Application note | 001106

Sample preparation

Determination of Polycyclic aromatic hydrocarbons in soils using the EXTREVA ASE Accelerated Solvent Extractor and GC-MS

Authors

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Keywords

Pressurized fluid extraction, PAHs, sample preparation, U.S. EPA Method 8270, environmental, soil, ISQ 7000 Single Quadrupole GC-MS

Goal

To demonstrate a method for the determination of Polycyclic aromatic hydrocarbons (PAHs) in soils using Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor, a newly developed and fully automated parallel extraction, and evaporation system.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants in the environment. PAHs are produced from the incomplete combustion of organic materials (e.g., coal, oil, petrol, and wood). Of the hundreds of known PAHs, sixteen have been designated high priority pollutants by the United States Environmental Protection Agency (U.S. EPA). Because of their tendency to bind to particulate matter they can be transported even long distances from the point of origin, settle out in soil and sediments, and act as contaminant sinks. The occurrence of PAHs in food and the environment is an increasing concern because of their toxicity, carcinogenicity, and mutagenicity.[1]
PAHs are typically determined after extraction from food, environmental or biological samples using analytical methods. The United States Environmental Protection Agency (U.S. EPA) Method 3500 provides general guidance on the selection of methods used in the quantitative extraction (or dilution) of samples for analysis by one of the semi-volatile or nonvolatile determinative methods.[2] Analysis of the extracts is described in Method 8100.[3] U.S. EPA Method 8270 provides procedures for the analysis of solid, water, and air samples for the detection and measurement of different groups of semi-volatile organic compounds, including PAHs, using GC-MS.[4]

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Techniques such as Soxhlet (U.S. EPA Method 3540), sonication (U.S. EPA Method 3550), and microwave extraction (U.S. EPA Method 3546) are presently used for extracting nonvolatile and semi-volatile organic compounds from solids such as soils, sludges, and wastes. Those techniques are very labor-intensive and suffer from high solvent consumption. Accelerated solvent extraction (U.S. EPA Method 3545) was developed to meet the new requirements for reducing solvent usage in the preparation of solid samples.[5] With accelerated solvent extraction, extractions can be completed in very short periods of time with minimal amounts of solvent compared to conventional sample extraction techniques such as Soxhlet and sonication.

The EXTREVA ASE system (Figure 1) is based on many proprietary technologies including gas-assisted solvent delivery[6] and parallel accelerated solvent extraction.[7] This fully automated system combines the extraction and evaporation capabilities in one instrument, and it can be conveniently used for extracting and concentrating/evaporating extracts from up to 16 solid and semi-solid samples.

Accelerated solvent extraction was originally developed to meet the new requirements for reducing solvent usage in the preparation of solid samples. With accelerated solvent extraction, extractions can be completed in very short periods of time and with minimal amounts of solvent compared to conventional samples extraction techniques such as Soxhlet and sonication. In this application note, the development of an analytical method using a fully automated solvent extraction system, the EXTREVA ASE system, and GC-MS for the determination of 16 PAHs in soil is presented.

Figure 1. EXTREVA ASE Accelerated Solvent Extractor

Experimental

Equipment and consumables

- EXTREVA ASE Accelerated Solvent Extractor (P/N 22184-60101)
- Thermo Scientific™ TRACE™ 1310 Gas Chromatograph
- Thermo Scientific™ ISQ™ 7000 Single Quadrupole Mass Spectrometer
- Thermo Scientific™ Rocket Synergy™ 2 Evaporator System
- Thermo Scientific™ Dionex™ ASE™ Collection Vials, 60 mL (P/N 048784)
- Clear collection bottles, 250 mL (P/N 056284)
- Thermo Scientific™ Dionex™ Cellulose Filter (P/N 056780)
- Concentration flask assembly 100 mL (P/N 22184-62235)
- Fisherbrand™ Robotic Screw Top Autosampler Vial, amber (P/N 03-391-9)
- Amber vial, Thermo National brand, Fisher# 03-377B, Thermo# C4000-2W, 9 mm glass screw with levels (Cap- Thermo Fisher# 03-379-123 Thermo# C5000-54A, 9 mm crew thread cap)
- Clear vial, Thermo National brand, Fisher# 03-377D, Thermo# C4000-1W 9 mm wide open with levels (Cap- Thermo Fisher# 03-379-123 Thermo# C5000-54A, 9 mm crew thread cap)

