

Analysis of Phenanthrols in Human Urine by Gas Chromatography-Mass Spectrometry: Potential Use in Carcinogen Metabolite Phenotyping

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Abstract

Phenanthrene is the simplest polycyclic aromatic hydrocarbon (PAH) containing a bay region, a feature closely associated with carcinogenicity. We have proposed that measurement of phenanthrene metabolites in human urine could be used to identify interindividual differences in metabolic activation and detoxification of PAH, and that these differences may be related to cancer susceptibility in smokers and other exposed individuals. Previously, we reported a method for quantitation of *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans*, *anti*-PheT) in human urine. *trans*, *anti*-PheT is the ultimate product of the diol epoxide metabolic activation pathway of phenanthrene. In this study, we have extended our carcinogen metabolite phenotyping approach by developing a method for quantitation of phenanthrols in human urine. PAH phenols such as phenanthrols are considered as detoxification products. After treatment of the urine by β -glucuronidase and arylsulfatase, a fraction enriched in phenanthrols was prepared by partitioning and solid phase extraction. The phenanthrols were silylated and analyzed by gas chromatography-positive

ion chemical ionization-mass spectrometry with selected ion monitoring. [*ring*-¹³C₆]3-phenanthrol was used as an internal standard. Accurate and reproducible quantitation of four phenanthrols, 1-HOPhe, 2-HOPhe, 3-HOPhe, and 4-HOPhe, was readily achieved. In smokers, mean levels of 1-HOPhe (0.96 ± 1.2 pmol/mg creatinine) and 3-HOPhe (0.82 ± 0.62 pmol/mg creatinine) were greater than those of 2-HOPhe (0.47 ± 0.29 pmol/mg creatinine), and 4-HOPhe (0.11 ± 0.07 pmol/mg creatinine). There were no significant differences between the levels of any of the phenanthrols in smokers and nonsmokers. Total levels of the quantified phenanthrols were highly correlated with those of 3-HOPhe. Ratios of phenanthrene metabolites representing activation and detoxification were calculated as *trans*, *anti*-PheT divided by 3-HOPhe. There was a 7.5-fold spread of ratios in smokers, and a 12.3-fold spread in nonsmokers, suggesting that this may be a useful parameter for distinguishing individual metabolic responses to PAH exposure. (Cancer Epidemiol Biomarkers Prev 2004;13(12):2167-74)

Introduction

Abundant evidence indicates that polycyclic aromatic hydrocarbons (PAH) are among the principal causative agents for lung cancer in smokers (1-3). Cigarette smoke fractions enriched in PAH are carcinogenic in laboratory animals, particularly when combined with tumor-promoting and co-carcinogenic agents in smoke (4). PAH-DNA adducts are present in the lungs of smokers, and in some cases, levels are higher than in nonsmokers (5). The spectrum of mutations found in

the p53 tumor suppressor gene from lung tumors of smokers is similar to that induced by diol epoxide metabolites of PAH in vitro (6). Other evidence from occupational studies strongly implicates PAH as the cause of lung and skin cancers due to exposure to coke oven emissions, coal tars, and soot (7-9).

PAH require metabolic activation to exert their carcinogenic effects (10-12). Although several metabolic activation pathways have been proposed, the strongest and most consistent evidence favors the view that bay region diol epoxide metabolites are in many cases major ultimate carcinogens of PAH (11-14). These diol epoxides, which react with DNA and are mutagenic and carcinogenic, are formed by a three-step sequence involving initial cytochrome P450-catalyzed epoxide formation, followed by epoxide hydrolase-catalyzed hydration, and finally, further epoxidation to the diol epoxide, catalyzed by cytochrome P450s and other enzymes. This sequence is illustrated in Fig. 1 for benzo(*a*)pyrene [B(a)P], a prototypic PAH. Although some of the diol epoxide reacts with DNA to initiate

Received 4/28/04; revised 7/2/04; accepted 7/15/04.

Grant support: Grant CA-92025 from the National Cancer Institute. S.S. Hecht is an American Cancer Society Research Professor, supported by ACS grant RP-00-138. Mass spectrometry and statistics were carried out in the core facilities of The Cancer Center, University of Minnesota, supported in part by National Cancer Institute grant CA-77598.

