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Ultra high performance liquid chromatography–atmospheric pressure photoionization-mass spectrometry for high-sensitivity analysis of US Environmental Protection Agency sixteen priority pollutant polynuclear aromatic hydrocarbons in oysters

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ABSTRACT

In response to Gulf of Mexico deepwater horizon oil spill, we have developed an atmospheric pressure photoionization (APPI) based ultra high performance liquid chromatography–mass spectrometry (UHPLC–MS) method for high-sensitivity analysis of United States Environmental Protection Agency (US EPA) 16 priority pollutant polynuclear aromatic hydrocarbons (PAHs) in oysters. Analyses were performed on an Agilent's Infinity 1290 UHPLC system coupled with a G6140A single quadrupole MS detector with Syagen's PhotoMate® APPI® source. Column separation was achieved using Zorbax Eclipse PAH column. Chlorobenzene was used as an APPI dopant for maximum overall sensitivity. Dynamic linear ranges were evaluated and found to cover 3.6–5.1 (Ave. 4.4) orders of magnitude with R^2 of at least 0.995. A quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction and cleanup procedure was used. The spike recoveries ranged from 77% to 110% with %RSD of 0.6–6.7 at spike concentrations below or substantially below the US Food and Drug Administration (FDA) level of concern in oysters. The on-column instrument detection limits (IDLs, 6σ S/N = 3) ranged from 8 to 106 pg with an average of 23 pg for 16 PAHs. The method detection limits (MDLs, 6σ S/N = 3) ranged from 0.013 to 0.129 ppm with an average of 0.040 ppm for all analytes. These MDLs were about 5 times to over 4 orders of magnitude lower than US FDA levels of concern in oysters.

1. Introduction

Atmospheric pressure photoionization (APPI) offers three major benefits for liquid chromatography–mass spectrometry (LC–MS) analysis of small molecules: (1) Ionizing both polar and non-polar small molecules simultaneously, allowing LC–MS users to analyze more compounds with a single injection [1,2]; (2) substantially reduced matrix effects or ion suppression relative to electrospray ionization (ESI), leading to simplified sample cleanup procedures, better analyte recoveries and data quality [1,3,4]; (3) up to five orders of magnitude dynamic linear range (vs. ESI: 2–3 orders), a preferred ionizer for quantitative analysis (Table 3) [5,6].

APPI has become the ionization source of choice for analysis of many small molecules not readily ionizable by ESI and atmospheric pressure chemical ionization (APCI). These compounds include polycyclic aromatic hydrocarbons (PAHs) [7–9], lipids [5,10–13], fat soluble vitamins [14,15], steroids [16,17], polymers [18,19], peptides [20,21], highly halogenated compounds [22,23], and many other chemicals with low proton affinities [24,25].

The advantages of APPI for LC–MS analysis of PAHs over other analytical techniques such as high performance liquid chromatography (HPLC) with UV detector (UVD) and fluorescence detectors (FLD), gas chromatography (GC) and GC–MS have been discussed in previous work [6,7]. Several research articles have been published describing APPI analysis of PAHs [7–9]. Moriwaki et al. described HPLC–APPI-MS analysis of 12 out of 16 United States Environmental Protection Agency (US EPA) PAHs in sediment using toluene as a dopant. Limits of detection (LODs) of 0.06–0.9 ppm were reported [8]. Itoh et al. evaluated the performance of APPI dopants for HPLC–APPI-MS analysis of US EPA sixteen priority pollutant PAHs using acetone, anisole, toluene and mixtures [26]. Toluene and anisole (99.5:0.5) mixture was found to offer the best performance relative to their individual component dopant, but no sample matrix was included in this study. Laszlo and Wenzl developed a HPLC–APPI-MS/MS method for the determination of 15 + 1 EU priority PAHs in edible oil [7]. Several APPI dopants were evaluated and anisole was selected as a dopant in the method.

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Low μg/kg (ppb) concentrations of LODs were achieved, meeting the requirement (0.3 ppb) set by EU food legislation. Smoker et al. described a simple and rapid HPLC–APPI-MS/MS method for analysis of US EPA sixteen priority pollutant PAHs in shrimp using toluene as a dopant [9]. A quick, easy, cheap, effective, rugged, and safe (QuEChERS) procedure was used for sample extraction and cleanup. The LODs were estimated to be 0.02–0.51 ppm with primary and secondary amine (PSA) cleanup.

