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Determination of naphthalene metabolites in human urine by liquid chromatography–mass spectrometry with electrospray ionization

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Abstract

The use of a liquid chromatography–electrospray mass spectrometry system was investigated for the quantitative analysis of naphthalene metabolites (α-naphthol, α-naphthylglucuronide and β-naphthylsulphate) in untreated urine samples. Chromatography was carried out under ion-suppressed reversed-phase conditions, by using high-speed (3 cm, 3 μm) columns and formic acid (2 m*M*) as a modifier in the mobile phase. The ionization was obtained in the negative-ion mode. Linearity, sensitivity and precision of the method were explored by operating in selected-ion monitoring mode. The method was applied to the quantitative analysis of naphthalene metabolites in untreated urine samples from workers in a naphthalene producing plant. Solid-phase extraction was used for sample clean-up and trace enrichment. Liquid chromatography–tandem mass spectrometry experiments were performed for confirmation purposes. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Naphthalene is a polycyclic aromatic hydrocarbon widely used as an intermediate in chemical and plastics industry, as well as in the manufacture of insecticides and fungicides. Occupational exposure to naphthalene is reported in the case of processing or handling of coal tar and mineral oil base products [1–3]. Moreover, naphthalene is the most abundant compound in creosote vapor [4], being the 10–16% (w/w) of creosote oil (used as wood preservative). Since household mothballs contain naphthalene, suspected cases of poisoning of infants have been reported [5]. High levels of naphthalene metabolites were found in the urine of psoriatic patients treated with coal tar pitch [6]. Due to its high volatility, naphthalene uptake occurs mostly via inhalation; the skin is another important route of uptake.

Naphthalene is metabolized by microsomal enzymes to naphthols and dihydrodiols, via the formation of an unstable epoxide [7]. 1,2-Dihydrodiol and α-naphthol were found to be metabolism products of human liver microsomes in vitro [8]. Studies in rats on the metabolism and excretion of naphthalene administered per os showed that it is excreted in the urine as dihydrodiol (80%) and α-naphthol or its glucuronide (4.6%) [9]. In human volunteers, excretion of α-naphthol was about 6.3–8.5% of the inhalation uptake of naphthalene [2]. Radiolabeled

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[^{14}C]naphthylsulphate and [^{14}C]naphthylglucuronide were found in the urine from mice treated with α-[^{14}C]naphthol [10]. Urinary α-naphthol has been proposed as a biomarker of occupational exposure to naphthalene [3], creosote [4] and the insecticide carbaryl [11]; recently, α-naphthol in combination with 1-hydroxypyrene has been proposed for the biological monitoring of exposure to complex mixtures of polycyclic aromatic hydrocarbons [2].

The analytical methods reported in the literature for the determination of α-naphthol in urine of workers exposed to naphthalene make use of chromatographic techniques, mainly capillary gas chromatography (GC) [4,12,13] or high-performance liquid chromatography (HPLC) with fluorescence detection (FLD) [14]. In both cases, a preliminary step, consisting of enzymatic hydrolysis (β-glucuronidase/arylsulphatase) [4] or/and acid (HCl, 100°C) hydrolysis [4,13] followed by liquid–liquid extraction is required prior to the analysis. In the case of HPLC–FLD procedure [14], a very critical step appears to be the extraction of 10 ml cyclohexane–isopropylalcohol with 300 μl of NaOH. When GC with electron-capture detection (ECD) or mass spectrometric (MS) detection are used, a further derivatization step is necessary [4,13]. All these sample manipulations make the analytical procedure difficult and time-consuming.

In the present work, a new method for the direct and simultaneous determination of naphthalene metabolites, i.e., α-naphthol, α-naphthylglucuronide and β-naphthylsulphate, is described. An HPLC–MS system with pneumatically assisted electrospray interface (ESI) was used for this purpose. Liquid chromatography was performed under ion-suppressed reversed-phase conditions, by using high speed (3 cm, 3 μm) columns and a mobile phase consisting of water–methanol in gradient elution mode. In order to suppress the ionization of the analytes, a concentration of 2 m*M* of formic acid was added to the eluent. The ionization of α-naphthol and the conjugated compounds was obtained in negative-ion mode. Off-line solid-phase extraction (SPE) was used for sample clean-up and trace enrichment. The specificity and selectivity of tandem-mass spectrometry (MS–MS) were used for confirmation purposes.

