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Note

Quantitative determination of styrene-7,8-oxide in blood by combined gas chromatography–multiple ion detection mass fragmentography

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Styrene (vinylbenzene) is a monomer widely used in the plastics and rubber industry, its production amounting to about 7 million tons per year. The metabolism of styrene has been extensively studied[1–5], and epoxidation at the 7,8-position has been suggested as the main biotransformation pathway[6–8]. Styrene-7,8-oxide is used in the polymer industry as a reactive diluent for the preparation of epoxy resins. This product binds covalently to cellular macromolecules[9], has mutagenic properties[10–12] and is considered as the metabolic intermediate responsible for styrene toxicity[13,14].

Interest in the biochemical mechanisms at the basis of styrene and styrene-7,-8-oxide toxicity and the lack of a suitable method for the determination of styrene-7,-8-oxide in biological specimens prompted us to study a gas chromatographic–mass fragmentographic procedure for the determination of this compound at the nanogram level.

EXPERIMENTAL

Chemicals

Styrene-7,8-oxide was supplied by Merck (Darmstadt, G.F.R.). *p*-Methylanisole, used as internal standard for quantitation, was obtained from Aldrich Europe (Beerse, Belgium).

All solvents were of analytical-reagent grade.

Animals

Male CD_2F_1 mice (body weight 20–22 g) were obtained from Charles River Italy (Calco, Como, Italy). Animals were given a single intraperitoneal injection of styrene-7,8-oxide (200 mg/kg dissolved in corn oil). Groups of fifteen mice were killed by decapitation 1, 3, 5, 7, 10, 15, 20, 30, 45 and 60 min after this treatment and blood samples were collected and immediately processed for styrene-7,8-oxide assay.

Micro-extraction procedure

To 1 ml of blood, 2 ml of a 1.15% potassium chloride solution and 200 μl of methylene chloride containing *p*-methylanisole (50 μg/ml or 200 ng/ml, depending on the expected concentration range for quantitation and the detector utilized) were added. The appropriate amount of *p*-methylanisole to be added to the 3 ml of

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biological sample was determined from a preliminary experiment to establish a suitable ratio between the peak areas. The tubes were capped, shaken on a rotary shaking system for 15 min and centrifuged at 1000 *g* for 2 min. The methylene chloride phase was transferred with a Pasteur pipette into capillary-ended glass tubes, which were further centrifuged at 4000 *g* for 10 min. Any traces of biological material and water were removed by aspiration and 2–5 μl of the methylene chloride phase were injected on to the gas chromatographic column.

Addition of styrene-7,8-oxide to styrene-7,8-oxide-free blood at concentrations from 10 ng/ml to 100 μg/ml resulted in extraction recovery of 93.5 $\pm$ 4.2%.

Gas chromatography

Gas chromatography was carried out on a Carlo Erba Model G1 instrument with a flame-ionization detector. The column was a glass tube, 2 m long and 4 mm I.D., packed with 100–120-mesh Gas-Chrom Q coated with 3% OV-17 (Applied Science Labs., State College, PA, U.S.A.). All newly prepared columns were conditioned at 280°C for 1 h without carrier gas and then for 12 h with a carrier gas flow-rate of 15 ml/min. During analysis, nitrogen was used as the carrier gas at a flow-rate of 35 ml/min; the air and hydrogen flow-rates were adjusted to give maximal detector response. The column oven temperature was 100°C, injection port heater temperature 150°C and flame-ionization detector temperature 250°C.

Mass spectrometry

An LKB 2091 mass spectrometer was used, equipped with a Model 2130 computer system for data acquisition and calculation. The gas chromatographic conditions were as described before except that helium was used as the carrier gas. Multiple ion detection mass fragmentography was performed at 70 eV, focusing the instrument on the ions at *m/e* 120 and 91 for styrene-7,8-oxide and *m/e* 122 and 107, characteristic of the *p*-methylanisole spectrum.

RESULTS AND DISCUSSION

Fig. 1 shows typical gas chromatograms, recorded with a flame-ionization detector, from the analysis of styrene-7,8-oxide in the blood of (A) untreated and (B) styrene-7,8-oxide-treated mice. *p*-Methylanisole was chosen as the internal standard for quantitative purposes because of its suitable retention time and its fragmentation pattern under electron impact.

The chemical nature of peaks (a) and (b) in the chromatograms was checked by mass spectrometry and the resulting mass spectra are shown in Fig. 2. For analysis at the nanogram level mass fragmentography was applied and Fig. 3 shows a typical fragmentogram obtained from mouse blood. Multiple ion monitoring was performed by focusing the mass spectrometer on the molecular ions of styrene-7,-8-oxide and *p*-methylanisole occurring at *m/e* 120 and 122, respectively, on the ion at *m/e* 107 (122–·CH_3) characteristic of the *p*-methylanisole spectrum, and on the ion at *m/e* 91, which is common to both the styrene-7,8-oxide and *p*-methylanisole spectra and arises as shown in Figs. 2 and 4.

