Beverages Applications Notebook

Coffee and Tea

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Introduction to Beverages

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

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

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

Thermo Scientific and Dionex Integrated Systems

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

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

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

UltiMate 3000 UHPLC+ Systems

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

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

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

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

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

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

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

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

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

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

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

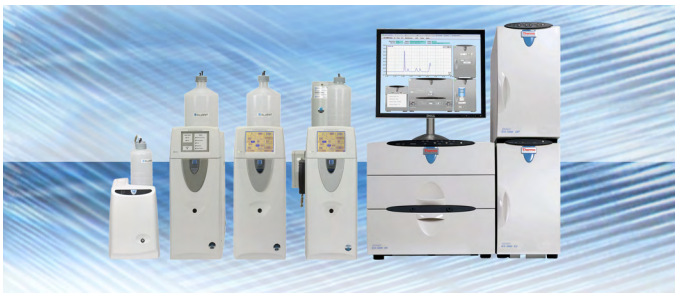
Dionex ICS-5000: Developed with flexibility, modularity, and ease-of-use in mind, the Dionex ICS-5000 combines the highest sensitivity with convenience

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

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

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

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



MS Instruments

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

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

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

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

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

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Chromeleon 7 Chromatography Data System Software

The fastest way to get from samples to results

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

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

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

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

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

Process Analytical Systems and Software

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

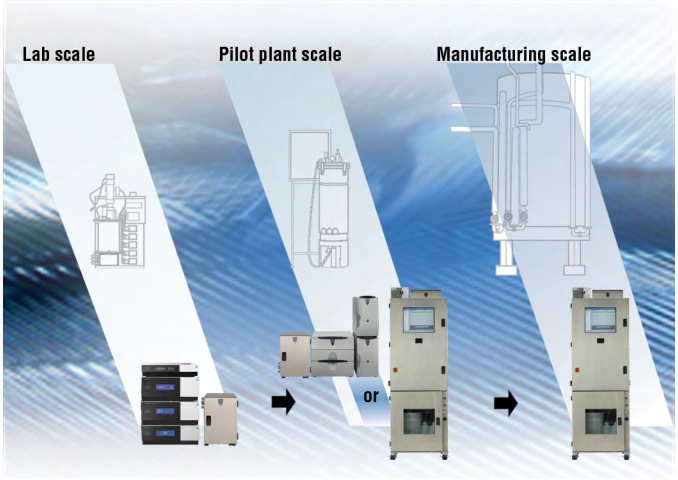
Our process analytical systems provide timely results by moving liquid chromatography-based measurements on-line. Information from the Thermo Scientific Dionex Integral process analyzer can help reduce process variability, improve efficiency, and reduce downtime. These systems provide comprehensive,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

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

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

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

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



Automated Sample Preparation

ACCELERATED SOLVENT EXTRACTORS

Two new solvent extraction systems with pH-hardened Dionium components

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

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SOLID-PHASE EXTRACTION SYSTEMS

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

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

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

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

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

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

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Analysis of Coffee and Tea

Carbohydrate in Coffee: AOAC Method 995.13 vs a New Fast Ion Chromatography Method

INTRODUCTION

Brewed coffee has emerged as one of the most consumed beverages in the world.[1] In addition, green coffee (unroasted beans) is one of the most traded agricultural commodities in the world.[2] Coffee is grown in over 70 countries, primarily in Latin America, Southeast Asia, and Africa. As of 2010, coffee production was approximately 134 million bags (each bag containing 60 kg), with Brazil and Columbia contributing to nearly 40% of the total.[3] The top coffee importing countries are the United States, Germany, Japan, France, Italy, Spain, Canada, and the United Kingdom.

In recent years, there has been growing interest in the physiology and biochemistry of green coffee beans and their role in the final roasted coffee quality. Drinks made from green coffee beans have been introduced in the market.[4] Green and roasted coffee are tested at several stages of its production and processing. Tests conducted on green coffee beans include tests for bean density, brightness, acidity, pH, moisture content, and total soluble solids. Tests performed on roasted coffee include tests for caffeine, chlorogenic acids, lipids, carbohydrates, total polyphenols, total proteins, and aflatoxins.

Coffee carbohydrates constitute the major part (at least 50% of the dry weight) of raw coffee beans. The carbohydrates in coffee contribute to the flavor of the beverage as they undergo complex changes (react with amino acids, i.e., the Maillard reaction) during the roasting process. They act as aroma binders, foam stabilizers, and also impart viscosity to the coffee beverage. Carbohydrates are also good tracers for assessing the authenticity of soluble (instant) coffee.[5]

Currently, the Association of Analytical Chemists (AOAC) official method 995.13[6]—which is based on high-performance anion-exchange (HPAE) chromatography with pulsed amperometric detection (PAD)—is used for determining the free and total carbohydrates in instant coffee. This method is also used by the British Standards Institution for testing coffee and coffee products.[7]

This study first tested the AOAC official method 995.13 on a Dionex ICS-3000 system. Carbohydrates in extracts from instant coffee and green coffee beans were separated on a CarboPac® PA1 column, and measured by electrochemical detection with disposable Au on polytetrafluoroethylene (PTFE) working electrodes. A few proposed modifications of the official method achieved separation of two pairs of sugars, which are otherwise difficult to resolve.

A fast method using the CarboPac SA10 column (with electrolytically generated eluent) was then tested for determining the common coffee carbohydrates.

This column has been shown to achieve fast, high resolution separation of mono- and disaccharides commonly found in food samples.[8] The CarboPac SA10 column is composed of a wide-pore macroporous substrate coated with a strong anion-exchange latex of nano-beads. The combination of the high capacity provided by the substrate and the new internal chemistry of the nano-bead functionality delivers high resolution and short analysis time for the common sugars of interest in food and beverages.

The testing here demonstrates the linearity, precision, and recovery of common coffee carbohydrates in samples ranging from instant coffee to green coffee beans. It compares the two methods and discusses their respective advantages and disadvantages. Note that the disposable electrodes used in these methods provide short equilibration times and greater electrode-to-electrode reproducibility compared to conventional electrodes. Additionally, compared to other disposable Au electrodes, the Au on PTFE electrodes have longer lifetimes and can operate at higher hydroxide concentrations.

Both the described methods provide good sensitivity, consistent response, and can be routinely used for sugar analysis in coffee applications. The fast method is recommended when rapid separation is desired, keeping in mind that two pairs of sugars (namely, rhamnose–galactose and fructose–ribose) are not resolved. In applications where all 11 common coffee carbohydrates need to be resolved, the AOAC official method 995.13 (with minor modifications) is recommended.

EQUIPMENT

Dionex ICS-5000 or ICS-3000 Ion Chromatography system including:

- Gradient or Isocratic Pump, with the vacuum degas option installed
- DC Detector/Chromatography Module
- Injection loop, 10 µL (for method based on AOAC official method 995.13)/injection valve with an internal 0.4 µL injection loop (P/N 074699) (for fast method)
- Electrochemical Detector (P/N 079830)
- Carbohydrate PTFE Disposable Au Working Electrodes (P/N 066480, package of 6)
- Ag/AgCl Reference Electrode (P/N 061879)
- PTFE gaskets, 2 mil (P/N 060141) or 15 mil (P/N 057364)
- Postcolumn Delivery Set with 125 µL reaction coil (P/N 53640)
- On Guard II Ag/H (P/N 057086), On Guard II RP (P/N 057083)

AS Autosampler

Chromeleon® Chromatography Data System (CDS) software

Eluent Organizer, including 2 L plastic bottles and pressure regulator

Polypropylene injection vials with caps (0.3 mL vial kit, P/N 055428)

Nalgene® 125 mL HDPE narrow-mouth bottles (VWR P/N 16057-062)

Nalgene 250 mL HDPE narrow-mouth bottles (VWR P/N 16057-109)

Nalgene 250 mL 0.2 µm nylon filter units (VWR P/N 28199-371)

Nalgene 1000 mL 0.2 µm nylon filter units (VWR P/N 28198-514)

REAGENTS AND STANDARDS

Reagents

Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better, filtered through a 0.2 µm filter immediately before use

Standards

Fucose (Sigma Cat. No. F2252)

Galactose (Sigma-Aldrich Cat. No. G-0625)

Mannose (Sigma-Aldrich Cat. No. M-6020)

Fructose (J.T. Baker Cat. No. M556-05)

Xylose (Sigma-Aldrich Cat. No. X107-5)

Sucrose (Sigma Cat. No. S-9378)

Glucose, monohydrate (J.T. Baker Cat. No. 1910-01)

Arabinose (Sigma-Aldrich Cat. No. A-3131)

Ribose (Sigma Cat. No. R7500)

Rhamnose (Sigma Cat. No. 3875)

Mannitol (Sigma-Aldrich Cat. No. M-9546)

CONDITIONS

Modified AOAC Official Method 995.13

Columns: CarboPac PA1 Analytical, 4 × 250 mm (P/N 035391)
CarboPac PA1 Guard, 4 × 50 mm (P/N 43096)

Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL (full loop)
Column Temp.:	25 ºC
Detector Temp.:	30 ºC
Back Pressure:	2400 psi
Eluent:	DI water from 0–50 min, 300 mM NaOH from 50–65 min DI water from 65–80 min (re-equilibration)
Postcolumn Base:	300 mM NaOH
Flow Rate for Postcolumn Base:	0.6 mL/min