Solvents and chemicals

- Dichloromethane, HPLC, ACROS Organics™, Fisher Chemical™ (P/N AC610050040)
- Acetone, Optima™, for HPLC and GC, Fisher Chemical™ (P/N A929-4)
- SV Calibration Mix #5/610 PAH Mix, Restek™ (P/N 31011)
- B/N Surrogate Mix (4/89 SOW), Restek (P/N 31024)
- Semi-volatile Internal Standard Mix, Restek (P/N 31206)
- Thermo Scientific™ Dionex™ ASE™ Prep Diatomaceous Earth (DE) Dispersant, 1 kg (P/N 062819)
- Clean loam soil (Sigma-Aldrich™ P/N CLNLOAM6)
- CRM soil, PAH, Sigma Aldrich (P/N CRM141-50G)

Extraction and concentration

The PAHs and surrogate standards (2-Fluorobiphenyl and *p*-Terphenyl-d14) were mixed and diluted with acetone-methylene chloride 1:1 (v/v) to produce a stock solution with a concentration of 5 µg/mL. Calibration standards with concentrations of 0.1, 0.2, 0.5, 0.75, 1.0, and 2.0 µg/mL were prepared by diluting the stock solution. The internal standard solution of naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12 had a concentration of 20 µg/mL. 20 µL were added to each calibration standard.

A cellulose filter was placed on top of a 10 mL body and the end cap was hand tightened. Two grams of clean loam soil were mixed in a glass beaker with an equal amount of diatomaceous earth (Dionex ASE Prep DE dispersant). The resulting mixture was carefully poured into the extraction cell and spiked with the appropriate amount of PAHs standard. Any empty volume was filled with Ottawa sand (or Dionex ASE Prep DE dispersant) while light tapping. After placing another cellulose filter on top of the cell body, the second end cap was hand tightened. The 100 ml extraction cell was instead prepared by first tightening the end cap with its body, followed by the insertion of the cellulose filter. Twenty grams of clean loam soil were mixed in a glass beaker with an equal amount of Dionex ASE Prep DE dispersant. The resulting mixture was carefully poured into the extraction cell and spiked with the PAHs standard. Any empty volume was filled with Ottawa sand (or Dionex ASE Prep DE dispersant) while light tapping. After placing another cellulose filter on top of the cell body, the second end cap was hand tightened. The Dionex ASE Prep DE dispersant, acting as a dispersant, plays a key role in preventing sample compaction during the compression phase and in ensuring efficient solvent contact with the sample. In the case of wet samples, it is highly recommended to either pre-dry the samples in the air or mix them in a 1:1 ratio with the proprietary Dionex™ ASE™ Prep Moisture Absorbing Polymer (P/N 083475) and the Dionex ASE Prep DE dispersant for optimum moisture removal under accelerated solvent extraction conditions.

The instrument was programmed according to the conditions reported in Table 1. Before proceeding to the extraction of the samples, the system was rinsed with the extraction solvent (acetone-methylene chloride 1:1, v/v). Acetone-methylene chloride 1:1 (v/v) was used during evaporation as a rinse solvent and 1.6 mL was added during evaporation. After concentration, the samples were added with internal standard and analyzed by GC-MS. The GC-MS conditions are summarized in Table 2.

Table 1. Extraction and concentration conditions for the EXTREVA ASE system

Extraction	
Cell type	Stainless steel
Cell size	10 mL and 100 mL
Oven temperature	100 ˚C
Purge time	45 s (10 mL cell); 180 s (100 mL cell)
Nitrogen flow (gas assisted extraction)	10 mL/min per channel
Cell fill volume	50%
Solvent flow rate	1.6 mL/min (10 mL cell); 1.1 mL/min (10 mL cell); 0.75 mL/min (100 mL cell)
Extraction solvent	Acetone-methylene chloride (1:1, v/v)
Extraction volume	~26 mL (10 mL cell); ~70 mL (100 mL cell)
Pre-run rinse	10 mL, acetone-methylene chloride (1:1, v/v)
Extraction time (four samples)	~10–15 min (10 mL cell); ~20 min (100 mL cell)
Concentration	
Mode	Fixed volume
Collection bottle	100 mL vial assembly
Final fixed volume	1 mL
Rinse solvent	Acetone-methylene chloride (1:1, v/v), 1.6 mL
Evaporation temperature	40 ˚C
Nitrogen flow rate	50 mL/min per channel
Vacuum	8 psi (414 torr/551 mbar)

