Analysis of Polycyclic Aromatic Hydrocarbons (PAH), Alkylated Derivatives, and Photo-degradation Products in Environmental and Food Samples using LC-FLD-MS/MS with Q TRAP® Technology

Takeo Sakuma[1], Daniel Leigh[2], Carmai Seto[1], André Schreiber[1], and Rebecca Wittrig[3]
[1]AB SCIEX Concord, Ontario (Canada); [2]AB SCIEX Warrington (UK); [3]Restek Corporation, Bellefonte, Pennsylvania (USA)

Overview

A new liquid chromatography (LC) method was developed that separates 26 polycyclic aromatic hydrocarbons (PAH), 6 alkylated derivatives, and 11 photo-oxidation products in a single LC run followed by sensitive fluorescence detection (FLD) and tandem mass spectrometry (MS/MS). Hybrid triple quadrupole linear ion trap (4000 Q TRAP® and QTRAP® 5500 systems) were operated in highly selective Multiple Reaction Monitoring (MRM) with automatic acquisition of fast and sensitive enhanced product ion (EPI) scan to enable quantitation and compound identification in the same analytical run.

Three ionization modes, Electrospray (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photo ionization (APPI) were examined. The developed method was applied to drinking water, atmospheric particulate, and seafood samples.

Introduction

There have been a number of maritime crude oil spills and explosions, like the 1989 Exxon Valdez accident in Prince William Sound Alaska releasing ~100 million gallons, the 2010 BP accident in the Gulf of Mexico releasing over 200 million gallons, and the 2011 Conoco-Phillips accident in North Eastern China, which contribute to the release of PAH and related compounds into the aquatic environment.

PAH, their alkylated derivatives, and photo-oxidation products are priority pollutants because of their carcinogenic, mutagenic, and teratogenic properties. Hence, the presence of PAH in sea water is of great concern for the aquatic environment and for the fishing and seafood producing industry, as PAH are bioavailable to fish and seafood, and thus enter dietary sources.

These PAH, alkylated derivatives and photo-oxidation products have a number of isomers and traditionally they are analyzed by GC or GC-MS. However, the analytical run time is significantly

long, because these isomers have to be chromatographically separated. In addition, some of the polar photo-degradation products are not amenable to GC.

In this study we investigated three LC-MS/MS ionization techniques and several small particle-size PAH LC columns to separate these isomers within a reasonable time and sensitivity.

The resulting LC-FLD-MS/MS method was successfully applied to the analysis of environmental and seafood samples.

Experimental

Chemicals

A number of PAH, alkylated derivatives, and photo-oxidation products were obtained from Restek (Bellefonte, PA), Sigma-Aldrich Canada (Oakville, ON) and Ultra Scientific (N. Kingstown, RI). The structures of these standards are shown in Figure 1 and Table 1.

