- Biogenic Amines
- Mycotoxins

Food Safety Applications Notebook

Environmental Contaminants

Thermo SCIENTIFIC

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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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- Fully UHPLC compatible advanced chromatographic techniques
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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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- Dionex ICS-1600: Standard integrated IC system
- Dionex ICS-1100: Basic integrated IC system
- Dionex ICS-900: Starter line IC system

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- Accelerate analyses and learn more from your data through dynamic, interactive displays
- Deliver customized reports using the built-in Excel-compatible speadsheet

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- Characterize your samples completely with multicomponent analysis
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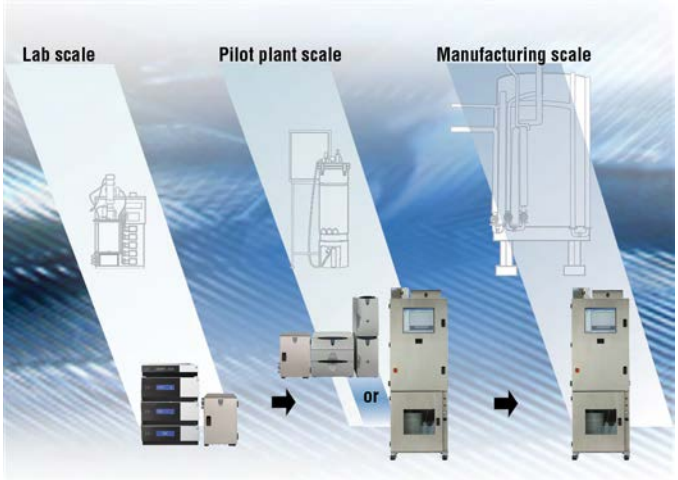
- Improve your process control with more timely results
- See more analytes with unique detection capabilities
- 25 years of experience providing on-line IC and HPLC capabilities to a wide range of industries
- The Thermo Scientific Integral Migration Path approach lets you choose the systems that best 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.

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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:

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- 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 Environmental Contaminants

Rapid Extraction and Determination of Arsenicals in Fish Tissue and Plant Material Using Accelerated Solvent Extraction (ASE®)

INTRODUCTION

The toxicity of arsenic is species dependent. Inorganic arsenic species such as arsenite (As[III]) and arsenate (As[V]) have been classified as carcinogens. Methylated forms such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) have recently been labeled as cancer promoters. Arsenobetaine (AsB), arsenocholine (AsC), and arseno sugars have been found to be relatively nontoxic.

Two major pathways for toxic arsenic exposure include drinking water and diet. Seafood (including fish and seaweed) accounts for the majority of ingested arsenic, most of which is nontoxic, however, fruits and vegetables grown in contaminated soils and sediments contribute another significant source.

Due to the variable levels of toxicity associated with arsenic species in foods, total arsenic determination is not sufficient to assess potential harmful contamination. Determination of individual arsenic species is necessary. This has increased the need to improve separation and detection methods for organometallic speciation. Unfortunately, the majority of organometallic sample preparation methods are still laborious and time consuming. The time discrepancies between the improved analytical methods and outdated sample preparation methods create bottlenecks which slow results of vital toxicological monitoring of food products used for human consumption.

To eliminate sample preparation bottlenecks, Accelerated Solvent Extraction (ASE) methods have been developed and proven to be an excellent alternative to the outdated sample preparation methods such as Soxhlet and sonication. ASE dramatically decreases the extraction time while providing good recoveries of arsenic species. ASE uses solvents at elevated temperature and pressure to increase the kinetics of the extraction process, resulting in faster, more efficient extractions.

This application note describes ASE methods for the extraction of various arsenic species from different food matrices, specifically fish tissue, ribbon kelp, and vegetables grown in contaminated soil. This note also includes references for analysis methods such as ICP-MS.

EQUIPMENT

Dionex ASE 200 Extractor with 11-mL stainless steel extraction cells (P/N 048765)

Dionex Cellulose Glass-fiber Filters (P/N 049458 or 047017)

Dionex Collection Vials (40 or 60 mL) (P/N 048783 or 048784)

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

Dionex SE 400 or SE 500 Solvent Evaporator (P/N 063221 or 063222)

REAGENTS

Methanol (HPLC grade)
HPLC water
Ottawa sand (Fisher Scientific)

EXTRACTION CONDITIONS

Fish Tissue[1]

Solvent:	Methanol 100%
Temperature:	100 °C
Pressure:	1500 psi
Cell Heat-up Time:	5 min
Static Time:	2 min
Flush Volume:	60%
Purge Time:	60 s
Cycles:	5
Total Time:	17 min
Total Solvent:	<30 mL

Ribbon Kelp[2]

Solvent:	30/70 (w/w) Methanol/ H_2O
Temperature:	Ambient
Pressure:	1500 psi
Cell Heat-up Time:	N/A
Static time:	1 min
Flush Volume:	90%
Purge Time:	120 s
Cycles:	3
Total Time:	7 min
Total Solvent:	<30 mL

Carrots[3]

Solvent:	Water
Temperature:	100 °C
Pressure:	1500 psi
Cell Heat-up Time:	5 min
Static Time:	1 min
Flush Volume:	100%
Purge Time:	90 s
Cycles:	3
Total Time:	18 min
Total Solvent:	<30 mL

SAMPLE PREPARATION

Freeze-dried samples are used for all the methods described in this document.

Fish Tissue[1]

Place a cellulose filter into an 11-mL extraction cell before loading the sample. Weigh approximately 0.1–0.3 g of freeze-dried fish tissue directly into the cell. Add Ottawa sand to the cell and mix with fish tissue using a stainless steel spatula. Cap the cell and prepare other samples in the same manner.

Ribbon Kelp[2]

Place a cellulose filter into an 11-mL extraction cell before loading the sample. Weigh approximately 0.25–0.5 g of freeze-dried kelp directly into the cell. Add Ottawa sand to the cell and mix with the dried kelp using a stainless steel spatula. Cap the cell and prepare other samples in the same manner. Note: Because the seaweed samples tend to swell when exposed to the solvent, the amount of sample used may need to be varied depending upon the type of seaweed being extracted.

Carrots[3]

Place a glass-fiber filter into an 11-mL extraction cell before loading the sample. Weigh approximately 0.25–1.0 g of freeze-dried carrot directly into the cell. Add Ottawa sand to the cell and mix with the dried carrot using a stainless steel spatula. Cap the cell and prepare other samples in the same manner. Note:
Because the carrot samples tend to swell when exposed to the solvent, the amount of sample used may need to be varied depending upon the type of plant material being extracted.

EXTRACTION PROCEDURE

Place the loaded cells onto the ASE 200 instrument. Label the appropriate number of collection vials and place them onto the vial carousel. Set up the method parameters that are suggested for the sample being extracted and push the START button to begin the extraction. When the extraction is complete the extracts should be treated as follows:

Fish Tissue Extracts[1]

Remove an aliquot of the extract from the collection vial and dilute 1:10 with water. Filter each diluted extract using a 0.45 µm nylon/glass syringe filter into an HPLC autosampler vial.

Ribbon Kelp Extracts[2]

Place the extracts onto the SE Evaporator and evaporate to dryness at 50 °C with a nitrogen stream. Redisolve each extract with 20 g of water. Filter an aliquot of each extract using a 0.45 µm nylon/glass syringe filter into an HPLC autosampler vial.

Carrot Extracts[3]

Filter each extract using a 0.45 µm nylon/glass syringe filter. Place an aliquot of the filtered extract into an HPLC autosampler vial.

Analytical Procedures

To determine the individual arsenic species in each extract, the authors used LC-ICP-MS. The LC-ICP-MS Methods for each sample can be found in literature references [1] for fish, [2] for kelp, and [3] for carrots.

RESULTS AND DISCUSSION

Fish Tissue[1]

The following certified reference materials (CRMs) were extracted for method validation: DORM-2 dogfish muscle (National Research Council, Ottawa Canada), BCR 627 tuna fish, and BCR 710 oyster tissue (Brussels, Belgium).

Subsamples of the different CRMs (n = 4–6) were extracted and diluted with water and analyzed via HPLC-ICP-MS.[1] Table 1 shows the results of AsB extracted by ASE as compared to the known CRM value. The results showed a 99.4% recovery for the DORM-2 samples, a 94.6% recovery for the BCR 627 samples and a 97.3% recovery for the BCR 710 samples.

Table 1. Results of ASE Extraction of Fish Tissue CRMs (n=6)

Data Obtained for AsB in Two Certified Reference Materials and a Candidate Reference Material* Extracted with ASE

	Measured Value	Certified Value
DORM-2 (dogfish muscle)	16.3 ± 0.9 (±1s)	16.4 ± 1.1 (±95% C.I.)
BCR 627 (tuna fish)	3.69 ± 0.21 (±1s)	3.90 ± 0.22 (±95% C.I.)
BCR 710** (oyster tissue)	31.8 ± 1.1 (±1s)	32.7 ± 5.1 (±1s)

* Expressed as mg/kg As, unless otherwise stated.
** Concentration as species. The data shown for this material is based on the consensus mean of the final certification round after the removal of statistical outliers.

Ribbon Kelp[2]

Ribbon kelp containing 3 arsenosugars (As 328, As 428, and As 392) was received from Puget Sound, WA. Portions of each kelp sample were digested with HNO_3 and H_2O_2 to determine the total As for comparison to the ASE method. The procedure to determine the As Total Digest concentration was a modification of US EPA method 200.3. Each digested sample was analyzed in triplicate.

In an effort to optimize the ASE method, several different ASE parameters were evaluated. It was determined that temperature and solvent mixture had the most dramatic effect on As recoveries. The tests conducted by the authors show a 19.7% increase in recoveries when increasing the temperature from ambient to 60 °C. Increasing the temp to 120 °C caused the extracts to become discolored. It was determined that this temperature was too high and the discoloration was due to the unknown thermal stability of the species. Further tests showed that extracting with 100% water gave the best recoveries, but caused the sample to swell excessively in the extraction cell. Using a mixture of water and methanol (30/70, v/v) gave similar results and eliminated sample swelling.

Table 2. Summary of ASE Average Recoveries for Arsenic Spiked on Carrots and Calculated by Direct Calibration (n=3)						
Standard Type	**Standard Concentration (ng/mL^{-1})**	**As (III)**	**As (v)**	**MMA**	**DMA**	**AsB**
Single	1	107 ± 10	106 ± 5	101 ± 2	91 ± 4	102 ± 4
Mixture of five	1	111 ± 8	121 ± 3	104 ± 5	109 ± 6	112 ± 3
Single	5	102 ± 8	102 ± 3	104 ± 3	94 ± 1	98 ± 1
Mixture of five	5	104 ± 9	108 ± 4	103 ± 7	102 ± 3	101 ± 1

Carrots[3]

Freeze-dried carrot samples that contained an undetectable total arsenic concentration were spiked with arsenic as a single species and with a mixed standard containing the following arsenic species: As(III), As(V), MMA, DMA and AsB. These were spiked at two different concentrations. Table 2 summarizes the average ASE recoveries.

CONCLUSIONS

The data presented in this application note demonstrate that ASE is an excellent technique for extracting arsenicals from food samples such as fish tissue and vegetables. Using ASE, we were able to rapidly extract arsenicals using a minimal amount of solvent while achieving excellent recoveries.

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Determination of Biogenic Amines in Alcoholic Beverages by Ion Chromatography with Suppressed Conductivity and Integrated Pulsed Amperometric Detections

INTRODUCTION

Biogenic amines are common in plants and animals, where they have important metabolic and physiological roles, such as the regulation of growth (putrescine, spermidine, spermine), control of blood pressure (indoleamines and histamine), and neural transmission (catecholamines and serotonin).[1,2] In foods and beverages, biogenic amines can be formed by the decarboxylation of amino acids from microbial activity.[3] Their presence in food is not only important from a toxicological view, but can also be used as an indicator of spoilage.[4] Biogenic amines, such as histamine, may be present before foods appear spoiled or have an unacceptable appearance.[5] The normal dietary intake of biogenic amines is not considered harmful because healthy individualscan readily metabolize the amines by acetylation and oxidation reactions mediated by the enzymes monoamine oxidase, diamine oxidase, and polyamine oxidase.[6] The consumption of an excess amount of these amines, however, can induce severe toxicological effects and produce various physiological symptoms, such as nausea, respiratory distress, headache, sweating, heart palpitations, and hyper- or hypotension.[7]

Malolactic fermentation or the action of yeasts in primary fermentation has been associated with the production of biogenic amines such as tyramine, putrescine, cadaverine, histamine, and phenylethylamine in wine samples.[2,8] Histamine can produce headaches, flushingof the face and neck, and hypotension, whereas some aromatic amines, such as tyramine and phenylethylamine, can cause migraines and hypertension.[1] The concentration and content of biogenic amines in wines are variable depending on the storage time and conditions, quality of raw materials, and possible microbial contamination during the winemaking process.[9] Putrescine, agmatine, spermidine, and spermine are considered natural beer constituents that primarily originate from malt. The presence of tyramine, cadaverine, and histamine, however, has been associated with the activities of contaminating lactic acid bacteria during the brewing process.[10]

The determination of biogenic amines presents a challenging analytical problem because they are usually hydrophobic, are poor chromophores, and often occur in low concentrations in complex matrices. Reversed-phase high-performance liquid chromatography (HPLC) combined with pre- or postcolumn chemical derivatization and UV or fluorescence detection is commonly used for determining biogenic amines in alcoholic beverages. *o*-Phthalaldehyde (OPA) combined with a thiol compound, such as 2-mercaptoethanol (MCE), is the most frequently reported derivatizing agent used to determine biogenic amines in wine[2,9,11–13] and beer[14,15] samples. Because OPA derivatives have limited stability, however, OPA-MCE postcolumn derivatization procedures are generally preferred over precolumn procedures.[16] Unfortunately, this chemical derivatization adds complexity to the analysis, requires additional skilled labor, and can sometimes produce by-product interferences.

Ion chromatography (IC) coupled to pulsed amperometric detection (PAD) or integrated pulsed amperometric detection (IPAD) after postcolumn base addition has been used for the determination of underivatized biogenic amines.[17–19] These procedures require high acid or salt gradients combined with an organic solvent to separate strongly retained amines, such as spermidine and spermine.[19] Organic solvents, however, such as acetonitrile, can produce undesirable decomposition by-products with amperometric detection, resulting in potential interferences.[20]

Consequently, the use of IC for the determination of biogenic amines has not been widely reported. This is at least partially due to the strong hydrophobic interactions between the protonated amines and stationary phases, resulting in long retention times and poor peak shapes. In addition, eluents required to separate these amines are often not compatible with suppressed conductivity, the simplest detection method for some of the major biogenic amines. The development of the IonPac® CS17, a weak carboxylic acid functionalized cation-exchange column that reduces the interactions of hydrophobic analytes,[21] allows the use of suppressed conductivity detection. This combination of column and detector was successfully applied to the determination of biogenic amines in fish[22] and meat[23] samples.

A newer cation-exchange column, the IonPac CS18, was specifically designed for the determination of small polar amines. This column has a slightly higher hydrophobicity than the CS17 and therefore improves the separation of close-eluting peak pairs, such as putrescine and cadaverine.

Suppressed conductivity detection is one of the simplest detection configurations, allowing the detection of most target biogenic amines. IPAD provides a broader selectivity, enabling the detection of all biogenic amines of interest. UV detection can provide selectivity towards aromatic compounds. Therefore all three detectors were employed and compared in this Application Note. The IonPac CS18 column was coupled to IPAD to detect biogenic amines in beer and wine samples prior to storage. Because relatively little information exists on the accumulation of biogenic amines in alcoholic beverages during storage, refrigerated samples were analyzed using suppressed conductivity detection coupled to IPAD. UV detection was used to confirm the presence of tyramine in some alcoholic beverages. Suppressed conductivity and IPAD were also compared in terms of linearity, detection limits, precision, and recovery of biogenic amines spiked in beer and wine samples.

EQUIPMENT

Dionex ICS-3000 system consisting of:

- DP Dual Pump with in-line degas option
- DC Detector/Chromatography module with conductivity and electrochemical cells
- Electrochemical cell consisting of a pH/Ag/AgCl reference electrode and a conventional Au electrode (PN 063722)
- EG Eluent Generator module
- EluGen® EGC II MSA cartridge (P/N 058902)
- AD25 UV/Vis Absorbance Detector with 10-mm cell
- Mixing Tee, 3-way, 1.5 mm i.d. (P/N 024314)
- Knitted Reaction Coil, 125 µL (P/N 053640)
- Two 4-L plastic bottle assemblies for external water mode of operation

Chromeleon® 6.7 Chromatography Management software

Centrifuge (Beckman Coulter, Brea, CA)

REAGENTS AND STANDARDS

Reagents

Deionized water, Type I reagent grade, 18 M -cm resistivity or better

Sodium hydroxide, 50% (w/w) (Fisher Scientific, SS254-1)

Methanesulfonic acid, 99% (Dionex Corporation, P/N 033478)

Standards

Dopamine hydrochloride (Sigma Chemical Co., H8502)

Serotonin hydrochloride, ≥98% (Sigma Chemical Co., H9523)

Tyramine, 99% (Aldrich Chemical Co., T90344)

Putrescine dihydrochloride, ≥98% (Sigma Chemical Co., P7505)

Cadaverine dihydrochloride, >98% (Sigma Chemical Co., C8561)

Histamine, ~97% (Sigma Chemical Co., H7125)

Agmatine sulfate, 97% (Aldrich Chemical Co., 101443)

ß-Phenylethylamine, 99% (Aldrich Chemical Co., 128945)

Spermidine trihydrochloride, >98% (Calbiochem, 56766)

Spermine tetrahydrochloride, ≥99% (Calbiochem, 5677)

CONDITIONS

Columns: IonPac CS18 Analytical, 2 × 250 mm (P/N 062878)
IonPac CG18 Guard, 2 × 50 mm (P/N 062880)

Eluent:* 3 mM MSA from 0–6 min, 3-10 mM from 6–10 min, 10–15 mM from 10–22 min, 15 mM from 22–28 min, 15–30 mM from 28–35 min, 30–45 mM from 35–45 min

Flow Rate: 0.30 mL/min

Temperature: 40 °C (lower compartment)
30 °C (upper compartment)

Inj. Volume: 5 µL

Detection**: Suppressed conductivity, CSRS® ULTRA II (2 mm), AutoSuppression® device, external water mode, power set at 40 mA and/or UV-Vis detection set at 276 nm

Background Conductance: 0.4–0.5 µS

Conductance Noise: 0.2–0.3 nS

System Backpressure: ~2500 psi

Postcolumn Addition

Detection: Integrated pulsed amperometry, conventional Au electrode

Postcolumn Reagent Flow: 100 mM NaOH at 0.24 mL/min

IPAD

Background: 40–50 nC

IPAD Noise: 60-70 pC (without suppressor installed)
~210 pC (with suppressor installed)

* The column was equilibrated at 3 mM MSA for 5 min prior to injection.

** This application note discusses three separate detection configurations: IPAD, suppressed conductivity-IPAD, and UV-IPAD.

Waveform

Time(s)	Potential vs pH (V)	Gain Region	Ramp	Integration
0.000	+0.13	Off	On	Off
0.040	+0.13	Off	On	Off
0.050	+0.33	Off	On	Off
0.210	+0.33	On	On	On
0.220	+0.55	On	On	On
0.460	+0.55	On	On	On
0.470	+0.33	On	On	On
0.536	+0.33	Off	On	Off
0.546	–1.67	Off	On	Off
0.576	–1.67	Off	On	Off
0.586	+0.93	Off	On	Off
0.626	+0.93	Off	On	Off
0.636	+0.13	Off	On	Off

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solution

Generate the methanesulfonic acid (MSA) eluent online by pumping high quality deionized water (18 M -cm resistivity or better) through the EGC II MSA cartridge. Chromeleon software will track the amount of MSA used and calculate the remaining lifetime.

Alternatively, prepare 10 mM MSA by carefully adding 0.961 g of concentrated MSA to a 1-L volumetric flask containing about 500 mL of deionized water. Dilute to the mark and mix thoroughly. Prepare 100 mM MSA by carefully adding 9.61 g of concentrated MSA to a 1-L volumetric flask containing about 500 mL of deionized water. Dilute to the mark and mix thoroughly. Degas the eluents and store in plastic labware. The 3 mM MSA eluent is then proportioned between 10 mM MSA and high quality deionized water. The gradient is proportioned between the 100 mM MSA solution and deionized water.

Postcolumn Base Addition Solution for IPAD

100 mM Sodium Hydroxide

Prepare 100 mM sodium hydroxide solution by adding 8 g of 50% w/w NaOH to ~800 mL of degassed deionized water in a 1000 mL volumetric flask and then dilute to volume. *Sodium hydroxide pellets, which are coated with a thin layer of sodium carbonate, must not be used to prepare this solution. The 100 mM NaOH solution should be stored under helium in a pressurized container at all times.*

STANDARD PREPARATION

Prapare biogenic amine stock standard solutions at 1000 mg/L each by dissolving 123.8 mg of dopamine hydrochloride, 100 mg of tyramine, 182.7 mg of putrescine dihydrochloride, 171.4 mg of cadaverine dihydrochloride, 96 mg of histamine, 120.7 mg of serotonin hydrochloride, 172.7 mg of agmatine sulfate, 100 mg of phenylethylamine, 175.3 mg of spermidine trihydrochloride, and 172.1 mg of spermine tetrahydrochloride in separate 100 mL volumetric flasks. Bring to volume with deionized water. Stock solutions should be stored at 4 °C and protected from light. Prepare working standard solutions for generating calibration curves with an appropriate dilution of the stock solutions in 3 mM MSA. These solutions should be prepared fresh weekly and stored at 4 °C when not in use.

SYSTEM PREPARATION AND SETUP

Integrated Pulsed Amperometric Detection

Do not use a continuously regenerated cation trap column (CR-CTC) with IPAD. Install the EGC II MSA cartridge in the EG-3000 and configure the setup of the cartridge with the Chromeleon server configuration. Connect the cartridge to the EG degas assembly and install sufficient backpressure tubing (~91.4 cm of 0.003" i.d.) in place of the column set to produce a system pressure of ~2000 psi at 1 mL/min. Condition the cartridge with 50 mM MSA for 30 min at 1 mL/min. Remove the backpressure tubing temporarily installed in place of the column set and install a 2 × 50 mm CG18 and a 2 × 250 mm CS18 column. Make the sure the backpressure is at an optimal pressure of ~2300 psi when 45 mM MSA is delivered at 0.30 mL/min. Install additional backpressure tubing between the EG degas and injection valve as necessary to achieve an optimal pressure reading. Connect the external water source outlet to the Regen In port of the EG degas and adjust the head pressure on the reservoir to deliver a flow rate of 0.5-1 mL/min (~10-15 psi for a 4 L bottle). Divert the column effluent to waste until the electrochemical cell is properly installed and ready for use. *It is important to verify the external water flow through the degas Regen channel to effectively remove gases generated by the MSA cartridge. Failure to properly remove oxygen from the EG will result in a significant decline in the electrochemical background signal.*

Calibrate the pH electrode according to the instructions provided by the Chromeleon software. Install the Au working electrode in the electrochemical cell and then install a short piece (~25 cm) of black tubing (0.010" i.d.) on the cell outlet. For delivery of the 100 mM NaOH postcolumn reagent, we highly recommend using the DP-3000 to have an accurate and consistent flow rate throughout the analyses. Alternatively, a pressurized reservoir may be used to deliver NaOH to the mixing tee. A comparison between the pump and reservoir resulted in nearly equivalent baseline noise, but the pump was found to deliver a more consistent flow, particularly at the low flow rate described in this application note.

Install sufficient backpressure tubing on the pump used for post column addition to achieve a system pressure of approximately 2000 psi when 100 mM NaOH is delivered at 0.24 mL/min. Connect the outlet of this pump to the mixing tee and install a 125 µL knitted reaction coil between the mixing tee and cell inlet. Set the flow rate at 0.24 mL/min for the postcolumn base addition and turn the pump on with the third port of the mixing tee plugged with a 1/4-28" fitting. Allow the NaOH to flow through the cell for about 10 min and then connect the column outlet to the third port of the mixing tee (previously plugged) while the analytical pump is still running. *Be sure to wear gloves to avoid exposure to MSA solution from the column outlet.*

Program the waveform in the Chromeleon software. Set the waveform mode and reference electrode to IntAmp and pH, respectively. After selecting the waveform, set the cell voltage to the ON position. *Make sure that flow is passing through the cell before turning the voltage to the ON position.* The pH recorded by the reference electrode in the electrochemical cell should be within 12.05–12.40 for the gradient described in this application. A significant deviation from this range may be an indication of excessive reference electrode wear (if addition of the NaOH has been verified), and therefore may require replacement (routinely every 6–12 months for the ICS-3000 cell). However, variations in the pH reading may occur depending on the accuracy of the NaOH concentration. The background should remain within the range 30–70 nC for the conditions described in this application document. Significantly higher or lower values may be an indication of electrode malfunction or contamination within the system.

When turning the system off be sure to disconnect the column outlet from the mixing tee while the pump is still running to prevent backflow of NaOH into the analytical column. Do not allow NaOH to enter the column as this can result in permanent damage.

Suppressed Conductivity–Integrated Pulsed Amperometric Detection

Suppressed conductivity detection can precede IPAD to obtain a dual determination of biogenic amines. Suppressed conductivity detection can also be used independently. Neither of these configurations, however, will allow the detection of dopamine, tyramine, or serotonin, which can be detected by using IPAD independently, or by using UV detection. Prepare the CSRS ULTRA II suppressor by hydrating the membranes with a disposable plastic syringe and push 3 mL of degassed deionized water through the Eluent Out port and 5 mL of degassed deionized water through the Regen In port. Allow the suppressor to stand for approximately 20 min to fully hydrate the suppressor screens and membranes. Install the CSRS ULTRA II suppressor for use in the external water mode by connecting the Regen Out of the suppressor to the Regen In of the EG degas and the Regen In of the suppressor to the external water source. Adjust the head pressure on the reservoir to deliver a flow rate of 1-3 mL/min (20–25 psi for a 4 L bottle). If IPAD is connected in series with the conductivity detector then install a short piece of 0.01" i.d. black tubing (5–6") on the cell outlet. *Do not install red tubing (0.005" i.d.) on the cell outlet because the combined pressure of the electrochemical cell and conductivity cell outlet tubing will result in backflow of NaOH through the suppressor and column. Backflow of NaOH can permanently damage the analytical column.* Connect the black tubing from the cell outlet to the mixing tee while flow is still on for both the postcolumn reagent and analytical column. Follow the setup instructions for the EG, column, and IPAD as previously described.

UV Absorbance–Integrated Pulsed Amperometric Detection

The UV absorbance detector was coupled to IPAD to gain further information on the presence of tyramine. Install the EG, column, and IPAD as previously described. Connect the column outlet to the UV detector cell inlet and the detector outlet to the mixing tee. Set the wavelength to 276 nm. Alternatively, UV can be

used in-line with suppressed conductivity detection to determine whether tyramine is present in the samples. In this configuration, the UV detector must be installed before the suppressor.

SAMPLE PREPARATION

Most alcoholic beverages were diluted two to five times with DI water before analysis. However, due to the formation of sediments in the California Cabernet Sauvignon red and rosé wine samples, centrifugation (6000 rpm, 4 °C, 30 min) was required. The California red wine was then diluted 1:5 with DI water and the rosé wine was injected directly without further preparation.

RESULTS AND DISCUSSION

Separation of Biogenic Amines

Figure 1 shows the separation of a standard mixture of biogenic amines with the column coupled directly to IPAD, suppressed conductivity, or UV detection using the gradient conditions described earlier. The separation was optimized to improve the resolution between histamine, serotonin, and agmatine. Dopamine, tyramine, and serotonin cannot be detected by suppressed conductivity detection because they are uncharged following suppression. Although dopamine, tyramine, and serotonin absorb at 276 nm, only tyramine was monitored by UV detection to confirm its presence in samples that had previously been identified with tyramine by IPAD.

Method Performance

The linearity, limits of detection, and precision of the method using suppressed conductivity detection, IPAD, and UV detection were examined. Dopamine, cadaverine, histamine, serotonin, spermidine, and spermine exhibited a linear peak area response in the range 0.10–5.0 mg/L. The linear range was 0.20–10 mg/L for tyramine, putrescine, and agmatine and 1–20 mg/L for phenylethylamine. The lower linear range limits for IPAD placed after the suppressor was slightly higher due to increased baseline noise. Calibration curves based on peak area response produced correlation coefficients

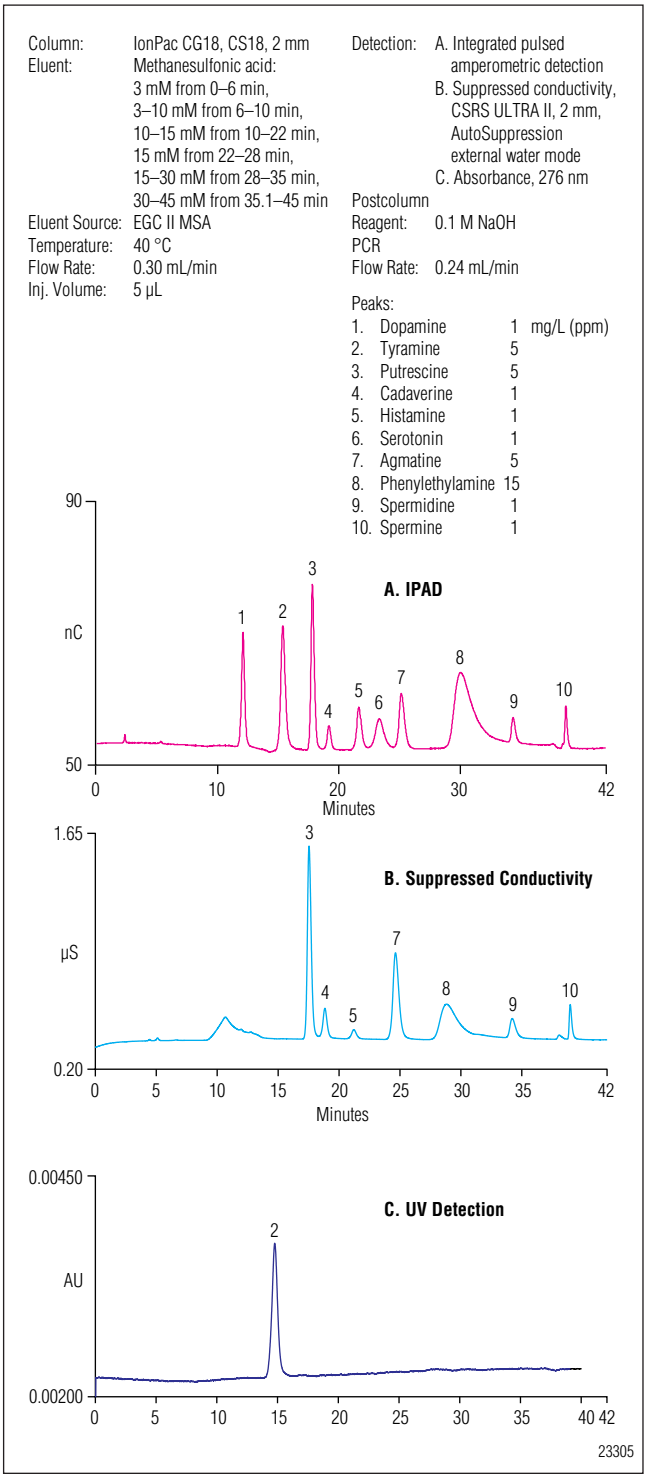

Figure 1. Separation of biogenic amines with (A) IPAD, or (B) suppressed conductivity detection. (C) Tyramine determined by UV detection.

Table 1. Linearity and Limits of Detection of Biogenic Amines												
	IPAD Only			Suppressed Conductivity Detection			IPAD (post suppression)			UV		
Analyte	Range (mg/L)	Linearity (r^2)	LOD (µg/L)	Range (mg/L)	Linearity (r^2)	LOD (µg/L)	Range v	Linearity (r^2)	LOD (µg/L)	Range (mg/L)	Linearity (r_2)	LOD (µg/L)
Dopamine	0.1–5	0.9999	20	—	—	—	—	—	—	—	—	—
Tyramine	0.2–10	0.9999	80	—	—	—	—	—	—	0.2–10	0.9997	110
Putrescine	0.2–10	0.9979	50	0.2–10	0.9986	3.5	0.2–10	0.9974	97	—	—	—
Cadaverine	0.1–5	0.9999	70	0.1–5	0.9997	5.3	0.25–5	0.9997	160	—	—	—
Histamine	0.1–5	0.9999	40	0.1–5	0.9998	18	0.1–5	0.9998	88	—	—	—
Serotonin	0.1–5	0.9998	70	—	—	—	—	—	—	—	—	—
Agmatine	0.2–10	0.9998	170	0.2–10	0.9999	9.0	0.5–10	0.9999	290	—	—	—
Phenyl-ethylamine	1–20	0.9999	400	1–20	0.9999	81	5--20	0.9999	1090	—	—	—
Spermidine	0.1–5	0.9999	80	0.1–5	0.9993	4.0	0.25–5	0.9996	140	—	—	—
Spermine	0.1–5	0.9996	50	0.1–5	0.9990	9.0	0.1–5	0.9998	90	—	—	—

between 0.997–0.999. The detection limits of the biogenic amines were determined by using a signal-to-noise ratio of 3. Table 1 summarizes the linearity and limits of detection (LOD) for the biogenic amines detected by IPAD, suppressed conductivity detection, IPAD (post-suppression), and UV detection. As shown, the LODs were significantly better for most of the biogenic amines detected by suppressed conductivity compared to IPAD. In addition, suppressed conductivity detection produced nearly an order of magnitude lower LODs than HPLC with fluorescence detection, while IPAD was comparable.[2,11] The improvement in sensitivity by suppressed conductivity detection is mainly due to the exceptionally low baseline noise of 0.2–0.3 nS and minimal baseline drift as result of electrolytically generating the MSA eluent online.

The peak area and retention time precisions for the biogenic amines were determined for the different detection configurations (IPAD, suppressed conductivity-IPAD, UV). A standard of biogenic amines containing 5 mg/L each of tyramine, putrescine, and agmatine and 1 mg/L dopamine, cadaverine, histamine, serotonin, spermidine, and spermine was used to determine precision. Replicate injections (n = 10) were performed and the retention time and peak area RSDs were calculated for each amine. Cation-exchange chromatography coupled to IPAD produced retention time and peak area precisions for 10 biogenic amines in the range 0.01–0.07% and 0.79–2.87%, respectively. For suppressed conductivity detection, retention time and peak area precisions for seven biogenic amines were in the range 0.01–0.04% and 0.24–1.29%, respectively. IPAD placed after the suppressor resulted in higher retention time and peak area precisions of 0–0.14% and 1.22–4.97%, respectively due to the increased baseline noise. The retention time and peak area precisions for tyramine detected by UV were 0.17% and 1.28%, respectively.

Determination of Biogenic Amines in Alcoholic Beverages with IPAD

Beer and wine samples can generate complex chromatograms with several unknown peaks that correspond to, or overlap with, the target biogenic amines. For alcoholic beverages, some of these unknowns may include free amino acids, aliphatic amines, aromatic amines, or possibly other components with similar functional groups that are detected electrochemically. The presence of an abundance of unknowns can often complicate the correct identification of the analytes of interest. The separation of the amino acid precursors to the biogenic amines of interest revealed several interferences for the determination of dopamine using the IonPac CS18 column. Therefore, the determination of dopamine by this method was not feasible. In addition, arginine interfered with tyramine, with only a 0.3 min difference in retention times. Further optimization of the gradient

conditions does yield a satisfactory arginine/tyramine resolution (6 mM MSA from 0–3.5 min, 6–27 mM from 3.5–11 min, 27 mM from 11–18 min, 27–45 mM from 18–35 min), but other biogenic amines of interest were not fully resolved by this method. Furthermore, changing the parameters for one sample type may not produce acceptable results for another sample type. Therefore, the presence or absence of tyramine producing a positive identification by IPAD was confirmed by UV detection.

Tyramine was initially detected in all alcoholic beverages by IPAD. These samples were considered suspect for tyramine due to the known interference with arginine and were therefore confirmed by UV absorbance detection. Confirmation of tyramine in the California Cabernet Sauvignon red wine sample by UV detection produced a positive identification for tyramine with a concentration of 2.6 mg/L and spiked recovery of 95% (Figure 2). The result from the same sample using IPAD was 5.6 mg/L, further indicating a high probability of an interferent in the sample. The eluent gradient conditions for the Pinot Grigio wine sample were altered to verify the detection of tyramine. The change in gradient conditions and a secondary confirmation by UV detection indicated that tyramine was not present. The Australian Cabernet Sauvignon red wine and rosé wine samples could not be confirmed by UV due to a very broad (~4 min) unknown interferent that eluted within the same retention time window as tyramine.

Tyramine has been reported as a major biogenic amine in Belgian beer samples, with concentrations of 28.7 ± 17.3 mg/L.[15] Tyramine levels detected in the beer samples by IPAD were within the concentration range of 10–17 mg/L. However, further investigation of these samples by UV detection revealed that no tyramine was present. In our study, all beer samples were also tested spiked with known tyramine concentrations, resulting in calculated recoveries in the range of 86–109%. The acceptable spiked recoveries calculated from these samples indicate that the unknown peak produces a similar electrochemical response to tyramine, further complicating the identification process. This demonstrates the benefit of using multiple detection systems for peak identification in complex matrices.

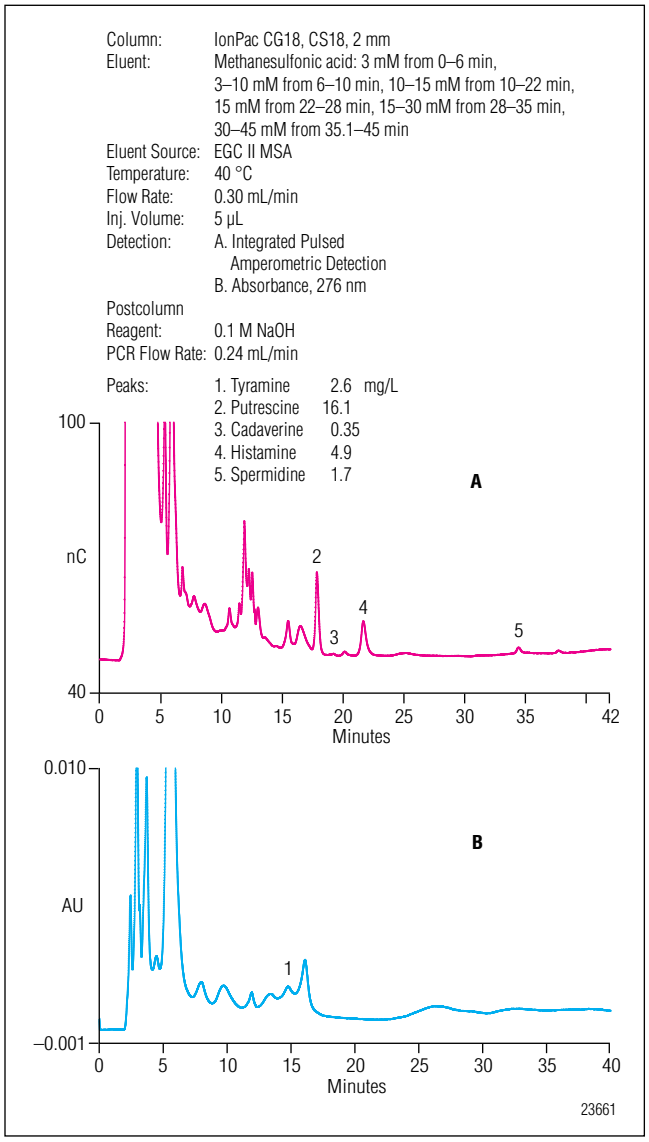

Figure 2. Determination of biogenic amines in a California Cabernet Sauvignon by (A) IPAD and (B) UV absorbance detection.

