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Determination of dihydroxynaphthalenes in human urine by gas chromatography–mass spectrometry

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Abstract

A gas chromatography–mass spectrometry (GC–MS) method was developed for measuring 1,2-dihydroxynaphthalene (1,2-DHN) and 1,4-dihydroxynaphthalene (1,4-DHN) in urine. The method involves enzymatic digestion of urinary conjugates to release the DHNs which were then analyzed as trimethylsilyl derivatives by GC–MS. For 1,2-DHN and 1,4-DHN, respectively, the assay limits of detection were 0.21 and 0.15 μg/l, the assay limits of quantitation were 0.69 and 0.44 μg/l, and the coefficients of variation were 14.7 and 10.9%. This method was successfully applied to determine urinary levels of 1,2-DHN and 1,4-DHN in coke workers (14 top workers and 13 side-bottom workers) and 21 matching control workers from the steel industry of northern China. The geometric mean (GM) levels of 1,2-DHN were approximately 100 and 30 times higher than those of 1,4-DHN in exposed and control subjects, respectively. The GM levels 1,2-DHN and 1,4-DHN were significantly higher for coke workers (1,2-DHN: top workers – 552 μg/l, side-bottom workers – 260 μg/l; 1,4-DHN: top workers – 3.42 μg/l, side-bottom workers – 3.56 μg/l) than for controls (1,2-DHN: 38.8 μg/l; 1,4-DHN: 1.21 μg/l) ($p \leq 0.0031$). In each exposure category, levels of the DHNs were marginally greater in smokers than in nonsmokers ($p = 0.0646$). Strong correlations were observed among 1,2-DHN and 1,4-DHN and previously measured urinary levels of naphthalene, 1-hydroxynaphthalene, and 2-hydroxynaphthalene in these subjects ($r_s \geq 0.623$; $p < 0.0001$). Also, levels of 1,2-DHN were significantly correlated with those of serum albumin adducts of l,2-naphthoquinone ($r_s = 0.492$, $p = 0.0004$). These results indicate that 1,2- and 1,4-DHN are good biomarkers for assessment of naphthalene exposure in coke workers. Since the DHNs are precursors of the naphthoquinones, which have been implicated as toxic products of naphthalene metabolism, measurements of urinary DHNs may have toxicological significance.

Keywords: 1,2-Dihydroxynaphthalene; 1,4-Dihydroxynaphthalene; Gas chromatography–mass spectrometry; Urine; Coke workers; Biomarker; Naphthalene

1. Introduction

Naphthalene is the most abundant member of the class of compounds known as polycyclic aromatic hydrocarbons (PAHs), which are produced primarily from incomplete combustion of hydrocarbon fuels [1,2]. Humans exposed to high levels of PAH mixtures have been shown to be at increased risk of cancer of the lung, skin, and bladder [3]. Although PAHs with the greatest carcinogenic potency tend to have four or more aromatic rings, naphthalene (with two rings) has produced respiratory-tract tumors in rats and mice of both sexes [4–7]. The demonstrated carcinogenicity of naphthalene in two mammalian species, coupled with the abundance of naphthalene in indoor and outdoor air (it is the only PAH to exist almost exclusively in the gas phase), recently motivated the International Agency for Research on Cancer (IARC) [8] and the U.S. Environmental Protection Agency (EPA) [9–11] to reclassify naphthalene as a possible human carcinogen.

Abbreviations: CYP, cytochrome P450; DHN, dihydroxynaphthalene; EI, electron impact; GC–MS, gas chromatography–mass spectrometry; GM, geometric mean; GSD, geometric standard deviation; LOD, limit of detection; PAH, polycyclic aromatic hydrocarbon; SIM, selected ion monitoring; TMS, trimethylsilyl

