



- Veterinary Drugs/Antibiotics
- Pesticides

# Food Safety Applications Notebook

## Agricultural Chemical Contaminants

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# Index of Analytes and Application Notes

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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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Dionex Products are now a part of the Thermo Scientific brand, creating exciting new possibilities for scientific analysis. Now, leading capabilities in liquid chromatography (LC), ion chromatography (IC), and sample preparation are together in one portfolio with those in mass spectrometry (MS). Combining Dionex's leadership in chromatography with Thermo Scientific's leadership position in mass spec, a new range of powerful and simplified workflow solutions now becomes possible.

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### *Best-in-class HPLC systems for all your chromatography needs*

Thermo Scientific Dionex UltiMate 3000 UHPLC<sup>+</sup> Systems provide excellent chromatographic performance while maintaining easy, reliable operation. The basic and standard analytical systems offer ultra HPLC (UHPLC) compatibility across all modules, ensuring maximum performance for all users and all laboratories. Covering flow rates from 20 nL/min to 10 mL/min with an industry-leading range of pumping, sampling, and detection modules, UltiMate™ 3000 UHPLC<sup>+</sup> Systems provide solutions from nano to semipreparative, from conventional LC to UHPLC.

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*Rapid Separation LC Systems:* The extended flow-pressure footprint of the RSLC system provides the performance for ultrafast high-resolution and conventional LC applications.

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- Thermo Scientific Dionex ICS-5000: The world's first capillary IC system
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- Dionex ICS-1100: Basic integrated IC system
- Dionex ICS-900: Starter line IC system

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- Self-cleaning ion source for low-maintenance operation

- Thermo Scientific Dionex Chromeleon Chromatography Data System software for single-point method setup, instrument control, and data management
- Compatible with existing IC and LC methods
- The complete system includes the MSQ Plus™ mass spectrometer, PC datasystem, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) probe inlets, and vacuum system

You no longer need two software packages to operate your LC/MS system. Chromeleon™ LC/MS software provides single-software method setup and instrument control; powerful UV, conductivity, and MS data analysis; and fully integrated reporting.

*MS Systems and Modules:* MSQ Plus Mass Spectrometer; MSQ18LA nitrogen gas generator; Thermo Scientific Dionex AXP-MS digital auxiliary pump



# Chromeleon 7 Chromatography Data System Software

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Discover Chromeleon software version 7, the chromatography software that streamlines your path from samples to results. Get rich, intelligent functionality and outstanding usability at the same time with Chromeleon software version 7—the Simply Intelligent™ chromatography software.

- Enjoy a modern, intuitive user interface designed around the principle of operational simplicity
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- Locate and collate results quickly and easily using powerful built-in database query features
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- Find everything you need to view, analyze, and report data in the Chromatography Studio

- Accelerate analyses and learn more from your data through dynamic, interactive displays
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Chromeleon software version 7 is a forward-looking solution to your long-term chromatography data needs. It is developed using the most modern software tools and technologies, and innovative features will continue to be added for many years to come.

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Chromeleon software version 7 ensures data integrity and reliability with a suite of compliance tools. Compliance tools provide sophisticated user management, protected database structures, and a detailed interactive audit trail and versioning system.





## Process Analytical Systems and Software

### *Improve your process by improving your process monitoring with a Thermo Scientific Dionex on-line IC or HPLC system*

Our process analytical systems provide timely results by moving liquid chromatography-based measurements on-line. Information from the Thermo Scientific Dionex Integral process analyzer can help reduce process variability, improve efficiency, and reduce downtime. These systems provide comprehensive, precise, accurate information faster than is possible with laboratory-based results. From the lab to the factory floor, your plant's performance will benefit from the information provided by on-line LC.

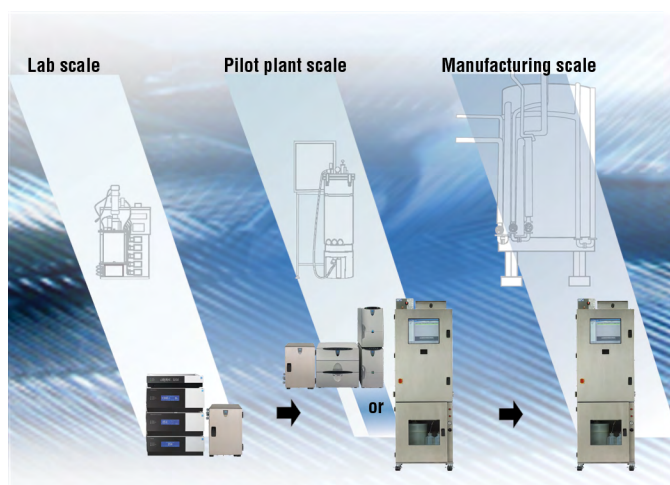
- Characterize your samples completely with multicomponent analysis
- Reduce sample collection time and resources with automated multipoint sampling
- Improve your process control with more timely results

