

## Sample preparation

## Determination of polychlorinated biphenyls (PCBs) in soils using a new fully automated parallel extraction and evaporation system and GC-MS

### Authors

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### Keywords

Pressurized fluid extraction, sample preparation, U.S. EPA Method 8082A, PCBs, soil, environmental, ISQ 7000 Single Quadrupole GC-MS

### Goal

To demonstrate a method for the determination of polychlorinated biphenyls (PCBs) in soils using the Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor, a newly developed and fully automated parallel extraction and evaporation system

### Introduction

Polychlorinated biphenyls (PCBs) belong to a broad family of synthetic organic chemicals known as chlorinated hydrocarbons. In the United States, PCBs were produced from 1929 until their manufacture was banned in 1979. These compounds have a range of toxicity and vary in consistency from thin, light-colored liquids to yellow or black waxy solids. Due to their non-flammability, chemical stability, high boiling point, and electrical insulating properties, PCBs were used in hundreds of industrial and commercial applications including electrical, heat transfer, and hydraulic equipment; as plasticizers in paints, plastics, and rubber products; in pigments, dyes, and carbonless copy paper; and in many other industrial applications. PCBs do not readily break down once in the environment. They can remain for long periods cycling between air, water, and soil. PCBs can be carried long distances and have been found in snow and sea water in areas far from where they were released into the environment. PCBs, especially the highly chlorinated congeners, tend to accumulate in the lipid-rich tissues of living organisms both by uptake from the environment over time (bioaccumulation) and along the food chain (biomagnification). A greater relative amount of PCBs is usually

found in adipose tissue and breast milk. Although PCBs are readily absorbed into the body, they are slowly metabolized and excreted. In general, less-chlorinated PCB congeners are more readily metabolized than highly chlorinated congeners. As a result of this preferential metabolism, highly chlorinated congeners tend to remain in the body longer than do less-chlorinated congeners.

Techniques such as Soxhlet United States Environmental Protection Agency (U.S. EPA) Method 3540,<sup>1</sup> sonication (U.S. EPA Method 3550)<sup>2</sup>, and microwave extraction (U.S. EPA Method 3546)<sup>3</sup> are presently used for the extraction of PCBs from soil prior to their analytical determination. Those techniques are, however, very labor intensive and suffer from high solvent consumption. Accelerated solvent extraction (U.S. EPA Method 3545A)<sup>4</sup> was developed to meet the new requirements for reducing solvent usage in the preparation of solid, soil, and sediment samples. With accelerated solvent extraction, extractions can be completed in very short periods of time and with minimal amounts of solvent compared to conventional sample extraction techniques such as Soxhlet and sonication.

The EXTREVA ASE Accelerated Solvent Extractor (Figure 1) is a newly developed system based on many proprietary technologies including gas-assisted solvent delivery<sup>5</sup> and parallel accelerated solvent extraction<sup>6</sup>. 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.



Figure 1. EXTREVA ASE Accelerated Solvent Extractor

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 sample extraction techniques such as Soxhlet and sonication. In this application note, the development of an analytical method using the EXTREVA ASE, a fully automated solvent extraction system, and a GC-MS for the determination of 17 PCBs in soil is presented.

## Experimental

### Equipment and consumables

- EXTREVA ASE Accelerated Solvent Extractor (P/N 22184-60101)
- Thermo Scientific™ TRACE™ 1310 Gas Chromatograph equipped with Thermo Scientific™ TraceGOLD™ TG-5MS GC Column (30 m × 0.25 mm × 0.25 μm, P/N 26098-1420)
- Thermo Scientific™ ISQ™ Single Quadrupole Mass Spectrometer
- 60 mL Thermo Scientific™ Dionex™ ASE™ vials (P/N 048784)
- 250 mL clear collection bottles (P/N 056284)
- Thermo Scientific™ Dionex™ Cellulose Filter (P/N 056780)
- 100 mL concentration flask assembly (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

- Hexane, HPLC, Fisher Chemical™ (P/N H302-4)
- PCB Congener Mix, Restek™ (P/N 32416)
- Decachlorobiphenyl (Internal Standard), Restek (P/N 32030)
- 2,4,5,6-Tetrachloro-*m*-xylene (Surrogate), Restek (P/N 32028)
- PCB mixed standards, Anpel (P/N CDAA-M-690047-HA-1mL)
- Diatomaceous Earth (DE) Dispersant for ASE, 1 kg Bottle (P/N 062819)
- Clean Loam Soil, Sigma-Aldrich™ (P/N CLNLOAM6)
- CRM soil, PCB, Sigma Aldrich (P/N CRM961)

