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Optimisation of solvent desorption conditions for chemical warfare agent and simulant compounds from Porapak Q™ using experimental design I. Methyl salicylate and di(propylene glycol) monomethyl ether

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Abstract

Factorial design (FD) was applied in order to develop an optimised method for the detection of chemical warfare (CW) agent simulant compounds on Porapak Q™. Application of FD allowed study of the adsorption/desorption mechanism of analytes. Di(propylene glycol) monomethyl ether (DPM) and methyl salicylate (MS) were selected for study as both compounds are employed in agent simulation trials but are currently analysed by different methods. An analytical method for simultaneous determination of both compounds was developed using solvent desorption. The optimised method identified non-polar interactions as the primary adsorption/desorption mechanism. Steel tubes were shown to be more suited for sampling of simulants, due to lower variability in recovery compared to glass tubes. Atmospheric detection limits for both simulants were estimated to be 0.2 mg m^{-3} allowing the trace analysis of these compounds by gas chromatography with flame ionisation detection (GC-FID).

Keywords: Optimisation; Solvent desorption; Experimental design; Factorial design; Methyl salicylate; Di(propylene glycol) monomethyl ether; Chemical warfare agents; Simulant compounds

1. Introduction

Due to the extreme toxicity of sulphur mustard and nerve agents, less toxic simulant compounds have been used in order to predict the behaviour of chemical warfare (CW) agents in development of defensive countermeasures.

Di(propylene glycol) monomethyl ether (DPM) and methyl salicylate (MS) are both used as CW simulants. The use of DPM as a G-agent series simulant stems from similarities in physical properties such as boiling point and vapour pressure. They are used to test equipment, such as the chemical agent monitor (CAM), in situations where live agent is not a viable option. DPM and MS have been shown to have substantially lower toxicity than the agents they simulate and give signals on CAM and other field portable military detection systems currently employed by UK armed forces. MS and DPM have recently been employed as sulphur mustard and G-agent series simulants to evaluate the use of atmospheric pressure chemical ionisation for CW detection [1].

The use of MS as a sulphur mustard (H) simulant is reasonable as both compounds show similar skin absorption characteristics [2] and physical properties. MS has historically been used at Porton Down for field trials and has also been used to study skin absorption of VX using swine as a model [3]. Compounds with a similar structure to sulphur mustard, e.g. 2-chloroethyl phenyl sulphide (CEPS) and 2-chloroethyl ethyl sulphide (CEES), have found wide application as sulphur mustard simulants [4–9] but may cause dermal burns and therefore MS is preferred.

Dimethyl methylphosphonate (DMMP) has found widespread application as a simulant for VX [8–10] due to similarities in structure. Diisopropyl methylphosphonate (DIMP) has been used as a G-agent series simulant [4–6]. Literature on the use, sampling and analysis of DPM is scarce, but DMP has found wide applicability in G-agent simulant trials at Porton Down. The main justification for its

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use is the similarity in physical properties with the G-agent series.

Sampling MS from the atmosphere has been accomplished by the use of passivated steel syringes [3] or sorbent tube sampling in active mode. Numerous sorbent materials have been evaluated for active sampling with porous polymeric sorbents, including Porapak Q™ and Tenax TA™ [10], proving the most suitable due to their large surface areas and excellent flow characteristics. Solvent elution prior to gas chromatographic determination has been employed using isopropyl alcohol (IPA) and acetone. Thermal desorption (TD) has also shown to be of great utility in analysis of CW agents and their simulants [10,11] but suffers from its 'one shot' nature with re-analysis of the sample not being possible. This situation will shortly be improved by introduction of new thermal desorption systems which allow the recollection of samples for replicate analysis.

DPM is currently analysed at Porton Down by sampling onto steel or glass tubes filled with Porapak Q™ with subsequent analysis by solvent or thermal desorption with gas chromatography and a flame ionisation detector (TD–GC-FID). This method allows analysis to approximately 10 ng on column in the case of thermal desorption.