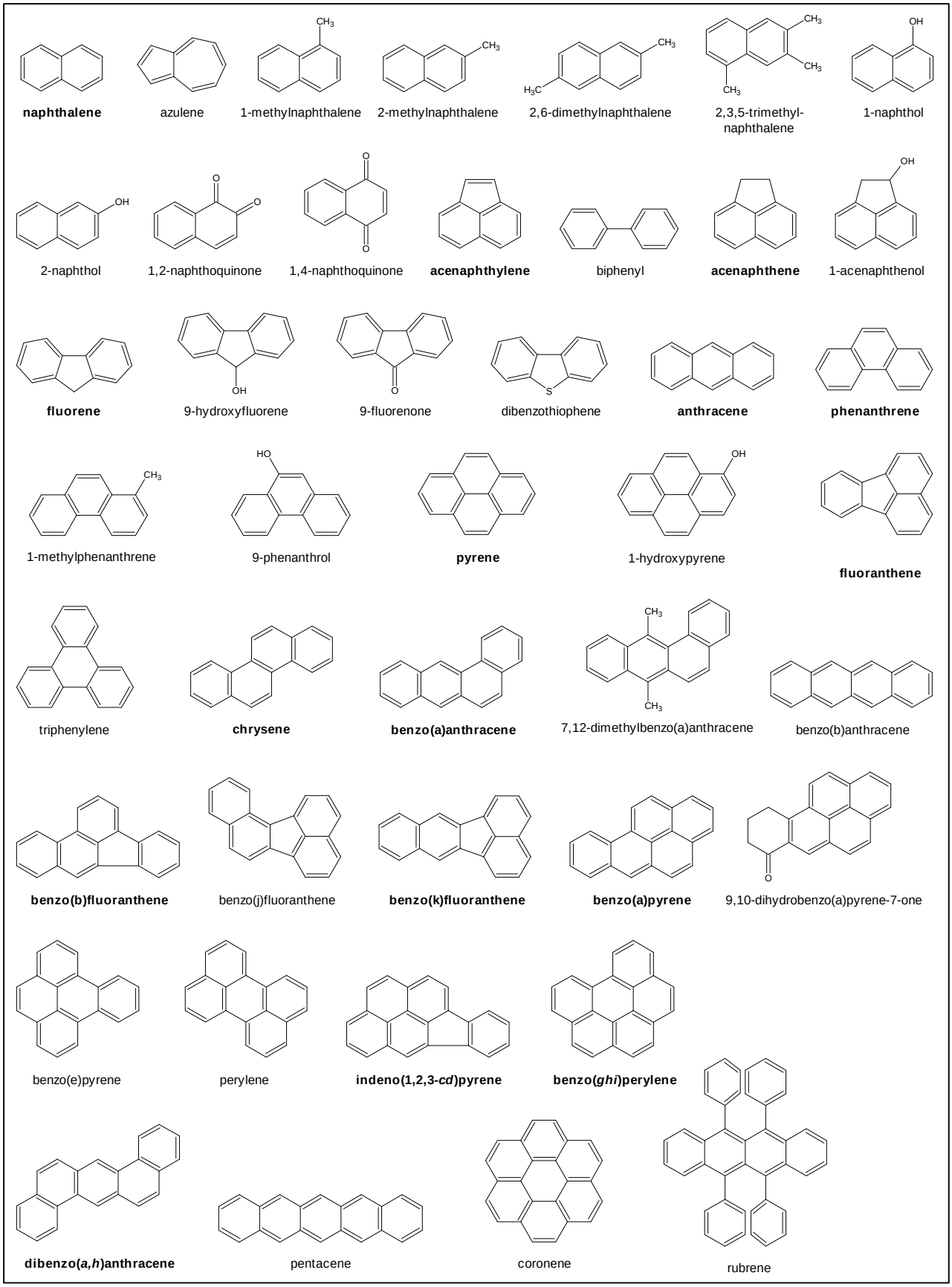


Figure 1. Structures of targeted PAH, alkylated derivatives, and photo-degradation products (Compounds in bold are the 16 EPA priority PAH.)

Table 1. MRM transitions used to detect PAH, alkylated derivatives, and photo-degradation products

