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Analysis of chemical warfare agents III. Use of bis-nucleophiles in the trace level determination of phosgene and perfluoroisobutylene

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Abstract

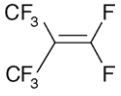
The reactivity of phosgene and perfluoroisobutylene (PFIB) towards 1,2-bis-nucleophiles was exploited to allow determination of these gases in air samples. 2-Aminothiophenol (ATP), 3,4-dimercaptotoluene (DMT) and 2-hydroxymethylpiperidine (HMP) were evaluated as bis-nucleophiles capable of forming thermally-stable derivatives with phosgene and PFIB when loaded with triethylamine onto Tenax TA. Experimental design was used to optimise thermal desorption conditions. Detection limits in the low ng m^{-3} range were observed for the five derivatives investigated. This work represents the most sensitive analytical method for trace level quantitation of phosgene and PFIB published to date.

Keywords: Chemical warfare agents; Central composite design; Derivatisation; Perfluoroisobutylene; Phosgene

1. Introduction and aim of investigation

Unequivocal identification and quantitation of toxic gases such as phosgene (carbonyl chloride) and perfluoroisobutylene (PFIB) is essential for occupational health monitoring and allegations of use in cases of contravention of the Chemical Weapons Convention [1]. Phosgene, deployed as a chemical warfare agent in World War I, is used industrially to manufacture many chemicals [2]. It forms during welding in air contaminated with chlorinated solvents [3] and during the decomposition of chloropicrin [4]. Perfluoroisobutylene has never been used in warfare and is a by-product of Teflon production [5]. Both gases contain two displaceable halogen atoms and are insoluble in water. They cause a delayed and potentially lethal build-up of fluid in the lungs when inhaled [6]. Pathological changes in poisoned animals are similar for both gases, suggesting a similar mechanism of action [7].

phosgene
bp 7°C



PFIB
bp 6°C

Analysis of phosgene [8–10] and PFIB [11] by gas chromatography (GC) is complicated by their low boiling points, which necessitates cryogenic cooling, and their susceptibility to hydrolysis, which liberates hydrogen halides that corrode GC columns. Determination of phosgene is possible using a pumped sorbent tube containing suitable packing – such as Tenax TA or XAD-2 – spiked with a derivatising reagent. Derivatives may be characterised by atomic emission detection, electron-capture detection (ECD) or flame ionisation detection. Perfluoroisobutylene and related fluoroalkenes may be analysed by GC using a Chromasil-310 packed column with ECD or negative ion chemical ionisation mass spectrometry [12]. Tenax TA and similar absorbents generally only retain heavier halogenated compounds. However, successful desorption of PFIB from charcoal and analysis by electron impact mass spectrometry has been reported [13]. Ion mobility spectroscopy also has potential for detecting and monitoring PFIB [14].

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To circumvent cryogenic trapping and corrosion problems, phosgene and perfluoroisobutylene can be derivatised and the derivatives analysed. Both gases react with nucleophiles in a stepwise fashion to give mono- then bis-substitution products; the latter are non-corrosive. Various reagents have been used to derivatise phosgene. Alcohols such as isopropanol react incompletely and have generally been rejected in favour of primary and secondary amines, which react completely to give urea derivatives with favourable properties. Amino alcohols are also useful reagents and give oxazolidines [15]. Oxazolidine derivatives have been formed by reaction of phosgene with 2-hydroxymethyl piperidine on XAD-2, the basis of the Occupational Safety and Health Administration (OSHA) method for occupational monitoring of phosgene [16]. Derivatisation of PFIB to aid its analysis has not been carried out, although the gas is reactive towards many nucleophiles [17]. With *ortho*-substituted phenylamines, cyclic products are obtained [18]. Table 1 summarises some derivatives of phosgene which have been used in quantitative and qualitative analysis. Direct comparison of limits of detection (LODs) between the derivatives and those obtained in this study is not possible as some of the literature methods lack detail concerning the measurement of the LODs.

Some of the reagents used to derivatise phosgene produce derivatives of low stability. To select the best reagent, the order of reactivity of nucleophiles, such as amines, alcohols and thiols, towards the analyte should be known. Bis-nucleophiles are more favourable than mono-nucleophiles as cyclic products form more easily than acyclic ones due to the neighbouring group effect. Experiments with phosgene and nucleophiles in water have established that primary amino and mercapto groups are highly reactive [26]; hydroxyl groups have much lower reactivity. The pattern of reactivity of PFIB is similar: amines and thiols react better than alcohols [27]. Based on these data, it might be expected that of all amino acids in the body, cysteine might react preferentially with both phosgene and perfluoroisobutylene. It has been shown that cysteine residues in albumin and haemoglobin are targeted by phosgene [28] and rodents can be protected from otherwise lethal doses of PFIB by pre-treatment with cysteine esters [29]. The formation of such adducts in vivo points to their high stability and cysteine-like compounds, especially 2-amino(alkane or arene)thiols, should be excellent derivatisation reagents.

This paper, the third on quantifying chemical warfare agents in environmental samples [30], describes the use of 3,4-dimercaptotoluene and 2-aminothiophenol to derivatise phosgene and perfluoroisobutylene in solution (to provide analytical standards) and on tubes packed with Tenax TA (for identification of the gases in atmospheric samples). Thermal desorption, instead of solvent desorption, as in the OSHA procedure which uses 2-hydroxymethylpiperidine on XAD-2 sorbent, is reported. After thermal desorption, products were analysed by gas chromatography-mass spectrometry (GC–MS) with selective ion monitoring (SIM). Central composite design (CCD) was used to optimise desorption parameters. Stability trials of derivatives at various temperatures, limits of detection, and quantitation of the two gases were carried out. Stability trials of