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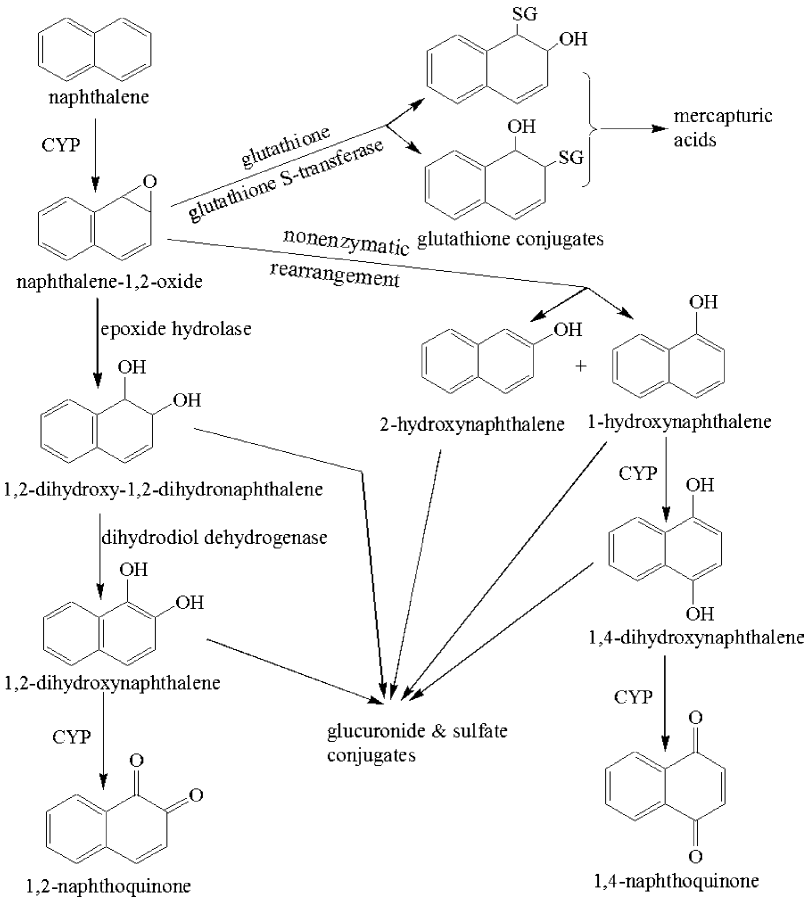

Fig. 1. Proposed metabolic pathways for naphthalene in human.

The mammalian metabolism of naphthalene is complicated [1,12]. As shown in Fig. 1, naphthalene is metabolized by various CYP enzymes (CYP 1A1, 1A2, 2A1, 2E1, 2F1, 2F2, CYP3A5, and CYP3A7) to naphthalene-1,2-oxide [7,13–15]. All other metabolites are thought to arise from naphthalene-1,2-oxide via the following pathways: spontaneous rearrangement to 1-hydroxynaphthalene and 2-hydroxynaphthalene, conjugation with glutathione and further rearrangement to the mercapturic acid, and conversion to 1,2-dihydroxy-1,2-dihydronaphthalene via epoxide hydrolase. Further CYP oxidation of 1-hydroxynaphthalene leads to 1,4-dihydroxynaphthalene (1,4-DHN) while reduction of 1,2-dihydroxy-1,2-dihydronaphthalene via dihydrodiol dehydrogenase leads to 1,2-DHN. The DHNs can be oxidized, either enzymatically or nonenzymatically, to 1,2- and 1,4-naphthoquinone, both of which are capable of binding with macromolecules [16–22].

In considering the mode of naphthalene's toxic action, attention has focused primarily upon the naphthoquinones and their precursors, the DHNs [16–22]. We have shown previously that levels of urinary naphthalene, urinary 1- and 2-hydroxynaphthalene, and albumin adducts of 1,2-naphthoquinone were all significantly increased in coke workers compared to control workers in the steel industry of northern China [2,16,23]. However, since analytical methods were not available to measure the DHNs, we could not complete our analysis of important urinary naphthalene metabolites in those samples. In the current study, we report a simple GC–MS method to quantify 1,2- and 1,4-DHN in urine and then we apply the assay to urine from the same 28 coke workers and 22 control workers, previously investigated. To our knowledge, this is the first report of measurements of urinary 1,2- and 1,4-DHN in humans.

2. Experimental

2.1. Chemicals and reagents

[$^2H_8$]Naphthalene (+98% isotopic purity) was obtained from Aldrich Chemical (Milwaukee, WI). 1,4- and 1,2-DHN were obtained from Tokyo Kasei Kogyo Co. (Toshima, Kita-Ku, Tokyo). β-Glucuronidase/aryl sulfatase (type H-2 from Helix pomatia; β-glucuronidase activity 98,000 units/ml and sulfatase activity 2400 units/ml) was purchased from Sigma Chemical Co. (St Louis, MO). Tri-Sil®-TBT reagent was obtained from Pierce Chemical (Rockford, IL). Magnesium sulfate, sodium dithionite, sodium acetate, anhydrous sodium sulfate, diethyl ether, acetonitrile, ethyl acetate, methanol and hexane were obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). Concentrated sulfuric acid, sodium chloride, ceric ammonium nitrate and L-ascorbic acid were purchased from Fisher Scientific Co. (Norcross, GA).

2.2. Synthesis of [$^2H_6$]1,4-DHN

The isotopically labeled internal standard, [$^2H_6$]1,4-DHN, was synthesized from [$^2H_8$]naphthalene via the corresponding quinone according to the method of Periasamy and Bhatt [24] with some modifications. Briefly, cerric ammonium nitrate (6.58 g) was dissolved in 170 ml acetonitrile and concentrated sulfuric acid (7:10, v/v), and [$^2H_8$]naphthalene (272 mg) was added. The mixture was stirred at room temperature for 6 h. The white precipitate was removed by filtration, dissolved in 100 ml of deionized water and the resulting solution was extracted three times with 100 ml of diethyl ether. Ether extracts were combined, dried with anhydrous magnesium sulfate, and filtered under nitrogen. Ether was removed on a rotatory evaporator to yield 318 mg (96%) of [$^2H_6$]1,4-naphthoquinone as an orange/brown solid.