Table 2. Conditions for the GC-MS

GC-MS conditions	
Injector	
Injector type	Programmable Temperature Vaporizer (PTV)
Liner	Thermo Scientific™ LinerGOLD™, PTV Split Liner with recessed gooseneck, 2 mm ID × 120 mm, P/N 45352070
PTV ramp	65 to 300 ˚C at 14.5 ˚C/s, hold for 50 min
Injection mode	Splitless
Splitless time	1 min
Injected volume	1.0 µL
GC	
Column	Thermo Scientific™ TRACE™ TR-5MS GC Column, 30 m × 0.25 mm × 0.25 µm
Carrier gas	Helium
Flow rate	1.2 mL/min, constant
Oven temperature	60 °C (hold for 1 min), ramp to 125 °C at 25 °C/min, ramp to 240 °C at 6 °C/min, ramp to 310 °C at 3 °C/min (hold for 4 min)
Mass spectrometer parameters	
Source temperature	275 °C
Ionization	EI
Electron energy	70 eV
Transfer line temperature	280 °C
Acquisition mode	Timed-SIM

Figure 2 shows the chromatogram of 0.5 µg/mL PAH standards under timed-SIM mode. The total analysis time is less than 51 min. A six-point calibration curve was used (0.1, 0.2, 0.5, 0.75, 1.0 and 2.0 µg/mL). Calibration curves were created by plotting concentrations versus peak area ratios of analyte to an internal standard. A linear regression or quadratic calibration curve was employed for quantification. The % errors between the measured amount the and true amount of each calibration point were less than 10% for all analytes.

Results and discussion

An increasing number of countries are establishing threshold values to monitor and evaluate the content of contaminants in soil. These values are subsequently applied to protect the environment and human health by restricting the reuse of soil and soil-like materials or by classifying them into landfill categories. Compliance control requires reliable and reproducible methods of sampling, sample pre-treatment prior to analysis, and analytical measurement to produce legally valid results. A schematic diagram of the EXTREVA ASE system is shown on Figure 3.