- See more analytes with unique detection capabilities
- 25 years of experience providing on-line IC and HPLC capabilities to a wide range of industries
- The Thermo Scientific Integral Migration Path approach lets you choose the systems that best meets your needs

The Integral Migration Path™ approach enables on-line IC/HPLC to generate timely, high-resolution information when monitoring a small-scale reactor in a process R&D lab, in a pilot plant, or improving current manufacturing plant processes. No matter what the application, the Integral™ process analyzer has the versatility to place a solution using on-line IC/HPLC, whenever and wherever it is needed.

*Integral:* The Integral Migration Path approach: System solutions wherever you need them: lab, pilot plant, or manufacturing

*Chromeleon Process Analytical (PA) Software:* Chromeleon PA software provides unique capabilities to support on-line IC or HPLC analysis



## Automated Sample Preparation

### ***ACCELERATED SOLVENT EXTRACTORS***

#### ***Two new solvent extraction systems with pH-hardened Dionium components***

We offer two solvent extraction systems. The Thermo Scientific Dionex ASE 150 Accelerated Solvent Extractor is an entry-level system with a single extraction cell, for

laboratories with modest throughput. The Dionex ASE™ 350 system is a sequential extraction system capable of automated extraction of up to 24 samples. Both systems feature chemically inert Dionium components that allow the extraction of acid- or base-pretreated samples.



## **SOLID-PHASE EXTRACTION SYSTEMS**

*Faster, more reliable solid-phase extraction while using less solvent*

The Thermo Scientific Dionex AutoTrace 280 Solid-Phase Extraction (SPE) instrument unit can process six samples simultaneously with minimal intervention. The instrument uses powerful pumps and positive pressure with constant flow-rate technology. Current analytical methods that require SPE sample preparation include gas chromatography (GC), GC-MS, LC, and LC-MS, IC and IC-MS. The Dionex AutoTrace™ 280 instrument is approved or adapted for U.S. EPA clean water methods and safe drinking water methods (600 and 500 series) and can extract the following analytes:

- 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



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## Analysis of Agricultural Chemical Contaminants

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# Accelerated Solvent Extraction (ASE<sup>®</sup>) 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  $\times$  0.25-mm i.d. RTX-5 capillary column (Restek Corporation). Set up a 1- $\mu$ L 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  $\mu$ L 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  $\mu$ L 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

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

| Compound           | Av. Recovery (%) | SD ( $\mu$ g/kg) | RSD (%) |
|--------------------|------------------|------------------|---------|
| $\alpha$ -BHC      | 100.3            | 2.3              | 2.3     |
| $\beta$ -BHC       | 102.2            | 2.3              | 2.3     |
| $\gamma$ -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 ( $\mu$ g/kg) | RSD (%) |
|--------------------|-------------------|------------------|---------|
| $\alpha$ -BHC      | 96.3              | 6.3              | 6.6     |
| $\beta$ -BHC       | 108.6             | 2.3              | 2.1     |
| $\gamma$ -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

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.

| Compound                  | Spike Level (µg/kg) | Recovery (%) |
|---------------------------|---------------------|--------------|
| <i>o</i> -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- <i>o,p</i>  | 48                  | 89.9         |
| Diclofop-methyl           | 36                  | 81.8         |
| Methoxychlor- <i>p,p'</i> | 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.

| 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](http://www.fishersci.com).  
 Restek Corporation, 110 Benner Cir., Bellefonte, Pennsylvania, 16823 USA, Tel.: 814-353-1300, [www.restekcorp.com](http://www.restekcorp.com).

