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Analysis of chemical warfare agents II. Use of thiols and statistical experimental design for the trace level determination of vesicant compounds in air samples

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Abstract

Thermal desorption with gas chromatography–mass spectrometry (TD–GC–MS) remains the technique of choice for analysis of trace concentrations of analytes in air samples. This paper describes the development and application of a method for analysing the vesicant compounds sulfur mustard and Lewisites I–III. 3,4-Dimercaptotoluene and butanethiol were used to spike sorbent tubes and vesicant vapours sampled; Lewisite I and II reacted with the thiols while sulfur mustard and Lewisite III did not. Statistical experimental design was used to optimise thermal desorption parameters and the optimum method used to determine vesicant compounds in headspace samples taken from a decontamination trial. 3,4-Dimercaptotoluene reacted with Lewisites I and II to give a common derivative with a limit of detection (LOD) of 260 $\mu g\,m^{-3}$, while the butanethiol gave distinct derivatives with limits of detection around 30 $\mu g\,m^{-3}$.

Keywords: Warfare agents; Derivatisation, GC; Central composite design; Thermal desorption; Lewisite; Sulfur mustard; Thiols

1. Introduction and aim of investigation

Sulfur mustard and Lewisite are blister agents that damage the skin [1–4]. Sulfur mustard was first used a chemical weapon in World War I. Since then there have been several recorded and suspected incidents of its use, including during the Iran–Iraq War in the 1980s [5–7]. Lewisite was produced by the USA [8,9] for deployment in Europe during World War I, but when Germany capitulated, the material was quickly transported by train to the East Coast and dumped in the Atlantic Ocean. Military use of Lewisite has not been proven, although Japan may have used it against China in 1937–1944 [10]. Lewisite comprises three compounds called Lewisite I (major component), Lewisite II and Lewisite III (minor component) [11]. Its blistering ability is believed to be due to reaction of Lewisites I–II and their hydrolysis products with sulfhydryl-containing proteins in skin [12–16]; Lewisite III is inert to nucleophiles and does not cause blisters. Both sulfur mustard and Lewisite are volatile liquids that readily evaporate and they feature in Schedule One of the Chemical Weapons Convention [17]. Methods for their analysis are becoming increasingly important as more effort is being spent on rendering safe old munitions and contaminated landfill sites [18]. Overshadowing environmental issues is the threat of their use by terrorists. Therefore, forensic methods for analysing these chemicals and their 'signatures' (e.g. impurities, hydrolysis products) are of paramount importance [19]. Being able to analyse for sulfur mustard and Lewisites I–III simultaneously is of interest as mustard–Lewisite mixtures have been stockpiled by Russia [20]; due to its low freezing point, the mixture remains a liquid in cold weather and at high altitudes.

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Sulfur mustard can be analysed without difficulty by gas chromatography (GC) unlike compounds with As–Cl bonds, which are much more reactive and corrosive. Analysis of Lewisites I and II by GC results in rapid deterioration of the column. Lewisite III may be analysed directly but is of lesser importance. The volatility of the Lewisite compounds decreases in the order I (bp 196 °C) < II (230 °C) < III (260 °C). Given that Lewsite I is likely to be the major constituent in weaponised Lewisite, and that it is the most volatile and hazardous component, more effort has gone into developing methods for its analysis relative to the other arsenicals. Lewisite I has been determined in atmospheric samples using flame spectrophotometry [21], with ethanolamine derivatisation [22], flow injection [23], atomic emission [24] and ion mobility spectroscopy [25]. These methods involve indirect determination and lack sensitivity. Tenax TA has been used to trap Lewisite I with derivatisation with methanethiol—the derivative was analysed by GC with flame photometric detection [26]. Other aliphatic thiols [27,28] and some dithiols [29,30] have been used to derivatise Lewisites I and II.

The first paper in this series reported a method for determination of Lewisite compounds in liquid hydrocarbon matrices using a series of normal aliphatic thiols [31]. We now report a method for the derivatisation of Lewisites I–II with butanethiol, pentanethiol, hexanethiol, heptanethiol, and 3,4-dimercaptotoluene on Tenax TA in the presence of sulfur mustard and Lewisite III. Although 3,4-dimercaptotoluene has been used to derivatise Lewisite I [29,32], derivatisation on-tube with thermal desorption has not been reported. We optimised thermal desorption parameters using statistical experimental design to allow analysis of trace levels of Lewisites I–III and sulfur mustard in air samples. Structures of the chemical warfare agents and thiol derivatives investigated in this study appear in Fig. 1. Both intact and hydrolysed Lewisite will be derivatised with thiols, but owing to the vapour pressure and polarity of the hydrolysed compound, the method is likely to only yield measurement of intact volatilised Lewisite present in the headspace of the reaction.

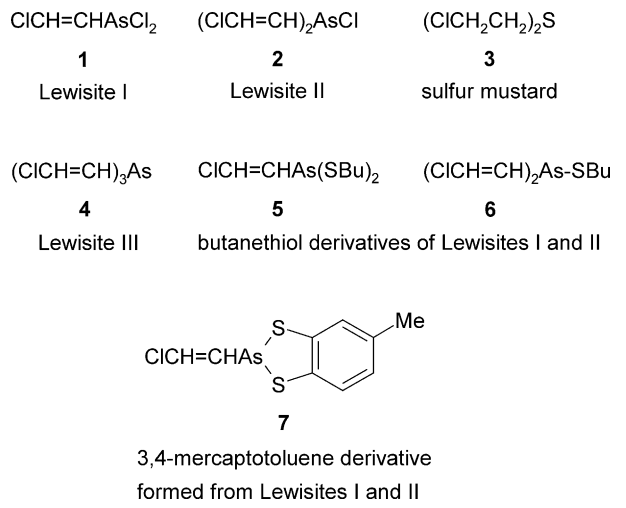

Fig. 1. Structures of vesicant compounds and their thiol derivatives.

