·Purge-and-trap method for the determination of styrene in urine

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Abstract

A purge-and-trap method for biological monitoring of styrene in urine was developed. Sorbent tubes packed with Tenax TA were used to trap styrene vapour purged from urine. Thermal desorption–gas chromatography was used for sorbent tubes analysis. The detection limit (0.70 μg/l), linearity range, recovery (>94% for spiked urine samples) and repeatability for urine from occupational exposed workers show the suitability of the method for the determination of styrene in urine. One specific advantage of this method is the possibility of storage of the charged sorbent tubes during long periods of time without a significant loss of styrene. This approach can be used, with slight modifications, for urinary determination of several others organic contaminants commonly present in occupational exposures.

1. Introduction

Styrene is a widely used solvent in several industrial activities such as fibreglass-reinforced plastics and boat building. It is a volatile compound that enters in the body mainly through the lungs or skin. General studies have been devoted to its uptake, distribution and elimination as urinary metabolites [1–3].

In general, styrene exposure is biologically monitored by measuring the urinary excretion of its two main metabolites, mandelic acid (MA) and phenylglyoxylic acid (PGA) [4,5]. However, this method shows a large inter-individual variability in the results caused by interferences in

the MA and PGA excretion by other solvents, drugs and alcohol consumption, differences between individual metabolisms, etc. [6].

Small amounts of styrene are eliminated in the urine. Thus, an index for the biological monitoring of styrene exposure that possibility avoids some of the problems mentioned could be the measurement of the urinary excretion of the compound. Although very low styrene concentrations in urine are expected, some current analytical methodologies could be used, and procedures based on the headspace determination of styrene in urine samples have been described [7–9]. Other methods, such as the purge-and-trap procedure, which can also measure very low styrene concentrations, may be an adequate alternative.

The aim of this work was to develop a method for the determination of styrene in urine by

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means of inert gas urine purging and styrene trapping in a solid sorbent. Subsequently, the styrene trapped in the sorbent can be determined by thermal desorption–gas chromatography. This procedure could be very useful for use in industrial hygiene and occupational toxicology.

2. Experimental

2.1. Sorbent selection

Gas–solid chromatography was used to determine the specific retention volumes of styrene on Tenax TA and Chromosorb 106 (SKC, Valley View, PA, USA). The adsorption measurements, made in the temperature range 373–513 K, were carried out using the system shown in Fig. 1. The sorbent (0.15 g) was packed into a stainless-steel tube (89 mm × 6.4 mm O.D.) and placed between the injector and the flame ionization detector of the gas chromatograph. Styrene vapour was generated by an on-line dynamic atmosphere and injected into the column by means of a 5-ml gas valve. Nitrogen was used as the carrier gas. Specific retention volumes (l/g) were calculated from the measured retention times.

2.2. Purge-and-trap method

Fig. 2 shows the scheme of the purge-and-trap device used. A sample of 10 ml of urine con-

Fig. 1. Schematic diagram of the system used to generate the controlled atmosphere and to obtain specific retention volumes: 1 = air intake; 2 = filter; 3 = humidifier; 4 = automatic injector; 5 = mixing chamber; 6 = sampling chamber; 7 = gas chromatograph; 8 = packed tube; 9 = integrator.
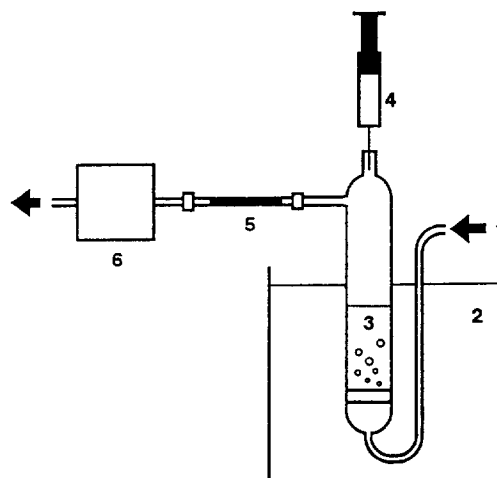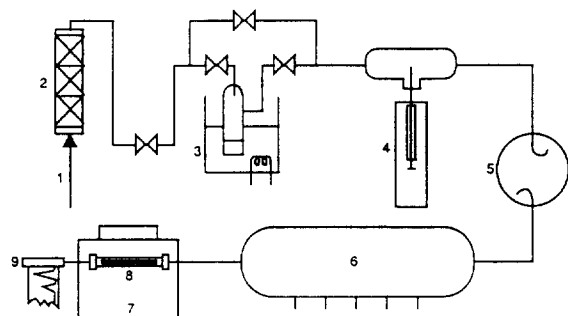

