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Sample preparation

# Method transfer to the EXTREVA ASE Accelerated Solvent Extractor

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## Keywords
Accelerated solvent extraction, pressurized fluid extraction, polyaromatic hydrocarbons (PAHs), sample preparation, environmental, soil, PLE

## Introduction
The Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor (Figure 1) is a system based on many proprietary technologies including gas-assisted solvent extraction and parallel accelerated solvent extraction. 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 per batch (i.e., 4 sequences of 4 sample cells in parallel). The EXTREVA ASE system combines elevated temperatures and pressurized gas with liquid solvents to achieve fast and efficient collection of analytes from various matrices. The system can use up to six different extraction solvents (or mixtures of them) and extract up to four cells in parallel. The gas-assisted solvent extraction comprises delivering a mixture of 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 enrichment/concentration process starts immediately after the completion of the extraction step without any analyst interaction, if no offline cleanup of extract needed. The extracts can be evaporated to dryness or concentrated in 2 mL vials to volumes as low as 0.3 mL, with the final volume controlled by artificial intelligence machine vision (Figure 2). Succinctly, the EXTREVA ASE system automates extraction, in-cell clean up, and evaporation, which frees up the analyst's time for other, more demanding tasks.

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A schematic diagram of the EXTREVA ASE Accelerated Solvent Extractor is shown in Figure 2.

This white paper describes the method transfer from a Thermo Scientific™ Dionex™ ASE 350 Accelerated Solvent Extractor to the EXTREVA ASE system, and provides instructions for new EXTREVA ASE system methods.

## Cell preparation, compact cell needed

Cell preparation for samples in the EXTREVA ASE system is similar when compared to the existing Dionex ASE 350 system. In short,

1. Close bottom end cap of the Thermo Scientific™ Dionex™ ASE™ Extraction Cell and add a filter. Note, depending on the cell size, the filter may be added before or after closing end caps.

2. Weigh samples and mix them with an adequate amount of sample modifier (e.g., dispersant or drying agent such as diatomaceous earth, DE). 1:1 or 1:2 sample to modifier ratios are usually recommended for most applications. Yet, ratio may change upon application.

3. Lightly tap the cell while loading samples, compact again, top off with clean sand or DE, and compact again.

4. Add filter to ASE cell and close top end cap.

**Important: A compact cell is needed for extraction in the EXTREVA ASE system.**

## Which options to choose for a particular application?

The EXTREVA ASE system workflows comprise three main options: extraction only, evaporation only, or extraction and evaporation combined (integrated) depending on the option of the instrument.

1. **Extraction only:** No evaporation. After extraction, the analyst may perform an offline cleanup, and, subsequently, perform sample enrichment. If evaporation is performed after cleanup, the analyst needs to manually load cells and evaporation bottles back onto the EXTREVA ASE system as per the evaporation workflow below.

2. **Evaporation only:** Evaporation has two modes of operation: evaporation to dryness or evaporation to a fixed volume. When evaporating to a fixed volume, the minimum and maximum volumes are 0.3 and 1.6 mL, respectively. When evaporating to dryness, automatic end point detection is not available; therefore, the analyst needs to optimize evaporation conditions, such as temperature, vacuum and nitrogen gas flow, in order to complete this process in the minimum amount of time while meeting analysis requirements.