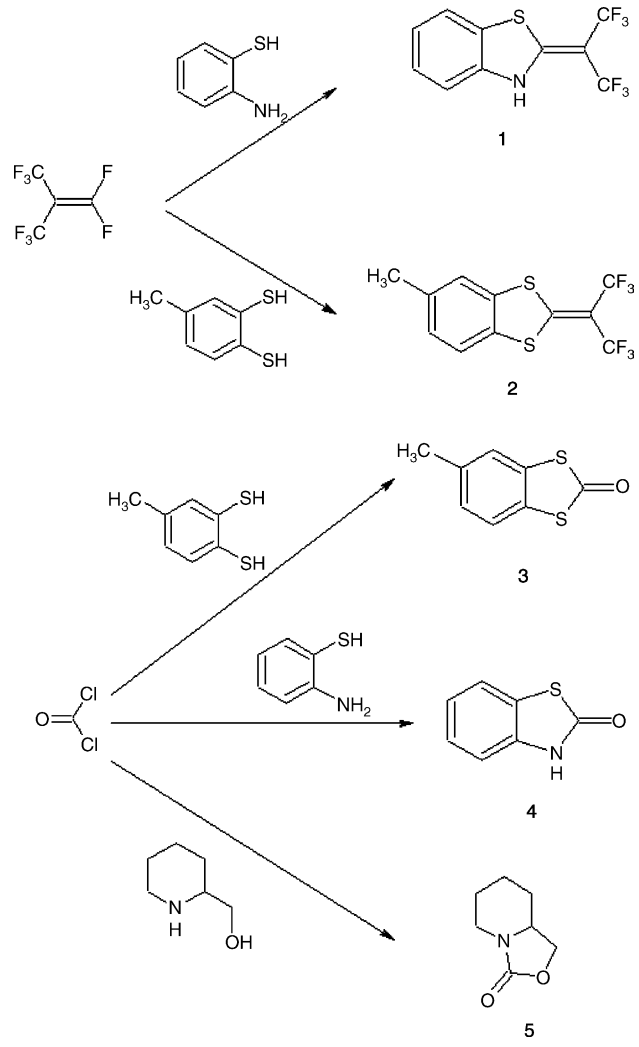


Fig. 1. Products from reaction of perfluoroisobutylene and phosgene with 2-aminothiophenol (ATP), 3,4-dimercaptotoluene (DMT) and 2-hydroxymethylpiperidine (HMP). The IUPAC numbering system in italics corresponds with NMR assignments.

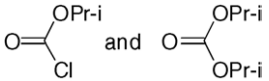
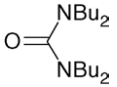
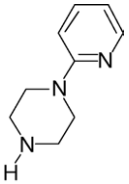
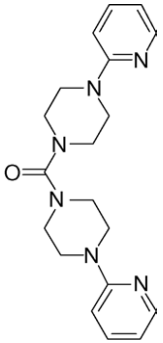
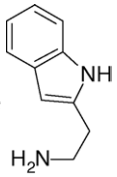
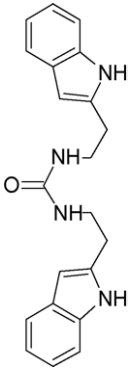
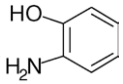
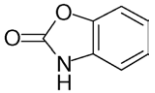
derivatives are especially important as, depending on the scenario, samples may be received for analysis as much as 2 weeks after the sampling event, e.g. after a challenge inspection by the OPCW. Throughout this time the samples may have been stored in a variety of environments representing differing storage temperatures. The work represents the first step towards developing a broad-spectrum derivatising agent capable of detecting many gases of military concern. Reactions studied are shown in Fig. 1.

2. Experimental

2.1. Materials

Phosgene at a concentration of 2.5 ppm in nitrogen was obtained from BOC (Southampton, UK). Perfluoroisopropylene of >99% purity was made at Dstl Porton Down using a published procedure [31] and standards of desired concentration prepared by dilution in a one litre polypropylene gas bag with a resealing syringe port (SKC, Dorset, UK). The bis-nucleophiles,

Table 1
Reagents and experimental parameters for the analysis of phosgene in various matrices

Reagent	Conditions	Structure of derivative	Method of detection	Limit of detection	Reference
Isopropanol $HOP_{r\text{-}i}$	Absorption in isopropanol		GC with electron capture detection	10^{-3} ppm	[19]
N,*N*-di(*n*-Butylamine) $HNBu_2$	Glass tubes packed with XAD 2 containing the reagent		Desorption with hexane and analysis by GC with FID	0.08 ppm (v/v)	[20]
			GC with AED and MS	Not stated	[21]
1-(2-Pyridyl)piperazine 	Glass tubes packed with Chromosorb containing 2.5% w/v reagent		Desorption with acetonitrile and analysis by reverse phase HPLC with UV detection	0.005 ppm	[22]
3-(2-Aminoethyl)indole 	Impinger containing solution of reagent		Reverse phase HPLC with detection by fluorescence emission and amperometric oxidation	0.04 mg m^{-3}	[23]
2-Aminophenol 	Phosgene solution mixed with reagent		GC with nitrogen-selective detection	1 ng ml^{-1}	[24]
			HPLC with UV detection	1 ppm in plastic packaging	[25]

triethylamine and hexane were obtained from Aldrich (Gillingham, UK) and used as received.

2.2. Synthesis of analytical standards

Analytical standards were prepared by adding perfluoroisobutylene or phosgene to the appropriate bis-nucleophilic thiol in diethyl ether in the presence of triethylamine at −78 °C. Under these conditions, reaction took place the instant the gas came into contact with the thiol. Products were isolated in greater than 99% purity according to analysis by NMR spectroscopy. NMR spectra were recorded using a Jeol Lambda 500 instrument (operating at 500 MHz for ^{1}H, 125 MHz for ^{13}C, and 470 MHz for ^{19}F spectra) in $CDCl_3$ solution, with $SiMe_4$ and $CFCl_3$ as internal and external references for ^{1}H and ^{19}F, respectively. Melting points were determined in an electrothermal apparatus and are uncorrected.