Table 2. Biogenic Amine Concentrations in Alcoholic Beverages Determined by IPAD[a]

Sample	Tyramine		Putrescine		Cadaverine		Histamine		Serotonin		Agmatine		Spermidine		Spermine	
	Amount Found[b] (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)
Wheat Beer #1	—[c]	—	6.2±0.2	87.1	<DL	—	0.19±0.01	99.9	<DL	—	8.7±0.2	96.8	<DL	—	<DL	—
Wheat Beer #2	<DL[d]	—	4.0±0.1	88.6	<DL	—	0.36±0.02	102.1	<DL	—	6.1±0.1	91.7	<DL	—	<DL	—
Lager Beer	<DL	—	1.9±0.1	90.1	<DL	—	0.39±0.02	104.0	<DL	—	14.4±0.3	95.6	<DL	—	<DL	—
California Cabernet Sauvignon	2.6±0.1[e]	94.7	16.1±0.0	85.3	0.35±0.05	92.9	4.9±0.1	90.6	<DL	—	<DL	—	1.7±0.1	104.1	<DL	—
Australian Cabernet Sauvignon	—	—	5.2±0.1	90.6	0.35±0.02	83.3	0.45±0.02	96.9	<DL	—	<DL	—	<DL	—	<DL	—
Rosé Wine	Int[f]	—	0.36±0.01	84.6	<DL	—	<DL	—	<DL	—	1.2±0.0	100.4	<DL	—	<DL	—
Pinot Grigio	<DL	—	1.3±0.0	97.0	0.68±0.01	94.4	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—

[a]Tyramine was determined by UV absorbance detection.
[b]Average concentration based on triplicate injections.
[c]Unconfirmed.
[d]<DL = less than the detection limit.
[e]Concentration determined after 6 weeks storage at 4 °C.
[f]Int = Chromatographic interference observed in UV detector.

Table 2 summarizes the results obtained for biogenic amines in alcoholic beverages using IPAD. Putrescine was the only biogenic amine detected in all wine samples, but the concentration varied considerably from 0.4–16 mg/L with higher concentrations detected in the red wines compared to the rosé and white wine samples. The highest concentration of histamine (4.9 mg/L) was detected in the California red wine and nearly an order of magnitude lower concentration was detected in the Australian red wine. No histamine was found in either the white or rosé wine samples. Similar results were reported for putrescine in Spanish and Portuguese red wines.[12, 13] Putrescine and histamine are generally found in higher concentrations in red wine where malolactic fermentation (MLF) occurs, compared to white or rosé wines where MLF does not naturally occur or takes place to a lower extent.[11] Histamine has also been found at higher concentrations in red wines with a lower total sulfur dioxide level.[24] Currently, there are no legal maximum tolerable limits for biogenic amines in wine. Although 2 mg/L histamine in wine has been suggested as a permissible limit,[3] many European countries have recommended limits in the range of 3–10 mg/L.[9] The histamine concentration found in the California red wine in this study was still significantly less than the 20 mg/L concentration described as producing physiological effects in humans.[11]

Cadaverine was detected at <1 mg/L in the red and white wine samples. Agmatine was only detected in the rosé wine at a concentration of 1.2 mg/L and spermidine was found in the California red wine with a concentration of 1.7 mg/L. Spermidine is a ubiquitous polyamine that is involved in a number of physiological processes, such as cell division, fruit development, and response to stress.[4] The occurrence of spermidine in wine may be derived from grapes or yeast lysis while the different quantities in different wines could be related to the harvest conditions, such as temperature, rain, and soil nutrients, among other possibilities.[13]

The total biogenic amine concentrations varied considerably among the four wines from 1.6–25.7 mg/L. The California red wine contained the highest total biogenic amine concentration while the white and rosé wines had almost equally low biogenic amine concentrations of 2 mg/L and 1.6 mg/L, respectively. Red wines commonly contain higher concentrations of amines as a result of the MLF process.[25] The recoveries of the biogenic amines were determined by spiking known concentrations of the target biogenic amines in the wine samples that resulted in calculated recoveries within 83–104% using the IonPac CS18 column coupled to IPAD.

The analysis of three different bottled beers revealed the presence of putrescine, histamine, and agmatine in all samples. The concentration ranges detected in the beer samples were 2–4 mg/L putrescine, 0.2–0.4 mg/L histamine, and 6–14 mg/L agmatine. Putrescine, agmatine, spermidine, and spermine are considered natural beer constituents that are present in malt and yeast at higher concentrations than in hops.[10] The putrescine concentrations in our beer samples were within the normal range of 0.2–8.0 mg/L reported for European beers.[26] Relatively little variability was observed for the histamine concentration between beer samples. The presence of histamine has previously been used as an indication of lactic acid bacteria contamination during the brewing process.[10] The histamine concentrations found in our samples were significantly lower relative to the other amines present and are not considered to represent any toxicological hazard. The total biogenic concentrations for each beer were not significantly different and were within the range 10–17 mg/L. Loret et. al. proposed a beer biogenic amine index (BAI) to assess the quality of the production process.[15] The BAI is calculated by taking the ratio of the biogenic amines of bacterial origin (i.e. tyramine, putrescine, cadaverine, histamine, phenylethylamine, and tryptamine) to the natural biogenic amine found in malt (agmatine). The BAIs calculated for our beer samples were <1, which indicates a non-contaminated fermentation process (high microbiological quality). Overall, recoveries for the spiked beer samples were within 87–104% using IPAD.

Determination of Changes in Biogenic Amine Concentrations in Alcoholic Beverages during Storage at 4 °C Using Suppressed Conductivity-IPAD

Table 3 summarizes the results obtained by suppressed conductivity-IPAD for most alcoholic beverages previously analyzed. The biogenic amine concentrations were determined after sample storage at 4 °C for up to three weeks. Nearly all amine concentrations increased after storage. Cadaverine, however, was not detected in the white wine after storage for one week. The most interesting result was the detection of agmatine and spermine that were not previously observed before storage. The detection of these amines was at least partially due to the improvement in sensitivity by suppressed conductivity resulting in about 5–10 times lower LODs for agmatine and spermine.

In the Australian red wine, spermidine increased from 0 to 1.4 mg/L after two weeks storage at 4 °C compared to no change in spermidine for the California red wine after three weeks storage. The putrescine concentration increased 20 to 36% for the three wine samples. Cadaverine increased 50 to 125% in the red wine samples, but completely diminished in the white wine sample. For histamine, the concentration increased 12% for the California red wine and 87% for the Australian red wine. The observed increases in putrescine, cadaverine, and histamine concentrations upon storage in our study were in agreement with previous findings for bottled wine samples stored at 4 °C, with the exception of the disappearance of cadaverine in our white wine sample.[27]

There are numerous variables that can affect the formation or degradation of biogenic amines by bacteria in wines. Therefore, it is difficult to determine the exact cause of the observed changes among the same or different types of wines. However, the increase in histamine at the beginning of the storage period is speculated to occur as a result of MLF and the decarboxylase activity of the microorganisms that remain in the wine.[27] Wine samples spiked with known quantities of the target biogenic amines produced recoveries within 88–122% using suppressed conductivity detection.

A direct comparison could not be made between the biogenic amines detected by suppressed conductivity and the amines detected by IPAD after suppression due to significant differences in the LODs. In terms of the biogenic amines that were detected in the wine samples by IPAD in this configuration, the concentration ranges were 1.5–22.1 mg/L putrescine, 0.67–5.4 mg/L histamine, and 1.5–2.0 mg/L spermidine. For suppressed conductivity detection, the concentrations were in the range 1.7–19.4 mg/L putrescine, 0.84–5.5 mg/L histamine, and 1.4–1.9 mg/L spermidine. Therefore, no significant differences in concentrations were observed between the two detectors in series. Configuring the electrochemical cell after the suppressor can be advantageous for monitoring method performance for biogenic amines detected at higher concentrations and can also be used to evaluate the performance of the Au electrode over time by comparing the concentrations determined by the two detectors in series.

Table 3. Biogenic Amine Concentrations in Stored Alcoholic Beverages Determined by Suppressed Conductivity Detection and IPAD

Suppressed Conductivity Detection

Sample	Putrescine		Cadaverine		Histamine		Agmatine		Spermidine		Spermine	
	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)
Wheat Beer #1[a]	6.4±0.0	96.0	0.28±0.02	91.4	0.54±0.02	95.4	9.1±0.0	102.3	0.45±0.01	101.0	0.47±0.02	113.0
Wheat Beer #2[b]	6.6±0.0	95.8	0.67±0.00	88.5	0.60±0.01	99.0	7.7±0.0	102.4	1.2±0.0	104.0	0.73±0.01	117.5
Lager Beer[c]	3.0±0.0	101.2	<DL[d]	—	0.72±0.03	98.2	14.9±0.1	104.8	0.14±0.01	104.3	0.33±0.02	—
California Cabernet Sauvignon[a]	19.4±0.1	97.6	0.79±0.00	103.1	5.51±0.06	103.7	0.37±0.00	89.3	1.9±0.0	101.6	0.19±0.01	121.9
Australian Cabernet Sauvignon[b]	7.1±0.1	95.8	0.53±0.01	88.5	0.84±0.03	99.0	0.23±0.02	95.8	1.4±0.0	104.0	0.21±0.02	100.0
Pinot Grigio[c]	1.7±0.0	103.5	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—

IPAD (post suppression)

Sample	Putrescine		Cadaverine		Histamine		Agmatine		Spermidine		Spermine	
	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)
Wheat Beer #1	6.2±0.1	96.0	<DL	—	<DL	—	8.7±0.2	94.0	0.42±0.00	100.6	0.48±0.01	97.3
Wheat Beer #2	5.8±0.1	95.6	<DL	—	<DL	—	7.2±0.1	87.2	1.2±0.0	95.4	0.67±0.03	110.8
Lager Beer	3.0±0.0	94.6	<DL	—	<DL	—	14.5±0.1	93.7	<DL	—	<DL	—
California Cabernet Sauvignon	22.1±0.4	106.5	<DL	—	5.4±0.2	99.3	<DL	—	2.0±0.1	100.6	<DL	—
Australian Cabernet Sauvignon	6.9±0.3	103.6	<DL	—	0.67±0.04	98.1	<DL	—	1.5±0.0	104.9	<DL	—
Pinot Grigio	1.5±0.1	100.7	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—

Stored at 4 °C for [a]3 weeks, [b]1 week, [c]2 weeks.
d<DL = less than the detection limit.

The biogenic amines in the beer samples were also determined by suppressed conductivity-IPAD after storage at 4 °C. The most significant changes in the biogenic amine concentrations after one to three weeks storage were the detection of cadaverine in the wheat beer and spermidine and spermine in all beer samples that were not detected prior to storage. As previously discussed, the detection of these amines is at least partially due to the improvement in sensitivity by suppressed conductivity detection. The wheat beer #2 produced the largest evolution of biogenic amines with increases in putrescine, histamine, and agmatine of 65%, 67%, and 26%, respectively. The range of concentration increase for all beer samples during storage was 0–65% for putrescine, 67-184% for histamine, and 26% for agmatine. Figure 3 shows a separation of biogenic amines determined in

wheat beer #2 using suppressed conductivity detection. Wheat beer #1 produced the most significant increase in histamine (184%) relative to the other beer samples. This observation is in agreement with a previous study that demonstrated a significant increase of histamine for a bottle beer sample stored at 21 °C for eight days.[28] The presence of lactic acid bacteria, primarily lactobacilli, has been demonstrated to be the primary cause for histamine increase in bottled beers over time.[28] Recalculating the BAI for all beers after storage at 4 °C results in an index value of <1 for wheat beer #1 and the lager beer and a value of 1.0 for wheat beer #2. According to the authors, a BAI between 1.0 and 10.0 would indicate that the beer had been produced by fermentation procedures that could be moderately contaminated by decarboxylating bacteria (intermediate level of microbiological quality).[15] The average amine recoveries for the spiked beer samples were in the range 88–118%. The calculated concentrations by IPAD in series with the suppressor were within ±12% of the concentrations determined by suppressed conductivity.

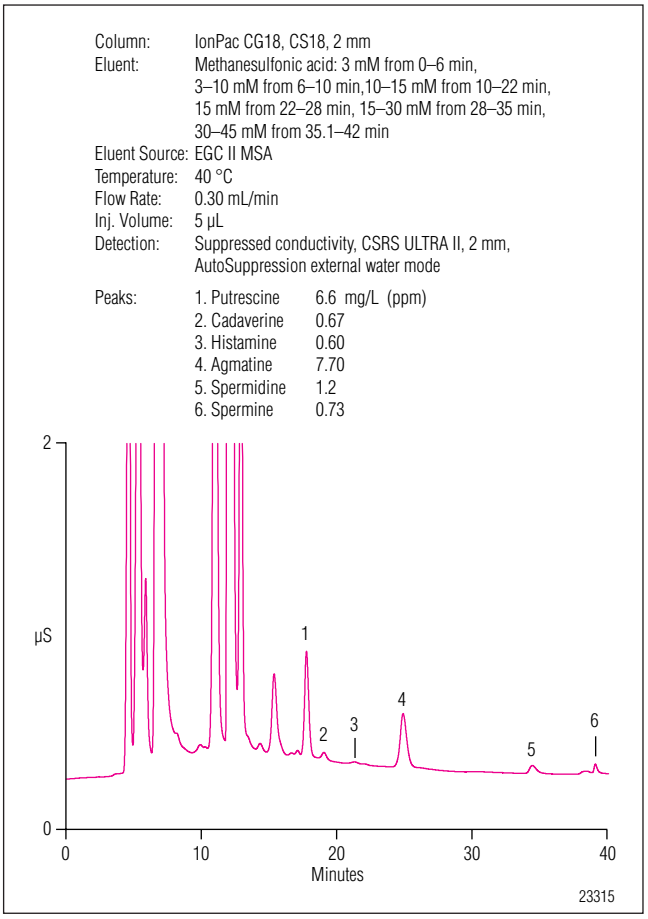

Figure 3. Determination of biogenic amines in wheat beer #2 using suppressed conductivity detection.

CONCLUSION

The described method demonstrates the use of the IonPac CS18 column for the separation of several target biogenic amines in alcoholic beverages. Simple MSA gradient conditions provide suitable compatibility for use with suppressed conductivity detection, IPAD, and UV detection to further examine and characterize the presence of biogenic amines in alcoholic beverages. Suppressed conductivity detection demonstrates good precision and recovery for many of the biogenic amines and superior sensitivity compared to previously reported methods in the literature. In addition, this detection technique provides the simplest approach for determining biogenic amines compared to methods requiring complex derivatizing procedures that are often prone to errors. IPAD provides a wider selectivity than suppressed conductivity and good sensitivity for many of the biogenic amines of interest. The addition of UV detection adds confidence to the analytical results by confirming the presence or absence of tyramine in the alcoholic beverages. The combination of three detection configurations described demonstrates the versatility and potential of cation-exchange chromatography for determining hydrophobic amines in complex matrices.

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Determination of Biogenic Amines in Fermented and Non-Fermented Foods Using Ion Chromatography with Suppressed Conductivity and Integrated Pulsed Amperometric Detections

INTRODUCTION

Biogenic amines are biologically active compounds with aliphatic (putrescine, spermidine, spermine), aromatic (dopamine, tyramine, phenylethylamine), or heterocyclic (histamine, serotonin) structures. Several biogenic amines have critical roles in human and animal physiological functions,[1] such as regulation of body temperature, stomach volume, stomach pH, and brain activity.[2] Polyamines such as putrescine, spermidine, and spermine are important in the synthesis of proteins, RNA, and DNA, and are therefore essential for cell proliferation and growth.[3,4] Several studies have revealed higher concentrations of biogenic amines in cancer patients compared to healthy individuals.[5] The inhibition of the biosynthesis of these amines in tumor-bearing patients is a major area of cancer therapy research. [3-5]

The formation of biogenic amines requires 1) free amino acids or proteins, 2) microorganisms that can decarboxylate amino acids, and 3) conditions that promote microbial activity.[6] Biogenic amines in food and food products are related to food spoilage and safety.[1] Consumption of low concentrations of biogenic amines in the average diet is not dangerous, but high concentrations can result in hypotension (histamine, putrescine, cadaverine), hypertension (tyramine), migraines (tyramine, phenylethylamine), nausea, rash, dizziness, increased cardiac output, and increased respiration.[6,7] Biogenic amines are known to occur in a wide variety of fermented and non-fermented foods, such as fish, meat, dairy, fruits, vegetables, and chocolate.[4] The determination of biogenic amines in food products is critical to assess potential health risks before consumption.

In the past three decades, several analytical methods have been introduced for the determination of biogenic amines in a wide variety of food and beverage matrices. These determinations are often accomplished by reversed-phase HPLC followed by UV or fluorescence detection. Because most biogenic amines lack a suitable chromophoric or fluorophoric group, however, either pre- or postcolumn chemical derivatization is required for detection. The most common derivatizing agents are dansyl chloride,[7,8-10] benzoyl chloride,[11-14] and o-phthalaldehyde (OPA).[8,15] These derivatization procedures are time-consuming, laborious, can produce potential by-product interferences, and sometimes under- or over-estimate the amount of amines.[9,16]

Pulsed amperometric detection (PAD) with a multi-potential waveform has demonstrated good sensitivity for the detection of underivatized biogenic amines. A conventional three-step waveform provides analyte detection by forming a gold oxide (AuO) layer, cleaning the electrode through exhaustive reduction of the electrode surface, reactivating the oxidative surface, and reducing the AuO back to Au.[17] The current generated from the initial oxidation of the gold surface contributes to the background, baseline noise, and baseline instability. To minimize this effect and enhance the amine signal on the gold oxide surface, integrated pulsed amperometric detection (IPAD) was introduced.[18] The advantage of using IPAD is that the current is continuously integrated during working electrode oxidation and during removal of the oxide surface, thus minimizing baseline disturbances.[18,19] IPAD has recently been used to detect biogenic amines in chocolate.[20]

Ion chromatography (IC) has not commonly been used for the determination of biogenic amines. This is due to the strong hydrophobic interactions between protonated amino groups and the stationary phase of many columns, resulting in long retention times and poor peak symmetry. To alleviate this problem, either high concentrations of an acidic eluent or an organic solvent are required to elute the amines from the column. Unfortunately, these eluents preclude the use of suppressed conductivity detection, the most common detection technique associated with IC. The introduction of a weak cation-exchange column, the IonPac® CS17, specifically designed for the separation of hydrophobic amines using a simple acidic eluent and no organic solvent, allows the determination of biogenic amines by suppressed conductivity detection.[21] The IonPac CS17 combined with suppressed conductivity detection has been used for the successful determination of biogenic amines in fish tissue[22] and meat[23] samples.

The stationary phase of the IonPac CS18 column is slightly more hydrophobic than that of the CS17 and therefore provides better resolution between close eluting peaks, such as putrescine and cadaverine. This column was used with suppressed conductivity and IPAD, configured separately and in tandem, for the determination of biogenic amines in selected food products. Because absorbance detection can provide selective detection of certain compounds that have aromatic character, such as tyramine, UV in combination with IPAD was also used to confirm or refute the presence of tyramine in suspect samples. The linear ranges, limits of detection, precisions, and recoveries of biogenic amines spiked in fermented (dairy, meat) and non-fermented (fish) products were analyzed and compared using suppressed conductivity detection and IPAD.

EQUIPMENT

Dionex ICS-3000 system consisting of:

- DP Dual Pump with in-line degas option
- DC Detector/Chromatography module (dual temperature zones) with conductivity and electrochemical cells
- The electrochemical cell consisted of a pH/Ag/AgCl reference electrode and a conventional Au electrode (PN 063722)
- EG Eluent Generator module
- EluGen® EGC II MSA cartridge (P/N 058902)
- AD25 UV-Vis Absorbance Detector with 10-mm cell
- Mixing tee, 3-way, 1.5 mm i.d. (P/N 024314)
- Knitted reaction coil, 125 µL (P/N 053640)
- Two 4-L plastic bottle assemblies for external water mode of operation

Chromeleon® 6.7 Chromatography Management Software

Blender (household or industrial strength type)

Centrifuge (Beckman Coulter, Brea, CA)

Vortex mixer (Fisher Scientific)

REAGENTS AND STANDARDS

Reagents

Deionized water, type I reagent grade, 18 MΩ-cm resistivity or better

Sodium hydroxide, 50% (w/w) (Fisher Scientific, SS254-1)

Methanesulfonic acid, 99% (Dionex Corporation, P/N 033478)

Trichloroacetic acid, ≥99.5% (Fluka Chemical Co., Sigma-Aldrich P/N 91228)

Standards

Dopamine hydrochloride (Sigma Chemical Co., H8502)

Serotonin hydrochloride, ≥98% (Sigma Chemical Co., H9523)

Tyramine, 99% (Aldrich Chemical Co., T90344)

Putrescine dihydrochloride, ≥98% (Sigma Chemical Co., P7505)

Cadaverine dihydrochloride, >98% (Sigma Chemical Co., C8561)

Histamine, ~97% (Sigma Chemical Co., H7125)

Agmatine sulfate, 97% (Aldrich Chemical Co., 101443)

ß-phenylethylamine, 99% (Aldrich Chemical Co., 128945)

Spermidine trihydrochloride, >98% (Calbiochem, 56766)

Spermine tetrahydrochloride, ≥99% (Calbiochem, 5677)

CONDITIONS

Columns:	IonPac CS18 Analytical, 2 × 250 mm (P/N 062878)
	IonPac CG18 Guard, 2 × 50 mm (P/N 062880)
Eluent:*	3 mM MSA from 0–6 min, 3–10 mM from 6–10 min, 10–15 mM from 10–22 min, 15 mM from 22–28 min, 15–30 mM from 28–35 min, 30–45 mM from 35.1–45 min
Eluent Source:	EG Eluent Generation module
Flow Rate:	0.30 mL/min
Temperature:	40 °C (lower compartment)
	30 °C (upper compartment)
Inj. Volume:	5 µL
Detection:**	Suppressed conductivity, CSRS® ULTRA II (2 mm), AutoSuppression® external water mode, power setting– 40 mA and/or UV-Vis detection set at 276 nm
Background Conductance:	0.4–0.5 µS
Conductance Noise:	0.2–0.3 nS
System Backpressure:	~2500 psi

**The column was equilibrated at 3 mM MSA for 5 min prior to each injection.*

***This application note discusses three separate detection configurations: IPAD, suppressed conductivity-IPAD, and UV-IPAD.*

Postcolumn Addition:

Detection:	Integrated pulsed amperometry, conventional Au electrode
Postcolumn Reagent Flow:	100 mM NaOH at 0.24 mL/min
IPAD Background:	40–50 nC
IPAD Noise:	60–70 pC (without suppressor installed)
	~210 pC (with suppressor installed)

Waveform

Time (s)	Potential (V vs. pH)	Gain Region	Ramp	Integration
0.000	+0.13	Off	On	Off
0.040	+0.13	Off	On	Off
0.050	+0.33	Off	On	Off
0.210	+0.33	On	On	On
0.220	+0.55	On	On	On
0.460	+0.55	On	On	On
0.470	+0.33	On	On	On
0.536	+0.33	Off	On	Off
0.546	-1.67	Off	On	Off
0.576	-1.67	Off	On	Off
0.586	+0.93	Off	On	Off
0.626	+0.93	Off	On	Off
0.636	+0.13	Off	On	Off

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solution

Generate methanesulfonic acid (MSA) online by pumping high quality deionized water (18 MΩ-cm resistivity or better) through the EGC II MSA cartridge. Chromeleon software will track the amount of MSA used and calculate the remaining lifetime.

Alternately, prepare 10 mM MSA by adding 0.961 g of concentrated MSA to a 1-L volumetric flask containing approximately 500 mL of deionized water. Bring to volume and mix thoroughly. Prepare 100 mM MSA by adding 9.61 g of concentrated MSA to a 1-L volumetric flask containing approximately 500 mL of deionized water. Bring to volume and mix thoroughly. Degas the eluents and store in plastic labware. The 3 mM MSA eluent is produced by proportioning between 10 mM MSA and deionized water. The gradient is proportioned between the 100 mM MSA solution and deionized water.

Postcolumn Base Addition Solution for IPAD

100 mM Sodium Hydroxide

Prepare 100 mM sodium hydroxide solution by adding 8 g of 50% w/w NaOH to approximately 800 mL of degassed deionized water in a 1-L volumetric flask and bring to volume. Sodium hydroxide pellets, which are coated with a thin layer of sodium carbonate, must not be used to prepare this solution. The 100 mM NaOH solution should be stored under helium in a pressurized container at all times.

Acid Extraction Solutions

100 mM Methanesulfonic Acid

Add 4.81 g of MSA to a 500-mL volumetric flask containing approximately 300 mL of deionized water. Bring to volume and mix thoroughly. Store solution in plastic labware.

5% and 1.5% Trichloroacetic Acid

Prepare 5% trichloroacetic acid (TCA) by adding 25 g of trichloroacetic acid to a 500-mL volumetric flask containing about 300 mL of deionized water. Bring to volume and mix thoroughly. Store the solution in plastic labware. Prepare 1.5% TCA by adding 30 mL of the 5% trichloroacetic acid solution to a 100-mL volumetric flask containing approximately 50 mL deionized water. Bring to volume and mix thoroughly. Store solution in plastic labware.

STANDARD AND SAMPLE PREPARATION

Standards

Prepare biogenic amine stock standard solutions at 1000 mg/L each by dissolving 123.8 mg of dopamine hydrochloride, 100 mg of tyramine, 182.7 mg of putrescine dihydrochloride, 171.4 mg of cadaverine dihydrochloride, 96 mg of histamine, 120.7 mg of serotonin hydrochloride, 172.7 mg of agmatine sulfate, 100 mg of phenylethylamine, 175.3 mg of spermidine trihydrochloride, and 172.1 mg of spermine tetrahydrochloride in separate 100-mL volumetric flasks. Bring each to volume with deionized water. Store stock solutions at 4 °C and protected from light. Prepare working standard solutions for generating the calibration curve with an appropriate dilution of the stock solutions in 3 mM MSA. These solutions should be prepared fresh weekly and stored at 4 °C when not in use.

Samples

Previous studies have found that TCA is a good acid for extracting fish and meat samples because it is highly effective for precipitating proteins.[9] The canned tuna and sausage samples were prepared by adding 5 g of ground sample to separate 50-mL centrifuge tubes, followed by 20 mL of 1.5% (sausage) or 5% (tuna) TCA. The mixtures were homogenized on a vortex mixer for 1 min and centrifuged at 6000 rpm for 20 min at 4 °C. The supernatants were decanted and filtered with a 0.2-µm filter into separate 50-mL volumetric flasks. An additional 20 mL aliquot of TCA was added to each tube and the extraction procedure was repeated. The supernatants were again filtered into their respective flasks, and each flask was brought to volume with deionized water. The canned tuna extract was further diluted 1:5 with deionized water before analysis.

The cheddar and Swiss cheese extracts were prepared as described above, except 100 mM MSA was used in place of TCA. Each extract was diluted 1:1 with deionized water before analysis.

SYSTEM PREPARATION AND SETUP

Integrated Pulsed Amperometric Detection

Do not use a continuously regenerated cation trap column (CR-CTC) with IPAD.

Install the EGC II MSA cartridge in the EG Eluent Generator module and configure the setup of the cartridge with the Chromeleon server configuration. Connect the cartridge to the EG degas assembly. In place of the column set, install sufficient backpressure tubing (~91.4 cm of 0.003 in. i.d.) to produce a system pressure of ~2000 psi at 1 mL/min. Condition the cartridge with 50 mM MSA for 30 min at 1 mL/min. Remove the backpressure tubing and install a 2 x 50 mm CG18 and 2 x 250 mm CS18 column. Confirm the backpressure is ~2300 psi when 45 mM MSA is delivered at 0.30 mL/min. Install additional backpressure tubing between the EG degas and injection valve as necessary to achieve this pressure. Connect the external water source outlet to the Regen In of the EG degas and adjust the head pressure on the reservoir to deliver a flow rate of 0.5-1 mL/min (10-15 psi for a 4 L bottle). Divert the column effluent to waste until the electrochemical cell is properly installed and ready for use.

It is important to verify the external water flow through the degas Regen channel to effectively remove gases generated by the MSA cartridge. Failure to properly remove oxygen from the EG will result in significant noise in the electrochemical background signal.

Calibrate the pH electrode according to the instructions provided by the Chromeleon software. Install the Au working electrode in the electrochemical cell and then install ~25 cm of black tubing (0.010 in. i.d.) on the cell outlet. For delivery of the 100 mM NaOH postcolumn reagent, we recommend using the DP Dual Pump to maintain an accurate and consistent flow rate. Alternatively, a pressurized reservoir may be used to deliver NaOH to the mixing tee. A comparison between the pump and reservoir resulted in nearly equivalent baseline noise, but the pump was found to deliver a more consistent flow, particularly at low flow rates.

Install sufficient backpressure tubing on the pump used for postcolumn addition to achieve a system pressure of approximately 2000 psi when 100 mM NaOH is delivered at 0.24 mL/min. Connect the outlet of this pump to the mixing tee and install a 125-µL knitted reaction coil between the mixing tee and cell inlet. Plug the third port of the mixing tee with a 1/4-28 in. fitting. Set the flow rate at 0.24 mL/min for the postcolumn base addition and turn the pump on. Allow the NaOH to flow through the cell for about 10 min and then connect the column outlet to the third port of the mixing tee (previously plugged) while the analytical pump is still running. *Be sure to wear gloves to avoid exposure to MSA solution from the column outlet.*

Using the Chromeleon software, set the waveform mode and reference electrode to IntAmp and pH, respectively. After selecting the waveform, set the cell voltage to the ON position. *Confirm that eluent is flowing through the cell before turning the voltage to the ON position.* The pH recorded by the reference electrode in the electrochemical cell should be within 12.05–12.40 for the gradient described in this application. If the pH deviates significantly from this range, first verify NaOH addition by testing the column effluent with pH indicating paper. Next check the accuracy of the NaOH concentration. Deviations may indicate excessive reference electrode wear and may require reference electrode replacement (routinely required every 6–12 months for the ICS-3000 cell). The background should remain within the range of 30–70 nC for the conditions described in this application document. Significantly higher or lower values may indicate electrode malfunction or contamination within the system. When turning the system off be sure to disconnect the column outlet from the mixing tee while the pump is still running to prevent backflow of NaOH into the analytical column. DO NOT allow NaOH to enter the column as this can result in permanent damage.

Suppressed Conductivity-Integrated Pulsed Amperometric Detection

Suppressed conductivity detection can precede IPAD for a dual detection method to determine biogenic amines. Alternatively, suppressed conductivity detection can be used independently from IPAD. Neither configuration, however, will allow the detection of dopamine, tyramine, or serotonin.

Prepare the CSRS ULTRA II suppressor by hydrating its membranes. Fill a disposable plastic syringe with degassed deionized water. Push 3 mL of the deionized water through the Eluent Out port and 5 mL through the Regen In port. Allow the suppressor to stand for approximately 20 min to fully hydrate the suppressor screens and membranes. Install the CSRS ULTRA II suppressor for use in the external water mode by connecting the Regen Out of the suppressor to the Regen In of the EG degas and the Regen In of the suppressor to the external water source. Adjust the head pressure on the reservoir to deliver a flow rate of 1–3 mL/min (20–25 psi for a 4-L bottle). If IPAD is connected in series with the conductivity detector then install 5–6 in. of 0.01 in. i.d. black tubing on the cell outlet. Do not install red tubing (0.005 in. i.d.) on the cell outlet because the combined pressure of the electrochemical cell and conductivity cell outlet tubing will result in backflow of NaOH through the suppressor and column. CAUTION: Backflow of NaOH can permanently damage the analytical column. Connect the black tubing from the cell outlet to the mixing tee while flow is still on for both the post-column reagent and analytical column. Follow the setup instructions for the EG, column, and IPAD as previously described.

UV Absorbance–Integrated Amperometric Detection

The UV absorbance detector was coupled to IPAD to gain further information on the presence of tyramine. Install the EG, column, and IPAD as previously described. Connect the column outlet to the UV detector cell inlet and the cell outlet to the mixing tee. Set the wavelength

to 276 nm. Alternatively, UV can be used in-line with suppressed conductivity detection to determine if tyramine is present in the samples. In this configuration, the UV detector must be installed before the suppressor.

RESULTS AND DISCUSSION

Separation and Detection of Biogenic Amines

The IonPac CS18 cation-exchange column is more hydrophobic and has a slightly lower exchange capacity (290 µequiv/column, 250 × 2 mm) than the IonPac CS17. This increased hydrophobicity enables better resolution of close eluting peaks, such as putrescine/cadaverine. Figure 1 shows the separation of biogenic amines with suppressed conductivity, integrated pulsed amperometric, and UV detections (not connected in series). Dopamine, tyramine, and serotonin cannot be detected by suppressed conductivity because they lack a positive charge after suppression. Therefore, IPAD was required to detect all 10 biogenic amines. Although dopamine, tyramine, and serotonin all absorb at 276 nm, only tyramine was monitored by UV detection to confirm its presence in samples that had previously been identified as containing tyramine by IPAD.

Electrolytically generated MSA eluent was used to simplify the method and streamline the process of developing an optimum gradient for the separation of the target biogenic amines. An electrolytically generated eluent has not been used with IPAD in previous studies due to the production of oxygen during generation of the MSA eluent. Dissolved oxygen in the eluent stream can result in significant changes in the background signal and therefore should be removed. Oxygen was removed by passing the eluent stream through the eluent channel and external water through the Regen channel of the EG degas device. This appeared to remove the oxygen created by the EG as no erratic changes in the background were observed. The EG simplified the method development by requiring only the addition of DI water, thus avoiding potential errors and inconsistencies that can occur when manually preparing eluents off-line.

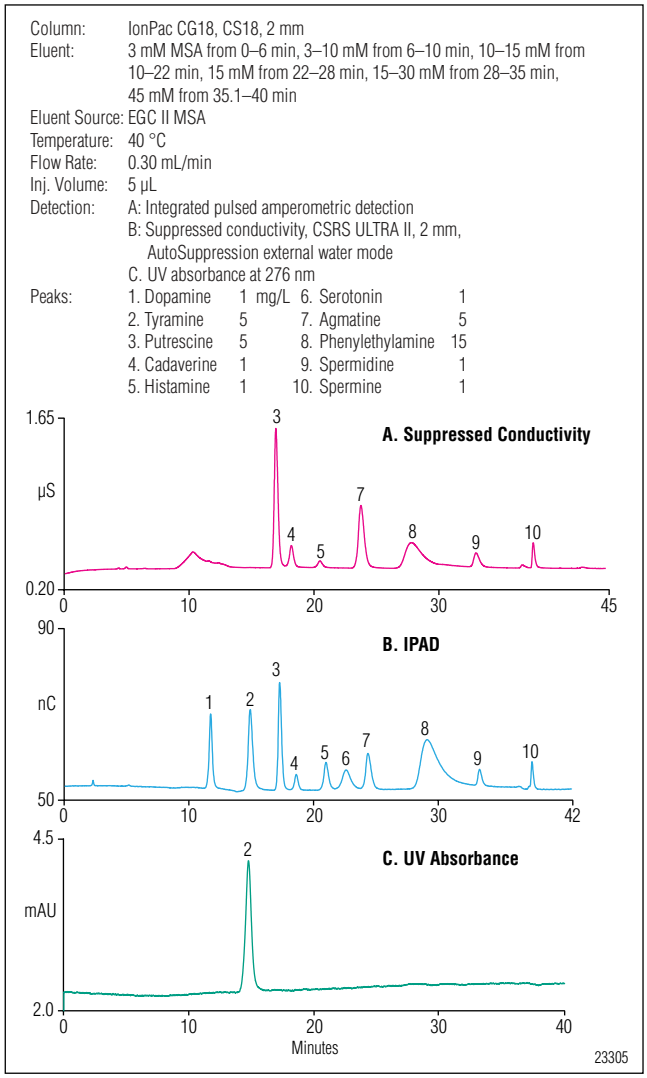

Figure 1. Separation of biogenic amines and detection by (A) suppressed conductivity, (B) IPAD, and (C) tyramine by UV detection.

