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Chromatography for Foods and Beverages Flavors, Colorants and Additives Analysis Applications Notebook

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Additives Including Colorants, Flavorants, and Preservatives

Introduction

Food additives are substances added to food to preserve flavor or enhance its taste, texture, and appearance. With the advent of processed foods many more additives are being used, both natural and artificial. Additives can maintain product quality and freshness (to stop deterioration, rancidity, and spoilage); improve or maintain the nutritional quality (to prevent diseases such as goiter, pellagra and rickets); make foods more appealing (make them look and taste good); and aid in the processing and preparation of foods.

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Analytical Technologies

High-Performance Liquid Chromatography

Thermo Scientific™ Vanquish™ UHPLC System and Thermo Scientific™ Dionex™ UltiMate™ 3000 UHPLC+ systems offer excellent chromatographic performance, operational simplicity and unrivaled flexibility. Choose from a wide range of standard and unique specialty detectors to extend your laboratory's analytical capabilities.

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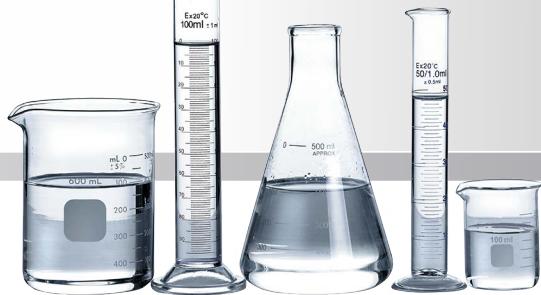
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Analytical Technologies

The Vanquish UHPLC System

The next generation in UHPLC innovations

The Vanquish system takes high-end UHPLC to a new level, offering more resolution while meeting the throughput demands of modern laboratories. The system is delivered better separations, more results and easier interaction, simultaneously, without compromise.



Delivering the new standard in UHPLC

- More powerful separations with 1500 bar of pump pressure at flow rates up to 5 mL/min
- Industry-leading flow and gradient precision
- Excellent injections up to 100 µL in 0.01 µL increments
- Automated workflows with barcode reading for simplified setup and tracking
- Maximum sample capacity with up to 23 well plates, or 8832 samples
- More confident separations with a wide temperature range of 5 °C to 120 °C for two thermostating modes and active column pre-heating for improved precision
- UV detection with linear response up to 3000 mAU and noise levels as low as 3 µAU
- Thermo Scientific™ LightPipe™ technology assures lowest peak dispersion with UV detection
- Available Vanquish Charged Aerosol detector for quantification of non-chromophoric compounds



Vanquish Diode Array Detector with LightPipe technology

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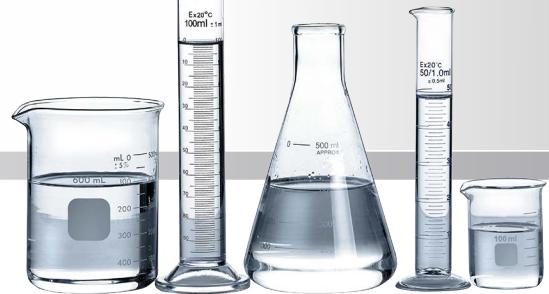
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UHPLC Portfolio

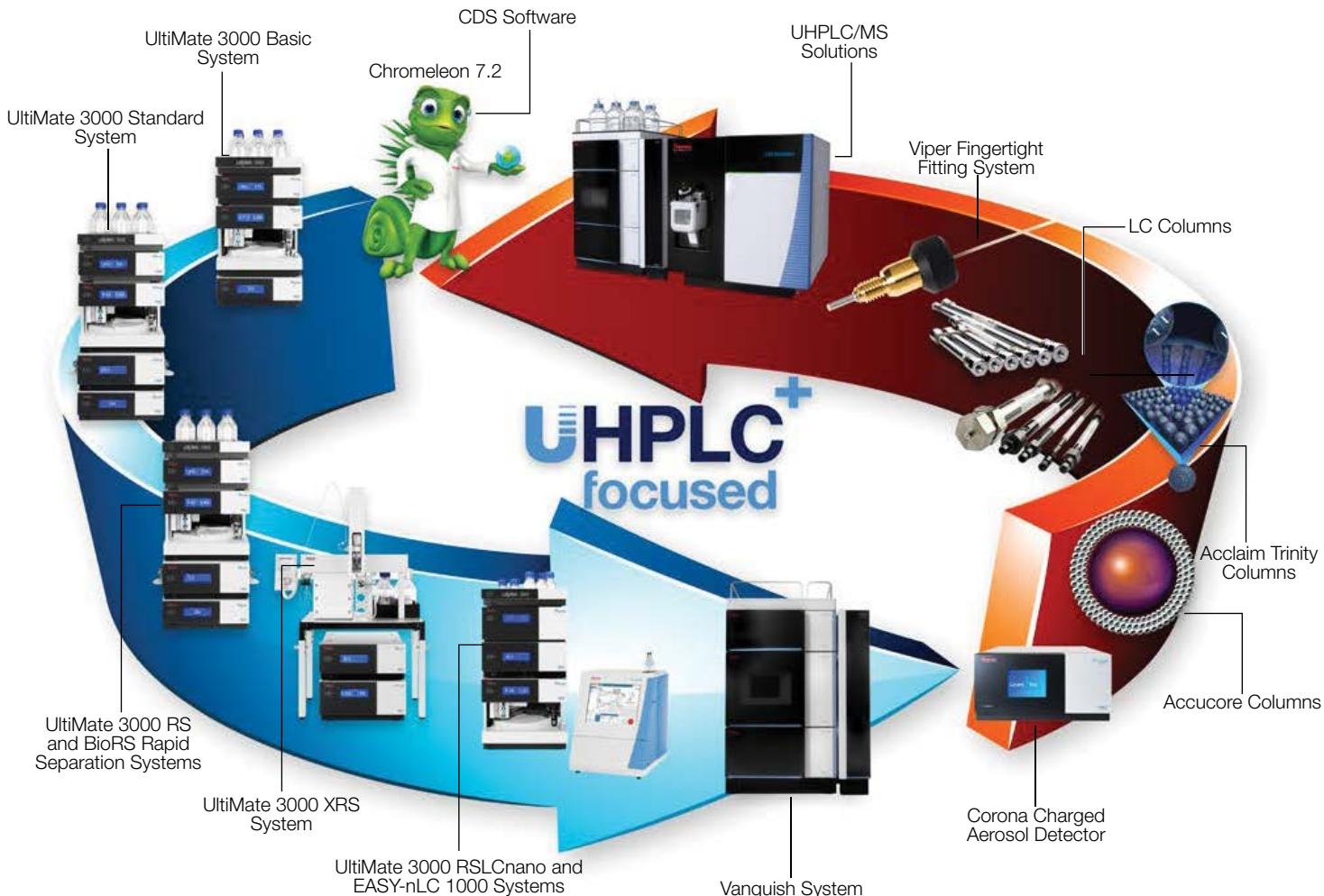


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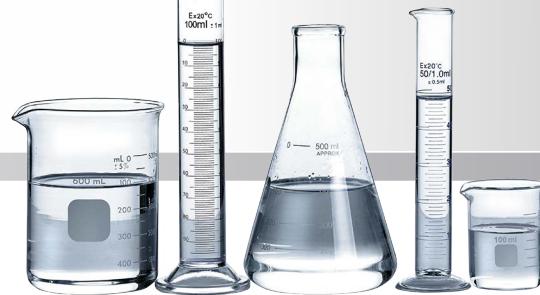
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UltiMate 3000 UHPLC⁺ Systems

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

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 chromatography (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™ fingertight fittings—the first truly universal, fingertight fitting system even at UHPLC pressures

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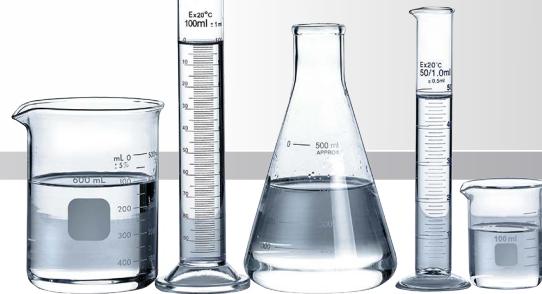
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UltiMate 3000 UHPLC⁺ Systems

We are uniquely focused on making UHPLC technology available to all users, all laboratories, and for all analytes.



Rapid Separation LC Systems

The extended flowpressure footprint of the RSLC system provides the performance for ultrafast high-resolution and conventional LC applications.



RSLC nano Systems

The Rapid Separation nano LC System (RSLC nano) 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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Advanced Detection Capabilities

Charged Aerosol Detection

Charged Aerosol Detection provides near universal detection independent of chemical structure for non- or semi-volatile analytes with HPLC and UHPLC. Thermo Scientific™ Dionex™ Corona™ Veo™ and Vanquish

Charged Aerosol detectors are ideally suited as a primary detector for any laboratory, while providing complementary data to UV or MS methods. No other LC detector available today can match the performance of a Corona Veo detector.

- High sensitivity – single-digit nanogram on column
- Consistent response – independent of chemical structure
- Wide dynamic range – to four orders of magnitude or greater
- Simple to use – easy to integrate with any HPLC/UHPLC system

Charged aerosol detectors give the simplicity, reproducibility and performance required for a full range of applications from basic research to manufacturing QC/QA. With charged aerosol detection you get predictable responses to measure analytes in direct proportion to their relative amounts for quantitation without actual standards.

This detector offers the flexibility to use reversed-phase gradients, as well as normal phase and HILIC modes of separation on any LC system. And, in many cases eliminates the need for derivatization or sample pre-treatment to provide real dilute-and-shoot simplicity.



Corona Veo Charged Aerosol Detector



Vanquish system with Charged Aerosol Detector

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Advanced Detection Capabilities

CoulArray Multi-electrode Array Detector

The Thermo Scientific™ Dionex™ CoulArray™ Multi-electrode Array detector is the only practical multi-channel electrochemical detection system that allows you to measure multiple analytes simultaneously, including those that are chromatographically unresolved. The CoulArray detector delivers the widest dynamic range of any available electrochemical detector with unmatched selectivity for detection of trace components in complex matrixes, even when used with aggressive gradients.

- Measures analytes from femtomole to micromole levels
- Greatly simplify sample preparation and eliminate interferences
- Simultaneously analyze multiple analytes in very complex samples
- Easily produce qualitative information for compound identification

Multiple system configurations offer 4, 8, 12, or 16 channels that can be upgraded anytime. The unique data acquisition and processing software uses automatic signal ranging and a unique patented baseline correction algorithms to provide identification and quantitation of single or multiple analytes and powerful 3D data for quick sample fingerprint confirmation with integration to pattern recognition platforms.

With the power of coulometric array technology, the CoulArray detector can give you the qualitative data of a optical PDA with 1,000 fold greater sensitivity to profile the characteristic qualities of products, determine integrity, identify adulteration and even evaluate competitors' products.



CoulArray Multi-electrode Array Detector

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Advanced Detection Capabilities

RefractoMax 521 Refractive Index Detector

The Thermo Scientific RefractoMax 521 Refractive Index Detector from ERC Inc. This detector, in combination with the UltiMate 3000 system, is the right choice for the isocratic analysis of sugars, polymers, and fatty acids. It features fast baseline stabilization and excellent reproducibility, combined with high sensitivity. The RefractoMax 521 is fully controlled by Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System Software (CDS), and can also operate in stand-alone mode.

- The detector is highly sensitive and applicable universally. It provides very stable baselines with a drift of 0.2 µRIU/h and a noise specification of 2.5 nRIU or less
- The optical bench, thermostatically regulated from 30 °C to 55 °C, and the superior signal-to-noise ratio ensure highly precise measurement results



RefractoMax 521 Refractive Index Detector

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UltiMate 3000 Diode Array and Multiple-Wavelength Detectors

The Thermo Scientific Dionex UltiMate DAD 3000 detector is a high-resolution, 1024-element diode array detector (DAD) available in Rapid Separation (200 Hz) and Standard (100 Hz) versions. It operates with Chromeleon CDS software to provide a variety of spectra views, including 3-D plotting and automated chromatogram handling. The high resolution and low-noise performance of the DAD-3000 family makes it ideal for the most sensitive and accurate library searches and peak purity analyses.

The detector is also available as a multiple wavelength detector (MWD) in Standard (100 Hz) and Rapid Separation (200 Hz) versions.

- Data collection at up to 200 Hz using a maximum of eight single-wavelength data channels and one 3-D field (3-D only with DAD-3000 (RS)) for best support of ultrafast separations
- Standard versions operate at up to 100 Hz data collection rate for optimum support of 62 MPa (9000 psi) UltiMate 3000 Standard systems
- Accurate compound confirmation with a 1024-element, high resolution photodiode array
- Flexibility in both UV and Vis applications with 190–800 nm wavelength range
- Low-noise over the full spectral range using deuterium and tungsten lamps
- Fast and accurate wavelength verification using a built-in holmium oxide filter

Advanced Detection Capabilities

- The detector can be upgraded with the UltiMate PCM 3000 for accurate monitoring pH gradients
- Excellent reliability and reproducibility with low baseline drift (typically < 500 µAU/h)
- Simplified routine maintenance with front access to pre-aligned cells and lamps
- ID chips on flow cells and lamps for identification and life-span monitoring
- Chromeleon CDS software for full control and flexible data handling
- Front-panel display for easy monitoring of detector status to maximize uptime
- Flow cells for semi-micro, semi-analytical, analytical, and semi-preparative applications
- Flow cells available in stainless steel and biocompatible versions



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Advanced Detection Capabilities

Features include:

- Detection Modes – choose from DC and PAD for optimum analyte response
- Choice of sensors – both coulometric and amperometric sensors to meet the demands of any application
- UHPLC compatibility – ultralow peak dispersion and high data acquisition rates for conventional or fast, high resolution chromatography
- Modularity – easily expandable to multiple independent sensors for unrivaled flexibility
- Autoranging – simultaneously measure both low and high levels of analytes without losing data
- SmartChip™ technology – easy operation with automatic sensor recognition, event logging and electrode protection



UltiMate 3000 Electrochemical Detector

Learn more at www.thermoscientific.com/ECDetection

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Advanced Detection Capabilities

UltiMate 3000 Fluorescence Detector

The Thermo Scientific Dionex UltiMate 3000 FLD-3000 is a high-sensitivity fluorescence detector series for UltiMate 3000 HPLC systems. It is available in Rapid Separation (RS) and Standard (SD) versions. The optics of the FLD-3000 series provide maximum stray-light suppression for best detection sensitivity. Operated with the Chromleon CDS software, the detector provides automated qualification, various tools for method development, and instrument wellness monitoring for ease of use, maximum uptime, and the highest degree of regulatory compliance.

- Data collection at up to 200 Hz for optimal support of even the fastest UHPLC separations (FLD-3400RS)
- Standard detectors operate at up to 100 Hz data rate for optimum support of 62 MPa (9,000 psi) UltiMate 3000 standard systems
- Lowest limits of detection with a Raman signal-to-noise ratio (S/N): > 550 ASTM (> 2100 using dark signal as noise reference)

- Unsurpassed reproducibility with active flow cell temperature control for stable fluorophore activity independent of changes in ambient temperature
- Long-life xenon flash lamp for highest sensitivity and long-term operation without the need for frequent lamp changing
- Optional second photomultiplier (PMT) for unique Dual-PMT operation, offering an extended wavelength range up to 900 nm without sacrificing sensitivity in the standard wavelength range
- Two-dimensional (2D) or three dimensional (3D) excitation, emission, or synchro scans to provide the highest degree of flexibility for method development or routine sample characterization
- Innovative Variable Emission Filter for real-time compound-related sensitivity optimization (FLD-3400RS only)
- Large front-panel display for easy monitoring of the detector status
- Two flow-cell sizes for easy optimization to application requirements: the 8 µL flow cell is ideal for trace analysis, and the 2 µL flow cell offers best peak resolution with narrow-bore HPLC and UHPLC columns



Ultimate 3000 Fluorescence Detector

Learn more at www.thermoscientific.com/liquidchromatography

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UltiMate 3000 Variable Wavelength Detectors

The Thermo Scientific Dionex UltiMate 3000 VWD-3000 is a variable wavelength detector (VWD) series for industry leading UV-Vis detection. The forward optics design and wide range of available flow cells ensure optimal performance over a flow rate range of five orders of magnitude. Automated qualification, performance optimization, and instrument wellness monitoring deliver maximum uptime, simplify work-flow, and give you full confidence in your analytical results. The detector is available in a standard 100 Hz (VWD-3100) and a 200 Hz Rapid Separation version (VWD-3400RS) for the most challenging UHPLC applications.

High-Performance UV-Vis Detection

- The VWD-3400RS variant provides data collection rates of up to 200 Hz for optimal support of today's and tomorrow's UHPLC separations
- The VWD-3100 standard detector operates at up to 100 Hz data rate for optimum support of 62 MPa (9000 psi) UltiMate 3000 Standard systems
- Superior detection of trace analytes with low noise (< -2.0 µAU) and drift (< 100 µAU/h)
- The detector's large linearity range of up to 2.5 AU is ideal for applications with widely varying analyte concentrations
- Up to four absorption channels (VWD-3400RS) and spectral scans support effective method development
- Active temperature control of optics and electronics for data acquisition independent of ambient conditions

Advanced Detection Capabilities

- Front panel access for quick and easy lamps and flow cells changes
- Automated qualification monitoring for full regulatory compliance
- Large front panel display for monitoring the detector status even from a distance
- Maximize uptime using predictive performance—based on monitoring the life cycle of detector lamps
- The detector can be upgraded with the Thermo Scientific Dionex pH/Conductivity Monitor (PCM-3000) for accurate and precise pH- and conductivity monitoring
- Unique 45 nL ultra-low dispersion UV monitor for dispersion-free UV detection in LC/MS



UltiMate 3000 VWD-3400 Variable Wavelength Detector.

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Analytical Technologies

Ion Chromatography

Thermo Scientific Dionex IC systems have led the analytical instrument industry for over 30 years with solutions that represent state-of-the art technological advancements and patented technologies.

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IC and RFIC Systems

Innovative Ion Chromatography Solutions

Our High-Pressure™ Ion Chromatography (HPIC™) systems include the Thermo Scientific Dionex ICS-5000+ HPIC system, which is optimized for flexibility, modularity, and ease-of-use, combining the highest chromatographic resolution with convenience. In addition, the Thermo Scientific Dionex ICS-4000 Capillary HPIC system is the world's first commercially available dedicated capillary high-pressure Reagent-Free™ (RFIC™) IC system. The Dionex ICS-4000 system is always ready for the next analysis, delivering high-pressure IC on demand.

Reagent-Free IC systems eliminate daily tasks of eluent and regenerant preparation in turn saving time, preventing errors, and increasing convenience. RFIC-EG systems use electrolytic technologies to generate eluent on demand from deionized water, and to suppress the eluent back to

pure water to deliver unmatched sensitivity. RFIC-ER systems are designed to use carbonate, carbonate/ bicarbonate, or MSA eluents for isocratic separations.

At the heart of our ion chromatography portfolio is a unique set of column chemistries that provide high selectivities and efficiencies with excellent peak shape and resolution. Thermo Scientific™ Dionex™ IonPac™ chromatography columns address a variety of chromatographic separation modes including ion exchange, ion exclusion, reversed-phase ion pairing, and ion suppression. Our column chemistries are designed to solve specific applications, and we offer a variety of selectivities and capacities for simple and complex samples. Additionally, our Dionex IonPac column line is available in standard bore, microbore and capillary formats for the ultimate application flexibility.



Thermo Scientific Dionex IC instrument family

Learn more at www.thermoscientific.com/IC

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Mass Spectrometry

We provide 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 ion chromatography family automatically removes mobile phase ions for effort-free transition to MS detection.

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Mass Spectrometry Instruments

- Chromeleon CDS 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 data system, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) probe inlets, and vacuum system

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



MSQ Plus Mass Spectrometer

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Chromatography Data Systems

Tackle chromatography management challenges with the world's most complete chromatography software. Whether your needs are simple or complex or your scope is a single instrument, a global enterprise, or anything in between – the combination of Chromleon CDS' scalable architecture and unparalleled ease-of use, makes your job easy and enjoyable with one Chromatography Data System for the entire lab.

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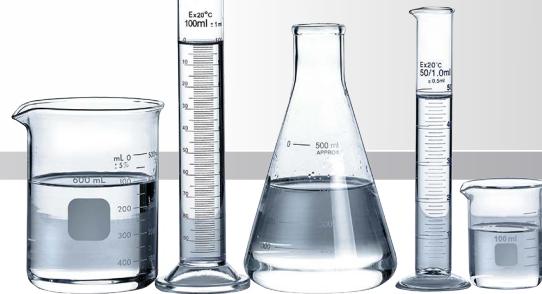
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The Fastest Way from Samples to Results

The 7.2 release of Chromeleon Chromatography Data System software is the first CDS that combines separation (GC/IC/LC) and Mass Spectrometry (MS) in an enterprise (client/server) environment. By extending Chromeleon 7.2 CDS beyond chromatography into MS, lab technicians can now streamline their chromatography and MS quantitation workflows with a single software package. MS support in Chromeleon 7.2 CDS is focused on routine and quantitative workflows, which provides access to rich quantitative data processing and automation capabilities — ultimately boosting your overall lab productivity and increasing the quality of your analytical results.

Chromeleon CDS 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 spreadsheet

Excel is a registered trademark of Microsoft Corporation.



Learn more at www.thermoscientific.com/Chromeleon

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Thermo Scientific Dionex process analytical systems provide timely results by moving chromatography-based measurements on-line.

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Process Analytical Systems and Software

Improved Process Monitoring with On-line Chromatography IC and LC Systems

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
- The Thermo Scientific Integral Migration Path approach lets you choose the systems that best meets your needs



Integral process analyzer

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Automated Sample Preparation

Solvent extractions that normally require labor-intensive steps are automated or performed in minutes, with reduced solvent consumption and reduced sample handling using the Thermo Scientific™ Dionex™ ASE™ Accelerated Solvent Extractor system or Thermo Scientific™ Dionex™ AutoTrace™ 280 Solid-Phase Extraction instrument.

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Accelerated Solvent Extractor System

Complete Extractions in Less Time Using Less Solvent

Thermo Scientific Dionex ASE systems extract of solid and semisolid samples using common solvents at elevated temperature and pressure. The Dionex ASE 150 and 350 systems feature pH-hardened pathways with Dionium™ components to support extraction of acidic or alkaline matrices, and combine pretreatment, solvent extraction, and cleanup into one step. Dionium is zirconium that has undergone a proprietary

hardening process that makes it inert to chemical attack by acids and bases at elevated temperatures.

Dionex ASE systems are dramatically faster than Soxhlet, sonication, and other extraction methods, and require significantly less solvent and labor. Accelerated solvent extraction methods are accepted and established in the environmental, pharmaceutical, foods, polymers and consumer product industries. Accelerated solvent extraction methods are accepted and used by government agencies worldwide.



Dionex ASE 150/350 and Dionex AutoTrace 280 SPE instruments

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Additives Including Colorants, Flavorants, and Preservatives

Colorants

Food colorings (also called color additives), are any dyes, pigments, or substances that are used to impart color when they are added to food or drink. Colorants are used to enhance colors that occur naturally, correct natural variation in color, and to make food more attractive and appetizing. Colorants are also used to offset the loss of color due to processing and storage.

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Artificial Colorants: Blue Dyes in Cereals

The FDA lists Food, Drugs, and Cosmetics (FD&C) certified color additives for use in foods in the United States. FD&C dyes are added to many popular breakfast cereal products to make them more visually appealing. This convenient and rapid assay uses ion-pair chromatography on a Thermo Scientific™ Acclaim™ C18 column to identify and quantify the dyes.

Pump: UltiMate LPG-3400
 Column: Acclaim 120 C18 3 µm, 3.0 x 75 mm
 Flow: 1.0 mL/min
 Temperature: UltiMate TCC-3100 at 30 °C
 Injection: UltiMate WPS-3000 SL sampler at 8 µL
 Mobile Phase: 677 g water, 0.97 g Na₂SO₄, 2.24 g KH₂PO₄, 3.20 g 55% Bu₄N OH (pH = 6.75), 250 g acetonitrile
 Detector: UltiMate WVD-3400, VIS at 427, 508, 625 nm, 2.5 Hz, 0.6 s time const.
 Sample Prep.: (1) Sort cereal by color
 (2) Grind to coarse powder
 (3) Extract 0.2 g of cereal with
 5 mL mobile phase
 (4) Filter through 0.1 µm membrane
 Peaks: 1. FD&C Blue #2
 2. FD&C Blue #1

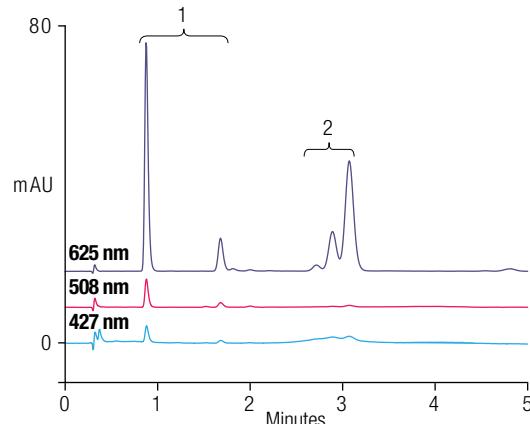
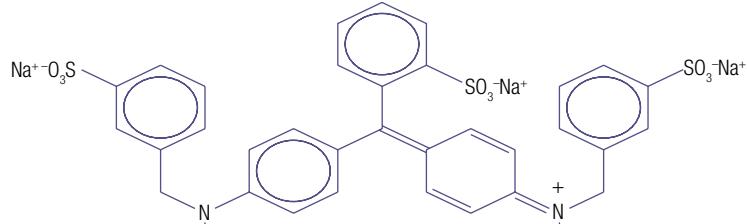
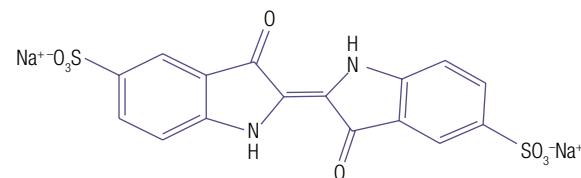


Figure 4-1. FD&C blue #1 and blue #2 in a blueberry-flavored cereal.



