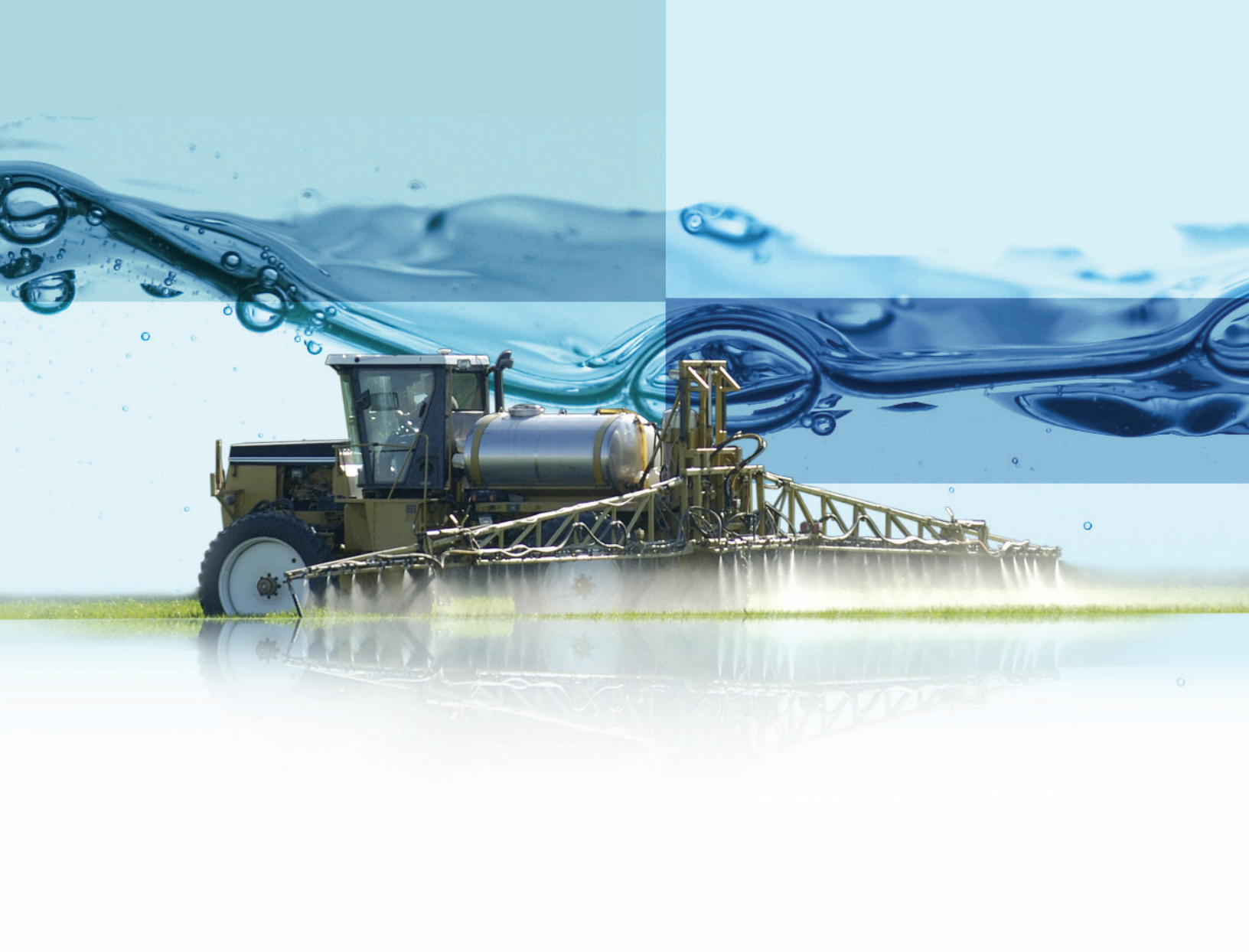


Environmental Water Applications Notebook

Pesticides • Herbicides • Emerging Contaminants

Thermo SCIENTIFIC

Table of Contents

Introduction to Environmental Water Analysis 3

Analysis of Pesticides and Herbicides 6

Rapid Determination of Persistent Organic Pollutants (POPs) Using Accelerated Solvent Extraction (ASE) 7

Rapid Determination of Organochlorine Pesticides in Animal Feed Using Accelerated Solvent Extraction (ASE) 15

Extraction of Chlorinated Pesticides Using Accelerated Solvent Extraction (ASE) 18

Extraction of Chlorinated Herbicides Using Accelerated Solvent Extraction (ASE) 21

Determination of Aniline and Nitroanilines in Environmental and Drinking Waters by On-Line SPE 24

Sensitive On-Line SPE–HPLC Determination of Paraquat and Diquat in Drinking and Environmental Waters 32

Quantitative Analysis of Nitrogen Mustard Hydrolysis Products as Ethanolamines 41

Sensitive and Fast Determination of Endothall in Water Samples by IC-MS/MS 48

Improved Separation of Diquat and Paraquat Using the Acclaim Mixed-Mode HILIC-1 Column 54

Determination of Glyphosate by Cation-Exchange Chromatography with Postcolumn Derivatization 56

Determination of *N*-Methylcarbamates by Reversed-Phase High-Performance Liquid Chromatography 61

Analysis of Emerging Contaminants 68

Determination of Linear Alkylbenzene Sulphonate in Treatment Plant Wastewater Streams Using On-Line Solid-Phase Extraction Followed by HPLC with Fluorescence Detection 69

Sensitive Determination of Microcystins in Drinking and Environmental Waters 79

Column Selection Guide 90

Column Selection Guide and Specifications 91

Transferring HPLC Methods to UHPLC 97

Easy Method Transfer from HPLC to RSLC with the Dionex Method Speed-Up Calculator 98

Thermo
SCIENTIFIC

Introduction to Environmental Water Analysis

Everyone in the global community is impacted by the quality of water resources. The water we drink must be free from harmful chemicals to ensure good health. The purity of ground and surface waters in our environment is critical to ensuring sustainable use. The water discharged by municipal wastewater treatment plants and industrial facilities must be monitored to ensure strict compliance with environmental guidelines. Process waters must be kept clean from contaminants to ensure product quality and acceptable exposure levels.

Thermo Fisher Scientific is committed to enhancing the quality of our global water resources. As innovation leaders in ion and liquid chromatography, our analytical instruments are used by government and industry to provide solutions for environmental water testing for a wide range of regulated and emerging inorganic elements and organic compounds.

As pioneers of suppression technology, we started a revolution in ion chromatography (IC) that increased the sensitivity and accuracy of ion determination. As constant innovators, we developed Reagent-Free™ (RFIC™) systems that set a new benchmark for ion analysis. Today, RFIC systems with eluent generation and eluent regeneration provide the ultimate in sensitivity and ease of use.

We also have a full high-performance liquid chromatography (HPLC) product line for the analysis of organic contaminants, from nano- to preparative-scale separation capabilities, including ultra HPLC (UHPLC).

In fact, we are the only separations science company that provides instrumentation, columns, and applications perfectly suited for both inorganic and organic contaminants.

THERMO SCIENTIFIC AND DIONEX INTEGRATED SYSTEMS

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

For more information on how the new line-up of Thermo Scientific products can expand your capabilities and provide the tools for new possibilities, choose one of our integrated solutions:

- Ion Chromatography and Mass Spectrometry
- Liquid Chromatography and Mass Spectrometry
- Sample Preparation and Mass Spectrometry

GROUND AND SURFACE WATER

Surface water is the largest source of fresh water used for human consumption. The U.S. Geological Survey implemented the National Water-Quality Assessment (NAWQA) Program in 1991 to develop long-term data on streams, rivers, groundwater, and aquatic systems. The data support national, regional, state, and local policies and decisions related to water-quality management. The NAWQA program is designed to answer the following questions:

- What is the condition of our nation's streams, rivers, and groundwater?
- How are these conditions changing over time?
- How do natural features and human activities affect these conditions, and where are those effects most pronounced?

Thermo Scientific has codeveloped several methods with the U.S. EPA Office of Ground Water and Drinking Water. This collaboration has strengthened with the development of unique technology, including electrolytic suppression and RFIC with eluent generation or regeneration.

DRINKING AND BOTTLED WATER

Currently, less than 1% of the planet's water is available for human consumption—making this valuable resource even more important. With surface water contamination and groundwater resources overexploited, the need for effective water analysis and monitoring has never been higher.

Regulatory agencies around the world have developed standards for water analysis and have provided guidance on water disinfection to assure drinking water quality. Thermo Scientific provides a variety of solutions for inorganic and organic drinking water contaminants.

WASTEWATER

Wastewater includes liquid waste from residences, industry, and agriculture, comprising a wide range of potential contaminants and concentrations. Industries discharge a variety of pollutants in their wastewater, including heavy metals, organic toxins, oils, nutrients, and solids, all of which endanger ecosystems and pose a threat to human health. In some areas, treated wastewater is recycled for irrigation purposes and even as drinking water. This reuse of water is gaining closer scrutiny as demand increases for water resources.

Treating and recycling wastewater requires careful analysis and monitoring, including the determination of low-level contaminants such as pharmaceuticals and personal care products (PCPs). Dionex HPLC and IC instruments are well suited to determine a wide range of nonpolar, polar, and ionic contaminants.

FAST WATER ANALYSIS

High-Throughput Solutions for Inorganic and Organic Contaminant Analyses

The Challenge:

Emerging contaminants, stricter regulations, growing municipalities and industries—all increase analytical laboratories' workloads, requiring processing of more samples and performing more tests in less and less time.

We have developed new technologies and methods to help labs and businesses increase their productivity and throughput for the analysis of inorganic and organic contaminants in a variety of water matrices.

Columns

Thermo Scientific Dionex IonPac Fast IC columns for anions, organic acids, oxyhalides, cations, and amines use the same proven chemistry in shorter column formats, decreasing run times by as much as three times while still retaining sufficient resolution.

Thermo Scientific Acclaim columns for organic contaminants use smaller particles that allow higher flow rates at standard pressures and compatibility with higher pressure systems. When used with the Thermo Scientific Dionex UltiMate 3000 rapid separation LC (RSLC) systems, these columns provide separation times as much as 30 times faster than standard columns and systems.

Inorganic Contaminants

The Thermo Scientific Dionex ICS-5000 capillary RFIC system provides IC on demand, reducing equilibration times and calibration requirements that save labor and increase throughput. The innovative Thermo Scientific Dionex IC Cube module, with half the connections of a standard IC configuration, makes plumbing and reconfiguring the system easier. Capillary Fast IC and monolith columns combine the speed of Fast IC with the convenience of IC whenever you need it—on demand. The simultaneous injection, sample, and standard preparation features of the Thermo Scientific Dionex AS-AP Autosampler, along with its AutoDilution capability, increase throughput, reduce manual labor, and decrease delays from out-of-range samples.

Organic Contaminants

UltiMate™ 3000 HPLC and RSLC systems are all UHPLC+ focused, enabling faster separations at standard HPLC system prices. From the economical Basic Automated system to the ×2 Dual RSLC system for high throughput, automated sample preparation, sample concentration, and matrix elimination, Thermo Scientific has the system to fit your needs and budget.

Thermo Scientific Dionex Chromeleon Chromatography Data System software version 7.1 streamlines your path from samples to results. eWorkflows guide the operator through a minimal number of choices needed to run that workflow, making configuration of even the most complex multidimensional analysis easy. Data analysis tools help users process chromatograms with minimal effort, report templates and audit trails, and help ensure regulatory compliance, and System Wellness tools increase up time.

Thermo Scientific is committed to enhancing the quality of our global water resources. Our analytical instruments are used by government and industry labs globally to provide services for environmental water testing for a wide range of regulated and emerging inorganic elements and organic compounds.

Analysis of Pesticides and Herbicides

Environmental Water Applications Notebook

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.

REFERENCES

1. "The Determination of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans in Soil and Sediment by GC-MS"; Method E3151B; Ontario Ministry of Environment and Energy, 1993.
2. U.S. Environmental Protection Agency. "Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue." Section 3.1.3; U.S. EPA Method 600/4-81-055; Cincinnatti, OH, 1981.
3. Richter, B.; Ezzell, J.; Felix, D. "Single Laboratory Method Validation Report: Extraction of TCL/PPL (Target Compound List/Priority Pollutant List) BNAs and Pesticides Using Accelerated Solvent Extraction (ASE) with Analytical Validation by GC/MS and GC/ECD" Document 116064.A; Dionex Corporation, June 16, 1994.
4. Dionex Corporation. Application Note 323; Sunnyvale, CA.
5. Preud'homme, H., Potin-Gautier, M., "Optimization of Accelerated Solvent Extraction for Polyhalogenated Dibenzo-*p*-Dioxins and Benzo-*p*-furans in Mineral and Environmental Matrixes Using Experimental Designs", *Anal. Chem.* **2003,** *75,* 6109–6118.
6. Saito, K., et al. "Extraction and Cleanup Methods of Dioxins in House Dust from Two Cities in Japan Using Accelerated Solvent Extraction and a Disposable Multi-Layer Silica-Gel Cartridge." *Chemosphere,* **2003,** *53,* 137–142.

Rapid Determination of Organochlorine Pesticides in Animal Feed Using Accelerated Solvent Extraction (ASE®)

INTRODUCTION

Animal feed contaminated with organochlorine pesticides (OCPs) has begun to attract worldwide attention. When ingested, the OCPs from animal feed tend to accumulate in certain animal products, especially those rich in fat, such as meat, milk, and butter. Because these types of animal products are widely consumed by humans, methods are needed that quickly extract and determine OCPs in the feeds of animals used to produce products for human consumption.

Traditional methods used to extract OCPs from animal feed require large amounts of organic solvents and take from one to several hours per extraction. Also, many of the traditional methods are very labor intensive and require constant analyst attention.

ASE was introduced in 1995 and is a proven, valuable technique for environmental laboratories. ASE is EPA approved under method 3545A. This technique uses high temperatures and pressures to increase the kinetics of the extraction process, thus decreasing the extraction time and solvent consumption. Also, because ASE is automated, it allows unattended extraction of up to 24 samples. In this application note, OCPs are extracted from certified reference material (CRM) BCR 115 (Institute for Reference Materials and Measurement, Geel Belgium), an animal feed containing certified levels of organochlorine pesticides.

EQUIPMENT

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

11-mL stainless steel extraction cells (P/N 055422)

Dionex cellulose filters (P/N 049458)

Dionex collection vials 40 mL (P/N 048783)

Analytical balance (accurate to the nearest 0.0001 g or better)

Laboratory grinder

Sand (Ottawa Standard, Fisher Scientific, Cat. No. S23-3 20-30 mesh)

Dichloromethane silica gel, 0.063–0.200 mm, water content 2.62% (Merck, Darmstadt, Germany)

S-X3 Bio-Beads (Bio Rad Laboratories)

REAGENTS

For reagents, use either:

Bulk Isolute Sorbent (International Sorbent Technology Ltd., UK)

Hydromatrix™ (Varian Associates)

STANDARD REFERENCE MATERIAL

CRM BCR 115 (Institute for Reference Materials and Measurement, Geel Belgium)*

*Similar standard reference materials may be substituted.

Solvents

Hexane

Acetone

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

EXTRACTION CONDITIONS

Solvent: Hexane: acetone (3:2)
Temperature: 100 °C
Pressure: 1500 psi
Static time: 9 min
Static cycles: 1
Flush: 60%
Purge: 60 s

SAMPLE PREPARATION

Each animal feed sample should be ground to a powder using a laboratory grinder. Weigh approximately 1.0 g of the powder and blend with 0.5 g of the Bulk Isolute Sorbent using a mortar and pestle. Transfer the mixture to an 11-mL stainless steel extraction cell containing a cellulose filter. Top off any void volume in the cell with Ottawa sand.

Table 1. Concentration Values (ng g^{-1}) and RSD (%) for the Extraction of CRM BCR 115

Compounds	Certified Value		ASE (n = 3)	
	C (ng g^{-1})	RSD (%)	C (ng g^{-1})	RSD (%)
α-HCH	*	*	21.5 ± 0.5	2.5
HCB	19.4 ± 1.4	7.2	20.6 ± 0.4	1.8
β-HCH	23 ± 3	13.0	26.0 ± 2.3	8.7
γ-HCH	21.8 ± 2	9.2	27.1 ± 1.4	5.3
Heptachlor	19 ± 1.5	7.9	20.0 ± 0.5	2.7
Aldrin	*	*	56.0 ± 3.1	5.5
p,p′–DDE	47 ± 4	8.5	54.6 ± 2.6	4.7
Dieldrin	18 ± 3	16.7	22.0 ± 0.6	2.6
Endrin	46 ± 6	13.0	52.1 ± 1.9	3.6
p,p′ –DDD	*	*	91.8 ± 2.6	2.8
o,p′ -DDT	46 ± 5	10.9	49.8 ± 0.5	1.1
p,p′ -DDT	*	*	59.4 ± 1.8	3.1

* Present but not certified.

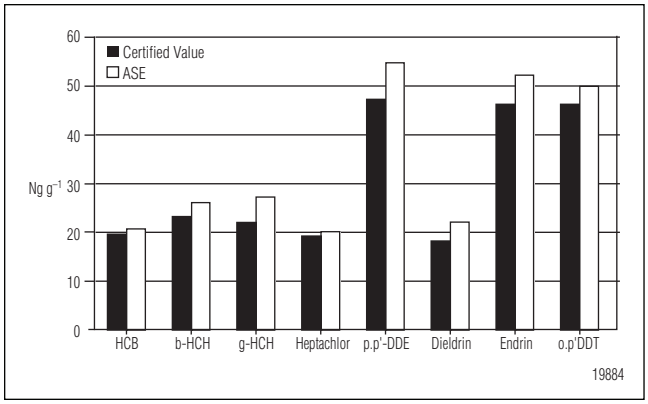


Figure 1. Graph of results from Table 1.

EXTRACTION PROCEDURE

Place the extraction cells onto the ASE 200. Label the appropriate number of collection vials and place these into the vial carousel. Set up the method suggested above and begin the extraction sequence. When the extractions are complete, the extracts can then be cleaned using silica gel adsorption followed by gel permeation chromatography (GPC) with *n*-hexane:dichloromethane (1:1) as the elution solvent.[1]

A two-step cleanup procedure based on silica gel adsorption followed by gel permeation chromatography (GPC) was optimized for the present determinations. An open glass cartridge (8-mm i.d., 6 mL) with a polyethylene frit at its bottom was packed with 1.5-g fresh dichloromethane silica gel and 1-g Na_2SO_4. The column bed was preconditioned with 50 mL *n*-hexane and compressed by a stream of N_2 (200 kPa). Thereafter, the concentrated raw extract was added onto the top of the silica gel column. The sample flask was rinsed with two 0.5-mL portions fo *n*-hexane-CH_2Cl (7+3, v/v) and this was added to the column bed. The analytes were eluted with 19 mL *n*-hexane-dichloromethane (7+3, v/v). The eluate was collected in a 50-mL pear-shaped flask and concentrated to 0.5 mL by means of a rotary evaporator.

The GPC column was prepared by weighting 6 g S-X3 bio-beads that were swelled in *n*-hexane-dichlorometrane (1 + 1, v/v) overnight, into a chromatographic column (15-mm i.d., 30 cm, 100 mL) with a reservoir, fused-in fritted disk, and Teflon® stopcock. The concentrated extract from the silica gel cleanup was applied onto the GPC column. The sample flask was rinsed twice with 0.5-µL elution solvent and

also applied on the GPC column. After permeation of the sample into the column bed, the separation was performed with an additional 35-mL *n*-hexane-dichloromethane 1 + 1 (v/v). The first 18.5 mL were discarded while the volume of 18.5–26.0 mL containing the analytes was collected. This eluate was concentrated to 1 mL by a rotary evaporator, blown to dryness under a gentle stream of N_2, dissolved in 250-µL cyclohexane, and transferred into a GC autosampler microvial for measurement.

Any efficient cleanup procedure may be substituted.

RESULTS AND DISCUSSION

Sample preparation is critical to good recoveries. Grind the samples to a uniform particle size to ensure proper permeation of the solvent into the matrix. It is important to remove the fat and lipids from the extracts so they are ready for GC-MS analysis.

The results of three extractions using ASE are compared to the certified values and listed in Table 1. Figure 1 shows these results graphically. The ASE results are in general agreement with the certified values, with the values of g-HCH and p,p –DDE slightly above the certified values. This slight difference is attributed to the higher temperatures and pressures of ASE, which increases the desorption of highly bound pesticides.

CONCLUSIONS

The extraction efficiency and reproducibility of ASE for extracting OCPs from animal feed was tested using an optimized method to extract a certified reference material (BCR 115). ASE provides a faster way to extract OCPs from animal feed than traditional techniques, such as Soxhlet, and ASE can accomplish these results using far less solvent.

ACKNOWLEDGEMENTS

We would like to acknowledge the work of S. Chen, M. Gfrerer, E. Lankmayr, X. Quan, and F. Yang at the University of Technology, Austria.

REFERENCES

1. Chen, S.; Gfrerer, M; Lankmayr, E.; Quan, X.; Yang, F. Optimization of Accelerated Solvent Extraction for the Determination of Chlorinated Pesticides from Animal Feed. *Chromatographia* **2003**, *58,* 631–636.

Extraction of Chlorinated Pesticides Using Accelerated Solvent Extraction (ASE®)

Meets the requirements of U.S. EPA Method 3545

INTRODUCTION

Accelerated Solvent Extraction (ASE) is a new extraction method that significantly streamlines sample preparation. A commonly used solvent is pumped 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.

Previously, the extraction of chlorinated pesticides from soils, sludge, and other solid wastes consumed large amounts of solvents. Soxhlet, for example, can use from 250 to 500 mL of solvent for most environmental samples. Recent and anticipated changes in environmental regulations will cause severe restrictions on the amount of solvent usage in laboratories worldwide. ASE was developed to meet the new requirements for reducing solvent usage in the preparation of solid waste samples.

ASE provides a more convenient, faster, and less solvent intensive method than previously available for the extraction of chlorinated pesticides from environmentally important samples. Recoveries of these analytes by ASE are equivalent to or better than other more solvent intense methods such as Soxhlet. ASE also avoids the problem of multiple washing procedures associated with sonication. ASE can extract a 10-g sample of a typical soil in about 12 min with a total solvent consumption of approximately 15 mL.

The procedures described in this application note meet the requirements for sample extraction as prescribed by EPA Method 3545. This method is applicable to the extraction of water-insoluble or slightly water-soluble volatiles and semivolatiles in preparation for gas chromatographic or GC/MS measurement. The method is applicable to the extraction of chlorinated pesticides from soils, clays, wastes, and sediments containing from 5 to 250 µg/kg of the target compounds.

EQUIPMENT

ASE 200 Accelerated Solvent Extractor with 11- or 22-mL stainless steel extraction cells

GC or GC/MS

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

SOLVENTS

Acetone (pesticide quality or equivalent)

Hexane (pesticide quality or equivalent)

ASE 200 CONDITIONS

Oven Temperature:	100 °C
Pressure:	10 MPa (1500 psi)
Oven Heatup Time:	5 min
Static Time:	5 min
Flush Volume:	60% of extraction cell volume
Solvent:	Acetone/hexane (1:1 v/v)

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 Perstorp Environmental Soxtec® (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 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 co-extractives. For the examples shown in this application note, extracts were analyzed by SW-846 Method 8080.

Note: All extractions and analytical work were performed by Mountain States Analytical, Inc. (Salt Lake City, Utah, USA).

SAMPLE PREPARATION

The sample is ground to 100–200 mesh (150–75 µm). Wet samples are mixed with either ASE Prep DE (diatomaceous earth), P/N 062819 (1:1, w/w), or air dried.[1] After grinding, a weighed sample is transferred to either a 11- or 22-mL extraction cell.

PROCEDURE

Mix sample thoroughly, especially composite samples. Dried sediment, soil, and dry waste samples should be ground or otherwise subdivided so that it passes 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 40-mL, precleaned, capped vials with septa. Set the method conditions on the ASE 200 system and initiate the run.

DISCUSSION AND RESULTS

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.[2] Results of this study are summarized in Tables 1 and 2 for chlorinated pesticides spiked at three different levels in three different soil types.

REFERENCES

1. U.S. Environmental Protection Agency. *U.S. EPA Method 600/4-81-055*, "Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue," Section 3.1.3.
2. Richter, B.; Ezzell, J.; Felix, D. "Single Laboratory Method Validation Report: Extraction of TCL/PPL (Target Compound List/Priority Pollutant List) BNAs and Pesticides Using Accelerated Solvent Extraction (ASE) with Analytical Validation by GC/MS and GC/ECD" Document 116064.A, Dionex Corporation, June 16, 1994.

Table 1. Average Recovery of Pesticides from Three Soil Types[a]—ASE Compared to Automated Soxhlet

Pesticide	Average Recovery (% of Soxhlet)
Alpha BHC	93.3
Gamma BHC - Lindane	95.6
Beta BHC	98.6
Heptachlor	88.0
Delta BHC	99.5
Aldrin	94.9
Heptachlor Epoxide	100.7
Gamma Chlordane	99.5
Alpha Chlordane	102.0
Endosulfan 1	100.3
p,p'-DDE	98.6
Dieldrin	101.2
Endrin	97.2
p,p-DDD	104.6
Endosulfan II	105.6
p,p'-DDT	74.9
Endrin Aldehyde	104.0
Endosulfan Sulfate	105.2
Methocychlor	79.6
Endrin Ketone	102.9

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

Table 2. Average RSD (%) for Chlorinated Pesticides

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

Extraction of Chlorinated Herbicides Using Accelerated Solvent Extraction (ASE®)

Meets the requirements of U.S. EPA Method 3545

INTRODUCTION

Accelerated Solvent Extraction (ASE) is a new extraction method that significantly streamlines sample preparation. A commonly used solvent is pumped 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.

Analysis of soils, sludge, and other solid wastes for chlorinated herbicides first requires extraction of the analytes from the matrix. Previously, this step usually required large amounts of solvents. Recent and anticipated changes in environmental regulations will cause severe restrictions on the amount of solvent usage in laboratories worldwide. For example, in the United States a recent executive order calls for a 50–90% reduction of solvent usage in all federal laboratories. ASE was developed by Dionex to meet the new requirements for reducing solvent usage in the preparation of solid waste samples.

The use of ASE in the extraction of chlorinated herbicides from solid wastes is more convenient, faster, and less solvent-intensive than previous methods. U.S. EPA Method 8150A for the analysis of chlorinated herbicides uses a wrist-shaking technique that requires 300 mL of acetone and diethyl ether following acidification to pH 2 with HCl. In the method reported in this application note, chlorinated herbicide recoveries by ASE are equivalent to recoveries from the wrist-shaking method recommended in Method 8150A. ASE can extract a 10-g sample of a typical soil in about 12 min with a total solvent consumption of approximately 15 mL.

The procedures described in this application note meet the requirements for sample extraction as prescribed by U.S. EPA Method 3545. This method is applicable to the extraction of water-insoluble or slightly water-soluble chlorinated herbicides in preparation for gas chromatographic measurements.

EQUIPMENT

ASE 200 Accelerated Solvent Extractor, with 11- or 22-mL stainless steel extraction cells

Gas chromatograph (GC) with ECD

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

SOLVENTS

Dichloromethane

Acetone

Phosphoric acid

ASE 200 CONDITIONS

Oven Temperature:	100 °C
Pressure:	14 MPa (2000 psi)
Oven	
Heatup Time:	5 min
Static Time:	5 min
Flush Volume:	60% of extraction cell volume
Nitrogen Purge:	1 MPa (150 psi) for 60 s
Solvent:	Dichloromethane/acetone (1:2, v/v), with 4% (v/v) H_3PO_4/H_2O (1:1)

SAMPLE INFORMATION

All spiked soils were prepared and certified by ERA (Environmental Resource Associates). Spiked samples were extracted both by the ASE 200 system and by the wrist-shaking technique. The extract was treated using the postextraction steps as outlined in U.S. EPA Method 8150A. These steps include treatment with acidic Na_2SO_4, base hydrolysis, concentration, and esterification with diazomethane. Extracts were analyzed by SW-846 Method 8150A using GC/ECD.

Note: All extractions and analytical work were performed by DataChem Laboratories (Salt Lake City, Utah, USA). Matrix blanks, spikes, and spike duplicates were included for each matrix.

SAMPLE PREPARATION

The sample is ground to 100–200 mesh (150–75 µm). Wet samples either are mixed with ASE Prep DE (diatomaceous earth), P/N 062819 (1:1, w/w) or are air dried.[1] After grinding, a weighed sample is transferred to either an 11- or 22-mL extraction cell.

PROCEDURE

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

Dried sediment, soil, and dry waste samples should be ground or otherwise subdivided to pass through a 1-mm sieve. Wet samples are mixed with ASE Prep DE until a free-flowing powder is obtained. Introduce a sufficient amount of sample into the grinding apparatus to yield 10–20 g after grinding. Dry the sample or mix. Air drying is not appropriate for the analysis of the more volatile chlorinated herbicides.

Place a cellulose disk at the outlet end of the extraction cell. Weigh 10 g of each sample into 11-mL extraction cells or 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 40-mL, precleaned, capped vials with septa. Set the method conditions on the ASE 200 system and initiate the run.

DISCUSSION AND RESULTS

Results from the extraction of two spiking levels in three different soil types are summarized in Tables 1 and 2. These results illustrate that the ASE technique obtains analyte recoveries equivalent to the Method 8150A wrist-shaking procedure for the extraction of chlorinated herbicides from solid waste.[2]

REFERENCES

1. "Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue"; U.S. EPA Method 600/4-81-055, Section 3.1.3.
2. Richter, B.; Ezzell, J.; Felix, D. "Single Laboratory Method Validation Report: Extraction of Organophosphorous Pesticides, Chlorinated Herbicides, and Polychlorinated Biphenyls Using Accelerated Solvent Extraction (ASE) with Analytical Validation by GC/NPD and GC/ECD"; Document 101124; Dionex Corporation: December 2, 1994.

Table 1. Average Recovery of Chlorinated Herbicides from Three Soil Types[a]—ASE Compared to Wrist-Shaking Method

Chlorinated Herbicide Target Compound	Average Recovery (% of Shaking Method)
2,4-D	116.2
2,4-DB	112.9
2,4,5-T	106.6
2,4,5-TP	117.4
Dalapon	101.8
Dicamba	108.1
Dichlorprop	107.7
Dinoseb	118.4

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

Table 2. Average Recovery and Precision for Extraction of Chlorinated Herbicides from Three Soil Types by ASE

Matrix[a]	ASE (% of Spike)	ASE[b] (RSD,%)	Shaking (% of Spike)	Shaking[b] (RSD,%)	ASE as % of Shaking
Clay (low)	36.1	54.5	42.2	25.2	89.8
Clay (high)	71.1	15.0	61.6	21.6	112.8
Loam (low)	56.7	11.2	36.9	78.7	126.6
Loam (high)	59.9	14.1	43.9	14.7	132.8
Sand (low)	51.1	12.6	49.7	13.3	111.2
Sand (high)	69.2	39.5	66.3	35.5	104.4

[a] Low spiking levels ranged from 50 to 500 µg/kg. High spiking levels ranged from 500 to 5000 µg/kg.

[b] Each precision (RSD,%) value is the average of seven replicate measurements for each compound, then averaged for all compounds.

Determination of Aniline and Nitroanilines in Environmental and Drinking Waters by On-Line SPE

INTRODUCTION

Aniline is an organic compound widely used in the polymer, rubber, pharmaceutical, and dye industries. Aniline and its derivatives (e.g., nitroanilines) are suspected carcinogens and are highly toxic to aquatic life. Therefore, it is necessary to establish sensitive, efficient, and simple methods for the determination of aniline and its derivatives in drinking and environmental waters.

The most common techniques for the determination of aniline and its derivatives in environmental and drinking waters are gas chromatography (GC)[1,2] and high-performance liquid chromatography (HPLC).[3–5] Capillary zone electrophoresis (CZE)[6] and spectrophotometric methods[7] have been reported as well. Because these compounds are thermolabile and polar, a derivatization step prior to GC analysis is often required, and most of these procedures are time consuming and complicated. Therefore, HPLC analysis is a good alternative to GC analysis because derivatization is not needed.

Normally, extraction processes for aniline and its derivatives from environmental and drinking water samples prior to HPLC analysis are required due to the limited sensitivity of direct injection for these samples, which have low concentrations of anilines. The typical extraction techniques are liquid-liquid extraction[8] and solid-phase extraction (SPE),[9] with SPE gaining favor either in the on-line or off-line mode. Compared to off-line SPE, on-line SPE offers the advantages of full automation, absence of operator influence, time savings, and strict process control.[10–12]

Here, an on-line SPE HPLC system is used to fulfill the simple and sensitive determination of aniline and four nitroanilines—*o*-nitroaniline, *m*-nitroaniline, *p*-nitroaniline, and *o,p*-dinitroaniline—in tap and pond water. The analyte structures are shown in Figure 1.

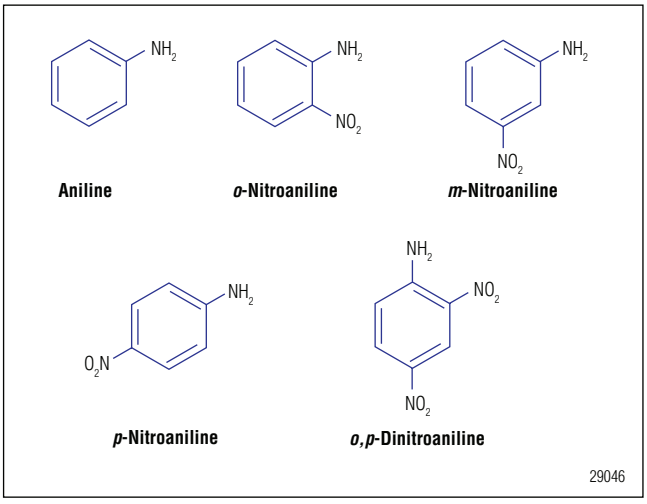


Figure 1. Structures of aniline and nitroanilines.

This on-line SPE HPLC system uses a Thermo Scientific Dionex SolEx™ HRP cartridge for the enrichment and a Thermo Scientific Acclaim™ 120 C18 column for the separation. The Thermo Scientific Dionex UltiMate™ 3000 Dual HPLC system provides an efficient platform to fulfill the on-line SPE and separation, and the system operates under automatic control of Thermo Scientific Dionex Chromeleon™ Chromatography Data System (CDS) software. The complete analysis requires only 15 min, and method detection limits (MDL) for these compounds are all less than 0.2 µg/L, which meets the requirement of United States Environmental Protection Agency (EPA) Method 8131 (GC method, MDLs range from 1.0 to 11 µg/L).[13]

EQUIPMENT

Dionex UltiMate 3000 HPLC system including:

- DGP-3600A pump with SRD-3600 solvent rack with degasser
- WPS-3000TSL semiprep autosampler with 2500 µL sample loop*
- TCC-3200 thermostatted column compartment equipped with one 2p–6p valve
- DAD-3000RS UV-vis detector

Chromeleon CDS software, Version 6.80, SR9

Orion 420A+ pH meter, Thermo Scientific

*The analytical version of the WPS-3000TSL autosampler can also be converted to the semipreparative version by installing the Semipreparative Conversion Kit (P/N 6822.2450) for large-volume injections for on-line SPE.

REAGENTS

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

Methanol (CH_3OH), HPLC grade (Cat.# AC610090040) Fisher Chemical

Acetonitrile (CH_3CN), HPLC grade (Cat.#AC610010040) Fisher Chemical

Phosphoric acid (H_3PO_4), analytical grade, SCRC, China

Dipotassium hydrogen phosphate (K_2HPO_4), analytical grade, SCRC, China

STANDARDS

Aniline, analytical standard, Fluka

o-Nitroaniline, 98%, Aldrich

m-Nitroaniline, 98%, Aldrich

p-Nitroaniline, 99%, Aldrich

o,p-Dinitroaniline, 98%, Aldrich

Accurately weigh ~50 mg of a standard and dilute in a 50 mL volumetric flask with methanol. The concentration of the standard is 1000 mg/L (stock standard solution 1). Pipet 50 µL of stock standard 1 into a 50 mL volumetric flask and dilute to the mark with methanol. The concentration of the standard is 1000 µg/L (stock standard solution 2). Prepare four working standard solutions for the calibration with 1, 10, 50, and 100 µg/mL concentrations by adding the proper amount of stock standard solution 2 and making dilutions with methanol.

Note: The concentration of the stock standard solution 1 is not 1000 mg/L because of the < 100% purity for the standards. So, the actual volume taken for the preparation of stock standard solution 2 must be, for example, 51 µL for *o*-nitroaniline with 98% purity.

SAMPLES

Tap water samples were collected at the Dionex Shanghai Applications Lab. Pond water samples were collected at Zhangjiang High-Tech Park located in the Pudong District of Shanghai, China.

These samples were filtered through a 0.45 µm membrane (Millex®-HN) prior to injection.

CHROMATOGRAPHIC CONDITIONS

SPE Cartridge:	Dionex SolEx HRP Cartridge, 12–14 µm, 2.1 × 20 mm (P/N 074400) Use V-3 Holder (P/N 074403)*
Analytical Column:	Acclaim 120 C18, 3 µm, 4.6 × 150 mm (P/N 059133)
Mobile Phase:	For on-line SPE: A: 10 mM phosphate buffer (pH 6.5) B: CH_3OH In gradient (Table 1)
For Separation:	A: H_2O B: CH_3CN In gradient (Table 1)
Valve-Switching:	Table 1
Flow Rate:	2.0 and 0.5 mL/min for on-line SPE 1.0 mL/min for separation
Inj. Volume:	5000 µL on the on-line SPE cartridge*
Column Temp.:	30 °C
UV Detection:	Absorbance at 230 nm

*Two consecutive injections of 2500 µL using the User Defined Program (UDP) injection mode controlled by Chromeleon CDS software

Table 1. Elution and Valve Switching for On-Line SPE and Separation							
Time (min)	Left Pump (for On-Line SPE)			Right Pump (for Separation)			Valve Switching
	Flow Rate (mL/min)	Solvent A 10 mM Phosphate Buffer (pH 6.5) (%)	Solvent B Methanol (%)	Flow Rate (mL/min)	Solvent A H_2O (%)	Solvent B Acetonitrile (%)	
0	2	90	10	1.0	70	30	1–2
2		90	10		70	30	6–1
3	0.5	30	70		—	—	1–2
10		30	70		45	55	—
11	2	90	10		30	70	—
13	—	—	—		30	70	—
15	—	—	—		70	30	—

RESULTS AND DISCUSSION

Selection of SPE Column

Considering the tolerance to large-volume injection of water samples, and the relative ease or difficulty of retention/elution of aniline and nitroanilines by SPE, two types of silica-based stationary phases (the Acclaim Mixed-Mode WCX-1 Guard and the Acclaim PA2 Guard) and two types of polymeric sorbents (the Dionex SolEx HRP Cartridge and the Thermo Scientific Dionex IonPac™ NG1 Guard) were evaluated as SPE columns. This evaluation followed the typical on-line SPE flow schematic shown in Figure 2. The chromatograms of aniline, *p*-nitroaniline, *m*-nitroaniline, *o*-nitroaniline, and *o,p*-dinitroaniline are shown in Figure 3.

