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Short communication

Liquid chromatography tandem mass spectrometry applied to quantitation of the organophosphorus nerve agent VX in microdialysates from blood probes☆,☆☆

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ABSTRACT

VX (O-ethyl-S-[2(di-isopropylamino)ethyl] methylphosphonothiolate) is a low volatility organophosphorus (OP) nerve agent and therefore the most likely route of exposure is via percutaneous absorption. Microdialysis has been used as a tool to study percutaneous poisoning by VX in the anesthetised guinea pig. A liquid chromatography tandem mass spectrometry (LC–MS–MS) method using positive electrospray ionisation (ESI) was used to quantitate VX in microdialysate samples collected from microdialysis probes, implanted into a blood vessel of anesthetised guinea pigs. The method resulted from modification of a LC–MS–MS method previously developed for the analysis of dermal microdialysates. Modification increased the sensitivity of the method, allowing quantitation of the trace levels of VX in blood microdialysates, over the range 0.002–1 ng/ml, with linear calibration. Quantitative results have been used to determine the time course of VX concentrations in the blood of guinea pigs following percutaneous poisoning.

1. Introduction

Organophosphorus (OP) nerve agents are a lethal class of chemical warfare agents. In the 1980s sarin (GB) was used in the Iraq–Iran war [1] and against Kurdish communities in Northern Iraq [2]. Despite use of OP nerve agents being prohibited under the terms of the Chemical Weapons Convention (CWC) 1997, there remains concern for their use by terrorists. Sarin was released in Matsumoto City by the Aum Shinrikyo Cult between 27th and 28th June 1994, and in the Tokyo subway on 20th March 1995 [3]. VX was also used for an assassination in Japan in 1994 [4].

Nerve agent poisoning can be treated by the use of oxime, anticholinergic and anticonvulsant drugs. Treatment for poisoning by the percutaneous route is particularly challenging as slower rates of absorption, later onset and longer duration of symptoms mean that careful timing of the administration of therapy is necessary to prevent lethality.

In vivo microdialysis has been used as a tool to measure the time course and penetration rate of VX, following percutaneous poisoning with VX in anesthetised guinea pigs [5]. Sensitive methods are required to determine VX concentrations in microdialysate samples. A previously reported LC–MS–MS method [5] was developed for the analysis of large numbers of samples from microdialysis experiments using linear dermal probes. That method has been modified in order to increase its sensitivity allowing quantitation of VX at the lower concentrations expected in blood probe microdialysate.

2. Experimental

2.1. Chemicals

VX (>95% purity, determined by ^{1}H and ^{31}P nuclear magnetic resonance) was synthesised at Dstl, Porton Down, UK. Ringer's solution (veterinary isotonic hydration solution containing sodium chloride 8.60 g/l, potassium chloride 0.30 g/l, and calcium chloride dihydrate 0.33 g/l in water) was supplied by Centaur Services Limited, Castle Cary, Somerset, UK. Trifluoroacetic acid (TFA) (spectrophotometric grade) and acetonitrile (Fluka, LC–MS grade) were purchased from Sigma Aldrich, Gillingham, Dorset, UK. A Barnstead EASYpure®II RF/UV Ultrapure water system provided deionised water. Blood probes were manufactured by Microbiotech/se AB, Stockholm, Sweden.

2.2. Preparation of standard solutions

Solutions of VX in Ringer's solution were prepared over the range 0.002–1 ng/ml by dilution of VX stock solutions (~1 mg/ml

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in isopropyl alcohol), and used to construct calibration curves for quantitation of blood microdialysates.

2.3. Sample preparation

Analytical samples consisted of *in vivo* microdialysate; Ringer's solution perfused through concentric microdialysis probes implanted in the carotid artery of an anesthetised guinea pig. Microdialysate was collected directly into 200 μl polypropylene autosampler vials. With a continuous dialysate flow rate of 1 μl/min, sampling was carried out over consecutive 30 min periods for up to 8 h. The sample volume collected was thus ~30 μl. Samples were frozen overnight and thawed prior to analysis. Approximately 40 samples from a single experiment with 2 animals required within day analysis. No sample preparation was required. Samples were vortexed and 5 μl aliquots injected for analysis by LC–MS–MS.