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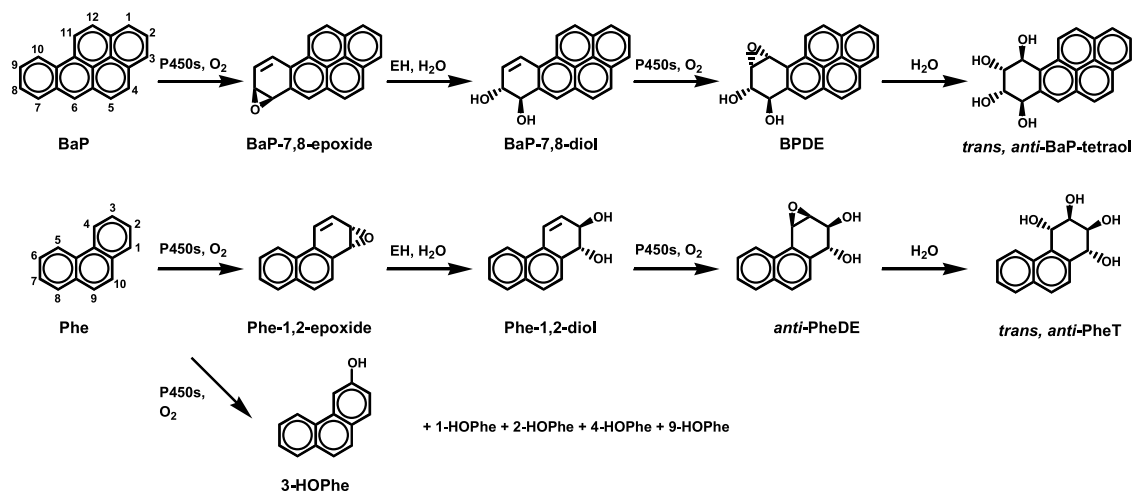


Figure 1. Metabolism of B(a)P and phenanthrene to bay region diol epoxides (BPDE and *anti*-PheDE) and tetraols (*trans*, *anti*-B(a)P-tetraol and *trans*, *anti*-PheT) and of phenanthrene to phenanthrols (1-HOPhe, 2-HOPhe, 3-HOPhe, 4-HOPhe, and 9-HOPhe).

carcinogenesis, most of it reacts with water to produce a tetraol. Numerous detoxification reactions compete with this metabolic activation pathway. Among these are phenol formation catalyzed by cytochrome P450s, reactions of the epoxides or diol epoxides with glutathione, catalyzed by glutathione S-transferases, detoxification of the diols by UDP-glucuronosyl transferases, and other pathways (2, 10). It is widely hypothesized that individual differences in activation versus detoxification of PAH and other carcinogens influence the likelihood of cancer development (15). This general hypothesis has been tested in many studies by assessing variants in one or more genes involved in carcinogen metabolism such as *CYP1A1*, *GSTs*, and *MPO* (16-22). The results of these studies have not shown consistently strong effects.

We have proposed that carcinogen metabolite phenotyping would be a more direct approach than the widely used genotyping strategy to determine the effects of metabolic differences on cancer susceptibility (23, 24). In pursuit of this idea, we developed a method to quantify *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans*, *anti*-PheT) in human urine (24). Phenanthrene is the simplest PAH with a bay region, a feature closely associated with carcinogenicity (11, 12). However, phenanthrene is generally considered to be noncarcinogenic (7, 25). As shown in Fig. 1, metabolism of phenanthrene to *trans*, *anti*-PheT closely mirrors that of B(a)P to *r*-7,*t*-8,9,*c*-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene [*trans*, *anti*-B(a)P-tetraol; ref. 26]. *trans*, *anti*-PheT, in contrast to *trans*, *anti*-B(a)P-tetraol, is readily quantified in human urine, and should represent the result of exposure to phenanthrene plus metabolic activation by the critical diol epoxide pathway (24). We have shown that levels of *trans*, *anti*-PheT in urine are strongly influenced by PAH exposure (24). Amounts of this metabolite were greatest in urine samples from psoriasis patients treated with a coal tar-containing

ointment, followed by urine samples from coke oven workers, smokers, and nonsmokers (24). Although these data indicate that *trans*, *anti*-PheT may be a good exposure marker for PAH, our major interest is the metabolic difference in individuals such as smokers with similar exposure. Therefore, we need to correct for exposure. In order to do this, we propose creating a metabolic activation/detoxification ratio. *trans*, *anti*-PheT would be in the numerator of this ratio, whereas a principal component of the denominator would be phenanthrols.

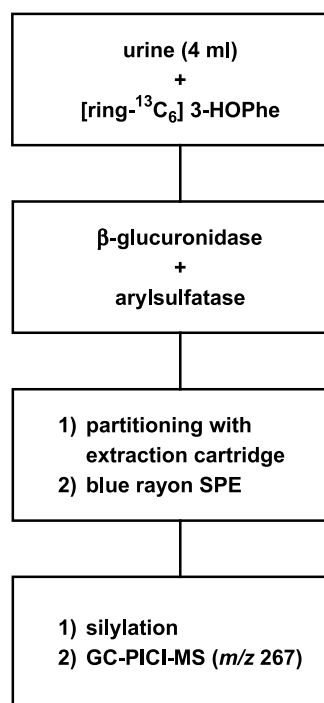


Figure 2. Analytic scheme for quantitation of phenanthrols in human urine.