2. Experimental methodology

2.1. Chemicals

Sulfur mustard [33] and Lewisites I–III were synthesised in-house [9] in an efficient, dedicated fume-cupboard. Heavy rubber gloves, a chemical-resistant Microgard smock (Orvec International, Hull, UK) and a face visor were worn. A perspex safety shield was used during distillations. Butanethiol, 3,4-dimercaptotoluene, methanol and triethylamine were obtained from Aldrich (Gillingham, UK), pentanethiol and hexanethiol from Acros Organics (Loughborough, UK) and heptanethiol from Lancaster Chemicals (Morecambe, UK). All were at least 95% pure and were used as received. Thermal desorption tubes packed with 100 mg Tenax TA were conditioned at 200 °C for 90 min, 250 °C for 30 min, then 340 °C for 30 min, in a flow of nitrogen (100 ml min^{-1}). About one quarter of the tubes were selected at random and desorbed in the thermal desorption system. No significant peaks above the baseline were observed; further cleaning was unnecessary.

2.2. Derivatisation procedure

2.2.1. Monothiol derivatives of Lewisites I and II (compounds **5** and **6**)

Tubes were spiked with 100 μl of a 1.5 μg ml^{-1} solution of thiol in methanol (150 ng on tube) using a vapour loading rig (Marks International, UK) at 50 ml min^{-1} nitrogen. Ten microliters of a 50 μg ml^{-1} solution of sulfur mustard and Lewisites I–III in methanol (500 μg on tube) and 10 μl of a 1.5 μg ml^{-1} solution of triethylamine in methanol were loaded onto the tube. Although it would have been preferable to use tubes loaded from a vapour generator, such loading methods generally show poor precision and this would have masked the influence of varying experimental parameters.

2.2.2. Dithiol derivative of Lewisites I and II (compound **7**)

Tubes were spiked with 10 μl of a 1.2 mg ml^{-1} solution of 3,4-dimercaptotoluene in methanol by vapour loading. 8.5 μl of a 1.2 mg ml^{-1} solution of Lewisite I in methanol was then injected. Loading conditions were identical to those used for monothiols.

2.3. Mathematical models and optimisation of thermal desorption parameters

2.3.1. Plackett–Burman design for compounds **3**–**6**

The theory and application of experimental design is reviewed elsewhere [34,35]. Initially a Plackett–Burman model (resolution III) was constructed for investigation of six parameters that would potentially influence the chosen response

Table 1
Plackett–Burman design matrix and corresponding p values ($p = 0.05$) for investigation of influence of desorption parameters on response of compounds **3**–**6**

Parameter	Level values			Significance (95% C.I.)			
	−1	0	+1	Compound **3**	Compound **4**	Compound **5**	Compound **6**
x_1	200	250	300	***0.01***	***0.01***	>0.05	>0.05
x_2	5	12.5	20	***0.02***	***0.04***	>0.05	>0.05
x_3	180	220	260	>0.05	***0.01***	***0.03***	***0.02***
x_4	200	250	300	>0.05	>0.05	>0.05	>0.05
x_5	Slow		Fast	>0.05	>0.05	>0.05	>0.05
x_6	37	93.7	150	>0.05	>0.05	>0.05	>0.05

x_1: desorption temperature (°C); x_2: desorption time (min); x_3: valve temperature (°C); x_4: trap high (°C); x_5: trap rate (°C s^{-1}); x_6: thiol mass (μg). 1 and -1 indicate a factorial point, 0 indicates a centre-point.

(peak area) of the four compounds. The design comprised six factors: desorption temperature (x_1), desorption time (x_2), valve temperature (x_3), trap high temperature (x_4), trap heating rate (x_5) and thiol mass (x_6). Although limited in power to detect higher order interactions, this design was used as a screening method to eliminate superfluous variables prior to development of a response surface design. A single replicate of each run was performed with two centre points added to detect curvature. The Plackett–Burman design employed is shown in Table 1.

2.3.2. Central composite design for compounds *3*–*6*

Response surface methodology allowed the response of the system (peak area) to be optimised with respect to three parameters: desorption temperature, desorption time and valve temperature. These factors were shown to influence the peak areas of the analytes from the Plackett–Burman design and hence warranted further investigation. A rotatable, orthogonal central composite design (CCD) allowed empirical relationships between system response and these parameters 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_{jk} the variable of system, and β_0, β_j, β_{jj} and β_{jk} are regression coefficients for constant, linear, square and interaction terms, respectively.

Regression coefficients were calculated by fitting the values of experimental parameters to the least squares regression line. A quadratic equation or an equation containing only significant terms resulted. This was then used to predict the response of the system for a given value of an experimental parameter. The experimental domain levels for the CCD appear in Table 2.

The CCD consists of a star design imposed through the centre of a factorial design. The three-factor design used in this investigation comprised a 2^3 factorial design (eight experiments), a star design (six experiments) and 6 centre points (six in factorial portion and zero in star portion). The resulting 20 experiments were run in a random order in one block. An α value of 1.68 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.

$$\alpha = \pm(N_F)^{1/4} = \pm 1.68 \quad (2)$$

where N_F is the number of experiments in factorial portion of design (8).

From the 20 experiments performed, a global optimum for compounds **3**–**6** was established. All model assumptions were confirmed using residual plots and normal probability plots. There was no evidence of lack of fit for any of the models subsequently described.

2.3.3. Central composite design for dithiol derivative (compound *7*)

Using the results obtained from the Plackett–Burman model, parameters x_1, x_2 and x_6 were chosen for optimisation. A three-factor CCD was selected to model the influence of desorption temperature, desorption time and weight of derivatising reagent on observed response of the dithiol derivative **7**. A face-centred model requiring 20 experiments was chosen (it was thought unnecessary to extend the domain to five points for each variable, a conclusion based on the results of optimisation for monothiol derivatives **5** and **6**). The experimental domain for this design, with $\alpha = 1$, is shown in Table 3.

