Food Safety Applications Notebook

Agricultural Chemical Contaminants

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Introduction to Food Safety

Food contamination stories in the news media have raised awareness of the fact that we live with a global food supply chain, and food safety is increasingly becoming an important concern. All types of fruits, vegetables, seafood, and meat can be purchased year round independent of the local growing season. For example, in many countries, well-stocked grocery stores carry cantaloupes from Guatemala, cucumbers from Mexico, shrimp from Vietnam, and fish from China. With fruit, vegetables, seafood, and meat traveling thousands of miles to reach far-flung destinations, and with poor or no knowledge of the agricultural practices, the need for food testing is increasingly important.

Thermo Fisher Scientific understands the demands of food safety related testing. Our separation and detection technologies, combined with experienced applications competence, and our best suited chemistries provide solutions for the analysis of inorganic ions, small drug molecules, pesticides to large components, such as polysaccharides. Your laboratory can now conduct reliable, accurate, and fast testing of food. This notebook contains a wide range of food safety related application notes that will help address your food safety issues.

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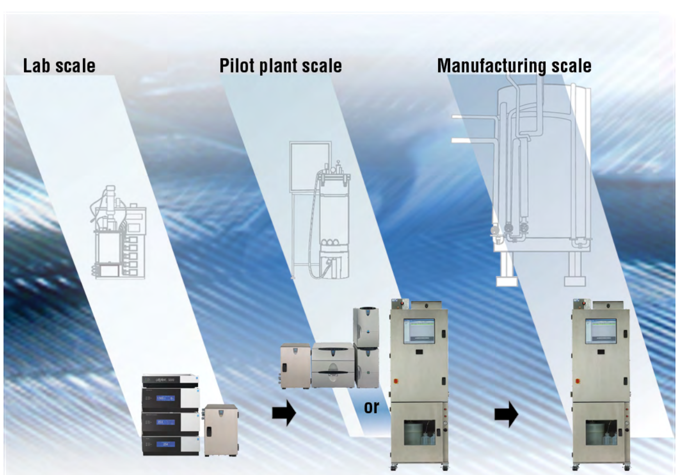

Automated Sample Preparation

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Two new solvent extraction systems with pH-hardened Dionium components

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- PCBs (polychlorinated biphenyls)
- OPPs (organophosphorus pesticides), OCPs (organochlorine pesticides), and chlorinated herbicides
- BNAs (base, neutral, acid semivolatiles)
- Dioxins and furans
- PAHs (polyaromatic hydrocarbons)
- Oil and grease or hexane extractable material

With SPE, large volumes of liquid sample are passed through the system and the compounds of interest are trapped on SPE adsorbents (cartridge or disk format), then eluted with strong solvents to generate an extract ready for analysis. Automated SPE saves time, solvent, and labor for analytical laboratories.

Dionex AutoTrace Systems: The new Dionex AutoTrace 280 system provides fast and reliable automated solid phase extraction for organic pollutants from liquid samples

Dionex AutoTrace Accessories: High-quality parts and accessories are available for Dionex AutoTrace 280 instruments

Analysis of Agricultural Chemical Contaminants

Accelerated Solvent Extraction (ASE®) of Pesticide Residues in Food Products

INTRODUCTION

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 these extractions 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 liquid solvents at elevated temperatures and pressures, which results in increased extraction kinetics. Extraction of samples ranging from 1 to 30 g typically requires 12–17 min and 15–50 mL of solvent.

In the environmental industry, ASE has been compared extensively to traditional preparation techniques, and has been found to generate similar extracts in a more efficient manner. ASE is now widely used in environmental applications to replace time- and solvent-intensive techniques such as Soxhlet and sonication. The principles of ASE technology are based on conventional liquid extraction theory, so the transfer of existing solvent-based extraction processes to ASE is simple. In addition, the ability to extract up to 24 samples unattended can result in a dramatic increase in laboratory efficiency.

EQUIPMENT

ASE 200 Accelerated Solvent Extractor equipped with 11-, 22-, or 33-mL cells

Dionex vials for collection of extracts (40 mL, P/N 049465; 60 mL, P/N 049466)

Cellulose filter disks (P/N 049458)

REAGENTS

Acetone, Optima grade (Fisher Scientific)

Acetonitrile, Optima grade (Fisher Scientific)

Hexane, Optima grade (Fisher Scientific)

ASE Prep DE (P/N 062819)

Sodium sulfate, anhydrous (Fisher Scientific) added after extraction

EXTRACTION CONDITIONS

Temperature:	100 °C
Pressure:	10 MPa (1500 psi)
Heatup Time:	5 min
Static Time:	5 min
Flush Volume:	60%
Purge Time:	100 s
Static Cycles:	1–2
Total Extraction Time:	14–18 min per sample
Total Solvent Used:	15–45 mL per sample

SAMPLE PREPARATION

Weigh dry samples (1–20 g) and add directly to extraction cells containing a cellulose extraction filter. Grind wet samples (1–10 g) and mix with 6 g of ASE Prep DE (diatomaceous earth) using a mortar and pestle. Rinse the mortar and pestle with 2–3 mL of the extraction solvent. Add this volume to the sample in the extraction cell.

EXTRACTION

Perform the sample extractions according to the outlined conditions. Following extraction, add 5 g of anhydrous sodium sulfate to the collection vial to absorb coextracted water. Shake the vial for 15 s and decant the water-free extract into a clean 60-mL vial. Rinse the original vial with 5 mL of the extraction solvent and decant this volume into a second vial. Concentrate the combined volume to approximately 10 mL under nitrogen.

ANALYTICAL

Analyze organochlorine pesticides using a gas chromatograph with a 30-m × 0.25-mm i.d. RTX-5 capillary column (Restek Corporation). Set up a 1-µL splitless injection volume with the injector at 275 °C and the electron capture detector (ECD) maintained at 300 °C with a nitrogen atmosphere. Program the run from 140 °C (3 min) to 265 °C at 10 °C/min. Quantify results using endosulfan I or endrin aldehyde as the internal standard. Pass pesticide extracts through carbon or C18 cleanup cartridges prior to analysis. Quantify results by GC analysis with ECD detection (U.S. EPA Method 8151) or GC with MS detection (U.S. EPA Method 8270).

RESULTS AND DISCUSSION

Samples (10 g) of raw potato and banana were spiked with 100 µL of a standard solution in hexane containing 12 organochlorine pesticides. Hexane with 10% acetone was chosen as the extraction solvent because it delivered good recoveries of the analytes with fewer interferences (coextractables) than a 1:1 mixture. Resulting extracts were clear (after sodium sulfate treatment) upon concentration and suitable for GC/ECD analysis. The necessity of the drying step limits the amount of raw sample that can be extracted to 10 g. Results are presented in Tables 1 and 2. These results represent three extractions with duplicate GC injections of each extract.

A 5-g sample of ground wheat grain was spiked with 100 µL of a standard solution containing 29 pesticides and herbicides at levels ranging from 8 to 102 ppb (see Table 3) and extracted at 100 °C with acetonitrile. Spike levels and recovery results are shown in Table 3. Recoveries ranged from 54.1 to 115.7%. The average recovery was 95.3% if the two outliers, dichlorvos and carbaryl, are excluded. Following the spike studies, 12 naturally incurred grain samples were extracted by the traditional wrist shaker extraction with acetonitrile, using postextraction solid phase extraction (SPE) cleanup, and by ASE using either acetone or acetonitrile as the extraction solvent. The ASE extraction took 12 min per sample and required 12–15 mL of solvent, while the shaker extraction took approximately 1 h per sample (including postextraction SPE cleanup on carbon or C18) and used 130 mL of acetonitrile per sample. The ASE extracts did not require postextraction processing.

Table 1. Recovery of Organochlorine Pesticides Spiked onto Raw Banana at the 100 ppm Level*

Compound	Av. Recovery (%)	SD (µg/kg)	RSD (%)
α-BHC	100.3	2.3	2.3
β-BHC	102.2	2.3	2.3
ϒ-BHC	98.9	3.2	3.2
Heptachlor	89.2	7.6	8.5
Aldrin	89.4	2.2	2.5
Heptachlor Epoxide	93.5	2.1	2.2
Dieldrin	93.7	1.6	1.7
4,4'-DDE	92.1	1.8	1.9
2,4'-DDD	95.4	2.5	2.6
Endrin	94.4	2.7	3.0
4,4'-DDD	88.0	2.7	3.0
4,4'-DDT	89.6	5.8	6.4

* N = 3

Table 2. Recovery of Organochlorine Pesticides Spiked onto Raw Potato at the 100 ppm Level*

Compound	Avg. Recovery (%)	SD (µg/kg)	RSD (%)
α-BHC	96.3	6.3	6.6
β-BHC	108.6	2.3	2.1
ϒ-BHC	97.4	6.6	6.8
Heptachlor	93.9	3.5	3.7
Aldrin	95.9	3.3	3.4
Heptachlor Epoxide	95.2	2.4	2.6
Dieldrin	97.1	0.55	0.57
4,4'-DDE	95.4	0.67	0.70
2,4'-DDD	95.7	0.85	0.89
Endrin	97.8	1.8	1.9
4,4'-DDD	93.7	1.8	1.9
4,4'-DDT	93.0	4.5	4.8

* N = 3

Table 3. Recovery of Spiked Pesticides from Wheat by ASE		
Compound	**Spike Level (μg/kg)**	**Recovery (%)**
o-Methoate	74	85.4
Trifluralin	44	99.6
Dichlorvos	18	60.5
Phorate	18	92.8
Demeton	38	96.7
Dimethoate	58	87.8
Carbofuran	22	96.6
Atrazine	14	92.8
Diazinon	26	96.9
Disulfoton	22	87.9
Triallate	68	87.8
Parathion-methyl	40	115.7
Chlorpyrifos-methyl	8	115.4
Carbaryl	92	54.1
Linuron	102	83.6
Malathion	22	104.5
Phorate-sulfone	32	105.7
Parathion	84	101.2
Endosulfan-alpha	56	94.1
Disulfoton-sulfone	98	77.1
Imazalil	40	108.8
Endosulfan-beta	68	93.3
Endosulfan sulfate	20	77.0
Methoxychlor-*o,p*	48	89.9
Diclofop-methyl	36	81.8
Methoxychlor-*p,p'*	50	114.9
Azinphos-methyl	56	94.2

Extraction results for two compounds identified in these extracts, methyl chlorpyrifos and malathion, are shown in Table 4. The detected amounts compared well between the two techniques, with the ASE values generally 10–20% higher. In all cases, samples with nondetectable levels (ND) were identified as such by both techniques. Acetonitrile and acetone appear to be good solvent choices for this application.

Table 4. Extraction of Incurred Pesticides in Wheat by ASE and Conventional Wrist Shaker Extraction

Sample No.	**Solvent**	**Sample Weight (g)**	**Methyl Chlorpyrifos (μg/kg)**		**Malathion (μg/kg)**	
			Wrist Shaker	**ASE**	**Wrist Shaker**	**ASE**
1	Acetone	20.31	70	90	40	50
2	Acetone	19.78	80	100	40	50
3	Acetone	20.91	50	60	60	70
4	Acetone	10.13	ND	ND	ND	ND
5	Acetone	10.24	30	70	40	100
6	Acetone	9.93	ND	ND	ND	ND
7	Acetone	5.32	ND	ND	ND	ND
8	Acetone	5.39	ND	ND	ND	ND
9	Acetonitrile	19.85	60	80	60	80
10	Acetonitrile	20.4	70	90	60	70
11	Acetonitrile	5.30	ND	ND	ND	ND

ND = Not Detected

CONCLUSION

Using ASE, pesticide residue analysis laboratories can increase sample throughput while reducing overall solvent usage. The simplicity of the ASE technique, combined with results showing excellent correlation to existing methods, have resulted in the rapid acceptance of ASE for environmental analysis. The promulgation of U.S. EPA Method 3545 now provides a means for environmental test laboratories to take full advantage of ASE technology. In addition to the wide range of target analytes covered under Method 3545 for organic pollutants in solid waste, ASE has been applied successfully to the extraction of total petroleum hydrocarbons (TPH), dioxins, and furans from a variety of matrices. ASE has also been applied to the extraction of explosives from soil, PCBs from fish and other marine tissues, and polyurethane foam (PUF) air sampling cartridges.