Table 1. Linearity and Limits of Detection of Biogenic Amines

	IPAD			Suppressed Conductivity Detection			IPAD (post-suppression			UV		
Analyte	**Range (mg/L)**	**Linearity (r^2)**	**LOD (µg/L)**	**Range (mg/L)**	**Linearity (r^2)**	**LOD (µg/L)**	**Range (mg/L)**	**Linearity (r^2)**	**LOD (µg/L)**	**Range (mg/L)**	**Linearity (r^2)**	**LOD (µg/L)**
Dopamine	0.1–5	0.9999	20	0.1–5	—	—	—	—	—	—	—	—
Tyramine	0.2–10	0.9999	80	0.2–10	—	—	—	—	—	0.2–10	0.9997	110
Putrescine	0.2–10	0.9979	50	0.2–10	0.9986	3.5	0.2–10	0.9974	97	—	—	—
Cadaverine	0.1–5	0.9999	70	0.1–5	0.9997	5.3	0.25–5	0.9997	160	—	—	—
Histamine	0.1–5	0.9999	40	0.1–5	0.9998	18.0	0.1–5	0.9998	88	—	—	—
Serotonin	0.1–5	0.9998	70	—	—	—	—	—	—	—	—	—
Agmatine	0.2–10	0.9998	170	0.2–10	0.9999	9.0	0.5–10	0.9999	290	—	—	—
Phenylethylamine	1–20	0.9999	400	1–20	0.9999	81.0	5–20	0.9999	1090	—	—	—
Spermidine	0.1–5	0.9999	80	0.1–5	0.9993	4.0	0.25–5	0.9996	140	—	—	—
Spermine	0.1–5	0.9996	50	0.1–5	0.9990	9.0	0.1–5	0.9998	90	—	—	—

Table 2. Intraday Retention Time and Peak Area Precisions of Biogenic Amines

	IPAD		Suppressed Conductivity Detection		IPAD (post-suppression		UV	
Analyte	**Retention time (RSD[a])**	**Peak Area (RSD**	**Retention time (RSD)**	**Peak Area (RSD**	**Retention time (RSD)**	**Peak Area (RSD**	**Retention time (RSD)**	**Peak Area (RSD)**
Dopamine	0.03	1.18	—	—	—	—	—	—
Tyramine	0.03	1.53	—	—	—	—	0.17	1.28
Putrescine	0.03	0.79	0.01	0.24	0.02	1.22	—	—
Cadaverine	0.04	2.86	0.01	1.29	0.06	4.97	—	—
Histamine	0.03	1.88	0.01	0.95	0.04	4.80	—	—
Serotonin	0.07	1.92	—	—	—	—	—	—
Agmatine	0.04	1.61	0.01	0.50	0.04	2.67	—	—
Phenylethylamine	0.07	1.94	0.04	0.29	0.14	1.83	—	—
Spermidine	0.03	2.87	0.01	0.53	0.04	3.97	—	—
Spermine	0.01	2.48	0.01	0.56	0.00	2.82	—	—

[a]RSD = relative standard deviation where n = 10

System Performance

The detection limits, linearity, reproducibility, and precision were measured over the course of 15 days to determine the robustness of this method. The calibration data and LODs for the three detection configurations are summarized in Table 1. The peak area and retention time relative standard deviations were determined for replicate injections of a standard biogenic amine solution containing 5 mg/L each of tyramine, putrescine, and agmatine and 1 mg/L each of dopamine, cadaverine, histamine, serotonin, spermidine, and spermine. Intraday precision was evaluated by performing 10 consecutive injections of the standard amine solution (Table 2). For a detailed description of these experiments and their results, please see Appendix A.

Determination of Biogenic Amines in Food Products with IPAD

The acid extraction of food samples not only removes biogenic amines but also extracts free amino acids, non-biogenic aliphatic and aromatic amines, and other electrochemically active components. This can produce complex chromatograms with a significant number of unknown peaks, some with retention times that overlap with or match the analytes of interest.

Most amino acids are weakly retained on the IonPac CS18 column and therefore do not interfere with many of the biogenic amines. Dopamine and tyramine, however, are also weakly retained. The determination of dopamine in food products was not feasible by this method due to several amino acids coeluting with

dopamine. Arginine, a direct amino acid precursor to agmatine, interfered with tyramine using the gradient conditions previously described. Several attempts were made to optimize the chromatographic conditions to resolve arginine and tyramine, but this decreased the resolution of other biogenic amines of interest. In addition, changing the parameters for one sample type may not yield satisfactory results for another sample type. Therefore, we recommend optimizing the gradient conditions for a particular sample type or combining two detectors in series, such as UV and IPAD. Because this document describes the determination of biogenic amines in a wide range of different food products, the use of UV was the most feasible approach for verifying the presence or absence of tyramine in samples that IPAD suggested contained tyramine.

A variety of fermented and non-fermented fresh and spoiled food products were assayed for the presence of biogenic amines by IPAD (Table 3). Tyramine, spermidine, and spermine were detected at concentrations of 33.4, 16.6, and 17.9 mg/kg, respectively, in unstored canned albacore tuna. After six weeks storage of the canned tuna at 4 °C, IPAD detected a tyramine concentration of 58.4 mg/kg compared to 33.4 mg/kg detected by UV. The higher concentration observed with the electrochemical detector was suspected to be due to an interferent. Analysis of this same sample by suppressed conductivity detection revealed an unknown peak within the same retention time window as tyramine. Because tyramine, like arginine, cannot be detected by suppressed conductivity, this confirmed the presence of an interferent. The unknown peak was detected by IPAD and suppressed conductivity, but did not absorb at 276 nm, so it could be an aliphatic amine.

Although histamine is the most critical biogenic amine implicated in food poisoning from fish, no histamine was detected in our sample using suppressed conductivity detection. According to the FDA Office of Regulatory Affairs, a "decomposed product (determined organoleptically) does not always produce histamine…"[25] To determine the potential for further increase of biogenic amines in the canned tuna, the product was stored at room temperature (~25 °C) for one week to allow spoilage. The spoiled tuna contained several chromatographic interferents for histamine, spermidine, and spermine and therefore these analytes could not be determined. However, putrescine could be determined and increased significantly from 0 to 102.9 mg/kg. Putrescine and cadaverine have been reported as indicators of seafood decomposition[25] and can enhance the toxicity of histamine by inhibiting the histamine metabolizing enzyme.[14]

Significant differences in biogenic amine concentrations were observed between the cheddar and

Table 2. Biogenic Amine Concentrations in Food Products Determined by IPAD[a]

Sample	Tyramine		Putrescine		Cadaverine		Histamine		Serotonin		Agmatine		Spermidine		Spermine	
	Amount Found[b] (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)
Canned Tuna	33.4±1.3[b]	95.3	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—	16.6±0.6	98.0	17.9±1.0	92.5
Spoiled Canned Tuna[c]	<DL[d]	—	103±1	109.7	<DL	—	int	—	<DL	—	<DL	—	<DL	—	<DL	—
Cheddar Chese	154±4[e]	100.6	<DL	—	<DL	—	12.5±0.04	92.3	<DL	—	<DL	—	<DL	—	<DL	—
Spoiled Cheddar Cheese[c]	2.6±0.1[e]	94	55.2±0.7	107.5	<DL	—	15.2±0.3	100.2	<DL	—	<DL	—	<DL	—	<DL	—
Swiss Cheese	706±4[e]	85.2	5.7±0.3	96.7	13.6±0.04	97.8	68.5±3.3	91.8	<DL	—	<DL	—	<DL	—	<DL	—
Spoiled Swiss Cheese[c]	1835±19[e]	102.6	3.4±0.2	105.9	82.2±2.5	101.4	7.2±0.3	100.2	<DL	—	<DL	—	<DL	—	<DL	—
Smoked Sausage	<DL	—	int[f]	—	<DL	—	<DL	—	<DL	—	8.9±0.5	103.2	8.9±0.4	99.9	58.1±0.7	95.7

[a]Tyramine determined by either UV or IPAD as noted.
[b]UV absorbance value determined after 6 weeks of storage at 4 °C.
[c]Stored at room temperature for one week.
[d]<DL = less than the detection limit.
[e]confirmed UV absorbance, value shown was determined by IPAD.
[f]int = chromatographic interference.

Swiss cheese samples. Tyramine and histamine were detected in cheddar cheese at concentrations of 154.1 and 12.5 mg/kg, respectively. In Swiss cheese, tyramine, putrescine, cadaverine, and histamine were detected at concentrations of 706, 5.7, 13.6, and 68.5 mg/kg, respectively. Upon spoilage after one week of storage at 25 °C, tyramine increased by approximately 320% in the cheddar cheese and 160% in the Swiss cheese. In the cheddar cheese, putrescine increased from undetected to 55 mg/kg, but no significant change in histamine was observed. For the spoiled Swiss cheese, putrescine decreased slightly from 5.7 to 3.4 mg/kg, histamine decreased from 68.5 to 7.2 mg/kg, and cadaverine increased from 13.6 to 82.2 mg/kg.

The accuracy of the tyramine results reported by IPAD was confirmed by reanalyzing the cheese samples (after one month storage at 4 °C) by combining UV and IPAD. Due to the increased storage time, microbial activity was observed in both cheese samples. For the refrigerated cheddar cheese sample, the calculated tyramine concentrations were 312 and 349 mg/kg for UV and IPAD, respectively. Tyramine concentrations determined in Swiss cheese by UV and IPAD were 716 and 747 mg/kg, respectively. The concentration differences of approximately ±10% suggested that the original determinations by IPAD were accurate. The microbial activities in the cheese samples were increased by storage at room temperature for one week to verify the absence of any potential tyramine interferents. Comparing UV to IPAD for the spoiled cheddar cheese sample, the tyramine concentrations were 1061 and 1063 mg/kg, respectively. For the spoiled Swiss cheese, the concentrations were 1139 and 1157 mg/kg, respectively for UV and IPAD. This provides further confirmation that the results originally reported using only IPAD for the previously spoiled cheeses were accurate. Figure 2 shows the confirmation of tyramine in spoiled Swiss cheese by IPAD and UV detection.

The polyamines spermidine and spermine, and to a lesser extent putrescine, are the primary amines reported in sausages.[26] In the smoked sausage sample, spermidine was the most abundant biogenic amine detected, followed by equal concentrations of agmatine and spermine. Agmatine is an intermediate product to putrescine from

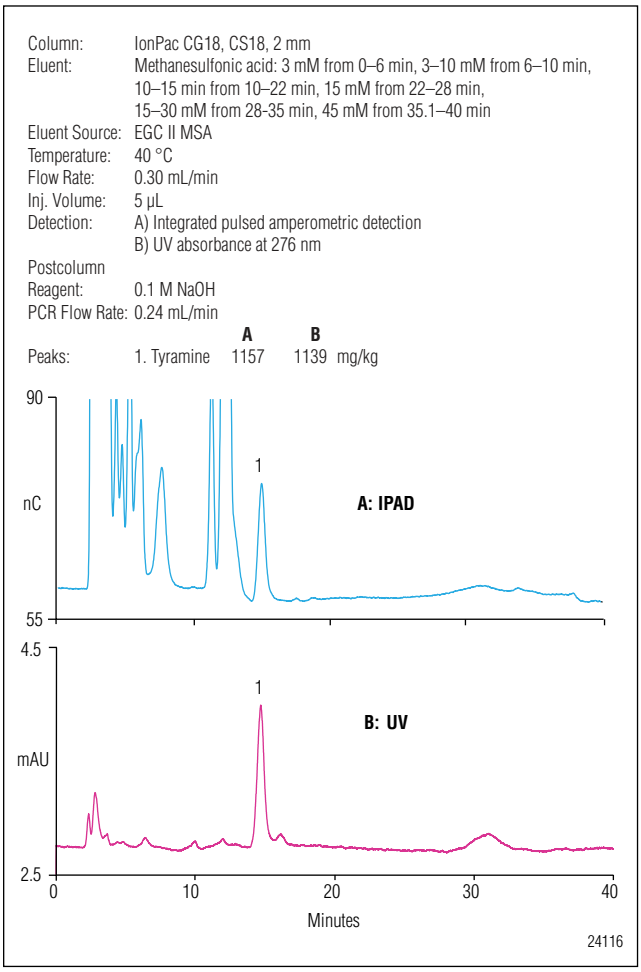

Figure 2. Determination of biogenic amines in spoiled Swiss cheese by (A) IPAD and (B) UV detection.

arginine and therefore trace amounts of putrescine may be expected in the sample. An initial analysis of the sausage extract by IPAD revealed significantly high concentrations of putrescine (>3000 mg/kg). Because most data in the literature report relatively trace concentrations of putrescine in sausages, further confirmation was required. Analysis by suppressed conductivity showed no interferent, indicating that an electrochemically active compound, one not detected by suppressed conductivity, has the same retention time, or coelutes with, putrescine. This was the only sample where an unknown peak was shown to interfere with the determination of putrescine.

Table 4. Biogenic Amine Concentrations in Food Products Determined by Suppressed Conductivity and IPAD

Suppressed Conductivity Detection

Sample	Putrescine		Cadaverine		Histamine		Agmatine		Spermidine		Spermine	
	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)
Canned Tuna[a]	<DL[b]	—	<DL	–	<DL	—	<DL	—	9.6±0.03	96.0	20.1±0.9	104.5
Spoiled Canned Tuna[c]	162.7±0.4	100.5	6.2±0.2	94.1	<DL	101.4	11.5±0.0	100.2	49.9±0.2	94.4	int[e]	—
Smoked Sausage[d]	0.65±0.0	100	<DL	—	<DL	—	8.2±0.0	101.4	7.6±0.1	101.4	46.6±0.1	104.0
Spoiled Sausage[c]	9.5±0.1	101.8	3.1±0.1	88.2	1.6±0.1	89.7	6.9±0.1	96.8	14.3±0.2	103.2	32.1±0.6	100.3

IPAD (post suppression)

Sample	Putrescine		Cadaverine		Histamine		Agmatine		Spermidine		Spermine	
	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)
Canned Tuna[a]	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—	22.1±0.6	100.8
Spoiled Canned Tuna[c]	148.7±0.6	93.0	<DL	—	<DL	—	<DL	—	49.1±2.8	90.3	int	—
Smoked Sausage[d]	<DL	—	<DL	—	<DL	—	<DL	—	7.1±0.2	106.4	46.1±0.6	104.0
Spoiled Sausage[c]	8.9±0.4	96.2	<DL	—	<DL	—	<DL	—	13.5±0.8	102.9	33.5±1.5	100.3

[a]Stored at 4 °C for three weeks.
[b]<DL = less than the detection limit.
[c]Stored at 25 °C for two weeks.
[d]Stored at 4 °C for two weeks.
[e]int = chromatographic interference.

Changes in Biogenic Amine Concentrations in Food Products during Storage at 4 and 25 °C Detected Using Suppressed Conductivity-IPAD

A selection of food products was used for the determination of biogenic amines by suppressed conductivity-IPAD (Table 4). Samples were stored at 4 °C for one to three weeks prior to analysis, with the exception of the spoiled canned tuna and spoiled sausage samples, which were stored at 25 °C for two weeks to allow spoilage. For the canned tuna stored at 4 °C, the spermidine concentration increased 73% compared to only a marginal change in the spermine concentration. Spoilage of the canned tuna resulted in the detection of putrescine, cadaverine, agmatine, and spermidine. An interferent precluded the detection of spermine. Putrescine and spermidine produced the most significant increases in concentration, from 0 to 162.7 mg/kg and 9.6 to 49.9 mg/kg, respectively. Cadaverine and agmatine were not previously detected in spoiled tuna using IPAD alone. It is unclear whether these biogenic amines were detected because of the improved sensitivity of suppressed conductivity or if they were generated during the additional week of storage at 25 °C compared to the initial study.

Storage of the smoked sausage at 4 °C for two weeks resulted in decreases in the concentrations of agmatine,

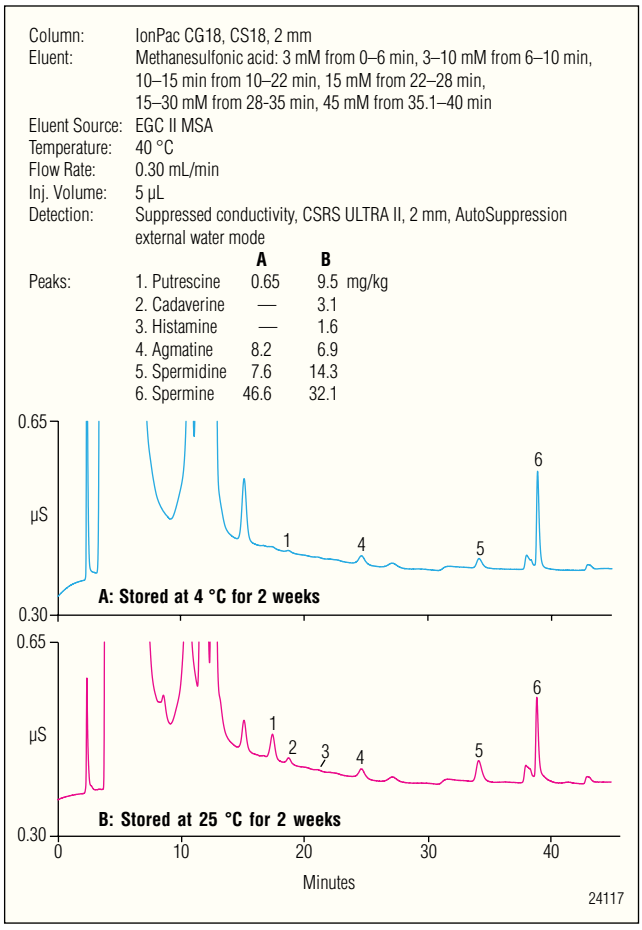

Figure 3. Determination of biogenic amines in spoiled sausage by IC with suppressed conductivity detection.

spermine, and spermidine. Similar concentrations for the physiological polyamines putrescine, spermidine, and spermine have been reported for fermented sausages.[27] Storage at 25 °C for two weeks produced significant changes in the content and concentrations of the biogenic amines. Figure 3 shows a separation of biogenic amines in spoiled sausage using suppressed conductivity detection. Cadaverine and histamine were not observed in the sausage stored at 4 °C, but evolved during storage at 25 °C. Vidal-Carou demonstrated that the histamine concentration in meat products increases more rapidly at room temperature than refrigerated temperature during the same time period.[28] Putrescine and spermidine increased significantly at room temperature whereas agmatine and spermine decreased. Similar trends for putrescine and spermine were observed by Bover-Cid et. al. in fermented sausages stored at 19 °C.[27] The presence of putrescine and cadaverine in meat products has been related to lysine and ornithine decarboxylase activity in Enterobacteriaceae. The concentrations of putrescine, spermidine, and spermine determined by IPAD following suppression were within the standard deviations of the concentrations determined by suppressed conductivity.

CONCLUSION

The IonPac CS18, a polymeric weak acid cation-exchange column, was used to separate biogenic amines in a variety of fermented and non-fermented food samples, with detection by IPAD, suppressed conductivity, and UV. The described method uses a simple electrolytically generated MSA eluent without requiring the use of solvents or aggressive eluent systems that have previously been reported. In addition, the method results in good precision and recovery over a wide range of sample matrices and avoids the need for complex and long derivatization procedures. The use of three different detection configurations provides additional information and confirms the identification of tyramine to increase confidence in the analytical results. Suppressed conductivity had exceptionally low LODs for the main biogenic amines of interest without chromatographic interferences from common cations and amines present in many of the food samples. In addition to the amines detected by conductivity, IPAD allows the detection of dopamine, serotonin, and tyramine, which can be confirmed with a UV detector.

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APPENDIX A: LINEARITY, PRECISION, AND REPRODUCIBILITY

Linear Ranges and Limits of Detection

The linear ranges for suppressed conductivity, IPAD, and UV detection were evaluated by tabulating peak area versus concentration. Calibration curves were prepared for each biogenic amine in 3 mM MSA using five increasing concentrations. Dopamine, cadaverine, histamine, serotonin, spermidine, and spermine were tested in the range of 0.10–5.0 mg/L. For tyramine, putrescine, and agmatine the linearity was determined in the 0.20–10 mg/L range. Phenylethylamine's linearity was determined in the range of 1–20 mg/L. The increase in baseline noise upon placing the electrochemical cell after the suppressor resulted in an increase in the lower linear range limits for some biogenic amines. The correlation coefficients (using a least squares linear regression fit) were between 0.997 and 0.999. The limits of detection (LODs) were determined based on the slopes of the calibration curves using three times the average baseline noise (S/N = 3). The LODs using suppressed conductivity, IPAD, and IPAD after suppression were in the ranges of 3.5–81 µg/L, 20–400 µg/L, and 88–1090 µg/L, respectively. Suppressed conductivity detection was approximately 2–20 times more sensitive than IPAD and the LODs increased by a factor of two when IPAD was placed after the suppressor. The calibration data and LODs for the three detection configurations are summarized in Table 1.

Precision and Reproducibility

The peak area and retention time relative standard deviations (RSDs) were determined for replicate injections of a standard biogenic amine solution containing 5 mg/L each of tyramine, putrescine, and agmatine and 1 mg/L each of dopamine, cadaverine, histamine, serotonin, spermidine, and spermine. Intraday precision was evaluated by performing 10 consecutive injections of the standard amine solution (Table 2). Retention time RSDs were 0.01–0.17% and the peak area precisions were 0.24–4.97%. In general, the peak area precisions were better using suppressed conductivity

detection and the highest RSDs were observed for IPAD installed after the suppressor. The higher RSDs for IPAD in this configuration are expected due to the increase in baseline noise caused by the suppressor.

The between-day precision was determined for the conventional gold electrode using IPAD coupled directly to the analytical column. Because a significant loss in peak area can indicate a reduction in the gold layer of the working electrode, the standard solution was injected intermittently over 15 days of continuous sample analysis to monitor the electrode response over time. Figure 4 shows the peak area trend for biogenic amines over 15 consecutive days. Dopamine, cadaverine, serotonin, spermidine, and spermine exhibited the least change in peak area responses for the first five consecutive days of analysis (71 total injections) with percent changes in the range of 0% to -6.5% (n = 25). The between-day precision for these biogenic amines during the same time period ranged from 2.0 to 6.7%. For tyramine, putrescine, and agmatine the changes in peak area response during the first five days were higher than the other amines, resulting in percent changes ranging from -9.3% to -14.8%.

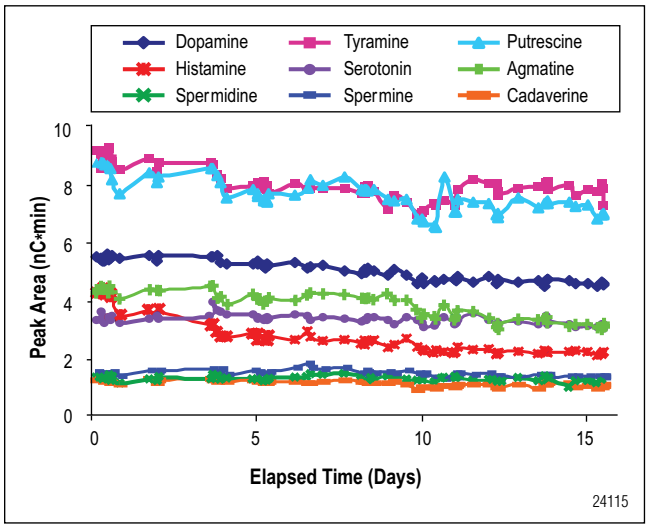

Figure 4. Peak area reproducibility of biogenic amines detected by IPAD over 15 days of continuous analysis.

The most significant decreases in peak area occurred after 96 h of continuous operation, with the exceptions of putrescine and histamine. These changed mainly during the first 24 h, with putrescine decreasing by 10% and histamine decreasing by 18%. Histamine had the most significant five day decrease in peak area response compared to all other biogenic amines, with a percent change of -38%.

Histamine's peak area decreases were less significant during the second week, with a percent change of -13%. Spermidine and spermine had the lowest change in response during the second week with a peak area standard deviation of ±2%. The peak area RSDs for these biogenic amines during this time span were 5.1% and 7.2%, respectively. The changes in peak area were greater for dopamine, cadaverine, and serotonin during the second week with a percent change in the range of -7.9% to -16.1%. The change in response for the third week was within ±5% for all biogenic amines except agmatine, which experienced a change in peak area response of -11.1%.

These results indicate that with continuous use of the gold working electrode, the recession of the gold layer will decrease over time, thereby reducing the loss in peak area response. In addition, previous studies have shown that a working electrode with significant gold recession can still generate accurate analytical results despite loss in sensitivity and changes in the slopes of the calibration curves.[24] It is unclear why histamine experienced a significantly greater loss in peak area response compared to other biogenic amines. To assure the integrity of the analytical results, a standard biogenic amine solution was injected before and after the sample replicates and the recoveries were calculated. A decrease in peak area response by 10–15% necessitated recalibration of the system before continuing with the analysis. Based on the results previously discussed, the system required calibration at least weekly. However, no samples required a repeat analysis due to a loss in peak area response.

The between-day precision for suppressed conductivity detection was determined over 12 days. The peak area RSDs over the 190 injections of samples or standards performed during that time ranged from 1.0 to 4.6% (n=44) for putrescine, cadaverine, histamine, agmatine, spermidine, and spermine. The lowest and highest RSD values were observed for agmatine and histamine, respectively. The Au working electrode was also monitored with the suppressor inline after previously being used continuously for three weeks. Because there was already a significant loss of gold, a smaller decrease in peak area was expected. The change in peak area response over the first five consecutive days for IPAD after suppression ranged from -5% to +5% with the exception of histamine peak area which decreased 16.6%. This is a significant improvement compared to earlier results due to prior use of the working electrode. The between-day peak area precision was in the range of 4.9% to 6.2% during this same time period for all of the biogenic amines except histamine (9.1%).

The between-day retention time precision was also evaluated for IPAD and suppressed conductivity-IPAD over 15 and 12 consecutive days, respectively. For IPAD, the retention time precision was in the range of 0.12 to 1.0% over the specified time. The percent change in retention time from the beginning to the end of this time period ranged from -0.3% to -4.3%. The between-day retention time precision for suppressed conductivity-IPAD was in the range of 0.04 to 0.39% with a decrease in retention time of <0.3% over 12 consecutive days.

Determination of Biogenic Amines in Fruit, Vegetables, and Chocolate Using Ion Chromatography with Suppressed Conductivity and Integrated Pulsed Amperometric Detections

INTRODUCTION

Biogenic amines play critical roles in human and animal physiological functions, and are related to food spoilage and safety.[1] Consumption of low concentrations of biogenic amines in the average diet is not dangerous, but consumption of high concentrations can result in hypotension (histamine, putrescine, cadaverine), hypertension (tyramine), migraines (tyramine, phenylethylamine), nausea, rash, dizziness, increased cardiac output, and increased respiration.[2,3] Biogenic amines are known to occur in a wide variety of foods, such as fish, meat, dairy, fruits, vegetables, and chocolate.[4] The determination of biogenic amines in food products is critical to assess potential health risks before consumption.

Determinations of biogenic amines are often accomplished by reversed-phase HPLC followed by UV or fluorescence detection. Because most biogenic amines lack a suitable chromophoric or fluorophoric group, however, either pre- or postcolumn chemical derivatization is required for detection. The most common derivatizing agents are dansyl chloride,[3,5-7] benzoyl chloride,[8-11] and o-phthalaldehyde (OPA).[5,12] These derivatization procedures are time-consuming, laborious, can produce potential by-product interferences, and sometimes under- or over-estimate the amount of amines.[6,13]

Application Note 183 (AN 183) describes the use of the IonPac® CS18, a weak acid cation-exchange column, with suppressed conductivity, integrated pulsed amperometric detection (IPAD), and UV for the detection of underivatized biogenic amines in meat and cheese. The CS18 allows separation of biogenic amines without the use of highly-concentrated acidic eluents or organic solvents while still providing resolution of closely eluting peaks such as putrescine and cadaverine. The milder separation conditions allow the use of suppressed conductivity to detect many underivatized biogenic amines. IPAD can detect these same amines, as well as those that lack a charge after suppression, and has recently been used to detect biogenic amines in chocolate.[14] UV can confirm the presence and concentrations of weakly-retained aromatic biogenic amines that coelute with other amines and amino acids. In this update, the procedures described in AN 183 are used to determine biogenic amine levels in kiwi fruit, spinach, and chocolate.

EQUIPMENT

Dionex ICS-3000 system consisting of:

- DP Dual Pump with in-line degas option
- DC Detector/Chromatography module (dual temperature zones) with conductivity and electrochemical cells
- Electrochemical cell consisting of a pH/Ag/AgCl reference electrode and a conventional Au electrode (PN 063722)
- EG Eluent Generator module
- EluGen® EGC II MSA cartridge (P/N 058902)
- AD25 UV-Vis Absorbance Detector with 10-mm cell
- Mixing tee, 3-way, 1.5 mm i.d. (P/N 024314)
- Knitted reaction coil, 125 µL (P/N 053640)
- Two 4-L plastic bottle assemblies for external water mode of operation

Chromeleon® 6.7 Chromatography Management Software

Blender (household or industrial strength type)

Centrifuge (Beckman Coulter, Brea, CA)

Vortex mixer (Fisher Scientific)

REAGENTS AND STANDARDS

Reagents

Deionized water, type I reagent grade, 18 MΩ-cm resistivity or better

Sodium hydroxide, 50% (w/w) (Fisher Scientific, SS254-1)

Methanesulfonic acid, 99% (Dionex Corporation, P/N 033478)

Standards

Dopamine hydrochloride (Sigma Chemical Co., H8502)

Serotonin hydrochloride, ≥98% (Sigma Chemical Co., H9523)

Tyramine, 99% (Aldrich Chemical Co., T90344)

Putrescine dihydrochloride, ≥98% (Sigma Chemical Co., P7505)

Cadaverine dihydrochloride, >98% (Sigma Chemical Co., C8561)

Histamine, ~97% (Sigma Chemical Co., H7125)

Agmatine sulfate, 97% (Aldrich Chemical Co., 101443)

ß-phenylethylamine, 99% (Aldrich Chemical Co., 128945)

Spermidine trihydrochloride, >98% (Calbiochem, 56766)

Spermine tetrahydrochloride, ≥99% (Calbiochem, 5677)

CONDITIONS

Columns:	IonPac CS18 Analytical, 2 × 250 mm (P/N 062878) IonPac CG18 Guard, 2 × 50 mm (P/N 062880)
Eluent:*	3 mM MSA from 0–6 min, 3–10 mM from 6–10 min, 10–15 mM from 10–22 min, 15 mM from 22–28 min, 15–30 mM from 28–35 min, 30–45 mM from 35.1–45 min
Eluent Source:	EG Eluent Generation module
Flow Rate:	0.30 mL/min
Temperature:	40 ºC (lower compartment) 30 ºC (upper compartment)
Injection Volume:	5 µL
Detection:**	Suppressed conductivity, CSRS® ULTRA II (2 mm), AutoSuppression® external water mode, power setting–40 mA and/or UV-Vis detection set at 276 nm
Background Conductance:	0.4–0.5 µS
Conductance Noise:	0.2–0.3 nS
System Backpressure:	~2500 psi

Postcolumn Addition:

Detection:	Integrated pulsed amperometry, conventional Au electrode
Postcolumn Reagent Flow:	100 mM NaOH at 0.24 mL/min
IPAD Background:	40–50 nC
IPAD Noise:	60–70 pC (without suppressor installed) ~210 pC (with suppressor installed)

**The column was equilibrated at 3 mM MSA for 5 min prior to each injection.*

***This application note discusses three separate detection configurations: IPAD, suppressed conductivity-IPAD, and UV-IPAD.*

Waveform

Time (s)	Potential (V vs. pH)	Gain Region	Ramp	Integration
0.000	+0.13	Off	On	Off
0.040	+0.13	Off	On	Off
0.050	+0.33	Off	On	Off
0.210	+0.33	On	On	On
0.220	+0.55	On	On	On
0.460	+0.55	On	On	On
0.470	+0.33	On	On	On
0.536	+0.33	Off	On	Off
0.546	-1.67	Off	On	Off
0.576	-1.67	Off	On	Off
0.586	+0.93	Off	On	Off
0.626	+0.93	Off	On	Off
0.636	+0.13	Off	On	Off

SYSTEM PREPARATION AND SETUP

Installation of the IPAD, suppressed conductivity, and UV detectors is described in detail in AN 183. When working with MSA and NaOH, be sure to wear gloves to prevent exposure. Follow all precautions to prevent backflow of NaOH, as this can result in permanent damage to the column.

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solution

Generate methanesulfonic acid (MSA) online by pumping high quality deionized water (18 MΩ-cm resistivity or better) through the EGC II MSA cartridge. Chromeleon software will track the amount of MSA used and calculate the remaining lifetime.

Alternately, prepare 10 mM MSA by adding 0.961 g of concentrated MSA to a 1-L volumetric flask containing approximately 500 mL of deionized water. Bring to volume and mix thoroughly. Prepare 100 mM MSA by adding 9.61 g of concentrated MSA to a 1-L volumetric flask containing approximately 500 mL of deionized water. Bring to volume and mix thoroughly. Degas the eluents and store in plastic labware. The 3 mM MSA eluent is produced by proportioning between 10 mM MSA and deionized water. The gradient is proportioned between the 100 mM MSA solution and deionized water.

Postcolumn Base Addition Solution for IPAD

100 mM Sodium Hydroxide

Prepare 100 mM sodium hydroxide (NaOH) solution by adding 8 g of 50% w/w NaOH to approximately 800 mL of degassed deionized water in a 1-L volumetric flask and bring to volume. NaOH pellets, which are coated with a thin layer of sodium carbonate, must not be used to prepare this solution. The 100 mM NaOH solution should be stored under helium in a pressurized container at all times.

Acid Extraction Solutions

100 mM Methanesulfonic Acid

Add 4.81 g of MSA to a 500-mL volumetric flask containing approximately 300 mL of deionized water. Bring to volume and mix thoroughly. Store solution in plastic labware.

5% and 1.5% Trichloroacetic Acid

Prepare 5% trichloroacetic acid (TCA) by adding 25 g of TCA to a 500-mL volumetric flask containing about 300 mL of deionized water. Bring to volume and mix thoroughly. Store the solution in plastic labware.

Prepare 1.5% TCA by adding 30 mL of the 5% TCA solution to a 100-mL volumetric flask containing approximately 50 mL deionized water. Bring to volume and mix thoroughly. Store solution in plastic labware.

STANDARD PREPARATION

Prepare biogenic amine stock standard solutions at 1000 mg/L each by dissolving 123.8 mg of dopamine hydrochloride, 100 mg of tyramine, 182.7 mg of putrescine dihydrochloride, 171.4 mg of cadaverine dihydrochloride, 96 mg of histamine, 120.7 mg of serotonin hydrochloride, 172.7 mg of agmatine sulfate, 100 mg of phenylethylamine, 175.3 mg of spermidine trihydrochloride, and 172.1 mg of spermine tetrahydrochloride in separate 100-mL volumetric flasks. Bring each to volume with deionized water. Store stock solutions at 4 °C and protected from light. Prepare working standard solutions for generating the calibration curve with an appropriate dilution of the stock solutions in 3 mM MSA. These solutions should be prepared fresh weekly and stored at 4 °C when not in use.

SAMPLE PREPARATION

Spinach

Spinach extracts were prepared by adding 5 g of ground sample to a 50-mL centrifuge tube, followed by 20 mL of 100 mM MSA. The mixture was homogenized on a vortex mixer for 1 min and centrifuged at 6000 rpm for 20 min at 4 ºC. The supernatant was decanted and filtered with a 0.2-µm filter into a 50-mL volumetric flask. An additional 20 mL aliquot of MSA was added to the tube and the extraction procedure was repeated. The supernatant was again filtered into the flask, and the flask was brought to volume with deionized water. The extract was further diluted 1:1 with deionized water before analysis.

Kiwi Fruit and Chocolate (70% Cocoa)

The kiwi fruit and 70% cocoa chocolate extracts were prepared by adding 5 g of ground sample to separate 50-mL centrifuge tubes followed by 10 mL of 100 mM MSA. The samples were extracted as described for the spinach and the undiluted extracts were analyzed.

Dark and Milk Chocolate

The chocolate extracts were prepared by adding 2 g of ground sample to separate 15-mL centrifuge tubes followed by 4 mL of 100 mM MSA. These mixtures were homogenized with a vortex mixer for 1 min and centrifuged at 6000 rpm for 30 min at 4 ºC. The supernatants were removed and filtered with a 0.2-µm filter into separate flasks and diluted 1:1 with deionized water.

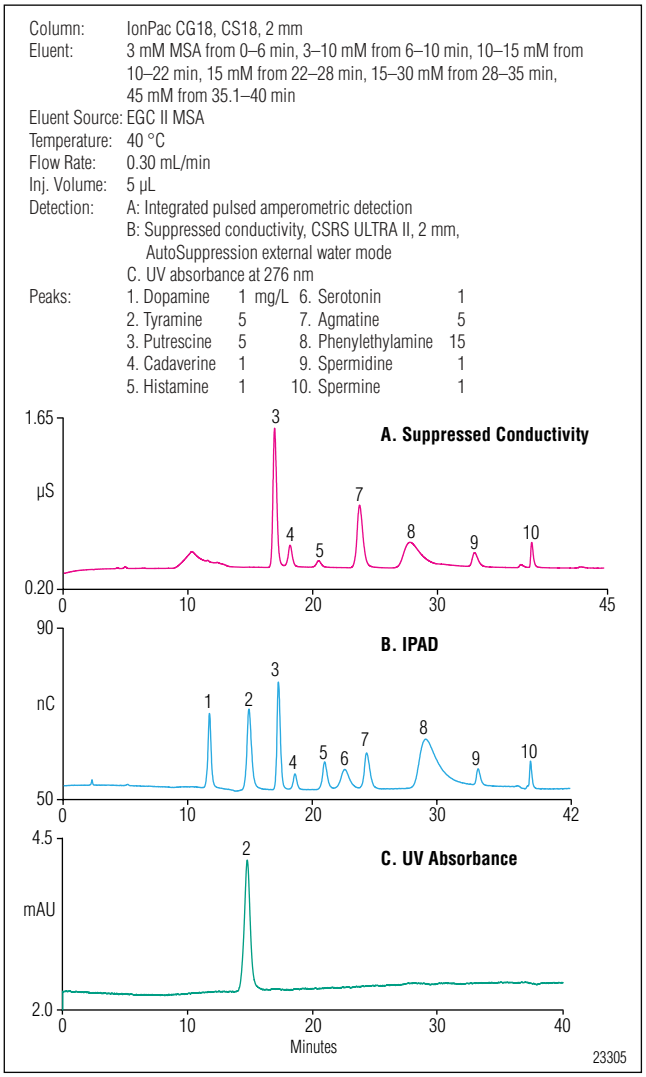

Figure 1. Separation of biogenic amines and detection by A) suppressed conductivity, B) IPAD, and C) tyramine by UV detection.

RESULTS AND DISCUSSION

Separation and Detection of Biogenic Amines

Figure 1 shows the separation of biogenic amines with suppressed conductivity, integrated pulsed amperometric, and UV detections (not connected in series). Dopamine, tyramine, and serotonin cannot be detected by suppressed conductivity because they lack a positive charge after suppression. Therefore, IPAD was required to detect all 10 biogenic amines. Tyramine was also monitored by UV detection to confirm its presence in samples that had previously been identified as containing tyramine by IPAD.

Electrolytically-generated MSA eluent was used to simplify the method and streamline the process of developing an optimum gradient for the separation of the target biogenic amines. An electrolytically generated eluent has not been used with IPAD in previous studies due to the production of oxygen by the generation of the MSA eluent. Dissolved oxygen in the eluent stream can result in significant changes in the background signal and therefore should be removed. Oxygen was removed by passing the eluent stream through the eluent channel and external water through the Regen channel of the EG degas device. This appeared to remove the oxygen created by the EG since no erratic changes in the background were observed. The EG simplified the method development by only requiring the addition of DI water, thus avoiding potential errors and inconsistencies that can occur when manually preparing eluents off-line.