Analyte	Q1	Q3 (quantifier)	Q3 (qualifier)	Analyte	Q1	Q3 (quantifier)	Q3 (qualifier)
naphthalene	128	78	102	*9-phenanthrol*	194	151	166
azulene	128	78	102	*fluoranthene*	202	150	200
naphthalene-d8	136	84	108	*pyrene*	202	150	200
1-methylnaphthalene	142	89	115	*1-hydroxypyrene*	219	190	201
2-methylnaphthalene	142	115	141	*chrysene*	228	200	226
1-naphthol	145	63	122	*triphenylene*	228	200	226
2-naphthol	145	63	122	*benzo(a)anthracene*	228	150	226
acenaphthylene	152	126	151	*benzo(b)anthracene*	228	202	226
acenaphthene	154	126	153	*chrysene-d12*	240	208	236
biphenyl	154	126	153	*benzo(b)fluoranthene*	252	224	250
2,6-dimethylnaphthalene	156	115	141	*benzo(j)fluoranthene*	252	224	250
1,2-naphthoquinone	159	103	131	*benzo(k)fluoranthene*	252	224	250
1,4-naphthoquinone	159	103	131	*benzo(a)pyrene*	252	224	250
acenaphthene-d10	164	132	162	*benzo(e)pyrene*	252	222	250
fluorene	166	115	165	*perylene*	252	224	250
2,3,5-trimethylnaphthalene	170	128	153	*7,12-dimethylbenzo(a)anthracene*	257	226	242
1-acenaphthenol	171	46	72	*perylene-d12*	264	232	260
anthracene	178	152	176	*9,10-dihydrobenzo(a)pyrene-7-one*	271	215	253
phenanthrene	178	151	176	*benzo(ghi)perylene*	276	248	274
9-fluorenone	181	127	152	*indeno(1,2,3-cd)pyrene*	276	246	274
9-hydroxyfluorene	182	165	95	*dibenzo(a,h)anthracene*	278	248	276
dibenzothiophene	184	139	152	*pentacene*	278	250	276
phenanthrene-d10	188	158	184	*coronene*	300	296	298
1-methylphenathrene	192	165	191	*rubrene*	533	377	455

Sample Preparation

Seafood samples were prepared by Restek as per NOAA Technical Memorandum NMFS-NWFSC-59.[1] An atmospheric particulate sample was collected with a polyurethane disk sampler and extracted with a Soxhlet tube by Environment Canada, Ontario Region as per Environment Canada Protocol No.EC-HAP-C-05.[2] Some carcinogenic PAH's were incubated to form Phase I metabolites in rat liver microsomes for 1 hour at 37°C under oxidative conditions.

LC

A Shimadzu NEXERA UHPLC system with a UV detector (SPD-20AV), a fluorescence detector (RF-20A XS) followed by MS/MS confirmation was used for analysis. For drinking water analysis a CTC PAL autosampler was used to enable large volume injection and extended wash cycles to reduce carry-over. Separation was achieved using an Inertsil ODS-P HP (250x2.1 mm) 3 µm column (GL Sciences, Tokyo, Japan), and a Kinetex C18 (50 x 2.1 mm) 2.7 µm column with a mobile phase of water and acetonitrile. The column oven temperature was set to 20°C. A 30 minute multi-step gradient was required to separate critical isomers (Table 2).

Table 2. LC gradient used for the separation of PAH, alkylated derivatives, and photo-degradation products

Time (min)	Flow (µL/min)	A (%)	B (%)
0.0	0.5	60	40
7.0	0.5	40	60
16.0	0.5	0	100
19.0	1.0	0	100
25.0	0.5	0	100
25.1	0.5	60	40
30.0	0.5	60	40

MS/MS

MS/MS detection was performed on an AB SCIEX 4000 Q TRAP® and QTRAP® 5500 equipped with Turbo V™ source. ESI, APCI, and APPI techniques were investigated during method development. Selective and sensitive MRM mode was used for detection. Two transitions were monitored for each analyte to allow quantitation and identification using the ratio of quantifier and qualifier ion (Table 1). In addition, EPI spectra were acquired to use the molecular fingerprint saved into the MS/MS spectrum to increase the confidence of identification.

Results and Discussion

An example LC-FLD-MS/MS run is presented in Figure 2. The separation of critical isomers is highlighted in Figure 3.

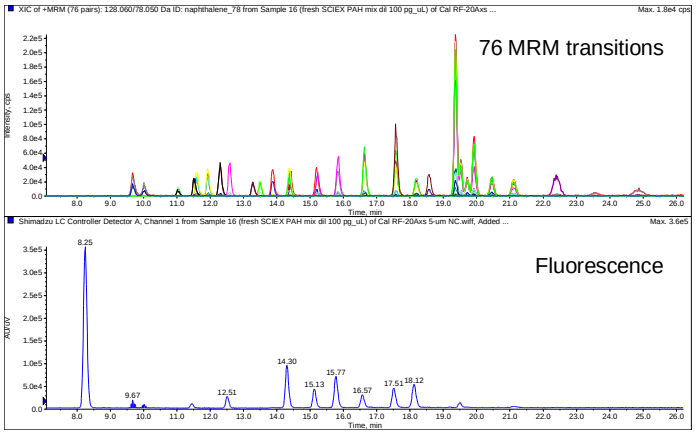


Figure 2: LC-MS/MS (top) and LC-FLD (bottom) analysis of PAH, alkylated derivatives, and photo-degradation products