2.3.4. Analysis of vesicant compounds and associated derivatives by TD–GC–MS

GC–MS was performed on a Hewlett-Packard 5890 GC system (series 2) interfaced to a Hewlett-Packard 5971A mass-selective detector. A DB5-MS capillary column (25 m × 0.25 mm, 0.25 μm) was used with an initial GC oven temperature of 40 °C maintained for 2 min then increased at a rate of 20 °C min^{-1} to 160 °C. A second ramp of 30 °C min^{-1} was employed to reach 310 °C and this temperature was held for 2 min. The MS instrument was operated in positive electron impact (EI) mode with an electron energy of 70 eV. Initially, the MS system was operated in scan mode between m/z 50 and 400 (2.16 cycles s^{-1}). Selected ion monitoring (SIM) experiments were conducted; diagnostic ions chosen (m/z) were as follows:

Table 2
Level values, p values and regression coefficients of CCD model for analysis of compounds **3**–**6** (significant p values at the 95% confidence level indicated in bold italics)

Parameter	Level values				Model parameters							
					p values ($p = 0.05$)				β			
	−1.68	−1	0	+1	+1.68	Compound **3**	Compound **4**	Compound **5**	Compound **6**	Compound **3**	Compound **4**	Compound **5**	Compound **6**
x_1	200	220	250	278	300	0.293	0.026	0.427	0.709	2538696	698478	703146	1751576
x_2	5	8	12.5	17	20	0.171	0.136	0.108	0.102	5883660	1323438	1139263	−5719993
x_3	180	196	220	244	260	0.280	0.743	0.347	0.181	−677797	−399789	60017	1101363
x_1^2						***0.083***[a]	0.352	***0.083***[a]	**0.003**	−4744.67	−1433	−1175.76	−3163.06
x_2^2						0.164	0.329	***0.073***[a]	0.155	164836	67022	54411.8	−54311.8
x_3^2						0.879	0.570	0.546	**0.030**	601.616	−1348.38	−597.181	−3137.17
x_1x_2						***0.099***[a]	***0.023***	**0.022**	0.796	−40168.6	−35232.1	−14876.9	−1885.42
x_1x_3						0.655	0.313	0.711	0.606	1905.72	2617.44	391.651	−708.161
x_2x_3						0.900	0.112	0.361	**0.004**	3538.42	28608.8	6542.4	32993.7

β = regression coefficient.
[a] Significant effect on **3** and **5** response at the 90% confidence level.

Compound **3** = 109, 111, 158, 160.
Compound **4** = 136, 138, 145, 258.
Compound **5** = 145, 203, 229, 286.
Compound **6** = 164, 204, 229, 314.

Following optimisation of thermal desorption parameters, tubes were desorbed and analysed by TD–GC–MS–SIM. A Perkin-Elmer ATD 400 system was used for thermal desorption of spiked tubes. ATD parameters were varied to optimise the system and are discussed later. A thermal desorption blank was run between each optimisation experiment where the parameters were all set at their maximum values.

For compounds **5** and **6**, a tube loading of 500 ng was used as described in Section 2.2.1. 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} gave approximately 50 ng of analyte on column resulting in a signal-to-noise ratio of about 30:1 for each analyte in the total ion chromatogram. Other parameters were set as follows: desorption time = 5 min, desorption temperature = 250 °C, valve temperature = 200 °C, line temperature = 225 °C, desorption flow = 50 ml min^{-1}, and outlet split flow = 10 ml min^{-1}.

2.3.5. Calibration and limits of detection (LODs)

A series of standard solutions containing compounds **3**–**6** were prepared in concentrations of 5, 10, 20, 40, 80 and 160 μg ml^{-1} in hexane. Ten microliters of each standard was injected and derivatised as described in Section 2.2. Derivatives were characterised from interpretation of EI fragmentation patterns and ions suitable for SIM selected. Calibration plots were linear for each thiol derivative.

Detection limits were calculated for GC–MS–SIM as described by Miller and Miller [36]. Each standard was run in triplicate and linear regression analysis performed to give regression coefficients for each derivative. In all equations, the intercept term was found to be non-significant ($p > 0.05$) and was excluded from the final equation. The limit of detection is given by Eq. (3). Standard error and slope terms refer to parameters calculated by performing linear regression analysis on each derivative concentration and resulting peak area. The standard deviation is that of the fitted line from the predicted

Table 3
Level values, p values and regression coefficients for CCD model for analysis of compound **7** (significant p values at the 5% level indicated in bold italics)

Parameter	Level values			Model parameters	
	−1	0	1	p values ($p = 0.05$)	β
x_1	200	275	350	***0.013***	−5.9e+6
x_2	5	12.5	20	0.105	8.6e+6
x_6	12	66	120	***0.022***	−9.7e+6
x_1^2				0.562	10071
x_2^2				0.552	1e+6
x_6^2				0.335	32853
x_1x_2				***0.019***	−2.7e+5
x_1x_6				0.067	28081
x_2x_6				***0.018***	−3.8e+5

line.

$$\text{LOD} = \frac{3\text{S.E.}}{\text{slope}} \quad (3)$$

2.3.6. Stability trials

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. The 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.

After optimisation of thermal desorption parameters, stability trials were carried out using three sets of six tubes loaded with 94 μg butanethiol and 200 ng of each compound loaded by vapour injection. Tubes spiked with compounds **3**–**6** were stored at room temperature (21 °C), in a refrigerator (2 °C) and in a freezer (−5 °C) for each of five time periods (1, 7, 14, 21 and 28 days). A similar procedure was adopted for dithiol derivative **7** with storage periods of 7, 14 and 30 days at room temperature or in a refrigerator.

2.3.7. Application of method to the quantitative determination of sulfur mustard and Lewisite compounds in a decontamination trial

The headspace above a sulfur mustard–Lewisite mixture was sampled during a decontamination trial. The trial involved the treatment of such mixtures at various temperatures with candidate hydrolysis-inducing compounds. Headspace samples were collected using a calibrated pump set at 1 l min^{-1} for a time of 2 min. The quantity of intact vesicant compounds in the headspace samples was compared to the quantity in the liquid and the degree of hydrolysis evaluated. Data from this trial cannot be fully discussed due to commercial restrictions, but a chromatogram of a headspace sample generated from the trial is included in Section 3.

3. Results

3.1. Investigation of the influence of desorption parameters on analyte response

Preliminary experiments with pentanethiol, hexanethiol and heptanethiol showed greatly reduced peak areas compared to butanethiol. The former thiols reacted with Lewisites I and II, as shown previously [31], the reduced peak area probably caused by incomplete desorption from the sorbent. For this reason, all subsequent experiments were performed using butanethiol (to form derivatives **5** and **6**) or 3,4-dimercaptotoluene (to form derivative **7**).

3.2. Butanethiol derivatives of Lewisites I and II (compounds *5* and *6*)

3.2.1. Plackett–Burman design

Initially a Plackett–Burman design was performed to assess which thermal desorption parameters were likely to influence the response observed for each compound. This enabled non-significant variables to be excluded from the subsequent central composite design (refer to Table 1 for *p* values for this design for six factors and four response signals).

