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Capillary electrophoresis and supercritical chromatography, complementary and alternative techniques for the determination of urinary metabolites of styrene

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Abstract

Two analytical methods without an extraction step were developed using capillary electrophoresis and supercritical fluid chromatography in order to determine phenylglyoxylic (PGA) and mandelic (MA) acids in urine, with minimum treatment and manipulation of biological samples. The urine was diluted ten-fold in acetonitrile and directly injected into the analytical systems after centrifugation. Analysis was performed by capillary electrophoresis on alkyl bonded phase capillary columns with sodium formiate ($4 \cdot 10^{-2}$ *M*)–isopropanol (9:1, v/v) as a buffer, and by supercritical fluid chromatography on a Diol bonded phase silica column with ethanol–water–methanesulphonic acid (97.5:2.4:0.1, v/v) as coeluent of CO_2. Detection of PGA and MA was performed by ultraviolet detection at 255 and 210 nm, respectively. The methods are in agreement, and are easily able to detect 5 mg/g creatinine for PGA, and 15 mg/g creatinine for MA, which are one twentieth of the lowest biological exposure index values.

Keywords: Styrene; Phenylglyoxylic acid; Mandelic acid

1. Introduction

Styrene is used extensively in industry for the production of plastics. Evaluation of the toxic effects of styrene on the nervous system, liver, lungs and kidneys [1] involve the monitoring of occupational exposure. To this end, the urinary styrene metabolites phenylglyoxylic acid (PGA) and mandelic acid (MA) are used as bioindicators. A biological exposure index (BEI) of 100 mg PGA/g creatinine and 300 mg MA/g creatinine for urine samples collected on the morning after exposure has been recommended [2,3].

The various methods used to determine PGA and MA in urine are well documented in the literature. Some of these methods have already been reviewed [4,5], and are essentially based on chromatographic techniques such as gas chromatography (GC) [6,7], high-performance liquid chromatography (HPLC) [8–10], and isotachophoresis (ITP) [11,12], with their respective advantages and drawbacks. The main disadvantage of these methods is often the need for an extraction step, with an additional derivatization procedure in the GC methods.

Although the detection limits are slightly better with HPLC than with ITP, the latter is attractive for analysis of compounds of interest in biological monitoring, as shown by Sollenberg [11,13,14].

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Capillary electrophoresis (CE), a recently developed technique related to ITP, seems to be more promising and quite powerful. Several CE methods describe the separation of aromatic acids [15–19], but few of them are currently available for the determination of metabolites in human urine following occupational exposure to aromatic solvents [18,19]. Bioanalytical applications of supercritical fluid chromatography have been reviewed [20,21], but no method has so far been proposed for the routine monitoring of workers exposed to chemicals.

In a previous paper [22], we described the use of CE and SFC, two techniques with different principles of separation, for the confirmation of the presence in a urine sample of 2-thiothiazolidine-4-carboxylic acid (TTCA), a urinary metabolite of CS_2. However, no direct qualitative or quantitative determination in urine was performed because of the lack of selectivity and sensitivity of the method. Nevertheless, our investigations led us to extend the field of application of these techniques to biological monitoring in order to reduce, or even eliminate the need for the pretreatment of the biological sample.

This paper describes the use of these two complementary and alternative techniques for the simultaneous determination of urinary PGA and MA. The methods presented here omit the liquid–liquid extraction step, and allow the direct injection of urine after a simple dilution in acetonitrile. The agreement between both methods was evaluated, and the advantages and drawbacks of each method are discussed.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical-reagent grade. Formic acid and perchloric acid were obtained from Merck (Nogent-sur-Marne, France), and buffer reagent (sodium formiate) was purchased from Fluka (St. Quentin-Fallavier, France). Water was purified by passage through a Millipore Milli-Q water treatment system. Organic solvents (acetonitrile, methanol, ethanol and isopropanol) of chromatographic grade were obtained from Merck. Carbon dioxide was of high purity (quality N45, Air Liquide, Paris, France). PGA, MA, hippuric acid (HA) and phthalic acid were obtained from Aldrich Chemicals (St. Quentin-Fallavier, France).

2.2. Equipment and chromatographic analyses

The quantitation of metabolites in human urine was performed by capillary electrophoresis and supercritical chromatography.

The CE apparatus, manufactured commercially by Thermo Separation Products, consisted of a modular system (SpectraPhoresis 100, ex Prime Vision system from Europhor, Toulouse, France), including a manual sampler that can be used to introduce the sample in hydrodynamic or electrokinetic mode, a regulated high-voltage power supply, and a variable-wavelength UV detector. The output signal was recorded with a computer (Ambra, Europhor) or a recorder (Kipp and Zonen, Touzart et Matignon, Vitry-sur-Seine, France). The CE column was a C_8 bonded-silica capillary (CELect-H175, Supelco Separation Technologies, St. Quentin-Fallavier, France) of 75 μm I.D., 363 μm O.D., and 80 cm total length.

