Application note | 001054

Sample preparation

Determination of organochlorine pesticides (OCPs) in soils using the EXTREVA ASE Accelerated Solvent Extractor and GC-ECD

Authors

Rahmat Ullah[1], Fabrizio Galbiati[1], Anzi Wang[1], Changling Qiu[1], Mingfang Wang[2], Yan Liu[1];
[1]Thermo Fisher Scientific, Sunnyvale, CA, USA; [2]Thermo Fisher Scientific, China Innovation Center, Shanghai, China

Keywords

Pressurized fluid extraction, U.S. EPA Method 8081B, automated sample preparation, environmental, TRACE 1310 Gas Chromatograph, electron capture detector (ECD)

Goal

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

Introduction

Organochlorine pesticides are a group of synthetic chlorinated hydrocarbon derivatives, which were commonly used to protect crops, livestock, buildings, and households from the damaging effects of insects. The wide structural variety and divergent chemical properties of organochlorides lead to a broad range of names, properties, and applications. OCPs have been banned in the United States (U.S.) and many other countries because their persistent presence in the environment and food pose a significant threat to human and animal health. OCPs are persistent and frequently can bioaccumulate in fat tissues of aquatic organisms such as fish and crustaceans. The undesirable characteristics of persistence, bioaccumulation, and biomagnification of the organochlorine compounds led to a drastic reduction in their use or replacement by less persistent chemical products. Thus, after the Stockholm Convention[1] in 2001, several organochlorine insecticides were banned or had their use restricted, especially in the European Union and the U.S., where none are currently authorized for agricultural use. The concentration of OCPs in food, environmental, or biological samples is typically determined using gas chromatographic methods. United States Environmental

Protection Agency (U.S. EPA) Method 8270 provides procedures for analysis of solid, water, and air samples for detection and measurement of different groups of semi volatile organic compounds, including OCPs, using gas chromatography-mass spectrometry (GC-MS).[2] Moreover, U.S. EPA Method 8081, provides validated procedures for determination of 28 OCPs using an electron capture detector (ECD).[3]

Techniques such as Soxhlet (U.S. EPA Method 3540), sonication (U.S. EPA Method 3550), and microwave extraction (U.S. EPA Method 3546) are currently 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 was 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 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[4] and parallel accelerated solvent extraction[5]. 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.[6] 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 a fully automated solvent extraction system, the EXTREVA ASE system, and GC/ECD for the determination of 20 OCPs in soil is presented.

Experimental

Equipment and consumables

- EXTREVA ASE Accelerated Solvent Extractor (P/N 22184-60101)
- Thermo Scientific™ TRACE™ 1310 Gas Chromatograph with Electron Capture Detector (ECD)
- 100 mL concentration flask assembly (P/N 22184-62235)
- 60 mL Thermo Scientific™ Dionex™ ASE™ Collection Vials (P/N 048784)
- 250 mL Thermo Scientific™ Dionex™ ASE™ Collection Bottles (P/N 056284)
- Thermo Scientific™ Dionex™ Extraction Cell Filters (P/N 056780 and P/N 068093)
- Fisherbrand™ 2 mL Screw Thread Autosampler Vials (P/N 03-391-9)
- Thermo Scientific™ Dionex™ ASE™ Prep DE Dispersant
- Fisher Chemical™ Ottawa Sand (P/N S23-3)

Solvents and chemicals

- Clean Loam Soil (Sigma-Aldrich™ P/N CLNLOAM6)
- Fisher Chemical™ Hexanes, Optima™ for HPLC and GC (P/N H303-4)
- Fisher Chemical™ Acetone, Optima™ for HPLC and GC (P/N A929-4)
- Organochlorine Pesticide Mix (Restek™ P/N 32415)
- Pesticide Surrogate Mix (Restek P/N 32000)
- Pesticide Internal Standard (Restek P/N 32091)

