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QUANTITATIVE DETERMINATION OF STYRENE IN BIOLOGICAL SAMPLES AND EXPIRED AIR BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY (SELECTED ION MONITORING)

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SUMMARY

Styrene, phenylethylene, is an oily liquid of commercial importance in the manufacture of various plastics, rubbers, resins, and insulating materials. A gas chromatographic-mass spectrometric method is described for the determination of low levels of styrene in biological samples. The procedure utilizes an internal standard and specificity is gained by monitoring selected ions.

INTRODUCTION

The toxicological properties of styrene have been described in several papers[1–4]. Numerous analytical methods for styrene have been described previously[5–7]. However, the lack of a satisfactory method for determining styrene exposure and the need to determine low concentrations in biological samples has prompted the development of a specific and sensitive gas chromatographic–mass spectrometric (GC–MS) method for the determination of styrene in various matrices, including blood, expired air, fat, liver, and kidney tissue. The method employs chlorobenzene as an internal standard and detection of styrene and chlorobenzene by selected-ion monitoring.

EXPERIMENTAL

Standards and reagents

Monomeric styrene and chlorobenzene were supplied by Dow Chem. (Midland, Mich., U.S.A.). Glass-distilled hexane and methanol were purchased from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). Various animal tissues for the preparation of standards were obtained by sacrificing and dissecting healthy adult Sprague–Dawley (Spartan strain) rats. Whole human blood for preparation of standards was donated by healthy male volunteers.

Apparatus

A Finnigan Model 3000D quadrupole mass spectrometer in conjunction with

a Model 6000 data computing system was used. GC separation was carried out with a glass column (3.7 ft. × 2 mm I.D.) packed with 3% SP-2100 on Supelcoport (80–100 mesh) (Supelco, Bellefonte, Pa., U.S.A.). Prior to use, the column was conditioned for 0.5 h at ambient temperature with a helium flow-rate of approximately 30 ml/min. The column temperature was increased to 100 at 10°/min and conditioned for an additional 2 h. Finally, the temperature was increased to 200 at 10°/min and conditioned for an additional 24 h. During analysis the column was maintained at 80° and just prior to each series of analyses the column was reconditioned for 5 min at ambient temperature and 15 min at 100° to remove traces of oxygen and water vapor. Helium carrier gas flow-rate was maintained at approximately 30 ml/min. The injection port was maintained at 190° and the molecular separator and transfer line to the MS were heated to 200°. The temperature of the ion source was 80°; the source pressure, $5 \cdot 10^{-5}$ torr; the ionization energy, 70 eV; and the filament emission current, 100 μA. The instrument was programmed to monitor the molecular ions of styrene (*m/e* 104) and [^{35}Cl]chlorobenzene (*m/e* 112). Peak areas were determined by electronic integration with background subtraction. Following each injection the peak area of the internal standard was assigned to be 100. The data system calculated the area of the styrene peak as a percent of the internal standard peak area, thus compensating for deviations in the injection volume.

Collection of styrene from expired air

Gas washing bottles containing all ground-glass fittings were modified by puncturing the bottom of the fritted gas dispersion tube. Two such bottles were connected in series and immersed in Dewar flasks containing dry ice. A piece of clean glass tubing with a ground-glass connector on one end was connected to the inlet of the first bottle, serving as a mouthpiece. The exit of the second bottle was connected, via Nalge® tubing, to a calibrated spirometer. Approximately 25 ml of hexane containing 1 ppm (μl/l) internal standard was added to each bottle. Subjects were instructed to inhale room air and exhale into the mouthpiece. This process was continued with subsequent breaths until about ten liters of expired air had passed through the bottles.

The solvent from each bottle was transferred to a separate tared vial, the vials reweighed, and stored in a freezer until analyzed. For the analysis, 5 μl of the hexane was injected directly on the GC column.

