DEVELOPMENT OF HYDROXYLATED POLYCYCLIC AROMATIC HYDROCERBON(OH-PAHs) ANALYTICAL METHOD IN BIOLOGICAL SAMPLE WITH UPLC/Q-TOF MS

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are known as environmental contaminants which occur in oil, coal and tar deposits, and are produced as byproducts of fuel burning. PAHs are also found in cooked foods. Some of PAHs may cause abnormal effect such as carcinogenicity, mutagenicity and teratogenicity. Some studies determined the residue levels of PAHs and metabolized PAHs (hydroxylated PAHs : OH-PAHs) in biological sample. Hydroxyl group of OH-PAHs has high polarity and boiling point, therefore OH-PAHs was made derivatization such as acetylation, trimethylsilylation and analyzed with GC-MS. Complicated preparations are needed for analyzing with GC-HRMS, beside that assining metabolized PAHs and derivatized OH-PAHs is difficult.

For solving analytical issue of measurement of metabolized environmental contaminants, our previous study determined elution order for 51 congeners of OH-PCB without derivatization and developed an analytical method for quantity to separate mixture of 6 major OH-PCBs in human blood, then applied analytical method to biological sample of human urine and blood with UPLC/QTof MS. As compound that might be hydroxypyrene was detected in urine sample, other OH-PAHs might be exist in biological sample. The present study aimed at developing analytical method of OH-PAHs that be able to separate each isomer, applying method to biological sample and measuring OH-PAHs for comprehensive evaluation of accumulation of environmental contaminants.

Materials and methods

OH-PAHs Standard Solution:

Ten standard solution of OH-PAHs (see Fig.1) were purchased from Cambridge Isotope Laboratories. Inc. and solution was diluted in acetone.

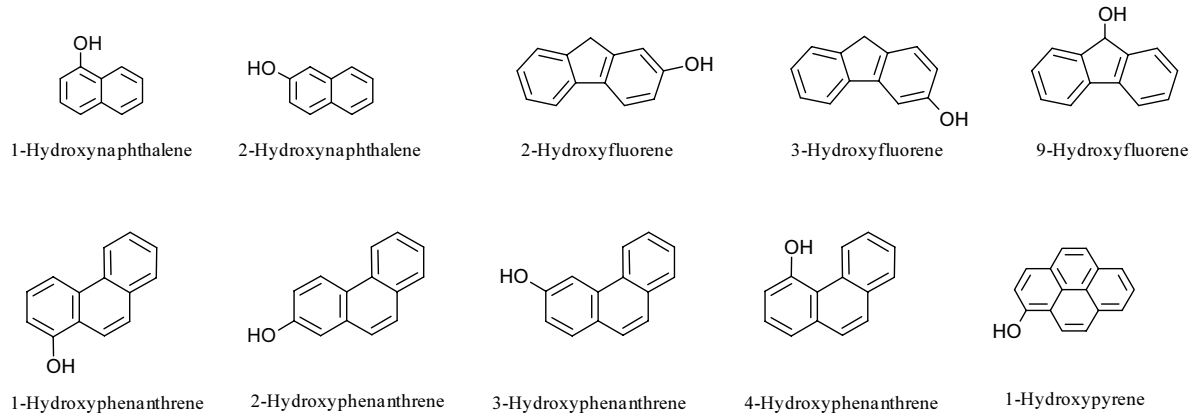

Fig.1 Ten compounds of OH-PAH

LC–TOF analysis:

Identification and quantification were performed using ultra performance liquid chromatography (UPLC: Waters Acquity UPLC system) and a high-resolution q-tof mass spectrometer (Xevo G2-S QTof MS) with a resolving power of more than 32500(Table 1)
Provide a succinct description of the materials and methods used in your research[2].

Table 1 Analytical conditions of UPLC/Q-Tof MS

UPLC			***MS***	
Instrument:	ACQUITY UPLC		Instrument:	Xevo G2-S Q-TOF
Column:	BEH C18 2.1ID X 150 mm, 1.7um		Ionization mode:	ESI negative
Flow rate:	0.5 mL/min.		Capillary:	1.5 kV
Column heater:	60 degree celsius		Sampling Cone:	40 V
Mobile Phese A:	5mM CH3COONH4 aq.		Source Temp:	120 degree celsius
Mobile Phese B:	THF/CH3CN (v/v: 1/4)		Desolvation Temp:	600 degree celsius
			Cone Gas Flow:	20 L/hr.
Gradient :			Desolvation Gas Flow:	800 L/hr.
Time(min)	%A	%B	Resolving Power:	<32,500
0.0	75	25		
17.0	25	75	Selected Ion:	
18.0	1	99	Hydroxynaphthalene	*m/z* =143.0497
18.5	75	25	Hydroxyfluorene	*m/z* =181.0653
Total Run Time:	20 min		Hydroxyphenanthrene	*m/z* =193.0653
			Hydroxypyrene	*m/z* =217.0653

Results and discussion

This method applied for human biological samples. Mass chromatogram and spectra of compound that might be hydroxypyrene or hydroxyfluoranthene in human urine sample are shown in Fig.1 and Fig. 2.