2.2.1. Caution

Owing to the high inhalation toxicities of perfluoroisobutylene and phosgene [32], the chemistry described requires a high level of awareness and experiments must be performed in an efficient fume-cupboard. The safest way to handle the gases is to inflate a polypropylene gas bag with a resealing syringe port via a lecture bottle and a short length of rubber tubing; a known volume of gas can then be removed from the bag, as required, using a large gas-tight syringe. Gas bags of one litre capacity were used (specification given in Section 2.1). Experiments were performed in a 250 ml round-bottomed flask sealed with a rubber septum. Addition of perfluoroisobutylene or phosgene was accomplished by first removing 300 ml of air from the flask using a gas-tight syringe. The correct volume of gas was removed from the polypropylene gas bag using another gas-tight syringe equipped with a wide-bore metal needle and introduced by cooling the flask to −78 °C and inserting the needle through the septum. The plunger was depressed and smoothly delivered the gas into the flask.

2.2.2. 2-[2,2,2-Trifluoro-1-(trifluoromethyl)ethyl]-1,3-benzothiazole (**1**)

PFIB (400 ml) was added to a stirred solution of 2-aminothiophenol (1.74 g, 16.22 mmol) and triethylamine (4.51 ml, 32.44 mmol) in diethyl ether (50 ml) at −78 °C. The insoluble thiolate salt, formed on mixing 2-aminobenzenethiol and triethylamine, reacted with perfluoroisobutylene and gave a colourless solution containing oily droplets of triethylamine hydrofluoride. After 4 h, water (25 ml) was added, the mixture transferred to a separating funnel, and the diethyl ether extract separated, dried ($MgSO_4$) and the solvent removed to give a solid. Chromatography on silica gel, eluting with hexane, gave the title compound as an orange solid (2.5 g, 54%), mp 100 °C; Lit. mp 100 °C [33]. ^{1}H NMR (signals at 4- and 7-H are broadened) δ = 8.15 (1H, d, J = 8 Hz, 4-H), 7.96 (1H, d, J = 8 Hz, 7-H), 7.57 (1H, dd, J = 7 and 1 Hz, 6-H), 7.51 (1H, dd, J = 8 and 1 Hz, 5-H), 4.88 (1H, septet, $^{3}J_{HF}$ = 8 Hz, CH). ^{13}C NMR δ = 153.6 (C=N), 152.2 (9-C), 135.8 (8-C), 126.9 (4-C), 126.7 (6-C), 124.*2* (5-C), 121.*8* (q, $^{1}J_{CF}$ = 279 Hz, CF_3), 121.7 (7-C), 53.*8* (septet, $^{3}J_{CF}$ = 31 Hz, CH). ^{19}F NMR δ = −64.1 (d, J = 7 Hz, CF_3).

2.2.3. 5-Methyl-2-[2,2,2-trifluoro-1-(trifluoromethyl) ethylidene]-1,3-benzodithiole (**2**)

PFIB (400 ml) was added to a stirred solution of 3,4-dimercaptotoluene (2.53 g, 16.22 mmol) and triethylamine (4.51 ml, 32.44 mmol) in diethyl ether (50 ml) at −78 °C. The yellow precipitation of triethylamine hydrochloride formed. The solution was allowed to warm to room temperature. After 4 h, water (25 ml) was added, the mixture transferred to a separating funnel, and the diethyl ether extract separated, dried ($MgSO_4$) and the solvent removed to give a solid. Chromatography on silica gel, eluting with hexane, gave the title compound as a white solid (1.1 g, 21%), mp 57 °C. ^{1}H NMR δ = 7.29 (1H, d, J = 8 Hz, *3*-H), 7.23 (1H, s, 6-H), 7.09 (1H, m, *4*-H), 2.36 (3H, s, CH_3). ^{13}C NMR δ = 159.*5* (=CS_2), 137.3 (4-C), 134.*3* (q, $^{5}J_{CF}$ = 3 Hz, 1-C), 130.9 (q, $^{5}J_{CF}$ = 3 Hz, 2-C), *128.0* (5-C), 122.7 (q, $^{1}J_{CF}$ = 273 Hz, *2x* CF_3), 121.8 (3-C), 121.2 (6-C), 100.0 (m, =*C*-CF_3), 21.0 (CH_3). ^{19}F NMR δ = −57.29 and −57.33 (both *complex* multiplets).

2.2.4. 5-Methyl-1,3-benzodithiol-2-one (**3**)

Phosgene (328 ml) was added to a stirred solution of 3,4-dimercaptotoluene (2.08 g, 13.31 mmol) and triethylamine (3.7 ml, 26.62 mmol) in diethyl ether (50 ml) at −78 °C. A white precipitate of triethylamine hydrochloride formed and the mixture almost set solid. After addition, the mixture was allowed to warm to room temperature and was left for 12 h. Chloroform (100 ml) and water (50 ml) were added and the yellow lower layer was separated, dried (Na_2SO_4) and filtered. Removal of solvent from the filtrate gave a yellow liquid. Chromatography on silica gel, eluting with 50:1 hexane–diethyl ether, followed by recrystallization from diethyl ether to which hexane was added, gave the title compound as fluffy white crystals (0.82 g, 34%), mp 55–56 °C. ^{1}H NMR δ = 7.35 (1H, d, J = 8 Hz, 3-H), 7.29 (1H, s, 6-H), 7.13 (1H, d, J = 8 Hz, 4-H), 2.39 (3H, s, CH_3). ^{13}C NMR δ = 190.5 (C=O), 137.2 (5-C), 132.52 (1-C), 129.*2* (2-C), 128.*1* (4-C), 123.30 (6-C), 122.7 (3-C), 21.1 (CH_3).

2.2.5. 2(3H)-Benzothiazolone (**4**)

Prepared using a similar process to that detailed above, the product having analytical properties identical to a commercial sample (Aldrich, Gillingham, UK). A mixed melting point test showed no depression, mp 137–140 °C.

2.2.6. 1-Aza-8-oxabicyclo[4.3.0.]nonan-9-one (**5**)

This derivative was prepared as described previously [16]. Although this is the OSHA method for analysis of phosgene using solvent desorption, its thermal desorption properties have not been reported and hence were evaluated.