The current method for determination of MS involves eluting steel or glass tubes filled with Porapak Q™ with 4 ml of IPA and analysing by high performance liquid chromatography with UV absorbance detection. The current LOD is approximately 100 ng ml^{-1}. The sorbent loading on steel and glass tubes is 50 and 75 mg, respectively.

DPM and MS are used as G and sulphur mustard simulant compounds in trials at Porton Down. The main areas of application are in developing protective clothing for UK armed forces, chemical agent detection capabilities (e.g. CAM) and air filtration systems in military hardware.

The above review of existing methodologies indicates that two different techniques are necessary to determine both simulants which are frequently used simultaneously in trials at Porton Down. A method to simultaneously determine both simulants by the same technique was developed in this study. The aim of this investigation was to develop and optimise a GC-FID analytical method which used solvent elution as the means for recovering MS and DPM from Porapak Q™ by application of factorial design (FD). Solvent elution was chosen to allow re-analysis of samples. Two solvents were investigated for elution, IPA and hexane, at different elution volumes for both types of tube available (glass and steel).

2. Experimental

2.1. Mathematical model

Three parameters were chosen for optimisation; tube type, solvent type and solvent volume. As two of the parameters are categorical variables as opposed to continuous numerical variables, a full factorial design (2^3) was used to create a series of 18 experiments to investigate the influence of the parameters (Table 1). Each point in the experimental design was replicated twice and experiments performed in a random order. The form of this model is shown in Eq. (1). The tube type was either steel or glass, the solvent used IPA or hexane, and the solvent volume 2 and 10 ml. A duplicated centrepoint was added to the design allowing a solvent volume of 6 ml to be included in the model thus allowing detection

Table 1
Factorial design and associated recoveries of simulant compounds from Porapak Q™

Std order	Run order	Solvent type	Solvent volume (ml)	Tube type	Recovery (%)			
					DPM 1	DPM 2	DPM 3	MS
3	1	IPA	10	Steel	72	73	73	73
5	2	IPA	2	Glass	93	93	94	36
14	3	Hexane	2	Steel	88	88	87	88
4	4	Hexane	10	Glass	107	104	101	96
12	5	Hexane	10	Glass	97	101	92	88
7	6	IPA	10	Steel	73	82	74	91
11	7	IPA	10	Glass	54	54	47	34
1	8	IPA	2	Glass	74	74	71	30
2	9	Hexane	2	Glass	93	93	90	80
6	10	Hexane	2	Steel	92	93	93	103
17	11	IPA	6	Steel	73	75	58	70
10	12	Hexane	2	Glass	86	87	84	76
9	13	IPA	2	Glass	83	83	83	36
8	14	Hexane	10	Steel	97	96	94	98
15	15	IPA	10	Steel	71	72	71	72
16	16	Hexane	10	Steel	94	96	93	88
18	17	Hexane	6	Glass	97	102	93	89
13	18	IPA	2	Steel	70	70	71	68

DPM 1–3: first, second and third eluting isomers of DPM. MS: methyl salicylate.

of curvature. The response variable, *Y*, was the percentage recovery of DPM or MS from each experiment.

$$\text{recovery (\%)} = \beta_0 + \beta_1 \text{ solvent type} + \beta_2 \text{ solvent volume} + \beta_3 \text{ tube type} \quad (1)$$

where $\beta_{0–3}$ are coefficients for experimental parameters.

2.2. Reagents

DPM was purchased from Aldrich (Gillingham, UK) at 97% purity as an unknown mix of isomers. MS was obtained from BDH (Poole, UK) at 99% purity. IPA and hexane (distol quality) were supplied by Fisher Scientific, UK and used without further purification. Steel tubes were packed in-house with 100 mg Porapak Q^{TM} (50/80 mesh) and Glass Porapak Q^{TM} tubes were purchased from SKC, UK and used without further conditioning.