The weakness of some of the previously published APPI work is that they do not cover a complete list of US EPA sixteen priority pollutant PAHs in the method, and/or that the selected dopants may not be optimal. In addition, conventional HPLC columns were used for separation instead of using ultra high performance liquid chromatography (UHPLC) column [7–9,26]. UHPLC offers superior on-column resolving power, sensitivity and speed of analysis. Smith et al. [27] and Robb et al. [28] did a thorough evaluation and performance comparison of APPI dopants among toluene, anisole, chlorobenzene and many other substituted benzenes for analysis of US EPA sixteen priority pollutant PAHs. They concluded that chlorobenzene was a preferred charge exchange dopant and offered better overall performance for these analytes especially for the lighter PAHs (e.g., naphthalene and acenaphthylene) with ionization energies (IE) close to anisole. Keeping that in mind, Cai et al. demonstrated the utility of UHPLC–APPI-MS/MS for high-throughput and high-sensitivity analysis of US EPA sixteen priority pollutant PAHs using chlorobenzene as a dopant [6]. Low picogram (pg) of instrument detection limits (IDL) was achieved, but unfortunately no sample matrix was included in this study at the time of method development.

In response to Gulf of Mexico deepwater horizon oil spill [29], we have developed an APPI based UHPLC–MS method for high-sensitivity analysis of PAHs in oysters. In this work, a single quadrupole mass detector (SQD) was used instead of triple quadrupole mass detector (TQD) [6]. In comparison with SQD, tandem mass spectrometer (MS/MS) is undoubtedly a preferred mass analyzer for trace analysis of these analytes in complex biological samples due to its higher mass selectivity and sensitivity. However, TQD may not be available in many routine environmental and food safety lab due to its higher cost. Therefore it is necessary to develop a SQD based analytical method so that APPI PAH analysis can be performed in those labs not accessible to MS/MS detector. To the best of our knowledge, this is the first APPI and UHPLC-SQD based method for trace analysis of US EPA sixteen priority pollutant PAHs in oysters. With the selectivity of APPI and superior resolving power of UHPLC column, a simple QuEChERS extraction and cleanup procedure was used. Although isobaric mass interference presents a big challenge using SQD as a mass analyzer, with a carefully designed gradient elution program, we were able to achieve method detection limits (MDLs) about 5 times to over 4 orders of magnitude lower than US FDA levels of concern in oysters [29].

2. Experimental

2.1. Chemicals and reagents

EPA 610 Polynuclear Aromatic Hydrocarbons Mix (Supelco Cat #, 48743) was purchased from Sigma Aldrich (Bellefonte, PA, USA). This stock solution was pre-dissolved in methanol: methylene chloride (1:1) and contained 16 PAHs (Table 1) with varied concentrations ranging from 100 ng/μL to 2000 ng/μL. HPLC water, acetonitrile, methanol and isopropanol (Optima grade, 0.2 μm prefiltered) were all purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Chlorobenzene (99.5% purity, P/N, AC40449-0010), used as an APPI dopant, was also purchased from Thermo Fisher Scientific (Pittsburgh, PA, USA).

2.2. Instruments

The LC system used was an Agilent's 1290 Infinity UHPLC system equipped with 1290 Thermostat (autosampler sample temperature control device, G1330B), Infinity DAD (UV diode-array detector, G4212A), Infinity binary pump (G4220A), Infinity TCC (LC column compartment temperature control device, G1316C), Infinity 1290 autosampler (G4226A), and Infinity 1290 flexible cube (autosampler needle wash and injection valve autoclean device, G4227A). The MS used was an Agilent's G6140A single quadrupole mass spectrometer with Syagen's PhotoMate® APPI® source. The data acquisition and processing software was LC/MSD ChemStation Rev. B.04.02 SP1. Column separation was performed using an Agilent's Zorbax Eclipse PAH, Rapid Resolution HT 2.1 mm × 50 mm, 1.8 μm, 600 bar UHPLC column (P/N, 959741-918). An Agilent's Zorbax Eclipse XDB-C8, 2.1 mm × 20 mm, 1.8 μm, 600 bar (P/N, 926700-906), was used as a guard column. The analytical column and guard column were connected by metal capillary tubing, 0.17 mm (I.D.) × 90 mm, 1/16 in. (O.D.), male/male (Agilent P/N, G1316-87300). An inline filter (2 mm frit inlet I.D., 0.2 μm pore size, Agilent P/N, 5067-1555) was installed before the guard column. Column separation was achieved using water/acetonitrile as a binary mobile phase system. The mobile phase solvents were 90:10 water/acetonitrile (A1) and 100% acetonitrile (B1).

