Harpur Hill, Buxton
Derbyshire, SK17 9JN
T: +44 (0)1298 218000
F: +44 (0)1298 218590
W: www.hsl.gov.uk

HEALTH & SAFETY LABORATORY

Development of Analytical Methods for Low Molecular Weight Isocyanates in Workplace Air

HSL/2007/47

Project Leader: **Duncan Rimmer**

Author(s): **John White, Ian Pengelly and Matthew Coldwell**

Science Group: **Health Improvement**

CONTENTS

EXECUTIVE SUMMARY

Objectives

To develop methods for the determination of low molecular weight isocyanates (NCO), especially isocyanic acid and methyl isocyanate, other mono-isocyanates and di-isocyanate monomers. These species are produced by thermal degradation of isocyanate derived polyurethanes at high temperatures. These high temperatures may be produced during machining of polyurethane painted parts e.g. in motor vehicle repair (MVR) shops or during flame bonding of polyurethane foams. The current method of analysis for airborne isocynates i.e. MDHS 25/3, was developed for the determination of the di-isocyanate monomers and their larger molecular weight oligomers and will not detect the lighter mono-isocyanates as these species will co-elute with the reagent peak.

Main Findings

Initially, two liquid chromatography (LC) methods were developed, one using an octadecylsilane (C18) column, the other a graphitised carbon (Hypercarb) column. Both methods used selected ion monitoring (SIM) mass spectrometry. Both methods had similar performance, with estimated limits of detection for the 1-(2-methoxyphenyl)piperazine (MP) derivatives of the isocyanates studied (isocyanic acid, methyl, ethyl, butyl isomers, diisopropylphenyl and hexyl) in the range of 0.1 to 1 ng NCO/ml. This corresponds to 0.013 to 0.13 μg NCO/m^3 for a 15 l (short term) sample or ~ 1/5,000th to 1/500th of the short term workplace exposure limit. The C18 method has the advantage of also being of use for the common di-isocyanate monomers with similar sensitivity.

Initial spiking experiments showed that a variety of monomeric isocyanates (ethyl, propyl, butyl isomers, diisopropylphenyl and hexyl) were effectively derivatized by a 1-(2-methoxyphenyl)piperazine (MP) coated filter (recoveries 90 – 126 %, %RSD 15 – 25 %, spiking range 70 to 200 ng NCO depending on isocyanates type.).

Propyl isocyanate MP was affected by a co-eluting interferent, probably phthalate, for both of the methods studied. This resulted in poor chromatography and a relatively high estimated limit of detection for the C18 method. Further work would be required to optimise the liquid chromatography (LC) conditions for this compound.

Spiking experiments using isocyanic acid MP and methyl isocyanate MP solutions were affected by a co-eluting interference with both of the methods described above. Various experiments were carried out to identify the source of this impurity. It was found that the impurity was an artefact of the filters used and that oven baking the filters at 400 ^{o}C prior to MP coating removed it for the solution spiking work.

Because of the failure of the two methods described above for isocyanic acid and methyl isocyanate, which are the principle low molecular weight isocyanates of interest, a third method, using the graphite column (Hypercarb 2) was developed. In this method methanol was used to improve LC selectivity. Acceptable performance was achieved with this method (recoveries 93 – 95%, repeatability 11 – 18% RSD, spiking range 25 to 106 ng NCO depending on isocyanate type).

Pyrolysis was carried out on various polyurethane materials. Analysis using the two SIM LC/MS methods gave peaks for parent di-isocyanate compound at 200 ^{o}C and for the parent di-isocyanate plus some breakdown products at 400 ^{o}C. This work shows that heating of polyurethane in the workplace can give rise to isocyanate exposure.

Both SIM methods gave early eluting peaks that could be incorrectly identified as isocyanic acid MP and so lead to a false estimation of worker exposure. This is believed to be because of a co-eluting interferent present in some polyurethanes. The Hypercarb 2 method was able to resolve this interference from the isocyanic acid MP peak and so enable accurate monitoring. Gas chromatography with mass spectrometric detection (GC/MS) was also used to assist in identification of the isocyanates species and other compounds produced on pyrolysis of various polyurethanes.

Analysis of simulated workplace samples (welding of polyurethane painted car parts) using the C18 and Hypercarb 2 methods was able to accurately determine the isocyanates produced.

The use of 1-(2-ethoxyphenyl)piperazine (EP) as a derivatizing agent was found to be a useful complementary approach to the standard MP derivatization procedure.

Recommendations

It is recommended that these methods are developed further i.e. investigation of other mass spectrometer designs e.g. ion-trap and other ionization sources e.g. atmospheric pressure chemical ionization (APCI) to see if these techniques provide any analytical benefits for these low molecular weight isocyanates.

The existing methods should be applied to workplace monitoring in areas where thermal degradation of polyurethanes is expected to occur e.g. MVR and flame lamination shops to assess the potential for worker exposure to these light isocyanates.

1 INTRODUCTION

Isocyanates (NCO) are potent respiratory sensitizers and the largest cause of occupational asthma in the UK. It has not been possible to establish a no-adverse-effect level for isocyanates. Isocyanates also cause irritation to the eyes, skin and respiratory system. The Health and Safety Executive (HSE) has published the method MDHS 25/3 which describes how airborne isocyanate exposure can be quantified (HSE, 1999).

The motor vehicle repair industry (MVR) is known to have high potential for exposure to free isocyanates, occurring during the spraying of isocyanate based 2-pack paints, finishing of the painted surfaces and car body repair work. Previous work looked at the potential of sanding (flatting) a painted surface (car door) to re-generate isocyanate from the cured paint. The conclusion of this work was that the temperatures generated during sanding were not high enough to re-generate isocyanate from the cured paint (Coldwell and White, 2003a). This finding was confirmed by further work carried out by HSL on sanding of painted car parts and curing of NCO painted car parts during the bake cycle (Coldwell and White, 2005).

A recent occupational health concern is the potential generation of " light" isocyanates (i.e. small molecular mass NCO, for example, methyl isocyanate, isocyanic acid and mono-isocyanates such as ethyl, butyl, hexyl, phenyl etc) during thermal degradation of isocyanate-derived materials. These NCO species may arise during finishing and machining processes that generate heat so leading to breakdown of the cured polyurethane and re-release of NCO e.g. grinding, welding and cutting of painted car parts in the motor vehicle repair industry and during flame bonding of polyurethane foams in the lamination and other industries. For these processes the worker is unlikely to be wearing the protective equipment required when working with uncured NCO and so could be exposed to hazardous levels of NCO. Henriks-Eckerman and others (Henriks-Eckerman et al, 2002) have shown that tasks in car body repair shops that generate high temperatures ie. grinding, welding, turning etc, can result in exposure to isocyanates, including the low molecular mass isocyanates isocyanic acid (ICA) and methyl isocyanate (MIC). High temperature degradation of polyurethanes giving low molecular mass isocyanates, has also been reported by Sennbro et al. (Sennbro et al, 2004). A more complete listing of thermal degradation related references (up to the beginning of 2003) is given elsewhere (White, 2003).

MDHS 25/3 was developed primarily for the determination of the di-isocyanate monomers and oligo-isocyanates commonly used in industry. MDHS 25/3 as routinely used is not suitable for the quantification of the low molecular mass isocyanates because of interference by the excess 1-(2-methoxyphenyl)piperazine (MP) reagent peak. The early eluting mono-isocyanate MP peaks will probably elute under the acetylated MP peak using the liquid chromatography (LC) conditions described in MDHS 25/3 and so will not be detectable by UV/vis or EC detectors..

The aim of this work was to extend the scope of MDHS 25/3 to cover the analysis of isocyanates that are likely to be formed by thermal degradation of isocyanate-based oligo-isocyanates e.g. polyurethanes. These decomposition products include the common di-isocyanate monomers and low molecular weight isocyanates. A listing of the di-isocyanates of interest that were studied here follows;

methylenebis (phenyl isocyanate), MDI, various isomers
toluene di-isocyanate, TDI, 2,4 and 2,6 isomers
1,6 – di-isocyanatohexane, HDI
isophorone di-isocyanate, IPDI, various isomers
hydrogenated MDI, HMDI, various isomers.

The mono-isocyanates (low molecular mass) of interest, studied here, are;

isocyanic acid
methyl isocyanate
ethyl isocyanate
propyl isocyanate
butyl isocyanate, n- and tert- isomers
hexyl isocyanate
phenyl isocyanate
diisopropylphenyl isocyanate.

MDHS 25/3 uses a mixture of electro-chemical (EC) and ultra-violet/visible (UV/vis) detectors to quantify and identify isocyanate-MP derivatives. The UV spectra of the aliphatic MP derivatives are very similar and hence not diagnostic and the EC detector provides no spectral data at all. It was therefore decided to develop a mass spectrometric (MS) method to take advantage of the specificity and sensitivity offered by this technique.

Previous liquid chromatography/mass spectrometry (LC/MS) work carried out at HSL and related references have been reported elsewhere (White et al, 2005; Unwin et al, 2007).

2 EXPERIMENTAL

2.1 PRELIMINARY LC/MS WORK

Initial work involved the preparation of 1-(2-methoxyphenyl)piperazine (MP) derivatives for the neat isocyanates. The neat isocyanates and MP reagent were purchased from Aldrich, UK. Ammonium formate, ammonium acetate, acetic acid, formic acid, hexanoic anhydride, trifluoroacetic acid anhydride and acetic anhydride were purchased from Aldrich, UK. LC solvents (acetonitrile and methanol) were purchased from Rathburns, UK and LC grade water was provided by a Millipore reverse osmosis system. 1-(2-ethoxyphenyl)piperazine (EP) was also prepared as described previously (Unwin et al, 2006) and was used to prepare ethyl isocyanate-EP and t-butyl isocyanate-EP derivatives for use as internal standards (ISTD). For MIC and ICA, the MP derivatives and deuterated MP derivatives (d3-MP) were purchased from Synthelec, Sweden. The d3-MP derivatives were used as internal standards.

These derivatives were then individually infused as acetonitrile solutions into the Applied Biosystems API 2000 triple quadrupole mass spectrometer (MS). Positive (+) and negative (-) ionization modes and electrospray (ES) and atmospheric pressure chemical ionization interfaces (APCI) were evaluated. Instrumental conditions such as lens voltages, nebuliser gas pressures, source temperatures and collision quadrupole settings were optimized for each isocyanate-MP derivative. The use of positive ionization electrospray was found to be the most favoured for the compounds under study, a finding in agreement with previous work (White et al, 2005).

These MS instrument conditions were then transferred to liquid chromatography-mass spectrometric (LC/MS) and tandem MS (LC/MS/MS) methods. The HSL LC/triple quadrupole MS used for these experiments consists of:

Applied Biosystems API 2000 triple quadrupole mass spectrometer (MS);
Agilent 1100 LC system - quaternary pump and autosampler.

LC optimization work looked at different column chemistries (CN, C18, C8 and graphite (Hypercarb), mobile phase compositions (acetonitrile/water/methanol with formic acid/ammonium formate and acetic acid/ammonium acetate buffers) and LC gradients.

2.2 DEVELOPMENT OF LC/MS AND LC/MS/MS METHODS

Three methods were developed, one is based on MDHS 25/3 in that it uses an octadecylsilane column (C18 4µm Genesis, 250 x 2 mm, Jones Chromatography) but uses a modified mobile phase and LC gradient. The other two methods use the more retentive Hypercarb column (graphitised carbon, 100 x 2 mm, Hypercarb, Thermo-Finnegan).

2.2.1 Modified MDHS 25/3 method

MDHS 25/3 specifies the use of a C18 column with an isocratic LC mobile phase run of ~ 50% acetonitrile/ ~50% sodium acetate buffer. An isocratic run is required because the electrochemical detector used in MDHS 25/3 cannot tolerate an LC gradient. Addition of more acetonitrile to the solvent will make the peaks elute quicker (e.g. at HSL, MP derivatized oligoisocyanates are commonly run at 60% acetonitrile/40% sodium acetate buffer).

