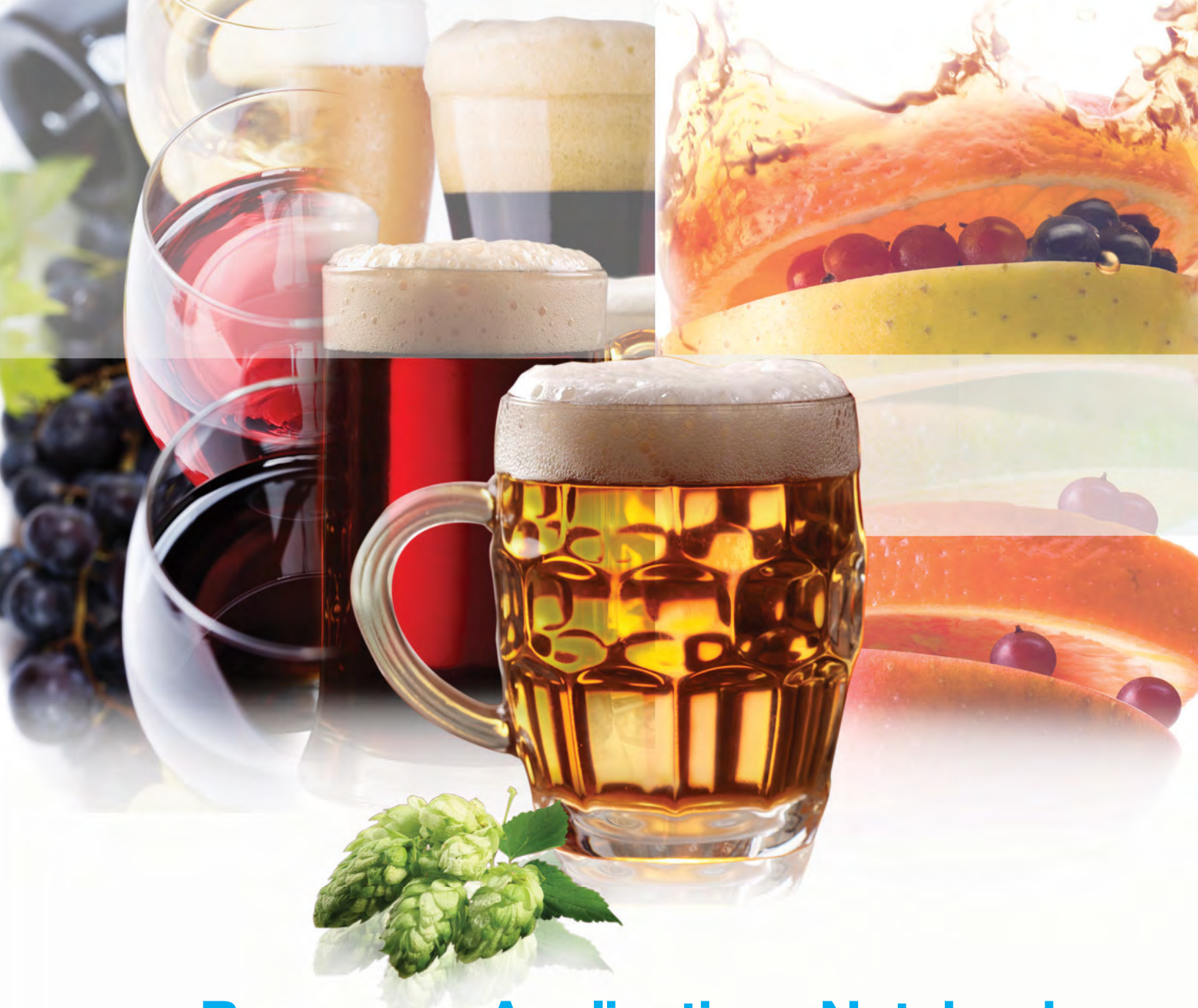

Beverages Applications Notebook

Alcoholic Beverages

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Introduction to Beverages

The global beverage industry is growing each year with the introduction of new products, such as vitamin-fortified water, energy drinks, anti-aging water, and herbal nutritional supplements. With this growth, come many more analytical challenges. These challenges are compounded by the continuing and new needs to analyze classic favorites such as sodas, fruit juices, milk drinks, alcoholic beverages, and bottled water. One such example would be the melamine contamination in milk and infant milk formula.

For all beverages, the compositional quality and safety must be monitored to help track contamination, adulteration, product consistency, and to ensure regulatory compliance from raw ingredients (water, additives, and fruits) to the final product.

Thermo Fisher Scientific is a recognized leader in providing analytical solutions for sample preparation, liquid chromatography for compositional testing, and chromatography data management for compliance and quality testing of beverages. From inorganic ions, organic acids, biogenic amines, glycols and alcohols, carbohydrates and sugar alcohols, to vitamins, additives, and sugar substitutes, we are unique in our commitment to provide fast, accurate testing and labeling information for all applications in this industry.

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 liquid chromatography (LC), ion chromatography (IC), and sample preparation are together in one portfolio with those in mass spectrometry (MS). Combining Dionex's leadership in chromatography with Thermo Scientific's leadership position in mass spec, a new range of powerful and simplified workflow solutions now becomes possible.

For more information on how the new lineup 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

UltiMate 3000 UHPLC+ Systems

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

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

- Superior chromatographic performance
- UHPLC design philosophy throughout nano, standard analytical, and rapid separation liquid chromotography (RSLC)
- 620 bar (9,000 psi) and 100 Hz data rate set a new benchmark for basic and standard analytical systems
- RSLC systems go up to 1000 bar and data rates up to 200 Hz
- ×2 Dual System for increased productivity solutions in routine analysis
- Fully UHPLC compatible advanced chromatographic techniques
- Thermo Scientific Dionex Viper and nanoViper–the first truly universal, fingertight fitting system even at UHPLC pressures

Thermo Fisher Scientific is the only HPLC company uniquely focused on making UHPLC technology available to all users, all laboratories, and for all analytes.

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

RSLCnano Systems: The Rapid Separation nano LC System (RSLCnano) provides the power for high-resolution and fast chromatography in nano, capillary, and micro LC.

Standard LC Systems: Choose from a wide variety of standard LC systems for demanding LC applications at nano, capillary, micro, analytical, and semipreparative flow rates.

Basic LC Systems: UltiMate 3000 Basic LC Systems are UHPLC compatible and provide reliable, high-performance solutions to fit your bench space and your budget.

IC and RFIC Systems

A complete range of ion chromatography solutions for all customer performance and price requirements

For ion analysis, nothing compares to a Thermo Fisher Scientific ion chromatography system. Whether you have just a few samples or a heavy workload, whether your analytical task is simple or challenging, we have a solution to match your needs and budget. And with your IC purchase, you get more than just an instrument—you get a complete solution based on modern technology and world-class support.

- Thermo Scientific Dionex ICS-5000: The world's first capillary IC system
- Dionex ICS-2100: Award-winning integrated Reagent-Free™ IC system
- Dionex ICS-1600: Standard integrated IC system
- Dionex ICS-1100: Basic integrated IC system
- Dionex ICS-900: Starter line IC system

Ranging from the Dionex ICS-900 to the ICS-5000, these IC systems cover the entire range of IC needs and budgets and come with superior support and service worldwide.

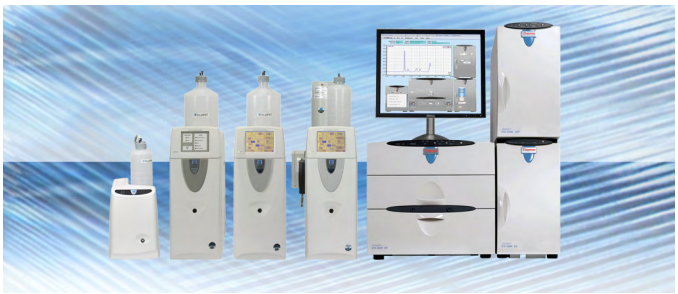
Dionex ICS-5000: Developed with flexibility, modularity, and ease-of-use in mind, the Dionex ICS-5000 combines the highest sensitivity with convenience

Dionex ICS-2100: An integrated Reagent-Free IC (RFIC™) system for electrolytically generated isocratic and gradient separations with conductivity detection, now with electrolytic sample preparation.

Dionex ICS-1600: The Dionex ICS-1600 combines high sensitivity with convenience. Now ready for eluent regeneration, with available dual-valve configuration for automated sample preparation.

Dionex ICS-1100: With dual-piston pumping and electrolytic suppression. Now ready for eluent regeneration, with available dual-valve configuration for automated sample preparation.

Dionex ICS-900: Can routinely analyze multiple anions and cations in 10–15 min—fully automated with Displacement Chemical Regeneration (DCR).



MS Instruments

Single-point control and automation for improved ease-of-use in LC/MS and IC/MS

Thermo Fisher Scientific provides advanced integrated IC/MS and LC/MS solutions with superior ease-of-use and modest price and space requirements. UltiMate 3000 System Wellness technology and automatic MS calibration allow continuous operation with minimal maintenance. The Dionex ICS-5000 instrument and the family of RFIC systems automatically remove mobile phase ions for effort-free transition to MS detection.

- Thermo Scientific MSQ Plus mass spectrometer, the smallest and most sensitive single quadrupole on the market for LC and IC
- Self-cleaning ion source for low-maintenance operation
- Thermo Scientific Dionex Chromeleon Chromatography Data System software for single-point method setup, instrument control, and data management
- Compatible with existing IC and LC methods
- The complete system includes the MSQ Plus™ mass spectrometer, PC datasystem, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) probe inlets, and vaccum system

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

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

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Chromeleon 7 Chromatography Data System Software

The fastest way to get from samples to results

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

- Enjoy a modern, intuitive user interface designed around the principle of operational simplicity
- Streamline laboratory processes and eliminate errors with eWorkflows, which enable anyone to perform a complete analysis perfectly with just a few clicks
- Access your instruments, data, and eWorkflows instantly in the Chromeleon Console
- Locate and collate results quickly and easily using powerful built-in database query features
- Interpret multiple chromatograms at a glance using MiniPlots
- Find everything you need to view, analyze, and report data in the Chromatography Studio
- Accelerate analyses and learn more from your data through dynamic, interactive displays
- Deliver customized reports using the built-in Excel-compatible speadsheet

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

The Cobra™ integration wizard uses an advanced mathematical algorithm to define peaks. This ensures that noise and shifting baselines are no longer a challenge in difficult chromatograms. When peaks are not fully resolved, the SmartPeaks™ integration assistant visually displays integration options. Once a treatment is selected, the appropriate parameters are automatically included in the processing method.

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

Process Analytical Systems and Software

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

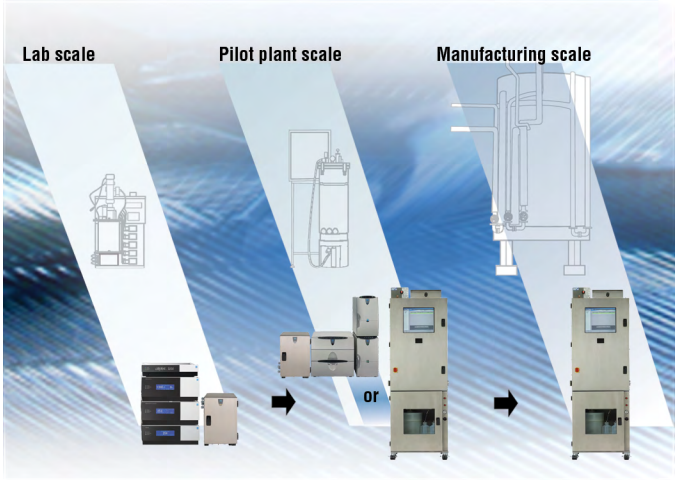
Our process analytical systems provide timely results by moving liquid chromatography-based measurements on-line. Information from the Thermo Scientific Dionex Integral process analyzer can help reduce process variability, improve efficiency, and reduce downtime. These systems provide comprehensive,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

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

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

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

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



Automated Sample Preparation

ACCELERATED SOLVENT EXTRACTORS

Two new solvent extraction systems with pH-hardened Dionium components

We offer two solvent extraction systems. The Thermo Scientific Dionex ASE 150 Accelerated Solvent Extractor is an entry-level system with a single extraction cell, for laboratories with modest throughput. The Dionex ASE™ 350 system is a sequential extraction system capable of automated extraction of up to 24 samples. Both systems feature chemically inert Dionium components that allow the extraction of acid- or base-pretreated samples.

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SOLID-PHASE EXTRACTION SYSTEMS

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

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

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

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

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

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

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Analysis of Alcoholic Beverages

Organic Acids in Wine

ANALYTES

In elution order: Citric, tartaric, malic, succinic, lactic, fumaric, acetic.

INTRODUCTION

Determining the levels of organic acids is an important part of fermentation monitoring during winemaking. The balance of the acids directly influences the flavor and color of the wine, and must also be carefully controlled to assure proper fermentation and to prevent spoilage.

The concentration of these organic acids are most important to the winery. In order of importance, they are:

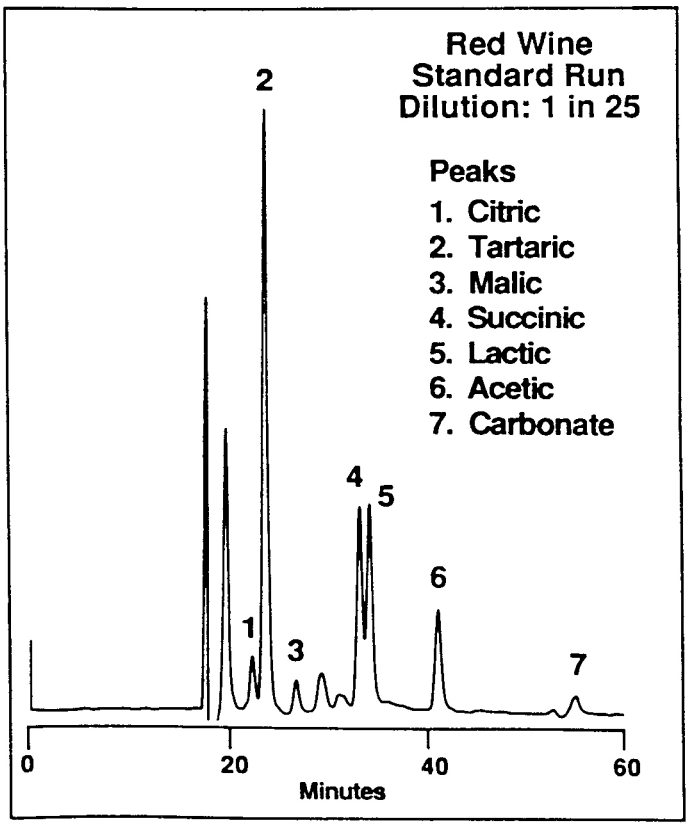
- Malic Acid – determined to measure the progress of malo-lactic fermentation.
- Acetic Acid – monitored to prevent spoilage; concentration must be determined for BATF regulations.
- Citric Acid – added to adjust acidity and to chelate metals; concentration must be determined for export limits.
- Tartaric Acid – added to lower the pH and adjust acidity; concentration is useful for deacidification and might be applied to cold stability testing.
- Fumaric Acid – added to prevent malo-lactic fermentation and adjust acidity (additions should be checked as fumaric is difficult to dissolve).

DISCUSSION OF METHOD

Organic acids in wine are determined by ion exclusion chromatography with chemical eluant suppression and conductivity detection. Since these organic acids do not contain UV chromophores, conductivity detection is far more sensitive than UV detection. Also, conductivity detection is more selective as there is no large phenolic front with red wines.

Included in the method are two sets of conditions. The Standard Run separates all the organic acids in 45 minutes. This run can be used for both routine monitoring and for enology research. The Fast Run elutes the acids in 15 minutes; however, lactic and malic acids are not resolved. (During both runs, carbonate elutes well after the last organic acid (acetic). It is not necessary to wait for carbonate to elute before the next injection is made as it will elute before the first peak in the second injection.)

After continued use, metals or other cations may build up on the column producing a decrease in resolution. The column can be regenerated by pumping 1 N HCl through the column for one hour, followed by a DI water rinse.



CONDITIONS

Standard Run

Separator Column:	Two HPICE-AS1 Columns in Series
Column Temperature:	40° C
Sample Loop Volune:	50 µL
Eluant:	2 mM Octanesulfonic Acid in 2% 2-propanol
Eluant Flow Rate:	0.5 mL/min.
Suppressor:	Anion MicroMembrane (AMMS-ICE)
Regenerant:	5 mM TBAOH
Regenerant Flow Rate:	2 to 3 mL/min.

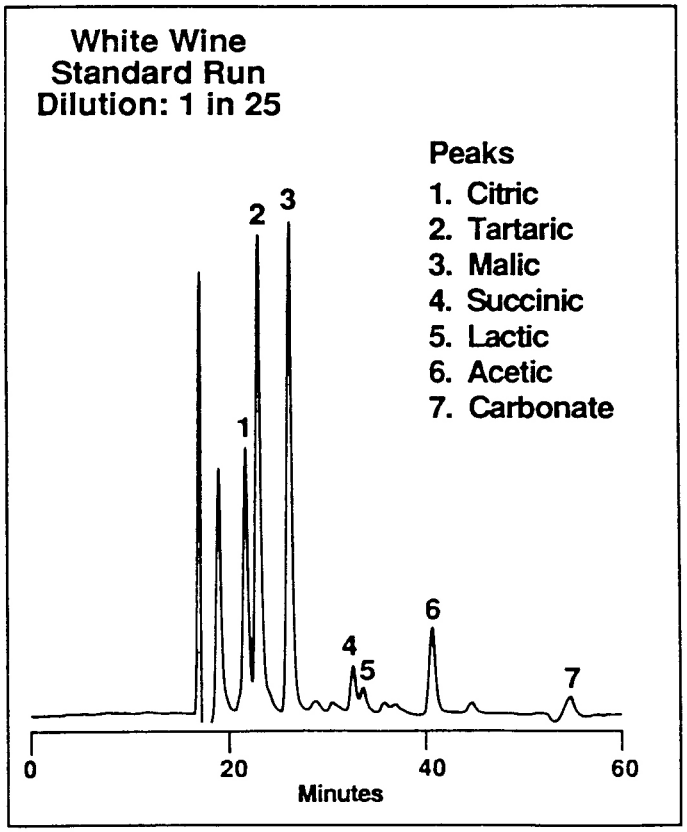
Conductivity Detector Range: 30 µS

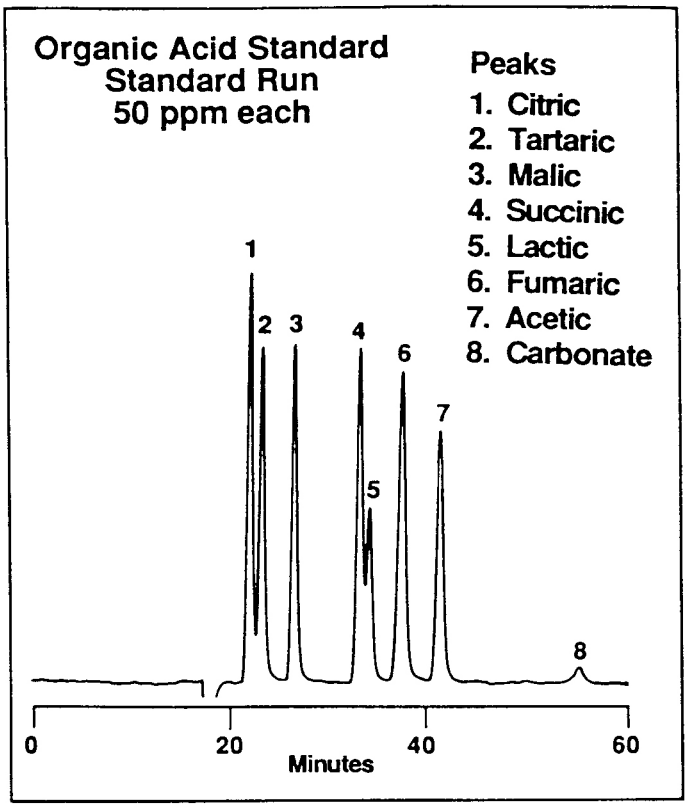
Expected Background Conductivity: 80 to 90 µS

Fast Run

same as standard run except the following:

Separator Column:	One HPICE-AS1
Column Temperature:	30° C
Eluant Flow Rate:	0.8 mL/min.





SOLUTIONS AND REAGENTS

Eluant: 2 mM Octanesulfonic Acid in 2% 2-propanol.

Dilute 20.0 mL of Dionex 0.10 M octanesulfonic acid MPIC-CR2 Reagent (P/N 35362) and 20 mL of high purity 2-propanol per liter of 18 M ohm DI water.

Regenerant: 5 mM TBAOH

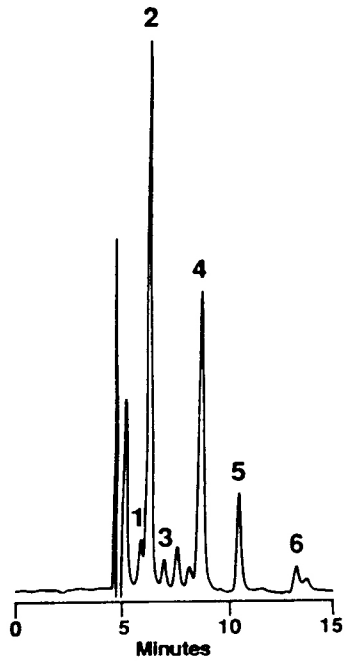
Dilute 10 mL of 55% TBAOH (tetrabutylammonium hydroxide) per 4 liters of 18 M ohm DI water.

Sample Preparation:

Filter wine through an 0.45 micron membrane and pass through a C18 pre-sep cartridge. The pre-sep cartridge should be activated with 5 mL methanol followed with 5 mL DI water. Dilute wine 1 in 33 or 1 in 25 with DI water.

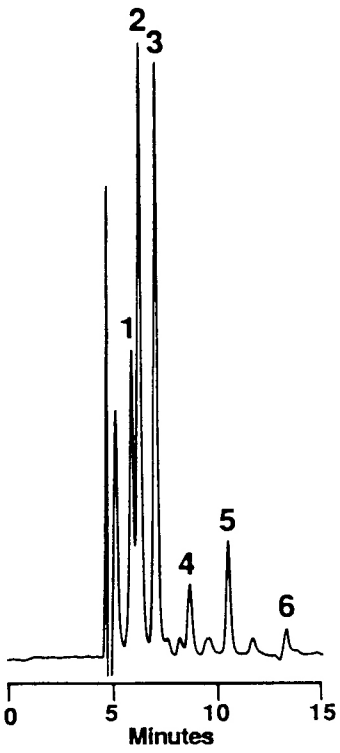
RECOMMENDED EQUIPMENT

Any Dionex Ion Chromatograph equipped with a Conductivity Detector: QIC, 2000i, 2000i/SP, or 4000i with Column Heater.



