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Application of capillary gas chromatography to the study of hydrolysis of the nerve agent VX in rat plasma

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Abstract

We present here a gas chromatography technique allowing the detection and quantification of VX [*O*-ethyl *S*-(2-diisopropylaminoethyl)methylphosphonothiolate] as well as its P–S bond hydrolysis product diisopropylaminoethanethiol directly from spiked rat plasma. This technique was applied to study VX hydrolysis in rat plasma. We observed that 53±4% of 374 μ*M* VX disappeared from spiked plasma after 2 h. VX disappearance was mainly related to enzymatic cleavage of the P–S bond (K_m=2.5 m*M* and V_{max}=13.3 nmol min^{-1} ml^{-1} of rat plasma). The activity was totally inhibited by 1 m*M* Hg^{2+} and was also inhibited by metal chelators.

Keywords: Diisopropylaminoethanethiol; Nerve agents; *O*-Ethyl *S*-(2-diisopropylaminoethyl)methylphosphonothiolate; VX, nerve agent

1. Introduction

Organophosphorus compounds (OPs) were developed as chemical warfare agents due to their highly toxic nature. As a matter of fact they produce a variety of toxic effects by irreversibly inhibiting AChE (acetylcholinesterase), an enzyme required for the normal functioning of the nervous system, and are consequently termed "nerve gases" [1–4]. Since most "nerve gases" are organophosphorus acid anhydrides, enzymatic hydrolysis of this chemical class of agents was abundantly studied and is now well documented [5–8]. Another generation of highly toxic AChE inhibitors is represented by phosphonothiolo compounds. The best known of this latter class is the "nerve" agent VX [*O*-ethyl *S*-(2-diisopropylaminoethyl)methylphosphonothiolate] [9] (see Fig. 1). VX presents two particularities: it is both the most powerful "nerve gas" to date [10–12] and one of the less biodegraded due to the stability of its P–S bond [12]. Recently and for the first time, VX biodegradation was demonstrated by a purified enzyme derived from *Pseudomonas diminuta* [13,14]. Nevertheless, until now the study of VX hydrolysis was impeded by the lack of analytical means to follow the fate of this compound in complex protein mixtures. We now present a new and fast procedure to extract, detect and quantify VX and its P–S bond hydrolysis product DPAT (diisopropylaminoethanethiol) (displayed in Fig. 1) in

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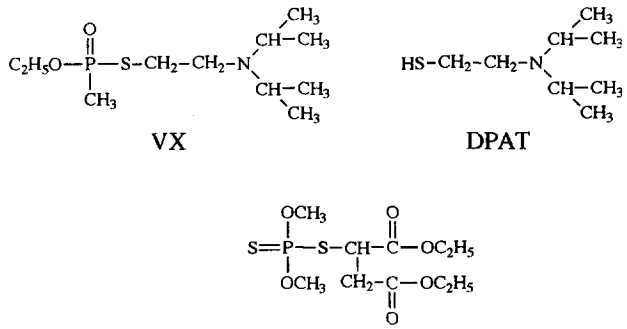

Fig. 1. Chemical structures of VX, DPAT, and malathion.

crude protein mixtures typified by rat plasma. In a second part, this procedure was applied to the study of VX disappearance in rat plasma demonstrating the existence of an organophosphorus hydrolase (OPH) displaying easily measurable activity toward VX.

2. Experimental

2.1. Materials and standard solutions

CAUTION. VX is extremely toxic! One should not attempt to synthesize or work with this compound unless proper training and adequate laboratory facilities have been acquired. VX and DPAT were obtained from the Centre d'Etudes du Bouchet, Department of Chemical Synthesis. The purity of these compounds was determined by gas chromatography (GC) and found to be better than 90%. Stock solutions of VX and DPAT were prepared in water–2-propanol (85:15) and 2-propanol respectively. Malathion, obtained from Sigma (St. Quentin Fallavier, France), was diluted in 2-propanol. Dithiothreitol (DTT) was from Fluka (St. Quentin Fallavier, France), hexane was purchased from Prolabo (Paris, France), ethyl acetate was from Baker (Noisy Le Sec, France) and 2-propanol from Merck (Nogent sur Marne, France). All other chemicals were from Sigma.

2.2. GC system

For separation of VX and/or DPAT, a CP 8CB fused-silica capillary column (25 m×0.32 mm I.D.) from Chrompack (Les Ulis, France) was installed in a Perkin-Elmer (St. Quentin en Yvelines, France) autosystem gas chromatograph. For identification of compounds, a Nermag R10-10S mass spectrometer was coupled to a Delsi DI700 gas chromatograph, both from Nermag Instruments (Suresnes, France).

