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Retrospective identification of chemical warfare agents by high-temperature automatic thermal desorption–gas chromatography–mass spectrometry

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Abstract

An automated thermal desorption (ATD)–gas chromatography–mass spectrometry method was developed for the analysis of selected chemical warfare (CW) agents. Suitable methods were developed for analytes of high volatility to low volatility. The less volatile CW agents required the purchase and installation of a high-temperature valve upgrade kit allowing valve temperatures of up to 260°C to be reached. The limit of detection was 50 ng on the tube for most CW agents in full scan. Chloropicrin exhibited some temperature dependence, with detection limits improving as ATD temperatures were decreased below 150°C. A sample storage trial was undertaken to establish the most suitable storage environment for CW agents adsorbed onto Tenax TA. Temperature and time of storage were found to influence recovery of analytes with best recoveries being observed after 1 day storage in a freezer (−12°C). This method was evaluated during a trial of procedures for sampling and identification of chemical agents at Porton Down, UK. Sulfur mustard was detected downwind of a simulated exploded munition.

Keywords: Chemical warfare agents; High-temperature automatic thermal desorption; Organophosphorus compounds

1. Introduction

The Chemical Weapons Convention (CWC) prohibits the development, production, stockpiling and use of chemical weapons. It entered into force on 29 April 1997 and is administered by the Organisation for the Prohibition of Chemical Weapons (OPCW), based in The Hague, The Netherlands.

Routine inspections are undertaken of facilities producing small quantities of scheduled chemicals for permitted purposes. There are also inspections of old chemical warfare (CW) weapons storage sites and destruction facilities in order to monitor the destruction of CW weapons. The treaty includes provision for verification of compliance. At any time a member state party can call for a challenge inspection upon another member state suspected of producing, storing or using chemical weapons.

During the course of inspections, there may be a requirement for the inspection team (IT) to resolve ambiguities. Therefore, samples may be taken for analysis, either on-site by the IT, using equipment they bring with them, or off-site to two OPCW designated laboratories for more difficult samples. The results of any analysis must be able to withstand

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international scrutiny, and therefore the OPCW has designated laboratories that have achieved suitable accreditation by a recognised body (such as the United Kingdom Accreditation Service). The laboratories must also successfully take part in proficiency tests set by the OPCW [1].

The Chemical and Biological Defence (CBD) Sector, Defence Evaluation and Research Agency, Porton Down, UK is one of the OPCW designated laboratories. The laboratory's main function is the analysis of CW agents, and their degradation products in a variety of vapour, liquid and solid matrices.

Sampling of vapours has traditionally been accomplished by drawing air through a tube loaded with an adsorbent material capable of trapping the analyte of interest. Analytes are then removed by solvent desorption and analysed by gas chromatography (GC) with a suitable detector. This approach suffers from a lack of sensitivity due to the elution volumes employed (1–4 ml), thus increasing detection limits. This problem may be circumvented by thermal desorption (TD) in which a greater proportion of the adsorbed analyte may be transferred to the GC system, offering considerable improvements in detection limits. The technique is particularly suited to volatile CW agents which, because of their high toxicity, require analytical methods capable of very low detection limits.

Automatic thermal desorption (ATD) is a technique used for the analysis of volatile compounds that have been adsorbed onto an adsorbent bed contained within a tube. Thermal desorption releases these volatile compounds by heating the adsorbent bed in a stream of helium. The volatile compounds are refocused onto a cold-trap and transferred to the GC system for analysis. ATD is a rapid means of delivering a volatile analyte collected on an air sampling tube to the analytical instrument [2]. Thermal desorption has many advantages over solvent extraction; the whole sample is analysed providing greater sensitivity, sample preparation and interfering solvent peaks are eliminated allowing better recovery of volatile compounds, and the sampling tubes can be reused [2,3]. However, ATD does have the disadvantage of being a one shot method and once analysed the sample is not available for re-analysis [2].

Sampling with Tenax TA and subsequent thermal desorption of compounds for GC–mass spectrometry (MS) analysis provides an effective method for identification of CW agents in air [4–6].

ATD is useful in determining background levels of CW agents in buildings [7], where CW agents may once have been manufactured or stored, in order to assess the risk of exposure and hence what level of protective clothing is required. ATD can also be beneficial in the retrospective identification of CW agents for proving allegations of use [8,9].