2. Experimental

2.1. Materials

α-Naphthol, α-naphthyl-β-D-glucuronide and β-naphthylsulphate were obtained by Sigma (Milan, Italy). All chemicals were of analytical-reagent grade and were used without further purification. Analytical solutions of all the compounds were prepared in 50% (v/v) aqueous methanol. Stock solutions stored in the dark at 4°C are stable for at least 1 month. HPLC-grade water and methanol were from Lab-Scan (Dublin, Ireland). Solvents were degassed before the use. Analytical grade formic acid, acetic acid and ammonium acetate were supplied by Carlo Erba (Milan, Italy). β-Glucuronidase/arylsulphatase from *Helix pomatia* was purchased from Boehringer Mannheim (Monza, Italy).

2.2. Sample collection and preparation

Fifteen urine samples from workers of a naphthalene producing plant were collected at the beginning (pre-shift) and at the end of the work week (end-of-shift) and frozen at −20°C prior LC–MS analysis. The sampling strategy was chosen according to the toxicokinetic properties of naphthalene. Basal urine samples were collected from occupationally non-exposed subjects, non-smokers ($n=13$). Samples were ultrafiltered on Centricon-10 centrifugal concentrators (Amicon, Beverly, MA, USA) and injected into the LC–MS system after acidification with 0.1 *M* formic acid.

Trace enrichment was performed by SPE using Bakerbond octadecyl C_{18} cartridges (J.T. Baker, Deventer, Netherlands) preconditioned with 1.0 ml of methanol and 2.0 ml of 2 m*M* aqueous formic acid. After acidification with 0.1 *M* formic acid, urine samples (2.0 ml) were loaded on the SPE cartridge and washed with 4.0 ml of 2 m*M* aqueous formic acid. Elution was obtained with 1.0 ml of methanol acidified with formic acid (2 m*M*). After evaporation to dryness with a nitrogen stream, samples were reconstructed with 0.5 ml of water–methanol (50:50, v/v). Samples were acidified with formic acid (0.1 *M*) and then 20 μl were injected in duplicate in the LC–MS system.

Several samples were hydrolyzed in order to convert conjugated metabolites into α-naphthol: 2.0 ml of urine were mixed with 4.0 ml of 0.5 *M* acetate buffer, pH 5.0, and 20 μl of enzyme (β-glucuronidase/arylsulphatase). The mixture was incubated for 24 h in a waterbath at 37°C, cooled to room temperature and applied to SPE as described above. Then 20 μl of the final eluate acidified with formic acid (0.1 *M*) were injected in the LC–MS system.

2.3. Liquid chromatography–mass spectrometry

LC–MS analyses were carried out on a PE-Sciex API 100 single-quadrupole mass spectrometer (Sciex, Tornhill, Canada) equipped with an atmospheric pressure ionization (API) source and an ionspray interface for pneumatically assisted electrospray. LC–MS–MS experiments were performed on a PE-Sciex API 365 triple-quadrupole mass spectrometer. The liquid chromatographic system consisted of a Perkin-Elmer series 200 dual solvent delivery system (Norwalk, CT, USA) equipped with an ASPEC XL Autosampler (Gilson, Villiers-le-Bel, France). A Power Macintosh 7200/120 computer was used for instrument control, data acquisition and processing.

Naphthalene metabolites were separated using Pecosphere 3×3 C_{18} CR (3 cm×4.6 mm, 3 μm) cartridges (PE Brownlee, Norwalk, CT, USA) under gradient elution conditions with a 2 m*M* aqueous formic acid–methanol mixture at a flow-rate of 0.9 ml/min. Elution programme: 33% methanol, hold for 1 min; from 33 to 80% methanol in 3 min (linear gradient); 80% methanol, hold for 3 min. The column effluent was connected to the fused-silica transfer line (1 m×100 μm) of the ESI interface (split ratio 1:50). Ionization was obtained in the negative ion (NI) mode. Optimization of the interface parameters was performed by infusing standard solutions of the analytes. Electrospray conditions: nitrogen curtain gas, 0.63 l/min; nebulizing gas (air), 1.02 l/min; ionspray voltage, −5400 V for β-naphthylsulphate, −4200 V for α-naphthylglucuronide and α-naphthol; orifice voltage, −40 V for β-naphthylsulphate and α-naphthylglucuronide, −70 V for α-naphthol; ring voltage, −200 V; scan range, *m/z* 100–330; scan rate, 0.63 s; $w_{1/2}$ 0.6 mass unit. Acquisition in the selection ion monitoring (SIM) mode was performed by monitoring the signal of the $[M-H]^-$ ions at *m/z* 223.0, 319.0 and 143.1 for β-naphthylsulphate, α-naphthylglucuronide and α-naphthol, respectively; width 1.0; dwell time 25 ms/channel.