2.3. APPI dopant

The most efficient charge exchange APPI dopant found so far for US EPA sixteen priority pollutant PAHs is chlorobenzene [1,27,28]. The flow rate was optimized and found to be 50–70 μL/min. Chlorobenzene was added by post column addition at a flow rate of 60 μL/min via a MicroTee assembly (P/N, P-890, Upchurch Scientific, WA, USA) and delivered using a LC pump (LabAlliance Series III, Scientific System, Inc., PA, USA). An inline check valve (P/N, CV-3340, Upchurch Scientific) was installed on the dopant flow line between MicroTee assembly and the dopant LC pump. A 1000 psi back pressure regulator (P/N, P-455, Upchurch Scientific) was installed on the dopant LC pump flow line to stabilize the dopant flow rate.

2.4. Autosampler parameters

Injection volume: 2 μL; Injection mode: Injection with needle wash; Needle wash mode: Flush port, Time: 10 s; Sample temp.: 4 °C.

2.5. Flexible cube parameters

Flexible cube cleaning: Enable needle seat back flush; Flexible cube settings: Solvent 1: CH_3CN, Start Cond. (A1): 90% water in CH_3CN, Flow: 4 mL/min, Duration: 30 s.

2.6. TCC parameters

LC column compartment temp: Left: 30 °C, Right: combined.

2.7. DAD data acquisition parameters

UV wavelength: 230 nm, Bandwidth: 4.0 nm; Reference wavelength: 360 nm; Reference bandwidth: 100 nm; Peak width: >0.025 min (0.5 s response time) (10 Hz).

2.8. MS data acquisition parameters and conditions

Table 1 shows the data acquisition parameters and conditions used for this analysis. Set Up MSD Signals (under ChemStation

Table 1
PAH data acquisition method.

Analyte	Major ion type	SIM ion (*m*/*z*)	Fragmentor (V)	Dwell time (ms)	%Rel dwell
Naphthalene	M^+	128.1	150	30	10
Acenaphthylene	M^+	152.1	160	30	10
Acenaphthene	M^+	154.1	140	30	10
Fluorene	M^+	166.1	140	30	10
Phenanthrene	M^+	178.1	160	30	10
Anthracene	M^+	178.1	160	30	10
Fluoranthene	M^+	202.1	170	30	10
Pyrene	M^+	202.1	170	30	10
Benzo[a]anthracene	M^+	228.1	170	30	10
Chrysene	M^+	228.1	170	30	10
Benzo[b]fluoranthene	M^+	252.1	180	30	10
Benzo[k]fluoranthene	M^+	252.1	180	30	10
Benzo[a]pyrene	M^+	252.1	180	30	10
Dibenzo[a,h]anthracene	M^+	278.1	190	30	10
Benzo[ghi]perylene	M^+	276.1	190	30	10
Indeno[1,2,3-cd]pyrene	M^+	276.1	190	30	10

menu \Instrument\): Peak width: 0.055 min (leading to a cycle time of 0.33 s/cycle); Ultra fast scan: On; Time filter: On; Scan data storage: Condensed; Mode: SIM; Polarity: positive. MSD Spray Chamber (under ChemStation menu \Instrument\More MSD\): Drying gas flow: 13 L/min; Nebulizer pressure: 50 psi; Drying gas temp.: 300 °C; Vaporizer temp.: 450 °C; Capillary voltage: 3000 V.

