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Quantitation of Polycyclic Aromatic Hydrocarbons (PAHs) in Water, Soil sediment and Fish tissue using GC-MS triple quad

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ABSTRACT
This paper discusses method of analysis for Polycyclic Aromatic Hydrocarbons (PAHs) including matrix specific sample extraction procedure for different matrices and final determination using hyphenated mass spectrometric technique of GC-MS/MS. In the present study, methods have been developed for Water, Fish tissue and Soil sediment by plotting calibration curve using respective matrix match standards so as to remove any interferences due to matrix effect. Extraction procedures for Fish tissue & Soil sediment being modified QuEChERS and for Water being liquid-liquid extraction. Chromatographic analysis was done using GC-MS triple quad with MRM mode for confirmatory analysis. With Instrument LOQ of 10ppb; optimized methodology gave results with quantitation limits of 0.2, 20 and 100ppb in water, soil sediment and fish tissue matrix respectively with %RSD (n=6) <15% for all 16 congeners of PAHs determined.
Keywords: Polycyclic Aromatic Hydrocarbons (PAH), GC-MS/MS, QuEChERS, Fish tissue, Soil sediment

INTRODUCTION

PAH falls under the category of persistent organic pollutants (POPs) and 16 of them have been classified by the US Environmental Protection Agency (US EPA) as priority pollutants owing to their carcinogenic and mutagenic behavior [1, 2]. This makes their analysis an important aspect to control their entry to food chain and for further remediation. In this study, focus is on determination of 16 of PAHs compounds namely– Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo[ghi]perylene, Benz[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd]Pyrene and Dibenz[a,h]anthracene using GC-MS/MS in Water, Soil sediment and Fish tissue.

Owing to the characteristic UV absorbance spectra for different isomers of PAHs and fluorescent behavior of the compounds [3], HPLC (High Performance Liquid Chromatography) with UV and fluorescence detector have long being associated and used for its determination. Though later on, GC-MS (Gas chromatography mass spectrometer) has emerged as the most suitable instrument technique for detection of PAHs in varied matrices especially in environmental and food samples owing to its high sensitivity with confirmation of reported results with high accuracy. GC-MS has offered the opportunity for the laboratories involved in testing of environmental and food samples to increase the selectivity for PAHs over that of classical detectors such as UV & Fluorescence, used with HPLC [4]. Along with appropriate sample preparation procedures involving matrix matched calibration curve, GC is definitely the preferred choice for detection of PAHs.

The use of GC coupled to Mass Spectrometry (MS) for the determination of PAHs is based on a favorable combination of greater selectivity, resolution, and sensitivity [5]. In comparison to GC-MS using SIM (Selected Ion Monitoring), GC-MS/MS (triple quadrupole) using MRM (Multiple Reaction Monitoring) provides greater selectivity and increased responses thus achieving lower LOQ (Limit of Quantitation) with ability to get over the matrix interferences due to sample, which in turn further enhances the capability and productivity of a chemical testing laboratory.

MATERIAL AND METHODS

Chemicals & Materials

Certified PAHs standard mixture solution of 2000 μg/ml containing each of the following compounds: Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo[ghi]perylene, Benz[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd]Pyrene and Dibenz[a,h]anthracene was provided from Restek. Purities of each PAHs compounds were between 98 to 99%. Working mixed standard solutions for plotting calibration curve were prepared by dilution of the stock solutions with Acetonitrile as solvent for Soil sediment & Fish tissue samples and Ethyl acetate as solvent for water samples.

Acetonitrile (Qualigens, Purity >99.8%), Dichloromethane (SDFCL, Purity > 99.9%) and Ethyl acetate (SDFCL, Purity > 99.9%) were the solvents used. Ultrapure water was produced by a Milli-Q system (Millipore Advantage A10). Anhydrous sodium sulphate (Purity-99%) was purchased from Merck. QuEChERS salts used includes: Magnesium sulphate heptahydrate (Agilent), Sodium chloride (Merck), Sodium citrate dibasic sesquihydrate (Sigma Aldrich), Sodium citrate tribasic dihydrate (Sigma Aldrich), Carbon 18 (Agilent) and Graphitized carbon black–GCB (Agilent).