Fast Method

Columns:	CarboPac SA10 Analytical, 4 × 250 mm (P/N 074641) CarboPac SA10 Guard, 4 × 50 mm (P/N 074902)
Flow Rate:	1.5 mL/min
Injection Volume:	0.4 µL (full loop)
Column Temp.:	45 ºC
Detector Temp.:	30 ºC
Back Pressure:	2500 psi
Eluent:	1 mM KOH
Eluent Source:	EGC II KOH with CR-ATC

Both

Detection:	PAD
Background:	30–70 nC
Noise:	30–60 pC
Working Electrode:	Carbohydrate PTFE Disposable Au Working Electrodes
Reference Electrode:	Ag/AgCl mode

Carbohydrate Waveform

Time (s)	*Potential (V)*	*Integration*
0.00	+0.1	
0.20	+0.1	Begin
0.40	+0.1	End
0.41	–2.0	
0.42	–2.0	
0.43	+0.6	
0.44	–0.1	
0.50	–0.1	

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solutions

Modified AOAC Official Method 995.13

Sodium Hydroxide, 1 M

It is essential to use high-quality water of high resistivity (18 MΩ-cm) containing as little dissolved carbon dioxide as possible. Biological contamination must be absent. Obtain source water using a water purification system consisting of filters manufactured without electrochemically active substances (e.g., glycerol). Filter prior to use through 0.2 µm porosity nylon under vacuum to remove particulates and reduce dissolved air. It is important to minimize contamination by carbonate, a divalent anion at high pH that binds strongly to the column, causing a loss of chromatographic resolution and efficiency. Commercially available sodium hydroxide pellets are covered with a thin layer of sodium carbonate and must not be used. A 50% (w/w) sodium hydroxide is much lower in carbonate and is the recommended source for sodium hydroxide.

Dilute 51.5 mL of a 50% (w/w) sodium hydroxide into 948.5 mL of thoroughly degassed water to yield a 1 M sodium hydroxide solution. Keep the eluents blanketed under 34–55 kPa (5–8 psi) of nitrogen at all times to reduce diffusion of atmospheric carbon dioxide and minimize microbial contamination.[9]

Fast Method

Potassium Hydroxide, 1 mM

Generate the potassium hydroxide (KOH) eluent online by pumping high-quality degassed deionized (DI) water through the EGC II KOH cartridge. Chromeleon software tracks the amount of KOH used and calculates the remaining lifetime. Although eluents can be prepared manually, if needed, Dionex strongly recommends running this application with eluents prepared by an eluent generator and does not recommend using manually prepared eluents. Consistent preparation of a 1 mM hydroxide eluent or a 10 mM hydroxide eluent (if proportioning is used) is difficult due to variable carbonate contamination. The impact of carbonate contamination is significant when using low-concentration hydroxide eluents. If eluents must be prepared manually, use NaOH rather than KOH and prepare according to the general instructions for hydroxide eluents in Dionex Technical Note 71.[9]

For this application, electrolytic eluent generation delivers superior performance and is used for all the fast method data in this study. Performance for this application with manually prepared eluents is not guaranteed.

Stock Standard Solution

Dissolve solid standards in DI water to prepare a 200 mg/mL stock solution for each of the 11 carbohydrates. Maintain the stock solution at –20 ºC until needed.

Mixed Carbohydrate Working Standard Solutions

Prepare the mixed carbohydrate working standards by diluting the stock solutions as required. Store working standards at 4 ºC. Make all dilutions gravimetrically to ensure high accuracy.

SAMPLE PREPARATION

Instant Coffee

Use soluble coffee without grinding or homogenization.

Free Carbohydrates

Weigh 300 mg of instant coffee to the nearest 0.1 mg into a 100 mL volumetric flask. Add 70 mL of DI water and shake the flask until dissolution is complete. Dilute the solution to volume with DI water. Filter 5–10 mL of solution through a C18 cartridge. Discard the first 1 mL. Pass the filtrate through a 0.2 µm membrane filter prior to injection.

Total Carbohydrates

Weigh 300 mg of instant coffee to the nearest 0.1 mg into a 100 mL volumetric flask. Add 50 mL of 1.0 M HCl and swirl the flask. Place the flask in a boiling water bath for 2.5 h (note: always keep the level of solution in the flask below that of water in the bath). Swirl the flask by hand every 30 min, then cool the flask to room temperature under tap water. Dilute the solution to 100 mL with DI water and filter through folded filter paper. Pass the filtrate (3–5 mL) through a disposable cation-exchange cartridge in the Ag form and a disposable cation-exchange cartridge in the hydronium form to eliminate the Cl- anion, neutralize the solution, and trap any Ag that might break through from the first cartridge (thus protecting the column and the working electrode). Discard the first 1 mL. Filter the remaining solution through a 0.2 µm membrane filter prior to LC injection.

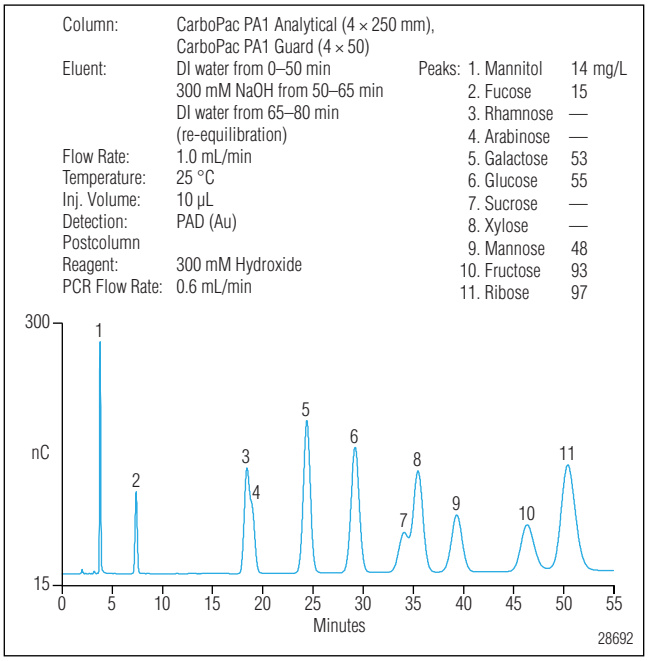

Figure 1. Chromatogram of mixed coffee carbohydrate standards, using the AOAC official method 995.13.

Green Coffee

Weigh 1g of green coffee beans and mix with 10 mL of DI water. Sonicate this solution for 15 min. Pass the supernatant through a 0.2 µm filter, and dilute further with water if needed. Use the sample within 24 h. (Extractions from ground green coffee beans, obtained using this procedure, gave similar results.)

RESULTS AND DISCUSSION

Modified AOAC Method 995.13

Separation

Figure 1 shows the separation of the carbohydrates present in a mix of standards. All the carbohydrates elute in 55 min with a total run time of 80 min (including column wash and equilibration steps). Note that the later eluting peaks are broader relative to the early eluting peaks, as expected from an isocratic method. Carbohydrate concentrations are calculated from the ratio of the peak response in the sample solution to that in the standard solution, and the concentration of the carbohydrate in the standard solution.[6]

Note that rhamnose–arabinose (Figure 1, peaks 3 and 4) and sucrose–xylose (Figure 1, peaks 6 and 7) are not completely resolved. The resolution issue for these sugars has been addressed in the official method.

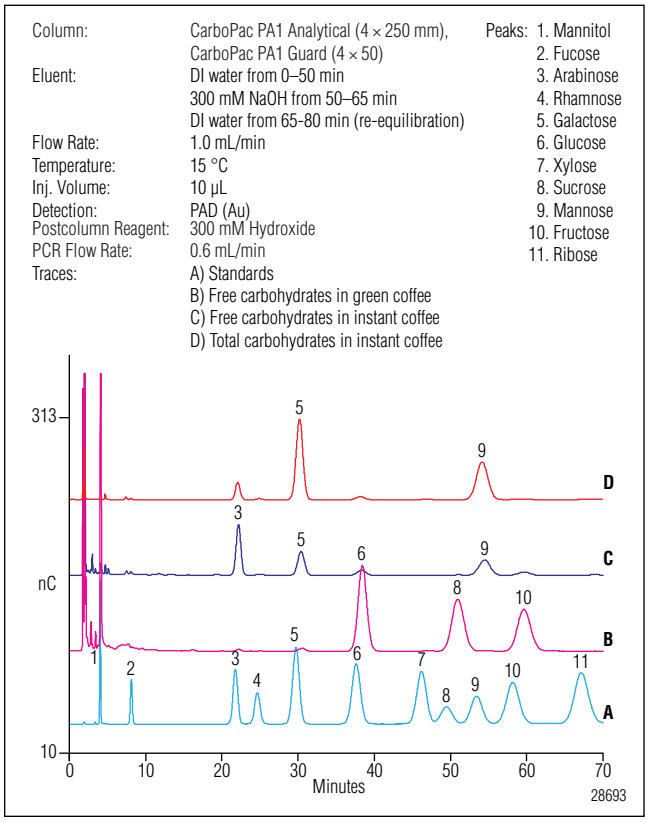

Figure 2. Chromatograms of mixed coffee carbohydrate standards (A), free carbohydrate in extract of green coffee beans (B), free carbohydrates in instant coffee (C), and total carbohydrates in instant coffee (D); using the modified AOAC official method 995.13 (T = 15 ºC).