2.3. Tube conditioning

Steel tubes were packed with 100 mg of Porapak Q^{TM} and conditioned for 1 h at 230 °C with a flow of 100 ml min^{-1} zero grade nitrogen. Glass Porapak Q^{TM} tubes were used without further conditioning.

2.4. Spiking of Porapak Q^{TM} tubes with DPM and MS

Tubes were spiked with 50 μl of a 1 mg ml^{-1} (50 μg on tube) hexane solution containing both DPM and MS using a liquid loading method described previously [10]. The standard was injected onto the front gauze for steel tubes or the front glass wool plug for glass tubes while drawing air through the tube at 100 ml min^{-1} for 10 s using a personal sampling pump. The tubes were eluted on the same day as they were spiked.

2.5. GC parameters

Analysis was carried out on a Hewlett-Packard 6890 GC with FID. A DB-FFAP (30 m × 0.53 mm × 0.5 μm) capillary column was used with a starting oven temperature of 40 °C which was held for 1 min, then increased by 20 °C min^{-1} until a temperature of 200 °C was reached. The final temperature was held for 1 min giving a total run time of 10 min. The FID was operated at 250 °C with gas flows of H_2 at 40 ml min^{-1}, air at 450 ml min^{-1} and N_2 at 45 ml min^{-1}.

2.6. Peak identification

The chromatogram for DPM exhibited three peaks (at 5.8, 5.9 and 6.1 min) due to the presence of three isomers, while MS gave a single peak (7.6 min). Single component standards of both analytes were analysed to identify the peaks by coincidence of retention time. A mixed 10 μg ml^{-1} standard was analysed by GC–MS and the peaks were also identified by comparison with an in-house library and spectral interpretation.

2.7. Calibration of GC-FID

Standards were prepared by diluting DPM and MS to give stock solutions in hexane. These were combined to give a 1 mg ml^{-1} stock in hexane. Two sets of standards were made from this stock solution, one in hexane and one in IPA. The concentrations of the standards were 0.5, 1, 5, 10, 20 and 30 μg ml^{-1}. These were injected in triplicate in the same run as the samples and the mean peak areas were plotted against concentration.

3. Results

3.1. Development of mathematical models for adsorption/desorption behaviour

The results of the factorial design employed in this study are summarised in Table 1.

Analysis of the FD indicated that volume of solvent used and tube type were insignificant for DPM isomers and MS. Solvent type was found to be the only parameter which influenced recovery of the compounds studied. As expected, two-way interactions and curvature were not observed between parameters. Therefore, Eq. (1) can be reduced and recoveries for each compound represented by a simple linear relationship as shown in Eqs. (2)–(5).

$$\text{recovery (\%) DPM 1} = 43.9 + (2.25 \times \text{solvent type}) \quad (2)$$

$$\text{recovery (\%) DPM 2} = 43.6 + (2.27 \times \text{solvent type}) \quad (3)$$

$$\text{recovery (\%) DPM 3} = 43.2 + (1.70 \times \text{solvent type}) \quad (4)$$

$$\text{recovery (\%) MS} = 30.9 + (12.72 \times \text{solvent type}) \quad (5)$$

where DPM 1–3 are first, second and third eluting isomers of DPM.

Table 1 shows that DPM recoveries range from 54 to 107%, 54 to 104%, and 47 to 101% for isomers 1, 2 and 3, respectively. MS recoveries ranged from 30 to 103% the with highest recoveries being obtained with hexane as solvent. Therefore MS recovery is greatly influenced by solvent polarity. The main effects plot for solvent volume for both compounds showed decreasing solvent polarity from IPA to hexane (48.4 and 31 kcal mol^{-1}), increases the recovery of DPM and MS. This indicates that the primary adsorption/desorption mechanism between analytes and sorbent is non-polar interactions. Hence hexane was chosen as the solvent of choice for elution. This solvent is also more suited than IPA for on column injection.