The use of MS as the detector allows the use of an LC gradient as this detector is relatively unaffected by changing solvent compositions through the run. However, involatile buffers such as sodium acetate are not recommended for use with MS as they precipitate out and clog up the MS skimmer stack. Additionally, ion formation in the MS can be assisted by optimizing the pH

of the mobile phase. For these reasons an LC gradient based on acetonitrile (+ 0.1% formic acid) and ammonium formate (5 mM in 0.1% formic acid) was used. The method developed is a combination of a selected ion monitoring method (for the mono-isocyanate-MP derivatives) and a multiple reaction monitoring method (MRM) (for the di-isocyanate monomer MP derivatives). A compromise set of MS conditions was used in both MS experiments (selected ion monitoring (SIM) and multiple reaction monitoring (MRM) modes). The instrumental conditions are given in Appendix 1.

2.2.2 Hypercarb method 1

A screening method for the MP derivatives of the mono-isocyanates was developed using the Hypercarb column with an LC gradient based on acetonitrile (+0.1% formic acid) and ammonium formate (5 mM in 0.1% formic acid). This method is a selected ion monitoring method using a set of compromise MS conditions over three time periods. The ions monitored are the protonated molecular ions. The instrumental conditions are given in appendix 2.

2.2.3 Hypercarb method 2

A multiple reaction monitoring mode method for the MP derivatives of the three mono-isocyanates of major interest (isocyanic acid, methyl isocyanate and ethyl isocyanate) was developed. This method monitors the daughter ions of specific fragmentations of the target compounds and so should be more specific and less susceptible to interferences. The instrumental conditions are given in Appendix 3.

2.3 RECOVERY AND SPIKING EXPERIMENTS

To assess the effectiveness of the methods developed above a series of recovery (filter spiking experiments) was carried out.

2.3.1 Experiment 1 – MDHS 25/3, glass fibre filters and acetic anhydride treatment

MP doped filters (glass fibre, prepared as described in MDHS 25/3) were spiked with ~0.1 mg of a series of underivatized isocyanates. The underivatized isocyanates used were;

ethyl isocyanate
butyl isocyanate, n- and tert- isomers
hexyl isocyanate
propyl isocyanate
diisopropylphenyl isocyanate.

The same filters were also spiked with ~0.1 mg of the MP derivatives of isocyanic acid and methyl isocyanate and with the MS internal standards (d3-isocyanic acid-MP, d3-methyl isocyanate-MP and ethyl isocyanate-EP).

These filters were then acetylated with 200 ml of acetic anhydride to react any excess MP reagent, dried down under nitrogen, desorbed in 2 mls of acetonitrile, sonicated for 15 minutes, filtered through a 0.2 mm syringe filter (filter work-up procedure as described in MDHS 25/3) and analysed using Hypercarb method 1 (section 2.2.2). The results of this work are given in table 1.

2.3.2 Experiment 2 – no acetic anhydride treatment

MP doped glass fibre filters were spiked with the MP derivatives of the isocyanates listed for experiment 1, but to assess the need for the acetylation step no acetic anhydride was added in the work-up process. The filter extracts were analysed using Hypercarb method 1. Internal standards were prepared as described for experiment 1. The results of this experiment are given in table 2.

2.3.3 Experiment 3 – trifluoro-acetic anhydride treatment and quartz filters

MP doped filters were spiked with the MP derivatives of the isocyanates listed for experiment 1. To assess the effect of work-up procedure and filter type the spiked filters were treated as follows:

a) MP doped glass fibre filter (GF/A) - treated with trifluoroacetic acid anhydride (TFAA) then worked-up as MDHS 25/3 – see experiment 1 (section 2.3.1);

b) MP doped glass fibre filter (GF/A) - no anhydride added;

c) MP doped quartz filter - no anhydride added.

The filter extracts were analysed using Hypercarb method 1. Calibration curves were prepared as described for experiment 2. Internal standards were as described for experiment 1 except t-butyl isocyanate-EP was used as an internal standard instead of ethyl isocyanate-EP. The results of this experiment are given table 3.

2.3.4 Experiment 4 – glass fibre filters, recrystallised MP, acetic anhydride treatment

The work described in experiment 1 (Section 2.3.1) was repeated with freshly prepared MP impregnated filters to see if the interferences noticed in the results so far were because of impurities in the MP reagent. The MP reagent was recrystallised before being used to dope the filters (GF/A). The results of this experiment are given table 4.

2.3.5 Experiment 5 – glass fibre filters, recrystallised MP, hexanoic anhydride and propionoic anhydride treatment

MP coated filters were spiked with isocyanic acid MP, methyl isocyanate MP and ethyl isocyanate MP and then treated with hexanoic anhydride. A second set of spiked filters were treated with propionoic anhydride (CH3(CH2)CO, mass 57 Thomsons (Th) a.k.a. mass units (m.u)., Daltons (Da) and m/z value (mass to charge ratio) to assess the affect of this anhydride. Both sets of filters were desorbed as described for experiment 1. The samples were analysed using MRM and SIM MS methods (Hypercarb 1 and Hypercarb 2). The results of this work are given in table 5.

2.3.6 Experiment 6 – freshly acetone washed glass fibre filters, recrystallised MP, hexanoic anhydride treatment – repeat of expt. 5

Freshly acetone washed GF/A filters were coated with MP reagent, spiked with isocyanic acid MP, methyl isocyanate MP and ethyl isocyanate MP and then treated with hexanoic anhydride before being desorbed as described for experiment 1. Three spiking levels were used. The samples were analysed using the MRM method (Hypercarb 2). The results of this work are given in table 6.

2.3.7 Experiment 7 – screening method for mono and di-isocyanates

MP doped glass fibre filters were spiked with the MP derivatives of the isocyanates listed for experiment 1. In addition, the following di-isocyanate - MP derivatives were spiked onto the filters;

methylenebis (phenyl isocyanate), MDI
toluene di-isocyanate, TDI, 2,4 and 2,6 isomers
1,6 - diisocyanatohexane, HDI
isophorone di-isocyanate, IPDI, mix of isomers
hydrogenated MDI, HMDI, mix of isomers.

These filters were processed as described for experiment 4 (no acetic anhydride) and analysed using the modified MDHS 25/3 method (C18 column). This column was used as the Hypercarb column was found to be too retentive for the di-isocyanate MP derivatives. Calibration curves were prepared as described for experiment 2. Internal standards were as described for experiment 3. The results of this experiment are given in table 7.

2.3.8 Experiment 8 – specific method – "Hypercarb 2" – Oven Baked Filters

The experiments described so far had not given acceptable results for isocyanic acid and methyl isocyanate MP derivatives (section 2.3.1 – 2.3.7). Unfortunately these are two of the main "light NCO" components of interest for thermal degradation studies (White, 2003; Unwin et al, 2005; Pengelly, 2002; Westberg et al, 2005; Boutin et al, 2006). To try to improve this situation an alternative method "Hypercarb 2" was developed specifically for these compounds. This method uses the more retentive Hypercarb (graphite) column and the weaker organic solvent methanol (as opposed to acetonitrile) in an attempt to give better separation.

The results obtained for experiments 4,5 and 6 and the scan runs suggested that an interferent on the acetone washed GF/A filters used for MP spiking was co-eluting with the isocyanic acid MP and methyl isocyanate MP peaks. To try to remove this interferent the glass fibre filters were baked in an oven at 400°C for ~2 hours and then, after cooling, doped with recrystallised MP.

The MP doped glass fibre filters were then spiked with the MP derivatives of isocyanic acid, methyl isocyanate and ethyl isocyanate and the internal standards d3-isocyanic acid-MP and d3-methyl isocyanate-MP. These filters were processed as described for experiment 7 (hexanoic anhydride treated) and analysed using Hypercarb method 2. Calibration curves were prepared as described for experiment 2. The results of this experiment are given in table 8.

2.4 SCAN EXPERIMENTS

To assist in the interpretation of the filter spiking results (sections 2.3.1 to 2.3.8) and the identification of any interfering peaks a selection of spiked filters (glass fibre and quartz, acetic anhydride treated, trifluoroacetic acid anhydride treated, hexanoic anhydride treated, propionoic anhydride treated, acetone washed and oven baked, etc.) were analysed using the LC/MS in scan mode. Blank MP impregnated filters and oven-baked blank MP impregnated filters were also analysed to assist in identification of the interference seen for the spiking experiments. The LC/MS was operated in positive ion electrospray mode, with a scan range of 50 to 700 Th.

2.5 THERMAL DEGRADATION OF POLYURETHANES AND OTHER MATERIALS

As discussed in the introduction, a cause of concern is the extent to which cured isocyanate based products e.g. polyurethane paints, foams etc., can re-release isocyanate on thermal degradation. This could lead to the exposure to isocyanate of workers who are unaware of this possibility and so are inadequately protected i.e. no local ventilation fitted or protective equipment worn. Previous work at HSL and elsewhere (White, 2003b; White et al, 2005b; Pengelly, 2002; Westberg et al, 2005; Boutin et al, 2006) on the pyrolysis of isocyanate foams and other isocyanate-based materials has shown the potential for re-release to occur if the temperature used is high enough.

As part of this study, a variety of isocyanate-based materials were heated at 200°C on a hotplate for 30 minutes and 400°C for 30 minutes. Any isocyanate containing fume or vapour emitted from these materials during heating was sampled via a glass funnel onto MP doped glass fibre filters at a flow rate of 2 l/min. These filters were then analysed by the methods developed above.

To simulate "real" samples, MP filter samples were taken during the automated spot welding of a polyurethane painted, metal, car part (samples taken by Ian Pengelly, HSL at the Welding Institute, UK). The samples were analysed using the three methods developed in sections 2.3.1 to 2.3.8 and the results of these experiments are given in tables 9 to 12.

2.6 INVESTIGATION OF AN INTERFERENCE SEEN DURING THE PYROLYSIS OF A POLYURETHANE FOAM

The work reported in sections 2.3.1 to 2.3.8 showed that the isocyanic acid-MP peak is susceptible to interferences from a variety of causes. GC/MS, pyrolysis GC/MS and scan LC/MS runs were carried out on the interference containing extracts to try to identify and so nullify these interferences. The results of this work are reported in section 3.4.

2.7 THE USE OF 1-(2-ETHOXYPHENYL)PIPERAZINE AS A DERIVATIZING AGENT FOR ISOCYANATES

1-(2-ethoxyphenyl)piperazine (EP) has recently been used as a derivatizing agent for NCO to produce internal standards (Unwin et al, 2006; White, 2006) for chromatography. GF/A filters were impregnated with EP to assess its use as a derivatizing agent for workplace NCO. NCO based samples were pyrolysed and sampled onto EP coated filters – see section 2.5 for experimental details of the pyrolysis. The results of this work are given in section 3.5.

3 RESULTS AND DISCUSSIONS

3.1 DEVELOPMENT OF LC/MS AND LC/MS/MS METHODS

3.1.1 Experiment 1 – MDHS 25/3, glass fibre filters and acetic anhydride treatment – Hypercarb 1 method

Table 1. Experiment 1 – MDHS 25/3, glass fibre filters and acetic anhydride treatment

Isocyanate spiked	Spike (mg NCO)	Recovery (%)	%RSD (10 filters)	n	% RSD (6 repeat injections)
isocyanic acid	0.197	---	---	10	---
methyl NCO	0.209	---	---	10	---
ethyl NCO	0.133	90	15	10	9
propyl NCO	0.111	8	28	5	13
diisopropylphenyl (DIPP)NCO	0.149	92	16	10	14
n–butylNCO	0.194	107	19	10	23
t–butylNCO	0.092	116	25	10	10
hexyl NCO	0.073	126	18	10	6

Notes

--- = No meaningful result obtained – see discussion

Ten filters were spiked for the determination of recovery and repeatability.

The repeat injection check is the %RSD found for six repeat injections of a single sample.

