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Journal of Pharmaceutical and Biomedical Analysis
15 (1997) 851–854

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Short communication

Occupational health monitoring using solid phase extraction of urine

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Received 3 July 1996

Keywords: Occupational health; Biological monitoring; Solid phase extraction; Determination of metabolites of C1- and C2-alkylbenzenes in urine

1. Introduction

For the evaluation of occupational exposure to chemical hazards, and for observance of occupational exposure limit values of individual workers, biological monitoring is an essential procedure. In comparison to air monitoring, biological monitoring involves either the estimation of chemicals in blood, the determination of their metabolites in urine or changes of various biochemical parameters as an early biological response to chemical exposure [1].

A significant problem in occupational health analysis is correct sample handling [1,2] and suitable analyte clean up for reliable analytical results. False analytical data are expensive [2–5] and can cause errors of more than 60% by the sampling processing [6] and additionally, the possible loss by bacterial or other decompositions of the analytes.

Sample clean up and preconcentration using liquid/liquid extraction is time consuming, expensive and can cause losses (i.e. decarboxylation of α-keto-acids) [7]. For a cost effective, simple to handle and reliable sample processing procedure solid phase extraction methods (SPE) [8] can be used for biomonitoring, with sealable columns for transportation of the analyte to the laboratory. This study deals with the application of SPE for biological monitoring in occupational medicine, using urine samples added to SPE-columns at the workplace to be processed for the following metabolites:

- hippuric acid, a biological marker for exposure to toluene, and also a physiological metabolite of benzoic acid,
- methylhippuric acids, markers of exposure to xylenes,
- mandelic-, phenylglyoxilic- and hippuric acid, markers of exposure to styrene and ethylbenzene respectively.

PII S0731-7085(96)01907-3

Table 1
Recovery rates and relative S.D. of serial replicates of the determination of mandelic acid (MA), phenylglyoxilic acid (PGA) and hippuric acid (HA) with and without SPE

Metabolite	Sample	Without SPE			After SPE		
		Number of replicates	Relative S.D. (%)	Recovery (%)	Number of replicates	Relative SD (%)	Recovery (%)
MA	Standard	15	6.83	100	10	6.27	98.0
	Urine	10	5.87	92.4	10	4.22	92.2
PGA	Standard	15	7.84	100	10	15.50	99.0
	Urine	10	9.80	95.8	10	7.48	99.9
HA	Standard	15	2.58	100	10	3.22	94.4
	Urine	10	3.52	97.1	10	4.05	101.0

For absolute concentrations in all Tables see text.

2. Materials and methods

2.1. Materials

Ammonium sulphate (Laborchemie, Apolda, Germany), methanol, acetonitrile and *n*-hexane (Merck, Darmstadt, Germany) were all analytical grade.

2.1.1. Standard solutions

Hippuric acid (76.9 mg 100 ml^{-1}) and *p*-methylhippuric acid (51.1 mg 100 ml^{-1}) were dissolved in a minimal volume of ethanol and made up to 100 ml with water. Mandelic acid (51.2 mg 100 ml^{-1}) and phenylglyoxilic acid (50.2 mg 100 ml^{-1}) respectively were dissolved in water and made up to 100 ml.

2.1.2. Spiked urines

For experiments pooled urines of unexposed persons were mixed with equal volumes of the standards. For SPE, reversed phase plastic cartridges with Luer connection fittings and stoppers (SILICAcart Separon-C18™, 60 μm from Tessek, Prague, Czech Republic or C18-Cartridges, from Dionex, Idstein, Germany) were used.

2.2. Methods

2.2.1. Conditioning of the columns

The columns were loaded with methanol (4 ml) for hippuric acid and methylhippuric acid and for mandelic acid, phenylglyoxilic acid and hippuric acid respectively with 2 ml each of methanol and acetonitrile and then conditioned with 2 ml 2.5% aqueous ammonium sulphate solution. Liquids were passed through the columns using vacuum (i.e. by SPE-equipment Dorcus™, Teesek, Prague).

2.2.2. Sample preparation

Before use we mixed equal volumes of the standards and urine samples with 5% aqueous ammonium sulphate solution. For these experiments, 1.0 ml of standards and spiked urine samples are used in the following concentrations: for standard solutions hippuric acid 384.5 mg l^{-1}, *p*-methylhippuric acid 255.5 mg l^{-1}, mandelic acid 256 mg l^{-1} and phenylglyoxilic acid 250.1 mg l^{-1}; for final concentrations of spiked urines; hippuric acid 657 mg l^{-1}, *p*-methylhippuric acid 255.5 mg l^{-1}, mandelic acid 256 mg l^{-1} and phenylglyoxilic acid 250.1 mg l^{-1}. The prepared standard solutions and urine samples were added to a conditioned SPE-column, the column washed with 2 ml 2.5% ammonium sulphate solution containing 200 μl *n*-hexane, and the column dried by passing about 80 ml air through the column. The SPE-column was then sealed with the stoppers and sent to the laboratory.