SUPPLIERS

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 800-766-7000, www.fishersci.com.

Restek Corporation, 110 Benner Cir., Bellefonte, Pennsylvania, 16823 USA, Tel.: 814-353-1300, www.restekcorp.com.

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)

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:	10 MPa (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

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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Agilent Technologies, 395 Page Mill Rd., Palo Alto, CA 94306 USA, Tel: 877-424-4536, www.agilent.com.

Bio-Rad Laboratories, 1000 Alfred Nobel Drive, Hercules, CA 94547 USA, Tel: (510) 724-7000, www.bio-rad.com.

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 800-766-7000, www.fishersci.com.

ACKNOWLEDGEMENTS

We acknowledge the work of Manfred Linker-haegner and his colleagues at Dr. Specht and Partner in Hamburg, Germany who performed the GPC cleanup and GC analysis of the extracts.

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

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)

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

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.

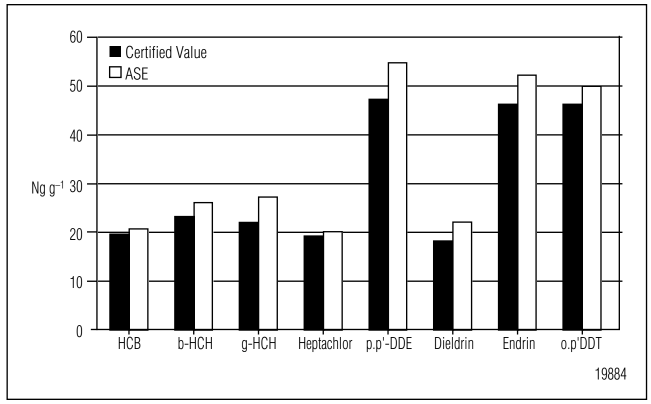

Figure 1. Graph of results from Table 1.

EXTRACTION PROCEDURE

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 Na_2SO_4. The column bed was preconditioned with 50 mL *n*-hexane and compressed by a stream of N_2 (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 fo *n*-hexane-CH_2Cl (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-dichlorometrane (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 N_2, dissolved in 250-µL cyclohexane, and transferred into a GC autosampler microvial for measurement.

Any 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.

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.

Extraction of Drugs from Animal Feeds Using Accelerated Solvent Extraction (ASE®)

INTRODUCTION

Maintaining adequate quality control of drug testing and production processes requires the ability to rapidly and efficiently extract prepared animal feed products. Current sample extraction techniques are labor and time intensive, and are often responsible for communication delays between manufacturing and quality control. Automation of the sample extraction process can accelerate the flow of information, free the analyst from the hands-on, repetitive nature of the work, and reduce potential exposure to hazardous solvents.

Accelerated Solvent Extraction (ASE) is an extraction technique developed to speed the extraction process and reduce the total amount of solvent. Conventional liquid solvents are used at elevated temperatures and pressures, which results in increased extraction kinetics. Extraction of sample sizes ranging from 1 to 30 g typically require 12–17 min and 15–50 mL of solvent.

ASE is widely used in the environmental industry to replace time- and solvent-intensive techniques such as Soxhlet and sonication extraction. Many features of the ASE system also make it attractive for use in pharmaceutical laboratories. Users can select organic and aqueous solvents to match the polarity of the extraction fluid to the target analytes. Extractions can be performed at temperatures ranging from ambient to 200 °C. Because the efficiency of a liquid extraction process is directly related to temperature, the user can select the most efficient temperature (maximum temperature below analyte degradation point), thereby reducing the time and the amount of solvent required. Finally, the ability to extract up to 24 samples, unattended, results in a dramatic increase in laboratory efficiency.

This Application Note gives two examples of how ASE can provide extraction efficiencies superior to other techniques. In the first example, ASE is used to extract an antischizophrenic agent from rodent feed used in drug testing. In the second example, ASE is used to extract Lasalocid, a veterinary medicinal added to poultry and cattle feed.

EQUIPMENT

Dionex ASE 200 Accelerated Solvent Extractor equipped with 11-, 22-, or 33-mL cells

Analytical balance

Dionex vials for extract collection (40 mL, P/N 49465; 60 mL, P/N 49466)

Cellulose filter disks (P/N 49458)

REAGENTS

Methanol (HPLC grade or better)

Acetic acid

EXTRACTION CONDITIONS

Rodent Feed:	**Antischizophrenic Agent**
Extraction Solvent:	Methanol
Temperature:	100 °C
Pressure:	1500 psi (10 MPa)
Heat-up Time:	5 min
Static Time:	5 min
Flush Volume:	60%
Purge Time:	100 s
Static Cycles:	1
Total Extraction Time:	12 min per sample

Total Solvent Use:	30 mL per sample
Poultry and Cattle Feed:	Veterinary Medicinal
Extraction Solvent:	Methanol + 0.3% Acetic acid
Temperature:	80 °C
Pressure:	1500 psi (10 MPa)
Heat-up Time:	5 min
Static Time:	5 min
Flush Volume:	60%
Purge Time:	100 s
Static Cycles:	1
Total Extraction Time:	12 min per sample
Total Solvent Use:	30 mL per sample

SAMPLE PREPARATION

Weigh and directly add dry, granular feed (1–20 g) to the ASE extraction cells containing a cellulose filter. Samples should be in the ground state (not pelleted). Sand (Fisher Scientific, P/N S23-3) can be used as a dispersant if sample particles tend to clump or adhere firmly.

ANALYTICAL

Feed sample extracts containing the antischizophrenic drug were sent to the manufacturer's facility for quantification. HPLC analysis was performed using an Astec Cyclobond® I 25 cm x 4.6 mm i.d. column at 5 °C and a Brownlee Labs™ Polypore® Phenyl RP (PRP-1) 3 cm × 4.6 mm i.d. pre-column held at ambient temperature. Columns were pur-chased from Alltech Associates, Inc. An isocratic mobile phase of acetonitrile:triethanolamine (97:3 v/v, pH 4.5) at 1.0 mL/min was used with UV detection at 280 nm.

Lasalocid feed sample extracts were analyzed by HPLC using a Phenomenex Ultracarb 5 ODS 25 cm × 4.6 mm i.d. C18 column with an IPA:water mobile phase (20:80) and UV detection at 248 and 318 nm.

RESULTS AND DISCUSSION

Feed samples were batch-fortified by the manufacturer's facility with an antischizophrenic drug at 10 and 0.2 g/kg. 10-g samples were extracted using ASE at 100 °C with a total extraction time of 12 min per sample and a total solvent volume of 30 mL of methanol per sample. Conventional wrist shaker extraction was performed for 30 min using acetonitrile, followed by filtering and volume adjustment. The method requires a total extraction time of approximately 55 min and uses 100–400 mL of solvent. Extraction of spiked placebo samples (blank samples spiked with the active compound) was performed by first loading the extraction cell and then adding the standard mixture directly to the top of the sample.

Table 1 compares the recovery and reproducibility generated for the target compound using ASE and the wrist shaker extraction method. The recovery of the ASE extractions is higher than the wrist shaker method for the two concentration-level samples. The ASE extracts were shipped to a different location for analysis, which may account for the greater RSD values. Although the higher temperatures used in ASE expedite the extraction process, there was concern that possible co-extractable materials could interfere with the chromatographic analysis of the active compound. Figure 1 compares the chromatographic analysis of ASE-generated extracts with standard and blank runs. No interferences were present in the analysis.

The chemical structure of Lasalocid is shown in Figure 2. This compound is currently licensed for veterinary use as an antibacterial (coccidiostat) in poultry and a growth promoter in cattle. The conventional method for the extraction of Lasalocid is either soaking the sample overnight or sonication for 30 min in 100–200 mL methanol. The ASE method requires only 12 min and 30 mL methanol containing 0.3% (v/v) acetic acid per sample. The results shown in Table 2 summarize extractions performed for poultry and cattle feed containing differing amounts of Lasalocid. Comparison of the average and standard deviations indicate that the two techniques generate equivalent results, with ASE recovery values between 96–105%, relative to the conventional method.

Table 1 Recovery of an Antischizophrenic Drug from 10 g of Rodent Feed using ASE and Wrist Shaker Extraction Methods

	Feed Level (% RSD)*	
Extraction Method	**0.2 g/kg**	**10 g/kg**
ASE (placebo spikes)	0.199 (4.1)	10.1 (4.2)
ASE	0.185 (4.2)	9.68 (4.4)
Wrist Shaker	0.170 (1.3)	9.43 (1.3)

*n=10

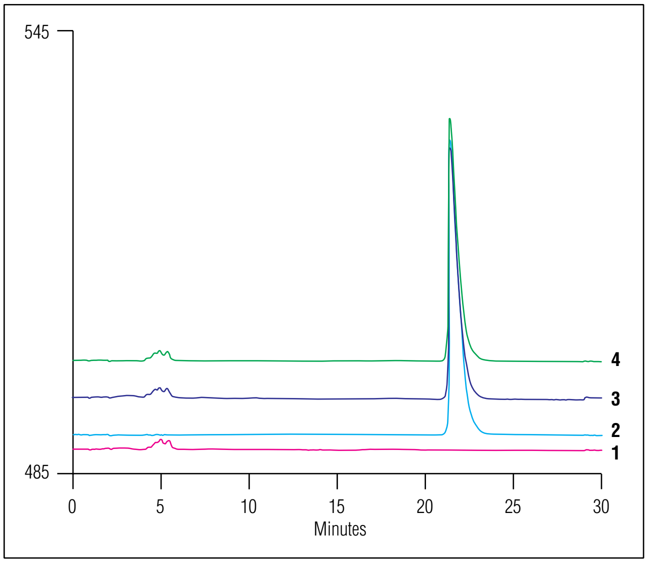

Figure 1. HPLC analysis of feed standards and extracts. (1) ASE extraction blank, (2) drug standard, (3) ASE extract of spiked standard, (4) ASE extraction of drug containing feed sample.

Table 2 Lasalocid Recovered from 10-g Samples of Poultry and Cattle Feed Taken from 12 Individual Lots by ASE and Sonication Extraction Methods

	Lasalocid Recovered (ppm)	
Sample #	**ASE**	**Sonication**
1	80.0	77.5
2	82.2	78.4
3	81.4	79.4
4	82.0	78.7
5	89.5	78.2
6	85.5	81.2
7	136.0	130.3
8	138.3	140.8
9	136.3	141.1
10	135.2	136.8
11	133.8	135.8
12	138.0	133.7

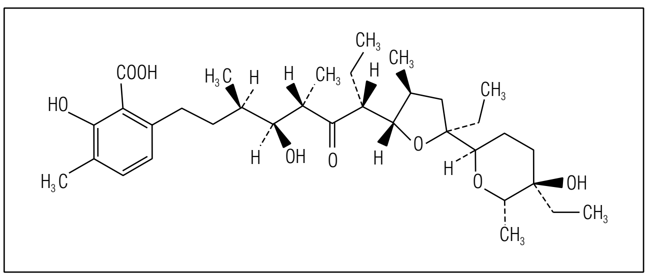

Figure 2. Lasalocid chemical structure.

CONCLUSION

Accelerated solvent extraction takes advantage of enhanced solubilization kinetics that occur at temperatures higher than are commonly used to perform liquid solvent extractions. As the efficiency of the extraction process improves, less solvent and time are required to complete the process. Because reducing solvent consumption and increasing sample throughput are important concerns to laboratories, ASE offers significant advantages for both production and research labs.

In this Application Note, ASE was compared to conventional solvent extractions of compounds added to animal feeds. ASE provides results comparable to conventional extraction methods while reducing the time and volume of extraction solvent typically associated with these analyses. Chromatographic profiles indicate ASE-generated extracts are nearly identical in composition to those generated by conventional techniques. In addition to savings in time and solvent consumption, ASE technology is automated to increase laboratory productivity.