Compounds **3** and **4** are significantly influenced by desorption temperature (x_1). Desorption time (x_2) influences the response of sulfur mustard **3** while butanethiol derivatives **5** and **6** are influenced by valve temperature (x_3). None of the compounds is influenced by cold trap high temperature (x_4), cold trap heating rate (x_5) or thiol mass (x_6). Therefore, x_4 and x_6 were set to their centre point values of 250 °C and 94 μg, respectively, while x_5 was set to high to ensure rapid transfer of analyte to the column. The value of 250 °C for x_4 ensured desorption of all analytes from the cold trap while operating at a modest temperature, thus prolonging the lifetime of the trap. The midpoint value of x_6 was adequate for complete derivatisation of Lewisites I and II and did not result in carryover of excess thiol.

Variables x_1, x_2 and x_3 were selected for further evaluation by CCD since the Plackett–Burman design highlighted only main effects—higher order effects and interactions were confounded.

3.2.2. Central composite design

A three-factor CCD was constructed with the domain shown in Table 2. Experiments were performed in one block with randomisation (Table 2 gives the *p* values for the analysis of each of the response variables using a full quadratic model and the corresponding regression coefficients).

There was no evidence of any influence of x_1x_3 for sulfur mustard (compound **3**) at the 95% confidence level. Therefore, a model was constructed for sulfur mustard at the 90% confidence level. Development of the model showed x_1^2 and the interaction term x_1x_2 significantly influenced the response of sulfur mustard. Increasing desorption temperature increased the response presumably by removing more sulfur mustard from the sorbent. Fig. 2a shows a characteristic saddle-shape indicative of second order (x_1^2) and interaction effects (x_1x_2) when two possible optimal regions exist at the edge of the experimental domain. Desorption of sulfur mustard is enhanced by increased desorption time and temperature.

The response of Lewisite III (compound **4**) shows a positive linear effect of x_1. Again increased desorption time is

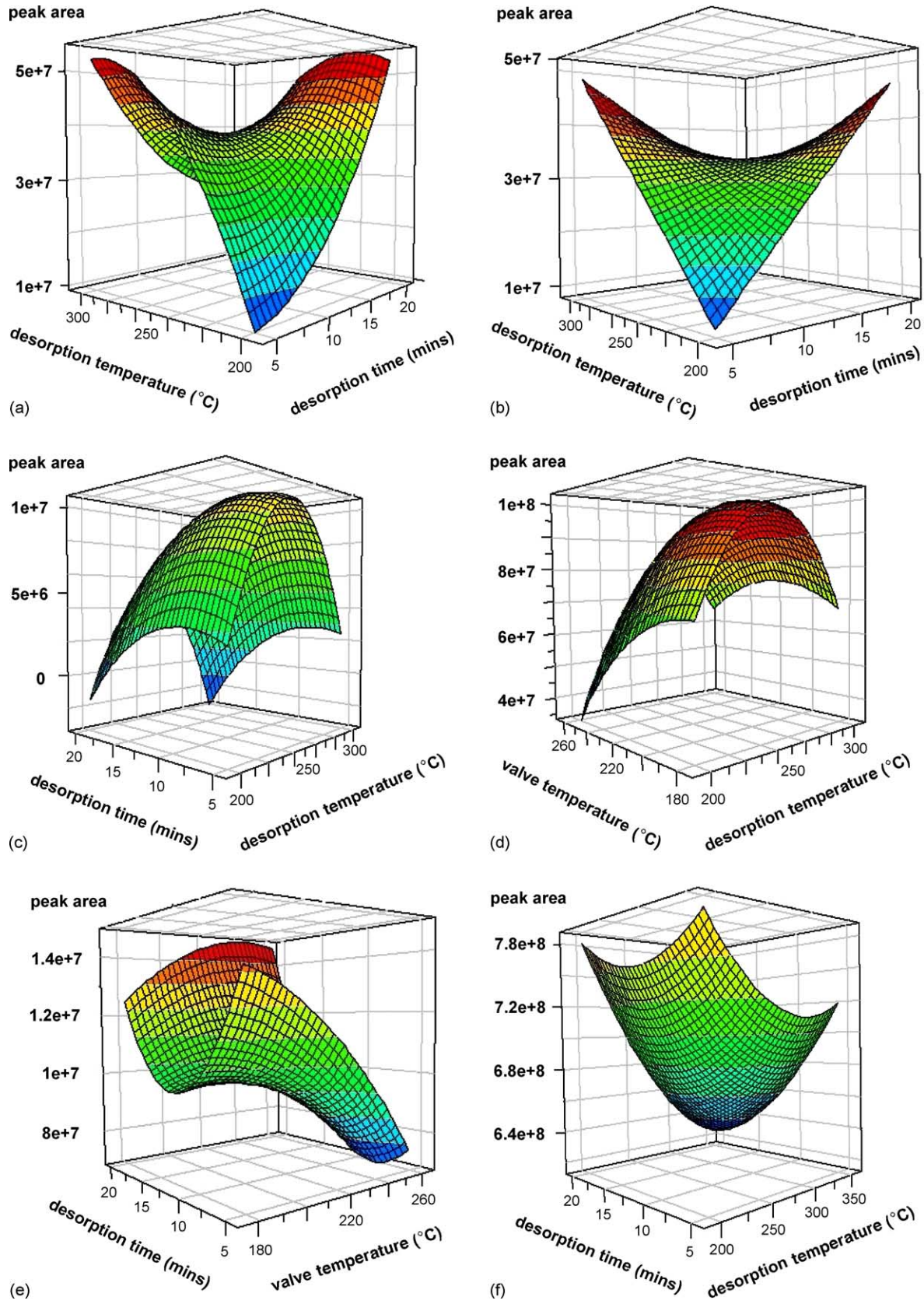

Fig. 2. Surface plot of peak areas of compounds **3**–**6** during optimisation experiments. (a) Compound **3** with desorption temperature and desorption time, (b) compound **4** with desorption temperature and desorption time, (c) compound **5** with desorption time and desorption temperature, (d) compound **6** with desorption temperature and valve temperature, (e) compound **6** with desorption time and valve temperature, (f) compound **7** with desorption temperature and desorption time.

favourable (Fig. 2b). Therefore, increased desorption time in conjunction with high desorption temperature should be employed. This is analogous behaviour to sulfur mustard.