For confirmation purposes, product-ion spectra of the $[M-H]^-$ molecules were obtained in the 50–330 mass range (0.61 s/scan) and the collision conditions were optimized by varying the collision energy between 5 and 50 eV. Selected reaction monitoring (SRM) experiments were performed following the reactions *m/z* 223.0→143.1, characteristic of β-naphthylsulphate, 319.0→175.1 and 319.0→143.1, characteristics of α-naphthylglucuronide, and 143.1→143.1, characteristic of α-naphthol, with a dwell time of 200 ms per transition. Nitrogen was used as the collision gas. The collision energy was 17 eV.

2.4. Preparation of calibration standards

In order to evaluate the linear dynamic range, calibration standards at 0.5, 1, 5, 10, 50, 100 and 500 mg/l were prepared by spiking blank urine with appropriate volumes of standard solutions containing α-naphthol, α-naphthylglucuronide and β-naphthylsulphate to achieve the required concentration. Injections were performed in triplicate. Integration of the LC–MS SIM peaks was performed by the PE-Sciex MACQUAN processing software. Calibration curves were constructed by plotting the peak area against concentration using an unweighted linear regression model ($n=21$). The detection limits of the compounds, defined as the amount injected giving a signal-to-noise ratio of 3, were determined by direct injections of standards. Repeatability (R.S.D.) was determined for each analyte at two concentration levels (1 and 10 mg/l) from six repetitive injections. Intra-day precision was determined at two concentration levels (1 and 10 mg/l) from six repetitive injections performed three times during a working day ($n=18$). Inter-day variability was evaluated on 5 consecutive working days from the analysis of six repetitive standard injections ($n=30$) at two concentration levels (1 and 10 mg/l).

Two working calibration standard sets were pre-

pared spiking blank urine at 0.05, 0.1, 0.5 and 1.0 mg/l, one for the quantitation of the analytes by direct injection in the LC–MS system, the other after enrichment on SPE cartridges. The calibration curves obtained were used to calculate concentrations of the analytes in samples analyzed with LC–MS and SPE–LC–MS.

3. Results and discussion

3.1. LC–MS of naphthalene metabolites

Recently, we have developed a method [17] for the analysis of conjugated metabolites in untreated urine samples, which makes use of high-speed (3 cm, 3 μm) columns and ion-suppressed reversed-phase chromatography, compatible with atmospheric pressure ionization techniques, both ESI and APCI. The advantages of using 3 cm columns are exalted in combination with MS or MS–MS detection: the use of high-speed columns allow steep gradients of elution and fast equilibration times, giving high sample throughput; on the other hand, the specificity and selectivity of mass spectrometry limit the need of complete chromatographic resolution of the analytes. Complete characterization of urinary naphthalene metabolites can be obtained in a 10-min run, without any sample manipulation, simply after filtration and acidification of the urine. Under the gradient elution conditions used (for details see Section 2), β-naphthylsulphate, α-naphthylglucuronide and α-naphthol elute at 1.8, 3.0 and 5.7 min, respectively.

The background subtracted full-scan (100–330) spectra of naphthalene metabolites are shown in Fig. 1. Negative-ion electrospray ionization of these metabolites (obtained at −50 V, orifice voltage) shows abundant deprotonated molecules $[M-H]^-$, at *m/z* 223.0, 319.0 and 143.1 for β-naphthylsulphate, α-naphthylglucuronide and α-naphthol, respectively. The ion at *m/z* 143.1, attributable to the deprotonated molecule of naphthol, is common also to the spectra of glucuronide- and sulphate-conjugates, and it is produced by the cleavage of the glucuronic or the sulphate–ester bond with retention of the negative charge on the aglycone moiety. Whereas fragmentation of sulphate-conjugate occurs by means of a neutral loss of sulphur trioxide (M−80), in the case of the glucuronide-conjugate, besides the loss of a neutral glucuronide moiety (M−176), a fragment-ion at *m/z* 175.1, attributable to the negatively charged glucuronic acid, is visible. This fragment ion is diagnostic for glucuronides [17]. Fragmentation of glucuronide- and sulphate-conjugates can be enhanced by increasing the voltage applied to the orifice, as shown in Fig. 2. No fragmentation was observed in the case of α-naphthol, even at high orifice voltages. For quantitative purposes, the signal of $[M-H]^-$ ions was chosen for the acquisition in SIM.

