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Short communication

Capillary gas chromatographic analysis of nerve agents using large volume injections

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Abstract

The use of large volume injections has been studied for the verification of intact organophosphorus chemical warfare agents in water samples. As the use of ethyl acetate caused severe detection problems new potential solvents were evaluated. With the developed procedure, the nerve agents sarin, tabun, soman, DFP and VX can be determined in freshly prepared water samples at ppt levels. Except for the nerve agent tabun all other agents added to the water samples were still present after 8 days at 20–60% levels, if the pH of the water sample is adjusted to ca. 5 shortly after sampling and adjusted to pH 7 for analysis.

Keywords: Large volume injection; Organophosphorus compounds; Sarin; Tabun; Soman; DFP; VX; Chemical warfare agents

1. Introduction

Since the seventies the TNO-Prins Maurits Laboratory (TNO-PML) has worked on analytical procedures for the determination of chemical warfare agents (CWA) and related compounds in environmental matrices. Procedures were developed for the determination of organophosphorus nerve agents and their polar degradation products in aqueous samples based on gas chromatography (GC), microcolumn liquid chromatography (μ-LC) using selective GC detectors and thermospray-LC–mass spectrometry (TS-LC–MS) [1–5]. These procedures could be used for the identification of chemical warfare agents in the event of a military conflict or for the verification of compounds relevant to the Chemical Weapons Convention, which prohibits the development, production, stockpiling and use of chemical weapons [6].

In the GC procedure, intact nerve agents were isolated from water by solid-phase extraction using XAD-4, followed by extraction with ethyl acetate [2]. To further concentrate the analytes the ethyl acetate extract was transferred onto a Tenax adsorption tube, which was analysed by thermal desorption cold trap-injection GC. With this set-up using a selective thermionic detector, nerve agents such as sarin, tabun and soman can be determined at the μg/l (ppb) level. However, transfer of the ethyl acetate extract onto a Tenax-tube is laborious and difficult to reproduce. Besides, thermal desorption is only suitable for rather volatile, thermostable com-

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pounds. Analysis of the nerve agent VX using thermal desorption resulted in a considerable decomposition. Therefore, a more straightforward large volume injection (200 μl) of the XAD-4 extract onto a capillary GC system was considered to be a good alternative.

Large volume on-column injections are based on the work by Grob and co-workers [7,8]. Several authors [9–13] have described the analysis of organophosphorus compounds in water by trace enrichment and large volume injections.

2. Experimental

2.1. Materials and standard solutions

Reagent grade water was obtained by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Ethyl acetate, methanol, isopropanol, *n*-hexane and *n*-pentane were of analytical grade and supplied by Merck (Darmstadt, Germany) and Aldrich (Milwaukee, USA). *n*-Pentane was additionally distilled in house using an automated distillation apparatus (Normschliff Geratebau, Wertheim, Germany). When injecting large volumes, special attention should be given to the quality of the solvent: not only the analytes but also the impurities in the solvent will be concentrated.

Sarin (isopropyl methylphosphonofluoridate), soman (1,2,2-trimethylpropyl methylphosphonofluoridate), tabun (ethyl N,N-dimethyl phosphoramidocyanidate), VX (O-ethyl S-2-diisopropylaminoethyl methylphosphonothioate and DFP (diisopropyl phosphorofluoridate) were synthesized at TNO-PML. A standard solution containing 2 mg of each sarin, soman, DFP and tabun, and 4 mg of VX in 10 ml isopropanol was prepared. The solution was stored in a refrigerator (ca. 4°C) and no degradation of the nerve agents was observed during the period of this study. Solid-phase extraction using XAD-4 was carried out with nerve agent concentrations of 10 μg/l water (10 ppb) and 100 ng/l water (100 ppt), respectively. Unless stated otherwise, experiments were carried out with freshly prepared dilutions of the CWA.

Since the procedure described earlier [2] gave some problems concerning irregular flow-rates during sorption, 50 mg Amberlite XAD-4 (Rohm and Haas Co., Philadelphia, PA, USA) was packed in stripped 3-ml BondElut columns (Varian, Cambridge, UK). On top of these columns a 10-ml Calibra glass syringe (Socorex, Renens, Switzerland) was placed, the needle of which pierced through a rubber stop fitted in the modified BondElut column. In this way 10 ml water containing the nerve agents was slowly pressed through the XAD-4 column. Subsequently, the XAD-4 was extracted with approximately 1 ml of solvent. The extracts were collected in glass tubes that were weighed before and after collection of the extract.