2.3. Plasma preparation

Plasma was obtained from male Wistar rats (200–250 g) originating from Janvier (Le Genest St Isle, France). The animals were kept on a 12 h light–dark cycle and given access to food and water ad libitum. Blood (8 ml/rat) was collected after cutting of the carotids. Plasma (around 4 ml/rat) was then prepared by centrifuging the blood at 4°C for 15 min at 3000 rpm on a Rottanta/RP (Hettich). Plasma (pH 7.8) from a minimum of four rats was pooled each time and was used fresh or stored at −18°.

Some plasma fractions were processed in order to be devoid of proteins. They were prepared from freshly collected plasma using Amicon MPS-1 ultrafiltration system (Epernon, France) with YMT membranes with a molecular mass cut-off of 30 000. These fractions are further termed deproteinated plasma in the text.

2.4. Incubation of DPAT

Fractions of 2 ml plasma or deproteinated plasma were thermostated at 37°C. At time $t=0$, DPAT was added to the fractions to get a final concentration of 310 μM. At time $t=120$ min, two 100-µl incubation samples were collected from fractions. DTT was

then added to the fractions to give a final concentration of 1 m*M* and was allowed to react for 1 min. At last, two other 100-μl incubation samples were collected. All incubation samples were diluted in 1 ml of 0.1 *M* Tris buffer, pH 9.4 as soon as they were collected. The sample–buffer mixtures obtained were subsequently assayed for DPAT as described in Section 2.7.

2.5. Incubation of VX

Incubations of VX with rat plasma were conducted under the same conditions used for DPAT except for the following features: (a) VX was added to plasma or deproteinated plasma to get a concentration of 374 μ*M* (100 μg/ml) unless specified otherwise; (b) Tris buffer used to dilute all samples collected contained 0.2 m*M* DTT; (c) sample–buffer mixtures obtained were first allowed to stand for 1 min before being assayed for VX and DPAT as described in Section 2.7.

2.6. AChE inhibition

AChE activity was inhibited by the selective and potent BW 284 C51 [1,5-bis(4-allyldimethyl-ammoniumphenyl)-pentan-3-one-dibromide] in some plasma fractions. To 4 ml of rat plasma were added 4 μl of inhibitor from a 10^{-2} *M* solution in water and the mixture allowed to stand for 20 min. AChE activity was determined with acetylthiocholine as substrate for the Ellman reaction [15] before and after incubation with the inhibitor. We obtained routinely a better than 90% inhibition of AChE activity by this procedure.

2.7. Detection procedure

VX and/or DPAT were extracted from sample–buffer mixtures using a liquid–liquid procedure with 1 ml of ethyl acetate–hexane (50:50). Aliquots of organic phases (475 μl) were transferred to Chromacol vials and 25 μl of the internal standard (I.S.) malathion (see Fig. 1) from a 60.6 μ*M* solution were then added to every vial with a Gilson Microman. A 1-μl aliquot was injected splitless in the capillary column. Chromatographic conditions were: injector temperature 260°C, detector temperature 300°C. The separation was carried out using temperature raises at a rate of 30°C/min with the following column temperature gradient: 100°C for 30 s, a linear increase to 210°C, 1 min isothermal, thereafter raised to 220°C, held for 1 min then increased to 300°C and maintained for 3 min to clean the column. The carrier gas inlet pressure was 84 kPa. The detector was a nitrogen–phosphorus detector operating under an air flow of 100 ml/min and a hydrogen flow of 1.5 ml/min. The bead was heated electrically to 1 mV.

2.8. Identification

Separation was accomplished on the same column used with the Perkin-Elmer autosystem and under the same chromatographic conditions. The mass spectrometer was operating with the following conditions: ion source temperature, 280°C; electron energy, 70 eV; ionization current, 0.2 mA; interface temperature, 200°C.

2.9. Quantification

Quantification was done by using standard curves obtained with serial dilution of VX or DPAT in deproteinated plasma. Samples of 100 μl from every concentration were diluted in 1 ml of 0.1 *M* Tris buffer pH 9.4 containing 0.2 m*M* DTT. Sample–buffer mixtures were allowed to stand for 1 min before being assayed for VX or DPAT as described in Section 2.7.