# Determination of Pesticides in Large-Volume Food Samples Using Accelerated Solvent Extraction (ASE<sup>®</sup>)

## 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.<sup>1,2</sup>

## 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<sub>2</sub>/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  $\mu\text{L}$  of a pesticide mixture at 0.2 mg/mL was added to the baby food for a final concentration of 50  $\mu\text{g}/\text{kg}$  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  $\times$  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 ( $= V_{\text{End}}$ ) 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  $\mu\text{m}$

Gases: Carrier: Helium, 10 mL/min  
Makeup: Helium, 15 mL/min  
Detector: Air, 100 mL/min  
Hydrogen, 75 mL/min

Temperatures: Oven: Initial 60  $^{\circ}\text{C}$  (hold for 1 min), heat rate 10  $^{\circ}\text{C}/\text{min}$  to 250  $^{\circ}\text{C}$  (hold for 10 min)

Injector: 250  $^{\circ}\text{C}$   
Detector: 240  $^{\circ}\text{C}$   
Injection Volume: 5  $\mu\text{L}$ , 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  $\mu$ m

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  $\mu$ L, 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.

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### **SUPPLIERS**

ABIMED Analysen-Technik GmbH, Raiffeisenstr. 3, 40764 Langenfeld, Germany, Tel: 02173 89 05 0, [www.abimed.de](http://www.abimed.de).

Agilent Technologies, 395 Page Mill Rd., Palo Alto, CA 94306 USA, Tel: 877-424-4536, [www.agilent.com](http://www.agilent.com).

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

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 800-766-7000, [www.fishersci.com](http://www.fishersci.com).

### **ACKNOWLEDGEMENTS**

We acknowledge the work of Manfred Linkerhaegner 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      |
| Fenclorophos           | 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       |
| Fenclorophos           | 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<sup>®</sup>)

## **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<sup>™</sup> (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<sup>-1</sup>) and RSD (%) for the Extraction of CRM BCR 115**

| Compounds  | Certified Value         |         | ASE (n = 3)             |         |
|------------|-------------------------|---------|-------------------------|---------|
|            | C (ng g <sup>-1</sup> ) | RSD (%) | C (ng g <sup>-1</sup> ) | 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.

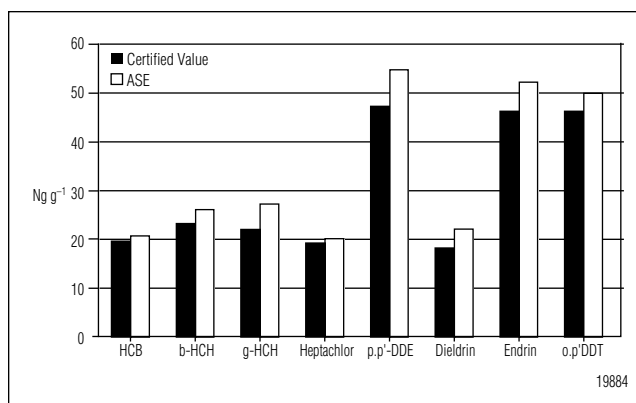


Figure 1. Graph of results from Table 1.

## 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.<sup>1</sup>

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<sub>2</sub>SO<sub>4</sub>. The column bed was preconditioned with 50 mL *n*-hexane and compressed by a stream of N<sub>2</sub> (200 kPa). Thereafter, the concentrated raw extract was added onto the top of the silica gel column. The sample flask was rinsed with two 0.5-mL portions of *n*-hexane-CH<sub>2</sub>Cl (7+3, v/v) and this was added to the column bed. The analytes were eluted with 19 mL *n*-hexane-dichloromethane (7+3, v/v). The eluate was collected in a 50-mL pear-shaped flask and concentrated to 0.5 mL by means of a rotary evaporator.

The GPC column was prepared by weighting 6 g S-X3 bio-beads that were swelled in *n*-hexane-dichloromethane (1 + 1, v/v) overnight, into a chromatographic column (15-mm i.d., 30 cm, 100 mL) with a reservoir, fused-in fritted disk, and Teflon<sup>®</sup> 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

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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<sub>2</sub>, dissolved in 250- $\mu$ L 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.