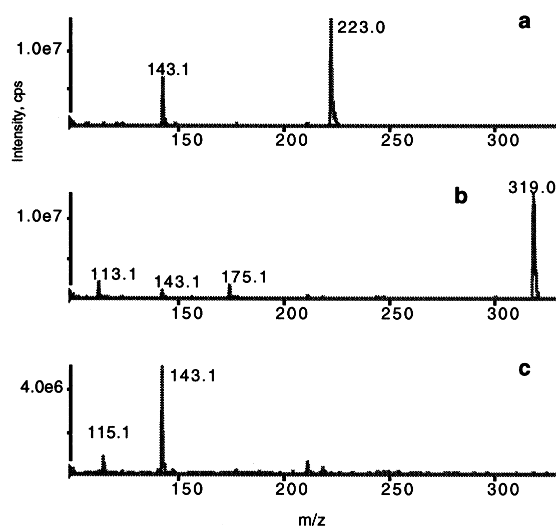

Fig. 1. Negative-ion ESI mass spectra of (a) β-naphthylsulphate, (b) α-naphthylglucuronide and (c) α-naphthol obtained in the 100–330 mass range, scan rate 0.63 s/scan.

The deprotonated molecule was chosen as precursor-ion in tandem MS experiments. The product-ion spectra of conjugates are characterized by the same fragments already observed in the case of orifice fragmentation. Even in the collision cell of a triple quadrupole, α-naphthol does not fragment without demolition of the molecule, owing to the high stability of the polycyclic aromatic structure. The effect of collision energy on the abundance of precursor ions and product ions of α-naphthylglucuronide and β-naphthylsulphate is shown in Fig. 3. For selected reaction monitoring experiments, the transitions *m/z* 223.0→143.1, characteristic of β-naphthylsulphate, 319.0→175.1 and 319.0→143.1, characteristics α-naphthylglucuronide, and

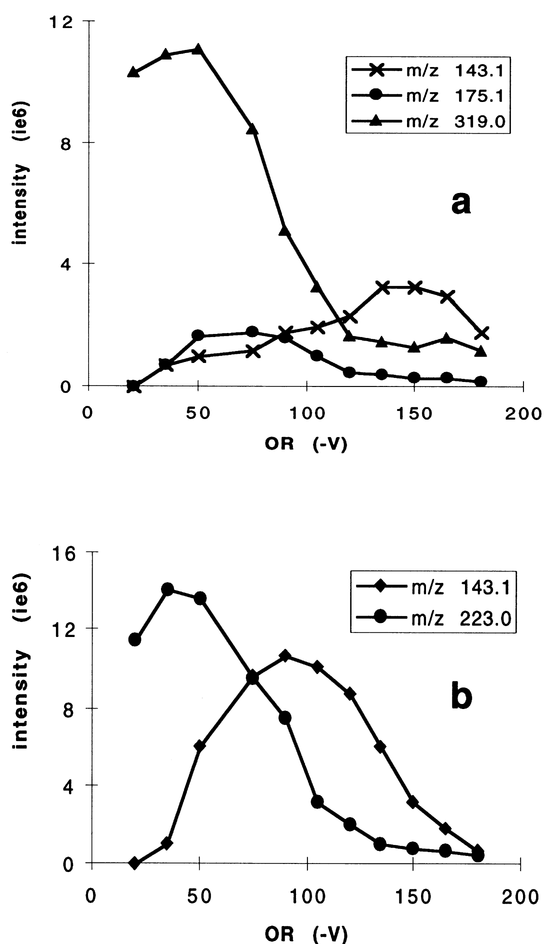

Fig. 2. Effect of the potential applied to the orifice on the intensity of the MS signal of (a) α-naphthylglucuronide and (b) β-naphthylsulphate.