3. Results and discussion

3.1. Initial studies

The extraction technique used for plasma was derived from that used to extract VX from aqueous samples [16]. All initial studies were performed in deproteinated plasma since VX was found to be metabolised in the presence of plasma proteins. Furthermore malathion was introduced after the extraction step since it was found to be also metabolised by rat plasma proteins. In addition, before extracting DPAT, it was found necessary to add DTT, a thiol reducing agent, to the deproteinated

Table 1
Characteristics of the calibration curves determined for VX and DPAT from deproteinated plasma

Compound	Linearity range (μM)	Slope	y-Axis intercept	Correlation coefficient
VX	37–1123	0.083	0.093	0.992
DPAT	15–621	0.151	−0.039	0.994

plasma samples. Indeed, it was known that DPAT was unstable in pHs ranging from 7 to 10 [17]. In these conditions, the thiol function of DPAT became oxided to give the corresponding disulfide namely bis(diisopropylaminoethane)disulfide (DPAD). The addition of DTT allowed us to obtain similar extraction yields for VX and DPAT amounting to 87±10 and 92±15% (n=6) respectively. GC conditions are suitable for following VX, DPAT and the disulfide DPAD and are reported in Section 2.7. Chromatograms obtained are given later in this paper.

3.2. Calibration curves and sensitivity

Standard curves were obtained with serial dilution of VX or DPAT in deproteinated plasma in the presence of a constant amount of I.S.. All concentrations were made in triplicate. VX and DPAT were extracted from deproteinated plasma as described in Section 2.7. The peak-area ratios VX/I.S. or DPAT/I.S. were respectively plotted versus the concentration ratios VX/I.S. or DPAT/I.S.. Calibration curves in the ranges 37–1123 μM (10–300 μg/ml) VX and 15–621 μM (2.5–100 μg/ml) DPAT were linear for deproteinated plasma. Table 1 lists slopes, y-axis intercepts and correlation coefficients obtained for VX and DPAT from deproteinated plasma. These calibration curves were used to quantify VX and DPAT concentrations in plasma or deproteinated plasma samples.

3.3. Assay precision and relative accuracy

The precision and the relative accuracy (recovery) of the method were examined by replicate analysis of freshly prepared deproteinated plasma samples spiked with known amounts of VX and DPAT. The precision [expressed as coefficient of variation (C.V.)] is defined as the degree of agreement between replicate measurements of the same quantity of VX or DPAT. The relative accuracy is defined as the degree of agreement between the measured value and the true value and is expressed as the percentage of the measured value/the true value (recovery). Measured values correspond to amounts of VX and DPAT extracted from deproteinated plasma, further diluted in the ethyl acetate–hexane (50:50) mixture and finally assayed as described in Section 2.7. Control concentrations were analysed six times in a single assay for both VX and DPAT. Table 2 gives details of C.V., mean concentrations found and recoveries of the method. The overall recoveries ranged from 86.1 to 99.5% for VX and from 82.2 to

Table 2
Precision (C.V.) and relative accuracy (recovery) of the method used to determinate concentrations of VX and DPAT from deproteinated plasma

Compound	Quality control concentration added (μM)	Concentration found (mean±S.D.) (μM) (n=6)	C.V. (%)	Recovery
VX	187	186±6	2.5	99.5
	374	322±8	2.5	86.1
	749	665±27	4.1	88.8
DPAT	155	157±10	6.9	101.3
	310	255±4	1.8	82.2
	621	612±51	8.3	98.5

101.3% for DPAT. Furthermore the within-run precision of the analysis method ranged from 2.5 to 4.1% for VX and from 1.8 to 8.3% for DPAT.

3.4. Detection and quantification limits

Limits of detection, for a signal-to-noise ratio of 3, and limits of quantification, for a signal-to-noise ratio of 10 were determined for VX and DPAT after extraction from deproteinated plasma fractions. Aliquots of organic phases containing VX or DPAT were not subjected to concentration prior to injection in GC. Values are gathered in Table 3.

3.5. Stability of DPAT in plasma

As for deproteinated plasma, it was found important to treat plasma samples with a good reducing agent (DTT) to allow quantitative extraction of the thiol DPAT. As a matter of fact, in the absence of DTT, mostly the disulfide DPAD was seen with variable reproducibility since DPAT was also found to react with free thiols of proteins. Fig. 2 shows the shift from DPAD to DPAT when an excess of DTT was supplied to plasma at time $t=120$ min. Results gathered in Table 4 showed that addition of DTT allowed the recovery of more than 95% of DPAT incubated at time $t=0$ in the presence of deproteinated plasma or plasma. These results indicated that: (a) DPAT incubated for 2 h in the presence of rat plasma did not undergo reactions other than an oxidation of the thiol function to react on itself or with free thiol groups from plasma proteins; (b) addition of DTT reduced DPAD or the reaction of DPAT with free thiols of plasma proteins to DPAT. Consequently, by adding DTT to samples collected at different times from incubations of VX in the presence of rat plasma we could stabilize DPAT in its reduced state before the extraction step.