Table 2
Recovery of simulants from tubes and summary of adequacy of FD model

Compound	Glass					Steel				
	Mean recovery (%)	*s*	95% CI	Predicted recovery (%)	Agreement with model (%)	Mean recovery (%)	*s*	95% CI	Predicted recovery (%)	Agreement with model (%)
DPM 1	83	6.9	8.6	96	86	84	4.1	4.3	94	90
DPM 2	83	7.3	9.1	96	86	84	4.4	4.2	94	89
DPM 3	82	8.3	10.4	94	87	86	4.3	4.5	90	96
MS	80	4.3	5.4	95	84	86	2.9	3.1	79	109

3.2. Optimisation of recovery and validation of experimental model

Eqs. (2)–(5) were used to obtain optimum conditions for desorption of DPM and MS from steel and glass tubes. An elution volume of 4 ml hexane was used in validation experiments as this volume allowed easy manipulation of samples while maintaining a reasonable concentration of analyte. The optimisation approach used was based on the desirability function which has been described and applied to similar studies [11,12]. Proposed conditions from the desirability optimisation were tested by performing replicate analysis (6×) on glass and steel spiked tubes. The predicted and actual recoveries for these experiments are shown in Table 2. The agreement between predicted and observed recoveries is greater than 84% for all compounds on both types of sorbent tube indicating the predictive models developed from the FD are adequate to explain the adsorption/desorption behaviour of DPM isomers and MS on Poropak Q^{TM}. Fig. 1 shows a typical chromatogram of a 1 l atmospheric sample containing both DPM and MS at 40 mg m^3. Fig. 2 displays the mean recovery of DPM and MS with associated 95% confidence intervals. A two-sided *t*-test was performed on each compound to compare recovery from steel and glass tubes. No significant difference was found in recovery ($P = 0.05$) for DPM isomers. Although the confidence limits overlap for MS on Fig. 2, there is a statistically significant difference ($P < 0.05$) for MS recoveries using steel tubes (86%) compared to glass tubes (80%). The overlap of the confidence limits is likely to be caused by the small number of samples used in validation experiments. The variability of

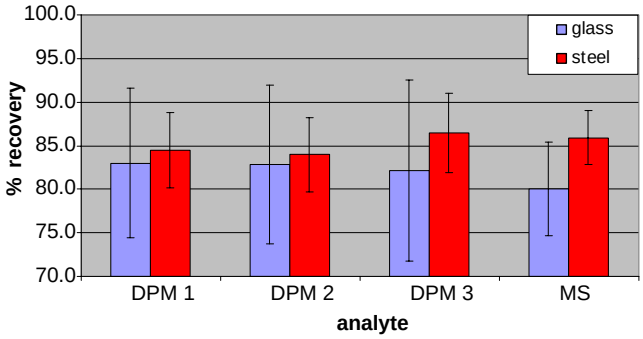

Fig. 2. Recovery of simulants from glass and steel spiked tubes.

recoveries on glass tubes for all analytes was approximately twice that of steel tubes. Therefore in order to maximise recoveries of all components, steel tubes should be used for collection of simulants and hexane employed as the elution solvent.

3.3. Limits of detection (LOD)

Detection limits were calculated as described previously [13]. Each standard was run in triplicate and linear regression analysis utilised to provide regression coefficients for each parameter. In all equations, the intercept term was found to be non-significant ($P > 0.05$) and hence excluded from the final equation. Therefore the limit of detection was given by Eq. (6).

