Food Safety Applications Notebook

Adulteration

Thermo SCIENTIFIC

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Introduction to Food Safety

Food contamination stories in the news media have raised awareness of the fact that we live with a global food supply chain, and food safety is increasingly becoming an important concern. All types of fruits, vegetables, seafood, and meat can be purchased year round independent of the local growing season. For example, in many countries, well-stocked grocery stores carry cantaloupes from Guatemala, cucumbers from Mexico, shrimp from Vietnam, and fish from China. With fruit, vegetables, seafood, and meat traveling thousands of miles to reach far-flung destinations, and with poor or no knowledge of the agricultural practices, the need for food testing is increasingly important.

Thermo Fisher Scientific understands the demands of food safety related testing. Our separation and detection technologies, combined with experienced applications competence, and our best suited chemistries provide solutions for the analysis of inorganic ions, small drug molecules, pesticides to large components, such as polysaccharides. Your laboratory can now conduct reliable, accurate, and fast testing of food. This notebook contains a wide range of food safety related application notes that will help address your food safety issues.

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- Dionex ICS-1100: Basic integrated IC system
- Dionex ICS-900: Starter line IC system

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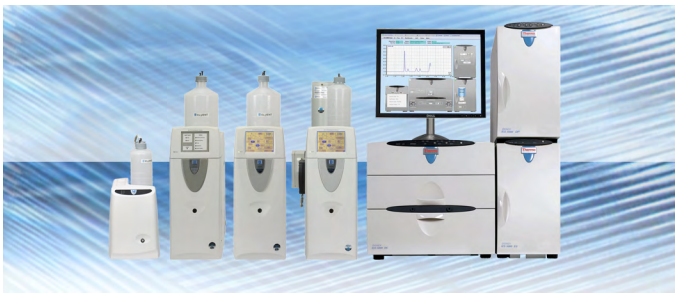
Dionex ICS-900: Can routinely analyze multiple anions and cations in 10–15 min—fully automated with Displacement Chemical Regeneration (DCR).



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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.

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

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

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

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

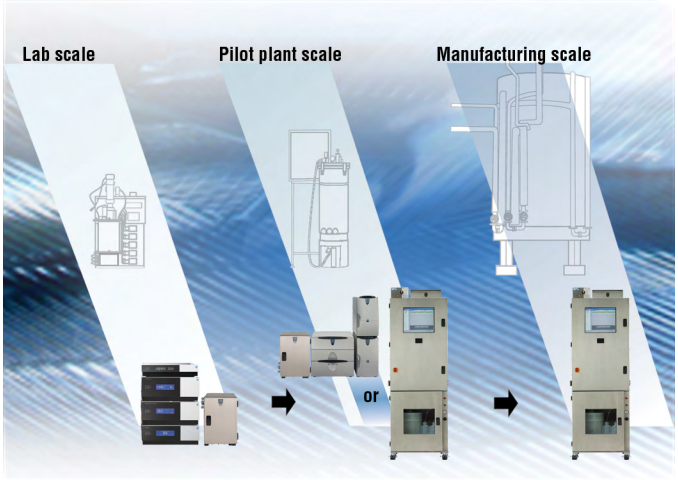
Our process analytical systems provide timely results by moving liquid chromatography-based measurements on-line. Information from the Thermo Scientific Dionex Integral process analyzer can help reduce process variability, improve efficiency, and reduce downtime. These systems provide comprehensive,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

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

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

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

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



Automated Sample Preparation

ACCELERATED SOLVENT EXTRACTORS

Two new solvent extraction systems with pH-hardened Dionium components

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

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SOLID-PHASE EXTRACTION SYSTEMS

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

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

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

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

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

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

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Adulteration

Rapid Determination of Melamine in Liquid Milk and Milk Powder by HPLC on the Acclaim Mixed-Mode WCX-1 Column with UV Detection

INTRODUCTION

Melamine (2,4,6-triamino-1,3,5-triazine, structure shown in Figure 1) is a chemical used in some plastics and fertilizer products. Recent investigations of death and health problems of babies in China have revealed that some baby foods (milk powder) have been contaminated by melamine. Some manufacturers illegally used melamine as an adulterant to increase the apparent protein content. Melamine was also used as an adulterant to increase the apparent protein content of animal feeds and there were news reports that melamine was found in eggs obtained from some markets.

The reported methods for quantitative determination of melamine include enzyme immunoassay (EIA), gas chromatography mass spectrometry (GC-MS), liquid chromatography mass spectrometry (LC-MS), and high-performance liquid chromatography (HPLC) with UV detection.[1-4] Standard methods enacted by the Chinese government for determining melamine in raw milk and dairy products included HPLC-UV, LC-MS, and GC-MS methods.[5] However, the high cost of operation and maintenance of GC/LC-MS systems as well as the labor intensive derivatization that GC-MS requires limits their use in milk product factories. The HPLC-UV method therefore is presently the popular choice for most factories. Because numerous batches of raw milk had to be monitored, another standard HPLC-UV method for rapidly determining melamine in raw milk was soon recommended;[6] however, its application is limited in the analysis of liquid milk products.

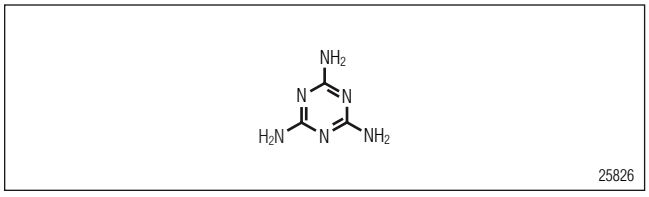

Figure 1. Structure of melamine.

In this Application Note (AN), we developed a simple HPLC method for rapid analysis of melamine in both liquid milk and milk powder samples. The separation was performed on the Acclaim® Mixed-Mode WCX-1 column[7] and UltiMate® 3000 HPLC system with UV detection using an acetate buffer and acetonitrile mobile phase. A sample analysis is completed within 10 min. The Acclaim Mixed-Mode WCX-1 column features a new mixed-mode silica-based packing material that incorporates both hydrophobic and weak cation-exchange properties, and demonstrates great potential for separating samples that contain a mixture of ionic and neutral compounds. Using an Acclaim Mixed Mode WCX-1 column with an UltiMate 3000 system allows a fast analysis of both liquid and powdered milk for melamine.

EQUIPMENT

Dionex UltiMate 3000 HPLC system consisting of:

- HPG 3400A pump
- WPS 3000TSL autosampler
- TCC-3000 thermostatted column compartment
- VWD-3400RS UV-vis Detector

Dionex Summit® UVD-340U Photodiode Array Detector

Chromeleon® 6.80 SP5 Chromatography Management Software

Kudos® SK3200LH Ultrasonic generator, Kudos Ultrasonic Instrumental Co., Shanghai, China

Mettler Toledo AL-204 Electoral o balance, Mettler-Toledo (Shanghai) Co., Shanghai, China

Anke® TGL-16B centrifuge, Anting Scientific Instrumental Factory, Shanghai, China

IKA® MS1 Minishaker, IKA Works, Guangzhou, China

REGENTS AND STANDARDS

Water, from Milli-Q® Gradient A 10

Methanol (CH_3OH), HPLC grade, Fisher

Acetonitrile (CH_3CN), HPLC grade, Fisher

Ammonium acetate (NH_4Ac), analytical grade, SCRC, China

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

Sodium 1-octane sulfate (98%), Baker Analyzed HPLC Reagent, USA

Melamine (99.0%), HPLC grade, Fluka

CHROMATOGRAPHIC CONDITIONS

Guard Column:	Acclaim® Mixed-Mode WCX-1, 5 µm, 4.3 × 10 mm, P/N 068354, with guard column holder, P/N 59526
Analytical Column:	Acclaim Mixed-Mode WCX-1, 5 µm, 4.6 × 250 mm, P/N 068352
Column Temp.:	30 °C
Mobile Phase:	Acetate buffer (mixture of 700 mL of 10 mM HAc and 300 mL of 10 mM NH4Ac, ~ pH 4.3) – CH_3CN (8 : 2, v/v)
Flow Rate:	1.0 mL/min
Inj. Volume:	20 µL
UV detection:	Absorbance at 240 nm

PREPARATION OF STANDARDS

Stock Standard Solution

Accurately weigh ~100 mg of melamine, dissolve in a 100 mL volumetric flask with aqueous methanol (50%, v/v). The melamine concentration is 1000 µg/mL

Working Standard Solutions

Prepare seven working standard solutions for calibration by adding defined volumes of the stock standard solution and diluting with the acetate buffer used in the mobile phase. The concentrations of melamine are 0.05, 0.1, 0.2, 1.0, 5.0, 20, and 40 µg/mL, respectively.

PREPARATION OF SAMPLES

Milk Powder Sample

Put an accurately weighed ~1 g of dried sample into a 15 mL centrifuge tube, and then add 10 mL water. After 1 min of vortex shaking, put in an ultrasonic bath for 30 min. Add 1 mL dilute HAc (3%, v/v) and store the solution at 4 °C for at least 30 min. After 15 min of centrifugation (setting = rpm ≥ 10000), move the supernatant to a 10-mL volumetric flask, and add water to the mark. Prior to injection, filter the solution through a 0.2 µm filter (Millex-HV).

Liquid Milk Sample

Directly add 1 mL dilute HAc (3%, v/v) to an accurately measured 10 mL of liquid milk sample in a 15 mL centrifuge tube and store the solution at 4 °C for at least 30 min. The remainder of the procedure is the same as that for milk powder.

Spiked Milk Powder and Liquid Milk Samples

Add 40 µL of the stock standard solution of melamine to the 15 mL centrifuge tubes together with the accurately weighed ~1 g of dried sample, and together with the accurately measured 10 mL of liquid milk sample, respectively. The remainder of the sample preparation procedure is the same as that for the milk powder and liquid milk samples.

