

# Determining Organophosphorus Pesticides in Foods Using Accelerated Solvent Extraction with Large Sample Sizes



The authors determined the presence of 26 organophosphorus pesticides at the 50- $\mu\text{g}/\text{kg}$  level in apple and carrot samples using accelerated solvent extraction. They cleaned up the extracts using gel permeation chromatography before analytical determination using gas chromatography with flame photometric detection. The average recovery from apples was 91%, with an average relative standard deviation (RSD) of 11.8% ( $n = 12$ ). The average recovery of all compounds from carrots was 89.7%, with an average RSD of 8.7% ( $n = 12$ ). These results with 30-g samples demonstrate that accelerated solvent extraction can be used in determining pesticide residues in foods.

**P**esticide residue analysis in crops and food products is performed routinely in regulatory and industrial laboratories around the world. Many of the traditional extraction procedures such as Soxhlet and sonication are time consuming and solvent intensive. In an earlier publication, Richter (1) showed that accelerated solvent extraction is equivalent to these extraction procedures for many analyte classes. Ezzell and colleagues (2) demonstrated that accelerated solvent extraction can be used to extract organophosphorus pesticides from soils and sediments. Obana and co-workers (3,4) described how accelerated solvent extraction can be used to extract organophosphorus pesticides and *N*-methylcarbamate pesticides from food samples. In their work, they used 5-g samples for the extractions. Many protocols call for larger sample sizes when determining pesticide residues in food materials.

The development of new instrumentation for accelerated solvent extraction allows the extraction of larger size samples. The increased sample size capacity expands the applicability of accelerated solvent extraction. This article will demonstrate the use of 30-g samples of fruits and vegetables in determining organophosphorus pesticides using accelerated solvent extraction.

## Experimental

**Apparatus and materials:** All extractions were performed using an ASE 300 accelerated solvent extraction system (Dionex Corp., Sunnyvale, California). The cells used had volumes of 100 mL. The extracts were collected in 250-mL collection bottles (I-Chem, New Castle, Delaware). All solvents used were analytical grade or better. The results of this study were obtained using apple and carrot baby food purchased at a local grocery store. We used baby food because of the strict requirements enforced for these products. The food was checked for pesticide residues by the manufacturer according to German law. In addition, these samples are already homogenized. Because no reference food materials with standardized levels of organophosphorus pesticides can be obtained, the food samples were fortified using solutions (AccuStandard, New Haven, Connecticut) that contained the pesticides at known levels.

**Sample preparation and extraction:** We weighed 30-g samples of carrots and apples and mixed the samples in beakers with 20 g of diatomaceous earth (Isolute HM-N, International Sorbent Technology, Tucson, Arizona) to make them easy to work with and easy to load into the extraction cells. The samples were loaded into 100-mL extraction cells that contained cellulose filter

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disks (Dionex). We took care to ensure that all of the samples were transferred from the beakers in which they were mixed to the appropriate extraction cells. Before closing each extraction cell, we added 7.5  $\mu\text{L}$  of a pesticide mixture at 0.2 mg/mL to the baby food samples for a final concentration of 50  $\mu\text{g}/\text{kg}$  of each pesticide on a sample mass basis. We extracted a total of 12 samples of each matrix. A cell full of diatomaceous earth was spiked with the standard pesticide mixture and extracted. In addition, we extracted a blank of each matrix and spiked the collected extract with the standard pesticide mixture to monitor analyte loss during sample cleanup and analysis. We extracted each sample using the following conditions: 100 °C temperature, 10 MPa (1500 psi) extraction pressure, 1:1 ethyl acetate–cyclohexane (v/v) extraction solvent, 5-min heat-up time, 5-min static time, 60% solvent flush, 180-s purge time, one static cycle, 17–20 min per sample, and 135–145 mL solvent per sample.

**Sample cleanup:** Because of the samples' high water content, the collected extracts contained two phases. The total volume of the obtained organic phase was filtered

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

We cleaned up a 5.0-mL aliquot ( $V_{R2}$ ) of the sample extract ( $V_{R1}$ ) using GPC. The automated gel permeation chromatograph (Clean-Up XL, Abimed Gilson, Langenfeld, Germany) was equipped with a 5-mL loop and 600 mm  $\times$  25 mm chromatographic column filled with 52-g Bio-Beads S-X3 (200–400 mesh) in a 33-cm gel bed length (Bio-Rad Laboratories GmbH, Munich,