There is no statistical evidence of a linear relationship between any of the factors in the case of the butanethiol derivative of Lewisite I (compound **5**). However, there is strong evidence of curvature in the relationship between x_1 (x_1^2) and x_2 (x_2^2). There is also a significant interaction between x_1 and x_2 which suggests that increased desorption time at elevated desorption temperatures greatly decreases the response (Fig. 2c). Increasing values of desorption temperature initially result in increased peak areas due to efficient desorption of the derivative from the sorbent. The response reaches a maximum at 250 °C, thereafter decreases, presumably due to decomposition of the derivative on the sorbent. Similar observations are noted for valve temperature suggesting thermal decomposition in the valve and during primary desorption above certain temperatures.

The response of the butanethiol derivative of Lewisite II (compound **6**) is influenced by x_1^2 and x_3^2 and the associated interaction term (x_2x_3). The influence of x_1^2 on the response shows an optimum at around 250 °C (Fig. 2d). The square term for valve temperature (x_3^2) also influences the response. Increased desorption times (x_2) are required to completely remove compound **6** from the sorbent which is consistent with this derivative having the highest molecular weight of the four analytes; more energy is required to remove it from the sorbent. This effect is related to the interaction term (x_2x_3) and acts to increase the response of compound **6** (Fig. 2e). The inverted saddle-shape is unusual and seems to indicate similar response when x_2 is 5 or 20 min. Confirmatory experiments at both values of x_2 revealed short desorption times and low valve temperatures are favourable for analysis of compound **6**.

Interestingly, as values of desorption temperature increase, there is a corresponding decrease in response (refer to Fig. 2a–e), indicating degradation of compounds **3**–**6**. This illustrates the importance of second order interactions. This is perhaps not surprising as the flow path of the thermal desorption unit and the sorbent tubes are made of stainless steel which may initiate or catalyse the thermal degradation.

3.2.3. Dithiol derivative of Lewisites I and II (compound 7)

Table 3 lists the parameter p values and regression coefficients obtained from the CCD for compound **7**. Parameters x_1 and x_6 influence the response of this compound by decreasing the peak area. The interaction x_1x_2 as shown in Fig. 2f suggests increased desorption times at high desorption temperatures results in an increased signal. This is similar behaviour to that of compounds **3** and **4** suggesting this derivative is more stable with respect to temperature than the monothiol derivatives **5** and **6**. There is no evidence of a linear effect for x_2 and square terms do not influence the response of compound **7** but interactions are important. The interaction x_1x_6 ($p = 0.05$) is marginal but was included in the model. Overall the results show the thermal desorption process of this compound should be performed at high temperatures resulting in a gradual accumulation of analyte in the cold trap over time.

Table 4
Optimised parameter values for maximisation of compound response

Parameter	Compound					
	3	**4**	**5**	**6**	**3–6**[a]	**3–6**[b]
x_1	235	300	300	250	250	280
x_2	20	5	5	5	5	5
x_3	260	200	200	180	200	180

[a] Indicates optimum conditions for maximum response of compound **5** in the presence of **3**, **4** and **6**.
[b] Indicates global compromised optimum conditions giving equal importance to all compounds.

3.3. Mathematical modelling and optimisation of system response

3.3.1. Compounds 3–6

Regression equations can be derived to explain response of each analyte using the regression coefficients in Table 2. The equations for the four analytes are described by Eqs. (4)–(7).

$$\text{Compound } \mathbf{3} = -2.7\text{e}^{+8} + (164836x_1^2) + (-40168x_1x_2) \quad (4)$$

$$\text{Compound } \mathbf{4} = -3.9\text{e}^{+7} + (1323438x_1) + (67022x_1^2) \quad (5)$$

$$\text{Compound } \mathbf{5} = -9.3\text{e}^{+7} + (54411x_1^2) + (-1175x_2^2) + (-14876x_1x_2) \quad (6)$$

$$\text{Compound } \mathbf{6} = -2.8\text{e}^{+8} + (-54311x_1^2) + (-3137x_2^2) + (-708x_2x_3) \quad (7)$$

A local maximum response for each compound within the experimental domain can be determined using the response optimisation approach [37]. This involves listing the low and high values for response and applying a maximisation algorithm. Optimum conditions for response of each compound were calculated using the desirability function (for results, refer to Table 4). The purpose of the present analysis was simultaneous quantitation of all four compounds (**3**–**6**), therefore a response optimisation was performed placing greatest emphasis on the butanethiol derivative of Lewisite I (compound **5**) since this compound gave the lowest average peak area throughout the experiment. Optimum conditions for simultaneous chromatographic determination of the four analytes placing greatest emphasis on compound **5** are shown in Fig. 3. A desorption temperature 250 °C results in complete desorption without thermally induced decomposition.

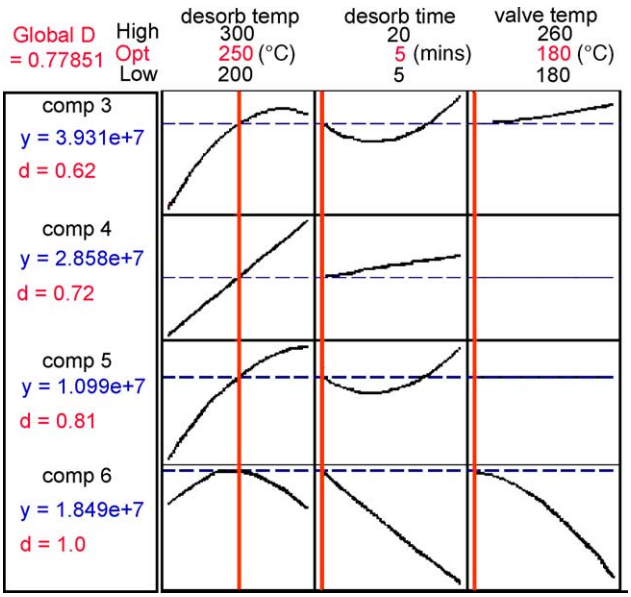

Fig. 3. Compromised global optimum for compounds **3**–**6** placing greatest priority on response maximisation of compound **6**.

A minimal desorption time of 5 min will also favour maximisation of this response. This is consistent with practical requirements; time should be minimised to give maximum throughput of samples and desorption temperature should be low to prolong the longevity of the sorbent.

