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Modern Mass Spectrometric Techniques Applied to Occupational Hygiene Analysis

By

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Abstract

The identification of chemical hazards is of significant importance in occupational hygiene. The concentration at which a chemical substance may be regarded as a hazard may be very low and as a result of this the demands on analytical techniques to determine the presence of a chemical substance can be great. Mass spectrometry is an analytical technique with a broad range of applications, described as the universal detector; the technique offers high sensitivity and high selectivity. Although an established technique in many areas only limited applications have been reported in the field of occupational hygiene analysis.

The aim of this work was to apply modern mass spectrometric techniques to analyse samples arising from occupational hygiene monitoring. Liquid Chromatography/Mass Spectrometry (LC/MS) was used with Electrospray Ionisation (ESI) and Atmospheric Pressure Chemical Ionisation (APCI). Nanospray ionisation was used for the direct infusion analysis of isocyanate samples. Matrix assisted Laser Desorption/Ionisation (MALDI) has been demonstrated as an ionisation technique suitable for application to occupational hygiene sample analysis. Tandem mass spectrometry (MS/MS) was used where necessary to identify unknown species.

Benzalkonium Chloride (BAC) has biocidal properties and for this reason the substance is used in many products such as in-can preservatives and ophthalmic cleaning systems. The phenyl substituent of BAC facilitates detection by spectrophotometric methods. Other quaternary ammonium compounds, that have no chromaphore, are used in preparations also containing BAC. The detection of these compounds can be a problem. In this work an LC/ESI/MS method is presented for the analysis of mixtures of BAC and other non-chromaphoric quaternary ammonium compounds. Excellent limits of detection were achieved e.g., 3 $ng.ml^{-1}$ for the C_{12} homologue of BAC. MS/MS and cone voltage dissociation techniques were used to identify an unknown quaternary ammonium compound.

Iodopropynylbutylcarbamate, a wood treatment agent with fungicidal properties, was analysed using LC/MS with APCI and ESI techniques. The effect of the cone voltage on the ionisation efficiency was studied, as were the effects of the wood treatment formulation matrix. Limits of detection of 3.19 $\mu g.ml^{-1}$ using APCI and 8.95 $\mu g.ml^{-1}$ using ESI were calculated.

Isocyanates are the number one cause of occupational asthma in the U.K. 1-(2-methoxyphenyl)piperazine derivatised isocyanate monomers and prepolymers were studied using LC/ESI/MS, nanospray and MALDI mass spectrometric techniques. Tandem mass spectrometry was used to facilitate structural elucidation of several derivatised prepolymeric isocyanate species.

The surfactant composition of a commercial sheep dip formulation was analysed using MALDI and LC/ESI/MS. The major surfactant constituent of the sheep dip formulation was identified as ethoxylated phenol. Mass spectrometry is shown to be a technique well suited to the analysis of nonionic surfactants

CONTENTS

This thesis describes the role of selected modern analytical techniques in the analysis of samples that result from occupational hygiene monitoring. The techniques used involve liquid chromatography coupled with UV/Vis absorbance and mass spectrometric detectors. The aforementioned techniques offer advantages which may be utilised in many fields including the analysis of occupational hygiene samples; however, the much praised mass spectrometric [1] detector has not been fully exploited in this field. The work presented is the result of three years work during which period the potential role of mass spectrometry for occupational hygiene analyses was investigated.

Occupational hygiene is concerned with the prevention and control of hazards arising from work activities [2]. The goals of occupational hygiene include the protection and promotion of workers' health and the protection of the environment [3]. Work is generally considered to be essential for life, development and personal fulfilment. Unfortunately, indispensable activities such as food production, extraction of raw materials, manufacturing of goods, energy production and services involve processes, operations and materials which can, to a greater or lesser extent, create hazards to the health of workers and those in nearby communities, as well as to the environment [4]. The exposure of individuals to hazards is minimised through adequate hazard control interventions, which not only protect workers' health but also seek to limit the damage to the environment which is often associated with industrialisation. It is in an employer's interest to provide a safe working environment for employees as the consequences of accidents can result not only in financial penalties but also damage the

employer's public image [5]. The need for occupational hygiene in the protection of workers' health cannot be overemphasised. The potential for harm associated with work involving chemical substances is great. In many cases the chemical hazard may not be apparent until harm is done. Often a feeling of discomfort can be an individual's first indication of a problem.

1.1.1 *Exposure*

Exposure to a toxicant poses a risk to the health of individuals who may or may not be aware of a health-affecting incident [6]. The toxic effects of an agent may be broadly defined as chronic or acute [7-9]. Acute effects are associated with incidents of abnormal contact with a high concentration of a hazardous substance. Such incidents can be the result of accidents (e.g. spillages or leakages), or a change from the normal working routine (e.g. maintenance) [7]. Chronic toxicity is the result of repeated exposure to a hazardous substance at a concentration sufficient to have a compounded detrimental affect to health. Chronic toxic effects can result from poor working practice (e.g. poor house keeping) or the failure to recognise a hazard in the workplace [7]. Conditions associated with exposure to toxic material include irritation, mutagenesis, teratogenesis, carcinogenesis, organ damage and reproductive constraints.

The main work-place exposure routes for chemical and biological agents are dermal and respiratory. Oral ingestion must also be considered as a less common but equally threatening mode of exposure [10]. Gases, vapours, aerosols, volatile solvents, liquids, dusts and fibres are common to many industrialised processes as raw materials or by-products of manufacturing processes. The skin and lungs may come into contact with

substances in all of these states and the substances can either be absorbed or cause local toxic effects. The degree of entry into the circulatory system is determined by many factors, including the toxic chemical's concentration, exposure duration, the solubility and reactivity of the substance in the blood and body tissues, and the excretion/secretion rate of the chemical from the body [11]. The inhalation of toxic substances into the air passages of the lungs and on into the circulatory system is a rapid and a direct means of entry into the bloodstream. The high rate of absorption is a result of the large surface area of the pulmonary alveoli, richly provided with capillaries [12].

Acute inhalation of solvents in large quantities can cause asphyxiation, unconsciousness or death [13]. Inhalation of large quantities of very irritant substances, such as methyl isocyanate, may cause immediate bronchoconstriction and pulmonary oedema leading to death [14]. Each of these pulmonary effects is usually the result of short term high level accidental exposure and these effects are less common than chronic industrial diseases. Over the long term, exposure to air-borne hazards can result in cancer or debilitating respiratory disease [15]. Sensitisation may also be a problem following inhalation exposure where it may lead to a systemic effect such as asthma [16]. Sensitisation is essentially an irreversible allergic reaction. A sensitised individual will exhibit symptoms of exposure at concentrations of a toxicant well below those of a non-sensitised individual. Sensitisation may affect not only the health of an employee but also their employment prospects [17].

Statistics published annually by the Health and Safety Executive, HSE, quote the number of new cases of occupational asthma in 2001 at 797 [18]. A Self-Reported

Work-Related Illness Survey conducted in 1995, SWI 95, [19], in which all cases were assessed, puts the number of persons suffering from asthma symptoms, which they believed to be work related, at 151,000. SWI 95 provides an interesting point of reference for all areas of occupational illness as it was a survey of the entire working population, and the focus of attention was not just new cases of illness. The average incidence of new cases of occupational asthma over three years (1998-2000) was 911, or around 3 cases per 100,000 [18] workers per year. Isocyanates are the most common agents responsible for occupational asthma [18]. The occupation with the highest incidence rate of occupational asthma reported to chest physicians was spray painters (72 cases per 100,000 workers per annum), where the estimated rate was over 30 times the national average [18]. The dangers associated with spray painting are well documented [20]. Occupational asthma has been defined by Bernstein as:

"A disease characterized by airflow limitation and/or airway hyper-responsiveness due to causes and conditions contributable to a particular occupational environment and not to stimuli encountered outside the workplace." [21].

Occupational asthma occurs when workplace exposures to particular substance/substances result in biological changes in a person's airways, known as a hypersensitive state, so that subsequent exposure to the same substance triggers an asthma attack [22]. The mechanism by which sensitization occurs varies from one substance to another; however, the fundamentals of the mechanism are now understood [23]. The range of industries which use substances with the potential to cause asthma is quite broad, and not all employees in these industries will necessarily be exposed. It is therefore difficult to estimate with any confidence the total number of workers at

risk. Monitoring of occupational asthma precursors in the workplace offers a means of reducing the number of cases by allowing measures to be taken to remove the source of the toxicant. It may be necessary for example to reassess the working practice and/or the design of a particular manufacturing process.

Meredith *et al.* [24] have estimated that the true incidence of occupational asthma diagnosed by consultant physicians may be as high as 1500-2000 or 6-8 cases per 100,000 workers, per year. The incidence of occupational asthma and its related precursors is a constant area of concern for employers and enforcement agencies alike. As a result of this, research in the area persists [25]. The need for sensitive and specific analytical methods for determining possible airborne toxicants and affiliates of respiratory dysfunction is extremely important.

Examples of airborne industrial toxicants are toluene diisocyanate [26-29], carbon monoxide [30, 31] and some fibrous materials such as asbestos [32]. Toluene diisocyanate is a commonly cited sensitising agent widely used in industry as a starting material in the manufacture of polyurethanes [26-29]. Carbon monoxide (CO) is a gas phase toxicant. Its toxicity is a consequence of tissue hypoxia created by the displacement of oxygen from haemoglobin and the subsequent impairment of oxygen release to the tissue [30]. Early symptoms of CO exposure include tightness across the forehead and a slight headache. The later symptoms of CO exposure involve severe headaches, vomiting, collapse of the lungs and ultimately death; which is essentially asphyxiation [30, 31]. Toxic substances that deposit in the lung can cause the irreversible formation of fibrous tissue, reducing the lung capacity. Asbestos and

mining dust are examples of such substances. For a review of asbestos toxicity see review [32].

The skin is composed of epidermis, dermis, and hypodermis layers. The relatively waterproof epidermis, or outer skin layer, prevents the diffusion of fluids and is tough enough to withstand abrasive contact. The dermis, which is thicker than the epidermis, provides tear resistance and flexibility to the skin. The even thicker hypodermis layer contains the blood vessels and nerves. The sweat glands penetrate both the dermis and epidermis [33]. Toxic substances have difficulty entering the body through the skin because of the epidermis layer [34]. Chemical diffusion through the epidermis layer depends upon the nature of the chemical substance as well as the skin thickness and its condition at the point of contact. Skin that has been damaged by physical abuse or chemical reaction is more vulnerable to toxic effects of a substance than undamaged skin as diffusion across the epidermis may occur more readily.

Skin contact with a hazardous substance is a common problem in many industrial processes [13]. The most prevalent occupational disease is dermatitis and this accounts for more working days lost in the U.K. than all other industrial diseases together [18]. Dermatitis is an inflammatory condition which can be caused by irritants or allergens [35]. The number of potential irritants is large and includes many different types of chemical substances such as acids, alkalis, metals, solvents and solid organic and inorganic chemicals [36]. There are a large number of possible interactions between potential irritants and the skin. Solvents will degrease skin, whereas acids and alkalis will denature skin proteins [37]. Skin sensitizers act via an immunological mechanism to cause contact dermatitis. The substance may pass through the epidermis and react

with proteins such as keratin, to produce an antigen. This "foreign", antigenic protein then initiates the production of antibodies. Re-exposure to the substance will then initiate an allergic reaction [38].

The best example of prevalence of work-related skin disease comes from the SWI 95 [19]. This provides an estimate of the prevalence of self-reported work-related skin disease of 66,000 workers. In terms of the current situation with regard to work-related skin disorders, figures provided by Occupational Physicians Reporting Activity (OPRA) and EPIDERM, a group responsible for the surveillance for occupational skin disease by dermatologists, report an estimated 4540 workers each year in the period 1998-2000 with work-related skin disorders. Approximately 78% of the reported cases were diagnosed as contact dermatitis [18]. EPIDERM and OPRA provide information on the incidence of work-related skin disease and contact dermatitis through voluntary surveillance schemes

The occupations estimated to be at highest risk in 1998-2000 according to dermatologists reporting to EPIDERM are hairdressers and barbers, grinding machine setters and operators, electroplaters, galvanizers and colour coaters, rubber process operatives and printers [18]. During 1998-2000, the most common agents associated with occupational skin disease cited both by dermatologists and occupational physicians are rubber chemicals and materials, followed by wet work, soaps and cleaners, respectively [18].

To reduce the risk and number of incidents of exposure, the HSE publishes guidelines for the safe use of specific substances. Exposure Assessment Documents are published

by the HSE and are aimed at a technical audience. They report scientific and technical information that has been collected by the HSE in respect of work on a specific substance or the results of work on a particular subject. Most of these documents will report the exposure assessment which contributes to the setting of an occupational exposure limit (OEL) for a specific substance. Two types of exposure limits are used: the maximum exposure limit (MEL) and the occupational exposure standard (OES) [39]. A description of the criteria used to determine which type of limit is applicable is given in HSE publications EH40, Occupational and Exposure Limits [36] and EH64, Summary Criteria for Occupational Exposure Limits [40].

1.1.2 *Legislation*

From its origins in the early nineteenth century the development of Health and Safety Legislation has been a constant process. The 1961 Factories Act [41] sought to consolidate previous legislation with the production of a single code of conduct that could be applied to the diverse factory industries developing at that time. The diverse nature of work activities had previously produced rules and regulations which were tailored to specific subsets of industry e.g. the Mines and Quarries Act 1954 [42] and the Agricultural (Poisonous Substances) Act 1952 [43] to protect miners and agricultural workers, respectively. The first all-encompassing act was published in 1974 i.e. The Health and Safety at Work Act (HASWA) [44].

HASWA [44] describes the role of both employer and employee in the acquisition and maintenance of a working environment which is both practical and conducive to a safe working practice. It describes the role of employees and employers in the maintenance

of a safe working environment. Employees are bound to obey the company health and safety policy and the company is bound to have a health and safety policy. Thus a safe working environment is created for all involved in the work activity. Examples of compliance include the responsibility of the employee to wear or use any personal protective equipment provided by the employer, and the responsibility of the employer to provide such equipment as is necessary. HASWA not only concerns itself with the physical safety of persons involved in a work task but also the premises on which that task is to be executed and also any waste products, emissions, from that task. Any substances that may be emitted as a result of a task must be rendered harmless and ineffective to avoid pollution of the external environment. Also, emission-related adjectives such as "noxious or offensive" are not confined to the description on a label, but include what is perceived by a user or third person to be noxious. An important statement in HASWA with regards the role of the employer to the employee is:

"It shall be the duty of every employer to ensure, so far is reasonably practicable, the health, safety and welfare at work of all his employees" [44].

This statement essentially describes the responsibility of the employer to the employee and emphasises the liability of the management of a company if the company is subject to a health and safety incident.

There are numerous Acts of parliament governing the safety of employees in the workplace. The Acts themselves contain every detail of how the content and context of an act will be enacted. Instructions to industry on how to implement the requirements of the Act are given in Regulations. Regulations can be divided into four

types; General in Scope, e.g. Reporting of Industrial Diseases and Dangerous Occurrences Regulations 1995 (RIDDOR) [45]; Health and Safety (First Aid) Regulations 1981 [46]; Limited in Scope, e.g. Control of Lead at Work Regulations 1980 [47]; Functional, e.g. Electricity at Work Regulations 1989 [48], Food Hygiene Regulations 1970 [49]; and Industry Related, e.g. Construction (Design and Management) 1994 (CONDAM) [50], Pottery (Health and Welfare) Special Regulations 1980 [51]. Practical guidance explaining how industry can conform to the detail of the respective regulations is provided in the form of Approved Codes of Practice (ACOPs), e.g. [52].

In the case of operations involving chemical substances health and safety regulations are reinforced by the Control of Substances Hazardous to Health Regulations (COSHH) [52]. Substances that may cause harm to health are subject to COSHH. The basic principle underlying the COSHH regulations is that the risks associated with the use of any substance hazardous to health must be assessed before it is used and the appropriate measures taken to control the risk. A hazardous substance is defined by the COSHH regulations as one which falls into any of five categories:

1. Any substance which is classified as toxic, very toxic, irritant, harmful, corrosive, flammable, explosive or radioactive under the Chemicals (Hazard Information and Packaging) Regulations 1996 (CHIP) [53].
2. Any substance which has a Maximum Exposure Limit (MEL) or Occupational Exposure Standard (OES).
3. Any micro-organism that creates a hazard to the health of any person.
4. Any dust particles in a substantial airborne concentration.

5. Any other substance not mentioned above that creates a comparable risk of ill-health effects to any person.

The COSHH Regulations cover any hazardous substance that is included in the five categories above, and includes any natural or artificial substance that is solid, liquid, dust, gas or vapour. An employer is required by the COSHH regulations to prevent, or if this is not reasonably practicable, adequately control, employees' exposure to hazardous substances. The occupational exposure limits set by the HSE are expressed as concentrations of a hazardous substance in the air, averaged over a specified period of time referred to as a time weighted average (TWA). Two time periods are used: 8 hour (based on average shift length), and 15 minute short term exposure limits (STEL) which are set up to help prevent effects, such as eye irritation, which may occur after just a few minutes exposure. OES are legally binding as they are approved by the Health and Safety Commission. A MEL is set for substance which may cause the most serious health effects, such as cancer and occupational asthma. To comply with COSHH, exposure should be reduced as far below the MEL as is reasonably practicable and should not exceed the MEL when averaged over the specified reference period. For substances given a short term MEL (15 minute reference period), the level of exposure should never be exceeded. The OEL values relate to effects resulting from inhalation. However, certain substances may rapidly penetrate intact skin, become absorbed into the body and exert, or contribute to, adverse health effects. When the effect of a substance on the skin is a significant hazard e.g., for strongly irritating substances, it is assigned the 'skin' notation (Sk), as well as an OES.

An OES is set at a level at which (based on current scientific knowledge) there is no indication of risk to the health of workers who are exposed to a particular substance on a regular basis. If exposure to a substance that has an OES is reduced at least to that level, then adequate control has been achieved. For some toxic substances, biological monitoring may also be appropriate as a means of assessing whether exposure is being taken into the body by breathing or through the skin [54]. Measurements of substances (or their by-products) in blood, urine or expired air are commonly used. Biological Monitoring Guidance Values (BMGV) are set to help employers, safety representatives and employees to interpret biological monitoring results but they have no legal status. MEL, OES and BMGV are set on the recommendations of the Health and Safety Commission (HSC) Advisory Committee on Toxic Substances (ACTS) and its Working group on the Assessment of Toxic Chemicals (WATCH). COSHH is omnipresent in industry with no employer, no matter how small the workforce, or whatever the industry, being exempt.

An example of substances with a MEL is the polychlorinated biphenyls (PCB); the maximum exposure limit for which is 0.1 mg. m^{-3} for an 8 hour (TWA) period. PCB are given the skin notation (Sk) reflecting their irritant status [36]. PCB are a group of highly lipophilic hepatotoxic compounds with the same general formula, $C_{12}H_{10-n}Cl_n$. PCB have been proven to be extremely toxic to aquatic organisms [55]. They have very high chemical, thermal and biological stability; low vapour pressure; and high dielectric constants. These properties have led to the use of PCB as cooling liquids in electrical equipment, particularly transformers and capacitors. Occupational exposure is most likely to occur during the removal, decontamination and disposal of PCB-containing transformers and capacitors. This work is generally carried out by specialist

companies or trained in-house staff. Regular exposure is therefore restricted to a small population. However, a minority of transformer and capacitor replacements is carried out by general electrical contractors. Occupational exposure to commercial PCB preparations can result in cutaneous effects such as chloracne and eczema.

Carbofuran and malathion, (Figure 1a and 1b), are examples of substances with OES: 0.1 $mg.m^{-3}$ and 10 $mg.m^{-3}$ for long term, 8hr, exposure, respectively. Carbofuran is a broad spectrum systemic carbamate insecticide, nematicide, and acaricide commonly used throughout the world [56, 36]. Malathion is an organophosphorus pesticide that is widely used for both domestic and commercial agricultural purposes. It is considered to be one of the safest organophosphate insecticides [57, 36]

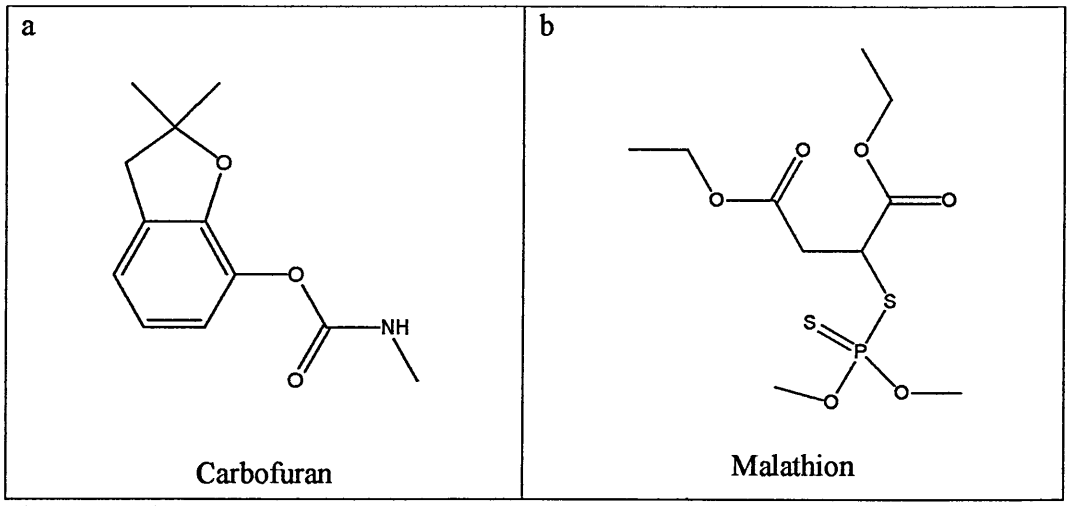


Figure 1. The structural formula of a common carbamate pesticide, carbofuran, and a common organophosphorus pesticide, malathion.

Pesticides are an instance of a class of compounds where separate legislation is used in conjunction with COSHH to enforce exposure limits. Pesticide legislation in the U.K. is in the form of The Food and Environment Protection Act 1985 (FEPA) [58] and Control of Pesticides Regulations 1986 (COPR) [59]. These Acts govern the

advertising, sale, supply, storage and use of pesticides. European legislation also heavily regulates the use of pesticides. The European Council (EC) Directive 91/414/EEC [60], the Plant Protection Directive, was introduced to harmonise national arrangements for the authorisation of plant protection products within the European Union. Under the provisions of the directive, individual member states are responsible for authorisation within their own territory of products containing active substances that appear in a list agreed at Community level. Only officially approved pesticides may be marketed in the United Kingdom. The official list of approved products, including all the above categories except those for experimental purposes, is the Ministry of Agriculture Food and Fisheries/Health and Safety Executive publication, *Pesticides 2001; Your Guide to Approved Pesticides* [61]. Pesticides are subject to exposure limits other than OES and MEL. Maximum Residue Levels (MRL) are levels set to prevent exposure to the number of pesticides which leave a residue even when used correctly [62]. MRL provide a check that products have been used as directed; they are not safety limits. However, they do take account of consumer safety because they are set at levels that ensure that normal dietary intake of residues presents no risk to health. Wide safety margins are built in and eating food containing residues above the MRL does not automatically imply a risk to health. Nevertheless, it is an offence to put into circulation any produce where the MRL is exceeded. Details of those MRL that have been set have been published in the Pesticides (Maximum Residue Levels in Crops, Foods and Feeding Stuffs) (England and Wales) Regulations 2002 [62]. An example of a pesticide MRL is 0.1 mg residue/kg food on fruits, vegetables, pulses, oilseeds, potatoes, tea and hops for atrazine. Atrazine is a triazine herbicide with residual and foliar activity (Figure 2). It has restricted permitted use [61-63].

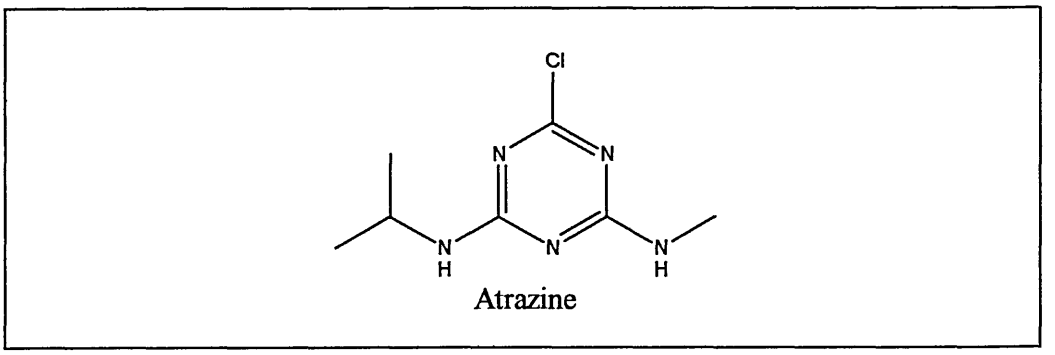


Figure 2. The structural formula of a common triazine herbicide, atrazine.

The European Commission passes down many laws in the form of European Community (EC) Directives that also act to provide a safe working environment. These Directives have no power over employers in the United Kingdom but are instructions to the governments of member states to implement a particular aspect of EC law [64]. In the United Kingdom, the power comes from the fact that the Government issues Regulations under an appropriate Act of Parliament. The European Directive 89/391/EEC [65] lays down the minimum requirements covering the prevention of occupational risks. The directive is backed by a number of 'daughter' directives which are specific to types of equipment and the requirements covering them. An example daughter directive is EC Directive 89/656/EEC covering the use of personal protective equipment, (PPE), in the workplace [66].

1.1.3 *Case Study*

Recent European legislation has isolated "Biocides" as a class of compounds which need to be regulated by member states. The Biocidal Products Directive (BPD) 98/8/EC [67] is the directive which is enforcing this regulation. The directive aims to

establish a single European market in biocides and to provide a high level of protection for people and the environment [69]. Biocidal products are defined as:

"Active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical and biological means" [67].

Biocides are essentially non-agricultural pesticides. This class of compounds was left unregulated by the Plant Protection Directive and so the biocide directive is necessary to cover this grey area. Biocidal products include such preparations as rodenticides, insecticides, avicides and moluscicides. The BPD is modelled on the Plant Protection Directive, despite the fact that biocidal product uses are quite different from crop protection applications [69].

Active substances in biocides have a desired effect, typically a chemical activity, e.g., disinfection of drinking water by killing bacteria and viruses in the water. Such desired effects are very important for general public health and without them significant public health problems may occur [70]. Chemical biocides also fulfil a key role in the preservation of products as diverse as cutting fluids, foods and beverages, cosmetics and pharmaceutical formulations and afford protection against spoilage in a wide range of industrial and environmental applications [71]. Biocides may exert both bacteriostatic and bactericidal effects, although the mechanism of action responsible for each may differ. Bacteriostatic events are generally considered to arise from some metabolic injury which is reversible upon removal or neutralisation of the biocide,

whereas bactericidal action results in irreparable and irreversible damage to a vital cellular structure or function [71].

One particular area where the negative effects of biocidal products are well documented is anti-foulant coatings. Biocides have been used for many years to prevent organisms such as bacteria, larve, macroalgae, barnacles and other invertebrates [72] from becoming encrusted on fresh and seawater marine structures, such as oil rigs supports, buoys, fish cages and ships hulls [73]. Chemicals including organo-mercury compounds, lead and arsenic, were historically used as anti-foulants. However, such compounds pose severe environmental and human health risks and were withdrawn voluntarily by the paint industry during the early 1960s [74]. A suitable replacement for these compounds was a group of organotin congeners and particularly tributyltin (TBT) [75]. The contamination generated by the direct introduction of biocides, mainly organotins, and more specifically of TBT into the aquatic environment from the leaching of anti-fouling paints, is well established [75, 76]. The introduction of organotin compounds into seawater has resulted in higher accumulation levels of organotin compounds in marine products [77]. The uptake mechanism is via food chain and/or direct absorption because of the high lipophilicities of the organotin congeners [78]. Despite various degradation pathways and the evidence of decreasing concentrations in the water column in some places around the world, TBT contamination is still considered to be one of the most important ecotoxicological problems of the last two decades [77].

The adverse effect of organotins on non-target aquatic organisms [78-82] combined with their persistence in the environment has led to regulations on the use of organotin

based anti-foulant paints in many countries. At the end of the 1980s, TBT and other organotin-based anti-foulants were banned by the European Union in small vessels (<25m) [83]. This ban was extended in 2002 to cover any organotin-based free association paint and any anti-foulant for use on, or as:

- All craft irrespective of their length intended for use in marine, coastal estuarine and inland waterways and lakes.
- Cages, floats, nets and any other appliances or equipment used for fish or shellfish farming.
- Any totally or partly submerged appliance or equipment.
- Constituents of preparations intended for use in the treatment of industrial waters [84].

Thus the use of organotin based anti-foulants has been effectively banned in the European Community.

In the absence of the anti-fouling potency of TBT, a copper compound such as cuprous oxide (Cu_2O), copper thiocyanate (CuSCN) or metallic copper is utilised as the principal biocide. Copper exhibits anti-fouling activity against organisms such as barnacles, tube worms and the majority of algal fouling species. However, several algal species (e.g. *Entermorpha spp., Ectocarpus spp., Achnanthes spp.*) show marked physiological tolerance to copper [85]. In order to achieve protection against these tolerant species, various booster biocides have been used in conjunction with copper to control copper-resistant fouling organisms.

Examples of organic booster biocides include chlorothalonil, Irgarol 1051, zinc pyrithione, Kathon 5287, TCMTB and TCMS pyridine (Figure 3). There is little known about the eco-toxicity, or environmental fate of these compounds and it is argued that the dissipation of the booster biocide from the surface to the surrounding waters, with subsequent exposure of non-target algae, could have similar consequences to those experienced with organotins. In the absence of organotin-based anti-fouling products concern has increased about the concentrations of copper in the aquatic environment.

Irgarol 1051 is added to anti-foulant products because of its high effectiveness as a growth inhibitor of marine and freshwater algae through interactions with their photosynthetic system [86]. There have been several studies involving the monitoring of Irgarol 1051 in coastal waters with positive results reported from the analyses of samples taken from the Mediterranean [87], English [88] and Scandinavian [89] coastal regions. These studies have found elevated levels of Irgarol 1051 contamination in areas of high recreational and commercial boating activity.

As a result of concerns regarding the levels of booster biocides in marine samples methods have been developed for the analysis of a broad spectrum of biocidal products in aqueous samples. Thomas reports methods for the analysis of Kathon 5287 and TCMTB, in aqueous samples [90] and Voulvoulis *et al.* have reported methods for the determination of chlorothalonil, Irgarol 1051, zinc pyrithione, Kathon 5287, TCTMB and TCMS Pyridine [91].

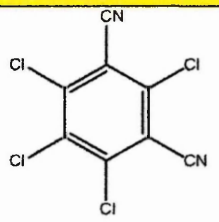
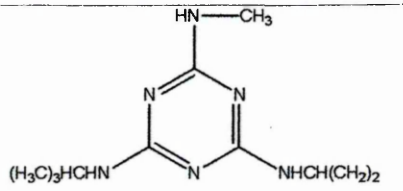
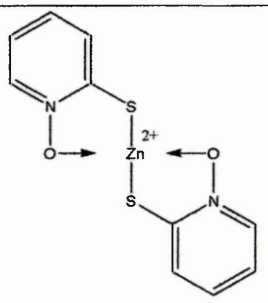
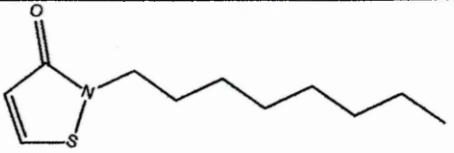
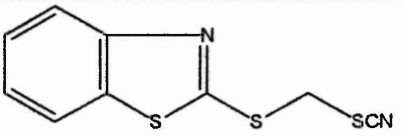
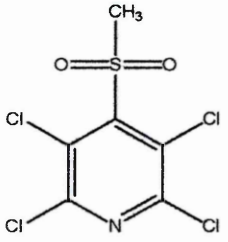
Common Name	Chemical Name	Structure
Chlorothalonil	2,4,5,6-tetracholoro-1,3,dicyanobenzene	
Irgarol 1051	Cyclopropyl-N'-(1,1-dimethylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine	
Zinc Pyrithione	Zinc pyridine-2-thiol-1-oxide	
Kathon 5287	2-n-octyl-4-isothiazolin-3-one	
TCMTB	2-thiocyanomethyl-benzothiazole	
TCMS Pyridine	2,3,5,6-tetrachloro-4-sulfuronyl pyridine	

Figure 3. Common name, chemical name and structural formula for several booster biocides. Booster biocides are used as replacements for the heavily regulated organotin compounds in the formulation of anti-foulant preparations.

The case of anti-foulant paints highlighted above is just one example of an area affected by the Biocidal Products Directive. With many other areas of industry relying on processes which are liable to bacterial infection with associated negative effects, it is important that methods are in place for the accurate determination of biocides in the working environment.

1.2 Conclusion

Exposure to chemical substances in the occupational environment and its adverse consequences is an issue of great importance. The exposure of an individual to a substance having a detrimental affect on their health is unpleasant and unnecessary. The close monitoring of the working environment and the encouragement of employers and employees to operate within a safe environment is one method of reducing cases of chemical exposure incidents. Using legal tools such as HASWA and COSHH it is possible to enforce safe working practice in the United Kingdom.

An understanding of the toxicity of chemical substance allows the HSE to establish occupational exposure limits (OEL). OEL are a method of controlling the exposure of individuals to a chemical substance. OEL are legally binding, and so can be viewed as another legal tool to improve safety in the workplace. In the case of pesticides exposure limits other than OEL are used. MRL are used to limit exposure to residual pesticides which may remain on a plant product after use.

Organotins have been used as an example to demonstrate the effectiveness of suitable regulations in dealing with a chemical hazard problem. In this case the link between

organotin anti-fouling products and their negative environmental effects has been well documented. The use of organotins has been extremely restricted in Europe. The common replacement for organotins is copper based anti-fouling products; the effects of these on the environment are now being studied.

The role of analytical chemistry in occupational hygiene monitoring is significant. The capability to detect a chemical substance in the working environment can prevent and where necessary identify a chemical hazard. The continuing development of analytical methods for the analysis of samples taken from occupational hygiene monitoring ensures that safe working practice is adhered to in all working environments.

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2.0 An Introduction to High Performance Liquid Chromatography and Mass Spectrometry

Liquid chromatography (LC) is a separation technique where analyte molecules are separated based on their interactions with two phases; a mobile liquid phase and a solid stationary phase.

2.1.1 *High Performance Liquid Chromatography*

High Performance Liquid Chromatography (HPLC) is an established separation technique with a wide range of modes of operation and varied applications. The technique is essentially a method for the separation of the components of a mixture by using the affinity of the individual components for either a solid stationary phase or a liquid mobile phase. The relative affinities of the components of a mixture for either phase will result in different rates of migration through the stationary phase. Compounds favouring the mobile phase will elute faster than those favouring the stationary phase. In liquid chromatography the stationary phase is packed into a column (commonly stainless steel) of varying dimensions depending upon the application. On a preparative scale, chromatography can be used to isolate individual fractions of mixtures and in this way can be considered similar to distillation processes (Column dimensions in this case are typically of the order 60 x 300 mm). On the analytical scale, chromatography offers a method for the identification of the components of a mixture by resolving the individual species present prior to detection by a suitable technique (Column dimensions here are typically $\leq$4.5 x $\leq$300 mm). The dynamic relationship between the solute species and the mobile and stationary phases is fundamental in the separation mechanism.

2.1.2 *Modes of Liquid Chromatography*

Chromatography can be controlled by the selection of the mechanism of interaction between the analyte and the stationary phase. This is termed the mode of the separation. The mode of chromatography selected for an analysis describes the best available conditions conducive to such interactions as are necessary to separate the desired components of a mixture. The elution of an analyte through the chromatographic system may be by either isocratic or gradient elution methods. Isocratic chromatography involves constant conditions for the separation. In gradient elution chromatography, solute elution is accomplished by varying the parameters of the solvent flow. Gradient chromatography elution in liquid chromatography commonly involves variation of the mobile phase composition from low elution strength to high elution strength. Gradient chromatography may also involve the variation of temperature or flow rate. The stationary phase in liquid chromatography is a major contributing factor to the separation process. (Further examination of the importance of stationary phase media is given in Section 2.1.4 *Chromatographic Theory*). The stationary phase in column chromatography may be referred to as the column packing although this is generally used to imply consideration of the interstitial uniformity of the stationary phase in the column.

2.1.3 *Partition Chromatography*

The term partition chromatography refers to the retention mechanism employed in the chromatographic separation process. There are two forms of partition chromatography, normal and reverse phase. In normal phase partition chromatography

the stationary phase is a polar, and the mobile phase a non-polar medium. The opposite is true for reversed phase partition chromatography. The origin of retention in normal-phase chromatography is the interaction of polar functional groups of analyte molecules with polar functional groups on the surface of the stationary phase. This interaction is mediated by the interaction of the mobile phase with both solute and stationary phase molecules. Hydrogen bonding and dipole-dipole interactions are important considerations in normal phase chromatography. In reversed phase chromatography the retention mechanism is dependent upon the hydrophobicity of the stationary phase and the solute molecule; however, dipole-dipole and hydrogen bonding interactions are also contributing factors to the separation mechanism. A strongly hydrophobic analyte will interact strongly with a hydrophobic stationary phase. The stationary phase in partition chromatography is commonly an organic moiety bound to a silica particle. Particulate silica acts as a centre for many of the organic moieties and provides a surface on which the retention mechanism may occur. The nature of the organic ligand is an important consideration in partition chromatography. Normal phase stationary phase media include polar functional groups such as aminopropyl and cyanopropyl. Reverse phase stationary phase media include hydrophobic moieties such as C_4, C_8 and C_{18} hydrocarbon chains. Interaction between the solute molecule and the stationary phase is further facilitated by acidic silanol groups on the stationary phase media. Thus pH is an important consideration in method development. The cross compatibility of the relative stationary phases is heavily weighted in favour of reverse phase chromatography. There are many solvents available for partition chromatography. The choice of solvent is dependent on the type of partition chromatography. Common examples of solvents used in partition chromatography (in order of increasing polarity) include n-hexane, toluene, acetone,

tetrahydrofuran (THF), acetonitrile, methanol and water. Reversed phase partition chromatography is more widely used than normal phase because it is applicable to a broader range of compounds.

2.1.4 *Chromatographic Theory*

The relationship between an analyte species and the chromatographic media is fundamental in the retention and elution of a chromatographic peak. The affinity of the analyte for the stationary or mobile phase determines the rate at which it is eluted from the column. This dynamic relationship is expressed as the partition coefficient, *K*, defined by Equation 1, that relates the concentration of the analyte in the mobile phase (*Cm*) to the concentration of the analyte in the stationary phase (*Cs*) thus defining the separation for a single point in time.

$$K = \frac{Cs}{Cm} \qquad \text{...Equation 1}$$

In the chromatographic process, the solute molecules are introduced to the mobile phase by a suitable injection device and are subsequently deposited at the head of the column by the mobile phase. As the band of solute molecules moves along the column, it gradually spreads out. Figure 1 illustrates this process. The solute molecules are injected at time = 0, and elute after time = 4. Since the solute molecules spread out as they travel through the column, some arrive at the end of the column earlier than the majority and some later. The detector records the dispersed band of

solute molecules as an "ideally" Gaussian peak; the greater the dispersion of the solute molecules, the broader the peak that the detector records.

The three processes that contribute to the dispersion of the solute molecules in the mobile phase are flow profile effects including eddy diffusion (*A*), longitudinal molecular diffusion (*B*), and resistance to mass transfers (*C*). The van Deemter equation (Equation 2) is a summation of the three effects and can be used to describe the theoretical plate height (*H*) and its relationship with the mobile phase velocity and the stationary phase.

$$H = A + \frac{B}{v} + Cv \qquad \text{...Equation 2}$$

Longitudinal molecular diffusion results from the tendency of solute molecules to migrate from the concentrated centre of the band toward the more dilute regions on the periphery. Flow profile broadening effects include the phenomenon arising from the multitude of pathways available to a solute molecule to find its way through the column. The lengths of these pathways may differ significantly, and the residence time in the column for molecules of the same species varies according to the lengths of the paths they take. Dispersion due to longitudinal molecular diffusion takes place both in and opposed to the direction of flow. Mass transfer refers to the flux of analyte molecules from one phase to another. The rate of mass transfer governs the dispersion of analyte molecules during the chromatographic process. If the rate of mass transfer is high then the eluting analyte molecules will maintain a uniform distribution in the column. A slow rate of mass transfer will result in an increase in the distribution of the velocities of the eluting analyte molecules. This increase in the distribution of the

velocities of the eluting analyte molecules has the result of broadening the chromatographic band.

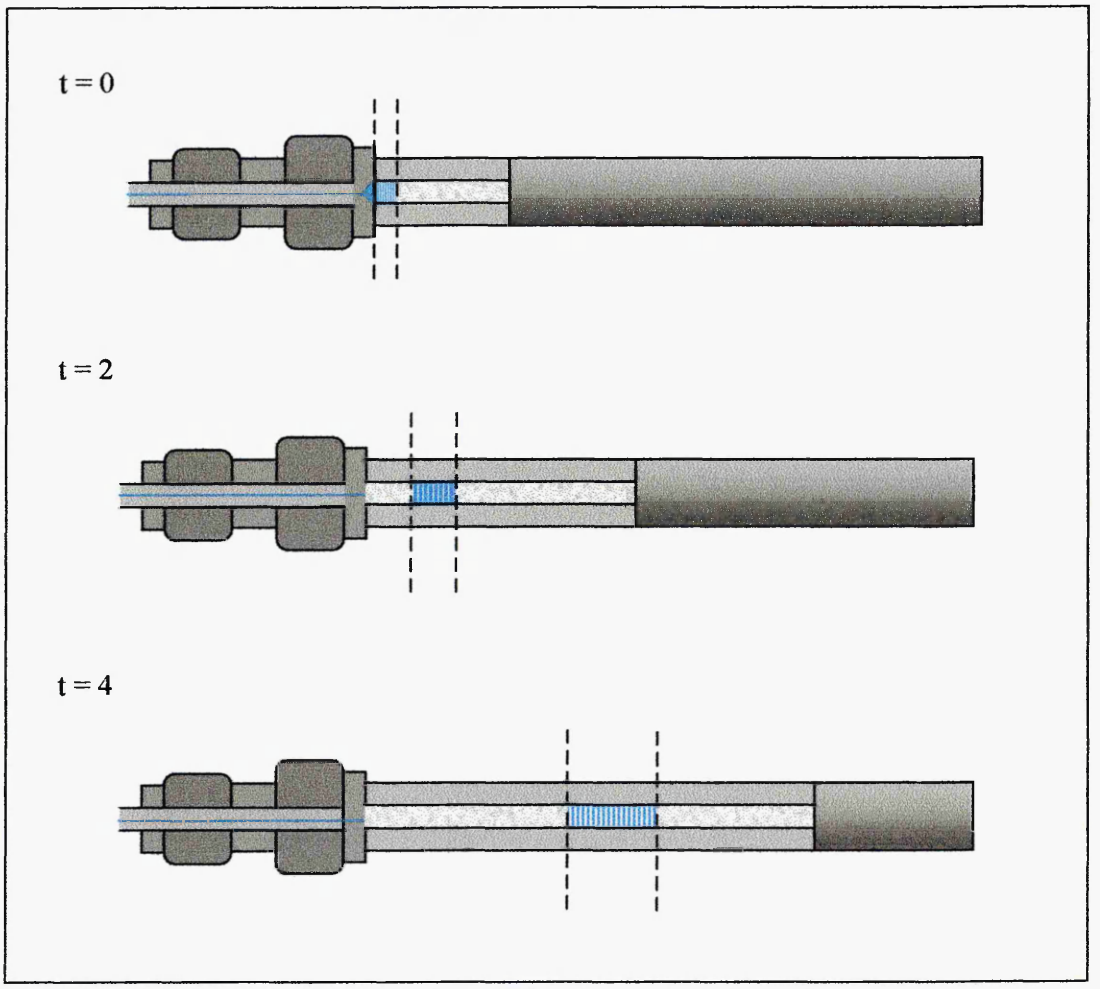

Figure 1. A schematic representation of the chromatographic solute elution process. The dashed lines show the broadening of the peak as it moves through the stationary phase packed column.