TD–GC–MS has been demonstrated to be suitable for measurement of volatile organic compounds (VOCs) in the environment [10–12] and organic nitriles [13]. However, no study to date has focused on a diverse range of CW agents.

Prior to reconfiguration, the ATD system used in this study was capable of analysing a wide range of CW and riot control agents, but problems were experienced with the less volatile agents. Of the 21 agents investigated (see Table 1), problems were experienced with the less volatile compounds 2-chlorobenzylidene malonitrile (CS), dibenzoxazepine (CR), ethyl *S*-2-diisopropyl aminoethylmethyl phosphonothioate (VX) and bis[2-(2-chloroethylthio)ethyl]ether (T).

CS and CR caused major carryover problems even at low levels of 50 ppb, while VX and T could not be analysed at these levels. The perceived problem was the ATD valve temperature being too low to prevent condensation of these analytes in the ATD valve and associated piping. These difficulties were overcome with the purchase of a high-temperature valve upgrade kit. The upgrade increased the valve and associated pipework temperature from 225 to 260°C.

2. Experimental

2.1. Materials

All solvents used (Distol quality) were obtained from Fisher Scientific (Loughborough, UK). 2,6-Dimethylphenol (DMP) and 5-chloro-2-methylanaline (CMA) were purchased from Aldrich (Dorset, UK). Deuterated dimethyl methylphosphonate (DMMP-d_6, >99%) was synthesized in the laboratory.

The agents investigated, comprising nerve agents,

Table 1
Compound names and abbreviations of all analytes used in this investigation

Compound name	Synonym/abbreviation
Ethyl *N*,*N*-dimethyl phosphoramidocyanidate	GA, tabun
Isopropyl methylphosphonofluoridate	GB, sarin
1,2,2-Trimethylpropyl methylphosphonofluoridate	GD, soman
Cyclohexyl methylphosphonofluoridate	GF, cyclohexyl-sarin
2-Methylcyclohexyl methylphosphonofluoridate	MeGF
Ethyl *N*,*N*-dimethylphosphoramido fluoridate	F-GA, fluoro-tabun
Ethyl *S*-2-diisopropylaminoethylmethyl phosphonothioate	VX
Diisopropyl phosphorofluoridate	DFP
Bis(2-chloroethyl) sulfide	H, mustard
Bis[2-(2-chloroethylthio)ethyl]ether	T
1,2-Bis(2-chloroethylthio)ethane	Q, sesquimustard
Bis(2-chloroethyl)ethylamine	HN1
Bis(2-chloroethyl)methylamine	HN2
Tris(2-chloroethyl)amine	HN3
Dichloronitrosomethane	CX, phosgene oxime
Ethyl iodoacetate	KSK
2-Chloroacetophenone	CN, CAP
α-Bromobenzyl cyanide	BBC
2-Chlorobenzylidene malonitrile	CS
Dibenzoxazepine	CR
Trichloronitromethane	PS, chloropicrin

vesicants and irritants are shown in Table 1. They were all synthesized in the laboratory, except for diisopropyl phosphorofluoridate (DFP), ethyl iodoacetate (KSK), 2-chloroacetophenone (CAP), and trichloronitromethane (chloropicrin), which were purchased from Aldrich.

ATD tube packing (Tenax TA) was obtained from Markes International (Mid-Glamorgan, UK).

2.2. Tube spiking methods

Two different ATD tube spiking methods were investigated. The first method (liquid spiking) involved connecting the ATD tube to a Casella sampling pump and drawing air through the tube at a nominal flow-rate of 1 l/min at room temperature. Analyte solutions were injected onto the tube with air drawn through the tube for a further 15 s. The second method (vapour spiking) required the ATD tube to be connected to a heated GC injector at 100°C, with a nitrogen flow of 100 ml/min. Analytes were injected in solvent and the vapour passed onto the ATD tube.

2.3. Instrument specifications

The ATD system employed was a Perkin-Elmer ATD 400. This was interfaced to a HP 5890 (Hewlett-Packard) GC system with detection accomplished by the use of a HP 5971 mass spectrometer.

The capillary column used was a DB5-MS 25 m×0.2 mm I.D. with 0.33 μm film thickness. The carrier gas was helium and flow was maintained at a constant pressure of 103.4 kPa giving an initial linear velocity of 37.7 cm/s (0.9 ml/min) set at 40°C. The mass spectrometer interface was maintained at 280°C.

