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Short communication

Purge-and-trap gas chromatographic determination of styrene in urine and blood
Application to exposed workers

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Abstract

A simple purge-and-trap gas chromatographic method with flame ionization detection was developed for the determination of styrene in urine and blood. Styrene present in a 5 ml sample at room temperature was swept by helium at 40 ml/min for 11 min, trapped on a Tenax trap, desorbed by heating, cryofocused, and injected by flash heating into a DB-5 capillary GC column. The oven temperature program was from 80°C, held for 8 min, to 120°C at 5°C/min, and then held for 2 min. The detector temperature was 250°C. The calibration curves were linear in the range of 2.5–15 ppb styrene in urine and 25–150 ppb in blood. The detection limits calculated were 0.4 μg/l in urine and 0.6 μg/l in blood. The coefficients of variations within the day and day-to-day were 3 and 3.1%, respectively, for 2.5 ppb of styrene in urine, and 1 and 1.6% for 25 ppb of styrene in blood. The results obtained from samples taken from workers exposed to styrene were reported.

Keywords: Styrene

1. Introduction

Styrene is a toxic volatile compound used in large quantities in the production of fibreglass reinforced polyesters. In the occupational environment, styrene is mainly absorbed by inhalation of its vapours, and it passes quickly into the blood. Distribution, biotransformation, and excretion of styrene has been reported in several toxicokinetic studies [1,2].

For assessment and prevention of the occupational risks of workers exposed to styrene, the most frequently used biological monitoring method in the past has been the determination of its two main metabolites in urine: phenylglyoxilic acid (PGA) and mandelic acid (MA) [3,4]. However, several studies show differences in MA and PGA excretion, possibly due to the non-specificity of these two metabolites, which can also be metabolically derived from other chemicals, or due to the interference from solvents [5–7]. So, other more specific indicators for styrene in blood and urine are preferred by some investigators. Styrene in urine, like urinary metabolites, is considered a weighted index of exposure, while that in venous blood represents instantaneous

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values, since it reflects the alveolar air concentration [8].

Many studies on the determination of volatile compounds in different biological media have been carried out by static headspace analysis, coupled to gas chromatography (GC) [5]. However, these techniques are not sensitive enough for the determination of styrene in urine, where only a very small fraction of styrene is expected to be found [9].

Dynamic sampling methods, such as purge-and-trap, coupled to GC are particularly suitable for the determination of volatile compounds at a low concentration. The described procedures often use hardly reproducible devices made in research laboratories and/or require less common spectrometric techniques for detection, such as mass spectrometry or Fourier transform infrared spectroscopy [10–14]. This work reports a simple method for the determination of styrene in urine and blood using a commercial purge-and-trap apparatus coupled to a gas chromatograph with a common flame-ionisation detector. This approach has been proved to be useful for the biological monitoring of industrial exposure to styrene.

2. Experimental

2.1. Urine and blood samples

Urine and blood samples were taken from 12 men working in a factory building boats of resin reinforced with glass fibre, and exposed to concentrations of styrene ranging from 1 to 179 mg/m^3 (arithmetic mean=72 mg/m^3) [7]. Samples were collected at the end of the 4-h working shifts in glass tubes stopped with Teflon screw caps and frozen at −25°C until analysis.

A 500-ppm standard stock solution was prepared directly by dilution of styrene (purity 99+%, Sigma-Aldrich, Steinheim, Germany) in methanol (for analysis and chromatography, Scharlau, Barcelona, Spain). A 1:100 dilution of this solution in a mixture of methanol–H_2O (50:50; v/v) was used as the working solution. Four standard samples covering the calibration ranges (2.5–15 ppb for the urine and 25–150 ppb for the blood) were prepared by adding aliquots of the working solution to human urine or blood samples free from styrene.

2.2. Instrumental

The purge-and-trap system was an O.I. Analytical Model 4560 (College Station, TX, USA) including an AutoInjector controlled by a Cryo-Focusing Module (CFM) (O.I. Analytical). We selected a trap mainly composed of Tenax (O.I. Analytical 10), because of its maximum temperature of use and its low capacity for adsorption of water vapour [11].

