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Chromatography for Foods and Beverages

Supplements Analysis Applications Notebook

From Raw Materials to Extracts and Natural Products

Thermo SCIENTIFIC

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Supplements

Introduction

Herbal supplements, made from plants, are used by some individuals to augment traditional medical treatments. Supplements vary greatly and can consist of raw material (e.g., fresh herb, dried leaf, powdered root, etc), extracts or tinctures, or even the refined purportedly active chemical, sometimes referred to as the natural product.

Herbal supplements have been used throughout history and their natural products (sometimes chemically modified) used in Western medicine (e.g., taxol from Pacific yew trees; digitalis from Foxgloves). Natural products scaffolds are also a valuable resource for drug development by the pharmaceutical industry.

Traditional Chinese Medicine (TCM) makes use of herbal supplements in addition to other substances derived from fungi, marine organisms, animals, and minerals.

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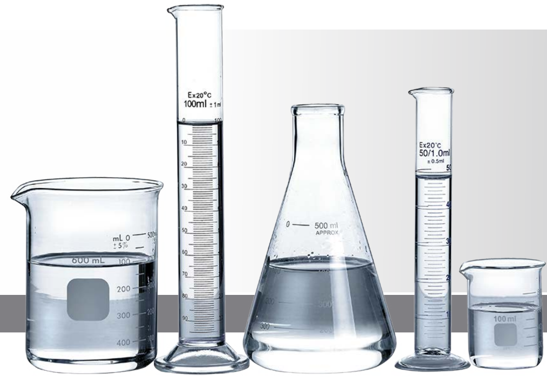

Analytical Technologies

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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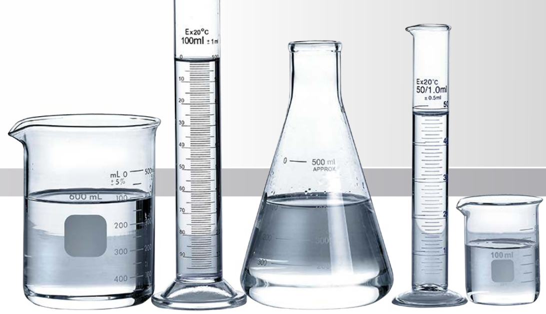

The Vanquish UHPLC System

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 delivers 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 ºC to 120 ºC for two thermostatting 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 LighPipe technology

Learn more at www.thermoscientific.com/vanquish

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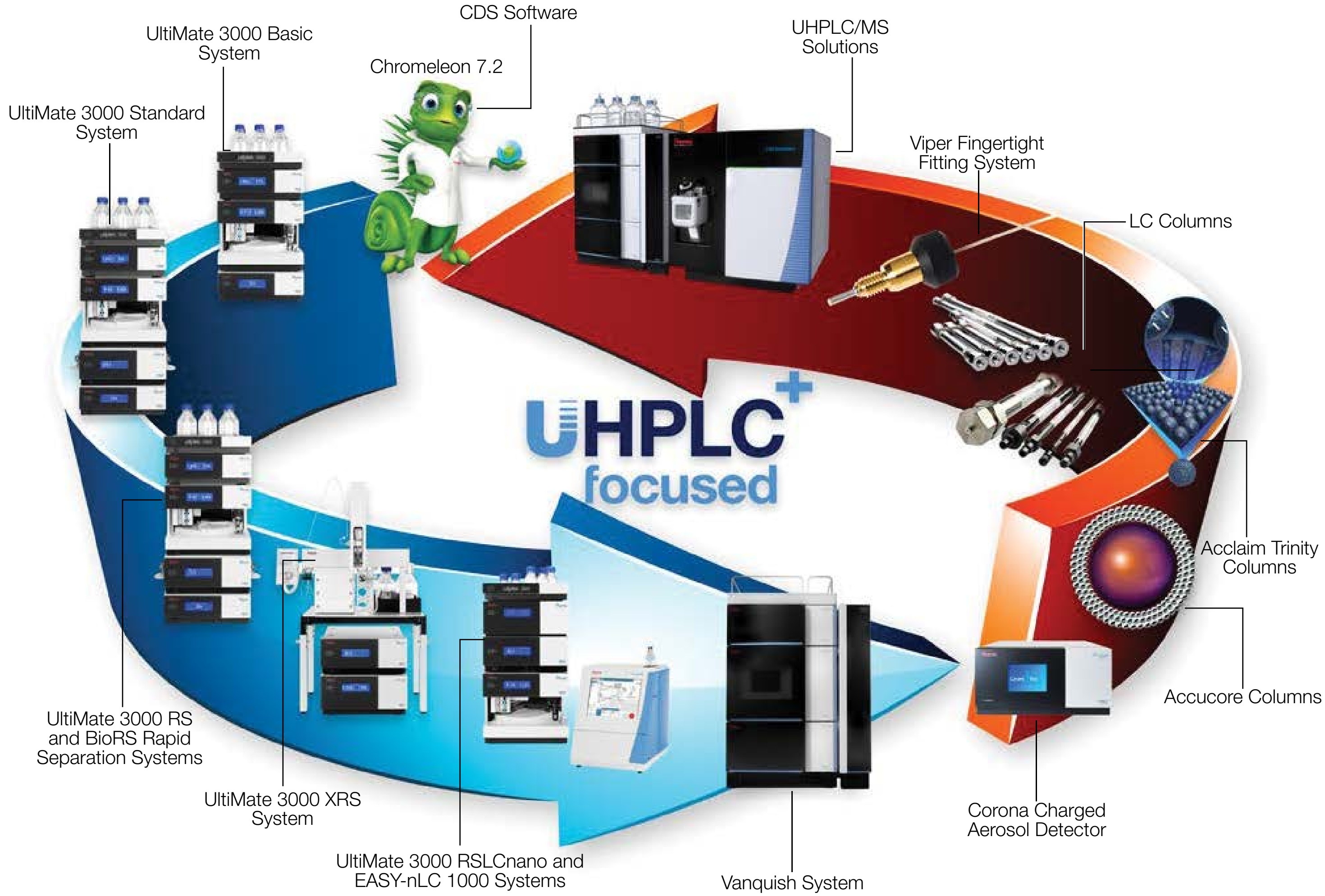
UHPLC Portfolio



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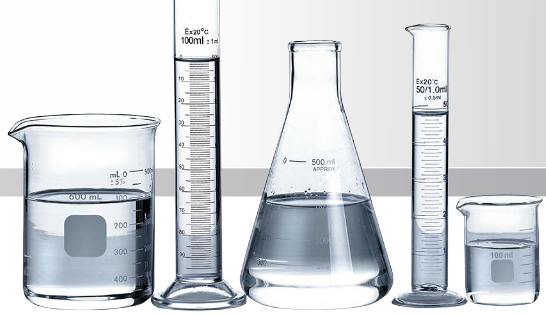

UltiMate 3000 UHPLC$^+$ Systems

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

Learn more at www.thermoscientific.com/liquidchromatography

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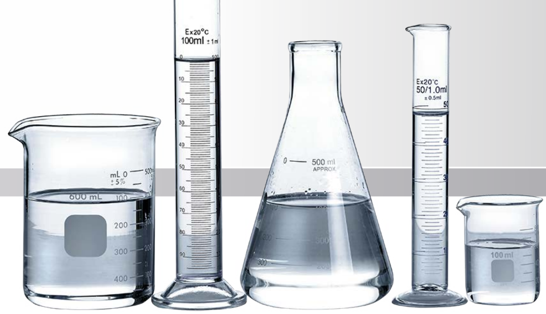

UltiMate 3000 UHPLC+ Systems

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.

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.

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

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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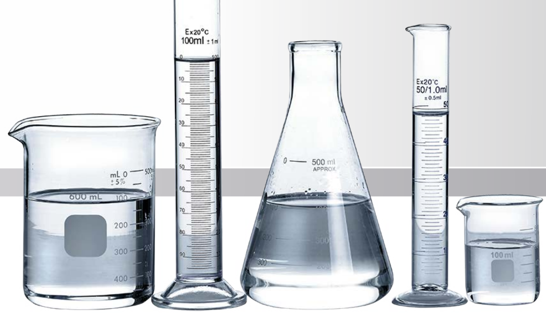

Advanced Detection Capabilities

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

Learn more at www.thermoscientific.com/CAD

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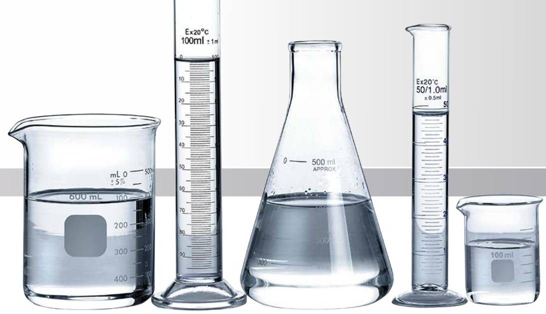

Advanced Detection Capabilities

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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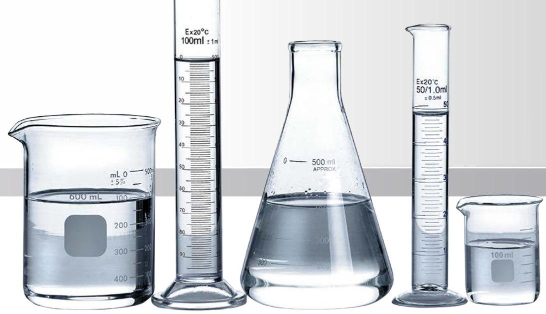

Advanced Detection Capabilities

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
- The extended flow rate range from 1 mL/min up to 10 mL/min and the operating range of 1.00 to 1.75 RIU enable the use of this detector for a wide range of applications
- Applications include the analysis of all compounds with low UV-Vis activity, such as alcohols, mono- and polysaccharides, esters, fatty acids, or polymers
- An Auto Set-up function automates purging, equilibration, autozero, and the control baseline stability and noise
- Operation with Chromeleon CDS makes the detector easy to use and ensures maximum productivity in instrument control, data processing, and reporting of results

RefractoMax 521 Refractive Index Detector

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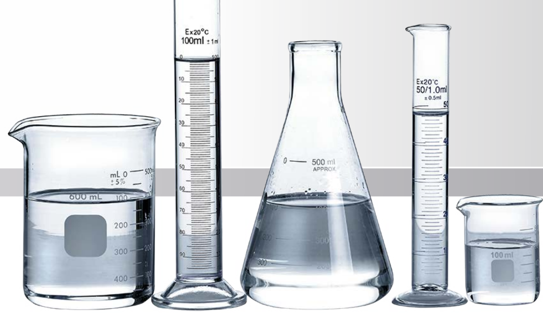

Advanced Detection Capabilities

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
- 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

UltiMate 3000 DAD-3000 Diode Array Detector

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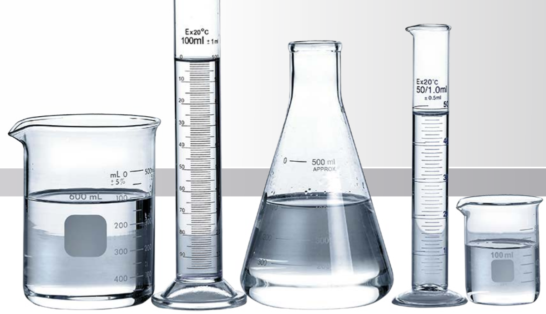

Advanced Detection Capabilities

UltiMate 3000 Electrochemical Detector

Electrochemical detection delivers high sensitivity for neurotransmitter analysis, simplicity and robustness for pharmaceutical or clinical diagnostics, and the selectivity for the characterization of complex samples such as natural products, biological tissues and fluids. For today's researcher, there is a continuing need for detecting vanishingly small quantities of analyte and often in complex samples. Because electrochemical detection measures only compounds that can undergo oxidation or reduction it is both highly sensitive and very selective.

The Thermo Scientific Dionex UltiMate 3000 Electrochemical Detector, designed by the pioneers of coulometric electrochemical detection, delivers state-of-the-art sensor technologies complete with an entire range of high performance and ultra-high performance LC systems optimized for electrochemical detection. The UltiMate 3000 ECD-3000RS takes electrochemical detection to the next level with UHPLC compatibility, total system integration, and selection of detection mode, all with unprecedented operational simplicity.

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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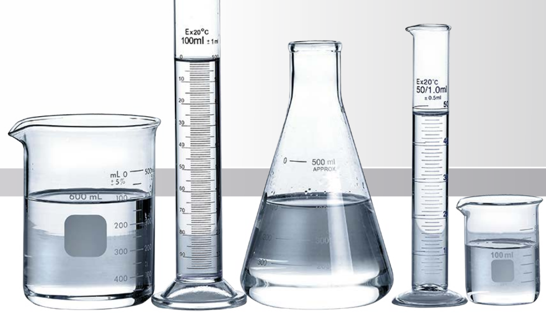

Advanced Detection Capabilities

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 Chromeleon 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

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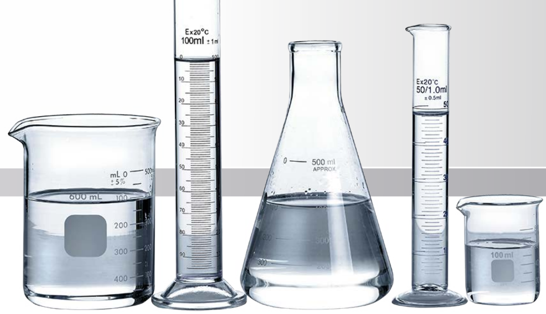

Advanced Detection Capabilities

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
- 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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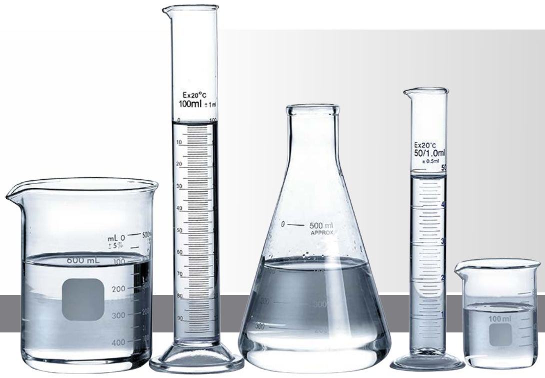

Analytical Technologies

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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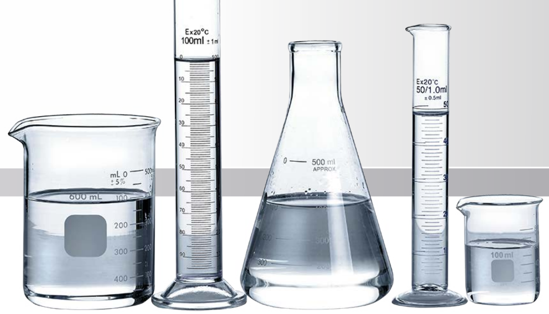

IC and RFIC Systems

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

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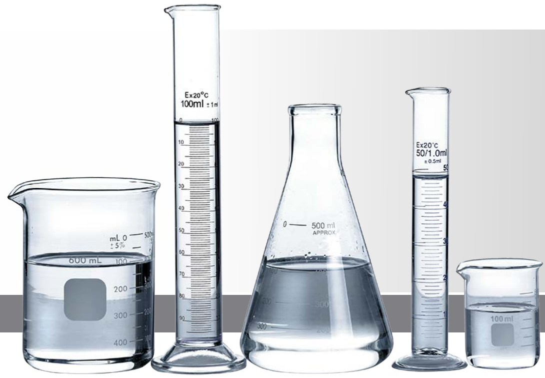

Analytical Technologies

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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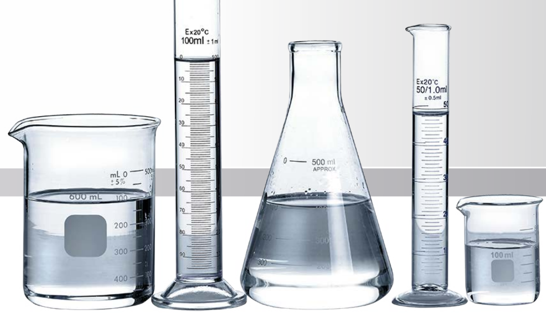

Mass Spectrometry Instruments

Single-Point Control and Automation

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 remove mobile phase ions for effort-free transition to MS detection.

- Thermo Scientific™ MSQ Plus™ mass spectrometer, the smallest and most sensitive single quadrupole on the market for LC and IC
- Self-cleaning ion source for low maintenance operation
- 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 vaccum 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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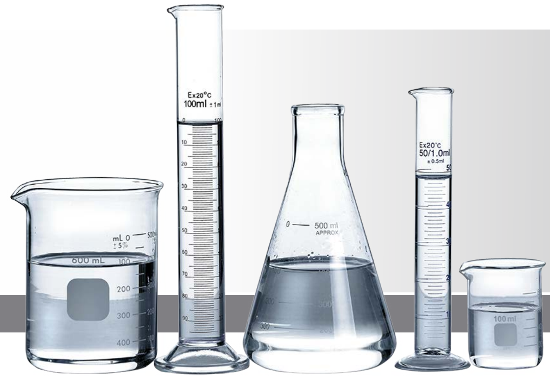

Analytical Technologies

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 Chromeleon 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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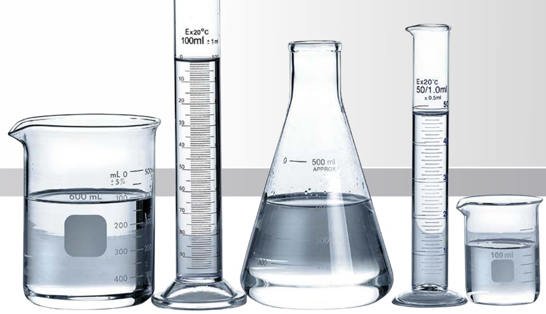

Chromeleon CDS Software

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.

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

Excel is a registered trademark of Microsoft Corporation.

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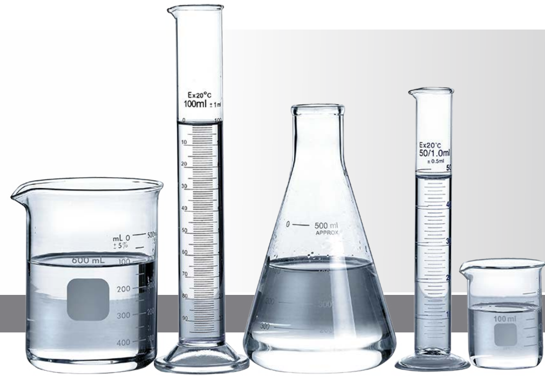

Analytical Technologies

Process Analytical Systems

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

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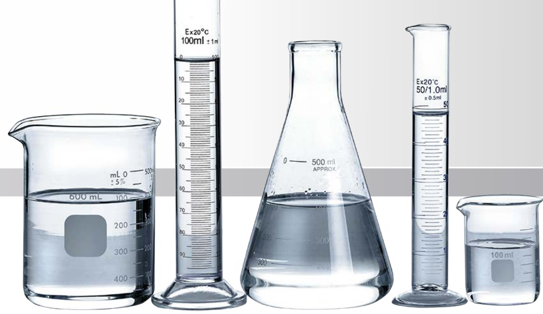

Process Analytical Systems and Software

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 analytzer

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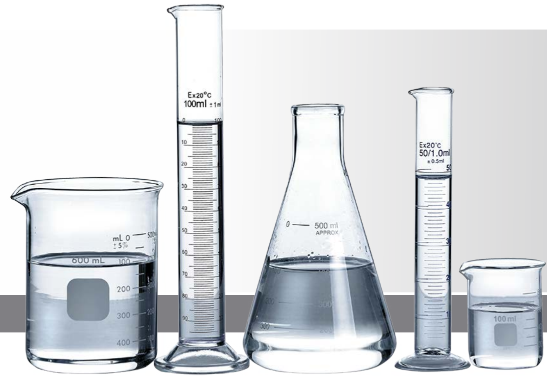

Automated Sample Preparation

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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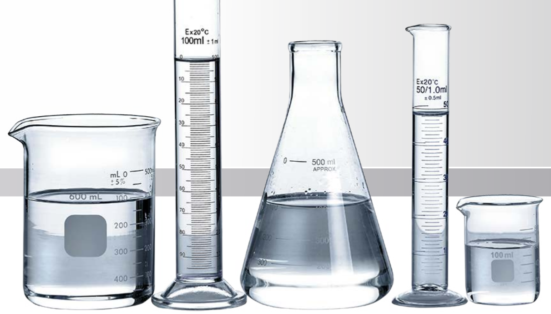

Accelerated Solvent Extractor System

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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Supplements

Blueberries are known to be high in anthocyanins.

Anthocyanins

Anthocyanins are water-soluble plant pigments widely present in fruits, vegetables, and flowers. Anthocyanins have gained considerable interest in the scientific community and consumer market due to their anti-inflammatory action and strong antioxidant and radiation-protection properties.

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Anthocyanins in Bilberry Extract

Bilberries are known to have a high anthocyanin content and are, therefore, one of the most expensive botanical ingredients in the health food industry. The high price of the extract makes bilberries more susceptible to adulteration. Analytical characterization and quantification methods for anthocyanins specific to bilberries are therefore required.

Chromatographic Conditions

System:	UltiMate 3000 RSLC System
Column:	Acclaim RSLC 120 C18 (2.1 × 100 mm, 2.2 µm)
Mobile Phase:	A) CH_3CN, B) DI water, C) 20% formic acid
Gradient:	C held constant at 10%, A from 0% to 8% from 11 to 42 min, held for 13 min, return to 0% in 5 min
Flow Rate:	0.5 mL/min
Injection Volume:	5 µL
Temperature:	40 °C
Sample:	NIST Bilberry Extract Standard Reference Material

Mass Spectrometric Conditions

System:	MSQ Plus single quadrupole mass spectrometer
Ionization interface:	ESI
Probe Temperature:	500 °C
Needle Voltage:	2000 V
Nebulizer Gas:	Nitrogen at 80 psi
Detection Mode:	SIM Refer to chromatogram for SIM Acquisitions

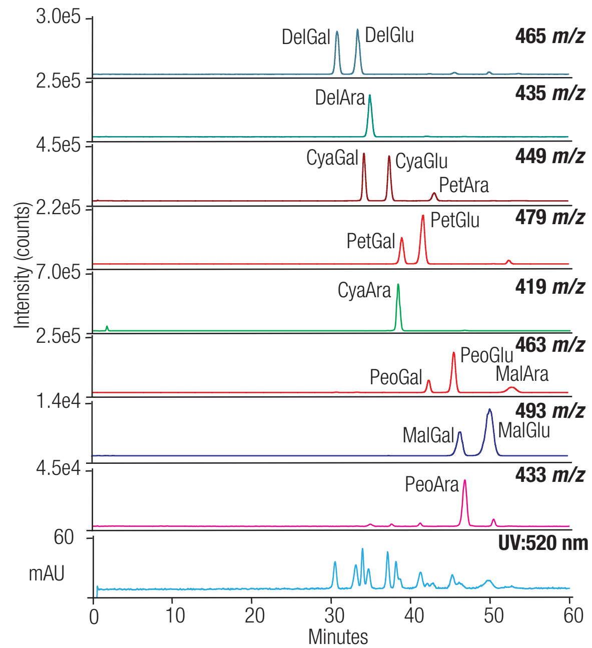

Figure 5-1. Determination of 15 anthocyanins in bilberry extract by LC-MS.