An important point of reference in chromatography is the column conditions. HPLC column conditions usually quoted are the column length, internal diameter (i.d.), stationary phase and stationary phase particle size. Reduction of the i.d. or bore, of the column is a useful method of reducing the total volume of the chromatographic column. Standard-bore columns are those with an internal diameter of about 4 or 5 mm, while narrow bore columns have an i.d. of about half that, around 2 mm. Micro-

bore columns have an i.d. of approximately 1 mm. Further reductions in column i.d. have produced capillary HPLC columns (i.d.<100 μm). The inherent benefits of reducing column i.d. are decreased solvent consumption and increased sensitivity. Capillary columns operate at flow rates substantially lower than those of conventional column chromatography (<10 $\mu l.min^{-1}$), hence the decrease in solvent consumption. The increase in sensitivity is because of the reduced volume of the column. The reduction in volume decreases the diffusion of the chromatographic band for samples that would in conventional chromatography be diffused, in the mobile phase, to such an extent that they would not be detected. Reductions in column i.d. require careful instrumental considerations. First, the liquid chromatograph must be capable of delivering low flow rates reproducibly and precisely. Second the system must have small internal volumes and be capable of producing small injection volumes (<5 μl). If these two conditions are not met, the advantages of using narrow bore columns are lost.

The size of the particles used in column packing is an integral part of the chromatographic dispersion process. Reducing particle size results in less dispersion of the sample in the column during the separation process. The flow path uniformity of solute molecules navigating a small particle is greater than that for a large particle. The result of greater uniformity in the flow path is a reduction in dispersion of the chromatographic peak which leads to a narrower and more favourable peak shape.

Dispersion in a HPLC system can be considered separately from the chromatographic interactions between solute molecules and the stationary phase. Instrumental factors are major contributors to band dispersion. These are primarily caused by system dead

volumes or extra-column volumes which are areas in a system where liquid flow is non-uniform. Extra column volumes are associated with areas such as connections and unions in system plumbing e.g. all connections from injector to column and from column to detector. Dispersion due to instrumental effects must be minimised to maintain the integrity of the chromatographic process.

The separation of two components is quantified by the peak resolution, (*Rs*). Resolution is defined by the ratio of the distance between two peak maxima, i.e. the distance between the two retention times, t_R and the arithmetic mean of the two peak widths, *w*. Peak resolution is described by Equation 3:

$$Rs = 2\frac{t_{R2} - t_{R1}}{w_1 + w_2} \qquad \text{...Equation 3}$$

A resolution of 1 will indicate the presence of two components although the peaks are not baseline separated. A resolution of 1.5 will clearly show two separate components of a mixture and is usually considered the upper limit of desired resolution. Resolution is a dimensionless parameter. When two peaks are just resolved to the baseline, the resolution, $Rs = 1.2$. When *Rs* is significantly less than 1.2, the peaks are not separated and this can interfere with quantitative analyses.

Resolution is based on both the distance between two peaks and their relative dispersion. The theoretical plate count (*N*) is a method of characterising the quality of chromatography by considering the dispersion of a single peak. The theoretical plate number, *N*, describes theoretically the number of sites of interaction between solute and stationary phase. The number of theoretical plates increases as a function of

increasing column length, mobile phase flow rate conditions, stationary phase quality and stationary phase packing. Whereas *N*, the theoretical number of plates, describes theoretically the number of interactions between solute and stationary phase, *H*, the height equivalent of a theoretical plate, describes theoretically the size of a single site of interaction on the column. To compare values of *H*, smaller values imply a larger number of plates in a column of fixed length. To allow inter-column comparisons a reduced parameter, *h*, can be used (Equation 4). This is a dimensionless form of the height equivalent to a theoretical plate. Reduced parameters allow comparison between columns under a broad range of mobile-phase conditions and over a range of particle sizes.

$$h = \frac{H}{d_P} \qquad \text{... Equation 4}$$

The retention factor is another method of comparing different columns, or the same column at different flow rates. The retention factor (*k'*) is measured as the retention time of an analyte (t_R) minus the retention time of a peak experiencing no interaction with the column stationary phase, an un-retained peak, (t_0) divided by the retention time of the un-retained peak (Equation 5).

$$k' = \frac{t_R - t_0}{t_0} \qquad \text{...Equation 5}$$

The retention factor (*k'*) is independent of the size of the column, and depends only on the mobile and stationary phases. Two components in a mixture cannot be separated

unless they have different k' values, the means of assessment being provided by the relative retention (α) also known as the separation factor as shown in Equation 7:

$$\alpha = \frac{k_1}{k_2} \qquad \text{...Equation 7}$$

If $\alpha = 1$ then no separation takes place as the retention times are identical. Relative retention is a measure of the chromatographic systems potential for separating two compounds, i.e., its selectivity.

2.2 Detectors for High Performance Liquid Chromatography

In the experimental work described in this thesis two of the many detectors available for HPLC were employed. These were the UV/Visible (UV/Vis) wavelength spectrophotometer and the mass spectrometer.

2.2.1 *UV/Visible Photometric Detectors for HPLC*

The function of a UV/Vis spectrophotometric system for HPLC is to determine the presence of individual components in an analytical sample based on the absorption of incident light on a sample in the liquid phase [1]. A single wavelength detector allows the analysis of the transmittance of only a single wavelength of light. Prior knowledge of the spectral characteristics of a target analyte allows the optimisation of the technique. Light is generated by a polychromatic light source, usually either a deuterium or tungsten lamp. The wavelength for the analysis is selected by a mono-

chromator. This may be a prism or a holographic diffraction grating. The monochromatic radiation then passes through the sample where some absorption may take place. The intensity of the transmitted radiation is recorded at the detector. The transmission of light through an absorbing liquid medium is governed by the Beer Lambert law. The Beer Lambert law, (Equation 8), states that the intensity of electromagnetic radiation transmitted through a sample at a given wavelength (A) is dependent on three parameters; *a*, molar absorption coefficient, *b*, molar concentration of the absorbing species, *c*, optical path-length (a constant).

$$A = abc \qquad \text{...Equation 8}$$

A single wavelength detector is limited in its ability to acquire absorbance data for only a single wavelength of light per chromatographic analysis. A photo-diode array (PDA) is a photometric detector capable of the simultaneous acquisition of multiple wavelengths of light within a given range for single analyses [2]. Light from a polychromatic light source is focused by a lens system and directed through the detector flow cell where wavelengths characteristic of the analyte and eluting medium are absorbed. The transmitted light is diffracted into a spectrum by a dispersion device and directed onto an array of photosensitive diodes. The immediate advantage of the simultaneous acquisition of multiple wavelengths of light, over a given range, is the ability to review acquired data identifying peaks which may have been previously un-recorded. The third dimension provided by the acquisition of complete spectral data for a single chromatographic analysis allows the extraction of a complete spectrum at any point in the analysis. This offers the possibility of spectral matching of detected

components of a sample against a reference library. Spectral data may also be used to give structural information about a detected peak.

2.2.2 *Liquid Chromatography/Mass Spectrometry (LC/MS)*

Mass Spectrometry is a technique used in the qualitative and quantitative identification of chemical and biological species using information obtained from the positive or negative ions produced by these species when they are subjected to a series of controlled conditions. Observations of the mass-to-charge (m/z) ratio of an ion can be used to elucidate molecular weight and structural information, ultimately allowing the determination of the atomic composition and the structure of a species. Determination of the mass-to-charge ratio is afforded by a mass analyser. A mass analyser is a device that uses the characteristics of an ion to determine its mass-to-charge ratio. Mass analysis is carried out in the presence of a high vacuum in order to reduce effects that could arise if gaseous phase interferences, such as water or carbon dioxide molecules, were present. These effects reduce the mean free path of an ion and reduce the efficiency and accuracy of the mass analysis process. Detection of ions is a result of physical processes involving contact between ions and one of several electronic amplification devices. The signals produced are interpreted by a data station and presented in the form of a mass spectrum [3].

Ion production takes place in the gas phase, by techniques operating at either low pressure (high vacuum) or atmospheric pressure. Ion production techniques may be classified as either 'soft' or 'hard'. Hard ionisation techniques (e.g. electron ionisation (EI)) are those techniques which involve gas phase interactions of sufficient energy to

result in bond dissociation in organic molecules Hard ionisation techniques produce a range of structurally diverse ions that can be used to elucidate the structure of a species. Soft ionisation is the term used to describe ionisation techniques that involve low levels of energy and primarily result in the formation of protonated molecules, $[M+H]^+$. Soft ionisation techniques (e.g. Electrospray (ESI), Atmospheric Pressure Chemical Ionisation (APCI), Chemical Ionisation (CI) and Matrix Assisted Laser Desorption Ionisation (MALDI)) are those ionisation techniques that result in minimal, if any, bond dissociation. Soft ionisation techniques require other mass spectrometric methods e.g. Collision Induced Dissociation (CID), to acquire structural information from an analysis. The analysis of mixtures with hard ionisation techniques without separation of the individual components of a sample prior to ionisation produces complex spectra that are difficult, if not impossible, to interpret. Separation of the components of a mixture is required if quality mass spectra are to be obtained for the individual components of mixtures. Gas Chromatography (GC) is a technique well suited to coupling with mass spectrometry as separation is carried out in the same phase as the ionisation technique i.e. the gaseous phase. However, gas chromatography can only be used for volatile species i.e. those species which exhibit limited intra-molecular attractive forces and species with low vapour pressure [4]. Liquid chromatography is a technique suited to the separation of those compounds for which the conditions of GC may be not ideal i.e. large molecules such as synthetic and biological polymers, and polar compounds. The coupling of a liquid chromatograph with a mass spectrometer presents a problem in terms of phase compatibility. Ions must be transferred from the liquid phase to the gas phase before mass spectrometric analysis can take place. Atmospheric pressure ionisation techniques have alleviated this problem in part, by removing the requirement for high pumping capacity in order

to maintain the high vacuum of the ionisation source. Matrix Assisted Laser Desorption Ionisation (MALDI) is a soft ionisation technique that can operate in a high vacuum [5], an intermediate vacuum [6] or in some cases at atmospheric pressure [7]. MALDI cannot be coupled on-line with separation techniques but for complex biological samples separation may be afforded off-line by electrophoresis techniques [8].

2.2.2.1 *Atmospheric Pressure Ionisation (API)*

The coupling of a liquid flow with a mass spectrometer is a problem if a high vacuum is to be maintained in the ion source, the part of the mass spectrometer where ions are produced. Coupling a high solvent flow would require an impractical pumping capacity. Operating the ionisation source at high pressure reduces the required pumping capacity to only that which is needed to remove the solvent vapour. Atmospheric pressure ionisation was originally demonstrated by Horning *et al.* [9]. It rapidly became the favoured method of coupling liquid flow methods to a mass spectrometer. There are two atmospheric pressure ionisation techniques which are widely used in mass spectrometry i.e. electrospray ionisation (ESI) [10] and atmospheric pressure chemical ionisation (APCI) [9].

2.2.2.2 *Electrospray Ionisation (ESI)*

Electrospray ionisation is a method of transferring ions from the condensed phase into the gaseous phase at atmospheric pressure. When a solution flows through a capillary in a large electric field a process of droplet dispersion is observed at the capillary outlet

and the liquid flow is nebulised. The first recorded application of this phenomenon, as a mechanism of ion formation for mass spectrometry, was reported by Dole [11, 12]. Dole recorded polymeric ions corresponding to m/z 51,000 and 411,000. Modern applications of electrospray ionisation are a result of the work of Yamashita and Fenn [13] who refined and successfully applied the technique to produce protonated molecules of n-dibutyl phthalate, 1,10-phenanthroline and a mixture of quaternary ammonium and phosphonium halides [13].

The mechanistic aspects of electrospray are a complex area and have been reviewed elsewhere [14]. For the purpose of this thesis the model will be described in a empirical manner in agreement with the ion evaporation model first suggested by Thomson and Iribarne [17, 18]. A high electric field applied to the spray capillary of the interface induces electrostatic repulsion of ions of the same polarity in the effluent. Positively charged ions are repelled away from the capillary surface and into the centre of the flow when a positive charge is placed on the capillary. The opposite is true for negative ions. The concentrated charge accumulates at the capillary outlet in the form of highly charged droplets. At a threshold value of the electric field the charged droplet form a Taylor cone [15]. A Taylor cone is a fine filamentous cone that sprays the liquid in a fine dispersion of micro droplets. The droplets undergo desolvation as they pass through the atmospheric pressure region of the source. The desolvation process acts to concentrate the charge in the droplets. When the electrostatic forces of repulsion within the droplet exceed the Rayleigh stability limit the droplet decomposes. The decomposition of a droplet in the ion source will ultimately yield ions. A nitrogen bath gas is introduced into the atmospheric pressure region of the source to produce turbulence in the ion formation region and prevent the formation of solvent clusters.

The presence of a bath gas and the resulting effects of shear forces on a droplet as it travels through the source are influential on the droplet disintegration process and have been discussed by Bruins [16].

The second physical stage of electrospray ionisation for mass spectrometric analysis is the sampling of the spray plume through a small orifice or supersonic nozzle into a region of low pressure. The resulting adiabatic expansion aids the production of a solvent free collimated ion beam. The entire ion production, ion sampling and ion analysis process is conducted across a pressure gradient. The electrospray process is conducted at atmospheric pressure and analysis is carried out in the high pressure of the mass analyser. The gradient is provided by a series of differentially pumped stages. Figure 2 shows the components and layout of an electrospray ionisation source.

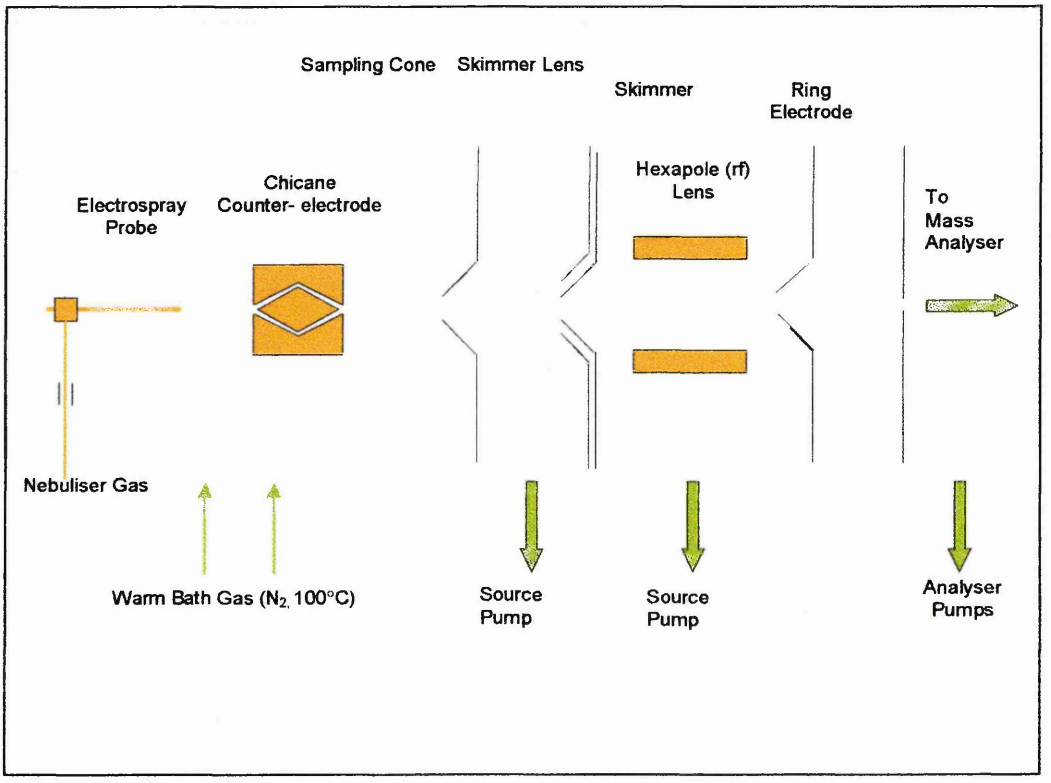

Figure 2. The components and layout of an electrospray ionisation source.

To fully realise Arpino's "impossible" [19] vision of coupling liquid chromatography with mass spectrometry the electrospray ionisation process would have to be capable of handling the flow rate from an LC separation. Conventional electrospray is compatible with solvent flow rates of approximately 5 $\mu l.min^{-1}$. Compatibility with conventional liquid chromatography was a result of developments by Henion *et al.* [20]. Pneumatically-assisted-electrospray or ionspray uses a sheath flow of nitrogen to aid the nebulisation process. At higher flow rates and/or percentages of water, electrospray produces a stream of larger droplets and these require larger voltages to induce ionisation. The combination of a large volume of solvent vapour and high electric field can initiate a corona discharge within the source. Also it may be a problem that the flow rate is such that the spray process is intermittent and sensitivity is low. The application of a nitrogen sheath flow alleviates this problem. Flow rates of 40 $\mu l.min^{-1}$ have been reported for ionspray allowing direct coupling with narrow bore and smaller columns (<2 mm) operating at their optimized flow rates. Turbo-ionspray [21] involves a greater sheath flow of nitrogen allowing coupling with flow rates of 0.2-2 $ml.min^{-1}$. Turbo-ionspray can be coupled directly with conventional normal bore columns (4.6 mm). Developments in the coupling of low flow rates with LC/MS have produced the nanospray source [22, 23]. The concentration sensitive nature of electrospray introduces an interesting opportunity to analyse sub femtomole (10^{-15} M) concentrations. The major instrumental difference between electrospray and nano-spray is the replacement of the stainless steel capillary of the probe with a much finer capillary (polyimide coated fused silica or glass capillary). The major obstacles in the development of the nanospray interface are the delicate nature of the fused silica capillary employed, and the requirement that it must be drawn or etched into a uniform conical tip. Issues are also encountered in maintaining the continuity of the electric

field applied to the capillary. Turbo-, pneumatically-assisted-electro-, electro- and nano-spray mass spectrometer interface technology have assisted the development of organic mass spectrometry and permitted the coupling of the technique with liquid separation techniques.

2.2.2.3 *Atmospheric Pressure Chemical Ionisation (APCI)*

Atmospheric pressure chemical ionisation (APCI) [9, 24] is a technique that is suitable for ionisation of some non-polar compounds not compatible with electrospray ionisation. The analyte is transferred from the liquid phase to the condensed phase via a heated nebulising probe. Ionisation is accomplished by the presence of a corona discharge needle situated in the ion source. The vaporised mobile phase acts as an ionising medium. Interactions between electrons and the solvent molecules produce an environment conducive to chemical ionisation type molecular interactions. Equations 9, 10 and 11 show a chemical ionisation mechanism for methanol, a common solvent in LC/APCI/MS.

$$CH_3OH + e^- \rightarrow CH_3OH^{\bullet+} + 2e^- \quad \text{...Equation 9}$$

$$CH_3OH^{\bullet+} + CH_3OH \rightarrow CH_3OH_2^+ + CH_3O^{\bullet} \quad \text{...Equation 10}$$

$$M + CH_3OH_2^+ \rightarrow MH^+ + CH_3OH \quad \text{...Equation 11}$$

Equation 9 is an example of electron ionisation. The methanol molecule interacts with an electron and a methanol radical cation is formed with the expulsion of an electron.

The radical cation formed interacts with a further methanol molecule to form a cation and a radical (Equation 10). The sample molecule interacts with the cation and proton transfer occurs (Equation 11). The protonated molecule provides molecular weight information for the sample. Direct interactions between electrons and the sample molecules are unlikely due to the relative concentrations of the sample molecules to the solvent molecules approximately 1:100,000. The robust nature of the heated probe in APCI has a distinct advantage for coupling with liquid chromatography since it can handle high solvent flow rates. APCI can operate efficiently with solvent flow rates of 1.0 $ml.min^{-1}$ and is compatible with conventional standard bore columns. This compatibility removes the introduction of dead volumes associated with a flow splitting device. A further advantage of the APCI interface is a reduction in sodium and potassium adducts often observed for electrospray ionisation. This may be a result of the high temperature and high sheath gas flow rate used that produce an ionisation process that is higher in energy than that associated with electrospray ionisation. The high energy of the system may be sufficient to dissociate any sodium and potassium adducts that may be formed, before they leave the ion source.

Sampling of the ions is as with electrospray i.e. with a small orifice or supersonic nozzle. Adiabatic expansion aids the removal of interfering solvent clusters. Passage from the nebuliser to the mass analyser is through a series of differentially pumped regions. For practical purposes the ionisation interface for APCI differs from that of electrospray only in the design in the probe and the corona discharge needle.

A technique widely used for the ionisation of in-volatile compounds is matrix assisted laser desorption ionisation (MALDI). It is a method of producing intact gas phase ions from an analyte-doped crystalline matrix. It was observed by Karas *et al.* [25] that small, non-absorbing molecules could be successfully laser desorbed intact if immersed in a surrounding matrix. It was, however, Tanaka who was the first to apply a matrix based laser desorption method to the analysis of large bio-molecules [26]. Conventional laser desorption ionisation (LDI) involves the direct irradiation of a sample with energetic laser light. The energy imparted ablates the sample and ionisation is achieved in the vapour phase above the sample surface. Molecules containing thermally labile bonds readily dissociate under the energetic conditions of Laser Desorption Ionisation (LDI). The application of a molecule-shielding matrix was developed to dissipate incidental energy around analyte species and prevent the direct exposure of the analyte to incident energy. In theory, under rapid heating conditions, organic compounds favour vaporisation reactions over decomposition reactions [27]. The crystalline matrix absorbs pulsed laser energy and the resulting fast energetic mechanism of equilibration results in sublimation and formation of a sample/ matrix plume. Ionisation is a result of CI-like interactions between photo-ionised matrix ions and analyte molecules above the sample surface. Common lasers in MALDI are nitrogen/UV lasers (wavelength = 337 nm), and CO_2/infra-red (IR) (wavelength = 10.6 μm) lasers. Nitrogen laser applications dominate as nitrogen lasers are cheaper than the IR lasers and are therefore more common. The wavelength of the laser light is important in determining the interaction between incident photons and the sample matrix. With UV photons initial interactions are electronic but as the

energy propagates through the condensed-phase medium collision dynamics assume a more substantial role [28]. With IR photons in MALDI, direct vibrational excitation occurs on irradiation. IR lasers can therefore be viewed as more efficient in their production of the gas phase sample plume. The laser energy is directed to the sample in short (1-10 ns) pulses. The pulse serves to avoid the pyrolytic decomposition of the sample and also proves a useful trigger for the detector, commonly time-of-flight, which is ideally suited to pulsed ionisation techniques. As with any ionisation technique if a sufficiently high amount of energy is input to the system bond dissociation will occur. This holds for MALDI and laser power optimisation is crucial for a successful analysis.

A compound suitable for application as a MALDI matrix must meet several requirements. It should be able to embed and isolate analytes (e.g. by co-crystallisation), be soluble in solvents compatible with the analyte, be vacuum stable, absorb the laser wavelength, cause desorption of the analyte upon laser irradiation, and promote analyte ionisation. Some common MALDI matrices are organic acids possessing a chromaphoric moiety. The chromaphore absorbs the laser light and aids the transfer of the sample to the vapour phase. The labile protons of the acid contribute to the chemistry of the gas phase ionisation mechanism. The matrix must be capable of absorbing the laser light. If it cannot then the laser energy will not produce the necessary energetic interactions to result in sublimation from the solid sample.

Matrix assisted laser desorption is a soft ionisation technique that has numerous applications [29, 30]. Its application to macro-molecules is common. For smaller

molecules its application is hindered by the presence of matrix ions in the low molecular weight region (<500 m/z) of an acquired spectrum [31].

2.2.3 Mass Analysers

Mass analysers aid in the acquisition of mass to charge ratio information of ions produced in the ion source. There are several techniques available for mass analysis, all developed around the manipulation of a particular property of a charged species. In this section two common mass analysers are examined to provide an understanding of the physical processes involved in mass analysis.

2.2.3.1 *Quadrupole Mass Filter* [32]

Quadrupole analysers are made of four parallel rods (with circular, hyperbolic or elliptical cross section) equidistant from a central axis. A potential is applied to the rods such that the opposite rods have the same polarity while adjacent rods have opposite polarity. The applied voltage has two components: a direct current (d.c.) component (U) and a radio frequency (r.f.) alternating component (V). Equation 12 describes the potential (ϕ) applied to the adjacent rods.

$$\phi_0 = U + V\cos\omega t \qquad \text{...Equation 12}$$

There is a 180° phase difference between the voltages applied to opposite rods. This aids ion transmission through the centre of the assembly with minimum displacement from the central axis. The successful transmission of an ion through a quadrupole is

governed by two mathematically derived parameters, a and q. These are described in equations 13 and 14.

$$a = \frac{8zU}{mr_0^{\,2}\omega^2} \qquad \text{...Equation 13}$$

$$q = \frac{4zV}{mr_0^{\,2}\omega^2} \qquad \text{...Equation 14}$$

Equations 14 and 15 can be combined to produce Equation 15.

$$\frac{a}{q} = \frac{2U}{V} \qquad \text{...Equation 15}$$

The stable trajectory of an ion through the rod assembly can be described by a stability plot, first described (Equation 15) by the physicist Mathieu in 1866 [32]. For certain values of U and V an ion entering the quadrupole will experience a stable oscillation such that the x, y displacement of the ion is not above or equal to r_0 The ion will pass unhindered to the detector.

To produce a mass spectrum, U and V must be varied, while the ratio U/V is kept essentially constant. The mass of the ions being analysed at any one time is proportional to V, and so a linear increase in V produces a linear increase in mass.

If the d.c. voltage is switched off, the quadrupole is described as operating in r.f. only mode, and all ions will perform stable oscillations if the r.f. voltage is sufficiently low. In cases such as this, the quadrupole is not being used as a mass analyser, but as a high

transmission lens, and as such has often been employed as a 'collision cell' situated between two different mass analysers in tandem mass spectrometers (See section 2.2.4.1) The quadrupole is an example of a direction focusing analyser.

2.2.3.2 *Time-of-Flight Mass Analyser* [33]

In a Time-of-Flight (ToF) mass analyser, ions are expelled from the source in packets or bundles by a pulsed electric field. The electric field pulse directs ions towards a field free drift tube, where they are velocity separated, and ultimately to the detector. All the ions expelled from the source experience the same electric field and therefore the same energy. This common energy forms the principle by which the ions are separated. If all ions leaving the source experience the same electric field then they have the same energy. Equation 16 describes the relationship between the kinetic energy (*K.E.*) of an ion in a constant electric field and the velocity of an ion. Ions of different mass experiencing the same electric field will exhibit different but constant velocities. For light ions the time taken to reach the detector will be smaller than that for heavy ions. A signal is triggered when the ions are pulsed out of the ion source and also when they arrive at the detector. This allows accurate determination of the time of flight and subsequent determination of mass.

$$\frac{mv^2}{2} = zeV \qquad \text{...Equation 16}$$

where m = the mass of an ion, v = the velocity of the ion, e = the charge of an electron (1.6×10^{-19} C), z = the number of charges on the ion, and V = the accelerating voltage.

The time (t) taken for an ion to traverse the flight tube, the time of flight, can be calculated from Equation 17.

$$t = \frac{l}{v} \qquad \text{...Equation 17}$$

where t = the flight time of the ion, v = the velocity of the ion and l = the length of the flight tube.

The relationship between the time of flight, and the mass of an ion is shown by rearrangement of equations 16 and 17 (Equation 18).

$$t^2 = \frac{m}{z}\left(\frac{l^2}{2Ve}\right) \qquad \text{...Equation 18}$$

where m = the mass of an ion, e = the charge on an electron, V = the accelerating voltage, t = the flight time of the ion, l = the length of the flight tube, and z = the number of charges on the ion.

Historically a limiting factor in the application of the time of flight mass analyser was resolution. Mass resolution is affected by factors that create a distribution in flight times among ions with the same mass-to-charge (m/z) ratio. For example not all ions will experience exactly the same accelerating potential, depending, for example, on precisely where they are formed. Ions can be formed at different times and those formed at different locations will travel somewhat different paths. All of these factors contribute to poor resolution. Improved resolution can be achieved by the application

of a reflectron or delayed extraction. The reflectron is an ion optic mirror-like device that corrects the energy dispersion of the ions leaving the source with the same m/z ratio. A reflectron has a retarding electric field placed at the end of the flight tube this acts to reverse the direction of arriving ions. Ions with greater kinetic energy penetrate further into the retarding electric field than those of the same m/z but with lower kinetic energy. The journey from the reflectron to the detector takes longer for those ions which have penetrated further into the reflectron. Thus ions of the same m/z will arrive at the same time. The overall effect is that the reflectron decreases the spread in the flight times for ions of the same m/z ratio but different kinetic energies, and so improves the resolution. With delayed extraction, the ions in the source are initially allowed to disperse in a near zero electric field, and then at an appropriate time some 0.5 to 10 microseconds later, an electric field is applied to the ions. More energetic ions, which have travelled longer distances from the point of formation, receive less energy from the applied field than less energetic ions. The applied electric field essentially cancels out any differences in kinetic energy between ions of the same m/z that occur during the ionisation process. For ions of the same m/z ratio but different energies velocity focusing is achieved and the ions of the same mass arrive simultaneously at the detector regardless of their initial velocities. Delayed extraction allows the optimisation of ToF analysis for ions of a selected mass. A disadvantage of delayed extraction is that the technique may be optimised for the extraction of only a single mass during an analysis. Only ions of the selected mass will exhibit improved resolution under the conditions of the pulsed extraction.

2.2.4 Molecular Dissociation Techniques

Soft ionisation techniques are useful in producing intact protonated molecules. However, little structural information is available from spectra acquired from such ions. There are several techniques available to provide additional structural information from ionic species.

2.2.4.1 *Tandem Mass Spectrometry*

Tandem mass spectrometry or MS/MS is any general method involving at least two stages of mass analysis, either in conjunction with a dissociation process or a chemical reaction that causes change in the mass or charge of an ion [34]. Figure 3 shows the scan modes available to a triple quadrupole instrument (adapted from [34]). The second quadrupole has no d.c. component and is therefore operated in r.f. only mode. Operating the second quadrupole in r.f. only mode allows the maximum transmission of ions through into the second stage of analysis.

2.2.4.2 *Cone Voltage Dissociation*

Cone voltage dissociation can be used to generate structural information when ESI or APCI techniques are used in conjunction with a single mass analyser. Electrospray and APCI liquid dispersion ionisation techniques are ideal for acquiring molecular weight information of an ionised species. The techniques produce little structural information. The coupling with tandem mass spectrometric techniques is one method of acquiring structural data for a molecule adduct. Cone Voltage dissociation is a

method of initiating bond CID of a molecular species within the ion source of a mass spectrometer [36]. Optimisation of the potentials across the transport region produces sufficient acceleration that energetic collisions in the high-pressure super-sonic expansion region result in bond dissociation.

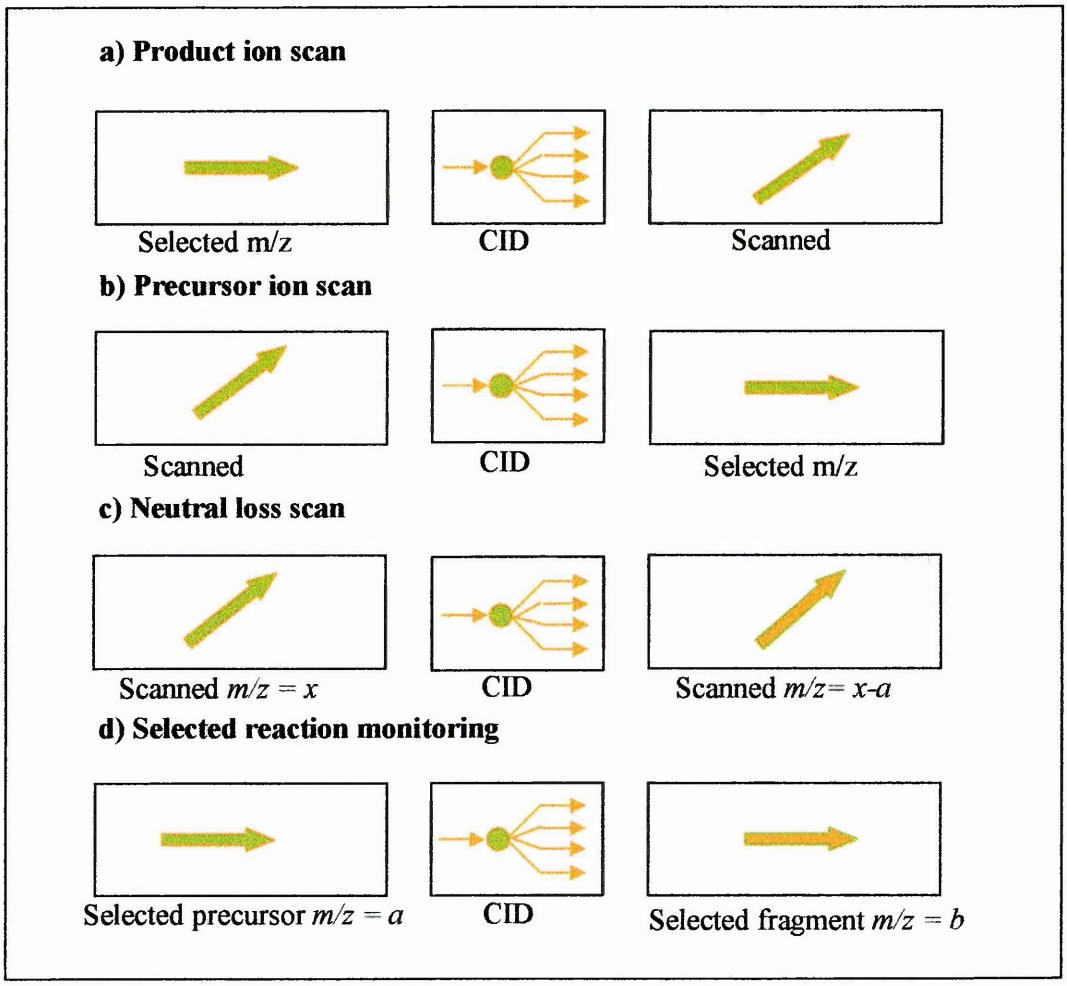

Figure 3. A schematic representation of the MS/MS modes of operation of a triple quadrupole mass spectrometer (Adapted from [34]).

The ion beam passes through the mass analyser and then is detected and transformed into a usable signal by a detector. Common detectors are multistage electron multipliers and micro-channel plate detectors. In multistage electron multipliers positive or negative ions hit a conversion dynode and cause the emission of several secondary particles. These particles include positive ions, negative ions, electrons and neutrals. When positive ions strike the negative high-voltage conversion dynode, the secondary particles of interest are negative ions and electrons. When negative ions strike the positive high-voltage conversion dynode, the secondary particles of interest are positive ions. These secondary particles are accelerated into the continuous dynode electron multiplier. They strike the cathode with sufficient energy to dislodge electrons. These electrons pass further into the electron multiplier, again striking the walls causing the emission of more and more electrons as they travel towards the ground potential. Thus a cascade of electrons is created that finally results in a measurable current at the end of the electron multiplier.

Micro-channel plate detectors consist of a large number of micro continuous dynode multipliers arranged in an array. This resembles an electronic version of a photographic plate. Each of the micro-channels (which are approximately 25 μm in diameter) is coated with a semi-conducting material and acts as a continuous dynode. The electron cascade is collected by an anode at the channel exit. The micro-channel plate detector system allows the simultaneous detection of ions of different masses and is therefore suitable for use in ToF mass analysers.

2.3 Conclusion

An overview of HPLC has been given with particular attention given to partition chromatography. Chromatographic theory and the affects of the variables in HPLC have been explained. The underlying theory of the two detectors used in this work, the UV/Vis spectrophotometer and the mass spectrometer, has been presented. Close attention has been given to the theoretical aspects of mass spectrometry. The coupling of a liquid flow with a mass spectrometer has been explained by examination of electrospray and atmospheric pressure chemical ionisation techniques. Matrix Assisted Laser Desorption/Ionisation (MALDI) has been introduced from a theoretical perspective. The mass analysers and detectors commonly used in mass spectrometry have been described.

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3.0 The Application of Liquid Chromatography Coupled with Electrospray Ionisation Mass Spectrometry and Tandem Mass Spectrometry to the Analysis of Alkylbenzyl and Dialkyldimethyl Quaternary Ammonium Biocides in Occupational Hygiene and Environmental Media.

3.1 Introduction

Benzalkonium chloride (BAC) [1] is a mixture of n-alkylbenzyldimethylammonium chloride homologues varying in n-alkyl chain length, where n represents an even number of carbons from C_8 to C_{18} (Figure 1a). The most commonly encountered homologues are C_{12}, C_{14} and C_{16} [2]. The biocidal properties of the individual homologues are known to be different [3]. BAC is widely used as an active substance in a variety of applications including anti-bacterial products, anti-fungal products, in-can preservatives, timber treatments, masonry biocides [4], medical disinfectants and ophthalmic systems [5,6]. The preparations used, which vary in individual homologue content, also often contain other ingredients (e.g. amines, steroids, alcohols etc.) that may co-elute and interfere with BAC determination. Other quaternary ammonium compounds (QAC) are also commonly used as biocides, and are collectively described as dialkyldimethylammonium compounds. One example is didecyldimethylammonium bromide (DDDMAB) (Figure 1b).

Several LC methods for the determination of BAC have been described with separation achieved on cyanopropylsilica (CPS) [3, 5-8], octadecylsilica (ODS) [9-12] or hydrophilic polymer [13] stationary phase columns. The phenyl substituent of BAC provides a suitable chromaphore for UV/Vis analysis but the dialkyldimethylammonium compounds such as DDDMAB have no chromaphore and so cannot be readily detected by this method. Indirect UV detection has been described for dialkyl QAC but this is not a very specific method of analysis and can be affected by co-eluting compounds. The detector most commonly used with high performance liquid chromatography (HPLC) for BAC has been UV/Vis, but

fluorimetric [13] and conductometric [14] methods have also been reported. The conductometric [14] detector has the advantage of being able to detect QAC with no UV absorbance, e.g. dodecyltrimethylammonium chloride. MS detection was applied to dialkyldimethyl QAC by Radke *et al.* working in the 0.4-140 $ng.ml^{-1}$ range [12]. Ferrer and Furlong report the application of ion trap LC/MS and LC/MS/MS to the analysis of BAC in water samples [15].

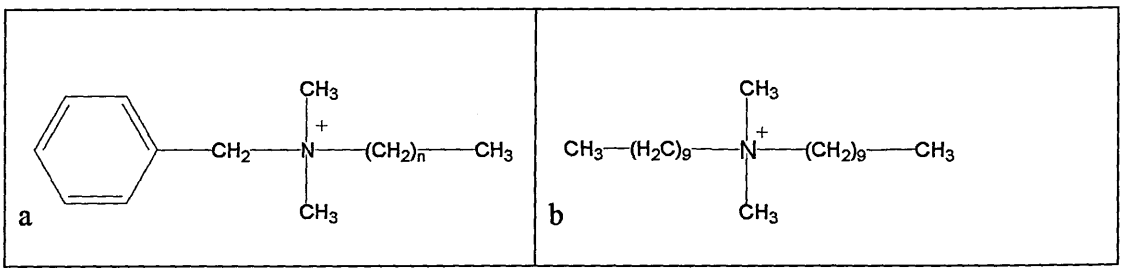

Figure 1. The structures of the cationic moiety of BAC (n represents an even number of carbons from C_8 to C_{18}) and DDDMAB.

Mass spectrometric (MS) detection offers several advantages over the previously described methods of detection, including increased sensitivity and specificity. The cationic nature of quaternary ammonium compounds makes them very amenable to liquid chromatography with positive ion electrospray ionisation (LC/ESI/MS). For BAC and dialkyldimethylammonium compounds, LC/ESI/MS offers the possibility of developing a method for simultaneous determination and quantification.

This work describes the application of LC/ESI/MS to the simultaneous determination of alkylbenzyl and dialkyl QAC. Structural determination is reported by the use of tandem mass spectrometry and cone voltage ion dissociation methods.

3.2.1 *Chemicals and Reagents*

Benzyldimethyldodecylammonium bromide (97%), benzyldimethyltetradecyl ammonium chloride (99%), benzyldimethylhexadecylammonium chloride (99%) and didecyldimethylammonium bromide (98%) were obtained from Sigma-Aldrich (Poole, Dorset, U.K.). HPLC grade acetonitrile and formic acid were obtained from Fischer Chemicals, (Loughborough, Leicester, U.K.). Ammonium acetate (HiPerSolv) was purchased from BDH, (Poole, Dorset, U.K.). Milli-Q water was used in all the experiments where necessary.

Stock standard solutions were prepared by dissolving 1mg of each standard compound in 1ml of acetonitrile. Working solutions and calibration standards for the individual compounds were prepared by serial dilution of the stock standards with acetonitrile. Concentrations of 0.5, 1, 5, 10, 50 $\mu g.ml^{-1}$ were prepared. Standards were analysed six times. A standard mixture was prepared using 1ml of the respective 50 $\mu g.ml^{-1}$ standards. Swab (cotton pad) and other occupational hygiene samples were collected as part of the Health and Safety Executive (HSE) routine programme of occupational hygiene monitoring and were desorbed with the mobile phase, as described previously for this type of work [16-17]. One sample of swimming pool water was taken as part of HSE enforcement activity. The swimming pool water sample was injected directly onto the column as received. All solutions were stored in glass vials at 4 °C. Homogenisation of all samples was achieved by sonication of the sample at 25 °C for 5 mins.

3.2.2 *Instrumentation*

The HPLC/UV/Vis system consisted of a Waters 610 *plus* solvent delivery system coupled with a Waters 717 auto-sampler and a Waters 996 photo-diode array detector. Data were recorded on a Dell PC equipped with Waters Millennium software (Waters Ltd., Watford, Hertfordshire, U.K.). The PDA was set to acquire wavelengths in the 210-350nm range with a sampling rate of 1, a resolution of 1.2 and a filter response of 1.0, (Photo diode array settings are quoted in the arbitrary units of Millennium Software).

The HPLC/MS system consisted of a Jasco PU-980 Intelligent HPLC pump with a LG-980-02 Ternary gradient unit (Jasco, Chelmsford Road, Great Dunmow, U.K.), a VG Quattro I mass spectrometer (Fisons Instruments, VG Biotech, Altrincham, Cheshire, U.K.) equipped with a pneumatically assisted electrospray (ESI) interface and a Rheodyne injection valve mounted in a gas flow regulating unit. The mass spectrometer was operated in the positive ion mode with the following working conditions; capillary voltage 3.95 kV, HV lens voltage 0.3 kV, cone voltage 32 V, Lens 3 potential 3 V, multiplier 550 V. The source temperature was 95 °C. The nitrogen nebuliser and curtain gas (BOC, Guildford, Surrey, U.K.) flows were 40 $l.h^{-1}$ and 350 $l.h^{-1}$, respectively. Data were recorded on a PC with Mass Lynx Software V2.0 (Micromass, Altrincham, Cheshire, U.K.). A flow rate of 100 $\mu l.min^{-1}$ from the electrospray probe was achieved by means of a 10:1 post-column T-piece/PEEK tubing split (Supelco, Poole, Dorset, U.K.).

Direct infusion MS experiments were performed using a Harvard 11 syringe driver (Merck, Poole, Dorset, U.K.) with a Hamilton 500 µl gas tight syringe (Supelco, Poole, Dorset, U.K.). PEEK fittings and tubing were purchased from Supelco and used throughout mass spectral data acquisition. The mass spectrometer conditions are described above.

3.2.3 *Analytical Procedure*

The chromatographic procedure employed was isocratic HPLC with a mobile phase of acetonitrile: 100 mM ammonium formate acidified with formic acid, pH 3.7, (55/45 v/v). The columns used were a Jones Chromatography, Genesis CN, 4 µ, 4.6 x 100 mm, (Phenomenex, Macclesfield, Cheshire, U.K.) and a Hypersil CN (CPS), 5µ, 4.6 x 125 mm, (Phenomenex, Macclesfield, Cheshire, U.K.). Chromatography was carried out with the column at room temperature. The flow-rate was 1.0 $ml.min^{-1}$ with an on column injection volume of 25 µl for the UV/Vis work and 5 µl for the MS work. UV chromatograms were extracted at a wavelength of 262 nm. This wavelength was chosen as the optimum for the working conditions by examination of the UV spectra of the homologues obtained using the UV/Vis detector.