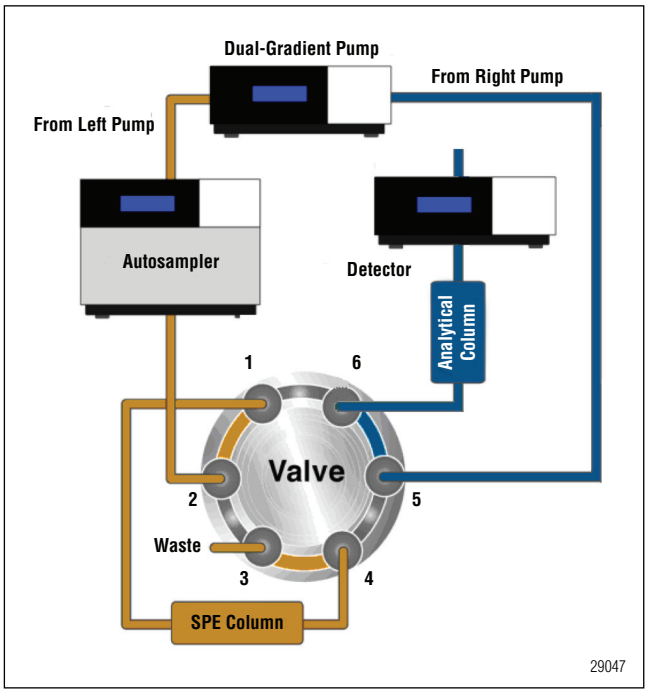


Figure 2. Flow schematic of on-line SPE.

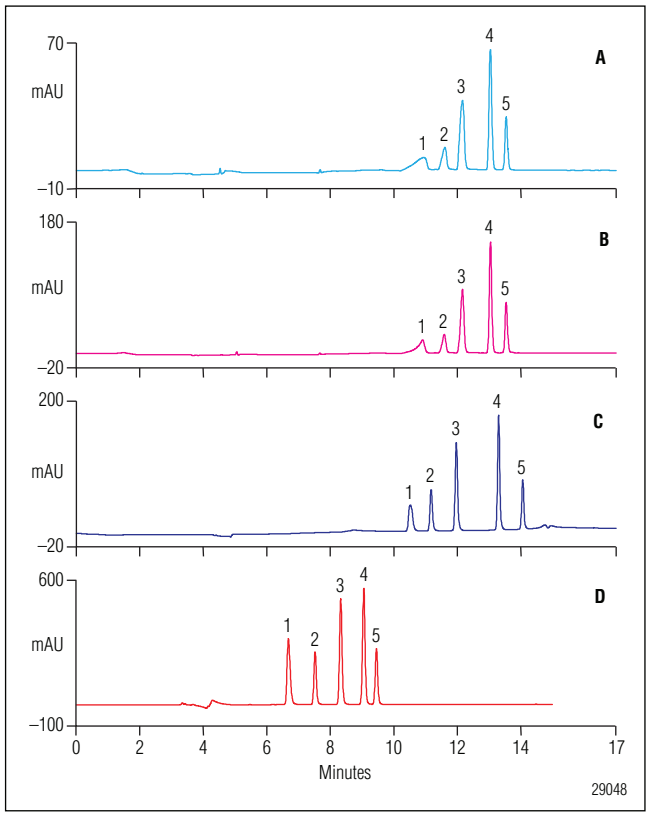


Figure 3. Chromatograms of aniline and nitroanilines (100 µg/L each) using different on-line SPE stationary phases (A) Dionex IonPac NG1 Guard, (B) Acclaim PA2 Guard, (C) Acclaim Mixed-Mode WCX-1 Guard, and (D) Dionex SolEx HRP Cartridge. See Table 2 for conditions.

As shown in Figure 3 A and B, severe band spreading for aniline (peak 1) was observed when using the Dionex IonPac NG1 Guard and the Acclaim PolarAdvantage II (PA2) Guard. This can be attributed to aniline's weak retention on these stationary phases, even using water as the mobile phase. During its enrichment in on-line SPE, aniline diffused on these SPE columns, resulting in severe band spreading on the analytical column even if using a reversed flush with organic mobile phase. Meanwhile, the weak retention of aniline on these stationary phases may result in its loss during the course of enrichment. Poor extraction efficiency, low to about 50%, was estimated by comparing the peak area obtained with on-line SPE to that obtained without SPE.

Although the peak shape improved using the Acclaim Mixed-Mode WCX-1 Guard (Figure 3C), a stationary phase that combines cation-exchange and RP properties, there was not a significant improvement in extraction efficiency. The Dionex SolEx HRP cartridge, packed with a divinylbenzene polymer with a hydrophilic bonded layer,[14] was thus selected based on its excellent retention properties of the analytes with different polarities. As shown in Figure 3D, good peak shape of aniline was observed; and the estimated extraction efficiency was > 95%. The peak shape and efficiency of *p*-nitroaniline were also improved using the Dionex SolEx HRP cartridge.

Table 2. Chromatographic Conditions for Figure 3

On-Line SPE Stationary Phase	**Dionex IonPac NG1 Guard (10 µm, 4 × 35 mm) and Acclaim PA2 Guard (5 µm, 4.6 × 10 mm)**		**Acclaim Mixed-Mode WCX-1 Guard (5 µm, 4.6 × 10 mm) and Dionex SolEx HRP Cartridge (12–14 µm, 2.1 × 20 mm)**	
Analytical Column		Acclaim 120 C18 (3 µm, 3.0 × 150 mm)	Acclaim 120 C18 (3 µm, 4.6 × 150 mm)	
Mobile Phase	For on- line SPE	50 mM NH_4 Ac-HAc (pH 4.6)/CH_3OH Gradient: CH_3OH, 0~2 min, 1%; 6~11 min, 70%; 11~17 min, 1.0%	10 mM phosphate buffer (pH 6.5/ CH_3OH Gradient: CH_3OH, 0~3 min, 0%; 7~14.5 min, 70%; 15.1~18 min, 0%	10 mM phosphate buffer (pH 6.5/ CH_3OH Gradient: CH_3OH, 0~2 min, 10%; 3~10 min, 70%; 11~15 min, 10%
	For separation	H_2O/CH_3OH Gradient: CH_3OH, 0~4 min, 5%; 10~17 min, 60%	H_2O/CH_3OH Gradient: CH_3OH, 0 min,10%; 2.5 min, 10%; 13~18 min, 70%; 23 min, 10%	H_2O/CH_3CN Gradient: CH_3CN, 0~2 min, 30%; 10 min, 55%; 11~13 min, 70%; 15 min, 30%
Flow Rate	For on- line SPE	0~2 min, 1.5 mL/min; 2.1~15 min, 0.5 mL/min; 17 min, 1.5 mL/min	0~3 min, 0.5 mL/min; 7~18 min, 1.0 mL/min; 18.1 min, 0.5 mL/min	0~2 min, 2.0 mL/min; 3~10 min, 0.5 mL/min; 11~15 min, 2 mL/min
	For separation	0.5 mL/min	1.0 mL/min	
Inj. Volume	5000 µL on the on-line SPE cartridge (two consecutive injections of 2500 µL using UDP injection mode)			
Column Temp.		30 °C	30 °C	
UV Detection	285 nm		230 nm	
Sample	Tap water spiked with anilines standards (100 µg/L each)			
Peaks	1) Aniline, 2) *p*-nitroaniline, 3) *m*-nitroaniline, 4) *o*-nitroaniline, 5) *o,p*-dinitroaniline			

Effect of Mobile Phase for On-Line SPE

The effect of mobile phase on on-line SPE was investigated. As shown in Figure 4, when using either water or phosphate buffer mobile phase containing 10% methanol for sample enrichment on the Dionex SolEx HRP cartridge, no difference was observed for the *p*-nitroaniline, *m*-nitroaniline, *o*-nitroaniline, and *o,p*-dinitroaniline peaks on the Acclaim 120 C18 analytical column. A tailing aniline peak was observed when using water; however, the peak became sharp and symmetrical when using phosphate buffer. So, a 10 mM phosphate buffer (pH 6.5) mobile phase was used for on-line SPE.

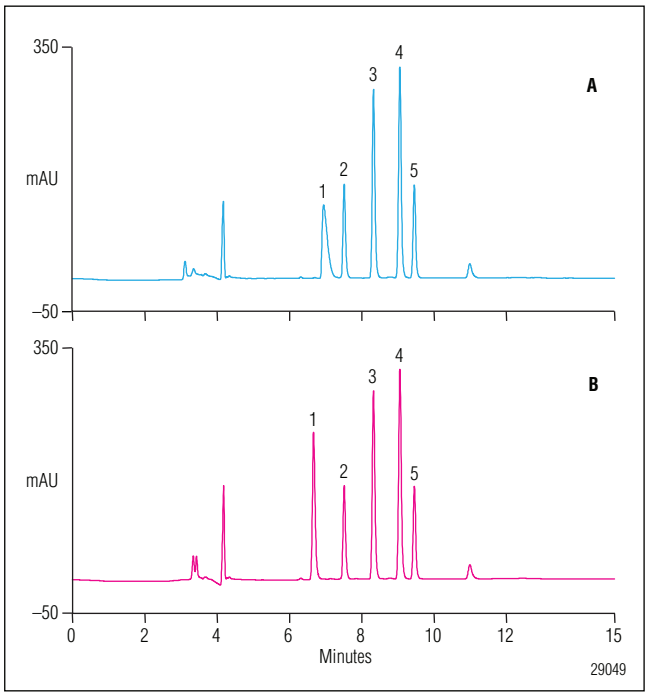


Figure 4. Chromatograms of aniline, p-*nitroaniline,* m-*nitroaniline,* o-*nitroaniline, and* o,p-*dinitroaniline using (A) H_2O/CH_3OH and (B) 10 mM phosphate buffer (pH 6.5)/ CH_3OH mobile phases for on-line SPE. See Table 3 for conditions.*

Table 3. Chromatographic Conditions for Figure 4

On-Line SPE Cartridge	Dionex SolEx HRP		
Analytical Column	Acclaim 120 C18		
Mobile Phase	For on-line SPE	H_2O/CH_3CN Gradient: CH_3CN, 0~2 min, 10%; 3~10 min, 70%; 11~15 min, 10%	10 mM phosphate buffer (pH 6.5/CH_3OH Gradient: CH_3OH, 0~2 min, 10%; 3~10 min, 70%; 11~15 min, 10%
	For separation	H_2O/CH_3CN Gradient: CH_3CN, 0~2 min, 30%; 10 min, 55%; 11~13 min, 70%; 15 min, 30%	
Flow Rate	For on-line SPE	0~2 min, 2.0 mL/min; 3~10 min, 0.5 mL/min; 11~15 min, 2 mL/min	
	For separation	1.0 mL/min	
Inj. Volume	5000 µL on the on-line SPE cartridge (two consecutive injections of 2500 µL using UDP injection mode)		
Column Temp.	30 °C		
UV Detection	230 nm		
Sample	Tap water spiked with aniline standards (100 µg/L for each)		
Peaks	1) Aniline, 2) *p*-nitroaniline, 3) *m*-nitroaniline, 4) *o*-nitroaniline, 5) *o,p*-dinitroaniline		

Method Reproducibility, Linearity, and Detection Limits

Method reproducibility was estimated by making five consecutive 5000 µL injections of mixed standards with a 10 µg/L concentration of each. Retention time and peak area reproducibilities are summarized in Table 4 and show good precision.

Table 4. Reproducibility for Peak Retention Time and Area

Analyte	Retention Time RSD	Peak Area RSD	Concentration of Standard (µg/L)
Aniline	0.022	0.300	10
p-Nitroaniline	0.031	0.183	
m-Nitroaniline	0.028	0.051	
o-Nitroaniline	0.026	0.123	
o,p-Dinitroaniline	0.039	0.160	

Table 5. Method Linearity Data and Method Detection Limits (MDL)					
Analyte	**Regression Equation**	**r**	**Range of Standards (µg/L)**	**MDL, µg/L**	
				Current Data	**Data Reported in EPA Method 8131**
Aniline	$A = 0.3686\ c - 0.1530$	0.9999	1–100	0.2	2.3
p-Nitroaniline	$A = 0.2290\ c - 0.0830$	1.0000		0.2	1.0
m-Nitroaniline	$A = 0.4770\ c + 0.0302$	1.0000		0.1	3.3
o-Nitroaniline	$A = 0.5286\ c - 0.0194$	1.0000		0.1	11.0
o,p-Dinitroaniline	$A = 0.2432\ c - 0.0252$	1.0000		0.2	8.9

Calibration linearity for aniline and nitroanilines was investigated by making three consecutive injections of a mixed standard prepared at four different concentrations. The external standard method was used to establish the calibration curve and to quantify these compounds in samples. Excellent linearity was observed from 1 to 100 µg/L when plotting concentration versus peak area, and the correlation coefficient was ≥ 0.9999 for each plot. The MDLs of each compound for UV detection were calculated using S/N = 3 (signal to noise), and all were ≤ 0.2 µg/L. Table 5 summarizes the method linearity and MDL data, which show excellent method linearity and sensitivity, with detection limits well below those defined in the EPA method.[13]

Sample Analysis

Chromatograms of tap and pond water samples, as well as the same samples spiked with aniline and related standards (1.0 µg/L each and 10 µg/L each, respectively), are shown in Figures 5 and 6, and the related data are summarized in Table 6. Recoveries for each standard in both sample sets ranged from 98 to 108% for the 10 µg/L standard spiked samples, and ranged from 93 to 147% for the 1 µg/L standard spiked samples. None of the samples had detectable aniline or nitroanilines.

The real samples may sometimes yield a false positive for aniline and/or one of the nitroanilines. An efficient and convenient way to determine if the peak is a target analyte is to compare the peak's UV spectrum to that of standards. Therefore, using a photodiode array detector for this analysis will help reduce the possibility of false positives.

When the pond water sample was analyzed, a small peak with retention time near that of aniline was found and labeled as aniline with a concentration 0.3 µg/L, similar to the estimated MDL of aniline (0.2 µg/L).

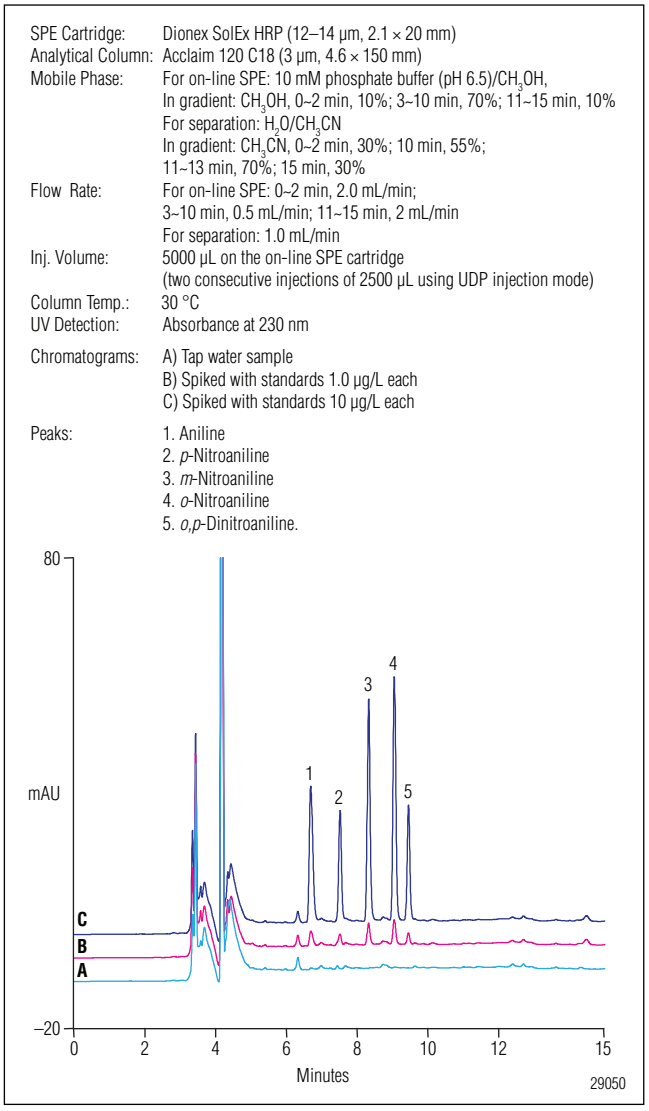


Figure 5. Chromatograms of (A) tap water sample, (B) the same sample spiked with 1.0 µg/L aniline and nitroanilines standard, and (C) spiked with 10 µg/L.

Comparison of the UV spectra shown in Figure 7 revealed that the peak was not aniline. The spike-recovery of aniline at 1.0 µg/L level in pond water, 147%, also suggests that there is interference.

SPE Cartridge:	Dionex SolEx HRP (12–14 µm, 2.1 × 20 mm)
Analytical Column:	Acclaim 120 C18 (3 µm, 4.6 × 150 mm)
Mobile Phase:	For on-line SPE: 10 mM phosphate buffer (pH 6.5)/CH_3OH, In gradient: CH_3OH, 0–2 min, 10%; 3–10 min, 70%; 11–15 min, 10% For separation: H_2O/CH_3CN In gradient: CH_3CN, 0–2 min, 30%; 10 min, 55%; 11–13 min, 70%; 15 min, 30%
Flow Rate:	For on-line SPE: 0–2 min, 2.0 mL/min; 3–10 min, 0.5 mL/min; 11–15 min, 2 mL/min For separation: 1.0 mL/min
Inj. Volume:	5000 µL on the on-line SPE cartridge (two consecutive injections of 2500 µL using UDP injection mode)
Column Temp.:	30 °C
UV Detection:	Absorbance at 230 nm
Chromatograms:	A) Pond water sample B) Spiked with standards 1.0 µg/L each C) Spiked with standards 10 µg/L each
Peaks:	1. Aniline 2. *p*-Nitroaniline 3. *m*-Nitroaniline 4. *o*-Nitroaniline 5. *o,p*-Dinitroaniline.

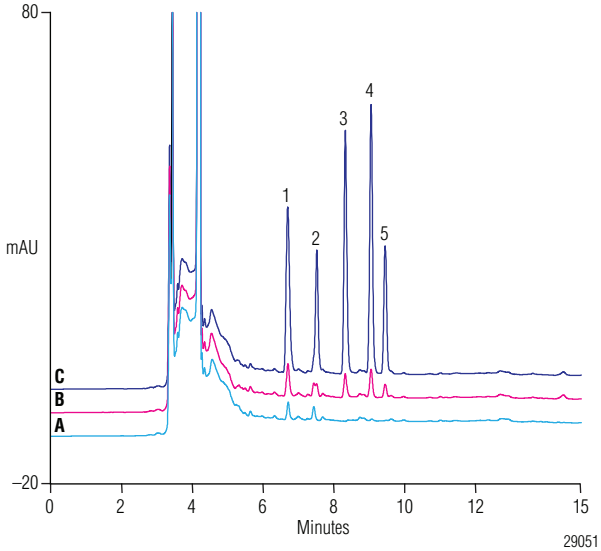


Figure 6. Chromatograms of (A) pond water sample, (B) the same sample spiked with 1.0 µg/L aniline and nitroanilines standard, and (C) spiked with 10 µg/L.

In addition, as shown in Figures 5 and 6, interference with retention time near that of *p*-nitroaniline (peak 2) was found. Although it was not labeled as *p*-nitroaniline, its presence affects the spike-recoveries of *p*-nitroaniline at the 1.0 µg/L level in both pond and tap waters samples (140% and 127%, respectively). This demonstrates that the limits of detection are often set by matrix interference instead of instrumental uncertainties in the analysis of environmental samples.

Table 6. Analysis Results of Anilines in Water Samples

Sample	Pond Water				
Analyte	**Detected (µg/L)**	**Added (µg/L)**	**Recovery (%)**	**Added (µg/L)**	**Recovery (%)**
Aniline	ND	1.0	147	10	104
p-Nitroaniline	ND		140		101
m-Nitroaniline	ND		94.2		99.7
o-Nitroaniline	ND		105		101
o,p-Dinitroaniline	ND		101		98.8
Sample	**Tap Water**				
Analyte	**Detected (µg/L)**	**Added (µg/L)**	**Recovery (%)**	**Added (µg/L)**	**Recovery (%)**
Aniline	ND	1.0	103	10	100
p-Nitroaniline	ND		127		108
m-Nitroaniline	ND		93.1		100
o-Nitroaniline	ND		109		102
o,p-Dinitroaniline	ND		103		100

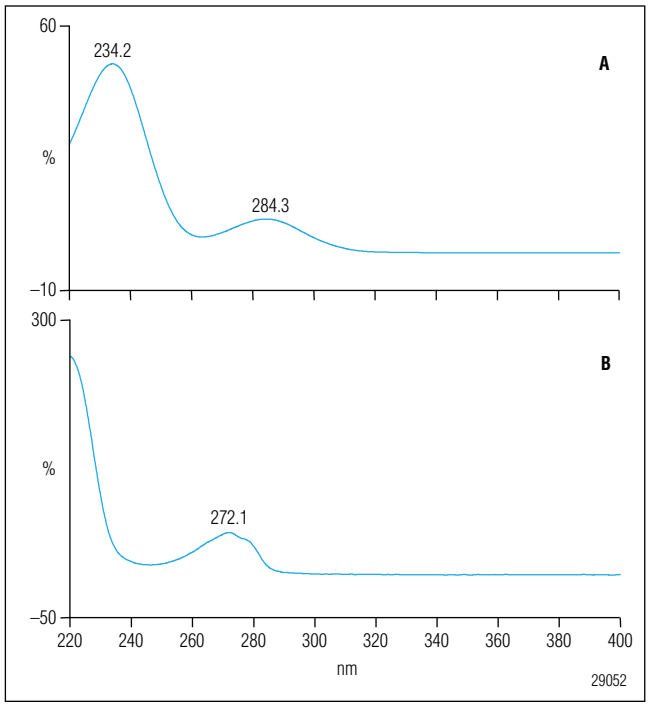


Figure 7. UV spectra of (A) aniline standard and (B) the putative aniline peak in a pond water sample.

CONCLUSION

This work describes an on-line SPE system using the Dionex SolEx HRP cartridge to enrich aniline and nitroanilines followed by HPLC with UV detection. The enrichment of aniline and nitroanilines in tap and pond water is sufficient, and baseline separation on the Acclaim 120 C18 column is achieved. The Dionex UltiMate 3000 Dual HPLC system provides an efficient platform to fulfill this on-line SPE, and the system operates under automatic control of Chromeleon CDS software. The determination of aniline and nitroanilines in tap and pond water is simple, rapid, and sensitive, and meets the MDL requirement of the EPA Method 8131. Although this work cannot be a substitute for the EPA method, it does demonstrate that these analytes can be determined by on-line SPE-HPLC while meeting the performance criteria of the EPA method.

REFERENCES

1. Brede, C.; Skjevrak, I.; Herikstad, H. Determination of Primary Aromatic Amines in Water Food Simulant Using Solid-Phase Analytical Derivatization Followed by Gas Chromatography Coupled with Mass Spectrometry *J. Chromatogr., A* **2003,** *983,* 35.
2. Chiang, J. S.; Huang, S. D. Simultaneous Derivatization and Extraction of Anilines in Waste Water with Dispersive Liquid–Liquid Microextraction Followed by Gas Chromatography–Mass Spectrometric Detection *Talanta* **2008,** *75,* 70.
3. Jen, J. F.; Chang, C. T.; Yang, T. C. On-Line Microdialysis–High-Performance Liquid Chromatographic Determination of Aniline and 2-Chloroaniline in Polymer Industrial Wastewater *J. Chromatogr., A* **2001,** *930,* 119.
4. Sarafraz-Yazdi, A.; Es'haghi, Z. Liquid–Liquid–Liquid Phase Microextraction of Aromatic Amines in Water Using Crown Ethers by High-Performance Liquid Chromatography with Monolithic Column *Talanta* **2005,** *66,* 664.
5. Zhao, L. M.; Zhu, L. Y.; Lee, H. K. Analysis of Aromatic Amines in Water Samples by Liquid–Liquid–Liquid Microextraction with Hollow Fibers and High-Performance Liquid Chromatography *J. Chromatogr., A* **2002,** *963,* 239.
6. Li, J.; Yuan, Z. B. Separation of Aniline Derivatives by Micellar Electrokinetic Capillary Chromatography *Chin. Chem. Lett.* **2004,** *15,* 947.
7. Gu, X. X.; Li, C. Y.; Qi, X.; Zhou, T. Z. Determination of Trace Aniline in Water by a Spectrophotometric Method After Preconcentration on an Organic Solvent-Soluble Membrane Filter *Anal. Lett.* **1997,** *30,* 259.
8. Wu, X. H.; Lei, Z. G.; Li, Q. S.; Zhu J. Q.; Chen, B. H. Liquid-Liquid Extraction of Low-Concentration Aniline from Aqueous Solutions with Salts *Ind. Eng. Chem. Res.* **2010,** *49,* 2581.
9. Patsias, J.; Papadopoulou-Mourkidou, E. Development of an Automated On-Line Solid-Phase Extraction–High Performance Liquid Chromatographic Method for the Analysis of Aniline, Phenol, Caffeine and Various Selected Substituted Aniline and Phenol Compounds in Aqueous Matrices *J. Chromatogr., A* **2000,** *904,* 171.
10. Thermo Fisher Scientific. *Determination of Phenols in Drinking and Bottled Mineral Waters Using Online Solid-Phase Extraction Followed by HPLC with UV Detection.* Dionex Application Note 191, LPN 1949, 2007, Sunnyvale, CA.
11. Thermo Fisher Scientific. *Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Edible Oils by Donor-Acceptor Complex Chromatography (DACC)-HPLC with Fluorescence Detection.* Dionex Application Note 196, LPN 1998, 2008, Sunnyvale, CA.
12. Thermo Fisher Scientific. *Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Tap Water Using On-Line Solid-Phase Extraction Followed by HPLC with UV and Fluorescence Detections.* Dionex Application Note 213, LPN 2126, 2009, Sunnyvale, CA.
13. *niline and Selected Derivatives by Gas Chromatography;* U.S. EPA Method 8131, U.S. Environmental Protection Agency: Cincinnati, OH, 1996.
14. Thermo Fisher Scientific. *SolEx HRP On-Line Sample SPE Concentration Cartridges.* LPN 2565, 2010, Sunnyvale, CA.

Sensitive On-Line SPE–HPLC Determination of Paraquat and Diquat in Drinking and Environmental Waters

INTRODUCTION

Mixtures of paraquat (1,1′-dimethyl-4,4′-dipyridylium dichloride) and diquat (1,1′-ethylene-2,2′-dipyridylium dibromide), quaternary ammonium herbicides, are widely used to control crop and aquatic weeds. Their structures are shown in Figure 1. Contamination of drinking water with paraquat and diquat is considered a risk factor for liver, heart, lungs, and kidney illnesses.

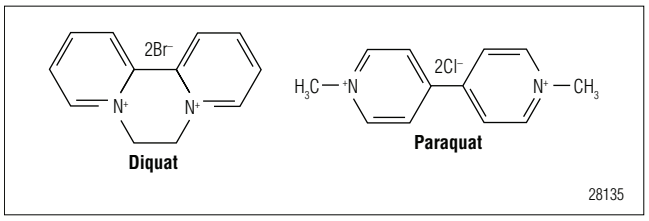


Figure 1. Structures of diquat and paraquat specified in the U.S. EPA Method 549.2.

The United States Environmental Protection Agency (U.S. EPA) specified a Maximum Contaminant Level Goal (MCLG) for diquat in drinking water of 20 µg/L[1] and the European Union (EU) published a general rule for pesticides in drinking water (98/83/EC).[2] This rule states that the maximum admissible concentration of each individual pesticide component is 0.1 µg/L, and the total concentration is not to exceed 0.5 µg/L. Therefore, simple and effective sample preparation and sensitive analytical methods are necessary for determining diquat and paraquat in environmental waters.

High-performance liquid chromatography (HPLC) is one commonly used method for the separation of diquat and paraquat. Their baseline separation is difficult on conventional reversed-phase (RP) columns (C18 or C8) due to their weak retention on those columns. Therefore, ion-pairing reagents are added to the mobile phase. This addition may also improve peak shape.[3–11]

The U.S. EPA published EPA Method 549.2 for monitoring diquat and paraquat in aqueous samples.[3] This method uses a C18 stationary phase with an ion-pairing reagent in the mobile phase and photodiode array detection.

Recently, a stationary phase that may be used in the hydrophilic interaction liquid chromatography (HILIC) mode was reported for the separation in the absence of an ion-pairing reagent.[12] An improved separation with resolution (R_s) of 3.2 was achieved using the Acclaim® Mixed-Mode HILIC-1 column.[13]

Solid phase extraction (SPE) is the typical method for sample extraction and enrichment in the analysis of diquat and paraquat in water samples by HPLC. Off-line SPE[14–16] is usually used, and EPA Method 549.2 also describes off-line SPE for water sample preparation, which is improved compared to Method 549.1, but still complex.[3] Compared to off-line SPE, on-line SPE offers the advantages of full automation, the absence of operator influence, time savings, and strict process control.[17–19] Several applications of on-line SPE to the determination of diquat and paraquat in water samples by HPLC have been reported.[20–22]

In the work shown here, an on-line SPE system is used to eliminate interferences sufficiently and fulfill the simple and sensitive determination of diquat and paraquat in tap and pond water. This on-line SPE system uses two SPE cartridges. One is the Acclaim Mixed-Mode WAX-1 cartridge for the elimination of anionic interferences; the other one is the Acclaim Mixed-Mode WCX-1 cartridge for the enrichment of diquat and paraquat, and the elimination of co-enriched cationic interferences.

The analysis is completed by baseline separation of diquat and paraquat on the Acclaim Trinity™ P1 column. The UltiMate® 3000 Dual HPLC system provides an efficient platform to fulfill the on-line SPE and separation, and the system operates under automatic control of Chromeleon® Chromatography Data System (CDS) software. The complete analysis only requires 16 min, and method detection limits (MDL) are 0.12 µg/L for diquat and 0.10 µg/L for paraquat, which meets the requirement of EPA Method 549.2 (0.72 µg/L for diquat and 0.68 µg/L for paraquat).

EQUIPMENT

Dionex UltiMate 3000 HPLC system including:

- DGP-3600A pump with SRD-3600 solvent rack with degasser
- WPS-3000TSL semiprep autosampler with 2500 µL sample loop*
- TCC-3200 thermostatted column compartment equipped with one 2p–6p valve
- DAD-3000RS UV-vis detector

Chromeleon CDS software, Version 6.80 SR9

Orion 420A+ pH meter, Thermo Scientific

*The analytical version of the WPS-3000TSL autosampler can also be converted to the semipreparative version by installing the Semipreparative Conversion Kit (P/N 6822.2450) for large-volume injections for on-line SPE.

REAGENTS

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

Methanol (CH_3OH), Fisher

Acetonitrile (CH_3CN), Fisher

Acetic acid (CH_3COOH), analytical grade, SCRC, China

Ammonium acetate (CH_3COONH_4), analytical grade, SCRC, China

Ammonium sulfate ($(NH_4)_2SO_4$), analytical grade, SCRC, China

Dimethyldichlorosilane (DMCO), analytical grade, SCRC, China

STANDARDS

Use the M-549.1 Diquat and Paraquat standard (1.0 mg/mL each, AccuStandard [Lot No. 6120096-1A]) for preparing a stock standard solution with 1.0 µg/mL each by dilution with deionized water. Prepare five working standard solutions for the calibration by adding the proper amount of stock standard solution and making dilutions with 25 mM ammonium acetate (pH 5.2, adjust with acetic acid).

Note: All glassware used for diquat and paraquat standards and in sample preparation should be soaked at least eight hours in a mixture of CH_3CN and DMCO (9:1, v/v) to avoid loss of diquat and paraquat.

SAMPLES

Tap water samples were collected at the Dionex Shanghai Applications Lab. Pond water samples were collected at Zhangjiang High-Science and Technology Park located in the Pudong District of Shanghai, China.

Add 0.77 g of ammonium acetate to 1 L of water sample, then adjust to pH 5.2 with acetic acid. Filter these samples through a 0.45 µm membrane (Millex-HN) prior to injection.

CONDITIONS

SPE Cartridge 1*:	Acclaim Mixed-Mode WAX-1 (guard), 5 µm, 4.6 × 10 mm (P/N 069704)
SPE Cartridge 2*:	Acclaim Mixed-Mode WCX-1 (guard), 5 µm, 4.6 × 10 mm (P/N 069705)
Analytical Column:	Acclaim Trinity P1, 3 µm, 3.0 × 50 mm (P/N 071388)
Column Temp.:	25 °C
Mobile Phase:	For on-line SPE: A: 250 mM ammonium acetate (pH 5.2, adjust with acetic acid) B: CH_3OH C: Water In gradient (Table 1) For separation: 500 mM ammonium sulfate–CH_3OH–water, 60:15:25, (v/v)
Valve Switching:	Table 1
Flow Rate:	0.7 mL/min for on-line SPE 0.6 mL/min for separation
Inj. Volume:	2500 µL on the on-line SPE cartridge 1
UV Detection:	Absorbance at 260 nm for paraquat and 311 nm for diquat

*Use the Acclaim Guard cartridge as the SPE cartridge, and use the V-2 Holder (P/N 069580).

RESULTS AND DISCUSSION

Column Selection

Diquat and paraquat are permanent cations.[23] This results in weak retention of diquat and paraquat on C18 or C8 stationary phases without using an ion-paring reagent in the mobile phase. The Acclaim Mixed-Mode HILIC-1 column has been reported for a baseline separation of diquat and paraquat.[13] The silica-based Acclaim Trinity P1 column—which provides multiple retention mechanisms including reversed-phase, anion-exchange, and cation-exchange[24]—has potential for the separation of diquat and paraquat. Therefore, these two columns were evaluated for use as the analytical column.

As for the selection of SPE cartridge, because diquat and paraquat are cations, the Trinity P1 cartridge and Mixed-Mode WCX-1 cartridges, with their cation-exchange and reversed-phase retention mechanisms, were evaluated for on-line SPE.

Table 1. Elution and Valve Switching for Target-Cut On-Line SPE and Separation

Time (min)	Left Pump (for SPE)				Right Pump (for separation)				Valve Switching
	Flow Rate (mL/min)	Solvent A Buffer (%)	Solvent B Methanol (%)	Solvent C Water (%)	Flow Rate (mL/min)	Solvent A Buffer (%)	Solvent B Methanol (%)	Solvent C Water (%)	Right
0.00	0.7	10	5	85	0.6	60	15	25	1-2
6.00		10	5	85					6-1
6.10		55	45	0					—
6.80		—	—	—					1-2
9.50		55	45	0					—
9.60		10	5	85					—
16.00		10	5	85					—

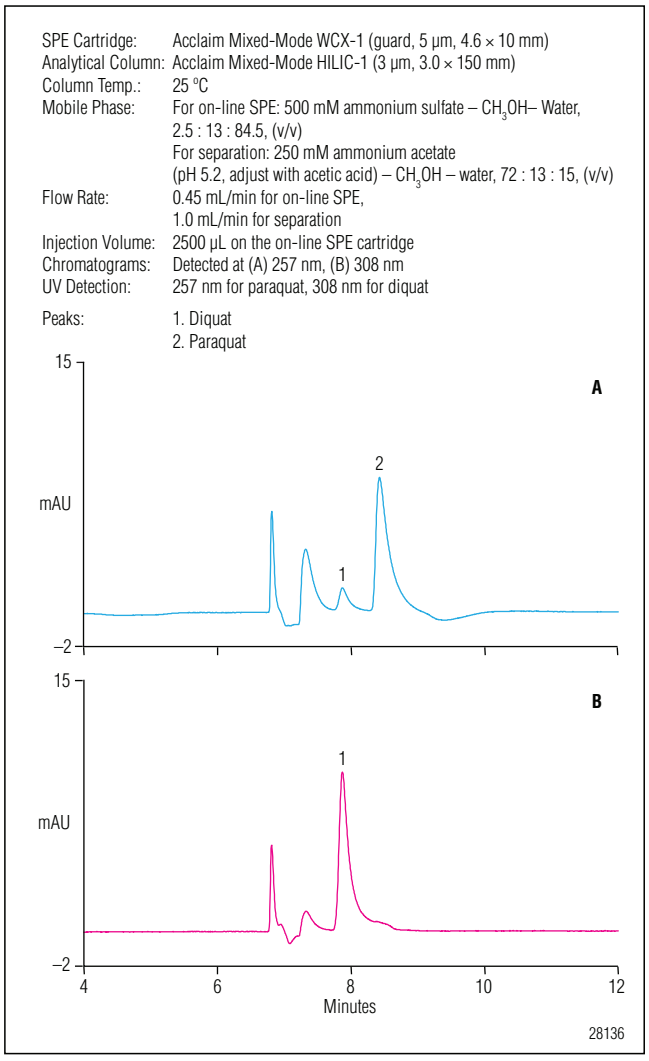


Figure 2. Chromatograms of a mixed diquat and paraquat standard detected at (A) 257 nm and (B) 308 nm using the traditional on-line SPE mode.

Figure 2 shows the chromatograms of diquat and paraquat standards using the Mixed-Mode WCX-1 as the SPE cartridge and the Mixed-Mode HILIC-1 as the analytical column. The flow schematic is shown in Figure 3A, which is a typical on-line SPE configuration. Although there was good separation of diquat and paraquat, both were subject to interferences even in the mixed diquat and paraquat standard.

Longer retention of diquat and paraquat would be beneficial to avoid interference from highly polar compounds. Experiments showed that the retention of diquat and paraquat on the Trinity P1 column was longer than that on the Mixed-Mode HILIC-1, which may reduce interferences from highly polar compounds. Therefore, a short Trinity P1 column was used as an on-line SPE cartridge and a longer Trinity P1 column was used as the analytical column.

Figure 4 shows the chromatograms of diquat and paraquat in a standard and in spiked tap and pond waters. Baseline separation and good peak asymmetry were observed when diquat and paraquat standards were injected (Figure 4A), but their determinations in real water samples were subject to interference (Figures 4B and C).

From the experiments shown in Figures 2 and 4, it was concluded that just using one SPE cartridge did not efficiently eliminate the interference caused by large concentrations of polar substances. Therefore, an Acclaim Mixed-Mode WAX-1 cartridge with anion-exchange and reversed-phase mechanisms was added following the autosampler (Figure 3B). This addition may retain anions and some non-polar substances to eliminate interferences.

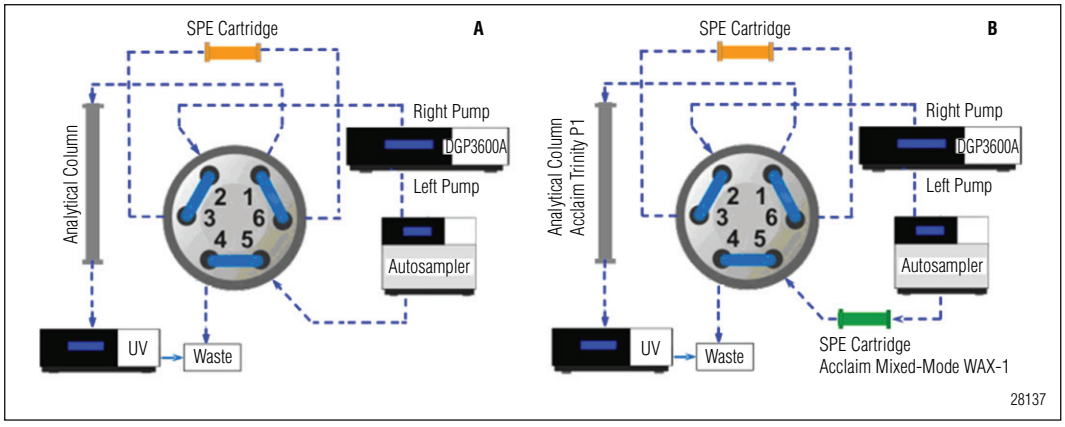


Figure 3. Flow schematic of on-line SPE in (A) traditional mode and (B) improved mode.