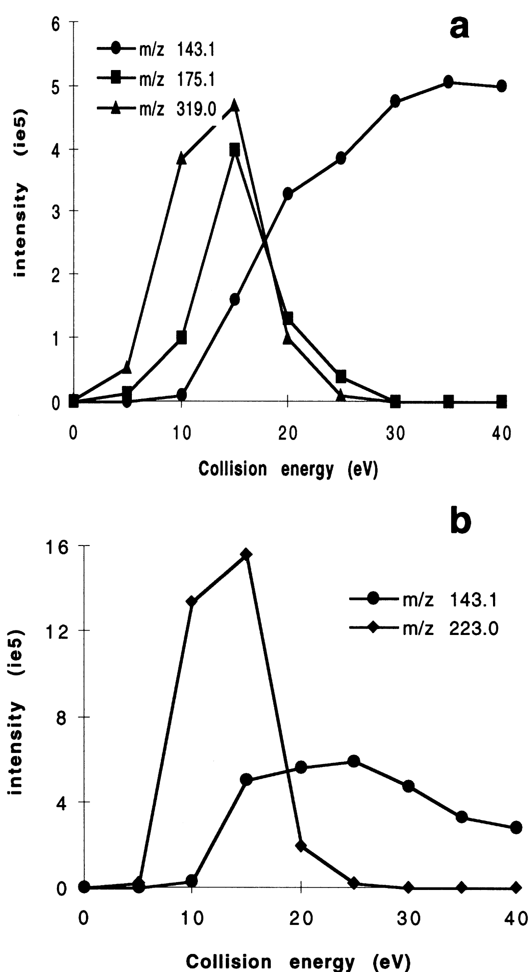

Fig. 3. Effect of the collision energy on the absolute abundance of $[M-H]^-$ (precursor) and product ions of (a) α-naphthylglucuronide (*m/z* 319.0→175.1 and *m/z* 319.0→143.1) and (b) β-naphthylsulphate (*m/z* 223.0→143.1).

143.1→143.1, characteristic of α-naphthol, were chosen.

3.2. Validation of the method

The applicability of the LC–MS method was evaluated after a careful study of the following parameters: linearity, limit of detection, precision and accuracy. The linearity of the method was explored for the three compounds, using spiked urine samples in the concentration range 0.5–500 mg/l. Regression data are reported in Table 1. Experimental data fitted a linear model, $y=a+bx$, unweighted. An excellent linearity of the method was observed, with correlation coefficients close to 1.000. The limits of detection (S/N=3), determined using the LC–MS system without any sample enrichment, were 0.1, 0.02 and 0.01 mg/l for α-naphthol, α-naphthylglucuronide and β-naphthylsulphate, respectively. Although neither biological exposure index (BEI) nor reference values have been proposed for α-naphthol, recent studies report mean values of 2.95 mg/l for subjects working at a creosote impregnation plant [4], 0.89 and 4.86 mg/l for coke plant workers (plants working with new technology and old technology, respectively) [3]. High α-naphthol concen-

Table 1
Calibration graph results for LC–ESI-MS of naphthalene metabolites[a]

Compound	Range (mg/l)	$a\cdot10^{-5}$ [b]	$b\cdot10^{-5}$ [b]	r^2	LOD[c]
α-Naphthol	0.5–500	–	0.40±0.03	0.999	0.1
α-Naphthylglucuronide	0.5–500	−1.13±0.23	4.06±0.21	0.999	0.02
β-Naphthylsulphate	0.5–500	3.75±0.81	9.01±0.03	0.999	0.01

[a] Calibration fitting: $y=a+bx$, (n=21).
[b] ± Values are confidence intervals for 95% probability level.
[c] Limit of detection (S/N=3) in mg/l under SIM conditions.

trations (0.4–34 mg/l) have been found in the urine from workers employed in the distillation of naphthalene oil [3]. A mean value of 0.12 mg/l is reported for non-exposed subjects [3], whereas other authors found concentration levels lower than 10 μg/l in the urine of occupationally non-exposed smoking referents [3,15,16]. As already observed [4], the variability of α-naphthol levels among reference groups may be due to the different analytical and pre-analytical methods used or to the different background exposure. Given these values it can be concluded that the method proposed is sensitive enough for the biological monitoring of occupational exposure to naphthalene. Moreover, the method might be useful even in the case of non-exposed subjects to determine reference values for the general population. In this case, the use of SPE appears to be useful to obtain better quantitation limits. If sample clean-up and trace enrichment require longer analysis time, the procedure can be easily automated and it allows more reliable results at low concentration of the analytes, besides granting longer column lifetime.

Repeatability, intra-day and inter-day precision, calculated as R.S.D. at two concentration levels (1 and 10 mg/l), are reported in Table 2.

Urinary concentrations of naphthalene metabolites after SPE enrichment were determined in samples using the following working calibrations: $y=(0.36+7.79x)\cdot10^5$ and: $y=(0.17+1.51x)\cdot10^6$ for α-naphthylglucuronide and β-naphthylsulphate, respectively.