Table 1. Linearity and Limits of Detection of Biogenic Amines												
	IPAD			Suppressed Conductivity Detection			IPAD (post-suppression			UV		
Analyte	Range (mg/L)	Linearity (r^2)	LOD (µg/L)	Range (mg/L)	Linearity (r^2)	LOD (µg/L)	Range (mg/L)	Linearity (r^2)	LOD (µg/L)	Range (mg/L)	Linearity (r^2)	LOD (µg/L)
Dopamine	0.1-5	0.9999	20	0.1-5	—	—	—	—	—	—	—	—
Tyramine	0.2-10	0.9999	80	0.2-10	—	—	—	—	—	0.2-10	0.9997	110
Putrescine	0.2-10	0.9979	50	0.2-10	0.9986	3.5	0.2-10	0.9974	97	—	—	—
Cadaverine	0.1-5	0.9999	70	0.1-5	0.9997	5.3	0.25-5	0.9997	160	—	—	—
Histamine	0.1-5	0.9999	40	0.1-5	0.9998	18	0.1-5	0.9998	88	—	—	—
Serotonin	0.1-5	0.9998	70	—	—	—	—	—	—	—	—	—
Agmatine	0.2-10	0.9998	170	0.2-10	0.9999	9.0	0.5-10	0.9999	290	—	—	—
Phenylethylamine	1-20	0.9999	400	1-20	0.9999	81	5--20	0.9999	1090	—	—	—
Spermidine	0.1-5	0.9999	80	0.1-5	0.9993	4.0	0.25-5	0.9996	140	—	—	—
Spermine	0.1-5	0.9996	50	0.1-5	0.9990	9.0	0.1-5	0.9998	90	—	—	—

Table 2. Biogenic Amine Concentrations in Food Products Determined by IPAD[a]																
Sample	Tyramine		Putrescine		Cadaverine		Histamine		Serotonin		Agmatine		Spermidine		Spermine	
	Amount Found[b] (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)
Chocolate (70% Cocoa)	<DL[b]	—	6.9±0.1	91.1	<DL	—	3.3±0.1	87.6	7.3±0.03	91.0	<DL	—	9.8±0.3	102.5	9.8±0.2	95.8
Dark Chocolate	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—	0.4±0.0	103.2	0.5±0.1	99.4
Milk Chocolate	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—
Spinach Leaves	<DL	—	7.8±0.1	107.6	<DL	—	61.0±1.5	93.8	<DL	—	<DL	—	48.5±1.8	102.0	6.6±0.3	98.2
Kiwi Fruit	<DL	—	3.1±0.1	96.1	<DL	—	1.9±0.1	91.3	9.2±0.3	95.4	<DL	—	7.5±0.1	105.5	1.9±0.1	98.0

[a]Tyramine determined by either UV or IPAD as noted.

[b]<DL = less than the detection limit.

System Performance

The linear ranges for suppressed conductivity, IPAD, and UV detection were evaluated by tabulating peak area versus concentration. Calibration curves were prepared for each biogenic amine in 3 mM MSA using five increasing concentrations. The calibration data and LODs for the three detection configurations are summarized in Table 1. For more details, and for reproducibility and precision measurements, see AN 183.

Determination of Biogenic Amines in Food Products with IPAD

Biogenic amine concentrations for the foods analyzed in this update are listed in Table 2. The total biogenic amine concentrations for chocolate containing 70% cocoa, dark chocolate, and milk chocolate were 37.1, 0.9, and 0 mg/kg, respectively. This suggests that most of the biogenic amines detected were derived from the cocoa present in the chocolate. The 70% cocoa sample contained putrescine, histamine, serotonin, spermidine, and spermine at concentrations of 6.9, 3.3, 7.3, 9.8, and 9.8 mg/kg, respectively (Figure 2). These values are in agreement with previously published analyses of amines in

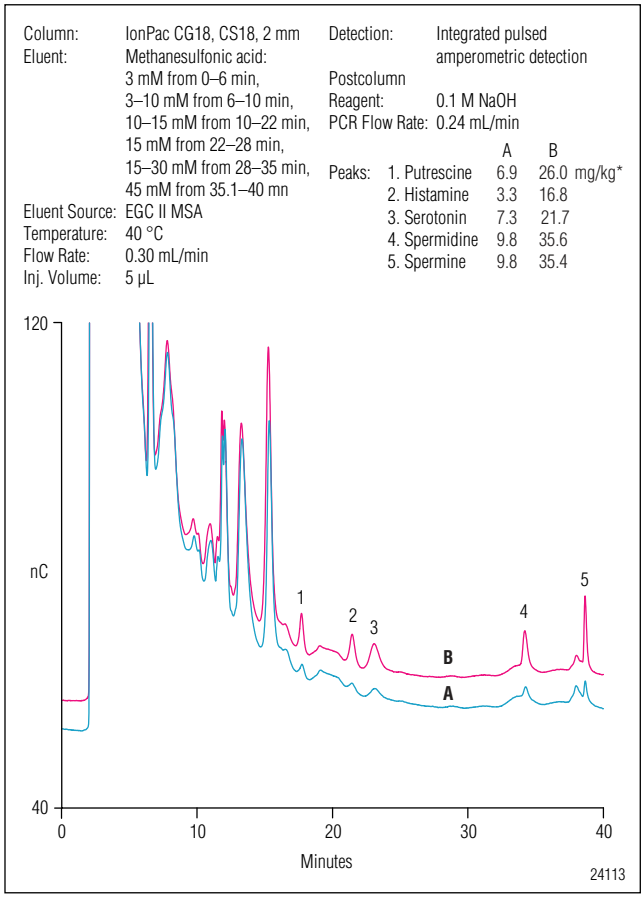

*Figure 2. Detection of biogenic amines in chocolate containing 70% cocoa by IPAD. (A) Unspiked sample. (B) Spiked sample. *Concentrations calculated based on a dilution factor of 1:22.*

chocolate with the exception of serotonin. Previous findings have reported serotonin in chocolate in the range 16–61 mg/kg.[14,15] Percent recoveries for biogenic amines spiked into the 70% cocoa sample ranged from 87.6 to 102.5% (Table 2).

Biogenic amines are also widespread in plant material that is commonly used for food, such as fruits and vegetables.[2] Limited information is available on the amine content in fruits. In this study, putrescine, histamine, serotonin, spermidine, and spermine were detected in kiwi fruit. Serotonin was detected at a concentration of 9.2 mg/kg, about 25% more than in the 70% cocoa sample. Putrescine, spermidine, and spermine are common polyamines that may serve specific protective roles in plants adapted to extreme environments.[13] Previous studies have indicated a range of 1–4 mg/kg serotonin in passion fruit.[16]

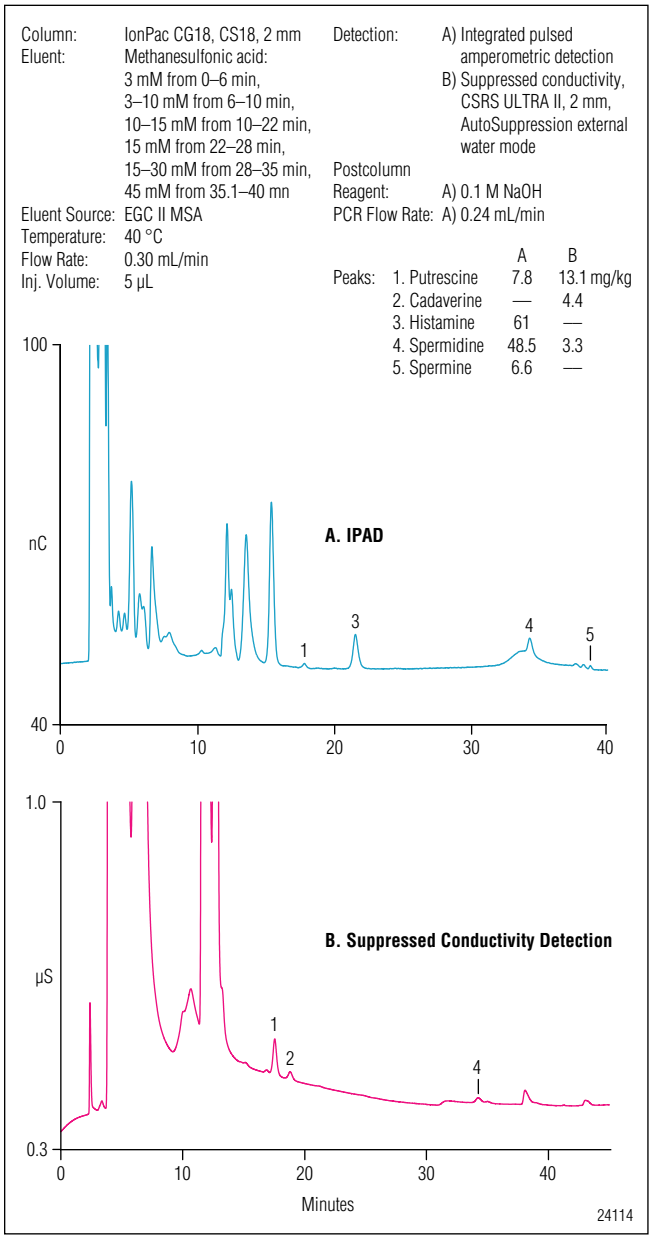

Figure 3: Separation of biogenic amines in spinach and detection by (A) IPAD (fresh sample) and (B) suppressed conductivity (after 3 weeks storage at 4 ºC).

In the spinach leaves, histamine and spermidine were the primary biogenic amines detected at concentrations of 61 and 48.5 mg/kg, respectively (Figure 3). The highest concentration of spermidine in any of the unstored food products analyzed (including those tested in AN 183) was found in spinach. High spermidine levels have been reported in other green vegetables.[5]

Table 3. Biogenic Amine Concentrations in Stored Alcoholic Beverages Determined by Suppressed Conductivity Detection and IPAD

Suppressed Conductivity Detection												
Sample	Putrescine		Cadaverine		Histamine		Agmatine		Spermidine		Spermine	
	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)
Spinach Leaves[a]	13.1±0.1	105.8	4.4±0.0	90.5	<DL[b]	—	<DL	—	3.3±0.1	88.5	<DL	—
Kiwi Fruit[c]	1.7±0.0	98.5	<DL	—	<DL	—	<DL	—	9.5±0.0	93.6	1.7±0.1	95.3
IPAD (post suppression)												
Sample	Putrescine		Cadaverine		Histamine		Agmatine		Spermidine		Spermine	
	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)
Spinach Leaves[a]	12.7±0.1	100.8	4.9±0.1	95.0	<DL	—	<DL	—	<DL	—	<DL	—
Kiwi Fruit[c]	<DL	—	<DL	—	<DL	—	<DL	—	8.0±0.8	102.7	1.5±0.0	95.3

[a]Stored at 4 °C for 3 weeks.

[b]<DL = less than the detection limit.

[c]Stored at 4 °C for 2 weeks.

Changes in Biogenic Amine Concentrations in Food Products during Storage at 4 °C Detected Using Suppressed Conductivity-IPAD

The kiwi fruit and spinach samples were reanalyzed after storage (Table 3). Significant changes in the biogenic amine content of spinach leaves were observed after three weeks refrigeration at 4°C. The spermidine concentration decreased from 48.5 to 3.3 mg/kg and histamine and spermine were completely degraded (Figure 3). The complete degradation of 61 mg/kg histamine presented the most interesting result. To confirm the accuracy of these results, the sample was reanalyzed using only IPAD, which confirmed the absence of histamine. Earlier studies have shown a change in the concentration of putrescine, spermidine, and spermine after three weeks refrigeration of some vegetable products.[17] Although Leuschner et. al. have demonstrated that some microbial species degrade histamine,[12] no data could be found on the correlation of histamine degradation in vegetables. Storage of the kiwi fruit for two weeks at 4 °C resulted in an approximately 82% decrease in putrescine, 25% increase in spermidine, and no change in the spermine concentration. Histamine was completely degraded in the kiwi fruit after storage.

CONCLUSION

The IonPac CS18, a polymeric weak acid cation-exchange column, was used to separate biogenic amines in a variety of food samples, with detection by IPAD, suppressed conductivity, and UV. The described method uses a simple electrolytically generated MSA eluent without requiring the use of solvents or aggressive eluent systems that have been reported previously. In addition, the method results in good precision and recovery over a wide range of sample matrices and avoids the need for complex and long derivatization procedures. The use of three different detection configurations provides additional information and confirms the identification of tyramine to increase confidence in the analytical results. Suppressed conductivity had exceptionally low LODs for the main biogenic amines of interest without chromatographic interferences from common cations and amines present in many of the food samples. In addition to the amines detected by conductivity, IPAD allows the detection of dopamine, serotonin, and tyramine, which can be confirmed with a UV detector.

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Selective Extraction of PCBs from Fish Tissue Using Accelerated Solvent Extraction (ASE®)

INTRODUCTION

Accelerated Solvent Extraction (ASE) is a new extraction method that significantly streamlines sample preparation. A solvent is delivered into an extraction cell containing the sample, which is then brought to an elevated temperature and pressure. Minutes later, the extract is transferred from the heated cell to a standard collection vial for cleanup or analysis. The entire extraction process is fully automated and performed in minutes for fast and easy extraction with low solvent consumption.

The analysis of extracts containing PCB contaminants from fish tissue and fish homogenates can be hindered by the presence of coextracted fatty materials that interfere with the chromatographic analysis. It is standard procedure to perform some form of cleanup to remove the coextracted lipids from such samples prior to analysis. These clean-up procedures include size-exclusion chromatography (SEC), column chromatography, and acid treatment. These procedures add time to sample preparation and increase the potential for analyte losses. As an alternative, selective extraction procedures have been developed using ASE.

The data presented in this application note demonstrate that selective extractions can be performed using ASE with the proper choice of solvent and sorbent in the extraction cell. Results are given for the recovery of PCBs from contaminated fish tissue showing that extracts can be obtained using ASE that do not require further cleanup prior to analysis by gas chromatography.

EQUIPMENT

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

Analytical balance

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

Cellulose filter disks (P/N 49458)

Gas chromatograph (GC) with electron capture detector (ECD)

SOLVENTS

Hexane (pesticide-grade or equivalent)

EXTRACTION CONDITIONS

Extraction Solvent:	Hexane
Temperature:	100 °C
Pressure:	10 MPa (1500 psi)
Heat Time:	5 min
Static Time:	5 min
Flush Volume:	60%
Purge Time:	90 s
Static Cycles:	2
Total extraction time:	17 min per sample

SAMPLE INFORMATION

The sample chosen for this study was obtained from the National Research Council of Canada (NRC-CNRC). Characterized as a ground whole Carp reference material for organochlorine compounds (CARP-1), the sample contains certified concentrations of 14 PCB congeners and 9 dioxin compounds. The moisture content is approximately 85%, and the lipid content approximately 4%.

SAMPLE PREPARATION

Sample preparation was performed by mixing 3 g of the homogenate with 15 g of ASE Prep DE (diatomaceous earth) (P/N 062819) in a mortar and pestle. Given the high water content of the sample and the nonpolar nature of the extraction fluid, complete drying of the sample is essential. A 33-mL extraction cell was loaded by inserting a disposable cellulose filter into the cell outlet, followed by 5 g of alumina (acid, Brockman activity I, 60-325 mesh). After the addition of the alumina, a second disposable cellulose filter was inserted. The sample/ASE Prep DE mixture was then added to the cell on top of the alumina. It is important that the orientation of the cell be maintained when it is loaded onto the extraction system.

PROCEDURE

After extraction, the extracts were then measured and analyzed by GC/ECD (U.S. EPA Method 8081). No cleanup was performed on the extracts from the "selectivity" experiments prior to GC analysis. This method is a dual-column GC method with electron capture detection (ECD). Extract analysis was per-formed by Mountain States Analytical Laboratory in Salt Lake City, Utah. Results are reported on a wet weight basis.

RESULTS AND DISCUSSION

Two different batches of homogenized tissue were extracted in triplicate and analyzed. Tables 1 and 2 show the data from these extractions. The certified values for the tissue are included for reference.

For comparison, additional samples from sample batch 2 were extracted nonselectively under the same ASE conditions, except that methylene chloride/acetone (1/1, v:v) was used as the extraction fluid. Extracts were passed over 2 g of sodium sulfate; the solvent was exchanged to hexane; and then mixed with an equal volume (10 mL) of sulfuric acid for fat removal. The extracts cleaned in this manner were analyzed, as were the "selective" extractions. The results of these extractions are given in Table 3.

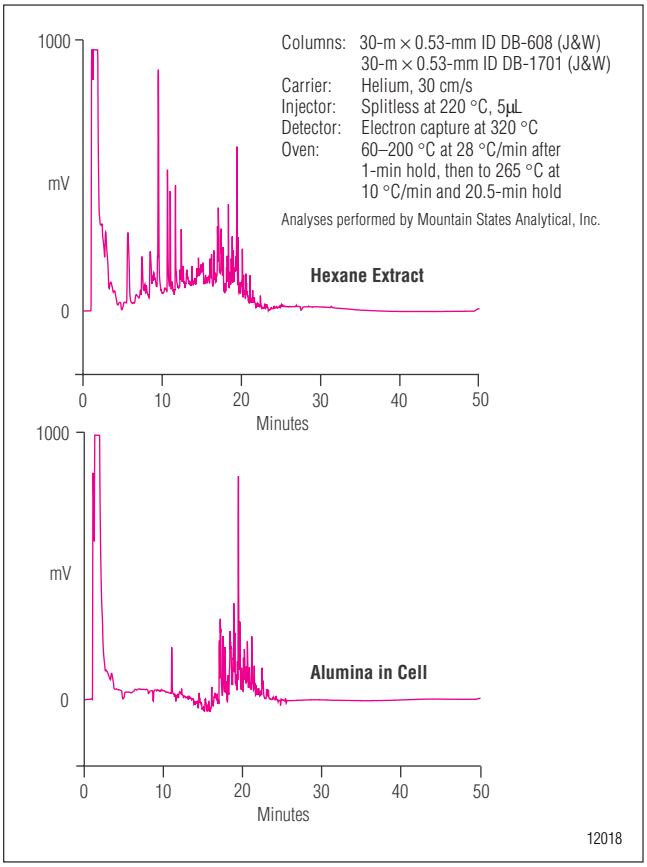

Figure 1. Chromatograms obtained from the "nonselective" ASE extraction of the fish tissue (top) and from the "selective" ASE extraction of a portion of the same sample (bottom).

Figure 1 compares chromatograms obtained from the "nonselective" hexane ASE extraction of the fish tissue with "selective" ASE extraction of a portion of the same sample. As can be seen, the use of alumina in the outlet of the extraction cell prevents lipids and other coextractable materials from coming out in the extract which would complicate the quantification of the analytes of interest due to chromatographic interferences.

As can be seen in Tables 1 and 2, the selective extraction using ASE gives acceptable results, and the need for additional cleanup such as sulfuric acid treatment or size exclusion chromatography is eliminated. Only one value obtained by ASE with selective extraction was below the 95% confidence interval and two values were above (Tables 1 and 2). In contrast, when using the conventional cleanup procedure with sulfuric acid, three values were low, and one was high (Table 3). In addition, the precision was superior for the samples that were extracted using the selective extraction procedure (Tables 1 and 2).

Table 1. Batch 1: Recovery of PCBs from Fish Tissue using Selective ASE
(Concentration Expressed as µg/kg)

Congener	Cert.* Value	Extract 1	Extract 2	Extract 3	Average	Standard Deviation	RSD (%)
52	124 ± 32	100	107	99	102	4.4	4.3
101/90	124 ± 37	101	103	100	101	1.5	1.5
105	54 ± 24	124	128	125	126**	2.1	1.7
118	132 ± 60	107	109	107	108	1.2	1.1
138/163/164	102 ± 23	48	48	48	48**	0.0	N/A
153	83 ± 39	48	48	48	48	0.0	N/A
170/190	22 ± 8	30	31	31	31	0.58	1.9
180	46 ± 14	65	62	64	64**	1.5	2.4
187/182	36 ± 16	30	30	30	30	0.0	N/A

* 95% confidence limits are given
** Values fall outside the 95% confidence limits

Table 2. Batch 2: Recovery of PCBs from Fish Tissue using Selective ASE
(Concentration Expressed as µg/kg)

Congener	Cert.* Value	Extract 1	Extract 2	Extract 3	Average	Standard Deviation	RSD (%)
52	124 ± 32	99	104	97	100	3.6	3.6
101/90	124 ± 37	93	100	93	95.3	4.0	4.2
105	54 ± 24	119	127	121	122**	4.2	3.4
118	132 ± 60	97	105	108	103	5.7	4.8
138/163/164	102 ± 23	41	44	40	42**	2.1	5.0
153	83 ± 39	41	44	40	42**	2.1	5.0
170/190	22 ± 8	28	31	28	29	1.7	3.4
180	46 ± 14	54	57	54	55	1.7	3.1
187/182	36 ± 16	35	38	35	36	1.7	4.7

* 95% confidence limits are given
** Values fall outside the 95% confidence limits

Table 3. Batch 2: Recovery of PCBs from Fish Tissue using Nonselective **ASE**
(Concentration Expressed as µg/kg)

Congener	Cert.* Value	Extract 1	Extract 2	Extract 3	Average	Standard Deviation	RSD (%)
52	124 ± 32	99	101	100	100	1.0	1.0
101/90	124 ± 37	145	138	134	139	5.6	4.0
105	54 ± 24	114	119	118	117**	2.6	2.2
118	132 ± 60	69	94	92	85	14	17
138/163/164	102 ± 23	54	37	37	43**	9.8	23
153	83 ± 39	54	37	37	43**	9.8	23
170/190	22 ± 8	42	ND	ND	14**	24	171
180	46 ± 14	64	58	57	60	3.8	6.4
187/182	36 ± 16	ND	39	47	29	25.1	87

* 95% confidence limits are given
** Values fall outside the 95% confidence limits

The amount of sample that can be selectively extracted is 1–4 g due to the necessity for sample drying and the volume of alumina in the extraction cell. If larger samples are required, up to 10 g (depending on the moisture content) can be nonselectively extracted. These samples should be prepared as described (smaller amounts of ASE Prep DE may be used), and extracted according to the conditions listed using hexane or methylene chloride/acetone (1:1) as the extraction fluid. In these cases, the fat will be coextracted, and standard extract cleanup steps and solvent exchanges will have to be employed. If the tissue is freeze dried or air dried, larger sample sizes may be used. Dried samples may be extracted without any pretreatment; however, mixing the sample with ASE Prep DE or sand may allow better penetration of the sample matrix. For selective extraction of dried tissues, add 2 g of alumina for every gram of sample (samples with higher fat content may require more alumina).

CONCLUSION

The method outlined in this application note demonstrates that selective extractions can be performed using ASE with the proper choice of solvent and sorbent in the extraction cell. In this case, the technique was used on fish tissue extracts containing PCB contaminants in which the selective extraction using ASE gives acceptable results, and the need for additional cleanup, such as sulfuric acid treatment or size-exclusion chromatography, is eliminated. Using this method, it is possible to decrease both the time for sample preparation and the potential for analyte losses.

Determination of PCBs in Large-Volume Fish Tissue Samples Using Accelerated Solvent Extraction (ASE®)

INTRODUCTION

Extraction and analysis of PCBs from fish and other marine tissues continues to be a necessary step in the monitoring of the aquatic food supply. Due to the large number of samples requiring analysis, automated extraction systems have proven useful in this area. ASE technology has been shown to produce good recoveries of naturally occurring PCBs from fish tissue samples,[1,2,3] and is approved for use in U.S. EPA SW-846 Method 3545 for the extraction of PCBs, OCPs, BNAs, OPPs, herbicides, and dioxins and furans.[4] ASE was designed to replace time-consuming and solvent-intensive methods such as Soxhlet and sonication in the environmental area. ASE operates at temperatures higher than those possible in traditional techniques, thus increasing the efficiency of the extraction process.

The continued need for lower analyte detection limits in monitoring the bioaccumulation of priority organic pollutants (POPs) has resulted in the use of large sample sizes. Many automated extraction systems are limited in their ability to extract large samples; however; the ASE 300 extraction system was designed for these larger sample size applications. With sample cell sizes of 34, 66, and 100 mL, it can extract 30-g samples of raw fish tissue under both selective and nonselective conditions. Under nonselective conditions, the extracts produced are similar in composition those from traditional methods and require the usual cleanup steps prior to GC analysis. Using selective ASE conditions, extracts can be produced that are free of coextracted lipid material. These sample extracts can be analyzed without laborious and time-consuming cleanup steps.

EQUIPMENT

Dionex ASE 300 Accelerated Solvent Extractor equipped with 100-mL cells

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

Cellulose filters (P/N 056780)

HP 6890 Gas Chromatograph equipped with an electron capture detector (ECD) (now Agilent Technologies)

REAGENTS AND STANDARDS

Methylene chloride (Optima Grade, Fisher Scientific)

ASE Prep DE (diatomaceous earth) (P/N 062819)

Alumina (basic, Brockman activity I, Fisher Scientific)

PCB standards (ULTRA Scientific)

SAMPLE PREPARATION

Thirty grams of raw fish tissue (cod fillet, purchased locally) was weighed out and spiked with 50 µL of a PCB congener standard solution (ULTRA Scientific) in hexane, containing 50–250 µg/mL individual PCB congeners. This resulted in a final sample concentration of 80–400 ng/g. The samples were mixed with 20 g of ASE Prep DE in a mortar and pestle and then loaded into 100-mL cells containing 10 g of alumina and a cellulose filter.

EXTRACTION CONDITIONS

Extraction Solvent:	Methylene chloride
Temperature:	125 °C
Pressure:	1500 psi (10 MPa)
Heatup Time:	5 min
Static Time:	3 min
Flush Volume:	60%
Purge Time:	120 s
Static Cycles:	3
Total Extraction Time:	18 min per sample
Total Solvent Use:	120–140 mL per sample

POST EXTRACTION

Sample extracts were dried by sodium sulfate treatment, concentrated to 10 mL under nitrogen, and analyzed by GC/ECD. Sodium sulfate treatment can be performed in traditional cartridges or funnels, or simply added to the extraction bottle and shaken.

QUANTIFICATION

Analysis was performed using an HP 6890 Series Gas Chromatograph equipped with an ECD. An RTX-1 30-m × 0.32-mm column was used. The injector was maintained at 280 °C and the detector at 300 °C. Temperature programming was performed from 100 to 300 °C (5 min) at 15 °C/min after a 1 min hold. Recoveries were determined by external standard calibration (three levels) with five replicate samples and duplicate GC injections.

RESULTS AND DISCUSSION

The fish tissue used in this study was cod fillet obtained from a local source. The sample had a fat content of 0.25% and a moisture level of 81%. The samples were premixed with 20 g of pelleted diatomaceous earth (ASE Prep DE) prior to cell loading. Extraction results are shown in Table 1. Average recovery for the nine PCB congeners was 96.9% with an average %RSD of 6.1 (n = 5). Selective extraction was performed by loading 10 g of alumina into the cell outlet. The alumina removes the coextracted lipid material from the extract as it passes from the cell. (The ratio of sample volume to alumina may have to be changed depending on the fat content of the tissue sample.) Generally, the alumina will retain approximately 75 mg of lipid per gram of material under the conditions described in this application note. Figure 1 shows comparative chromatograms. Chromatogram A shows a PCB standard analysis and chromatogram B shows a tissue extract produced using the conditions outlined. The material present in the nonselective (no alumina treatment) ASE extract (chromatogram C) would have to be removed by a cleanup procedure such as sulfuric acid treatment or gel permeation chromatography (GPC) after traditional extraction methods.

Table 1. Recovery of Spiked PCB Congeners from 30-g Fish Tissue Samples Using Selective

Cogener	BZ #	Spike (µg)	% Recovery	% RSD
2-Chlorobiphenyl	1	2.5	99.8	3.0
2,3-Dichlorobiphenyl	5	2.5	103.8	8.8
2,4,5-Trichlorobiphenyl	29	2.5	107.1	3.1
2,2',4,6-Tetrachlorobiphenyl	50	5	98.4	2.4
2,2',3,4,5'-Pentachlorobiphenyl	87	5	92.3	7.9
2,2',4,4',5,6'-Hexachlorobiphenyl	154	5	89.0	5.9
2,2',3,4',5,6,6'-Heptachlorobiphenyl	186	7.5	91.1	8.5
2,2',3,3',4,5',6,6-Octachlorobiphenyl	200	7.5	96.0	6.5
Decachlorobiphenyl	209	12.5	94.2	8.7

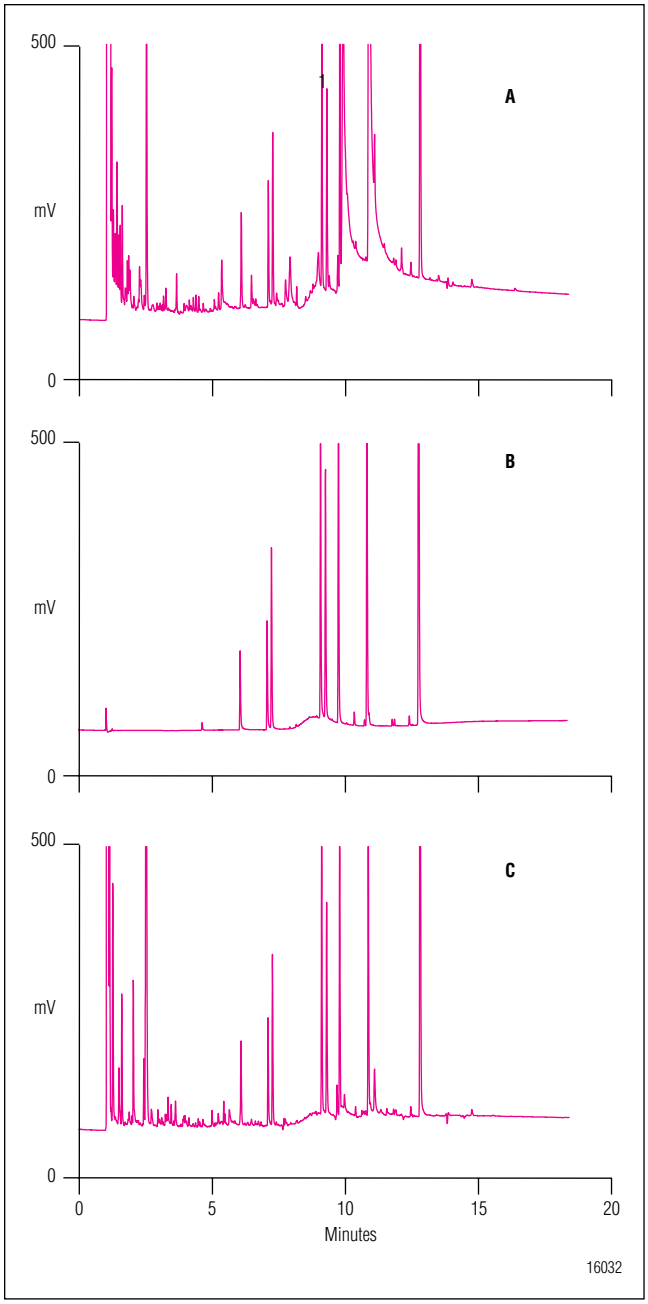

Figure 1. GC/ECD analysis of (A) PCB congener standards, (B) ASE fish tissue extract, and (C) ASE fish tissue extract produced nonselectively.

CONCLUSION

The data presented in this application note indicate that ASE provides good recovery and precision for the extraction of PCBs from 30-g fish tissue samples. Using the selective method described, tissue extracts can be produced that can be immediately dried and concentrated, eliminating the traditional cleanup steps normally required in this analysis. Using ASE, extraction times can be reduced and the sample preparation process automated to make more efficient use of laboratory resources.

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3. Ezzell, J.; Richter B.; and Francis, E. "Selective Extraction of PCBs from Fish Tissue Using Accelerated Solvent Extraction". Amer. Environ. Lab. December **1996**, 12–13.
4. "Test Methods for Evaluating Solid Waste, Method 3545". U.S. EPA SW-846, Update III. Fed. Regist. Vol. 62, 114: 32451. U.S. GPO: Washington, DC, June 13, 1997.

SUPPLIERS

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

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

ULTRA Scientific, 250 Smith St., North Kingstown, RI 02852 USA, Tel: 800-338-1754, www.ultrasci.com.

Rapid Determination of Persistent Organic Pollutants (POPs) Using Accelerated Solvent Extraction (ASE®)

INTRODUCTION

The United Nations Environmental Program (UNEP) has been implemented in an effort to combat the release of selected persistent organic pollutants (POPs). POPs are found in environmental samples such as soils, sludges, solid and semisolid waste, and sediments. POPs are also found in biological samples such as human breast milk, and fish tissue. UNEP is interested in eliminating POPs from the environment because these compounds are considered toxic, carcinogenic, and mutagenic, and degrade slowly in the environment, posing a threat to the global environment. The following compounds are listed by UNEP to be POPS:

- **Pesticides:**
  Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor, Mirex, and Toxaphene
- **Industrial chemicals:**
  Hexachlorobenzene (a type of BNA), and polychlorinated biphenyl (PCB)
- **Chemical by-products (Dioxins):**
  Polychlorinated dibenzo-p-dioxins (PCDD)

Accelerated Solvent Extraction (ASE) is equivalent to U.S. EPA Methods 3540, 3541, 3550, and 8151 for the extraction of organochlorine pesticides (OCPs), organophosphorous pesticides (OPPs), semivolatiles or base neutral acids (BNAs), chlorinated herbicides, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). ASE complies with U.S. EPA Method 3545A for these compounds. ASE is an extraction technique that significantly streamlines sample preparation. This technique uses extraction solvents at elevated temperatures and pressure to increase the kinetics of the extraction process. The high pressure allows the solvent to be used above its boiling point, keeping it in a liquid state, and thus decreases the amount of time and solvent required to extract the desired analyte from the sample matrix. ASE replaces extraction techniques such as Soxhlet, sonication, and wrist-shaker with equivalent or better results.

This application note describes methods and results for extraction of the POPs listed above, with tables comparing ASE to traditional extraction methods.

EQUIPMENT

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

Use either:

22-mL Stainless Steel Extraction Cells (P/N 048764)

11-mL Stainless Steel Extraction Cells (P/N 048765)

33-mL Stainless Steel Extraction Cells (P/N 048766)

Cellulose Filters (P/N 049458)

Collection Vials 60 mL (P/N 048784) or Collection Vials 40 mL (P/N 048783)

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

ASE Prep DE (diatomaceous earth) (P/N 062819)

SOLVENTS

Hexane

Dichloromethane

Acetone

Toluene

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

EXTRACTION CONDITIONS

Pesticides and PCBs (8081/8082)

Solvent:	Hexane/acetone (1:1), (v/v)
Temperature:	100 °C
Pressure:	1500 psi
Static Time:	5 min
Static Cycles:	1–2
Flush:	60%
Purge:	60–120s

Hexachlorobenzene (8270)

Solvent:	Dichloromethane/acetone (1:1), (v/v)
Temperature:	100 °C
Pressure:	1500 psi
Static Time:	5 min
Static Cycles:	1–2
Flush:	60%
Purge:	60–120 s

Dioxins (PCDD) (8290)

Solvent:	Toluene (100%) or toluene/acetic acid (5%, v/v) if HCl pretreatment currently used
Temperature:	175–200 °C
Pressure:	1500 psi
Static time:	5–15 min
Static cycles:	2–3
Flush:	60–70%
Purge:	60–120 s

SAMPLE INFORMATION AND EXTRACTION PROCEDURES

Pesticide Sample Information

Spiking concentrations ranged from 5 to 250 µg/kg. All spiked soils were prepared and certified by ERA (Environmental Resource Associates, Arvada, Colorado, USA). Spiked samples were extracted both by the ASE 200 system and by a Soxtec™ system (automated Soxhlet). Matrix blanks, spikes, and spike duplicates were included for the low-level spikes; matrix spikes were included for all other concentrations. Collected extracts from the ASE 200 were approximately 13–15 mL from the 11-mL extraction cells and approximately 26–30 mL from the 22-mL cells. Extracts can be further cleaned up or directly analyzed depending on the extent of interfering coextractables. For the examples shown in the application note, extracts were analyzed by SW-846 Method 8080. All extractions and analytical work were performed by an independent testing laboratory, Mountain States Analytical, Inc. (Salt Lake City, Utah, USA.)

Pesticide Extraction Procedure

Mix sample thoroughly, especially composite samples. Dried sediment, soil, and dry waste samples should be ground or otherwise subdivided to pass through a 1-mm sieve. Introduce sufficient sample into the grinding apparatus to yield at least 10–20 g after grinding. Air-dry the sample at room temperature for 48 h in a glass tray or on hexane-cleaned aluminum foil, or dry the sample by mixing with ASE Prep DE until a free-flowing powder is obtained. Air-drying is not appropriate for the analysis of the more volatile organochlorine pesticides (e.g., the BHCs), because of losses during the drying process. For sediment and soils (especially gummy clay) that are moist and cannot be air-dried because of loss of volatile analytes, mix 5–10 g of sample with an equal amount of ASE Prep DE in a small beaker using a spatula. Use this approach for any solid sample that requires dispersion of the sample particles to ensure greater solvent contact throughout the sample mass.

Gummy, fibrous, or oily materials not amenable to grinding should be cut, shredded, or otherwise separated to allow mixing and maximum exposure of the sample surfaces for the extraction. If grinding of these materials is preferred, the addition and mixing of ASE Prep DE with the sample (1:1, w/w) may improve grinding efficiency. The professional judgment of the analyst is required for handling such difficult matrices.

Place a cellulose disk at the outlet end of the extraction cell. Weigh approximately 10 g of each sample into 11-mL extraction cells, or approximately 20 g into 22-mL cells. For samples mixed with ASE Prep DE, transfer the entire contents of the beaker to the extraction cell. Surrogate spikes and matrix spikes may be added to the appropriate sample cells.

Place extraction cells into the autosampler tray and load the collection tray with the appropriate number (up to 24) of 60-mL, precleaned, capped vials with septa. Set the method conditions on the ASE 200 system and initiate the run.

PCB Sample Information

Sewage sludge was obtained from the Fresenius Institute (Taunusstein, Germany). Oyster tissue samples were obtained from the National Oceanographic and Atmospheric Administration (NOAA) Laboratory (Seattle, Washington, USA). The river sediment is a standard reference material, SRM 1939 (National Institute of Science and Technology, Gaithersburg, Maryland, USA). Contaminated soil used in this study was a certified reference material (CRM911-050) purchased from Resource Technology Corporation (Laramie, Wyoming, USA).

PCB Extraction Procedure

Samples should be dried and ground. Before filling the cell, a cellulose disk should be placed in the outlet end of the cell. Samples that contain water (greater than 10%) should be mixed in equal proportions with ASE Prep DE.