Initial mass spectrometric data acquisition was via sample infusion (50 $\mu g.ml^{-1}$) performed at a flow of 5.0 $\mu l.min^{-1}$ using scan acquisition mode over a range of 50-400 m/z. Data acquisition was in centroid mode with a cycle time of 2 secs and an interscan time of 0.1s. The total run time was 2 mins. The formulations and swimming pool samples were analysed using selected-ion recording (SIR) mode with a dwell time of 0.5 secs and an inter channel delay of 0.02 secs and a mass span of ±0.25

Da. The mass spectrometer conditions were optimised by tuning on the protonated molecules, $[M+H]^+$, of acetonitrile and formic acid 42 and 47 m/z respectively. MS/MS acquisitions were performed via direct infusion of the standard material of DDDMAB and a diluted formulation. The product ion spectra were recorded with first quadrupole at a low resolution setting on the software allowing the highest number of precursor ions through into the collision cell. The third quadrupole was optimised for unit resolution. Argon collision gas was introduced into the collision cell to a density that reduced the precursor signal by approximately 50%. MS/MS data acquisition was in the continuum mode to provide improved sensitivity. Collision energy values of 100 eV were noted as being sufficient to achieve structural dissociation yielding ions from which structures of the parent molecule could be suggested. Cone voltage bond dissociation was achieved by increasing the value of the cone voltage while infusing the sample. Dissociation was monitored using the real time display of the tune page. Values of 60 V+ were found sufficient to produce ion dissociation for the compounds included in this report.

3.3 Results

3.3.1 *Application of LC/MS*

To demonstrate the applicability of the LC/MS technique, a standard mixture of the BAC homologues and DDMAB was analysed. Ions of m/z 304, 332, 360 and 326 were monitored corresponding to the BAC C_{12}, BAC C_{14}, BAC C_{16} and DDDMAB cationic species. The order of elution was: BAC C_{12}, BAC C_{14}, DDDMAB and BAC C_{16}. The Selected ion recording (SIR) method permits the previously unachievable

simultaneous quantitative determination of all four compounds. Figure 2 shows two chromatograms acquired from the analysis of a standard mixture of the benzyl and dialkyl QAC. Operating the mass spectrometer in SIR mode offers an increase in sensitivity and also permits the quantitation of the individual analytes without chromatographic resolution (i.e. $Rs \geq 1$ of the individual component peaks. Figure 3 shows the single channels of a mass chromatogram recorded for a standard mixture of BAC C_{12}, BAC C_{14}, BAC C_{16} and DDDMAB. The ammonium formate in the mobile phase facilitated a low pH and improved peak resolution through its silanophilic interactions with the column packing. The BAC retention mechanism was greatly influenced by pH with low pH providing the optimum peak shape. The limits of detection (LOD) were determined as being 3, 4, 4 and 4 $ng.ml^{-1}$ for C_{12}, C_{14}, C_{16} and DDDMAB using the statistical method suggested by Miller and Miller [18]. This is a sensitivity increase of three orders of magnitude compared with the UV/Vis method for the BAC homologues. For DDDMAB it allows the determination of a species that previously would not have been recorded by the UV/Vis method.

3.3.2 *Evaluation of Linear Response of LC/MS Method*

A linearity study was performed for the LC/MS method. A series of BAC, C_{12}, C_{14}, C_{16}, and DDDMAB standards was prepared at 5 concentrations (0.5, 1, 5, 10, 50 $\mu g.ml^{-1}$) and analysed in triplicate. Calibration curves were constructed by linear regression between peak area and compound concentration. Linear regression values were recorded; DDDMAB: 0.9966, BAC C_{12}: 0.9683, BAC C_{14}: 0.9519 and BAC C_{16}: 0.9878 and noted as being rather poor. This is probably a result of the absence of an internal standard in this method.

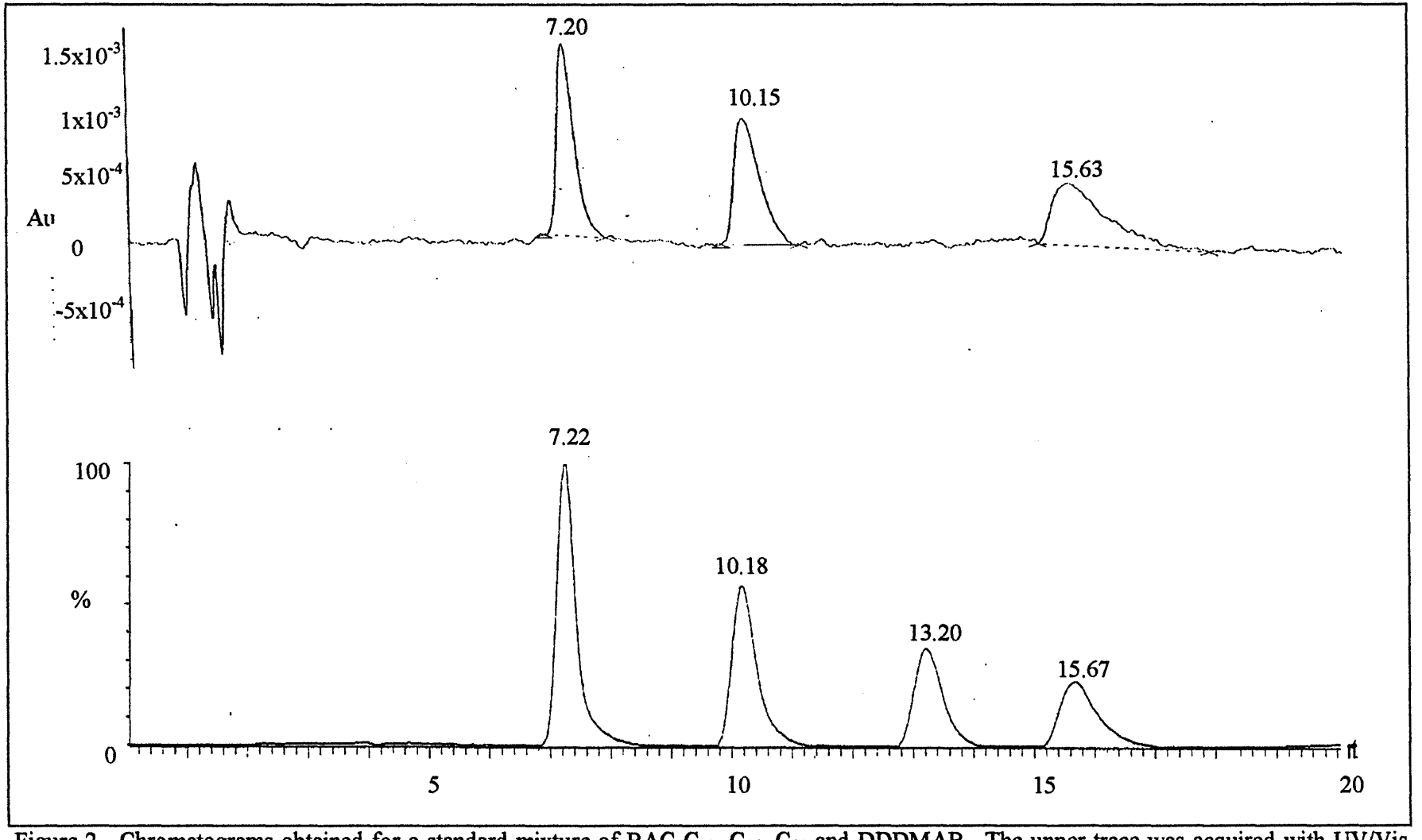

Figure 2. Chromatograms obtained for a standard mixture of BAC C_{12}, C_{14}, C_{16} and DDDMAB. The upper trace was acquired with UV/Vis detection, the lower with MS detection.

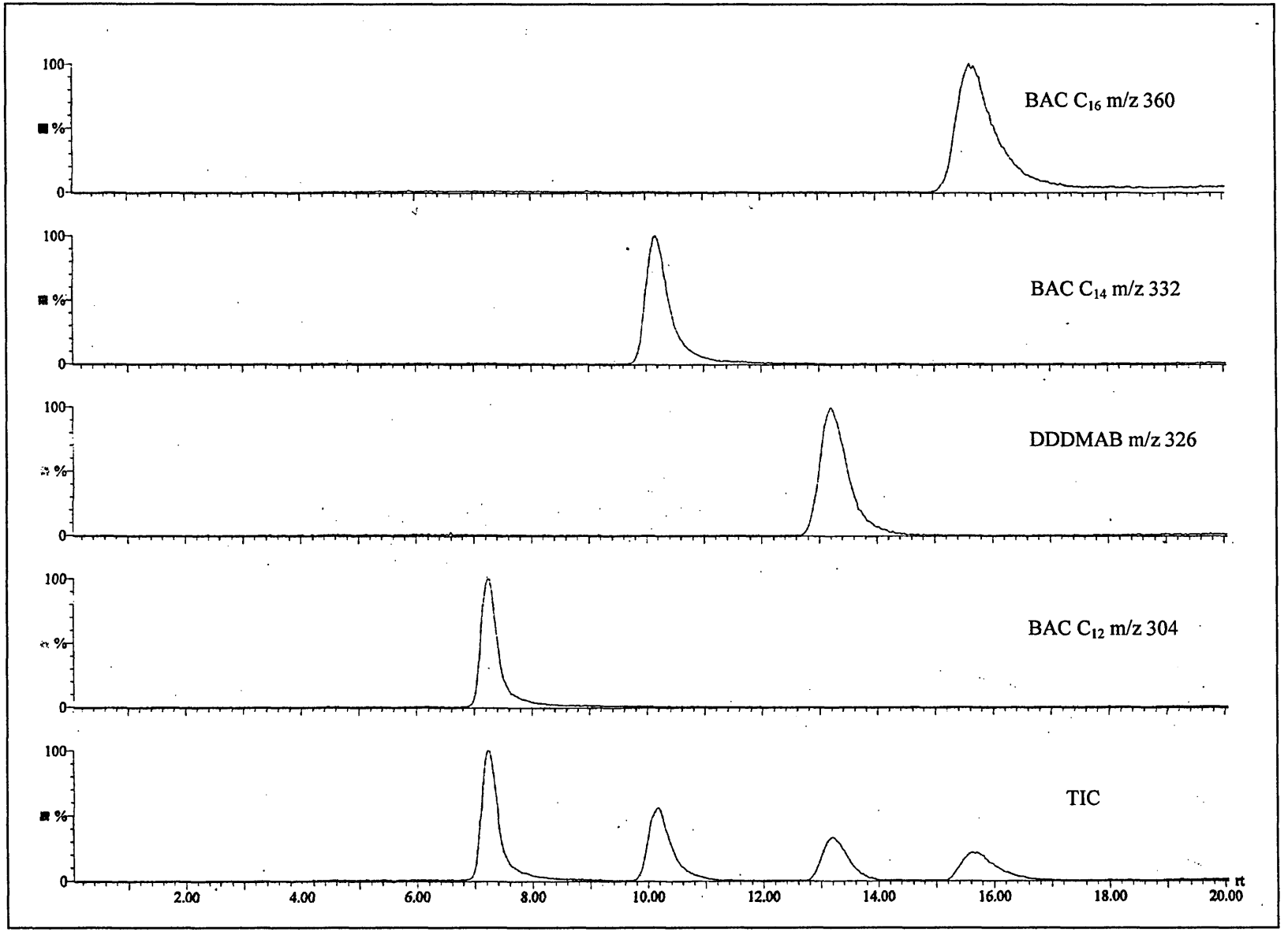

Figure 3. A Mass chromatogram acquired for a standard mixture of BAC C_{12}, C_{14}, C_{16} and DDDMAB. Peaks are annotated with the appropriate name and m/z value. The TIC is the total ion chromatogram.

Figure 4 shows mass chromatograms of a swab sample taken during occupational hygiene monitoring of a worker spraying a product known to contain BAC. The sampling procedures and guidance on occupational hygiene monitoring, have been published elsewhere [16]. No interference effects were observed from the desorption solvent. Six channels were monitored using selective in recording (SIR), corresponding to the C_8-C_{18} alkyl chain lengths of BAC. The six resolved peaks are clearly visible in the total ion chromatogram (TIC). The presence of the individual homologues provided by MS detection operating in SIR mode is important for correctly identifying the preparation used. Different BAC containing products contain different amounts of the C_8 to C_{18} homologues. For some preparations a homologue may be absent. Identification is possible by UV/Vis but relies on retention time only. UV/Vis is much less sensitive than ESI/MS. Using ESI/MS, quantitation of the C_{12}, C_{14} and C_{16} homologues was possible. The following concentrations were calculated for the BAC C_{12}, C_{14}, C_{16} homologues present in the formulation: BAC C_{12}: 6.41 $mg.ml^{-1}$, BAC C_{14}: 2.25 $mg.ml^{-1}$ and BAC C_{16}: 1.66 $mg.ml^{-1}$. This application highlights the sensitive and selective detection offered by LC/ESI/MS without time consuming sample clean up and pre-concentration methods.

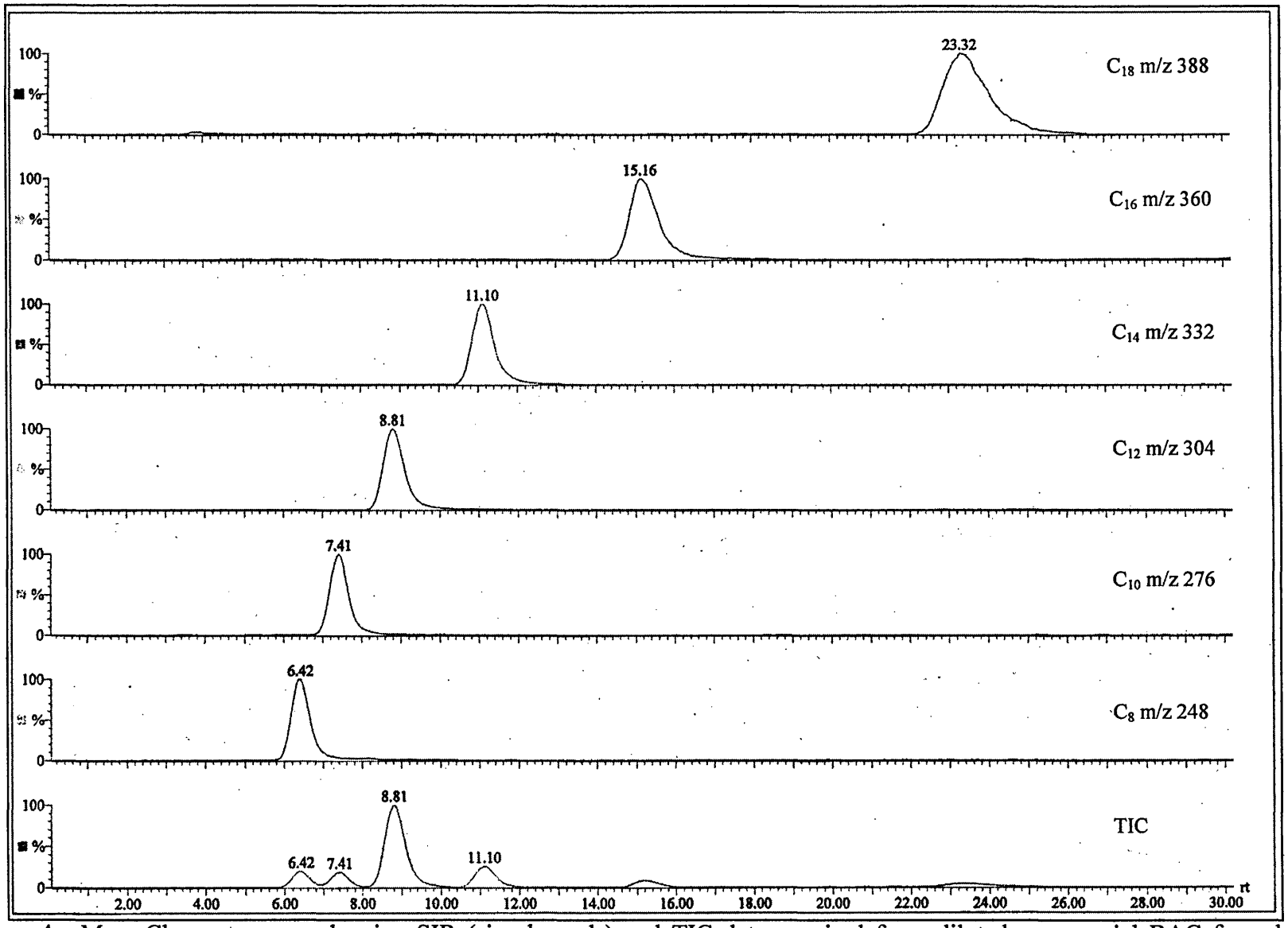

Figure 4. Mass Chromatograms showing SIR (six channels) and TIC data acquired for a diluted commercial BAC formulation. (Note, differences in retention time between the commercial formulation and the standards is caused by the use of the Jones Chromatography CPS (Cyano), 100 x 2.1 mm column for the standards and the Hypersil CPS (Cyano), 4.6 x 125 mm column for the formulation.)

The ingestion of swimming pool water treated with a BAC containing algaecide commercial product had been proposed as a cause of ill health amongst pupils at a school. Samples of the algaecide concentrate and the pool water were taken and analysed for BAC by LC/UV/Vis and LC/ESI/MS methods. BAC C_{12} and C_{14} homologues were identified in the samples. Quantitation of the individual peaks yielded C_{12} and C_{14} concentrations of 19.169 $mg.ml^{-1}$ and 2.163 $mg.ml^{-1}$ for the concentrated sample, and 13.036 $\mu g.ml^{-1}$ and 3.125 $\mu g.ml^{-1}$ for the pool water. LC/MS provided a rapid sensitive and selective method of analysis for this sample.

3.3.4 *Structural Elucidation of Quaternary Ammonium Compounds*

Biocidal QAC are frequently reported as simply "dialkyl" on product labels. More information about the actual species present is required especially for forensic purposes. Initial work focused on the application of MS/MS to DDDMAB. Dissociation was noted about the quaternary nitrogen with the corresponding loss of the C_{10} alkyl chain (140 Da). The alkyl chain values of m/z = 57, 71 and 85 were also recorded, corresponding to C_4, C_5 and C_6 alkyl chain lengths, respectively. It was found that the bond dissociation produced by MS/MS could be reproduced on a single quadrupole by using an increased cone voltage. A cone voltage of 60V was found sufficient to produce the same bond dissociation information as had been achieved with MS/MS. Table I shows the data collected by cone voltage and collision induced dissociation techniques. Abundance values are quoted to the nearest integer.

m/z	Proposed Structure	Abundance (% Full Scale)	
		MS/MS	Cone Voltage
326	$CH_3(CH_2)_9N^+CH_3)_2(CH_2)_9CH_3$	100	100
186	$CH_3(CH_2)_9N^+CH_3)_2$	16	17
85	$[C_6H_{13}]^+$	2	2
71	$[C_5H_{11}]^+$	3	3
57	$[C_4H_9]^+$	19	18

Table I. Dissociation data obtained for didecyldimethylammonium bromide using cone voltage and MS/MS techniques.

3.3.5 *Application of Dissociation Methods to Real Samples*

A sample of a commercially available surface biocide concentrate containing a dialkyldimethyl QAC as the active substance was obtained. The dilute sample was initially analysed and the weight of the unknown QAC cation was determined as 270 Da. The sample was subjected to MS/MS and cone voltage bond dissociation. The data are given in Table II. The unknown QAC cation was determined as being of the dioctyldimethylammonium type.

m/z	Proposed Structure	Abundance (% Full Scale)	
		MS/MS	Cone Voltage
270	$CH_3(CH_2)_7N^+(CH_3)_2(CH_2)_7CH_3$	100	100
157	$CH_3(CH_2)_7N^+(CH_3)_2$	16	15
71	$[C_5H_{11}]^+$	2	2
57	$[C_4H_9]^+$	3	3

Table II. Dissociation data obtained for an unknown dialkyl QAC using cone voltage and MS/MS techniques.

3.4 Conclusion

The work shown here demonstrates a novel application of LC/ESI/MS to the determination of dialkyl and benzyl QAC. The sensitivity and selectivity advantages of MS detection are shown with respect to UV absorbing and non-UV absorbing QAC. Cone voltage and MS/MS ion dissociation methods have been applied to known and unknown dimethyl QAC showing the similarities in data obtained by the respective techniques. The cationic moiety of an unknown QAC was determined as being of the dioctyldimethylammonium type. The LC/ESI/MS method developed has been successfully applied to the analysis of real samples with the requirement for minimum sample preparation demonstrated.

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4.0 The Analysis of Iodopropynylbutylcarbamate (IPBC) by LC/ESI/MS and LC/APCI/MS.

Iodopropynylbutylcarbamate (IPBC) (Figure 1) is a fungicidal agent that was developed to replace pentachlorophenol and the sodium salts of tri-, tetra, and pentachlorophenol due to the ill effects of the phenol species on man and the environment.

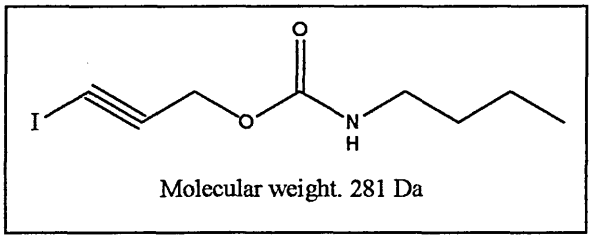

Figure 1. Iodopropynylbutylcarbamate

IPBC has been used as a consumer and industrial fungicide in the production of paints, vinyl wallpapers, wall paper adhesives, metal working fluids, and cosmetics [1]. The author is aware of one other group which has applied LC/MS to IPBC analysis. Zirrolli *et al.* [2] reported a 100 fold increase in sensitivity (10 $\mu g.ml^{-1}$) for LC/MS analysis, using electrospray ionisation, over their LC/UV method. Gas chromatography combined with flame ionisation detection (FID) and mass spectrometry (MS) has also been used to detect IPBC. Nakashima *et al.* [3] report the simultaneous analysis of three organoiodine antimicrobial ingredients including IPBC in commercially available antimicrobial/deodorant products using a GC/FID. A GC/MS method has been reported by Horn *et al.* for application to the analysis of wood preservative formulations [4]. There are twenty nine entries for HSE approved IPBC containing products in the 2001 HSE/MAFF pesticides guide [5]. With only three analytical methods for the determination of IPBC reported in the literature, and a

substantial number of approved products, it is a fair assessment to report that there are currently surprisingly few analytical methods for IPBC reported in the literature.

4.2 Experimental

4.2.1 *Chemicals and Reagents*

HPLC grade acetonitrile and acetic acid were obtained from Fischer Chemicals, (Loughborough, Leicester, U.K.). Ammonium acetate (HiPerSolv) was purchased from BDH, (Poole, Dorset, U.K.). Milli-Q water was used in all the experiments where necessary. The IPBC (certified as 99%) was obtained from Sigma-Aldrich (Loughborough, Leicester, U.K.).

A standard stock solution, 1000 $\mu g.ml^{-1}$, was prepared by weighing 10mg of IPBC into a 10 mL volumetric flask, and dissolving the IPBC solid to volume with methanol. Five working standards were prepared by dilution of the stock standard with methanol. The concentrations of the IPBC standard solutions were 0.5, 1, 10, 50, 100 $\mu g.ml^{-1}$. All solutions were stored in glass vials at 4°C. Homogenisation of all samples was achieved by sonication of the sample at 25°C for 30 secs.

4.2.2 *Instrumentation*

The HPLC/MS system consisted of a Jasco PU-980 Intelligent HPLC pump with a LG-980-02 Ternary gradient unit (Jasco, Chelmsford Road, Great Dunmow, U.K.), a VG TRIO 2000 mass spectrometer (Fisons Instruments, VG Biotech, Altrincham,

Cheshire, U.K.) equipped with pneumatically assisted electrospray (ESI) and atmospheric pressure chemical ionisation (APCI) interfaces. The injector used for the analysis was a Perkin-Elmer ISIS-200 sample introduction station (Perkin-Elmer, Beaconsfield, Buckinghamshire, U.K.). Data were recorded on a PC with Mass Lynx Software Version 3.2 (Micromass, Altrincham, Cheshire, U.K.). A flow rate of 10 $\mu l.min^{-1}$ from the electrospray probe was achieved by means of a 20:1 post- column T-piece/PEEK tubing split (Supelco, Poole, Dorset, U.K.). The nitrogen nebuliser and curtain gas were supplied by BOC (Guildford, Surrey, U.K.).

Direct infusion MS experiments were performed using a Harvard 11 syringe driver (Merck, Poole, Dorset, U.K.) with a Hamilton 500 µl gas tight syringe (Supelco, Poole, Dorset, U.K.). PEEK fittings and tubing were purchased from Supelco and used throughout mass spectral data acquisition. The flow rate used for direct infusion experiments was 7 $\mu l.min^{-1}$.

4.2.3 *Analytical Procedure*

Direct infusion experiments were performed over a range of cone voltages, 5 V increments in cone voltage were used, across the 5-60 V range. Samples for direct infusion analysis were prepared in the HPLC mobile phase at a concentration of 50 $\mu g.ml^{-1}$. The direct infusion mass spectrometer conditions were as those used for LC/MS experiments (see below).

The chromatographic procedure employed was isocratic with a 60/40, methanol/0.01 M ammonium acetate (pH 5 with acetic acid) at a flow rate of 1.0 $ml.min^{-1}$. The

column was a Waters Spherisorb S3 ODS2 cartridge column (4.6 x 100mm). Chromatography was carried out with the column at room temperature.

LC/MS experiments were performed in positive ion mode with the following working conditions;

ESI: Capillary 3.9 kV, Cone 23 V, Counter Electrode 0.07 kV, Lens 2 -252 V, Lens 3, -11 V, T 97 °C, LM Res 14.0, HM Res 14.0, Ion Energy 2.3, IE Ramp 1.0, Multiplier 685, Nebulising Gas 40 $l.hr^{-1}$, Drying gas 250 $l.hr^{-1}$.

APCI: Corona Voltage 3.9 kV, Counter Electrode 0.22 kV, Cone 23V, Lens 2 -154V, Lens 3 -10V, T 89 °C, LM Res 13.5, HM Res 13.0, Ion Energy 2.3, IE Ramp 1.0, Multiplier 650, Nebulising gas Instrument pre-set, drying gas 500 $l.hr^{-1}$.

LC/MS experiments were conducted in single ion recording (SIR) mode, and the ion corresponding to the protonated IPBC molecule, m/z 282, was monitored. The SIR settings were: Dwell time 0.2secs, Inter channel delay 0.02 secs, Repeats 1, Span 0.5 m/z.

4.3.1 *Optimisation of Spray Ionisation Parameters*

Initial experimental observations showed that IPBC was liable to bond dissociation and adduct formation during spray ionisation processes. To allow closer consideration of adduct and dissociation product ion formation, a series of experiments was conducted during which the cone voltage of the ion source was varied in increments of 5V. Direct infusion experiments allowed the observation of both the ESI and APCI ionisation behaviour of IPBC to be studied rapidly. A 100 $\mu g\ ml^{-1}$ IPBC standard was used for the cone voltage experiments. In the case of APCI it was necessary to use the standard solution as a mobile phase with a HPLC pump as the delivery system in order to achieve the required flow rate of 1.0 $ml.min^{-1}$. The cone voltage was varied and the abundances of the most abundant ions were recorded. The molecular weight of IPBC is 281 Da. Under ESI and APCI conditions we expect to see the protonated molecule, $[M+H]^+$, m/z 282. The ions which dominated the electrospray spectra were 156, 282 and 304 corresponding to $[M-I+2H]^+$, $[M+H]^+$ and $[M+Na]^+$, respectively. Figure 1 shows a graph of ion abundance as a function of cone voltage for m/z 156, 282 and 304 acquired using electrospray ionisation. The formation of metal adducts with carbamates under electrospray conditions is a phenomenon that has been reported previously [6] and was not unexpected. Dissociation of the carbon-iodine bond was noted. Below 5 V, acquisition of ions was not possible as the voltage was insufficient to produce ion transmission across the ion source. From Figure 1 the optimum ion abundance for the $[M+H]^+$ species of IPBC was noted as occurring after a cone voltage of 23 V. For this reason the value of 23 V was used for the cone voltage setting in the

LC/MS experiments. Solvent ions of the type $[(CH_3OH)_n+H]^+$ were recorded at low cone voltage values (5-10 V), where n = 2, 3, 4, 5 giving m/z 65, 97, 129, 161, respectively. Generally, the abundances of the solvent adduct ions are high at low cone voltage values. These adduct ions are decomposed by collisions with vapour phase molecules in the interface at the higher cone voltages. Sodium adducts are more resistant to decomposition and were even persistent at higher cone voltage values. The $[M+Na]^+$ ion was noted as being more stable at higher cone voltages than the $[M+H]^+$ species. The $[M+H]^+$ ion signal was questionable (Signal/noise = 3) after 50 V while the sodium adduct was still prominent in the spectra. It is also noted with interest that the formation of sodium adducts does not initiate until after 25 V cone voltage.

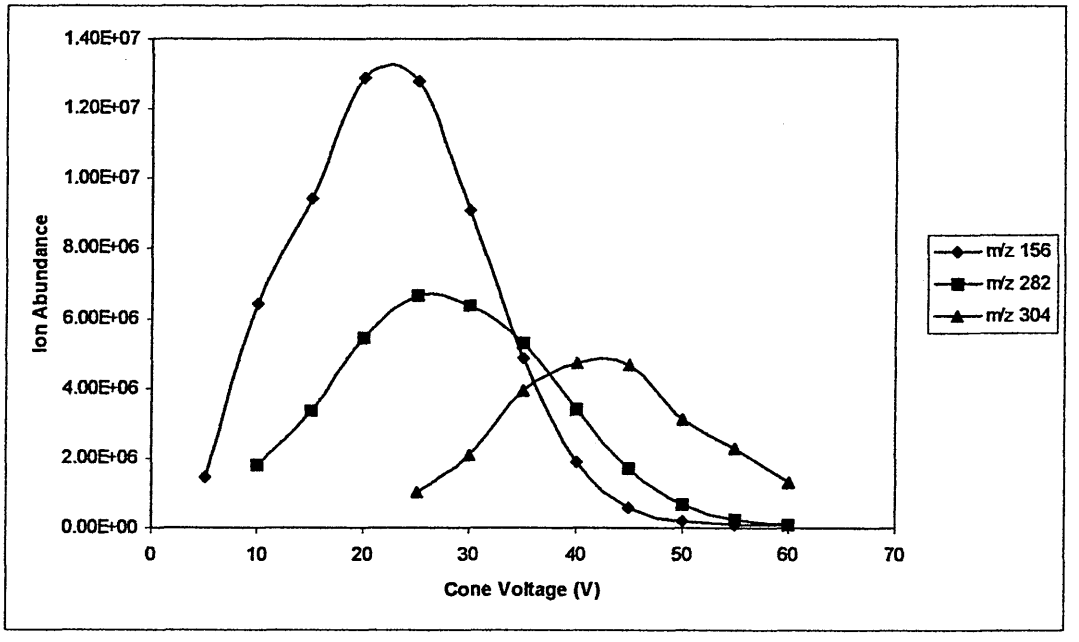

Figure 2. A graph showing the relationship between cone voltage and ion abundance for the ions corresponding to $[M-I+2H]^+$, m/z 156, $[M+H]^+$, m/z 282, $[M+Na]^+$, m/z 304 acquired using electrospray ionisation.

Optimisation of the cone voltage for APCI experiments produced much simpler spectra than those recorded using the electrospray source. APCI can be considered as a more energetic process than electrospray, exposing molecules to conditions of higher

temperature and a more turbulent gas phase atmosphere. The solvent clusters observed using electrospray were not present even at lower cone voltages (5-10V). The presence of the IPBC sodium adduct, $[M+Na]^+$; m/z 304, was not significant with APCI. Although present, the sodium adduct was of substantially lower abundance than that recorded using electrospray ionisation, (signal/noise<3). APCI conditions were optimized with consideration of only the $[M-I+2H]^+$, and $[M+H]^+$; m/z 156 and 282, ions. Figure 2 shows the relationship between m/z 156 and 282. It is noted that 23 V is, as for electrospray, the optimum cone voltage. An interesting observation was made that the applied cone voltage required to successfully achieve ion transmission across the source was at least 15 V, 10V higher than required for electrospray.

4.3.2 *Evaluation of Linear Response of LC/ESI/MS and LC/APCI/MS Methods*

Linearity studies were performed for the ESI and APCI LC/MS methods. IPBC standards were prepared at six concentrations (0.5, 1, 5, 10, 50, 100 $\mu g.ml^{-1}$) and analysed six times. Calibration curves were constructed by linear regression between peak area and compound concentration. Linear regression values were recorded as: ESI: 0.9932, APCI: 0.9991.

4.3.3 *Determination of LOD for LC/APCI/MS and LC/ESI/MS Methods*

Limits of detection (LOD) were determined using statistical methods described by Miller and Miller [7]. The APCI method was determined as being more sensitive than the ESI method. The limits of detection values for both methods were of the same order of magnitude. The LOD were 3.19 and 8.95 $\mu g.ml^{-1}$ for APCI and ESI methods,

respectively. A possible explanation of the increased sensitivity for the APCI method is the flow rate compatibility of the interface with the chromatographic method. Electrospray experiments require the splitting of the column effluent. This splitting introduces a dead volume to the chromatographic fluidics and was noted as a source of chromatographic band broadening. APCI allows direct coupling of the chromatographic column with the interface and requires no split, thus negating the dead volumes of a flow splitter and producing no degradation in peak shape.

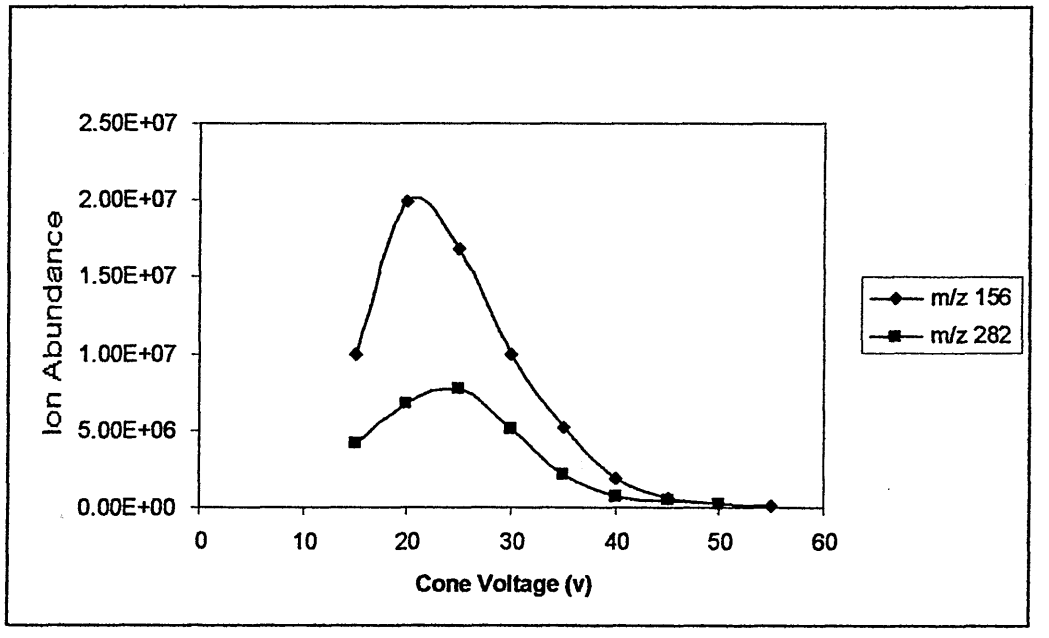

Figure 3. A graph showing the relationship between cone voltage and ion abundance for the ions corresponding to $[M-I+2H]^+$, m/z 156, $[M+H]^+$, m/z 282 acquired using APCI ionisation.

4.3.4 *Application of LC/ESI/MS and LC/APCI/MS to the Analysis of IPBC Containing Professional use Products*

The ionisation methods ESI and APCI were used in the LC/MS analysis of four samples of IPBC containing professional use products. The samples covered four

different products and three different manufacturers. The four products were selected for analysis by the HSL. Table 1 shows the details of the products.

Samples were prepared to contain an approximate concentration of 10 $\mu g.ml^{-1}$ IPBC (methanol solvent, using manufacturers' quoted values) and analysed six times. Results were evaluated using a calibration curve over the range 0.5-100 $\mu g.ml^{-1}$ (plotted from peak areas acquired in SIR mode monitoring m/z 282). The results were interpreted to give a % IPBC composition value for the formulation analysed. The results acquired in ESI and APCI modes are shown in tables 2 and 3, respectively.

Product name	Abbreviation	Manufacturer	% IPBC w/w
Timber Preservative M9	M9	Remtox, U.K.	6.3
Omega Biocide	Omega	Restoration, U.K	5
Fungicidal Wall Solution	Remtox	Remtox, U.K.	1
Trisol 23	Trisol	Triton Chemicals, U.K.	5

Table 1. Product name, product abbreviation, manufacturer information and IPBC content (as quoted by the manufacturer) for the four IPBC containing professional use products used in the work presented in this thesis.

Product	% IPBC w/w	RSD
M9	5.95	0.015
Omega	3.88	0.034
Remtox	0.74	0.015
Trisol	3.63	0.11

Table 2. Results acquired for the quantification of IPBC in professional use products using LC/MS with ESI ionisation. RSD is the relative standard deviation.

Product	% IPBC w/w	RSD
M9	6.37	0.035
Omega	4.57	0.018
Remtox	0.83	0.022
Trisol	4.18	0.020

Table 3. Results acquired for the quantification of IPBC in professional use products using LC/MS with APCI ionisation. RSD is the relative standard deviation

4.3.5 *Analysis of Matrix Effects*

The results acquired from the analysis of commercial formulations warranted further examination of the LC/MS methods that had been developed as a means of quantitatively analysing IPBC. Matrix effects are a phenomenon often associated with poor recoveries or low yields in quantitative analysis [8] and so a study was undertaken to examine the effect of the formulation matrix on the analysis of IPBC. The method of standard addition was chosen as being suitable for determining the matrix effects involved in the analysis of IPBC formulations. Sample solutions were prepared as in the previous quantification experiment. The samples were spiked with a known concentration of IPBC standard solution (10 $\mu g.ml^{-1}$) and analysed using the ESI and APCI LC/MS methods. The spiked samples were analysed six times. The data were analysed and percentage recoveries calculated for ESI and APCI methods (Table 4 and 5).

Product	M9	Omega	Remtox	Trisol
Average Recovery (%)	70.30	81.66	69.05	83.21
Stdev.	5.26	7.49	5.18	5.83
RSD	0.075	0.089	0.075	0.069

Table 4. Average Recovery, standard deviation (Stdev.) and relative standard deviation (RSD) results for matrix effect experiments described in section 4.3.5 using LC/MS with ESI ionisation.

Product	M9	Omega	Remtox	Trisol
Average Recovery (%)	80.32	84.35	70.56	84.87
Stdev.	7.39	8.30	2.21	1.69
RSD	0.092	0.106	0.031	0.020

Table 5. Recovery, standard deviation (Stdev.) and relative standard deviation (RSD) results for matrix effect experiments described in section 4.3.5 using LC/MS with APCI ionisation.

Tables 4 and 5 show an interesting pattern of recoveries. The recoveries from the electrospray experiments being lower than those acquired using APCI. This is in agreement with the quantitation data where the electrospray experiments yielded lower results than APCI. These results would indicate that the formulation matrix does have an effect on the ionisation efficiency of the electrospray process.

4.3.6 *Comparison of ESI and APCI Ionisation Methods for the LC/MS Analysis of IPBC in Professional use Products*

To compare the application of ESI and APCI as ionisation methods employed for the analysis of IPBC in professional use products, a statistical test was employed (t-test). This method of analysis essentially compares the means of results obtained using the two different ionisation techniques and reports the statistical significance of any difference between them. The method was applied to the results obtained from the

analysis of the commercial formulations (section 4.3.4). Table 6 shows the results obtained from the t-test analysis.

Product	M9	Omega	Remtox	Trisol
Pooled Stdev.	0.285	0.199	0.123	0.483
t	0.849	2.004	0.421	0.658

Table 6. *t*- test results obtained from the analysis of IPBC containing professional use products using ESI and APCI ionisation methods.

There are five degrees of freedom, so the critical value, $t = 2.57$. The experimental values recorded for t are less than the critical value and so differences between the results are not significant at the 5% level and the null hypothesis is accepted. There is no difference between the LC/APCI/MS and LC/ESI/MS methods for the determination of IPBC when applied to the analysis of commercial formulations containing IPBC.

4.4 Conclusion

A LC/MS method has been developed for the analysis of IPBC in commercial products using ESI and APCI ionisation methods. The LOD for the method using each interface has been calculated and both are lower than had been previously achieved using LC/UV/Vis. Differences in LOD methods have been explained and attributed to experimental conditions. The linearity of the method has been shown to be good over a practical working concentration range. The developed method has been successfully applied to the quantitative analysis of commercial IPBC-containing products. The application of a statistical t-test to data obtained from both methods of ionisation shows no significant difference between the two methods. However, it has also been shown

that a lower LOD is achievable using APCI and so in the case of forensic work this method, would favoured by the author. The implication of this work for occupational hygiene analysis is the ability to analyse IPBC, with lower limits of detection and in the presence of non-chromaphoric co-formulants, using LC/MS.

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5.0 The Qualitative Determination of Isocyanate Derivatives by LC/MS, Nanospray/MS/MS and MALDI/MS/MS

Isocyanates (NCOs) are a group of highly reactive compounds widely used in industry. Mono-functional NCOs are used as intermediates in the production of herbicides, crop-protection agents and anti-diabetic drugs [1]. The most important class of NCOs for industrial purposes is the di-functional NCOs. Diisocyanates are widely used in the manufacture of polyurethane products such as flexible and rigid foams, fibers, coatings such as paints and varnishes, and elastomers [2]. Diisocyanates are increasingly used in motor vehicle manufacture (manufacture of interior fittings and trim) and body repair industries and in the production of building insulation materials [2]. A urethane is the product of the reaction between an isocyanate and an alcohol. Figure 1 shows the reaction for the formation of a urethane. Polyurethanes are manufactured using poly-functional NCOs and poly-functional alcohols, polyols.

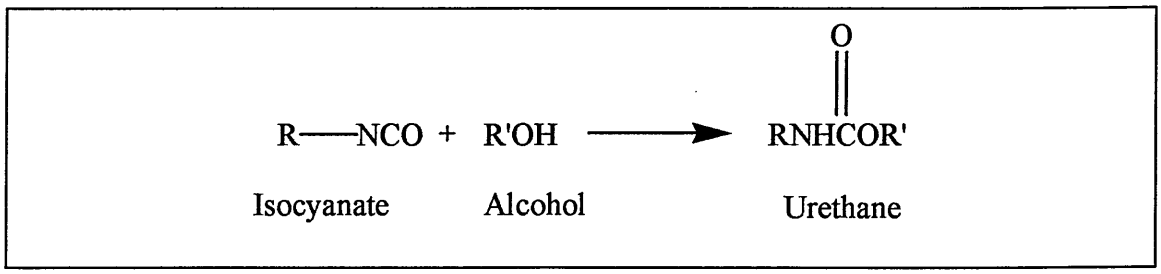


Figure 1. The formation of a urethane from the reaction of an isocyanate with an alcohol.