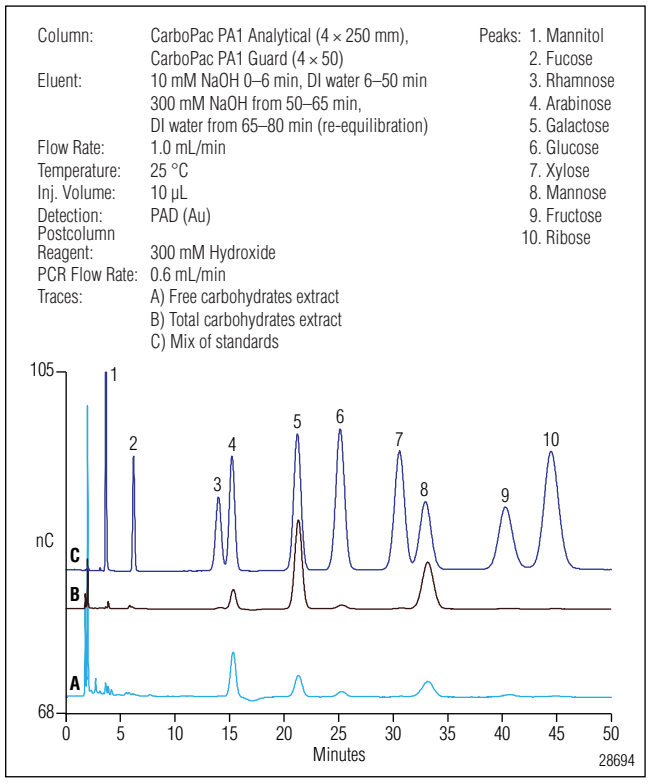

Figure 3. Chromatograms of free carbohydrates extract from instant coffee (A), total carbohydrates extract from instant coffee(B), and mixed carbohydrate standards (C); using the modified AOAC official method 995.13 (10 mM hydroxide for 6 min, and sucrose not included in mix of standards).

If the rhamnose–arabinose peaks are not resolved, the method suggests excluding rhamnose from the mixed standard solution. For the other pair, the AOAC method recommends 2–3 injections of the specified carbohydrates standard solution or an increase of the re-equilibrium time in order to achieve a good separation of glucose, sucrose, and xylose.

As an alternative, the column temperature may be lowered to 15 °C (referred to here as modification 1) to achieve separation of all 11 carbohydrates in the mixed standard solution. Note that the run time is increased (Figure 2), and all the sugars now elute in 70 min. In addition, arabinose elutes before rhamnose, and xylose before sucrose, compared to the elution order at 25 °C, suggesting that the interaction of these sugars with the stationary phase at low temperature (15 ºC) is different than at 25 °C.

Figure 2 also shows representative chromatograms of extracts from green coffee beans (B), and extracts of free carbohydrates (C), and total carbohydrates (D) from instant coffee.

The primary carbohydrates present in extracts from green coffee beans (B) were mannitol, glucose, sucrose, and fructose. In comparison, in the instant coffee sample tested, the major free carbohydrates were arabinose, galactose, and mannose, and the minor sugars were glucose and fructose. In the extract for total carbohydrates from instant coffee, the sugars were mainly arabinose, galactose, and mannose in the sample tested. This gives an indication of how the sugars present in green coffee have changed during roasting and other heat treatment processes (e.g., extraction, spray drying).

The first set of co-eluting peaks, rhamnose and arabinose, was also resolved by modifying the conditions of the mobile phase: by eluting with 10 mM hydroxide for the first 6 min, then switching to DI water (i.e., using a step change; Figures 3 and 4). Note that only the mobile phase was modified; all other chromatography conditions were the same as in AOAC method 995.13. Because extracts from instant coffee typically do not contain sucrose (Figure 2, C and D), sucrose can be eliminated from the mix of standards that will be used when analyzing samples from instant coffee (Figure 3 C).

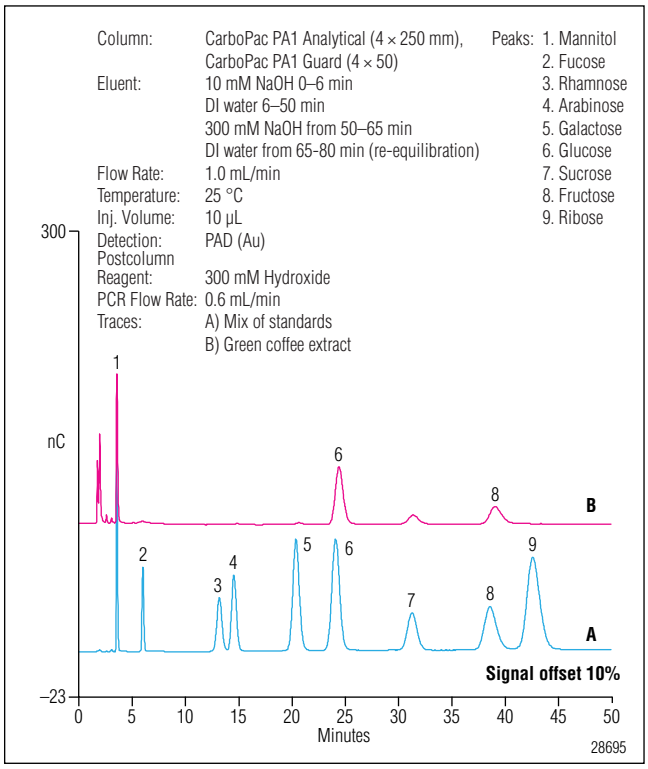

Figure 4. Chromatograms of mixed coffee carbohydrate standards (A), free carbohydrates extract from green coffee beans (B); using the modified AOAC official method 995.13 (10 mM hydroxide for 6 min, and xylose and mannose not included in mix of standards).

For determining sugars in instant coffee, the suggested changes (referred to as modification 2) to the official method include: (a) elution with 10 mM hydroxide for the first 6 min, then switch to DI water, and (b) exclude sucrose from the mix of standards. Note that flavored instant coffees are more likely to contain sucrose, so modification 1 will be more appropriate.

Typically, green coffee samples do not contain xylose and mannose (Figure 2 B). When analyzing green coffee samples, exclude xylose and mannose from the mix of standards (Figure 4 A). Similar to the original AOAC official method 995.13, all the sugars elute in 50 min. The suggested method changes (referred to as modification 3) for analyzing extracts from green coffee include: (a) elution with 10 mM base for the first 6 min, followed by DI water, and (b) exclusion of xylose and mannose from the mix of standards, while maintaining all other chromatography conditions in AOAC method 995.13.

In summary, it may be difficult to achieve baseline resolution of some of the peaks using the official method. Three modifications have been proposed: (1) a lower column temperature to resolve the 11 common coffee carbohydrates in all samples (the caveat being increased run time); (2) for instant coffee samples, exclusion of sucrose in the mix of standard, and eluting with a step gradient with 10 mM base for the first 6 min, and water thereafter; and (3) for green coffee samples, avoiding xylose and mannose in the mix of standards and eluting with a step gradient with 10 mM base for the initial 6 min, followed with water.

Table 1. Precisions for Coffee Carbohydrates Using Modified[a] AOAC Official Method 995.13

Analyte	Concentration Used for Precision Injections (mg/L)	RT Precision RSD	Peak Area Precision RSD
Mannitol	15	0.20	4.49
Fucose	15	0.24	4.69
Rhamnose	35	0.30	4.66
Arabinose	40	0.40	4.83
Galactose	50	0.42	4.72
Glucose	55	0.46	4.82
Sucrose	45	0.68	5.15
Xylose	55	0.42	4.88
Mannose	45	0.44	4.87
Fructose	90	0.47	4.45
Ribose	90	0.48	4.66

[a]Column temperature = 15 °C

Table 2. Precisions for Coffee Carbohydrates Using Modified[a, b] AOAC Official Method 995.13

Analyte	Concentration Used for Precision Injections (mg/L)	RT Precision RSD	Peak Area Precision RSD
Mannitol	15	0.19	1.79
Fucose	15	0.75	2.09
Rhamnose	35	1.84	3.44
Arabinose	40	1.05	2.57
Galactose	50	0.71	1.05
Glucose	55	0.80	2.05
Sucrose[b]	45	0.31	1.79
Xylose[b]	55	0.33	4.96
Mannose[b]	45	0.50	4.20
Fructose	90	0.72	1.17
Ribose	90	0.66	1.27

[a]10 mM NaOH in the eluent in the first 6 min, followed by water; all other chromatography conditions same as AOAC Method 995.13

[b]Exclusion of sucrose from the mix of standards when analyzing instant coffee samples, and xylose and mannose when analyzing green coffee samples

Precision

The peak area and retention time (RT) precisions (RSDs) for six replicate injections of a mixture of sugar standards for the AOAC official method 995.13 with modification 1 (i.e., with column temperature 15 ºC) are listed in Table 1. The retention time precisions ranged from 0.2–0.68% and the average peak area precision was 4.7%. The precisions for the official method with proposed modifications 2 and 3 are presented in Table 2. In these configurations, the RT precisions were in the range 0.19–1.84%, and the peak area precisions were 1.05–4.96%.

Accuracy

The accuracy of the method was evaluated by measuring recoveries in spiked coffee samples (Tables 3–5). Samples were spiked with analytes at a level that was 50–100% of the amount determined in the original sample. Recoveries were calculated from the difference in response between the spiked and unspiked samples. The average recovery for the sugars (using modification 2 with the official method) in the instant coffee samples ranged from 70–116%. For green coffee samples (using the official method with modification 3), the average recovery ranged from 73–95%. The between-day recovery precision for the coffee sugars in the spiked samples averaged 12% over three days. These recovery values indicate that the modified methods are accurate for analyzing coffee carbohydrates.