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# Extraction of Drugs from Animal Feeds Using Accelerated Solvent Extraction (ASE<sup>®</sup>)

## 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 x 4.6 mm i.d. pre-column held at ambient temperature. Columns were purchased 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 x 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**

| Extraction Method    | Feed Level (% RSD)* |            |
|----------------------|---------------------|------------|
|                      | 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

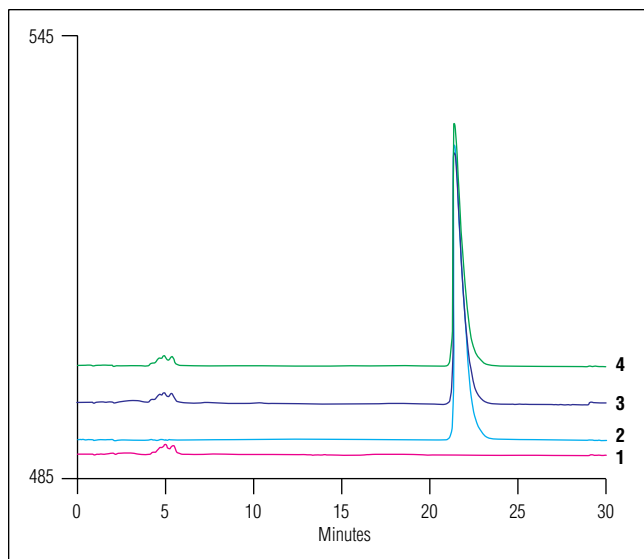


Figure 1. HPLC analysis of feed standards and extracts. (1) ASE extraction blank, (2) drug standard, (3) ASE extract of spiked standard, (4) ASE extraction of drug containing feed sample.

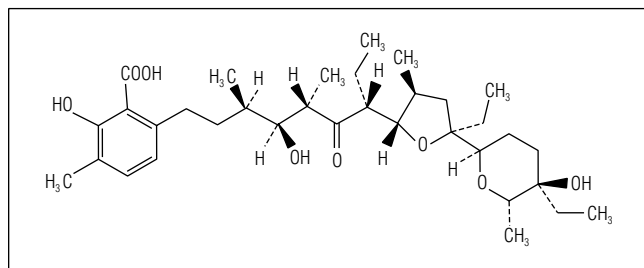


Figure 2. Lasalocid chemical structure.

## 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.

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

| Sample # | Lasalocid Recovered (ppm) |            |
|----------|---------------------------|------------|
|          | 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      |

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<sup>®</sup>)

## **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), Sulfamethoxypyridazine (SMT), Sulfachloropyridazine (SCP), Sulfamethoxypyridazine (SMP), Sulfadiazine (SDZ), Sulfamerazine (SMR), Sulfamethazine (SMZ), Sulfasomidine (SIM), Sulfamonomethoxine (SMM), Sulfadimethoxine (SDM), Sulfaquinoxaline (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.<sup>1</sup>

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

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**

| Analyte | Bovine Meat |           | Porcine Meat |           | Poultry Meat |           |
|---------|-------------|-----------|--------------|-----------|--------------|-----------|
|         | 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**

| Analyte | Raw Meat  |           | Baby Food |           |
|---------|-----------|-----------|-----------|-----------|
|         | 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.<sup>1</sup>

## 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](http://www.sigmaaldrich.com).

Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823 USA, Tel: 800-356-1688, [www.restekcorp.com](http://www.restekcorp.com).

Sigma-Aldrich Chemical Company, 3050 Spruce St., St. Louis, MO 63103 USA, Tel: 800-325-3010, [www.sigmaaldrich.com](http://www.sigmaaldrich.com).

## REFERENCES

- 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.<sup>1</sup> 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.<sup>2-4</sup>

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,<sup>5,6</sup>

accelerated solvent extraction (ASE),<sup>7,8</sup> liquid-liquid extraction,<sup>9</sup> and cloud-point extraction (CPE).<sup>10</sup> 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<sup>11</sup> by the Association of Official Analytical Chemists (AOAC) in the United States, and the European equivalent, EN 15662.<sup>12</sup>

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<sub>4</sub>; 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<sup>®</sup> 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

Chromleon® 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.<sup>3</sup>

## **REAGENTS AND STANDARDS**

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

Methanol (CH<sub>3</sub>OH), Fisher

Acetonitrile (CH<sub>3</sub>CN), Fisher

Potassium dihydrogen citrate (KC<sub>6</sub>H<sub>7</sub>O<sub>7</sub>), 98%, Fluka

Sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>), 98%, Fluka

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

Boric acid, 99.5%, Fluka

*o*-Phthalaldehyde (OPA, C<sub>8</sub>H<sub>6</sub>O<sub>2</sub>), 99%, Pickering

β-Mercaptoethanol, 99%, SCRC, China

Magnesium sulfate (MgSO<sub>4</sub>), 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<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 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  $\mu$ L 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.<sup>1</sup>

### 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.<sup>1</sup> To review the postcolumn chemistry and see a diagram of the postcolumn system configuration, see EPA Method 531.2.<sup>1</sup>