The gas chromatograph was a Hewlett-Packard 5890 (HP) (Palo Alto, CA, USA), equipped with a flame-ionisation detector (FID) for quantification and a mass selective detector (HP 5988A), which we used in this study only for identification purposes.

2.3. Procedure

A sample of 5 ml of urine or blood (with EDTA as an anticoagulant) with a drop (50 μl) of a solution of Antifoaming Respumit (Bayer Hispania, Barcelona, Spain) in water (1:4) was introduced into a 25-ml sparger cell. We used two cells and two corresponding modes for purging gas introduction: through a frit or by a needle. In the urine samples the frit mode is advantageous, but with blood, the needle mode is more convenient because of the sample density and viscosity. In this case, the cell has to be unscrewed after each run to remove the sample, while in the frit mode it is removed automatically through the needle.

The flow-rate of helium during the purge was 40 ml/min and the system pressure was held between 6 and 11 p.s.i.. Samples were purged at 20°C for 11 min, and meanwhile the volatile organic compounds were retained in the Tenax trap at 20°C. Thereafter, volatiles were desorbed at 180°C for 4 min, swept by the carrier gas flowing in the trap in an opposite direction to the adsorption and transferred through a heated transfer line at 180°C to the CFM, where they were condensed onto a short capillary silica tube in liquid nitrogen at −100°C. Finally, the samples were injected into the capillary GC system by an automatic flash heating of the capillary tubing at 210°C. The trap was cleaned after each run by heating it at 200°C for 20 min. The interference from water

vapour produced by the samples in concentration, separation and detection processes was minimised.

A DB-5 capillary column (50 m×0.32 mm I.D., 0.52 μm film thickness) (J&W Scientific, Folsom, CA, USA) was used for GC analysis. The carrier gas and the make-up gas was helium (99.999%) at flow-rates of 1.5 and 38 ml/min, respectively. The oven temperature was held at 80°C for 8 min, then increased by 5°C/min to 120°C, where it was held for 2 min; the injection temperature was 210°C (temperature of CFM at inject step), and the detector temperature was 250°C.

The mass spectra were obtained at an ionisation voltage of 70 eV and an ion source temperature of 200°C and were recorded in the total ion scan mode from 20 to 250 amu.

3. Results and discussion

Figs. 1 and 2 show, respectively, the chromatograms of urine and blood samples taken from the same worker exposed to styrene. In spite of the complexity of the signals, the peak of styrene was