2.2. Gas chromatographic system

Fused-silica retention gaps (20 m × 0.53 mm I.D.) deactivated either with diphenyl tetramethyl disilazane (DPTMDS, "phesil", Fisons Instruments, Milan, Italy), or with cyano/phenyl/methyl-siloxane (Chrompack, Middelburg, Netherlands) were used. The retention gap was coupled to a CP-Sil 19 fused-silica capillary column (50 m × 0.32 mm I.D., 0.4 μm film thickness, Chrompack) by a press-fit connector (Hewlett-Packard, Wilmington, DA, USA). The retention gap and the column were installed in a Fisons Instruments (Milan, Italy) Model 5300 Mega series gas chromatograph. The retention gap was connected to the on-column injector and the end of the column was coupled to a Gerstel (Brielle, Netherlands) effluent splitter, which in turn was connected to a flame ionisation detector (FID) and to a thermionic detector (TID) (ratio 1:1). The detector temperatures were 240°C. The column head pressure was set to 200 kPa to give a flow-rate close to the optimum flow-rate of the analytical column.

All experiments were performed with an injection volume of 200 μl. With a GC-TID detection limit of less than 50 pg, an injection volume of 200 μl taken from a total volume of 1 ml extract which originates from 10 ml water, corresponds with less than 25 ppt organophosphorus CWA in water.

Injections of the extracts were performed by means of a 500-μl syringe (500F-GSG; SGE,

Ringwood, Vic., Australia) placed on an infusion pump (Harvard Apparatus, Edenbridge, Kent, UK), which was able to produce a constant injection flow-rate. To the needle of the syringe a piece of deactivated fused-silica tubing (0.4 m × 0.1 mm I.D.) was connected by means of a Valco 1/32″ zero-dead volume union (VICI AG, Schenkon, Switzerland). The tubing has to be disconnected to enable filling of the syringe. After filling, the tubing was reconnected and gently pushed through the on-column injector into the column. After injection the tubing was removed from the injector.

3. Results and discussion

The experiments were carried out by using the conventional retention gap technique, because of its ease of handling [7,14]. The length and diameter, 20 m × 0.53 mm, of the retention gap were selected to allow injection of up to 500 μl of organic solvents of different polarities under various temperature conditions without severe risk of damaging the analytical column. The apparent film thickness [7] of the retention gap should be very thin compared to the film thickness of the analytical column.

With a commercially available cyano/phenyl/methyl deactivated retention gap (20 m × 0.53 mm), broad and distorted peaks were observed. Based on the dimensions of the gap and of the analytical column, the maximum tolerable film thickness for the retention gap was calculated [7]. If 20% of band broadening is allowed, the apparent film thickness of the retention gap amounts to 1.93 nm. According to the specifications of the manufacturer of the retention gap, it has a film thickness of 50 nm, which makes it rather a column with a very thin film of stationary phase, than a retention gap.

With a different, "phesil", retention gap the problem of broadened peaks was not observed. It proved to be robust, since it could be used for over two years before it showed any distortion of the peaks due to activity (tailing peaks).

The experiments were started with ethyl acetate as this was the solvent used in the previous study [2]. Unfortunately, injection of large volumes of ethyl acetate proved to give reversible but severe negative effects on the performance of the thermionic detector. The use of methanol, being a good solvent for XAD-4 desorption, turned out to be impossible because of the disastrous effect large volumes of methanol appeared to have on the CP-Sil 19 stationary phase. After injection a saw-tooth baseline was observed, which remained for hours.

Finally, a mixture of solvents was investigated. It is well-known from liquid chromatography [15] that the addition of 1 to 5% of a polar solvent to an apolar solvent gives a large increase in the solvent strength (ϵ°). The ϵ° (Al_2O_3) values for *n*-pentane, ethyl acetate and methanol are 0.0, 0.58 and 0.95, respectively. The addition of 5% (mole fraction 0.13) of methanol to *n*-pentane results in a value of ϵ° of 0.57, which is comparable with that of ethyl acetate. Picogram quantities of CWA spiked on XAD-4 were desorbed by *n*-pentane–methanol (95:5, v/v) and 1-ml portions of the extract were collected. The CWA appeared to be contained in the first portion.

The solvent mixture is a minimum homoazeotropic system. From published data on the influence of pressure on boiling point and composition of the azeotropic mixture [16,17] it was calculated that at a pressure of 300 kPa the azeotropic boiling point is 63°C and the mole fraction of methanol is 0.26.

The injection of relatively small amounts of methanol (5% of 200 μl = 10 μl) proved to have no adverse effects on the analytical column.