3. **Extraction and evaporation combined (integrated):** Extraction followed by evaporation either to dryness (such as fat extraction) or fixed volume (such as PAH, OCP, and PCB) can be pursued based on the need of a particular application or target analytes.
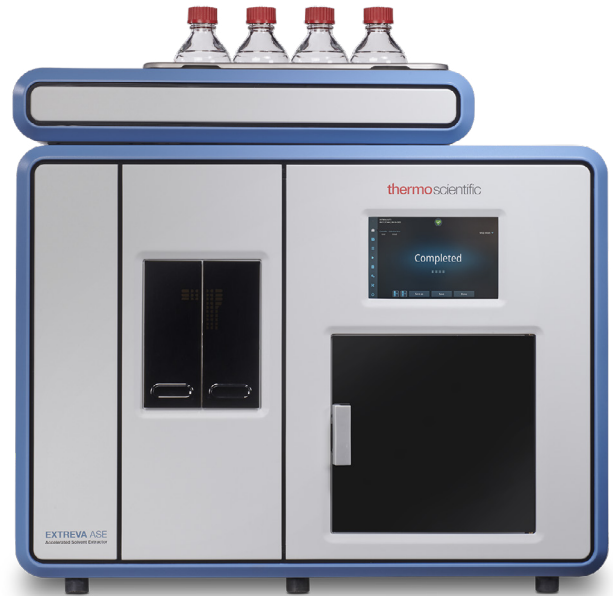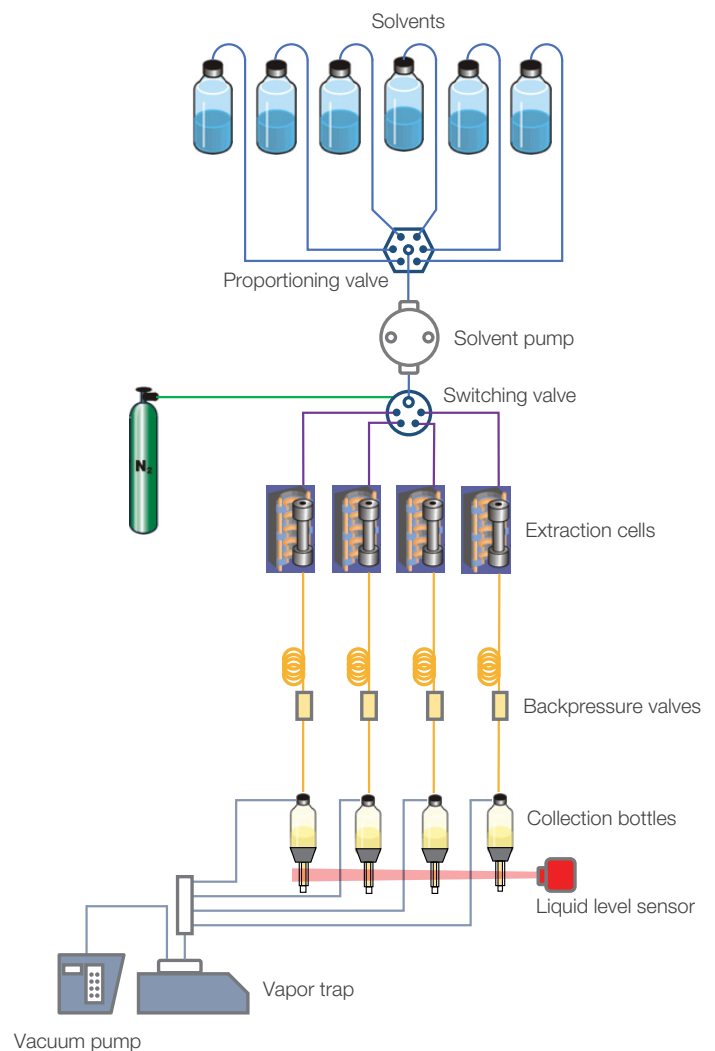


Figure 1. EXTREVA ASE Accelerated Solvent Extractor



Figure 2. Schematic diagram of the EXTREVA ASE Accelerated Solvent Extractor

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## Where should I start?

It is important to emphasize that the EXTREVA ASE system has far more features than the Dionex ASE 350 system, and a direct method transfer from the Dionex ASE 350 system to the EXTREVA ASE system is not available. Thus, optimizations of the EXTREVA ASE system method will most likely be required to attain the best possible results. New and existing analysts are invited to review ASE system application notes, EXTREVA ASE system application notes, prior ASE system SOPs, and published literature on ASE systems as guidelines about the most logical parameters of the EXTREVA ASE system (Table 1). Some of the following parameters including solvent composition, solvent amount, temperature, and extraction time are critical to constructing a method for the EXTREVA ASE system. Table 2 can be used as a guideline to create an EXTREVA ASE system method based on prior information.

## Polycyclic aromatic hydrocarbons (PAHs) application

Analysts can rely on existing/legacy or available knowledge to construct the evaporation part of the EXTREVA ASE system method. Information from existing Thermo Fisher Scientific/ Dionex ASE application notes regarding extraction and analysis of PAHs were utilized to make the compatible EXTREVA ASE system method presented herein. The conditions listed in Table 2 can be utilized to set up either extraction, evaporation, or integrated extraction-evaporation workflows.

