Beverages Applications Notebook

Fruit Juice

Thermo SCIENTIFIC

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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

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

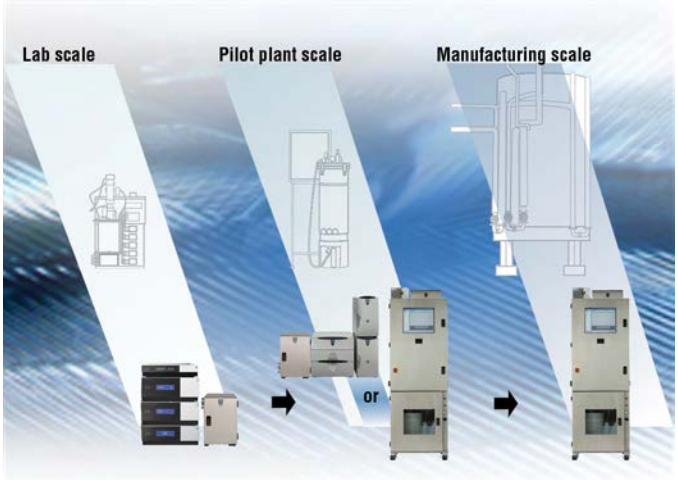
Our process analytical systems provide timely results by moving liquid chromatography-based measurements on-line. Information from the Thermo Scientific Dionex Integral process analyzer can help reduce process variability, improve efficiency, and reduce downtime. These systems provide comprehensive, 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.

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 Fruit Juice

Analysis of Fruit Juice Adulterated with Medium Invert Sugar from Beets

INTRODUCTION

Fruit juice adulteration presents an economic and regulatory problem. The United States orange juice industry estimates that orange juice sales gross more than one billion dollars annually.[1] The most common forms of adulteration include simple dilution and blending of inexpensive and synthetically produced juices into the more expensive ones. The source of sweetener can be other juices or sugar derived from fruits or vegetables. One adulterant currently in use is partially inverted sucrose, wherein about one-half of the sucrose has been hydrolyzed to glucose and fructose. This ratio of approximately 1:1:2 (glucose : fructose : sucrose) closely matches the ratio found in orange juice. Figures 1 and 2 show chromatograms of pure orange juice and medium invert sugar samples, respectively. When cane sugar is the source of inverted sucrose, Stable Isotope Ratio Analysis (SIRA) can be used to identify adulterated juices because the ratio of ^{13}C to ^{12}C is different for sugars in orange juice and cane sugar.[2] Beets, on the other hand, produce sugar via a metabolic pathway different from cane and similar to that of many fruits, so that the ratio of ^{13}C to ^{12}C is about the same for sugars in orange juice and beet sugar. This fact renders SIRA inadequate for detecting adulteration by beet sugar.

Recently, investigators using high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) have discovered several components in beet medium invert sugar (BMIS) that are not present in orange juice.[1,3,4] Swallow, Low, and Petrus have suggested that a pattern of late-eluting components appearing at about 60 minutes be used to identify adulteration (Method A herein). Tsang and coworkers have used raffinose — a trisaccharide of D-glucose, D-fructose, and D-galactose — as a marker for orange juice adulteration (Method B).[3,4] A third method (presented herein as Method C), similar to that of Swallow, et al., uses only one analytical column and also exhibits a pattern of late-eluting components indicative of adulteration by BMIS.

Conditions and illustrative chromatograms for each method are included in this application note. The selectivity of anion exchange chromatography, especially for oligosaccharides, and the sensitivity and specificity of pulsed amperometric detection make HPAE-PAD uniquely suited to this analysis. For further information about HPAE-PAD, please refer to Dionex Technical Note 20.[5]

EQUIPMENT

Any Dionex chromatography system consisting of:

Advanced Gradient Pump (AGP)

Liquid Chromatography Module

Pulsed Electrochemical Detector or Pulsed Amperometric Detector

Dionex AI-450 Chromatography Workstation

CONDITIONS

Experimental conditions are presented in Table 1.

DISCUSSION

Methods A (Figs. 3 and 4) and C (Figs. 5 and 6) rely on the analyst's ability to discern normal concentrations of these late-eluting components from elevated concentrations caused by adulteration. Raffinose is not found in pure orange juice (Fig. 7), so its presence indicates BMIS adulteration, though not necessarily an exact measure of the extent of adulteration (as determined by Method B). The chromatogram in Figure 8 shows the presence of raffinose in BMIS.† A sample of pure orange juice which had been 12% adulterated with BMIS (Fig. 9), was determined to contain 220 ng/mL of raffinose.

Table 1 Experimental Conditions

Method A (As described by Swallow, et al.[1])

Column:	2 CarboPac™ PA1 (4 x 250 mm)
Eluent 1:	0.1 M Sodium hydroxide
Eluent 2:	0.1 M Sodium hydroxide, 0.1 M sodium acetate
Eluent 3:	0.3 M Sodium hydroxide

Gradient:

Time	%E1	%E2	%E3
0-4 min	100	0	0
4-20	100-97	0-3	0
20-50	97-0	3-100	0
50-60	0	100	0
60	0	0	100

All gradient steps are linear (AGP curve 5).

Flow Rate:	0.70 mL/min
Inj. Vol.:	100 µL
Expected Pressure:	1400–2000 psi (10–14 MPa)
Postcolumn Reagent:	0.3 M Sodium hydroxide
Postcolumn Flow Rate:	0.8 mL/min
Detection:	Pulsed amperometry, gold working electrode

PAD Settings:

t(ms)	E(volt)*
120	0.05
120	0.80
420	–0.60

Sample Prep.: As described in ref. 1.

Method B (Raffinose as Adulteration Marker)

Column:	CarboPac PA1 (4 x 250 mm)
Eluent:	0.10 M Sodium hydroxide
Flow Rate:	1.0 mL/min
Inj. Vol.:	50 µL
Expected Pressure:	700–1000 psi (5–7 MPa)
Detection:	Pulsed amperometry, gold working electrode

PED program 1, or PAD Settings:

t(ms	E(volt)*
480	0.05
120	0.60
60	–0.60

*Potentials are referenced to Ag/Ag(I).

Sample Prep.: Centrifuge at 16,000 G for 15 min. Dilute supernatant to 1/100 original concentration with deionized water. Filter through a 0.2-µm filter.

Method C (One-Column Alternative to Method A)

Column:	CarboPac PA-100 (4 x 250 mm)
Eluent 1:	0.15 M Sodium hydroxide
Eluent 2:	0.15 M Sodium hydroxide, 0.15 M sodium acetate

Gradient:

Time	%E1	%E2	Curve
0-1 min	99	1	5
1-20	99-0	1-100	9

Equilibrate 10 minutes at starting conditions before each injection.

Flow Rate:	1.0 mL/min
Inj. Vol.:	25 µL
Expected Pressure:	700–1000 psi (5–7 MPa)
Detection:	Pulsed amperometry, gold working electrode

PED program 1, or PAD Settings:

t(ms)	E(volt)*
480	0.05
120	0.60
60	–0.60

Sample Prep.: Dilute sample to 1/10 original concentration with deionized water. Filter through a 0.2-µm filter.

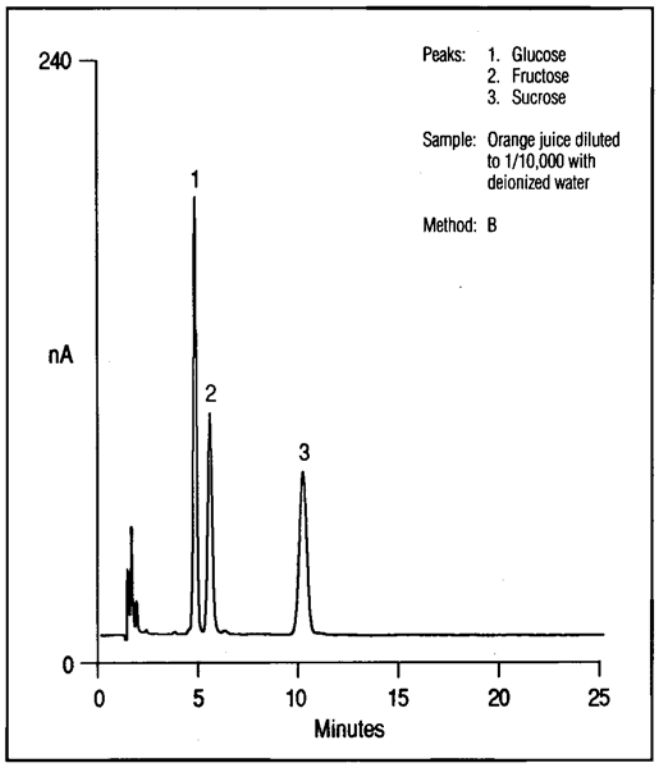


Figure 1 *Orange juice analyzed by Method B.*

Each lot of BMIS may vary slightly in raffinose content and in the content of the unidentified late-eluting components. These facts make the precise determination of the extent of adulteration difficult, but any of these methods can be used to estimate adulteration levels above about 5%.

Method A requires extensive sample preparation. The elapsed time for preparing a sample is 3 to 5 days. In contrast, Methods B and C require less than 30 minutes per sample. In each case, sample throughput can be improved by preparing several samples in parallel.

REFERENCES

1. Swallow, K.W.; Low, N.H.; Petrus, D.R. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 341.
2. Doner, L.W.; White, J.W. *Science* **1977**, *197*, 891.
3. Tsang, W.S.C.; Cargel, G.L.R.; Clarke, M.A. *Proceedings of the 1990 Sugar Processing Research Conference* **1991**, *368*.
4. Tsang, W.S.C.; Clarke, M.A.; Cargel, G.L.R. *Publ. Tech. Pap. Proc. Annu. Meet. Sugar Ind. Technol.* **1991**, *50*, 13.
5. Dionex Technical Note 20: "Analysis of Carbohydrates by Anion Exchange Chromatography with Pulsed Amperometric Detection."

[†]A sample of medium invert sugar derived from beets (BMIS) was graciously supplied by the American Crystal Sugar Company.

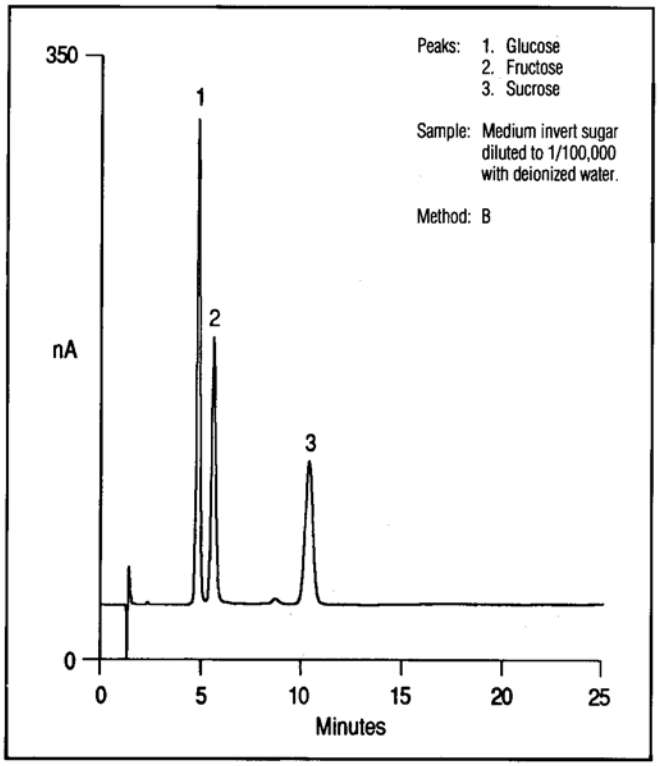


Figure 2 *Medium invert sugar analyzed by Method B. This profile looks similar to the profile for pure orange juice in Figure 1.*

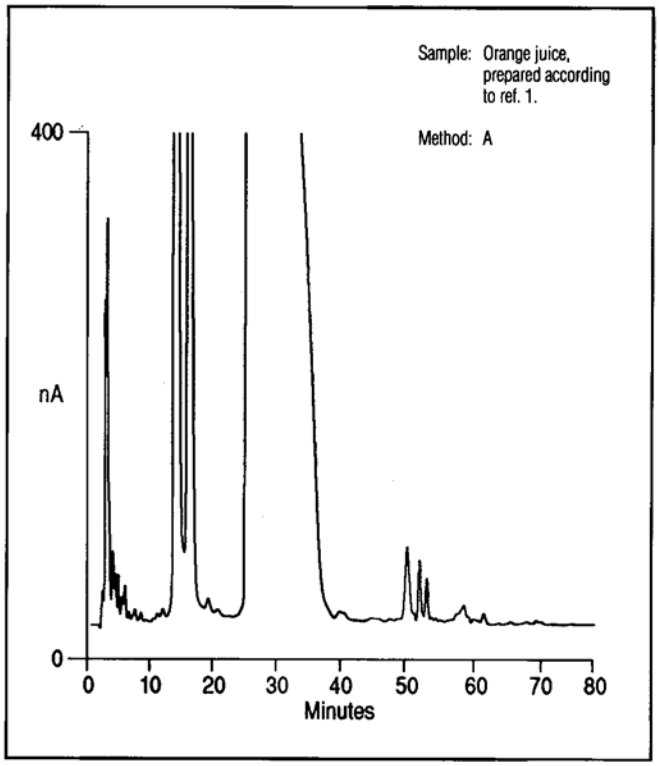


Figure 3 *Orange juice analyzed by Method A.*

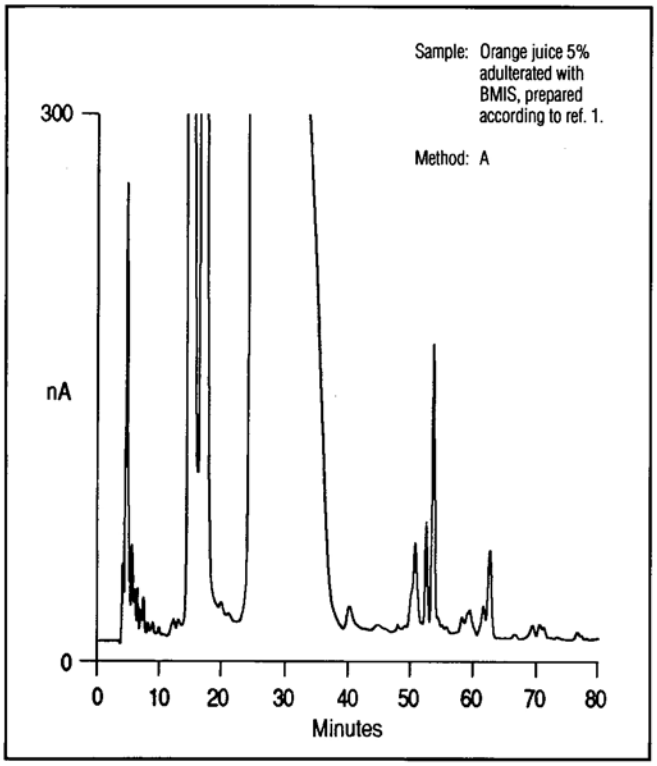


Figure 4 *Orange juice adulterated with medium invert sugar, analyzed by Method A. Note the late-eluting fingerprint between 50 and 60 minutes.*