Hexamethylene diisocyanate (HDI), 4,4'-methylene biphenyl diisocyanate (MDI), 2,4-and 2,6-toluene diisocyanate (2,4-and 2,6-TDI) (Figure 2) account for more than 90% of the commercial use of diisocyanates in the United Kingdom [3]. NCO prepolymers are derivatives of NCO monomers that have similar chemistry to the parent molecule but

exhibit a lower vapour pressure as a result of their greater size. HDI, an aliphatic NCO, is commonly produced in biuret form i.e. three molecules of HDI bound together [4] (Figure 3). NCO prepolymers and monomers are commonly encountered in the same environment. In statistics published by the Health and Safety Executive (HSE) NCOs are cited as the primary cause of occupational asthma in the United Kingdom [5]. Bernstein has defined occupational asthma as:

"a disease characterized by airflow limitation and/ or air way hyper-responsiveness due to causes and conditions attributable to a particular occupational environment and not to stimuli encountered outside the workplace" [6].

The Trades Union Congress (TUC) has predicted that the economy of the United Kingdom could lose ten million pounds over the next ten years as a result of occupational asthma [7]. Although NCO are not produced in the United Kingdom, the country currently (2002) imports between 90,000 and 100,000 tonnes of NCO per year for consumption by its industry. The exposed occupational population is about 25,000 workers [3]. The relationship between NCOs and adverse health effects is in fact, very well established [8-12]. Exposure to airborne monomeric NCOs is known to cause a range of respiratory disorders in laboratory animals and in humans [13]. In the case of pulmonary exposure the immune response produces symptoms such as wheezing, coughing, chest tightness and general breathing difficulties. Additional symptoms include runny eyes and noses, or even life threatening anaphylactic shock [14]. The inhalation of aerosols of non-volatile NCOs species has also been observed to cause the same type of respiratory effects as inhalation

of monomeric NCO vapours [15]. The degree of injury as a result of exposure to NCOs is proportional to the type and level of exposure [14]. Repeated exposure to low concentrations of NCO over a period of several months or years can lead to progressive impairment of pulmonary function with considerable shortness of breath and stress on the heart. More seriously, a "sensitised" condition can arise such that asthmatic symptoms are produced almost immediately on exposure to relatively low concentrations that do not affect other non-sensitised workers [16]. Sensitisation may be the result of a single case of high exposure (e.g. a leak or spillage) or prolonged exposure to low levels of NCO [17]. It has been shown that the effects of isocyanate sensitisation are not monomer-specific, that is an individual sensitised by exposure to one type of NCO will exhibit the symptoms of sensitisation in an atmosphere of a different NCO [18]. The accurate determination of NCOs in the working environment is a matter of concern.

The degree of exposure of an individual to NCOs in the work-place is determined to a large extent by the physical form of the NCOs. Low molecular weight, high vapour pressure NCOs are readily volatilized and present a threat to a worker's health. 2,4- and 2,6-Toluene diisocyanate are examples of such NCOs [9, 11]. Toluene diisocyanate is commonly used in the manufacture of polyurethane foams. Foam blocks are cut to size using industrial cutting devices and these impart their mechanical energy to the block being fashioned causing heating. This can lead to the emission of NCOs and other polyurethane degradation products, such as polyols, into the local environment [19]. Low vapour pressure isocyanates e.g., the biuret form of HDI (Figure 3), are less readily volatilized and so are perceived to reduce the potential threat to a worker's health [12].

However, consideration of the way in which NCO containing products are used and the mechanical processes to which they are subjected, suggests that emerge even low vapour pressure NCOs have the potential to cause harmful effects. HDI for example is less sensitive to light than its aromatic counterparts and is widely used for surface coating and finishing [20]. Such coatings are often applied in the form of an aerosol and hence the low volatility monomers and prepolymers present in the bulk product are converted into a respirable form [21, 22].

In order to prevent working conditions which present a hazard to workers' health the HSE has classified NCOs as toxic substances and they have been assigned a MEL of 0.02 $mg.m^{-3}$ for an 8 hr Time Weighted Average (TWA) or 0.01 $mg.m^{-3}$ for a 15 minute reference period [23]. The concentrations quoted for the MEL values are for total NCO group concentration.

A large number of analytical methods capable of determining diisocyanate monomers both qualitatively and quantitatively have been reported. As a result of the workplace occurrence of complex mixtures of NCOs of undetermined composition, and their related adverse health effects, analytical methods must be capable of identifying and quantifying not only those NCO which are well documented and for which reference materials are available, but, also other NCO species for which reference materials are not available.

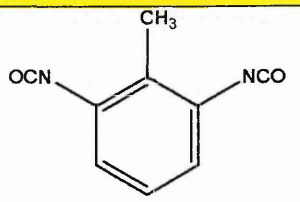
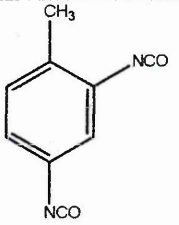
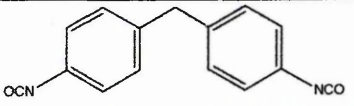
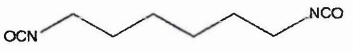
Compound Name	Abbreviation	Mw.	Structural Formula
2,6-Toluene diisocyanate	2,6-TDI	174.2	
2,4-Toluene diisocyanate	2,4-TDI	174.2	
4,4'-Methylenebiphenyl diisocyanate	MDI	250.3	
Hexamethylene diisocyanate	HDI	168.2	

Figure 2. Compound name, abbreviation, molecular weight (Mw.) and structural formula for four common NCO monomers.

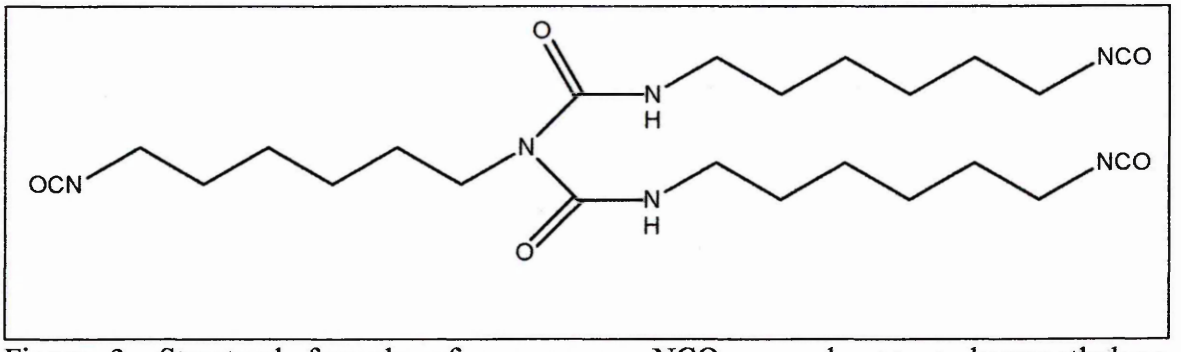


Figure 3. Structural formula of a common NCO pre-polymer, a hexamethylene diisocyanate biuret adduct [4].

Sample analysis is further complicated by the reactivity of the NCO functional group which leads to the loss of sample components in the course of sampling and analysis e.g. NCOs will react with polyols and water to produce urethanes and amines. Problems regarding NCO reactivity can be overcome by derivatising the NCO during collection.

Chemisorption combined with transformation of NCO groups into stable forms is used for the pre-concentration of NCOs from air [24]. The derivatising reagent used must be capable of transforming the NCO into a stable species, thus reducing error in measurements taken from monitoring surveys. Early analytical methods for the analysis of NCOs used acid hydrolysis to convert diisocyanates to diamines. In such methods, once collected, the amines are subsequently diazotized and coupled with 1-naphthylethylenediamine to form a stable diazo compound. The diazo compound can be analysed spectrophotometrically [25]. The acid hydrolysis reaction takes place in an impinger device through which the sampling atmosphere is drawn by a suitable pumping device [25]. Such methods of analysis have several problems including poor specificity and poor ruggedness.

The characteristics of suitable derivatising reagents for NCO sampling have been described by Streicher *et al.* [26]. A derivatising reagent for total NCO functional group analysis must posses the following characteristics:

1. It must react rapidly with all NCO species in the sampling device to prevent losses of NCO functional group to competing reactions with polyols or water.
2. NCOs derivatised with this reagent must be detectable at very low levels.
3. NCOs derivatised with this reagent must give a detector response that is independent of the structure.

The reaction of isocyanates with amines to produce urea derivatives has found favour as a method of forming stable isocyanate derivatives (Figure 4). Also modern spectroscopic techniques i.e. UV and fluorescence, have eliminated the need for several chemical reactions (reduction of the nitro group, diazotization, and coupling) prior to the detection of a derivatised isocyanate.

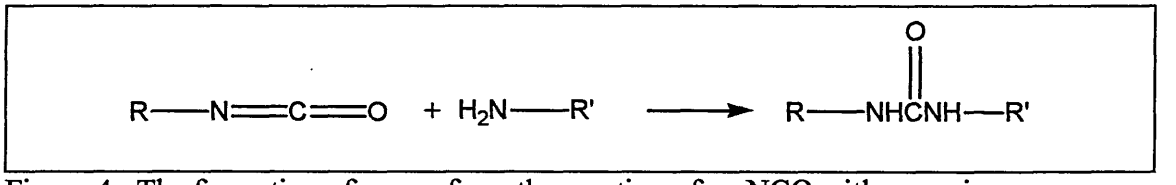


Figure 4. The formation of a urea from the reaction of an NCO with an amine.

Several reagents (Figure 5), based on the formation of urea derivatives have been successfully applied to NCO derivatisation. Dunlap *et al.* reported the use of N-4-nitrobenzyl-n-propylamine (nitro reagent) [27] to form stable urea derivatives of NCOs. Although since its development many analytical methods have been reported which use nitro reagent, problems have been reported with the effects of excess nitro reagent on HPLC column packings (although these problems have been overcome by acetylating the excess reagent, by addition of acetic anhydride) [28-30,]. Other problems encountered in the use of nitro reagent are the sensitivity of the compound to oxidising and reducing atmospheres, the chemistry necessary to prepare the reagent and its stability once formed [30]. A limitation of this method is that it is only capable of the quantification of NCO monomers. The analysis of bulk formulation samples is carried out by preparing standards derived from a bulk material of which the NCO composition is supposedly already known. Such methodology does not account for the formation of reactive intermediates or transformation products when air monitoring samples are considered.

Hardy and Walker introduced 1-(2-pyridyl)piperazine (PP) [30] as a reagent for derivatisation of NCOs. This piperazine-based reagent showed rapid reaction rates with aliphatic and aromatic NCOs. In addition the substituted ureas formed possess significantly higher molar absorptivities in the UV region than those derived from nitro reagent [31].

MAMA or 9-(methylaminomethyl)anthracene was developed by Elwood *et al.* [31] as a suitable reagent for NCO derivatisation. MAMA derivatives exhibit strong absorbance and fluorescence characteristics. MAMA has recently been used for total NCO determination by HPLC with UV and/or fluorescence detection [32]. In these studies it was found that the UV responses of MAMA derivatised NCO are independent of the NCO structure.

It was also found that the total NCO group content of several products containing oligomeric NCOs could be accurately determined after derivatisation with MAMA and analysis by HPLC with UV at two different wavelengths. Whilst fluorescence detection gives better sensitivity, comparisons of fluorescence response of several monomers derivatised with MAMA have shown a very large dependence on NCO structure. This variability compromises the ability to correctly identify by fluorescence NCO species for which standards are not available, and makes accurate quantification of these species by fluorescence impossible.

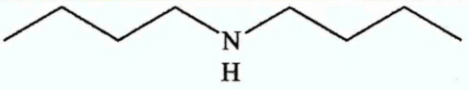
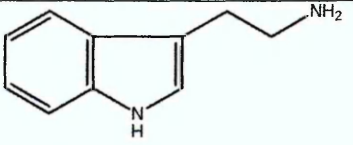
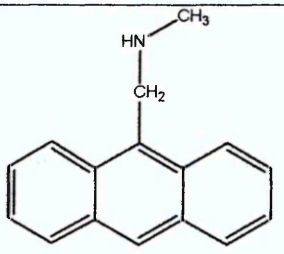
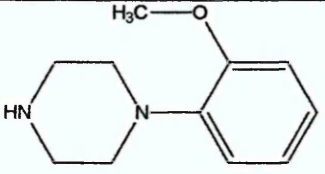
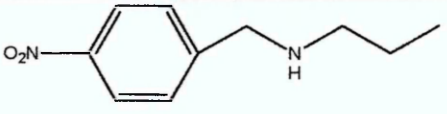
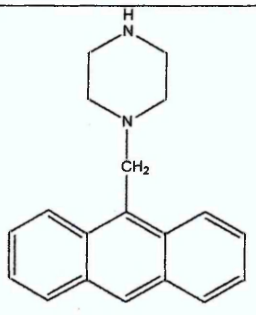
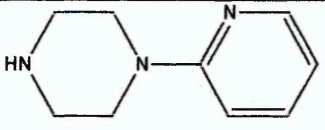
Compound Name	Abbreviation	Mw.	Structural Formula
Dibutylamine	DBA	129.2	
Tryptamine	Trypt	160.2	
9-(methylaminomethyl) anthracene	MAMA	221.3	
1-(2-methoxyphenyl) piperazine	MP	192.3	
N-4-nitrobenzyl-n-propylamine	Nitro	194.2	
1-(9-anthracylmethyl)-piperazine	MAP	276.4	
1-(2-pyridyl)piperazine	PP	163.2	

Figure 5. The chemical names, common abbreviations, molecular weights (Mw.) and structural formulae for some common NCO derivatising reagents

To identify and directly quantify each individual NCO species in a sample using chromatographic techniques, there needs to be some characteristic other than chromatographic retention time on which to base identification. Identification could be achieved with a single detector if that detector responded only to the derivatised NCO. Unfortunately, even when detectors generally considered highly selective (such as fluorescence detectors) are used, absolute selectivity cannot be achieved. For instance, even though relatively few potential interferents are likely to fluoresce, if these compounds are present at reasonably high concentrations, they could produce a significant signal indistinguishable from that of a derivatised NCO. If as an alternative two detectors are used in series, the ratio of detector responses for a particular analyte is constant and is therefore characteristic of a particular analyte. This is called the detector response ratio method.

The detector response ratio concept was first used to identify NCOs in Health and Safety Executive Method for the determination of Hazardous Substances 25 (MDHS 25) [33]. In this method NCO species are derivatised with 1-(2-methoxyphenyl)piperazine (2MP) and the derivatives analysed by HPLC with UV and electrochemical (EC) detection in series. This is the method used in the United Kingdom to determine compliance with the total NCO group exposure standard. Unfortunately, in a study of oligomeric NCO species derived from 2,4-toluene diisocyanate (2,4-TDI), it was noted that neither the UV nor the EC response was proportional to the number of derivatised NCO groups. The failure of the UV response to be proportional to the number of NCO groups resulted from the fact that only a small portion of the UV response was attributable to the derived NCO group. As a

result, it was concluded that identification based on the detector response ratio is not reliable, particularly when the analyte has a strong source of UV absorbance other than the derivatised NCO group [34]. Moreover, EC detectors have been found to be relatively unstable and this instability has been shown to contribute to the variability of the detector response ratios in the National Institute for Occupational Safety and Health (NIOSH, Ohio, U.S.A) method 5521[35].

Rudzinski *et al.* report derivatisation of NCOs with 1-(9-anthracylmethyl)-piperazine (MAP) [36]. The anthracene moiety facilitates sensitive detection by either fluorescence or UV. It is postulated that the greater distance between the point of isocyanate attachment and the chromaphore/fluorophore minimises the dependence of UV and fluorescence response on the nature of the NCO species. MAP has been shown to exhibit rapid reaction rates with NCOs. Fast derivatisation reactions reduce the probability of reaction of the NCO with polyol or water interferents. MAP-derivatised NCOs have the same fluorescence and UV characteristics as MAMA-derivatised NCOs [36].

Another analytical method for the determination of the total NCO groups utilises derivatisation with tryptamine (Trypt) followed by HPLC with fluorescence and EC detection. Suggested by Wu *et al.* [37], it was found that the response is fairly independent of NCO structure for Trypt-derivatised isocyanates in both fluorescence and EC detectors. As a consequence of the structure independent response, and the selectivity and sensitivity of the fluorescence detector, NIOSH researchers have developed an impinger sampling method using Trypt as the derivatising reagent [38].

Dibutylamine (DBA) has been used to form a stable derivative urea with NCOs. DBA was investigated with aim of producing a reagent with improved selectivity towards NCOs and improved stability to allow prolonged storage of derivatised NCO [39]. The stability of MAMA, MAP and 2MP derivatives, and the effect of this on the storage of derivatised samples has been questioned [39]. DBA is particularly amenable to the electrospray ionisation technique and so is favoured for LC/ESI/MS analysis of samples. DBA also has the advantage of having a negligible response to photometric detection and therefore does not interfere with quantitation by LC/UV. If a diode-array detector is employed, UV spectra can be obtained with negligible response from the derivatising reagent.

5.2 Experimental

5.2.1 *Equipment*

Direct infusion and LC/MS experiments were performed on a Quattro I triple quadrupole mass spectrometer (Fisons Instruments, VG Biotech, Altrincham, Cheshire, U.K.) equipped with a pneumatically assisted electrospray (ESI) interface. Direct infusion MS experiments were performed using a Harvard 11 syringe driver (Merck, Poole, Dorset, U.K.) with a Hamilton 500 µl gas tight syringe (Supelco, Poole, Dorset, U.K.). The HPLC system used for the analyses was a Jasco PU-980 Intelligent HPLC pump with a LG-980-02 Ternary gradient unit (Jasco, Chelmsford Road, Great Dunmow, U.K.). The column used for the separation was a Hypersil C18 (2.1 x 100 mm) (Hypersil Thermo Hypersil-Keystone, Cheshire, U.K.). A Rheodyne injection valve mounted in a gas flow regulating

unit was used for sample introduction. A home made flow splitter was made from a T-piece/ and PEEK tubing (Supelco, Poole, Dorset, U.K.). Nanospray infusion experiments were performed on a Q/ToF Ultima (Micromass, Altrincham, Cheshire, U.K.). MALDI experiments were performed on a LaserTof 1500 mass spectrometer (SAI, Manchester, U.K.) which uses a N_2 laser at 337 nm. MALDI/MS/MS experiments were performed on a Q-Star, quadrupole time of flight mass spectrometer (Applied Biosystems, Foster City, U.S.A.)

5.2.2 *Operating Conditions*

The LC/MS experiments were performed with the following operating conditions: capillary voltage 3.95 kV, HV lens voltage 0.3 kV, cone voltage 30 V, Lens 3 potential 3 V, multiplier 550 V, source temperature was 95 °C. The nitrogen nebuliser and curtain gas (BOC, Guildford, Surrey, U.K.) flows were 40 $l.h^{-1}$ and 350 $l.h^{-1}$, respectively. The collision gas used was argon (BOC, Guildford, Surrey, U.K.). Data was recorded on a PC with Mass Lynx Software V2.0 (Micromass, Altrincham, Cheshire, U.K.). The HPLC flow rate was 200 $\mu l.min^{-1}$. This flow was split post column and 10 $\mu l.min^{-1}$ was directed to the mass spectrometer. The chromatographic conditions employed gradient elution; and the gradient program is shown in Table 1. The injection volume used was 5 µl. Data were acquired in scan mode. The analytical run time was 40 mins and after each run the column was allowed to re-equilibrate for 10 mins. To ensure column and analytical integrity the analysis of bulk formulation samples was bracketed with the analysis of blank samples.

Time (mins)	Flow rate ($ml.min^{-1}$)	%A	%B
0	1	60	40
10	1	60	40
10.5	1	70	30
15	1	100	0
25	1	100	0
30	1	60	40

Table 1. The Gradient elution program used for LC/ MS analysis of isocyanate derivatives, A = acetonitrile, B = 0.1M ammonium acetate pH 4.7 (Acetic Acid).

5.2.3 *Chemicals*

NCO monomers (HDI, 2,4-TDI, 2,6-TDI and MDI) as 1-(2-methoxyphenyl)piperazine derivatives were provided by the Health and Safety Laboratory (HSL, Sheffield, U.K.). HPLC grade acetonitrile, methanol and trifluoroacetic acid (TFA) were obtained from Fischer Chemicals, (Loughborough, Leicester, U.K.). Ammonium acetate (HiPerSolv) was purchased from BDH, (Poole, Dorset, U.K.). Milli-Q water was used in all the experiments where necessary. 2,5-Dihydroxybenzoic acid (DHB) was purchased from Sigma-Aldrich (Poole, Dorset, U.K.).

5.2.4 *Derivatisation of Isocyanates*

Bulk NCO formulations were prepared for analysis by derivatisation of the bulk material with 1-(2-methoxyphenyl)piperazine. The derivatisation method is reported in MDHS 25/3 [33].

5.2.5 *Working Solutions*

Stock solutions of 2,4-TDI, 2,6-TDI, MDI and HDI as urea derivatives were prepared at a concentration of 100 $\mu g.ml^{-1}$ of the white urea derivative solid in acetonitrile. Bulk formulations were prepared by dissolving the derivatised material in acetonitrile to achieve a concentration of 100 $\mu g.ml^{-1}$ of the white urea derivative. This concentration varied with the product type because of solubility issues with the large urea derivatives (>500 Da) of isocyanates.

5.2.6 *MALDI Matrix Preparation*

The matrix used for the MALDI analysis was 2,5,-dihydroxybenzoic acid dissolved in 0.1 % trifluoroacetic acid (TFA) in methanol (50 $mg.ml^{-1}$). 200 µl of the sample and 50 µl of the matrix were mixed and 1µl of the resulting solution was placed on the stainless steel target of the instrument for analysis. MALDI experiments were carried out using a 20 kV extraction voltage. All spectra were the result of the cumulative acquisition of 20 shots.

5.3.1 *Initial Direct Infusion Experiments*

To investigate the electrospray ionisation behaviour of derivatised isocyanate species direct infusion experiments were performed on derivatised isocyanate monomers, and bulk isocyanate formulations. The monomers investigated were MDI, HDI, 2,4-TDI and 2,6-TDI. Table 2 shows the ions acquired from the direct infusion analysis of the four monomeric NCOs used in this study.

Following the success of the initial experiments, the method was applied to the analysis of bulk NCO formulations. Figure 7a shows an example mass spectrum acquired from a derivatised trimeric HDI bulk formulation sample. The structure of the derivatised HDI trimer is shown in Figure 6. The electrospray mass spectrum shown in Figure 7a contains several peaks corresponding to ions of m/z values higher than those of the derivatised HDI trimer which, to the author's knowledge, had previously not been reported. Figure 7b is such an example and shows a magnification of the high mass region, (1545-1645 m/z), of the spectrum shown in Figure 7a.

The observation of high mass ions was common in the analysis of derivatised bulk NCO formulations. The determination of the structural properties of these species is an important area of concern. The main concern is whether or not the high mass ions are NCO species.

Derivatised NCO Monomer	m/z	Proposed Ion
HDI	193	$[MP+H]^+$
	361	$[M+H-MP]^+$
	553	$[M+H]^+$
	746	$[M+2H+MP]^+$
MDI	193	$[MP+H]^+$
	443	$[M+H-MP]^+$
	318	$[M+2H]^{2+}$
	635	$[M+H]^+$
	827	$[M+2H+MP]^+$
2,4-TDI	193	$[MP+H]^+$
	366	$[M+H-MP]^+$
	559	$[M+H]^+$
	751	$[M+H+MP]^+$
2,6-TDI	193	$[MP+H]^+$
	559	$[M+H]^+$
	751	$[M+H+MP]^+$

Table 2. Ions observed from the direct infusion electrospray ionisation experiments of derivatised NCO monomers. M is the corresponding NCO monomer, MP is the derivatising reagent and H is hydrogen.

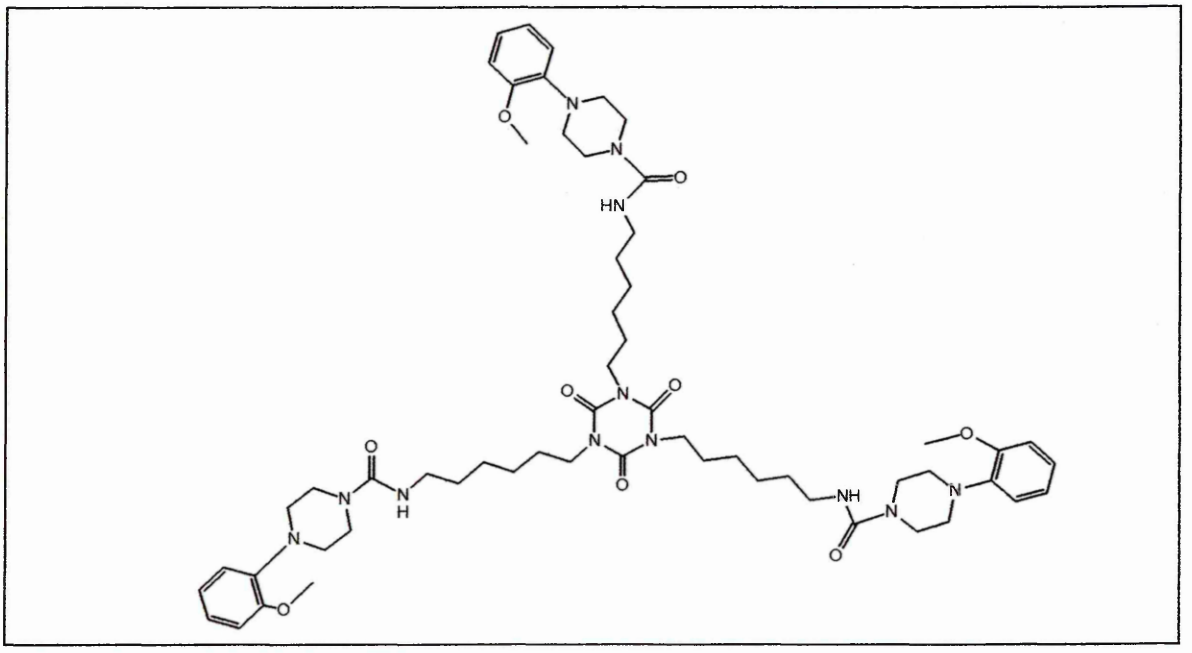


Figure 6. The structural formula of a derivatised trimeric HDI species [40]. The molecular weight of the derivatised species is 1081 Da.

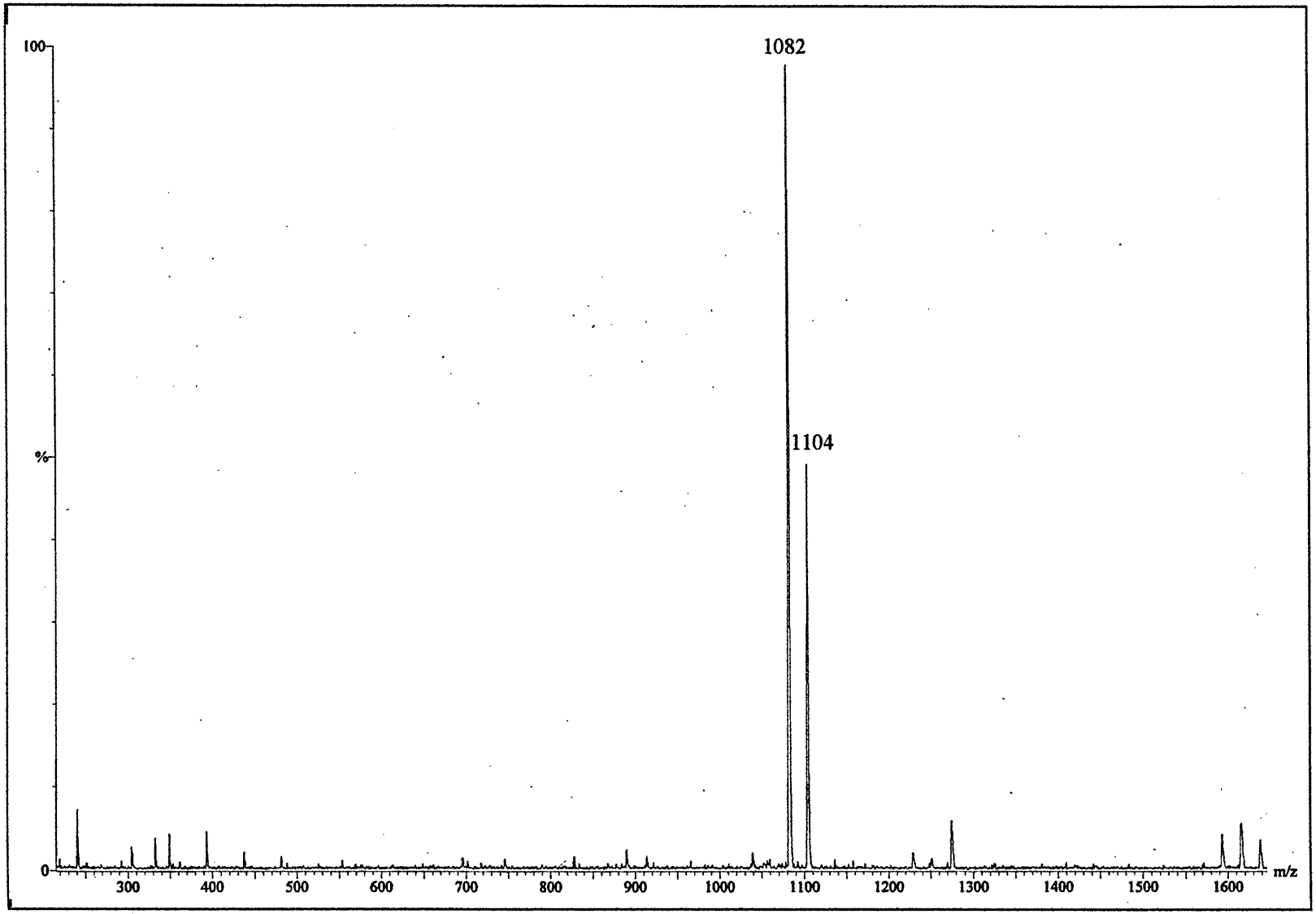


Figure 7a. An electrospray mass spectrum acquired from the direct infusion of an MP derivatised HDI bulk formulation. The ion dominating the spectrum, m/z 1082, is a protonated derivatised trimeric HDI molecule, $[M+H]^+$, shown in Figure 6. The ion at m/z 1104 is a sodium adduct of the derivatised trimeric HDI, $[M+Na]^+$.

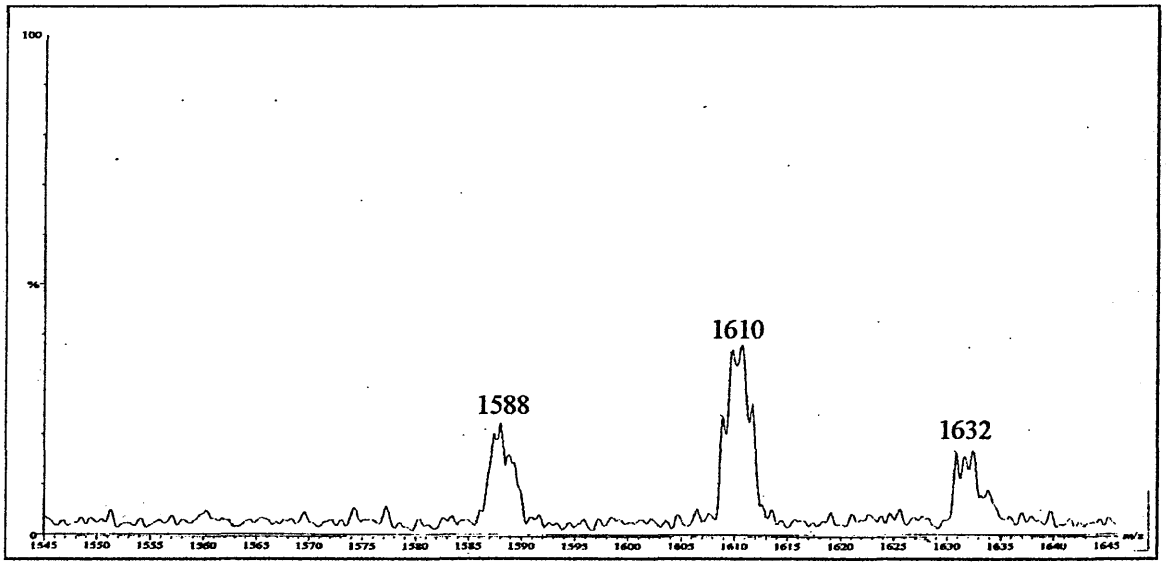


Figure 7b. A mass spectrum showing the high mass region highlighted in Figure 7a

5.3.2 *Application of LC/MS to the Analysis of Derivatised Isocyanates*

The sensitive and specific nature of liquid chromatography/mass spectrometry, (LC/MS), makes it an ideal analytical technique for the analysis of mixtures of NCOs and their related products. Previous work using LC/MS for NCO determination has focused on the analysis of DBA derivatised NCOs. The work presented here differs in that the methods developed are for the 2MP derivatised NCOs and are to be used in conjunction with the existing HSE approved method MDHS 25/3 as a qualitative method. The mass region of interest for the analysis of bulk NCO formulations was determined by initial direct infusion experiments on the samples of interest (as described in Section 5.3.1). To improve the transfer of larger species through the interface the cone voltage was increased from 30 V to 90 V after 8 mins of the chromatographic run.

Figure 8 shows the result from the LC/MS analysis of an aged derivatised trimeric HDI sample (100 $\mu g.ml^{-1}$ of white urea solid). The peaks eluting between 3 and 6 minutes, and observable in the mass chromatograms at m/z 193 and 1082, correspond to the protonated excess 2MP derivatising reagent and the protonated molecules of the derivatised trimeric HDI species (Figure 6), respectively. These species were expected and their structures were known prior to the analysis [40]. However, unlike previous LC/UV/Vis analysis, where the presence of the trimeric HDI molecule is confirmed by the retention time combined with prior knowledge of the sample's origin, here it is confirmed with highly specific mass to charge ratio information. The peaks eluting between 9 and 22 mins are of great interest. Their presence has previously been suspected from LC/UV/Vis data but the structures of these species are unknown. The extracted mass chromatograms m/z 1632, 1610, 1324, 1588 and 1335 show some interesting features. M/z 1632 and 1610 appear to "track" each other in the region between 9 and 12 mins suggesting that they are related. The most likely explanation is that m/z 1632 is the sodiated adduct of a molecule of molecular weight 1609 and m/z 1610 is the protonated molecule of the same compound.

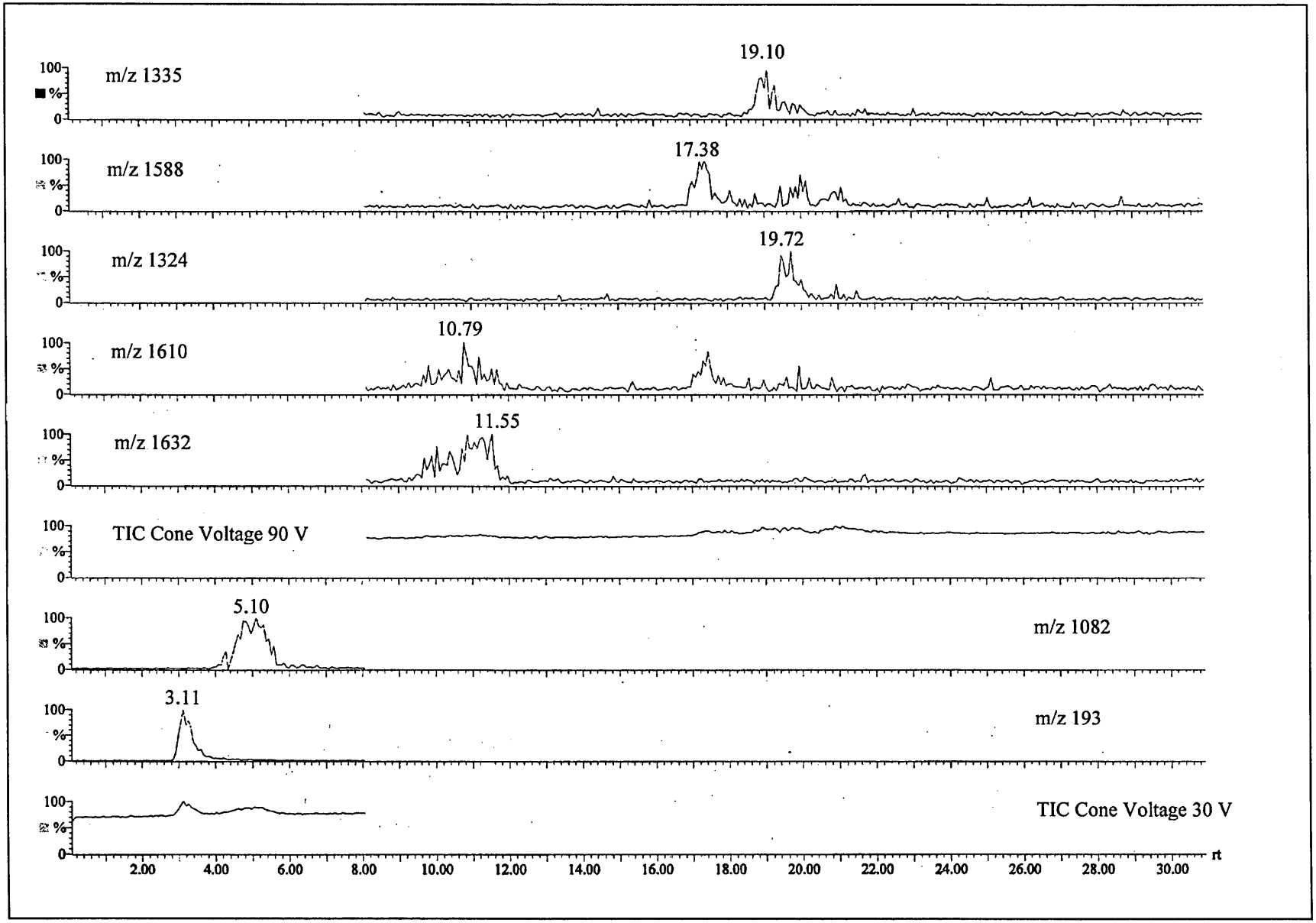


Figure 8. Mass Chromatograms showing the separation of an aged derivatised HDI sample. The TIC is shown (Cone voltage 30 and 90 V) and seven channels of the most abundant ions are shown. The column used for the separation was a Hypersil C18 (2.1 x 100 mm). The flow rate was 200 $\mu l.min^{-1}$ and the gradient conditions used for the separation are shown in Table 1.

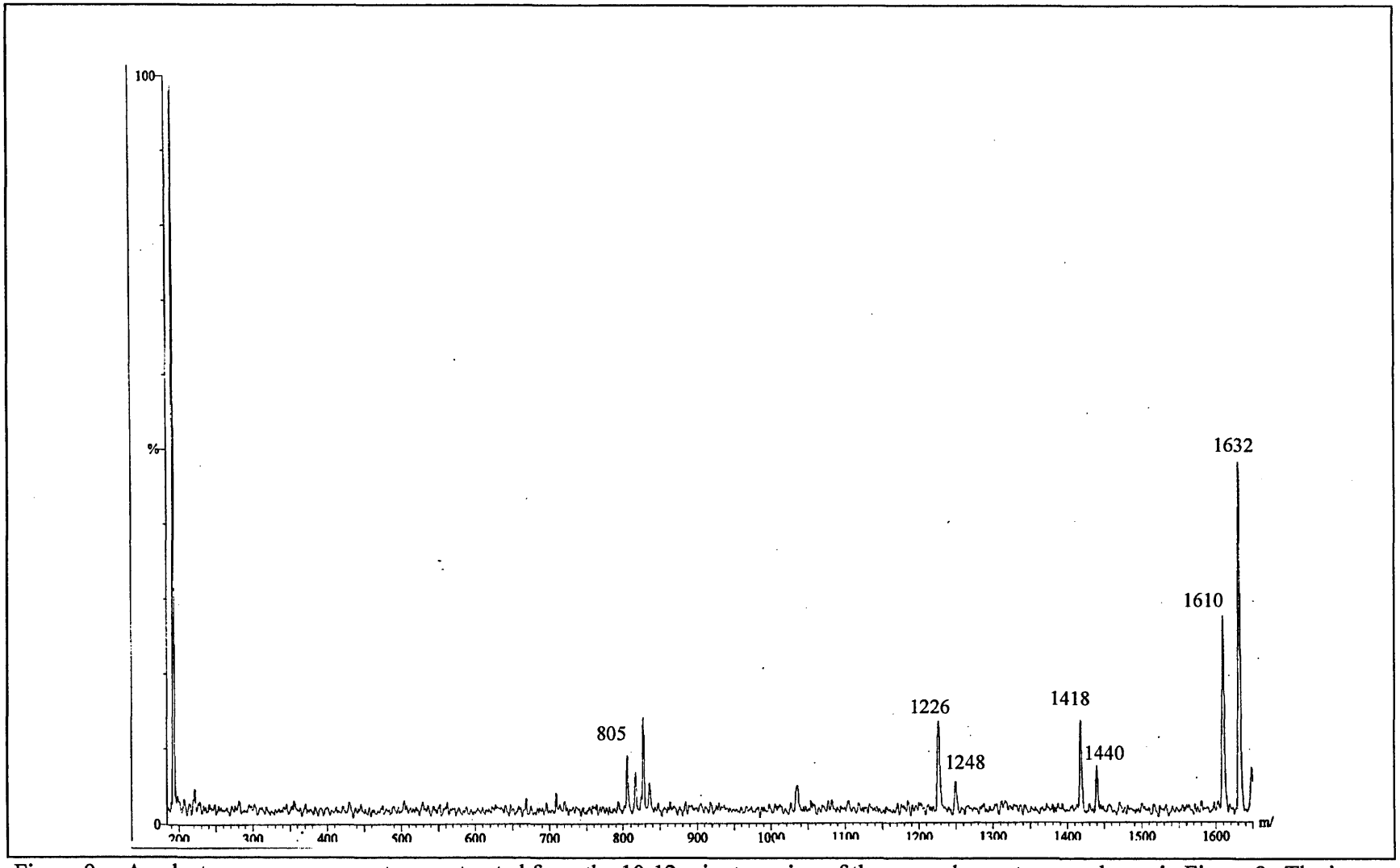


Figure 9a. An electrospray mass spectrum extracted from the 10-12 minute region of the mass chromatogram shown in Figure 8. The ions labelled on the mass spectrum are assigned in Table 3. The cone voltage was 90 V.

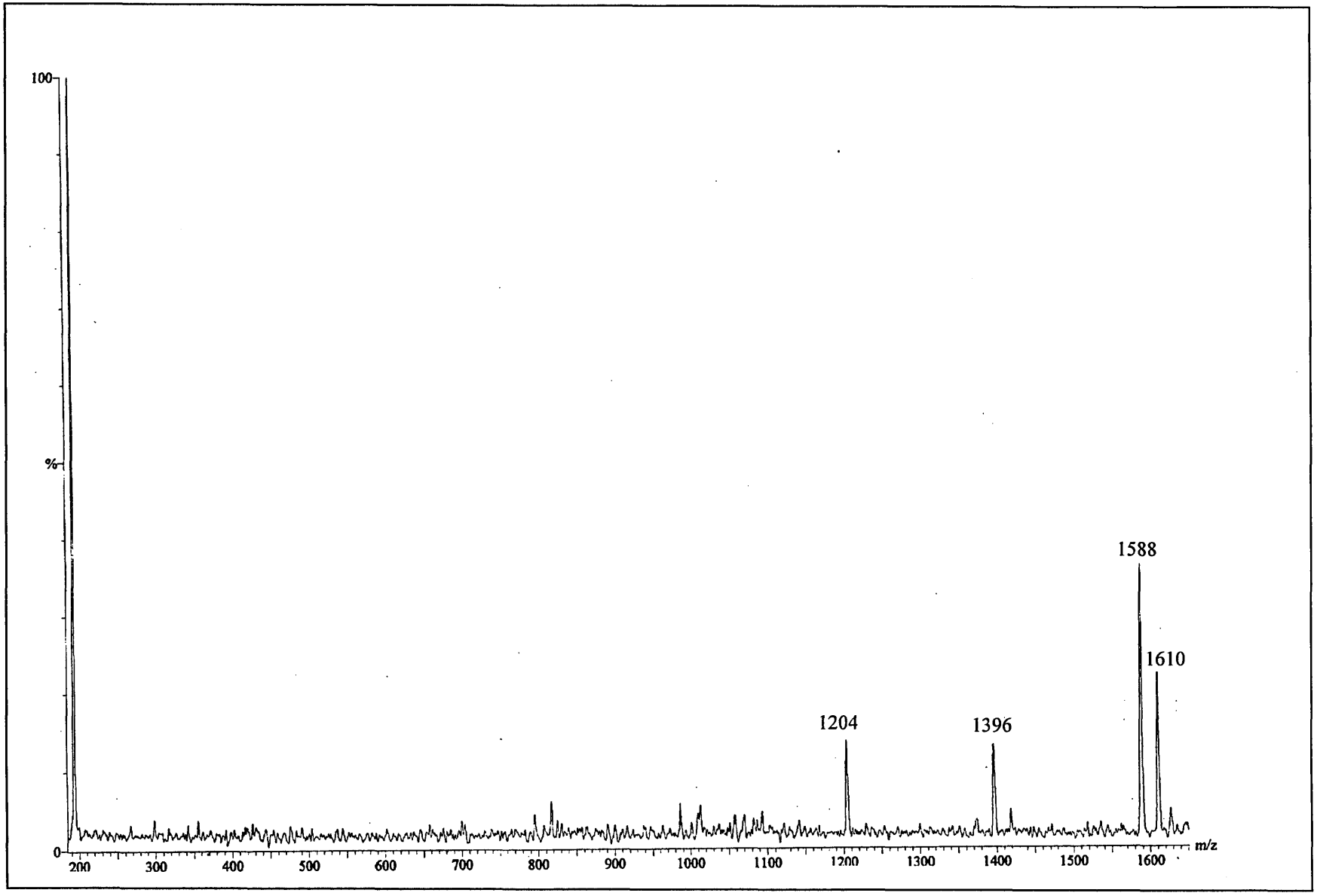


Figure 9b. An electrospray mass spectrum extracted from the 10-12 minute region of the mass chromatogram shown in Figure 8. The ions labelled on the mass spectrum are assigned in Table 4. The cone voltage was 90 V.