Standards were prepared by adding styrene neat to 10-l Saran bags containing 10 l room air and gently heating and agitating to volatilize the styrene. The contents of the bags were then expelled into the trapping system described above.

Extraction of styrene from whole blood

Because preliminary studies on the partitioning of styrene between erythrocytes and plasma indicated the presence of significant styrene in the erythrocytes, the method was designed to measure styrene in whole blood. Venous blood was collected in stoppered tubes containing EDTA anticoagulant. Approximately 5 ml of blood was transferred to a tared vial. The vial was immediately reweighed and 1.0 ml of hexane containing 1 ppm internal standard was added. The vial was shaken vigorously for 2 min and allowed to stand until the organic layer separates. Injections of the hexane layer are made directly on the GC column.

TABLE I

RECOVERY OF STYRENE FROM WHOLE BLOOD, FAT, LIVER AND KIDNEY

Sample	*Recovery (%) ± standard deviation*	*Concentration range*
Whole blood	92.8 ± 13.1	1 ppb–10 ppm
Fat	89.6 ± 14.6	3–300 ppm
Liver	96.5 ± 4.6	3–300 ppm
Kidney	97.3 ± 5.4	3–300 ppm

The recovery of styrene from spiked whole blood standards was linear in the concentration range from 1 ppb* to 10 ppm and is given in Table I.

Extraction of styrene from fat, liver and kidney

Approximately 1-g samples of fat, liver, or kidney were added to tared vials. The vials were reweighed prior to the addition of 10 ml of hexane containing 1 ppm internal standard. The tissues were homogenized in the hexane at low speed with a Brinkmann Polytron® Homogenizer (Brinkmann Instruments, Westbury, N.Y., U.S.A.). When necessary, samples were centrifuged prior to injection of the hexane on the GC column.

Standards were prepared by introducing styrene directly into the hexane prior to homogenization and by permitting the homogenized samples to equilibrate 4 h prior to analysis. The recoveries of styrene from fat, liver and kidney were linear in the concentration range from 3 to 300 ppm and are given in Table I.

RESULTS AND DISCUSSION

The volatility of styrene and chlorobenzene make these compounds especially suitable for analysis by GC–MS. The molecular ions are of sufficient intensity under

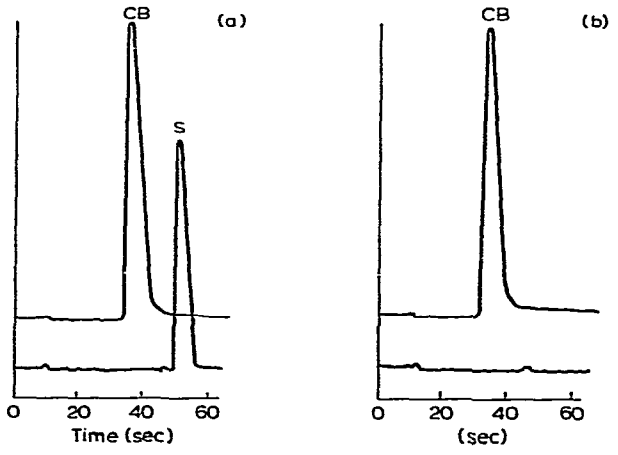

Fig. 1. Representative selected-ion chromatograms. (a) Chlorobenzene (CB) and styrene (S) in biological sample extract following styrene exposure. (b) Control biological sample extract.

* Throughout this article the American billion (10^9) is meant.

electron impact conditions to permit quantitation of low levels when these ions are selectively monitored. Of particular significance is the low level of interference from endogenous substances observed when selected ions are monitored. Typical selected-ion chromatograms of styrene and chlorobenzene in a biological sample following styrene exposure and a control biological sample are shown in Fig. 1. The identities of the peaks were verified by GC–MS, monitoring total ions.