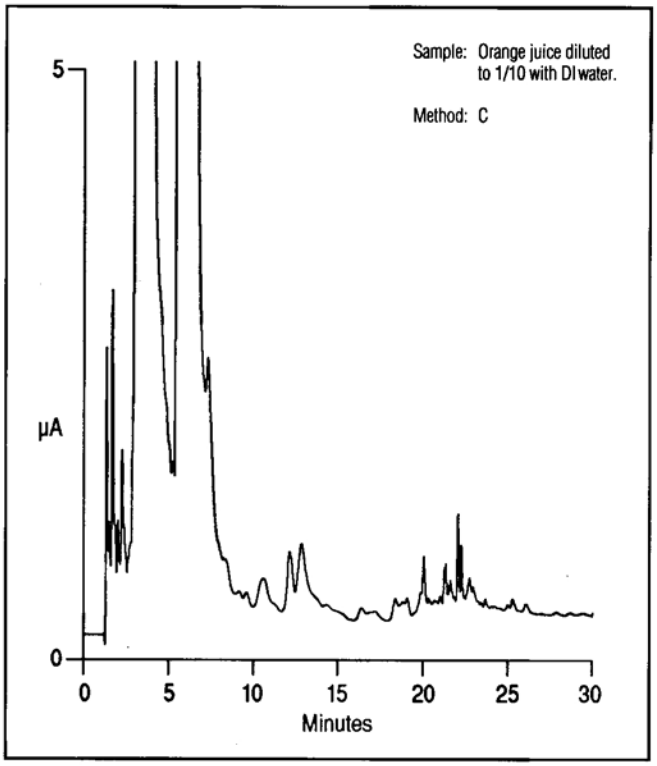


Figure 5 *Orange juice analyzed by Method C.*

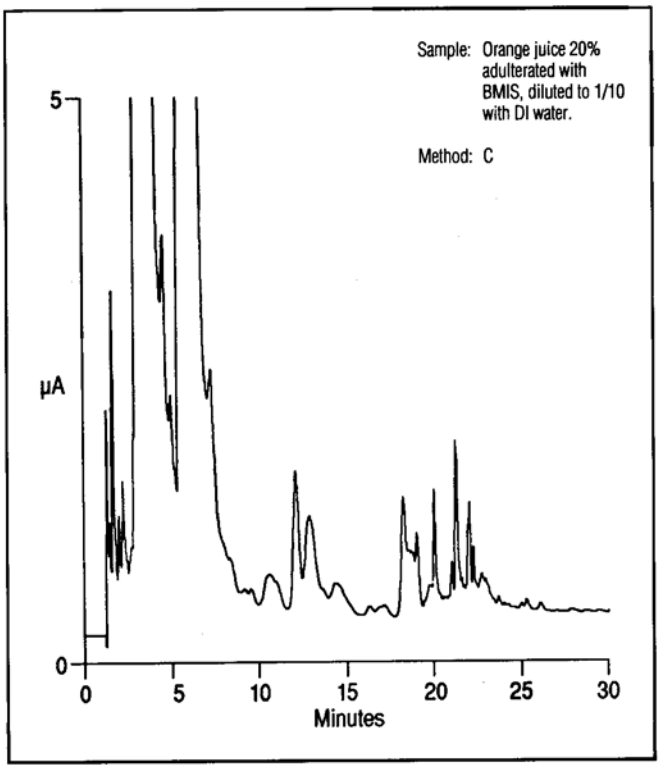


Figure 6 *Orange juice adulterated with medium invert sugar, analyzed by Method C. Note the late-eluting fingerprint between 18 and 24 minutes.*

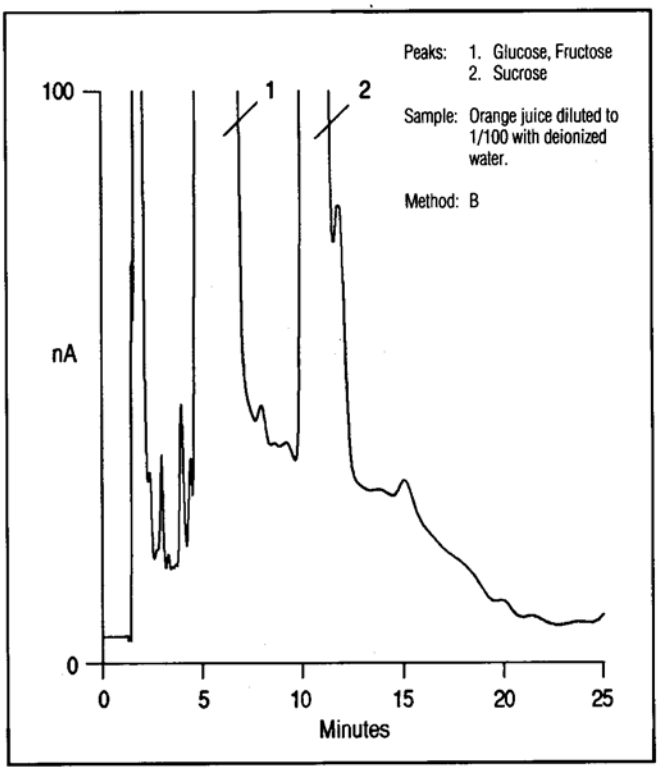


Figure 7 *Orange juice analyzed by Method B. Note the lack of any peaks eluting at 20 minutes.*

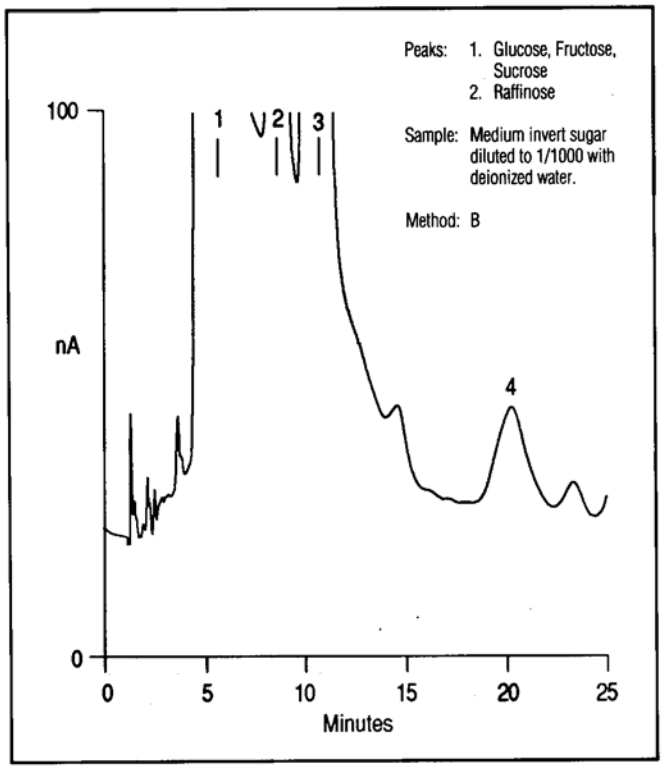


Figure 8 *Medium invert sugar analyzed by Method B. Note the raffinose peak eluting at approximately 20 minutes.*

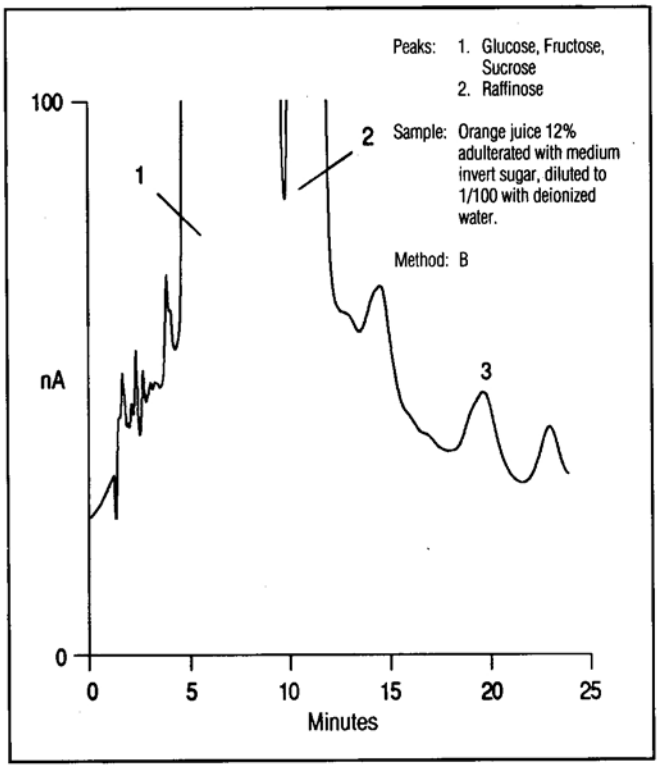


Figure 9 *Orange juice adulterated 12% with medium invert sugar, analyzed by Method B. Adulteration can be detected by the presence of raffinose.*

Determination of Sugar Alcohols in Confections and Fruit Juices by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection

INTRODUCTION

Sugar alcohols are used in confectionary products because they impart a sweet taste without the calories associated with sugars. Sorbitol (60% as sweet as sucrose)[1] and mannitol are sugar alcohols commonly used as replacements for sucrose in dietetic candy. However, their use in foods is regulated because they exhibit laxative and diuretic properties.

Sugar alcohols, also called alditols, are the reduced forms of monosaccharide aldoses. For example, D-glucose can be reduced to glucitol (sorbitol).[2] See Figure 1.

The CarboPac™ MA1 has unique selectivity for sugar alcohols. As a high-performance, high-capacity anion exchange column, the CarboPac MA1 permits sugar alcohols to be resolved at elevated sodium hydroxide concentrations. Unlike metal-loaded columns (often used for sugar alcohol analysis), the CarboPac MA1 operates at ambient temperature and employs sodium hydroxide eluent for high pH separations and maximum sensitivity using pulsed amperometric detection (PAD). Also unlike metal-loaded columns, which elute large saccharides first and sugar alcohols last, the elution order using the CarboPac MA1 column is determined by pK_a values. The sugar alcohols with higher pK_a values elute first, then monosaccharides and disaccharides that have lower pK_as (see Figure 2).

Carbohydrates (e.g., alditols and aldoses) are weak acids that ionize at pH 12 to 14. At these pH levels, carbohydrates can be separated by anion-exchange mechanisms. Sugar alcohols have higher pK_a values than mono- and disaccharides, thus higher pH eluents are required to separate them from one another by anion exchange. The CarboPac MA1 column uses a higher concentration of sodium hydroxide (typically up to 600 mM) for its eluent than the other CarboPac columns.

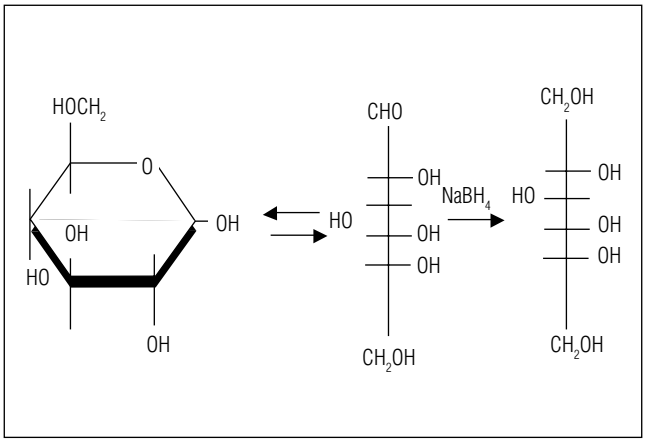


Figure 1. D-Glucose reduced to glucitol (sorbitol).

This application note focuses on sugar alcohols found in confections and fruit juices. However, the CarboPac MA1 is also useful for sugars found in physiological fluids, living tissues, and for reduced carbohydrate moieties of glycoconjugates (e.g., β-elimination reaction products).

EQUIPMENT

Dionex chromatography system consisting of:
Gradient Pump
Liquid Chromatography Module
Pulsed Electrochemical Detector
Dionex PeakNet or AI-450 Chromatography Workstation

REAGENTS AND STANDARDS

18 MΩ-cm Deionized water

Sodium hydroxide solution, 50% (w/w), low carbonate

Standards consisting of: Inositol, Xylitol, Sorbitol, Mannitol, Glucose, Fructose, Sucrose, Dulcitol, Arabinose, Mannose, Xylose, and Galactose

CONDITIONS

Column:	CarboPac MA1 (4 x 250 mm)
Expected Operating Pressure:	5.5 to 7.6 MPa (800 to 1100 psi)
Injection Volume:	10 µL
Eluents:	A: Deionized water B: 1.0 M Sodium hydroxide
Flow Rate:	0.4 mL/min
Detection:	Pulsed amperometry, gold working electrode

ED40 Settings as follows*:

t (ms)	E (volts)	Integration (s)
400	+0.05	0.2–0.4
200	+0.75	
400	–0.15	

*See Technical Note 21 for a discussion of pulse potentials, including settings to use with a PAD.[3]

PREPARATION OF REAGENTS AND STANDARDS

Eluent A: Deionized Water

Vacuum degas 1 L of 18 MΩ-cm deionized water.

Eluent B: 1 M Sodium Hydroxide

Dilute 52 mL of sodium hydroxide solution in 1.0 L of degassed 18 MΩ-cm deionized water. Use a sodium hydroxide solution that is 50% (w/w) and contains low carbonate. Sodium hydroxide pellets are coated with a layer of carbonate and will not produce acceptable eluents.

SAMPLE PREPARATIONS

"Sugarless" Hard Candy

Dissolve one candy drop (weighing 3.4 g) in 10 mL of deionized water and dilute 1:1000 with 18 MΩ-cm deionized water.

Apple Juice

Dilute apple juice 1:1000 with 18 MΩ-cm deionized water.

Chewing gum extract

Divide one stick of chewing gum (weighing 2.7 g) into small pieces (of approximately 3 x 3 mm), and sonicate gum in 10 mL of deionized water for 10 minutes. Then pass the supernatant through an OnGuard A (prepare by passing 5 mL of deionized water through the cartridge at a flow rate of 2-mL/min; discard the first 3 mL of the sample), to remove anions and pass it through a 0.45-µm filter to remove particulates. Dilute the filtrate 1:1000 with 18 MΩ-cm deionized water.

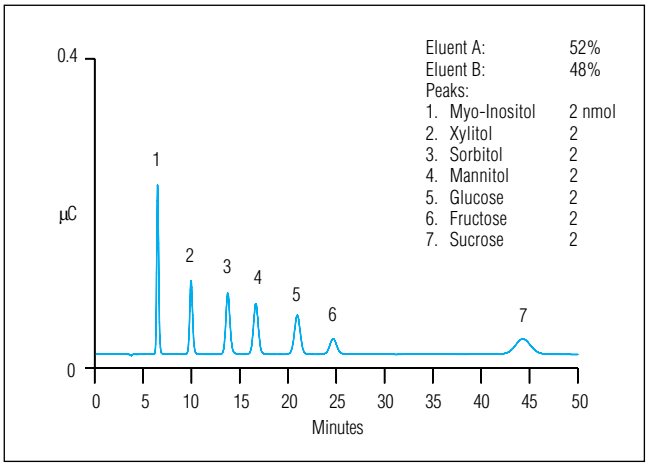


Figure 2. Sugar alcohols with high pK_a values elute first.

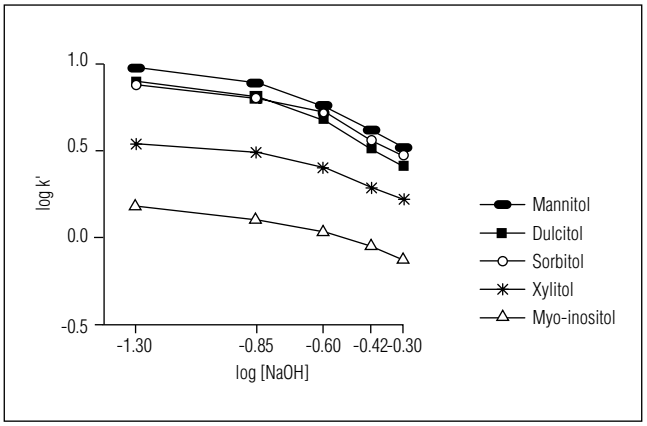


Figure 3A. Log capacity factor vs. log sodium hydroxide concentration of some sugar alcohols.