2.3. Preparation of standards for method development and calibration studies

Tenax TA was obtained from Markes International (Pontyclun, UK) and each thermal desorption tube packed with 100 mg of sorbent. Tubes were conditioned at 100, 200 and 350 °C for 2 h prior to spiking. About one-quarter of the tubes were

selected at random and desorbed in a Perkin Elmer ATD 400 system. No significant peaks above the baseline were observed and further cleaning was unnecessary. Solutions of standards **1**–**5** were prepared in hexane. Tubes were spiked with 10 μl of a 50 μg ml^{-1} solution of compounds **1**–**5** in hexane (500 ng on tube) by vapour or liquid loading. Vapour loading was performed using a vapour loading rig (Markes International, UK) at a flow rate of 50 ml min^{-1} nitrogen. The spiked tubes were employed in the thermal desorption optimisation study. All calibration levels were repeated six times and the arithmetic mean of the response plotted against concentration to produce calibration plots.

2.4. Development of the mathematical models

The theory and application of experimental design have been reviewed [34,35]. Response surface methodology allowed the response of the system (peak area) to be optimised with respect to four parameters: desorption temperature, desorption time, valve temperature and transfer line temperature. These factors are usually varied to optimise thermal desorption systems. A rotatable orthogonal CCD allowed empirical relationships between system response and these factors to be elucidated. A mathematical model for a four variable CCD can be described by Eq. (1).

$$Y = \beta_0 + \sum \beta_j X_j + \sum \beta_{jj} X_j^2 + \sum \beta_{jk} X_j X_k \quad (1)$$

where Y is the response of system; $X_{j,k}$ the variable of system; β_0, β_j, β_{jj}, β_{jk} are the regression coefficients for constant, linear, square and interaction terms, respectively.

Regression coefficients are calculated by fitting the values of experimental parameters to the least squares regression line. A quadratic equation or an equation containing only significant terms results. This can then be used to predict the response of the system at given levels of experimental factors.

The CCD consists of a star design imposed through the centre of a factorial design. The four factor design used in this investigation comprised a 2^4 factorial design (16 experiments), a $2 \times 4 + 1$ star design (nine experiments) and six centre points (four in factorial portion and two in star portion). The resulting 30 experiments were run in a random order in three blocks (three consecutive days). An α value of 2 was used to ensure rotatability and orthogonality of the design as calculated by Eq. (2). The upper and lower limits of each factor were placed on the axial points of the design. Thirty experiments were performed and a global optimum for compounds **1**–**4** established.

$$\alpha = \pm(N_F)^{1/4} = \pm 2 \quad (2)$$

where N_F is the number of experiments in factorial portion of design [16].

2.4.1. Mathematical model for compounds (*1*–*4*)

A CCD was constructed for investigating the effect of desorption temperature, desorption time, valve temperature and line temperature on the response (peak area) of compounds **1**–**4**. Experimental domain parameters used for optimisation of **1**–**4** appear in Table 2.

Table 2
Experimental domain for CCD for optimisation of compounds **1**–**4**

Parameter	Levels				
	−2	−1	0	+1	+2
x_1 desorption time (min)	5	9	13	16	20
x_2 desorption temperature (°C)	150	200	250	300	350
x_3 valve temperature (°C)	120	155	190	225	260
x_4 line temperature (°C)	120	146	173	199	225

2.4.2. Mathematical model for compound (*5*)

CCDs are relatively inefficient for studying less than three factors and we used the more efficient Box-Behnken design for compound **5**. Only two factors that could influence its response were studied; desorption time and desorption temperature (fewer factors were studied for compound **5** as our primary aim was to examine the behavior of compounds **1**–**4**). The experimental domain for the Box-Behnken design is shown in Table 3.

2.5. Experimental parameters for central composite design experiments

GC–MS was performed on a Hewlett Packard 5890 GC (series 2) interfaced to a Hewlett Packard 5971A mass selective detector (MSD). A DB-1701 capillary column (25 m × 0.25 mm, 0.25 μm) was used with an initial GC oven temperature of 40 °C which was maintained for 1 min then increased at 20 °C min^{-1} to 280 °C and held for 1 min. The MSD was operated in electron ionisation mode with an electron energy of 70 eV. Initially, it was operated in scan mode between m/z 50 and 400 (2.16 cycles s^{-1}). Selective ion monitoring (SIM) experiments were carried out. Ions chosen were m/z 285, 266 and 216 (compound **1**), 316, 297, 266 and 247 (compound **2**), 182, 154 and 121 (compound **3**) and 151, 123 and 96 (compound **4**), 141, 100 and 83 (compound **5**). Compounds **1**–**4** were determined in a single chromatographic run whereas compound **5** was determined as a single analyte using the above method.

After optimisation of thermal desorption parameters, tubes were desorbed using the Perkin Elmer ATD 400 instrument and analysed by GC–MS-SIM. ATD parameters were varied to optimise the system and are discussed in subsequent sections. A head pressure of 14.7 psi set at 40 °C (31 cm s^{-1}) and an outlet split flow of 10 ml min^{-1} were employed for all experiments. The outlet split gave approximately 50 ng on the analytical column (from a tube loaded with 500 ng each of compounds **1**–**5**)

Table 3
Experimental domain for CCD for optimisation of compound **5**

Parameter	Levels		
	−1	0	+1
x_1 desorption time (min)	200	260	320
x_2 desorption temperature (°C)	5	17.5	30

Table 4
Thermal desorption parameters employed in analysis[a] using the ATD 400

Parameter	Value	Parameter	Value
Desorption temperature	250 °C	Desorption flow	53 ml min^{-1}
Desorption time	13 min	Trap low	−20 °C
Valve temperature	260 °C	Trap high	300 °C
Line temperature	225 °C	Trap hold	3 min
Outlet split flow	66 ml min^{-1}	Carrier gas pressure	14.7 psi

[a] Employing these flow rates, 0.67% of the sample reached the capillary column.

resulting in a signal-to-noise ratio of about 30:1 for the total ion chromatogram. A thermal desorption blank was run between each optimisation experiment where all parameters were set at the maximum values.

2.6. Calibration of GC–MS

Tenax tubes were loaded with a hexane solution of compounds **1**–**5** using vapour loading ($n = 6$). The mean peak area for the replicates (SIM) was plotted against ng injected on the column. Injection volumes of 1, 2.5, 5, 10, 20, 40, 60, 80 and 100 μl of 100 ng μl^{-1} standard were used. Weighted least squares regression [36] was used as examination of the residual plots revealed non-uniform variance over the calibration range. Thermal desorption parameters used in this part of the study are shown in Table 4.

