

Rapid Determination of Organochlorine Pesticides in Animal Feed Using Accelerated Solvent Extraction (ASE)

INTRODUCTION

Animal feed contaminated with organochlorine pesticides (OCPs) has begun to attract worldwide attention. When ingested, the OCPs from animal feed tend to accumulate in certain animal products, especially those rich in fat, such as meat, milk, and butter. Because these types of animal products are widely consumed by humans, methods are needed that quickly extract and determine OCPs in the feeds of animals used to produce products for human consumption.

Traditional methods used to extract OCPs from animal feed require large amounts of organic solvents and take from one to several hours per extraction. Also, many of the traditional methods are very labor intensive and require constant analyst attention.

ASE was introduced in 1995 and is a proven, valuable technique for environmental laboratories. ASE is EPA approved under method 3545A. This technique uses high temperatures and pressures to increase the kinetics of the extraction process, thus decreasing the extraction time and solvent consumption. Also, because ASE is automated, it allows unattended extraction of up to 24 samples. In this application note, OCPs are extracted from certified reference material (CRM) BCR 115 (Institute for Reference Materials and Measurement, Geel, Belgium), an animal feed containing certified levels of organochlorine pesticides.

EQUIPMENT

Dionex ASE 200 Accelerated Extractor* with Solvent Controller (P/N 048765)

11-mL stainless steel extraction cells (P/N 055422)

Dionex cellulose filters (P/N 049458)

Dionex collection vials 40 mL (P/N 048783)

Analytical balance (accurate to the nearest 0.0001 g or better)

Laboratory grinder

Sand (Ottawa Standard, Fisher Scientific, Cat. No. S23-3 20–30 mesh)

Dichloromethane silica gel, 0.063–0.200 mm, water content 2.62% (Merck, Darmstadt, Germany)

S-X3 Bio-Beads (Bio Rad Laboratories)

**ASE and 350 can be used for equivalent results*

REAGENTS

For reagents, use either:

Bulk Isolute Sorbent (International Sorbent Technology Ltd., UK)

Hydromatrix™ (Varian Associates)

STANDARD REFERENCE MATERIAL

CRM BCR 115 (Institute for Reference Materials and Measurement, Geel Belgium)*

**Similar standard reference materials may be substituted.*

Solvents

Hexane

Acetone

(All solvents are pesticide-grade or equivalent and available from Fisher Scientific).

EXTRACTION CONDITIONS

Solvent: Hexane: acetone (3:2)

Temperature: 100 °C

Pressure: 1500 psi*

Static Time: 9 min

Static Cycles: 1

Flush: 60%

Purge: 60 s

*Pressure studies show that 1500 psi is the optimum extraction pressure for all ASE applications.

SAMPLE PREPARATION

Each animal feed sample should be ground to a powder using a laboratory grinder. Weigh approximately 1.0 g of the powder and blend with 0.5 g of the Bulk Isolute Sorbent using a mortar and pestle. Transfer the mixture to an 11 mL stainless steel extraction cell containing a cellulose filter. Top off any void volume in the cell with Ottawa sand.

Table 1. Concentration Values (ng g⁻¹) and RSD (%) for the Extraction of CRM BCR 115

Compounds	Certified Value		ASE (n=3)	
	C (ng g ⁻¹)	RSD (%)	C (ng g ⁻¹)	RSD (%)
α-HCH	*	*	21.5 ± 0.5	2.5
HCB	19.4 ± 1.4	7.2	20.6 ± 0.4	1.8
β-HCH	23 ± 3	13.0	26.0 ± 2.3	8.7
γ-HCH	21.8 ± 2	9.2	27.1 ± 1.4	5.3
Heptachlor	19 ± 1.5	7.9	20.0 ± 0.5	2.7
Aldrin	*	*	56.0 ± 3.1	5.5
p,p' -DDE	47 ± 4	8.5	54.6 ± 2.6	4.7
Dieldrin	18 ± 3	16.7	22.0 ± 0.6	2.6
Endrin	46 ± 6	13.0	52.1 ± 1.9	3.6
p,p' -DDD	*	*	91.8 ± 2.6	2.8
o,p' -DDT	46 ± 5	10.9	49.8 ± 0.5	1.1
p,p' -DDT	*	*	59.4 ± 1.8	3.1

*Present but not certified.

