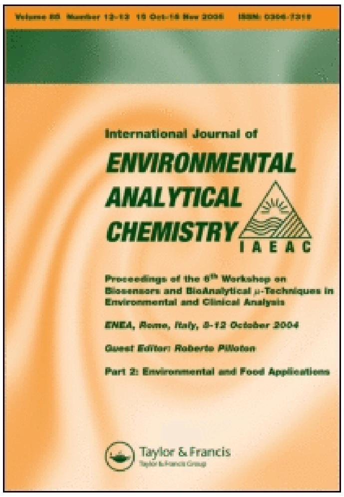

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Use of Solid-Phase Extraction in Determination of Chemical Warfare Agents

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USE OF SOLID-PHASE EXTRACTION IN DETERMINATION OF CHEMICAL WARFARE AGENTS

Part I Evaluation of the Solid-Phase Extraction Technique

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This paper gives a comprehensive evaluation of the solid-phase extraction technique for isolation of chemical warfare agents and related compounds from aqueous solutions. Several factors which may affect the amounts recovered by this method were investigated. These included sorbent type, amount of sorbent, pretreatment of the sorbent, type of eluting solvent, amount of eluting solvent and washing procedures. In addition, the degradation of compounds retained on the sorbent has been evaluated at different temperatures.

Among several non-polar sorbents, octadecylsilane (C_{18}) was found to give the highest yields. The amounts recovered depended on the sample volume and the amount of sorbent used. The sorbent may be washed with water after application of the samples in order to remove polar impurities. Methanol and acetone were most effective in eluting the compounds from the C_{18} sorbent, but isopropanol, dichloromethane, chloroform and diethylether could also be used.

In order to prevent degradation of unstable compounds during storage and transport, the cartridges should be kept as cool as possible, and preferably in a freezer or a box filled with dry ice. This is especially important if the time between application and elution is more than 24 h.

KEY WORDS: Chemical warfare agents, determination, aqueous samples, solid phase extraction, gas chromatography.

1 INTRODUCTION

The Conference on Disarmament which takes place in Geneva includes negotiations on a chemical weapons convention. This convention has the intention to prohibit development, production, stockpiling and use of chemical warfare agents. The effectiveness of a Chemical Weapons Convention will to a large extent depend on the possibility of verifying alleged violation of the convention.

The use of small cartridges filled with a sorbent (i.e. solid-phase extraction) for preparation of different types of samples has grown substantially during the last few years. These cartridges have the advantage of being small, easy to handle, rapid in use and inexpensive. They can be obtained from several manufacturers, and racks for simultaneously preparation of up to 24 samples are available. A review article dealing with extraction of organic compounds from water by use of solid-phase extraction has been published[1], and the solid-phase extraction technique has also been used for isolation of chemical warfare agents from aqueous samples at some laboratories[2–7].

This paper describes the experiments carried out with the aim to optimize the use of solid-phase extraction for isolation of chemical warfare agents and related compounds from aqueous solutions.

In addition to type of sorbent, several factors are important in obtaining optimal conditions for a solid-phase extraction procedure. After application of the sample, the sorbent may be washed with one or more solvents to remove impurities. Washing solvents that remove most of the impurities without eluting the compounds should be selected. The next step is to elute the compounds with an organic solvent in the smallest possible volume to avoid dilution. A solvent should be chosen in which the solutes are soluble, and which is acceptable in the final analysis. If the applied cartridges are to be stored or transported, the storage temperature and maximum time before analysis have to be investigated.

2 EXPERIMENTAL

2.1 *Gas chromatographic analyses*

Most of the quantitative measurements were carried out using a Hewlett-Packard model 5880 gas chromatograph equipped with a flame ionization detector and a 3-ft glass column packed with Carbowax 20M on 80–100 mesh Chromosorb W AW or a 30 m × 0.535 mm I.D. megabore column with 1.0 μm DB-17 liquid phase. Some of the analyses have also been performed on a Carlo Erba HRGC-5160 gas chromatograph equipped with a 30 m × 0.242 mm I.D. capillary column with 0.25 μm DB-WAX liquid phase and a flame ionization detector.

2.2 *Equipment and chemicals*

The sorbents investigated were C_{18} (octadeclysilane), C_8 (octylsilane), C_2 (ethylsilane), PH (phenylsilane), CH (cyclohexylsilane) and CN (cyanopropylsilane) packed in small cartridges from Analytichem International. For simultaneous preparation of up to ten samples, a rack called Vac-Elut from the same company was used.

The chemical warfare agents ethyl *N*,*N*-dimethylphosphoramidocyanidate (Tabun, GA), isopropyl methylphosphonofluoridate (Sarin, GB), pinacolyl methylphosphonofluoridate (Soman, GD), *O*-ethyl S-2-diisopropylaminoethyl methylphosphonothiolate (VX) and bis(2-chloroethyl)sulphide (mustard gas, H) and the phosphonate esters diisopropyl methylphosphonate (DIPMP), isopropyl methyl methylphosphonate (IPMMP) and methyl 1,2,2-trimethylpropyl methylphosphonate (MTMP) were sythensized and purified (>95%) in our laboratory. Hexadecane from Koch–Light Laboratories was used as internal standard in the gas chromatographic analyses and the organic solvents used were Uvasol or LiChrosolv grade from Merck.