## Extraction, concentration, and measurement

The PCB Congener Mix and surrogate standard (2,4,5,6-Tetrachloro-*m*-xylene) were used for the calibration of extracts from the spiked clean loam soil. They were mixed and diluted with hexane to produce a stock solution with a concentration of 1 µg/mL. Calibration standards with concentrations of 0.005, 0.02, 0.05, 0.10, 0.20, 0.3 µg/mL were prepared by diluting the stock solution. The internal standard solution of decachlorobiphenyl had a concentration of 10 µg/mL. 20 µL were added to each calibration standard. The PCB mixed standards (Anpel P/N CDAA-M-690047-HA-1mL) and surrogate standard (2,4,5,6-Tetrachloro-*m*-xylene) were used for the calibration of the extracts from the Certified Reference Materials (CRM). The calibration and internal standards were prepared using the same method as the standards for the clean loam soil.

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 with the appropriate amount of PCBs standard. Any empty volume was filled with Ottawa sand (or ASE Prep DE) 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 ASE Prep DE. The resulting mixture was carefully poured into the extraction cell and spiked with the PCBs standard and surrogate. Any empty volume was filled with Ottawa sand (or ASE Prep DE) 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, 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 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 Thermo Scientific™ Dionex™ ASE™ Prep Moisture Absorbing Polymer (P/N 083475) and ASE Prep DE 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 (hexane). Hexane 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 evaporation 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) 0.35 mL/min (10 mL cell) 0.75 mL/min (100 mL cell)
Extraction solvent	Hexane
Extraction volume	~26 mL (10 mL cell, flow rate 1.6 mL/min) ~15 mL (10 mL cell, flow rate 0.35 mL/min) ~70 mL (100 mL cell, flow rate 0.75 mL/min)
Pre-run rinse	10 mL, Hexane
Extraction time (four samples)	~10 min (10 mL cell, flow rate 1.6 mL/min) ~15 min (10 mL cell, flow rate 0.35 mL/min) ~20 min (100 mL cell, flow rate 0.75 mL/min)
Concentration	
Mode	Fixed volume
Collection bottle	100 mL vial assembly
Final fixed volume	1 mL
Rinse solvent	Hexane, 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 conditions				Mass spectrometer parameters	
Injector		GC			
Injector type	Split/Splitless injector	Column	Thermo Scientific TraceGOLD TG-5MS GC column (30 m × 0.25 mm × 0.25 μm, P/N 26098-1420)	Source temperature	300 °C
Liner	Thermo Scientific™ LinerGOLD™ SSL Splitless Liner, Deactivated, 4 mm × 6.3 mm × 78.5 mm, P/N 453A1925-UI			Ionization	EI
		Carrier gas	Helium	Electron energy	70 eV
Injector temperature	250 °C	Flow rate	1.2 mL/min, constant	Transfer line temperature	305 °C
Injection mode	Splitless	Oven temperature	100 °C (hold for 2 min), ramp to 220 °C at 25 °C/min, ramp to 280 °C at 5 °C/min, ramp to 310 °C at 20 °C/min (hold for 2 min)	Acquisition mode	Timed-SIM
Splitless time	1 min				
Split flow	50 mL/min				
Purge flow	5 mL/min				
Injected volume	2.0 μL				

Figure 2 shows a chromatogram of a 0.20 μg/mL PCB standard under timed-SIM mode. The total analysis time is less than 22 min. A six-point calibration curve was used (0.005, 0.02, 0.05, 0.10, 0.20, 0.30 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.