RESULTS AND DISCUSSION

Optimized Procedure for Preparing Milk and Milk Powder Samples

The common procedure for preparing milk and milk powder samples for melamine consists of two steps, sample extraction and cleaning the sample extract on an activated SPE column; and then drying the cleaned extract with N_2 at 50 °C.[5] A simpler preparation procedure for use with an ion-exchange (IEX) analysis method was reported;[6] however, it is only for liquid milk samples. Therefore it is necessary to find an efficient and simple way to prepare both milk and milk powder samples.

The optimized procedure in this AN is simple and efficient, and does not require clean up by SPE and sample drying. It requires only precipitation with dilute acetic acid, subsequent centrifugation and filtration, and is suitable for both liquid milk and milk powder products. Additionally, this procedure is also suitable for the reversed-phase ion-pair chromatography method (RP-PIC). Figure 2 shows that this sample preparation method yields good chromatography for melamine using either the Acclaim Mixed-Mode WCX-1 column method or the RP-PIC method with the Acclaim 120 C18 column, as no interfering matrix peaks elute in the retention time range of melamine.

Optimized Chromatographic Conditions

Melamine is a hydrophilic compound that is poorly retained on a typical RP column (e.g. C18 or C8 column). Most RP methods for melamine use an ion-pairing reagent. With an ion-pairing reagent in the mobile phase, e.g. octane sulfate, melamine is well retained. However, the ion-pairing reagent may coat the RP stationary phase, changing the retention property of RP column, which may not be desired if the column is used for other methods. The RP-PIC method is also not compatible with MS detection. We therefore attempted to separate the cationic melamine on the Acclaim Mixed-Mode WCX-1 column using an ammonium acetate buffer as the eluent.

The Acclaim Mixed-Mode WCX-1 column features a mixed mode silica-based packing material that incorporates both hydrophobic and weak cation-exchange properties. Mobile phase pH affects the charge and hydrophobicity of the stationary phase. At a pH below the pKa of the stationary phase carboxylate group, the cation-exchange functionality is OFF so that hydrophobic interaction is the primary retention mechanism. At a pH above the pKa of the stationary phase carboxylate group, the cation-exchange functionality is ON so that both cation-exchange and hydrophobic interaction contribute to retention depending on the structures of analytes. Our experiments revealed that melamine does not have good retention when the pH is lower than 3.5 and higher than 5.0. Ionic strength is crucial for changing retention of charged molecules. An increase in ionic strength results in a retention decrease for melamine based on its basicity. Hydrophobic retention is markedly affected by the organic modifier composition of the mobile phase. In general, all types of molecules (acids, bases, and neutrals) are less

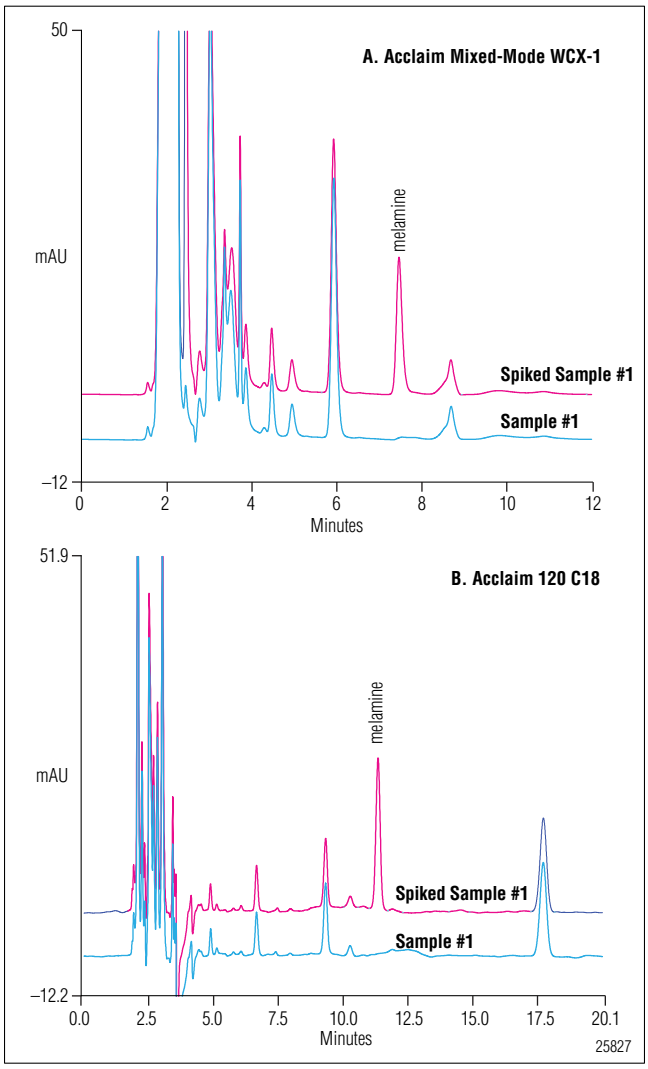

Figure 2. Chromatograms of a milk sample and the same sample spiked with melamine on (A) an Acclaim Mixed-Mode WCX-1 column, and (B) an Acclaim 120 C18 column. Chromatograms: 1, sample #1; and 2, sample #1 spiked with 4 µg/mL melamine. Melamine is detected by absorbance at 240 nm.

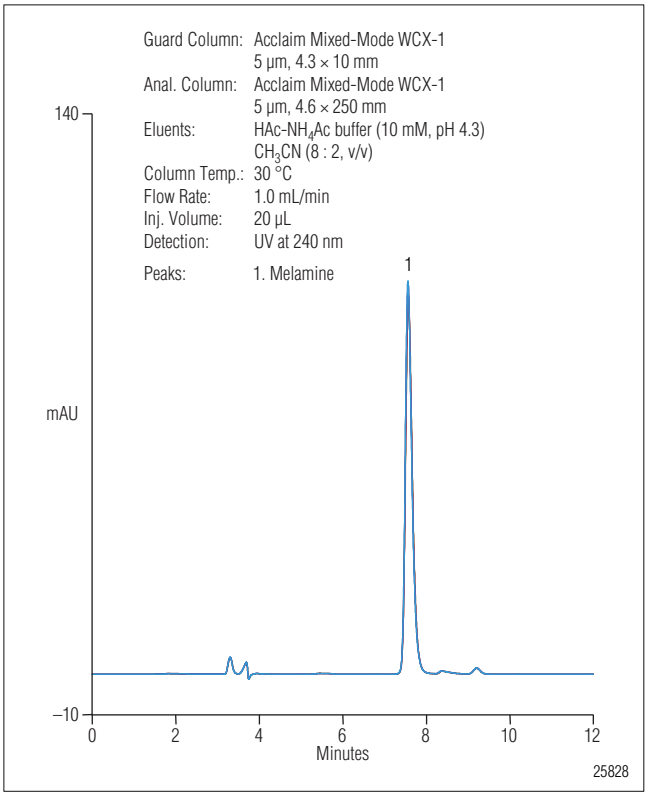

Figure 3. Overlay of chromatograms of five consecutive injections of a 20 µg/mL melamine standard.

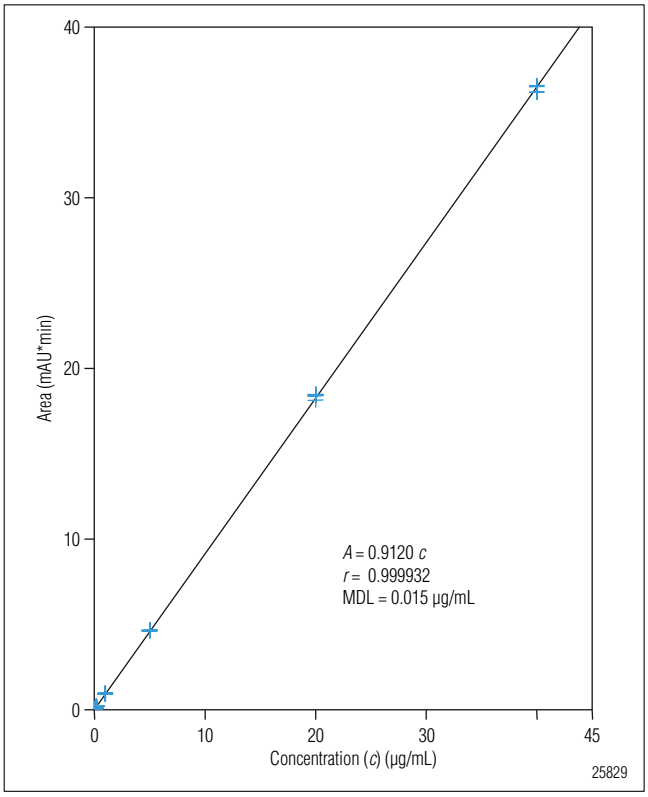

Figure 4. Calibration curve for melamine.

retained with an increase in the organic content of the mobile phase, though to different extents, when other conditions (e.g. ionic strength, pH, temperature, etc.) remain constant.[7]

The optimized mobile phase for these samples is 10 mM ammonium acetate pH 4.3, 20% CH_3CN. For more complex samples, a higher buffer capacity may be required. In these situations, increase the concentration of ammonium acetate. This may result in a decrease in melamine retention time, but it may be possible to restore it by decreasing the percent of CH_3CN in the mobile phase.

Chromatographic Performance

Figure 3 shows overlay chromatograms of five consecutive injections of a 20 µg/mL melamine standard. Note the good reproducibility of retention time and peak area. Calibration linearity for melamine was investigated by making five replicate injections of each standard prepared at seven different concentrations. The external standard method was used to establish the calibration curve and to quantify melamine in samples. As shown in Figure 4, excellent linearity was achieved throughout the range from 0.05 to 40 µg/mL. The linearity equation of melamine is as follows, with the curve forced through the origin.

$$A = 0.9120\ c$$

Here, A stands peak area, and c stands for melamine concentration (µg/mL). The correlation coefficient (r) is 0.9999.

Method reproducibility was estimated by making ten consecutive injections of a 1 µg/mL standard. The RSD for retention time was 0.037, and the RSD for peak area was 0.472.