Hippuric acid/*p*-methylhippuric acid and mandelic acid/ phenylglyoxilic acid/hippuric acid are then eluted from the SPE-column with 1.80 ml 50% aqueous methanol and the volume made up

Table 2
Time depending (day to day control) recovery rates and Relative S.D. of replicates of the determination of MA, PGA and HA in spiked urine with and without SPE

Metabolite	Sample	Number of replicates (d/d)	Relative S.D. (%)	Recovery (%)
MA	Standard without SPE	20	3.98	100
	Urine without SPE	20	6.38	99.1
	Urine after SPE	20	5.57	98.4
PGA	Standard without SPE	20	6.52	100
	Urine without SPE	20	6.16	102
	Urine after SPE	20	6.98	100
HA	Standard without SPE	20	(8.77)[a]	(101.5)[a]
	Urine without SPE	20	5.35	98.3[b]
	Urine after SPE	20	4.65	97.5[b]

[a] In relation to the serial standard value in Table 1.
[b] Corresponding to the actual standard value.

to 2.0 ml. After centrifugation we spotted 5 or 10 μl of the solution onto the silica gel plate. TLC determination of the metabolites was performed for hippuric acid and *p*-methylhippuric acid photometrically after reaction with *p*-dimethylamino benzaldehyde, for mandelic acid derivative spectroscopically and for phenylglyoxilic acid photometrically at 255 nm as described in [9].

3. Results and discussion

For assessment of the suitability of the SPE for biological monitoring, control experiments were carried out for relative recovery and relative S.D. The standards and spiked urines were determined as serial replicates (Table 1) and in day to day control (Table 2). Furthermore, the recovery rates of analytes were determined using conditioned columns that had been stored closed for different periods before using the sample (Table 3). Columns were also stored for different periods after they had been loaded with sample (Table 4) for simulation of the conditions encountered in practice. The results show no significant differences in the analytical results in all cases. This shows that sample clean up using SPE-columns is reproducible for the purposes of biological monitoring. An earlier published SPE method

Table 3
Recovery rates of MA, PGA, HA and *p*-methylhippuric acid (MHA) using conditioned SPE-columns after storage until loading with urine samples

Application after storing (days)	Recovery of MA in urine (%)	Recovery of PGA in urine (%)	Recovery of HA in urine (%)	Recovery of MHA in urine (%)
1	94.0	100.0	97.5	99.4
2	91.8	99.7	103.5	103.5
3	90.5	99.5	94.7	96.9
4	92.8	104.0	95.1	95.9
5	97.4	101.0	91.8	96.8
7	96.6	103.0	86.6	93.0
32	97.6	103.0	86.6	93.0
42	100.0	90.0	93.4	98.4

Standards used without SPE.

Table 4
Recovery rates of MA, PGA, HA and MHA after loading the conditioned SPE columns with urine samples and storage up to elution

Elution of metabolites in days after loading the sample	Recovery of MA in urine (%)	Recovery of PGA in urine (%)	Recovery of HA in urine (%)	Recovery of *p*-MHA in urine (%)
0	100.0	100.0	100.0	100.0
1	97.4	101.0	93.6	95.8
2	119.0	103.0	97.7	95.8
3	122.0	99.7	97.4	95.6
6	111.0	113.0	100.8	100.9
8	115.0	101.0	101.2	101.4
9	120.0	101.0	99.5	96.0
10	104.0	100.0	95.4	92.0
13	112.0	103.0	95.3	92.4

Standards used without SPE.

for mandelic acid and phenylglyoxilic acid after conditioning the column with methanol and a phosphate solution for washing [10] were less satisfactory because losses of between 28 and 50% occurred for both acids. The best recovery was found using an ammonium sulphate concentration of 2.15–2.5%. Higher concentrations of ammonium sulphate have a lower pH-value and can decarboxylate the phenylglyoxilic acid [7]. Loading the column with methanol only increases the loss of analytes.

The use of *n*-hexane added to the ammonium sulphate solution is very helpful to displace water from the cartridge and improves the air-drying stage. Additionally, preliminary studies, show that SPE may be also used for phenolic metabolites (phenol, *o*-, *p*-resol and *o*-, *p*-nitrophenol tested) in urine, if ammonium sulphate solution in 10 mM sulfuric acid is used.

It would be an advantage if SPE cartridges were manufactured to permit them to be sealed after sampling.

In conclusion, the application of SPE can be used in occupational health analysis for sampling at the workplace followed by clean up and analyte measurement in the laboratory.

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