2.7. Stability trials

Compounds **1–4** are stable and do not deteriorate over a period of one year when pure and stored in a refrigerator. After optimising the thermal desorption parameters, stability trials were carried out using three sets of six Tenax TA tubes loaded with 10 μg each of compounds **1**–**4** by vapour injection. Tubes were spiked and stored at room temperature (21 °C), in a refrigerator (2 °C) and in a freezer (−15 °C) for each of four time periods (1, 7, 14 and 28 days). The stability trial for compound **5** was performed in a similar manner with storage at room temperature and in a refrigerator being evaluated for 7, 14 and 28 days. Percentage recoveries and peak areas were normally distributed and no transformation of data was necessary prior to statistical manipulation. Each set of recovery data was examined for outliers by Dixon's *Q*-test. Normality of the recoveries was confirmed using the Anderson–Darling normality test at the 95% confidence level. Normalised data had a $p > 0.05$ confirming normal distribution. Analysis of variance (ANOVA) was performed using a two-way general linear model for compound, storage conditions and storage time and the associated interaction terms at the 95% confidence level. Bonferroni simultaneous confidence intervals were also generated as part of the ANOVA to allow comparison of multiple sample means. An Anderson–Darling normality test at the 95% confidence level on the residuals of the fitted ANOVA model was used to validate the model. The error bars on plots constructed from storage stability trial data represent the 95% confidence limits and therefore may be expected to be large due to the relatively low number of replicates performed.

2.8. Quantitation of phosgene in a synthetic gas mixture

During the course of the study, the ATD 400 system gave an intermittent fault leading to a substantial delay in completion of the study. Therefore, the Ultra Unity thermal desorption system (Markes Internation, Pontyclun, UK) was used for this part of the study. The main advantage of this system is in the electronic pressure control module which allows constant carrier gas flow. Therefore, the findings of the initial optimisation study using the ATD 400 were transferred to the Ultra Unity and desorption conditions employed for this part of the study detailed in Table 5. The result of employing constant flow (1 ml min^{-1}) was to decrease the retention times of the analytes appreciably. Also the GC oven program was altered to give a more rapid analysis. An initial oven temperature of 40 °C for 1 min was employed, then ramped at 30–170 °C for 1 min. A second ramp of 40–280 °C was employed and held for 1 min where the run terminated. The retention times for compounds **1**–**4** were 6.0, 8.47, 8.6 and 9.35 min, respectively. The decrease in retention time of compounds **1**–**4** using the Unity system as compared to the ATD 400 system illustrates the advantage of employing constant flow in the analysis. Compound **5** was not analysed by this system.

The phosgene content of a 1 l air sample was determined using DMT and ATP. Tenax TA tubes were loaded with 100 μg each of derivatising agent and triethylamine using a vapour loading rig. Phosgene samples were prepared from serial dilutions of phosgene in litre volumes of laboratory air (only 100 μl of phosgene was added to a litre gas bag of air and the increase in volume was negligible). Two phosgene concentrations were used, 44 and 4.4 ng l^{-1}, which combined with a 1:10 split on the ATD, gave 4.4 and 0.44 ng injected onto the column respectively. The equivalent experiment was not carried out for PFIB due to availability of suitable standards at the time of the study. Quantitation was carried out against tubes spiked with pure compounds **3** and **4**. Calibration levels of 0.1, 0.5, 1, 5, 10, 50, 100 and 250 ng on tube were used, which with a 1:10 split gave a calibration range of 0.01–25 ng on column. Each calibration level was measured in triplicate and the mean SIM peak area of replicates plotted against ng injected on the column. Phosgene samples were passed through the derivatising agent-spiked

Table 5
Thermal desorption parameters employed in analysis of phosgene in a synthetic mixture using the Ultra Unity

Parameter	Value	Parameter	Value
Desorption temperature	250 °C	Desorption flow	50 ml min^{-1}
Desorption time	13 min	Trap low	10 °C
Flow path temperature	215 °C	Trap high	225 °C
		Trap hold	3 min
Outlet split flow	9 ml min^{-1}	Initial carrier gas pressure (set at 40 °C)	16.5 psi

Tenax TA tube using an air sample pump set at a flow rate of 1000 ml min^{-1}.

3. Results

3.1. Optimisation of thermal desorption parameters

Peak areas were used as a measure of response for each compound. Compounds **1**–**3** were unaffected by variation of experimental parameters, suggesting that they were sufficiently volatile to pass through the TD system even at low temperatures. They also appeared to be thermally stable as evidenced by uniform response at −2 and +2 temperature levels. The response of compound **4** was affected by valve and line temperature. The coefficients suggest that raising both temperatures increases the observed signal, implying that the derivative condenses at low valve and line temperatures, and is thermally stable. These temperatures should therefore be set to their +2 values. Since none of the analytes was influenced by desorption time or temperature, these values were set to their centre point values (13 min and 250 °C, respectively). Thermal desorption parameters used in subsequent analysis appear in Table 4. Compound **5** was found to be significantly influenced by desorption time only, with 30 min producing the maximum peak area.

3.2. Peak identification and integration

The only significant peaks in chromatograms corresponded to compounds **1**–**5** and were identified by interpretation of fragmentation patterns. Fig. 2 shows the 100 ng on-column standard of compounds **1**–**4** in scan mode. Electron ionisation fragmentation patterns for **1**–**5** appear in Fig. 3a–e, respectively.