2.9. QuEChERS sample extraction

Blank live oysters were purchased from a local grocery store. The oysters were prepared and homogenized based on the US FDA described procedure [29]. Ten (10) g of homogenized oysters was transferred to a 50 mL BD falcon centrifuge tube (VWR P/N, 21008-940). For a spike recovery test, 100 μL known concentrations of PAH analytes (dissolved in acetonitrile) was spiked at this point. Two ceramic homogenizers (Agilent P/N, 5982-9313) were added and the sample was vortexed for 1 min. Seven mL HPLC water was added and the sample was vortexed for 1 min. Fifteen mL of acetonitrile was added and the sample was vortexed for 1 min. The content of the QuEChERS salt pouch (Agilent P/N, 5982-6555) was added to the tube. Each pouch contains 6 g of $MgSO_4$ and 1.5 g of NaCl. The mixture was immediately and vigorously shaken for 1 min, and then the sample was centrifuged for 5 min at 6000 rpm (4180 rcf or relative centrifuge force).

2.10. Sample cleanup by dispersive-SPE

Five hundred microliters of clear supernatant were pipetted into a 2 mL dispersive-SPE (d-SPE) centrifuge tube containing 50 mg of PSA and 150 mg of $MgSO_4$ (Agilent P/N, 5982-5022). The sample was vortexed for 1 min and centrifuged for 0.5 min at 6000 rpm. Using a 1-mL syringe (Agilent P/N, A6401), the supernatant was filtered through a 17 mm, 0.2 μm PTFE membrane syringe filter (Agilent P/N, A4135) into a micro-V injection vial (Agilent P/N, 5184-3550). The samples were ready for UHPLC–APPI-MS analysis.

3. Results and discussion

3.1. UHPLC column separation and analysis

With the resolving power of Rapid Resolution HT Eclipse PAH column, combined with an ultra fast scan rate of MS used, we were able to achieve mass separation and on-column analysis of sixteen PAHs (including five groups of isobaric PAH analytes) within 2.5 min using a steep mobile phase gradient elution program, together with a flow rate gradient program (results not shown). However, this is not practical for analysis of PAHs in complex biological samples such as oysters when single quadrupole MS detector is used. A longer and shallower gradient elution program is necessary in order to separate isobaric mass matrix interference peak responses from target analytes. The gradient program is presented in Table 2. The gradient elution is started at 100% A1 (i.e., 90% water in acetonitrile). This is necessary to separate isobaric mass interferences from target analytes with *m*/*z* 178, 202 and 228. The initial flow rate is governed by the column back pressure rating and the complexity of sample matrix. Due to the limitation of back pressure rating of 600 bar of the used column, the initial mobile phase flow rate was set at a rate, which gave approximately 400 bar back pressure (about 67% of maximum back pressure). This work was completed prior to the availability of the Zorbax RRHD Eclipse PAH, 2.1 mm × 50 mm, 1.8 μm, 1200 bar column (Agilent P/N, 959757-918). As the gradient elution proceeds, the organic content of mobile phase on column increases, leading to a decreased column back pressure. In order to increase on-column sample throughput and sensitivity (narrower peak responses) for later eluters, a flow rate gradient program is applied. With such a high mobile phase flow rate, column re-equilibrium time is not required between injections.

The flow rate gradient was programmed in such a way that the LC separation was performed with a back pressure of about 400–500 bar. This is necessary for continuing analysis of complex sample matrix where the column back pressure builds up over time as injection proceeds. Column back pressure buildup can be a big problem for UHPLC analysis of complex sample matrixes. To ensure a smooth and continuing analysis, the column must be flushed with a high flow rate of mobile phase (A1), acetonitrile (B1), methanol (A2) or isopropanol (B2) for a prolonged period of time whenever not in use. If this does not significantly lower column back pressure, detach the guard column (XDB C8) from the analytical column and back flush each individual column with a high flow rate of these solvents.

Lower column temperature (15–20 °C) offers higher LC resolving power than higher column temperature for these PAH analytes. However, due to the limitation of back pressure rating of the used

Table 2
H_2O/ACN gradient elution program.