In a separatory funnel, [$^2H_6$]1,4-naphthoquinone (164 mg) was placed with 751 mg sodium dithionite dissolved in 5 ml of water. Then 4.75 ml of diethyl ether was added to this mixture and the solution was shaken periodically for 30 min. After removing the aqueous layer, the organic phase was washed once with 5 ml of saturated brine solution containing 100 mg of sodium dithionite. Diethyl ether was removed under nitrogen and the brown solid of [$^2H_6$]1,4-DHN was recovered at 63% yield. The final product was characterized by gas chromatography–electron ionisation–mass spectrometry (GC–EI–MS) after converting it to the corresponding trimethylsilyl (TMS) derivative.

2.3. Subjects and urine collection

Urine from unexposed volunteers was obtained for method development and calibration purposes and stored at −80 °C prior to use. Spot urine samples were obtained from 28 coke workers (2 women and 26 men, 17 of whom were smokers) from a steel-producing complex in northern China at the end of a workweek (4 days of 8 h/day) and from 22 office and hospital workers (3 women and 19 men, 3 of whom were smokers) from the same geographic area. Of the coke workers, 15 were top workers and 13 were side or bottom workers. The range of ages of control subjects (18–50 years old) was similar to that of the coke workers (29–51 years old). Urine samples were maintained at −80 °C for up to 42 months prior to analysis. Data on cigarette smoking were obtained by questionnaire.

2.4. Optimization of the assay

Since 1,2- and 1,4-DHN are easily oxidized to the corresponding quinones, ascorbic acid was used in all solutions to minimize oxidative losses. Stock solutions were prepared in methanol that had been saturated with ascorbic acid. For optimization of the amount of ascorbic acid to use in the assay, sufficient ascorbic acid was added to 2 ml aliquots of volunteer urine to reach concentrations of between 0 and 130 mM. The treated urine at pH, ca. 5 was incubated with β-glucuronidase/sulfatase at 37 °C for 3 h and then carried through the assay procedure.

In a separate experiment, the optimal digestion time was determined by incubating urine containing 65 mM ascorbic acid with β-glucuronidase/sulfatase at 37 °C for periods ranging between 0 and 21 h and then carried through the assay as described below to obtain optimal yields of 1,2- and 1,4-DHN. In this case, the internal standard was added after the incubation step.

2.5. Application of the assay

After optimization of the assay, 1,2- and 1,4-DHN were measured in experimental samples as follows. To 2 ml aliquots of urine in 8 ml glass vials, 100 μl of 250 mg/ml ascorbic acid in water, 20 μl of 5N sodium acetate and 20 μl of a 10 μg/ml of [$^2H_6$]1,4-DHN in methanol (saturated with ascorbic acid) were added to form a medium with 65 mM ascorbic acid, pH, ca. 5. Following addition of 20 μl of β-glucuronidase/sulfatase, the samples were incubated at 37 °C for 4 h and then extracted two times with 4 ml of ethyl acetate. After drying the organic layer with anhydrous sodium sulfate it was removed under nitrogen and the residue was reconstituted in 400 μl of ethyl acetate and transferred to a 500 μl glass insert in a 2 ml auto-sampler vial. Samples were dried under nitrogen and 185 μl of hexane and 15 μl of Tri-Sil-TBT reagent were added. The vials were capped immediately and incubated at 75 °C for 30 min. One microliter aliquots of these solutions were analyzed by GC–EI–MS as described below.

Standard curves were prepared by spiking urine from office clerk volunteers with 1,2- and 1,4-DHN over the range of 10–1000 μg/l and 1–100 μg/l, respectively, and carrying out the assay as described for the samples. Quantitation of both 1,2- and 1,4-DHN was based on peak areas relative to the isotopically labeled internal standard, [$^2H_6$]1,4-DHN.

Experimental samples were processed by unique identification numbers so that the analyst was not aware of the exposure or smoking status of each subject.

2.6. Gas chromatography–mass spectrometry

Samples were analyzed with an Agilent 6890N GC coupled to an Agilent 5973N mass selective detector. Separation was achieved on a DB-5 fused silica capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, Folsom, CA) with helium as the carrier gas at a constant flow rate of 1.0 ml/min. Samples were injected in splitless mode. The temperatures of the GC injection port and MS transfer line were 280 °C. The GC oven was held at 75 °C for 2 min, ramped at 8 °C/min to 195 °C where it was held for 20 min. Late eluting compounds were removed by ramping the oven at 25 °C/min rate to 280 °C where it was held for 20 min. The EI electron energy was 70 eV and ion source and quadrupole temperatures were 200 and 100 °C, respectively. The TMS derivatives of [$^2H_6$]1,4-, 1,2- and 1,4-dihydroxynaphthalenes (TMS-[$^2H_6$]1,4-, -1,2 and -1,4-DHN) were characterized by scanning the mass spectrometer from 50 to 700 amu. Quantitation of 1,2- and 1,4-DHN was achieved in selected ion monitoring mode by monitoring ions m/z 304 (TMS-1,2- and -1,4-DHN) and m/z 310 (TMS-[$^2H_6$]-

1,4-DHN). The retention times for TMS-1,2- and 1,4- DHN and [$^2H_6$]-1,4-DHN were 25.08, 26.80 and 26.69 min, respectively.