FDC Blue #1, Brilliant Blue, E133



FDC Blue #2, Indigocarmine, E132



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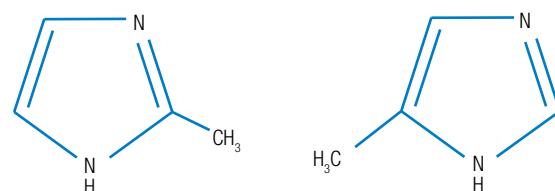
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The 2- and 4-methylimidazoles (2-MI and 4-MI) are undesired byproducts from the manufacturing of the caramel color ingredient used to darken food products such as carbonated beverages and soy sauces. Studies from the National Toxicology Program (NTP) and other researchers have concluded that there is clear evidence of both chemicals' carcinogenicity. California's Office of Environmental Health Hazard Assessment (OEHHA) listed 4-MI as a carcinogen in January 2011 with a proposed No Significant Risk Level (NSRL) of 16 µg per person per day. In February 2011, a group of scientists from the Center for Science in the Public Interests filed a petition with the U.S. FDA to bar the use of caramel coloring containing 2-MI and 4-MI, which is used for "purely cosmetic purposes." On the other hand, the European Food Safety Authority concluded that exposure to 4-MI from caramel coloring is not of concern. Although the issue is controversial, it seems prudent to take precautions, especially for sensitive populations susceptible to excessive exposure. Therefore, a quantitative analytical measurement method for both compounds is needed for exposure assessment.



2-methylimidazole
Chemical Formula: $C_4H_6N_2$
Exact Mass: 82.05
Molecular Weight: 82.10

4-methylimidazole
Chemical Formula: $C_4H_6N_2$
Exact Mass: 82.05
Molecular Weight: 82.10

Figure 4-3. Chemical structures for caramel colorants.

Column: Dionex IonPac CS19 (2 × 250 mm)
Flow: 0.25 mL/min
Temperature: 40 °C
Injection Volume: 5 µL
Eluent Source: Dionex EGC II MSA cartridge
Eluent: Isocratic 1.7 mM MSA, gradient to 7 mM MSA
From 9 to 13 min, isocratic to 25 min, back to 1.7 mM MSA at 25.1 min
Detection: Suppressed Conductivity, Thermo Scientific™ Dionex™ CSRS 300, AutoSuppression, recycle mode

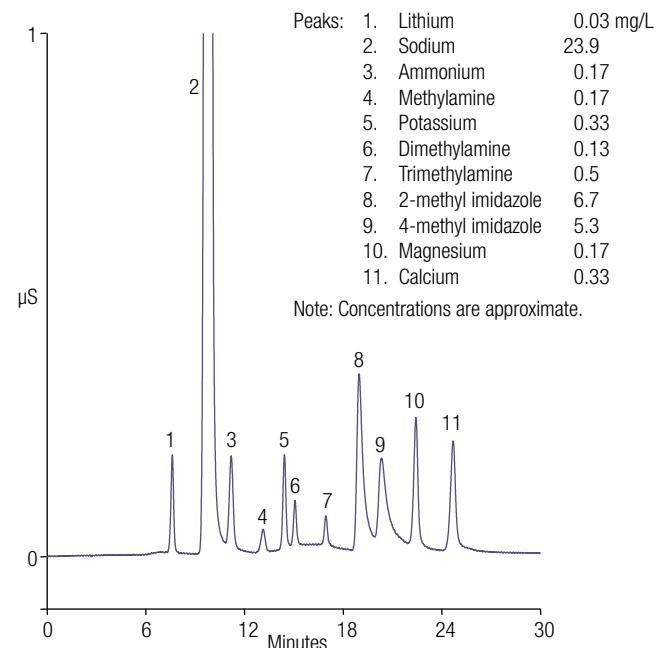


Figure 4-4. Gradient separation of six common cations, methylamines and imidazoles using the Dionex IonPac CS19 2 mm column.

Download the Poster Note: Simultaneous Quantification of 2- and 4-Methylimidazoles in Carbonated Beverages by Ultra High-Performance Liquid Chromatography Tandem Mass Spectrometry

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Artificial Colorants: General Dye Method

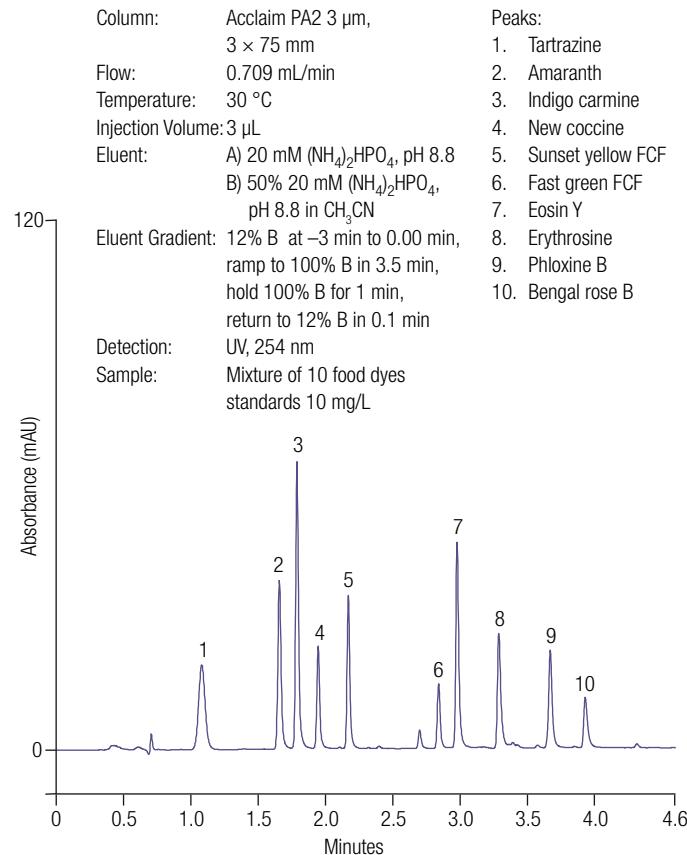


Figure 4-5. Chromatogram of the standard mixture of 10 dyes.

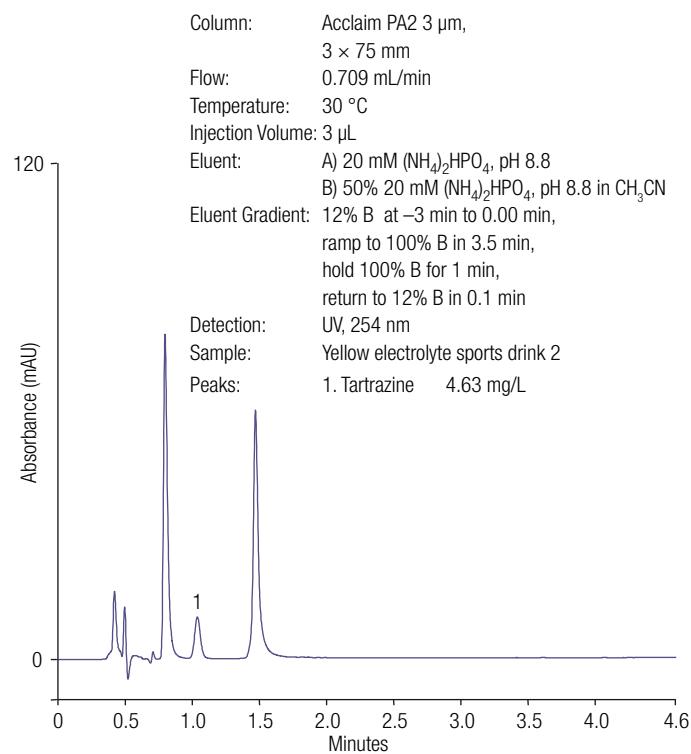


Figure 4-6. Chromatogram of an electrolyte sports drink.

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Artificial Colorants: General Dye Method

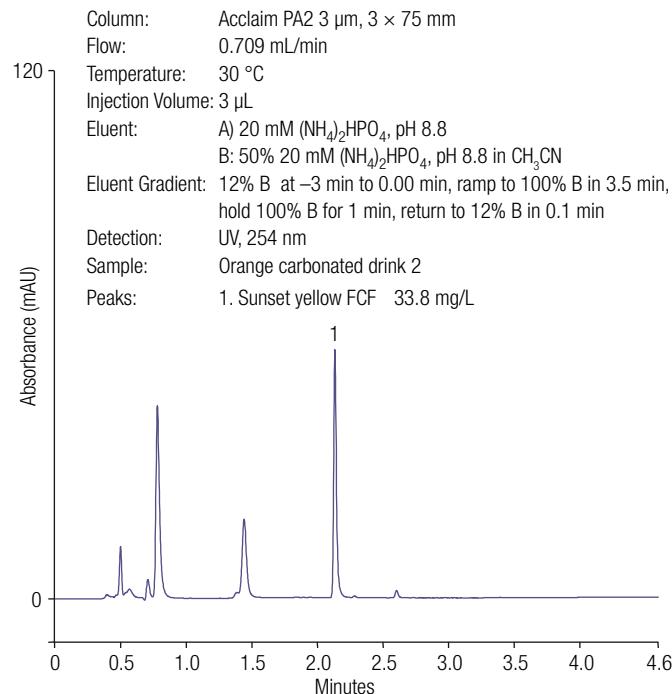


Figure 4-7. Chromatogram of an orange carbonated drink.

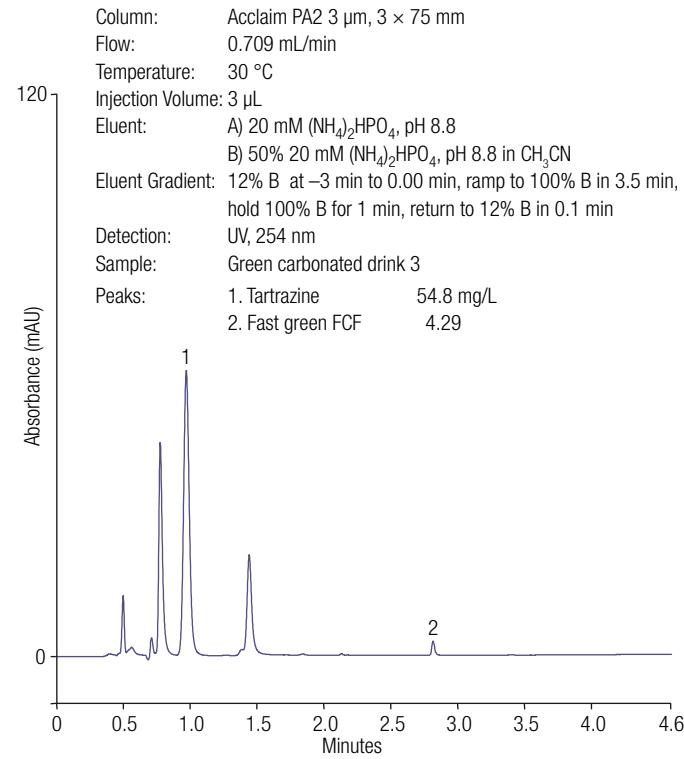


Figure 4-8. Chromatogram of a green carbonated drink.



Download Application Note 245: Fast HPLC Analysis of Dyes in Foods and Beverages

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Artificial Colorants: General Dye Method

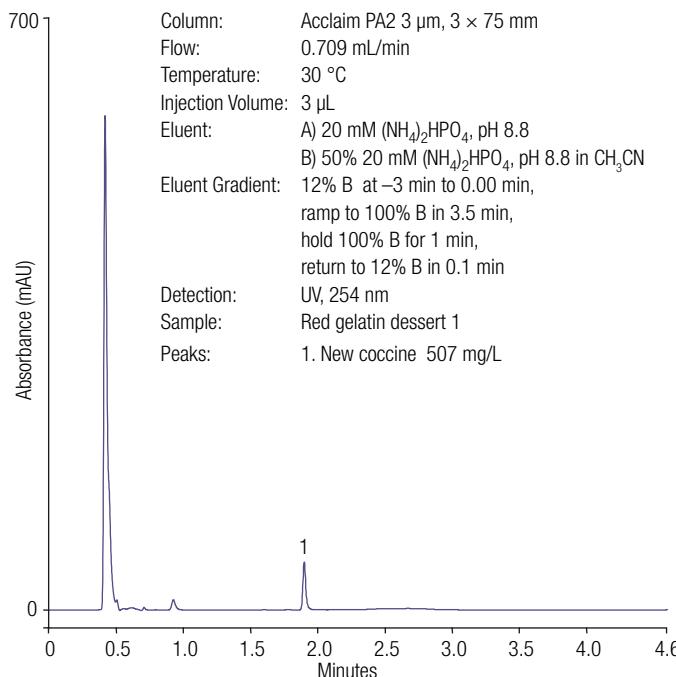


Figure 4-9. Chromatogram of a red gelatin dessert sample.



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Artificial Colorants: Global Method for Permitted and Banned Food Dyes

Colors are food additives that are regulated in the EU, USA, and China. Only approved food colors can be used and others are banned such as some azo compounds, because of their potential carcinogenicity. The so-called Sudan colors are usually used for coloring oils or as additives in shoe polish, but have been used illegally in foods. They are classified as class 3 carcinogens by the International Agency for Research on Cancer (IARC). Despite considerable publicity these banned colors are still found in foodstuffs, e.g. chili and chili containing products, according to recent RASFF alerts. Directive 2005/402/EC introduced emergency measures that has required certification of each product exported to the EU to be certified to be free of Sudan colors.

Method 52212 enables the measurement of both regulated water-soluble and banned fat-soluble colors and can be applied to liquid, semi-liquid and solid food samples. The method combines cloud point extraction and

Column: Thermo Scientific™ Hypersil GOLD™, 150 x 2.1 mm, 1.9 µm
Tray Temperature Control: 22 °C
Column Oven Temperature: 22 °C
Injection Volume: 10 µL
Injection Mode: Partial loop
Mobile Phase: A: water (0.1 % formic acid); B: acetonitrile (0.1% formic acid)

Gradient:	Time	A[%]	B[%]
	0	99	1
	5	99	1
	10	30	70
	15	25	75
	35	25	75
	35.01	99	1
	42	99	1

LC-MS to quantify banned azo colors (Sudan I-IV, Sudan red G, Sudan red B, Sudan red 7B, Sudan black B, metanil yellow and rhodamine B), at an LOD of 0.5 mg/kg. Simultaneously, regulated food colors (sunset yellow, allura red, tartrazine and erythrosine) can be analyzed in liquid, semi-liquid and solid food and beverages.

Table 4-1. Limits of detection and limits of quantification (LODs and LOQs) of the LC-MS method for different matrices

Analyte	Liquid Matrix (wine)		Semi-liquid Matrix (sauce)		Solid Matrix (chili)	
	LOD [mg/kg]	LOQ [mg/kg]	LOD [mg/kg]	LOQ [mg/kg]	LOD [mg/kg]	LOQ [mg/kg]
Allura Red	47.0	140.0	5.0	15.0	13.0	38.0
Sunset Yellow	50.0	150.0	12.0	35.0	8.3	25.0
Tartrazine	**	**	40.0	120.0	**	**
Erythrosine	30.0	90.0	30.0	90.0	30.0	90.0
Metanil Yellow	*	*	0.5	1.5	0.3	0.9
Rhodamine B	*	*	0.03	0.1	0.03	0.1
Sudan I	*	*	0.5	1.5	0.4	1.3
Sudan II	*	*	0.3	0.9	0.3	0.9
Sudan III	*	*	0.2	0.6	0.2	0.6*
Sudan IV	*	*	0.1	0.3	0.1	0.3
Sudan Red G	*	*	0.5	1.5	0.4	1.2
Sudan Red B	*	*	0.5	1.5	0.5	1.5
Sudan Red 7B	*	*	0.5	1.5	0.5	1.5
Sudan Black B 1 st	*	*	0.03	0.1	0.03	0.1
Sudan Black B 2 nd	*	*	0.3	0.9	0.3	0.9

* Colors were not spiked because not relevant to food

** Compound not applicable for this matrix

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Artificial Colorants: Global Method for Permitted and Banned Food Dyes

MS analysis is carried out using a MSQ Plus LC single quadrupole mass spectrometer. Data acquisition and processing was performed using Thermo Scientific™ Xcalibur™ software. The MS conditions were as follows:

Ionization: Electrospray
Polarity: Positive negative switching
Probe Temperature: 450 °C
Cone Voltage: 150 V
Scan Mode: Multi ion monitoring (MIM)

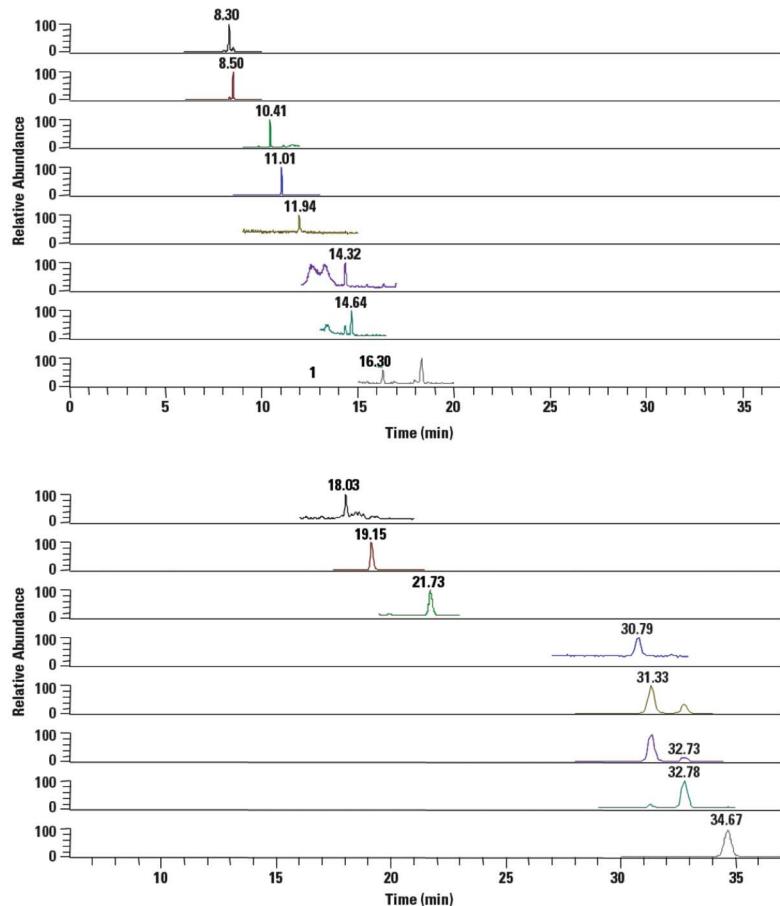


Figure 4-10. LC-MS chromatogram: separation and detection of all colors in sauce matrix.

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Artificial Colorants: Red Dyes in Cereals

Pump: UltiMate LPG-3400
Column: Acclaim 120 C18 3 μ m, 3.0 \times 75 mm
Flow: 1.0 mL/min
Temperature: UltiMate TCC-3100 at 30 °C
Injection: UltiMate WPS-3000 SL sampler at 8 μ L
Mobile Phase: 677 g water, 0.97 g Na₂SO₄, 2.24 g KH₂PO₄, 3.20 g 55% Bu₄NOH (pH = 6.75), 250 g acetonitrile
Detector: UltiMate VWD-3400, VIS at 427, 508, 625 nm, 2.5 Hz, 0.6 s time const.
Sample Prep.:
(1) Sort cereal by color
(2) Grind to coarse powder
(3) Extract 0.2 g of cereal with 5 mL mobile phase
(4) Filter through 0.1 μ m membrane
Peaks: 1. FD&C Red #40

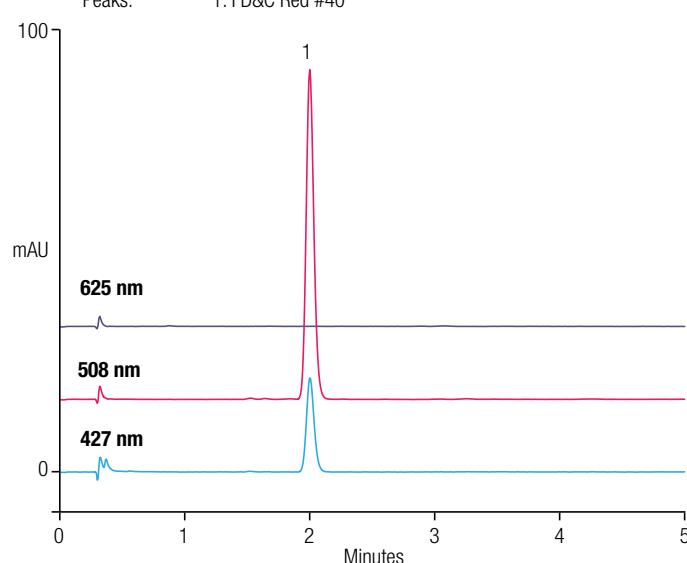


Figure 4-11. FD&C red #40 used in a cherry-flavored cereal.

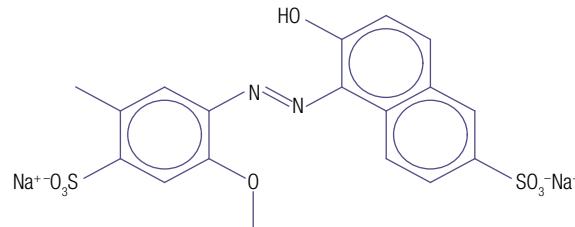


Figure 4-12. FD&C Red #40 dye structure.



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Artificial Colorants: Sudan Dyes in Chili Oil

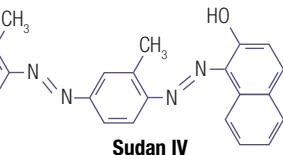
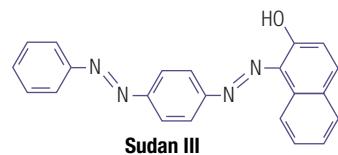
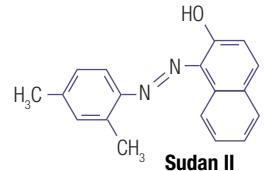
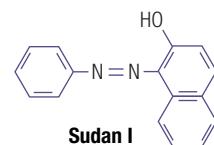


Figure 4-13. Structures of Sudan dyes I, II, III, and IV.

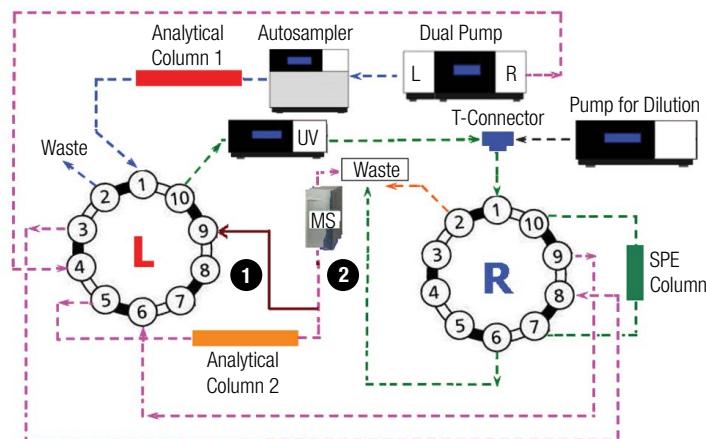


Figure 4-14. Flow schematics for a two-dimensional HPLC with online SPE intercolumn trapping system. Flow path 1 is configured for method development using a UV-vis detector; flow path 2 is configured for the determination of Sudan dyes in chili oil using an MS detector.