Fig. 2. Schematic diagram of the purge-and-trap system: 1 = purge gas intake; 2 = thermostated bath; 3 = urine sample; 4 = syringe for addition of urine sample; 5 = sorbent tube; 6 = digital flow meter.

taining 1.5 ml of methanol, to minimize foam production, was purged with helium at a flow-rate of 45 ml/min for 11 min at a bath temperature of 27°C. Standard glass tubes for an ATD 50 thermal desorption system (Perkin-Elmer, Beaconsfield, UK) (89 mm × 6.4 mm O.D.), packed with 150 mg of 20–40-mesh Tenax TA, conditioned prior to use, were used as a trap. The tubes were fitted to the system by means of PTFE ferrules held with 1/4-in. Swagelock connections.

During storage, the tubes were protected with caps. The extraction conditions were optimized by determining the recovery for spiked urine samples using the simplex method [10].

2.3. Analysis

The analysis of sorbent tubes containing the styrene vapour from urine samples was performed by means of coupled thermal desorption–gas chromatographic techniques. The sorbent tubes were desorbed with a Perkin-Elmer ATD 50 automatic thermal desorption system, directly connected to a Perkin-Elmer Model 8700 gas chromatograph by a heated transfer line.

Thermal desorption was carried out in two stages: first, the tube was heated with the carrier gas flowing through it, transferring the desorbed

vapour from the sorbent tube to a cooled trap (packed with Tenax TA). Subsequently, when the entire sample had been collected, the trap was rapidly heated to desorb the volatile materials, which were rapidly injected into the GC column via the heated transfer line. Desorption was carried out with nitrogen as carrier gas (68.95 kPa) at an oven temperature of 200°C, a 10-min desorption time, trap lower and upper temperatures of −30 and 300°C, respectively, and a transfer line temperature of 120°C.

GC analysis was performed with a fused-silica capillary column (25 m × 0.2 mm I.D.) of cross-linked free fatty acid phase (FFAP) of 0.3-μm film thickness (Hewlett-Packard, Palo Alto, CA, USA), using nitrogen at 68.95 kPa as the carrier gas. The oven and flame ionization detector temperatures were 120 and 200°C, respectively.

Calibration standard tubes were prepared by injecting known volumes of several styrene solutions in methanol, covering the calibration range. An additional flow of nitrogen (40 ml/min) was passed through both samples and calibration tubes for 3 min before the thermal desorption. With this procedure, methanol sorbed in the tube was purged, avoiding interferences.

2.4. Recovery tests

The studies of recovery were carried out with urine samples spiked with low concentrations of styrene at two levels of styrene concentrations

(Table 1). In both cases, identical volumes of methanolic styrene solution, measured with the same microsyringe, were also injected into sorbent tubes and into aluminium-sealed vials with 10 ml of urine sample, respectively. Sets of seven sorbent tubes and urine samples were prepared for each level.

Styrene was extracted from urine samples according to the above purge-and-trap procedure. For every set of spiked urine samples, additional non-spiked urine samples were also extracted as blanks. Both sorbent tubes directly injected and trap tubes collected after the purge were thermally desorbed and analysed.

2.5. Storage

A set of spiked urine samples of 0.112 μg/ml were prepared in 10-ml aluminium-sealed vials; two samples were analysed immediately and the rest were stored at −25°C. Subsequently, two samples each time were analysed after 5, 20 and 60 days.