Table 3
Limits of detection and quantification of VX and DPAT from deproteinated plasma

Compound	Limit of detection (μM)	Limit of quantification (μM)
VX	0.2	0.5
DPAT	0.3	0.6

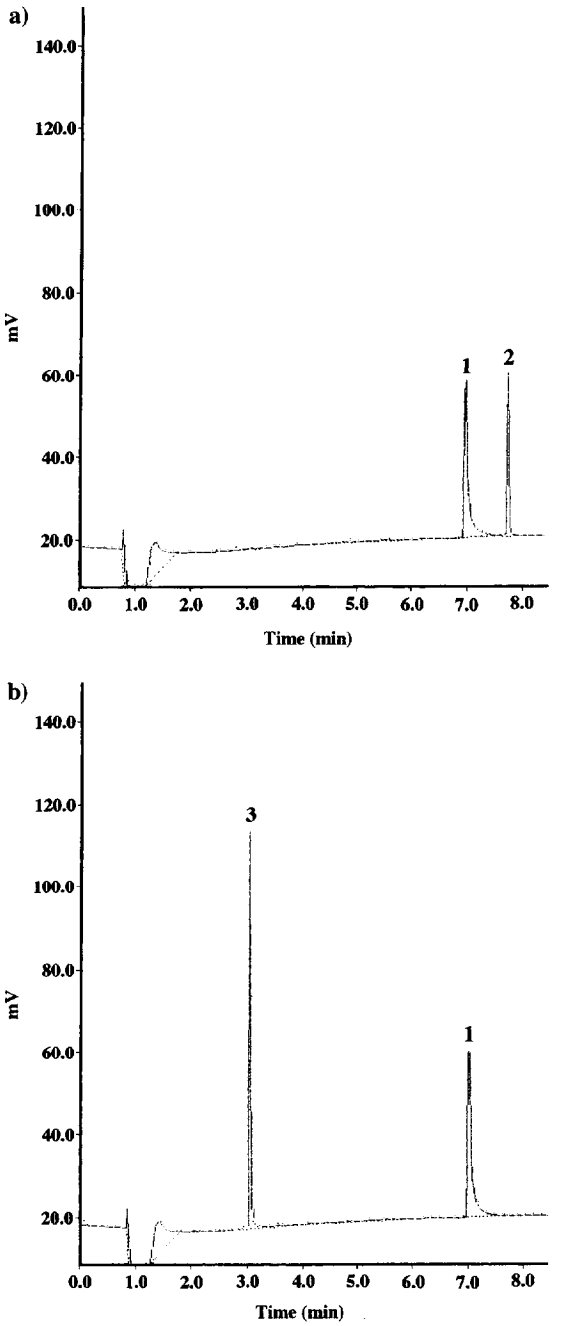

Fig. 2. Chromatograms of 310 μM DPAT incubated in the presence of rat plasma at 37°C as described in Section 2 at time $t=120$ min before (a) and after (b) addition of 1 mM DTT in the plasma fraction. Peaks: 1=3 μM malathion (I.S.); 2= bis(diisopropylaminoethane)disulfide (DPAD); 3= diisopropylaminoethanethiol (DPAT). One microlitre was injected splitless. Attenuation: 4. Further chromatographic conditions as mentioned in Section 2.

Table 4
DPAT recovery in plasma or deproteinated plasma fractions[a]

Time (min)	DPAT in plasma (μM)	DPAT in deproteinated plasma (μM)
0	310	310
120	n.d.	n.d.
120+DTT[b]	304±30	301±35

[a] Incubations of 310 μM DPAT in the presence of either plasma or deproteinated plasma fractions. At time $t=120$ min, two samples were collected from each fraction, 1 mM DTT was then added to the fractions and two other samples were taken. These latter samples correspond to time $t=120$ min+DTT. All samples were further diluted in Tris buffer pH 9.4 and were assayed for DPAT as described in Section 2.7.
[b] Values are means±S.D. of three separate experiments.
n.d. not detected.