LIST OF SUPPLIERS

Fisher Scientific, 711 Forbes Avenue, Pittsburgh, Pennsylvania, 15219-4785, USA. Tel.: 1-800-766-7000

Alltech Associates, Inc., 2051 Waukegan Road, Deerfield, Illinois, 60015, USA. Tel.: 1-800-255-8324

Phenomenex, 2320 W. 205th Street, Torrance, California, 90501, USA. Tel.: 1-310-212-0555

Rapid Determination of Sulfonamide Residues in Animal Tissue and Infant Food Containing Animal Products Using Accelerated Solvent Extraction (ASE®)

INTRODUCTION

Veterinary drugs containing antimicrobial agents are often administered to livestock for the treatment or prevention of disease and, at low levels, to promote growth in food-producing animals. Recently, government agencies have discovered that some livestock companies are abusing the use of these antimicrobial drugs, by administering them at higher than recommended levels to promote faster growth. This practice can result in unwanted residues of these drugs in meat and meat products eaten by humans. Negative health effects in humans have been traced to the consumption of these antimicrobial drugs and their metabolites. Therefore, screening for these types of residues in animal tissue and meats has become a priority, not only in the United States, but in Europe as well.

Sulfonamides are one class of antimicrobial agent used widely in the livestock industry to promote growth. Sulfonamides are often overused because they are inexpensive and readily available. Short-life sulfonamides are mixed with the feed several times per day to prevent bacterial contamination, while the long-life sulfonamides are injected into the animals at high levels to increase animal growth. American and European institutions have established maximum residue levels (MRLs) to regulate the amount of veterinary medicinal product residues allowed in meat and meat products used for human consumption.

This application note shows that ASE is an excellent technique for the extraction of sulfonamides from meat and baby food containing meat products.

EQUIPMENT

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

11-mL Stainless Steel Extraction Cells (P/N 055422)

Glass-Fiber Filters (P/N 049458)

Collection Vials, 40 mL (P/N 048783)

Analytical Balance (to read to nearest 0.0001 g or better)

Standard-Grade tissue homogenizer

Centrifuge (any standard laboratory centrifuge capable of at least 10,000 rpm)

Freezer capable of –18 °C

REAGENTS

C18 resin (can be purchased from any reputable manufacturer like Supelco or Restek)

STANDARD REFERENCE MATERIAL

Sulfamethoxazole (SMX), Sulfamoxole (SMO), Sulfapyradine (SPD), Sulfamethoxypiridazine (SMT), Sulfachloropyridazine (SCP), Sulfamethoxypiridazine (SMP), Sulfadiazine (SDZ), Sulfamerazine (SMR), Sulfamethazine (SMZ), Sulfasomidine (SIM), Sulfamonomethoxine (SMM), Sulfadimethoxine (SDM), Sulfaquinozaline (SQX)

SAMPLES

Crude Meat Samples

Bovine:	Tissues of veal, tender beef, and beef
Porcine and Poultry:	Ham and chicken

Baby Food Samples

Containing:	Bovine (veal and beef), porcine (pork products and ham), poultry (chicken and turkey)

SOLVENTS

HPLC-grade water

EXTRACTION CONDITIONS

Solvent:	HPLC-grade water
Temperature:	160 °C
Pressure:	1500 psi
Static time:	5 min
Static cycles:	1
Flush:	60%
Purge:	60 s

SAMPLE PREPARATION

Baby Food Samples

Mix 2 g of baby food with 4 g of C18 material using a mortar and pestle until the entire mixture is of uniform consistency. Transfer this mixture to an 11-mL cell containing a glass-fiber filter.

Crude Meat Samples

Homogenize the meat samples using any standard tissue homogenizer. This task should be done with deionized (DI) water added to the sample, starting the homogenizer at 5000 rpm and increasing to 25,000 rpm for 15 min. Evaporate excess water. Weigh out approximately 2 g of homogenized tissue and mix with 4 g of C18 resin until the entire mixture is of uniform consistency. Transfer this mixture to an 11-mL cell containing a glass-fiber filter.

Extraction Procedure

Place the cell onto the ASE 200 cell tray. Label the appropriate number of collection vials and place these into the vial carousel. Set up and load the method suggested above. Then press start. After the extraction, allow the sample vials to cool in a freezer at –18 °C for one hour to precipitate the coextracted lipids. Centrifuge the vials for 5 min at 10,000 rpm. Inject 100 µL of the supernatant for analysis.

Analytical Procedure

For the data shown in Table 1, all analyses were performed using LC-MS/MS (Perkin-Elmer Series 200 with a PE Sciex API 2000 tandem triple-quadrupole mass spectrometer with a TurboIonSpray® source operated in the positive ionization mode). The HPLC column was an Alltima™ 25 cm × 4.5 i.d. column filled with 5 µm C-18 reverse phase packing. For additional details, consult Reference 1.

RESULTS AND DISCUSSION

When compared with diatomaceous earth, C18 material was the best agent for dispersing the meat samples because it retained more of the lipids from the sample matrix, giving cleaner extracts.[1]

Temperature tests were conducted and the best recoveries of all sulfonamides were at 160 °C with no negative effects on analyte stability.[1]

Recovery levels were tested on bovine, porcine, and poultry samples. Table 1 lists the recovery results with the limits of detection (LODs) and limits of quantitation (LOQs) listed in Table 2.

CONCLUSION

For several reasons, ASE technology has proven advantages for extracting sulfonamides from meat. First, extraction times of ~15 min when using ASE, second, ASE uses between 25–30 mL of solvent, and third, because of the efficiency offered by the increased temperature and pressure, ASE is able to extract polar compounds at acceptable recovery levels such as sulfonamides using water as the extraction solvent, which cuts solvent purchase and disposal costs.

Table 1. Recoveries (%) of Sulfonamides from Various Meat Matrices Spiked with 100 ppb Standards						
	Bovine Meat		Porcine Meat		Poultry Meat	
Analyte	**Raw Meat**	**Baby Food**	**Raw Meat**	**Baby Food**	**Raw Meat**	**Baby Food**
SPD	89 (5)	90 (4)	92 (5)	94 (3)	91 (4)	93 (3)
SDZ	92 (4)	91 (3)	93 (4)	95 (4)	94 (3)	94 (4)
SMX	92 (4)	94 (3)	94 (5)	93 (3)	96 (4)	95 (3)
SMR	99 (4)	101 (4)	98 (5)	99 (4)	98 (6)	100 (5)
SMO	70 (6)	71 (4)	72 (5)	74 (5)	76 (5)	75 (5)
SMT	86 (6)	87 (6)	90 (5)	92 (5)	91 (4)	93 (4)
SIM	94 (4)	97 (4)	98 (5)	100 (4)	99 (3)	98 (4)
SMZ	88 (5)	91 (5)	90 (5)	89 (4)	92 (4)	95 (4)
SMP	85 (6)	84 (4)	85 (5)	85 (5)	85 (5)	87 (3)
SMM	90 (6)	92 (5)	92 (5)	91 (5)	94 (5)	95 (5)
SCP	79 (4)	83 (5)	85 (5)	84 (6)	82 (6)	88 (4)
SQX	81 (5)	84 (4)	82 (6)	82 (5)	85 (5)	87 (5)
SDM	85 (6)	88 (5)	90 (5)	92 (5)	93 (4)	91 (4)

The numbers in parenthesis equal total extractions performed.

Table 2. LODs and LOQs of the Method for Analyzing Sulfonamides in Beef, Raw Meat, and Baby Food				
	Raw Meat		Baby Food	
Analyte	**LOD (ppb)**	**LOQ (ppb)**	**LOD (ppb)**	**LOQ (ppb)**
SPD	1.4	4.2	1.2	3.5
SDZ	1.6	4.8	0.8	2.4
SMX	2.6	7.8	1.4	4.2
SMR	1.9	5.7	1.5	4.5
SMO	1.1	3.3	1.1	3.3
SMT	1.1	3.3	0.4	1.2
SIM	1.7	5.1	1.7	5.1
SMZ	2.1	8.3	1.6	4.8
SMP	0.7	2.1	0.4	1.2
SMM	0.9	2.7	0.9	2.7
SCP	1.3	3.7	0.5	1.5
SQX	1.2	3.8	1.2	3.8
SDM	0.8	1.8	0.5	1.5

LOD = Three times the noise level of the baseline in the chromatogram (S/N = 3)
LOQ = Three times the LOD. The noise level depends on the matrix, therefore there are different LODs for different samples.[1]

ACKNOWLEDGEMENTS

We would like to acknowledge the work of Alessandra Gentili and colleagues at the University "La Sapienza" Department of Chemistry, Roma, Italy.

LIST OF SUPPLIERS

Supelco Inc, Supelco Park Bellefonte, PA 16823 USA,Tel: 814-359-3441, www.sigmaaldrich.com.

Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823 USA, Tel: 800-356-1688, www.restekcorp.com.

Sigma-Aldrich Chemical Company, 3050 Spruce St., St. Louis, MO 63103 USA, Tel: 800-325-3010, www.sigmaaldrich.com.

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1. Gentili, A., Perret, D., Marchese, S., Sergi, M., Olmi, C., and Curini, R. "Accelerated Solvent Extraction and Confirmatory Analysis of Sulfonamide Residues in Raw Meat and Infant Foods by Liquid Chromatography Electrospray Tandem Mass Spectrometry", *J. Agric. Food Chem*, **2004**, *52*, 4614-4624.

Faster Yet Sensitive Determination of *N*-Methylcarbamates in Rice, Potato, and Corn by HPLC

INTRODUCTION

The *N*-methylcarbamates and the *N*-methylcarbamoximes are among the most widely used pesticides in agriculture. Because these pesticides may create health problems—including issues impacting the central nervous and reproductive systems—concerns over the presence of carbamate residues in water, crops, and food products have promoted increased awareness and testing for these compounds.

For the detection of carbamate residues in environmental waters, the United States Environmental Protection Agency (U.S. EPA) provides guidelines for monitoring the presence of carbamate pesticides and related compounds in raw surface water using EPA Method 531.2.[1] This method uses high-performance liquid chromatography (HPLC) with fluorescence detection (FD) following postcolumn derivatization to enhance method sensitivity and selectivity compared to UV absorbance detection. Dionex has published detailed methods that are consistent with EPA Method 531.2.[2-4]

For the detection of carbamate residues in food matrices, sample preparation is key for a sensitive determination. Several methods currently exist for the extraction from a variety of different food matrices, such as using a solid-phase extraction (SPE) column,[5,6] accelerated solvent extraction (ASE),[7,8] liquid-liquid extraction,[9] and cloud-point extraction (CPE).[10] The work shown here uses a two-step sample preparation method that first uses a salting-out extraction to extract the target analytes, then a dispersive solid-phase extraction (dSPE) to remove sugars, lipids, organic acids, sterols, proteins, and pigments. Similar methods are now available, such as AOAC 2007.01 Method[11] by the Association of Official Analytical Chemists (AOAC) in the United States, and the European equivalent, EN 15662.[12]

This study describes a faster yet sensitive method for the determination of carbamates (those specified in EPA Method 531.2) in rice, potato, and corn (maize). The sample preparation method uses a salting-out extraction step with acetonitrile, NaCl, and $MgSO_4$; and a dSPE cleanup step using primary secondary amine (PSA) resin to extract the carbamates and remove interfering substances from these crop samples. The separation is performed on an Acclaim® Carbamate column with detection by a FLD-3400RS fluorescence detector. The chromatography method is based on a reversed-phase separation of the carbamates with subsequent derivatization by *o*-Phthalaldehyde (OPA) followed by FD.

EQUIPMENT

Dionex UltiMate® 3000 HPLC system including:

HPG-3400A Pump with SRD-3400 Solvent Rack with Degasser

WPS-3000 Autosampler

TCC-3000 Thermostatted Column Compartment

FLD-3400 Fluorescence Detector

Chromeleon® Chromatography Data System (CDS) software Version 6.80 SR9

Pickering PCX 5200 Derivatization Instrument, Pickering Laboratories, Inc. CA, U.S.A.