During the *in vivo* determinations no interference from endogenous substrates was observed and good linearity of the detector response (flame ionization or mass

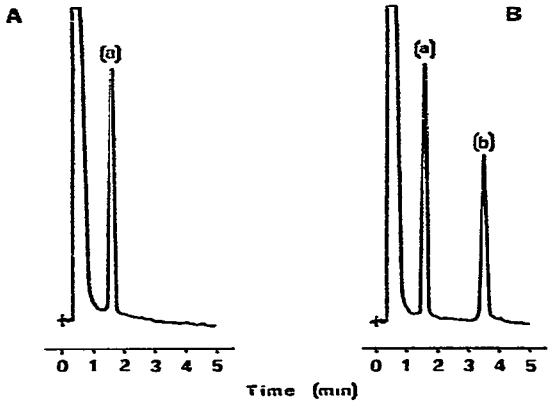

Fig. 1. Gas chromatographic analysis with flame-ionization detection. (A) Chromatogram corresponding to analysis of the blood of an untreated mouse. (B) Chromatogram corresponding to analysis of the blood of a styrene-7,8-oxide-treated mouse. Peaks: a = *p*-methylanisole; b = styrene-7,8-oxide.

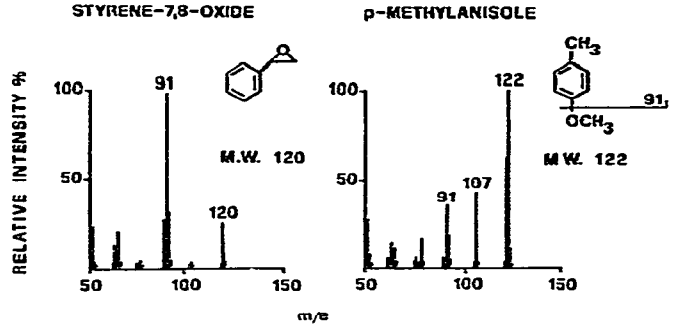

Fig. 2. Mass spectra of styrene-7,8-oxide and *p*-methylanisole obtained at 70 eV.

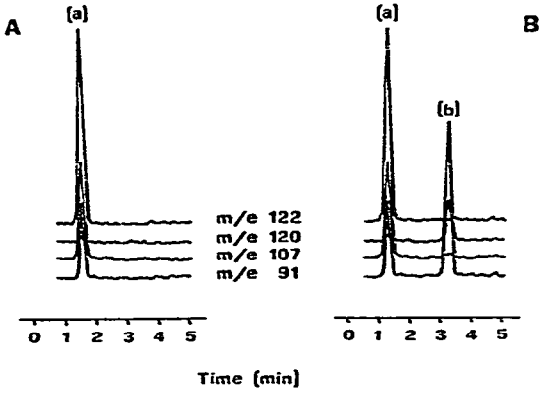

Fig. 3. Gas chromatographic–multiple ion detection mass fragmentographic analysis. (A) Mass fragmentogram corresponding to analysis of the blood of an untreated mouse. (B) Mass fragmentogram corresponding to analysis of the blood of a styrene-7,8-oxide-treated mouse. Peaks: a = *p*-methylanisole; b = styrene-7,8-oxide.

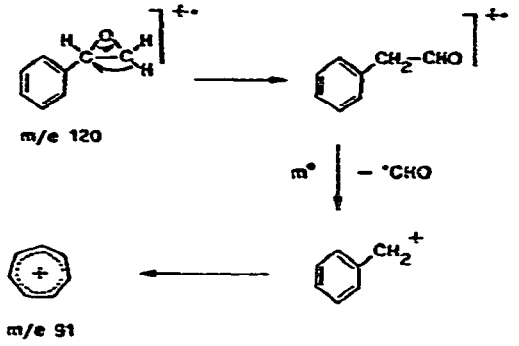

Fig. 4. Rationale for the loss of 29 a.m.u. from the molecular ion (*m/e* 120) of styrene-7,8-oxide.

fragmentography) was obtained over a range of styrene-7,8-oxide concentrations from 10 ng to 100 μg/ml in blood.

The validity of the procedure described for the determination of styrene-7,-8-oxide in biological specimens was checked by studying the distribution of this compound in mouse blood. Mice were chosen because they have been defined as one of the most vulnerable animal species to the toxic effects of styrene and styrene-7,-8-oxide, on the basis of their hepatic non-protein sulphydryl content depression[15] and their capacity to toxify and detoxify styrene[16].

Fig. 5 gives a semilogarithmic plot of the time course of styrene-7,8-oxide mouse blood concentrations. Styrene-7,8-oxide is rapidly absorbed, reaching a peak concentration of 40 ± 7 μg/ml at 7 min, after which the compound rapidly disappears until at 60 min it is no longer detectable. The parameters describing the kinetics of styrene-7,8-oxide in mouse blood are reported in the legend to Fig. 5, and were calculated by the method of residuals[17].