The detection limit of melamine was calculated using the equation:

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

S = standard deviation of replicate analyses

n = number of replicates

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

Using the same 10 injections of 1 µg/mL standard the calculated MDL was 0.015 µg/mL.

Sample Analysis

Five samples, including three milk powder samples (#1 - #3) and two liquid milk samples (#4 and #5), were analyzed using the sample preparation method and WCX chromatography method described in this AN. No melamine was detected in #1. Melamine was found in the other milk and milk powder samples. The results were summarized in Table 1 and Figure 5 shows the chromatograms of these samples. We also spiked samples #1 and #4 with melamine before sample preparation and found that melamine was sufficiently recovered from both.

Comparison of Sample Preparation and Analysis Methods

The three milk powder samples, #1–#3, and three additional milk powder samples, #6– #8, were prepared with the sample preparation method presented in the application note and the procedure for milk powder samples that uses an activated SPE column (detailed in reference 5). These samples were then analyzed using an Acclaim 120 C18 column under the chromatographic conditions in Ref. 5 and on the Acclaim Mixed-Mode WCX-1 column under the chromatographic conditions described in this AN. This allowed an evaluation of both the simplified sample preparation procedure and the chromatography procedure in this AN. Table 2 shows the results for all six samples prepared using both sample preparation procedures and analyzed by both chromatography methods. The results show good agreement for all six samples using either of the two sample preparation methods or chromatography procedures.

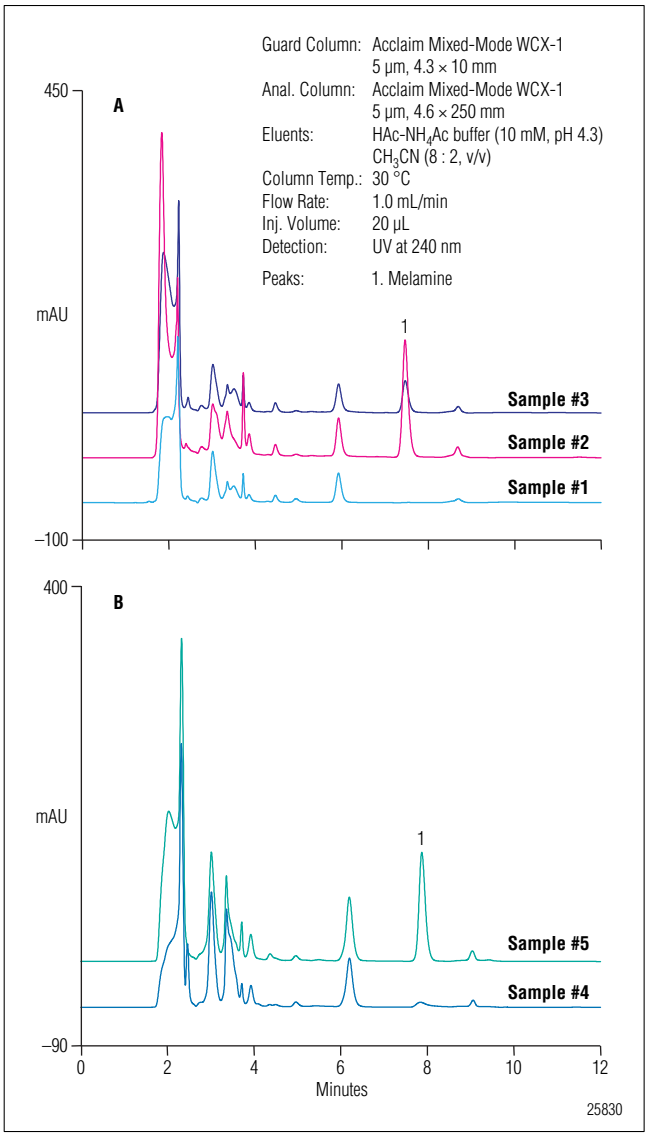

Figure 5. Chromatograms of (A) milk powder samples #1– #3, and (B) liquid milk samples #4 and #5.

Table 1.Sample Analysis Results

	Milk Powder						Liquid Milk				
	#1				#2	#3	#4				#5
	Detected (mg/Kg)	Added (mg/Kg)	Found (mg/Kg)	Recovery (%)	Detected (mg/Kg)	Detected (mg/Kg)	Detected (mg/L)	Added (mg/L)	Found (mg/L)	Recovery (%)	(mg/L)
Melamine	n.a.	40	35	88	262	67	1.8	4.0	3.6	90	22

Note: * Injections were made for each sample

** Found = Measured value of spiked sample - Measured value of sample

The complicated matrix of milk products may sometimes yield in a false positive for melamine. An efficient way to determine if the peak is melamine is by comparison of the peak's UV spectrum to that of melamine. When we analyzed sample #8, using the sample preparation method described in Ref. 5 and the Mixed-Mode WCX-1 column method, a small peak with retention time near that of melamine was found, and labeled as melamine with a concentration was 3.3 mg/Kg. The other sample preparation method and other chromatography method did not detect melamine in this sample. We reanalyzed the sample after substituting the VWD UV-Vis detector with a photodiode array detector. Comparison of the UV spectra, shown in Figure 6 revealed that the peak was not melamine. Using a photodiode array detector for this analysis will help reduce the possibility of false positives for melamine.

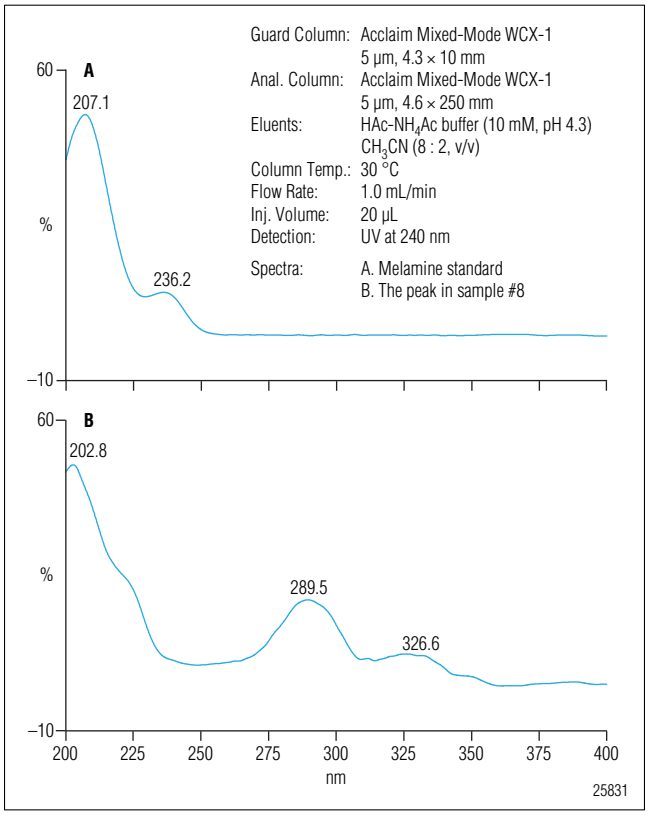

Figure 6. UV-spectra of (A) melamine standard and (B) the putative melamine peak in sample #8.

CONCLUSION

This application note describes an efficient and simple method for preparing liquid milk and milk powder samples coupled to an HPLC method for rapid analysis of melamine in these samples. The Acclaim Mixed-mode WCX-1 column exhibits good retention of melamine, using ammonium acetate buffer and acetonitrile as the mobile phase. This mobile phase should make this method compatible with MS detection.

Table 2. Comparison of Milk Powder Sample Analysis Results Using Different Sample Preparation Methods and HPLC Analysis Methods

	Melamine Concentration using the RP-PIC method (mg/Kg)[1]		Melamine Concentration using the Acclaim Mixed-Mode WCX-1 Column (mg/Kg)[2]	
Sample #	Prepared following the description in this AN	Prepared following the description in Ref. 5	Prepared following the description in this AN	Prepared following the description in Ref. 5
-	n.a.	n.a.	n.a.	n.a.
-	274	229	262	229
-	67	68	67	68
-	2.2	2.3	2.4	2.8
-	n.a.	n.a.	n.a.	n.a.
-	n.a.	n.a.	n.a.	n.a.

Note: 1. Using the chromatographic conditions described in Ref. 5.
2. Using the chromatographic conditions described in this AN.

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Determination of Melamine in Milk Powder by Reversed-Phase HPLC with UV Detection

INTRODUCTION

Recent investigations of death and health problems of babies in China have revealed that some baby foods (milk powder) have been contaminated by melamine (structure shown in Figure 1). Some manufacturers illegally used melamine as an adulterant to increase the apparent protein content. Standard methods enacted by the Chinese government for determining melamine in raw milk and dairy products included HPLC-UV, LC-MS, and GC-MS methods.[1] However, the high cost of operation and maintenance of GC/LC-MS systems as well as the labor intensive derivatization that GC-MS requires limits their use in the milk product factories. The HPLC-UV method therefore is presently the popular choice for most factories. In this method, melamine is separated on a C_8 or C_{18} column using an ion pair buffer (mixture of citric acid and sodium 1-octane sulfonate) and acetonitrile mobile phase.

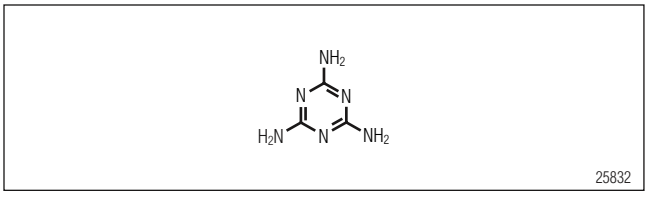

Figure 1. Structure of melamine.

In this Application Note (AN), we determined melamine in milk powder samples following the regulated method.[1] Melamine was separated from other components in the powdered milk samples using an Acclaim® 120 C18 column and an UltiMate® 3000 HPLC system with UV detection. The results of method detection limits (MDL), recovery, and permitted detection deviation match the requirements in the regulated method.