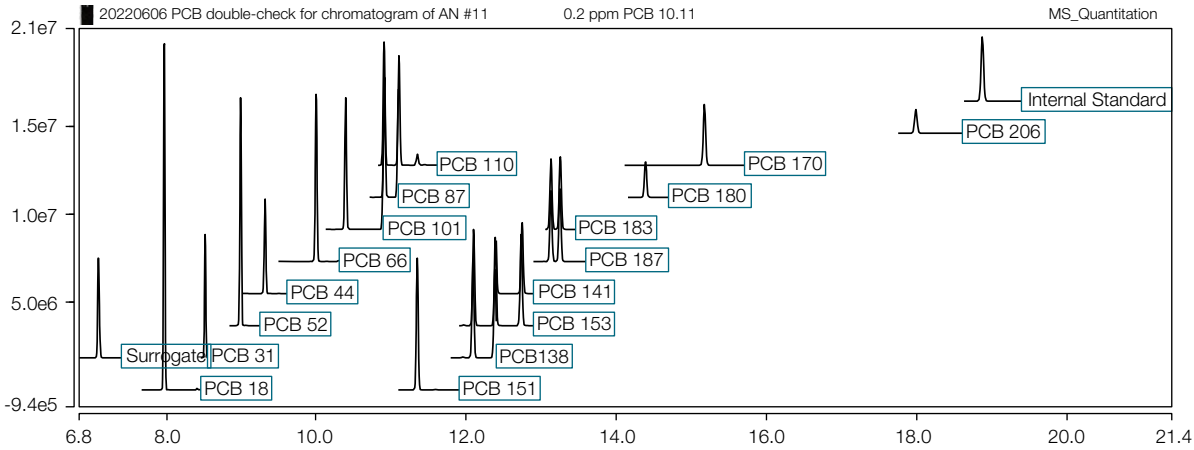
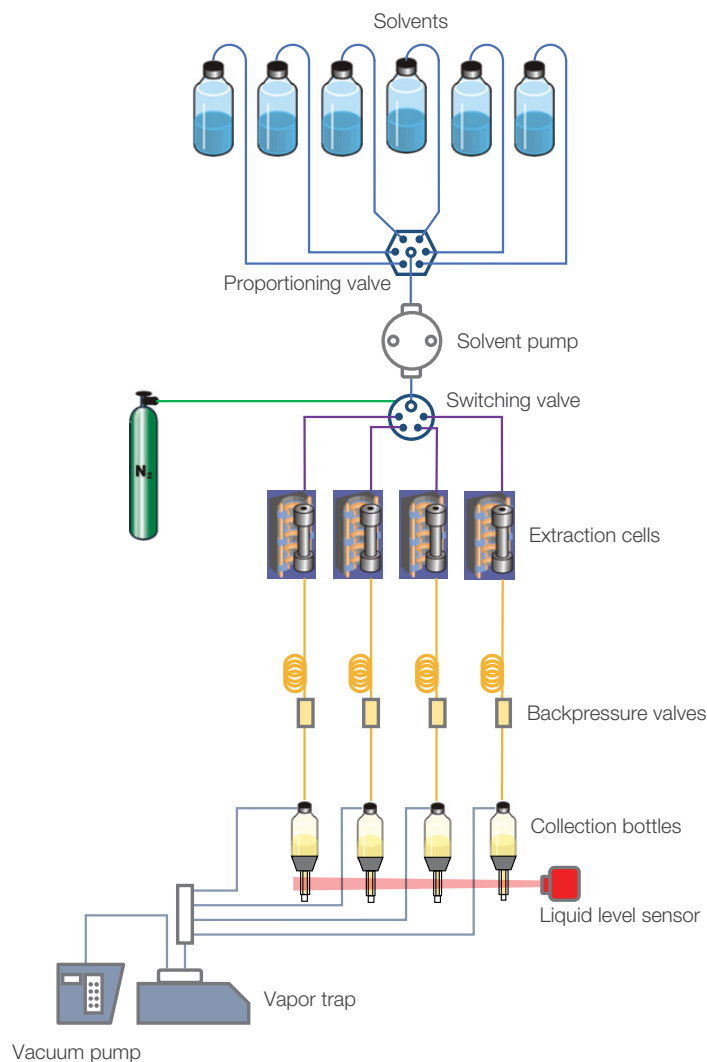


Figure 2. GC-MS chromatogram of PCBs (timed-SIM mode) at 0.20 μg/mL standard solution

