Analysis of Polycyclic Aromatic Hydrocarbons in Soil with AgilentSampliQ QuEChERS AOAC Kit and HPLC-FLD

An HPLC-Florescence Detection (FLD) method was developed and validated for the determination of sixteen polycyclic aromatic hydrocarbons (PAHs) in soil. The analysed PAHs include naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fln), pyrene (Pyr),1,2-benza[α]anthracene (BaA), chrysene (Chr), benzo[e]pyrene (BeP), benzo[e]acenaphthylene (BeA), benzo[k]fluoranthene (BkF), dibenzo[α,h]anthracene (DahA), benzo[g,h,i]perylene (Bghi)P and indeno[1,2,3-cd]pyrene (InP). The method employs aquick, easy, cheap, effective, rugged and safe (QuEChERS) multiresidue sample preparationprocedure adopted from the Association of Analytical Communities (AOAC) Official method 2007.01 for extraction and cleanup. The analytes were separated onan Agilent ZORBAX Eclipse PAH column (4.6 mm × 50 mm, 1.8 µm) by gradient elutionwith a binary system of acetonitrile - water with subsequent fluorescence detectionset at appropriate excitation and emission wavelengths. The analyte recoveries ranged from 86.0% to 99.2% with relative standard deviations ranging from 0.6% to 1.9% at three different fortification levels. The limits of detection and quantification ranged from 0.005 to 0.78 and 0.02 to 1.6 ng/g, respectively.

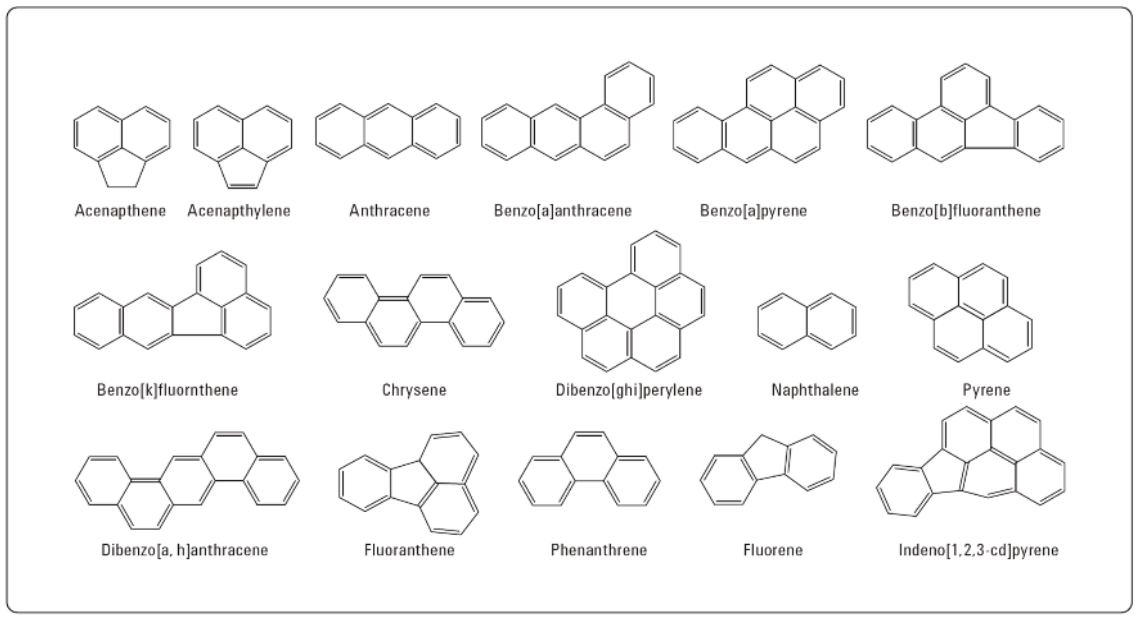

Figure 1: Chemical structures for the polycyclic aromatic hydrocarbons used in the study.