A combined mass spectrum of the 10-12 min region of Figure 8, (Figure 9a), confirms the presence of the sodium adduct, (m/z 1632), of a species corresponding to a protonated molecule at m/z 1610. Each protonated molecule in the spectrum has a "satellite" peak corresponding to the sodiated species. Table 3 is an interpretation of the major ions in Figure 9a.

m/z	Suggested Ion of Derivatised Isocyanate
805	$[M+2H]^{2+}$
1226	$[M+H-2MP]^{+}$
1248	$[M+Na-2MP]^{+}$
1418	$[M+H-MP]^{+}$
1440	$[M+Na-MP]^{+}$
1610	$[M+H]^{+}$
1632	$[M+Na]^{+}$

Table 3. Peak assignment for the ions labeled in Figure 9a. M corresponds to some unidentified species, H represents a hydrogen, Na sodium and MP the derivatising reagent 1-(2-methoxyphenyl) piperazine.

m/z	Suggested Ion of Derivatised Isocyanate
1204	$[M+H-2MP]^{+}$
1396	$[M+H-MP]^{+}$
1588	$[M+H]^{+}$
1610	$[M+Na]^{+}$

Table 4. Peak assignment for the ions labeled in Figure 7b. M corresponds to some unidentified species, H represents a hydrogen, Na sodium and MP the derivatising reagent 1-(2-methoxyphenyl) piperazine

If we examine the mass chromatogram in Figure 8, it is apparent that m/z value 1610 is present twice. Two peaks are visible on the mass chromatogram, eluting at retention times 9-12 mins and 17-18 mins, corresponding to m/z 1610. The mass spectrum produced by combining the area between 17 and 18 mins is shown in Figure 9b. From Figure 9b it is possible to postulate that the ion observed at m/z 1610 in the 17-18 mins region is a

sodium adduct of the molecule whose protonated species, labelled as m/z 1588, which also elutes in the same time period. Table 4 is an interpretation of the major ions in Figure 9b.

The validity of the observation of peaks at high mass in the mass chromatogram was demonstrated by the LC/MS analysis of blank samples, these samples showed no peaks and so the potential hypothesis of sample carry over, which one may suspect when dealing with large molecules, was dismissed. Also, in cases of column contamination it is not uncommon to observe shifting retention times, as the contaminants move through the column. A shift in retention times was not observed in these experiments. These data were of sufficient interest to warrant further investigation.

5.3.3 *MS/MS Direct Infusion Triple Quadrupole*

The elucidation of the structure of an ion from an electrospray mass spectrum is not always possible. Using MS/MS it is possible to obtain structural information from ions produced by electrospray ionisation. Previous work [40] had given a structural formula for a trimeric HDI species (molecular weight 510.3 Da) that would correspond to the ion at m/z 1082 as a protonated MP derivative, observed in the LC/MS experiments (see figure 6). It was decided that MS/MS experiments on the protonated molecule of the derivatised HDI trimer would be a useful place to begin the work. Preliminary assumptions could be made about any possible patterns and relationships in the data which could then be applied to the MS/MS analysis of unidentified derivatised isocyanate species. Table 5 is the interpretation of the mass spectrum in Figure 10.

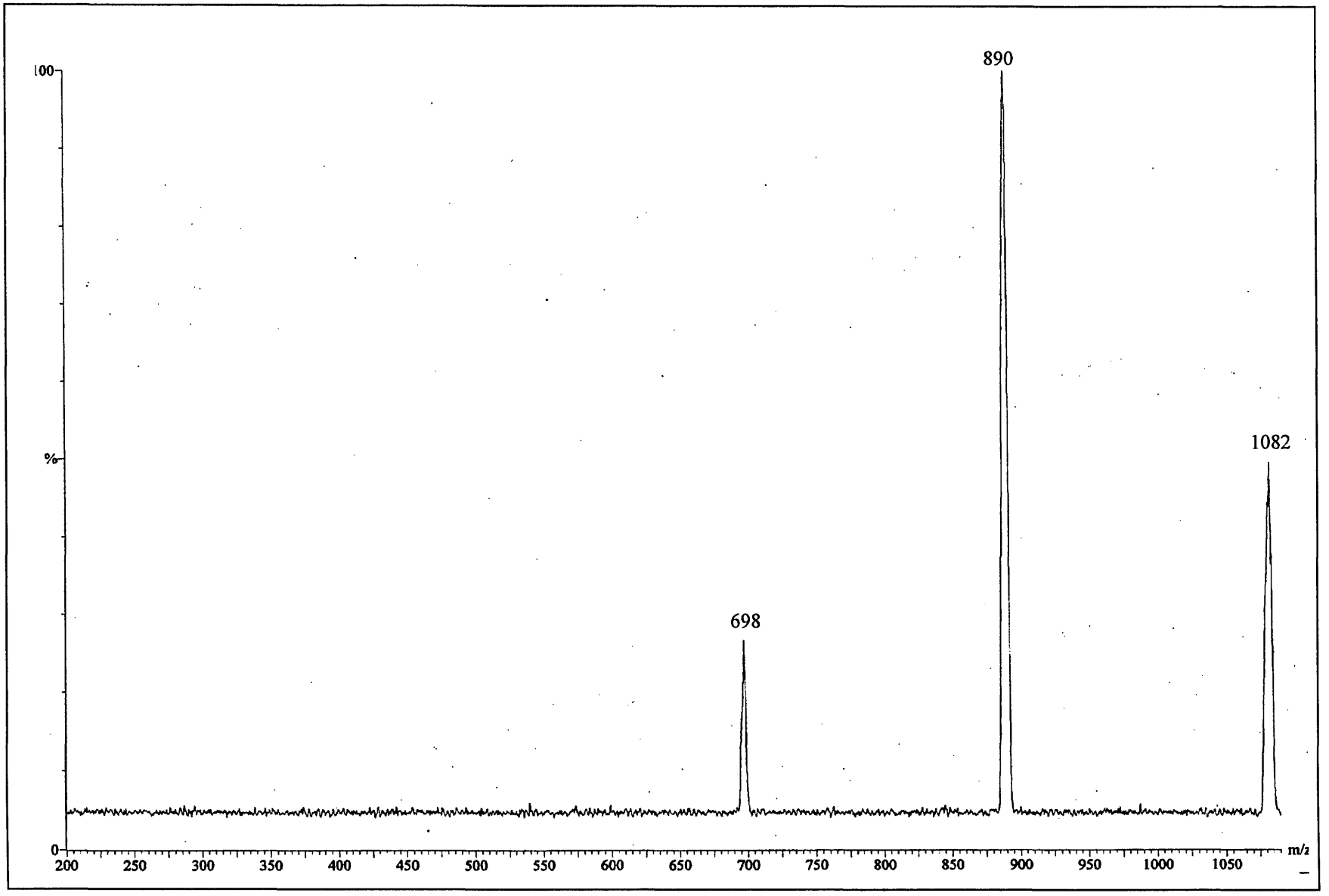


Figure 10. A mass spectrum acquired from product ion experiments on a protonated derivatised trimeric HDI species, m/z 1082. The spectrum shows the neutral loss 192 Da and 384 Da from the precursor ion corresponding to the loss of one and two derivatising reagent groups, respectively. The spectrum was acquired using a Quattro I mass spectrometer.

m/z	Proposed Ion
698	$[M+H-2MP]^+$
890	$[M+H-MP]^+$
1082	$[M+H]^+$

Table 5. Peak assignment for the ions labeled in Figure 10, M corresponds to a trimeric HDI species, H represents a hydrogen, Na sodium and MP the derivatising reagent 1-(2-methoxyphenyl) piperazine

An ion at m/z 193 was observed in all the derivatised isocyanate MS/MS experiments shown in this thesis. M/z 193 is a result of a hydrogen rearrangement and subsequent alpha, (α), cleavage of the bond between the isocyanate carbonyl carbon and the derivatising reagent amine. Figure 11 shows examples of the hydrogen rearrangement and bond dissociation with charge retention by the derivatising reagent, (a), or the isocyanate species (b). When the charge on the precursor ion is retained by the derivatising reagent during bond dissociation an ion is observed at m/z 193. The ion at m/z 193 is not shown in Figure 10 as it dominates the spectrum and overshadows the high mass ions of interest.

The loss of 192 Da is noted in the MS/MS spectrum in Figure 10, and can be used as a predictive tool for the determination of isocyanate functionality. A species is not observed that corresponds to the HDI trimer minus three derivatising reagent groups, m/z 506. An explanation for the absence of the ion at m/z 506 could be that the instrument is not sensitive enough to detect this ion if it is formed, or that the ion is not formed at all.

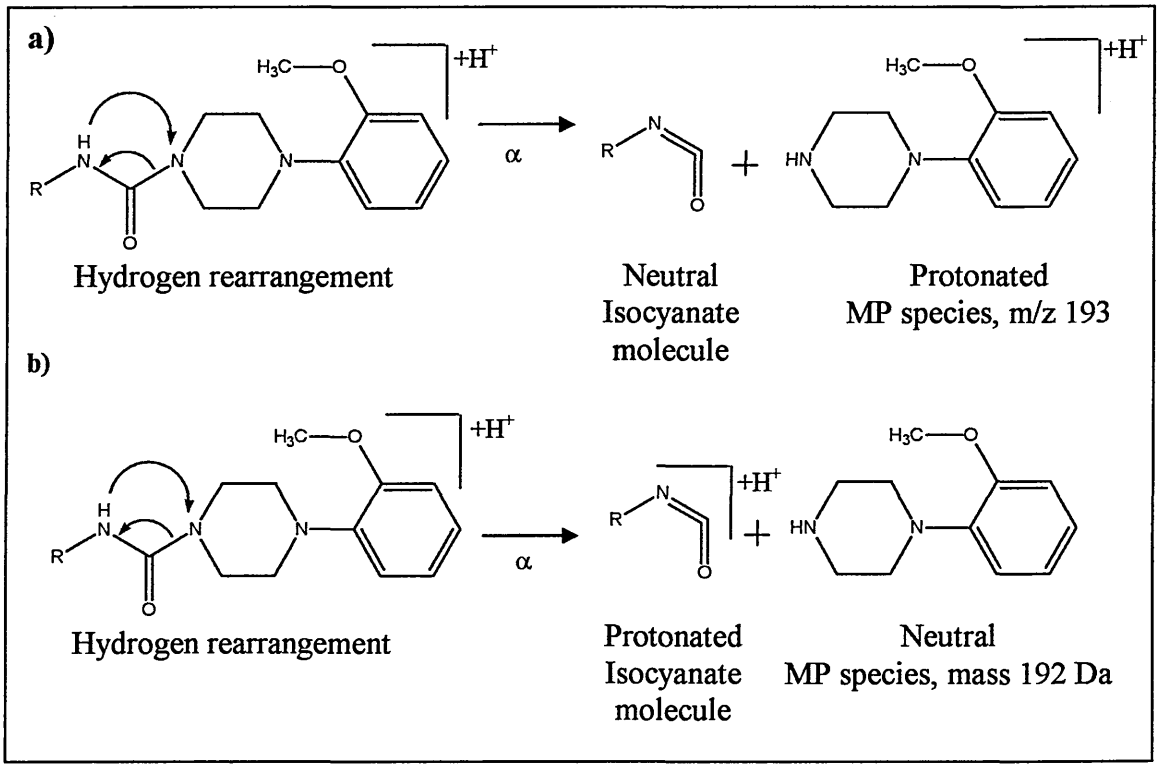


Figure 11. Mechanisms for a) The formation of an ion corresponding to m/z 193, b) The neutral loss of an MP species corresponding to mass loss of 192 Da in the electrospray MS/MS analysis of 1-2(methoxyphenyl)piperazine derivatised NCOs. In each case, R represents the isocyanate moiety and α refers to alpha clevage.

5.3.4 *MS/MS Nanospray Q/ToF Ultima*

Nanospray MS/MS was applied to the analysis of derivatised NCO monomers and bulk formulations. The concentration-dependent nature of the electrospray ionisation process [41] makes nanospray ideal for the analysis of samples where the species of interest are present at low concentration. Figure 12 is an example of the dissociation spectra observed from product ion experiments on the ion corresponding to the protonated derivatised HDI trimer, m/z 1082, using the Q/ToF Ultima mass spectrometer. The Q/ToF Ultima is a significantly more sensitive instrument than the VG Quattro. The use of a more sensitive instrument should facilitate the detection of an ion formed from the HDI trimer as a result

of the loss of three derivatising reagent groups, m/z 506, if such an ion was formed. An ion of m/z 506 was not detected. We can therefore say that this ion is not formed during product ion experiments on the derivatised HDI trimer.

Using the Q/ToF Ultima instrument it was also possible to isolate the ion corresponding to m/z 1610 previously observed using LC/MS (section 5.3.2). (Product ion experiments were not possible on the ion corresponding to m/z 1610 using the VG Quattro as a result of the low sensitivity of this instrument). The product ion spectrum acquired for the ion corresponding to m/z 1610 is shown in Figure 12. The spectrum shows three neutral losses of 192 Da from the precursor ion, m/z 1610. Comparison of this result with the previous MS/MS experiment on the protonated derivatised HDI trimer could indicate a species with four isocyanate functional groups; this is assuming that the loss of the fourth derivatising reagent is not observed. The spectrum in Figure 12 also shows an ion at m/z 1082 with a satellite peak at m/z 1104. The dissociation pattern observed from the previous product ion analysis of m/z 1082 is present in the m/z 1610 product ion spectrum. The possibility that the ion of m/z 1610 is an adduct of the ion observed at m/z 1082 has to considered, i.e., the ion of m/z 1610 could possibly be an ion of the type $[M+X]^+$, where M is the derivatised HDI trimer and X is one or more gas phase interferents present during the formation of the precursor ion. Structural interpretation of the ions in Figure 14 can be found in Table 11; the presence of m/z 1082 cannot be accounted for.

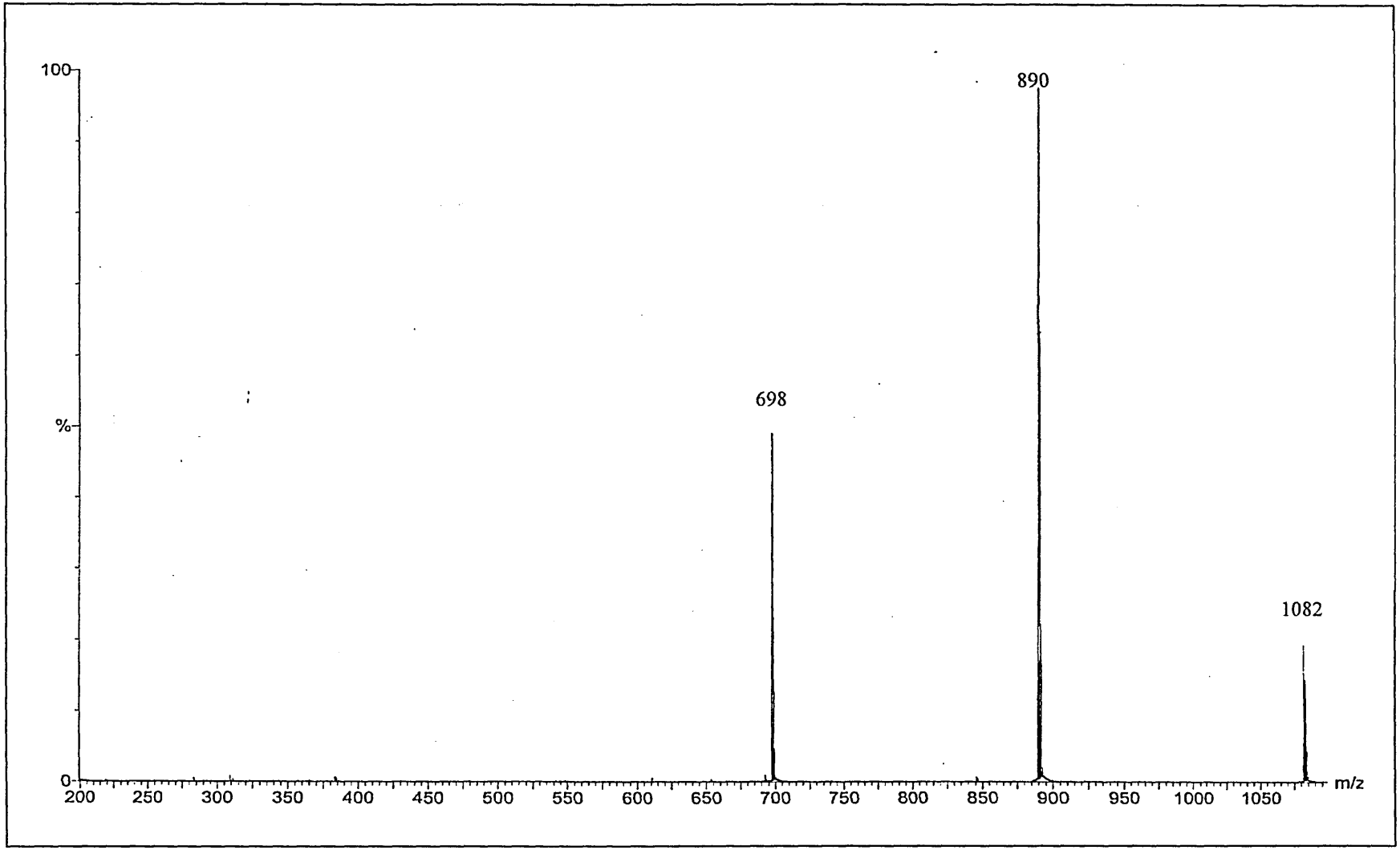


Figure 12. A mass spectrum acquired from the product ion MS/MS analysis of a derivatised HDI trimer, m/z 1082 using a Q/ToF Ultima instrument.

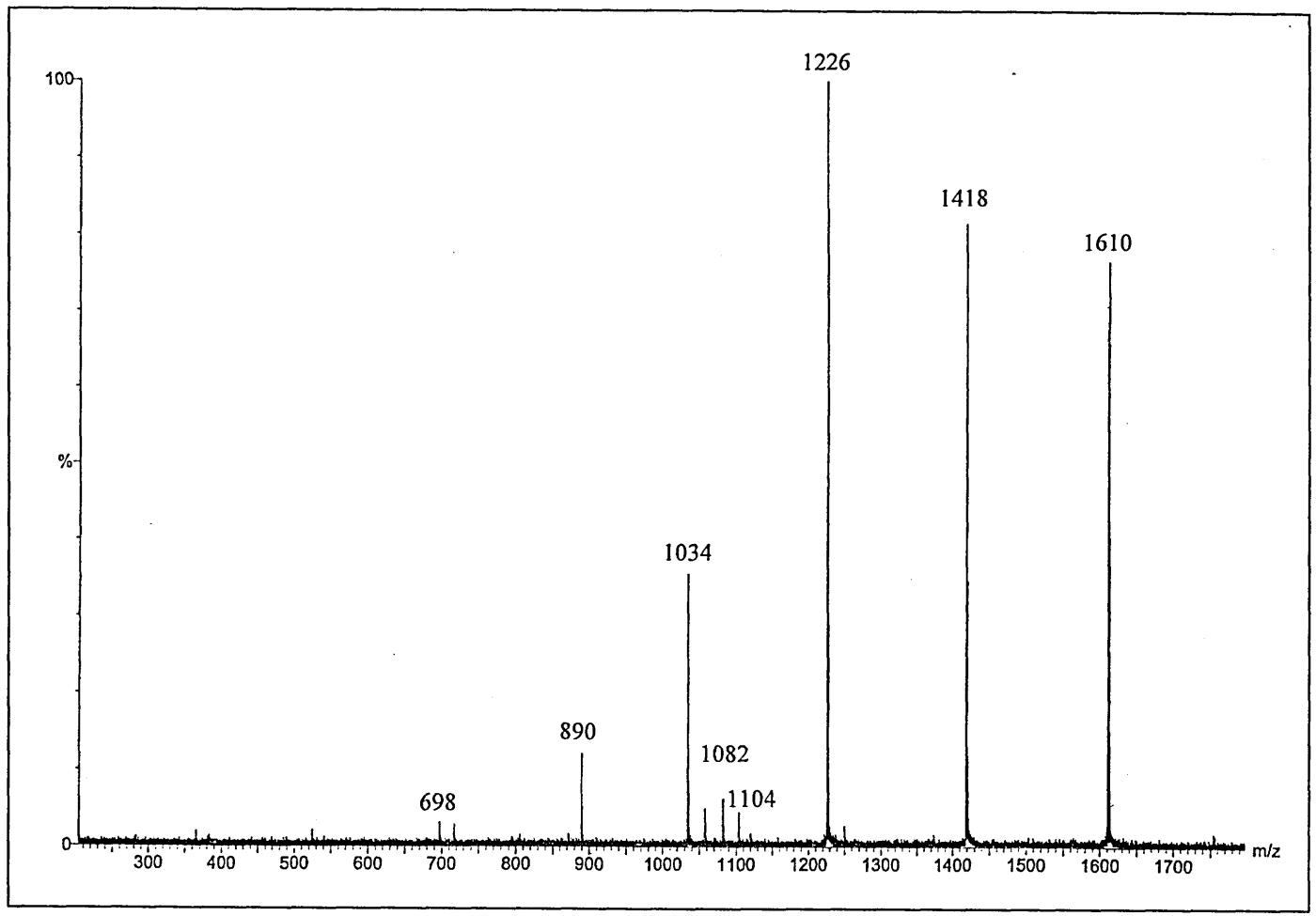


Figure 13. Mass spectrum acquired from product ion experiments on the HDI prepolymer ion corresponding to m/z 1610 using a Q/ToF Ultima instrument.

The presence of high molecular weight, possibly polymeric, species suggested that it might be of interest to analyse the samples by MALDI. MALDI is commonly applied to the analysis of large synthetic polymeric molecules [42]. Previous work by Carr *et al.* had shown that the direct analysis of underivatised isocyanates was not possible [43]. Initial experiments involved the analysis of derivatised monomeric NCO, HDI, MDI, 2,4-TDI and 2,6-TDI. These data are summarised Table 6; matrix ions are excluded as they are omnipresent in MALDI mass spectra. Protonated molecules were readily observed for the individual monomers. Sodium adducts were also observed for each NCO monomer species studied. Figures 14 and 15 show MALDI mass spectra for derivatised MDI and 2,4-TDI NCO monomers. Following the successful analysis of monomeric NCO, the MALDI method was applied to the analysis of derivatised bulk formulations. Figure 16 shows a MALDI mass spectrum acquired from the derivatised HDI sample which had been previously analysed using LC/MS (as described in Section 5.3.2). One major limitation of the application of MALDI to the analysis of complex mixtures is the absence of a separation technique prior to ionisation. Thus peak assignment can be a difficult exercise with questionable results. One method of overcoming this problem is the introduction of MS/MS techniques. The use of MS/MS helps the assignment of peaks and introduces a greater degree of specificity to the analysis of complex mixtures by MALDI.

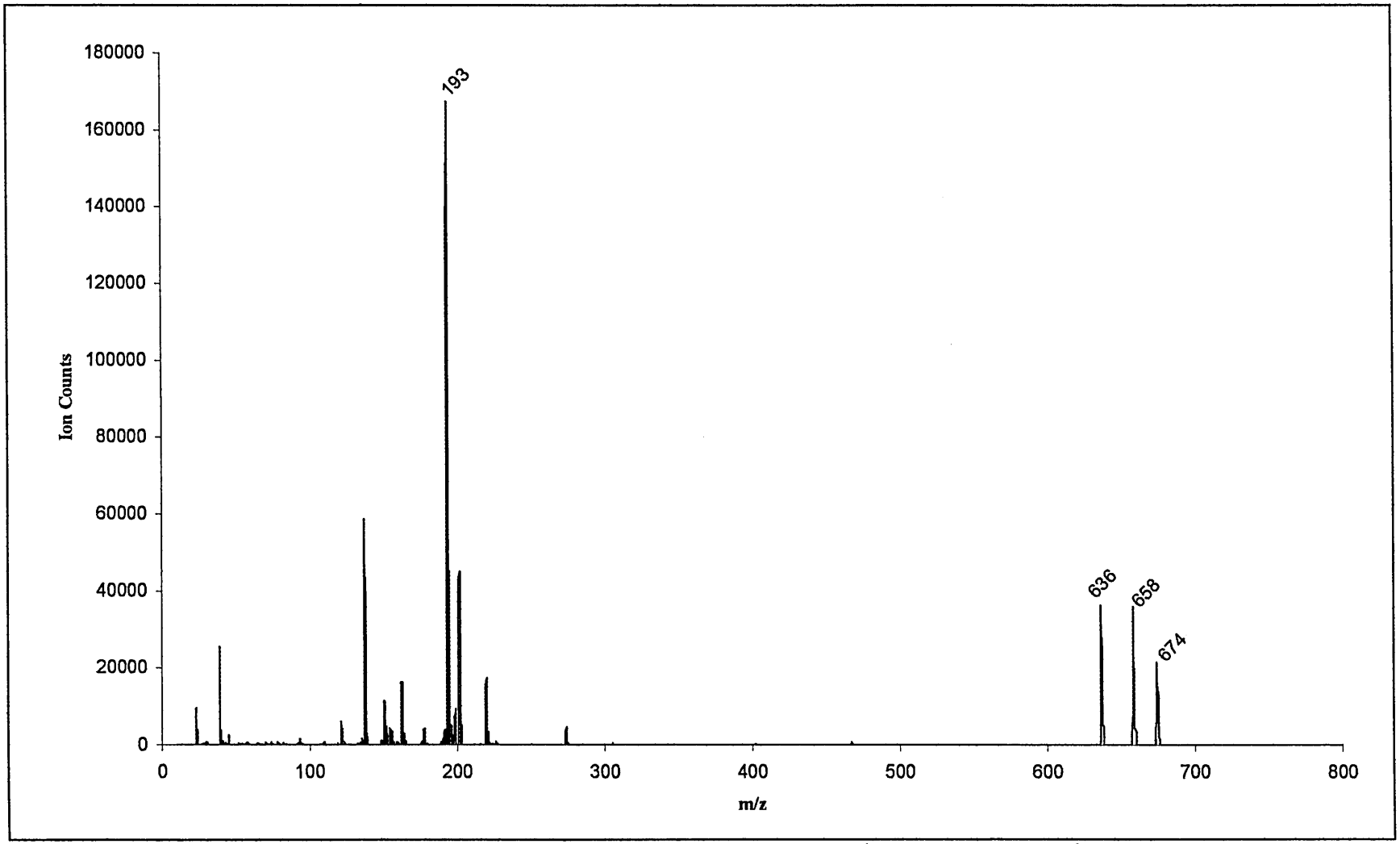


Figure 14. A MALDI mass spectrum acquired from the analysis of MDI-2MP (10 $\mu g.ml^{-1}$) in DHB (50 $mg.ml^{-1}$). The spectrum was acquired in positive ion mode with a 20 kV extraction voltage. The mass spectrometer used for the acquisition was an SAI LaserToF 1500, (SAI, Manchester, U.K.)

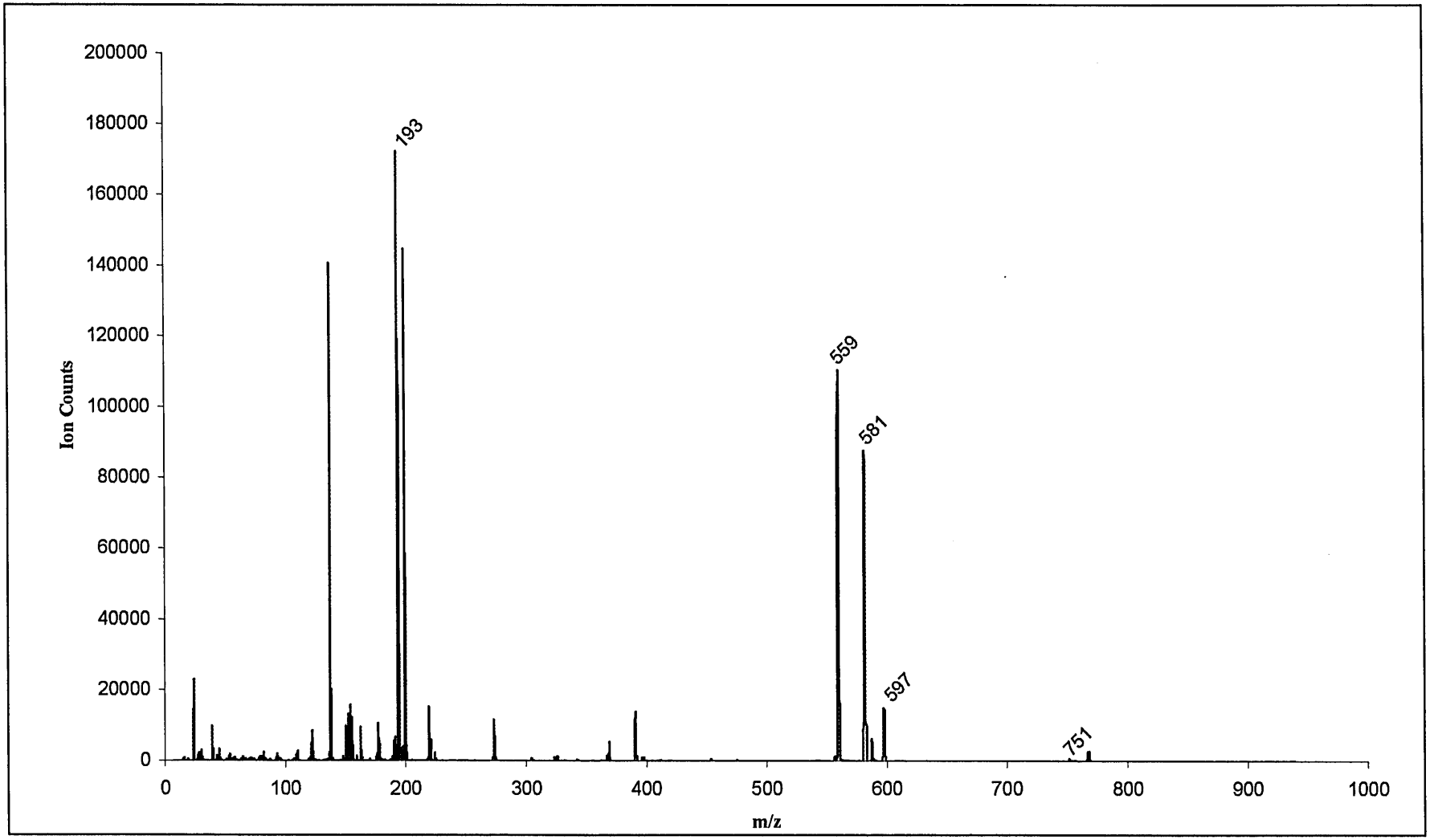


Figure 15. A MALDI mass spectrum acquired from the analysis of 2,4-TDI-2MP (10 $\mu g.ml^{-1}$) in DHB (50 $mg.ml^{-1}$). The spectrum was acquired in positive ion mode with a 20 kV extraction voltage. The mass spectrometer used for the acquisition was an SAI LaserToF 1500, (SAI, Manchester, U.K.)

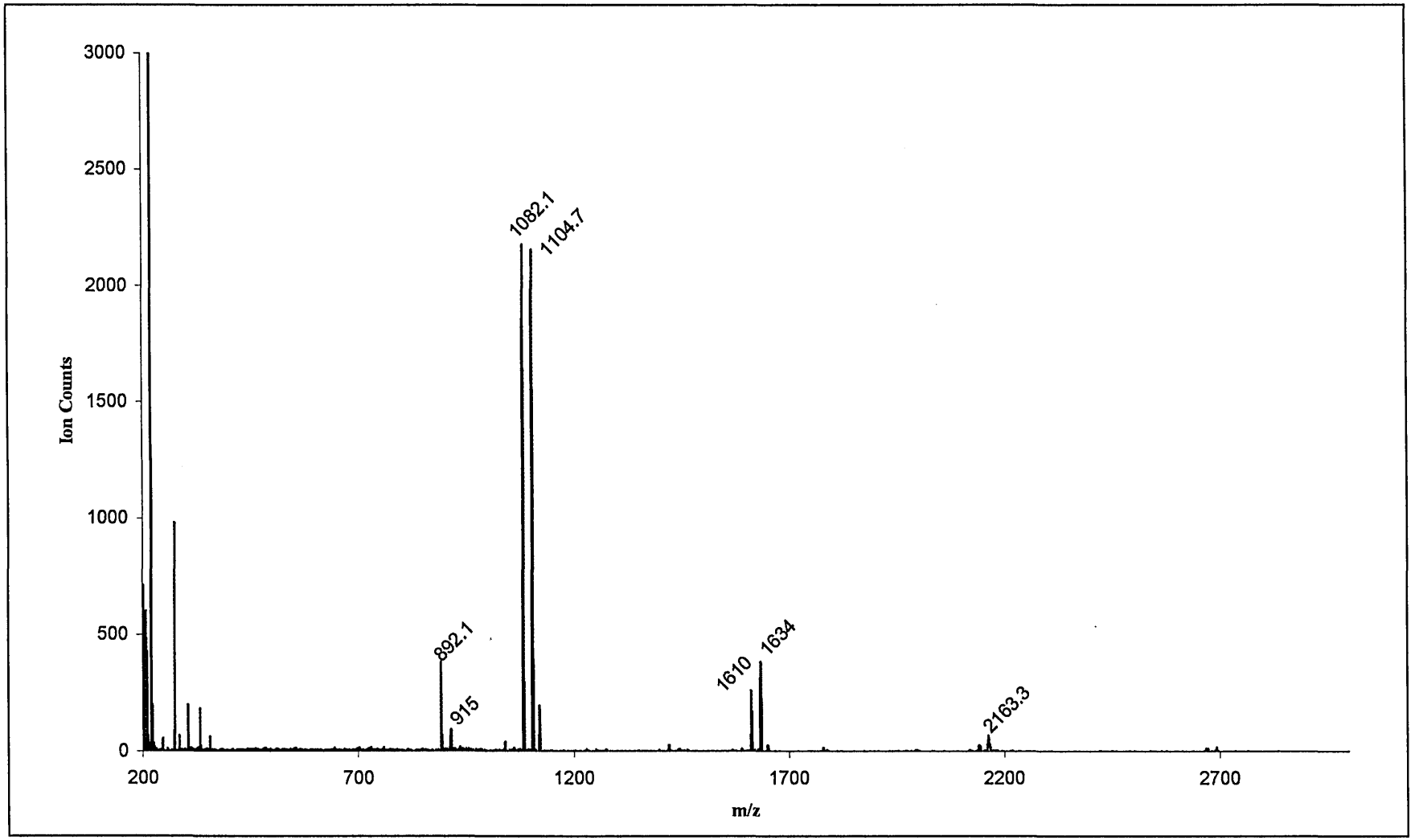


Figure 16. A MALDI mass spectrum acquired from the analysis of a derivatised HDI prepolymer sample (10 $\mu g.ml^{-1}$) in DHB (50.$mg.ml^{-1}$). The spectrum was acquired in positive ion mode with a 20 kV extraction voltage. The mass spectrometer used for the acquisition was an SAI LaserToF 1500, (SAI, Manchester, U.K.)

NCO Monomer	m/z	Suggested Ion of Derivatised Isocyanate
HDI	193	$[MP+H]^+$
	361	$[M+H-MP]^+$
	383	$[M+Na-MP]^+$
	553	$[M+H]^+$
	575	$[M+Na]^+$
	591	$[M+K]^+$
	745	$[M+MP]^+$
MDI	193	$[MP+H]^+$
	636	$[M+H]^+$
	658	$[M+Na]^+$
	674	$[M+K]^+$
2,4-TDI	193	$[MP+H]^+$
	369	$[M-MP]^+$
	391	$[M+Na-MP]^+$
	559	$[M+H]^+$
	581	$[M+Na]^+$
	597	$[M+K]^+$
	751	$[M+MP]^+$
2,6-TDI	193	$[MP+H]^+$
	369	$[M-MP]^+$
	391	$[M+Na-MP]^+$
	559	$[M+H]^+$
	581	$[M+Na]^+$
	597	$[M+K]^+$
	751	$[M+MP]^+$

Table 6. Summary of data obtained from the analysis of four 2MP derivatised NCO monomers by MALDI/MS using DHB matrix

5.3.6 *Application of MALDI/MS/MS to the Analysis of Derivatised Isocyanates*

MALDI/MS/MS data was acquired. Bulk formulations of MDI, HDI and 2,4-TDI were analysed and ions of interest subjected to CID. Product ion data were obtained for ions corresponding to m/z 1447.6 and 1640.8 for 2,4-TDI; m/z 1412.8 and 1605.9 for MDI; and m/z 1610 for HDI. In the following section the respective spectra are presented with their interpretation.

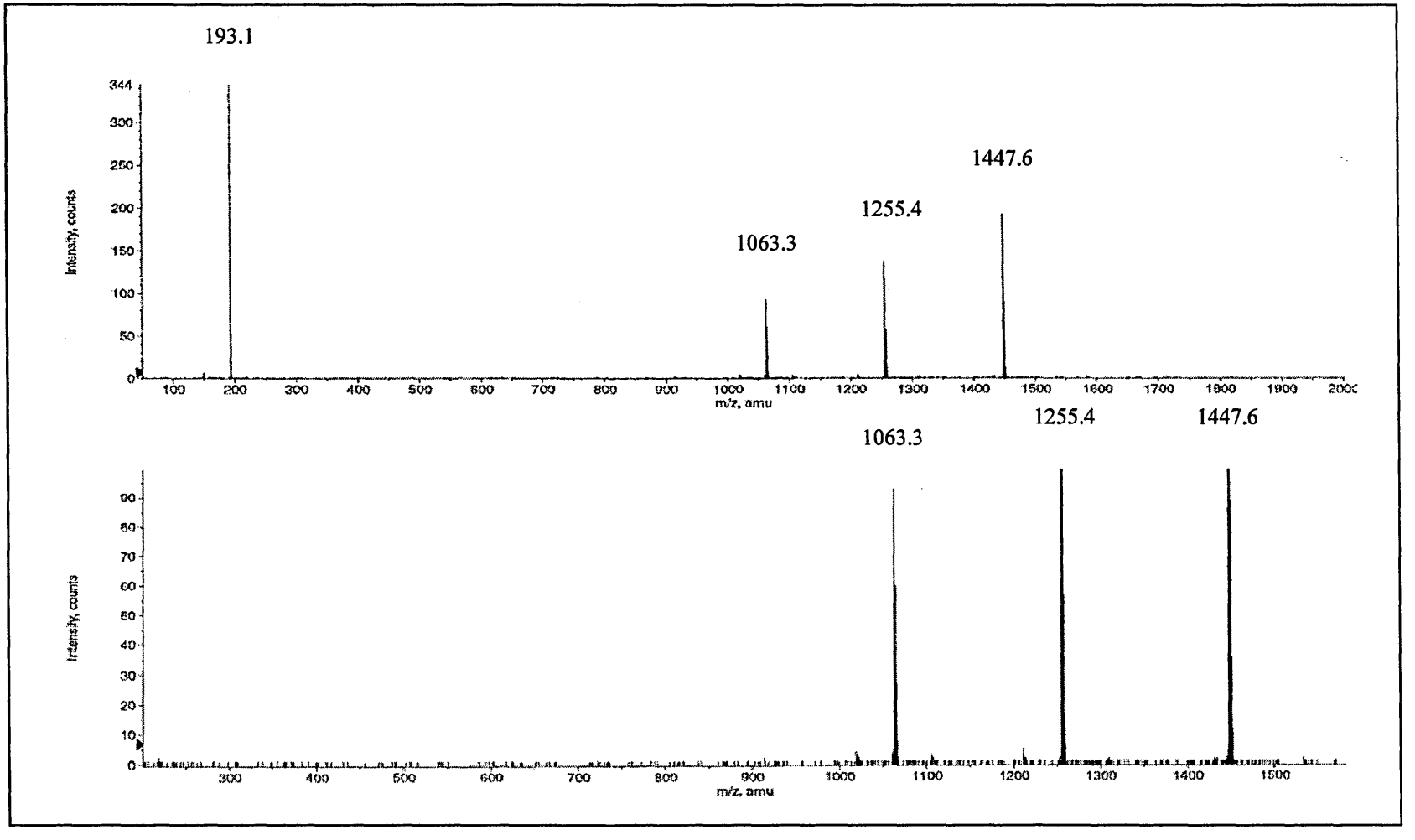


Figure 17. MALDI/MS/MS product ion spectra for the dissociation of a 2,4-TDI related precursor ion corresponding to m/z 1447. The upper spectrum shows the presence of the rearrangement/α-cleavage product m/z 193.