3.3. Application

The method proposed was applied for the determination of free α-naphthol and naphthyl-conjugates in the urine of workers exposed to naphthalene in order to evaluate its suitability for the biological monitoring of occupational exposure to this compound. Because no reference values for α-naphthol are available in the literature, thirteen urine samples from non-exposed subjects were analyzed by LC–ESI-MS prior to and after concentration on SPE cartridges. A representative LC–MS chromatogram of a urine sample (non-smoker subject) obtained in SIM mode after SPE is shown in Fig. 4a. In the case of non-smoker subjects, trace amounts of naphthalene metabolites, β-naphthylsulphate (Fig. 4b) and α-naphthylglucuronide (Fig. 4c), were found (0.032–0.078 mg/l of total metabolites, 0.012–0.036 mg/g creatinine). Owing to the elution of many matrix interferents in the region of the chromatogram

Table 2
Repeatability, intra-day and inter-day precision of the LC–ESI-MS method, calculated at two concentration levels (1 and 10 mg/l) of urinary naphthalene metabolites

Compound	Concentration (mg/l)	Repeatability (%)	Intra-day precision (%)	Inter-day precision (%)
α-Naphthol	1	3.9	6.6	8.8
	10	2.7	5.2	7.1
α-Naphthylglucuronide	1	3.6	5.2	7.8
	10	2.2	4.3	6.3
β-Naphthylsulphate	1	2.6	5.3	7.5
	10	1.9	3.7	6.1

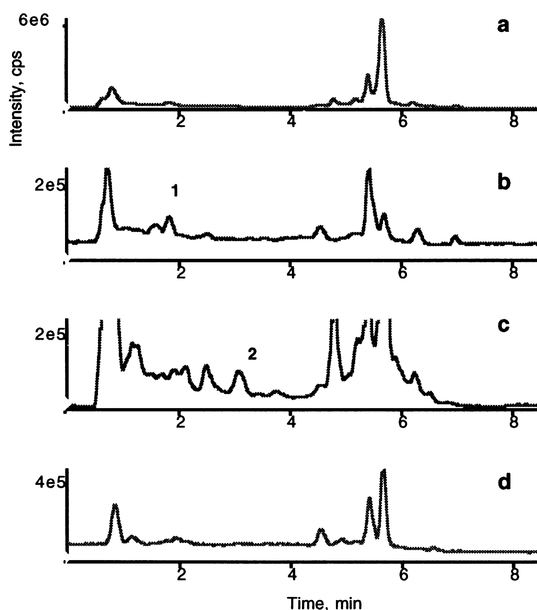

Fig. 4. (a) LC–ESI-MS SIM chromatogram of a basal urine sample from a non-exposed subject (non-smoker) after solid-phase extraction and mass chromatograms of *m*/*z* (b) 233.0, (c) 319.0 and (d) 143.1. Peaks identification: 1=β-naphthylsulphate, 2=α-naphthylglucuronide. Chromatographic conditions: see Section 2.

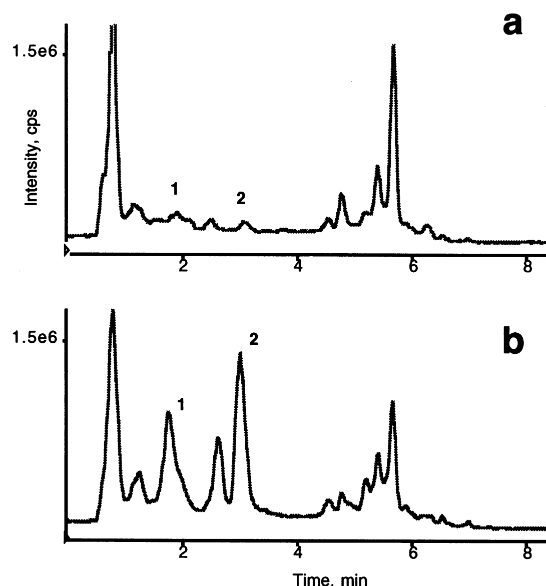

Fig. 5. LC–MS SIM chromatogram of a urine sample from a occupationally exposed subject (a) pre-shift and (b) end-of-shift sample after solid-phase extraction. Peaks: 1=β-naphthylsulphate, 2=α-naphthylglucuronide. Chromatographic conditions: see Section 2.

between 5 and 6 min, which also show fragment-ions at *m*/*z* 143.1, the LC–SIM-MS method lacks the specificity for the determination of α-naphthol in urine (Fig. 4d). This is a general problem related with the single-ion monitoring determination of low-mass analytes, for which more specificity and selectivity could be obtained using tandem MS. Nevetherless, α-naphthol is expected to be excreted as a conjugate.