Column	Agilent ZORBAX Eclipse PAH C18 4.6 × 50 mm, 1.8 µm	
Flow rate	0.8 mL/min	
Column temperature	18 °C	
Injection volume	5 µL	
Mobile phase	A = Deionized H_2O	B = CH_3CN
Gradient	T (min)	% B
	0	60
	1.5	60
	7	90
	13	100
Detection	UV at 230 nm (Acy) and varying fluorescence excitation (Ex) and emission (Em) wavelengths	
Wavelengths:		
Time (min)	**Ex/Em wavelengths (nm)**	**PAH detected**
0 – 5 (dark blue)	260/352	Nap, Ace, Flu, Phe, Chr
0 – 14 (red)	260/420	Ant, Pyr, BeP, DahA, BghiP
0 – 14 (light blue)	260/460	Fln, 1,2-BaA,BeA, BkF, InP

Table 1: HPLC Conditions Used for Separation of PAHs

Polyaromatic hydrocarbons, or poly-nuclear aromatic hydrocarbons (PAHs), are fused ring aromatic compounds classified by the number of carbon rings as well as their carcinogenicity.

The two and three ring PAHs are non-carcinogenic, while several of the four, five and six ring PAHs are carcinogenic. The four ring PAHs, chrysene and benzo-[a]anthracene, the five ring PAHs, benzo[a]pyrene, benzo[b]fluoranthene, benzo [k] fluoranthene and dibenzo [a,h] anthracene, and the six ring PAH, indeno [1,2,3-cd] pyrene, are carcinogenic PAHs. Benzo [a] pyrene is the most potent carcinogen among the PAHs [1].

The US-EPA and EU lists sixteen of these PAHs as hazardous compounds [2]. Generally PAHs are lipophilic compounds that show a high affinity for organic matter and their determination in soil always requires powerful extraction techniques to release the strongly sorbed contaminants from the soil material [3]. Several extraction methods (soxhlet, liquid-liquid or solid phase extraction) for sample preparation of soil have been investigated and most of these involved an evaporation step which leads to the loss or low recoveries of the volatile PAHs such as naphthalene [4].

The AOAC QuEChERS method has been widely applied in the analysis of pesticides in food since it was introduced by United States Department of Agriculture (USDA) scientists [5].

More recently, the technique has branched out into new application areas outside of food safety. In general, there are two major steps: extraction and dispersive SPE cleanup. The method uses a single step buffered acetonitrile extraction while simultaneously salting out water from the aqueous sample using anhydrous magnesium sulfate ($MgSO_4$) to induce liquid-liquid partitioning. After removing an aliquot from an organic layer, for further cleanup, a dispersive solid phase extraction (dSPE) is conducted using a combination of primary secondary amine (PSA) sorbent to remove fatty acids from other components and anhydrous $MgSO_4$ to reduce the remaining water in the extract. Other sorbents, such as graphitized carbon black (GCB), may be added to remove pigments and sterol, or C18 to remove lipids and waxes.

This application note presents a method for the analysis of PAHs at trace levels in soil with HPLC-Fluorescence detection (FLD). The HPLC methods are useful for PAH analysis since UV and fluorescence detection offer enhanced selectivity over other techniques such as GC with flame ionization detection [6]. The method includes sample preparation with SampliQ AOAC Buffered Extraction kit (p/n 5982-5755) and SampliQ AOAC Fatty Dispersive SPE 15 ml kit (p/n 5982-5156). Chemical structures of the PAHs in this study are shown in Figure 1.

Experimental

Reagents and Chemicals

All reagents were analytical or HPLC grade. Acetonitrile (CH_3CN) and PAHs were purchased from Sigma-Aldrich (St. Louis, MO, USA). The water used was from a MilliQ system (Milford, Mass, USA). The mobile phase was filtered through a Whatman membrane filter (47 mm diameter and 2 µm pore size).

Standard Solutions

Standard stock solutions (1 mg/mL) were prepared by dissolving 10 mg of the desired PAH in 10 mL CH_3CN and stored at –20°C. All working solutions were prepared fresh daily by serial dilution with CH3CN.

Equipment and Material

The analysis was performed on an Agilent 1200 Series HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump and a fluorescence detector (FLD) set at varying excitation and emission wavelengths (Table 1). The selection of the excitation and emission wavelengths for fluorescence detection was based on the optimum responses for the various PAHs. Since acenaphthylene does not fluoresce, UV detection at 230nm was used. Separation of the compounds was achieved on an Agilent ZORBAX Eclipse PAH column (4.6 mm × 50 mm, 1.8 µm), p/n 959941-918. The data was processed by HPLC 2D Chemstation software.

