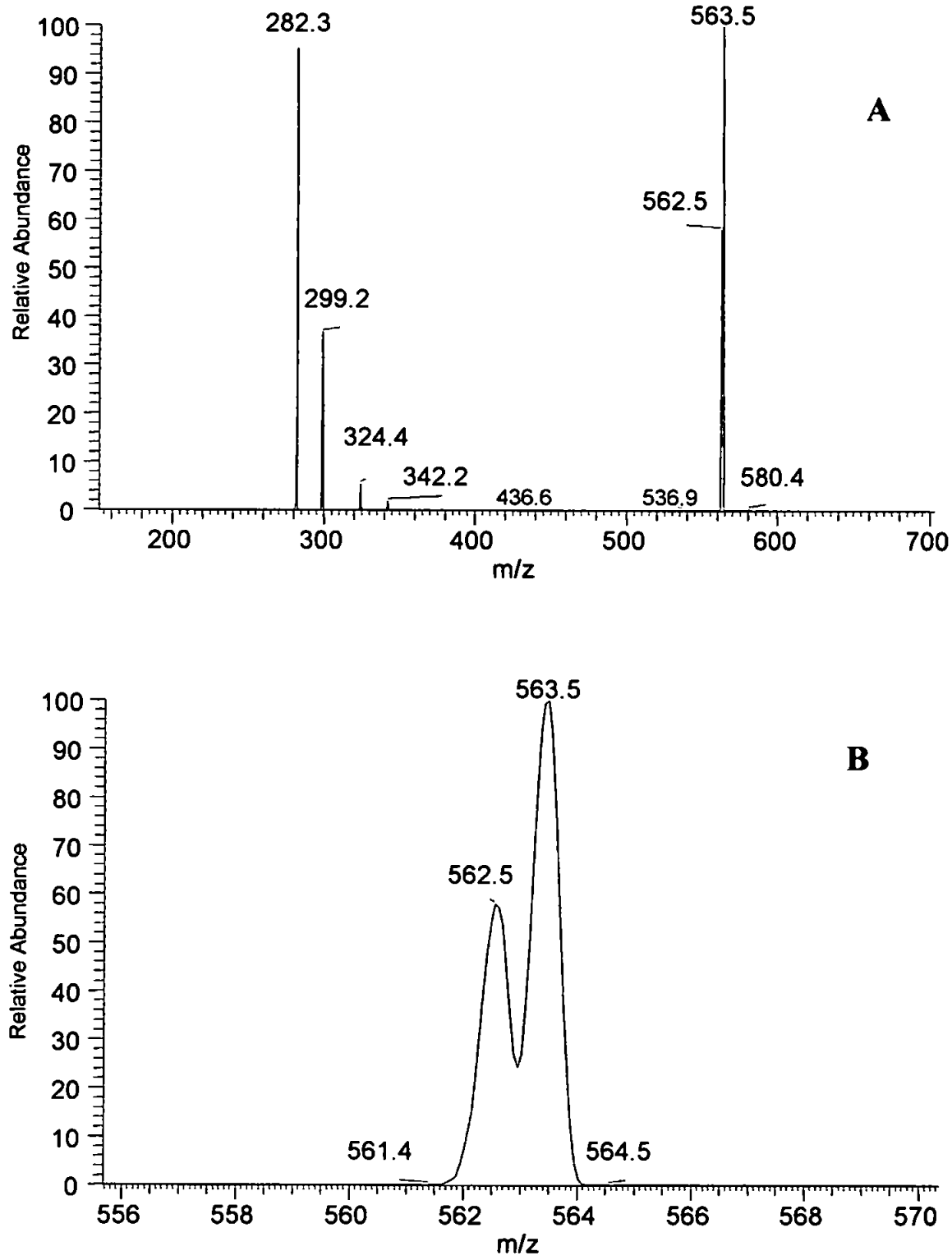


Figure 5.23 ESI-MS^2 CID mass spectrum (A) of the palmitic-diamide precursor ion, *m/z* 580.6 (40 % AA) in the full-scan spectrum (Figure 5.20B). (B) Shows an enlarged view of ion at *m/z* 563.6.

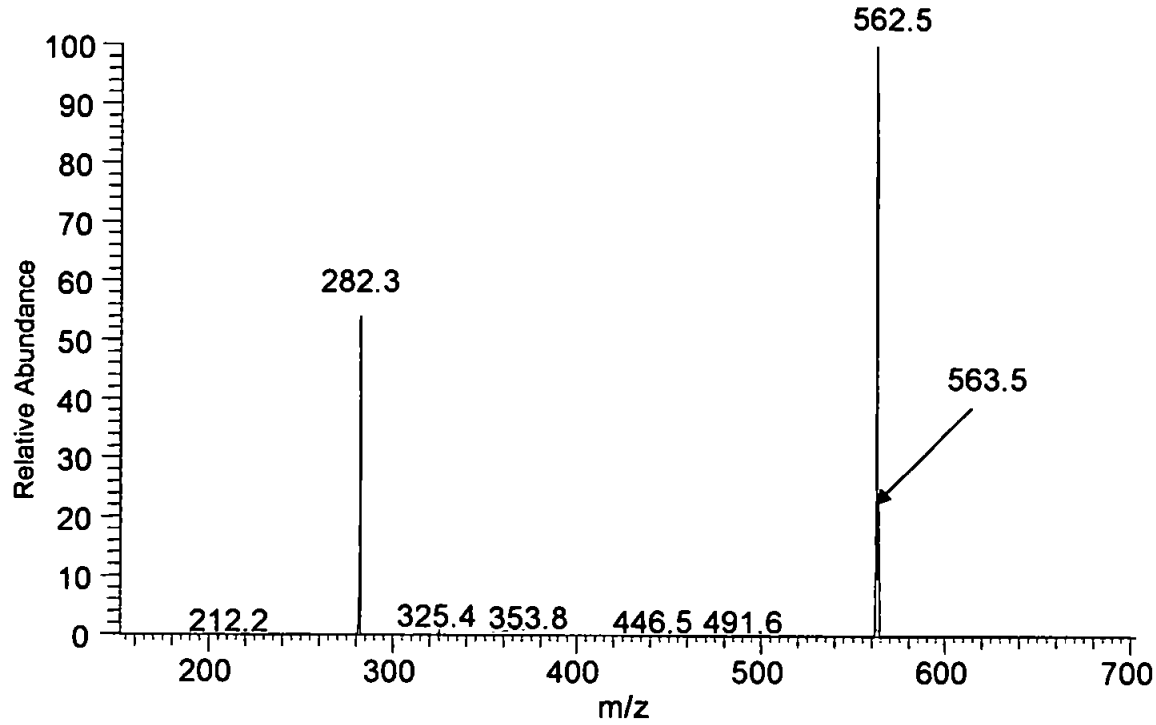


Figure 5.24 ESI-MS^3 CID mass spectrum of the product ion, *m/z* 563.5 (35 % AA) in the MS^2 spectrum (Figure 5.23).

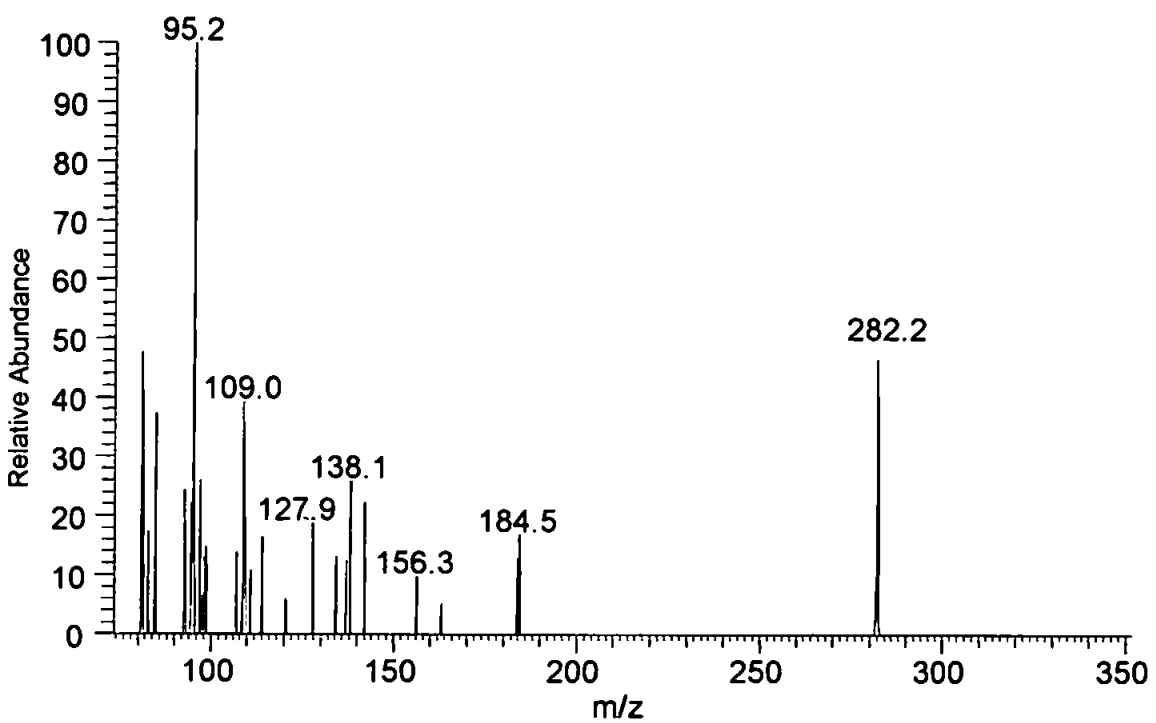


Figure 5.25 ESI-MS^4 CID mass spectrum of the product ion, *m/z* 282.3 (45 % AA) in the MS^3 spectrum (Figure 5.24).

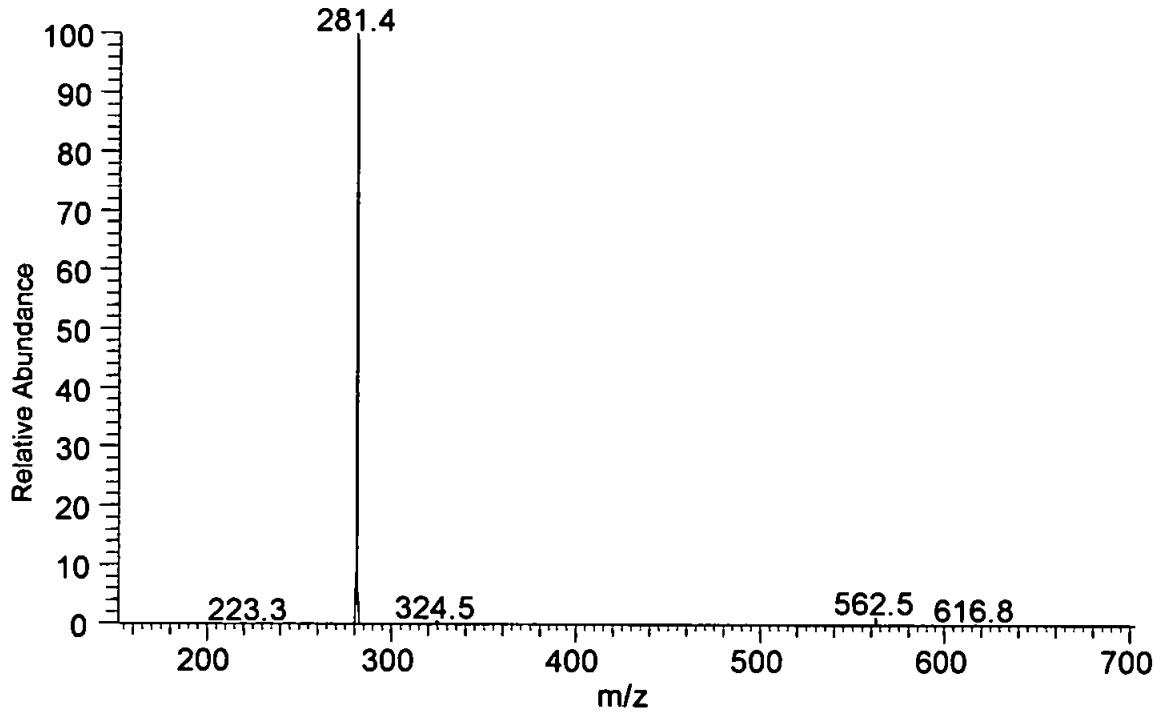


Figure 5.26 ESI-MS^3 CID mass spectrum of the product ion, *m/z* 562.5 (42 % AA) in the MS^2 spectrum (Figure 5.23).

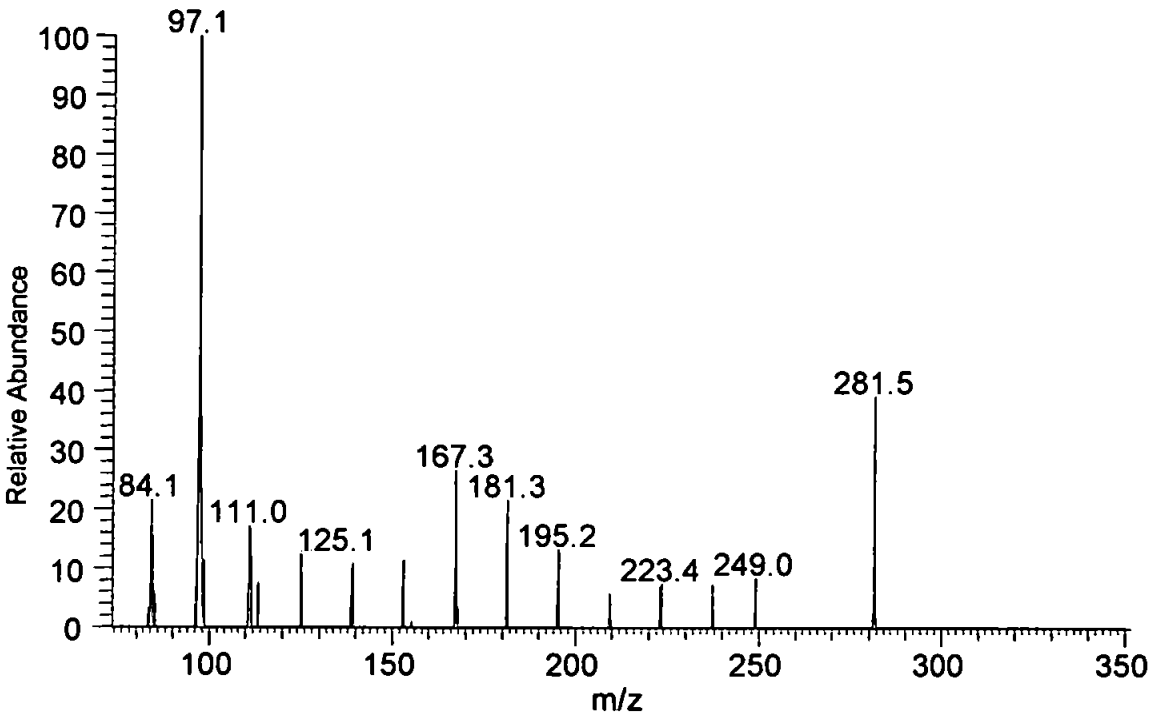


Figure 5.27 ESI-MS^4 CID mass spectrum of the product ion, *m/z* 281.3 (53 % AA) in the MS^3 spectrum (Figure 5.26).

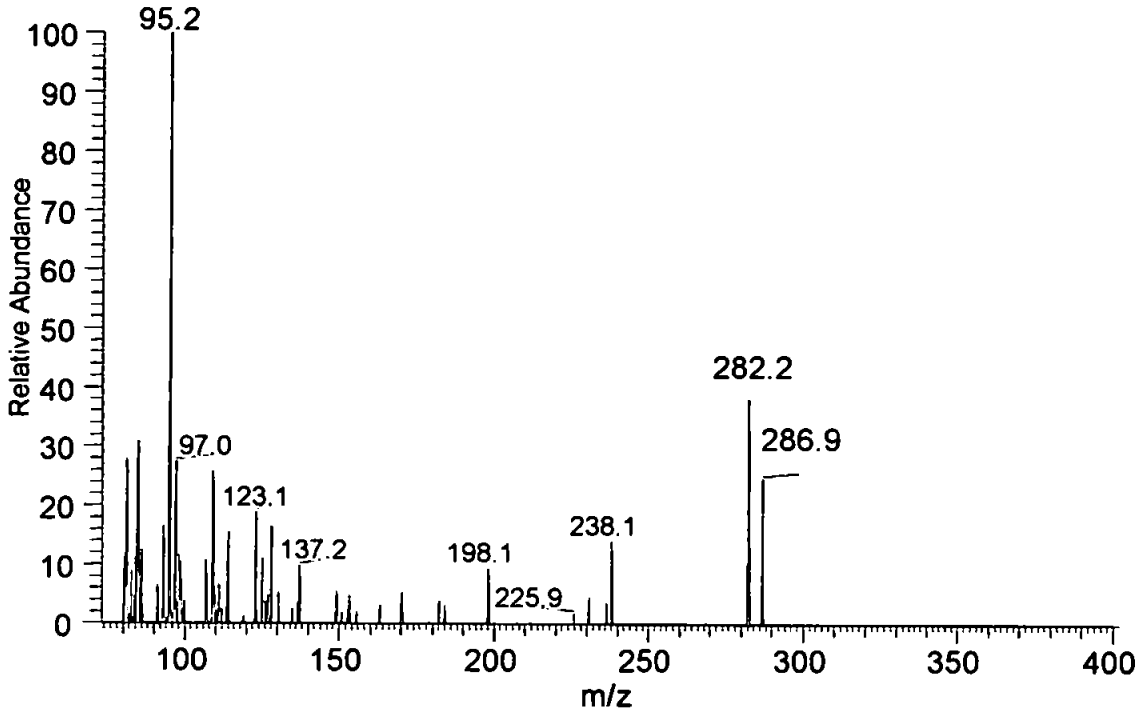


Figure 5.28 ESI-MS^3 CID mass spectrum of the product ion, *m/z* 282.3 (47 % AA) in the MS^2 spectrum (Figure 5.23).

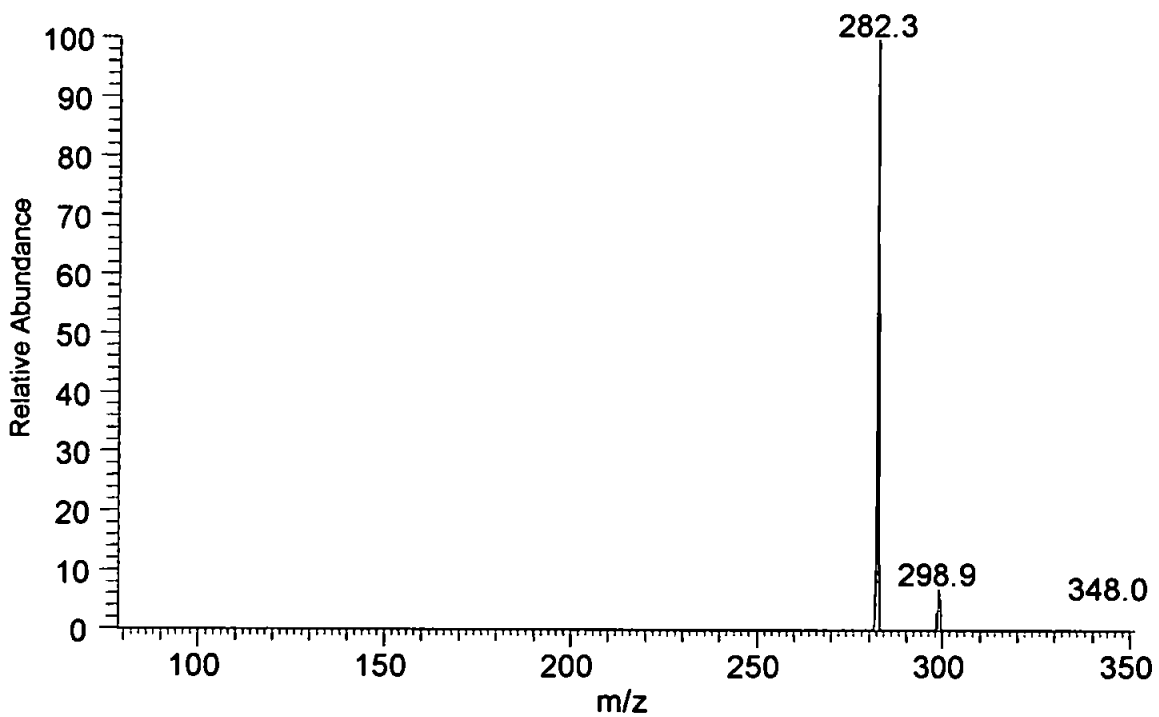


Figure 5.29 ESI-MS^3 CID mass spectrum of the product ion, *m/z* 299.2 (28 % AA) in the MS^2 spectrum (Figure 5.23).

From all the MS^n CID spectra obtained for the protonated 1,2- and/or 1,3-diamides (*m/z* 580.6 (palmitic derived) and *m/z* 632.6 (oleic derived)) precursor ions (Figure 5.23A and Figure A.2 (Appended)), two ion trap CID fragmentation pathways can be deduced (Figure 5.30). It is proposed that one pathway is derived from the 1,2-diamide which would have a distinct MS^2 CID product ion, from loss of ammonia (MS^2b; Figure 5.30). The second pathway for 1,3-diamides would not result in MS^2 CID loss of ammonia, but would involve production of M-18 ion *via* loss of water with cyclisation (MS^2a; Figure 5.30). It is also suggested that the MS^2 CID product ions MS^2c and MS^2d ((Figure 5.30) might be more abundant from the symmetrical 1,3-diamides. Evidence to support the presence of both 1,2- and 1,3-diamides can be shown in the HPLC/ESI-MS *m/z* 580.6 (palmitic-diamide) extracted mass chromatograms (Figure 5.5 and Figure 5.11) which show LC resolution of two compounds. When the relative abundance of the components is low, some resolution of the two diamides is observed. At higher abundances the peaks co-elute. An improvement in the HPLC/ESI-MS resolution of the 1,2- and 1,3-diamides should confirm this proposal and allow online HPLC/ESI-MS^n analysis of the individual compounds to be carried out.

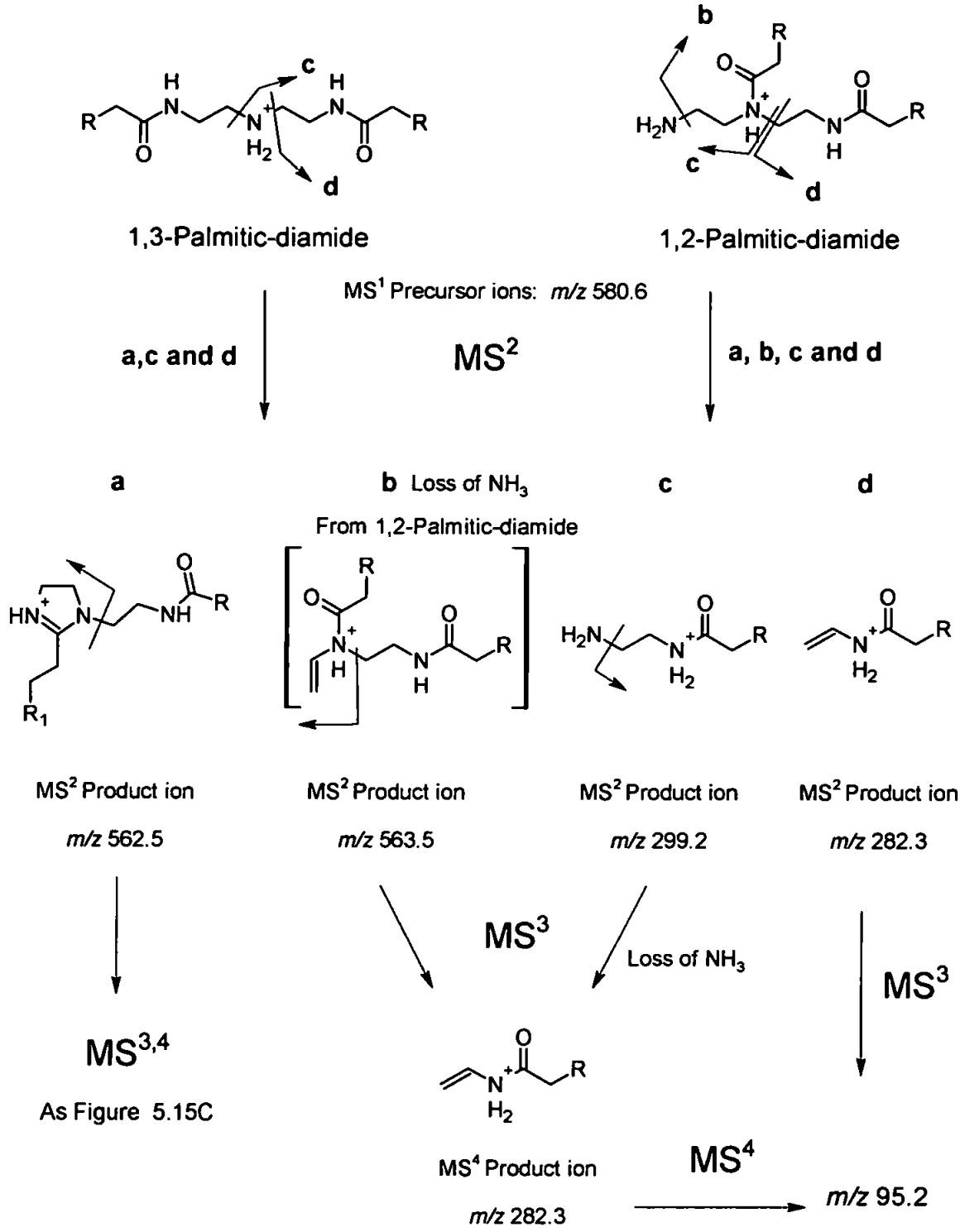


Figure 5.30 Proposed CID fragmentation pathway of compounds assigned as 1,2- and 1,3-diamides.

