METHOD DEVELOPMENT FOR QUANTITATION OF POLYCYCLIC AROMATIC HYDROCARBONS AND THEIR ALKYLATED CONGENERS IN ORGANIC TISSUE BY TWO-DIMENSIONAL GAS CHROMATOGRAPHY HIGH RESOLUTION TIME OF FLIGHT MASS SPECTROMETRY

Ifeoluwa G Idowu[1], Wesley Johnson[1], Olga Francisco[1], Philippe Thomas[2], Chris Marvin[3], Jorg Stetefeld[1] and Gregg T Tomy[1]

[1]Centre for Oil and Gas Research and Development, Department of Chemistry, University of Manitoba, Winnipeg, Manitoba R3T 2N2 Canada

[2]Environment and Climate Change Canada, National Wildlife Research Centre, Ottawa, Ontario K1A 0H3 Canada

[3]Environment and Climate Change Canada, National Water Research Institute, Burlington, Ontario L7R 4A6 Canada

1 INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in nature with vast structural diversity; consisting of two or more aromatic rings in their structures, having different source materials; biogenic, petrogenic or pyrogenic[1]. Their alkylated substituted homologues possess vast structural diversity and congeners with different molecular weights. Few studies have established their distribution, resistance to weathering, diffusion across biological membranes and enhanced toxicity compared to their parent compound[2, 3 4].

There have been successful methods for quantifying the 16 priority parent PAHs prioritized by the E.U. and U.S. Environmental Protection Agency (EPA) but the accurate determination of the alkyl substituted PAHs is often challenging to achieve. Studies have confirmed their abundance more than the parent compound in crude oil[5, 6]. There are also some reports that suggest that the alkyl-PAHs (APAHs) are more toxic than the parent analogue. For example, 5-methyl chrysene has been shown to be more carcinogenic than chrysene[7]. Further, ring positioning of the alkylated group results in different biological effects [7]. Thus, the need to expand the EPA list to include other polycyclic aromatic compounds, particularly, the APAHs will improve environmental risk assessments[4].

Chromatographic separation of APAH congeners is often challenging with present day chromatography. This is due to each alkyl-substituted group possessing many constitutional isomers[8]. Also, quantitation is often underestimated because it has been done mostly with respect to the response factors of their respective parent compounds[7].

This study employs the two-dimensional gas chromatography coupled with a high-resolution time-of-flight mass spectrometer (GCxGC HRTOFMS) to separate and detect APAH congeners. The developed method was applied to the quantitation of APAHs in a NIST standard reference material.

2 EXPERIMENTAL

2.1 Materials

All organic solvents used were of high-purity (Optima grade) and obtained from Fischer Chemical. Individual alkyl-PAHs, C_1-naphthalene (1-methylnaphthalene; 2-methynaphthalene), C_2-naphthalene (1,6-dimethylnaphthalene; 2,6-dimethylnaphthalene), C_3-naphthalene (1,6,7-trimethylnaphthalene), C_4-naphthalene (1,2,5,6-tetramethylnaphthalene), C_1-chrysene (3-methylchrysene; 6-methylchrysene), C_1-pyrene (1-methylpyrene; 4-methylpyrene), C1-fluoranthene (1-methylfluoranthene; 3-methylfluoranthene), C_1-anthrancene (2-methylanthracene), C_1-phenanthrene (1-methylphenanthrene; 2-methylphenanthrene; 3-methylphenanthrene; 9-methylphenanthrene) standards were purchased from Accustandard Inc. and Caledon Laboratory Chemicals. Sixteen

(16) unsubstituted PAH native mix and deuterium mass labeled d_{10} anthracene were purchased from Accustandard Inc. while their surrogates mixture were from Cambridge Isotope Laboratories Inc. All standards were >98% purity. In-house PAH and APAH standards mix in hexane were prepared at varying concentrations as required. The NIST standard reference material (SRM-2974A) was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). The suite of isotope labeled internal standard used as recovery internal standard (RIS) were d_8 naphthalene, d_8 acenaphthylene, d_{10} acenaphthene, d_{10} Fluorene, d_{10} Phenanthrene, d_{10} pyrene, d_{12} benz(a)anthracene, d_{12} chrysene, d_{12} benzo(b)fluoranthene, d_{12} benzo(k)fluoranthene, d_{12} benzo(a)pyrene, d_{12} indeno(1,2,3-cd) pyrene, d_{14} dibenz(a,h)anthracene, d_{14} benzo(g,h,i)perylene. d_{10} anthracene was used as the instrument performance internal standard (IPIS).