Extraction, concentration, and measurement

The pesticide and surrogate standards (decachlorobiphenyl and 2,4,5,6-tetrachloro-*m*-xylene) were mixed and diluted with hexane to produce a stock solution. Calibration standards with concentrations of 0.01, 0.02, 0.05, 0.1 and 0.2 µg/mL were prepared diluting the stock solution. The internal standard solution of pentachloronitrobenzene had a concentration of 2 µg/mL and was added to each of the standards. 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 pesticide standard. Any empty volume was filled with Ottawa sand or Dionex ASE Prep DE dispersant while lightly tapping the cell. 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 with the appropriate tool. 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 pesticide standard. Any empty volume was filled with 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 by 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 (hexane-acetone 1:1, v/v). Hexane was used for solvent exchange (10 mL were added to the collection vessel before evaporation and 1.6 mL were added during evaporation). After concentration, the samples were added with internal standards and analyzed by GC-ECD. The GC-ECD 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	1.1 mL/min (10 mL cell); 0.75 mL/min (100 mL cell)
Extraction solvent	Acetone-Hexane (1:1)
Extraction volume	~26 mL (10 mL cell); ~70 mL (100 mL cell)
Extraction time (four samples)	~15 min (10 mL cell); ~20 min (100 mL cell)
Rinse	Prerun, 10 mL, Acetone-Hexane (1:1)
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-ECD

GC conditions	
Injector	
Injector type	Programmable Temperature Vaporizer (PTV)
Liner	Topaz liner, Split PTV, 2 mm × 2.75 mm × 120 mm
PTV ramp	75 ˚C to 225 ˚C at 5 ˚C/s, hold for 10 min
Injected volume	1.0 µL
GC	
Column	Rtx-CLPesticides (30 m × 0.25 mm × 0.25 µm)
Carrier gas	Helium
Flow rate	2 mL/min, constant
Oven temperature	120 °C (hold for 0.3 min), ramp to 190 °C at 4 °C/min, ramp to 300 °C at 18 °C/min (hold for 3 min)
Detector	
Detector type	Electron Capture Detector (ECD)
Detector temperature	310 °C
Makeup gas flow	15 mL/min

A five-points calibration curve was used (0.01, 0.02, 0.05, 0.1 and 0.2 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 measured amount 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.

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 increase 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. A schematic diagram of the EXTREVA ASE system is shown on Figure 2.

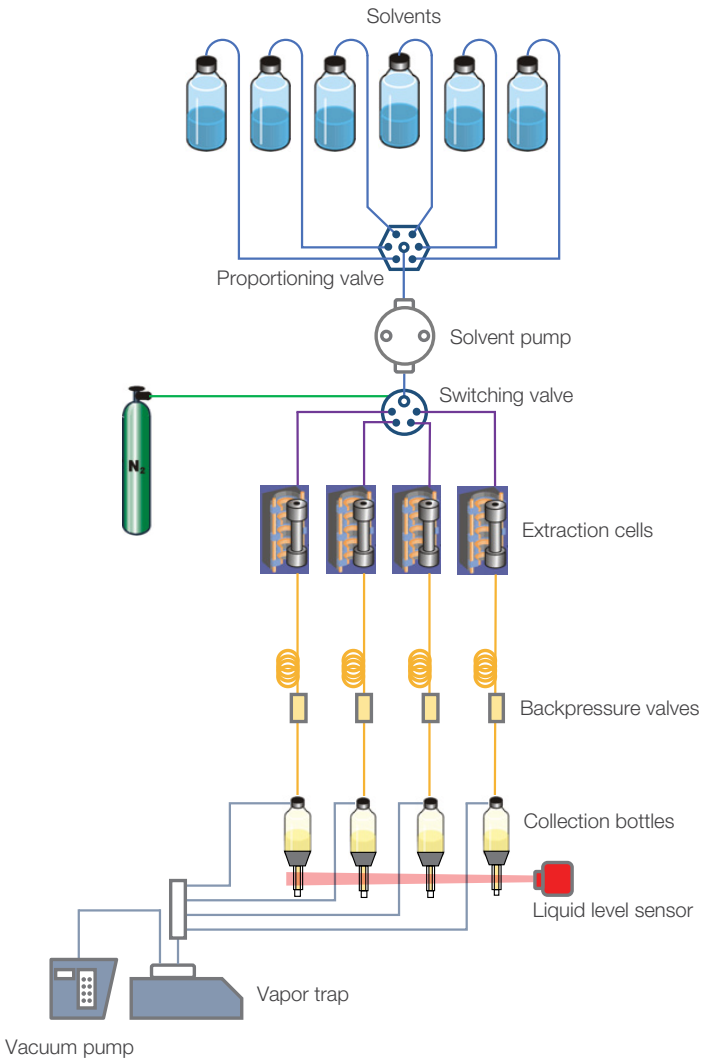

Figure 2. Schematic diagram of the EXTREVA ASE system

Extraction and concentration

The recoveries studies for the complete extraction and evaporation workflow were made using a 25 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 on Table 3 and Figure 3. All recoveries were between 80% and 115%, thus demonstrating the high extraction efficiency and the minimal loss of the most volatile compounds. These results met the recommended acceptance criteria of 70–130% from the U.S. EPA[7] and even the more severe 80–120% from other worldwide regulations. The RSD was below 8% 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 25 µg/kg spike level