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Download Application Brief 134: LC-MS Analysis of Anthocyanins in Bilberry Extract

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Anthocyanins in Bilberry Extract

Column: Acclaim 120, C18, 2.2 µm, Analytical (2.1 × 150 mm)
Eluent: A: 10% Formic Acid
B: 10% Formic Acid, 22.5% Methanol, 22.5% Acetonitrile
Gradient: 0.0–12.0 min, 9% B
12.0–25.0 min, 35% B
25.0–50% B Step change
Hold at 50% B for 5 min
0.0–35.0 min, 9% B
Flow Rate: 0.475 mL/min
Injection Volume: 2.0 µL
Temperature: 35 °C
Detection: Absorbance, vis, 520 nm
Sample: 25 mg/mL Cranberry Extract

Peaks:	Conc. (µg/mL)
1. Dp3Gal	0.019
2. Dp3Glu	0.029
3. Cy3Gal	0.080
4. Cy3Glu	0.038
5. Cy3Ara	—)
6. Peo3Gal	0.018
7. Peo3Glu	—
8. Mal3Gal	0.070
9. Peo3Ara	0.160

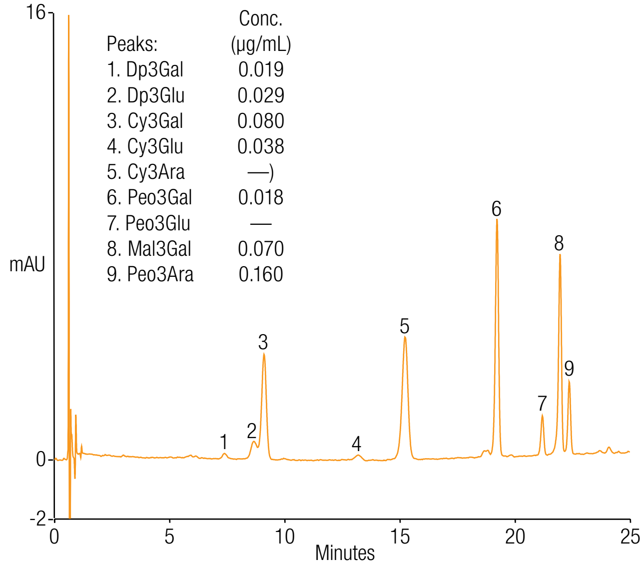

Figure 5-2. Separation of anthocyanins in cranberry extract standard using an Acclaim 120, C18 column

Column: Accucore C18, 2.6 µm, Analytical (2.1 × 150 mm)
Flow: 0.65 mL/min
Temperature: 42 °C
Injection Volume: 2.0 µL
Eluent: A: 10% Formic Acid
B: 10% Formic Acid, 22.5% Methanol, 22.5% Acetonitrile
Gradient: 0.0–15.0 min, 10% B
Detection: Absorbance, vis, 520 nm
Sample: 25 mg/mL Cranberry Extract

Peaks:	Conc. (µg/mL)	Peaks:	Conc. (µg/mL)
1. Dp3Gal	0.021	6. Cy3Ara	0.157
2. Dp3Glu	0.037	7. Peo3Gal	0.176
3. Unknown	—	8. Peo3Glu	—
4. Cy3Gal	0.090	9. Peo3Ara	0.170
5. Cy3Glu	0.041	10. Mal3Gal	0.088

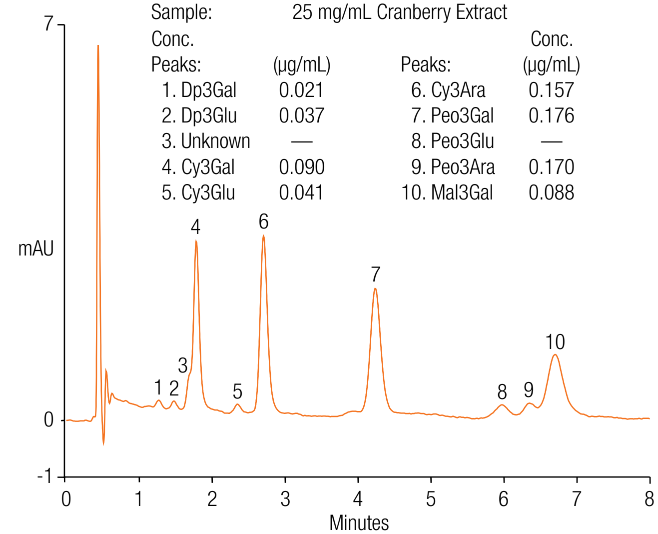

Figure 5-3. Separation of anthocyanins in cranberry extract using an Accucore C18 column.

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Download Application Note 1042: Rapid Separation of Anthocyanins in Cranberry and Bilberry Extracts Using a Core-Shell Particle Column

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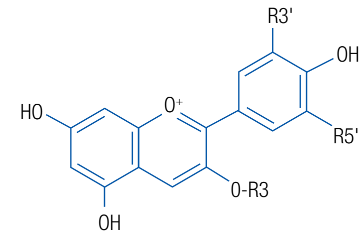
Anthocyanins in Bilberries Using UHPLC

Bilberry (*Vaccinium myrtillus L.*) is a low-growing shrub that is native to temperate regions of North America and Europe. The shrubs are closely related to the native North American wild blueberries, but one characteristic difference is that bilberry plants produce single or paired berries on the bush, unlike blueberries that grow in clusters. In addition, bilberries are smaller, darker, hard, less juicy, easier to transport, and have a different flesh color than blueberries. The two berries also have different phytochemical profiles, with the anthocyanin content of fresh bilberry fruits being almost 4× higher than that of blueberries. Bilberries – known to have a high anthocyanin content – cannot be cultivated, are hard to harvest and process, and therefore are one of the most expensive botanical ingredients in the health food industry. The high price of the extract makes it more susceptible to adulteration.

Bilberry extracts are widely used in nutritional supplements and pharmaceuticals for improving visual acuity and treating circulatory disorders. Chemical and pharmacological studies have identified anthocyanins as the main components responsible for the therapeutic effect of the extracts that are used in these supplements. Clinical trials on therapeutic products using bilberry extracts have shown that a 36% anthocyanin level is effective in the treatment of peripheral vascular disease and venous sensitivity.

Application Note 281 describes a sensitive, fast, and accurate HPLC method to determine anthocyanins in bilberry products. The method uses a high-resolution silica-based 2.2 µm Thermo Scientific™ Acclaim™ RSLC 120 C18 column and a wavelength of 520 nm to separate, detect, and quantify anthocyanins in several commercially available bilberry nutritional supplements.



R3'	R5'	R3	Anthocyanin
H	H	Glucose/Arabinose/Galactose	Pelargonidin 3-Glucose/Arabinose/ Galactose
OH	H	Glucose/Arabinose/Galactose	Cyanidin 3-Glucose/Arabinose/Galactose
OH	OH	Glucose/Arabinose/Galactose	Delphinidin 3-Glucose/Arabinose/ Galactose
OCH_3	OH	Glucose/ Arabinose/Galactose	Petunidin 3-Glucose/Arabinose/ Galactose
OCH_3	H	Glucose/Arabinose/Galactose	Peonidin 3-Glucose/Arabinose/Galactose
OCH_3	OCH_3	Glucose/Arabinose/Galactose	Malvidin 3-Glucose/Arabinose/Galactose

Figure 5-4. Basic structure of anthocyanins.

Download Application Note 281: Rapid and Sensitive Determination of Anthocyanins in Bilberries Using UHPLC

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Anthocyanins in Bilberries Using UHPLC

Column: Acclaim RSLC 120, C18, 2.2 µm, Analytical (2.1 × 150 mm)
Flow: 0.475 mL/min
Temperature: 35 °C
Injection. Volume: 2.0 µL
Eluent: A: 10% Formic acid
B: 10% Formic acid, 22.5% methanol, 22.5% CH_3CN
Gradient: 0.0–12.0 min, 9% B
12.0–25.0 min, 35% B
25.0–30.0 min, 50% B
30.0–35.0 min, 9% B
Detection: Absorbance, vis 520 nm
Sample: 125 µg/mL
15 Anthocyanin standard

Peaks:	Conc. (µg/mL)
1. Dp3Gal	—
2. Dp3Glu	20.1
3. Cy3Gal	11.0
4. Dp3Ara	—
5. Cy3Glu	12.3
6. Pet3Gal	—
7. Cy3Ara	—
8. Delphinidin	—
9. Pet3Glu	9.91
10. Peo3Gal	3.91
11. Pet3Ara	—
12. Peo3Glu	—
13. Mal3Gal	8.76
14. Peo3Ara	12.7
15. Cyanidin	—
16. Mal3Glu	—
17. Mal3Ara	—
18. Petunidin	—
19. Peonidin	—
20. Malvidin	—

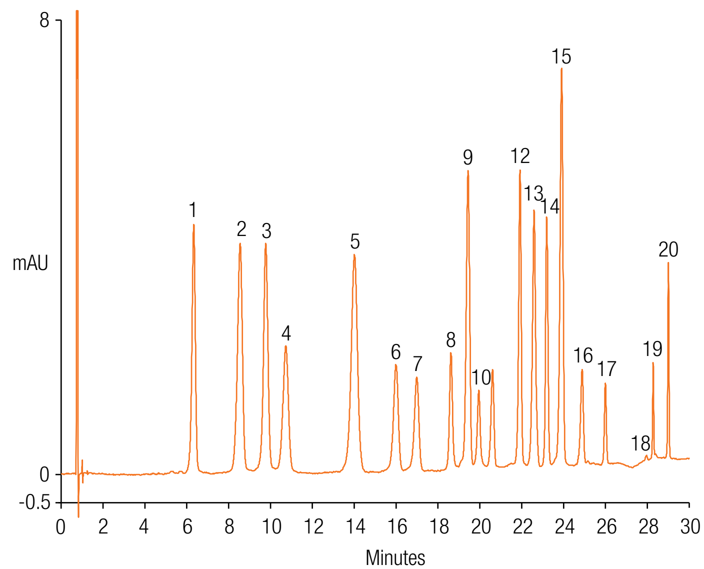

Figure 5-5. Separation of 15 anthocyanins and five anthocyanidins on the Acclaim RSLC 120 C18 column.

Table 5-1. Determination of anthocyanins in NIST and USP reference samples

Analyte	NIST (µg/mL)	USP (µg/mL)
Dp3Glu	14.9	12.2
Cy3Gal	8.01	7.00
Cy3Glu	7.59	6.61
Pet3Glu	6.08	5.14
Peo3Ara	5.76	5.22
Peo3Gal	1.53	1.45
Mal3Gal	2.58	2.24

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Anthocyanins in Bilberries Using UHPLC

Column:	Acclaim RSLC 120, C18, 2.2 µm, Analytical (2.1 × 150 mm)
Flow:	0.475 mL/min
Temperature:	35 °C
Injection Volume:	2.0 µL
Eluent:	A: 10% Formic acid B: 10% Formic acid, 22.5% methanol, 22.5% CH_3CN
Gradient:	0.0–12.0 min, 9% B 12.0–25.0 min, 35% B 25.0–30.0 min, 50% B 30.0–35.0 min, 9% B
Detection:	Absorbance, vis 520 nm
Sample:	Nutritional Supplement A

Peaks:	Conc. (µg/mL)
1. Dp3Gal	—
2. Dp3Glu	21.7
3. Cy3Gal	11.9
4. Dp3Ara	—
5. Cy3Glu	10.6
6. Pet3Gal	—
7. Cy3Ara	—
8. Delphinidin	—
9. Pet3Glu	8.76
10. Peo3Gal	3.31
11. Pet3Ara	—
12. Peo3Glu	—
13. Mal3Gal	6.26
14. Peo3Ara	8.88
15. Cyanidin	—
16. Mal3Glu	—
17. Mal3Ara	—
18. Petunidin	—
19. Peonidin	—
20. Malvidin	—

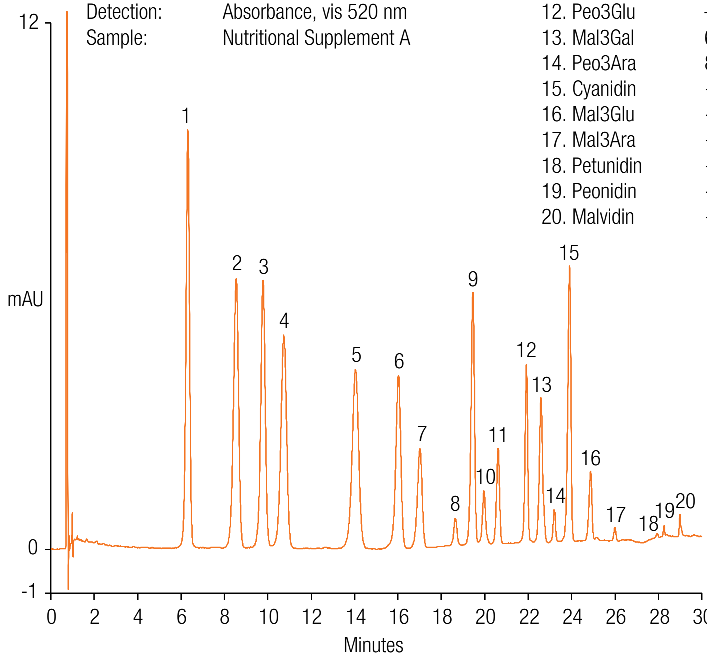


Figure 5-6. Separation of anthocyanins in a sample of bilberry-based Nutritional Supplement A.

Column:	Acclaim RSLC 120, C18, 2.2 µm, Analytical (2.1 × 150 mm)
Flow:	0.475 mL/min
Temperature:	35 °C
Injection Volume:	2.0 µL
Eluent:	A: 10% Formic acid B: 10% Formic acid, 22.5% methanol, 22.5% CH_3CN
Gradient:	0.0–12.0 min, 9% B 12.0–25.0 min, 35% B 25.0–30.0 min, 50% B 30.0–35.0 min, 9% B
Detection:	Absorbance, vis 520 nm
Sample:	Nutritional Supplement B

Peaks:	Conc. (µg/mL)
1. Dp3Gal	—
2. Dp3Glu	20.8
3. Cy3Gal	10.2
4. Dp3Ara	—
5. Cy3Glu	9.99
6. Pet3Gal	—
7. Cy3Ara	—
8. Delphinidin	—
9. Pet3Glu	8.40
10. Peo3Gal	1.12
11. Pet3Ara	—
12. Peo3Glu	—
13. Mal3Gal	3.27
14. Peo3Ara	6.96
15. Cyanidin	—
16. Mal3Glu	—
17. Mal3Ara	—
18. Petunidin	—
19. Peonidin	—
20. Malvidin	—

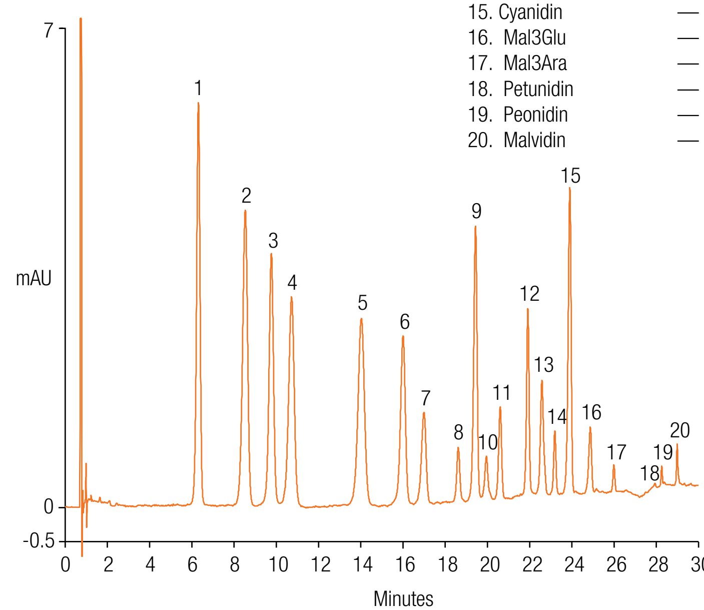


Figure 5-7. Separation of anthocyanins in a sample of bilberry-based Nutritional Supplement B.

Supplements

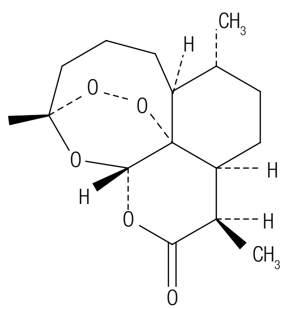
Wormwood grass.

Artemisinin

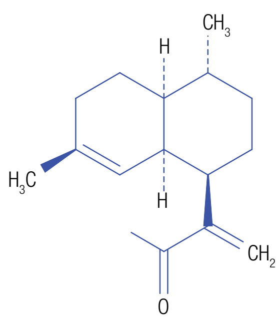
Artemisinin, a sesquiterpene lactone, is the key ingredient obtained from the species *Artemisia annua,* or sweet wormwood, a plant indigenous to Asia, with a long history of use as a highly effective antimalarial remedy.

Artemisinin

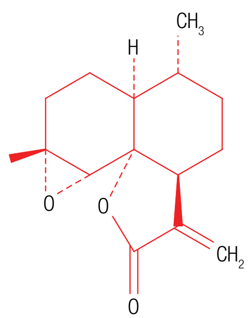
The artemisinin compound was first isolated in 1972; investigators at the Walter Reed Army Institute of Research located and crystallized the active component in 1984. However, in China, the leaves of the sweet worm-wood have been used for several centuries as an antiparasitic agent and a treatment for othersystemic disorders, including certain types of cancer.



Artemisinin



Artemisinic Acid



Artannuin B

Figure 5-8. Structures of purported natural products found in sweet wormwood.

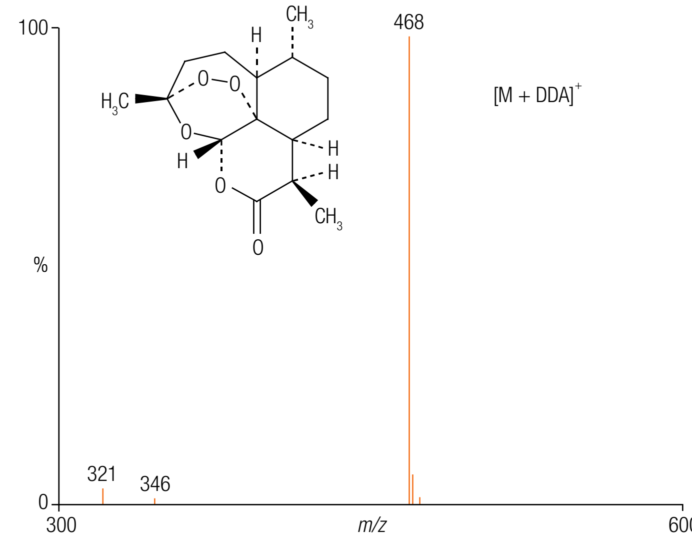


Figure 5-9. MS Spectrum of artemisinin-dodecylamine adduct, ESI positive, needle voltage: 4 kV, cone voltage: 50 V.

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Artemisinin

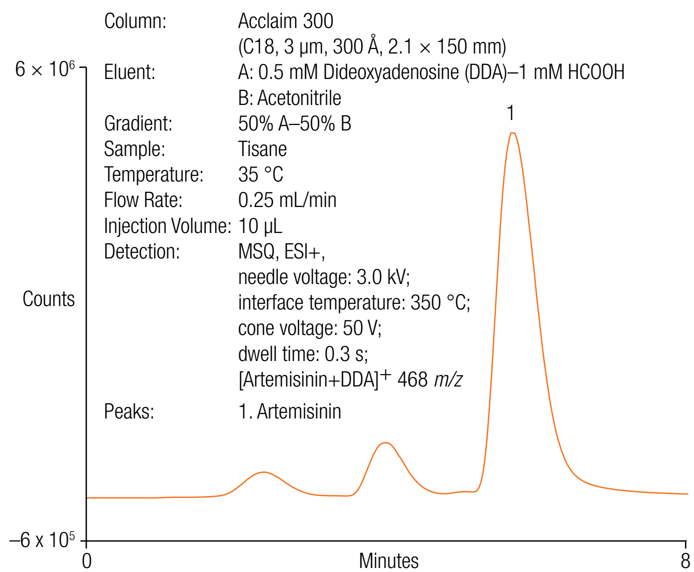

Figure 5-10. Antimalarial drug, artemisinin in pharmaceutical formulation (Tisane).

HPLC- Charged Aerosol Detection Parameters

Mobile Phase:	60% aqueous acetonitrile, pH 3 with 0.1% TFA
Flow Rate:	2mL/min
Column:	C18 4.6 × 75 mm, 3 µm
Column Temp.:	Ambient
Injection Volume:	10 µL
Peaks:	1. Arteannuin B 2. Artemisinin 3. Artemisinic acid

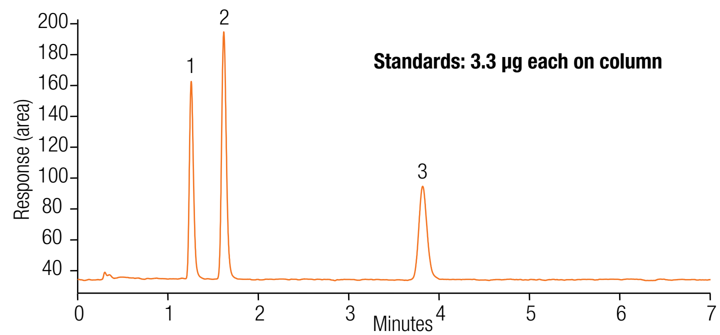

Figure 5-11. HPLC-CAD analysis of Arteannuin B, Artemisinin and Artemisinic Acid.

Did You Know?

Therapies that combine antimalaria drugs with Artemisinin are not only the preferred treatment, but are also very well received by patients.

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Ashwagandha

Withania somnifera is a woody shrub in the nightshade family (*Solanacea*) native to India. Known as ashwagandha, it is used in Ayurvedic medicine as a strengthening tonic and to cool the body, typically by drinking a cup of hot milk containing the powdered root.

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Ashwagandha

Purported active compounds in ashwagandha include steroidal lactones isolated from the root, some of which are well resolved by HPLC (below). Figure 5-12 shows that charged aerosol detection measures several compounds not readily seen by UV absorbance at 230 nm.

Trivia Question

Q: Do you know what are the most popular Ayurvedic Herbs?

A: The Top 10 Ayurvedic Herbs are:

- Turmeric
- Garlic
- Aloe vera
- Ginger
- Tulsi or Holy Basil (Tulsi)
- Amla (Indian Gooseberry)
- Ashwagandh or Winter Cherry
- Jatamansi or Indian Spikenard
- Neem
- Bhrahmi

HPLC System:	UltiMate 3000 RS system
Detection:	Diode Array Detector DAD-3000RS and Corona *ultra* RS Charged Aerosol Detector Nebulizer temperature: 25–35 °C Power function: 1.00 Data collection rate: 20 Hz
Column:	Accucore C8, 4.6 × 150 mm; 2.6 µm
Column Temp.:	45 °C
Flow Rate:	2.0 mL/min
Mobile Phase:	A. Deionized water
Mobile Phase:	B. Acetonitrile, Optima LCMS
Gradient:	20% B to 50% B in 6 min; to 95% B in 10 min; hold 10 min
Injection Volume:	1 µL

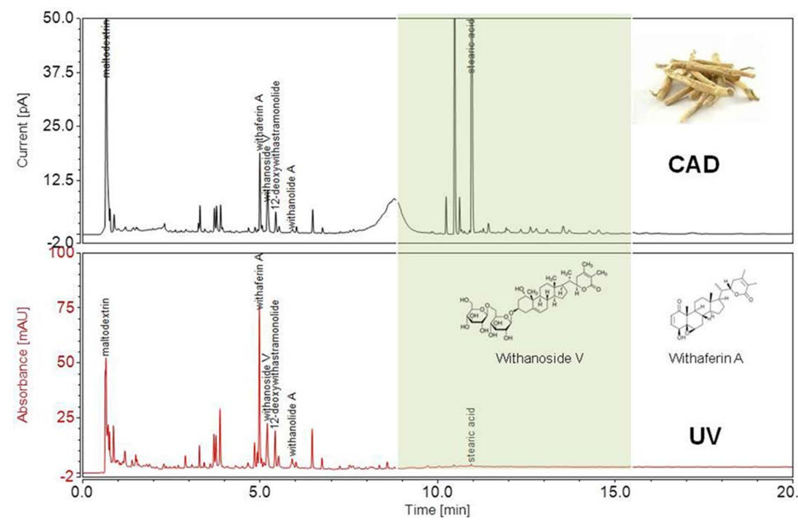

Figure 5-12. Comparison between charged aerosol detection and UV absorbance detection for HPLC separation of ashwagandha extract.

