Journal of Chromatography, 467 (1989) 129–138
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 238

GAS CHROMATOGRAPHIC DETERMINATION OF METHYLPHOSPHONIC ACIDS BY METHYLATION WITH TRIMETHYLPHENYLAMMONIUM HYDROXIDE

J. Aa. TØRNES* and B. A. JOHNSEN
Norwegian Defence Research Establishment, Division for Environmental Toxicology, P.O. Box 25, N-2007 Kjeller (Norway)
(First received October 3rd, 1988; revised manuscript received December 29th, 1988)

SUMMARY

A method is described for the isolation of decomposition products of nerve agents from aqueous solutions and their determination by gas chromatography. The methylphosphonic acids were retained on an aminopropyl ion exchanger and eluted with trimethylphenylammonium hydroxide, which also acted as a methylating reagent. The procedure was applied to environmental samples in a field exercise in which samples contaminated with the nerve agents sarin and soman were exposed to the prevailing weather for periods of up to 4 weeks. The methylphosphonic acids were detected in all the samples examined.

INTRODUCTION

In connection with the verification of the use of chemical warfare agents, additional information can be obtained from the determination of the degradation products of unstable compounds such as nerve agents. Hence it is important to have methods available for the determination of such compounds. The nerve agents isopropyl methylphosphonofluoridate (sarin, GB), 1,2,2-trimethylpropyl methylphosphonofluoridate (soman, GD) and ethyl S-2-diisopropylaminoethyl methylphosphonothiolate (VX) are hydrolysed to methylphosphonic acids according to the scheme outlined in Fig 1.

The methylphosphonic acids are polar, non-volatile compounds, and should therefore be well suited for determination by high-performance liquid chromatography (HPLC)[1–3]. The problem, however, is that no sensitive detectors for compounds such as methylphosphonic acids are available. These compounds have therefore often been determined by gas chromatography (GC) with prior derivatization. The most common derivatization methods have been the formation of trimethylsilyl (TMS) ethers or *tert.*-butyldimethylsilyl (t-BDMS) ethers from the acids[4,5] or methylation with diazomethane[6,7]. Experiments carried out in our laboratory have shown that the formation of t-BDMS ethers gave poor yields, especially from methylphosphonic acids with small alkyl groups. In addition, the silyl ethers were unstable and sensitive to moisture.

0021-9673/89/$03.50

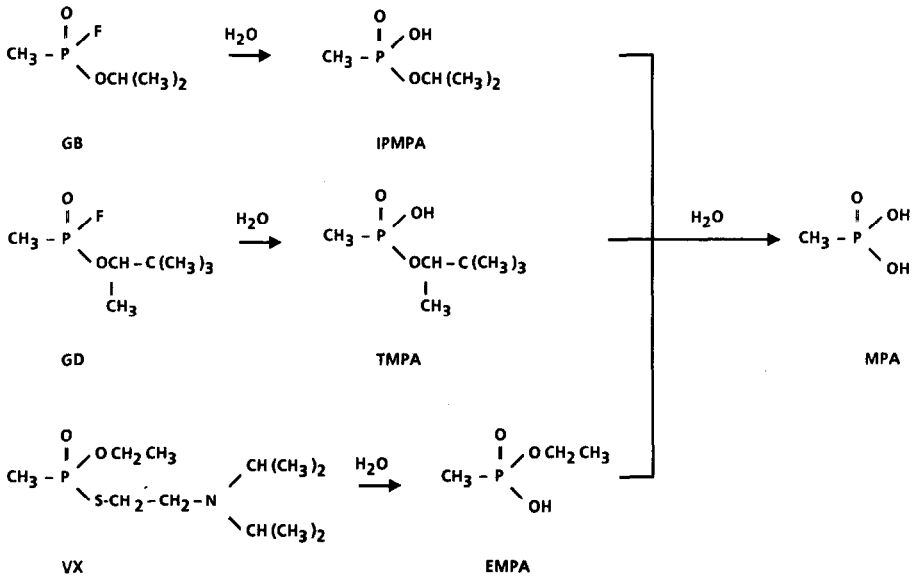

Fig. 1. Reaction scheme for hydrolysis of the nerve agents sarin (GB), soman (GD) and VX.