To evaluate the existence of exposure-related changes in a larger group, fifteen workers of a naphthalene producing plant were recruited for this study: pre-shift (i.e., at the beginning of the week) and end-of-shift (after a 4-day shift) samples were collected and analyzed by LC–MS and SPE–LC–MS. As an example, Fig. 5 shows the LC–MS analysis of a urine sample from a worker (non-smoker) involved in the distillation of naphthalene (a) pre-shift and (b) after 4 days of exposure. From the comparison of chromatograms, it should be noticed that lower concentrations of naphthalene metabolites are detectable at the beginning of the work week with respect to the end-of-shift analysis. Both β-naphthylsulphate and α-naphthylglucuronide are easily visible in the latter chromatogram at 1.8 and 3.0 min, respectively. The peak at 2.6 min, whose *m*/*z* is 319.0, might be attributable to the β-isomer of naphthylglucuronide.

To confirm this hypothesis and to verify the specificity of the LC–MS method, SRM experiments were performed on urine samples, untreated and after SPE. Fig. 6 shows the case of a worker involved in naphthalene distillation. Separate profiles for the transitions 319.0→143.1 and 319.0→175.1 of α-naphthylglucuronide are shown and similar ratios were found for both the peaks at 2.6 and 3.0, proving that also the former peak is a glucuronide of naphthol, i.e. the β-isomer. In the case of sulphate-conjugate, only one peak was obtained for the isomers probably coeluted.

As already observed above, α-naphthol cannot be detected in single-quadrupole MS due to the lack of specificity of the method for this analyte with respect to the matrix interferent compounds. Moreover, neither in the interface (at the orifice) nor in the collision cell of the triple quadrupole fragmentation

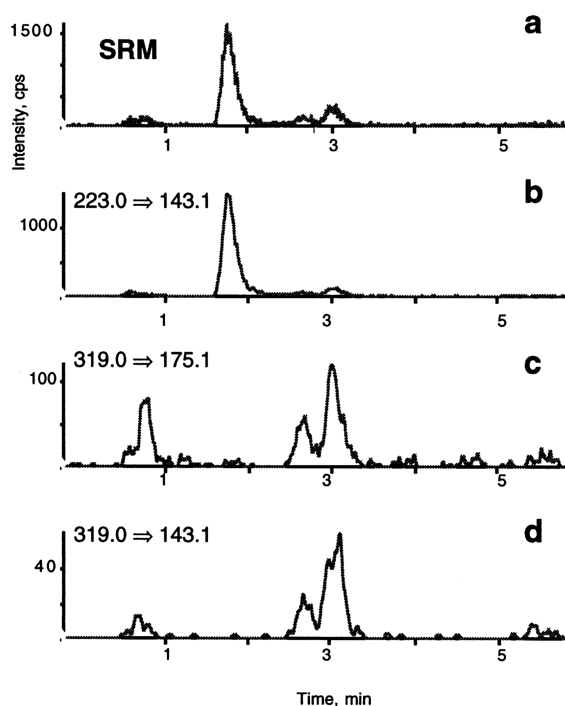

Fig. 6. (a) LC–MS–MS SRM chromatogram of a urine sample from a worker exposed to naphthalene and separate traces for the transitions, (b) *m/z* 223.0→143.1, (c) *m/z* 319.0→175.1, (d) *m/z* 319.0→143.1.

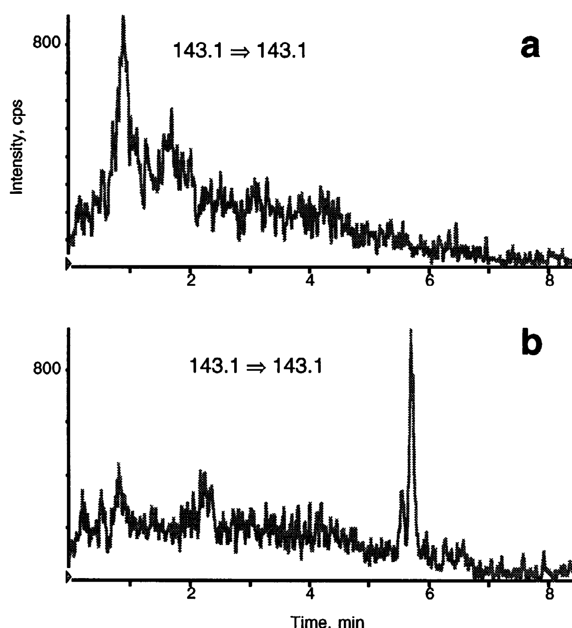

Fig. 7. LC–MS–MS SRM chromatogram (143.1→143.1) of a urine sample from a worker exposed to naphthalene (a) untreated and (b) after enzymatic hydrolysis. Largest peak=α-naphthol.