2.3 *Isolate adsorption*

2.3.1 Introduction The choice of sorbents for preparation of different samples depends on three factors: (a) the compounds that are to be retained on the sorbent, (b) the matrix from which the compounds are to be extracted, and (c) the aim of the sample preparation.

When these three factors are taken into consideration, the most promising method for the extraction of the selected chemical warfare agents and related compounds from aqueous solutions appears to be non-polar extraction with non-polar sorbents. The chemical compounds investigated have a non-polar alkyl chain, are not highly soluble in water and, in addition, the gas chromatographic analysis requires non-aqueous solvents.

2.3.2 Pretreatment of the sorbent Wetting of the sorbent is necessary to obtain reproducible interactions between the sorbent and the isolates (i.e. the compounds to be retained). This is especially important for the most non-polar sorbents, such as C_{18}. Wetting is performed by passing 1–2 bed volumes of a suitable organic solvent through the sorbent. One bed volume is the amount of solvent required to fill all the internal pores and interstitial spaces in a given size sorbent bed. For sorbents with 40 μm particles and 60 Å pores, bed volumes are about 120 μl per 100 mg sorbent[8]. Any solvent can be used that will wet both the polar silica surface and the bonded functional groups, and that is miscible with the solvent used for the samples. Methanol is an effective wetting agent, and is used in the experiments described in this paper.

After wetting, the excess of methanol should be removed by passing 10–20 bed volumes of the solvent used to prepare the samples (for example water) through the cartridges. Cartridges filled with 100 mg of a sorbent with 40 μm particles and 60 Å pores should therefore be washed with 1.2–2.4 ml water. Once wetted, the sorbent should not be allowed to become desolvated by excessive drying before application of the sample.

Experiments have been carried out to see whether the yield of chemical warfare agents was enhanced if some water was left on top of the sorbent bed before the sample was applied. Cartridges containing 100 mg of C_{18} sorbent were pre-wetted with 0.5 ml methanol and then washed with 2 ml water. 10 ml water samples containing GA, GB, GD and H were applied to the cartridges when about 1 ml of the washing water remained on top of the sorbent bed. For comparison, similar samples were applied to cartridges with no water on top of the bed. The compounds were eluted from the cartridges with 300 μl chloroform and analyzed by gas chromatography. No significant differences in yields were observed for GA, GB or GD. For H, the yields were slightly higher from the cartridges with water remaining on top of the bed.

2.3.3 Efficiency of different sorbents The ability of various non-polar sorbents to retain the chemical warfare agents GA, GB, GD, VX, H, and the related compounds DIPMP, IPMMP and MTMP has been investigated. The cartridges containing

100 mg sorbent were pre-wetted with 0.5 ml methanol and 2.0 ml water, and 10 ml cold aqueous solutions (+4°C) containing 1 mg of each compound were passed through the cartridges. The flow rates used were 2–5 ml/min. The cartridges were then washed with 3 ml cold water, and both aqueous phases were collected and extracted with 1 ml $CHCl_3$. The chloroform extracts were analyzed by gas chromatography to determine the amount of the compounds not retained on the cartridges. The results (mean values) shown in Table 1 are the total amounts found in the two extracts. These results show that most of the compounds were not sufficiently retained on cartridges filled with the sorbents C_2, CH, CN and PH. These sorbents were therefore not acceptable for isolating the group of compounds investigated.

The amounts of the selected analytes which could be eluted from the different sorbents by means of chloroform were also investigated. This solvent was selected since earlier experiments had shown that chloroform was a good solvent for the compounds in question. Cold aqueous solutions of the compounds were passed through cartridges packed with different sorbents as described above. Two replicates were then eluted with 300 μl chloroform and analyzed by gas chromatography. The amounts of the compounds that could be eluted from the cartridges represented by the mean values are shown in Table 2. These results show that C_{18} and C_8 were the most efficient sorbents for isolation of the selected compounds when chloroform was used as eluting solvent. In these experiments, C_8 was slightly better than C_{18} for GA and H, whereas C_{18} gave higher yields for the phosphonate esters (DIPMP, IPMMP, MTMP). No significant differences were observed for the other compounds.

For some of the combinations of sorbent type and chemical compound, the amounts not retained on the cartridges (Table 1) and the amounts eluted from the cartridges (Table 2) do not add up to 100%. This can be explained by the fact that the extraction efficiencies with chloroform were not 100% and that the sorbent amount and elution volume had not been optimized at the time this experiment was carried out. Nevertheless, the results from this experiment give an indication of which sorbent types should be selected for further investigation.

Table 1 Percentages of applied compounds not retained on different sorbents.

Agent	*Percentages not retained on cartridges of*					
	C_{18}	C_8	C_2	*CH*	*CN*	*PH*
GA	17	20	75	42	70	44
GB	36	38	64	55	68	50
GD	—	0.1	19	0.9	72	4
VX	—	—	—	0.2	×	0.6
H	2	3	19	4	13	3
DIPMP	—	—	21	16	41	34
IPMMP	18	17	33	34	26	0.1
MTMP	—	—	5	3	62	7

—, concentration below detection limit.
×, no analysis carried out.

Table 2 Percentages of applied compounds eluted from different sorbents with 300 μl chloroform.