**Table 1. Application notes using the Dionex 350 sytems and earlier ASE units and their corresponding target analyte groups**

| Analyte groups | Literature code | Title |
|---|---|---|
| Polycyclic aromatic hydrocarbons (PAHs) application | AN313 | Extraction of PAHs from Environmental Samples by Accelerated Solvent Extraction (ASE) |
| | AU313 | Accelerated Solvent Extraction—GC-MS Analysis and Detection of Polycyclic Aromatic Hydrocarbons in Soil |
| | AN341 | Extraction of Base/Neutrals and Acids (BNAs) from Large-Volume Samples Using Accelerated Solvent Extraction (ASE) |
| | AN317 | Extraction of BNAs (Bases, Neutrals, and Acids) Using Accelerated Solvent Extraction (ASE) |
| | AN1025 | Simultaneous Extraction of PAHs and PCBs from Environmental Samples Using Accelerated Solvent Extraction |
| Organochlorine pesticides (OCPs) application | AN320 | Extraction of Chlorinated Pesticides Using Accelerated Solvent Extraction (ASE) |
| | AN332 | Accelerated Solvent Extraction of Pesticide Residues in Food Products |
| | AB152 | Extraction of Organochlorine Pesticides from Oyster Tissue Using Accelerated Solvent Extraction |
| Polychlorinated biphenyls (PCBs) application | AN316 | Extraction of PCBs from Environmental Samples Using Accelerated Solvent Extraction |
| | CAN121 | Determination of Polychlorinated Biphenyls (PCBs) in Soils and Solid Waste by Accelerated Solvent Extraction and GC-MS/MS |
| | AN342 | Determination of PCBs in Large-Volume Fish Tissue Samples Using Accelerated Solvent Extraction (ASE) |
| | AN1025 | Simultaneous Extraction of PAHs and PCBs from Environmental Samples Using Accelerated Solvent Extraction |
| Fat extraction application | AN321 | Determination of Unbound Fat in Various Food Matrices Using Accelerated Solvent Extraction |
| | AN340 | Determination of Fat in Dried Milk Products Using Accelerated Solvent Extraction (ASE) |
| | AN329 | Determination of Total Fat in Powdered Infant Formula Using Accelerated Solvent Extraction (ASE) |
| | AU194 | Determination of Existent and Potential Sulfate and Total Inorganic Chloride in Denatured Ethanol by Direct Injection Using an RFIC System |
| Extractables and leachables application | AN1108 | Comparison of Soxhlet and Accelerated Solvent Extraction for Leachable and Extractable Analysis of Packing Material |

**Table 2. Extraction and evaporation conditions/parameters for PAH application in the EXTREVA ASE system following a method transfer of the Dionex ASE 350 system**