0.1 mg NCO/ml for a 15 l air sample corresponds to ~13 mg NCO/m^3 (2 ml desorption volume). The short term limit value (STEL) is 70 mg NCO/m^3.

These results are acceptable for the majority of compounds spiked. The fact that the recoveries for most NCO species are ~100% shows that the MP coated filter and "field desorption" in MP solution is effective. Methyl NCO and ICA were spiked onto the filters as the MP derivatives. For methyl NCO MP (MIC) and isocyanic acid MP (ICA) acceptable calibration curves could not be obtained probably because of interference by a co-eluting compound causing ion suppression and mis-identification. For propyl NCO MP the peak co-eluted with a large broad peak, probably caused by phthalates that eluted with the LC gradient leading to signal suppression and poor integration.

Some of these samples were run in scan mode to try to identify the interferences (see section 2.4). A large peak with a major ion of 236 Th was seen at a retention time of ~6.3 minutes, this is the acetylated MP reagent.

The results of experiment 1 show that the work-up procedure is affecting the final chromatogram. It was thought that part of the problems seen for the early eluting isocyanic acid MP and methyl isocyanate MP peaks could be because of interference from the early eluting (acetylated) excess MP reagent peak. The acetylated MP peak has a m/z+ of 236 (acetyl group - CH3CO, mass 43 Th)(Th = Thomsons, equivalent to m/z+, m.u. (mass units) and Da, Daltons). This is isobaric (i.e. of equal mass) with the protonated isocyanic acid MP ([HNCO-MP+H]+, 236 Th) and so could lead to an isobaric interference in the chromatogram.

It was therefore decided to carry out an experiment without acetic acid acetylation of the filter to see if the acetylation step could be dispensed with.

3.1.2 Experiment 2 – Hypercarb 1 method, no acetic anhydride treatment

Table 2. Experiment 2 – no acetic anhydride treatment

Isocyanate MP derivative spiked	Low level spike			High level spike		
	Spike µg NCO	% Recovery	% RSD	Spike µg NCO	% Recovery	%RSD
isocyanic acid	0.004	---	---	0.040	---	---
methyl NCO	0.006	85	46	0.066	98	8
ethyl NCO	0.003	91	9	0.048	106	7
propyl NCO	0.004	---	---	0.038	---	---
DIPP NCO	0.01	90	6	0.116	107	2
n-butyl NCO	0.012	95	7	0.127	105	4
t-butyl NCO	0.01	90	5	0.124	107	2
hexyl NCO	0.005	98	8	0.056	108	2

Notes

--- = No meaningful result obtained – see discussion

Ten filters were spiked for the determination of recovery and repeatability (n = 10).

The majority of the results are acceptable at both spiking levels showing that the NCO-MP derivatives are being efficiently desorbed from the filters. Again the isocyanic acid MP calibration was meaningless probably because of a co-eluting interference. However as these samples were not acetylated with acetic anhydride this interference is not caused solely by the acetylated MP peak as suggested in section 3.1.1. The peak for propyl NCO MP at 278 Th was interfered with by a co-eluting phthalate peak. Analysis of the filters, using the Hypercarb

column without anhydride treatment, therefore appears to be acceptable for ethyl NCO, DIPP NCO, the butyl NCO isomers and hexyl NCO.

Analysis of some of the samples in full scan mode showed that the unacetylated MP was eluting as a very broad peak over the range 3 to 9 minutes. In workplace samples, this peak could lead to interference and ion suppression of the analyte peaks. This suggests that some treatment of the filters is necessary to sharpen up the excess MP reagent peak so other anhydrides were considered i.e. trifluoro-acetic anhydride.

3.1.3 Experiment 3 – Hypercarb 1 method, trifluoro-acetic anhydride treatment and quartz filters

Table 3. Experiment 3 – Tri-fluoro acetic acid treatment, No treatment and Quartz filters

isocyanate MP derivative spiked	Quartz filter no anhydride			GF/A no anhydride			GF/A trifluoroacetic anhydride treated		
	Spike µg NCO	% Recovery	% RSD	Spike µg NCO	% Recovery	% RSD	Spike µg NCO	% Recovery	% RSD
isocyanic acid	0.063	176	35	0.063	79	51	0.063	---	---
methyl NCO	0.095	63	1	0.095	72	5	0.095	---	---
ethyl NCO	0.065	62	6	0.065	83	2	0.065	---	---
propyl NCO	0.115	52	15	0.115	75	7	0.115	---	---
DIPP NCO	0.153	77	4	0.153	75	3	0.153	---	---
n–butyl NCO	0.17	83	5	0.17	86	5	0.17	---	---
t–butyl NCO	0.163	189	21	0.163	82	3	0.163	---	---
hexyl NCO	0.073	66	5	0.073	74	6	0.073	---	---

Notes

--- = No meaningful result obtained – see discussion

Seven filters were spiked for each filter/treatment type (n = 7).

The trifluoroacetic acid anhydride treated filters gave no meaningful results. Full scan MS runs on TFAA treated filters, spiked with high levels of NCO-MP derivatives, showed that the TFAA-MP peak had been moved over to ~21 minutes but that the ion counts for the NCO-MP derivatives were much lower than for untreated filters, probably because the TFAA is acting to suppress ion formation and so lowering sensitivity. However scan mode MS of the filters showed that the TFAA treatment was successful in removing the excess MP reagent peak to a region of the chromatogram where it did not interfere with the early eluting peaks.

When no anhydride was added the quartz filter results were inferior to the GF/A results. The GF/A results were acceptable except for isocyanic acid that again showed interference from other early eluting compounds leading to a poor %RSD. The t-butyl NCO-EP was successful as an internal standard, eluting at ~18.5 minutes and having a mass of 306 Th.

These results suggest that the GF/A filter should be used as the filter of choice. The untreated filters gave acceptable results but with slightly lower than ideal recoveries. It was decided that treatment using a different anhydride was worth investigating and that recrystallization of the MP reagent used to coat the filters might lead to less interferences and so better results.

3.1.4 Experiment 4 – Hypercarb 1 method, glass fibre filters, recrystallised MP, acetic anhydride treatment

Table 4. Experiment 4 – glass fibre filters, recrystallised MP, acetic anhydride treatment, Hypercarb 1 method

Isocyanate MP derivative spiked	Spike μg NCO	% Recovery	% RSD	Estimated Limit of detection S/N=3 (solution) ng NCO/ml
isocyanic acid	0.030	65	28	0.1
methyl NCO	0.090	63	10	0.9
ethyl NCO	0.062	100	4	0.4
propyl NCO	0.110	230	64	0.1
DIPP NCO	0.145	105	9	0.2
n–butyl NCO	0.165	110	7	0.2
t–butyl NCO	0.155	87	17	0.1
hexyl NCO	0.069	101	3	0.6

Notes

Ten filters were spiked for the determination of recovery and repeatability (n = 10).

These results are acceptable except for the isocyanic acid, methyl NCO and propyl MP derivative results. The early eluting isocyanic acid and methyl NCO MP derivatives give lower than ideal recoveries and the isocyanic acid results are imprecise (high %RSD). However these results are an improvement on the previous work (sections 3.1.1 to 3.1.3).

As seen for experiment 1, propyl NCO-MP is affected by a phthalate peak eluting with the solvent gradient and requires a different solvent gradient programme to determine effectively. The wide variation in recovery found for experiments 1 and 4 show that these propyl NCO-MP results are meaningless.

For isocyanic acid and methyl isocyanate MP derivatives these results are an improvement on those obtained in experiment 1 suggesting that recrystallising the MP reagent has minimised the co-eluting impurities. A detection limit of 0.2 ng NCO/ml corresponds to ~ 0.027 mg NCO/m^3 for a 15 litre sample, the STEL is 70 mg NCO/m^3 for a 15 litre sample. As isocyanic acid has been suggested as a major product of polyurethane thermal decomposition attention was paid to improving the method for this compound. An example chromatogram for a spiked filter is shown in Figure 1.

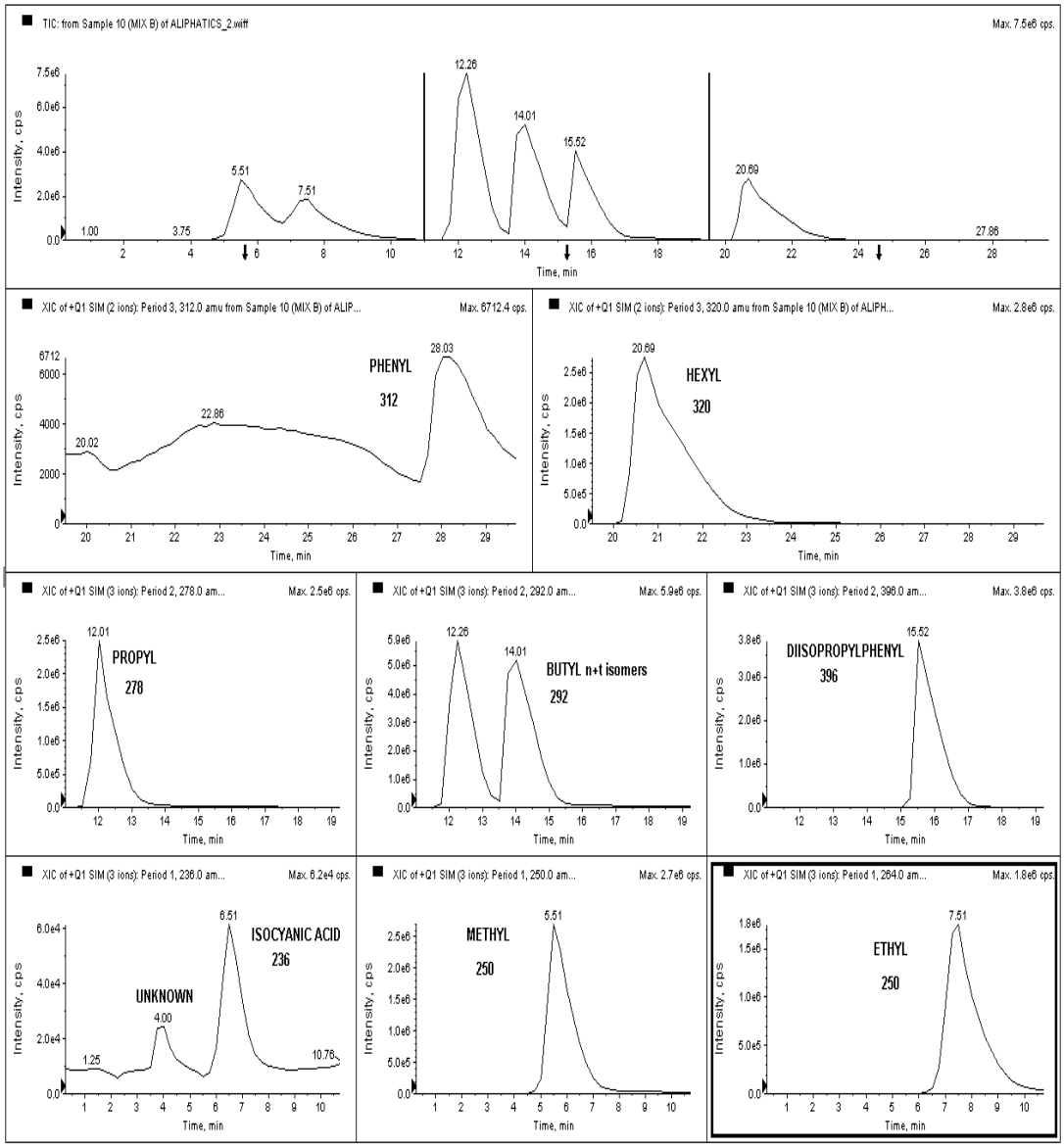

Figure 1. Example Total Ion (top) and Selected Ion (lower 3) chromatograms for Hypercarb 1 method (SIM run of spiked filter).