## 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 Accelerated Solvent Extractor is shown in Figure 3.



**Figure 3. Schematic diagram of the EXTREVA ASE Accelerated Solvent Extractor**

The EXTREVA ASE system is a fully automated sample preparation platform, designed for extracting and concentrating organic compounds from a variety of solid and semi-solid 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 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 100 µg/kg fortified soil sample. 10 mL and 100 mL cells were used, and the conditions are reported on Table 1. The results are summarized in Table 3 and Figure 4. All recoveries were between 77.0% and 100.9%, thus demonstrating the high extraction efficiency and the minimal loss of the most volatile compounds like PCB18. These results met the recommended acceptance criteria of 70–130% from the U.S. EPA for all compounds. It also met even the more severe 80–120% of other worldwide regulations. The relative standard deviation (RSD) was well below 20% for all compounds, suggesting good channel-to-channel and run-to-run reproducibility for both extraction and evaporation. A solvent flow reduction from 1.6 mL/min (Method 1) to 0.35 mL/min (Method 2) only minimally affected the recovery rates while reducing by 50% the solvent consumption (10 mL cell).

Table 3. Average recoveries and RSDs for the 100 µg/kg spike level

Compound	10 mL – Method 1		10 mL – Method 2		100 mL	
	Average recovery % (n=12)	RSD	Average recovery % (n=12)	RSD	Average recovery % (n=12)	RSD
PCB 18	82.8	7.4	79.2	3.8	77.0	2.6
PCB 31	88.2	7.3	81.4	4.9	80.4	2.8
PCB 52	90.4	7.8	82.5	3.7	82.0	2.5
PCB 44	93.7	6.3	87.5	4.3	82.4	2.8
PCB 66	91.6	6.2	87.6	3.1	87.7	2.6
PCB 101	92.6	5.1	86.3	5.0	86.5	2.6
PCB 87	94.3	5.2	87.1	2.6	88.7	2.6
PCB 110	95.5	5.2	86.8	2.9	86.9	2.2
PCB 151	92.9	4.0	84.6	3.3	88.9	1.9
PCB 153	91.4	3.6	90.7	6.2	92.8	2.2
PCB 141	95.6	3.0	90.7	4.0	93.4	2.3
PCB 138	99.4	4.7	94.6	4.4	94.8	2.3
PCB 187	95.4	6.8	94.8	3.5	92.9	2.5
PCB 183	94.9	6.5	89.2	4.2	92.8	2.6
PCB 180	97.4	4.6	93.7	4.4	101	4.7
PCB 170	92.7	8.1	98.0	4.4	99.8	2.0
PCB 206	85.8	8.7	93.7	4.3	97.0	2.1