Five phenanthrols, 1-HOPhe, 2-HOPhe, 3-HOPhe, 4-HOPhe, and 9-HOPhe, could be formed from phenanthrene (Fig. 1), and all have been previously identified in human urine (27, 28). Several methods have been published for quantitation of phenanthrols in human urine. These used either high-performance liquid chromatography [(HPLC); refs. 29-34], gas chromatography [GC; refs. 35, 36], or GC-mass spectroscopy (GC-MS; refs. 37-42] for detection and quantitation. We have developed a GC-positive ion chemical ionization-MS (GC-PICI-MS) method for quantitation of phenanthrols in human urine and have applied it to smokers and nonsmokers. Ratios of *trans*, *anti*-PheT/phenanthrols were also determined.

Materials and Methods

Apparatus. GC-PICI-MS was carried out with a Hewlett Packard (Agilent, Wilmington, DE) Model 5973 instrument equipped with a 0.25 mm (inside diameter) \times 30 m, 0.15 μ m film thickness, DB-17MS column (Agilent) and a 0.53 mm (inside diameter) \times 2 m deactivated fused silica precolumn. Removal of solvents was carried out with a SVC-200 Speedvac (Thermo Savant, Holbrook, NY).

Materials. 1-HOPhe and 2-HOPhe were synthesized (43). 3-HOPhe (Chiron AS, Trondheim, Norway), 4-HOPhe (Chiron), and 9-HOPhe (Sigma-Aldrich, St. Louis, MO) were either synthesized (43) or purchased. [*ring*- $^{13}\text{C}_6$]3-Phenanthrol was procured from Cambridge Isotope Laboratories (Cambridge, MA). 1-Hydroxybenz(*a*)anthracene was obtained from the National Cancer Institute Chemical Carcinogen Reference Standard Repository, Kansas City, MO. Stock solutions were prepared in acetonitrile.

β -Glucuronidase and arylsulfatase (from *Helix pomatia*) were obtained from Roche Diagnostics Corp., Indianapolis, IN. bis-Trimethylsilyltrifluoroacetamide containing 1% trimethylchlorosilane was purchased from Regis Technologies, Inc., Morton Grove, IL. Chem-Elute diatomaceous earth partitioning cartridges (5 mL) were

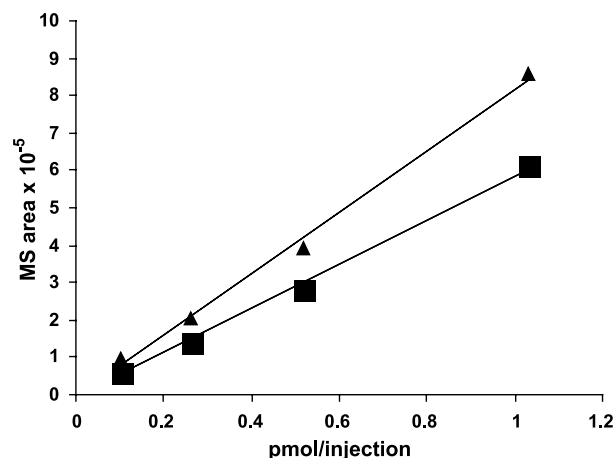


Figure 3. Calibration curves for 3-TMSOPhe (▲) and [*ring*- $^{13}\text{C}_6$]3-TMSOPhe (■).

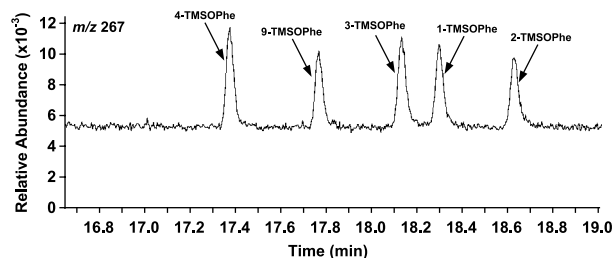


Figure 4. GC-PICI-MS chromatogram, with selected ion monitoring at *m/z* 267, of a mixture of standard TMSOPhe. The amount of each HOPhe which was silylated and injected was 2.5 pg. TMSOPhe used are indicated on the chromatogram. The analysis was carried out on a 0.25 mm (inside diameter) \times 30 m, 0.15 μ m film thickness, DB-17MS column. See Materials and Methods for further details.

obtained from Varian, Inc. (Walnut Creek, CA). Blue rayon was obtained from Sigma-Aldrich and 100 mg was placed in an empty 6 mL Bond Elute solid phase extraction cartridge (Varian) between two polyethylene frits.