2.2 Sample Preparation, Extraction of fish tissue samples for PAH Quantitation

Before the extraction of fish tissue samples, scales were removed and dissected using knife. Approximately 20g of fish tissue sample was homogenized using Homogenizer. Accurately weighed 1g of homogenized sample was weighed and extracted with 10ml of acetonitrile and a salt mixture of 4g anhydrous $MgSO_4$, 1g NaCl, 1g Sodium citrate dibasic sesquihydrate and 0.5g of sodium citrate tribasic dihydrate, after 10 minutes of vigorous shaking using multitube vortexer followed by centrifugation at 8000 rpm for 10 minutes. Further dispersive clean-up was given to 5ml of supernatant organic layer with 900mg of anhydrous $MgSo_4$, 300mg of C18 and 45mg of GCB, followed by vortex for 2 minutes and centrifugation at 10000 rpm for 5 minutes. Supernatant thus received was made up to 1ml with solvent (Acetonitrile) and passed through 0.45μm membrane filter prior to injection on GC-MS/MS.

2.3 Sample Preparation, Extraction of Soil sediment samples for PAH Quantitation

Soil sediment sample was pulverized and homogenized using Mixer-Grinder. Accurately weighed 5g of homogenized sample was weighed and extracted the same way as for fish sample mentioned above. Supernatant thus received was made up to 1ml with solvent (Acetonitrile) and passed through 0.45μm membrane filter prior to injection on GC-MS/MS.

Extraction of Water samples for PAH Quantitation

Water sample was vigorously shaken before drawing sample for analysis to ensure homogeneity of sample. Measured volume of 100ml was extracted with 30ml of Dichloromethane (extracting solvent) in a 1-liter glass separating funnel for Liquid–Liquid Extraction (LLE). Samples were shaken vigorously and kept undisturbed for visible phase separation of organic and aqueous layers. The organic layer was collected into a different glass bottle. Extraction process was repeated with two more portions of 30ml dichloromethane so as to ensure complete extraction of residues into organic phase. Organic phase portions collected in glass bottle was passed through anhydrous sodium sulphate to ensure removal of any traces of moisture. Dichloromethane extracts were evaporated to dryness carefully using Rotary evaporator and water bath preset at 40°C. 2ml of ethyl acetate was added to evaporating bottle to dissolve residues and passed through 0.45μm membrane filter prior to injection on GC-MS/MS.

Calibration Standard Preparation

Matrix matched calibration standards is the best solution to overcome the matrix effect when employing PAH determination method for different matrices. For this, PAH free matrix were arranged for Soil sediment, Water and Fish tissue and were initially analyzed for presence of any PAH compounds against solvent linearity. These matrices were treated the same way as samples to obtain respective matrix blanks. Respective matrices were then fortified with Standard mix of PAHs in the range of 10 to 200 ppb to be used as matrix-matched calibration standards.

GC-MS/MS Method

For detection and quantitation of the 16 PAH congeners, the programmed chromatographic conditions were first optimized. The program started from a full Q3 scan analysis and subsequent peak identification using Library search. Every compound peak revealed specific mass spectra and most intense ion for that compound. Based on the most intense ion, Product Ion Scan (PIS) method was run to get product ions with different intensities. Then final optimization of method was developed where these ions were subjected to increasing collision energy (CE). Operating conditions of GC-MS/MS for PAHs, thus optimized are mentioned in Table1 (Operating Parameters) and Table2 (MRM transitions).

Table1: Optimized GC-MS/MS operating parameters for analysis of PAHs

Model & Make of GC-MS/MS	Shimadzu, TQ-8040		
Column used	Non-polar column, 5%-phenyl methyl polysiloxane (60m length x 250µm inner diameter x 0.25µm film thickness)		
Carrier gas	Helium		
Column flow (ml/min.)	1.5		
Column oven initial temperature (°C)	70		
Column oven temperature programming	Rate (°C/min.)	Temp. (°C)	Hold time (min.)
	-	70	1
	20	150	2
	5	300	20
Injector mode	Split-less		
Injection temperature(°C)	280		
Ion source temperature(°C)	230		
Interface temperature(°C)	280		

Table2: Optimized MRM transitions with Collision Energy (CE) for 16 PAH (Polycyclic Aromatic Hydrocarbons) congeners using GC-MS/MS