EQUIPMENT

Dionex UltiMate 3000 HPLC system consisting of:
- HPG 3400A pump
- WPS 3000TSL autosampler
- TCC-3000 thermostatted column compartment
- VWD-3400 UV-vis detector

Chromeleon® 6.80 SP5 Chromatography Data System

Kudos® SK3200LH ultrasonic generator, Kudos Ultrasonic Instrumental Co., Shanghai, China

Mettler Toledo AL-204 Electoral o balance, Mettler (Shanghai) Co., Shanghai, China

Anke® TGL-16B centrifuge, Anting Scientific Instrumental Factory, Shanghai, China

IKA® MS1 Minishaker, IKA Works, Guangzhou, China

Strata™-x-c SCX SPE (Phenomenex) column

SE-506 Nitrogen Purge Instrument, Shine Tech., Beijing, China

REAGENTS AND STANDARDS

Water, from Milli-Q® Gradient A 10

Methanol (CH_3OH), HPLC grade, Fisher

Acetonitrile (CH_3CN), HPLC grade, Fisher

Trichloroacetic acid, analytical grade, SCRC, China

Citric acid ($C_6H_8O_7 \cdot H_2O$), analytical grade, SCRC, China

Sodium 1-octane sulfonate (98%), Baker Analyzed @ HPLC Reagent, USA

Melamine (99.0%), HPLC grade, Fluka

Nitrogen (N_2, 99.999%), Lumin Gas Works, Shanghai, China

Ammonia solution (25%–28%), analytical grade, SCRC, China

CHROMATOGRAPHIC CONDITIONS

Guard Column:	Acclaim 120 C18, 5 µm, 4.3 × 10 mm, P/N 059446, with guard column holder, P/N 59526
Analytical Column:	Acclaim 120 C18, 5 µm, 4.6 × 250 mm, P/N 059149
Mobile Phase:	Buffer (dissolve 2.10 g citric acid and 2.16 g sodium 1-octanesulfonate in 980 mL H_2O, adjust pH value to 3.0 with 1 M NaOH solution, add water to the mark of 1000-mL volumetric flask)—CH_3CN (92 : 8, v/v)
Column Temp.:	40 °C
Flow Rate:	1.0 mL/min
Inj. Volume:	20 µL
UV Detection:	Absorbance at 240 nm

PREPARATION OF STANDARDS

Stock Standard Solution

Accurately weigh ~100 mg of melamine, dissolve in a 100-mL volumetric flask with aqueous methanol (50%, v/v). The concentration of melamine is 1000 µg/mL.

Working Standard Solutions

Prepare seven working standard solutions for calibration by adding defined volumes of the stock standard solution and diluting with mobile phase. The concentrations of melamine are 0.2, 0.5, 2.0, 20, 25, 50 and 100 µg/mL, respectively.

SAMPLE PREPARATION

Sample Extraction

Put an accurately weighed ~2 g of dried sample to a 50-mL centrifuge tube, and then add 15 mL aqueous trichloroacetic acid (1%, v/v) and 5 mL acetonitrile. After 1 min of vortex shaking, put in an ultrasonic bath for 30 min, and then shake for 10 min. After 10 min of centrifugation (setting = rpm ≥ 10,000), move the supernatant to a 25-mL volumetric flask while passing through filter paper, and add the 1% aqueous trichloroacetic acid to the mark.

Cleaning Sample Extract on an SCX SPE Column

Prior to use, the SPE column should be activated by passing 3 mL CH_3OH and 5 mL H_2O in turn.

Mix 5 mL of the sample extract and 5 mL water, move them to the activated SCX SPE column. Wash the SPE column with 3 mL methanol and 3 mL water, respectively, then elute with 6 mL of aminated methanol solution (mixture of 5 mL ammonia solution and 95 mL methanol). Dry the collected eluent with N_2 at 50 °C, dissolve the residue in 1-mL mobile phase, and then vortex the solution for 1 min. Prior to injection, filter the solution through a 0.2-µm filter (Millex®-HV).

Spiked Milk Powder Samples

Add 20 µL of stock standard solution of melamine to the 50-mL centrifuge tubes together with the accurately weighed ~2 g of dried sample. The remainder of the sample preparation procedure is the same as that for the milk powder sample.

RESULTS

Method Reproducibility

Figure 2 shows an overlay of chromatograms of seven melamine standards with different concentrations. The RSD for retention time is 0.143%.

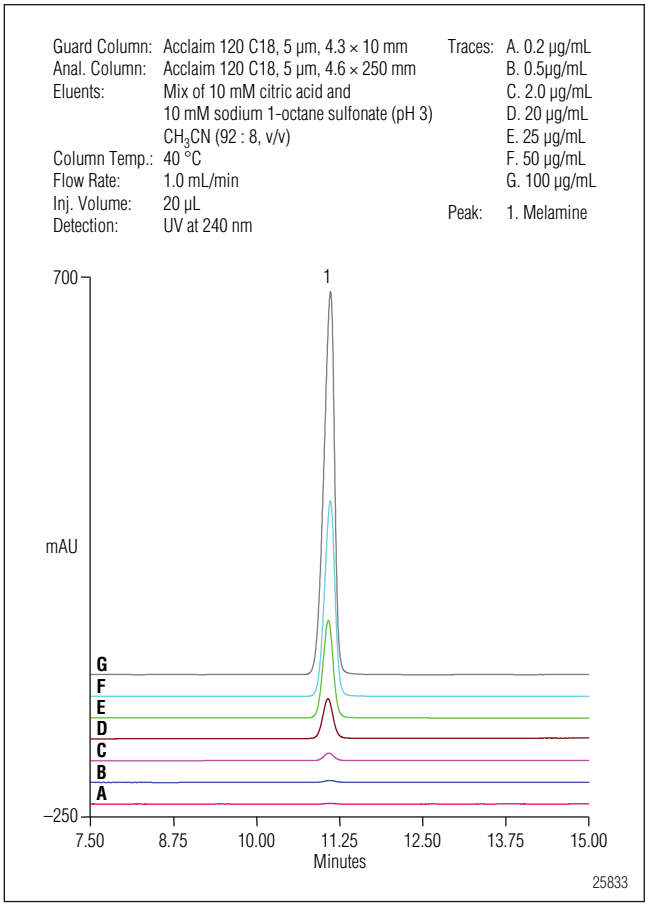

Figure 2. Overlay of chromatograms of seven melamine standards with different concentrations.

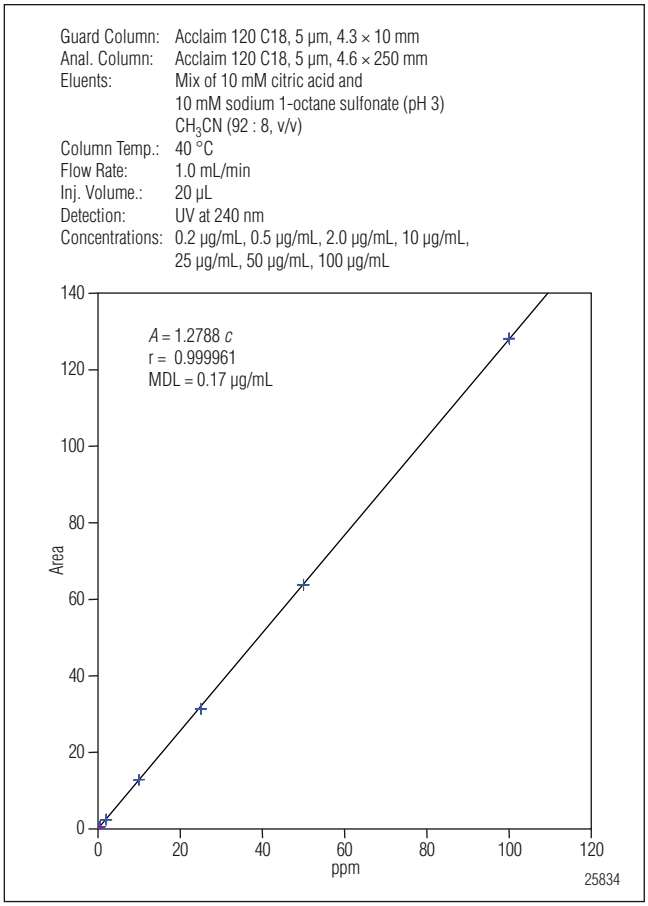

Figure 3. Calibration curve for melamine.

Linearity and Detection Limit

Calibration linearity for melamine was investigated by making three replicate injections of each standard prepared at seven different concentrations. The external standard method was used to establish the calibration curve and to quantify melamine in samples. As shown in Figure 3, excellent linearity was achieved throughout the range from 0.2 to 100 µg/mL. The linearity equation of melamine is as follows with the curve forced the through origin.

$$A = 1.2788\ c$$

Where, A stands peak area, and c stands melamine concentration (µg/mL). The correlation coefficient (r) is 0.999961.

Method detection limit (MDL) of melamine was calculated by using S/N = 3, where S = signal, N = noise. The calculated value of MDL is 0.17 µg/mL.

Sample Analysis

Five milk powder samples (1–5) obtained from a manufacturer were analyzed. Melamine was found in samples #1 through #4. An amount of melamine less than the MDL was detected (0.12 µg/mL) in sample 5 and therefore can not be reliably identified as melamine. The results are summarized in Table 1. Figure 4 is an overlay chromatograms of these samples.

Table 1. Sample Analysis Data								
Sample #	**1**				**2**	**3**	**4**	**5**
Melamine	**Detected (µg/mL)**	**Added (µg/mL)**	**Found (µg/mL)**	**Recovery (%)**	**Detected (µg/mL)**	**Detected (µg/mL)**	**Detected (µg/mL)**	**Detected (µg/mL)**
	0.83	4.0	3.2	80	0.58	0.61	3.6	0.12

Notes: 1. 3 injections were made for each sample
2. Spiked sample was prepared according to the description in the sample section.
3. The detected amount in the list is for the prepared sample (diluted sample).
4. The amount in original sample should be calculated by multiplying "Diluted fold", which is 2.5 in this Application Note.
5. Found = Measured Value of spiked sample - Measured Value of sample.