Urine Samples

Analysis of Urine for Phenanthrols. A 4 mL urine sample was placed in a 15 mL centrifuge tube and 1.5 ng (5.5 pmol) [*ring*- $^{13}\text{C}_6$]3-HOPhe was added. The pH was adjusted to 5 by addition of 1.0 mL of sodium acetate buffer (0.5 mol/L, pH 5). Then 10 μ L of β -glucuronidase and arylsulfatase (equivalent to 1,000 units β -glucuronidase and 8,000 units arylsulfatase) were added and the mixture was incubated overnight with shaking at 37°C. The sample was loaded directly on a Chem-Elute cartridge and allowed to stand for 2 minutes. The cartridge was eluted with three 8-mL portions of toluene into a 50 mL centrifuge tube. To the eluant was added 5.5 mL of 0.11 mM aqueous $\text{Na}_2\text{S}_2\text{O}_4$. The toluene was removed on the Speedvac, leaving approximately 5.5 mL of the aqueous solution. This required about 3.5 hours. The Speedvac was then vented with nitrogen. Methanol (0.5 mL) was added to the aqueous solution. A blue rayon solid phase extraction cartridge (100 mg) was prewashed with 5 mL methanol and 10 mL water. The sample was applied dropwise to the top of the column. The column was washed with 7 mL of methanol/water (25:75), which was discarded. It was then washed with 8 mL of methanol into a 15 mL centrifuge tube. This fraction contained the phenanthrols. The solution was concentrated to dryness on the Speedvac, overnight in the dark, with no heat applied. The Speedvac was vented with nitrogen. The residue was transferred into a microvial with three 50- μ L portions of methanol. The sample was concentrated to dryness on the Speedvac with venting by nitrogen. To this residue, 0.6 ng 1-hydroxybenz(*a*)anthracene was added in 3 μ L acetonitrile as external standard and 9 μ L bis-trimethylsilyltrifluoroacetamide containing 1% trimethylchlorosilane. The resulting solution was heated at 60°C for 60 minutes with mixing. It was then analyzed by GC-PICI-MS using splitless injection of 2 μ L samples at 270°C and selected ion monitoring at *m/z* 267 (silylated phenanthrols), *m/z* 273 (internal standard), and *m/z* 317 (external standard). The GC oven was programmed as follows: 80°C for

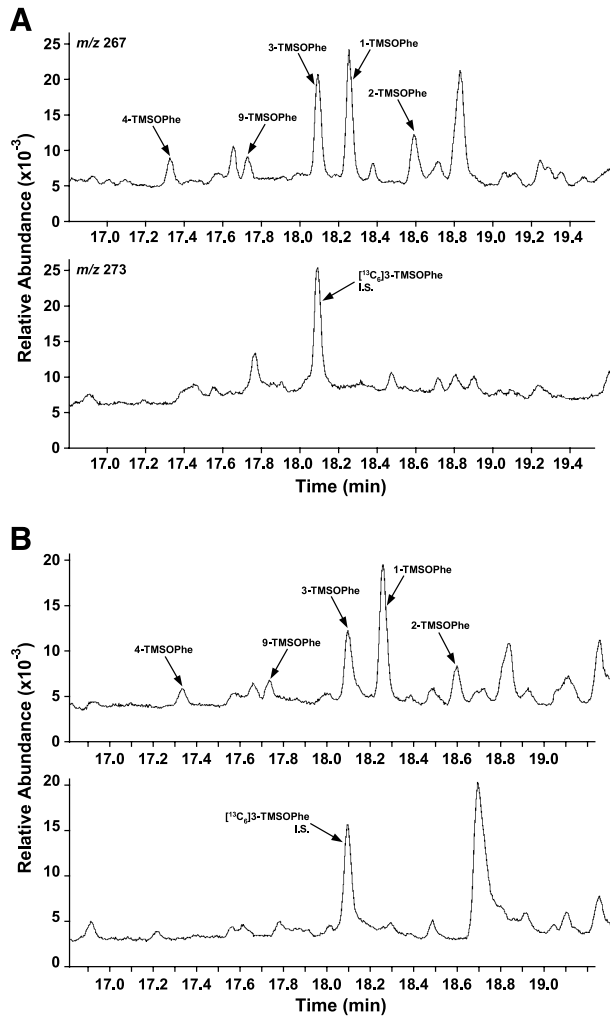


Figure 5. GC-PICI-MS chromatogram, with selected ion monitoring at m/z 267, of TMSOPhe in the urine of (A) a smoker and (B) a nonsmoker. TMSOPhe used are indicated on the chromatogram. *Bottom*, results of selected ion monitoring at m/z 273, which is $[M + 1]^+$ of the internal standard, $[ring-^{13}C_6]3-TMSOPhe$. 9-TMSOPhe was not quantified for the reasons discussed in the text.