Experiments were carried out in the anodic mode (cathode at the inlet) and at ambient temperature, with a constant voltage of 15 kV being applied. The electrolyte system consisted of a mixture (90:10, v/v) of a sodium formiate buffer ($4 \cdot 10^{-2} M$), adjusted to pH 3.7 with formic acid, and of isopropanol. The electric current was approximately 34–40 μA. Samples were introduced into the capillary by suction using a weak vacuum for 4 s. The wavelengths of the UV detector were either 255 or 210 nm. Between two successive injections, the capillary was washed with running buffer during 3 min. The cathodic buffer vial was changed every four runs.

For the analysis of urinary PGA and MA in an unknown sample, the retention time was compared with that of an external standard, and the method of peak-area measurement was used for quantitative assessment. The analysis of each sample was completed within ca. 30 min.

The SFC system consisted of two HPLC chromatographic pumps (Shimadzu LC9 A, Touzart et Matignon). The first one was modified for SFC with pump-heads cooled at 5°C. The second was used for

the distribution of the organic modifier. Sample injection was performed with a manual valve (Rheodyne Model 7125, Touzart et Matignon) equipped with a 20-μl sample loop. Temperature was maintained with a column thermostat (Spark Holland SpH 99, Milton Roy, Paris, France), and pressure was controlled using a manually adjustable back-pressure regulator (Tescom Model 26-1722-24-084, Milton Roy) located downstream of the UV detector (Milton Roy Model SM 4000) equipped with an high pressure cell. The output signal was recorded with an integrator (Milton Roy CI 4100) or a recorder (Kipp and Zonen). The analytical column, a 150 × 3.2 mm I.D. stainless-steel tube filled with LiChrosorb Diol 5 μM (Merck) or Rosil Polyol (Research Separations Laboratories, Eke, Belgium), was packed in our laboratory at $4 \cdot 10^7$ Pa; a mixture of 2-propanol–95% ethanol–toluene (1:1:1, v/v) was used as the slurry solvent, and methanol followed by water was used as the displacement liquid. The column and back pressure regulator temperature was 30°C. The liquid CO_2 flow-rate was 1.75 ml/min at 5°C, and the organic modifier (methanol–water–methanesulphonic acid; 95:5:0.01; v/v) flow-rate was 0.2 ml/min. The outlet pressure was regulated at $1.25 \cdot 10^7$ Pa, which corresponded to an inlet pressure of $1.4 \cdot 10^7$ Pa. Detection was performed at 255 nm and 210 nm.

As for CE, the method of external standardization was used, but peak-height measurement was chosen for quantitative assessment. The analysis of each sample was completed within about 12 min.

2.3. Urine collection and pretreatment

The urine samples were collected in polyethylene bottles and refrigerated. If urine samples were not analysed within 48 h after collection, they were aliquoted and kept frozen at −20°C. For CE analysis, 0.1 ml human urine was diluted ten-fold in acetonitrile, the mixture was vortex-mixed for 0.5 min, and centrifuged at 2000 *g* for 1 min. The acetonitrile (0.5 ml) was transferred from the microcentrifuge tube to a vial used for the CE injection.

For SFC analysis, two pretreatments were performed: the first was the same as used for CE, with the exception of acetonitrile which was acidified (0.04 *M* HCl); the second consisted of a "salting out" pretreatment of urinary sample prior to injection onto the chromatographic system in order to reduce the introduction of undesirable compounds into the column. The "salting out" procedure was performed as follows: after the dilution of the urinary sample with acidified acetonitrile (1:10) as previously described, an excess of sodium chloride was added (about 25 mg for 0.1 ml of urine), and the mixture was vortex-mixed briefly (0.5 min) and centrifuged at 2000 *g* for 1 min. The acetonitrile layer was directly injected onto the SFC column.

2.4. Standards preparation

Stock solutions of PGA and MA were initially dissolved in a mixture of acetonitrile–water (1:9, v/v) and further diluted with artificial urine prepared following the method of Wielders and Mink [23]. The standard concentrations ranged from 1.5 mg/l to 375 mg/l for PGA and 4.5 mg/l to 1125 mg/l for MA. Before their injection onto the chromatographic systems, these standards were dissolved in acetonitrile for CE analysis, and acidified acetonitrile for SFC analysis, shaken and centrifuged in the same way as for urine samples.

2.5. Creatinine determination

Jaffé colorimetric determinations were performed on a Cobas-Bio Analyser (Roche Instruments, Neuilly-sur-Seine, France), and based on a kinetic method.