2.4. Chromatographic and mass spectrometric conditions

A ThermoFinnigan Surveyor autosampler and pump were used to carry out the LC separation. The analytical column was a Luna $C_{18}(2)$, 150 mm × 2.0 mm ID, 3 μm particle size (Phenomenex, Macclesfield, UK). Isocratic chromatographic conditions were used with mobile phase consisting of 0.05% TFA in water and 0.05% TFA in acetonitrile, mixed on-line in the ratio 80:20 (v/v), at a flow rate of 0.2 ml/min. Total run time is 6 min.

The detection system used was a ThermoFinnigan TSQ Quantum triple quadrupole mass spectrometer, operating in positive ESI mode, with orthogonal spray. Single reaction monitoring (SRM) was used to monitor the transition *m/z* 268.2 to *m/z* 128.2 for VX. Full scan ESI and product ion spectra are shown in Fig. 1. The important modification made to the method was the incorporation of the divert valve in the TSQ Quantum instrument, which was used to divert the LC flow to waste for the first 2 min of each run. This ensured the diversion of unretained material and minimised the build up of salts from Ringer's solution sample matrix.

2.5. Linearity

Linearity of calibration curves (peak area vs. VX concentration) was assessed over the calibration range 0.002–1 ng/ml VX by comparing the correlation coefficient (R^2) for six separate curves.

2.6. Accuracy and precision

A 0.1 ng/ml VX QC standard was analysed in the middle and at the end of the analytical run. Concentrations of the QC standards were determined by comparing peak areas obtained for QCs with peak areas of 0.1 ng/ml calibration standards analysed at the start of the analytical run. The accuracy and precision (RSD) of the QC concentrations relative to the calibration standards were calculated for 6 separate analytical runs and averages calculated across the 6 data sets to assess the intra- and inter-run accuracy and precision of the method, see Table 1.

2.7. Stability

Solutions of VX in Ringer's solution have been shown to be stable over 14 days when stored at −20 °C [5].

2.8. Assessment of matrix effects of microdialysate

Previous investigations into analyses of microdialysates using ESI have shown that due to the matrix ion suppression may be observed [6]. Using microdialysis buffer (Ringer's solution) for preparation of standards for quantitation ensures that any matrix effect due to buffer components that is experienced in sample analysis is replicated during the analysis of standards. Comparisons between the analysis of VX standards in Ringer's solution and standards in control dermal microdialysate have been made and show no significant differences. Therefore sample components arising from dermal microdialysis have no significant effect upon quantitation [5]. However, matrix effects of blood microdialysates could not be assessed as insufficient control blood microdialysate was available (~30 μl from each experiment) for comparative studies. Although, analysis of control blood microdialysate samples collected prior to the animal being exposed to VX, confirmed the absence of any detectable interferences.

3. Results and discussion

The LC–MS–MS method was rapid with retention time for VX of ~3.5 min and total run time 6 min. Lack of sample preparation and use of isocratic chromatographic conditions, requiring no equilibration time, facilitated within day analysis of the entire experimental set of around 40 blood microdialysates together with calibration and QC standards and blanks. The method was suitable for use with low sample volumes (~30 μl), allowing repeat analysis if required.

Calibration curves for VX concentrations ranging from 0.002 to 1 ng/ml showed good linearity, R^2 was ≥0.999 for each of six separate curves. Some variation in the slopes of the curves was seen due to the varying condition of the instrument, given that analysis of the six calibration curves was spread over a number of weeks.