### 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  $\mu$ L of 1  $\mu$ g/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  $\mu$ L of supernatant to a 1.5 mL vial, then add 900  $\mu$ L 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 ( $\mu$ g/mL) | Volume of Stock Standard of Carbamate Mixture ( $\mu$ L) | Stock Standard of BDMC (SUR) ( $\mu$ g/mL) | Volume of Stock Standard of BDMC (SUR) ( $\mu$ L) | Final Volume of Calibration Standard (mL) | Final Concentration of Carbamate Standard ( $\mu$ g/L) | Final Concentration of BDMC (SUR) ( $\mu$ g/L) |
|---|--|--|---|---|--|--|
| 1.0   | 6.25   | 1.0  | 50.0  | 25  | 0.25   | 2.0  |
|   | 12.5   |  |   |   |  |  |
|   | 25.0   |  |   |   |  |  |
|   | 50.0   |  |   |   |  |  |
|   | 200  |  |   |   |  |  |



## CONDITIONS

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

| Time (min) | Flow Rate (mL/min) | Methanol (%) | H <sub>2</sub> O (%) |
|------------|--------------------|--------------|----------------------|
| -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<sub>4</sub> 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.<sup>13</sup>

### 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.<sup>13</sup> 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<sub>4</sub> to remove the residual water as completely as possible. Therefore, use two additions of MgSO<sub>4</sub> 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.

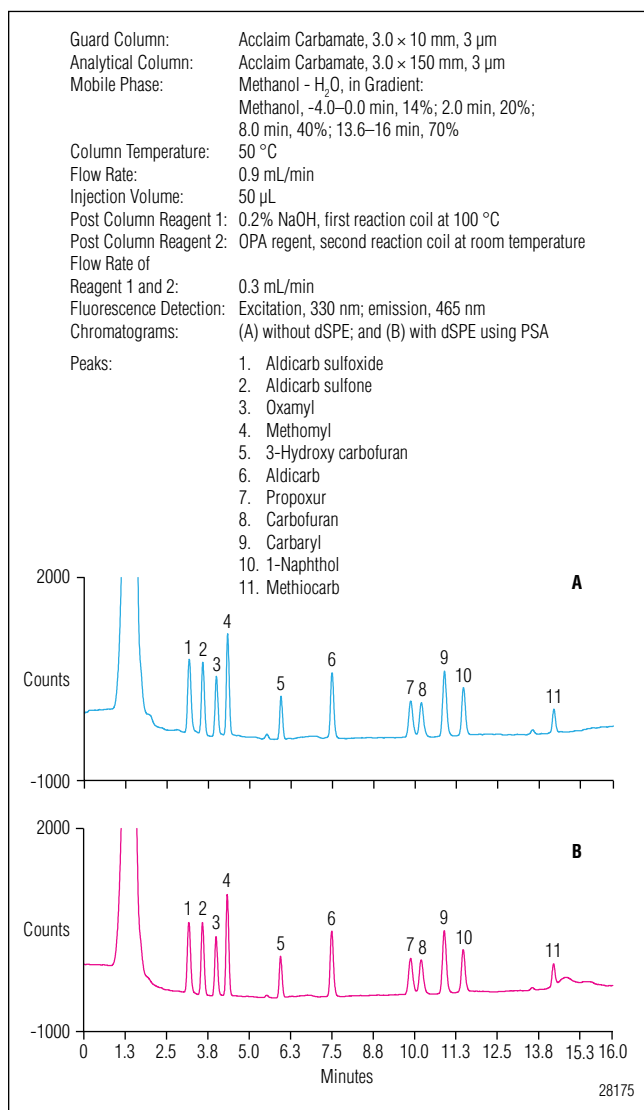


Figure 1. Chromatograms of extracts of carbamate standard-spiked rice samples (2 μg/L each) after acetonitrile and salt-out extractions (A) without and (B) with dSPE using PSA.