Compound Name	RT (min.)	Quantifier ion transition	CE	Qualifier ion transition	CE
Naphthalene	8.1	128.10>102.00	20	128.10>128.10	10
Acenaphthylene	11.8	152.10>151.10	30	152.10>152.10	10
Acenaphthene	12.2	153.10>152.10	20	153.10>153.10	10
Fluorene	13.9	166.10>165.10	20	166.10>166.10	10
Phenanthrene	17.7	178.10>152.10	20	178.10>178.10	10
Anthracene	17.9	178.10>152.10	35	202.10>202.10	10
Fluoranthene	22.9	202.10>199.90	35	202.10>202.10	10
Pyrene	23.9	202.10>199.90	30	228.10>228.10	10
Benz[a]anthracene	29.5	228.10>226.00	35	252.10>252.10	10
Chrysene	29.7	228.10>226.00	35	252.10>252.10	10
Benzo[b]fluoranthene	34.3	252.10>249.90	35	276.10>276.10	10
Benzo[k]fluoranthene	34.4	252.10>249.90	35	278.10>278.10	10
Benzo[a]Pyrene	35.6	252.10>249.90	35	276.10>276.10	10
Indeno[1,2,3-cd]Pyrene	40.9	276.10>274.00	20	128.10>128.10	10
Dibenz[a,h]anthracene	41.1	278.10>275.90	30	152.10>152.10	10
Benzo[ghi]perylene	42.5	276.10>274.00	20	153.10>153.10	10

In this study, to combat the matrix effect due to different matrices studied, matrix matched calibration standards on instrument were run in the range of 10 to 200ppb for Water & Soil sediment and 10 to 1000ppb for Fish tissue.

RESULTS
GC-MS/MS MRM method thus formed was run and gave acceptable separation of the 16 compounds of PAH with retention times in the range of 8.1 to 42.7 minutes. (Figure1)
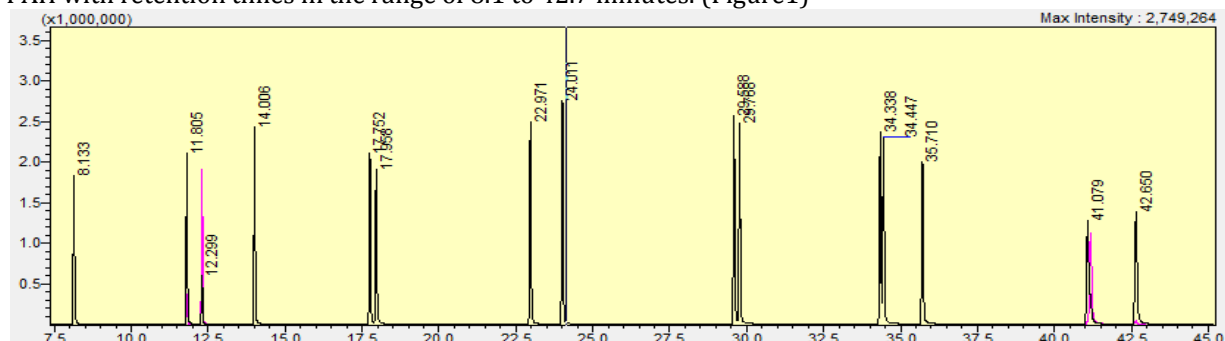

Figure1: Total ion chromatogram (TIC) for 16 PAHs of 200ppb mixed standard with their respective Retention Time (RT) and following order of elution - Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo[ghi]perylene, Benz[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd]Pyrene and Dibenz[a,h]anthracene

Limit of Quantitation (LOQ) in different matrix were established based on Maximum Residue Limit (MRL) for PAHs such that LOQ attained in the method developed is sufficiently low for the method to be used for monitoring purposes. LOQ of 0.2, 20 and 100 ppb were achieved in water, Soil sediment and fish tissue matrix respectively, Instrument LOQ being 10 ppb. %RSD obtained for 6 replicates at LOQ level for all the 16 PAHs is <15%. Calibration ranges in sample, respective regression equation, coefficient of regression (R²), method LOQ and % RSD at LOQ level of concentration for 16 PAH compounds are mentioned in Table 3, 4 & 5 for Water, Soil sediment and Fish tissue respectively.