3. Results and discussion

3.1. Capillary electrophoresis

Among the CE-based methods currently available for the determination of aromatic carboxylic acids, the published chromatographic conditions were generally not satisfactory for the screening of metabolites in human urine of unexposed subjects. The main reasons were that the limit of quantitation was not low enough, and that there was a lack of specificity particularly in the case of "concentrated" urines. Consequently, a new CE method was developed on the basis of our previous results, using an alkyl bonded fused-silica capillary as the separation col-

umn, with 40 m*M* sodium formiate buffer at pH 3.6–3.8. With the bonded capillary, PGA and MA migrate toward the anode before the electroosmotic flow. This electroosmotic flow was also reversed, and injection was carried out at the cathodic electrode. The pH dependence of separation and retention time (t_R) of solutes was examined over the pH range 3.0–4.0. It was observed that optimum resolution was obtained at ca. pH 3.7 with isopropanol as an organic modifier. PGA ($t_R \approx 15$ min) was eluted faster than both MA ($t_R \approx 22$ min) and HA (hippuric acid, $t_R \approx 28$ min); the latter being a known endogenous compound of urine, with high-level concentrations in the range of 0.5 to 1.5 g/g creatinine. So, HA can be used to provide information or as a comparison point with regard to the respective migration times of these acids. The migration times of carboxylic acids increased with a decrease of pH (pH range, 3.0–4.0), and with an increase in the organic modifier concentration, and decreased in the opposite cases. This effect was greater for MA and HA than for PGA, probably due to their higher pK_a values.

Peak-area measurements, normalized by dividing them by their migration time, were used for quantitative peak analysis. The concentrations were determined by external standardization. Peak-height measurement gave erroneous results due to sample matrix effects on the peak width or solute overload. This was observed during the definition of the calibration curves as the solute standard concentration was increased.

The linearity of the method using peak-area measurement was evaluated between 1 and 200 mg/l. The corresponding regression equations are $y = -207 + 569.9x$ for PGA, and $y = -397 + 1236.2x$ for MA, with a correlation coefficient greater than 0.999 for the two compounds. However, when peak-height measurement was used, the method was linear only between 0.1 and 25 mg/l for both compounds. Peak-height measurement was therefore preferred to peak-area measurement for trace determination, when concentrations of metabolites in urine were lower than 25 mg/g creatinine (creatinine 1 g/l), and especially for PGA.

It is known that the capillary temperature greatly affects electrophoresis results. As the instrument used in this study had no temperature control system, except for forced cooling of the capillary by a fan, the influence of the temperature of the laboratory on the repeatability of analysis was investigated. The results obtained with a standard solution with air temperature variation of 8°C in the laboratory for a period of 8 h, showed that the migration time was greatly affected (C.V. = 10%, $n = 8$). In contrast, results obtained in a low fluctuating air temperature (23±1°C) showed higher repeatability (C.V. = 3%, $n = 8$).

Phthalic acid ($t_R \approx 19$ min), was shown to be a suitable internal standard for the correction of any variation in migration times, and was used to reduce instrumental and injection bias. PGA and MA were detected in the same run with shifting wavelengths at 255 nm and 210 nm, respectively.

The minimum detection limits for the determination of PGA and MA standards were 0.10 and 0.15 mg/l ($S/N > 3$), respectively. For ten-fold diluted human urine, the quantitation limit was estimated to be 1.0 and 1.5 mg/g creatinine (creatinine 1 g/l) ($S/N > 3$). To check the specificity of the method, measurements of urinary PGA and MA were performed on the urine of workers who had not been exposed to styrene or ethylbenzene. The background concentration of PGA and MA in the urine of 79 persons ranged from 0.2 mg/g to 11.7 mg/g creatinine and 0.3 mg/g to 37.2 mg/g creatinine, respectively, with an arithmetic mean of 1 mg/g (S.D. = 1.8) and 1.4 mg/g creatinine (S.D. = 4.2). These values are in agreement with those found in the literature [7,10].

Various amounts of PGA and MA were added to urine samples. The recovery of the compounds from the spiked urine samples, ranging from 95.0 to 103.3%, is shown in Table 1.

Fig. 1 shows the electropherograms of PGA and MA standards, a blank human urine and spiked urine samples with PGA (150 and 15 mg/l) and MA (450 and 45 mg/l). These values approximately correspond to the lowest BEI (urinary creatinine = 1.54 g/l) for urine samples collected on the morning after exposure, and one tenth of these values, respectively. Fig. 1b and Fig. 1c show the electropherograms obtained using the analytical conditions for the limit of quantitation of the technique. Fig. 1d shows the urine fingerprint with PGA and MA concentrations close to the values of BEIs; the marked emergence of

Table 1
PGA and MA recovery from spiked blank urine samples ($n=5$)

Concentration (mg/l)	Recovery (%)		S.D. (%)	
	CE	SFC	CE	SFC
PGA				
15	97	92	3.2	1.1
30	95	96	2.8	1.1
150	95	98	2.9	0.5
300	98	94	5.1	0.8
MA				
45	103	96	3.1	1.2
90	99	98	7.5	1.8
450	98	98	3.5	1.2
900	97	97	5.6	1.3

the peaks of these acids compared to other compounds in the urine can be seen clearly.