### 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.

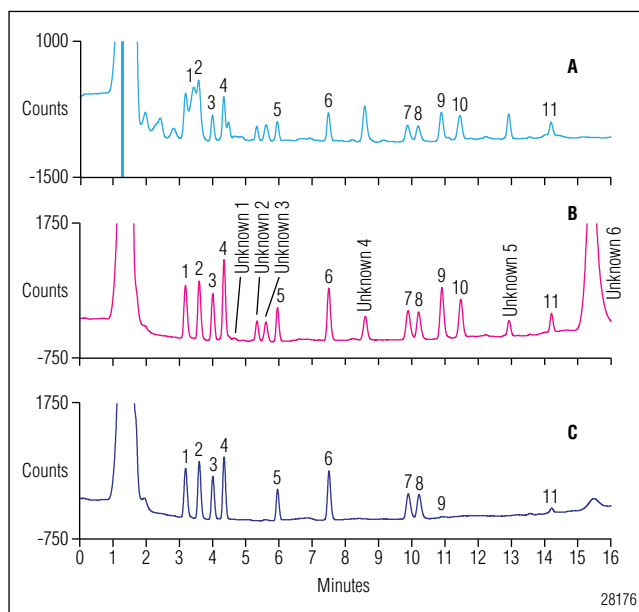


Figure 2. Chromatograms of extracts of carbamate standard-spiked corn samples (2 μg/L each) after acetonitrile and salt-out extractions (A) without and with dSPE using (B) PSA and (C) activated carbon. Other conditions are the same as in Figure 1.

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.

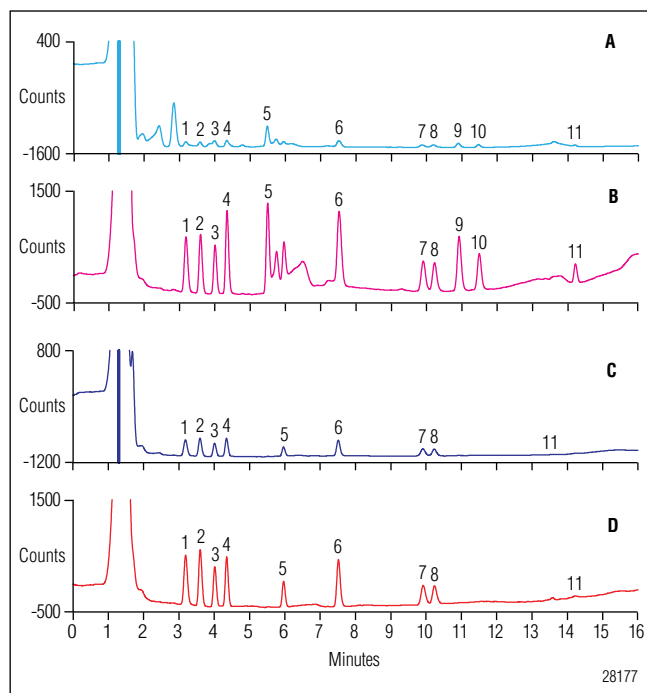


Figure 3. Chromatograms of extracts of carbamate standard-spiked potato samples (2 µg/L each) after acetonitrile and salt-out extractions (A) without and with dSPE using (B) PSA, (C) activated carbon, and (D) mixture of PSA and activated carbon (1:1, w/w). Other conditions are the same as in Figure 1.

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.

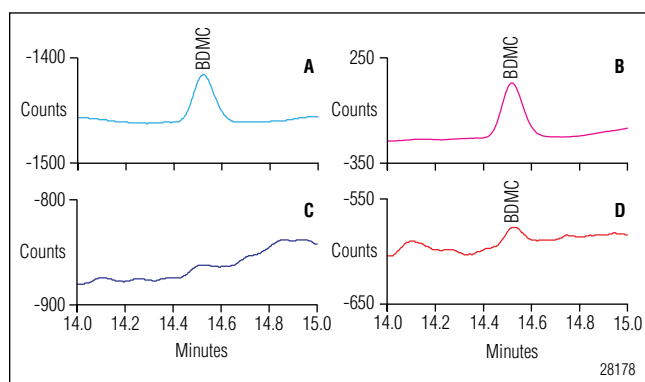


Figure 4. Chromatograms of BDMC (SUR) after acetonitrile and salt-out extractions (A) without and with dSPE using (B) PSA, (C) activated carbon, and (D) mix of PSA and activated carbon (1:1, w/w). Other conditions are the same as in Figure 1.

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<sup>1</sup> 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       |               |                   |
|                      | PSA<br>50 mg           | PSA<br>100 mg | Difference<br>(%) | Dried        |               |                   | Fresh        |               |                   | PSA<br>50 mg | PSA<br>100 mg | Difference<br>(%) |
|                      |                        |               |                   | PSA<br>50 mg | PSA<br>100 mg | Difference<br>(%) | PSA<br>50 mg | PSA<br>100 mg | Difference<br>(%) |              |               |                   |
| 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:  $\text{Difference} = (A_{100 \text{ mg of PSA}} - A_{50 \text{ mg of PSA}}) / A_{50 \text{ mg of PSA}}$

$A_{100 \text{ mg of PSA}}$  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  $\geq 1.5$ .

