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Accelerating the quantitation of polycyclic aromatic hydrocarbons (PAHs) in soil samples using the EXTREVA ASE system

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Keywords

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Goal

To develop a method for the determination of polycyclic aromatic hydrocarbons (PAHs) from soil samples using the Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of toxic organic compounds formed through the incomplete combustion of carbon-based materials. Their widespread presence in environmental and food matrices, as well as their known carcinogenic and mutagenic properties, make them a high-priority target for regulatory monitoring. Accurate detection and quantification of PAHs demand not only high sensitivity and selectivity, but also an analytical workflow that meets rigorous compliance standards.

Regulatory methods, such as US EPA Method 8270E and US EPA Method 3500C, establish the framework for PAH analysis via gas chromatography-mass spectrometry (GC-MS), defining both the target compound list and the procedural steps required for reliable quantitation.[1,2] These methods rely on a sequence of critical operations, including the following: solvent extraction to isolate PAHs from complex matrices, filtration through sodium sulfate to remove moisture and particulates, evaporation to concentrate analytes, and final volume adjustment to ensure consistency across analytical batches. This workflow is not only method-critical, but it is also highly labor-intensive. Each step demands meticulous execution to maintain data integrity, regulatory compliance, and reproducibility.

The complexity and number of operations place a substantial burden on analysts, increasing the risk of variability and limiting throughput.

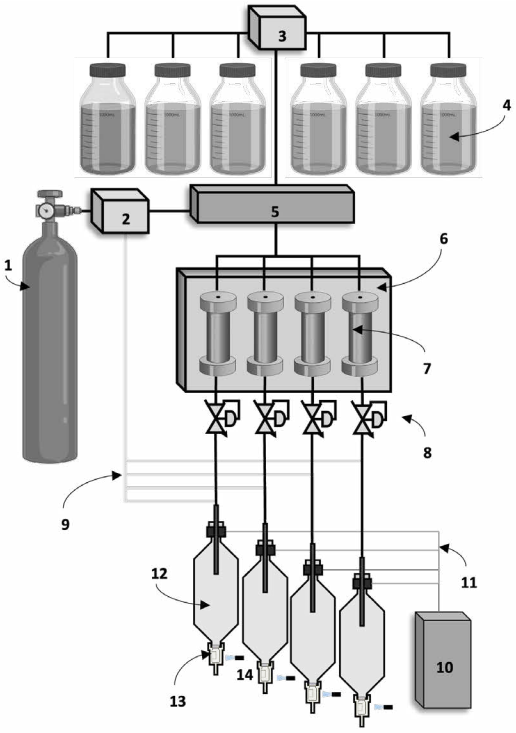
Accelerated solvent extraction (ASE; US EPA Method 3545A[3-4]), introduced in the late 1990s, streamlined the extraction of nonvolatile and semi-volatile organic compounds from solid matrices such as soils and sediments. Compared to traditional techniques like Soxhlet, ASE significantly reduced solvent use and processing time, establishing a new standard for efficiency. However, its workflow remained constrained by single-sample processing and manual handling. Furthermore, analysts were still required to transfer extracts to separate enrichment systems and manually manage evaporation and reconstitution. This sustained the complexity and labor demands of that segment of the workflow and continued to limit overall throughput.

To address persistent limitations in sample preparation workflows, Thermo Fisher Scientific introduced the EXTREVA ASE system in 2022 (Figure 1), a fully integrated platform that automates both extraction and evaporation within a single instrument. This system incorporates gas-assisted dynamic solvent extraction (GA-dASE),[5-6] parallel ASE, and endpoint level detection (Figure 1; objects 5, 6, and 14), enabling a streamlined, start-to-finish process with minimal user intervention. The EXTREVA ASE system delivers heated solvents, selectable from up to six reservoirs (Figure 1, object 4), in combination with nitrogen gas into stainless-steel sample cells (Figure 1, object 7) that are housed within a temperature-controlled oven (Figure 1, object 6). Operating at a working pressure of approximately 200 psi (~14 bar), regulated via a back pressure valve (Figure 1, object 8), the system initiates GA-dASE. Once the pressure threshold is exceeded, excess extract is routed through dedicated channels into collection bottles equipped with 2 mL vial adaptors (Figure 1, object 12).