Extraction and cleanup were achieved with Agilent SampliQ Buffered QuEChERS AOAC Extraction kit, p/n 5982-5755 and SampliQ QuEChERS AOAC Dispersive SPE kit, p/n 5982-5058 (Agilent Technologies).

Instrument conditions

HPLC conditions

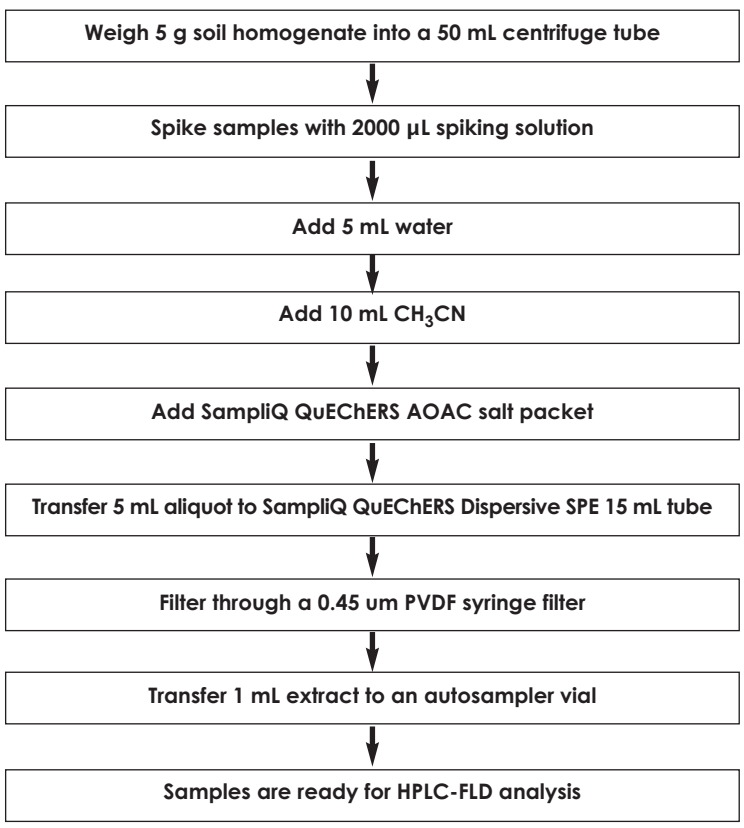

Figure 2: Flow chart of QuEChERS AOAC sample preparation procedure.

Sample preparation

The soil sample was collected from the local botanical garden in Grahamstown, South Africa, air dried at ambient temperature and then sieved to obtain a homogeneous sample.

Extraction

A 5g sample of soil homogenate was placed into a 50mL centrifuge tube from the SampliQ QuEChERS AOAC Extraction kit. Samples were spiked with appropriate spiking solutions to yield the best working solutions for recoveries and reproducibility studies. A 2000µL volume of spiking solution was added to all samples except the blank. Next, 5.0mL of water was added to the tube, and the tube shaken vigorously for 1 min. A 10 mL amount of CH_3CN was then added, followed by an Agilent SampliQ QuEChERS AOAC extraction salt packet (p/n 5082-5755), which contained 6g of anhydrous $MgSO_4$ and 1.5g of anhydrous NaOAc. The sample tubes were hand shaken vigorously for 1 min then further centrifuged at 4000rpm for 5min.

Dispersive-SPE Cleanup

A 6.0 mL aliquot of the upper ACN layer was transferred into a SampliQ QuEChERS AOAC Dispersive SPE 15 mL tube. This SPE tube contained 400 mg of PSA and 1200mg of anhydrous $MgSO_4$. After one minute of shaking, the tubes were centrifuged at 4000rpm for 5 min. A 4 mL aliquot of the extract was filtered through a 0.45µm PVDF syringe filter, then 1000µL extract was placed in an autosampler vial for HPLC-FLD analysis.

Results and Discussion

Chromatographic analysis

The separation of the 16 PAHs was obtained on the Agilent ZORBAX Eclipse PAH column (4.6mm × 50mm, 1.8µm) by gradient elution with a binary system of acetonitrile – water. The chromatogram of the standard mixture is presented in Figure 3. A chromatogram of the blank soil extract is presented in Figure 4 while overlay chromatograms of the spiked soil sample at level 1 (Table 3) are shown in Figure 5.