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Artificial Colorants: Sudan Dyes in Chili Oil

Column: Analytical column 1 (for the first dimension)
Acclaim PA2, 3 μ m, 3.0 \times 150 mm (P/N 063705)
On-line SPE
Acclaim 120 C18, 5 μ m, Guard Cartridge, 4.6 \times 10 mm (P/N 069580)
Analytical column 2 (for the second dimension)
Acclaim RSLC 120 C18, 2.2 μ m, 2.1 \times 100 mm (P/N 068982)
Flow: For analytical column 1 (the first dimension), 0.6 mL/min
For on-line SPE, 1.0 mL/min
For analytical column 2 (the second dimension), 0.3 mL/min
Column Temp.: 30 °C
Injection Volume: 20 μ L on the analytical column 1
Mobile Phase: For analytical column 1 (the first dimension)
A: Water
B: CH₃CN
C: CH₃OH/THF, 1:1 (v/v)
In gradient: -3.5 ~ -0.5 min, B 50%, C 20%; 5.5 min, B 50%,
C 50%; 11.0 ~ 14.0 min, B 20%, C 80%; 14.1 ~ 23 min, B 50%, C 20%
For on-line SPE
0.1% FA in water, isocratic
For analytical column 2 (the second dimension):
A: Water
B: CH₃CN
C: 0.1% FA in CH₃CN
In gradient: -3.5 ~ 7.0 min, B 50%; 16.0 ~ 22.4 min,
B 90%; 22.5 ~ 23.0 min, B 50%.
A and C maintain 40% and 10%, respectively.
UV-vis Detection: Absorbance at 500 nm
Chromatograms: (A) Sudan dye standards (2 mg/L for each Sudan dye)
(B) Chili oil sample, spiked with 6 mg/L of each Sudan dye standard
Peaks:
1. Sudan dye I
2. Sudan dye II
3. Sudan dye III
4. Sudan dye IV

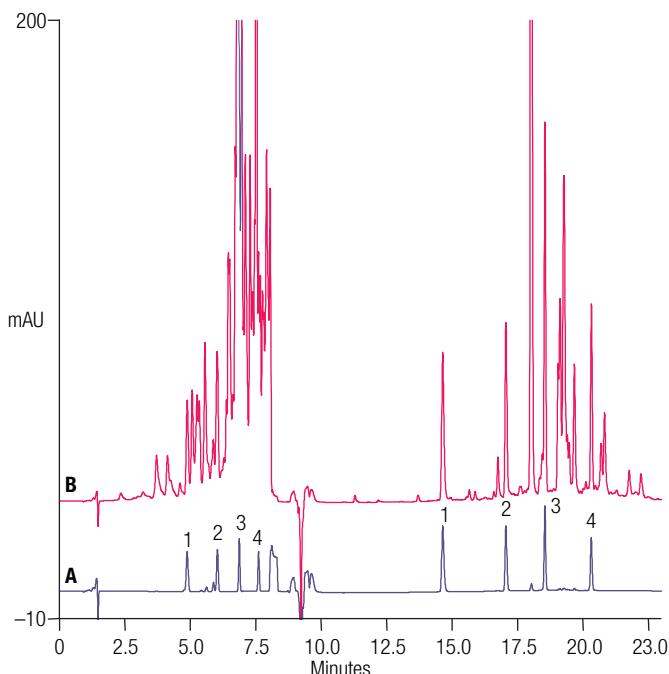


Figure 4-15. Chromatograms of (A) Sudan dye standards (2 mg/L for each Sudan dye), and (B) chili oil sample, spiked with 6 mg/L of each dye standard using the two-dimensional HPLC with on-line SPE intercolumn trapping system with UV-vis detection. The second dimension separation starts at 10 min.

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- Column: Analytical column 1 (for the first dimension)
Acclaim PA2, 3 µm, 3.0 × 150 mm (P/N 063705)
On-line SPE
Acclaim 120 C18, 5 µm, Guard Cartridge, 4.6 × 10 mm (P/N 069580)
Analytical column 2 (for the second dimension)
Acclaim RSLC 120 C18, 2.2 µm, 2.1 × 100 mm (P/N 068982)
- Flow: For analytical column 1 (the first dimension), 0.6 mL/min
For on-line SPE, 1.0 mL/min
For analytical column 2 (the second dimension), 0.3 mL/min
- Column Temp.: 30 °C
- Injection Volume: 20 µL on the analytical column 1
- Mobile Phase: For analytical column 1 (the first dimension)
A: Water
B: CH₃CN
C: CH₃OH/THF, 1:1 (v/v)
In gradient: -3.5 ~ -0.5 min, B 50%, C 20%; 5.5 min, B 50%, C 50%; 11.0 ~ 14.0 min, B 20%, C 80%; 14.1 ~ 23 min, B 50%, C 20%
For on-line SPE
0.1% FA in water, isocratic
For analytical column 2 (the second dimension):
A: Water
B: CH₃CN
C: 0.1% FA in CH₃CN
In gradient: -3.5 ~ 7.0 min, B 50%; 16.0 ~ 22.4 min, B 90%; 22.5 ~ 23.0 min, B 50%.
A and C maintain 40% and 10%, respectively.
- MS Detection: ESI mode, positive scan, probe temp at 450 °C, needle voltage at 4000 V, SIM at 249 m/z for Sudan dye I, 277 m/z for Sudan dye II, 353 m/z for Sudan dye III, and 381 m/z for Sudan dye IV; cone voltage at 35 V for Sudan dye I and II, 50 V for Sudan dye III and IV; nebulizer gas, Nitrogen at 75 psi
- Chromatograms: (A) blank
(B) Sudan dye standards (5 µg/L for Sudan dyes II and III, and 15 µg/L for Sudan dyes I and IV)
(C) chili oil sample
(D) Chili oil sample, spiked with 10 µg/L of Sudan dye II and III, and 30 µg/L of Sudan dye I and IV standards
- Peaks: 1. Sudan dye I
2. Sudan dye II
3. Sudan dye III
4. Sudan dye IV

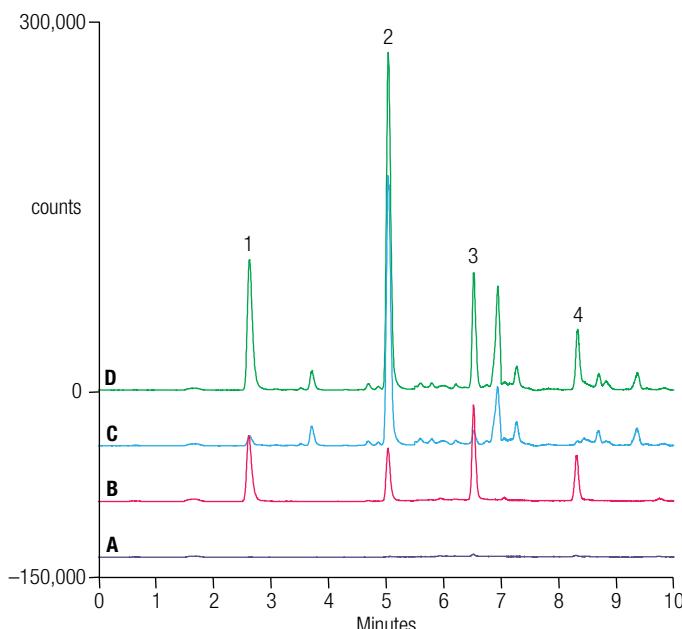


Figure 4-16. MS TIC chromatograms of (A) blank (CH₃CN), (B) mixed Sudan dye standards (5 µg/L for Sudan dye II and III, and 15 µg/L for Sudan dye I and IV), (C) chili oil sample, and (D) the same sample spiked with 10 µg/L of Sudan dyes II and III, and 30 µg/L of Sudan dyes I and IV using the two-dimensional HPLC with on-line SPE intercolumn trapping system with MS detection.

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Artificial Colorants: Sudan Dyes in Curry Paste

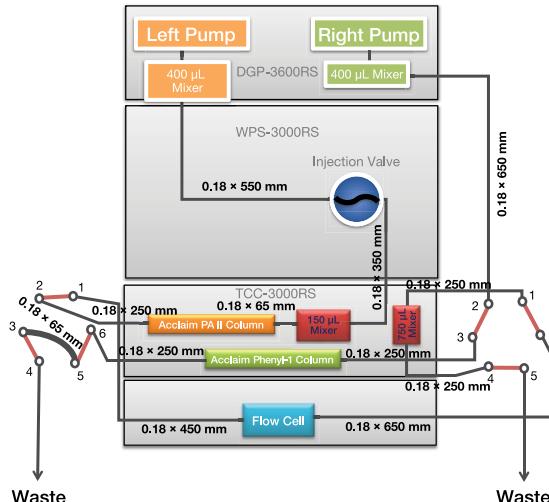


Figure 4-17. System configuration.

Table 4-2. Gradient program and valve switching.

First Dimension (Left Pump)					Valve Switching			Second Dimension (Right Pump)				
Time (min)	Flow Rate (mL/min)	% A (Water)	% B (CH_3CN)	% C ($\text{C}_2\text{H}_5\text{OH}$)	Time	Right Valve Position	Left Valve Position	Time (min)	Flow Rate (mL/min)	% A (Water)	% B (CH_3CN)	% C (CH_3OH)
0.00	1.0	80	20	0	0.00	1-2	1-2	0.00	1.0	85	0	15
2.00	1.0	5	95	0	6.773	6-1	1-2	13.0	1.0	85	0	15
13.5	1.0	5	95	0	7.042	1-2	1-2	14.0	1.0	0	0	100
14.00	0.7	0	5	95	7.947	6-1	1-2	15.0	1.0	0	0	100
19.00	0.7	0	5	95	8.164	1-2	1-2	16.0	1.0	0	45	55
20.0	0.7	5	95	0	9.583	6-1	1-2	25.0	1.0	0	45	55
22.0	0.7	5	95	0	9.811	1-2	1-2	—	—	—	—	—
22.5	1.0	80	20	0	11.990	6-1	1-2	—	—	—	—	—
25.0	1.0	80	20	0	12.290	1-2	1-2	—	—	—	—	—
—	—	—	—	—	13.500	6-1	6-1	—	—	—	—	—

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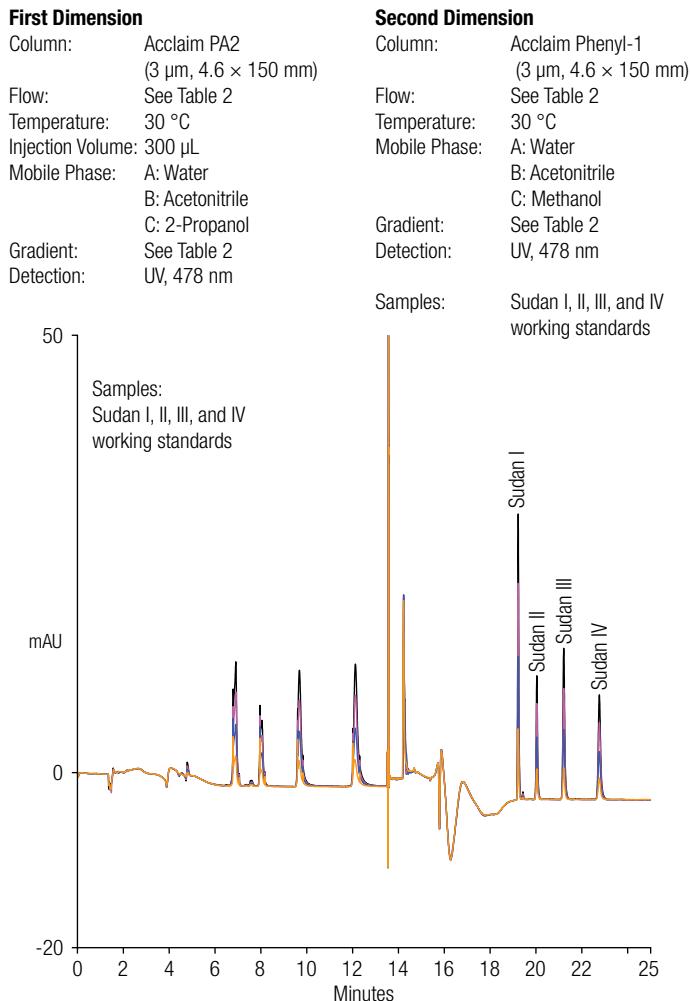


Figure 4-18. Overlay of chromatograms of working standards.

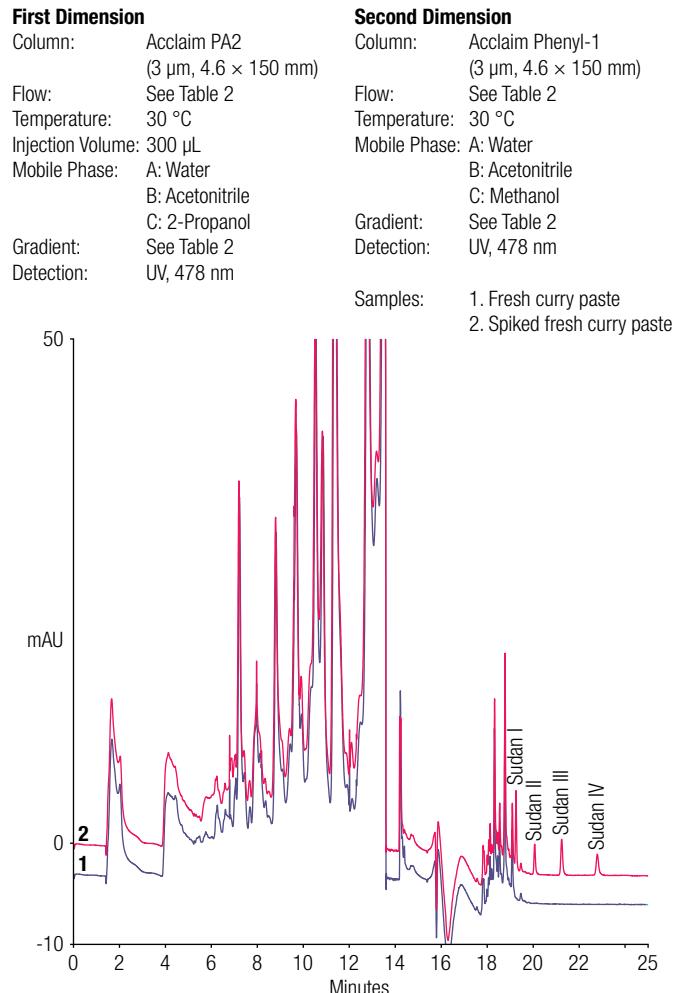


Figure 4-19. Overlay chromatograms of fresh curry paste and spiked fresh curry paste samples.

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Artificial Colorants: Sudan Dyes in Paprika

Sudan dyes are a class of synthetic dyes that are mainly used for industrial applications such as the coloring of plastic. These dyes are banned as a food-coloring agent because they are classified as carcinogens. For economical reasons, however, Sudan dyes are sometimes illegally used to color food to improve its appearance. Therefore, methods are needed to determine if food products have been adulterated with these dyes.

There have been cases of adulteration of capsicum products with Sudan Orange and other illegal dyes that resulted in large-scale product recalls. The chromatography shown here cleanly resolves the Sudan dyes while compressing the natural pigments into a well-resolved band. The example shown here has >85% recovery for all dyes.



Column: Acclaim 120 C18, 5 µm, 4.6 × 150 mm

Flow: 1.25 mL/min

Temperature: 30 °C

Injection: Summit ASI-100, 15 µL

Pump: Summit P680 DGP

Mobile Phase: (A) Water

(B) 1:1 tetrahydrofuran:methanol

Gradient: Times 0 7.0 7.1 13

%A 20 8 0 0

%B 80 92 100 100

Detector: Diode array, 500 nm, and spectra 200–595 nm

Samples: (A) Control paprika without artificial coloring

(B) Paprika spiked with approx. 25 mg/kg of each dye

Peaks:

1. Sudan I
 2. Sudan II
 3. Sudan III
 4. Sudan IV
 5. Oil Red O (2 peaks)
- (Natural pigments 8–12 min)

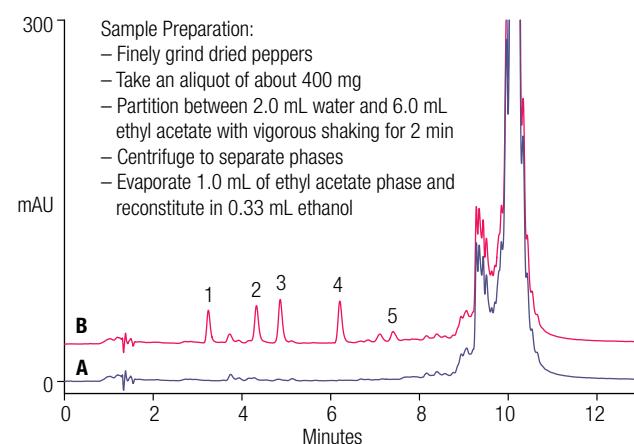


Figure 4-20. Overlay chromatograms of control and paprika spiked with sudan dyes.

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Artificial Colorants: Yellow Dyes in Cereals

Pump: UltiMate LPG-3400
Column: Acclaim 120 C18 3 µm, 3.0 × 75 mm
Flow: 1.0 mL/min
Temperature: UltiMate TCC-3100 at 30 °C
Injection: UltiMate WPS-3000 SL sampler at 8 µL
Mobile Phase: 677 g water, 0.97 g Na₂SO₄, 2.24 g KH₂PO₄, 3.20 g 55% Bu₄NOH (pH = 6.75), 250 g acetonitrile
Detector: UltiMate VWD-3400, VIS at 427, 508, 625 nm, 2.5 Hz, 0.6 s time const.
Sample Prep.:
(1) Sort cereal by color
(2) Grind to coarse powder
(3) Extract 0.2 g of cereal with 5 mL mobile phase
(4) Filter through 0.1 µm membrane

Peaks:
1. FD&C Yellow #6

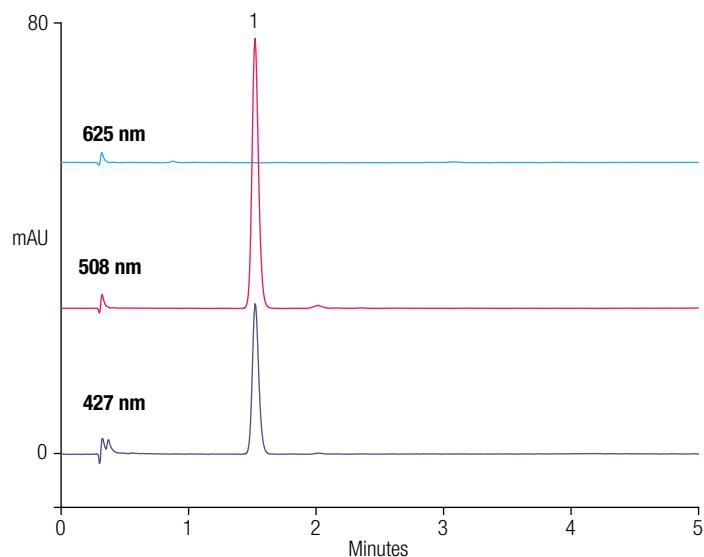


Figure 4-21. FD&C yellow #6 used in an orange-flavored cereal.

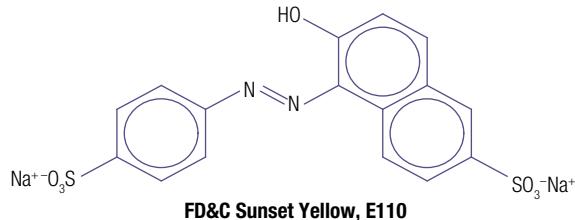


Figure 4-22. FD&C yellow #6 dye structure.



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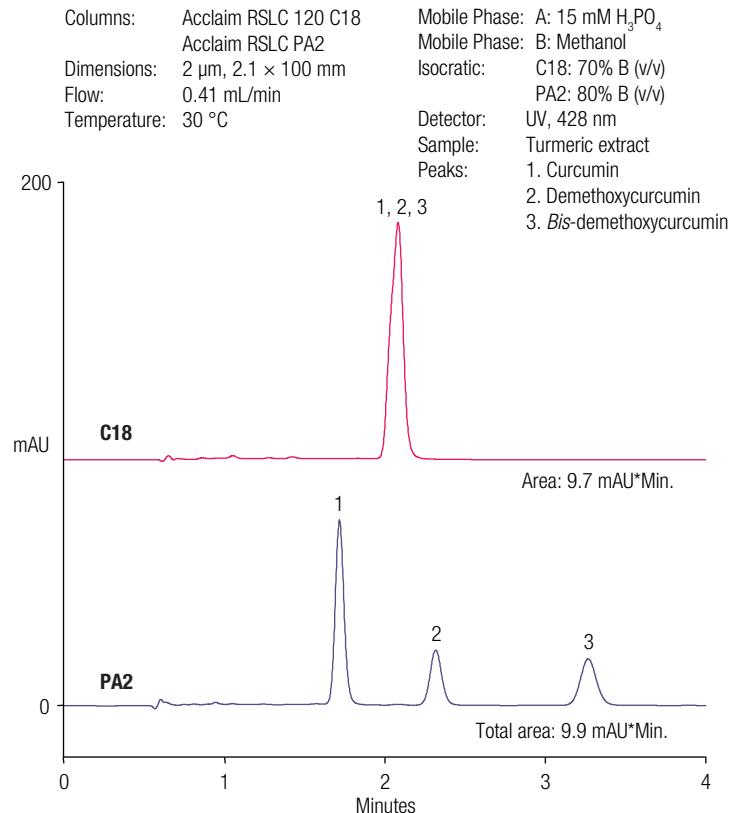
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Natural Colorants: Turmeric by UV

Pigments in Turmeric

Turmeric (*Curcuma longa*) is used as a spice, a natural yellow food color, an herbal medicine, and even a colorimetric reagent. This versatile plant has three major yellow pigments, but they are not well resolved on C18 columns. Using the alternate selectivity of the Acclaim PolarAdvantage II columns gives excellent separation.



Did You Know?

- Turmeric is a member of the ginger family.
- It is used to color foods yellow (designated E100) and as a fabric dye, although it is not very light stable.
- It is used in Ayurvedic medicine to treat stomach and liver ailments and is being investigated in Western medicine as a treatment to Alzheimer's, diabetes, and other diseases



Figure 4-23. Improved resolution of curcuminoids using an Acclaim PA2 column.

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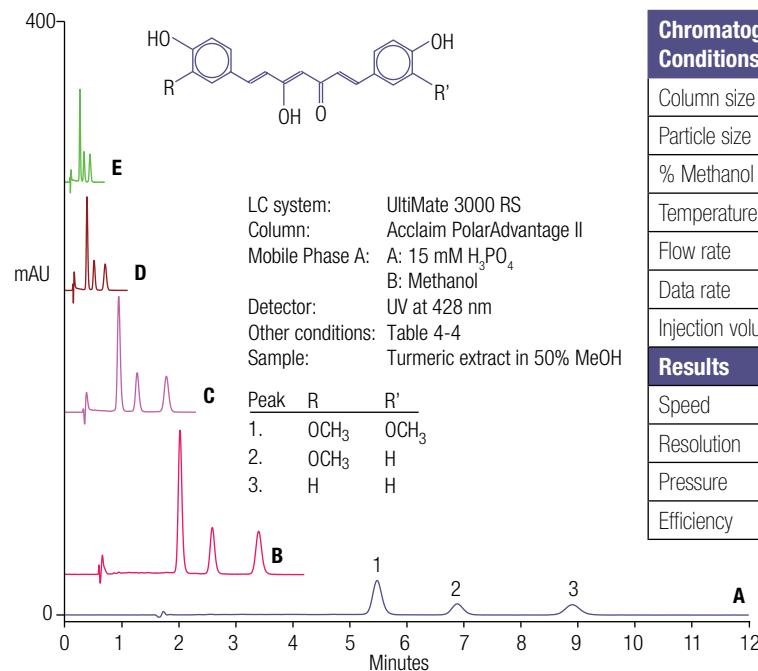
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Natural Colorants: Turmeric by UV

Chromatogram Conditions	A	B	C	D	E
Column size	4.6 × 150 mm	3.0 × 75 mm	2.1 × 50 mm	2.1 × 150 mm	2.1 × 150 mm
Particle size	4.5 µm	3.0 µm	2.2 µm	2.2 µm	2.2 µm
% Methanol	80%	80%	80%	80%	75%
Temperature	30 °C	30 °C	30 °C	30 °C	45 °C
Flow rate	1.00 mL/min	0.61 mL/min	0.41 mL/min	1.00 mL/min	1.50 mL
Data rate	2 hz	4 hz	4 hz	10 hz	20 hz
Injection volume	3.0 µL	3.0 µL	1.2 µL	1.2 µL	1.2 µL
Results					
Speed	1 time	3 times	4 times	11 times	20 times
Resolution	4.2 (R1,2)	4.2 (R1,2)	3.2 (R1,2)	2.3 (R1,2)	2.0 (R1,2)
Pressure	89 bar	140 bar	174 bar	417 bar	529 bar
Efficiency	5270 Th. Plates	4860 Th. Plates	2370 Th. Plates	1150 Th. Plates	1160 Th. Plates

Figure 4-24. Separation of pigments in turmeric. The table shows increasing degrees of acceleration by using smaller particle sizes, shorter columns, faster flow rates, and higher temperatures. With a speed-up factor as high as 20, and a solvent savings of 93%, a baseline-resolved chromatogram is still obtained.

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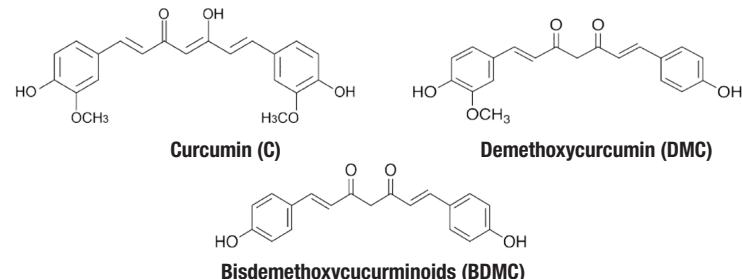


Figure 4-25. Chemical structure of curcuminoids.

Method for Figures 4-26, 4-27, and 4-28

Liquid Chromatography

An UltiMate 3000 HPLC system consisting of:

- LPG-3400BM Pump
- WPS-3000 TBSL Autosampler
- TCC-3000RS Thermostatted Column Compartment
- ECD-3000RS Electrochemical Detector with 6011RS ultra Coulometric Analytical cell, E1 = E2 = 700 mV
- FLD-3400RS Fluorescence Detector with Dual PMT, Excitation 426 nm, Emission 539 nm
- VWD-3100 Variable Wavelength Detector, 426 nm

Data Analysis and Processing

Chromatographic data were collected using Chromeleon CDS 6.8 (SR9)

Standard Preparation

Stock solution of C, DMC, and BDMC were prepared in methanol at 1 mg/mL. Working standards were prepared by dilution of stock solution in mobile phase.

Sample Preparation

Turmeric powder, yellow curry powder, and a pellet curry sauce sample were prepared at 1 mg/mL in methanol, then vortex-mixed and sonicated for 10 min. The solution was filtered through a 0.22 µm centrifuge tube filter and diluted with mobile phase prior to analysis.