3.1.5 Experiment 5 – Hypercarb 1 and Hypercarb 2 methods, glass fibre filters, recrystallised MP, hexanoic anhydride and propionic anhydride treatment

Table 5. Experiment 5 – glass fibre filters, recrystallised MP reagent, Hypercarb 1 method

Treatment	Result ng NCO/ml	MRM (Hypercarb 2)		
		ICA	MIC	ETHYL
Hexanoic anhydride	mean (s.d.)	73(33)	30(7)	34(5)
	%RSD	45	22	15
	spike (ng)	76	43	36
	% Rec	96	70	94
Propionic anhydride	mean (s.d.)	89(49)	23(17)	42(14)
	%RSD	55	75	33
	spike (ng)	76	43	36
	% Rec	117	53	117
Treatment	Result ng NCO/ml	SIM (Hypercarb 1)		
		ICA	MIC	ETHYL
Hexanoic anhydride	mean (s.d.)	89(37)	58(12)	36(4)
	%RSD	41	20	10
	spike (ng)	76	43	36
	% Rec	117	135	100
Propionic anhydride	mean (s.d.)	41(40)	22(14)	130(53)
	%RSD	98	64	41
	spike (ng)	76	43	36
	% Rec	54	51	361

Notes

Seven filters were spiked for the determination of recovery and repeatability (n = 7).

To alter the retention time of the excess reagent peak and change its mass, thereby eliminating any interference, the filters were treated with hexanoic or propionic anhydride instead of acetic anhydride. This will add either the hexanoyl group (CH3(CH2)4CO-), mass 99 Th) or the propionyl group (C2H5CO-, mass 57 Th) instead of the acetyl. This will give the hexanoyl-MP excess reagent peak with an m/z+ of 292 Th or the propionyl-MP excess reagent peak with an m/z+ of 250 Th. The propionyl-MP and hexanoyl-MP will also be more strongly retained on the Hypercarb LC column because of the longer alkyl chains on the hexanoyl and propionyl groups and so elute later than the acetyl-MP.

The ICA-MP results, for the hexanoic anhydride treatment, have acceptable recoveries but again poor repeatability. The results for the two methods (MRM and SIM) are in reasonable agreement. The propionic anhydride treatment has results with poor repeatability and poor agreement between the two methods.

The MIC-MP results have better repeatability but more variable recoveries between the two methods for the hexanoic anhydride treatment than those for the propionic anhydride treatment. The propionic anhydride recoveries for both methods were ~50% with high %RSD.

The ethyl NCO-MP results have acceptable recoveries with slightly high %RSD for the hexanoic anhydride treatment using the two methods. The propionic anhydride treatment results for ethyl NCO MP are unacceptable (variable recoveries and poor repeatability). High

recoveries (>100%) usually are because of a co-eluting peak being falsely identified as an NCO-MP derivative. Low recoveries may be because a co-eluting peak is suppressing formation of the analyte ion so leading to under reporting. The SIM method should be more sensitive but more susceptible to interferences than the MRM method.

These results showed that the hexanoic anhydride treatment may be of some benefit for the isocyanic acid-MP and methyl NCO-MP results. It was decided to repeat the hexanoic anhydride treatment with freshly prepared MP impregnated filters (see Section 3.1.6).

3.1.6 Experiment 6 – Hypercarb 2 method, acetone washed glass fibre filters, recrystallised MP, hexanoic anhydride treatment

Table 6. Experiment 6 – freshly acetone washed glass fibre filters, recrystallised MP, hexanoic anhydride treatment – repeat of expt. 5

Treatment	Result ng NCO/ml	MRM (Hypercarb 2)		
		ICA	MIC	ETHYL
Hexanoic anhydride	HIGH			
	mean (s.d.)	> 5000	353(12)	362(14)
	%RSD	not calculated	5	4
	spike (ng)	637	353	402
	% Recovery	---	100	91
Hexanoic anhydride	MEDIUM			
	mean (s.d.)	1118(407)	202(9)	259(25)
	%RSD	36	5	10
	spike (ng)	412	193	232
	% Recovery	271	105	112
Hexanoic anhydride	LOW			
	mean (s.d.)	>1000	24(2)	29(2)
	%RSD	not calculated	10	8
	spike (ng)	134	25	30
	% Recovery	---	96	97

Notes

--- = No meaningful result obtained – see discussion

Ten filters were spiked for each level for the recovery and repeatability experiments (n = 10).

The treatment of the filters with an alternative anhydride to acetyl appeared to have some benefits so the hexanoic anhydride treatment experiment was repeated with a freshly prepared batch of filters. The methyl NCO-MP and ethyl NCO-MP results are acceptable for all spiking levels. The ICA-MP results were badly affected by a co-eluting interferent. The isocyanic acid MP results again have unacceptable %RSD and % recovery.

The work reported in sections 3.1.1 to 3.1.6 allows the following conclusions to be made.

An acceptable screening method for the majority of the mono isocyanates (MIC, ethyl, propyl, DIPP, butyls and hexyl) is feasible. The isocyanic acid MP results are affected by co-eluting impurities. Alternative anhydride treatment of the filters, recrystallization of the MP reagent prior to coating the GF/A filters and acetone washing of the blank filters prior to coating with MP decreased but did not totally remove these impurities. Further work on ICA-MP is reported in section 3.1.8. A partial validation and determination of estimated limits of detection for the other isocyanates is reported in section 3.1.7.

3.1.7 Experiment 7 – MDHS 25/3, C18 screening method for mono and di-isocyanates

Table 7. Experiment 7 – Screening method for mono- and di-isocyanates

Isocyanate MP derivative spiked	Spike μg NCO	% Recovery	% RSD	Est. LOD (solution) S/N=3 ng NCO/ml
isocyanic acid	0.030	78	54	2
methyl NCO	0.090	84	21	0.04
ethyl NCO	0.062	89	14	2
propyl NCO	0.110	104	13	46
DIPP NCO	0.145	119	11	2
n- and t–butyl NCO	0.32	92	11	2
hexyl NCO	0.069	101	3	0.4
phenyl NCO	0.064	102	5	1
HDI	0.033	114	12	1
MDI	0.088	114	7	2
T-2,4-DI	0.031	96	14	2
T-2,6-DI	0.031	92	21	2
IPDI mix of isomers	0.231	119	10	1
HMDI mix of isomers	0.278	116	7	13

Notes

Ten filters were spiked for the determination of recovery and repeatability (n = 10).

A screening method based on MDHS 25/3 and the work reported in sections 3.1.1 to 3.1.4 was developed (C18 column/SIM detection - see appendix 1). For most of the commonly occurring mono- and di-isocyanates measured using this screening method acceptable recoveries and repeatabilities were obtained. The relatively high LOD estimated for propyl NCO-MP is because this peak elutes on the phthalate "hump" mentioned earlier. As seen for the other method, isocyanic acid MP derivative gives low recoveries (< 80%) and poor %RSDs (> 50%). A detection limit of ~1ng NCO/ml corresponds to ~0.13 mg NCO/m^3 for a 15 litre sample (i.e. ~ 1/500th of the STEL). For the mono-NCO the LOD found for the “C18” method (table 7) were slightly higher than those found using the method “Hypercarb 1” (table 4) but the “C18” has the advantage of being able to monitor the common di-isocyanates in the same run. An example chromatogram is given in Figure 2.

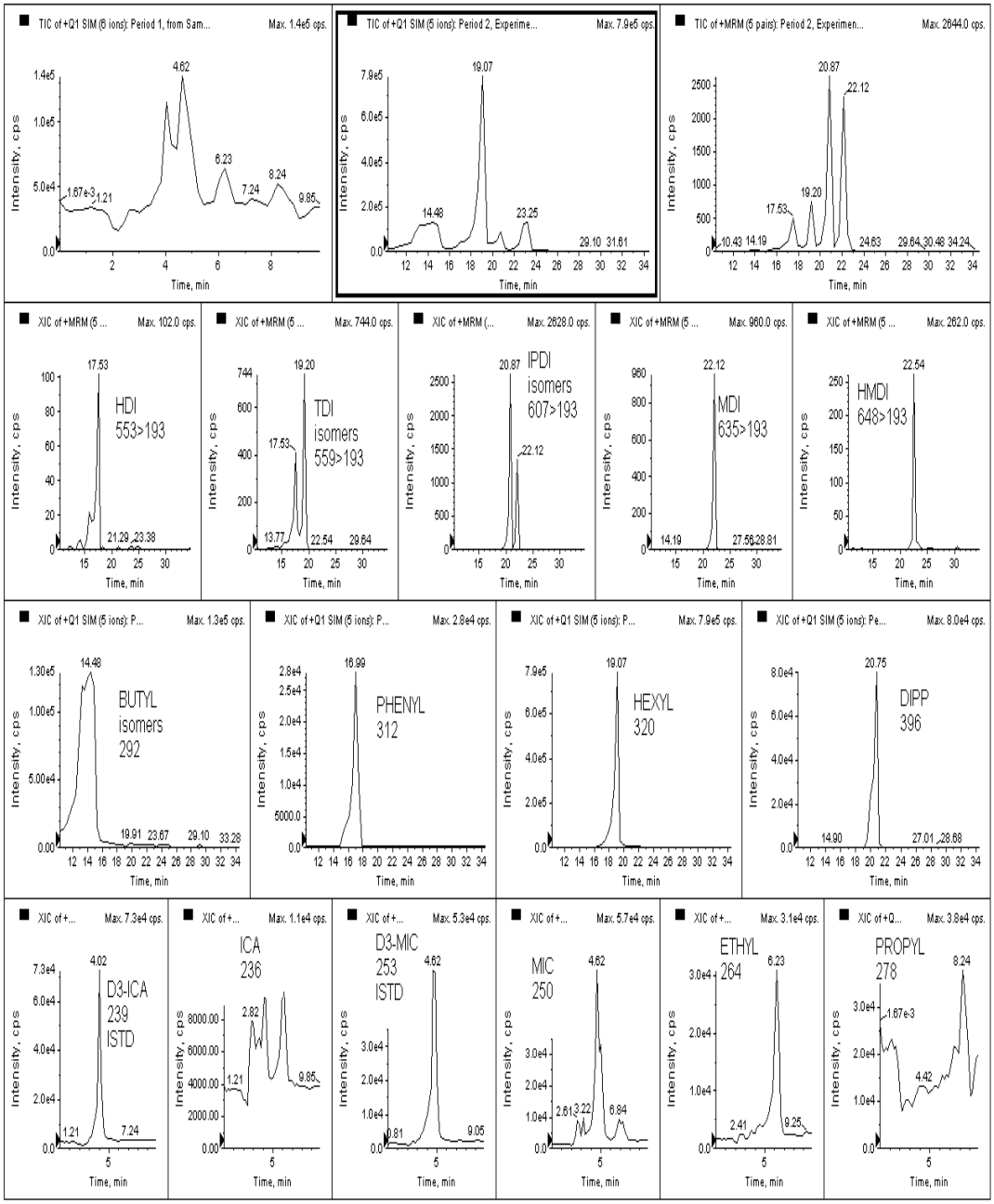

Figure 2. Example chromatograms for C18 screening method (spiked filter)

Total ion traces (top) Multiple Reaction Monitoring (MRM) traces (second from top) and Selected Ion Monitoring (SIM) traces (lowest and second lowest) .

3.1.8 Experiment 8 – Hypercarb 2 method, oven baked (400°C) filters

Table 8. Experiment 8 – specific method – "Hypercarb 2" – Oven Baked Filters

Treatment	Result ng NCO/ml	MRM		
		ICA	MIC	ETHYL
Hexanoic anhydride	HIGH LEVEL SPIKE			
	mean (s.d.)	580(80)	240(20)	282(25)
	%RSD	15	8	9
	spike (ng)	537	253	302
	% Recovery	108	95	94
Hexanoic anhydride	LOW LEVEL SPIKE			
	mean (s.d.)	98(18)	24(3)	29(4)
	%RSD	18	11	15
	spike (ng)	106	25	30
	% Recovery	93	93	95
Est. LOD (ng NCO/ml)		3	4	3

Notes

Ten filters were spiked for the determination of recovery and repeatability.