Time (min)	%A1	%B1	Time (min)	Flow (mL/min)
0	100	0	0	0.4
0.5	60	40	14	0.679
14	18.6	81.4	18	0.8
14.25	0	100	18.01	0.4
18	0	100		
18.01	100	0	Column temp: 30 °C	

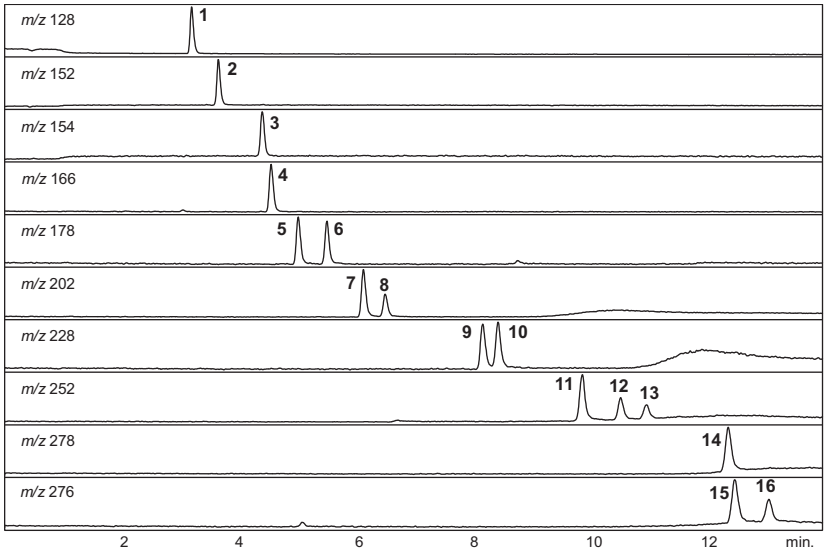

Fig. 1. Mass chromatograms of PAH calibration standard. Injection: 2 μL. (1) Naphthalene (1.33 ng); (2) acenaphthylene (2.67 ng); (3) acenaphthene (1.33 ng); (4) fluorene (0.267 ng); (5) phenanthrene (0.133 ng); (6) anthracene (0.133 ng); (7) fluoranthene (0.267 ng); (8) pyrene (0.133 ng); (9) benzo[a]anthracene (0.133 ng); (10) chrysene (0.133 ng); (11) benzo[b]fluoranthene (0.267 ng); (12) benzo[k]fluoranthene (0.133 ng); (13) benzo[a]pyrene (0.133 ng); (14) dibenzo[a,h]anthracene (0.133 ng); (15) benzo[ghi]perylene (0.267 ng); (16) indeno[1,2,3-cd]pyrene (0.133 ng).

column, the column temperature was set at 30 °C. Fig. 1 shows selected ion monitoring (SIM) traces of sixteen PAHs in calibration standard mix with on-column injection amounts ranging from 0.133 ng to 2.67 ng. Fig. 2 shows SIM traces of sixteen PAHs in PAHs spiked oyster sample with spike concentrations ranging from 0.1 to 2 ppm (Level 1 in Table 4). These results show that all the PAHs including isomers were well separated by retention time or by mass and were quantified in approximately 14 min. The instrument cycle time for this analysis was approximately 20 min, including the time required for post data acquisition column flushing, injector needle and needle seat cleaning. Unspiked blank oyster sample gave no isobaric mass interferences for most of target analytes except for *m*/*z* 178, 202 and 228 with trace amounts of background contributions (chromatograms not shown). This was reflected by slightly elevated spike recoveries for phenanthrene, fluoranthene, pyrene and benzo[a]anthracene at low level spike concentrations (Level 1 in Table 4).

3.2. Evaluation of linearity

A series of calibration standards were prepared in acetonitrile from the EPA 610 PAH mix by a serial dilution method with a dilution factor of 2. These prepared calibration standards covered concentration ranges of 5 orders of magnitude. Triplicate analyses were performed for each standard with 2 μL of injection volume. The calibration standards were analyzed in an injection sequence starting from the lowest (e.g., 0.76–15.3 pg/μL) to the highest (100,000–2,000,000 pg/μL) concentrations. A total of 18 calibration standards were analyzed including the stock solution injected for the data points with the highest concentrations. Table 3