2.7. Recoveries, precision, limits of detection

To estimate the recoveries of 1,2- and 1,4-DHN from urine, triplicate samples of 1,2- and 1,4-DHN were prepared by spiking urine obtained from a human volunteer at concentrations of 10, 50, 200 and 1000 μg/l and 1, 5, 20 and 100 μg/l, respectively, The precision was estimated by assaying 22 duplicate samples from coke workers and controls whose exposures covered a wide range. Limits of detection and limits of quantitation for 1,2- and 1,4-DHN were calculated based on a signal to noise ratio of three and 10, respectively. Levels of the DHNs were not adjusted for urinary creatinine in this study due to earlier evidence from the same urine samples that creatinine adjustment led to larger standard errors in multivariate analyses of hydroxynaphthalene levels [23]. Since we compare levels of DHNs with those of the hydroxynaphthalenes from the earlier study, we did not adjust DHNs for creatinine in the current investigation.

2.8. Statistical analysis

All statistical analyses were performed with SAS system software (V 9.12, SAS Institute, Cary, NC, USA). In all cases a *p*-value of 0.05 (two-tailed) was considered significant. Spearman coefficients were estimated to investigate correlations (in natural scale, using PROC CORR of SAS) among the DHNs and other analytes, which had been measured previously in the same subjects [16]. All other analyses were performed after logarithmic transformation of data to remove heteroscedasticity and to satisfy normality assumption. Two samples (one control and one exposed) were lost during the assay and hence 21 control and 27 exposed subjects were available for data analysis. The coefficients of variation (CV) of the assay for 1,2- and 1,4-DHN were estimated as $CV_a = \sqrt{e^{S_w^2} - 1}$, where s_w^2 represents the estimated within-sample variance component from ANOVA of the logged levels observed in 22 duplicate urine samples from exposed and control workers [25]. Two-way ANOVA was applied to test for differences in mean (logged) levels of urinary 1,2- and 1,4-DHN in subjects categorized by work location and smoking status (using PROC GLM of SAS).

3. Results and discussion

3.1. Characterization of TMS derivatives of [$^2H_6$]1,4-DHN, 1,4-DHN and -1,2-DHN

The mass spectra of the TMS derivatives of [$^2H_6$]1,4-DHN, 1,4-DHN and 1,2-DHN are shown in Fig. 2A–C, respectively. Since the molecular ion at *m/z* 310 (Fig. 2A) or *m/z* 304 (Fig. 2B and C) was prominent in each spectrum, these ions were chosen for quantitation of 1,2- and 1,4-DHN in samples.