5.3.4 *Fourier transform infrared (FT-IR) spectroscopy analysis of the solvent and solid phase synthesis products*

Fourier transform infrared spectroscopy has been widely[54,75,77,78,89,94] used to identify and to characterise imidazoline and its related amides. Identifications have been based on the characteristic absorption frequencies of amides 1680 – 1640 cm^{-1} (C=O stretch; Amide I band) and 1560 – 1530 cm^{-1} (NH bend; Amide II band)) and imidazolines (1610 - 1600 cm^{-1}; >C=N stretch).[124] The FT-IR spectra of the solvent and solid phase crude and recrystallised products (Appended; Figures A.8 – A.12) showed the respective characteristic bands for amides and imidazolines (Table 5.4). No appreciable differences could be determined between the comparable solvent and solid phase 2:1-PI products. Although the HPLC/ESI-MS mass chromatograms and MS^n spectra of the crude 2:1-PI (solvent synthesised) and palmitic-diamide (solid phase synthesised) products showed *ca* 50 % each of the 1:1 and 2:1 molar ratio of fatty acid/DETA imidazoline or diamide products, very little difference could be observed between IR spectra of the crude and recrystallised products. The only significant difference was a very weak peak at 1506 cm^{-1} in the spectra of both the crude 2:1-PI and diamide products which was not observed in the spectra of the recrystallised products. This peak is in the range for NH_4^+ deformation (1530 - 1490 cm^{-1}; amine salt)[124] and both crude products were shown by HPLC/ESI-MS to contain amine compounds (1:1-PI and palmitic-monoamide, solvent and solid phase crude products respectively). The peak could be evidence for the fatty acid salt of 1:1-PI or palmitic-monoamide, although the corresponding $-COO^-$ anti-symmetrical[124] stretch peak between 1600 - 1590 cm^{-1} is not observed.

Table 5.4 Fourier transform infrared spectra, characteristic absorption frequencies observed for the solvent and solid phase synthesis products of thermal reaction of palmitic acid with DETA

Characteristic Absorption band	Amide		Imidazoline
SOLVENT SYNTHESIS	C=O (υ/cm^{-1})	N-H (υ/cm^{-1})	>C=O (υ/cm^{-1})
Crude 2:1-PI	1645	1550	1608
Recrystallised 2:1-PI	1639	1552	1606
SOLID PHASE SYNTHESIS			
Crude palmitic-diamide	1639	1562	
Recrystallised –palmitic-diamide	1639	1565	
Recrystallised 2:1-PI	1643	1550	1608

5.3.5 *^{1}H Nuclear magnetic resonance (NMR) spectroscopy analysis of the solvent and solid phase synthesis products*

^{1}H Nuclear magnetic resonance spectroscopy (^{1}H NMR) has been used to characterise the structures of imidazolines and amides.[75,77,78,89] The spectrum (Appended; Figure A.13) of the 2:1-PI product of the recrystallised solid phase synthesis (Section 5.2.5) and the spectrum of the 2:1-PI produced by solvent synthesis (Chapter 4, section 4.2.4.3; not shown) were similar and the chemical shifts were in general agreement with those for 2:1-imidazoline published previously.[75,77,78,89] The characteristic proton of the amide group is observed as a broadened signal at 6.5 ppm. The CH_2-N=C protons of the imidazoline ring are observed as a multiplet about 3.7 ppm, whilst the resonance of the ring CH_2-N< protons (multiplet) overlap with the >N-CH_2-CH_2-N- protons (triplets) between 3.0 and 3.5 ppm.

^{1}H NMR spectra of the solid-phase synthesised palmitic-diamide (Section 5.2.5; Appended; Figure A.14) and a oleic-diamide formed from hydrolysis (during storage) of

2:1-oleic imidazoline (Chapter 4, section 4.3.8, Appended; Figure A.7) are consistent with a 1,3-diamide (structure V, Figure 3.1).[77,78,89] The amide protons are observed as a broadened signal at 6.5 and 6.0 ppm (1,3-palmitic and 1,3-oleic diamide respectively). The CH_2–CO and –CH_2-N-CH_2- protons are observed as triplets about 2.11 (2.20) and 2.66 (2.7) ppm respectively and the –CH_2-N-CO protons as a quartet at 3.3 (3.2) ppm. Interpretation of ^{1}H NMR spectrum (not shown) of the 2:1-PI product from the solvent synthesis (section 5.2 4) was not possible due to impurities (as shown by HPLC/ESI-MS) and further purification is required for ^{1}H NMR structural interpretation.

5.3.6 *Reaction intermediates and pathways of solvent and solid phase thermal reaction of palmitic acid with DETA*

The aim of repeating the synthesis originally described by Wu and Herrington[89] was predominantly to obtain pure palmitic-diamide and 2:1-PI. Subject to improved recrystallisation procedures, this was achieved. It was apparent however, that the mechanisms proposed by Wu and Herrington for the solvent-based and solid phase reactions require modification in the light of the results obtained herein. Only the final recrystallised reaction products were analysed by Wu and Herrington by FT-IR, ^{1}H NMR, MS, NP-HPLC and Shiff base reactions by UV. Neither crude final products or reaction sub-samples were examined. The important mono-amide and 1:1-imidazoline intermediates were therefore not detected. Mono-amide and 1:1-imidazoline intermediates were detected herein by HPLC/ESI-MS and ESI-MS^n analysis of the crude and recrystallised products of the solvent and solid phase syntheses.

An important consideration in determining the reaction mechanisms and intermediates is the relative reactivities of the primary and secondary amino groups of DETA. In general, the secondary amino group of polyamines has higher nucleophilicity with most

electrophilic reagents[93]. However, the primary amino groups can be more reactive due to steric factors of the carbonyl group to attack by the nucleophile[75,93]. Even though MS^n analysis of the palmitic and oleic diamide products (Section 5.3.3.3) showed evidence for a primary amine group (1,2-diamine), currently the HPLC/ESI-MS and MS^n methods do not distinguish between primary, 1-monoamine or secondary, 2-monoamine intermediates.

Without solvent the thermal reaction of fatty acid with DETA appears to proceed *via* three main stages; *viz*, salt, mono/diamide and imidazoline formation (Figure 5.31). Each stage requires higher temperatures to overcome the intermolecular bonding and to allow the correct orientations of the reaction centres and configuration of the products. In the first stage, if the fatty acid and DETA are added together at the melting point of the fatty acid (63 °C, palmitic acid) a spontaneous salt formation is observed as a gel with liberation of heat. The salt/gel is rapidly overcome as the temperature is increased to *ca* 150 °C and is not observed if the reaction is carried out with the reagents preheated to *ca*150 °C. Salt formation will also occur at room temperature. The second and third stages are temperature-dependent. The lower temperature (*ca* 150 °C) mono/diamide formation stage and at higher temperature and under vacuum (*ca* 240 °C, 30 mm Hg) or long reaction times[75-77,89,90] cyclisation to imidazoline. In monoamide formation, the reacting centres in the salt are already in close proximity and the amide product can maintain a relatively linear configuration. Therefore the inter-molecular bonding to be overcome to allow elimination of a water molecule and amide bond formation is not too high. If the fatty acid/DETA ratio is 2:1, a diamide formation can occur. For cyclisation of the mono/diamides to imidazolines, a much greater rearrangement of amides configuration is required to allow the reacting centres to be positioned correctly and to allow subsequent elimination of a second water molecule. Therefore there is a much higher energy requirement to overcome inter-molecular bonding.

In contrast to the solid phase, when the reaction of fatty acid with DETA is carried out in solvent (at suitable concentrations to ensure full solvation), the alkyl chain van der Waals inter-molecular interactions are dispersed by much weaker solvent-molecule interactions thereby eliminating steric effects. Thus a lower temperature is required to obtain the correct molecular configurations for the reactions. Analysis of reaction sub-samples (Chapter 4; Sections 4.3.4, 4.37 and 4.3.8) showed only small amounts of the amides relative to imidazolines, even after only a short reaction time (1 hr). This indicates that due to the low steric interactions, cyclisation of the amides is rapid and imidazolines are the preferred products. Also, the ratio of acid to DETA, the order of addition of reagents (*i.e.* DETA to acid or acid to DETA) and time over which reagents are added, may influence final ratio of 1:1- and 2:1-imidazoline products. This is due to competition, and the relative reactivity of the respective amine groups, of the DETA, monoamide and 1:1-imidazoline products for available acid.

Revised reaction schemes for solid phase and solvent based thermal reactions of fatty acids with DETA based on the foregoing HPLC/ESI-MS experimental results are shown in Figures 5.31 and 5.32. It is not known from this study whether the amides are primary, secondary or secondary/primary amides. It is believed that the initial temperature, fatty acid/DETA ratios and the method and time spans over which the reagents are mixed may be the most important factors in determining the final ratio of products in both solid phase and solvent based syntheses. An even more detailed study of these factors needs to be carried out in order to arrive at more efficient syntheses of the desired products.

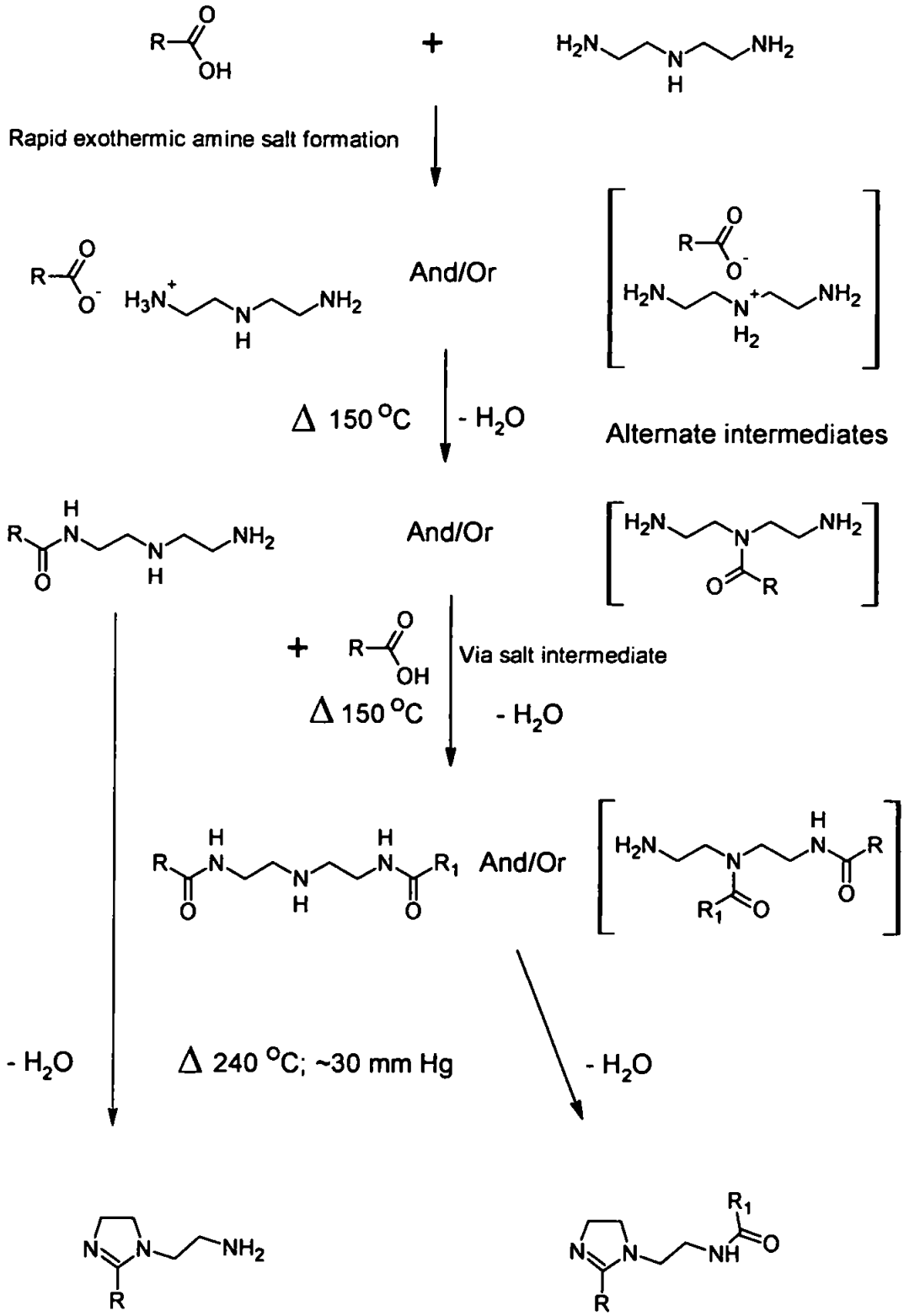


Figure 5.31 Proposed reaction mechanism for the solid phase thermal reaction of fatty acids with DETA.

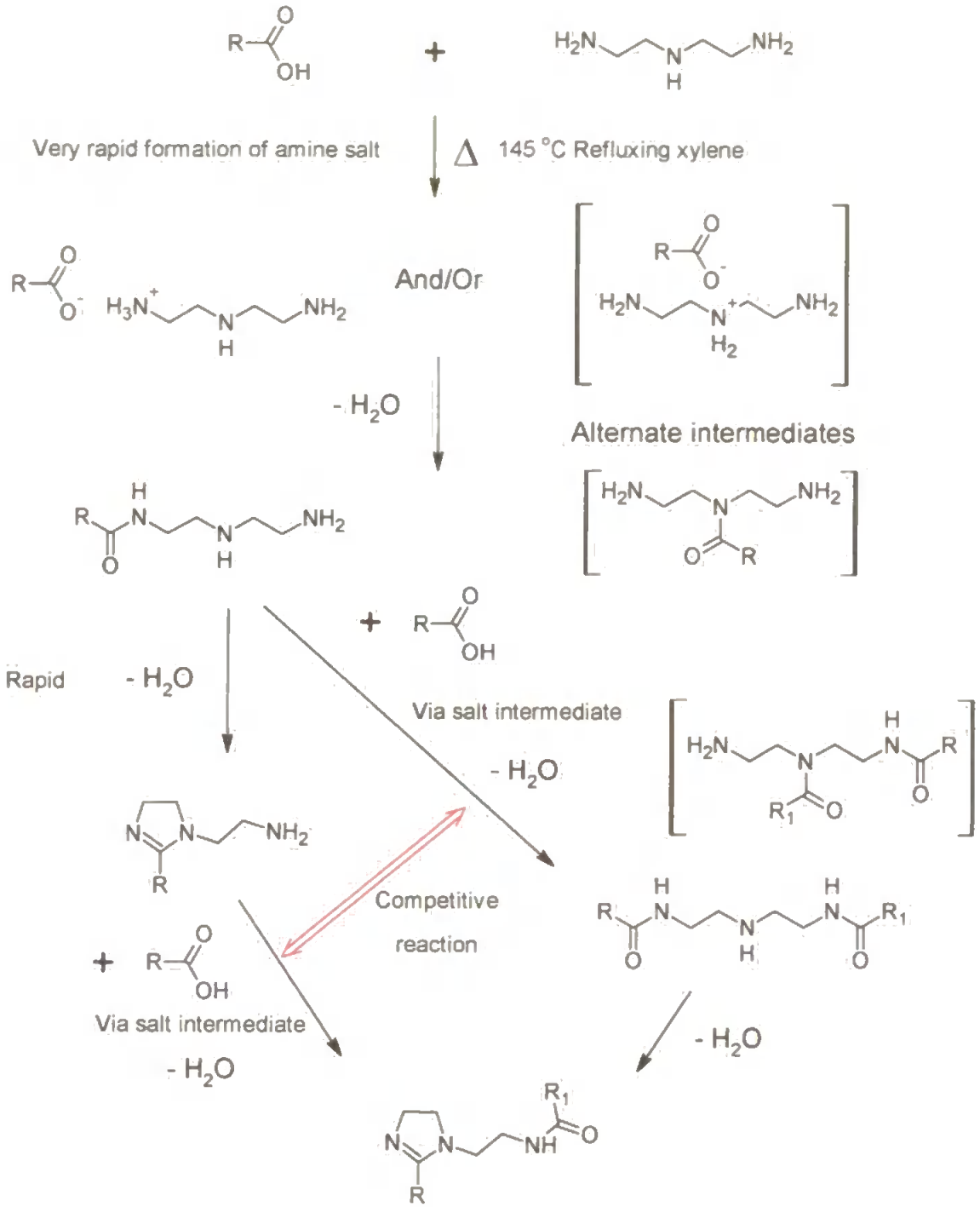


Figure 5.32 Proposed reaction mechanism for the solvent based thermal reaction of fatty acids with DETA.

5.4 Conclusions

The thermal synthesis of 2:1-palmitic imidazoline proceeds *via* two different pathways depending on whether the reaction is carried out in the solid phase (Figure 5.31) or diluted in refluxing xylene solvent (Figure 5.32). The reaction products may be influenced by the method by which the reagents are added and the time span of their addition. Reaction intermediates and final products (1,1- and 2:1-palmitic imidazolines and mono and diamides) were identified and characterised by HPLC/ESI-MS and by MS^n. CID MS^n fragmentation pathways were determined for 1,1- and 2:1-palmitic imidazolines and 1,2-/1,3-palmitic and oleic diamides.

CHAPTER SIX

Conclusions and Future Work

This chapter summarises the conclusions from the results presented and discussed in Chapters 2 – 5. Proposals are suggested for work which would develop further the solid-phase extraction (SPE), high performance liquid chromatography (HPLC) and ESI-MS^n methods used herein for the study of imidazoline-based and other oilfield chemicals. Such developments would also aid further studies of the synthesis of imidazolines and related amides by thermal reactions of fatty acids with diethylenetriamine (DETA).

6.1 Conclusions

The primary aim of this study was to investigate new analytical methods for monitoring the environmental fate of the diverse polar organic chemicals used during the offshore production of crude oils. Methods have been devised for the extraction, chromatographic separation, detection and quantitation of some of these chemicals from environmental matrixes. At the inception of the research, electrospray ionisation mass spectrometry (ESI-MS) was an emerging technique that appeared to have attributes compatible with the detection of some of these compounds. Use of quadrupole ion trap (QIT) mass spectrometers for multi-stage mass spectrometry (MS^n, n = 1 – 10) seemed particularly attractive. A range of speciality oilfield chemicals (corrosion inhibitors, scale inhibitors, biocides and demulsifiers) were therefore investigated by ESI-MS^n. Using appropriate model compounds, compatible liquid chromatography (LC) methods and chromatographic phases were developed for the extraction, separation and quantitation of speciality oilfield chemicals from environmental matrixes. The developed methods were applied to the study of real environmental samples as follows:

A range of speciality oilfield chemicals (corrosion inhibitors and demulsifiers; Chapter 2) in which the identities of the active compounds were unknown, was examined by infusion ESI-MS^n (positive and negative ion) on a QIT instrument (LCQ™, ThermoFinnigan). Full scan (MS) and collision induced dissociation (CID) multi-stage mass spectra (MS^n) were obtained from the products. Analysis of spectra allowed identification of alkylbenzenesulfonates, di-[alkyldimethylammonium-ethyl]ethers, alkylbenzyl-dimethylammonium and imidazoline compounds. CID MS^n fragmentation pathways were determined for the compounds identified (Figures 2.2, 2.6, 2.8 and 2.10 respectively). The

identification of alkylbenzenesulfonates, alkylbenzyldimethylammonium cations and imidazolines was confirmed by comparison of the mass spectral fragmentation pathways with those of authentic compounds. The MS^3 product ions for both the alkylbenzenesulfonates (MS^3 product ion *m/z* 119) and alkylbenzyl-dimethylammonium cations (MS^3 product ion *m/z* 58) identified in the fragmentation pathways had not been reported previously to the present author's knowledge. An authentic di-[alkyldimethylammonium-ethyl]ether was not commercially available and future studies might include synthesis and co-chromatography of the compound.

Polar organic fractions (dichloromethane extracted) of real North Sea oilfield Produced Water, were examined by infusion ESI-MS^n (positive and negative ion). Alkylbenzenesulfonates similar to those identified in the studied oilfield products were observed in the Produced Water by negative ion MS and confirmed by MS^n. The positive ion mass spectra indicated the presence of diamides produced from hydrolysis of 2:1-imidazoline corrosion inhibitor compounds and polyethoxylated compounds typical of those used in commercial demulsifiers. The complexity of the spectra highlighted the requirement for analytical separation of components prior to ESI-MS analysis in order to more accurately identify individual components.

Examination of the oilfield chemicals and Produced Water extracts showed that ESI-MS^n was a powerful technique for identification and characterisation of polar chemicals. The particular advantages of the ion trap multi-stage ESI-MS method were: the possibility of both positive and negative ion detection (*e.g.* for quaternary amines and sulfonates respectively); the ability through the so-called 'ZoomScan™' facility to determine the accurate mass differences between ion pairs, thus allowing differentiation of the multiple charge state of the ions and collision induced dissociation multi-stage mass spectrometry which allowed fragmentation pathways to be studied, thereby allowing previously

unknown compounds to be identified and unambiguous identification by comparison with authentic compounds.

Imidazoline compounds are widely used in speciality oilfield corrosion inhibitor products and were shown in Chapter 2, to be amiable to ESI-MS detection and MS^n characterisation. As such, they were identified as suitable representative compounds for further investigation by liquid chromatography (LC) coupled with ESI-MS and solid phase extraction (SPE) methods for their determination in environmental matrixes.

In conjunction with a HPLC/ESI-MS method developed in a further part of the study (Chapter 4, see below), a SPE method for the extraction of imidazoline compounds from crude oils was developed (Chapter 3). A semi-quantitative but sensitive and specific method, involving SPE with a silica phase, followed by HPLC/ESI-MS, was developed for the determination of individual imidazolines at low (<10) parts per million concentrations in crude oils. Whilst the method requires further optimisation due to the high experimental losses of imidazolines during work-up, it nonetheless represents a considerable improvement on previous techniques and should prove valuable for operational and environmental studies of corrosion inhibitor and surfactant behaviour.

Pure authentic compounds were required to develop the SPE and HPLC methods and to investigate the relative ESI-MS responses of imidazolines and amides for quantitation. Therefore, syntheses of 1:1- and 2:1-palmitic and oleic acid derived imidazoline compounds (Chapter 4) were carried out by solid-phase and solvent-based thermal reactions of diethylenetriamine (DETA) with palmitic and oleic acids. Although a simple synthesis and purification method for pure compounds was not optimised, reaction sub-samples and products were successfully used to develop a high performance liquid

chromatography (HPLC) separation with coupled ESI-MS detection (Chapter 4) and a SPE of 2:1-palmitic and oleic acid-derived imidazoline from crude oil (Chapter 3).

A poly(styrene-divinylbenzene) HPLC phase (Chapter 4) in conjunction with a gradient elution method using trifluoroacetic acid (TFA) ion-pair reagent with modified methanol (or ethanol) and water eluents, was determined to be the optimum for analytical separation with ESI-MS detection of palmitic and oleic acid-derived 2:1-imidazolines, 1:1-imidazolines, monoamide and diamide compounds (Chapter 4; compound structures are given in Chapter 3, Figure 3.1). The HPLC/ESI-MS responses of palmitic and oleic 2:1-imidazolines (2:1-PI and 2:1-OI respectively) were investigated and detection limits of 0.01μg mL^{-1} 2-1-PI and OI standard (signal/noise ≥ 3) were determined in the full scan range *m/z* 100 – 1000. These concentrations are much lower than those previously published for imidazolines (50μg mL^{-1} and 150 μg mL^{-1}).[52,88] The HPLC/ESI-MS method was used to separate the imidazoline and amide components of a commercial imidazoline product (Chapter 4). The presence and separation of a range 1:1- and 2:1-imidazolines and the corresponding monoamides and diamides derived from C_{18} acids with up to three degrees of unsaturation ($C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$) and C_{20} acids with up to three degrees of unsaturation ($C_{20:0}$, $C_{20:1}$, $C_{20:2}$ and $C_{20:3}$). Separation of structural isomers of the compounds was also shown.

When syntheses of model imidazoline compounds by thermal reaction of DETA with fatty acids were carried out (Chapter 4), it became apparent that the developing HPLC/ESI-MS method was a powerful technique for the study of this important reaction. Analyses of reaction sub-samples and products highlighted the contradictory results in the published literature and specifically the existence of 1:1-imidazoline and monoamide intermediates was shown. Due to the requirement for efficient syntheses of high purity model compounds

an investigation of solid-phase and solvent-based thermal reaction of DETA with palmitic acid was carried out (Chapter 5). It was shown that the thermal synthesis of 2:1-palmitic imidazoline proceeds *via* two different pathways depending on whether the reaction is carried out in the solid phase (Figure 5.31) or diluted in refluxing xylene solvent (Figure 5.32). Reaction intermediates and final products (1:1- and 2:1-palmitic imidazolines and mono and diamides) were identified and characterised by HPLC/ESI-MS and by MS^n. CID MS^n fragmentation pathways were determined for 1:1- and 2:1-palmitic imidazolines and 1,2-/1,3-palmitic and oleic diamides.