Mettler Toledo AL204 Laboratory Balance, Mettler Toledo (Shanghai) Co., Shanghai, China

Anke TDL 80-2B centrifuge, Anting Scientific Instrumental Factory, Shanghai, China

Anke TDL 16B centrifuge, Anting Scientific Instrumental Factory, Shanghai, China

IKA MS1 Minishaker, IKA Works, Guangzhou, China

Note:

Prior to the use of HPLC and Pickering PCX 5200 Derivatization Instruments, use pure methanol to wash the system.

The pressure limit of the Pickering PCX 5200 Derivatization Instrument needs to be increased to 350 bar to prevent shutdown during derivatization.

For more details on using the Pickering PCX 5200 Derivatization Instrument, see AN 96.[3]

REAGENTS AND STANDARDS

Deionized water, Milli-Q® Gradient A10, Millipore Corporation

Methanol (CH_3OH), Fisher

Acetonitrile (CH_3CN), Fisher

Potassium dihydrogen citrate ($KC_6H_7O_7$), 98%, Fluka

Sodium thiosulfate ($Na_2S_2O_3$), 98%, Fluka

Sodium hydroxide solution, (NaOH), 50%, Fluka

Boric acid, 99.5%, Fluka

o-Phthalaldehyde (OPA, $C_8H_6O_2$), 99%, Pickering

β-Mercaptoethanol, 99%, SCRC, China

Magnesium sulfate ($MgSO_4$), analytical grade, SCRC, China

Sodium chloride (NaCl), analytical grade, SCRC, China

Primary secondary amine (PSA) Bonded Silica, Supelco

Activated carbon, SCRC, China

EPA Method 531.2 Carbamate Pesticide Calibration Mixture, Restek, 100 µg/mL (P/N 257974)

4-Bromo-3,5-dimethylphenyl-*N*-methylcarbamate standard (BDMC), Restek, 100 µg/mL (P/N 32274)

PREPARATION OF REAGENTS AND STANDARDS

Reagent Water

Use deionized water from a Milli-Q Gradient A10, 18 MΩ-cm resistivity or better.

Preserved Reagent Water

Dissolve 4.6 g of potassium dihydrogen citrate and 40 mg of $Na_2S_2O_3$ in a 50 mL beaker with reagent water, transfer this solution to a 500 mL volumetric flask, and bring to volume with reagent water. Prior to use, filter the solution through a 0.45 µm filter.

Stock Standard Carbamates Calibration Mixture

Pipet 10 µL of EPA 531.2 carbamate calibration mixture (100 µg/mL) into a 1 mL vial and add 990 µL of methanol. The concentration for each carbamate in the stock standard mixture is 1.0 µg/mL.

1-Naphthol is naturally fluorescent. It may assist in troubleshooting postcolumn chemistry issues because it will be the only peak present in a chromatogram when the postcolumn system is not functioning properly.

Stock Standard of BDMC (Surrogate Analyte, SUR)

Pipet 10 µL of BDMC (100 µg/mL) into a 1 mL vial and add 990 µL of methanol. The concentration of the stock standard solution is 1.0 µg/mL.

Working Standard Solutions for Calibration

Prepare five working standard solutions by adding the quantities of carbamate mixture stock standard solutions listed in Table 1 to 25 mL volumetric flasks. Add 50 µL of the stock standard solution of BDMC into each flask. Bring to volume with preserved reagent water.

Sodium Hydroxide Hydrolysis Reagent (Postcolumn Reagent 1)

Sodium hydroxide, 0.2%: Dilute 4 mL of 50% w/w NaOH solution to 1 L with reagent water. The concentration of the hydrolysis solution can dramatically affect the analyte response. Filter and degas with nitrogen just before use.[1]

OPA Reagent (Postcolumn Reagent 2) for Postcolumn Derivatization

To prepare boric acid buffer: dissolve 3.0 g of boric acid in approximately 800 mL of reagent water in a 1 L volumetric flask. Add 1.2 mL of a 50% (w/w) NaOH solution. Bring the volume to 1.0 L with reagent water. Filter and degas prior to preparation of postcolumn reagent 2.

Dissolve 100 mg of OPA in 5–10 mL of methanol and add to 1 L of boric acid buffer, then add 1 mL of 2-mercaptoethanol. This solution is postcolumn reagent 2.[1] To review the postcolumn chemistry and see a diagram of the postcolumn system configuration, see EPA Method 531.2.[1]

SAMPLES AND SAMPLE PREPARATION

Three kinds of crop samples—rice, potato, and dried and fresh corn—were purchased from a market located in Zhangjiang High-Tech Park, Shanghai.

Mill the samples to powder or mash using a food processor. Put an accurately weighed ~5 g of milled sample into a clean 15 mL centrifuge tube, then add 5.0 mL of acetonitrile and 10 µL of 1 µg/mL BDMC (SUR). After 1 min of vortexing, add ~2 g $MgSO_4$ and ~0.5 g NaCl, then vortex for 1 min. Centrifuge for 10 min (rpm 3000), pipet 1.00 mL of supernatant into a 1.5 mL centrifuge tube, then add ~100 mg $MgSO_4$ and ~50 mg PSA. After 1 min of vortexing followed by 5 min of centrifugation (10,000 rpm), pipet 100 µL of supernatant to a 1.5 mL vial, then add 900 µL of preserved reagent water. Vortex this sample for a few seconds prior to analysis by HPLC.

Table 1. Preparation of Calibration Curve Standards

Stock Standard of Carbamate Mixture (µg/mL)	Volume of Stock Standard of Carbamate Mixture (µL)	Stock Standard of BDMC (SUR) (µg/mL)	Volume of Stock Standard of BDMC (SUR) (µL)	Final Volume of Calibration Standard (mL)	Final Concentration of Carbamate Standard (µg/L)	Final Concentration of BDMC (SUR) (µg/L)
1.0	6.25	1.0	50.0	25	0.25	2.0
	12.5				0.50	
	25.0				1.00	
	50.0				2.00	
	200				8.00	

CONDITIONS

Guard Column:	Acclaim Carbamate, 3.0 × 10 mm, 3 µm, P/N 072929 (Use Holder V2, P/N 069580)
Analytical Column:	Acclaim Carbamate, 3.0 × 150 mm, 3 µm, P/N 072926
Column Temp.:	50 °C
Mobile Phase:	Methanol–water, in gradient (Table 2)
Flow Rate:	0.9 mL/min
Inj. Volume:	50 µL
Postcolumn Reagent 1:	0.2% NaOH, first reaction coil at 100 °C
Postcolumn Reagent 2:	OPA regent, second reaction coil at room temperature
Flow Rate of Reagent 1 and 2:	0.3 mL/min
Fluorescence:	Excitation: 330 nm Emission: 465 nm Data Collection Rate: 5 Response Time: 4 Sensitivity: 7 Lamp Mode: High Power PMT (Photomultiplier Tube): Pmt1 Filter Wheel: 280 nm

Table 2. Gradient for the Separation of Carbamates

Time (min)	Flow Rate (mL/min)	Methanol (%)	H_2O (%)
-4	0.9	14	86
0		14	86
2		20	80
8		40	60
13.6		70	30
16		70	30

RESULTS AND DISCUSSION

Sample Preparation

The sample preparation uses two steps. One extraction step that is based on partitioning using salting-out extraction involving equilibrium between aqueous and organic layers, and then a second step, dSPE, that involves further cleanup using various combinations of salts and porous sorbents.

Acetonitrile was used because it is a good solvent for carbamates, and NaCl was used for the salting-out extraction. To remove residual water, $MgSO_4$ was used. The authors chose PSA for the dSPE step to remove sugars and fatty acids, and also because it was reported to be a good choice for the determination of carbamate and organophosphorus pesticides in fruits and vegetables.[13]

Effect of Water in Extracts

The presence of water in the extract may affect the adsorptivity of PSA in the dSPE step, resulting in poor removal of coextracted interferences.[13] The experiments showed that the more residual water in the extract, the more coextracted interferences were left after dSPE with PSA. A simple way to resolve this problem is to add enough $MgSO_4$ to remove the residual water as completely as possible. Therefore, use two additions of $MgSO_4$ during sample preparation.

Determining the Amount of PSA for Sample Preparation

Enough PSA is required to absorb as much of the co-extracted interferences as possible. Therefore, the effects of PSA on the determination of crop samples spiked with carbamate standards were investigated. Experiments showed that there was no significant difference for peak area of each carbamate after dSPE using 50 and 100 mg of PSA, respectively. Thus, use 50 mg of PSA for the dSPE sample preparation step.

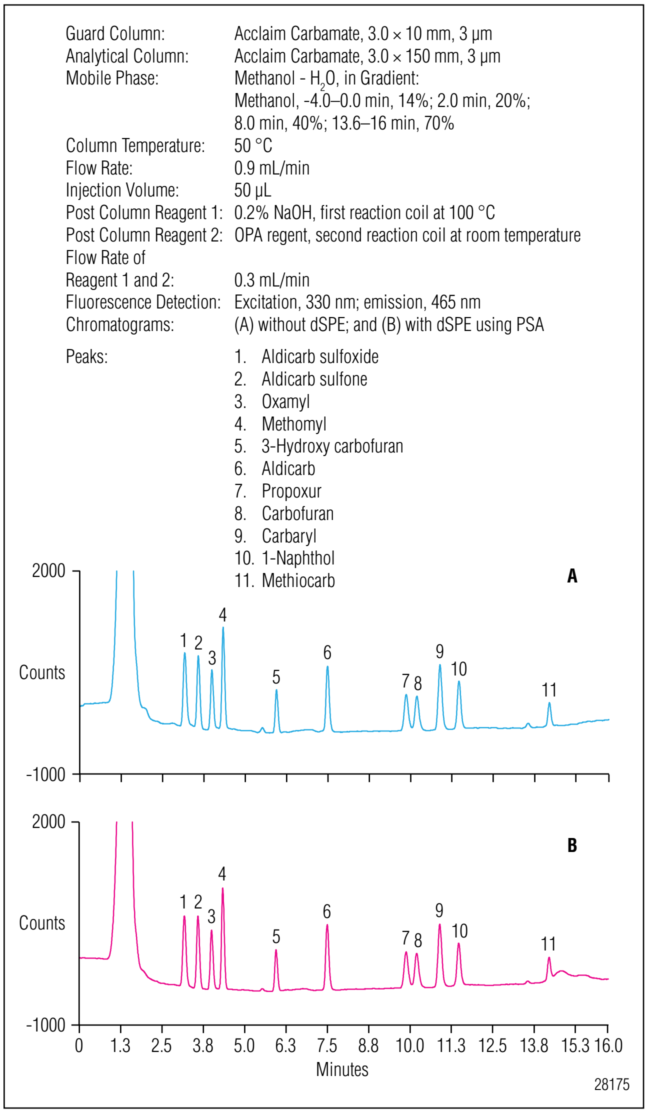

Figure 1. Chromatograms of extracts of carbamate standard-spiked rice samples (2 µg/L each) after acetonitrile and salt-out extractions (A) without and (B) with dSPE using PSA.

Choice of Sorbent

For some crop samples, such as rice, the coextracted substances were sufficiently removed after acetonitrile and salt-out extractions, and hence the dSPE cleanup step was not required. As shown in Figure 1, there is no significant difference between the chromatograms of the extracts of carbamate standard-spiked rice samples obtained by acetonitrile and salt-out extractions with and without using dSPE.