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Supplements

Bacopa bitter herbs.

Bacopa

In Ayervedic medicine Brahmi (*Bacopa monnieri*) is purported to enhance mind power (Medhya effect) and improve all aspects of mental functioning, including comprehension, memory, and recollection.

Bacopa

Among the many bioactive ingredients found in *Bacopa monnieri* are the triterpene saponins separated below, including Bacoside A3, Bacopaside II, Bacopaside X, and Bacopasaponin C. Compared to HPLC with low-wavelength UV detection (220 nm), HPLC with charged aerosol detection offers improved sensitivity.

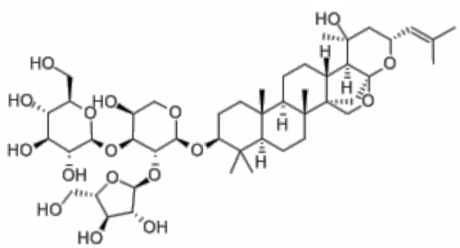

Figure 5-13. Structure of Bacopaside X.

System:	UltiMate 3000 RS with Diode Array Detector DAD-3000RS and a Corona Veo Charged Aerosol Detector: Evaporation temp.: 35 °C Power function: 1.00 Data collection rate: 2 Hz
Column:	Accucore column C18, 2.1 × 150 mm; 2.6 µm
Column Temp.:	45 °C
Flow Rate:	0.50 mL/min
Mobile Phase A:	Deionized water
Mobile Phase B:	Acetonitrile, Fisher Scientific Optima LCMS
Gradient:	28% B
Injection Volume:	1 µL

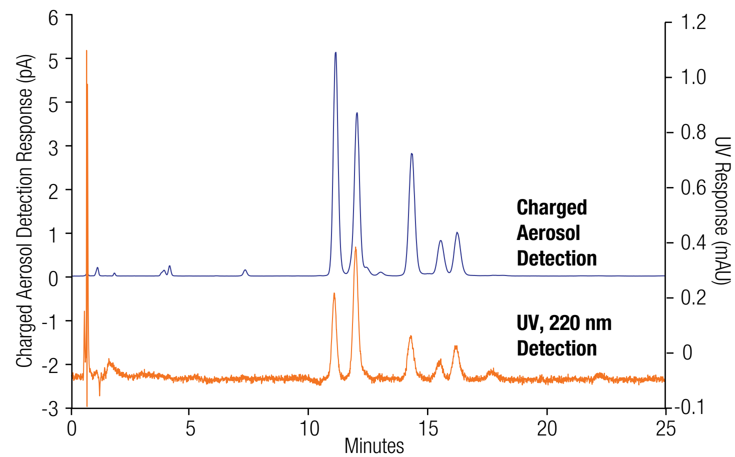

Figure 5-14. HPLC-Charged Aerosol analysis of commercially available of *Bacopa monnieri* extract (containing Bacoside A3, Bacopaside II, Bacopaside X, and Bacopasaponin C). The first two peaks are Bacopaside A3 and Bacopaside II. The later eluting peaks include the isomers Bacopasaponin C and Bacopaside X.

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Whole black cohosh.

Black Cohosh

Extracts of black cohosh (*Cimicifuga racemosa*) have been used since the 1950s to relieve symptoms of menopause, including hot flashes, and a number of clinical trials have supported this use. In addition, research has found that black cohosh may improve neurovegatative symptoms and psychological complaints associated with hormonal deficiencies in women, and may offer an alternative to traditional hormone therapy. Although the mechanism of action of black cohosh is not yet well understood, it is believed that the triterpene glycosides present in black cohosh (including 27-deoxyactein, actein, cimiracemoside F, and others) are bioactive.

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Black Cohosh

Quantitation of the triterpene glycosides is challenging because many do not possess a chromophore that absorbs above 200 nm. As a result, high-performance liquid chromatography-evaporative light scattering detection (HPLC-ELSD) has become the most accepted technique for the quantitation of the triterpene glycosides in black cohosh. This technique is used in the Institute for Nutraceutical Advancement (INA) Method 113.001. However, ELSD suffers from poor sensitivity, highly non-linear calibration curves, and poor reproducibility.

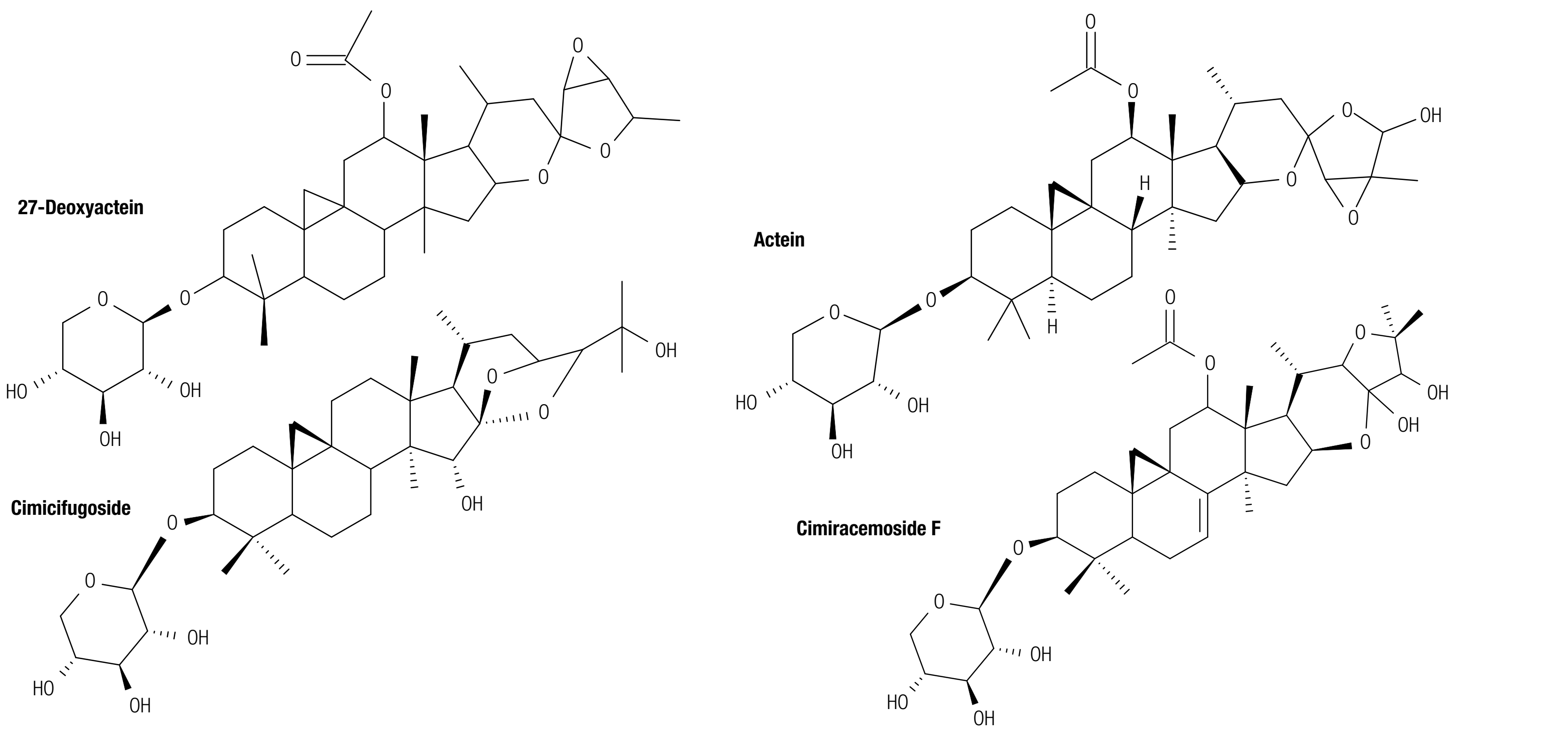

Figure 5-15. Chemical structures of the triterpene glycosides present in black cohosh.

Black Cohosh

Although evaporative light scattering detection has become the most accepted technique for the quantitation of the triterpene glycosides in black cohosh, the method suffers from poor sensitivity and highly nonlinear calibration curves. The Corona Charged Aerosol detection shows less complex calibration curves and is more sensitive than ELSD.

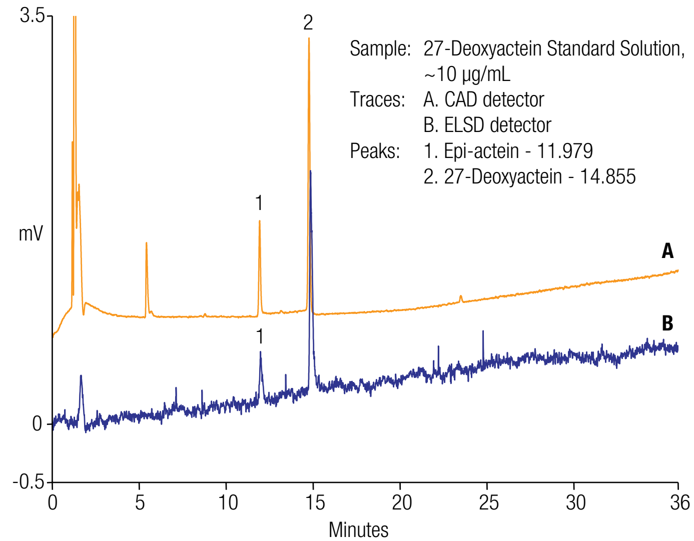

Figure 5-16. Overlay of chromatograms of 27-Deoxyactein standard solution.

Did You Know?

Other names for black cohosh include black snakeroot, bugwort, rattleroot, rattleweed, and bugbane.

Download Customer Application Note 113: Determination of Triterpene Glycosides in *Cimicifuga racemosa* (Black Cohosh) by HPLC-CAD

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Black Cohosh

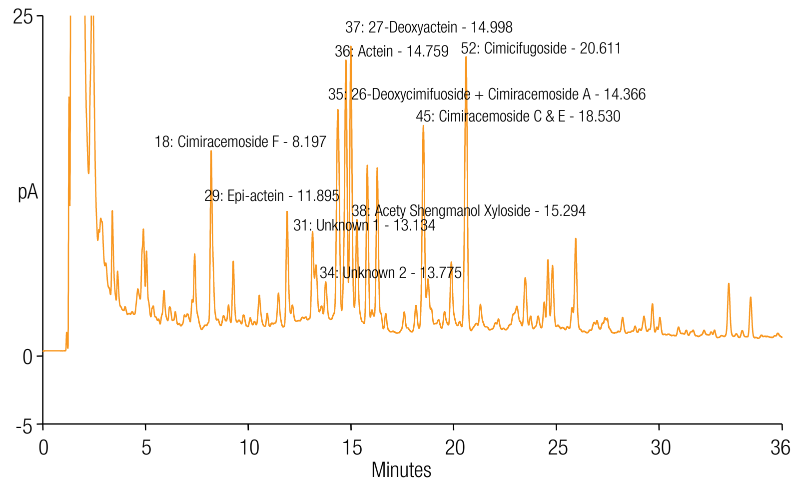

Figure 5-17. Black cohosh sample chromatogram using the Corona Charged Aerosol Detector.

Did You Know?

Data from the National Health and Nutrition Survey (2003 to 2006) suggest that about one-half of American adults use dietary supplements and 20 percent use a supplement with at least one botanical ingredient. Between 1994 and 2008, the number of dietary supplement products on the market increased from 4,000 to 75,000.

System:	Thermo Scientitfic Dionex Summit*
Column:	Fused-Core C18, 2.7 µm, 4.6 × 150 mm
Flow:	1.00 mL/min
Injection Volume:	10 µL
Mobile Phase:	A: 0.1% Formic acid in water B: Acetonitrile
Gradient:	30% B to 40% B from 0–12 min; 4 0% B to 60% B from 12–36 min
Detection:	Charged Aerosol Detection

*Equivalent or improved results can be obtained using an UltiMate 3000 HPLC System

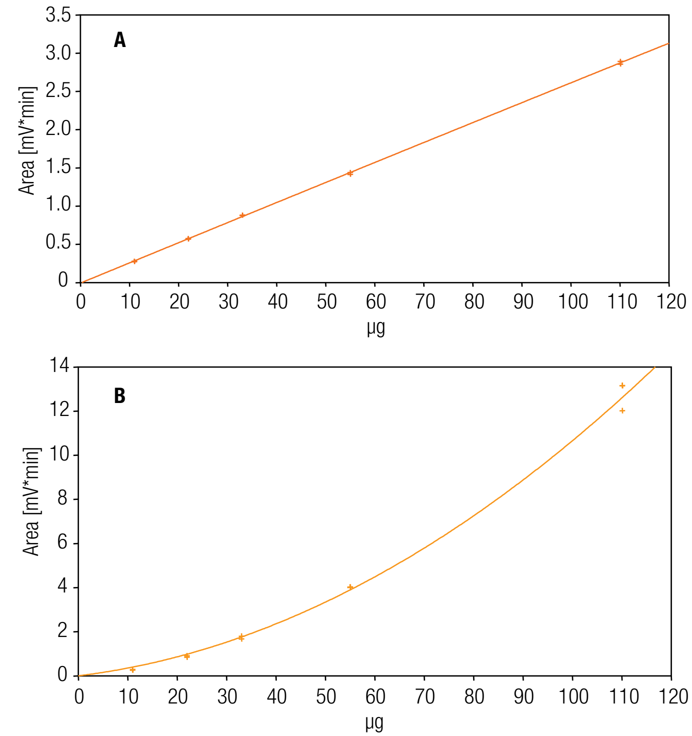

Figure 5-18. The 27 deoxyactein calibration curves using (A) the Corona CAD detector and (B) an ELSD detector.

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Boswellia serrata.

Boswellic Acids in *Boswellia*

Boswellia is a plant that contains triterpenoid compounds called boswellic acids. Boswellic acid and its derivatives have anti-carcinogenic, anti-tumor, and blood lipid lowering activities. Dried extracts of the resin of the *Boswellia serrata* tree have been used since antiquity in India to treat inflammatory conditions.

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Boswellic Acids in *Boswellia*

Column:	Reversed Phase, 150 × 4.6 mm
Flow:	1.50 mL/min
Temperature:	25 °C
Injection Volume:	50 µL
Mobile Phase:	A: Acetonitrile:Water: Phosphoric Acid, 85% (80:20:0.1, v/v/v) B: Acetonitrile
Gradient:	100%A–100%B over 15 min, hold at 100% B for 5 min
Detection:	UV, 210 nm
Sample Preparation:	Approximately 500 mg of sample is extracted with 100 mL of acetonitrile and a 10-fold dilution made with acetonitrile
Peaks:	1. 11-keto-β-Boswellic acid 2. 3-acetyl-11-keto-β-Boswellic acid 3. α-Boswellic acid 4. β-Boswellic acid 5. 3-acetyl-α-Boswellic acid 6. 3-acetyl-β-Boswellic acid

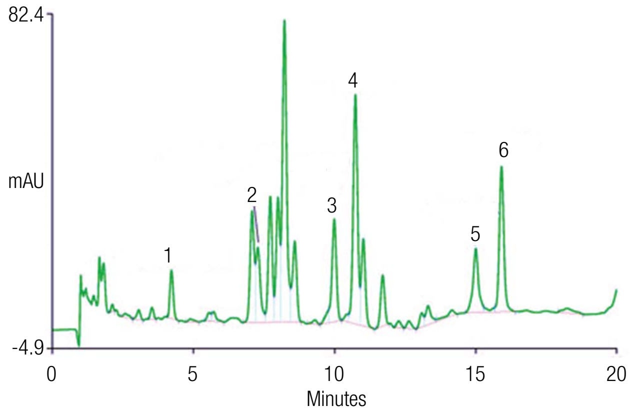


Figure 5-19. Determination of boswellic acids in *boswellia* extracts, UV at 210 nm.

Column:	Reversed Phase, 150 × 4.6 mm
Flow:	1.50 mL/min
Temperature:	25 °C
Injection Volume:	50 µL
Mobile Phase:	A: Acetonitrile:Water: Phosphoric Acid, 85% (80:20:0.1, v/v/v) B: Acetonitrile
Gradient:	100%A–100%B over 15 min, hold at 100% B for 5 min
Detection:	UV, 250 nm
Sample Preparation:	Approximately 500 mg of sample is extracted with 100 mL of acetonitrile and a 10-fold dilution made with acetonitrile
Peaks:	1. 11-keto-α-Boswellic acid 2. 3-acetyl-11-keto-α-Boswellic acid

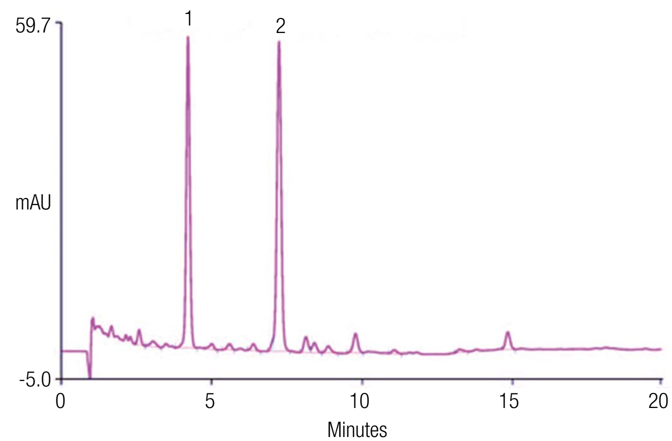


Figure 5-20. Determination of boswellic acids in *boswellia* extracts, UV at 250 nm.

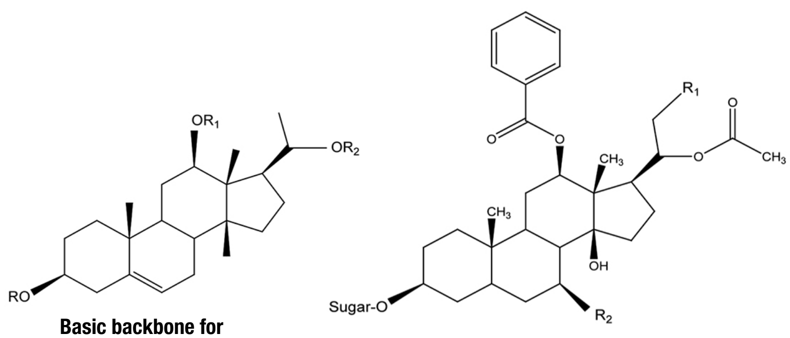
Caralluma cactus

Caralluma

The edible cactus *Caralluma fimbriata* is used throughout India both as a food and to suppress hunger and enhance endurance.

Caralluma

The active ingredients in *Caralluma fimbriata* are believed to include oxypregnane glycosides. As with the triterpenoids in black cohosh, many of the oxypregnane glycosides are not well detected by low-wavelength UV absorbance. Charged aerosol detection measures the analytes with higher sensitivity than UV while also showing less baseline rise from the mobile phase gradient.



Basic backbone for pregnase-based molecules

Example pregane glycoside from caralluma

Figure 5-21. Chemical structures of active ingredients in caralluma.

System:	UltiMate 3000 RS with Diode Array Detector DAD-3000RS and a Corona Veo Charged Aerosol Detector Evaporation temperature: 35 or 50 °C Power function: 1.00 Data collection rate: 2 Hz
Column:	Accucore C8 column, 4.6 × 150 mm; 2.6 µm
Column Temp.:	45 °C
Flow Rate:	2.0 mL/min
Mobile Phase A:	Deionized water
Mobile Phase B:	Acetonitrile, Fisher Scientific Optima LCMS
Gradient:	40% B to 47% B in 2.5 min; to 95% B in 12.5 min; hold 10 min
Injection Volume:	10 µL

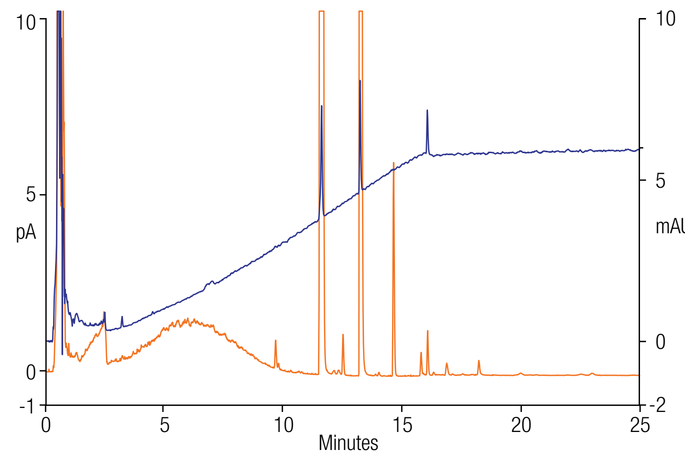


Figure 5-22. HPLC-Charged Aerosol analysis of oxypregnane glycosides in *Caralluma fimbriata* leaf extract.

Download the Poster Note: Novel, Universal Approach for the Measurement of Natural Products in a Variety of Botanicals and Supplements, Part 2

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Caulis Lonicerae

Caulis lonicerae, the dried rattan of *Lonicera japonica* (*Caprifoliaceae* or honeysuckle family), is an important traditional Chinese medicine used for the treatment of such ailments as acute fever, headache, respiratory infection, and epidermic diseases.

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Caulis Lonicerae

Active Components of Caulis Lonicerae

The major active components in Caulis lonicerae are loganin, sweroside, chlorogenic acid, caffeic acid, rutin, and galuteolin. The Pharmacopoeia of the People's Republic of China (PPRC) 2010 regulates Caulis lonicerae with different high performance liquid chromatography methods for the determination of loganin (using a phenyl stationary phase) and chlorogenic acid (using a C18 phase), respectively.Therefore, the PPRC quality control (QC) protocols for Caulis lonicerae are inconvenient (requiring two methods) and inadequate (determining only two target components). Although there are other methods to determine sweroside, caffeic acid, rutin, galuteolin, and up to three other minor active components of Caulis lonicerae on a C18 stationary phase using HPLC, these methods require long separation times (≥ 25 min) and have insufficient peak resolution between loganin and sweroside.

Figure 5-23. Caulis lonicerae plants; *Lonicera japonica* (left) and dried rattan (right).