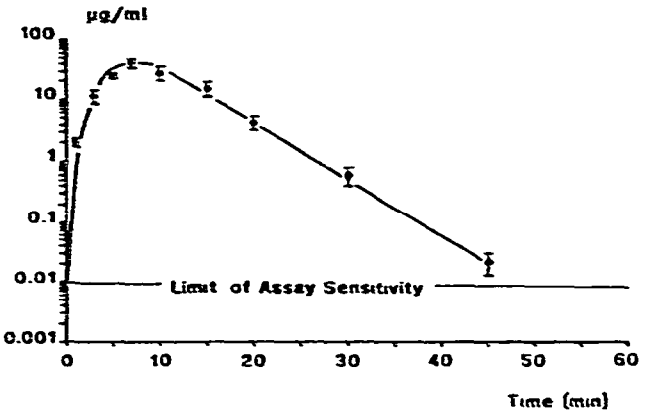

Fig. 5. Blood concentrations of styrene-7,8-oxide at different times after intraperitoneal injection of 200 mg/kg. Each point is the average of fifteen determinations. Kinetic parameters: K_a, rate constant of absorption = 0.288 min^{-1}; K_{el}, rate constant of elimination = 0.202 min^{-1}; $T_{1/2}$, half-life = 3.4 min; C_e, concentration extrapolated at time 0 = 224.1 μg/ml; V_d, apparent volume of distribution = 2.99 l/kg; *AUC*, area under the curve = 329.4 μg/ml·min.

The short *in vivo* half-life of styrene-7,8-oxide provides further evidence of its lability. It is, in fact, well known that apart from its chemical reactivity towards cellular molecules and/or macromolecules, styrene-7,8-oxide is also a good substrate for epoxide hydrase and glutathione transferases[18–20], two ubiquitous enzymatic activities.

In conclusion, the simple and rapid method reported here is specific and sensitive enough for application in experimental toxicological studies and may even prove useful for monitoring human exposure.

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REFERENCES

1 A. M. El Masri, J. N. Smith and R. T. Williams, *Biochem. J.*, 68 (1958) 199.
2 S. P. James and D. A. White, *Biochem. J.*, 104 (1967) 914.
3 H. Ohtsuji and M. Ikeda, *Toxicol. Appl. Pharmacol.*, 18 (1971) 321.
4 Z. Bardodej, *Scand. J. Work Environ. Health*, *4*, suppl. 2 (1978) 95.
5 C. Pantarotto, R. Fanelli, F. Bidoli, P. Morazzoni, M. Salmona and K. Szczawinska, *Scand. J. Work Environ. Health*, 4, suppl. 2 (1978) 67.
6 K. C. Leibman and E. Ortiz, *Biochem. Pharmacol.*, 18 (1969) 552.
7 K. C. Leibman and E. Ortiz, *J. Pharmacol. Exp. Ther.*, 173 (1970) 242.
8 K. C. Leibman, *Environ. Health Perspect.*, 11 (1975) 115.
9 J. Marniemi, E. M. Suolinna, N. Kaartinen and H. Vainio, in V. Ullrich, I. Roots, A. Hildebrand, R. W. Estabrook and A. Conney (Editors), *Microsomes and Drug Oxidations*, Pergamon Press, Oxford, 1977, p. 698.
10 N. Loprieno, A. Abbondandolo, R. Barale, S. Baroncelli, S. Bonatti, G. Bronzetti, A. Cammellini, C. Corsi, G. Corti, D. Frezza, C. Leporini, A. Mazzaccaro, R. Nieri, D. Rosellini and A. M. Rossi, *Mutat. Res.*, 40 (1976) 317.
11 H. Vainio, R. Pääkkönen, K. Rönnholm, V. Raunio and O. Pelkonen, *Scand. J. Work Environ. Health*, 2 (1976) 147.
12 C. De Meester, F. Poncelet, M. Roberfroid, J. Rondelet and M. Mercier, *Mutat. Res.*, 56 (1977) 147.
13 H. Härkönen, *Scand. J. Work Environ. Health*, 4, suppl. 2 (1978) 104.
14 H. Vainio, *Chem. Biol. Interactions*, 22 (1978) 117.
15 H. Vainio and A. Mäkinen, *Res. Commun. Chem. Pathol. Pharmacol.*, 17 (1977) 115.
16 L. Cantoni, M. Salmona, T. Facchinetti, C. Pantarotto and G. Belvedere, *Toxicol. Lett.*, 2 (1978) 179.
17 D. S. Riggs, *The Mathematical Approach to Physiological Problems*, Williams & Wilkins, Baltimore, 1963, p. 193.
18 F. Oesch and J. Daly, *Biochim. Biophys. Acta*, 227 (1971) 692.
19 F. Oesch, *Biochem. J.*, 139 (1974) 77.
20 T. Hayakawa, R. A. Lemahieu and S. Udenfriend, *Arch. Biochem. Biophys.*, 162 (1974) 223.