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  $\leq 0.07\%$  for retention time and  $\leq 3.0\%$  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  $\leq 7.0\%$ , demonstrating sufficient reproducibility for the sample preparation method.

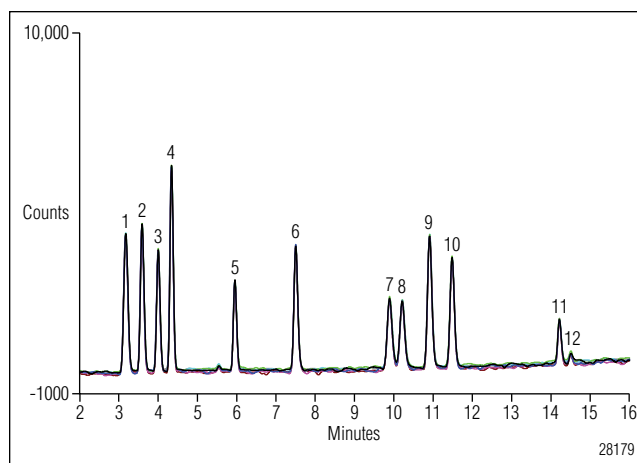


Figure 5. Overlays of chromatograms of seven consecutive injections of carbamate standards (8 µg/L each). Other conditions are the same as in Figure 1.

**Table 4. Method Linearity Data and Method Detection Limits (MDL)**

| Carbamate           | Regression Equation   | r <sup>2</sup> | MDL                                      |  |
|---------------------|-----------------------|----------------|--|--|
|                     |                       |                | In Well-Prepared Sample Solution<br>µg/L | Equivalent in the Original Sample<br>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.

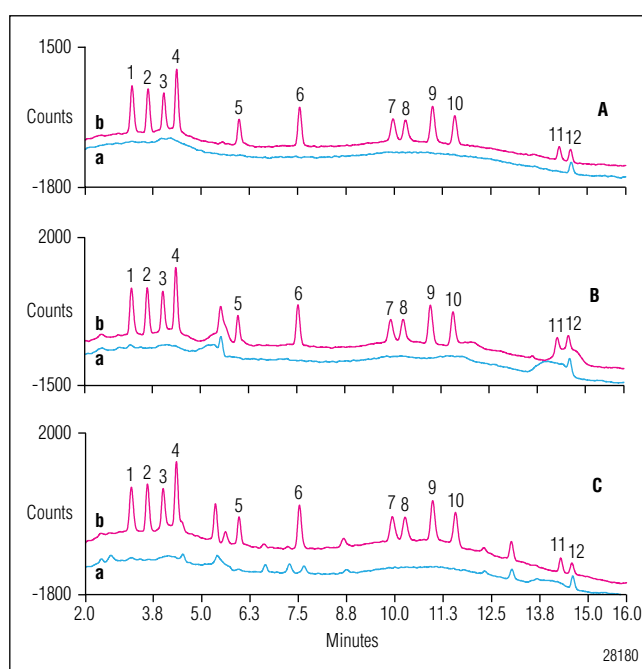


Figure 6. Overlays of chromatograms of crop samples spiked with 2.0 µg/L of BDMC (SUR, peak 12), and the same samples spiked with a carbamate standard mixture with 2.0 µg/L for each carbamate. Chromatograms, (a) crop samples, (b) carbamate standard-spiked crop samples; samples, (A) rice, (B) potato, 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.<sup>15</sup> 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