SPE Cartridge: Acclaim Trinity P1 (guard, 5 µm, 4.6 × 10 mm)
Analytical Column: Acclaim Trinity P1 (3 µm, 3.0 × 150 mm)
Column Temp.: 25 °C
Mobile Phase: For on-line SPE: 500 mM ammonium sulfate – CH_3OH– Water, 55 : 10 : 35, (v/v)
For separation: 250 mM ammonium acetate (pH 5.2, adjust with acetic acid) – CH_3OH – water, 60 : 40, (v/v)
Flow Rate: 0.6 mL/min for on-line SPE
Injection Volume: 2500 µL on the on-line SPE cartridge
UV Detection: 257 nm for paraquat, 308 nm for diquat
Panels: (A) Diquat and paraquat standards (20 µg/L each)
(B) Pond water spiked with diquat and paraquat standards (5 µg/L each)
(C) Tap water spiked with diquat and paraquat standards (5 µg/L each

Peaks: 1. Diquat
2. Paraquat

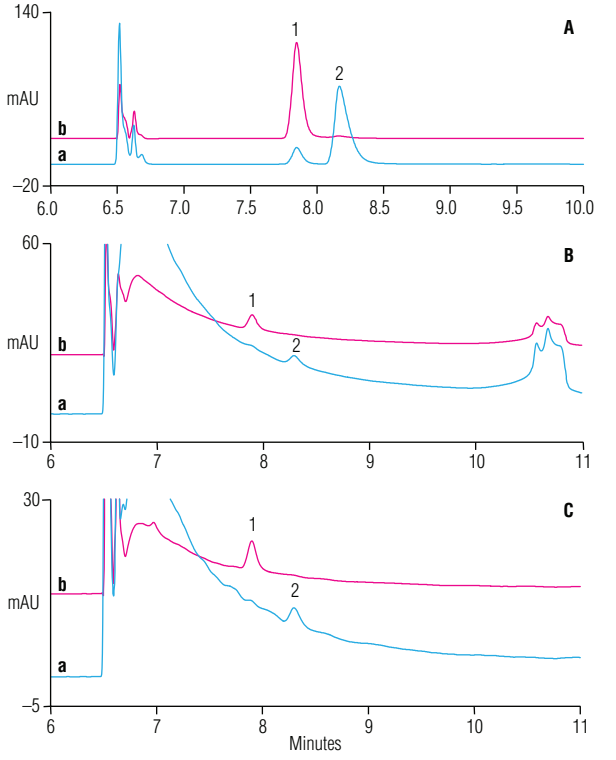


Figure 4. Chromatograms of (A) diquat and paraquat standards (20 µg/L each); (B) pond water and (C) tap water samples, both spiked with diquat and paraquat standards (5 µg/L each), using traditional on-line SPE mode. The (a) trace shows detection at 257 nm and the (b) trace at 308 nm.

Although the Trinity P1 cartridge and Mixed-Mode WCX-1 both can be used as SPE cartridges for the enrichment of diquat and paraquat, the latter was selected due to the observation that less co-enriched compounds enable easier elution of diquat and paraquat.

For the separation column, the Trinity P1 column was selected due to its longer retention for diquat and paraquat. As shown in Figure 5, using the improved on-line SPE mode (Figure 3B), baseline separation with good peak symmetry was observed not only when diquat and paraquat standards were injected (Figure 5A) but also in

SPE Cartridge 1: Acclaim Mixed-Mode WAX-1 (guard), 5 µm, 4.6 × 10 mm
SPE Cartridge 2: Acclaim Mixed-Mode WCX-1 (guard), 5 µm, 4.6 × 10 mm
Anal. Column: Acclaim Trinity P1, 3 µm, 3.0 × 50 mm
Mobile Phase: For on-line SPE,
A: 250 mM CH_3COONH_4 (pH 5.2)
B: MeOH
C: Water
Gradient: 0.0 – 6.0 min, A: 10%, B 5%; 6.1 – 9.5 min, A: 55%, B: 45%; 9.6 – 16 min, A: 10%, B: 5%
For separation, 500 mM $(NH_4)_2SO_4$ – CH_3OH –Water, 60 : 15 : 25, (v/v)
Column Temp.: 25 °C
Flow Rate: 0.7 mL/min for on-line SPE; 0.6 mL/min for separation
Inj. Volume: 2500 µL onto SPE cartridge 1
Detection: At 260 nm for paraquat, 311 nm for diquat
Panels: (A) Diquat and paraquat standards (2 µg/L each)
(B) Tap water spiked with diquat and paraquat standards (2.5 µg/L each)
(C) Pond water spiked with diquat and paraquat standards (2.5 µg/L each)

Peaks: 1. Diquat
2. Paraquat

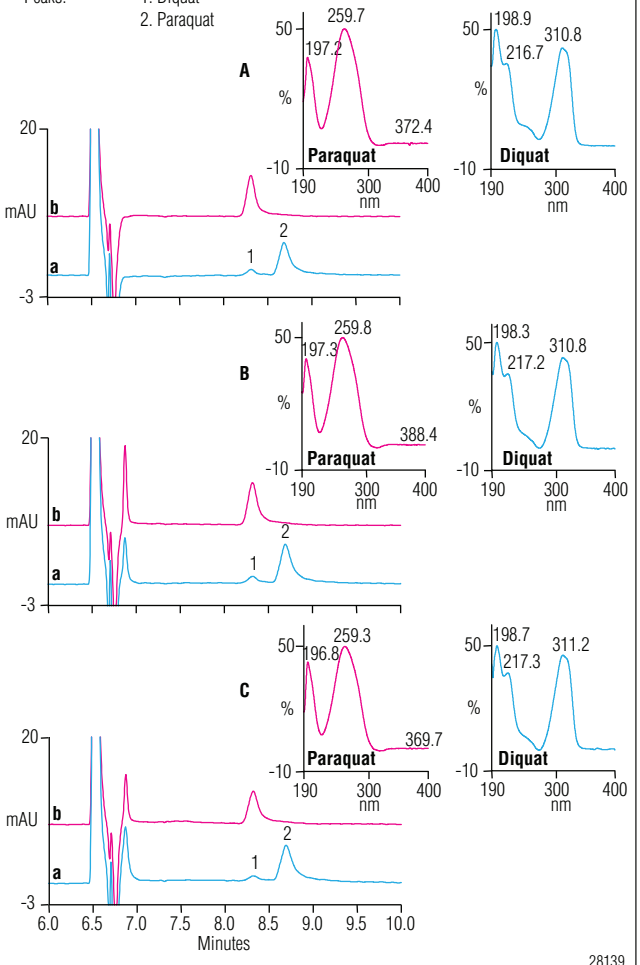


Figure 5. Chromatograms of (A) diquat and paraquat standards (2 µg/L each); (B) tap water and (C) pond water samples, both spiked with diquat and paraquat standards (2.5 µg/L each), using the improved on-line SPE mode. The (a) trace shows detection at 260 nm and the (b) trace at 311 nm.

the analysis of tap and pond water samples (Figures 5B and C). This demonstrated an efficient and simple on-line SPE HPLC method for the determination of diquat and paraquat in real water samples.

Configuration of the Improved On-Line SPE Method

As shown in the flow schematic in Figure 3B, the filtered sample was injected directly onto the system and delivered to SPE cartridge 1 (Mixed-Mode WAX-1) (1-2 position of the valve) using the left pump. This was for the elimination of anionic interferences. The cationic compounds—including diquat and paraquat—passed through, while anionic compounds and some non-polar interferences were retained. The compounds that passed through SPE cartridge 1 were delivered to SPE cartridge 2 (Mixed-Mode WCX-1) for enrichment of diquat and paraquat.

The analytical column was simultaneously equilibrated using the right pump. After the analytes were bound to SPE cartridge 2, the cartridge switched into the analytical flow path (6-1 position of the valve), and the enriched diquat and paraquat were separated on the analytical Acclaim Trinity P1 column.

The SPE cartridge 1 was simultaneously eluted in a gradient using the left pump to send the retained interferences to waste. After diquat and paraquat were completely eluted from SPE cartridge 2, the SPE cartridge 2 switched out of the analytical flow path and back to the SPE flow path (1-2 position of the valve), and those cationic compounds that were still retained were eluted to waste. Afterwards, both SPE cartridges 1 and 2 were re-equilibrated for the next injection.

Method Reproducibility, Linearity, and Detection Limits

Method reproducibility was estimated by making nine consecutive 2500 µL injections of a pond water sample spiked with a 2.5 µg/L of diquat and paraquat standard. Retention time and peak area reproducibilities are summarized in Table 2 and show good precision.

Calibration linearity for diquat and paraquat was investigated by making three consecutive injections of a mixed standard prepared at five different concentrations. The external standard method was used to establish the calibration curve and to quantify these herbicides in samples. Excellent linearity was observed from 1.0 to 20 µg/L when plotting concentration vs peak area.

Detection limits were calculated using the equation:

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

Where S represents Standard Deviation (SD) of replicate analyses, n represents number of replicates, and $t_{(n-1,\ 1-\alpha=0.99)}$ represents Student's value for the 99% confidence level with n – 1 degrees of freedom. Method detection limits (MDL) were estimated using six consecutive injections of a drinking water sample spiked with 2.5 µg/L of each diquat and paraquat standard to determine S. Table 3 summarizes the method linearity and MDL data, which show excellent method linearity and sensitivity with detection limits well below those defined in the EPA method.

Table 2. Reproducibility of Peak Retention Time and Area

Analyte	Retention Time RSD	Peak Area RSD	Concentration of Standard (µg/L)
Diquat	0.020	2.41	2.5
Paraquat	0.024	3.70	2.5

Table 3. Method Linearity Data and Method Detection Limits (MDL)

Analyte	Regression Equation	r^2	Concentration Range of Standards (µg/L)	MDL, µg/L	
				Current Data	Data reported in EPA Method 549.2
Diquat	$A = 0.3582\ c - 0.0195$	0.9997	1.0–20.0	0.10	0.72
Paraquat	$A = 0.4755\ c - 0.2741$	0.9989		0.12	0.68

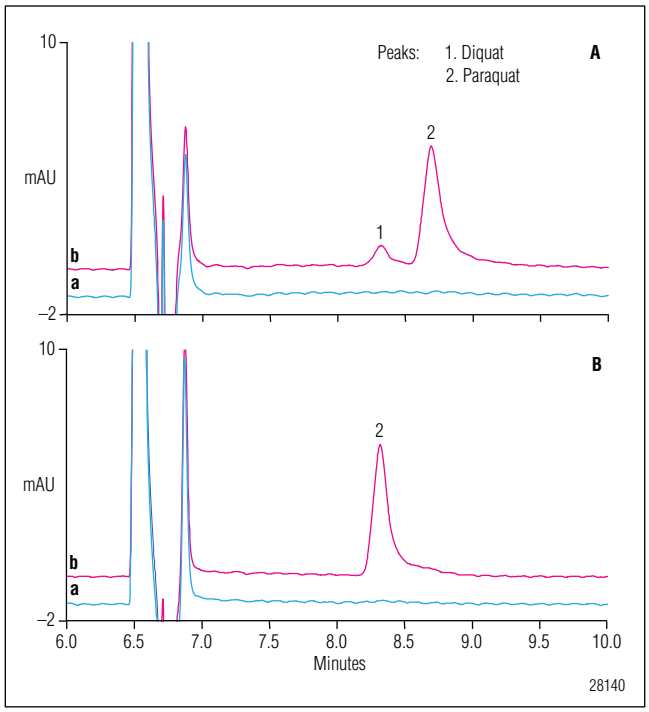


Figure 6. Determination of diquat and paraquat in tap water using the improved on-line SPE mode. Chromatograms: (a) tap water and (b) the same sample spiked with diquat and paraquat standards (2.5 µg/L each) with Panel A showing detection at 260 nm and Panel B at 311 nm. Other conditions are the same as in Figure 5.

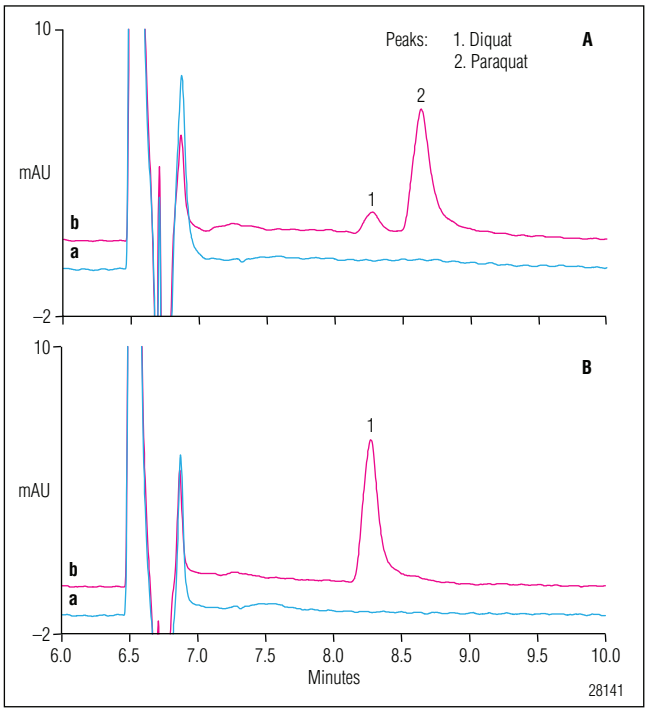


Figure 7. Determination of diquat and paraquat in pond water using the improved on-line SPE mode. Chromatograms: (a) pond water and (b) the same sample spiked with diquat and paraquat standards (2.5 µg/L each) with Panel A showing detection at 260 nm and Panel B at 311 nm. Other conditions are the same as in Figure 5.

Table 4. Analysis Results of Diquat and Paraquat in Water Samples

Sample	Tap Water				Lake Water			
Analyte	**Detected (µg/L)**	**Added (µg/L)**	**Found (µg/L)**	**Recovery (%)**	**Detected (µg/L)**	**Added (µg/L)**	**Found (µg/L)**	**Recovery (%)**
Diquat	ND	2.5	2.45	98	ND	2.5	2.40	96
Paraquat	ND	2.5	2.40	96	ND	2.5	2.36	94

Sample Analysis

Chromatograms of tap and pond water samples, as well as the same samples spiked with a diquat and paraquat standard (2.5 µg/L each), are shown in Figures 6 and 7; the related data are summarized in Table 4. None of the samples had detectable diquat and paraquat. Recoveries for each standard in both samples ranged from 94 to 98%, thus indicating that the analysis method is accurate.

CONCLUSION

This work describes an on-line SPE system using two SPE cartridges to eliminate anionic interferences and enrich diquat and paraquat. The elimination of interferences in tap and pond water is sufficient, and baseline separation of diquat and paraquat on the Acclaim Trinity P1 column is achieved.

Efficient and sensitive analyses are achieved with the UltiMate 3000 Dual HPLC with on-line SPE configuration controlled by Chromeleon CDS software. The determination of diquat and paraquat in tap and pond water is simple, rapid, and sensitive.

REFERENCES

1. *Basic Information about Regulated Drinking Water Contaminants;* U.S. Environmental Protection Agency: Cincinnati, OH, 1997. http://water.epa.gov/drink/contaminants/basicinformation/diquat.cfm.
2. Quality of Water Intended for Human Consumption, L 330/42, *Official Journal of the European Council Directive 98/83/EC (En),* European Communities, November 1998.
3. *Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High-Performance Liquid Chromatography with Ultraviolet Detection;* U.S. EPA Method 549.2, Revision 1.0; U.S. Environmental Protection Agency: Cincinnati, OH, 1997.
4. Waters Corporation, *Paraquat/Diquat.* Waters Column, Applications Notes, 1996, *4* (1), Milford, MA.
5. Agilent Technologies, *Analysis of Paraquat and Diquat by HPLC.* Publication Number 5966-1875E, 1997, Palo Alto, CA.
6. Hara, S.; Saski, N.; Takasa, D.; Shiotsuka, S.; Ogata, K.; Futagami, K.; Tamura, K. Rapid and Sensitive HPLC Method for the Simultaneous Determination of Paraquat and Diquat in Human Serum. *Anal. Sci.* **2007,** *23,* 523.
7. Lee, X.P.; Kumazawa, T.; Fujishiro, M.; Hasegawa, C.; Arinobu, T.; Seno, H.; Ishii, A.; Sato, K. Determination of Paraquat and Diquat in Human Body Fluids by High-Performance Liquid Chromatography/Tandem Mass Spectrometry. *J. Mass Spectrom.* **2004,** *39,* 1147.
8. Ito, M.; Hori, Y.; Fujisawa, M.; Oda, A.; Katsuyama, S.; Hirose, Y.; Yoshioka, T. Rapid Analysis Method for Paraquat and Diquat in the Serum Using Ion-Pair High-Performance Liquid Chromatography. *Biol. Pharm. Bull.* **2005,** *28,* 725.
9. Aramendía, M.A.; Borau, V.; Lafont, F.; Marinas, A.; Marinas, J.M.; Moreno, J.M.; Porras, J.M; Urbano, F.J. Determination of Diquat and Paraquat in Olive Oil by Ion-Pair Liquid Chromatography–Electrospray Ionization Mass Spectrometry (MRM). F*ood Chem.* **2006,** *97,* 181.
10. Lee, H.S.; Kim, K.; Kim, J.H.; Do, K.S.; Lee, S.K. On-Line Sample Preparation of Paraquat in Human Serum Samples Using High-Performance Liquid Chromatography with Column Switching. *J. Chromatogr., B* **1998,** *716,* 371.
11. Castro, R.; Moyano, E.; Galceran, M. T. On-Line Ion-Pair Solid-Phase Extraction–Liquid Chromatography–Mass Spectrometry for the Analysis of Quaternary Ammonium Herbicides. *J. Chromatogr., A* **2000,** *869,* 441.
12. Waters Corporation, *Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection (EPA Method 549.2).* 2008, Milford, MA.
13. Dionex Corporation, *Improved Separation of Diquat and Paraquat Using the Acclaim Mixed-Mode HILIC-1 Column.* Application Brief 114, LPN 2577, 2010, Sunnyvale, CA.
14. Raquel, R.O.; Beatriz, C.G.; Concepcion, P.L.; Jesús, S.G.; Manuel, A.E. Simultaneous Determination of the Herbicides Diquat and Paraquat in Water. *J. Chromatogr. Sci.* **2006,** *44,* 539.
15. Tuzimski, T. Determination of Pesticides in Water Samples from the Wieprz-Krzna Canal in the Leczynsko-Wlodawskie Lake District of Southeastern Poland by Thin-Layer Chromatography with Diode Array Scanning and High-Performance Column Liquid Chromatography with Diode Array Detection. *J. AOAC Int.* **2008,** *91,* 1203.
16. Taguchi, V.Y.; Jenkins, S.W.D.; Crozie, P.W.; Wang, D.T. Determination of Diquat and Paraquat in Water by Liquid Chromatography-(Electrospray Ionization) Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **1998,** *9,* 830.
17. Dionex Corporation, *Determination of Phenols in Drinking and Bottled Mineral Waters Using Online Solid-Phase Extraction Followed by HPLC with UV Detection.* Application Note 191, LPN 1949, 2007, Sunnyvale, CA.

18. Dionex Corporation, *Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Edible Oils by Donor-Acceptor Complex Chromatography (DACC)-HPLC with Fluorescence Detection.* Application Note 196, LPN 1998, 2008, Sunnyvale, CA.
19. Dionex Corporation, *Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Tap Water Using On-Line Solid-Phase Extraction Followed by HPLC with UV and Fluorescence Detections.* Application Note 213, LPN 2126, 2009, Sunnyvale, CA.
20. Ibáñez, M.; Picó, Y.; Mañes, J. On-Line Liquid Chromatographic Trace Enrichment and High-Performance Liquid Chromatographic Determination of Diquat, Paraquat and Difenzoquat in Water. *J. Chromatogr., A* **1996,** *728,* 325.
21. Ren, J.; Huang, C.L.; Zhao, G.D.; Jiang, K. Determination of Herbicides in Drinking Water by Solid Phase Extraction-Liquid Chromatography-Mass Spectrometry. *Chin. Anal. Chem.* **2001,** *29,* 876
22. Chen, Z.L.; Megharaj, M.; Naidu, R. On-Line Solid Phase Extraction of Pesticide Residues in Natural Water, Coupled with Liquid Chromatography and UV Detection, Using Various Sorbents. *J. Liquid Chromatogr. & Rel. Tech.* **2002,** *25,* 1779. http://www.informaworld.com/smpp/title~db=all~content=t713597273~tab=issueslist~branches=25 - v25.
23. Li, H.M.; Sun, S.W.; Shi, X.F. Analysis of Diquat and Paraquat by High Performance Liquid Chromatography. *Chin. J. Anal. Chem.* **2007,** *35,* 1499.
24. Dionex Corporation, *Acclaim Trinity P1 LC Column,* Sunnyvale, CA. http://www.dionex.com/en-us/products/columns/lc/specialty/acclaim-trinity/lp-81754.html (accessed Jan 15, 2011).

Quantitative Analysis of Nitrogen Mustard Hydrolysis Products as Ethanolamines

INTRODUCTION

Ethanolamines have been used as bio- and environmental markers for nitrogen mustards (HN1, HN2, and HN3), which are listed on the Chemical Weapons Convention Schedule of Chemicals[1] to monitor potential exposures. Direct quantification of exposure to HN1, HN2, and HN3 is difficult due to their reactivity, extent of metabolism, and short half-life.[2] Nitrogen mustards readily react with biomolecules and are found in urine as the hydrolysis products: *N*-methyldiethanolamine (MDEA), *N*-ethyldiethanolamine (EDEA), and triethanolamine (TEA).[3]

Over half a million tons of ethanolamines are produced annually and used as emulsifying agents, detergents, ingredients in bactericides and cosmetics, and also in the pesticide manufacturing process.[4] Inefficient removal and/or inappropriate disposal of ethanolamimes may cause adverse effects to the environment.

To monitor human and environmental exposure to nitrogen mustard, and also the removal of ethanolamines from industrial discharged waste, a quantitative analytical method is desired.

Reported methods for ethanolamines analysis include GC or LC separation with MS detection.[8] The GC-MS methods involve labor-intensive derivatization which limits throughput, and reported LC methods usually suffer from poor retention and chromatographic separation with reversed-phase (RP) columns. A fast LC-MS/MS method reported the total separation of MDEA, EDEA, and TEA.[6] However, the estimated retention factor (k) for the first-eluted TEA was less than one, making the method subject to possible interference from sample matrices, which was confirmed in the same report.

This study reports a rapid separation liquid chromatography (RSLC) tandem mass spectrometric (MS/MS) method for quantitative analysis of ethanolamines in environmental water samples. An Acclaim® Trinity™ P1 Mixed-Mode column featuring reversed-phase, anion-exchange, and cation-exchange retention mechanisms was used to provide retention and resolution for all analytes within 5 min. The MS detector was operated in multiple reaction monitoring (MRM) mode, and an isotope labeled internal standard (IStd) was used to provide selective and sensitive detection and to ensure quantification accuracy.

EQUIPMENT

Dionex UltiMate® 3000 RSLC system including:

- DGP-3600RS dual gradient pump
- WPS-3000TRS autosampler
- TCC-3200RS column oven

CONDITIONS

Column: Acclaim Trinity P1 (2.1 × 100 mm, 3 µm, P/N 071389)

Mobile Phase: Isocratic, 90% Acetonitrile; 5% DI water; 5% Ammonium formate 100 mM, pH 3.7

Flow Rate: 0.6 mL/min

Temperature: 20 °C

Inj. Volume: 20 µL

MASS SPECTROMETRIC CONDITIONS

System: AB SCIEX 4000 QTRAP® mass spectrometer

Interface: TurboV™ source with ESI

Curtain Gas (CUR): 15 psi

Collision Gas (CAD): Medium

IonSpray Voltage (IS): 4500 V

Temperature (TEM): 700 °C

Ion Source Gas 1 (GS1): 50 psi

Ion Source Gas 2 (GS2): 20 psi

Ihe: On

Acquisition Mode: Multiple reaction monitoring (MRM); refer to Table 1 for details on MRM scan parameters

Software: Analyst® 1.5 with DCMS^{Link}™ 2.7.0 for Analyst. DCMS^{Link} is a Chromeleon®-based software module providing the interface for controlling a wide range of Dionex chromatography instruments from different mass spectrometer software platforms.

Table 1. MRM Scan Parameters of Studied Analytes

Peak No.	Analyte	ID	Q1 (*m/z*)	Q3 (*m/z*)	Time (ms)	DP (V)	CE (V)	CXP (V)	t_R (min)
1	*N*-ethyldiethanolamine	EDEA-1	134.1	116.0	75	51	21	8	1.8
		EDEA-2	134.1	72.0	25	51	25	4	
2	*N*-methyldiethanolamine	MDEA-1	120.1	102.0	75	46	19	8	2.3
		MDEA-2	120.1	58.0	25	46	27	4	
3	Triethanolamine	TEA-1	150.0	132.0	75	61	19	10	2.7
		TEA-2	150.0	88.0	25	61	23	6	
4	Diethanolamine	DEA-1	106.1	88.0	350	66	19	6	3.6
		DEA-2	106.1	70.0	50	66	21	4	
5	Diethanolamine-d_8	DEA-IS	114.1	78.0	100	53	24	6	3.6
6	Ethanolamine	EA-1	62.0	44.1	350	46	15	6	4.0
		EA-2	62.0	45.0	50	46	19	6	

The 1st MRM of each analyte was used for quantiation, and the 2nd MRM was used for confirmation only.

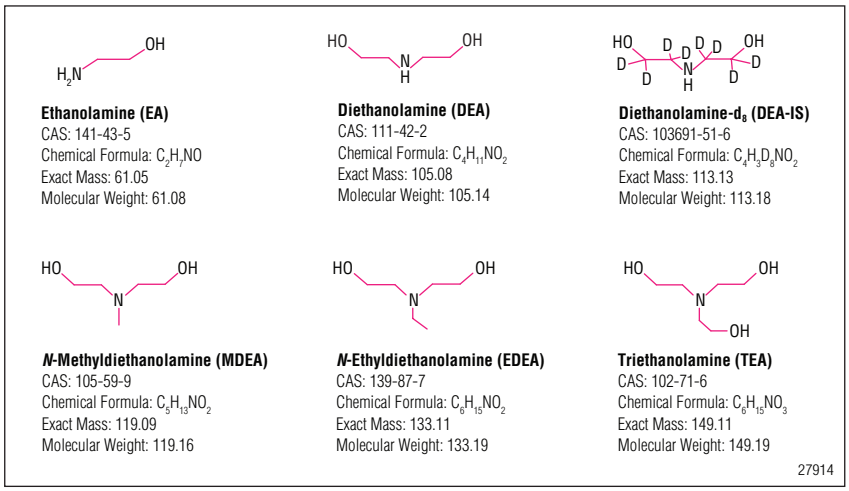


Figure 1. Chemical structures of studied compounds.

PREPARATION OF SOLUTIONS AND REAGENTS

Chemical and Reagents

Standards of studied analytes were purchased from Sigma-Aldrich: ethanolamine (EA, CAS: 141-43-5, Aldrich: 411000), diethanolamine (DEA, CAS: 111-42-2, Fluka: 31589), *N*-methyldiethanolamine (MDEA, CAS: 105-59-9, Aldrich: 471828), *N*-ethyldiethanolamine (EDEA, CAS: 139-87-7 Aldrich: 112062), triethanolamine (TEA, CAS: 102-71-6, Fluka: 90279). Isotope labeled internal standard (IStd) diethanolamine-d_8 (DEA-IS) was purchased from C/D/N Isotopes (CAS: 103691-51-6, D-5308). Figure 1 shows the chemical structures and related information.

Ammonium formate was purchased from Aldrich (516961). Acetonitrile was obtained from Burdick & Jackson (HPLC grade, AH015-4). Deionized water (18.2 MΩ-cm resistance) used in this study was obtained from a Millipore water station.

All chemicals were dissolved in deionized (DI) water to prepare individual primary stock solutions at 1000 µg/mL (ppm). Working stock solutions were prepared for each analyte by diluting primary stock solutions in DI water to 1 ppm, 100 ppb, 10 ppb, and 1 ppb to prepare calibration standards. A working stock solution for the internal standard was prepared at 100 ppb in deionized water for the preparation of calibration standards and to spike unknown samples. Calibration standards were prepared in DI water at 8 levels: 0.05 ppb, 0.1 ppb, 0.5 ppb, 1 ppb, 2 ppb, 5 ppb, 10 ppb, and 20 ppb. Each level contains all five target analytes with internal standard spiked at 1 ppb.

Sample Preparation

Surface water samples were collected in HDPE plastic bottles and stored under refrigeration at 4 °C until analysis. An aliquot of each water sample was spiked with internal standard at 1 ppb in a 1.5 mL autosampler vial and analyzed directly (filter the surface water samples when necessary, e.g., if suspended particles are observed).

RESULTS AND DISCUSSION

Chromatography

As shown in Figure 2, all five target analytes were separated to baseline within 4.5 min. A retention factor (k') of 3.3 for the first-eluted EDEA indicated sufficient retention for all analytes and thus ensured the separation of targeted analytes from early eluting species. Different from general RP columns, the Acclaim Trinity P1 Mixed-Mode column features RP and ion exchange mechanisms, thus providing unique selectivity for ionizable organics. For the mixed-mode column, eluent strength is affected by organic modifier composition, buffer type, buffer pH, and buffer concentration. Refer to the column manual for more information on method development and modification. The conditions described in the experimental section were developed to achieve sufficient retention and total resolution for all target analytes with consideration of method throughput. Although the separation was completed within 4.5 min, the total run time was set at 8 min to elute any possible strongly retained species and thus improve method ruggedness.

Mass Spectrometry

The aim of this study was to develop a selective and sensitive method for the direct analysis of trace level ethanolamines in environmental water samples, therefore, MS/MS instrumentation was selected for its sensitivity and ability to provide trace level detection. In addition, the selectivity of MS/MS instrumentation allows minimal sample preparation and cleanups. The MS/MS instrument was tuned and run in MRM mode. With continuous infusing of individual standards, each target analyte showed a strong protonated molecular ion $[M+H]^+$ in positive ESI mode, and was used as the Q1MS precursor ion for MRM experiments. Product ions were selected using the Compound Optimization option from the instrument operating software. The three most intense MRM transitions were selected as MRM candidates for further selectivity evaluation. The MRM selectivity was evaluated by analyzing individual standards with respect to chromatographic separation and MS/MS detection with MRM candidate transitions.

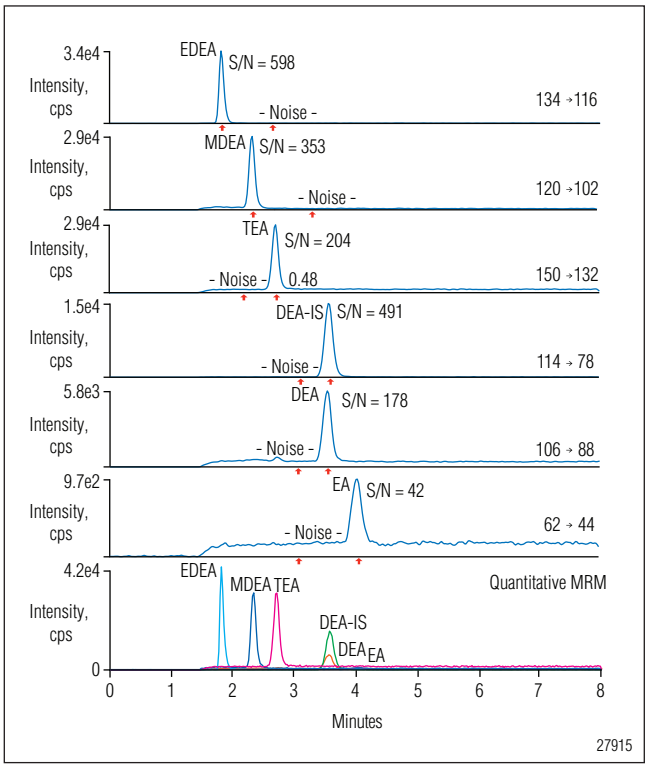


Figure 2. MRM chromatograms of five ethanolamines by RSLC-MS/MS on an Acclaim Trinity P1 column with 0.5 ppb of each analyte.

The two final MRM transitions were selected that showed specific MS peaks with better intensity. It is worth noting that interference was observed for both DEA MRM channels from TEA and EDEA; and this can be explained by the source region fragmentation of TEA and EDEA: $[M\text{-}C_2H_3OH+H]^+$, and $[M\text{-}C_2H_4+H]^+$, respectively, which have the identical *m/z* as the precursor ion of DEA at 106 *m/z*. This observation also indicated that chromatographic separation for EDEA, TEA, and DEA are crucial for quantification accuracy. The scan time for MRM scans was optimized to focus on quantitative MRMs and MRMs with less intensity in order to achieve better signal-to-noise ratio (S/N) for those mentioned MRMs, thus providing balanced overall method performance. The detailed MRM scan parameters are listed in Table 1. The TurboV with ESI ionization source parameters were optimized by a series of runs with varying parameter settings, and the optimum settings are listed in the experimental section.

Method Performance

As mentioned earlier, the selectivity for this method was evaluated by observing the specific MRM peaks at the specific retention times for each analyte. Although interference was observed in both MRM channels for DEA, ($[M\text{-}C_2H_3OH+H]^+$ from TEA, and $[M\text{-}C_2H_4+H]^+$ from EDEA), as seen in Figure 3, these interference peaks were chromatographically separated and thus did not affect the method selectivity for the accurate quantification of DEA. Carryover was evaluated by injecting a reagent blank (DI water) after the highest calibration standard at 20 ppb. No quantifiable peak was observed at the specific retention time for each analyte thus indicating no observed carryover for this method.

Linearity was evaluated and calibration curves were generated with duplicate assays of eight calibration standards from 0.05 ppb to 20 ppb using isotope labeled DEA-d_8 as the internal standard. Linear regression was used to fit all experimental data with 1/x weighting factor. Excellent linearity was achieved from 0.05 ppb (except EA from 0.2 ppb) to 20 ppb with correlation of determination (r) greater than 0.999 for each analyte.

Figure 4 shows the calibration curve for DEA as an example. Run-to-run precision and accuracy was evaluated by seven replicate assays of the 0.5 ppb standard and measured by RSD and %Accuracy (calculated by Observed Amount/Specified Amount × 100%). Method detection limit (MDL) was statistically calculated for each analyte using the standard deviation obtained from the seven replicate analysis of a 0.5 ppb standard following this equation: MDL = s × *t* where s is the standard deviation and *t* is the Student's *t* at 99% confidence interval. Excellent precision was observed with RSDs ranging from 3.26% (MDEA) to 5.49% (TEA). The calculated MDL ranged from 0.050 ppb (MDEA) to 0.092 ppb (TEA).

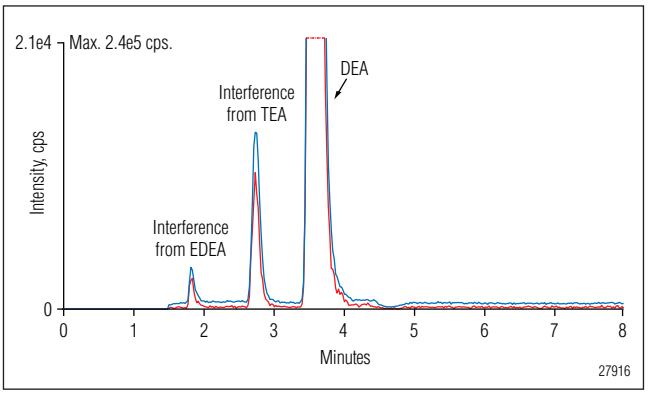


Figure 3. Chromatographically separated MRM interferences for DEA.

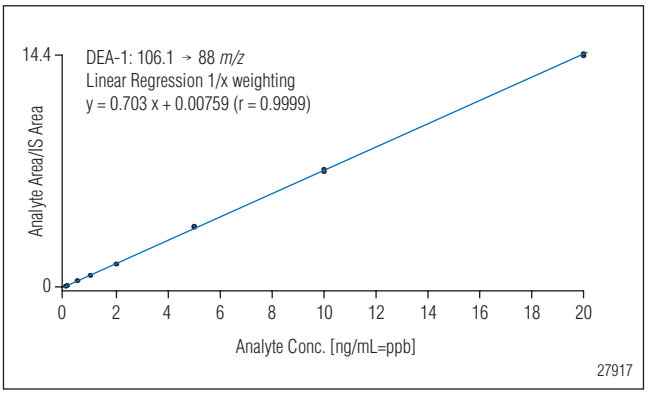


Figure 4. Calibration curve of DEA (106.1→88.0 m/z*) from 0.05 to 20 ppb using isotope labeled DEA-d8 as internal standard.*

Table 2. Calibration, RSD, Detection, and Reporting Limits									
Analytes	**Calibration**	**r**	**Mean[a]**	**Accuracy[a] (%)**	**RSD[a]**	**MDL[a]**	**LLOQ (ppb)**	**S/N at LLOQ**	**LRL[b]**
EDEA	y = 4.64x + 0.00242	0.9993	0.463	97.57	3.58	0.052	0.05	106.8	0.052
MDEA	y = 4.73x - 0.0109	0.9996	0.484	96.77	3.26	0.050	0.05	54.5	0.050
TEA	y = 4.22x + 0.169	0.9994	0.530	106.0	5.49	0.092	0.05	26	0.092
DEA	y = 0.703x + 0.00759	0.9999	0.508	101.5	3.85	0.061	0.05	24.9	0.061
EA	y = 0.217x - 0.00756	0.9990	0.510	101.9	5.34	0.085	0.20	13.1	0.20

All concentrations were in the unit of ppb.

[a] Calculated based on seven replicate assays of a standard at 0.5 ppb.

[b] LRL, Lowest reporting limit: the lowest concentration can be reported by this method, and is the higher concentration between MDL and LLOQ.

The lower limit of quantification (LLOQ) was determined as the lowest calibration standard consistently showing S/N greater than 10. The LLOQ for all analytes were reported as 0.05 ppb, except EA, which was 0.2 ppb. Figure 5 shows the MRM chromatograms of each analyte at LLOQ. The lowest reporting limit (LRL) for each analyte is the lowest concentration that can be reported by this method and was determined as the higher concentration between MDL and LLOQ. The results for method performance evaluations are summarized in Table 2.

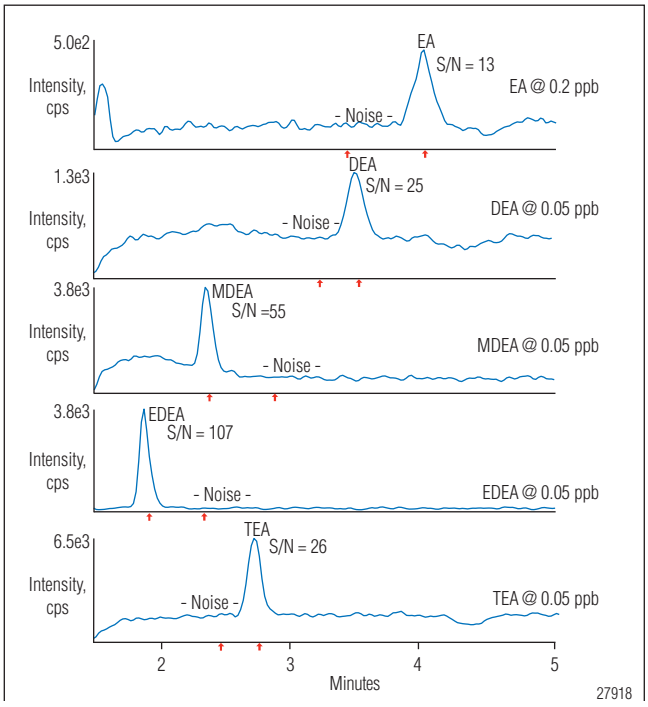


Figure 5. MRM chromatograms of five ethanolamines at LLOQ.

