

Poster Reprint

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Analysis of Polycyclic Aromatic Hydrocarbons (PAH) and Hydroxylated PAH Metabolites in Plasma and Urine Using High-Resolution GC/Q-TOF

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are products of incomplete combustion of organic materials and present ubiquitously in the environment. PAHs are well-know carcinogens, and the primary sources of human exposure to PAHs are environmental, dietary as well as occupational [1]. Hydroxylated metabolites of the PAHs are present at trace levels in biological matrices, and can be used as biomarkers of the recent exposure to these compounds [1]. Because of high analytical sensitivity requirements from the analytical methods for PAH metabolite analysis, Triple Quadrupole GC/MS instruments are preferred for their targeted analysis [2]. To add capability for untargeted analysis in addition to a highly sensitive targeted approach for detection of the persistent organic pollutants and their metabolites, use of a high resolution accurate mass 7250 GC/Q-TOF system was evaluated in the current study. Thus, we have applied a targeted approach for the detection of PAHs and PAH metabolites, and an untargeted approach to discover other biologically relevant compounds in human urine and plasma extracts.

Experimental



Experimental

Six milliliters of pooled plasma and urine samples were extracted with an equal volume of hexane/acetone (1:1, v/v). To these, 1.1 g magnesium sulfate and 1.1 g sodium chloride was added to assist in phase separation. The extracts were spiked with various concentrations of PAH and hydroxy-PAH standards, ranging from 0.2 ppb to 2 ppm, as well as deuterated internal standards. Dried samples were derivatized with a mixture of MSTFA/pyridine and analyzed using an Agilent 7890B GC system coupled to a high resolution 7250 GC/Q-TOF, equipped with an Electron Ionization (EI) source allowing low-energy ionization (Figure 1). Instrument parameters are shown in Table 1.



GC and MS Conditior	าร:
Column	DB-5MS UI, 30 m, 0.25 mm, 0.25 µm
Injection volume	1 μL
Injection mode	Splitless
Split/Splitless inlet temperature	270°C
Oven temperature program	70°C for 1 min 20°C/min to 270°C, 10°C/min to 307°C, 40°C/min to 325°C, 4 min hold
Carrier gas	Helium at 1.2 mL/min constant flow
Transfer line temperature	280°C
Ionization mode	Standard EI at 70 eV Low Electron Energy EI at 15 eV and 12 eV
Source temperature	280°C (200°C for low electron energy)
Quadrupole temperature	150°C
Mass range	50 to 650 m/z
Spectral acquisition rate	5 Hz

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The data were processed using MassHunter Qualitative Analysis (B.08) as well as Quantitative Analysis (B.09) software. Unknowns Analysis was further used for the untargeted identification of additional PAH-like compounds and other compounds of potential interest not found in the target list.

Table 1. GC/Q-TOF conditions

Quantitation and qualitative screening approach

As a first step, an accurate mass Personal Compound Database and Library (PCDL) containing PAHs and hydroxylated PAHs in their derivatized form, (Table 2) was constructed (Figure 2) and used for automated creation of a quantitative method.