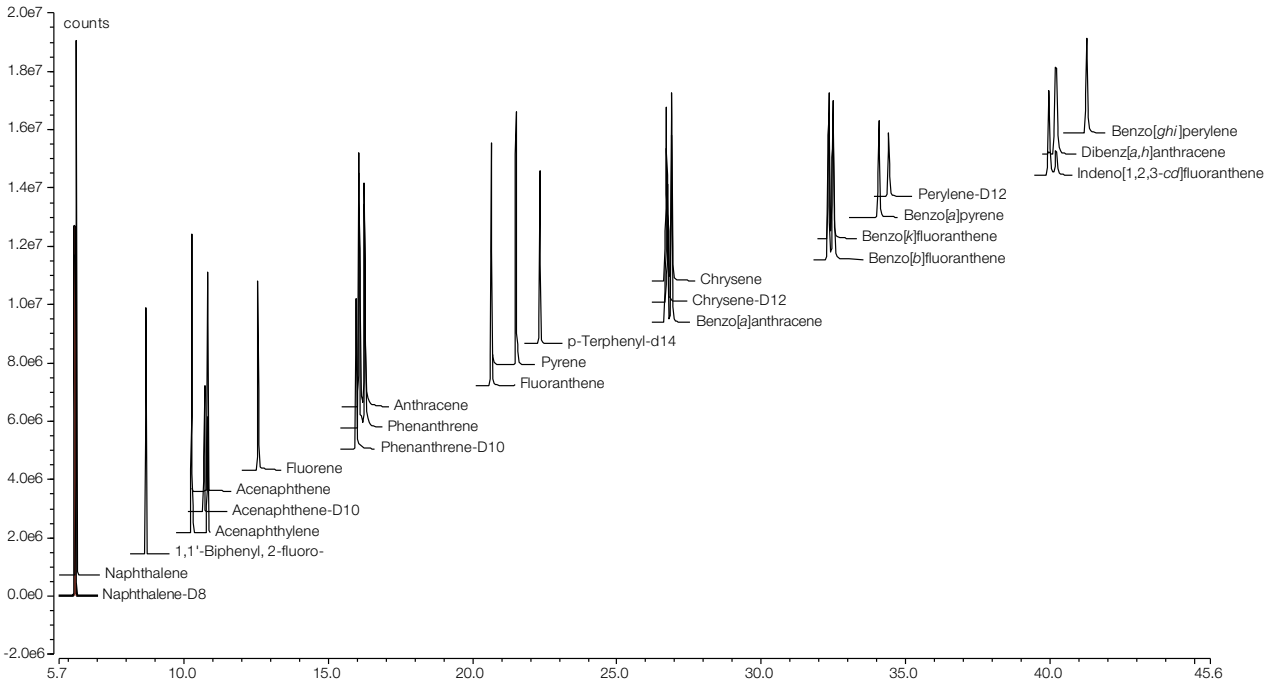

Figure 2. GC-MS chromatograms of PAHs (timed-SIM mode) at 0.5 µg/mL standard solution

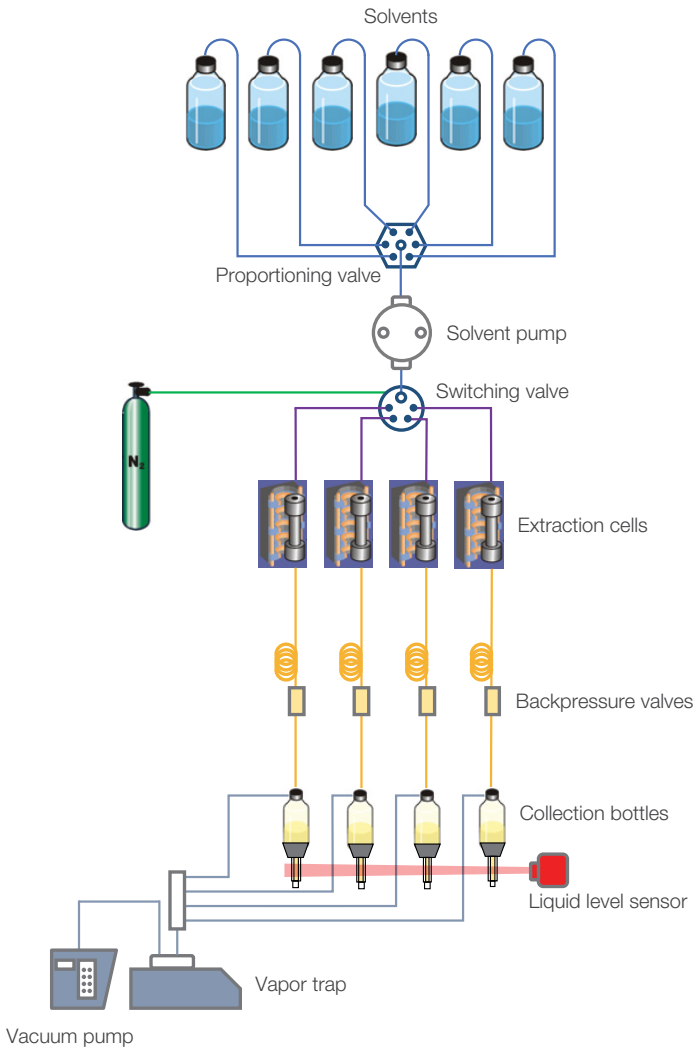

Figure 3. Schematic diagram of the EXTREVA ASE system

The EXTREVA ASE system is a fully automated sample preparation platform, designed for extracting and concentrating organic compounds from a variety of solid and semisolid matrices. The system can use up to six different extraction solvents (or mixtures of them) and extract up to four cells in parallel. The newly developed gas-assisted solvent extraction basically consists of the addition of the hot extraction solvents and nitrogen gas to the stainless-steel cell to reach the working pressure of 200 psi (~14 bar). The combined effect of temperature and pressure greatly increases the efficiency of the extraction process, significantly reducing the amount of time and solvent required for extraction when compared to traditional techniques such as Soxhlet. The evaporation process starts immediately after the completion of the extraction step without any user interaction. The extracts can be evaporated to dryness or concentrated in 2 mL vials, with the final volume controlled by artificial intelligence machine vision.

Extraction and evaporation

Combining two sample preparation instruments into one, the EXTREVA ASE system performs extraction as well as evaporation for organic compounds—all in one seamless operation. The recoveries studies for the complete extraction and evaporation workflow were made using a 250 µg/kg fortified soil sample. 10-mL and 100-mL cells were used, and the conditions are reported in Table 1. The results are summarized in Table 3 and Figure 4. All recoveries were between 77% and 113%, thus demonstrating the high extraction efficiency and the minimal loss of the most volatile compounds like naphthalene. These results met the recommended acceptance criteria of 70–130% from the U.S. EPA[8] for all compounds. It also met even the more severe 80-120% of other worldwide regulations. The RSD was below 20% for all compounds, suggesting good channel-to-channel and run-to-run reproducibility for both extraction and evaporation.