6.2 Future work

The use and discharge of chemicals used in the United Kingdom offshore oil and gas industry (The Offshore Chemical Regulations 2002)[125] have recently changed from a voluntary scheme (the Oslo Paris Commission (OSPAR) Harmonised Offshore Chemicals Notification Format (HOCNF)) to compulsory regulation (OSPAR Harmonised Mandatory Control Scheme (HMCS)). The scheme requires chemicals to be ranked by a Hazard Quotient (HQ: predicted environmental concentration/predicted no effect concentration (PEC/PNEC ratio)) determined by chemical hazard assessment and risk management models (e.g. CHARM) based on the toxicity, bioaccumulation potential and biodegradability of each chemical. Such mathematical computer models predict the environmental concentrations/discharges based on theoretical calculations (e.g. CHARM) or at best, based on laboratory and some field studies for a limited range of chemicals (e.g. PROTEUS[126]). A major problem for real predictions and assessments of these chemicals has been the lack of suitable analytical techniques. However, new methods such as those described in this thesis may allow the detailed characterisation of oilfield chemicals and

determination of their fates in the marine environment and 'sea-truthing' of the data currently used by mathematical models such as CHARM and PROTEUS. This is important as it has been shown that oilfield discharges (e.g. Produced Water) can produce undesirable biological impacts on marine organisms[41,47,55,56,127,128] and large amounts (estimated 313,790 tonnes of oilfield chemicals used and 106,592 tonnes discharged) of oilfield chemicals were used and discharged by the UK sector in 2000.[51] The requirement for better analytical techniques to allow investigation of oilfield chemicals is expected to increase. Thus, improvements to developed methods such as those described in this thesis may further advance the study of these and similar chemicals.

The HPLC separation of imidazoline and amide compounds developed could be improved by changing the column used in this study (PRP-1, 5 μ, 4.1 x 50 mm; Hamilton) to a more efficient 3μ PRP-1 phase with a change in column dimensions (2.1 x 100 mm). Decreasing the internal diameter (from 4.1 to 2.1mm) allows the use of 150 - 200 μL min^{-1} (*cf.* 1000μL min^{-1}) flow rates which do not require splitting prior to the ESI source. This alleviates peak broadening due to the couplings required for splitting the post column eluent flow and allows the entire sample injected on column to enter the ESI source. The advantages of this change have been shown in a separate study by the present author[129] for siderophore compounds (iron complexed and non-complexed rodotoluate, ferrioximine and ferrichrome) in a modification of the developed HPLC/ESI-MS method. The use of inert polyetheretherketone (PEEK) columns in place of stainless steel columns may also improve peak broadening and resolution as imidazolines have a high surface activity with iron. Indeed, the stainless steel tubing and components of the auto-sample used in the present study could be substituted with a metal free sample injector system. Further investigations of ion-pair reagents and eluent modifiers (such as pentafluoropropionic acid) may improve separations. Improved resolution of the 1-/2-monoamides and 1,2-/1,3-

diamides would allow HPLC/ESI-MS identification MS^n characterisation of these isomers in reaction intermediates, products and environmental samples.

A more detailed study of the solid-phase and solvent-based thermal reaction of DETA with fatty acid could be carried out with HPLC/ESI-MS, ESI-MS^n and other spectrometric methods of analysis. An emphasis on the important initial method of addition, time span, temperature and concentration of reagents may provide a better understanding and method for efficient synthesis of desired products, for what is still a debatable reaction.

The SPE method developed requires further development to improve the efficiency of imidazoline extraction from crude oils. Specifically the loss of sample by sorption to surfaces during sample handling procedures requires investigation. Comparisons with other solid phase supports (such as DIOL, CN and NH_2) which are less sensitive to effects of water wet oils may be more efficient. The high organic content required to elute imidazolines and amides from the poly(styrene-divinylbenzene) HPLC phase suggests that the phase may be suitable for solid phase extraction of these compounds from environmental waters such as oilfield Produced Water and marine samples.

The specific identity of several ethoxylated compounds observed by ESI-MS in oilfield products (Chapter 2) was not established during this study due to the limited information on the product composition and the limited time available. Further studies of these specific products are required to establish their identities and subsequently their extraction, chromatographic separation from and quantitation in environmental matrixes.

ESI has been shown to be suitable for the detection of some polar compounds in oilfield chemicals however; it may not be applicable to many other compounds or be the most sensitive method of detection. Atmospheric pressure chemical ionisation (APCI) and

atmospheric pressure photoionisation (APPI; an emerging ionisation process for interface with liquid chromatography/mass spectrometry[130,131]) may be more appropriate for other compounds of interest in oilfield products. A study comparing ESI, APCI and APPI detection for compounds in oilfield chemicals would enable the most appropriate method for each compound or group of compounds to be determined. A comparison of ionisation interfaces would be greatly enhanced if the separate ionisation interfaces were combined into one interface. Currently, interfaces that allow software switching from ESI to APCI[132,133] (and *vice versa*) and APCI to APPI[130] are becoming commercially available. However there does not appear to be reports of dual ESI/APPI or triple ESI/APCI/APPI interfaces. The combination of ESI and APPI as a single mass spectrometer interface could be a very powerful detection system for highly polar and non-polar compounds in a single chromatographic separation. A study to investigate the feasibility of a combined ESI/APPI could be carried out.

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APPENDIX

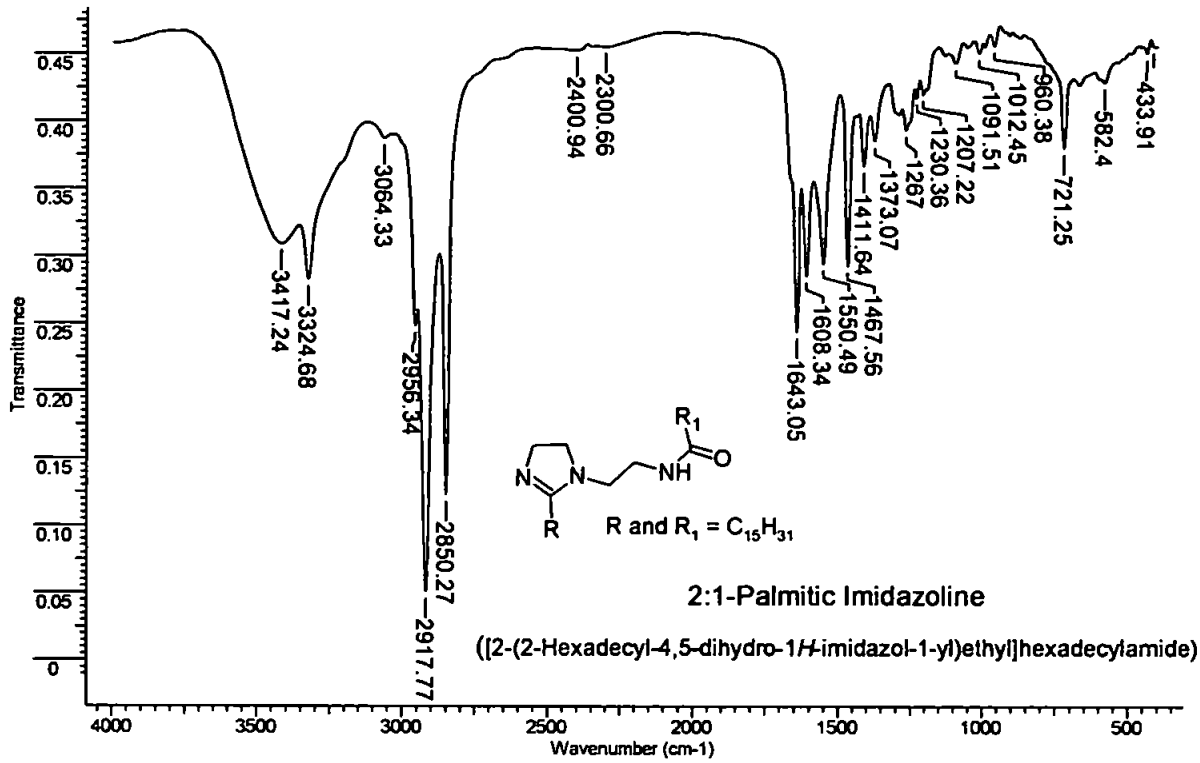


Figure A.1 Fourier transform infrared spectrum of 2:1-palmitic imidazoline synthesised by thermal reaction of palmitic acid with diethylenetriamine refluxed in xylene solvent (Chapter 4; Section 4.2.5.3): (υ/cm^{-1}); 1643, 1550 (-NH-C=O), 1608 (C=N).

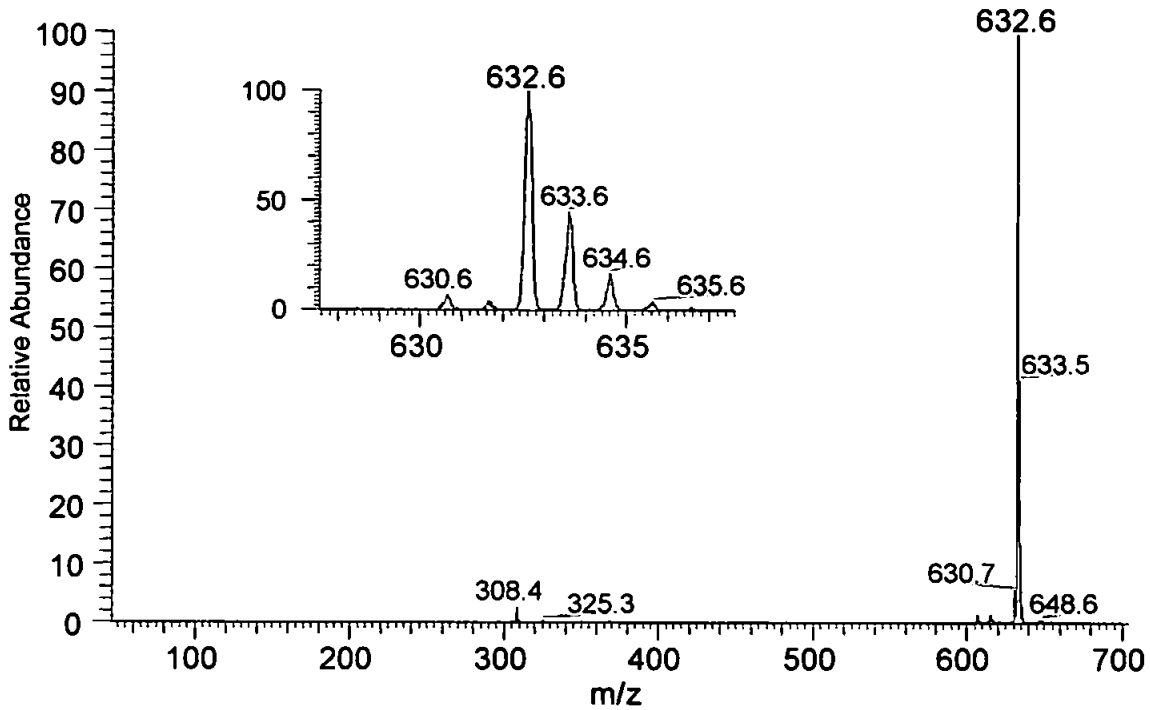


Figure A.2 Infusion ESI-MS mass spectrum of oleic-diamide product (Chapter 4, section 4.3.8). Positive ion full scan spectra: (auto-tuned on ion *m/z* 632.6, *m/z* 50 – 700, capillary voltage (+16.03 V), 1st octapole (-3.15 V), 2nd octapole (-6.44 V) and tube lens (-21.17 V). Inset is the ZoomScan of ion *m/z* 632.6.

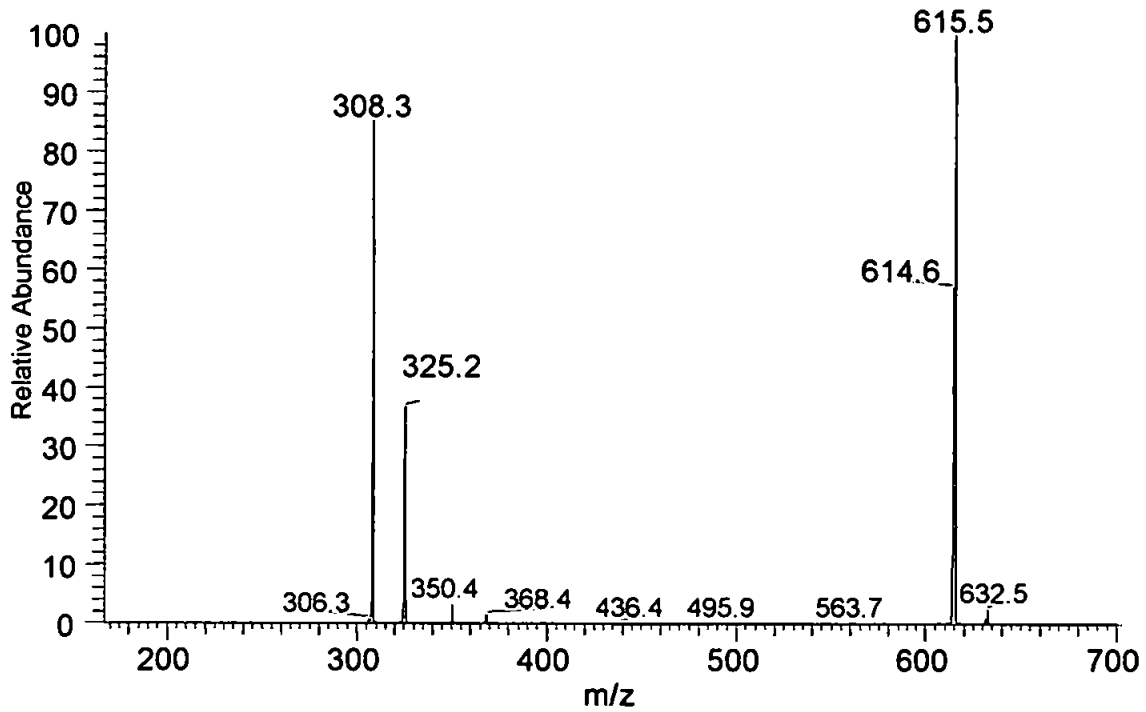


Figure A.3 ESI-MS^2 CID mass spectrum of the oleic-diamide precursor ion, *m/z* 632.6 (39 % AA) in the full-scan spectrum (Figure A.2).

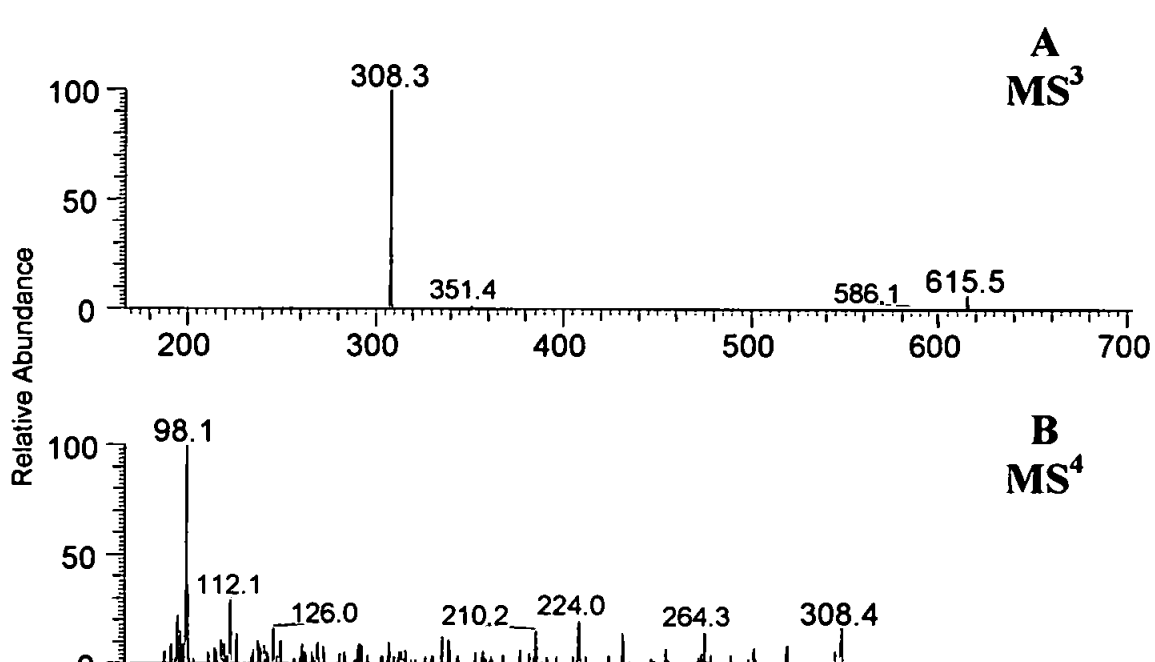


Figure A.4 ESI-MS^n mass spectra of the oleic-diamide MS^2 product ion (*m/z* 615.6) in the MS^2 spectrum Figure A.3. **(A)** MS^3 on precursor ion *m/z* 6.15.6 (36 % AA), **(B)** MS^4 on product ion *m/z* 308.3 (43 % AA).

Figure A.5 ESI-MS^3 mass spectrum of the product ion (*m/z* 325.2; AA 29 %) in the oleic-diamide MS^2 spectrum of product ion (*m/z* 615.6) in the MS^2 spectrum Figure A.3. MS^4 on the *m/z* 308.3 product ion is identical to Figure A.4B.

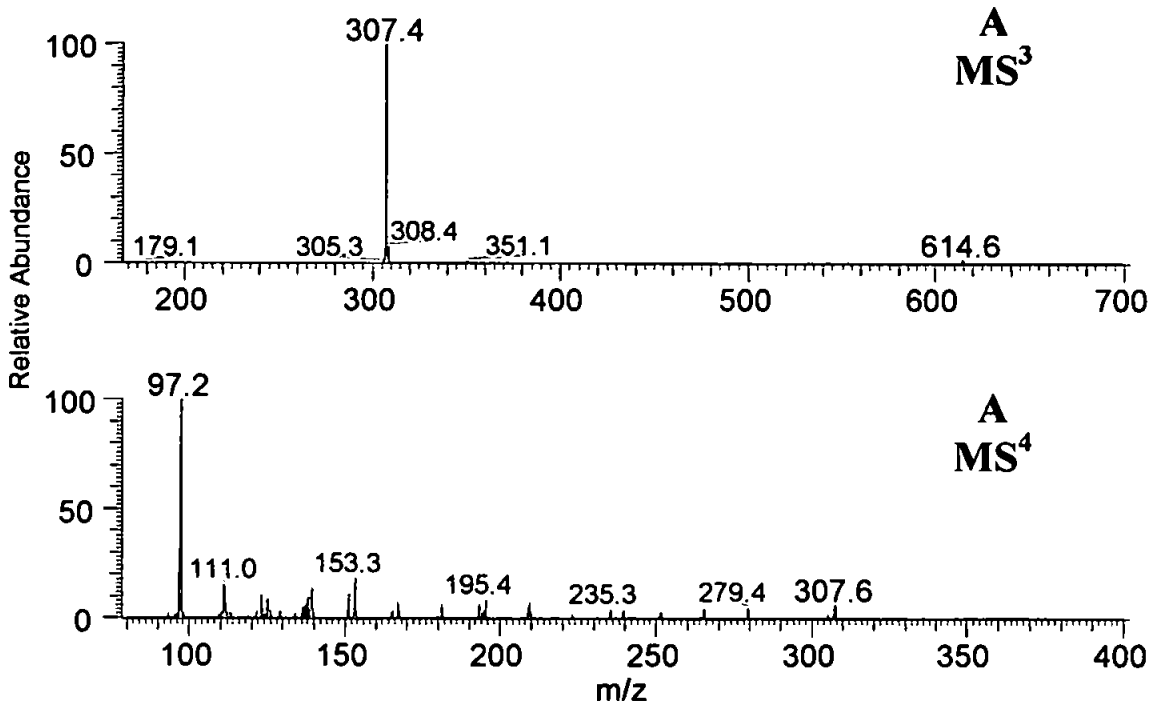


Figure A.6 ESI-MS^n mass spectra of the oleic-diamide MS^2 product ion (*m/z* 614.6) in the MS^2 spectrum Figure A.3. **(A)** MS^3 on precursor ion *m/z* 6.14.6 (41 % AA), **(B)** MS^4 on product ion *m/z* 307.4 (51 % AA).

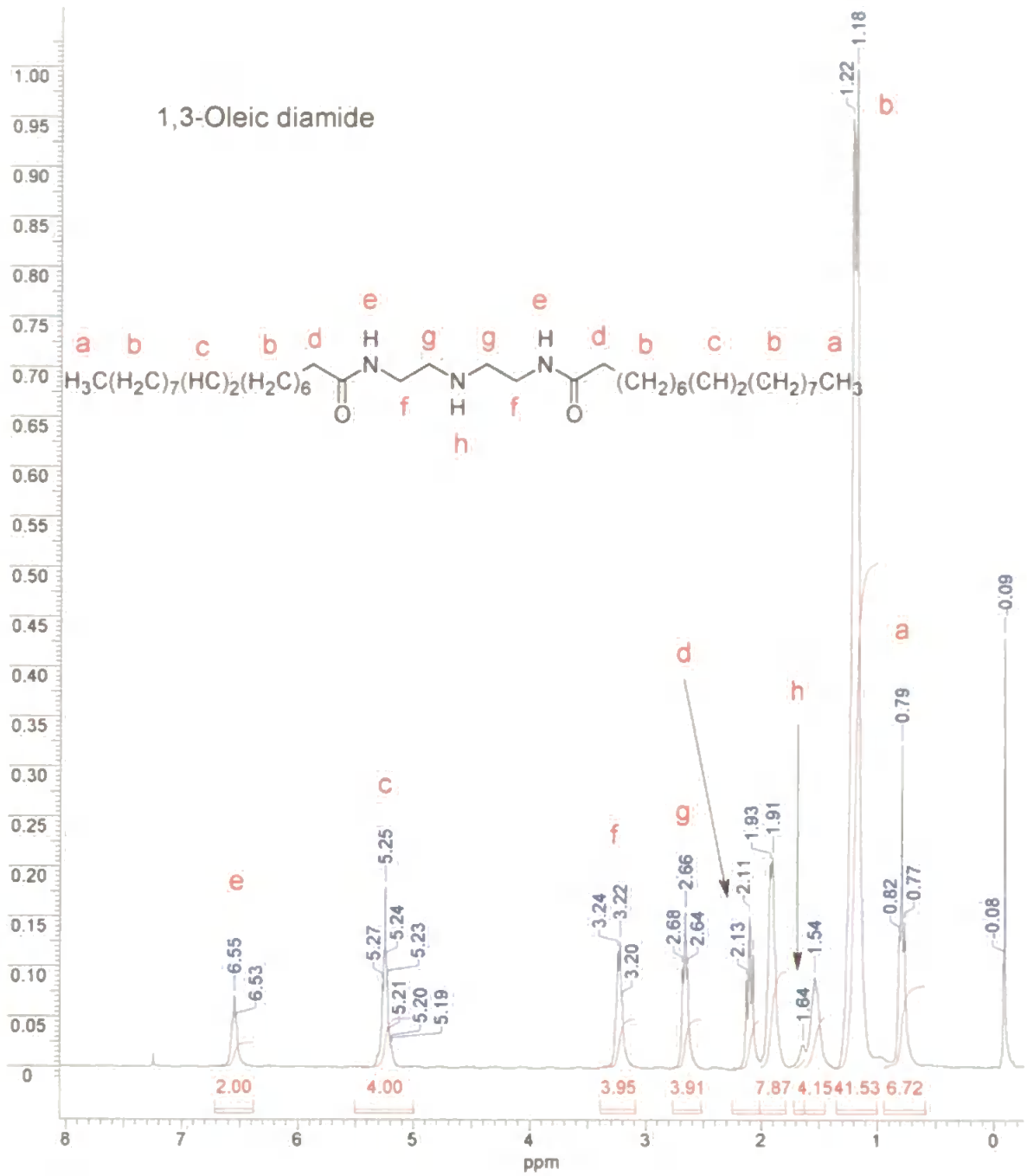


Figure A.7 ^{1}H NMR spectrum of the 1,3-oleic diamide product -diamide formed from hydrolysis (during storage) of 2:1-oleic imidazoline (Chapter 4, section 4.3.8). $CDCl_3$, 270 MHz.

