ISOTOPE DILUTION QUANTITATION OF PAH METABOLITES AT TRACE LEVELS USING A GC-MSMS MRM METHOD

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Introduction
Humans are exposed to polycyclic aromatic hydrocarbons (PAHs) from various occupational, environmental, medicinal, and dietary sources. PAH metabolites in human urine can be used as biomarkers to assess exposure to PAHs. PAH metabolites have been detected in human urine as a number of hydroxylated PAHs. Metabolites are found at low levels in human urine even in persons without apparent occupational or smoking exposure. Although measurement of these metabolites is used in assessing recent exposure to PAHs, their value as predictive markers of biological effect or health outcomes is currently being evaluated. In order to perform comprehensive epidemiological studies where multiple metabolites of several PAHs can be measured and compared in low-dose urine samples, fast and robust methods are needed to measure many analytes.

Tab.1: PAH-OH metabolites investigated

No.	*Parent PAH*	*Metabolite/Analyte*	*Abbreviation*	*M (underiv.)*	*M (MSTFA)*
1	Naphthalene	1-hydroxynaphthalene	1-nap	144,0575	216,0970
2	Naphthalene	2-hydroxynaphthalene	2-nap	144,0575	216,0970
3	Fluorene	2-hydroxyfluorene	2-fluo	182,0732	254,1127
4	Fluorene	3-hydroxyfluorene	3-fluo	182,0732	254,1127
5	Fluorene	9-hydroxyfluorene	9-fluo	182,0732	254,1127
6	Phenanthrene	1-hydroxyphenanthrene	1-phen	194,0732	266,1127
7	Phenanthrene	2-hydroxyphenanthrene	2-phen	194,0732	266,1127
8	Phenanthrene	3-hydroxyphenanthrene	3-phen	194,0732	266,1127
9	Phenanthrene	4-hydroxyphenanthrene	4-phen	194,0732	266,1127
10	Phenanthrene	9-hydroxyphenanthrene	9-phen	194,0732	266,1127
11	Fluoranthene	3-hydroxyfluoranthene	3-flran	218,0732	290,1127
12	Pyrene	1-hydroxypyrene	1-pyr	218,0732	290,1127
13	Benzo(c)phenanthrene	1-hydroxybenzo(c)phenanthrene	1-bcp	244,0888	316,1283
14	Benzo(c)phenanthrene	2-hydroxybenzo(c)phenanthrene	2-bcp	244,0888	316,1283
15	Benzo(c)phenanthrene	3-hydroxybenzo(c)phenanthrene	3-bcp	244,0888	316,1283
16	Chrysene	1-hydroxychrysene	1-chry	244,0888	316,1283
17	Chrysene	2-hydroxychrysene	2-chry	244,0888	316,1283
18	Chrysene	3-hydroxychrysene	3-chry	244,0888	316,1283
19	Chrysene	4-hydroxychrysene	4-chry	244,0888	316,1283
20	Chrysene	6-hydroxychrysene	6-chry	244,0888	316,1283
21	Benzo(a)anthracene	1-hydroxybenz(a)anthracene	1-baa	244,0888	316,1283
22	Benzo(a)anthracene	3-hydroxybenz(a)anthracene	3-baa	244,0888	316,1283
23	Benzo(a)anthracene	9-hydroxybenz(a)anthracene	9-baa	244,0888	316,1283
24	Benzo(a)pyrene	3-hydroxybenzo(a)pyrene	3-bap	268,0888	340,1283
25	Benzo(a)pyrene	7-hydroxybenzo(a)pyrene	7-bap	268,0888	340,1283

Materials and Methods
A multiple reaction monitoring (MRM) GC-MSMS method using the Thermo Fisher Triple quadrupole mass spectrometer TSQ Quantum GC has been evaluated for low level quantitation. 17 carbon 13 labeled standards have been added for isotope-dilution calibration and quantitation. Using this method, we measured 25 metabolites, representing 9 parent PAHs, with detection limits in the low pg/uL range.