Analysis of Water Samples

This method was used to analyze local municipal water samples, a local creek water sample, and a Nevada lake water sample. Following the procedures in the Experimental section, these samples were spiked with the internal standard and analyzed directly. None of the tested samples showed concentrations of target analytes above the lowest reporting limit, and thus the local creek water and the Nevada lake water were used as blank matrices to evaluate the method recovery. Each matrix was spiked with ethanolamines at three levels: 0.5 ppb, 5 ppb, and 20 ppb with three replicates at each level, and the internal standard was spiked at 1 ppb.

Table 3. Recoveries of Ethanolamines in Two Water Matrices												
Analyte	Matrix A: Local Creek Water						Matrix B: Nevada Lake Water					
	0.5 ppb		5 ppb		20 ppb		0.5 ppb		5 ppb		20 ppb	
	% RVY[a]	% RSD	% RVY[a]	% RSD	% RVY[a]	% RSD	% RVY[a]	% RSD	% RVY[a]	% RSD	% RVY[a]	% RSD
EDEA	136.0	0.78	135.6	1.81	131.3	1.34	133.8	1.76	123.9	1.03	118.3	1.71
MDEA	145.9	1.87	137.2	1.83	142.2	2.34	140.7	0.70	127.5	1.97	127.8	2.01
TEA	83.9	2.08	86.8	1.83	95.0	1.58	79.3	4.92	85.4	0.81	93.7	0.82
DEA	98.5	1.54	101.8	0.52	100.2	0.29	103.3	3.89	100.5	0.83	101.8	0.28
EA	37.8	1.83	61.9	1.66	70.0	0.71	N/A	N/A	20.2	8.65	22.8	1.08

[a]Recovery, mean of three replicate recoveries calculated by Observed Amount /Specified Amount × 100%.

As summarized in Table 3, consistent recoveries were observed for most analytes at different levels in two different matrices. However, differences in concentration and matrices showed significant effects on the recovery of EA: 37.8% for 0.5 ppb vs 61.9% for 5 ppb and 70.0% for 20 ppb in Matrix A; not detected for 0.5 ppb vs 20.2% for 5 ppb and 22.8% for 20 ppb in Matrix B.

Higher recovery was observed for samples spiked at higher levels, and prepared in Matrix A. It is worth noting that the recovery for DEA was observed near 100%, indicating the benefit of using isotope labeled analogues as an internal standard correcting the matrix effect on that specific analyte. The deviation of recoveries from 100% indicates the different extents of matrix effects on each analyte, i.e., significant relative signal enhancement for EDEA and MDEA.

It was also noticed that EA exhibited short-term instability, although the samples were placed in the thermally controlled autosampler at 10 °C and sheltered from light. The duplicate assays of a batch of samples run on the following day of the sample preparation showed no detectable EA, suggesting immediate analysis after sample preparation is required. The stability of prepared sample in target matrices should also be evaluated to avoid degradation.

CONCLUSION

An RSLC-MS/MS method for quantitative analysis of five ethanolamines was developed and described. By using a mixed-mode analytical column and selective MRM MS/MS detection, this method showed significant improvements over previously reported methods with minimum sample preparation, total chromatographic resolution, capability of sub-ppb level quantification, and high throughput. Application of this method to the analysis of surface waters was demonstrated and showed no quantifiable amounts above the LRLs. Matrix effects and recovery were evaluated using two surface water matrices and the results indicated better quantitation accuracy for DEA by using an isotope labeled analogue as an internal standard.

REFERENCES

1. Conference on Disarmament. The Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction. United Nations Document CD/1170, Geneva, Switzerland, 1992.
2. Black, R.M.; Read, R.W., *J. Chromatogr., A* **1998**, *449*, 261–270.
3. Opresko, D.M.; Young, R.A.; Faust, R.A.; Talmage, S.S.; Watson, A.P.; Ross, R.H.; Davidson, K.A.; King, J., In Reviews of Environmental Contamination and Toxicology, Vol. 156, G.W. Ware, Ed. Springer, New York, NY, 1998, 127–133.
4. Edser, C., *Focus on Surfactants* **2004**, *7,* 1–2.
5. Ohsawa, I.; Seto, Y.; *J. Chromatogr., A* **2006**, *1122*, 242–248.
6. Lemire, S.W.; Barr, J.R.; Ashley, D.L.; Olson, C.; Hayes, Y., *J. Anal. Toxicol*. **2004**, *28,* 320–326.
7. Campo, P.; Suidan, M.; Chai, Y.; Davis, J. *Talanta* **2010***, 80*, 1110–1115.
8. Peru, K.; Headley, J.; Doucette, W.; *Rapid Commun. Mass Spectrom.* **2004**, *18,* 1629–1634.
9. Dionex Document No. 065306-01, *Product Manual for Acclaim Trinity P1 Columns,* available online at http://www.dionex.com/en-us/webdocs/75675-Man-065306-01-ACCLAIM-TRINITY-P1-Feb09.pdf

Sensitive and Fast Determination of Endothall in Water Samples by IC-MS/MS

INTRODUCTION

Endothall is a widely used herbicide for both terrestrial and aquatic weeds. Major uses of endothall include defoliation of cotton, the control of aquatic weeds and algae, and as a dessicating agent for lucerne and potatoes. Human exposure to endothall in excess of the maximum contamination level (MCL) may cause gastrointestinal problems. Endothall is regulated by the United States Environmental Protection Agency (EPA) with an MCL at 0.1 mg/L or 100 ppb for drinking water;[1] and the California EPA developed the Public Health Goal (PHG) of 0.58 mg/L or 580 ppb for this compound.[2]

Current analytical methods for quantitation of endothall in water samples are described in EPA method 548.1[3] as gas chromatography with mass spectrometry or flame ionization detection (GC-MS or GC-FID). These methods involve ion-exchange solid phase extraction, sample enrichment, and dimethyl ester derivatization, followed by a 20 min GC separation and MS or FID detection.

This study describes the direct analysis of trace-level endothall in water samples by ion chromatography mass spectrometry (IC-MS). Water samples were directly injected without labor-intensive sample preparation and chromatographic separation was achieved in 10 min, thus significantly improving method throughput. An MS/MS instrument was operated in selected reaction monitoring (SRM) mode requiring minimal sample cleanup and ensuring highly sensitive (low ppb) and selective quantitation. Isotope labeled glutaric acid (glutaric acid–d_6) was used as an internal standard to ensure quantitation accuracy. This method has been successfully used for quantification of endothall in various water matrices including fresh creek water, lake water, and high salt content lake water. Method performance parameters such as linearity, calibration range, precision, accuracy, detection limits, and recovery were evaluated and will be presented in this application note.

EQUIPMENT

Dionex ICS-2000 or ICS-2100 Reagent-Free™ Ion Chromatography (RFIC™) system

TSQ Quantum Access™ triple quadrupole mass spectrometer

XCalibur® 2.1 with integrated Dionex Chromatography Mass Spectrometry Link (DCMS^{Link} ™) 2.8 for instrument control, data acquisition and processing, and report generation

Two Dionex AXP-MS auxiliary pumps

CHROMATOGRAPHIC CONDITIONS

Column: IonPac® AS16 and AG16 hydroxide selective anion-exchange columns (2 mm)

Column Temp.: 30 °C

Flow Rate: 400 µL/min

Eluent Source: EGC II KOH with Continuously-Regenerated Anion Trap Column (CR-ATC), 2 mm

Eluent: Hydroxide gradient

Time/min	*Conc./mM*
–4.0	15
0.0	15
5.0	15
6.0	80
9.0	80
9.5	15
10.0	5

Solvent: 200 µL/min acetonitrile delivered by an AXP-MS pump

Detection: 1st detector: Suppressed Conductivity with Anion Self-Regenerating Suppressor® (ASRS®), 2 mm (external water at 0.5 mL/min delivered by an AXP-MS pump)
2nd detector: TSQ Quantum Access Mass Spectrometer

MASS SPECTROMETRIC CONDITIONS

Interface: Negative Electrospray Ionization (ESI)

Spray Voltage: 3500 V

Sheath Gas: 50 Arbitrary units

Auxiliary Gas: 30 Arbitrary units

Capillary Temp.: 350 °C

Collision Gas: Argon at 1.5 mTorr

SRM Acquisition: 4.2 to 6 min

Operating Mode: Selected Reaction Monitoring (SRM)

Analyte	*Parent Ion (m/z)*	*Product Ion (m/z)*	*Collision Energy (V)*
Endothall-1	185	141	17
Endothall-2	185	123	19
Glutaric Acid-d_6	137	74	21

REAGENTS AND STANDARDS

Endothall standard solution, 1 mg/mL in methanol (AccuStandard P/N P-183S-10XT)

Isotope labeled internal standard (IStd) glutaric acid-d_6 (C/D/N Isotopes P/N D-5227)

Seven anion standard solution with various concentrations from 20 ppm (fluoride) to 150 ppm (sulfate and phosphate) (Dionex P/N 056933)

Acetonitrile (HPLC grade, Burdick & Jackson)

Deionized (DI) water with 18.2 MΩ-cm resistance (Millipore Coporation)

The chemical structures of endothall and isotope labeled internal standard glutaric acid-d_6 are shown in Figure 1.

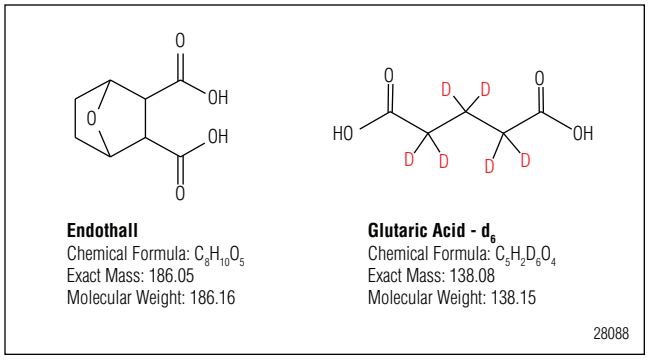


Figure 1. Chemical structures of studied compounds.

STANDARD PREPARATION

Dilute endothall standard in DI water to 10 ppm and 1 ppm as the two working standards to prepare calibration standards. Dilute the internal standard stock solution (1000 ppm) to 10 ppm for calibration standard preparation and for spiking unknown samples.

Prepare calibration standards by serial dilution from the working standards at 10 concentrations: 1 ppb, 2 ppb, 5 ppb, 10 ppb, 20 ppb, 50 ppb, 100 ppb, 200 ppb, 500 ppb, and 1000 ppb with the IStd spiked at 100 ppb for each concentration with the presence of diluted (100× dilution) seven common anions (fluoride 0.2 ppm, chloride 0.3 ppm, nitrite 1 ppm, bromide 1 ppm, nitrate 1 ppm, phosphate 1.5 ppm, and sulfate 1.5 ppm).

SAMPLE PREPARATION

Ground water samples were collected from a local creek and Lake Tahoe west shore. A lake water sample with a high salt content was kindly provided by a customer. These samples were spiked with IStd at 100 ppb and injected directly for ion chromatography tandem mass spectrometry (IC-MS/MS) quantification. (For samples with observed particulates, filtration is required prior to injection.) The salty water sample was diluted with DI water at a 1 to 10 ratio (v/v) due to the observed high concentration of ionic species.

RESULTS AND DISCUSSION

Chromatography

As shown in Figure 1, endothall is a dicarboxylic acid. IC is the preferred separation technique for ionic or ionizable compounds; therefore, it was selected in this study for the separation of endothall from interferences. For IC-MS analysis, the major interferences are inorganic matrix ions such as chloride and sulfate, and other ionizable organics at high concentration. The goals of chromatographic separation were to achieve sufficient chromatographic resolution for endothall from common anions, and to have a short run time to improve method throughput.

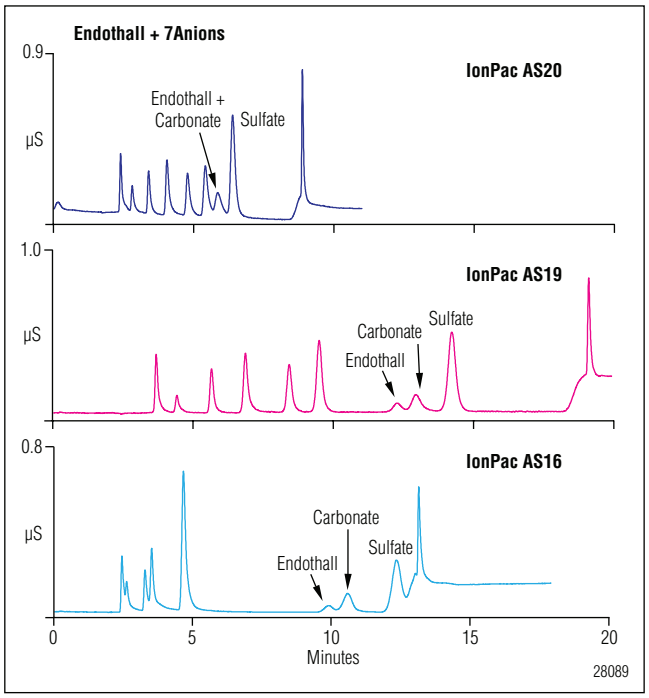


Figure 2. Column selection for endothall separation.

In this study, several high-capacity anion-exchange columns were evaluated for their selectivity; namely, the IonPac AS20, AS19, and AS16 columns. The IonPac AS16 column exhibited the best performance by: 1) providing a wide elution window for endothall between chloride and sulfate, and 2) requiring substantially less chromatographic run time than the IonPac AS19 column. Note that the IonPac AS19 column would be a better choice for simultaneous quantification of endothall and seven anion profiling, as it provided complete chromatographic resolution for all analytes (Figure 2).

CHROMATOGRAPHIC CONDITIONS

System:	ICS-2000 or ICS-2100 RFIC system
Column:	IonPac AS16 and AG16 (2 mm)
Column Temp.:	30 °C
Flow Rate:	400 µL/min
Eluent Source:	EGC II KOH with CR-ATC (2 mm)
Eluent:	Hydroxide gradient

Time/min	*Conc./mM*
–4.0	15
0.0	15
5.0	15
6.0	80
9.0	80
9.5	15
10.0	5

Solvent:	200 µL/min acetonitrile delivered by an AXP-MS pump
Detection:	1st detector: Suppressed Conductivity using ASRS 300 (2 mm) (external water at 0.5 mL/min delivered by an AXP-MS pump) 2nd detector: TSQ Quantum Access Mass Spectrometer

MASS SPECTROMETRIC CONDITIONS

System:	TSQ Quantum Access triple quadrupole mass spectrometer
Interface:	Negative Electrospray Ionization (ESI)
Spray Voltage:	3500 V
Sheath Gas:	50 Arbitrary units
Auxiliary Gas:	30 Arbitrary units
Capillary Temp.:	350 °C
Collision Gas:	Argon at 1.5 mTorr
SRM Acquisition:	4.2 to 6 min
Operating Mode:	Selected Reaction Monitoring (SRM)

Analyte	*Parent Ion (m/z)*	*Product Ion (m/z)*	*Collision Energy (V)*
Endothall-1	185	141	17
Endothall-2	185	123	19
Glutaric Acid-d_6	137	74	21

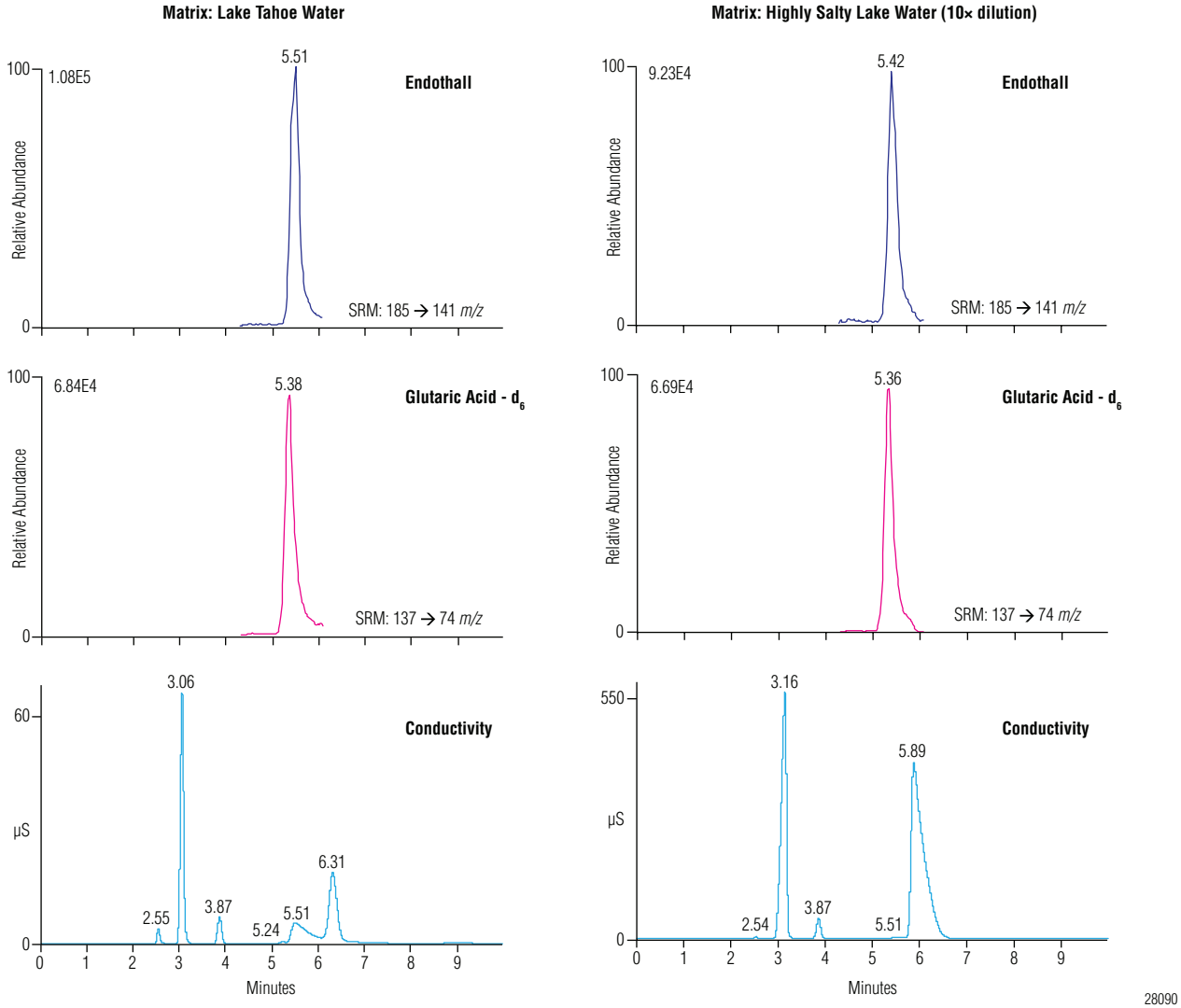


Figure 3. Conductivity and SRM chromatograms of endothall spiked in different samples. Left, 5 ppb endothall spiked in Lake Tahoe water; right, 50 ppb endothall in salty lake water with 10-fold dilution.

Mass Spectrometry

A TSQ Quantum Access triple quadrupole mass spectrometer was selected in this study for sensitivity and selectivity, which allowed minimum sample preparation such as cleanup and enrichment. A matrix diversion valve was used to divert high concentrations of inorganic anions to waste to prevent MS entrance fouling and to maintain long-term system stability. It is important to adjust the diversion window for specific matrices to ensure the analyte signal is collected by the MS and inorganic salts are diverted to waste.

As seen in Figure 3, two different matrices, Lake Tahoe water (LTW) and highly salty lake water (HSW), were spiked with endothall at 5 ppb and analyzed by this method. The HSW is a highly salty water sample (~1000 ppm sulfate, estimated by conductivity peak area), and the retention time of endothall in this 10× diluted matrix was slightly shifted (0.09 min earlier than in the LTW matrix). In addition, the retention time for sulfate shifted significantly earlier by 0.42 min; thus, the diversion window had to be adjusted accordingly to avoid MS entrance contamination. In rare situations where dilution of the original sample is not applicable, such as when endothall is present in very low concentration in a high salt matrix, the gradient program will have to be adjusted, i.e. decrease the gradient slope from 5 to 9 min, in order to increase the resolution of endothall from sulfate (as seen in Figure 2).

The quantitation of endothall was carried out in SRM mode, the precursor ion was observed as the deprotonated molecular ion $[M-H]^-$ at 185 *m/z*, and the predominant product ion was observed as $[M-H-CO_2]^-$ at 141 *m/z* and used as the quantitative SRM transition; another characteristic product ion was also observed as $[M-H-CO_2-H_2O]^-$ at 123 *m/z* with less intensity and was used as a confirmative SRM transition. Isotope labeled glutaric acid-d_6 was used as the internal standard due to its similarity in chemical structure and chromatographic retention to endothall.

Because the IC eluent is 100% aqueous post-suppression, acetonitrile was introduced as the desolvation solvent to assist desolvation/ionization and was delivered by an auxiliary pump at 0.2 mL/min.

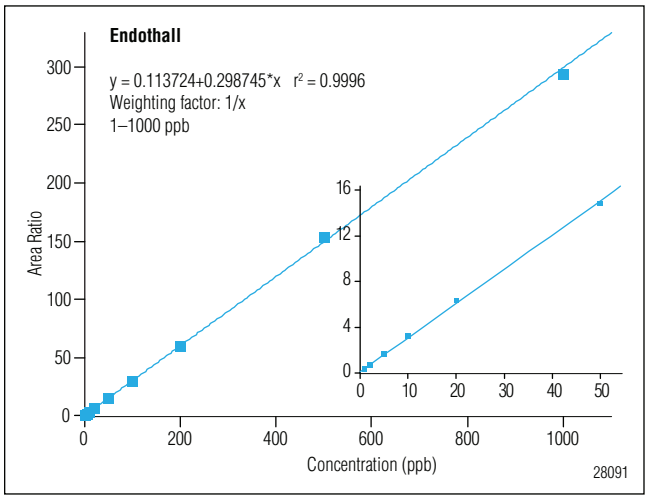


Figure 4. Calibration curve of endothall using glutaric acid-d_6 as an internal standard.

Method Performance

Typical conductivity and SRM chromatograms are shown in Figure 3. Sulfate, chloride, and nitrate were observed as the main anionic species in tested ground water, and were chromatographically separated from the target analyte, endothall, and the internal standard, glutarate-d_6. The MS/MS instrument operated in SRM mode provided sensitive and selective detection: each target analyte was observed as a single peak in each SRM channel with excellent intensity.

This method was used to determine endothall in various water samples mentioned in the Sample Preparation section, and no quantifiable level of target analyte was observed in any of the tested samples. Thus, these three matrices were used to evaluate the recovery from matrices.

Method performance was evaluated against quality parameters such as linearity, reproducibility, precision, accuracy, detection limits, and recovery from matrix. Calibration was performed by analyzing calibration standards at 10 concentrations from 1 to 1000 ppb using internal calibration with IStd at 100 ppb in each standard. Excellent linearity was achieved through three orders of magnitude with the coefficient of determination (r^2) at 0.9996. The calibration curve is shown in Figure 4, and the insert shows the calibration curve at lower concentrations.

Table 1. Recovery of Endothall from Three Matrices						
Sample	**5 ppb**			**500 ppb**		
	Mean	**Standard Deviation**	**% Recovery**	**Mean**	**Standard Deviation**	**% Recovery**
Creek Water	5.00	0.15	100	551	31.6	110
LTW	5.20	0.08	104	540	7.32	108
HSW	4.76	0.06	95.1	535	50.3	107

Precision and accuracy were evaluated by seven replicate assays of a standard at 5 ppb, with the calculated mean at 4.89 ppb (97.8% accuracy) with standard deviation at 0.18 (3.63% RSD). The method detection limit (MDL) was calculated following the equation MDL= s × *t*, where s is the standard deviation and *t* is the Student's *t* at 99% confidence interval. The calculated MDL is 0.56 ppb in DI water (n =7).

Recovery was evaluated by spiking three samples (Creek Water, LTW, and a HSW [10-fold dilution])at two levels: 5 ppb and 500 ppb with IStd spiked at 100 ppb. The results are summarized in Table 1.

CONCLUSION

A fast and sensitive IC-MS/MS method was presented for the quantitative determination of trace level endothall in environmental water samples. The detection limit was estimated at 0.56 ppb, and linear response was observed from 1 ppb to 1000 ppb. The significantly improved sensitivity enables direct analysis of water samples without labor intensive sample enrichment and derivatization. The total chromatographic run time was halved from the 20 min GC run in EPA Method 548.1.

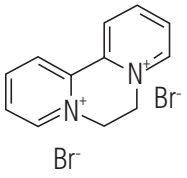
REFERENCES

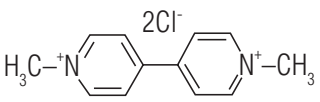
1. United States Environmental Protection Agency, *Basic Information about Endothall in Drinking Water*. http://water.epa.gov/drink/contaminants/basicinformation/endothall.cfm (accessed September, 2010).
2. Pesticide and Environmental Toxicology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, *Public Health Goal for ENDOTHALL in Drinking Water,* December 1997.
3. United States Environmental Protection Agency, *Method 548.1: Determination of Endothall in Drinking Water by Ion-Exchange Extraction, Acidic Methanol Methylation and Gas Chromatography/Mass Spectrometry.* Available from http://www.epa.gov/waterscience/methods/method/files/548_1.pdf (accessed January 31, 2011).

Improved Separation of Diquat and Paraquat Using the Acclaim Mixed-Mode HILIC-1 Column

INTRODUCTION

Mixtures of diquat and paraquat–quaternary ammonium herbicides–are widely used to control crop and aquatic weeds. The structures of these herbicides are shown below. High-performance liquid chromatography (HPLC) is one commonly used method for the determination of diquat and paraquat. The U.S. Environmental Protection Agency (EPA) has published EPA Method 549.2, a method for the analysis of these herbicides in aqueous samples.[1]





Paraquat

The separation of diquat and paraquat is difficult due to their very weak retention on a conventional reversed-phase (RP) C18 column; therefore, ion-pairing reagents are added to the mobile phase.[1-4] These reagents are also added to improve peak shape.[5] A stationary phase that may be used in the hydrophilic interaction liquid chromatography (HILIC) mode can be used for this separation in the absence of an ion-pairing reagent.[6] However, the only separation that shows a baseline separation of diquat and paraquat is the one reported in reference 5 that uses a special column and a commercial buffer.

The Acclaim® Mixed-Mode HILIC-1 column, based on high-purity spherical silica functionalized with a silyl ligand containing both hydrophilic and hydrophobic functionalities, may be used either in HILIC mode (high organic conditions) or RP mode (high aqueous conditions). In HILIC mode, this column has been used for the determination of urea and allantoin in cosmetics.[7]

The work shown here describes an efficient method for the baseline separation of diquat and paraquat with improved peak shape. Experiments performed on an Acclaim Mixed-Mode HILIC-1 column (3.0 × 150 mm, 3 µm) show that when increasing the pH value of mobile phase buffer from 3.5 to 5.5 or decreasing the proportion of organic mobile phase (methanol), both retention time (t_R) and peak resolution (R_s) increase, whereas peak symmetry (A_s) decreases. This method uses the column in RP mode with an ammonium formate (160 mM, pH 4.7)–methanol (87:13, v/v) mobile phase to separate diquat and paraquat. Figure 1 shows the chromatogram with baseline separation (R_s = 3.2).

EQUIPMENT

Dionex UltiMate® 3000 RSLC system, including:

- HPG 3400RS Pump
- WPS 3000RS Autosampler
- TCC-3000RS Thermostatted Column Compartment
- DAD-3000RS UV-vis Detector
- Chromeleon® Chromatography Data System (CDS) software Version 6.80 SR9

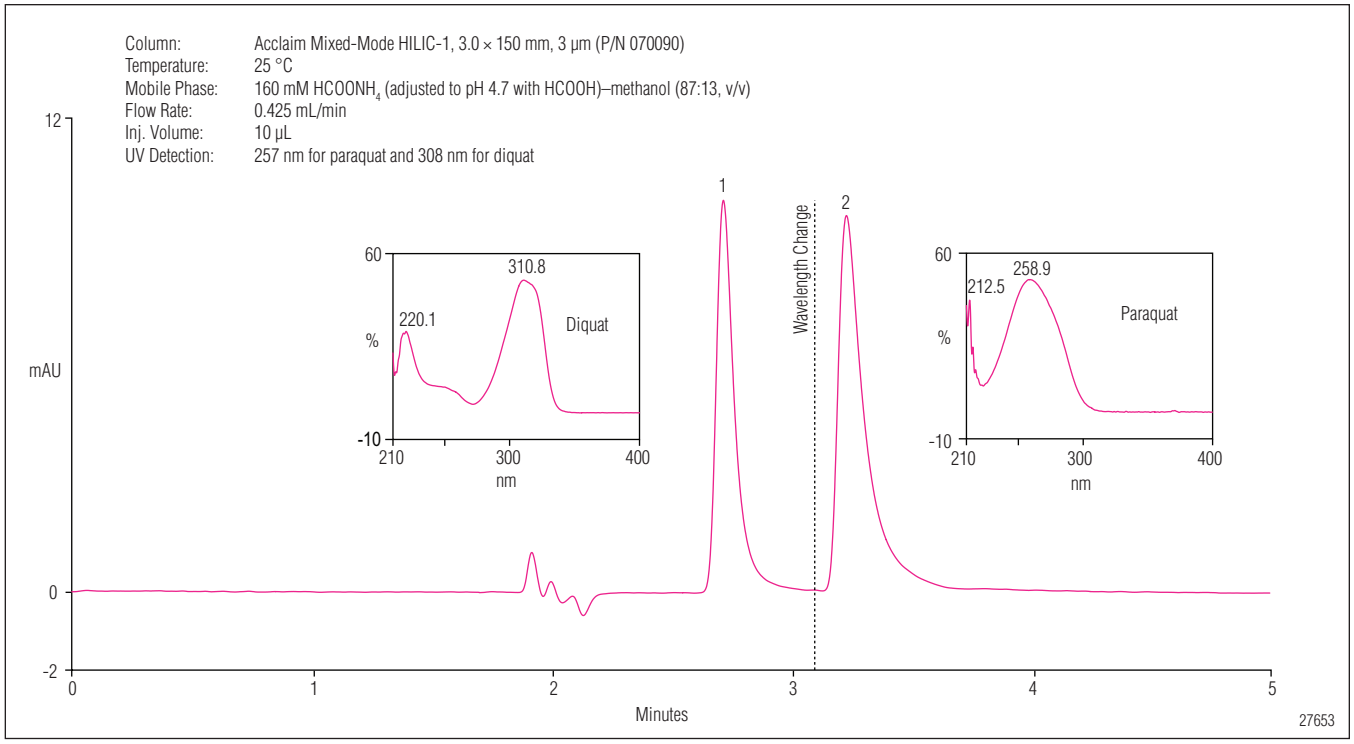


Figure 1. Chromatogram of diquat (peak 1) and paraquat (peak 2) (1.0 µg/mL each) with the UV spectrum for each.

REFERENCES

Environmental Protection Agency Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection; U.S. EPA Method 549.2, Revision 1.0; U.S. Environmental Protection Agency: Cincinnati, OH, 1997.

Waters Corporation, *Paraquat/Diquat.* Waters Column, Applications Notes, 1996, 4 (1), Milford, MA.

Agilent Technologies, *Analysis of Paraquat and Diquat by HPLC.* Publication Number 5966-1875E, 1997, Palo Alto, CA.

Hara, S.; Saski, N.; Takasa, D.; Shiotsuka, S.; Ogata, K.; Futagami, K.; Tamura, K. Rapid and Sensitive HPLC Method for the Simultaneous Determination of Paraquat and Diquat in Human Serum. *Anal. Sci.*, **2007**, 23, p 523.

Restek Corporation, *Simple, Sensitive HPLC/UV Analysis for Paraquat and Diquat, Using High-Recovery Solid Phase Extraction and an Ultra Quat HPLC Column.* Applications Note 580006, 2006, Bellefonte, PA.

Waters Corporation, *Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection (EPA Method 549.2).* 2008, Milford, MA.

Dionex Corporation, *Determination of Urea and Allantoin in Cosmetics Using the Acclaim Mixed-Mode HILIC Column*. Application Note, LPN 2098, 2008, Sunnyvale, CA.

Determination of Glyphosate by Cation-Exchange Chromatography with Postcolumn Derivatization

INTRODUCTION

Glyphosate is a broad-spectrum herbicide with low mammalian toxicity. The herbicide's widespread use makes it a possible contaminant in ground water and eventually drinking water. The U.S. EPA has established Method 547 to monitor glyphosate in drinking water.[1]

Alternative chromatographic methods for the analysis of glyphosate, whether gas or liquid chromatography, usually require precolumn derivatization of the glyphosate.[2–6] Precolumn derivatization is tedious and can be subject to interferences. Nonchromatographic methods, such as differential pulse polarography, demonstrate poor recoveries and inadequate detection limits.[7]

This application note details a convenient chromatographic method for the analysis of glyphosate and its primary metabolite, aminomethylphosphonic acid (AMPA). Since glyphosate has been shown to rapidly decompose in chlorinated water, AMPA is the species most likely to be found in drinking water matrices.

Sample Preparation and Preservation

Drinking water samples should be collected in clean glass bottles and sealed using caps with PTFE-faced silicone septa. Samples should be filtered prior to injection and can be analyzed without any further treatment.

Summary of Chromatographic Method

The method achieves a high degree of sensitivity and selectivity by combining postcolumn derivatization with fluorescence detection. It is designed to be consistent with U.S. EPA Method 547.

After direct injection of the sample onto the column, glyphosate and AMPA are separated with a potassium phosphate buffer. After separation, the analytes pass through a postcolumn reaction system where they are reacted to form fluorescent derivatives, which are then quantified using fluorescence detection.

The analytical column specified in this application note is quality controlled by the vendor to ensure reliable glyphosate analysis. The reagents specified are also quality controlled to ensure freedom from background interferences. When analyzing a 50 µL drinking water sample, as shown in Figure 1, method detection limits meet or exceed those cited in U.S. EPA Method 547. See the *Results* section for method performance data.

EQUIPMENT

Dionex DX 500 HPLC system consisting of:
- GP40 Gradient Pump
- Postcolumn Derivatization Module (Pickering PCX-5100, Pickering Laboratories)
- Jasco® FP-920 Fluorescence Detector (Jasco)
- Jasco Y-46 Emission Filter (Jasco)
- Eluent Organizer

PeakNet Chromatography Workstation with
- UI20 Universal Interface

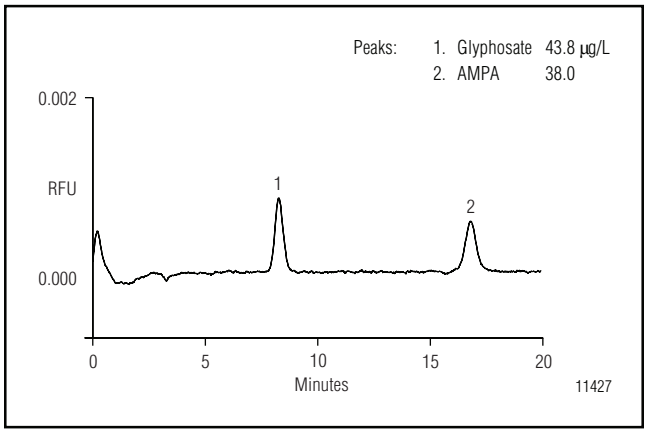


Figure 1 *Drinking water fortified with glyphosate and AMPA. Injection volume = 50 µL.*

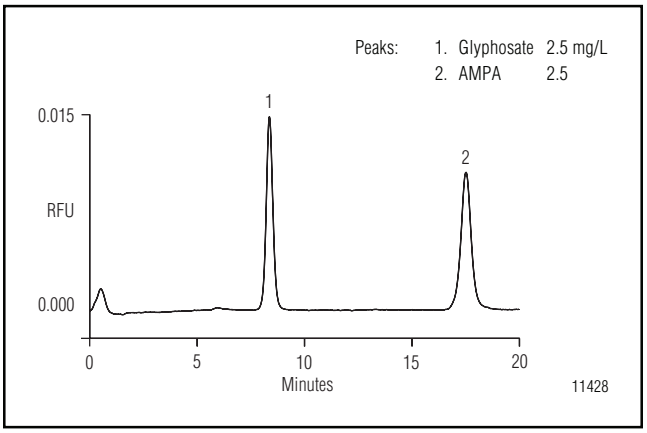


Figure 2 *10-µL injection of glyphosate and AMPA standards.*

REAGENTS

5 mM Potassium phosphate, pH 2.0 (Glyphosate Eluent, Pickering P/N K200)

5 mM Potassium hydroxide (Glyphosate Column Regenerant, Pickering P/N RG019)

Hypochlorite diluent (Pickering P/N GA116)

5% Sodium hypochlorite solution

Sodium borate buffer diluent (Pickering *o*-Phthalaldehyde Diluent, P/N GA104)

OPA, Chromatographic grade (Pickering *o*-Phthalaldehyde, P/N O120)

Methanol, Optima™ grade or equivalent (Fisher Scientific)

Mercaptan reagent (Thiofluor™, Pickering P/N 3700-2000)

PREPARATION OF REAGENTS AND STANDARDS

Oxidizing Reagent (Reagent 1):

Pour one bottle of the pre-prepared hypochlorite diluent into a clean reagent reservoir that has been rinsed with methanol. Add 100 µL of the 5% hypochlorite solution (household bleach has been found to be suitable) and swirl to mix. This amount may require adjustment as follows to optimize detector response: after the chromatographic system is fully equilibrated, inject 10 µL of glyphosate test mixture (Pickering P/N 1700-0080), as shown in Figure 2. If area counts for AMPA and glyphosate differ significantly, add 5% sodium hypochlorite solution to the oxidizing reagent in 20-µL increments until the peak areas are approximately equal.

OPA Reagent (Reagent 2):

Pour the contents of the OPA diluent into a clean reagent bottle that has been rinsed with methanol. Sparge the diluent for approximately 10 minutes to remove any oxygen.

The remaining steps should be accomplished quickly since the prepared reagents are sensitive to oxygen and light: weigh approximately 100 mg of *o*-phthalaldehyde into a small beaker, dissolve in 10 mL of methanol, and add to the OPA diluent. Rinse the beaker with 1 or 2 mL of methanol and add the rinsate to the diluent. Add 2 g of Thiofluor to the reagent bottle, replace the cap, and sparge for 1 or 2 additional minutes. Swirl the bottle gently to ensure complete mixing.