Agent	*Percentages recovered from cartridges of*					
	C_{18}	C_8	C_2	*CH*	*CN*	*PH*
GA	75	89	7	48	2	28
GB	31	32	1	8	0.2	3
GD	94	100	93	85	0.3	77
VX	90	97	21	35	×	35
H	28	43	16	31	9	28
DIPMP	96	50	34	72	0.3	26
IPMMP	73	32	2	4	—	—
MTMP	100	76	67	94	0.5	82

—, concentration below detection limit.
×, no analysis carried out.

2.3.4 Extended investigation of the C_{18} and C_8 sorbents The experiments described in Ch. 2.3.3 showed that the C_{18} and C_8 sorbents were best suited for extraction of the selected compounds from aqueous solutions. These two sorbents were therefore further examined.

It is often of interest to extract the compounds from larger sample volumes than 10 ml. The extraction yields by using C_{18} and C_8 sorbents from different sample volumes were therefore examined. 1 mg of the agents GA, GB, GD and H was added to 10, 100 or 500 ml water and passed through the cartridges containing 100 mg sorbent as described earlier, at a flow rate of about 5 ml/min. The cartridges were then eluted with 300 μl chloroform and analyzed by gas chromatography. The results given in Table 3 show a significant drop in recovery when the sample volume was increased. Use of cartridges containing more than 100 mg of sorbent should therefore be considered when samples volumes larger than 10 ml are to be handled. Table 3

Table 3 Percentages of applied compounds eluted from 100 mg of C_{18} and C_8 sorbents with 300 μl chloroform.

Agent	*Sorbent*	*Percentages recovered from sample volumes of*		
		10 ml	*100 ml*	*500 ml*
GA	C_{18}	75	20	2
	C_8	89	12	2
GB	C_{18}	31	13	2
	C_8	32	8	2
GD	C_{18}	94	92	68
	C_8	100	82	58
H	C_{18}	28	46	2
	C_8	43	21	2

also shows that the C_{18} sorbent was more efficient than the C_8 sorbent when used for sample volumes of 100 ml and 500 ml.

The fact that addition of sodium chloride to the sample often enhances the extraction yield by making the water phase less polar is a well known phenomenon in solvent extraction[9]. It was therefore suggested that the same principle could be used for solid-phase extraction as a more efficient means of retaining the compounds on the sorbent. This was investigated in experiments where 5 g of sodium chloride were added to 50-ml water samples containing 1 μl or 0.1 μl of the agents GA, GB, GD, VX and H and passed through 100 mg C_{18} sorbent previously wetted with 0.5 ml methanol and 2 ml water. The compounds were then eluted from the cartridges with 300 μl chloroform and analyzed by gas chromatography. For comparison, samples without sodium chloride were prepared and analyzed in the same way. This experiment showed that for the more water soluble compounds like GB and GA, the yields were slightly enhanced by addition of sodium chloride. For GD, VX and H, no effect was observed.

2.3.5 Amount of sorbent The results in Ch. 2.3.4 show that cartridges with 100 mg sorbent were not efficient enough in retaining the chemical warfare agents from sample volumes in excess of 100 ml. Cartridges containing 200, 500 and 1000 mg sorbent are available, and were tested with larger sample volumes. The cartridges were pre-wetted with 1 ml methanol and 4 ml water, 2.5 ml methanol and 10 ml water and 5 ml methanol and 20 ml water, respectively. Samples of 50, 100 and 500 ml water containing 1 mg of the compounds GA, GB, GD and H were then passed through the cartridges. The volumes of chloroform used to elute the compounds were 500 μl for the 200 and 500 mg cartridges, and 1500 μl for the cartridges containing 1000 mg sorbent. The results are given in Table 4; five replicates were analyzed for each combination of agent, sample volume and amount of sorbent. The results show that when 50-ml sample volumes are used, a minimum of 200 mg sorbent is required. For GA and GB even larger amounts of sorbent could be used. For 100 ml sample volumes, 500 mg sorbent is optimal for GA, GD and H, whereas 1000 mg could be used for GB. For 500 ml samples, cartridges containing 1000 mg sorbent are required for all compounds investigated.

2.3.6 Flow rate through the sorbent The efficiency of a given solid-phase extraction technique may depend on the flow rate of the sample and other solvents through the sorbent bed. The maximum allowable flow rate through the cartridges is to some degree a function of how strongly the compounds are retained to the sorbent, and on the amount of sorbent used. In general, the flow rate through 100 mg sorbent should not exceed 5–10 ml/min. Ion exchange is a slower process than polar and non-polar extraction, and a maximum of 5 ml/min is suggested for ion-exchange processes in the literature[10].

Some investigations were carried out to determine the dependence of the flow rate on the yields for GA, GB, GD and H. The yields did not vary significantly when the flow rate was varied from 0.8 ml/min to 7 ml/min which was the maximum flow rate that could be obtained with the Vac-Elut rack used.

Table 4 Percentages of compounds recovered after application of different volumes of aqueous samples to different amounts of C_{18} sorbent.