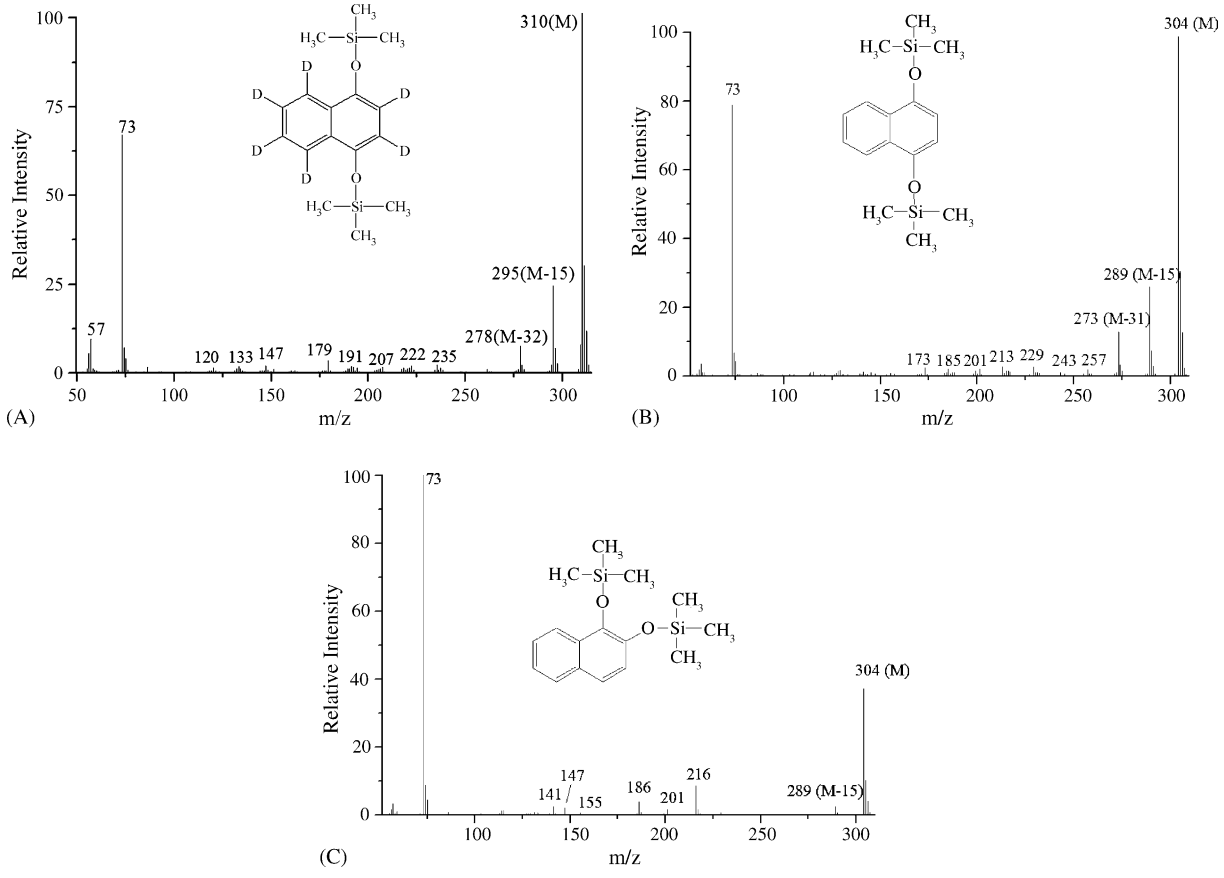

Fig. 2. GC–EI mass spectra of TMS derivatives of (A) [$^2H_6$]1,4-DHN; (B) 1,4-DHN and (C) 1,2-DHN.

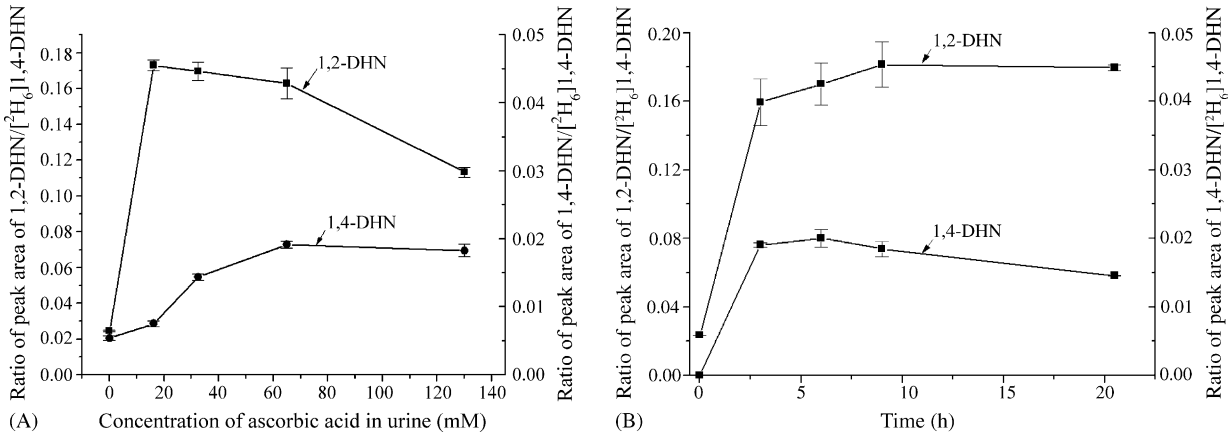

Fig. 3. (A) Effect of ascorbic acid concentration on urinary levels of 1,2-DHN and 1,4-DHN. Two milliliters of urine from a human volunteer were incubated at pH, ca. 5 with varying amounts of ascorbic acid and with glucuronidase/sulfatase at 37 °C for 3 h. (B) Effect of incubation time on the levels of 1,2-DHN and 1,4-DHN. Two milliliters of urine from a human volunteer containing 65 mM ascorbic acid were incubated for different periods of time at pH, ca. 5 with glucuronidase/sulfatase.

3.2. Optimization of the assay

The use of ascorbic acid to minimize oxidative losses of the DHNs in the assay was optimized by monitoring the release of 1,2- and 1,4-DHN from their glucuronide and sulfate conjugates in samples of volunteer urine. As shown in Fig. 3A, the level of 1,2-DHN released from the conjugates was maximal at 16 mM ascorbic acid while that of 1,4-DHN was maximal at 65 mM. Since the yield of 1,2-DHN from its conjugates diminished markedly at ascorbic acid concentrations above 65 mM, this concentration was chosen for the assay. With 65 mM ascorbic acid in urine the yield of 1,2-DNH was about 95% of its maximum level.