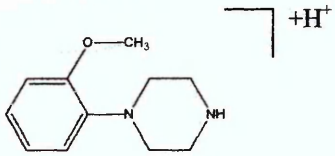
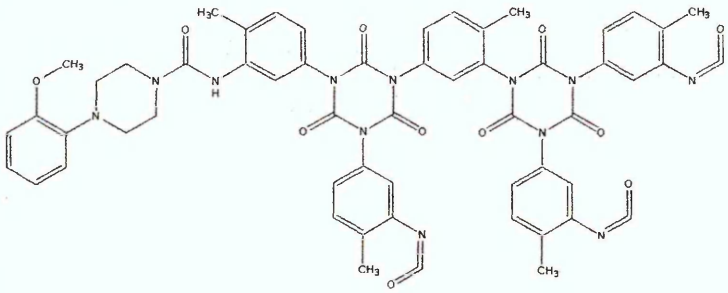
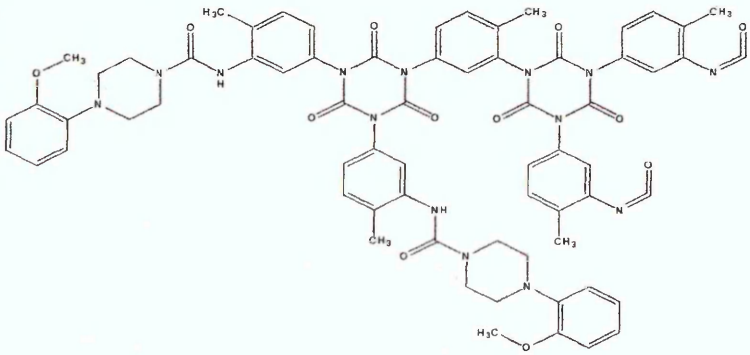
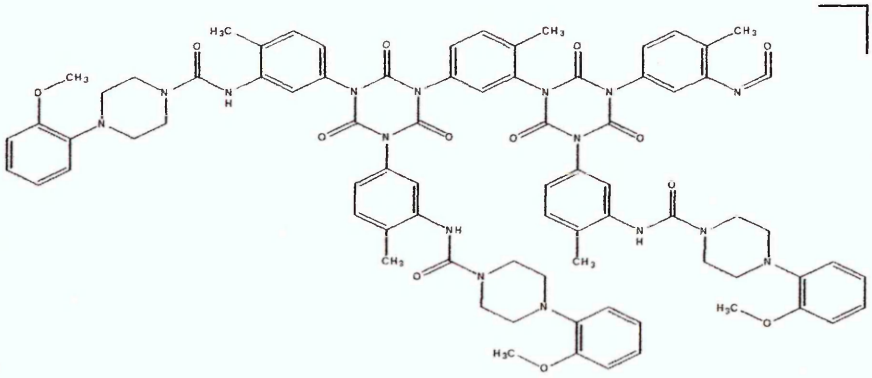
m/z	Proposed Structures
193.1	
1063.3	
1255.4	
1448.6	

Table 7. Product ions and proposed structures from product ion experiments on a 2,4-TDI related species corresponding to m/z 1447.6, acquired using MALDI/MS/MS

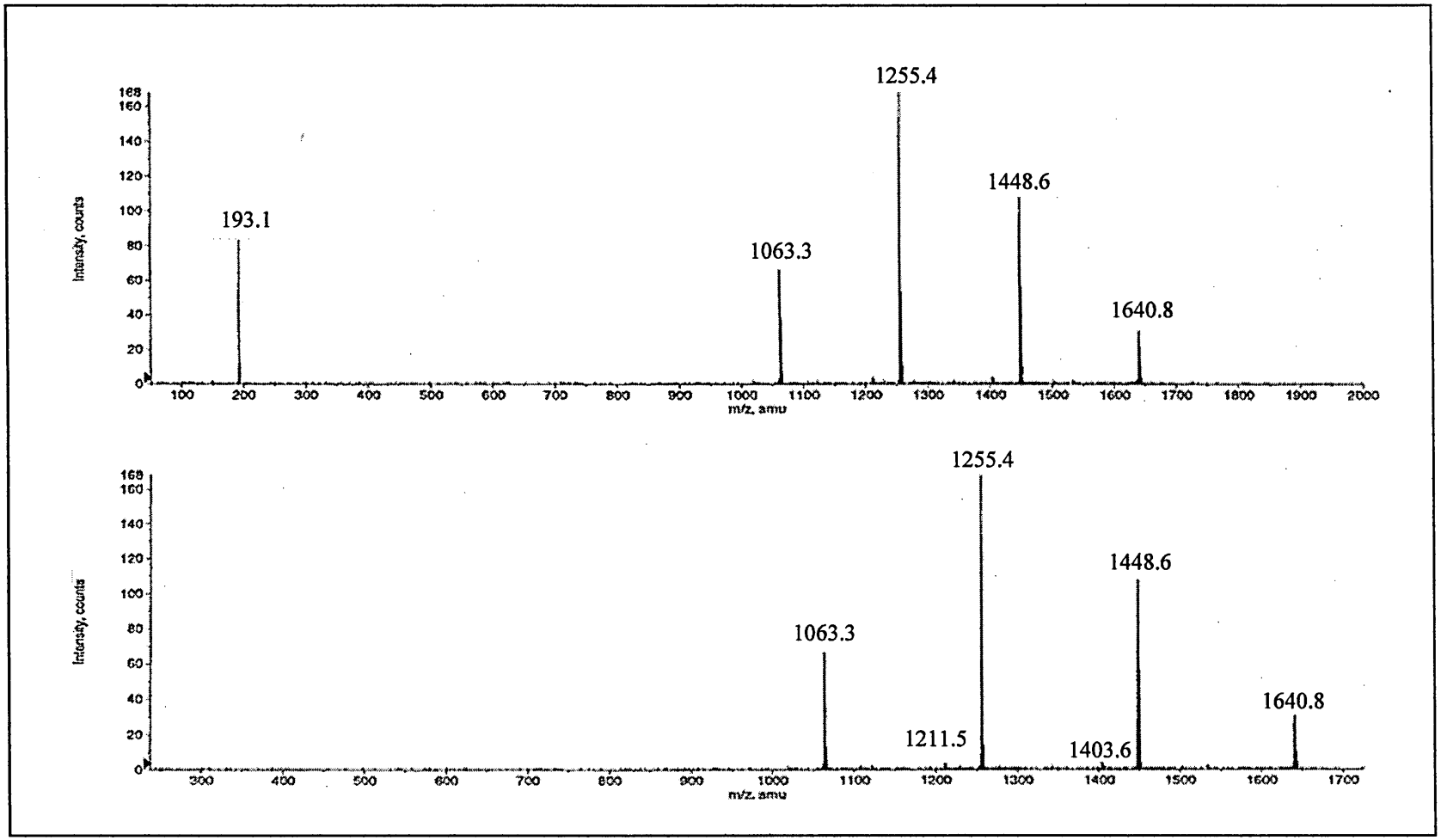


Figure 18. MALDI/MS/MS product ion spectra for the dissociation of a 2,4-TDI related precursor ion corresponding to m/z 1640.8 The upper spectrum shows the presence of the rearrangement/α-cleavage product m/z 193.

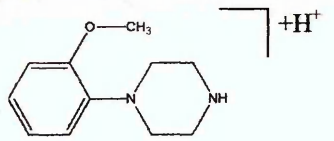
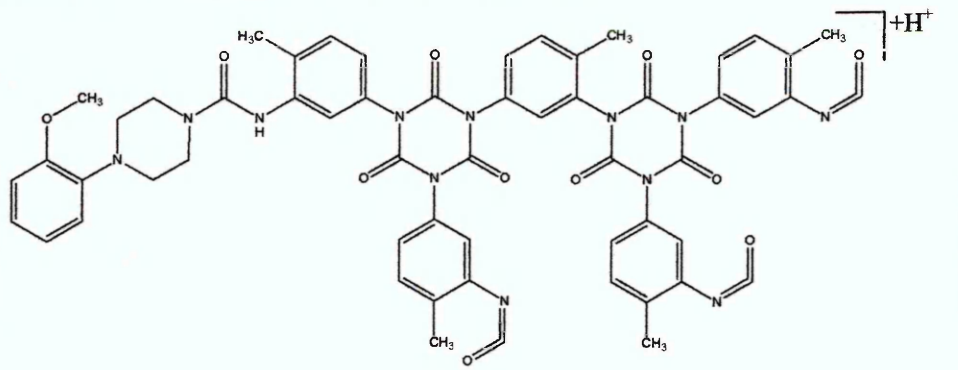
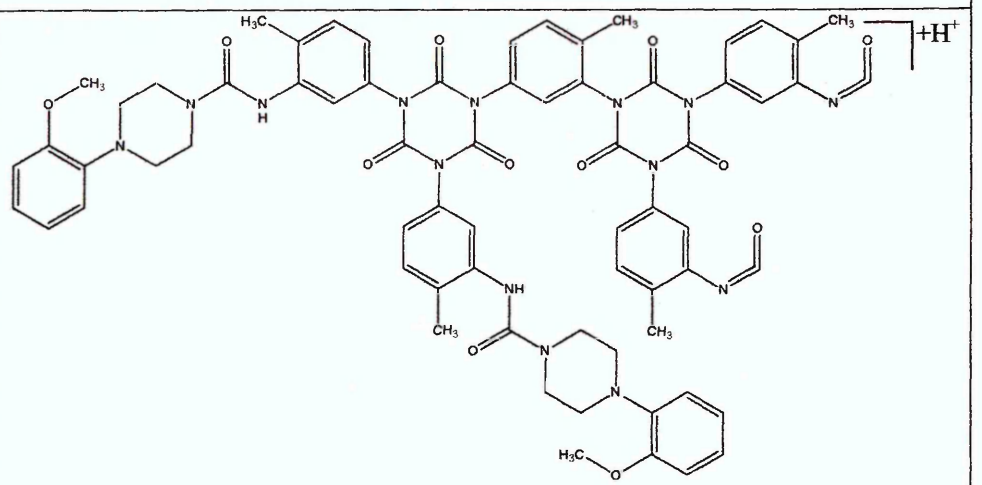
m/z	Proposed Structure
193.1	
1063.3	
1255.5	

Table 8. Product ions and proposed structures from product ion experiments on a 2,4-TDI related species corresponding to m/z 1639.8, acquired using MALDI/MS/MS

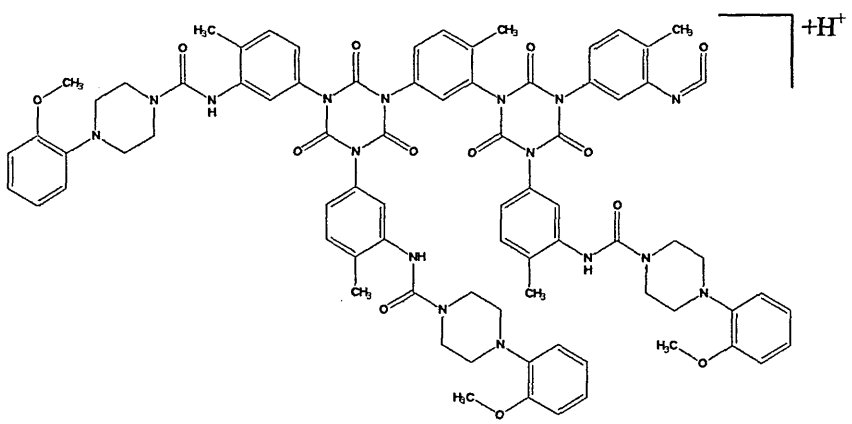
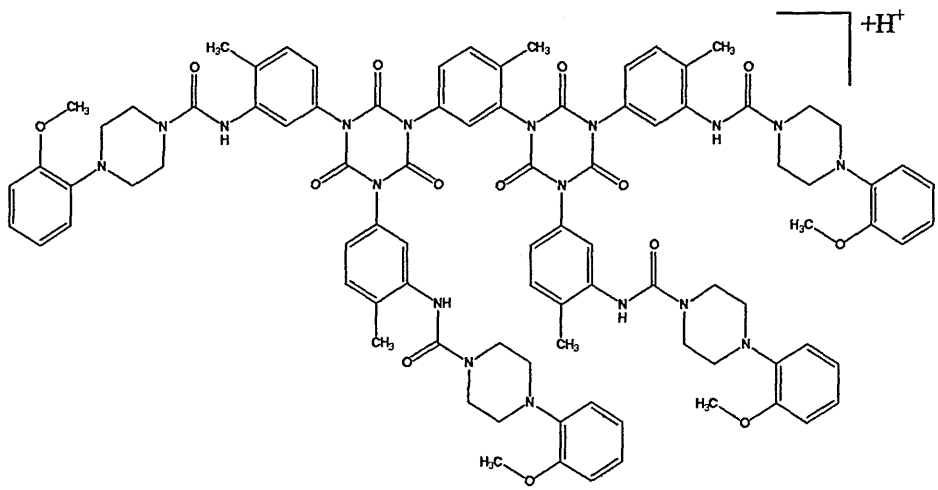
1448.6	
1639.8	

Table 8. Continued.

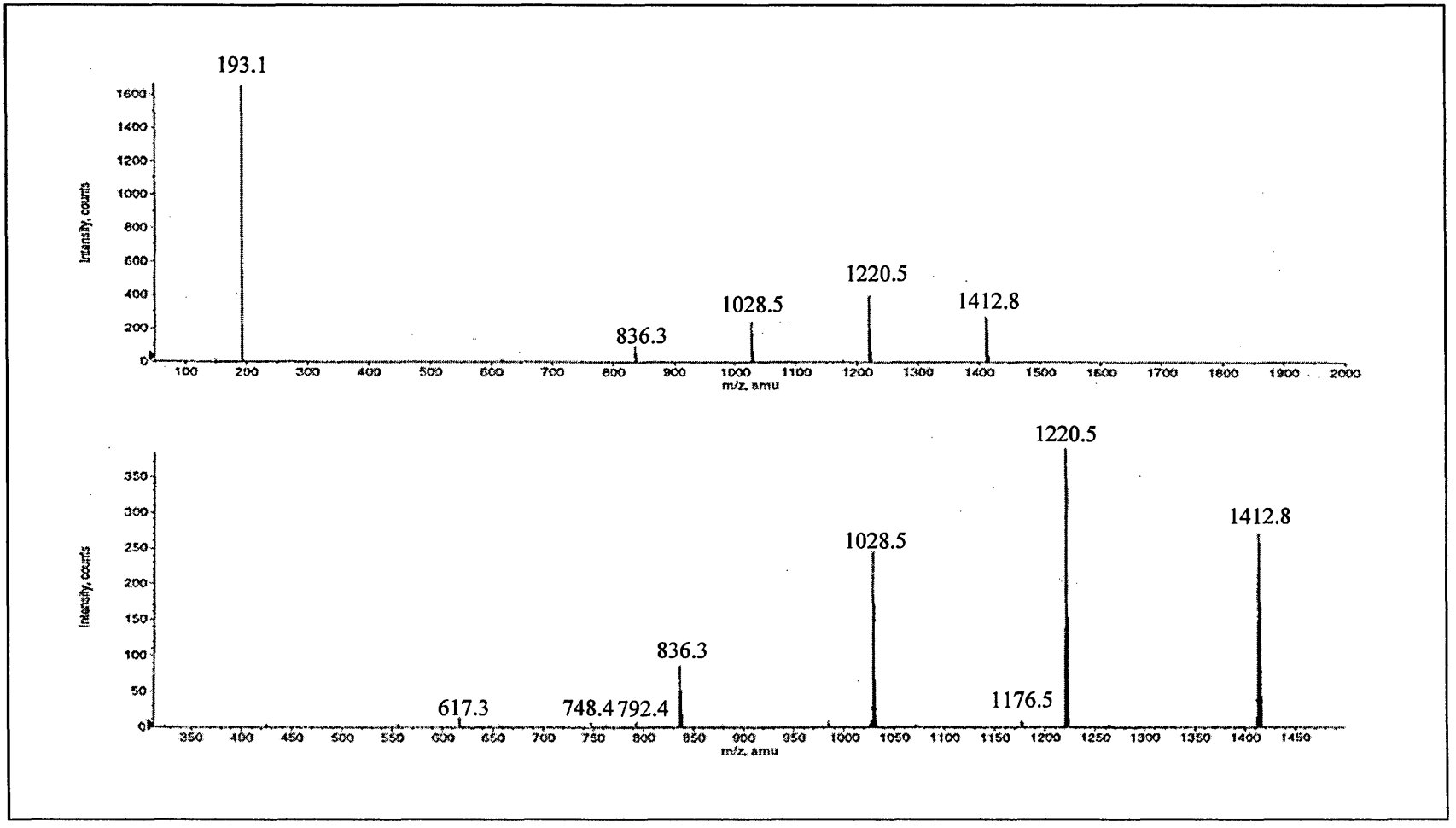


Figure 19. MALDI/MS/MS product ion spectra for the dissociation of an MDI related precursor ion corresponding to m/z 1412.8 The upper spectrum shows the presence of the rearrangement/α-cleavage product m/z 193.

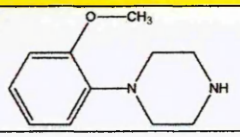
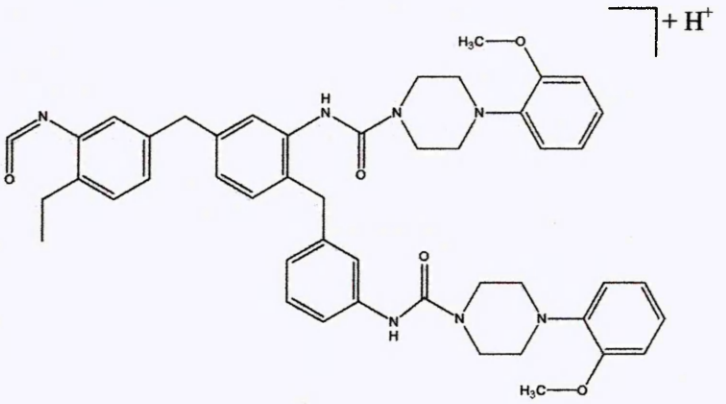
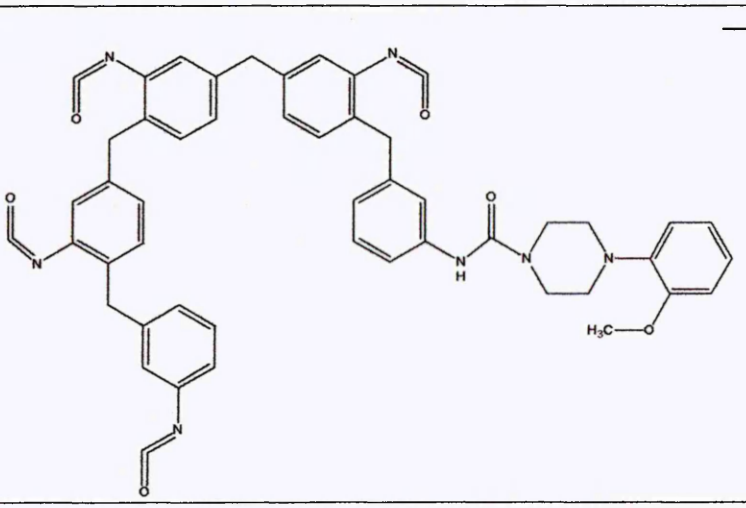
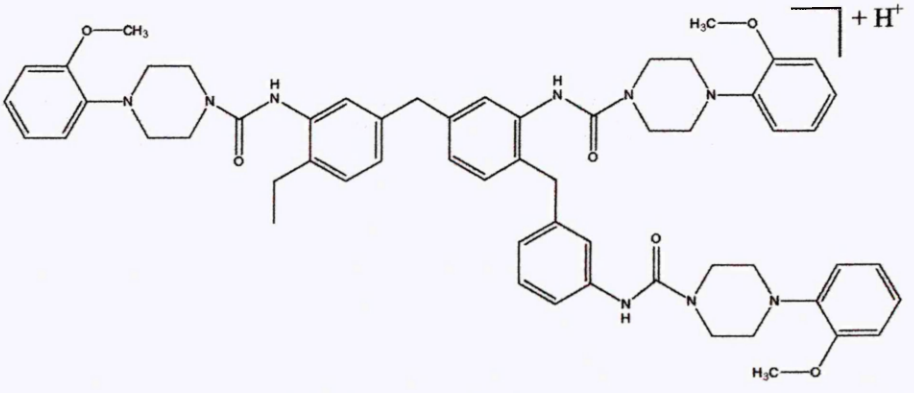
m/z	Proposed Structure
193.1	
792.4	
836.4	
984.5	

Table 9. Product ions and proposed structures from product ion experiments on a MDI related species corresponding to m/z 1412.8, acquired using MALDI/MS/MS

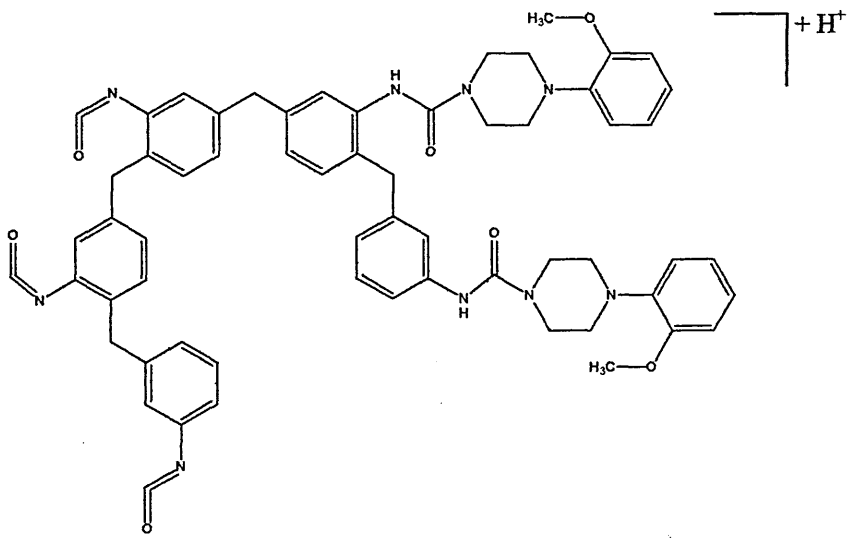
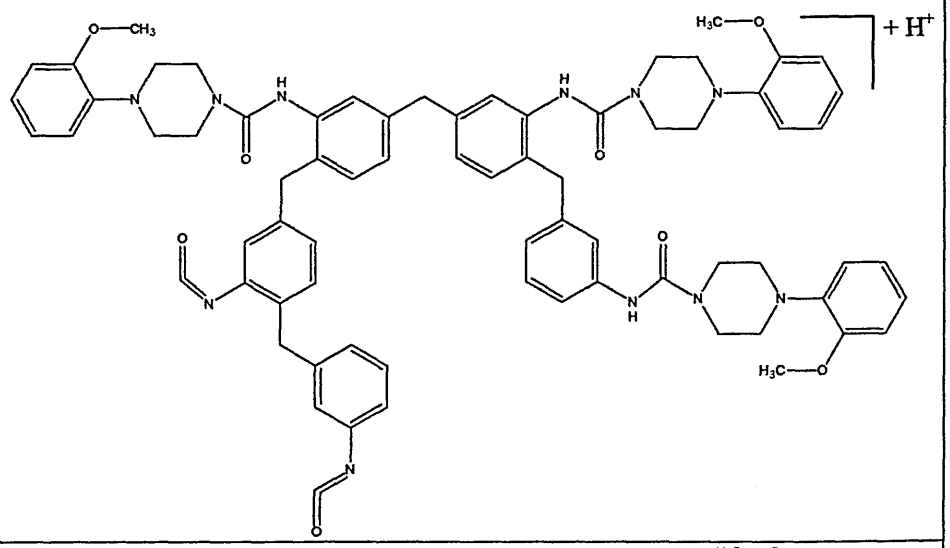
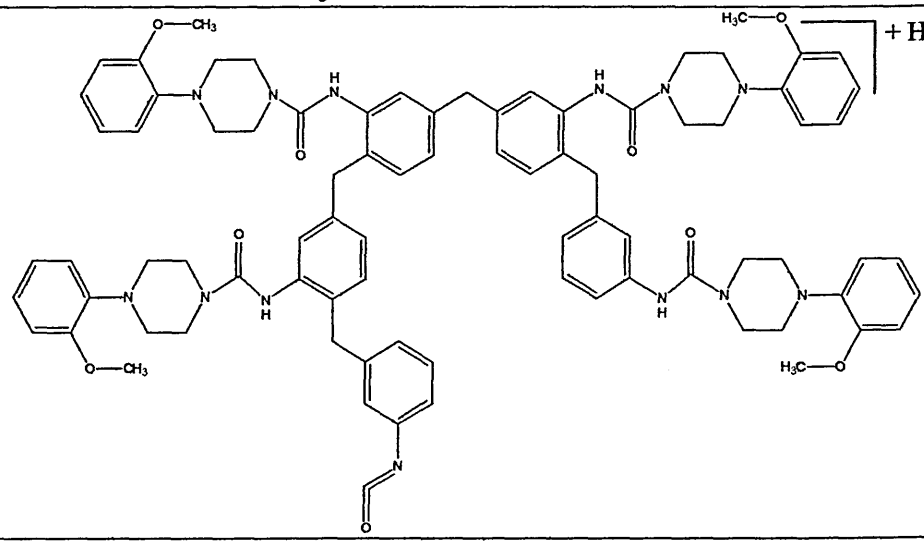
1028.5	
1220.5	
1412.8	

Table 9. Continued.

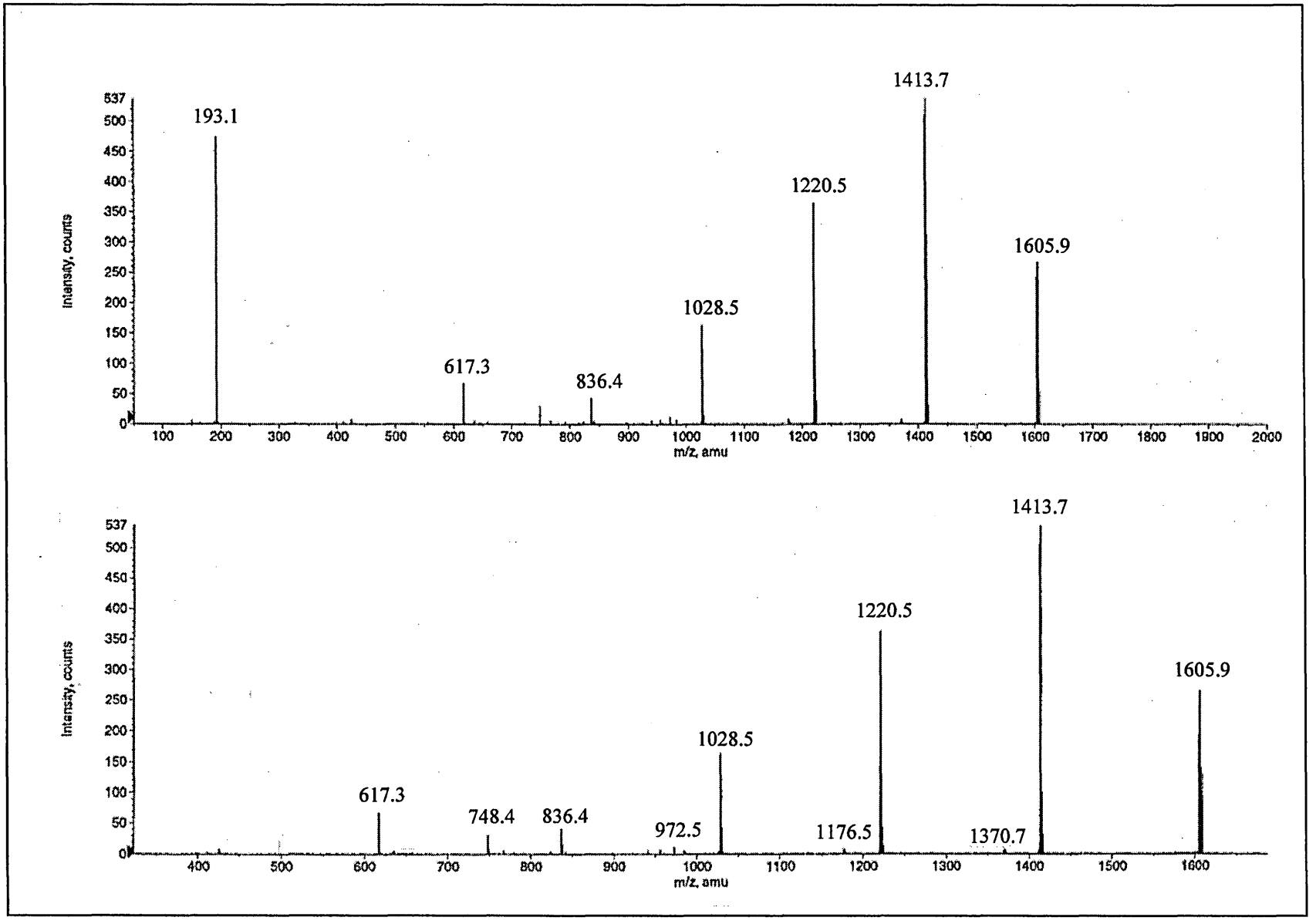


Figure 20. MALDI/MS/MS product ion spectra for the dissociation of an MDI related precursor ion corresponding to m/z 1605.9 The upper spectrum shows the presence of the rearrangement/α-cleavage product m/z 193.

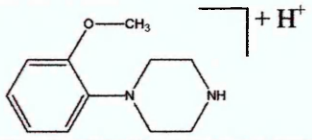
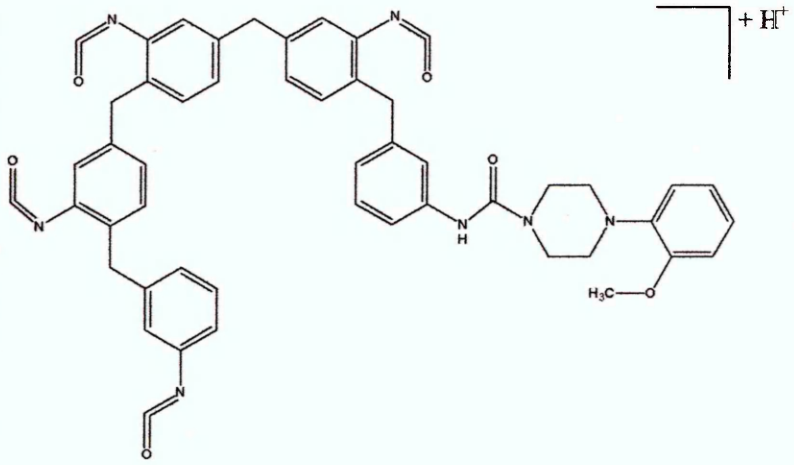
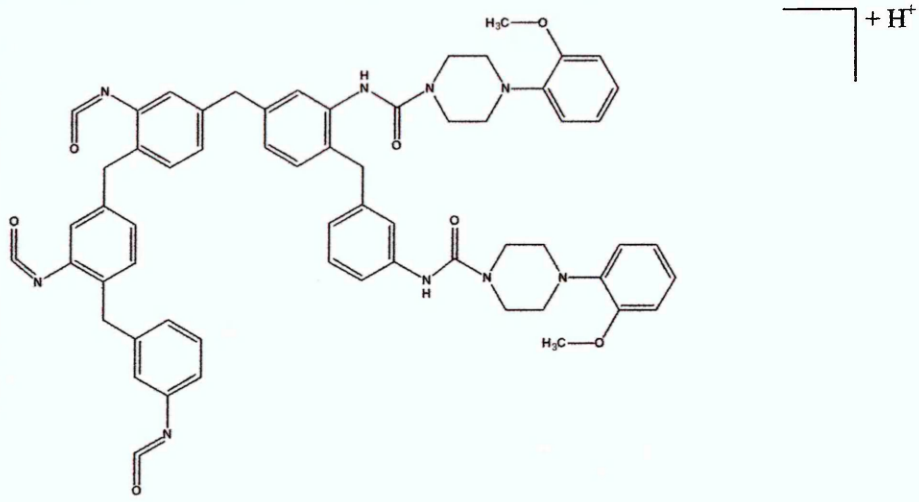
m/z	Proposed Structure
193.1	
836.4	
1028.5	

Table 10. Product ions and proposed structures from product ion experiments on a MDI related species corresponding to m/z 1605.9, acquired using MALDI/MS/MS

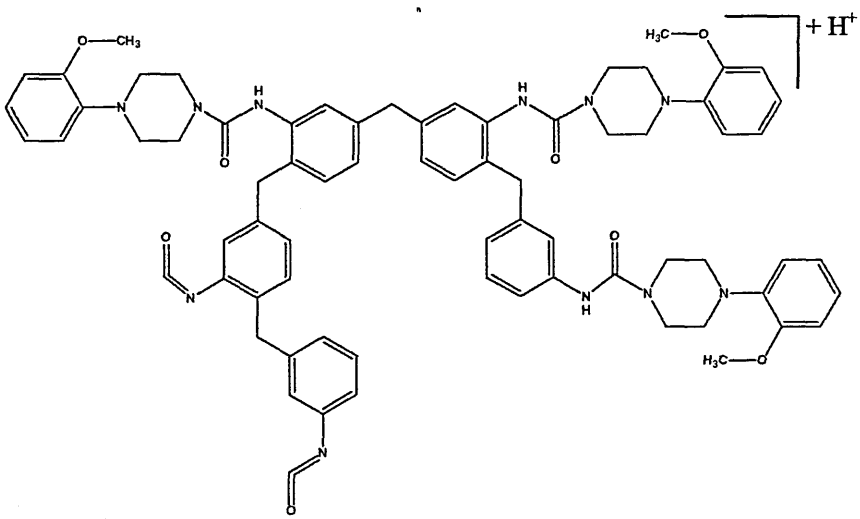
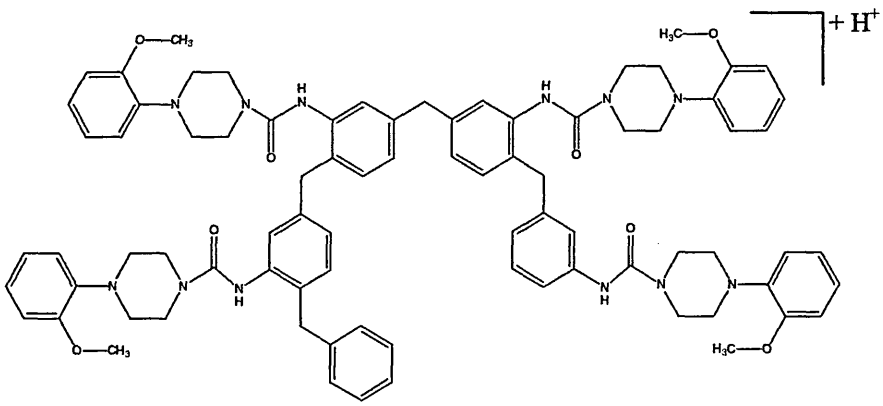
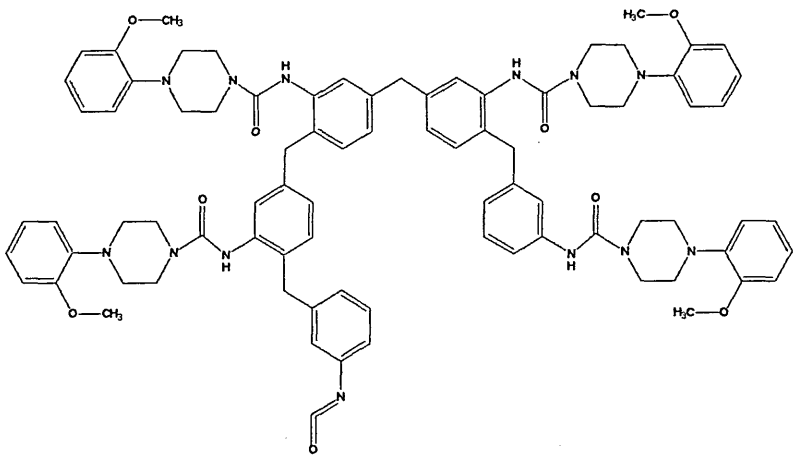
1220.5	
1370.7	
1413.7	

Table 10. Continued

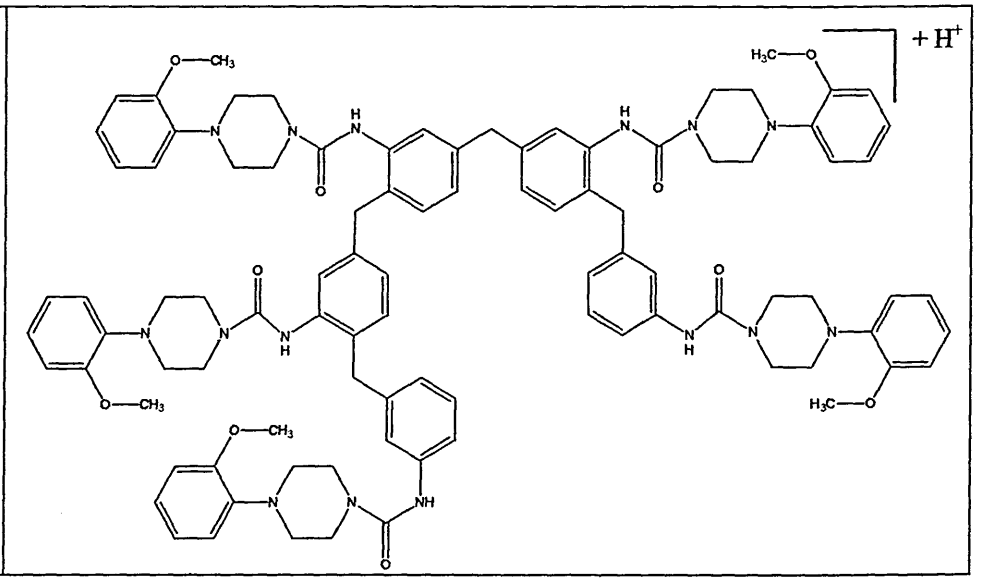
1605.9	

Table 10. Continued.

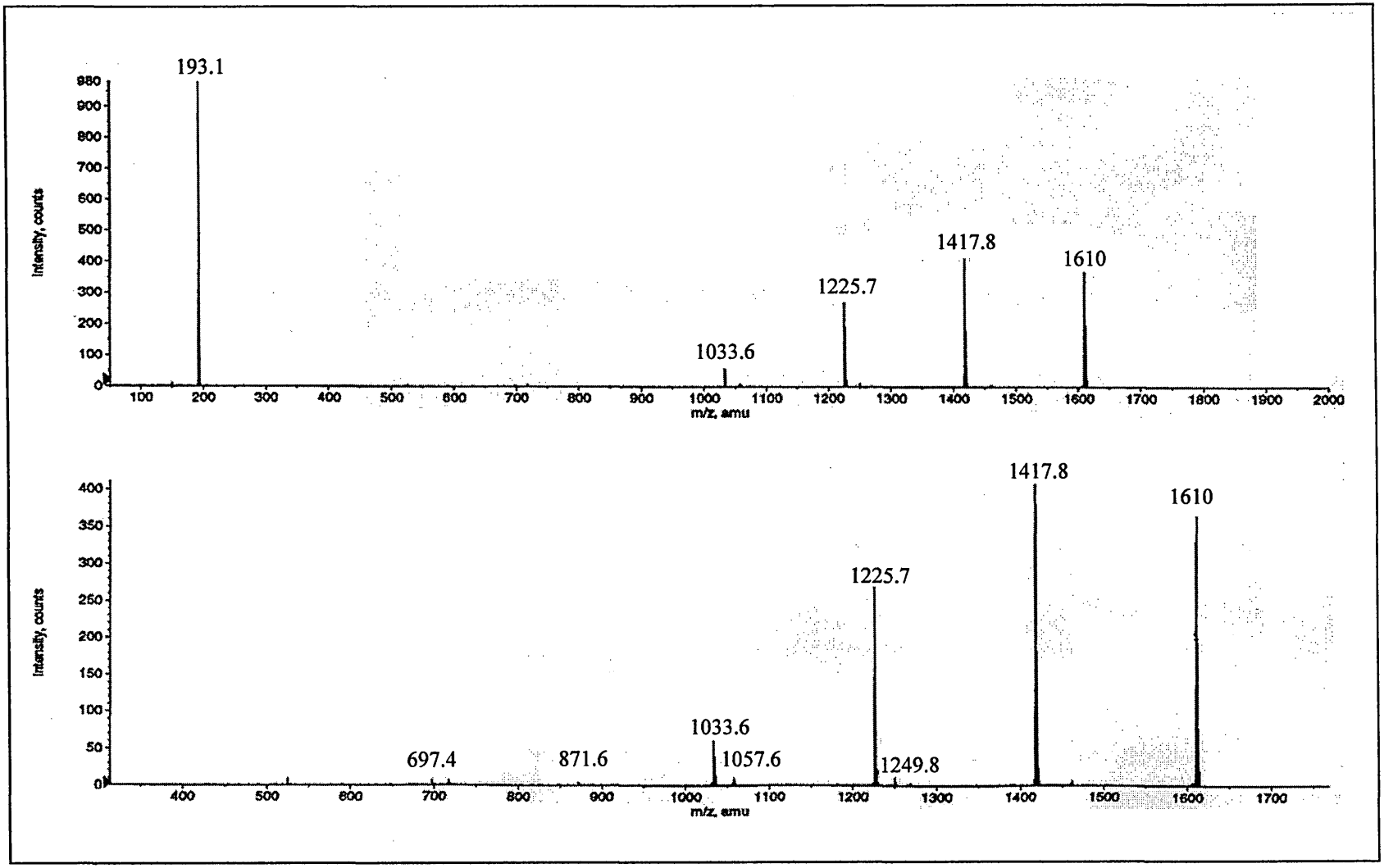


Figure 21. MALDI/MS/MS product ion spectra for the dissociation of an HDI related precursor ion corresponding to m/z 1610. The upper spectrum shows the presence of the rearrangement/α-cleavage product m/z 193

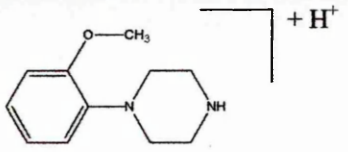
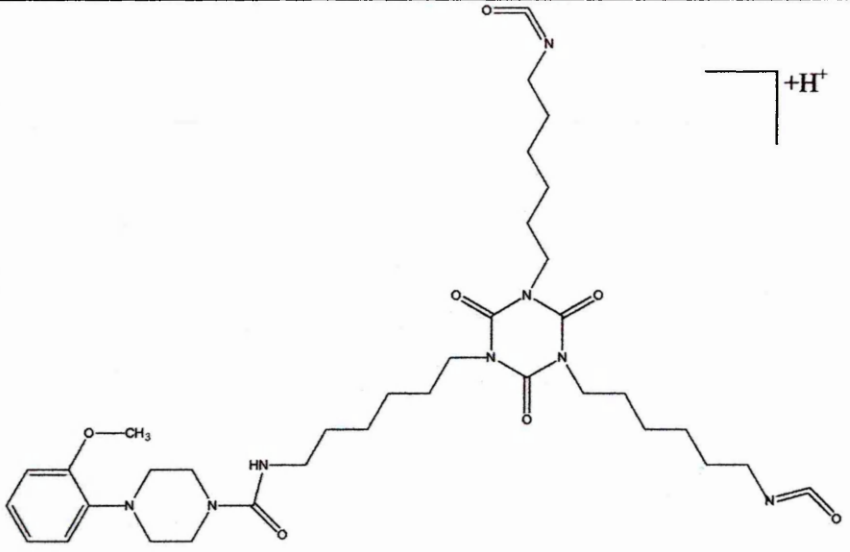
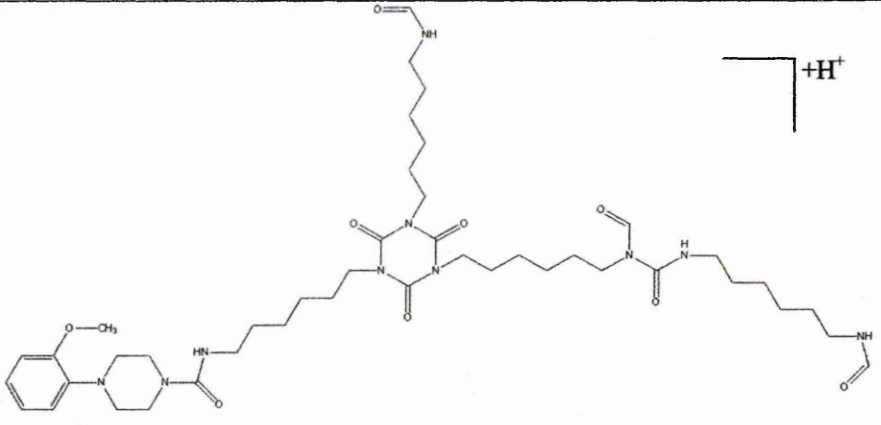
m/z	Proposed Structure
193.1	
697.4	
871.6	

Table 11. Product ions and proposed structures from product ion experiments on a derivatised HDI related species corresponding to m/z 1610, acquired using MALDI/MS/MS

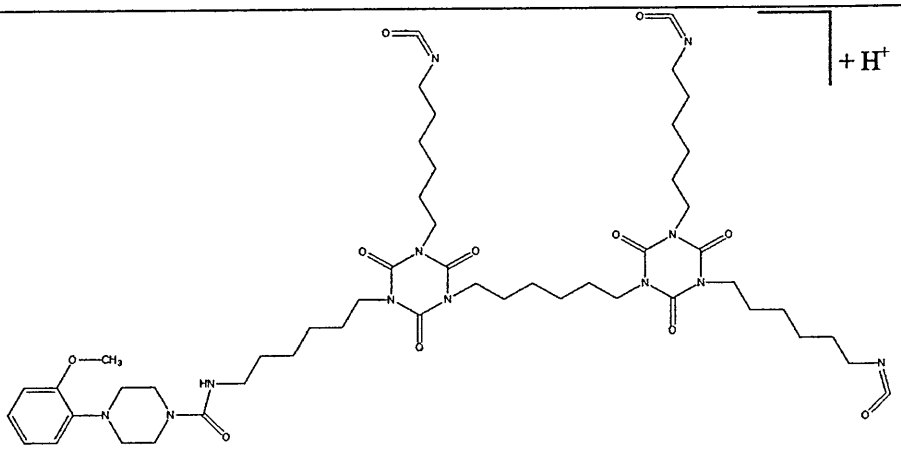
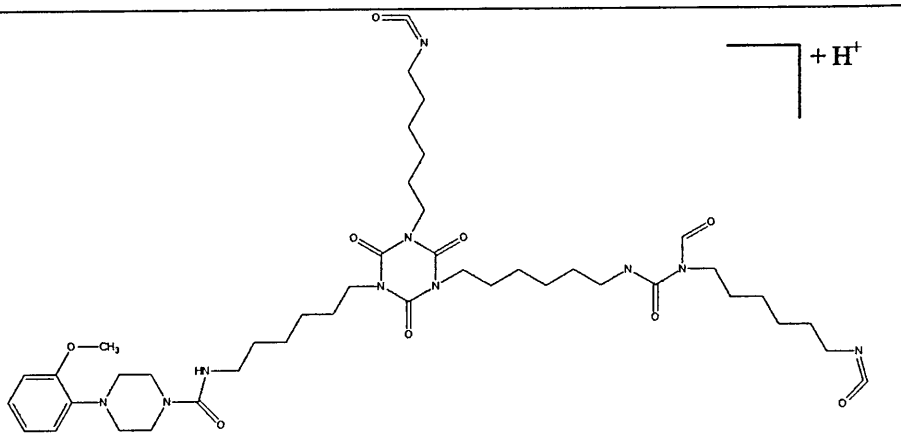
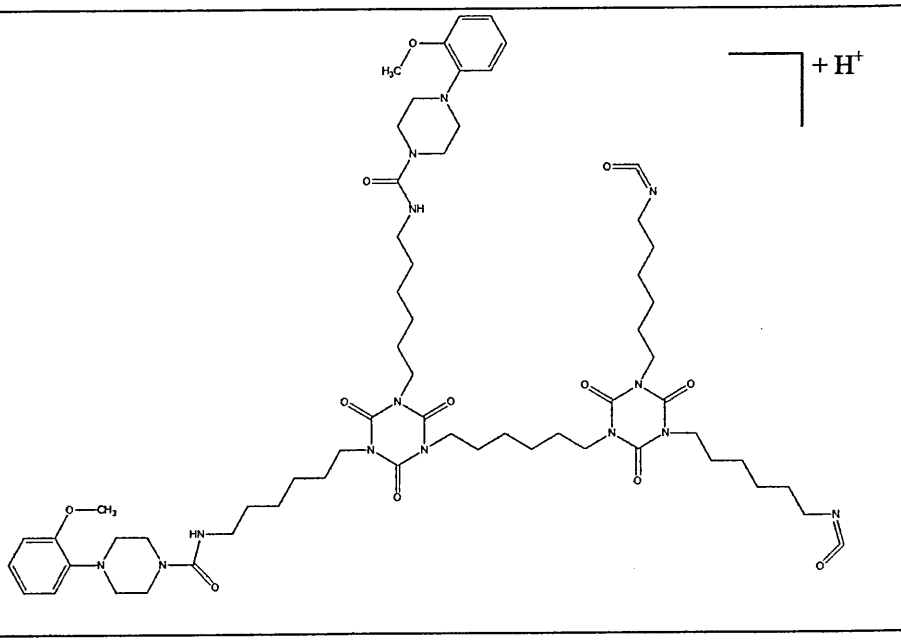
1033.6	
1057.6	
1225.7	

Table 11. Continued.