The instrument supports simultaneous extraction of up to four solid or semisolid samples using a multi-cell oven and accommodates batch processing of up to sixteen samples via four independent trays. Each tray holds four sample cells, allowing sequential extraction across the batch. Upon completion of extraction, the system transitions directly into automated evaporation, driven by controlled heating, vacuum pulling (Figure 1, object 10), and gas flow across the container surface to efficiently remove excess solvent without compromising target analyte integrity. The process is precisely regulated by endpoint level sensing for accurate volume control (Figure 1, object 14). It requires no user input and can be configured to evaporate samples to dryness or concentrate them to a defined volume, so the samples are ready for downstream chromatographic analysis.

Turnaround time (TAT) is a critical performance metric for laboratories under increasing pressure to deliver results rapidly and reliably. Medium-to-large labs often face the challenge of processing up to 100 samples within a standard 8-hour shift, making workflow efficiency essential. Emerging extraction technologies aim to meet this demand by targeting streamlined sample preparation times of approximately four hours per instrument for up to 24 samples. While the EXTREVA ASE system already delivers exceptional reproducibility and enhanced operator productivity, our ongoing R&D efforts have identified procedural bottlenecks that could unnecessarily extend batch TAT and limit throughput. To address these, we implemented parallelization of initial preparation steps, significantly reducing the time required to initiate extraction. Additionally, optimizing extraction conditions under dynamic gas assistance has enabled exhaustive analyte recovery with substantially less solvent, thereby shortening sequence completion times due to reduced evaporation requirements. To further accelerate method performance, we upgraded the evaporation hardware. The latest EXTREVA ASE system now features a more powerful mass flow controller, enabling higher gas flow rates during evaporation. Enhancements to the endpoint level sensing system have also improved monitoring speed and accuracy, ensuring precise extract volumes even under rapid evaporation conditions.

In this application note, we present results from PAH determination in soil samples, demonstrating extraction recoveries, blank carry-over following high-concentration samples, and TAT reproducibility. The EXTREVA ASE system sets a new benchmark for laboratories seeking high throughput, robust reproducibility, and operational efficiency, delivering precise, reliable results with minimal user intervention.



1. Nitrogen cylinder. 2. Gas manifold. 3. Solvent pump and mixing valves. 4. Solvent bottles. 5 Gas/Liquid manifold and mixing chamber. 6. Sample cells-oven. 7. Sample cells. 8. Back pressure valve. 9. Evaporation line. 10. Vacuum pump. 11. Vacuum path. 12. Evaporation bottle with adaptor for 2 mL vial. 13. Vial for GC/LC analysis. 14. Level sensing system for unattended end-point evaporation.

Figure 1. Schematic diagram of the EXTREVA ASE accelerated solvent extractor.

Equipment and consumables

- EXTREVA ASE Accelerated Solvent Extractor (P/N B51004598)
- Thermo Scientific™ TRACE™ 1310 Gas Chromatograph
- Thermo Scientific™ ISQ™ Single Quadrupole Mass Spectrometer
- Thermo Scientific™ Dionex™ Cellulose Filter (P/N 056780)
- Thermo Scientific™ Dionex™ ASE™ 10 mL Stainless Steel Extraction Cells (P/N 068087)
- Thermo Scientific™ SureSTART™ 2 mL Glass Screw Top Vials, Amber Glass (P/N CHSV9-20PT) and Thermo Scientific™ SureSTART™ 9 mm Screw Caps (P/N 6ASC9SPI1)
- Diatomaceous Earth (DE) Dispersant for ASE, 1 kg bottle (P/N 062819)
- Fisher Chemical™ Ottawa Sand (P/N S23-3)
- Sigma-Aldrich™ Clean Loamy Soil (P/N CLNLOAM6-100G)

Reagents and standards

- Thermo Scientific™ Chemicals Dichloromethane, for HPLC (P/N 610050040)
- Fisher Chemical™ Optima™ Hexanes for HPLC and GC (P/N H303-4)
- Restek™ SV Calibration Mix #5/610 PAH Mix (P/N 31011)
- Restek™ B/N Surrogate Mix (4/89 SOW) (P/N 31024)
- Restek™ Semi-Volatile Internal Standard Mix (P/N 31206)
- Sigma-Aldrich™ CRM Soil, PAH (P/N CRM141-50G)

Extraction, concentration, and measurement

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 25 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 was 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 (ASE Prep DE). The resulting mixture was carefully poured into the extraction cell and spiked, either at 250 µg/kg (recovery

tests and TAT reproducibility) or 12,500 µg/kg (carry-over tests) for the PAHs. Any empty volume in the cell body was filled with ASE Prep DE while lightly tapping to ensure the material settled. Another cellulose filter was placed on top of the cell body, and the second end cap was hand tightened. The ASE Prep DE acts as a dispersant and plays a key role in preventing sample compaction during the compression phase and ensuring efficient solvent contact with the sample. In the case of wet samples, it is highly recommended to either pre-dry the samples with air or to mix them in a 1:1 ratio with Thermo Scientific™ Dionex™ ASE™ Prep Moisture Absorbing Polymer (P/N 083475) and the ASE Prep DE for optimum moisture removal under ASE conditions.