Table 3. Carbohydrate Recoveries in an Extract of Total Carbohydrates from Instant Coffee (n = 3 days) Using Modified[a,b] AOAC Official Method 995.13

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	97.3	105	107.5	9.3
Fucose	99.5	82.0	82.1	9.1
Rhamnose	106	101	71.1	14.0
Arabinose	91.5	186	88.8	15.0
Galactose	102	817	114.4	15.4
Glucose	92.8	113	84.6	9.9
Xylose	129	106	76.2	14.2
Mannose	200	819	59.8	18.0
Fructose	103	89.7	87.1	12.4
Ribose	98.4	79.1	80.3	5.8

[a]10 mM NaOH in the eluent in the first 6 min, followed by water; all other chromatography conditions same as AOAC Method 995.13

[b]Exclusion of sucrose from the mix of standards

Table 4. Carbohydrate Recoveries in an Extract of Free Carbohydrates from Instant Coffee (n = 3 Days) Using Modified[a,b] AOAC Official Method 995.13

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	39.5	47.7	116.1	18.1
Fucose	41.4	29.3	71.3	11.1
Rhamnose	45.1	40.5	89.5	6.8
Arabinose	36.6	61.0	77.9	20.6
Galactose	45.2	56.3	83.5	15.5
Glucose	42.2	43.5	92.4	9.6
Xylose	41.2	43.0	104.4	7.9
Mannose	41.2	58.7	83.3	19.2
Fructose	39.2	44.2	94.8	11.0
Ribose	49.9	43.2	85.1	17.3

[a]10 mM NaOH in the eluent in the first 6 min, followed by water; all other chromatography conditions same as AOAC Method 995.13

[b]Exclusion of sucrose from the mix of standards

Table 5. Carbohydrate Recoveries in an Extract of Free Carbohydrates from Green Coffee (n = 3 Days) Using Modified[a,b] AOAC Official Method 995.13

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	42.9	41.3	76.6	6.2
Fucose	95.2	90.7	95.4	12.8
Rhamnose	111	83.5	75.6	8.6
Arabinose	97.7	81.1	83.0	2.5
Galactose	104	97.8	92.2	7.6
Glucose	101	129	88.7	23.1
Sucrose	88.4	233	69.5	32.1
Fructose	106	140	73.3	14.9
Ribose	109	90.7	83.3	5.6

[a]10 mM NaOH in the eluent in the first 6 min, followed by water; all other chromatography conditions same as AOAC Method 995.13

[b]Exclusion of xylose and mannose from the mix of standards

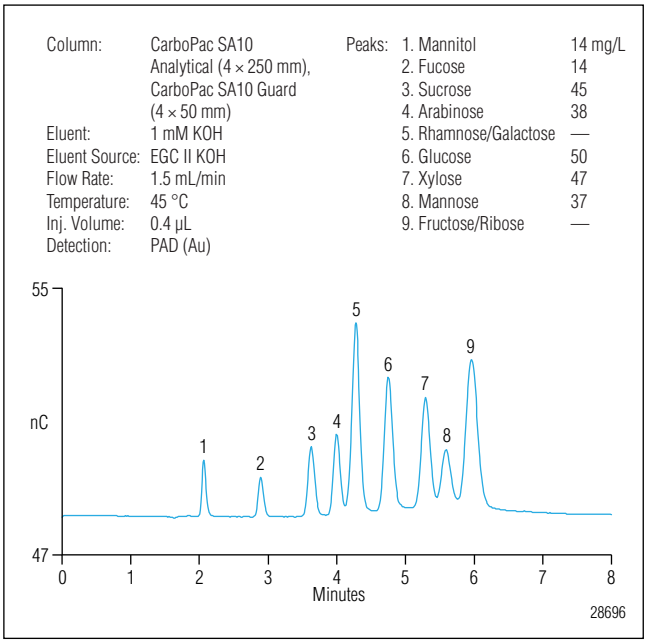

Figure 5. Chromatogram of mixed coffee carbohydrate standards using the fast method.

Fast Method

Separation

The mixture of coffee carbohydrate standards separated on a CarboPac SA10 column is shown in Figure 5. All the sugars elute within 8 min. This is significantly faster than the other methods used for analyzing common sugars in food and beverages. However, note that two pairs of sugars co-elute under the current configuration. These are rhamnose–galactose and fructose–ribose (Figure 5, peaks 5 and 9).

Peaks (mg/mL)	Standard (A)	Instant Free (D)	Instant Total (C)	Green (B)
1. Mannitol	—	0.017	0.006	0.054
2. Fucose	0.040	0.001	—	—
3. Sucrose	0.046	—	—	0.315
4. Arabinose	0.042	0.041	0.126	—
5. Rhamnose and Galactose	—	—	—	—
6. Glucose	0.047	—	0.032	0.059
7. Xylose	0.045	—	—	—
8. Mannose	0.041	0.028	0.603	0.001
9. Fructose and Ribose	—	—	—	—

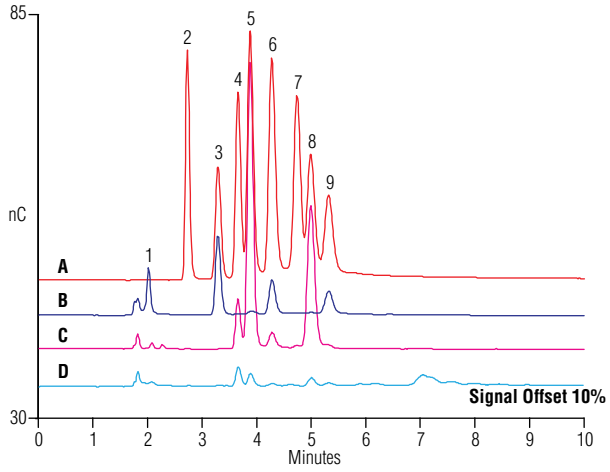

Figure 6. Chromatograms of a mixture of coffee carbohydrate standards (A), free carbohydrates from green coffee beans (B), free carbohydrates (C), and total carbohydrates (D) extract from instant coffee; using the fast method. Chromatographic conditions same as listed in Figure 5.

Figure 6 shows representative chromatograms for extracts from green coffee beans and extracts from instant coffee. The green coffee sample has mannitol, sucrose, glucose, and fructose (assignment is based on the knowledge that green coffee samples have minimal or no ribose), whereas the instant coffee samples have arabinose, galactose (assignment is based on the knowledge that instant coffee samples have minimal or no rhamnose), glucose, and mannose.

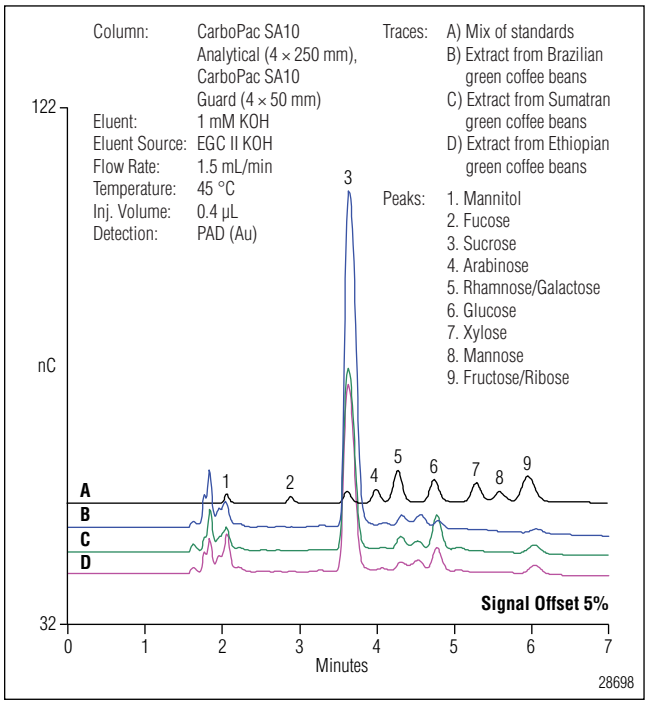

Figure 7. Chromatograms of a mix of coffee carbohydrate standards (A), free carbohydrates in green coffee beans (ground green coffee beans dissolved in water), Brazilian beans (B), Sumatran beans (C), and Ethiopian beans (D).

Figure 7 shows extracts from three kinds of green coffee beans, all of which contain mannitol, sucrose, glucose, and fructose.

Linearity and Precision

The linearity of the method was determined by injecting calibration standards in triplicate, covering the expected range of the sugars of interest in the samples (ranging from 5–900 mg/L) (Table 6). The coefficients of determination obtained from the calibration curves were between 0.9942–0.9998, using least squares regression fits.