For detection and quantification, the fluorescence detector was set at varying emission wavelengths (Table 1) to accommodate the diverse absorption intensities of the PAHs. The overlays of Figures 3 and 5 are color-coded according to the chosen excitation and emission wavelengths. The dark blue portion of the chromatogram used the following excitation /emission wavelengths: 260-nm/352-nm; the red portion: 260-nm/420-nm and the light blue portion: 260-nm/440-nm. However, due to lack of a fluorophore, UV detection at 230nm was employed for acenaphthylene.

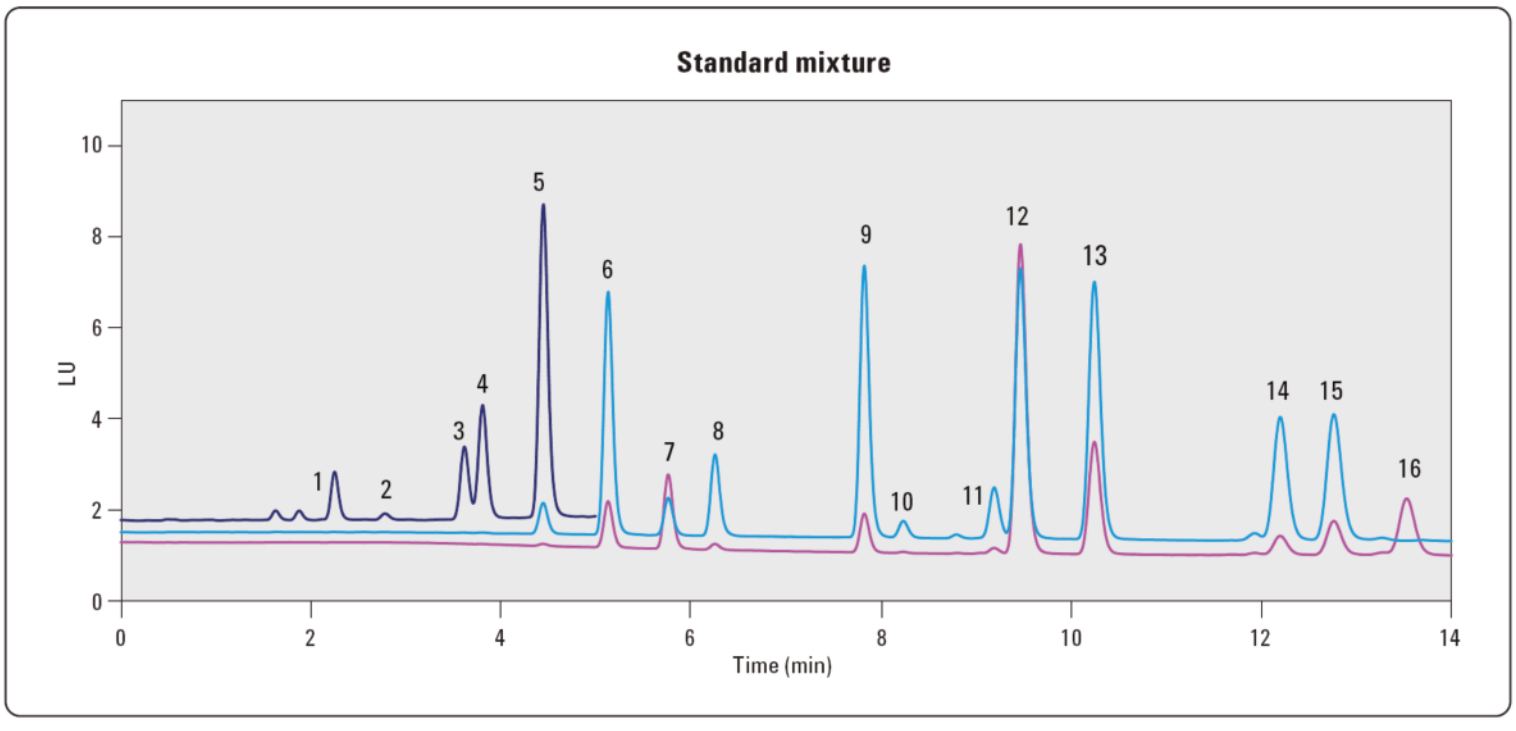

Figure 3: Overlay HPLC – FLD chromatograms of the standard mixture containing: 1. Nap 2. Acy 3. Ace 4. Flu 5. Phe 6. Ant 7. Fln 8. Pyr 9. BaA 10. Chr 11. BeP 12. BeA 13. BkF 14. DahA 15. BghiP 16. InP. The concentration of the PAHs was 1-mg/mL. The blue portion of the chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm; the red portion: 260-nm/420-nm and the light blue portion: 260-nm/440-nm. However, due to lack of a fluorophore, UV detection at 230 nm was employed for acenaphthylene. Chromatographic conditions are shown in Table 1.

