Determination of Pesticides in Large-Volume Food Samples Using Accelerated Solvent Extraction (ASE)

INTRODUCTION

Pesticide residue analysis in crops and food products is routinely performed in regulatory and industrial laboratories around the world. Many of the traditional procedures used to perform the extractions for these analyses are time consuming and solvent intensive. Accelerated Solvent Extraction (ASE) is an extraction technique that speeds the extraction process and reduces the total amount of solvent used. The system uses conventional solvents at elevated temperatures and pressures, which results in improved extraction kinetics. The extraction of samples ranging from 1 to 30 g typically requires 12–17 min and 15–50 mL of solvent.

Extraction of samples up to 30 g have been reported using the Dionex ASE 200 extractor with an upper limit sample cell size of 33 mL. However, for many pesticide residue analyses, this volume is insufficient. Food samples such as fruit and vegetables have very high water contents and must be mixed with desiccants such as sodium sulfate to achieve quantitative pesticide recovery. In this case, the actual weight of the sample extracted will be much less than 30 g. The Dionex ASE 300 has the capability to extract samples with volumes as large as 100 mL. This capability allows the direct extraction of food and vegetable samples with weights in the 30 to 50-g range. This application note reports on the use of the ASE 300 for the determination of organophosphorus pesticides (OPPs) in fruits and vegetables. ASE has previously been compared to more traditional extraction procedures for the determination of OPPs in soils.[1, 2]

EQUIPMENT

ASE 300 Accelerated Solvent Extractor* with 34-, 66-, or 100 mL extraction cells

Dionex vials (250 mL) for collection of extracts (P/N 056785)

Cellulose filter disks (P/N 056780)

**ASE 150 and 350 can be used for equivalent results*

REAGENTS

Acetone, Optima grade (Fisher Scientific)

Methylene chloride, Optima grade (Fisher Scientific)

Ethyl acetate, Optima grade (Fisher Scientific)

Hexane, Optima grade (Fisher Scientific)

Cyclohexane, Optima grade (Fisher Scientific)

ASE Prep DE (diatomaceous earth)

Sodium sulfate, anhydrous (Fisher Scientific)

EXTRACTION CONDITIONS

Temperature:	100 °C
Pressure:	1500 psi*
Solvent:	Ethyl acetate/cyclohexane or $MeCl_2$/acetone (1:1, v/v)
Heatup Time:	5 min
Static Time:	5 min
Flush Volume:	60%
Purge Time:	180 s
Static Cycles:	1–2
Total Extraction Time:	14–20 min per sample
Total Solvent:	135–145 mL per sample

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

SAMPLE PREPARATION

The results of this study were obtained using baby food purchased at a local grocery store. Baby food was used because of the strict requirements enforced for these products, and it was assumed that no pesticide residues were present above the detection levels. In addition, these samples are already homogenized. Samples of 30 g of carrots and apples were weighed out. For this study, 7.5 µL of a pesticide mixture at 0.2 mg/mL was added to the baby food for a final concentration of 50 µg/kg on a sample mass basis. The samples were mixed with enough ASE Prep DE to make them easy to work with and easy to load into the extraction cells, usually around 1:1 (w/w). (If surrogates are used, they should be added to the sample prior to loading the extraction cells).

For samples other than baby food, blend or chop the food samples to produce a uniform homogenate. (A blender or food processor can be used.) Then weigh a 30–50 g portion of the homogenate and mix with ASE Prep DE.

Place a cellulose filter disk in the outlet end of each extraction cell. Carefully transfer the samples to the extraction cells, ensuring that each sample is completely removed from the container in which it was mixed with the ASE Prep DE. Load the extraction cells and collection vials into the ASE 300 and perform the extraction according to the conditions listed.

ANALYTICAL

The total volume of the organic phase obtained was filtered through sodium sulfate (50 g) into a 500-mL round-bottom flask. The filter and flask were rinsed four times with approximately 20-mL portions of ethyl acetate/cyclohexane (1/1). The filtrate was evaporated to a watery residue (not to dryness). Exactly 5 mL of ethyl acetate was added to the evaporation residue. The residue was dissolved completely, immersing the flask in an ultrasonic bath. Approximately 5 g of a mixture of sodium sulfate/sodium chloride (1:1 w/w) was added and swirled. Then exactly 5 mL of cyclohexane was added to obtain a total volume of 10.0 mL (= V_{R1}) and swirled vigorously again. The solution was allowed to stand so the salt mixture could settle to the bottom of the flask. This solution was then ready for cleanup by gel permeation chromatography (GPC).