| Extraction | | | |
|---|---|---|---|
| Items/parameters | EXTREVA ASE system | Dionex ASE 350 system | Comments |
| Pre-run rinse/ Post-run rinse | 10 mL, Methylene chloride - acetone (1:1, v/v, 50%:50%, v/v). The solvent goes to waste. | Pre-run rinse | An analyst can choose the option of a pre-run or post-run rinse with a certain amount of solvent to rinse each of the fluidic pathways to waste. |
| Cell type | Stainless steel | Stainless steel | Thermo Scientific™ Dionex™ ASE™ 350 Stainless Steel Extraction Cells are compatible with the EXTREVA ASE system. The EXTREVA ASE instrument is not acid/base compatible. |
| Cell size | 1, 5, 10, 22, 34, 66, 100 mL | 10, 34, 66, 100 mL | Method development examples are provided for 10 and 100 mL cells. The Dionex ASE 350 instrument does not require the analyst to specify cell size, the instrument recognizes the size and there is no solvent flow rate to choose from. But with the EXTREVA ASE system, prior to starting the analysis, the analyst needs to specify in the user interface the cell size and corresponding solvent flow rate based on solvent. The EXTREVA ASE system corroborates that the cell size listed by the analyst is correct and proceeds with the analysis. |
| Extraction solvent | Methylene chloride - acetone (1:1, v/v, 50%:50%, v/v) | Methylene chloride - acetone (1:1, v/v, 50%:50% v/v) | Analysts can start the EXTREVA ASE system using the recommended ASE solvent. For PAHs, this solvent composition is widely used and cited in the existing application notes. Analysts can choose either ratio or percent, but the use of percent is recommended. |
| Extraction volume | 26 and 65 mL for 10 and 100 mL cell sizes, respectively | 28, 60, and 140 mL for 10, 34 and 100 mL cell sizes, respectively | Analysts can start using the same solvent amount as used with the ASE system. Extraction volume in the EXTREVA ASE system is greatly influenced by flow and total extraction time. It is recommended to start with the same extraction volume as the Dionex ASE 350 system and optimize to a lower volume. |
| Extraction pressure | 200 psi (default) | 1500 psi (default) | Extraction pressure in the EXTREVA ASE system is set to approximately 200 psi and this pressure is maintained (200–240 psi) across the entire process by the apparatus. |
| Extraction temperature | 100℃ | 100℃ | Analysts can start using temperature information recommended for the ASE system. Temperatures ranging between 40 and 200°C can be selected for the EXTREVA ASE system. Analysts can also choose "No oven heating". |
| Heating time | NA | 5 min chosen by Dionex ASE 350 instrument based on an algorithm for 100℃ extractions. Analysts cannot choose. | In the case of the EXTREVA ASE system, there is no "heating time" option as there is with the Dionex ASE 350 system. Besides, the EXTREVA ASE system has a solvent preheater before the oven, thus, the solvent is preheated before entering the cells. |
| Cell fill volume | 50% | NA | In most cases filling 50% (+5 mL preheater dead volume) of the cell volume has shown to deliver the best results for PAHs application. Analysts can select a range between 10 and 70%. In the case of the Dionex ASE 350 system, the cell is filled until the cell reaches a pressure of 1,500 psi and the analyst has no control over the amount of solvent that gets in contact with the sample. |
| Solvent flow rate (Dynamic extraction) | 1.1 mL/min (10 mL cell), 0.50 mL/min (100 mL cell) for a target of 65 mL solvent | NA | In the case of the Dionex ASE 350 system, the extraction is static and there is no flow of solvent during the extraction process. The analyst can select solvent flow rates ranging between 0.1 and 8 mL/min for the EXTREVA ASE system. The analyst needs to optimize this flow rate in order to meet the analytical requirements of the method. In the case of PAHs we have found that 1.1 mL/min for 10 mL cells provided great recoveries (AN001106). |
| Gas flow rate (gas-assisted extraction, dynamic extraction) | 10 mL/min per channel (Nitrogen is utilized for the EXTREVA ASE system) | NA | The EXTREVA ASE system uses gas assisted extraction (dynamic extraction). |
| Extraction time | ~15 min (10 mL cell) and ~20 min (100 mL cell) for four samples at the same time; parallel extraction. | 23 min per sample for 10 mL cell (i.e., 3 cycles of 5 min static extractions plus 5 min heating plus 2–3 min filling and purge) | Extraction for the EXTREVA ASE system is conducted in parallel, while extraction for the Dionex ASE 350 system is performed sequentially. |

**Table 2. Extraction and evaporation conditions/parameters for PAH application in the EXTREVA ASE system following a method transfer of the Dionex ASE 350 system** *(continued)*