2.6. Repeatability in exposed subjects urine samples

A repeatability test was carried out on urine samples from three occupationally exposed workers, analysing three aliquots of 10 ml from the same urine sample of every exposed worker. The aliquots were placed in 10-ml aluminium-sealed vials until extraction and analysis. All the

Table 1
Recoveries for spiked urine samples

Level 1		Level 2	
Standard (μg)	Urine (μg)	Standard (μg)	Urine (μg)
1.342	1.313	0.76	0.71
1.333	1.380	0.82	0.70
1.353	1.194	0.76	0.73
1.359	1.253	0.82	0.75
1.361	1.401	0.80	0.77
1.428	1.306	0.78	0.77
1.384	1.276	0.79	0.73
Mean = 1.366	Mean = 1.303	Mean = 0.79	Mean = 0.74
(S.D. = 0.03; R.S.D. = 2.19%)	(S.D. = 0.07; R.S.D. = 5.37%)	(S.D. = 0.02; R.S.D. = 2.53%)	(S.D. = 0.03; R.S.D. = 4.05%)
Recovery = 0.95		Recovery = 0.94	

samples were extracted and analysed in the same way.

3. Results and discussion

The plots of the logarithm of the measured retention volume against the reciprocal of the absolute temperature of the chromatographic column for the two sorbents checked are shown in Fig. 3. From these plots, the specific retention volume at 20°C can be calculated, being 4626 and 188 l/g for Chromosorb 106 and Tenax TA, respectively. The strength of a sorbent should be expressed as the retention volume at 20°C and, if this value exceeds 100 l/g, the sorbent is satisfactory for sampling [11]. Following this guideline, both sorbents are adequate.

Tenax TA was selected because its maximum temperature of use is higher than that of Chromosorb 106 and it needs a shorter time for quantitative thermal desorption. In addition, Tenax TA has a low capacity for adsorption of water vapour [12], which is very important because the purge gas from urine samples will contain high levels of humidity.
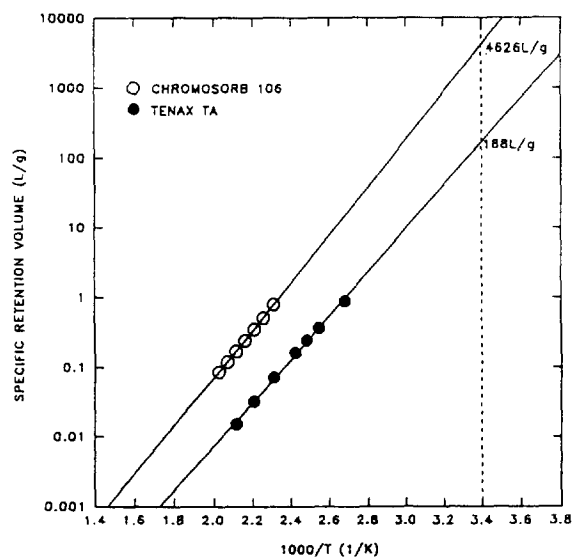
The calibration line obtained was $y =$ $-0.0575 + 34.6466x$; $r = 0.9998$ ($x = \mu g$ of styrene, $y =$ area). The detection limit of the technique was 0.70 $\mu g/l$ for urine samples analysed according to the previously described protocol. The linearity range is adequate for analysing urine samples from subjects occupationally exposed to environmental concentrations ranging from 0.1 to 4 times the current TLV-TWA of the ACGIH (50 ppm), according to the correlation between environmental and urine styrene levels found by Gobba et al. [8].

The recoveries of styrene at two different concentrations in urine samples are shown in Table 1. Mean recoveries of 0.95 and 0.94 were obtained for the upper and lower levels of styrene tested, respectively. This makes possible the use of the described methodology for the direct determination of styrene in urine, avoiding calculation based on partition coefficients which is necessary when using headspace techniques. The relative standard deviation (R.S.D.) obtained at both levels was less than 2.52% and 5.37% ($n = 7$) for standard tubes and urine samples, respectively.