Compound	Average recovery (%) (10 mL cell, n = 12)	RSD	Average recovery (%) (100 mL cell, n = 12)	RSD
α-BHC	86.7	3.7	91.7	7.6
γ-BHC	86.4	3.3	95.6	3.9
β-BHC	97.8	5.1	104.8	3.9
δ-BHC	90.6	3.5	98.9	4.8
Heptachlor	100.2	4.4	105.6	6.1
Aldrin	83.9	2.8	92.3	3.9
Heptachlor epoxide	85.8	2.8	96.0	4.1
trans-Chlordane	89.3	2.7	97.4	3.9
cis-Chlordane	93.5	4.0	96.7	4.6
4,4'-DDE	85.5	2.9	93.9	5.0
Endosulfan I	87.9	2.7	96.9	4.5
Dieldrin	87.4	3.4	96.3	4.6
Endrin	96.2	4.3	112.3	5.8
4,4'-DDD	89.1	3.2	92.3	5.1
Endosulfan II	89.3	3.5	91.7	7.2
4,4'-DDT	87.3	3.1	94.7	4.7
Endrin aldehyde	86.1	4.0	82.5	7.6
Methoxychlor	95.4	1.9	97.7	5.2
Endosulfan sulfate	94.6	2.5	102.3	4.7
Endrin ketone	89.5	2.4	95.9	5.0

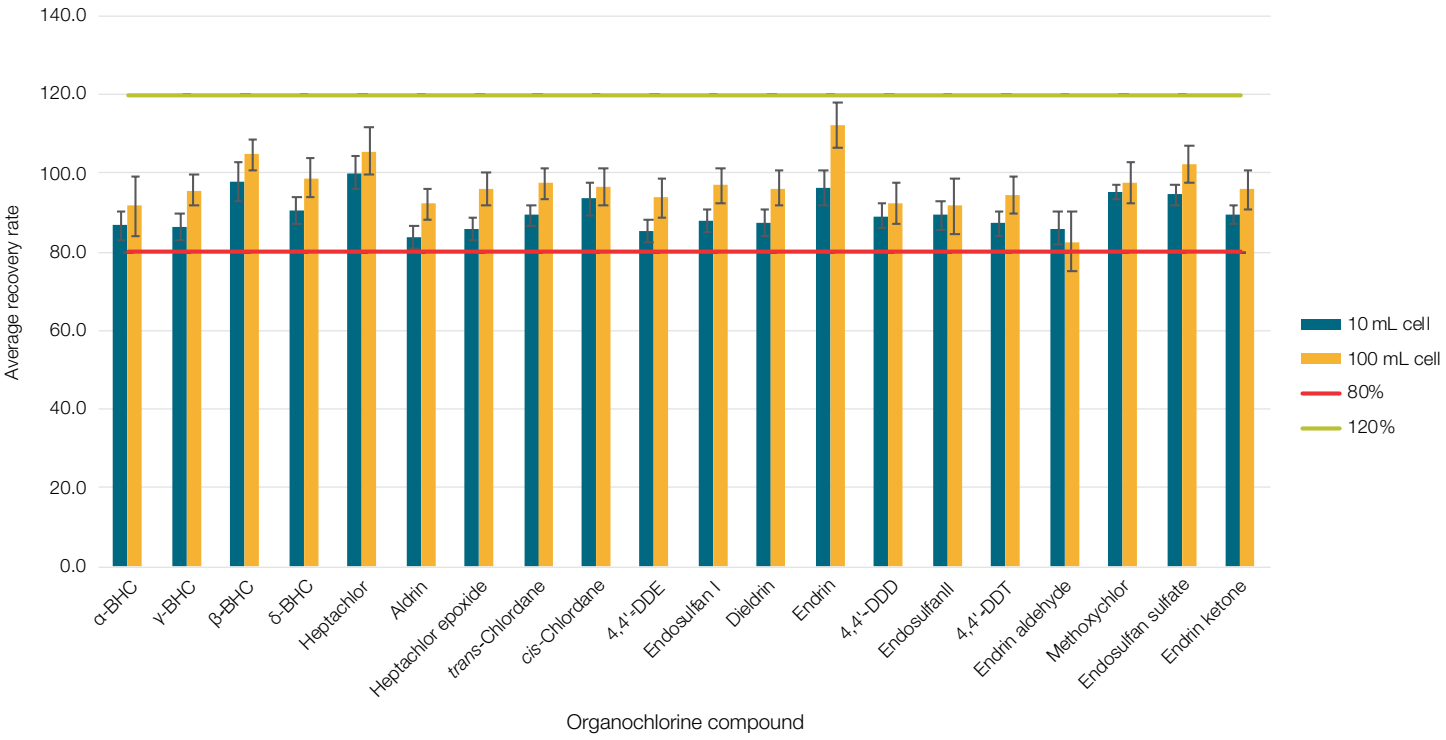

Figure 3. Average recovery rates for the 25 µg/kg spike level with the 80 and 120% limits