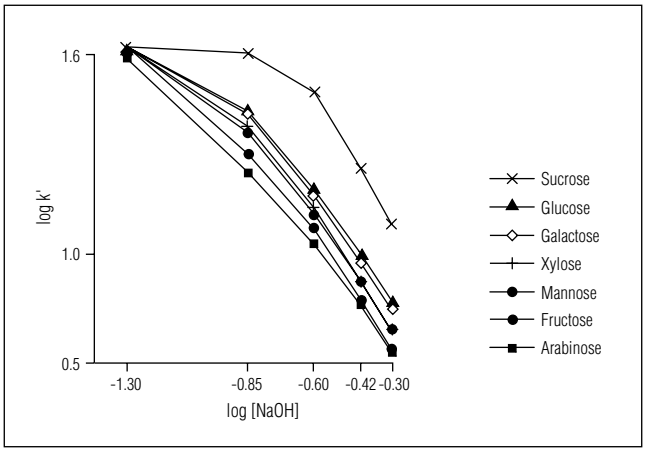


Figure 3B. Log capacity vs. log sodium hydroxide concentration of selected carbohydrates.

DISCUSSION AND RESULTS

The CarboPac MA1 allows 0 to 1 M sodium hydroxide eluent concentrations. This is important for the separation of sugar alcohols because their pK_as are high and each is unique. Separation on the CarboPac MA1 can be achieved by choosing an eluent pH near the pK_a value of a sugar alcohol. Altering an eluent's pH by varying the sodium hydroxide concentration changes the effective charge on the compounds. This in turn can change the elution order so that those sugar alcohols of interest will be resolved from one another. Figures 3A and 3B show capacity factors vs. eluent concentration for some common sugar alcohols. It is possible to predict which eluent strength will work best for a particular separation.

Figure 4 shows a chromatogram of "sugarless" hard candy. This particular candy is sold as dietetic candy. Its major PAD-active, water-soluble components are sorbitol and mannitol. The 3.4-g candy drop contained 2.70 g of sorbitol and 50.5 mg of mannitol.

Figure 5 shows a chromatogram of diluted apple juice. Glucose, fructose, and sucrose are found in all fruit juices. Sorbitol is found in apples, pears, and plums, among other fruits.[4] An 8-ounce (237-mL) serving of this apple juice contains 1.86 g of sorbitol, 8.22 g of glucose, 18.8 g of fructose, and 7.76 g of sucrose.

Figure 6 shows a chromatogram of chewing gum extract. The peaks are glycerol, sorbitol, mannitol, and hydrogenated glucose syrup, respectively. The 2.7-g stick of chewing gum contains 218 mg of glycerol, 1140 mg of sorbitol, and 280 mg of mannitol. Hydrogenated glucose syrup was not quantitated.

Operators of refractive index (RI) detection may notice the low detection limits presented in Table 1. Pulsed amperometric detection (PAD) is routinely able to achieve these low levels. Pulsed amperometric detectors apply a potential at an electrode to oxidize the carbohydrate. This not only allows for low detection limits, but also increases specificity and freedom from matrix interferences.

PAD utilizes a repeating sequence of three potentials. The durations of these three potentials are optimized for carbohydrates. If a single potential is used, the peak heights will steadily decrease as the electrode surface fouls. For further details concerning pulsed sequences for PAD, refer to Dionex Technical Note 21.[3]

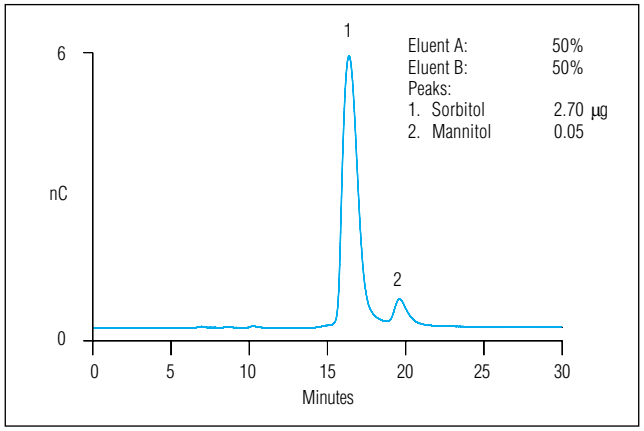


Figure 4. "Sugarless" hard candy containing sorbitol and mannitol.

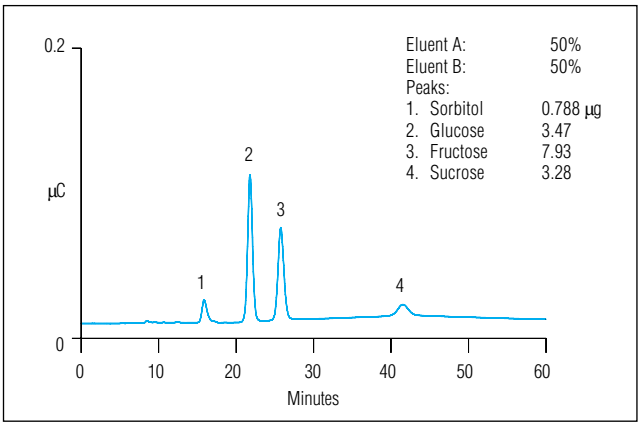


Figure 5. Diluted apple juice containing sorbitol.

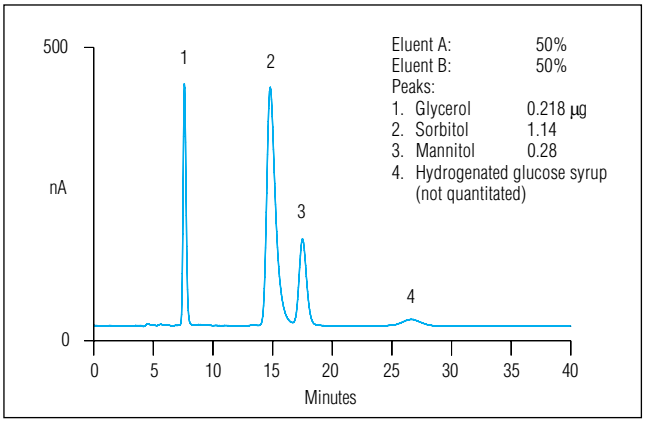


Figure 6. Chewing gum extract containing glycerol, sorbitol, and mannitol.

To drive the oxidation reaction at the working electrode of the pulsed amperometric detector, the eluent must be greater than pH 12. Dionex CarboPac columns are designed using a polymer-base for stability and durability in the 0 to 14 pH range. For further details concerning carbohydrate determination with PAD, refer to Dionex Technical Note 20.[5]

CONCLUSION

The Dionex CarboPac MA1 is the preferred column for the analysis of sugar alcohols. It yields excellent resolution and allows for changes in carbohydrate selectivity; the column permits changes in the eluent concentration and hence pH levels. The CarboPac MA1 is used for pulsed amperometric detection of sugar alcohols with high sensitivity and specificity without derivatization or the addition of a postcolumn reagent. This column operates at ambient temperatures to promote ease-of-use and an increased lifetime. As with other CarboPac columns, the MA1 demonstrates long-term reproducibility and durability.

PRECAUTIONS

The CarboPac MA1 has an operational flow rate range of 0.2–0.5 mL/min. The maximum flow rate possible without irreversible compression of the column resin is 0.8 mL/min. The maximum pressure that the column can withstand without irreversible compression of the macroporous resin is 13.8 MPa (2000 psi).

Metal should be eliminated from the flow path prior to column use. If an autosampler is used, all flow paths should be metal-free. Metal contamination of the analytical column can result in poor peak efficiency and/or symmetry, which may lead to poor reproducibility.

If a PED is used, the Ag/AgCl reference electrode is preferred over the combination pH-Ag/AgCl reference electrode. Sodium hydroxide eluents above 200 mM will cause the delicate glass bulb of the electrode to become brittle and break.

Table 1 Detection limits and linearity data for the carbohydrates in Figure 1

Standard	r^2 [a]	Method Detection Limit (pmol)[b]
myo-inositol	0.9995	2
xylitol	0.9953	4
sorbitol	0.9951	5
mannitol	0.9995	4
glucose	0.9984	10
fructose	0.9995	10
sucrose	0.9999	10

[a] The coefficients of determination were calculated over the range of 10 to 30,000 picomoles or 3 orders of magnitude.
[b] The method detection limits of myo-inositol, xylitol, sorbitol, and mannitol were determined at three times the noise.

REFERENCES

1. Shaw, P.E. *CRC Handbook of Sugar Separations in Foods by HPLC;* CRC Press, Boca Raton, Florida, 1988.
2. Solomons, T.W.G. *Organic Chemistry*, 2nd ed., John Wiley & Sons, New York, 1980.
3. Dionex, Technical Note 21: "Optimal Settings for Pulsed Amperometric Detection of Carbohydrates using the Dionex Pulsed Electrochemical Detector (PED-2) and the Pulsed Amperometric Detector (PAD-2)".
4. Coppola, E.D. *Food Technology.* **1984**, *38(4)*, 88-91.
5. Dionex, Technical Note 20: "Analysis of Carbohydrates by Anion Exchange Chromatography with Pulsed Amperometric Detection".

Determination of Organic Acids in Fruit Juices

INTRODUCTION

Organic acids are important in characterizing the flavor of fruit juices. Their presence and concentration determine tartness and other flavor attributes. In some cases, it is necessary to determine organic acids to assess whether an expensive juice has been illegally adulterated with a cheaper juice. Because organic acid profiles are distinct to each type of fruit juice,[1] evidence of tampering can be evaluated by comparing the known juice fingerprint to that of the suspected adulterated juice. Organic acid profiles can also determine juice freshness or spoilage.[2] Masson used ion chromatography to determine the organic acids in grape juice.[3]

The IonPac® AS11-HC is the ideal column for anion chromatographic determination of organic acids in fruit juices. These organic acids are present in concentrations ranging from less that 1 mg/L to hundreds of milligrams per liter. The AS11-HC is a high-capacity anion-exchange column that is solvent-compatible, allowing for the addition of organic solvents to enhance performance. The column's high capacity yields an improved separation of lactate, acetate, and formate. The column packing of the AS11-HC consists of 9.0-µm diameter macroporous resin beads, functionalized with quaternary ammonium groups.

The AS11-HC uses hydroxide eluent gradients and can be used with the EG50 Eluent Generator. Through electrolysis, the EG50 produces high-purity, carbonate-free potassium hydroxide (KOH) eluent. This on-line generation of KOH eliminates carbonate contamination and therefore increases baseline stability and chromatographic reproducibility, making peak integration more accurate. This application note shows how organic acids can be determined in fruit juices at low to high mg/L concentrations using a simple dilution, an IonPac AS11-HC, EG50-generated eluent gradients, and suppressed conductivity detection. The fruit juices analyzed in this application note are orange, grape, apple, and cranberry.

EQUIPMENT

Dionex DX-600 IC system consisting of:

- GP50 Gradient Pump
- EG50 Eluent Generator
- LC30 Chromatography Oven
- CD25 Conductivity Detector
- AS40 Autosampler
- Chromeleon® Chromatography Workstation
- ASRS® ULTRA Anion Self-Regenerating Suppressor

SYSTEM SETUP

To ensure a quiet baseline, the following steps must be taken during the system setup. Add 1000 psi of backpressure to the degas module on the eluent generator. (See the EG50 manual, P/N 031908, for details on adding backpressure to the degas module.) The final system backpressure should be approximately 2900 psi, but should not exceed 3000 psi. Install a conditioned ATC after the proportioning valve. A rise in background during gradient elution is observed if the ATC needs regeneration. For instruction on ATC conditioning and regeneration, see the EG50 manual (P/N 031908). Prior to sample analysis, determine a system blank by analyzing a 10-µL injection of deionized water using the chromatographic method described below. An equilibrated system has a background conductance of 1–4 µS with the peak-to-peak noise typically 10–20 nS, and no peaks eluting with the same retention time as an analyte of interest.

PREPARATION OF STANDARDS

One thousand mg/L standards of 30 organic acids and inorganic anions were prepared using the compounds and masses listed in Table 1. The mixed standard whose separation is shown in Figure 1 was prepared by mixing appropriate volumes of the 1000 mg/L standards. To determine method linearity for organic acids, representative monovalent, divalent, and trivalent organic acids were analyzed. These organic acids were quinate, tartrate, and citrate, respectively. A 10,000 ppm solution of quinate was diluted to prepare the following standards: 1, 2, 5, 10, and 20 mg/L. A 1000 ppm solution of tartrate was diluted to prepare 50, 75, 100, 150, and 200 mg/L standards. To prepare citrate standards of 75, 100, 200, 300, and 400 mg/L, a 10,000 ppm solution was used. Deionized water (DI H_2O), Type I reagent-grade, 18 MΩ-cm resistance or better was used to feed the EG50, prepare all standards, and dilute samples.

Table 1. Amounts of Compounds Used to Prepare 1 L of 1000 mg/L Anion Standards

Anion	Compound	Mass (g)
Quinate	Quinic acid	1.000
Fluoride	Sodium fluoride	2.210
Lactate	Lithium lactate	1.067
Acetate	Sodium acetate, trihydrate	2.305
Glycolate	Glycolic acid	1.000
Propionate	Sodium propionate	1.315
Formate	Sodium formate	1.511
Butyrate	Sodium butyrate	1.250
Pyruvate	Pyruvic acid	1.000
Valerate	Valeric acid	1.000
Galacturonate	D-Galacturonic acid, monohydrate	1.000
Bromate	Sodium bromate	1.179
Chloride	Sodium chloride	1.648
Trifluoroacetate	Trifluoroacetatic acid	1.000
Bromide	Sodium bromide	1.288
Nitrate	Sodium nitrate	1.371
Glutarate	Glutaric acid	1.000
Succinate	Sodium succinate	1.396
Malate	L-Malic acid	1.000
Malonate	Malonic acid	1.000
Tartrate	Sodium tartrate	1.311
Maleate	Maleic acid	1.000
Sulfate	Sodium sulfate	1.479
Oxalate	Sodium oxalate	1.522
Fumarate	Fumaric acid	1.000
Phosphate	Potassium phosphate, monobasic	1.433
Citrate	Citric acid	1.000
Isocitrate	Isocitric acid trisodium dihydrate	1.306
Cis-aconitate	*Cis*-aconitic acid	1.000
Trans-aconitate	*Trans*-aconitic acid	1.000

SAMPLES

Samples were filtered (0.45 µM filter IC Acrodisk®, Gelman P/N 4483; or Anotop™ IC, Whitman P/N 68099232) and diluted 1:10 prior to analysis.

CONDITIONS

Columns:	IonPac AS11-HC Analytical, 4 mm (P/N 052960) IonPac AG11-HC Guard, 4 mm (P/N 052962)
Eluent:	Potassium hydroxide gradient: 1 mM from 0–8 min 1 mM to 30 mM, 8–28 min 30 mM to 60 mM, 28–38 min Methanol: 10%, 0–38 min
Eluent Source:	EG50
Flow Rate:	1.5 mL/min
Temperature:	30 °C
Detection System:	Suppressed conductivity, ASRS ULTRA 4 mm, AutoSuppression®, external water mode (10 mL/min)
Backpressure:	2900 psi
Background Conductance:	1–4 µS
Degas Setting:	30 s every 2 min
Injection Volume:	10 µL

RESULTS AND DISCUSSION

Figure 1 shows the separation of 30 organic acids and inorganic anions in a single injection of a fruit juice standard. Of these 30 analytes, only the two pairs (formate/butyrate and tartrate/malonate) are not resolved. This separation demonstrates that monovalent (e.g., quinate), divalent (e.g., tartrate), and trivalent (e.g., citrate) organic acids can be separated with this method. The stable baseline is due to the use of the EG40 for KOH eluent generation. The responses of quinate, tartrate, and citrate were measured over the concentration ranges described in the "Preparation of Standards" section, and good linearity was obtained (r^2 = 0.999, 0.993, and 0.998, respectively) for each class of organic acid (mono-, di-, and trivalent). Using an IonPac AS11 column, Masson also found good linearity of organic acid standards ($r^2 = (0.990)^3$. These studies show that this method can be used to measure a wide range of organic acids in fruit juices with a single sample injection.