The results for the stored samples are plotted in Fig. 4, where the mean values for two samples are plotted against time of storage in days. Significant losses are detected in the urine samples analysed after 60 days. However, this procedure made it possible to store the sorbent tubes obtained after the purge, instead of urine samples. In sorbent tubes collected directly from atmospheres of known styrene concentration, there were no significant losses after storage at 4°C for more than 60 days [13]. In addition, reference materials for aromatic hydrocarbons in Tenax are stored for long periods of time without significant losses.
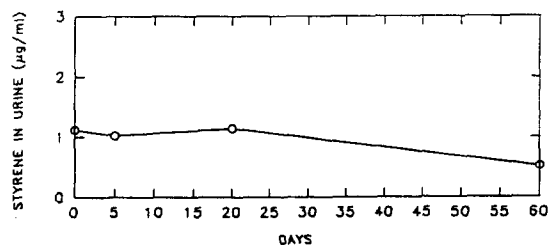

Fig. 3. Specific retention volumes for Chromosorb 106 and Tenax TA.

Fig. 4. Recovery from spiked urine samples stored at −25°C.

Table 2
Repeatability of the determination of styrene in urine samples from occupationally exposed workers

Subject	Styrene concentration (μg/ml)	R.S.D. (%)
1	0.117, 0.121, 0.124	2.9
2	0.092, 0.096, 0.096	2.4
3	0.031, 0.028, 0.029	5.2

Table 2 shows the results for three aliquots of urine samples from three subjects occupationally exposed to styrene in different fibreglass-reinforced plastics industries. The R.S.D.s for the aliquots analysed ranged from 2.9% to 5.2% ($n = 3$). A representative chromatogram of the
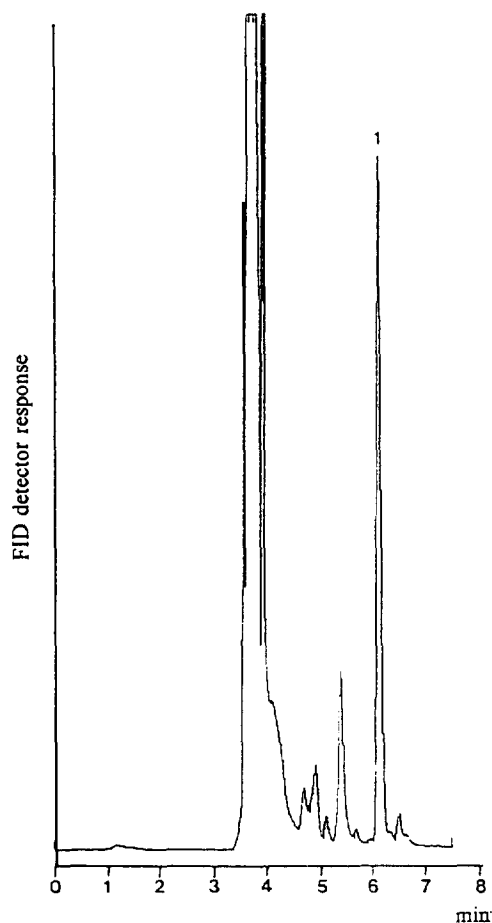

Fig. 5. Chromatogram of urine sample from subject No. 3 in Table 3, occupationally exposed to styrene (1).

urine samples corresponding to subject No. 3 is shown in Fig. 5.

With the described procedure, typical concentrations of styrene in urine of occupationally exposed workers can be easily determined for comparison with the proposed biological equivalent exposure limits (BEEL) for urinary styrene, corresponding to the current TLV–TWA of the ACCIH, of 80-μg/l [14].

4. Conclusions

A procedure for the direct determination of styrene in urine, based on concentration in a solid sorbent, was developed, involving the use of a purge-and-trap device and subsequent analysis by thermal desorption–gas chromatography.

The detection limits and linearity range of this method, using a flame ionization detector, are similar to those for other alternatives, based on the headspace method and mass spectrometric detection [8]. In addition, this procedure can improve the storage conditions, if the urine samples have been previously purged, because the concentration measurements of samples from the sorbent tubes are not significantly affected by long delay periods between sampling and analysis. Hence the present purge-and-trap method appears to be particularly useful when the sampling conditions do not allow immediate measurements.

The results show that this method for styrene determination in urine on a purge-and-trap method, may be useful for biological monitoring of occupational exposure to this contaminant. The same methodology can be used, with slight modifications, for urinary determination of several other organic contaminants commonly present in occupational exposures.

Acknowledgement

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