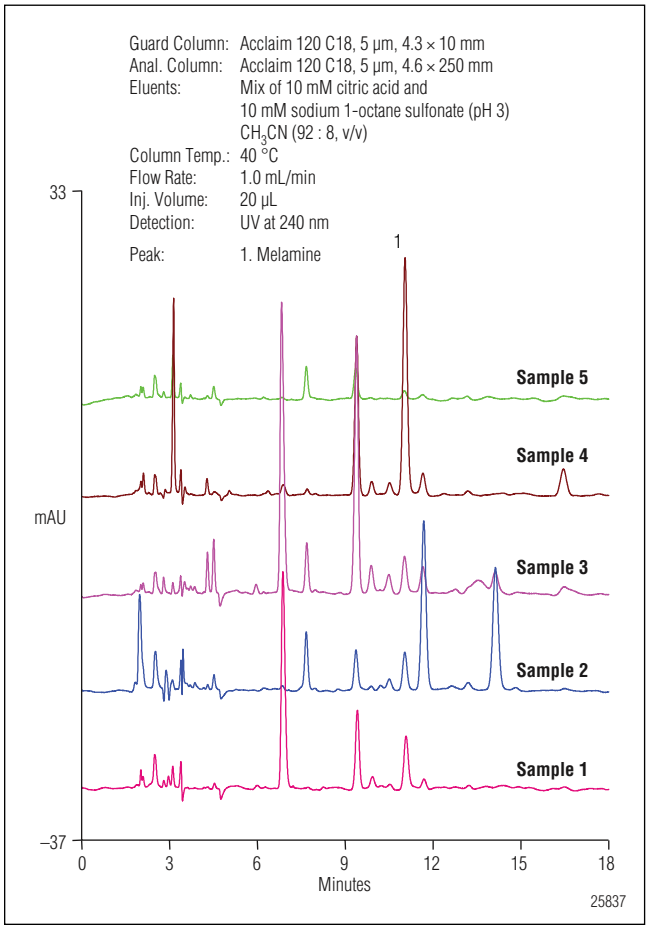

Figure 4. Chromatograms of five milk powder samples.

CONCLUSION

The determination of melamine in milk powder was performed on the Acclaim 120 C18 column and UltiMate 3000 HPLC system with UV detection following the regulated method.[1] Table 2 lists the comparison of chromatographic performance data obtained in this experiment to the requirements in the standard method, which demonstrates that the results match the requirements in the regulated method.

Table 2. Method Performance		
	Method in this Application Note	**Requirement in the Regulated Method**
Correlation coefficient (r)	0.999961	\
Linearity range (µg/mL)	0.2 ~ 100	0.8 ~ 80
Recovery (%)	80	80 ~ 110

Note: Regulated method, see Ref. 1.
\ Not specified.

REFERENCE

1. Determination of Melamine in Raw Milk and Dairy Products, *GB/T* 22388 **2008**.

Determination of Melamine in Milk by Ion Chromatography with UV Detection

INTRODUCTION

In 2008, melamine was found as a contaminant of milk and milk-containing products after the discovery of melamine contamination of pet food. These contaminations resulted in infant and pet deaths. Both deliberate contaminations originated in China, with some contaminated products exported to neighboring and more distant countries. Melamine was added to both products to increase their apparent protein content, as it was determined by a nonspecific total nitrogen test and melamine has a large amount of nitrogen per unit mass.

Dionex has designed two reversed-phase HPLC methods to determine the melamine adulteration of liquid and powdered milk.[1, 2] One method is an ion-pairing HPLC method using an Acclaim®120 C18 column and the other method uses the Acclaim Mixed-Mode WCX column, where both the hydrophobic and cationic properties of melamine are used to affect the separation. As a cation, melamine can be separated by cation-exchange chromatography and therefore be determined by Ion Chromatography (IC). This Application Note (AN) shows how melamine can be determined in milk, powdered milk, and a milk-containing candy by IC using an IonPac® CS17 column and UV detection at 240 nm. This gives the analyst another chromatographic option, providing a selectivity that may be needed for some samples, or a way to increase sample throughput with existing laboratory instrumentation.

EQUIPMENT

Dionex ICS-3000 consisting of:

- DP Dual Pump
- DC Detector/Chromatography module with dual temperature zone equipped with
- 6-port valve (injection valve)
- AM Automation Manager equipped with,
- 10-port valve (high pressure valve)
- EG Eluent Generator
- AS Autosampler
- PDA-3000 Photodiode Array Detector*

Chromeleon® 6.8 Chromatography Data System

* The Dionex VWD detector can also be used for this application. The photodiode array detector is required to confirm the melamine peak identity with the peak purity option.

Conditions

Column:	IonPac CS17 Analytical, 4 × 250 mm (P/N 060557)
Guard:	IonPac CG17 Guard, 4 × 50 mm (P/N 060560)
Concentrator:	IonPac TCC-LP1, 4 × 35 mm (P/N 046027)
Eluent Source:	EGC II MSA (P/N 058902) with CR-CTC (P/N 066262)
Gradient:	See chromatogram
Flow Rate:	Pump 1: 1.0 mL/min Pump 2: 1.0 mL/min
Inj. Volume:	See chromatogram
Pressure:	~2100 psi
Detection:	UV at 240 nm

REAGENT AND STANDARDS

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

Melamine (Sigma-Aldrich)

Acetic acid (Labscan)

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solution

The eluent generator produces the eluent using the EluGen EGC II MSA cartridge and deionized water supplied by the pump, with the eluent concentration controlled by the Chromeleon software. Backpressure tubing must be added to achieve 2000–2500 psi backpressure that will allow the EG degasser to function properly. See the *ICS-3000 Ion Chromatography System Operator's Manual,* (P/N 065031-03) for instructions on adding backpressure.[3]

Standard Solutions

Stock Standard Solutions (1000 mg/L)

To prepare the 1000 mg/L melamine standard, dissolve 0.1 g of melamine in 100 mL of deionized water.

Secondary Standards

Prepare a 1 mg/L secondary standard from the stock standard. From this secondary standard, prepare the standard calibrations and MDL standards as follows.

1. Calibration standards

To prepare melamine standards at concentrations of 25, 50, 100, 200, 400, and 800 µg/L, add the appropriate volumes of 1 mg/mL standard to separate 100 mL volumetric flasks. For example, add 5.0 mL of 1 mg/L standard for the 50 µg/L standard. Bring to volume with deionized water. These six standards were used to calibrate one of the IC methods presented here, and for the second method, we prepared an additional 12.5 µg/L standard and calibrated with seven standards.

2. MDL standard

Prepare a 25 µg/L melamine standard by adding 2.5 mL of 1 mg/L melamine standard to a 100 mL volumetric flask and bring to volume with deionized water.

SAMPLE PREPARATION

OnGuard RP Preparation

Flush the OnGuard RP cartridge with 5 mL of methanol and then with 10 mL of deionized water at about 4 mL/min.

Liquid Milk Preparation

1. Mix 10 mL of liquid milk and 8 mL of deionized water.
2. Add 2 mL of 3% acetic acid and mix.
3. Pass the sample through a Whatman 2V filter.
4. Pass 5 mL of the filtered sample through a prepared OnGuard RP cartridge, discarding the first 3 mL and collecting the remaining sample into an AS vial.

Milk Powder and Candy Sample Preparation

Add about 5 g of sample to a 50 mL volumetric flask, dissolve, and bring to volume with deionized water. Take 10 mL of this sample and prepare by the same method as the liquid milk sample.

Samples Spiked with Melamine

To prepare a 50 µg/L melamine spiked sample, in step 1 of the sample preparation instead of adding 8 mL of deionized water to the sample, add 1 mL of 1 mg/L melamine secondary standard and 7 mL of deionized water. For the 100 µg/L standard, use 2 mL of 1 mg/L melamine and 6 mL deionized water.

Calculating Amount of Melamine in the Milk-Containing Candy Sample

The sample preparation involved dissolving 5.079 g of candy in 50 mL DI water and diluted 1:1.

Amount of melamine in 100 µL of the prepared candy sample:

$= (13.78\ \mu g/L) \times (L/1{,}000{,}000\ \mu L) \times 100\ \mu L$
$= 13.78 \times 10^{-4}\ \mu g$

Amount of candy in the 100 µL injection:
$5.079 g/50\ mL \times ((mL/1000\ \mu L)/2) \times 100\ \mu L$
$= 5.079 \times 10^{-3}\ g$

Amount of melamine per g of candy:
$= 13.78 \times 10^{-4}\ \mu g / 5.079 \times 10^{-3}\ g$
$= 0.27\ \mu g/g$

RESULTS AND DISCUSSION

Melamine is a cation and, therefore, can be separated from other compounds by cation-exchange chromatography. The IonPac CS17 column was designed for the separation of hydrophobic amines like melamine. While melamine is a cation at neutral pH, it is not fully ionized at pH 7 and therefore, suppressed conductivity does not provide a sensitive detection method for this compound. Sensitivity can be increased using the salt converter cation self-regenerating suppressor. However, more sensitivity and selectivity for melamine was found by using absorbance detection at 240 nm.

Milk and milk-based products can be difficult for chromatographic methods due to the large variety of compounds present that can interfere with the analytes of interest. To determine melamine, we used a sample preparation technique first developed for the IC determination of iodide in milk.[4] Despite this sample preparation, we found it difficult to determine melamine in the sample. Therefore, we first loaded the sample loop installed on the AM-HP1 with the prepared sample. Then, using deionized water, we moved the sample onto the cation-exchange concentrator installed on the injection valve, and then eluted from the concentrator, directly onto the IonPac CS17 column set. Figure 1 shows the schematic of this system configuration and Table 1 shows the valve programming that allows the sample delivery to the concentrator while washing unbound compounds to waste, and subsequent chromatography. This method ultimately proved more successful for melamine determination.