Tab. 2: ^{13}C-labeled PAH-OH standards

No.	*Parent PAH*	*Metabolite/Analyte*	*Abbreviation*	*M (MSTFA)*
1	Naphthalene	1-hydroxynaphthalene$^{13}C_6$	1-napC13	222,1172
2	Naphthalene	2-hydroxynaphthalene$^{13}C_6$	2-napC13	222,1172
3	Fluorene	2-hydroxyfluorene$^{13}C_6$	2-fluoC13	260,1328
4	Fluorene	3-hydroxyfluorene$^{13}C_6$	3-fluoC13	260,1328
5	Fluorene	9-hydroxyfluorene$^{13}C_6$	9-fluoC13	260,1328
6	Phenanthrene	1-hydroxyphenanthrene$^{13}C_6$	1-phenC13	272,1328
7	Phenanthrene	2-hydroxyphenanthrene$^{13}C_6$	2-phenC13	272,1328
8	Phenanthrene	3-hydroxyphenanthrene$^{13}C_6$	3-phenC13	272,1328
9	Phenanthrene	4-hydroxyphenanthrene$^{13}C_6$	4-phenC13	272,1328
10	Phenanthrene	9-hydroxyphenanthrene$^{13}C_6$	9-phenC13	272,1328
11	Fluoranthene	3-hydroxyfluoranthene$^{13}C_6$	3-flranC13	296,1328
12	Pyrene	1-hydroxypyrene$^{13}C_6$	1-pyrC13	296,1328
13	Benzo(c)phenanthrene	1-hydroxybenzo(c)phenanthrene$^{13}C_6$	1-bcpC13	322,1485
18	Chrysene	3-hydroxychrysene$^{13}C_6$	3-chryC13	322,1485
20	Chrysene	6-hydroxychrysene$^{13}C_6$	6-chryC13	322,1485
21	Benzo(a)anthracene	1-hydroxybenz(a)anthracene$^{13}C_6$	1-baaC13	322,1485
24	Benzo(a)pyrene	3-hydroxybenzo(a)pyrene$^{13}C_3$	3-bapC13	343,1380

The PAH-OH metabolites given in Tab.1 and the ^{13}C labeled standards in Tab.2 were analyzed with the TSQ Quantum GC. A set of 8 standards (named STD1 to STD8), a PAH-OH blank and two quality control samples (QC A and B) were measured.

Tab. 3: Analytical Conditions

Trace GC Ultra:	
Injector:	Split/splitless, 260 °C
	Splitless injection, 1 uL
	Split time 1.2 min
	Split flow 70 mL/min
GC column:	30m x 0.25mm ID x 0.25 film (5% phenyl dimethylsiloxane)
Carrier gas	He, constant flow 0.9 mL/min
Oven temp. program:	85 °C, 1 min
	15 °C/min, 165 °C
	3 °C/min, 195 °C
	20 °C/min, 250 °C
	1.5 °C/min, 260 °C
	30 °C/min, 310 °C, 4 min
TSQ Mass Spectrometer:	
Ionisation mode	EI, 70 eV
Emmission current:	100 uA
Ion volume	CEI volume
Q1 mass resolution:	0.7 Da
Collision gas:	Ar, 1.0 – 1.5 mTorr

Results and Discussion

Full scan spectra have been recorded and investigated for suitable SRM transitions in a scan range of 80 – 500 Da with a scan time of 0.4 s. The Q1 peak width was set to 0.7 Da.

Product ion experiments were performed with the molecular ions of the PAH-OH metabolites applying different collision energies and different collision gas pressures. The collision energy range used was 10 - 20 eV, and Ar collision gas pressures of 1.0 and 1.5 mTorr.

Common conditions for SRM experiments showed that under these MS/MS conditions the loss of 15 Da is the most intense fragmentation for most PAH-OH metabolites. This fragmentation was therefore used as the

quantitation transition. The loss of 31 Da, which is generally detected with lower intensity, was used as the identifier transition. Exceptions have been 9-FLUO and 3-FLUO with the loss of 89 Da (quantitation). The loss of 15 u was used as the identifier. With 3-BAP the loss of 16 Da (quantitation) was slightly more intense then the loss of 15 Da (identifier). Collision energies used in the final method have been between 15 and 20 eV at a collisions gas pressure between 1.0 mT and 1.5 mT has been applied. For the data acquisition 7 retention time segments were used.