2 minutes, then 10°C per minute to 160°C, then 7°C per minute to 225°C, then held for 2 minutes, then 15°C per minute until 310°C. The carrier gas was helium, at a constant flow rate of 1 mL/min. The MS transfer line temperature was 310°C. MS conditions were as follows: reagent gas, methane; source temperature, 250°C; quadrupole temperature, 106°C; electron multiplier voltage, 3,000 volts.

Other Analyses. *trans*, *anti*-PheT was determined as described previously (24). Creatinine was assayed by Fairview-University Medical Center Diagnostic Laboratories (Minneapolis, MN) using Vitros CREA slides.

Statistical Analysis. The Wilcoxon rank sum test and the Spearman rank correlation test, respectively, were used to compare levels of the different phenanthrols and to determine correlations among the phenanthrols.

Results

The method for analysis of phenanthrols in human urine is summarized in Fig. 2. $[ring-^{13}C_6]3-HOPhe$ was added to the urine as an internal standard. Treatment with β -glucuronidase plus arylsulfatase effected hydrolysis of conjugates, releasing the phenanthrols. A phenanthrol-containing fraction was obtained by partitioning with toluene, using a diatomaceous earth-based partitioning cartridge. The eluant from this step was further purified on a copper phthalocyanine rayon (blue rayon) solid phase extraction cartridge. This material binds planar compounds such as phenanthrols, which ultimately were eluted with methanol. The resulting fraction was silylated, converting the phenanthrols to their trimethylsilyl derivatives [phenanthrol trimethylsilyl ether (TMSOPhe)], which were analyzed by GC-PICI-MS.

A full scan PICI MS of 4-TMSOPhe showed a base peak at m/z 267 $[M + H]^+$ and ions at m/z 251 $[M - CH_3]^+$ (relative intensity 23) and m/z 295 $[M + C_2H_5]^+$ (relative intensity 29). MS of the other TMSOPhe isomers were similar. Selected ion monitoring at m/z 267 gave a detection limit of 12.9 fmol TMSOPhe (signal/noise, 2:1) on column. A calibration curve for derivatization and detection of 0.129 to 1.03 pmol of 3-HOPhe and $[ring-^{13}C_6]3-HOPhe$ is shown in Fig. 3.

Baseline separation of the TMSOPhe isomers was readily achieved, as shown in Fig. 4. Typical GC-PICI-MS selected ion monitoring traces of TMSOPhe isomers in the urine of a smoker and a nonsmoker are shown in Fig. 5A, B. The indicated peaks at the *top* are the five TMSOPhe isomers, whereas those in the *bottom* are the internal standard, $[ring-^{13}C_6]3-TMSOPhe$. The identities of the peaks were confirmed by their retention times and by their relative intensities using selected ion monitoring of various ions, either under PICI-MS or electron impact-MS conditions (data not shown).

The accuracy of the method was determined by the standard addition method. Various levels of HOPhe isomers were added to duplicate aliquots of a urine sample from a nonsmoker. Analysis produced the results illustrated in Fig. 6 for 3-HOPhe, showing excellent agreement between the measured and added amounts. The y intercept (0.48 pmol/mL) agreed with the quantified amount of 3-HOPhe in this sample before the additions (0.44 pmol/mL). The added and measured amounts of 3-HOPhe were highly correlated ($r = 0.999$).

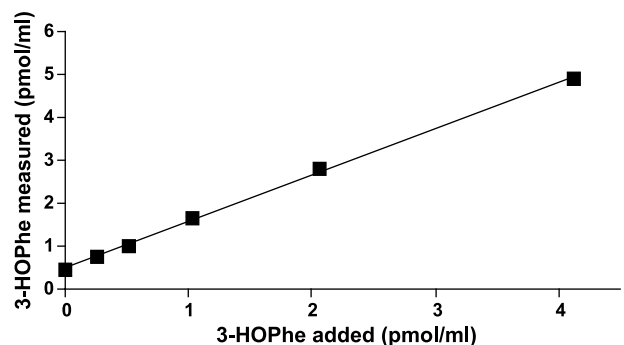


Figure 6. Relationship between levels of 3-HOPhe added to a nonsmoker's urine and measured amounts, $r = 0.999$.

Similar relationships between added and measured amounts were found for the other phenanthrols (1-HOPhe, $r = 0.999$; 2-HOPhe, $r = 0.999$; 4-HOPhe, $r = 0.998$), except 9-HOPhe. Difficulties were encountered in reproducible quantitation of 9-HOPhe. Recoveries of added 9-HOPhe were zero when precautions against oxidation, such as venting with nitrogen rather than air after solvent removal steps, were not taken. However, even when these precautions were followed, quantitation of 9-HOPhe was poor using this method and it was not pursued further.