Agent	*Sample volume (ml)*	*Percentages recovered from cartridges filled with*		
		200 mg	*500 mg*	*1000 mg*
	50	74 ± 11	87 ± 8	90 ± 11
GA	100	48 ± 2	87 ± 11	89 ± 11
	500	8 ± 1	32 ± 4	77 ± 13
	50	46 ± 5	71 ± 3	93 ± 11
GB	100	23 ± 2	70 ± 8	91 ± 10
	500	3.6 ± 0.5	12 ± 1	29 ± 3
	50	95 ± 7	81 ± 14	88 ± 10
GD	100	84 ± 4	80 ± 16	90 ± 13
	500	74 ± 8	76 ± 9	96 ± 5
	50	26 ± 9	25 ± 4	20 ± 4
H	100	39 ± 9	37 ± 12	49 ± 9
	500	11 ± 3	34 ± 15	53 ± 11

2.3.7 Discussion A study of different sorbents for isolation of selected chemical warfare agents and related compounds from aqueous solutions showed that the C_{18} and C_8 sorbents are most efficient. C_8 was slightly more efficient for Tabun and mustard gas from small sample volumes (10 ml), whereas C_{18} gave the highest yields for the phosphonate esters. When the volume of the samples exceeded 10 ml, the C_{18} cartridges were more efficient than C_8 for all agents.

For sample volumes larger than 10 ml, the efficiency of the cartridges containing 100 mg sorbent was markedly reduced and the use of more sorbent improved the amounts recovered. For sample volumes of 50, 100 and 500 ml, cartridges containing 200, 500 and 1000 mg should be used, respectively. These figures are valid only for relatively clean samples. For samples with much organic matter, the amount of sorbent should be increased due to possible overloading of the sorbent capacity[8].

Before application of the samples, the sorbent should be wetted with methanol and the excess methanol removed with water. For some compounds (e.g. H) slightly higher yields could be obtained if a small amount of water was left on top of the sorbent bed before the sample was applied.

Addition of sodium chloride to the samples before application to the cartridges slightly increased the yields for the more water-soluble agents like GB and GA.

2.4 *Isolate elution*

2.4.1 Introduction Elution is a process by which an isolate is removed from the sorbent bed on which it has been retained. Unlike chromatography, solid-phase extraction is based on the principle that the isolate is retained so strongly that it does not move until the elution solvent is introduced. The elution solvent should elute the compounds from the sorbent within a small volume. Elution should require no more than five bed volumes of organic solvent (see Ch. 2.3.2).

Before elution, the sorbent may be washed with a solvent to remove most of the impurities. Normally, the most efficient solvent in removing the impurities without eluting the compounds should be selected.

2.4.2 *Evaluation of different solvents* Several solvents were investigated to find the most efficient one for eluting the chemical warfare agents from 100 mg of the C_{18} sorbent in 300 μl (2.5 bed volumes). Aqueous samples containing GA, GB, GD, H, VX and MTMP were passed through C_{18} cartridges, and then eluted with dichloromethane, chloroform, carbon tetrachloride, diethyl ether, acetone, ethyl acetate, hexane, 2-propanol or methanol. The results from the gas chromatographic analyses of these samples are given in Table 5; three replicates were analyzed for each solvent. The data show that the arithmetic means of the yields are correlated with the polarity of the solvents: highly polar solvents give high yields and apolar solvents low yields. Methanol and acetone have the best properties as general eluting solvents, but the yields with isopropanol, dichloromethane, chloroform and diethyl ether are also high. With ethylacetate, the yields are high for all the agents except H, for which it is very low. Carbon tetrachloride and especially hexane show very poor eluting properties for the investigated compounds.

2.4.3 Elution volume The efficiency of different volumes of chloroform in eluting the analytes from the C_{18} sorbent was investigated. 10 ml of aqueous samples containing the chemical warfare agents GA, GB, GD, VX and H together with the phosphonate ester MTMP were passed through cartridges containing 100, 200, 500 and 1000 mg C_{18} sorbent. The compounds were then eluted with different volumes of chloroform, and the amount recovered were determined by gas chromatography. Five replicates were analyzed for each combination of elution volume and amount of sorbent; the results are shown in Table 6. They show that the amounts of chloroform required to elute optimal amounts of the compounds from cartridges filled with 100 mg sorbent were 300 μl for GA, GB, GD and MTMP, 500 μl for VX and 1 ml for H. 500 μl should be used for the 200 mg sorbent, 800 μl for the 500 mg sorbent and 1.5 ml for the 1000 mg sorbent. The compounds VX and MTMP were only investigated with cartridges filled with 100 mg sorbent.

2.4.4 Washing solvents In order to remove as much sample impurities as possible, the sorbent may be washed with a solvent after the sample has been applied. Retention of the isolates on the sorbent is generally strong enough that 10–20 bed volumes of an appropriate washing solvent can be passed through the sorbent without eluting the isolates. As the evaluation of eluting solvents (Ch. 2.4.2) demonstrated, only small amounts of the compounds were eluted with hexane. This solvent could therefore be used to remove apolar compounds from the cartridges. In addition, highly polar impurities could be removed by washing with water. Some experiments were carried out to find what proportion of the compounds was washed out by 15 bed volumes

Table 5 Percentages chemical warefare agents removed from 100 mg C_{18} cartridges with 300 μl volumes of different eluting solvents.

Agent	*Per cent of agent eluted from C_{18} cartridge with*								
	Isopropanol	*$CHCl_3$*	*Methanol*	*Acetone*	*Hexane*	*Ethyl acetate*	*Diethyl ether*	*Dichloromethane*	*CCl_4*
GA	70	69	91	93	14	79	65	78	71
GB	53	55	74	66	4	53	48	54	53
GD	76	62	100	96	7	82	100	86	48
H	18	14	15	19	16	6	12	28	18
VX	100	99	95	90	0.2	49	29	43	3
MPMP	98	36	98	100	0.5	71	56	54	4
Mean	69	55	79	77	7	57	52	57	33

Table 6 The effect of the elution volume on the recovery of chemical warefare and similar agents from cartridges filled with different amounts of C_{18} sorbent.