The infusion pump was set to move the injection syringe plunger at a rate of 200 μl in 150 s (80 μl/min). The sample was introduced at a temperature which is below the solvent boiling point at the column inlet pressure [18]. After the injection of the sample, the oven temperature was raised to the solvent evaporation temperature to speed up solvent evaporation [7]. Several sample injection and solvent evaporation temperatures were tested close to the boiling point of the azeotropic mixture.

Based on the results, final conditions were selected as summarised in Table 1.

As an example, the resulting chromatogram of the GC analysis of 200 μl of a solution containing the five nerve agents under study in *n*-

Table 1
Final experimental conditions

Solvent	*n*-Pentane–methanol (95:5, v/v)
Injection volume	200 μl
Injection rate	200 μl/150 s
Retention gap	20 m × 0.53 mm I.D., DPTMDS-deactivated
Analytical column	50 m × 0.32 mm I.D., CP-Sil 19, 0.4 μm film thickness
Column pressure	200 kPa
Oven temperature	60°C (3 min), 5°C/min → 70°C (7 min), 10°C/min → 180°C (0 min), 15°C/min → 240°C (12 min)

pentane–methanol (95:5, v/v) is presented in Fig. 1. The injected amounts were 0.4 ng for sarin, DFP, soman and tabun, and 0.8 ng for VX. The tailing of the solvent peak is probably caused by the graphite ferrules in the effluent splitter.

Applications

The isolation of the nerve agents sarin, DFP, soman, tabun and VX from water on XAD-4 was performed as described previously [2]. Experiments with water samples [concentration 10 μg/l (10 ppb) and 100 ng/l (100 ppt), respectively] performing subsequent isolation on XAD-4, extraction by 1 ml *n*-pentane–methanol (95:5, v/v) and GC analysis gave recoveries of 30–50% for sarin, soman and tabun, and of 80–100% for DFP and VX. This is in agreement with the previous results [2].

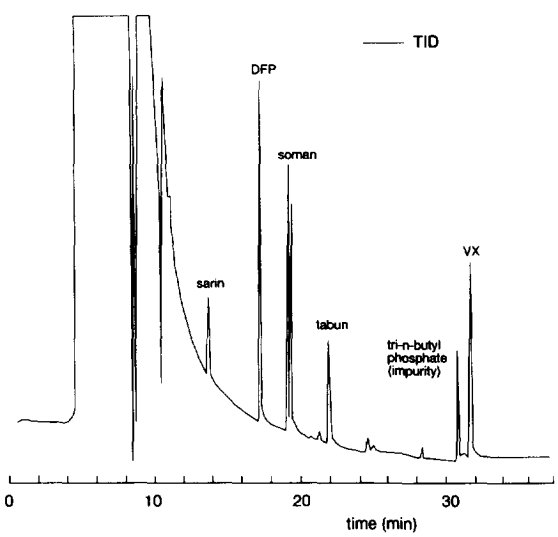

Fig. 1. Injection of 200 μl of a solution containing sarin, DFP, soman, tabun and VX in *n*-pentane–methanol (95:5, v/v). For conditions see Table 1.

Nerve agents are readily hydrolysed in water depending on the pH and the presence of other ions [19]. This means that samples should be analysed shortly after sampling and, to reduce the hydrolysis of nerve agents it is advisable to acidify the water samples to pH 4–5 directly after sampling. However, the pH should be adjusted to pH 6–7 before extraction, because at pH 4–5 VX ($pK_a = 8.5$ [20]) is protonated and remains in the water.

When an XAD-4 column had been used a number of times, the recovery for all nerve agents except VX decreased. Finally, only VX was recovered at 80–100%. Therefore, one should be careful to reuse XAD-4 columns. Apparently, rinsing XAD-4 with Milli-Q water, methanol and ethyl acetate was not sufficient to recondition the sorbent.

4. Conclusions

Large volume on-column injection proved to be a good alternative for the thermal desorption of a Tenax adsorption tube in the analytical procedure for the determination of intact organophosphorus chemical warfare agents in water by GC. Using XAD-4 as sorbent and *n*-pentane–methanol (95:5, v/v) as extraction solvent, the recoveries found were in agreement with those obtained previously [2]. Moreover, the nerve agent VX could be determined employing the new procedure.

Compared to the usual gas chromatographic

injection volume of 1 μl, large volume on-column injection of 200 μl gives a 200 times improvement of the detection limit. Starting with a water sample of 10 ml, ppt detection of organophosphorus nerve agents can be performed by large volume on-column injection and thermionic detection.

The low detection limit of this procedure (ppt level) makes it possible to verify the presence of nerve agents in water, even after they have substantially degraded. To analyse the polar decomposition products, techniques like CE, μ-LC and/or TS-LC–MS should be used.

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