The use of diazomethane as a derivatization reagent gives high yields, but is not recommended because of its explosive and carcinogenic properties. Another disadvantage of diazomethane is that it is unstable and has to be prepared freshly every day.

A method that has been used for the methylation of acids that are difficult to esterify, *e.g.*, barbituric acids and sterically hindered carboxylic acids[8–13], has now been applied to the methylphosphonic acids. This method is based on formation of ion pairs of the acids with trimethylphenylammonium hydroxide (TMPAH) and subsequent methylation in the injection port of the gas chromatograph. This derivatization method is fast and gives stable methyl esters in quantitative yields from isopropyl methylphosphonic acid (IMPA), 1,2,2-trimethylpropyl methylphosphonic acid (TMPA) and methyl methyl phosphonic acid (MMPA), even from aqueous solutions of the acids.

The methylphosphonic acids have pK_a values of about 2.5 and are totally dissociated in neutral aqueous solutions. It should therefore be possible to retain the acids on anion-exchange cartridges. Hydrolysed sarin (IPMPA) and hydrolysed soman (TMPA) were applied to different sorts of anion exchangers and the results are reported in this paper.

Experiments have shown that large excess of the reagent should be used in order to obtain quantitative yields from the derivatization with TMPAH. This problem has been solved by using TMPAH as the solvent to elute the methylphosphonic acids from the ion-exchange cartridges.

EXPERIMENTAL

Sorbents and chemicals

The ion exchangers used were aminopropyl (NH_2), diethylaminopropyl (DEA) and trimethylaminopropyl (SAX) from Analytichem International. In addition, octadecylsilane (C_{18}) cartridges from the same company were used in the field experiments. All cartridges contained 100 mg of packing material.

The TMPAH derivatization reagent was a 0.1 *M* solution in methanol from Kodak. Other chemicals used were Uvasol-grade methanol and chloroform from Merck.

Isopropyl methylphosphonic acid, 1,2,2-trimethylpropyl methylphosphonic acid and methyl methylphosphonic acid were synthesized and purified (>95%) as described in the literature[14,15].

Chromatographic equipment and conditions

The analysis of the hydrolysed and derivatized nerve agents was performed on a Packard Model 438 gas chromatograph coupled to a LKB 2091 mass spectrometer. A 3-ft glass column packed with SP1200–1% H_3PO_4 liquid stationary phase on 80–100-mesh Chromosorb W AW was used for separation. The column temperature was maintained at 120°C for 2 min and then raised at 16°C/min to 170°C. The temperature of the transfer line between the gas chromatograph and the mass spectrometer was set at 250°C and the injector temperature at 300°C. The compounds were detected by selected ion monitoring (SIM) and the mass fragments used for detection were *m/z* 111 (100%) and 137 (32%) for methylated IPMPA, *m/z* 138 (100%) and 111 (86%) for methylated TMPA, *m/z* 94 (100%) for methylated MMPA and *m/z* 57 (100%) for decane. SIM curves of a mixture of IPMPA and TMPA methylated with TMPAH together with decane as internal standard are shown in Fig 2.