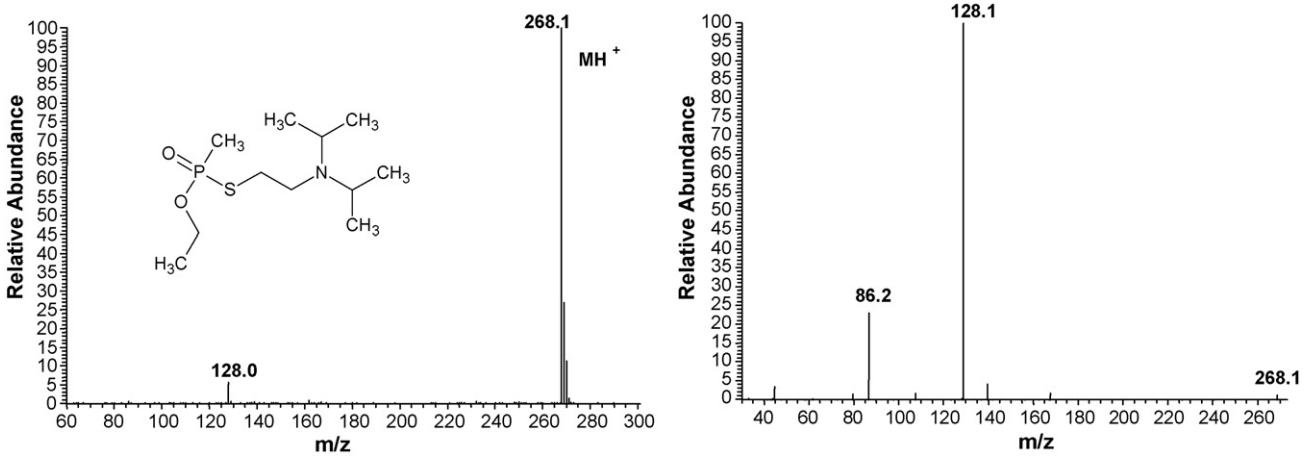

Fig. 1. Full scan ESI (left) and product ion spectra of *m/z* 268 (right) for VX.

Table 1
Shows intra- and inter-run accuracy and precision data for 6 individual analyses.

Analytical run number and position		VX conc. (ng/ml) (QC peak area/cal std peak area × 0.1)	Accuracy (%)	Precision RSD (%)
1	Middle	0.090	89.6	5.8
	End	0.092	91.8	
2	Middle	0.089	89.4	10.1
	End	0.082	81.8	
3	Middle	0.104	103.5	2.3
	End	0.104	104.4	
4	Middle	0.104	104.1	2.9
	End	0.106	105.8	
5	Middle	0.099	99.4	0.9
	End	0.101	101.1	
6	Middle	0.090	90.0	6.9
	End	0.088	88.2	
	Inter-run average		95.8	4.8

Slope RSD was 11.6% for six calibrations. A typical curve equation was $y = 1209850x$, and the intercept for the curves was shown to be not significant (P was not <0.05 at 95% confidence interval), and therefore was taken to be zero.

Accuracy of the method was good, ranging from 81.8 to 105.8%, with a mean accuracy of 95.8% for the 0.1 ng/ml QCs from six analytical runs. Precision of the method was also good, intra-run precision showed RSDs ranging between 0.9 and 10.1% for six analyses, and the average RSD for QCs across the six analytical runs was 4.8%, see Table 1.

A high level of sensitivity was achieved with signal to noise (S/N) ratio >20:1 at 0.002 ng/ml, Fig. 2. Incorporation of the divert valve into the method helped to eliminate build up of buffer salts from samples in the source, reducing background noise and providing this high level of sensitivity. This modification has lowered the limit of quantitation for VX in microdialysates from 0.1 ng/ml [5], to 0.002 ng/ml.

Importantly, this modified method is also highly selective, ensuring no interfering peaks endogenous to the matrix are present in blanks (fresh Ringer's solution), Fig. 2, and control blood microdialysates, Fig. 3.

Critically, this method has facilitated the analysis of small volume blood microdialysate samples, with low VX concentrations and enabled concentration–time relationships to be established following exposure to VX in the anesthetised guinea pig, Fig. 4.

In vitro experiments to assess microdialysis probe efficiency [5], will be used to establish the efficiency of the blood probe. This will allow actual VX concentrations in the blood to be established using blood microdialysate concentrations at sampled time points. This is something which could not easily be achieved by taking blood samples. Given the small blood volume of the guinea pig (~6 ml), fewer time points could be sampled and sample volumes would be low. The complex matrix and low blood sample volumes present problems for quantitative analysis, and may make low levels of quantitation hard to achieve. Sample preparation may also be time consuming. Microdialysis is a continuous sampling technique and removes the need for taking blood samples. Blood microdialysate

