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THE DETERMINATION OF O,S,S-TRIMETHYLPHOSPHORODITHIOATE IN THE PLASMA AND VARIOUS TISSUES OF RATS USING HIGH-RESOLUTION GAS CHROMATOGRAPHY WITH NITROGEN–PHOSPHORUS DETECTION

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SUMMARY

A method has been developed for the determination of O,S,S-trimethylphosphorodithioate in the plasma, lung, liver, brain and thymus of rats using high-resolution gas chromatography. The organophosphorus compound was extracted from the biological sample with ethyl acetate and analysed on a Carbowax 20M fused-silica capillary column with a nitrogen-phosphorus specific detector. O,S,S-Triethylphosphorodithioate was used as an internal standard added to the sample before extraction. The sensitivity of the method allowed the compound to be measured in 0.1-ml aliquots of plasma or in 20-mg wet weight of tissue down to a level of 5 ng/sample. The method has been applied to a pharmacokinetic study in the rat after an oral or intravenous dosage with 25 mg/kg of O,S,S-trimethylphosphorodithioate.

INTRODUCTION

O,S,S-Trimethylphosphorodithioate (OSS-Me) is one of a number of minor impurities found in technical formulations of malathion[1,2] which has been shown to potentiate the toxicity of this insecticide in the rat[1–3]. By inhibiting rat liver carboxylesterase it prevents the normal metabolism of malathion and results in the increased formation of a toxic metabolite malaoxon[4]. When administered on its own to rats at an LD_{50} dose OSS-Me causes two distinct toxic effects. Initially, over the first 24 h, it produces a typical cholinergic response from which the animal recovers. A second phase of toxicity is evident between days 3 and 6, which is related to an effect on the lung. The LD_{50} of this compound is related to this second phase of toxicity[5].

As a contribution to a better understanding of the toxicity of OSS-Me in the rat, a study of the distribution and kinetic behaviour of this organophosphorus compound has been made. For the purpose of this study an assay procedure was required which would allow measurements to be made at low levels in small samples of plasma or tissue. For this reason we have developed a method based on the use of high-

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resolution capillary gas chromatography with a sensitive and selective thermionic nitrogen–phosphorus detector (NPD).

MATERIALS AND METHODS

Reagents

OSS-Me and O,S,S-triethylphosphorodithioate (OSS-Et), prepared by the method of Aldridge *et al.*[3], were obtained from Dr. J. W. Miles, Bureau of Tropical Diseases, Centre for Disease Control, Atlanta, GA, U.S.A. The purity of these compounds was checked by capillary gas chromatography with flame ionization detection and was found to be greater than 98%. Standard stock solutions (1 mg/ml) prepared in ethyl acetate were stable for several months when stored at 4°C. Ethyl acetate (BDH, Poole, Great Britain) was of Analar grade and was used without further purification.

Animal studies

Female LAC Porton derived Wistar rats (body weight 170–200 g; 8–10 weeks old) were used throughout. OSS-Me was dissolved in glycerol formal (Fluorochem, Glossop, Great Britain) to obtain a dosage volume of 1 ml/kg, and this was administered either intravenously or orally at a dosage of 25 mg/kg. Oral dosing was carried out by oesophogeal intubation and intravenous dosing by injection into a lateral tail vein following prewarming of the tail at 48°C.

At the pre-determined times rats were bled out from the heart under deep ether anaesthesia and tissue obtained for analysis. Blood samples collected in heparinized capillary tubes were centrifuged immediately after collection and the plasma stored at −5°C until analysed. Tissues were removed from the rat within 1–2 min of bleeding out and were immediately frozen in liquid nitrogen. The frozen tissues were weighed, and homogenized in ice-cold distilled water using a Ystral X10/20 homogenizer to give a 20% homogenate.