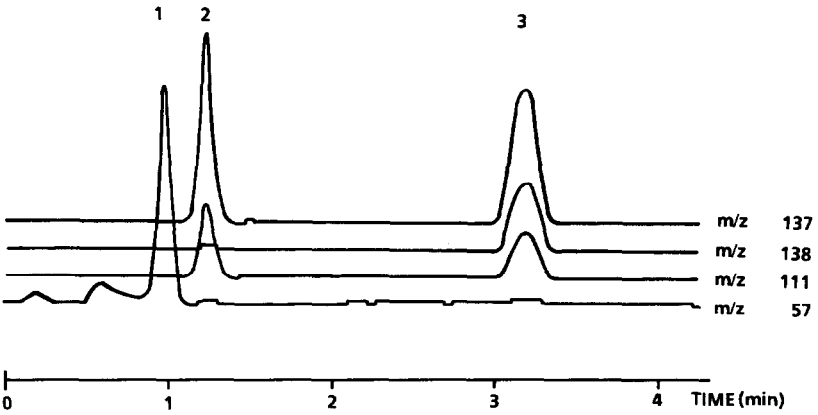

Fig. 2. SIM curves for a sample containing 10 μg/ml of IPMPA (2) and TMPA (3) methylated with TMPAH, together with decane (1) as internal standard. The chromatographic conditions are given in the text.

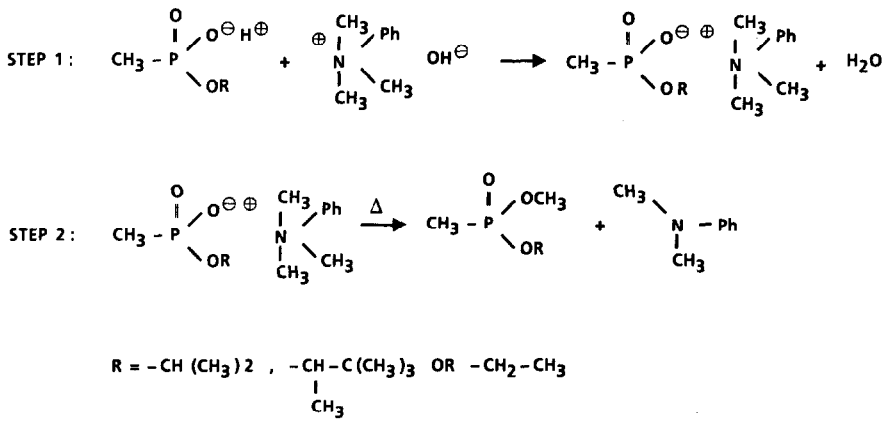

Fig. 3. Reaction scheme for methylation of methylphosphonic acids with TMPAH.

Derivatization

A derivatization method applicable to GC should be fast and non-hazardous when a large number of samples are to be handled. The methylation by TMPAH meets these requirements, and it can even be used with aqueous samples.

When a solution of methylphosphonic acids is mixed with TMPAH in methanol, the methylation of the acids takes place in the injection port of the gas chromatograph at high temperature after the injection[8–13] (Fig 3). The temperature in the injector is an important factor in obtaining the maximum yields. To find the optimum conditions, 50 μl of an aqueous solution of 1 mg/ml of IPMPA were mixed with 300 μl of 0.1 *M* TMPAH in methanol and 1 -μl aliquots of the mixture were injected on to the gas chromatograph with injector temperatures varying from 160 to 300°C. A plot of the peak area *versus* the injector temperature (Fig 4) shows that temperatures in excess of 260°C should be used. In the following experiments the injector temperature was set at 300°C.

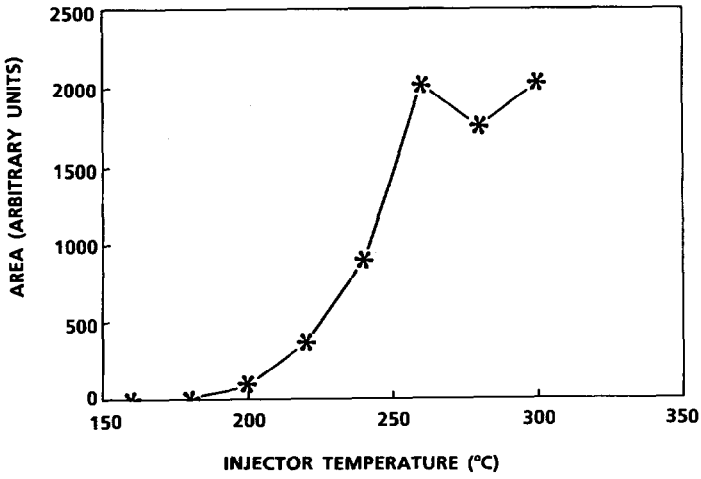

Fig. 4. Plot of the peak area *versus* injector temperature for a mixture of IPMPA and TMPAH.