| Extraction (continuation) | | | |
|---|---|---|---|
| Items/parameters | EXTREVA ASE system | Dionex ASE 350 system | Comments |
| Rinse (% of cell size) | NA | 60% (recommended, range 0–150%). The solvent goes to the collection bottle. | The EXTREVA ASE system does not use a solvent rinse function during the extraction step (also known as "flush function" on older Dionex ASE systems). Therefore, please do not confuse the "Rinse/Flush" feature on the Dionex ASE 350 system with the "Pre-run rinse" or "Post-run rinse" functions listed above for the EXTREVA ASE system. |
| Purge time | 45 s (10 mL cell) and 180 s (100 mL cell) | 45–180 s based on cell size | Analysts need to optimize the purge in seconds. Recommended values are in the table. It is recommended to start with the Dionex ASE 350 system start times. Purge is performed for up to 4 cells in parallel. |
| Total analysis time (extraction; four samples) | ~35 min (10 mL cell) and ~45 min (100 mL cell) | 30 min each one ×4 | In the case of the EXTREVA ASE system, the total analysis of 4 samples includes mechanical movement, extraction, and purge. In the case of the Dionex ASE 350 system, the total analysis time includes the heating, extraction, and purge. |
| **Evaporation** | | | |
| Evaporation mode | Evaporation to dryness or evaporation to fixed volume | NA | There are two modes in the EXTREVA ASE system: evaporation to fix volume and evaporation to dryness. Evaporation to fix volume was used in this PAH application. |
| Collection bottle | 100 mL vial assembly (100-2 mL) | NA | Either a 100-2 or 60-2 mL evaporation flask can be used. In the example presented herein, 100-2 mL evaporation flasks were used. If the solvent is greater than 65 mL, then use a 250-2 mL flask. |
| Final fixed volume | 1 mL | NA | Evaporation to fix volume of 1 mL was used in this PAH application with fully automated level sensing. A 2 mL GC autosampler vial was used. **The GC autosampler vial must have a 9 mm opening.** |
| Pre-rinse solvent | None used, unchecked | NA | With this option, the analyst may add a solvent of choice into the evaporation flask before evaporation. It was not used in the PAH application (AN001106). However, a 10 mL hexane (for solvent exchange from hexane/acetone to predominantly hexane) can be added in the OCP application (AN001054) by checking the Pre-rinse feature and specifying the solvent bottle(s) and composition. |
| Rinse solvent | Methylene chloride - acetone (1:1, v/v, 50%:50%, v/v), 1.6 mL | NA | Analysts can choose the same extraction solvent or any other solvent (to exchange such as in OCP application AN001054) to automatically rinse the inner walls of the evaporation flask at the near end of the evaporation. |
| Evaporation temperature | 40°C | NA | Analysts can choose evaporation temperature (range 40–100°C) depending on volatilities of analyte and boiling point of solvent(s). |
| Nitrogen flow rate (entered as total flow) | 200 mL/min for 4 channels; (i.e., 50 mL/min per channel) | NA | Analysts must enter the total nitrogen flow rate for the number of channel/samples being used as there is one mass flow controller hence total flow. For example, 200 mL/min for 4 channels (50 mL/min per channel). If there are 3 channels/sample, then use 150 mL/min. |
| Vacuum | 8 psi (414 torr/551 mbar) | NA | The vacuum level is optimized for PAH application at 8 psi for not losing any volatile compounds. If the vacuum pull is strong (less than 8 psi) there is a possibility of losing volatile/semi-volatile early eluting peaks in PAH application. However, a strong vacuum pull (1–2 psi) can be used for dryness application where no fear of losing any volatile/semi-volatile analytes. |

Note: The vapor trap and vacuum pump should be on when running an extraction and evaporation method.

## Method parameter adjustment: Flow rate calculation

Table 3 presents an example of an EXTREVA ASE system method developed based on the conditions taken from the Dionex ASE 350 instrument. Extraction flow during the dynamic extraction is driven by the total extraction volume and the extraction time defined by the analyst.

The detailed sample preparation is outlined in the EXTREVA ASE system PAH application note (AN 001106). Soil samples were prepared in both 10 mL and 100 mL cells with the same level of contamination for both the Dionex ASE 350 system plus the Thermo Scientific™ Rocket™ Evaporator System (n = 4) and the EXTREVA ASE system runs (n = 4 × 3 = 12). The recovery 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 2. The results are shown in Table 4.

All recoveries were between 78% and 108% for the EXTREVA ASE system, thus demonstrating the high extraction efficiency and the minimal loss of the most volatile compounds like naphthalene in the EXTREVA ASE system. These results met the recommended acceptance criteria of 70–130% from the Unites States Environmental Protection Agency (US EPA) for all compounds. It also met even more severe 80–120% of other worldwide regulations for all the analytes except Acenaphthene (78%). The RSD was below 10% for all compounds, suggesting good channel-to-channel and run-to-run reproducibility for both extraction and evaporation and which was better than the Dionex ASE 350/Rocket Evaporator systems. Moreover, comparable analyte recoveries were observed between the Dionex ASE 350 and the EXTREVA ASE systems as evident in Table 4. The side-by-side results signify the equivalency of using the EXTREVA ASE system to pursue a similar workflow as in Dionex ASE 350/Rocket Evaporator systems with respect of performance.