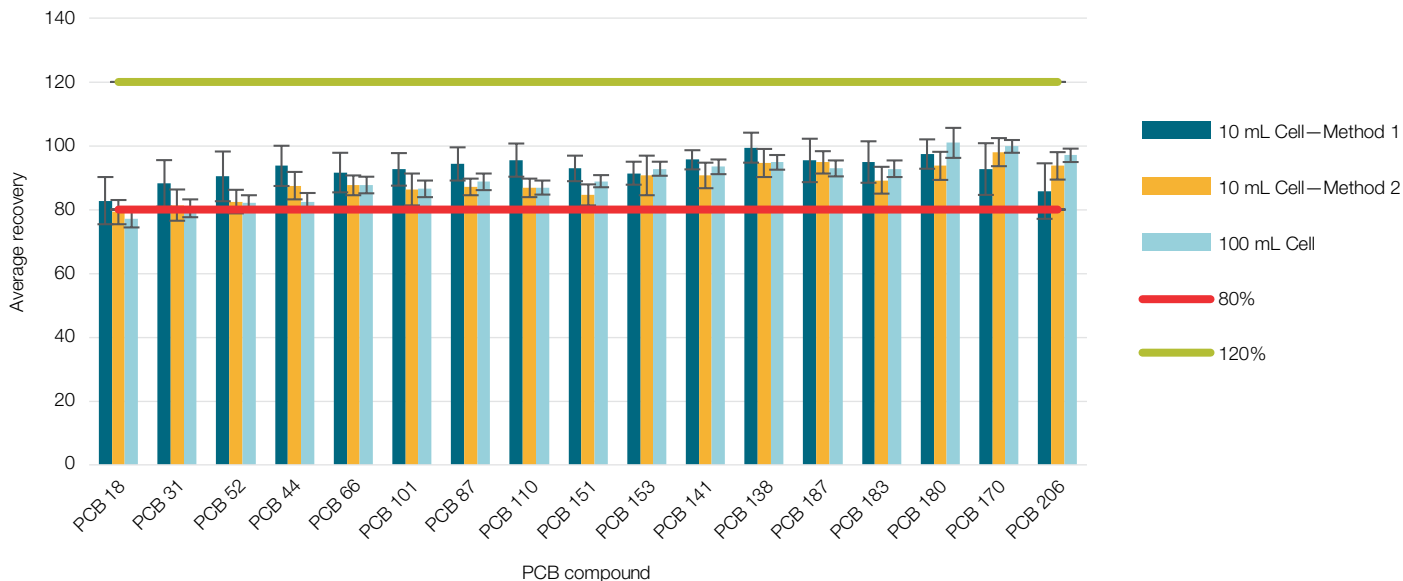


Figure 4. Average recoveries for the 100 µg/kg spike level

## Concentration

A separate study was conducted to evaluate the influence of the evaporation step on the global extraction process. Twelve samples simulating extracts from 10 mL and 100 mL cells (solution A and solution B) were prepared by diluting the stock solution with hexane to volumes of 28 mL and 70 mL, corresponding to final concentrations of 7.1 µg/L and 28.6 µg/L respectively. The resulting solutions were concentrated to 1 mL using the conditions outlined on Table 1. The recoveries of all PCB analytes were in the range of 84.2–112.6%, showing very low analyte losses from the evaporation process even for the more volatile

compounds (Table 4 and Figure 5). These results met the recommended acceptance criteria of 70–130% from the U.S. EPA and even the more severe 80–120% from other worldwide regulations within experimental error. The calculated RSDs were all less than 10%, 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 as necessary in the method. Depending on chemical properties of the analytes, solvent exchange may reduce sample breakdown or boost recovery.

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

Compound	Average recovery (%) (10 mL cell, n=12) Solution A	RSD	Average recovery (%) (100 mL cell, n=12) Solution B	RSD
PCB 18	92.1	8.0	85.9	5.0
PCB 31	84.2	5.7	87.3	5.5
PCB 52	95.6	7.3	90.0	3.9
PCB 44	85.2	4.9	87.4	6.5
PCB 66	101	6.2	91.4	3.2
PCB 101	94.6	5.2	91.1	3.0
PCB 87	96.3	6.2	93.2	2.6
PCB 110	97.4	5.6	92.3	2.9
PCB 151	93.4	5.7	91.6	2.6
PCB 153	100	6.4	92.7	2.8
PCB 141	100	6.4	93.0	2.3
PCB 138	102	6.7	93.2	2.7
PCB 187	100	6.7	93.4	2.3
PCB 183	99.7	6.4	92.9	2.2
PCB 180	113	6.0	90.2	5.3
PCB 170	104	6.1	93.9	2.7
PCB 206	103	6.1	93.1	3.2