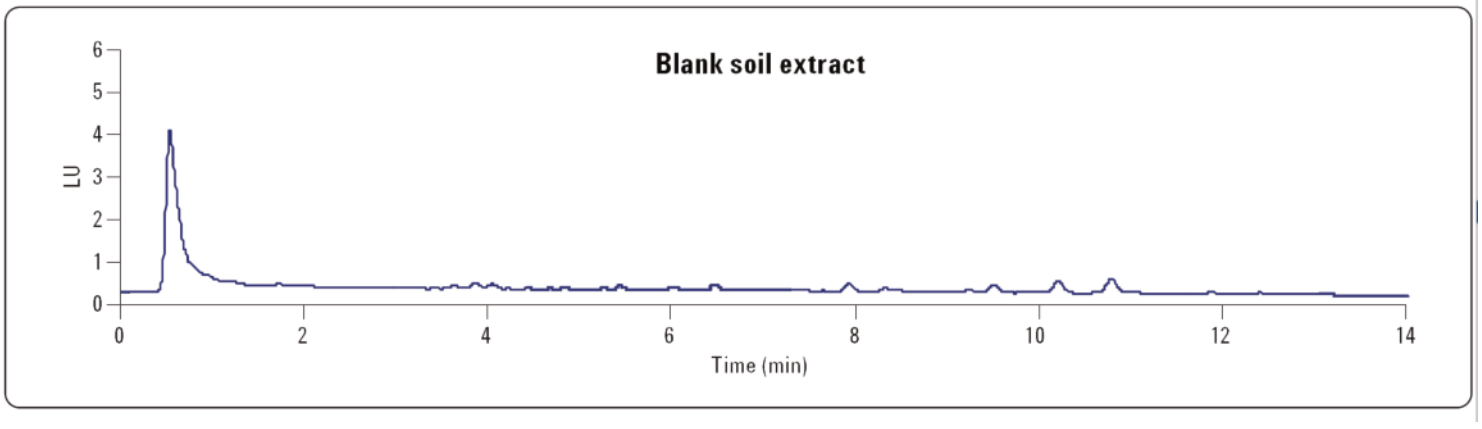

Figure 4: Chromatogram of the blank soil extract. Chromatographic conditions are shown in Table 1. The baseline chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm. The other excitation/emission conditions showed no other interferences.