Table 6. Linear Range and Precisions for Coffee Carbohydrates Using the Fast Method

Analyte	Range (mg/mL)	Coeff. Of Determ. (r^2)	Concentration Used for Precision (mg/L)	RT (min)	Retention Time Precision (RSD)	Peak Area (nC*min)	Peak Area Precision (RSD)
Mannitol	0.005–0.2	0.9992	15	2.06	0.21	0.16	1.35
Fucose	0.006–0.2	0.9998	15	2.89	0.15	0.13	3.25
Sucrose	0.01–0.8	0.9959	45	3.61	0.19	0.29	3.28
Arabinose	0.018–0.3	0.9997	40	3.99	0.13	0.33	4.24
Glucose	0.013–0.9	0.9963	55	4.74	0.20	0.75	3.64
Xylose	0.01–0.74	0.9967	55	5.28	0.18	0.71	4.64
Mannose	0.006–0.7	0.9942	45	5.58	0.15	0.86	3.85

Table 7. Carbohydrate Recoveries in an Extract of Total Carbohydrates from Instant Coffee (n = 3 Days) Using the Fast Method

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	105	109.9	105.9	12.6
Fucose	93.7	100.7	107.7	6.9
Sucrose	85.9	109.5	127.7	5.1
Arabinose	94.4	368.3	101.3	7.1
Glucose	97.8	161.5	114.5	7.8
Xylose	91.5	109.8	120.5	11.5
Mannose	620	1658	74.2	7.9

Table 8. Carbohydrate Recoveries in an Extract of Free Carbohydrates from Instant Coffee (n = 3 Days) Using the Fast Method

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	41.1	39.0	85.4	1.4
Fucose	39.7	39.9	81.4	13.1
Sucrose	38.1	33.6	102.4	14.2
Arabinose	49.9	84.1	98.0	9.8
Glucose	42.4	33.0	78.9	24.9
Xylose	46.4	37.1	80.0	16.0
Mannose	38.7	69.0	131.9	9.1

The peak area and RSDs were determined for seven replicate injections of a mixture of sugar standards. The concentrations of the carbohydrates in the mix of standards used for precision are listed in Table 6. The RSDs ranged from 0.13–0.21%. The peak area precisions were in the range 1.35–4.65%. These precisions suggest that the method based on separation with the CarboPac SA10 column can be used for the determination of coffee carbohydrates.

The two methods (the AOAC official method and the fast method) gave similar intra-day and between-day (over three days) RT and peak area precisions (data not shown).

Table 9. Carbohydrate Recoveries in an Extract of Free Carbohydrates from Green Coffee (n = 3 Days) Using the Fast Method

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	96.1	139	81.3	8.3
Fucose	96.8	83.2	86.5	8.6
Sucrose	163	385	73.9	7.3
Arabinose	92.2	87.9	97.4	16.9
Glucose	111	136	83.5	9.5
Xylose	109	80.7	75.3	14.4
Mannose	103	78.8	78.0	17.8

Accuracy

The accuracy of the method was evaluated by measuring recoveries in spiked coffee samples (Tables 7–9). Samples were spiked with analytes at a level that was 50–100% of the amount determined in the original sample. Recoveries were calculated from the difference in response between the spiked and unspiked samples. Intra-day carbohydrate concentration RSDs for coffee extracts were in the range of 0.2–1.8%. The average recovery for the sugars in the three types of coffee samples (free and total carbohydrates extract from instant coffee and free carbohydrate extract from green coffee) ranged from 74–127%. The between-day recovery precision for the coffee sugars in the spiked samples ranged from 1.5–24% (average 10%) over three days. These recoveries fall within the accepted range for food matrices.

CONCLUSION

This study describes HPAE-PAD methods for the determination of carbohydrates in extracts from instant coffee and green coffee beans. Two methods (the AOAC official method 995.13 and a fast method using the CarboPac SA10 column) were compared. The former method has a longer run time (80 min) compared to the fast method (10 min). For certain sugars that might be difficult to resolve with the official method, minor modifications are suggested. The fast method, proposed for determining coffee carbohydrates, resolves 7 of the 11 coffee carbohydrates in 8 min (two additional peaks are coelutions of two pairs of carbohydrates) and needs only the addition of DI water for continuous operation.

Both methods have high precisions and acceptable recoveries for the carbohydrates in instant and green coffee extracts. In addition, disposable gold working electrodes provide consistently high detector response for both methods, assuring greater instrument-to-instrument and lab-to-lab reproducibility. In summary, both the AOAC official method 995.13 (with suggested modifications) and the fast method are sensitive, accurate, reliable, and differ primarily in their total analysis time and peak resolutions for coffee carbohydrate determinations.

LIST OF SUPPLIERS

VWR1310 Goshen Parkway
West Chester, PA 19380, U.S.A.
Tel: 800-932-5000

Sigma-Aldrich Chemical Co.
P.O. Box 2060
Milwaukee, WI 53201, U.S.A.
Tel: 800-558-9160.

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Sensitive Determination of Catechins in Tea by HPLC

INTRODUCTION

Consumption of tea has become increasingly popular in North America and tea is currently one of the most consumed non-alcoholic drinks worldwide.[1-3] Studies have shown that tea (*Camellia sinensis*) provides several health benefits, such as reduction of cholesterol and obesity, and protection against cardiovascular disease and cancer. Catechins are powerful antioxidants found in tea that are thought to provide several of these health benefits. Figure 1 shows the structures of the most abundant catechins found in tea.

The composition of catechins in commercial teas varies based on the species, season, horticultural conditions, and most importantly, the degree of oxidation during the manufacturing process.[4] There are four major varieties of teas: white, green, oolong, and black. Although all teas are derived from the same *Camellia sinensis* plant, the processing methods for each tea are different. For example, white tea is naturally dried using either sun drying or steaming methods before being minimally processed to prevent oxidation. These processing methods protect the tea flavor and preserve the high catechin concentrations.

Green tea represents about 20% of the total tea production. It is primarily popular in Japan and parts of China, but its popularity is growing in other parts of the world due to its wide availability and reported health benefits. The production process for green tea is similar to white tea, and therefore, it also contains a relatively high concentration of catechins.

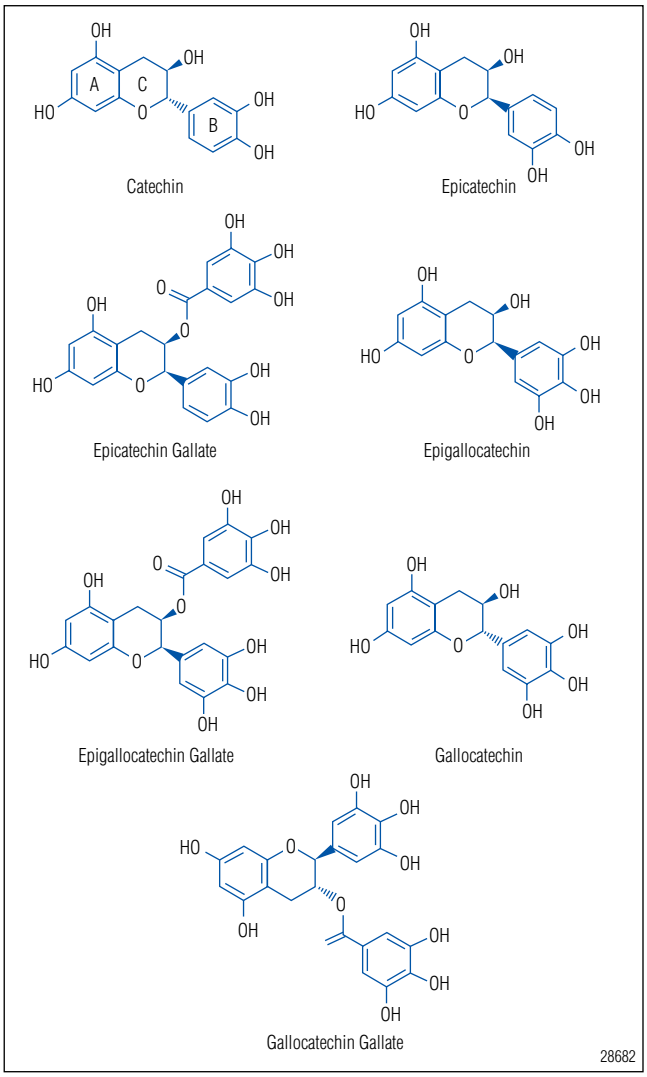

Figure 1. Structures of catechins in Camellia sinensis.

Black tea represents approximately 78% of the global tea production and is the most common type of tea in the United States and Europe.[5] It is made by completely oxidizing the harvested leaves for several hours before drying. Oxidation imparts a dark coloration to the tea and also triples the caffeine content.[5] Due to variability in the composition of tea catechins and their potential health benefits, it is critical to establish a simple and reliable analytical method for the determination of these compounds in different tea products.

This work describes a sensitive, fast, and accurate high-performance liquid chromatography (HPLC) method to determine catechins in tea. The most abundant catechins in tea products include catechin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), gallocatechin (GC), gallocatechin gallate (GCG), and epigallocatechin gallate (EGCG). The method uses a high-resolution silica-based 2.2 µm Acclaim® C18 RSLC column and a wavelength of 280 nm to separate, detect, and quantify catechins in white, green, black, and a blended white and green tea.

In addition, this work evaluated two standard reference materials (SRMs) provided by the National Institute of Standards and Technology (NIST) as part of a collaborative study. The control material (SRM 3255) was a spray-dried green tea extract and the sample (SRM 3256) contained ground and homogenized green tea tablets.

The method demonstrates good sensitivity, enabling the detection of a wide variety of catechins with concentrations ranging from 2.46 mg/g for catechin to 80.8 mg/g for EGCG, and has a total run time of less than 20 min. The reported limits of detection (LODs) using the method range from 0.20 µg/mL for EG and catechin to 1.17 µg/mL for GC, and limits of quanititation (LOQs) range from 0.59 µg/mL for EC to 3.56 for GC. The method described here is ideal for simple, sensitive, accurate, rapid, and routine analysis of catechins in different tea products.