Different detection and ionization techniques were investigated and compared regarding their selectivity, sensitivity, and linear dynamic range. Benzo(a)pyrene was used as a model compound for this study (Table 3).

The UV detection at 254 nm suffered from high background and low specificity. FLD using optimized excitation wavelength and emission wavelength offered highest sensitivity, with a limit of detection (LOD) of 0.1 ng/mL, but selectivity was limited in comparison to MS/MS detection.

ESI was found to give highest sensitivity of all ionization techniques, however, ESI showed strong matrix effects. APPI was investigated using different dopants, including toluene, anisol, and chlorobenzene. Chlorbenzene was found to give highest sensitivity for benzo(a)pyrene, however, background noise was increased and thus LOD was higher in comparison to ESI and APCI. Higher selectivity and sensitivity were achieved using APCI. In addition, APCI offers the advantage of increased robustness and minimal matrix effects.

As a result APCI was chosen for further LC-MS/MS analysis. The example calibration line presented in Figure 4 highlights the excellent linearity and reproducibility.

LC-FLD and LC-MS/MS are complementary techniques for high sensitivity for quantitation with very little matrix interferences. While LODs of the quantitation of PAH are very similar using both techniques LC-MS/MS is able to ionize polar metabolites and degradation products more efficiently, thus, allowing quantitation at lower levels.

Table 3. Limits of detection (LOD) and linearity of different detection and ionization techniques of benzo(a)pyrene using the 4000 Q TRAP® system using an injection volume of 10 µL

Detection by	LOD (ng/mL)	Linearity	Additional comment
UV	10	10 – 1000	high background
FLD	0.1	0.1 – 1000	
ESI	0.1	0.1 – 100	matrix effects
APCI	1.0	1 – 1000	
APPI	10	10 – 1000	high background