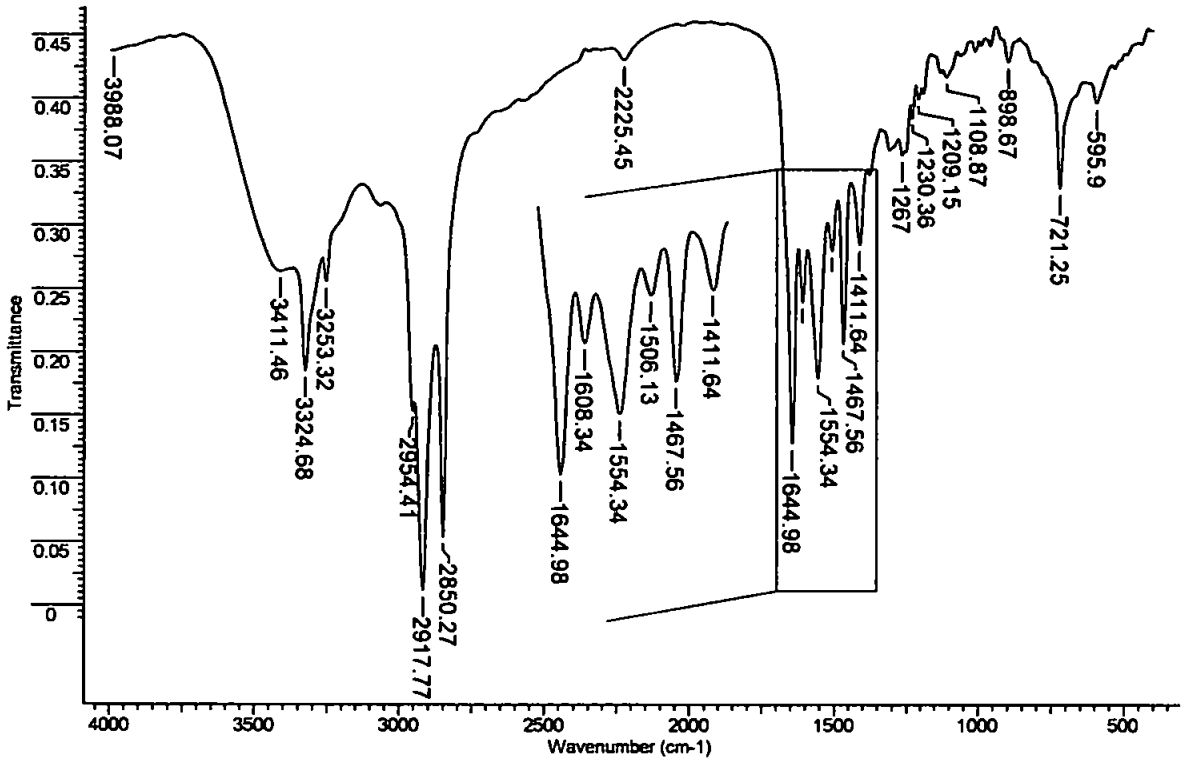


Figure A.8 Fourier transform infrared spectrum of the crude 2:1-palmitic imidazoline product of thermal reaction of palmitic acid with DETA in xylene solvent (Chapter 5; Section 5.2.4): (υ/cm^{-1}); 1645, 1550 (-NH-C=O), 1608 (C=N).

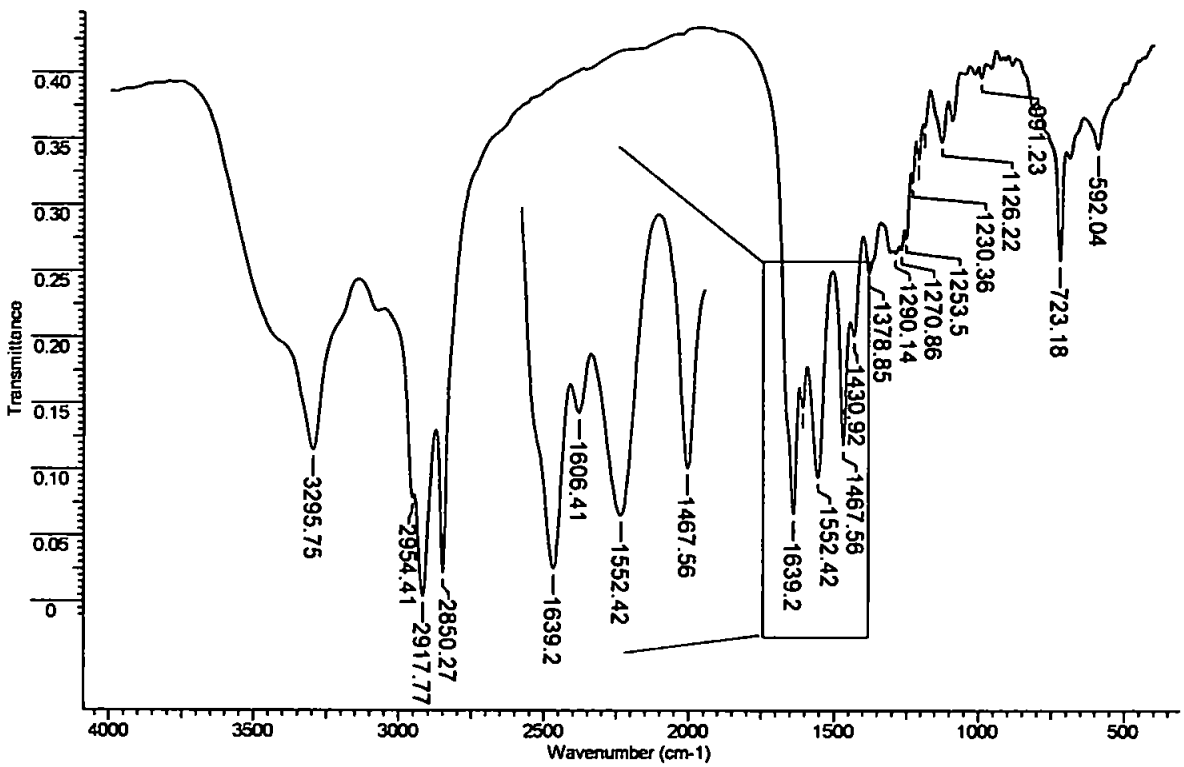


Figure A.9 Fourier transform infrared spectrum of the recrystallised 2:1-palmitic imidazoline product of thermal reaction of palmitic acid with DETA in xylene solvent (Chapter 5; Section 5.2.4): (υ/cm^{-1}); 1639, 1552 (-NH-C=O), 1606 (C=N).

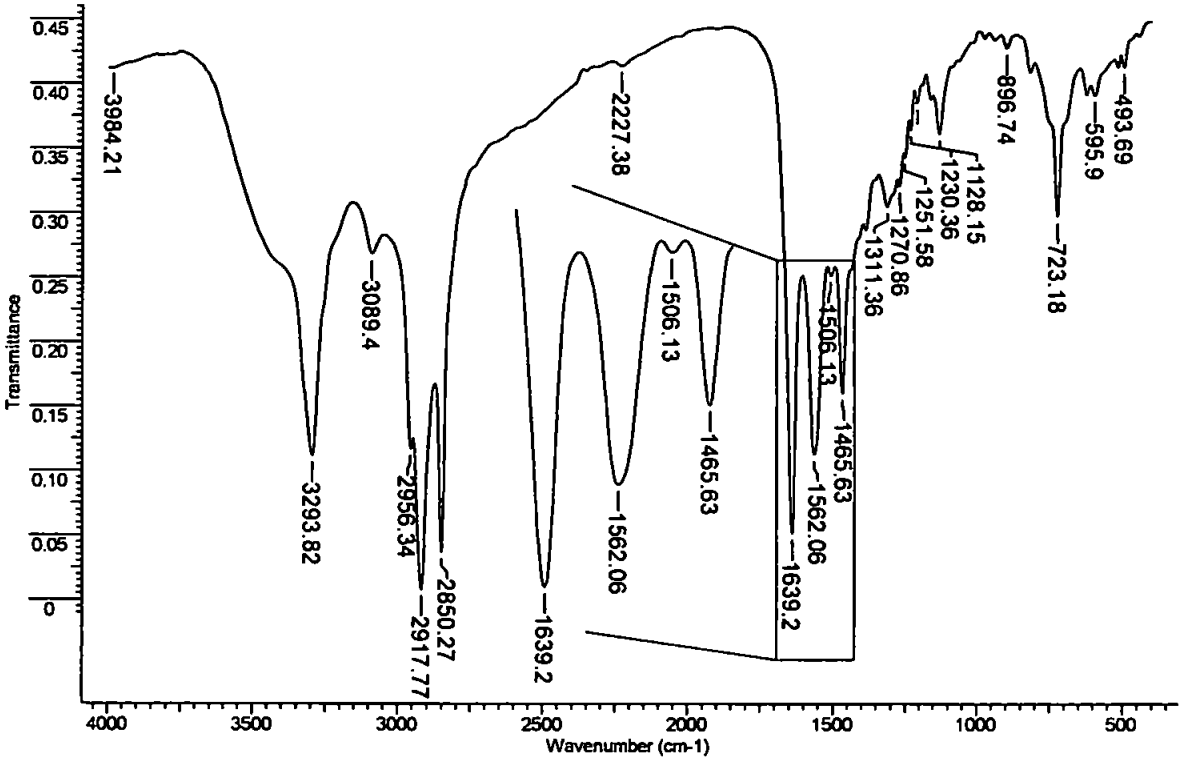


Figure A.10 Fourier transform infrared spectrum of the crude palmitic-diamide product of solid phase thermal reaction of palmitic acid with DETA (Chapter 5; Section 5.2.5): (υ/cm^{-1}); 1639, 1562 (-NH-C=O).

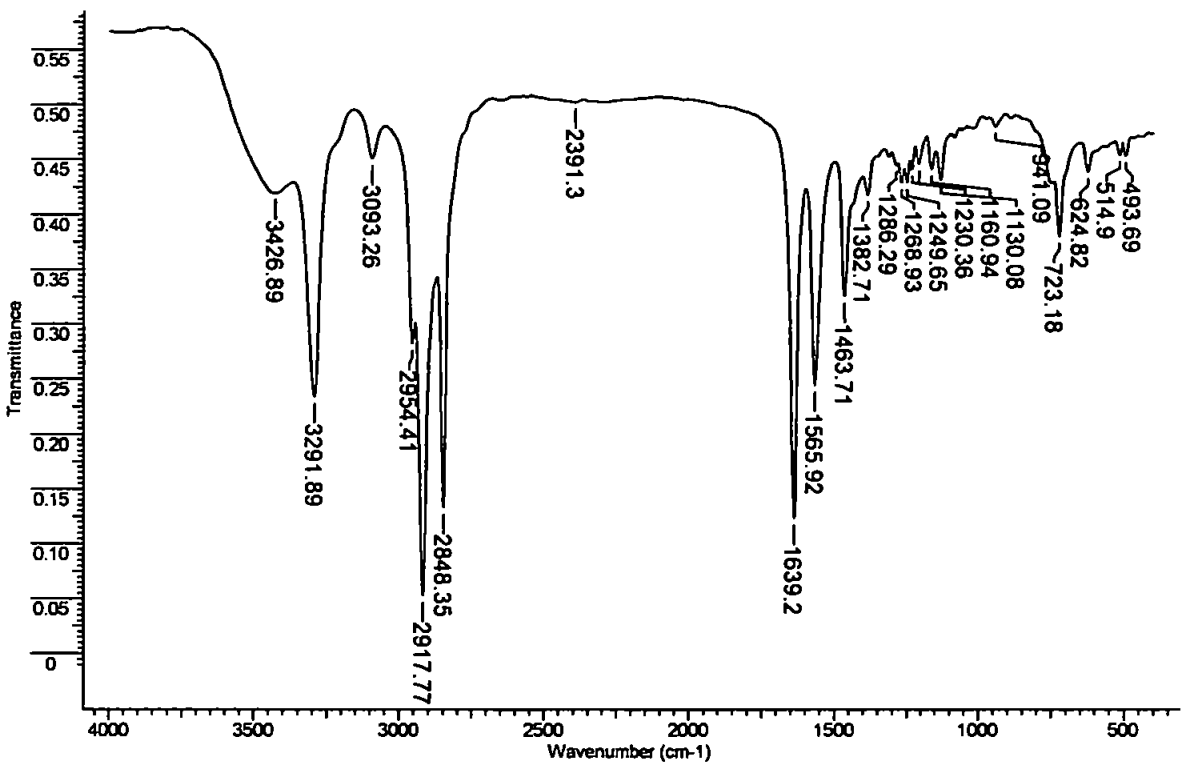


Figure A.11 Fourier transform infrared spectrum of the recrystallised palmitic-diamide product of solid phase thermal reaction of palmitic acid with DETA (Chapter 5; Section 5.2.5): (υ/cm^{-1}); 1639, 1566 (-NH-C=O).

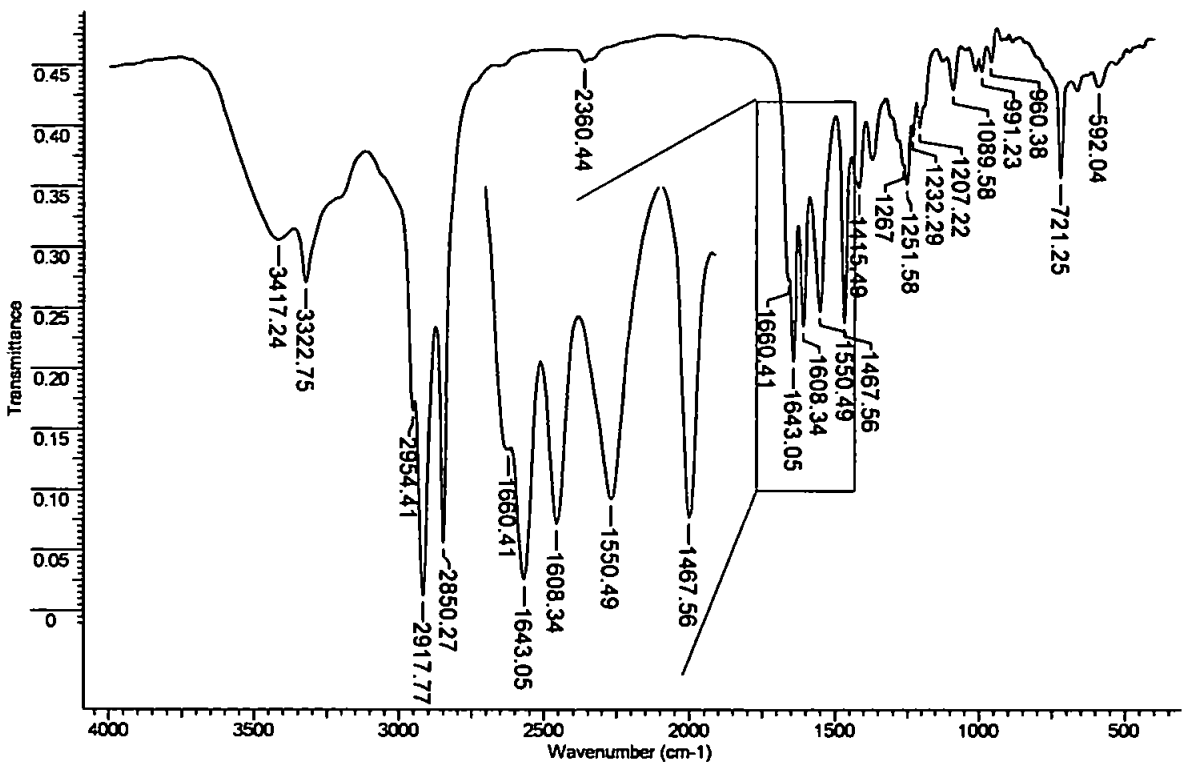


Figure A.12 Fourier transform infrared spectrum of the recrystallised 2:1-palmitic imidazoline product of solid phase thermal reaction of palmitic acid with DETA (Chapter 5; Section 5.2.5): (υ/cm^{-1}); 1643, 1550 (-NH-C=O), 1608 (C=N).

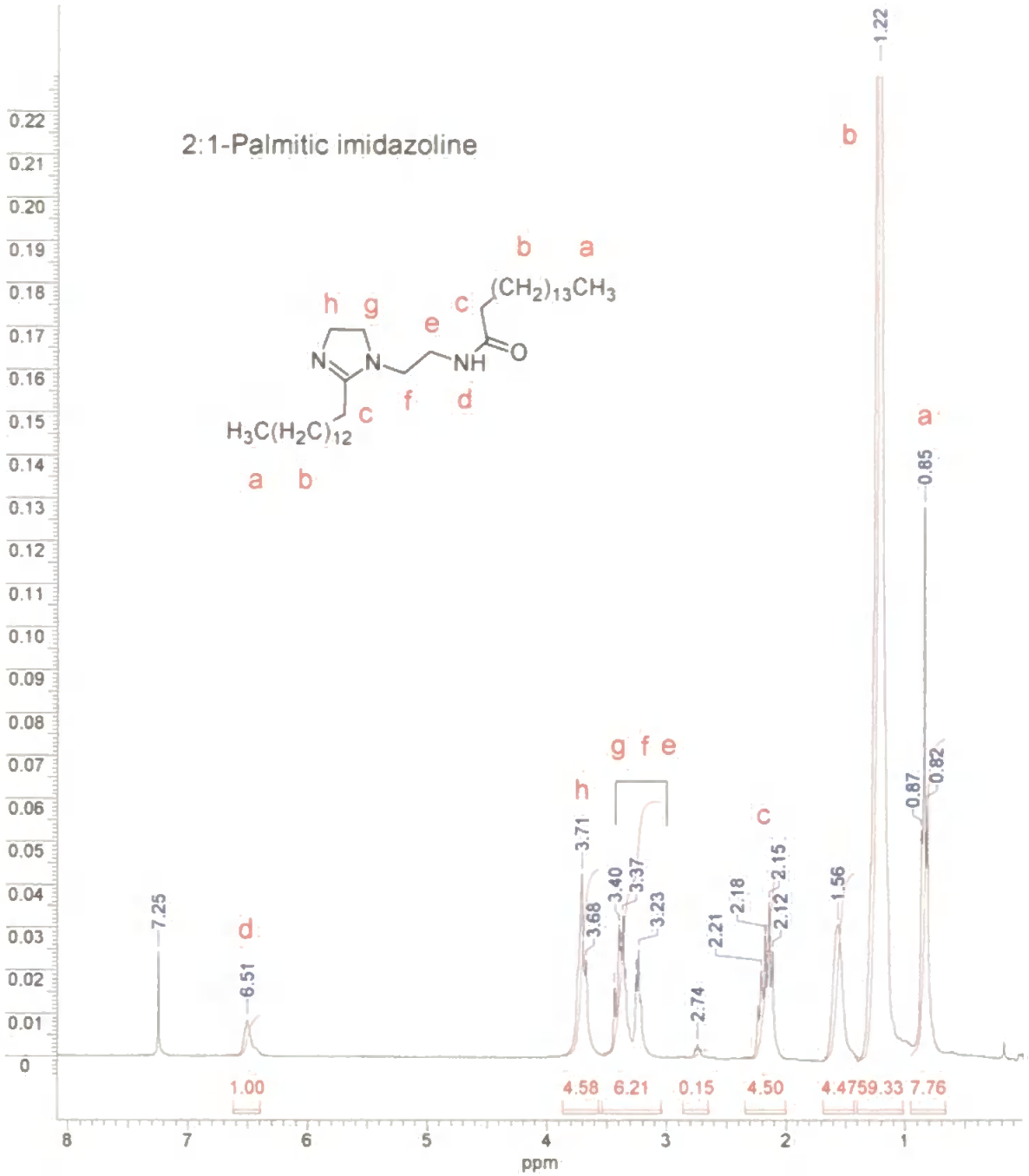


Figure A.13 ^{1}H NMR spectrum of the 2:1-Palmitic imidazoline product the solid phase synthesis (Section 5.2.5). $CDCl_3$, 270 MHz.

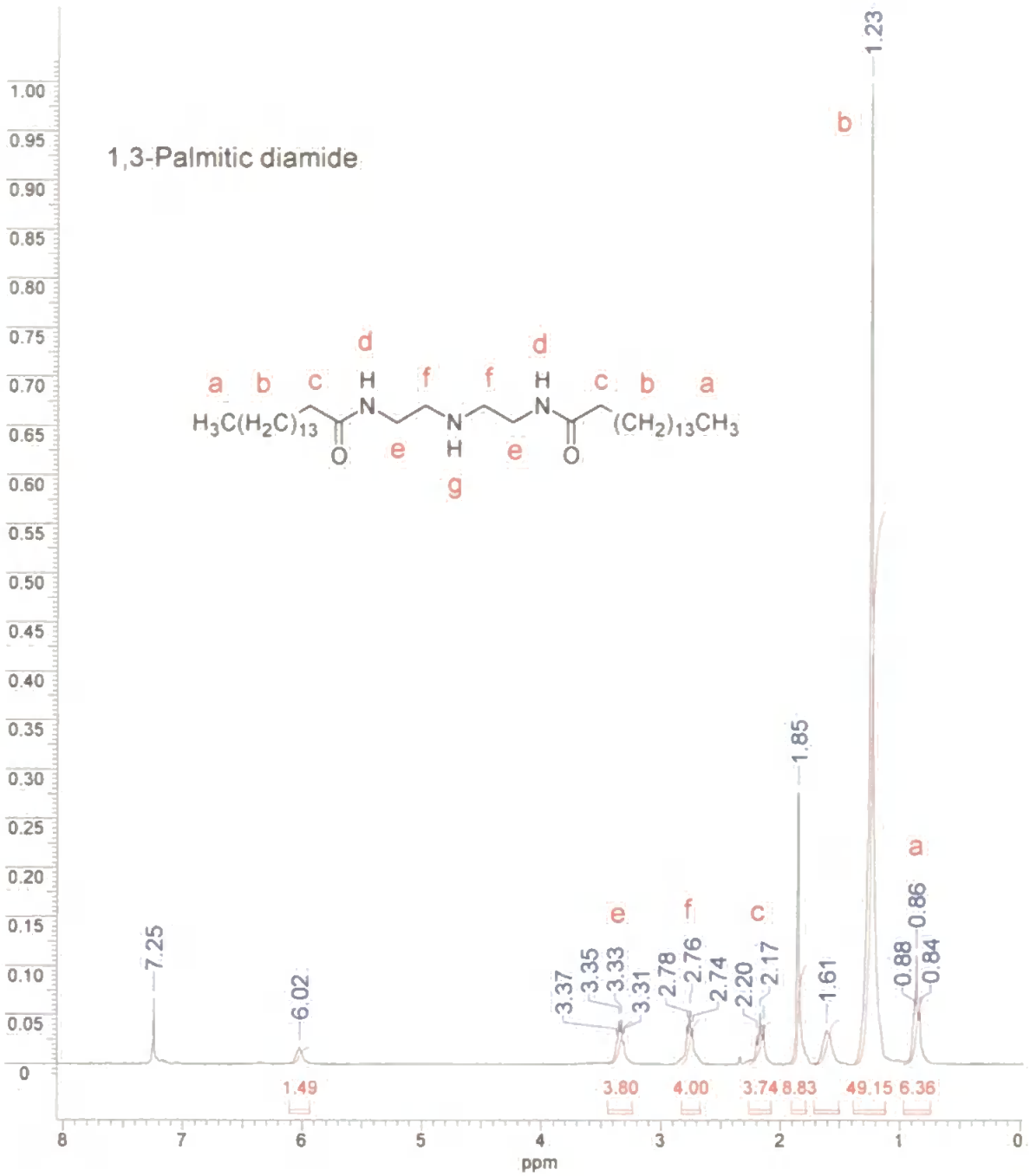


Figure A.14 ^{1}H NMR spectrum of the 1,3-Palmitic diamide product of solid phase synthesis (Section 5.2.5). $CDCl_3$, 270 MHz.

RESEARCH & DEVELOPMENT
BULLETIN
AstraZeneca
Global Safety, Health & Environment Department
Number 100 – September 2002

Analysis of Highly Polar Low Molecular Weight Organic Molecules: Part 1 - HPLC Separation using a Novel Hypercrosslinked Resin

Background

The aim of this industry sponsored PhD project was to research new approaches to the problem of pre-concentration, separation and identification of polar or ionisable organic molecules from aqueous media.

The practical work, which has now been completed, was divided into two areas of study: The investigation of novel materials for the isolation and separation of model compounds, and the use of multi-stage mass spectrometry techniques to provide both molecular weight and specific structural information for the identification of unknowns in complex mixtures.

Examples of the latter are described in a second R&D bulletin (Ref 101).

The development of HPLC methods involving reverse phase silica gel columns was a major advance in solving the difficult problem of separating polar organic molecules with high efficiency. However, many low molecular weight highly polar molecules, are still difficult to separate on commonly available phases such as octadecyl (C18) bonded silica, even in highly aqueous mobile phases. One reason for this is the relatively weak hydrophobic interaction between the polar molecule and the C18 bonded layer, dihydroxybenzenes, (Figure 1, compounds 2, 4 and 5) being a typical example, producing very short retention times.

Polymeric phases, based on neutral polystryrene resins, have been investigated as alternative reverse phase substrates to bonded silica gels for polar molecule separations.

Although in some cases there has been an improvement in separations, many of these polymeric phases give asymmetrical peak shapes with poor resolution. Nonetheless, a recent development involving hypercrosslinked polystyrene resins appears to have overcome this problem and shows great potential for the efficient separation of highly polar compounds.

Normally, a high degree of crosslinking would make a dense non-porous substrate with poor separating capabilities.

However, a special polymerisation process, first pioneered in Russia, produces highly porous particles even when the degree of crosslinking approaches 100%.

A research collaboration with Moscow University provided a number of novel phases for investigation. One of these phases, a hypercrosslinked resin called MN200 (originally used for the large scale treatment of waste waters containing polar compounds), was found to have an unusually strong affinity for a range of highly polar phenolic compounds. The use of this phase with an LCMS-compatible water/methanol mobile phase provided very promising high efficiency separations without the usual requirement for inorganic buffers.

Separation of phenols using MN200 column

An example of the isocratic separation of eight phenolic compounds is shown in Figure 1. These

straZeneca Global Safety Health & Environment Department Brixham Devon TQ5 8BA UK
el +44 1803 882882 Fax +44 1803 882974 Intranet website global.she.astrazeneca.net

compounds are usually poorly retained and therefore difficult to resolve by conventional reverse phase LC.