Agent	*Sorbent (mg)*	*Percentages recovered from C_{18} cartridges with Volume of chloroform (μl)*						
		200	*300*	*500*	*800*	*1000*	*1200*	*1500*
GA	100	81 ± 7	93 ± 8	87 ± 13	x	92 ± 9	x	x
	200	x	84 ± 18	74 ± 11	84 ± 16	89 ± 10	84 ± 18	x
	500	x	40 ± 5	87 ± 8	79 ± 15	x	x	x
	1000	x	x	x	70 ± 9	86 ± 9	x	90 ± 11
GB	100	59 ± 6	70 ± 11	75 ± 8	x	74 ± 17	x	x
	200	x	33 ± 3	46 ± 5	56 ± 14	52 ± 8	53 ± 17	x
	500	x	17 ± 4	71 ± 3	73 ± 8	x	x	x
	1000	x	x	x	38 ± 8	78 ± 15	x	93 ± 11
GD	100	91 ± 14	95 ± 10	95 ± 4	x	96 ± 7	x	x
	200	x	76 ± 9	95 ± 7	96 ± 5	98 ± 5	91 ± 18	x
	500	x	21 ± 4	81 ± 7	81 ± 14	x	x	x
	1000	x	x	x	58 ± 10	83 ± 13	x	88 ± 10
H	100	10 ± 3	12 ± 5	12 ± 4	x	27 ± 12	x	x
	200	x	25 ± 7	26 ± 9	34 ± 11	17 ± 9	25 ± 7	x
	500	x	13 ± 4	25 ± 4	16 ± 5	x	x	x
	1000	x	x	x	18 ± 4	21 ± 8	x	20 ± 4
VX	100	10 ± 3	15 ± 4	33 ± 9	x	33 ± 23	x	x
MTMP	100	91 ± 7	96 ± 5	98 ± 3	x	98 ± 3	x	x

x, no analysis carried out.

of water. Samples of 50 ml water containing GA, GB, GD and H were applied to pre-wetted cartridges with 100, 200, 500 and 1000 mg C_{18} sorbent. The sorbents were then washed with 2, 4, 10 and 20 ml water, respectively and the water extracted with chloroform and analyzed. The compounds were also eluted from the cartridges by means of chloroform and analyzed, and the yields compared to similar treatment without washing. This experiment showed that no agents could be detected in the washing water, and no significant differences (95% level) between the two treatments were observed.

2.4.5 Discussion Methanol and acetone are the most effective in eluting the selected chemical warfare agents and related compounds from the C_{18} cartridges. Since most of the chemical warfare agents are unstable with respect to nucleophilic attack by hydroxy groups as are present in alcohols, acetone is the preferred of these two. One problem encountered with the more polar solvents is that they are miscible with water. This could cause hydrolysis of the compounds if some water is left in the sorbent before elution. Chloroform was therefore chosen as eluting solvent for most of the experiments in this paper. If the samples are analyzed shortly after elution, this problem is reduced and acetone would be the most suitable eluting solvent. Acetone should also be used with selective detectors like the nitrogen-phosphorous and electron-capture detectors. The optimal volumes of chloroform needed for elution were 300, 500, 800 and 1500 ml for the cartridges containing 100, 200, 500 and 1000 mg C_{18} sorbent, respectively. Larger volumes could be used, but this resulted in lower concentrations in the eluate due to dilution of the compounds. When optimal elution volumes were used, the yields were about 90% for GA, GD and MTMP and 30% for H and VX. For GB, the maximum yield varied from about 60% for the 200 mg sorbent to about 90% on the 1000 mg sorbent.

The cartridges may be washed with 10–20 bed volumes of hexane or water after application in order to remove organic or highly polar impurities, respectively, from the samples. Less than 10% loss was observed by hexane washing, except for GA and H where the losses were about 15%. No loss of isolate was observed with water as washing solvent.

2.5 *Storage and transport of sorbents*

2.5.1 Introduction In verification of alleged use of chemical warfare agents, the sample preparation and transport can be simplified by applying the samples to the sorbent close to the contaminated area. This minimizes the need for equipment and chemicals, and only the cartridges filled with sorbent have to be transported back to the laboratory for analysis[2,3,4]. In order to prevent degradation of the compounds on the sorbent, the effect of storage time and temperature during transport had to be investigated.

2.5.2 Experimental The effect of storage time and temperature on the recoveries of chemical warfare agents from the C_{18} sorbent was studied. Samples of 50 ml tap water at 20°C spiked with 1 mg of the chemical warfare agents GA, GB, GD, VX and H were passed through 100 mg C_{18} cartridges which had been pre-wetted with 0.5 ml methanol and 2 ml distilled water. After application of the samples, air was passed through the cartridges (about 1.5 l/min) for 10 min to remove most of the water from the sorbent. About 50 mg of water were removed from each cartridge by this procedure. It had already been shown that this air flow did not remove any of the compounds from the cartridges. The cartridges were stored for different periods of time of up to 7 days in a freezer at $-24.5°C$, in a refrigerator at $+2.5°C$ or at room temperature at $+21°C$. The cartridges were then eluted with 300 μl chloroform and analyzed by gas chromatography; five replicates were analyzed for each temperature in each time period. Plot of percentage yields against storage time for the three storage temperatures are given in Figure 1.