The precision of the method was determined by replicate analysis ($N = 6$) of a nonsmoker's urine. The results were [mean \pm SD in pmol/mL urine (relative standard deviation)]: 1-HOPhe, 0.93 ± 0.069 (7.4); 2-HOPhe, 0.58 ± 0.079 (14); 3-HOPhe, 0.44 ± 0.025 (5.7); 4-HOPhe, 0.16 ± 0.036 (22). Recoveries of internal standards averaged 36%. The detection limits for 1-HOPhe, 2-HOPhe, 3-HOPhe, and 4-HOPhe were 0.05 pmol/mL urine.

Urine samples were obtained from 25 smokers (12 male). They smoked 12 ± 10 cigarettes per day and

Table 1. Phenanthrols in the urine of smokers and nonsmokers

Subject no.	pmol/mg creatinine				<i>trans, anti</i> -PheT	
	1-HOPhe	2-HOPhe	3-HOPhe	4-HOPhe	3-HOPhe	Total HOPhe*
Smokers						
1	0.26	0.26	0.38	0.04	2.1	0.86
2	0.36	0.39	0.44	0.09	3.6	1.2
3	0.58	0.48	0.57	0.12	2.6	0.86
4	0.32	0.34	0.62	0.05	2.6	1.2
5	0.27	0.20	0.40	0.04	5.6	2.5
6	0.82	0.44	0.77	0.18	3.9	1.4
7	0.69	0.47	0.65	0.09	4.9	1.7
8	0.43	0.45	0.77	0.08	1.2	0.55
9	0.57	0.45	0.45	0.10	3.2	0.92
10	0.98	0.54	0.56	0.15	1.7	0.42
11	1.1	1.5	0.82	0.32	1.7	0.37
12	0.40	0.14	0.40	0.04	9.0	3.7
13	0.35	0.38	0.45	0.09	2.4	0.84
14	0.84	0.93	1.1	0.19	4.1	1.5
15	4.1	1.0	3.3	0.16	1.5	0.56
16	0.77	0.43	0.74	0.14	4.4	1.6
17	1.2	0.27	1.0	0.01	5.5	2.3
18	0.39	0.35	0.39	0.05	3.4	1.1
19	5.4	0.64	1.9	0.07	1.5	0.35
20	0.81	0.29	0.63	0.11	4.2	1.4
21	0.24	0.25	0.30	0.07	5.7	2.0
22	0.79	0.39	0.82	0.08	3.5	1.4
23	0.45	0.08	0.86	0.06	2.9	1.7
24	0.87	0.55	1.2	0.20	2.4	1.0
25	1.1	0.48	1.0	0.13	3.8	1.5
Mean \pm SD	0.96 ± 1.2	0.47 ± 0.29	0.82 ± 0.62	0.11 ± 0.07	3.4 ± 1.9	1.3 ± 0.76
Range	0.24-5.4	0.08-1.5	0.30-3.3	0.01-0.32	1.2-9.0	0.35-3.7
Nonsmokers						
1	0.15	0.09	0.15	0.05	3.3	1.1
2	0.24	0.20	0.45	0.04	3.3	1.6
3	0.57	0.47	0.20	0.08	4.4	0.70
4	0.11	0.12	0.17	0.03	3.7	1.6
5	1.9	0.77	2.7	0.27	1.7	0.81
6	0.47	0.34	0.47	0.10	1.8	0.61
7	0.43	0.46	0.43	0.18	3.8	1.1
8	0.55	0.40	1.1	0.07	2.8	1.4
9	0.76	0.70	0.36	0.08	3.0	0.60
10	0.60	0.58	0.68	0.15	3.4	1.1
11	0.24	0.30	0.19	0.07	2.0	0.48
12	0.46	0.40	0.81	0.10	NA	NA
13	0.48	0.42	0.60	0.11	4.3	1.6
14	1.5	0.75	1.8	0.22	7.9	3.4
15	0.24	0.43	0.70	0.16	2.7	1.2
16	1.5	0.53	0.81	0.15	0.64	0.17
17	0.27	0.29	0.34	0.09	4.0	1.4
18	0.35	0.23	0.52	0.14	2.3	0.97
19	0.92	0.43	0.87	0.63	NA	NA
20	0.59	0.13	0.43	0.01	3.5	1.3
21	0.52	0.45	1.1	0.11	2.2	1.1
Mean \pm SD	0.61 ± 0.47	0.40 ± 0.19	0.71 ± 0.61	0.14 ± 0.13	3.2 ± 1.5	1.2 ± 0.67
Range	0.11-1.9	0.09-0.77	0.15-2.7	0.01-0.63	0.64-7.9	0.17-3.4

*Total HOPhe = 1-HOPhe + 2-HOPhe + 3-HOPhe + 4-HOPhe.