Natural Colorants: Turmeric by UV, FLD, and ECD

Analytical Column: Acclaim RSLC PA2, 2.1 × 50 mm, 3 µm
Flow: 0.4 mL/min
Injection Volume: 2 µL
Mobile Phase: 25% 100 mM phosphate buffer, pH 3, 75% methanol

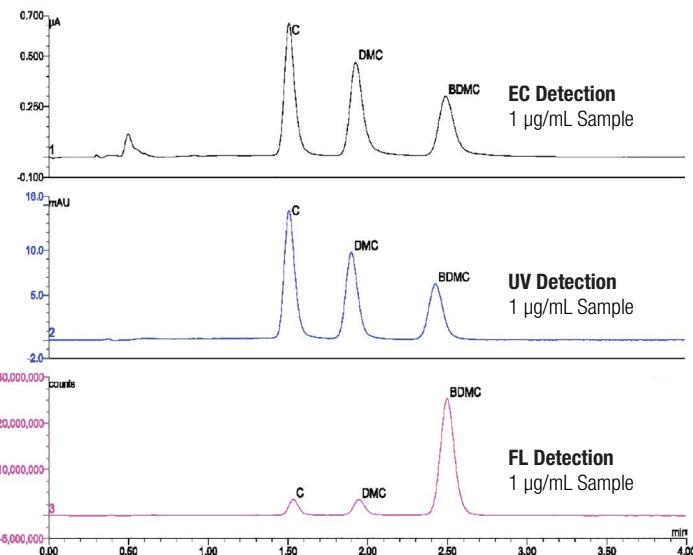


Figure 4-26. Chromatograms showing separation of curcuminoids in 1 µg/mL standard using EC, UV, or FL detection

Table 4-3. Limits of detection (LoD) of the three curcuminoids using UV, FL, and EC detection

Detector	LoD (pg on column)	
	C	DMC
UV	10	20
FL	20	20
EC	2	2

Download the Poster Note: The Quantitative Analysis of Curcuminoids in Food and Food Additives Using Rapid HPLC With Electrochemical, UV, or Fluorescence Detection

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Choline

Glucosamine

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Analytical Column: Acclaim RSLC PA2, 2.1 × 50 mm, 3 µm
Flow: 0.4 mL/min
Injection Volume: 2 µL
Mobile Phase: 25% 100 mM phosphate buffer, pH 3, 75% methanol

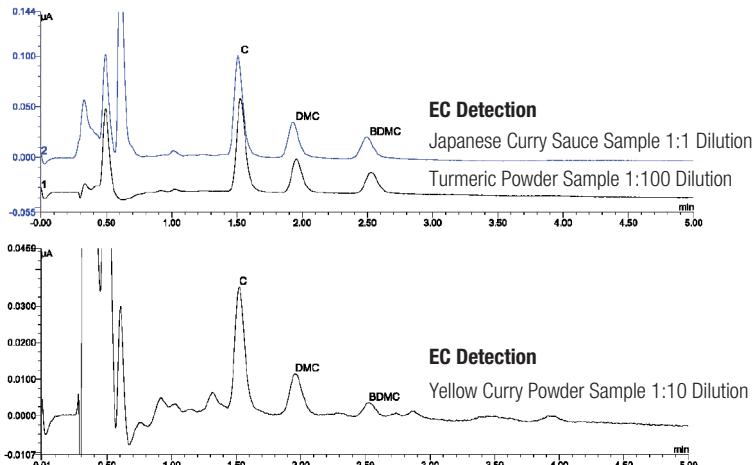


Figure 4-27. EC chromatogram of turmeric powder, yellow curry powder, and a Japanese curry sauce sample.

Table 4-4. Curcuminoid content in tested samples (ng/mg sample).

Sample Name	C	DMC
Japanese curry sauce	127	57.5
Turmeric powder	17,255	7,797
Yellow curry powder	1,804	813

Natural Colorants: Turmeric by UV, FLD, and ECD

Curcuminoids can be measured using HPLC with UV, FL, or EC detection. However, EC detection is the preferred approach for measurement of degradation and related products, and also has the sensitivity and selectivity for measurement of these analytes *in vivo*.

Analytical Column: Acclaim RSLC PA2, 2.1 × 50 mm, 3 µm
Flow: 0.4 mL/min
Injection Volume: 2 µL
Mobile Phase: 25% 100 mM phosphate buffer, pH 3, 75% methanol

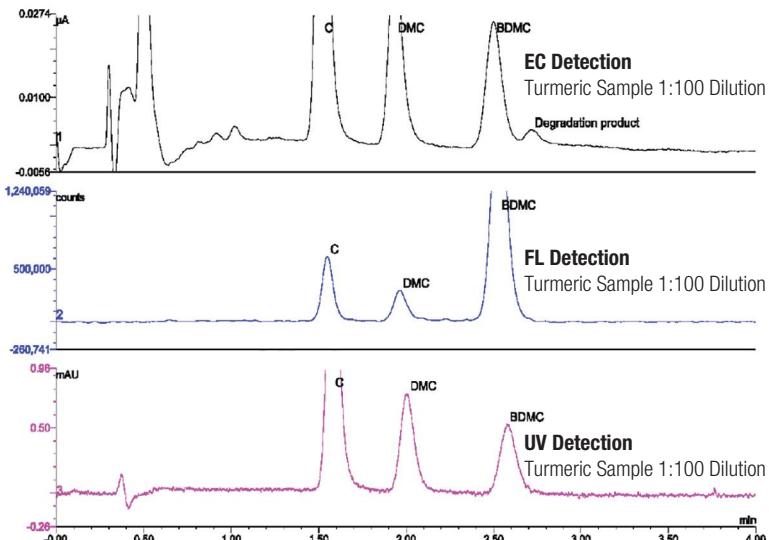


Figure 4-28. Chromatograms showing a degradation product of curcuminoids determined by EC, but not by UV or FL detection.

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Flavorants

A flavorant is defined as an additive, either artificial or natural, that is used to give foods and beverages flavor. Flavorants include chemicals that can impart or enhance a particular taste e.g., sweet, sour, tangy, savory, or bitter.

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Although not an additive, *per se*, Amadori products add to flavor and appearance. Amadori products are reactive amino acid sugar conjugates formed by the Maillard reaction of an α -hydroxy carbonyl moiety of a reducing sugar and an amino group of an amino acid. This reaction can be initiated even under mild conditions; food products stored at room temperature may eventually turn brown due to the reaction of reducing sugars with amino acids. However, during high-temperature processing of food, further degradation of Amadori products, formed in the early phase of the Maillard reaction, results in the formation of complex mixtures of heterocyclic, polymeric, and other compounds. These products are directly responsible for the distinctive aroma and brown color of baked and roasted food products.



Flavorants: Amadori Compounds

Column: Acclaim PA (3 μ m, 120 , 2.1 \times 150 mm)

Flow: 0.25 mL/min

Temperature: 30 °C

Injection Volume: 10 μ L

Eluent: 40 mmol/L formic acid in water

Detection: MSQ, ESI+, needle voltage: 3.0 kV;
probe temperature: 350 °C; cone voltage: 50 V;
dwell time: 1 μ s; SIM as indicated, time window: 0—15 min

Peaks:

1. Fru-Gly
2. Fru-Pro
3. Fru-Glu

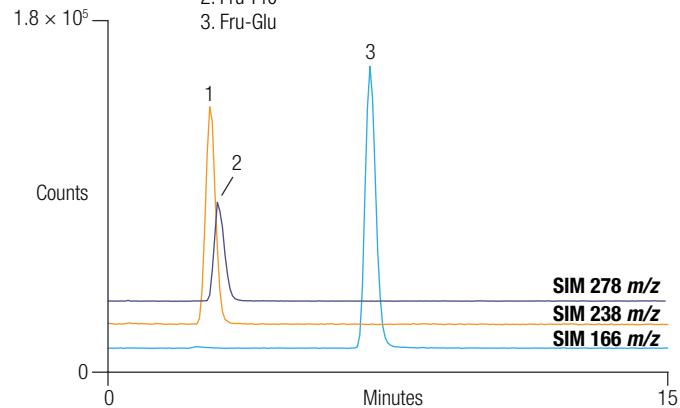


Figure 4-29. SIM chromatogram of different amadori compounds 1) fructosyl-glycine; 2) fructosyl proline and 3) fructosyl-glutamate.

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Chili peppers (*Capsicum* sp.) are very popular ingredients in cooking worldwide. The active principles (mainly capsaicin and dihydrocapsaicin) are present in variable amounts depending on the growth conditions, so a reliable quality control method is needed. HPLC has displaced the older Scoville test based on sensory evaluation. Chilies also contain pigments, vitamins, flavonoids, and many other substances. Therefore, the ideal method should have high-resolution, high-throughput, and easy, sample cleanup. The use of the 2.2 µm Acclaim RSLC column in 2.1 mm i.d. format allows fast analysis time with reduced solvent consumptions. Shown here are two examples of separation of capsaicin and dihydrocapsaicin in dried pasilla chilies and habanero sauce. The Acclaim RSLC 120 C18 column provides excellent resolution and fast analysis at the same time, even with a very minimal sample cleanup.

Did You Know?

- The perceived heat of a chili is measured in Scoville units.
- Pure capsaicin is equivalent to 16M Scoville units.
- Resiniferatoxin, found in resin surge is equivalent to 15B Scoville units and actually can inflict chemical burns.

Flavorants: Capsaicin by UV

LC System: UltiMate 3000 RS
Column: Acclaim RSLC 120 C18, 2.2 µm, 2.1 x 100 mm
Flow: 0.80 mL/min
Temperature: 60 °C
Injection Volume: 2 µL; bypass mode at 0.25 min, reset at -1.0 min.
Mobile Phases: A: 2 mM NH₄OAc, pH 5.4
B: Acetonitrile
Time (min) 0.3 0.0 0.25 3.0 3.01 5.0
%A 55 55 55 30 0 0
%B 45 45 45 70 100 100
Detection: UV at 280 nm (shown); spectra 220-800 nm
10 Hz data rate, 0.3 sec time constant
Samples:
A. Natural capsaicin 50 µg/mL in EtOH (denatured 3A)
B. Dried pasilla chili

- Extract 0.5 g ground chili with 20 mL EtOH and sonication.
- To a 4.5 mL aliquot, add 1.0 mL heptane, 1.0 mL water, and shake.
- Analyze lower (ethanol) layer

C. Habanero sauce

- Mix 4.5 g of sauce and 16 mL of EtOH with sonication.
- To a 5 mL aliquot, add 1.0 mL heptane and shake.
- Analyze lower layer.

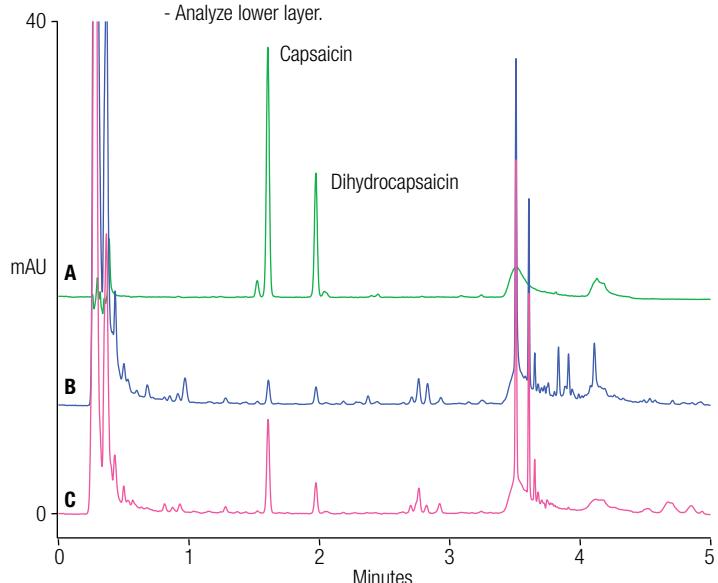


Figure 4-30. Capsaicin in chilies on Acclaim RSLC 120 C18 column.

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Flavorants: Characteristics of Capsaicin

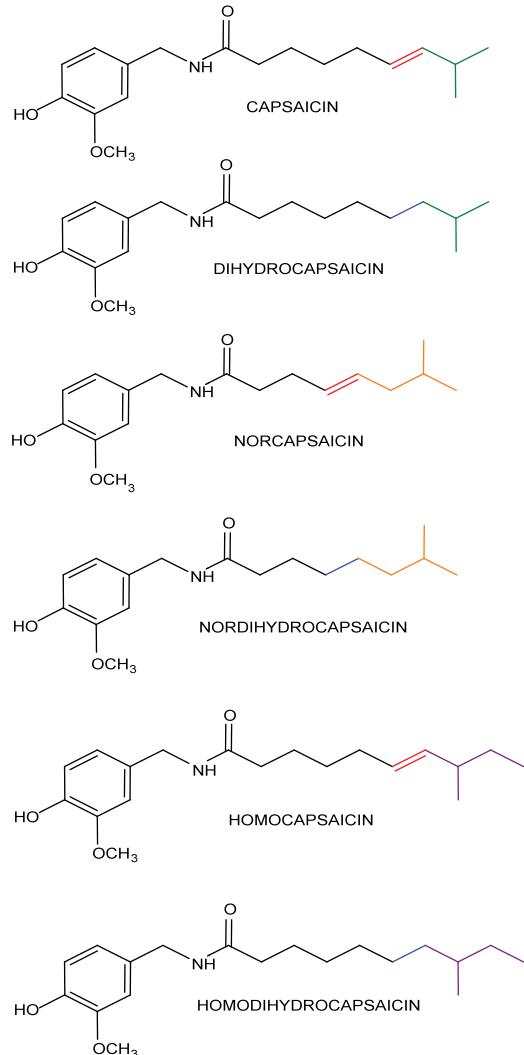


Figure 4-31. Chemical structures of different capsaicin compounds.

Table 4-5. Heat ranking of different chili peppers.

Scoville Heat Units	Examples
1,500,000–2,000,000	Trinidad Moruga Scorpion
855,000–1,463,700	Naga Viper pepper, Infinity Chilli, Bhut Jolokia chili pepper, Trinidad Scorpion Butch T pepper, Bedfordshire Super Naga, 7-Pot Chili
350,000–580,000	Red Savina habanero
100,000–350,000	Habanero chili, Scotch bonnet pepper, Datil pepper, Rocoto, Piri Piri Ndungu, Madame Jeanette, Peruvian White Habanero, Jamaican hot pepper, Guyana Wiri Wiri, Fatalii
50,000–100,000	Byadgi chili, Bird's eye chili (aka. Thai Chili Pepper), Malagueta pepper, Chiltepin pepper, Piri piri (African bird's eye), Pequin pepper, Siling Labuyo (native chili cultivar from the Philippines)
30,000–50,000	Guntur chili, Cayenne pepper, Ají pepper, Tabasco pepper, Cumari pepper (<i>Capsicum Chinense</i>)
10,000–23,000	Serrano pepper, Peter pepper, Aleppo pepper
3,500–8,000	Espelette pepper, Jalapeño pepper, Chipotle, Guajillo pepper, New Mexican peppers, Hungarian wax pepper, Tabasco sauce
1,000–2,500	Anaheim pepper (cultivar of New Mexican peppers), Poblano pepper, Rocotillo pepper, Peppadew, Sriracha sauce, Gochujang
100–900	Pimento, Peperoncini, Banana pepper, Cubanelle
No significant heat	Bell pepper, Aji dulce

Did You Know?

- Capsaicin gives chilies their burning taste
- The seeds themselves do not contain capsaicin. The highest concentration of capsaicin can be found in the white pith of the inner wall where the seeds are attached.

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Flavorants: Capsaicin by ECD

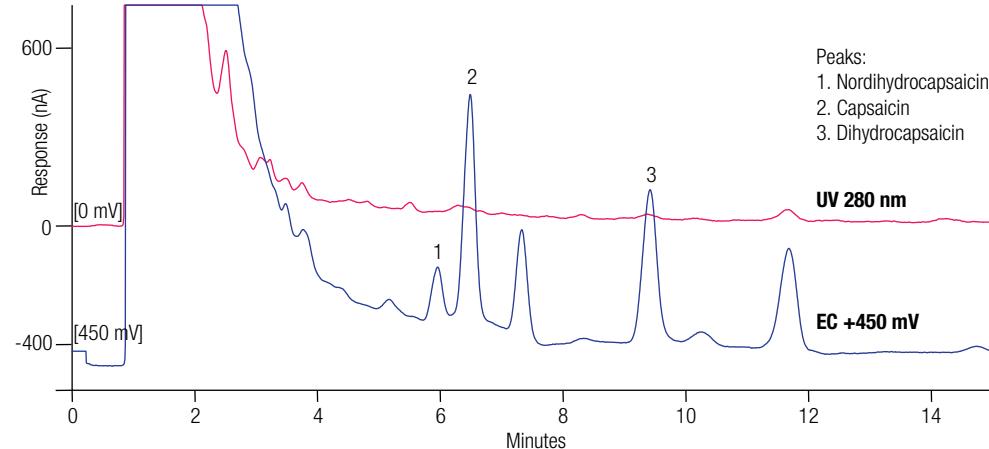


Figure 4-32. Targeted analysis of an American Spice Trade Association chili sample showing the resolution of capsaicinoids and the superior sensitivity of electrochemical detection.

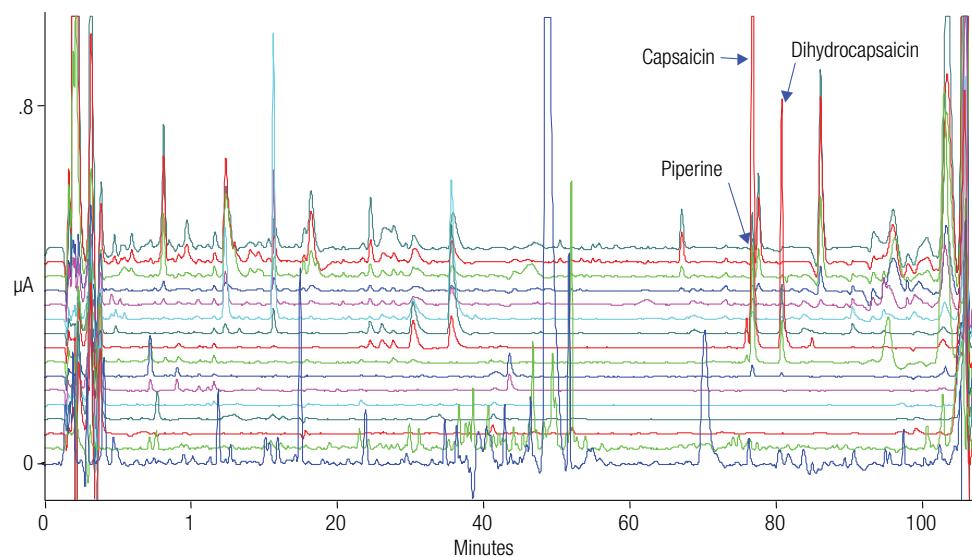


Figure 4-33. Gradient array chromatogram of chili extract.

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Flavorants: Quinine in Tonic Water

Quinine is a toxic bitter alkaloid extracted from the bark of the cinchona tree (*Cinchona* spp.). Quinine was the first medication to successfully treat malaria. Its addition to tonic water gave rise to the classic summer cocktail—gin and tonic—which proved an easy way for British colonists in India to take a mild dose of medicine. Today, quinine is still added to tonic water, but at a much lower level.

Did You Know?

Quinine is naturally fluorescent, causing tonic water to glow blue when exposed to UV light. Too much quinine causes quinism also known as cinchonism. Symptoms include dizziness, tinnitus and vision problems amongst others. Pilots in the armed forces are advised not to consume tonic water 3 days before flying and not to consume more than 36 oz of tonic water per day.

Column: Acclaim PA C16, 5 μ m,
4.6 \times 150 mm
Mobile Phase: A. 896 mL water, 100 mL acetonitrile,
1.40 mL methanesulfonic acid,
0.80 mL acetic acid,
2.00 mL diethylamine
B. 70:30 v:v acetonitrile:water
Gradient: Time 0.0 10.5 10.6 15.0
%A 100 100 50 50
%B 0 0 50 50
Flow: 1.0 mL/min
Temperature: 30 °C
Inj. Volume: 25 μ L
Detection: UV at 235 nm
Sample Prep.: Filter

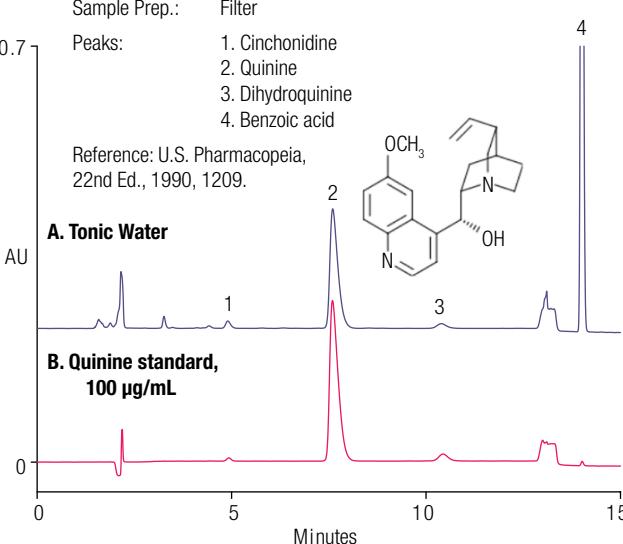


Figure 4-34. The Acclaim PolarAdvantage column can be used to rapidly determine the flavor additive quinine, as well as preservatives including benzoic acid.

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Flavorants: Quinine in Tonic Water

Quinine can be determined by HPLC with UV or fluorescence detection with high sensitivity. For peak confirmation, MS is used.

Column: Acclaim 120 C18, 3 μ , 120 Å, 2.1 × 150 mm
Temperature: 35 °C
Flow: 0.25 mL/min
Injection Volume: 10 μ L
Eluent: 20% methanol, 10% acetonitrile, 70% water
Detection: 1. UV: 254 nm; 3-D: 200–800 nm
2. MSQ, ESI+, needle voltage: 3.0 kV;
probe temperature: 350 °C;
cone voltage: 50 V; dwell time: 0.2 s;

SIM: 324.7–325.7 m/z
Peaks:
1. Quinine 325 m/z

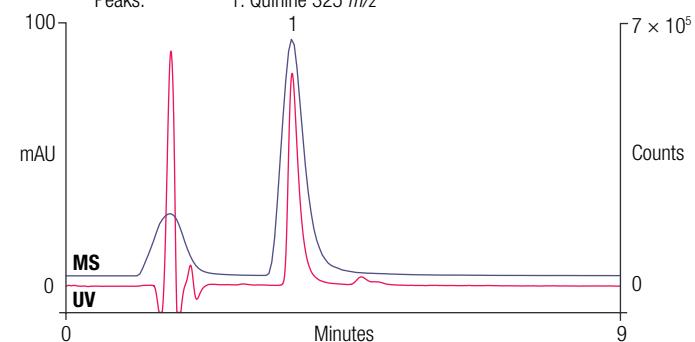


Figure 4-35. Comparison between UV and SIM chromatograms for quinine determination in tonic water.

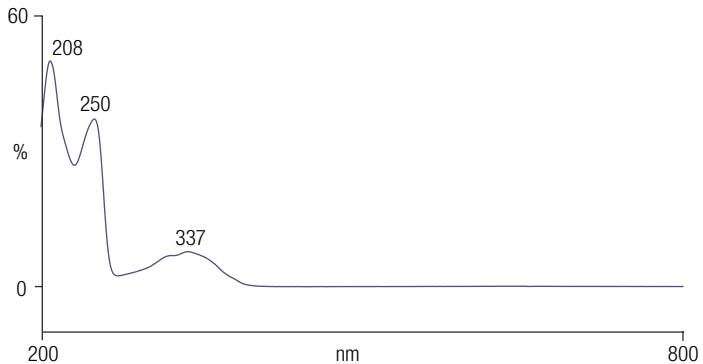


Figure 4-36. UV spectrum of quinine.

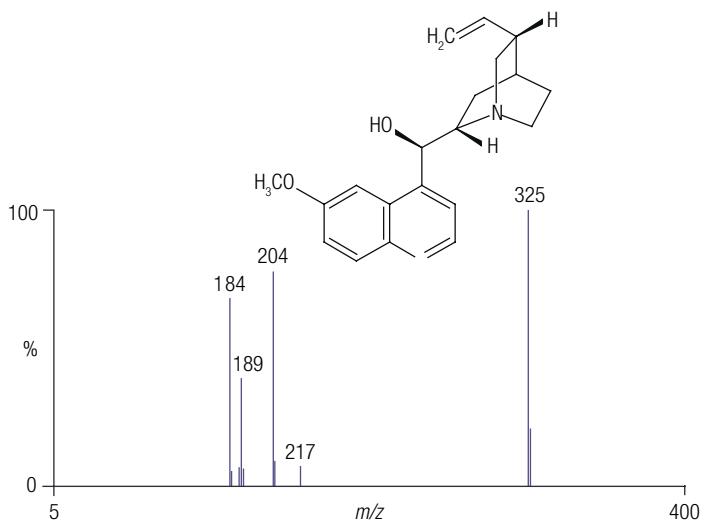


Figure 4-37. MS spectrum of quinine, ESI positive; cone voltage, 75 V; scan time, 1 s; mass range, 100–800 m/z.

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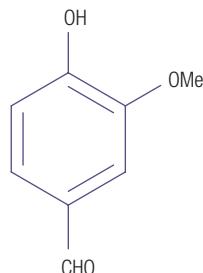
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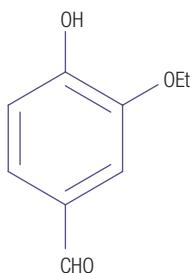
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Artificial vanilla flavor is significantly less expensive to manufacture than natural vanilla. Its main components are vanillin and ethyl vanillin. Coumarin is considered an adulterant in artificial vanilla, and its use is illegal in the United States. The Acclaim Polar Advantage (PA) II column demonstrates excellent selectivity for this matrix, with separation and detection of the components complete in approximately 3.5 minutes.