The optimum valve temperature for compound **5** was calculated as 180 °C. However, this resulted in intermittent valve failure and hence a valve temperature of 200 °C was employed. From the predictive model this represented a decrease of 13% for the signal of compound **5**. This decrease in response and hence sensitivity was deemed acceptable as desirable LODs were still achieved with greater instrument reliability.

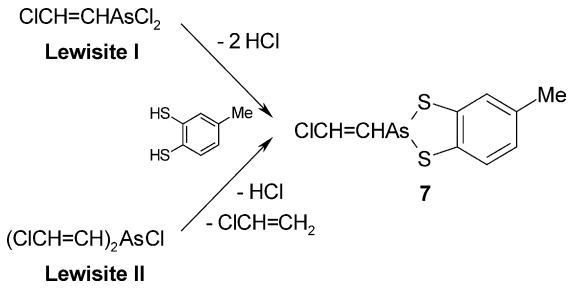

Fig. 5. Lewisites I and II form a common adduct with 3,4-dimercaptotoluene.

Reference to Fig. 3 indicates the desirability (*d*) for compounds **3**, **4** and **6** was modest as expected. A compromise optimisation resulted in a decrease in response of 12% for compound **3** when compared to optimum desorption temperature of 280 °C. Again this was deemed acceptable for this analysis. Table 4 also indicates the optimum conditions for determination of the compounds simultaneously by placing equal importance on maximising all responses. The total ion chromatogram employing GC–MS–SIM of compounds **3**–**6** using these compromise optimum conditions is shown in Fig. 4.

3.3.2. *Compound 7*

High-desorption temperatures result in increased response of this compound and hence should be set at the upper limit of 350 °C. The interaction x_1x_2 increases response with longer desorption times being favoured. Fig. 2f summarises the influence of desorption time and desorption temperature on response. The regression model obtained for compound **7** is shown in Eq. (8). Optimum conditions were a desorption temperature of 350 °C for 20 min using 12 μg of thiol.

$$\text{Compound } \mathbf{7} = 1.5 \times 10^9 + (-5.9 \times 10^6 x_1) + (-1.1 \times 10^7 x_6) + (3.1 \times 10^5 x_1 x_2) + (-4.1 \times 10^5 x_1 x_6) \quad (8)$$

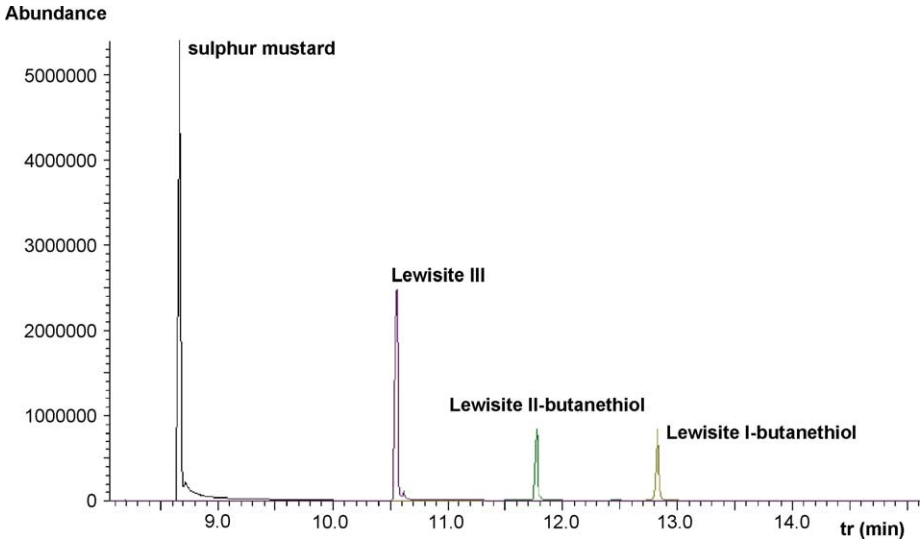

Fig. 4. Extracted ion chromatogram of compounds **3**–**6** (5 ng each on column) analysed by placing greatest emphasis on maximising signal of compound **5**.