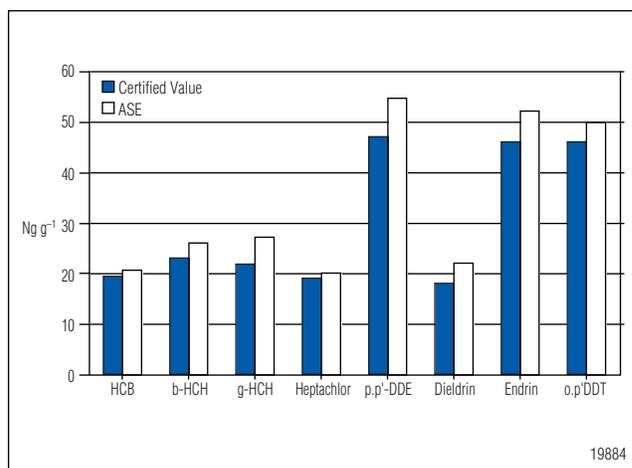


Figure 1. Graph of results from Table 1.

EXTRACTION

Place the extraction cells onto the ASE 200. Label the appropriate number of collection vials and place these into the vial carousel. Set up the method suggested above and begin the extraction sequence. When the extractions are complete, the extracts can then be cleaned using silica gel adsorption followed by gel permeation chromatography (GPC) with *n*-hexane: dichloromethane (1:1) as the elution solvent.¹

A two-step cleanup procedure based on silica gel adsorption followed by gel permeation chromatography (GPC) was optimized for the present determinations. An open glass cartridge (8 mm i.d., 6 mL) with a polyethylene frit at its bottom was packed with 1.5 g fresh dichloromethane silica gel and 1 g Na₂SO₄. The column bed was preconditioned with 50 mL *n*-hexane and compressed by a stream of N₂ (200 kPa). Thereafter, the concentrated raw extract was added onto the top of the silica gel column. The sample flask was rinsed with two 0.5 mL portions of *n*-hexane-CH₂Cl (7+3, v/v) and this was added to the column bed. The analytes were eluted with 19 mL *n*-hexane-dichloromethane (7+3, v/v). The eluate was collected in a 50 mL pear-shaped flask and concentrated to 0.5 mL by means of a rotary evaporator.

The GPC column was prepared by weighting 6 g S-X3 bio-beads that were swelled in *n*-hexane-dichloromethane (1 + 1, v/v) overnight, into a chromatographic column (15 mm i.d., 30 cm, 100 mL) with a reservoir, fused-in fritted disk, and Teflon[®] stopcock. The concentrated extract from the silica gel cleanup was applied onto the GPC column.

The sample flask was rinsed twice with 0.5 µL elution solvent and also applied on the GPC column. After permeation of the sample into the column bed, the separation was performed with an additional 35 mL *n*-hexane-dichloromethane 1 + 1 (v/v). The first 18.5 mL were discarded while the volume of 18.5–26.0 mL containing the analytes was collected. This eluate was concentrated to 1 mL by a rotary evaporator, blown to dryness under a gentle stream of N₂, dissolved in 250 µL cyclohexane, and transferred into a GC autosampler microvial for measurement.

An efficient cleanup procedure may be substituted.

RESULTS AND DISCUSSION

Sample preparation is critical to good recoveries. Grind the samples to a uniform particle size to ensure proper permeation of the solvent into the matrix. It is important to remove the fat and lipids from the extracts so they are ready for GC-MS analysis.

The results of three extractions using ASE are compared to the certified values and listed in Table 1. Figure 1 shows these results graphically. The ASE results are in general agreement with the certified values, with the values of *g*-HCH and *p,p*-DDE slightly above the certified values. This slight difference is attributed to the higher temperatures and pressures of ASE, which increases the desorption of highly bound pesticides.

CONCLUSIONS

The extraction efficiency and reproducibility of ASE for extracting OCPs from animal feed was tested using an optimized method to extract a certified reference material (BCR 115). ASE provides a faster way to extract OCPs from animal feed than traditional techniques, such as Soxhlet, and ASE can accomplish these results using far less solvent.

ACKNOWLEDGEMENTS

We would like to acknowledge the work of S. Chen, M. Gfrerer, E. Lankmayr, X. Quan, and F. Yang at the University of Technology, Austria.

UNIVERSITY OF TECHNOLOGY, AUSTRIA. REFERENCES

1. Chen, S.; Gfrerer, M.; Lankmayr, E.; Quan, X.; Yang, F. Optimization of Accelerated Solvent Extraction for the Determination of Chlorinated Pesticides from Animal Feed. *Chromatographia* **2003**, 58, 631–636.

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LPN 1575-02 PDF 4/11
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