Gel Permeation Chromatography

A 5.0-mL aliquot (= V_{R2}) of the sample extract (= V_{R1}) was cleaned up using GPC. The automated gel permeation chromatograph (Clean-Up XL, ABIMED Gilson, D-40736, Langenfeld, Germany) was equipped with a 5-mL loop and chromatographic column (600 × 25 mm i.d.) filled with 52-g Bio-Beads S-X3 (200–400 mesh), 33-cm gel bed length (Bio-Rad Laboratories, D-80901 Munich, Germany). A solvent of ethyl acetate/cyclohexane (1:1, v/v) at 5 mL/min was used.

The conditions for gel permeation chromatography were:

Dump: 17 min (to discard 85 mL)

Collect: 22 min (to collect 100 mL)

The collected fraction containing the pesticides was concentrated to about 4 mL using rotary evaporation and was then made up to a volume of 5.0 mL (= V_{End}) with ethyl acetate and analyzed by gas chromatography with flame photometric detection (FPD).

Detection

Chromatographic Conditions—DB 5

Pesticide determination was by gas chromatography using flame photometric detection._

Gas Chromatograph:	HP 5890 (Hewlett-Packard) with Autosampler HP7673 and FPD (phosphorus mode, 526 nm) (now Agilent Technologies)	
Column:	30-m fused silica capillary column DB-5 (J&W); internal diameter 0.53 mm, film thickness 1.5 µm	
Gases:	Carrier:	Helium, 10 mL/min
	Makeup:	Helium, 15 mL/min
	Detector:	Air, 100 mL/min Hydrogen, 75 mL/min
Temperatures:	Oven:	Initial 60 °C (hold for 1 min), heat rate 10 °C/min to 250 °C (hold for 10 min)
	Injector:	250 °C
	Detector:	240 °C
Injection Volume:	5 µL, splitless	
Integrator:	HP ChemStation (software A.05.04)	

Chromatographic Conditions—DB 1701

The following conditions were for the determination of Sulfotep, Chlorpyrifos, and Parathion (-ethyl).

Gas		
Chromatograph:	HP 5890 (Hewlett-Packard) with Autosampler HP7673 and FPD (phosphorus mode, 526 nm) (now Agilent Technologies)	
Column:	15-m fused silica capillary column DB-1701 (J&W); internal diameter 0.53 mm, film thickness 1 µm	
Gases:	Carrier:	Helium, 8 mL/min
	Makeup:	Helium, 15 mL/min
	Detector:	Air, 100 mL/min
		Hydrogen, 75 mL/min
Temperatures:	Oven:	Initial 60 °C (hold for 1 min), heat rate 10 °C/min to 260 °C (hold for 10 min)
	Injector:	250 °C
	Detector:	240 °C
Injection Volume:	5 µL, splitless	
Integrator:	HP ChemStation (software A.05.04)	

RESULTS AND DISCUSSION

Tables 1 and 2 show the analysis results of the ASE 300 extracts of fortified apple and carrot samples. The recovery using ASE averaged 91% for the 26 compounds with average RSD of 11.8% (n = 12) from apples. The recovery using ASE averaged 89.7% for all compounds with an average RSD of 8.7% (n = 12) from carrots. These recovery and precision values are well within acceptable performance limits of other extraction techniques. As a control, blank sample extracts from each matrix were fortified with the pesticide standard following ASE extraction. Compounds that exhibited lower recovery in Table 2 (Demeton-O, 65%; Demeton-S, 59%; and Disulfoton, 63%) also exhibited lower recovery in the test samples (83%, 66%, and 82% respectively). This result indicates that these compounds are lost during the postextraction cleanup steps or in the GC analysis.

CONCLUSION

These results confirm that pesticide residues can be easily extracted from large-volume food samples using the ASE 300. Traditional extraction methods would take from one to several hours for each sample and several hundred milliliters of solvent would be used for each sample. With the ASE 300, these samples can be extracted in about 15 min each with about 160 mL of solvent for each sample. In addition, the ASE 300 can extract up to 12 samples sequentially without user intervention.

REFERENCES

1. Dionex Corporation. “Extraction of Organophosphorous Pesticides Using Accelerated Solvent Extraction (ASE)”. Application Note 319; Sunnyvale, CA.
2. Ezzell, J. L.; Richter, B. E.; Felix, W. D.; Black, S. R.; Meikle, J. E. “A Comparison of Accelerated Solvent Extraction with Conventional Solvent Extraction for Organophosphorus Pesticides and Herbicides.” LC-GC, **1995**, 13, 390–398.