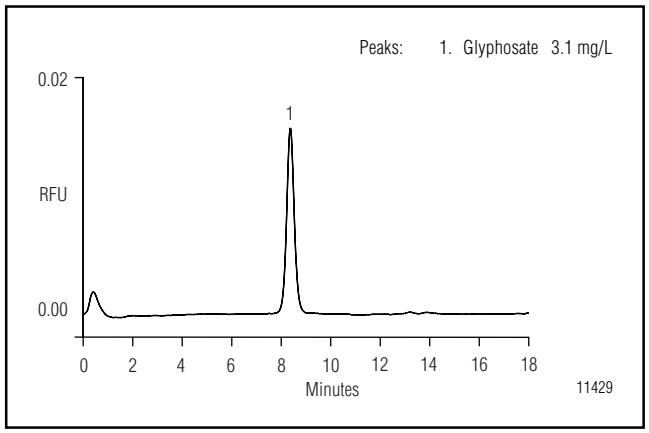


Figure 3 *A 1/2500 dilution of Roundup. Injection volume = 10 µL.*

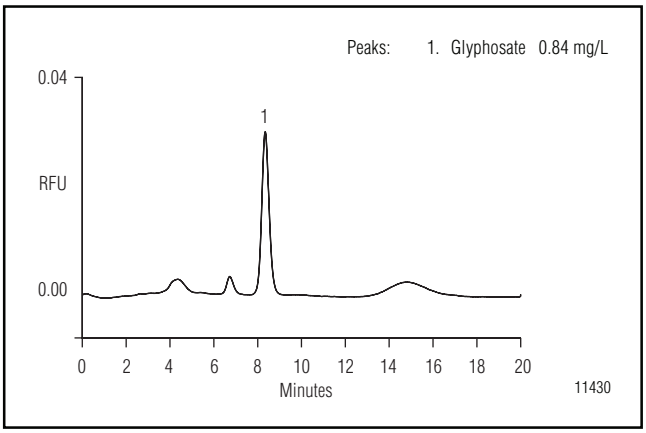


Figure 4 *Glyphosate in a milkweed sample. Total concentration of glyphosate = 23 µg/g of plant material. Injection volume = 50 µL.*

CONDITIONS

Column:	Glyphosate Column, cation exchange, 4 mm x 150 mm x 8 µm (Pickering P/N 1954150)
Guard:	Glyphosate Guard Column, cation exchange, 3 mm x 20 mm x 8 µm (Pickering P/N 1953020)
Column Temp.:	55 ˚C
Eluents:	(A) Potassium phosphate (P/N K200) (B) Potassium hydroxide (P/N RG019)

Gradient:

Time (min)	A (%)	B (%)
0	100	0
15	100	0
15.01	0	100
17	100	0
25	100	0

Flow Rate:	0.4 mL/min
Postcolumn Reagent 1:	Oxidizing reagent at 36 ˚C
Flow Rate:	0.3 mL/min
Reagent 2:	OPA reagent, ambient
Flow Rate:	0.3 mL/min
Fluorescence:	Excitation: 330 nm Emission: >460 nm (cut-off filter)

DISCUSSION AND RESULTS

Figure 3 shows the analysis of glyphosate in Roundup®, a commercially available herbicide. The Roundup was diluted 1/2500 in deionized water and 50 µL was injected directly onto the column. The concentration of glyphosate in this formulation was found to be about 0.8%.

Roundup was then applied to a milkweed plant. After approximately 6 hours, a 7.2-g sample of the milkweed was homogenized with 200 mL of the potassium phosphate eluent in a blender. The sample was centrifuged at 5000 rpm for 15 minutes, and the supernatant filtered through a 0.45-µm filter (Gelman Acro™ LC13, P/N 4453). A sample size of 50 µL was injected into the system and the results are shown in Figure 4.

Postcolumn Chemistry[8]

The postcolumn system features two reagent pumps, two reactors (one heated), and a column oven. A built-in pressure switch shuts down the reagent pumps if it senses that the analytical pump pressure has dropped below 3.4 MPa (500 psi). This feature prevents back-flow of postcolumn reagents onto the analytical column. A schematic diagram of the system hardware is shown in Figure 5.

After it is eluted from the column, the glyphosate is oxidized by hypochlorite at 36 ˚C to form glycine. The glycine is then derivatized with *o*-phthalaldehyde and *N,N*-dimethyl-2-mercaptoethylamine hydrochloride

(Thiofluor) to form a highly fluorescent isoindole compound. AMPA reacts directly with the OPA reagent to form a similar isoindole, as shown in Figure 6.

The Thiofluor reagent is a solid that may be substituted for the liquid 2-mercaptoethanol that is traditionally used for this application. The advantage of Thiofluor is that it is much more stable in solution and is relatively odorless.

Method Detection Limits

The method detection limit for a 50-µL injection of glyphosate in reagent water is 1.8 µg/L, which is less than the 6.0 µg/L MDL for a 200-µL injection volume cited in U.S. EPA Method 547. Dionex recommends an injection of ≤50 µL to preserve peak shapes for all possible matrices; however, 200-µL injection volumes can be used if the pH of the sample is adjusted to ≤2.

Linearity

Glyphosate and AMPA standards of 0.05, 0.5, 2, 4, 6, 8, and 10 mg/L were injected in duplicate for this study. The method was found to be linear for glyphosate over the range tested.

AMPA is not linear over this range ($r^2 = 0.9983$), but a quadratic fit of the data gave an r^2 value of 0.99999. For more accurate work, a quadratic fit should be employed.

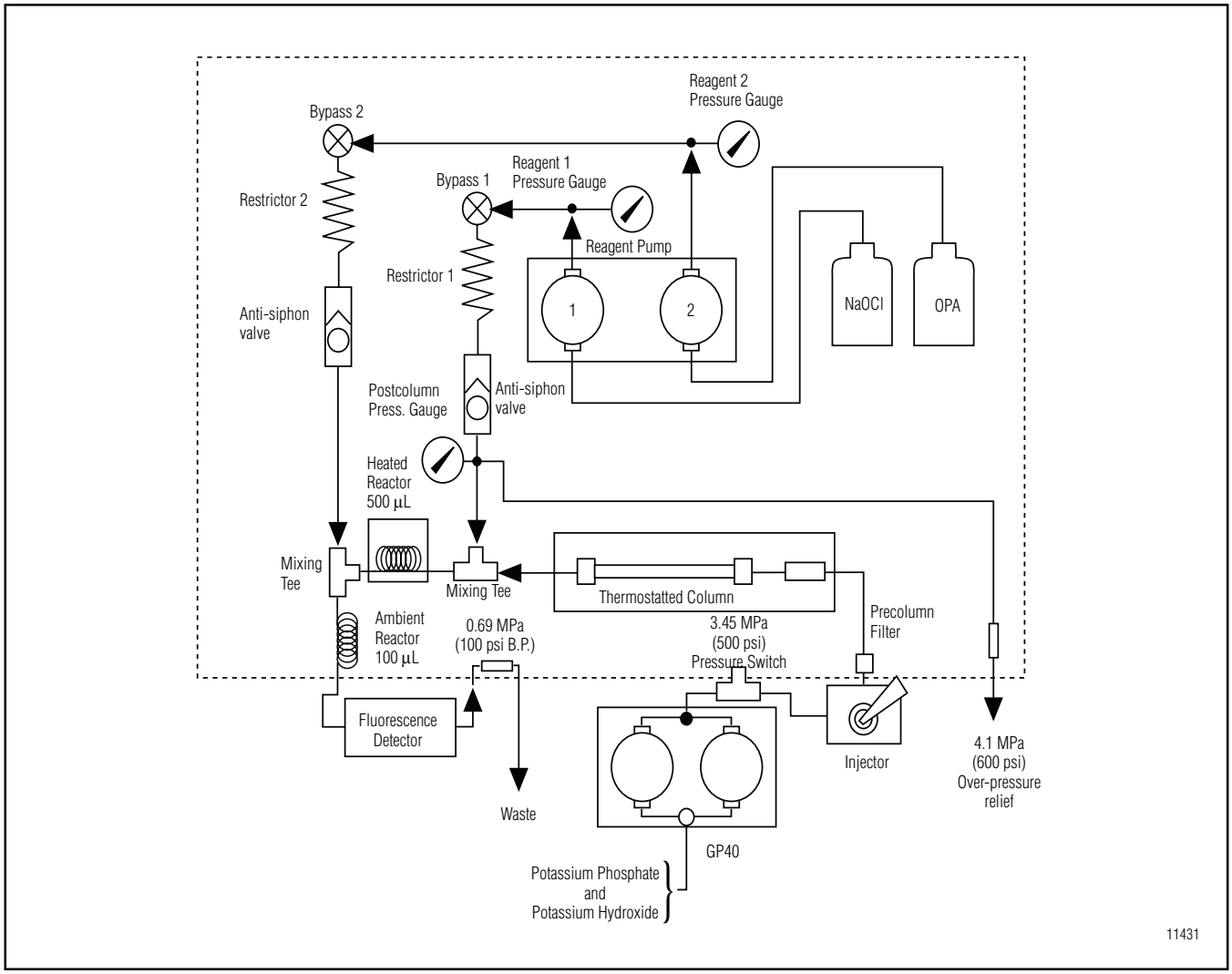


Figure 5 *Schematic of glyphosate analysis system. The chromatography column and postcolumn reactor are represented by the portion of the diagram inside the dotted line.*

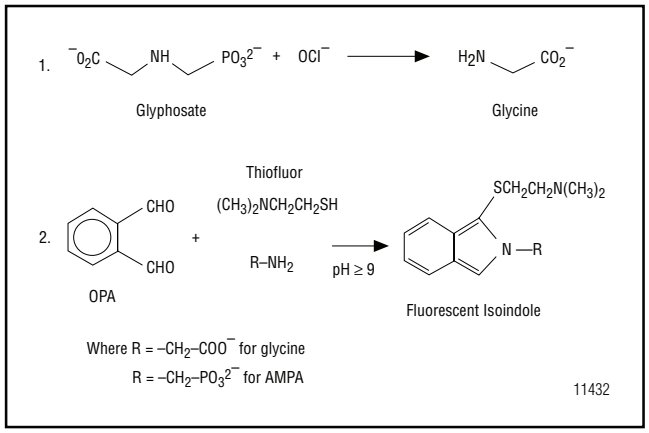


Figure 6 *Postcolumn reaction chemistry of glyphosate and AMPA.*

PRECAUTIONS

Contamination

This method is sensitive to amines from fingerprints and other sources of contamination. We recommend using gloves while preparing reagents and rinsing reagent bottles and eluent lines with methanol before use.

The U.S. EPA method calls for the use of calcium hypochlorite in the oxidizing reagent. The substitution of sodium hypochlorite, however, is equally effective and reduces the chance of plugging the postcolumn reactor.

Reagent Compatibility

The cation-exchange column is *not* solvent compatible and care should be taken to ensure that methanol from the postcolumn system does not back up into the analytical column.

The column regenerant (Eluent B) is strongly alkaline and all system components should be compatible with high pH. Many standard injection valve rotor seals are made of Vespel® or other incompatible polymers. For this application, the seal should be made of Tefzel®.

Reagent Storage

Oxidizing Reagent, when kept under helium, can be used for about three days. After this period, fresh reagent should be prepared.

OPA reagent is oxygen sensitive. If stored under helium, it is stable for up to one week.

Aqueous samples and standards should be kept refrigerated until they are ready to use.

REFERENCES

1. U.S. EPA Method 547, "Analysis of Glyphosate in Drinking Water by Direct Aqueous Injection HPLC with Postcolumn Derivatization," Cincinnati, OH, 1990.
2. Tsunoda, N. *J. Chromatogr.* **1993**, *637*, 167–173.
3. Eberbach, P.L.; Douglas, L.A. *J. Agric. Food Chem.* **1991**, *39*, 1776–1780.
4. Tomita, M.; Okuyama, T.; Nigo, Y.; Uno, B.; Kawai, S. *J. Chromatogr.* **1991**, *571*, 324–330.
5. Kataoka, H.; Horii, K.; Makita, M. *Biosci. Biotech. Biochem*. **1991**, *55*, 195–198.
6. Tomita, M.; Okuyama, T.; Watanabe, S.; Uno, B.; Kawai, S. *J. Chromatogr.* **1991**, *566*, 239-243.
7. Friestad, H.; Bronstad, J. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 76–79.
8. *PCX 5100 Postcolumn Derivatization Instrument User's Manual*, Pickering Laboratories, Mountain View, CA, 1993.

LIST OF SUPPLIERS

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

Jasco, 8649 Commerce Drive, Easton, Maryland, 21601-9903, USA, 1-800-333-5272.

Pickering Laboratories, 1951 Colony Street, Suite S, Mountain View, California, 94043, USA, 1-800-654-3330.

Determination of *N*-Methylcarbamates by Reversed-Phase HPLC

INTRODUCTION

N-methylcarbamates and *N*-methylcarbamoyloximes are among the most widely used pesticides in the world. U.S. EPA method 531.2 provides guidelines for monitoring these compounds in ground and surface waters as well as drinking water.[1] Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) is the preferred method for separating carbamates. Most alternate methods for analysis have significant limitations. Gas chromatographic analysis has proven unsuccessful due to degradation of the analyte compounds during vaporization. HPLC with UV detection does not offer the sensitivity or specificity required for the sample matrices of interest. HPLC with positive mode electrospray ionization mass spectrometry has been shown to be a promising alternative, but it increases the expense and expertise required for the analysis.

Postcolumn derivatization with fluorescence detection after a reversed-phase HPLC separation is consistent with U.S. EPA Method 531.2. This method delivers highly sensitive determinations of carbamate compounds.

SUMMARY OF CHROMATOGRAPHIC METHOD

After direct injection of the sample onto the HPLC column, carbamates are separated using a water/methanol/acetonitrile gradient. After separation, they pass through a postcolumn reaction system where they are derivatized with a fluorescent reagent, then quantified using fluorescence detection. The Acclaim® 120 C18 column provides reliable separation of the analyte compounds. The reagents specified are quality controlled by the manufacturer to ensure minimal background interference.

INSTRUMENTATION

Dionex Summit® HPLC system* consisting of:

- DPG-680 Dual Pump
- ASI-100 Automated Sample Injector
- TCC-100 Thermostatted Column Compartment
- RF-2000 Fluorescence Detector
- Chromeleon® 6.80 Chromatography Workstation
- Pickering PCX 5200 Derivatization Instrument

*This application can also be performed on an UltiMate® 3000 HPLC with the following components:

- DPG 3600A Dual Pump SRD 3600
- WPS-3000TSL Automated sample injector
- TCC-3200 Thermostatted Column Compartment
- RF-2000 Fluorescence Detector

REAGENTS AND STANDARDS

Water, Milli-Q water from Milli-Q Gradient A10
Methanol (CH_3OH), Fisher, HPLC grade
Acetonitrile (CH_3CN), Fisher, HPLC grade
Potassium dihydrogen citrate ($KC_6H_7O_7$), Fluka, ≥ 98%
Sodium thiosulfate ($Na_2S_2O_3$), Fluka, ≥ 98%
Hydrolysis Reagent (0.2% NaOH), Pickering, chromatographic grade (P/N CB130)
o-Phthalaldehyde (OPA, $C_8H_6O_2$), Pickering, chromatographic grade, (P/N O120)
OPA Diluent (0.4% sodium borate solution), Pickering, chromatographic grade (P/N CB910)
Mercaptan Reagent, (Thiofluor™ $(CH_3)_2NCH_2CH_2SH•HCl$), Pickering, chromatographic grade (P/N 3700-2000)
531.2 Carbamate Pesticide Calibration Mixture, Restek, 100 µg/mL (P/N 257974)
4-Bromo-3, 5-dimethylphenyl-N-methylcarbamate standard, Restek, 100 µg/mL (P/N 32274)

PREPARATION OF REAGENTS AND STANDARDS

Reagent WaterStock Standard Solutions

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

Preserved Reagent Water

Dissolve 4.7 g potassium dihydrogen citrate and 160 mg $Na_2S_2O_3$ in a 50 mL beaker with reagent water, transfer this solution to a 500 mL volumetric flask and bring to volume with reagent water. Prior to use, filter the solution through a 0.45-µm filter.

Stock Standard Solutions of Carbamate Pesticide Calibration Mixture

Pipet 10 µL and 100 µL 531.2 carbamate pesticide calibration mixture (100 µg/mL) into two 1 mL vials, add 990 µL to vial one and 900 µL methanol to vial two. The concentrations of stock standard solutions of the calibration mixture are 1.0 µg/mL and 10 µg/mL, respectively.

Stock Standard Solution of 4-Bromo-3,5-dimethylphenyl-*N*-methylcarbamate Standard (Surrogate Analyte, SUR)

Pipet 100 µL 4-bromo-3, 5-dimethylphenyl-N-methylcarbamate standard (100 µg/mL) into a 1 mL vial, and add 900 µL methanol. The concentration of the stock standard solution of the standard is 10 µg/mL.

Table 1: Preparation of calibration curve standards

Stock std. of carbamate cal. mixture (µg/mL)	Vol. of stock std. of carbamate cal. mixture (µL)	Vol. of 10 µg/mL stock std. of SUR (µL)	Final vol. of cal. std. (mL)	Final conc. of cal. std. (µg/L)	Final conc. of SUR (µg/L)
1.0	5.0	5.0	25	0.20	2.00
1.0	12.5	5.0	25	0.50	2.00
1.0	25.0	5.0	25	1.00	2.00
10.0	5.0	5.0	25	2.00	2.00
10.0	12.5	5.0	25	5.00	2.00
10.0	25.0	5.0	25	10.0	2.00

Working Standard Solutions

Prepare six working standard solutions by adding the quantities of carbamate mixture stock standard solutions listed in Table 1 to separate 25 mL volumetric flasks. Add 5 µL of the stock standard solution of 4-bromo-3, 5-dimethylphenyl-N-methylcarbamate into each flask. Bring to volume with preserved reagent water.

Sodium Hydroxide Hydrolysis Reagent (Post Column Reagent 1)

Decant the hydrolysis reagent into a clean reagent reservoir that has been rinsed with methanol. Because high-purity sodium hydroxide is difficult to purchase as well as prepare, we strongly recommend the use of this reagent for optimum system performance. This reagent also contains an antifouling additive to prevent mineral buildup inside the reactor.

OPA Reagent (Postcolumn Reagent 2)

Decant the contents of the OPA diluent into a clean reagent bottle that has been rinsed with methanol. Sparge the diluent for approximately 10 min to remove oxygen.

Note: The remaining steps should be accomplished quickly because the prepared reagents are sensitive to oxygen and light. Weigh approximately 100 mg of *o*-phthalaldehyde into a small beaker, dissolve in 10 mL methanol, and add to the OPA diluent. Rinse the beaker with 1 to 2 mL of methanol and add this to the diluent. Add 2 g of Thiofluor to the reagent bottle, replace the cap, and sparge for 1 to 2 min. Swirl the bottle gently to ensure complete mixing.

Tap Water Sample Preparation

The tap water sample was obtained at the Dionex (Shanghai) Application Lab located in the Pudong District, Shanghai, China.

Place 2.32 g potassium dihydrogen citrate and 80 mg $Na_2S_2O_3$ in a 500 mL beaker, then add approximately 250 mL tap water and mix. The treated tap water sample should be stored in the dark at -10°C until analyzed. Prior to use, filter through a 0.45-µm filter.

CONDITIONS

Column:	Acclaim® 120 C18, 3 µm, 4.6 × 150 mm (P/N 059133)
Column temperature:	42 °C
Mobile phase:	A: water, B: acetonitrile, C: methanol

Gradient:

Time (min)	A (%)	B (%)	C (%)	Curve
0.0	85	0	15	
3.0	85	0	15	5
14.0	60	20	20	1
15.3	40	30	30	5
21.0	40	30	30	5
22.0	85	0	15	5
30.0	85	0	15	5

Flow rate:	1.0 mL/min
Injection volume:	250 µL
Post column reagent 1:	0.2% sodium hydroxide, first reaction coil at 100 °C
Flow rate of reagent 1:	0.3 mL/min
Post column reagent 2:	OPA reagent, second reaction coil at ambient temperature
Flow rate of reagent 2:	0.3 mL/min
Fluorescence:	Excitation: 330 nm Emission: 465 nm Response: 2 (0.5 s) Sensitivity: 2 (medium) Gain: 1 (×1)

RESULTS AND DISCUSSION

Postcolumn Chemistry

The postcolumn system features two reagent pumps, two reactors (one heated), and a column oven. A built-in pressure switch will shut down the reagent pumps when it senses that the analytical pump pressure has dropped below 3.4 Mpa (500 Psi). This feature prevents backflow of sodium hydroxide onto the analytical column when the analytical pump fails to pump or deliver mobile phase. A schematic diagram of the system hardware is shown in Figure 1.

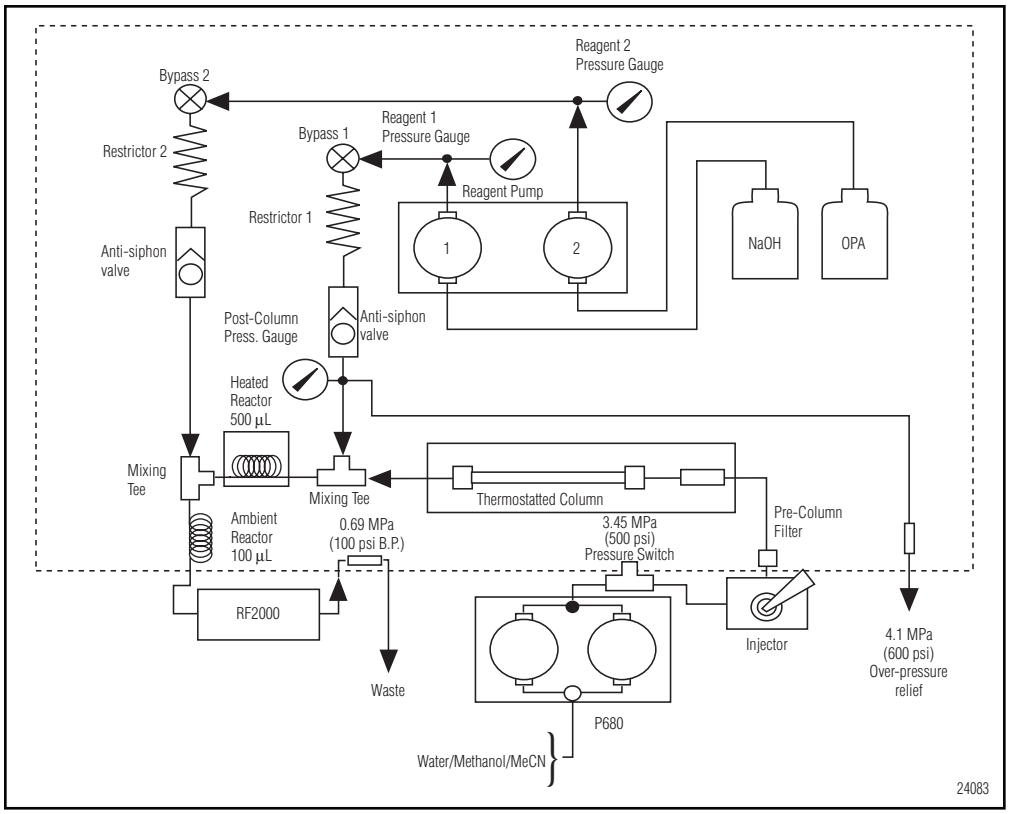


Figure 1. Schematic of carbamate analysis system setup. The chromatography column and postcolumn reactor system are represented by the portion of diagram inside the dotted line.

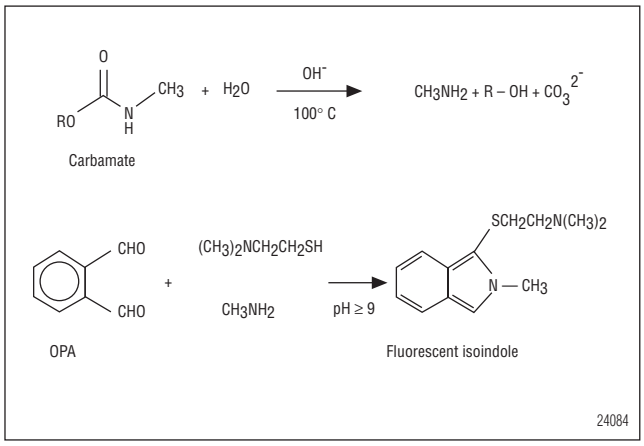


Figure 2. Postcolumn reaction chemistry of carbamate.

After separation on the C18 column, carbamates are hydrolyzed by sodium hydroxide at 100° C. The resulting methylamines are then reacted with o-phthalaldehyde and N, N'-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor) to form a fluorescent isoindole compound. The details of this chemistry are shown in Figure 2.

Note: The Thiofluor reagent replaces 2-mercaptoethanol, a reagent sometimes used for this application. The advantage of using Thiofluor is that it is more stable and relatively odorless.

To assist in troubleshooting postcolumn chemistry issues, 1-naphthol is included in some standard carbamate mixtures, as shown in Figure 3 (peak 10). This compound is naturally fluorescent; therefore it will be the only peak present in a chromatogram when the postcolumn system is not functioning properly.

Resolution and Reproducibility

Figure 3 illustrates good separation of the carbamates listed in U. S. EPA Method 531.2 using the Acclaim 120 C18. Resolution for all peaks is much better than the values required in the EPA Method (≥ 1.0).

Reproducibility was estimated by making 7 replicate injections of a calibration standard with concentration of 1.0 µg/L. The values of relative standard deviation (RSD) of each carbamate for retention time and for peak area are listed in Table 2.

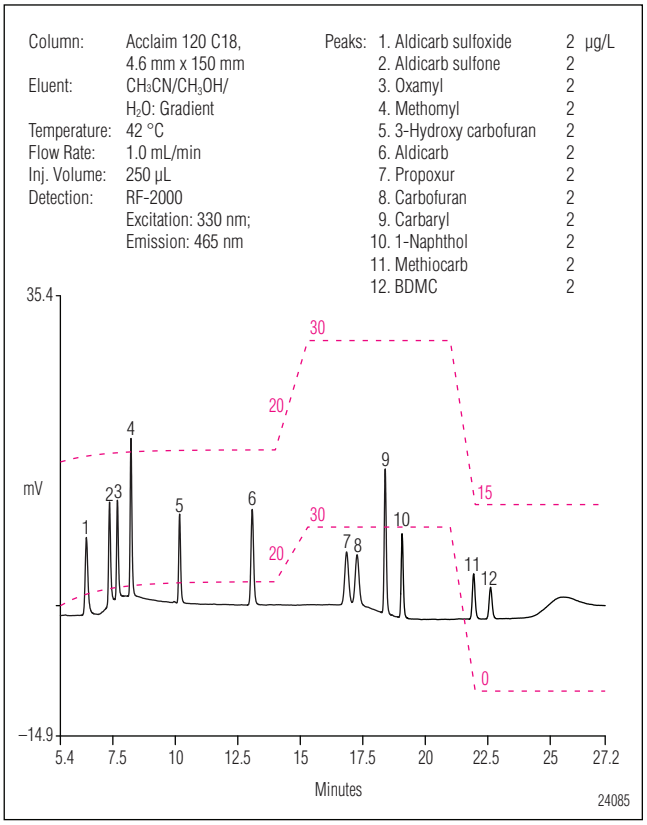


Figure 3. Standard mixture of 10 carbamates (2 µg/L) plus two reference compounds (peaks 10 and 12) with 250 µL injection.

Table 2: Reproducibility of retention time and peak areas for ten carbamates and two reference components

Carbamates	Retention Time RSD (%)	Peak Area RSD (%)
Aldicarb sulfoxide	0.11	3.09
Aldicarb sulfone	0.08	1.39
Oxamyl	0.08	1.04
Methomyl	0.06	0.99
3-Hydroxycarbofuran	0.06	1.39
Aldicarb	0.05	1.06
Propoxur	0.07	2.04
Carbofuran	0.08	2.98
Carbaryl	0.04	0.83
1-Naphthol	0.03	0.79
Methiocarb	0.03	2.78

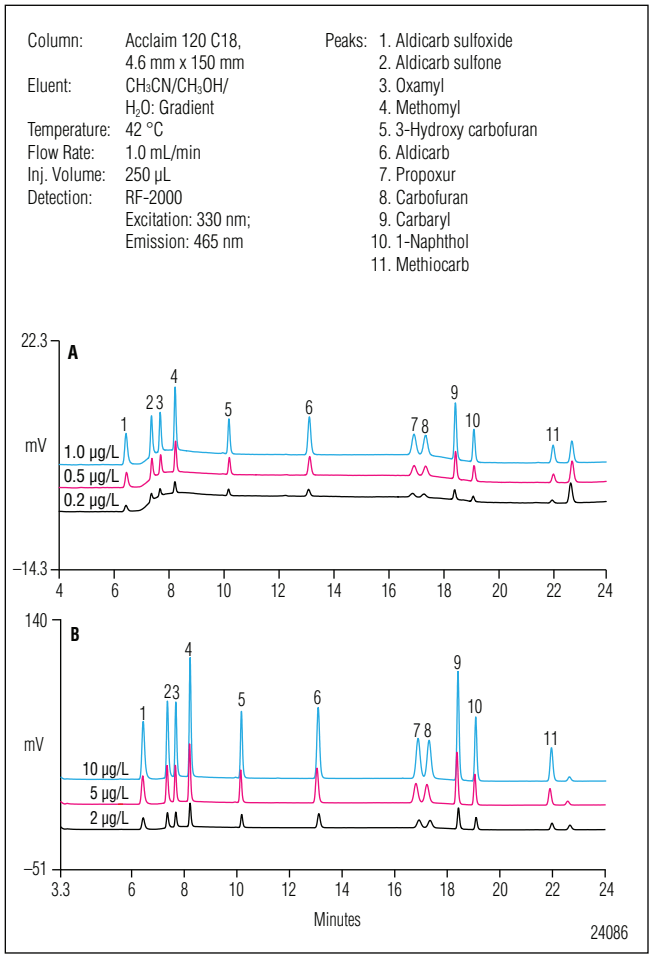


Figure 4. An overlay of chromatograpms of carbamates with concentrations of (A) 0.2, 0.5 and 1.0 µg/L, respectively; and (B) 2.0, 5.0 and 10 µg/L.

Linearity and Detection Limits

Figure 4 shows an overlay of chromatograms of the serial standard solutions of carbamates. Calibration linearity for the determination of carbamates by this method was investigated by making replicate injections (n = 7) of serial standard solutions of carbamates at six different concentrations. Detection limits of carbamates were calculated by using the equation found in EPA 531.1 and 531.2:

Detection limit = $St_{(n-1,\ 1-\alpha=0.99)}$

Where:

S = standard deviation of replicate analyses

$t_{(n-1,\ 1-\alpha=0.99)}$ = Student's value for the 99% confidence level with n -1 degrees of freedom,

n = number of replicates

Table 3: Method linearity data and method detection limits (MDL)

Carbamates	Correlation coefficient (R)	Method 531.1 MDL requirements (µg/L)	Method 531.1 MDL (µg/L)	Dionex Method MDL (µg/L)
Aldicarb sulfoxide	0.9992	2.0	0.59	0.018
Aldicarb sulfone	0.9995	2.0	1.00	0.046
Oxamyl	0.9994	2.0	0.86	0.035
Methomyl	0.9995	0.5	0.29	0.028
3-Hydroxycarbofuran	0.9994	2.0	1.90	0.036
Aldicarb	0.9995	1.0	0.22	0.032
Propoxur	0.9994	1.0	1.00	0.031
Carbofuran	0.9993	1.5	0.52	0.059
Carbaryl	0.9995	2.0	1.30	0.026
Methiocarb	0.9993	4.0	1.90	0.041

* *When n = 10,* $t_{(n-1,\ 1-\alpha=0.99)} = 3.17$

Table 4: Tap water sample analysis

Carbamates	Detected (µg/L)	Added 1 (µg/L)	Found (µg/L)	RSD (%)	Recovery (%)	Added 2 (µg/L)	Found (µg/L)	RSD (%)	Recovery (%)
Aldicarb sulfoxide	0.0	0.20	0.20	6.25	102	10.00	10.66	4.14	107
Aldicarb sulfone	0.0	0.20	0.21	8.42	103	10.00	10.65	4.60	107
Oxamyl	0.0	0.20	0.19	13.4	93	10.00	10.60	4.52	106
Methomyl	0.0	0.20	0.18	5.11	92	10.00	10.88	4.61	109
3-Hydroxycarbofuran	0.0	0.20	0.19	4.25	94	10.00	10.46	4.43	105
Aldicarb	0.0	0.20	0.18	6.16	88	10.00	10.88	4.30	109
Propoxur	0.0	0.20	0.19	6.92	92	10.00	10.56	4.67	106
Carbofuran	0.0	0.20	0.21	8.11	103	10.00	10.60	4.78	106
Carbaryl	0.0	0.20	0.17	6.28	87	10.00	10.54	4.85	105
1-Naphthol	0.0	0.20	0.20	4.47	99	10.00	10.22	4.52	102
Methiocarb	0.0	0.20	0.20	4.82	100	10.00	10.46	4.80	105

Ten replicate injections of reagent water fortified with 0.2 µg/L carbamate standard were used in this method. Table 3 summarizes the data, which show excellent method linearity and sensitivity, with detection limits well below those defined in the EPA method. (The improved detection limits are largely due to improvements in fluorescence detector sensitivity and reversed-phase column technology since the original EPA work was completed. The EPA method used a 5 µm 4.6 x 250 mm column while this method used a 3 µm 4.6 x 150 mm column to yield more efficient peaks.) These improved limits easily allow the analyst to reach the minimum reporting limits (3-5 times the MDL) of the original method.

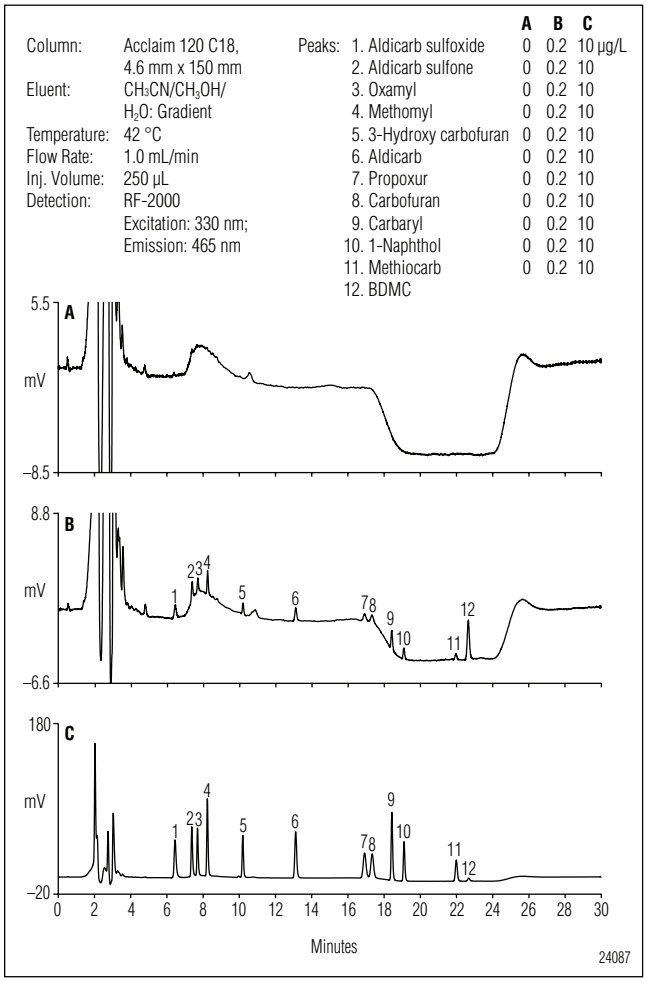


Figure 5. Chromatograms (A) a tap water sample, (B) tap water (A) + 0.2 µg/L carbamate standard mix (C) tap water (A) + 10 µg/L carbamate standard mix.

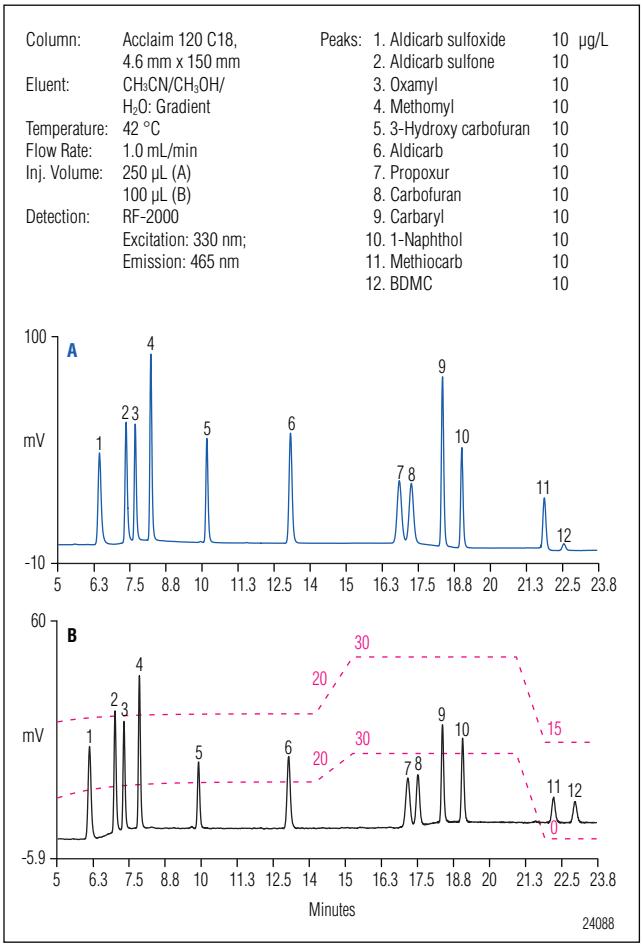


Figure 6. Chromatograms of a standard mixture of 10 carbamates (2.5 µg/mL) plus two reference compounds (peaks 10 and 12) on (A) Summit and (B) UltiMate Systems, respectively, under the same chromatographic condition except for injection volume, (A) 250 µL and (B) 100 µL.

Tap Water Sample Analysis

Figure 5 compares the chromatograms of an unadulterated tap water sample with two samples spiked with 0.2 µg/L and 10 µg/L carbamate standards. No detectable levels of carbamates were found in the tap water. The related data are summarized in Table 4. This data shows excellent spike recovery for each carbamate compound.

Chromatography with the UltiMate 3000 HPLC system

This application can also be performed on the UltiMate 3000 HPLC system. Figure 6 shows the chromatograms of a 10 µg/L carbamate standard on the Summit and UltiMate 3000 systems, respectively, using the same chromatographic conditions except for injection volume. (The UltiMate 3000 used a 100 µL sample loop while the Summit used a 250 µL sample loop.) The UltiMate 3000 can be equipped with a 250 µL sample loop (P/N 6820.2422) and 250 µL syringe (P/N 6822.0003). Good separation of carbamates is achieved on both HPLC systems.

CONCLUSION

This application note describes an optimized method for determining carbamates on a Dionex HPLC system with an Acclaim 120 C18 column (3µm). The method meets or exceeds the chromatographic requirements of the U. S. EPA 531.2 monograph method for carbamates, demonstrating that it is ideally suited for determining these compounds in drinking water.

PRECAUTIONS

Mobile Phase Precautions

- Avoid touching the interior of the mobile phase reservoirs and the dip tubes; the amino acids present in fingerprints will cause contamination. Gloves are suggested.
- Do not leave caps and lines exposed to the atmosphere. To fill the reservoir, transfer caps and lines into a spare bottle or an Erlenmeyer flask filled with deionized water.
- Change the water in the solvent reservoir every 3 to 4 days to prevent possible bacterial growth.
- Do not purge the system with 100% acetonitrile as this reagent can promote precipitation of borate salt in the reactor.