Concentration

A separate study was conducted to evaluate the influence of the evaporation step on the global extraction process. A set of 12 samples simulating extracts coming from 10 and 100 mL cells (defined as solution A and solution B) were prepared by diluting with acetone-hexane 1:1 the stock solution to a final volume of 38 mL and 80 mL, corresponding to a final concentration of 1.3 µg/L and 6.3 µg/L respectively. The resulting solutions were concentrated to 1 mL using the conditions outlined on Table 1. The recoveries of all OCP analytes were in the range of 85 to 110%, showing very low analyte losses from the evaporation process (Table 4 and Figure 4). These results met the recommended acceptance criteria of 70–130% from the U.S. EPA[7] and even the more severe 80–120% from other worldwide regulations. The calculated relative standard deviations (RSD) were all below 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 in the method. Depending on chemical properties of the analytes, solvent exchange may reduce sample breakdown or boost recovery. In this study, the majority of acetone in the extracting solvent for OCP samples was replaced with hexane during the evaporation process.

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
α-BHC	87.7	3.7	93.3	6.6
γ-BHC	87.2	4.0	93.1	6.6
β-BHC	101.4	8.0	99.3	6.4
δ-BHC	95.3	5.6	98.1	7.0
Heptachlor	91.5	3.5	87.3	6.1
Aldrin	90.1	4.9	90.3	6.6
Heptachlor epoxide	92.9	3.8	95.2	7.1
trans-Chlordane	94.3	4.1	96.9	7.3
cis-Chlordane	101.3	3.8	97.7	7.2
4,4'-DDE	97.4	2.8	96.0	7.4
Endosulfan I	98.5	2.8	95.4	7.9
Dieldrin	98.4	3.0	94.8	7.4
Endrin	103.2	5.9	102.2	8.5
4,4'-DDD	103.4	3.6	91.5	7.6
Endosulfan II	101.5	4.1	97.4	7.7
4,4'-DDT	96.9	2.5	94.9	8.3
Endrin aldehyde	103.2	4.5	88.0	9.0
Methoxychlor	108.0	4.1	96.5	6.8
Endosulfan sulfate	100.7	2.9	102.2	6.7
Endrin ketone	99.7	1.9	97.1	6.9