Selection of ion exchangers

The ion exchanger selected for the preparation of samples containing methylphosphonic acids for GC analysis should be able to retain acids containing different alkyl chains with high effciency. In order to take full advantage of the methylating reagent TMPAH, the methylphosphonic acids should also be eluted from the selected ion exchanger with this reagent with high efficiency.

In order to find the best ion exchanger, the recoveries of hydrolysed sarin (IPMPA) and hydrolysed soman (TMPA) from different anion exchangers were investigated. Volumes of 10 ml of aqueous solutions of 1 mg of IPMPA and 1 mg of TMPA were passed through cartridges packed with NH_2, DEA or SAX ion exchangers that had previously been wetted with 0.5 ml of methanol and 2 ml of distilled water. The aqueous phases were collected in order to check the amounts of the acids not retained on the cartridges. Volumes of 50 μl of these aqueous phases were mixed with 300 μl of TMPAH in methanol and 1 μl of the mixture was injected on to the gas chromatograph; 20 μl of a 0.2% solution of decane were added to the mixture as an internal standard before injection. The methylphosphonic acids were eluted from the cartridges with 300 μl of TMPAH (pH 13.0), internal standards were added and the samples were injected into the gas chromatograph. Seven replicates were analysed for each sample, and the amounts of acids recovered from the aqueous phases and from the TMPAH eluates together with standard deviations are shown in Table I. According to these results, the NH_2 and the DEA cartridges gave the best recoveries from the eluate. The best results were obtained with the NH_2 cartridge, which was selected for further investigations.

TABLE I

RECOVERIES OF IPMPA AND TMPA FROM THE AQUEOUS PHASE AND THE ELUATE FROM DIFFERENT ION EXCHANGERS

Recoveries ± S.D. (n = 7).

Agent	*Phase*	*Recoveries from cartridges (%)*		
		NH_2	*DEA*	*SAX*
IPMPA	Aqueous phase	6±3	5±3	11±3
	Eluate	88±7	73±3	27±9
TMPA	Aqueous phase	10±5	45±19	1±1
	Eluate	75±6	62±8	4±2

Optimization of the preparation procedure

The capability of a cartridge containing 100 mg of NH_2 sorbent to retain methylphosphonic acids from various sample volumes was investigated. Volumes of 10, 50 and 100 ml of aqueous solutions of 1 mg of IPMPA and 1 mg of TMPA were passed through NH_2 cartridges, eluted with 300 μl of TMPAH and analysed as described above. The recoveries, which are given in Table II, showed a significant decrease with increasing sample volume. This decrease is probably due to inefficient retention of the methylphosphonic acids from large sample volumes, because experiments have

TABLE II

RECOVERIES OF IPMPA AND TMPA FROM NH_2 CARTRIDGES WITH DIFFERENT SAMPLE VOLUMES

Sample volume (ml)	*Recovery (%)*	
	IPMPA	*TMPA*
10	88	75
50	78	59
100	57	40

shown that the recoveries did not vary significantly with sample concentration. Therefore, if sample volumes in excess of 100 ml are to be handled, cartridges containing larger amounts of packing material should be applied.