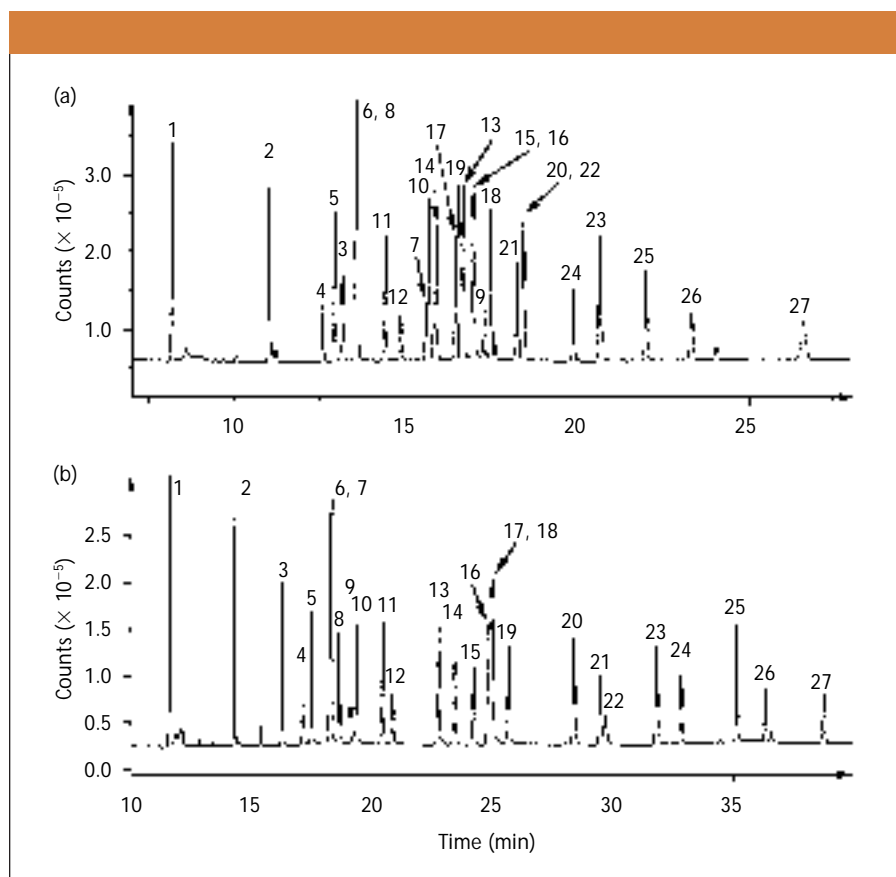
Germany). We used a solvent of 1:1 (v/v) ethyl acetate–cyclohexane at a flow rate of 5 mL/min. The GPC conditions were a 17-min dump to discard 85 mL and a 22-min collection time to collect 110 mL. The collected fraction containing the pesticides was concentrated using rotary evaporation to approximately 4 mL and then made up to a volume of 5.0 mL ( $V_{\text{end}}$ ) with ethyl acetate and analyzed by gas chromatography (GC) with flame photometric detection (FPD).

**Chromatographic conditions:** Some of the organophosphorus pesticides were coeluted, so we had two sets of conditions for the determination of all pesticide levels in the extracts. We used an Agilent model 5890 gas chromatograph equipped with a model 7673 autosampler and a flame photoionization detector (all from Agilent Technologies, Palo Alto, California) operated in phosphorus mode at 526 nm. We used a 30 m  $\times$  0.53 mm, 1.5- $\mu\text{m}$   $d_f$  DB-5 fused-silica capillary column (J&W Scientific, Folsom, California). We used helium as the carrier and make-up gases at flow rates of 10 and 15 mL/min, respectively; we used air at 100 mL/min and hydrogen at 75 mL/min as the detector gases. The oven temperature program started at 60 °C with a 1-min hold and increased to 250 °C at 10 °C/min with a 10-min hold. The injector and detector temperatures were 250 and 240 °C, respectively. The injection volume was 5  $\mu\text{L}$ , splitless. We used Agilent ChemStation software A.05.04 (Agilent) for integration.

For the determination of sulfotep, chlorpyrifos, and parathion-ethyl, we modified the conditions slightly. We used a 15 m  $\times$  0.53 mm, 1- $\mu\text{m}$   $d_f$  DB-1701 fused-silica capillary column (J&W Scientific). We used helium as the carrier gas at a flow rate of 8 mL/min. The oven temperature program started at 60 °C with a 1-min hold and increased to 260 °C at 10 °C/min with a 10-min hold.

## Results

Figures 1a and 1b show typical chromatograms obtained from extractions of the apple samples on the DB-1701 and DB-5 columns, respectively. The extractions from the carrot samples looked essentially identical. Tables I and II show the analysis results of the extracts of fortified apple and carrot samples. The recovery using accelerated solvent extraction averaged 91% for the 26 compounds, with an average RSD of 11.8% ( $n = 12$ ) from apples. The recovery using accelerated solvent extraction averaged 89.7% for all compounds with an average



**Figure 1:** Gas chromatograms of apple extract obtained using accelerated solvent extraction. Column: (a) DB-1701, (b) DB-5. See text for GC conditions. See Tables I and II for compound identification.