Surprisingly, the bonded capillary columns were shown to be particularly stable. Up to two thousand injections, using 1300–1400 urine samples, were performed with the same column without loss of resolution. A reduction of retention time (about 20%) occurred progressively after one year of use. The great disadvantage of this type of capillary is the very long stabilization time (up to 48 h). The capillary was conditioned by making successive runs with the running buffer until the drift of the baseline became acceptable at 210 nm (less than 10^{-3} AU/h), in order to quantify MA. The retention times were similar between two C_8 bonded capillaries, and a decrease in the retention times of the studied compounds was observed with the use of a shorter alkyl chain length of the bonded capillary, i.e. C_1 bonded capillary.

3.2. Supercritical fluid chromatography

In order to confirm the results obtained with the CE method, the use of a complementary technique was envisaged. Based on previous work performed in our laboratory [22], SFC was evaluated as a potential alternative separation technique for the

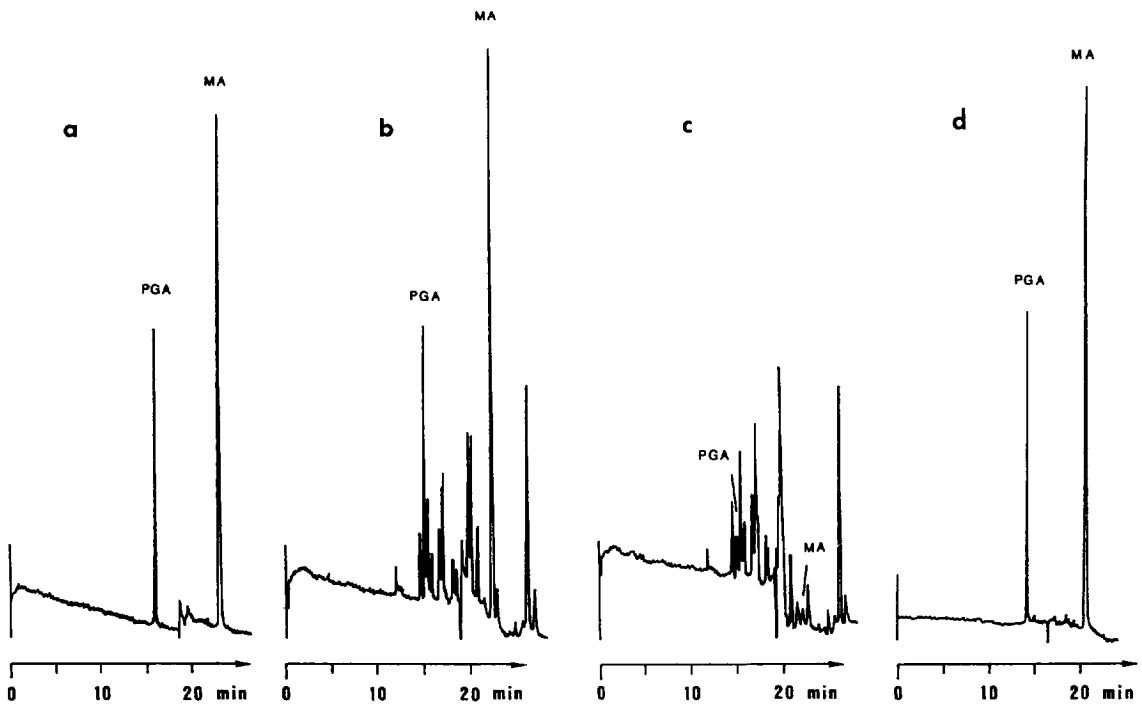

Fig. 1. Electropherograms of: (a) standard containing 1.5 mg/l of PGA and 4.5 mg/l of MA; (b) urine sample spiked with 15 mg/l of PGA and 45 mg/l of MA and then ten-fold diluted; (c) blank urine sample ten-fold diluted, 1.7 mg/l of PGA and 1.6 mg/l of MA; (d) urine sample spiked with 150 mg/l of PGA and 450 mg/l of MA and then ten-fold diluted, attenuation ×12. Electrophoretic conditions are detailed in Section 2. UV: absorbance range 0.005 full scan; $t=0$ min, $\lambda=255$ nm; $t=19$ min, $\lambda=210$ nm.

analysis of urinary aromatic acids. During preliminary assays, PGA, MA and HA were effectively separated by SFC with a CO_2 mobile phase modified with aliphatic alcohols. But, the acid solutes exhibited poor peak shapes, primarily due to solute ionization. Berger and Deye [24,25] were able to prevent this peak distortion by suppressing solute ionization through the use of coeluents modified with low concentrations of acidic additives, such as citric or trifluoroacetic acid. Nevertheless these additives turned out to be unusable for our application due to their non-negligible UV absorbance at 210 nm, which debases the limit of detection by increasing the background and noise. Consequently, methanesulphonic acid was chosen to increase the eluent strength and to cover the active sites of the HPLC phase.