Vanillin
Mw = 152.15



Ethyl Vanillin
Mw = 166.17

Figure 4-38. Chemical structures for vanillin and ethyl vanillin.

Flavorants: Vanilla (Artificial)

Column: Acclaim PA2, 3 μ m 3.0 \times 33 mm

Flow: 1.00 mL/min

Temperature: TCC-100 at 30 °C

Injection: ASI-100 sampler at 4 μ L

Mobile Phase: 25:75:0.2 Methanol:water:phosphoric acid

Detector: UVD 340U at 254 nm, spectra 220–350 nm

Samples: A. Artificial vanilla flavor, diluted 5 \times

B. Standards, 330 μ g/mL in 2% EtOH

Peaks:

1. Vanillin

2. Coumarin

3. Ethyl vanillin

4. Benzoic acid

Notes:

- Coumarin is an illegal additive

- Benzoic acid is a preservative

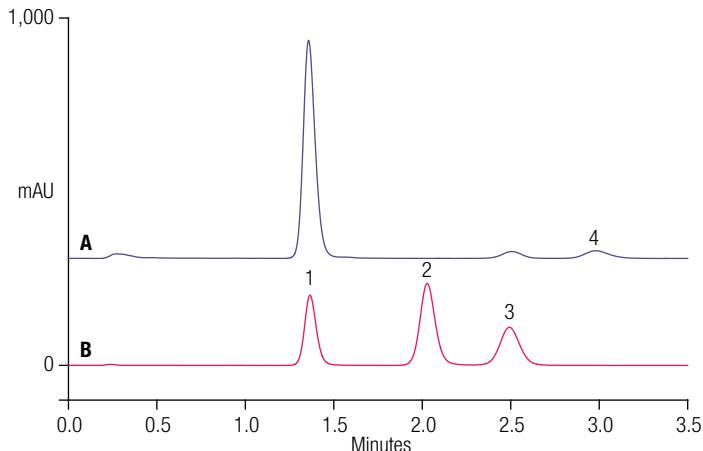


Figure 4-39. Components of artificial vanilla flavor on an Acclaim PA2 column.

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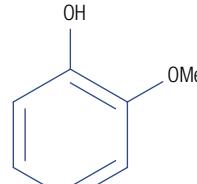
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Artificial Vanilla Precursors

Vanillin was first synthesized from eugenol (found in oil of clove) and later synthesized from lignin-containing sulfite liquor, a byproduct of wood pulp processing in paper manufacture. While some vanillin is still made from lignin wastes, today most synthetic vanillin is synthesized in a two-step process from the petrochemical precursors: guaiacol and glyoxylic acid.



Guaiacol
Mw = 124.14



Glyoxylic Acid
Mw = 74.04

Figure 4-40. Structures of guaiacol and glyoxylic acid.

Flavorants: Vanilla (Artificial)

Column: Acclaim PA2
3 x 75 mm, 3 μ m
Temperature: 30 °C
Flow: 0.5 mL/min
Injection Volume: 1 μ L
Eluent: A. Water
B. Acetonitrile
C. 10% Acetic acid
Detection: UV, at 280 nm

Sample: A. Solution of second step reaction (oxidation)
B. Solution of first step reaction (condensation)

Peaks: 1. Impurity
2. Ethyl vanillin
3. Impurity
4. Byproduct
5. Ethyl vanillyl mandelic acid
6. Byproduct
7. Ethyl vanillin
8. Guaethol

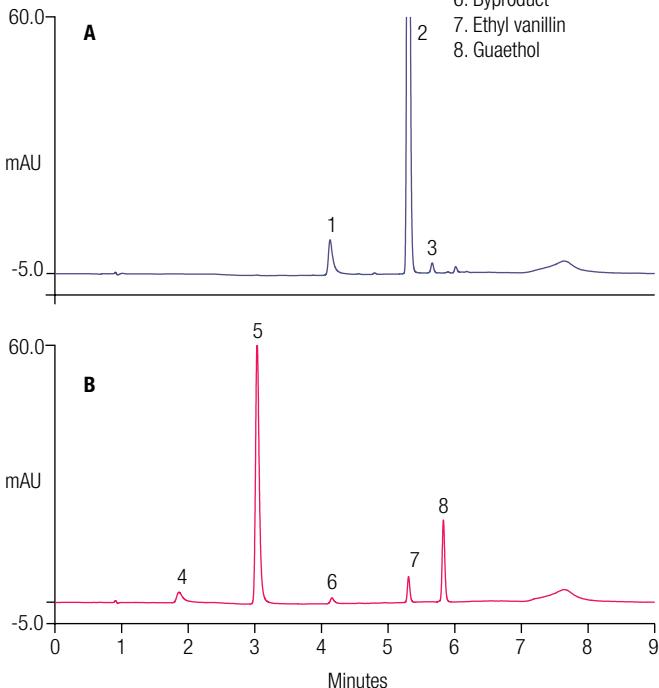


Figure 4-41. Chromatograms of solutions of ethyl vanillin synthesis process A) solution of the second step of the synthetic process. Peak 2 is ethyl vanillin, and peaks 1 and 3 are impurities, and B) solution of the first step of the synthetic process.

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Flavor Components of Vanilla

Natural vanilla (*Vanilla planifolia*, *V. tahitensis*, and *V. pompona*) contains many flavor components, but only four are used in official methods to assess the quality of the vanilla. The AOAC Official Method 990.25 specifies a 4.6 x 250 mm column packed with C8, 10 µm irregular particles—a column that has only about 7000 plates, and takes over an hour to run. This figure shows the AOAC conditions implemented on Acclaim C18 columns. The RSLC column has the same number of plates and better selectivity than the originally specified column, and runs in minutes. Using well-known geometric scaling rules, the assay can be accelerated six-fold. Doubling the flow rate accelerates the analysis twelve-fold, with no sacrifice in the quality of the chromatogram.

Did You Know?

- Natural vanilla is obtained from the cured fruit of an orchid vine.
- Mexican *V. planifolia* flowers can only be pollinated by a specific *Melipone* bee found in Mexico (*abeja de monte* or mountain bee). Vines transplanted to Europe, flowered but failed to produce fruit unless hand pollinated.



Flavorants: Vanilla (Natural)

Column: A: Acclaim 120 C18, 5 µm, 4.6 x 150 mm
B, C: Acclaim RSLC C18, 2 µm, 2.1 x 50 mm
Flow: A: 1.00 mL/min
B: 0.41 mL/min
C: 0.82 mL/min
Temperature: 20 °C
Injection Volume: A: 10 µL
B: 1.2 µL
C: 1.2 µL
Mobile Phase: 200 mM HOAc in 10% (v/v) MeOH

Detector: UV, 254 nm,
A: 1 Hz data rate
B: 5 Hz data rate
C: 10 Hz data rate
Peaks: 1. p-Hydroxybenzoic acid
2. p-Hydroxybenzaldehyde
3. Vanillic acid
4. Vanillin
Sample: Commercial vanilla extract in 40% ethanol, filtered
Reference: AOAC Official Method 990.25

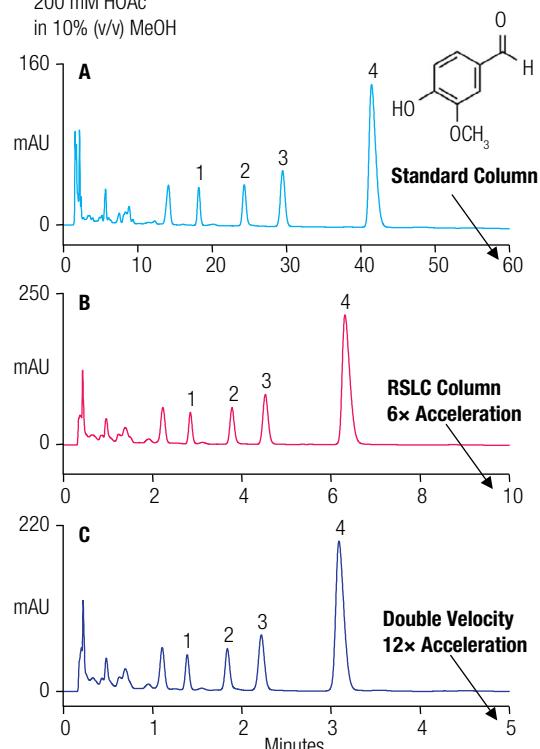


Figure 4-42. Major improvement in sample throughput when using UHPLC conditions.

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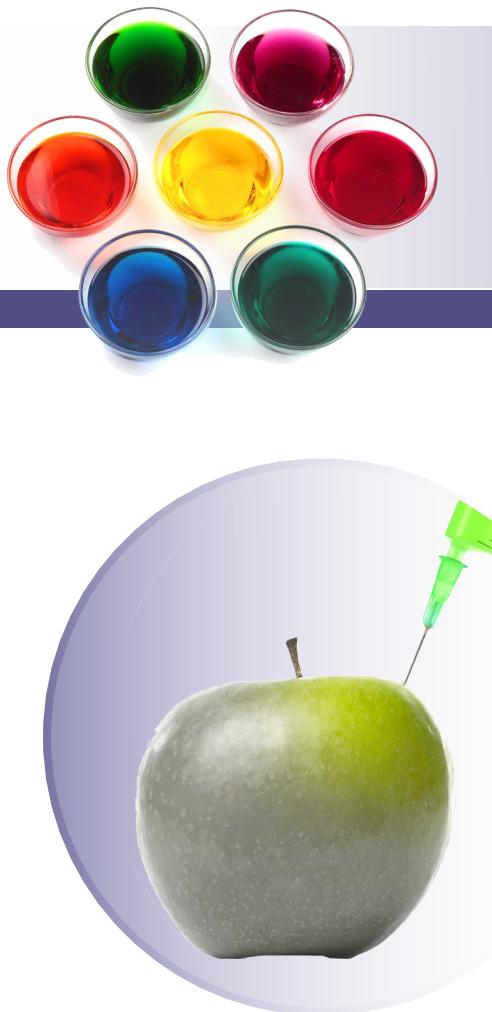
Benzene

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There are a wide variety of chemicals that can be added to foods and beverages. These include the addition of stimulants (e.g., caffeine), fortification by inclusion of micronutrients (e.g., choline, iodide), and the use of additives to improve appearance and texture of the product (e.g., polyphosphates).

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Caffeine

Caffeine is a bitter xanthine alkaloid found in varying quantities in the seeds, leaves, and fruit of some plants, where it acts as a natural pesticide. It is most commonly consumed by humans in infusions extracted from the seed of the coffee plant, the leaves of the tea bush, and from products that are derived from the kola nut.

Caffeine acts as a central nervous system stimulant, temporarily warding off drowsiness and restoring alertness.

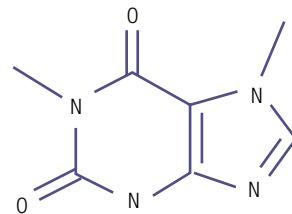


Figure 4-43. Structure of caffeine.

Did You Know?

- Unlike humans caffeine is considerably more toxic to various animals, such as dogs and birds. This is at least partly due to their poorer ability to metabolize the compound.
- Chocolate, derived from cocoa beans, typically contains only a small amount of caffeine, along with the other stimulants theobromine and theophylline. A typical 28-gram serving of a milk chocolate bar has about as much caffeine as a cup of decaffeinated coffee. Some dark chocolate contains as much as 160 mg per 100 g, about double the caffeine content by weight of the strongest caffeinated drip coffee.

Caffeine in Beverages

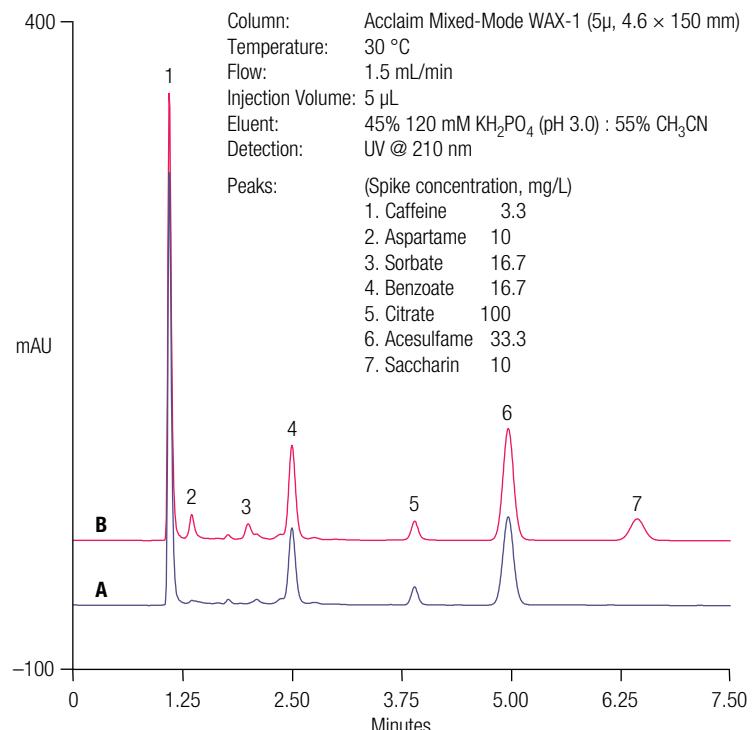


Figure 4-44. Overlay of chromatograms of a lemon-lime carbonated beverage, diluted ten-fold (A) and the same sample spiked (B).

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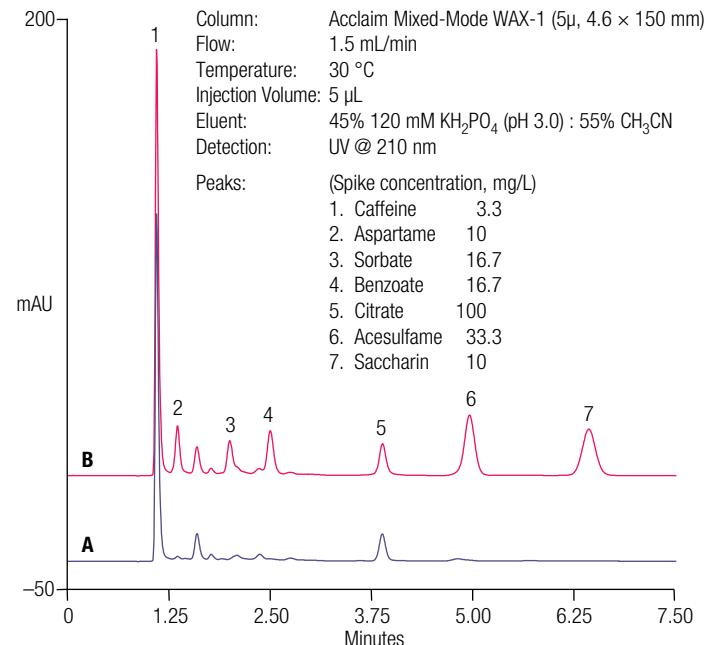


Figure 4-45. Overlay of chromatograms of cola #2, (A) diluted five-fold and (B) the same sample spiked.

Column: Acclaim RSLC 120, C18, 2.2 μ m, analytical (2.1 x 150 mm)

Flow: 0.45 mL/min

Temp.: 25 °C

Injection Volume: 1.0 μ L

Eluent: A: 0.1% TFA, 5% acetonitrile

B: 0.1% TFA in acetonitrile

Gradient: 100% A, 0.0–1.2 min:

0 to 28.5% B, 1.2–15.5 min:

Hold at 28.5% B for 1.5 min

Detection: Absorbance, UV 280 nm

Sample: 1:20 Dilute white tea

Peaks: 1. Gallic Acid — mg/g*

2. Gallocatechin 15.77

3. Epigallocatechin 16.51

4. Caffeine —

5. Catechin 3.12

6. Epicatechin 2.73

7. Epigallocatechin Gallate 42.60

8. Gallocatechin Gallate 8.83

9. Epicatechin Gallate 8.96

*Calculated concentration

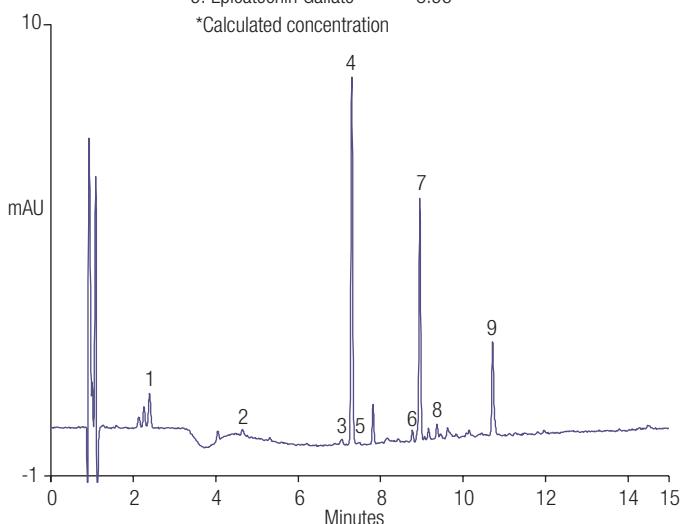


Figure 4-46. Overlay of chromatograms of cola #2, (A) diluted five-fold and (B) the same sample spiked. Separation of catechins in white tea using the Acclaim RSLC 120, C18, 2.2 μ m column.

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Choline is a water-soluble micronutrient vital to cell membrane integrity, support of methyl group metabolism, and nervous system activity. It is present as free choline in small quantities in a wide variety of foods and frequently found in its esterified forms. Choline can also be found in fortified foods and dietary supplements; e.g., choline is a required additive in many infant formulas. It is therefore important to determine the choline content of common foods.

Application Update 189 describes an improved IC method for determination of choline in infant formula and other food samples (e.g., egg powder and soy flour) using an RFIC system with suppressed conductivity detection.



Columns: Dionex IonPac CG19 and CS19, 2 mm
Flow: 0.25 mL/min
Injection Volume: 5 μ L
Eluent Source: Dionex EGC III MSA cartridge with CR-CTC II trap column
Eluent: 6.4 mM MSA
Detection: Suppressed conductivity, Dionex CSRS 300 suppressor, 2 mm, 4 mA, recycle mode

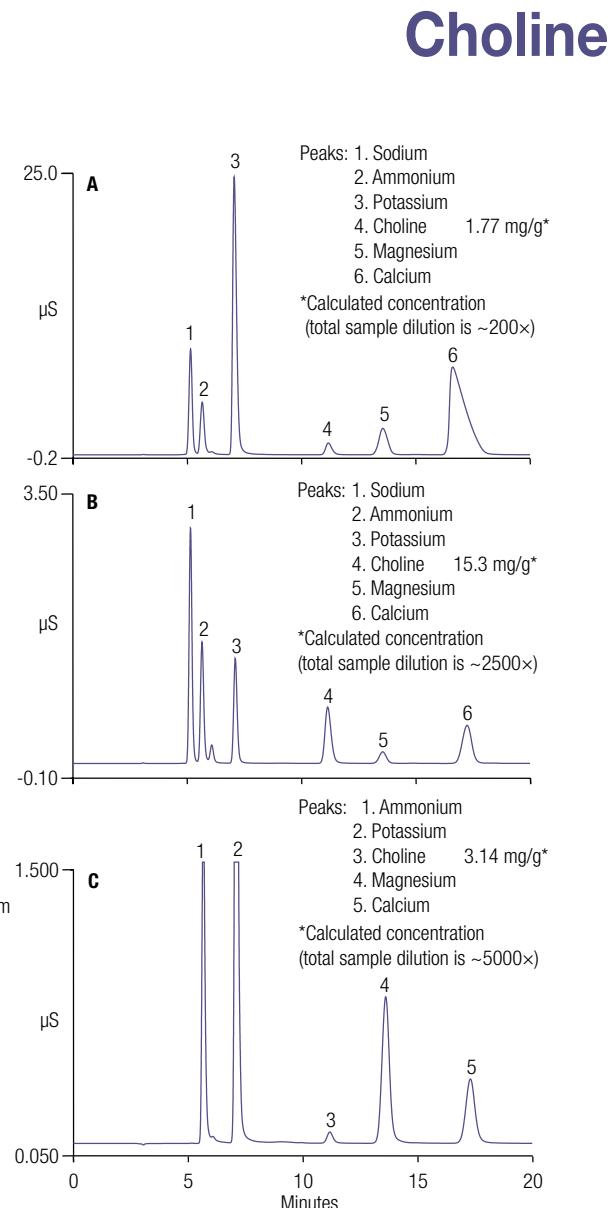


Figure 4-47. A) Determination of choline in infant formula, B) determination of choline in egg powder, and C) determination of choline in soy flour.

[Download Application Update 189: Determination of Choline in Infant Formula and Other Food Samples by IC](#)

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Glucosamine

Glucosamine (GlcN), an amino sugar, occurs naturally in the human body. It is a major structural component in the biosynthesis of glycosaminoglycans, compounds involved in normal joint function. Use of GlcN as a dietary supplement in the management of osteoarthritis has attracted considerable attention. Results of the 2002 National Health Interview Survey showed that GlcN was one of the five nonvitamin, nonmineral herbal products/dietary supplements most frequently used by adults in the U.S.A. While the principal use for GlcN dietary supplements is for arthritis management, especially in older adults, its use as a preventive measure to maintain health and in veterinary medicine also has been reported.

The 1994 Dietary Supplement Health and Education Act granted the US FDA authority to prescribe good manufacturing practices for dietary supplements. The final rule, published in June, 2007, established regulations requiring current good manufacturing practices (cGMP) for dietary supplements. Using the cGMP regulation model for foods, the rule ensures that dietary supplements are produced in a quality manner, do not contain contaminants or impurities, and are accurately labeled.

Application Note 197 describes the use HPAE-PAD chromatography for the sensitive, selective and direct-detection of GlcN.

Column: Thermo Scientific™ Dionex™ CarboPac™ PA20, 3 mm
Flow: 0.5 mL/min
Temperature: 30 °C
Injection Volume: 10 µL
Eluent: 20 mM KOH
Detection: PAD, disposable Au electrode
Sample: Dietary supplements, about 10 µM (1.8 µg/mL)

Peaks:

1. Propylene glycol	8. Mannitol
2. Glycerol	9. Glucosamine
3. myo-Inositol	10. Glucose
4,5. Unknown	11. Fructose
6. Sorbitol	12. Sucrose
7. Unknown	

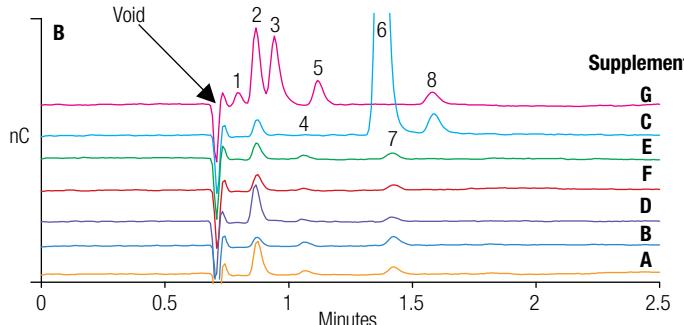
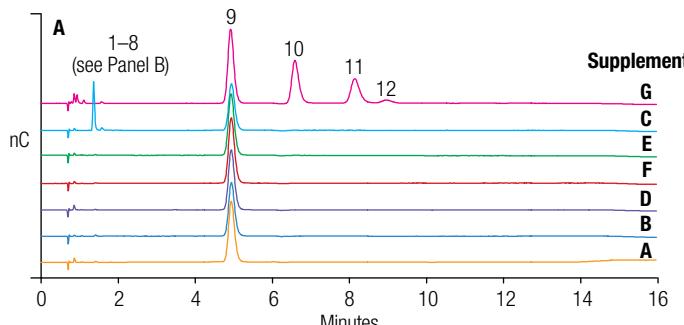


Figure 4-48. HPAE-PAD analysis of GlcN-containing dietary supplements. Seven dietary supplement samples diluted to approximately 10 µM (1.8 µg/mL) GlcN, 10-µL injection. A) Full chromatogram. B) Expanded early RT region of the chromatogram.

Download Application Note 197: Determination of Glucosamine in Dietary Supplements using HPAE-PAD

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Iodide

Iodide in Infant Formulas

Iodine is an important micronutrient essential for the production of thyroid hormones that are involved in the regulation of many key biochemical reactions. Iodine deficiency can lead to varying degrees of growth and developmental abnormalities in children and adults, including such illnesses as goiter and cretinism. However, an excess of iodine can also lead to thyroid disorders, especially in infants. As iodine is primarily absorbed from our diet, supplementation of iodine in food is a common practice.

The concentration of iodine in iodine-fortified foods is often regulated and monitored. Infant formula is the most highly regulated consumer food product on the market today. The Infant Formula Act of 1980 specifies minimum and maximum amounts of several nutrients, and authorizes the U.S. FDA to create and enforce standards for commercial infant formulas.

Application Note 37 includes an acetic acid digestion method for iodide extraction, coupled with an IC-PAD method for iodide detection. The IC method coupled with electrochemical detection enables selective and sensitive determination of iodide in complex matrices. An acid digestion procedure to extract iodate was optimized for milk- and soy-based infant formulas. In addition, sample preparation conditions to convert iodate to iodide for determining total iodine (i.e., iodide and iodate) are described. This method is shown to be fast, robust, and sensitive.