**Table I:** Pesticide recoveries from 50- $\mu$ g/kg carrot samples

Compound	Peak Number	Average Recovery (%)	RSD (%)*
Dichlorvos	1	82	8
Mevinphos	2	94	8
Tetraethyl pyrophosphate	3	90	14
Demeton-O	4	97	11
Ethoprop	5	89	8
Sulfotep	6	85	6
Monocrotophos	7	—	—
Phorate	8	88	7
Demeton-S	9	93	18
Dimethoate	10	125	9
Diazinon	11	86	7
Disulfoton	12	97	11
Parathion-methyl	13	87	8
Fenclorpos	14	84	7
Malathion	15	86	8
Fenthion	16	88	7
Chlorpyrifos	17	83	8
Parathion-ethyl	18	84	12
Trichloronat	19	86	7
Tetrachlorvinphos	20	85	7
Prothiofos	21	84	7
Merphos	22	87	9
Fensulfothion	23	91	8
Sulprofos	24	86	8
EPN	25	89	7
Azinphos-methyl	26	95	9
Coumaphos	27	90	7
<b>Average</b>	—	<b>89.7</b>	<b>8.7</b>

\*  $n = 12$ .**Table II:** Pesticide recoveries from 50- $\mu$ g/kg apple samples

Compound	Peak Number	Average Recovery (%)	RSD (%)*
Dichlorvos	1	87	12
Mevinphos	2	100	12
Tetraethyl pyrophosphate	3	121	16
Demeton-O	4	65	19
Ethoprop	5	95	12
Sulfotep	6	95	10
Monocrotophos	7	—	—
Phorate	8	86	9
Demeton-S	9	59	18
Dimethoate	10	128	15
Diazinon	11	93	10
Disulfoton	12	63	18
Parathion-methyl	13	95	10
Fenclorpos	14	91	10
Malathion	15	94	15
Fenthion	16	86	8
Chlorpyrifos	17	91	10
Parathion-ethyl	18	99	11
Trichloronat	19	89	10
Tetrachlorvinphos	20	91	9
Prothiofos	21	85	11
Merphos	22	82	10
Fensulfothion	23	98	11
Sulprofos	24	80	10
EPN	25	97	11
Azinphos-methyl	26	98	11
Coumaphos	27	98	8
<b>Average</b>	—	<b>91</b>	<b>11.8</b>

\*  $n = 12$ .

RSD of 8.7% ( $n = 12$ ) from carrots. These recovery and precision values are well within acceptable performance limits of other extraction techniques. It is unclear why demeton-O, demeton-S, and disulfoton demonstrated low recovery from apples but not from carrots.

As a control, we fortified blank sample extracts from each matrix with the pesticide standard after accelerated solvent extraction. We observed low recoveries for these three compounds from the control standards for both carrots and apples but good recoveries for these compounds directly from carrots. Although the recovery of these compounds is low from apples, it is still within acceptable limits compared with standard extraction procedures.

The results for monocrotophos are not reported in Tables I and II because the recovery values obtained were near 200%. Additional work using GC-mass spectrometry (MS) determined that the reported concentration of monocrotophos in the spiking solution we obtained from the vendor was too low. It is unclear if this low concentration was due to an error in the production of the standard or a loss of analyte while in solution. For this reason, we reported no results for monocrotophos even though this compound appears in the chromatograms in Figures 1a and 1b.

## Conclusions

Our results confirm that pesticide residues can be extracted easily from large-volume food samples using accelerated solvent extraction. Traditional extraction methods would require from one to several hours for each sample and use several hundred milliliters of solvent for each sample. With accelerated solvent extraction, these samples can be extracted in less than 20 min each with approximately 140 mL for each sample. In addition, we were able to extract as many as 12 samples sequentially without user intervention. This ability to extract large sample sizes opens up new application areas for accelerated solvent extraction technology that were not amenable to this methodology in the past because of limited sample size.

## References

- (1) B.E. Richter, *Current Trends and Developments in Sample Preparation*, LCGC 17(6S), S22–S28 (1999).
- (2) J.L. Ezzell, B.E. Richter, W.D. Felix, S.R. Black, and J.E. Meikle, LCGC 15(5), 390–398 (1995).
- (3) M. Okihashi, H. Obana, and S. Hori, *Analyst* 123, 711–714 (1998).
- (4) H. Obana, K. Kikuchi, M. Okiashi, and S. Hori, *Analyst* 122, 217–220 (1997). ■