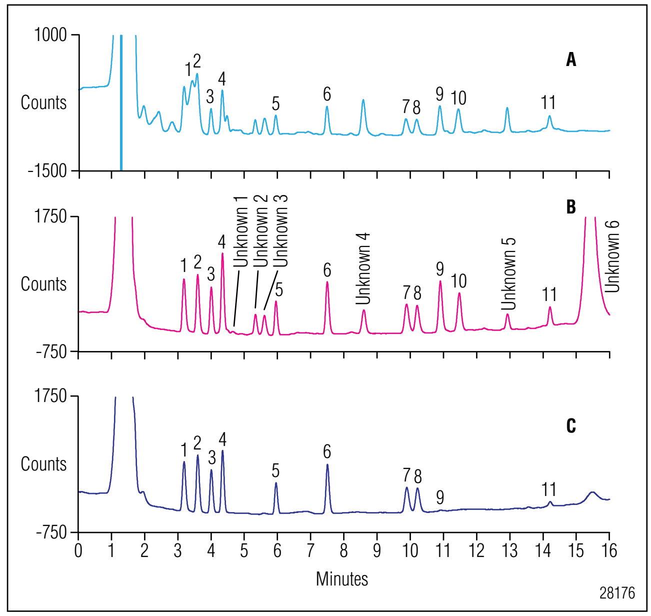

Figure 2. Chromatograms of extracts of carbamate standard-spiked corn samples (2 µg/L each) after acetonitrile and salt-out extractions (A) without and with dSPE using (B) PSA and (C) activated carbon. Other conditions are the same as in Figure 1.

For other crop samples, such as corn and potato, experiments demonstrated that after acetonitrile and salt-out extractions, coextracted interferences were still present in the extracts. Figure 2(A) shows a chromatogram of the extract of a carbamate standard-spiked corn sample obtained by acetonitrile and salt-out extractions. The coextracted interferences with retention times 3 ~ 5 min may interfere with the determination of aldicarb sulfoxide, aldicarb sulfone, oxamyl, and methomyl (peaks 1 to 4). Use dSPE with PSA or activated carbon to try to remove these interferences. As shown in Figure 2(B), after dSPE with PSA, good separation of these carbamates was observed. This can be attributed to the efficient removal of these interferences by PSA treatment. When using activated carbon instead of PSA, better removal of interferences was obtained. As shown in Figure 2(C), the coextracted substances with retention times 3 ~ 5 min were removed, and the unknown peaks in Figure 2(B) were removed as well; however, carbaryl (peak 9) and 1-naphthol (peak 10) were lost, and methiocarb (peak 11) was not fully recovered after dSPE with activated carbon, demonstrating that they were absorbed by activated carbon.

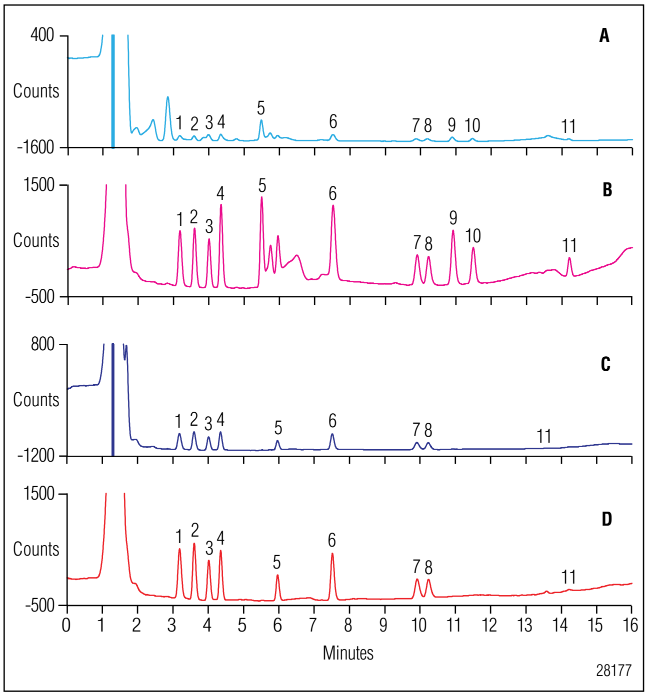

Figure 3. Chromatograms of extracts of carbamate standard-spiked potato samples (2 µg/L each) after acetonitrile and salt-out extractions (A) without and with dSPE using (B) PSA, (C) activated carbon, and (D) mixture of PSA and activated carbon (1:1, w/w). Other conditions are the same as in Figure 1.

Figure 3(A) shows a chromatogram of an extract of a potato sample obtained by acetonitrile and salt-out extractions and spiked with carbamate standards. It appears that large amounts of coextracted polar substances with retention times 1 ~ 2 min may interfere with the determination of carbamates (i.e., there are low responses for the carbamates). After dSPE with PSA, as shown in Figure 3(B), much higher responses were observed; however, the presence of the substances with retention times 5 ~ 7 min probably interfere with the determination of 3-hydroxycarbofuran (peak 5).

When using activated carbon, as shown in Figure 3(C), these coextracted interferences were removed, but the polar substances with retention times 1 ~ 2 min still remained and resulted in low responses of all carbamates.

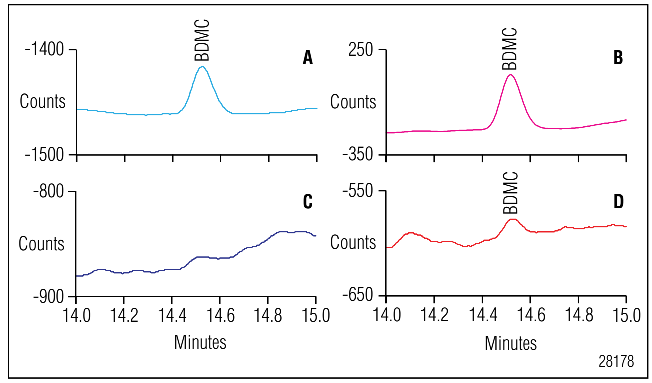

Figure 4. Chromatograms of BDMC (SUR) after acetonitrile and salt-out extractions (A) without and with dSPE using (B) PSA, (C) activated carbon, and (D) mix of PSA and activated carbon (1:1, w/w). Other conditions are the same as in Figure 1.

As with the corn samples, carbaryl (peak 9) and 1-naphthol (peak 10) were lost, and methiocarb (peak 11) exhibited poor recovery after dSPE with activated carbon. If the carbamates, except for those absorbed by activated carbon, were the target analytes, the mixture of PSA and activated carbon would be a better choice (Figure 3[D]).

The mixed sorbent treatment removed most of the interferences and the analyte responses were good. Unfortunately, the carbamate BDMC, whose addition was specified in EPA Method 531.2[1] as an SUR for quantification, was also absorbed completely by activated carbon (Figure 4). Therefore, only PSA was used for dSPE for all samples in this study (Table 3).

Effect of Sample Dilution

Experiments showed that the peak shapes of aldicarb sulfoxide, aldicarb sulfone, oxamyl, and methomyl (peak 1 ~ 4) were asymmetrical when the extract (extracted by acetonitrile) was injected directly, which can be attributed to the significant difference of solvent strength between the sample solvent (acetonitrile) and mobile phase (methanol–water). To acquire ideal peak shape, the extract was diluted with preserved reagent water (see the section on Preparation of Reagents and Standards). Accordingly, the injection volume was increased to 50 µL to maintain the detection limits.

Table 3. Peak Areas of Carbamates in Crop Samples Spiked with Carbamate Standards (2 µg/L for each), after dSPE Using Different Amounts of PSA

Carbamate	Peak Area (Counts*min)											
	Rice			Corn (Maize)						Potato		
				Dried			Fresh					
	PSA 50 mg	PSA 100 mg	Difference (%)	PSA 50 mg	PSA 100 mg	Difference (%)	PSA 50 mg	PSA 100 mg	Difference (%)	PSA 50 mg	PSA 100 mg	Difference (%)
Aldicarb sulfoxide	121.3	127.2	4.9	125.9	128.7	2.2	128.9	138.5	7.5	123.7	127.2	2.8
Aldicarb sulfone	115.0	117.3	2.0	117.3	116.7	-0.5	123.0	128.1	4.1	109.8	116.6	6.2
Oxamyl	87.30	98.21	12	100.3	97.11	-3.2	105.8	108.6	2.6	95.33	103.5	8.6
Methomyl	138.9	157.3	13	135.3	150.6	11	145.9	156.4	7.2	153.5	162.0	5.5
3-Hydroxy carbofuran	57.30	63.81	11	66.42	67.47	1.6	67.28	70.86	5.3	65.34	68.03	4.1
Aldicarb	106.9	118.7	11	120.0	123.7	3.1	123.1	133.7	8.6	111.1	126.8	14
Propoxur	70.28	75.01	6.7	78.08	74.39	4.7	77.58	84.89	9.4	73.14	79.67	8.9
Carbofuran	62.44	69.14	11	68.36	69.55	1.7	74.53	80.85	8.5	68.46	72.67	6.1
Carbaryl	113.5	126.5	11	124.4	123.1	1.0	129.6	137.7	6.2	121.3	134.0	10
1-Naphthol	86.46	61.96	-28	104.0	45.73	-56	137.6	120.4	-12.5	99.22	89.02	-10
Methiocarb	32.01	36.81	15	34.68	34.68	0.0	34.37	38.16	11	36.23	41.20	14

*Note: The difference was calculated by using the following equation: Difference = $(A_{100\text{ mg of PSA}} - A_{50\text{ mg of PSA}}) / A_{50\text{ mg of PSA}}$

$A_{100\text{ mg of PSA}}$ stands for the average of peak area (n = 7) of each carbamate obtained by using 100 mg of PSA in dSPE, and $A_{50\text{ mg of PSA}}$ stands for that obtained by using 50 mg of PSA.

Separation and Reproducibility

Figure 5 illustrates good separation of the carbamates listed in EPA Method 531.2 using the Acclaim Carbamate column, which is designed for the baseline separation of these carbamates. Resolution (R_s) for all peaks is ≥1.5.

Reproducibility of the separation method was estimated by making seven replicate injections of a calibration standard with a concentration of 8.0 µg/L for each carbamate. The RSD value of each carbamate was ≤0.07% for retention time and ≤3.0 % for peak area.

The reproducibility of the sample preparation method was evaluated by making injections of carbamate standard-spiked crop samples from five separate sample preparations. The value of relative standard deviation (RSD) of each carbamate for peak area was ≤7.0 %, demonstrating sufficient reproducibility for the sample preparation method.

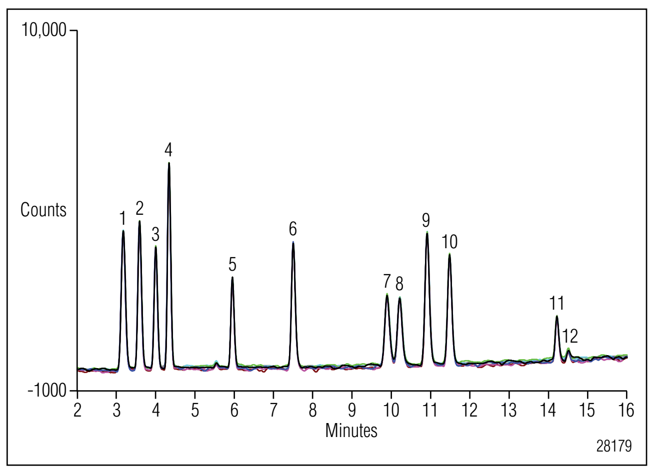

Figure 5. Overlays of chromatograms of seven consecutive injections of carbamate standards (8 µg/L each). Other conditions are the same as in Figure 1.