Column:	Dionex IonPac AG11/AS11, 4 mm	Samples:	Iodide (mg/L)
Flow:	1.5 mL/min	Standard	0.040
Temperature:	30 °C	Infant Formula Brand 1	0.027
Injectio Volume:	100 µL	Infant Formula Brand 2	0.042
Eluent:	50 mM Nitric acid	Infant Formula Brand 3	0.023
Detection:	PAD	Infant Formula Brand 4	0.035
Electrode:	Ag	Soy Infant Formula	0.046

Peaks:
1. Iodide
2. Thiocyanate

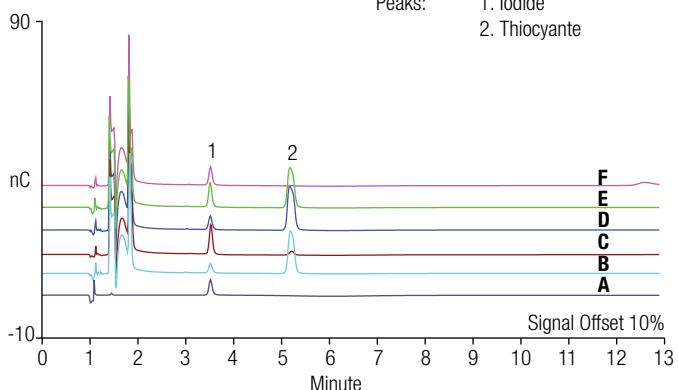


Figure 4-49. Determination of iodide in (A) DI water, (B-E) milk-based infant formulas, and (F) soy-based infant formula.

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Iodide

Iodide in Iodized Table Salt

Iodine is an essential element naturally present in seawater and seafood. The most common forms of natural iodine in the diet are iodide and iodate, with additional iodized organic compounds providing a small fraction of bioavailable iodine. To combat disease, table salt is routinely iodized. Iodization levels vary greatly and can range from 5–100 µg/g (ppm) of iodine in salt depending on the country of manufacture and the storage conditions. Potassium iodide and potassium iodate are safe sources of dietary iodine used to iodize salt and prevent iodine deficiencies. In the United States and Canada, potassium iodide is frequently used to iodize salt whereas in many other countries, potassium iodate is preferred due to its greater stability.

Iodide can be oxidized to iodine under many conditions, including exposure to humidity, reaction with existing moisture present in the salt, exposure to sunlight, and exposure to heat. This conversion is also catalyzed by metal ions, particularly ferrous ions. Iodine readily sublimates, and is therefore easily lost from iodized salt. However, iodate is comparably more stable and is not lost by such pathways.

Application Note 236 describes a High Performance Liquid Chromatography-photodiode array UV detection method to determine iodide and iodate in synthetic sea salt, and table salt. This method is specific, sensitive, and rapid.

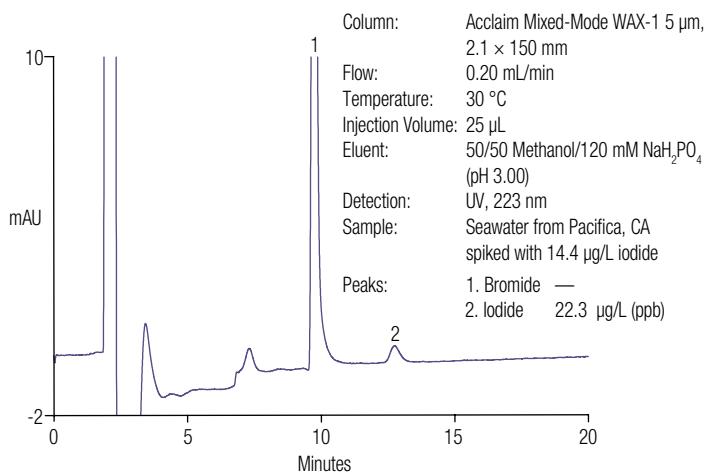


Figure 4-50. Separation of iodide and iodate in synthetic sea salt.

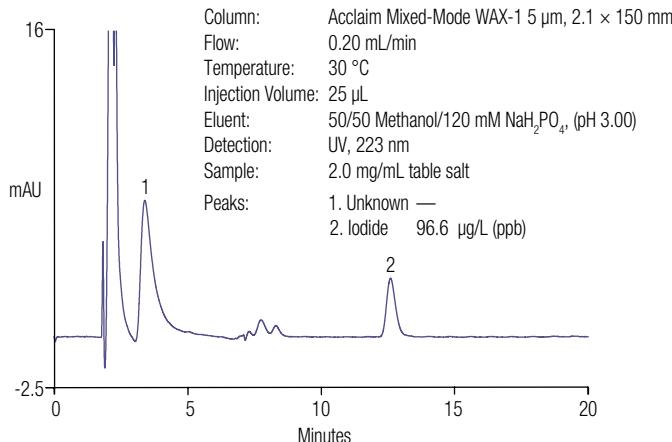


Figure 4-51. Separation of iodide in table salt on the Acclaim Mixed- Mode WAX-1 column.

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Polyphosphates are legally added to some food products such as meat, fish, and seafood. In the seafood industry, polyphosphates are used in both fresh and frozen products to increase their water-binding capacity. This improves the appearance and texture of the product and also increases the weight of the seafood. Polyphosphates are legal, but some countries require that polyphosphate usage be declared and may also limit the amount that can be added. Citric acid is sometimes added to shrimp as a preservative to maintain an acidic environment.

Application Note 1007 describes the simultaneous determination of mono-, di-, and tri-phosphates and citrate in shrimp by ion chromatography.



Polyphosphates

Column: Dionex IonPac AS11 Analytical, 4 × 250 mm
Dionex IonPac AG11 Guard, 4 × 50 mm
Flow: 1.00 mL/min
Column Tem.: 35 °C
Tray Temp.: 10 °C
Injection Volume: 25 µL
Eluent Source: Dionex EGC III KOH, gradient mode
Gradient: 30 mM from -5 to 3 min, 45 mM from 3.1 to 7 min
50 mM from 7.5 to 13 min, (gradient curve 5)
Pressure: ~2000 psi
Detection: Suppressed conductivity, Dionex ASRS 300 suppressor, 4 mm, with external water mode, Thermo Scientific Dionex SRS suppressor, current 130 mA
Sample: Single phosphate, polyphosphates, and citrate standard

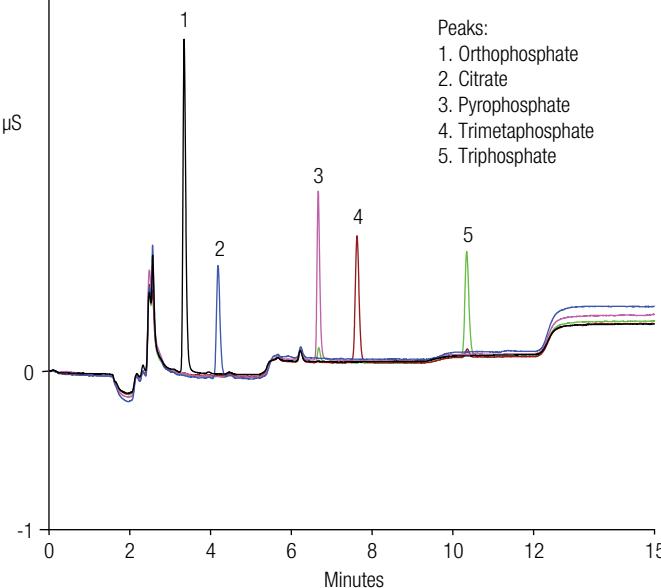


Figure 4-52. Overlay of chromatograms of single standard injections of a phosphate, three polyphosphates, and citrate.

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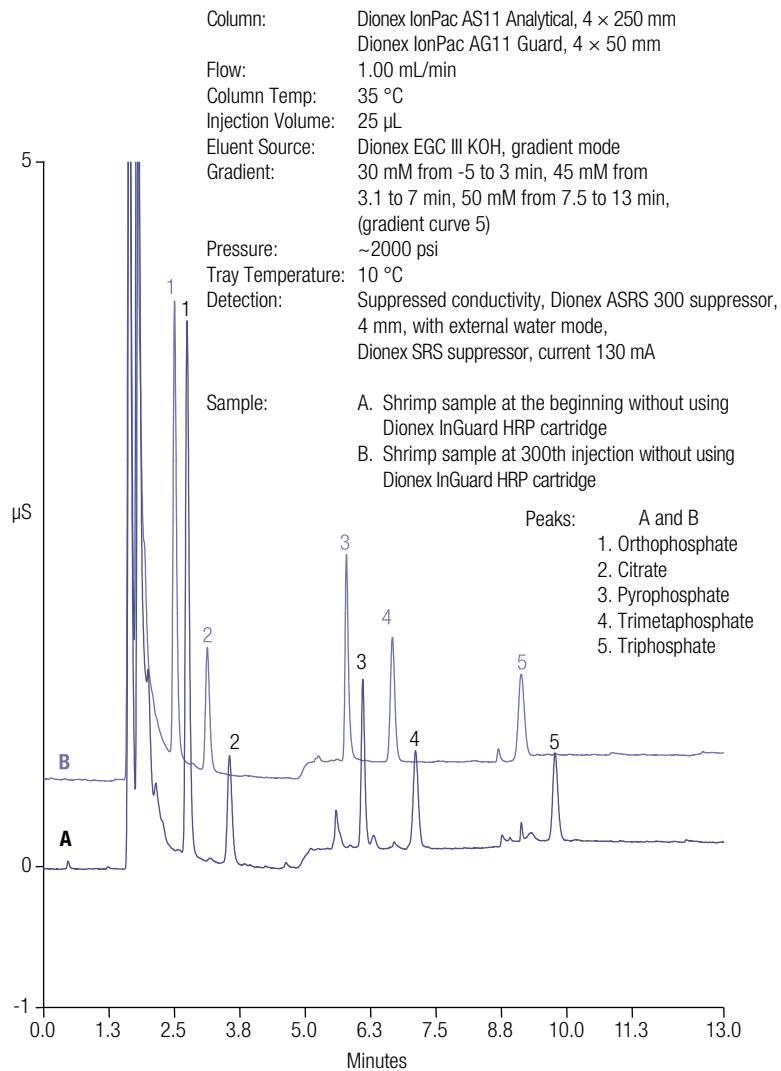


Figure 4-53. Overlay of chromatograms of a shrimp sample at the first and 300th injection using a Dionex InGuard HRP cartridge showing the value of sample preparation prior to IC analysis.

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Preservatives

Artificial preservatives are man-made. They are used in foods to prevent undesirable chemical changes, reduce the risk of foodborne infections, decrease microbial spoilage, and preserve fresh attributes and nutritional quality.

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Preservatives: Structures

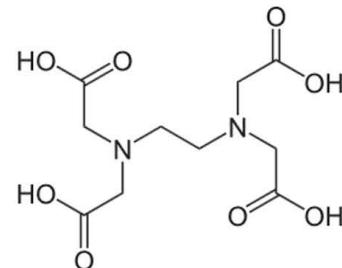


Figure 4-54A. **EDTA**. Ethylenediaminetetraacetic acid (EDTA) is an antioxidant that acts by sequestering transition metal ions (e.g., iron). EDTA is added to some food as a preservative or stabilizer to prevent catalytic oxidative decoloration. In soft drinks containing ascorbic acid and sodium benzoate, EDTA helps to prevent the formation of benzene, a carcinogen.

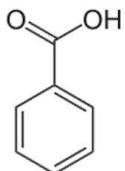


Figure 4-54B. **Benzoic acid** (E210) and its salts are preservatives. Sodium benzoate (E211) is bacteriostatic and fungistatic under acidic conditions. It is commonly added to acidic foods such as salad dressings, carbonated drinks, jams, pickles, and fruit juices.

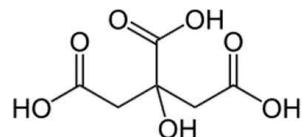


Figure 4-54C. **Citric acid**, like EDTA, acts as an antioxidant through sequestration of transition metal ions. However, due to its chemical structure, it is far less effective in this role than EDTA. Citric acid is added to caramel to prevent re-crystallization of sucrose, to ice cream as an emulsifying agent to keep fats from separating, and is generally used as a substitute for fresh lemon juice.

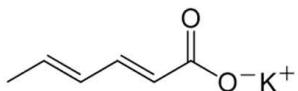


Figure 4-54D. **Potassium sorbate** (E202) is a preservative used to inhibit molds and yeasts in many foods, such as cheese, wine, yogurt, dried meats, soft drinks, fruit drinks, and baked goods. It is also added to herbal dietary supplement products to increase shelf life.

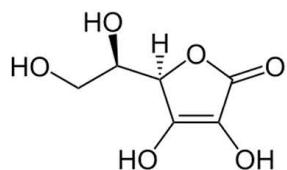


Figure 4-54E. **Erythorbic acid** (E315), previously referred to as isoascorbic acid, is a stereoisomer of ascorbic acid (vitamin C). It is a vegetable-derived food additive produced from sucrose and is widely used as an antioxidant in processed foods.

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Since the early 1900s, benzoate has been widely used worldwide as a preservative due to its antimicrobial properties combined with its low toxicity and taste. The soft drink industry is the largest user of benzoate as a preservative due to the amount of high fructose corn syrup in many carbonated beverages.

Application Note 165 describes a simple and reliable ion chromatography method for the direct determination of benzoate in liquid food products. This method can be used to ensure that the concentration of benzoate in a sample is within product and regulatory specifications.

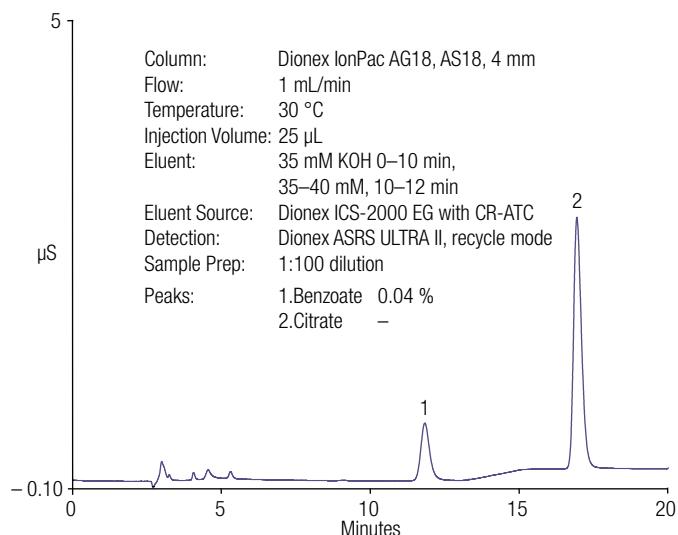


Figure 4-55. Determination of benzoate in flavored soda.

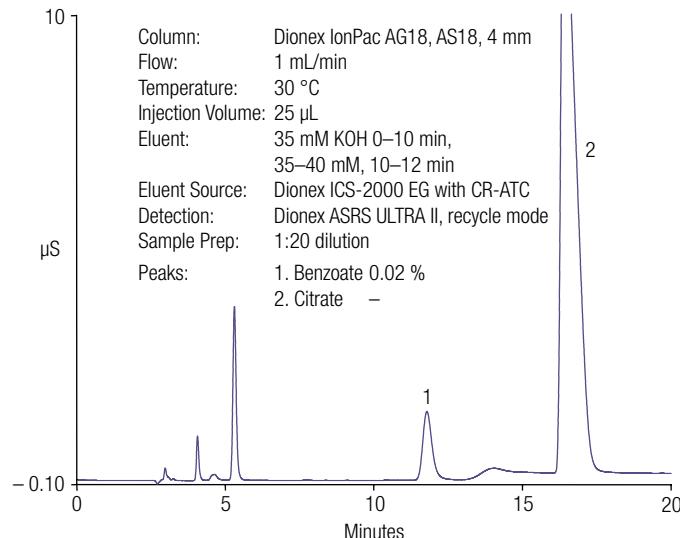


Figure 4-56. Determination of benzoate in diet soda.

Preservatives: Benzoate

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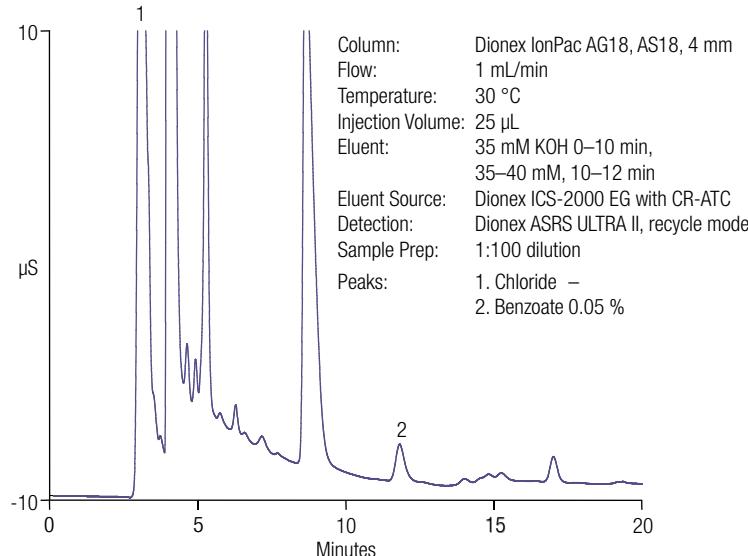


Figure 4-57. Determination of benzoate in soy sauce.



Preservatives: Benzoate

Column: Dionex IonPac AG18, AS18, 4 mm
Flow: 1 mL/min
Temperature: 30 °C
Injection Volume: 25 µL
Eluent: 35 mM KOH 0–10 min,
35–40 mM, 10–12 min
Eluent Source: Dionex ICS-2000 EG with CR-ATC
Detection: Dionex ASRS ULTRA II, recycle mode
Sample Prep: 1:100 dilution
Peaks:
1. Benzoate 0.05 %
2. Citrate –

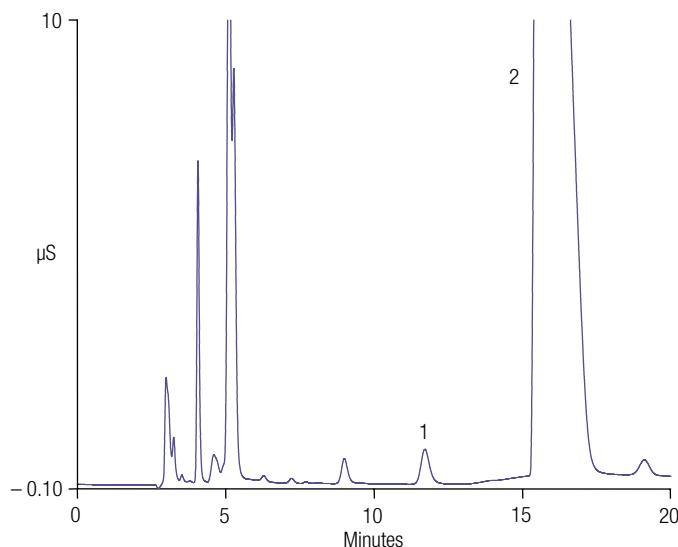


Figure 4-58. Determination of benzoate in lemon juice.

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Nitrate and nitrite are usually added to processed meat products to protect against microorganisms that can cause food poisoning, such as *Clostridium botulinum*. However, nitrite can react with secondary amines to form nitrosoamines, a class of carcinogenic compounds, in food products or in the digestive system. Nitrate, although more stable than nitrite, can act as a reservoir for nitrite. Also, nitrate can readily be converted into nitrite by microbial reduction. Thus, both nitrate and nitrite must be monitored to ensure the quality and safety of meat products.

Application Note 112 describes an accurate and sensitive method in which nitrate and nitrite are extracted from meat products and then determined directly using anion exchange chromatography with UV detection. Commercially available ham and salami were used as model samples.



Preservatives: Nitrate and Nitrite

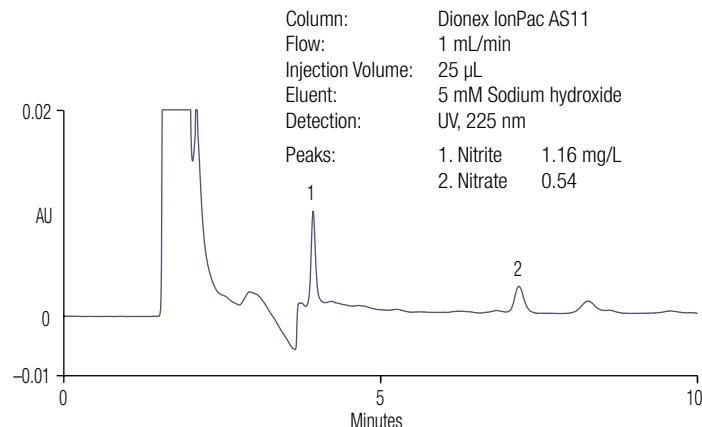


Figure 4-59. Separation of nitrate and nitrite from ham.

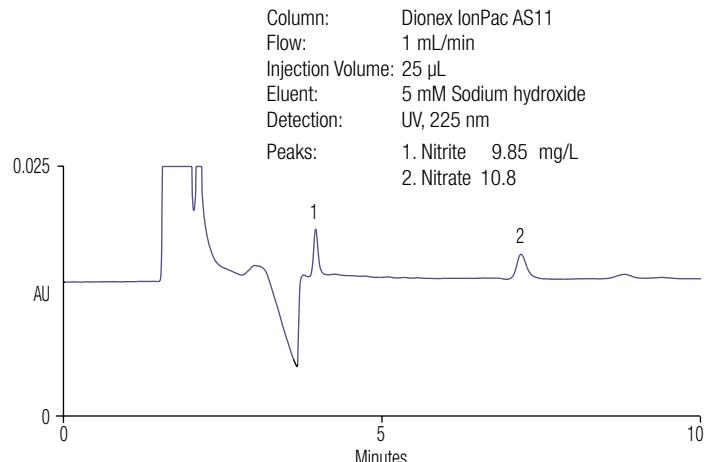


Figure 4-60. Separation of nitrate and nitrite from salami.

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In the food and beverage industries, sulfites are a group of compounds that includes sulfur dioxide and sulfite salts. Sulfites can occur naturally in some foods and beverages due to fermentation. For centuries, sulfiting agents—such as sodium sulfite, sodium bisulfite, and sodium metabisulfite—have been used as preservatives to prevent microbial spoiling and browning reactions in a wide variety of food and beverage products.

Conditions for Figures 4-61–4-64

Columns: Dionex IonPac ICE-AS1 Guard, 4 × 50 mm (P/N 067842)
Dionex IonPac ICE-AS1 Analytical, 4 × 250 mm (P/N 064198)

Temperature: 25 °C (upper compartment, detector)
30 °C (lower compartment, column)

Eluent: 20 mM MSA

Tray Temp: 4 °C

Detection: PAD, disposable Pt working electrode

System Backpressure: ~1760 psi

Background: 50–60 nC

Noise: 0.04 nC peak-to-peak

Run Time: 25 min

Waveform for the ED:

Time (s)	Potential (V)	Last Step*	Ramp*	Gain Region*	Integration
0.00	0.8	Off	On	Off	Off
0.40	0.8	Off	On	On	On
0.60	0.8	Off	On	On	Off
0.61	1.2	Off	On	Off	Off
0.70	1.2	Off	On	Off	Off
0.71	0.1	Off	On	Off	Off
1.00	0.1	On	On	Off	Off

*These settings are required in the Dionex ICS-3000/5000 system but not used in older Dionex systems; the reference electrode is in AgCl mode (Ag/AgCl reference electrode).

Preservatives: Sulfites

Sulfites have been implicated as the cause of allergic reactions that range in severity from minor to life threatening. Sulfites also have been reported as the cause of some asthmatic responses in certain people. Therefore, since 1986, the U.S. Food and Drug Administration (FDA) has required labeling of any food or beverage containing a sulfite concentration >10 ppm.

Application Note 54 describes an improved ion-exclusion chromatography method for the determination of total and free sulfite in foods and beverages, that does not have the analytical problems associated with AOAC Methods 990.28 and 990.31.

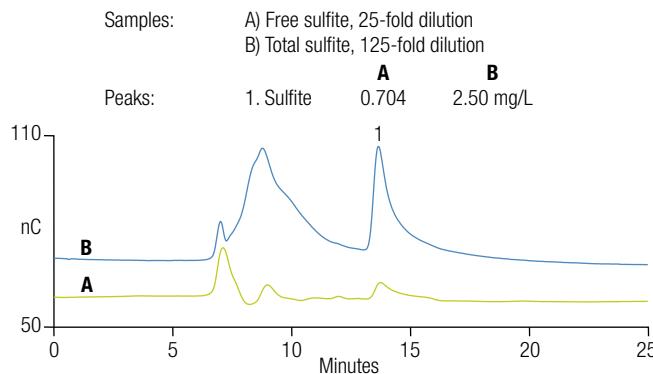


Figure 4-61. Chromatograms of A) total and B) free sulfite in red wine. A 15% signal offset has been applied.

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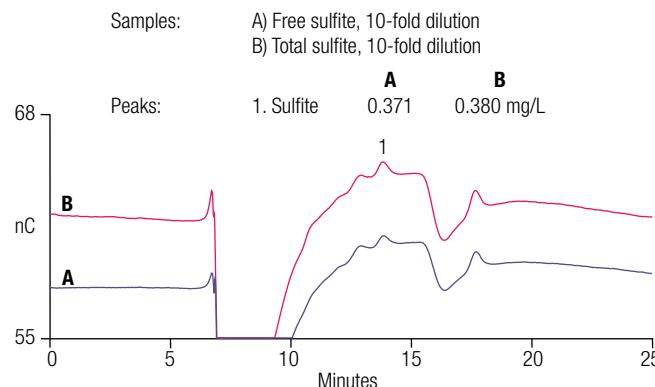


Figure 4-62. Chromatograms of A) free and B) total sulfite in coconut water.