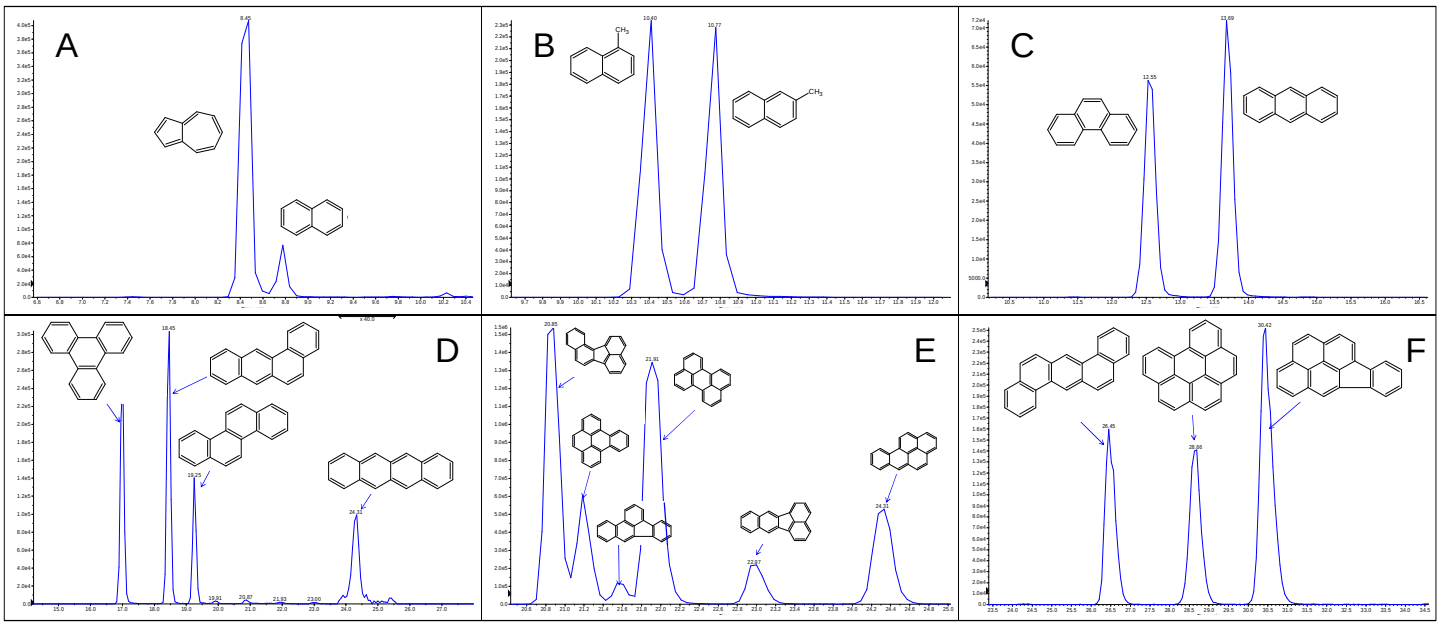


Figure 3. Separation of critical isomers using the developed LC method

A: $C_{10}H_8$ azulene, naphthalene

B: $C_{11}H_{10}$ 1-methylnaphthalene, 2-methylnaphthalene

C: $C_{14}H_{10}$ anthracene, phenanthrene

D: $C_{18}H_{12}$ benzo(a)anthracene, benzo(b)anthracene, chrysene, triphenylene

E: $C_{20}H_{12}$ benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(e)pyrene, perylene

F: $C_{22}H_{12}$ benzo(ghi)perylene, indeno(1,2,3-cd)pyrene, $C_{22}H_{14}$ dibenzo(a,h)anthracene

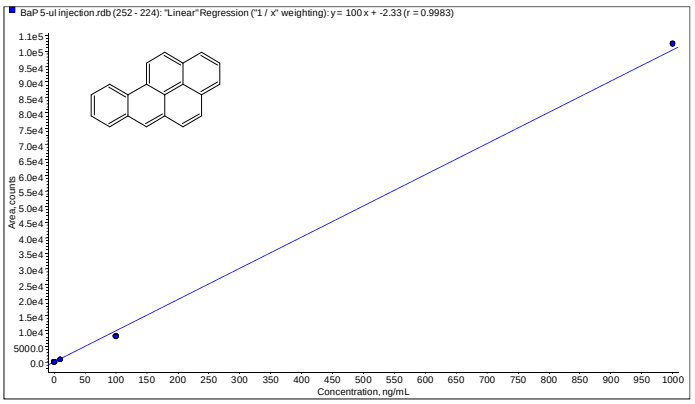


Figure 4. Calibration line of benzo(a)pyrene using triplicate injections into LC-MS/MS with APCI

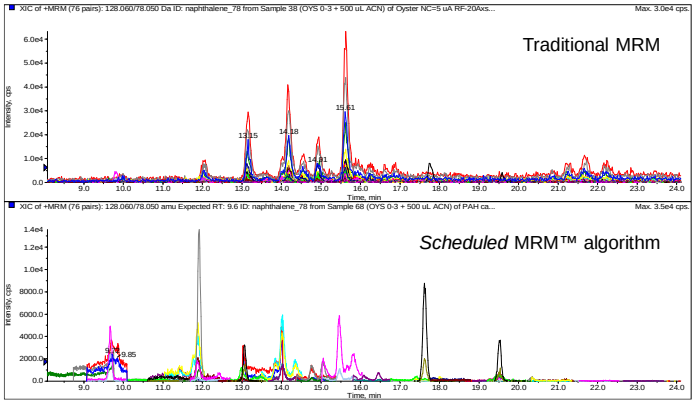


Figure 5. Analysis of an oyster extract using traditional MRM and the *Scheduled* MRM™ algorithm

Examples of using the developed method for the analysis of PAH, alkyl derivatives, and photo-oxidation products in a variety in environmental and food samples are presented in Figures 5 to 7.