Table 4. Method Linearity Data and Method Detection Limits (MDL)				
Carbamate	Regression Equation	r^2	MDL	
			In Well-Prepared Sample Solution µg/L	Equivalent in the Original Sample µg/Kg
Aldicarb sulfoxide	$A = 2.4522\ c - 0.0994$	0.9971	0.08	0.8
Aldicarb sulfone	$A = 2.2352\ c - 0.0862$	0.9973	0.07	0.7
Oxamyl	$A = 1.7381\ c - 0.0523$	0.9972	0.06	0.6
Methomyl	$A = 2.7759\ c - 0.0117$	0.9975	0.09	0.9
3-Hydroxycarbofuran	$A = 1.2364\ c - 0.0627$	0.9965	0.09	0.9
Aldicarb	$A = 2.0374\ c + 0.0498$	0.9977	0.10	1.0
Propoxur	$A = 1.3220\ c + 0.0094$	0.9978	0.09	0.9
Carbofuran	$A = 1.2588\ c - 0.0315$	0.9973	0.04	0.4
Carbaryl	$A = 2.5121\ c - 0.0995$	0.9973	0.09	0.9
1-Naphthol	$A = 1.9981\ c + 0.0008$	0.9979	0.11	1.1
Methiocarb	$A = 0.6360\ c + 0.0069$	0.9976	0.09	0.9

Note: The single-sided Student's test method (at the 99% confidence limit) was used for determining MDL, where the standard deviation (SD) of the peak area of seven injections is multiplied by 3.50 to yield the MDL.

Linearity and Detection Limits

Calibration linearity for the determination of carbamates by this method was investigated by making seven replicate injections of serial standard solutions of carbamates at five different concentrations from 0.25 to 8 µg/L.

Detection limits of carbamates were calculated using the equation:

$$\text{Detection limit} = S t_{(n-1,\ 1-\alpha = 0.99)}$$

The symbol S represents Standard Deviation (SD) of replicate analyses, n represents number of replicates, and $t_{(n-1,\ 1-\alpha=0.99)}$ represents Student's value for the 99% confidence level with n – 1 degrees of freedom. Seven replicate injections of extract of rice sample spiked with 2 µg/L of carbamate standard mixture were used to determine the minimum detection limits. Table 4 summarizes the calibration and MDL data, showing excellent method linearity and sensitivity.

Rice, Potato, and Corn Sample Analysis

Figure 6 shows the chromatograms of rice, potato, and fresh corn samples; the related data is summarized in Table 5, showing satisfactory spike recovery for each carbamate. No detectable levels of carbamates were found in rice and potato samples.

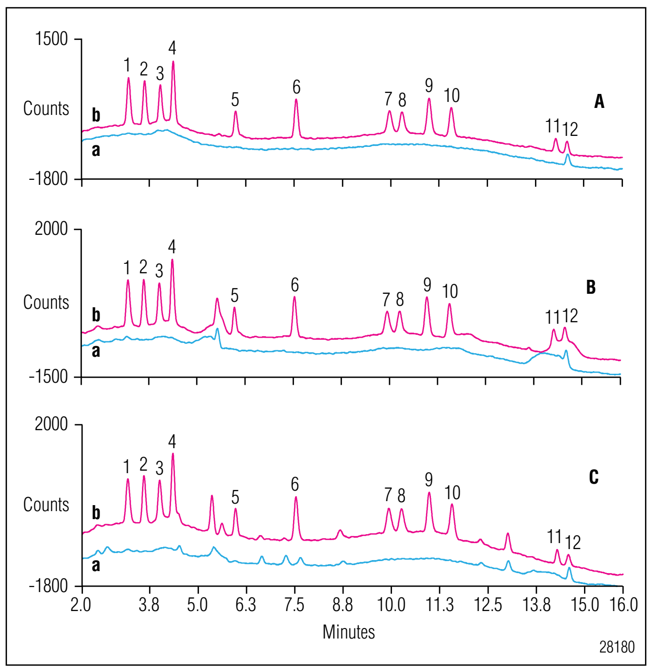

Figure 6. Overlays of chromatograms of crop samples spiked with 2.0 µg/L of BDMC (SUR, peak 12), and the same samples spiked with a carbamate standard mixture with 2.0 µg/L for each carbamate. Chromatograms, (a) crop samples, (b) carbamate standard-spiked crop samples; samples, (A) rice, (B) potato, and (C) fresh corn (maize). Other conditions are the same as in Figure 1.

Table 5. Crop Sample Analysis

Carbamate	Rice				Potato				Fresh Corn (Maize)				
	Detected (µg/L)	Added (µg/L)	Found (µg/L)	Recovery (%)	Detected (µg/L)	Added (µg/L)	Found (µg/L)	Recovery (%)	Detected (µg/L)		Added (µg/L)	Found (µg/L)	Recovery (%)
									By FD	Confirmed by MS			
Aldicarb sulfoxide	ND*	2.0	1.81	90	ND	2.0	1.87	94	0.10	ND	2.0	1.82	91
Aldicarb sulfone	ND		1.72	86	ND		1.81	90	ND	Detected		1.88	94
Oxamyl	ND		1.82	91	ND		1.99	99	ND			2.05	102
Methomyl	ND		1.78	89	ND		2.06	103	ND			1.77	89
3-Hydroxy-carbofuran	ND		1.68	84	ND		1.98	99	ND	Detected		1.74	87
Aldicarb	ND		1.85	93	ND		1.97	98	0.29	Detected		1.73	87
Propoxur	ND		1.87	93	ND		1.96	98	ND			2.06	103
Carbofuran	ND		1.87	93	ND		1.88	94	ND			1.89	95
Carbaryl	ND		1.68	84	ND		1.77	89	ND			1.85	92
1-Naphthol	ND		1.58	79	ND		1.85	93	ND			1.80	90
Methiocarb	ND		1.79	90	ND		1.97	94	ND			1.95	97

Note: ND = not detected

When the fresh corn sample was analyzed, two small peaks with retention times near that of aldicarb sulfoxide (peak 1) and aldicarb (peak 6) were found and labeled, as the two carbamates with concentrations were 0.10 and 0.29 µg/L, respectively (Figure 6[C]).

A complicated matrix may sometimes yield false positives for carbamates. An efficient way to determine if the peaks are carbamates is by using mass spectrometry (MS) detection. Based on the LC-MS method described in Reference 14, LC-MS results revealed that the peak with retention time near peak 1 was not aldicarb sulfoxide, and the one near peak 6 was aldicarb with estimated concentration of 0.30 µg/L, which is similar to the concentration determined using FD.

The EPA's August 2010 risk assessment indicates that aldicarb no longer meets the Agency's rigorous food safety standards and may pose unacceptable dietary risks, especially to infants and young children. The Agency is initiating action to terminate uses of aldicarb, and also plans to revoke aldicarb tolerances. Bayer CropScience plans to stop marketing aldicarb worldwide by 2014.[15] Therefore, simpler, efficient, and sensitive methods for the determination of aldicarb in soil, crops, environmental water, and food products are desired. Additionally, although aldicarb sulfone (peak 2) and 3-hydroxycarbofuran (peak 5) were not found in the fresh corn sample using FD, they were detected using LC-MS.

CONCLUSION

This testing describes an effective method for the determination of carbamates in rice, potato, and corn on an UltiMate 3000 HPLC system with an Acclaim Carbamate column and FD following postcolumn derivatization. Acetonitrile extraction and dSPE cleanup using a PSA was used to isolate the carbamates and remove the interference substances from the crop samples prior to HPLC analysis. The prepared samples yielded accurate results using the method described here.

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Column Selection Guide

Silica Columns

			Acclaim 120 C18	Acclaim 120 C8	Acclaim 300 C18	Acclaim Polar Advantage (PA)	Acclaim Polar Advantage II (PA2)	Acclaim Phenyl-1	Acclaim Trinity P1	Acclaim Mixed-Mode WAX-1	Acclaim Mixed-Mode WCX-1	Acclaim Mixed-Mode HILIC-1	Acclaim HILIC-10	Acclaim Organic Acid	Acclaim Surfactant	Acclaim Explosives E1	Acclaim Explosives E2	Acclaim Carbamate	Example Applications
			Reversed-Phase (RP)						Mixed-Mode			HILIC		Application-Specific					
General Applications	Neutral Molecules	High hydrophobicity	√	√	√	√	√	√	√	√	√	√	√						Fat-soluble vitamins, PAHs, glycerides
		Intermediate hydrophobicity	√	√	√	√	√	√	√	√	√	√							Steroids, phthalates, phenolics
		Low hydrophobicity	√			√	√					√	√						Acetaminophen, urea, polyethylene glycols
	Anionic Molecules	High hydrophobicity	√	√	√	√	√	√	√	√	√	√							NSAIDs, phospholipids
		Intermediate hydrophobicity	√	√	√	√	√	√	√	√		√							Asprin, alkyl acids, aromatic acids
		Low hydrophobicity				√			√	√		√	√						Small organic acids, e.g. acetic acids
	Cationic Molecules	High hydrophobicity	√	√	√	√	√	√		√	√	√							Antidepressants
		Intermediate hydrophobicity	√	√	√	√	√	√	√		√	√							Beta blockers, benzidines, alkaloids
		Low hydrophobicity	√			√			√		√	√	√						Antacids, pseudoephedrine, amino sugars
	Amphoteric/ Zwitterionic Molecules	High hydrophobicity	√	√	√	√	√	√	√	√	√	√							Phospholipids
		Intermediate hydrophobicity	√	√	√	√	√	√			√								Amphoteric surfactants, peptides
		Low hydrophobicity				√	√		√	√	√	√	√						Amino acids, aspartame, small peptides
	Mixtures of Neutral, Anionic, Cationic Molecules	Neutrals and acids	√			√	√		√	√									Artificial sweeteners
		Neutrals and bases	√			√	√		√		√								Cough syrup
		Acids and bases				√			√										Drug active ingredient with counterion
		Neutrals, acids, and bases				√			√										Combination pain relievers
Specific Applications	Surfactants	Anionic	√	√	√	√	√								√				SDS, LAS, laureth sulfates
		Cationic													√				Quats, benzylalkonium in medicines
		Nonionic	√	√	√	√	√					√			√				Triton X-100 in washing tank
		Amphoteric	√	√	√	√	√								√				Cocoamidopropyl betaine
		Hydrotropes													√				Xylenesulfonates in handsoap
		Surfactant blends													√				Noionic and anionic surfactants
	Organic Acids	Hydrophobic							√	√				√					Aromatic acids, fatty acids
		Hydrophilic							√	√				√					Organic acids in soft drinks, pharmaceuticals
	Environmental Contaminants	Explosives														√	√		U.S. EPA Method 8330, 8330B
		Carbonyl compounds															√		U.S. EPA 1667, 555, OT-11; CA CARB 1004
		Phenols	√			√													Compounds regulated by U.S. EPA 604
		Chlorinated/Phenoxy acids				√													U.S. EPA Method 555
		Triazines	√			√													Compounds regulated by U.S. EPA 619
		Nitrosamines				√													Compounds regulated by U.S. EPA 8270
		Benzidines	√			√													U.S. EPA Method 605
		Perfluorinated acids				√													Dionex TN73
		Microcystins	√																ISO 20179
		Isocyanates					√					√							U.S. OSHA Methods 42, 47
		Carbamate insecticides																√	U.S. EPA Method 531.2
	Vitamins	Water-soluble vitamins				√	√		√										Vitamins in dietary supplements
		Fat-soluble vitamins	√	√	√	√	√	√		√									Vitamin pills
	Pharmaceutical Counterions	Anions							√	√									Inorgaic anions and organic acids in drugs
		Cations							√		√								Inorgaic cations and organic bases in drugs
		Mixture of Anions and Cations							√										Screening of pharmaceutical counterions
		API and counterions							√										Naproxen Na^+ salt, metformin Cl^- salt, etc.