Quantification of Sewage Sludge, Oyster Tissue, and River Sediment

Sample extracts from ASE were prepared for analysis by passing through silver nitrate/sulfuric acid loaded silica gel and alumina columns, followed by concentration to 1 mL for GC analysis. PCB analyses were performed by gas chromatography with ECD using a 30-m × 0.25-mm i.d., Rtx-5 (Restek, Bellefonte, Pennsylvania, USA) or equivalent column. Injector and detector were maintained at 300 °C. The GC oven was programmed from 100–300 °C at 10 °C/min following a 5-min hold. External standards were used for calibration.

Quantification of Soil (CRM911-050)

PCB analyses of the soil extracts were performed according to U.S. EPA SW-846 Method 8080. The ASE 200 extracts were diluted to 25 mL prior to analysis by GC. Injection was through a split/splitless injector in a GC with dual-electron capture detectors. Two capillary columns, a 30-m × 0.53-mm i.d. DB-608 and a 30-m × 0.53-mm i.d. DB-1701 (J&W Scientific, Folsom, California, USA) provided primary and confirmation data, respectively. Both columns were joined with a fused-silica "Y" connector (Restek). The remaining part of the "Y" was connected to a 5-m section of deactivated 0.53-mm i.d. fused-silica capillary tubing that acted as a guard column. The end of this guard column was inserted into the GC injector. Dual confirmation of the analytes was achieved with a single 5-µL injection. The injector was maintained at 220 °C and both detectors were operated at 320 °C. The oven was programmed from 60–200 °C at 28 °C/min after a 1-min hold, then 265 °C at 10 °C/min with a hold of 20.5 min. Helium was used as the carrier gas at a linear velocity of approximately 30 cm/s.

Hexachlorobenzene Sample Information

Spiking concentrations ranged from 250 to 12,500 µg/kg for the semivolatiles (BNA compounds). All spiked soils were prepared and certified by ERA (Environmental Resource Associates). Samples were ground to 100–200 mesh (150–75 µm). Wet samples were mixed with either ASE Prep DE (10-g sample to 10-g ASE Prep DE), or air-dried. After grinding, a weighed sample was transferred to either a 11- or 22-mL extraction cell.

Spiked samples were extracted both by the ASE 200 system and by a Perstorp Environmental Soxtec (automated Soxhlet). Extracts were analyzed by SW-846 Method 8270A.

Note: All extractions and analytical work were performed by Mountain States Analytical, Inc. (Salt Lake City, Utah, USA). Matrix blanks, spikes, and spike duplicates were included for the low-level spikes; matrix spikes were included for all other concentrations.

Hexachlorobenzene Extraction Procedure

The procedure used in this application note follows the detailed method as described under the U.S. EPA SW-846 Method 3545A.

Mix sample thoroughly, especially composite samples. Dried sediment, soil, and dry waste samples should be ground or otherwise subdivided to pass through a 1-mm sieve. Introduce a sufficient amount of sample into the grinding apparatus to yield at least 10–20 g after grinding. Air-dry the sample at room temperature for 48 h in a glass tray or on hexane-cleaned aluminum foil, or dry the sample by mixing with ASE Prep DE until a free-flowing powder is obtained. Air-drying is not appropriate for the analysis of the more volatile organochlorine pesticides (e.g., the BHCs), or the more volatile of the semivolatile organics because of losses during the drying process.

Gummy, fibrous, or oily materials not amenable to grinding should be cut, shredded, or otherwise separated to allow for mixing and maximum exposure of the sample surfaces for extraction. If grinding of these materials is preferred, the addition and mixing of ASE Prep DE with the sample (1:1, w/w) may improve grinding efficiency.

For sediment and soils (especially gummy clay) that are moist and cannot be air-dried because of loss of volatile analytes, mix 5–10 g of sample with an equal amount of ASE Prep DE in a small beaker using a spatula. Use this approach for any solid sample that requires dispersion of the sample particles to ensure greater solvent contact throughout the sample mass.

Place a cellulose disk into the extraction cell. Weigh approximately 10 g of each sample into an 11-mL extraction cell or approximately 20 g into a 22-mL extraction cell. Transfer the entire contents of the beaker to the extraction cell. Surrogate spikes and matrix spikes may be added to the appropriate sample cells.

Place extraction cells into the autosampler tray and load the collection tray with the appropriate number (up to 24) of 40-mL, precleaned, capped vials with septa. Set the method conditions on the ASE 200 system and initiate the run.

Collected extracts will be approximately 13–15 mL from the 11-mL extraction cells and 26–30 mL from the 22-mL size cells. The extract is now ready for cleanup or analysis depending on the extent of interfering coextractables.

Dioxins (PCDD) Sample Information

Two different sample sets were investigated: one from Germany that included chimney brick, urban dust, and fly ash, and a second from Canada that included four sediment samples. A sediment sample (EC-2) containing high ng/kg levels (ppt) of PCDDs and PCDFs was obtained from the National Water Research Institute (867 Lakeshore Road, P.O. Box 5050, Burlington, Ontario, L7R 4A6, Canada). A low-level sediment sample (HS-2) was obtained from the National Research Council Institute for Marine Biosciences (1411 Oxford Street, Halifax, Nova Scotia, B3H 3Z1, Canada). Both samples are being investigated as potential standard reference materials and were used as received. Two other sediment samples, Parrots Bay and Hamilton Harbor, were extracted. Both of these samples have high levels of coextractable materials.

Dioxins (PCDD) Extraction Procedure

Table 1 lists the conditions used for the extraction of the chimney brick and urban dust by Soxhlet and ASE.

The procedure for the extraction of fly ash was slightly different than the procedure for the other matrices. Before solvent extraction, the samples were treated with 6 M HCl for 30 min and then rinsed thoroughly with distilled water. The extractions by both Soxhlet and ASE were then performed as listed in Table 1. One additional set of extractions was performed on fresh fly ash samples. Instead of pretreatment with HCl, 5% (v/v) glacial acetic acid was added to the toluene for the ASE extraction. All other conditions were held constant.

Table 1. Extraction Conditions for Chimney Brick and Urban Dust

Condition	Soxhlet	ASE
Sample Size	4–10 g	4–10 g
Solvent	Toluene, 250 mL	Toluene 15 mL
Temperature	<111 °C	150 °C
Pressure	Atmospheric	1500 psi
Time	18 h	5-min heatup
Cycles		5-min static, 2 or 3 cycles
Analytical	GC/MS	GC/MS

Quantitation and Sample Cleanup

Cleanup on the chimney brick, urban dust, and fly ash sample extracts was performed by using a chromatographic column packed with multiple layers of silica gel and alumina, in accordance with the German method VDI 3499.

Extracts from the sediment samples were cleaned up using a dual-stage open column chromatography procedure consisting of modified silica and alumina stationary phases.

Samples were further cleaned up using an automated HPLC carbon-based method to remove diphenylether interferences. Complete details of the analytical procedure are available in reference 1.

Analysis by GC/MS and GC/MS/MS

Extracts of the chimney dust, urban dust, and fly ash samples were analyzed by GC-LRMS with an HP 5890 Series II GC, HP MSD 5972. The column used for the chromatography was a 60-m × 0.25-mm i.d. × 0.15-µm film thickness J&W DB-Dioxin column.

Sediment sample extracts were analyzed by GC/MS/MS (Varian 3400 GC, Finnigan MAT TSQ® 70 triple-stage quadrapole mass spectrometer, and ICIS II data system) or GC/HRMS (HP 5890 Series II GC, VG Autospec at 10000 resolution, and OPUS data system). All extracts were separated using a 60-m × 0.25-mm i.d. × 0.25-µm film thickness J&W DB-5 fused-silica capillary column.

Standards

An internal standard solution containing 10 reference compounds, including $^{13}C_{12}$-2,3,7,8-T_4CDD was used for the chimney dust, urban dust, and fly ash samples. No cleanup standard was used. Samples were reconstituted with a recovery standard solution (100 µL) containing $^{13}C_{12}$-1,2,3,4-T_4CDD at 25 ng/mL.

For the sediment samples, standard PCDD/PCDF mixtures were prepared from stock solutions obtained from either Cambridge Isotope Laboratories, Inc. or Wellington Laboratories. The internal quantitation standard contained 15 $^{13}C_{12}$-2,3,7,8-substituted PCDDs and PCDFs. The compounds used are those congeners listed in the data tables. Following extraction, the samples were spiked with a cleanup standard ($^{37}Cl_4$-2,3,7,8-T_4CDD) to differentiate between losses occurring at the extraction and cleanup stages. Prior to injection, the samples were reconstituted with a recovery standard solution (10 µL) containing $^{13}C_{12}$-1,2,3,4-T_4CDD and $^{13}C_{12}$-1,2,3,7,8,9-H_6CDD at 100 pg/µL in nonane.

RESULTS AND DISCUSSION

Pesticides

Tables 2 and 3 shows examples of extraction of selected environmental samples, including both spiked and incurred samples, are shown. These examples illustrate the effectiveness of the ASE technique in obtaining recoveries of analytes equivalent to Soxtec. Tables 2 and 3 summarize the results of this study for chlorinated pesticides spiked at three different levels, in three different soil types.

Table 2. Average Recovery of Pesticides from Three Soil Types[a] — ASE Compared to Automated Soxhlet

Pesticide	Average Recovery (% of Soxhlet)
Heptachlor	88.0
Aldrin	94.9
Gamma Chlordane	99.5
Alpha Chlordane	102.0
Dieldrin	101.2
Endrin	97.2
p,p'-DDT	74.9

[a] Averages from extraction of sand, loan, and clay soils.

Table 3. Average RSD (%) for Chlorinated Pesticides

Matrix	ASE	Automated Soxhlet
Clay	5.0	9.7
Loam	7.8	6.2
Sand	12.0	10.1

PCBs

Results from extractions of sewage sludge, oyster tissue, river sediment, and soil are shown in Tables 4 through 7. These tables show the average recoveries and RSDs (%) for PCB congener content of these matrices. Recoveries for all compounds with the exception of one (PCB 153 from the river sediment) are above 77% of the certified or Soxhlet comparison values. Interferences in the river sediment extract prevented quantification of two low-molecular-weight PCB congeners (PCB 28 and PCB 52).

The results demonstrate the effectiveness of ASE as a sample preparation method. ASE provides extracts with minimal solvent usage and significant time reduction compared to other extraction methods. Results are comparable to the traditional Soxhlet extraction method. ASE meets the requirements for PCB analysis as described in U.S. EPA SW-846 Method 3545A.

Table 4. PCB Recoveries from Sewage Sludge[a]

PCB Congener	Average Recovery, n = 6 (as % of Soxhlet)	RSD (%)
PCB 28	118.1	2.5
PCB 52	114.0	4.7
PCB 101	142.9	7.4
PCB 153	109.5	5.8
PCB 138	109.6	3.9
PCB 180	160.4	7.5

[a] Analyte concentration range: 160–200 µg/kg per component

Table 5. PCB Recoveries from Oyster Tissue[a]

PCB Congener	Average Recovery, n = 6 (as % of Soxhlet)	RSD (%)
PCB 28	90.0	7.8
PCB 52	86.9	4.0
PCB 101	83.3	1.5
PCB 153	84.5	3.5
PCB 138	76.9	3.0
PCB 180	87.0	4.3

[a] Analyte concentration range: 50–150 µg/kg per component

Table 6. PCB Recovery from River Sediment (SRM 1939)[a]

PCB Congener	Average Recovery, n = 6 (as % of Soxhlet)	RSD (%)
PCB 101	89.2	3.7
PCB 153	62.3	4.1
PCB 138	122.1	2.3
PCB 180	111.5	5.9

[a] Analyte concentration range: 170–800 µg/kg per component

Table 7. Recovery of Arochlor 1254 from Soil (CRM911-050)

Run Number	Arochlor Found (µg/kg)
1	1290.0
2	1365.8
3	1283.4
4	1368.6
Average	1327.0 (99.0%)
RSD	3.51%

Hexachlorobenzene

This application note shows the effectiveness of the ASE technique in obtaining recoveries of Hexachlorobenzene equivalent to Soxtec. Tables 8 and 9 summarize the results for Hexachlorobenzene at three different spiking levels, in three different soil types, that were extracted according to the method presented. ASE recoveries and RSD (%) values were all within the range expected from Soxhlet extractions.

Table 8. Average Recovery of Hexachlorobenzene from Three Soil Types[a]—ASE Compared to Automated Soxhlet	
BNA Target Compound	**Average Recovery (% of Soxhlet)**
Hexachlorobenzene	93.7

[a] Averages from extraction of sand, loam, and clay soils

Table 9. Average RSD (%) for BNA for Three Soil Types		
Matrix	**ASE**	**Automated Soxhlet**
Clay	9.1	9.6
Loam	16.1	15.2
Sand	13.4	17.1

Dioxins (PCDD)

Ground Chimney Brick and Urban Dust

Table 10 shows the results from the ground chimney brick and urban dust as selected congeners and as the total of the isomers. The toxicity equivalent is calculated by adding the weighted factors of each isomer's toxicity. One is calculated according to a formula from the North Atlantic Treaty Organization (NATO) and the other is from the German health organization BgVV. The results show that ASE is equivalent to the Soxhlet method with respect to recovery of these compounds.

Fly Ash

Table 10 lists the results from the extractions of the fly ash. The units for this sample are µg/kg because the sample was so highly contaminated. ASE results are equivalent to those from Soxhlet extractions when the HCl/water pretreatment was used.

High-Level Sediment Samples

Table 10 presents a comparison of average results for the Soxhlet and ASE methods for the high-level sediment sample (EC-2). The data compare very favorably.

The data for sample HS-2 also shows a favorable comparison trend (Table 10).

Table 10. Comparison of Soxhlet vs ASE—Total[a] Polychlorinated Dibenzo-*p*-dioxins		
Sample Matrix	**Soxhlet (ng/kg)**	**ASE (ng/kg)**
Chimney Brick	8040	8170
Urban Dust	1110	1159
Fly Ash (µg/kg)	93,200	107,900
Sediment (EC-2)	6750	6840
Sediment (HS-2)	11,731	12,783
Hamilton Harbor Sediment	4283	4119
Parrots Bay Sediment	2836	2444

[a]Total of tetra, penta, hexa, hepta, and octachlorodibenzo-*p*-dioxins

Highly Contaminated Sediment Samples

The ASE technique was also evaluated with two sediment samples containing high levels of coextractables and oil (Table 10). Aliquots of these samples were taken from a larger container as quantitatively as possible, but were not nearly as homogeneous as the rigorously prepared reference materials. Generally, the data compare favorably between ASE and Soxhlet for the recovery of PCDDs from these heavily contaminated sediments.

CONCLUSION

The data shows that ASE is essentially equivalent to classical extraction procedures such as Soxhlet for the extraction of POPs from environmental matrices. In addition to being equivalent to Soxhlet, ASE can perform the extractions in a fraction of the time and with much less solvent.

SUPPLIERS

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

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

National Water Research Institute, 867 Lakeshore Road, Burlington, Ontario L7R 4A6 Canada.

National Research Council Institute for Marine Biosciences, 1411 Oxford Street, Halifax Nova Scotia, B3H 3Z1 Canada.

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

ACKNOWLEDGMENTS

We would like to thank David S. Waddell, Tereza Gobran, and Vin Khurana from the Ontario Ministry of Environment and Energy, Ontario, Canada, and of Dr. Scheutwinkel and Dr. Mattulat from Dr. Scheutwinkel GmbH, Berlin, Germany, for their contributions.

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Determination of Perchlorate in Vegetation Samples Using Accelerated Solvent Extraction (ASE®) and Ion Chromatography

INTRODUCTION

Perchlorate (ClO_4^-) is an environmental contaminant that has been found in drinking, ground, and surface waters in several states within the United States. Most of the contaminated sites have been traceable to sources near military installations or manufacturing sites where perchlorate salts are used to manufacture rocket propellant, munitions, or fireworks. The solubility, mobility, and persistence of perchlorate have resulted in the contamination of drinking water, soil, and vegetation in several areas.

Perchlorate has been shown to present a health-based risk to humans.[1] Exposure to perchlorate disrupts uptake of iodide by the thyroid gland. For this reason, the EPA has placed this anion on its Contaminant Candidate List (CCL) for drinking water. The EPA has not established any enforceable health regulations for perchlorate in drinking water or related matrices. Nevertheless, states such as California and Massachusetts have set individual action levels restricting the amount of perchlorate in drinking water.

Many scientists have shown that plants grown with perchlorate tainted water become contaminated with perchlorate.[2] The determination of perchlorate in water at the low part per billion (ppb) level can be challenging, however, sample preparation for water samples is generally not considered extremely difficult. The sample preparation necessary to measure perchlorate levels in vegetation is much more challenging and tedious. Analytical protocols for perchlorate typically begin with some type of liquid-solid extraction. High speed blending and ultrasonication extraction are the most common methods of removing perchlorate from soil or vegetation samples. These methods are labor intensive, yet simple and easy to use, but are not efficient enough to extract tightly bound ions such as perchlorate from complex vegetation or other biosolid matrices. Additionally, these techniques often require post extraction cleanup steps such as solid phase extraction (SPE) using different absorbents. Accelerated solvent extraction (ASE) has been shown to overcome complex analyte-matrix interactions and was successfully applied to the extraction of perchlorate from several matrices. In addition to automating the extraction procedure, the ASE technique coupled with Dionex OnGuard® resins produces a clean extract that can be directly injected into an ion chromatograph.

ASE extracts solid samples rapidly using minimal amounts of solvent. A typical 5-g sample of soil or plant material would require approximately 10 to 100 times the weight of the sample in water. Compared to other manual based extraction methods, ASE also provides a significant reduction in time and labor. ASE extractions are typically complete in about 10 to 15 min. Recoveries and precision (RSD) are comparably better than blending or sonication techniques. Furthermore, ASE can be completely automated and provide in-cell cleanup to remove potential interferences.

With the ASE technique, solvent is pumped through the sample from top to bottom in a stainless steel extraction chamber. After solvent is introduced, the sample is heated. Pressure is used to maintain the solvent as a liquid. Typical ASE extraction temperatures range from 80 °C to 120 °C, depending on the sample, with a maximum temperature of 200 °C. ASE uniquely combines dynamic and static extraction methods, resulting in an efficient extraction in a relatively short period of time. At the end of an ASE method, a solvent flush followed by a gas purge separates the solvent and analytes from the sample. Because elevated extraction temperatures are used in ASE, analyte diffusion rates are accelerated compared to soaking, sonication, or blending extraction methods. Higher temperatures also act to overcome the enthalpy associated with adsorption of the analytes onto sites at the matrix surface or the intracell or interstitial spaces of vegetation material.

This application note provides the details of using ASE for the determination of perchlorate in soil, milk, and several plant matrices. The method provides a rapid means of extracting perchlorate from all of the aforementioned matrices using only water as an extraction solvent. The benefits of this method are simplicity, speed of analysis, and automation. ASE allows the rapid extraction and in-line cleanup of a large number of samples with minimal labor. ASE technology allows automated, uninterrupted extractions of up to 24 samples for the ASE 200 (sample sizes less than 3 g) and twelve samples for the ASE 300 (sample sizes greater than 3 g). Computer control of all extraction parameters is available for both instruments.

EQUIPMENT

ASE 200 or ASE 300 system
- 60-mL collection vials (Dionex P/N 048784)
- 250-mL collection bottles (Dionex P/N 056284)
- Glass fiber filters (P/N 047017 for ASE 200, P/N 056781 for ASE 300)
- OnGuard II Sample Pretreatment Cartridges
  - Ag (P/N 057089)
  - Ba (P/N 057093)
  - H (P/N 057085)
  - RP (P/N 057083)
- ASE® Prep DE (P/N 062819)
- Analytical balance with 0.1 mg resolution

Dionex ICS-2500 chromatography system consisting of:
- GP50 Gradient Pump with vacuum degas option
- EG50 Eluent Generator with EluGen® EGC II NaOH cartridge (P/N 058908)
- AS40 Autosampler
- LC30 Chromatography Oven
- CD25 Conductivity Detector with conductivity cell
- Chromeleon® 6.6 Chromatography Management Software (Service Pack 3)

CONDITIONS

Chromatographic Conditions

Columns:	IonPac® AS16 Analytical, 2 × 250 mm (P/N 55376) IonPac AG16 Guard, 2 × 50 mm (P/N 055379) IonPac Cryptand C1 Concentrator, 4 × 35 mm (P/N 062893)
Eluent:	0.50, 65, and 100 mM NaOH
Flow Rate:	0.25 mL/min
Temperature:	35 °C
Backpressure:	2300 psi
Detection:	Suppressed conductivity, ASRS® ULTRA II, external water ymode, 100 mA current
Run Time:	46 min

ASE Extraction Conditions for Perchlorate

Extraction Solvent: Water
Pressure: 1500 psi
Temperature: 80 ˚C
Equilibration Time: 5 min
Extraction Time: 5 min (static)
Solvent Flush: 30% (of cell volume)
Nitrogen Purge: 120 s (after extraction)
Extraction Cycles: 3
Cell Sizes: 33 mL and 100 mL

ASE Sample Preparation

Due to the large amount of matrix interferences seen in the initial work done with alfalfa, it was decided to incorporate the use of Dionex OnGuard H (Hydronium), Ag (Silver), Ba (Barium), and RP (Poly-divinylbenzene) pretreatment cartridges into the extraction cells. These cartridges contain ion-exchange resins, which remove alkali earth metals, halides, sulfates and hydrophobic compounds from the sample. It was suspected that large amounts of chloride and sulfate ions were seen in the initial extractions of alfalfa and spinach, hence the need for ion-exchange resins. Basic alumina (Fisher Scientific—used as received) was also added to the extraction cell. The use of the OnGuard cartridge resins along with the basic alumina greatly reduced the amount of interferences detected in the resulting extracts. In these experiments, the cartridges are opened and the resins are scooped out into the extraction cells. The chromatograms shown in Figure 1 compare ASE alfalfa extracts obtained using no in-line cleanup (green) and OnGuard resins combined with basic alumina (blue) in the ASE extraction cell.

Prior to extraction, the 100-mL cells are prepared from bottom to top as follows: two GFB filters, 3.0 g of OnGuard H, a GFB filter, 6.0 g of OnGuard Ag, a GFB filter, 3.0 g OnGuard Barium, a GFB filter, 18 g basic alumina, a GFB filter, 1.8 g OnGuard RP, a glass fiber filter and then fill the remainder of the cell with Dionex ASE Prep DE. The 33-mL cells are prepared in the same manner with proportionally less of each resin.

To ensure clean resins, each prepared cell was extracted under the same ASE conditions as the samples. During this step, the resins and the ASE Prep DE were cleaned of any potential interference with the perchlorate ion. Ten g of the "clean" ASE Prep DE was mixed with 5 g of sample. The resulting mixture was then ground in a mortar and pestle and added back into the cell prior to extraction.

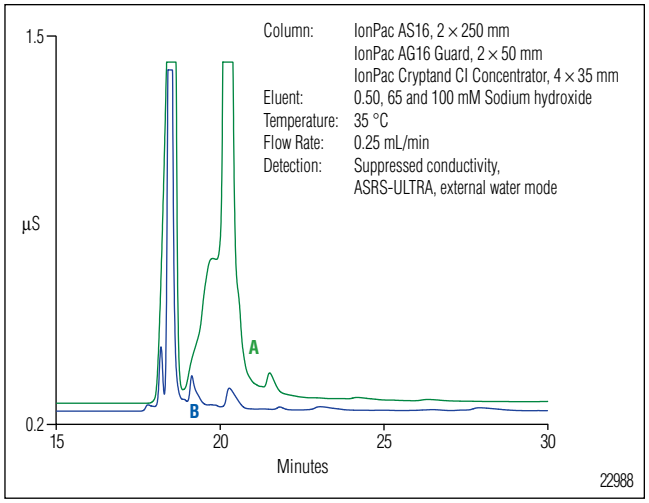


Figure 1. Alfalfa extracts obtained using (A) no in-line cleanup and (B) OnGuard resins combined with basic alumina in the ASE extraction cell.

Prior to analysis, each of the extracts was filtered using a 0.2 µm polyethersulfonate syringe filter.

PREPARATION OF SOLUTIONS AND REAGENTS

Reagents and Standards

Deionized water (DI H_2O), Type I reagent grade, 18 Ω-cm resistance or better
Sodium perchlorate, 98% ACS reagent grade or better (Aldrich)
ACS reagent grade sodium salts (Mallinckrodt, Fisher)
Sodium Hydroxide (NaOH) 50% w/w (Fisher Scientific)

Stock Perchlorate Standard Solution

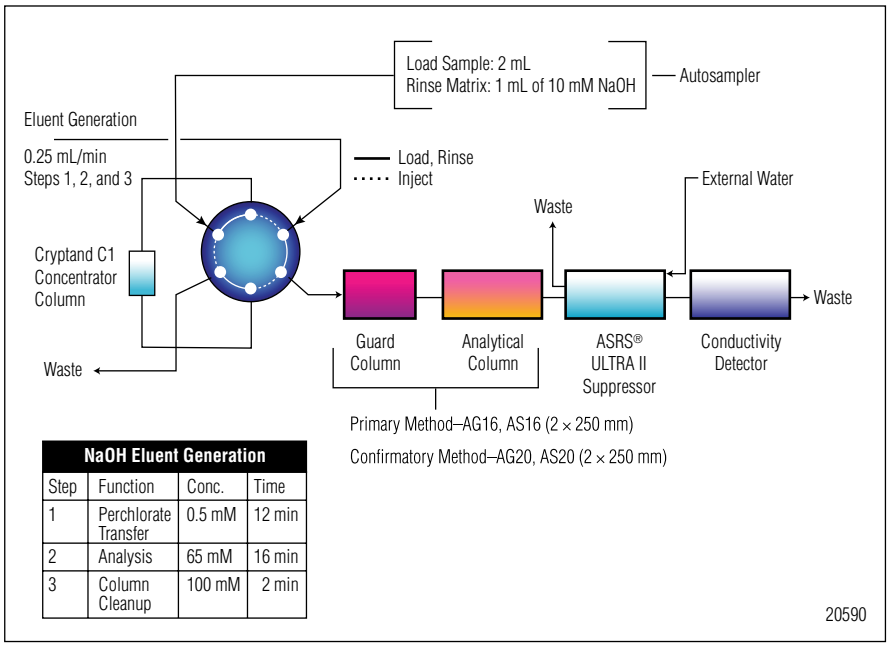
Dissolve 0.3078 g of sodium perchlorate in 250 mL of deionized water for a 1000 mg/L standard solution. This stock standard is stable for at least one month when stored at 4 °C.

Stock Synthetic Sample Matrix Stock Solution

Dissolve 8.6 g of sodium bicarbonate, 9.3 g of sodium sulfate, and 10 g of sodium chloride in 250 mL of deionized water for a 25.0 g/L stock solution. One mL of this Laboratory Synthetic Sample Matrix Stock Solution (LSSMSS) is then added to all calibration standards. Next, 62.5 mL of the LSSMSS is diluted to 250 mL to give a solution with a concentration of 6.25 g/L. The resulting solution (Laboratory Synthetic Sample Matrix Fortification Solution, LSSMFS) is added to all field samples to give a final concentration of 100 mg/L for the sodium compounds.

Working Standard Solutions

Prepare working standards at lower concentrations by diluting the appropriate volumes of the 1000 mg/L stock standard with deionized water. These working standards are prepared at 10.0 mg/L and 1.0 mg/L. Dilutions of these standards are then used to prepare the calibration standards. Calibration standards were prepared at 1, 2, 5, 10, 25, 50 and 100 µg/L for the initial work and then at 5, 10, 20, 50, 100 and 200 µg/L for the replicate studies of corn, melon, and spinach. One mL of the LSSMSS is also added to each calibration standard. The calculated correlation coefficient for one of the calibration curves used for analysis of the vegetation extracts was 0.9986.



NaOH Eluent Generation

Step	Function	Conc.	Time
1	Perchlorate Transfer	0.5 mM	12 min
2	Analysis	65 mM	16 min
3	Column Cleanup	100 mM	2 min

Figure 2. Schematic of the system configuration for the extraction of perchlorate in vegetable samples.

SYSTEM PREPARATION AND SETUP

Samples

Milk, melon, spinach, alfalfa, and corn samples were obtained from a local grocery store. The soil was purchased from Wibby Environmental (Golden, CO). Representative samples (5 g) were placed into a mortar with 10 g of ASE Prep DE (cleaned as detailed above), ground with a pestle, and then added to the ASE extraction cell. The mixture was then spiked with the appropriate amount of perchlorate standard. The cells were allowed to stand overnight at 4 ºC. The final volume of each of the resulting extracts was then adjusted to either 40 mL (if the 33-mL ASE cells were used) or 100 mL (if 100-mL ASE cells were used).

It was also possible to eliminate part of the so-called matrix effect associated with plant or fruit matrices with the AS40 Autosampler and Cryptand preconcentration column. Two sample vials are prepared for use with the AS40. One contains 2 mL of sample that had been spiked with the LSSMFS and the second contains 1 mL of 10 mM sodium hydroxide. The sodium ion from the LSSMFS reacts with the Cryptand column to retain perchlorate. As the sodium ion concentration increases, the capacity of the Cryptand column to retain perchlorate also increases. The sodium hydroxide solution washes away any contaminants from the preconcentration column. The perchlorate is then eluted onto the analytical column for analysis. A schematic of this system configuration is shown in Figure 2.

RESULTS AND DISCUSSION

Initial ASE studies were performed with soil, alfalfa, corn, and milk. The samples were prepared as described above in the "Experimental" section. Each sample matrix was extracted in replicates of five. The recovery data and reproducibilities for each set of extractions are shown in Table 1.

Table 1. Recovery Data for ASE Extraction of Perchlorate (n=5) of µg/L Anions in Ultrapure Water

Matrix	Perchlorate (ppb)	%Recovery*	%RSD
Soil	50	106	7.89
Alfalfa	50	94.2	8.24
Corn	50	88.7	8.86
Milk	25	118.7	1.57

*Analysis was performed using EPA Method 314.1 with a Dionex ICS-2500 ion chromatography system.

Figure 3 compares chromatograms of a 3-g soil sample that had been spiked with perchlorate and extracted with water. The resulting perchlorate concentration is 50 ppb (ng/g). The chromatogram of a soil "blank" ASE extract is overlaid with the spiked sample to show that there are no interferences with the perchlorate peak.

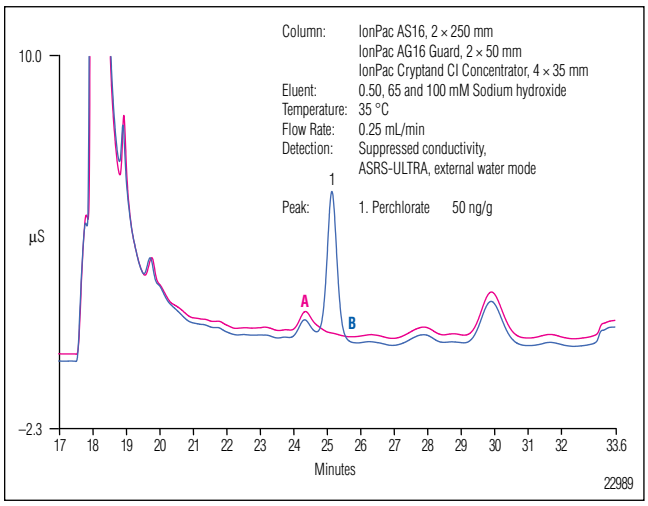


Figure 3. Chromatograms of (A) a soil "blank" obtained using ASE, and (B) a 3-g soil sample spiked with perchlorate and extracted with water.

Figure 4 shows the chromatogram resulting from an ASE extract of a 5-g melon sample spiked with perchlorate. The resulting perchlorate concentration is 10 ppb (ng/g). The chromatogram of a "blank" melon extract is overlaid with the spiked sample to show that there are no extraneous peaks in the "blank" extract that interfere with the perchlorate peak.

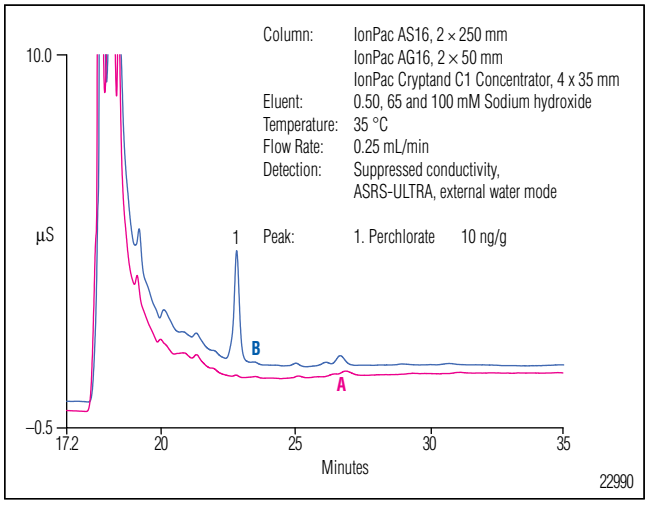


Figure 4. Chromatograms of (A) a melon "blank" obtained using ASE, and (B) a 5-g melon sample spiked with perchlorate.

Figure 5 shows the chromatogram of a 5-g spinach sample spiked with perchlorate. The resulting perchlorate concentration is also 10 ppb (ng/g). The chromatogram of a spinach "blank" extract is again overlaid with the spiked sample to show that there are no interferences with the perchlorate peak.

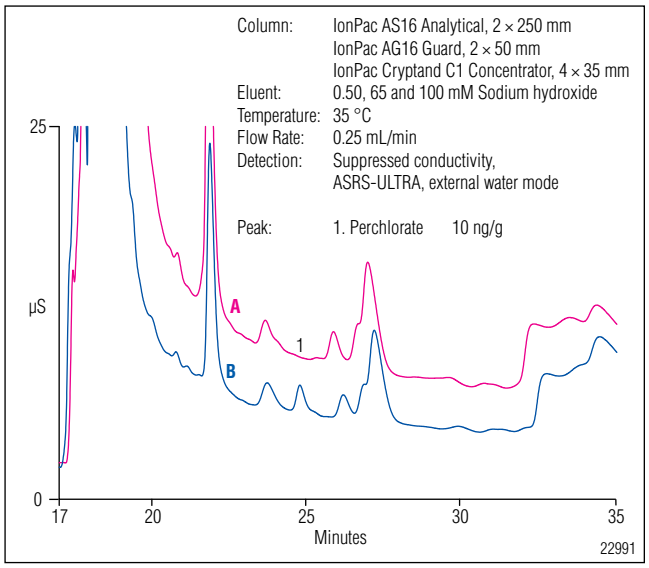


Figure 5. Chromatograms of (A) a spinach "blank" obtained using ASE, and (B) a 5-g spinach sample spiked with perchlorate.

As a result of the experiments summarized in Table 1, we decided to continue the recovery studies at a lower spike level. A more detailed study was done for corn, melon, and spinach. Each sample matrix was spiked at three different levels of perchlorate (10, 50, and 200 ppb) and the extractions were done in replicates of seven. The results from these experiments are summarized in Table 2.

Table 2. Recovery and Reproducibility Data for ASE Extraction of Perchlorate

Matrix	Perchlorate (ppb)	%Recovery*	%RSD
Melon	10	110	2.48
	50	96.8	2.54
	200	103	5.51
Corn	10	102	5.36
	50	88.7	8.86
	200	95.7	6.80
Spinach	10	106	5.40
	50	101	7.17
	200	97.9	6.53

*Analysis was performed using EPA Method 314.1 with a Dionex ICS-2500 ion chromatography system.

Figure 6 shows a graph summarizing and comparing the data obtained from the study. There appears to be no matrix or concentration effect under the conditions tested.

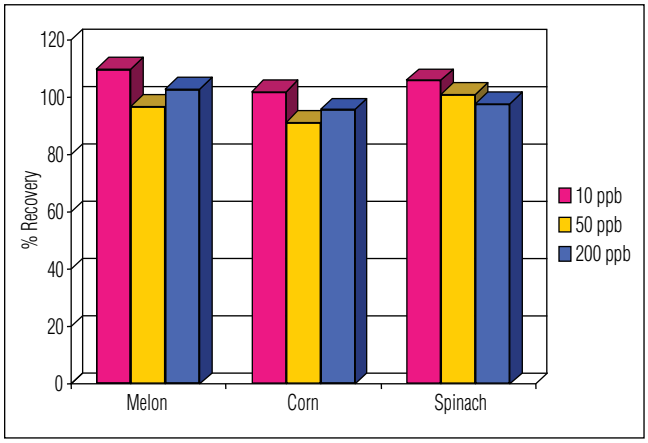


Figure 6. Bar graph summarizing the percent recovery data of perchlorate extracted from spiked samples of melon, corn, and spinach using ASE.

The method performance of the method was also evaluated by calculating the method detection limit (MDL). This was done by multiplying the standard deviation of the seven replicates of the low-level samples by 3.143 (as per EPA guidelines). The reliable quantization limit (RQL) was calculated by multiplying the MDL by 4. Table 3 summarizes these results.

Table 3. Summary of the Method Performance

Matrix	Avg. Recovery (%, n=21)	*MDL (µg/kg)	*RQL (µg/kg)
Melon	103.3	0.72	2.9
Corn	96.3	1.4	5.6
Spinach	101.6	2.0	8.0

*Analysis was performed using EPA Method 314.1 with a Dionex ICS-2500 ion chromatography system.

CONCLUSION

The ASE method detailed in this application note provides a fast and efficient extraction of perchlorate from various food and soil samples. The extracted samples can be analyzed directly using IC coupled with a conductivity detector. As can be seen from the recovery and reproducibility data mentioned above, the results from the ASE extraction are very similar, if not better than the popular ultrasonication methods. Using ASE saves time, solvent, and labor when compared to manual extraction techniques. This also demonstrates that it is possible to achieve ppb level of detection from vegetation samples with very little sample cleanup prior to analysis.

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Determination of Phenols in Drinking and Bottled Mineral Waters Using Online Solid-Phase Extraction Followed by HPLC with UV Detection

INTRODUCTION

Phenolic compounds are subject to regulation as water pollutants due to their toxicity. The European Community (EC) Directive specifies a legal tolerance level of 0.5 µg/L for each phenol in water intended for human consumption[1] and Japan's Ministry of Health, Labour, and Welfare specifies a maximum contaminant level (MCL) of 5 µg/L for phenols in drinking water.[2] The U.S. EPA specifies a MCL of 1 µg/L for pentachlorophenol,[3] and eleven common phenols are on the U.S. EPA priority pollutants list.[4] The structures for these common phenols are shown in Figure 1. The method typically used for determining phenols is gas chromatography (GC) combined with flame ionization detection (FID)[5,6] or mass spectrometric detection (GC–MS).[7-9] However, liquid chromatography (LC) methods combined with UV/DAD,[10] electrochemical,[11] and fluorescence[12] detections are finding increased application, particularly due to nonvolatiles in many samples that can poison GC columns.