The effect of incubation time for enzymatic digestion was investigated in a parallel experiment. As shown in Fig. 3B, levels of 1,2- and 1,4-DHN increased with increasing incubation time up to about 4 h whereupon those of 1,4-DHN began to decrease. Using 4 h for enzymatic digestion the recoveries of 1,2- and 1,4-DHN were about 90 and 96% of their maximum values, respectively.

3.3. Calibration, recoveries, coefficients of variation and limits of detection

Standard curves were prepared by adding sufficient quantities of 1,2- and 1,4-DHN to volunteer urine to give 10–1000 μg/l and 1–100 μg/l, respectively, and then carrying the samples through the assay. Calibration curves of both DHNs were linear with values of R^2 of about 0.998. Recoveries of spiked urine samples were 90–100% for 1,2-DHN at levels of 100–1000 μg/l and 96–103% for 1,4-DHN at levels of 1–100 μg/l. The CVs of the method, based on 22 duplicate assays of experimental samples, were 14.7 and 10.9%, respectively, for 1,2- and 1,4-DHN. Limits of detection (LOD) for 1,2- and 1,4-DHN were 0.21 and 0.15 μg/l, respectively, based on a signal-to-noise ratio of three. Limits of quantitation (LOQ) for 1,2- and 1,4-DHN were 0.69 and 0.44 μg/l, respectively, based on a signal-to-noise ratio of 10.

3.4. Urinary levels of 1,2- and 1,4-DHN among coke workers and controls

This assay was used to determine the urinary levels of 1,2- and 1,4-DHN among coke workers and controls from a steel factory in northern China. Both 1,2- and 1,4-DHN were detected in all subjects. Figs. 4 and 5 show the typical GC–EI–MS chromatograms obtained from the urine samples of a control and a side-bottom worker, respectively, where the levels of 1,2- and 1,4-DHN are 8.65 and 0.69 μg/l for the control worker and 550 and 7.03 μg/l for the side-bottom worker.

Table 1 shows summary statistics of urinary 1,2- and 1,4-DHN for subjects categorized as controls, side-bottom workers, and top workers based upon their primary work locations. The levels of 1,2-DHN were about 30- and 100-fold higher than those of 1,4-DHN in controls and coke workers, respectively, indicating that the pathway(s) leading to the production of 1,2-DHN was more efficient than that leading to 1,4-DHN in humans (see Fig. 1). Comparing across exposure categories, levels of 1,2-DHN increased in the order: control workers < side-bottom workers < top workers, which was expected on the basis of previous analyses of urinary naphthalene and naphthols in these workers [23,26]. However, 1,4-DHN levels were essentially the same for side-bottom workers and top workers. Levels of urinary DHNs were significantly greater in both side-bottom workers and top workers than in control workers ($p \leq 0.0031$). However, the levels of DHNs were not significantly different between side-bottom workers and top workers ($p \geq 0.1216$). The GM level of 1,2-DHN in controls was 184 times its LOD while that of 1,4-DHN was eight times its LOD. This suggests that the assay has sufficient sensitivity to be generally useful for monitoring populations without occupational sources of exposure to PAHs.

Because cigarette smoking is a prominent source of environmental exposure to naphthalene [1], the effect of smoking status on levels of urinary DHNs was investigated. As shown in Fig. 6, the levels of 1,2- and 1,4-DHN were higher for smokers than for nonsmokers in each category, however, the difference

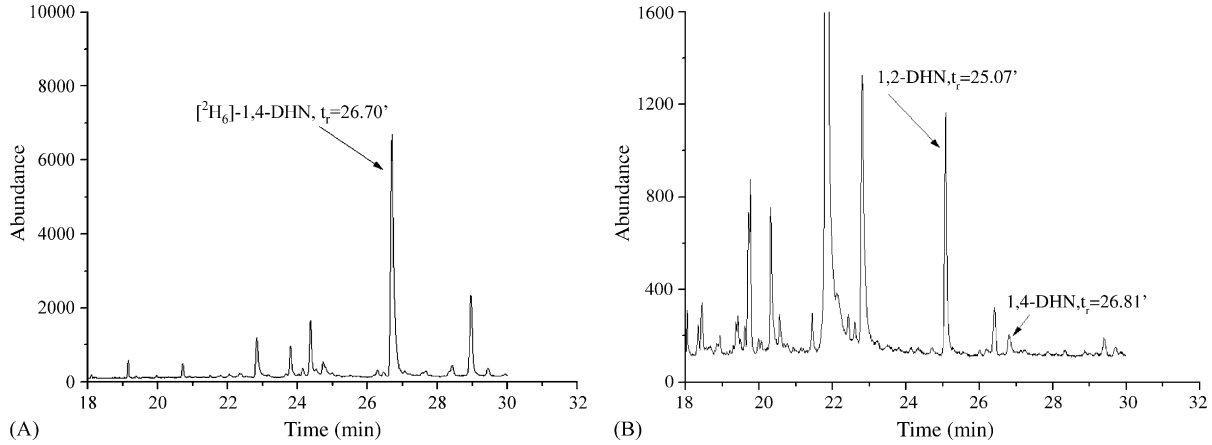

Fig. 4. GC–EI–MS chromatogram obtained in selected ion monitoring mode of a urine sample from a control worker; the estimated concentration of 1,2-DHN was 8.65 μg/l and of 1,4-DHN was 0.69 μg/l.