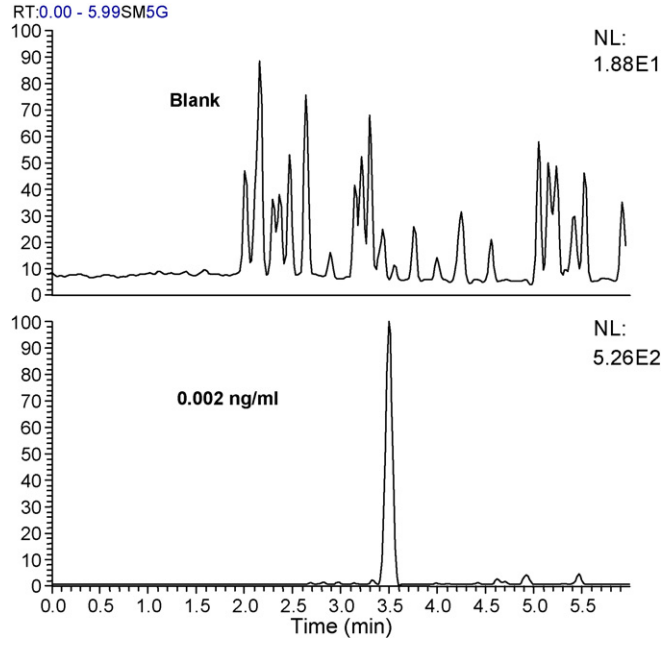

Fig. 2. LC–MS–MS chromatogram showing 0.002 ng/ml VX standard and Ringer's solution blank.

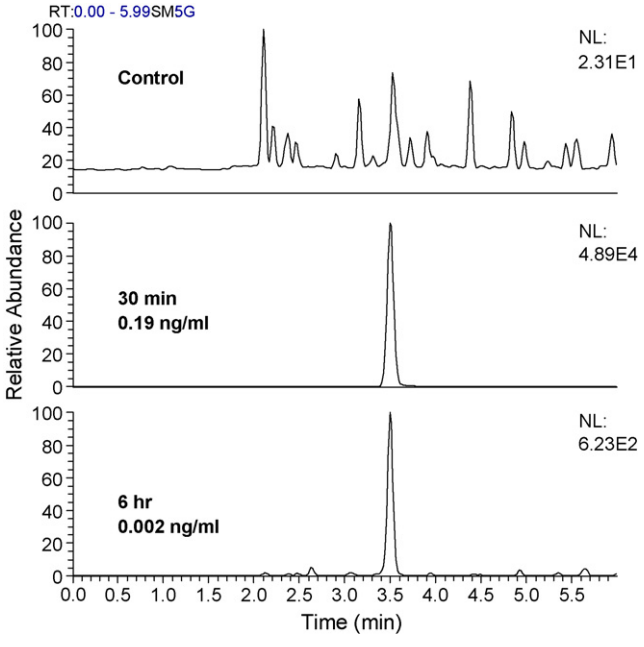

Fig. 3. Blood microdialysates from an anesthetised guinea pig exposed percutaneously to VX (296 μg/kg) with control blood microdialysate.

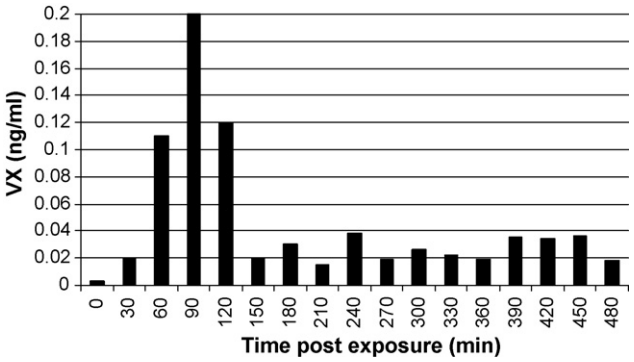

Fig. 4. Concentration–time plot showing VX concentration determined in blood microdialysates following percutaneous VX exposure (296 μg/kg) in an anesthetised guinea pig.

is a good sample matrix for analysis by LC–MS–MS. It is relatively clean, requires no sample preparation and therefore allows very quick and highly sensitive analysis, reaching very low limits of quantitation.

4. Conclusion

A rapid LC–MS–MS method has been developed to facilitate the analysis of microdialysate samples from blood probes following percutaneous exposure to VX in the guinea pig. No sample preparation is required and the method is compatible with low sample volumes. The method is highly sensitive and selective, with good accuracy and precision, allowing accurate quantitation of VX in blood microdialysates over the range 0.002–1 ng/ml. This method allows the determination of VX concentrations in blood of exposed animals without the need to take blood samples.

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