As reported in section 3.1.6 the results for ethyl NCO-MP and methyl NCO-MP are acceptable.

The results for isocyanic acid MP are now also acceptable and a major improvement on the previous runs (sections 3.1.1 to 3.1.6). This suggests that the major source of interferent for this analyte was caused by a semi-volatile material present in the GF/A filters and that this interferent can be removed by oven baking the filters prior to spiking but not by acetone washing. Scan runs on these samples gave clean blank filter extracts (acetonitrile and methanol). A small peak eluting near the ICA-MP peak was seen in some of the samples. This is investigated further in section 3.4. The instrumental conditions of the method "Hypercarb 2" are give in appendix 3. Optimisation of the MRM conditions for isocyanic acid MP and methyl NCO MP may improve (lower) the estimated limits of detection. An example chromatogram of a spiked filter obtained using this method is given in Figure 3.

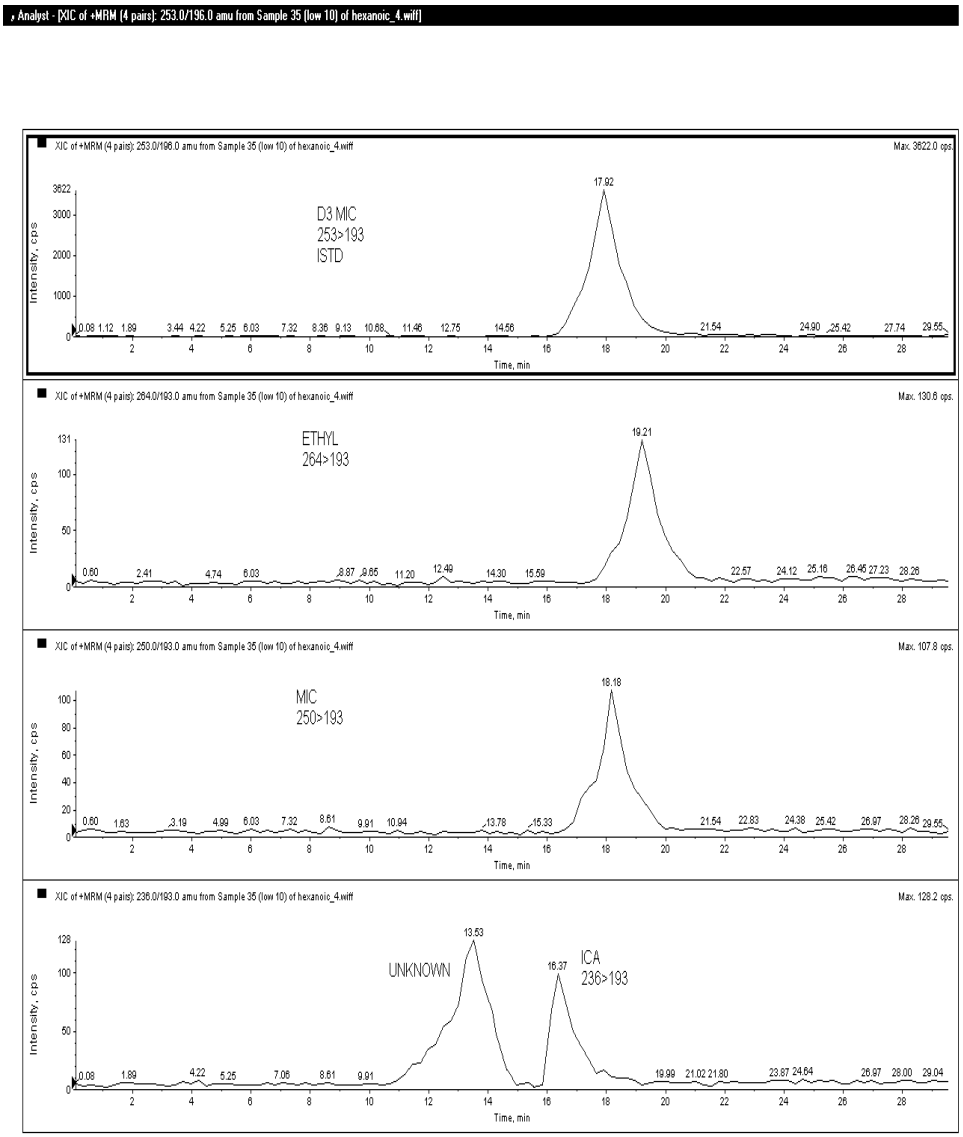

Figure 3. Example MRM chromatograms for Hypercarb 2 method (spiked filter).

3.2 SCAN EXPERIMENTS

Scan experiments on selected filters were carried out after each experiment to clarify the results and inform decisions on further work required. The results of these runs are discussed in the sections describing relevant experiments.

3.3 THERMAL DEGRADATION OF POLYURETHANES

Table 9. Hypercarb method 1, heated at 400°C for 30 minutes

Isocyanate MP derivative detected (ng NCO)	NCO containing material			
	spray foam (insulation)	flexible foam (cushion)	rigid foam (packaging)	paint (top-coat/lacquer)
ICA	*8,981*	*18,519*	*27,199*	*1,060*
MIC	ND	ND	ND	ND
ethyl	ND	ND	ND	ND
butyl NCO (n- & t- isomers)	108	ND	ND	71
hexyl NCO	ND	ND	ND	60

Numbers in italics – see section 3.4 and discussion

Table 10. C18 method (MDHS 25/3), heated at 200°C for 30 minutes

Isocyanate MP derivative detected (ng NCO)	NCO containing material			
	spray foam (insulation)	flexible foam (cushion)	rigid foam (packaging)	paint (top-coat/lacquer)
ICA	ND	ND	ND	ND
MDI	158	ND	ND	ND
HDI	ND	ND	ND	201
TDI (2,4- & 2,6- isomers)	ND	149	ND	ND

Table 11. C18 method (MDHS 25/3), heated at 400°C for 30 minutes

Isocyanate MP derivative detected (ng NCO)	NCO containing material			
	spray foam (insulation)	flexible foam (cushion)	rigid foam (packaging)	paint (top coat/lacquer)
ICA	*8,800*	*13,513*	*21,622*	*1,027*
butyl NCO (n- & t- isomers)	19	ND	ND	ND
MDI	1,117	ND	ND	ND
TDI (2,4- & 2,6- isomers)	40	2,115	ND	ND
HDI	ND	ND	ND	2,170
IPDI (2 isomers)	ND	ND	ND	180
HMDI	ND	ND	ND	10
phenyl	19	ND	ND	ND
hexyl	ND	ND	ND	115

Numbers in italics – see section 3.4 and discussion.

The Hypercarb 1 and C18 (MDHS 25/3) methods gave similar isocyanic acid MP results for the 400°C tests (Tables 9 and 11). Both give values, which if genuine, indicate high values of ICA produced for some of the materials tested (i.e. a value of ~10,000 ng NCO over the sampling

period taken corresponds to ~167 mg NCO/m3) - however see section 3.4 for a further discussion of these results. This finding is interesting as other workers have reported large but extremely variable amounts of isocyanic acid found during cutting and grinding of polyurethane painted car parts (Boutin et al, 2006).

Although it should be recognised that these are "worst case" values using a very small sampling chamber, these results (if accurate – see section 3.4) suggest that ICA could be a problem in the workplace. However, the isocyanic acid MP peaks seen in these samples all have a tailing shape, this could be because the LC column is overloaded or it could be that they are caused by a co-eluting interferent i.e. not by ICA-MP. Further work to verify these results is described in section 3.4.

The Hypercarb 1 method does not give peaks for the di-isocyanate MP derivatives as these are strongly retained on the porous graphite stationary phase (LC column). Small amounts of butyl and hexyl NCO are reported in table 9 suggesting some breakdown is taking place or these very small peaks may be artefacts.

Comparing tables 10 and 11, C18 column, pyrolysis at 200°C and 400°C shows that the lower temperature is giving ~ 10x lower values of di-isocyanate and no ICA was detected. These results are discussed further in section 3.4. The results in table 11 show some small peaks for breakdown products (butyl, hexyl and phenyl NCO) and quite large peaks (~1,000 of ng NCO) for the di-isocyanates on which these samples are presumably based i.e. spray foam – MDI, cushion foam – TDI and topcoat/lacquer – HDI. Pyrolysis GC/MS confirmed these attributions and breakdown products.

The results for the topcoat/lacquer are particularly interesting. Small peaks corresponding to the other aliphatic NCO were identified (IPDI and HMDI). These are not listed as present in the formulation and so may be impurities in the bulk material, from an external source or artefacts i.e. falsely identified peaks.

From other work carried out at HSL it is known that this formulation is oligo-HDI (mainly biuret and isocyanurate) with a very small monomeric HDI content (< 0.1%). However scan and precursor ion scan runs on this sample found no oligomeric HDI but did find monomeric HDI, a finding that is in agreement with the results in tables 9 to 11. This suggests a scheme in which these oligo-HDI thermally breakdown, initially to the monomeric HDI and then if heated further to smaller mono-NCO and ICA. This could provide a means of differentiating between NCO exposure due to thermal breakdown of cured NCO (“indirect”) and "direct" exposure i.e. if the airborne samples contain oligo-HDI with very little mono-HDI this NCO exposure is "direct" e.g. paint spraying, whereas if the airborne samples contain little oligo-HDI with mainly mono-HDI (and possibly lighter mono-NCO) this NCO exposure is "indirect" e.g. thermal degradation of polyurethanes caused by heating during welding and grinding.

A further possibility occurs; if these differences in NCO species present in the workplace atmosphere are carried through into the body, i.e. by conjugation with serum proteins etc., it may be possible to use these different NCO-protein conjugates, i.e. mono-HDI-protein, biuret-HDI-protein, isocyanurate-HDI-protein etc. to differentiate between exposure sources by biological monitoring. The current method used at HSL hydrolyses the NCO-protein conjugates to the simple amine, i.e. all HDI species to HDA, and so any exposure source information is lost.

Table 12. Spot welding of polyurethane coated car part - pooled data from Hypercarb 2 and C18 methods

Isocyanate MP derivative detected	[NCO] ng/sample
C18 results (MDHS 25/3)	
IPDI (2 isomers)	19,740
TDI (2 isomers)	DETECTED - NOT QUANTIFIED
HDI	NOT DETECTED
Hypercarb 2 results	
ethyl NCO	30
MIC	DETECTED - NOT QUANTIFIED
ICA	NOT DETECTED

Table 12 gives the results of some simulated spot welding experiments analysed using the methods, "Hypercarb 2" (for ICA, MIC and ethyl NCO) and C18 (modified MDHS 25/3, for a range of mono- and di-NCO).

The C18 method gave 2 large peaks for the IPDI isomers and small amounts of TDI were detected.

The "Hypercarb 2" method gave small peaks for ethyl and MIC but no peak for ICA-MP. Eluting close to the ICA-MP peak is a large peak. ICA-MP was spiked into a portion of the extract to verify that the large peak was indeed an interferent. For the undiluted extract, this peak dominated the chromatogram and the peak due to the spiked ICA-MP appeared as a shoulder. Diluting the extract 100x gave baseline resolution between the ICA-MP and the interferent and so the ICA-MP peak could be quantified. The unspiked sample extract was diluted 100x and this solution verified that there was no ICA-MP in the extract. This interferent is investigated further in section 3.4. A comparison of the chromatograms obtained for an air sample taken during spot welding of a polyurethane painted car part using the Hypercarb 2 and C18 methods is given in Figure 4.