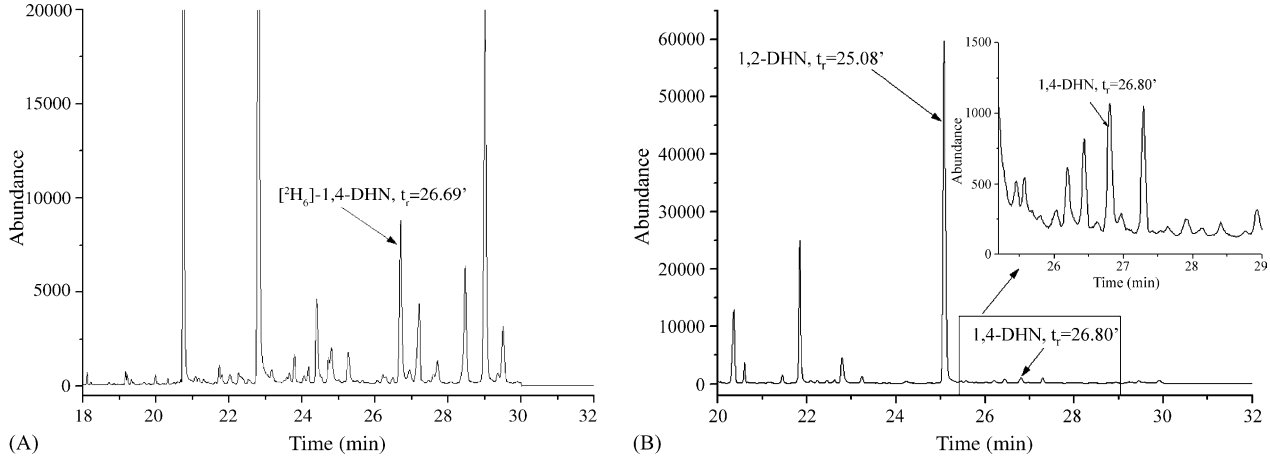

Fig. 5. GC–EI–MS chromatogram obtained in selected ion monitoring mode of a urine sample from a side-bottom worker; the estimated concentration of 1,2-DHN was 550 μg/l and of 1,4-DHN was 7.03 μg/l.

between smokers and non-smokers was only marginally significant ($p = 0.0646$).

Since we had previously measured urinary levels of naphthalene [26], 1- and 2-hydroxynaphthalene [23], and levels of serum albumin adducts of 1,2- and 1,4-naphthoquinone [16] in the same subjects, the correlations among these biomarkers with 1,2- and 1,4-DHN are summarized in Table 2. Both 1,2- and 1,4-DHN were highly correlated with all of the other urinary biomarkers ($r_s \geq 0.623$; $p < 0.0001$). Also, the levels of 1,2-DHN were significantly correlated with those of albumin adducts of 1,2-naphthoquinone ($r_s = 0.492$, $p = 0.0004$) (Table 2). As 1,2-DHN is thought to be the immediate precursor of 1,2-naphthoquinone, this result is reasonable. Also, since 1,2-naphthoquinone is thought to be a toxic metabolite of naphthalene [12], this suggests that urinary measurements of 1,2-DHN have toxicological significance. It is also noteworthy that the levels of 1,2-DHN were marginally more correlated with those of 1-hydroxynaphthalene ($r_s = 0.920$) than of 2-hydroxynaphthalene ($r_s = 0.870$); this was not anticipated based upon the accepted metabolic scheme for naphthalene (Fig. 1). Levels of 1,4-DHN were not correlated with those of the albumin adduct of 1,4-naphthoquinone ($r_s = -0.0702$).

Table 1
Summary of urinary 1,2-dihydroxynaphthalene and 1,4-dihydroxynaphthalene among categories of workers in the Chinese steel industry (controls, side-bottom and top workers)

Urinary analytes	Exposure category		
	Controls ($n = 21$)	Side-bottom workers ($n = 13$)	Top workers ($n = 14$)
1,2-Dihydroxynaphthalene GM ± GSD (μg/l)	38.8 ± 2.31	260 ± 2.98*	452 ± 2.28*
1,4-Dihydroxynaphthalene GM ± GSD (μg/l)	1.21 ± 2.53	3.58 ± 2.22*	3.42 ± 3.11*

* p-value < 0.05 compared to controls.