An experiment has been carried out in order to investigate the influence of water remaining in the sorbent on the stability of chemical agents during storage of the cartridges. 10-ml water samples containing 1 mg of GA, GB, GD and H were passed through pre-wetted C_{18} cartridges as described earlier. An air stream was passed through one group of the cartridges for 10 min in order to remove most of the water, while another group was not treated for water removal. The cartridges were then stored for 1, 2, 3, 4 and 7 days before elution and analysis. No significant increase (95% level) in the amounts recovered were observed in the samples treated for water

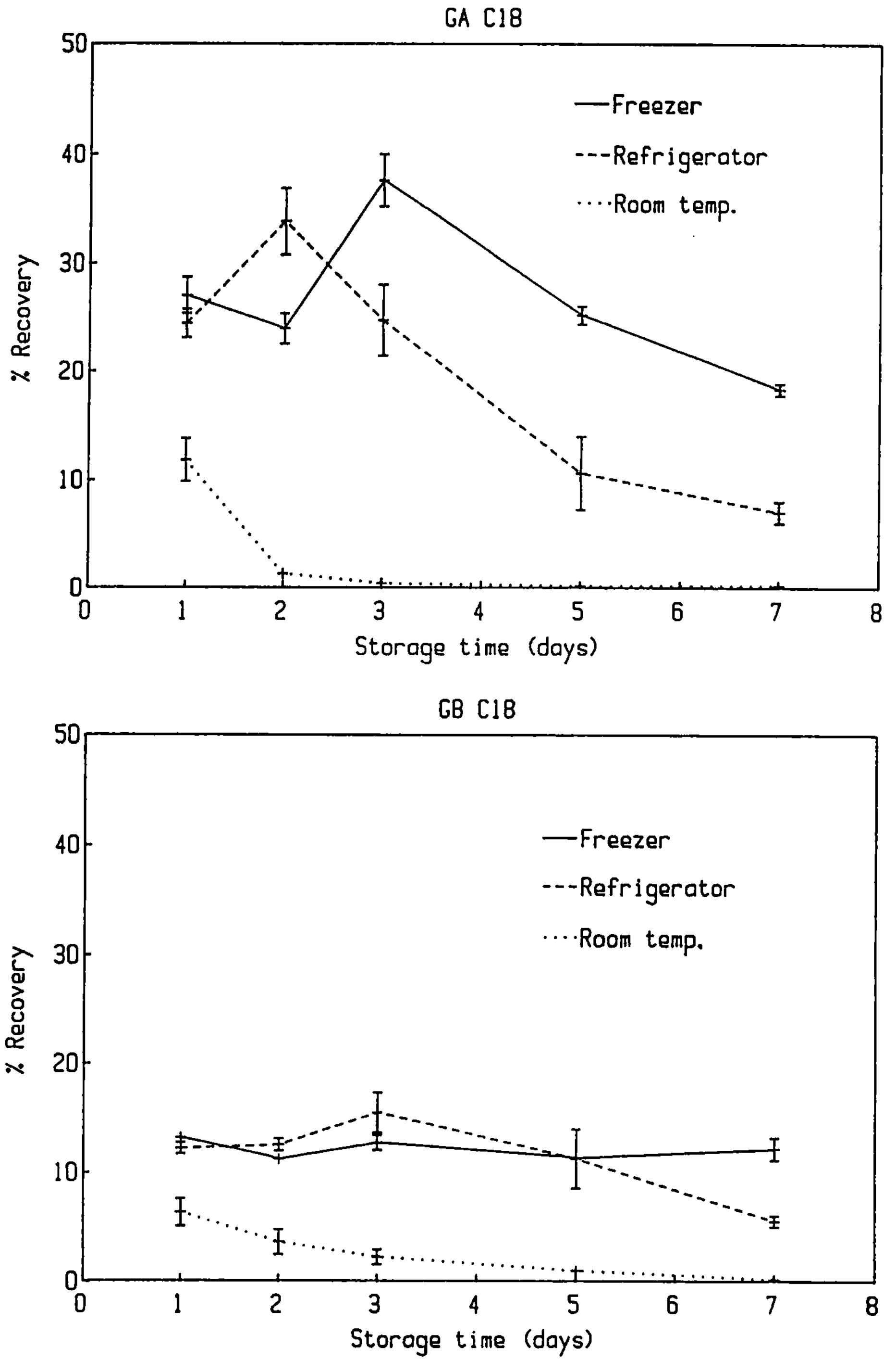

Figure 1 Percentages of (1) GA, (2) GB, (3) GD, (4) VX and (5) H recovered after storage for different periods at room temperature (+21°C), in a refrigerator (+2.5°C) and in a freezer (−24.5°C).