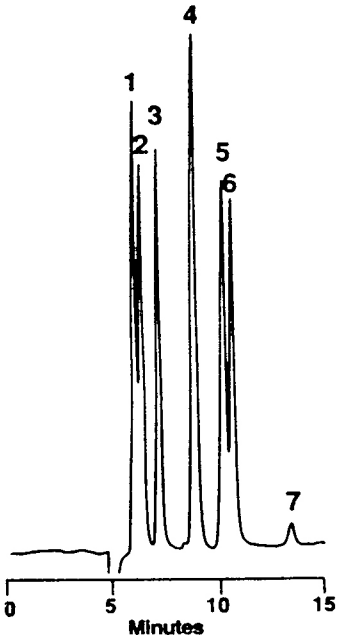
Red Wine
Fast Run
Dilution: 1 in 33.3

Peaks
1. Citric
2. Tartaric
3. Malic
4. Lactic & Succinic
5. Acetic
6. Carbonate



White Wine
Fast Run
Dilution: 1 in 25

Peaks
1. Citric
2. Tartaric
3. Malic
4. Succinic & Lactic
5. Acetic
6. Carbonate



Organic Acid Standard
Fast Run
50 ppm each

Peaks
1. Citric
2. Tartaric
3. Malic
4. Succinic & Lactic
5. Fumaric
6. Acetic
7. Carbonate

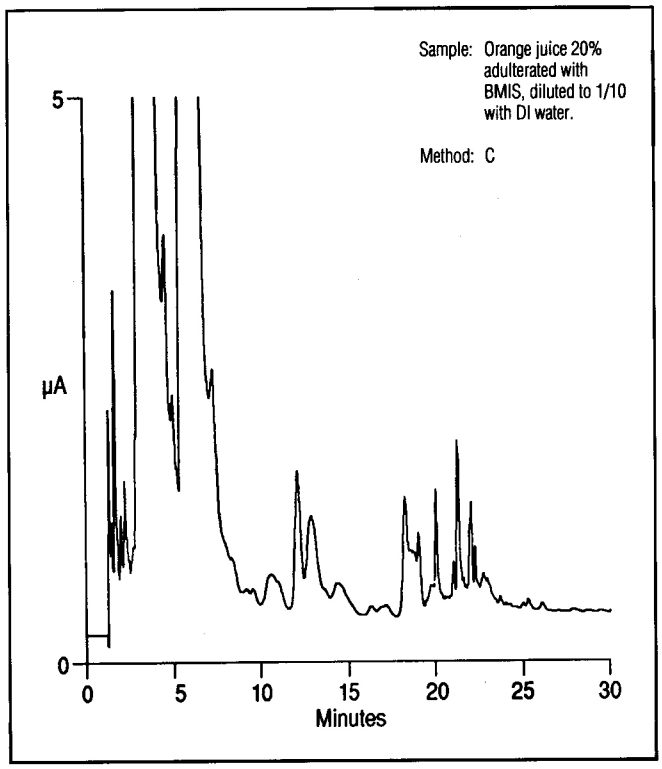


Figure 6 *Orange juice adulterated with medium invert sugar, analyzed by Method C. Note the late-eluting fingerprint between 18 and 24 minutes.*

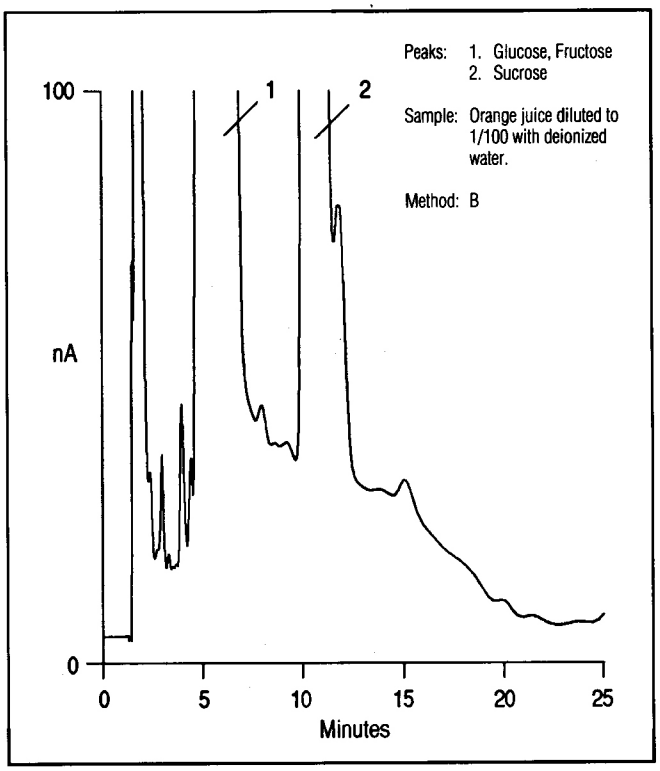


Figure 7 *Orange juice analyzed by Method B. Note the lack of any peaks eluting at 20 minutes.*

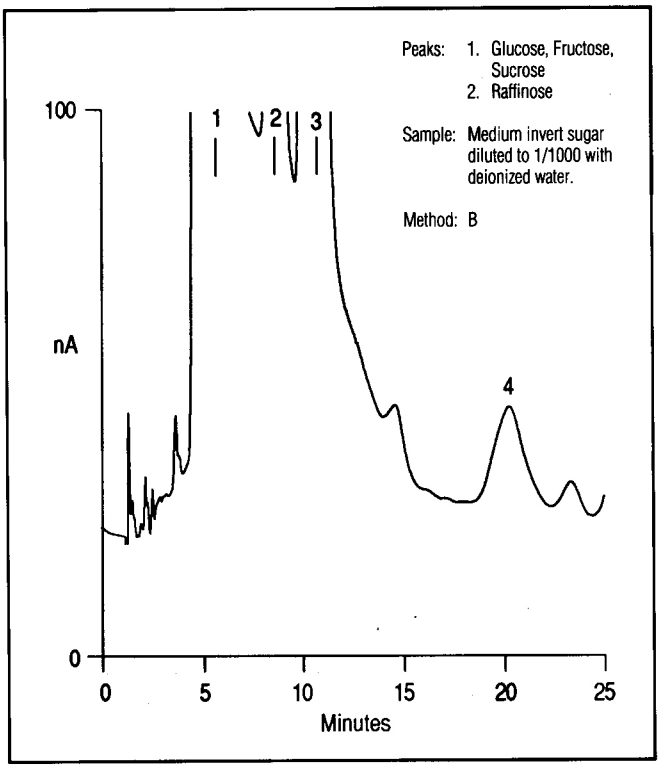


Figure 8 *Medium invert sugar analyzed by Method B. Note the raffinose peak eluting at approximately 20 minutes.*

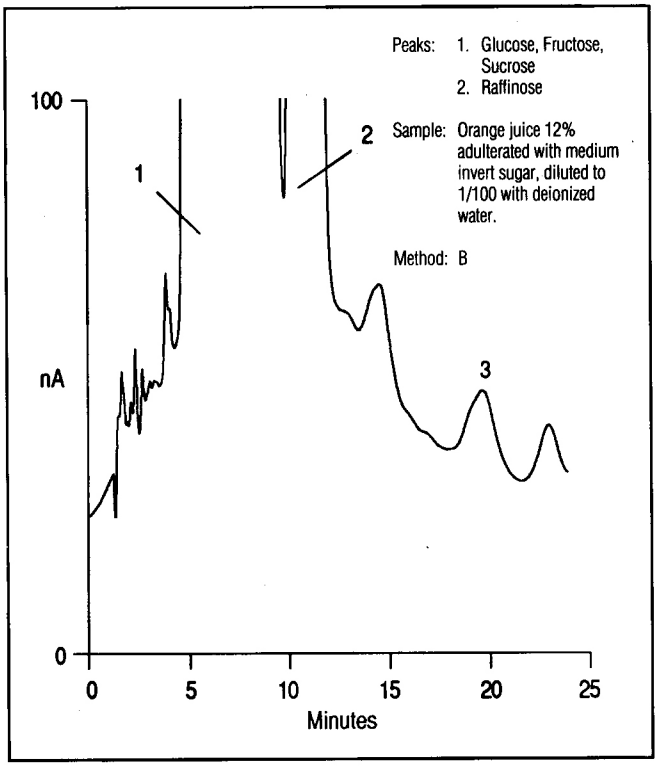


Figure 9 *Orange juice adulterated 12% with medium invert sugar, analyzed by Method B. Adulteration can be detected by the presence of raffinose.*

Ion Chromatography: A Versatile Technique for the Analysis of Beer

INTRODUCTION

Ion chromatography is an efficient technique for the analysis and quantification of ions in solution. Although there are several techniques that have been used for the analysis of beer — including gas chromatography, HPLC, enzyme-based methods, and wet chemical methods — ion chromatography is rapidly becoming the method of choice.

The compounds of interest for the beer industry range — from inorganic ions, organic acids, and hop bittering principles that contribute to the overall taste and bitterness of the beverage — to proteins, carbohydrates, and alcohols that are monitored to determine the extent of fermentation. The finished beer product may be analyzed to determine the concentration of added preservatives and colorants, in addition to ensuring manufacturing authenticity.

The first step in the beer making process involves soaking barley, and sometimes other grains, in warm water. Enzymes present in the barley break down starch from the grains, producing mostly glucose, maltose, and other oligo- and polysaccharides. This process is called mashing, and the resulting solution is called sweet wort. The sweet wort is then treated with hops, thereby producing hopped wort. Yeast is added and the smaller saccharides are fermented to produce alcohol. Because of the different concentrations, chemical behavior, and molecular mass ranges of the various components in beer, their isolation and determination can be difficult. Ion chromatography, using polymer-based resins, provides a means to monitor many of these important compounds during the brewing process and in the final product.

This application note describes the use of ion-exchange or ion-exclusion chromatography for the determination of five classes of compounds of interest to the brewing industry, including: carbohydrates, alcohols, organic acids, inorganic anions, and inorganic cations. One of two forms of electrochemical detection is used, pulsed amperometry or conductivity detection.

EQUIPMENT

A Dionex chromatographic system consisting of:
- Gradient Pump
- Chromatography Enclosure
- Electrochemical Detector with pulsed amperometry and conductivity modes
- Eluent Organizer

PeakNet Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water, 17.8 MΩ-cm or better

Carbohydrate Analysis

Sodium acetate (Sigma)

Sodium hydroxide solution, 50% (w/w) (Fisher Scientific)

Anion Analysis

Sodium hydroxide solution, 50% (w/w) (Fisher Scientific)

Methanol (EM Science)

Alcohol Analysis

Perchloric acid (Fisher Scientific)

Cation Analysis

Methanesulfonic acid (Fluka Chemika-BioChemika)

Organic Acid Analysis

0.1 M Tetrabutylammonium hydroxide (TBAOH) (Dionex P/N 39602)

Heptafluorobutyric acid (Fluka Chemika-BioChemika)

PREPARATION OF SOLUTIONS AND REAGENTS

1.00 M Sodium Acetate

Weigh out 82.0 g of anhydrous sodium acetate and place into a 1-L volumetric flask. Add approximately 600 mL of 17.8 MΩ-cm deionized water and swirl to dissolve. When the salt has dissolved completely, fill up to the mark with 17.8 MΩ-cm deionized water. Filter the resulting solution through a 0.2-µm filter.

500 mM Sodium Hydroxide

Weigh 974 g (974 mL) of 17.8 MΩ-cm deionized water into an eluent reservoir bottle. Degas the water for approximately 10 minutes. Tare the bottle on the balance and add 40.0 g (26.2 mL) of 50% (w/w) sodium hydroxide directly to the bottle. Quickly transfer the eluent bottle to the instrument and pressurize it with helium.

100 mM Sodium Hydroxide

Weigh 992 g (992 mL) of 17.8 MΩ-cm deionized water into an eluent reservoir bottle. Degas the water for approximately 10 minutes. Tare the bottle on the balance and add 8.00 g (5.25 mL) of 50% (w/w) sodium hydroxide directly to the bottle. Quickly transfer the eluent bottle to the instrument and pressurize it with helium.

1.00 mM Sodium Hydroxide

Place 990 g (990 mL) of 17.8 MΩ-cm deionized water into an eluent reservoir bottle. Degas the water for approximately 10 minutes. Pipette 10.0 mL of the 100 mM sodium hydroxide solution directly into the bottle. Quickly transfer the eluent bottle to the instrument and pressurize it with helium.

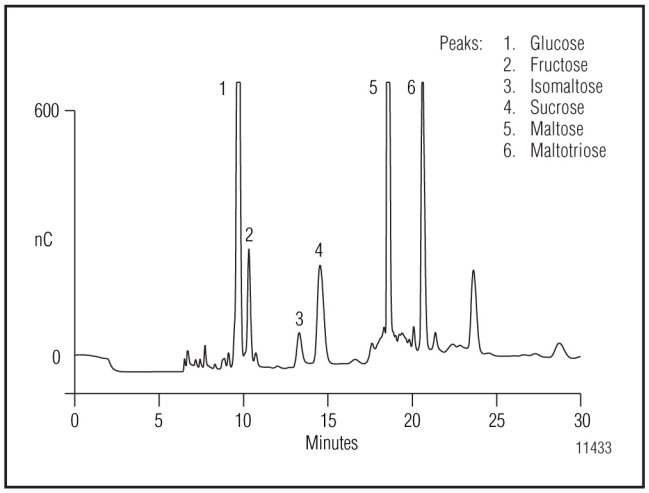


Figure 1 *Separation of fermentable sugars in wort by ion-exchange chromatography with pulsed amperometric detection. Experimental conditions are listed in Table 2. The sample was diluted 1:10 before injection.*

100 mM Methanesulfonic Acid

Weigh out 9.61 g of methanesulfonic acid (MSA). Carefully add this amount to a 1-L volumetric flask containing about 500 mL of deionized water. Dilute to the mark and mix thoroughly.

100 mM Perchloric Acid

Dilute 8.60 mL of perchloric acid into 992 mL of 17.8 MΩ-cm deionized water.

0.8 mM Heptafluorobutyric Acid

Heptafluorobutyric acid (perfluorobutyric acid) is supplied by FLUKA in 10.0-mL bottles. Dilute the entire contents of one 10.0-mL bottle in 1.00 L to obtain a 77.2 mM stock solution. Dilute 10.4 g of the stock solution in 1.00 L to obtain the 0.800 mM working eluent.

5 mM Tetrabutylammonium Hydroxide

Dilute 50 mL of the Dionex 0.1 M TBAOH ion-pairing reagent (P/N 35360) to 1 L with 17.8 MΩ-cm deionized water. Prepare several liters of the regenerant.

disaccharides, and trisaccharides. The experimental conditions used for the separations shown in Figures 1 and 2 are presented in Table 2.

The carbohydrates of most importance to the brewing industry are the fermentable sugars. In general, saccharides larger than DP3 are not fermentable; however, they will contribute to the caloric value as well as to the overall flavor of the beer and its ability to form a head. Figure 1 shows the separation of fermentable sugars (≤ DP3) in a hopped wort sample. These are the sugars that are converted by yeast to alcohol. If this figure is compared with the separation shown in Figure 2, the difference between the finished beer and a beer during production is evident. As expected, the concentration of fermentable sugars is greater in the wort than in the final beer product.

The complex sugars, starches, and dextrins, which are broken down by enzymes to produce a wort of high fermentability, are built up from glucose sub-units. Maltose is the simplest of the complex sugars and is formed by two glucose molecules joined together by an α-1,4 linkage. Following convention, chains are named according to the number of glucose units that have been incorporated. Thus, maltotetraose (DP4), for example, is a chain of four glucose units linked together by α-1,4 linkages.

Figure 3 shows the separation of maltose oligosaccharides in beer from DP3 to DP10, using the CarboPac PA-100. Excellent resolution of maltose oligomers up to DP15 is possible, allowing for rapid profiling. As indicated in the experimental conditions listed in Table 3, the eluent contained sodium acetate in addition to sodium hydroxide. The sodium acetate increases the eluent strength, which reduces the retention time of the oligosaccharides. Separation is possible in the absence of sodium acetate, but the run times are prohibitively long.

Alcohol Analysis

Usually, the only two alcohols present in high concentrations in beer are ethanol and glycerol. Glycerol is an important component in beer; it has a considerable effect on flavor and is sweeter than glucose. Ethanol and glycerol can be separated by ion-exclusion chromatography, then detected with pulsed amperometry using the waveform described in Table 4 with integration performed from 0.05 to 0.25 seconds.

Table 3 Experimental conditions for the separation of oligosaccharides in beer using the CarboPac PA-100

Column:	CarboPac PA-100				
Eluent 1:	Deionized water				
Eluent 2:	500 mM Sodium hydroxide				
Eluent 3:	1 M Sodium acetate				
Gradient:	Time	E1	E2	E3	Comments
	Initial	57	33	10	Reequilibrate
	1.50	57	33	10	Inject
	6.50	57	33	10	Gradient Start
	31.50	42	33	25	Gradient End
Flow Rate:	1.0 mL/min				
Inj. Volume:	10 µL				
Detection:	Pulsed amperometry, gold electrode (see Table 1 for waveform)				

Table 4 ED40 waveform for the analysis of alcohols by ion-exclusion chromatography using a platinum electrode

Time (s)	Potential (V)
0.00	0.30
0.05	0.30
0.25	0.30
0.26	1.25
0.60	1.25
0.61	0.10
1.00	0.10

Table 5 Experimental conditions used for the separation of glycerol and ethanol by ion-exclusion chromatography using the IonPac ICE-AS6 column

Column:	IonPac® ICE-AS6
Eluent:	100 mM Perchloric acid
Flow Rate:	2.0 mL/min
Inj. Volume:	10 µL
Detection:	Pulsed amperometry, platinum electrode (see Table 4 for waveform)

disaccharides, and trisaccharides. The experimental conditions used for the separations shown in Figures 1 and 2 are presented in Table 2.

The carbohydrates of most importance to the brewing industry are the fermentable sugars. In general, saccharides larger than DP3 are not fermentable; however, they will contribute to the caloric value as well as to the overall flavor of the beer and its ability to form a head. Figure 1 shows the separation of fermentable sugars (≤ DP3) in a hopped wort sample. These are the sugars that are converted by yeast to alcohol. If this figure is compared with the separation shown in Figure 2, the difference between the finished beer and a beer during production is evident. As expected, the concentration of fermentable sugars is greater in the wort than in the final beer product.

The complex sugars, starches, and dextrins, which are broken down by enzymes to produce a wort of high fermentability, are built up from glucose sub-units. Maltose is the simplest of the complex sugars and is formed by two glucose molecules joined together by an α-1,4 linkage. Following convention, chains are named according to the number of glucose units that have been incorporated. Thus, maltotetraose (DP4), for example, is a chain of four glucose units linked together by α-1,4 linkages.

Figure 3 shows the separation of maltose oligosaccharides in beer from DP3 to DP10, using the CarboPac PA-100. Excellent resolution of maltose oligomers up to DP15 is possible, allowing for rapid profiling. As indicated in the experimental conditions listed in Table 3, the eluent contained sodium acetate in addition to sodium hydroxide. The sodium acetate increases the eluent strength, which reduces the retention time of the oligosaccharides. Separation is possible in the absence of sodium acetate, but the run times are prohibitively long.

Alcohol Analysis

Usually, the only two alcohols present in high concentrations in beer are ethanol and glycerol. Glycerol is an important component in beer; it has a considerable effect on flavor and is sweeter than glucose. Ethanol and glycerol can be separated by ion-exclusion chromatography, then detected with pulsed amperometry using the waveform described in Table 4 with integration performed from 0.05 to 0.25 seconds.

Table 3 Experimental conditions for the separation of oligosaccharides in beer using the CarboPac PA-100

Column:	CarboPac PA-100				
Eluent 1:	Deionized water				
Eluent 2:	500 mM Sodium hydroxide				
Eluent 3:	1 M Sodium acetate				
Gradient:	Time	E1	E2	E3	Comments
	Initial	57	33	10	Reequilibrate
	1.50	57	33	10	Inject
	6.50	57	33	10	Gradient Start
	31.50	42	33	25	Gradient End
Flow Rate:	1.0 mL/min				
Inj. Volume:	10 µL				
Detection:	Pulsed amperometry, gold electrode (see Table 1 for waveform)				

Table 4 ED40 waveform for the analysis of alcohols by ion-exclusion chromatography using a platinum electrode

Time (s)	Potential (V)
0.00	0.30
0.05	0.30
0.25	0.30
0.26	1.25
0.60	1.25
0.61	0.10
1.00	0.10

Table 5 Experimental conditions used for the separation of glycerol and ethanol by ion-exclusion chromatography using the IonPac ICE-AS6 column

Column:	IonPac® ICE-AS6
Eluent:	100 mM Perchloric acid
Flow Rate:	2.0 mL/min
Inj. Volume:	10 µL
Detection:	Pulsed amperometry, platinum electrode (see Table 4 for waveform)

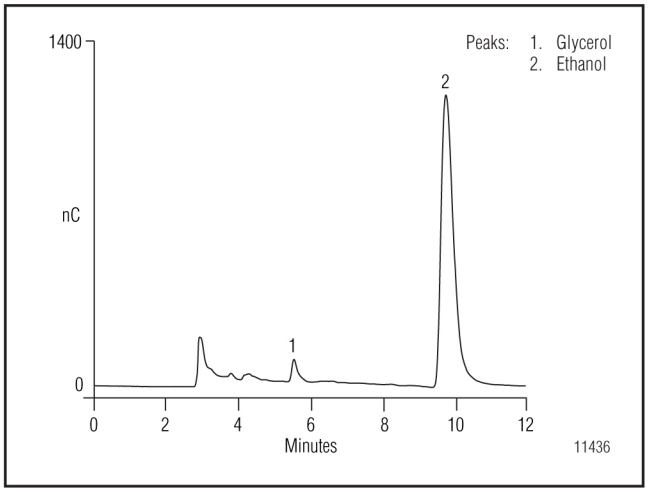


Figure 4 *Separation of glycerol and ethanol in an American beer by ion-exclusion chromatography with pulsed amperometric detection. Experimental conditions as listed in Table 5. The sample was diluted 1:10 before injection.*

The IonPac ICE-AS6 column is an ion-exclusion column that successfully separates alcohols. The experimental conditions used for the separation of glycerol and ethanol are listed in Table 5. Figure 4 shows the separation of ethanol and glycerol in beer by ion-exclusion chromatography using pulsed amperometric detection with a platinum electrode.

Organic Acid Analysis

The measurement of organic acids, in all phases of beer production, can be used to help track metabolic products of fermentation and to correlate beer flavor trends. One way to separate organic acids is with ion-exclusion chromatography using suppressed conductivity detection. The IonPac ICE-AS6 column is an ion-exclusion column designed for the efficient separation of low molecular weight aliphatic organic acids including hydroxy-substituted organic acids, in addition to species such as aliphatic alcohols and glycols. Using this separation mechanism, weakly ionized species are separated based on differences in their pK_as. Strong inorganic acid anions are not retained by the stationary phase and elute in the void volume of the column. The standard eluent for use with the IonPac ICE-AS6 is 0.4 mM heptafluorobutyric acid (perfluorobutyric acid). Other monoprotic acids can be used; however, the background conductivity will be higher. The experimental conditions are listed in Table 6.

Table 6 Experimental conditions for the separation of organic acids in beer by ion-exclusion chromatography using the IonPac ICE-AS6 column

Column:	IonPac ICE-AS6
Eluent:	0.8 mM Heptafluorobutyric acid
Flow Rate:	1.0 mL/min
Inj. Volume:	25 µL
Detection:	Suppressed conductivity, AMMS™-ICE
Regenerant:	5 mN Tetrabutylammonium hydroxide at 5 mL/min

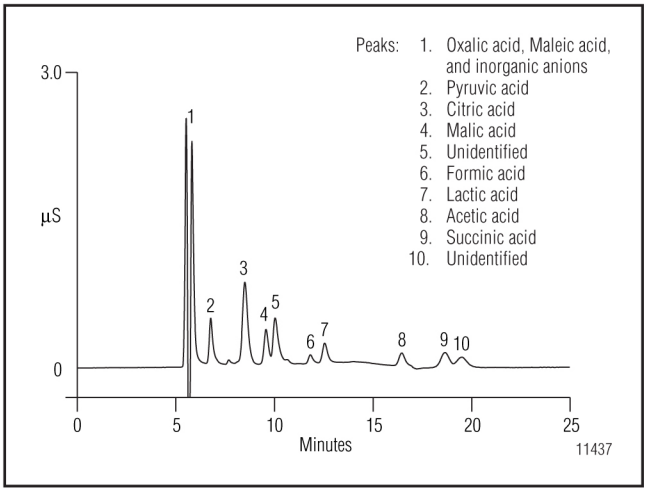


Figure 5 *Separation of organic acids in a British stout. Experimental conditions are listed in Table 5. The sample was diluted 1:40 before injection.*

Figure 5 shows the separation of a series of organic acids in a stout. The sample was degassed and diluted 1:40 prior to injection. Oxalic and maleic acids are eluted on either side of the 'water dip,' masked by strong acid anions such as fluoride and chloride. Pyruvate, citrate, malate, formate, lactate, acetate, and succinate, however, are all baseline resolved. The presence of acetate may provide evidence of oxidation, while pyruvic acid is present as an intermediate product in the conversion of glucose to alcohol. Lactate is produced by lactic acid bacteria that convert glucose and other sugars to lactic acid, so it is kept to a minimum in most beers. A few unidentified peaks are also resolved.

Inorganic Anions

Inorganic anions are introduced into beer from the brewing water and have an important impact on the flavor of beer. Thus, the water can be monitored by ion chromatography to ensure purity and consistency. Despite the deliberate addition of high levels of some anions such as sulfate (Burtonization), excessive amounts of sulfate and chloride, for example, can have a detrimental effect on the flavor of the beer. In addition, high concentrations of other anions such as nitrate (if it is converted to nitrite) can harm the yeast during the fermentation process. Therefore, monitoring the anion profile is an important quality control step in the brewing industry.

Inorganic anions are separated by anion-exchange chromatography and monitored by suppressed conductivity detection; Table 7 lists the experimental conditions. When performing gradient elution on the IonPac AS11 column, a hydroxide eluent system is used instead of a carbonate eluent because of the low background conductivity of hydroxide. An Anion Trap Column (ATC) should be installed between the gradient pump and the injection valve to minimize baseline shifts resulting from the elution of anionic contaminants in the eluent.

Figure 6 shows the simultaneous separation of a mixture of inorganic and organic anions in an American ale using the IonPac AS11 column. The sample was degassed and diluted 1:40 prior to injection. The sodium hydroxide concentration in Eluent 2 is weak enough that not only is fluoride eluted after the void, but several weakly retained monovalent organic acids are also resolved. The addition of methanol to the eluent modifies the selectivity of the column for the more hydrophobic anions, thus allowing resolution between succinate and malate and also between tartrate and maleate, which would otherwise coelute. Thus, using the conditions described in Table 6, it is possible to separate not only the strong acid anions, but also a variety of weak organic acids. To obtain a flat baseline for this chromatogram, the baseline subtraction option in the PeakNet software was used. Reproducibility for this method is on the order of 0.5% or better for retention times and 2% or better for peak areas with good linearity (r^2=0.999) over the range tested (1.5 orders of magnitude).