Method detection limits (MDLs) of LC techniques employing direct injection of samples are too high for the detection of the low levels allowed in natural waters. Therefore, water samples require preconcentration before analysis. Solid-phase extraction (SPE) is one of the most important techniques for sample enrichment, because it overcomes many of the disadvantages of liquid-liquid extraction. Unfortunately, preparing individual samples is time consuming, and a new SPE cartridge must be used for each sample.

The expense of using multiple SPE cartridges and the associated manual labor can be eliminated with online SPE combined with HPLC. This technique delivers a simple, rapid, and accurate means for determining phenols at low concentrations in real samples.[13,14] The UltiMate® 3000 was designed to easily execute more advanced HPLC methods, such as parallel LC, 2-D LC, and online SPE/HPLC. An UltiMate 3000 together with an autosampler capable of injecting large volumes can be used to execute an online SPE method to determine phenols in drinking and bottled waters. A method using one pump channel of a dual pump system instead of the large volume injector can also be used to achieve online SPE, as described in the Appendix.

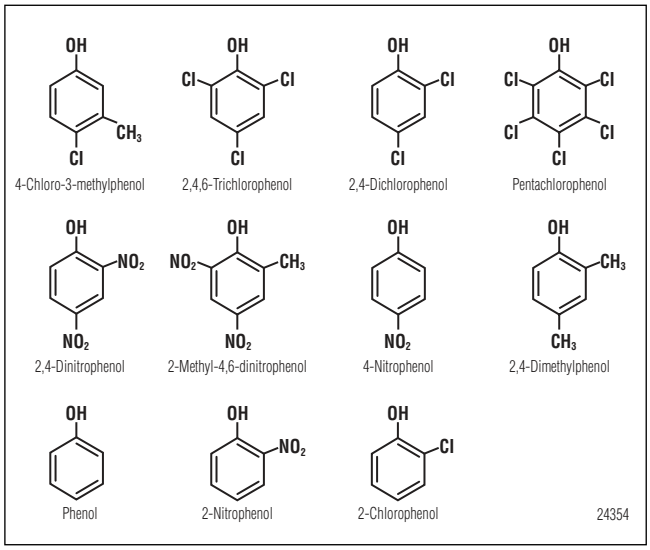

Figure 1. Structures of the 11 phenols specified in the U.S. EPA priority pollutants list.

This application note details an online SPE method followed by HPLC with UV detection for determining the 11 phenols specified in U.S. EPA Priority Pollutants List at the concentrations required by world regulatory agencies. Phenols from drinking and bottled waters are trapped on an IonPac® NG1, a small polymeric reversed-phase column, then separated on a polar-embedded reversed-phase column, the Acclaim® PA. This automated method is a cost-effective way to determine phenols in drinking and bottled water samples.

EQUIPMENT

Dionex UltiMate 3000 HPLC system consisting of:

DGP 3600M dual gradient pump

SRD 3600 solvent rack with integrated vacuum degasser

TCC-3200 Thermostatted Column Compartment with two two-port, six-position (2P-6P) valves

VWD-3400 Variable Wavelength Detector

AS-HV High-Volume Autosampler*

Chromeleon® Chromatography Management Software, version 6.80

*See Precautions.

REAGENTS AND STANDARDS

Use only ACS reagent grade chemicals for all reagents and standards.

Deionized (DI) water from a Milli-Q® Gradient A10 water purification system

Methanol (CH3OH), HPLC grade (Fisher)

Acetonitrile (CH3CN), HPLC grade (Fisher)

Glacial acetic acid (HAc), analytical reagent-grade (Shanghai Chemical Reagent Company)

Ammonium acetate (NH4Ac), analytical reagent-grade (Shanghai Chemical Reagent Company)

Methanesulfonic acid (MSA), > 99.5% (Aldrich)

Trifluoroacetic acid (TFA), > 99% (Aldrich)

604 Phenols Calibration Mix (Restec) 2000 µg/mL in methanol, consisting of:

4-chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol, phenol, and 2,4,6-trichlorophenol

CONDITIONS

Solid-Phase Extraction

Column:	IonPac NG1, 5 µm, 4 × 35 mm (P/N 039567)
Mobile Phases for SPE (Left Pump):	A. 0.2 mM MSA B. CH_3CN
Flow Rates:	Rinse: 1 mL/min with 100% B Loading: 2 mL/min with 100% A Phenol Elution: 1 mL/min with 15% B
Inj. Volume:	10 mL
Column Temperature:	40 °C

The total time for on-line SPE is 14 min. For the detailed program see Table 1A.

Analytical

Column:	Acclaim PA, 5 µm, 4.6 × 150 mm (P/N 061320)
Mobile Phases for Analysis (Right Pump):	A. 25 mM HAc / 25 mM NH_4Ac (1.45 : 1, v/v) B. CH_3CN
Gradient:	25 to 70% B in 17.5 min
Flow Rate:	1 mL/min
Inj. Volume:	10 mL
Temperature:	40 °C
Detection:	UV, 280 nm

Total analysis time is 18 min. During SPE, the column is equilibrated for the next separation prior to injection while online SPE is occurring. For the detailed program see Table 1B.

Table 1A. Left Pump Program (Loading Pump Used for SPE) A = 0.2 mM MSA, B = Acetonitrile

Time (min)	Commands	Comments
Preparation	ValveLeft = 6_1, ValveRight = 6_1	
–14.0	Flow = 1000 [µL/min] %B = 100.0, %C = 0.0, Curve = 5	Rinse the SPE column (NG1) using 100% CH_3CN, about 3 min.
–11.5	Flow = 1000 [µL/min] %B = 100.0, %C = 0.0, Curve = 5	
–11.0	Flow = 1000 [µL/min] %B = 1.0, %C = 0.0, Curve = 5	Equilibrate the SPE column.
–8.5	Flow = 2000 [µL/min] %B = 1.0, %C = 0.0, Curve = 5	Load sample from the loop to SPE column at 2 mL/ min, about 5 min.
–3.5	Flow = 2000 [µL/min] %B = 1.0, %C = 0.0, Curve = 5	
–3.0	Flow = 1000 [µL/min] %B = 15.0, %C = 0.0, Curve = 5	Wash the SPE column.
0.2	Flow = 0 [µL/min] %B = 0.0, %C = 0.0, Curve = 5	
3.5	Flow = 200 [µL/min] %B = 100.0, %C = 0.0, Curve = 5	SPE column switches back to the system. Begin to wash the SPE column to prepare for loading the next sample.

Table 1B. Right Pump Program (Analytical Pump) A = 25 mM HAc/NH_4Ac, B = Acetonitrile

Time (min)	Commands	Comments
Preparation	ValveLeft = 6_1, ValveRight = 6_1	
–14.0	Flow = 200 [µL/min] %B = 100.0, %C = 0.0, Curve = 5	Wash the analytical column.
–13.0	Flow = 200 [µL/min] %B = 25.0, %C = 0.0, Curve = 5	
–7.0	Flow = 200 [µL/min] %B = 25.0, %C = 0.0, Curve = 5	
–5.0	Flow = 1000 [µL/min] %B = 25.0, %C = 0.0, Curve = 5	Begin to equilbrate the analytical column using initial conditions for 5 min. Injections at 0 min.
17.5	Flow = 1000 [µL/min] %B = 70.0, %C = 0.0, Curve = 5	17.5 min gradient
18.0	Flow = 1000 [µL/min] %B = 100.0, %C = 0.0, Curve = 5	Begin the column wash.

PREPARATION OF STANDARDS

The preparation of standards for calibration is based on the requirements of EPA Method 604.6

Stock Standard Solution 1

Add 9.95 mL methanol using a graduated 5-mL pipette (two times) to a 10-mL vial, and add 50 µL of the 604 Phenols Calibration Mix (2000 µg/mL) using a 250-µL syringe. The concentration of stock standard solution 1 is 10 µg/mL.

Stock Standard Solution 2

Add 900 µL methanol to a 10-mL vial using a 5-mL graduated pipette, and add 100 µL of stock standard solution 1 using a 250-µL syringe. The concentration of stock standard solution 2 is 1 µg/mL.

Working Standard Solutions

Add 50, 100 and 200 µL of stock standard solution 2 into three separate 100-mL volumetric flasks, using a 250-µL syringe. Bring each to volume with a 0.2 mM MSA solution containing 1% methanol. The concentrations of these solutions are 0.5, 1.0 and 2.0 µg/L.

Add 50, 100 and 200 µL of stock standard solution 1 into three separate 100-mL volumetric flasks, using a 250-µL syringe. Bring each to volume with a 0.2 mM MSA solution containing 1% methanol. The concentrations of these solutions are 5, 10 and 20 µg/L.

SYSTEM SETUP

Figure 2A is a schematic of the devices used for the determination of phenols using online solid-phase extraction (SPE) followed by HPLC with UV detection. The AS-HV has a peristaltic pump that can draw samples from sample bottles through a movable needle. This needle can sample from 15 different 100 mL sample bottles in the sample tray. The movement of the AS-HV is controlled by Chromeleon software. The AS-HV uses the left valve of the TCC-3200 as a sample valve and the right valve as an online SPE switching valve. Figure 2B shows the diagram for programming the large volume injection using the AS-HV. The program for the AS-HV is listed in Table 2. Tables 1A and 1B list the programs for the left (SPE) and right (analytical) UltiMate pumps.

SAMPLE PREPARATION

For the present analysis, tap water was collected at the Dionex Shanghai Applications Lab located in the Pudong District, Shanghai, China. One bottle of pure distilled drinking water and two brands of bottled mineral drinking water (named mineral drinking water 1 and 2, respectively) were purchased from a local supermarket.

Bottled pure distilled drinking water, bottled mineral drinking waters 1 and 2, and tap water samples were prepared by filtering 495 mL of each through 0.45 µm filters into four 500-mL bottles and adding 5 mL methanol and 56 µL MSA to each. The final concentration of MSA in the samples was approximately 2 mM.

Spiked samples were prepared from the above solutions. The procedures for preparation of spiked water samples are shown in Table 3.

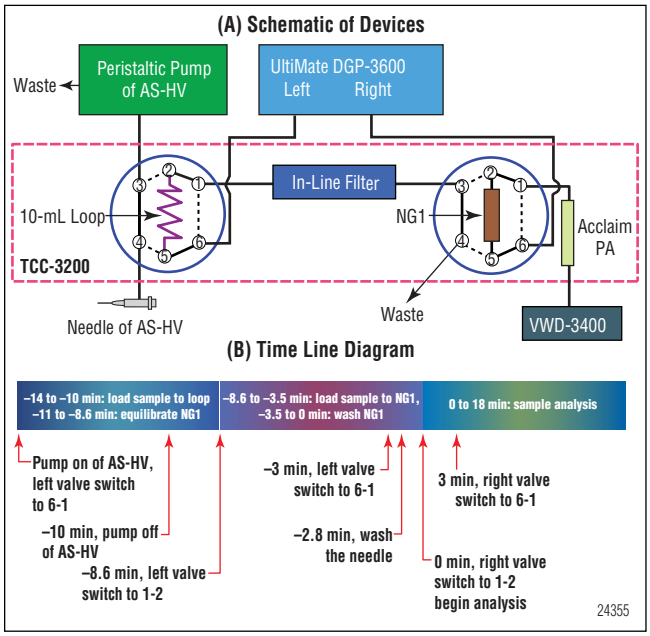

Figure 2. A) Schematic of devices for determination of phenols using online solid-phase extraction (SPE) followed by HPLC with UV detection. B) Time line diagram for programming the high-volume injection using the AS-HV.

Table 2. AS-HV Program

Time (min)	Commands	Comments
Preparation	Y_Axis = AIM_sampler.position X_Axis = AIM_sampler.position Needle = 157, Go To Position	Find position from CM sequence. Set the needle's height and enter the sample bottle.
–14.0	Pump On	Begin to load sample from the bottle. The flow rate of the peristaltic pump is about 3.3 mL/min.
–10.0	Pump Off Needle Home	End sample loading. After sample loading, sample loop switches inline with the SPE column.
–2.8	AIM Sampler, Wash = On, Pump On	Wash the sampling needle and the sample loop in preparation for the next injection.
3.0	Pump Off, Needle Home	End of AS-HV wash.

Table 3. Preparation of Spiked Water Samples		
Samples prepared with 1% methanol and 2 mM MSA	**Amount of added stock standard solution 1 (µL)**	**Phenol concentration (µg/L)**
Distilled drinking water	50	5
Mineral drinking water 1	100	10
Mineral drinking water 2	100	10
Tap water	150	15

RESULTS AND DISCUSSION

Optimization of the Online SPE Method

Different concentrations of acids (HAc or MSA) mixed with methanol or acetonitrile were investigated as wash solutions to elute phenols concentrated on the SPE column. Experiments demonstrated that compared to the acid/methanol solutions, acid/acetonitrile solutions yielded higher peak efficiency, and 0.2 mM MSA/acetonitrile yielded the lowest background.

Figure 3 shows an overlay of chromatograms of phenols spiked into tap water samples, eluted from the SPE column using acetonitrile solutions with different concentrations, and then separated on an Acclaim PA column. More impurities and a high background (poor baseline) were obtained when using acidified water only (Chromatogram A). Although fewer impurities and a lower background were found when using a 20% acetonitrile solution, the recovery of early eluting phenols was reduced (Chromatogram D). Therefore, a 15% acetonitrile solution was selected to ensure recovery of all phenols (Chromatogram C).

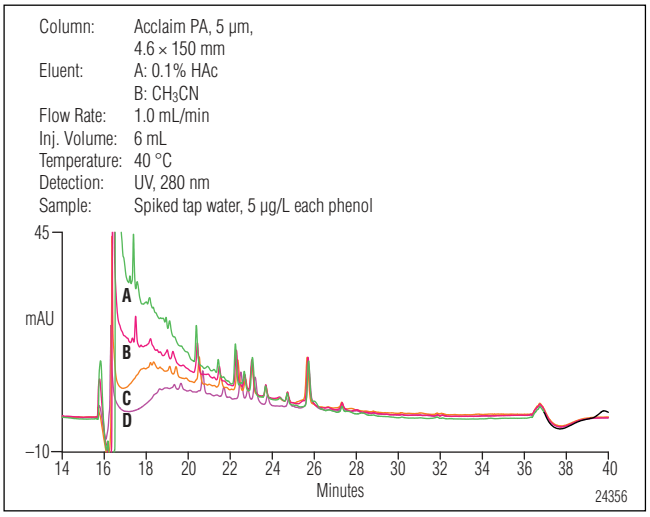

Figure 3. Overlay of chromatograms of tap water samples spiked with 5 µg/L of each phenol, and washed from the IonPac NG1 SPE column using acetonitrile solutions with different concentrations: A) 0% CH_3CN, B) 10% CH_3CN, C) 15% CH_3CN, D) 20% CH_3CN.

Effect of Acidic Solution and Its Concentration in the Mobile Phase on Retention of Phenols

Several acid solutions[15-17] can be used as mobile phases to separate phenols. As shown in Figure 4, good separation of the phenols can be obtained when using methanesulfonic acid (MSA), trifluoroacetic acid (TFA), acetic acid (HAc), or an acetic acid-ammonium acetate buffer (HAc-NH_4Ac).

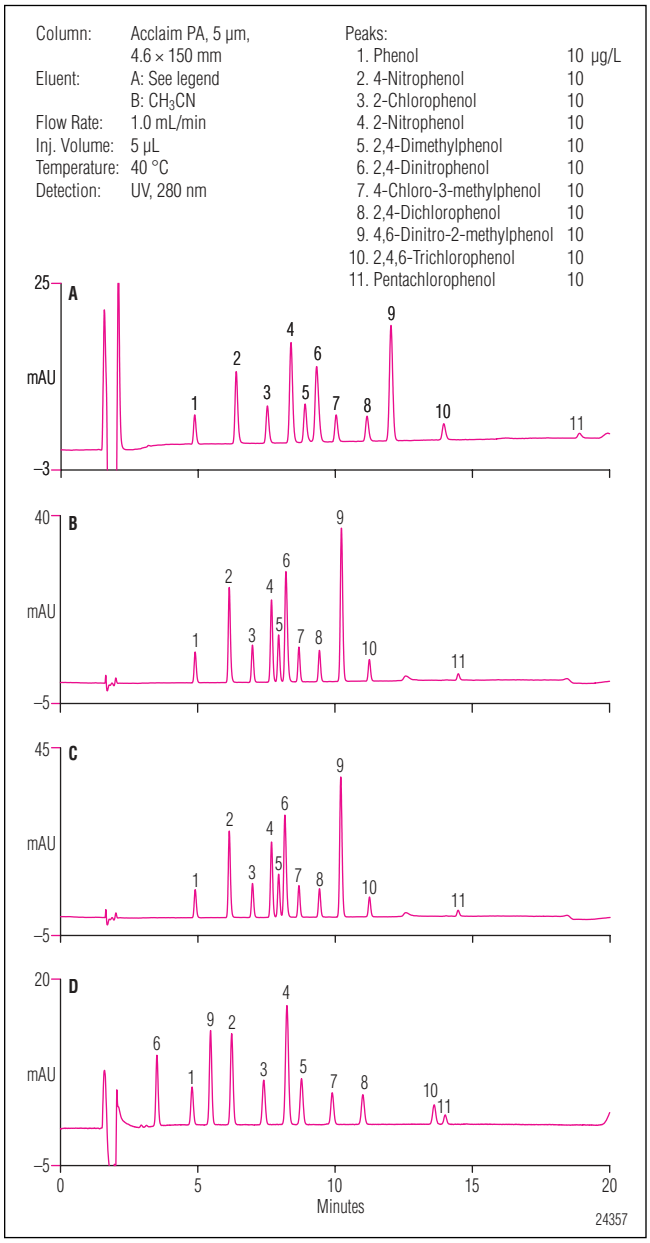

Figure 4. Chromatograms of 10 µg/L phenol working standard separated using acetonitrile as mobile phase B and different acid solutions as mobile phase A: A) 0.1 mM MSA, B) 0.1% TFA, C) 0.1% HAc, D) 25 mM HAc/NH_4Ac.

The effect of changing the mobile phase acid concentration on retention of phenols was investigated. As shown in Figure 5, the retention time of most phenols changed slightly, but that of a few phenols changed significantly with mobile phases and concentrations. When MSA concentration was increased from 0.1 mM to 3.0 mM, the retention time of 2,4-dinitrophenol shifted considerably. The retention time of 4,6-dinitro-2-methylphenol also decreased slightly (Figure 5A). When HAc concentration was increased from 0.03% to 2.0%, the same pattern of retention change was observed (Figure 5B). Substituting TFA for HAc yielded similar results, therefore those data have been omitted.

Changing the proportions of the 25 mM HAc/NH_4Ac buffer had a stronger effect on the retention times of 2,4-dinitrophenol and 4,6-dinitro-2-methylphenol than changing the concentrations of the acid solutions. The retention times of 2,4,6-trichlorophenol and pentachlorophenol also shifted more with changes in the buffer than with changes in the acid concentration (Figure 5C).

Selection of Mobile Phase

HAc, MSA, and TFA solutions all yielded good separation of the eleven phenols specified in U.S. EPA Method 604. When the concentration of acid in the mobile phase was lower, the separation was much better, but the retention times of a few phenols were sensitive to small changes in acid concentration, resulting in unsatisfactory method reproducibility. Therefore, HAc/NH_4Ac buffer was selected as the mobile phase for separating phenols, because it delivered good separation and reproducibility. From Figure 5C, we can predict all eleven phenols will be well resolved using the buffer at about a 1.5:1 (v/v) ratio of the two 25 mM components.

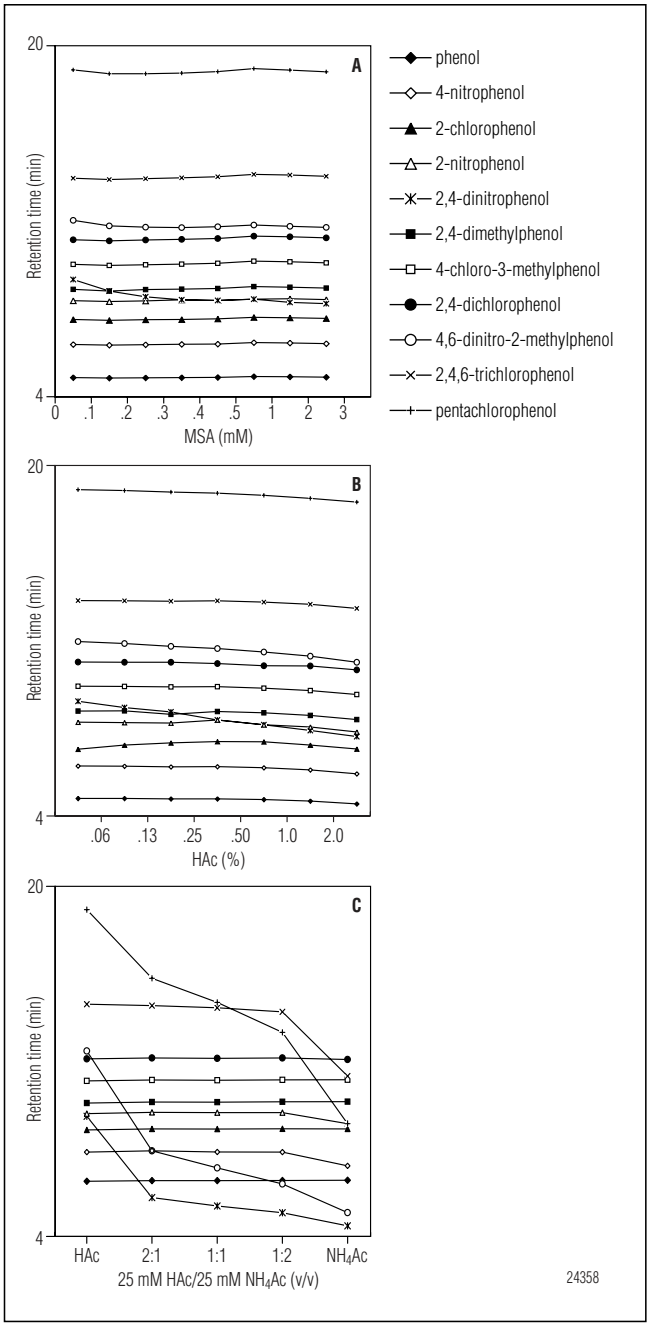

Figure 5. Effect of changing acid concentration in the mobile phase on retention time. A) MSA from 0.1 to 3.0 mM, B) HAc from 0.03 to 2.0%, C) 25 mM HAc-NH_4Ac buffer from 100% HAc to 100% NH_4Ac (v/v).

Table 4. Retention Time Reproducibility, Peak Area Reproducibility, and Comparison of Detection Limits for the 11 Phenols on the U. S. EPA Priority Pollutants List

Phenol	RT RSD[a] (%)	Area RSD[a] (%)	MDL[b] (µg/L)	MDL (µg/L) obtained by GC-FID in EPA 604	MDL (µg/L) obtained by GC-ECD in EPA 604
2,4-Dinitrophenol	0.292	1.358	0.46	13.0	0.63
Phenol	0.240	5.584	0.87	0.14	2.2
4,6-Dinitro-2-methylphenol	0.164	0.647	0.40	16.0	not detected
4-Nitrophenol	0.155	0.432	0.42	2.8	0.70
2-Chlorophenol	0.122	1.659	0.41	0.31	0.58
2-Nitrophenol	0.092	1.487	0.41	0.45	0.77
2,4-Dimethylphenol	0.089	0.462	0.30	0.32	0.68
4-Chloro-3-methylphenol	0.085	0.477	0.31	0.36	1.8
2,4-Dichlorophenol	0.072	0.731	0.08	0.39	not detected
2,4,6-Trichlorophenol	0.056	0.717	0.20	0.64	0.58
Pentachlorophenol	0.064	8.599	0.93	7.40	0.59

[a]Seven injections of the 2 µg/L working standard solution.

[b]The single-sided Student's *t* test method (at the 99% confidence limit) was used for estimating MDL, where the standard deviation (SD) of the peak area of seven injections is multiplied by 3.14 (at n = 7) to yield the MDL.

Reproducibility, Detection Limits, and Linearity

The reproducibility was estimated by making seven replicate injections of the 2 µg/L calibration standard. Table 4 summarizes the retention time and peak area precision data. The method detection limits (MDLs) of the phenols are also listed in Table 4, as are the MDLs reported for the GC method in U.S. EPA Method 604. The MDLs of the on-line SPE-HPLC method are similar to and in most cases better than those achieved using GC, without the labor and cost of liquid/liquid extraction or manual SPE.

Calibration linearity for the determination of phenols was investigated by making replicate injections of a mixed standard of phenols prepared at six different concentrations. The external standard method is used in EPA Method 604. Therefore, we used it to calculate the calibration curve and for sample analysis. Table 5 lists the data from the calibration as reported by Chromeleon.

Table 5. Calibration Data and Linearity of the 11 Phenols

Phenol	r^2	RSD (%)
2,4-Dinitrophenol	0.9998	1.73
Phenol	0.9984	4.29
4,6-Dinitro-2-methylphenol	0.9998	1.69
4-Nitrophenol	0.9997	1.79
2-Chlorophenol	0.9996	2.22
2-Nitrophenol	0.9992	3.03
2,4-Dimethylphenol	0.9999	1.33
4-Chloro-3-methylphenol	0.9998	1.42
2,4-Dichlorophenol	0.9998	1.33
2,4,6-Trichlorophenol	0.9999	1.28
Pentachlorophenol	0.9965	6.07

Sample Analysis

To achieve satisfactory chromatography of phenols in the tap and mineral water samples, these samples should be acidified to approximately pH 3.5 prior to analysis. Figure 6 shows the chromatograms of spiked mineral water sample acidified to pH 7 and pH 3 with MSA, respectively. The peak shapes of 2,4-dinitrophenol, 4,6-dinitro-2-methylphenol, and 4-nitrophenol are superior at pH 3.

For different water samples, the amount of acid required to achieve a pH < 4.5 varies. For example, 6 µL MSA (about 0.2 mM final concentration) was added to the 500 mL pure distilled water sample solution (495 mL distilled water + 5 mL methanol) to yield a pH of approximately 3.9. For the tap water and mineral water samples, much more MSA was needed because these samples contain ions that are capable of buffering the MSA, most notably bicarbonate (Table 6). Therefore, approximately 56 µL MSA (about 2 mM final concentration) was added to the tap and mineral water samples to achieve pH values ranging from 2.5 to 4.5.

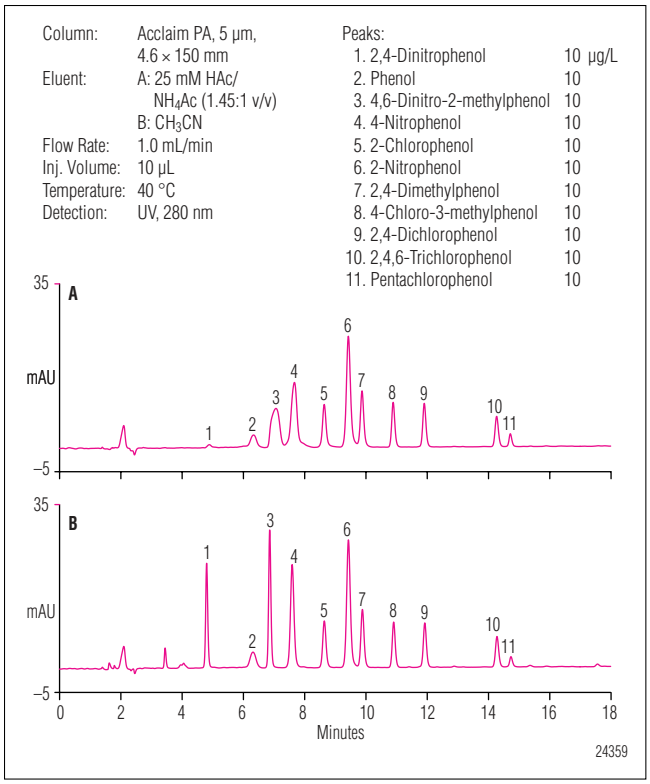

Figure 6. Chromatograms of bottled mineral drinking water 1 spiked with 10 µg/L phenols and acidified with MSA to A) pH 7, and B) pH 3.

Table 6. Listed Amounts of Ions in Bottled Mineral Drinking Waters

Labeled Contents	Bottled Mineral Drinking Water 1 (mg/L)	Bottled Mineral Drinking Water 2 (mg/L)
Na^+	≥0.8	4–12
K^+	≥0.35	0.3–1.0
Ca^{2+}	≥4	not reported
Mg^{2+}	≥0.5	0.3–0.5
Zn^{2+}	not reported	0.25
Sr^{2+}	not reported	0.14
$HSiO_2$	≥1.8	71.6
HCO_3^-	not reported	14
pH (25 °C)	7.35 ± 0.5	7.0–8.0

Table 7. Bottled Mineral Drinking Water Analytical Results								
Phenol	**Bottled mineral drinking water 1[a]**				**Bottled mineral drinking water 2[b]**			
	Unspiked (µM)	**Added (µM)**	**Found (µM)**	**Recovery (%)**	**Unspiked (µM)**	**Added (µM)**	**Found (µM)**	**Recovery (%)**
2,4-Dinitrophenol	ND[c]	10	9.44	94.4	ND	10	9.57	95.7
Phenol	ND	10	11.9	119	0.37	10	10.0	100
4,6-Dinitro-2-methylphenol	ND	10	9.56	95.6	ND	10	9.57	95.7
4-Nitrophenol	ND	10	10.2	102	ND	10	10.0	100
2-Chlorophenol	ND	10	10.4	104	ND	10	9.02	90.2
2-Nitrophenol	ND	10	11.9	119	ND	10	10.9	109
2,4-Dimethylphenol	ND	10	10.5	105	ND	10	9.97	99.7
4-Chloro-3-methylphenol	ND	10	9.56	95.6	ND	10	9.40	94.0
2,4-Dichlorophenol	ND	10	9.75	97.5	ND	10	9.05	90.5
2,4,6-Trichlorophenol	ND	10	10.1	101	0.75	10	9.55	95.5
Pentachlorophenol	0.73	10	9.67	96.7	ND	10	9.60	96.0

[a]One unspiked sample of mineral drinking water 1 was prepared and two injections were made. One spiked sample was prepared and four injections were made.

[b]One unspiked sample of mineral drinking water 2 was prepared and three injections were made. One spiked sample was prepared and five injections were made.

[c]ND = not detected.

Bottled Mineral Drinking Water

Two brands of bottled mineral drinking water were analyzed. Table 6 shows the contents listed on the labels of each. Figures 7 and 8 show chromatograms of the bottled mineral water samples and the same samples spiked with phenols. The results are summarized in Table 7. Low concentrations of two phenols were detected in the unspiked mineral water 2 sample and a low concentration of one phenol in the unspiked mineral water 1. Good recoveries were obtained for all eleven phenols.

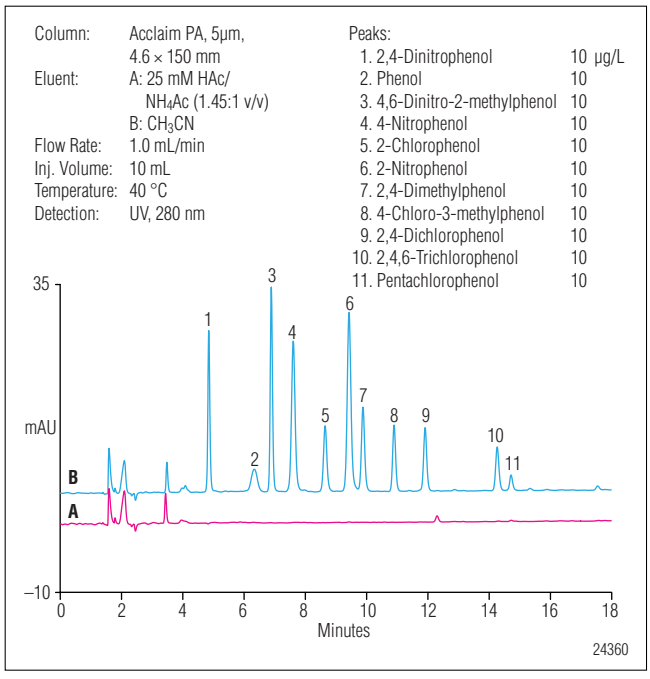

Figure 7. Overlay of chromatograms of bottled mineral drinking water 1, A) unspiked, and B) spiked with 10 µg/L phenols.

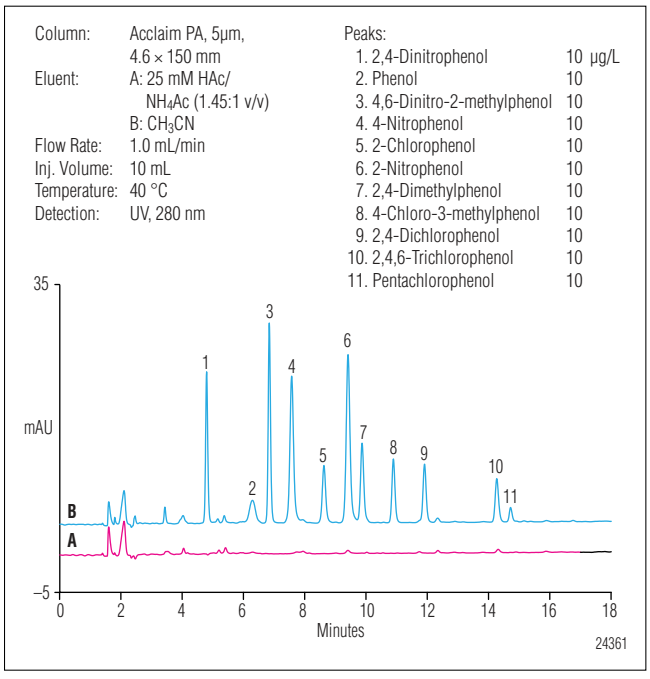

Figure 8. Overlay of chromatograms of bottled mineral drinking water 2, A) unspiked, and B) spiked with 10 µg/L phenols.

Table 8. Bottled Pure Distilled Drinking Water and Tap Water Analytical Results								
Phenol	**Pure distilled water[a]**				**Tap water[b]**			
	Unspiked (µM)	**Added (µM)**	**Found (µM)**	**Recovery (%)**	**Unspiked (µM)**	**Added (µM)**	**Found (µM)**	**Recovery (%)**
2,4-Dinitrophenol	ND[c]	5	4.95	99.0	2.11	15	10.4	70.0
Phenol	ND	5	4.84	96.8	0.41	15	14.2	94.7
4,6-Dinitro-2-methylphenol	ND	5	5.02	100	ND	15	15.1	101
4-Nitrophenol	ND	5	5.09	102	0.80	15	15.2	101
2-Chlorophenol	ND	5	5.22	104	<MDL[d]	15	11.50	76.7
2-Nitrophenol	ND	5	5.30	106	ND	15	14.0	93.3
2,4-Dimethylphenol	ND	5	5.19	104	1.63	15	15.0	100
4-Chloro-3-methylphenol	ND	5	5.07	101	<MDL	15	14.5	96.4
2,4-Dichlorophenol	ND	5	4.98	99.6	ND	15	14.1	94.0
2,4,6-Trichlorophenol	ND	5	5.20	104	0.65	15	14.6	97.0
Pentachlorophenol	ND	5	4.99	99.8	1.13	15	14.2	94.5

[a]One unspiked sample of pure distilled drinking water was prepared and five injections were made. One spiked sample was prepared and four injections were made.

[b]One unspiked sample of tap water was perpared and two injections were made. One spiked sample was prepared and five injections were made.

[c]ND = not detected..

[d]<MDL = lower than method detection limit.

Bottled Pure Distilled Drinking Water

Figure 9 shows chromatograms of pure distilled drinking water and the same water spiked with phenols. The results are summarized in Table 8. No phenols were found in the unspiked sample, and recovery of all phenols in the spiked sample was excellent.

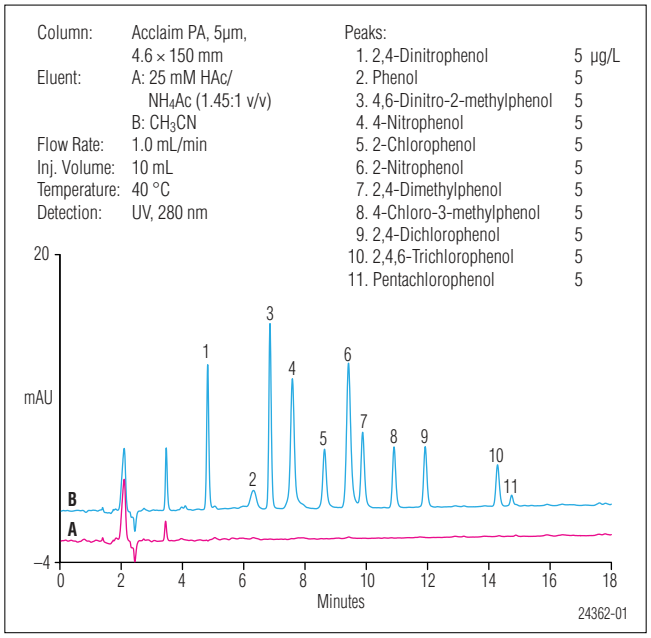

Figure 9. Overlay of chromatograms of pure distilled drinking water, A) unspiked, and B) spiked with 5 µg/L phenols.

Tap Water

Figure 10 shows chromatograms of tap water and the tap water spiked with phenols. The results are summarized in Table 8. Low concentrations of several phenols were detected and some peaks were detected with peak areas that yielded concentrations below the estimated MDL.

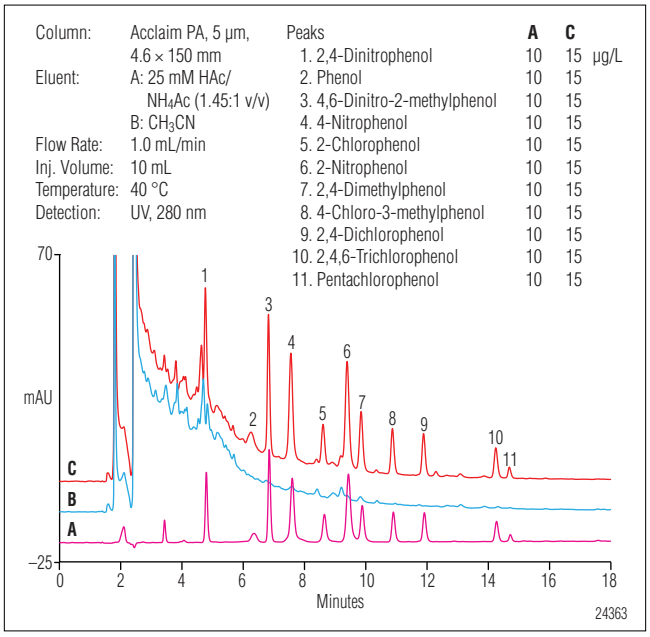

Figure 10. Overlay of chromatograms of A) the 10 µg/L phenol standard, B) unspiked tap water, and C) tap water spiked with 15 µg/L phenols.