Column Maintenance and Precautions

- If the column backpressure is high, isolate the source—guard, analytical column, or the 0.5-µm in-line filter. Replace items causing the increased pressure.
- At shutdown, flush the column with pure methanol; do not store the column in water.
- Organic contaminants may be removed from the column by first washing with methanol, followed by dichloromethane, followed with a final methanol rinse.
- Never disconnect any fitting between the HPLC pump and the column until the postcolumn system has been shut down and depressurized by loosening the fitting at the "To Detector" port.
- Replace any fittings that leak between the HPLC pump and the column in order to prevent backflow in the event of an unattended shutdown.
- When removing the column, disconnect the outlet fitting first.

Reagent, Sample and Standard Precautions

- Always wear gloves when preparing reagents. Both the hydrolysis reagent and Thiofluor cause skin irritation. Fingerprints also contaminate reagents.
- The hydrolysis reagent is stable and can be replaced as it is used. The OPA reagent is sensitive to oxidation, and degrades over time. It should be prepared fresh for optimum sensitivity.
- Thiofluor is extremely hygroscopic. Store in a tightly closed container.
- Filter all samples through a 0.45 µm membrane filter. Some samples may require even more thorough filtration, e.g. 0.2 µm, especially if colloids are present.
- Aqueous samples must always be properly buffered. Consult EPA Method 531.2 for details.

REFERENCES

1. Measurement of *N*-methylcarbamoyloximes and *N*-methylcarbamates in Water by Direct Aqueous Injection HPLC with Postcolumn Derivatization" U.S. EPA Method 531.2, Revision 1.0, U.S. Environmental Protection Agency: Cincinnati, OH, 2001.
2. PCX 5200 Postcolumn Analysis of Carbamate, Application Manual, Version 3.0, Pickering Laboratories: Mountain View, CA, 2003.

Analysis of Emerging Contaminants

Environmental Water Applications Notebook

Determination of Linear Alkylbenzene Sulphonate in Treatment Plant Wastewater Streams Using On-Line Solid-Phase Extraction Followed by HPLC with Fluorescence Detection

INTRODUCTION

Surfactants are the major active ingredients of laundry detergents. During the washing process, they lower the surface tension of water and loosen and release stains from fabric. Historically, the first surfactant used in laundry cleaning was soap (mixture of animal fat and ashes). Soap is not stable in hard or acid waters. It readily precipitates with divalent metal ions and is, thus, soluble in alkaline soft waters. Other surfactants, including anionic, cationic, non-ionic, and amphoteric surfactants have been developed to improve cleaning performance. Linear alkylbenzene sulphonate (LAS, CAS 68411-30-3) is an anionic surfactant. It was introduced in 1964 as the readily biodegradable replacement for highly-branched alkylbenzene sulphonate (ABS). The commercial product, the subject of this application note (AN), is a mixture of closely-related isomers and homologues, each containing an aromatic ring sulphonated at the para position and attached to a linear alkyl chain (Figure 1).

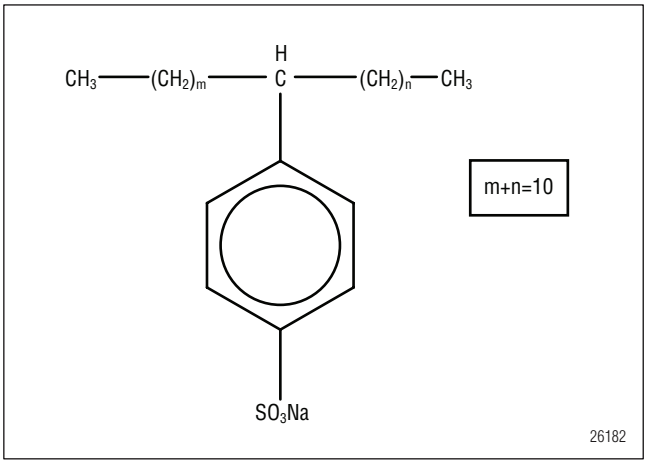


Figure 1. LAS chemical structure (C13 homologue).

The linear alkyl chain typically consists of: 10–13 carbon units, approximately in the mole ratio C10:C11:C12:C13 = 13:30:33:24; an average carbon number near 11.6; and a content of the most hydrophobic 2-phenyl isomers in the 18–29% range.[1] Thus, commercial LAS consists of more than 20 individual components. The ratio of the various homologues and isomers representing different alkyl chain lengths and aromatic ring positions along the linear alkyl chains is relatively constant across the various household applications. The global production of LAS is 2.2 x 10^6 t/yr[1]. The present risk assessment is based on recent environmental safety data and updates the previous LAS terrestrial risk assessments in the literature.[1-9]

LAS surfactants have a strong affinity for sorption to sediments. However, data regarding the fate and effects in sediments following release into the environment have not been reported in great detail.[10] The concentration range of LAS in wastewater treatment plant effluent is 19,000–71,000 ng/L while the total removal in wastewater treatment plants for LAS is 95–99%.[11,12] Approximately a 20% loss over a 4.3 min residence time in stream surface water has been observed. Hence, the half-life (DT50) of LAS in river water is conservatively set to <12 h.[1,13] However, in some situations, continuous inputs to the environment (for example, via wastewater treatment plants effluent, other known and unknown point sources, runoff etc.) of compounds that biodegrade rapidly may replace dissipated material resulting in chronic low exposures.

Sanderson et al. (2005) demonstrated the adapted analytical method for the AS/AES homologues and low likelihood of risks.[10] However, it was also concluded that additional monitoring with more sampling locations, in combination with both biota and habitat up- and downstream of wastewater treatment plants, was needed to better elucidate the potential risks. Figure 2 shows the movement of LAS in the environment.

According to the Italian official methods, the determination of surfactants in industrial discharges or surface waters must be carried out by colorimetric methods. Recently, the European Community introduced a new regulation on detergents (Regulation EC No. 648/2004 of the European Parliament and the Council of 31 March, 2004) which establishes standard methods for detergents.[14] This regulation states that for those

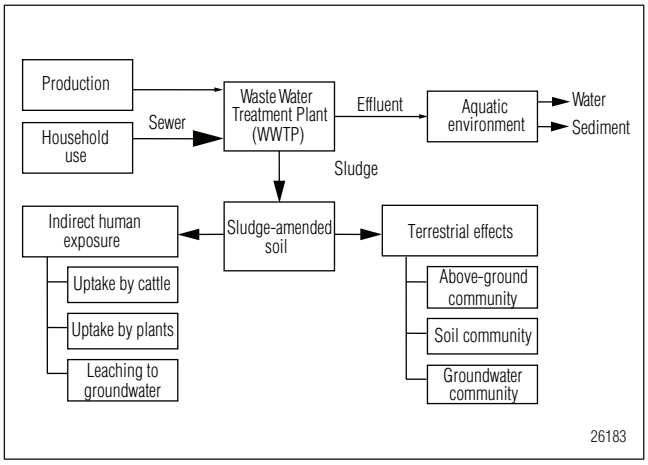


Figure 2. Fate of LAS in the environment.

surfactants not reacting with colorimetric methods (MBAS for anionic, BiAS for non-ionic, and BBAS for cation surfactants) *"if it seems more appropriate for reasons of efficiency or precision, appropriate specific instrumental analyses such as high performance liquid chromatography (HPLC) or gas chromatography (GC) are to be applied."* This is the first statement in favor of the introduction of chromatographic methods for surfactant determination. The LAS surfactants are very diffuse in discharges because they replace alkylphenolethoxylates in domestic detergents which have have been banned for many years.

In 2000, the European Union published the third draft of a future sludge directive entitled *Working Document on Sludge* (Third Draft, 27 April, 2000) adopted in the Italian regional legislation *(Regione Emilai Romagna, Determinazione del Direttore generale ambiente, difesa del suolo e della costa n. 11046 del 29/0//2005)*, where more restricted concentration limit values of some organic compounds are included for the first time.[15] Some of the organic compounds included are LAS surfactants. According to the directive draft, the concentration of LAS has to be lower than 2600 mg/kg SS (Suspended Solids) for land application of sludge. The control of final concentration of LAS in sludges in the wastewater treatment plants (WWTPs) needs continuous monitoring in the effluents in each step of the depuration process. This monitoring can be accomplished by developing an automated chromatographic system for LAS analysis that should not require a manual concentration step.

The determination of LAS can be used as an indicator of the effectiveness of the depuration process. The importance of monitoring the LAS concentration is mainly due to the agricultural destination of resulting sludge, which is regulated for LAS content in some nations.[16] Using sewage sludges for agricultural purposes is an economical pathway for disposal. If the LAS limit is exceeded, the sludge must be delivered to an incinerator to burn at 10 times the cost.

In the recent past, several analytical methods have been described in the literature for the determination of LAS in environmental samples, mainly in agricultural soils and sediments.[17] Most of these methods are based on determination by liquid chromatography with ultraviolet diode array (UV-DAD), fluorescence (FLD), or mass spectrometric detectors.[18-21] The 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 on-line SPE combined with HPLC. This technique delivers a simple, rapid, and accurate means for determining phenols at low concentrations in real samples.[22]

The Dionex UltiMate® 3000 system was designed to easily execute more advanced HPLC methods, such as parallel LC, 2D-LC, and on-line SPE/HPLC. An UltiMate 3000 system, together with an autosampler capable of injecting large volumes, can be used to execute an on-line SPE method to determine LAS in water streams. This AN details an on-line SPE method, followed by HPLC with fluorescence detection, for determining LAS at the concentrations required by world regulatory agencies.

The LAS from river and water streams of a depuration plant are trapped on an IonPac® NG1, a small polymeric reversed-phase column, and then separated on a speciality polar-embedded reversed-phase column, the Acclaim® Surfactant column. This automated method is a cost-effective way to determine LAS in river and water stream samples and follow their fate after water treatments. The integrated on-line SPE solution proposed uses an easy instrumentation setup through Chromeleon® software. This combination represents a simple and economically convenient analytical solution, even for routine laboratory operators. Compared to any alternative system, a Dionex dual pump system will save money and reduce maintenance costs. Also, the elimination of the disposable cartridge in the off-line SPE procedure will save money, solvents, and workload. The same system can be easily configured to perform other common, direct, or SPE-based HPLC applications, such as the determination of phenols or polycyclic aromatic hydrocarbons (PAHs).

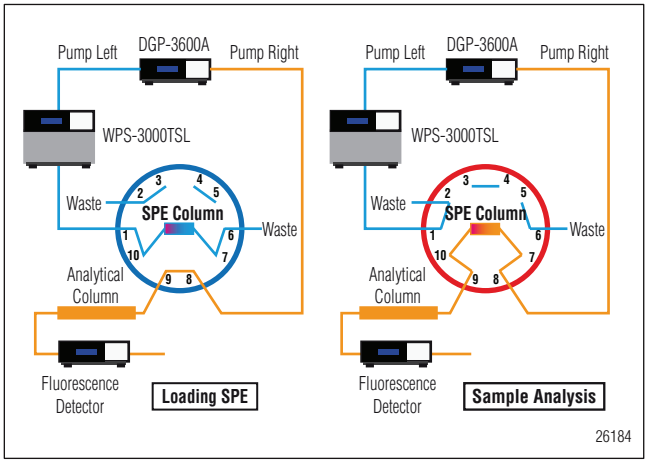


Figure 3. Schematic of devices for determination of LAS using on-line SPE followed by HPLC with fluorescence detection.

EQUIPMENT

Dionex UltiMate 3000 HPLC system consisting:

- DGP-3600A dual gradient pump
- SRD-3600 solvent rack with integrated vacuum degasser
- WPS-3000 TSL equipped with 500 µL loop
- TCC-3200 Thermostatted Column Compartment with one two-port, ten-position (2P–10P) valve
- RF 2000 Fluorescence Detector
- Chromeleon Chromatography Data System, 6.80 SP4

REAGENTS AND STANDARDS

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

Deionized (DI) water ASTM grade I (18.2 MΩ-cm) from a Barnstad or other water purification system

Acetonitrile (CH_3CN), HPLC grade (Merck, Darmstadt)

Ammonium acetate (CH_3COONH_4), analytical reagent grade

The starting standard solution contains 1463 mg/L of $C_{10\text{-}13}$ LAS (Sigma-Aldrich) in methanol. To this standard solution, bisphenol A, nonyphenol, nonyphenol-1-ethoxylated, and nonyphenol-2-ethoxylated were added for determination of additional information not considered in this paper. This information does not interfere with the LAS determination.

CONDITIONS

Solid-Phase Extraction

Column: IonPac NG1, 5 µm, 4 × 250 mm (P/N 039567)

Mobile Phases for SPE (Left Pump): A. Water

Flow Rate: Rinse/Loading 2 mL/min with 100% A

Temperature: 30 °C

Inj. Volume: 0.5–5 mL

For the detailed program see Tables 1A-C.

Analytical

Column: Acclaim Surfactant, 5 µm, 4.6 × 250 mm (P/N 063203)
Acclaim Surfactant Guard 4.3 × 10 mm (P/N 063215)

Mobile Phases for Analysis (Right Pump): A. CH_3CN
B. 100 mM CH_3COONH_4 (pH 5 with HCl)

Gradient: 50 to 15% B in 20 min

Flow Rate: 1 mL/min

Temperature: 30 °C

Detection: Fluorescence, 0 min: Ex 230 nm Em 302 nm; 18 min: Ex 221 nm Em 284 nm

Total analysis time is 45 min. For the detailed program see Table 1B.

Table 1A. List of Default Conditions for SPE and Analytical Separation

Default Conditions	Sampler.TempCtrl = On Sampler.Temperature.Nominal = 10.0 [°C] ColumnOven.TempCtrl = On ColumnOven.Temperature.Nominal = 30.0 [°C] PumpLeft.%A.Equate = Water PumpRight.%A.Equate = ACN PumpRight.%B.Equate = NH_4AcOH 100 mM pH5 PumpRight.%C.Equate = "%C" InjectMode = UserProg SyncWithPump = On Emission.ExWavelength = 230 [nm] Emission.EmWavelength = 302 [nm] Emission.Sensitivity = Med PumpLeft.Flow = 2.000 [ml/min] PumpLeft.%B = 0.0 [%] PumpLeft.%C = 0.0 [%] ValveRight = 10_1 WashVolume = 1000.000 [µl] WashSpeed = 30.000 [µl/s] PrepVial= Position+1

Table 1B. List of Analytical Conditions for LAS Determination		
–10.000	Sampler.TempCtrl = On Sampler.Temperature.Nominal = 10.0 [°C] ColumnOven.TempCtrl = On	Begin to equilibrate the analytical column using initial conditions for 10 min Injections at 0 min.
0.000	UV.Autozero Wait AZ_Done Emission.Autozero PumpRight.Flow = 1.000 [mL/min] PumpRight.%B = 50.0 [%] PumpRight.%C = 0.0 [%] Wait ColumnOven.Ready and Sampler.Ready Inject Emission.AcqOn PumpRight.Flow = 1.000 [mL/min] ValveRight = 1_2 PumpRight.%B = 50.0 [%] PumpRight.%C = 0.0 [%]	Starts SPE column rinse
5.200	ValveRight = 10_1	Start SPE column rinse
18.000	Emission.ExWavelength = 221 [nm] Emission.EmWavelength = 284 [nm]	Wavelengths optimized for LAS
20.000	PumpRight.Flow = 1.000 [mL/min] PumpRight.%B = 15.0 [%] PumpRight.%C = 0.0 [%]	Gradient end
30.000	PumpRight.Flow = 1.000 [mL/min] PumpRight.%B = 15.0 [%] PumpRight.%C = 0.0 [%] Emission.AcqOff ReleaseExclusiveAccess End	Isocratic step

Table 1C. Program for Multiple Sample Loading Onto SPE Column Using a WPS-3000 Autosampler Equipped with 500 µL Loop			
500 µL	UdpDraw	From=SampleVial, Volume=500.000, SyringeSpeed=12.000, SampleHeight=2.000	Single step for 500 µL sample loading onto SPE column
	UdpMixWait	Duration=5	
	UdpInjectValve	Position=Inject	
	UdpMoveSyringe	Unload=500.000, SyringeSpeed=12.000	
	UdpInjectValve	Position=Load	
			To be repeated according to the final desired volume
5000 µL	UdpDraw	From=SampleVial, Volume=500.000, SyringeSpeed=12.000, SampleHeight=2.000	10th step for 5000 µL of sample loaded onto SPE column
	UdpMixWait	Duration=5	
	UdpInjectValve	Position=Inject	
	UdpMoveSyringe	Unload=500.000, SyringeSpeed=12.000	
	UdpInjectValve	Position=Load	
Final step	UdpInjectMarker		Placed at the end of any repetitive sequence last step

Table 2. Range and Determined Homologues Average Distribution of LAS in Working Standard (n=5)

	C_{10}	C_{11}	C_{12}	C_{13}
Range %	5–15	30–40	20–40	15–30
Average found %	13	33	31	23

RESULTS AND DISCUSSION

Optimization of the On-line SPE Method

The linearity of the on-line SPE procedure for the determination of C_{10-13} LAS was investigated by loading the SPE cartridge with different volumes of the C_{10-13} LAS standard prepared at the same concentration. The resulting chromatograms are shown in Figure 4. Linearity results are summarized in Table 3.

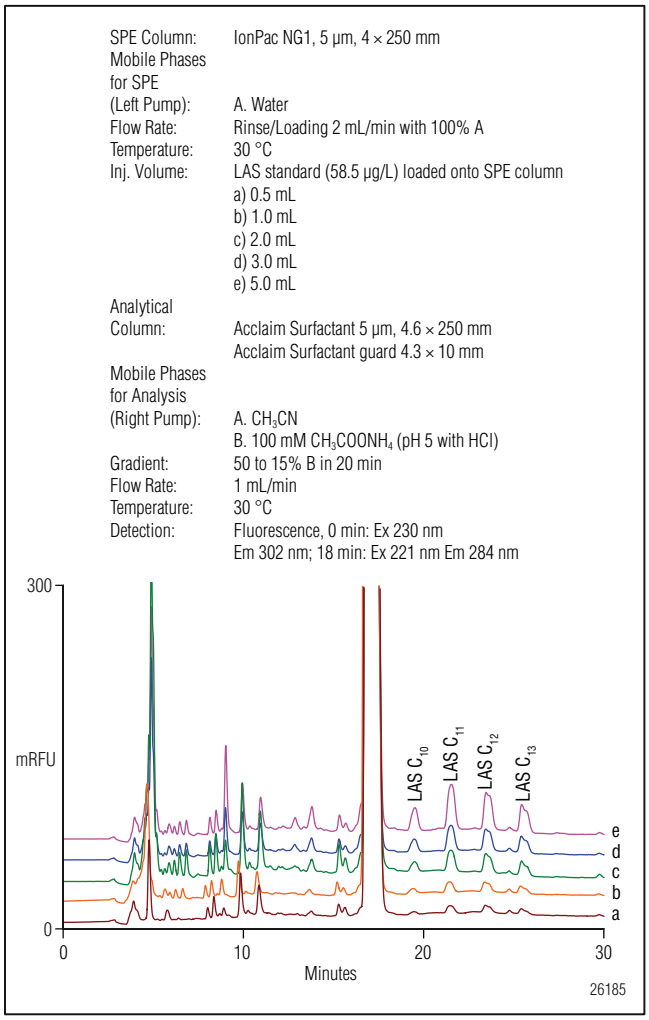


Figure 4. An overlay of chromatograms of different volumes of the same C_{10-13}.

Reproducibility, Detection Limits, and Linearity

The wide range of LAS concentrations in the analyzed samples collected in the inlet and outlet of the WWTP and the limited dynamic range of fluorescence detection, require the use of two different loading volumes for the SPE device, 0.5 and 5 mL respectively, and subsequent use of two calibration curves for 0.5 and 5 mL volumes. The reproducibility was estimated by making five replicate injections of an inlet sample and three replicates of a low concentration sample. Table 4 summarizes the retention time and peak area precision data for both samples. The method detection limits (MDLs) of the C_{10-13} LAS using the on-line SPE-HPLC are listed in Table 5. The calibration linearity for the determination of C_{10-13} LAS was investigated by making replicate injections of C_{10-13} LAS prepared at five different concentrations. The external standard method is used to calculate the calibration curve and for sample analysis. Table 3 lists the data from the calibration as reported by the Chromeleon software.

Sample Analysis

To achieve satisfactory chromatography of C_{10-13} LAS in water samples, the samples should be filtered prior to analysis, and analyzed in a short time to avoid degradation processes due to the dissolved organic matter.

Figure 5 shows an example of an inlet stream of an urban and industrial wastewater treatment plant. The concentration of the total C_{10-13} LAS in the sample was 8689 µg/L. The chromatogram of the effluent stream of the same plant is shown in Figure 6. The C_{10-13} LAS concentration in the sample was reduced to 740 µg/L. Another example of an urban waste depuration plant effluent analysis is shown in Figure 7. This situation is far above the quantification limit and the C_{10-13} LAS concentration in the sample was as low as 37.1 µg/L.

SPE Column:	IonPac NG1, 5 µm, 4 × 250 mm
Mobile Phases for SPE (Left Pump):	A. Water
Flow Rate:	Rinse/Loading 2 mL/min with 100% A
Temperature:	30 °C
Inj. Volume:	LAS standard (58.5 µg/L) loaded onto SPE column 0.5 mL
Analytical Column:	Acclaim Surfactant, 5 µm, 4.6 × 250 mm Acclaim Surfactant guard 4.3 × 10 mm
Mobile Phases for Analysis (Right Pump):	A. CH_3CN B. 100 mM CH_3COONH_4 (pH 5 with HCl)
Gradient:	50 to 15% B in 20 min
Flow Rate:	1 mL/min
Temperature:	30 °C
Detection:	Fluorescence, 0 min: Ex 230 nm Em 302 nm; 18 min: Ex 221 nm Em 284 nm

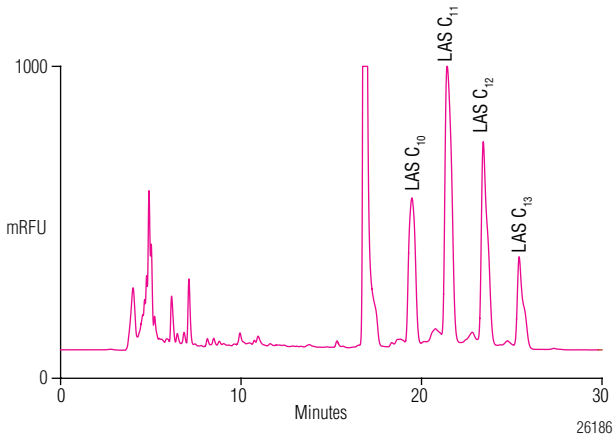


Figure 5. Chromatogram of the inlet stream of an urban and industrial wastewater treatment plant. The total LAS concentration is 8689 µg/L.

SPE Column:	IonPac NG1, 5 µm, 4 × 250 mm
Mobile Phases for SPE (Left Pump):	A. Water
Flow Rate:	Rinse/Loading 2 mL/min with 100% A
Temperature:	30 °C
Inj. Volume:	LAS standard (58.5 µg/L) loaded onto SPE column 5.0 mL
Analytical Column:	Acclaim Surfactant, 5 µm, 4.6 × 250 mm Acclaim Surfactant guard 4.3 × 10 mm
Mobile Phases for Analysis (Right Pump):	A. CH_3CN B. 100 mM CH_3COONH_4 (pH 5 with HCl)
Gradient:	50 to 15% B in 20 min
Flow Rate:	1 mL/min
Temperature:	30 °C
Detection:	Fluorescence, 0 min: Ex 230 nm Em 302 nm; 18 min: Ex 221 nm Em 284 nm

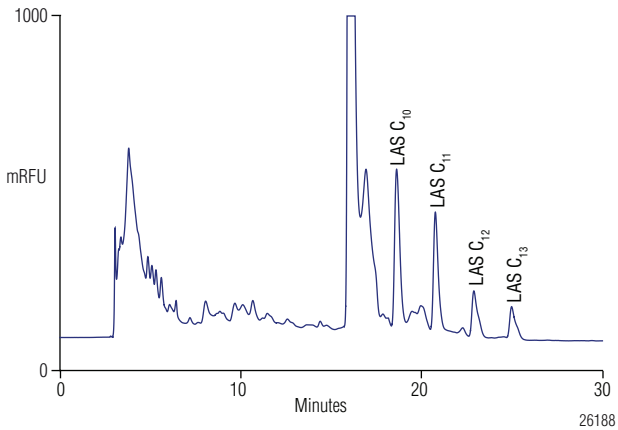


Figure 6. Chromatogram of the outlet stream of an urban and industrial wastewater treatment plant of Figure 5. The total LAS concentration is 740 µg/L.

Table 3. LAS Calibration at Two Different SPE Volumes and Five Different Concentrations Each

SPE Loaded Volume	C_{10}			C_{11}			C_{12}			C_{13}		
	r^2	Offset	Slope	r^2	Offset	Slope	r^2	Offset	Slope	r^2	Offset	Slope
0.5 mL	0.9967	-0.3170	0.1343	0.9964	0.5495	0.1137	0.9962	0.6692	0.1117	0.9935	0.6836	0.1108
5 mL	0.9996	1.2408	1.1660	0.9988	4.2647	0.9655	0.9988	4.4579	0.8101	0.9975	3.3943	0.6974

Table 4. Retention Time and Peak Area Precisions for C_{10-13} LAS at Two Different SPE Loading

SPE Loaded Volume	C_{10}		C_{11}		C_{12}		C_{13}	
	RT RSD	Area RSD	RT RSD	Area RSD	RT RSD	Area RSD	RT RSD	Area RSD
0.5 mL (n=5)	0.24	1.72	0.22	1.33	0.16	1.39	0.12	3.25
5 mL (n=3)	0.95	11.1	0.03	9.9	0.04	15.1	0.03	15.4

Table 5. Detection Limits for C_{10-13} LAS at Two Different SPE Loading Volumes								
SPE Loaded Volume	C_{10}		C_{11}		C_{12}		C_{13}	
	LOD (µg/L)	RSD	LOD (µg/L)	RSD	LOD (µg/L)	RSD	LOD (µg/L)	RSD
0.5 mL (n=5)	5.1	3.29	14.2	3.89	15.4	1.1	12.3	3.86
5 mL (n=3)	0.19	11.4	0.51	12.1	0.46	12.4	0.31	15.3

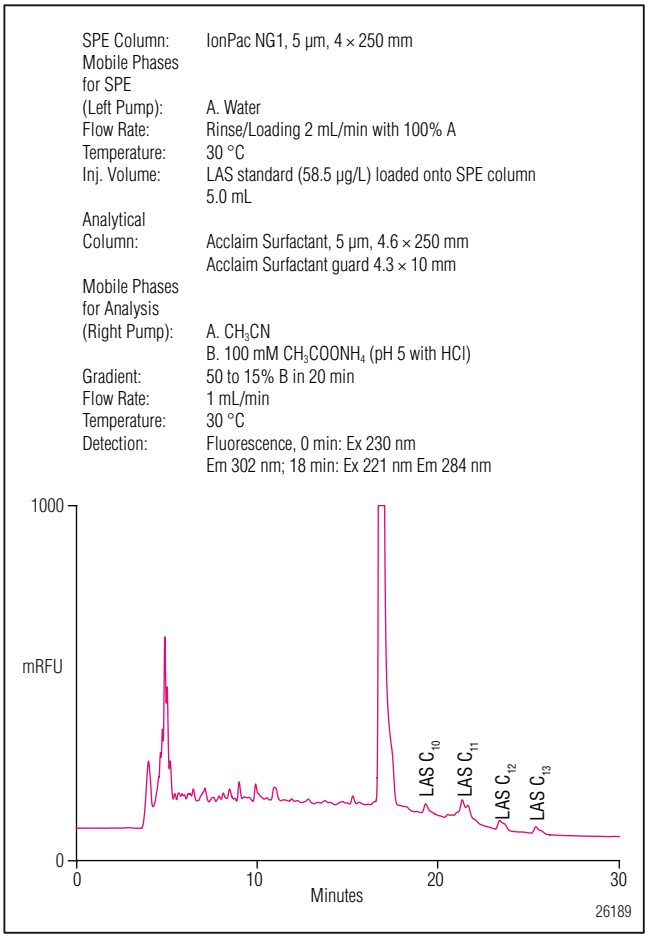


Figure 7. Chromatogram of the outlet stream of an urban wastewater treatment plant. The total LAS concentration is 37.1 µg/L.

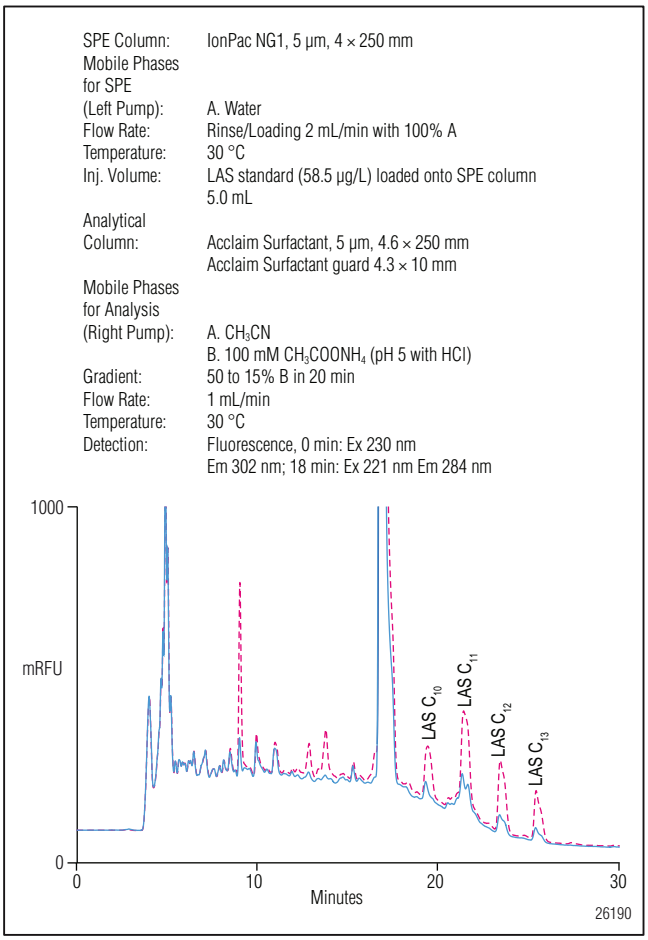


Figure 8. Chromatograms of the outlet stream of an urban wastewater treatment plant at low C_{10-13} LAS concentration and same sample spiked with 117 µg/L of C_{10-13} LAS.

Recovery

A recovery study was performed by spiking a low concentration sample with 117 µg/L of C_{10-13} LAS and doing replicate analyses. Figure 8 shows the comparison between a low concentration sample (Figure 7) and the same sample spiked with 117 µg/L of C_{10-13} LAS. The average recovery was better that 104% with a RSD of 14% (n=3).

CONCLUSION

The successful analysis of all the water samples above demonstrates that on-line SPE with a dual UltiMate system can determine the LAS without laborious off-line sample preparation. The on-line SPE method with fluorescence detection has very good reproducibility and selectivity with detection limits of 0.9 µg/L for total C_{10-13} LAS using a 5 mL sample loaded onto the SPE column.

PRECAUTIONS

Method interferences may be caused by surfactants in solvents, reagents, glassware, and other sample processing hardware. Clean and rinse all glassware scrupulously with high-purity water and use high-purity reagents and solvents to minimize interference problems. Samples must be filtered and analyzed in a short time to avoid degradation due to dissolved organic matter.

REFERENCES

1. HERA, 2004. Human and Environmental Risk Assessment of Ingredients of Household Cleaning Products: Report for LAS. http://www.heraproject.com.
2. Mieure, J.P.; Waters, J.; Holt, M.S.; Matthijs, E. Terrestrial Safety Assessment of Linear Alkylbenzene Sulphonate *Chemosphere* **1990**, *21*, 251–262.
3. Kloepper-Sams, P.; Torfs, F.; Feijtel, T.C.J.F.; Gooch, J. Effects Assessment for Surfactants in Sludge-Amended Soils: a Literature Review and Perspective for Terrestrial Risk Assessment. *Sci. Tot. Environ.* **1996**, *185*, 171–175.
4. De Wolf, W.; Feijtel, T.C.J.F. Terrestrial Risk Assessment for Linear Alkylbenzene Sulfonate (LAS) in Sludge-Amended Soils. *Chemosphere* **1998**, *36* (6), 1319–1343.
5. Jensen, J. Fate and Effects of Linear Alkylbenzene Sulphonates (LAS) in the Terrestrial Environment-A Review *Sci. Tot. Environ.* **1990**, *226*, 93–111.
6. Jensen, J.; Lokke, H.; Holmstrup, M.; Krogh, P.H.; Elsgaard, L. Effects and Risk Assessment of Linear Alkylbenzene Sulfonates in Agricultural Soil. 5. Probabilistic Risk Assessment of Linear Alkylbenzene Sulfonates in Sludge-Manded Soils. *Environ. Toxicol. Chem.* **2001**, *20* (8), 1690–1697.
7. Fendinger, N.J.; Versteeg, D.J.; Weeg, E.; Dyer, S.; Rapaport, R.A. Environmental Behavior and Fate of Anionic Surfactants. In: Baker LA, editor. Environmental Chemistry of Lakes and Reservoirs. ACS Advances in Chemistry Series American Chemical Society **1994**, 528–57.
8. Versteeg, D.J.; Rawlings, J.M. Bioconcentration and Toxicity of Dodecylbenzene Sulfonate (C12LAS) to Aquatic Organisms Exposed in Experimental Streams. *Arch. Environ. Contam. Toxicol.* **2003**, *44*, 237–46.
9. Temara, A.; Carr, G.; Webb, S.; Versteeg, D.J.; Feijtel, T. Marine Risk Assesment: Linear Alkylbenzensulphonates (LAS) in North Sea. *Marine Pollution Bulletin* **2001** *42*(8), 635–42.
10. Sanderson, H.; Price, B.B.; Dyer, S.D.; DeCarvalho, A.J.; Robaugh, D.; Waite, S.W. et al. Occurrence and Impact Screening of Alkyl Sulfates and Alkyl Ethoxysulfates in River Sediments. *Sci. Total Environ.* **2005**.
11. DK-EPA. Environmental Project 615: Environmental and health assessment of substances in household detergents and cosmetic detergent products **2001**. http://www.mst.dk/udgiv/Publications/2001/87-7944-596-9/html/helepubl_eng.htm.
12. Matthijs, E.; Holt, M.S.; Kiewiet, A.; Rijs, G.B.J. Environmental monitoring for Linear Alkylbenzene Sulfonate, Alcohol Ethoxylate, Alcohol Ethoxy Sulfate and Soap. *Environ. Toxicol. Chem.* **1999**, *18,* 2634–44.

13. Guckert, J.B.; Walker, D.D.; Belanger, S.E. Environmental Chemistry for a Surfactant Ecotoxicology Study Supports Rapid Degradation of C12 Alkyl Sulfate in a Continuous-Flow Stream Mesocosm. *Environ. Chem. Toxicol.* **1996**, *15*, 262–9.

14. http://ec.europa.eu/enterprise/chemicals/legislation/detergents/docs/faq_detergent_regulation_march2009.pdf

15. http://ec.europa.eu/environment/waste/sludge/pdf/sludge_en.pdf

16. Schowanek, D.; David, H. et al. Probabilistic Risk Assessment for Linear Alkylbenzene Sulfonate (LAS) in Sewage Sludge Used on Agricultural Soil *Regulatory Toxicology and Pharmacology* **2007**, *49*, 245–259

17. Nimer M.; Ballesteros, O.; Navalón, A.; Crovetto, G.; Verge, C.; López, I.; Berna, J.L. Vílchez, J.L. *Anal. Bioanal. Chem.* **2007**, *387,* 2175.

18. Comellas L.; Portillo, J.L.; Vaquero, M.T. *J. Chromatogr. A* **1993**, *657*, 25.

19. Morales-Muñoz S.; Luque-García, J.L.; Luque de Castro, M.D. *J. Chromatogr. A* **2004**, *1026*, 41.

20. Lara-Martín P.A.; Gómez-Parra, A.; González-Mazo, E. *J. Chromatogr. A* **2006**, *1114*, 205.

21. Helaleh, M.I.H.; Al-Omair, A.; Nisar, A.; Gevao, B.; *J. Chromatogr. A* **2005**, *1083*, 153.

22. Dionex Corporation. *Determination of Phenols in Drinking and Bottled Mineral Waters Using Online Solid-Phase Extraction Followed by HPLC with UV Detection.* Application Note 191, LPN 1949-02, Sunnyvale, CA, 2008.

Sensitive Determination of Microcystins in Drinking and Environmental Waters

INTRODUCTION

Waterblooms of cyanobacteria (blue-green algae) can produce potent toxins that have become a severe problem for eutrophic aquatic environments. Hepatotoxins are among the primary toxins produced by these species growing in lakes, ponds, and rivers used as drinking water sources. Microcystins (structures shown in Figure 1) are hepatotoxins that exhibit tumor-promoting activity and are among the most commonly found cyanobacteria toxins. Microcystin contamination of drinking water at low nanomolar concentrations is considered a risk factor for cancer, and microcystin-LR has been associated with most of the incidents of toxicity involving microcystins. Therefore, the World Health Organization (WHO) has proposed a provisional guideline concentration of 1.0 µg/L for microcystin-LR in drinking water.[1]

The analytical approaches commonly used for microcystins include bioassay, chemical, and biochemical methods. Bioassays have been used in screening but were found to be non-specific and/or more time consuming. Biochemical methods, such as enzyme-linked immunosorbent assay (ELISA) and protein phosphatase inhibition assay (PPIA), are advantageous as screening methods due to their high sensitivity and ability to quickly treat a large number of samples; the disadvantage of these methods, however, is that they provide poor identification and have the potential for false positives. Reversed-phase high-performance liquid chromatography (HPLC) with UV detection, liquid chromatography mass spectrometry (LC-MS), and capillary electrophoresis are chemical methods that have been used for the identification and quantification of microcystins.[2]

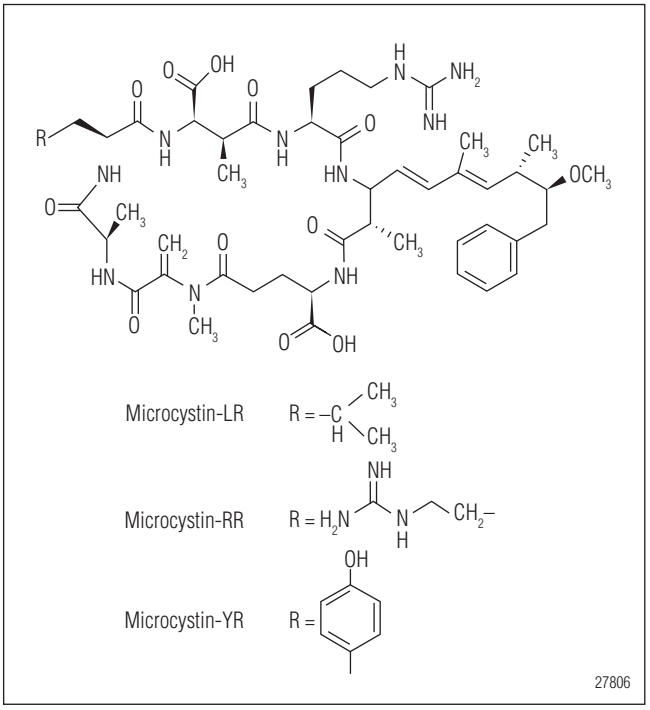


Figure 1. Structures of microcystins.