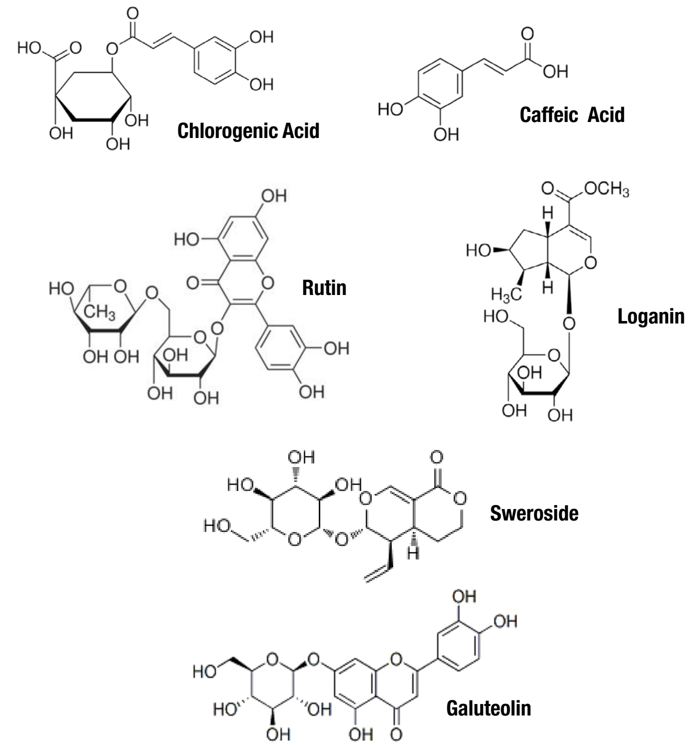

Figure 5-24. Chemical structures of compounds found in Caulis lonicerae.

Download Application Note 299: HPLC Analysis of Six Active Components of Caulis Lonicerae Using a Phenyl-1 Column

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Caulis Lonicerae

Column: Acclaim Phenyl-1 4.6 × 150 mm, 3 µm
Flow: 1.0 mL/min
Temperature: 30 °C
Injection Volume: 5 µL
Mobile Phase: Acetonitrile, 0.4% formic acid aqueous (v/v)
Gradient: Acetonitrile, -2–0 min, 17%; 0–4 min, 17–30%; 4–6 min, 30–45%; 6–10 min, 45%
Detection: UV, 236 nm
Scans: A. Caulis lonicerae sample
B. mixture of standards, 10 µg/mL

Peaks:
1. Loganin
2. Sweroside
3. Chlorogenic acid
4. Caffeic acid
5. Rutin
6. Galuteolin

UV spectra:
A1. loganin standard (Peak 1)
A2. Peak 1 of sample
B1. sweroside standard (Peak 2)
B2. Peak 2 of sample
C1. chlorogenic acid standard (Peak 3)
C2. Peak 3 of sample
D1. caffeic acid standard (Peak 4)
D2. Peak 4 of sample

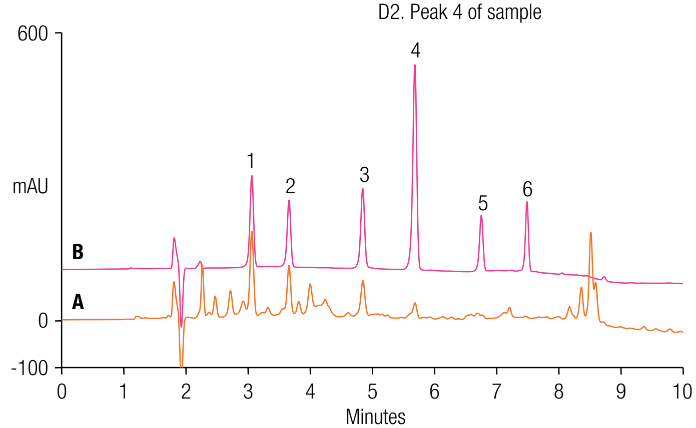

Figure 5-25. Chromatograms and UV spectra of A) a Caulis lonicerae sample, and B) a mixture of standards (10 µg/mL each).

Did You Know?

Caulis lonicerae, also known as Japanese Honeysuckle Stem, is often used as an anti-inflammatory and anti-spasm agent in traditional Chinese medicines.

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Chlorophyll present in plants.

Chlorophyll

Chlorophyll is a green pigment found in most plants, algae, and cyanobacteria. Chlorophyll is vital for photosynthesis, which allows plants to obtain energy from light. Chlorophyll a and chlorophyll b are present in plants. There are many claims that are made about the healing properties of chlorophyll but most have been disproved.

Chlorophyll

Because of their high hydrophobicity, chlorophylls can be analyzed by HPLC using a reversed-phase column under normal-phase condition (high organic solvent). The Acclaim RSLC PA2 column offers suitable selectivity for high-speed, baseline separation of chlorophylls in spinach leaf.

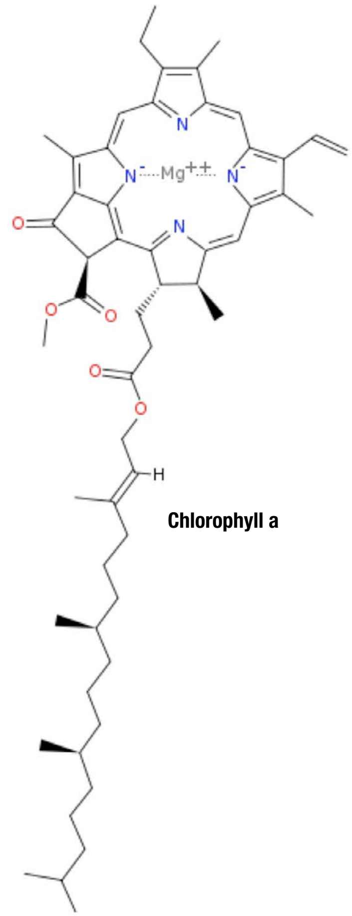

Figure 5-26. Structure of Chlorophyll a.

Column:	Acclaim RSLC PolarAdvantage II (PA2), 2.2 µm
Dimension:	2.1 × 50 mm
HPLC System:	UltiMate 3000 RS
Mobile Phase:	50/50/0.1 v/v/v CH_3CN/MeOH/2M NH_4OAc pH 5.4
Flow Rate:	0.60 mL/min
Pressure:	300 bar
Injection Volume:	2 µL
Temperature:	30 °C
Detection:	Visible at 445 amd 655 nm (shown); spectra 220-800 nm 10 Hz data rate, 0.5 sec time constant
Sample:	Acetone extract of spinach leaf

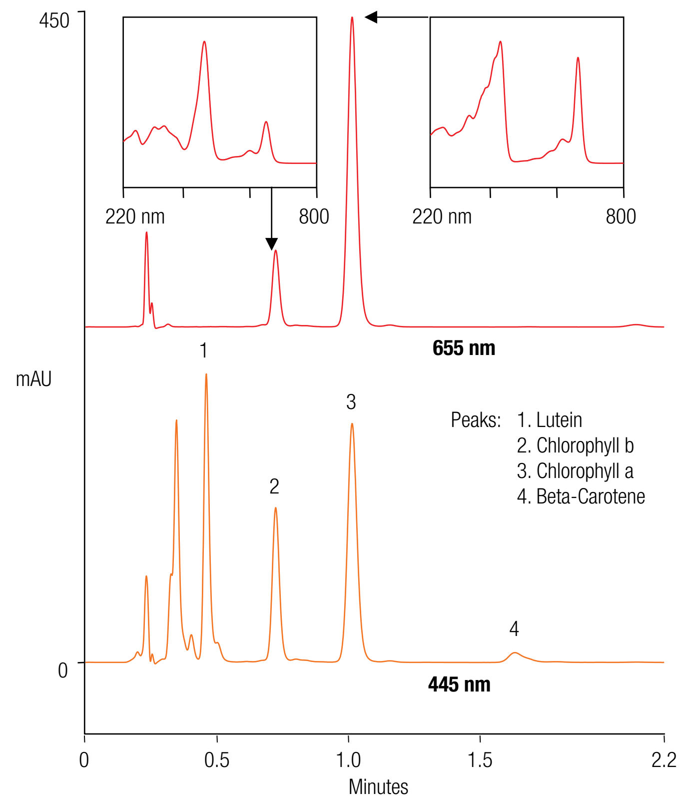

Figure 5-27. Chlorophyll on Acclaim RSLC PolarAdvantage II column..

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Viola

Cyclotides

Cyclotides typically contain 28-37 amino acids that forms a cyclised peptide backbone "locked" in place by three disulfide bonds. This "cystine knot" gives cyclotides great stability. Hundreds of cyclotides are thought to exist in nature, with dozens being found in each plant.

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Cyclotides

The fact that cyclotides contain a conserved core of peptides and a series of hypervariable loops suggest that these peptides may play an important role in nature including protecting the plant from pests and pathogens. Cyclotides have been reported to have a wide range of biological activities including insecticidal, anti-microbial, anti-tumor, anti-HIV, and uterotonic activities. Although cyclotides can be measured with HPLC-UV absorbance detection, the use of HPLC with electrochemical detection provides superior sensitivity.

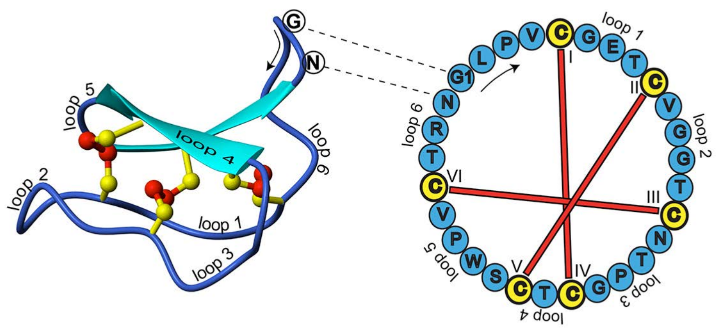

Figure 5-28. Structure and sequence of the prototypic cyclotide kalata B1.

Column:	Dionex Acclaim C18, 2.2µ, 3 × 75 mm
Column Temp:	45 °C
Mobile phase:	A. 50 mM ammonium formate, pH 4.4 B. 65% MeCN, 15% water, 20% 100 mM ammonium formate at pH 4.4
Gradient:	0–15 min, 30–100% B; 15–20 min, 100% B
Flow Rate:	0.5 mL/min
UV:	UV1 280 nm, UV2 218 nm
EC:	Dionex 6041RS cell with boron-doped diamond electrode, +1500 mV
Peak:	1. Cycloviolacin O2

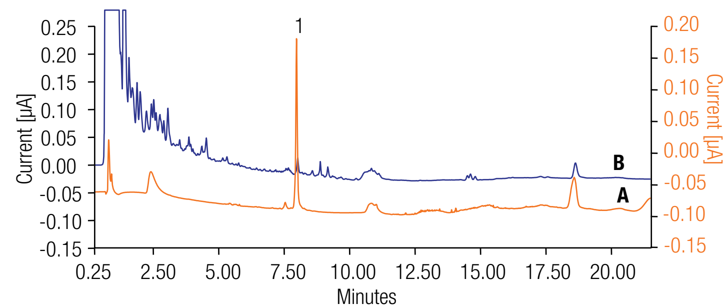

Figure 5-29. Overlay of chromatograms for the purified peptide (A) (10 µL of 100 µg/mL) and plant extract (B) (10 µL of 5 mg/mL).

Download Poster Note: Cystine, an Essential Determinant of Protein Tertiary Structure, Is Also a Target for Electrochemical Manipulation

Echinacea flowers.

Echinacea

Herbalists consider echinacea one of the best blood purifiers and an effective antibiotic. Constituents of echinacea include polysaccharides, polyacetylenes, sesquiterpenes, polyphenols, and more. It has remained one of the top selling herbal remedies in the United States.

Echinacea

Column:	C18, 150 × 4.6 mm
Flow:	1.50 mL/min
Temperature:	25 °C
Injection Volume:	25 µL
Mobile Phase:	A: Acetonitrile:Water, 85% (90:10 v/v) B: Acetonitrile:Water:Phosphoric Acid, 85% (25:75:0.1, v/v/v)
Gradient:	100%A—100%B over 20 minutes, hold at 100% B for 2 min
Detection:	UV, 330 nm
Sample Preparation:	Approximately 250 mg of sample is extracted with 100 mL of acetonitrile:water (10:90, v/v)
Peaks:	1. Cattaric acid 2. Chlorogenic acid 3. Echinacoside 4. Cichonic acid

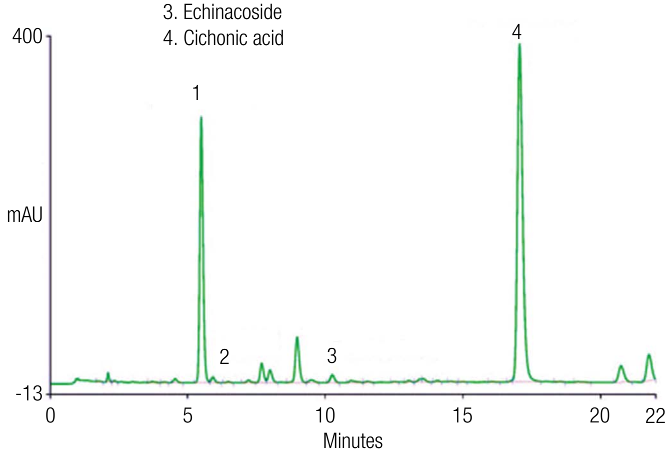

Figure 5-30. Determination of phenolic compounds in echinacea extracts.

Did You Know?

Use of dietary and herbal supplements has grown dramatically in recent years in the United States. In 2007, according to the National Center for Complementary and Alternative Medicine, $14.8 billion was spent on non-vitamin, non-mineral, natural products, such as fish oil, glucosamine, and Echinacea.

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Food plants from the Apiaceae family.

Falcarinols

Food plants in the *Apiaceae* (formerly *Umbelliferae*) family (e.g., carrots, parsley, and celery) contain a group of bioactive C17-polyacetylene compounds, sometimes referred to as the polyacetylenic oxylipins. These compounds have been shown to be highly toxic toward bacteria and fungi and to exhibit a diverse range of biological activities in mammals, both beneficial (e.g., their cytotoxicity is proposed to reduce the risk of developing cancer) and detrimental (e.g., occupational allergic contact dermatitis).

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Falcarinols

Three polyacetylenic oxylipins—falcarinol, falcarindiol, and falcarindiol-3-acetate—are natural pesticides produced by carrots in response to fungal diseases, and have recently garnered significant media attention.

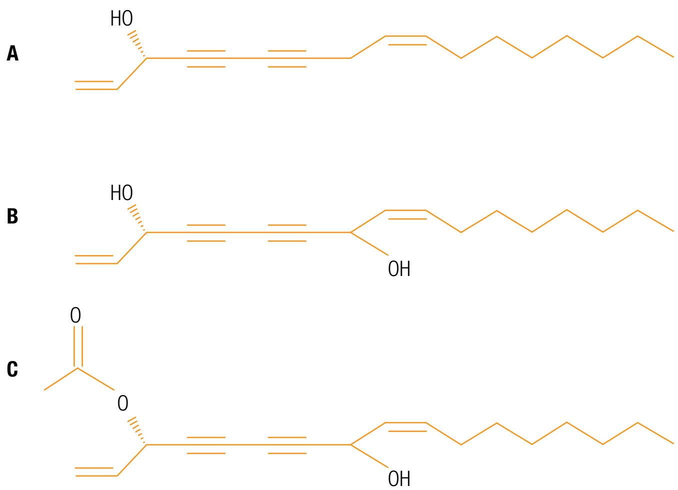

Figure 5-31. Structures of (A) falcarinol, (B) falcarindiol, and (C) falcarindiol-3-acetate.

System:	UltiMate 3000 HPLC
Column:	Acclaim RSLC 120 C18, 2.2 µm, 2.1 × 150 mm
Flow:	0.65 mL/min
Temperature:	50 °C
Injection Volume:	5 µL at 10°C
Mobile Phase:	A: Methanol/water/acetic acid (500:500:4); B: Acetone/methanol/tetrahydrofuran/acetic acid (500:375:125:4)
Gradient:	Mobile Phase B, 0–1 min, 0%, 1–10 min, 40%, 10–15 min, 60%, 15 min, 100%, 15–20 min, 0%
Detection:	Charged Aerosol
Peaks:	1. Falcarindiol 7. Falcarindiol-3-acetate 12. Falcarinol *Remaining peaks are unknown

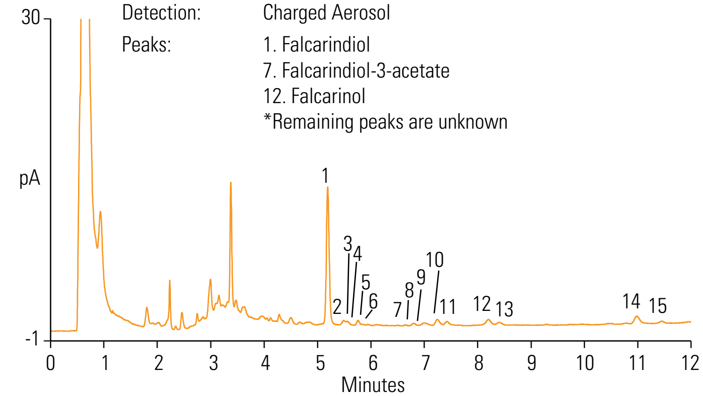

Figure 5-32. Charged aerosol detection showed good sensitivity with minor interference from other analytes

Download the Poster Note: Simple and Direct Analysis of Falcarinol and Other Polyacetylenic Oxylipins in Carrots by Reversed-Phase HPLC and Charged Aerosol Detection

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Giant Knotweed Rhizome

Giant Knotweed Rhizome

Giant knotweed rhizome, the dried rhizome and root of *Polygonum cuspidatum* Sieb. et Zucc. is a common medicinal plant in China. Chinese Pharmacopeia Edition 2005 (Ch. P 2005) regulates its use as an herbal medicine.

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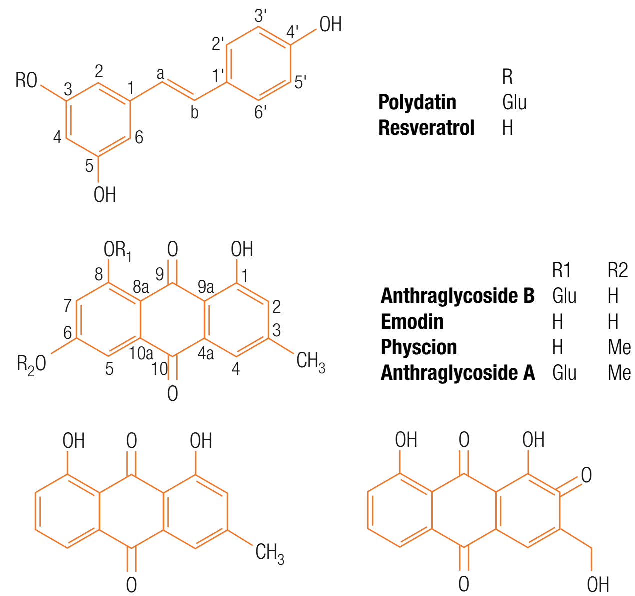
Giant Knotweed Rhizome

Giant knotweed rhizome is used with the belief that it cures angiocardiopathy, skin inflammations, liver diseases, reduces fever, and relieves arthritis pain etc. There are many compounds that are considered active components in giant knotweed rhizome, including anthraquinones (for example, anthraglycoside A, anthraglycoside B, emodin, physcion, rhein, and chrysophanol) and stilbenes (for example, resveratrol and polydatin).



Chrysophanol **Rhein**

Figure 5-33. Chemical structures of active components found in giant knotweed rhizome.

Giant Knotweed Rhizome

Guard Column: Acclaim 120 C18, 5 µm, 4.3 × 10 mm
Anal. Column: Acclaim 120 C18, 5 µm, 4.6 × 250 mm
Eluent: (A) CH_3CN
(B) 20 mM NH_4Ac
Gradient: See Table 1 in below Application Note
Column Temp.: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 5 µL
Detection: UV at 254 nm

C-grams: A. H_2O
B. Mixed Standard
C. #1
D. #2
E. #3
F. #4

Peaks: 1. Polydatin
2. Resveratrol
3. Anthraglycoside B
4. Anthraglycoside A
5. Rhein
6. Emodin
7. Chrysophanol
8. Physcion
a–g. Anthraquinones

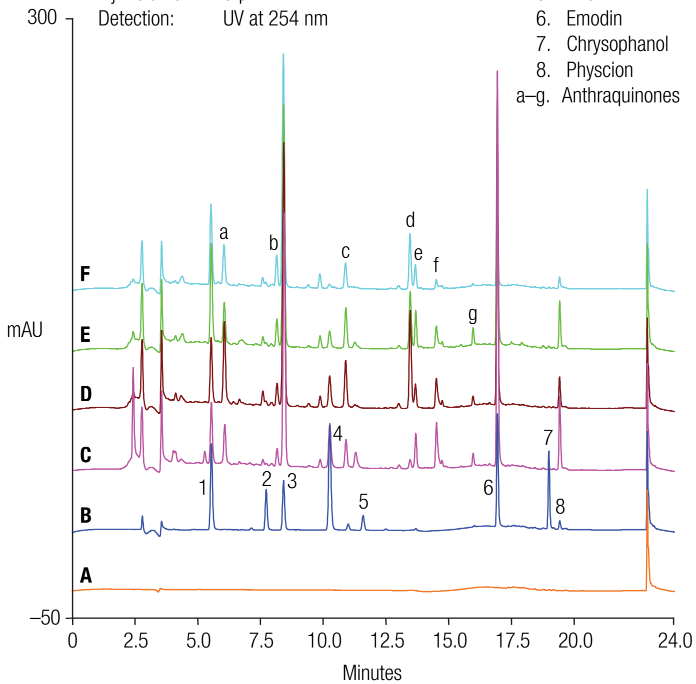


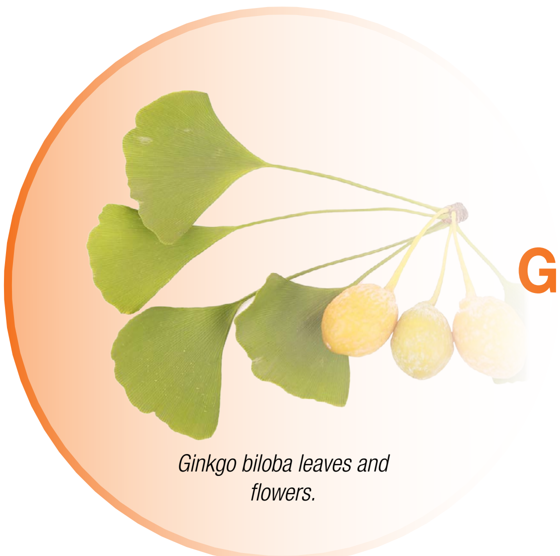
Figure 5-34. Overlay of chromatograms of water (chromatogram A), the mixed standard (chromatogram B), and samples 1-4 (chromatograms C–F, respectively).

Did You Know?

Giant knotweed rhizome, also known as Huzhang, or tiger cane, has been used in traditional Chinese medicines since the first century BC.

Download Application Note 232: Determination of Anthraquinones and Stilbenes in Giant Knotweed Rhizome by HPLC with UV Detection

Supplements



Ginkgo biloba leaves and flowers.

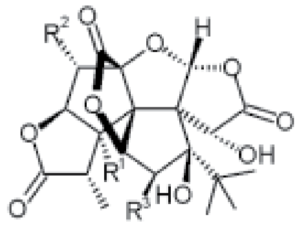
Ginkgo

Ginkgo biloba is thought to possess nootropic activity, and is taken to improve memory and enhance concentration. Sesquiterpenoid bilobalide and numerous diterpenoid ginkgolides are believed to be the active ingredients.