EXPERIMENTAL

Dionex UltiMate® 3000 Rapid Separation LC System

SRD-3600 Solvent Rack with 6 degasser channels (P/N 5035.9230) and Eluent Organizer, including pressure regulator and 2 L glass bottles for each pump (eluents were maintained under helium or nitrogen headspace from 5–8 psi)

DGP 3600RS Pump (P/N 5040.0066)

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

Sample Loop, 25 µL (P/N 6820.2415)

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 120 C18, 2.2 µm, RSLC column, 2.1 × 150 mm (P/N 059130)

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

Viper™ fingertight fitting system, SST Flex. –Cap., i.d. × L, 0.13 × 250 mm (P/N 6040.2325)

Viper fingertight fitting system, SST Flex. –Cap., i.d. × L, 0.13 × 350 mm (P/N 6040.2335)

Viper fingertight fitting system, SST Flex. –Cap., 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)

Borosilicate glass scintillation vials with closures attached, 20 mL (VWR P/N 66022-129)

REAGENTS AND STANDARDS

Reagent grade water, Type I, 18 MΩ-cm resistance or better, filtered through a 0.2 µm filter immediately before use (referred to here as deionized [DI] water)

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

Trifluoroacetic acid (TFA), 98% pure (Pierce P/N 208901)

Epigallocatechin (Chromadex P/N ASB-00005145-010)

Epicatechin gallate (Chromadex P/N ASB-00005135-010)

Catechin (Chromadex P/N ASB-00003310-010)

Epicatechin (Chromadex P/N ASB-00005125-010)

Epigallocatechin gallate (Chromadex P/N ASB-00005150-010)

Gallocatechin (Sigma P/N G6657)

Gallocatechin gallate (Sigma P/N G6782)

SAMPLES

NIST SRM 3255: *Camellia sinensis* extract used as a control material

NIST SRM 3256: Green tea-containing tablets

White tea

White tea blended with green tea

Green tea brand A

Green tea brand B

Black tea

CONDITIONS

Column:	Acclaim 120 C18, 2.2 µm (2.1 × 150 mm)
Flow Rate:	0.450 mL/min
Inj. Volume:	1.0 µL
Tray Temp.:	4 ºC
Detection:	Absorbance, UV, 280 nm
Column Temp.:	25 ºC
Eluents:	A: 0.1% TFA, 5% acetonitrile B: 0.1% TFA in acetonitrile
System Backpressure:	~6025–6200 psi during the gradient

Gradient Conditions:

Time (min)	*A %*	*B %*
0.0	100.0	0.0
1.2	100.0	0.0
15.5	71.5	28.5
17.0	71.5	28.5
17.0	100.0	0.0
25.0	100.0	0.0

PREPARATION OF SOLUTIONS AND REAGENTS

Trifluoroacetic Acid (0.1%) in Acetonitrile (5%)

Transfer 100 mL of acetonitrile into a glass 2 L volumetric flask containing approximately 1700 mL of DI water. Add 2 mL of TFA to the volumetric flask. Bring to volume using DI water and invert flask several times to mix.

Trifluoroacetic Acid (0.1%) in Acetonitrile

Transfer 900 mL of acetonitrile into a glass 1 L volumetric flask, then add 1 mL of TFA to the flask. Bring to volume using acetonitrile and invert flask several times to mix.

Formic Acid (0.05%) in 70% Methanol (Extraction Solvent)

Transfer 700 mL of methanol into a glass 1 L volumetric flask and add 500 µL of formic acid. Bring to volume using DI water and invert several times to mix.

Standard Concentrates (1 mg/mL)

Catechin standards of EGC, ECG, catechin, EC, EGCG, GC, and GCG were prepared by accurately weighing 1–2 mg of solid and adding 1–2 mL of 0.05% formic acid in 70% acetonitrile to make a stock solution of 1.0 mg/mL for each individual catechin. The stocks were prepared in 1.5 mL glass vials, vortexed to mix, and stored at -40 °C until needed. All standard concentrates can be stored for up to six months at -40 °C when protected from light.

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 0.05% formic acid in 70% acetonitrile. To prepare mixed catechin standards, combine appropriate volumes of the individual stock catechin standards in a glass vial containing the appropriate volume of 0.05% formic acid in 70% methanol. Diluted intermediate standards are stable for 3 months at -40 °C and working and mixed standards are stable for 4 weeks at 2–4 °C.

SAMPLE PREPARATION

Two SRMs used in this study—control material (SRM 3255) and sample (SRM 3256)—were provided by NIST as part of a collaborative study. All commercial tea samples were purchased locally.

NIST Control Material

Prepare the NIST control material by weighing 20 mg of solid, then adding 7 mL of the extraction solvent. Vortex the mixture, sonicate for 90 min, and centrifuge at 5000 RPM for 10 min. Collect the supernatant in a glass vial and add another 7 mL of the solvent to the pellet. Vortex the mixture, sonicate for 90 min, and centrifuge at 5000 RPM for 10 min at 4 ºC. Add the supernatant to the first 7 mL to make a total volume of 14 mL. Filter the samples using 0.2 µm cellulose acetate sterile syringe filters, and dilute 1:5 in the extraction solvent prior to analysis.

Commercial Tea Samples and NIST SRM 3256

Prepare all samples by weighing 60 mg of solid and adding 7 mL of the extraction solvent. Vortex the mixture, sonicate for 90 min, and centrifuge at 5000 RPM for 10 min. Collect the supernatant in a glass vial and add another 7 mL of the solvent to the pellet. Vortex the mixture, sonicate for 90 min, and centrifuge at 5000 RPM for 10 min. Add the supernatant to the first 7 mL to make a total volume of 14 mL. Filter the samples using 0.2 µm cellulose acetate sterile syringe filters and dilute 1:5 or 1:20 in the extraction solvent prior to analysis, depending on the sample type.

RESULTS AND DISCUSSION

Separation of Catechin Standards

The initial investigation for the separation of catechins used a 2.2 µm RSLC Acclaim 120 C18 column in the 2.1 × 150 mm format. This column format was chosen to increase sample throughput and reduce sample and eluent consumption. Shorter column formats were also evaluated, but the 2.1 × 150 mm format was chosen because this column provided the best resolution of the target compounds.

Figure 2 shows a chromatogram of a mixed standard containing the predominant catechins in tea. In addition, free gallic acid and moderate amounts of caffeine are naturally present in tea; and therefore were included in the mixed standard. The retention times of gallic acid, GC, EGC, caffeine, catechin, EC, EGCG, GCG, and ECG are 2.15, 4.60, 7.00, 7.25, 7.40, 8.70, 8.90, 9.30, and 10.7 min, respectively. All catechins are well resolved and the total analysis time is less than 20 min.

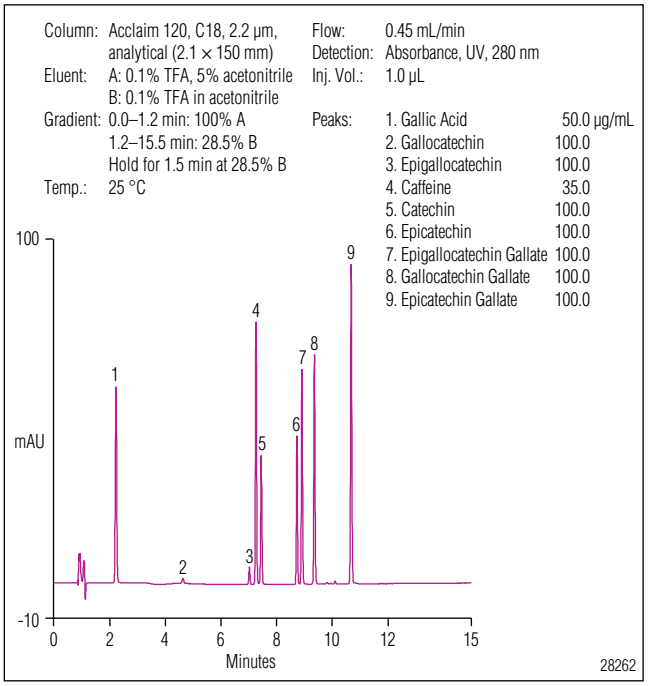

Figure 2. Separation of a mixed catechin standard on the Acclaim C18 RSLC column.

Preliminary Sample Analysis

Prior to analyzing commercial tea samples, a NIST sample and control were evaluated for their catechin profiles using the method described here. The sample and control were prepared as described in the Sample Preparation section. The chromatography demonstrated that all peaks were resolved, suggesting that the method can be used for further system suitability studies.

System Suitability

The linearity, LODs, and LOQs were evaluated to determine the suitability of the method for this analysis. To determine the appropriate calibration ranges for the target compounds, each sample was analyzed and compared to a mixed catechin standard. EGCG, catechin, GCG, GC, EGC, ECG, and EC exhibited a linear peak area response in the ranges summarized in Table 1.