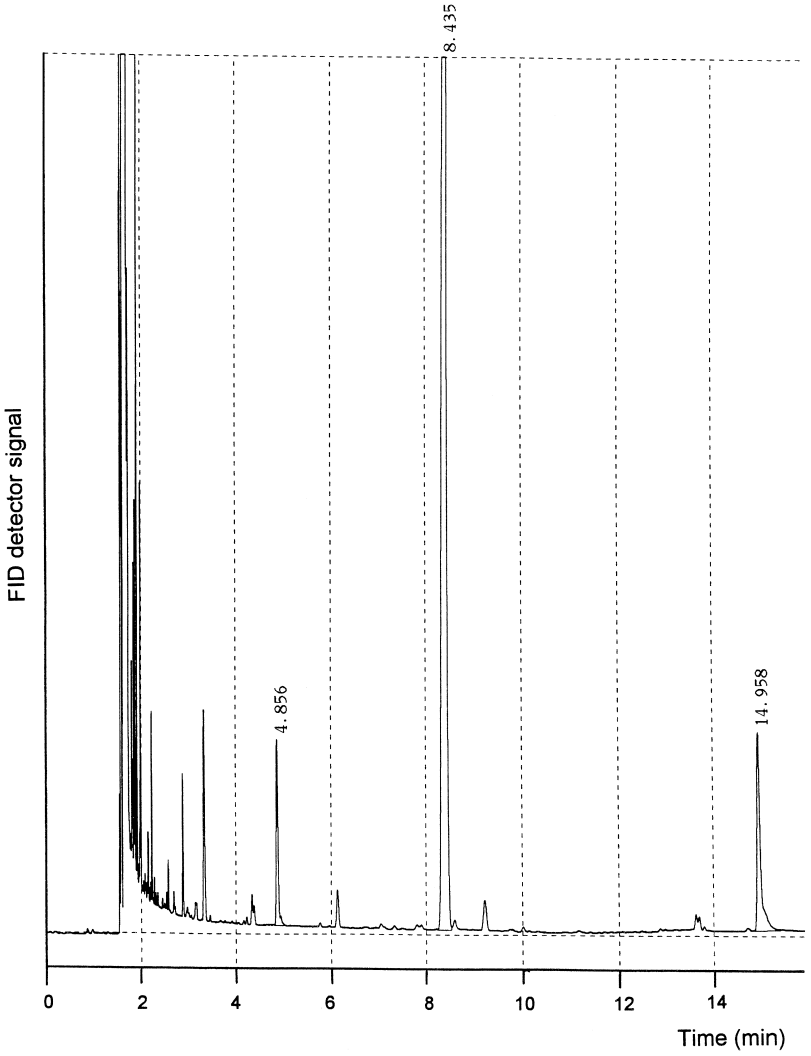

Fig. 1. Chromatogram of urine sample from an exposed worker (worker 2 in Table 2).

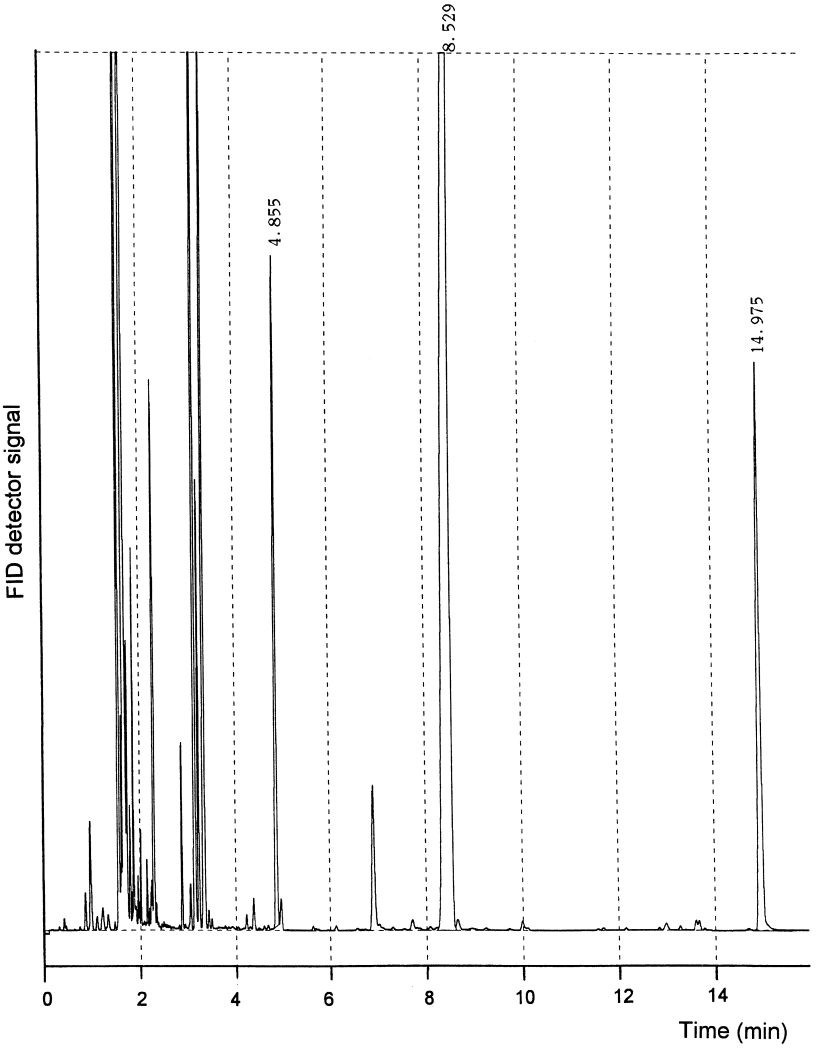

Fig. 2. Chromatogram of blood sample from an exposed worker (worker 2 in Table 2).

clearly identified on the basis of its GC retention time (4.87 min) and its mass spectrum compared with that of standard samples. Two peaks from the antifoaming agent, at near 8.5 and 15.0 min, were identified as hexamethylcyclotrisiloxane and octamethylcyclotetrasiloxane, respectively, by comparison with the NBS library spectra stored in the HP chemstation. These compounds were not removed from the trap in the baking cleaning step, causing artifacts which, however, do not interfere with the identification of styrene.