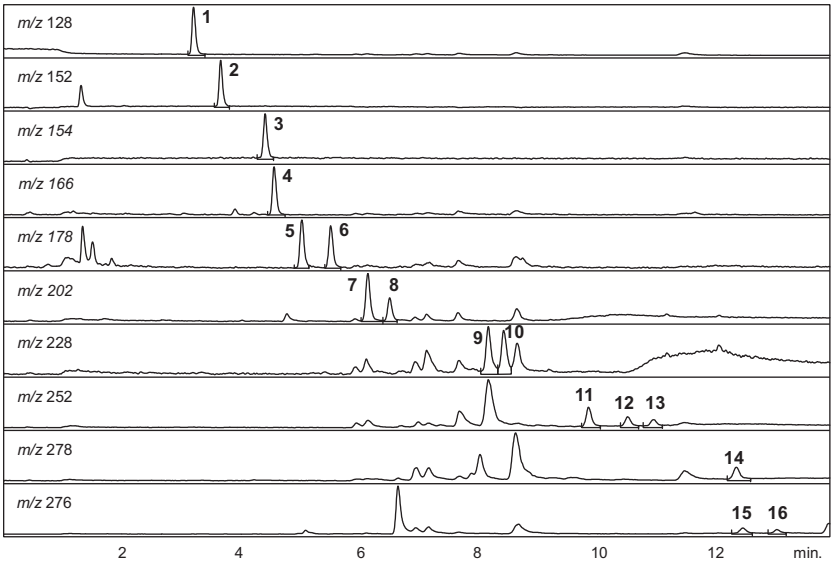

Fig. 2. Mass chromatograms of PAHs in oysters. 10 g PAH spiked oyster sample. Spike conc. equivalent to Level 1 in Table 4. Injection: 2 μL. (1) naphthalene (1 ppm); (2) acenaphthylene (2 ppm); (3) acenaphthene (1 ppm); (4) fluorene (0.2 ppm); (5) phenanthrene (0.1 ppm); (6) anthracene (0.1 ppm); (7) fluoranthene (0.2 ppm); (8) pyrene (0.1 ppm); (9) benzo[a]anthracene (0.1 ppm); (10) chrysene (0.1 ppm); (11) benzo[b]fluoranthene (0.2 ppm); (12) benzo[k]fluoranthene (0.1 ppm); (13) benzo[a]pyrene (0.1 ppm); (14) dibenzo[a,h]anthracene (0.1 ppm); (15) benzo[ghi]perylene (0.2 ppm); (16) indeno[1,2,3-cd]pyrene (0.1 ppm).

Table 3
PAH dynamic linear ranges.

Analyte	Linear range (pg)	Equation	R^2	Linear (order)
Naphthalene	15.3–125,000	$y = 79.58x + 82682$	0.995	3.9
Acenaphthylene	30.5–125,000	$y = 77.56x + 87926$	0.997	3.6
Acenaphthene	30.5–125,000	$y = 78.28x + 77830$	0.997	3.9
Fluorene	3.1–50,000	$y = 92.78x + 14843$	0.999	4.2
Phenanthrene	1.5–200,000	$y = 83.97x + 13784$	0.996	5.1
Anthracene	3.1–200,000	$y = 87.17x + 15345$	0.995	4.8
Fluoranthene	6.1–50,000	$y = 87.17x + 15345$	0.999	3.9
Pyrene	3.1–200,000	$y = 106.6x + 13793$	0.997	4.8
Benzo[a]anthracene	3.1–200,000	$y = 97.12x + 15946$	0.996	4.8
Chrysene	3.1–200,000	$y = 114.6x + 15542$	0.997	4.8
Benzo[b]fluoranthene	6.1–100,000	$y = 114.5x + 84139$	0.995	4.2
Benzo[k]fluoranthene	6.1–200,000	$y = 109.1x + 16702$	0.997	4.5
Benzo[a]pyrene	12.2–200,000	$y = 94.33x + 19431$	1.000	4.2
Dibenzo[a,h]anthracene	3.1–50,000	$y = 222.6x + 72588$	0.996	4.2
Benzo[ghi]perylene	6.1–400,000	$y = 75.88x + 28538$	0.995	4.8
Indeno[1,2,3-cd]pyrene	6.1–200,000	$y = 106.8x + 85494$	0.999	4.5

shows the linear ranges, linear regression equations, and correlation coefficients (R^2) in terms of analyte peak area as a function of absolute injection amount (pg) on column. These results show that UHPLC–APPI-MS offered dynamic linear ranges covering 3.6–5.1 (Ave. 4.4) orders of magnitude with R^2 of at least 0.995 under tested conditions.