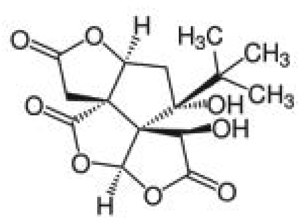
Ginkgo

Ginkgolide

	R1	R2	R3
A	OH	H	H
B	OH	OH	H
C	OH	OH	OH
J	OH	H	OH
M	H	OH	OH



Ginkgolide



Bilobalide

Figure 5-35. Chemical structures of ginkgolide and bilobalide.

Charged aerosol detection is able to detect numerous non-volatile compounds in a *Ginko biloba* extract.

Column:	Accucore C8, 4.6 × 150 mm, 2.6 µm
Flow:	2.0 mL/min
Column Temp.:	45 °C
Injection Volume:	5 µL
Mobile Phase:	A: Deionized water
	B: Acetonitrile, Fisher Scientific™ Optima LCMS
Gradient:	20% B to 50% B in 6 min; to 95% B in 10 min; hold 10 min
Sample Prep.:	Sonicate

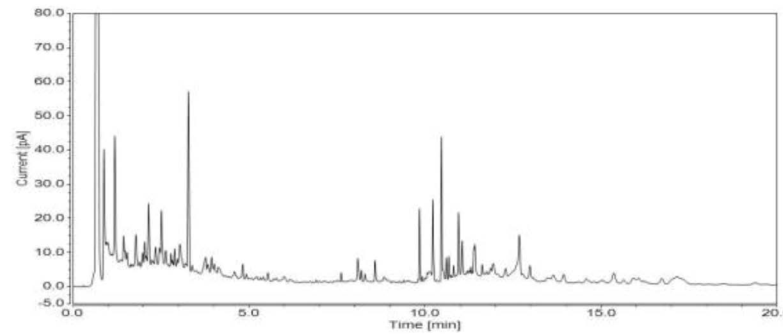


Figure 5-36. HPLC-Charged Aerosol detection of *Ginkgo biloba* extract.

Download Poster Note: Novel, Universal Approach for the Measurement of Natural Products in a Variety of Botanicals and Supplements, Part 2

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Ginseng root.

Ginseng

Asian ginseng (*Panax ginseng*) is used traditionally as a tonic to reduce the effects of stress, counteract fatigue, and increase stamina. The main bioactive ingredients found in *Panax ginseng* and a related species, *Panax quinquefolius* (American ginseng), are triterpene saponis, commonly referred to as ginsenosides. There are seven major ginsenosides present in Panax ginseng: the protopanazatriols (Rg1, Re, and Rf0 and the protopaxadiols (RB1, Rc, Rb2, and Rd). Panax quinquefolius contains the same ginsenosides, with the exception of Rf.

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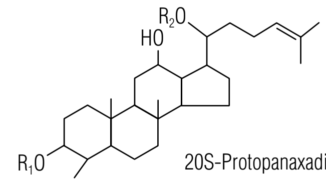
Ginseng

Most methods for the analysis of ginsenosides use gradient elution reversed-phase high-performance liquid chromatography) RP-HPLC with low-wavelength UV detection (203–205 nm) because the ginsenosides do not strongly absorb above 205 nm. This often results in strongly sloping baselines that complicate integration, and interferences from minor components that have stronger UV chromophores than the ginsenosides.

The Corona Charged Aerosol Detector offers an alternative to low-wavelength UV detection. Charged aerosol detection does not overrespond to strong chomophores, reducing interferences. Baseline slopes are not as pronounced or as variable, making peak area determinations more reliable.

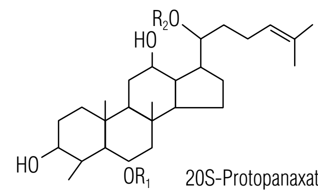
A



20S-Protopanaxadiol (R_1=R_2=H)

Ginsenoside	R_1	R_2
Rb1	- Glc (2-1) Glc	- Glc (2-1) Glc
Rb2	- Glc (2-1) Glc	- Glc (6-1) Glc
Rb3	- Glc (2-1) Glc	- Glc (6-1) Xyl
Rc	- Glc (2-1) Glc	- Glc (6-1) Araf
Rd	- Glc (2-1) Glc	- Glc
Rh2	- Glc	- Glc
Rg3	- Glc (2-1) Glc	- H

B



20S-Protopanaxatriol (R_1=R_2=H)

Ginsenoside	R_1	R_2
Re	- Glc (2-1) Rha	- Glc
Rg1	- Glc	- Glc
Rg2	- Glc (2-1) Rha	- H
Rf	- Glc (2-1) Glc	- H
Rh1	- Glc	H
R1	- Glc (2-1) Xyl	- Glc

Figure 5-37. Structure of 15 ginsenosides.

Download Customer Application Note 112: Determination of Ginsenosides in Panax ginseng by HPLC-Charged Aerosol Detection

Ginseng by Charged Aerosol Detection

System:	UltiMate 3000 RS system with Diode Array Detector DAD-3000RS and a Corona *ultra* RS Charged Aerosol Detector Nebulizer temperature: 25–35 °C Power function: 1.00 Data collection rate: 20 Hz
Column:	Fused-Core C18 HPLC Column, 3.0 × 100 mm, 2.7 µm
Column Temp:	30 °C
Flow Rate:	0.67 mL/min
Mobile Phase A:	Water
Mobile Phase B:	Acetonitrile
Gradient:	15% B to 35% B in 30 minutes
Injection Volume:	20 µL

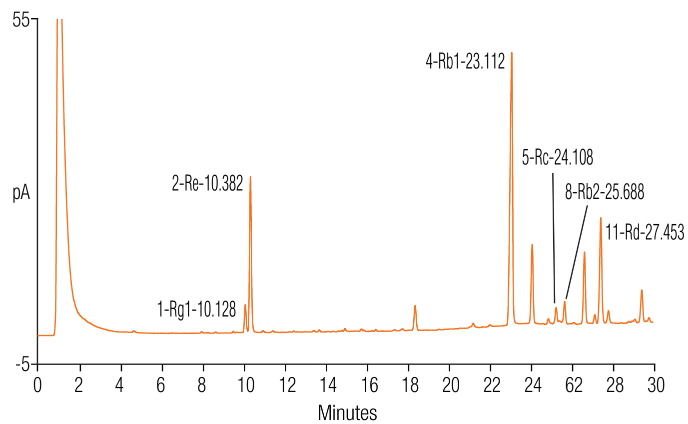


Figure 5-38. *Panax ginseng* sample chromatogram

System:	UltiMate 3000 RS system with Diode Array Detector DAD-3000RS and a Corona *ultra* RS Charged Aerosol Detector: Nebulizer temperature: 25–35 °C Power function: 1.00 Data collection rate: 20 Hz
Column:	Fused-Core C18 HPLC Column, 3.0 × 100 mm, 2.7 µm
Column Temp:	30 °C
Flow Rate:	0.67 mL/min
Mobile Phase A:	Water
Mobile Phase B:	Acetonitrile
Gradient:	15% B to 35% B in 30 minutes
Injection Volume:	20 µL

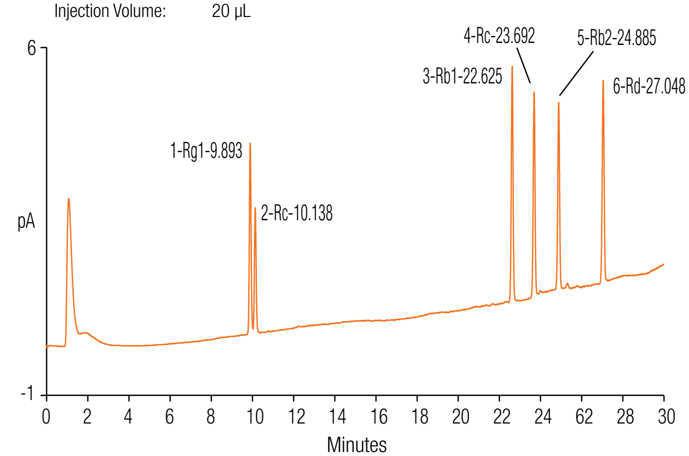


Figure 5-39. Calibration solution #5 chromatogram (4 µg/mL of each ginsenoside)

Table 5-2. Results of the ginsenoside analysis.

Ginsenoside	Amount (%)
Rg1	0.0895
Re	1.16
Rb1	2.36
Rc	0.402
Rb2	0.0505
Rd	0.618
Total	4.68

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Ginseng by UV

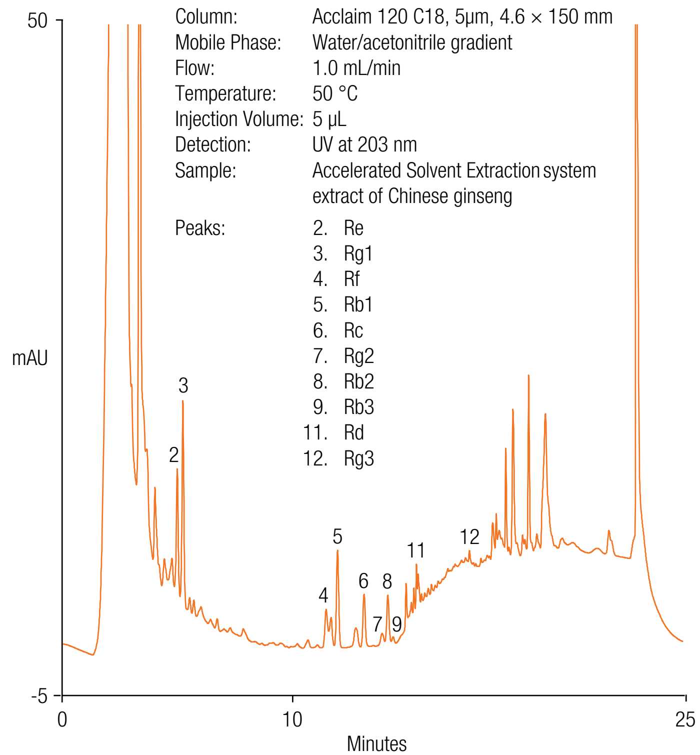

Figure 5-40. Ginsenosides from panax ginseng on an Acclaim 120 C18 column.

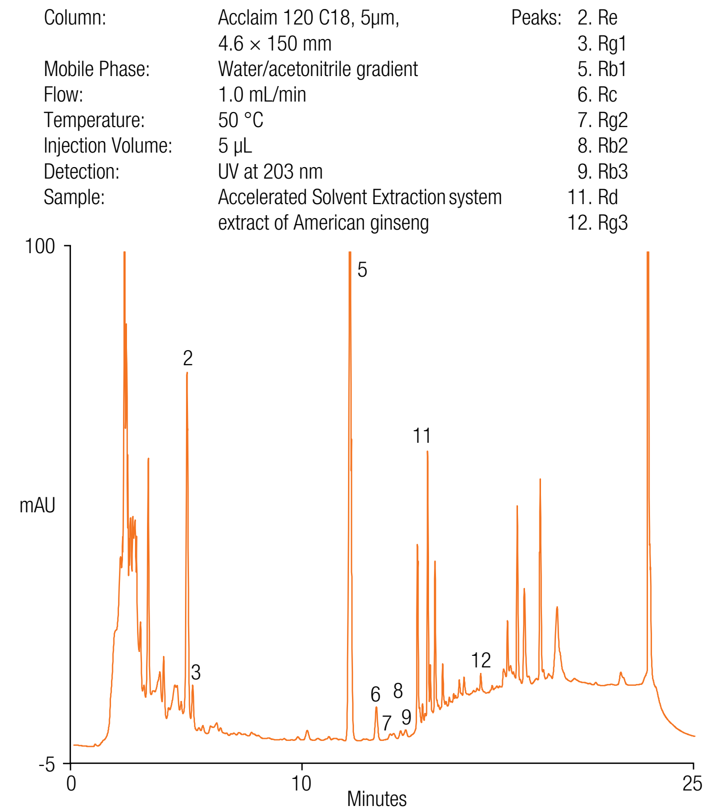

Figure 5-41. Ginsenosides from American ginsengon an Acclaim 120 C18 column.

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Download Application Note 192: Rapid Analysis of Ginseng Using Accelerated Solvent Extraction and HPLC

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Ginseng by UV

Eleutherosides in Siberian Ginseng Extracts

Eleutherosides are glycosides with aromatic alcohol aglycones. They are found in Siberian ginseng, and are considered to be responsible for the plants adaptogenic properties.

Trivia Question

Q: Do you know what the most frequently used Chinese Medicinal Herbs are?

A:The Top 10 Chinese Medicinal Herbs are:

- Ginseng
- Angelica Sinesis or Dang Gui
- Mushroom
- Goji or Wolfberry
- Coptis Chinensis (Chinese goldthread)
- Licorice Root
- Astragalus
- Ephedra Sinica (Ma Huang)
- Bupleurum

Column 1:	Acclaim 120 C18, 150 mm × 4.6 mm
Flow:	1.00 mL/min
Temperature:	25 °C
Injection Volume:	20 µL
Mobile Phase:	A: Water:Acetonitrile (90:10, v/v) B: Water:Acetonitrile (60:40, v/v)
Gradient:	100% A—100%B over 25 min
Detection:	UV, 220 nm
Sample Preparation:	Approximately 1 g of sample is extracted with 100 mL of mobile phase A
Peaks:	1. Eleutheroside B 2. Eleutheroside E 3. Eleutheroside E1

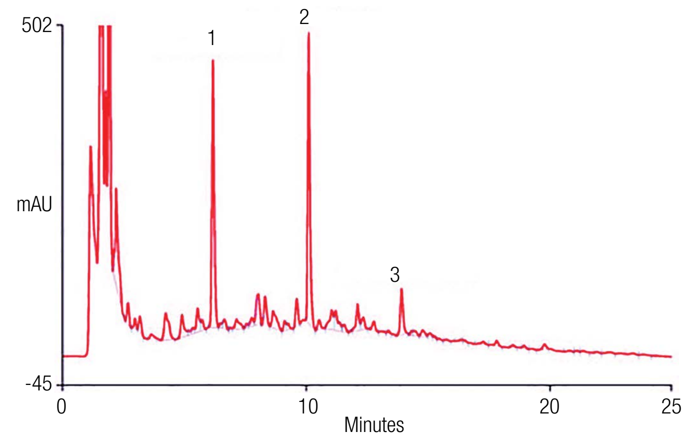

Figure 5-42. Determination of eleutherosides in Siberian ginseng extracts.

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Gotu Kola

Gota Kola leaves.

Centella asiatica (commonly called gotu kola) is a small herbaceous annual plant that is native to India, Sri Lanka, northern Australia, and other parts of Asia and the western Pacific. It is used as a medicinal herb in Ayurvedic medicine as well as traditional Chinese medicine for a wide variety of conditions, such as improving memory, blood flow, as a wound-healing agent, and as a topical application for skin conditions such as ulcers, wounds, and eczema.

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Gotu Kola

The chemical compounds of interest in gotu kola are usually considered to be the ursane- and oleanane-type triterpenes and triterpene glycosides. Although low-wavelength UV can be used to measure these compounds, it suffers from sensitivity and baseline issues – these can be readily overcome by the charged aerosol detector. Interestingly, Figure 5-44 also shows that the number of potential interferences are reduced when using the charged aerosol detector, probably as the sample has a number of volatile compounds that absorb low-wavelength UV.

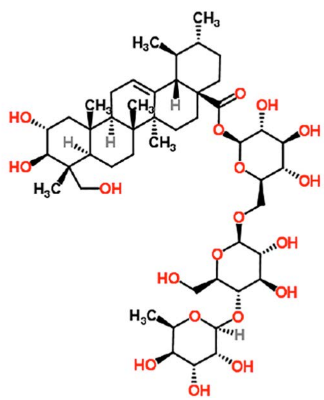
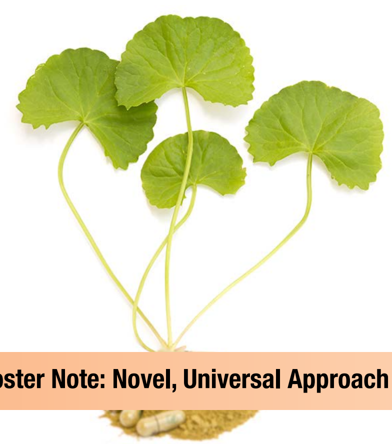


Figure 5-43. Asiaticoside structure.



System: UltiMate 3000 RS with Diode Array Detector DAD-3000RS and a Corona Veo Charged Aerosol Detector:
Evaporation temp: 35 or 50 °C
Power function: 1.00
Data collection rate: 2 Hz
Column: Fused-Core C18 HPLC Column, 3.0 × 100 mm, 2.7 µm
Column Temp: 35 °C
Flow Rate: 0.64 mL/min
Mobile Phase: A. 0.1% formic acid in Water
B. Acetonitrile
Gradient: 18% B to 22% B in 8 min;
22% B to 45% B from 8 min to 17 min;
45% B to 80% B from 17 min to 23 min
Injection Volume: 5 µL
Peaks: 1. Asiaticoside B
2. Madecassoside3
3. Asiaticoside
4. Madecassic Acid
5. Terminolic Acid
6. Asiatic Acid

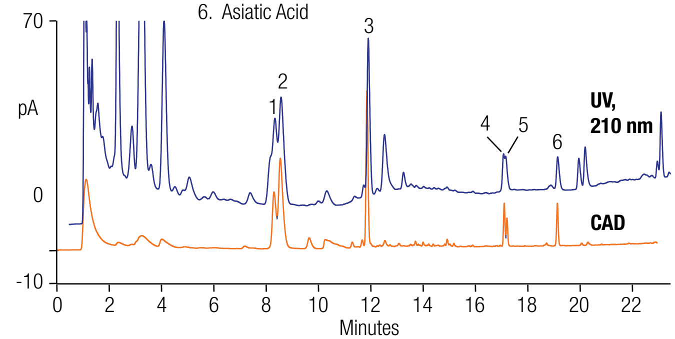


Figure 5-44. Comparison of UV and charged aerosol detection response for gotu kola extract.

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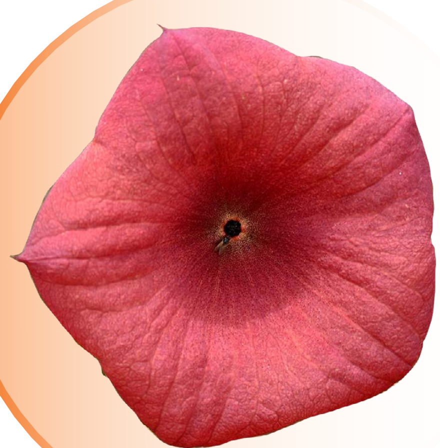

Hoodia cactus flower.

Hoodia

Hoodigosides are oxypregnane steroidal glycosides abundant in *Hoodia gordonii* and related plants native to the deserts of southwestern Africa. This plant is used traditionally to ease hunger during long hunting expeditions and enjoys wide use today in dietary supplements purported to aid in appetite suppression and weight loss.

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Hoodia

This application highlights the superior sensitivity of HPLC with charged aerosol detection for determination of hoodigosides in plant extracts. Eight hoodigosides isolated from dried plant material are separated within 15 min on an UltiMate 3000 RSLC system paired with a Thermo Scientific™ Accucore™ C18 analytical column. The Accucore column delivers superb resolution with low backpressure and the charged aerosol detector provides sensitive detection of all non-volatile analytes.

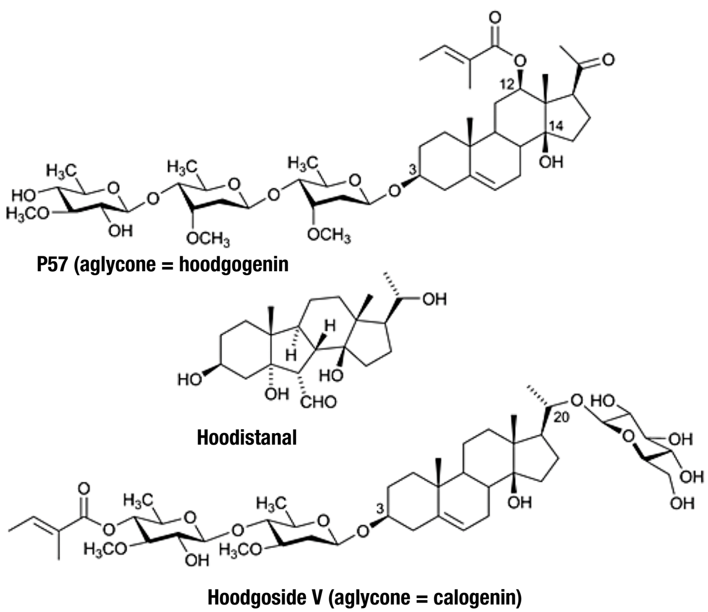

Figure 5-45. Chemical structures of hoodigosides.

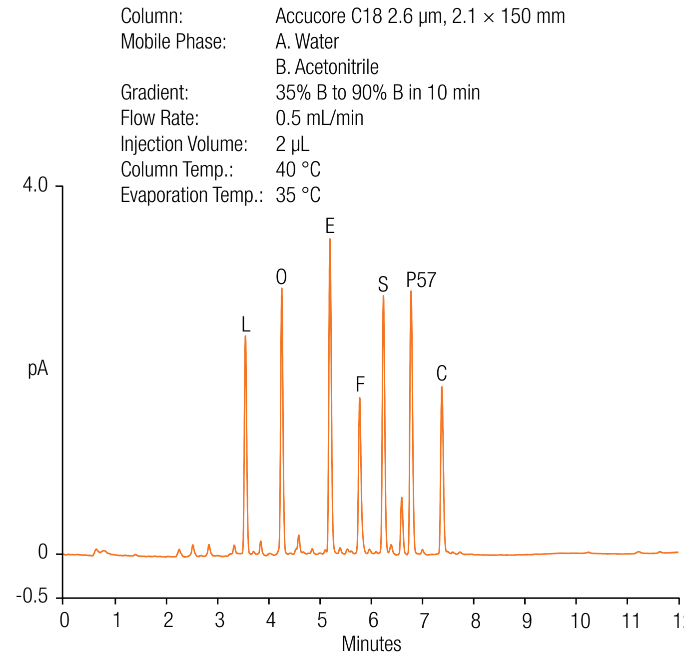

Figure 5-46. Analysis of *Hoodia gordonii* hoodigosides in a supplement (10 ng/µL).

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Kava root powder.

Kava Kava

Kavalactones are the active ingredient in the roots of the Kava (or Kava Kava), a shrub commonly found on many of the Pacific Islands. It is considered to have muscle relaxant, anticonvulsive, and anesthetic properties. Kava preparations are sold in the U.S. as capsules or teas.

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Kava Kava

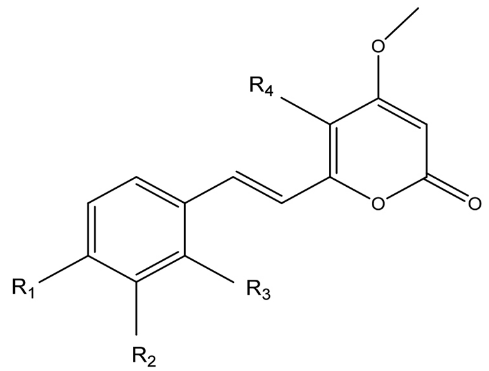

Figure 5-47. Kavalactone basic structure.