3.5.1. Application

The extraction and detection procedures were used to assay VX degradation in rat plasma. As a matter of fact when VX was incubated in the presence of rat plasma, we observed a decrease of VX concentration versus time. We proposed the hypothesis that VX could undergo an enzymatic degradation by an OPH as what was found for soman [7,9,18], (see Chapters 5 to 9 of [18], sarin and tabun previously [8], (see Chapter 7 of [18]). VX detoxication had also been previously reported in *P. diminuta* [13,14].

Fig. 3 shows chromatograms from incubation of VX in the presence of plasma at times $t=0$ (A) and $t=120$ min (B). The mass spectrum of VX is presented also (C). Peak 1 increased over time of incubation and presented a retention time and relative intensity mass fragments identical to those of authentic DPAT (D). This finding indicated that VX was undergoing hydrolysis of its P–S bond in rat plasma thus giving the thiol product DPAT. Fig. 4 describes the fate of 240 μM VX incubated either in the presence of rat plasma or deproteinated plasma as a function of time. We observed a low and almost linear loss of VX in the absence of proteins while it rapidly disappeared in the presence of plasma (Fig. 4, top). The loss of VX was related to the increase of DPAT and mainly depended on the presence of plasma proteins (Fig. 4, bottom). We determined that 53±4% ($n=8$) of VX was remaining when incubated at the concentration of 374 μM for 2 h in the presence of rat plasma. After quantification of DPAT formed over six separate experiments, we determined the relative contribution of the P–S bond hydrolysis in regard to VX decrease. We found that rupture of the P–S bond was accounting for 72±2% of VX disappearance when incubated for 120 min in the presence of rat plasma. Since DPAT was found in VX solutions as an impurity (see Fig. 3A) we subtracted DPAT concentrations determined at time $t=0$ from those found at time $t=120$ min.

The results presented in Fig. 4 showed that hydrolysis was mainly dependent on the presence of plasma proteins. The contribution of spontaneous hydrolysis (in the presence of deproteinated plasma) to the total VX degradation is reported in Table 5. This spontaneous VX hydrolysis was further subtracted from every determination performed in the presence of plasma.

Due to the strong affinity of VX for AChE ($k_i = 1.4 \cdot 10^8\ M^{-1}\ min^{-1}$) [10], we assessed the possibility that hydrolysis could take place after its covalent binding to the active site of this enzyme as it is well described for soman-inhibited AChE going to the "aged" state [19–22]. For this reason, some plasma fractions were preincubated with BW 284 C51, a selective and potent inhibitor of AChE [23]. Results of VX incubations with these AChE inhibited plasma fractions are also presented in Table 5. They demonstrated that VX hydrolysis was not dependent on a previous covalent binding to the AChE active site but was more probably due to an OPH.

Many OPHs able to metabolise OPs have been found to need metals to be active [12–14]. Consequently, we studied the sensitivity of VX enzymatic hydrolysis to different agents. The following metal chelators were assayed: EDTA, 1-10-Ø (1,10-phenanthroline) and 8-OHQ-5-SA (8-hydroxyquinoline-5-sulfonate), the two latter ones being transition element chelators. Inhibition by the heavy metal Hg^{2+} was tested as well. All inhibitors were added to the plasma fractions at time $t=0$ and VX disappearance and formed DPAT were determined at time $t=120$ min. Results are shown in Fig. 5. EDTA completely inhibited enzymatic hydrolysis of VX since no DPAT could be detected after 2 h. Inactivation by 1-10-Ø was severe but not complete while 8-OHQ-5-SA displayed little impact on VX P–S bond hydrolysis. At the same time no decrease of VX was detected over 2 h when EDTA was used. Addition of Hg^{2+} to the incubations resulted in a complete inhibition of