It was expected that the recoveries of methylphosphonic acids from large sample volumes could be enhanced if lower flow-rates are used. Ion-exchange interaction is a slower process than polar and non-polar interactions and a maximum flow-rate of 5 ml/min has been suggested in the literature[16]. To verify this, 50 ml of aqueous solutions containing 1 mg of IPMPA were passed through NH_2 cartridges at different flow-rates from 1.7 to 10 ml/min. The methylphosphonic acids were eluted and analysed by GC as above. These experiments showed no significant differences in recoveries with the different flow-rates investigated.

Investigations did not show significant differences in the amounts recovered when the volume of the elution solvent was varied from 300 μl to 1 ml. However, 300 μl was considered to be the minimum amount required to elute the acids from the cartridges in a reproducible manner, and was therefore used in the following studies.

Field experiments

The procedure for the determination of hydrolysed sarin and soman was applied in a field exercise in order to evaluate the usefulness of the method. Amounts of 1 mg of sarin and soman were added to samples of 50 ml of water or about 10 g of grass, sand or soil, and the samples were exposed to the prevailing weather for periods of up to 4 weeks. The concentrations of both nerve agents and the methylphosphonic acids were measured after 1, 2 and 4 weeks. The temperature and relative humidity were recorded. The mean temperature was 15°C during the first week, 10°C during the second week and about 15°C during the third and fourth week. The relative humidity varied from about 30% during the day to about 95% during the night.

In order to determine both nerve agents and methylphosphonic acids in the same samples, C_{18} cartridges were connected with the NH_2 cartridges. The nerve agents were retained on the C_{18} cartridges on top [17,18], whereas the acids passed through and were retained on the NH_2 cartridges at the bottom.

Samples of about 10 g of grass, sand or soil were extracted with 50 ml of water by shaking for 1 min. The aqueous phases, together with the 50-ml water samples, were then filtered and passed through the cartridges which were pre-wetted with 1 ml of methanol and 5 ml of water[17,18]. The C_{18} and NH_2 cartridges were separated before the compounds were eluted. The nerve agents were eluted from the C_{18} car-

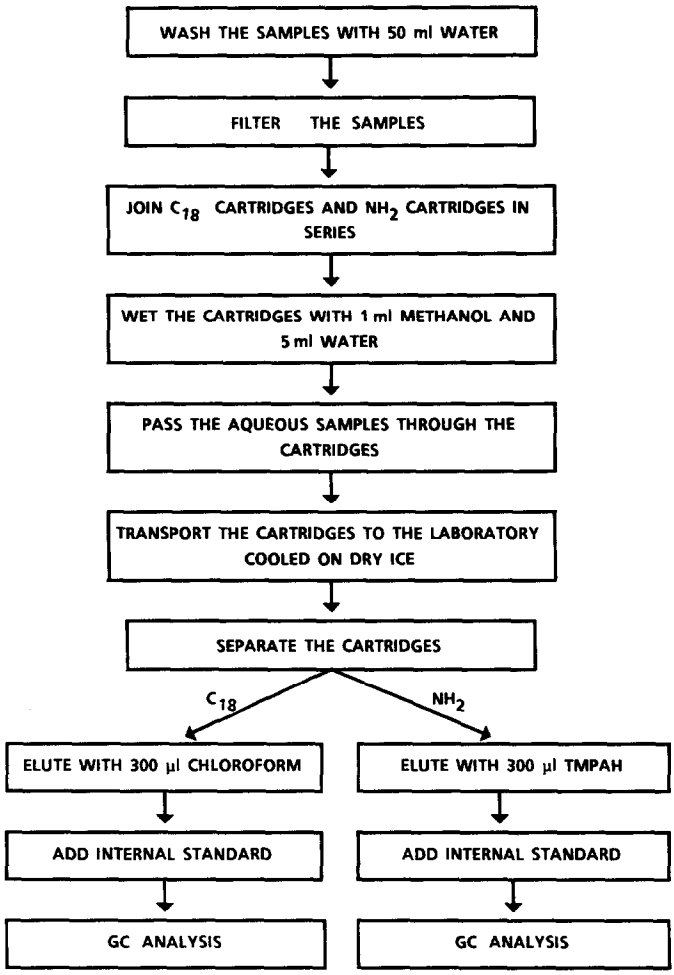

Fig. 5. Flow chart showing the procedure for the determination of nerve agents and methylphosphonic acids from environmental samples.