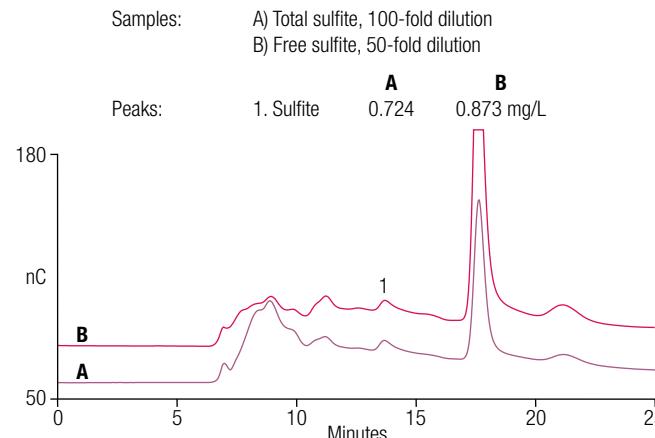


Figure 4-63. Chromatograms of A) total and B) free sulfite in red wine. A 15% signal offset has been applied.

Preservatives: Sulfites

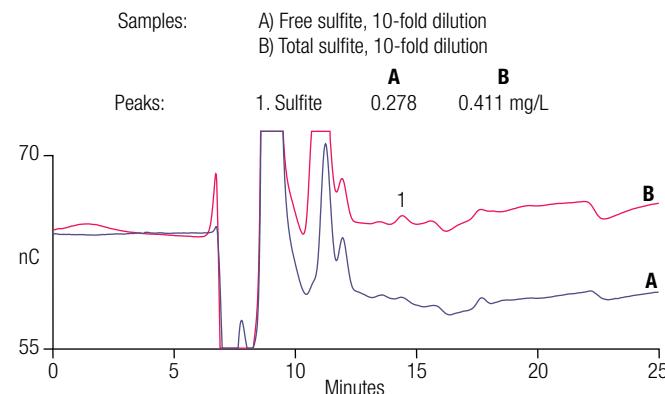


Figure 4-64. Chromatograms of A) free and B) total sulfite in coconut water.



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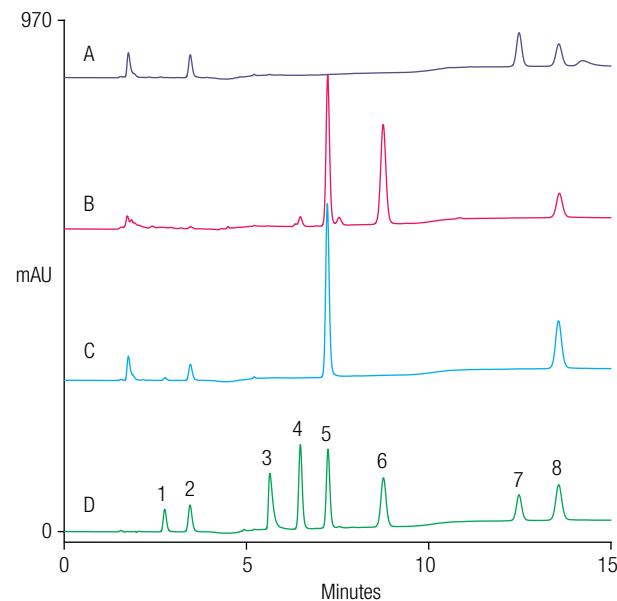
Preservatives: Soft Drinks

Soft drink formulations often contain additives such as artificial sweeteners, preservatives, citric acid and caffeine. This analysis determines eight major ingredients in a single run. The Acclaim OA column is uniquely suited because of its aqueous compatibility for the separation of organic acids, and its hydrophobic selectivity for the separation of preservatives.

Column: Acclaim OA 5 μm , 4.0 \times 150 mm
Flow: 0.8 mL/min
Temperature: 30 °C
Injection Volume: 10 μL
Mobile Phase: (A) 14.2 g/L Na₂SO₄ + 0.550 mL/L CH₃CO₂H in water
(B) Methanol
Gradient: Time 0.0 1.0 2.0 6.5 7.5 15.0
%A 100 100 69 69 60 60
%B 0 0 31 31 40 40
Detection: UV at 210 nm; optional 230, 262 nm
Samples: (A) Minute-Maid® Orange Soda
(B) Diet Coke® Soda
(C) Mountain Dew® Soda
(D) Standards
Minute-Maid and Diet Coke are registered trademarks of Coca-Cola. Mountain Dew is a registered trademark of Pepsico.
Sample Prep.: Filter and dilute 5 \times with mobile phase A
Peaks:

1. Erythorbic acid	50* $\mu\text{g/mL}$
2. Citric acid	500
3. Acesulfame-K	50
4. Aspartylphenylalanine	50
5. Caffeine	12
6. Aspartame	50
7. Sorbate potassium	50
8. Benzoic acid	50

*Listed concentrations are for the standards (chromatogram D).



Note: For alternate calibration mixes, use ascorbic acid instead of erythorbic acid, or use saccharin instead of aspartame.

Figure 4-65. Soft drink ingredients using the Acclaim OA column.

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Soft drinks can contain many additives including caffeine, preservatives, artificial sweeteners, and other flavor components. Since many of these compounds are weak acids, they are retained on the Acclaim Mixed-Mode WAX-1 column. The pH may be lowered to favor reversed-phase

retention, or raised to promote anion-exchange retention. In this way, the separation can be adjusted to the particular requirements of a specific beverage formulation.

Column: Acclaim Mixed-Mode WAX-1, 5 μ m
Flow: 1 mL/min
Temperature: 30 °C
Injection Volume: 2.5 μ L
Dimension: 4.6 x 150 mm
Mobile Phase: (A) 55/45 v/v Acetonitrile/
0.2 M phosphate buffer, pH6.0
(B) 57/43 v/v Acetonitrile/
0.12 M phosphate buffer, pH2.9
Detection: UV @ 210 nm
Peaks:

1. Caffeine	100 μ g/mL
2. Aspartame	100
3. Acesulfame, potassium	100
4. Saccharin	100
5. Sorbate, potassium	100
6. Benzoic acid	100
7. Citric acid	300

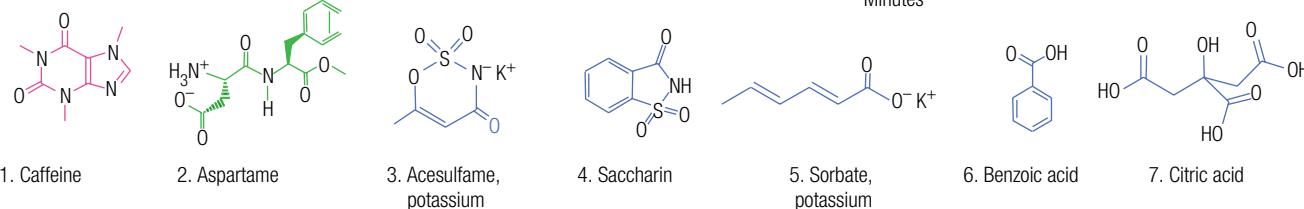


Figure 4-66. Analysis of additives used in soft drinks on Acclaim Mixed-Mode WAX-1 column.

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Rapid analysis is required for quality control testing in highly productive, modern factories. Using the additional leverage provided by its multifunctional chemistry, the Acclaim Mixed-Mode WAX-1 column can be optimized for a 3-minute assay of cola drinks.

Column: Acclaim Mixed-Mode WAX-1,
5 μ m, 4.6 \times 150 mm
Flow: 2 mL/min
Temperature: 30 °C
Injection Volume: 2.5 μ L
Mobile Phase: 57/43 v/v Acetonitrile/
120 mM phosphate buffer, pH 2.9
Detection: UV @ 210 nm
Sample: Direct injection of degassed sample

Peaks: 1. Caffeine
2. Aspartame
3. Sorbate
4. Benzoate
5. Citrate
6. Acesulfame

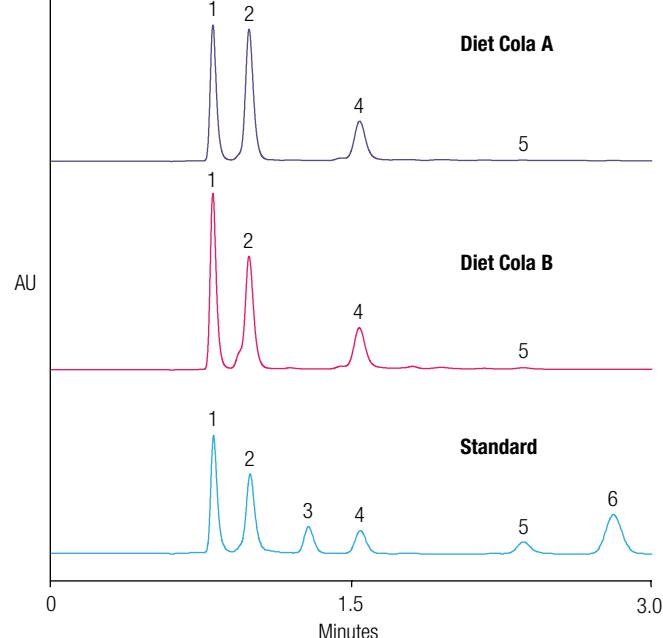


Figure 4-67. Rapid analysis of soft drinks with the Acclaim Mixed-Mode WAX-1 column.

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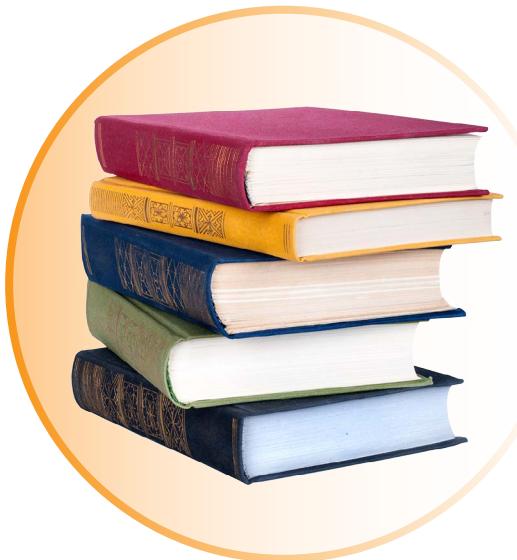
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Technical Collateral and Peer Reviewed Journals

Here you'll find a multitude of references using our HPLC, ion chromatography and sample preparation solutions.

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Peer Reviewed Journals: HPLC and UHPLC Methods

Carbohydrates

Title	Authors	Publication	Publication Date
Carbohydrate and oligosaccharide analysis with a universal HPLC detector.	Asa, D.	American Laboratory 38, 16.	2006
Determination of levoglucosan in atmospheric aerosols using high performance liquid chromatography with aerosol charge detection.	Dixon, R. W.; Baltzell, G.	J. Chromatogr., A. 1109 (2), 214–221	2006 Mar 24
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1,2-alpha-L-Fucosynthase: A glycosynthase derived from an inverting alpha-glycosidase with an unusual reaction mechanism	Wada, J.; Honda, Y.; Nagae, M.; Kato, R.; Wakatsuki, S.; Katayama, T.; Taniguchi, H.; Kumagai, H.; Kitaoka, M.; Yamamoto, K.	<i>FEBS Lett.</i> 582 (27), 3739–3743	2008 Nov 12
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Characterization of phenolic compounds in strawberry (<i>Fragaria x ananassa</i>) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity	Aaby, K.; Ekeberg, D.; Skrede, G.	<i>J. Agric. Food Chem.</i> 55 (11), 4395–4406	2007 May 30
Analysis of flavonoids and other phenolic compounds using high-performance liquid chromatography with coulometric array detection: relationship to antioxidant activity	Aaby, K.; Hvattum, E.; Skrede, G.	<i>J. Agric. Food Chem.</i> 52 (15), 4595–4603	2004 Jul 28
Aqueous extract of <i>Astragalus Radix</i> induces human natriuresis through enhancement of renal response to atrial natriuretic peptide	Ai, P.; Yong, G.; Dingkun, G.; Qiuyu, Z.; Kaiyuan, Z.; Shanyan, L.	<i>J. Ethnopharmacol.</i> 116 (13), 413–421	2008 Mar 28
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Recent methodology in ginseng analysis	Baek, S.; Bae, O.; Park, J.	<i>J. Ginseng Res.</i> 36 (2), 119–134	2012 Apr
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Comprehensive analysis of polyphenols in 55 extra virgin olive oils by HPLC-ECD and their correlation with antioxidant activities	Bayram, B.; Esatbeyoglu, T.; Schulze, N.; Ozcelik, B.; Frank, J.; Rimbach, G.	<i>Plant Foods Hum. Nutr. (N. Y., NY, U.S.)</i> 67 (4), 326–336	2012 Dec
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Utilization of RP-HPLC fingerprinting analysis for the identification of diterpene glycosides from <i>Stevia rebaudiana</i>	Chaturvedula, V.; Prakash, I.	<i>Int. J. Res. Phytochem. Pharmacol.</i> 1 (2), 88–92	2011 Jun 9
Acid and alkaline hydrolysis studies of stevioside and rebaudioside A	Chaturvedula, V.; Prakash, I.	<i>J. Appl. Pharm. Sci.</i> 1 (8), 104–108	2011 Oct
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alpha-Lipoic acid in dietary supplements: development and comparison of HPLC-CEAD and HPLC-ESI-MS methods	Durrani, A. I.; Schwartz, H.; Schmid, W.; Sontag, G.	<i>J. Pharm. Biomed. Anal.</i> 45 (4), 694–699	2007 Nov 30
Comparison between evaporative light scattering detection and charged aerosol detection for the analysis of saikogenins	Eom, H. Y.; Park, S. Y.; Kim, M. K.; Suh, J. H.; Yeom, H.; Min, J. W.; Kim, U.; Lee, J.; Youm, J. R.; Han, S. B.	<i>J. Chromatogr. A.</i> 1217 (26), 4347–4354	2010 Jun 25
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Development and validation of HPLC-DAD-CAD-MS3 method for qualitative and quantitative standardization of polyphenols in <i>Agrimoniae eupatoriaie herba</i> (Ph. Eur.)	Granica, S.; Krupa, K.; Klebowska, A.; Kiss, A. K.	<i>J. Pharm. Biomed. Anal.</i> 86, 112–122	2013 Dec
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Urinary 3-(3,5-dihydroxyphenyl)-1-propanoic acid, an alkylresorcinol metabolite, is a potential biomarker of whole-grain intake in a U.S. population	Guyman, L. A.; Adlercreutz, H.; Koskela, A.; Li, L.; Beresford, S. A.; Lampe, J. W.	<i>J. Nutr.</i> 138 (10), 1957–1962	2008 Oct
Multidimensional LC x LC analysis of phenolic and flavone natural antioxidants with UV-electrochemical coulometric and MS detection	Hájek, T.; Skerlová, V.; Cesla, P.; Vynuchalová, K.; Jandera, P.	<i>J. Sep. Sci.</i> 31 (19), 3309–3328	2008 Oct
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RP-HPLC analysis of phenolic compounds and flavonoids in beverages and plant extracts using a CoulArray detector	Jandera, P.; Skeifíková, V.; Rehová, L.; Hájek, T.; Baldriánová, L.; Skopová, G.; Kellner, V.; Horna, A.	<i>J. Sep. Sci.</i> 28 (9–10), 1005–1022	2005 Jun
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HPLC analysis of rosmarinic acid in feed enriched with aerial parts of <i>Prunella vulgaris</i> and its metabolites in pig plasma using dual-channel coulometric detection	Jirovský, D.; Kosina, P.; Myslínová, M.; Stýskala, J.; Ulrichová, J.; Simánek V.	<i>J. Agric. Food Chem.</i> 55 (19), 7631–7637	2007 Sep 19
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Sensitive electrochemical detection method for alpha-acids, beta-acids and xanthohumol in hops (<i>Humulus lupulus L.</i>)	Kac, J.; Vovk, T.	<i>J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.</i> 850 (1–2), 531–537	2007 May 1
Determination of phenolic compounds and hydroxymethylfurfural in meads using high performance liquid chromatography with coulometric-array and UV detection	Kahoun, D.; Rezková, S.; Veskrnová, K.; Královský, J.; Holcapek, M.	<i>J. Chromatogr., A</i> 1202 (1), 19–33	2008 Aug 15
Analysis of terpene lactones in a Ginkgo leaf extract by high-performance liquid chromatography using charged aerosol detection	Kakigi, Y.; Mochizuki, N.; Ichio, T.; Hakamatsuka, T.; Goda, Y.	<i>Biosci., Biotechnol., Biochem.</i> 74 (3), 590–594	2010
Linear aglycones are the substrates for glycosyltransferase DesVII in methymycin biosynthesis: analysis and implications	Kao, C.; Borisova, S.; Kim, H.; Liu, H.	<i>J. Am. Chem. Soc.</i> 128 (17), 5606–5607	2006 May 3

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Certification of a pure reference material for the ginsenoside Rg1	Kim, D.; Chang, J.; Sohn, H.; Cho, B.; Ko, S.; Nho, K.; Jang, D.; Lee, S.	<i>Accredit. Qual. Assur.</i> 15 (2), 81–87	2009 Sep
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Transepithelial transport of rosmarinic acid in intestinal Caco-2 cell monolayers	Konishi, Y.; Kobayashi, S.	<i>Biosci., Biotechnol., Biochem.</i> 69 (3), 583–591	2005 Mar
Effects of various doses of selenite on stinging nettle (<i>Urtica dioica L.</i>)	Krstofova, O.; Adam, V.; Babula, P.; Zehnalek, J.; Beklova, M.; Havel, L.; Kizek, R.	<i>Int. J. Environ. Res. Public Health</i> 7 (10), 3804–3815	2010 Oct
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Determination of volatile phenols in wine using high-performance liquid chromatography with a coulometric array detector	Larcher, R.; Nicolini, G.; Puecher, C.; Bertoldi, D.; Moser, S.; Favaro, G.	<i>Anal. Chim. Acta</i> 582 (1), 55–60	2007 Jan 16

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The analysis of lipids via HPLC with a charged aerosol detector	Moreau, R. A.	<i>Lipids</i> 41 (7), 727–34	2006 Jul
Lipid analysis via HPLC with a charged aerosol detector	Moreau, R. A.	<i>Lipid Technol.</i> 21 (8–9), 191–194	2009 Oct 23
Extraction and analysis of food lipids	Moreau, R. A.; Winkler-Moser, J. K.	Chapter 6 in <i>Methods of Analysis of Food Components and Additives</i> , Second Edition; Ötles, S., Ed.; Taylor & Francis Group, LLC: Boca Raton, FL.; 115–134	2011 Nov
Aerosol based detectors for the investigation of phospholipid hydrolysis in a pharmaceutical suspension formulation	Nair, L.; Werling, J.	<i>J. Pharm. Biomed. Anal.</i> 49 (1), 95–99	2009 Jan 15
Structure/function relationships of adipose phospholipase A2 containing a cys-his-his catalytic triad	Pang, X. Y.; Cao, J.; Addington, L.; Lovell, S.; Battaile, K. P.; Zhang, Rao, J. L.; Dennis, E. A.; Moise, A. R.	<i>J. Biol. Chem.</i> 287 (42), 35260–35274	2012 Oct 12
Simultaneous assessment of lipid classes and bile acids in human intestinal fluid by solid-phase extraction and HPLC methods	Persson, E.; Löfgren, L.; Hansson, G.; Abrahamsson, B.; Lennernäs, H.; Nilsson, R.	<i>J. Lipid Res.</i> 48 (1), 242–251	2007 Jan

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Peer Reviewed Journals: HPLC and UHPLC Methods

Lipids

Title	Authors	Publication	Publication Date
The use of charged aerosol detection with HPLC for the measurement of lipids	Plante, M.; Bailey, B.; Acworth, I.	<i>Methods Mol. Biol.</i> (Totowa, NJ, U.S.) 579, 469–482	2009
Comparison between charged aerosol detection and light scattering detection for the analysis of Leishmania membrane phospholipids	Ramos, R. G.; Libong, D.; Rakotomanga, M.; Gaudin, K.; Loiseau, P. M.; Chaminade, P.	<i>J. Chromatogr. A.</i> 1209 (1–2), 88–94	2008 Oct 31
Authentication of geographical origin of palm oil by chromatographic fingerprinting of triacylglycerols and partial least square-discriminant analysis	Ruiz-Samblás, C.; Arrebola-Pascual, C.; Tres, A.; van Ruth, S.; Cuadros-Rodríguez, L.	<i>Talanta.</i> 116, 788–793	2013 Nov 15
Simple and precise detection of lipid compounds present within liposomal formulations using a charged aerosol detector	Schönherr, C.; Touchene, S.; Wilser, G.; Peschka-Süss, R.; Francese, G.	<i>J. Chromatogr. A.</i> 1216 (5), 781–786	2009 Jan 30
Determination of intraluminal individual bile acids by HPLC with charged aerosol detection	Vertzoni, M.; Archontaki, H.; Reppas, C.	<i>J. Lipid Res.</i> 49 (12), 2690–2695	2008 Dec
Neurolipids and the use of a charged aerosol detector	Waraska, J.; Acworth, I.	<i>Am. Biotechnol. Lab.</i> 26 (1), 12–13	2008



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Product Number	Technique	Title
AB 119	UV	Rapid Separation of Paclitaxel and Related Compounds in Paclitaxel Injection
AB 134	MS	LC-MS Analysis of Anthocyanins in Bilberry Extract
AB 139	UV	Separation of Schizandrin, Schizandrin A, and Schizandrin B in a Tablet Sample
AB 153	UV	Save the Flavor – Robust Iso- α -Acids Assaying in Beer within Ten Minutes
AB 155	UV	Monitor the Brewing Process with LC-Transformation of Hop alpha-Acids into Beer Iso-alpha-Acids
AN 109	FLD	Determination of Glyphosate by Cation-Exchange Chromatography with Postcolumn Derivatization
AN 156	UV	The Everlasting Paradigm-Keep Beer Tradition or Prevent Beer from a Skunk Off-Flavor?
AN 196	FLD	Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Edible Oils by Donor-Acceptor Complex Chromatography (DACC)-HPLC with Fluorescent Detection
AN 207	UV	Chromatographic Fingerprinting of <i>Flos Chrysanthema indicum</i> Using HPLC
AN 213	UV/FLD	Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Tap Water Using on-Line Solid-Phase Extraction Followed by HPLC with UV and Fluorescence Detection
AN 216	UV	Determination of Water- and Fat-Soluble Vitamins in Functional Waters by HPLC with UV-PDA Detection
AN 224	UV	Determination of Melamine in Milk Powder by Reversed-Phase HPLC with UV Detection
AN 232	UV	Determination of Anthraquinones and Stilbenes in Giant Knotweed Rhizome by HPLC with UV Detection
AN 236	UV	Determination of Iodide and Iodate in Seawater and Iodized Table Salt by HPLC-UV Detection
AN 245	UV	Fast Analysis of Dyes in Foods and Beverages
AN 251	UV	Determination of Water- and Fat-Soluble Vitamins in Nutritional Supplements by HPLC with UV Detection
AN 252	UV	HPLC Assay of Water-Soluble Vitamins, Fat-Soluble Vitamins, and a Preservative in Dry Syrup Multivitamin Formulation
AN 261	UV	Sensitive Determination of Microcystins in Drinking and Environmental Waters
AN 264	UV	Fast Determination of Anthocyanins in Pomegranate Juice
AN 266	FLD	Determination of Sialic Acids Using UHPLC with Fluorescence Detection
AN 272	FLD	Faster Yet Sensitive Determination of N-Methylcarbamates in Rice, Potato, and Corn by HPLC
AN 275	UV	Sensitive Determination of Catechins in Tea by HPLC
AN 287	UV	Two-Dimensional HPLC Combined with On-Line SPE for Determination of Sudan Dyes I–IV in Chili Oil
AN 292	UV	Determination of Aniline and Nitroanilines in Environmental and Drinking Waters by On-Line SPE
AN 293	CAD and UV	Steviol Glycoside Determination by HPLC with Charged Aerosol and UV Detections Using the Acclaim Trinity P1 Column
AN 299	UV	HPLC Analysis of Six Active Components of <i>Caulis Ilicicerae</i> Using a Phenyl-1 Column
AN 1008	UV	Determination of Nitidine Chloride, Toddalolactone, and Chelerythrine Chloride by HPLC

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Product Number	Technique	Title
AN 1020	EC, UV	Chalcinoids and Bitter Acids in Beer by HPLC with UV and ECD
AN 1023	UV	Determination of Sudan Dyes I–IV in Curry Paste
AN 1026	CAD	Fatty Acid Esters at Low Nanogram Levels
AN 1027	CAD	Ginseng
AN 1028	CAD	Ginkgo biloba
AN 1029	CAD	Black Cohosh
AN 1030	CAD	Soy Saponins
AN 1032	CAD	Unsaturated Fatty Acid: Arachidonic, Linoleic, Linolenic and Oleic Acids
AN 1033	CAD	Corn Syrup
AN 1034	CAD	Honey Sugars
AN 1035	CAD	Phenolic Acids
AN 1036	CAD	Water-Soluble Antioxidants: Ascorbic Acid, Glutathione and Uric Acid
AN 1037	CAD	Artificial Sweeteners-Global Method
AN 1039	CAD	Simultaneous Measurement of Glycerides (Mono-, Di- and Triglycerides) and Free Fatty Acids in Palm Oil
AN 1040	CAD	Analysis of Commercially Available Products Containing Stevia
AN 1041	CAD	Phytosterols
AN 1042	UV	Rapid Separation of Anthocyanins in Cranberry and Bilberry Extracts Using a Core-Shell Particle Column
AN 1045	UV	Determination of Phthalates in Drinking Water by UHPLC with UV Detection
AN 1046	UV	Determination of Phenylurea Compounds in Tap Water and Bottled Green Tea
AN 1055	CAD	Determination of Virginiamycin, Erythromycin, and Penicillin in Dried Distillers Grains with Solubles
AN 1063	ECD	Targeted Analyses of Secondary Metabolites in Herbs, Spices, and Beverages Using a Novel Spectro-Electro Array Platform
AN 1064	ECD	Product Authentication and Adulteration Determination Using a Novel Spectro-Electro Array Platform
AN 1067	UV	Determination of Carbendazim in Orange Juice
AN 1069	UV	Two-Dimensional HPLC Determination of Water-Soluble Vitamins in a Nutritional Drink
AN 1070	UV	Determination of Inositol Phosphates in Dried Distillers Grains and Solubles
AN 20583	UV	Determination of Catechins and Phenolic Acids in Red Wine by Solid Phase Extraction and HPLC
AN 20610	UV	Fast Analysis of Coffee Bean Extracts Using a Solid Core HPLC Column
AN 20663	CAD	Comparative Analysis of Cooking Oils Using a Solid Core HPLC Column
AN 20847	CAD	Analysis of a Sports Beverage for Electrolytes and Sugars Using Multi-Mode Chromatography with Charged Aerosol Detection