Table 3. Average recovery rates for the 250 µg/kg spike level

Compound	Average recovery (%) (10 mL cell, n = 36)	RSD	Average recovery (%) (100 mL cell, n = 35)	RSD
Naphthalene	78.3	8.7	81.0	2.3
Acenaphthylene	85.4	8.7	88.6	3.3
Acenaphthene	77.5	9.7	83.5	0.8
Fluorene	79.4	4.0	85.1	4.9
Phenanthrene	81.6	3.7	88.7	0.2
Anthracene	85.1	8.5	90.3	0.3
Fluoranthene	91.2	2.1	100.7	2.0
Pyrene	92.4	6.4	101.3	3.0
Benzo[*a*]anthracene	98.0	9.5	113.1	3.3
Chrysene	90.9	1.9	98.5	2.0
Benzo[*b*]fluoranthene	90.9	17.4	103.1	5.3
Benzo[*k*]fluoranthene	85.1	18.7	100.7	4.2
Benzo[*a*]pyrene	93.0	2.1	106.6	1.7
Indeno[1,2,3-*cd*]fluoranthene	92.0	8.6	81.0	2.3
Dibenz[*a,h*]anthracene	90.3	9.5	88.6	3.3
Benzo[*ghi*]perylene	89.1	10.0	83.5	0.8