CONCLUSION

The successful analysis of all the water samples above demonstrates that online SPE with a dual UltiMate system can determine the 11 phenols designated on the EPA Priority Pollutants List without laborious offline sample preparation. The online SPE method with UV detection has very good reproducibility, with detection limits similar to and in many cases superior to the GC methods described in EPA Method 604.

PRECAUTIONS

Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware. Clean all glassware scrupulously and use high purity reagents and solvents to minimize interference problems.

Samples must be acidified to about pH 3.5 with MSA before large volume injections, especially for the mineral drinking water and tap water samples. If not, the determination of 2,4-dinitrophenol, 4,6-dinitro-2-methylphenol and 4-nitrophenol can be affected.

The tubing and sample loop of the AS-HV are not compatible with high concentration organic solvents. Change the sample loop and the tubing used to connect the loop to the sample valve to either stainless steel or PEEK™.

APPENDIX

Using One Pump Channel of a Dual Pump System Instead of the High-Volume Autosampler

If only a few samples need to be analyzed for phenols, it is possible to use one pump channel of a dual pump system instead of the AS-HV autosampler for sample injection. This configuration is shown in Figure 11. Figure 11A shows the system schematic and Figure 11B shows the program.

Place the sample in an eluent bottle and use one pump of the dual pump system to deliver the sample to the SPE column at a defined flow rate for a set amount of time. Bypass the degasser with the eluent lines used to deliver sample to minimize carryover between injections. Clean eluent lines thoroughly with 100% organic solvent and pure water prior to using this pump channel for other applications.

Use the left pump as the SPE pump and channel C of the left pump as an injector. Pump the sample for 6 min at 1 mL/min to deliver 6 mL of sample to the SPE column. Use channels A (0.2mM MSA) and B (acetonitrile) of the left pump to rinse the SPE column and elute the captured phenols. Use the second (right) pump to deliver the gradient to separate the phenols on the Acclaim PA column. Figure 12 shows a chromatogram of the separation of phenols in a spiked tap water sample using this setup.

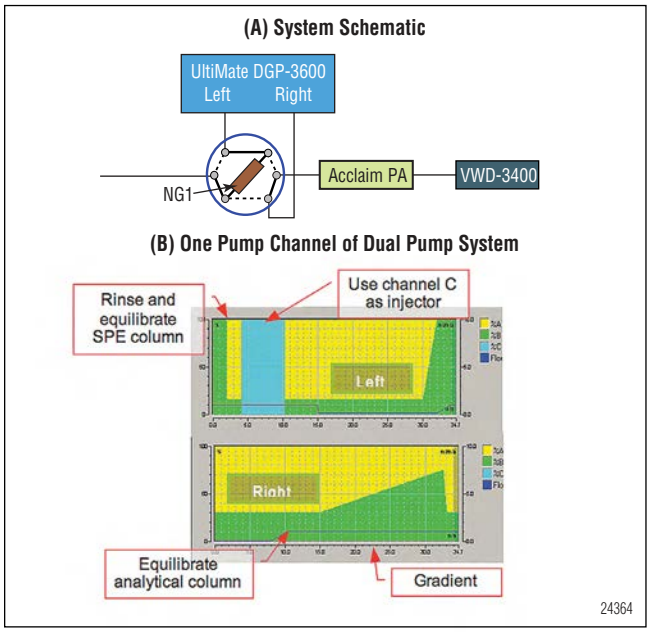

Figure 11. A) System schematic and B) program for using one pump channel of a dual pump system in place of the AS-HV Autosampler.

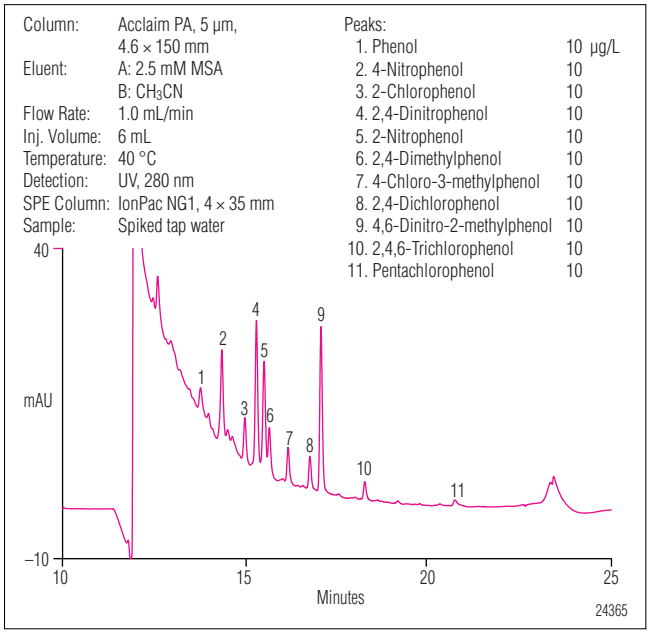

Figure 12. Chromatogram of a tap water sample spiked with 10 µg/L phenols, using one pump channel of a dual pump system instead of the AS-HV Autosampler.

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Extraction of Zearalenone from Wheat and Corn by Accelerated Solvent Extraction (ASE®)

INTRODUCTION

Zearalenone (ZON) is a mycotoxin produced by the Fusarium fungus. ZON can be found in a wide variety of plants and soils, and can have negative health effects on animal husbandry and humans. Traditional methods for extracting ZON from soils or animal feed include wrist shaking or blending. These methods normally take 30–60 min per sample with constant lab technician attendance. Because of the time-consuming nature of these traditional extraction techniques, many sample prep labs experience large bottlenecks that hinder the flow of samples to the analytical lab.

Accelerated Solvent Extraction (ASE) is a proven extraction technology that not only helps to eliminate these bottlenecks by decreasing the extraction time, but requires far less technician attendance because it is an automated system. ASE uses increased temperatures to speed up the extraction process, while incorporating high pressure to maintain the solvents in their liquid states at these elevated temperatures. Because of the increased temperatures and pressures, ASE can perform extractions in less than half the time traditional extraction methods require and can do these extractions using very small amounts of solvent.

EQUIPMENT

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

22-mL stainless steel extraction cells (P/N 048764)

Dionex Cellulose Filters (P/N 049458)

Dionex Collection Vials, 60 mL (P/N 048784)

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

Sand (Ottawa Standard, Fisher Scientific, Cat. No. S23-3)

Laboratory grinder or blender (Fisher Scientific)

Tyler Sieve 0.5 mm (Fisher Scientific)

PTFE Syringe Filter 0.45 µm (Fisher Scientific)

REAGENTS

Dionex ASE Prep DE (P/N 062819)

SOLVENTS

Methanol

Acetonitrile

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

EXTRACTION CONDITIONS

Solvent:	50% methanol, 50% acetonitrile
Temperature:	80 °C
Pressure:	1500 psi
Heatup time:	5 min
Static time:	5 min
Static cycles:	2
Flush:	75%
Purge:	100 s
Total extraction time:	15 min
Volume of solvent used:	5–35 mL

SAMPLE PREPARATION

Grind samples using a laboratory grinder to a powder that can pass through a 0.5-mm sieve. Weigh 5 g of the sample powder into a small beaker and mix thoroughly with 3 g of ASE Prep DE. Mixing the sample with ASE Prep DE ensures a porous mixture that allows the solvent to flow easily through the sample. Add the sample mixture to a 22-mL stainless steel extraction cell containing a cellulose filter. Fill any void volume with Ottawa sand and screw on the end cap.

EXTRACTION PROCEDURE

Place the cells onto the ASE 200. Label the appropriate number of collection vials and place these into the extractor. Set up the method suggested above and begin the extraction. When the extraction is complete, the extract can be diluted to any desired final volume or concentrated for samples containing low levels of contamination. Finally, filter a portion of the extract into an autosampler vial through a 0.45-µm PFTE filter and analyze using LC-MS.[1]

RESULTS AND DISCUSSION

Sample preparation is critical to good recoveries. It is important to grind the samples to a uniform particle size to ensure proper permeation of the solvent into the matrix. Proficiency tests were performed using corn and wheat samples spiked with a ZON standard at 400 ng/g, which showed that MeOH-ACN(1:1) at 80 °C using a 5-min static cycle was optimum for quantitatively extracting all of the ZON from the sample. Because ZON reference material is still not commercially available, two samples used in an international proficiency study were analyzed to evaluate the parameters chosen for the ASE instrument. The samples were extracted in triplicate for both matrices. The results, shown in Table 1, indicate that ASE can provide better results than traditional methods for the extraction of ZON from wheat and corn.

Table 1. Results of Extraction of ZON from Wheat and Corn Using ASE

Sample	Target Value (ng/g)	Average Recovery (ng/g) n=3	Percent Recovery	Percent RSD
Wheat	112	132	118	5.2
Corn	285	305	107	2.2

CONCLUSIONS

These results confirm that ASE is comparable to traditional extraction methods for the extraction of Zearalenone from wheat and corn. The extraction times of traditional extraction methods usually range from 30 to 60 min per sample, and require large amounts of solvent and constant technician attendance. ASE reduces the extraction time to ~15 min per sample and uses only 25–35 mL of solvent. In addition, the ASE 200 can automatically extract up to 24 samples sequentially without user intervention.

ACKNOWLEDGEMENT

We would like to acknowledge the work of Lea Pallaroni and Christoph von Holst from the European Commission, Institute for Reference Materials and Measurements, Retieseweg, Belgium.

SUPPLIER

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

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Time Savings and Improved Reproducibility of Nitrate and Nitrite Ion Chromatography Determination in Milk Samples

INTRODUCTION

Cow's milk is of particular dietary value to infants, small children, and expectant mothers as it is an important source of calories, minerals (including calcium), fat-soluble vitamins A, D, E, and K, and protein. Because of its nutritional value, it is imperative that the commercial milk supply be free of contaminants such as nitrate and nitrite. The excessive consumption of nitrate can lead to underoxygenation of the blood and, consequently, underoxygenation of the tissues, which can cause numerous health problems, the most severe of which is death. With a much smaller total blood volume, infants and small children are more severely impacted than adults when consuming the same nitrate-contaminated product.

The most likely source of nitrate in the blood stream is drinking water. Drinking water can become contaminated in areas where there has been excessive application of nitrate-based fertilizers and where sodium or potassium nitrate is used in canisters designed to kill rodents. For an infant, the water used to prepare infant formula (baby food), the water consumed by the nursing mother, or the water consumed by dairy cattle whose milk is used to prepare milk-based infant formulas, are possible sources of nitrate. For most children, infant formula and mother's milk will eventually be replaced by cow's milk.

Nitrite is also a concern because it is easily oxidized to nitrate. Excessive consumption of nitrite and nitrate also has been implicated as a cause of other health problems.[1] For these reasons, the United States Environmental Protection Agency (U.S. EPA) regulates the amount of nitrite and nitrate in drinking water and has published an ion chromatography (IC) method for the determination of these two anions, along with fluoride, chloride, bromide, sulfate, and phosphate.[2] For the same reasons, the concentration of nitrite and nitrate should also be determined in milk.

The IC analytical technique is the most commonly used for simultaneously measuring nitrite and nitrate in samples. These two anions can be detected either by suppressed conductivity detection or by their absorbance at 210 nm.[3] Unfortunately, milk samples cannot be injected directly onto the IC system to measure nitrite and nitrate because the milk fat will foul and eventually poison the column, and milk proteins will interfere with the chromatography and compromise the detection of nitrite and/or nitrate by either suppressed conductivity or absorbance detection. Even after one or more sample preparation steps, the remaining protein or other anionic molecules can interfere with nitrite and nitrate determination, or foul the column. The analyst must remove as many interfering compounds from the milk as possible while still achieving full recovery of nitrite and nitrate.

In this study, a milk sample is subjected to an acid precipitation step prior to loading the sample into an autosampler vial. The remainder of the sample preparation is completed in-line with an InGuard® HRP sample preparation cartridge. This saves the analyst time and reduces the possibility of sample contamination. Nitrite and nitrate are then separated on an IonPac® AS20 column set and detected by suppressed conductivity detection using a Reagent-Free™ IC (RFIC™) system. The RFIC system prepares the hydroxide eluent with high fidelity, which augments method reproducibility. The InGuard cartridge must be changed every 100 injections, which allows the column set to be used for approximately1000 sample injections while still accurately determining the nitrite and nitrate contents of the milk sample with only minimal off-line sample preparation.

EQUIPMENT

Dionex ICS-3000 system* including:

DP Dual Pump

DC Detector/Chromatography module with dual-temperature zone equipped with two 6-port valves and a conductivity detector

EG Eluent Generator

AS Autosampler

EWP Electrolytic Water Purifier (P/N 071553)

AXP Auxiliary Pump (P/N 063973)

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

*This application can also be executed on an ICS-5000 system.

REAGENTS AND STANDARDS

Deionized water (DI), Type I reagent grade, 18 MΩ-cm resistivity or better

Concentrated acetic acid (CH_3COOH, Labscan)

Sodium Nitrite ($NaNO_2$, Fluka)

Sodium Nitrate ($NaNO_3$, Fluka)

Samples

Instant Powder Milk Sample #1 (containing 6.203% fat)

Instant Powder Milk Sample #2 (containing 1.799% fat)

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solution

The eluent generator produces the eluent using the EluGen EGC II KOH cartridge and DI water supplied by the pump, with the eluent concentration controlled by the Chromeleon software. Backpressure tubing must be added to achieve 2000–2500 psi backpressure that will allow the EG degasser to function properly. See the ICS-3000 Ion Chromatography System Operator's Manual (P/N 065031-03) for instructions on adding backpressure.

Acetic Acid (3%)

Add 3 mL of concentrated acetic acid to approximately 50 mL of DI water in a 100 mL volumetric flask. Dilute to volume with DI water and mix.

Standard Solutions

Nitrite Stock Standard Solution (1000 mg/L)

Dissolve 0.150 g of sodium nitrite in a 100 mL volumetric flask with DI water.

Nitrate Stock Standard Solution (1000 mg/L)

Dissolve 0.137 g of sodium nitrate in a 100 mL volumetric flask with DI water.

Nitrite Standard Solution (10 mg/L)

Dilute 1 mL of 1000 mg/L nitrite standard in a 100 mL volumetric flask with DI water.

Nitrate Standard Solution (20 mg/L)

Dilute 2 mL of 1000 mg/L nitrate standard in a 100 mL volumetric flask with DI water.

Calibration Standard and Sample Preparation

Prepare calibration standard solutions by adding a known amount of standard solution into the sample during sample preparation. Weigh 1 g of milk powder into a 100 mL bottle, then add the appropriate volumes of 10 mg/L nitrite and 20 mg/L nitrate solutions to produce each calibration standard. Table 1 lists the volumes to be added of each standard and the subsequent concentrations in the sample.

Table 1. Volumes of 10 mg/L Nitrite, 20 mg/L Nitrate, 3% Acetic Acid, and DI Water Used for Each Preparation

Sample	Volume of Added Standard and Resulting Concentration (mL, mg/L)		Volume of 3% Acetic Acid Added (mL)	Volume of DI Water Added (mL)
	10 mg/L Nitrite	20 mg/L Nitrate		
Blank (no milk)	—	—	1	49.0
Unspiked*	—	—	1	49.0
Spiked 1*	0.1, 0.02	0.1, 0.04	1	48.8
Spiked 2*	0.2, 0.04	0.2, 0.08	1	48.6
Spiked 3*	0.4, 0.08	0.4, 0.16	1	48.2
Spiked 4*	0.8, 0.16	0.8, 0.32	1	47.4

*The preparation is for 1 g of milk sample. The total volume of the final samples is 50 mL.

Add the appropriate amount of DI water to bring the volume of each sample to 49 mL (Table 1), shake, and put in an ultrasonic bath for 10 min. Add 1 mL of 3% acetic acid and shake to precipitate protein. Let the sample sit for 20 min. Use a 3 mL syringe to remove 3 mL of sample solution and filter with a 0.45 µm syringe filter washed with DI water before use. Discard the first 1.5 mL of sample and collect the remaining sample into a 1.5 mL glass vial. Rinse the vials with DI water prior to adding sample. The sample solutions to which known amounts of standard are added are referred to as Spiked 1, Spiked 2, Spiked 3, and Spiked 4. Sample solution without added standard solution is referred to as Unspiked.

Spiked Sample Preparation for Recovery and MDL Studies

Prepare spiked samples for recovery and MDL studies in the same manner as described above. For the recovery study, prepare the spiked sample to yield the same concentration as Spiked 1. Due to the nitrate present in the sample, spike only nitrite into the sample for the MDL study. Spike in an amount to yield 0.01 mg/L after preparation.

Table 2. Gradient Program and Valve Switching

Time (min)	Eluent Conc. (mM)	Inject-Valve_1	Inject-Valve_2	Remark
-20.0	50	Inject	Inject	Wash column and concentrator
-7.1	50	Inject	Inject	
-7.0	7	Inject	Load	
-5.0	7	Load, Inject*	Load	Load sample and then begin in-line sample preparation
0.0	7	Inject	Inject	Begin separation
25.0	7	Inject	Inject	
25.1	50	Inject	Inject	

*InjectValve_1 is controlled by the AS so that the program clock will be held during loading of the sample into the sample loop. After loading, InjectValve_1 is immediately switched to the inject position and the program resumes.

CHROMATOGRAPHIC CONDITIONS

Column:	IonPac AS20 Analytical, 4 × 250 mm (P/N 063148) IonPac AG20 Guard, 4 × 50 mm (P/N 063154)
InGuard:	InGuard* HRP, 9 × 24 mm (P/N 074034)
Concentrator:	IonPac UTAC-LP1, 4 × 35 mm (P/N 063079)
Eluent Source:	EGC II KOH (P/N 058900) with CR-ATC (P/N 060477)
Gradient:	See Table 2
Flow Rate:	1.0 mL/min
Sample Volume:	25 µL
Column Temp.:	30 °C (both zones of the DC are set to 30 °)
Detection:	Suppressed conductivity ASRS® 300, 4 mm (P/N 064554), External water mode (AXP flow rate 1 mL/min), 125 mA

*Prewash the InGuard cartridge in the IC system with water for a few minutes before use.

RESULTS AND DISCUSSION

Milk is a challenging sample because it has high concentrations of protein and fat. The protein can consume column capacity and interfere with the detection of nitrite and nitrate either by suppressed conductivity or UV absorbance detection. The fat can damage the column by a number of mechanisms including the generation of excessive backpressure. Therefore, removing the protein and fat from the sample is required for a successful application and for extending column lifetime. In traditional sample treatment, off-line sample treatment with an OnGuard® RP cartridge should be done before sample injection. There are some disadvantages to off-line sample treatment. It requires analyst time and the sample can be contaminated. OnGuard cartridges are designed for a single use and, therefore, each study requires multiple cartridges. InGuard cartridges are designed for on-line sample treatment during which multiple injections can be made on a single InGuard cartridge. With on-line sample preparation, analyst time is reduced, sample contamination is minimized, and the cost of sample analysis is reduced.

In this application, protein was first precipitated using acetic acid and then fat was removed on-line with an InGuard HRP cartridge. The goal was to have the InGuard cartridge last for at least 50 sample injections. In this study, the InGuard cartridge was changed after 100 milk sample injections (previously treated with acetic acid). The InGuard cartridge was changed before failure to ensure that the column was protected. Without protein precipitation prior to sample injection, high backpressure caused the InGuard cartridge to fail after less than 50 injections, and the chromatography was compromised by a noisy baseline. The goal of this challenging application was to have the column set withstand 500 sample injections before failure. With this sample treatment, approximately 1000 milk sample injections were made before nitrite had too great a loss of retention to be resolved from other peaks. Figure 1 shows the loss of retention time of nitrite and nitrate after approximately 800 sample injections, which still yielded acceptable resolution of nitrite from the unknown peak.

Column:	IonPac AS20 Analytical, 4 × 250 mm IonPac AG20 Guard, 4 × 50 mm
InGuard:	HRP, 9 × 24 mm
Concentrator:	IonPac UTAC-LP1, 4 × 35 mm
Eluent Source:	EGC II KOH with CR-ATC
Gradient:	*Time (min)* *Eluent Conc. (mM)* -20 50 -7.1 50 -7.0 7 -5.0 7 0.0 7 25.0 7 25.1 50
Flow Rate:	1.0 mL/min
Inj. Volume:	25 µL
Column Temp.:	30 °C
Detection:	Suppressed conductivity, ASRS 300, 4 mm with external water mode, current 125 mA
Sample:	A. Spiked Milk Sample at start B. Spiked Milk Sample after 800 sample injections

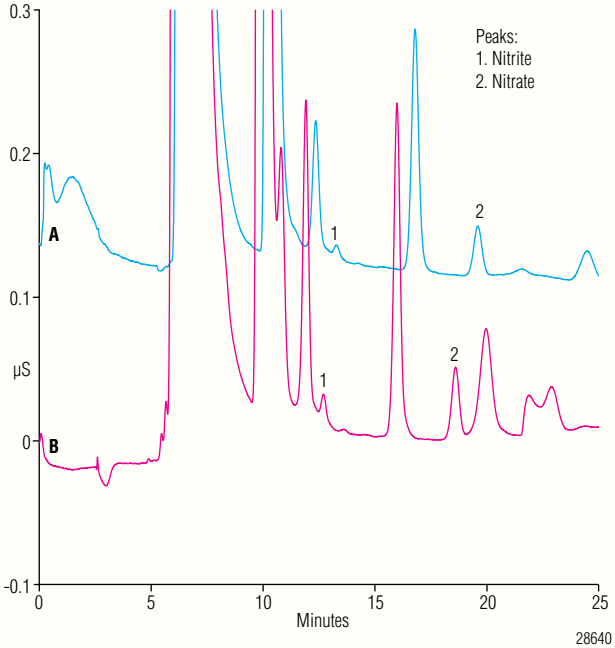

Figure 1. Chromatogram of Spiked Milk Sample #2 at the start and after 800 sample injections.

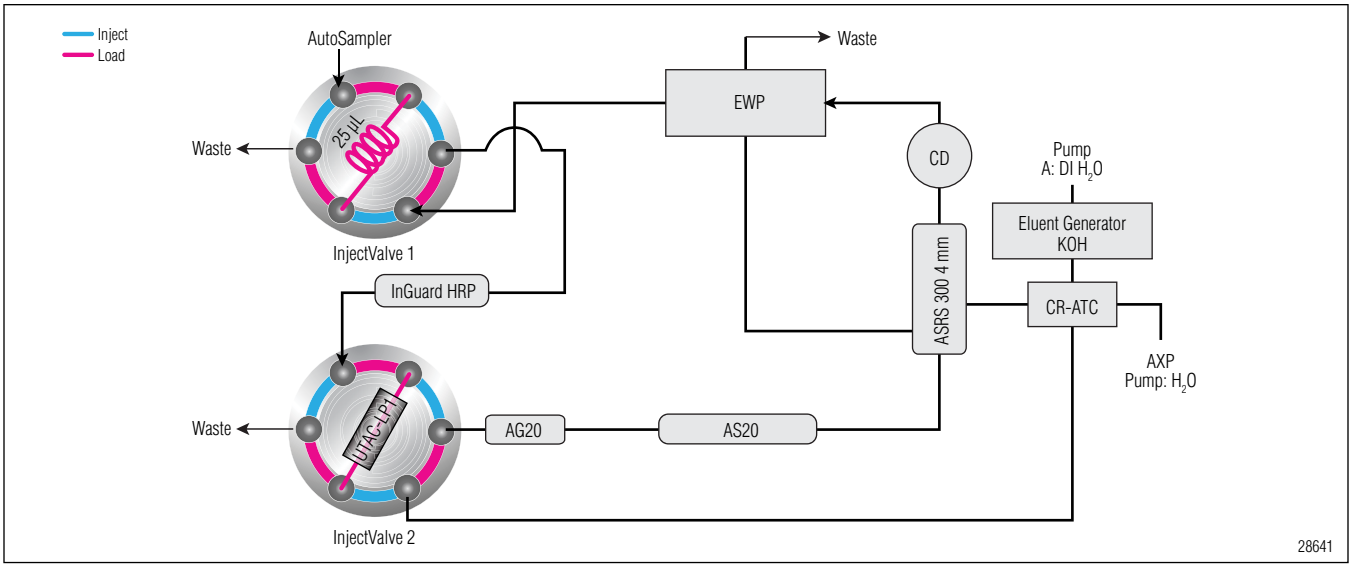

Figure 2. System configuration (both valves are in the load position).

Figure 2 shows the configuration of the system. After protein precipitation with 3% acetic acid, 25 µL of the sample were injected. Water from the outlet of the conductivity cell, purified by the EWP, was used to flush the sample from the sample loop to the InGuard cartridge. The sample compounds not bound by the InGuard cartridge, including nitrite and nitrate, were collected on the concentrator. The concentrator was then eluted onto the IonPac AS20 column set to separate nitrite and nitrate from the other bound sample components. Other hydroxide selective columns—including the IonPac AS11, AS11-HC, AS15, AS18, and AS19 columns—were tested, but the resolution on the AS20 column and its high capacity made it the most suitable column for this application.

To achieve the highest retention time reproducibility, this application was configured on an RFIC system. This system eliminates the labor and possible error of manual hydroxide eluent preparation. After a sample injection, the column and concentrator must be washed with 50 mM KOH for 13 min. This will remove the anionic compounds that were not eluted during the separation. The suppressed conductivity detection was configured with external water mode so that the effluent from the conductivity cell could be a source of water to move the sample from the sample loop to the InGuard cartridge.

When this configuration is used to execute the method in Table 2 on a blank sample (the acetic acid used for protein precipitation), the result is shown in Figure 3. The large peak between approximately 6 and 11 min is

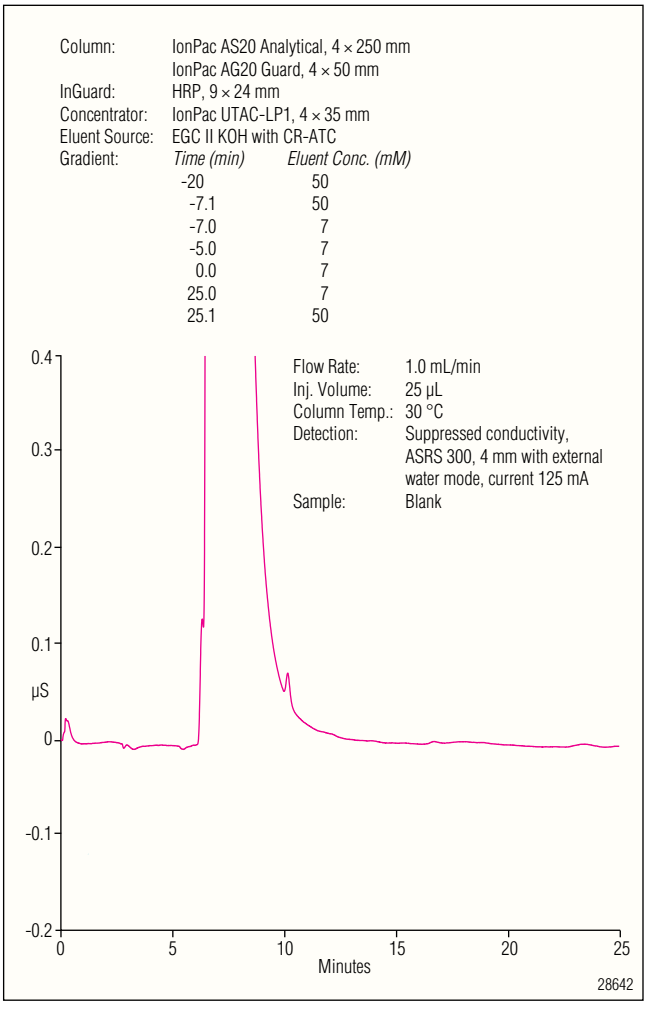

Figure 3. Chromatogram of an acetic acid blank.

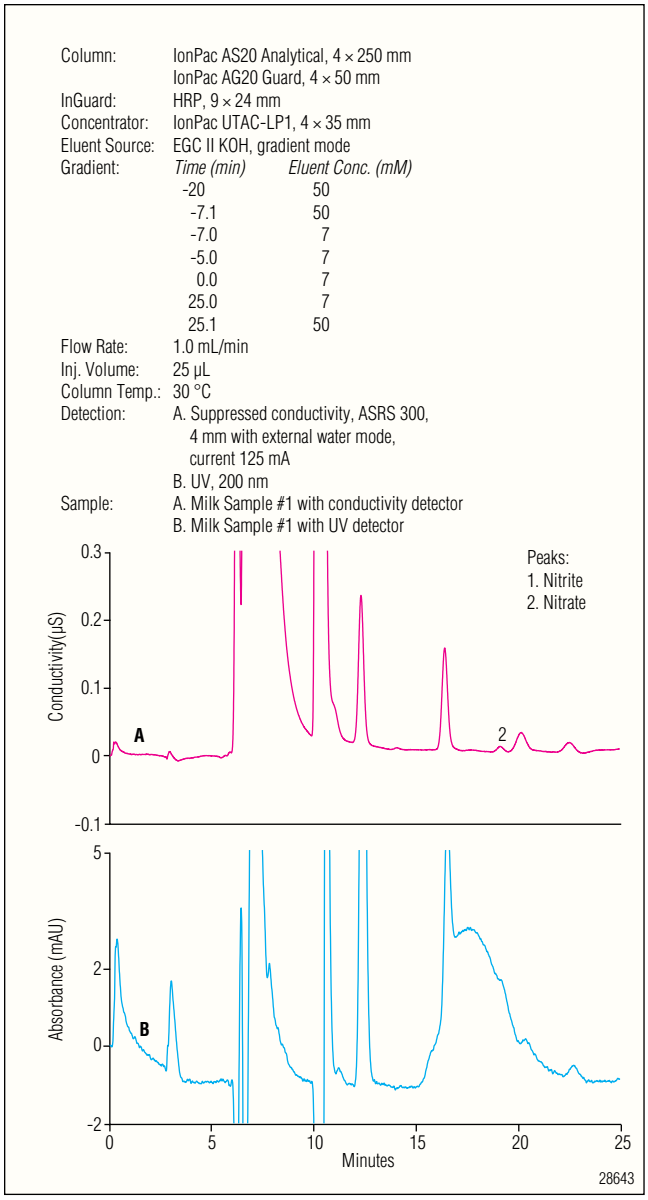

Figure 4. Overlay of chromatograms of Milk Sample #1 with (A) suppressed conductivity detection and (B) UV detection.

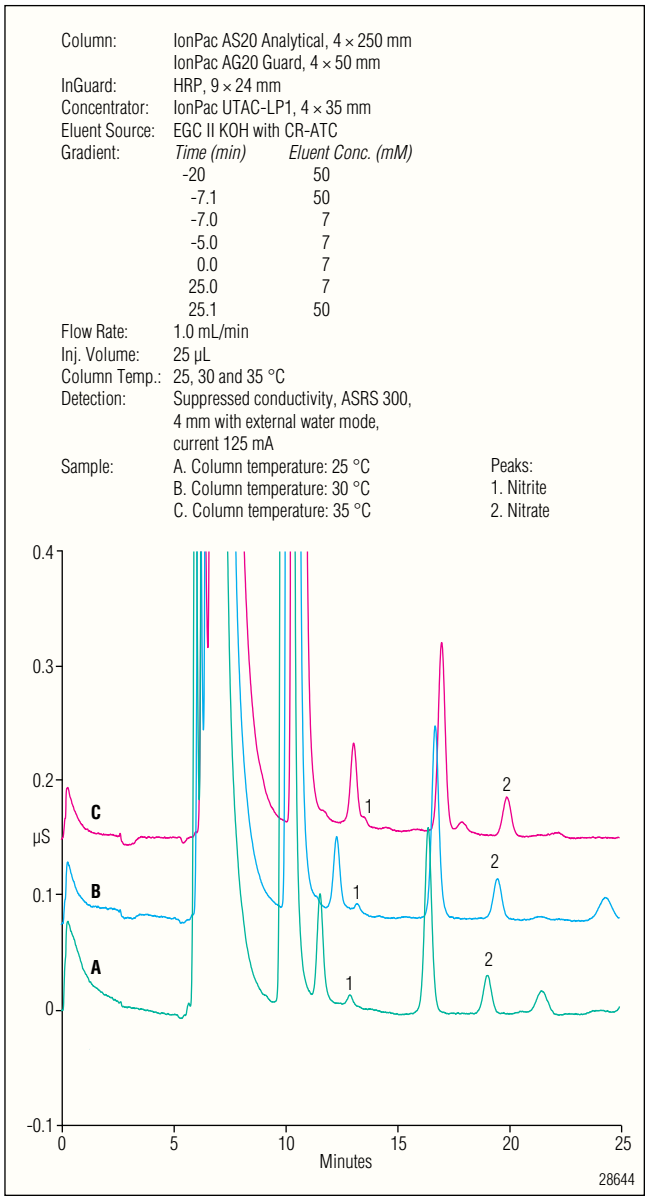

Figure 5. Overlay of chromatograms of prepared Milk Sample #2 analyzed at different column temperatures.

acetate, which does not interfere with nitrite or nitrate detection. A UV detector is placed after the conductivity detector to determine the best mode of detection for this analysis. Figure 4 shows chromatograms of Milk Sample #1 with conductivity and UV detections. The nitrate present in Sample #1 is difficult to determine with the UV detector, whereas it is readily determined by the conductivity detector.

This study showed that column temperature control is important to the success of this application. Specifically, if the column temperature was too high, the desired separation was not achieved. Figure 5 shows the effect of column temperature on the separation of nitrite and nitrate in milk. At 35 °C, nitrite co-elutes with an unknown peak in the milk sample. For the remainder of the study, the column temperature was set at 30 °C, but 25 °C also yielded a good separation. These separation conditions were successful for the two samples studied. Some milk samples may require an adjustment of eluent concentration and/or column temperature to resolve nitrite and/or nitrate from unknown sample components.

Table 3. Calibration Results								
Analyte	**Sample #1**				**Sample #2**			
	Points	**r^2**	**Offset**	**Slope**	**Points**	**r^2**	**Offset**	**Slope**
Nitrite	4	0.9990	-0.0002	0.1463	4	0.9991	-0.0008	0.1224
Nitrate	5	0.9995	0.0023	0.1268	5	0.9993	0.0101	0.1189

The method of standard additions was chosen for this application. Calibration standards were prepared in the sample and the added standard concentration was plotted versus the measured signal. Using this calibration curve, the amount of endogenous analyte in the sample can be determined. Two brands of milk purchased in a local supermarket are referred to as Sample #1 and Sample #2. Figure 6 shows the calibration chromatograms obtained for Sample #1 (chromatography of the calibration for Sample #2 is similar to Sample #1). Table 3 shows calibration results for both samples.

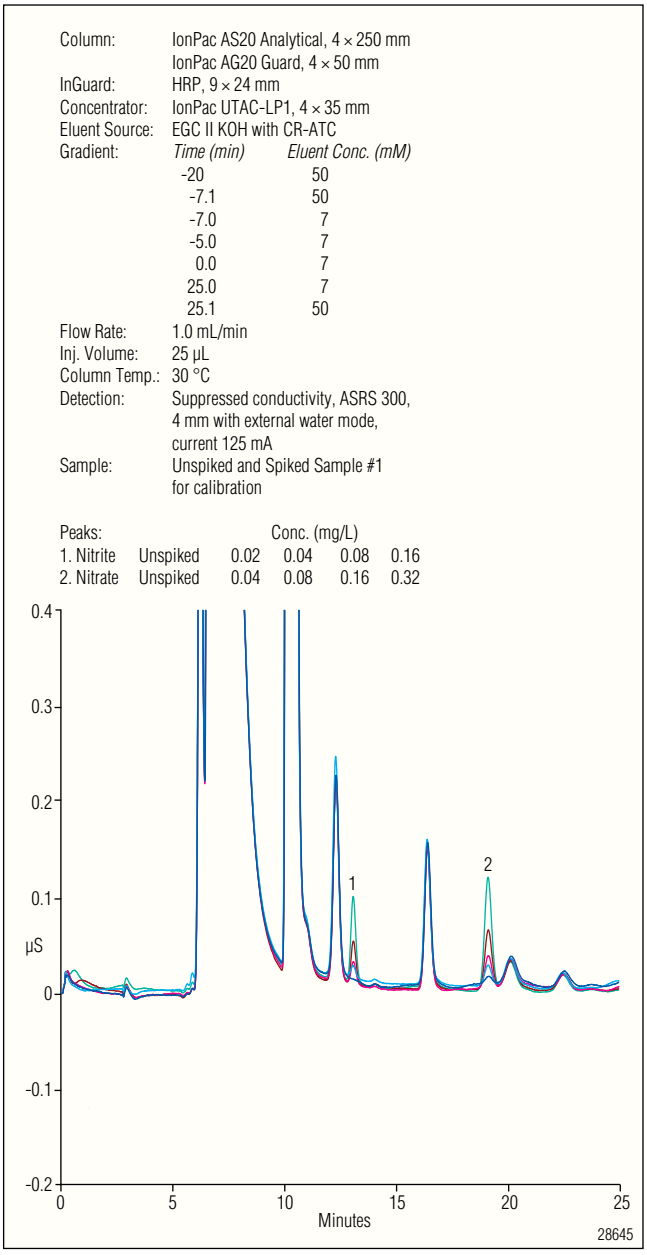

Figure 6. Overlay of five chromatograms of calibration standards of Sample #1 (chromatography of calibration standards for Sample #2 is similar).