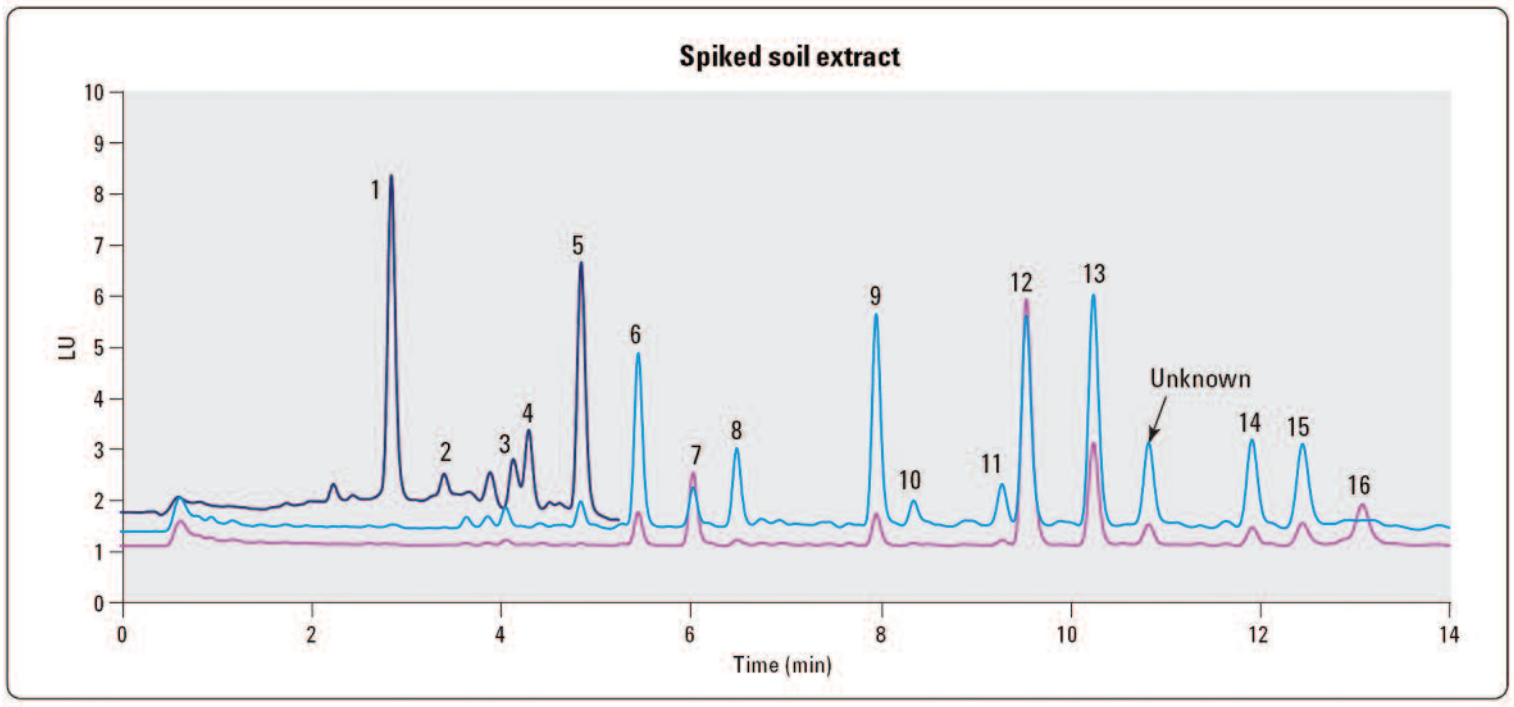

Figure 5: Overlay HPLC – FLD chromatograms of the spiked soil sample containing: 1. Nap 2. Acy 3. Ace 4. Flu 5. Phe 6. Ant 7. Fln 8. Pyr 9. BaA 10. Chr 11. BeP 12. BeA 13. BkF 14. DahA 15. BghiP 16. InP. The spiking level for this sample was a level 1 (see Table 3). The blue portion of the chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm; the red portion: 260-nm/420-nm and the light blue portion: 260-nm/440-nm. However, due to lack of a fluorophore, UV detection at 230 nm was employed for acenaphthylene. Chromatographic conditions are shown in Table 1.

QuEChERS extraction

The use of CH_3CN as an extracting solvent in a salting-out condition, without the need to add co-solvents, attained high extraction yields as shown by the recoveries in Table 4. In addition, the CH_3CN solvent is compatible with the HPLC – FLD procedure in this application note. Therefore, no evaporation or reconstitution solvent was required. This is particularly important for the PAHs since some of these compounds (naphthalene, acenaphthene and fluorene) are extremely volatile and may be lost during an evaporation step [7].

Linearity, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Linearity

The linear calibration curves were obtained by plotting the peak area for each analyte versus its concentration. Curves were generated by spiking the sample blanks at a concentration range of 0 – 300 ng/g.

Limits of Detection and Quantification

The limits of detection and quantification were evaluated from the concentration of sulfonamides required to give a signal-to-noise ratio of 3 and 10 respectively. Table 2 shows the regression equation, correlation coefficients, and very limits of detection and quantification.

Recovery and Reproducibility

The recovery and reproducibility (RSD) were evaluated on spiked samples at three different levels as shown in Table 3. The analysis was performed in replicates of six (n = 6) at each level. Table 4 shows the recoveries and RSD values for the sixteen polycyclic aromatic hydrocarbons.

Conclusions

A simple and fast multiresidue method based on SampliQ QuEChERS AOAC and HPLC-FLD has been developed for the simultaneous determination of sixteen polycyclic aromatic hydrocarbons at parts-per-billion levels in soil. High recoveries with excellent RSD were attained, therefore it is concluded that the method is applicable for quality control PAHs in real samples.