The stability of the standard working solution was tested by comparing a fresh 5-ppb styrene standard in urine with those prepared 10, 20 and 40 days before and stored at −4°C in dark recipients, in order to prevent polymerisation of the styrene. As a result, the working solution is stable at least for 10 days in these conditions.

Table 1 shows the areas of styrene, obtained by analysing three aliquots of each standard sample covering the calibration ranges in urine and blood and where the within the day precision of measurements, can be calculated. The calibration curves are linear in the range tested and can be described by the

Table 1
Areas of the peaks obtained to construct the calibration curves for styrene in blood and urine

Concentration of styrene in urine (ppb)	Area of styrene in urine	Concentration of styrene in blood (ppb)	Area of styrene in blood
2.5	19 841	25	274 618
	20 874		296 855
	19 692		292 851
5	39 258	50	629 441
	39 982		629 961
	37 415		560 024
10	87 192	100	1 359 729
	74 359		1 241 604
	80 197		1 445 720
15	128 065	150	2 281 240
	142 441		2 293 243
	134 011		2 243 479

equations: $y = 9148x - 5717$; $r = 0.993$ for the urine and $y = 15\,879x - 161\,070$; $r = 0.996$ for the blood (x = concentration of styrene (ppb), y = area).

The limits of detection were calculated following the IUPAC recommendation [15] as the value corresponding to three times the standard deviation of the blank obtained from seven measurements. The values obtained were 0.4 μg/l of styrene in urine and 0.6 μg/l in blood.

The precision tests were carried out on standard samples of 2.5 ppb of styrene in urine and 25 ppb in blood. The coefficients of variation within the day were 3% for urine and 1% for blood. The respective values for day-to-day, at the same concentrations, from six analysis were quite similar: 3.1 and 1.6%, respectively. The detection limits and the variation coefficients obtained are significantly lower than those reported using static headspace methods with FID detection [5] and similar to those obtained with mass spectrometric detection [8] or using discontinuous purge-and-trap methods with an additional concentration step [11].

Table 2 shows the concentrations of styrene in urine and blood of workers, as well as in environmental air to which they were exposed. Chromatograms shown in Figs. 1 and 2 refer to worker 2 in Table 2. Styrene contents in unknown samples were calculated by interpolating in the respective cali-

Table 2
Levels of environmental exposure and concentrations of styrene in urine and blood obtained from occupationally exposed workers

Worker	Styrene concentration in urine (μg/l)	Styrene concentration in blood (μg/l)	Styrene concentration in environmental air (mg/m^3)
1	9.8	88.0	141.4
2	7.9	82.3	86.2
3	3.1	27.5	62.6
4	15.3	166.8	130.0
5	7.5	73.6	119.6
6	5.4	101.1	69.0
7	5.7	96.4	71.8
8	3.0	53.0	93.8
9	4.9	57.4	36.2
10	2.5	35.8	65.9
11	4.5	79.8	67.5
12	9.6	186.7	189.3

bration curves. The concentrations obtained in urine and blood samples must be considered valid because they are within the range of applicability of the proposed method, and the method is based in consistently linear calibration curves constructed with the same matrix. The results properly reflect the differences found in the levels of environmental styrene in a range of concentrations encompassing the TLV-TWA of the ACGIH (85 mg/m^3) [16].

The whole determination of styrene in either a urine or blood sample is finished in about 35 min, including the extraction and concentration process and the chromatographic determination (the trap cleaning takes place during the GC separation). Therefore, routine control of exposure to styrene can be carried out in a reasonably short time using this approach.

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