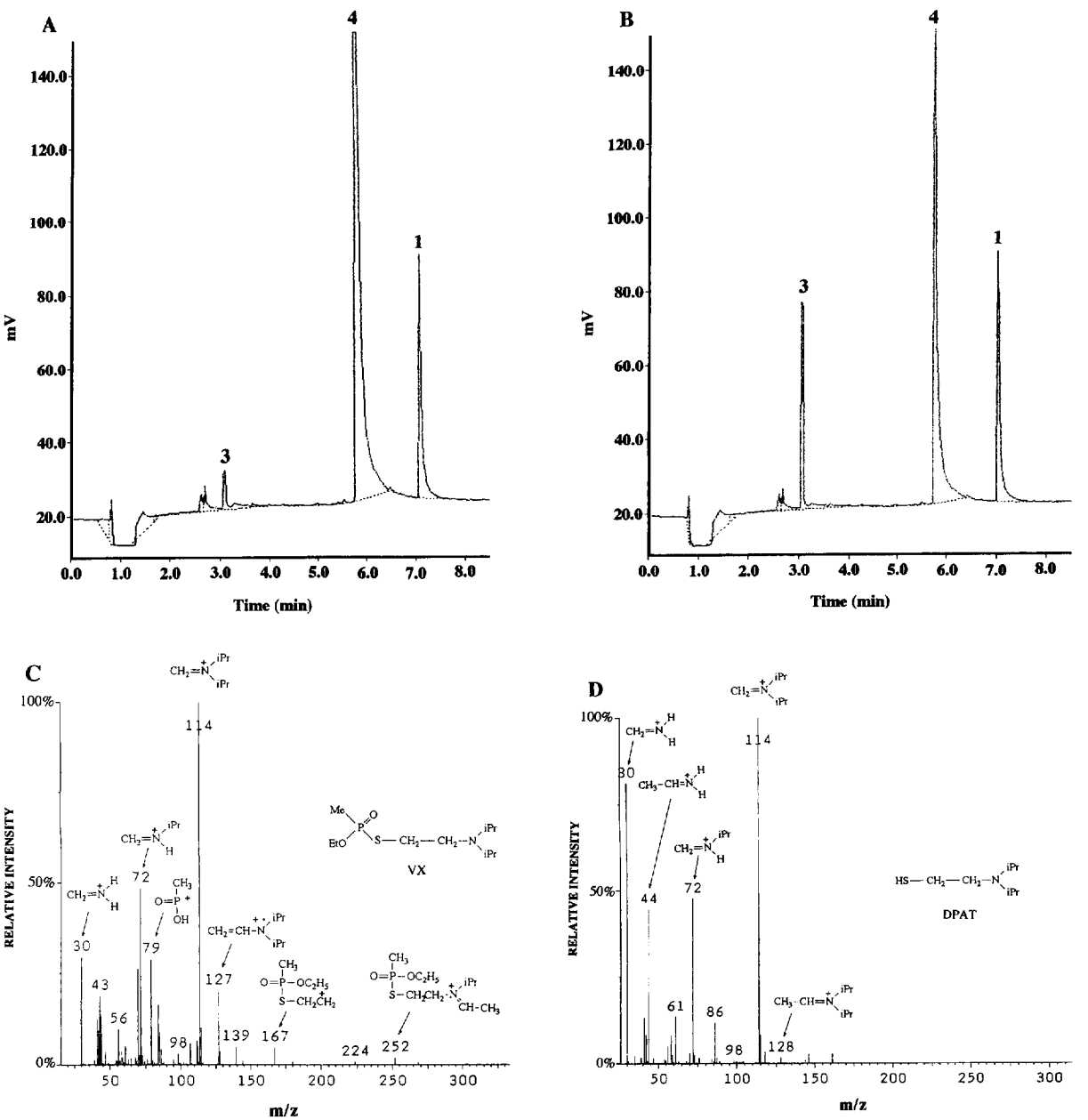

Fig. 3. Chromatographs of 374 μ*M* VX incubated in the presence of rat plasma at 37°C (see Section 2) at time $t=0$ (A) and time $t=120$ min (B). One microlitre was injected splitless. Attenuation: 4. Further chromatographic conditions as mentioned in Section 2. Mass spectra of authentic VX (C) and authentic DPAT (D). Peaks: 1=3 μ*M* malathion (I.S.); 3=DPAT; 4=VX. Me, methyl; Et, ethyl; iPr, isopropyl.

formed DPAT and no VX decrease was observed as found with EDTA. Thus, no decrease in VX concentration was observed over time with EDTA and Hg^{2+} which were able to totally inhibit DPAT enzymatic formation. Since we calculated that the P–S bond hydrolysis product DPAT was only amounting to 72% of total VX disappearance, we also demonstrated that the remaining 28% VX loss is