Column:	Reversed Phase, 250 × 4.6 mm
Flow:	1.00 mL/min
Temperature:	40 °C
Injection Volume:	5 µL
Mobile Phase:	Acetonitrile:Isopropyl Alcohol:0.1% Phosphoric Acid in Water (20:16:64, v/v)
Detection:	UV, 246 nm
Sample Preparation:	Approximately 100 mg of sample is extracted with 50 mL of acetonitrile
Peaks:	1. Methysticin 2. Dihydromethysticin 3. Kavain 4. Dihydrokavain 5. Yangonin 6. Desmethoxyyangonin

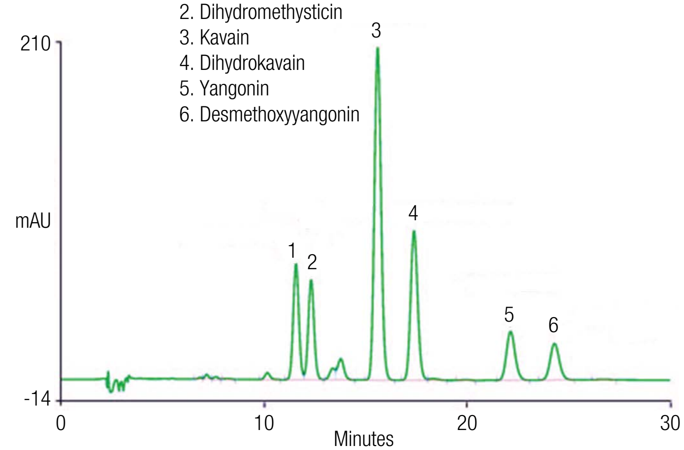

Figure 5-48. Determination of kavalactones in Kava Kava extracts using UV absorption detection at 246 nm.

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Supplements

Mangosteens.

Mangostins

The juice of the fruits of mangosteen *(Garcinia mangostana* L.; Clusiaceae) has become a popular botanical dietary supplement in the United States, due to its purported role in promoting overall health.

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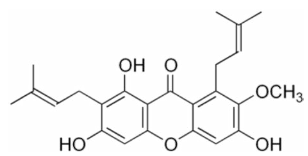
Mangostins

The primary ingredient alpha-mangostin, a xanthonoid, is perceived to have a number of biological properties including antioxidant, anticancer anti-bacterial, and anti-inflammatory activities.



Alpha-mangostin

Figure 5-49. Chemical structure of alpha-magostin.

Pump:	3000 DPG-3600RS
Autosampler:	3000 WPS-3000TRS
Detector:	DAD-3000RS, 320 nm
	Corona Veo, evaporation 50 °C, 5 s filter, 20 Hz, PFV 1.0
Column Thermostat:	3000 TCC-3000RS
Column:	Acclaim 120 C18, 2.2 µm, 2.1 × 100 mm
Temperature:	30 ºC
Mobile Phase A:	Water
Mobile Phase B:	Acetonitrile
Gradient:	0–20 min, 50–90% B; hold at 90% B for 5 min
Flow Rate:	0.5 mL/min
Injection Volume:	10 µL
Sample:	ASE extraction of mangosteen pericarp powder
Equipment:	ASE 200 Accelerated Solvent Extractor with solvent controller
	10 mL stainless extraction cells
	Cellulose filters
	Amber collection vials, 40 mL

ASE method:	Sample size ~0.5g
Solvent:	95% ethanol
Temperature:	80 °C
Static Time:	5 min
Static Cycles:	4
Flush:	60%
Purge:	90 s

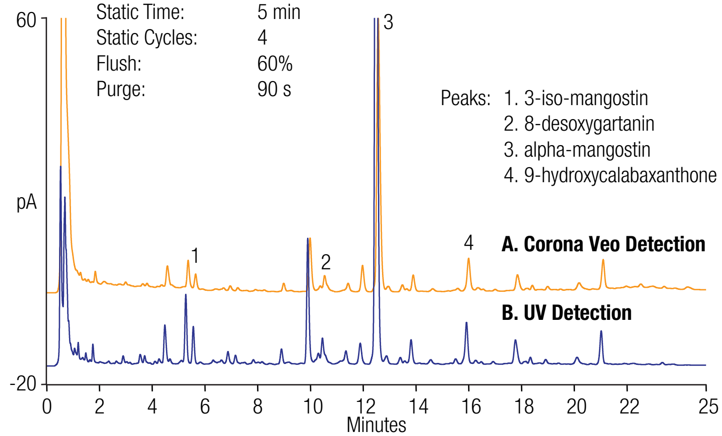


Figure 5-50. Comparison of UV and Corona Veo detection for xanthones in the same mangosteen pericarp extract sample.

Download Poster Note: Fast Analysis of Selected Xanthones in Mangosteen Pericarp Using Accelerated Solvent Extraction and UHPLC

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Milk thistle flowerhead.

Milk Thistle

The milk thistle plant is native to Mediterranean Europe. *Silybum marianium* has long been used as an herbal remedy to promote liver health. The seeds, root, and milky sap of the milk thistle contain an antioxidant flavonolignan complex known as the silymarin group.

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Milk Thistle

The standard method for determination of silymarins in milk thistle fruit and powdered extracts, Institute for Nutraceutical Advancement (INA) Method 115. 00, was improved by using a higher efficiency Acclaim RSLC 120 C18 2.2 µm column. The Corona Veo detector provided more uniform response for all nonvolatile analytes than did an UV absorbance detector.

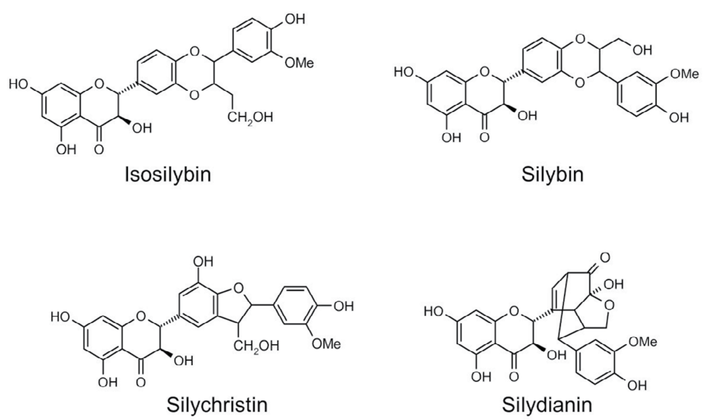

Figure 5-51. Chemical structures of the silymarin group.

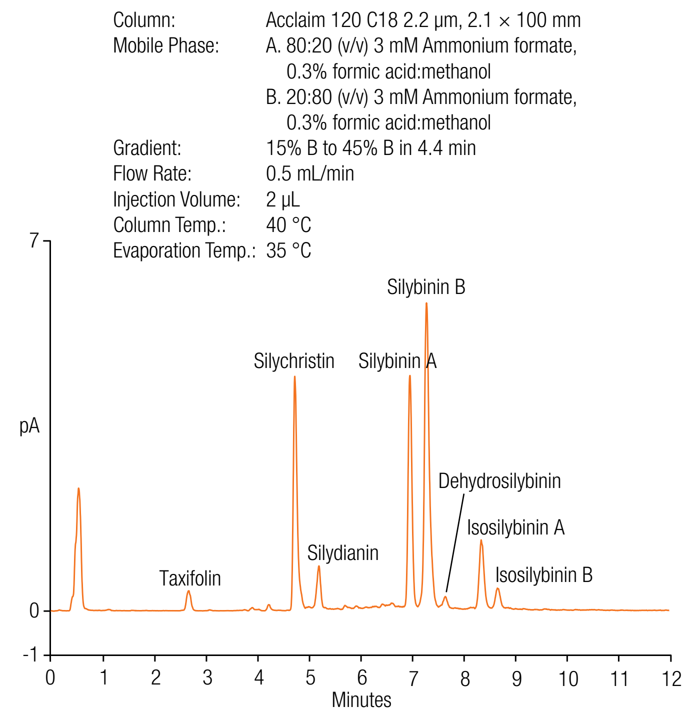

Figure 5-52. Analysis of milk thistle supplement (560 ng Silymarin/µL) by HPLC with Charged Aerosol detection.

Did You Know?

According to the National Center for Complementary and Alternative Medicine, in 2007 in the United States approximately one-third of total out-of-pocket spending on prescription drugs was spent on natural products. Of that total, $4.4 billion was spent on herbal supplements.

Download Poster Note: Novel, Universal Approach for the Measurement of Natural Products in a Variety of Botanicals and Supplements, Part 2

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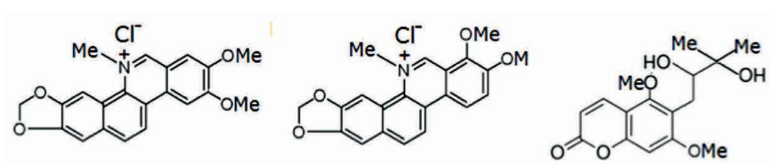
Chinese herbal medicine.

Nitidine Chloride, Toddalolactone, and Chelerythrine Chloride

Zanthoxylum nitidum (Roxb.) DC is an important traditional Chinese medicine. The Pharmacopeia of the People's Republic of China (PPRC) 2010 regulates this dried root as an herbal medicine. *Zanthoxylum nitidum* var. fastuosum is another plant in the same genus used in Chinese folk medicine.

Nitidine Chloride, Toddalolactone, and Chelerythrine Chloride

Nitidine is the specific active component in the dried root version of *Zanthoxylum nitidum* (Roxb.) DC, and nitidine chloride is reported to be beneficial for both killing and constraining the growth of cancerous cells. *Zanthoxylum nitidum* var. fastuosum is another plant in the same genus as *Zanthoxylum nitidum* (Roxb.) DC. Although *Zanthoxylum nitidum* var. fastuosum is not regulated in the PPRC, its dried root is still used in Chinese folk medicine because some of its reported medical benefits, such as promotion of blood circulation, pain relief, treatment of gastric ulcers, and reduction of inflammation, are the same as those reported for *Zanthoxylum nitidum* (Roxb.) DC.7



Nitidine Chloride **Chelerythrine Chloride** **Toddalolactone**

Figure 5-53. Chemical structures of Nitidine Chloride, Chelerythrine Chloride and Toddalolactone.

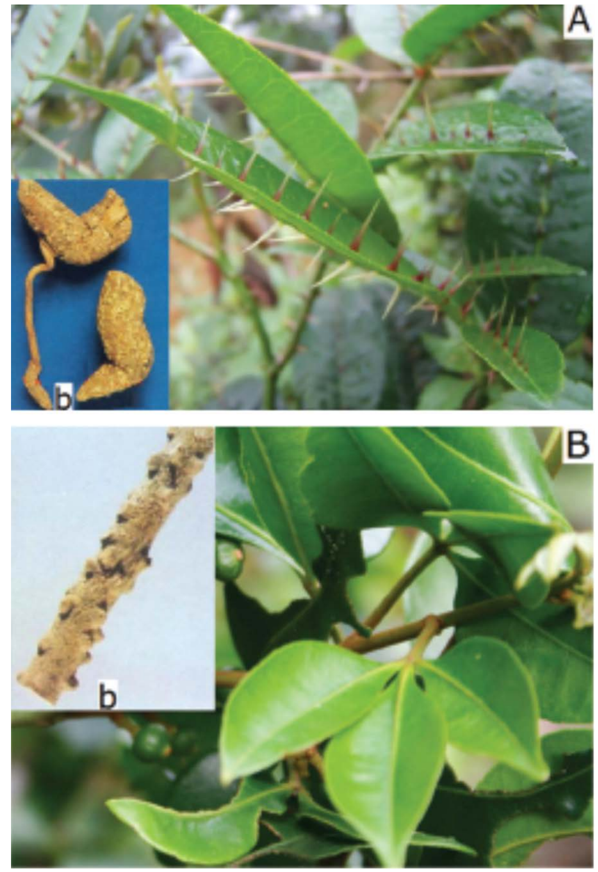


Figure 5-54. Top: (A) *Zanthoxylum nitidum (Roxb.)* DC and (b) root and Bottom: (A) *Zanthoxylum nitidum* var. fastuosum and (b) root.

Download Application Note 1008: Determination of Nitidine Chloride, Toddalolactone, and Chelerythrine Chloride by HPLC

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Nitidine Chloride, Toddalolactone, and Chelerythrine Chloride

Column:	Acclaim LC PA, 3 µm, Analytical, 2.1 × 150 mm
Mobile Phase:	25 mM ammonium acetate (pH 4.5 with acetic acid)/acetonitrile
Gradient:	Acetonitrile: 0 min, 20%; 8 min, 30%; 15 min, 70%; 15.5–18 min, 20%
Flow Rate:	0.6 mL/min
Injection Volume:	5 µL
Temperature:	30 °C
Detection:	UV absorbance at 273 nm

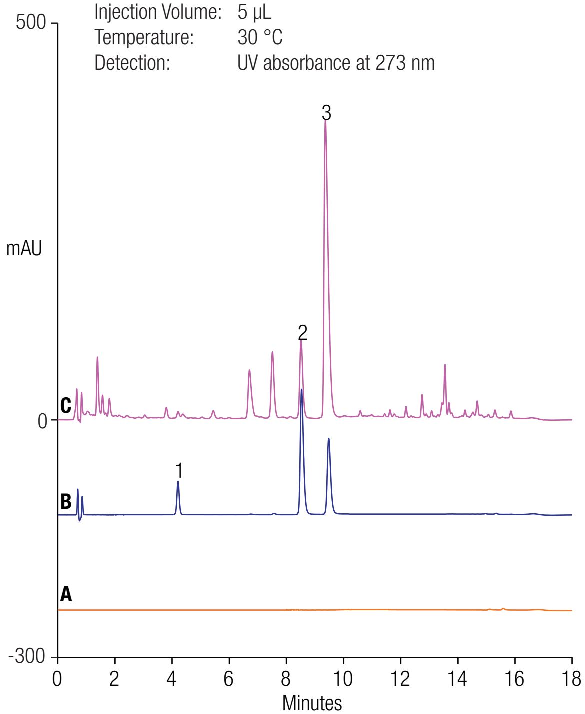

Figure 5-55. Chromatogram of a *Zanthoxylum nitidum* (Roxb.) DC sample.

Column:	Acclaim LC PA, 3 µm, Analytical, 2.1 × 150 mm
Mobile Phase:	25 mM ammonium acetate (pH 4.5 with acetic acid)/acetonitrile
Gradient:	Acetonitrile: 0 min, 20%; 8 min, 30%; 15 min, 70%; 15.5–18 min, 20%
Flow Rate:	0.6 mL/min
Injection Volume:	5 µL
Temperature:	30 °C
Detection:	UV absorbance at 273 nm

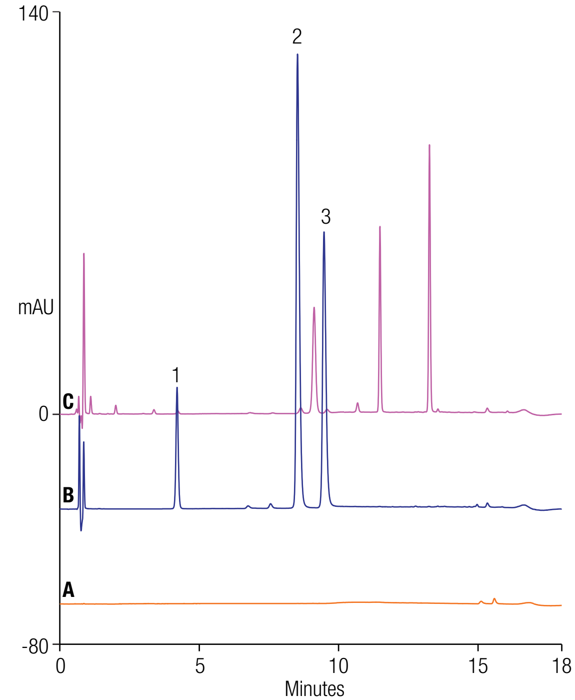

Figure 5-56. Analysis of a nitidum toothpaste sample.

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Dried fruit.

Phenolic Acids

Phenolic acids are found throughout the plant kingdom where they serve various roles, from structural to protective. Many phenolic acids occur naturally, and are categorized based on whether they contain a hydroxycinnamic acid or hydroxybenzoic acid backbone. Many studies suggest that consumption of foods containing phenolic acids may offer health benefits. Fruits, and especially dried fruits (where nutrients are concentrated during processing), are good sources of phenolic acids.

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Phenolic Acids

Research interests in phenolic acids also arise for food quality because they are associated with color, sensory qualities, and nutritional and antioxidant properties. Recent interest has focused on their antioxidant properties and potential health implications.

Instrumentation for Figures 5-57 and 5-58

- Ultimate 3000 HPLC System with dual ternary pump, ASI-100 autosampler, TCC-100 column temperature compartment, and UVD340U detector
- MSQ Plus single quadruple mass spectrometer with electrospray ionization (ESI) interface
- Chromeleon (6.8, SP3) CDS software and Xcalibur (1.4)

Chromatographic Conditions

Column:	Acclaim Mixed-Mode WAX-1 (150 × 2.1 mm, 5 µm)
Column Temp.:	50 °C
Mobile Phase:	A: CH_3CN 83% B: NH_4OAc 200 mm pH 4.6 5% C: H_2O 12%
Flow Rate:	0.5 mL/min
Injection Volume:	5 µL

Mass Spectrometric Conditions

Ionization Interface:	Electrospray ionization (ESI)
Detection Mode:	Selected ion monitoring (SIM)
Needle Temp.:	500 °C
Needle Voltage:	2000 V
Cone Voltage:	60 V
Span:	0.5 amu
Dwell Time:	0.2 s for each SIM
N2 Nebulizer Gas Pressure:	80 psi
Scan Events:	See Table 1 for details.

#	Name	R.T.	SIM	#	Name	R.T.	SIM
1.	Sinapic acid	5.7	223.1	9.	Benzoic acid	10.3	121.0
2.	Ferulic acid	6.2	193.1	10.	*o*-coumaric acid	10.9	163.1
3.	Veratric acid	6.7	181.1	11.	*m*-coumaric acid	11.6	163.1
4.	*p*-coumaric acid	7.3	163.1	12.	Caffeic acid	11.8	179.0
5.	Vanillic acid	7.5	167.0	13.	Salicylic acid	12.5	137.1
6.	Syringic acid	7.5	197.1	14.	Protocatechuic acid	14.0	153.0
7.	*p*-hydroxybenzoic acid	8.1	137.1	15.	Gentisic acid	16.3	153.0
8.	Cinnamic acid	8.8	147.1				

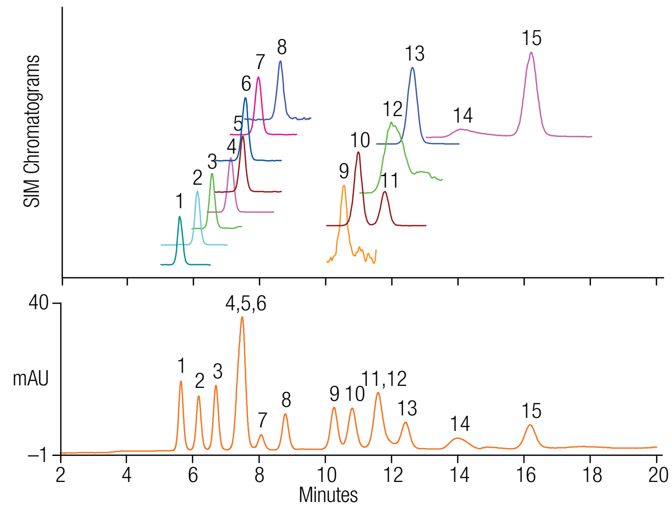

Figure 5-57. SIM and UV chromatograms of 15 predominant naturally occurring phenolic acids. SIM chromatogram is normalized to 100% of the greatest peak in each channel.

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Phenolic Acids

Chromatographic Conditions

Column:	Acclaim Mixed-Mode WAX-1 (150 × 2.1 mm, 5 µm)
Column Temp.:	50 °C
Mobile Phase:	A: CH_3CN 83% B: NH_4OAc 200 mm pH 4.6 5% C: H_2O 12%
Flow Rate:	0.5 mL/min
Injection Volume:	5 µL

Mass Spectrometric Conditions

Ionization Interface:	Electrospray ionization (ESI)
Detection Mode:	Selected ion monitoring (SIM)
Needle Temp.:	500 °C
Needle Voltage:	2000 V
Cone Voltage:	60 V
Span:	0.5 amu
Dwell Time:	0.2 s for each SIM
N2 Nebulizer Gas Pressure:	80 psi
Scan Events:	See Table 1 for details.

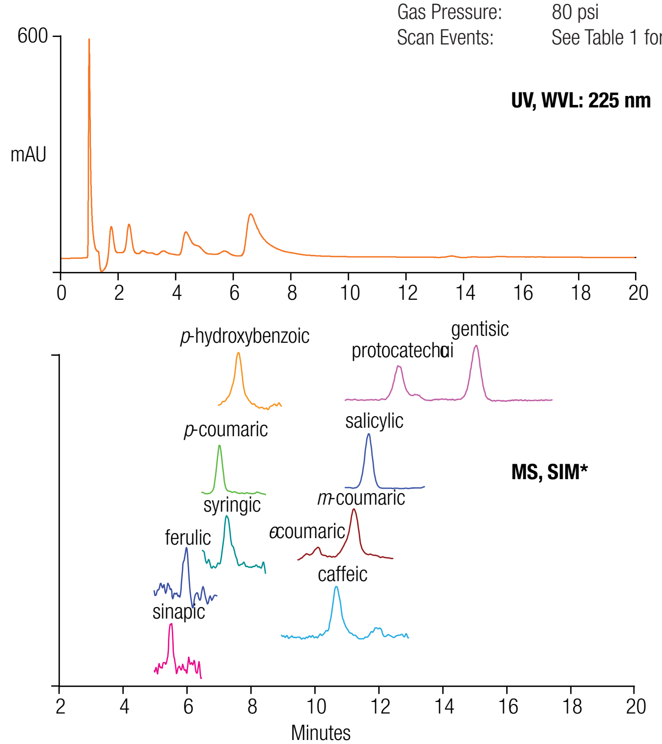

Figure 5-58. SIM and UV chromatograms of phenolic acids in green tea.

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Phenolic Acids

ORAC

Oxygen radical absorbance capacity (ORAC) is a method of determining the effective antioxidant strength of fruits, vegetables and spices. Often tables of data are presented (e.g., Table 5-3) ranking foods based on their antioxidant capacity.

Originally, the United States Department of Agriculture (USDA) published extensive tables of ORAC data for numerous foods and beverages but in 2012 withdrew its web publication of ORAC values due to the absence of scientific evidence that ORAC has any biological significance.

Table 5-3. Ranking of antioxidant capacity of fruits and vegetables based on ORAC testing.

Rank	Food item	Serving Size	Total Antioxidant Capacity per Serving Size
1	Small Red Bean (dried)	Half cup	13,727
2	Wild blueberry	1 cup	13,427
3	Red kidney bean (dried)	Half cup	13,259
4	Pinto bean	Half cup	11,864
5	Blueberry (cultivated)	1 cup	9,019
6	Cranberry	1 cup (whole)	8,983
7	Artichoke (cooked)	1 cup (hearts)	7,904
8	Blackberry	1 cup	7,701
9	Prune	Half cup	7,291
10	Raspberry	1 cup	6,058
11	Strawberry	1 cup	5,938
12	Red Delicious apple	1 whole	5,900
13	Granny Smith apple	1 whole	5,381
14	Pecan	1 ounce	5,095
15	Sweet cherry	1 cup	4,873
16	Black plum	1 whole	4,844
17	Russet potato (cooked)	1 whole	4,649
18	Black bean (dried)	Half cup	4,181
19	Plum	1 whole	4,118
20	Gala apple	1 whole	3,903

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Pommegranate, a source of phytoestrogens.