The control of microcystins at 1.0 µg/L levels requires sensitive analytical methods and HPLC methods have been widely used for this purpose. Solid-phase extraction (SPE) is one of the main methods for sample extraction and preconcentration; however, the authors of Reference 3 suggest that the typically used SPE stationary phase (C18) does not supply good selectivity for trace analysis.[3] Immunoaffinity columns (IAC) modified with anti-microcystin-LR monoclonal antibodies on polypropylene stationary phases have been used for

extraction with good selectivity for the HPLC analysis of microcystins,[3-5] but extensive use of this method is limited because an IAC is not commercially available for this application.

The authors have reported a simple, fast, and effective target-cut on-line SPE method followed by HPLC with UV detection on an UltiMate® 3000 HPLC system consisting of a dual gradient pump, autosampler, and column oven equipped with one 2p–6p valve for the determination of trace amounts of vitamin B_{12} added to beverages.[6, 7] This on-line SPE method is different from the typical one. The bound analyte on the SPE column is selectively eluted from the SPE column using a mobile phase gradient, just like the first dimension of a two-dimensional chromatography system. This reduces the number of interferences for sample analysis. While the SPE process is running, the analytical column is equilibrating. Just before the front portion of the analyte peak elutes from the SPE column, the SPE column is switched into the analytical flow path. As soon as the analyte is completely eluted from the SPE column, the SPE column is switched out of the analytical flow path and back to the SPE flow path. Therefore, only those interferences co-eluting with the analytes will enter the analytical column; thus, more interferences are removed. The volume of analyte cut from the SPE column is separated on the analytical column and detected by the UV detector. This target-cut on-line SPE method with dual function (analyte capture and partial separation) operates under automatic control of Chromeleon® Chromatography Data System (CDS) software and offers full automation, absence of operator influence, and strict process control, compared to a typical off-line SPE method.[8]

Here, the target-cut on-line SPE method followed by HPLC with UV detection was applied to the determination of three microcystins (-LR, -RR, and -YR) in drinking, tap, and lake water. The three target analytes were co-eluted from the first column using chromatographic conditions that eliminated as many interferences as possible; then the analytes were sent to the analytical flow path and separated on the second column using the same type of stationary phase under different chromatographic conditions. This design takes advantage of the separation power of both columns and may eliminate interferences more efficiently than typical on- and off-line SPE methods. An additional dual-valve design is easy to use and convenient for method development.

The UltiMate 3000 ×2 Dual HPLC system provides an efficient platform to fulfill the requirements of these designs. Sub-µg/L concentrations of microcystins-LR, -RR, and -YR spiked in water samples were determined, which exceeds the WHO requirement.

EQUIPMENT

Dionex UltiMate 3000 HPLC system including:

- DGP-3600A pump with SRD 3600 solvent rack with degasser
- WPS-3000TSL semiprep autosampler (with 2500 µL sample loop)*
- TCC-3200 Thermostatted Column Compartment equipped with two 2p–6p valves
- VWD-3400RS UV-vis detector
- Chromeleon software
- Orion 420A+ pH meter, Thermo Scientific

*The analytical version of the WPS-3000TSL Autosampler can also be converted and used for large-volume injection for on-line SPE. The procedure is the same as specified in Reference 6.

REAGENTS

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

Acetonitrile (CH_3CN) and methanol (CH_3OH), HPLC grade, Fisher

Potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), and phosphoric acid (H_3PO_4), 85% (analytical grade), SCRC, China

STANDARDS

100 µg of microcystins-LR (CAS 101043-37-2), -RR (CAS 111755-37-4), and -YR (CAS 101064-48-6), respectively, ≥ 95% (HPLC), Alexis Corporation

Prepare stock standard solutions with 50 µg/mL concentrations by dissolving the standards with 2000 µL of methanol. Prepare the standard solutions used for the calibration curve by making appropriate dilutions of the stock standard solutions with water.

SAMPLES

Tap water samples were collected at the Dionex Shanghai Applications Lab. The lake water sample was collected at Zhangjiang High-Science and Technology Park located in the Pudong District of Shanghai, China. Bottled spring water samples were purchased from a supermarket in Shanghai. These samples were filtered through a 0.45 µm membrane (Millex-HN) prior to injection.

CHROMATOGRAPHIC CONDITIONS

On-Line SPE

Column: Acclaim® PA2, 3 µm, 3.0 × 33 mm (P/N 066276)

Analytical

Column: Acclaim PA2, 3 µm, 3.0 × 150 mm (P/N 063705)

Column Temp.: 40 °C

Mobile Phase: For SPE:
A: 22.5 mM KH_2PO_4–2.5 mM K_2HPO_4 buffer (dissolve ~ 3.1 g of KH_2PO_4 and 0.44 g of K_2HPO_4 in 1 L of water)
B: CH_3CN
In gradient (Table 1)

For separation:
A: 0.05% (v/v) H_3PO_4 (dilute 0.6 mL of 85% H_3PO_4 to 1 L with water)
B: CH_3CN
In gradient (Table 1)

Valve-Switching: Table 1

Flow Rate: 0.7 mL/min for both SPE and separation

Injection Vol.: 2500 µL on the SPE column

UV Detection: Absorbance at 240 nm

Table 1. Gradients and Valve Switching for Target-Cut On-Line SPE and Separation

Time (min)	Right Pump (for Separation)			Left Pump (for On-Line SPE)			Valve Switching	
	Flow Rate (mL/min)	Solvent A Buffer (%)	Solvent B CH_3CN (%)	Flow Rate (mL/min)	Solvent A Buffer (%)	Solvent B CH_3CN (%)	Left	Right
0.00	0.7	85	15	0.7	80	20	6-1	1-2
5.00		—	—		80	20		—
6.95		—	—		—	—		6-1
7.00		85	15		65	35		—
7.35		—	—		—	—		1-2
7.50		—	—		20	80		—
8.50		—	—		20	80		—
8.60		—	—		80	20		—
12.0		41	59		—	—		—
12.1		85	15		—	—		—
15.0		85	15		80	20		—

Table 2. Gradient and Valve Switching for Traditional On-Line SPE and Separation

Time (min)	Right Pump (for Separation)			Left Pump (for On-Line SPE)			Valve Switching
	Flow Rate (mL/min)	Solvent A Buffer (%)	Solvent B CH_3CN (%)	Flow Rate (mL/min)	Solvent A Buffer (%)	Solvent B CH_3CN (%)	
0.00	0.7	80	20	0.7	80	20	1-2
5.00		80	20				6-1
6.00		—	—				1-2
9.00		50	50				
9.10		25	75				
11.0		25	75				
11.1		80	20				
12.0		80	20				

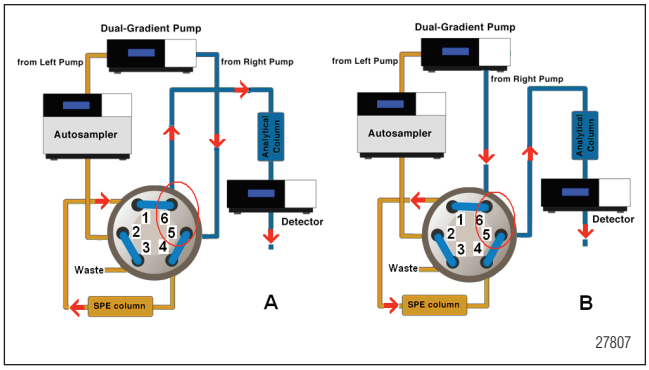


Figure 2. Flow schematics for A) traditional and B) target-cut on-line SPE methods equipped with one 2p–6p valve for sample preparation and analysis.

RESULTS AND DISCUSSION

Retention Behavior of Microcystins-RR, -YR, and -LR on the Acclaim PA2 Column

The Acclaim Polar Advantage II (PA2) is a polar-embedded column designed for enhanced hydrolytic stability within a wide range of pH values (pH 1.5 to 10), and compatibility with 100% aqueous mobile phases, overcoming the limitations of conventional C8 and C18 reversed-phase columns.

Effect of Buffer pH Value

The pH value of the mobile phase buffer may affect the retention of microcystins-RR, -YR, and -LR. Changes in their retention behavior on the Acclaim PA2 stationary phase were investigated. Experiments showed that when the buffer pH value decreased from pH 6.5 to 2.7, the retention time of microcystins-YR and -LR increased and the resolution between them improved, whereas the retention time of microcystin-RR did not change. The three microcystins were separated at a pH value lower than 2.5. They co-eluted at approximately pH 6.0.

Thus, for the requirements addressed here, the PA2 column is a good choice as an SPE column for concentrating the three microcystins from large-volume water samples (tap water and beverages) and co-eluting them using mobile phase buffer with a high pH value (~ 6.0). The PA2 column is also a good choice as an analytical column for the separation using a mobile phase buffer with a low pH value.

Effect of Column Temperature

The effect of column temperature on the retention of microcystins-RR, -YR, and -LR on the Acclaim PA2 stationary phase was investigated. Increasing column temperature may shorten the retention time, and is a benefit to the separation of microcystins-YR and -LR, which have close retention times. For example, resolution (R_s) between the two compounds increased from 0.50 to 1.94 when the column temperature increased from 25 to 40 ºC.

Comparison of Traditional and Target-Cut On-Line SPE Methods

The commonly used on-line SPE flow scheme (Figure 2A) couples the SPE column directly with the analytical HPLC column using one six-port (2p–6p) column valve. The filtered sample is injected directly onto the system and delivered to the SPE column for enrichment (1-2 position) using the left pump; the analytical column is equilibrated with the right pump at the same time. After the analytes are bound to the SPE column and impurities are washed out, the SPE column is switched into the analytical flow path to elute the bound analytes (6-1 position), then the analytes are separated on the analytical column and detected by the UV detector.

For the target-cut on-line SPE method, a small change in the flow scheme of the traditional on-line SPE mode reverses the flush direction on the SPE column (Figure 2B) and creates an on-line SPE system that can have a dual function to eliminate interferences more efficiently. The SPE process in this mode is different from that described in the traditional method. The bound analyte on the SPE column is selectively eluted from the SPE column using a mobile phase gradient, just like the first dimension of a two-dimensional chromatography system. As the SPE process (position 1-2) is running, the analytical column is equilibrating. Just before the front portion of the analyte peak elutes from the SPE column, the SPE column is switched into the analytical flow path (position 6-1). As soon as the analyte is completely eluted from the SPE column, the SPE column is switched out of the analytical flow path and back to SPE flow path (position 1-2). Therefore, only those interferences co-eluting with the analytes will enter the analytical column; thus, more interferences are removed.

SPE Column:	Acclaim PA2, 3 µm, 3.0 × 33 mm
Anal. Column:	Acclaim PA2, 3 µm, 3.0 × 150 mm
Column Temp.:	40 °C
Eluent for SPE:	CH_3CN–phosphate buffer (22.5 mM KH_2PO_4–2.5 mM K_2HPO_4) A. Isocratic: 20% CH_3CN: 80% phosphate buffer (v/v) B. In gradient: CH_3CN, 0–5 min, 20%; 7.0 min, 35%; 7.5–8.5 min, 80%; 8.6–15 min, 20%
Eluent for Separation:	CH_3CN–0.05% (v/v) H_3PO_4, in gradient A. CH_3CN, 0–5.0 min, 20%; 9.0 min, 50%; 9.1–11 min, 75%; 11.1–12 min, 20% B. CH_3CN, 0–7.0 min, 15%; 12 min, 59%; 12.1–15 min, 15%
Flow Rates:	0.7 mL/min for SPE and separation
Injection Vol.:	2500 µL on SPE column
Detection:	UV at 240 nm

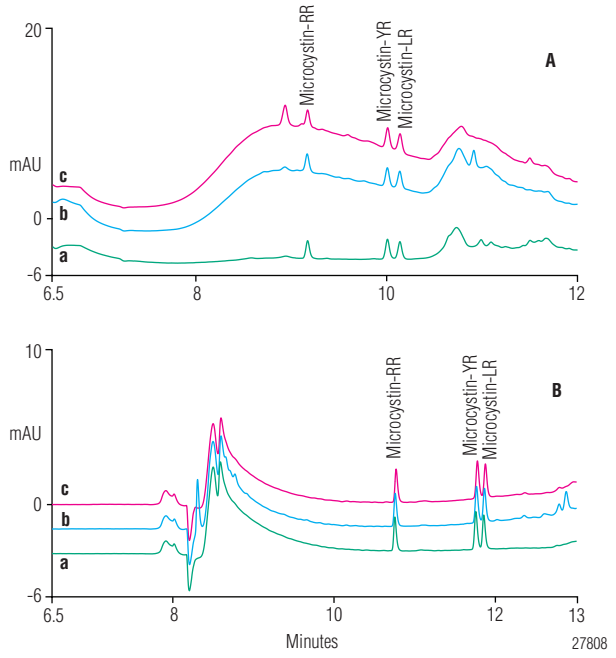


Figure 3. Chromatograms of a) bottled spring water, b) tap water, and c) lake water spiked with 1 µg/L each of microcystin-RR, -YR, and -LR standard using A) traditional and B) target-cut on-line SPE methods.

Figure 3 shows chromatograms of three types of water samples spiked with 1.0 µg/L each of microcystin-RR, -YR, and -LR standard using the traditional and target-cut on-line SPE methods, respectively. Tables 1 and 2 list the gradients and valve-switching times. Comparison of the two on-line SPE methods for analysis of different water samples demonstrates that the target-cut method may flush far fewer interferences to the analytical flow path, which is more efficient for analysis of the three microcystins in different water samples, whereas the traditional on-line SPE method is merely acceptable for the water samples.

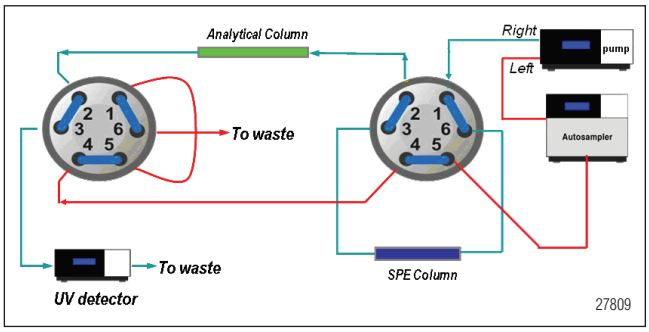


Figure 4. Flow schematic for the target-cut on-line SPE method equipped with two 2p–6p valves.

Position of Left Valve	Position of Right Valve	Description
1-2	1-2	Determine switching time of right valve during method development
6-1	1-2	Load sample and analysis
6-1	6-1	Transfer analytes from SPE column to analytical column

In practice, an additional 2p–6p valve may be used to construct a two-valve (2p–6p) system for convenient method development. The flow schematic of the two-valve configuration is shown in Figure 4. The left valve can be used to switch the SPE column or separation column into the flow path of the detector.

Evaluation of Microcystins Extraction Using the Target-Cut On-Line SPE Method

Configuration of Target-Cut Method

This newly developed on-line SPE method with dual function (analyte capture and partial separation) automatically controlled by Chromeleon software was used for analysis of vitamin B_{12}.[6,7] In that application, it was easy to configure the instrument and set the method parameters for target-cut mode because there was only a single target analyte and the same mobile phases were used for SPE and separation.

For samples containing more than one target analyte, the choice of target-cut method parameters is important for the success of the on-line SPE method. In theory, the ideal approach would be to cut the analytes one by one from the first stationary phase (SPE column) to the second stationary phase (analytical column), thereby minimizing the interferences entering the analytical flow path. This approach is not recommended, however, because it may result in a complicated valve-switching process and affect the separation on the analytical column.

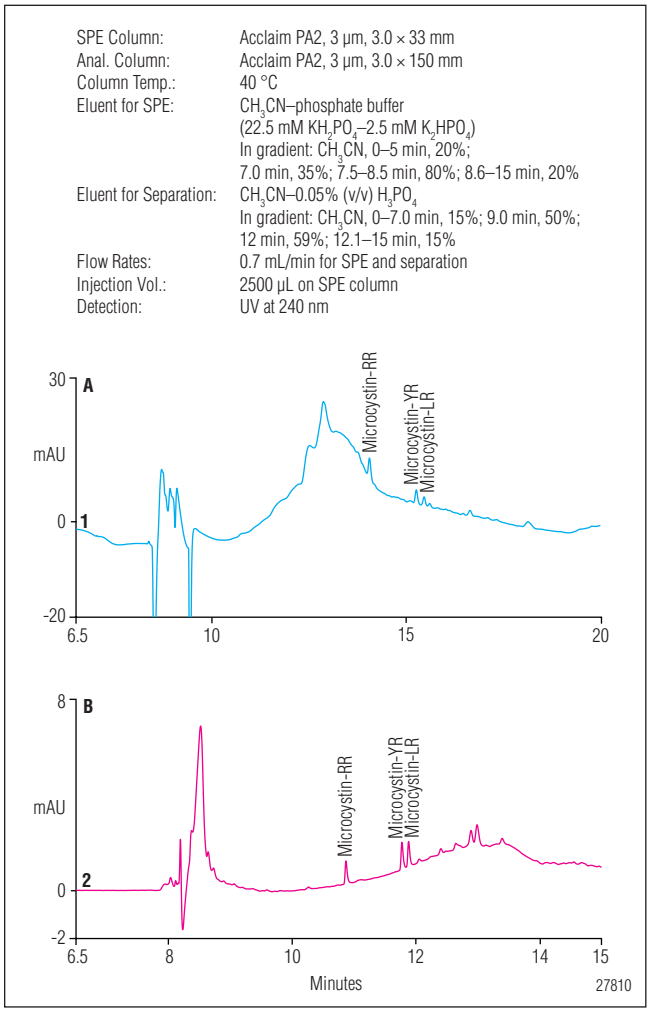


Figure 5. Chromatograms of a tap water sample spiked with 0.5 µg/L each of microcystin-RR, -YR, and -LR standard using different target-cut modes. A) Valve-switching starts from microcystin-RR and ends at microcystin-LR when they are eluted from the SPE column. B) The three microcystins elute together from the SPE column.

A simpler approach is to start the target-cut when the front shoulder of the first analyte peak is just eluting from the SPE column, then end when the tail of the last analyte peak elutes from the SPE column. This target-cut method is suitable for analytes with similar retention on the SPE column. For example, on the Acclaim PA2 SPE column, the retention times of microcystins-YR and -LR are similar but significantly different from that of microcystin-RR. For the determination of microcystins-RR, -YR, and -LR in a spiked tap water sample, the volume of cut analytes separated on the analytical column (Acclaim PA2 column) was large. As shown in Figure 5A, with the target-cut method, a large amount of interferences were still cut to the analytical flow path, which resulted in interference with the determination of microcystins at sub-µg/L concentrations.

The appropriate target-cut method for a sample containing several target analytes is to use a mobile phase that will elute the analytes together (as one chromatographic peak) from the SPE column and then send them to the analytical flow path. Because the volume of cut target analytes is much smaller than that obtained by the alternate method, the co-eluted interferences may be much less; if so, the elimination of interferences will be more efficient.

Using the same determination of microcystins-RR, -YR, and -LR in a spiked tap water sample, Figure 5B shows the target-cut method with a CH_3CN–phosphate buffer (pH 6.0) mobile phase to elute analytes from the SPE column, and the analytical column using CH_3CN–0.05% H_3PO_4 (v/v, pH 2.2) mobile phase. Figure 5B shows that this approach does, in fact, have fewer interferences. Note that if the valve-switching times are inaccurate, the difference between the two mobile phases may affect separation of the three microcystins. Therefore, correctly setting valve-switching times is key to success of the target-cut on-line SPE method.

Determination of Valve-Switching Times

Based on the target-cut method in which all three analytes are eluted from the SPE column together, the valve-switching times for the extraction of microcystins-RR, -YR, and -LR can be estimated using the following equation, which was applied to vitamin B_{12} analysis.[6]

$$t_{valve\text{-}switching\ 2} = t_{valve\text{-}switching\ 1} + (v_1/v_2) \times w_h$$

Where $t_{valve\text{-}switching\ 1}$ represents the first valve-switching time when the front shoulder of the analyte peak is just eluting from the SPE column at the flow rate for SPE; $t_{valve\text{-}switching\ 2}$ represents the second valve-switching time when the SPE column is switched out of the analytical flow path; v_1 and v_2 represent the flow rates for SPE and separation, respectively; and w_h represents baseline peak width (min) of analytes on the SPE column.

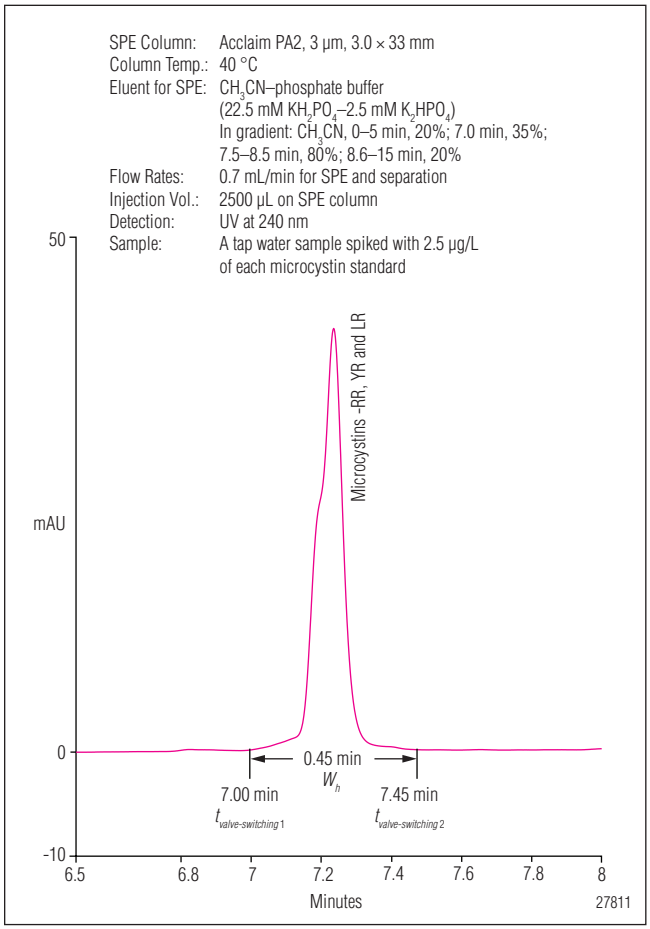


Figure 6. Chromatography to determine valve-switching time for the target-cut on-line SPE method based on the configuration showed in Figure 4.

Figure 6 shows the chromatogram of co-eluted microcystins-RR, -YR, and -LR on the SPE column. The front shoulder of the peak eluting from the SPE column at 0.7 mL/min (v_1) appears at 7.00 min ($t_{valve\text{-}switching\ 1}$). The peak is detected by the UV detector and the baseline peak width on the SPE column is 0.45 min (w_h). When the flow rate for the separation on the analytical column is also 0.7 mL/min (v_2), the second valve-switching time ($t_{valve\text{-}switching\ 2}$) calculated using the equation is 7.45 min.

The authors tried to have a slightly earlier $t_{valve\text{-}switching\ 1}$ and a delay in $t_{valve\text{-}switching\ 2}$ (0.10 min) to avoid losing microcystins when using different mobile phases for SPE and separation. Experiments showed that a 0.10 min delay in $t_{valve\text{-}switching\ 2}$ had no obvious effect, but 0.10 min earlier in $t_{valve\text{-}switching\ 1}$ resulted in the loss of microcystin-RR.

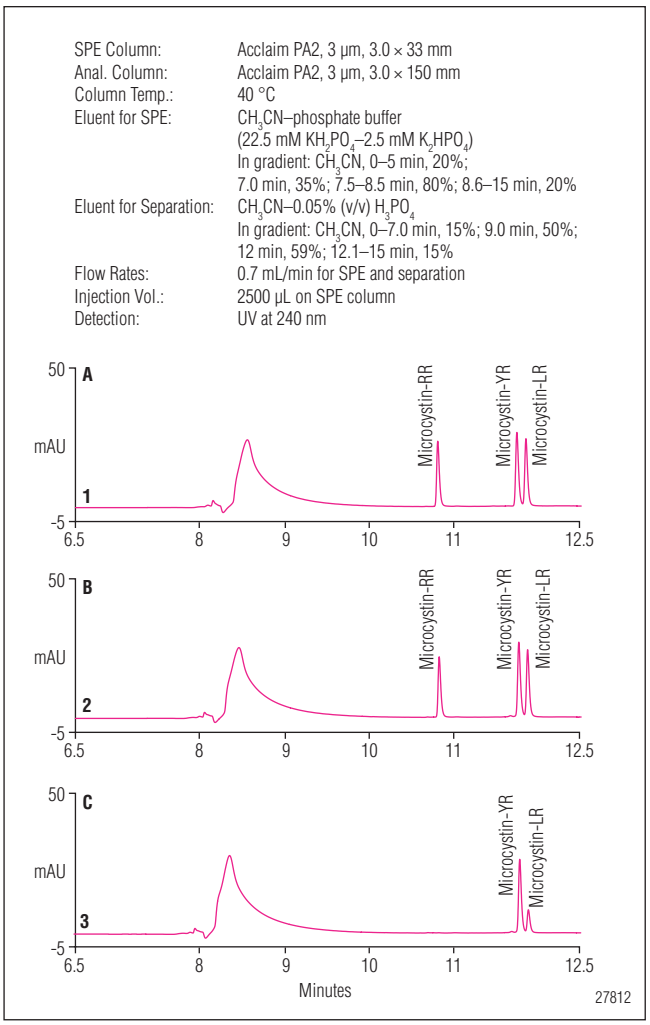


Figure 7. Chromatograms of a mixture of microcystin-RR, -YR, and -LR standards with concentration 1.0 µg/L for each extracted at different valve-switching times: A) $t_{valve\text{-}switching\ 1}$ = 7.00 min, B) $t_{valve\text{-}switching\ 1}$ = 6.90 min, and C) $t_{valve\text{-}switching\ 1}$ = 6.80 min.

As shown in Figure 7, when $t_{valve\text{-}switching\ 1}$ = 7.00 min, all three microcystins were well retained; with 0.10 min earlier ($t_{valve\text{-}switching\ 1}$ = 6.90 min), a small part of microcystin-RR was lost; and with just 0.20 min earlier ($t_{valve\text{-}switching\ 1}$ = 6.80 min), microcystin-RR was lost completely, and more than half of microcystin-LR was lost as well. The authors hypothesize that this analyte loss was due to the cut volume obtained by using the slightly earlier time (0.2 min), which brought mobile phase of higher pH value (pH 6.0) and higher proportion of organic solvent (CH_3CN) to the analytical flow path before the analytes; this resulted in a change of the intrinsic equilibrium of the analytical column that significantly affected analyte retention. Therefore, the control of valve-switching time $t_{valve\text{-}switching\ 1}$ must be accurate.

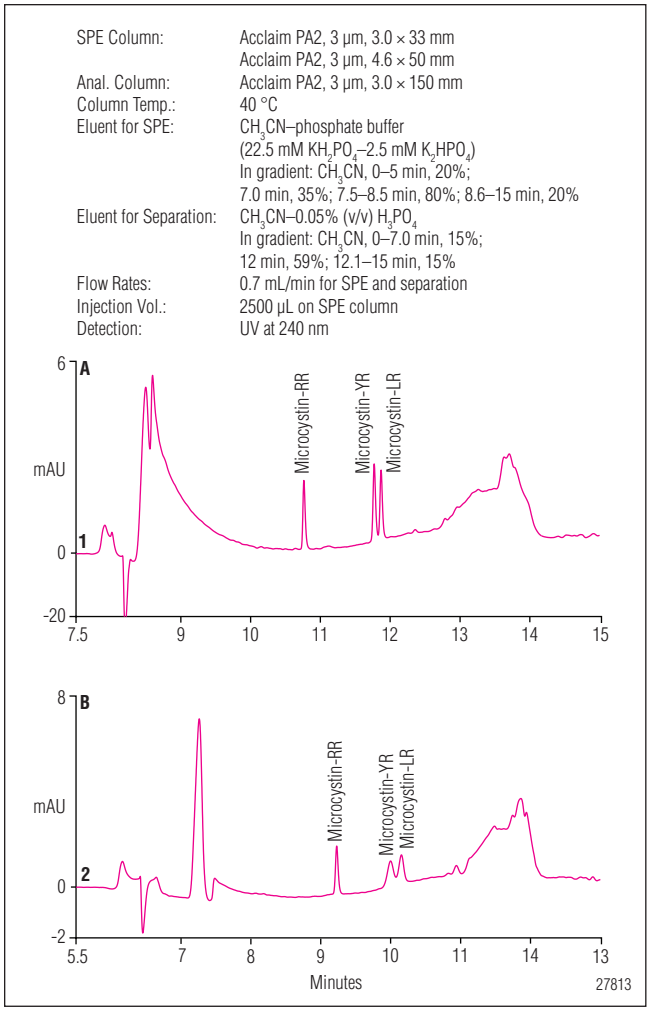


Figure 8. Chromatograms of a lake water sample spiked with 1.0 µg/L each of microcystin-RR, -YR, and -LR standard using different size SPE columns: A) Acclaim PA2, 3 µm, 3.0 × 33 mm column, and B) Acclaim PA2, 3 µm, 4.6 × 50 mm column with the target-cut on-line SPE method in Table 1.

Selection of SPE Column Format

The effect of SPE column size on elimination of impurities using the target-cut on-line SPE method was investigated. Two Acclaim PA2 columns with different sizes, 4.6 × 50 mm and 3.0 × 33 mm, were used for SPE. As shown in Figure 8, interference elimination was slightly better on the larger column, which can be attributed to separation on the larger column being more efficient than that on the smaller one; therefore, fewer impurities enter the analytical flow path. However, the larger column did have a significant effect on separation on the analytical column due to more mobile phase being cut from SPE to the analytical flow path, which resulted in poor peak shape and less detection sensitivity for microcystins-YR and -LR. Therefore, the 3.0 × 33 mm Acclaim PA2 column was selected as the SPE column for this application.

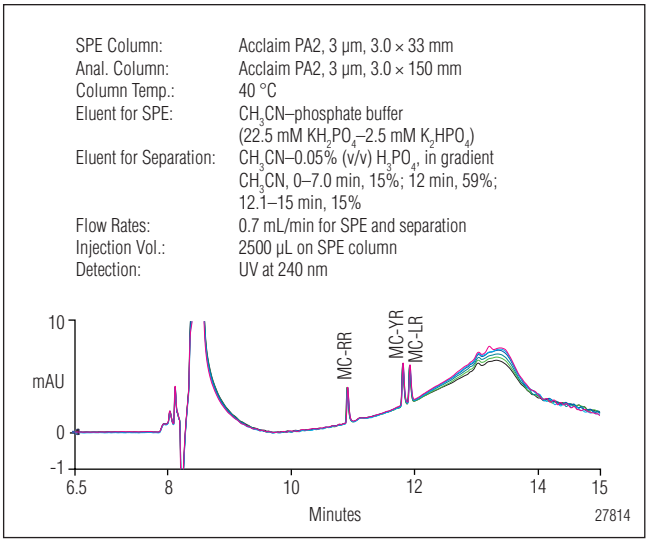


Figure 9. Overlay of chromatograms of six consecutive injections of a drinking water sample spiked with 0.5 µg/L each of microcystin-RR, -YR, and -LR standard using the target-cut on-line SPE method in Table 1.

Table 3. Reproducibility for Peak Retention Time and Area

Microcystins	Retention Time RSD	Peak Area RSD	Concentration of standard (µg/L)
RR	0.037	1.53	0.5
YR	0.028	1.59	0.5
LR	0.029	1.13	0.5

Method Reproducibility, Linearity, and Detection Limits

Method reproducibility was estimated by making six consecutive 2500 µL injections of a drinking water sample spiked with 0.5 µg/L of each microcystin standard. Retention time and peak area reproducibilities are summarized in Table 3 and show good precision. Figure 9 shows an overlay of chromatograms for the six consecutive injections.

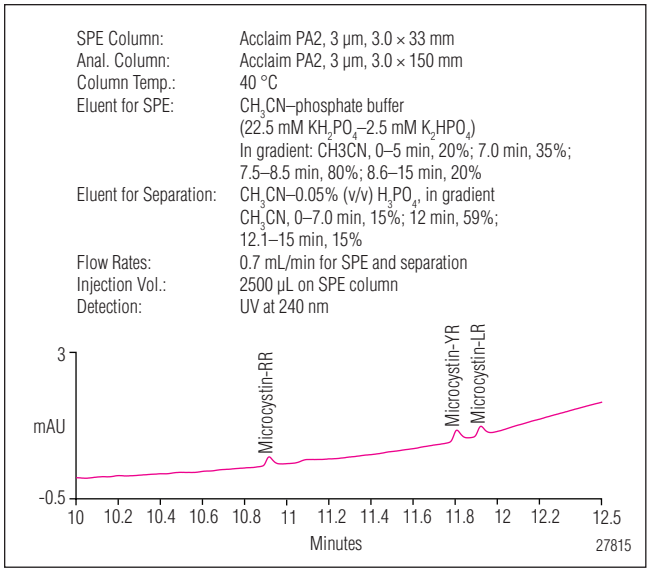


Figure 10. Chromatogram of a mixed solution with concentrations of 0.1 µg/L each of microcystin-RR, -YR, and -LR standard using the target-cut on-line SPE method in Table 1.

Calibration linearity for microcystins-RR, -YR, and -LR was investigated by making three consecutive injections of a mixed standard prepared at five different concentrations. The external standard method was used to establish the calibration curve and to quantify these microcystins in samples. Excellent linearity was observed from 0.1 to 10 µg/L when plotting concentration versus peak area. Figure 10 shows a chromatogram of the three microcystins with concentrations of 0.1 µg/L each. Table 4 reports the data from the calibration as calculated by Chromeleon software.

Detection limits were calculated using the equation:

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

Where S represents Standard Deviation (SD) of replicate analyses, n represents number of replicates, $t_{(n-1,\ 1-\alpha=0.99)}$ represents Student's value for the 99% confidence level with n – 1 degrees of freedom.

Method detection limits (MDL) were estimated using six consecutive injections of drinking water sample spiked with 0.5 µg/L of each microcystin standard to determine S (Table 4).

Table 4. Calibration Data and MDLs for Microcystins-RR, -YR, and -LR

Microcystin	Regression Equations	r (%)	Range of Standards µg/L	RSD for Calibration Curve	MDL* (µg/L)
RR	A = 0.0844 c - 0.0027	99.997	0.1–10	0.91	0.028
YR	A = 0.1054 c - 0.0022	99.994		1.25	0.028
LR	A = 0.0942 c + 0.0030	99.994		1.21	0.019

Note. * The single-sided Student's test method (at the 99% confidence limit) was used for determining MDL, where the standard deviation (SD) of the peak area of six injections is multiplied by 4.03 to yield the MDL.

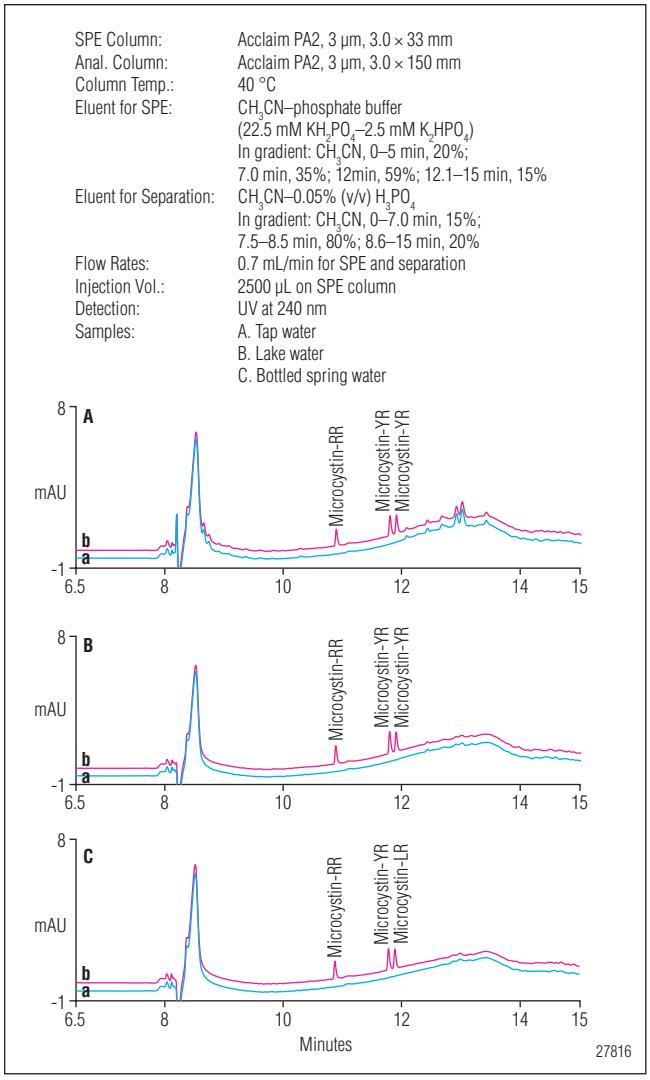


Figure 11. Overlay of chromatograms for a) water sample and b) the same sample spiked with 0.5 µg/L each of microcystin-RR, -YR, and -LR standard using the target-cut on-line SPE method in Table 1.

Sample Analysis

Figure 11 shows chromatograms of tap water, lake water, and bottled spring water samples, as well as the same samples spiked with 0.5 µg/L of each microcystin standard. None of the three samples had detectable microcystins. Recoveries for each standard in all three samples ranged from 92 to 100%, thus indicating that the analysis method is accurate (Table 5).

CONCLUSION

This work describes a target-cut on-line SPE method that can fully recover low concentrations (< 1 µg/L) of three microcystins (-RR, -YR, and -LR) when added to three different water samples. These concentrations are less than the maximum concentrations recommended by WHO. This method is fully automated and easily configured on an UltiMate 3000 ×2 Dual HPLC system.

Table 5. Analysis Results of Microcystins-RR, -YR, and -LR in the Samples

Sample	Tap Water				Lake Water				Bottled Spring Water			
Microcystin	Detected (µg/L)	Added (µg/L)	Found (µg/L)	Recovery (%)	Detected (µg/L)	Added (µg/L)	Found (µg/L)	Recovery (%)	Detected (µg/L)	Added (µg/L)	Found (µg/L)	Recovery (%)
RR	ND	0.50	0.48	96	ND	0.50	0.55	110	ND	0.50	0.49	98
YR	ND	0.50	0.46	92	ND	0.50	0.51	102	ND	0.50	0.48	96
LR	ND	0.50	0.48	96	ND	0.50	0.51	102	ND	0.50	0.49	98

Note: * ND = not detected

REFERENCES

Cyanobacterial Toxins: Microcystin-LR in Drinking-Water; Background Document for Development of WHO Guidelines for Drinking-Water Quality: World Health Organization, 2003.

Lee, H.S.; Jeong, C.K.; Lee, H.M.;Choi, S.J.; Do, K.S.; Kim, K.; Kim, Y.H. On-Line Trace Enrichment for the Simultaneous Determination of Microcystins in Aqueous Samples Using High-Performance Liquid Chromatography with Diode-Array Detection. ***J. Chromatogr. A*** **1999**, ***848***, 179.