The column temperature programme was as follows: initial temperature 40°C (held for 2 min), increased at 20°C/min to 160°C, then increased at 30°C/min to 310°C and held at the final temperature for 5.00 min. Solvent delay was set at 4.50 min and the mass spectrometer was operated in full scan mode (40–550 amu).

2.4. Identification standards

The method is semi-quantitative; standards were

Table 2
ATD parameters for all analytes listed in Table 1 except for PS

Parameter	Value
Line temperature	225°C
Oven temperature	350°C
Desorb time	10 min
Valve temperature	260°C
Injection per tube	1
Trap fast	Yes
Cycle	No
Trap low	−30°C
Trap high	300°C
Hold	1 min
In split	No
Out split	Yes (15 ml/min)
Recycle	No
Purge	1 min
Purge flow	50 ml/min
Minimum pressure	34.5 kPa
Pressure	103.4 kPa
Standard injection	0.0

used for identification of retention time, mass spectra and linearity of response. Stock solutions of all the agents were made up as mixtures of each class, nerve agents, vesicants and irritants. Each mixture was made up with hexane to 50 μg/ml.

Volumes of 1, 2 or 5 μl of the solutions were used in order to produce 50, 100, or 250 ng of each analyte on the ATD tube.

Table 3
ATD parameters for PS

Parameter	Value
Line emperature	100°C
Oven temperature	150°C
Desorb time	10 min
Valve temperature	150°C
Injection per tube	1
Trap fast	Yes
Cycle	No
Trap low	−30°C
Trap high	300°C
Hold	1 min
In split	No
Out split	Yes (15 ml/min)
Recycle	No
Purge	1 min
Purge flow	50 ml/min
Minimum pressure	34.5 kPa
Pressure	103.4 kPa
Standard injection	0.0

2.5. Method development for CW agents

The three classes of compounds, nerve agents, vesicants and irritants, were investigated by TD–GC–MS. ATD tubes were spiked with 50, 100, or 250 ng on the tube with a mixture of either (a) nerve agents, (b) vesicants or (c) irritants. Ten replicate ATD sample tubes were spiked and analysed by TD–GC–MS. The TD parameters for all CW agents except PS are shown in Table 2.

2.5.1. Method development for chloropicrin (PS)

ATD tubes were spiked with 50, 100 and 250 ng on tube of PS. A thermal desorption method for PS was developed by varying the oven, valve and line temperature. Ten replicate ATD tubes were spiked and analysed by TD–GC–MS. The TD parameters for PS are shown in Table 3.

2.6. Analysis of all CW agents

Prior to analysis of each batch of samples, the mass spectrometer was tuned according to the manufacturers' instructions. A blank ATD tube and a CW Test Mix spiked tube were run on the ATD system and analysed by GC–MS.

2.7. Storage trial

GA, VX, H, PS and CS were selected for use in the storage trial according to their CW agent classification and their volatility. GA (medium volatility) and VX (low volatility) were selected from the nerve agent group, H (medium volatility) from the vesicants group and PS (high volatility) and CS (low volatility) from the irritants group. ATD tubes were spiked with 100 ng on tube of the above analytes. Five replicate ATD tubes were spiked and stored at room temperature (25°C), in a refrigerator (2°C) and in a freezer (−12°C). These tubes were analysed at 1, 7 and 28 day intervals. The PS spiked tubes were analysed using the specifically developed method shown in Table 3. The desorbed tubes were compared with a standard tube which was spiked on the day of analysis. The ends of the ATD tubes were fitted with PTFE ferrules and 1/4 in. Swagelok fittings during storage (1 in.=2.54 cm).

2.8. Statistical methodology

Analyte concentrations and peak areas were found to be normally distributed and hence no transformation of data was performed prior to statistical manipulation. Each set of concentration data was examined for outliers by the Dixon's *Q*-test. This resulted in two observations being excluded from statistical analysis. Normality of the concentration data was confirmed using the Anderson–Darling normality test at the 95% confidence level [14]. Normalised data had a $p>0.05$ confirming normal distribution.

Analysis of variance (ANOVA) was performed to establish which experimental factors influenced recovery of analytes from ATD tubes by fitting a general linear model to a two-way ANOVA which also included a second order interaction term. ANOVA was performed at the 95% confidence level. Bonferroni simultaneous confidence intervals were also generated as part of the ANOVA to allow comparison of multiple sample means.