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

Analyte	Range (µg/mL)	Coeffic. of Determin. (r^2)	LOD (µg/mL)	LOQ (µg/mL)	RSD	
					Ret. Time* (n=30)	Peak Area* (n=30)
Gallocatechin	3.56–75	0.9993	1.17	3.56	0.11	1.17
Epigallocatechin	1.8–50	0.9993	0.59	1.80	0.18	1.45
Catechin	0.78–50	0.9992	0.20	0.78	0.13	1.19
Epicatechin	0.59–50	0.9999	0.20	0.59	0.06	1.51
Epigallocatechin Gallate	1.17–150	0.9994	0.39	1.17	0.04	1.00
Gallocatechin Gallate	1.2–10	0.9998	0.39	1.20	0.02	1.37
Epicatechin gallate	1.56–50	0.9995	0.39	1.56	0.02	1.30

*EGC, Catechin, EGCG, EC, GCG, and ECG at concentrations of 15, 1, 30, 1, 2, and 3 µg/mL, respectively, were used for precision studies

The LODs for the catechins were determined based on the concentration of the analyte that provides a peak height of 3× the measured noise (S/N = 3). The LOQs were determined as the concentration of the analyte that provides a peak height of 10× the measured noise (S/N = 10). The LODs ranged from 0.20 µg/mL for EC to 1.17 µg/mL for GC, and the LOQs ranged from 0.59 µg/mL for EC to 3.56 µg/mL for GC. Retention time precisions of the standards were excellent, with RSDs ranging from 0.02% for ECG to 0.18% for EGC. This demonstrated good precision of the gradient delivered by the DPG 3600RS pump. Peak area precision ranged from 1.00% for EGCG to 1.51% for EC. Peak height precision ranged from 0.25% for EGC to 1.70% for GC over 30 runs.

Sample Analysis

Catechin concentrations were determined in a NIST control and sample prior to the analysis of commercial teas. The samples and control were prepared as described in the Sample Preparation section. Table 2 summarizes the catechin concentrations in the NIST controls and samples with a comparison to the certified values.

The concentrations for all the catechins in the control were consistent with the certified NIST values. EGCG is the catechin present at the highest concentration, contributing to 60% of the total catechin content based on the determination presented here, and 58% based on the NIST certified value.

Table 2. Determination of Catechins in a 1:5 Diluted NIST Control and Reference Sample

Analyte	NIST Control (mg/g)	NIST Control Certified Value (mg/g)	NIST Sample (mg/g)	NIST Sample Certified Value (mg/g)
Gallocatechin	22.8 ± 1	24 ± 1	7.84	7.60
Epigallocat-echin	84.7 ± 1	88 ± 3	29.6	30.7
Catechin	9.70 ± 0.5	9.8 ± 0.4	2.46	2.60
Epicatechin	47.3 ± 1	46 ± 2	11.9	12.0
Epigallocat-echin Gallate	427.3 ± 12	417 ± 16	80.8	71.1
Gallocatechin Gallate	40.9 ± 1	38 ± 3	4.46	4.60
Epicatechin Gallate	76.8 ± 2	94 ± 5	17.4	17.1
Total Catechins	709.6 ± 12.5	716.8 ± 27.4	154.5	145.7

The total catechin content calculated using the method described here was determined to be 709.6 mg/g, compared to the NIST certified value of 716.8 ± 27 mg/g. This agreement of the control results with the NIST values confirms that the method is accurate for the determination of catechins.

A NIST reference sample was also evaluated using this method. The individual determined catechin concentrations for the NIST sample ranged from 2.46 mg/g for catechin to 80.8 mg/g for EGCG, compared to certified values of 2.60 mg/g for catechin to 71.7 mg/g for EGCG. The total catechin content is 154.5 mg/g, compared to the certified value of 145.7 mg/g, which is within 6% of the certified value.

Several different brands of teas were evaluated for their catechin content. The samples investigated in this study included two different types of green tea, white tea, a blend of white tea with green tea, and black tea. White tea is minimally processed, so it is expected to be very high in catechins. Figure 3 shows the separation of catechins in white tea. Concentrations ranged from 2.73 mg/g for EC to 42.6 mg/g for EGCG. The total catechin content in this sample was 98.5 mg/g.

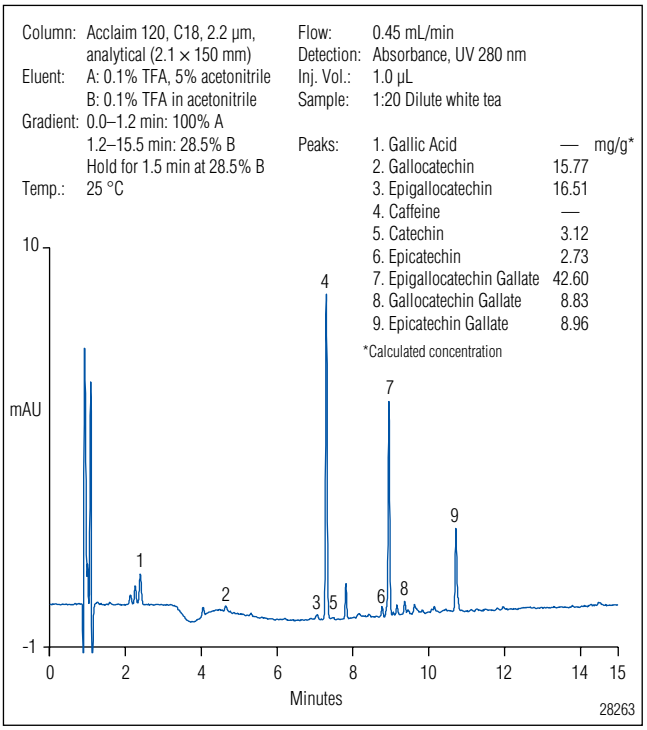

Figure 3. Separation of catechins in a 1:20 diluted sample of white tea.

Figure 4 shows the separation of catechins present in the two different commercially available green teas. The catechin concentrations ranged from 3.45 mg/g for catechin to 64.0 mg/g for EGCG in brand A green tea. In brand B, the concentrations ranged from 3.57 mg/g for catechin to 60.6 mg/g for EGCG. The health benefits of consuming green tea are attributed to the high concentrations of catechins, which account for approximately 30% of the dry weight of green tea leaves.

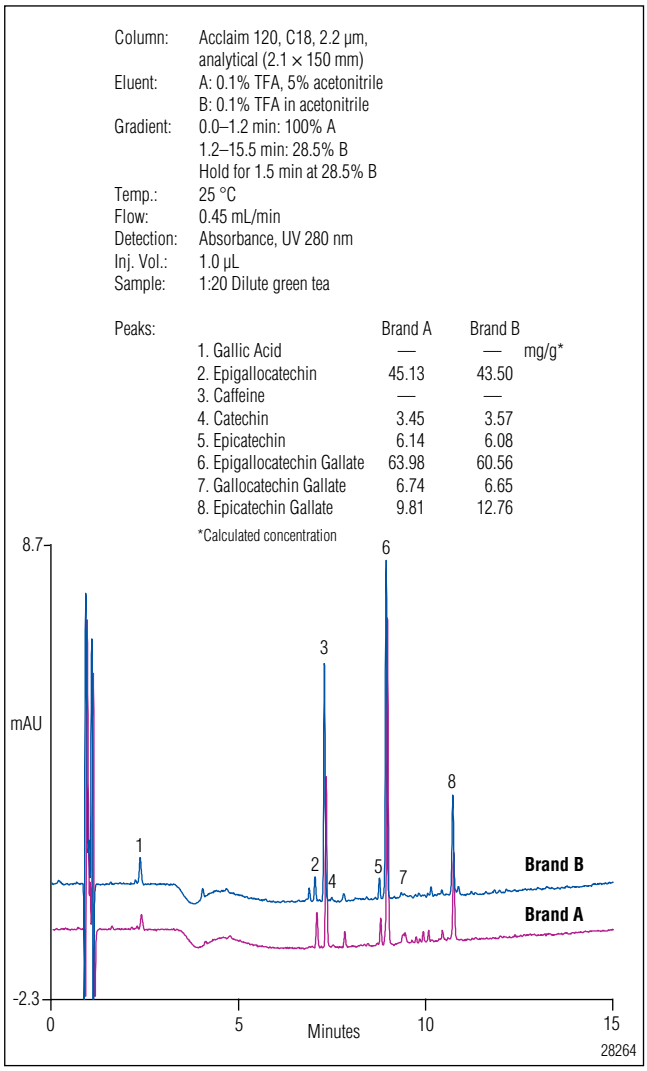

Figure 4. Comparison of catechins in a two different brands of green tea (diluted 1:20).

As shown in Figure 4, the most abundant catechin is EGCG, which is about 50% of the total catechin content. One cup of green tea may contain 100–200 mg of EGCG. The concentrations of individual catechins were determined to be similar for both brands of the green tea, with the exception of ECG. Brand A green tea had 9.81 mg/g and brand B had 12.8 mg/g of ECG. The total catechin content were determined to be similar for both green tea samples with the total concentrations determined at 135.3 mg/g and 133.1 mg/g for brands A and B, respectively. The data shows that the total catechin concentration in the white tea is unexpectedly lower than the total catechin concentrations in the green tea products that were evaluated in this study. However, additional white tea samples were not analyzed to determine if this was representative of white tea products.

The majority of tea produced in the world is black tea, but it is also reported to contain the lowest concentration of catechins due its additional processing. In this study, the content in black tea ranged from 2.25 mg/g for EC to 27.8 mg/g for EGC (Figure 5). Unlike the other teas studied, the EGC concentration in black tea is higher than the EGCG content. The total catechin content is 63.3 mg/g, which is nearly 50% less than the total catechins in the green tea products analyzed.