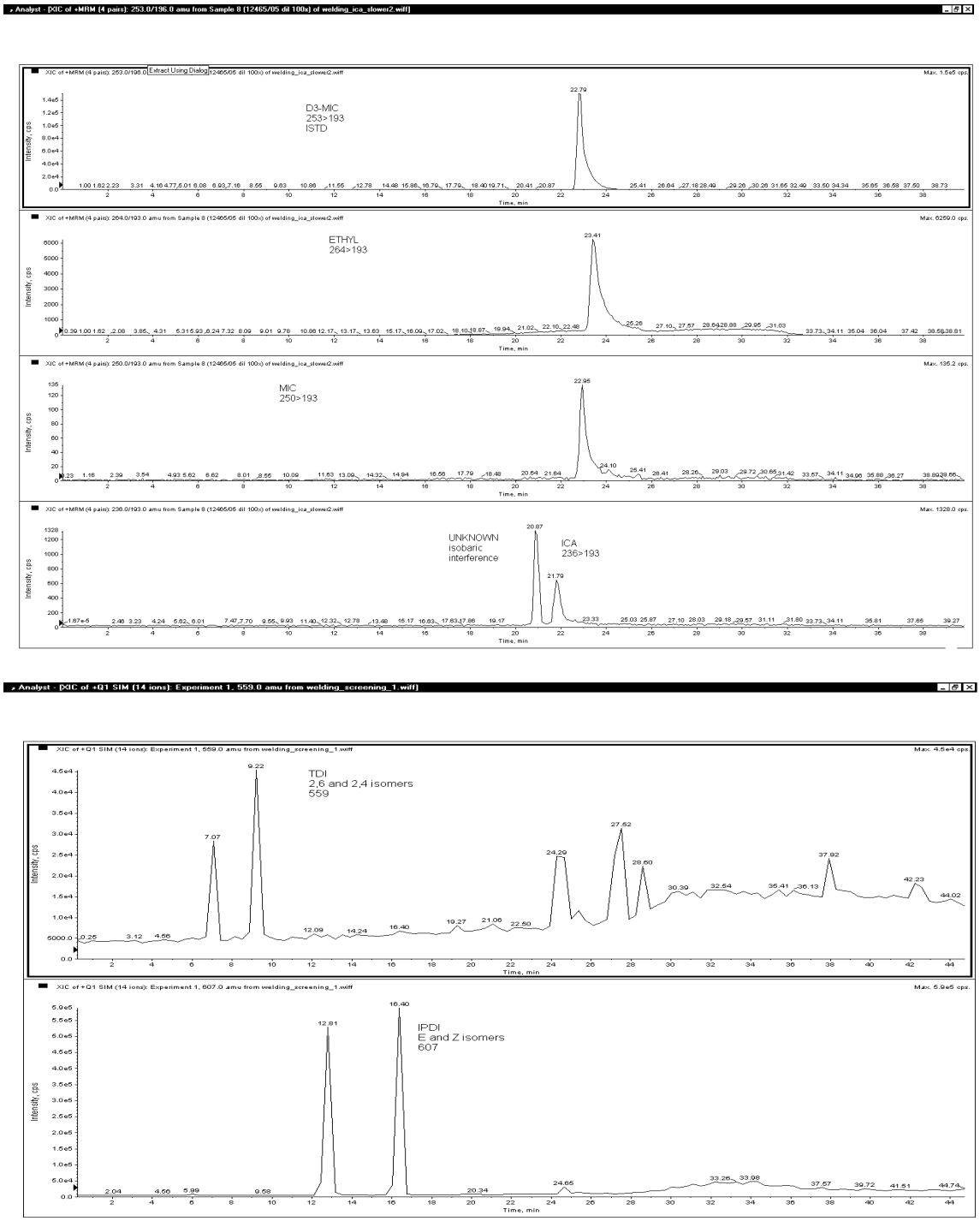

Figure 4. Comparison of chromatograms obtained using Hypercarb 2 (upper) and C18 (lower) methods for an air sample taken during spot welding of polyurethane painted car part.

3.4 INVESTIGATION OF AN INTERFERANCE SEEN DURING THE PYROLYSIS OF A POLYURETHANE FOAM

GC/MS, pyrolysis GC/MS and scan mode LC/MS experiments were carried out on some of the extracts that may contain an interferent from the thermal degradation experiments (section 3.3) and also on freshly pyrolysed “neat" materials. The "Hypercarb 2" method was also used to specifically look for ICA-MP in the extracts. A comparison of the results obtained with this method and with the “Hypercarb 1” and “C18” methods is given in table 13.

Table 13. Comparison of ICA-MP results Obtained Using the Three Methods under Evaluation for Several Pyrolysed Polyurethane Materials

[ICA-MP] detected (ng NCO)	NCO containing material			
	spray foam (insulation)	flexible foam (cushion)	rigid foam (packaging)	Paint (top coat/lacquer)
Hypercarb 2	352	ND	N/A	1,075
Hypercarb 1 (table 9)	*8,981*	*18,519*	*27,199*	1,060
C18 (table 11)	*8,800*	*13,513*	*21,622*	1,027

Numbers in italics – results found to be in error because of co-eluting interferences

For the flexible (cushion) foam the large peak tentatively reported as ICA-MP in tables 9 and 11 was definitely identified as an interferent by LC/MS/MS ("Hypercarb 2" method), GC/MS and scan mode LC/MS. Using the "Hypercarb 2" method the peak elutes at a different retention time to that of the ICA-MP standards. No ICA-MP peak was seen in this extract. The interferent peak has a different LC peak shape and MS spectrum to a ICA-MP standard solution when analysed by scan mode LC/MS.

The interferent peak has a different MS spectrum and retention time to ICA-MP when analysed by GC/MS. Figure 5 gives the GC/MS spectrum of this interferent produced by pyrolysing a portion of the foam and sampling onto an uncoated GF/A filter. Unfortunately the GC/MS NBS 75k and Wiley MS libraries did not contain a match for this interferent so it could not be positively identified. The mass spectrum shows a series peaks corresponding to losses of ~14 Th fragments, these are most likely to be -CH2- and suggest an aliphatic compound. Nine of these peaks were identified in the spectrum (77, 91, 106, 120, 134, 149, 162, 176 and 191 Th). If it is assumed that the highest mass ion 234 Th) is the molecular ion (M+. for a "standard" odd-electron ion produced by electron-impact ionisation) then the Nitrogen rule suggests that this even mass ion will contain an even number of nitrogens i.e. 0, 2, 4 etc. The ions present at 92, 106, 120 and 134 suggest an –O-phenyl-R structure may be present and this suggestion is supported by the 77 ion which is commonly the C_6H_5+ fragment. However despite this information further work would be required to identify the interferent.

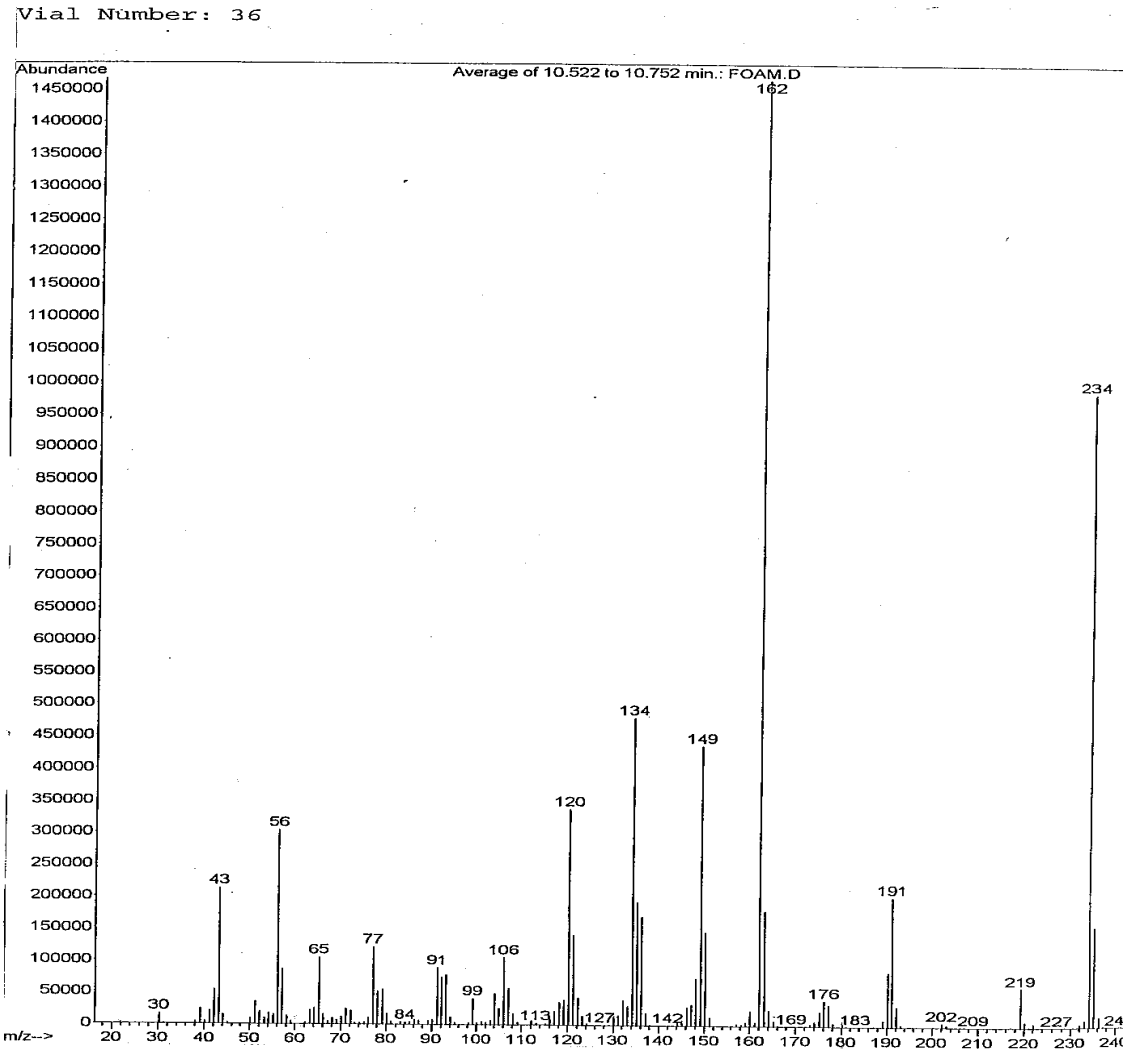

Figure 5. GC/MS spectrum of isobaric interference.

GC/MS of the ICA-MP derivative using the same GC/MS system and method used for the interferent gave a chromatogram dominated by a large peak at ~8.0 minutes. This peak had a mass spectrum identified as 1-(2-methoxyphenyl)piperazine ie. the major ions were 150 and 192 and no molecular ion at 235 was seen. The spectrum shown in figure 5 shows how this molecule could interfere with the ICA-MP MRM peak (236>193 transition) because of the "spillover" from the peaks at 234 and 191, especially if these peaks give rise to protonated species by LC/MS.

Further work would be required to characterise this interferent and this was not carried out because of time constraints. Potential means of decreasing or eliminating the effect of this interferent are discussed below and in section 3.5. Identification of the interferent would be useful as it may be possible to poredict which polyurethanes will give rise to it on heating.

The scan mode LC/MS run of the pyrolysed lacquer extract showed a single peak with the correct retention time for ICA-MP (as verified by comparison with the standards). A value of

1,075 ng NCO per sample was calculated. This is in good agreement with the results obtained for the other methods suggesting that the interferent noticed for the other samples is not present in this case.

For the insulation (spray) foam, the method "Hypercarb 2" resolved the large peak seen with the “Hypercarb 1” and “C18” methods into 2 peaks, one of which was identified as ICA-MP the other as an interferent. Figures 6a and 6b give a comparison of the chromatograms obtained using the C18 (SIM) and Hypercarb 2 method (MRM). This figure shows the importance of good chromatography to accurately quantify ICA-MP.

A value of 352 ng NCO per sample was calculated, this is about 30x less than the values given in Tables 9 and 11. Interestingly, this run suggested a difference in the ratios for the 235 Th and 236 Th peaks seen in the ICA-MP and interferent LC/MS traces. This could be used to discriminate between the genuine peak and interferent. Scan mode LC/MS of these 2 peaks was carried out which suggested that the interferent was the same compound as seen in the flexible foam.