Aguete, E.C.; Gago-Martínez, A.; Leão, J.M.; Rodríguez-Vázquez, J.A.; Menàrd, C.; Lawrence, J.F. HPLC and HPCE Analysis of Microcystins RR, LR, and YR Present in Cyanobacteria and Water by Using Immunoaffinity Extraction. ***Talanta***, **2003**, ***59***, 697.

Kondo, F.; Matsumoto, H.; Yamada, S.; Tsuji, K.; Ueno, Y.; Harada, K. Immunoaffinity Purification Method for Detection and Quantification of Microcystins in Lake Water. ***Toxicon***. **2000**, ***38***, 813.

Tsutsumi, T.; Nagata, S.; Hasegawa, A.; Ueno, Y. Immunoaffinity Column as Clean-Up Tool for Determination of Trace Amounts of Microcystins in Tap Water. ***Food Chem. Toxicol.*** **2000,** ***38***, 593.

Dionex Corporation, ***Determination of Vitamin B_{12} in Beverages Using On-Line SPE Followed by HPLC with UV Detection.*** Application Note 256, LPN 2574, 2010, Sunnyvale, CA.

Dionex Corporation, ***Determination of Water- and Fat-Soluble Vitamins by HPLC.*** Technical Note 89, LPN 2598, 2010, Sunnyvale, CA.

Sun, Q.L; Zhuang, S.Q. ***Determination of Vitamin B_{12} Using UPLC***; Application Summary for Waters Corporation: Milford, MA, 2010.

Column Selection Guide

Environmental Water Applications Notebook

Column Selection Guide

Silica Columns

			Reversed-Phase (RP)						Mixed-Mode			HILIC		Application-Specific						
			Acclaim 120 C18	Acclaim 120 C8	Acclaim 300 C18	Acclaim Polar Advantage (PA)	Acclaim Polar Advantage II (PA2)	Acclaim Phenyl-1	Acclaim Trinity P1	Acclaim Mixed-Mode WAX-1	Acclaim Mixed-Mode WCX-1	Acclaim Mixed-Mode HILIC-1	Acclaim HILIC-10	Acclaim Organic Acid	Acclaim Surfactant	Acclaim Explosives E1	Acclaim Explosives E2	Acclaim Carbamate	Example Applications
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	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

umn	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
ac 2A	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
ac I-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
ac I	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
ac	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
ac 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
ac 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
ac A-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
ac n	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
ac	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
ac A	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
ac	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	-

Transferring HPLC Methods to UHPLC

Environmental Water Applications Notebook

Easy Method Transfer from HPLC to RSLC with the Dionex Method Speed-Up Calculator

INTRODUCTION

The goal of every chromatographic optimization is a method that sufficiently resolves all peaks of interest in as short a time as possible. The evolution of packing materials and instrument performance has extended chromatographic separations to new limits: ultrahigh-performance liquid chromatography (UHPLC).

The new Dionex UltiMate® 3000 Rapid Separation LC (RSLC) system is ideal for ultrafast, high-resolution LC. The RSLC system was designed for ultrafast separations with flow rates up to 5 mL/min at pressures up to 800 bar (11,600 psi) for the entire flow-rate range. This industry-leading flow-pressure footprint ensures the highest flexibility possible; from conventional to ultrahigh-resolution to ultrahigh-speed methods. The RSLC system, with autosampler cycle times of only 15 seconds, oven temperatures up to 110 °C, and data collection rates up to 100 Hz (even when acquiring UV-Vis spectra), sets the standard for UHPLC performance. Acclaim® RSLC columns with a 2.2 µm particle size complete the RSLC dimension.

A successful transfer from an HPLC method to an RSLC method requires recalculation of the chromatographic parameters. Underlying chromatographic principles have to be considered to find the appropriate parameters for a method transfer. With the Method Speed-up Calculator, Dionex offers an electronic tool that streamlines the process of optimum method transfer. This technical note describes the theory behind the Method Speed-Up Calculator and the application of this interactive, multi-language tool, illustrated with an exemplary method transfer from a conventional LC separation to an RSLC separation. You may obtain a copy of this calculator from your Dionex representative.

METHOD SPEED-UP STRATEGY

The purpose of method speed-up is to achieve sufficient resolution in the shortest possible time. The strategy is to maintain the resolving power of the application by using shorter columns packed with smaller particles. The theory for this approach is based on chromatographic mechanisms, found in almost every chromatography text book. The following fundamental chromatographic equations are applied by the Method Speed-Up Calculator for the method transfer from conventional to ultrafast methods.

The separation efficiency of a method is stated by the peak capacity *P*, which describes the number of peaks that can be resolved in a given time period. The peak capacity is defined by the run time divided by the average peak width. Hence, a small peak width is essential for a fast method with high separation efficiency. The peak width is proportional to the inverse square root of the number of theoretical plates N generated by the column. Taking into account the length of the column, its efficiency can also be expressed by the height equivalent to a theoretical plate *H*. The relationship between plate height H and plate number *N* of a column with the length L is given by Formula 1.

Formula 1: $$N = \frac{L}{H}$$

Low height equivalents will therefore generate a high number of theoretical plates, and hence small peak width for high peak capacity is gained. Which factors define *H*? For an answer, the processes inside the column have to be considered, which are expressed by the Van Deemter equation (Formula 2).

Formula 2: $$H = A + \frac{B}{u} + C \cdot u$$

The Eddy diffusion *A* describes the mobile phase movement along different random paths through the stationary phase, resulting in broadening of the analyte band. The longitudinal diffusion of the analyte against the flow rate is expressed by the term *B*. Term *C* describes the resistance of the analyte to mass transfer into the pores of the stationary phase. This results in higher band broadening with increasing velocity of the mobile phase. The well-known Van Deemter plots of plate height *H* against the linear velocity of the mobile phase are useful in determining the optimum mobile phase flow rate for highest column efficiency with lowest plate heights. A simplification of the Van Deemter equation, according to Halász[1] (Formula 3), describes the relationship between column efficiency (expressed in plate height *H*), particle size d_p (in µm) and velocity of mobile phase *u* (in mm/s):

Formula 3: $$H = 2 \cdot d_p + \frac{6}{u} + \frac{d_p^2 \cdot u}{20}$$

The plots of plate height H against velocity u depending on the particle sizes dp of the stationary phase (see Figure 1, top) demonstrate visually the key function of small particle sizes in the method speed-up strategy: The smaller the particles, the smaller the plate height and therefore the better the separation efficiency. An efficiency equivalent to larger particle columns can be achieved by using shorter columns and therefore shorter run times.

Another benefit with use of smaller particles is shown for the 2 µm particles in Figure 1: Due to improved mass transfer with small particle packings, further acceleration of mobile phases beyond the optimal flow rate with minimal change in the plate height is possible.

Optimum flow rates and minimum achievable plate heights can be calculated by setting the first derivative of the Halász equation to zero. The optimal linear velocity (in mm/s) is then calculated by Formula 4.

Formula 4: $$u_{opt} = \sqrt{\frac{B}{C}} = \frac{10.95}{d_p}$$

The minimum achievable plate height as a function of particle size is calculated by insertion of Formula 4 in Formula 3, resulting in Formula 5.

Formula 5: $$H_{min} \approx 3 \cdot d_p$$

Chromatographers typically prefer resolution over theoretical plates as a measure of the separation quality. The achievable resolution *R* of a method is directly proportional to the square root of the theoretical plate number as can be seen in Formula 6. *k* is the retention factor of the analyte and *k* the selectivity.

Formula 6: $$R = \frac{1}{4} \cdot \sqrt{N} \cdot \frac{k_2}{1 + k_2} \cdot \frac{\alpha - 1}{\alpha}$$

If the column length is kept constant and the particle size is decreased, the resolution of the analytes improves. Figure 1, bottom, demonstrates this effect using 5 µm and 2 µm particles.

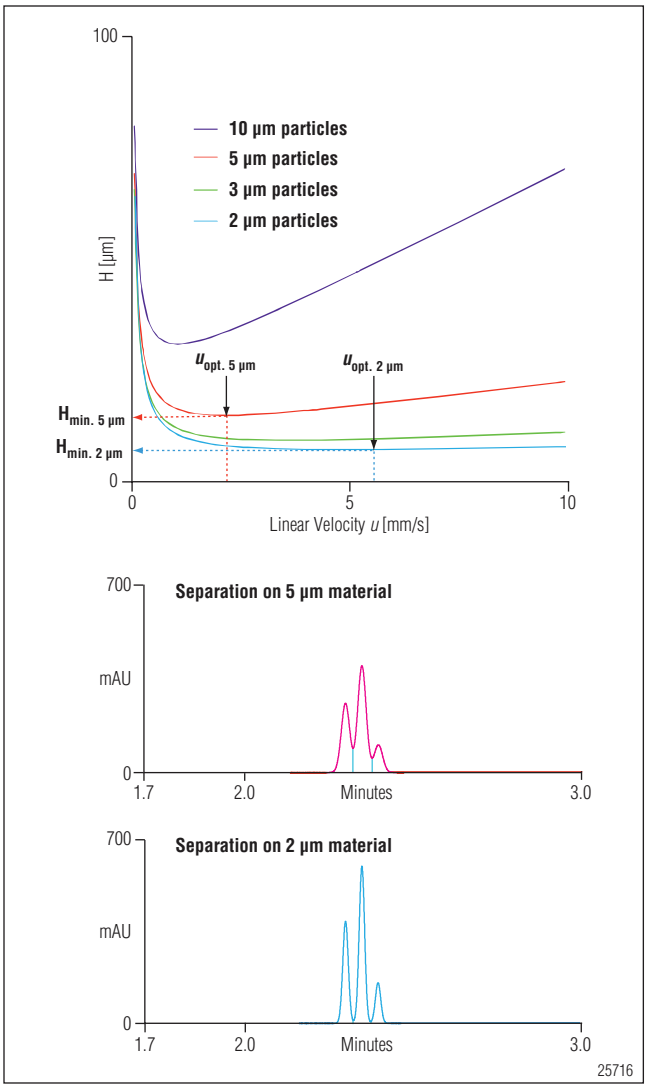


Figure 1. Smaller particles provide more theoretical plates and more resolution, demonstrated by the improved separation of three peaks (bottom) and smaller minimum plate heights H *in the Van Deemter plot (top). At linear velocities higher than uopt,* H *increases more slowly when using smaller particles, allowing higher flow rates and therefore faster separations while keeping separation efficiency almost constant. The speed-up potential of small particles is revealed by the Van Deemter plots (top) of plate height* H *against linear velocity u of mobile phase: Reducing the particle size allows higher flow rates and shorter columns because of the decreased minimum plate height and increased optimum velocity. Consequently, smaller peak width and improved resolution are the result (bottom).*

When transferring a gradient method, the scaling of the gradient profile to the new column format and flow rate has to be considered to maintain the separation performance. The theoretical background was introduced by L. Snyder[2] and is known as the gradient volume principle. The gradient volume is defined as the mobile phase volume that flows through the column at a defined gradient time t_G. Analytes are considered to elute at constant eluent composition. Keeping the ratio between the gradient volume and the column volume constant therefore results in a correct gradient transfer to a different column format.

Taking into account the changed flow rates F and column volume (with diameter d_c and length L), the gradient time intervals t_G of the new methods are calculated with Formula 7.

Formula 7: $$t_{G,new} = t_{G,old} \cdot \frac{F_{old}}{F_{new}} \cdot \frac{L_{new}}{L_{old}} \cdot \left(\frac{d_{c,new}}{d_{c,old}} \right)^2$$

An easy transfer of method parameters can be achieved by using the Dionex Method Speed-Up Calculator (Figure 2), which incorporates all the overwhelming theory and makes manual calculations unnecessary. This technical note describes the easy method transfer of an example separation applying the calculator. Just some prerequisites described in the following section have to be taken into account.

PREREQUISITES

The Method Speed-Up Calculator is a universal tool and not specific for Dionex products. Nevertheless, some prerequisites have to be considered for a successful method transfer, which is demonstrated in this technical note by the separation of seven soft drink additives.

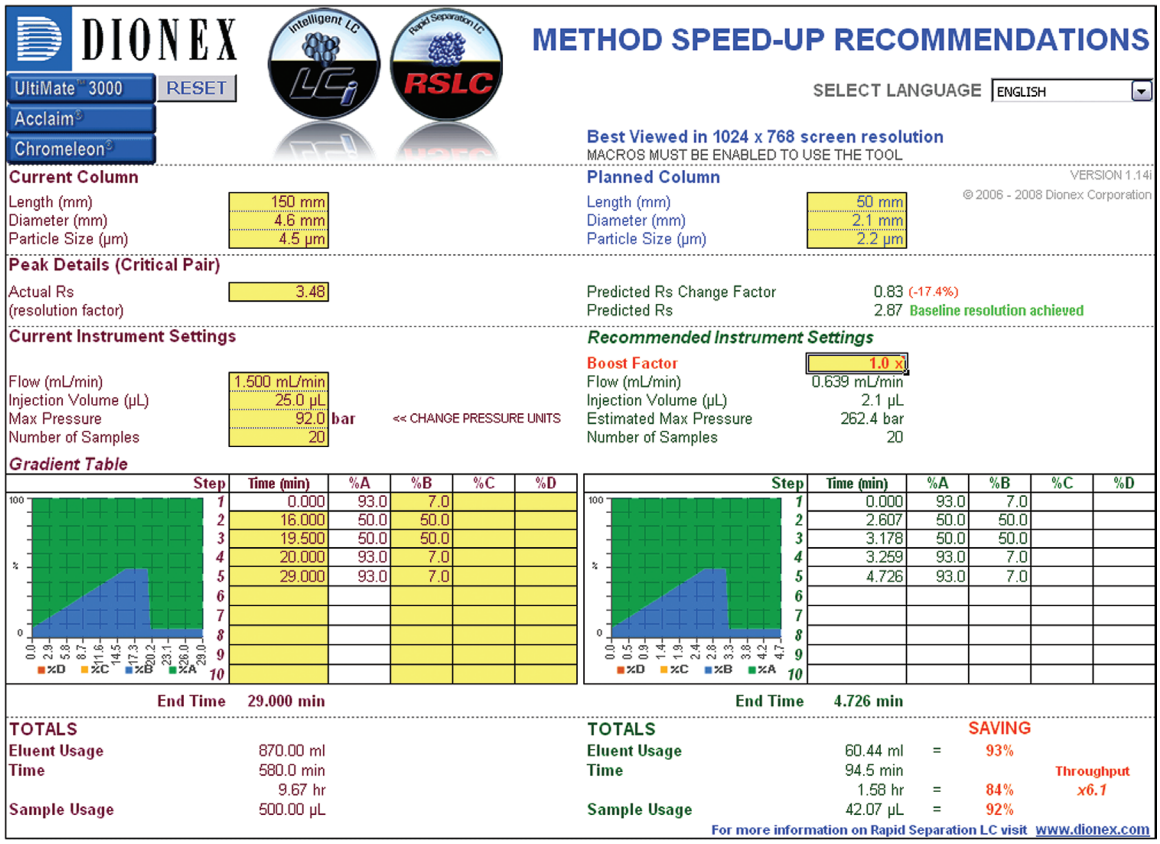


Figure 2. The Dionex Method Speed-Up Calculator transfers a conventional (current) HPLC method to a new (planned) RSLC method.

Column Dimension

First, the transfer of a conventional method to an RSLC method requires the selection of an adequate column filled with smaller particles. The RSLC method is predicted best if the selectivity of the stationary phase is maintained. Therefore, a column from the same manufacturer and with nominally identical surface modification is favoured for an exact method transfer. If this is not possible, a column with the same nominal stationary phase is the best choice. The separation is made faster by using shorter columns, but the column should still offer sufficient column efficiency to allow at least a baseline separation of analytes. Table 1 gives an overview of the theoretical plates expected by different column length and particle diameter size combinations using Dionex Acclaim column particle sizes. Note that column manufacturers typically fill columns designated 5 µm with particle sizes 4–5 µm. Dionex Acclaim 5 µm columns are actually filled with 4.5 µm particles. This is reflected in the table.

Table 1. Theoretical Plates Depending on Column Length and Particle Diameter (Calculated Using Formula 5)

	Theoretical Plates N		
Particle size	4.5 µm	3 µm	2.2 µm
Column length: 250 mm	18518	27778	37879
150 mm	11111	16667	22727
100 mm	7407	11111	15152
75 mm	5555	8333	11364
50 mm	3703	5556	7576

If the resolution of the original separation is higher than required, columns can be shortened. Keeping the column length constant while using smaller particles improves the resolution. Reducing the column diameter does not shorten the analysis time but decreases mobile phase consumption and sample volume. Taking into account an elevated temperature, smaller column inner diameters reduce the risk of thermal mismatch.

System Requirements

Smaller particles generate higher backpressure. The linear velocity of the mobile phase has to be increased while decreasing the particle size to work within the Van Deemter optimum. The UltiMate 3000 RSLC system perfectly supports this approach with its high maximum operation pressure of 800 bar (11,600 psi). This maximum pressure is constant over the entire flow rate range of up to 5 mL/min, providing additional potential to speed up applications even further by increasing the flow rate.

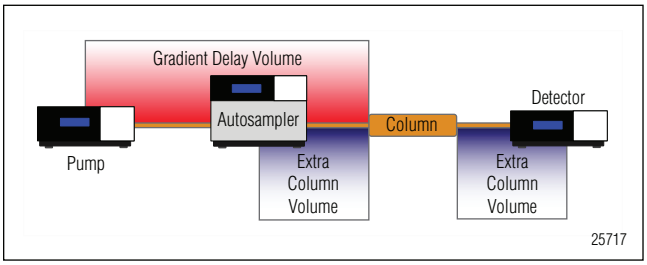


Figure 3. Gradient delay volume and extra column volume of an HPLC system. Both play an important role in method speed-up.

For fast gradient methods, the gradient delay volume (GDV) plays a crucial role. The GDV is defined as the volume between the first point of mixing and the head of the column. The GDV becomes increasingly important with fast, steep gradients and low flow rate applications as it affects the time taken for the gradient to reach the head of the column. The larger the GDV, the longer the initial isocratic hold at the beginning of the separation. Typically, this leads to later peak elution times than calculated. Early eluting peaks are affected most. In addition, the GDV increases the time needed for the equilibration time at the end of a sample and therefore increases the total cycle time. A general rule is to keep the gradient steepness and the ratio of GDV to column volume constant when transferring a standard method into a fast LC method. This will maintain the selectivity of the original method.[3]

The GDV can be adjusted to the column volume by installing appropriate mixer kits to the RSLC pump (see Table 2), which contributes most to the GDV. Typically, 100 µL or 200 µL mixers are good starting points when operating a small volume column in an RSLC system.

Another option is to switch the sample loop of the split-loop autosampler out of the flow path. The GDV is then reduced by the sample loop volume in the so-called bypass mode. The GDV of a standard sample loop of the RSLC autosampler is 150 µL, the micro injection loop has a 50 µL GDV.

Table 2. Mixer Kits Available for UltiMate 3000 RSLC System to Adapt GDV of Pump

Mixer Kit	GDV pump
Mixer kit 6040.5000	35 µL
Static mixer kit 6040.5100	100 µL
Static mixer kit 6040.5150	200 µL

Besides the gradient delay volume, the extra column volume is an important parameter for fast LC methods. The extra column volume is the volume in the system through which the sample passes and hence contributes to the band broadening of the analyte peak (Figure 3). The extra column volume of an optimized LC system should be below $^{1}/_{10}$th of the peak volume. Therefore the length and inner diameter of the tubing connections from injector to column and column to detector should be as small as possible. Special care has to be taken while installing the fittings to avoid dead volumes. In addition, the volume of the flow cell has to be adapted to the peak volumes eluting from the RSLC column. If possible, the flow cell detection volume should not exceed $^{1}/_{10}$th of the peak volume.

Detector Settings

When transferring a conventional method to an RSLC method, the detector settings have a significant impact on the detector performance. The data collection rate and time constant have to be adapted to the narrower peak shapes. In general, each peak should be defined by at least 30 data points. The data collection rate and time constant settings are typically interrelated to optimize the amount of data points per peak and reduce short-term noise while still maintaining peak height, symmetry, and resolution.

The Chromeleon® Chromatography Management Software has a wizard to automatically calculate the best settings, based on the input of the minimum peak width at half height of the chromatogram. This width is best determined by running the application once at maximum data rate and shortest time constant. The obtained peak width may then be entered into the wizard for optimization of the detection settings. Refer to the detector operation manual for further details.

METHOD SPEED-UP USING THE CALCULATOR

Separation Example

Separation was performed on an UltiMate 3000 RSLC system consisting of a HPG-3200RS Binary Rapid Separation Pump, a WPS-3000RS Rapid Separation Well Plate Sampler with analytical sample loop (100 µL), a TCC-3000RS Rapid Separation Thermostatted Column Compartment with precolumn heater (2 µL), and a VWD-3400RS Variable Wavelength Detector with semi-micro flow cell (2.5 µL). Chromeleon Chromatography Management Software (version 6.80, SR5) was used for both controlling the instrument and reporting the data. The modules were connected with stainless steel micro capillaries, 0.01" ID, $^{1}/_{16}$" OD when applying the conventional LC method, 0.007" and 0.005" ID, $^{1}/_{16}$" OD when applying the RSLC methods. A standard mixture of seven common soft drink additives was separated by gradient elution at 45 °C on two different columns:

- Conventional HPLC Column: Acclaim 120, C18, 5 µm, 4.6 × 150 mm column, (P/N 059148)
- Rapid Separation Column: Acclaim RSLC 120, C18, 2.2 µm, 2.1 × 50 mm column (P/N 068981).

The UV absorbance wavelength at 210 nm was recorded at 5 Hz using the 4.6 × 150 mm column and at 25 Hz and 50 Hz using the 2.1 × 50 mm column. Further method details such as flow rate, injection volume, and gradient table of conventional and RSLC methods are described in the following section. The parameters for the method transfer were calculated with the Dionex Method Speed-Up Calculator (version 1.14i).

The conventional separation of seven soft drink additives is shown in Figure 4A. With the Method Speed-Up Calculator, the method was transferred successfully to RSLC methods (Figure 4B and C) at two different flow rates. The easy method transfer with this universal tool is described below.

Column Selection for Appropriate Resolution

The column for method speed-up must provide sufficient efficiency to resolve the most critical pairs. In this example, separating peaks 5 and 6 is most challenging. A first selection of the planned column dimensions can be made by considering the theoretical plates according to Table 1. The 4.6 × 150 mm, 5 µm column is actually filled with 4.5 µm particles. Therefore, it provides 11,111 theoretical plates. On this column, the

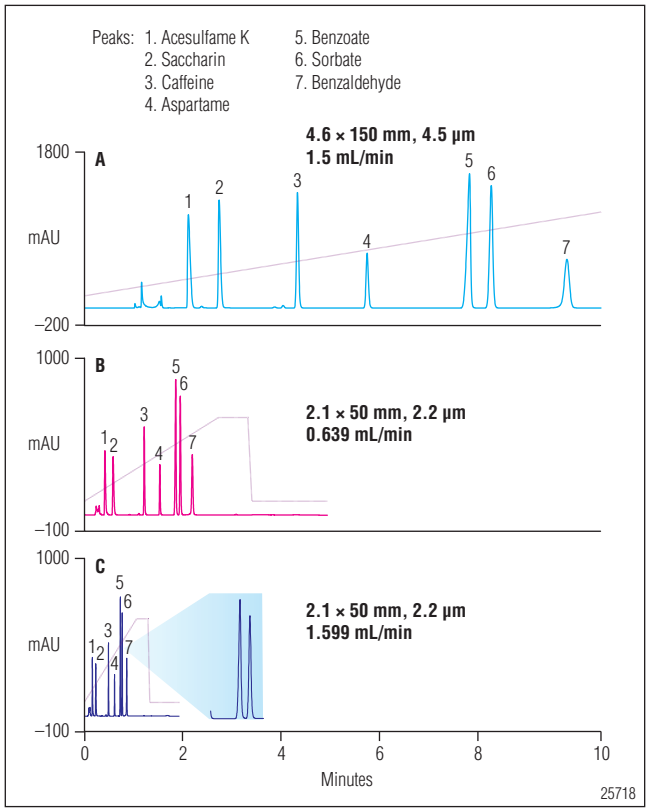


Figure 4. Method transfer with the Method Speed-Up Calculator from A) a conventional LC separation on an Acclaim 5 µm particle column, to B) and C) RSLC separations on an Acclaim 2.2 µm particle column.

resolution is $R_{(5,6)}$=3.48. This resolution is sufficiently high to select a fast LC column with fewer theoretical plates for the speed up. Therefore, a 2.1 × 50 mm, 2.2 µm column with 7579 plates was selected.

The first values to be entered into the yellow field of the Method Speed-Up Calculator are the current column dimension, planned column dimension, and the resolution of the critical pair. To obtain the most accurate method transfer, use the particle sizes listed in the manufacturer's column specifications sheet instead of the nominal size, which may be different. Dionex Acclaim columns with a nominal particle size of 5 µm are actually filled with 4.5 µm particles, and this value should be used to achieve a precise method transfer calculation. This has a positive impact on the performance and pressure predictions for the planned column. Based on the assumption of unchanged stationary phase chemistry, the calculator then predicts the resolution provided by the new method (Figure 5).

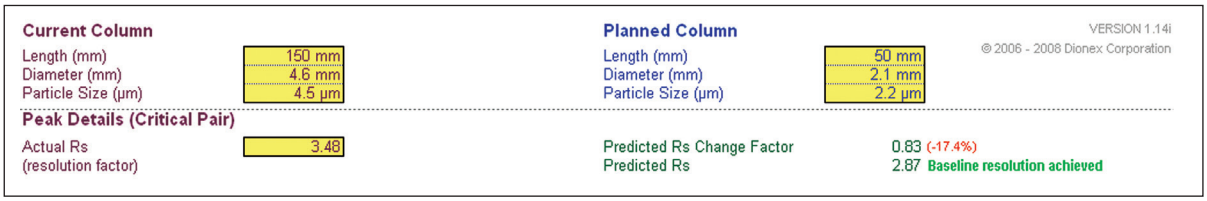


Figure 5. Column selection considering the resolution of the critical pair.

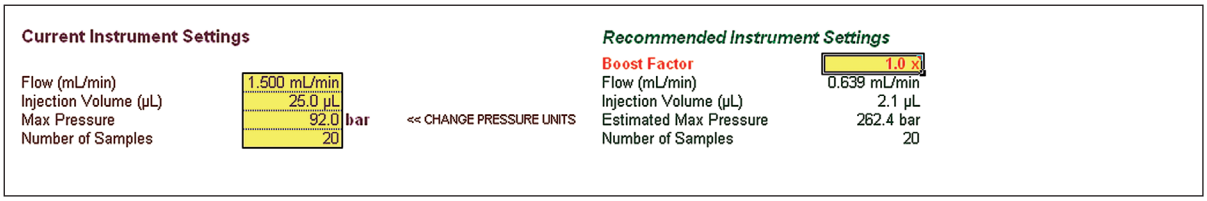


Figure 6. The flow rate, injection volume and backpressure of the current method are scaled to the new column dimension.

In the example in Figure 5, the predicted resolution between benzoate and sorbate is 2.87. With a resolution of $R \geq 1.5$, the message "Baseline resolution achieved" pops up. This indicates that a successful method transfer with enough resolution is possible with the planned column. If R is smaller than 1.5, the red warning "Baseline is not resolved" appears. Note that the resolution calculation is performed only if the boost factor BF is 1, otherwise it is disabled. The function of the boost factor is described in the Adjust Flow Rate section.

Instrument Settings

The next section of the Method Speed-Up Calculator considers basic instrument settings. These are flow rate, injection volume, and system backpressure of the current method (Figure 6). In addition to these values, the detector settings have to be considered as described in the earlier section "Detector Settings". Furthermore, the throughput gain with the new method can be calculated if the number of samples to be run is entered.

Adjust Flow Rate

As explained by Van Deemter theory, smaller particle phases need higher linear velocities to provide optimal separation efficiency. Consequently, the Dionex Method Speed-Up Calculator automatically optimizes the linear velocity by the ratio of particle sizes of the current and planned method. In addition, the new flow rate is scaled to the change of column cross section if the column inner diameter changed. This keeps the linear velocity of the mobile phase constant. A boost factor (*BF*) can be entered to multiply the flow rate for a further decrease in separation time. If the calculated resolution with $BF=1$ predicts sufficient separation, the method can be accelerated by increasing the boost factor and therefore increasing the flow rate. Figure 1 shows that applying linear velocities beyond the optimum is no problem with smaller particle phases, as they do not significantly loose plates in this region. Note that the resolution calculation of the Method Speed-Up Calculator is disabled for $BF \neq 1$.

For the separation at hand, the flow rate is scaled from 1.5 mL/min to 0.639 mL/min when changing from an Acclaim 4.6 × 150 mm, 4.5 µm column to a 2.1 × 50 mm, 2.2 µm column (see Figure 6), adapting the linear velocity to the column dimensions and the particle size. The predicted resolution between peak 5 and 6 for the planned column is $R=2.87$. The actual resolution achieved is $R=2.91$, almost as calculated (chromatogram B in Figure 4).

A Boost Factor of 2.5 was entered for further acceleration of the method (Figure 7). The method was then performed with a flow rate of 1.599 mL/min, and resolution of the critical pair was still sufficient at $R=2.56$ (see zoom in chromatogram C in Figure 4).

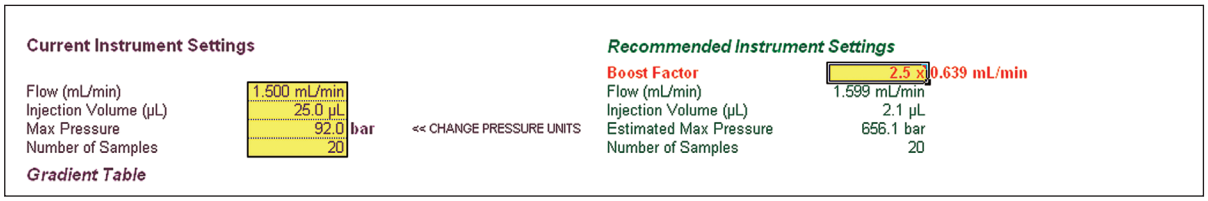


Figure 7. The new flow rate is further accelerated by applying the Boost Factor of 2.5.

Scale Injection Volume

The injection volume has to be adapted to the new column dimension to achieve similar peak heights by equivalent mass loading. Therefore the injection plug has to be scaled to the change of column cross section. In addition, shorter columns with smaller particles cause a reduced zone dilution. Consequently, sharper peaks compared to longer columns are expected. The new injection volume $V_{inj,new}$ is then calculated by Formula 8, taking a changed cross section and reduced band broadening by changed particle diameter into account.

Formula 8: $$V_{inj,new} = V_{inj,old} \cdot \left(\frac{d_{c,new}}{d_{c,old}}\right)^2 \cdot \sqrt{\frac{L_{new} \cdot d_{p,new}}{L_{old} \cdot d_{p,old}}}$$

Generally, it is recommended that a smaller flow cell be used with the RSLC method to minimize the extra column volume. Also, the difference in path length of different flow cell sizes has to be taken into account while scaling the injection volume. In the example of the soft drink analysis, the injection volume is scaled from 25 µL to 2.1 µL when replacing the Acclaim 4.6 × 150 mm, 4.5 µm column with a 2.1 × 50 mm, 2.2 µm column (see Figure 6).

Predicted Backpressure

Speeding-up the current method by decreasing particle size and column diameter and increasing flow rate means elevating the maximum generated backpressure. The pressure drop across a column can be approximated by the Kozeny-Carman formula.[4] The pressure drop of the new method is predicted by the calculator considering changes in column cross section, flow rate, and particle size and is multiplied by the boost factor. The viscosity of mobile phase is considered constant during method transfer. The calculated pressure is only an approximation and does not take into account nominal and actual particle size distribution depending on column manufacturer. If the predicted maximum pressure is above 800 bar (11,600 psi) the warning "Exceeds pressure limit RSLC" is shown, indicating the upper pressure limit of the UltiMate 3000 RSLC system. However, in the case the method is transferred to a third party system, its pressure specification has to be considered.

In the example of the soft drink analysis, the actual pressure increases from 92 bar to 182 bar with *BF*=1 on the 2.1 × 50 mm column, and to 460 bar for the RSLC method with *BF*=2.5. The pressures predicted by the Method Speed-Up Calculator are 262 bar and 656 bar, respectively. The pressure calculation takes into account the change of the size of the column packing material. In a speed up situation, the pressure is also influenced by other factors such as particle size distribution, system fluidics pressure, change of flow cell, etc. When multiplication factors such as the boost factor are used, the difference between calculated and real pressure is pronounced. The pressure calculation is meant to give an orientation, what flow rates might be feasible on the planned column. However, it should be confirmed by applying the flow on the column.

Adapt Gradient Table

The gradient profile has to be adapted to the changed column dimensions and flow rate following the gradient-volume principle. The gradient steps of the current method are entered into the yellow fields of the gradient table. The calculator then scales the gradient step intervals appropriately and creates the gradient table of the new method.

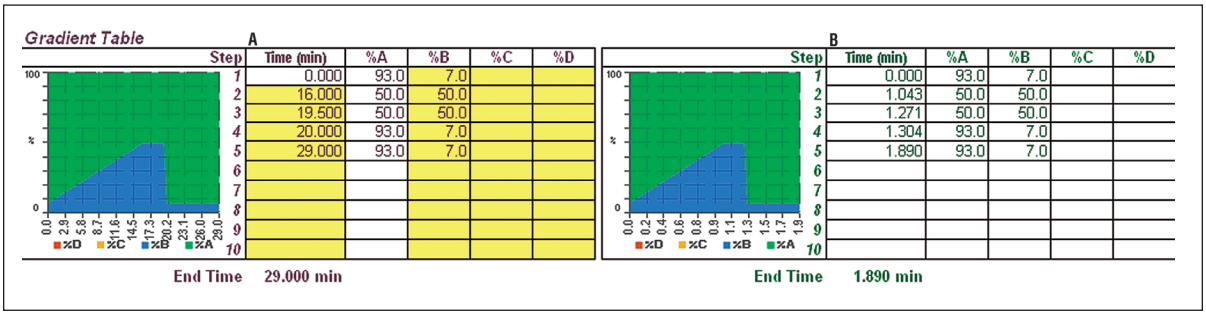


Figure 8. The gradient table of the current method (A) is adapted to the boosted method (B) according to the gradient-volume principle.

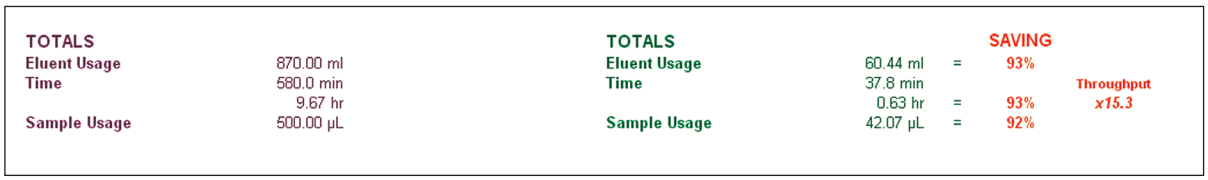


Figure 9. The absolute values for analysis time, eluent usage, and sample usage of the current (purple) and planned (green) method are calculated by the Method Speed-Up Calculator. The savings of eluent, sample, and time due to the method transfer are highlighted.

The adapted gradient table for the soft drink analysis while using a boost factor *BF*=1 is shown in Figure 8. According to the gradient-volume principle, the total run time is reduced from 29.0 min to 4.95 min by taking into account the changed column volume from a 4.6 × 150 mm, 5 µm (4.5 µm particles entered) to a 2.1 × 50 mm, 2.2 µm column and the flow rate reduction from 1.5 mL/min to 0.639 mL/min. The separation time was further reduced to 1.89 min by using boost factor *BF*=2.5. Gradient time steps were adapted accordingly. The comparison of the peak elution order displayed in Figure 4 shows that the separation performance of the gradient was maintained during method transfer.

Consumption and Savings

Why speed-up methods? To separate analyte peaks faster and at the same time reduce the mobile phase and sample volume consumption. Those three advantages of a method speed-up are indicated in the Method Speed-Up Calculator sheet right below the gradient table. The absolute values for the time, eluent, and sample usage are calculated taking the numbers of samples entered into the current instrument settings section of the calculation sheet into account (see Figure 6).

Regarding the soft drink analysis example, geometrical scaling of the method from the conventional column to the RSLC method means saving 93% of eluent and 92% of sample. The sample throughput increases 6.1-fold using *BF*=1. The higher flow rate at *BF*=2.5 results in a 15.3-fold increased throughput compared to the conventional LC method (Figure 9).

CONCLUSION

Fast method development or increased sample throughput are major challenges of most analytical laboratories. A systematic method speed-up is accomplished by reducing the particle size, shortening the column length, and increasing the linear velocity of the mobile phase. The Dionex Method Speed-Up Calculator automatically applies these rules and scales the conventional LC parameters to the conditions of the RSLC method. The interactive electronic tool is universally applicable. New instrument settings are predicted and gradient tables are adapted for optimum performance for the new method. The benefit of the method transfer is summarized by the integrated calculation of savings in time, eluent and sample. In addition, users can benefit from getting results earlier and thereby reducing the time to market. The Dionex Method Speed-Up Calculator is part of Dionex's total RSLC solution, which further consists of the industry leading UltiMate 3000 RSLC system, powerful Chromeleon Chromatography Management Software, and high-efficiency Acclaim RSLC columns.

REFERENCES

1. Halász, I.; Endele, R.; Asshauer, J. *J. Chromatogr., A* **1975,** *112,* 37–60.
2. Snyder, L.R.; Dolan, J.W.; Grant, J.R. *J. Chromatogr., A* **1979,** *165,* 3–30.
3. Schellinger, A.P.; Carr, P.W. *J. Chromatogr., A* **2005,** *1077,* 110-119.
4. Bear, J. *Dynamics of Fluids in Porous Media;* Dover: Mineola, NY, 1988.

Analyst is a registered trademark of Applied Biosystems.
Hydromatrix is a trademark of Varian Associates, Inc.
Jasco is a registered trademark of Jasco, Inc.
Milli-Q and Millex are registered trademarks of Millipore Corporation.
QTRAP is a registered trademark and TurboV is a trademark of AB SCIEX.
Roundup is a registered trademark of Monsanto Company.
Soxtec is a trademark of FOSS.
Thiofluor is a trademark of Pickering Laboratories, Inc.
Vespel and Tefzel are registered trademarks of E. I. du Pont de Nemours & Co.

All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

Dionex Products: 1228 Titan Way, PO Box 3603, Sunnyvale, CA 94088-3603, (408) 737-0700
North America: U.S./Canada (847) 295-7500
South America: Brazil (55) 11 3731 5140
Europe: Austria (43) 616 51 25, Benelux (31) 20 683 9768 (32) 3 353 4294
Denmark (45) 36 36 90 90, France (33) 1 39 30 01 10, Germany (49) 61125 991 0
Ireland (353) 644 0064, Italy (39) 02 51 62 1267, Sweden (46) 8 473 3380,
Switzerland (41) 62 205 9966, United Kingdom (44) 1276 691722
Asia Pacific: Australia (61) 2 9420 5233, China (852) 2428 3282, India (91) 22 2764 2735,
Japan (81) 6885 1213, Korea (82) 2 2653 2580, Singapore (65) 6289 1190,
Taiwan (886) 2 875 6655

www.thermoscientific.com/dionex

AN70051_E 04/12S

Now sold under the
Thermo Scientific brand

Thermo
SCIENTIFIC