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# Silica Columns

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



# 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<br>4 × 250 mm | Carbonate      | Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water.  | 6 µm              | 55%                    | -              | -                  | 80 µeq<br>320 µeq     | Alkyl quaternary ammonium   | Ultralow       |
| IonPac AS22       | 2 × 250 mm<br>4 × 250 mm | Carbonate      | Recommended column for fast analysis of common inorganic anions.  | 6.5 µm            | 55%                    | -              | -                  | 52.5 µeq<br>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<br>4 × 250 mm | Hydroxide      | Recommended column for trace perchlorate prior to suppressed conductivity detection.  | 7.5 µm            | 55%                    | -              | -                  | 77.5 µeq<br>310 µeq   | Alkanol quaternary ammonium | Ultralow       |
| IonPac AS19       | 2 × 250 mm<br>4 × 250 mm | Hydroxide      | Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water.  | 7.5 µm            | 55%                    | -              | -                  | 60 µeq<br>350 µeq     | Alkanol quaternary ammonium | Low            |
| IonPac AS18       | 2 × 250 mm<br>4 × 250 mm | Hydroxide      | Recommended column for the analysis of common inorganic anions.   | 7.5 µm            | 55%                    | 65 nm          | 8%                 | 75 µeq<br>285 µeq     | Alkanol quaternary ammonium | Low            |
| IonPac AS17-C     | 2 × 250 mm<br>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<br>30 µeq     | Alkanol quaternary ammonium | Low            |
| IonPac AS16       | 2 × 250 mm<br>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<br>170 µeq   | Alkanol quaternary ammonium | Ultralow       |
| IonPac AS15       | 2 × 250 mm<br>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<br>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<br>4 × 250 mm | Carbonate      | Moderate capacity for fast analysis of common inorganic anions.   | 9 µm              | 55%                    | -              | -                  | 16 µeq<br>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<br>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<br>52 µeq      | Alkyl quaternary ammonium   | Medium         |
| IonPac AS11-HC         | 2 × 250 mm<br>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<br>290 µeq   | Alkanol quaternary ammonium | Medium-Low     |
| IonPac AS11            | 2 × 250 mm<br>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<br>45 µeq      | Alkanol quaternary ammonium | Very Low       |
| IonPac AS10            | 2 × 250 mm<br>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<br>170 µeq   | Alkyl quaternary ammonium   | Low            |
| IonPac AS9-HC          | 2 × 250 mm<br>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<br>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<br>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<br>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<br>4 × 250 mm | MSA                        | Recommended column for hydrophobic and polyvalent amines (biogenic amines and diamines)   | 7 µm              | 55%                    | -               | -                  | 0.363 µeq<br>1.45 µeq                            | Carboxylic acid                                     | Very Low       |
| IonPac CS16       | 3 × 250 mm<br>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<br>8.4 µeq                               | Carboxylic acid                                     | Medium         |
| IonPac CS15       | 2 × 250 mm<br>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<br>2.8 µeq                               | Carboxylic acid/<br>phosphonic acid/<br>crown ether | Medium         |
| IonPac CS14       | 2 × 250 mm<br>4 × 250 mm | MSA                        | Aliphatic amines, aromatic amines, and polyamines plus mono- and divalent cations.  | 8.5 µm            | 55%                    | -               | -                  | 0.325 µeq<br>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/<br>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/<br>phosphonic acid                 | Medium         |
| IonPac CS12A      | 2 × 250 mm<br>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<br>2.8 µeq                               | Carboxylic acid/<br>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<br>4 × 250 mm | Pyridine dicarboxylic acid | Recommended column for transition and lanthanide metals analysis. Aluminum analysis.  | 9 µm              | 55%                    | 140 nm<br>75 nm | 10%<br>20%         | 0.02 µeq/<br>0.005 µeq/<br>0.04 µeq/<br>0.01 µeq | Sulfonic acid/<br>alkanol<br>quaternary<br>ammonium | -              |

## Ion-Exclusion Columns

| Column            | Format                   | Primary Eluent          | Application  | Particle Diameter | Substrate Crosslinking | Latex Diameter | Latex Crosslinking | Capacity (per column) | Functional Group             | Hydrophobicity |
|-------------------|--------------------------|-------------------------|--|-------------------|------------------------|----------------|--------------------|-----------------------|------------------------------|----------------|
| IonPac ICE-AS1    | 4 × 250 mm<br>9 × 250 mm | Heptafluorobutyric acid | Organic acids in high ionic strength matrices. Fast separation of organic acids. | 7.5 µm            | 8%                     | -              | -                  | 5.3 µeq<br>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 <sup>2</sup> /g) | Total Carbon Content |
|--------------------------------|----------------------------|----------|-------------|------------------|----------------|---------------|---------------------------------|-----------------------|----------------------------------|----------------------|
| Mixed-Mode WAX                 | Proprietary alkyl amine    | na       | Proprietary | Ultrapure silica | Spherical      | 5 µm          | <10 ppm                         | 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         |                  |                | 3 and 5 µm    |                                 | 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. Incompatible 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 functional 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 Å, surface area 100 m <sup>2</sup> / 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 agglomerated 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 compositional analysis                    | 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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