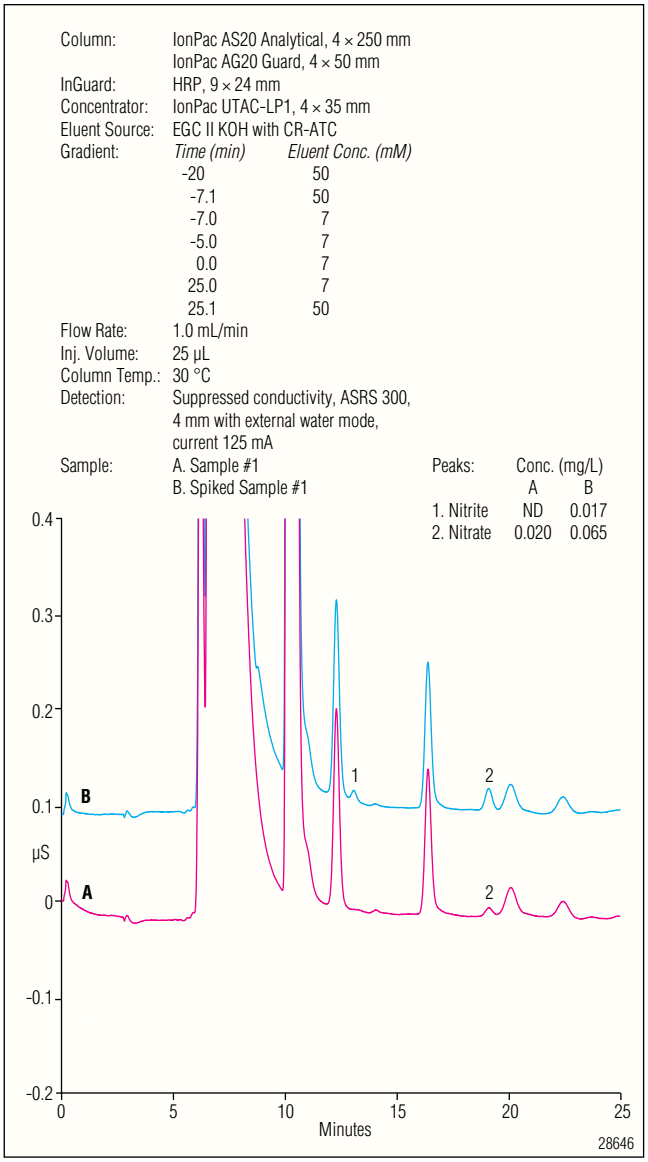

Figure 7. Overlay of chromatograms of Sample #1 and Spiked Sample #1.

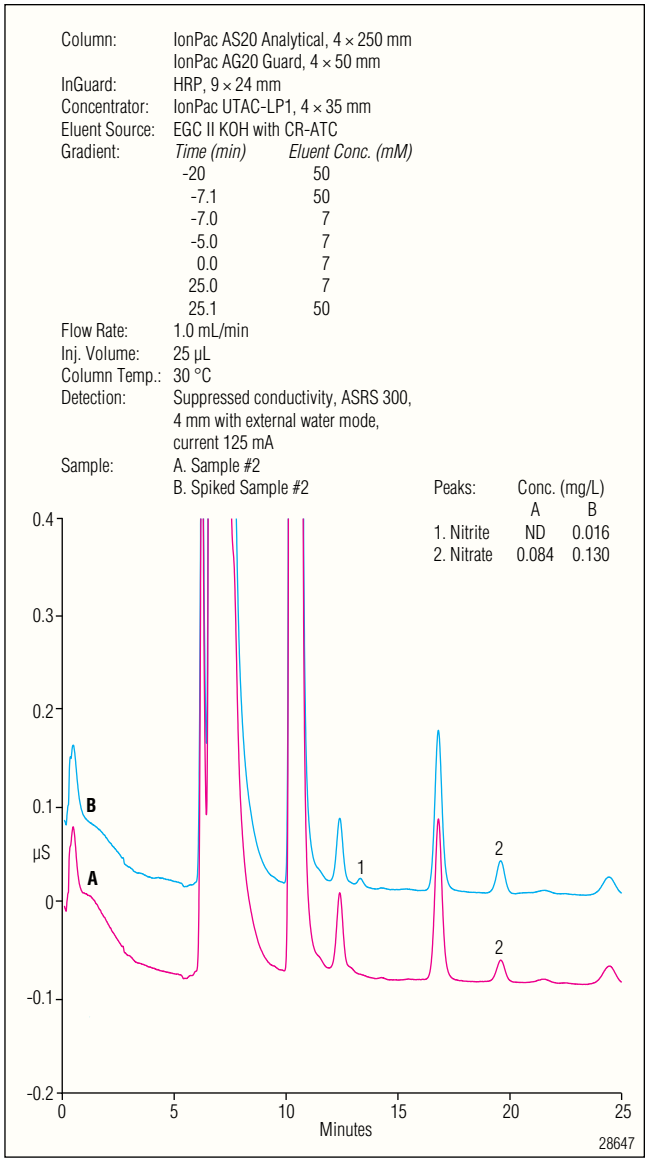

Figure 8. Overlay of chromatograms of Sample #2 and Spiked Sample #2.

To evaluate recovery, spiked samples were prepared to yield known concentrations of 0.02 mg/L nitrite and 0.04 mg/L nitrate; recoveries were calculated using the calibration curves prepared for each samples. Nitrate was found at 0.020 and 0.084 mg/L in Samples #1 and #2, respectively, and nitrite was absent in both samples.

Figures 7 and 8 show the chromatography from the spike recovery experiments. Note the difference in the number, size, and retention times of the unknown peaks in Samples #1 and #2. This again suggests that chromatography may need to be optimized for individual milk samples.

Table 4. Concentrations of Nitrite and Nitrate Determined in Sample #1, Spiked Sample #1, Sample #2, and Spiked Sample #2								
Injection No.	Concentration in Milk Sample #1 (mg/L)				Concentration in Milk Sample #2 (mg/L)			
	Sample		Spiked Sample (Spiked 0.02 mg/L Nitrite and 0.04 mg/L Nitrate)		Sample		Spiked Sample (Spiked 0.02 mg/L Nitrite and 0.04 mg/L Nitrate)	
	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate
1	ND	0.021	0.016	0.065	ND	0.084	0.016	0.126
2	ND	0.022	0.017	0.065	ND	0.085	0.016	0.127
3	ND	0.019	0.017	0.065	ND	0.084	0.016	0.135
Average	ND	0.020	0.017	0.065	ND	0.084	0.016	0.130
RSD f	ND	6.29	2.87	0.18	ND	0.93	2.84	3.81
Recovery (%)	—	—	84.0	111	—	—	80.0	113

The sample analysis and recovery results are shown in Table 4. To assess method sensitivity, the method detection limit (MDL) was determined. Due to the nitrate present in the sample, only nitrite was spiked into the sample to yield a concentration of 0.01 mg/L. The endogenous concentration of nitrate was used to estimate the MDL. Seven injections were made and the single-sided Student's *t* test at a 99% confidence level used to estimate the MDLs. This resulted in MDLs for nitrite and nitrate of 0.002 mg/L and 0.005 mg/L, respectively. During this study, the conductivity background and baseline noise were approximately 0.37 µS and 0.25 nS, respectively. Chromatography of one of the seven injections from the MDL study is shown in Figure 9.

CONCLUSION

This application demonstrates the determination of nitrite and nitrate in milk by IC with suppressed conductivity detection using in-line sample preparation. This method uses a simple acid precipitation followed by additional in-line automated sample preparation to prepare the sample prior to chromatography. The prepared sample is separated on the high-capacity IonPac AS20 column to resolve nitrite and nitrate from the remaining sample components. The RFIC system automatically prepares the separation eluent to achieve high separation reproducibility. The automated sample and eluent preparation saves time and improves the reproducibility of the analysis.

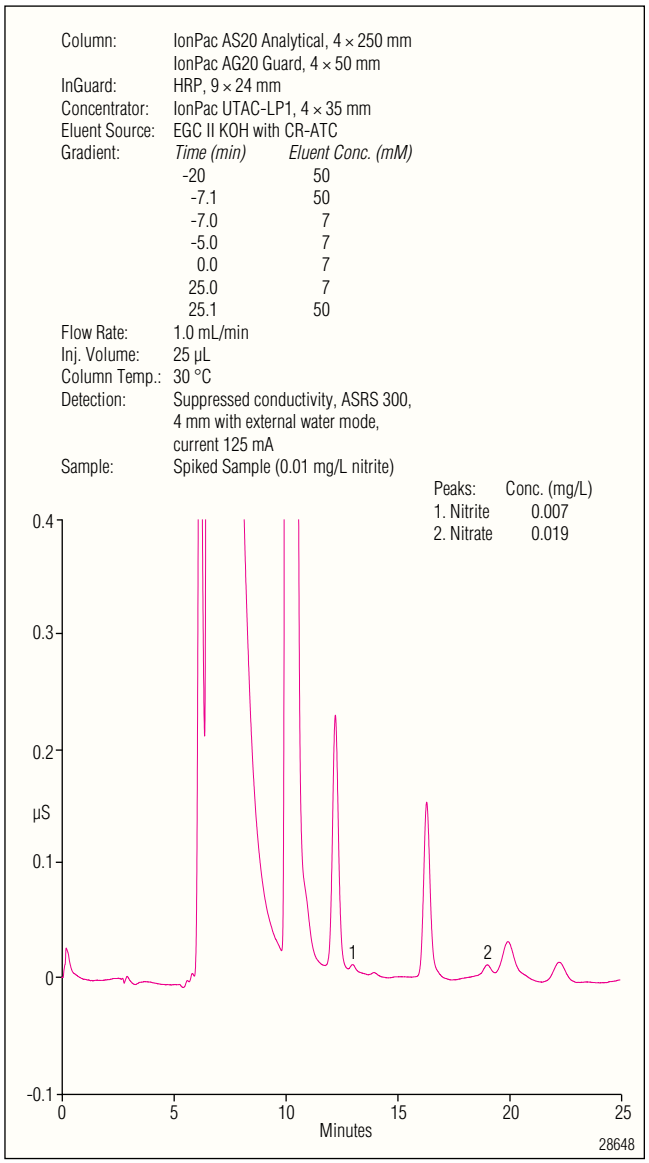

Figure 9. Chromatogram of a spiked sample for the MDL study.

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Nitrates and Nitrites, TEACH Chemical Summary, United States Environmental Protection Agency. http://www.epa.gov/teach/chem_summ/Nitrates_summary.pdf (accessed April 21, 2011).

The Determination of Inorganic Anions in Water by Ion Chromatography; Method 300.0; United States Environmental Protection Agency: Cincinnati, Ohio, 1993.

Dionex Corporation, *Determination of Nitrite and Nitrate in Drinking Water Using Ion Chromatography with Direct UV Detection.* Application Update 132, LPN 034527, 1991, Sunnyvale, CA.

Extraction of Contaminants, Pollutants, and Poisons from Animal Tissue Using Accelerated Solvent Extraction (ASE)

INTRODUCTION

Accelerated Solvent Extraction (ASE®) uses solvents at elevated temperatures and pressures to extract organic materials from solid and semisolid samples in a fraction of the time required by traditional extraction procedures. ASE is approved under EPA Method 3545A for extraction of organochlorine pesticides (OCPs), semivolatile compounds (BNAs), polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins and furans (PCDDs and PCDFs), and polynuclear aromatic hydrocarbons (PAHs) from samples such as soils and sediments.

Extensive documentation is available describing accelerated solvent extraction of environmental contaminants from soils and sediment samples.[1,2,3,4] ASE can also be used also to extract organic materials from matrices such as milk, foodstuffs, plant material, plasma, serum, and tissue. This Application Note details procedures for extracting the following contaminants from animal tissues:

- Dioxins/Furans
- Polybrominated Flame Retardants (PBDE)
- PCBs
- Pesticides
- PAHs
- Organotin

EQUIPMENT

Dionex ASE 200 Accelerated Solvent Extractor with ASE Solvent Controller (P/N 059087)

11-mL stainless steel extraction cells (P/N 049560) or

22-mL stainless steel extraction cells (P/N 049561) or

33-mL stainless steel extraction cells (P/N 049562)

Cellulose filters (P/N 049458)

Collection vials 40 mL (P/N 048783) or

Collection vials 60 mL (P/N 048784)

Dionex SE 500 Solvent Evaporation system (P/N 063221)

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

Tissue homogenizer (Buchi B-400 or equivalent)

Freeze drier (for PCB extraction)

Centrifuge (for organotin extraction)

Mechanical shaker (for organotin extraction)

ANALYTES

Dioxins/Furans

Extraction of pesticides was performed using a modification of the method described by Raccanalli, et al.[1]

Chemicals and Reagents

Toluene (Pesticide-Free, Reagent Grade, Fisher Scientific)

Acidified silica (40% H_2SO_4) alumina and carbon

Native and $^{13}C_{12}$-labeled PCDD and PCDF standards (Cambridge Isotope Laboratories.)

ASE Prep DE (P/N 062819)

Samples

Fish tissue (Certified Reference Material (CRM) CARP-1, National Research Council, Halifax Nova Scotia, Canada)

Sample Preparation

Prepare the ampule of CRM fish tissue according to the manufacturer's directions. Weigh out the desired amount of tissue sample and mix with ASE Prep DE (1:1). Transfer the sample mixture to a 22-mL stainless steel extraction cell containing a cellulose filter. Spike the sample mixture with a series of 13 C_{12}-labeled 2,3,7,8 PCDD/F substituted isomers as internal standards.

ASE Conditions

Pressure: 1500 psi*

Temperature: 175 °C

Solvent: 100% Toluene

Static Time: 10 min

Static Cycles: 2

Flush: 60%

Purge: 60 sec

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

Extraction

Place the extraction cells containing the tissue samples into the ASE 200 carousel. Label 60-mL collection vials and place them on the vial carousel. Configure the ASE method as listed above, and press Start to begin extraction. Once the extraction is complete, remove the extracts and perform a solvent transfer by evaporating the toluene then dissolving with hexane.

Cleanup

Clean the extracts by using any automated offline cleanup system available from various vendors. Alternately, offline cleanup can be eliminated by using the proper in-cell cleanup procedure described in Dionex Technical Note 210. Analyze using high resolution gas chromatography/high resolution mass spectrometry.[1]

Results and Discussion

Table 1 shows comparative results between the ASE and Soxhlet methods for extraction of dioxins and furans from fish tissue. Total extraction time for ASE was 30 min per sample using approximately 50 mL of toluene, as compared to Soxhlet extraction, which required a total time of 36 hours per batch and 300 mL of toluene per sample.

Table 1. PCDDs/PCDFs in Fish Tissue Samples (ng/kg or ppt) Using ASE[1]

Compound	Soxhlet	ASE	Certified
2,3,7,8-TCDD	7.6	7.6	6.6
1,2,3,4,8-PCDD	4.3	4.3	4.4
1,2,3,4,7,8-HCDD	1.4	1.4	1.9
2,3,4,7,8-TCDF	13.4	12.6	11.9
1,2,3,7,8-PCDF	5.4	5.1	5.0
1,2,3,4,7,8-HCDF	12.5	12.2	12.2
OCDD	12.4	6.4	6.3
Total TEQ	21.4	21.1	21.0

PBDEs

Extraction of PBDEs was performed using a modification of the method described by Oros, et al.[2]

Chemicals and Reagents

Acetone (HPLC grade, Fisher Scientific)

Dichloromethane (DCM) (HPLC grade, Fisher Scientific)

ASE Prep DE (P/N 062819)

Bio Beads®

Samples

Freshwater mussel, oysters, and clam samples were collected from estuary sites near San Francisco, California.

Sample Preparation

Rinse the bivalve samples with reagent grade water to remove any extraneous material. Shuck each sample into a homogenizing container and homogenize. Weigh 1–5 g of tissue homogenate and dry in a 70 °C oven for 48 hours to determine moisture content. Weigh 10 grams of the dried tissue homogenate and mix with 7 g ASE Prep DE until the mixture is homogenous and free flowing. Transfer the sample mixture to a 33-mL stainless steel extraction cell containing a cellulose filter.

ASE Conditions

Pressure:	1500 psi
Temperature:	100 °C
Solvent:	Acetone:DCM (1:1)
Static Time:	5 min
Static Cycles:	1
Flush:	60%
Purge:	60 sec

Extraction

Place the extraction cells with samples onto the ASE 200 carousel. Label 60-mL vials and place them on the vial carousel. Configure the ASE method listed above and save as "Method 1." Create a Schedule to extract each cell twice using Method 1. (Using this method, the ASE will extract the same cell twice, but will deliver each extract to separate vials.) When both extractions are complete, combine the extracts from the two vials and evaporate to approximately 0.5 mL, then dilute to 10 mL with DCM. Remove a 2-mL aliquot for lipid determination if needed. Clean the remaining extract using gel permeation chromatography (70 g Bio-Beads in 100% DCM) and fractionate using Florisil® or using in-cell cleanup as described in Dionex Technical Note 210. Perform a solvent exchange for a final volume of sample in 2 mL isooctane. Analyze by gas chromatography.[2]

Results and Discussion

The data from ASE extraction of mussel, clam, and oyster tissue collected at various sites from the San Francisco area over a two year period shows a range of 9–106 ppb PBDE dry weight. For quality assurance, cleaned sample extracts and blanks were spiked with surrogate recovery standards prior to extraction with ASE. Certified Reference Materials (CRM) for PBDE were not available at the time of testing and so were not used. Replicates of the spiked matrix and field samples were analyzed and determined to be within the accepted RSD of <20%.

PCBs

Extraction of PCBs was performed using a modification of the method described by Gomez-Ariza, et al.[3]

Chemicals and Reagents

Dichloromethane (DCM) (HPLC grade, Fisher Scientific)

Pentane (HPLC grade, Fisher Scientific)

Florisil (U.S. Silica Company)

Ottawa sand (Fisher Scientific)

Samples

Eggs of the Common Spoonbill (Platalea leucorodia) and freshwater oysters (species unidentified) were collected from Marshes National Park, Huelva, Spain.

Assorted clams and fish were collected from multiple areas around the south coast of Spain. Assorted mussels (species unidentified) were purchased from a local market in southern Spain.

Sample Preparation

Freeze dry samples and grind to a particle size of 100 µm. (The authors chose to freeze dry the tissue samples prior to extraction and mix the samples with ASE Prep DE at a 1:1 ratio.) Prepare the 22-mL extraction cell by inserting two cellulose filters into the outlet followed by 6 g of Florisil.* Weigh 2 g of freeze-dried sample and mix with Florisil (1:2) using a mortar and pestle, until sample is homogenous and free-flowing. Load the sample mixture into the extraction cell on top of the Florisil (Figure 1). Fill the remainder of the cell with Ottawa sand.

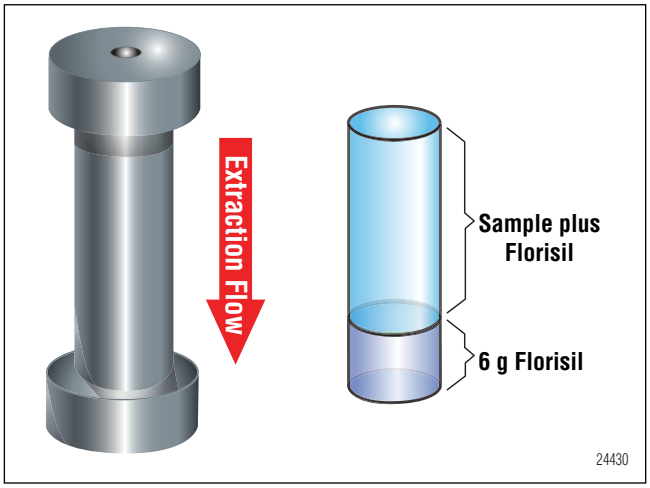

Figure 1. Schematic of in-cell cleanup.

*The addition of the Florisil to the extraction cell or in-cell cleanup as described in Dionex Technical Note 210 may eliminate the need for a post-extraction cleanup step.

ASE Conditions

Pressure:	1500 psi
Temperature:	40 °C
Solvent:	DCM: Pentane (15:85, v/v)
Static Time:	10 min
Static Cycles:	2
Flush:	150%
Purge:	60 sec

Extraction

Place the extraction cells containing the tissue samples onto the ASE 200 carousel. Label 60-mL vials and place them on the vial carousel. Enter the ASE method listed above and begin the extraction. Once complete, remove the extracts and concentrate to dryness using the SE 500. Dissolve the residue in 100 µL of hexane containing an internal standard. Analyze by gas chromatography with electron capture detection.[3]

Results and Discussion

Table 2 shows recovery data of ASE with in-cell cleanup compared to Soxhlet extraction of PCB from spoonbill eggs. The Soxhlet extraction required a post-extraction cleanup step using Florisil due to high concentrations of lipids present in the extracts. Total extraction time for ASE was 25 min using approximately 40 mL of solvent per sample. Extraction time using Soxhlet was 13 hours per batch with 150 mL of solvent used per sample.

Table 2. PCB Concentration Found in Spoonbill Eggs Using Using ASE and Soxhlet

PCB	Recovery ASE vs Soxhlet
1	96.47
11	106.67
29	98.91
47	97.78
87	98.72
99	100.36
101	97.44
105	97.48
118	97.67
121	92.47
128	102.78
136	110.59
138	106.15
153	97.12
156	106.02
170	113.36
180	97.44
183	97.02
185	87.36
194	92.02
206	106.59
209	103.53
Avg.	100.18

Pesticides

Extraction of pesticides was performed using a modification of the method described by Curren, et al.[4]

Chemicals and Reagents

Ethanol (HPLC grade, Fisher Scientific)

Methanol (HPLC grade, Fisher Scientific)

Amberlite XAD-7 HP resin (Supelco)

Atrazine standard (Chem Service Inc.)

Water (HPLC grade, Fisher Scientific)

Samples

Beef kidney (purchased at a local retail outlet in Salt Lake City, UT)

Sample Preparation

Homogenize kidney samples using a standard tissue homogenizer or blender. Freeze each sample homogenate until ready for analysis.

Prepare the XAD-7 resin prior to preparing the kidney samples for extraction using the following steps:

- Weigh an appropriate amount of dry XAD-7 resin into a clean beaker and cover with methanol for 15 min.
- Decant methanol and soak resin in water for 5 min.
- Rinse resin with water at least three times or until all methanol has been removed.

Allow the kidney sample to thaw slightly and weigh approximately 0.5 g sample and 1 g ASE Prep DE in a plastic weighing dish. Spike the sample with a 1 µL aliquot of atrazine (or other pesticide) standard prepared with methanol (2 µg/g). Allow 30 min for the standard to permeate the meat sample. Add an additional 1 g ASE Prep DE to the sample. Place the contents of the plastic weigh dish into a mortar and add 1–2 g prepared XAD-7 HP resin. Grind the entire mixture using a mortar and pestle until the sample is homogenous and free-flowing. Transfer the sample mixture to an 11-mL extraction cell containing a cellulose filter.

ASE Conditions

Pressure: 1500 psi
Temperature: 100 °C
Solvent: Ethanol/Water (30:70, v/v)
Static Time: 5 min
Static Cycles: 3
Flush: 50%
Purge: 60 sec

Extraction

Place the extraction cells containing the kidney sample mixture onto the ASE 200 carousel. Label 40-mL collection vials and place them onto the vial carousel. Enter the ASE method listed above and begin the extraction. When complete, use Solid Phase Microextraction (SPME) to sample the extracts directly by placing a 1.5 mL aliquot in a sampling vial. SPME adsorption time is 30 minutes. Analyze the SPME fibers by gas chromatography.

Results and Discussion

Table 3 summarizes the recovery data for kidney samples spiked with different concentrations of atrazine. The data shows the described extraction method produced excellent recoveries of pesticide from animal tissue.

Table 3. Percent Recovery for Atrazine in Beef Kidney

	% Recovery[a] (%RSD)		
Atrazine in Beef Kidney (µg/g)	**Sample 1**	**Sample 2**	**Sample 3**
2	104 (14)	103 (8)	104 (7)
0.2	115 (19)	127 (3)	90 (3)

[a]n = 3

PAHs

Extraction of PAHs was performed using a modification of the method described by Yusa, et al[5]

Chemicals and Reagents

Dichloromethane (DCM) (HPLC grade, Fisher Scientific)

Acetone (HPLC grade, Fisher Scientific)

Sodium sulfate (Anhydrous, Fisher Scientific)

Samples

Mussels (SRM 2977, NIST)

Sample Preparation

Homogenize tissue. Store sample at -18° C until ready to analyze. Thaw tissue, weigh out approximately 4 g of sample and grind with ASE Prep DE (1:1) using a mortar and pestle until the sample is homogenous and free flowing. Add this sample mixture to a 22-mL cell containing a cellulose filter.

ASE Conditions

Pressure: 1500 psi
Temperature: 125 °C
Solvent: DCM: Acetone (50:50, v/v)
Static Time: 5 min
Static Cycles: 1
Flush: 60%
Purge: 60 sec

Extraction

Place the extraction cells containing the sample onto the ASE 200 carousel. Place labeled 60-mL vials onto the vial carousel and begin the extraction using the ASE parameter listed above. Once complete, add 1–2 g sodium sulfate to the extraction vials and shake. Evaporate the solvent to approximately 0.5 mL using the SE 400 or SE 500. Dilute to 5 mL with DCM. Clean the extracts using gel permeation chromatography (GPC).[5]* Analyze by HPLC-Fl to remove interferances from lipids.

*It may be possible to eliminate post-extraction GPC cleanup by using an adsorbent such as Florisil in the bottom of the extraction cell with sample added to the top.

Results and Discussion

Table 4 shows ASE data compared to MSE (methanol solvent extraction) for extraction of PAHs from mussel tissue. Total extraction time for ASE was 10 min per sample, with approximately 25 mL solvent used. ASE completed the extraction 24 times faster than the MSE method and used 1/12 the amount of solvent.

Table 4. Extraction of PAH from SRM 2977: Comparison of ASE and MSE

		ASE	MSE	ASE	MSE
	Certified Concentration[a,b]	Mean[a]	Mean[a]	% Recovery	% Recovery
Compound					
Anthracene	8 ± 4	7.6	7.5	95.0	93.75
Fluoranthene	35.1 ± 3.8	33.3	35	94.87	99.72
Pyrene	78.9 ± 3.5	76.4	72	96.83	91.25
Benzo (a) anthracene	20.34 ± 0.78	19.5	20	95.87	98.33
Chrysene	49 ± 2	47	46	95.92	93.88
Benzo (e) pyrene	13.1 ± 1.1	13	11	99.24	83.97
Benzo (b) fluoranthene	11.01 ± 0.28	10.8	10	98.09	90.83
Benzo (k) fluoranthene	4 ± 1	3.9	3.8	97.50	95.0
Benzo (g,h,i) perylene	9.53 ± 0.43	9.1	8	95.49	83.95
Indeno (1,2,3-e,d) pyrene	4.84 ± 0.81	4.6	4.4	95.04	90.91

(n = 5). [a]Concentrations in µg/Kg dry weight:
[b]The dispersion is expressed as expanded uncertainty.

Organotin

Chemicals and Reagents

Extraction of organotin was performed using a modification of the method described by Wasik, et al.[6]

Acetic acid (HPLC grade, Fisher Scientific)
Sodium acetate (Analytical grade, Sigma-Aldrich)
Tropolone (Analytical grade, Sigma-Aldrich)
Methanol (HPLC grade, Fisher Scientific)
Hexane (HPLC grade, Fisher Scientific)
Sodium Sulfate (Analytical grade, Fisher Scientific)
Aluminum Oxide (3% water, Sigma-Aldrich)
Tetrabutyltin (TetraBT) (Internal Standard, Sigma-Aldrich)
Sodium tetraethylborate ($NaBEt_4$, Derivatization agent Sigma-Aldrich)

Prepare the stock solution of the internal standards (TetraBT) by dissolving in methanol to the desired concentration, and storing in the dark at 4 ° C. Prepare an acetic acid/sodium acetate buffer solution to pH 5.

Samples

Certified Reference Material NIES No. 11 (freeze-dried fish tissue sample, certified for total tin and TBT (tributyltin chloride)

Sample Preparation

Weigh approximately 1 g of sample into a beaker and mix with 9 g Ottawa sand. Add the sample mixture to an 11-mL cell containing a cellulose filter.

ASE Conditions

Pressure:	1500 psi
Temperature:	125 °C
Solvent:	1 mol acetic acid + 1 mol sodium acetate + 0.3 g tropolone per 1 mL methanol/ water mixture (90% v/v methanol)
Static Time:	3 min
Static Cycles:	4
Flush:	20%
Purge:	100 sec

Extraction

Place the extraction cells containing the sample mixture onto the ASE 200 carousel, and place labeled 40-mL vials onto the vial carousel. Enter the ASE method listed above and begin the extraction. Once extraction is complete, transfer approximately 10 mL of the extract into a glass, round-bottomed centrifuge vial using a volumetric pipette. Add 10 mL of acetic acid/sodium acetate buffer solution (pH=5), 2 mL hexane, and 4 µL TetraBT standard solution (internal standard). Secure the centrifuge cap tube and shake the sample mechanically for 10 min at 520 cpm. Next, centrifuge the sample at 4400 rpm for 3 min. Using a pipette, decant approximately 1.5 mL of the top hexane layer and clean by passing through a column made from a Pasteur pipette closed with a piece of silanized glass wool and packed with 1 g Al_2O_3 (3% water) and a 1 mL layer of anhydrous Na_2SO_4 on the top. Elute the organotin compounds with 10 mL hexane and evaporate to 1 mL using the SE 500. Decant a 2 µL aliquot for analysis by gas chromatography.

RESULTS AND DISCUSSION

The ASE recoveries show the sum of TBT and DBT to be very close to the TBT certified value (96%) whereas the sum of MPhT, DphT, and TPhT is only 76% of the TPhT reference value. This may indicate that a significant amount of TPhT was degraded to inorganic tin and was thereby not detectable using this method.

CONCLUSIONS

The recovery data presented in this Application Note show ASE to be an excellent alternative to traditional methods for extraction of various environmental contaminants from animal tissues. ASE gives comparable or better results while providing a faster, easier way to prepare tissue samples for analysis.

LIST OF MANUFACTURERS

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Thermo
SCIENTIFIC

Column Selection Guide

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

Polymer Columns

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

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

Column Specifications

IC Anion Columns

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
IonPac AS24	2 × 250 mm	Hydroxide	Recommended column for haloacetic acids prior to MS or MS/MS detection	7 µm	55%	-	-	140 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS23	2 × 250 mm 4 × 250 mm	Carbonate	Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water.	6 µm	55%	-	-	80 µeq 320 µeq	Alkyl quaternary ammonium	Ultralow
IonPac AS22	2 × 250 mm 4 × 250 mm	Carbonate	Recommended column for fast analysis of common inorganic anions.	6.5 µm	55%	-	-	52.5 µeq 210 µeq	Alkyl quaternary ammonium	Ultralow
IonPac AS21	2 × 250 mm	Hydroxide	Recommended column for trace perchlorate prior to MS or MS/MS detection	7.0 µm	55%	-	-	45 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS20	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for trace perchlorate prior to suppressed conductivity detection.	7.5 µm	55%	-	-	77.5 µeq 310 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS19	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water.	7.5 µm	55%	-	-	60 µeq 350 µeq	Alkanol quaternary ammonium	Low
IonPac AS18	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for the analysis of common inorganic anions.	7.5 µm	55%	65 nm	8%	75 µeq 285 µeq	Alkanol quaternary ammonium	Low
IonPac AS17-C	2 × 250 mm 4 × 250 mm	Hydroxide	Trace anions in HPW matrices. Carboxylated resin, no sulfate blank. Low capacity for fast analysis of common inorganic anions using gradient elution with the Eluent Generator.	10.5 µm	55%	75 nm	6%	7.5 µeq 30 µeq	Alkanol quaternary ammonium	Low
IonPac AS16	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for hydrophobic anions including iodide, thiocyanate, thiosulfate, and perchlorate. Polyvalent anions including: polyphosphates and polycarboxylates	9 µm	55%	80 nm	1%	42.5 µeq 170 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS15	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for trace analysis of inorganic anions and low molecular weight organic acids in high purity water matrices.	9 µm	55%	-	-	56.25 µeq 225 µeq	Alkanol quaternary ammonium	Medium-High
IonPac AS15-5mm	3 × 150 mm	Hydroxide	Fast run, high capacity for trace analysis of inorganic anions and low molecular weight organic acids in high purity water matrices.	5 µm	55%	-	-	70 µeq	Alkanol quaternary ammonium	Medium-High
IonPac AS14A- 5 µm	3 × 150 mm	Carbonate	Recommended column for fast analysis of common inorganic anions.	5 µm	55%	-	-	40 ueq	Alkyl quaternary ammonium	Medium
IonPac AS14A	4 × 250 mm	Carbonate	For analysis of common inorganic anions.	7 µm	55%	-	-	120 µeq	Alkyl quaternary ammonium	Medium
IonPac AS14	2 × 250 mm 4 × 250 mm	Carbonate	Moderate capacity for fast analysis of common inorganic anions.	9 µm	55%	-	-	16 µeq 65 µeq	Alkyl quaternary ammonium	Medium-High
IonPac AS12A	2 × 200 mm 4 × 200 mm	Carbonate	Moderate capacity for analysis of inorganic anions and oxyhalides. Trace chloride and sulfate in high carbonate matrices.	9 µm	55%	140 nm	0.20%	13 µeq 52 µeq	Alkyl quaternary ammonium	Medium
IonPac AS11-HC	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for the determination of organic acids and inorganic anions in uncharacterized samples.	9 µm	55%	70 nm	6%	72.5 µeq 290 µeq	Alkanol quaternary ammonium	Medium-Low
IonPac AS11	2 × 250 mm 4 × 250 mm	Hydroxide	Low capacity for fast profiling of organic acids and inorganic anions in well-characterized samples.	13 µm	55%	85 nm	6%	11 µeq 45 µeq	Alkanol quaternary ammonium	Very Low
IonPac AS10	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for the analysis of inorganic anions and organic acids in high nitrate samples.	8.5 µm	55%	65 nm	5%	42.5 µeq 170 µeq	Alkyl quaternary ammonium	Low
IonPac AS9-HC	2 × 250 mm 4 × 250 mm	Carbonate	High-capacity column for inorganic anions and oxyhalides. Trace bromate in drinking water.	9 µm	55%	90 nm	18%	48 µeq 190 µeq	Alkyl quaternary ammonium	Medium-Low
IonPac AS9-SC	4 × 250 mm	Carbonate	Low capacity for fast analysis of inorganic anions and oxyhalides. Specified column in US EPA Method 300.0 (B).	13 µm	55%	110 nm	20%	30-35 µeq	Alkyl quaternary ammonium	Medium-Low
IonPac AS4A-SC	2 × 250 mm 4 × 250 mm	Carbonate	Low capacity for fast analysis of common inorganic anions. Specified column in U.S. EPA Method 300.0 (A).	13 µm	55%	160 nm	0.50%	5 µeq 20 µeq	Alkanol quaternary ammonium	Medium-Low
IonPac Fast Anion IIIA	3 × 250 mm	Hydroxide	Recommended column for phosphoric and citric acids in cola soft drinks.	7.5 µm	55%	-	-	55 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS7	4 × 250 mm	Specialty Eluents	Polyvalent anions including chelating agents, polyphosphates and polyphosphonates. Cyanide, sulfide, hexavalent chromium, and arsenic speciation.	10 µm	2%	530 nm	5%	100 µeq	Alkyl quaternary ammonium	Medium-High
IonPac AS5A	4 × 150 mm	Hydroxide	Low capacity for fast profiling of organic acids and inorganic anions in well-characterized samples.	5 µm	2%	60 nm	4%	35 µeq	Alkanol quaternary ammonium	Low
IonPac AS5	4 × 250 mm	Hydroxide	Metal-EDTA complexes, metal-cyanide complexes, and oxyanions.	15 µm	2%	120 nm	1%	20 µeq	Alkanol quaternary ammonium	Low

IC Cation Columns

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

Ion-Exclusion Columns

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

Acclaim General and Specialty Columns

Column	Bonded Phase	USP Type	Endcapped	Substrate	Particle Shape	Particle Size	Metal Impurity (ppm) Na, Fe, AL	Average Pore Diameter	Surface Area (m^2/g)	Total Carbon Content
Mixed-Mode WAX	Proprietary alkyl amine	na	Proprietary	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. Incompatable with alcohols and MeOH	3000 psi (21 MPa)	2–12.0
ProPac SCX-10	Strong Cation Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter nonporous substrate to which is grafted a polymer chain bearing sulfonate groups.	55%	3 mg/ mL lysozyme	0.2–2.0 mL/min	80% ACN, acetone, MeOH	3000 psi (21 MPa)	2–12.0
ProPac SCX-20									
ProPac WAX-10	Weak Anion Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter non-porous substrate to which is grafted a polymer chain bearing tertiary amine groups.	55%	5 mg/ mL BSA/ mL	0.2–2.0 mL/min	80% ACN, acetone, MeOH,	3000 psi (21 MPa)	2–12.0
ProPac SAX-10	Strong Anion Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter non-porous substrate with grafted polymer chain bearing quaternary ammonium groups.	55%	15 mg/ mL BSA	0.2–2.0 mL/min	80% ACN, acetone, MeOH	3000 psi (21 MPa)	2–12.0
ProSwift RP-1S	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith Standard permeability	5.5 mg/mL Insulin	2–4 mL/min	Most common organic solvents	2800 psi (19.2 Mpa)	1–14
ProSwift RP-2H	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith High permeability	1.0 mg/mL Lysozyme	1–10 mL/min	Most common organic solvents	2800 psi (19.3 Mpa)	1–14
ProSwift RP-4H									
ProSwift RP-3U	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith Ultrahigh permeability	0.5 mg/mL Lysozyme	1– 16 mL/min	Most common organic solvents	2800 psi (19.3 Mpa)	1–14
ProSwift SAX-1S	Strong Anion Exchange	Fast protein separation with good resolution using Anion Exchange	Monolith; polymethac-rylate 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; polymethac-rylate with sulfonic acid fuctional group	Monolith Standard permeability	30 mg/mL Lysozyme	0.5–1.5 mL/min (4.6 mm)	Most common organic solvents	1000 psi (4.6 mm)	2–12.0
ProSwift WAX-1S	Weak Anion Exchange	Fast protein separation with good resolution using Anion Exchange	Monolith; polymethacrylate with tertiary amine (DEAE) functional group	Monolith Standard permeability	18 mg/mL BSA	0.5–1.5 mL/min (4.6 mm), 0.05–.25 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProSwift WCX-1S	Weak Cation Exchange	Fast protein separation with good resolution using Cation Exchange	Monolith; polymethacrylate with carboxylic acid (CM) functional group	Monolith Standard permeability	23 mg/mL Lysozyme	0.5–1.5 mL/min (4.6 mm), 0.05–.20 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProPac IMAC-10	Immobilized Metal Affinity	High resolution separation of certain metal-binding proteins and peptides	10 µm diameter non-porous polystyrene divinylbenzene substrate with poly (IDA) grafts.	55%	>60 mg lysozyme/ mL gel (4 x 250 mm)	1.0 mL/min	EtOH, urea, NaCl, non- ionic detergents, glycerol, acetic acid, guanidine HCl	3000 psi (21MPa)	2–12
ProSwift ConA-1S									
ProPac HIC-10	Reversed-Phase	Protein separation using hydrophobic interaction with salt gradient elution	Spherical 5 µm, ultrapure silica, 300 A, surface area 100 m^2/ g,	n/a	340 mg lysozyme per 7.8 x 75 mm column	1.0 mL/ min	2M Ammonium sulfate/ phosphate salts, organic solvent for cleanup	4,000 psi	2.5–7.5

Carbohydrate

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

DNA

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

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