Table 7 Experimental conditions for the separation of inorganic anions in beer using the IonPac AS11 column

Column:	IonPac AS11 Analytical (4 mm) IonPac AG11 Guard (4 mm) IonPac ATC-1 Anion Trap				
Eluent 1:	Deionized water				
Eluent 2:	1 mM Sodium hydroxide				
Eluent 3:	100 mM Sodium hydroxide				
Eluent 4:	Methanol				
Gradient:	Time	E1	E2	E3	E4
	Initial	80	20	—	—
	3.00	80	20	—	—
	5.00	66	20	—	14
	18.00	42	—	38	20
	18.01	80	20	—	—
Flow Rate:	2 mL/min				
Inj. Volume:	25 µL				
Detection:	Suppressed conductivity, ASRS™, AutoSuppression™ recycle mode				

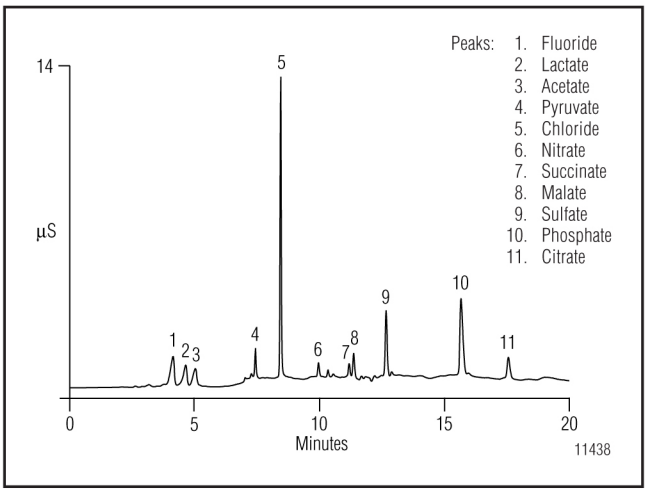


Figure 6 *Separation of inorganic anions and organic acids in an American ale by ion-exchange chromatography. Experimental conditions are listed in Table 6. The sample was diluted 1:40 before injection.*

Table 8 Experimental conditions for the separation of inorganic cations in beer using the IonPac CS12 column

Column:	IonPac CS12 Analytical (4 mm)				
	IonPac CG12 Guard (4 mm)				
	IonPac CTC-1 Cation Trap				
Eluent 1:	Deionized water				
Eluent 2:	100 mM Methanesulfonic acid				
Gradient:	Time	E1	E2	E3	E4
	Initial	84	16	—	—
	5.00	84	16	—	—
	5.01	60	40	—	—
	10.00	60	40	—	—
	10.01	84	16	—	—
Flow Rate:	1.0 mL/min				
Inj. Volume:	25 µL				
Detection:	Suppressed conductivity, CSRS™, AutoSuppression recycle mode				

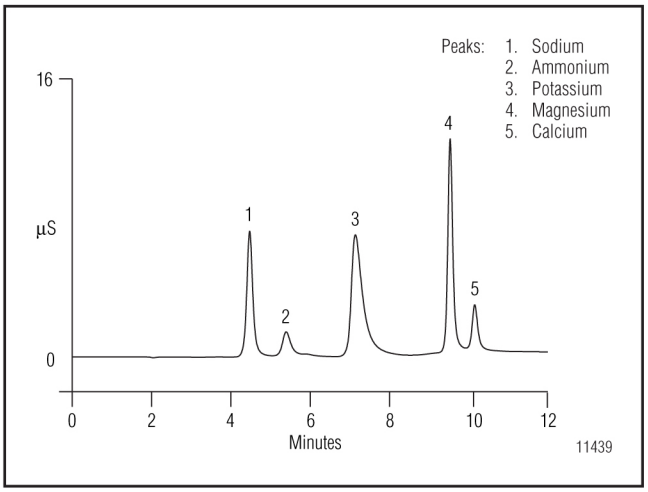


Figure 7 *Separation of inorganic cations in a 1:40 dilution of an American lager by ion-exchange chromatography using the IonPac CS12 column. Experimental conditions are listed in Table 8.*

The first inorganic anion to be eluted is fluoride, which is often added to municipal water supplies to prevent tooth decay and is harmless for brewing purposes. Chloride is eluted next, and at levels above 250 mg/L it has been found to enhance the sweetness of beer. However, it may also hamper yeast flocculation. Nitrate was once thought of as a problem in the brewing process, but it has since been discovered that it is the nitrite produced from nitrate that affects yeast metabolism to cause weak and incomplete fermentation. Sulfate is found naturally in water but imparts a sharp, dry edge to well hopped beers and is therefore kept to a minimum. Finally, phosphate is present in the malt and buffers the mash at a slightly acidic pH.

Inorganic Cations

As is the case with the inorganic anions, most of the inorganic cations are introduced into the beer from the water supply. The four most abundant cations in beer are sodium, potassium, calcium, and magnesium. Some of these cations affect the pH of the mash, while others affect the flavor of the beer. Other metals such as lead, copper, and zinc are also monitored to ensure their absence since most of them are poisonous at any significant level. This application note focuses on the alkali and alkaline earth metals.

Inorganic cations are separated by ion-exchange chromatography and monitored by suppressed conductivity detection, as described in Table 8. The gradient allows for the separation of barium and strontium in addition to the five cations shown in Figure 7. A step change at 5 minutes from the weak eluent to a stronger eluent allows for the elution of sharp peaks for the divalent cations. The reproducibility of this method is on the order of 0.5% or better for retention times and 2% or better for peak areas. Linearity is good over the range tested (2 orders of magnitude) with a coefficient of determination, $r^2 = 0.999$ or better, for all analytes except ammonium. If it is not important to monitor for barium or strontium, the conditions can be changed to allow for isocratic elution of the five cations shown in Figure 7 (≤ 10 minutes). If isocratic elution is desired, then the concentration eluent should be 20 mM methanesulfonic acid.

Figure 7 shows the separation of the main cations in beer using the IonPac CS12 column. The sample was degassed and diluted 1:40 prior to injection. Sodium was the first peak to be eluted. At a concentration of 75–150 mg/L, it gives a round smoothness to the beer when combined with chloride. If too much sulfate is present, however, sodium gives an unpleasant harshness to the flavor. Potassium is the next metal to be eluted, and like sodium it can impart a slightly salty flavor to the beer. It also inhibits the action of certain enzymes in mash. Magnesium is an important nutrient for yeast at levels around 10–20 mg/L, but imparts a sharp, bitter-sour flavor at levels much higher than 20 mg/L. Calcium is perhaps the most important

metal, since it reacts with phosphate in the malt to lower the pH of the mash and wort. It also assists enzyme action, but has no effect on the flavor of the beer.

CONCLUSION

Carbohydrates, alcohols, organic acids, and inorganic anions and cations can all be separated on various ion-exchange or ion-exclusion columns and detected by pulsed amperometric or suppressed conductivity detection. Although many of these constituents can be determined individually using unrelated analytical techniques, reduced analysis time and equipment costs can be realized by using one instrument with multi-species capability. Ion chromatography is a versatile technique that meets many of the analytical requirements of the beer making process.

LIST OF SUPPLIERS

EM Science, P.O. Box 70, 480 Democrat Road, Gibbstown, New Jersey, 08027, USA, 1-800-222-0342.

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

Fluka Chemika-BioChemika, Fluka Chemie AG, Industriestrasse 25, CH-9471 Buchs, Switzerland, +81 755 25 11.

Sigma Chemical Company, P.O. Box 14508, St. Louis, Missouri, 63178, USA, 1-800-325-3010.

Determination of Inorganic Anions and Organic Acids in Fermentation Broths

INTRODUCTION

Fermentation broths are used in the manufacture of biotherapeutics and many other biologically derived products using recombinant genetic technology. Broths are also used for the production of methanol and ethanol as alternative energy sources to fossil fuels. In addition, many food and beverage products such as alcoholic beverages, vinegars, fermented vegetables, sauces, and dairy products are all prepared by controlled fermentation processes. Fermentation monitoring is also important in detection of spoilage of fruit juices and food products. Recently, attention has been given to characterizing the ingredients of fermentation broths because carbon sources and metabolic by-products can impact the yield of the desired products. Carbohydrates (glucose, lactose, sucrose, maltose, etc.) are carbon sources essential for cell growth and product synthesis, while alcohols (ethanol, methanol, sugar alcohols, etc.), glycols (glycerol), and organic anions (acetate, lactate, formate, etc.) are metabolic by-products, many of which reduce desired yields. Fermentation broths are complex mixtures of nutrients, waste products, cells and cell debris, and desired products, such as antibiotics. Many of these ingredients are nonchromophoric and cannot be detected by absorbance.

Organic and inorganic anions are ionic and therefore can be determined by ion chromatography using suppressed conductivity detection. Suppressed conductivity is a powerful detection technique with a broad linear range and very low detection limits. Nonionic compounds are not detected. Suppression lowers the background conductivity caused by the eluent and effectively increases the conductivity of the analyte.[1, 2] Anion-exchange chromatography is a technique capable of separating complex mixtures of organic acids and inorganic anions. For complex samples like fermentation broths, the high resolving power of ion-exchange chromatography and the specificity of suppressed conductivity allow the determination of ionic fermentation broth ingredients, with little interference from other broth ingredients.[3–5] Although biosensor and flow-injection analysis methods are commonly used to evaluate fermentation broths,[6, 7] these techniques cannot simultaneously determine multiple compounds. Gel permeation chromatography with refractive index detection, and anion-exchange chromatography with UV-VIS detection, have been used for analysis of fermentation broths, but both are limited by poor selectivity and sensitivity.[8, 9] Anion-exchange chromatography with suppressed conductivity monitors, by direct injection, a large number of different compounds simultaneously, using a single instrument and chromatographic method.[10]

This application note describes the use of two different anion-exchange columns, with suppressed conductivity detection, to analyze common organic and inorganic anions in yeast and bacterial fermentation broths. The yeast Saccharomyces cerevisiae in yeast extract-peptone-dextrose (YPD) broth and the bacteria *Escherichia coli* in Luria-Bertani (LB) broth are common fermentation broth cultures and represent eukaryotic and prokaryotic systems. Both fermentation broth cultures are complex and contain undefined media ingredients, and thus are a great challenge for most separation and detection technologies. These formulations also contain carbohydrates, sugar alcohols, alcohols, and glycols that have been analyzed using the CarboPac™ PA1, PA10, and MA1 anion-exchange columns with pulsed amperometric detection.[11]

In the methods outlined in this application note, the selectivities of the IonPac® AS11 and IonPac AS11-HC anion-exchange columns are compared for the determination of anionic analytes in fermentation broths. The IonPac AS11 column packing consists of an alkanol quaternary ammonium latex bonded to a microporous crosslinked ethylvinylbenzene core. The AS11-HC (high capacity) latex is bonded to a macroporous crosslinked ethylvinylbenzene core. Due to the greater surface area of its core, the AS11-HC has six times more anion-exchange capacity than the AS11. Both columns are designed for separation of organic and inorganic anions using sodium hydroxide gradients. Organic solvents can be added to eluents to modify the selectivity of these columns.

Expected detection limits, linearity, selectivity, stability, and precision for organic and inorganic anions in fermentation broths are reported for the IonPac AS11 and AS11-HC columns using the Dionex DX-500 BioLC® system with suppressed conductivity detection.

EQUIPMENT

Dionex DX-500 BioLC system consisting of:

- GP40 Gradient Pump with degas option
- ED40 Electrochemical Detector
- LC30 or LC25 Chromatography Oven
- AS3500 Autosampler

PeakNet Chromatography Workstation

REAGENTS AND STANDARDS

Reagents

Sodium hydroxide, 50% (w/w) (Fisher Scientific and J. T. Baker)

Deionized water, 18 MΩ-cm resistance or higher was used for preparing all standards and eluents. Water that was used to prepare YPD broth was filter sterilized by passage through a 0.2-µm filter.

Standards

Lactic acid (Fisher Scientific)
Succinic acid (Aldrich Chemical Co.)
Pyruvic acid, sodium salt (Fisher Scientific)
DL-Isocitric acid, trisodium salt (Sigma Chemical Co.)
n-Butyric acid, sodium salt (Sigma Chemical Co.)
Sodium formate (Fisher Scientific)
Phenylacetic acid (Sigma Chemical Co.)
Propionic acid, sodium salt (Sigma Chemical Co.)
Maleic acid, disodium salt (Sigma Chemical Co.)
Oxalic acid, sodium salt (Fluka Chemika)
L-Malic acid (Eastman Chemical Co.)
Pyrophosphoric acid (Fluka Chemika)
Trichloroacetic acid (Fluka Chemika)
Chloroacetic acid (Aldrich Chemical Co.)
Glycolic acid (Sigma Chemical Co.)
L-Glutamic acid (Sigma Chemical Co.)
Fumaric acid (Fluka Chemika)
D-Gluconic acid, sodium salt (Sigma Chemical Co.)
Oxalacetic acid (Sigma Chemical Co., and Fluka Chemika)
Methylmalonic acid (Sigma Chemical Co.)
5-Keto-D-Gluconic acid, potassium salt (Sigma Chemical Co.)
2-Keto-D-Gluconic acid, hemicalcium salt (Sigma Chemical Co.)
Valeric acid (Aldrich Chemical Co.)
Isovaleric acid (Sigma Chemical Co.)
Isobutyric acid (Sigma Chemical Co.)
Sodium bromate (Fluka Chemika)
Sodium arsenate, dibasic, 7-hydrate (J.T. Baker Chemical Co.)
Sodium acetate, anhydrous (Fluka Chemika)
Sodium fluoride (Fisher Scientific)
Sodium nitrate (Fisher Scientific)
Sodium chloride (Fisher Scientific)
Potassium phosphate, dibasic, anhydrous (Fisher Scientific)
Citric acid, monohydrate (Fisher Scientific)
Sodium bromide (Aldrich Chemical Co.)
Sodium sulfate, anhydrous (EM Science)
Sodium carbonate, monohydrate (Fisher Scientific)

Table 1. Chromatographic Conditions

Conditions		
	System 1	**System 2**
Column:	IonPac AS11 Analytical (P/N 44076) IonPac AG11 Guard (P/N 44078) ATC-1 Anion Trap Column (P/N 37151)	IonPac AS11-HC Analytical (P/N 52960) IonPac AG11-HC Guard (P/N 52962) ATC-1 Anion Trap Column (P/N 37151)
Flow Rate:	2.0 mL/min	1.5 mL/min
Injection Volume:	10 µL	10 µL
Oven Temperature:	Ambient	30 °C
Detection (ED40):	Suppressed conductivity, ASRS®, AutoSuppression® recycle mode, 300 mA	Suppressed conductivity, ASRS, AutoSuppression recycle mode, 300 mA
Eluents:	A: Water B: 5 mM sodium hydroxide C: 100 mM sodium hydroxide	A: Water B: 5 mM sodium hydroxide C: 100 mM sodium hydroxide
Gradient:	0.5–38 mM sodium hydroxide: 0.5 mM sodium hydroxide, hold for 2.5 min; 0.5–5 mM sodium hydroxide in 3.5 min; 5–38 mM sodium hydroxide in 12 min.	1–60 mM sodium hydroxide: 1 mM sodium hydroxide, hold for 8 min; 1–15 mM sodium hydroxide in 10 min; 15–30 mM sodium hydroxide in 10 min. 30–60 mM sodium hydroxide in 10 min; 60 mM sodium hydroxide, hold for 2 min.
Method:	Time (min) A (%) B (%) C (%) 0.0 90 10 0 2.5 90 10 0 6.0 0 100 0 18.0 0 62 38 18.1 90 10 0 25.0 90 10 0	Time (min) A (%) B (%) C (%) 0.0 80 20 0 8.0 80 20 0 18.0 85 0 15 28.0 70 0 30 38.0 40 0 60 40.0 40 0 60 40.1 80 20 0 50.0 80 20 0
Typical Background Conductivity:	0.5 mM sodium hydroxide: 0.5–1 µS 38 mM sodium hydroxide: 2–3 µS	1 mM sodium hydroxide: 0.5–1 µS 60 mM sodium hydroxide: 2–3 µS
Typical System Operating Backpressure:	12.4 Mpa (1800 psi)	15.2 Mpa (2200 psi)

Culture and Media

Bacto YPD Broth (DIFCO Laboratories, Cat# 0428-17-5)

Bacto Yeast Extract (DIFCO Laboratories, Cat# 0127-15-1)

Bacto Peptone (DIFCO Laboratories, Cat# 0118-15-2)

LB Broth (DIFCO Laboratories, Cat# 0446-17-3)

Yeast, *S. cerevisiae;* Bakers Yeast type II (Sigma Chemical Co., Cat# 45C-2)

Bacteria, *E. coli* (donated by SRI International)

CONDITIONS

See "Conditions" (Table 1).

PREPARATION OF SOLUTIONS AND REAGENTS

Sodium Hydroxide Eluents

5 mM Sodium Hydroxide

It is essential to use deionized water of high resistance (18 MΩ-cm) that is as free of dissolved carbon dioxide as possible. Carbonate is formed in alkaline eluents from carbon dioxide. Carbonate, a divalent anion at

high pH, binds strongly to the columns and causes a loss of chromatographic resolution and efficiency. Carbonate can be removed by placing an anion trap column (ATC-1, P/N 37151) between the pump and the injection valve. Commercially available sodium hydroxide pellets are covered with a thin layer of sodium carbonate and should not be used. A 50% (w/w) sodium hydroxide solution is much lower in carbonate and is the preferred source for sodium hydroxide.

Dilute 0.524 mL of 50% (w/w) sodium hydroxide solution into 2000 mL of thoroughly degassed water to yield 5 mM sodium hydroxide. Keep the eluents blanketed under 5–8 psi (34–55 kPa) of helium at all times.

100 mM Sodium Hydroxide

Follow the same precautions described above for the 5 mM sodium hydroxide eluent. Dilute 10.4 mL of 50% (w/w) sodium hydroxide solution into 1990 mL of thoroughly degassed water to yield 100 mM sodium hydroxide. Keep the eluents blanketed under 5–8 psi (34–55 kPa) of helium at all times.

Stock Standards

Solid standards were dissolved in water to 10 g/L anionic concentrations. These were combined and further diluted with water to yield the desired stock mixture concentrations. The solutions were kept frozen at –20 °C until needed. For determinations of linear range, combine 10-g/L solutions of chloride, bromide, and citrate to make a 1-mg/L standard mix solution. Dilute with water to concentrations of 800, 600, 400, 200, 100, 80, 60, 40, 20, 10, 4, and 1 µg/L. Standard solutions of acetate, bromide, nitrate, sulfate, phosphate, and citrate were also prepared for estimating lower detection limits and linearity at concentrations of 10, 8, 6, 4, 2, 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.08, 0.06, 0.04, 0.02, and 0.01 mg/L. Chloride was prepared at concentrations of 2.4, 1.9, 1.5, 1.0, 0.49, 0.24, 0.19, 0.15, 0.098, 0.048, 0.024, 0.019, 0.014, 0.0097, 0.0048, and 0.0024 mg/L.

SAMPLE PREPARATION

Yeast Fermentation Broth Culture—Standard Media

In a sterile 500-mL Erlenmeyer flask, dissolve 10 g of Bacto YPD Broth (DIFCO Laboratories, Cat# 0428-17-5) in 200 mL filter-sterilized water. Bacto YPD Broth contains 2 g Bacto Yeast Extract, 4 g Bacto Peptone, and 4 g dextrose (glucose) per 10 g. Dissolve 1.0 g yeast (*S. cerevisiae;* Bakers Yeast type II; Sigma Chemical Co., Cat# 45C-2) in the YPD broth. Cap the flask with a vented rubber stopper. Incubate the culture in a 37 °C shaking water bath (500–600 rpm) for 24 h, removing aliquots at designated time points and placing them on ice. For this study, samples were taken after the addition of yeast at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 24-h intervals. The incubation starts when yeast is added to the medium. Aliquots are centrifuged at 14,000 × g for 10 min and diluted 10- and 100-fold in purified water. Diluted supernatant (10 µL) was analyzed directly.

Heat-inactivated yeast fermentation broth supernatant was spiked with anions for the recovery and stability study. To inactivate the culture, broth supernatant was diluted 10-fold, and heated in boiling water for 10 min. An aliquot of heat-inactivated supernatant was then diluted another 10-fold using 100 µg/mL lactate, acetate, formate, pyruvate, sulfate, oxalate, phosphate, and citrate. The final concentration of each anion was 10 µg/mL. Another aliquot of heat-inactivated yeast culture supernatant was diluted 100-fold in water, serving as an unspiked "blank".

E. Coli Fermentation Broth Culture—Standard Media

LB Broth is dissolved to a concentration of 25 g/L with water, heated to a boil, and autoclaved for 15 minutes at 121 psi. A liter of LB broth contains 10 g of tryptone, 5 g yeast extract, and 10 g of sodium chloride per 25 g. The culture was incubated and sampled as described for the yeast standard media.

RESULTS AND DISCUSSION

Selectivity

IonPac AS11

Figure 1A shows the separation of the common fermentation broth anions using an IonPac AS11 column set with a 0.5–38 mM NaOH gradient (Table 1, System 1) flowing at 2.0 mL/min. The organic and inorganic anions were well-resolved. The analytes were eluted from the column in less than 20 min. The retention times of the anions in Figure 1A are listed in Table 2. In general, monovalent anions eluted first, followed by di- and trivalent anions.

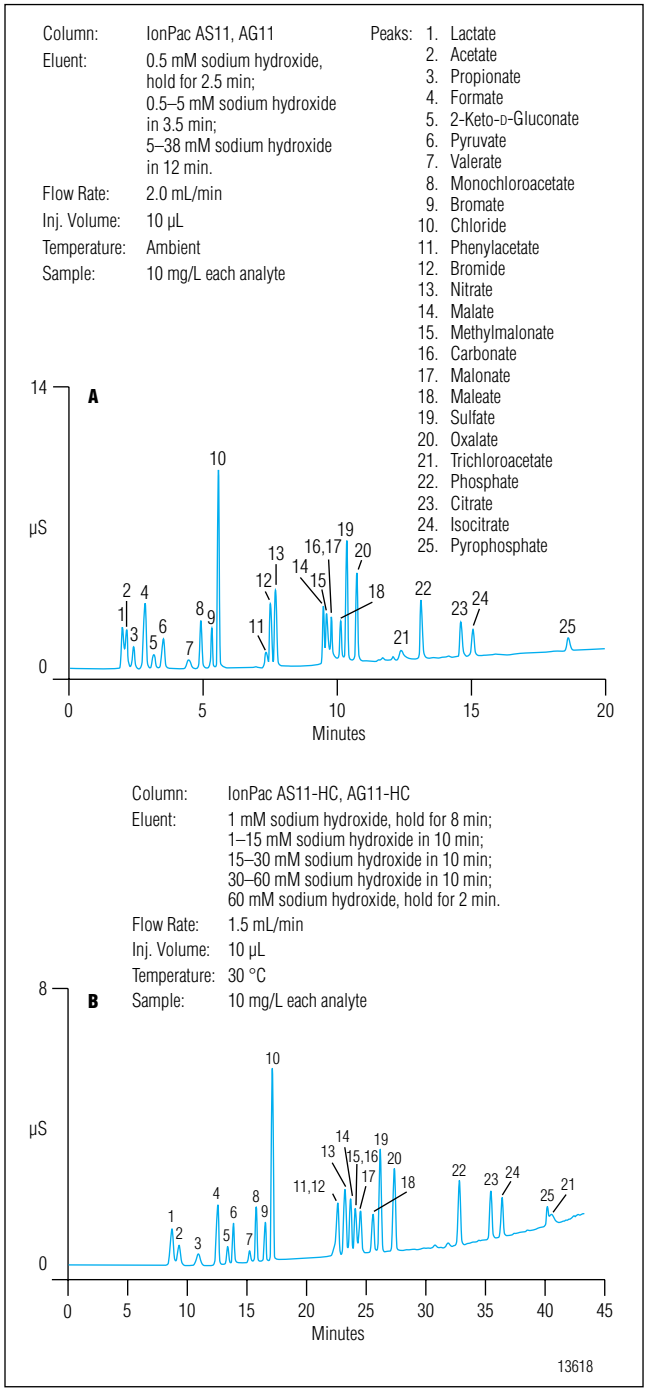


Figure 1. Common organic and inorganic anions found in fermentation broths analyzed on the IonPac AS11 and AS11-HC columns with suppressed conductivity.

Table 2. Retention Times for Common Organic and Inorganic Anions

Analyte	Retention Times (Minutes) IonPac AS11/AG11	IonPac AS11-HC/AG11-HC
Fluoride	2.3	8.7
Gluconate	2.3	8.2
Lactate	2.5	8.8
Acetate	2.6	9.5
Glycolate	2.6	9.4
Propionate	2.9	11.0
Isobutyrate	3.2	12.3
Formate	3.4	12.4
Butyrate	3.6	12.8
2-Keto-D-Gluconate	4.0	13.1
Pyruvate	4.3	13.5
Isovalerate	4.3	13.7
Valerate	5.1	14.8
Monochloroacetate	5.5	15.3
Bromate	5.8	16.1
Chloride	6.1	16.7
Phenylacetate	8.0	21.7
Bromide	8.2	21.9
5-Keto-D-Gluconate	8.3	20.1
Nitrate	8.4	22.4
Glutarate	N/A	22.5
Succinate	10.1	22.9
Malate	10.1	23.0
Carbonate	N/A	23.5
Methylmalonate	10.2	23.4
Malonate	10.4	23.8
Maleate	10.7	24.9
Sulfate	11.0	25.4
Oxalate	11.4	26.6
Fumarate	11.4	26.8
Oxalacetate	12.2	29.2
Trichloroacetate	13.5	39.0
Phosphate	13.9	31.8
Arsenate	15.1	33.9
Citrate	15.5	34.4
Isocitrate	16.0	35.3
Pyrophosphate	19.7	39.1

N/A - Not available

IonPac AS11-HC

Figure 1B shows the analysis of common fermentation broth anions using the IonPac AS11-HC column. Analytes were eluted using a 1–60 mM sodium hydroxide gradient (Table 1, System 2) flowing at 1.5 mL/min. A stronger eluent was needed to elute anions from this column due to its higher capacity. The higher capacity improves resolution of early eluting peaks. For example, lactate and acetate are better resolved on the AS11-HC than the AS11. The elution order of the AS11-HC is similar to the AS11. Table 2 also summarizes the retention times of different anions on the AS11-HC column. These results demonstrate that the AS11-HC column has slightly different selectivity than the AS11. For example, the AS11 column elutes phenylacetate, bromide,

Table 3. Estimated Lower Detection Limits (10-µL Injection)

	System 1 AS11	
	ng	µg/L
Acetate	2	200
Chloride	0.5	50
Bromide	4	400
Nitrate	3	300
Sulfate	1	100
Phosphate	4	400
Citrate	4	400

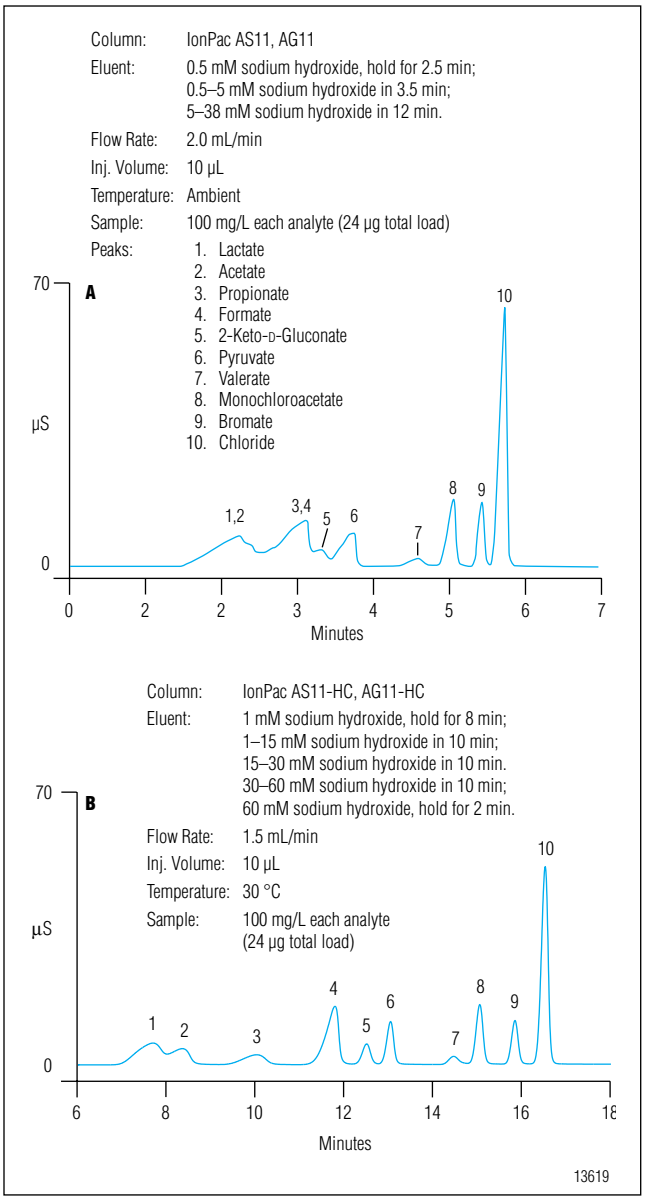


Figure 2. Separation of early eluting organic and inorganic anions at high levels (24 µg total load) using the IonPac AS11 and AS11-HC.

and nitrate several minutes before malate; the AS11-HC elutes these compounds much closer to malate. Also, trichloroacetate elutes before phosphate on the AS11, but elutes after pyrophosphate on the AS11-HC. The high capacity of the AS11-HC permits larger sample loads.