tridges with 300 µl of chloroform and the methylphosphonic acids were eluted from the NH_2 cartridges with 300 µl of TMPAH in methanol. Decane was added as internal standard and 1-µl samples were injected on to the gas chromatograph. The overall preparation procedure is summarized in Fig. 5.

RESULTS AND DISCUSSION

Evaluation of ion exchangers

The investigations described above showed that the NH_2 anion exchanger was the most efficient for the isolation of hydrolysed sarin (IPMPA) and hydrolysed soman (TMPA) from aqueous solutions. Cartridges containing 100 mg of NH_2 sorbent were selected for further evaluations and showed a recovery of 88% for IPMPA

and 75% for TMPA from sample volumes of 10 ml. The investigations showed further that when the sample volumes exceeded 100 ml, the recoveries decreased to below 50% and cartridges containing larger amounts of sorbent should be considered.

Although a flow-rate through the ion-exchange cartridges of 5 ml/min has been suggested[16], no significant differences in the amounts of methylphosphonic acids recovered were observed when the flow-rate was varied between 1.7 and 10 ml/min, indicating that the flow-rate is not critical.

Derivatization

Elution of the methylphosphonic acids with TMPAH and subsequent methylation in the injection port of the gas chromatograph with TMPAH as derivatization reagent were selected as the best derivatization method. The minimum amount of TMPAH required to elute the acids from the NH_2 cartridges in a reproducible manner was found to be 300 μl and was therefore used in the analysis.

Detection limits

The detection limits for hydrolysed sarin (IPMPA) and hydrolysed soman (TMPA) were established by preparing and analysing sample blanks and samples containing known amounts of the methylphosphonic acids in the same way as the real samples. The detection limits were taken as the concentrations that gave a signal-to-

TABLE III

RESULTS FROM FIELD EXPERIMENTS

Agent	*Sample*	*Amount recovered (μg) after exposure for*		
		1 week	*2 weeks*	*4 weeks*
GB	Water	80	50	0.4
	Grass	0.02	0.04	–[a]
	Sand	0.1	0.2	–
	Soil	0.08	0.08	–
GD	Water	290	90	0.1
	Grass	0.5	0.07	–
	Sand	0.1	0.2	–
	Soil	0.1	–	–
IPMPA	Water	3	2	1
	Grass	0.6	2	1
	Sand	2	1	2
	Soil	0.7	1	NA[b]
TMPA	Water	2	1	0.9
	Grass	0.3	0.8	2
	Sand	0.9	0.3	2
	Soil	0.2	0.3	NA

[a] –, Concentration below detection limit.
[b] No analysis carried out.

noise ratio of 3. The minimum amounts that could be detected in a 50-ml sample volume were 100 ng for both IPMPA and TMPA when a mass spectrometer with SIM was applied. These limits could be lowered if a capillary column was used and the eluates were concentrated prior to analysis.

Results from field experiments

The amounts of nerve agents and methylphosphonic acids recovered from the different samples after exposure to the prevailing weather for up to 4 weeks are given in Table III. The recoveries shown were calculated relative to standard samples containing known amounts of the compounds and prepared in the same way as the unknown samples. Hydrolysed soman was to some extent retained on the C_{18} cartridges, leading to reduced recoveries. This did not occur with hydrolysed sarin. More recent work showed that a better approach would be to place the NH_2 cartridge on top of the C_{18} cartridge. Sarin and soman were not retained on the NH_2 cartridge and could be collected on the C_{18} cartridge at the bottom, whereas the hydrolysed nerve agents were collected on the NH_2 cartridge.