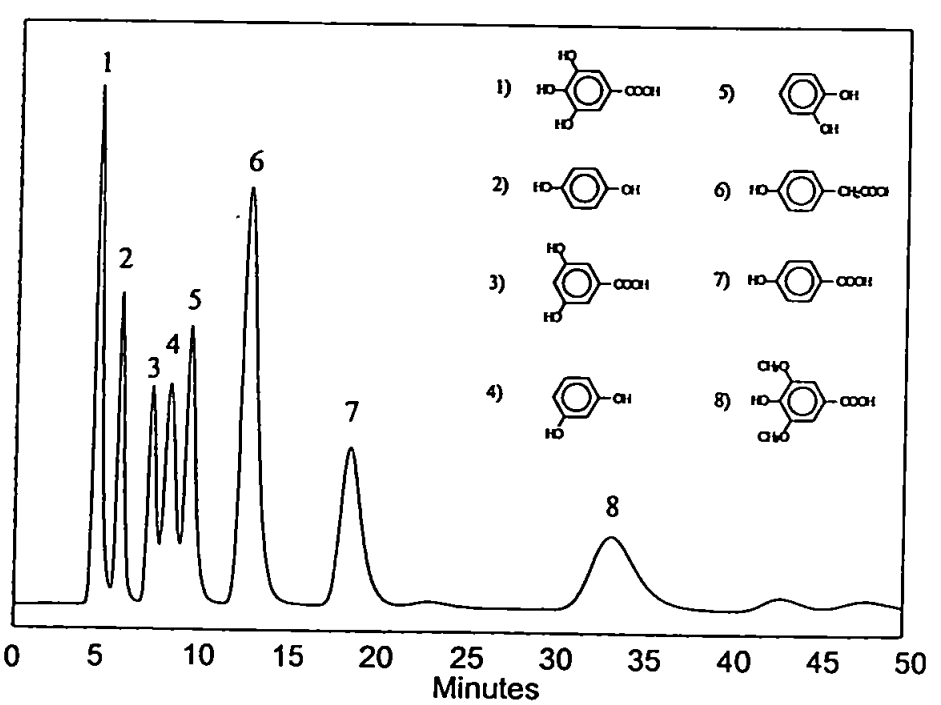
The separation is exceptional in that good retention and resolution was obtained with a high degree of symmetry in peak shape, using a short column (100 mm). The separation was achieved with high percentages of methanol, which is beneficial to LC-MS sensitivity, a technique of major importance for identifying unknown polar compounds in environmental water samples.

Gradient elution could be used to decrease the retention time and peak width of the later eluting peaks (6-8) and may be considered to separate much more complex mixtures of polar compounds.

One of these materials, the hypercrosslinked resin MN200, was found to have an unusually strong affinity for highly polar phenolic compounds and provided an improved separation when compared to existing methods. The use of MN200 as a SPE packing material for the extraction of polar organics from aqueous samples should be evaluated in a future project.

Additionally, because polar compounds are highly retained when using a mainly aqueous mobile phase, the MN200 should be ideally suited for use as a solid phase extraction (SPE) packing material for the extraction/concentration of polar organics from aqueous samples.

Figure 1. The separation of eight phenolic compounds using a 5 µm MN200 (100 x 2.1 mm) column at a flow rate of 0.15 ml min^{-1}



Conclusion

Collaboration with the Moscow University allowed the evaluation of novel LC packings, previously unavailable in Europe. One of these materials, the hypercrosslinked resin MN200, was found to have an unusually strong affinity for highly polar phenolic compounds and provided an improved separation when compared to existing methods. The use of MN200 as a SPE packing material for the extraction of polar organics from aqueous samples should be evaluated in a future project.

This bulletin was prepared by Malcolm Hetheridge, Paul McCormack[1], Phil Jones[1] and Prof. Pavel Nestorenko[2]. [1] University of Plymouth and [2] Moscow University as part of the AstraZeneca Global SHE Research & Development Programme

RESEARCH & DEVELOPMENT
BULLETIN
AstraZeneca
Global Safety, Health & Environment Department
Number 101 – September 2002

Analysis of Highly Polar Low Molecular Weight Organic Molecules: Part 2 - New Methods for the Identification of Polar Chemicals in Complex Mixtures

INTRODUCTION

The use of liquid chromatography-mass spectrometry (LC-MS) has increased in importance over recent times. Unfortunately though, LC-MS spectra often only provide the molecular weight of an unknown chemical and reveal little structural information. Methods such as LC-MS-MS are more powerful and in our studies we have employed the latest multistage MS techniques.

Multiple stage mass spectrometry (MS^n) with both positive and negative ion detection allows high specificity detection and characterisation of a wide range of polar and charged molecules. For example, linear alkylbenzenesulfonates (LAS), alkyldimethylbenzylammonium compounds, the previously poorly characterised corrosion inhibitors, 2-alkylimidazolines and a series of di-[alkyldimethylammonium-ethyl]ethers, were all identified and characterised in commercial formulations and/or marine 'produced' waters (Ref 1 and 2). The technique will benefit the future study of environmental fate and effects of these and similar polar compounds.

EXAMPLES

Two features of the LC-MS analysis: zoom scan and MS^n are illustrated here:

A positive ESI full scan mass spectrum of a sample containing alkyl di-quaternary salt, showed two distinctive odd *m/z* ion series, Figure 1. The ions in the most intense series, centred on *m/z* 249, differed from each other by *m/z* 14, whilst those in the series around *m/z* 533 differed by *m/z* 28. The charge state of ions in each series were elucidated by the use of the so-called 'ZoomScan'. This allows resolution of isotope peaks for up to +4 charge-state ions. Thus, ions in the first series *eg* *m/z* 249.4 showed mass differences of 0.5 Da (Fig. 1B) indicating these to be doubly charged ions ($M^{2+}/2$). By contrast, analysis of the higher mass ion series eg *m/z* 533.4 showed a mass differences of 1.0 Da (Figure. 1C) consistent with singly charged ions ($M^{+}/1$). This quickly allowed the molecular weight of these compounds to be determined. Structures were subsequently identified by $LC\text{-}MS^n$ analysis.

Figure 1. Mass Spectrum and 'Zoom Scan' Spectra of quaternary amine salts

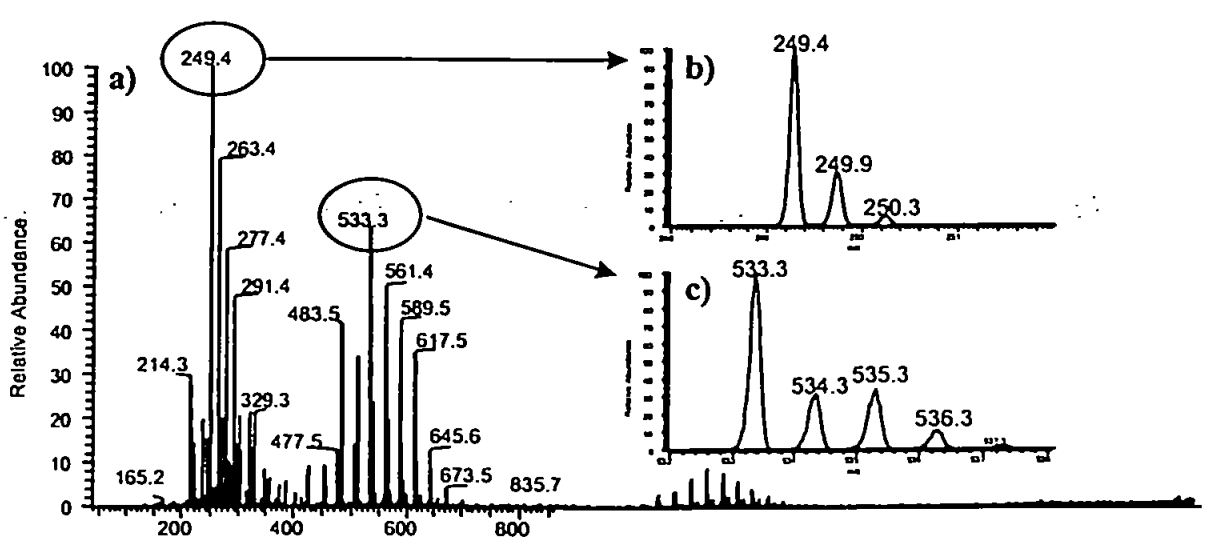


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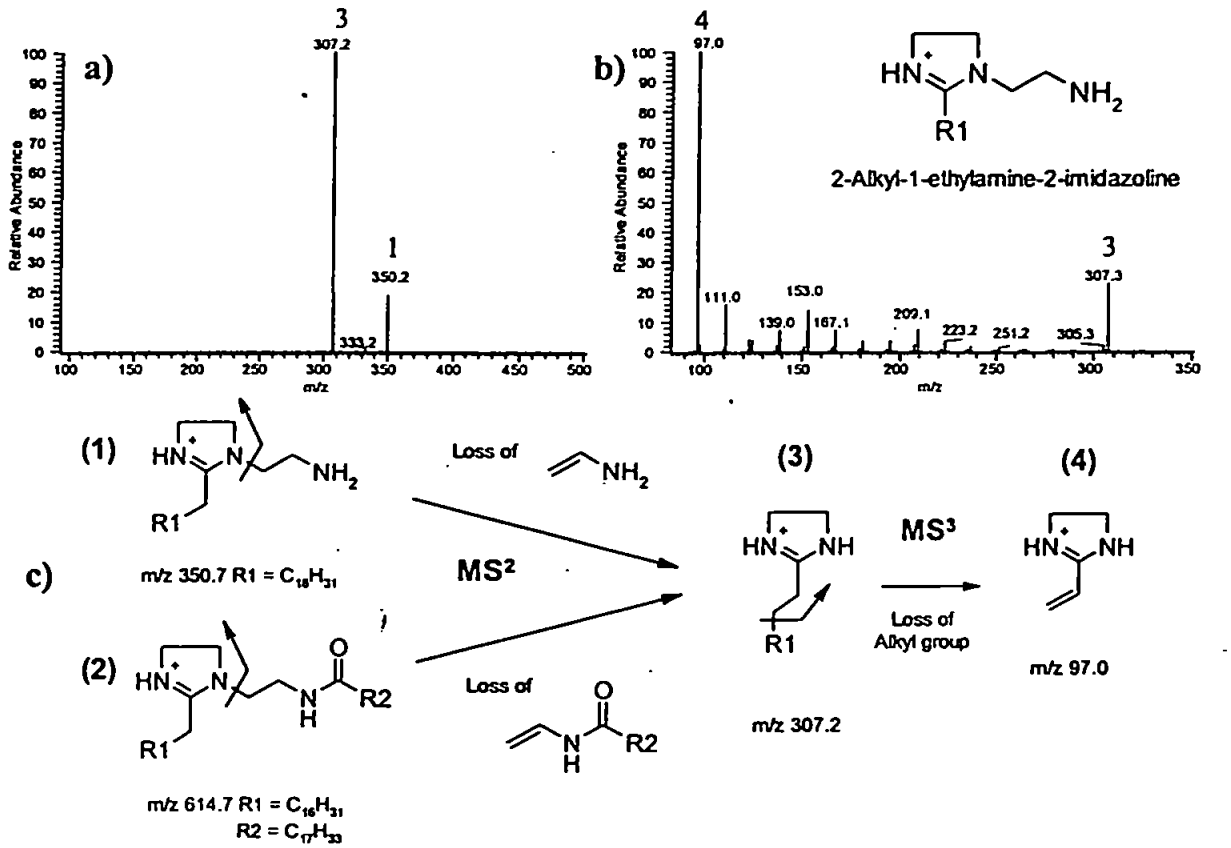
Figure 2. MS^n spectra and Fragmentation pathways for Imidazoline analogues



The LC-MS^n capability is illustrated by the characterisation of the highly polar, "sticky", 2-alkylimidazoline corrosion inhibitors (Figure 2), identified in formulated product and in environmental samples.

The molecular ion, in this example m/z 350, is fragmented to m/z 307 (Figure 2a) by loss of ethenamine (43 Da). The fragment ion, m/z 307 is then isolated and fragmented to give further structural information eg loss of alkyl side chain, Figure 2b. In this way, a fragmentation pathway can be developed (Figure 2c), to allow a firm identification to be made.

Few other techniques provide this level of information for complex mixtures of polar chemicals at sub parts per million concentrations, and therefore the LC-MS^n method promises to be a boon to analytical chemists for the characterisation of many polar chemicals.

Conclusion

An additional benefit from this study was the considerable expertise gained in the interpretation of sequential fragmentation patterns provided by the MS^n technique. The development of this particular MSMS expertise at Plymouth University, will provide an invaluable foundation for future collaborative projects.

References

1 McCormack *et al* (2001). *Wat. Res.* 35 (15) 3567-3578.

2 McCormack *et al* (2002). *Rapid com. mass spec* 16, 705-712.

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[1] University of Plymouth as part of the AstraZeneca Global SHE Research & Development Programme.

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Liquid chromatography/electrospray ionisation mass spectrometric investigations of imidazoline corrosion inhibitors in crude oils

P. McCormack, P. Jones and S. J. Rowland*

Petroleum and Environmental Geochemistry Group, Department of Environmental Sciences, Plymouth Environmental Research Centre, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK

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The so-called imidazolines (2-alkyl-1-[ethylalkylamide]-2-imidazolines and 2-alkyl-1-ethylamine-2-imidazolines) are a group of surface-active compounds, complex mixtures of which are used by various industries as surfactants and corrosion inhibitors. Although their industrial synthesis was reported over 100 years ago, few methods for the determination of individual imidazolines in mixtures, including industrial matrices such as crude oils, have been reported. Here we demonstrate that spiking of crude oils with synthetic imidazolines followed by solid-phase extraction and liquid chromatography/electrospray ionisation multistage mass spectrometry (LC/ESI – MS^n) allows an estimation of low (<10) parts per million concentrations of individual imidazolines in crude oils. Whilst non-optimised at present, the method is a significant advance and may prove useful not only for improving an understanding of the mechanisms of industrial imidazoline synthesis and for monitoring downhole and topside oilfield operations, but also for the determination of the fate of imidazoline-based oilfield corrosion inhibitors and surfactants in the environment.

The advent of electrospray ionisation mass spectrometry (ESI-MS) has seen application of the technique to the identification of a growing number of polar chemicals in environmental and industrial matrices.[1-5] Coupled with liquid chromatography (LC), ESI-MS has been used for the determination of chemicals as diverse as positively charged quaternary benzalkonium chlorides ('Quats') and anions of linear alkylbenzene sulphonates ('LAS'). For example, Gough and Langley[3] demonstrated application of LC/ESI-MS techniques to the detection of a variety of polar organics in oilfield chemical formulations and oilfield produced water (PW) including Quats (used as biocides) and corrosion inhibitors such as the so-called imidazolines (2-alkyl-1-[ethylalkylamide]-2-imidazolines (e.g. I, III) and 2-alkyl-1-ethylamine-2-imidazolines (II)). Used with multistage ion trap MS (MS^n), we were able to employ electrospray ionisation, not only in the detection of Quats, LAS and imidazolines in oilfield chemicals and PW, but were also able to assign unknowns such as a di(alkyldimethylammonium ethyl) ether in the mixtures by elucidation of the MS fragmentation pathways in up to five MS steps[6] (viz: ESI-MS^5). Clearly, ESI-MS^n has great potential for the determination of numerous chemicals which were previously outside the analytical window of most methods.

*Correspondence to: S. J. Rowland, Petroleum and Environmental Geochemistry Group, Department of Environmental Sciences, Plymouth Environmental Research Centre, University of Plymouth Drake Circus, Plymouth PL4 8AA, UK.
E-mail: srowland@plym.ac.uk

However, although detection of oilfield chemicals has been reported, few authors have reported ESI-MS methods for the quantitation of polars such as imidazolines;[3,7] and there are no reports of their determination in complex industrial matrices such as crude oil. For imidazolines this is important for the assessment of the efficiency of both downhole inhibitor addition (so-called squeezing)[8] and to topside operations where control of dosing rates for the protection of metalwork reduces over-dosing and consequent economic and environmental costs. Whilst methods such as thin-layer chromatography have been used previously to detect imidazolines in crude oils,[8] such methods at best only allow total imidazolines to be determined.

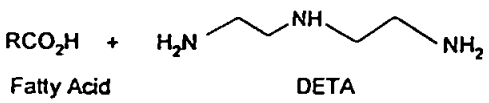
In the present study we report the use of multistage LC/ESI-MS for the identification and estimation of individual imidazolines in crude oils. We have synthesised individual imidazolines and used these to dose crude oil at known concentrations to try to measure the efficiency of the recovery of the compounds by solid-phase extraction (SPE) methods and to calibrate the LC/ESI-MS responses of the individual compounds.

MATERIALS AND METHODS

Chemicals

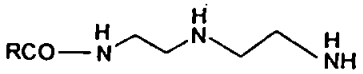
Ultrapure water was obtained from an Elgastat filtration system (Elga, High Wycombe, UK). All solvents used were HPLC grade except glass-distilled diethyl ether (ether; Rathburn Walkerburn, UK and BDH, Poole, UK). Hyper-

REACTANTS

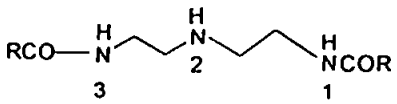


Fatty Acid DETA

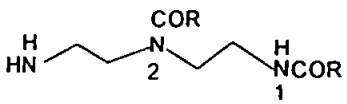
INTERMEDIATES & PRODUCTS



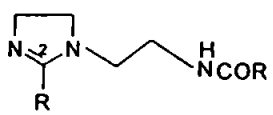
IV Monoamide



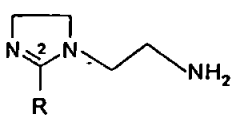
V 1,3 Diamide



VI 1,2 Diamide



I R=n-$C_{15}H_{31}$ III R=n-$C_{17}H_{33}$ "2:1 imidazolines"



II "1:1 imidazolines"

solv-grade methanol (MeOH), propan-2-ol (IPA), ammonia (0.88), trifluoroacetic acid (TFA) and xylene (sodium-dried) were from BDH. Dichloromethane (DCM), ethanol (EtOH), hexane, acetone and toluene were from Rathburn. Sodium acetate (AcNa, 99%) was Aristar grade from BDH. Diethylenetriamine (DETA, 98.5%) was supplied by ACROS Organics, Ltd, (Geel, Belgium) and palmitic and oleic acids (90%) were from Aldrich Chemical Company Inc., (Milwaukee, WI, USA).

The crude oils were Middle Eastern crudes. The corrosion inhibitor active ingredients were from a commercial supplier.

Synthesis of 2-alkyl-1-[ethylalkylamide]-2-imidazolines

2-Pentadecyl-1-[*N*-ethylhexadecylamide]-2-imidazoline (2:1-PI; I) and 2-heptadecenyl-1-[*N*-ethyloctadecenylamide]-2-imidazoline (2:1-OI; III) were synthesised by both solid-phase and solvent-based reactions from palmitic and oleic acids and DETA by previously published methods.[9,10] Stock solutions were prepared in MeOH and diluted as required. Calibration solutions were prepared by serial dilution.

Solid phase extraction (SPE)

Imidazolines were isolated from hexane or from crude oils (1 g) dissolved in hexane (1 mL) by use of an Isolute vacuum manifold and Isolute Si SPE cartridges (1 g × 6 mL). Briefly the procedure was: hexane preconditioning, sample loading, hexane elution, ether elution, vacuum drying (few seconds), imidazoline elution with IPA/ammonia (*cf.* Ref. 8). Imidazoline fractions were blown to dryness (nitrogen), reconstituted to 1 mL and transferred to 2-mL glass autosampler vials via a 1-mL glass syringe and expelled through a 0.2-μm PVDF syringe filter.

High performance liquid chromatography (LC)

LC was carried out using a P580A binary pump (Dionex-Softron GmbH, Germering, Germany) with 1 mL min^{-1} flow rate, split ~200 μL high pressure micro-splitter valve (Upchurch Scientific Ltd, Oak Harbor, WA, USA) to the mass spectrometer and the residue to a UV6000LP diode array detector (Thermoquest, San Jose, CA, USA). 20-μL sample injections were made with an ASI 100 autosampler (Dionex-Softron GmbH, Germering, Germany). A gradient elution was carried out as described previously using modified MeOH and water solvent and a 5-μm, 50 × 4.6 mm reverse phase column.[3]

Mass spectrometry (MS)

Mass spectrometry was carried out using a ThermoQuest Finnigan Mat LCQ (San Jose, CA, USA) ion trap mass spectrometer fitted with an electrospray interface. Data were acquired and processed with Xcalibur 1.0 software. Instrument tuning and mass calibration was carried out and checked using the automatic calibration procedure (tuning and calibration solutions caffeine (Sigma, St. Louis, MO, USA), MRFA (Finnigan Mat, San Jose, CA, USA) and Ultramark 1621 (Lancaster Synthesis Inc., Widham, NH, USA) in methanol/water/acetic acid (50:50:1, v/v/v)). Instrument method optimisation was carried out by infusing 1M sodium acetate at 1μL min^{-1} into a 200 μL min^{-1} eluent flow from the LC system by way of the built-in syringe pump, a Hamilton 1725N (250 μL) syringe (Reno, CA, USA) and a PEEK Tee union (Upchurch Scientific Ltd, Oak Harbor, WA, USA). The automatic tune function was used on a suitable sodium trifluoroacetate adduct ion. For the positive ion full scan range m/z [100–1000] tuned on adduct ion m/z 563 the following instrument parameters were used: source voltage, +4.5 kV; capillary voltage +20V; tube lens offset, +10.00V; capillary temperature, 220°C; nitrogen sheath gas flow rate, 60 (arbitrary units) and nitrogen auxiliary gas 20 (arbitrary units).

RESULTS AND DISCUSSION

Synthesis of 2-alkyl-1-[ethylalkylamide]-2-imidazolines

Ever since the initial synthesis of 'imidazolines' described by Hofmann[11] in 1888, there has been debate concerning the mechanism of the reaction between fatty acids and triamines.[9,10,12,13] Indeed, even the existence of the compounds has been questioned.[14] Yet the subject is of major importance to the efficient deployment of anti-corrosion measures since

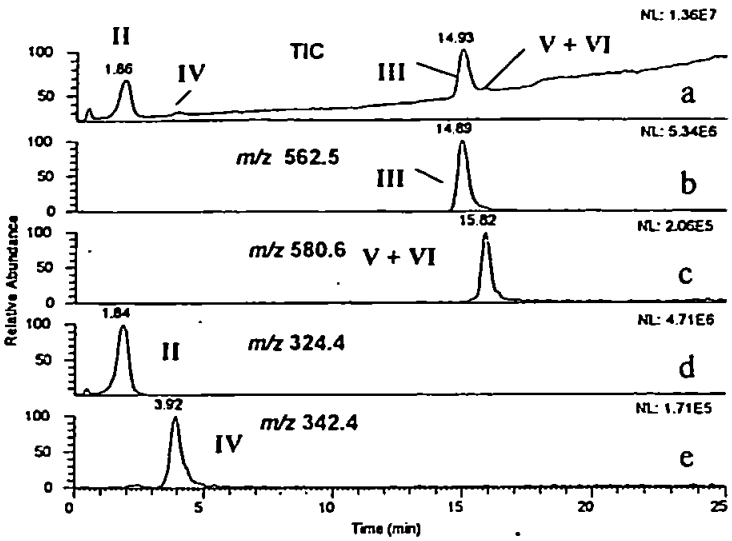


Figure 1. LC/ESI-MS mass chromatograms illustrating the composition of the crude reaction products of imidazoline synthesis (7h; boiling xylene);[10] reactants oleic acid and diethylenetriaine (DETA), i.e. R = $C_{17}H_{33}$). Mass chromatograms are: (a). Total ion current illustrating presence of all products II–VI. (b)–(e). Molecular ion mass chromatograms for III, V + VI, II and IV, respectively. The presence of co-eluting diamides can be demonstrated by MS^2 and MS^3 as shown in Fig. 2. R = $C_{17}H_{33}$.

the composition of the synthetic mixtures controls their self-assembly as monolayers on metal surfaces and hence their efficacy.[15]

Much of the confusion seems to have arisen from the existence of different mechanisms in the solid-phase and solvent-based reactions.[10] However, attempts to elucidate the mechanisms have also been hampered by the lack of sensitive, specific methods for identification of the reactants, intermediates and products in the complex mixtures resulting from such reactions. Thus, even recent studies[10] have required isolation and recrystallisation of the chemicals at each stage, followed by spectroscopic examination by nuclear magnetic resonance spectroscopy and other techniques. Powerful though these methods are, they are not sensitive to (perhaps important) minor products and intermediates, most of which are in any case removed by recrystallisation procedures. This may have led to some of the misleading conclusions concerning the mechanisms. It is not the purpose of this report to detail our investigations of the mechanisms, which will reported elsewhere, but an example of an LC/ESI-MS mass chromatogram of a reaction mixture will suffice to illustrate the utility of the present method. Thus, Fig. 1 shows the presence of monoamide [IV], so-called 1,3- [V] and 1,2-diamides [VI]* and 2-alkyl-1-ethylamine-2-imidazolines [II] in addition to the 2-alkyl-1-[ethylalkylamide]-2-imidazolines [e.g. III] in the crude synthetic products of the Dean and Stark reaction between oleic acid and DETA after 7 h. The identities of the major compounds were established either by co-chromatography with the isolated, recrystallised products, which were also characterised by infrared spectroscopy, or by interpretation of the multistage collision induced mass spectra, as we have reported previously.[6]

As an illustration, Fig. 2(a) shows the MS^1 mass spectrum of the reaction products of palmitic acid and DETA. Figure 2(b) shows the MS^2 CID mass spectrum of *m/z* 580.6 which is the mass expected for the 1,3- and 1,2-diamides [V,VI]. Although both amides are isobaric, only the 1,2-diamide can lose ammonia by MS^2 CID to give the *m/z* 563.5 product ion observed in Fig. 2(b). MS^3 CID of the *m/z* 563.5 involves an approximately symmetrical cleavage to *m/z* 282.3 (Fig. 2(c)), which on MS^4 CID produces a *m/z* 95.2 ion (Fig. 2(d)). The *m/z* 562.5 MS^2 CID product ion also observed in Fig. 2(b) (inset) can be accounted for by loss of water and cyclisation to form the quite stable protonated 2-alkyl-1-[ethylalkylamide]-2-imidazoline ion. Indeed, this is confirmed by MS^3 and MS^4 CID spectra (spectra not shown) product ions corresponding to our previously proposed ion trap CID fragmentation pathway for 2-alkyl-1-[ethylalkylamide]-2-imidazoline.[6] An ion at *m/z* 562.5 is also observed as the base peak in the MS^3 CID spectrum (Fig. 2(c)), which might be construed as evidence of loss of a hydrogen radical from *m/z* 563.5 (and therefore loss of a hydroxyl radical rather than ammonia from the diamide parent). However, loss of hydrogen radical following loss of hydroxyl was ruled out on the basis of further detailed MS experiments which are not discussed here. We suspect the *m/z* 562 ion is in fact not a CID product ion at all since it was present at 0% activation amplitude (AA) and the relative abundance remained constant at 31, 33 and (the

* The trivial names 1,2- and 1,3- diamides are in common use. The numbers refer to amide formation at the first and second or first and third nitrogen atoms, respectively, as shown in structures V and VI.