Methanol, ethanol or isopropanol can be used interchangeably as the organic modifier, the retention time of solutes becoming slightly longer with an increase of the chain length. Nevertheless, isopropanol was preferred during the analysis of blank urines because interferences were minimized. PGA ($t_R \approx 1.9$ min) was eluted faster than both MA ($t_R \approx 4.8$ min) and HA ($t_R \approx 12$ min), and the migration time of carboxylic acids became longer with a decrease in the concentration of the organic modifier.

The results of quantitative analyses obtained using peak-area and peak-height measurements are in agreement, but peak-height measurements were generally used for the determination of the concentrations by external standardization. The linearity of the method was evaluated between 1.5 and 375 mg/l for PGA and 4.5 and 1125 mg/l for MA with peak-height response. The resulting regression equations are $y = 1.037 + 0.221x$ for PGA and $y = -6.221 + 1.167x$ for MA, with a correlation coefficient greater than 0.999 for both compounds.

The minimum detection limits for the determination of PGA and MA standards were 0.07 and 0.4 mg/l ($S/N > 3$) respectively; and for a human urine diluted ten-fold, the quantitation limits were estimated as 0.7 and 4 mg/g creatinine (creatinine 1 g/l) ($S/N > 3$).

To check the specificity of the method, measurements of urinary MA and PGA were performed on the urine of workers not exposed to styrene or ethylbenzene. A total of 62 urine samples was analysed, and the PGA and MA background levels ranged from 0.2 mg/g to 2 mg/g creatinine and 1.2 mg/g to 13 mg/g creatinine, respectively, with arithmetic means of 0.6 mg/g (S.D. = 0.4) and 5.3 mg/g (S.D. = 2.1) creatinine.

Various quantities of PGA and MA were added to urine samples. The recovery of the compounds from these spiked urine samples ranged from 91.7 to 98.2% (Table 1). The concentrations were determined by external standardization, with and without "salting out" pretreatment. No internal standardization method was performed because of the difficulty of finding an appropriate internal standard. "Salting out" pretreatment of a urinary sample was performed prior to injection onto the chromatographic system in order to restrict the formation of a layer of salts and adsorption of polar urinary compounds onto the stationary phase, and also to protect the column and increase its lifetime. The procedure is easy and fast. PGA and MA losses were less than 5 and 2%, respectively, in comparison to the simple acetonitrile dilution procedure.

Fig. 2 shows the SFC chromatograms corresponding to the same samples as Fig. 1. Fig. 2b and Fig. 2c show the chromatograms obtained using the analytical conditions for the limit of quantitation of the technique. Fig. 2d shows the urine fingerprint with PGA and MA concentrations close to the values of BEIs; the marked emergence of the peaks of these acids compared to other compounds in urine can be seen clearly .

Various HPLC phases were tested: silica, diol, dimethylamino, nitro and cyano phases. The best compromise in terms of both retention time and resolution was obtained with the silica and diol phases. Under the same analytical conditions, little difference in the retention time was observed between diol bonded phases obtained from various manufacturers.

3.3. Comparison of both techniques

For the two techniques, the within-day and between-day precision was established on a sample of pooled urine (0.85 g/l creatinine) to which PGA (15, 30, 150 and 300 mg/l) and MA (45, 90, 450 and 900 mg/l) were added. The average values, the standard deviations and the variation coefficients are reported