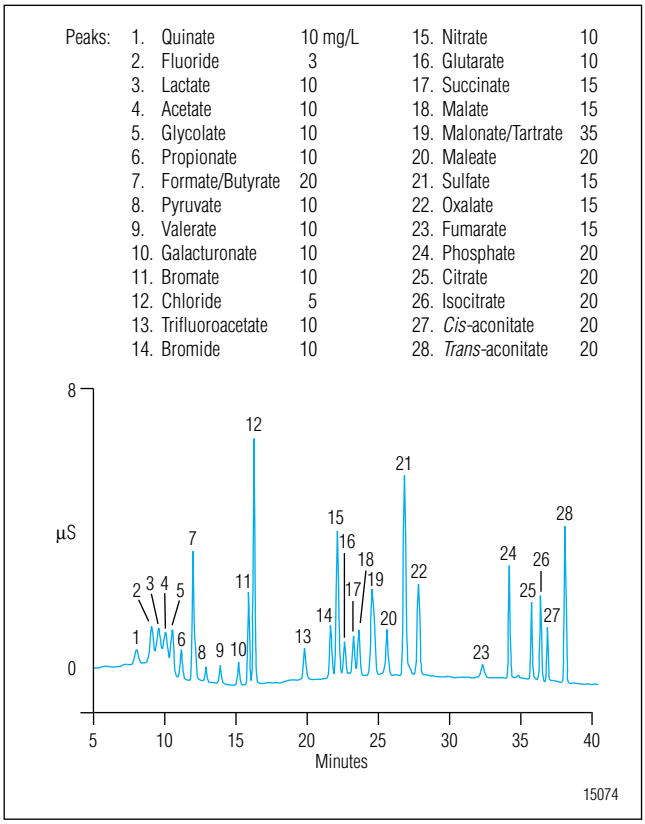


Figure 1. Standard for fruit juice analysis.

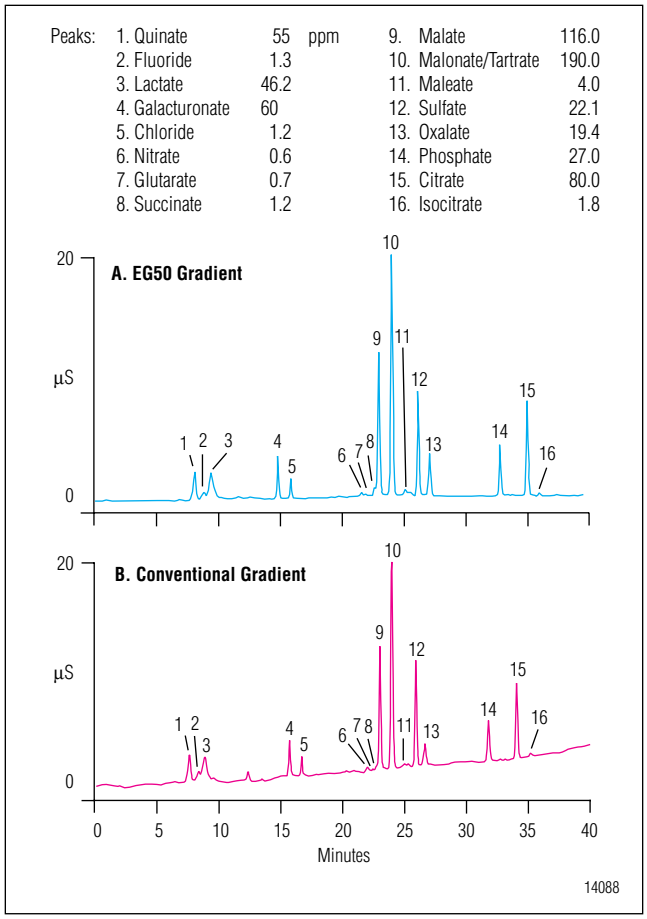


Figure 2. Determination of anions and organic acids in grape juice.

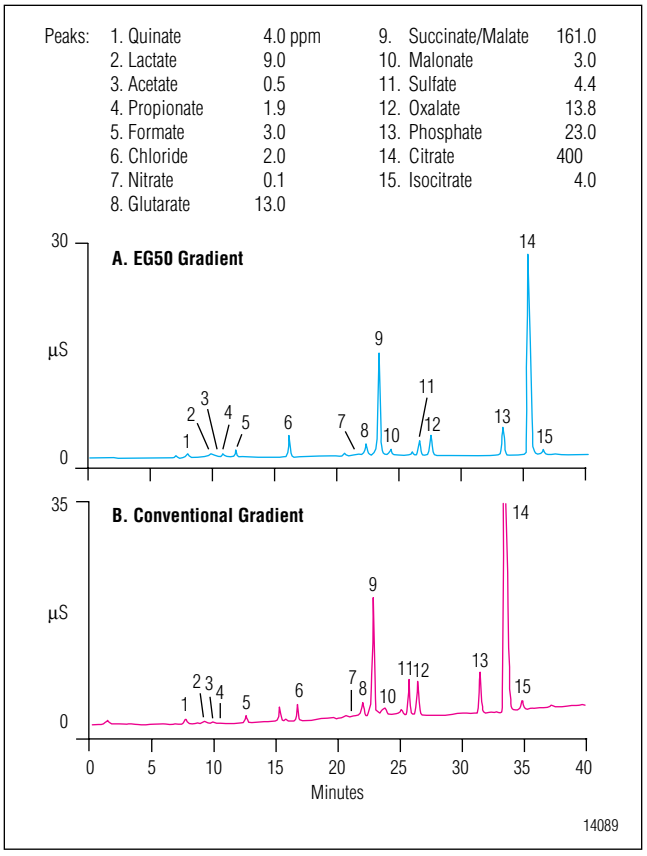


Figure 3. Determination of anions and organic acids in orange juice.

Figures 2 and 3 show analyses of grape and orange juices using this method. These figures also show a comparison of EG50-generated eluent and conventional eluent preparation. The baselines of the chromatograms that used the EG50 are noticeably flatter and more stable than those from the chromatograms that used manually prepared eluents. Figure 2 shows that the major organic acids in grape juice are malate, tartrate, and citrate, as has been reported by others.[2–4] Figure 3 shows that orange juice has, as expected, a high concentrate of citric acid (citrate ion).

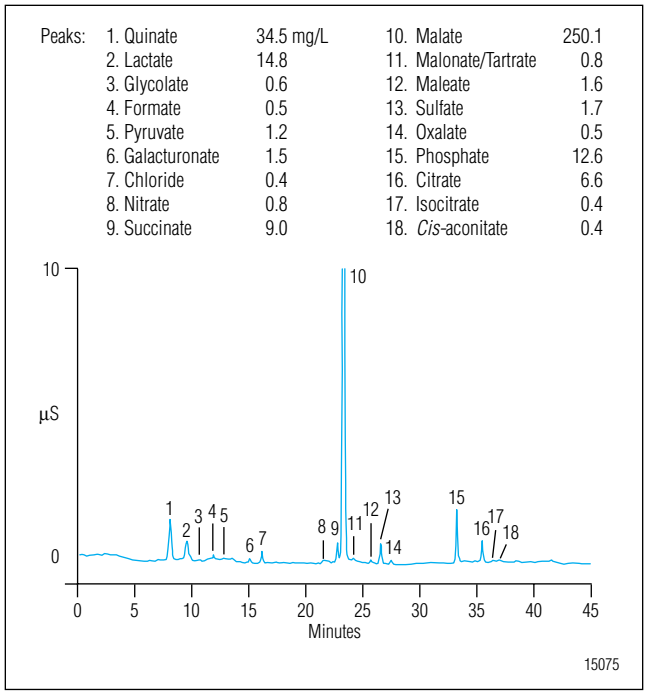


Figure 4. Determination of anions and organic acids in apple juice.

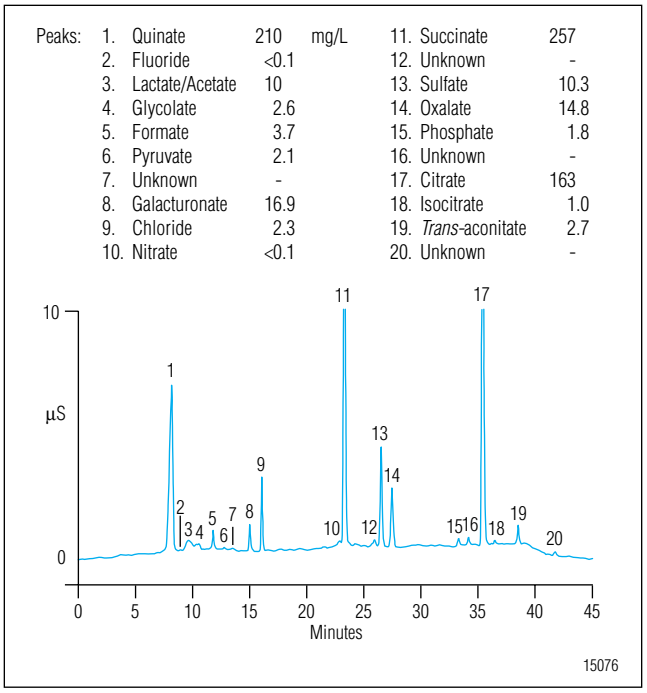


Figure 5. Determination of anions and organic acids in cranberry juice cocktail.

Figures 4 and 5 show organic acid determination in apple juice and cranberry juice cocktail, respectively. Notice that both lactic and acetic acids are low in both figures. Elevated levels of lactic and acetic acids may be caused by microbiological spoilage,[4,5] so it is important to monitor the concentrations of these organic acids as a measure of product quality. Malate is the major constituent of apple juice. In cranberry juice cocktail, there are high concentrations of succinate and quinate, which provide a tart taste. Oxalate and citrate are also present at high levels. Note the presence of galacturonate in cranberry juice cocktail and grape juice. The presence of galacturonate can be attributed to the degradation of pectins in the skins of fruit.[4] Freshly squeezed juices generally show lower levels of galacturonate. Note the lack of galacturonate in the orange juice.

SUMMARY

The method described in this application note can be used to determine organic acids in fruit juices. This method uses the EG50 to generate high-purity, carbonate-free eluents to suppress baseline drift and therefore improve retention time and integration reproducibility. The IonPac AS11-HC is the ideal column for this method because its high capacity improves separation of a wide range of organic acids.

REFERENCES

1. Henshall, A. *Cereal Foods World* **1998,** *43(2)*, 98–103.
2. Dong, M. *LC-GC*, **1998**, *16(12)*, 1092–1097.
3. Masson, P. *J. of Chromatogr., A* **2000**, *881*, 387–394.
4. Saccani, G. et al. *J. of Chromatogr., A* **1995**, *706*, 395–403.
5. Trifiro, A. et al. *J. of Chromatogr., A* **1997**, *770*, 243–252.

Determination of Azide in Aqueous Samples by Ion Chromatography with Suppressed Conductivity Detection

INTRODUCTION

Sodium azide is a white crystalline solid that is highly toxic when ingested or inhaled. The salt readily dissolves in water to yield the azide anion (N_3^-). Contact with water or acid causes release of hydrazoic acid (HN_3), a gas with a sharp, disagreeable odor that is considerably more toxic than the salt. Azide anion prevents the cells of the body from using oxygen, inhibiting the function of cytochrome oxidase by binding irreversibly to the heme cofactor.[1] Fatal doses occur with exposures of 700 mg (10 mg/kg), but exposure to smaller doses can cause eye and skin irritation, headache, nausea, shortness of breath, dizziness, blurred vision, low blood pressure, or kidney damage.

Sodium azide is best known as the chemical that triggers automobile airbag inflation. An electrical discharge triggered by automobile impact causes sodium azide to explode and release nitrogen gas inside the airbag. The nationwide production of sodium azide has increased to 6000 tons per annum, chiefly as a result of increased automobile airbag production. Sodium azide is also used as a chemical preservative in hospitals and laboratories, in agriculture for pest control, in pharmaceutical manufacturing, and in detonators and other explosives. Azide is also of interest to forensic investigators. Several people were poisoned in Japan in 1998 when azide was added into tea and orange juice. Ion chromatography was a key tool in diagnosing the cause of this poisoning.

Several methods are available to determine azide anion or hydrazoic acid. To determine sodium azide and hydrazoic acid in workplace atmospheres, an air sample is collected on an impregnated filter, desorbed into an aqueous sodium carbonate/bicarbonate solution, separated on an IonPac® AS9 column, and detected by UV absorbance at 210 nm (OSHA ID-211).[3] To determine azide anion in bodily fluids, Kruszyna described an approach based on trapping the hydrazoic acid sparged from an acidified sample, followed by ion chromatographic (IC) analysis of the trapping fluid.[4] Other methods include GC-MS of the pentafluorylbenzyl derivative,[5] and a micro diffusion extraction combined with spectrophotometry using the König cyanide reaction and ferric azide complex formation in conjugation with cerium azide oxido-reduction.[6]

This application note describes how to routinely monitor for azide anion in aqueous samples including water, food products, bodily fluids, and biological buffers by using a Reagent-Free™ IC (RFIC™) system. The azide anion is determined in 35 min by using a 4-mm IonPac AS15 column, isocratic potassium hydroxide provided automatically by an eluent generator, and suppressed conductivity detection. The IC method allows direct injection of the sample, avoiding laborious derivatization or sample preparation, and is highly sensitive, providing a detection limit for azide in reagent water of 50 µg/L.

EQUIPMENT

A Dionex ICS-3000 chromatography system consisting of:

Dual Pump (DP)

Conductivity Detector (CDS)

Eluent Generator (EG)

AS Autosampler (AS)

Chromeleon® Chromatography Workstation with Chromeleon 6.7 Chromatography Management Software

Note: This application can be performed on any Dionex RFIC system.

CONSUMABLES

EluGen® Hydroxide Cartridge (EGC II KOH) (P/N 058900)

ASRS® ULTRA II, 4 mm (P/N 061561)

Continuously Regenerating Anion Trap Column (CR-ATC) (P/N 060477)

Syringe filters (Gelman IC Acrodisc® 0.2 µm, PN 4483)

CONDITIONS

Columns:	IonPac AS15 Analytical, 4 × 250 mm (P/N 053940)
	IonPac AG15 Guard, 4 × 50 mm (P/N 053942)
Eluent:	42 mM potassium hydroxide (KOH)
Flow Rate:	1.2 mL/min
Temperature:	30 ˚C
Injection:	25 µL
Detection:	Suppressed conductivity, ASRS ULTRA II (4 mm), recycle mode
Power Setting:	125 mA
Background	
Conductance:	1–2 µS
Noise:	< 5 nS/min peak-to-peak
Backpressure:	~2300 psi
Run Time:	35 min

REAGENTS AND STANDARDS

Reagent grade water Type I, 18 MΩ-cm resistance or better, filtered through a 0.2-µm filter immediately before use

Seven Anion Standard II (P/N 57590)

Sodium azide (Sigma, P/N S-8032)

Sodium citrate (Sigma, P/N S-4641)

Fumaric acid (Fluka, P/N 47900)

Sodium chloride (JT Baker, P/N 4058-05)

Potassium chloride (Mallinckrodt, P/N 6858)

Sodium phosphate dibasic (Aldrich, P/N 21988-6)

Potassium phosphate monobasic (Fisher, P/N P285-500)

Sodium oxalate (Fluka, PN 71800)

Orange juice (Minute Maid® original, 100% Pure squeezed, P/N 0548 CT800)

Black tea (Lipton® 100% Natural Tea, P/N 83004654)

Green tea (Bigelow® P/N 44093EBE5)

Lyophilized citrated plasma (Sigma, P/N P9523)

ELUENT SOLUTION

Generate the 42 mM KOH eluent on-line by using the EG Eluent generator system with an EGC II-KOH cartridge. Fill the plastic eluent reservoir with reagent water and maintain an inert helium atmosphere of 3–5 psi in the eluent reservoir.