The developed analytical protocol was applied for determination of 16 PAHs in water, Soil sediment and fish tissue samples to assess method efficiency. It was seen that water and soil sediment samples were found to be free from PAH's with LOQ of 0.2ppb (Figure2) and 20ppb, but fish tissue samples reportedly were found to have PAH's namely – Naphthalene, Acenaphthene, Fluorene, Phenanthrene, Fluoranthene, Pyrene, Benzo[ghi]perylene, Benz[a]anthracene and Chrysene. This is probably because of hydrophobicity of PAH's.

Table3: Retention time and Calibration data in Water obtained using matrix matched standards for 16 selected PAHs using GC-MS/MS

Compound Name	RT (min.)	Regression equation (n=5)	R²	Instrument LOQ (ppb)	Method LOQ (ppb)	%RSD at LOQ (n=6)
Naphthalene	8.1	y=5171.2x+12617.6	0.9996	10	0.2	1.3
Acenaphthylene	11.8	y=5714.7x+1504.4	0.9998	10	0.2	1.0
Acenaphthene	12.3	y=12850.6x+4364.0	0.9998	10	0.2	1.1
Fluorene	14.0	y=18736.2x+1576.8	0.9998	10	0.2	1.6
Phenanthrene	17.8	y=7105.98x+3882.4	0.9999	10	0.2	1.0
Anthracene	18.0	y=5588.2x+4628.8	0.9999	10	0.2	3.6
Fluoranthene	23.0	y=9636.5x+6340.1	0.9999	10	0.2	1.6
Pyrene	24.0	y=11404.4x+7010.0	0.9999	10	0.2	2.7
Benz[a]anthracene	29.6	y=15468.9x+46964.5	0.9999	10	0.2	0.9
Chrysene	29.8	y=15737.5x+17950.1	0.9999	10	0.2	1.2
Benzo[b]fluoranthene	34.4	y=11229.8x+42493.5	0.9999	10	0.2	1.2
Benzo[k]fluoranthene	34.5	y=11171.5x+12436.1	0.9999	10	0.2	2.4
Benzo[a]Pyrene	35.7	y=10545.2x+34939.2	0.9999	10	0.2	2.6
Indeno[1,2,3-cd]Pyrene	41.1	y=12798.1x+85021.0	0.9995	10	0.2	2.5
Dibenz[a,h]anthracene	41.2	y=9612.0x+78368.8	0.9989	10	0.2	3.7
Benzo[ghi]perylene	42.7	y=12332.0x+56139.3	0.9999	10	0.2	2.4

Table4: Retention time and Calibration data in Soil sediment obtained using matrix match standards for 16 selected PAHs using GC-MS/MS

Compound Name	RT (min.)	Regression equation (n=5)	R²	Instrument LOQ (ppb)	Method LOQ (ppb)	%RSD at LOQ (n=6)
Naphthalene	8.1	y=3042.3x+18456.2	0.9998	10	20	4.8
Acenaphthylene	11.8	y=3347.9x+2772.4	0.9999	10	20	6.9
Acenaphthene	12.3	y=7735.9x+7825.0	0.9999	10	20	6.7
Fluorene	14.0	y=11464.6x+12526.3	0.9999	10	20	6.6
Phenanthrene	17.7	y=4141.6x+9036.4	0.9999	10	20	6.8
Anthracene	17.9	y=3236.0x+3546.4	0.9999	10	20	4.0
Fluoranthene	22.9	y=5718.3x+7438.8	0.9999	10	20	8.0
Pyrene	24.0	y=6857.6x+8414.6	0.9999	10	20	8.4
Benz[a]anthracene	29.6	y=9873.5x+2778.6	0.9999	10	20	8.8
Chrysene	29.7	y=10116.8x+16494.6	0.9999	10	20	8.2
Benzo[b]fluoranthene	34.3	y=7409.5x+2398.1	0.9999	10	20	11.8
Benzo[k]fluoranthene	34.4	y=7381.3x+3612.6	0.9999	10	20	10.4
Benzo[a]Pyrene	35.7	y=7016.3x+6838.1	0.9999	10	20	12.9
Indeno[1,2,3-cd]Pyrene	41.0	y=8801.1x+21979.6	0.9996	10	20	12.8
Dibenz[a,h]anthracene	41.1	y=6757.9x+25765.0	0.9991	10	20	14.3
Benzo[ghi]perylene	42.6	y=8372.1x+9976.9	0.9998	10	20	13.4

