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

Sofia Nieto¹, Michael Armstrong², Anthony Macherone¹, Marc Elie², Richard Reisdorph², Nathan Eno¹ and Nichole Reisdorph²

¹Agilent Technologies Inc., Santa Clara, CA ;

²University of Colorado School of Pharmacy, Aurora, CO

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are products of incomplete combustion of organic materials and present ubiquitously in the environment. PAHs are well-known 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

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.

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.

Experimental



Figure 1. Agilent 7250 GC/Q-TOF

GC and MS Conditions:

Column	DB-5MS UI, 30 m, 0.25 mm, 0.25 μ m
Injection volume	1 μ L
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

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.

Compound name	RT	Formula of derivatized compound	m/z of derivatized 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

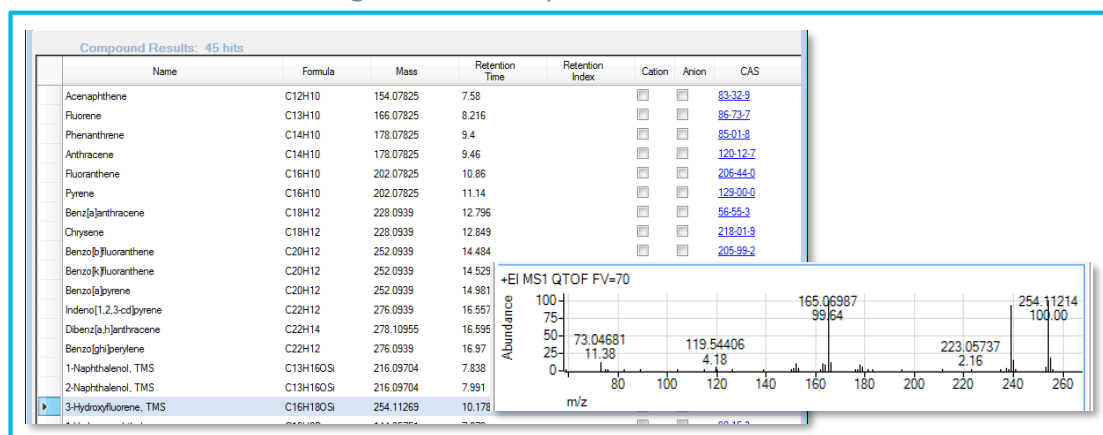


Figure 2. Accurate Mass in PCDL format created for PAHs and hydroxylated PAH metabolites.

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.

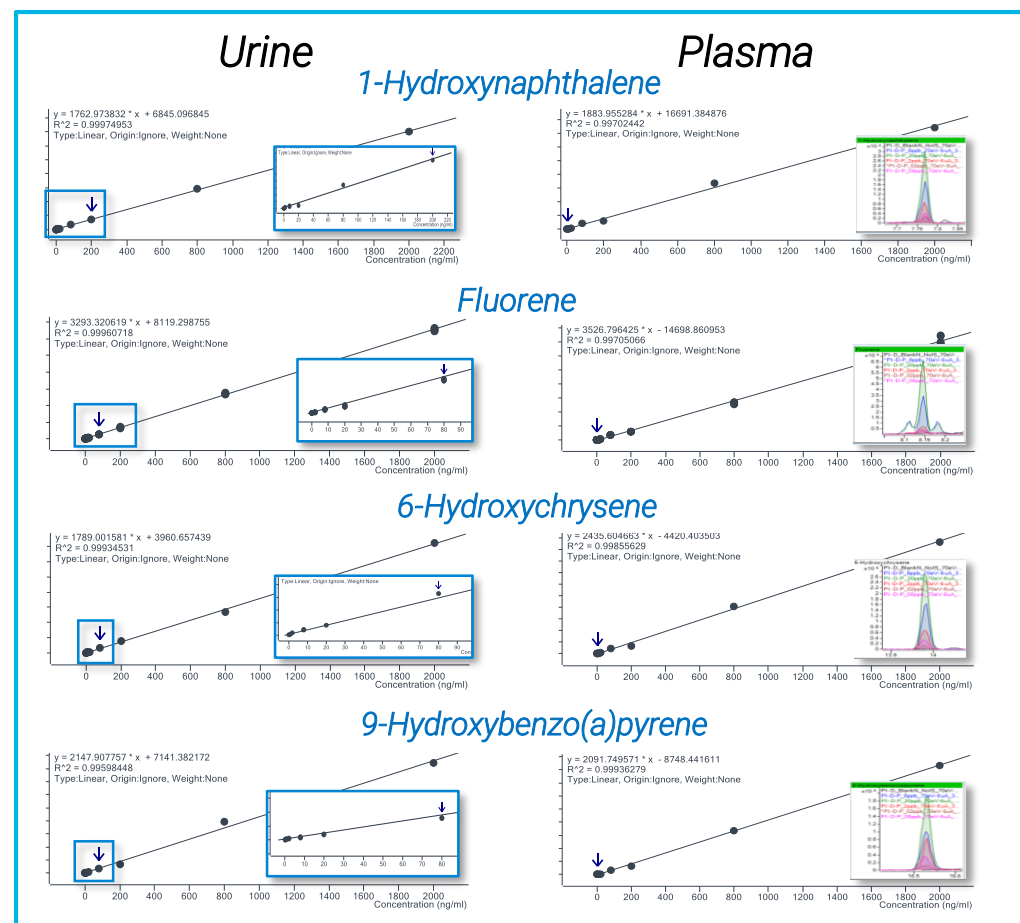


Figure 3. Calibration curves examples for both urine and plasma matrices (0.2-2000 ng/mL). EIC overlay for concentrations 0.2-20 ng/mL is shown on the right.