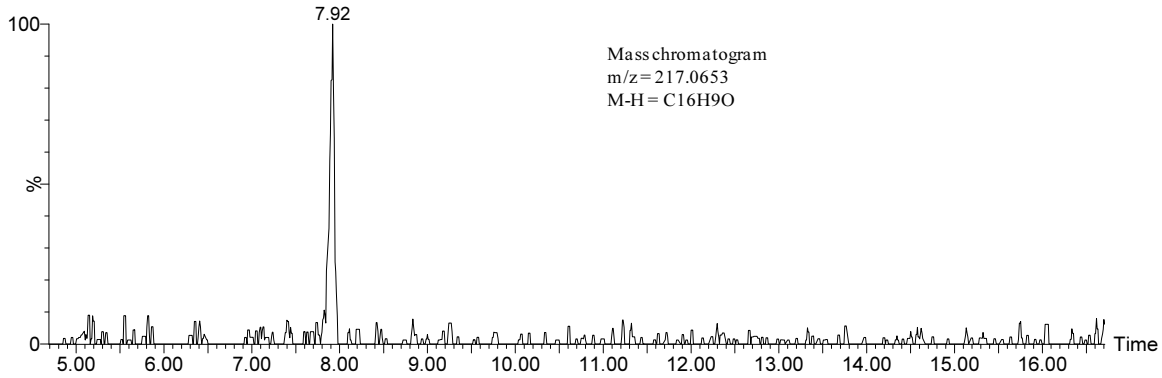

Fig.1 Mass chromatogram of *m/z* = 217.0653 in urine sample

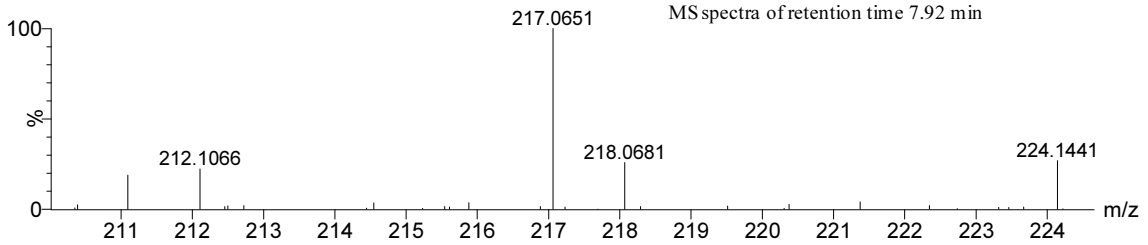

Fig.2 MS spectra of retention time 7.92 min in urine sample

Result of elemental composition of *m/z*=217 in urine sample is shown in Fig. 3. Composition was decided by accurate mass and isotope pattern. There were two candidates gratifying number of element, carbon, hydrogen, nitrogen and oxygen. This compound was decided C16H10O same as composition of hydroxypyrene and hydroxyfluoranthene by accurate mass and isotope pattern.

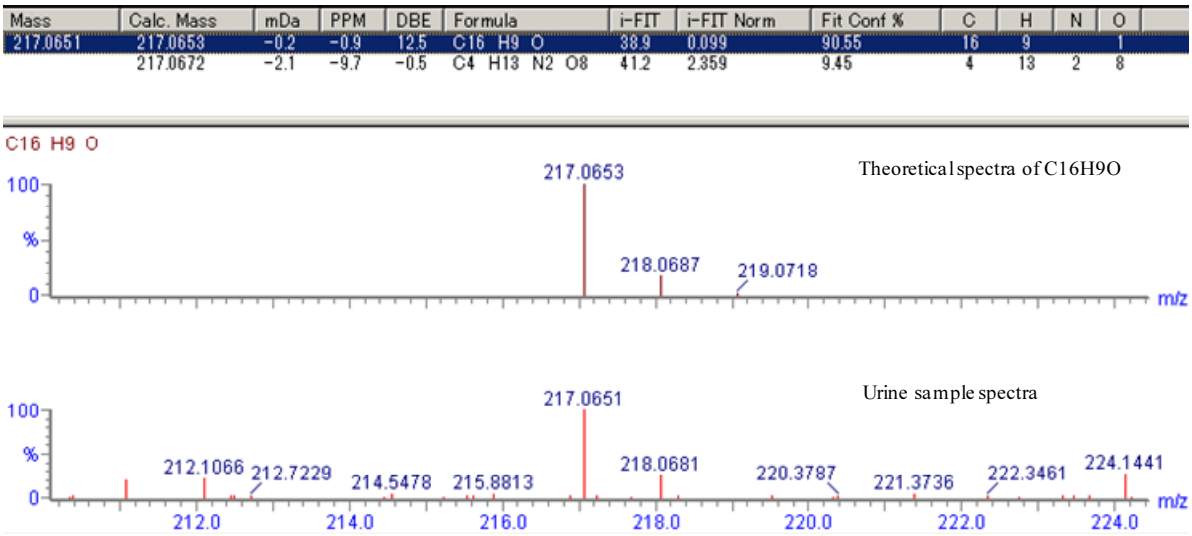

Fig.3 Result of elemental composition of spectra in urine sample at retention time of 7.92 min

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