Table 2. Correlations among total and individual phenanthrols in smokers and nonsmokers

	<i>r</i> (<i>P</i>)			
	1-HOPhe	2-HOPhe	3-HOPhe	4-HOPhe
total HOPhe* (smokers, <i>N</i> = 25)	0.96 (<0.0001)	0.81 (<0.0001)	0.89 (<0.0001)	0.66 (<0.0003)
total HOPhe* (nonsmokers, <i>N</i> = 21)	0.85 (<0.0001)	0.83 (<0.0001)	0.88 (<0.0001)	0.73 (<0.0001)

*Total HOPhe = 1-HOPhe + 2-HOPhe + 3-HOPhe + 4-HOPhe.

had been smoking 24 ± 14 years (mean \pm SD). The results of the analyses for phenanthrols are summarized in Table 1. Mean levels of 1-HOPhe (0.96 ± 1.2 pmol/mg creatinine) and 3-HOPhe (0.82 ± 0.62 pmol/mg creatinine) were similar. The mean levels of these phenanthrols were significantly greater than that of 2-HOPhe (0.47 ± 0.29 pmol/mg creatinine, $P < 0.001$). The mean level of 4-HOPhe (0.11 ± 0.07 pmol/mg creatinine) was consistently and significantly less than those of the other three phenanthrols ($P < 0.0001$).

Twenty-one nonsmokers (12 male) participated in the study. The results were similar to those obtained in smokers (Table 1). Mean levels of 1-HOPhe (0.61 ± 0.48 pmol/mg creatinine) and 3-HOPhe (0.71 ± 0.61 pmol/mg creatinine) were similar and significantly ($P < 0.002$) greater than that of 2-HOPhe (0.40 ± 0.19 pmol/mg creatinine). The mean level of 4-HOPhe (0.14 ± 0.13 pmol/mg creatinine) was significantly less than those of the other phenanthrols ($P < 0.0001$). There was no significant difference between levels of any of the phenanthrols in smokers and nonsmokers.

Correlations of individual phenanthrols with total phenanthrols (excluding 9-HOPhe) are summarized in Table 2 and are illustrated for 3-HOPhe in Fig. 7A, B. All correlations were highly significant.

Because urine samples are often kept at room temperature for some time before freezing, we investigated the stability of the phenanthrols under these conditions. Levels of phenanthrols were the same at the time of collection and after storage at room temperature for 4, 12, 24, and 48 hours.

Ratios of *trans, anti*-PheT to 3-HOPhe and total phenanthrols (excluding 9-HOPhe) are summarized for smokers and nonsmokers in Table 1. In smokers, there was a 7.5-fold spread of *trans, anti*-PheT/3-HOPhe ratios and 4 of 25 subjects had ratios greater than the mean plus 1 SD. In nonsmokers, the corresponding figures were a 12.3-fold spread and 1 of 21 subjects with ratio greater than the mean plus 1 SD.

Discussion

We have developed a straightforward method for quantitation of phenanthrols in human urine. There are several advantages in our method. First, the purification procedure leading to a fraction enriched in phenanthrols is relatively simple, involving two solid phase extraction steps using commercially available materials. Second, the GC resolution of the individual

phenanthrols is outstanding, allowing quantitation of individual isomers (Fig. 4). Third, the specificity and sensitivity of GC-PCI-MS allows positive identification of specific phenanthrols, by retention time and selected ion monitoring at $[M + H]^+$, m/z 267 (Fig. 5). Finally, the use of a ^{13}C -labelled internal standard, [*ring*- $^{13}\text{C}_6$] 3-HOPhe, monitored at its $[M + H]^+$, m/z 273 (Fig. 5), provides reliable quantitation.

Among the GC-MS methods described in the literature, some use fairly simple enrichment procedures but provide limited quantitative data on a range of phenanthrols in urine and are consequently difficult to compare with our method (39-42). The method described by Jacob et al. (38) is operationally more complex than ours, but does yield comparable data for individual phenanthrols (Table 3). Although GC-MS requires derivatization, it is probably superior to currently available HPLC methods because of its higher resolving power and specificity, allowing reliable quantitation of individual phenanthrols. Data reported in the literature for individual phenanthrols, as measured by HPLC, are summarized in Table 3. Levels seem to be somewhat higher than ours although relative amounts of the individual phenanthrols are similar to our data.