Table5: Retention time and Calibration data in Fish tissue obtained using matrix matched standards for 16 selected PAHs using GC-MS/MS

Compound Name	RT (min.)	Regression equation (n=7)	R²	Instrument LOQ (ppb)	Method LOQ (ppb)	%RSD at LOQ (n=6)
Naphthalene	8.1	y=2592.0x+14582.1	0.9996	10	100	5.5
Acenaphthylene	11.8	y=3061.3x+5785.3	0.9999	10	100	6.6
Acenaphthene	12.3	y=6882.0x+5717.6	0.9999	10	100	4.5
Fluorene	14.0	y=10227.5x+39648.6	0.9999	10	100	5.6
Phenanthrene	17.8	y=3936.2x+27290.3	0.9997	10	100	5.0
Anthracene	18.0	y=3195.0x+35491.5	0.9993	10	100	10.2
Fluoranthene	23.0	y=5780.5x+36231.1	0.9997	10	100	5.4
Pyrene	24.0	y=6900.8x+50476.9	0.9996	10	100	5.5
Benz[a]anthracene	29.5	y=10344.0x+110888	0.9993	10	100	7.3
Chrysene	29.8	y=10168.4x+73869.5	0.9996	10	100	6.6
Benzo[b]fluoranthene	34.4	y=7372.5x+49943.6	0.9998	10	100	8.4
Benzo[k]fluoranthene	34.5	y=7328.2x+32876.8	0.9998	10	100	8.7
Benzo[a]Pyrene	35.7	y=7190.2x+63055.4	0.9996	10	100	9.6
Indeno[1,2,3-cd]Pyrene	41.1	y=7929.1x+15044.9	0.9997	10	100	11.7
Dibenz[a,h]anthracene	41.2	y=7788.7x+130337.1	0.9990	10	100	14.0
Benzo[ghi]perylene	42.7	y=8616.4x+84134.0	0.9996	10	100	11.6
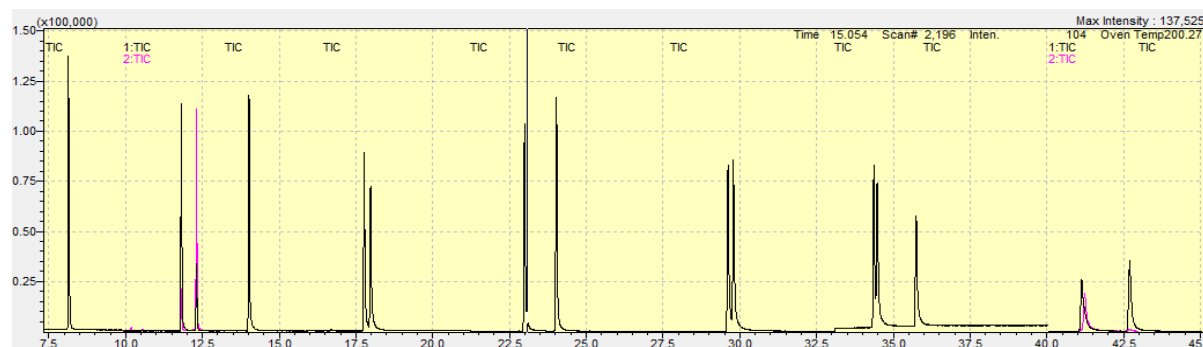
Figure 2: Total ion chromatogram (TIC) for 16 PAHs at level of Limit of Quantitation (LOQ) for Water matrix i.e. 0.2ppb, showing good signal-to-noise (S/N) ratios.

DISCUSSION

QuEChERS methodology for sample preparation has been long used for analysis of pesticide residues in different matrices. In this study, QuEChERS technique is observed to give equally good results for PAH extraction and matrix cleanup in case of Soil sediment and fish tissue. On the other hand, GC-MS triple quad instrument has provided better selectivity and increased sensitivity, thus achieving LOQs to a level that regulatory requirements are met. The method enabled efficient extraction and quantitation and thus is suitable for laboratories engaged in routine analysis of large number of sample volumes.

Dietary intake of contaminated foods especially aquatic products is considered a major source of human exposure to PAHs. This study can be taken further to application in different food matrices so as to assess bioaccumulation of these persistent pollutants in aquatic ecosystem for further control and remediation.

ACKNOWLEDGMENTS

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