Sample preparation

To a 0.1-ml aliquot of rat plasma or tissue homogenate, contained in a 1.5-ml polypropylene microcentrifuge tube (Alpha Laboratories, Eastleigh, Hants, Great Britain), was added 10 μl of the internal standard solution of OSS-Et in ethyl acetate (50 μg/ml). The sample was extracted with 500 μl of ethyl acetate by Vortex mixing for 30 sec, followed by centrifugation for 2 min at 2000 *g*. The solvent layer was then transferred, using a Pasteur pipette, into a similar centrifuge tube for analysis by gas chromatography.

Gas–liquid chromatography

Analyses were carried out on a Packard Becker 419 gas chromatograph equipped with dual flame ionization detectors. One of these detectors was replaced by a Perkin-Elmer alkali-bead NPD. The existing liquid injection system on this instrument was modified for the split–splitless injection of samples into a capillary column.

The gas chromatographic separation was made using a fused-silica capillary (20 m × 0.3 mm I.D.) coated in the authors' laboratory with Carbowax 20M by the static coating procedure to give a film thickness of 0.4 μm. The flexibility of fused

silica allowed the end of the column to be located just below the tip of the detector jet. No make-up gas was used to the detector. Helium was employed as carrier gas with a flow-rate, measured at 180°C, of 6 ml/min. The column was operated isothermally at an oven temperature of 180°C. The NPD was operated at an air flow-rate of 120 ml/min and a hydrogen flow-rate of 4 ml/min. The rubidium silicate bead temperature was adjusted according to the sensitivity required for the analysis. The amplifier attenuation was 8×10^{-11} A and the recorder was set at 20 mV. The 1-μl samples were injected using a split ratio of 10:1.

Calibration curves

Standard calibration curves were prepared by analysing 0.1-ml aliquots of blank rat plasma or tissue homogenates containing known amounts (100, 400, 600, 800, 1000, 1600 and 2000 ng) of OSS-Me and 500 ng of the internal standard (OSS-Et). The peak height ratios OSS-Me:OSS-Et were plotted against the concentration of OSS-Me. The concentration of OSS-Me in the unknown samples was derived from regression equations obtained from the standard curve. When the concentration levels were expected to be less than 500 ng/ml sample, one-tenth of the above amounts of OSS-Me and internal standard were used for the calibration curve.

RESULTS AND DISCUSSION

The alkali-bead NPD, when operated in the nitrogen–phosphorus mode, is a very stable and reliable detector which exhibits a very high sensitivity of detection for many organophosphorus compounds[6,7]. When used with a capillary column, as little as 1 pg of OSS-Me and OSS-Et can be detected with a signal-to-noise ratio of 20:1. The selectivity of this detection system allowed OSS-Me and the added internal standard OSS-Et to be determined in ethyl acetate extracts of plasma and tissue homogenates at very low levels. No concentration or purification of the solvent extract was found to be necessary.

Fig. 1 shows a typical trace from the analysis of a 0.1-ml plasma sample from a rat following an oral dose of 25 mg/kg OSS-Me. Similar contaminant-free tracings

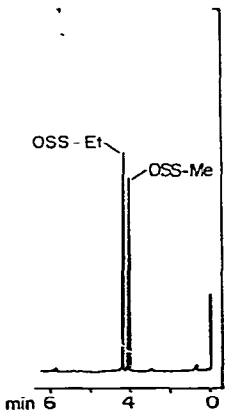

Fig. 1. Gas chromatogram of an extract from a rat plasma sample containing 4 μg of OSS-Me per ml of plasma. For the gas chromatographic conditions see text.

were obtained from tissue samples. The analysis of blank rat plasma and tissue homogenates gave very few background peaks, none of which interfered with the measurement of the peak derived from either OSS-Me or the internal standard.

A representative calibration curve for quantitation in plasma is shown in Fig. 2. Good linearity was obtained over this concentration range and over the lower range between 100 ng/ml and 2 μg/ml.