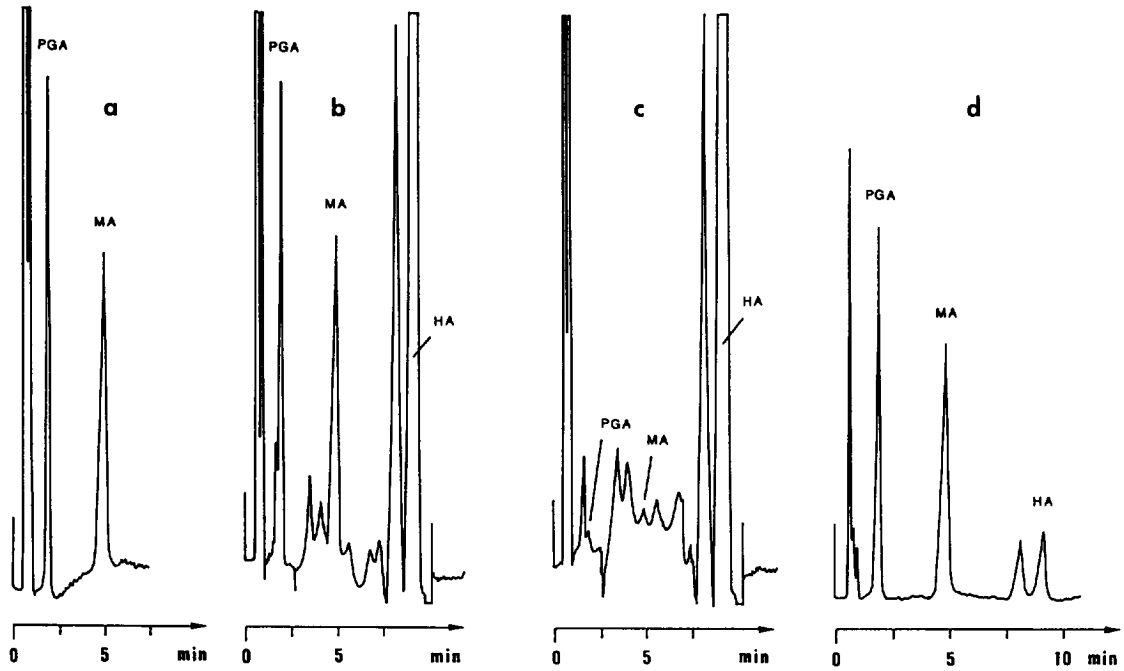

Fig. 2. SFC chromatograms of the same samples as in Fig. 1: (a) standard of 1.5 mg/l of PGA and 4.5 mg/l of MA (b) urine sample spiked with, 15 mg/l of PGA and 45 mg/l of MA and then ten-fold diluted; (c) blank urine sample ten-fold diluted, less than 0.6 mg/l of PGA and 3 mg/l of MA; (d) urine sample spiked with 150 mg/l of PGA and 450 mg/l of MA and then ten-fold diluted, attenuation ×12. Chromatographic conditions are detailed in Section 2. UV: absorbance range 0.01 full scan; t=0 min, λ=255 nm; t=2.5 min, λ=210 nm; t=7.5 min, λ=230 nm.

in Table 2. Variation coefficients lower than 10% were obtained with both methods.

In order to compare the CE method with the SFC method, 167 urine samples from workers exposed to styrene during their occupation were analysed by both techniques. Results are compared by using product–moment correlation (r). Fig. 3 and Fig. 4 show plots of the results of the CE method versus

Table 2
Within-day and between-day variation at four concentration levels of urinary samples spiked with PGA and MA

Concentration (mg/l)	CE						SFC					
	Within-day (n=5)			Between-day (n=10)			Within-day (n=5)			Between-day (n=10)		
	$\bar{x}$ (mg/l)	S.D. (mg/l)	C.V. (%)	$\bar{x}$ (mg/l)	S.D. (mg/l)	C.V. (%)	$\bar{x}$ (mg/l)	S.D. (mg/l)	C.V. (%)	$\bar{x}$ (mg/l)	S.D. (mg/l)	C.V. (%)
PGA												
15	14.5	0.5	3.5	13.2	1.6	12.0	13.5	0.2	1.2	12.7	0.9	7.0
30	28.5	0.8	2.8	26.9	1.9	7.0	27.8	1.1	4.0	25.9	2.2	8.5
150	143	4.4	3.0	137	7.6	5.5	139	4.2	3.0	140	3.1	2.2
300	293	15.2	5.2	298	15.5	5.2	272	5.2	1.9	277	6.4	2.3
MA												
45	46.5	1.4	3.1	45.4	2.6	5.7	44.6	0.5	1.1	45.1	2.2	4.9
90	88.7	6.8	7.7	89.4	4.8	5.4	88.4	1.3	1.5	88.9	1.7	1.9
450	450	17.6	3.9	435	17/.1	3.9	441	5.7	1.3	449	9.8	2.1
900	877	50.1	5.7	887	42.9	4.8	876	11.7	1.3	886	13.6	1.5