Compound **1** shows a molecular ion at *m*/*z* 285 with other abundant diagnostic ions at *m*/*z* 266 (–F), 216 (–CF_3) and 69 (CF_3). Compound **2** behaves similarly, showing a molecular ion at *m*/*z* 316 and fragment ions at *m*/*z* 297 (–F), 266 (–CF_2) and 247 (–CF_3). Compound **3** gives a molecular ion at *m*/*z* 182 and diagnostic fragment ions at *m*/*z* 154 (–CO) and *m*/*z* 121 (HOCS). Compound **4** gives a molecular ion at *m*/*z* 151 and other ions at *m*/*z* 123 (–CO) and 96 (C_5H_4S, ring-opened fragment). Compound **5** shows a molecular ion peak at *m*/*z* 141 with other ions at *m*/*z* 100 (loss of propenyl radical from molecular ion, C_2H_5) and a base peak at *m*/*z* 83 (neutral loss of CH_2OCO from molecular ion).

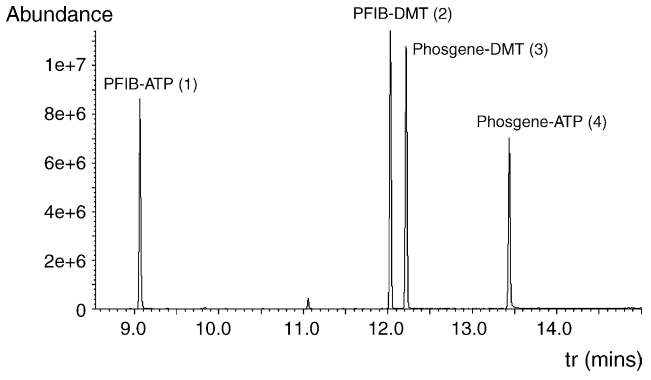


Fig. 2. Total ion chromatogram of 100 ng on column of compounds **1**–**4**. N.B. ATD 400 TD system used.

3.3. Linearity and limits of detection (LODs)

Weighted linear regression was performed on the total peak area of SIM chromatograms for each compound. This methodology gives a more accurate estimate of regression parameters when the variance of the points from the fitted line is not uniform over the calibration range. Intercepts were non-significant for all compounds ($p > 0.05$) and were removed from the regression equation. *F*-test *p* values were $\ll 0.05$ and r^2 values > 0.999 confirming linearity of the calibration range. The conditions employed in this analysis resulted in 0.67–67 ng of each compound being transferred to the column. Calculated LODs for compounds **1**–**4** were 0.02, 0.19, 0.03 and 0.03 ng on-column, respectively. A total volume of 10 l (1 l min^{-1} for 10 min) is normally adopted for sampling and hence the LODs correspond to atmospheric detection limits of 0.002, 0.019, 0.003 and 0.003 ng, respectively (2, 19, 3 and 3 ng m^{-3}). The corresponding LOD for compound **5** was 0.2 ng on-column or 20 ng m^{-3} for atmospheric samples, a vast increase in sensitivity compared to the OSHA solvent desorption method (LOD = 14 μg m^{-3}) [23].

3.4. Storage stability

For compounds **1**–**4** only linear terms (storage time and storage conditions) were significant ($p < 0.05$) by ANOVA analysis. Insignificant differences were observed for individual compounds up to 14 days after spiking (Fig. 4). Recoveries of compounds **1–3** dropped to about 70% after 21 days before decreasing to under 20% after 28 days. Compound **4** showed no significant reduction of recovery except after 28 days.

There was no significant difference of recoveries for compounds **2**–**4** under the conditions studied ($p > 0.05$). However compound **1** was recovered to a greater extent after storage at reduced temperature (refrigerator or freezer). The data suggest that analysis with sensitivity adequate for most applications will be possible after storage at room temperature for 21 days.

Storage time influenced the recovery of compound **5** with storage temperatures having no significant influence. After storage at room temperature or in a refrigerator, compound **5** could be recovered quantitatively after 7 and 14 days. Recovery fell to 40% after 28 days under both storage conditions. Compound **5** showed the poorest precision across all storage times as indicated by the 95% confidence limit.

3.5. Quantitation of phosgene in a synthetic gas mixture

The mean SIM peak areas of the analytes were compared against the linear regressions of the calibration. A very small degree of carryover was noted with phosgene-DMT and -ATP derivatives appearing in the blanks as shown in Fig. 5. For quantitation, the value in the blank was removed from the determined value of the sample to give a final quantity of phosgene as the derivative in the sample. Weighted linear regression for