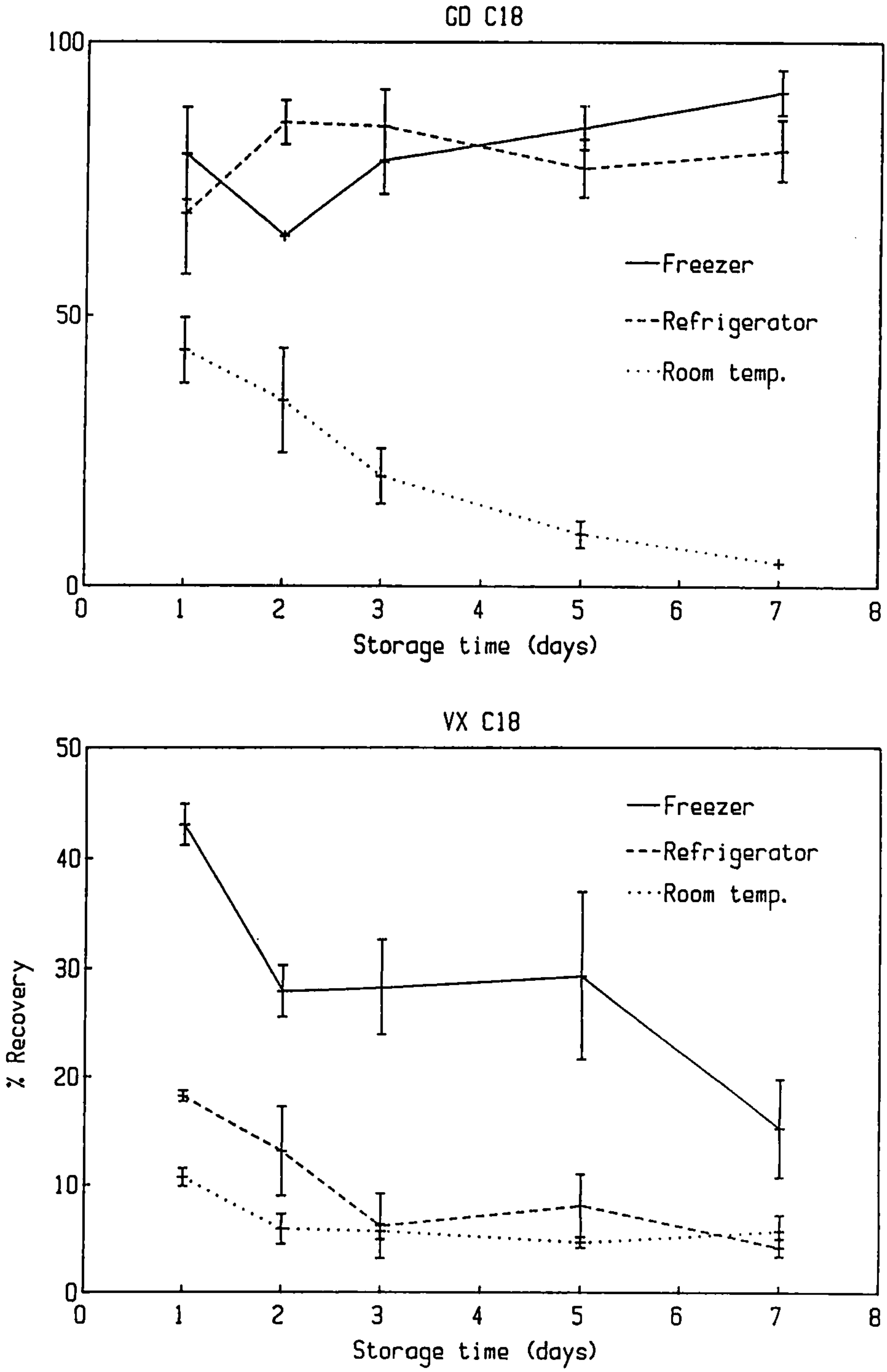

Figure 1 (*Continued*)

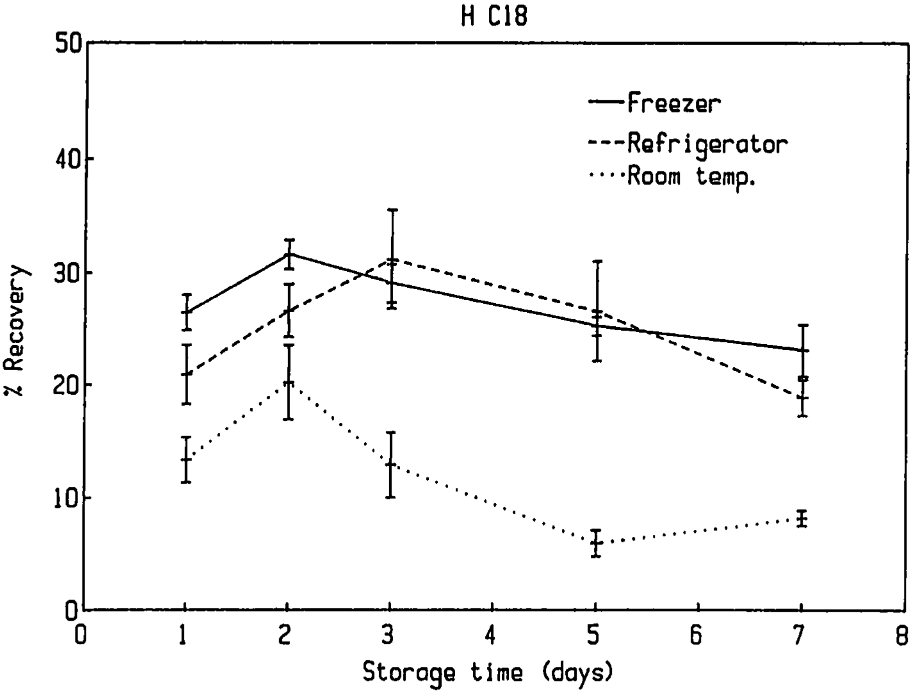

Figure 1 (*Continued*)

removal compared to the control samples. This shows that passing an air stream through the sorbent bed did not remove all the water contained in the sorbent.