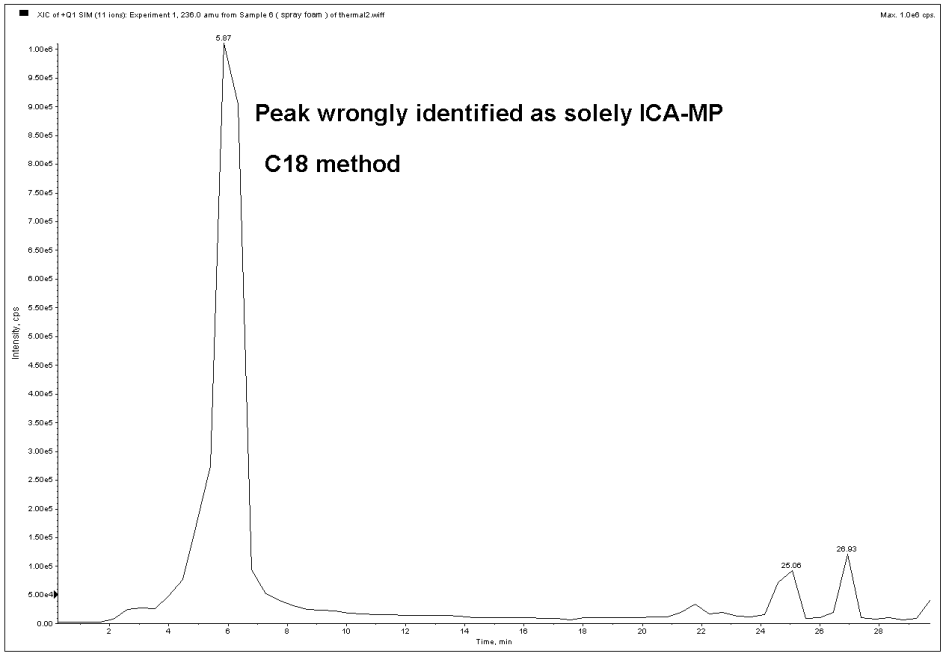

Figure 6a. Comparison of chromatograms obtained for the peaks identified as ICA-MP using the C18 SIM method for pyrolysed insulation (spray) foam.

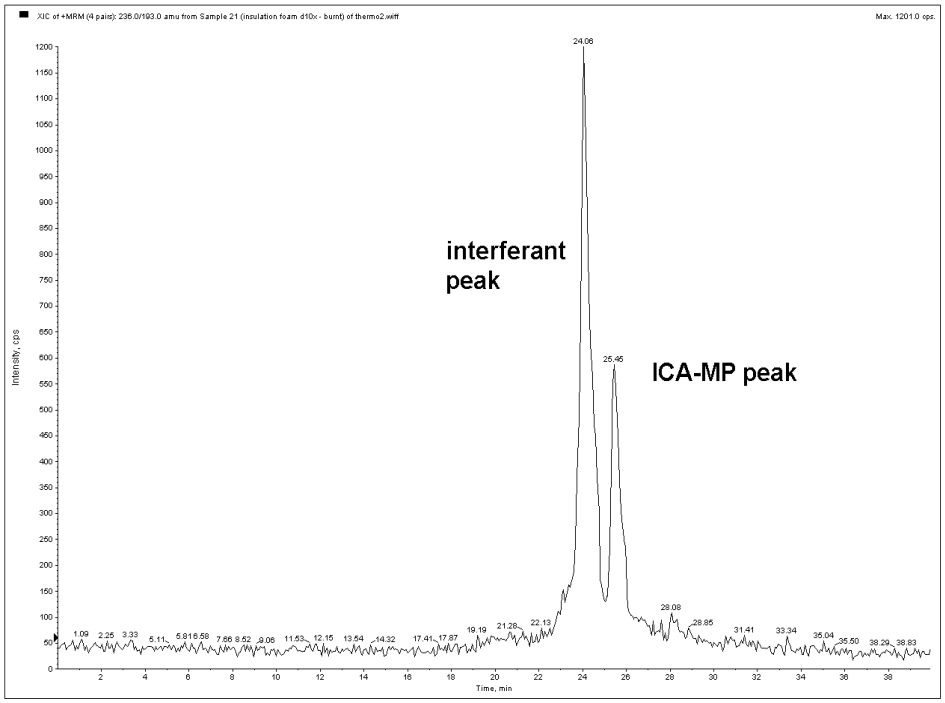

Figure 6b. Comparison of chromatograms obtained for the peaks identified as ICA-MP using the Hypercarb 2 MRM method for pyrolysed insulation (spray) foam.

The rigid (packaging) foam extract was not analysed using the "Hypercarb 2" method as this sample was taken with a conventionally prepared (i.e. not oven baked) MP impregnated filter and so was known to contain the interferent.

In summary, the isocyanic acid MP peak can be affected by a variety of interferences e.g. acetylated MP, impurities from the MP reagent and GF/A filters and compounds produced on pyrolysis of NCO based materials. Oven baking at 400°C has been shown to remove the interference present on the MP impregnated filters prior to sampling (see section 3.1.8).

However the work reported above shows that thermal degradation of some polyurethane materials can give rise to a compound with a potentially interfering mass at 235 Th. The problem is that the ICA-MP peak has a relatively low molecular mass (236 Th) and gives a limited fragmentation pattern with few major daughter ions. This makes obtaining a specific set of MS conditions for this compound difficult, even using MRM or precursor ion scan methods. The "Hypercarb 2" method was capable of separating the ICA-MP peak away from the interferent and illustrates the requirement for good chromatography even when using MS to analyse these complex samples.

Altering the resolution on the Q1 and Q3 quadrupoles in the MRM method i.e from low to high, may decrease the effect of the interfering peak but would also decrease the signal intensity. Ion trap MS, a different MS source and analyser design, with the ability to generate full scan spectra from a particular fragment ion and the ability to carry out MS^n may be less susceptible to interferences for ICA-MP and further work to investigate this and to develop the method reported here is suggested.

A further option would be the use of a higher resolution MS instrument i.e. time-of-flight MS (TOF-MS). The higher mass resolution of the TOF-MS design may enable the ICA-MP and interferent peaks to be separated (e.g. isocyanic acid - $^{1}H^{14}N^{12}C^{16}O$ accurate mass = 1.008 +

14.003 + 12 + 15.995 = 43.006, acetyl - $^{12}C^{16}O^{12}C^{1}H3$ accurate mass = 12 + 15.995 + 12 + 3x1.008 = 43.019 – difference in mass is 0.013 Th in 236 (nominal mass of ICA-MP) or 55ppm). This is well within the range of a modern medium resolution, TOF-MS.

3.5 THE USE OF 1-(2-ETHOXYPHENYL)PIPERAZINE AS A DERIVATIZING AGENT FOR ISOCYANATES

The use of EP as a derivatization agent should be complimentary to that of MP because the EP derivatives will have different LC retention times so making it unlikely that a peak that co-elutes with MP derivative will interfere with the corresponding EP derivative. The EP derivatives also have different m/z+ values i.e. [MP+ H]+ = +193 Th and [EP+ H]+ = +207 Th so an interference that is isobaric with the MP derivative will not be isobaric with the EP derivative. This approach may be particularly useful in diminishing the analytical problems found for the ICA-MP derivative.

The NCO-EP derivatives obtained from the pyrolysis of the lacquer and insulation foam samples were analysed using the MS scan method and an MRM method set up for the EP derivatives. Figure 7 shows an extracted ion chromatogram obtained for the LC/MS scan mode run of an air sample obtained during the pyrolysis of spray (insulation) foam.

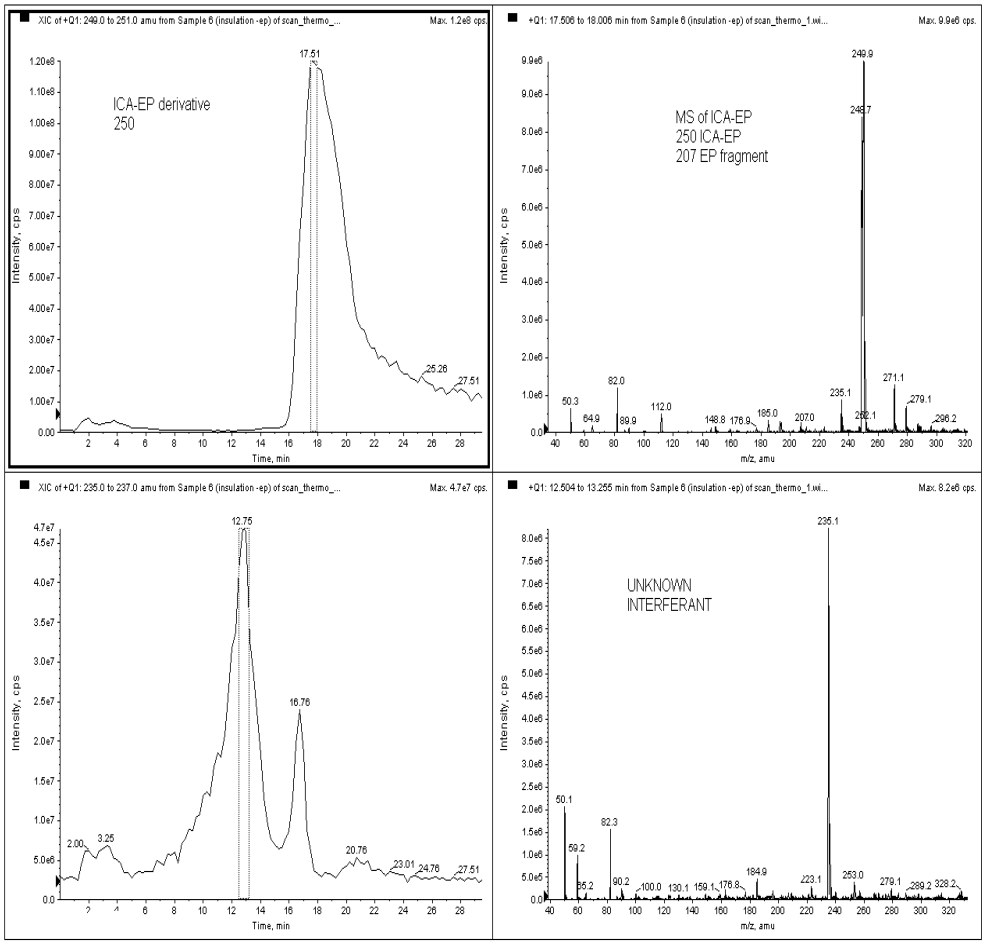

Figure 7. Chromatogram of air sample obtained on an EP coated GF/A during the pyrolysis of spray (insulation) foam – Scan mode MS.

Figure 7 shows that using the EP reagent has moved the ICA-EP (upper extracted ion chromatogram) peak away from the interference (lower extracted ion chromatogram). In addition the ICA-EP peak has a protonated molecular ion of 250 Th i.e. no longer isobaric with the interference mass at 235 Th. Figure 7 clearly shows the interference peaks at 235 Th (lower extracted ion chromatogram). The mass spectra of the interferent and ICA-EP are also shown for comparison. Interestingly the fact that the interferent mass has remained 235 Th suggests that the interferent does not react with MP or EP reagents used to coat the filter. This finding is in agreement with the observations made in section 3.4 (see Figure 5). A MRM method based on the loss of EP from ICA-EP i.e. 250>207 transition may therefore be able to resolve this interference problem entirely.

4 CONCLUSIONS

Methods for the determination of monomeric NCO MP derivatives have been developed. Initial spiking experiments gave acceptable (~100%) and reproducible recoveries (9 – 23%) for various underivatized NCO monomers spiked onto MP coated filters (experiment 1).