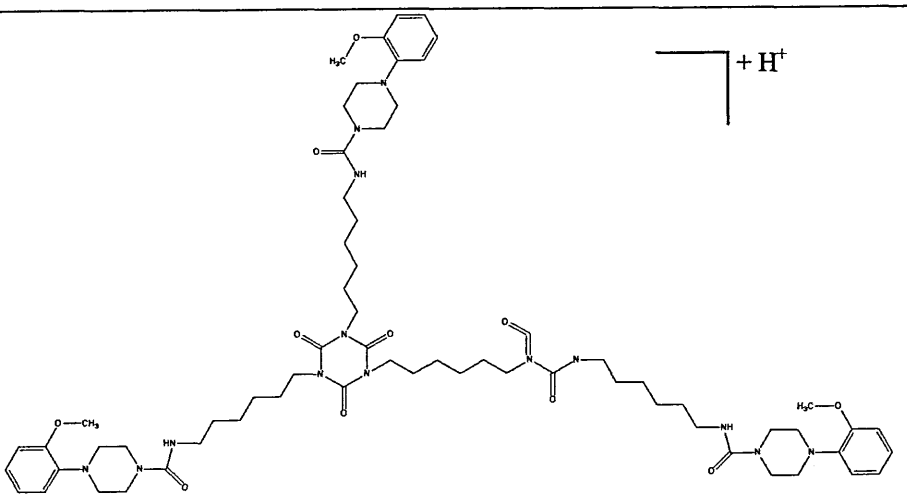
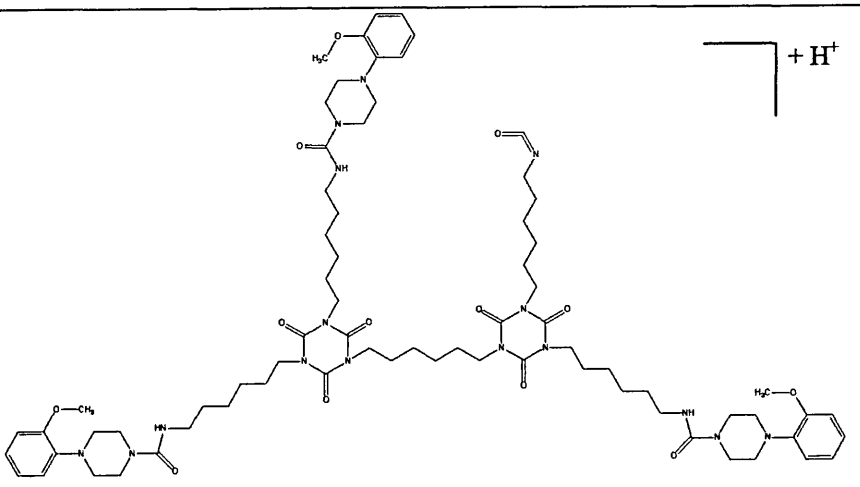
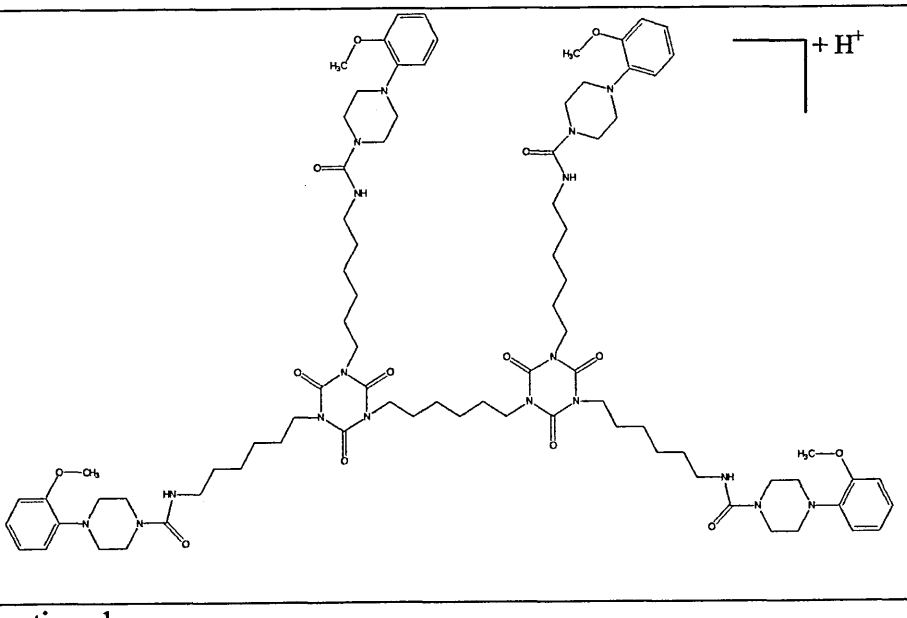
1249.8	
1417.80	
1610	

Table 11. Continued.

The labile nature of the MP/NCO bond provides us with a hypothesis for the determination of NCO functionality. A product ion corresponding to the loss of one derivatising group (192 Da), by the mechanism shown in Figure 11, corresponds to the presence of one NCO functional group. The hypothesis fails when we examine Figure 10. A derivatised HDI trimer precursor ion, m/z 1082, containing three NCO functional groups, dissociates to produce ions corresponding to the loss of one or two, but not three, derivatising reagents. A species retaining one derivatising reagent may be electronically and structurally stable enough to avoid further dissociation. The instrument used in the first product ion experiments was not sufficiently sensitive enough for these conclusions to be unequivocally made i.e. the loss of three NCO groups and the formation of an ion of m/z 506 could not be dismissed, since the reason it was not observed could have been instrumental. Figure 12 is an example of product ion data acquired for the derivatised HDI trimer, m/z 1082, using a more sensitive instrument. An ion corresponding to the HDI trimer minus three derivatising reagent groups, i.e. m/z 506, is not observed in Figure 12. This evidence would indicate that counting the total number of losses of the derivatising reagent as a means of determining the total NCO functionality of a species does not work. It may be possible to postulate that the number of NCO groups is equal to the number of losses of derivatising reagent plus 1 or, total NCO functional groups = (number observed losses 192)+1. To test the hypothesis, data from product ion experiments for NCO monomer related precursor ions are examined below:

2,4-TDI: MALDI/MS/MS was applied to two 2,4-TDI related ions and the ion at m/z 1447.6 was determined as a product of the ion m/z 1640.8. The determination of this relationship produces a potential diagnostic ion, m/z 1447.6, characteristic of the ion corresponding to 1640.8 identified in Table 8. Figure 18 shows the peaks at m/z 1255.4 and 1448.6; both have satellite peaks with a mass 44 Da less. This is a loss of 236 Da, or multiple thereof, from the precursor and cannot be explained. The product ion mass spectrum produced by the ion of m/z 1640.8 shows three losses of 192 Da, a result of bond dissociation reactions of the type shown in Figure 11. The species is characterised as having four NCO groups, the total NCO functional groups = (number observed losses 192)+1 theory is true in this instance. The theory fails, however, when we apply it to m/z 1447.6, a dissociation product.

MDI: MALDI/MS/MS was applied to two MDI related ions, m/z 1412.8 and 1605.9. The spectrum produced by product ion experiments on m/z 1412.8. Figure 19 shows the characteristic loss of three MP reagent groups, a result of bond dissociation reactions of the type shown in Figure 11. The loss of three derivatising reagent groups would suggest a four NCO functional group species. The structure for the ion corresponding to m/z 1412.8 is shown in Table 9. Figures 19 and 20 shows ions at a relatively low mass and structures have been proposed for these. The ion at m/z 1412.8 shows a loss of 236 Da; this loss was also observed for product ion experiments on derivatised 2,4-TDI related ions. The mechanism for the formation of a neutral species corresponding to 236 Da is not known. The mass spectrum in Figure 20 acquired as a result of product ion experiments on a derivatised MDI related precursor, m/z 1605.9, shows multiple losses of 192 and 236

Da. There are four losses of 192 from the parent molecule that would suggest a five NCO functional group species. The structure derived for the ion at m/z 1605.9 is shown in Table 10 and is a five functional NCO species. The total NCO functional groups = (number observed losses 192)+1 theory is true for the product ions, m/z 1412.8 and 1605.9.

HDI: The spectrum in Figure 21 is the result of product ion experiments on the ion corresponding to m/z 1610. Product ion experiments on this ion have been presented earlier, using nanospray ionisation (Figure 13). Structures have been assigned for the ions in Figure 21 and these can also be applied to Figure 13. The data in Figure 21 shows the characteristic loss of multiples of 192 and 236 Da. A potential structure for the ion at m/z 1610 has been proposed in Table 11. The spectrum in Figure 21 shows the loss of three derivatising reagent groups by the mechanism shown in Figure 11. This would suggest a four NCO functional group containing species. The structure of the precursor ion derived from this spectrum (shown in Table 11) is in agreement with the total NCO functional groups = (number observed losses 192)+1 theory.

Mass spectrometry has been demonstrated as a powerful tool in the analysis of NCO in many forms. Using mass spectrometry, many interesting questions have been raised and the scope for future scientific research is immense. The potential for the determination of the number of NCO functional groups by analysis of the product ion mass spectra and calculation of the losses of the derivatising reagent has great potential and further method development will no doubt result in some interesting science. The loss of 236 Da from the product ions can be explained by further work in this area. Interesting future work will focus on the determination of the structural characteristics of the low mass ions observed in derivatised NCO product ion experiments. Accurate mass measurement applied to product ion species sub 600 Da will give elemental composition data, thus reducing the difficulty in structural elucidation. Also the readiness of the urea bond to cleave and yield the derivatising reagent as a charged species (Figure 11) raises the possibility of a quantitative method based on product ion analysis of a species, using MP as the quantifying medium. The role of NCO in occupational asthma gives this and future work extreme relevance in the modern working environment. Although a structure has been postulated for the ion at m/z 1610 the problem highlighted in Section 5.3.2 has not been conclusively addressed and the possibility of two species corresponding to m/z 1610 cannot be ruled out.

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6.0 Analysis of the Surfactant Composition of a Commercial Sheep Dip Formulation

The purpose of this work was to determine the surfactant composition of a commercial sheep dip formulation. Sheep dipping is an agricultural activity used to clean sheep of ectoparasites which if left untreated can become a major problem for parties involved. Sheep dip formulations are used in conjunction with some form of water bath to immerse the subject and expose it various cleaning agents. Insect ectoparasites of sheep fall broadly into three groups depending on the time of year when they are most prevalent. In the spring, ticks are the main sheep ectoparasite problem while in summer fly strike by blow flies becomes more important and in the autumn and winter the control of lice and keds is necessary [1]. Sheep dip formulations incorporate a suitable active ingredient to aid in the removal of these pests. Commonly organophosphorus compounds are used. The incorporation of surfactants into sheep dipping formulations aids in the cleaning process. There are broadly speaking two classes of surfactant: nonionic and ionic. Further differentiation is made between anionic and cationic surfactants.

Nonionic surfactants are being used increasingly as an effective and less environmentally harmful alternative to their ionic equivalents for a range of industrial applications. Three nonionic surfactants are commonly used in commercial formulations: alkylphenol ethoxylate (APE), primary alcohol ethoxylate (PAE) and secondary alcohol ethoxylate (SAE), (Figure 1). PAE and SAE may exist as a mixture of isomers and homologues of the hydrocarbon chain, combined with a series of homologues of the polyethoxylate chain differing from each other simply by one ethylene oxide unit, as is the case for NPE, PAE

and SAE. The ratio of the average number of ethylene oxide units to the hydrocarbon chain length gives the surfactant its characteristic properties.

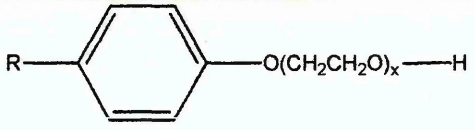
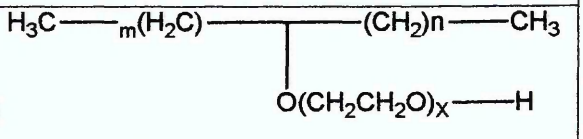
Chemical Name	Abbreviation	Structural Formula
Alkyl phenol ethoxylate	APE	 $x \geq 1$
Primary alcohol ethoxylate	PAE	$H_{2m+1}+C_m$——$O(CH_2CH_2O)_x$——H $x \geq 1$
Secondary alcohol ethoxylate	SAE	 $x \geq 1$

Figure 1. Chemical name, abbreviation and structural formula for three classes of common nonionic surfactants.

Almost all commercial and industrial surfactant formulations are made of mixtures of different types of surfactants. Therefore the determination of mixtures of surfactants is not only important in order to understand specific properties for industrial applications but also for environmental monitoring.

Nonylphenol ethoxylates (NPE) are an important class of nonionic surfactants. They have been used commercially for many years as emulsifiers and solubilisers in pharmaceutical and agrochemical formulations, cosmetics and various biotechnological processes due to their favorable physiochemical characteristics. One of the major uses of NPE is as

surfactants in industrial cleaners [2]. NPE have attracted a great deal of attention because of their potentially adverse environmental effects. Their anaerobic degradation to 4-nonylphenol (NP), a persistent, lipophilic toxicant, has been linked to feminisation of male fish located downstream of sewage treatment plants [3, 4].

There are numerous methods used for the determination of surfactants. Commonly a separation technique is coupled with a suitable mode of detection. HPLC methods have been reported with UV [5], fluorescence [6], evaporative light scattering [7], refractometric [8] and mass spectrometric [9] detection methods. The application of GC/MS has been reported by Castillo *et al.* [10]. Capillary electrophoresis (CE) with UV detection has been reported as a technique suitable for surfactant analysis [11].

Surfactant analysis has been reported without separation techniques but with the direct application of MALDI/MS and ESI/MS techniques. Thompson *et al.* produced MALDI spectra for three classes of surfactant: nonionic, anionic and cationic, using 2,5 dihydroxybenzoic acid (DHB) as the matrix in all three cases [12]. Willets *et al.* quantitatively analysed nonionic surfactants in surface water using MALDI/MS. Internal standard calibration was achieved by using octylphenol ethoxylate as the internal standard [13]. Benomar *et al.* used MALDI in conjunction with LC/ESI/MS to analyse nonylphenol and octylphenol ethoxysulphonates [14]. Direct analysis of the photocatalytic degradation of wool scouring formulations using ESI/MS, without prior separation, was reported by Sherrard *et al.* [15]. Socher used ESI/MS with CID to characterise ethoxylated surfactants [16]. In the work reported in this thesis, MALDI/MS,

ESI/MS and LC/ESI/MS have been used to characterise the surfactant composition of a commercial surfactant formulation.

6.2 Experimental

6.2.1 *Chemicals and Reagents*

HPLC grade methanol and trifluoroacetic acid (TFA) was purchased from Fischer Chemicals, (Loughborough, Leicester, U.K.). Lithium Chloride was purchased from BDH, (Poole, Dorset, U.K.). Milli-Q water was used in all the experiments where necessary. MALDI matrix 2,5-dihydroxybenzoic acid (DHB) was purchased from Sigma-Aldrich (Poole, Dorset, U.K.). Paracide sheep dip formulation was provided by the Health and Safety Laboratory (HSL, Sheffield, U.K.).

6.2.2 *Instrumentation*

The LC/MS system consisted of a Jasco PU-980 Intelligent HPLC pump with a LG-980-02 Ternary gradient unit (Jasco, Great Dunmow, U.K.), a VG Quattro I mass spectrometer (Fisons Instruments, VG Biotech, Altrincham, Cheshire, U.K.) equipped with a pneumatically assisted electrospray (ESI) interface and a Rheodyne injection valve mounted in a gas flow regulating unit. The mass spectrometer was operated in the positive ion mode with the following working conditions; capillary voltage 3.85 kV, HV lens voltage 0.3 kV, cone voltage 30 V, Lens 3 potential 3 V, multiplier 550 V, source

temperature was 95 °C. The nitrogen nebuliser and curtain gas (BOC, Guildford, Surrey, U.K.) flows were 40 $l.h^{-1}$ and 350 $l.h^{-1}$, respectively. Data was recorded on a PC with Mass Lynx Software V2.0 (Micromass, Altrincham, Cheshire, U.K.). A flow rate of 100 $\mu l.min^{-1}$ from the electrospray probe was achieved by a 10:1 post-column T-piece/PEEK tubing (Supelco, Poole, Dorset, U.K.), flow splitter.

Direct infusion MS experiments were performed using a Harvard 11 syringe driver (Merck, Poole, Dorset, U.K.) with a Hamilton 500 µl gas tight syringe (Supelco, Poole, Dorset, U.K.). PEEK fittings and tubing were purchased from Supelco and used throughout mass spectral data acquisition.

MALDI/MS experiments were performed on a Kratos MALDI IV (Kratos Analytical Ltd., Manchester, U.K.) mass spectrometer which uses a nitrogen laser at 337 nm. Experiments were carried out using a 20 kV extraction voltage. All spectra were the result of the cumulative acquisition of 20 shots. Data was processed with Microsoft Excel.

6.2.3 *Analytical Procedure*

A Paracide standard (0.01 mg) was prepared in methanol (10 ml). Direct infusion experiments were performed at a flow rate of 5 $\mu l.min^{-1}$. The chromatographic procedure was isocratic elution with a mobile phase of 50/50 methanol/water. The column used was a Hypersil C18 3.5µ (4.5 x 150mm) (Thermo Hypersil-Keystone, Cheshire, U.K.). The

flow-rate was 1.0 $ml.min^{-1}$ with an on column injection volume of 5 µl. Direct infusion experiments were performed at a flow rate of 5.0 $\mu l.min^{-1}$.

The MALDI matrix 2,5-dihydroxybenzoic acid (DHB), (10 $mg.ml^{-1}$), was prepared in methanol containing 0.1% trifluoroacetic acid (TFA). MALDI analysis was carried out by mixing 200 µl of the Paracide solution with 1 ml of the matrix solution. For experiments involving Li^+ ions, LiCl in methanol (10 $mg.ml^{-1}$) was used as the Li^+ source.

6.3 Results and Discussion

6.3.1 *Application of MALDI/MS to surfactant analysis*

Initial experiments were performed using MALDI/MS. This allowed the acquisition of data over a very large mass range without concerns regarding multiple charging which one associates with ESI/MS. It was expected that mass spectra would be complex and so any steps that could be taken to reduce this complexity were taken. Figure 2 shows a MALDI mass spectrum acquired for the analysis of the dilute Paracide sample. DHB matrix was used as this has been previously demonstrated as a suitable matrix for surfactant analysis [13].

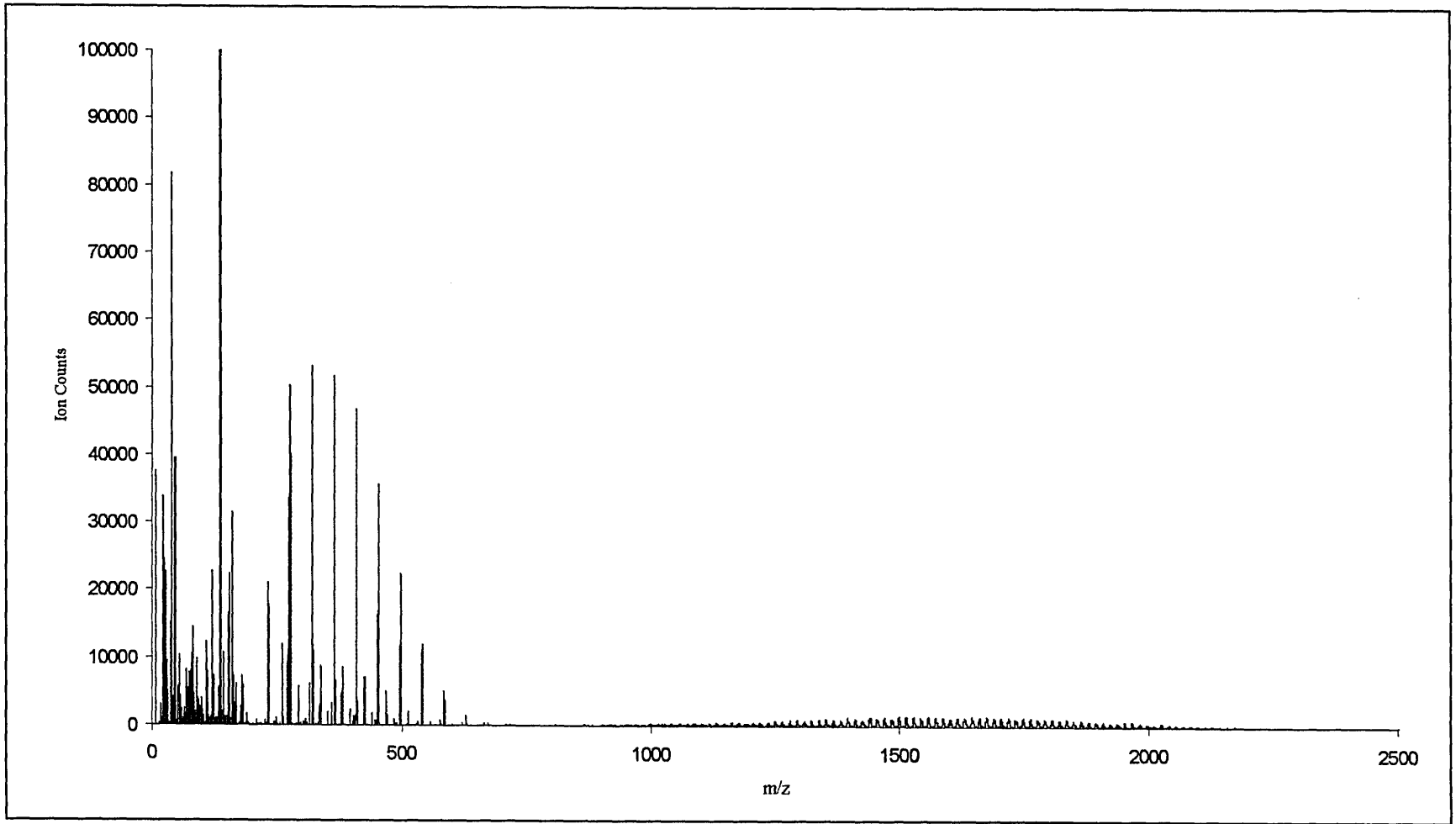


Figure 2. A MALDI/MS mass spectrum acquired from the analysis of Paracide (100 $\mu g.ml^{-1}$) suspended in DHB (10 $mg.ml^{-1}$). The spectrum was acquired in positive ion mode with a 20 kV extraction voltage.

The mass spectrum in Figure 2 shows a large number of ions in the high mass region m/z 1500- 2000. This is suspected to be epoxidised linseed oil, a known additive in sheep dipping formulations. Closer examination of the high mass region of Figure 2 (Figure 3) does not show the characteristic fingerprint of an ethoxylated or propyloxylated surfactant spectrum i.e. mass differences of 44 or 58, respectively. These ions are assumed to be a co-formulant and not part of the surfactant composition of the Paracide formulation.

In this study data for the presence of surface active agents is in the 0-1000 m/z region of Figure 2; this area is highlighted in Figure 4. Initial inspection of Figure 4 reveals the presence of a series of ethoxylated phenol homologues characterised by a series of sodium and potassium adduct ions separated by 44 Da. The sodium and potassium adducts are indicated on Figure 4 by * and **, respectively (see also Table 1 for further interpretation). The complexity of spectra produced using MALDI to analyse surfactant mixtures can be reduced by the addition of a lithium salt. The high electrophilicity of lithium cations will prevent the formation of salt adducts with salt cations other than itself, providing lithium cations are present in sufficient concentration. Figure 5 shows the result of the analysis of a Paracide sheep dip sample with the addition of lithium chloride. The addition of lithium cations in the Paracide solution was found to significantly reduce the complexity of the mass spectrum. The series of ions which dominate the spectrum in Figure 5 correspond to lithium adducts of ethoxylated phenol homologues. The addition of lithium to the sample had no effect on the complexity of the high mass region of the spectrum. As opposed to the diagnostic mass differences between a protonated molecule and a sodium or potassium adduct, +22 and +38 Da, respectively, in the case of lithium adducts a mass difference of +6 Da is observed.

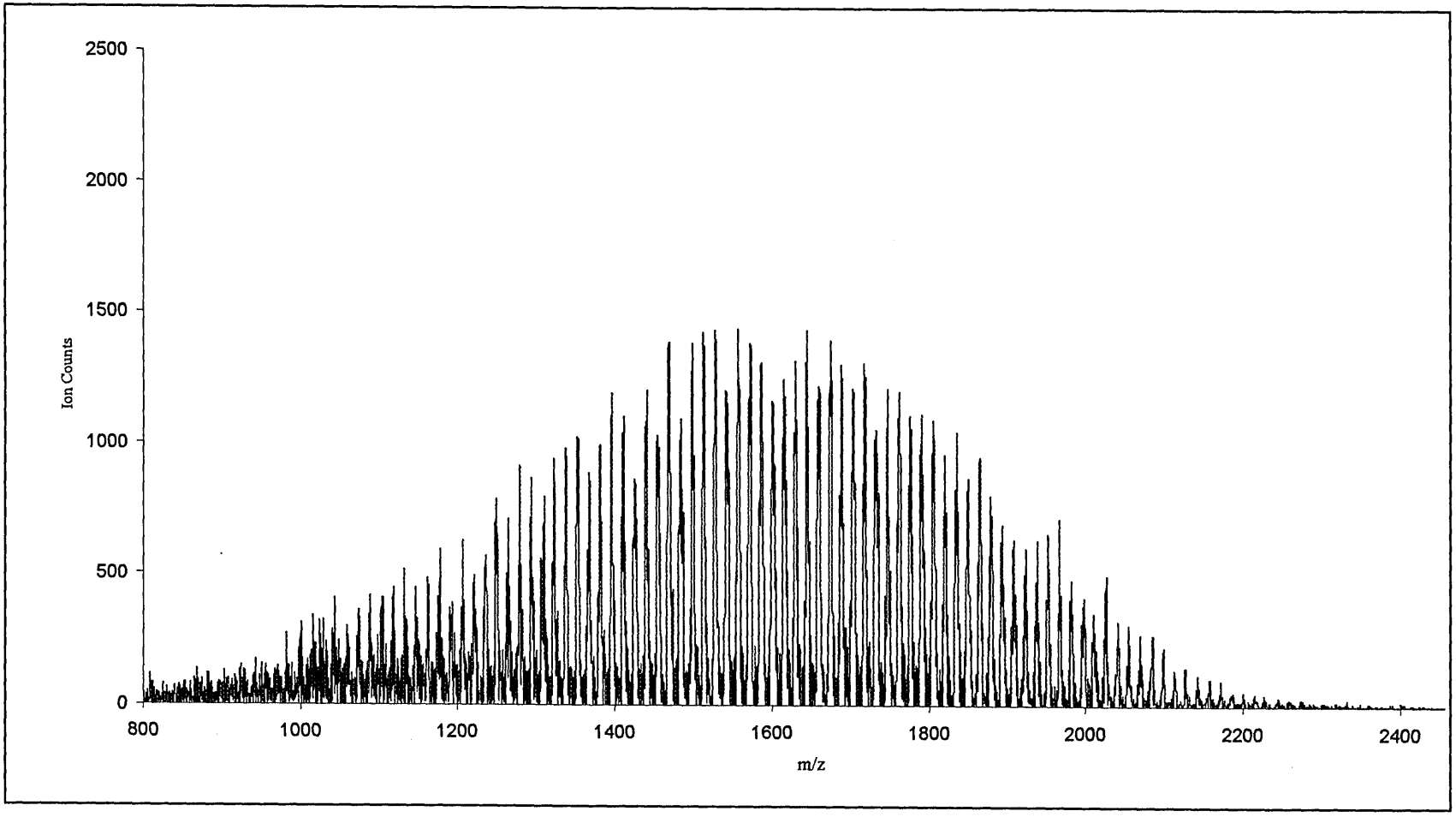


Figure 3. The high mass region of Figure 2 showing the mass spectrum of what is suspected to be expoxidised linseed oil, a common co-formulant in sheep dip formulations.

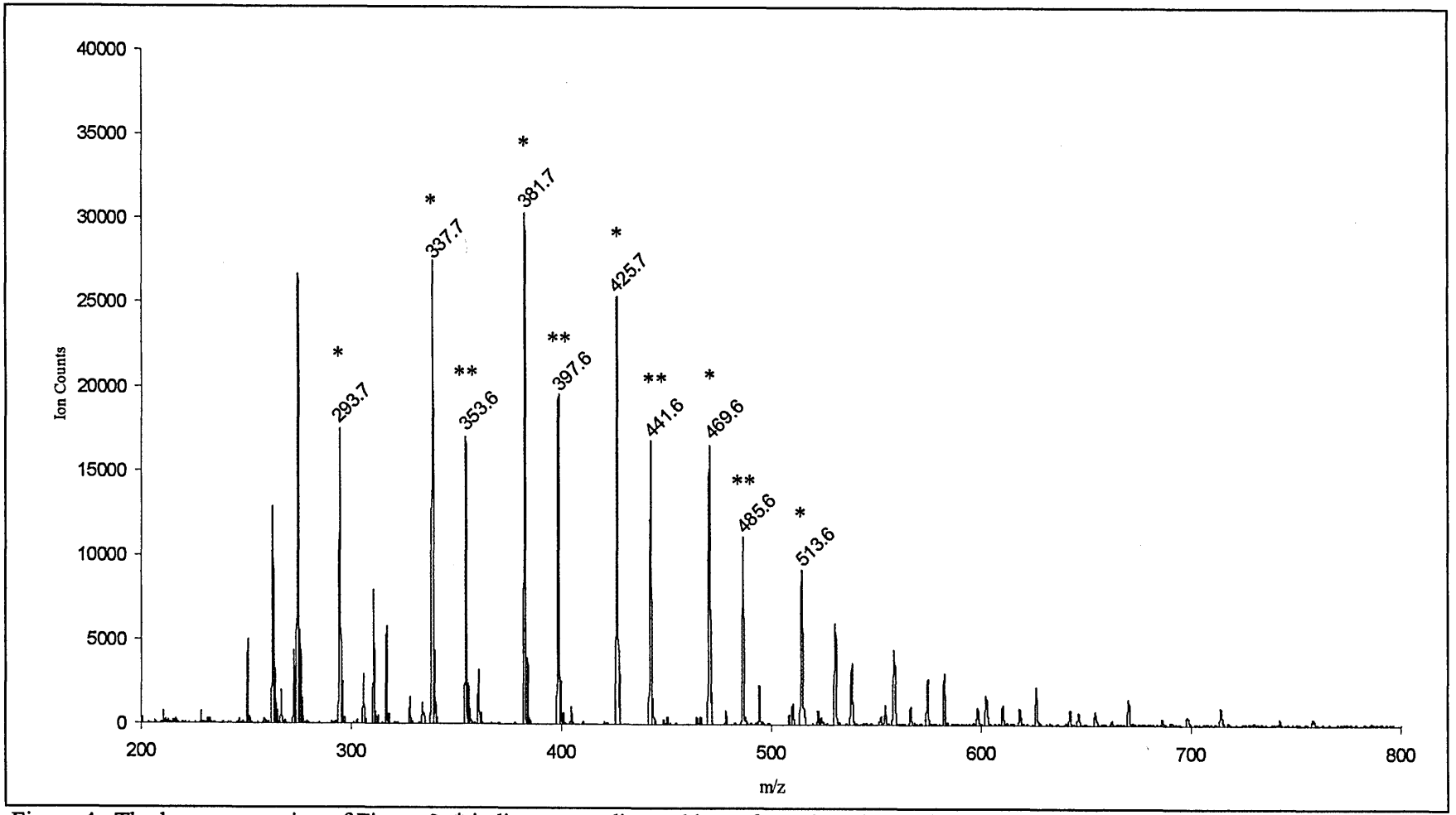


Figure 4. The low mass region of Figure 2, * indicates a sodium adduct of an ethoxylated phenol homologue, ** indicates a potassium adduct of an ethoxylated phenol homologue

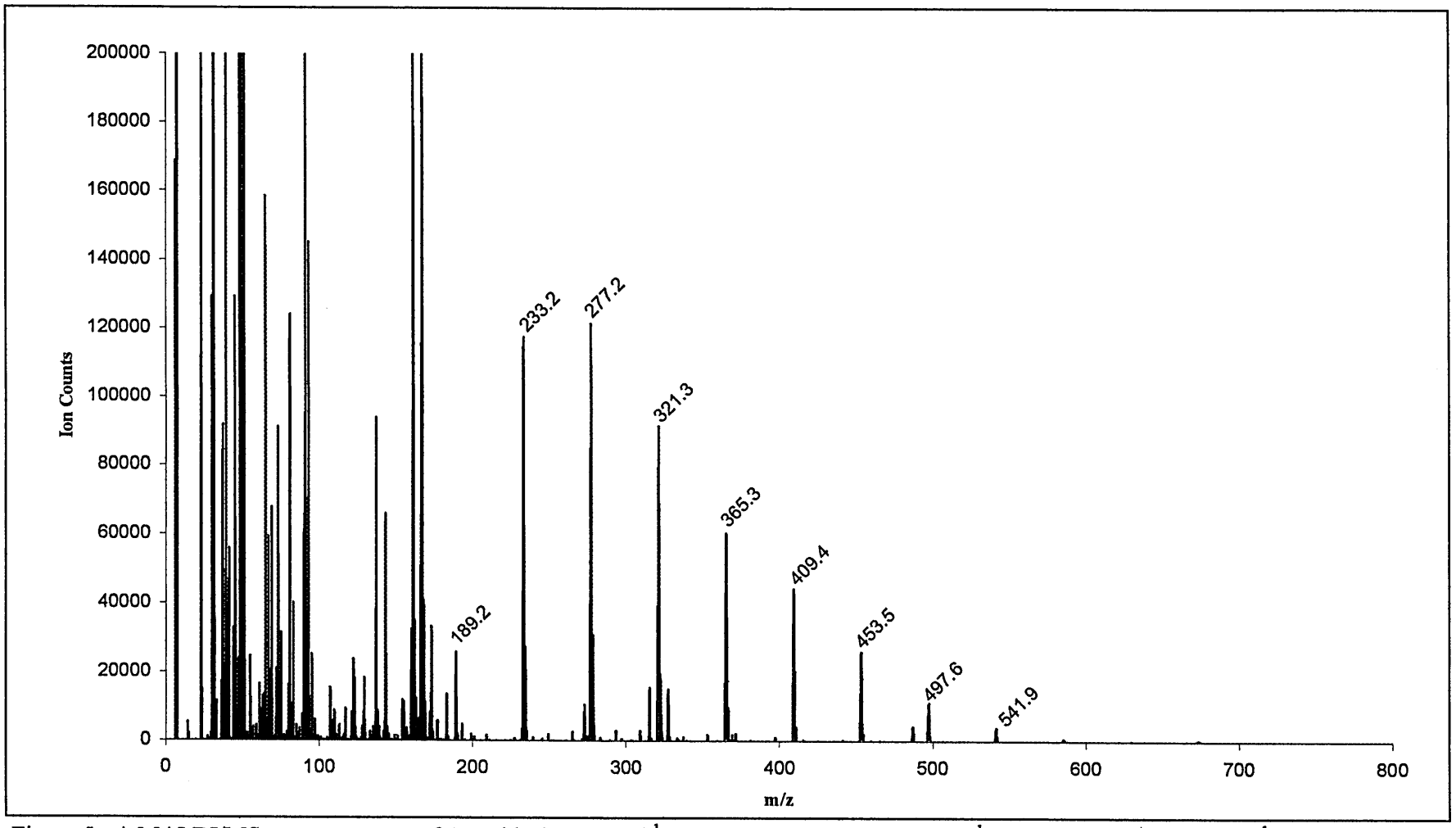


Figure 5. A MALDI/MS mass spectrum of Paracide (100 μg.ml^{-1}) suspended in DHB (10 mg.ml^{-1}) doped with Li^+ (10 mg.ml^{-1}). The ions labelled are lithium adducts, $[M+Li]^+$, of ethoxylated phenol homologues.

Ethoxylate Units	m/z			
	$[M+H]^+$	$[M+Na]^+$	$[M+K]^+$	$[M+Li]^+$
4	271	293	309	277
5	315	337	353	321
6	359	381	397	365
7	403	425	441	409
8	447	469	485	453
9	491	513	529	497
10	535	557	573	541
11	579	601	617	585

Table 1. m/z values for the protonated molecule, sodium, potassium and lithium adducts for a series of ethoxylated phenol homologues.

6.3.2. *Application of ESI/MS to the Analysis of Surfactants*

Figure 6 shows the result of a direct infusion ESI/MS analysis of a dilute Paracide sheep dip formulation sample. The series which dominates the spectrum corresponds to the sodium adducts of ethoxylated phenol homologues. Another homologues series can be identified in the spectrum in Figure 6. Labelled, m/z", these are unidentified.

6.3.3. *Application of LC/ESI/MS to the Analysis of Surfactants*

Figure 7 is a mass chromatogram acquired from the separation of a Paracide sheep dip sample using LC/ESI/MS. The data shown in Table 2 shows the retention time and corresponding number of ethylene oxide units for all the ethylene oxide homologues detected in the Paracide analysis.

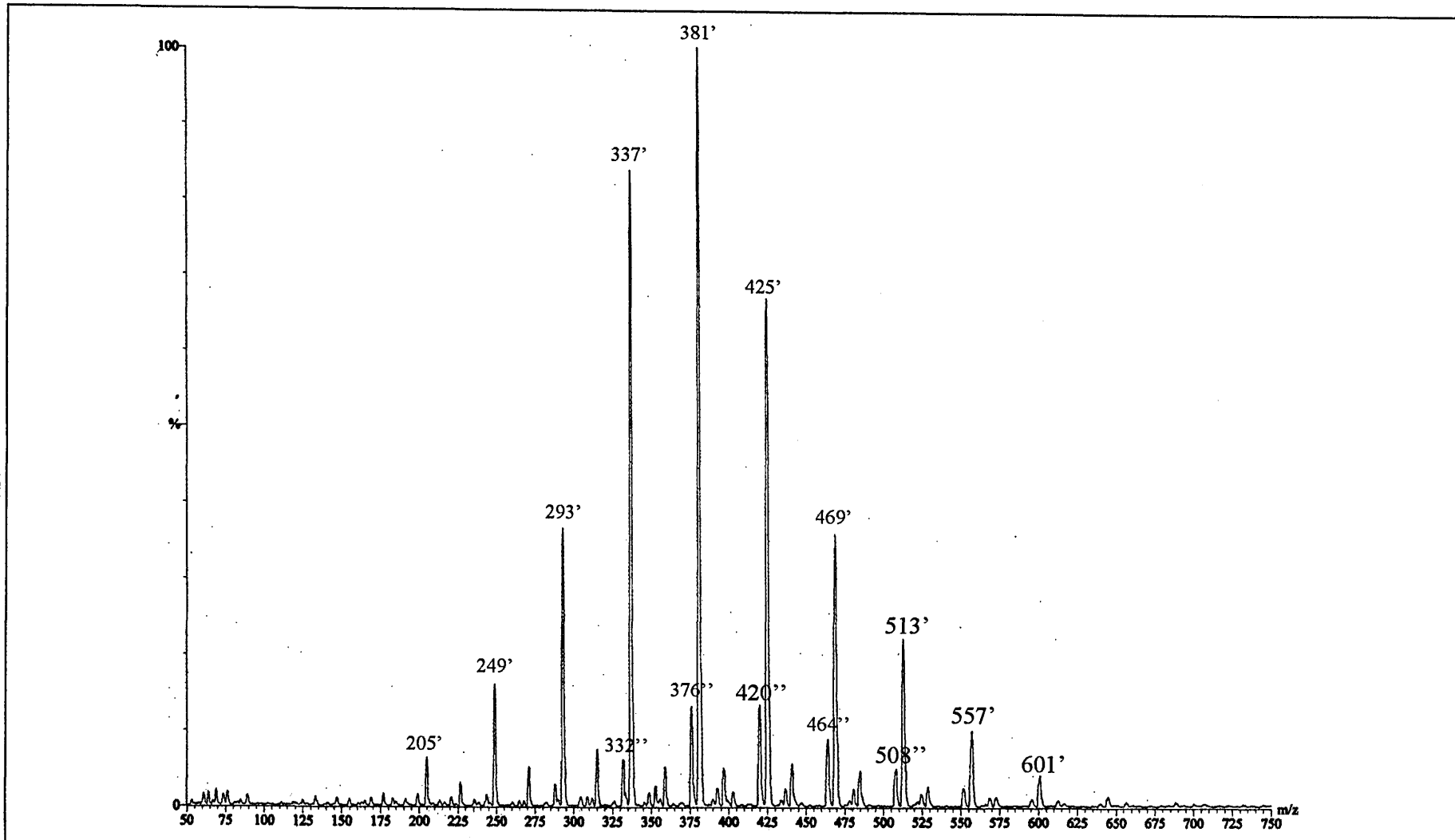


Figure 6. An ESI/MS mass spectrum acquired from the analysis of a dilute Paracide sample. The flow rate was 5 $\mu l.min^{-1}$ and the cone voltage was 30 V. The peaks labelled, m/z', are the sodium adducts of ethoxylated phenol homologues. The peaks labelled, m/z'', are unidentified.