Figure 2A shows early-eluting peaks from the analysis of 24-µg sample of fermentation broth anions analyzed on the AS11, and Figure 2B shows the same analysis on the AS11-HC. At this sample load, the AS11 is overloaded.

Detection Limits

The detection limits for a 10-µL injection of representative fermentation broth anions, in the absence of broth matrix, using the AS11 column, are shown in Table 3. The detection limit is defined as the minimum concentration required to produce a peak height signal-to-noise ratio of 3. The detection limit can be further decreased by increasing the injection volume above the 10-µL injection volume used for this application note. If increasing injection volume also increases sample load beyond the AS11 column capacity, the higher capacity AS11-HC can overcome this limitation. The detection limit can be further decreased by using smoothing algorithms available in PeakNet software and by using external water mode.

Linearity

Chloride, bromide, and citrate standards ranging from 1–1000 mg/L (10–10,000 ng) were injected (in triplicate) on the AS11 column. For these analytes, the peak area response was found to be linear over this range ($r^2 \geq 0.999$). Acetate, nitrate, sulfate, and phosphate were investigated over the concentration range of 0.1–12 mg/L (1–120 ng) and showed high linearity ($r^2 \geq 0.999$). Broad linear ranges help reduce the need to repeat sample analyses when components vary greatly in concentration. Representative calibration curves for the AS11 column is presented in Figure 3.

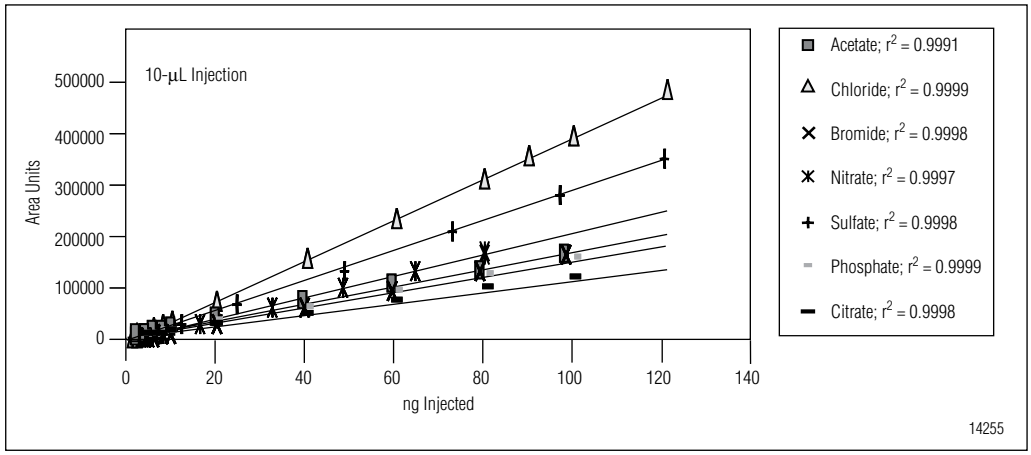


Figure 3. Method linearity for IonPac AS11 with suppressed conductivity detection.

Precision and Stability

The peak area and retention time RSDs were determined for replicate injections of common anions spiked into yeast fermentation broth. Anion standards were added to heat-inactivated S. cerevisiae fermentation broth culture supernatant to yield 10 mg/L spike concentrations and then analyzed repeatedly for 96 h (10-µL injections) on the AS11-HC column. Statistics for this experiment are presented in Table 4. Figures 4 and 5 show peak areas and retention times for every injection of this experiment. Peak area RSDs were 0.4–2.1% over 96 h. Retention time RSDs ranged from 0.3–0.7%. Retention times shifted slightly at 45 h (Figure 5) when the 100 mM sodium hydroxide eluent was replenished during the study. These results demonstrate that changing eluents can affect retention time precision.

Recovery from Sample Matrix

After correction for endogenous amounts, the measured levels of selected anions spiked into a heat-inactivated yeast fermentation broth culture were compared to their expected levels. These results are presented in Table 5, and show good recovery of anions from the yeast fermentation broth.

Table 4. Peak Area Precision (RSD, %)

Analyte	Last 8 Hours	First 48 Hours	Second 48 Hours	96 Hour Period
Lactate	0.2	0.4	0.3	0.5
Acetate	0.2	0.7	0.4	0.6
Formate	0.1	0.3	0.2	0.4
Pyruvate	0.5	0.5	0.7	0.8
Chloride	0.6	0.5	0.4	0.5
Sulfate	0.3	1.2	1.3	1.3
Oxalate	0.4	0.8	0.5	1.2
Phosphate	0.7	1.6	0.5	1.9
Citrate	0.3	1.8	0.6	2.1

Retention Time Precision (RSD, %)

Analyte	Last 8 Hours	First 48 Hours	Second 48 Hours	96 Hour Period
Lactate	0.1	0.2	0.2	0.3
Acetate	0.1	0.2	0.1	0.3
Formate	0.1	0.2	0.1	0.3
Pyruvate	0.0	0.2	0.1	0.3
Chloride	0.0	0.2	0.1	0.4
Sulfate	0.0	0.3	0.1	0.7
Oxalate	0.0	0.3	0.1	0.7
Phosphate	0.0	0.3	0.1	0.7
Citrate	0.0	0.3	0.0	0.7

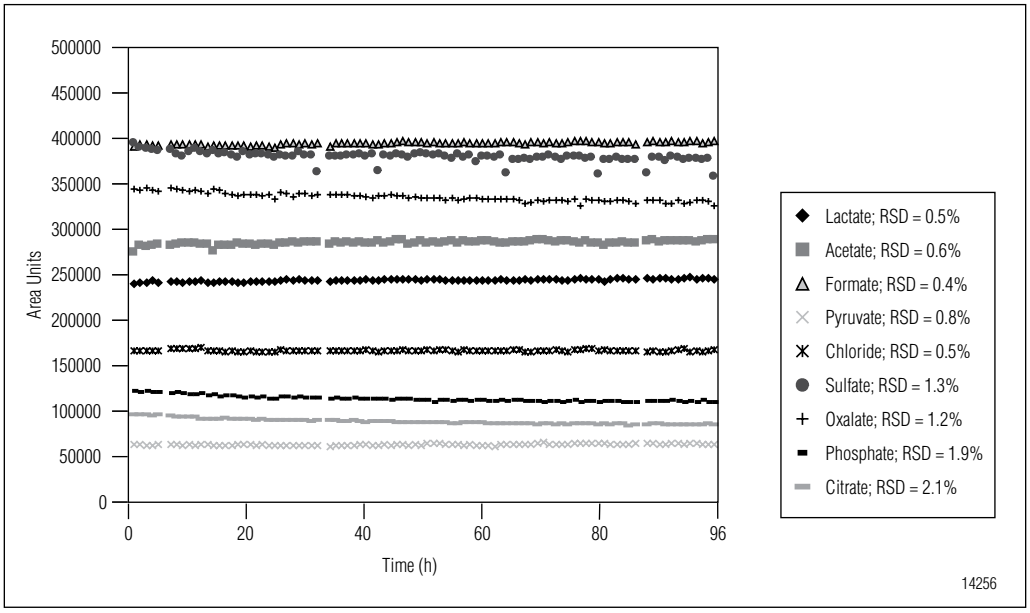


Figure 4. Peak Areas during 4 day repetitive analysis of heat-inactivated yeast fermentation broth.

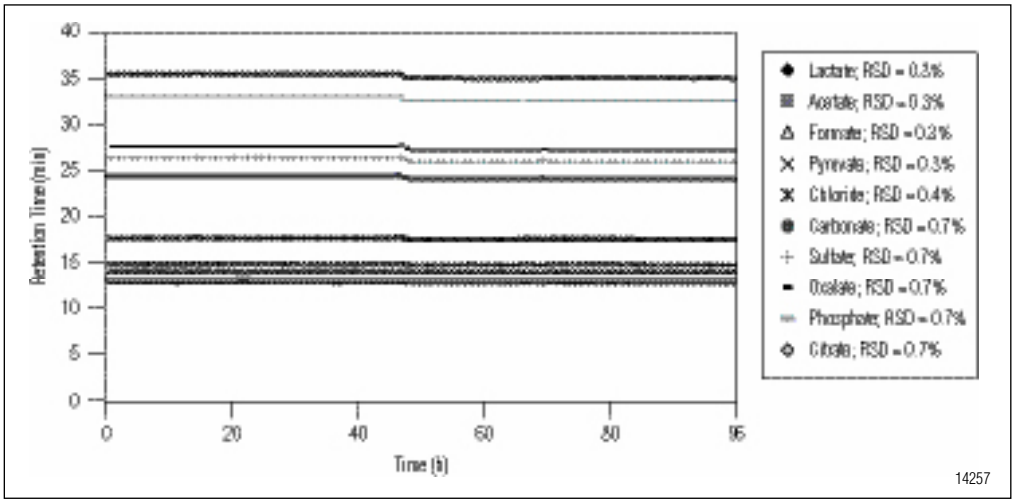


Figure 5. Retention times during 4 day repetitive analysis of heat-inactivated yeast fermentation broth.

Table 5. Recovery of Anions in the Yeast Fermentation Broth

Analyte	Percent Recovery
Lactate	100
Acetate	88
Formate	101
Pyruvate	99
Sulfate	101
Phosphate	100
Citrate	84

Yeast *(S. cerevisiae)* Culture

Yeast were grown in Bacto YPD broth at 37 °C for up to 24 h. Figure 6 shows the separation of fermentation broth ingredients in a yeast culture at the beginning (Figure 6A) and after 24 h (Figure 6B) of incubation. Lactate, acetate/glycolate, formate, valerate, methylmalonate, and citrate increased during the 24-h incubation. Table 6 lists the measured concentrations of these and other analytes during the 24-h incubation. Between 7 and 24-h, no additional time points were taken; however, substantial increases in the levels of lactate, acetate/glycolate,

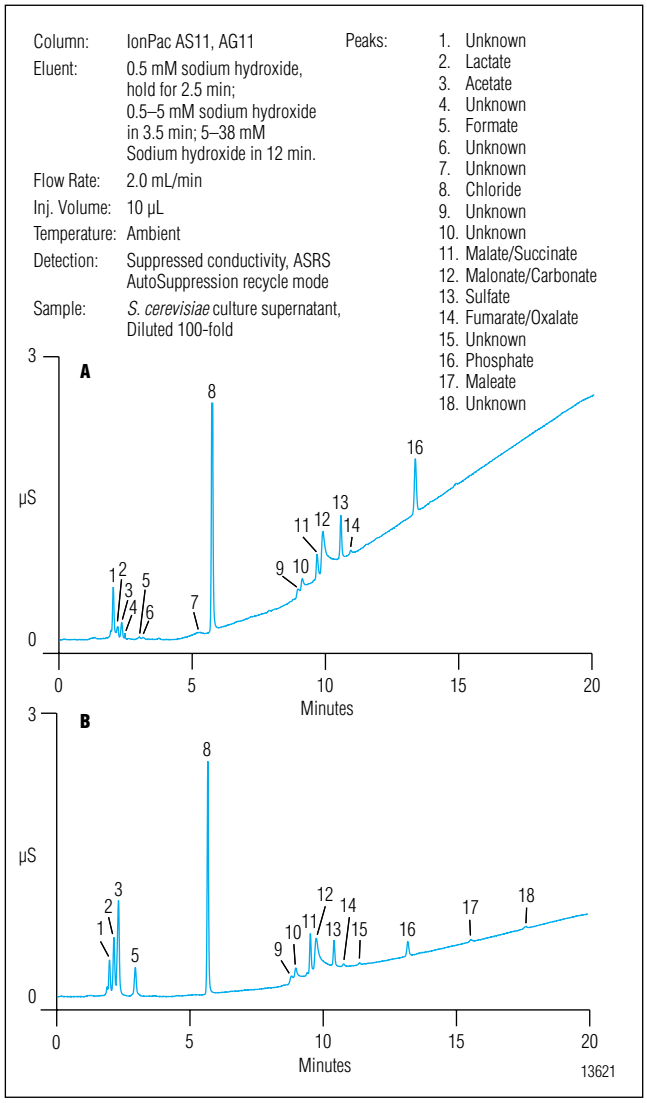


Figure 6. S. cerevisiae *fermentation broth culture (100-fold dilution) using the IonPac AS11 column at 0 h (A) and 24 h (B) of incubation.*

formate, and valerate occur. Some anions remained constant throughout the 24-h incubation, including chloride, malonate, sulfate, and oxalate/fumarate. Phosphate concentration decreased, presumably due to incorporation into the biomass (e.g., DNA, RNA, membrane phospholipids, etc.). At least 10 unidentified peaks were observed. The area units for eight of these peaks changed over the course of the incubation period. Changes in lactate, acetate, and formate concentrations are expected as a result of normal metabolic processes. Trending can be used to track culture status.

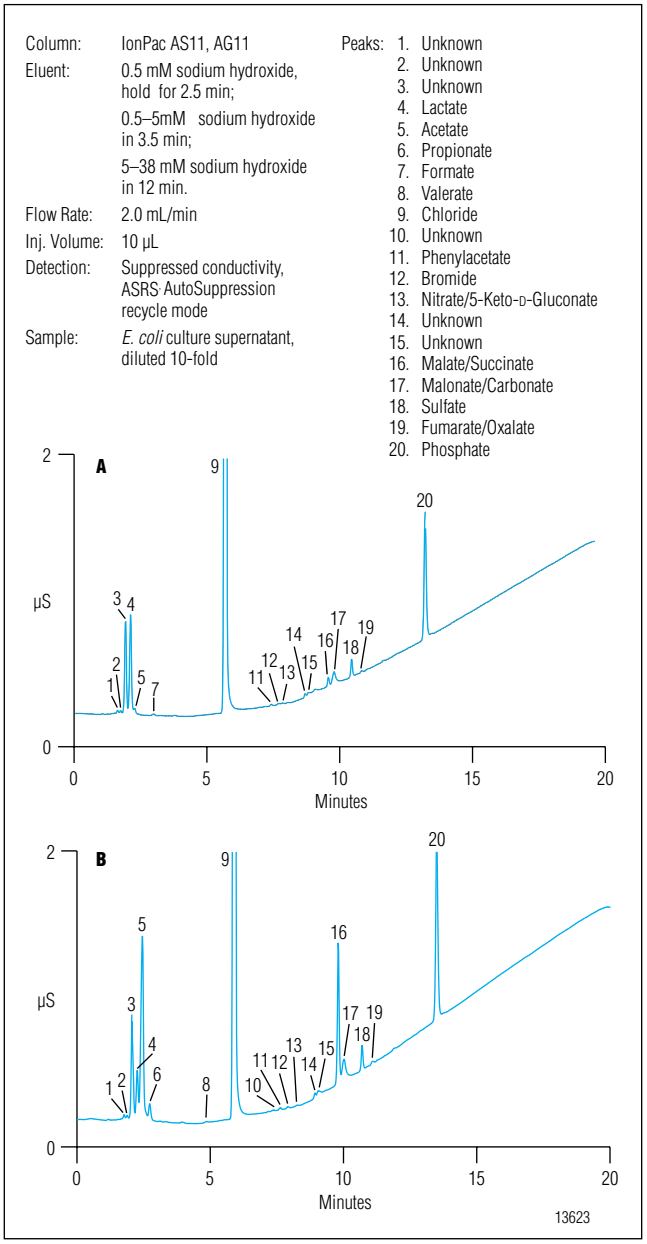


Figure 7. E. coli *fermentation broth culture using the IonPac AS11 column at 0 h (A) of incubation and the AS11 column at 24 h (B) of incubation.*

Bacteria (*E. coli*) Culture

Bacteria (*E. coli*) was grown on LB broth for 24 h at 37 ºC. Figure 7A shows the anions present in this broth at the beginning of the culture, and Figure 7B shows anions after 24 h.

Table 6. Anions in Yeast Fermentation Broth During in 24 h Incubation

		Broth Concentration (µg/mL)									
	Incubation Time (h)	0	0.5	1	2	3	4	5	6	7	24
Anions	**Lactate**	59	67	66	70	88	85	84	89	90	338
	Acetate	72	122	153	187	199	222	227	247	235	704
	Propionate	11	10	4	9	4	6	4	7	4	11
	Formate	7	10	11	13	11	14	6	7	6	21
	2-Keto-D-Gluconate	4	9	2	10	0	0	0	11	4	2
	Pyruvate	10	14	19	24	14	16	17	17	17	6
	Valerate	0	0	0	0	0	5	5	4	11	24
	Chloride	348	345	353	320	355	356	357	354	371	347
	Malate	0	7	13	9	8	15	15	12	15	11
	Methylmalonate	100	125	169	180	224	248	247	237	253	229
	Malonate	428	452	569	476	474	547	563	531	577	563
	Sulfate	68	68	79	63	67	65	64	63	61	57
	Oxalate	12	14	14	10	12	13	12	16	15	11
	Phosphate	165	124	92	62	55	57	58	58	59	62
	Citrate	0	0	0	13	15	12	13	13	10	0

To examine anions at lower concentrations, injections of a more concentrated culture are needed and the AS11-HC column is the best choice. Yeast fermentation broth (diluted only 10-fold) was analyzed by both the AS11 and AS11-HC columns, and is presented in Figures 8A and 8B, respectively. The AS11 column did not resolve the first unknown peak from lactate, while the AS11-HC did. Lactate and acetate were better resolved on the AS11-HC. Butyrate was resolved from formate on the AS11-HC column. Furthermore, many of the trace components that could not be measured using a 100-fold dilution could be measured with a 10-fold diluted sample using the AS11-HC column. Concentrations of anions were determined at different time points during incubation. After 24 h, lactate decreased, while acetate increased in concentration. Malate/succinate increased over this period. Chloride remained unchanged. Peaks having retention times equal to propionate and valerate were present after 24 h of incubation.

CONCLUSION

These results show that both yeast and bacterial culture fermentation broths can be analyzed for anion composition using ion chromatography and suppressed conductivity. Two columns (IonPac AS11 and AS11-HC) are available for fermentation broth analysis of organic acids and inorganic anions. The AS11-HC permits higher sample loading due to higher capacity. The high capacity of the column is able to resolve lactate, acetate, and formate. Complex mixtures of organic and inorganic anions can be monitored simultaneously during fermentation, providing the analyst with some of the information needed to optimize the fermentation.

REFERENCES

1. Dionex Corporation, "Ion Chromatography in the Pharmaceutical Industry". Application Note 106; Sunnyvale, CA
2. Rabin, S.; Stillian, J.R.; Barreto, V.; Friedman, K.; and Toofan, M.J. "New membrane-based electrolytic suppressor device for suppressed conductivity detection in ion chromatography" *J. Chromatogr.* **1993**, *640,* 97–109.

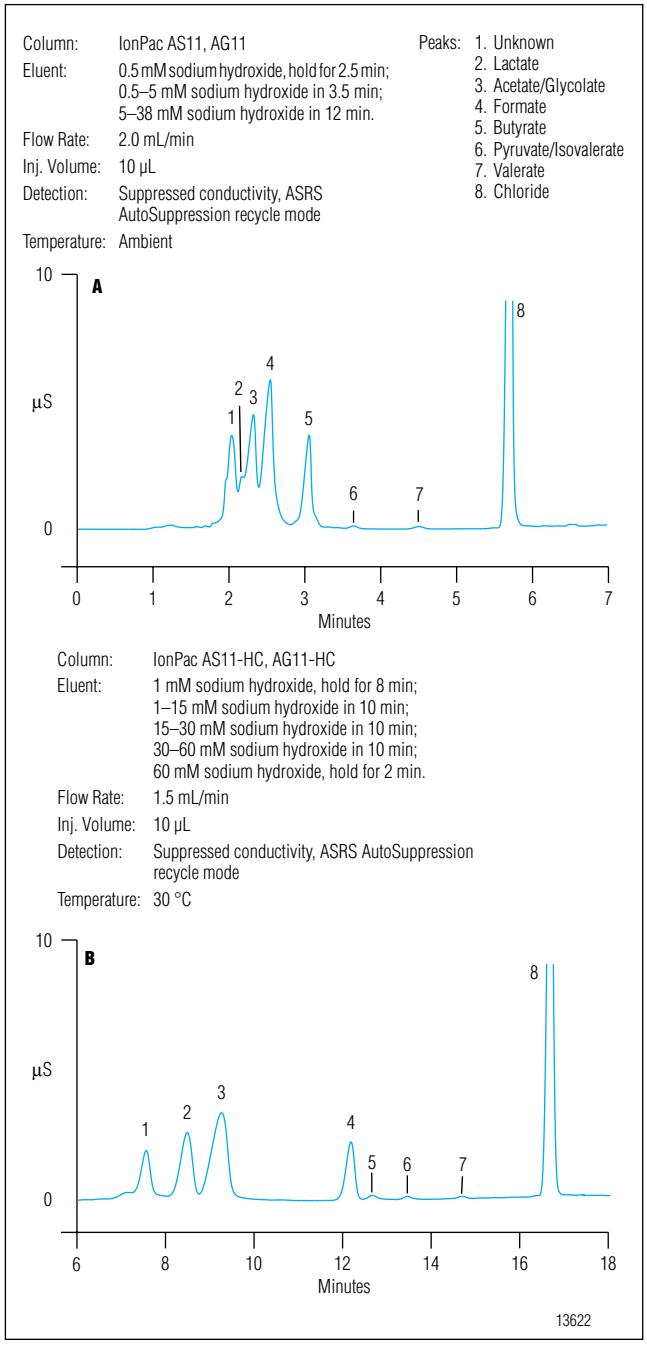


Figure 8. S. cerevisiae *fermentation broth culture (10-fold dilution) using the IonPac AS11 and AS11-HC column at 24 h of incubation.*

3. Robinett, R. S. R.; George, H. A.; Herber, W.K., "Determination of inorganic cations in fermentation and cell culture media using cation-exchange liquid chromatography and conductivity detection". *J. Chromatogr., A* **1995**, *718,* 319–327.
4. Joergensen, L.; Weimann, A.; Botte, H.F. "Ion chromatography as a tool for optimization and control of fermentation processes". *J. Chromatogr.,* **1992**, *602,* 179–188.
5. Loconto, P.R.; Hussain, N. "Automated coupled ion exclusion-ion chromatography for the determination of trace anions in fermentation broth". *J. Chromatogr. Sci.* **1995**, *33,* 75–81.
6. Forman, L. W.; Thomas, B. D.; Jacobson, F. S. "On-line monitoring and control of fermentation processes by flow-injection analysis". *Anal. Chim. Acta* **1991**, *249,* 101–111.
7. Huang, Y. L.; Khoo, S. B.; Yap, M. G. S. "Flow-injection analysis-wall-jet electrode system for monitoring glucose and lactate in fermentation broths". *Anal. Chim. Acta* **1993**, *283,* 763–771.
8. Schugerl, K.; Brandes, L.; Xiaoan, W.; Bode, J.; Ree, J. I.; Brandt, J.; Hitzmann, B. "Monitoring and control of recombinant protein production". *Anal. Chim. Acta* **1993**, *279,* 3–16.
9. Gey, M.; Nagel, E.; Weissbrodt, E.; Stottmeister, U. "Fast liquid chromatographic determination of organic acids in fermentation media with short glass columns". *Anal. Chim. Acta* **1988**, *213,* 227–230.
10. Buttler, T.; Gorton, L.; Marko-Varga, G. "Characterization of a sampling unit based on tangential flow filtration for on-line bioprocess monitoring". *Anal. Chim. Acta* **1993**, *279,* 27–37.
11. Dionex Corporation, "Determination of carbohydrates, alcohols, and glycols in fermentation broths". Application Note 122; Sunnyvale, CA