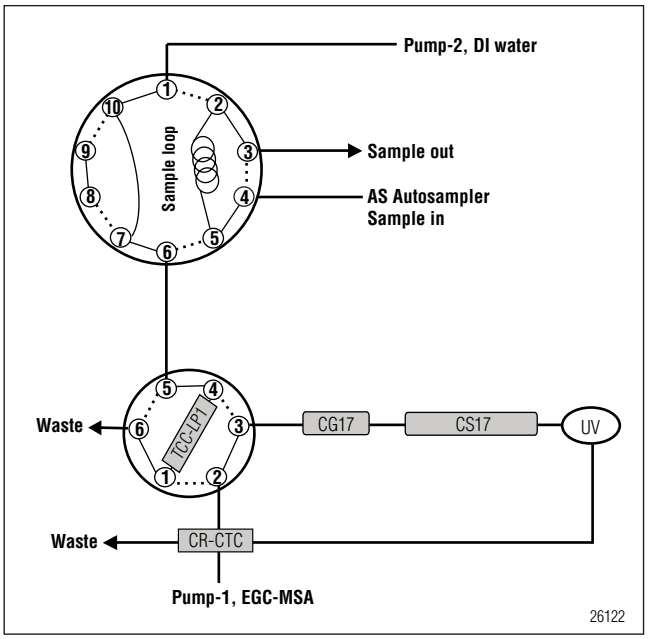

Figure 1. System configuration schematic.

Table 1. Valve Switching Program

Retention Time (min)	AM_HP1	Injection Valve	Note
–5.0	A	Load	
–3.0	B	Load	AS loads the sample to sample loop before the AM_HP1 switches from A to B.
0.0	A	Inject	
End run	A	Inject	The end of runtime depends on the gradient.

Prior to quantitative sample analysis, we calibrated the method as described in the section *Secondary Standards,* earlier in this application note. Figure 2 shows the chromatography from the calibration, which was linear with a correlation coefficient of 0.9998. To estimate the minimum detection limit, we made seven injections of the 25 µg/L standard. Figure 3 shows the seven injections along with the blank, an injection of water. The blank shows that there are no peaks from the water or chromatography system interfering with melamine determination. Table 2 shows the data from the MDL experiment and that the MDL estimate was 4.4 µg/L.

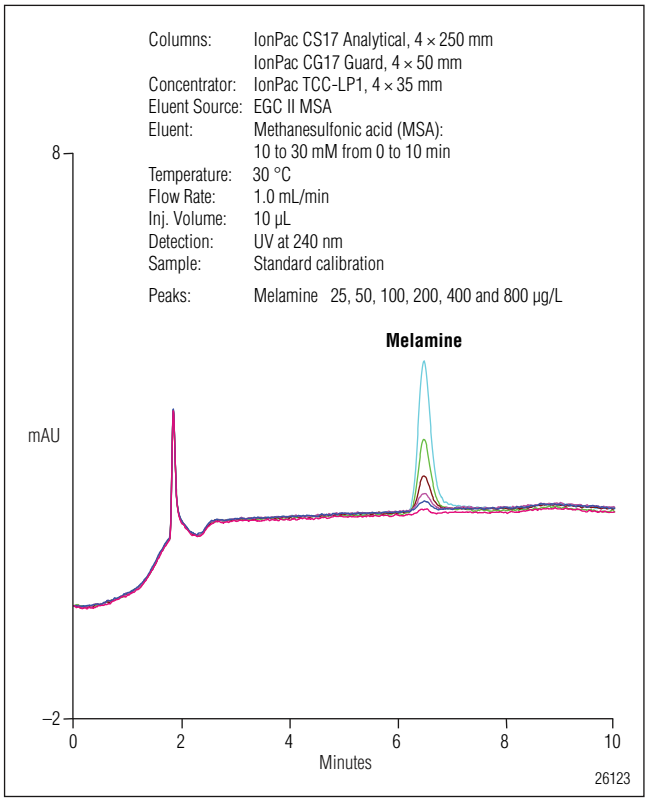

Figure 2 Chromatograms of six melamine standards used for calibration.

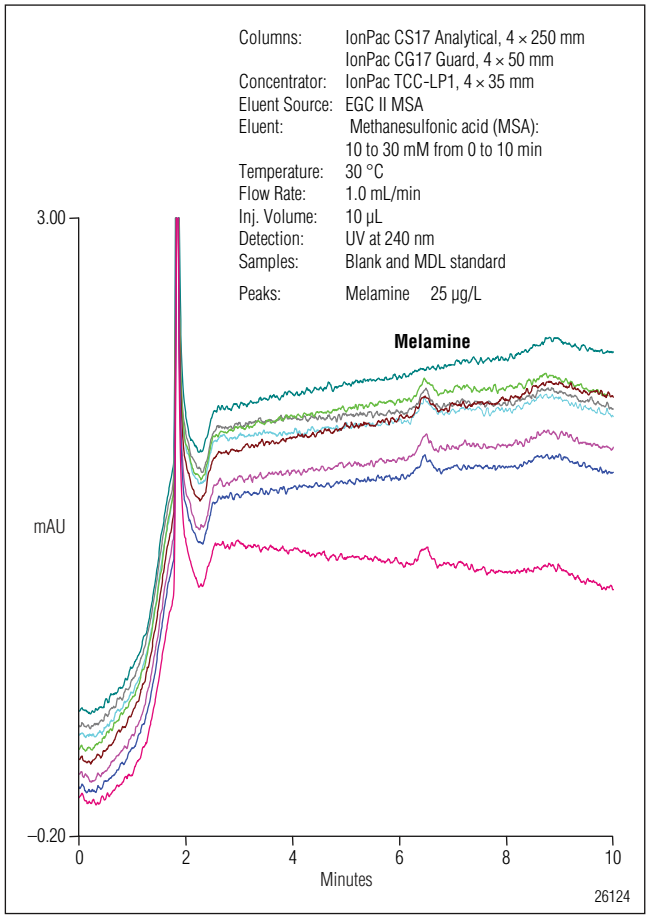

Figure 3. Chromatograms of a water injection (blank) and seven consecutive injections of 25 µg/L melamine.

After method qualification, we evaluated the melamine content of milk, milk powder, and a milk-containing candy. Only the candy was known to contain melamine. Figures 4 and 5 together with Tables 3 and 4 show the results of the determinations of melamine in milk and milk powder. The analysis shows that neither sample contained melamine.

To demonstrate that melamine was not lost during sample preparation, melamine was added to each sample prior to sample preparation. One portion of each sample was spiked with 50 µg/L melamine and a second portion was spiked with 100 µg/L melamine. The chromatography in Figures 4 and 5 and the quantitative results in Tables 3 and 4 show that melamine was recovered from both samples with recoveries greater than 90%.

Table 2. Data from Seven Consecutive Injections of 25 µg/L Melamine

Injection No.	Height (mAU)
1	0.0906
2	0.1071
3	0.0959
4	0.0924
5	0.0948
6	0.0969
7	0.0999
Average:	0.0968
RSD:	5.63
MDL (µg/L):	4.4

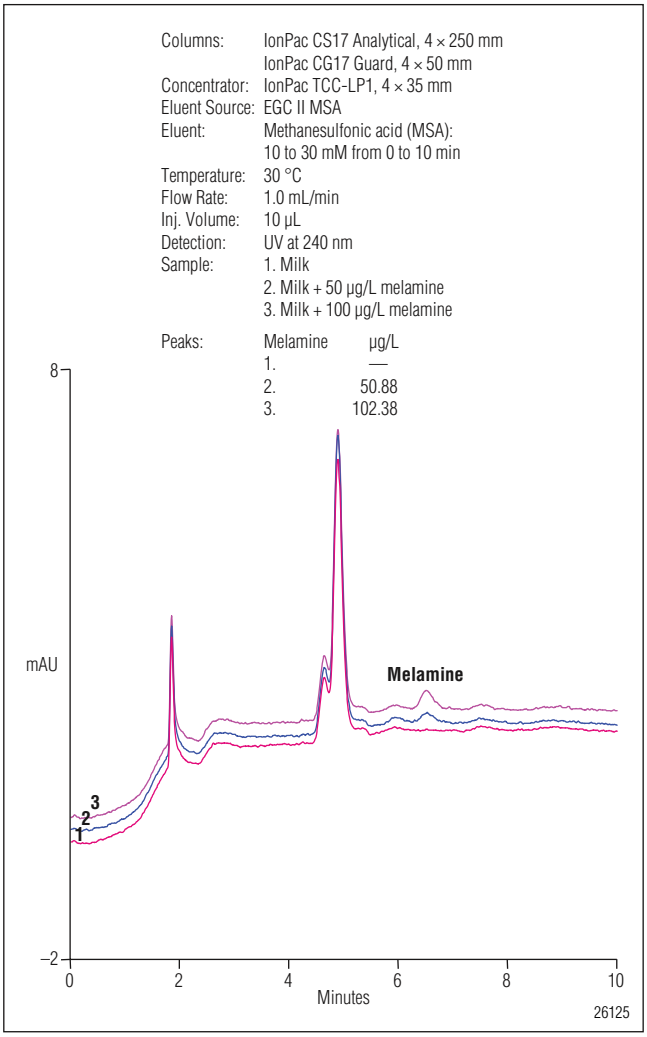

Figure 4. Chromatograms of milk and milk spiked with melamine. Milk (1); milk + 50 µg/L melamine (2) ; and milk + 100 µg/L melamine (3).