2.5.3 Discussion The results given in Figure 1 show that all compounds investigated were rapidly degraded on cartridges stored at room temperature. For GA and GB, less than 5% was recovered after two days of storage. For GD, VX and H, the degradation was slower, but the yields had dropped to less than 10% after five days.

For the compounds GB, GD and H, a refrigerator and a freezer were comparably suited for storing cartridges for periods of up to five days, and the amounts recovered were relatively constant. For GA, the yield began to drop after three days storage both in the freezer and in the refrigerator, but the degradation was faster in the refrigerator. For VX, degradation was fast the first two days both in a refrigerator and at room temperature, but the concentration seemed to level off at about 5%. If the cartridges need to be stored for more than seven days, a freezer should be used for all compounds investigated. For shorter storage times, a refrigerator may be used. The samples should also be kept cool under transport to the laboratory, and a box filled with dry ice (−78.5°C) or other cooling devices such as an isolated bag with cooling elements may be used, especially if the transport takes more than a few hours.

Passing an air stream through the cartridges was not efficient enough in drying the sorbent bed. The remaining water can cause degradation of the compounds during

Table 7 Amounts of compounds recovered from water at different concentrations.

Agent	*Per cent recovered at concentration of* 20 $\mu g/ml$	20 ng/ml
GA	86 ± 11	56 ± 8
GB	54 ± 22	64 ± 5
GD	98 ± 3	68 ± 8
VX	36 ± 12	×
H	12 ± 1	11 ± 2
DIPMP	87 ± 10	46 ± 4

×, no investigation carried out.

transport and storage and eventually phase separation if, for example, chloroform is used as an eluting solvent.

2.6 *Method validation*

The efficiency of the overall sample preparation procedure was established for GA, GB, GD and VX, H and DIPMP from water. The yields for all the agents, except VX, were established at concentrations of both 20 μg/ml and 20 ng/ml. The recovery of VX was only established at the 20 μg/ml level. After adding the analytes to 50 ml water, the sample was placed in a closed vial for 30 min and then passed through 200 mg C_{18} sorbent which previously had been wetted with 1 ml methanol and 4 ml water. The compounds were immediately eluted from the cartridges with 600 μl chloroform, internal standard was added and the sample analyzed by gas chromatography; five replicate experiments were conducted for each combination of chemical agent and concentration. The figures in Table 7 show yields above 85% for GA, GD and DIPMPa at the 20 μg/ml concentration. The yields for H are low, but as shown in Table 6, these can be improved by using larger elution volume. The yields were lower for all agents except GB and H at the 20 ng/ml level compared to the 20 μg/ml level.

3 CONCLUSIONS

This paper describes the development of a sample preparation procedure to be used in screening aqueous samples suspected to be contaminated by chemical warfare agents. The procedure has therefore been made as general as possible.

The non-polar octadecylsilane (C_{18}) sorbent was chosen since it gave the highest yields. The amount of sorbent needed depends on the sample volume, and some suggestions are given in Table 8. After application of the sample, the sorbent should be washed with water in order to remove most of the polar impurities. If necessary,

Table 8 Optimal values of various parameters for solid-phase extraction of chemical wareface agents from aqueous samples.

Sample volume (ml)	*Sorbent amount (mg)*	*Pretreatment*		*Water wash (ml)*	*Elution (μl)*
		MeOH (ml)	*Water (ml)*		
10	100	0.2	2.5	2.5	300
50	200	0.3	5.0	5.0	500
100	500	1.0	12	10	800
500	1000	2.0	25	25	1500

the sorbent may also be washed with hexane to remove organic impurities. Some suggestions about the required volume of washing solvent are included in the table. The yields are almost independent of sample flow rate through the sorbent, but speeds of 5–10 ml/min are suggested. Methanol and acetone are the most efficient solvents for eluting the investigated compounds from the C_{18} sorbent. Methanol should, however, be avoided because of possible reaction with the chemical warfare agents through nucleophilic attack. Some water always remains in the sorbent after sample application; this will be eluted by acetone together with the compounds and cause hydrolysis. As an alternative, therefore, chloroform is often used as eluting solvent. The amounts of chloroform or acetone needed for elution are given in Table 8.

The figures in this table should be treated with some care. The amounts of sorbent needed to retain compounds from the different sample volumes are only valid for relatively clean samples. For samples containing high proportions of organic matter, the amount should be increased. Larger sorbent amounts should also be used to isolate water-soluble agents like GB. Higher yields can be obtained by using larger volumes of eluting solvent, but this will result in lower concentrations of the compounds in the eluate.

The cartridges should be kept cool during transport and storage to prevent degradation of the compounds. Temperatures below 0°C such as in a freezer or a box filled with dry ice should be used if the storage or transport lasts for more than 24 h. For shorter time periods, temperatures as in a refrigerator may be acceptable.

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