LIST OF SUPPLIERS

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Eastman Chemical Company, 1001 Lee Road, Rochester, NY, 14652-3512 USA, Tel: 1-800-225-5352, www.eastman.com

EM Science, P.O. Box 70, 480 Democrat Road, Gibbstown, NJ, 08027 USA, Tel: 1-800-222-0342, www.emscience.com

Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA 15219-4785 USA, Tel: 1-800-766-7000, www.fischersci.com

Fluka Chemika, Fluka Chemie AG, Industriestrasse 25, CH-9471 Buchs, Switzerland, Tel: 081 755 25 11, www.sigmaaldrich.com

Sigma-Aldrich Chemical Company, P.O. Box 14508, St. Louis, MO 63178 USA, Tel.: 1-800-325-3010, www.sigmaaldrich.com

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

INTRODUCTION

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

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

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

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

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

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

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

EQUIPMENT

Dionex ICS-3000 system consisting of:

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

Chromeleon® 6.7 Chromatography Management software

Centrifuge (Beckman Coulter, Brea, CA)

REAGENTS AND STANDARDS

Reagents

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

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

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

Standards

Dopamine hydrochloride (Sigma Chemical Co., H8502)

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

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

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

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

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

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

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

Spermidine trihydrochloride, >98% (Calbiochem, 56766)

Spermine tetrahydrochloride, ≥99% (Calbiochem, 5677)

CONDITIONS

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

Postcolumn Addition

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

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

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

Waveform

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

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solution

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

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

Postcolumn Base Addition Solution for IPAD

100 mM Sodium Hydroxide

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

STANDARD PREPARATION

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

SYSTEM PREPARATION AND SETUP

Integrated Pulsed Amperometric Detection

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

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

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

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

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

Suppressed Conductivity–Integrated Pulsed Amperometric Detection

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

UV Absorbance–Integrated Pulsed Amperometric Detection

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

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

SAMPLE PREPARATION

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

RESULTS AND DISCUSSION

Separation of Biogenic Amines

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

Method Performance

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

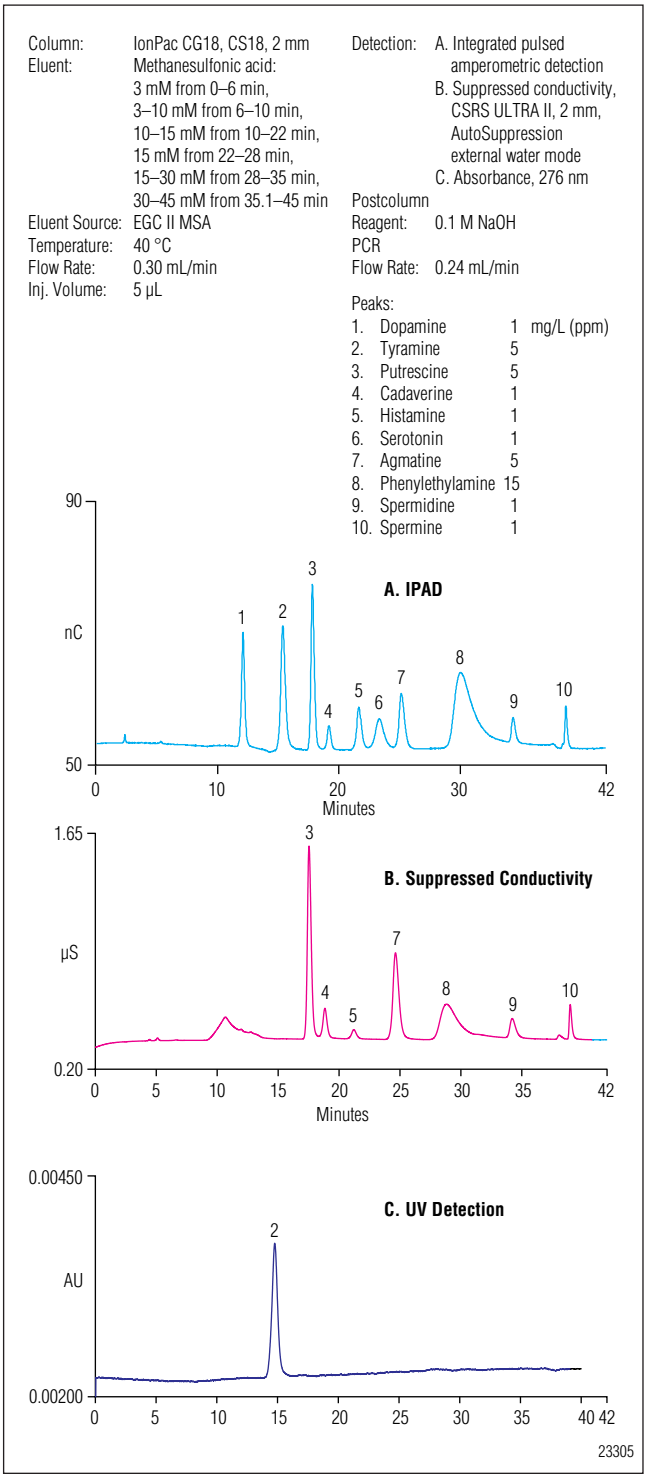

Figure 1. Separation of biogenic amines with (A) IPAD, or (B) suppressed conductivity detection. (C) Tyramine determined by UV detection.

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

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

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

Determination of Biogenic Amines in Alcoholic Beverages with IPAD

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

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

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

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

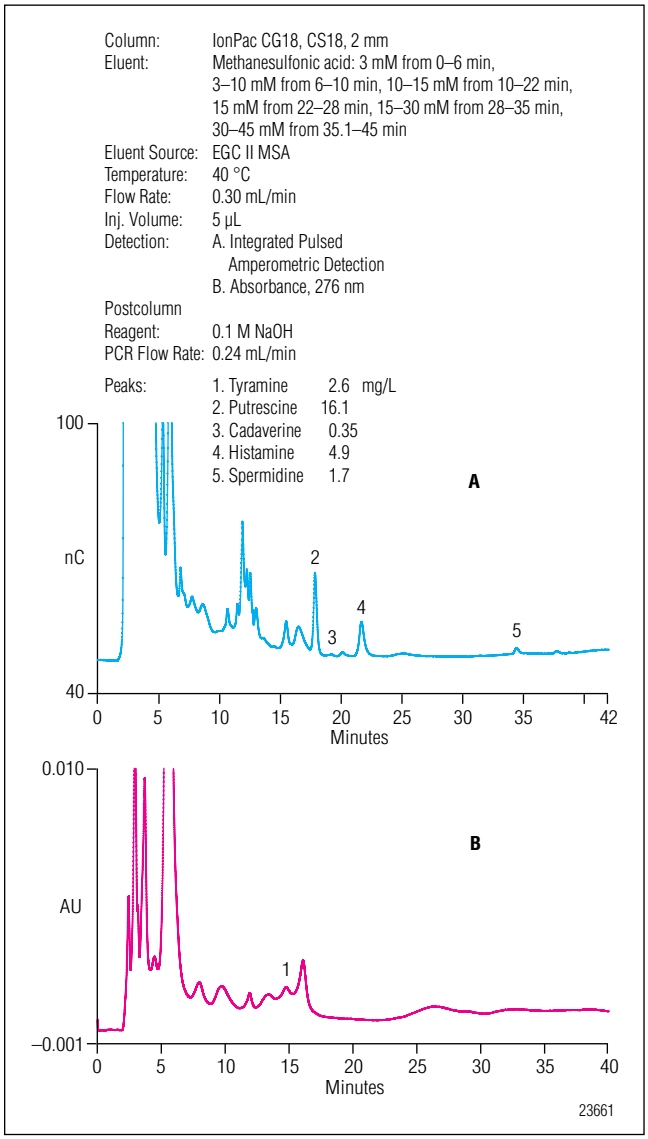

Figure 2. Determination of biogenic amines in a California Cabernet Sauvignon by (A) IPAD and (B) UV absorbance detection.

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

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

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

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

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

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

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

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

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

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

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

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

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

Suppressed Conductivity Detection

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

IPAD (post-suppression)

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

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

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

trescine, 67-184% for histamine, and 26% for agmatine. Figure 3 shows a separation of biogenic amines determined in wheat beer #2 using suppressed conductivity detection. Wheat beer #1 produced the most significant increase in histamine (184%) relative to the other beer samples. This observation is in agreement with a previous study that demonstrated a significant increase of histamine for a bottle beer sample stored at 21 °C for eight days.[28] The presence of lactic acid bacteria, primarily lactobacilli, has been demonstrated to be the primary cause for histamine increase in bottled beers over time.[28] Recalculating the BAI for all beers after storage at 4 °C results in an index value of <1 for wheat beer #1 and the lager beer and a value of 1.0 for wheat beer #2. According to the authors, a BAI between 1.0 and 10.0 would indicate that the beer had been produced by fermentation procedures that could be moderately contaminated by decarboxylating bacteria (intermediate level of microbiological quality).[15] The average amine recoveries for the spiked beer samples were in the range 88–118%. The calculated concentrations by IPAD in series with the suppressor were within ±12% of the concentrations determined by suppressed conductivity.

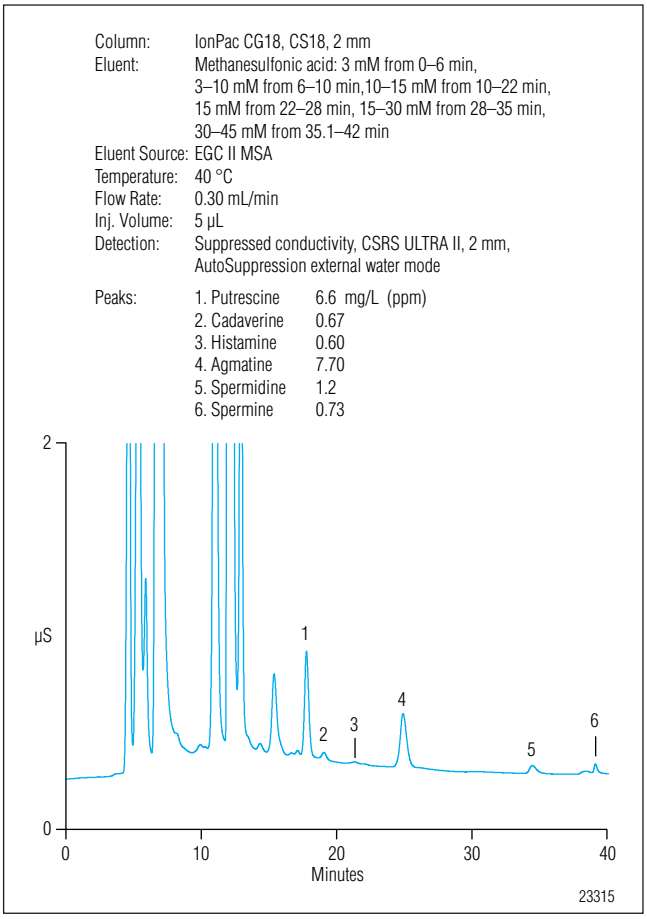

Figure 3. Determination of biogenic amines in wheat beer #2 using suppressed conductivity detection.

CONCLUSION

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

REFERENCES

1. Santos, M.H.S. Biogenic Amines: Their Importance in Foods. *Int. J. Food Microbiol.* **1996,** *29,* 213–231.
2. Soleas, G.J.; Carey, M.; Goldberg, D.M. Method Development and Cultivar-Related Differences of Nine Biogenic Amines in Ontario Wines. *Food Chem.* **1999,** *64,* 49–58.
3. Smith, T.A. Amines in Food. *Food Chem.* **1980-81,** *6,* 169–200.
4. Halász, A.; Baráth, Á.; Simon-Sarkadi, L.; Holzapfel, W. Biogenic Amines and their Production by Microorganisms in Food. *Trends Food Sci. Technol.* **1994,** *5,* 42–49.
5. Shalaby, A.R. Significance of Biogenic Amines to Food Safety and Human Health. *Food Res. Int.* **1996,** *29,* 675-690.
6. Bardócz, S. Polyamines in Food and their Consequences for Food Quality and Human Health. *Trends Food Sci. Technol.* **1995,** *6,* 341–346.

7. ten Brink, B.; Damink, C.; Joosten, H.M.L.J.; Huis in 't Veld, J.H.J. Occurrence and Formation of Biologically Active Amines in Foods. *Int. J. Food Microbiol.* **1990,** *11,* 73–84.
8. Gardini, F.; Zaccarelli, A.; Belletti, N.; Faustini, F.; Cavazza, A.; Martuscelli, M.; Mastrocola, D.; Suzzi, G. Factors Influencing Biogenic Amine Production by a Strain of Oenococcus Oeni in a Model System. *Food Contr.* **2005,** *16,* 609–616.
9. Vidal-Carou, M.C.; Lahoz-Portolés, F.; Bover-Cid, S.; Mariné-Font, A. Ion-Pair High-Performance Liquid Chromatographic Determination of Biogenic Amines and Polyamines in Wine and Other Alcoholic Beverages. *J. Chromatogr. A* **2003,** *998,* 235–241.
10. Kalac, P.; Krízek, M. A Review of Biogenic Amines and Polyamines in Beer. *J. Inst. Brew.* **2003,** *109,* 123–128.
11. Marcobal, A.; Polo, M.C.; Martín-Álvarez, P.J.; Moreno-Arribas, M.V. Biogenic Amine Content of Red Spanish Wines: Comparison of a Direct ELISA and an HPLC Method for the Determination of Histamine in Wines. *Food Res. Int.* **2005,** *38,* 387–394.
12. Leitão, M.; Marques, A.P.; San Romão, M.V. A Survey of Biogenic Amines in Commercial Portuguese Wines. *Food Contr.* **2005,** *16,* 199–204.
13. Bover-Cid, S.; Iquierdo-Pulido, M.; Mariné-Font, A.; Vidal-Carou, M.C. Biogenic Mono-, Di-, and Polyamine Contents in Spanish Wines and Influence of a Limited Irrigation. *Food Chem.* **2006,** *96,* 43–47.
14. Glória, M.B.A.; Izquierdo-Pulido, M. Levels and Significance of Biogenic Amines in Brazilian Beers. *J. Food Compos. Anal.* **1999,** *12,* 129–136.
15. Loret, S.; Deloyer, P.; Dandrifosse, G. Levels of Biogenic Amines as a Measure of the Quality of the Beer Fermentation Process: Data From Belgian Samples. *Food Chem.* **2005,** *89,* 519–525.
16. Campíns-Falcó, P.; Molins-Legua, C.; Sevillano-Cabeza, A.; Genaro, L.A.T. *o*-Phthalaldehyde-*N*-Acetylcysteine Polyamine Derivatives: Formation and Stability in Solution and in C18 Supports. *J. Chromatogr. B* **2001,** *759,* 285–297.
17. Hoekstra, J.C.; Johnson, D.C. Comparison of Potential-Time Waveforms for the Detection of Biogenic Amines in Complex Mixtures Following Their Separation by Liquid Chromatography. *Anal. Chem.* **1998,** *70,* 83–88.
18. Draisci, R.; Giannetti, L.; Boria, P.; Lucentini, L.; Palleschi, L.; Cavalli, S. Improved Ion Chromatography-Integrated Pulsed Amperometric Detection Method for the Evaluation of Biogenic Amines in Food of Vegetable or Animal Origin and in Fermented Foods. *J. Chromatogr. A* **1998,** *798,* 109–116.
19. Pineda, R.; Knapp, A.D.; Hoekstra, J.C.; Johnson, D.C. Integrated Square-Wave Electrochemical Detection of Biogenic Amines in Soybean Seeds Following Their Separations by Liquid Chromatography. *Anal. Chim. Acta* **2001,** *449,* 111–117.
20. Campbell, D.L.; Carson, S.; Van Bramer, D. Improved Determination of Alkanolamines by Liquid Chromatography with Electrochemical Detection. *J. Chromatogr.* **1991,** *546,* 381–385.
21. Rey, M.; Pohl, C. Novel Cation-Exchange Column For the Separation of Hydrophobic and/or Polyvalent Amines. *J. Chromatogr. A* **2003,** *997,* 199–206.
22. Cinquina, A.L.; Calí, A.; Longo, F.; De Santis, L.; Severoni, A.; Abballe, F. Determination of Biogenic Amines in Fish Tissues by Ion-Exchange Chromatography with Conductivity Detection. *J. Chromatogr. A* **2004,** *1032,* 73–77.
23. Saccani, G.; Tanzi, E.; Pastore, P.; Cavalli, S.; Rey, M. Determination of Biogenic Amines in Fresh and Processed Meat by Suppressed Ion Chromatography-Mass Spectrometry Using a Cation-Exchange Column. *J. Chromatogr. A* **2005,** *1082,* 43–50.
24. Vidal-Carou, M.C.; Codony-Salcedo, R.; Mariné-Font, A. Histamine and Tyramine in Spanish Wines: Relationship with Total Sulfur Dioxide Level, Volatile Acidity and Malo-lactic Fermentation Intensity. *Food Chem.* **1990,** *35,* 217–227.
25. Anli, R.E.; Vural, N.; Yilmaz, S.; Vural, Y.H. The Determination of Biogenic Amines in Turkish Red Wines. *J. Food Compos. Anal.* **2004,** *17,* 53–62.
26. Kalac, P.; Hlavatá, V.; Krízek, M. Concentrations of Five Biogenic Amines in Czech Beers and Factors Affecting Their Formation. *Food Chem.* **1997,** *58,* 209–214.
27. Marco, A.G.; Azpilicueta, C.A. Amine Concentrations in Wine Stored in Bottles at Different Temperatures. *Food Chem.* **2006,** 99, 680-685.
28. Kalac, P.; Savel, J.; Krízek, M.; Pelikánová, T.; Prokopová, M. Biogenic Amine Formation in Bottled Beer. *Food Chem.* **2002,** *79,* 431–434.

Determination of Glycols and Alcohols in Fermentation Broths Using Ion-Exclusion Chromatography and Pulsed Amperometric Detection

INTRODUCTION

Fermentation with yeast, bacteria, or other microorganisms has been used for centuries to produce alcoholic beverages, bread, cheese, yogurt, and feed stock for animals. Fermentation with other microorganisms has more recently been used to produce antibiotics such as penicillin and pharmaceutical compounds, enzymes, amino acids, and organic acids,[1-4] as well as ethanol for fuel or fuel additives.[2,3]

Fermentation broths are complex mixtures of microorganisms and both organic and inorganic compounds. Ionic compounds, carbohydrates, and amino acids are essential for cellular growth and structure. Organic acids, alditols (sugar alcohols), glycols, alcohols, and other compounds are metabolic byproducts.[2,3]

To optimize growth and yields, it is crucial to monitor fermentation broths for both cellular fuel sources as well as metabolic byproducts. Many of these carbohydrates, amino acids, anions, and organic acids have been successfully monitored in fermentation media and broths using ion chromatography (IC) (Dionex Application Notes 122,[5] 123,[6] and 150[7]).

For beer and wine, the absence or presence of aldehydes and glycols affects their quality and flavor. Alcohols and glycols have been determined using ion-exclusion chromatography with an IonPac® ICE-AS1, a perchloric acid eluent, and pulsed amperometric detection (PAD) with a platinum working electrode and a three-potential waveform.[8] However, perchloric acid is not an optimal eluent, as perchlorate poses a health risk to women of childbearing age and children by disrupting the uptake of iodide by the thyroid gland and causing hypothyroidism and birth defects.[9] Perchlorate is highly regulated in the U.S. because of these health risks. To avoid the use of perchlorate, an ion-exclusion method was developed that uses a more stable and environmentally benign acid, methanesulfonic acid.

Disposable electrodes and an optimized waveform[10] have also been developed to further improve amperometric detection of a variety of analytes. The disposable platinum electrode used in this application is easy to install, does not require reconditioning or polishing, allows faster equilibration, and provides lower detection limits than a conventional platinum electrode. The waveform and electrode used here allow fast, accurate determinations of alcohols and glycols in fermentation media and alcoholic beverage samples through ion-exclusion chromatography with PAD.

EQUIPMENT

Dionex ICS-3000 Reagent-Free™ IC (RFIC™) system consisting of:

- SP Single Pump module, gradient pump with degas option
- DC Detector/Chromatography module, single or dual zone
- ED Electrochemical Detector (P/N 079830)
- AS Autosampler with sample tray temperature controlling option and 1.5 mL sample tray

An electrochemical cell containing a combination pH-Ag/AgCl reference electrode (cell and reference electrode, P/N 061756, reference electrode P/N 061879) and a disposable platinum (Pt) working electrode (package of 6 electrodes, P/N 064440)

Knitted reaction coil, 375 µL, (P/N 043700) with two PEEK™ unions (¼-28 thread female to 10-32 thread female, P/N 042806)

Chromeleon® Chromatography Management Software, version 6.8

1.5-mL glass sample vials, with caps and slit septa (vial kit, P/N 055427)

PEEK tubing:

- Red (0.127-mm or 0.005-in i.d.) tubing, used for eluent connections from Inj. Valve 1 to column and cell.
- Black (0.25-mm or 0.010-in i.d.) tubing used for eluent connections from Pump 1 to Inj. Valve 1 and the waste line from the cell to waste container (5 ft, P/N 052306)
- Green (0.76-mm or 0.030-in i.d.) tubing, waste line to AS Autosampler (5 ft, P/N 052305)

Heated water bath (VWR Scientific 1200 series)

Shaker table (Lab Line)

Centrifuge (Eppendorf 5400 series)

Sterile assembled micro-centrifuge tubes with screw cap, 1.5 mL (Sarstedt 72.692.005)

REAGENTS AND STANDARDS

Reagents

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

Deionized water, Type 1 reagent-grade, 18 MΩ-cm resistivity or better, freshly degassed by vacuum filtration

L-Arabitol (Aldrich, P/N A3506)

meso-Erythritol, HPLC grade, (1,2,3,4-butanetetrol; Fluka BioChemika, P/N 45670)

Ethanol, denatured, (VWR, P/N JT9401-1, 90% pure)

Ethylene glycol (1,2-ethanediol; VWR, P/N JT9300-33)

Galactitol (dulcitol; Aldrich P/N D0256)

myo-Inositol (Aldrich, P/N I5125)

D-Mannitol (Aldrich, P/N 240184)

Methanesulfonic acid (Aldrich, P/N 64280; Dionex, P/N 033478)

Methanol (VWR, P/N JT9070-33)

1,2,3-Propanetriol (glycerol, glycerin; VWR, P/N JT2142-1)

2-Propanol (isopropyl alcohol, VWR, P/N BDH1133-4LG)

1,2-Propanediol (propylene glycol, Aldrich, P/N 241229)

pH 7 and pH 4 (yellow, blue) buffer solutions, NIST traceable (VWR International, P/N 34170-130, 34170-127)

Ribitol (adonitol; Aldrich, P/N A5502)

D-Sorbitol, HPLC (D-glucitol; Fluka BioChemika Ultra, P/N 85529)

Fermentation Medium

BD™ Bacto™ Yeast Extract-Peptone-Dextrose (YPD) Broth (BD Diagnostic, P/N 242820; VWR, P/N 90003-284)

Samples

Wyeast "German Ale," "Bohemian Lager," and "American Wheat" *Saccharomyces cerevisiae* samples purchased from Hop Tech Home Brewing (Dublin, CA, USA, P/N 1007XL, 2124XL, and 1010XL, respectively)

S. cerevisiae samples incubated from a Bacto YPD fermentation broth

American, German, and British beer and American wine beverages

CONDITIONS

Column:	IonPac ICE-AS1 Analytical, 4 × 250 mm (P/N 064198)
Flow Rate:	0.2 mL/min
Eluent:	100 mM Methanesulfonic acid
Column Temperature:	30 ºC
Oven Temperature:	30 ºC
Tray Temperature:	10 ºC
Inj. Volume:	10 µL (PEEK sample loop, P/N 042949), full loop injection
Detection:	Pulsed amperometric detection (PAD)
Waveform:	See Table 1.
Reference Electrode:	pH-Ag/AgCl electrode (P/N 061879) in AgCl mode
Working Electrode:	Disposable platinum working electrode (P/N 064440, package of six)
Typical Background:	60–90 nC versus Ag/AgCl
Typical System Backpressure:	~800 psi
Noise:	<10 pC
Typical pH:	1.0
Run Time:	30 min
Syringe Speed:	4
Flush Volume:	250 µL

Table 1. Waveform[10]

Time (sec)	Potential vs Ag/AgCl (V)	Gain Region	Integration	Ramp
0.00	+ 0.30	Off	Off	Ramp
0.31	+ 0.30	On	Off	Ramp
0.32	+ 1.15	On	Off	Ramp
0.64	+ 1.15	On	On (Start)	Ramp
0.66	+ 1.15	On	Off (End)	Ramp
0.67	- 0.30	On	Off	Ramp
1.06	- 0.30	Off	Off	Ramp
1.07	+ 0.30	Off	Off	Ramp

PREPARATION OF SOLUTIONS AND STANDARDS

Eluent (100 mM Methanesulfonic Acid)

An eluent generator cannot be used for this application because the pressure limitations of the ICE-AS1 (see Precautions at the end of this note). When manually preparing eluents, it is essential to use high quality, Type 1 water, 18 MΩ-cm resistivity or better, that contains as little dissolved gas as possible. Dissolved gases can increase noise levels. Degas the deionized water before eluent preparation. Prepare freshly degassed deionized water weekly for the AS Autosampler flush solution.

Mix 994 g of degassed Type 1 deionized water with 9.6 g of methanesulfonic acid (MSA) in a 1-L glass eluent bottle. Swirl gently to mix. Connect the prepared eluent to Eluent A line from Pump 1 and place the eluent bottle under ~4–5 psi of helium or other inert gas. Swirl the eluent bottle to thoroughly mix the eluent. Prime the pump with the new eluent.