Alternatively, prepare 42 mM NaOH by pipetting 2.92 g of 50% (w/w) aqueous NaOH from the middle portion of the reagent bottle into a 1-L volumetric flask containing about 900 mL of degassed reagent water. Do not shake the 50% (w/w) NaOH bottle or pipette from the top of the solution where sodium carbonate may have formed. Dispense the aliquot of NaOH below the surface of the water to avoid introducing carbon dioxide from the air into the eluent. Bring to volume with degassed reagent water, mix, and place the eluent in a plastic eluent reservoir under an inert helium atmosphere of 3–5 psi to minimize carbonate contamination.

Note: Atmospheric carbon dioxide readily dissolves in dilute basic solutions, forming carbonate. Carbonate contamination of eluents can cause higher variance in retention time and lower sensitivity. Use of an EG system can improve retention time stability and sensitivity.

STOCK STANDARD SOLUTIONS

Caution: Sodium azide is toxic. Consult the MSDS and wear proper protective gear to avoid inhalation, ingestion, or skin contact with sodium azide. Sodium azide

can react with heavy metal ions like copper, silver, or lead to form explosive metal azides. Do not pour concentrated azide solutions down the sink. Sodium azide can also explode when heated. DO NOT dry sodium azide in an oven.

Prepare a 1000 mg/L stock standard solution of azide anion by dissolving 0.155 g of sodium azide in reagent grade water in a 100-mL volumetric flask. Bring to volume with reagent grade water and store the stock solutions in high-density polyethylene or polypropylene bottles at 4 ˚C.

WORKING STANDARD SOLUTIONS

To prepare azide working standards, use a calibrated pipette to deliver the appropriate volume of the 1000 mg/L stock standard into a volumetric flask and bring to volume with reagent grade water. Method linearity was determined by diluting the stock solution to working standard solutions of 10, 5, 2, 1, 0.5, 0.25, 0.1, 0.05, and 0.025 mg/L.

To prepare mixed standards containing azide and other anions, combine appropriate volumes of the azide stock standard with the Seven Anion Standard II solution in a volumetric flask, and bring to volume with reagent water. Single-component anion standards may be used instead of Seven Anion Standard II.

SYSTEM PREPARATION AND SETUP

Verify that the pump flow rate is within specifications and recalibrate if necessary. Verify that the conductivity cell constant is within specifications and recalibrate if necessary. Consult the pump or detector manuals for procedural details.

Install the EG and condition the EluGen II KOH cartridge as directed in the manual by running a gradient from 1 to 60 mM KOH in 20 min, then 60 mM for 40 min at 1 mL/min. (For instructions on installation and use, see the ICS-3000 IC system installation instructions, Document No. 065032).

Install and configure the autosampler. Use a calibrated sample loop in the full loop mode to obtain the best accuracy and precision. If you must make partial loop injections, program a sample volume that is less than half the volume of the installed sample loop, and program a cut volume of 8 µL. This injection procedure should provide peak area precision of <1% RSD.

Install a 1-mL sample syringe and set the syringe speed to 3. Enter the correct sample loop size and sample syringe volume in the AS Plumbing Configuration Screen. Refer to the ICS-3000 Ion Chromatography system installation instructions, Document No. 065032 for details.

Install a 4 × 50 mm IonPac AG15 and a 4 × 250 mm IonPac AS15 column. Make sure that the system pressure displayed by the pump is at least 2300 psi when 42 mM KOH is delivered at 1.2 mL/min so the degas assembly can effectively remove electrolysis gas from the eluent. If necessary, install backpressure coils supplied with the EG ship kit to adjust the system pressure to between 2300 and 2800 psi. Because the system pressure can rise over time, trim the backpressure coil as necessary to maintain system pressure under 3000 psi. Do not exceed 3000 psi or the degas assembly tubing may rupture.

Prepare the ASRS ULTRA II for use by hydrating the eluent chamber. Pump approximately 5 mL of regenerant reagent water through the Regen In port. Pump approximately 5 mL of reagent water through the Eluent In port. Allow the suppressor to sit for approximately 20 min to fully hydrate the suppressor screens and membranes. Install the ASRS ULTRA II in the recycle mode by following the Installation and Troubleshooting Instructions for the ASRS ULTRA II, Document No. 031956.

Equilibrate the column with 42 mM KOH eluent for 60 min, and analyze a system blank of reagent water. A well-equilibrated system should have a conductance between 1–2 µS and peak-to-peak noise of <5 nS/min.

Inject a Seven Anion standard spiked with 10 mg/L azide. The column is equilibrated when two consecutive injections of the standard produce the same retention time for azide. Confirm that the resulting chromatogram resembles the chromatogram of the 10 mg/L standard shown in Figure 1.

INTERFERENCE STUDIES

To determine whether other anions interfere with azide determination, single-component standard solutions containing 10 mg/L of the following anions were injected: fluoride, chloride, nitrite, sulfate, oxalate, fumarate, nitrate, adipate, and bromide. Also, a mixed standard was injected containing 10 mg/L each azide, fumarate, and phosphate, 5 mg/L each chloride, nitrite, sulfate, oxalate, bromide, and nitrate, and 1 mg/L fluoride.

SAMPLES

Samples can be directly injected after minimal sample preparation. The samples must be diluted (1:10 or 1:50 in reagent water) and filtered to remove particulates, otherwise, overloading of the AS15 column by the matrix ions will cause the azide peak to appear shorter and broader. The azide peak will be harder to reliably integrate, and low concentration detection limits cannot be achieved.

For this application, phosphate-buffered saline solution (PBS) was prepared by adding 1.00 g NaCl, 0.025 g KCl, 0.18 g Na_2HPO_4, and 0.03 g KH_2PO_4 to about 50 mL reagent grade water in a 100-mL volumetric flask, swirling to dissolve, and bringing to volume with reagent grade water. The PBS was diluted 50-fold with reagent grade water and filtered through a syringe filter before injection. For spike recovery measurements, the PBS was spiked with 10 mg/L sodium azide before dilution and filtration.

Green tea and black tea were prepared by steeping a teabag in about 100 mL of hot reagent grade water for 10 min. After cooling, the infusion was diluted 10-fold with reagent grade water and filtered before injection. For spike recovery measurements, the tea was spiked with 10 mg/L sodium azide before dilution and filtration.

Orange juice was diluted 10-fold with reagent grade water and filtered before injection. For spike recovery measurements, the orange juice was spiked with 10 mg/L sodium azide before dilution and filtration.

Human urine was diluted 10-fold with reagent grade water and filtered before injection. For spike recovery measurements, the urine was spiked with 10 mg/L sodium azide before dilution and filtration.

Lyophilized, citrated human plasma was reconstituted by adding 10 mL of reagent water to one vial and gently mixing to completely dissolve the powder. This reconstituted plasma was then diluted 10-fold with reagent grade water and filtered prior to use. For spike recovery measurements, the plasma was spiked with 10 mg/L sodium azide before dilution and filtration.

RESULTS AND DISCUSSION

Table 1 summarizes the calibration data for typical calibration curves obtained by injecting calibration standards at 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2, 5, and 10 mg/L. The calibration curve in reagent grade water is linear, with a correlation coefficient of 0.9998. Figure 1 shows an ion chromatogram of a 10 mg/L azide standard along with several commonly occurring anions, obtained by using the optimized conditions described above.

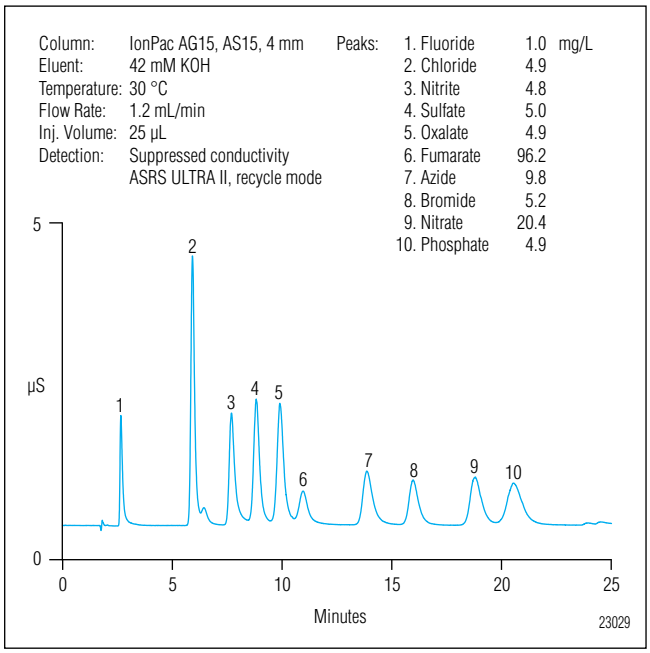


Figure 1. Determination of a 10 mg/L azide standard along with several commonly occurring anions.

Table 1. Linear Range and Detection Limits of Azide in Various Matrices

Matrix	Range (mg/L)	R^2	MDL Standard (µg/L)	% RSD	*Calculated MDL (µg/L)
Reagent Water	0.025 – 10	0.9999	100	9.8	51
Black Tea	0.025 – 10	0.9989	500	13.0	140
Green Tea	0.025 – 10	0.9996	500	6.1	68
Orange Juice	0.025 – 10	0.9940	500	12.3	155
PBS	0.025 – 10	0.9924	500	6.3	87
Urine	0.025 – 10	0.9995	500	9.5	133
Plasma	0.025 – 10	0.9978	250	2.2	34

*The MDLs were calculated as MDL = (t) × (SD) Where t=Student's t value for a 99% confidence interval and a standard deviation estimate with n – 1 degrees of freedom [t = 3.14]

The method detection limit (MDL) for azide was determined by making seven injections of a low-level solution fortified with azide at a level that yielded a signal-to-noise of about 6–9. The concentration values determined from the calibration curve were used to calculate the MDL.

The MDL for azide in reagent grade water was determined by making seven replicate injections of reagent water fortified with azide at 100 ppb. The calculated MDL from this work is given in Table 1. The MDL is defined as the minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero. It is basically a measure of the precision of preparing and analyzing low-level samples according to the method. The MDL for azide in reagent water obtained by this method is 50 µg/L.

Azide was determined in several different matrices that varied in ionic strength and the presence of possible interferences. Some of the samples exemplify matrices of forensic interest, for example orange juice, tea, urine, and plasma.

For each matrix, precision, recovery, and freedom from interferences were measured. Precision and recovery were measured by spiking 1 mg/L azide into seven matrices; reagent water, black tea, green tea, orange juice, PBS, urine, and plasma. Recovery for all matrices was greater than 80%. Precision varied from 2 to 8% for all the matrices. The results are shown in Table 2.

Both green tea and black tea were diluted 1:10. Figure 2 shows the determination of 10 mg/L of azide spiked into green tea. Fluoride, chloride, sulfate, and oxalate anions were observed. Figure 3 shows the determination of 10 mg/L of azide spiked into orange juice. Orange juice contains phosphate, sulfate, fluoride, and fumarate. These compounds do not interfere with azide.

Table 2. Recovery of Azide from Various Matrices

Matrix	Amount Added (mg/L)	Recovery (%) (n=3)	Precision (RSD) (n=3)
Reagent Water	1	100	4.4
Black Tea	1	91	5.4
Green Tea	1	104	1.9
Orange Juice	1	87	8.0
PBS	1	98	4.1
Urine	1	108	3.7
Plasma	1	117	2.2

Sodium azide is commonly used as a preservative in aqueous laboratory reagents and biological fluids. Azide was spiked into PBS and plasma in order to help characterize sodium azide in some fluids commonly used in biological laboratories. PBS contains large amounts

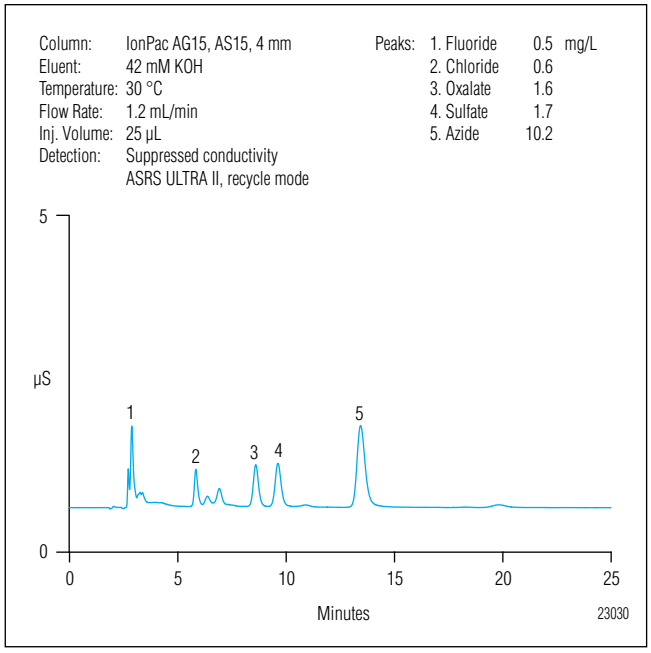


Figure 2. Determination of 10 mg/L azide spiked into 10-fold diluted green tea.

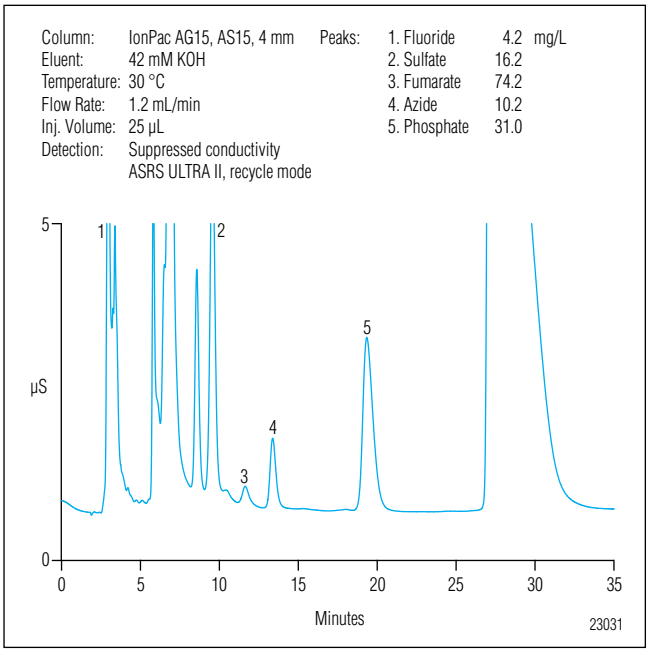


Figure 3. Determination of 10 mg/L azide spiked into 10-fold diluted orange juice.

of chloride and phosphate that can interfere with the integration of azide if it is not diluted. Figure 4 shows the analysis of dilute PBS samples spiked with azide and reveals that phosphate and chloride do not interfere with the determination of azide.

Forensic analysis may require that urine and plasma be analyzed for azide. Figure 5 shows the determination of 10 mg/L azide spiked into urine. None of the component anions interfere with azide quantification. Figure 6 shows the determination 10 mg/L azide spiked into citrated plasma. None of the component anions interfered with the integration of azide under the conditions used for the study.