Figure 5 shows LC-MS/MS chromatograms of the analysis of an oyster extract. The acquisition of traditional MRM mode is compared to an acquisition using the *Scheduled* MRM™ algorithm. Target analytes are monitored only in short time windows around the expected retention time using the *Scheduled* MRM™ algorithm. As a result the scheduling

decreases the number of concurrent MRM transitions, allowing both the cycle time and the dwell time to be optimized for highest sensitivity, accuracy, and reproducibility. In addition, data exploration is easier because of a more selective acquisition removing many of the matrix interferences.

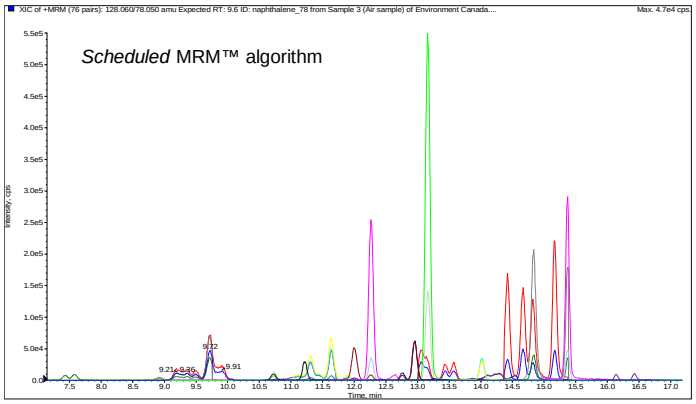


Figure 6. Detection of PAH, alkyl derivatives, and photo-oxidation products in an atmospheric particulate sample

Figure 6 shows a chromatogram of the detection of target analytes in an atmospheric particulate sample collected with a polyurethane disk sampler and after Soxhlet extraction. The ratio of two MRM transition monitored for each compound was used for identify target analytes with high confidence.

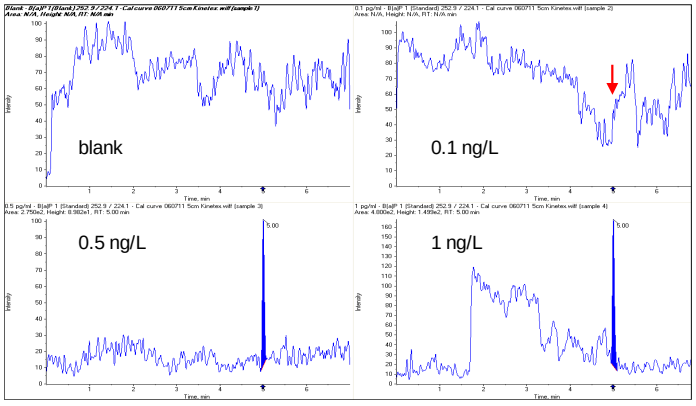
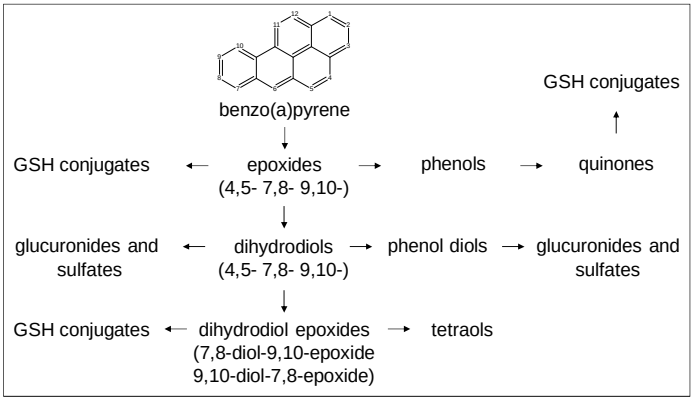


Figure 7. Parts-per-trillion level (ppt) detection of benzo(a)pyrene in drinking water. The direct injection of 1 mL into LC-MS/MS allowed quantitation with an LOD of less than 1 ng/L using the QTRAP® 5500 system.