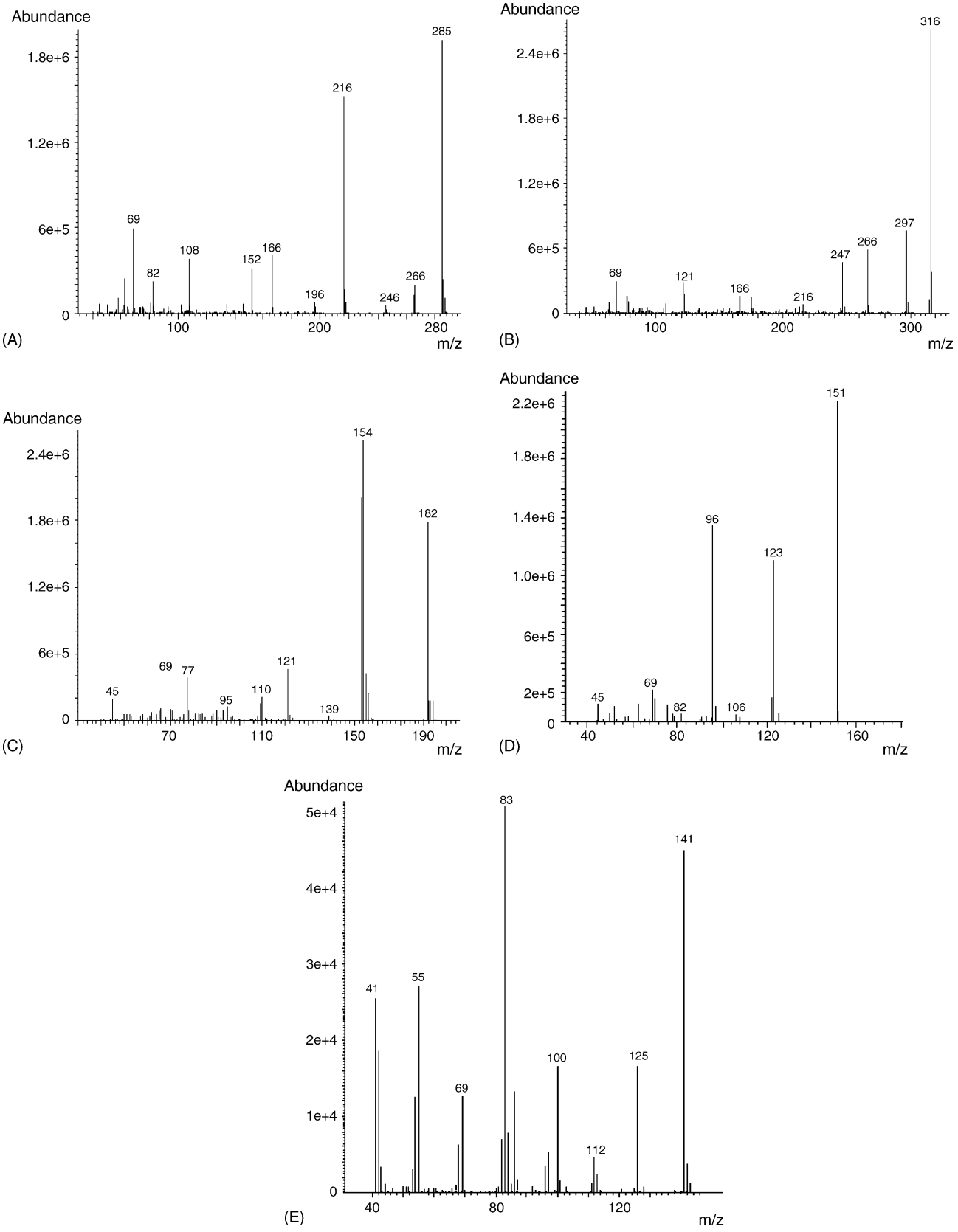


Fig. 3. Electron ionisation mass spectra for (A) PFIB-ATP **1**, (B) PFIB-DMT **2**, (C) phosgene-DMT **3**, (D) phosgene-ATP **4** and (E) phosgene-2HMP **5**.

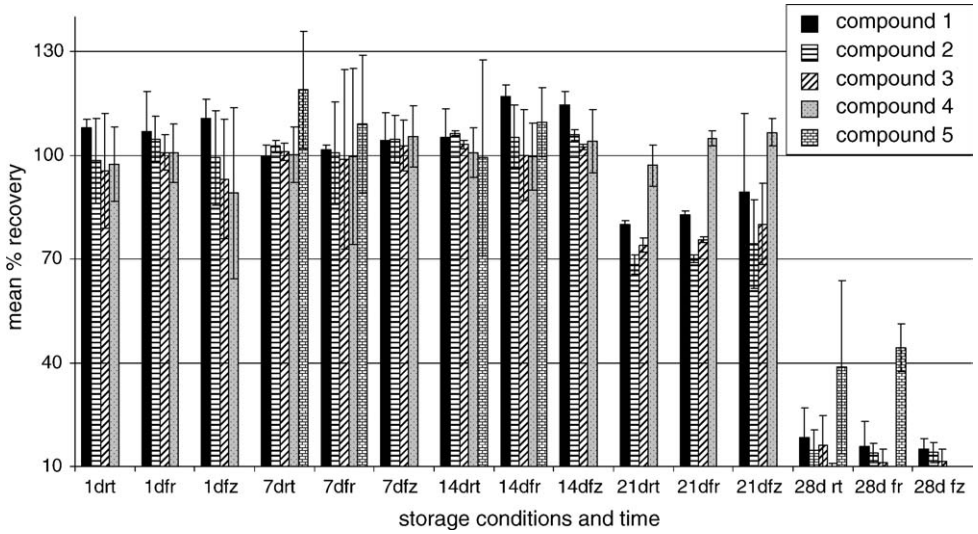


Fig. 4. Percentage recoveries of compounds **1**–**4** after storage for 1, 14, 21 and 28 days at room temperature (rt, 21 °C), refrigerator (fr, 2 °C) and freezer (fz, −15 °C). Error bars indicate the 95% confidence limits.

the calibration found the intercepts for both analytes to be non-significant ($p > 0.05$) and they were removed from the regression equation. Both DMT and ATP phosgene derivatives gave an excellent linear range ($r^2 > 0.999$) with a calculated lower LOD of ∼1.6 ng on column. This was above the lower sample concentration but quantitation was still possible by subtracting the blank peak area from that of the sample.

The loading of the DMT derivatives on the tube was 0.69 and 4.6 ng for the low and high concentration samples, respectively. The final loading on the tube for the ATP derivatives was 1.17 and 4.6 ng for the low and high concentration samples, respectively.

Fig. 5 shows the blank and sample total ion chromatograms of a SIM determination for phosgene content of an air sample using ATP as the derivatising reagent. The blank in this case was generated using laboratory air with no phosgene added. The total mass of phosgene in the 1 l air sample was 4.4 ng (a concentration of 4.4 ng l^{-1}). The entire one litre volume was sampled as described in Section 2.8. Fig. 5 clearly shows a signal greater than three times the signal-to-noise ratio of the blank.

The determined concentrations for the phosgene-DMT derivative were 0.62 ng and 4.13 ng for the low and high loading levels of phosgene respectively. This represents a >90% agreement with the sampled concentrations. The reproducibility was ±20% for this derivative. The phosgene-ATP derivative concentrations were determined to be 1.05 and 4.1 ng, respectively for low and high phosgene concentrations which represents >88% agreement with the sampled concentrations. Again, the reproducibility was determined to be ±20% for this derivative. Given the small number of replicates ($n = 3$) and possible fluctuations in ambient temperature, the results were deemed acceptable.