Standard Preparation

To prepare separate 100 mM stock solutions of *meso*-erythritol, glycerol, propylene glycol, ethanol, *myo*-inositol, D-mannitol, D-sorbitol, galactitol, ribitol, L-arabitol, ethylene glycol, methanol, and isopropyl alcohol, weigh the amount of reagent grade compound stated in Table 2 into individual 20-mL glass scintillation bottles. Add degassed deionized water to a total weight of 20.00 g. The stock standards are stable for more than a month when refrigerated.

Working Standards

To prepare 25, 50, 100, 200, 400, 800, 1600, 3200, and 6400 µM working standards of *meso*-erythritol, glycerol, propylene glycol, and ethanol from the 100 mM stock standards, pipette 5, 10, 20, 40, 80, 160, 320, 640, and 1280 µL, respectively, of each stock standard into 20-mL glass scintillation bottles. Dilute these working standards to 20.00 g total weight with degassed deionized water. The working standards should be prepared weekly.

Standards for Retention Time Determination and Alditol and Glycol Separation Experiments

Dilute the 100 mM stock standards of *myo*-inositol, D-mannitol, D-sorbitol, galactitol, ribitol, L-arabitol, ethylene glycol, methanol, isopropyl alcohol, *meso*-erythritol, glycerol, propylene glycol, and ethanol to 50 µM (10 µL of stock in 20.00 g total with deionized water) for retention time determination experiments. Prepare combined 50 µM standards of *myo*-inositol, D-mannitol, D-sorbitol, galactitol, ribitol, L-arabitol, and *meso*-erythritol in a similar way. Prepare combined 25, 50, and 100 µM ethylene glycol and propylene glycol standards in a similar way, by diluting 5, 10, and 20 µL of stock solutions to 20.00 g total weight with deionized water.

SAMPLE PREPARATION

Bacto YPD Broth Medium Preparation

To prepare the Bacto Yeast Extract-Peptone-Dextrose (YPD) broth, dissolve 10.0 g in 200 mL of aseptically prepared deionized water (0.2 µm, nylon). The Bacto YPD broth contains a 1:2:2 ratio of Bacto Yeast Extract, Bacto Peptone, and dextrose. The growth medium was used for fermentation experiments and as a matrix blank.

Fermentation Samples

Fermentation Samples for Dilution Experiments

To prepare fermentation broth samples for the dilution experiments, first prepare the matrix control sample according to the instructions in AN 122.[5] Add 20–25 g of American Wheat *S. cerevisiae* mixture to 200 g of Bacto YPD broth. Immediately heat-quench 1-mL aliquots of this time = zero fermentation broth in boiling water for 10 min and then centrifuge them at 14,000 x g for 10 min. Transfer the supernatant to another vial and dilute with purified (0.2-µm nylon filter) deionized water according to dilution experiments.

Table 2. Amount of Compound Used to Prepare 20.00 g of Individual 100 mM Stock Standard Solutions

Compound	Formula Weight (g/mole)	Mass (g)
myo-Inositol ($C_6H_{12}O_6$)	180.16	0.360
D-Mannitol [$CH_2OH(HO\text{-}CH)_2(CHOH)_2CH_2OH$]	182.17	0.364
D-Sorbitol [D-glucitol, $CH_2OH(CHOH)(HO\text{-}CH)(CHOH)_2CH_2OH$]	182.17	0.364
Galactitol [dulcitol, $CH_2OH(HO\text{-}CH)(CHOH)_2(HO\text{-}CH)CH_2OH$]	152.17	0.364
D-Ribitol (adonitol, 1,2,3 4 5-pentanol) [$CH_2OH(HO\text{-}CH)(CHOH)(HO\text{-}CH)CH_2OH$]	152.15	0.304
L-Arabitol [$CH_2OH(CHOH)(HO\text{-}CH)_2CH_2OH$]	152.15	0.304
meso-Erythritol [1,2,3,4-butanetetrol, $HO\text{-}CH_2(CHOH)_2CH_2OH$]	122.12	0.244
Glycerol (1,2,3-propanetriol, $HO\text{-}CH_2CHOHCH_2OH$)	92.09	0.184
Ethylene glycol (1,2-ethanediol, $HO\text{-}CH_2CH_2OH$)	62.07	0.124
Propylene glycol (1,2-propanediol, $CH_3CHOHCH_2OH$)	76.09	0.152
Methanol (CH_3OH)	32.04	0.064
Denatured ethanol, anhydrous 90% (CH_3CH_2OH)	62.07	0.138
Isopropyl alcohol (2-propanol, $CH_3CHOHCH_3$)	60.09	0.120

Fermentation Samples for Robustness Experiments

To prepare samples (50 µM glycerol, propylene glycol, and ethanol in the control fermentation broth) for robustness experiments, first prepare the fermentation broth as prepared for the dilution experiments. Pipette 66.7 µL of diluted (300-fold) supernatant from the centrifuged, heat-quenched fermentation broth into a 20-mL scintillation bottle and dilute to 20.00 g with purified deionized water. Pipette 5.0 µL of 100 mM glycerol, propylene glycol, and ethanol stock standards into a 20-mL glass scintillation bottle and dilute to 10.00 g total weight with 300-fold dilution fermentation broth.

Fermentation Samples for Growth Experiments

To prepare fermentation samples for growth experiments, add 20–25 mL of wine or beer *S. cerevisiae* mixture to 200 mL of Bacto YPD medium (see Medium Preparation, above) in a sterile 500-mL Erlenmeyer flask. Cap the flask with a rubber stopper prepared with an air line pointed downward. Incubate for 26–28 h at 37 °C in a shaking (500–600 rpm) water bath. Remove 1-mL aliquots at selected time points (0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 22, 23, 24, 25, 26, 27, and 28 h), heat-quench, centrifuge, and dilute the supernatant 300-fold with purified deionized water in the same way as previously discussed. Spike recovery of select *S. cerevisiae* samples incubated in the Bacto YPD medium are prepared in a similar way as the spike recovery samples for the control fermentation broth.

Beverage Samples

To prepare the wine samples, centrifuge a 1-mL aliquot at 14,000 x g for 10 min. Transfer the supernatant to another vial and dilute with purified deionized water according to the dilution experiments described in the Results and Discussion section under Beverage Samples. The beer samples are first degassed with ultrasonic agitation and applied vacuum, and treated in the same manner as the wine beverage samples.

To prepare the 40 µM glycerol and 400 µM ethanol spike recovery samples for wine, pipette 4 and 40 µL, respectively, of 100 mM stock standards into a 20-mL glass scintillation bottle, and dilute to 10.00 g total weight with a 400-fold dilution of the supernatant from the centrifuged sample. Prepare the spike recovery samples for beer in the same way with a 400-fold dilution of the supernatant from the centrifuged, degassed sample.

SYSTEM PREPARATION AND SETUP

The setups for the individual modules, components, and system are thoroughly described in the *ICS-3000 Operator's Manual,*[12] *ICS-3000 Installation Manual,*[13] *AS Autosampler Operator's Manual,*[14] and the Chromeleon Help menus.

Plumbing the Chromatography System

Connect black PEEK (0. 254-mm or 0.010-in i.d.) tubing from Pump 1 to position P on Inj. Valve 1 inside the DC module. Connect red PEEK (0.127-mm or 0.005-in i.d.) tubing from Inj. Valve 1, position C to the heat exchanger. Install the IonPac ICE-AS1 column, according to the *IonPac ICE-AS1 Product Manual,*[15] by connecting the red PEEK tubing exiting the System 1 heat exchanger to the column and another 5 cm of red PEEK tubing to the free end the column.

Install a 375-µL knitted reaction coil between the end of the column and the cell to minimize dissolved oxygen from the sample and, therefore, the oxygen dip in the chromatogram. First connect both ends of the reaction coil to the large end (¼-28 thread female) of two unions (¼-28 thread female, 10-32 thread female).

Caution: tighten to finger tight only. Do not over tighten. Over tightening this connection can break the thermoformed end of the knitted reaction coil.

Connect the free end of the 5 cm of red PEEK tubing from the column to a free end of one of the unions. The free end of the remaining union will be connected to the cell (with another 5 cm of red PEEK tubing) after the combination pH/Ag/AgCl electrode is calibrated. The waste line from the cell will also be installed after the cell is assembled and installed. Install a 10-µL loop in DC Inj. Valve 1, in both L positions. Connect the AS Autosampler Injection Port tubing and the green PEEK (0.76-mm or 0.030-in i.d.) tubing waste line to DC Inj. Valve 1 positions S and W, respectively.

Configuring the AS Autosampler

Configure the AS Autosampler and connect the Sample Prep and Sample syringes according to the *AS Autosampler Operator's Manual.*[14] Enter the loop size (10 µL) in Loop Size V1, on the AS front panel, under Menu and Plumbing Configuration. Select the syringe sizes of the sample prep and the sample syringe from the pull down menus, under Menu and System Parameters. Also select Normal sample mode and enable Wait function, under Menu and System Parameters.

Configuring the System

Install the ED module in the middle DC chamber, above Inj. Valve 1 before turning on the DC and configuring the system. Do not remove or install the ED module while the DC is turned on. Turn on the AS Autosampler and DC and SP modules and wait until the AS Autosampler finishes its startup process. To configure the system, open and start the Chromeleon Server Monitor program, and then open and start the Chromeleon Server Configuration program. Create a timebase (named "Fermentation" for this discussion) if one is not already present for this system, and then add the devices: ICS-3000 SP pump module, DC module, and

AS Autosampler. Assign Pump 1 to the timebase (right click on SP module, select Properties, select Devices tab, and select the timebase on the pull-down menu for the pump). Verify that the AS device has the same options (e.g., SamplePreparation, Temperature Tray Control, etc.) listed on the AS Autosampler module. Save and check the configuration before leaving the program.

Configuring a Virtual Channel to Monitor pH

It is useful to monitor and record the pH during sample analyses. To record periodic pH measurements, manually enter log commands into the program (see Program section).

To continuously record the pH during sample determinations, create a virtual channel in Server Configuration. Open the Server Configuration program, right click on the timebase and select Add Device, Generic, and Virtual Channel Driver. Right click on the newly created Virtual Channel Driver device, and select Properties and the General tab. The Device Name should be automatically entered as VirtualChannel_01. Select the Signal tab, and select and double click on VirtualChannel_01 to open the Signal Configuration window. Enter pH for Unit, 1.0 for Factor, click on Analog for Type, and enter pH.value for Formula. Save and check the configuration before leaving the program. The pH virtual channel becomes one of the available signal channels. More information can be found in the Chromeleon Help program.

Amperometry Cell

Calibration, handling, and installation tips for the reference electrode and Certified Disposable Platinum working electrodes are thoroughly described in the System Preparation and Setup section of this application note, the *ICS-3000 Operator's Manual,*[12] and the product[16] and installation[17] manuals for disposable electrodes. To calibrate the combination pH-Ag/AgCl reference electrode, remove the storage cap from the reference electrode but leave the storage cap o-ring in place on top of the reference electrode. The storage cap o-ring will be used again when the reference electrode is removed and sealed into the storage cap. It does not interfere with the installation of the reference electrode. Rinse the KCl storage solution off the reference electrode, pat dry, and place the reference electrode in pH 7 buffer.

Open Chromeleon and connect to the Fermentation timebase. Click on the Chromeleon Panel icon, expand the timebase panel, and select the EC Detector tab. Connect the blue lead of the reference electrode to the ED black port. Check the cell on/off button to ensure that the cell is turned off. (The pH electrode remains active regardless of the cell power.) Click on the Calibration button which opens the ED Wellness Panel. Follow the calibration instructions in the Instructions button or in the *ICS-3000 Operator's Manual.* Wait for the pH reading to stabilize, then press the pH Offset Cal button and wait while it calculates the pH offset. After it is finished, remove the reference electrode, rinse, and pat it dry. Place the reference electrode in pH 4 buffer and wait until the reading is stable. Enter 4.00 in the pH Slope Buffer value, press the pH Slope Cal. button, and wait while it calculates the slope and intercept. When it is finished, save, upload the new calibration values, and close the ED Wellness Panel.

Assembling the Electrochemical Cell

Check that the reference electrode o-ring on the bottom of the reference electrode is in place and install one if it is missing or damaged. Gently screw the reference electrode into the electrochemical cell body. Tighten finger tight to a snug fit. (Do not use tools.) Install the disposable platinum electrode in the electrochemical cell, according to the *Disposable Platinum Electrode Installation Guide for ED* shipped with the electrodes. Install the electrochemical cell into the ED. Connect the yellow and blue leads on the cell to the yellow and black ports on the ED (Figure 1).[12] Connect the free end of union-knitted reaction coil to another 5-cm length of red PEEK tubing. Connect the remaining end to the cell inlet and direct the black cell outlet tubing to waste. Loosen the waste line until after the pump has started to prevent trapping bubbles in the cell (a source of high noise). Retighten the fitting when eluent is observed in the outlet.

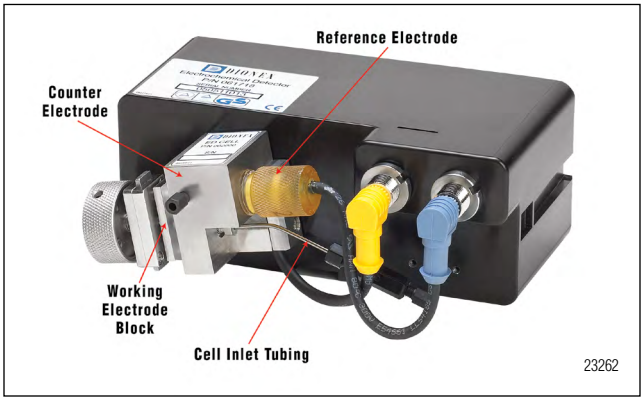

Figure 1. Amperometry cell.

In this application, the working electrode is a disposable platinum working electrode. When used with a recommended waveform and integration, the disposable platinum working electrodes have a background specification of 0 to 200 nC against the reference electrode in AgCl mode. Typically, the background will stabilize within 10 min. However, the pump may cause minute fluctuations in the background for up to an hour after installation. For trace analysis it is advisable to allow for an hour of equilibration before running samples.

Program

To make a new program, use the Program Wizard to enter the parameters from Table 1 and the Conditions section. In the EDet 1 Mode Options tab, select Integrated Amperometry. If a Virtual Channel was created in Configuration, the pH channel will be present in the Acquisition Options (see the section Configuring Virtual Channel to Monitor pH.) Select the pH, Pressure, and EDet1 detector channels. If a Virtual Channel was not created, select the Pressure and EDet1 detector channels. In the Pump_1_Pressure Options, select Auto for Step and check Average. In the EDet1 Options, select On for the amperometry cell, select all channels, enter 1.00 (Hz) for Data Collection Rate, 0 and 3 for pH Lower and Upper Limit, and enter the waveform (Table 1) in the Waveform selector. After the EDet1 Options tab, a Virtual Channel Options tab should appear with the same parameters as those entered when the Virtual Channel was configured: pH.value in Formula, Analog for Type, and Select Auto and Average for Step. Enter the title of the program and select Review Program. The new program will open in command mode in a new window.

To improve the signal to noise response for the detector signals, add signal averaging commands into the program. Find the following two commands in the program, and enter four empty lines. Using Control, Command, EDet1, ED_1, enter the commands Average, On and Step, Auto. Enter the same commands for ED_1_total. Check the program, using the Control, Check commands, and Save and Close the program.

```
EDet1.Mode =            IntAmp
EDet1.CellControl =     On
ED_1.Average =          On
ED_1.Step =             Auto
ED_1_total.Average =    On
ED_1_total.Step =       Auto
```

To record periodic pH measurements (optional), manually enter log commands into the program using Control, Command. Select System, Log, EDet1, pH, and Value, enter the retention time, and press Execute. Repeat the process for each pH reading. Save and close the program.

RESULTS AND DISCUSSION

Separation

Ion-exclusion chromatography uses a fully sulfonated resin with a strong acid eluent to exclude strongly ionic compounds by Donnan exclusion and large compounds by steric exclusion. Small neutral compounds are separated by adsorption partition.[18] Alcohols and glycols are neutral compounds and therefore not subject to Donnan exclusion. Organic acids are protonated by the strong acid eluent to neutral compounds. The stronger acids elute earlier than the weaker acids, therefore the compounds elute in the order of their pK_a. Carbohydrates are typically neutral aliphatic or cyclic poly-hydroxyl compounds. Most of the carbohydrates have little adsorption to the column and are found in the exclusion volume. Smaller linear carbohydrates are slightly adsorbed and elute early. Thus, ion-exclusion chromatography excludes strongly ionic compounds, dissacharides, polysaccharides, and some hexoses and pentoses that are typically present in high concentrations in the sample matrix. This allows better resolution of alcohols and glycols in the sample.

Figure 2 shows the separation of a *meso*-erythritol [$C_4H_6(OH)_4$], glycerol [$C_3H_5(OH)_3$], propylene glycol [$C_3H_6(OH)_2$], methanol (CH_3OH), ethanol (C_2H_5OH), and isopropanol (C_3H_7OH) standard in deionized water using 100 mM MSA. Generally, the steric interferences of the hydroxyl group(s) have a stronger influence than compound size on the elution order. That is, alditols with six hydroxyl groups elute first, followed by alditols with five and then four hydroxyl groups, glycols with three and then two hydroxyl groups, and alcohols with one hydroxyl group. Within the group, the compounds elute from most polar to least polar. For example, the alcohols elute in the following order: methanol, ethanol, and propanol.

The separation of a seven alditol standard (D-ribitol, D-sorbitol, L-arabitol, D-mannitol, galactitol, *myo*-inositol, *meso*-erythritol, 50 µM each) was evaluated to determine method suitability for alditol determinations. As expected, all of the alditols eluted early and were poorly resolved (not shown) from the other enantiomers with the exception of *meso*-erythritol. *myo*-Inositol (8.0 min) was barely resolved from the exclusion volume peak

(7.0 min), followed by D-mannitol (8.3 min), D-sorbitol and galactitol (8.4 min), D-ribitol (8.6 min), L-arabitol (8.8 min), and *meso*-erythritol (9.2 min). This method can detect alditols but can not resolve them with this separation. A HPAE-PAD carbohydrate method with a gold working electrode has already established acceptable resolution and quantification for alditols and should be used for their determination.[5]

The separation of ethylene glycol and propylene glycol in 25, 50 and 100 µM standards was also evaluated. Ethylene glycol and propylene glycol have a 0.4 min difference in retention time, 12.1 and 12.5 min, respectively (not shown), and they co-elute as a bimodal peak. When both are present, the bimodal peak can be forced into two separate peaks, allowing the quantification of each peak. This practice does increase the uncertainty associated with the two determined values. Ethylene glycol, propylene glycol, and diethylene glycol can be fully resolved by combining an ion-exclusion guard column (IonPac ICE-AS1, 2 x 50 mm as the guard column) with cation-exchange separation (IonPac CS14, 2 x 250 mm), using the same eluent conditions, waveform, and Pt working electrode conditions, as described in this application.[10]

Waveform

Alcohols and glycols are detected with a three-potential waveform, using E_1, E_2, and E_3. These voltages are applied at the designated times during a 1.07-min waveform. E_1, the initial potential, is + 0.30 V vs Ag/AgCl and maintained from 0.00 to 0.31 min. E_2 is the oxidation cleaning potential, +1.15 V vs Ag/AgCl from 0.32 to 0.64 min with detection (integration of current) occurring from 0.64 to 0.66 min. E_3 is the reductive cleaning potential, -0.30 V vs Ag/AgCl from 0.67 to 1.06 min. At 1.06 to 1.07 min, the potential reverts to the E_1 potential. Most of the period at E_2 and all of E_3 clean and restore the working electrode. The detection and integration occurs at the end of E_2.

Method Qualification

Prior to determining *meso*-erythritol, glycerol, propylene glycol, and ethanol concentrations in a fermentation broth, the alcohol and glycol method was qualified by determining linearity over a 250-fold concentration range, typical noise, estimated limits of detection, reproducibility, and robustness. The linearity of peak response was determined by measuring *meso*-erythritol, glycerol, propylene glycol, and ethanol in five

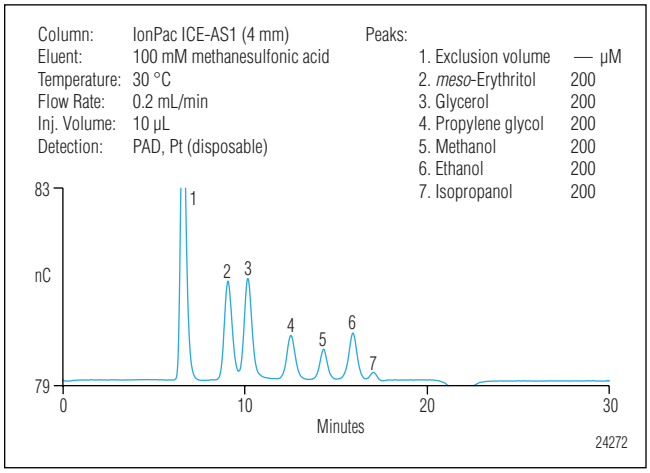

Figure 2. Separation of meso*-erythritol, glycerol, propylene glycol, ethanol, methanol, and isopropanol.*

replicates each of nine standards (25, 50, 100, 200, 400, 800, 1600, 3200, and 6400 µM). The calibration results showed a quadratic relationship for *meso*-erythritol, glycerol, and propylene glycol and a linear relationship for ethanol over this concentration range, $r^2 > 0.999$ (not shown). *meso*-Erythritol, glycerol, and propylene glycol exhibit linear behavior from 25 to 2000 µM.

The noise over two 60-min runs, when no sample was injected, was determined for each of three disposable electrodes by measuring the noise in 1-min intervals from 5 to 60 min. The noise value determined by this experiment was 8.0 ± 2.7 pC (n=10). The noise was similar for all three disposable platinum working electrodes. The method detection limit (MDL) was defined as the standard with the peak height three times the noise level. For this application with a 10-µL injection, the estimated limits of detection for *meso*-erythritol, glycerol, propylene glycol, and ethanol were 1.9, 1.6, 4.2, and 4.5 µM, or 2, 2, 4, and 2 ng, respectively. The signal-to-noise ratios for *meso*-erythritol, glycerol, propylene glycol, and ethanol in the 25 µM combined standard were 11.6 ± 0.1, 13.4 ± 0.2, 5.5 ± 0.1, and 4.4 ± 0.1 (n=5), respectively.

Before determining reproducibility and robustness, the 200-, 300-, 400-, 500-, and 1000-fold dilution levels of a heat-quenched, American Wheat *S. cerevisiae* fermentation broth were evaluated. The results showed that the 500- and 1000-fold dilutions had small and poorly defined peaks, while the 200-fold dilutions had column overload. The 300-fold and 400-fold dilutions exhibited the best chromatography, and the 300-fold dilution was selected for the fermentation experiments (Figure 3).

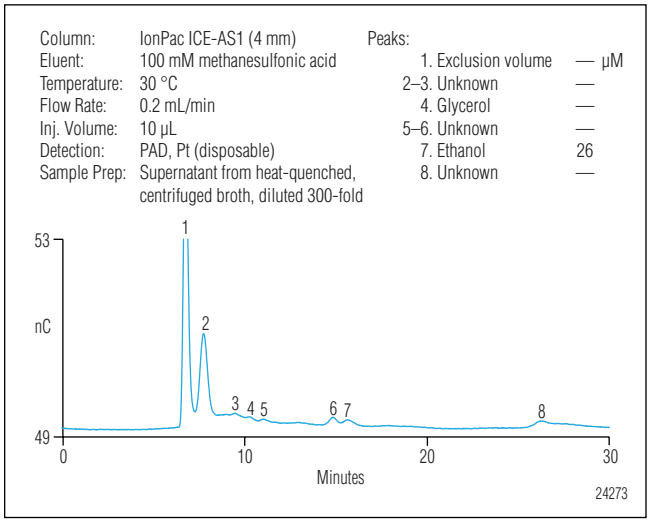

Figure 3. American wheat S. cerevisiae *fermentation broth.*

The retention time and peak area reproducibilities of glycerol, propylene glycol, and ethanol were measured over 250 injections (~125 h) to determine the reproducibility and robustness of the method. A 300-fold dilution of heat-quenched American Wheat beer *S. cerevisiae* in Bacto YPD growth medium without incubation was spiked with 50 µM glycerol, propylene glycol, and ethanol and was analyzed. One deionized water injection was inserted between groups of six sample injections. The results (Figures 4–5) showed that retention time and peak areas were stable over the entire experiment (125 h). The peak areas for glycerol, propylene glycol, and ethanol had small negative drifts over the five days, -0.2, -4.3, and -1.3%, respectively. The peak areas were also affected when the eluent was changed during the experiment (Figure 5). To determine single day reproducibility, peak areas were averaged for each spiked compound, not including the data immediately after the eluent change. These calculations showed that the peak area reproducibilities were less than 2% RSD for glycerol and ethanol, and less than 8% RSD for propylene glycol (Table 3).