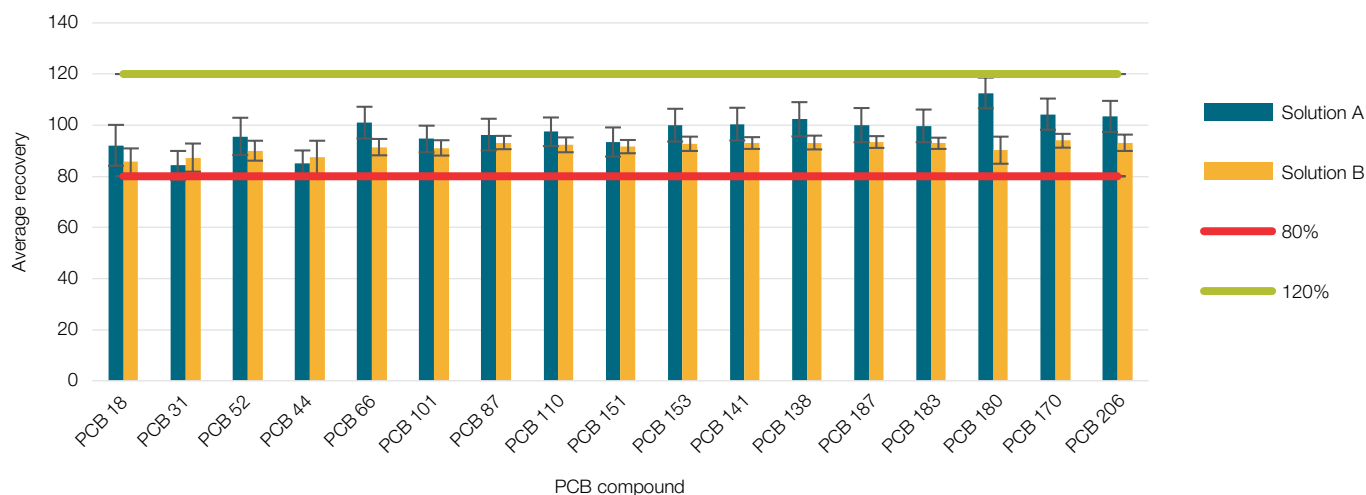


Figure 5. Average recoveries for the concentration of the solutions A and B

## Carryover

With the small amount of solvent used relative to the sample size, carryover or cross-contamination could be of potential concern with the EXTREVA ASE Accelerated Solvent Extractor. To investigate these concerns, a heavily fortified soil sample (1400 µg/kg) 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 0.50% for all analytes. These results demonstrate 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.

The quality of the above-mentioned results was confirmed by the extraction of PCB Certified Reference Material (CRM). A 10 mL cell was used, and the conditions are reported on Table 1 (Method 2). 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 5. Average recoveries and carryover from soil samples with high spike level**

Compound	Average recovery (%) (10 mL cell, n=12)	RSD	Average carryover (%) (10 mL cell, n=12)
PCB 18	77.0	10.5	0.02
PCB 31	80.5	9.2	0.05
PCB 52	84.3	8.0	0.04
PCB 44	80.4	9.2	0.06
PCB 66	90.6	5.5	0.05
PCB 101	90.0	5.4	0.05
PCB 87	91.1	5.0	0.05
PCB 110	91.3	5.1	0.06
PCB 151	95.8	3.5	0.05
PCB 153	95.9	3.8	0.04
PCB 141	97.4	3.4	0.03
PCB 138	98.0	3.5	0.04
PCB 187	99.7	3.7	0.06
PCB 183	97.7	3.6	0.07
PCB 180	94.0	4.6	0.20
PCB 170	106	5.2	0.05
PCB 206	101	3.6	0.12



**Table 6. Average recoveries of certified soil sample**

PCB compound	Certified value µg/kg	Acceptance range µg/kg	Average recovery and RSD (10mL cell, n=12)	
			Avg (n=12) µg/kg	RSD (n=12)
PCB 28	64.7 ± 17.3	12.8 to 117	60.4	4.20
PCB 52	155 ± 37	45.1 to 265	136	3.90
PCB 101	37.3 ± 11.5	2.6 to 71.9	38.5	4.50
PCB 81	44.3 ± 8.7	18.2 to 70.4	44.9	4.30
PCB 77	115 ± 22	48.6 to 181	107	4.00
PCB 123	35.6 ± 6.2	16.9 to 54.3	33.7	5.10
PCB 118	120 ± 22	53.9 to 186	113	4.00
PCB 114	234 ± 50	85.2 to 382	227	3.70
PCB153	147 ± 46	8.8 to 285	158	4.10
PCB105	40.8 ± 8.6	14.9 to 66.7	40.1	4.70
PCB138	112 ± 32	17.2 to 207	118	4.50
PCB126	280 ± 61	97.5 to 462	270	3.60
PCB167	122 ± 25	47.9 to 197	123	4.40
PCB156	193 ± 38	79.5 to 307	157	3.70
PCB157	216 ± 50	67.7 to 365	180	3.50
PCB180	146 ± 40	26.9 to 266	124	4.60
PCB169	66.7 ± 13.3	26.8 to 107	58.3	5.20
PCB189	123 ± 24	51.3 to 195	104	4.20

## Conclusions

This application note described a method for the determination of PCBs in soil matrices using the Thermo Scientific EXTREVA ASE Accelerated Solvent Extractor, Thermo Scientific TRACE 1310 Gas Chromatograph, and Thermo Scientific ISQ Single Quadrupole Mass Spectrometer. Good recoveries and reproducibilities 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 for 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 the

Chromeleon CDS software for complete walkaway efficiency. Overall, the Thermo Scientific EXTREVA ASE Accelerated Solvent Extractor demonstrated efficient, reliable, and high-throughput performance to tackle challenging PCB applications.

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