Figure 7 shows the analysis of drinking water samples spiked with low levels of benzo(a)pyrene using the AB SCIEX QTRAP® 5500 system. A Phenomenex Kinetex C18 (50 x 2.1mm) column with a 5.5 min gradient was used to speed up the analytical run time.

The direct injection of 1 mL of sample was used successfully to quantify PAH with an LOD below 1 ng/L. The Signal-to-noise (S/N) at a concentration of 1ng/L was >10 using a 3x standard deviation algorithm. Repeat injections (n=5) at the 1 ng/L concentration level resulted in less than 5% coefficient of variation. Excellent accuracy (91 to 114%) and linearity (r = 0.9995) was found over the concentration range of interest 1 to 100 ng/L besides the injection of a large volume of 1 mL.



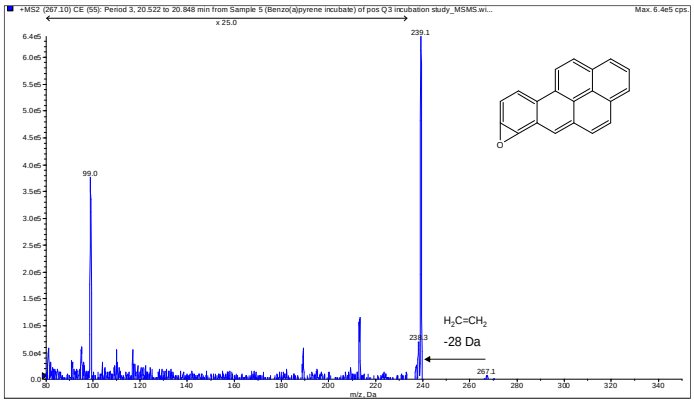


Figure 8. Metabolism of benzo(a)pyrene (top, adapted from IRAC 2010) and identification of benzo(a)pyreneepoxide using full scan MS/MS scanning using the QTRAP® 5500 system after incubation (note: the position of the epoxy group cannot be confirmed with MS/MS)

Figure 8 shows an example spectrum of a benzo(a)pyrene sample incubated with rat liver microsomes for 1 hour at 37°C under oxidative conditions to produce *in vitro* metabolites.

Summary

A study was conducted to develop a robust and sensitive LC-FLD-MS/MS method using the AB SCIEX QTRAP® 5500 system for selective and sensitive MS/MS detection of PAH, alkylated derivatives, and photo-oxidation products. Three ionization modes, ESI, APCI, and APPI were investigated. APCI was found to give best results with respect to sensitivity, robustness and smallest matrix effects.

Two LC methods were developed, one to separate isomers and the other one to quickly quantify benzo(a)pyrene by direct injection of drinking water.

The method was successfully applied to the analysis of environmental and seafood samples. In addition, microsome incubations were performed to study the metabolism of PAH.

Acknowledgement

The authors wish to thank Jack Cochran and Rebecca Wittrig of Restek (Bellefonte, PA) for seafood extracts, Susan Genualdi and Mahiba Shoeib of Environment Canada for atmospheric samples, Curtis Campbell and Masatoshi Takahashi of Shimadzu (Columbia, MD) for providing the NEXERA UHPLC system.

References

[1] NOAA Technical Memorandum NMFS-NWFSC-59: 'Extraction, Cleanup, and Gas Chromatography/Mass Spectrometry Analysis of Sediments and Tissues for Organic Contaminants' (2004)

[2] Environment Canada Protocol No.EC-HAP-C-05

[3] International Agency for Research on Cancer: 'IARC Monographs on the Evaluation of Carcinogenic Risks to Humans' 92 (2010) 518-519

Publication number: 4520411-01

Headquarters
500 Old Connecticut Path, Framingham, MA 01701 USA
Phone 508-383-7700
www.absciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
www.absciex.com/offices