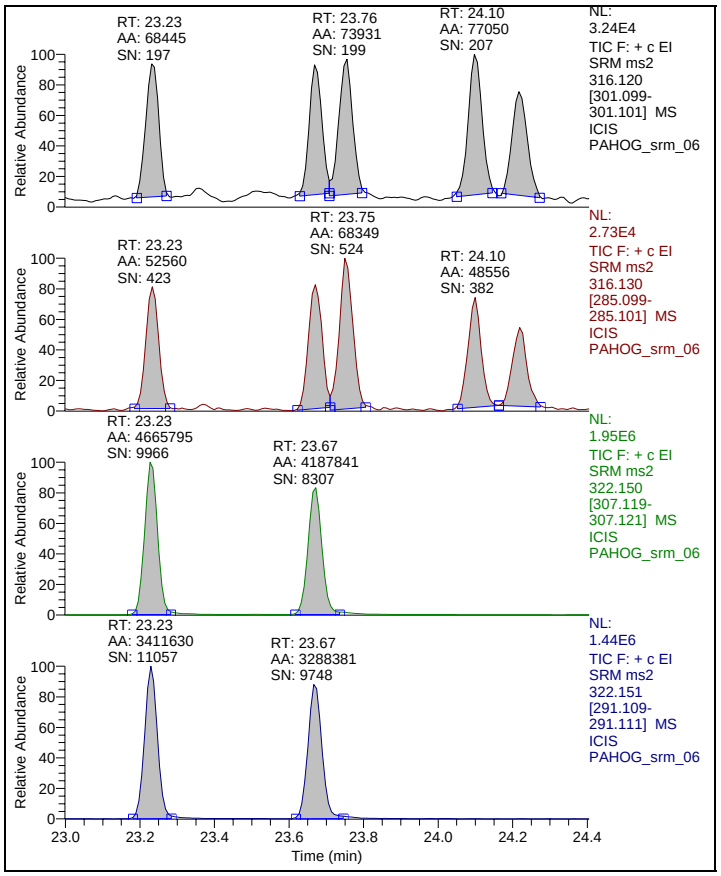

Fig. 1: 3-BCP, 3-CHRY, 1-CHRY, 3/9-BAA, 2-CHRY: STD 1 (1 pg/uL level)

Figure 1 shows the elution profile of the Benzo(c)phenanthrene (BCP-3), Chrysene (1/2-CHRY), Benzo(a)anthracene (3/9-BAA) metabolites with the common precursor mass m/z 316.1283. The labeled internal standards are detected using precursor ion m/z 322.1485. The metabolite compounds can be detected at the 1 pg/uL level with high signal/noise level of 200:1.

The quantitative calibration was achieved covering 3 orders of magnitude from 1pg to 1000 pg with good linearity and a regression coefficient of 0.9998, see Fig. 2.

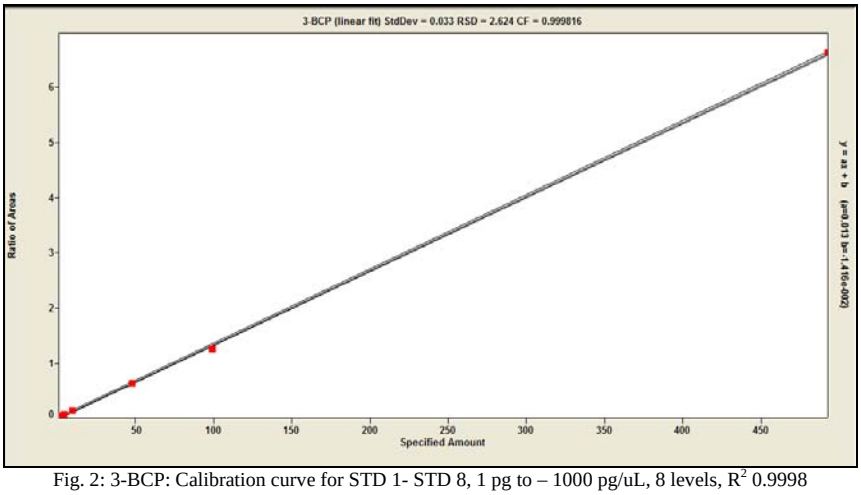

Fig. 2: 3-BCP: Calibration curve for STD 1- STD 8, 1 pg to – 1000 pg/uL, 8 levels, R^2 0.9998

With the applied GC-MSMS technique a sensitive and reliable detection and quantitation of the investigated 25 PAH-OH metabolites at trace levels can routinely be achieved.

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention. This abstract should not be taken as an endorsement of the products mentioned.

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