These experiments showed that after exposure for 4 weeks under summer conditions the nerve agents could only be detected in water samples. The amounts of methylphosphonic acids were fairly constant and could easily be detected after 4 weeks in all samples. In this field experiment, only one sample was analysed for each agent in each time period and it was therefore not possible to make a statistical evaluation of the results.

CONCLUSIONS

The results from the field exercise have shown that the determination of breakdown products can be an important method when alleged use of nerve agents has to be verified under summer conditions. The hydrolysed sarin and soman were easily detected after exposure to the prevailing weather for 4 weeks, whereas sarin and soman were only found close to the detection limits after 2 weeks.

Although several methods have been proposed for the determination of hydrolysed nerve agents, the present procedure has several advantages. The sample preparation is based on the same technique as used by the Norwegian Defence Research Establishment for isolation of nerve agents[17,18]. The method does not require large amounts of equipment or chemicals, and can easily be performed by untrained personnel under field conditions. The transport of the samples to the laboratory is simple as only small cartridges have to be transported. The derivatization with TMPAH is a fast and non-hazardous technique which produces stable methyl esters in high yields. Its combined use as eluent and derivatization reagent makes TMPAH especially advantageous.

REFERENCES

1 P. C. Bossle, J. J. Martin, E. W. Sarver and H. Z. Sommer, *J. Chromatogr.*, 267 (1983) 209.
2 P. C. Bossle, D. J. Reutter and E. W. Sarver, *J. Chromatogr.*, 407 (1987) 399.
3 A. Verweij, paper presented at the *2nd. Symposium on the Chemistry and Fate of Organophosphorus Compounds, Barcelona, Spain, November 10–12, 1987.*
4 D. J. Harvey and M. G. Horning, *J. Chromatogr.*, 79 (1973) 65.

5 J. G. Purdon, J. G. Pagotto and R. K. Miller, *Report DREO-936*, Defence Research Establishment Ottawa, Ottawa, 1985.
6 A. Verweij, C. E. A. M. Degenhardt and H. L. Boter, *Chemosphere*, 3 (1979) 115.
7 A. Verweij, H. L. Boter and C. E. A. M. Degenhardt, *Science*, 204 (1979) 616.
8 E. W. Robb and J. J. Westbrook, *Anal. Chem.*, 35 (1963) 1644.
9 J. MacGee, *Clin. Chem.*, 17 (1971) 587.
10 V. Prelog and M. Piantanida, *Z. Physiol. Chem.*, 244 (1936) 56.
11 R. C. Fuson, J. Corse and E. C. Horning, *J. Am. Chem. Soc.*, 61 (1939) 1290.
12 E. Brochmann-Hanssen and T. O. Oke, *J. Pharm. Sci.*, 58 (1969) 370.
13 G. W. Stevenson, *Anal. Chem.*, 38 (1966) 1948.
14 A. M. de Roos, *Recl. Trav. Chim. Pays-Bas*, 78 (1959) 145.
15 Houben-Weyl, *Metoden der organischen Chemie — Organische Phosphorverbindungen, Teil 1*, Georg Thieme, Stuttgart, 1963, pp. 387–414.
16 *Sorbent Extraction Technology — Handbook*, Analytichem International, Harbor City, CA, 1985, p. 23.
17 B. A. Johnsen, J. A. Tørnes, T. Axelsson and P. J. Karlsen, *Handling of Samples Contaminated with Chemical Warfare Agents: Part 5, FFI/RAPPORT-86/6005*, Norwegian Defence Research Establishment, Kjeller, 1986.
18 B. A. Johnsen, J. A. Tørnes, A. M. Opstad and E. Odden, *Development of Procedures for Verification of Alleged Use of Chemical Warfare Agents: Part 6, FFI/RAPPORT-87/6010*, Norwegian Defence Research Establishment, Kjeller, 1987.