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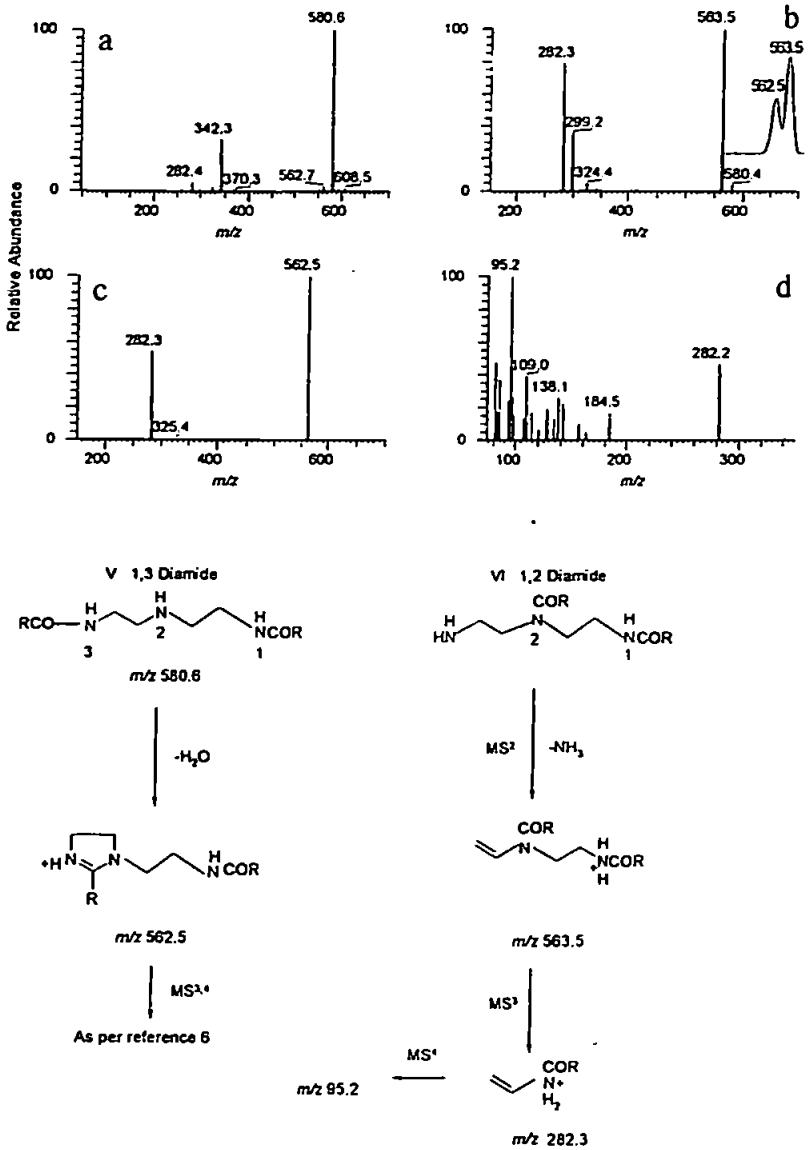

Figure 2. Multistage mass spectra and proposed fragmentation pathway of compounds assigned as 1,2- and 1,3-diamides [*m/z* 580.6; VI, V]. The diamides were re-crystallised from the solid-phase thermal reaction products of palmitic acid with DETA (i.e. R = $C_{15}H_{31}$). (a). Full scan *m/z* 50–700 mass spectrum of mixture of co-eluting 1,2- and 1,3-diamides (VI,V). (b). MS^2 spectrum of daughter ions of *m/z* 580.6. Insert shows the formation of both *m/z* 563.5 attributed to loss of ammonia from the 1,2-diamide (VI) and *m/z* 562.5 attributed to loss of water from the 1,3-diamide (V). This demonstrates the presence of both V and VI in the reaction products. (c). MS^3 spectrum of product ions of *m/z* 563.5 (1,2-diamide). (d). MS^4 spectrum of product ions of *m/z* 282.3 (1,2-diamide).

optimum) 35% AA. Rather we suggest that the *m/z* 562.5 ion was coincidentally isolated with the *m/z* 563.5 ion. Unfortunately, attempts to prove this by reducing the isolation width from the normal *m/z* 1 to *m/z* 0.9 resulted in lower relative abundances for all ions and even lower isolation width values resulted in trapping of too few ions and signal instability. However, in a similar experiment with a higher purity oleic acid derived diamide (*m/z* 632.6), the corresponding M-18 ion (*m/z* 614.6 *cf.* 562.5) was not present in the MS^3 spectrum which supports our suggestion that 562.5 was not a CID product ion.

In summary, from all the MS^n spectra, two distinct ion trap CID fragmentation pathways can be identified (Fig. 2). It is proposed that one pathway is derived from the 1,2-diamide by MS^2 CID loss of ammonia. The second pathway is MS^2 CID loss of water with cyclization from the 1,3-diamide. Clearly, the LC/ESI-MS^n method is very useful, particularly for examination of complex mixtures, and

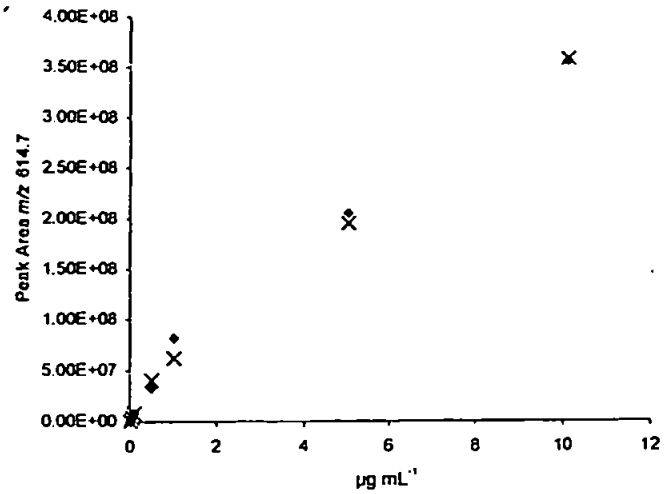

Figure 3. Mass spectral ion current response (*m/z* 614.7) of duplicate solutions of 2:1-OI [III] in methanol (0.01 to 10.0 µg mL^{-1}).

allows the reaction progress to be monitored almost in real time.

Calibration of LC/ESI-MS response of 2-alkyl-1-[ethylalkylamide]-2-imidazolines

The use of the LC/ESI-MS method to measure the concentrations of imidazolines in mixtures accurately, requires calibration of the MS response. This was achieved for 2-pentadecyl-1-[*N*-ethylhexadecylamide]-2-imidazoline (2:1-PI, I) and 2-heptadecenyl-1-[*N*-ethyloctadecenylamide]-2-imidazoline (2:1-OI, III) by measuring the molecular-ion (*m/z* 562.6 and 614.7, respectively) current response of a series of standard solutions (0.010 to 10.0 µg mL^{-1}) of each in methanol (e.g. Fig. 3). The response was curvilinear and was subject to considerable day-to-day variation, thus illustrating the necessity for the synthetic compounds.

Recovery experiments

Imidazolines are very prone to strong sorption to surfaces. Indeed, it this property which has led to their extensive deployment as corrosion inhibitors. The compounds coat the surfaces of metal oxides, reducing further access to water and other corrosive substances, the imidazoline group serving as a sufficiently strong Lewis base to displace water from the Lewis acid sites of the oxide surface.[14–19] This sorption behaviour may also influence the quantitative recovery of imidazolines during analytical chemical procedures. The compounds may sorb to particles in the oil matrix (for example) and also to glassware, metal syringe needles, solid chromatography sorbents and other apparatus used in isolation methods. Ultrasonic dichloromethane extraction of freeze-dried sediments spiked with (unstipulated concentrations of) 2:1-PI and 2:1-OI imidazolines[7] resulted in recoveries of >90% but we feel this may be concentration and matrix dependent, especially if active Lewis acid sites are present on mineral surfaces. To try to assess losses during our procedures we also undertook a series of recovery experiments.

Isolation and LC/ESI-MS determination of 2:1-PI and 2:1-OI from hexane

A 1:1 mixture containing 1 µg mL^{-1} ^{hexane} each of pure synthetic 2:1-PI and 2:1-OI was added to hexane and the imidazolines isolated from the SPE column in IPA/ammonia after sequential elution as detailed above in Materials and Methods. The experiment was conducted in duplicate. LC/ESI-MS determination revealed a mean recovery of 42 and 24% PI and OI, respectively, relative to the response obtained for the 1:1 mixture without SPE treatment. Obviously losses of the imidazolines to the apparatus are considerable and this method requires further optimisation to reduce the losses.

Isolation and LC/ESI-MS determination of 2:1-PI and 2:1-OI from crude oil

A 1:1 mixture containing 1 µg mL^{-1} ^{oil} each of pure synthetic 2:1-PI and 2:1-OI was also added to untreated (water-wet), Middle Eastern crude oil. The imidazolines were isolated from the spiked crude via SPE with IPA/ammonia. The experiment was conducted in duplicate. LC/ESI-MS determination revealed only 37 and 25% (mean 31%) recovery of 2:1-PI and 4 and 2% (mean 3%) recovery of the added di-unsaturated 2:1-OI at this spiking concentration relative to the responses obtained for the 1:1 mixture without SPE treatment. From the further reduction in recovery of the imidazolines from wet crude compared with their recovery from hexane (above; viz reduced from means of 42% to 31% PI and 24% to 3% OI) it appears that small amounts (ca. 1 µg) of imidazolines not only sorb strongly to some of the operational surfaces (most likely the metal syringe needles used for sample transfer), but probably also react with the oil and/or are hydrolysed by the water in the oil, which is known to be a rapid process.[12,13] The differences in PI and OI recovery probably reflect the different physical properties of the compounds (aqueous solubility, hydrolysis rates, sorptivity). Alkyl chain length is known to influence anti-corrosion behaviour (longer chains increasing inhibition)[17] suggesting that the sorptivities of the imidazolines do differ.

Nonetheless, despite these obvious disadvantages, the spiking method followed by SPE and LC/ESI-MS appears to be both more specific and more sensitive than those published previously for determination of imidazolines in crude oil (e.g. Ref. 8) and with further optimisation should be an important advance.

LC/ESI-MS characterisation of a commercial imidazoline-based corrosion inhibitor

The utility of ESI-MS^2 for the characterisation of imidazoline corrosion inhibitors has been demonstrated previously[3,7] and we have recently shown how multistage ESI-MS (MS^4) is an even more powerful method for confirmation of imidazoline structures.[6] Gough and Langley[3] also demonstrated LC/ESI-MS characterisation of 100 ppm concentrations of spiked imidazolines in production fluids (e.g. brines) from West Africa and the North Sea. The detection and measurement of a 2:1-OI imidazoline [III] was clearly shown in the former, whilst, in the latter, the 1,3-diamide [V] and a monoamide [IV] were present, possibly due to hydrolysis in the brine of

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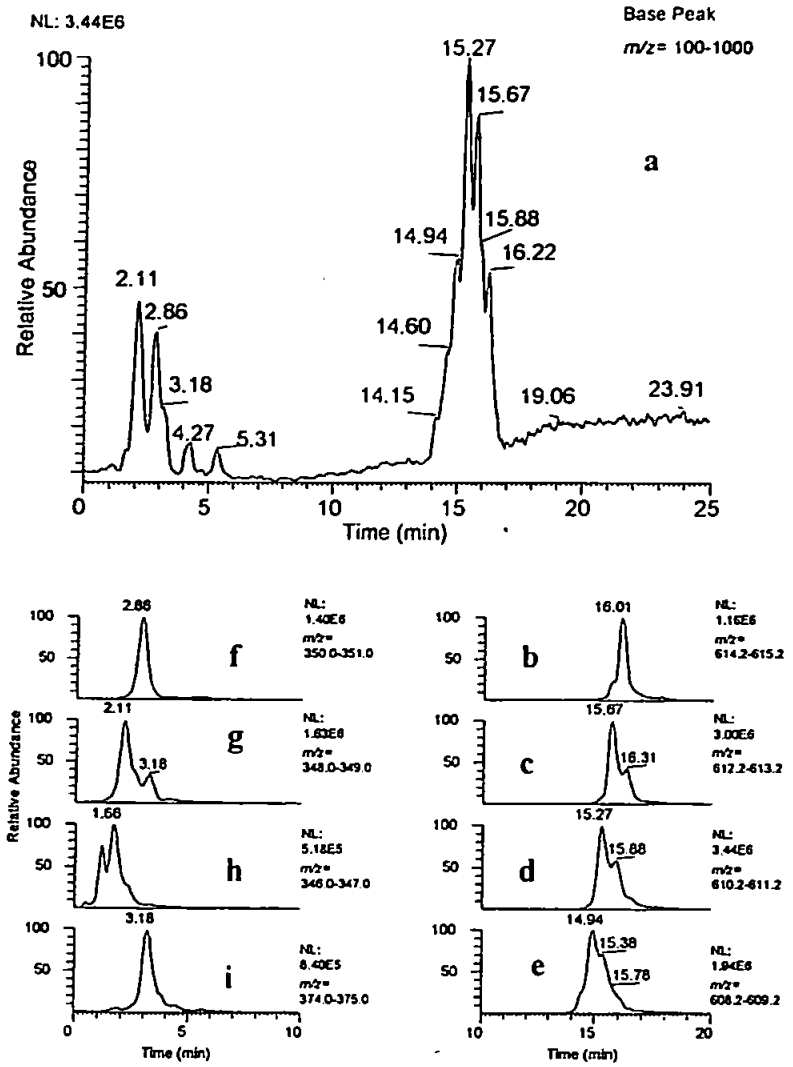

Figure 4. LC/ESI-MS mass chromatograms illustrating the composition of the imidazolines used in manufacture of a commercial corrosion inhibitor. (a). Base peak *m/z* 100–1000 ion current chromatogram. (b). 2:1-OI (*m/z* 614) (c). 2-Heptadecenyl-1-[*N*-ethyloctadecadienylamide]-2-imidazoline, 2-heptadecadienyl-1-[*N*-ethyloctadecenylamide]-2-imidazoline (*m/z* 612) (d). 2-Heptadecadienyl-1-[*N*-ethyloctadecadienylamide]-2-imidazoline, 2-heptadecatrienyl-1-[*N*-ethyloctadecenylamide]-2-imidazoline and/or 2-heptadecenyl-1-[*N*-ethyloctadecatrienylamide]-2-imidazoline (*m/z* 610) (e). 2-Heptadecadienyl-1-[*N*-ethyloctadecatrienylamide]-2-imidazoline and 2-heptadecatrienyl-1-[*N*-ethyloctadecadienylamide]-2-imidazoline (f)–(i). 2-Alkyl-1-ethylamine-2-imidazolines (e.g. II; *m/z* 350).

OI [III] and 2-alkyl-1-ethylamine-2-imidazolines [II] present in the synthetic inhibitor.

Prior to determination of an imidazoline-based corrosion inhibitor in a crude oil (below) we also characterised the imidazolines of a commercial corrosion inhibitor by LC/ESI-MS (Fig. 4(a); 50 μg mL^{-1} in MeOH). The presence of not only 2:1-OI (*m/z* 614; Fig. 4(b)), but also 2-heptadecenyl-1-[*N*-ethyloctadecadienylamide]-2-imidazoline (*m/z* 612; Fig. 4(c)), 2-heptadecadienyl-1-[*N*-ethyloctadecenylamide]-2-imidazoline (*m/z* 612; Fig. 4(c)), 2-heptadecadienyl-1-[*N*-ethyloctadecadienylamide]-2-imidazoline, 2-heptadecatrienyl-1-[*N*-ethyloctadecenylamide]-2-imidazoline and/or 2-heptadecenyl-1-[*N*-ethyloctadecatrienylamide]-2-imidazoline(*m/z* 610; Fig. 4(d)) was revealed by mass chromatography (Fig. 4). Indeed, the latter compounds with tetraunsaturation in the alkyl chains were the major components (e.g. Fig. 4(a); retention time 15.27). Similarly, the presence of the 2-alkyl-1-ethylamine-2-imidazolines [e.g. II; *m/z* 350] could be demonstrated (Figs 4(f)-(i)).

LC/ESI-MS determination of OI-based corrosion inhibitor in crude oil

A crude oil known to have been produced during use of an unsaturated acid-based corrosion inhibitor was examined by

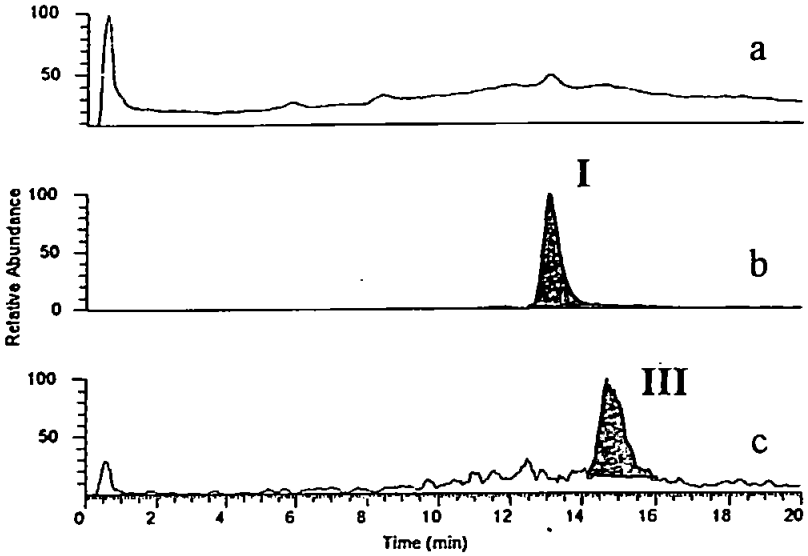

Figure 5. LC/ESI-MS mass chromatograms illustrating the presence in a Middle Eastern crude oil of 2:1-OI [III; *m/z* 614.7]. The concentration of OI could be estimated by comparison of the relative mass ion current responses of 2:1-OI (*m/z* 614.7) and 2:1-PI [I; *m/z* 562.6] added to the oil at 1 μg mL^{-1} ^{oil}. The selectivity of the SPE isolation method combined with the resolving power of LC and the mass selectivity and sensitivity of ESI-MS makes this potentially a powerful method for the determination of 'imidazolines' in crude oil.

the SPE and LC/ESI-MS method. An aliquot of synthetic 2:1-PI (5 μg mL^{-1} ^{oil}) was added as an internal reference (Fig. 5). Obviously, given the 10:1 differential recovery of 2:1-PI over 2:1-OI (above), PI is far from an ideal internal reference, but it probably represents the most appropriate surrogate available at present. Possibly synthesis of polydeuterated OI compounds chosen to have non-isobaric molecular masses with the analytes would produce better internal standards for future quantitative studies. Comparison of the integrated peak areas from mass chromatography for 2:1-OI (*m/z* 614.7; 313 relative response units) and 2:1-PI (*m/z* 562.6; 20 relative response units) allowed an estimation of the concentration of 2:1-OI in the oil (<0.1 μg mL^{-1} ^{oil}). If the recovery of OI is only 10% of that of PI (as was found above at 1 μg mL^{-1} ^{oil}) then this value should be raised to <1 μg mL^{-1} ^{oil} of the individual 2-heptadecenyl-1-[*N*-ethyloctadecenylamide]-2-imidazoline (2:1-OI; III). Although this method is therefore only approximate and certainly requires further optimisation and replication, previous determinations of individual imidazolines in crude oil have not proved possible at all and determinations of even >25ppm concentrations of total imidazolines are not routine.[8] The method is two orders of magnitude more sensitive than most and will be important for operational and environmental uses. Indeed, given the complexity of the crude oil substrate used herein compared to produced waters and sediments,[3,6,7] the method may prove particularly useful for studies of topside discharges and environmental samples.

CONCLUSIONS

A semi-quantitative but sensitive and specific method, involving SPE followed by LC/ESI-MS^n, has been developed for the determination of individual imidazolines at low (<10) parts per million concentrations in crude oils. Whilst the method requires further optimisation due to the high experimental losses of imidazolines during work-up, it nonetheless represents a considerable improvement on previous techniques and should prove valuable for operational and environmental studies of corrosion inhibitor and surfactant behaviour.

Acknowledgements

We thank AstraZeneca Brixham Environmental Laboratory and the University of Plymouth for a studentship, the NERC for financial support for ESI-MS and Dr M. J. Hetheridge of AstraZeneca Brixham Environmental Laboratory for advice on LC/ESI-MS. We thank an anonymous industrial sponsor for provision of chemicals and an anonymous referee for helpful suggestions which improved the original manuscript.

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ANALYSIS OF OILFIELD PRODUCED WATERS AND PRODUCTION CHEMICALS BY ELECTROSPRAY IONISATION MULTI-STAGE MASS SPECTROMETRY (ESI-MS^n)

P. McCORMACK[1], P. JONES[1], M. J. HETHERIDGE[2] and S. J. ROWLAND[1]*

[1]Plymouth Environmental Research Centre, Department of Environmental Sciences, University of Plymouth, Drake circus, Plymouth Devon, PL4 8AA, UK and [2]Brixham Environmental Laboratory, AstraZeneca, Freshwater Quarry, Brixham, TQ5 8BA, UK

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Abstract—Large quantities of diverse polar organic chemicals are routinely discharged from oil production platforms in so-called produced waters. The environmental fate of many of these is unknown since few methods exist for their characterisation. Preliminary investigations into the use of multistage electrospray ionisation ion trap mass spectrometry (ESI-MS^n) show its potential for the identification and quantification of compounds in specialty oilfield chemicals (corrosion inhibitors, scale inhibitors, biocides and demulsifiers) and produced waters. Multiple stage mass spectrometry (MS^n) with both positive and negative ion detection allows high specificity detection and characterisation of a wide range of polar and charged molecules. For example, linear alkylbenzenesulfonates (LAS), alkyldimethylbenzylammonium compounds, 2-alkyl-1-ethylamine-2-imidazolines, 2-alkyl-1-[*N*-ethylalkylamide]-2-imidazolines and a di-[alkyldimethylammonium-ethyl]ether were all identified and characterised in commercial formulations and/or North Sea oilfield produced waters. The technique should allow the marine environmental effects and fates of some of these polar compounds to be studied. © 2001 Elsevier Science Ltd. All rights reserved

Key words—produced waters, oilfield chemicals, electrospray-mass spectrometry, corrosion inhibitor, multi-stage mass spectrometry

INTRODUCTION

When crude oil is produced from offshore oilfields it is associated with varying proportions of water called formation waters (Warren and Smalley, 1994). In the early stages of oil production, the water content is usually low whereas later the proportions can rise to as high as 80% (Tibbetts *et al.*, 1992). Once the water has been separated from the oil it is known as produced water (PW) and this is discharged into the sea (Ray and Engelhart, 1992; Reed and Johnsen, 1996). PW discharges on the UK continental shelf rose from about 55 million tonnes in 1984 to about 145 million tonnes in 1994 and since 1994 have increased still further (Stagg *et al.*, 1996).

The oil recovery, production and separation processes involve addition of a diverse mixture of oilfield chemicals (OCs) to the oil–water mixtures. For example, these chemical mixtures are formulated to act as scale inhibitors to prevent mineral scale deposition blocking pipework, corrosion inhibitors to prevent pipe work from attack by the salt water and dissolved gases, biocides to prevent bacterial degradation of the oil and other products, and emulsion breakers to facilitate oil–water separation (e.g. Reed and Johnsen, 1996). Some or all of these OCs may be discharged to the marine environment along with the PW (van Zwol, 1996; Slager *et al.*, 1992; Stephenson *et al.*, 1994; Flynn *et al.*, 1996). In the North Sea an estimated 5934 tonnes of OCs were discharged in 1989 along with an estimated 84097 tonnes of drilling chemicals (Hudgins, 1994).

Although the syntheses of the individual components of the formulated oilfield chemicals rely substantially on well-known chemical reactions, the products from individual companies may differ due to blending in order to achieve different activities and functions. Many of the organic chemicals in the OCs are polar, hydrophilic compounds, which are not amenable to routine analysis (Martin and Valone, 1985; Gough *et al.*, 1997; Gough and Langley, 1999). Establishment of such methods is therefore important to the OC manufacturers, to the oilfield operators and to scientists concerned with the fate and effects, if any, of PW and OCs in the marine environment (e.g. Gamble *et al.*, 1987; Washburn *et al.*, 1999).