3.3. Spike recovery test

Ten grams of homogenized blank oysters was respectively spiked with 100 μL of 10 times dilution of EPA 610 PAH mix stock solution, leading to PAH spike concentrations of 0.1–2 ppm in oysters (Level 1 in Table 4) and with 100 μL of 2 times dilution of EPA 610 PAH mix, resulting in PAH spike concentrations of 0.5–10 ppm in oysters (Level 2 in Table 4). The level 1 spike concentrations (0.1–2 ppm in oysters) were below or substantially below the US FDA level of concern in oysters [29]. Level 2 was five times the concentration of level 1. Triplicate extractions and analyses were performed for each spike level. The samples were extracted using the QuEChERS extraction and d-SPE cleanup procedure described in Section 2. Once the linearity was established, the spike recovery extracts were analyzed by a single point calibration method using their matching calibration standards with 1500× dilution of EPA 610 PAH mix stock solution for level 1 and 300× dilution for level 2. The spike recoveries ranged from 77% to 110% with %RSD of 0.6–6.7 for level 1 and 71% to 92% with %RSD of 0.3–4.3 for level 2 (Table 4).

Extensive work has been performed to investigate the performance of QuEChERS extraction and d-SPE cleanup procedure for oysters. Extraction was performed with no water, 5 mL, 7 mL and 10 mL water added and comparable spike recoveries were obtained. The cleanest extract was obtained with water added prior to salt extraction. It is believed that many water-soluble matrix interferences partitioned into the aqueous phase, leading to a cleaner extract for analysis. Excess amount of the salts $MgSO_4$ and NaCl are present in the initial extraction in order to compensate for varying amounts of water associated with the native oysters. We noticed that higher molecular weight PAHs especially benzo[ghi]perylene (m/z 276) tended to be lost more than its isobaric mass PAH (indeno[1,2,3-cd]pyrene) during extraction. Spike recoveries were also compared between 10 mL and 15 mL acetonitrile, respectively partitioned with 7 mL of water. It was determined that 15 mL acetonitrile yielded a few percent higher recovery for higher molecular weight PAH analytes.

Several individual solid phase extraction sorbents were evaluated in the d-SPE for matrix clean-up. These sorbents include GCB (graphitized carbon black), PS-DVB (polystyrene-divinyl benzene), C18, C18EC, PSA, Florisil and Silica. As predicted, GCB and PS-DVB strongly retained PAH analytes, leading to extremely poor recoveries. The use of either C18 or C18EC lowered the spike recoveries of higher molecular weight PAHs. Recoveries decreased as the molecular weight increased. PSA, Florisil and Silica gave excellent overall recoveries for all PAHs. These three sorbents were further tested for d-SPE cleanup efficiencies. Silica was found to be not as efficient in removing isobaric mass matrix interferences as PSA and Florisil. PSA and Florisil offered comparable cleanup performances. However, for method simplicity PSA was used as d-SPE sorbent in the finalized method.

3.4. Instrument detection limits

The instrument detection limits (IDLs in pg, 6σ S/N = 3) on column based on the gradient elution program (Table 2) were calculated from low picogram injection amounts using low level calibration standards. The S/N ratios were calculated using ChemStation software based on six times the standard deviation of manually selected background noise region for each analyte. The on-column IDLs and near-to-IDL injection amounts used to generate IDLs are presented in Table 5. The results show that the IDLs ranged from 8 pg to 106 pg with an average of 23 pg. The first three eluters (naphthalene, acenaphthylene and acenaphthene) gave higher IDLs due to elevated background noise from acetonitrile/water mobile phase. This is probably due to the fact that these earlier eluters have lower masses, appearing in a mass region which generally has a higher chemical background in LC–MS. Removing these three compounds yields an average IDL of 12 pg for the rest of target analytes. These results show that Agilent's Infinity 1290 UHPLC system coupled with G6140A SQ MSD offered low picogram on-column detection limits for all sixteen PAH analytes under the tested conditions.

3.5. Method detection limits

The method detection limits (MDLs in ppm, 6σ S/N = 3) were calculated from PAH peak area responses generated from triplicate extractions and analyses of low level spike samples with near-to-MDL spike concentrations (0.02–0.2 ppm, Table 5). These calculated MDLs ranged from 0.013 ppm for fluorene and dibenzo[a,h]anthracene to 0.129 ppm for acenaphthylene with an average MDL of 0.040 ppm for all 16 compounds. Background subtraction was performed for those analytes with trace amount of

Table 4
PAH spike recoveries in oysters.