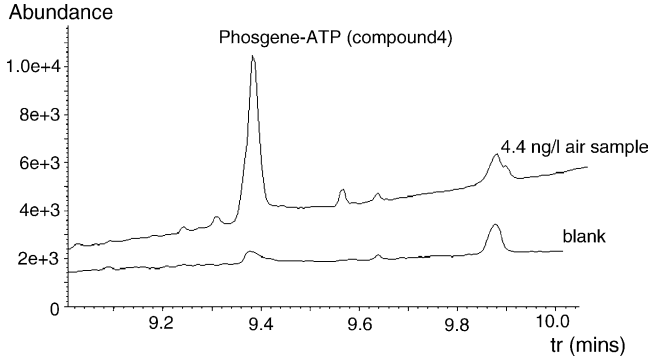


Fig. 5. Total ion chromatogram of SIM determination of phosgene in a 1 litre air sample using ATP as the derivatising agent (4.4 ng l^{-1}). N.B. Ultra Unity TD system used.

4. Conclusions

This study has illustrated the utility of bis-nucleophiles for the determination of lung-damaging gases in the atmosphere. The limits of detection of phosgene in atmospheric samples are superior to those reported elsewhere [16,17]. A dramatic improvement in detection limit was obtained by employing thermal desorption in a modification of the OSHA method for phosgene determination. PFIB reacted with 2-aminobenzenethiol and 3,4-dimercaptotoluene to produce derivatives with characteristic molecular ions/base peaks – m/z 285 and 316 respectively – under electron ionisation. The results were consistent with theory demonstrating the thermal desorption approach should be inherently more sensitive since no dilution of the samples occurs prior to analysis. Another advantage of the formation of derivatives containing nitrogen and sulphur atoms, is the potential to employ FPD or NPD detection t to allow the analytes to be analysed selectively in future.

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References

[1] Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction, Organisation for the Prohibition of Chemical Weapons (OPCW), The Hague, 1994.
[2] R.D. Ashford, Ashford's Dictionary of Industrial Chemicals, Wavelength Publications, London, 1994.
[3] M.S. Nieuwenhuizen, E.R. Groeneveld, AIHAJ 61 (2000) 539.
[4] B. Muir, W.A. Carrick, D.B. Cooper, Analyst 127 (2002) 1198.
[5] J. Patocka, J. Bajgar, The Applied Science and Analysis (ASA) Newsletter, pp. 1057–9419, available online at http://www.asanltr.com/ASANews-98/pfib.html.
[6] M.P. Maidment, D.G. Upshall, J. Appl. Toxicol. 12 (1992) 393.
[7] R.F.R. Brown, P. Rice, Int. J. Exp. Path 72 (1991) 437.
[8] A. Baiker, H. Geisser, W. Richarz, J. Chromatogr. 147 (1978) 453.
[9] A. Kuessner, J. Chromatogr. 204 (1981) 159.
[10] C.G. Esposito, D. Lillian, G.E. Podolak, R.M. Tuggle, Anal. Chem. 49 (1977) 1774.
[11] N.D. Kagramanov, P.S. Zuev, A.A. Kutin, I.O. Bragilevskii, A.K. Malt'sev, O.M. Nefedov, Izv. Akad. Nauk. SSSR, Ser. Khim 1 (1989) 37 (English translation).
[12] M.L. Shih, J.R. Smith, J.D. McMonagle, Anal. Lett. 23 (1990) 1711.
[13] M.C. Biesinger, N.S. McIntyre, S.J. Splinter, S. Liang, Carbon 35 (1997) 463.
[14] A.J. Bell, C.J. Hayhurst, C.A. Mayhew, P. Watts, Int. J. Mass Spectrom. Ion Proc. 140 (1994) 133.
[15] O. Gyllenhaal, J. Vessman, J. Chromatogr. 395 (1987) 445.
[16] Occupational and Safety and Health Administration (OSHA) method number 61 for phosgene analysis, US Department of Labour.
[17] Yu.V. Zeifman, E.G. Ter-Gabrielyan, N.P. Gambaryan, I.L. Knunyants, Russian Chem. Rev. 53 (1984) 256.
[18] H. Hirada, S. Mizutaki, S. Hayashi, N. Ishikawa, J. Fluorine Chem. 12 (1978) 211.
[19] J.A. Dahlberg, I.B. Kihlman, Acta Chem. Scand. 24 (1970) 644.
[20] J.P. Hendershott, Am. Ind. Hyg. Assoc. J. 47 (1986) 742.
[21] K. Shoene, H.-J. Bruckert, J. Steinhanses, Fresenius J. Anal. Chem. 345 (1993) 688.
[22] R.J. Rando, H.G. Poovey, S.-N. Chang, J. Liq. Chromatogr. 16 (1993) 3291.
[23] W.S. Wu, V. Gaind, Analyst 118 (1993) 1285.
[24] O. Gyllenhaal, Analyst 108 (1983) 978.
[25] M. Gmeiner, P.G. Demertzis, R. Franz, Deut. Lebensm. -Rundsch. 94 (1998) 41.
[26] A.M. Potts, F.P. Simon, R.W. Gerrard, Arch. Biochem. 24 (1949) 329.
[27] M.P. Maidment, S.K. Patel, D.G. Upshall, C.M. Timperley, J. Appl. Toxicol. 19 (1999) 113.
[28] D. Noort, A.G. Hulst, A. Fidder, R.A. van Gurp, L.P.A. de Jong, H.P. Benschop, Chem. Res. Toxicol. 13 (2000) 719.
[29] A.F. Lailey, L. Hill, I.W. Lawston, D. Stanton, D.G. Upshall, Biochem. Pharmacol. 42 (1991) S47.
[30] B. Muir, B.J. Slater, D.B. Cooper, C.M. Timperley, J. Chromatogr. A 1028 (2004) 313.
[31] J.A. Young, T.M. Reed, J. Org. Chem. 32 (1967) 1682.
[32] C.M. Timperley, J. Fluorine Chem. 125 (2004) 685.
[33] C.M. Timperley, J. Fluorine Chem. 94 (1999) 37.
[34] R.G. Brereton, Chemometrics: Application of Mathematics and Statistics to Laboratory Systems, Ellis Horwood, Chichester, 1990.
[35] L. Davies, Efficiency in research, in: Development and Production: The Statistical Design and Analysis of Chemical Experiments, Royal Society of Chemistry, Cambridge, 1993.
[36] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, third ed., Ellis Horwood, Chichester, 1993.