The instrument was programmed according to the conditions reported in Table 1. Before proceeding with the extraction of the samples, the system was rinsed with the extraction solvent (hexane-methylene chloride 1:4, v/v). Hexane was used during evaporation as a rinse solvent, and 3 mL was added before the final evaporation step to rinse the collection bottle walls. After concentration, the samples were added to the internal standard mix and analyzed by GC-MS.[7] The GC-MS conditions are summarized in Table 2.

Figure 2 shows the chromatogram of a 0.5 µg/mL PAH standard under timed-SIM mode. The total analysis time is less than 45 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 internal standard. A linear regression or quadratic calibration curve was employed for quantification. The % errors between the measured amount and the true amount of each calibration point were less than 10% for all analytes.

Extraction and evaporation

The recovery studies for the extraction and evaporation workflow were made using a 250 µg/kg fortified soil sample. 10 mL cells were used, and the conditions are reported in Table 1. The results are summarized in Table 3 and Figure 3. All recoveries were between 74% and 98%, 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 US EPA 3545A for all compounds. The RSD was below 20% for all compounds, suggesting good channel-to-channel and run-to-run reproducibility for extraction and evaporation.

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

Extraction	
Cell type	Stainless steel
Cell size	10 mL
Oven temperature	100 ˚C
Purge time	30 s
Nitrogen flow (gas-assisted extraction)	20 mL/min per channel
Cell fill volume	50%
Solvent flow rate	0.5 mL/min
Extraction solvent	Hexane:Methylene chloride (1:4, v/v)
Extraction volume	~12.5 mL (~7.5 mL real volume)
Pre-run rinse	10 mL, Hexane:Methylene chloride (1:4, v/v)
Extraction time (four samples)	5 min
Enrichment	
Mode	Fixed volume
Collection bottle	250 + 2 mL vial assembly
Final fixed volume	1 mL
Rinse solvent	Hexane, 3.0 mL
Evaporation temperature	40 ˚C
Nitrogen flow rate	200 mL/min per channel (800 mL total)
Vacuum	8 psi (420 torr)

Table 2. Chromatographic and mass spectrometer conditions.

Injector	
Injector type	Programmable temperature vaporizer (PTV)
Liner	Thermo Scientific™ LinerGOLD™ PTV Split Liner with Recessed Gooseneck, 2 mm ID x 120 mm, P/N 45352070
PTV ramp	65 ˚C to 300 ºC at 14.5 ˚C/s, hold for 50 min
Injection mode	Splitless
Splitless time	1 min
Injected volume	1.0 µL
GC	
Column	Trace TR-5MS, 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