Analyte	FDA level of concern (ppm)	Level 1			Level 2		
		Spike conc. (ppm)	% Rec.	Rec.% RSD	Spike conc. (ppm)	% Rec.	Rec.% RSD
Naphthalene	133	1	92	1.5	5	91	1.1
Acenaphthylene	NA	2	89	4.2	10	92	3.1
Acenaphthene	NA	1	93	4.8	5	90	1.6
Fluorene	267	0.2	91	3.7	1	89	4.2
Phenanthrene	2000[a]	0.1	96	3.7	0.5	91	0.3
Anthracene		0.1	91	1.6	0.5	92	1.7
Fluoranthene	267	0.2	110	1.3	1	91	1.8
Pyrene	200	0.1	98	3.3	0.5	91	2.6
Benzo[a]anthracene	1.43	0.1	100	4.9	0.5	90	1.8
Chrysene	143	0.1	87	4.0	0.5	81	1.0
Benzo[b]fluoranthene	1.43	0.2	86	1.3	1	84	1.5
Benzo[k]fluoranthene	14.3	0.1	93	0.6	0.5	84	3.2
Benzo[a]pyrene	0.143	0.1	93	6.7	0.5	88	4.3
Dibenzo[a,h]anthracene	0.143	0.1	90	4.8	0.5	81	2.4
Benzo[ghi]perylene	NA	0.2	77	0.6	1	71	2.2
Indeno[1,2,3-cd]pyrene	1.43	0.1	84	4.4	0.5	75	2.0

FDA level of concern from Ref. [29]; NA, not available; %Rec, average of triplicate extractions and analyses.
[a] The sum of level of concern for phenanthrene and anthracene.

Table 5
Instrument detection limits and method detection limits.

Analyte	Retention time (min)	IDL injection amount (pg)	IDL[a] (pg)	MDL spike conc. (ppm)	MDL[a] (ppm)
Naphthalene	3.320	61.0	50.9	0.1	0.028
Acenaphthylene	3.794	122.1	105.6	0.2	0.129
Acenaphthene	4.564	61.0	60.4	0.1	0.102
Fluorene	4.724	12.2	11.7	0.02	0.013
Phenanthrene	5.065	12.2	10.2	0.1	0.040
Anthracene	5.551	12.2	10.4	0.1	0.030
Fluoranthene	6.177	12.2	9.3	0.2	0.030
Pyrene	6.546	6.1	8.0	0.1	0.023
Benzo[a]anthracene	8.211	12.2	14.8	0.1	0.030
Chrysene	8.468	12.2	13.9	0.1	0.031
Benzo[b]fluoranthene	9.896	24.4	12.9	0.2	0.021
Benzo[k]fluoranthene	10.555	12.2	11.9	0.1	0.021
Benzo[a]pyrene	10.991	12.2	12.1	0.1	0.027
Dibenzo[a,h]anthracene	12.387	12.2	10.3	0.1	0.013
Benzo[ghi]perylene	12.489	24.4	14.1	0.2	0.057
Indeno[1,2,3-cd]pyrene	13.069	12.2	11.2	0.1	0.040

[a] IDLs and MDLs calculated from $6 \times \sigma$ S/N of 3, average of triplicate analyses.

isobaric mass interferences or background level contributions and the affected MDLs were adjusted and elevated accordingly. These background levels were generally near or below MDLs (6σ S/N = 3). In comparison with the US FDA PAH levels of concern in oysters [29] listed in Table 4, these MDLs ranged from about 5 times lower for benzo[a]pyrene to more than 4 orders of magnitude lower for phenanthrene and anthracene.

4. Conclusions

In this work, we have developed an UHPLC–APPI-MS method for analysis of US EPA sixteen priority pollutant PAHs in oysters. The MDLs were about 5 times to over 4 orders of magnitude lower than US FDA levels of concern in oysters [29] depending on specific compounds. With a single quadrupole MS detector used for this method, isobaric mass interference presented a big challenge for analysis of 16 PAHs in complex biological samples. This was overcome with a carefully designed gradient elution program in combination with superior resolving power of UHPLC column. However, in order to separate isobaric mass interferences from target analytes, a longer and shallower gradient elution program must be used, which compromises on-column sample throughput relative to MS/MS detector [6].

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