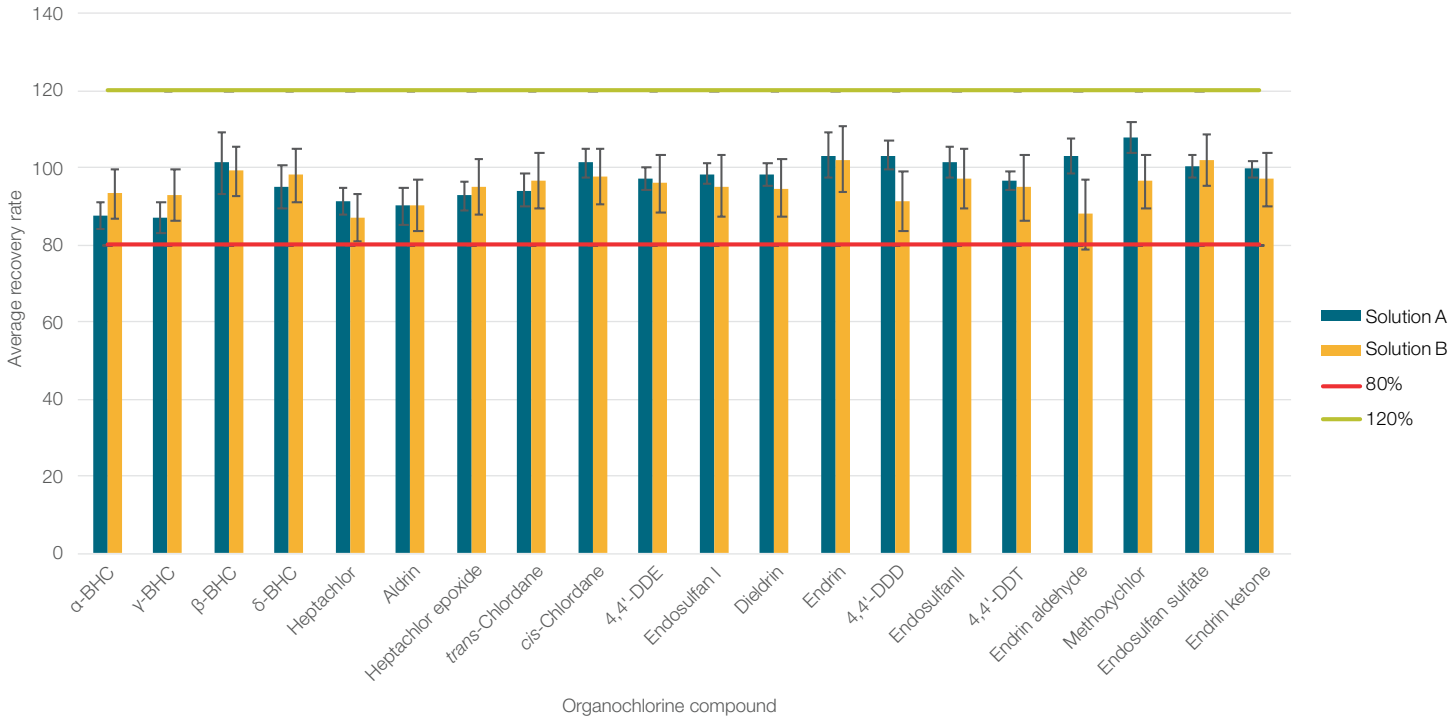

Figure 4. Average recovery rates for the concentration of the solutions A and B

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 (250 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 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.

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

OCP	Average recovery % (10 mL, n = 4)	RSD %	Average carryover % (10 mL, n = 4)
α-BHC	81.7	7.9	0.00
γ-BHC	83.1	6.5	0.19
β-BHC	93.9	5.7	0.07
δ-BHC	89.6	5.0	0.09
Heptachlor	90.1	7.0	0.33
Aldrin	86.9	6.9	0.00
Heptachlor epoxide	92.6	5.7	0.01
trans-Chlordane	92.9	5.0	0.00
cis-Chlordane	93.5	5.6	0.05
4,4'-DDE	86.6	5.8	0.06
Endosulfan I	90.6	5.1	0.00
Dieldrin	94.4	4.8	0.01
Endrin	102.2	4.3	0.02
4,4'-DDD	91.0	3.9	0.00
Endosulfan II	89.8	4.0	0.43
4,4'-DDT	91.7	3.8	0.02
Endrin aldehyde	83.8	5.1	0.03
Methoxychlor	98.6	4.4	0.14
Endosulfan sulfate	97.5	3.5	0.03
Endrin ketone	95.0	3.6	0.03

Thermal degradation test

Because soil samples were typically extracted at elevated temperature (>100 °C), thermally labile analytes may partially degrade during the extraction phase. Out of the 20 OCP analytes used in this study, endrin and 4,4′-DDT are the least thermally stable compounds. Therefore, soil samples spiked with endrin and 4,4'-DDT at 25 µg/kg were extracted to evaluate the presence of thermal degradation products. Table 6 shows the thermal degradation results when the soil sample was extracted at 100 °C and 150 °C. All breakdown percentages were well below the 15% criteria suggested by the U.S. EPA. For endrin, a 3.1% breakdown occurred in the GC injection port, as evidenced by injecting a 0.05 ppm QC standard. DDT breakdown was very low for both the soil samples and the QC standard. In summary, the newly developed gas assisted extraction method employed by the EXTREVA ASE system does not cause significant breakdown for OCP analytes during the extraction process.

Table 6. Endrin and DDT breakdown at different extraction temperatures

	Average breakdown (%)	
Extraction temperature	Endrin	DDT
100 °C	4.0	1.5
150 °C	3.2	1.0

Conclusion

This application note reports a method that can be used to determine the levels of 20 OCPs in soil matrices using the Thermo Scientific EXTREVA ASE Accelerated Solvent Extractor system and the Thermo Scientific™ TRACE™ 1310 Gas Chromatograph. Good recoveries and reproducibility were observed for all analytes extracted from spiked soil matrices. Carryover between consecutive runs were minimal after solvent rinse, and thermal degradation of DDT and endrin were low under typical extraction conditions. 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 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 organochlorine pesticides applications.

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