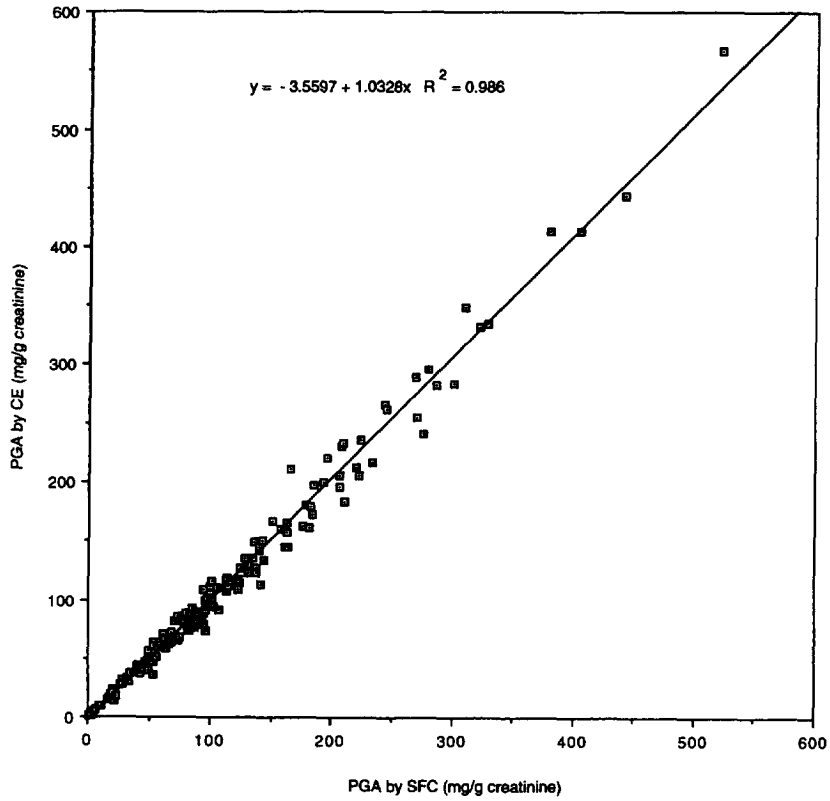

Fig. 3. Comparison of PGA measurements of 162 urine samples by CE and SFC methods.

results of the SFC method for PGA and MA, respectively. Correlation coefficients are greater than 0.993 for the two metabolites in a concentration range of 2 to 570 mg/g creatinine for PGA, and 5 to 1890 mg/g creatinine for MA. A small "rotational" bias is observed for the two compounds due to a difference between the standard solutions which were made independently by two analysts. The higher C.V.s found for the CE method can be attributed to the weaker precision of the CE technique. The within-day and the between-day precision of the solely hydrodynamic injection step and stability of separation, assessed by repeated analysis of standards, was respectively less than 4% and 8%. These results were implicitly included in the data of Table 1 and Table 2.

An analysis of the same blank urine sample, particularly "concentrated", was performed by CE, SFC and HPLC using the analytical conditions for the quantitation limit of each technique. The results are shown in Fig. 5. HPLC analysis was performed following conditions described by Mürer et al. [10], with the exception of the UV detector, which was not a diode array, but rather a simple variable-wavelength UV detector. If the HPLC method is well suited for styrene exposure assessment, the quantitation of MA is difficult below the limit of detection (about 10 mg/l according to the data of Mürer et al.) with a simple variable-wavelength UV detector. CE and SFC, the most efficient techniques, were shown to be complementary. The quantitation limit of MA is better using CE than SFC, and conversely for PGA. Due to the rapidity with which the results are obtained by SFC, less than 5 min for PGA and MA, this technique should preferably be used when relatively high styrene concentrations are expected [exceeding about 40 mg/m^3 in the atmosphere, which is one fifth of the French atmospheric styrene