PAH	Regression equation	R2	LOD	LOQ
Naphthalene	Y = 0.0266x + 0.1568	0.9992	0.48	1.6
*Acenaphthylene	Y = 0.0580x – 0.1323	0.9991	0.06	0.20
Acenaphthene	Y = 0.0176 x + 0.0122	0.9995	0.12	0.41
Fluorene	Y = 0.0358x – 0.1701	0.9991	0.24	0.79
Phenanthrene	Y = 0.1097x - 0.4277	0.9994	0.07	0.22
Anthracene	Y = 0.0884x – 0.096	0.9993	0.18	0.60
Fluoranthene	Y = 0.0273x – 0.0069	0.9997	0.07	0.24
Pyrene	Y = 0.0284x – 0.1041	0.9993	0.005	0.02
1,2-Benzanthracene	Y = 0.0120x – 0.0249	0.9994	0.78	0.26
Chrysene	Y = 0.0067x + 0.0165	0.9992	0.007	0.02
Benzo[e]pyrene	Y = 0.017x – 0.0252	0.9995	0.008	0.03
Benz[e]acenaphthylene	Y = 0.1304x + 0.0727	0.9993	0.03	0.11
Benzo[k]fluoranthene	Y = 0.052x + 0.0165	0.9993	0.06	0.21
Dibenzo[a,h]anthracene	Y = 0.062x – 0.0346	0.9994	0.18	0.6
Benzo[g,h,i]perylene	Y = 0.0599x + 0.0779	0.9995	0.18	0.81
Indeno[1,2,3-cd]pyrene	Y = 0.0352x – 0.1588	0.9992	0.05	0.59
* UV detection at 230 nm				

Table 2: Linearity, LOD and LOQ for the Sixteen Polycyclic Aromatic Hydrocarbons

PAH	Spiking level (ng/g)		
	1	2	3
Naphthalene	20	100	200
*Acenaphthylene	20	100	200
Acenaphthene	10	50	100
Fluorene	10	50	100
Phenanthrene	10	50	100
Anthracene	10	50	100
Fluoranthene	10	50	100
Pyrene	10	50	100
1,2-Benzanthracene	5	20	50
Chrysene	10	50	100
Benzo[e]pyrene	5	20	50
Benz[e]acenaphthylene	5	20	50
Benzo[k]fluoranthene	5	20	50
Dibenzo[a,h]anthracene	5	20	50
Benzo[g,h,i]perylene	5	20	50
Indeno[1,2,3-cd]pyrene	5	20	50
* UV detection at 230 nm			

Table 3: PAHs Spiking Levels

PAH	Level of spiking (ng/g) (n = 6)					
	1		2		3	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
Naphthalene	96.5	0.7	86.2	1.4	92.8	1.4
*Acenaphthylene	87.3	0.7	90.0	1.3	91.7	1.6
Acenaphthene	91.0	1.8	89.2	1.1	89.7	1.4
Fluorene	95.2	0.8	91.4	1.3	86.0	1.2
Phenanthrene	93.0	1.0	94.6	0.7	98.1	0.9
Anthracene	91.9	1.1	90.0	0.8	97.6	0.7
Fluoranthene	93.5	1.7	94.7	1.3	87.9	1.5
Pyrene	96.3	1.3	89.4	0.9	91.2	1.9
1,2-Benzanthracene	92.9	1.7	87.8	1.5	92.8	0.7
Chrysene	98.0	1.4	92.4	1.2	95.8	1.0
Benzo[e]pyrene	97.2	1.0	97.5	0.7	90.3	0.8
Benz[e]acenaphthylene	93.2	0.9	93.1	0.6	98.0	0.7
Benzo[k]fluoranthene	94.1	1.1	97.6	0.7	91.4	1.1
Dibenzo[a,h]anthracene	89.2	1.0	99.2	1.7	90.8	1.3
Benzo[g,h,i]perylene	91.0	0.9	96.7	0.8	97.3	1.6
Indeno[1,2,3-cd]pyrene	86.0	1.2	97.8	0.8	94.3	1.3
* UV detection at 230 nm						

Table 4: Recoveries and RSDs for the Sixteen Polycyclic Aromatic Hydrocarbons in Soil Sample (n = 6)

AUTHOR DETAILS

Bellah O. Pule,
Lesego C. Mmualefe,
Nelson Torto
Department of Chemistry
Rhodes University
P. O. Box 94,
Grahamstown 6140
South Africa
Web: www.agilent.com/chem

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