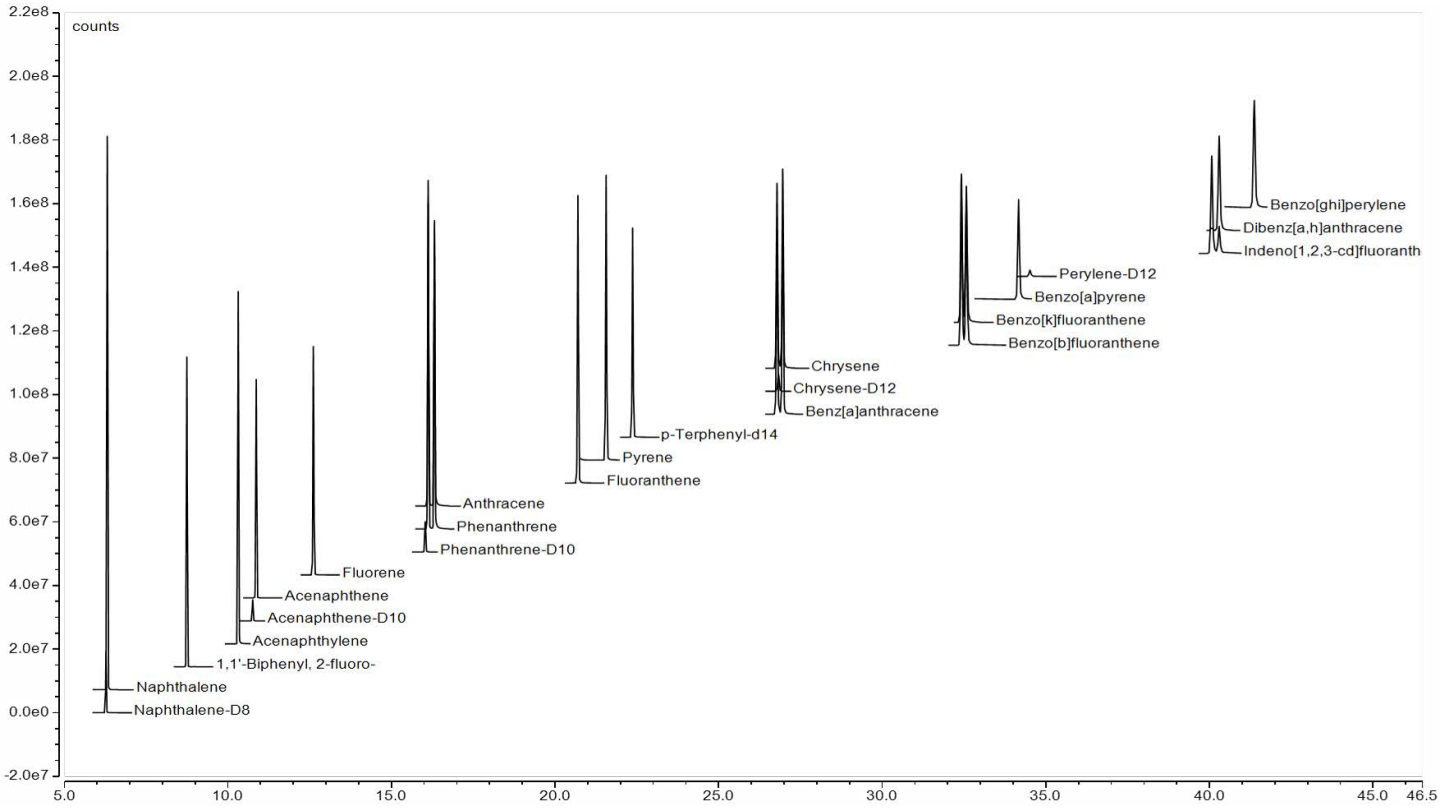


Figure 2. GC-MS chromatograms of PAHs (timed-SIM mode) at 0.5 µg/mL standard solution. All solvents are optima-grade or equivalent and available from Thermo Fisher Scientific.

Carryover

With the small amount of solvent used relative to the sample size, carryover or cross-contamination could be concerning with the EXTREVA ASE system. To investigate these, a heavily fortified soil sample (12,500 µg/kg)[5] was extracted and concentrated under the conditions reported in 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. The results of the carryover test are shown in Table 4. The carryover percentage was calculated by comparing the peak area ratio of the analyte between the spiked samples and the blanks. Carryover was less than 0.5% for all analytes. These results demonstrate that the rinse implemented between the extractions was effective in minimizing carryover or cross-contamination. Moreover, the rinse volume can be adjusted to accommodate different sample sizes, matrices, and concentrations.

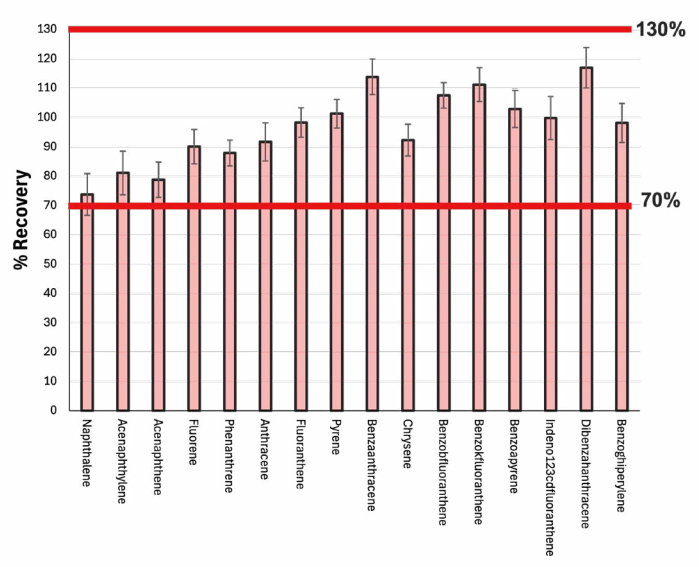


Figure 3. Average recovery rates for PAHs from soil samples.