2.2 Sample preparation

Sample preparation included accelerated solvent extraction (ASE), gel permeation chromatography (GPC) and Silica/Alumina column clean-up.

2.2.1 Accelerated Solvent Extraction

Approximately 1.5 grams (*n=3*) of organic sample was accurately weighted and mixed with diatomaceous earth and transferred to an ASE extraction cell size, 34 mLs, and spiked with suite of isotope labeled internal standards (10 uL of 10 ng/uL) as recovery internal standard (RIS) prior to extraction. Extracts were reduced and then transferred to a glass test-tube and further reduced in volume to 6 mLs using a gentle stream of UHP nitrogen. One mL of the extract was removed and transferred to a pre-weighed aluminum boat that was previously rinsed with hexane. Lipids content was determined using the equation:

$$\% \text{ lipid} = \frac{weight\ of\ lipid\ in\ 1ml\ x\ 6ml}{weight\ of\ whole\ sample}\ x\ 100$$

2.2.2 Automated Gel Permeation Chromatography

Removal of lipids from the extracted was achieved using a J_2-scientific AccuPrepMPS™ automated GPC. Five mL of the extract was loaded onto the head of GPC column packed with 60 g of S-X3 Biobeads, obtained from Bio-Rad Laboratories, USA, Extracts were then accurately made up to 1 mL and further cleaned up using silica/alumina column chromatography.

2.2.3 Silica/Alumina Column Chromatography

Adsorption chromatography was used to further clean-up the lipid-free extract. One mL of the sample extract was applied to the head of a column packed with Silica/ Alumina and a mobile phased of DCM: Hexane (1:1, v/v) mixture and a volume of 25 mL was used to elute all the PAHs and APAHs.

The extract was concentrated by rotary evaporation to ~ 5 mL and further reduced (1 mL) under a gentle stream of UHP nitrogen. The final solution was fortified with an instrument performance internal standard (IPIS, 10uL of 10 ng/uL solution) containing d_{10}-anthracene. The extract was then stored at 4°C in an amber GC vial prior to instrumental analyses.

2.3 GCxGC-HRTOFMS condition

An Agilent 7890 GC equipped with secondary oven, modulator operated at -80°c and a split/splitless injector with a time of flight mass spectrometer fitted with electron ionization (EI) source was used for the MS acquisition. A RESTEK Rxi-5ms ultra inert column (30 m×0.25 mm×0.25 µm) was the first column followed by a Rxi-17SilMS in the dimension and the carrier gas was helium at a constant flow of 1.2 mL/min. 1 µL was injected with an autoinjector into splitless injector at a temperature of 250°C. The oven temperature was held at 80 °C for 1 min then raised to 210 °C at 35 °C/min, further ramped up to 260 °C at 3 °C/min, and finally held for 5 min at 10 °C/min to 315 °C. Transfer line temperature was set to 300 °C and source temperature at 250 °C.

3.3.1 HRTOFMS Analysis

Target analytes (5 ng/µL) were prepared by diluting the stock standard solutions in a hexane to identify the appropriate confirmation ions (Table 1). These standards were then run in full-scan mode from m/z 50 to 350 to select the precursor ion for each homologue.