Phytoestrogens

Phytoestrogens are widely occurring plant-derived chemicals that have estrogenic activity in animals (nonsteroidal estrogens). There are three major classes including coumestans, isoflavones, and lignans. Phytoestrogens also show antioxidant, anti-inflammatory, antiviral, and antifungal properties.

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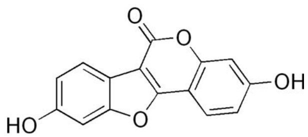
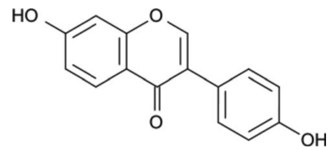
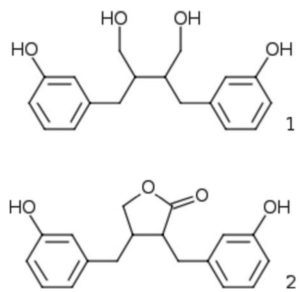
Phytoestrogens



A. Coumestrol (a coumestan)



B. Daidzein the aglycone of daidzin (an isoflavone)

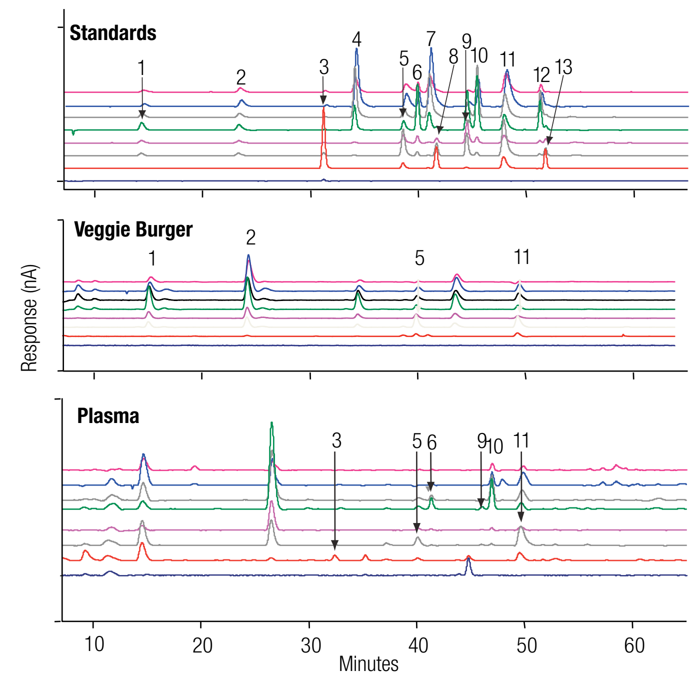


C. Enterodiol (1) and Enterolactone (2) are mammalian lignans

Figure 5-59. Coumestans: (A) Coumestrol is the most highly studied coumestan. The primary source is legumes, especially alfalfa.(B) Isoflavone is a type of flavonoid. The primary source is soybeans. Daidzein, genistein, and equol are of high interest. (C) The plant precursor lignans secoisolariciresinol and matairesinol are converted to their active mammalian forms enterodiol and enterolactone following consumption. Their primary source is flaxseed.

Column: C18, 150 × 4 mm
Mobile Phase: 50 mM sodium acetate, pH 4.8 with acetic acid/methanol/acetonitrile, 40:40:20 (v/v/v)
Gradient: Initial conditions of 20% mobile phase B and a linear gradient to 100% B over 25 min; 5 min hold at initial conditions
Flow Rate: 0.6 mL/min
Temperature: 37 °C
Potentials: 120, 320, 380, 440, 500, 560, 620, and 680 mV vs Pd

Peaks:
1. Daidzin
2. Genistein
3. Secoisolariciresinol
4. Dihydrodaidzen
5. Daidzein
6. Enterodiol
7. Matairesinol
8. Dihydrogenistein
9. Equol
10. Enterolactone
11. Genistein
12. *O*-Desmethylangolensin
13. Anhydrosecoisolaresinol



Thanks to Drs. Nurmi and Adlercreutz

Figure 5-60. Global phytoestrogen method.

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Phytoestrogens

Soy Isoflavones

Column:	Acclaim 120 C18, 150 × 4.6 mm
Flow:	1.20 mL/min
Temperature:	45 °C
Injection Volume:	10 µL
Mobile Phase:	A: 0.1% Phosphoric Acid in Water B: Acetonitrile
Gradient:	100%A–90%B over 40 minutes
Detection:	UV, 260 nm
Sample Preparation:	Approximately 5 mg of each reference standard is dissolved in dimethylsulfoxide and water:acetonitrile (60:40, v/v)
Peaks:	1. Daidzin 2. Glycitin 3. Genistin 4. Daidzein 5. Glycitein 6. Genistein

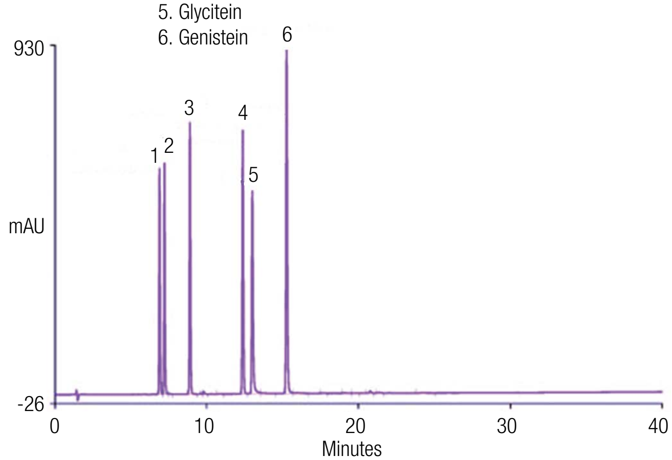

Figure 5-61. Determination of soy isoflavones in soy extracts using HPLC with UV detection.

Column:	C18 4.6 × 35 mm; 3 µm
Mobile Phase:	15% ACN in 0.1% Acetic Acid
Flow Rate:	1.5 mL/min
Column Temp.:	Ambient
Injection Volume:	10 µL (2 µg on column)
Detection:	Charged Aerosol
Peaks:	1. Daidzin 2. Glycitin 3. Genistin

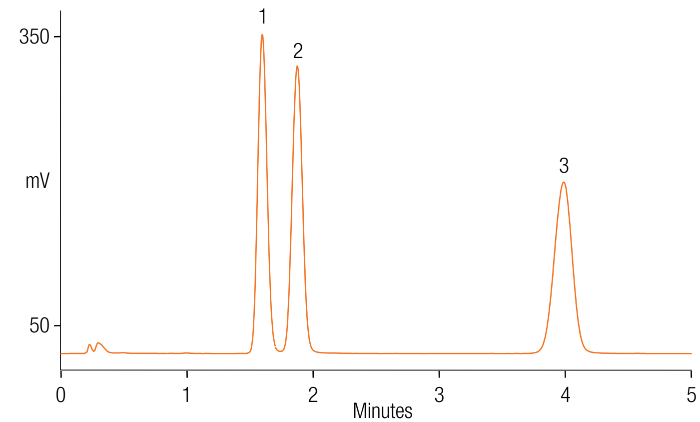

Figure 5-62. Analysis of soy isoflavones using HPLC with Charged Aerosol detection.

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Phytoestrogens

Soy Isoflavones Aglycones

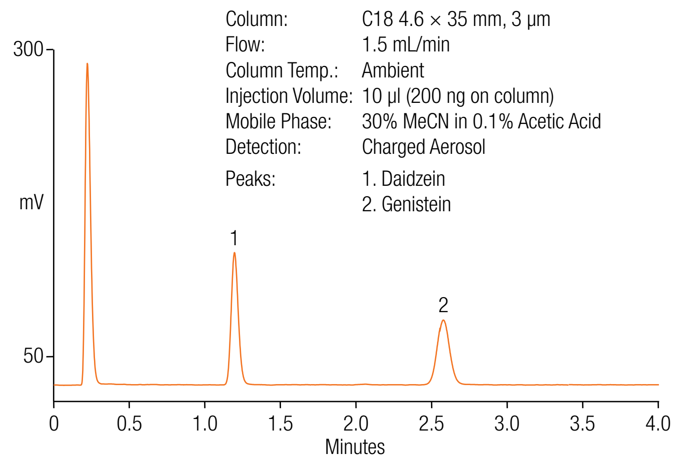

Figure 5-63. Analysis of soy isoflavone aglycones using Charged Aerosol detection.

Did You Know?

Soybeans were first domesticated in China around the 11th century BC. Soybean oil accounts for about 80% of all the vegetable oils and animal fats (lard, butter, etc.) consumed in the U.S. each year. In 2007, farmers in the US planted about 70 million acres of soybeans – accounting for 46 percent of the world's soybean production.

Coumestans and Lignans

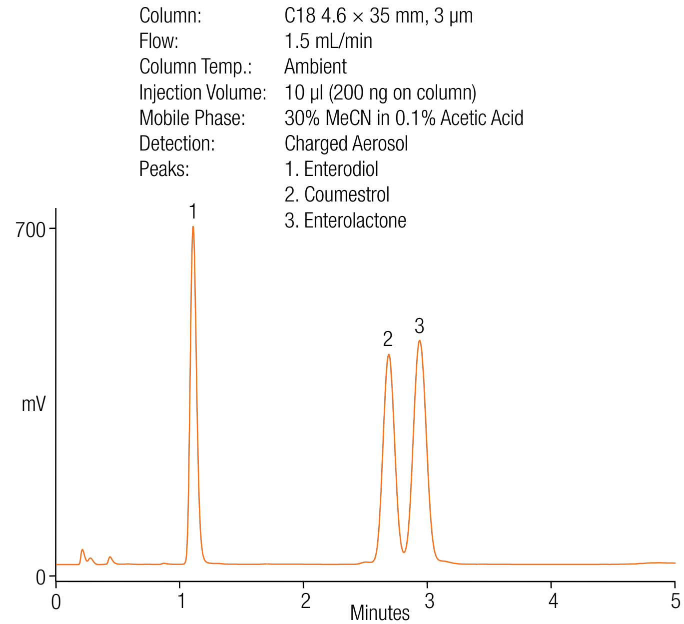

Figure 5-64. Analysis of coumestans and ligans using Charged Aerosol detection.

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Phytoestrogens

Isoflavones in Red Clover

Red clover is rich in isoflavones that may have useful pharmaceutical properties. Accelerated Solvent Extraction is a convenient, automated process that uses heat and pressure in a closed cell to extract samples with reduced time and solvent consumption. The high resolution of the Acclaim C18 column gives a clean separation even with this complex sample matrix.

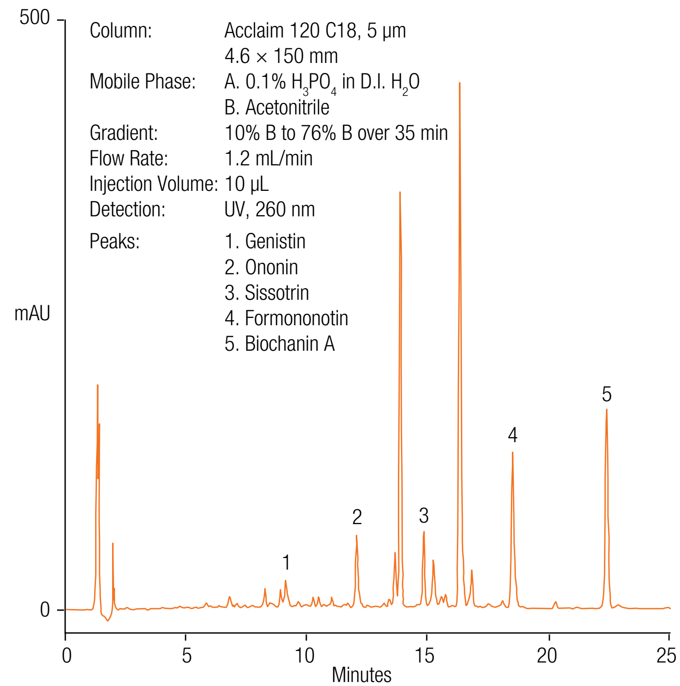

Figure 5-65. Isoflavones in red clover. Samples were prepared by Accelerated Solvent Extraction.

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Phytosterols

Phytosterols are a group of naturally occurring steroid alcohols found in plants, which can occur in foods as the free form or as esters with fatty acids/cinammic acid or glycosides. They are key structural components of plant cell membranes, assuming the role that cholesterol plays in mammalian cells.

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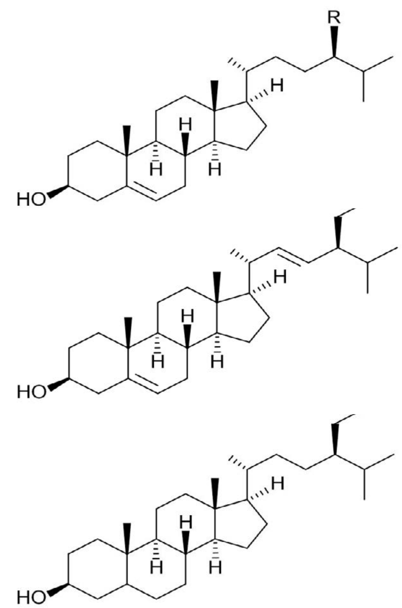
Phytosterols

There is considerable interest in phytosterols as dietary supplements as they are reported to lower cholesterol levels and also have a positive impact on cardiovascular diseases. However, recent research suggests that phytosterol supplementation may aggravate atherosclerosis and lead to aortic valve stenosis.

The chemical structures of five standards, cholesterol, campesterol, stigmasterol, β-sitosterol, and stigmastanol, are shown. These compounds lack a good chromophore, requiring UV detection below 210 nm, or they need to be derivatized with TMS for GC analysis. Phytosterols measured by gas chromatography is time consuming since it requires saponification of the sample, several extractions steps, and then derivatization. Poster Note 70986 presents a simplified method using reversed phase, high-performance liquid chromatography (HPLC) and electrochemical detection using a boron doped diamond electrode The latter technique requires careful selection of internal standards, complete extractions, and good reaction yields for quantitative results. These processes can significantly add to the time required and analysis costs for sample analysis.



Cholesterol, R = H
Campesterol, R = CH_3
b-Sitosterol, R = CH_2CH_3

Stigmasterol

Stigmastanol

Figure 5-66. Structures of five phytosterols

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Phytosterols

Conditions for Figures 5-67 and 5-68

Column:	Accucore C8, 2.6 µm, 2.1 × 100 mm		
Pump Flow Rate:	1.00 mL/min		
Column Temperature:	50.0 °C		
Mobile Phase A:	0.1% trifluoroacetic acid, 50 mM lithium perchlorate in water		
Mobile Phase B:	0.1% trifluoroacetic acid, 50 mM lithium perchlorate in acetonitrile		
Injection Volume:	2 µL standards and samples		
Gradient:	Time	%A	%B
	-5.00	22.0	78.0
	0.00	22.0	78.0
	5.00	10.0	90.0
	5.50	22.0	78.0
	6.50	22.0	78.0
Cell Potential:	6041RS ultra Amperometric Analytical Cell with BDD electrode at +1900 mV		
Clean Cell Potential:	+1950 mV		
Cell Clean Duration:	20.0 s		
Filter Constant:	2.0 s		
Sample Prep:	5–20 µL whole blood + 500 µL Mobile Phase B, mix and spin for 10 minutes at 13,000 RPM. The supernatant was transferred into an autosampler vial and placed onto the autosampler. tray		

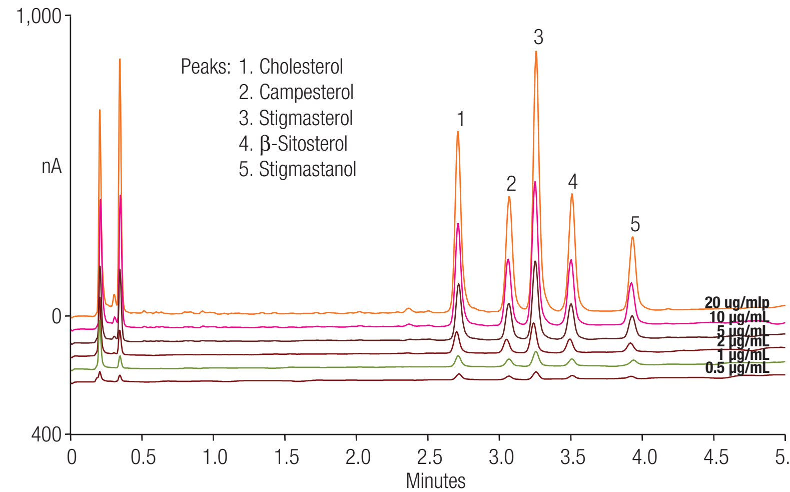

Figure 5-67. Overlay of analytical standards (1–40 ng on column).

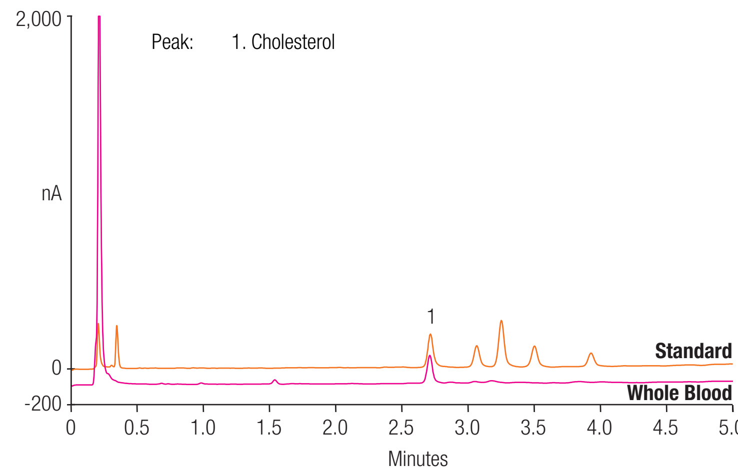

Figure 5-68. Overlay of standard vs. whole blood.

Download the Poster Note: Rapid and Direct Analysis of Free Phytosterols by Reversed Phase HPLC with Electrochemical Detection

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Red wine is a major source of polyphenols.

Polyphenols

Polyphenols, a large family containing over 5000 compounds, are widely distributed in plants.

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Polyphenols

For convenience polyphenols can be categorized as follows:

- Anthoxanthins including Flavones (e.g., tangeritin) and Flavonols (e.g., quercetin)
- Flavanones (e.g., hesperetin)
- Flavanonols (e.g., taxifolin)
- Flavans – Flavan-3-ol (e.g., Catechin) and its polymers (proanthocyanidins) are the most common
- Anthocyanidins (aglycones of anthocyanins)
- Isoflavonoids e.g., the isoflavone genistein

Polyphenols are the major antioxidants consumed in human diets. The main dietary sources are polyphenol-rich foods and beverages, which include fruits, fruit juices, tea, coffee, and red wine. Studies suggest that diets abundant in fruits and vegetables may provide protection against certain diseases, such as cardiovascular disease and cancer.

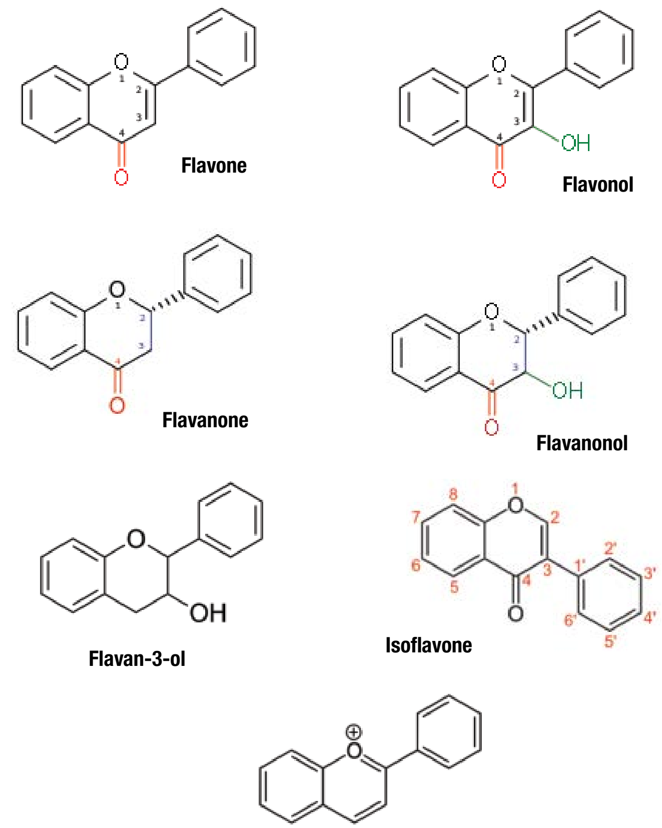

Figure 5-69. Chemical structures of various polyphenols.

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Polyphenols: Global Method

Column:	Acclaim 120, C18, 3 µm Analytical (3.0 × 150 mm) P/N 063691
Mobile Phase A:	20 mM Sodium Phosphate Monobasic, 3% Acetonitrile, 0.2% Tetrahydrofuran, pH 3.35
Mobile Phase B:	20 mM Sodium Phosphate Monobasic, 50% Acetonitrile, 10% Tetrahydrofuran, pH 3.45
Mobile Phase	C: 90% Methanol
Gradient:	0–2 min, 2% B, 3% C; 30 min, 97% B, 3% C; 45 min, 97% B, 3% C; Curve 7 (concave)
Flow Rate:	0.65 mL/min
Injection Volume:	20 µL
Detection:	UV; Channel 1, 218 nm; Channel 2, 240 nm; Channel 3, 254 nm; Channel 4, 275 nm
EC Detector Parameters:	16 Channel Array from 0 to +900 mV in 60 mV increments

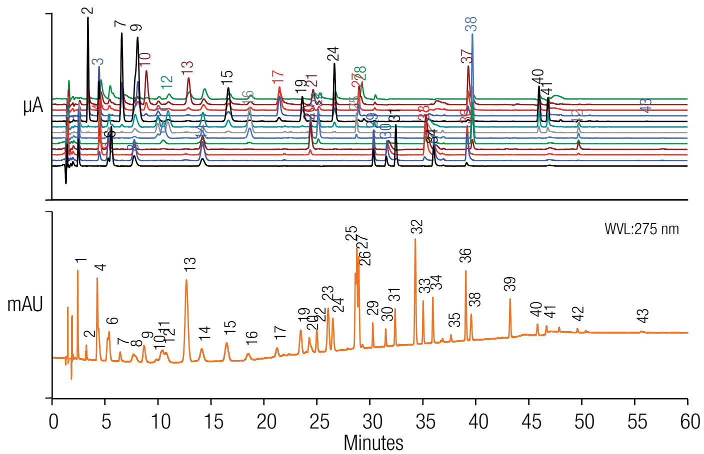

Figure 5-70. Gradient HPLC with spectro-electroarray detection.

Table 5-4. Peak identifications for Figures 5-71 through 5-73.