Polymer Columns

		IonPac AS23	IonPac AS22	IonPac AS22-Fast	IonPac AS14/A	IonPac AS12A	IonPac AS9/HC/SC	IonPac AS4A/SC	IonSwift MAX-100	IonPac AS24	IonPac AS21	IonPac AS20	IonPac AS19	IonPac AS18	IonPac AS18-Fast	IonPac AS17-C	IonPac AS16	IonPac AS15	IonPac AS11(-HC)	IonPac AS10	IonPac AS7	IonPac AS5	IonPac Fast Anion IIIA	OmniPac PAX-100	OmniPac PAX-500
ANIONS	Inorganic Anions	√	√	√	√	√	√	√	√	√		√	√	√	√	√		√	√	√					
	Oxyhalides	√				√	√			√			√												
	Bromate	√					√			√			√												
	Perchlorate										√	√					√								
	Organic Acids								√							√		√	√	√					
	Phosphoric/Citric Acids																						√		
	Poly/High-Valence Anions								√			√					√		√		√	√			
	Hydrophobic Anions								√			√					√		√						
	Hydrophobic/Halogenated Anions								√			√							√					√	
	Anionic Neutral Molecules									√	√	√	√												√
CATIONS	Inorganic Cations																								
	Sodium/Ammonium																								
	Amines/Polyvalent Amines																								
	Aliphatic/Aromatic Amines																								
	Alkanol/Ethhanolamines																								
	Biogenic Amines																								
	Transition/Lanthanide Metals																								
	Hydrophobic Cations																								
	Cationic Neutral Molecules																								
BIO-MOLECULES	Amino Acids																								
	Phosphorylated Amino Acids																								
	Amino Sugars																								
	Oligosccharides																								
	Mono-/Di-Saccharides																								
	Glycoproteins																								
	Alditols/Aldoses mono/di Saccharides																								
	ds Nucleic Acids																								
	Single-Stranded Oligonucleotides																								
	Peptides																								
	Proteins																								
	Metal-binding Proteins																								
	Monoclonal antibodies																								
ORGANIC MOLECULES	Aliphatic Organic Acids																								
	Alcohols																								
	Borate																								
	Large Molecules, Anions																								
	Small Molecules																								
	Small Molecules/LC-MS																								
	Polar/Non-Polar Small Molecules																								
	Hydrophobic/Aliphatic Organic Acids																								
	Surfactant Formulations																								
	Explosives/EPA 8330																								
MODE	Anion Exchange / Carbonate	√	√	√	√	√	√	√																	
	Anion Exchange / Hydroxide								√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		
	Cation Exchange																								
	Multi-Mode																							√	√
	Affinity																								
	Ion Exclusion																								
	Reversed Phase																								
	Anion Exchange/Other																								

	IonPac CS18	IonPac CS17	IonPac CS16	IonPac CS15	IonPac CS14	IonPac CS12A	IonPac CS11	IonPac CS10	IonPac CS5A	OmniPac PCX-100	OmniPac PCX-500	AminoPac PA10	AminoPac PA1	CarboPac PA200	CarboPac PA100	CarboPac PA20	CarboPac PA10	CarboPac PA1	CarboPac MA1	DNAPac PA200	DNAPac PA100	ProPac WAX/SAX	ProPac WCX/SCX	ProPac IMAC	ProPac HIC	ProPac PA1	ProSwift	IonPac ICE-AS6	IonPac ICE-AS1	IonPac ICE-Borate	IonPac NS1
Inorganic Anions																															
Oxyhalides																															
Bromate																															
Perchlorate																															
Organic Acids																															
Phosphoric/Citric Acids																															
Poly/High-Valence Anions																															
Hydrophobic Anions																															
Hydrophobic/Halogenated Anions																															
Anionic Neutral Molecules																															
Inorganic Cations	√	√	√	√	√	√	√	√																							
Sodium/Ammonium			√	√		√																									
Amines/Polyvalent Amines	√	√																													
Aliphatic/Aromatic Amines		√			√	√																									
Alkanol/Ethhanolamines	√	√		√																											
Biogenic Amines	√	√																													
Transition/Lanthanide Metals									√																						
Hydrophobic Cations	√	√			√					√																					
Cationic Neutral Molecules											√																				
Amino Acids												√	√																		
Phosphorylated Amino Acids												√																			
Amino Sugars												√		√	√	√	√														
Oligosccharides														√	√			√	√												
Mono-/Di-Saccharides																√	√	√	√												
Glycoproteins																					√	√	√				√				
Alditols/Aldoses mono/di Saccharides																√	√	√	√												
ds Nucleic Acids																				√	√										
Single-Stranded Oligonucleotides																				√	√						√				
Peptides																								√	√	√	√				
Proteins																						√	√	√	√	√	√				
Metal-binding Proteins																								√							
Monoclonal antibodies														√	√			√		√	√	√	√	√	√	√	√				
Aliphatic Organic Acids																												√	√		√
Alcohols																												√	√		
Borate																														√	
Large Molecules, Anions																															√
Small Molecules																															
Small Molecules/LC-MS																															
Polar/Non-Polar Small Molecules																															
Hydrophobic/Aliphatic Organic Acids																															
Surfactant Formulations																															√
Explosives/EPA 8330																															
Anion Exchange / Carbonate																															
Anion Exchange / Hydroxide														√	√	√	√	√	√												
Cation Exchange	√	√	√	√	√	√	√	√	√														√			√	√				
Multi-Mode										√	√																				
Affinity																								√							
Ion Exclusion																												√	√	√	
Reversed Phase																									√	√	√				√
Anion Exchange/Other												√	√							√	√	√				√	√				

Column Specifications

IC Anion Columns

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
IonPac AS24	2 × 250 mm	Hydroxide	Recommended column for haloacetic acids prior to MS or MS/MS detection	7 µm	55%	-	-	140 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS23	2 × 250 mm 4 × 250 mm	Carbonate	Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water.	6 µm	55%	-	-	80 µeq 320 µeq	Alkyl quaternary ammonium	Ultralow
IonPac AS22	2 × 250 mm 4 × 250 mm	Carbonate	Recommended column for fast analysis of common inorganic anions.	6.5 µm	55%	-	-	52.5 µeq 210 µeq	Alkyl quaternary ammonium	Ultralow
IonPac AS21	2 × 250 mm	Hydroxide	Recommended column for trace perchlorate prior to MS or MS/MS detection	7.0 µm	55%	-	-	45 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS20	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for trace perchlorate prior to suppressed conductivity detection.	7.5 µm	55%	-	-	77.5 µeq 310 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS19	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water.	7.5 µm	55%	-	-	60 µeq 350 µeq	Alkanol quaternary ammonium	Low
IonPac AS18	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for the analysis of common inorganic anions.	7.5 µm	55%	65 nm	8%	75 µeq 285 µeq	Alkanol quaternary ammonium	Low
IonPac AS17-C	2 × 250 mm 4 × 250 mm	Hydroxide	Trace anions in HPW matrices. Carboxylated resin, no sulfate blank. Low capacity for fast analysis of common inorganic anions using gradient elution with the Eluent Generator.	10.5 µm	55%	75 nm	6%	7.5 µeq 30 µeq	Alkanol quaternary ammonium	Low
IonPac AS16	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for hydrophobic anions including iodide, thiocyanate, thiosulfate, and perchlorate. Polyvalent anions including: polyphosphates and polycarboxylates	9 µm	55%	80 nm	1%	42.5 µeq 170 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS15	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for trace analysis of inorganic anions and low molecular weight organic acids in high purity water matrices.	9 µm	55%	-	-	56.25 µeq 225 µeq	Alkanol quaternary ammonium	Medium-High
IonPac AS15-5mm	3 × 150 mm	Hydroxide	Fast run, high capacity for trace analysis of inorganic anions and low molecular weight organic acids in high purity water matrices.	5 µm	55%	-	-	70 µeq	Alkanol quaternary ammonium	Medium-High
IonPac AS14A- 5 µm	3 × 150 mm	Carbonate	Recommended column for fast analysis of common inorganic anions.	5 µm	55%	-	-	40 ueq	Alkyl quaternary ammonium	Medium
IonPac AS14A	4 × 250 mm	Carbonate	For analysis of common inorganic anions.	7 µm	55%	-	-	120 µeq	Alkyl quaternary ammonium	Medium
IonPac AS14	2 × 250 mm 4 × 250 mm	Carbonate	Moderate capacity for fast analysis of common inorganic anions.	9 µm	55%	-	-	16 µeq 65 µeq	Alkyl quaternary ammonium	Medium-High

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
IonPac AS12A	2 × 200 mm 4 × 200 mm	Carbonate	Moderate capacity for analysis of inorganic anions and oxyhalides. Trace chloride and sulfate in high carbonate matrices.	9 µm	55%	140 nm	0.20%	13 µeq 52 µeq	Alkyl quaternary ammonium	Medium
IonPac AS11-HC	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for the determination of organic acids and inorganic anions in uncharacterized samples.	9 µm	55%	70 nm	6%	72.5 µeq 290 µeq	Alkanol quaternary ammonium	Medium-Low
IonPac AS11	2 × 250 mm 4 × 250 mm	Hydroxide	Low capacity for fast profiling of organic acids and inorganic anions in well-characterized samples.	13 µm	55%	85 nm	6%	11 µeq 45 µeq	Alkanol quaternary ammonium	Very Low
IonPac AS10	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for the analysis of inorganic anions and organic acids in high nitrate samples.	8.5 µm	55%	65 nm	5%	42.5 µeq 170 µeq	Alkyl quaternary ammonium	Low
IonPac AS9-HC	2 × 250 mm 4 × 250 mm	Carbonate	High-capacity column for inorganic anions and oxyhalides. Trace bromate in drinking water.	9 µm	55%	90 nm	18%	48 µeq 190 µeq	Alkyl quaternary ammonium	Medium-Low
IonPac AS9-SC	4 × 250 mm	Carbonate	Low capacity for fast analysis of inorganic anions and oxyhalides. Specified column in US EPA Method 300.0 (B).	13 µm	55%	110 nm	20%	30-35 µeq	Alkyl quaternary ammonium	Medium-Low
IonPac AS4A-SC	2 × 250 mm 4 × 250 mm	Carbonate	Low capacity for fast analysis of common inorganic anions. Specified column in U.S. EPA Method 300.0 (A).	13 µm	55%	160 nm	0.50%	5 µeq 20 µeq	Alkanol quaternary ammonium	Medium-Low
IonPac Fast Anion IIIA	3 × 250 mm	Hydroxide	Recommended column for phosphoric and citric acids in cola soft drinks.	7.5 µm	55%	-	-	55 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS7	4 × 250 mm	Specialty Eluents	Polyvalent anions including chelating agents, polyphosphates and polyphosphonates. Cyanide, sulfide, hexavalent chromium, and arsenic speciation.	10 µm	2%	530 nm	5%	100 µeq	Alkyl quaternary ammonium	Medium-High
IonPac AS5A	4 × 150 mm	Hydroxide	Low capacity for fast profiling of organic acids and inorganic anions in well-characterized samples.	5 µm	2%	60 nm	4%	35 µeq	Alkanol quaternary ammonium	Low
IonPac AS5	4 × 250 mm	Hydroxide	Metal-EDTA complexes, metal-cyanide complexes, and oxyanions.	15 µm	2%	120 nm	1%	20 µeq	Alkanol quaternary ammonium	Low