Table 1: Expected and observed m/z values of quantifying ions of selected PAHs and APAHs

Target Analyte	Formula	Expected ion	Observed ions	Mass Accuracy
3-Methylphenanthrene	$C_{15}H_{12}$	192.09335	192.09314	-1.11
2-Methylphenanthrene	$C_{15}H_{12}$	192.09335	192.09312	-1.19
9-Methylphenanthrene	$C_{15}H_{12}$	192.09335	192.09314	-1.11
1-Methylphenanthrene	$C_{15}H_{12}$	192.09335	192.09314	-1.11
4-Methylpyrene	$C_{17}H_{12}$	216.09335	216.09302	-1.55
1-Methylpyrene	$C_{17}H_{12}$	216.09335	216.09293	-1.93
Fluoranthene	$C_{16}H_{10}$	202.07770	202.07760	-0.49
Phenanthrene	$C_{14}H_{10}$	178.07770	178.07766	-0.26
Pyrene	$C_{16}H_{10}$	202.07770	202.07762	-0.42
Benz(*a*)anthracene	$C_{18}H_{12}$	228.09335	228.09333	-0.08
Benzo(*b*)fluoranthene	$C_{20}H_{12}$	252.09335	252.09345	0.37
Benzo(*k*)fluoranthene	$C_{20}H_{12}$	252.09335	252.09347	0.47

4.0 Preliminary result

A certified reference material (CRM 2974a), an organic tissue, was used to confirmed the developed method. Table 2 shows the result of the quantitation of APAHs and PAHs present in the standard material.

Target Analyte	**CRM 2974a Certified Mass Fraction (µg/kg)**	**Measured Mass Fraction (µg/kg)**	**Measurement Bias (%)**
3-Methylphenanthrene	24.10 ± 1.4	18.15 ± 1.03	24.69
2-Methylphenanthrene	28.20 ± 2.6	25.15 ± 1.03	10.82
9-Methylphenanthrene	15.90 ± 1.3	16.36 ± 1.02	2.89
1-Methylphenanthrene	17.60 ± 1.6	20.95 ± 0.46	19.03
4-Methylpyrene	19.77 ± 0.89	12.14 ± 0.47	38.59
1-Methylpyrene	10.69 ± 0.83	10.31 ± 0.46	3.55
Fluoranthene	287.00 ± 34	220.00 ± 9.20	23.35
Phenanthrene	74.40 ± 4.7	63.27± 3.04	14.96
Pyrene	166.00 ± 21	129.76 ± 4.64	22.29
Benz(*a*)anthracene	31.10 ±3.9	19.00 ± 1.61	38.91

Benzo(*b*)fluoranthene	41.50 ± 2.6	34.22 ±1.55	17.54
Benzo(*k*)fluoranthene	18.95 ± 0.54	22.07 ± 1.42	16.46

5.0 Future work

Evaluation of the chromatographic separation of 18 alkylated PAH has been done in 1D mode using the GCxGCTOFMS. Additional 18 targeted APAH compounds of environmental significance are being worked on at the moment. Co-elution was observed for 1&3-methylfluoranthenes, typical of APAHs in one dimensional chromatographic method. The co-elution of the fluoranthenes and others that could evolve from the additional compounds will be teased out using the system in 2D mode.

REFERENCES

1. Abdel-Shafy, H. I.; Mansour, M. S. (2016) *Egyptian Journal of Petroleum, 25*, (1), 107-123.
2. Wang, Z.; Fingas, m. F. (2003) Development of oil hydrocarbon fingerprinting and identification techniques. *Marine Pollution Bulletin, 47*, 423-452.
3. Wang, Z.; Fingas, M.; Blenkinsopp, S.; Sergy, G.; Landriault, M.; Sigouin, L.; Foght, J.; Semple, K.; Westlake, D. (1998) *Journal of Chromatography A, 809*, (1), 89-107.
4. Andersson, J. T.; Achten, C. (2015) *Polycyclic Aromatic Compounds***,** *35*, (2-4), 330-354.
5. Youngblood, W.; Blumer, M. (1975) *Geochimica et Cosmochimica Acta***,** *39*, (9), 1303-1314.
6. Wang, Z.; Fingas, M.; Lambert, P.; Zeng, G.; Yang, C.; Hollebone, B. (2004) *Journal of Chromatography A, 1038*, (1), 201-214.
7. Yang, C.; Zhang, G.; Wang, Z.; Yang, Z.; Hollebone, B.; Landriault, M.; Shah, K.; Brown, C. E. (2014) *Analytical Methods 6*, (19), 7760-7771.
8. Wesley Johnson, Ifeoluwa G Idowu., Olga Francisco, Chris Marvin, Phil Thomas, Jorg Stetefeld and Gregg T Tomy (2017) *In preparation*.