We did not observe a significant difference in levels of urinary phenanthrols in smokers and nonsmokers. This is consistent with the results of Jacob et al. (38), using GC-MS, but differs from those reported by Angerer et al. (27) and Heudorf and Angerer (31), who used HPLC and found significantly higher levels of 2-HOPhe, 3-HOPhe, and 4-HOPhe in smokers than in nonsmokers (Table 3; ref. 27). Both Heudorf and Angerer (31), based on phenanthrols only, and Jacob et al. (38), based on phenanthrols plus phenanthrene dihydrodiols, observed a lower ratio of 1,2-oxidation/3,4-oxidation in smokers versus nonsmokers. This was attributed to induction of 3,4-oxidation, perhaps through cytochrome P450 1A2, by cigarette smoke. In our data, the ratio 1- + 2-HOPhe/3 + 4-HOPhe was 1.5 in smokers and 1.2 in nonsmokers. Thus, we saw no difference in this ratio in smokers and nonsmokers, which is different from the previous reports.

We propose to use *trans, anti*-PheT, the end product of the diol epoxide metabolic activation pathway of phenanthrene, as a biomarker of PAH metabolic activation. We hope to incorporate this biomarker into molecular epidemiology studies of smokers to determine

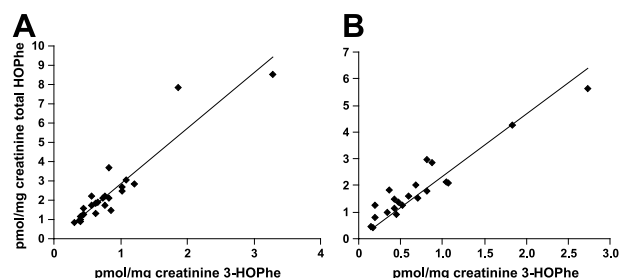


Figure 7. Relationship between the sum (1-HOPhe + 2-HOPhe + 3-HOPhe + 4-HOPhe) and 3-HOPhe in the urine of (A) smokers ($N = 25$, $r = 0.89$) and (B) nonsmokers ($N = 21$, $r = 0.88$).

Table 3. Comparison of phenanthrol levels reported previously with those reported here

	pmol/mg creatinine*			
	1-HOPhe	2-HOPhe	3-HOPhe	4-HOPhe
Smokers				
GC-MS, Jacob et al. (38) [†]	1.3	0.99	1.9	0.30
HPLC, Angerer et al. (27) [†]	1.7	1.3 [‡]	1.9	0.31
HPLC, Heudorf and Angerer (31)	1.6	1.2 [‡]	1.8	0.24
This study	0.96	0.47	0.82	0.11
Nonsmokers				
GC-MS, Jacob et al. (38) [†]	1.7	0.89	1.2	0.28
HPLC, Angerer et al. (27) [†]	1.6	0.97 [‡]	0.97	0.13
HPLC, Heudorf and Angerer (31)	1.7	1.0 [‡]	1.1	0.22
This study	0.61	0.40	0.71	0.14

*Mean \pm SD except Angerer et al. (27), median.

[†]Based on 1.2 g creatinine excreted per 24 hours.

[‡]Includes 9-HOPhe.

whether those individuals who are more efficient in metabolic activation of phenanthrene are at higher risk for cancer. However, *trans*, *anti*-PheT is affected by both exposure and metabolic activation. Our major goal in the present study was to develop a method to quantify phenanthrols. Levels of phenanthrols could then be used to correct for exposure. We propose that a ratio of *trans*, *anti*-PheT to phenanthrols would accomplish this correction. Because cytochrome P450s are involved in the metabolism of phenanthrene to both *trans*, *anti*-PheT and phenanthrols, one possible result could have been that *trans*, *anti*-PheT/phenanthrol ratios would not differ among individuals. However, we did observe a 7.5-fold spread of ratios in smokers and a 12.3-fold spread in nonsmokers, suggesting that this may be a useful parameter for distinguishing individuals. Further research is required to determine the frequency distribution of the ratio in large numbers of subjects and to investigate its longitudinal stability in a given subject.

In summary, we have developed a convenient, accurate, and precise MS method for the quantitation of phenanthrols in human urine. We anticipate the application of this method as one component of a carcinogen metabolite phenotyping approach to determine whether differences in PAH metabolism affect cancer risk.

Acknowledgments

We thank Patrick Kenney for technical assistance, Peter Villalta, Analytical Biochemistry Shared Resource, for assistance with mass spectrometry, Yan Zhang, Biostatistics Shared Resource, for statistical assistance, and Bob Carlson for editorial assistance.

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BLOOD CANCER DISCOVERY

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Cancer Epidemiol Biomarkers Prev 2004;13:2167-2174.

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