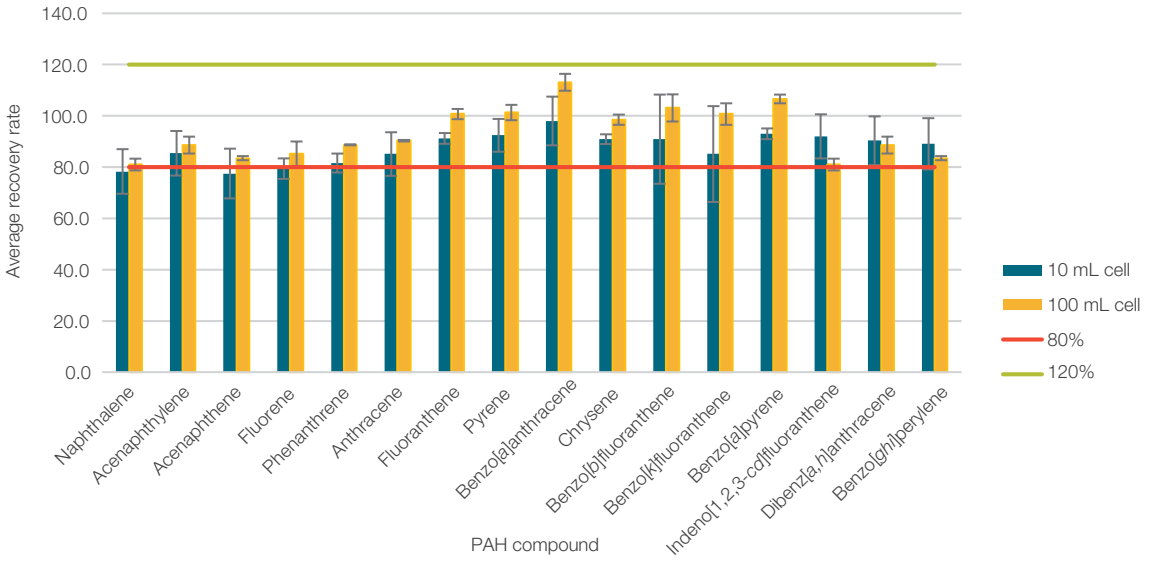

Figure 4. Average recovery rates for the 250 µg/kg spike level

Concentration

A separate study was conducted to evaluate the influence of the evaporation step on the global extraction process. A set of 36 and 35 samples simulating extracts coming from 10- and 100-mL cells (Solution A and Solution B) were prepared by diluting with acetone-methylene chloride 1:1 the stock solution to the volume of 28 mL and 70 mL, corresponding to a final concentration of 18 µg/L and 71 µg/L respectively. The resulting solutions were concentrated to 1 mL using the conditions outlined on Table 1. The recoveries of all PAH analytes were in the range of 77–105%, showing very low analyte losses from the evaporation process even for the more volatile compounds like naphthalene (Table 4 and Figure 5). These results met the recommended acceptance criteria of 70–130% from the U.S. EPA[4] and even the more severe 80–120% from other worldwide regulations within experimental error. The calculated relative standard deviations (RSD) were all below 15%, demonstrating good reproducibility from the evaporation system. In addition, the EXTREVA ASE system supports solvent exchange through solvent addition and solvent rinse functions. The volume and solvent ratio can be readily adjusted in the method. Depending on chemical properties of the analytes, solvent exchange may reduce sample breakdown or boost recovery.

Table 4. Average recovery rates for the concentration of the solutions A and B

Compound	Average recovery (%) (10 mL cell, n = 12)	RSD	Average recovery (%) (100 mL cell, n = 12)	RSD
Naphthalene	78.7	1.1	79.5	4.9
Acenaphthylene	81.7	5.4	82.3	4.2
Acenaphthene	76.8	1.7	79.7	6.8
Fluorene	78.4	2.6	80.2	1.7
Phenanthrene	79.0	2.7	85.1	6.2
Anthracene	80.3	2.0	85.3	7.1
Fluoranthene	86.3	1.0	93.1	4.5
Pyrene	85.5	2.1	95.2	6.0
Benzo[*a*]anthracene	97.6	2.0	99.8	3.0
Chrysene	86.8	0.9	98.3	4.6
Benzo[*b*]fluoranthene	81.4	12.9	103.3	1.6
Benzo[*k*]fluoranthene	80.6	13.3	103.0	1.7
Benzo[*a*]pyrene	92.1	1.6	104.7	1.5
Indeno[1,2,3-*cd*]fluoranthene	86.6	4.8	99.2	2.5
Dibenz[*a,h*]anthracene	83.9	6.7	96.6	1.7
Benzo[*ghi*]perylene	84.3	7.1	98.8	3.7

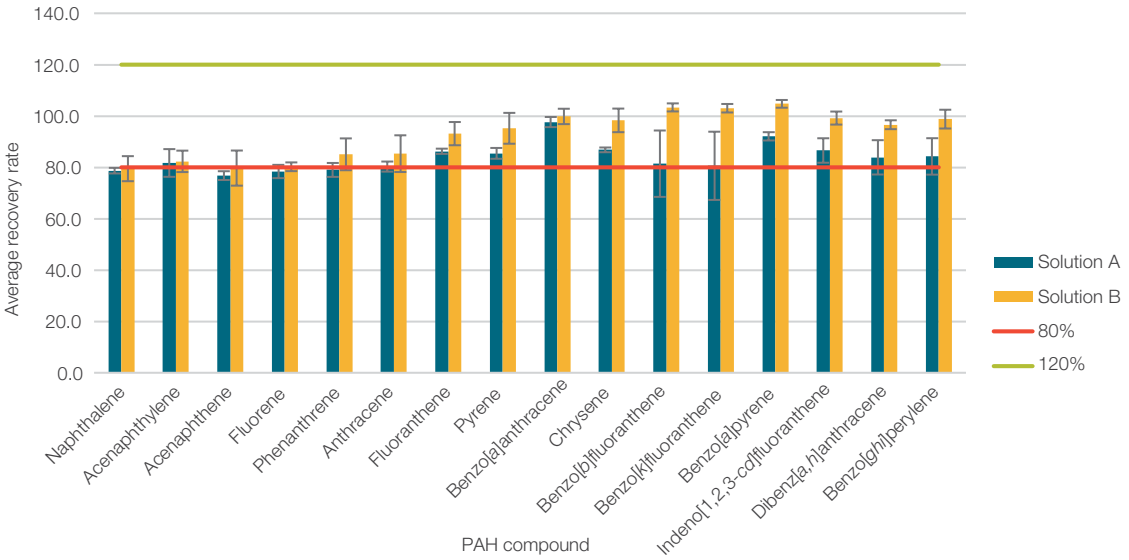

Figure 5. Average recovery rates for the concentration of the solutions A and B

Carryover

With the small amount of solvent used relative to the sample size, carryover or cross-contamination could be of potential concerns with the EXTREVA ASE system. To investigate these concerns, a heavily fortified soil sample (12500 µg/kg)[5] was extracted and concentrated under the conditions reported on Table 1. A second extraction was performed under the same conditions but using a new cell filled with Ottawa sand. Between the two extractions, each flow path channel was rinsed with 10 mL of solvent. Results of the carryover test are shown in Table 5. The carryover percent was calculated by comparing the peak area ratio of the analyte between the spiked samples and the blanks. Good recoveries were observed, and carryover was less than 0.5% for all analytes. These results demonstrates that the rinse implemented between the extractions was effective for minimizing carryover or cross-contamination. Moreover, the rinse volume can be adjusted to accommodate different sample sizes, matrices, and concentrations.