SUPPLIERS

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Table 1. Percent Recovery of Organophosphorus Pesticides from Apple Puree Fortified at 50 ppb															
Apples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Mean (%)**	**SD**	**RSD (%)**
Dichlorvos/Naled	76	80	93	82	80	97	102	95	97	90	67	92	87	10	12
Mevinphos	91	96	105	93	90	108	115	111	110	104	71	106	100	12	12
TEPP	117	141	124	120	96	126	144	137	150	107	79	115	121	20	16
Demeton-O	64	78	47	44	64	67	83	71	75	51	64	77	65	12	19
Ethoprophos (Ethoprop)	84	86	105	91	87	106	110	106	103	91	70	97	95	11	12
Sulfotep	94	100	101	95	88	102	105	101	108	87	72	90	95	10	10
Phorate	80	84	85	77	83	88	100	93	95	83	71	89	86	8	9
Demeton-S	60	68	45	46	72	55	65	55	72	41	63	73	59	11	18
Dimethoate	128	125	146	133	106	141	148	140	149	115	81	121	128	19	15
Diazinon	87	92	101	86	86	99	107	101	104	91	73	93	93	9	10
Disulfoton	59	73	46	44	66	59	78	63	75	52	66	80	63	11	18
Parathion-methyl	91	95	104	89	88	103	108	104	101	94	70	95	95	10	10
Fenchlorphos	82	89	101	86	85	100	103	99	96	91	71	93	91	9	10
Malathion	87	96	106	97	84	106	116	104	105	82	62	89	94	14	15
Fenthion	82	89	87	79	83	91	98	93	94	82	71	90	86	7	8
Chlorpyrifos	89	97	94	82	84	101	99	100	95	89	70	87	91	9	10
Parathion-ethyl	100	104	105	99	87	104	109	106	118	92	75	91	99	11	11
Trichloronat	80	91	98	83	82	99	98	95	96	90	68	90	89	9	10
Tetrachlorvinphos	87	90	100	87	85	95	100	97	98	91	71	94	91	8	9
Prothiofos	76	87	93	78	78	93	93	91	97	85	64	90	85	9	11
Merphos	78	79	91	76	74	88	89	88	96	82	63	83	82	9	10
Fensulfothion	91	92	113	93	90	106	110	106	105	100	71	95	98	11	11
Sulprofos	70	85	80	70	76	86	92	82	88	76	64	85	80	8	10
EPN	96	103	100	97	88	105	111	103	111	89	70	93	97	11	11
Azinphos-methyl	95	99	106	87	84	105	111	104	111	99	75	104	98	11	11
Coumaphos	95	98	102	92	89	102	110	102	106	101	80	96	98	8	8

Table 2. Percent Recovery of Organophosphorus Pesticides from Carrot Puree Fortified at 50 ppb															
Carrots	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Mean (%)**	**SD**	**RSD (%)**
Dichlorvos/Naled	85	87	80	84	86	86	75	84	83	90	74	67	82	6	8
Mevinphos	99	96	95	98	101	100	83	96	92	101	85	77	94	7	8
TEPP	76	110	98	78	107	106	75	79	100	82	87	83	90	13	14
Demeton-O	91	93	82	96	100	110	89	109	108	112	95	80	97	11	11
Ethoprophos (Ethoprop)	93	92	89	95	95	95	78	89	91	97	82	73	89	7	8
Sulfotep	91	85	79	86	90	89	75	82	91	89	84	80	85	5	6
Phorate	91	88	85	89	92	96	79	88	90	96	83	75	88	6	7
Demeton-S	87	85	80	72	98	110	91	117	104	119	97	63	93	17	18
Dimethoate	145	130	113	122	129	138	103	125	131	127	116	114	125	11	9
Diazinon	88	85	86	89	88	89	77	87	89	97	80	75	86	6	7
Disulfoton	90	87	82	94	105	112	89	109	106	112	95	81	97	11	11
Parathion-methyl	90	88	86	92	91	95	79	92	90	96	78	72	87	7	8
Fenchlorphos	89	87	82	88	88	88	75	84	86	94	79	74	84	6	7
Malathion	98	88	83	86	86	89	73	78	93	93	84	81	86	7	8
Fenthion	90	86	86	92	95	90	79	89	95	97	82	76	88	6	7
Chlorpyrifos	85	84	79	90	90	89	77	82	86	93	77	68	83	7	8
Parathion-ethyl	93	89	82	95	92	87	75	78	92	89	60	78	84	10	12
Trichloronat	88	87	85	88	92	87	74	87	90	94	80	76	86	6	7
Tetrachlorvinphos	86	86	83	88	89	91	77	88	89	94	79	74	85	6	7
Prothiofos	81	86	81	87	86	89	76	85	90	94	81	71	84	6	7
Merphos	87	87	87	89	94	93	73	85	92	100	82	72	87	8	9
Fensulfothion	92	91	93	97	97	97	83	91	95	100	83	76	91	7	8
Sulprofos	84	84	81	88	92	93	77	88	90	99	82	71	86	7	8
EPN	98	87	87	94	95	93	77	88	94	92	85	80	89	6	7
Azinphos-methyl	103	98	93	101	103	97	84	94	102	105	86	75	95	9	9
Coumaphos	91	91	89	94	95	94	81	91	93	99	84	78	90	6	7

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