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).

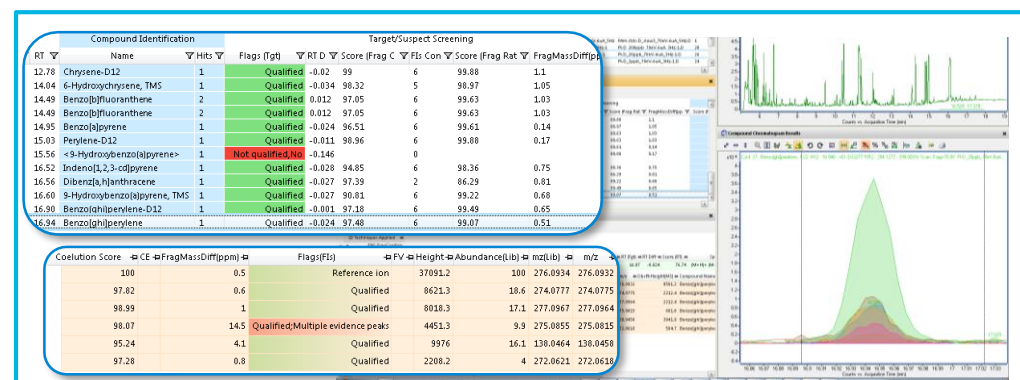


Figure 4. Example of screening results using PCDL MassHunter Qualitative software

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 5A). 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).

Compound/concentration, ng/mL	Mass Accuracy (ppm) per concentration							LOD (ng/mL) per matrix		
	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
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
1-Hydroxynaphthalene	0.74	0.49	0.48	0.52	0.50	0.74	0.86	1-Hydroxynaphthalene	0.48	0.47
2-Hydroxynaphthalene	0.95	0.51	0.54	0.47	0.43	0.76	1.07	2-Hydroxynaphthalene	0.28	0.20
Fluorene	0.92	1.23	1.07	0.39	0.77	0.96	1.45	Fluorene	1.12	0.26
Phenanthrene	0.64	0.45	0.88	1.19	1.34	0.64	0.61	Phenanthrene	0.35	0.18
Anthracene	0.50	0.44	0.84	1.35	1.28	0.80	0.46	Anthracene	0.36	0.26
1,6-Dihydroxynaphthalene	0.73	1.26	0.62	0.36	0.62	0.40	0.56	1,6-Dihydroxynaphthalene	0.34	0.16
2,7-Dihydroxynaphthalene	0.35	0.55	0.78	1.05	0.86	0.41	0.53	2,7-Dihydroxynaphthalene	0.13	0.10
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
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
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
Benzo(a)anthracene	0.58	0.48	0.33	0.58	0.59	1.11	1.71	Benzo(a)anthracene	0.16	0.23
Chrysene	0.93	0.66	0.41	0.56	0.44	0.92	1.51	Chrysene	0.26	0.09
6-Hydroxychrysene	0.37	0.25	0.62	0.53	0.36	0.20	0.60	6-Hydroxychrysene	0.14	0.16
Benzo(b)fluoranthene	1.14	0.46	0.49	0.70	0.74	0.92	0.98	Benzo(b)fluoranthene	0.38	0.26
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
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
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
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

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.

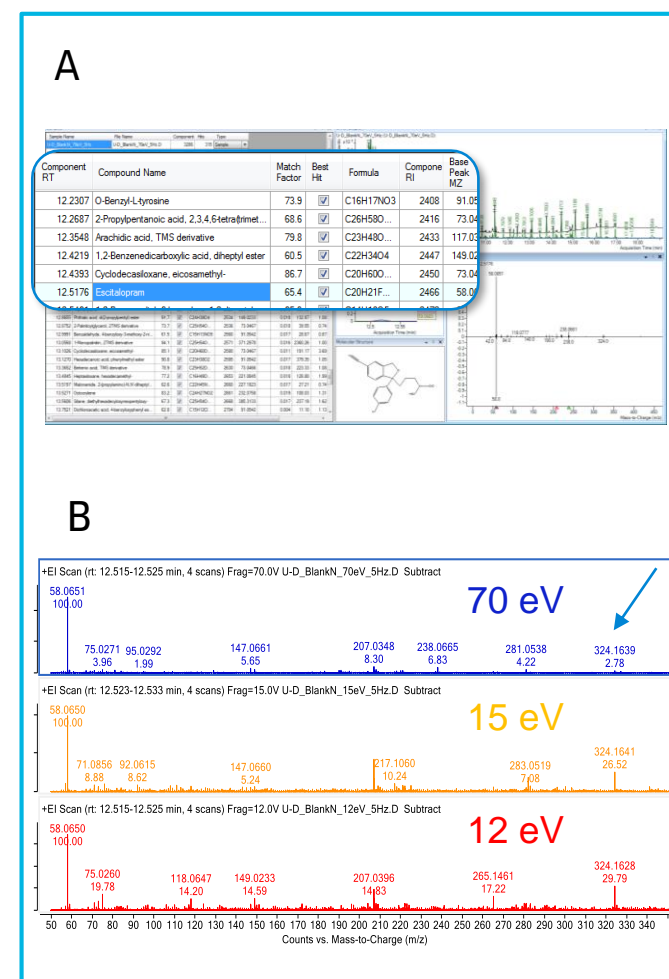


Figure 5. Unknowns Analysis results showing an example of a tentatively identified compound in urine matrix using NIST17.L (A) as well as 70 eV and low electron energy spectra for the tentatively identified compound (B).

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.

References

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