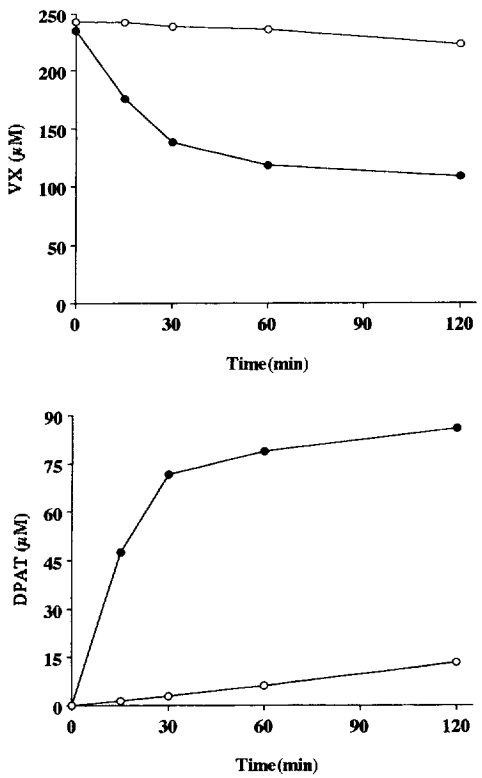

Fig. 4. VX disappearance (top) and DPAT formation (bottom) when 240 μ*M* VX was incubated at 37°C in the presence of rat plasma (●) or deproteinated plasma (○) fractions. Samples of 100 μl of each fraction were taken in duplicate at different times and diluted in 1 ml of 0.1 *M* Tris buffer pH 9.4 containing 0.2 m*M* DTT. VX and metabolite DPAT were further extracted from the sample–buffer mixtures and aliquots of organic phases obtained were then injected splitless in GC as described in Section 2. Values are means of two separate experiments.

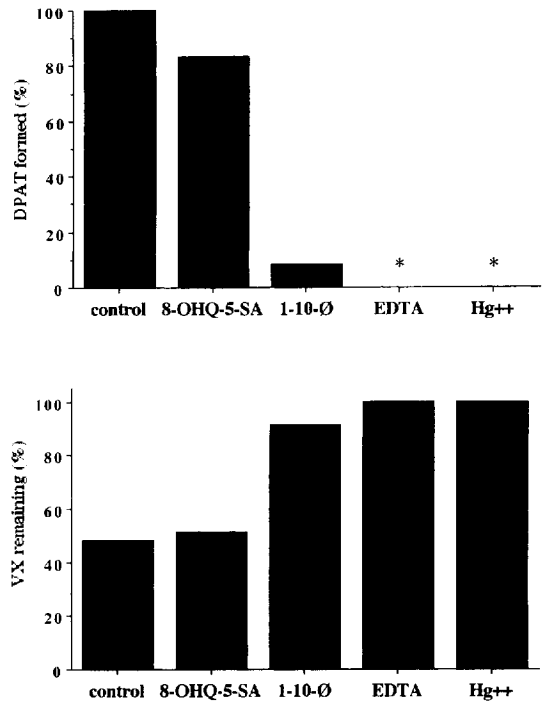

Fig. 5. Effect of metal chelators and the heavy metal Hg^{2+} on VX hydrolysis. DPAT formation compared to control (top) and VX disappearance compared to $t=0$ (bottom) in the same experiments after 2 h when VX was incubated at 37°C with rat plasma in the absence (controls) or in the presence of inhibitors. Control incubations: 374 μ*M* VX as substrate in rat plasma, at time $t=120$ min, samples were collected in duplicate and processed as mentioned in Section 2. VX and DPAT recovered at time $t=120$ min were respectively 187 μ*M* (50%) and 133 μ*M* (100%). Putative inhibitors were added to the plasma fractions at time $t=0$ to give final concentrations of 10 m*M* for 8-OHQ-5-SA, 1-10-Ø and EDTA and 1 m*M* for Hg^{2+} and DTT. These fractions were then processed at time $t=120$ min as control ones. Values are means of two separate experiments. * not detected.

Table 5
DPAT formation[a] under different conditions

Condition	DPAT[b] (μ*M*)	Percentage
Plasma	128±8 (3)	100
Deproteinated plasma[c]	16±0 (3)	12.6±0.4
AChE inhibited[d]	148 (2)	116

[a] After 2 h of incubation of 374 μ*M* VX in the presence of rat plasma at 37°C as described in Section 2.
[b] Values are means±S.D. (number of experiments) when possible.
[c] Deproteinated plasma was obtained from fresh ultrafiltrated plasma as described in Section 2.
[d] Plasma fractions were preincubated for 20 min at room temperature in the presence of the selective AChE inhibitor BW 284 C51 before addition of VX.

also inhibited by EDTA and Hg^{2+}. This finding strongly suggests that the VX disappearance which is not related to DPAT formation could also be due to VX hydrolysis but of a bond other than the P–S one. In this case a good candidate would be cleavage of the P–O-ethyl bond [17,24] thus giving the toxic S-[(2-diisopropylamino)ethyl]methylphosphonothioic acid [11]. We determined that this latter hydrolysis product was not extracted by our procedure by using the authentic reference (data not shown). Whether the two kinds of hydrolysis process are dependent on the same OPH is unknown.