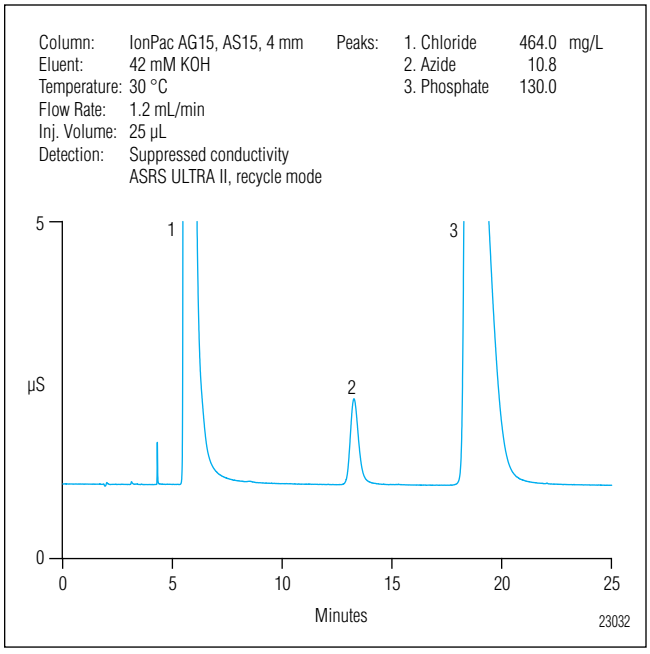


Figure 4. Determination of 10 mg/L azide spiked into 50-fold diluted PBS.

INTERFERENCES

None of the anions tested, at concentrations up to 10 mg/L, interfered with the determination of azide. Figure 1 shows that azide is well resolved from all of these anions under the conditions shown.

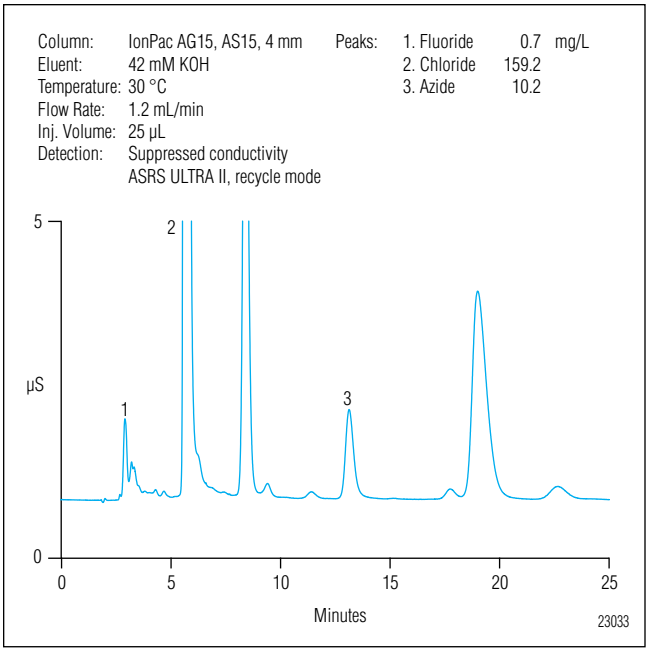


Figure 5. Determination of 10 mg/L azide spiked into 10-fold diluted urine.

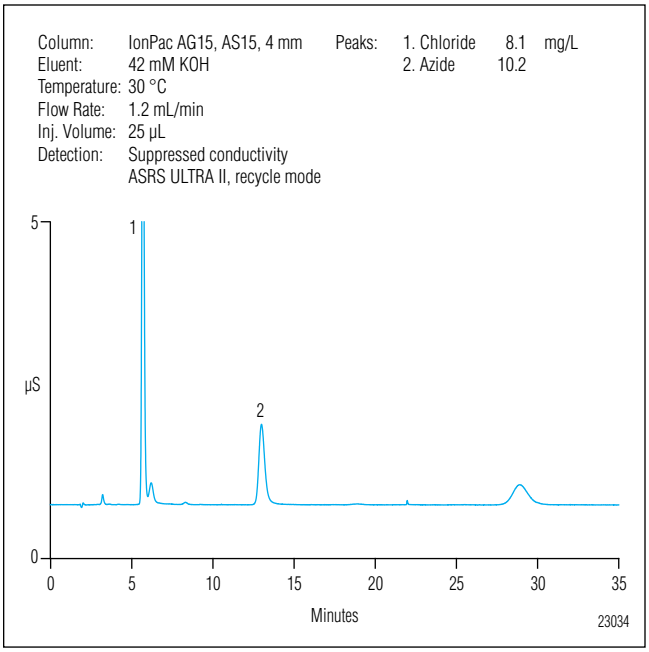


Figure 6. Determination of 10 mg/L azide spiked into 10-fold diluted plasma.

SUGGESTIONS FOR BEST PERFORMANCE

These results were obtained by using a 4-mm AG15/AS15 column set with a 25-µL injection of sample. Peak area precision and accuracy depend on autosampler performance. Replace the water in the flush reservoir daily with freshly filtered and degassed water. Inspect the AS daily for bubbles in the sample syringe or its tubing. Purge to remove any bubbles by following the instructions in the AS manual.

Strongly retained compounds from injected samples can accumulate on the column and degrade its performance. Signs of a fouled column include loss of capacity, loss of resolution, shortened retention times, higher noise and background, spurious peaks, and peak tailing. To remove low valency, hydrophilic contaminant ions, flush the column with a 0.42 M hydroxide solution (i.e., a 10X concentrate of the most concentrated eluent used in the application). To remove high valency, hydrophobic contaminants, flush the AS15 column with 0.2 N HCl in 80% CH_3CN. (For more information on column troubleshooting and cleanup, see the Installation Instructions and Troubleshooting Guide for the IonPac AS15 Analytical Column, Document No. 031362.)

Some samples contain particulates that will plug the column and increase the backpressure. Use a guard column to protect the analytical column; change the guard column if such a sample causes a sudden increase in total backpressure to greater than 3000 psi.

Caution: Sodium azide is toxic. Consult the MSDS and wear proper protective gear to avoid inhalation, ingestion, or skin contact with sodium azide. Sodium azide can react with heavy metal ions like copper, silver, or lead to form explosive metal azides. Do not pour concentrated azide solutions down the sink. Sodium azide can also explode when heated. DO NOT dry sodium azide in an oven.

SUMMARY

The resulting MDL for azide in several matrices ranges from 51 µg/L in reagent grade water to 155 µg/L in orange juice. Calibration is linear over the range of 0.025–10 mg/L in reagent grade water as well as other matrices and quantitative recoveries were obtained for azide spiked at 1 mg/L concentrations. The method provides acceptable performance, in terms of peak shape and recovery, in the presence of high amounts of chloride, sulfate, oxalate, and phosphate.

REFERENCES

1. *Fed. Regist.* **1989,** *54* (12), 2540.
2. Chang, S.; Lamm. S. H. Human Health Effects of Sodium Azide Exposure: a Literature Review and Analysis. *Int. J Toxicol.* **2003**, *22* (3), 175–186.
3. Ku, J. C. *Sodium Azide and Hydrazoic Acid in Workplace Atmospheres*. OSHA_SLTC Method ID-211. U.S. Department of Labor, Occupational Safety and Health Administration, 1992.
4. Kruszyna, R.; Smith R. P.; Kruszyna, H. Determining Sodium Azide Concentration in Blood by Ion Chromatography. *J. Foren. Sci.* **1998**, *43*, 192-194.
5. M. Kikuchi, M. Sato, T. Ito and M. Honda.Application of a New Analyical Method Using Gas Chromatography and Gas Chromatography-Mass Spectrometry for the Azide Ion to Human Blood Samples of an Actual Case. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2001,** *752* (1), 149–157.
6. Tsuge,K.; Kataoka, M.; Seto, Y. Rapid Determination of Cyanide and Azide in Beverages by Microdiffusion Spectrophotometric Method. *J. Anal. Toxicol.* **2001**, *25* (4), 228–236.

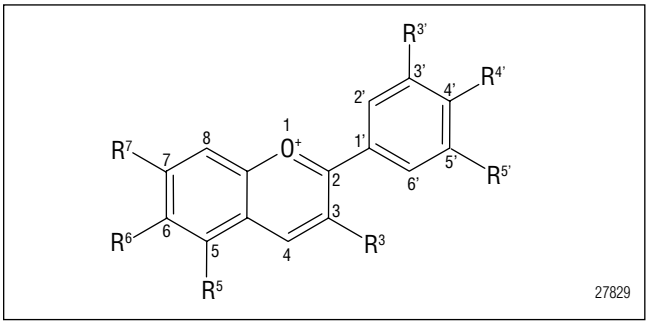
Fast Determination of Anthocyanins in Pomegranate Juice

INTRODUCTION

Anthocyanins are a subclass of molecules known as flavonoids that are responsible for the brilliant red, orange, and blue colors of most fruits and flowers. Anthocyanidins lack the sugar component of the parent anthocyanin. Six of the anthocyanidins that occur most commonly in nature are pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin. Anthocyanins are the mono and diglycosylated forms of anthocyanidins with substitutions at the 3 and 5 positions (Figure 1).[1] The most common carbohydrates encountered on anthocyanins include glucose, galactose, rhamnose, and arabinose.

Due to their strong antioxidant properties, anthocyanins are of considerable interest to the scientific community and consumer market. The naturally electron-deficient chemical structure of anthocyanins makes them highly reactive toward free radicals and, consequently, makes them powerful natural antioxidants. Increased understanding of their health benefits has led to a growing interest in determining anthocyanins in foods, nutraceuticals, and natural products.[2, 3]

Major sources of anthocyanins include blueberries, cherries, raspberries, bilberries, strawberries, black currants, purple grapes, and pomegranates. Pomegranate juice (PJ) has been reported to contain 3× more antioxidant activity than green tea and higher total polyphenol concentrations, compared to common fruit juices (e.g., orange, grapefruit, grape, cranberry, pineapple, and apple). Due to the increased health consciousness of consumers, combined with the potential health benefits of PJ, the demand for PJ and pomegranate-related products has grown rapidly in recent years.



R3'	R5'	Anthocyanidin	R3	R5	Anthocyanin
H	H	Pelargonidin	Glucose		Pelargonidin 3-glucoside
H	H	Pelargonidin	Glucose	Glucose	Pelargonidin 3,5-diglucoside
OH	H	Cyanidin	Glucose		Cyanidin 3-glucoside
OH	H	Cyanidin	Glucose	Glucose	Cyanidin 3,5-diglucoside
OH	OH	Delphinidin	Glucose		Delphinidin 3-glucoside
OH	OH	Delphinidin	Glucose	Glucose	Delphinidin 3,5-diglucoside
OCH_3	OH	Petunidin	Glucose		Petunidin 3-glucoside
OCH_3	OH	Petunidin	Glucose	Glucose	Petunidin 3,5-diglucoside
OCH_3	H	Peonidin	Glucose		Peonidin 3-glucoside
OCH_3	H	Peonidin	Glucose	Glucose	Peonidin 3,5-diglucoside
OCH_3	OCH_3	Malvidin	Glucose		Malvidin 3-glucoside
OCH_3	OCH_3	Malvidin	Glucose	Glucose	Malvidin 3,5-diglucoside

Figure 1. Basic structure of anthocyanins.

Pomegranate is extensively cultivated worldwide and has become a high-value crop for juice production. The retail market now contains numerous pomegranate-related products such as juices, smoothies, flavored waters, and sports and energy drinks.[4] From 2006 to 2008, nearly 320 products containing pomegranate or pomegranate flavoring were launched and PJ currently remains one of the most popular drinks in the *super juice* category.[5]

Due to the high demand for pomegranates outstripping the supply, adulteration of PJ has become widespread. The United States Food and Drug Administration (U.S. FDA) has proposed a working definition of economic adulteration as "The fraudulent, intentional substitution or addition of a substance in a product for the purpose of increasing the apparent value of the product or reducing the cost of its production (i.e., for economic gain)." According to the U.S. FDA, the globalization of the food market has raised economic adulteration as a key concern because imports have increased annually by approximately 14% since 1997.[6] Manufacturers have attempted to extend the limited supply of PJ by blending with filler ingredients such as cane sugar, corn syrup sweeteners, and lower-quality juices containing sorbitol, malic acid, and sucrose (e.g., grape, apple, and blackberry).[7]

To establish an authentication criterion, an International Multidimensional Authenticity Specifications algorithm was developed based on the analysis of commercial juice samples from 23 manufacturers in the United States, Iran, Turkey, Azerbaijan, Syria, India, and China.[8] There is universal agreement that the anthocyanin profile in PJ consists of a constant group of six anthocyanins, regardless of the origin. However, the anthocyanin concentrations can vary depending on the geographic source of the PJ. The anthocyanin profile is one of several chemical analyses that are required to determine the authenticity of PJ. Additional chemical profiling methods include measuring other polyphenols (i.e., ellagitannins), monosaccharides (e.g., fructose and glucose), organic acids, amino acids, and potassium in PJ samples. Determinations of monosaccharides, organic acids, and punicalagins in fruit juices have been previously described in AN 82, 143, and CAN 106, respectively.[9-11]

The method described here is a sensitive, fast, and accurate way to determine anthocyanins in commercially available fruit juices using a simple dilution. Anthocyanins were separated using a 2.2 µm, Acclaim® RSLC 120, C18 rapid separation liquid chromatography column and detected at a visible wavelength of 540 nm. The silica-based column used in this application is designed for rapid, high-resolution separations, which is compatible with ultrahigh pressure instrumentation. The six anthocyanins of interest were separated in <5 min in various beverages that included PJ, grape juice, simulated adulterated PJ, pomegranate cherry juice, and pomegranate wildberry juice.

EQUIPMENT

Dionex UltiMate® 3000 RSLC system including:

SRD-3600 Solvent Rack with 6 degasser channels (P/N 5035.9230)

Eluent Organizer, including pressure regulator and 2 L glass bottles for each pump, eluents maintained under helium or nitrogen head space (5–8 psi)

HGP 3400RS Pump (P/N 5040.0046)

WPS-3000TRS Well Plate Sampler (P/N 5840.0020)

TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)

DAD-3000RS Photodiode Array Detector (P/N 5082.9920)

Semi-Micro Flow Cell for DAD-3000 and MWD-3000 Series, SST, 2.5 µL volume, 7 mm path length (P/N 6080.0300)

CONSUMABLES

Acclaim RSLC 120, C18, 2.2 µm Analytical column, 2.1 × 150 mm (P/N 071399)

Centrifuge equipped with a 10-place, aluminum fixed-angle rotor (Beckman Spinchron R, GS-6R Series, Beckman Coulter, P/N 358702 or equivalent)

Viper™ SST fingertight fitting including capillary for 10-32 fitting, i.d. × L 0.13 × 250 mm (P/N 6040.2325)

Viper SST fingertight fitting including capillary for 10-32 fitting, i.d. × L 0.13 × 350 mm (P/N 6040.2335)

Viper SST fingertight fitting including capillary for 10-32 fitting, i.d. × L 0.18 × 450 mm (P/N 6040.2365)

Static mixer, mixing volume: 350 µL (P/N 6040.0040)

Glass injection vials with caps and septa, 1.5 mL (P/N 055427)

REAGENTS AND STANDARDS

Reagent-grade water, Type I, 18 MΩ-cm resistance or better, filtered through a 0.2 µm filter immediately before use

Acetonitrile, HPLC-Grade (Honeywell P/N AH015-4)

Formic Acid, 98% Pure (Fluka P/N 06440)

Delphinidin 3-glucoside (Cerilliant P/N 89627)

Delphinidin 3,5-diglucoside (Cerilliant P/N 89626)

Cyanidin 3,5-diglucoside (Cerilliant P/N 89615)

Cyanidin 3-glucoside (Cerilliant P/N 89616)

Pelargonidin 3-glucoside (Cerilliant P/N 89753)

Pelargonidin 3,5-diglucoside (Cerilliant P/N 80334)

SAMPLES

100% Pomegranate juice

100% Grape juice

Simulated adulterated pomegranate juice

Pomegranate cherry juice

Pomegranate wildberry juice

CONDITIONS

Conditions for a 2.1 × 150 mm Column

Columns: Acclaim RSLC 120, C18, 2.2 µm Analytical, 2.1 × 150 mm (P/N 071399)

Flow Rate: 0.475 mL/min

Injection Volume: 0.5 µL

Tray Temp.: 4 ºC

Detection: Absorbance, visible, 540 nm

Column Temp.: 30 ºC

Eluents: A: 9% Acetonitrile, 10% formic acid
B: 36% Acetonitrile, 10% formic acid

System Backpressure: 6025–6200 psi over the gradient

Gradient Conditions:

Time (min)	Flow (mL/min)	% A	% B
0.0	0.475	100.0	0.0
0.9	0.475	100.0	0.0
8.0	0.475	71.5	28.5
10.0	0.475	71.5	28.5

Conditions for a 4.6 × 250 mm Column

Columns: Acclaim 120, C18, 5.0 µm Analytical, 4.6 × 250 mm (P/N 059149)

Flow Rate: 1.0 mL/min

Injection Volume: 5 µL

Gradient Conditions:

Time (min)	Flow (mL/min)	% A	% B
0.0	1.0	100.0	0.0
2.5	1.0	100.0	0.0
30.0	1.0	71.5	28.5
45.0	1.0	71.5	28.5

PREPARATION OF SOLUTIONS AND REAGENTS

9% Acetonitrile, 10% Formic Acid

Transfer 200 mL of acetonitrile into a glass 2 L volumetric flask containing approximately 700 mL of deionized water. Mix by inverting the volumetric flask, bring to volume with deionized water, and mix again. Remove 200 mL of the mix and dispose in organic waste, then add 200 mL of formic acid to the volumetric flask and invert to mix.