		Earmula of darivatized	m/z of			
Compound name	RT		derivatized			
		compound	compound			
Naphthalene-d8	5.485	C10D8	136.1123			
Naphthalene	5.507	C10H8	128.0621			
Acenaphthylene	7.366	C12H8	152.0621			
Acenaphthene-d10-IS	7.54	C12D10	164.1405			
Acenaphthene	7.58	C12H10	154.0777			
Fluorene	8.216	C13H10	166.0777			
1-Hydroxynaphthalene	7.838	C13 H16 O Si	216.0965			
1-Hydroxynaphthalene-d7	7.799	C13H9D7OSi	223.1404			
2-Hydroxynaphthalene	7.99	C13 H16 O Si	216.0965			
Phenanthrene-d10	9.37	C14D10	188.1405			
Phenanthrene	9.4	C14H10	178.0782			
Anthracene	9.46	C14H10	178.0782			
Fluoranthene	10.86	C16H10	202.0777			
Pyrene	11.14	C16H10	202.0777			
3-Hydroxyfluorene	10.18	C16H18OSi	254.1121			
2-hydroxyfluorene-d9	10.24	C16H9D9OSi	263.1686			
2-hydroxyfluorene	10.29	C16H18OSi	254.1121			
4-Hydroxyphenanthrene	10.76	C17H18OSi	266.1121			
1,6-Dihydroxynaphthalene	9.75	C16H24O2Si2	304.1309			
3-Hydroxyphenanthrene-d9	11.038	C17H9D9OSi	275.1686			
3-Hydroxyphenanthrene	10.94	C17H18OSi	266.1121			
2,7-Dihydroxynaphthalene	9.87	C16H24O2Si2	304.1309			
1-Hydroxyphenanthrene	11.083	C17H18OSi	266.1121			
9-hydroxyphenanthrene-d8	10.899	C17H10D8OSi	274.1624			
9-Hydroxyphenanthrene	11.12	C17H18OSi	266.1121			
Benzo(a)anthracene	12.796	C18H12	228.0934			
Chrysene-d12	12.81	C18D12	240.1687			
Chrysene	12.849	C18H12	228.0934			
1-hydroxypyrene-d9	12.761	C19H9D9OSi	299.1686			
1-Hydroxypyrene	12.816	C19H18OSi	290.1121			
Benzo(b)fluoranthene	14.484	C20H12	252.0934			
Benzo(k)fluoranthene	14.529	C20H12	252.0934			
Benz(a)pyrene	14.981	C20H12	252.0934			
Perylene-d12	15.06	C20D12	264.1687			
3-Hydroxychrysene-d11	14.521	C21H9D11OSi	327.1968			
6-Hydroxychrysene	14.08	C21H20OSi	316.1278			
Indeno(1,2,3-cd)pyrene	16.557	C22H12	276.0934			
Dibenz(a,h)anthracene	16.595	C22H14	278.1091			
Benzo(ghi)perylene-d12	16.924	C22D12	288.1687			
Benzo(ghi)perylene	16.97	C22H12	276.0934			
3-Hydroxybenzo(a)pyrene-d11	16.614	C23H9D11OSi	351.1968			
9-Hydroxybenzo(a)pyrene	16.634	C23H20OSi	340.1278			
7,8-Hydroxybenzo(a)pyrene	17.89	C26H28O2Si2	428.1622			

Table 2. List of targeted compounds

Name	Formula	Mass	Retention Time	Retention Index	Cation	Anion	CAS				
Acenaphthene	C12H10	154.07825	7.58				<u>83-32-9</u>				
Fluorene	C13H10	166.07825	8.216				<u>86-73-7</u>				
Phenanthrene	C14H10	178.07825	9.4				<u>85-01-8</u>				
Anthracene	C14H10	178.07825	9.46				120-12-7				
Fluoranthene	C16H10	202.07825	10.86				206-44-0				
Pyrene	C16H10	202.07825	11.14				<u>129-00-0</u>				
Benz[a]anthracene	C18H12	228.0939	12.796				<u>56-55-3</u>				
Chrysene	C18H12	228.0939	12.849				<u>218-01-9</u>				
Benzo[b]fluoranthene	C20H12	252.0939	14.484				205-99-2				
Benzo (k)fluoranthene	C20H12	252.0939	14.529 +ELM	STOTOE EV-70							
Benzo[a]pyrene	C20H12	252.0939	14.981	100 1				tot oconz			0544
Indeno[1,2,3-cd]pyrene	C22H12	276.0939	16.557 ²	75				99.64			204.1
Dibenz[a,h]anthracene	C22H14	278.10955	16.595 2	50- 72 04001							
Benzo[ghi]perylene	C22H12	276.0939	16.97 📮	25- 11.38		119.	54406			223.0573	7
1-Naphthalenol, TMS	C13H16OSi	216.09704	7.838	0-4		4	. 18	عيراه اللارب		2.16	ا اس
2-Naphthalenol, TMS	C13H16OSi	216.09704	7.991	80	10	0 1	140 140	160 180	200	220	240
3-Hvdroxyfluorene, TMS	C16H18OSi	254,11269	10.178	m/z							

The examples of matrix-matched calibration curves for PAHs and their hydroxylated metabolites are shown in Figure 3.

In most cases, the calibration curves were linear up to 2000 ng/mL. However, in a few cases, non-linearity was observed above approximately 1000 ng/mL.



Figure 3. Calibration curves examples for both urine and plasma matrices (0.2-2000 ng/mL). EIC overlay for concentrations 0.2-20 ng/mL is shown on the right.

Mass accuracy (Table 3) is shown for PAH and OH-PAH in plasma matrix across a concentration range of 2-2000 ppb and on average was below 1 ppm.

LOD for both urine and plasma matrices were calculated based on 5 replicate injections and are shown in Table 4.

In addition to quantitation, the PCDL was also utilized in quick qualitative screening using MassHunter Qualitative Analysis (shown in Figure 4).



Figure 2. Accurate Mass in PCDL format created for PAHs and hydroxylated PAH metabolites.