Table 5. Average recoveries and carryover from soil samples with high spike level

Compound	Average recovery (%) (10 mL cell, n = 4)	RSD	Average carryover (%) (10 mL cell, n = 4)
Naphthalene	78	2.0	0.01
Acenaphthylene	85	2.3	0.01
Acenaphthene	84	2.6	0.01
Fluorene	85	2.4	0.01
Phenanthrene	92	2.4	0.01
Anthracene	98	2.1	0.01
Fluoranthene	102	3.2	0.02
Pyrene	99	2.2	0.02
Benzo[*a*]anthracene	104	1.8	0.02
Chrysene	100	2.2	0.02
Benzo[*b*]fluoranthene	101	1.2	0.02
Benzo[*k*]fluoranthene	100	1.4	0.01
Benzo[*a*]pyrene	100	2.3	0.01
Indeno[1,2,3-*cd*]fluoranthene	92	2.4	0.01
Dibenz[*a,h*]anthracene	88	2.1	0.01
Benzo[*ghi*]perylene	91	2.4	0.01

The quality of the above-mentioned results was confirmed by the extraction of PAH Certified Reference Material (CRM). A 10-mL cell was used, and the conditions are reported on Table 1. The results are summarized on Table 6. All the results were within the suggested acceptance range of the accompanying certificate, thus confirming the excellent efficiency of the combined extraction and evaporation features of the EXTREVA ASE system.

Table 6. Average recoveries of certified soil sample

PAH compound	Certified value	Acceptance range	Average recovery and RSD (10 mL cell, n = 12)	
	µg/kg	µg/kg	Avg (n=12) µg/kg	RSD (n=12)
Naphthalene	494 ± 38	164 to 824	362	6.76
Acenaphthylene	630 ± 38	328 to 933	490	1.58
Acenaphthene	651 ± 64	141 to 1162	502	1.25
Fluorene	157 ± 19	10.7 to 303	140	3.07
Phenanthrene	290 ± 26	65.2 to 516	283	0.58
Anthracene	612 ± 51	173 to 1051	447	2.76
Fluoranthene	333 ± 25	119 to 547	349	0.95
Pyrene	202 ± 20	35.7 to 369	240	2.21
Benzo[*a*]anthracene	329 ± 20	158 to 500	404	1.22
Chrysene	146 ± 12	49.8 to 241	168	4.45
Benzo[*b*]fluoranthene	69.9 ± 4.5	32.6 to 107	79	1.74
Benzo[*k*]fluoranthene	266 ± 21	95.0 to 437	251	1.41
Benzo[*a*]pyrene	223 ± 17	83.5 to 363	206	4.34
Indeno[1,2,3-*cd*]fluoranthene	88.8 ± 8.3	19.5 to 158	106	6.50
Dibenz[*a,h*]anthracene	193 ± 16	74.4 to 312	230	1.95
Benzo[*ghi*]perylene	224 ± 22	44.3 to 404	274	1.49

Conclusion

This application note described a method for the determination of 16 PAHs in soil matrices using the EXTREVA ASE system and TRACE 1310 Gas Chromatograph and ISQ 7000 Single Quadrupole Mass Spectrometer. Good recoveries and reproducibility were observed for all analytes. Carryover between consecutive runs was minimal. By combining two sample preparation instruments into one, the EXTREVA ASE system performs both extraction and evaporation of organic compounds in one seamless operation. Offering the full benefits of automation and an easy "load-and-go" start process, the EXTREVA ASE system saves time, reduces errors and solvent usage, enables unattended operations, and significantly increases analytical throughput. The system can be controlled using the integrated user interface or remotely through Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software for complete walkaway efficiency. Overall, the EXTREVA ASE system demonstrated efficient, reliable, and high-throughput performance to tackle challenging PAH applications.

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