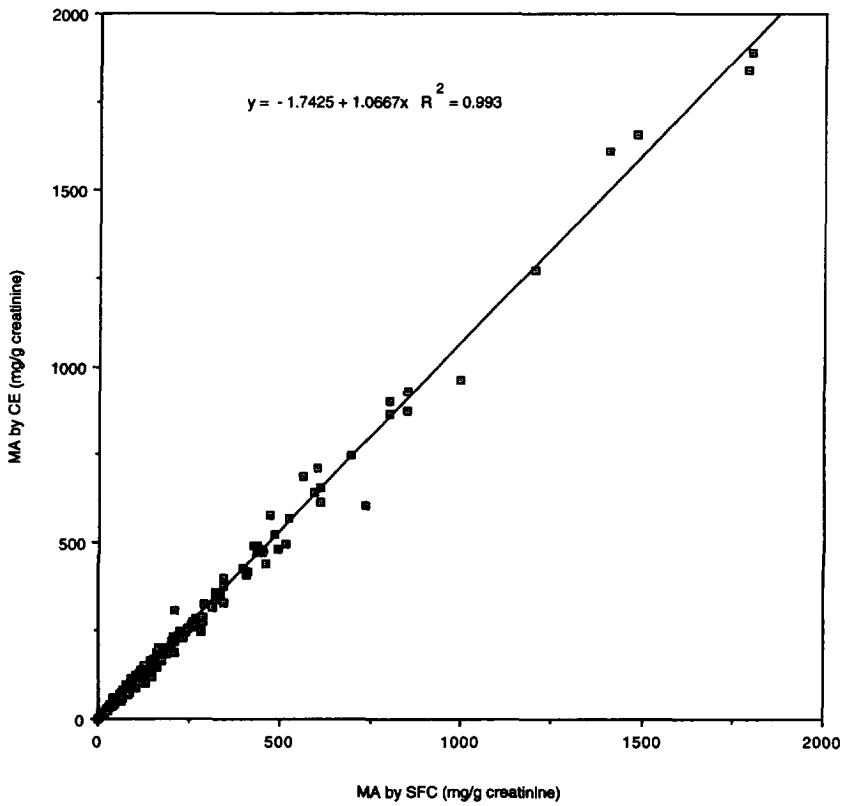

Fig. 4. Comparison of MA measurements of 162 urine samples by CE and SFC methods.

Valeur Moyenne d'Exposition (VME)]. Considering the selectivity and the sensitivity of CE, and most particularly for MA, this technique could be used for assessment of exposure to low airborne styrene concentrations (i.e. environmental concentrations), or when it is necessary to confirm the results obtained with SFC.

4. Conclusion

Due to the values of the biological exposure index of PGA and MA, and the UV absorbance of these substances, concentration levels as low as one twentieth can easily be evaluated with the two methods proposed in this work. The results of this study showed that SFC and CE are in agreement for the analysis of PGA and MA, and confirmed that CE is an attractive technique for biological monitoring of exposure to industrial chemicals. Further investigations are in progress to extend the use of this technique in the field of industrial hygiene to the screening of other solvent metabolites.

Acknowledgments

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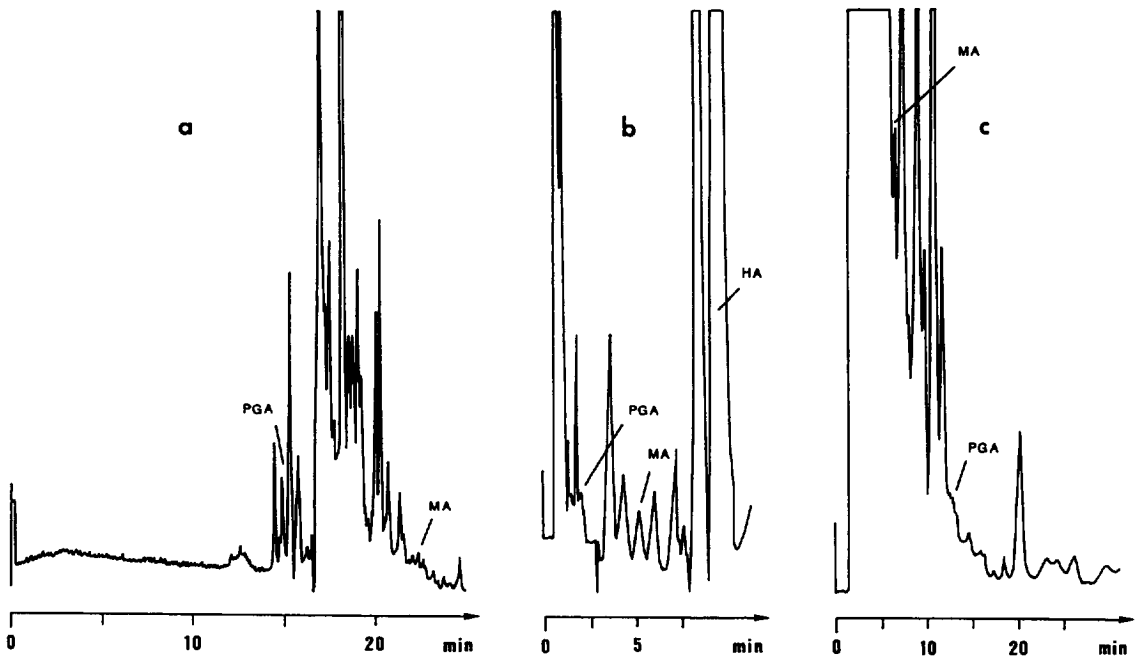

Fig. 5. Comparison between an electropherogram: (a) (2.4mg/l of PGA 1.3 mg/l of MA), a SFC chromatogram; (b) less than 1.5 mg/l of PGA, 8.0 mg/l of MA) and a HPLC chromatogram; (c) (less than 4.5 mg/l of PGA, MA not quantifiable below the limit of detection, 10 mg/l) of the same blank urine sample (dilution 1:10) obtained following the analytical conditions used for the determination of the respective limit of quantitation of each technique. For (a) and (b), electrophoretic and chromatographic conditions are the same as in Fig. 1 and Fig. 2, and for (c), HPLC chromatographic conditions were those used by Mürer et al. [10], with the exception of the UV detector.

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