Table 3. Five Days Peak Area Reproducibilities for Glycerol, Propylene Glycol, and Ethanol Spiked into 300-Fold Diluted Sample *S. Cervisiae* Incubated in Bacto YPD Fermentation Broth

Day	Glycerol (nC-min)	Propylene Glycol (nC-min)	Ethanol (nC-min)
1	0.193 ± 0.003	0.073 ± 0.005	0.457 ± 0.006
2	0.200 ±0.004	0.074 ± 0.005	0.453 ±0.008
3	0.196 ± 0.003	0.073 ± 0.004	0.454 ± 0.006
4	0.195 ± 0.004	0.071 ± 0.006	0.452 ± 0.006
5	0.193 ± 0.004	0.070 ± 0.005	0.451± 0.006

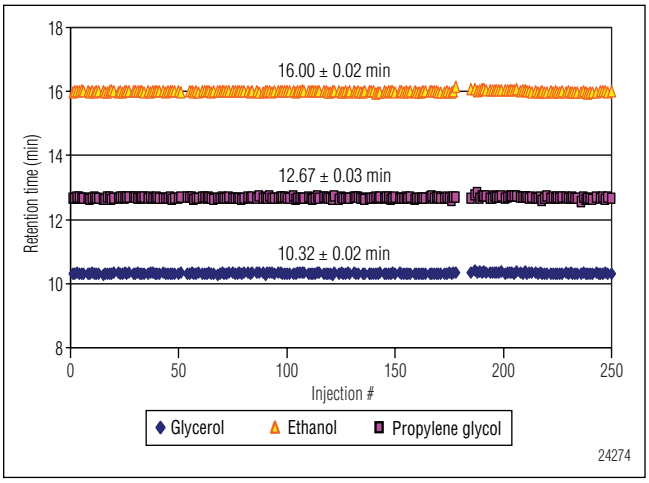

Figure 4. Retention time stability of 50 µM glycerol, propylene glycol, and ethanol spiked into a 300-fold dilution of the supernatant from heat quenched and centrifuged American Wheat S. cerevisiae *incubated in Bacto YPD.*

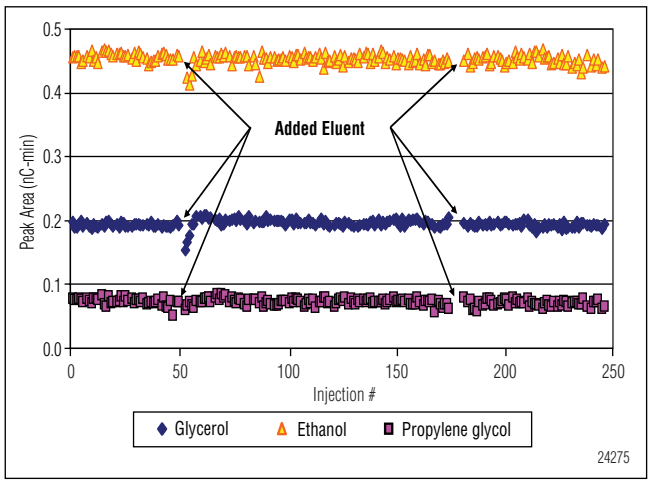

Figure 5. Peak area stability of 50 µM glycerol, propylene glycol, and ethanol spiked into a 300-fold dilution of the supernatant from heat quenched and centrifuged American Wheat S. cerevisiae *incubated in Bacto YPD.*

Disposable Platinum Working Electrodes

To determine the life of the platinum working electrodes with this method and waveform, the peak responses of glycerol, propylene glycol, and ethanol were determined in a 50 µM standard during the course of the experiments. Three disposable platinum working electrodes from the same lot were evaluated. Original response was measured after the newly installed electrode was allowed to equilibrate for 1 h. The working electrode was considered unacceptable and replaced with a fresh electrode when the peak response fell to 80% of the original response. The three working electrodes had similar initial responses for glycerol (0.1931 ± 0.004, 0.1891 ± 0.013, and 0.1940 ± 0.002 nC-min), propylene glycol (0.0720 ± 0.005, 0.0730 ± 0.003, and 0.0720 ± 0.003 nC-min), and ethanol [0.4564 ± 0.007, 0.4558 ± 0.007, and 0.4543 ± 0.006 nC-min (n=5)]. The electrodes showed good reproducibility within the same lot, <1.4% RSD. All three electrodes exceeded the two-week specification.

Fermentation Broth Samples

German Ale and Bohemian Lager *S. cerevisiae* were evaluated for the presence of *meso*-erythritol, glycerol, propylene glycol, and ethanol after incubation for 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 22, 23, 24, 25, 26, 27, and 28 h in Bacto YPD medium at 37 °C. *meso*-Erythritol and propylene glycol were not detected at any time during the fermentation. Figures 6 and 7 show the total concentration of glycerol and ethanol over the 28 h. Both fermentation broths had similar growth curves for glycerol and ethanol. As expected, the ethanol concentration grew exponentially. The glycerol concentration was below the quantification level of the 400-fold dilution incubation samples from the start of the incubation to 10 h. When the next samples were taken at 22 and 23 h, the glycerol concentration had increased to 21.0 ± 0.2 and 40.4 ± 0.8 µM in the 300-fold dilution German ale and Bohemian lager *S. cerevisiae* fermentation broths, respectively (Figure 8).

To determine recovery of glycerol, propylene glycol, and ethanol in broths that had undergone active fermentation, we spiked 60 mM glycerol and 100 mM propylene glycol and ethanol in 300-fold diluted, centrifuged, and heat-quenched samples from German ale and Bohemian lager (Figure 9) *S. cerevisiae* incubated 5 and 26 h, and 7 and 27 h, respectively.

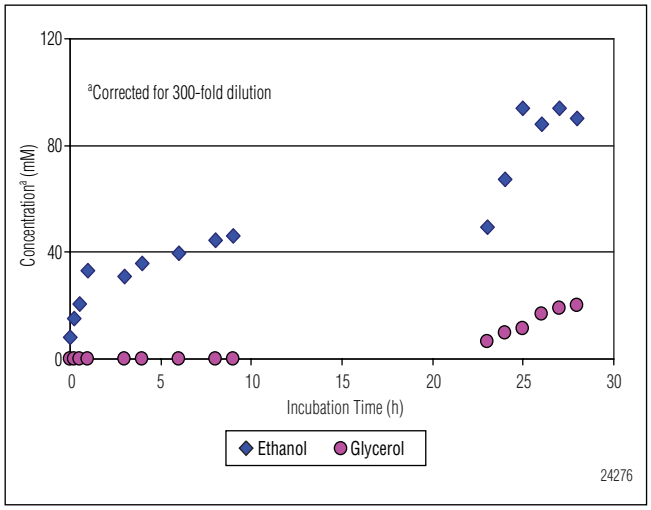

Figure 6. Total ethanol and glycerol concentrations during incubation of German ale S. cerevisae *in Bacto YPD growth medium.*

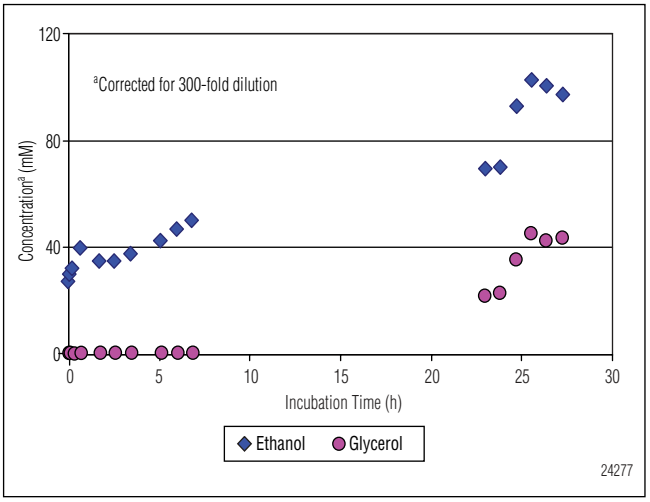

Figure 7. Total ethanol and glycerol concentrations during incubation of Bohemian lager S. cerevisae *in Bacto YPD growth medium.*

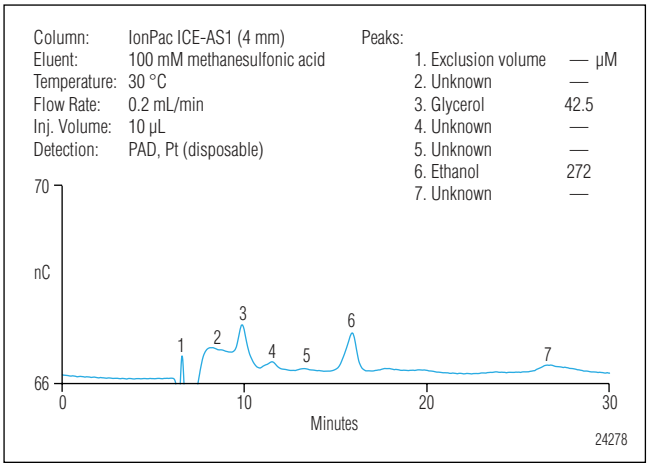

Figure 8. Bohemian lager S. cerevisae *incubated in Bacto YPD medium for 22 h.*

Table 4. Recovery of Glycerol, Propylene Glycol, and Ethanol Spiked into 300-Fold Diluted Samples of S. Cervisiae Incubated in Bacto YPD Fermentation Medium

		Glycerol			Propylene Glycol			Ethanol		
***S. Cerevisiae* Sample**	**Incubation (h)**	**Unspiked (µM)**	**Spiked[a] (µM)**	**Recovered (%)**	**Unspiked (µM)**	**Spiked[a] (µM)**	**Recovered (%)**	**Unspiked (µM)**	**Spiked (µM)**	**Recovered[a] (%)**
Brand A German Ale	5	ND	55.8 ± 1.8	99.8	ND	105.9 ± 2.5	96.2	291.4 ± 0.7	375.4 ± 3.1	94.5
Brand A German Ale	26	55.3 ± 0.1	112.4 ± 1.0	101.1	ND	107.9 ± 1.6	98.0	293.8 ± 6.3	402.3± 0.6	100.6
Brand A Bohemian Lager	7	ND	56.1 ± 0.5	100.4	ND	105.2 ± 2.7	95.5	347.9 ± 1.3	442.9 ± 1.3	97.6
Brand A Bohemian Lager	27	120.3 ± 2.3	178.5 ± 2.4	101.3	ND	104.6 ± 1.0	95.0	358.6 ± 2.6	456.3 ± 1.4	97.4

n = 2 for each sample.

[a]Added 55.9 ± 0.2 µM glycerol, 110.1 ± 1.4 µM propylene glycol, and 106.0 ± 1.3 µM ethanol for spike recovery experiments

The results (Table 4) show good recoveries for glycerol, propylene glycol, and ethanol in the range of not detected to 180 µM glycerol, not detected to 55 µM propylene glycol, and 300 to 400 µM ethanol.

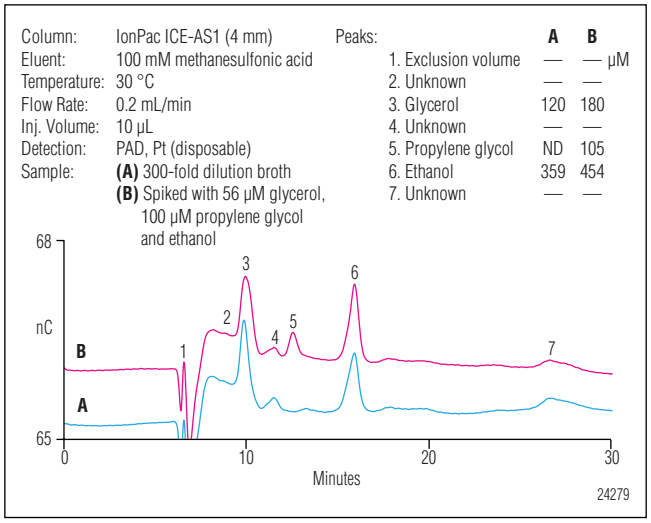

Figure 9. Bohemian lager S. cerevisae *incubated in Bacto YPD medium for 27 h.*

Beverage Samples

This chromatographic method was also applied to two California wine samples (Chardonnay and Cabernet Sauvignon) and three beer samples (American hefeweizen, German lager, and British ale). To evaluate the optimum dilution, 300-, 400-, 500-, and 1000-fold dilutions of the Chardonnay and British ale samples were tested. Both beverages had a large ethanol peak regardless of dilution. The glycerol was at a measurable concentration in the 300- and 400-fold dilution Chardonnay samples and below the limit of quantification at all tested dilution levels for the British ale. To quantify the glycerol in the wine samples and to minimize column overload, the 400-fold dilution concentration was selected for the beverage samples.

To evaluate the recovery of glycerol and ethanol in the 400-fold dilution of the five wine and beer samples, 40 µM glycerol and 400 µM ethanol were spiked into each and the concentrations of glycerol and ethanol determined in both the unspiked and spiked diluted beverage samples. The results (Table 5) show good recovery for glycerol and ethanol for all samples, ranging from 93.3 to 109.6%. Figures 10 and 11 show the chromatograms of a 400-fold dilution of British ale spiked with 40 µM glycerol and 400 µM ethanol and a 400-fold dilution of Chardonnay without additional glycerol and ethanol.

Table 5. Recovery of 40 µM Glycerol and 400 µM Ethanol Spiked into 400-Fold Diluted Samples of Wine and Beer						
	Glycerol			Ethanol		
400-fold Diluted Beverage Sample	Unspiked (µM)	Spiked (µM)	Recovered (%)	Unspiked (µM)	Spiked (µM)	Recovered (%)
Domestic Chardonnay	399 ± 4	430 ± 5	97.9	6799 ± 67	7183 ± 51	99.8
Domestic Carbernet Sauvignon	374 ± 6	409 ± 6	98.8	6391 ± 49	6801 ± 114	100.1
German Lager	ND	39.9 ± 2	99.8	2257 ± 32	2671 ± 26	100.5
Domestic Hefeweizen	ND	39.6 ± 1	100.5	2365 ± 78	2765 ± 14	100.0
British Brown Ale	ND	40.5 ± 1	101.3	1924 ± 34	2296 ± 43	98.9

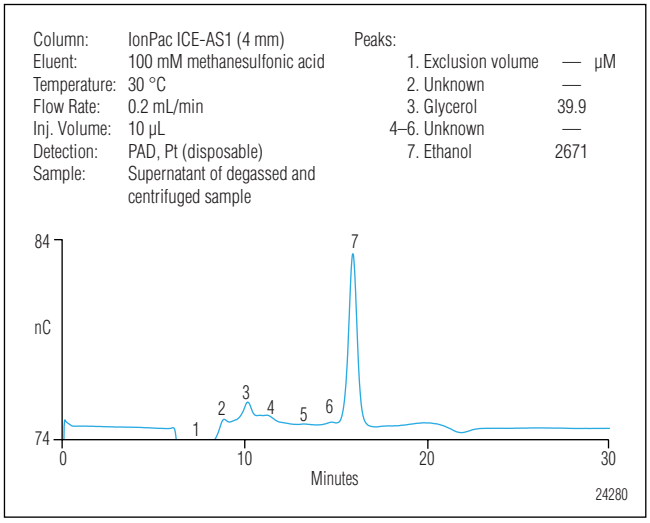

Figure 10. 40 µM glycerol and 400 µM ethanol spiked into a 400-fold dilution of German lager.

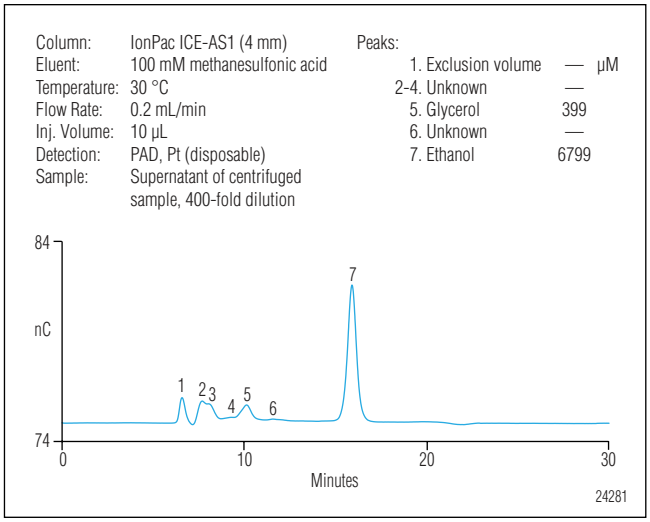

Figure 11. Glycerol and ethanol in Chardonnay.

CONCLUSION

The method presented in this application note can determine glycols and alcohols in beverages and complex matrices such as fermentation broths using ion-exclusion chromatography and PAD with a waveform optimized for a Pt disposable working electrode. This method offers a sensitive direct detection of alcohols without the need for sample preparation other than dilution and centrifugation.

Many of the compounds of interest in fermentation broths can now be determined using methods described in Dionex application notes: anions and organic acids [Application Note 123 (AN 123)],[6] carbohydrates and alditols (AN 117 and AN 122),[8,5] amino acids (AN 150),[7] cations using an method developed for water analysis (AN 141),[11] and glycols and alcohols using this note.

PRECAUTIONS

The IonPac ICE-AS1 column should not be used with system backpressures greater than 1000 psi. The eluent generator requires a system backpressure greater than 2000 psi and, therefore, can not be used as the eluent source for this application. Do not remove or install the ED module while the DC module is turned on. These power surges could cause internal damage to the ED module. Glass vials and bottles are required for this application.

REFERENCES

1. Slator, A. The Rate of Fermentation by Growing Yeast Cells. *Biochem. J.,* **1913**, *7* (2), 197–203.
2. Hough, J. S. *The Biotechnology of Malting and Brewing*; Cambridge University Press: New York, 1985.
3. Fix, G., Ph.D. *Principles of Brewing Science,* 2nd ed.; Brewers Publications: Boulder, CO, 1999.

4. Weiss, J. Ion Chromatography in the Pharmaceutical Industry. In *Handbook of Ion Chromatography,* 3rd ed.; Wiley-VCH, Weinheim, Germany, 2004; pp 772–781.
5. Dionex Corporation. *Determination of Carbohydrates, Alcohols, and Glycols in Fermentation Broths*; Application Note 122, LPN 1030: Sunnyvale, CA, 2004.
6. Dionex Corporation. *Determination of Inorganic Anions and Organic Acids in Fermentation Broths*; Application Note 123, LPN 1029: Sunnyvale, CA, 2004.
7. Dionex Corporation. *Determination of Amino Acids in Cell Cultures and Fermentation Broths*; Application Note 150, LPN 1538: Sunnyvale, CA, 2003.
8. Dionex Corporation. *Quantification of Carbohydrates and Glycols in Pharmaceuticals*; Application Note 117, LPN 957: Sunnyvale, CA, 2000.
9. *Health Implications of Perchlorate Ingestion*; Board on Environmental Studies and Toxicology, Division on Earth and Life Studies, National Research Council of the National Academies, National Academies Press: Washington, D. C., 2005.
10. Cheng, J.; Jandik, P.; Liu, X.; Pohl, C. *New Waveform for Direct and Sensitive Amperometric Detection of Alcohols, Alkenes, Alkynes, Aldehydes, Carboxylic Acids, Ethers, Ketones, Nitriles, Phenols, Thioethers, and Sulfoxides with Disposable Platinum Electrodes.* Paper presented at the 19th Annual International Ion Chromatography Symposium (IICS 2006), Pittsburgh, PA, 2006.
11. Dionex Corporation. *Determination of Inorganic Cations and Ammonium in Environmental Waters by Ion Chromatography Using the IonPac CS16 Column*; Application Note 141, LPN 1404: Sunnyvale, CA, 2001.
12. Dionex Corporation. *Operator's Manual for ICS-3000 Ion Chromatography System*; Document No. 065031: Sunnyvale, CA, 2005.
13. Dionex Corporation. *Installation Manual for ICS-3000 Ion Chromatography System*; Document No. 065032: Sunnyvale, CA, 2005.
14. Dionex Corporation. *Operator's Manual for AS Autosampler*; Document No. 065051: Sunnyvale, CA, 2005.
15. Dionex Corporation. *Product Manual for IonPac ICE-AS1*; Document No. 031181: Sunnyvale, CA, 2006.
16. Dionex Corporation. *Product Manual for Disposable Electrodes*; Document No. 065040: Sunnyvale, CA, 2006
17. Dionex Corporation. *Installation Guide for Disposable Platinum Electrode for ED (IC-3000)*; Document No. 065139: Sunnyvale, CA, 2006.
18. Weiss, J., Ion-Exclusion Chromatography (HPICE). In *Handbook of Ion Chromatography,* 3rd ed.; Wiley-VCH, Weinheim, Germany, 2004; pp 359–391.

SUPPLIERS

BD Laboratories, 1 Becton Drive, Franklin Lakes, NJ 07417 USA, 1-201-847-6800, www.bd.com/

Fermentation™ Solutions, 2511 Winchester Blvd, Campbell, CA 95008 USA, 1-408-871-1400 www.fermentationsettlement.com

Fisher Scientific International Inc., Liberty Lane, Hampton, NH 03842 USA, 1-800-766-7000 www.fisherscientific.com

HopTech Homebrewing Supplies, 6398 Dougherty Road, #7, Dublin, CA 94568 USA, 1-925-875-0246 http://www.hoptech.com

Sarstedt Inc., 1025, St.James Church Road, P.O. Box 468, Newton, NC 28658-0468 USA, 1-828-465-4000, www.sarstedt.com

Sigma-Aldrich Corp., St. Louis, MO 63103 USA 1-800-325-3010, www.sigmaaldrich.com

U.S. Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD, 20852-1790 USA, 1-800-227-8772 www.usp.org

VWR International, Inc., Goshen Corporate Park West, 1310 Goshen Parkway, West Chester, PA 19380 USA, 1-800-932-5000, www.vwrsp.com

Higher Resolution Separation of Organic Acids and Common Inorganic Anions in Wine

INTRODUCTION

The flavors imparted by wine are in part due to its organic acid composition. Tartaric, citric, and malic acids are the three major organic acids found naturally in wines. The maturation of a wine can be followed by changes in organic acid composition. For example, as many red wines age, the concentration of free tartaric acid decreases as it precipitates by binding with other components of the wine.

Organic acids also contribute to the overall acidity and tartness of a wine and can contribute flavors that are either pleasing or undesirable. For example, malic acid can impart a green apple flavor, whereas excessive acetic acid will impart an unwanted vinegar flavor. Malolactic fermentation is a winemaking technique popular for the production of some chardonnays. In this process, the malic acid is converted to lactic acid by bacteria, either naturally or by the specific introduction of the bacteria, to produce a wine with a lower acidity and different taste.

Although organic acids in wine can be determined by ion-exclusion chromatography, the peak capacity of most ion-exclusion columns is low and some organic acids are not well resolved, even when two columns are placed in series.[1] The ion-exclusion separation also does not allow the simultaneous determination of inorganic anions.

Ion chromatography (IC) with suppressed conductivity detection is an excellent way to separate a large variety of organic acids and detect them with high sensitivity along with inorganic anions. Masson used IC to determine organic acids and inorganic anions in grape juices used to make wine.[2] Dionex has also demonstrated that IC can be used to determine organic acids and inorganic anions in a variety of fruit juices, including grape juice.[3,4]

The method described here shows a higher resolution separation of the organic acids and inorganic acids found in wine than previously reported.[1] This approach is applied to the separation and detection of organic acids and inorganic anions in four red and white wine samples.

EQUIPMENT

Dionex ICS-3000 or ICS-5000 system including:

DP or SP Pump

DC Detector/Chromatography module with dual-temperature zone equipped with 6-port valve (Injection valve)

AS Autosampler

AXP Pump

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

REAGENTS AND STANDARDS

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

Sodium fluoride (NaF, Fluka)

Sodium acetate ($C_2H_3O_2Na$, Fluka)

Lactic acid 85% ($C_3H_6O_3$, APS)

Formic acid ($C_2H_2O_2$, Merck)

Shikimic acid 99% ($C_7H_{10}O_5$, Sigma)

Sodium chloride (NaCl, Fluka)

Sodium nitrite ($NaNO_2$, Fluka)

Sodium bromide (NaBr, Fluka)

Sodium nitrate ($NaNO_3$, Fluka)

Succinic acid ($[C_2H_3O_2]_2$, Ajax)

Malic acid ($C_6H_6O_5$, Ajax)

Tartaric acid ($[C_2H_3O_3]_2$, Ajax)

Sodium sulfate (Na_2SO_4, Fluka)

Oxalic acid ($C_2H_2O_4$*$2H_2O$, Merck)

Sodium hydrogen orthophosphate (Na_2HPO_4, Fluka)

Citric acid ($C_6H_8O_7$, Ajax)

Sodium hydroxide solution (400 g/L) (NaOH, KANTO)

Methanol (CH_3OH, RCI Labscan)

Ethanol (C_2H_5OH, RCI Labscan)

Sulfuric acid (98%) (H_2SO_4, RCI Labscan)

PREPARATION OF SOLUTIONS AND REAGENTS

Eluents

Sodium Hydroxide (0.1 M)

Dilute 10 mL of 400 g/L sodium hydroxide solution to 1 L in a 1 L volumetric flask with DI water and mix.

Sodium Hydroxide (1 M)

Dilute 100 mL of 400 g/L sodium hydroxide solution to 1 L in a 1 L volumetric flask with DI water and mix.

Methanol (12%)/Ethanol (16%) in DI water

Mix 120 mL of methanol, 160 mL of ethanol, and 720 mL of DI water in a 1L bottle and degas.

Sulfuric Acid Stock Solution (1 N)

Add approximately 700 mL of DI water into a 1 L volumetric flask and slowly add 50.04 g of 98% sulfuric acid into the same flask. Bring the volume to 1 L with DI water.

Sulfuric Acid (20 mN)

Dilute 20 mL of 1 N sulfuric acid stock solution in a 1 L volumetric flask with DI water.

Standard Solutions

Stock Standard Solutions

For each stock standard, dissolve the weight of salt or acid solution shown in Table 1 in a 100 mL volumetric flask with DI water.