Previously, fast atom bombardment—mass spectrometry (FAB-MS) has been used to good effect to

*Author to whom all correspondence should be addressed. Tel.: +44-1752-233013; fax: +44-1752-233035; e-mail: s.rowland@plymouth.ac.uk

partially characterise OCs in PW (Tibbetts *et al.*, 1992) and other spectroscopic methods have been used in attempts to characterise oilfield chemicals such as imidazoline corrosion inhibitors (Martin and Valone, 1985). Recently, electrospray ionisation mass spectrometry (ESI-MS) has also proved to be a powerful method for the identification of some constituents of OCs (Gough and Langley, 1999). Here we report that improved characterisation of numerous polar organic chemicals in OCs and in PW samples from North Sea oilfields is possible by use of both positive and negative ion multistage MS with ES ionisation ((±)ESI-MS^n). The method promises to be useful for monitoring the fate of OCs in oilfield operations and in the marine environment.

EXPERIMENTAL

Chemicals

Ultra pure water was obtained from an Elgastat (Elga, High Wycombe, UK) filtration system. All solvents used were of HPLC grade. Methanol and acetonitrile (Hypersolv) were obtained from BDH (Poole, UK), and dichloromethane (DCM) from Rathburn (Walkerburn, UK.). Dodecylbenzenesulfonic acid (DBSA) sodium salt was purchased from Aldrich (Dorset, UK.) and benzyldimethyltetradecylammonium chloride from Fluka (Buchs, SW).

PW samples were supplied by various North Sea oilfield operators and specialty OCs by various manufacturers.

Mass spectrometry

Mass spectrometry analysis was carried out using a ThermoQuest Finnigan Mat LCQ (San Jose, CA, USA.) bench top mass spectrometer fitted with an electrospray interface. Navigator 1.1 software was used for the PW and DBSA samples with a full scan range of *m/z* 50–1850. All other samples were run with Xcalibur 1.0 software in the normal full scan range (*m/z* 50–2000) (following an instrument hardware upgrade). Instrument tuning and mass calibration was carried out and checked using the automatic calibration procedure (tuning and calibration solution-caffeine, MRFA, and Ultramark 1621 in methanol : water : acetic acid (50 : 50 : 1, v/v/v)). Infusions of PW extracts and OC solutions were carried out using a built in syringe pump with a Hamilton 1725N (250 µl) syringe (Reno CA, USA.). Analytes were infused at 3 µl min^{-1}. Source voltage, (±) 4.5 kV; capillary Voltage (±) 0–50 V (auto tune function on ion of interest); capillary temperature, 200°C; nitrogen sheath gas flow rate, 40 (arbitrary units). (±) MS^n analysis of selected ions was performed in the ion trap by collision-induced dissociation (CID) with helium. MS^n ion isolation widths, relative activation amplitudes and activation Q_z were optimised to obtain high response and stability of the base peak fragment ion. High-resolution zoomscans (ZS) were recorded for all ions of interest. All spectral data was recorded and averaged over a 1-min acquisition time.

Produced waters

PW samples were extracted with DCM (~50 ml). One extract (PW60) was blown to dryness with nitrogen and the residue re-dissolved in 500 µl methanol and 250 µl Ultra pure water (PW60MW). A DCM extract from a different PW (PW90) was reduced to ~1 ml using a Kuderna Danish controlled evaporation and the remaining extract was divided into two portions (~0.5 ml) in pre-weighed vials and blown down to dryness with nitrogen. The dry residues were re-dissolved in methanol (1 ml; PW90 M) and acetonitrile (1 ml; PW90A). A further 900 µl ACN was added to 100 µl of PW90 A for analysis.

Stock solutions of standard compounds

Dodecylbenzenesulfonic acid (DBSA) sodium salt (50 mg) was prepared by dissolution in Ultra pure water and made up to 50 ml. Benzyldimethyltetradecylammonium (BDMTDA) chloride (50 mg) was made up to 50 ml with methanol. Dilutions of the stock were made using the required solvents.

Oilfield chemicals

Stock solutions of commercial products were prepared as follows: Corrosion inhibitor CI-D2 (50 µl) was diluted to 50 ml with MeOH : H_2O (1 : 1, v/v); Corrosion inhibitor CI-A3 (25 mg) was made up to 50 ml with MeOH; Corrosion inhibitor CI-B1 (116 mg) was made up to 100 ml with MeOH; Corrosion inhibitor CI-C3 (100 µl) was made up to 10 ml with MeOH; Demulsifier DM-C2 (25 mg) was made up to 50 ml with MeOH. Dilutions of the stock solutions were made using the required solvents.

RESULTS AND DISCUSSION

Produced waters

Discharged PW is a complex mixture of liquid and particulates and comprises inorganic and organic compounds of natural origin, applied production chemicals (OCs), and residues of other platform effluents and deck washes. For an initial evaluation of ESI-MS^n as an analytical technique for PW analysis, DCM extracts were used to obtain polar organic fractions. After removal of DCM, the extracts were re-dissolved in various solvents (e.g. methanol, acetonitrile or methanol/water) as it is well known that electrospray ionisation of compounds varies for different solvents. Negative and positive ion full scan spectra [*m/z* 50–1850], ZS and CID MS^n spectra of significant ions were acquired in order to provide data that could be used to identify the unknown extracted compounds.

In general, negative ion spectra of PW extracts were relatively simple with low baseline noise. Ions at *m/z* 297, 311, 325 and 339 were the only significant ions in extracts dissolved in methanol/water and methanol. The spectrum of an extract dissolved in acetonitrile (Fig. (1A)) also contained these ions but in addition a series of ions differing by 14 Da in three clusters were apparent at *m/z* 423, 529 and 635. Compounds that correspond to ions in the clusters are still to be identified but CID MS^n analysis of the ions at *m/z* 297, 311, 325 and 339 (base peak) indicated a common structure differing only by 14 Da. Thus, CID MS^2 of precursor ions *m/z* 297, 311, 325 and 339 produced neutral fragment losses to give a common product ion *m/z* 183 in each case (e.g. Fig. 2(A)). CID MS^3 of the *m/z* 183 product ion in each case produced a fragment ion *m/z* 119, representing loss of a neutral 64 Da fragment (Fig. 2(B)). Further fragmentation (MS^4) of the *m/z* 119 ions produced no detectable ions.

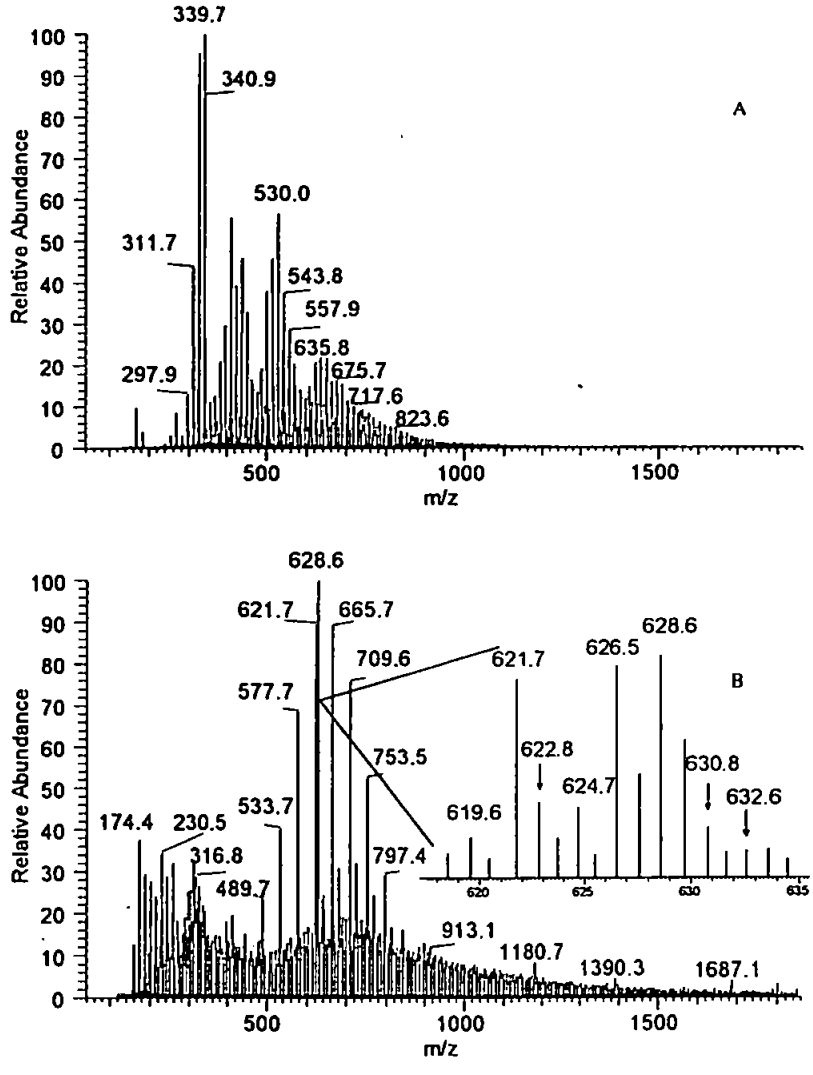


Fig. 1. Negative (A) and positive (B) ion ESI-MS mass spectra of produced water extracts: (A) ACN solvent and (B) MeOH solvent.

The precursor ions *m/z* 297, 311 325, 339 and the CID MS^2 product ion *m/z* 183 are consistent with those expected of linear alkylbenzenesulfonates (LAS) (Fig. 2(C), (i)) (Lyon *et al.*, 1998; Schöder, 1996; GonzalezMazo and GomezParra, 1996; GonzalezMazo *et al.*, 1997; Reemtsma, 1996; Rieu *et al.*, 1999). The MS^3 fragment ion *m/z* 119 produced from the CID of the *m/z* 183 product ion via a neutral loss of 64 Da is consistent with loss of sulfur dioxide (SO_2) from an ethylenebenzenesulfonate ion (Fig. 2(C), (ii)) to yield an ethylenephenoxy ion (Fig. 2(C), (iii)). The fragmentation pathway shown in Fig. 2(C) could be deduced for the MS^3 CID of LAS. To our knowledge the MS^3 *m/z* 119 fragment ion produced by loss of SO_2 from the CID of the MS^2 *m/z* 183 product ion has not been reported previously. In order to confirm that the peaks are due to alkylbenzenesulfonates a commercial sample of dodecylbenzenesulfonic acid sodium salt was also examined. The negative ion full scan spectrum showed the same four main ions (*m/z* 297, 311, 325 and 339), whilst MS^n produced identical fragment ion losses from each precursor ion to those shown in the PW components. The negative ion mass spectrum of an oilfield demulsifier DM-C2 (Fig. 3(A)) also contained ions at *m/z* values consistent with the presence of alkylbenzenesulfonates. MS^n analysis showed fragmentations identical to the dodecylbenzenesulfonic acid sodium salt. Thus, ESI-MS^n allows unambiguous identification of linear alkylbenzenesulfonates in both PW and OCs.

The positive ion spectra of the PW extracts were more complex than the negative ion spectra and the spectrum of the extract dissolved in methanol was very 'noisy'. This is expected, as the ionisation efficiency of many compounds is greater in

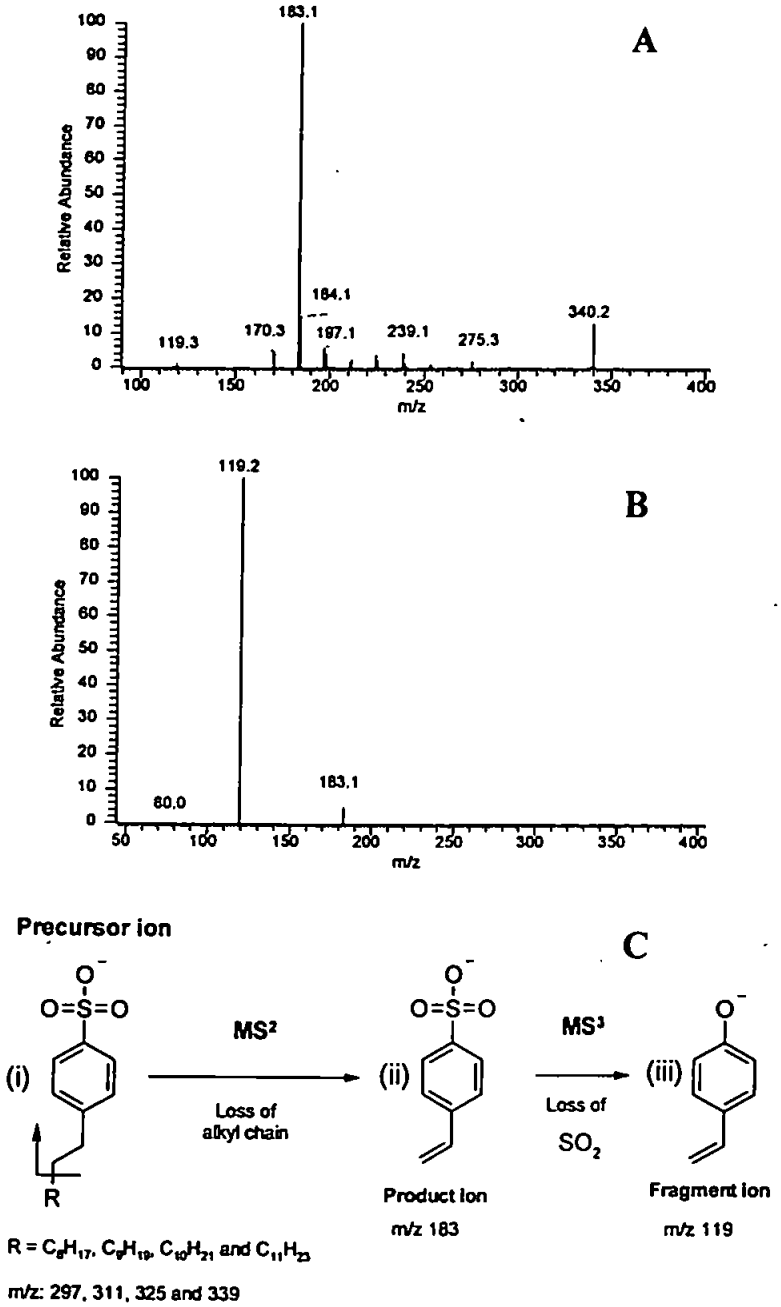


Fig. 2. Negative ion ESI-MS^n spectra of precursor ion *m/z* 339.7 shown in Fig. 1(A) for produced water and CID MS^n fragmentation pathway of alkylbenzenesulfonates: (A) MS^2 spectrum of the precursor ion *m/z* 339.8; (B) MS^3 spectrum of the *m/z* 183 product ion and (C) CID MS^n fragmentation pathway of alkylbenzenesulfonates. MS^2 cleavage of the alkyl chain is at the same position for all homologues producing identical product ions.

methanol/water mixtures rather than pure methanol. The spectra of extracts dissolved in methanol (Fig. 1(B)) and acetonitrile were very similar with a base peak ion at *m/z* 628 with a distribution of ions differing by 2 Da. This is consistent with the presence of two homologous series of compounds, one containing a further degree of unsaturation. The spectra are consistent with a diamidoamine synthesised from tall oil fatty acids (TOFA) and diethylenetriamine (DETA) (Gough and Langley, 1999). Such amides are commonly produced during the manufacture of imidazoline corrosion inhibitors. Also imidazoline compounds readily hydrolyse to their amide precursor compounds on exposure to air (Lomax, 1996) and might therefore be produced in PW from hydrolysis of an imidazoline-based pro-

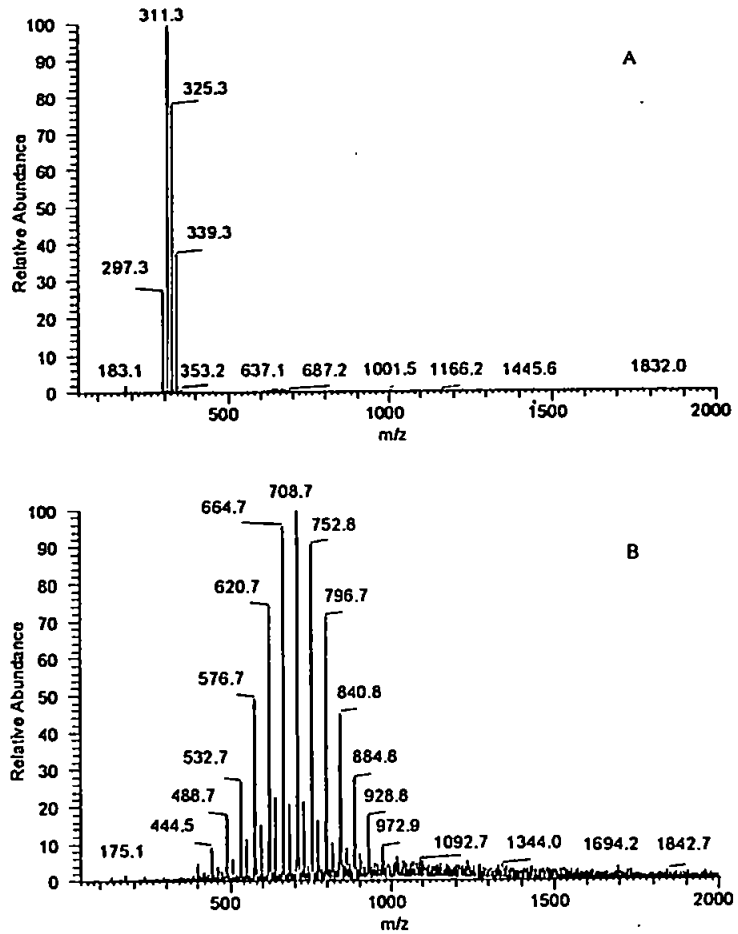


Fig. 3. Negative (A) and positive (B) ion ESI-MS mass spectra of DM-C2 oilfield demulsifier (7 : 3 v/v MeOH : H_2O).

duct. Ions at *m/z* 489.7, 533.7, 577.7, etc. differing by 44 Da (Fig. 1(B)) are tentatively attributed to polyethoxylated compounds, typical of those found in commercial de-emulsifiers (Lomax, 1996).

Oilfield chemicals (OCs)

A wide range of OCs are used in offshore oil and gas production. Most are complex mixtures derived from impure raw materials (Hudgins, 1992). Furthermore, many commercial products are blends of two or more chemical types. For reasons of commercial confidentiality, the specific chemicals and quantities contained in oilfield products are not generally made public and only the legally required health and safety data are normally specified on material safety data sheets (MSDS). The information given regarding the active chemicals is generally restricted to the class of compounds (e.g. amines, quaternary amines, imidazolines, polycarboxylates, phosphonates) and the solvents (aqueous, methanol or aromatic solvents). Without detailed knowledge of the OC compositions or the availability of suitable analytical methods, it is difficult for the fate of OCs in operational processes or in environmental scenarios to be followed. ESI-MS^n methods begin to allow such knowledge to be assembled, particularly if the mass spectra can be interpreted to identify unknown constituents, as illustrated in the following experiments in which samples of commercial OCs were dissolved and diluted in appropriate solvents and analysed by direct infusion ESI-MS^n.

Corrosion inhibitor CI-D2: CI-D2 is a commercial oilfield corrosion inhibitor stated to contain (MSDS) fatty amine quaternary salts (10–30%) in water and methanol (10–30%). A +ESI full scan mass spectrum (Fig. 4(A)) of CI-D2 dissolved in methanol : water (1 : 1, v/v) solvent, showed three distinctive odd *m/z* ion series. Ions in the most intense series centred on *m/z* 249 differed from each other by *m/z* 14, whilst those in the series around *m/z* 533 and 1131 differed by *m/z* 28. These differences between the series were

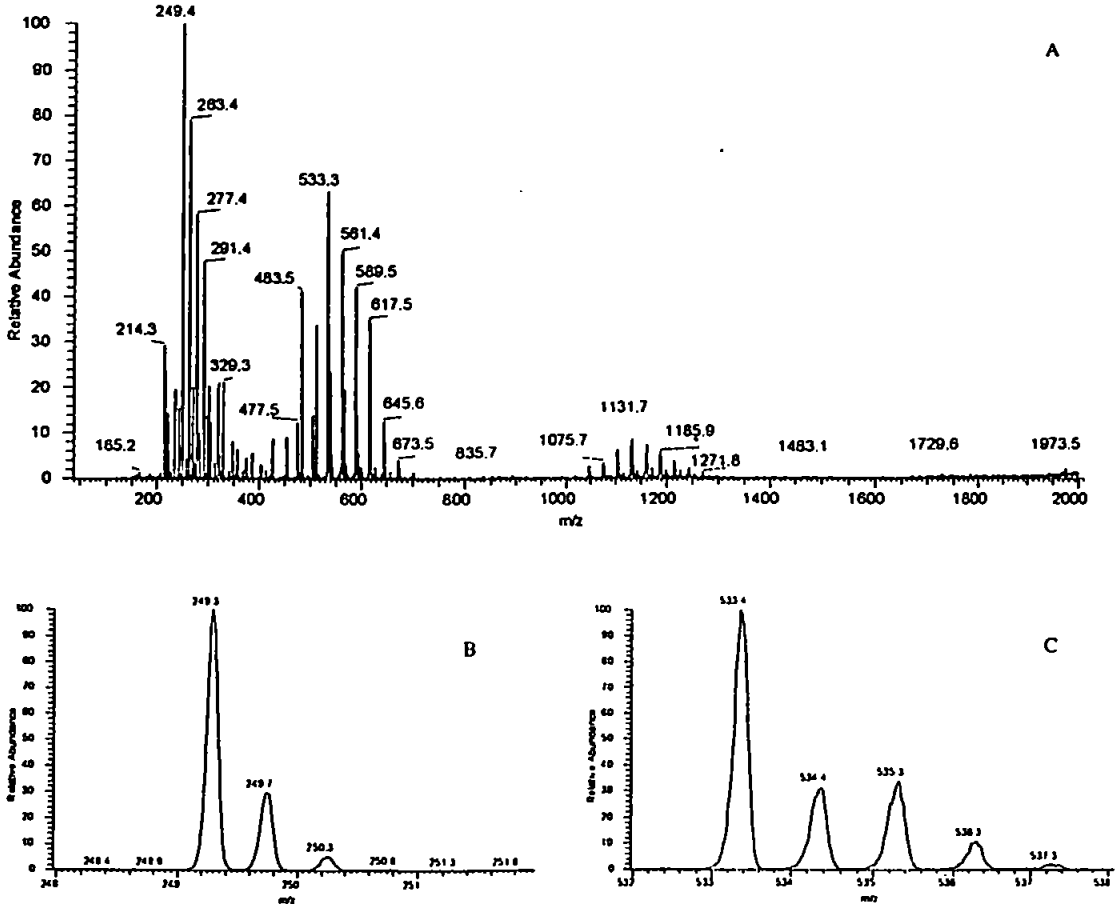


Fig. 4. Positive ion ESI-MS mass spectra of CI-D2 oilfield corrosion inhibitor (1 : 1 v/v MeOH : H_2O): (A) Full scan *m/z* 50–2000; (B) ZoomScan *m/z* 249.4 and (C) ZoomScan *m/z* 533.4.

elucidated by the use of the so-called 'zoomscan' (ZS) facility on individual ions in each series. ZS allows resolution of isotope peaks for up to +4 charge-state ions. Thus, pairs of ions in the series at *m/z* 221.4–305.4 (e.g. *m/z* 249.4 and 249.7) showed mass differences of 0.4 Da (Fig. 4(B)). The *m/z* 249.3/249.7 ion ratio was consistent with that expected for $^{12}C/^{13}C$ isotopes. There is no evidence for the presence of chlorine or bromine. The 0.4 Da mass differences suggest the *m/z* 221.4–305.4 ions are doubly charged ions ($M^{2+}/2$). By contrast, ZS analysis of the ion series *m/z* 477.5–645.5 shows mass differences of 1.0 Da between ion clusters (e.g. *m/z* 533.4/534.4; Fig. 4(C)) consistent with singly charged ions ($M^{+}/1$). However, whilst the *m/z* 533/534 ratio is also consistent with that expected for $^{12}C/^{13}C$ isotopes, the 3 : 1 ratio of *m/z* 533 and 535 is also indicative of the presence of a chlorine atom (viz: $^{35}Cl/^{37}Cl$). An odd *m/z* value for the ions indicates that an even number of nitrogen atoms is present. As fatty amine quaternary salts are expected from the MSDS description, identification of the compound as a di-quaternary salt is consistent with all the spectral data (viz: ion series at *m/z* 221–305 due to M^{2+}; *m/z* 533 $[M^{2+}+Cl^{-}]^{+}$ and *m/z* 1131 $[2M^{2+}+3Cl^{-}]^{+}$).

In an attempt to identify the chemical more completely, MS^n analysis of the ions observed in the full scan spectrum was carried out. Highly reproducible product ion spectra were obtained for ions attributed to M^{2+} and $[M^{2+}+Cl^{-}]^{+}$ with up to five CID fragmentation steps (MS^5; Fig. 5). A fragmentation pathway and a molecular structure for the precursor ion at *m/z* 533.5, consistent with the MS^n data (Fig. 5(A)–(D)) is shown in Fig. 6. The structure is consistent with known synthetic routes to *bis*-quaternary ammonium compounds, involving reaction of *β*-dichloroethylether with alkyl dimethylamine (Linfield, 1970). Thus, if the fatty amine was derived from a coco or palm oil source (cf Gough and Langley, 1999) then all the possible ions resulting from combinations of $C_{8,10,12,14,16,18}$ alkyl groups can be identified in the mass spectrum (Table 1).