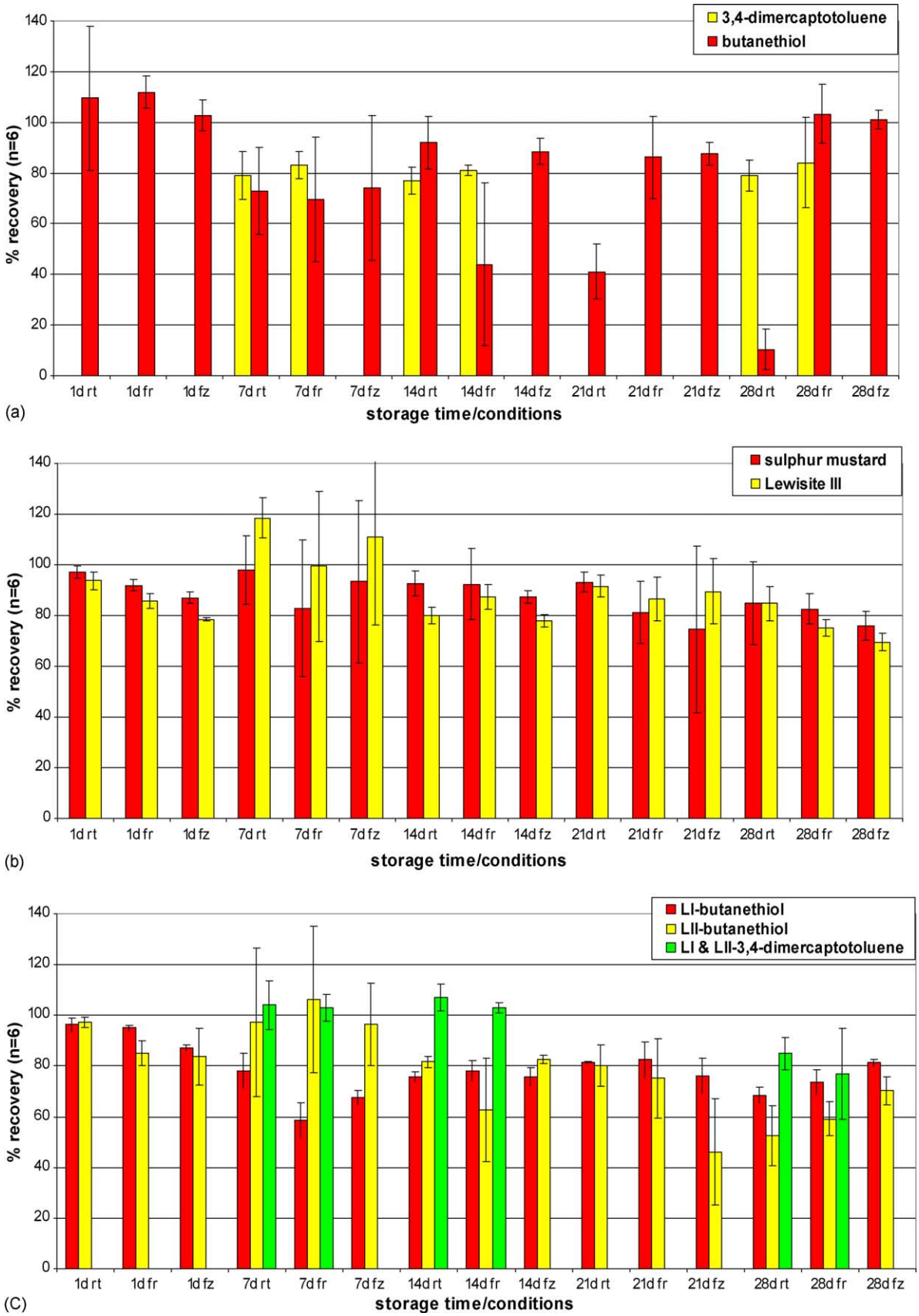

Fig. 6. Summary of storage stability trials for compounds **3**–**6** and associated derivatising reagents. (a) 3,4-Dimercaptotoluene and butanethiol. (b) Sulfur mustard and Lewisite III. (c) Lewisite I and II–butanethiol derivatives.

Compound **7** was analysed in a different experimental run from compounds **3** to **6** as injecting two thiols onto a Tenax TA tube would have given complex results. Derivatisation of Lewisites I and II with 3,4-mercaptotoluene using splitless or on-column injection, or on-tube, gave a common product, compound **7**. Although expected for Lewisite I, this outcome was not expected for Lewisite II, and suggests that a second nucleophilic attack can take place with loss of chloroethene. The reaction is most likely driven by the formation of two strong As–S bonds and a stable five-membered ring (Fig. 5) [38–40]. In the context of this paper, the reaction of Lewisites I and II with 3,4-mercaptotoluene might be of use when total Lewisite is of interest, but the overestimation of concentration of Lewisite I and underes-

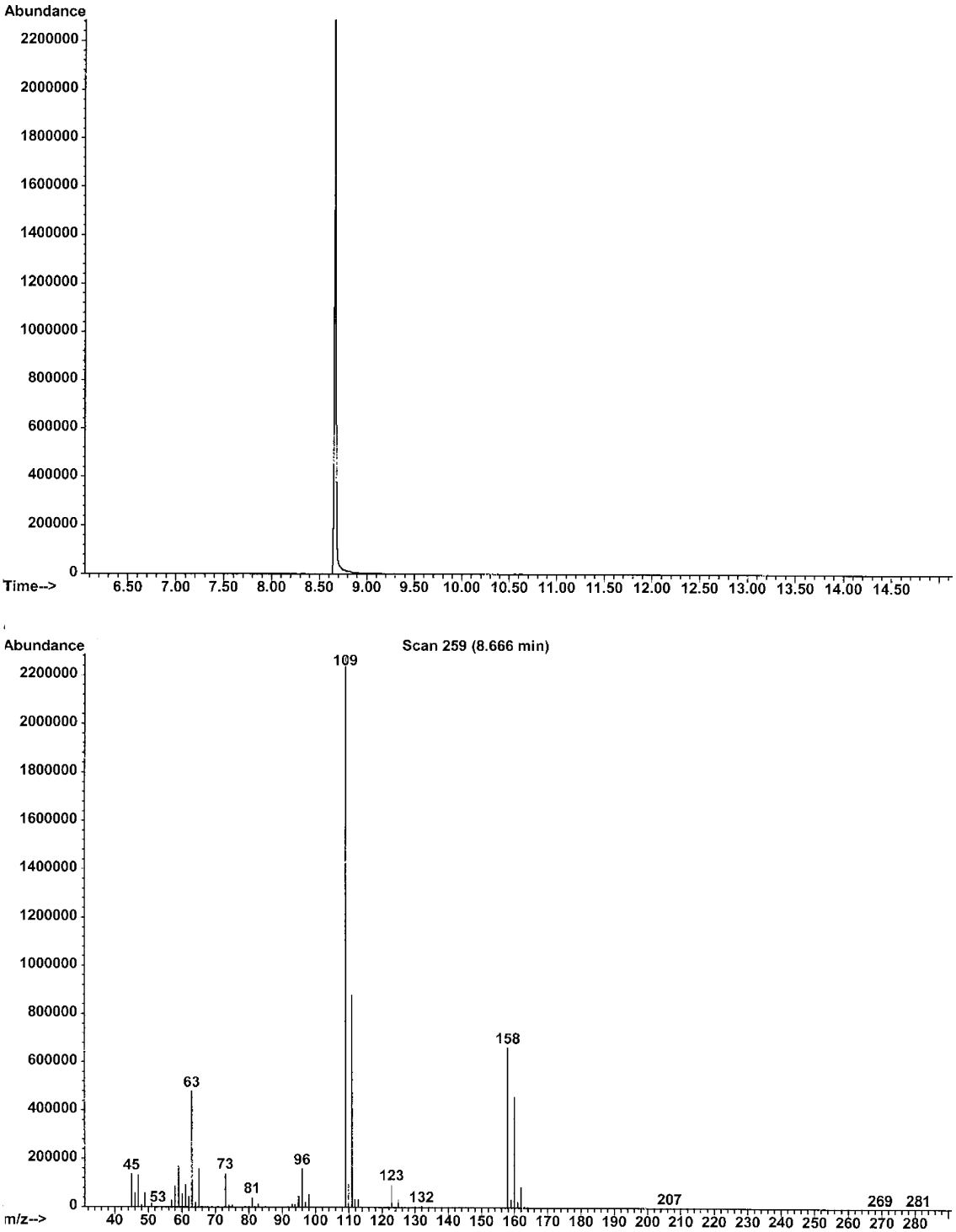

Fig. 7. Total ion chromatogram and mass spectrum of a headspace sample taken during a decontamination trial showing the presence of sulfur mustard.

timation of concentration of Lewisite II must be acknowledged.