Two SIM methods were developed based on graphite (Hypercarb) and octadecylsilane (C18) stationary phase chemistries. The C18 method is a development of the existing HSL method, MDHS 25/3. Both methods gave similar performance with estimated limits of detection of ~ 0.1 to 1 ng NCO/ml depending on NCO species (see Tables 4 and 7). The C18 method has the advantage of also being able to determine the common di-isocyanates as well as the mono-isocyanates. The di-isocyanates are strongly retained on the graphite column and did not elute. Both methods gave poorer results for propyl NCO MP because of the phthalate interference mentioned previously. Both methods gave poor results for methyl NCO MP and isocyanic acid MP because of co-elution with an unknown isobaric impurity.

Various attempts were made to try remove these co-eluting interferences. The MP reagent was re-crystallised before use (experiment 4), the GF/A filters were acetone washed prior to adding the MP reagent (experiment 6) and a variety of pre-desorption treatments were investigated (hexanoic anhydride, propionic anhydride, trifluoroacetic anhydride and no anhydride – experiments 2,3,5 and 6). Although these alternative treatments removed the potential for isobaric interferences the recovery and repeatability data obtained for methyl NCO MP and isocyanic acid MP were not satisfactory.

A combination of baking the filters at 400°C prior to coating with and MP reagent and treatment of the filters after sampling with hexanoic anhydride was found to be successful in reducing the interferences to acceptable levels. A method using the graphite column and the MS in MRM mode gave acceptable recoveries (~100%), repeatabilities (~10 – 20 %RSD) and estimated limits of detection of ~ 3 ng NCO/ml. This corresponds to ~ 0.4 mg NCO/m3 for a 15 l air sample, or ~ 1/200th of the STEL (short-term workplace exposure limit).

Thermal degradation experiments were carried out on various polyurethane materials. At 200°C a peak belonging to the parent monomer was detected. At 400°C other peaks were seen presumably caused by breakdown of the parent monomer and the polyurethane. Both the C18 and Hypercarb SIM method gave similar results. Both of these methods gave large, early eluting peaks with the correct mass for isocyanic acid MP. However these peaks had poor peak shapes. Using MRM and a different mobile phase gradient (method Hypercarb 2) this large peak was separated into two peaks, one of which was identified as isocyanic acid MP and the other was an unknown interferent. Pyrolysis GC/MS and LC/MS scan mode runs using both MP and EP derivatized NCO were used to confirm this conclusion. This finding emphasises the need for caution when using MS to identify peaks and the need for good chromatography. Failure to correctly identify the isocyanic acid MP peak could lead to large errors (over-estimation) in the evaluation of worker exposure.

The MRM and C18 methods were used to characterise the vapours produced from a simulated workplace exposure, ie. spot welding of a polyurethane coated piece of metal (Table 12). These methods produced data that agreed with results obtained for pyrolysis GC/MS of a fragment of the polyurethane coating. Further work is suggested to extend these methods and gather more information on the exposure of workers to thermal degradation products of polyurethanes.

5 REFERENCES

HSE, (1999) Methods for the Determination of Hazardous Substances, MDHS 25/3, Organic Isocyanates in Air, HSE Books ISBN 0-7176-1668-1, available at http://www.hse.gov.uk/pubns/mdhs/pdfs/mdhs25-3.pdf.

M. Boutin, A. Dufresne, C. Ostiguy and J. Lesage (2006), Determination of Airborne Isocyanates Generated During the Thermal Degradation of Paint in Car Body Shops, *Ann. Occup. Hyg.* **40**(4), 381-393.

M. Coldwell and J. White, (2003) Sanding of Isocyanate Based Paints, Part 1, HSL Report OMS/2003/06.

M. Coldwell and J. White, (2005) Measurement of Isocyanate During Sanding and the Bake cycle – Results December 2004 and March 2005, HSL Report OMS/2005/10, available at http://www.hse.gov.uk/research/hsl_pdf/2005/hsl0559.pdf.

M. Henriks-Eckerman, J. Välimaa, C. Rosenberg, K. Peltonen and K. Engström, (2002) Exposure to Airborne Isocyanates and Other Thermal Degradation Products at Polyurethane-Processing Workplaces. *J. Envir. Monit.* **4**, 717-721.

I. Pengelly, (2002) Pyrolysis Screening Methods, HSL report OMS/2002/02.

C.J. Sennbro, C.H. Lindh, A. Östin, H. Welinder, B. A. G. Jönsson and H. Tinnerberg, (2004) A survey of Airborne Isocyanate Exposure in 13 Swedish Polyurethane Industries, *Ann. Occup. Hyg*. **48**(5), 405-414.

J. Unwin D. Dabill, C. Keen, I. Pengelly, J. Saunders, J. White, A. Simpson and M. Coldwell, (2006) Investigation of Complex Harmful Substances – Call-off contract 2003/05, HSL report OMS/2005/11, available at http://www.hse.gov.uk/research/hsl_pdf/2006/hsl06106.pdf.

J. Unwin and others, (2007) Organic Measurement Core Activity (2005–2007) - Methods for Hazardous Substances, HSL report AS/2007/02.

H. Westberg, H. Löfstedt, A. Seldén, B-G Lilja and P. Nayström, (2005) Exposure to Low Molecular Weight Isocyanates and Formaldehyde in Foundries using Hot Box Core Binders, *Ann. Occup. Hyg*. **49**(8), 719-725.

J. White, (2003) The Determination of Isocyanates in the Presence of Amines, HSL Report OMS/2003/10.

J. White, H.Corns, K. Jones, I.Pengelly and J. Cuthbert, (2005) New Instrumental Techniques – Call-off contract 2003 - 2005, HSL report OMS/2005/17.

J. White, (2006) MDHS 25 Revisited; Development of MDHS 25/3, the Determination of Organic Isocyanates in Air, *Ann. Occup. Hyg.* **50**(1), 15-27.

6 APPENDICES

Appendix 1 - Modified MDHS 25/3 method

LC column - C18 4 μm Genesis, 250 x 2 mm, Jones Chromatography
Gradient elution, 150 ml/min, injection volume 25 ml.

equilibrate at initial conditions for 5 min.

T (min.)	0	5	17.5	27	27.5	35
%B	40	40	80	80	40	40

%A = 5 mM ammonium formate/ 0.1% formic acid pH~ 3.8
%B = acetonitrile/ 0.1% formic acid

MS - "simultaneous" SIM and MRM

Period 1 (SIM – mono-NCO) (0 to 10 minutes)
Dwell time per ion - 100 msec

Ions(Th)	236	250	264	278
NCO-MP	Isocyanic acid (ICA)	Methyl (MIC)	Ethyl	Propyl

MS settings

Declustering Potential – orifice plate (DP)	80 V	Focussing ring Potential (FP)	400 V
Entrance Potential – Q_o lens (EP)	10 V	Curtain gas (CUR)	20 psi
Electrospray needle Potential (IS)	5200 V	Source Temperature (TEM)	550 ^oC
Nebulizer gas (GSG1)	35 psi	Nebulizer gas (GSG2)	35 psi
Deflector plate potential (DF)	–250 V	Electron multiplier (CEM)	2000 V
Ion energy (IE1)	1 V		

Period 2 – experiment 1 (SIM – mono-NCO) (10 – 35 minutes)
Dwell time per ion - 100 msec

Ions(Th)	292	312	320	396
NCO-MP	Butyl (n+t isomers)	Phenyl	Hexyl	Diisopropylphenyl (DIPP)
Ions(Th)	239	253	306	
NCO-MP	d3-ICA(ISTD1)	d3-MIC(ISTD2)	t-Butyl-EP (ISTD 3)	

MS settings

Declustering Potential – orifice plate (DP)	80 V	Focussing ring Potential (FP)	400 V
Entrance Potential – Q_o lens (EP)	10 V	Curtain gas (CUR)	20 psi
Electrospray needle Potential (IS)	5200 V	Source Temperature (TEM)	550 ^oC
Nebulizer gas (GSG1)	35 psi	Nebulizer gas (GSG2)	35 psi
Deflector plate potential (DF)	–250 V	Electron multiplier (CEM)	2000 V
Ion energy (IE1)	1 V		

Period 2 – experiment 2 (MRM – di-NCO) (10 – 35 minutes)
Dwell time per transition – 500 msec

MRM	607>193
NCO-MP	isophorone di-isocyanate (E+Z isomers) - IPDI
MRM	648>193
NCO-MP	4,4'-methylenebis(cyclohexyl isocyanate) - HMDI

MRM	559>193
NCO-MP	toluene di-isocyanate (2,4 and 2,6 isomers) - TDI
MRM	635>193
NCO-MP	methylenebis(phenyl isocyanate) (2,2',2,4' and 4,4' isomers) - MDI
MRM	553>193
NCO-MP	1,6- diisocyanatohexane - HDI

MS settings

DP	80 V	FP	400 V	EP	10 V
TEM	550 °C	IS	5200 V	CUR	20 psi

CAD (Collisionally Activated Dissociation) cell gas density (CAD) 6 psi
Collision cell energy – Q_2 LINAC (CE) 50 V
Collision cell exit potential (CXP) 10 V
Collision cell entry potential (CEP) fixed on API 2000

GSG1	35 psi	GSG2	35 psi	DF	–2500 V	CEM	2000 V	IE1	1 V

Appendix 2 - Hypercarb method 1 - SIM

LC column – Graphite Hypercarb, 100 x 2 mm, Thermo-Finnegan
Gradient elution, 200 ml/min, injection volume 25 ml

equilibrate at initial conditions for 5 min.

T (min.)	0	5	6	20	21.5	30
%B	40	40	90	90	40	40

%A = 5 mM ammonium formate/ 0.1% formic acid pH~ 3.8
%B = acetonitrile/ 0.1% formic acid

MS – SIM (+Q1 SIM, 3 time periods)

Period 1 (0 to 12.2 minutes)

Ion	236	250	264	239	253
NCO-MP	ICA	MIC	Ethyl	d3-ICA	d3-MIC
Dwell (msec)	5000	1000	1000	500	500

Period 2 (12.2 to 21.2 minutes)

Ion	396	292	278
NCO-MP	DIPP	Butyl	Propyl
Dwell (msec)	2500	2500	2500

Period 3 (21.2 to 30 minutes)

Ion	320
NCO-MP	Hexyl
Dwell (msec)	5000

MS settings (for explanation of abbreviations – see Appendix 1)

DP	70 V	FP	400 V	EP	10 V
CUR	20 psi	IS	5200 V	TEM	550 °C

GSG1	30 psi	GSG2	30 psi	DF	–2500 V	CEM	2000 V	IE1	1 V

Appendix 3 - Hypercarb method 2 - MRM

LC column – Graphite Hypercarb, 100 x 2 mm, Thermo-Finnegan
Gradient elution, 150 ml/min, injection volume 25 ml

equilibrate at initial conditions for 5 min.

T (min.)	0	3	8	20	21	30
%B	60	60	85	85	60	60

%A = 10 mM ammonium formate/ 0.1% formic acid pH~ 3.8
%B = methanol/ 0.1% formic acid

MS – MRM

Dwell time per transition – 5,000 msec except for ISTD (d3-MIC) 500 msec

MRM	236>193
NCO-MP	isocyanic acid (ICA)
MRM	250>193
NCO-MP	methyl isocyanate (MIC)
MRM	264>193
NCO-MP	ethyl isocyanate
MRM	253>196
NCO-MP	d3-MIC

MS settings (for explanation of abbreviations – see Appendix 1)

DP	30 V	FP	350 V	EP	5 V
TEM	550 ^{o}C	IS	5200 V	CUR	30 psi

CAD (Collisionally Activated Dissociation) cell gas density (CAD) 6 psi
Collision cell energy – Q_2 LINAC (CE) 20 V
Collision cell exit potential (CXP) 20 V
Collision cell entry potential (CEP) fixed on API 2000

GSG1	35 psi		GSG2	35 psi	DF	–2500 V	CEM	2000 V	IE1 1 V