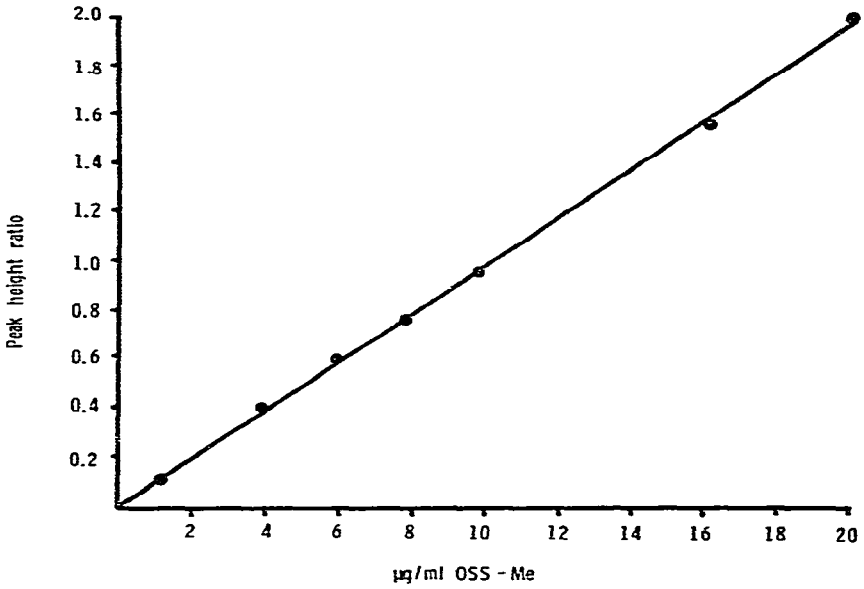

Fig. 2. Calibration curve for the analysis of OSS-Me in plasma using OSS-Et as internal standard.

A single extraction with ethyl acetate gave a high recovery of both OSS-Me and the internal standard. Using OSS-Et as an internal standard the mean absolute recovery of OSS-Me from spiked rat plasma was 87.5% (S.D. ± 4.3%) and from a tissue homogenate 89.6% (S.D. ± 3.4%). The accuracy of the method was determined from recovery experiments of authentic OSS-Me added to blank plasma and lung samples at concentrations of 4, 8 and 16 μg/ml plasma or tissue homogenate (1 g of wet weight tissue homogenate made up to 5 ml with distilled water). The precision of the method was determined from replicate analyses at these same concentrations. The results from these analyses are given in Table I.

The sensitivity of the method can, if required, be improved by reducing the volume of ethyl acetate used for extraction and also by injecting the extract into the capillary column using the splitless rather than the split injection technique. The lower limit of detection is in the order of 50 ng/ml plasma or 250 ng/g tissue.

A number of rat plasma samples were analysed immediately after collection and then following their storage at 4°C for two weeks. No significant difference in the results was obtained. Extracted samples were also stable when stored under these same conditions. Tissues were immediately immersed in liquid nitrogen after their removal from the animal to prevent possible enzyme degradation. After homogenization, these samples were also stable when stored at 4°C.

TABLE I

PRECISION AND ACCURACY OF THE METHOD FROM THE REPLICATE ANALYSES OF OSS-Me ADDED TO BLANK PLASMA AND LUNG HOMOGENATE SAMPLES

Each value is the mean of six determinations.

Amount added (μg/ml)	*Plasma*		*Tissue*	
	Amount found (μg/ml ± S.D.)	*Recovery (% ± S.D.)*	*Amount found (μg/ml ± S.D.)*	*Recovery (% ± S.D.)*
0.4	0.43 ± 0.01	108.3 ± 2.36	0.42 ± 0.03	105.0 ± 6.27
0.8	0.84 ± 0.02	105.4 ± 2.76	0.83 ± 0.06	103.7 ± 7.60
1.6	1.58 ± 0.05	98.9 ± 3.26	1.56 ± 0.07	97.7 ± 4.41

This method has been applied to a study of the pharmacokinetics in the rat after oral and intravenous injection of OSS-Me. Following oral administration, peak concentrations of the organophosphorus compound were found in the plasma between 30 min and 1 h after dosage. The concentration fell to approximately one-tenth of these levels 5 h later, but measurable amounts were still present after 24 h (see Fig. 3). In the liver, lung, brain and thymus the concentration–time profile was similar, showing that the compound was evenly distributed throughout the animal.