3.3.3. Evaluation of model accuracy

Mathematical models for the response of each compound described in Section 3.2 were evaluated to establish prediction accuracy. Five Tenax TA tubes were spiked with 12 μg of thiol and 800 ng of compounds **3**–**6** and the tubes desorbed using the optimum conditions established in Section 3.2. The predicted response of each compound employing these conditions was obtained from the appropriate model, and predicted and observed values compared. Model accuracy was found to be 73, 95, 76 and 72% for compounds **3**, **4**, **5** and **6**, respectively. These values proved that the CCD models gave an acceptable estimate of compound response. The accuracy of the model with respect to compound **7** was not evaluated.

3.3.4. Limits of detection and linearity

All calibration plots were linear over the calibration range. LODs were calculated as described in Section 2.3.5. Linear regression analysis and application of Eq. (3) yielded detection limits of 12, 11, 15, and 19 ng on column for compounds **3**–**6**, respectively. In a 2 l air sample and employing a split flow of 10 ml min^{-1}, atmospheric detection limits were calculated as 24, 35, 28, and 29 μg m^{-3}, respectively. These figures, even for the small volume of air sampled, represent superior LODs to methods described previously. The LOD for the 3,4-dimercaptotoluene derivative **7** was established as 260 μg m^{-3}, considerably higher than that of the butanethiol derivatives **5** and **6**. The precision of replicates at all concentration levels was excellent with coefficients of variation typically less than 5%. Establishment of breakthrough volumes for the analytes will potentially allow more air to be sampled and perhaps improve LODs.

3.3.5. Storage stability trial

Tubes were spiked and stored as described in Section 2.3.6. Percentage recoveries of compounds **3**–**7**, butanethiol and 3,4-dimercaptotoluene under each storage condition over the five time periods evaluated are shown in Fig. 6a–c. Although butanethiol was added in excess, this species was monitored to observe any formation of dibutyl disulfide. Fig. 6 does not show the percent recovery of dibutyl disulfide, which was detected in samples. With the exception of dibutyl disulfide, storage conditions were not statistically significant in the ANOVA for any compound. This indicates that butanethiol, 3,4-dimercaptotoluene and compounds **3**–**7** are stable when stored under each condition. Dibutyl disulfide showed an increase over the time period with most being observed after 21 and 28 days. This was pronounced in samples stored at room temperature and was also coincident with the minimum recovery of butanethiol. This supports the thesis of slow conversion to the disulfide on storage at ambient temperature.

Storage time was shown to significantly affect recovery of analytes. Greater than 90% of sulfur mustard (compound **3**) was recovered from 1 to 21 days after spiking. Recovery decreased to 81% after 28 days. Lewisite III (compound **4**) showed analogous behaviour with recovery falling to a mean of 76% after 28 days. An initial recovery of 93% for the butanethiol derivative of Lewisite I (compound **5**) decreased to 75% after 7 days with this percentage recovery being exhibited for the remainder of the storage trial. A significant decrease in recovery of the butanethiol derivative of Lewisite II (compound **6**) from a mean of 88% after 1 day to 64% after 21 days was observed.

The 3,4-mercaptotoluene derivative of Lewisites I and II (compound **7**) was not significantly affected by storage time. Recovery of 3,4-dimercaptotoluene decreased to around 80% after 7 days and remained at this level for the duration of the study. This may imply that formation the dithiol analogue of 3,4-dimercaptotoluene is less favoured than the corresponding butanethiol analogue.

3.3.6. Application of method to the quantitative determination of sulfur mustard and Lewisite compounds in a decontamination trial

Vesicant compounds were detected and quantitated in headspace samples, using butanethiol derivatisation, within a few days of the experiments being conducted. Depending on the conditions, compounds **3**–**6** were observed in the samples. Fig. 7 illustrates a total ion chromatogram of a headspace sample taken during the decontamination study. The chromatogram shows the presence of sulfur mustard but the absence of lewisite compounds. Lewisite compounds were only detected at trace levels in this study as the hydrolysis rate of Lewisite is 50 times greater than that of sulfur mustard. As expected, the concentration of each compound in the headspace was influenced by the temperature and candidate hydrolysis-inducing reagent.

4. Conclusions

Sequential application of statistical experimental design has allowed TD–GC–MS method to be developed for analysis of sulfur mustard and Lewisites I–III in ultra-trace amounts in air samples. Butanethiol derivatives of Lewisites I and II showed significant thermal-induced decomposition in the thermal desorption unit. Decomposition occurred during tube desorption (derivative of Lewisite I and II) or when passing through the heated valve (derivative of Lewisite II). A short desorption time was adopted (5 min) to reduce this effect for the butanethiol derivative of Lewisite I and II which led to a compromise desorption method. However, limits of detection of about 30 μg m^{-3} were achievable in a 2 l air sample, approximately 10 times greater than that of the 3,4-dimercaptotoluene derivative of Lewisites I and II.

Storage conditions did not influence the recovery of any compound in this study. Sulfur mustard was quantitatively recovered (>90%) from 1 to 21 days after spiking. The recovery decreased significantly ($p < 0.05$) to 81% after 28

days. Lewisite III showed analogous behaviour with recovery falling to a mean value of 76% after 28 days. The initial recovery of 93% for the butanethiol derivative of Lewisite I decreased to 75% after 7 days with this percentage recovery being exhibited for the remainder of the storage trial. A statistically significant decrease in the recovery of the butanethiol derivative of Lewisite II from a mean value of 88% after 1 day to 64% after 21 days was noted. The Lewisite–3,4-dimercaptotoluene derivative was not influenced by storage condition with 80% being recovered after 28 days. Therefore, the above time periods should be acknowledged when the method is used for atmospheric sampling. This will ensure adequate sample will be present on the tube when received by the laboratory for analysis.

The optimised analytical method was employed in a decontamination trial to allow successful quantitation of vesicant species in headspace/air samples.

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