16.90	Benzo(ghi)pervlene-	D12	1	Qualified -0.001	7.18	6	99.49	0.65				
6.94	Benzo[ghi]perylene		1	Qualified -0.024	7.48	6	99.07	0.51			28-	
					D hittense App						24-	
6	oelution Score 🛛 🖨 Cl	E -⇔FragMas	sDiff(ppm) 🕁	Flags(FIs)	4	⊐ FV +¤ Height +₽	Abundance(Lib) 🖨	mz(Lib) +∈	m/z 🖡	and the and the loss set a la	2	
	100		0.5	Ref	erence ion	37091.2	100	276.0934	276.0932	wy with Rephysical a Compound Name	1.0-	
	97.82		0.6		Qualified	8621.3	18.6	274.0777	274.0775	163032 1585.3 decorpt/perject 163075 3232.4 decorpt/perject	12-	
	98.99		1		Qualified	8018.3	17.1	277.0967	277.0964	17.0014 J212.4 Becauge/geodes 15.0023 GRL4 Becauge/geodes	0.0-	
L .	98.07		14.5	Qualified;Multiple evid	ence peaks	4451.3	9.9	275.0855	275.0815	15959 19459 beautyvijeener 15968 SIAT beautyvijeene	04-	
	95.24		4.1		Qualified	9976	16.1	138.0464	138.0458		-	
	97.28		0.8		Qualified	2208.2	4	272.0621	272.0618		2.4 10.00 10.00	when many who make when when when when when when when the tribut tribut to
					-	-			1.11	(W)		Covin vs. Acquisition Time (set)

Figure 4. Example of screening results using PCDL MassHunter Qulitative software

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Unknowns identification and confirmation of tentative hits

The untargeted workflow using Unknowns Analysis and NIST 17.L library identified several additional PAH-like compounds and other xenobiotics, including 1-methyl-2-(phenylmethyl) benzene, benzophenone, pentochlorophenol, Escitalopram, octocrylene, 1,12-dimethyl benz(a)anthracene and dibenz(a,j)acridine in urine and cotinine (biomarker of exposure to tobacco smoke [3]) and 1-naphthoic acid in plasma (Figure 5Å). Accurate mass information as well as retention indices were utilized to confirm the candidate hit's identity. A proprietary Electron Ionization (EI) source design allowed for low energy ionization to assist in the confirmation of candidate molecular ions (Figure 5B).