Corrosion inhibitors CI-C3 and CI-B1: CI-C3 and CI-B1 are commercial oilfield corrosion inhibitors stated (MSDS) to comprise: ethoxylated amines and

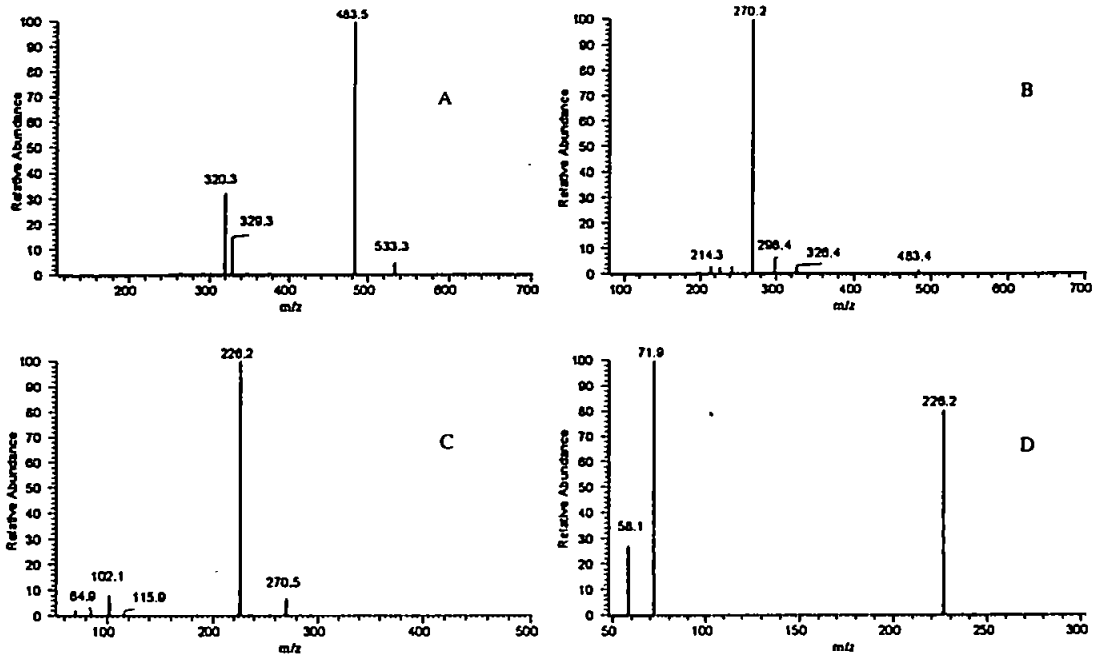


Fig. 5. Positive ion ESI-MS^n mass spectra of precursor ion *m/z* 533.4 in spectrum Fig. 5 for CI-D2 oilfield corrosion inhibitor: (A) MS^2 on precursor ion *m/z* 533.4; (B) MS^3 on product ion *m/z* 483.4; (C) MS^4 on product ion *m/z* 270.2 and (D) MS^3 on product ion *m/z* 226.2.

quaternary compounds (5–10%), butyl glycol (20–30%) and monoethylene glycol (20–30%) in water (CI-C3) and benzyl chloride quat amine (5–10%) and methanol (1–5%) (CI-B1). Positive ion ESI-MS spectra suggest that both formulations contain quaternary amines corresponding to alkylbenzyldimethylammonium (benzalkonium) compounds (Figs 7(A) and (B)). The spectra show that the alkyl chain source is different for each product, with CI-C3 having a C_{12} (base peak), C_{14}, C_{16} and C_{18} distribution whilst CI-B1 has only two ions for C_{16} and C_{18} (base peak). This has been shown previously by ESI-MS (Gough and Langley, 1999). The more powerful multi-stage MS^n analysis of the benzalkonium ions produced highly reproducible fragmentations with masses differing only by the alkyl chain lengths. Thus, CID MS^2 of the benzalkonium precursor ion produced two fragment ions (Fig. 8(A)) with the base peak *m/z* corresponding to a neutral loss of 92.0 Da, consistent with fragmentation of the benzyl moiety with a proton migration to effect loss of a methylbenzene and yield an iminium ion (Fig. 8(C)). The fragment at *m/z* 91.0 is consistent with a benzyl cation (tropylium ion) commonly seen in electron ionisation-MS of aromatic compounds, and a neutral loss of alkyldimethylamine (Fig. 8(C)). Both of the MS^2 fragment ions result from cleavage of the same nitrogen–benzyl bond. CID MS^3 of the MS^2 iminium ion (Fig. 8(B)) results in loss of an alkene molecule via a 1,5 proton shift McLafferty rearrangement (De Hoffmann *et al.*, 1996) to yield a *m/z* 58 iminium ion (Fig. 8(C)). MS^n analysis of authentic benzyldimethyltetradecylammonium chloride (BDMTDACl) produced identical fragmentation spectra to those for the benzalkonium ions in the corrosion inhibitors. The reproducibility of the CID MS^n spectra allows unambiguous identification of the benzalkonium ions.

Corrosion inhibitor CI-C3 also contained ethoxylated amines (MSDS). Ions due to these are also seen in the (+)ESI-MS spectrum (Fig. 7(A)) as at least three series of ions differing by 44 Da (*viz*: corresponding to $-C_2H_4O-$) between *m/z* 500–1300. Although the MSDS for corrosion inhibitor CI-B1 does not state that it contains ethoxylated compounds there are clearly several series of ions differing by 44 Da in the spectrum and some have similar *m/z* values to those seen in CI-C3 and in the demulsifier DM-C2 (Fig. 3(B)). The even *m/z* ions infer an odd number of nitrogen atoms in the compounds and the presence of several series suggests that both CI-B1 and DM-C2 contain ethoxylated amines. Series differing by 44 Da are also observed in positive ion ESI-MS spectra of the produced waters (e.g. Fig. 1(B)), suggesting these demulsifiers may be discharged into the environment. However in the PW the ethoxylated compounds differed by 1 Da (reliably measured by ZS) from those in DM-C2 and CI-C3.

Corrosion inhibitor CI-A3: Corrosion inhibitor CI-A3 is a commercial oilfield corrosion inhibitor with a stated composition (MSDS) comprising 10–15%

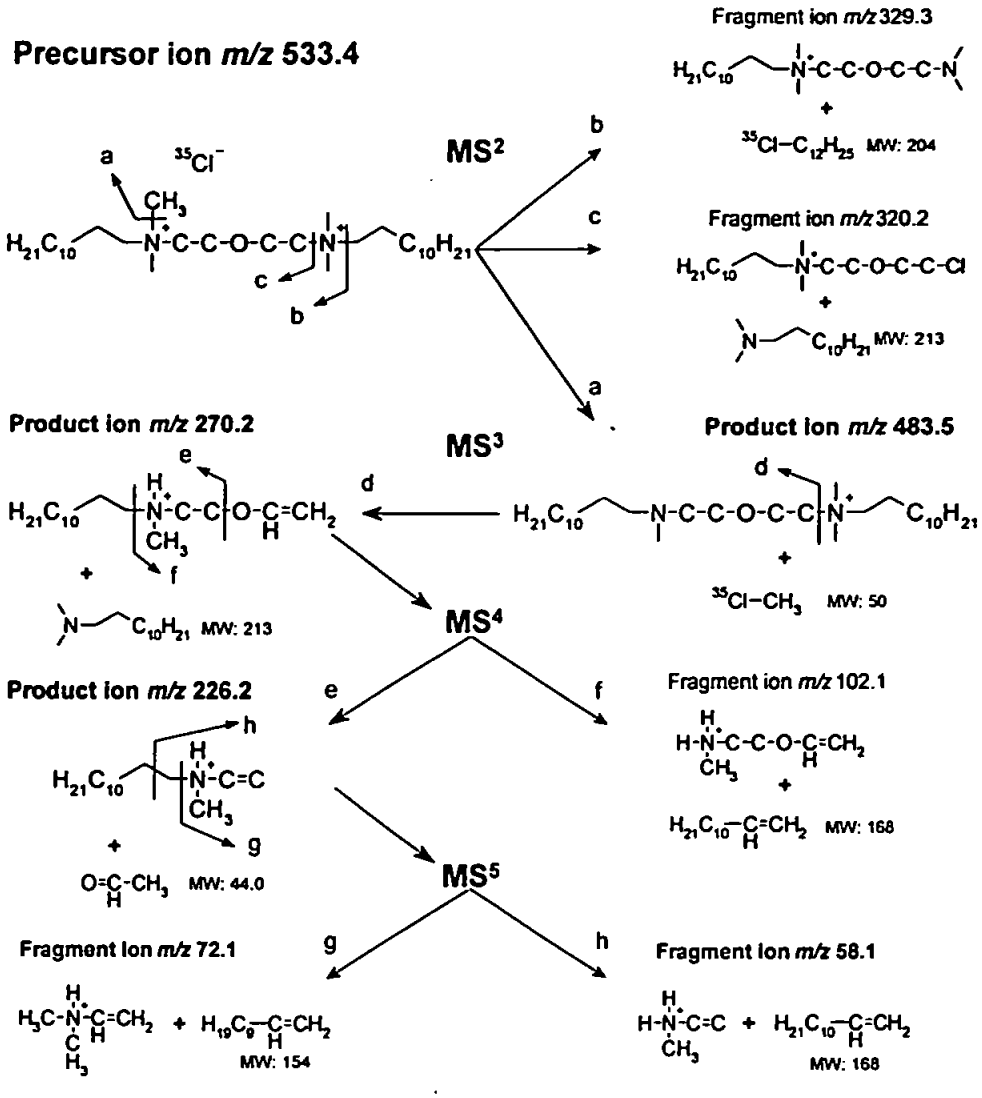
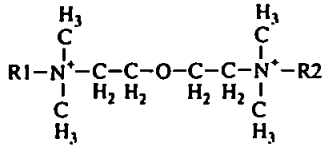


Fig. 6. Positive ion CID ESI-MS^n fragmentation pathway of precursor ion *m/z* 533.4 in spectrum Fig. 4(A) and MS^n spectra Fig. 5(A)–(D) for CI-D2 oilfield corrosion inhibitor.

Table 1. Isotopic Masses for $[M^{2+} + {}^{35}Cl^-]^+$ where R1 and R2 are alkyl moieties derived from coco or palm oils



R1/R2	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}
C_8	421.4	449.4	477.5	505.5	533.5	561.5
C_{10}	449.4	477.5	505.5	533.5	561.5	589.6
C_{12}	477.5	505.5	533.5	561.5	589.6	617.6
C_{14}	505.5	533.5	561.5	589.6	617.6	645.6
C_{16}	533.5	561.5	589.6	617.6	645.6	673.7
C_{18}	561.5	589.6	617.6	645.6	673.7	701.7

solvent naphtha (petroleum), 1–5% butoxyethanol and 20–40% long chain alkyl imidazoline. A positive ion ESI full scan spectrum (Fig. 9) of a solution of CI-A3, contained two prominent sets of ions at *m/z* 350 and 612. These are near identical to those reported by Gough and Langley (1999) for TOFA/DETA-derived imidazolines. Gough and Langley attribute the spectra to protonated 2-alkyl-1-ethylamine-2-imidazolines and 2-alkyl-1-[*N*-ethylalkylamide]-2-imidazolines (Fig. 9). The peak distributions differ by 2 Da in each group, which is consistent with a natural distribution of unsaturated acids expected from reactants derived from a natural source such as a Tall Oil. MS^2 analysis of the precursor ions *m/z* 350.7 and 614.7 (Fig. 10(A) and (C)), corresponding to imidazolines with alkyl moieties $R_1 = C_{16}H_{31}$ and $R_2 = C_{17}H_{33}$, shows the formation of a protonated 2-alkylimidazoline moiety

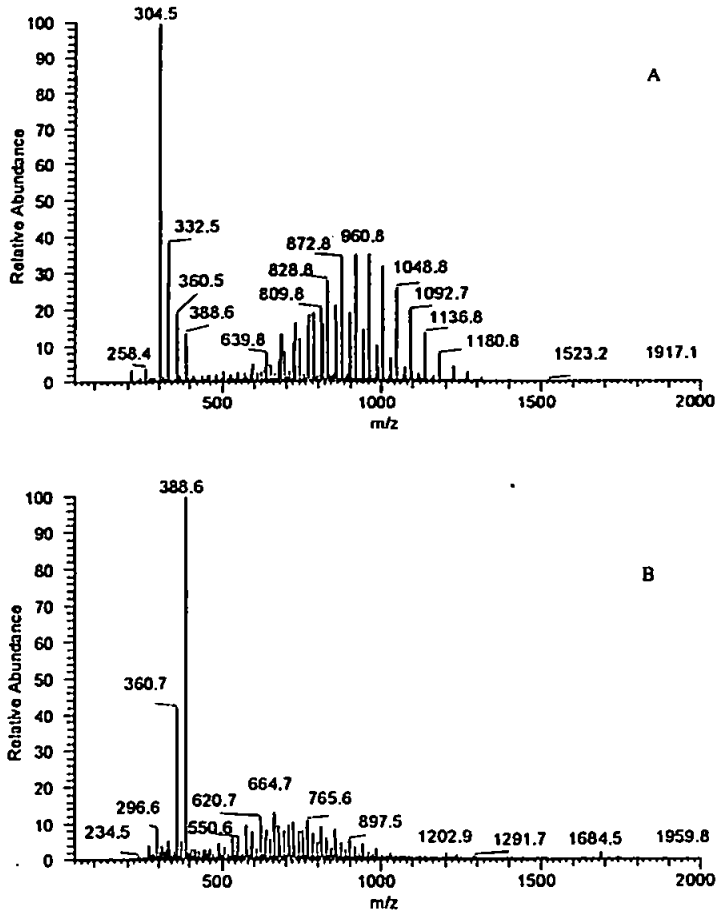


Fig. 7. Positive ion ESI mass spectra of oilfield corrosion inhibitors (A) CI-C3 and (B) CI-B1 (1:1 v/v MeOH:H_2O).

(Fig. 10(E)) as the base peak ion for both ions *via* losses of etheneamine and N-ethene-alkylamide, respectively. MS^3 fragmentation of the product ion *m/z* 307 (Fig. 10(B) and (D)) produced identical spectra consistent with the loss of the alkyl chain to give a protonated 2-etheneimidazoline (Fig. 10(E)). Further fragmentation produced no ions above the *m/z* 50 lower mass limit of the instrument. Spectra were obtained for other ions in the two groups producing product ions that varied by 2 Da, confirming the differences were due to the degree of unsaturation of the alkyl chains. A fragmentation pathway that is consistent with the structures of the imidazoline compounds is given in Fig. 10(E).

CONCLUSIONS

Examination of a range of oilfield chemicals (OCs) and of oilfield platform produced waters (PW) by electrospray-ion trap-mass spectrometry has demonstrated that the technique is very powerful for the identification of polar chemicals used as demulsifiers, corrosion inhibitors and biocides, for example.

A range of imidazolines, alkylbenzene sulfonates, quaternary ammonium compounds (quats) and ethoxylates have all been identified, some for the first time in OCs and PW.

The particular advantages of the ion trap multistage MS method are: the possibility of both positive and negative ion detection (e.g. for quats and sulfonates, respectively); the ability through the so-called 'ZoomScan' facility to determine the accurate mass differences between ion pairs, thus allowing differentiation of the multiple charge state of the ions; the collision-induced dissociation multistage mass spectrometry allowing fragmentation pathways to be studied in up to five steps, thereby allowing previously unknown compounds to be identified.

Coupled with liquid chromatography, the ESI-MS^n method promises to be valuable for studies of the operational use and the environmental fate of a range of OCs in oil production chemicals and produced waters.

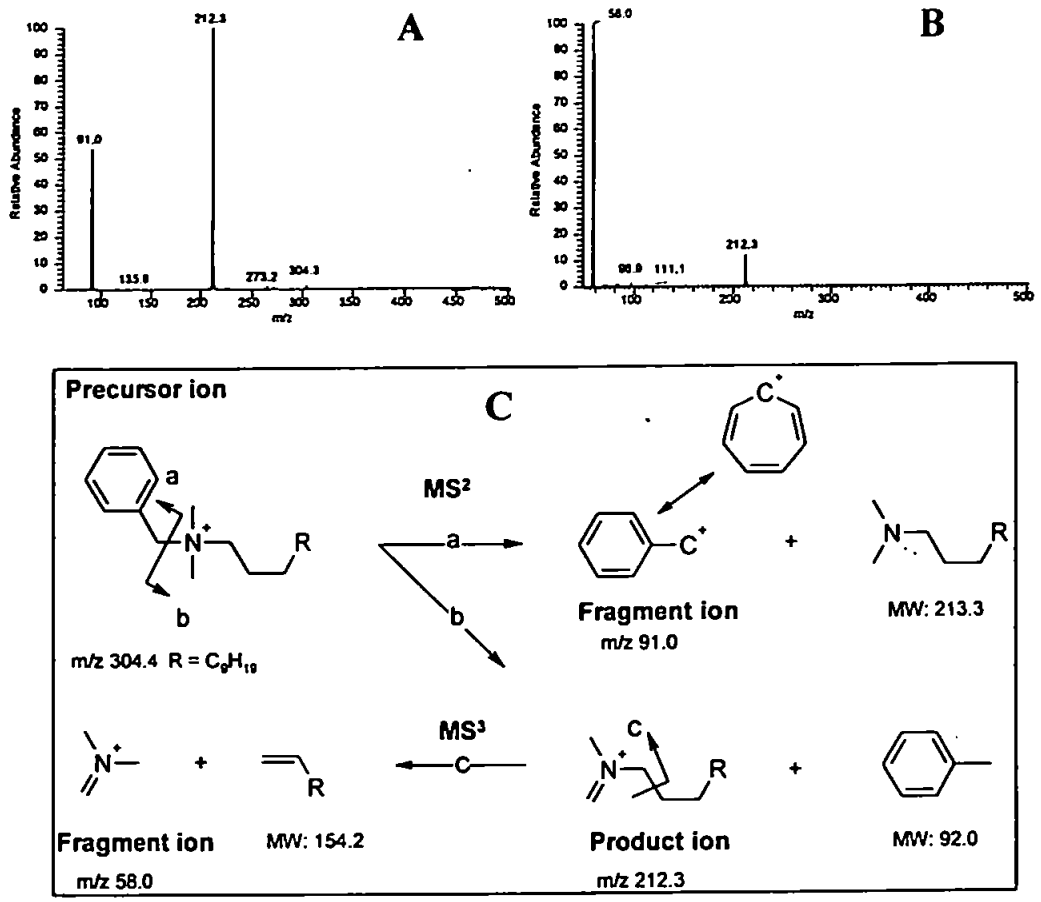


Fig. 8. Positive ion ESI-MS^n mass spectra of ion *m/z* 304.4 in spectrum Fig. 7 and CID MS^n fragmentation pathway for CI-C3 oilfield corrosion inhibitor: (A) MS^2 on precursor ion *m/z* 304.4; (B) MS^3 on product ion *m/z* 212.3; (C) CID fragmentation pathway for alkylbenzyldimethylammonium ions.

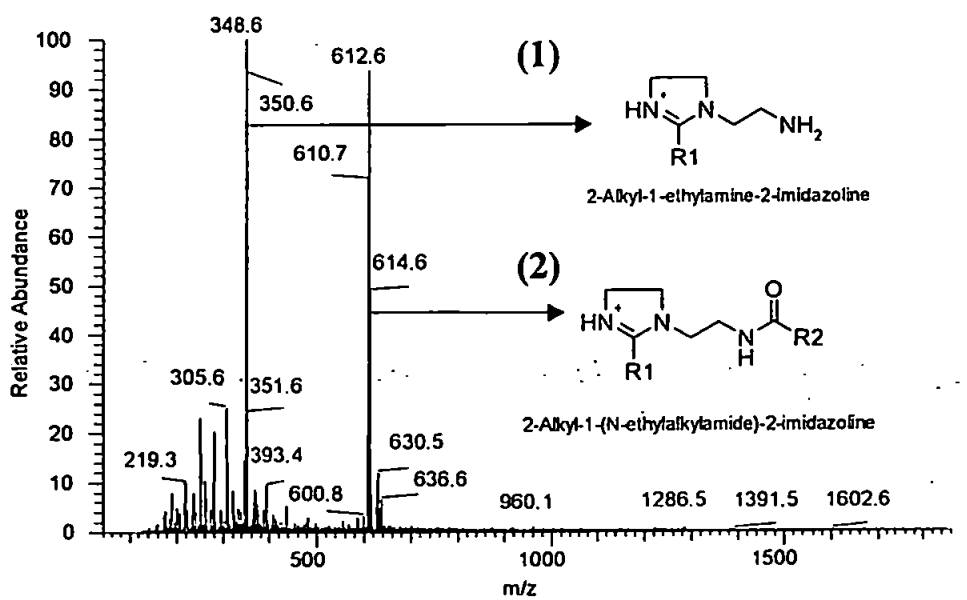


Fig. 9. Positive ion ESI-MS mass spectrum of CI-A3 oilfield corrosion inhibitor (90:10:0.1 v/v/v MeOH:H_2O:AcOH).

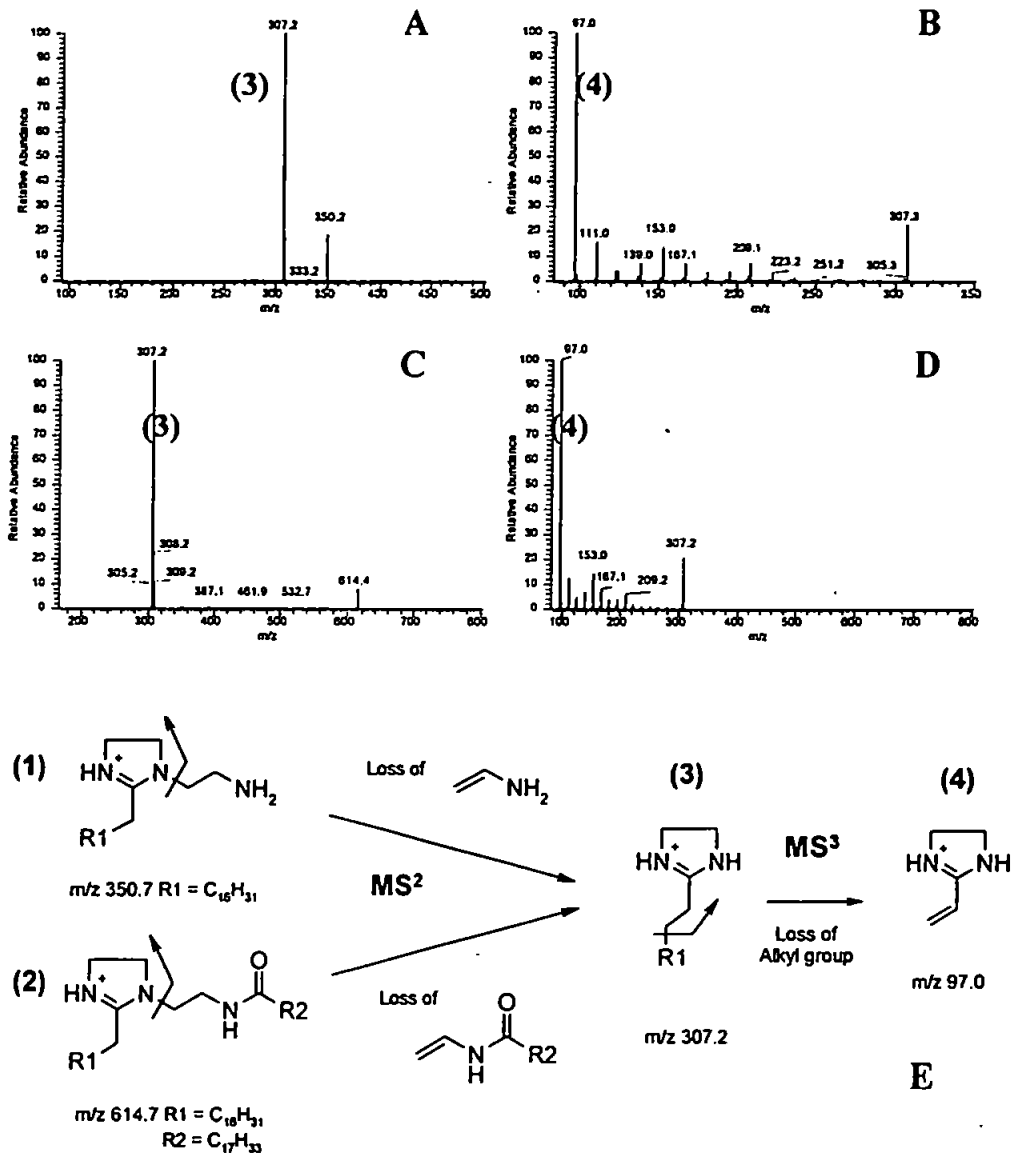


Fig. 10. Positive ion ESI-MS^n mass spectra of ions *m/z* 350 and 614 shown in Fig. 9 and CID MS^n fragmentation pathway for CI-A3 oilfield corrosion inhibitor: (A) MS^2 on precursor ion *m/z* 350.7; (B) MS^3 on product ion *m/z* 307.2 in A; (C) MS^2 on product ion *m/z* 614.7; (D) MS^3 on product ion *m/z* 307.2 in C and (E) proposed CID fragmentation pathway.

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