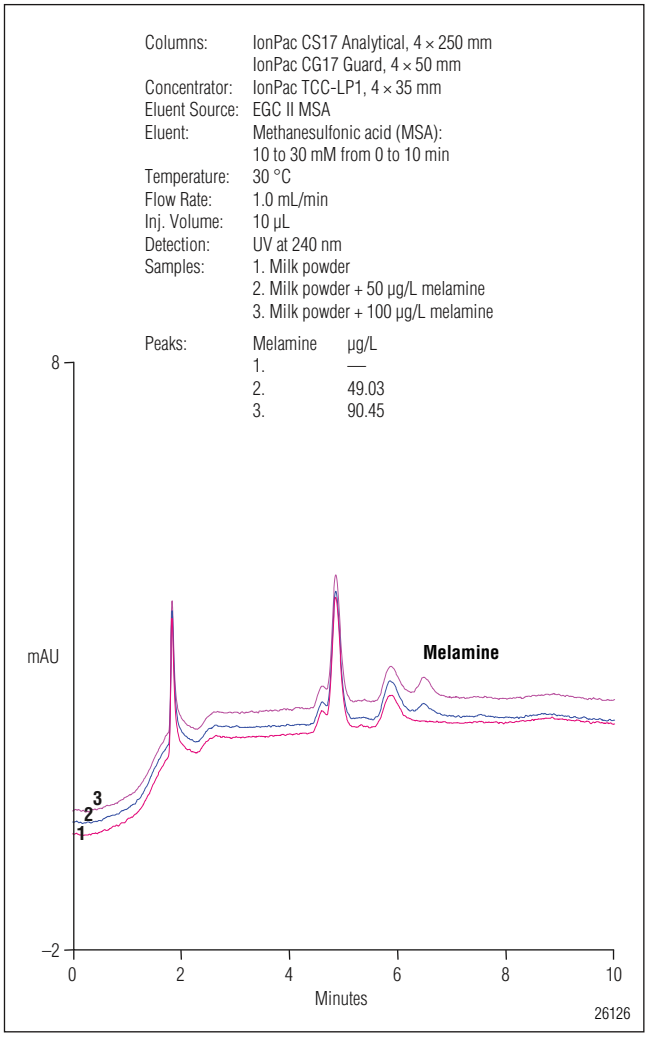

Figure 5. Chromatograms of milk powder and milk powder spiked with melamine. Milk (1); milk + 50 µg/L melamine (2); and milk + 100 µg/L melamine (3).

Table 3. Recovery of Melamine in the Milk Sample

Injection #	Amount (µg/L)		
	Liquid Milk	Liquid Milk + 50 µg/L Melamine	Liquid Milk + 100 µg/L Melamine
1	ND	53.58	96.20
2	ND	53.12	110.31
3	ND	44.42	93.54
4	ND	52.00	100.33
5	ND	51.27	111.51
Average:	NA	50.88	102.38
RSD:	NA	7.32	7.98
% Recovery:	NA	101.8	102.4

Table 4. Recovery of Melamine in the Milk Powder Sample

Injection #	Amount (µg/L)		
	Milk Powder	Milk Powder + 50 µg/L Melamine	Milk Powder + 100 µg/L Melamine
1	ND	49.56	92.30
2	ND	56.27	89.74
3	ND	45.07	88.04
4	ND	49.16	88.74
5	ND	45.07	93.44
Average:	NA	49.03	90.45
RSD:	NA	9.35	2.57
% Recovery:	NA	98.06	90.45

The analysis of the melamine-containing candy sample proved more difficult. Melamine was not completely resolved from another peak. This was not observed in the milk and milk powder samples. To resolve these two peaks, we changed the mobile phase composition from a 10 min 10–30 mM MSA gradient to 5 mM MSA for 20 min. Due to the use of a RFIC system, this mobile phase change and other changes made to arrive at the final method did not require the preparation of new eluents. We simply used the Chromeleon chromatography workstation to instruct the eluent generator to prepare a new mobile phase.

Our initial chromatography of the candy sample also suggested that there was only a small amount of melamine in the sample. Therefore, when we calibrated the system for the new separation method, we added a lower concentration standard (12.5 µg/L) to the calibration and increased the injection volume from 10 to 100 µL. The calibration was linear with a correlation coefficient of 0.9997.

Figure 6 and Table 5 show the results of the analysis of the candy sample for melamine. The candy sample contained melamine with a concentration of about 14 µg/L in the prepared sample, or 0.27 µg/g in the candy. To assess the accuracy of this determination, we prepared two spiked candy samples with (a) a 10 µg/L spike, and (b) a 20 µg/L spike. Melamine was recovered from both samples suggesting that the method is accurate. After installing the photodiode array detector on our system, we also confirmed that the melamine peak in the candy sample was a spectral match to the melamine standard.

This IC method accurately determined melamine in milk, milk powder, and a milk-containing candy after a simple sample preparation. As this method uses a RFIC system, the analyst does not have to prepare eluents and can easily change the mobile phase for samples where unknown peaks coelute with melamine.

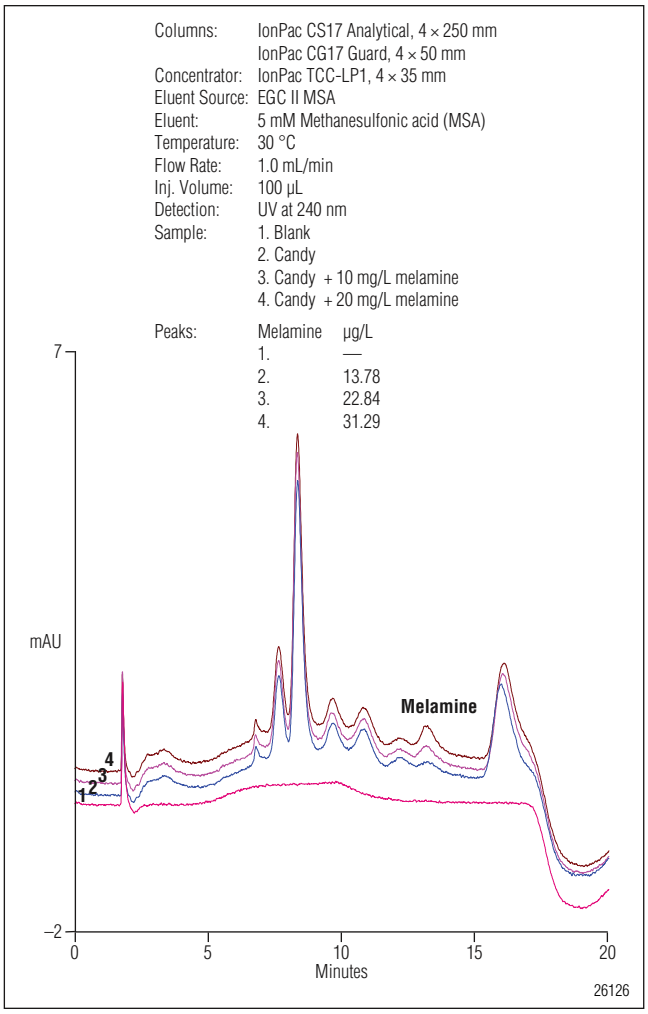

Figure 6. Chromatograms of milk-containing candy and candy spiked with melamine. Blank (1); candy (2); candy + 10 µg/L melamine (3); and candy + 20 µg/L melamine (4).

Table 5. Recovery of Melamine in the Candy Sample

Injection #	Amount (µg/L)		
	Candy	Candy + 10 µg/L Melamine	Candy + 20 µg/L Melamine
1	14.27	23.16	28.86
2	15.31	25.46	31.47
3	12.70	21.96	31.23
4	12.49	21.59	32.56
5	14.10	22.05	32.32
Average:	13.78	22.84	31.29
RSD:	8.51	6.91	4.69
% Recovery:		90.66	87.55

REFERENCES

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2. *Determination of Melamine in Milk Powder by Reversed-Phase HPLC with UV Detection.* Application Note 224 (LPN 2184, March 2009), Dionex Corporation, Sunnyvale, CA.
3. *ICS-3000 Ion Chromatography System Operator's Manual,* Document No. 065031-03. Dionex Corporation, Sunnyvale, CA
4. *Determination of Iodide in Milk Products.* Application Note 37 (LPN 0702-03, October, 2004), Dionex Corporation, Sunnyvale, CA.

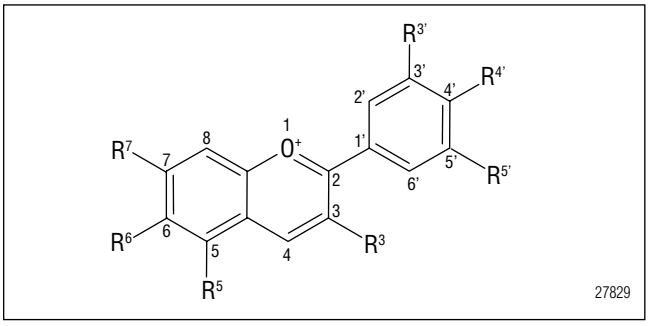
Fast Determination of Anthocyanins in Pomegranate Juice

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.

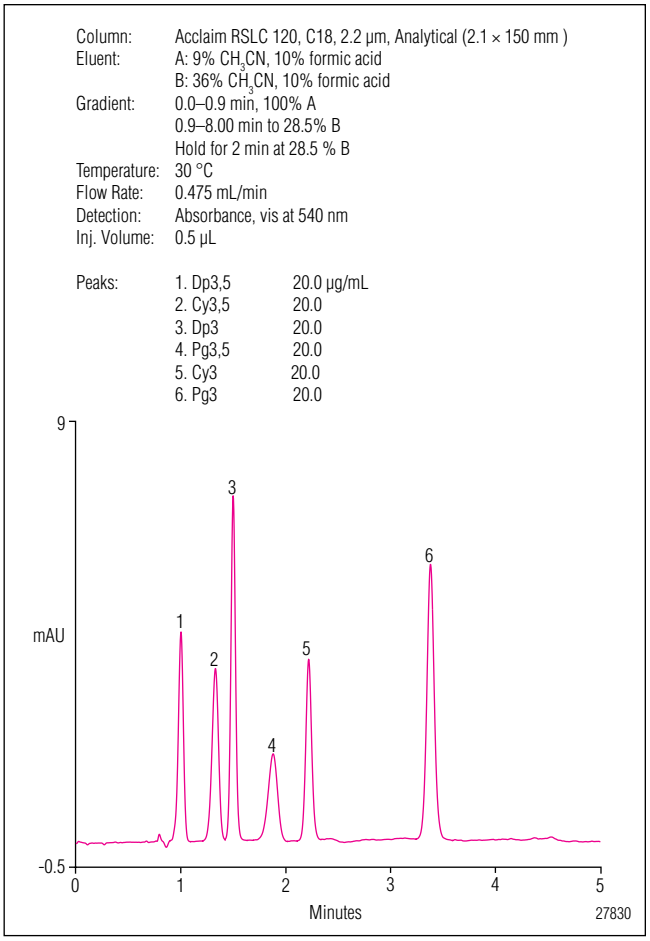


Figure 2. Separation of a mixed anthocyanin standard using the Acclaim RSLC 120 C18 column.