$$\text{LOD} = \frac{3 \times \text{std error}}{\text{slope}} \quad (6)$$

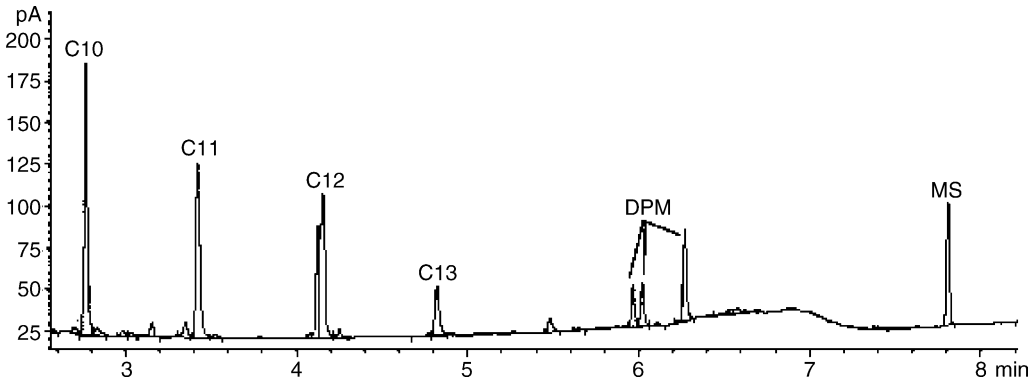

Fig. 1. GC-FID chromatogram showing the presence of DPM isomers and MS in an atmospheric sample (40 mg m^3).

Linear regression analysis was performed on calibration data for each DPM isomer and MS. A nominal instrumental LOD of 0.83 and 0.90 $\mu g\,ml^{-1}$ was established for DPM isomers and MS, respectively. This corresponds to an instrumental LOD of 2 μg on tube which, in a 10 l air sample ($1\,l\,min^{-1}$), gives an atmospheric LOD of $0.2\,mg\,m^{-3}$. The breakthrough volumes of MS and DPM will be evaluated and reported in due course to ensure this sampling rate is suitable. A storage study of MS spiked tubes is underway and will be reported in due course.

4. Conclusions

A sensitive method for determining CW agent simulants has been developed using solvent desorption. FD was applied to investigate the parameters influencing recovery of simulants from glass and steel tubes packed with Porapak Q™. The models developed were adequate to explain the adsorption/desorption of simulants (>84% agreement) with both processes being governed primarily by non-polar interactions. Thus hexane was found to yield higher recoveries of both simulant compounds.

A comparison of recovery of simulants from steel and glass tubes was performed with DPM being recovered equally well for both tube types. Steel tubes gave higher recoveries of MS compared to glass tubes. Therefore sampling 10 l of air on steel tubes gave an atmospheric limit of detection for both simulants of $0.2\,mg\,m^{-3}$.

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References

[1] L. Charles, L.S. Ritter, G.R. Cooks, Anal. Chem. 73 (2001) 5061.
[2] J.E. Riviere, C.E. Smith, K. Budsaba, J.D. Broks, E.J. Olajos, H. Salem, N.A. Monteiro-Riviere, J. Appl. Toxicol. 21 (2001) 91.
[3] E.J. Scott Duncan, A. Brown, P. Lundy, T.W. Sawyer, M. Hamilton, I. Hill, J.D. Conley, J. Appl. Toxicol. 22 (2002) 141.
[4] M.V. Buchanan, R.L. Hettich, J.H. Xu, L.C. Waters, A. Watson, J. Hazard. Mater. 42 (1995) 49.
[5] R.A. Jenkins, M.V. Buchanan, R. Merriweather, R.H. Ilgner, T.M. Gayle, A.P. Watson, J. Hazard. Mater. 37 (1994) 303.
[6] W.H. Griest, R.S. Ramsey, C.H. Ho, W.M. Caldwell, J. Chromatogr. 600 (1992) 273.
[7] C.C. Chong, S.R. Long, Appl. Opt. 29 (33) (1990) 4981.
[8] D.A. Jaeger, C.L. Schilling, A.K. Zelenin, B. Li, E. Kubicz-Loring, Langmuir 15 (1999) 7180.
[9] G.W. Wagner, P.W. Bartram, Langmuir 15 (1999) 8113.
[10] W.A. Carrick, D.B. Cooper, B. Muir, J. Chromatogr. A 925 (1–2) (2001) 241.
[11] B. Muir, W.A. Carrick, D.B. Cooper, Analyst 127 (2002) 1198.
[12] A.M. Carro, R.A. Lorenzo, Analyst 126 (2001) 1005.
[13] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, third ed., Ellis Horwood, Chichester, 1993.