	Mas	ss Acc	uracy (ppm) p	ber cor	ncentra	ation	LOD (ng/mL) per matrix			
Compound/concentration, ng/mL	2	8	20	80	200	800	2000	Compound name	Urine Plasma		
Naphthalene	0.47	0.56	0.47	1.02	0.55	1.48	0.84	Naphthalene	0.52 0.38		A
Acenaphthylene	0.63	0.50	0.18	0.40	0.19	0.45	0.79	Acenaphthylene	0.42	0.74	
Acenaphthene	0.93	0.52	0.97	1.85	1.37	2.16	3.37	Acenaphthene	0.98	1.28	Tender film Servert film an Servert film an Servert film an Servert film and Servert film a
1-Hydroxynaphthalene	0.74	0.49	0.48	0.52	0.50	0.74	0.86	1-Hydroxynaphthalene	0.48	0.47	Component Compound Name Match Best Formula Compone Base RI Peak RI Peak
2-Hydroxynaphthalene	0.95	0.51	0.54	0.47	0.43	0.76	1.07	2-Hydroxynaphthalene	0.28	0.20	12 2307 O-Bencyl-Lynosine 73.9 ✔ C16H17N03 2408 91.05 12 2687 2-Propylgentanoic acid, 2,3,4.6+etrat/met 68.6 ✔ C25H580 2416 73.04
Fluorene	0.92	1.23	1.07	0.39	0.77	0.96	1.45	Fluorene	1.12	0.26	12.3548 Arachidic acid, TMS derivative 79.8 ☑ C23H480 24/3 117.03
Phenanthrene	0.64	0.45	0.88	1.19	1.34	0.64	0.61	Phenanthrene	0.35	0.18	12 4393 Cyclodecasloxane, elcosamethyl- 86.7 2 C20H600 2450 73.04 *** 12 5176 Elcotelogram 65.4 2 C20H21F 2466 58.0
Anthracene	0.50	0.44	0.84	1.35	1.28	0.80	0.46	Anthracene	0.36	0.26	Coll Description D* Coll Description D* Coll Description D* Coll Description D* D* <th< td=""></th<>
1,6-Dihydroxynaphthalene	0.73	1.26	0.62	0.36	0.62	0.40	0.56	1,6-Dihydroxynaphthalene	0.34	0.16	Unit Surgery & Ready & Surgery & Control Str.
2,7-Dihydroxynaphthalene	0.35	0.55	0.78	1.05	0.86	0.41	0.53	2,7-Dihydroxynaphthalene	0.13	0.10	1020 Server 20 Telever 10 20 20 Control 20 10 20 20 20 20 20 20 20 20 20 20 20 20 20
3-Hydroxyfluorene	0.59	0.59	0.79	0.82	0.43	0.40	0.34	3-Hydroxyfluorene	0.22	0.36	
2-hydroxyfluorene	0.96	0.54	0.66	0.73	0.51	0.49	0.34	2-hydroxyfluorene	0.25	0.21	
4-Hydroxyphenanthrene	0.70	0.90	0.34	0.36	0.34	0.38	0.59	4-Hydroxyphenanthrene	0.39	0.42	_
Fluoranthene	0.41	0.56	0.37	0.52	0.73	0.73	0.71	Fluoranthene	0.30	0.19	В
3-Hydroxyphenanthrene	0.78	0.39	0.38	0.36	0.43	0.21	0.38	3-Hydroxyphenanthrene	0.60	0.27	
1-Hydroxyphenanthrene	0.46	0.37	0.47	0.55	0.59	0.37	0.35	1-Hydroxyphenanthrene	0.34	0.30	1 +EI ccan (t): 12.515-12.525 min, 4 scans) Frag=70.00 U-U_Blankx_/08U_0H2.U Subtract
9-Hydroxyphenanthrene	0.52	0.65	0.37	0.74	0.46	0.26	0.45	9-Hydroxyphenanthrene	0.50	0.33	
Pyrene	0.35	0.25	0.36	0.40	0.42	0.70	0.51	Pyrene	0.42	0.10	75.0271 95.0292 147.0661 207.0348 238.0665 281.0538 324.1639 3.96 1.99 5.65 8.30 6.83 4.22 2.78
Benzo(a)anthracene	0.58	0.48	0.33	0.58	0.59	1.11	1.71	Benzo(a)anthracene	0.16	0.23	+EI Scan (rt: 12.523-12.533 min, 4 scans) Frag=15.0V U-D_BlankN_15eV_5Hz.D Subtract
Chrysene	0.93	0.66	0.41	0.56	0.44	0.92	1.51	Chrysene	0.26	0.09	15 eV
6-Hydroxychrysene	0.37	0.25	0.62	0.53	0.36	0.20	0.60	6-Hydroxychrysene	0.14	0.16	- 324.1641 71.0856 92.0615 147.0660 217.1080 283.0519 26.52
Benzo(b)fluoranthene	1.14	0.46	0.49	0.70	0.74	0.92	0.98	Benzo(b)fluoranthene	0.38	0.26	0.00 0.02 524 10.2
Benzo(k)fluoranthene	0.59	0.62	0.93	0.75	0.99	0.21	0.55	Benzo(k)fluoranthene	0.38	0.27	
Benz(a)pyrene	0.53	0.91	0.32	0.28	0.42	0.44	0.76	Benz(a)pyrene	0.61	0.30	- 75.0260 119.0547 140.023 207.0206 265.1461 20.70
Indeno(1,2,3-cd)pyrene	0.29	0.43	0.48	0.62	0.44	0.47	0.78	Indeno(1,2,3-cd)pyrene	0.65	0.35	- 19.78 14.20 14.59 14.59 14.83 17.22
Dibenz(a,h)anthracene	0.57	0.42	0.50	0.47	0.47	0.98	1.06	Dibenz(a,h)anthracene	0.51	0.09	50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 Counts vs. Mass-to-Charge (m/z)
9-Hydroxybenzo(a)pyrene	0.68	0.67	0.84	0.65	0.49	0.45	0.51	9-Hydroxybenzo(a)pyrene	0.47	0.34	
Benzo(ghi)perylene	0.88	0.76	0.61	0.65	0.43	0.61	0.35	Benzo(ghi)perylene	0.89	0.36	
7,8-Hydroxybenzo(a)pyrene	2.57	1.17	0.98	0.57	0.81	0.48	0.93	7,8-Hydroxybenzo(a)pyrene	6.65	3.63	Figure 5. Unknowns Analysis

Table 3. Mass accuracy in plasma observed for PAHs and hydroxylated PAH metabolites across concentrations of 2-2000 ppb.

Table 4. LOD calculated for PAH and PAH metabolites in plasma and urine matrices based on 5 replicate injections.

results showing an example of a tentatively identified compound in urine matrix using NIST17.L (A) as well as 70 eV and low electron energy spectra for the tentatively identified compound (B).

Conclusions

Targeted quantification and untargeted screening for PAHs, their metabolites, and other substances in complex biological matrices was successfully performed using an HRMS GC/Q-TOF.



¹ Strickland MK, Kang D, and Sithisarankul P. Environ Health Perspect., 1996, 104(Suppl 5): 927–932. ² Gupta P, et al., Journal of analytical toxicology. April 2015, 39(5). ³ Benowitz NL, Epidemiol Rev. 1996;18(2):188-204.

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