The inhibitions we determined in this study are in good agreement with the respective strengths of the three metal chelators used in the assays. Indeed EDTA is a much more potent metal chelator than 1-10-Ø and 8-OHQ-5-SA. In this regard EDTA was already found to completely inhibit both the squid OPAA (organophosphorus acid anhydrolase) and a *Stearothermophilus* OPAA [25]. However, Hoskin et al. [14] reported an almost complete inhibition of Tetriso (a close analogue of VX) hydrolysis by the OPH isolated from *P. diminuta* with both 1-10-Ø and 8-OHQ-5-SA. In the same study, EDTA displayed little impact on Tetriso hydrolysis. The authors concluded that a possible explanation of this ambiguity could be the lack of purity of their OPH preparation [14].

In order to determine enzymatic hydrolysis characteristics, VX was incubated with rat plasma at concentrations ranging from 187 to 1123 μ*M* (50 to 300 μg/ml), the initial rates of DPAT formation were monitored and the results gathered in Fig. 6. The Michaelis–Menten constant (K_m) and the maximum rate (V_{max}) of the enzyme responsible for VX P–S bond hydrolysis were calculated from the computer-generated slope and *y*-axis intercept of Fig. 6 and gave K_m=2.5 m*M* and V_{max}=13.3 nmol per min per ml. These results indicated that the OPH responsible for VX P–S bond hydrolysis displayed both low affinity toward VX since the K_m was in the millimolar range and also low maximum hydrolytic rate. These two latter results illustrate the difficulties encountered by living organisms in dealing with phosphonothiolo compounds.

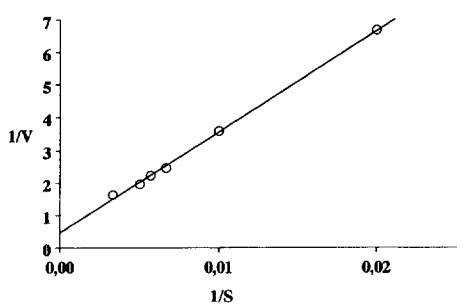

Fig. 6. Reciprocal plot of the hydrolysis of VX by the OPH deriving from rat plasma. On the abscissa, 0.01 represents 374 μ*M* VX; on the ordinate, 3 represents 2 nmol VX hydrolysed per min per ml of plasma.

4. Conclusions

We have developed a new and fast GC technique to detect VX, the most lethal chemical warfare compound, and the product resulting from its P–S bond rupture from complex protein mixtures. Advantages of this technique are the following: (a) detection of both compounds is direct and simultaneous; (b) the number of steps is limited thus minimising losses (a single extraction, no sample concentration, a single injection); (c) we obtained reproducible results over experiments. This technique was further applied to the study of VX hydrolysis in rat plasma demonstrating that an OPH was responsible for VX P–S bond degradation. Sensitivity of VX enzymatic hydrolysis to metal chelators in rat plasma was as follow: EDTA>1-10-Ø≫8-OHQ-5-SA. Both EDTA, the more potent metal chelator used in this study and Hg^{2+} produced a complete inhibition of VX hydrolysis. These results demonstrate the requirement of a metal ion for enzyme activity. The affinity of the OPH from rat plasma toward VX was weak as indicated by the high K_m of 2.5 m*M*. Furthermore, maximum hydrolytic rate (V_{max}=13.3 nmol per min per ml) was quite low.

5. List of abbreviations

OPs	organophosphorus compounds
AChE	acetylcholinesterase
VX	*O*-ethyl *S*-(2-diisopropylaminoethyl)-methylphosphonothiolate
DPAT	diisopropylaminoethanethiol
OPH	organophosphorus hydrolase
DTT	dithiothreitol
I.S.	internal standard
BW 284 C51	1,5-bis(4-allyldimethyl-ammoniumphenyl)-pentan-3-one-dibromide
DPAD	bis(diisopropylaminoethane)-disulfide
Soman	1,2,2-tri-methylpropylmethyl-phosphonofluoridate

1-10-Ø	1,10-phenanthroline
8-OHQ-5-SA,	8-hydroxyquinoline-5-sulfonate
Sarin	isopropylmethylphosphonofluoridate
Tabun	ethyl-*N*-dimethylphosphoramido-cyanidate

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