Table 3. EXTREVA ASE system parameters based on ASE system conditions and desired extraction time

| Case | Cell size (mL) | Target volume (mL) | Volume % of ASE method | Cell filling % | Cell filling volume (mL) | Pre-heater volume (mL) | Remaining volume (mL) | Extraction time (min) | Extraction flow (mL/min) |
|------|----------------|--------------------|-----------------------|----------------|--------------------------|------------------------|----------------------|----------------------|--------------------------|
| 1 | 10 | 26 | 100 | 50 | 5 | 5 | 16 | 15 | 1.1 |
| 2 | 100 | 65 | 50 | 50 | 50 | 5 | 10 | 20 | 0.50 |

Note: For bigger cells, it is recommended to use less solvent amount than the standard amount used with the Dionex ASE 350 system without compromising the analyte recovery.

Table 4. PAH recoveries for total workflow (extraction and evaporation) in the Dionex ASE 350/Rocket Evaporator and the EXTREVA ASE systems.

| PAHs-Recovery | | | | | | | | |
|---------------|---|---|---|---|---|---|---|---|
| | | 10 mL | | | | 100 mL | | |
| | | Dionex ASE 350/Rocket Evaporator systems | | EXTREVA ASE system | | Dionex ASE 350/Rocket Evaporator systems | | EXTREVA ASE system | |
| Compound | | % | RSD (%) | % | RSD (%) | % | RSD (%) | % | RSD (%) |
| 1 | Naphthalene | 81.1 | 9.5 | 82.1 | 3.8 | 95.6 | 0.5 | 96.0 | 5.3 |
| 2 | Acenaphthylene | 83.5 | 10.8 | 85.4 | 3.3 | 104.0 | 1.5 | 103.2 | 4.9 |
| 3 | Acenaphthene | 77.9 | 10.3 | 78.0 | 2.9 | 93.1 | 2.2 | 95.2 | 5.4 |
| 4 | Fluorene | 80.9 | 10.1 | 82.0 | 3.5 | 95.2 | 0.9 | 97.0 | 5.4 |
| 5 | Phenanthrene | 78.3 | 10.5 | 81.3 | 3.6 | 90.9 | 1.0 | 94.3 | 5.5 |
| 6 | Anthracene | 81.8 | 11.6 | 86.1 | 3.1 | 94.3 | 1.7 | 94.5 | 3.8 |
| 7 | Fluoranthene | 92.3 | 11.1 | 96.5 | 3.9 | 102.7 | 1.9 | 103.4 | 6.0 |
| 8 | Pyrene | 85.2 | 10.2 | 90.7 | 3.6 | 101.2 | 1.3 | 100.4 | 5.7 |
| 9 | Benz[a]anthracene | 100.3 | 8.2 | 106.8 | 5.8 | 103.7 | 1.0 | 105.5 | 5.1 |
| 10 | Chrysene | 90.2 | 9.3 | 95.1 | 5.0 | 95.1 | 1.6 | 97.4 | 5.2 |
| 11 | Benzo[b]fluoranthene | 83.7 | 9.5 | 88.4 | 6.0 | 97.5 | 3.0 | 103.1 | 5.3 |
| 12 | Benzo[k]fluoranthene | 80.0 | 8.0 | 84.5 | 6.2 | 95.5 | 2.1 | 101.4 | 5.1 |
| 13 | Benzo[a]pyrene | 100.2 | 13.2 | 104.9 | 5.2 | 106.9 | 3.7 | 108.2 | 5.1 |
| 14 | Indeno[1,2,3-cd]fluoranthene | 98.8 | 7.3 | 101.7 | 5.8 | 105.5 | 3.1 | 105.2 | 4.4 |
| 15 | Dibenz[a,h]anthracene | 95.9 | 7.6 | 98.7 | 5.6 | 101.6 | 3.4 | 103.2 | 4.5 |
| 16 | Benzo[ghi]perylene | 91.8 | 7.1 | 94.7 | 5.8 | 93.9 | 3.2 | 96.0 | 5.3 |

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