After intravenous injection the plasma and tissue concentrations at 30 min corresponded to those observed 60 min after oral administration, but from then on the disappearance curves were similar. The only exception was liver, where peak levels after intravenous dosing were *ca.* 90% lower. The calculated half-life values were *ca.* 60 min. More details from these kinetic studies will be given in a future publication.

In conclusion, the method described in this publication has proved to be ac-

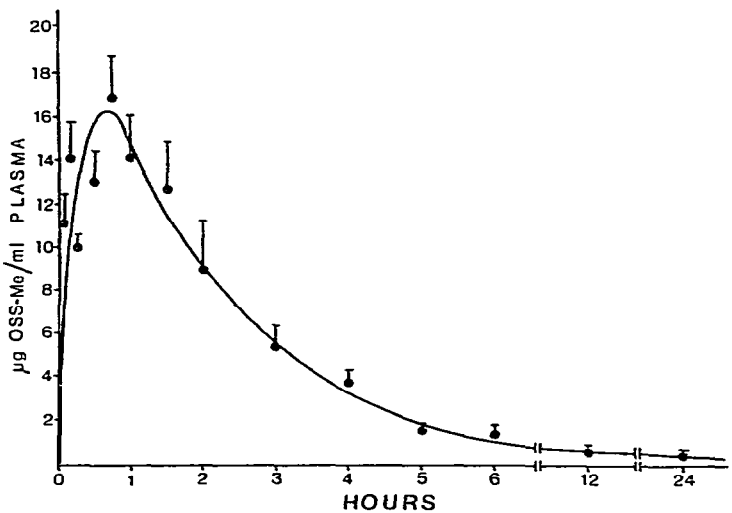

Fig. 3. Plasma concentration–time profiles in the rat after oral dosage of 25 mg/kg. Levels are presented as mean values ± standard error of the mean (n = 4).

curate and reliable for quantifying OSS-Me in small sample volumes of rat plasma and tissue. The employment of a high-resolution capillary column enhances both the sensitivity and specificity of the method. Since the assay involves only a single solvent extraction and a short analysis time on the column, it is simple and rapid.

Using the appropriate internal standard this assay procedure can be used to determine and study the kinetics of other organophosphorus triesters, including O,S,S-trimethylphosphorothioate and O,O,S-trimethylphosphorodithioate, which also occur as toxic impurities in technical malathion.

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REFERENCES

1 G. Pellegrini and R. Santi, *J. Agr. Food Chem.*, 20 (1972) 944.
2 N. Umetsu, F. H. Grose, R. Allahyari, S. Abu-El-Haj and T. R. Fukuto, *J. Agr. Food Chem.*, 25 (1977) 946.
3 W. N. Aldridge, J. W. Miles, D. L. Mount and R. D. Verschoyle, *Arch. Toxicol.*, 42 (1979) 95.
4 W. C. Dautermann and A. R. Main, *Toxicol. Appl. Pharmacol.*, 9 (1966) 408.
5 R. D. Verschoyle, W. N. Aldridge and J. R. P. Cabral, in B. Holmstedt, R. Lauwerys, M. Mercier and M. Roberfroid (Editors), *Mechanisms of Toxicity and Hazard Evaluation*, Elsevier/North Holland Biomedical Press, Amsterdam, 1980, p. 631.
6 B. Kolb and J. Bischoff, *J. Chromatogr. Sci.*, 12 (1974) 625.
7 B. Kolb, M. Auer and P. Pospisil, *J. Chromatogr.*, 134 (1977) 65.