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

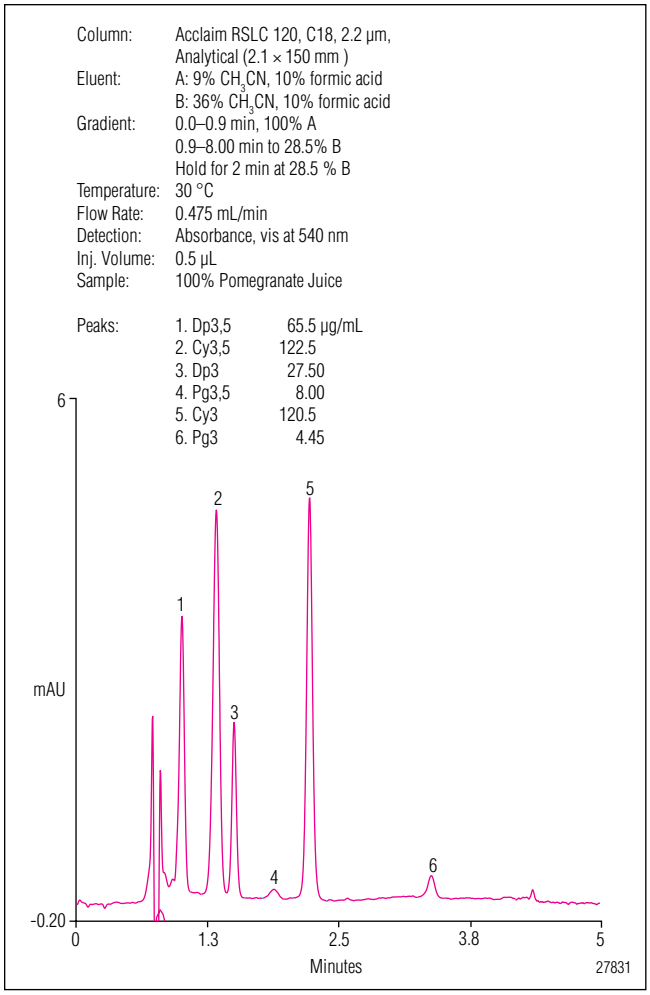


Figure 3. Separation of anthocyanins in a pomegranate juice sample.

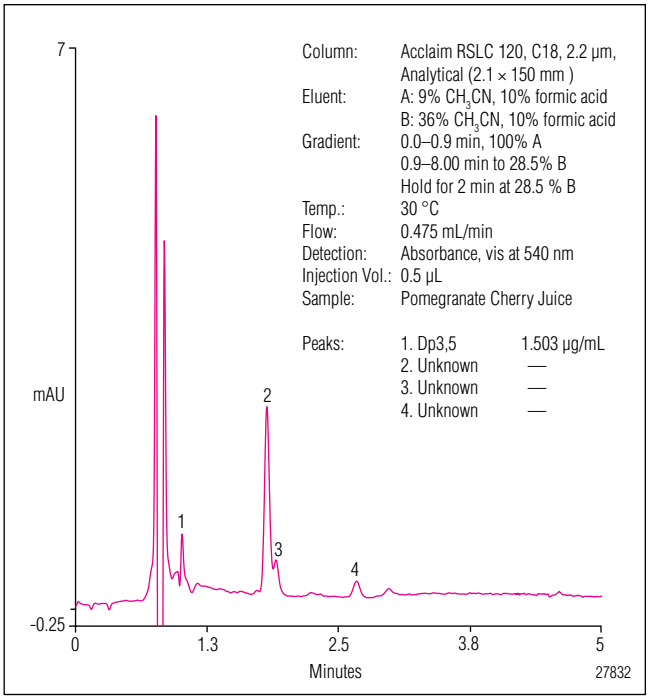


Figure 4a. Determination of anthocyanins in pomegranate cherry juice.

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.

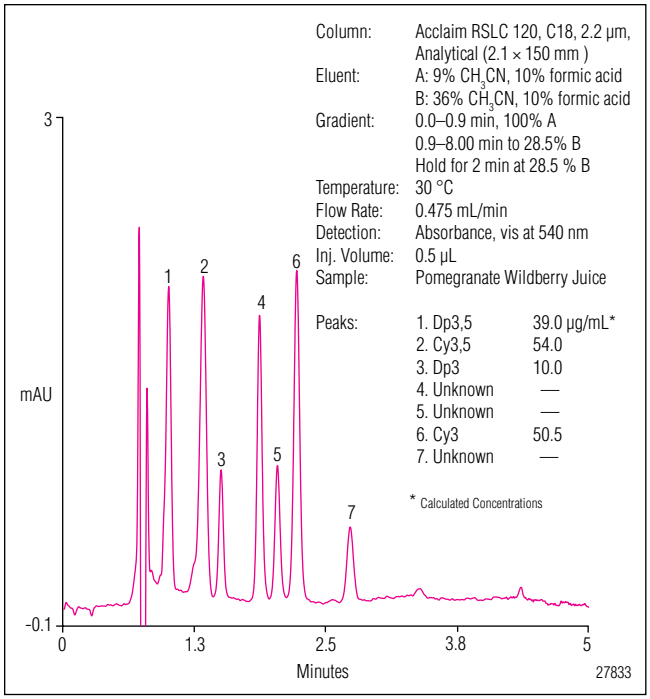


Figure 4b. Determination of anthocyanins in pomegranate wildberry juice.

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.

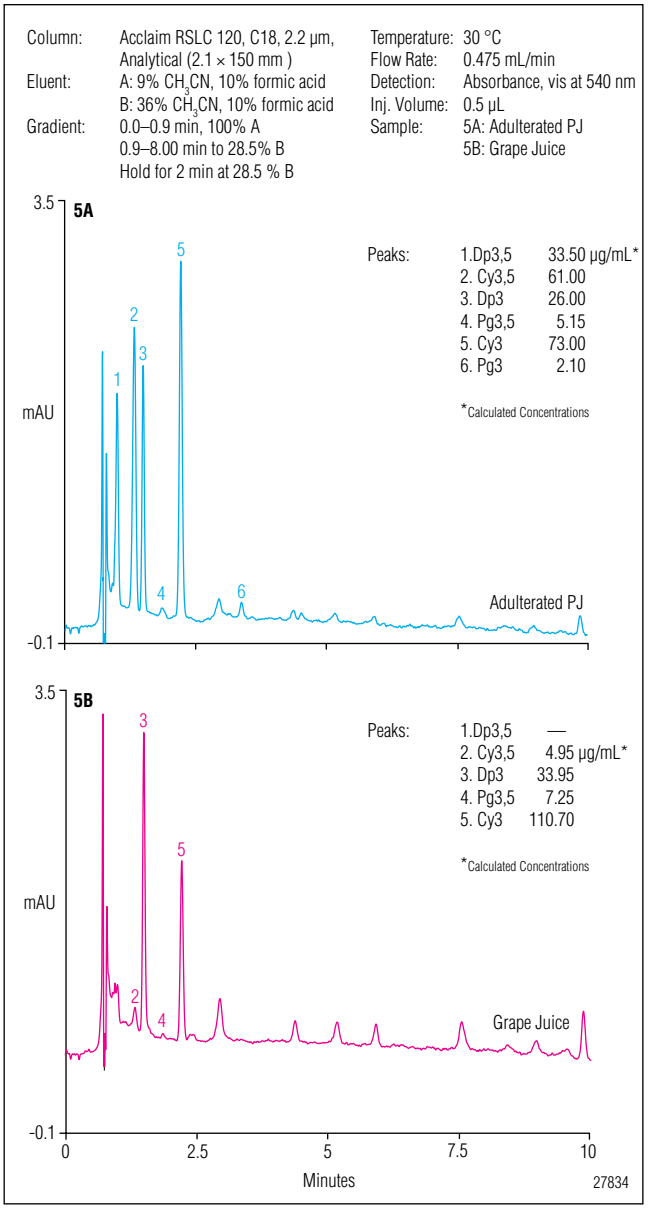


Figure 5. Separation of anthocyanins in simulated adulterated pomegranate juice (5A) overlayed with a separation of anthocyanins in grape juice (5B).

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.

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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	Hydro-phobicity
IonPac ICE-AS1	4 × 250 mm 9 × 250 mm	Heptafluorobutyric acid	Organic acids in high ionic strength matrices. Fast separation of organic acids.	7.5 µm	8%	-	-	5.3 µeq 27 µeq	Sulfonic acid	Ultra Low
IonPac ICE-AS6	9 × 250 mm	Heptafluorobutyric acid	Organic acids in complex or high ionic strength matrices.	8 µm	8%	-	-	27 µeq	Sulfonic and carboxylic acid	Moderate
IonPac ICE-Borate	9 × 250 mm	MSA/ Mannitol	Trace concentrations of borate	7.5 µm	8%	-	-	27 µeq	Sulfonic acid	Ultra Low

Acclaim General and Specialty Columns

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

Bio Columns

Protein

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

Carbohydrate

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

DNA

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

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