DEVELOPMENT OF A COMPREHENSIVE ANALYTICAL METHOD FOR REGULATED POLYCYCLIC AROMATIC HYDROCARBONS

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Introduction

Several polycyclic aromatic hydrocarbons (PAHs) have carcinogenicity and/or mutagenicity[1], and have known as representative air pollutants. For these reasons, PAHs have regulated in a part of countries such as USA, Germany and China. The accurate evaluation of regulated compounds is essential because the regulations have been tightened and a novel regulation could be established in near future. However, a number of previous studies have targeted 16 compounds (16 EPA)[2] designated by United States Environmental Protection Agency (USEPA), and little is known about unregulated compounds. In addition, the chromatographic separation for even 16 EPA is incomplete, which lead to overestimation of 16 EPA concentrations. For example, it has been found that chrysene is unable to separate from several other isomers of chrysene. Actually, it has reported[3] that chrysene was found in shellfish samples at higher concentrations compared with those of benz[*a*]anthracene, benzo[*b*]fluoranthene and benzo[*a*]pyrene designated as PAH4 by EU[4]. To eliminate the overestimation, we developed a comprehensive analytical method for 29 PAHs including compounds regulated by USEPA as well as EU[5] and German Product Safety Commission (AfPS)[6] and for 26 halogenated PAHs (XPAHs).

In this study, we analyzed the dried bonito as an example to validate the developed analytical method. The dried bonito is a traditional Japanese smoked food. It is well known that PAHs adhere to the dried bonito by the smoke process[7], which leads to frequently exceeding the regulatory limit of PAH4 by EU[8].

Materials and methods

Chemicals

As target compounds, 29 PAHs and 26 XPAHs listed in Table 1 were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan) or Sigma-Aldrich Co. (St. Louis, MO, USA) or were synthesized in our laboratory by standard methods[9]. As shown in Table 1, several unregulated PAHs were also included because the peaks of these compounds could overlap with the peaks of regulated compounds. Isotope-labeled [$^{13}C_6$]phenanthrene, [$^{13}C_6$]fluoranthene, [$^{13}C_6$]chrysene and [$^{13}C_4$]benzo[*a*]pyrene were used as recovery standards for PAHs. [$^{13}C_6$]1-chloropyrene, [$^{13}C_6$]7-chlorobenz[*a*]anthracene, [$^{13}C_6$]7,12-dichlorobenz[*a*]anthracene and [$^{13}C_6$]7-bromobenz[*a*]anthracene for XPAHs, and [$^{2}H_{10}$]phenanthrene, [$^{2}H_{10}$]fluoranthene and [$^{2}H_{12}$]Benzo[*a*]pyrene were used as internal standards for both PAHs and XPAHs. The labeled compounds were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). The analytical-grade solvents (dichloromethane, hexane and toluene) for extraction and purification were purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan).

Optimization of analytical conditions

The analysis was performed by gas chromatography/high-resolution mass spectrometry (GC-HRMS; JMS-700 V, JEOL, Tokyo, Japan) and gas chromatography/triple quadrupole mass spectrometry (GC-MS/MS; GC 7890B/MS 7010B, Agilent Technologies, Santa Clara, CA, USA). Gas chromatographic separation was accompanied with a 60 m BPX-DXN (0.25 mm id; Kanto Chemical CO., Tokyo, Japan) for GC-HRMS and a 60 m Rxi-PAH (0.25 id×0.10 μm film thickness, RESTEK) for GC-MS/MS. Analytical conditions of GC-HRMS were set according to an established method[10]. Briefly, the column oven temperature program was as follows: hold at 130 °C for 1 min, ramp to 250 °C at 5 °C min^{-1}, then ramp to 320 °C at 10 °C min^{-1}, and hold at 320 °C for 18 min. The mass spectrometer was operated in an electron-impact ionization, and selected-ion-monitoring mode at a resolution of R > 10,000 (10% valley definition). On the other hand, column oven temperature program of GC-MS/MS was as follows: hold at 110 °C for 1.6 min, ramp to 175 °C at 30 °C min^{-1}, then ramp to 265 °C at 1.6 °C min^{-1}, ramp to 350 °C at 4 °C min^{-1}, and hold at 350 °C for 8.7 min. The mass spectrometer was operated in an electron-impact ionization, and multiple-reaction-monitoring mode. Both analytical conditions were compared in order to separate all peaks, and optimal conditions were used for subsequent analysis.