3. Results and discussion

3.1. Tube spiking methods

The performance of the two different tube spiking methods was compared. The first technique (liquid spiking) was injection of compound solutions onto tubes with air drawn through at a nominal flow-rate of 1 l/min for ca. 15 s. This was compared to injecting the compounds using a heated GC injector (100°C) with a nitrogen flow of 100 ml/min for ca. 15 s (vapour spiking). A two-sided *t*-test was performed with the null hypothesis that no difference existed between observed mean peak area of any compound by either spiking method. Values of the Student *t* statistic were below the critical *t* value and therefore the null hypothesis was retained. No significant difference in mean peak area was found for any of the compounds by both spiking methods ($p>0.05$). Therefore, all experimental work was carried out using the liquid spiking method. This was a convenient technique since little apparatus was required and it could be performed in a fume cupboard thus minimising exposure to CW agents during spiking.

3.2. Results of CW agents

3.2.1. All analytes excluding chloropicrin

All compounds in the three classes were detected at all concentrations, 50, 100 and 250 ng on the tube. The S/N ratio at 50 ng on the tube was $\geq$4:1. Relative standard deviations (RSDs) for all compounds at 50, 100 and 250 ng on the tube are shown in Table 4. A TD–GC–MS extracted ion chromatogram of all the G and V agents at 50 ng on the tube is displayed in Fig. 1.

3.2.2. Results of chloropicrin

PS, the most volatile of the agents, was not

Table 4
RSD of peak areas for all G and V, vessicant and irritant agents investigated at 50, 100 and 250 ng on the tube

Analyte	RSD (%) at the stated test mass (ng)		
	50	100	250
G and V agents			
GB	21	14	19
F-GA	10	11	13
DEP	9	12	11
GD (isomer 1)	17	10	15
GD (isomer 2)	13	12	15
GA	8	14	7
GF	11	9	11
Me-GF	11	10	8
VX	30	20	6
Vessicants			
H	17	16	15
T	30	23	9
Q	19	11	7
HN1	22	21	9
HN2	28	16	15
HN3	17	10	11
Irritants			
PS	40[a]	37	17
CX	34	42	22
CSK	17	37	8
CN	13	37	7
BBC	25	40	16
CS	16	14	7
CR	13	17	7

[a] On modification of the method (Section 3.2.3), RSD at 50 ng was reduced to 17%.