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

Compound	Average recovery (%) (10 mL cell, n = 8)	RSD
Naphthalene	74	7
Acenaphthylene	81	7
Acenaphthene	79	6
Fluorene	90	6
Phenanthrene	88	4
Anthracene	92	7
Fluoranthene	98	5
Pyrene	101	5
Benz[a]anthracene	114	6
Chrysene	92	5
Benzo[b]fluoranthene	108	4
Benzo[k]fluoranthene	111	6
Benzo[a]pyrene	103	6
Indeno[1,2,3-cd]fluoranthene	100	7
Dibenz[a,h]anthracene	117	7
Benzo[ghi]perylene	98	7

Table 4. Average carryover from soil samples with high spike level.

Compound	Average Carryover % (10 mL cell, n = 8)
Naphthalene	0.01
Acenaphthylene	0.01
Acenaphthene	0.01
Fluorene	0.01
Phenanthrene	0.03
Anthracene	0.02
Fluoranthene	0.04
Pyrene	0.03
Benz[a]anthracene	0.05
Chrysene	0.03
Benzo[b]fluoranthene	0.02
Benzo[k]fluoranthene	0.03
Benzo[a]pyrene	0.01
Indeno[1,2,3-cd]fluoranthene	0.01
Dibenz[a,h]anthracene	0.01
Benzo[ghi]perylene	0.02

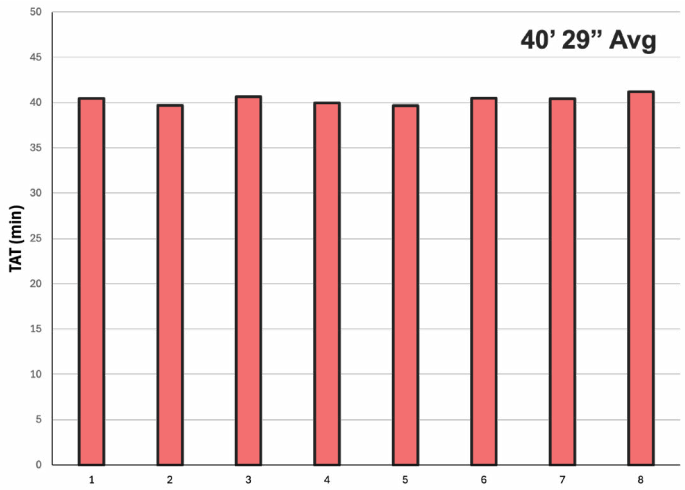


Figure 4. TAT reproducibility towards determination of PAHs in soil samples.

Turnaround time improvements

Eight replicate tests of the method have not only confirmed the precision of the process with a relative standard deviation (RSD) under 1.3%, but they have also highlighted its rapidity, with an average analysis time of approximately 40 min (n=8; 4 sample batches; see Figure 4). This swift turnaround enables an expedited response to environmental monitoring and contamination assessments. The ability to reliably reproduce results in such a short timeframe is a testament to the instrument’s robustness. This also demonstrates its potential to disrupt a field’s use of several manual steps. When compared to the prior version of the instrument, TAT was decreased threefold.

Validation against CRM

The quality of the above-mentioned results was confirmed by the extraction of PAH Certified Reference Material (CRM). A 10-mL cell was used with the conditions outlined in Table 1, and the results are summarized in Table 5. All the results were within the suggested acceptance range of the accompanying CRM certificate, thus confirming the excellent efficiency of the combined extraction and evaporation features of the improved EXTREVA ASE system.

Table 5 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
Benz[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	6.5
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

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 extractions, and increases analytical throughput. By refining the extraction and evaporation processes, we aimed to further streamline the analytical workflow, ensuring a more efficient approach to sample preparation and analysis.

This application note reports the advantages of using the recently enhanced EXTREVA ASE system towards the rapid determination of PAHs in soil samples. The method presented here offers a fast and accurate analysis of 16 PAHs in less than 40 min with high reproducibility and data integrity. The EXTREVA ASE system is a powerful tool for environmental monitoring and assessment, as it enables rapid and reliable detection of PAHs in soil samples according to US EPA Method 3545A. The EXTREVA ASE system is a novel solution that can streamline the workflows and improve the productivity of analytical laboratories.

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