Verification of a pretreatment method

Since target compounds in this study involved higher molecular weight PAHs than 16 EPA, it is necessary to validate a pretreatment method for the higher molecular weight PAHs. The recovery rates of target PAHs were confirmed using an established method in our previous study[11] with some modification. Briefly, a silica gel cartridge (Supelclean LC-Si, 2 g, Supelco, St. Louis, MO, USA) was connected to an activated carbon cartridge (Carboxene 1016, 200 mg, Supelco), and the cartridges were washed with 20 mL of 10% dichloromethane/hexane. The silica gel cartridge was then removed, and the activated carbon cartridge was

reversed and eluted with 240 mL of toluene. After elution with 120 mL, we fractionated every 40 mL until 240 mL.

Table 1: Carcinogenicity and regulatory status of target PAHs

Compound	Abbreviation	Rings	IARC group	CAS number	Regulation		
					USEPA	EU	AfPS
naphthalene	Nap	2	2B	91-20-3	○		○
acenaphthylene	Acl	3		208-96-8	○		○
acenaphthene	Ace	3	3	83-32-9	○		○
fluorene	Fle	3	3	86-73-7	○		○
phenanthrene	Phe	3	3	85-01-8	○		○
anthracene	Ant	3	3	120-12-7	○		○
fluoranthene	Flu	4	3	206-44-0	○		○
pyrene	Pyr	4	3	129-00-0	○		○
benzo[*a*]fluorene	BaFL	4	3	238-84-6			
benzo[*c*]fluorene	BcFL	4	3	205-12-9		○	
benz[*a*]anthracene	BaA	4	2B	56-55-3	○	○	○
triphenylene	Tpl	4	3	217-59-4			
chrysene	Chr	4	2B	218-01-9	○	○	○
tetracene	Tec	4		92-24-0			
cyclopenta[*cd*]pyrene	CPcdP	5	2A	27208-37-3		○	
benzo[*b*]fluoranthene	BbF	5	2B	205-99-2	○	○	○
benzo[*k*]fluoranthene	BkF	5	2B	207-08-9	○	○	○
benzo[*j*]fluoranthene	BjF	5	2B	205-82-3		○	○
benzo[*e*]pyrene	BeP	5	3	192-97-2			○
benzo[*a*]pyrene	BaP	5	1	50-32-8	○	○	○
dibenz[*a,c*]anthracene	DBacA	5	3	215-58-7			
dibenz[*a,h*]anthracene	DBahA	5	2A	53-70-3	○	○	○
indeno[1,2,3-*cd*]pyrene	IcdP	6	2B	193-39-5	○	○	○
benzo[*ghi*]perylene	BghiPE	6	3	191-24-2	○	○	○
dibenzo[*a,l*]pyrene	DBalP	6	2A	191-30-0		○	
dibenzo[*a,e*]pyrene	DBaeP	6	3	192-65-4		○	
dibenzo[*a,i*]pyrene	DBaiP	6	2B	189-55-9		○	
dibenzo[*a,h*]pyrene	DBahP	6	2B	189-64-0		○	
coronene	Cor	7	3	191-07-1			

Validation of a developed analytical method

The dried bonito samples were homogenized with anhydrous sodium sulfate. The homogenized samples were then extracted with 250 mL of dichloromethane in a soxhlet extraction apparatus for 16 h after spiking with 2 ng of each recovery standard. The extracts were purified and fractionated using an activated carbon cartridge connected to a silica gel cartridge. The cartridges were washed with 20 mL of 10% dichloromethane/hexane. The silica gel cartridge was then removed, and the activated carbon cartridge was reversed and eluted with 120 mL of toluene. The toluene fraction containing PAHs and XPAHs was spiked with 2 ng of each internal standard and then concentrated to 100 μL.

Results and discussion

Optimization of analytical conditions

A part of the chromatogram (especially around chrysene) obtained by analysis of a PAH standard solution using target analytical columns are shown in Figure 1. The peaks of chrysene, benz[*a*]anthracene, triphenylene, and tetracene were co-eluted in the chromatogram analyzed by GC-HRMS using BPX-DXN (Figure 1-A). On the other hand, in the chromatogram analyzed by GC-MS/MS using Rxi-PAH (Figure 1-B), the peaks of these compounds were fully resolved. The chromatgraphic separation by GC is essential because chrysene, triphenylene, and tetracene have the same molecular weight. Analytical conditions of GC-MS/MS such as analytical column and column oven temperature program were evaluated as optimal and used for subsequent analysis. After optimizing the analytical parameters of GC-MS/MS such as fragmentation pattern and collision energy, the limit of quantification (LOQ) of target compounds by GC-HRMS and GC-MS/MS was also compared. The LOQs was 0.016 pg-6.8 pg for GC-HRMS and 0.021 pg-0.26 pg for GC-MS/MS. From this

result, it was revealed GC-MS/MS had 7.6-26 times higher sensitivities for PAHs and XPAHs than those of GC-HRMS.