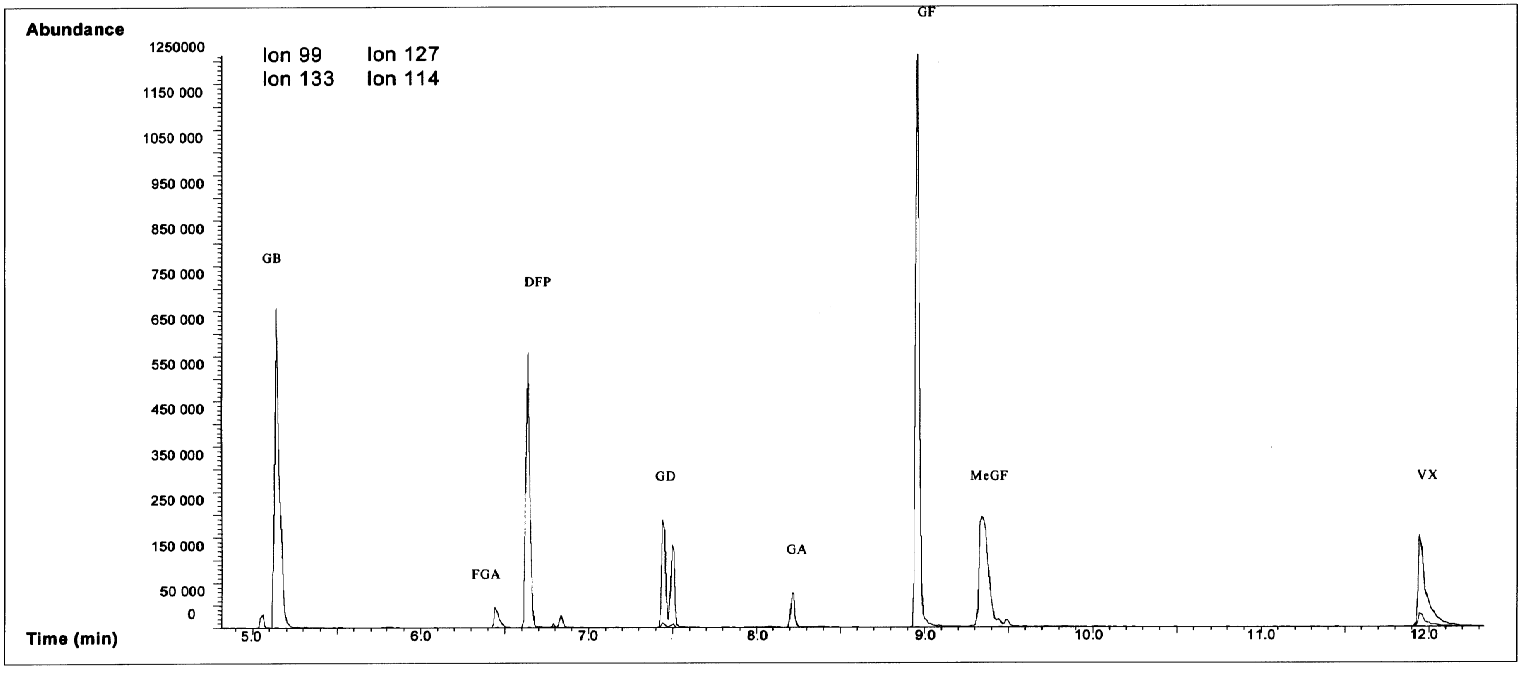

Fig. 1. TD–GC–MS extracted ion chromatogram of the G and V agents at 50 ng on the tube.

initially detected at 50 ng on tube and the response at 250 ng on tube was low compared to other irritant compounds. It was believed this might have been due to slippage through the system or instability at high temperatures.

3.2.3. Results of varying ATD parameters on the analysis of PS

The ATD parameters oven, valve and line temperature were reduced sequentially. Reducing the oven and valve temperature from 350 to 150°C and 225 to 150°C, respectively, failed to increase peak area of PS above baseline for 50 ng on tube. Maintaining the oven and valve temperatures at reduced levels and reducing the line temperature from 150°C to 100°C resulted in detection of PS well above baseline levels at 50 ng on tube. The average peak area for PS at 50 ng on tube using this modified method was $1.3 \cdot 10^6$ with an RSD of 17%. This represents a signal-to-noise ratio of about 10:1.

3.3. Storage trial results

3.3.1. PS storage trial results

Replicate injections of PS showed poor reproducibility with respect to peak area. This was thought to be due to a non-optimal ATD method. Subsequently the variation in peak area data meant that a meaningful interpretation of the storage trial data was impossible. An optimum ATD method for PS is currently being developed using central composite design and the results will be published shortly.

3.3.2. GA, VX, H and CS storage trial results

ANOVA was performed to determine the effect of storage time and storage environment on recovery of GA, VX, H and CS. ANOVA revealed that storage time and conditions significantly influenced recovery ($p<0.05$). All analytes showed similar patterns. Plots of residuals for the ANOVA model of each analyte were found to be normally distributed by the Anderson–Darling normality test ($p>0.05$) confirming the validity of the model.

Storage at room temperature resulted in lower recoveries than storage at reduced temperatures. No significant difference in recovery was observed between refrigerator and freezer storage. A storage time of 1 day resulted in the greatest recovery of each analyte with decreasing recoveries being observed after 7 and 28 days.

The following trends can be deduced for each compound:

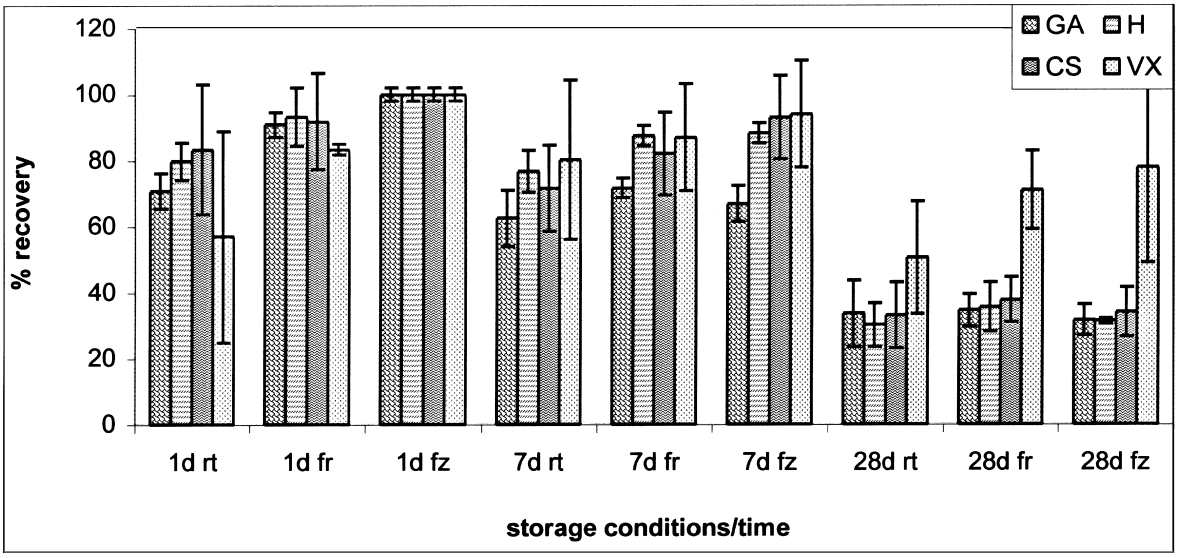

Fig. 2. Agent recovery and associated SDs during storage trials. Key: d = day(s); rt = room temperature storage; fr = fridge storage; fz = freezer storage. Error bars indicate 1σ.

Storage environment	fridge = freezer > room temperature
Storage time (days)	1 day > 7 days > 28 days

Therefore, prior to analysis, tubes should be stored for minimal time in a refrigerator or freezer.

Percentage recoveries of each analyte under different storage conditions at 1, 7 and 28 days after spiking are shown in Fig. 2.

4. Validation of the ATD method during an authentic CW agent sampling and analysis trial

An exercise to validate sampling and analysis procedures for the UK SIBCRA Team (Sampling and Identification of Biological, Chemical and Radiological Agents) was carried out on the ranges at CBD, Porton Down. The team, working in a toxic chemical environment, exercised their protocols for sample collection, decontamination and transportation to the analytical laboratory. The scenario simulated bomb craters caused by exploding sulfur mustard munitions. A crater inner surface was contaminated with liquid sulfur mustard, whilst the crater lip and surrounding ground was contaminated with a simulant, methyl salicylate. Meteorology conditions at the time of the exercise were 80% humidity, 9°C and a wind speed of 12 knots.

Pumped air samples were taken, at a height of 0.1 m and a distance of 0.5 m downwind of the crater onto Tenax adsorbent tubes. The air was sampled for approximately 15 min at a flow-rate of 1 l/min. The tubes were then sealed with end caps, decontaminated on their outer surface before packaging for transportation to the laboratory. Soil and vegetation samples from the area surrounding the crater were also taken for analysis.

Analysis of the adsorbent tubes using the method described in this paper gave a positive identification for sulfur mustard, together with methyl salicylate, which was also sampled onto the tube. The results from the soil and vegetation showed only the presence of methyl salicylate.

5. Conclusion

All the investigated compounds were recovered without loss between 50 and 250 ng on the tube. ATD has shown it is a viable method for semi-quantitative determination of a wide spectrum of CW agents. With optimised desorption conditions for chloropicrin and running MS in the selected ion mode (SIM), this method has the potential to be a quantitative method for a wide range of CW agents.

The important ATD parameters for the CW agents except chloropicrin were found to be: line temperature 225°C, oven temperature 350°C and valve temperature 260°C.

The important ATD parameters for chloropicrin were found to be: line temperature 100°C, oven temperature 150°C and valve temperature 150°C.

Results of the GA, VX, H and CS storage trial showed that prior to analysis, sampled ATD tubes should be stored for minimal time in a refrigerator or freezer.

The results of the chloropicrin storage trial showed poor reproducibility with respect to peak area, which was thought to be due to a non-optimal ATD method. Subsequently, that meant that a meaningful interpretation of the storage trial data was impossible. An optimum ATD method for chloropicrin is currently being developed using central composite design and the results will be published in due course.

In general, ATD has been demonstrated to have a wide range of applicability for CW agents from low volatility to high volatility. It is a simple and rapid method, with desorption and analysis complete in approximately 30 min.

This method has demonstrated its applicability to sampling and identification of chemical agents, and to the OPCW during a deposition trial of sulfur mustard at CBD, Porton Down, when sulfur mustard was detected downwind of a simulated exploded munition.

For situations where safety information is required, or where chemical data is necessary for retrospective identification purposes where other samples are unavailable, ATD would be an ideal method for the OPCW to adopt for the analysis of vapour samples.

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