36% Acetonitrile, 10% Formic Acid

Transfer 400 mL of acetonitrile into a glass 1 L volumetric flask containing approximately 400 mL of deionized water. Mix by inverting the volumetric flask, bring to volume with deionized water, and mix again. Remove 100 mL of the mix and dispose in organic waste, then add 100 mL of formic acid to the volumetric flask and invert to mix.

Standards

All standard concentrates can be stored for up to 6 months at -40 °C protected from light. Diluted intermediate standards are stable for 3 months at -40 °C and working and mixed standards are stable for 4 weeks at 2 to 4 °C.

1 mg/mL Standard Concentrates

Prepare anthocyanin standards of delphinidin 3,5-diglucoside (Dp3,5), cyanindin 3,5-diglucoside (Cy3,5), delphinidin 3-glucoside (Dp3), pelargonidin 3,5-diglucoside (Pg3,5), cyanidin 3-glucoside (Cy3), and pelargonidin 3-glucoside (Pg3) by weighing 1 to 2 mg of solid and adding 1 to 2 mL of mobile phase A to make a stock solution of 1.0 mg/mL for each individual anthocyanin. Prepare the stocks in 10 mL glass vials, vortex to mix, and store at -40 °C until needed.

Working Standards and Standards for Method Linearity

To prepare working standards, use a calibrated pipette to deliver the appropriate volume of the 1 mg/mL stock standard into a glass vial containing the appropriate volume of mobile phase A. For method linearity studies, the following standards were used: 160, 80, 40, 20, 10, 5, 2.5, 1.25, 0.62, and 0.31 µg/mL.

Mixed Standards

To prepare mixed anthocyanin standards, combine appropriate volumes of the individual stock anthocyanin standards into a glass vial containing the appropriate volume of mobile phase A.

SAMPLE PREPARATION

Centrifuge all samples at 5000 rpm for 10 min. Aspirate the supernatant and store in a glass vial at -40 °C until needed. Prepare a 1:5 dilution of the supernatant of all the juices (with the exception of pomegranate cherry) in mobile phase A prior to analysis. The anthocyanin content of pomegranate cherry is low; therefore, sample dilution is not required.

RESULTS AND DISCUSSION

Separation of Anthocyanin Standards

The initial investigation for the separation of anthocyanins was evaluated using a 5 µm Acclaim 120 C18 column in the 4.6 × 250 mm format (gradient specified in the Conditions section). To increase sample throughput and reduce sample and eluent consumption, this application was transferred to an UltiMate 3000 RSLC system. The *Dionex Method Speed-Up Calculator* was used to accelerate the method by using an RSLC column format (2.2 µm, 2.1 × 150 mm).

Figure 2 shows a chromatogram of a mixed anthocyanin standard with all six anthocyanins using a 2.1 × 150 mm column. The retention times of Dp3,5, Cy3,5, Dp3, Pg3,5, Cy3, and Pg3 are 1.02, 1.34, 1.51, 1.90, 2.23, and 3.42 min, respectively. All anthocyanin compounds are well separated and the analysis time is <8 min, compared to approximately 30 min when using the larger column format and larger particle diameter. The accelerated method saves 40 mL of solvent per injection.

System Suitability

The linearity, limits of detection (LOD), and limits of quantification (LOQ) were evaluated to determine suitability of the method for this analysis. Dp3,5, Cy3,5, Dp3, Pg3,5, Cy3, and Pg3 exhibited a linear peak area response in the range of 0.31 to 160 µg/mL, which produced correlation coefficients between 0.9984 and 0.9996 (Table 1). The LOD for the anthocyanins were determined based on the concentration of the analyte that provides a peak height of 3× the measured noise (S/N = 3), whereas the LOQ was determined as the concentration of the analyte that provides a peak height of 10× the measured noise (S/N = 10). The LODs ranged from 0.12 µg/mL for Dp3 to 0.37 µg/mL for Pg3,5, whereas the LOQs ranged from 0.63 µg/mL for Dp3 to 1.25 µg/mL for Pg3,5. Retention time precisions of the standards were excellent, with RSDs ranging from 0.06% for Dp3,5 to 0.12% for Cy3,5. This demonstrates good precision of the gradient delivered by the HPG-3400RS. Peak area precision ranged from 1.45% for Dp3 to 1.82% for Dp3,5, whereas peak height precision ranged from 1.19% for Cy3 to 1.85% for Pg3,5 over 30 runs at a 10 µg/mL concentration.

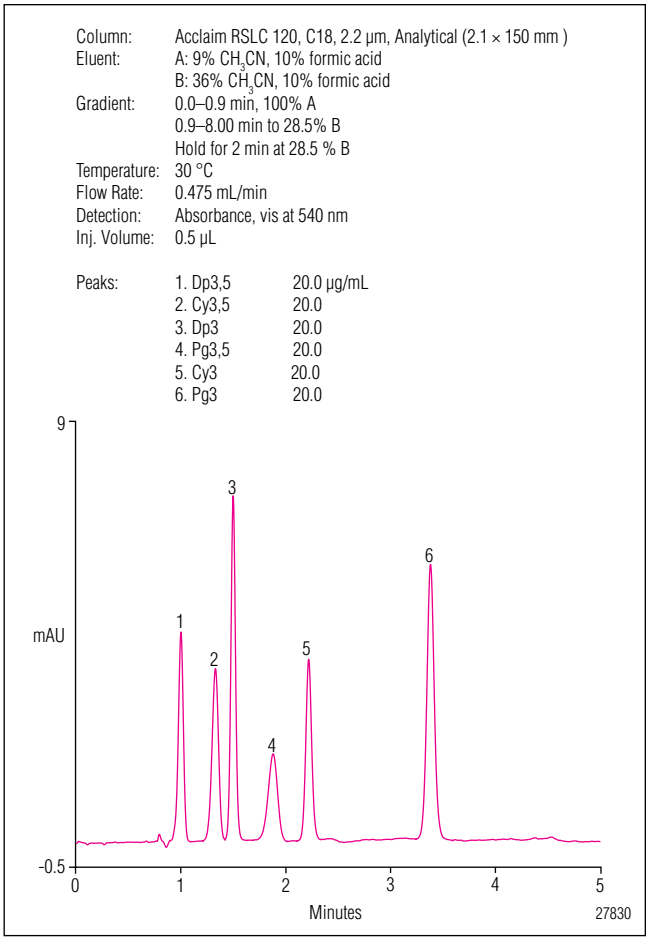


Figure 2. Separation of a mixed anthocyanin standard using the Acclaim RSLC 120 C18 column.

Table 1. Data for Linearity, LOD, and LOQ of Anthocyanins

Analyte	Range (µg/mL)	Correlation Coefficient r^2	LOD (µg/mL)	LOQ (µg/mL)	RSD		
					Ret. Time* (n=30)	Peak Area* (n=30)	Peak Height (n=30)
Dp3,5	0.31-160	0.9992	0.21	0.66	0.06	1.82	1.40
Cy3,5	0.31-160	0.9995	0.19	1.25	0.12	1.60	1.45
Dp3	0.31-160	0.9996	0.12	0.63	0.06	1.45	1.35
Pg3,5	0.31-160	0.9984	0.37	1.25	0.07	1.80	1.85
Cy3	0.31-160	0.9994	0.15	1.25	0.06	1.46	1.19
Pg3	0.31-160	0.9996	0.20	0.63	0.09	1.70	1.50

*Analyte concentrations for precision = 10 µg/mL

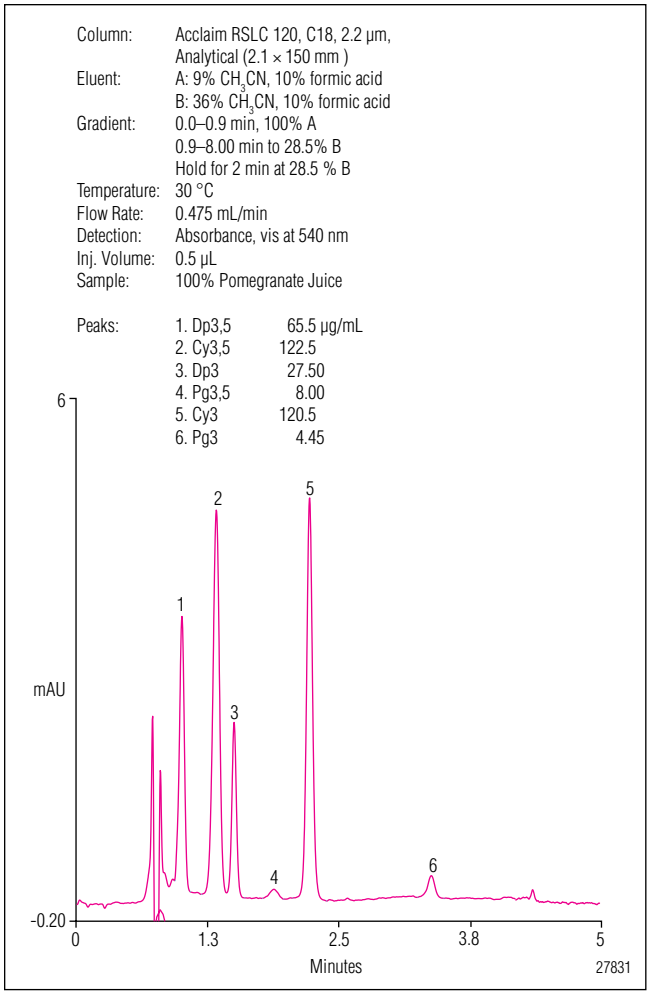


Figure 3. Separation of anthocyanins in a pomegranate juice sample.

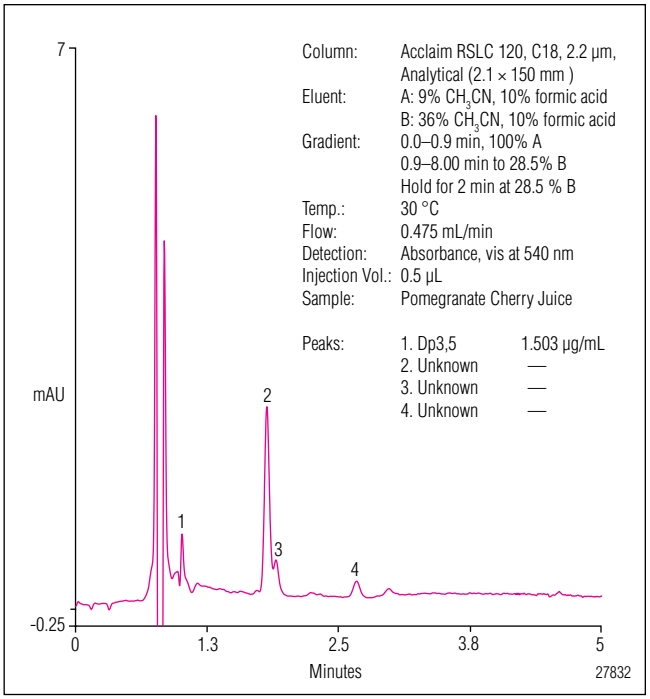


Figure 4a. Determination of anthocyanins in pomegranate cherry juice.

Sample Analysis

This method was applied to determination of the six common anthocyanins that are expected in PJ. The samples investigated in this study included 100% PJ, 100% grape juice, pomegranate cherry juice, pomegranate wildberry, and simulated adulterated PJ. The 100% PJ was used as a reference sample to compare its anthocyanin profile and concentrations to other juices on the market that feature pomegranate on the label. Figure 3 shows the separation of the six signature anthocyanins present in 100% PJ. This confirms previous reports that claim the presence in pomegranates of six anthocyanins that can be isolated and identified from different cultivars.[8]

The pomegranate cherry and pomegranate wildberry juices do not have a claim that states 100% PJ. Therefore, all six anthocyanins were not expected to be detected in these samples. Figure 4a shows the separation of anthocyanins present in pomegranate cherry juice. When this fruit juice was diluted, the anthocyanin concentrations were below the LODs; therefore, the juice was not diluted prior to analysis. A low concentration of Dp3,5 (1.50 µg/mL) was observed in the undiluted juice. No other anthocyanins were observed in pomegranate cherry juice, which implies that very little PJ was added to this juice blend.

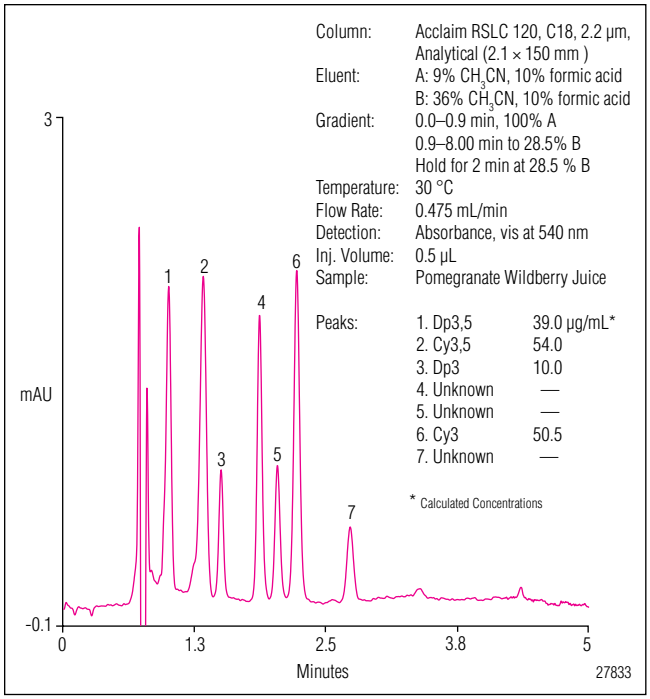


Figure 4b. Determination of anthocyanins in pomegranate wildberry juice.

Figure 4b shows a good separation of the anthocyanins in pomegranate wildberry juice. The four detected anthocyanins in pomegranate wildberry juice are Dp3,5 (39.0 µg/mL), Cy3,5 (54.0 µg/mL), Dp3 (10.0 µg/mL), and Cy3 (50.5 µg/mL), which indicates a significant proportion of PJ was added to the product. However, Pg3,5 and Pg3 were not detected in this sample, although these anthocyanins typically are present at significantly lower concentrations in 100% PJ. Therefore, it is possible that these anthocyanins were present but at concentrations that were < LODs.