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Product Number	Technique	Title
AN 70158	CAD	Novel Universal Approach for the Measurement of Natural Products in a Variety of Botanicals and Supplements
AN 70277	CAD	Simultaneous Analysis of Glycerides and Fatty Acids in Palm Oil
AU 144	UV	Determination of Hexavalent Chromium in Drinking Water Using Ion Chromatography
AU 170	UV	Fast Determination of Vanillin and its Synthesis Precursor by HPLC
AU 182	CAD	Measuring Lactose in Milk: A Validated Method
AU 184	CAD, UV	Mogroside V Determination by HPLC with Charged Aerosol and UV Detection
CAN 106	UV	Determination of the Punicagins Found in Pomegranate by High Performance Liquid Chromatography
CAN 111	CAD	Determination of Triterpenes in <i>Centella asiatica</i> (Gotu Kola) by HPLC-CAD
CAN 112	CAD	Determination of Ginsenosides in Panax ginseng by HPLC-CAD
CAN 115	FLD	Clean-Up and Analysis of Aflatoxins and Ochratoxin A in Herbs and Spices
LPN 2062	MS	Profiling Analysis of 15 Prominent Naturally Occurring Phenolic Acids by LC-MS
LPN 2069	FLD	Fast and Effective Determination of Aflatoxins in Grains or Food Using Accelerated Solvent Extraction followed by HPLC
LPN 2421	UV	Achieving Maximum Productivity by Combining UHPLC with Advanced Chromatographic Techniques
LPN 2818	CAD	Analysis of Fat-Soluble Vitamins and Antioxidants in Supplements by RP-HPLC
LPN 2870	FLD	Benefits of High-Speed Wavelength Switching in UHPLC Methods Using Fluorescence Detection
LPN 2930	CAD	Determination of the Composition of Natural Products by HPLC with Charged Aerosol Detection
LPN 2923	CAD	Simple and Direct Analysis of Falcarinol and Other Polyacetylenic Oxylipins in Carrots by Reversed-Phase HPLC and Charged Aerosol Detection
LPN 2931	CAD	Quantification of Underivatized Omega-3 and Omega-6 Fatty Acids in Foods by HPLC CAD
LPN 2932	ECD	A Versatile Detector for the Sensitive and Selective Measurement of Numerous Fat-Soluble Vitamins and Antioxidants in Human Plasma and Plant Extracts
LPN 2934	CAD	Sensitive Analysis of Commonly Used Artificial and Natural Sweeteners Including Stevia and Their Impurities and Degradation Products
LPN 2991	CAD	Evaluation of Methods for the Characterization and Quantification of Polysorbates and Impurities Along with Other Surfactants and Emulsifiers Used in the Food and Pharmaceutical Industries
PN 70026	CAD	Carbohydrate Analysis Using PAD, FLD, CAD and MS Detectors
PN 70037	CAD	Sensitive HPLC Method for Triterpenoid Analysis Using Charged Aerosol Detection with Improved Resolution
PN 70055	CAD	Direct Analysis of Surfactants using HPLC with Charged Aerosol Detection
PN 70138	UV	Rapid Determination of Polyphenol Antioxidants in Green Tea and Cranberry Extract Using Core Shell Columns
PN 70538	CAD	Analysis of Silicone Oils by HPLC-CAD
PN 70540	CAD, ECD	Profiling <i>Hoodia</i> Extracts by HPLC with CAD, ECD, Principal Component Analysis

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Product Number	Technique	Title
AB 127	IC-PAD	Determination of Carbohydrates in Fruit Juice Using Capillary High-Performance Anion-Exchange Chromatography
AB 135	IC-SC	Determination of Anions and Organic Acids in Brewed Coffee Samples Using Capillary IC
AB 137	IC-SC	Determination of Inorganic and Organic Acids in Apple and Orange Juice Samples Using Capillary IC
AN 25	IC-SC	Determination of Inorganic Ions and Organic Acids in Non-Alcoholic Carbonated Beverages
AN 37	IC-PAD	Determination of Iodide and Iodate in Soy- and Mil-Based Infant Formulas
AN 46	IC-PAD	Ion Chromatography: A Versatile Technique for the Analysis of Beer
AN 54	IC-PAD	Determination of Total and Free Sulfite in Foods and Beverages
AN 67	IC-PAD	Determination of Plant-Derived Neutral Oligo- and Polysaccharides
AN 81	IC-SC	Ion Chromatographic Determination of Oxyhalides and Bromide at Trace Level Concentrations in Drinking Water Using direct Injection
AN 82	IC-PAD	Analysis of Fruit Juice Adulterated with Medium Invert Sugar from Beets
AN 87	IC-PAD	Determination of Sugar Alcohols in Confections and Fruit Juices by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection
AN 101	IC-SC	Trace Level Determination of Bromate in Ozonated Drinking Water Using Ion Chromatography
AN 112	IC-UV	Determination of Nitrate and Nitrite in Meat Using High-Performance Anion-Exchange Chromatography
AN 121	IC-SC	Analysis of Low Concentrations of Perchlorate in Drinking Water and Ground Water by Ion Chromatography
AN 123	IC-SC	Determination of Inorganic Anions and Organic Acids in Fermentation Broths
AN 133	IC-SC	Determination of Inorganic Anions in Drinking Water by Ion Chromatography
AN 136	IC-SC and IC-UV	Determination of Inorganic Oxyhalide Disinfection Byproduct Anions and Bromide in Drinking Water Using Ion Chromatography with the Addition of a Postcolumn Reagent for Trace Bromate Analysis
AN 140	IC-SC	Fast Analysis of Anions in Drinking Water by Ion Chromatography
AN 143	IC-SC	Determination of Organic Acids in Fruit Juices
AN 149	IC-SC	Determination of Chlorite, Bromate, Bromide, and Chlorate in Drinking Water by Ion Chromatography with an On-Line-Generated Postcolumn Reagent for Sub- μ g/L Bromate Analysis
AN 150	IC-PAD	Determination of Amino Acids in Cell Cultures and Fermentation Broths
AN 154	IC-SC	Determination of Inorganic Anions in Environmental Waters Using a Hydroxide-Selective Column
AN 155	IC-PAD	Determination of Trans-Galactooligosaccharides in Foods by AOAC Method 2001.02

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Product Number	Technique	Title
AN 165	IC-SC	Determination of Benzoate in Liquid Food Products by Reagent-Free Ion Chromatography
AN 167	IC-SC	Determination of Trace Concentrations of Oxyhalides and Bromide in Municipal and Bottled Waters Using a Hydroxide-Selective Column with a Reagent-Free Ion Chromatography System
AN 168	IC-UV	Determination of Trace Concentrations of Disinfection By-Product Anions and Bromide in Drinking Water Using Reagent-Free Ion Chromatography Followed by Postcolumn Addition of Iol-Dianisidine for Trace Bromate Analysis
AN 169	IC-SC	Rapid Determination of Phosphate and Citrate in Carbonated Soft Drinks Using a Reagent-Free Ion Chromatography System
AN 172	IC-SC	Determination of Azide in Aqueous Samples by Ion Chromatography with Suppressed Conductivity Detection
AN 173	IC-PAD	Direct Determination of Cyanide in Drinking Water by Ion Chromatography with Pulsed Amperometric Detection (PAD)
AN 178	IC-SC	Improved Determination of Trace Concentrations of Perchlorate in Drinking Water Using Preconcentration with Two-Dimensional Ion Chromatography and Suppressed Conductivity Detection
AN 182	IC-SC and IC-PAD	Determination of Biogenic Amines in Alcoholic Beverages by Ion Chromatography with Suppressed Conductivity and Integrated Pulsed Amperometric Detections
AN 183	IC-SC and IC-PAD	Determination of Biogenic Amines in Fermented and Non-Fermented Foods Using Ion Chromatography with Suppressed Conductivity and Integrated Pulsed Amperometric Detections
AN 187	IC-SC	Determination of sub- μ g/L Bromate in Municipal Waters Using Preconcentration with Two-Dimensional Ion Chromatography and Suppressed Conductivity Detection
AN 188	IC-PAD	Determination of Glycols and Alcohols in Fermentation Broths Using Ion-Exclusion Chromatography and Pulsed Amperometric Detection
AN 197	IC-PAD	Determination of Glucosamine in Dietary Supplements Using HPAE-PAD
AN 227	ICE-PAD	Determination of Total Cyanide in Municipal Wastewater and Drinking Water Using Ion-Exclusion Chromatography with Pulsed Amperometric Detection (ICE-PAD)
AN 248	IC-PAD	Determination of Lactose in Lactose-Free Milk Products by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection
AN 253	IC-PAD	HPAE-PAD Determination of Infant Formula Sialic Acids
AN 270	IC-PAD	Determination of Hydroxymethylfurfural in Honey and Biomass
AN 273	IC-SC	Determination of Organic Acids in Fruit Juices and Wines by High-Pressure IC
AN 279	IC-SC	Time Savings and Improved Reproducibility of Nitrate and Nitrite Ion Chromatography Determination in Milk Samples
AN 280	IC-PAD	Carbohydrates in Coffee: AOAC Method 995.13 vs a New Fast Ion Chromatography Method
AN 295	IC-SC	Determination of Phytic Acid in Soybeans and Black Sesame Seeds
AN 1007	IC-SC	Determination of Mono-, Di-, and Triphosphates and Citrate in Shrimp by Ion Chromatography

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Product Number	Technique	Title
AN 1044	IC-SC	Determination of Anions in Dried Distillers Grains with Solubles
AN 1068	IC-SC	Determination of Organic Acids in Fruit Juices and Wines by High-Pressure IC
AU 132	IC-UV	Determination of Nitrite and Nitrate in drinking Water by Ion Chromatography with Direct UV Detection
AU 144	IC-UV	Determination of Hexavalent Chromium in Drinking Water Using Ion Chromatography
AU 148	IC-SC	Determination of Perchlorate in Drinking Water Using Reagent-Free Ion Chromatography
AU 150	IC-PAD	Determination of Plant-Derived Neutral Oligo- and Polysaccharides Using the CarboPac PA200
AU 151	IC-PAD	Determination of Sucralose in Reduced- Carbohydrate Colas using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection
AU 189	IC-SC	Determination of Choline in Infant Formula and Other Food Samples by IC
LPN 2982	IC-SC	Determination of Inorganic Anions and Organic Acids in Beverages Using a Capillary IC on a Monolith Anion-Exchange Column
PN 70743	IC-SC	Determination of Perchlorate Levels in Food and Soil Samples Using Accelerated Solvent Extraction and Ion Chromatography
TN 20	IC-PAD	Analysis of Carbohydrates by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD)
TN 126	IC-SC	Determination of Organic Acids in Beer Samples Using a High-Pressure Ion Chromatography System
TN 135	IC-PAD	Determinations of Monosaccharides and Disaccharides in Beverages by Capillary HPAE-PAD

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Accelerated, microwave-assisted, and conventional solvent extraction methods affect anthocyanin composition from colored grains	Abdel-Aal el-SM; Akhtar, H.; Rabalski, I.; Bryan, M.	<i>J. Food Sci.</i> 79 (2), C138–46	2014 Feb
Multi-residue method for the analysis of pesticide residues in fruits and vegetables by accelerated solvent extraction and capillary gas chromatography	Adou, K.; Bontoyan, W. R.; Sweeney, P. J.	<i>J. Agric. Food Chem.</i> 49 (9), 4153–4160	2001 Sep
The development of an optimized sample preparation for trace level detection of 17α-ethinylestradiol and estrone in whole fish tissue	Al-Ansari, A. M.; Saleem, A.; Kimpe, L. E.; Trudeau, V. L.; Blais, J. M.	<i>J. Chromatogr. B Analys. Technol. Biomed. Life Sci.</i> 879 (30), 3649–52	2011 Nov
Determination of polyphenolic profiles of basque cider apple varieties using accelerated solvent extraction	Alonso-Salces, R. M.; Korta, E.; Barranco, A.; Berrueta, L.A.; Gallo, B.; Vicent, F.	<i>J. Agric. Food Chem.</i> 49 (8), 3761–376	2001
Pressurized liquid extraction for the determination of polyphenols in apple	Alonso-Salces, R. M.; Korta, E.; Barranco, A.; Berrueta, L. A.; Gallo, B.; Vicente, F.	<i>J. Chromatogr. A.</i> 933 (1–2), 37–43	2001 Nov
Methods for extraction and determination of phenolic acids in medicinal plants: a review	Arceusz, A.; Wesolowski, M.; Konieczynski, P.	<i>Nat. Prod. Commun.</i> 8 (12), 1821–9	2013 Dec
Study of an accelerated solvent extraction procedure for the determination of acaricide residues in honey by high-performance liquid chromatography-diode array detector	Bakkali, A.; Korta, E.; Berrueta, L. A.	<i>J. Food Protection</i> 65 (1), 161–166	2002
Pressurized liquid extraction of medicinal plants	Benthin, B.; Danz, H.; Hamburger, M.	<i>J. Chromatogr. A.</i> 837 (1-2), 211–9	1999 Apr
Comparison of the chemical composition of extracts from <i>Scutellaria lateriflora</i> using accelerated solvent extraction and supercritical fluid extraction versus standard hot water or 70% ethanol extraction	Bergeron, C.; Gafner, S.; Clausen, E.; Carrier, D. J.	<i>J. Agric. Food Chem.</i> 53 (8), 3076–80	2005 Apr
Polybrominated diphenyl ethers (PBDEs) in Mediterranean mussels (<i>Mytilus gallo-provincialis</i>) from selected Apulia coastal sites evaluated by GC-HRMS	Bianco, G.; Novario, G.; Anzilotta, G.; Palma, A.; Mangone, A.; Cataldi, T. R.	<i>J. Mass Spectrom.</i> 45 (9), 1046–55	2010 Sep
Free and bound phenolic compounds in barley (<i>Hordeum vulgare L.</i>) flours. Evaluation of the extraction capability of different solvent mixtures and pressurized liquid methods by micellar electrokinetic chromatography and spectrophotometry	Bonoli, M.; Marconi, E.; Caboni, M. F.	<i>J. Chromatogr. A.</i> 19; 1057 (1-2), 1–12	2004 Nov
Pressurized liquid extraction of lipids for the determination of oxysterols in egg-containing food	Boselli, E.; Velazco, V.; Caboni, M. F.; Lercker, G.	<i>J. Chromatogr. A.</i> 11; 917 (1-2), 239–44	2001 May
Optimisation of accelerated solvent extraction of cocaine and benzoylecgonine from coca leaves	Brachet, A.; Rudaz, S.; Mateus, L.; Christen, P.; Veuthey, J-L.	<i>J. Sep. Sci.</i> 24 (10-11), 865–873	2001 Nov

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Multi-residue determination of 130 multiclass pesticides in fruits and vegetables by gas chromatography coupled to triple quadrupole tandem mass spectrometry	Cervera, M.I.; Medina, C.; Portolés, T.; Pitarch, E.; Beltrán, J.; Serrahima, E.; Pineda, L.; Muñoz, G.; Centrich, F.; Hernández, F.	<i>Anal. Bioanal. Chem.</i> 397 (7), 2873–91	2010 Aug
Influence of extraction methodologies on the analysis of five major volatile aromatic compounds of citronella grass (<i>Cymbopogon nardus</i>) and lemongrass (<i>Cymbopogon citratus</i>) grown in Thailand	Chanthai, S.; Prachakoll, S.; Ruangviriyachai, C.; Luthria, D. L.	<i>J. AOAC Int.</i> 95 (3), 763–72	2012 May-Jun
Accelerated solvent extraction of vitamin K₁ in medical foods in conjunction with matrix solid-phase dispersion	Chase, G. W.; Thompson, B.	<i>J. AOAC Int.</i> 83 (2), 407–10	2000
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A multi-residue method for the analysis of organophosphorus residues in cooked and polished rice using accelerated solvent extraction and dispersive-solid phase extraction (D-SPE) technique and uncertainty measurement	Sanyal, D.; Rani, A.; Alam, S.	<i>J. Environ. Sci. Health, B</i> 44 (7), 706–16.	2009 Sep
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HPLC analysis of kaempferol and quercetin derivatives isolated by different extraction techniques from plant matrix	Skalicka-Wozniak, K.; Szypowski, J.; Głowniak, K.	<i>J. AOAC Int.</i> 94 (1), 17–21.	Jan-Feb 2011
Statistical evaluation of fatty acid profile and cholesterol content in fish (common carp) lipids obtained by different sample preparation procedures	Spiric, A.; Trbovic, D.; Vranic, D.; Djinovic, J.; Petronijevic, R.; Matekalo-Sverak, V.	<i>Anal. Chim. Acta</i> 672 (1-2), 66–71.	2010 Jul
Application of accelerated solvent extraction in the analysis of organic contaminants, bioactive and nutritional compounds in food and feed	Sun, H.; Ge, X.; Lv, Y.; Wang, A.	<i>J. Chromatogr., A</i> 1237, 1–23.	2012 May
Development of an accelerated solvent extraction, ultrasonic derivatization LC-MS/MS method for the determination of the marker residues of nitrofurans in freshwater fish	Tao, Y.; Chen, D.; Wei, H.; Yuanhu, P.; Liu, Z.; Huang, L.; Wang, Y.; Xie, S.; Yuan, Z.	<i>Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.</i> 29 (5), 736–45.	2012
Simultaneous determination of lincomycin and spectinomycin residues in animal tissues by gas chromatography-nitrogen phosphorus detection and gas chromatography-mass spectrometry with accelerated solvent extraction	Tao, Y.; Chen, D.; Yu, G.; Yu, H.; Pan, Y.; Wang, Y.; Huang, L.; Yuan, Z.	<i>Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.</i> 28 (2), 145–54.	2011 Feb
Determination of 17 macrolide antibiotics and avermectins residues in meat with accelerated solvent extraction by liquid chromatography-tandem mass spectrometry	Tao, Y.; Yu, G.; Chen, D.; Pan, Y.; Liu, Z.; Wei, H.; Peng, D.; Huang, L.; Wang, Y.; Yuan, Z.	<i>J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.</i> 897, 64–71.	2012 May
Determination of seven toxaphene congeners in ginseng and milkvetch root by gas chromatography tandem mass spectrometry	Tian, S.; Mao, X.; Miao, S.; Jia, Z.; Wang, K.; Ji, S.	<i>Se Pu</i> 30 (1), 14–20.	2012 Jan

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Title	Authors	Publication	Publication Date
A consecutive preparation method based upon accelerated solvent extraction and high-speed counter-current chromatography for isolation of aesculin from <i>Cortex fraxinus</i>	Tong, X.; Zhou, T; Xiao, X.; Li, G.	J. Sep. Sci. 35 (24), 3609–14	2012 Dec
Characterization of anthocyanins and anthocyanidins in purple-fleshed sweetpotatoes by HPLC-DAD/ESI-MS/MS	Truong, V. D.; Deighton, N.; Thompson, R. T.; McFeeters, R. F.; Dean, L. O.; Pecota, K. V.; Yencho, G. C.	J. Agric. Food Chem. 58 (1), 404–10	2010 Jan
Fat extraction from acid- and base-hydrolyzed food samples using accelerated solvent extraction	Ullah, S. M.; Murphy, B.; Dorich, B.; Richter, B.; Srinivasan, K.	J. Agric. Food Chem. 59 (6), 2169–74.	2011 Mar
Analysis of zearalenone in cereal and swine feed samples using an automated flow-through immunosensor	Urraca, J. L.; Benito-Peña, E.; Pérez-Conde, C.; Moreno-Bondi, M. C.; Pestka, J. J.	J. Agric. Food Chem. 53 (9), 3338–3344	2005
Accelerated solvent extraction and gas chromatography/mass spectrometry for determination of polycyclic aromatic hydrocarbons in smoked food samples	Wang, G.; Lee, A. S.; Lewis, M.; Kamath, B.; Archer, R. K.	J. Agric. Food Chem. 47 (3), 1062–6.	1999 Mar
Subcritical water extraction of alkaloids in <i>Sophora flavescens</i> Ait. and determination by capillary electrophoresis with field-amplified sample stacking	Wang, H.; Lu, Y.; Chen, J.; Li, J.; Liu, S.	J. Pharm. Biomed. Anal. 58, 146–51.	2012 Jan
Evaluation of Soxhlet extraction, accelerated solvent extraction and microwave-assisted extraction for the determination of polychlorinated biphenyls and polybrominated diphenyl ethers in soil and fish samples	Wang, P.; Zhang, Q.; Wang, Y.; Wang, T.; Li X.; Ding, L.; Jiang, G.	Anal. Chim. Acta. 663 (1), 43–8.	2010 Mar
Determination of ten pesticides of pyrazoles and pyrroles in tea by accelerated solvent extraction coupled with gas chromatography-tandem mass spectrometry	Xu, D.; Lu, S.; Chen, D.; Lan, J.; Zhang, Z.; Yang, F.; Zhou, Y.	Se Pu.; 31 (3), 218–22.	2013 Mar
Online cleanup of accelerated solvent extractions for determination of adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), and adenosine 5'-monophosphate (AMP) in royal jelly using high-performance liquid chromatography	Xue, X.; Wang, F.; Zhou, J.; Chen, F.; Li, Y.; Zhao, J.	J. Agric. Food Chem. 57 (11), 4500–5.	2009 Jun
Identification and quantitation of eleven sesquiterpenes in three species of <i>Curcuma</i> rhizomes by pressurized liquid extraction and gas chromatography-mass spectrometry	Yang, F. Q.; Li ,S.; Chen, Y.; Lao, S. C.; Wang, YT.; Dong, T. T. X.; Tsim, K. W. K.	J. Pharm. Biomed. Anal. 39 (3/4), 552–558	2005 Sep
Dispersive solid-phase extraction cleanup combined with accelerated solvent extraction for the determination of carbamate pesticide residues in <i>Radix glycyrrhizae</i> samples by UPLC-MS-MS	Yang, R. Z.; Wang, J. H.; Wang, M. L.; Zhang, R.; Lu, X. Y.; Liu, W. H.	J. Chromatogr. Sci. 49 (9), 702–8.	2011 Oct
Simultaneous determination of amitraz and its metabolite residue in food animal tissues by gas chromatography-electron capture detector and gas chromatography-mass spectrometry with accelerated solvent extraction	Yu, H.; Tao, Y.; Le, T.; Chen, D.; Ihsan, A.; Liu, Y.; Wang, Y.; Yuan, Z.	J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 878 (21), 1746–52.	2010 Jul
Simultaneous determination of fluoroquinolones in foods of animal origin by a high performance liquid chromatography and a liquid chromatography tandem mass spectrometry with accelerated solvent extraction	Yu, H.; Tao, Y.; Chen, D.; Pan, Y.; Liu, Z.; Wang, Y.; Huang, L.; Dai, M.; Peng, D.; Wang, X.; Yuan, Z.	J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 885-886, 150–9.	2012 Feb

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Title	Authors	Publication	Publication Date
Determination of pentachlorophenol residue in meat and fish by gas chromatography-electron capture detection and gas chromatography-mass spectrometry with accelerated solvent extraction	Zhao, D.	<i>J. Chromatogr. Sci.</i>	2013 May
Response surface modeling and optimization of accelerated solvent extraction of four lignans from <i>fructus schisandrae</i>	Zhao, L. C.; He, Y.; Deng, X.; Yang, G. L.; Li, W.; Liang, J.; Tang, Q. L.	<i>Molecules</i> . 17 (4), 3618–29	2012 Mar
Determination of acetanilide herbicides in cereal crops using accelerated solvent extraction, solid-phase extraction and gas chromatography-electron capture detector	Zhang, Y.; Yang, J.; Shi, R.; Su, Q.; Yao, L.; Li, P.	<i>J. Sep. Sci.</i> 34 (14), 1675–82	2011 Jul
Application of accelerated solvent extraction coupled with high-performance counter-current chromatography to extraction and online isolation of chemical constituents from <i>Hypericum perforatum</i> L	Zhang, Y.; Liu, C.; Yu, M.; Zhang, Z.; Qi, Y.; Wang, J.; Wu, G.; Li, S.; Yu, J.; Hu, Y.	<i>J. Chromatogr., A.</i> 1218 (20), 2827–34	2011 May
Analysis of volatile components in Qingshanlvshui tea using solid-phase microextraction/accelerated solvent extraction-gas chromatography-mass spectrometry	Zhan, J.; Lu, S.; Meng, Z.; Xiang, N.; Cao, Q.; Miao, M.	<i>Se Pu.</i> 26 (3), 301–5.	2008 May



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Technical Collateral: Sample Preparation Methods

Product Number	Technique	Title
AN 326	HPLC-UV	Extraction of Drugs from Animal Feeds Using Accelerated Solvent Extraction (ASE)
AN 335	HPLC-UV	Accelerated Solvent Extraction (ASE) of Active Ingredients from Natural Products
AN 356	IC-conductivity	Determination of Perchlorate in Vegetation Samples Using Accelerated Solvent Extraction and Ion Chromatography
AN 357	HPLC	Extraction of Phenolic Acids from Plant Tissue Using Accelerated Solvent Extraction (ASE)
AN 363	HPLC	Extraction of Herbal Marker Compounds Using Accelerated Solvent Extraction Compared to Traditional Pharmacopoeia Protocols



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