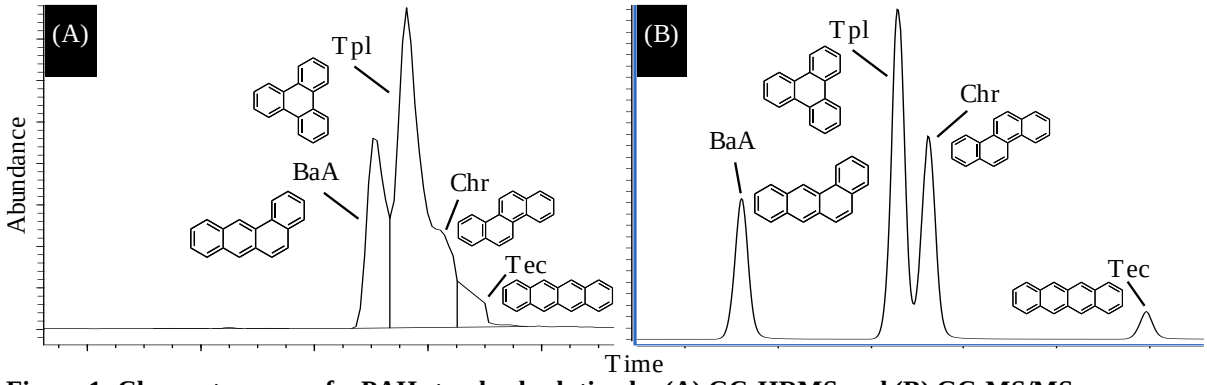

Figure 1: Chromatograms of a PAH standard solution by (A) GC-HRMS and (B) GC-MS/MS

Verification of a pretreatment method

The result of the recovery test for target compounds in this study is shown in Figure 2. The recovery rates were over 72% for target compounds except for 2 rings-PAHs/-XPAHs. 2 rings-PAHs/-XPAHs, that is, Nap and its halogenated derivatives (1-BrNap and 1,4-Br_2Nap) had low recovery rates (5-59%) in the pretreatment processes. Nap has the lowest boiling point among target PAHs and XPAHs, and only Nap has classified into volatile organic compounds (VOCs). In addition, 19% of Nap, 40% of 1-BrNap and 20% of 1,4-Br_2Nap were detected in 10% dichloromethane/hexane fraction, suggested these compounds were passed through the activated carbon cartridge, despite the fact that the ratio of the other compounds were below 10% in 10% dichloromethane/hexane fraction. For these reasons, lower recovery rates of 2 rings-PAHs/XPAHs could be caused by the vaporization during the concentration processes and passing through the activated carbon cartridge during the purification processes. The good recovery rate of Nap was obtained by purification using only silica gel cartridge in previous study[12] and our test. It could not be necessary to use the activated carbon cartridge if the interferences in the samples were negligible in GC-MS/MS analysis.

Although the elution of 6 and 7 rings-PAHs from the activated carbon cartridge is generally more difficult than that of other compounds, the recovery rates of 6 and 7 rings-PAHs in the 3 fractions were 6% and 9%, respectively, suggested that the recovery rates are negligible. Thus, elution volume from the activated carbon cartridge was set to be 120 mL, and it was certified the pretreatment method used in our previous study is also available for target compounds in this study.