IC Cation Columns

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
IonPac CS18	2 × 250 mm	MSA	Recommended column for polar amines (alkanolamines and methylamines) and moderately hydrophobic and polyvalent amines (biogenic and diamines). Nonsuppressed mode when extended calibration linearity for ammonium and weak bases is required	6 µm	55%	-	-	0.29 µeq	Carboxylic acid	Medium
IonPac CS17	2 × 250 mm 4 × 250 mm	MSA	Recommended column for hydrophobic and polyvalent amines (biogenic amines and diamines)	7 µm	55%	-	-	0.363 µeq 1.45 µeq	Carboxylic acid	Very Low
IonPac CS16	3 × 250 mm 5 × 250 mm	MSA	Recommended column for disparate concentration ratios of adjacent-eluting cations such as sodium and ammonium. Can be used for alkylamines and alkanolamines.	5 µm	55%	-	-	3.0 µeq 8.4 µeq	Carboxylic acid	Medium
IonPac CS15	2 × 250 mm 4 × 250 mm	MSA	Disparate concentration ratios of ammonium and sodium. Trace ethanolamine in high-ammonium or high- potassium concentrations. Alkanolamines.	8.5 µm	55%	-	-	0.7 µeq 2.8 µeq	Carboxylic acid/ phosphonic acid/ crown ether	Medium
IonPac CS14	2 × 250 mm 4 × 250 mm	MSA	Aliphatic amines, aromatic amines, and polyamines plus mono- and divalent cations.	8.5 µm	55%	-	-	0.325 µeq 1.3 µeq	Carboxylic acid	Low
IonPac CS12A-MS	2 × 100 mm	MSA	IC-MS screening column for fast elution and low flow rates required for interfacing with IC-MS	8.5 µm	55%	-	-	0.28 µeq	Carboxylic acid/ phosphonic acid	Medium
IonPac CS12A-5 µm	3 × 150 mm	MSA	Recommended column for high efficiency and fast analysis (3 min) of mono- and divalent cations.	5 µm	55%	-	-	0.94 µeq	Carboxylic acid/ phosphonic acid	Medium
IonPac CS12A	2 × 250 mm 4 × 250 mm	MSA	Recommended column for the separation of mono- and divalent cations. Manganese morpholine, alkylamines, and aromatic amines.	8.5 µm	55%	-	-	0.7 µeq 2.8 µeq	Carboxylic acid/ phosphonic acid	Medium
IonPac CS11	2 × 250 mm	HCl + DAP	Separation of mono- and divalent cations. Ethanolamines if divalent cations are not present.	8 µm	55%	200 nm	5%	0.035 µeq	Sulfonic acid	Medium
IonPac CS10	4 × 250 mm	HCl + DAP	Separation of mono- and divalent cations.	8.5 µm	55%	200 nm	5%	0.08 µeq	Sulfonic acid	Medium
IonPac CS5A	2 × 250 mm 4 × 250 mm	Pyridine dicarboxylic acid	Recommended column for transition and lanthanide metals analysis. Aluminum analysis.	9 µm	55%	140 nm 75 nm	10% 20%	0.02 µeq/ 0.005 µeq 0.04 µeq/ 0.01 µeq	Sulfonic acid/ alkanol quaternary ammonium	-

Ion-Exclusion Columns

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydro-phobicity
IonPac ICE-AS1	4 × 250 mm 9 × 250 mm	Heptafluorobutyric acid	Organic acids in high ionic strength matrices. Fast separation of organic acids.	7.5 µm	8%	-	-	5.3 µeq 27 µeq	Sulfonic acid	Ultra Low
IonPac ICE-AS6	9 × 250 mm	Heptafluorobutyric acid	Organic acids in complex or high ionic strength matrices.	8 µm	8%	-	-	27 µeq	Sulfonic and carboxylic acid	Moderate
IonPac ICE-Borate	9 × 250 mm	MSA/ Mannitol	Trace concentrations of borate	7.5 µm	8%	-	-	27 µeq	Sulfonic acid	Ultra Low

Acclaim General and Specialty Columns

Column	Bonded Phase	USP Type	Endcapped	Substrate	Particle Shape	Particle Size	Metal Impurity (ppm) Na, Fe, AL	Average Pore Diameter	Surface Area (m^2/g)	Total Carbon Content
Mixed-Mode WAX	Proprietary alkyl amine	na	Proprietary			5 µm		120 Å	300	na
Mixed-Mode HILIC	Proprietary alkyl diol	na	Proprietary			5 µm		120 Å	300	na
Mixed-Mode WCX	Proprietary alkyl carboxyl	na	Proprietary			5 µm		120 Å	300	na
Organic Acid (OA)	Proprietary	na	Yes			5 µm		120 Å	300	17%
Surfactant and Explosives E1/2	Proprietary	na	Yes			5 µm		120 Å	300	na
120 C18	C18	L1	Yes			2, 3 and 5 µm		120 Å	300	18%
120 C8	C8	L7	Yes	Ultrapure silica	Spherical	3 and 5 µm	<10 ppm	120 Å	300	11%
300 C18	C18	L1	Yes			3 µm		300 Å	100	7%
Polar Advantage	Sulfamido C16	na	Yes			3 and 5 µm		120 Å	300	17%
Polar Advantage II	Amide C18	na	Yes			2, 3 and 5 µm		120 Å	300	17%
HILIC	Proprietary hydrophilic		Yes			3 µm		120 Å	300	
Phenyl-1	Proprietary alkyl phenyl		Yes			3 µm		120 Å	300	
Carbamate	Proprietary alkyl group		Yes			3 and 5 µm		120 Å	300	
Trinity			Yes					120 Å	300	

Bio Columns

Protein

Column	Phase	Target Applications	Base Matrix Material	Substrate Crosslinking	Capacity	Recommended Flow Rate	Solvent Compatibility	Maximum Backpressure	pH Range
MAbPac SEC-1									
MAbPac SCX-10									
ProPac WCX-10	Weak Cation Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10-µm diameter nonporous substrate to which is grafted a polymer chain bearing carboxylate groups.	55%	6 mg/ mL lysozyme	0.2–2 mL/min	80% ACN, acetone. Incompatable with alcohols and MeOH	3000 psi (21 MPa)	2–12.0
ProPac SCX-10	Strong Cation Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter nonporous substrate to which is grafted a polymer chain bearing sulfonate groups.	55%	3 mg/ mL lysozyme	0.2–2.0 mL/min	80% ACN, acetone, MeOH	3000 psi (21 MPa)	2–12.0
ProPac SCX-20									
ProPac WAX-10	Weak Anion Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter non-porous substrate to which is grafted a polymer chain bearing tertiary amine groups.	55%	5 mg/ mL BSA/ mL	0.2–2.0 mL/min	80% ACN, acetone, MeOH,	3000 psi (21 MPa)	2–12.0
ProPac SAX-10	Strong Anion Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter non-porous substrate with grafted polymer chain bearing quaternary ammonium groups.	55%	15 mg/ mL BSA	0.2–2.0 mL/min	80% ACN, acetone, MeOH	3000 psi (21 MPa)	2–12.0
ProSwift RP-1S	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith Standard permeability	5.5 mg/mL Insulin	2–4 mL/min	Most common organic solvents	2800 psi (19.2 Mpa)	1–14
ProSwift RP-2H	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith High permeability	1.0 mg/mL Lysozyme	1–10 mL/min	Most common organic solvents	2800 psi (19.3 Mpa)	1–14
ProSwift RP-4H									
ProSwift RP-3U	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith Ultrahigh permeability	0.5 mg/mL Lysozyme	1– 16 mL/min	Most common organic solvents	2800 psi (19.3 Mpa)	1–14
ProSwift SAX-1S	Strong Anion Exchange	Fast protein separation with good resolution using Anion Exchange	Monolith; polymethacrylate with quaternary amine functional group	Monolith Standard permeability	18 mg/mL BSA	0.5–1.5 (4.6 mm), 0.05–.25 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProSwift SCX-1S	Strong Cation Exchange	Fast protein separation with good resolution using Cation Exchange	Monolith; polymethacrylate with sulfonic acid fuctional group	Monolith Standard permeability	30 mg/mL Lysozyme	0.5–1.5 mL/min (4.6 mm)	Most common organic solvents	1000 psi (4.6 mm)	2–12.0

Column	Phase	Target Applications	Base Matrix Material	Substrate Crosslinking	Capacity	Recommended Flow Rate	Solvent Compatibility	Maximum Backpressure	pH Range
ProSwift WAX-1S	Weak Anion Exchange	Fast protein separation with good resolution using Anion Exchange	Monolith; polymethacrylate with tertiary amine (DEAE) functional group	Monolith Standard permeability	18 mg/mL BSA	0.5–1.5 mL/min (4.6 mm), 0.05–.25 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProSwift WCX-1S	Weak Cation Exchange	Fast protein separation with good resolution using Cation Exchange	Monolith; polymethacrylate with carboxylic acid (CM) functional group	Monolith Standard permeability	23 mg/mL Lysozyme	0.5–1.5 mL/min (4.6 mm), 0.05–.20 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProPac IMAC-10	Immobilized Metal Affinity	High resolution separation of certain metal-binding proteins and peptides	10 µm diameter non-porous polystyrene divinylbenzene substrate with poly (IDA) grafts.	55%	>60 mg lysozyme/ mL gel (4 x 250 mm)	1.0 mL/min	EtOH, urea, NaCl, non- ionic detergents, glycerol, acetic acid, guanidine HCl	3000 psi (21MPa)	2–12
ProSwift ConA-1S									
ProPac HIC-10	Reversed-Phase	Protein separation using hydrophobic interaction with salt gradient elution	Spherical 5 µm, ultrapure silica, 300 A, surface area 100 m^2/ g,	n/a	340 mg lysozyme per 7.8 x 75 mm column	1.0 mL/ min	2M Ammonium sulfate/ phosphate salts, organic solvent for cleanup	4,000 psi	2.5–7.5

Carbohydrate

Column	Target Applications	Base Matrix Material	Substrate Crosslinking	Latex Crosslinking	Capacity	Recommended Eluents	Recommended Flow Rate	Solvent Compatibility	Maximum Backpressure	pH Range
CarboPac MA1	Reduced mono- and disaccharide analysis.	7.5 µm diameter macroporous substrate fully functionalized with an alkyl quaternary ammonium group	15%	No latex	1450 µeq (4 × 250 mm)	Hydroxide	0.4 mL/min	0%	2000 psi (14 MPa)	0–14
CarboPac PA1	General purpose mono-, di-, and oligosaccharide analysis	10 µm diameter nonporous substrate agglomerted with a 500 nm MicroBead quaternary ammonium functionalized latex	2%	5%	100 µeq (4 × 250 mm)	Hydroxide, acetate/ hydroxide	1.0 mL/min	0–5%	4000 psi (28 MPa)	0–14
CarboPac PA10	Monosaccharide compositonal anaylysis	10 µm diameter nonporous substrate agglomerated with a 460 nm MicroBead di-functionalized latex	55%	5%	100 µeq (4 × 250 mm)	Hydroxide, acetate/ hydroxide	1.0 mL/min	0–90%	3500 psi (24.5 MPa)	0–14
CarboPac PA20	Fast mono-, and disaccharide analysis	6.5 µm diameter nonporous substrate agglomerated with a 130 nm MicroBead quaternary ammonium functionalized latex	55%	5%	65 µeq (3 × 150 mm)	Hydroxide, acetate/ hydroxide	0.5 mL/min	0–100%	3000 psi (21 MPa)	0–14
CarboPac PA100	Oligosaccharide mapping and analysis	8.5 µm diameter nonporous substrate agglomerated with a 275 nm MicroBead di-functionalized latex	55%	6%	90 µeq (4 × 250 mm)	Hydroxide, acetate/ hydroxide	1.0 mL/min	0–90%	4000 psi (28 MPa)	0–14
CarboPac PA200	High resolution oligosaccharide mapping and analysis	5.5 µm diameter nonporous substrate agglomerated with a 43 nm MicroBead quaternary ammonium functionalized latex	55%	6%	35 µeq (3 × 250 mm)	Hydroxide, acetate/ hydroxide	0.5 mL/min	0–100%	4000 psi (28 MPa)	0–14

DNA

Column	Target Applications	Base Matrix Material	Substrate Crosslinking	Latex Crosslinking	Capacity	Recommended Eluents	Recommended Flow Rate	Solvent Compatibility	Max. Backpressure	pH Range
DNAPac PA100	Single stranded DNA or RNA oligonucleotides, restriction fragments, glycoprotein isoforms.	13-µm diameter nonporous substrate agglomerated with a 100-nm MicroBead alkyl quaternary ammonium functionalized latex.	55%	5%	40 µeq	Chloride, acetate, bromide, perchlorate: in lithium sodium or ammonium forms	1.5 mL/min	0–100%	4000psi (28MPa)	2–12.5
DNAPac PA200	High resolution single stranded DNA or RNA oligonucleotides, restriction fragments, glycoprotein isoforms.	8-µm diameter nonporous substrate agglomerated with a 130-nm MicroBead alkyl quaternary ammonium functionalized latex.	55%	5%	40 µeq	Chloride, acetate, bromide, perchlorate: in lithium sodium or ammonium forms	1.2 mL/min	0–100%	4000psi (28MPa)	2–12.5
DNASwift										

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