Table 1. Masses of Compounds Used to Prepare 100 mL Stock Standard Solutions

Anion	Compound	Stock Concentration (mg/L)	Weight (g)
Fluoride	Sodium fluoride	500	0.111
Acetate	Sodium acetate ($C_2H_3O_2Na$)	1000	0.139
Lactate	Lactic acid 85% ($C_3H_6O_3$)	2000	0.235
Formate	Formic acid ($C_2H_2O_2$)	1000	0.100
Shikimate	Shikimic acid 99% ($C_7H_{10}O_5$)	1000	0.101
Chloride	Sodium chloride (NaCl)	1000	0.165
Nitrite	Sodium nitrite ($NaNO_2$)	1000	0.150
Bromide	Sodium bromide (NaBr)	1000	0.129
Nitrate	Sodium nitrate ($NaNO_3$)	1000	0.137
Succinate	Succinic acid $(C_2H_3O_2)_2$	1000	0.100
Malate	Malic acid ($C_6H_6O_5$)	2000	0.200
Tartarate	Tartaric acid $(C_2H_3O_3)_2$	2000	0.200
Sulfate	Sodium sulfate (Na_2SO_4)	1000	0.148
Oxalate	Oxalic acid ($C_2H_2O_4$*$2H_2O$)	1000	0.140
Phosphate	Sodium hydrogen phosphate (Na_2HPO_4)	2000	0.298
Citrate	Citric acid ($C_6H_8O_7$)	1000	0.100

Table 2. Concentrations of Working Standards and Their Preparation										
Anion	Calibration Standard Concentration (mg/L)					Volume of Stock Standard Solution (mL)				
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 1	Level 2	Level 3	Level 4	Level 5
Fluoride	0.05	0.1	0.25	0.5	0.75	0.01	0.02	0.05	0.10	0.15
Acetate	0.6	1.2	3.0	6.0	9.0	0.06	0.12	0.30	0.60	0.90
Lactate	1.0	2.0	5.0	10	15	0.05	0.10	0.25	0.50	0.75
Formate	0.1	0.2	0.5	1.0	1.5	0.01	0.02	0.05	0.1	0.15
Shikimate	0.4	0.8	2.0	4.0	6.0	0.04	0.08	0.20	0.40	0.60
Chloride	0.2	0.4	1.0	2.0	3.0	0.02	0.04	0.10	0.20	0.30
Nitrite	0.2	0.4	1.0	2.0	3.0	0.02	0.10	0.20	0.20	0.30
Bromide	0.1	0.2	0.5	1.0	1.5	0.01	0.05	0.05	0.10	0.15
Nitrate	0.1	0.2	0.5	1.0	1.5	0.01	0.05	0.05	0.10	0.15
Succinate	0.6	1.2	3.0	6.0	9.0	0.06	0.12	0.30	0.60	0.90
Malate	1.0	2.0	5.0	10	15	0.05	0.10	0.25	0.50	0.75
Oxalate	0.1	0.2	0.5	1.0	1.5	0.01	0.02	0.05	0.10	0.15
Phosphate	1	2	5	10	15	0.05	0.10	0.25	0.50	0.75
Citrate	0.5	1	2.5	5	7.5	0.05	0.10	0.25	0.50	0.75

Working Standard Stock Solutions

For each calibration level, add the volumes of stock standard solutions listed in Table 2 to a 100 mL volumetric flask and bring to volume with DI water.

Spiking Standard Stock Solutions

Prepare each spiking standard stock solution at 10× the spiked concentration by adding the volumes of stock standard solutions listed in Table 3 to a 100 mL volumetric flask, then bring to volume with DI water.

Sample Preparation

Dilute a 1 mL wine sample with DI water in a 100 mL volumetric flask and treat the sample with an OnGuard® II RP cartridge. Discard the first 3 mL, then collect the sample in an autosampler vial. For more information about using the OnGuard II RP cartridge, please refer to the OnGuard II Cartridges Product Manual.[5]

Spiked Sample Preparation

Prepare spiked sample in the same manner as in the Sample Preparation section. Add 10 mL of the appropriate spiking stock standard solution (Table 3) to the same volumetric flask as the wine before bringing the volume to 100 mL with DI water.

Table 3. Concentrations and Preparation of the Spiking Stock Standard Solutions (100 mL)

Anion	Spiking Stock Standard Concentration (mg/L)		Volume of Stock Standard Solution (mL)	
	Red Wine 1 and 2, and White Wine 1	White Wine 2	Red Wine 1 and 2, and White Wine 1	White Wine 2
Fluoride	1	1	0.20	0.20
Acetate	10	10	1.0	1.0
Lactate	20	20	1.0	1.0
Formate	1	1	0.10	0.10
Shikimate	5	5	0.50	0.50
Chloride	5	5	0.50	0.50
Nitrite	5	5	0.50	0.50
Bromide	2	2	0.20	0.20
Nitrate	1	1	0.10	0.10
Succinate	20	20	2.0	2.0
Malate	10	10	0.50	0.5
Oxalate	1	1	0.10	0.10
Phosphate	10	10	0.50	0.50
Citrate	5	20	0.50	2.0

CONDITIONS

Columns:	OmniPac® PAX-100 Analytical, 4 × 250 mm (P/N 042150) OmniPac PAX-100 Guard, 4 × 50 mm (P/N 042151)
Trap Column:	IonPac® ATC-HC, 9 × 75 mm (P/N 059604)
Eluent:	A: DI water B: 12% Methanol/16% ethanol in DI water C: 0.1 M Sodium hydroxide D: 1.0 M Sodium hydroxide
Gradient:	See Table 4
Temperature:	30 °C
Injection Vol:	25 µL
Flow Rate:	1.0 mL/min
Detection:	Suppressed Conductivity
Suppressor:	AMMS® 300, 4 mm (P/N 064558)
Regenerant:	20 mN H_2SO_4, delivered by AXP pump

Table 4. Gradient

Time	%A	%B	%C	%D
–15.0	80	0	0	20
–10.0	80	0	0	20
–9.5	0	97	3	0
0.0	0	97	3	0
3.5	0	97	3	0
3.6	0	92	8	0
9.0	0	92	8	0
28.0	0	0	100 (Curve 7)	0
35.0	0	0	100	0

RESULTS AND DISCUSSION

Separation

The IonPac AS11 and AS11-HC columns are well known for their ability to separate organic acids and inorganic anions in a wide range of products including grape and other fruit juices.[3,4] A set of nine organic acids and seven common anions were chosen to represent anions most likely to be in a wine sample. Attempts to achieve adequate resolution of this set of analytes on the IonPac AS11 or AS11-HC columns, both with and without added methanol, were unsuccessful (Figure 1). In particular, it was difficult to resolve acetate, shikimate, and lactate as well as succinate and malate.

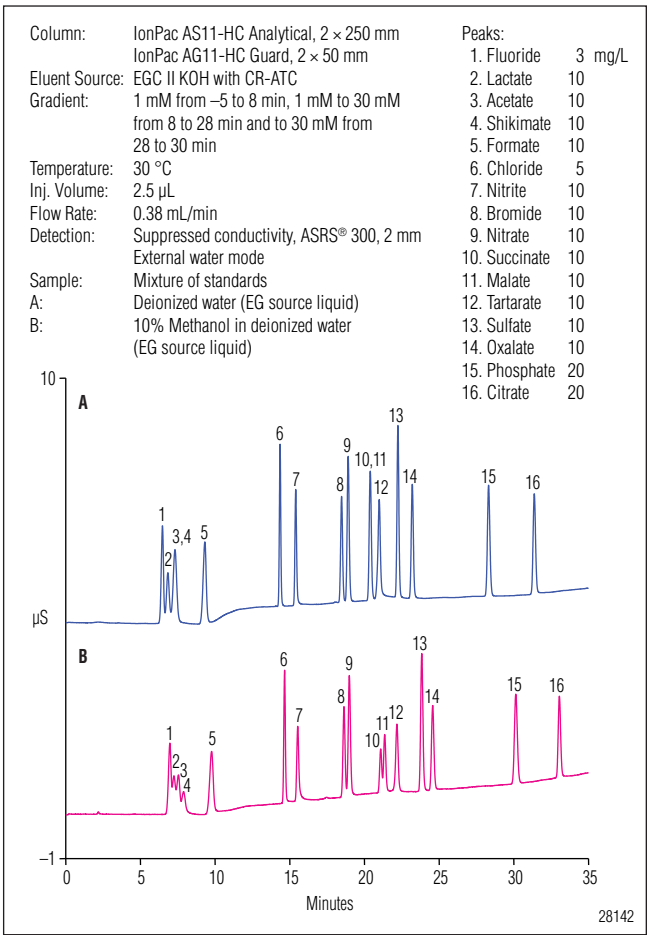

Figure 1. Chromatogram of a mixture of standards with and without adding methanol to the eluent.

The OmniPac PAX-100 column was then used in an attempt to achieve a better separation of the organic acids in wine. Using a sodium hydroxide eluent containing methanol and ethanol with gradient elution, the 16 compounds were resolved in 35 min (Figure 2). Applying these and similar conditions to the IonPac AS11 or 11-HC columns did not yield a similar separation. Because organic solvents were used in the separation on the OmniPac column, chemical regeneration was required for suppressed conductivity detection. For some applications, the separation without added organic solvent shown in Figure 1A may be adequate. That separation can be conveniently executed with a Reagent-Free™ IC (RFIC™) system where only DI water needs to be added to the system for the chromatography.

Method Calibration

Before sample analysis, the method was calibrated using five mixed standards with different concentrations of each of the 16 anions. Concentrations were chosen based on a preliminary analysis of the samples. Three injections of each level were made to construct the calibration plot. Table 5 shows the calibration results.

		Peaks:		
Column:	OmniPac PAX-100 Analytical, 4 × 250 mm			
	OmniPac PAX-100 Guard, 2 × 50 mm	1. Fluoride	0.75	mg/L
Trap Column:	IonPac ATC-HC, 9 × 75 mm	2. Lactate	9.00	
Eluent:	A: DI water	3. Acetate	15.0	
	B: 12% Methanol/16% Ethanol in DI water	4. Formate	1.50	
	C: 0.1 M Sodium hydroxide	5. Shikimate	6.00	
	D: 1 M Sodium hydroxide	6. Chloride	3.00	
Gradient:	See Table 4	7. Nitrite	3.00	
Temperature:	30 °C	8. Bromide	1.50	
Inj. Volume:	25 µL	9. Nitrate	1.50	
Flow Rate:	1.0 mL/min	10. Succinate	9.00	
Detection:	Suppressed conductivity	11. Malate	15.0	
Suppressor:	AMMS 300, 4 mm	12. Tartarate	22.5	
Sample:	Mixture of standards	13. Sulfate	7.50	
Regenerant:	20 mN H_2SO_4	14. Oxalate	1.50	
		15. Phosphate	15.0	
Sample:	Calibration standard, Level 5	16. Citrate	7.50	

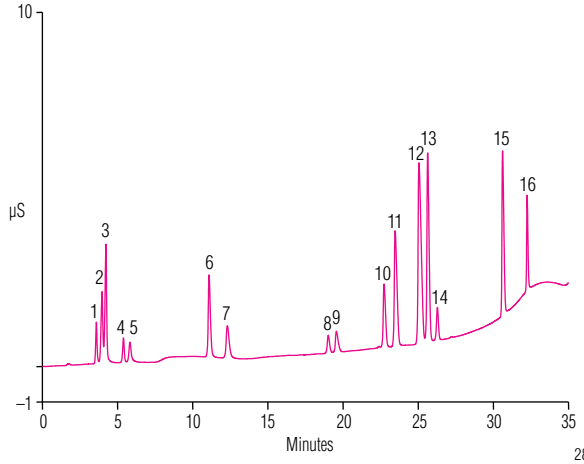

Figure 2. Chromatogram of the Level 5 Calibration Standard. Note: the rise and subsequent fall in the baseline between 7 and 12 min is present in the blank chromatogram (not shown).

Table 5. Calibration Standard Concentration and Calibration Results

Anion	Concentration (mg/L)					# Points	Calibration Result		
	Level 1	Level 2	Level 3	Level 4	Level 5		r^2	Offset	Slope
Fluoride	0.05	0.1	0.25	0.5	0.75	15	0.9984	0.0000	0.1647
Acetate	0.6	1.2	3.0	6.0	9.0	15	0.9973	0.0101	0.0306
Lactate	1.0	2.0	5.0	10	15	15	0.9993	0.0042	0.0287
Formate	0.1	0.2	0.5	1.0	1.5	15	0.9977	–0.027	0.0591
Shikimate	0.4	0.8	2.0	4.0	6.0	15	0.9979	0.0004	0.0169
Chloride	0.2	0.4	1.0	2.0	3.0	15	0.9995	–0.0011	0.1261
Nitrite	0.2	0.4	1.0	2.0	3.0	15	0.9996	0.0029	0.0685
Bromide	0.1	0.2	0.5	1.0	1.5	15	0.9995	0.0002	0.0557
Nitrate	0.1	0.2	0.5	1.0	1.5	15	0.9991	–0.0019	0.0816
Succinate	0.6	1.2	3.0	6.0	9.0	15	0.9993	0.0153	0.0527
Malate	1.0	2.0	5.0	10	15	15	0.9998	–0.0040	0.1161
Oxalate	0.1	0.2	0.5	1.0	1.5	15	0.9996	–0.0007	0.0785
Phosphate	1	2	5	10	15	15	0.9995	–0.0133	0.0466
Citrate	0.5	1	2.5	5	7.5	15	0.9998	0.0006	0.0368

Sample Analysis

Four wine samples were purchased at a local supermarket for this analysis. These included two different brands of red wine and two different brands of white wine (referred to as Red Wine 1, Red Wine 2, White Wine 1, and White Wine 2). Wine samples were diluted with DI water and treated with the OnGuard II RP cartridge before the analysis. This treatment removes hydrophobic components that could possibly foul the column. Figure 3 shows an overlay of the chromatograms of each of the four wine samples. Three injections of each wine sample were made to check the repeatability of the injection. The results of the wine sample analysis are shown in Table 6.

To judge the accuracy of this method, a spiking stock standard solution (Table 3) was added to the wine samples during sample preparation. Three injections of each spiked wine sample were made with the results shown in Table 7. The averaged value of three injections was used for recovery calculation. The recovery results of spiked sample analysis are shown in Table 8 and suggest the method is accurate. Overall, the data suggest that this is an effective method for determining the important organic acids and inorganic anions in wine.

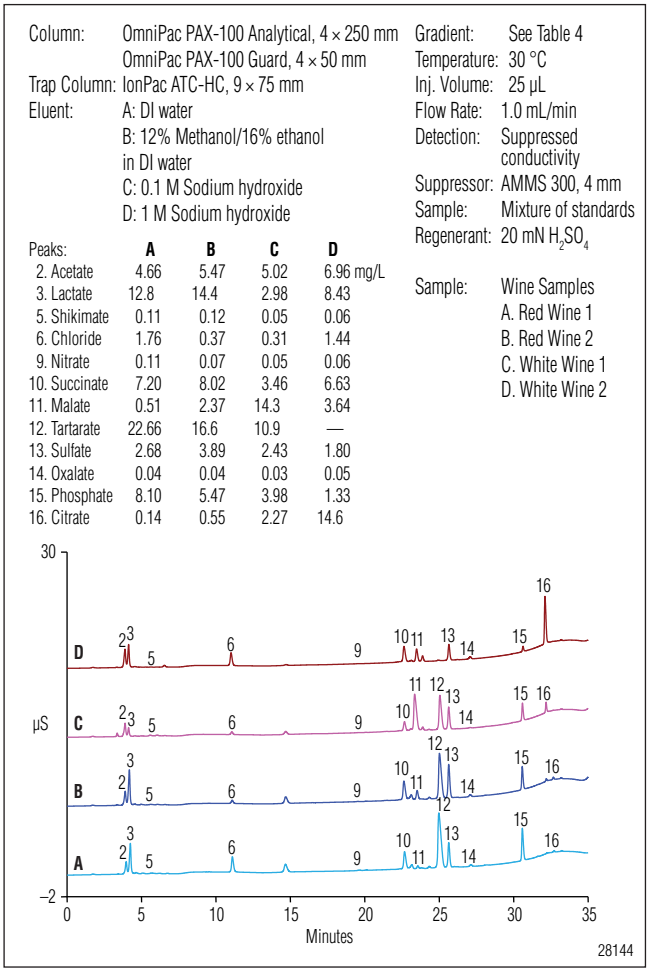

Figure 3. Chromatograms of the four wine samples.

Table 6. Amount of Anions and Organic Acids in Wine Samples (100 × Dilution)

Analyte	Red Wine 1		Red Wine 2		White Wine 1		White Wine 2	
	Average (mg/L)	RSD (n=3)	Average (mg/L)	RSD (n=3)	Average (mg/L)	RSD (n=3)	Average (mg/L)	RSD (n=3)
Fluoride	—	—	—	—	—	—	—	—
Acetate	4.66	0.91	5.47	0.52	5.02	1.51	6.96	0.60
Lactate	12.8	1.42	14.4	0.87	2.98	2.27	8.43	0.65
Formate	–	–	—	—	—	—	—	—
Shikimate	0.11	2.242	0.12	3.53	0.05	4.72	0.06	9.91
Chloride	1.76	1.20	0.37	0.45	0.31	2.05	1.44	0.80
Nitrite	—	—	—	—	—	—	—	—
Bromide	—	—	—	—	—	—	—	—
Nitrate	0.11	0.92	0.07	0.70	0.05	2.31	0.06	10.48
Succinate	7.20	1.07	8.02	0.35	3.46	1.41	6.63	1.22
Malate	0.51	1.41	2.37	0.57	14.3	1.35	3.64	1.37
Tartarate	22.6	0.96	16.6	0.34	10.9	1.47	—	—
Sulfate	2.68	1.09	3.89	0.45	2.43	1.80	1.80	1.34
Oxalate	0.04	0.77	0.04	1.79	0.03	4.77	0.05	1.98
Phosphate	8.10	0.95	5.47	0.50	3.98	1.68	1.33	2.16
Citrate	0.14	1.04	0.55	0.76	2.27	1.59	14.6	1.43

Table 7. Amount of Anions and Organic Acids in Spiked Wine Samples (100 × Dilution)								
Analyte	Spiked Red Wine 1		Spiked Red Wine 2		Spiked White Wine 1		Spiked White Wine 2	
	Average (mg/L)	RSD n=3	Average (mg/L)	RSD n=3	Average (mg/L)	RSD n=3	Average (mg/L)	RSD n=3
Fluoride	0.11	1.81	0.11	2.19	0.10	1.42	0.08	5.04
Acetate	5.71	1.02	6.51	0.63	5.93	0.39	7.83	1.22
Lactate	14.4	0.75	16.2	0.90	4.85	0.07	10.1	0.57
Formate	0.10	3.18	0.09	1.12	0.08	0.59	0.12	2.34
Shikimate	0.68	2.80	0.59	9.35	0.63	2.54	0.44	8.71
Chloride	2.30	0.69	0.86	0.70	0.80	1.13	1.91	0.55
Nitrite	0.39	1.34	0.42	2.17	0.40	0.62	0.45	2.29
Bromide	0.21	2.07	0.20	2.35	0.20	1.08	0.19	1.43
Nitrate	0.22	2.38	0.17	2.83	0.14	0.87	0.15	2.01
Succinate	8.87	0.70	9.64	0.08	5.34	0.59	8.29	1.28
Malate	1.51	0.68	3.49	0.42	16.0	0.66	4.57	1.19
Tartarate	24.4	0.81	18.5	0.13	12.8	1.20	1.91	1.07
Sulfate	3.68	1.05	4.90	0.11	3.37	1.62	2.69	1.08
Oxalate	0.13	2.19	0.13	0.61	0.13	2.56	0.14	0.71
Phosphate	9.22	0.86	6.48	0.45	5.01	0.77	0.08	5.04
Citrate	0.60	1.27	1.10	5.28	2.83	2.68	7.83	1.22

Spiked concentration for all samples: Fluoride 0.1 mg/L, Acetate 1.0 mg/L, Lactate 2.0 mg/L, Formate 0.1 mg/L, Shikimate 0.5 mg/L, Chloride 0.5 mg/L, Nitrite 0.5 mg/L, Bromide 0.2 mg/L, Nitrate 0.1 mg/L, Succinate 2.0 mg/L, Malate 1.0 mg/L, Tartarate 2.0 mg/L, Sulfate 1.0 mg/L, Oxalate 0.1 mg/L, Phosphate 1.0 mg/L, Citrate 0.5 mg/L (2.0 mg/L for White Wine 2)

Table 8. Recovery Results for Wine Samples				
Analyte	Red Wine 1 Recovery (%)	Red Wine 2 Recovery (%)	White Wine 1 Recovery (%)	White Wine 2 Recovery (%)
Fluoride	110	110	100	80.0
Acetate	105	104	91.0	87.0
Lactate	80.0	90.0	93.5	83.5
Formate	100	90.0	80.0	120
Shikimate	114	94.0	116	76.0
Chloride	108	98.0	98.0	94.0
Nitrite	78.0	84.0	90.0	90.0
Bromide	105	100	100	95.0
Nitrate	110	100	90.0	90.0
Succinate	84.5	81.0	94.0	83.0
Malate	100	112	85.0	93.0
Tartarate	90.0	95.0	95.0	95.5
Sulfate	100	101	94.0	89.0
Oxalate	90.0	90.0	100	90.0
Phosphate	112	101	103	83.0
Citrate	92.0	110	112	80.0

REFERENCES

1. Dionex Corporation, *Organic Acids in Wine*. Application Note 21, LPN 03205-02, 1997, Sunnyvale, CA.
2. Masson, P. Influence of Organic Solvents in the Mobile Phase on the Determination of Carboxylic Acids and Inorganic Anions in Grape Juice by Ion Chromatography. *J. Chromatogr., A* **2000**, **881**, 387–394.
3. Dionex Corporation, *Determination of Organic Acids in Fruit Juices*. Application Note 143, LPN 1415, 2003, Sunnyvale, CA.
4. Dionex Corporation, *Determination of Organic Acids in Cranberry and Bilberry Extracts*. Application Brief 112, LPN 2560, 2010, Sunnyvale, CA.
5. Dionex Corporation, *Product Manual for OnGuard Cartridges*. Document No. 031688-06, 2004, Sunnyvale, CA.

Thermo
SCIENTIFIC

Column Selection Guide

Silica Columns

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

Polymer Columns

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

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

Column Specifications

IC Anion Columns

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

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

IC Cation Columns

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

Ion-Exclusion Columns

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

Acclaim General and Specialty Columns

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

Bio Columns

Protein

Column	Phase	Target Applications	Base Matrix Material	Substrate Crosslinking	Capacity	Recommended Flow Rate	Solvent Compatibility	Maximum Backpressure	pH Range
MAbPac SEC-1									
MAbPac SCX-10									
ProPac WCX-10	Weak Cation Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10-µm diameter nonporous substrate to which is grafted a polymer chain bearing carboxylate groups.	55%	6 mg/ mL lysozyme	0.2–2 mL/min	80% ACN, acetone. Incompatable with alcohols and MeOH	3000 psi (21 MPa)	2–12.0
ProPac SCX-10	Strong Cation Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter nonporous substrate to which is grafted a polymer chain bearing sulfonate groups.	55%	3 mg/ mL lysozyme	0.2–2.0 mL/min	80% ACN, acetone, MeOH	3000 psi (21 MPa)	2–12.0
ProPac SCX-20									
ProPac WAX-10	Weak Anion Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter non-porous substrate to which is grafted a polymer chain bearing tertiary amine groups.	55%	5 mg/ mL BSA/ mL	0.2–2.0 mL/min	80% ACN, acetone, MeOH,	3000 psi (21 MPa)	2–12.0
ProPac SAX-10	Strong Anion Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter non-porous substrate with grafted polymer chain bearing quaternary ammonium groups.	55%	15 mg/ mL BSA	0.2–2.0 mL/min	80% ACN, acetone, MeOH	3000 psi (21 MPa)	2–12.0
ProSwift RP-1S	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith Standard permeability	5.5 mg/mL Insulin	2–4 mL/min	Most common organic solvents	2800 psi (19.2 Mpa)	1–14
ProSwift RP-2H	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith High permeability	1.0 mg/mL Lysozyme	1–10 mL/min	Most common organic solvents	2800 psi (19.3 Mpa)	1–14
ProSwift RP-4H									
ProSwift RP-3U	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith Ultrahigh permeability	0.5 mg/mL Lysozyme	1– 16 mL/min	Most common organic solvents	2800 psi (19.3 Mpa)	1–14
ProSwift SAX-1S	Strong Anion Exchange	Fast protein separation with good resolution using Anion Exchange	Monolith; polymethac-rylate with quaternary amine functional group	Monolith Standard permeability	18 mg/mL BSA	0.5–1.5 (4.6 mm), 0.05–.25 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProSwift SCX-1S	Strong Cation Exchange	Fast protein separation with good resolution using Cation Exchange	Monolith; polymethac-rylate with sulfonic acid fuctional group	Monolith Standard permeability	30 mg/mL Lysozyme	0.5–1.5 mL/min (4.6 mm)	Most common organic solvents	1000 psi (4.6 mm)	2–12.0
ProSwift WAX-1S	Weak Anion Exchange	Fast protein separation with good resolution using Anion Exchange	Monolith; polymethacrylate with tertiary amine (DEAE) functional group	Monolith Standard permeability	18 mg/mL BSA	0.5–1.5 mL/min (4.6 mm), 0.05–.25 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProSwift WCX-1S	Weak Cation Exchange	Fast protein separation with good resolution using Cation Exchange	Monolith; polymethacrylate with carboxylic acid (CM) functional group	Monolith Standard permeability	23 mg/mL Lysozyme	0.5–1.5 mL/min (4.6 mm), 0.05–.20 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProPac IMAC-10	Immobilized Metal Affinity	High resolution separation of certain metal-binding proteins and peptides	10 µm diameter non-porous polystyrene divinylbenzene substrate with poly (IDA) grafts.	55%	>60 mg lysozyme/ mL gel (4 x 250 mm)	1.0 mL/min	EtOH, urea, NaCl, non- ionic detergents, glycerol, acetic acid, guanidine HCl	3000 psi (21MPa)	2–12
ProSwift ConA-1S									
ProPac HIC-10	Reversed-Phase	Protein separation using hydrophobic interaction with salt gradient elution	Spherical 5 µm, ultrapure silica, 300 A, surface area 100 m^2/ g,	n/a	340 mg lysozyme per 7.8 x 75 mm column	1.0 mL/ min	2M Ammonium sulfate/ phosphate salts, organic solvent for cleanup	4,000 psi	2.5–7.5

Carbohydrate

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

DNA

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

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