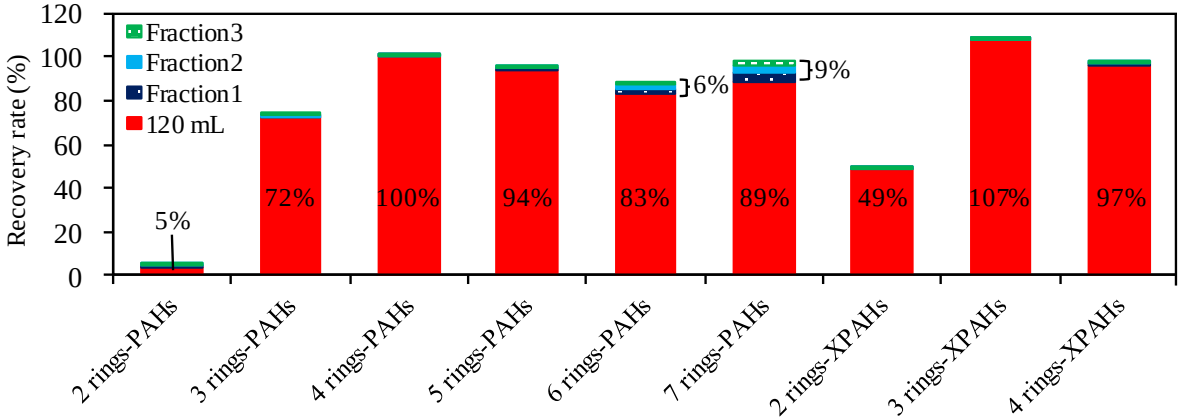

Figure 2: Recovery rates of PAHs and XPAHs in the purification process

Validation of a developed analytical method

A part of the chromatogram (especially around chrysene) obtained by analysis of dried bonito sample are shown in Figure 3. Chr and Tpl were found from the dried bonito sample, which means that the concentration of Chr would be overestimated by 35% if the peaks of Chr and Tpl were co-eluted. CPcdP classified as group 2A by the International Agency for Research on Cancer (IARC) was also detected because of the improvement of resolution and sensitivity for target compounds. As a result, BaA was slightly overestimated by 4% due to the co-elution of BaA and CPcdP peaks. In addition, the concentration of BaP was also overestimated by 100% due to the co-elution of BaP and BeP peaks. A previous study reported[13] that PAH concentrations in dried bonito

samples were 24.9 μg/kg for BaA, 47.6 μg/kg for Chr, 9.4 μg/kg for BbF, and 8.5 μg/kg for BaP. Sum of these concentrations was 90.4 μg/kg, which exceeded more than 3 times the regulatory limit (30 μg/kg) of PAH4 by EU[8]. However, the concentrations of Chr and BaP could be overestimated because the peak separation described in the previous study was insufficient. The method developed in the present study would be useful for determining the accurate concentrations of PAHs including a number of isomers. Besides, it was revealed that comprehensive investigation including XPAHs is necessary because a number of XPAHs were detected in our sample. Further studies are needed to identify and quantify the PAHs and XPAHs.

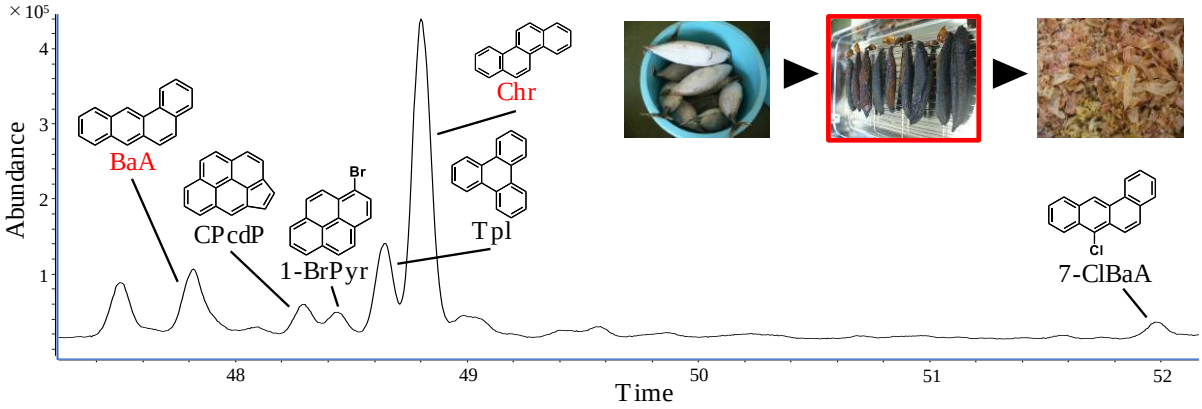

Figure 3: A part of the chromatogram of a dried bonito extract (red-letter: a kind of the PAH4 by EU)

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References

1. International Agency for Research on Cancer (2010) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* 92: 1-853.
2. United States Environmental Protection Agency (1999) *Compendium Method TO-13A*: 1-78.
3. Ni HG, Guo JY (2013) *J. Agric. Food Chem.* 61: 2013-2018.
4. Commission Regulation (EU) No 835/2011 (2011) *Official Journal of the European Union* L215: 4-8.
5. Commission Regulation (EU) No 1881/2006 (2006) *Official Journal of the European Union* L364: 1-26.
6. German Product Safety Commission (2014) *AfPS GS 2014:01 PAK*: 1-12
7. Kikugawa K, Kato T, Hayatsu H (1985) *Mutat. Res.* 158: 35-44
8. Commission Regulation (EU) No 2015/1125 (2015) *Official Journal of the European Union* L184: 7-10.
9. Ohura T, Sawada K, Amagai T, Shinomiya M (2009) *Environ. Sci. Technol.* 43(7): 2269-2275.
10. Miyake Y, Tokumura M, Wang Q, Amagai T, et al. (2017) *Environ. Sci. Technol.* 51: 14100-14106.
11. Wang Q, Miyake Y, Tokumura T, Amagai T, et al. (2018) *Sci. Total. Environ.* 625: 633-639
12. Ohura T, Horii Y, Yamashita N (2018) *Environ. Pollut.* 232: 367-374
13. Ishizaki A, Saito K, Hanioka N, Narimatsu S, Kataoka H (2010) *J. Chromatogr. A* 1217: 5555-5563