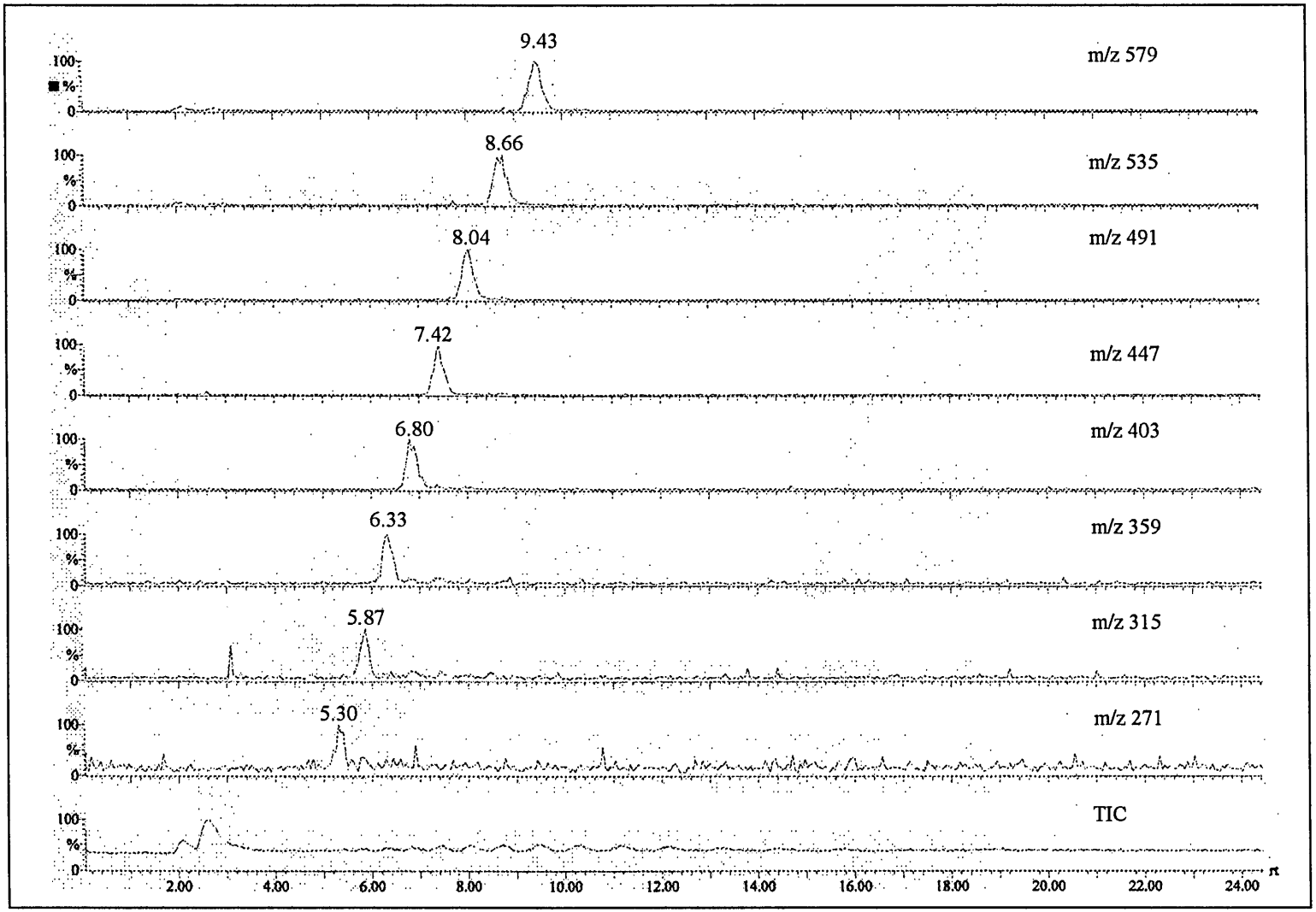


Figure 7. A mass chromatogram acquired from the analysis of a series of ethoxylated phenol homologues in a commercial sheep dip formulation, Paracide. The mobile phase was 50/50 methanol/water. The column was a Hypersil C18 3.5μ (4.5 x 100 mm) (Thermo Hypersil-Keystone, Cheshire, U.K.). The HPLC flow-rate was 1.0 ml.min^{-1}.

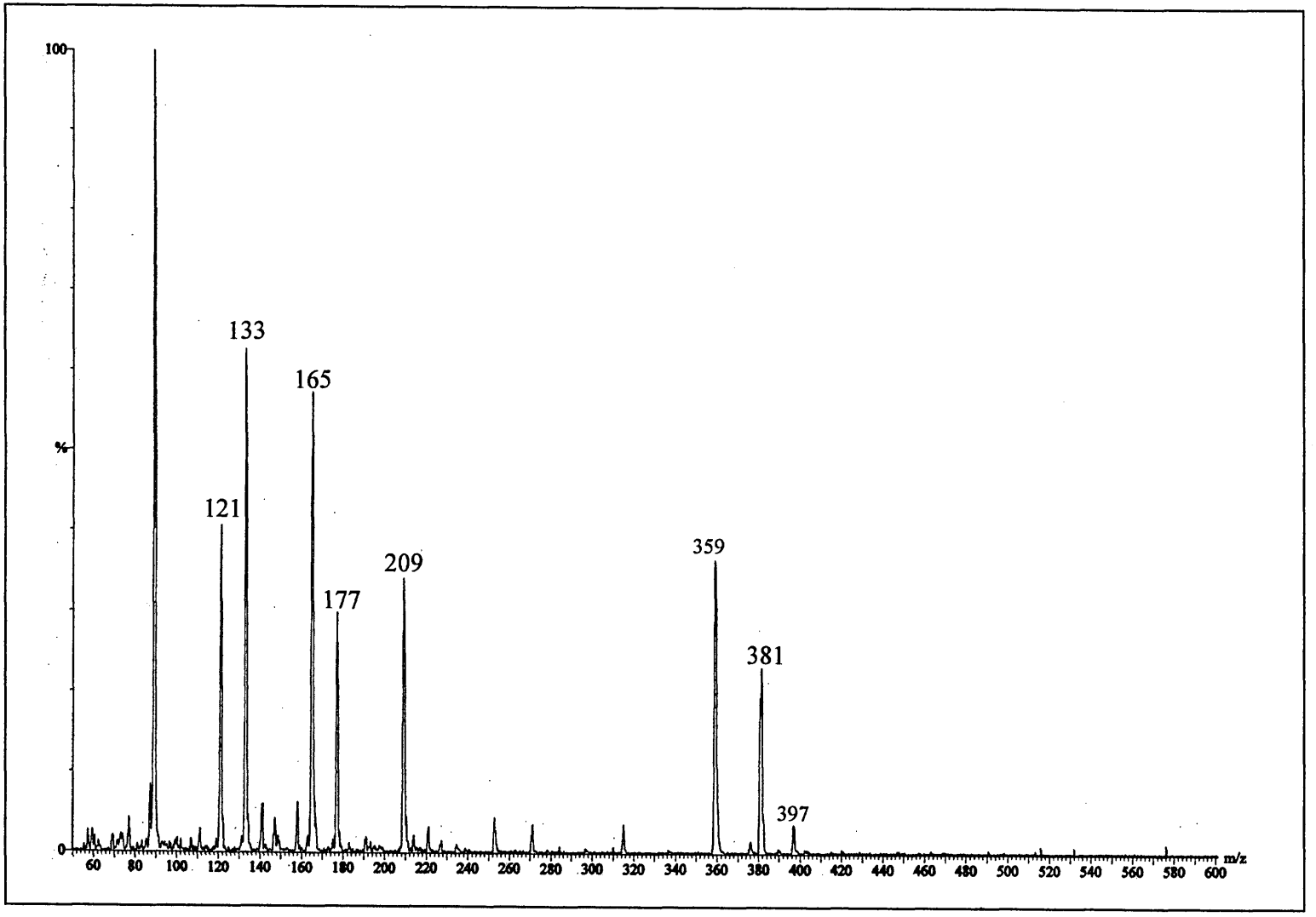


Figure 8. A mass spectrum extracted from the mass chromatogram shown in Figure 8. The spectrum was extracted at 6.33 mins. The peak at m/z 359 represents a protonated ethoxylated phenol homologue (n = 6), the neighbouring peaks at m/z 381 and 397 are the sodium and potassium adducts of the same ethoxylated phenol homologue.

Retention Time (mins)	m/z	Ethoxylate Units
5.30	271	4
5.87	315	5
6.33	359	6
6.80	403	7
7.42	447	8
8.04	491	9
8.66	535	10
9.43	579	11
10.03	623	12
10.72	667	13
11.33	711	14

Table 2. Retention time data, corresponding m/z values and the number of ethylene oxide units for the separation of ethoxylated phenol standard.

Figure 8 is an example of a mass spectrum extracted from the mass chromatogram shown in Figure 7. The spectrum was extracted at 6.33 mins. The spectrum shows a protonated ethoxylated phenol homologue molecule with n = 6 ethylene oxide units, m/z 359. Sodium and potassium adducts of the homologue are also present and were identified in mass spectra extracted for all the ethoxylated phenol homologues separated using LC/ESI/MS. The spectra also contain interesting ions at masses lower than the protonated ethoxylated phenol homologue; m/z 121, 133, 165, 177 and 209. Plomley identified similar peaks to these as dissociation products when analysing nonylphenol ethoxylates using LC/ESI/MS. Figure 9 shows the structures of the ions of m/z values 121, 133 and 165. The ions at m/z 177 and 209 are potentially the species at m/z 133 and 165 with an additional ethylene oxide unit.

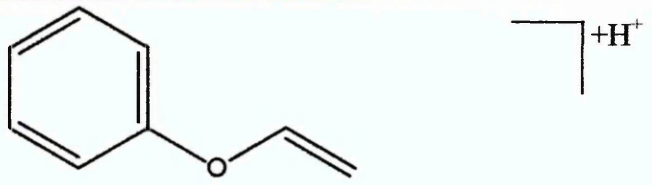
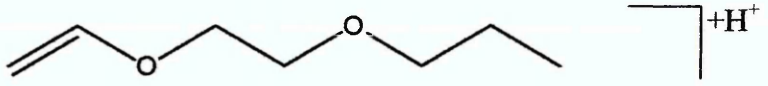
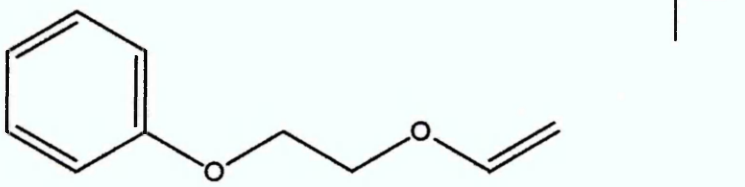
m/z	Proposed Structure
121	
133	
165	

Figure 9. Proposed structures for the ions of m/z 121, 133 and 165 identified in Figure 8, dissociation products from ethoxylated phenol homolgues.

6.4 Conclusion

Modern mass spectrometric techniques have been applied to the analysis of a complex surfactant mixture. M/z data acquired using MALDI/MS, ESI/MS and LC/ESI/MS techniques has facilitated the identification of the primary surfactant component of a commercial sheep dip formulation. The primary surfactant of Paracide was identified as being ethoxylated phenol. A series of ions recorded using ESI/MS could not be identified. The direct analysis approach of MALDI has been demonstrated as a useful tool in surfactant analysis, with the addition of lithium ions being a suitable means of reducing the complexity of surfactant spectra.

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7.0 Conclusion and Future Work

Occupational hygiene is a complex subject with an important primary goal of protecting the health of workers and those involved in work processes. In many cases occupational hygiene has direct implications on areas of environmental concern outside the workplace. The work described in this thesis has introduced, in many cases for the first time, mass spectrometry as an analytical tool to aid in the analysis of samples arising as a result of occupational hygiene monitoring. It is through the monitoring of the workplace environment that conditions can be improved for workers. Sensitive analytical methods allows exposure limits to be reduced and enforced. Mass spectrometry has been demonstrated as a sensitive analytical detection method that is capable of application to the field of occupational hygiene sample analysis. Liquid chromatography is a separation technique that has many advantages. The limiting factor in the successful detection of the separated components of a mixture can be the mode of detection used for an analysis. Mass spectrometric detection is a universal detector which when coupled with liquid chromatography offers one of the most broadly applicable analytical techniques for determination of organic, and in, some cases inorganic species.

The application of LC/MS to the analysis of benzalkonium chloride and related quaternary compounds presented in this thesis (Chapter 3, *The Application of Liquid Chromatography Coupled with Electrospray Ionisation Mass Spectrometry and Tandem Mass Spectrometry to the Analysis of Alkylbenzyl and Dialkyldimethyl Quaternary Ammonium Biocides in Occupational Hygiene and Environmental Media*) demonstrates the advantages of LC/MS as an analytical technique. Previous studies

involving the application of HPLC to the separation of quaternary compounds using non-mass spectrometric methods had been limited by some functional characteristic of the target species. In this work that disadvantage was the absence of a chromaphore on the dialkyldimethyl quaternary ammonium cations. The charged nature of quaternary compounds makes them extremely amenable to electrospray ionisation and this has led to very low limits of detection for the compounds involved in the study (3 $ng.ml^{-1}$ for the C_{12} homologue of BAC). Also, unlike other detection methods where the determination of structural characteristics is limited to some property of the target compound e.g. a particular wavelength of UV/Vis absorption can be related to some functionality. Mass spectrometric methods allow a more complete determination of structural characteristics. Collision induced dissociation allows species of interest to be isolated and subjected to conditions which yield important structural information. In the benzalkonium chloride study using CID it was possible to a identify quaternary ammonium cation in a commercial biocidal product.

In many cases, the advantage of using LC/MS may simply be the low limits of detection obtainable that have implications in forensic work where low concentrations are to be considered. The work in Chapter 4, *The Analysis of Iodopropynylbutylcarbamate (IPBC) by LC/ESI/MS and LC/APCI/MS,* is an example of the low limits of detection obtainable with LC/MS. Also in Chapter 4, the matrix effects involved in the analysis by LC/MS of IPBC-containing professional use products were examined. Matrix effects may have detrimental effects on the ionisation efficiency of the electrospray and atmospheric pressure chemical ionisation processes. These effects were proved to be of no consequence for this work when tested with a

null hypothesis. The effects of matrix composition on ionisation efficiency remains of interest as other workers have reported less positive results.

The application of mass spectrometric techniques to the analysis of NCO species was a very encouraging experience. Here data obtained for the first time really put into perspective the advantages of mass spectrometry and as a result of this work another research project has been funded to examine the area in more detail with particular focus on the application of MALDI/MS to the analysis of NCO prepolymers. It is hoped that direct examination of sampling media, Whatman 5μm filter papers, using novel scanning laser methods will allow for the first time a direct examination of the efficiencies of various sampling methods and sampling head designs. The representation of a sample rich filter paper in three dimensions and subsequent examination of the data will yield some of the most informative analytical data ever acquired in the field of NCO analysis. CID techniques and accurate mass measurement will allow work to progess in the identifiaction of high mass derivatised NCOs.

The work presented with respect to surfactants used in sheep dipping formulations demonstrated the pairing of LC/MS and MALDI/MS techniques. The techniques are shown to pair well with each other and, although previously demonstrated elsewhere for other groups of surfactants, the application of both techniques to ethoxylated phenol analysis is novel. In terms of the development of a quantitative method for the analysis of surfactants used in sheep dipping formulations, matrix effects would have to be examined and the potential of APCI in this method would be again be interesting to explore.

The potential role of LC/MS in the field of occupational hygiene has been explored and several interesting topics have been investigated. Further work in this area will no doubt result in the development of routine methods for The Health and Safety Executive MDHS series.

Appendix 1

Presentations and Conferences Attended

Oral Presentations

The Application of LC/MS and LC/MS/MS to the Qualitative Analysis of Polymeric Isocyanates. British Mass Spectrometry Society (BMSS), 25th Annual Meeting, University of Southampton, 9th-12th September 2001

Poster Presentations

Application of LC/MS to the Analysis of Selected Biocides: A Survey of Occupational Exposure to Metal Working Fluids. British Mass Spectrometry Society (BMSS), 26th Annual Meeting, Loughborough University, 8th-11th September 2002

The Application of MALDI/MS and MALDI/MS/MS to the Qualitative Determination of Isocyanate Derivatives. Royal Society of Chemistry Analytical Division, Analytical Research Forum (Incorporating R&D Topics), Kingston University, 15th–17th July 2001

The Application of LC/MS and LC/MS/MS to the Qualitative Determination of Polymeric Isocyanate Derivatives. Royal Society of Chemistry Analytical Division, Analytical Research Forum (Incorporating R&D Topics), University of East Anglia, 16th-18th July 2001

Meetings Attended

British Mass Spectrometry Society (BMSS), 26th Annual Meeting, University of Loughborough, 8th-11th September 2002.

British Mass Spectrometry Society (BMSS), 25th Annual Meeting, University of Southampton, 9th-12th September 2001.

Royal Society of Chemistry Analytical Division, Analytical Research Forum (Incorporating R&D Topics), Kingston University, 15th-17th July 2002.

Royal Society of Chemistry Analytical Division, Analytical Research Forum (Incorporating R&D Topics), University of East Anglia, 16th-18th July 2001

Chromatography-Mass Spectrometry: The Ultimate Combination? Guys Hospital, London, 8th March 2002.

Life Scientists' Annual Careers Seminar, Royal Academy of Engineering, 9th November 2001

Discovering New Leads and Getting them Towards Market – Faster. A Waters Sponsored Pharmaceutical and Pharmacogenics Seminar, RAF Museum, Hendon, 17th October 2001.

Current Developments and Future Prospects in Environmental Monitoring, University of Northumbria, 24th January 2001.

Biotechnology Yes, Entrepreneurial Biotechnology Competition, Cambridge, 10th October 2000.

Appendix 2

Publications

Determination of benzyl and dialkyldimethyl quaternary ammonium biocides in occupational hygiene and environmental media by liquid chromatography with electrospray ionisation mass spectrometry and tandem mass spectrometry.

M.J.Ford, L.W. Tetler, J. White, D. Rimmer. *Journal of Chromatography A*, **952**, (2002), 165-172

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Journal of Chromatography A, 952 (2002) 165–172

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Determination of alkyl benzyl and dialkyl dimethyl quaternary ammonium biocides in occupational hygiene and environmental media by liquid chromatography with electrospray ionisation mass spectrometry and tandem mass spectrometry

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This paper is dedicated to the memory of Dr. Lee Tetler.

Abstract

A new method for the simultaneous qualitative and quantitative determination of alkyl benzyl and dialkyl quaternary ammonium compounds (QACs) has been developed. Analysis is by reversed-phase high-performance liquid chromatography coupled with electrospray ionisation mass spectrometry. QACs are extremely amenable to the electrospray ionisation technique (limit of detection of BAC C_{12} homologue 3 ng ml^{-1}). The selectivity of mass spectrometric detection allows simultaneous determination of benzyl and dialkyl dimethyl ammonium compounds. The method was successfully applied to the analysis of real samples (occupational hygiene sampling devices, products and swimming pool water). Structural information was obtained by MS–MS and cone voltage ion dissociation techniques. Ion dissociation enabled the structural elucidation of an unknown quaternary ammonium compound present in a commercial formulation.

Keywords: Mass spectrometry; Alkyl benzyl quaternary ammonium compounds; Dialkyl dimethyl quaternary ammonium compounds; Quaternary ammonium compounds

1. Introduction

The Biocidal Products Directive (BPD) [1] aims to establish a single European market for biocides and to ensure that a high level of protection is provided for users, the public and the environment. A biocide is defined as a product that is intended to kill or exert some controlling effect on harmful organisms by chemical or biological means. Biocidal products have a wide range of uses such as general disinfectant products, preservatives, pest control and anti-fouling products. There are currently many biocidal products on the UK market employing a variety of active substances (ASs) and preparations

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[2]. To aid potential enforcement of the Biocidal Products Regulations, in Great Britain, there is a requirement for analytical methods for the qualitative and quantitative determination of active substances used in biocidal products. Analysis is also required to monitor occupational and environmental exposure arising from biocide use.

Benzalkonium chloride (BAC) [3] is a mixture of *n*-alkyl benzyldimethyl ammonium chloride homologues varying in *n*-alkyl chain length, where *n* represents an even number of carbons from C_8 to C_{18}. The most commonly encountered homologues are C_{12}, C_{14} and C_{16} [4], the biocidal properties of the individual homologues are known to be different [5]. BAC is widely used as an active substance in a variety of applications including anti-bacterial products, anti-fungal products, in-can preservatives, timber treatments, masonary biocides [2], medical disinfectants and ophthalmic systems [6,7]. The preparations used, which vary in individual homologue content, also often contain other ingredients (e.g., amines, steroids, alcohols, etc.) and these can interfere with BAC determination. Other quaternary ammonium compounds (QACs) are also commonly used as biocides, and are collectively described as dialkyl dimethyl ammonium compounds. One example is didecyl dimethyl ammonium bromide (DDDMAB).

Several liquid chromatography (LC) methods for the determination of BAC have been described with separation achieved on cyanopropylsilica (CPS) [5–9], octadecylsilica (ODS) [10–13] or hydrophilic polymer [14] stationary phase columns. The phenyl substituent of BAC provides a suitable chromophore for UV–Vis analysis but the dialkyl dimethyl ammonium compounds such as DDDMAB have no chromophore and so cannot be readily detected by this method. Indirect UV detection has been described for dialkyl QACs but this is not a very specific method of analysis and can be affected by co-eluting compounds. The detector most commonly used for BAC has been UV–Vis but fluorimetric [14] and conductometric [15] methods have been reported. The conductometric [15] detector has the advantage of being able to detect QACs with no UV absorbance, QACs such as dodecyl trimethyl ammonium chloride. Mass spectrometry (MS) was applied to dialkyl dimethyl QACs by Radke et al. working in the 0.4–140 ng ml^{-1} range [13].

MS detection offers several advantages over the previously described methods of detection including increased sensitivity and specificity. The cationic nature of quaternary ammonium compounds makes them very amenable to positive ion liquid chromatography with electrospray ionisation (LC–ESI-MS). For BAC and dialkyl dimethyl ammonium compounds, LC–ESI-MS offers the possibility of a method of simultaneous determination and quantification.

This paper describes the application of LC–ESI-MS to the simultaneous determination of alkylbenzyl and dialkyl QACs. Structural determination is reported by the use of tandem mass spectrometry and cone voltage ion dissociation methods.

2. Experimental

2.1. Chemicals and reagents

Benzyldimethyl dodecyl ammonium bromide (97%), benzyldimethyl tetradecyl ammonium chloride (99%), benzyldimethyl hexadecyl ammonium chloride (99%) and didecyl dimethyl ammonium bromide (98%) were obtained from Sigma–Aldrich (Poole, UK). HPLC-grade acetonitrile and formic acid were obtained from Fischer (Loughborough, UK). Ammonium acetate (HiPerSolv) was purchased from BDH (Poole, UK). Milli-Q water was used in all the experiments where necessary.

Stock standard solutions were prepared by dissolving 1 mg of each standard compound in 1 ml of acetonitrile. Working solutions and calibration standards for the individual compounds were prepared by serial dilution of the stock standards with acetonitrile. Concentrations of 0.5, 1, 5, 10, 50 μg ml^{-1} were prepared. Standards were analysed ×6. A standard mixture was prepared using 1 ml of the 50 μg ml^{-1} respective standards. Commercial products containing QACs were purchased from high street retail outlets and were diluted 1:100 with acetonitrile prior to LC–ESI-MS analysis. Swab (cotton pad) and other occupational hygiene samples were collected as part of the Health and Safety Executive (HSE)

routine programme of occupational hygiene monitoring and were desorbed with the mobile phase, as for this type of work [16,17]. One sample of swimming pool water was taken as part of HSE enforcement activity. The swimming pool water sample was injected directly onto the column as received. All solutions were stored in glass vials at 4 °C. Homogenisation of all samples was achieved by sonication of the sample at 25 °C for 5 min.

2.2. Instrumentation

The HPLC–UV–Vis system consisted of a Waters 610 plus solvent delivery system coupled with a Waters 717 autosampler and a Waters 996 diode array detector. Data was recorded on a Dell personal computer equipped with Waters Millennium software (Waters, Watford, UK). The diode array detection (DAD) system was set to acquire wavelengths in the 210–350 nm range with a sampling rate of 1, a resolution of 1.2 and a filter response of 1.0 (photo diode array settings are quoted in the arbitrary units of Millennium Software).

Direct infusion MS experiments were performed using a Harvard 11 syringe driver (Merck, Poole, UK) with a Hamilton 500 μl gas tight syringe (Supelco, Poole, UK). Polyether ether ketone (PEEK) fittings and tubing were purchased from Supelco and used throughout mass spectral data acquisition.

The HPLC–MS system consisted of a Jasco PU-980 Intelligent HPLC pump with an LG-980-02 ternary gradient unit (Jasco, Great Dunmow, UK), a VG Quattro I mass spectrometer (Fisons Instruments, VG Biotech, Altrincham, UK) equipped with a pneumattically assisted electrospray (ESI) interface and a Rheodyne injection valve mounted in a gas flow regulating unit. The mass spectrometer was operated in the positive ion mode with the following working conditions; capillary voltage 3.95 kV, HV lens voltage 0.3 kV, cone voltage 32 V, lens 3 potential 3 V, multiplier 550 V, source temperature was 95 °C. The nitrogen nebuliser and curtain gas (BOC, Guildford, UK) flows were 40 and 350 l h^{-1}, respectively. Data were recorded on a personal computer with Mass Lynx Software V2.0 (Micromass, Altrincham, UK). A flow-rate of 100 μl min^{-1} from the electrospray probe was achieved by means of a 10:1 post-column T-piece/PEEK tubing split (Supelco).

2.3. Analytical procedure

The chromatographic procedure employed was isocratic with a mobile phase of acetonitrile–100 m*M* ammonium formate acidified with formic acid, pH 3.7 (55:45, v/v). The columns used were a Jones Chromatography, Genesis CN, 4 μm, 100×4.6 mm (Phenomenex, Macclesfield, UK) and a Hypersil CN (CPS), 5 μm, 125×4.6 mm (Phenomenex). Chromatography was carried out with the column at room temperature. The flow-rate was 1.0 ml min^{-1} with an on-column injection volume of 25 μl for the UV–Vis work and 5 μl for the MS work. UV chromatograms were extracted at a wavelength of 262 nm, this wavelength was chosen to be the optimum for the working conditions by examination of the UV spectra of the homologues obtained using the UV–Vis detector.

Initial mass data acquisition was via sample infusion (50 μg ml^{-1}) performed at 5.0 μl min^{-1} using scan acquisition mode over a range of 50–400 *m/z*. Data acquisition was in centroid mode with a cycle time of 2 s and an interscan time of 0.1 s, the run time was 2 min. The formulations and swimming pool samples were analysed using the selected-ion recording (SIR) mode with a dwell time of 0.5 s and an inter channel delay of 0.02 s and a mass span of ±0.25 Da. The mass spectrometer conditions were optimised by tuning on the protonated molecules, $[M+H]^+$, of acetonitrile and formic acid 42 and 32 *m/z*, respectively. MS–MS acquisitions were performed via direct infusion of the standard material of DDDMAB and a diluted formulation. The product ion spectra were recorded with Q1 at low resolution allowing the highest number of precursor ions through into the collision cell. Q3 was optimised for unit resolution. Collision gas was introduced into Q2 to a density that reduced the precursor signal by approximately 50%. MS–MS data acquisition was in the continuum mode to provide improved sensitivity. Collision energy values of 100 were noted as being adequate to achieve structural eluding ion dissociation. Cone voltage bond dissociation was achieved

by increasing the value of the cone voltage whilst infusing the sample. Dissociation was monitored using the real time display of the tune page. Values of 60 V+ were found sufficient to produce ion dissociation for the compounds included in this report.

3. Results

3.1. Application of LC–ESI-MS

To demonstrate the applicability of the LC–ESI-MS technique, the standard mixture of the BAC homologues and DDMAB was analysed. Ions 304, 332, 360 and 326 *m/z* were monitored corresponding to the BAC C_{12}, BAC C_{14}, BAC C_{16} and DDDMAB cationic species. The order of elution is: BAC C_{12}, BAC C_{14}, DDDMAB and BAC C_{16}. The SIR method permits the previously unachievable simultaneous quantitative determination of all four compounds. Fig. 1 shows two chromatograms acquired from the analysis of a standard mixture of the benzyl and dialkyl QACs. Operating the mass spectrometer in SIR mode offers an increase in sensitivity and also permits the quantitation of the individual analytes without resolution ≥1 of the chromatographic peaks. Fig. 2 shows the single channels of a mass chromatogram recorded for a standard mixture of BAC C_{12}, BAC C_{14}, BAC C_{16} and DDDMAB, structures are shown. The ammonium formate in the mobile phase facilitated a low pH and improved peak resolution through its silanophilic interactions with the column packing. The BAC retention mechanism was greatly influenced by pH with low pH providing the optimum peak shape. The limits of detection (LODs) were determined as being 3, 4, 4 and 4 ng ml^{-1} (S/N=3) for C_{12}, C_{14}, C_{16} and DDDMAB, respectively. This is a sensitivity increase of three orders magnitude when compared to the UV–Vis method for the BAC homologues. For DDDMAB it allows

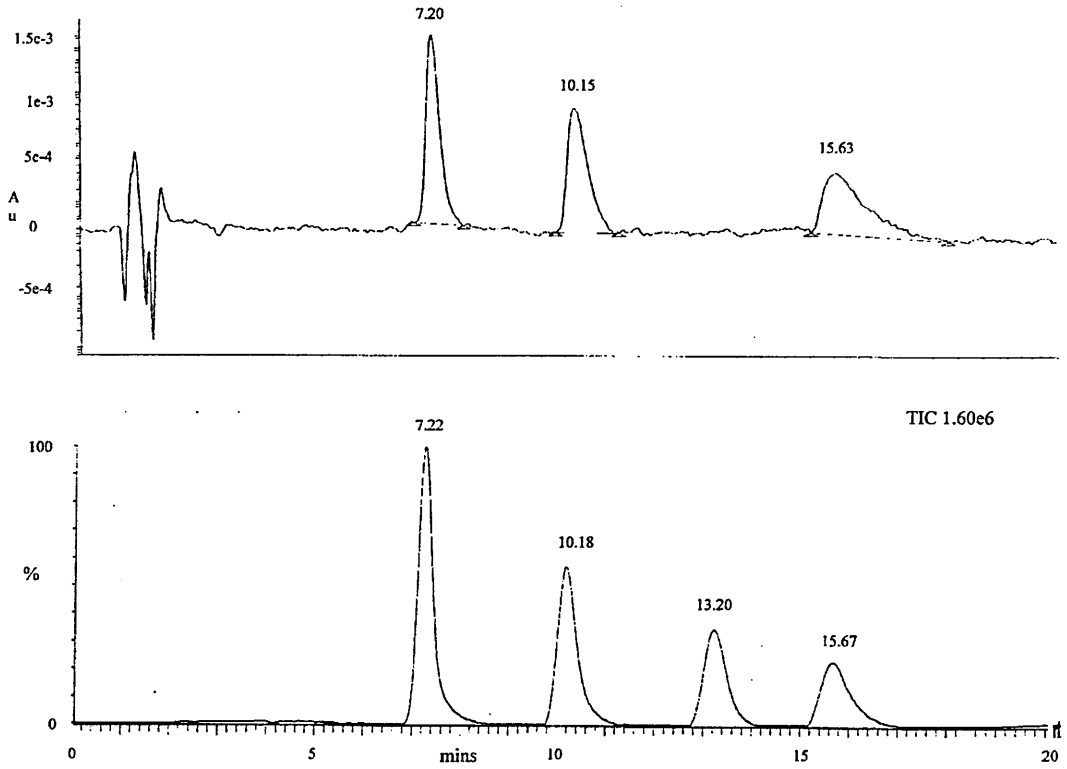

Fig. 1. Chromatograms obtained for a standard mixture of BAC C_{12}, C_{14}, C_{16} and DDDMAB. The upper trace was acquired with UV–Vis detection, the lower MS detection.

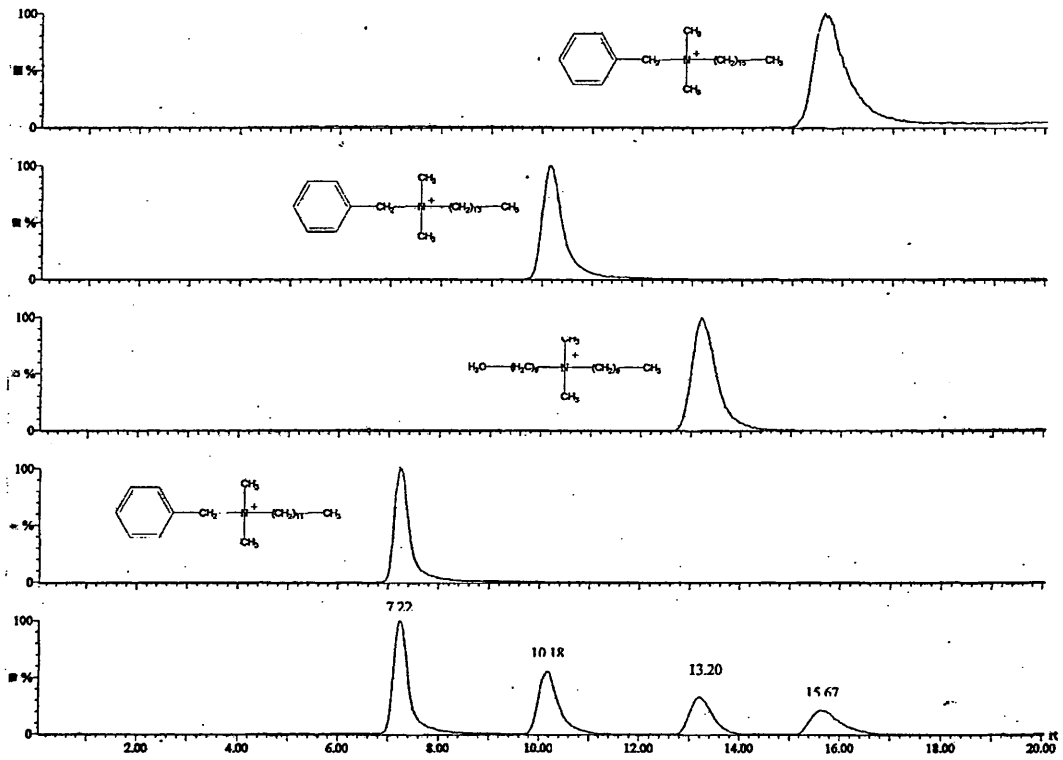

Fig. 2. A mass chromatogram acquired for a standard mixture of BAC C_{12}, C_{14}, C_{16} and DDDMAB peaks are annotated with the appropriate molecular structure.

the determination of a species that previously would not have been recorded by the UV–Vis method.

3.1.1. Evaluation of linear response of the LC–ESI-MS method

A linearity study was performed for the LC–MS method. A series of BAC, C_{12}, C_{14}, C_{16}, and DDDMAB standards were prepared at five concentrations (0.5, 1, 5, 10, 50 $\mu g\ ml^{-1}$). Six replicates were analysed at each concentration value. The Average relative standard deviation (RSD) calibration data was BAC C_{12}: 16.3% BAC C_{14}: 19.25% BAC C_{16}: 19.98% DDDMAB: 15.61%. Calibration curves were constructed by linear regression between peak area and compound concentration. Linear regression values were recorded; DDDMAB: 0.9966, BAC C_{12}: 0.9683, BAC C_{14}: 0.9519 and BAC C_{16}: 0.9878 and noted as being rather poor, this may be due to the use of manual injection (Rheodyne valve) in these experiments or because the LC–ESI-MS system used for this work is less reproducible than the LC–UV–Vis system.

3.1.2. Application of the LC–ESI-MS method to occupational hygiene, concentrates and forensic samples

Fig. 3 shows a mass chromatogram of a swab sample taken during occupational hygiene monitoring of a worker spraying a product known to contain BAC. The sampling procedures and other guidance on occupational hygiene monitoring have been published elsewhere [16,17]. No interferences effects were observed from either the desorption solvent. SIR was used to monitor six channels, corresponding to the C_8–C_{18} alkyl chain lengths of BAC, the six resolved peaks are clearly visible in the total ion chromatogram (TIC). The molecular mass information provided by MS is important for correctly identifying the preparation used as different BAC containing products contain different amounts of the

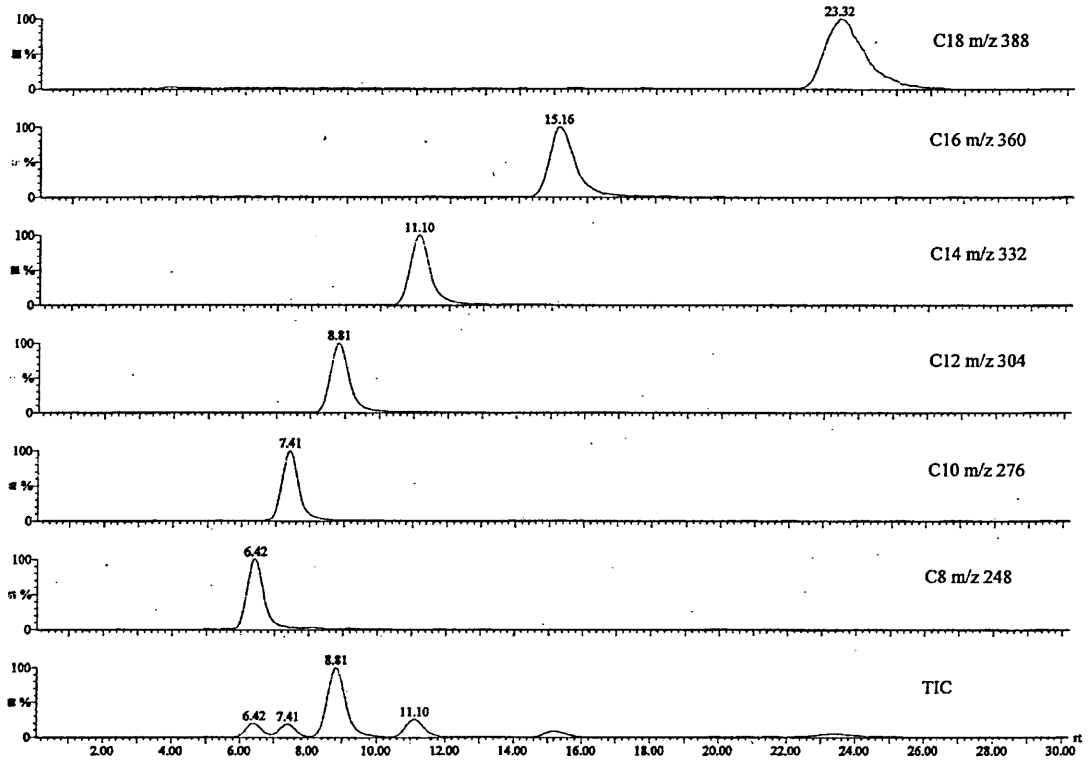

Fig. 3. A mass chromatogram showing SIR (six channels) and TIC data acquired for a diluted commercial formulation. Note: Differences in retention time between the commercial formulation and the standards caused by the use of the Jones Chromatography CPS (cyano), 100×2.1 mm column for standards and the Hypersil CN (CPS), 5 μm, 125×4.6 mm for formulations.

C_8 to C_{18} homologues. For some preparations a homologue may be absent. Identification is possible by UV–Vis but relies on retention time only and is much less sensitive than ESI/MS. Quantification of the C_{12}, C_{14} and C_{16} homologues was possible. The following concentrations were calculated for the BAC C_{12}, C_{14}, C_{16} homologues present in the formulation. BAC C_{12}: 6.41 mg ml^{-1}, BAC C_{14}: 2.25 mg ml^{-1} and BAC C_{16}: 1.66 mg ml^{-1}. The application highlights the sensitive and selective detection offered by LC–ESI-MS without time consuming sample clean up and pre-concentration methods.

Ingestion of swimming pool water treated with a BAC containing algaecide had been proposed as a cause of ill health amongst pupils at a school. Samples of the algaecide concentrate and the pool water were taken and analysed for BAC by both LC–UV–Vis and LC–ESI-MS. BAC C_{12} and C_{14} homologues were identified in the samples. Quantification of the individual peaks yielded C_{12} and C_{14} concentrations of 19.169 mg ml^{-1} and 2.163 mg ml^{-1} for the concentrated sample, and 13.036 μg ml^{-1} and 3.125ug ml^{-1} for the pool water, respectively. LC–ESI-MS provided a rapid sensitive and selective method of analysis for this sample.

3.2. Structural elucidation of quaternary ammonium compounds

Biocidal QACs are frequently reported as simply "dialkyl" on product labels. More information about the actual species present is required especially for

Table 1
Dissociation data obtained for didecyl dimethyl ammonium bromide

m/z Value	Fragment	Abundance (% full scale)	
		MS–MS	RSD
326	$CH_3(CH_2)_9N^+(CH_3)_2(CH_2)_9CH_3$	100	100
186	$CH_3(CH_2)_9N^+(CH_3)_2$	16	17
85	$C_6^+H_{13}$	2	2
71	$C_5^+H_{11}$	3	3
57	$C_4^+H_9$	19	18

forensic purposes. Initial work focused on the application of MS–MS to DDDMAB. Dissociation was noted about the quaternary nitrogen with the corresponding loss of a C_{10} alkyl chain (*m/z* 145). The alkyl chain values of *m/z* 57, 71 and 85 were also recorded, corresponding to C_4, C_5 and C_6 alkyl chain lengths, respectively. It was found that the bond dissociation produced by MS–MS could be reproduced on a single quadrupole by using an increased cone voltage. A cone voltage of 60 V was found sufficient to produce the same bond dissociation information achieved with MS–MS. Table 1 shows the data collected by cone voltage and collision induced dissociation techniques. Abundance values are quoted to the nearest integer.

3.2.1. Application of fragmentation methods to real samples

A sample of a commercially available surface biocide concentrate containing a dialkyl dimethyl QAC as the active substance was obtained. The dilute sample was initially analysed and the relative molecular mass (M_r) of the unknown QAC determined as 270. The sample was subjected to MS–MS and cone voltage bond dissociation; the data is given in Table 2. The unknown QAC cation was determined as being of the dioctyl dimethyl ammonium type.

4. Conclusions

The work shown here demonstrates a novel application of LC–ESI-MS to the determination of dialkyl and benzyl QACs. The sensitivity and selectivity advantages of MS detection are shown with respect to UV absorbing and non-UV absorbing QACs. Cone voltage and MS–MS ion dissociation methods have been applied to known and unknown dimethyl QAC showing the similarities in data obtained by the respective techniques. The unknown QAC was determined as being of the dioctyl dimethyl ammonium type. The LC–ESI-MS method developed has been successfully applied to the analysis of real samples with the requirement for minimum sample preparation demonstrated.

Table 2
Dissociation data obtained for an unknown dialkyl QAC

m/z Value	Fragment	Abundance (% full scale)	
		MS–MS	RSD
270	$CH_3(CH_2)_7N^+(CH_3)_2(CH_2)_7CH_3$	100	100
14	$CH_3(CH_2)_7N^+(CH_3)_2$	16	15
71	$C_5^+H_{11}$	2	2
57	$C_4^+H_9$	3	3

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