Grape juice is one of several juices used to adulterate PJ. Therefore, a 50:50 mixture of grape and 100% PJ was used in this study to simulate an adulterated sample.[7] Figure 5B shows a separation of grape juice with the presence of Cy3,5, Dp3, Cy3, and Pg3,5. Several other later-eluting unknown peaks are also present.

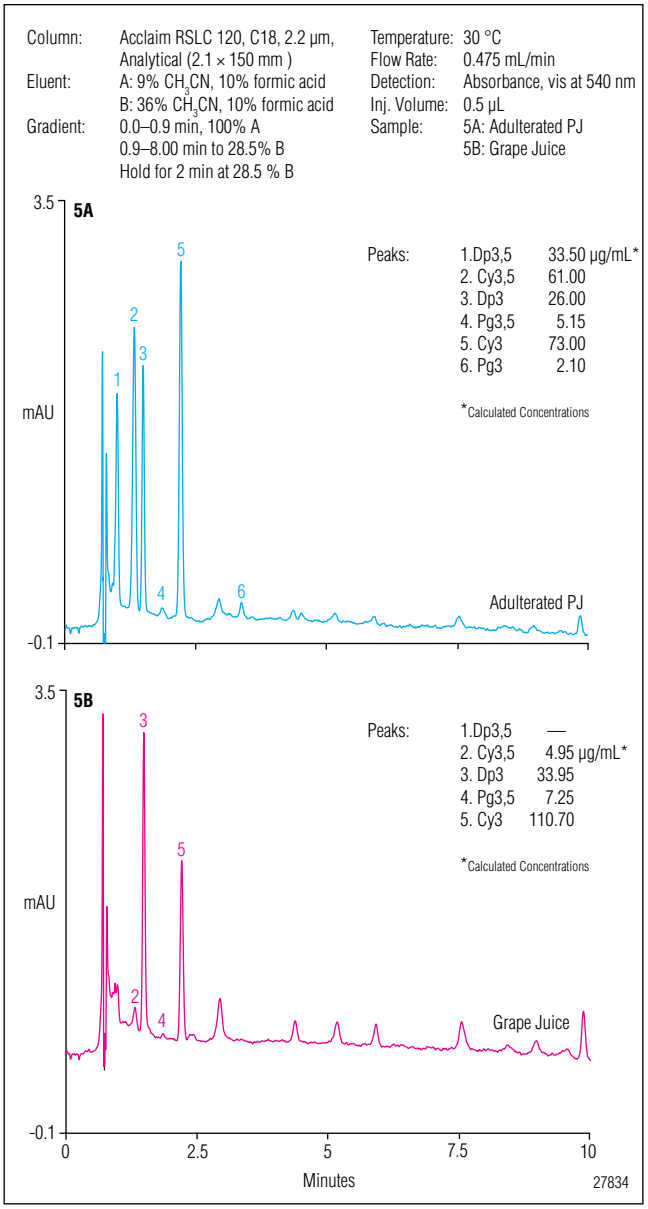


Figure 5. Separation of anthocyanins in simulated adulterated pomegranate juice (5A) overlayed with a separation of anthocyanins in grape juice (5B).

Grape juice contains four of the six anthocyanins present in PJ but at much lower concentrations. Simulated adulterated PJ was prepared by combining PJ and grape juice, then diluting 1:5 in mobile phase A prior to analysis. The chromatogram in Figure 5A shows a separation of Dp3,5, Cy3,5, Dp3, Pg3,5, Cy3, and Pg3 at concentrations of 33.5 µg/mL, 61.0 µg/mL, 26.0 µg/mL, 5.15 µg/mL, 73.0 µg/mL, and 2.10 µg/mL, respectively. The adulterated juice shows all of the signature anthocyanins and several other late-eluting peaks not characteristic of PJ. The anthocyanin content of the adulterated juice is also lower than that of PJ, as expected.

Table 2. Sample Analysis for Intraday and Between-Day Precision						
Sample	**Analyte**	**Amount µg/mL**	**Intraday Precision RSD**			**Between-Day Precision**
			Ret. Time* (n=3)	**Peak Area* (n=3)**	**Peak Height* (n=3)**	**Peak Area* (n=3, over 3 days)**
1:5 Dilute 100% Pomegranate Juice	Dp3,5	13.0	0.010	1.61	0.81	2.10
	Cy3,5	23.2	0.010	0.18	0.16	1.18
	Dp3	5.35	0.006	1.09	0.69	1.65
	Cy3	22.8	0.178	1.41	1.28	2.16
	Pg3,5	1.03	0.085	0.86	0.81	1.48
	Pg3	0.85	0.056	1.16	1.08	1.67
1:5 Dilute 100% Grape Juice	Cy3,5	0.86	0.148	1.17	1.04	1.87
	Dp3	6.24	0.125	1.07	1.19	1.83
	Cy3	6.72	0.004	0.89	0.84	1.42
1:5 Dilute Simulated Adulterated Pomegranate Juice	Dp3,5	6.99	0.010	0.70	0.70	1.05
	Cy3,5	12.1	0.007	0.65	1.11	1.38
	Dp3	5.29	0.006	1.04	1.12	1.86
	Cy3	14.5	0.082	1.43	1.92	2.51
	Pg3,5	0.58	0.371	1.07	0.83	1.39
	Pg3	0.43	0.096	0.62	1.16	1.87
1:5 Dilute Pomegranate Wildberry Juice	Dp3,5	8.24	0.010	1.34	1.57	1.74
	Cy3,5	11.2	0.007	1.98	1.31	2.28
	Dp3	2.10	0.006	1.71	1.20	2.52
	Cy3	10.5	0.088	2.71	2.72	2.68
Pomegranate Cherry Juice	Dp3,5	1.55	0.012	2.65	2.30	4.16

Sample Precision and Accuracy

Five different kinds of juice were analyzed over three days to evaluate the precision of the method. Representative data from each of the juices are presented in Table 2. Intraday retention time RSDs ranged from 0.004% for Cy3 in grape juice to 0.317% for Pg3,5 (n=3) in simulated adulterated PJ. Intraday peak area RSDs ranged from 0.62% for Pg3 in simulated adulterated PJ to 2.71% for Cy3 in pomegranate wildberry juice (n=3). The between-day peak area RSDs ranged from 1.05% for Dp3,5 in adulterated pomegranate juice to 4.16% for Dp3,5 in pomegranate cherry juice (n=3). The imprecision observed in pomegranate cherry juice was attributed to the increased background noise and low concentration of Dp3,5, which made quantification challenging. Recovery studies were performed on all five fruit juices by spiking in known amounts of the six anthocyanins.

Table 3. Recovery of Anthocyanins in Various Matrices

Sample	Analyte	Amount (µg/mL)	Amount Spiked (µg/mL)	Recovery %
1:5 Dilute 100% Pomegranate Juice	Dp3,5	13.2	15.0	101.8
	Cy3,5	23.8	25.0	98.0
	Dp3	6.50	5.0	106.9
	Pg3,5	1.04	1.0	104.1
	Cy3	23.6	25.0	102.2
	Pg3	0.88	1.0	108.3
1:5 Dilute 100% Grape Juice	Dp3,5	< LOD	2.5	104.6
	Cy3,5	1.05	1	105.1
	Dp3	5.12	5	89.5
	Pg3,5	< LOD	1	87.3
	Cy3	4.24	5	85.5
1:5 Dilute Simulated Adulterated Pomegranate Juice	Dp3,5	6.5	10.0	102.6
	Cy3,5	12.8	10.0	80.1
	Dp3	5.48	5.0	103.4
	Cy3	14.9	10.0	97.2
	Pg3,5	0.56	0.75	102.0
	Pg3	0.43	0.75	87.6
Pomegranate Cherry Juice	Dp3,5	1.55	1.0	64.2
	Cy3,5	< LOD	10.0	110.0
	Dp3	< LOD	5.0	81.1
	Pg3,5	< LOD	1.0	97.0
	Cy3	< LOD	5.0	106.0
	Pg3	< LOD	1.0	93.7
1:5 Dilute Pomegranate Wildberry Juice	Dp3,5	9.15	7.5	75.7
	Cy3,5	8.39	10.0	84.4
	Dp3	1.70	2.0	73.9
	Pg3,5	< LOD	5.0	89.7
	Cy3	8.37	5.0	94.1
	Pg3	< LOD	2.0	70.3

Table 3 summarizes the amounts spiked and the calculated recoveries. Recoveries ranged from 64.2% for Dp3,5 in pomegranate cherry juice to 108.3% for Pg3 in PJ. Recoveries were low for Dp3,5 in the pomegranate cherry because of increased background noise and low concentration of Dp3,5.

Table 4. Peak Purity Results for PJ and Other PJ Blends

Sample	Analyte	Match	PPI (nm)	RSD PPI %
1:5 Dilute 100% Pomegranate Juice	Dp3,5	924	505	0.94
	Cy3,5	995	503	0.33
	Dp3	993	509	0.27
	Cy3	931	442	0.99
	Pg3,5	793	493	3.91
	Pg3	931	483	0.31
1:5 Dilute Simulated Adulterated Pomegranate Juice	Dp3,5	988	400	0.88
	Cy3,5	989	358	0.87
	Dp3	949	505	0.91
	Cy3	914	495	0.89
	Pg3,5	995	318	0.20
	Pg3	829	468	2.86
1:5 Dilute Pomegranate Wildberry Juice	Dp3,5	951	395	0.89
	Cy3,5	873	465	1.69
	Dp3	960	334	0.98
	Cy3	994	387	0.69
Pomegranate Cherry Juice	Dp3,5	992	319	0.17

Application of UV Spectral Information to Determine Purity

Spectral scanning was used for the analysis of the standard mix of anthocyanins. High match values of the standards suggested that the peaks were pure and the peak spectra were loaded to the spectral library to identify anthocyanins in different fruit juices. Table 4 displays the match factor and the peak purity index (PPI) values of different anthocyanins in four different fruit juices. The match factor expresses the similarity of two spectra (one from the standard and one from the sample). The match factor also refers to the correlation between the spectrum at its peak maximum and the leading and tailing edges. A 100% peak match indicates that the peak start and end do not deviate from the spectrum at the peak maximum, therefore resulting in a perfect match score of 1000. The match values for all anthocyanins were more than 900, with the exceptions of Pg3,5 in PJ, Pg3 in simulated adulterated PJ, and Cy3,5 in pomegranate wildberry juice. Therefore, the anthocyanins separated in all four fruit juices showed high spectral matches with the exception of three anthocyanins, each in only one sample. This suggests that matrix-related interfering peaks may have

co-eluted with the peaks for Pg3,5 in PJ, Pg3 in simulated adulterated PJ, and Cy3,5 in pomegranate wildberry juice, thereby causing the match score to be low.

PPI is another measure for evaluating spectral purity. It represents the wavelength where the areas of the spectrum to the left and right are identical and, therefore, independent of the concentration. In the case of a pure peak, the individual PPI values result in a rectangular curve. The height of each single rectangle corresponds to the value of the central wavelength. The deviation from the rectangle shape can be mathematically expressed by the relative standard deviation of the PPI value. Low RSDs represent good spectral purity, which were observed for all the anthocyanins in all four fruit juices with the exception of Pg3,5 in PJ, Pg3 in simulated adulterated PJ, and Cy3,5 in pomegranate wildberry juice. The PPI values further confirm that some matrix interferences caused the PPI and match scores to be low for Pg3,5, Pg3, and Cy3,5 in PJ, simulated PJ, and pomegranate wildberry juice, respectively. A closer visual inspection of Pg3,5 peak in PJ chromatogram, Pg3 peak in simulated PJ chromatogram, and the Cy3,5 peak in pomegranate wildberry juice chromatogram reveals that all three peaks show a good amount of tailing or fronting, which correlates to the high PPI RSDs.

CONCLUSION

This work describes a sensitive and accurate method to separate and quantify anthocyanins in different fruit juices with a simple dilution of the sample. The method uses a high-resolution, silica-based, Acclaim RSLC C18 column and absorbance detection at a visible wavelength of 540 nm to separate and detect anthocyanins in < 5 min. Several fruit juices with varying concentrations of anthocyanins ranging from 122.5 µg/mL of Cy3,5 in PJ to 1.5 µg/mL of Dp3,5 in pomegranate cherry juice were determined by this method.

PRECAUTIONS

Supplier PhytoLab recommends dissolution of the anthocyanin standards in methanol acidified with 0.01% HCl; however, this experiment showed that using mobile phase A for standard dilution resulted in better peak shapes (tailing was observed with acidified methanol), retention time, peak area, and peak height precisions.

REFERENCES

1. Einbond, L. S.; Reynertson, K. A.; Luo, X.; Basile, M. J.; Kennelly, E. J. Anthocyanin Antioxidants From Edible Fruits. *Food Chem*. **2004**, 23–28.
2. Lee, HS. Characterization of Major Anthocyanins and the Color of Red-Fleshed Budd Blood Orange (Citrus sinensis). *J. Agric. Food Chem*. **2002**, *50*, 1243–6.
3. Lee, J.; et al. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorant, and Wines by pH Differential Method: Collaborative Study. *J. of AOAC International* **2005**, *88 (5)*, 1269–1278.
4. Fuhrman, E. A Wonderful World: POM Wonderful Takes Pomegranate to Places It Has Never Gone Before, *Beverage Industry Magazine*, July 15, 2008. Available at http://www.bevindustry.com/Articles/Cover_Story/BNP_GUID_9-5-2006_A_1000000000000383065
5. Zegler, J. Ingredient Spotlight: Pomegranate Packs a Punch, *Beverage Industry Magazine*, January 11, 2010. Available at http://www.bevindustry.com/Articles/Departments/BNP_GUID_9-5-2006_A_1000000000000727599
6. Lutter, R. *Addressing Challenges of Economically-Motivated Adulteration*, Presented at the Public Meeting on Economically Motivated Adulteration, College Park, MD, 2009.
7. Murray, N. Pomegranate: New Target for Crooks. *Food News*, Oct 27, 2006.
8. Zhang, Y.; Krueger, D.; Durst, R.; Lee, R.; Wang, D.; Seeram, N.; Herber, D. International Multidimensional Authenticity Specification (IMAS) Algorithm for Detection of Commercial Pomegranate Juice Adulteration. *J. Agric. Food Chem*. **2009**, *57 (6)*, 2550–2557.
9. Dionex Corporation, *Analysis of Fruit Juice Adulterated with Medium Invert Sugar From Beets*. Application Note 82, LPN 034751, 1992, Sunnyvale, CA.
10. Dionex Corporation, *Determination of Organic Acids in Fruit Juices*. Application Note 143, LPN 1415, 2003, Sunnyvale, CA.
11. Dionex Corporation, *Determination of Punicalagins found in Pomegranate by High Performance Liquid Chromatography*. Customer Application Note 106, LPN 2281, 2009, Sunnyvale, CA.

SUPPLIERS

Sigma-Aldrich, 3050 Spruce Street, St. Louis,
MO 63103, U.S.A. Tel: 800-521-8956.
www.sigmaaldrich.com

Sarstedt Inc., 1025 St. James Church Road,
P.O. Box 468, Newton NC 28658-0468, U.S.A.
Tel: +1 828 465 4000.
www.sarstedt.com

Praxair Specialty Gases and Equipment,
39 Old Ridgebury Road, Danbury, CT 06810-5113
U.S.A. Tel: 877-772-9247.
www.praxair.com

PhytoLab GmbH & Co. KG, Dutendorfer Str. 5-7,
91487 Vestenbergsgreuth, Germany.
Tel: +49 9163 88-330.
www.phytolab.com

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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