of α-naphthol could be obtained. The specificity necessary for the detection of α-naphthol could be obtained by using the collision cell as a filter, inducing the fragmentation of all the interferents but not of α-naphthol and by monitoring the 143.1→143.1 transition. Fig. 7 shows the results of this SRM experiment obtained in the case of (a) non-hydrolyzed and (b) enzymatically hydrolyzed urine sample from an occupationally exposed worker. Neither α-naphthol nor interferents are present prior to hydrolysis, whereas after hydrolysis the peak of the target analyte is visible at 5.7 min. The other peak at 5.5 min might be attributable to the other isomer, β-naphthol.

Urinary concentrations of naphthalene metabolites found in pre-shift and end-of-shift samples are summarized in Table 3. The total amount of metabolites was calculated taking into account the β-naphthylglucuronide concentration as well. No free α-naphthol was found in the non-hydrolyzed urine of the fifteen occupationally exposed subjects. Quantitative analysis performed by tandem mass spectrometry on several samples confirmed the results obtained in single-quadrupole MS. Significant increases of naphthalene metabolite concentrations ($P=.026$, paired *t*-test) were observed after exposure to naphthalene. Moreover, whereas no significant changes were observed in a sub-group of smoker subjects, non-smokers show significant changes between post-shift and pre-shift values ($P=.004$, paired *t*-test). Confounding effects due to smoking habits are reported also by other authors [13], especially in the case of low naphthalene exposure. The total concentration of metabolites in the pre-shift urine was 0.049–0.181 mg/l (0.045–0.130 mg/g creatinine) for smokers and 0.020–0.075 mg/l (0.012–0.057 mg/g creatinine) for non-smokers. At the end of the work week, the range of concentrations was 0.107–0.754 mg/l (0.075–0.503 mg/g creatinine) and 0.076–0.201 mg/l (0.032–0.157 mg/g creatinine) for smokers and non-smokers, respectively. It should be noticed that β-naphthylglucuronide is absent in the pre-shift urine of non-smoker subject.

Table 3
Urinary concentrations of naphthalene metabolites in workers exposed to airborne concentrations ranging from 0.1 to 0.7 mg/m^3 (n=15)

Compound	Urinary concentrations (mg/l)				Values expressed as a function of creatinine			
	Pre-shift		End-of-shift		Pre-shift		End-of-shift	
	Median	Range	Median	Range	Median	Range	Median	Range
β-Naphthylsulphate	0.016	0.004–0.049	0.030	0.014–0.121	0.010	0.006–0.024	0.024	0.009–0.070
β-Naphthylglucuronide	0.062	0.000–0.083	0.086	0.013–0.147	0.040	0.000–0.060	0.076	0.013–0.134
α-Naphthylglucuronide	0.058	0.006–0.089	0.084	0.021–0.448	0.035	0.003–0.056	0.053	0.021–0.299
Total	0.092	0.020–0.181	0.192	0.076–0.754	0.061	0.012–0.130	0.155	0.032–0.503

In the naphthalene plant where the present study has been carried out, exposure levels found were considerably lower than those reported in a creosote impregnation plant [4] and coke plants [3]. Naphthalene production in this plant is based on modern technology and most of the processes are done outdoors. Data gathered from an environmental monitoring programme show that airborne concentrations of naphthalene are in the range from 0.1 to 0.7 mg/m^3.

4. Conclusions

The LC–ESI-MS method shows adequate sensitivity and reliability for the quantitative analysis of naphthalene conjugated metabolites (i.e., β-naphthylsulphate and α-naphthylglucuronide) in human urine. The use of LC–ESI-MS–MS enabled demonstration of the absence of free α-naphthol in non-hydrolyzed urine and identification of unknown metabolites, like β-naphthylglucuronide. The LC–ESI-MS method, proposed for the determination of naphthyl-conjugates in untreated urine samples, is therefore suitable for the biological monitoring of occupational exposure to naphthalene, creosote and carbaryl. SPE for sample clean-up and trace enrichment is recommended when low occupational exposure occurs or reference values for the general population have to be determined.

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