The efficiency of trapping styrene from expired air was obtained from the different amounts in the primary and secondary traps. When bag standards theoretically containing from 10 ppb–10 ppm (0.043 to 42.6 μg styrene/l air) were analyzed the amount of styrene in the secondary trap ranged from less than detectable to 10% of that in the primary trap. One can conclude that each trap is removing approximately 90% of the available styrene and that two traps in series recover effectively all styrene. In an additional experiment a third trap was added and the resultant increase in recovery was less than 1%.

Lower detection limits for styrene in expired air were governed in part by the volume of air samples. Between 5 and 10 l was found to be a practical working volume, normally requiring less than 3 min to collect. Using approximately 25 ml of trapping solvent per trap, the lower limit of detection (signal-to-noise ratio = 2) was 5 ppb styrene in expired air (22 ng/l).

In blood samples the presence of a small peak resulting from an endogenous substance limited the detection of styrene to concentrations above 2 ppb (ng/ml). In four subjects tested, the mean area of this peak corresponded to the equivalent of 0.7 ppb styrene.

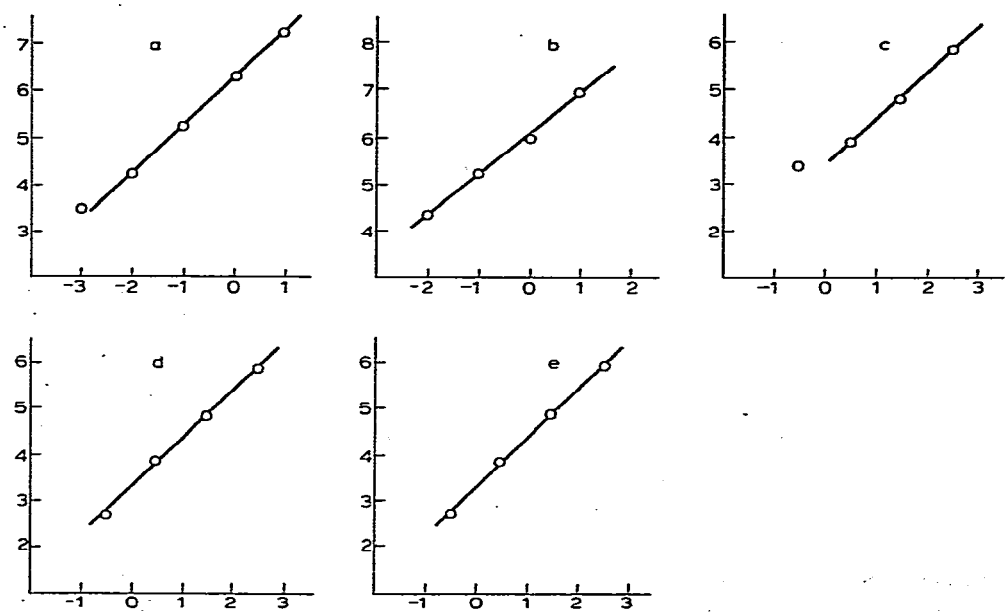

Fig. 2. Linearity of peak area (log mV) with styrene concentration (log ppm). (a) Whole blood extracts. (b) Expired air trap solvent. (c) Fat extracts. (d) Liver extracts. (e) Kidney extracts.

In fat, liver and kidney samples, the addition of larger amounts of hexane effectively diluted the samples. The detection limit for liver and kidney samples was 50 ppb (signal-to-noise ratio = 2). In fat an endogenous background peak limited detection to samples with concentrations above 200 ppb.

The linearity of the method has been demonstrated from 10 ppb–10 ppm in blood and expired air, from 1–100 ppm in fat, and from 300 ppb–300 ppm in liver and kidney, as shown in Fig. 2.

The method described is sensitive and specific and could easily be adapted to analyze styrene in other biological tissues. The procedures are satisfactory for measurements of pharmacokinetic parameters in humans and animals. Initial results indicate that the level of styrene in expired air may be a good index of human exposure[3].

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