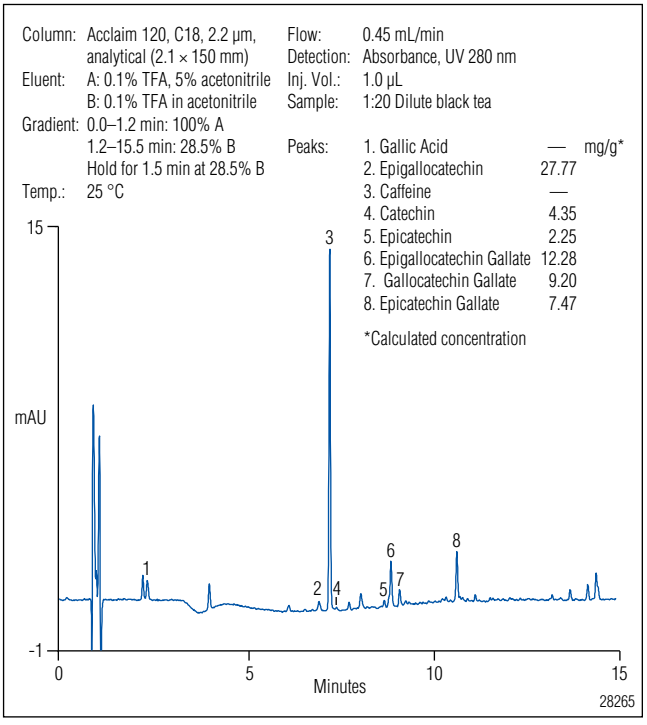

Figure 5. Separation of catechins in a 1:20 diluted sample of black tea.

Table 3. Intraday and Between-Day Precision						
Sample	Analyte	Amount (mg/g)	Intraday Precision RSD (n=3)			Between-day Precision
			Retention Time	Peak Area	Peak Height	Peak Area (n=9 over 3 days)
White Tea	GC	15.8	0.02	0.56	0.90	1.30
	EGC	16.5	0.03	1.04	0.98	2.13
	Catechin	3.12	0.06	0.78	0.89	1.24
	EC	2.73	0.02	1.18	0.83	1.51
	EGCG	42.6	0.02	0.91	0.35	1.74
	GCG	8.83	0.02	0.54	0.85	1.83
	ECG	8.96	0.02	1.06	0.54	2.03
White/Green Tea Blend	EGC	26.7	0.10	1.16	0.50	1.44
	Catechin	2.63	0.14	1.27	1.58	1.49
	EC	2.88	0.08	1.63	1.35	2.15
	EGCG	30.7	0.06	1.17	0.90	1.66
	GCG	4.40	0.02	1.40	1.18	1.62
	ECG	7.44	0.02	0.93	0.74	1.88
Black Tea	EGC	27.8	0.05	1.00	1.39	1.15
	Catechin	4.35	0.09	0.61	0.51	1.08
	EC	2.25	0.03	0.47	0.70	1.07
	EGCG	12.3	0.02	1.33	1.65	1.42
	GCG	9.20	0.05	1.76	1.57	1.97
	ECG	7.47	0.03	1.66	1.37	1.72
Green Tea Brand A	EGC	45.1	0.12	1.10	0.57	1.71
	Catechin	3.45	0.09	1.16	0.87	1.93
	EC	6.14	0.04	1.34	0.98	1.72
	EGCG	64.0	0.02	1.30	1.00	1.91
	GCG	6.74	0.36	1.35	1.09	1.52
	ECG	9.81	0.01	0.33	1.37	1.04
Green Tea Brand B	EGC	43.5	0.01	0.43	0.48	1.92
	Catechin	3.57	0.01	1.49	1.69	1.50
	EC	6.08	0.01	0.25	0.41	1.81
	EGCG	60.6	0.01	0.45	0.36	1.93
	GCG	6.65	0.01	0.66	0.27	1.14
	ECG	12.8	0.01	0.92	0.50	1.08

Sample Precision and Accuracy

Five different samples of teas were analyzed over three days to evaluate the method precision. Representative data from each of the teas are summarized in Table 3. For all the samples analyzed in this study, the intraday retention time RSDs ranged from 0.01% for several catechins to 0.36% for GCG. Intraday peak area RSDs ranged from 0.25% for EC to 1.76% for GCG. The between-day peak area RSDs ranged from 1.04% for ECG to 2.15% for EC.

The accuracy of the method was confirmed by determining catechin concentrations in the NIST control and comparing against the NIST certified values. The NIST certified values for the control and the samples were in agreement with the values reported in this study. Recovery studies were performed on all five tea samples by spiking known amounts of the seven catechins to determine method accuracy.

Table 4. Recovery of Catechins in Different Commercial Tea Products

Sample	Analyte	Amount Spiked µg/mL	% Recovery
White Tea	GC	4.0	103.5
	EGC	4.0	92.1
	Catechin	1.0	99.8
	EC	1.0	112.0
	EGCG	10.0	93.3
	GCG	3.0	105.1
	ECG	3.0	84.2
White/Green Tea Blend	GC	1.0	93.4
	EGC	6.5	91.3
	Catechin	1.0	94.3
	EC	1.0	96.4
	EGCG	10.0	99.1
	GCG	1.0	97.6
	ECG	2.0	93.6
Black Tea	GC	1.0	94.0
	EGC	7.0	93.3
	Catechin	1.0	96.8
	EC	0.5	90.2
	EGCG	3.0	101.0
	GCG	2.0	102.3
	ECG	2.0	102.0
Green Tea Brand A	GC	1.0	85.4
	EGC	15.0	99.9
	Catechin	1.0	100.5
	EC	1.5	90.9
	EGCG	15.0	99.9
	GCG	2.0	95.5
	ECG	3.0	96.5
Green Tea Brand B	GC	1.0	84.2
	EGC	15.0	94.4
	Catechin	1.0	89.8
	EC	2.0	95.1
	EGCG	15.0	96.8
	GCG	2.0	93.8
	ECG	3.0	91.7

Table 4 summarizes the amounts spiked and the calculated recoveries. Recoveries ranged from 84.2% for ECG to 112% for EC.

CONCLUSION

This work describes a simple, sensitive, rapid, and accurate method to separate and quantify catechins in different commercially available teas with a simple solvent extraction. The method uses a high-resolution silica-based Acclaim RSLC C18 column and absorbance at a wavelength of 280 nm to separate and detect catechins in less than 20 min. Catechin concentrations in NIST controls and samples were determined using the described method, and the values reported were in agreement with certified NIST values. The catechin concentrations varied over a wide range in the samples, from 427.3 mg/g of EGCG in the NIST control to 2.25 mg/g of EC in black tea. The method described here is ideal for routine screening and quantification of catechins in different tea products.

PRECAUTIONS

Trifluoroacetic acid is corrosive, causes burns, and is harmful if swallowed, inhaled, or absorbed through skin. This material is extremely destructive to the upper respiratory tract, eyes, and skin. In case of contact, immediately flush eyes or skin with plenty of water for at least 15 min while removing contaminated clothing and shoes. Wash clothing before reuse. If inhaled, quickly move the exposed person to a source of fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. If the product is swallowed, do not induce vomiting. Give large quantities of water. Never give anything by mouth to an unconscious person. In all cases, call a physician immediately. Please read the material safety data sheets (MSDS) prior to handling and contact a licensed waste disposal organization to ensure all disposals are in accordance with existing federal, state, and local environmental regulations.

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1. Castro, J.; Pregibon, T.; Chumanov, K.; Marcus, R. K. Determination of Catechins and Caffeine in Proposed Green Tea Standards Reference Materials by Liquid Chromatography-Particle Beam/Electron Ionization Mass Spectrometry (LC-PB/EIMS). *Talanta* **2010**, *82* (5), 1687–95.
2. Kajimoto, O.; Kajimoto, Y.; Yabune, M.; Nozawa, A.; Nagata, K.; Kakuda, T. Tea Catechins Reduce Serum Cholesterol Levels in Mild and Borderline Hypercholesterolemia Patients. *J. Clin. Biochem. Nutr.* **2003**, *33*,101–111.
3. Bu-Tian, Ji et al. Green Tea Consumption and the Risk of Pancreatic and Colorectal Cancers. *Int. J. Cancer* **1997**, *70*, 255–258.
4. Zuo, Y.; Chen, H.; Deng, Y. Simultaneous Determination of Catechins, Caffeine and Gallic Acids in Green, Oolong, Black and Pu-erh Teas using HPLC with a Photodiode Array Detector. *Talanta* **2002**, *57*, 307–316.
5. Jian-Min, Yuan et al. Urinary Biomarkers of Tea Polyphenols and Risk of Colorectal Cancers in the Shanghai Cohort Study. *Int. J. Cancer* **2007**, *120*, 1344–1350.

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, Dansbury, CT 06810-5113. U.S.A. Tel: 877-772-9247. www.praxair.com

ChromaDex Inc., 10005 Muirlands Blvd, Suite G, First Floor, Irvine, CA 92618, U.S.A. Tel: 949-419-0288. www.chromadex.com

Thermo
SCIENTIFIC

Column Selection Guide

Silica Columns

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

Polymer Columns

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

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

Column Specifications

IC Anion Columns

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

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

IC Cation Columns

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

Ion-Exclusion Columns

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

Acclaim General and Specialty Columns

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

Bio Columns

Protein

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

Carbohydrate

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

DNA

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

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