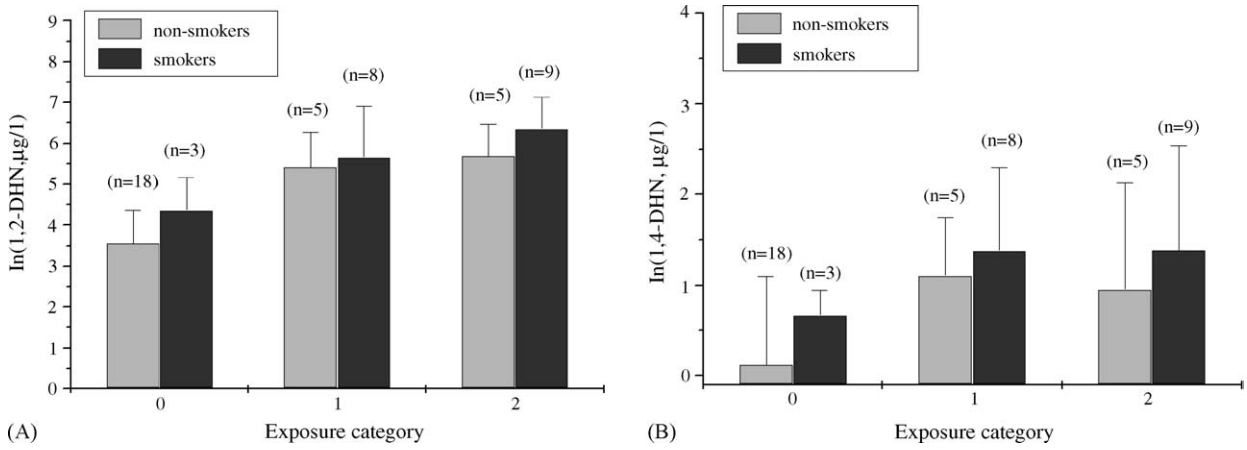

Fig. 6. Mean levels of logged concentrations of urinary 1,2-DHN and 1,4-DHN aggregated by exposure category (0, controls; 1, side-bottom workers; 2, top workers). Error bars represent the standard deviation based on the logged data.

Table 2
Correlation among urinary biomarkers for coke workers and controls

Analytes	1-Hydroxy-naphthalene	2-Hydroxy-naphthalene	1,2-NPQ	1,4-NPQ	1,2-DHN	1,4-DHN
Naphthalene[a]	0.621[b] <0.0001 $n = 50$	0.640[b] <0.0001 $n = 50$	0.460[b] 0.0008 $n = 50$	0.256[b] 0.0722 $n = 50$	0.623[b] <0.0001 $n = 48$	0.697[b] <0.0001 $n = 48$
1-Hydroxy-naphthalene[a]		0.904[b] <0.0001 $n = 50$	0.381[b] 0.0063 $n = 50$	−0.0496[b] 0.7323 $n = 50$	0.920[b] <0.0001 $n = 48$	0.749[b] <0.0001 $n = 48$
2-Hydroxy-naphthalene[a]			0.385[b] 0.0058 $n = 50$	0.0839[b] 0.5624 $n = 50$	0.870[b] <0.0001 $n = 48$	0.740[b] <0.0001 $n = 48$
1,2-NPQ[c,d]				0.457[b] 0.0008 $n = 50$	0.492[b] 0.0004 $n = 48$	0.346[b] 0.0161 $n = 48$
1,4-NPQ[c,d]					−0.0632[b] 0.6697 $n = 48$	−0.0702[b] 0.6352 $n = 48$
1,2-DHN[c,a]						0.722[b] <0.0001 $n = 48$

[a] Units for all urinary biomarkers are μg/l.
[b] Spearman correlation coefficients and *p*-values are shown for each comparison based on natural scale data.
[c] 1,2-DHN and 1,4-DHN represent 1,2- and 1,4-dihydroxynaphthalene, respectively; 1,2-NPQ and 1,4-NPQ represent albumin adducts of 1,2- and 1,4-naphthoquinone.
[d] Units for albumin adducts of 1,2- and 1,4-naphthoquinone are pmol/g of albumin.

4. Conclusion

A GC–EI–MS method has been used to quantify levels of 1,2- and 1,4-DHN in human urine. This method was successfully applied to determine urinary levels of these metabolites in coke workers and controls from the steel industry in northern China. The level of 1,2-DHN was significantly higher than that of 1,4-DHN in both control and exposed subjects. The levels 1,2-DHN and 1,4-DHN were significantly higher for coke workers than for controls, as anticipated in the Chinese coke workers, and were marginally greater in smoking subjects. DHNs were also highly correlated with the other urinary biomarkers and protein adducts of 1,2-naphthoquinone in same subjects. Our results show the potential of using DHNs, either alone or in combination with other available biomarkers, to assess the uptake and metabolism of naphthalene in humans.

Acknowledgements

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