Peak	Compound	Peak	Compound
1	Gallic Acid	23	Rutin
2	4-Hydroxybenzyl Alcohol	24	Ethyl Vanillin Bourbonal
3	p-Aminobenzoic Acid	UV3	Methoxybenzaldehyde
4	3,4-Dihydroxybenzoic Acid	25	4-Hydroxycoumarin
5	Gentisic Acid	26	Hesperidin
6	2-Hydroxybenzyl Alcohol	27	Naringin
7	4-Hydroxybenzoic Acid	28	Rosemarinic Acid
8	Chlorogenic Acid	29	Fisetin
9	p-Hydroxyphenylacetic Acid	30	Myricetin
10	Catechin Hydrate	31	trans-Resveratrol
11	Vanillic Acid	UV4	Cinnamic Acid
12	4-Hydroxybenzaldehyde	32	Luteolin
13	Syringic Acid	33	cis-Resveratrol
14	Caffeic Acid	34	Quercetin Dihydrate
15	Vanillin	UV5	Apigenin
16	Syringaldehyde	35	Kaempferol
17	Umbelliferone	36	Isorhamnetin
18	p-Coumaric Acid	37	Eugenol
UV1	3,4-Dimethoxybenzoic Acid	38	Isoxanthohumol
19	Salicylic Acid	UV6	Chrysin
20	Sinapic Acid	39	Carvacrol
21	Ferulic Acid	40	Thymol
22	Ellagic Acid Dihydrate	41	Carnosol
UV2	Coumarin	42	Xanthohumol
		43	Carnosic Acid

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Polyphenols

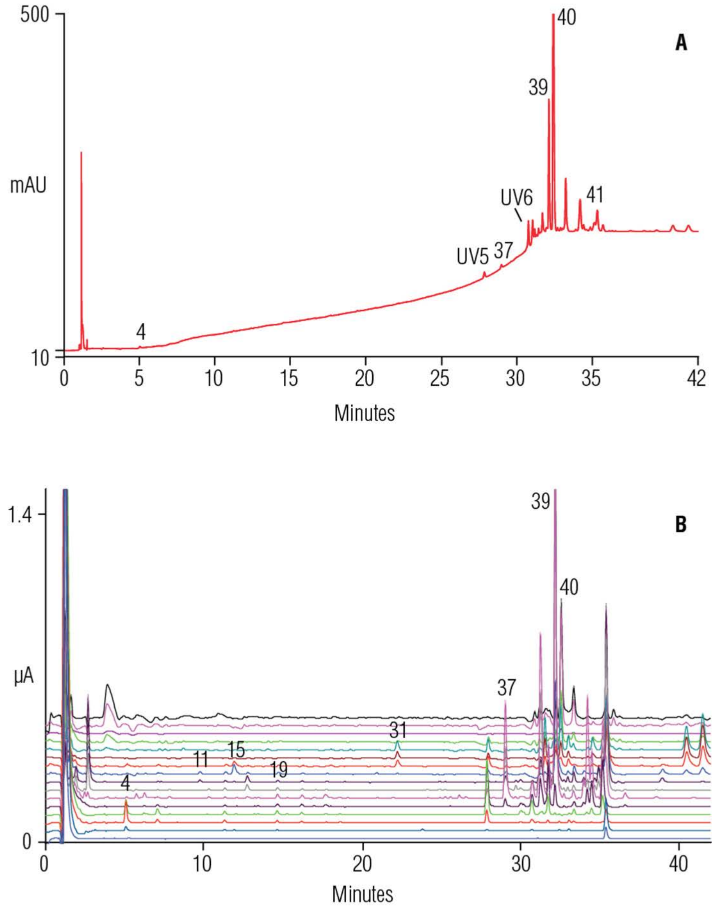

Figure 5-71. Nutmeg analyzed using UV detection at 210 nm (A) and EC detection (B). See Figure 5-70 for conditions and Table 5-4 for peak identifications.

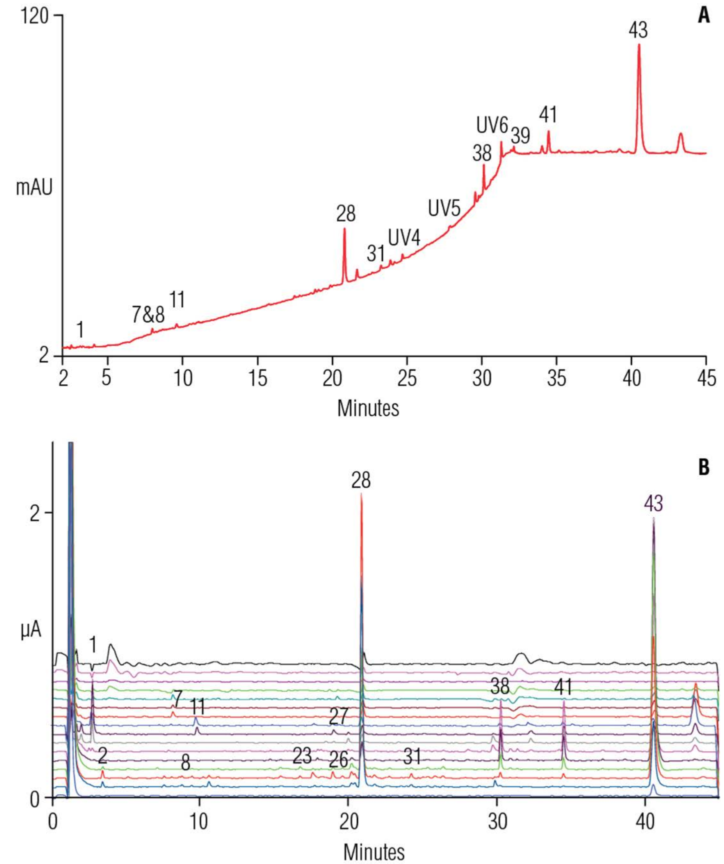

Figure 5-72. Rosemary analyzed using UV detection at 218 nm (A) and EC detection (B). See Figure 5-70 for conditions and Table 5-4 for peak identifications.

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Pomegranates.

Punicalagins

Antioxidants found in fruits and vegetables or the juices of fruits and vegetables are known to provide our bodies with many health benefits. Pomegranate is one such fruit that contains high levels of antioxidants. Studies have shown that pomegranate contains more antioxidants than green tea, cranberries, and even red wine.

Punicalagins

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Punicalagins From Pomegranate

The antioxidants in pomegranates include polyphenols, such as tannins and anthocyanins. The most abundant polyphenols in pomegranate juice are the hydrolyzable tannins called punicalagins.

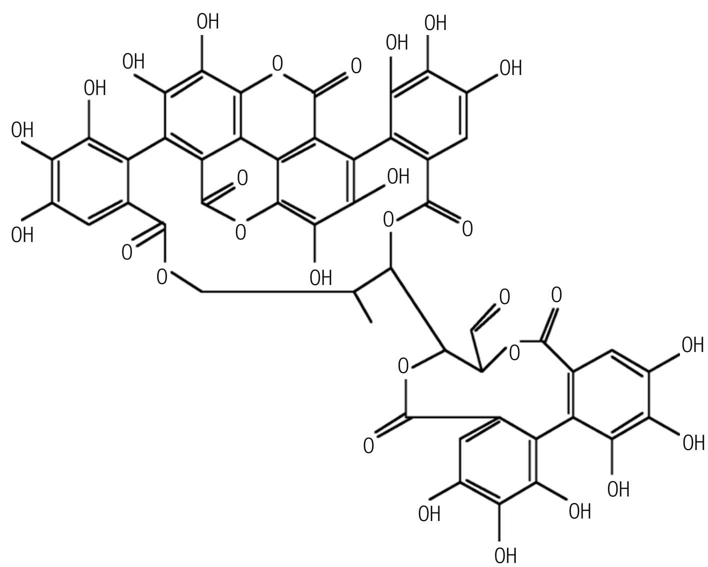

Figure 5-73. Structure of punicalagin A.

Instrument: Thermo Scientific Dionex HPLC System
Detection: UV-vis
Mobile Phase A: 1% Formic Acid in Milli-Q Water
Mobile Phase B: Acetonitrile
Gradient Program:

Time (min)	%A	%B
0	95	5
18	85	15
20	35	65
25	95	5
30	95	5

Column: Acclaim Polar Advantage PA II
150 × 3.0 mm, 3 µm particle
Flow Rate: 1.0 mL/min
Temperature: 30 °C
Injection Volume: 10 µL
UV Detection: 260 nm
Peaks: 1. Punicalagin B
2. Punicalagin A

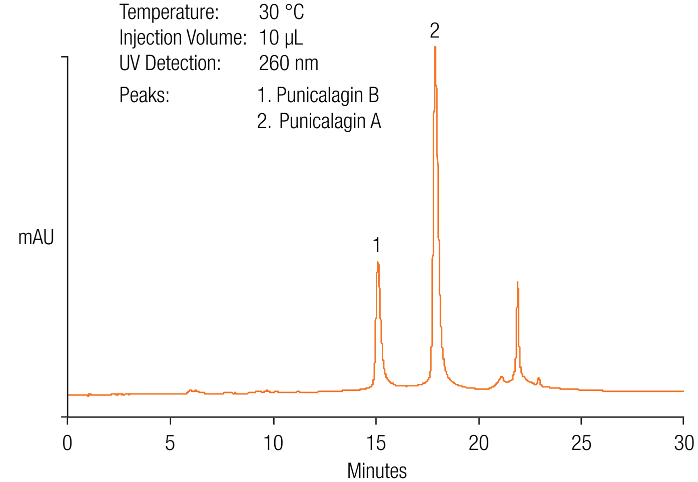

Figure 5-74. Punicalagins mixed standard.

Download Customer Application Note 106: Determination of the Punicalagins Found in Pomegranate by HPLC

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Resveratrol

Resveratrol (3,5,4’-trihydroxy-*trans*-stilbene) is a stilbenoid. It is a phytoalexin produced by some plants when they are attacked by pathogens such as bacteria and fungi. Resveratrol is found in skin of red grapes and in other fruits.

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Resveratrol

The major commercial source of resveratrol is from the roots of Japanese knotweed (Polygonum cuspidatum). Resveratrol is purported to have a number of health benefits including life extension and protection against cardiovascular disease, cancer, and diabetes.

Buyer Beware!

When buying dietary supplements, the manufacturer may not fully disclose the content or form of the active ingredient. In these examples, one vendor supplied resveratrol, two others suppled mixtures of resveratrol and polydatin, and one provided nothing at all.

Column: Acclaim 120 C18, 3 µm, 3.0 × 250 mm
Pump: UltiMate HPG3400 RS
Mobile Phases: A. Methanol
B. 0.02% H_3PO_4 (v/v)

Gradient:

Time:	–8	0	2	12	13
A.	40	40	40	75	90
B.	60	60	60	25	10

Flow: 0.60 mL/min
Temperature: TCC-3100 at 25 °C
Injection Volume: WPS-3000 RS sampler at 2 µL
Detector: DAD-3000 RS UV at 310 nm
Spectral confirmation, 200–450 nm

Chromatograms:
A. Brand #1; 17 mg resveratrol
B. Brand #2; 25 mg total resveratrol
C. Brand #3; 16 mg total resveratrol
D. Resveratrol standard; 50 µg/mL
E. Brand #4; <0.06 mg resveratrol

Sample Preparation:
1. Weigh contents of capsule
2. Take aliquot of 1/10
3. Add 1.0 mL 0.02% H_3PO_4 and 4.0 mL MEOH
4. Sonicate 10 min then filter

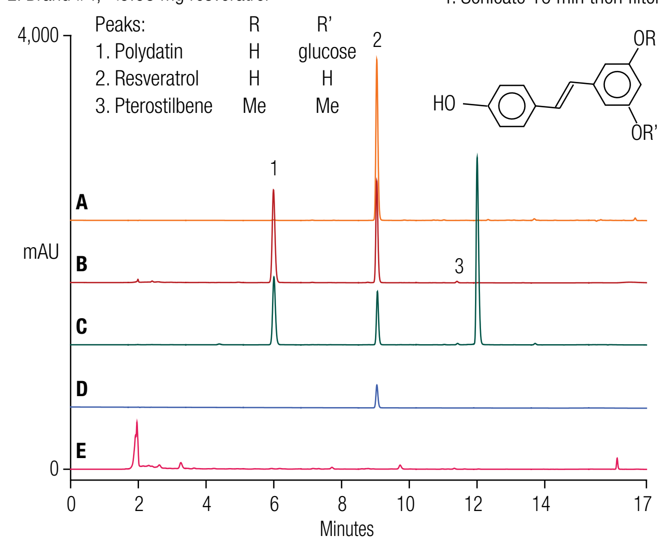

Figure 5-75. Resveratrol in dietary supplements on an Acclaim 120 C18 column.

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Supplements

Schizandrin and Schizandrin A and B

Schisandra chinensis supplement

Schisandra chinensis (Turcz.) Baill is an important traditional Chinese medicine believed to be an anticarcinogen and provide hepatoprotection, among other attributes.

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Schizandrin and Schizandrin A and B

The major active components of *Schisandra chinensis (Turcz.) Baill* are lignanoids, and the three major lignanoids are schizandrin, schizandrin A, and schizandrin B. Hugan tablets, which contain *Schisandra chinensis (Turcz.) Baill*, are a traditional Chinese medicine for hepatoprotection.The Pharmacopoeia of the People's Republic of China (PPRC) 2010 regulates its quality control with a UHPLC method for the determination of schizandrin, schizandrin A, and schizandrin B.

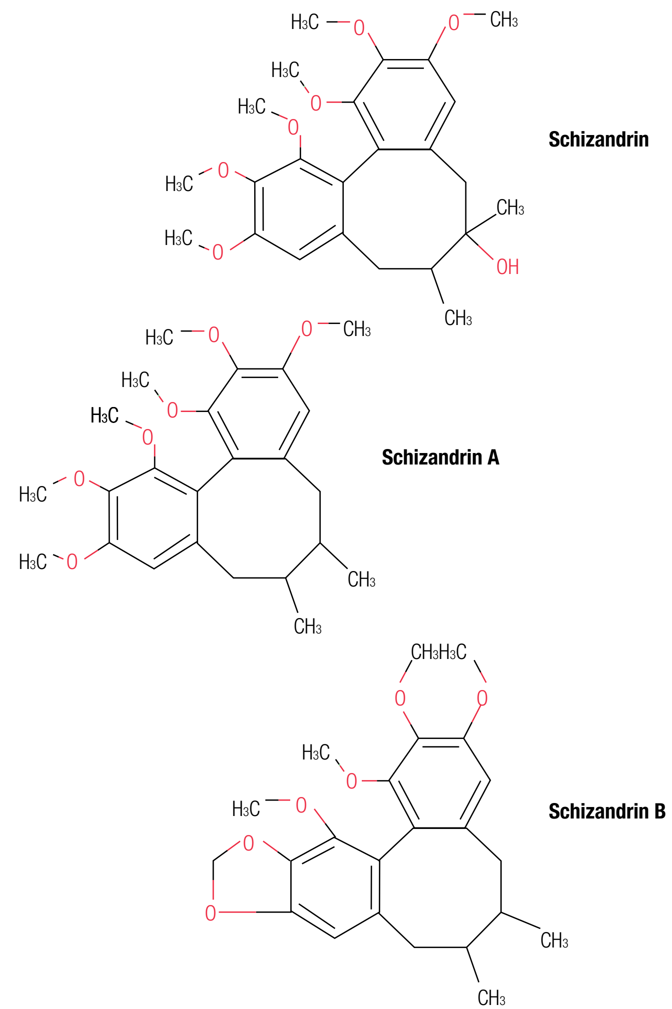

Figure 5-76. Chemical structures of Schizandrin, Schizandrin A and Schizandrin B.

Download Application Brief 139: Separation of Schizandrin, Schizandrin A, and Schizandrin B in a Tablet Sample

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Schizandrin and Schizandrin A and B

Column:	Acclaim RSLC 120 C18 (2.1 × 100 mm, 2.1 µm)
Mobile phase:	CH_3CN/H_2O, in gradient: CH_3CN: 0–3 min, 45%; 3–5 min, 45–80%; 15.1 min, 80–100%; 17 min, 100%
Flow Rate:	0.4 mL/min
Injection Volume:	2 µL
Temperature:	40 °C
Detection:	UV at 250 nm
Chromatograms:	A) Standards B) Tablet sample

Peaks:
1. Schizandrin
2. Schizandrin A
3. Schizandrin B

UV spectra:
A1 peak 1 of standard
A2 peak 2 of standard
A3 peak 3 of standard
B1 peak 1 of sample
B2 peak 2 of sample
B3 peak 3 of sample

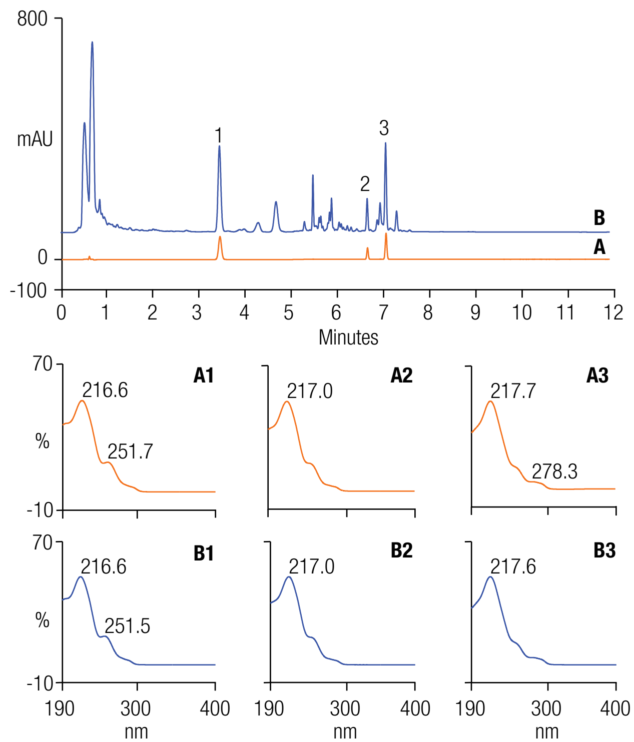

Figure 5-77. Chromatograms of a schizandrin, schizandrin A, and schizandrin B mixed standard and a Hugan tablet sample.

Did You Know?

There are two kinds of schizandra plants, one producing red berries and the other black. The red berries are the fruits you see at the Chinese herbalist's shop; the black ones rarely make it out of Asia.The fruits are said to have five distinct flavors: sweet, sour, pungent, bitter, and salty. This feature of the plant gave rise to its name in Chinese, wu wei tsu, the five-flavored plant.

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St. John's Wort.

St. John's Wort

St. John's wort, *Hypericum perforatum,* is a flowering plant species of the genus *Hypericum,* and is used as a treatment for depression.

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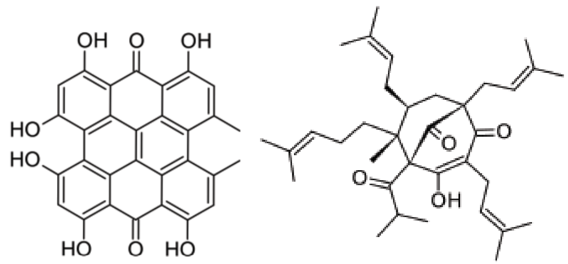
St. John's Wort

A number of compounds are purported to be responsible for the activity of St. John's wort including hypericin (a naphthodianthrone) and hyperforin



Hypericin Hyperforin

Figure 5-78. Chemical structures of active compounds in St. John's wort.

Trivia Question

Q: Do You Know What the Most Commonly Used Herbs Are?

A: The Top 10 most commonly used herbs are:

- Echinacea (Echinacea angustifolia)
- Evening primrose oil (Oenothera biennis)
- Feverfew (Tanacetum parthenium)
- Garlic (Allium sativum)
- Ginger (Zingiber officinale)
- Ginkgo biloba (Ginkgo biloba)
- Ginseng (Panax ginseng)
- Green tea extract(Camellia sinensis)
- St. John's wort (Hypericum perforatum)
- Saw palmetto (Serenoa repens)

Column:	Acclaim 120, C18, 3 µm Analytical (3.0 × 150 mm) P/N 063691
Flow:	0.65 mL/min
Injection Volume:	20 µL
Mobile Phase A:	20 mM Sodium Phosphate Monobasic, 3% Acetonitrile, 0.2% Tetrahydrofuran, pH 3.35
Mobile Phase B:	20 mM Sodium Phosphate Monobasic, 50% Acetonitrile, 10% Tetrahydrofuran, pH 3.45
Mobile Phase	C: 90% Methanol
Gradient:	0–2 min, 2% B, 3% C; 30 min, 97% B, 3% C; 45 min, 97% B, 3% C; Curve 7 (concave)
Detection:	UV; Channel 1, 218 nm; Channel 2, 240 nm; Channel 3, 254 nm; Channel 4, 275 nm
EC Detector Parameters:	16 Channel Array from 0 to +900 mV in 60 mV increments

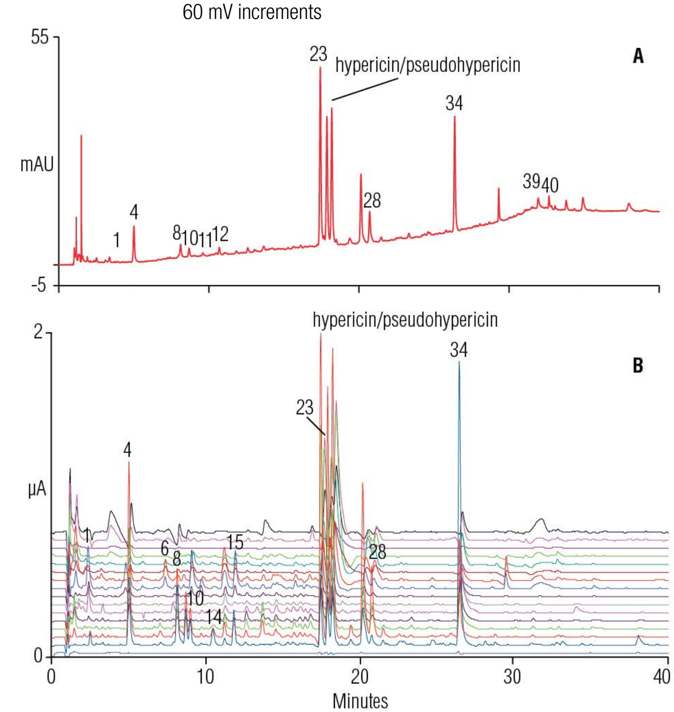


Figure 5-79. St. John's wort analyzed using UV detection at 254 nm (A) or EC detection (B). Peak identities shown in Table 5-4. on next page.

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St. John's Wort

Table 5-5. Peak identifications for Figure 5-79.

Peak	Compound	Peak	Compound
1	Gallic Acid	23	Rutin
2	4-Hydroxybenzyl Alcohol	24	Ethyl Vanillin Bourbonal
3	p-Aminobenzoic Acid	UV3	Methoxybenzaldehyde
4	3,4-Dihydroxybenzoic Acid	25	4-Hydroxycoumarin
5	Gentisic Acid	26	Hesperidin
6	2-Hydroxybenzyl Alcohol	27	Naringin
7	4-Hydroxybenzoic Acid	28	Rosemarinic Acid
8	Chlorogenic Acid	29	Fisetin
9	p-Hydroxyphenylacetic Acid	30	Myricetin
10	Catechin Hydrate	31	trans-Resveratrol
11	Vanillic Acid	UV4	Cinnamic Acid
12	4-Hydroxybenzaldehyde	32	Luteolin
13	Syringic Acid	33	cis-Resveratrol
14	Caffeic Acid	34	Quercetin Dihydrate
15	Vanillin	UV5	Apigenin
16	Syringaldehyde	35	Kaempferol
17	Umbelliferone	36	Isorhamnetin
18	p-Coumaric Acid	37	Eugenol
UV1	3,4-Dimethoxybenzoic Acid	38	Isoxanthohumol
19	Salicylic Acid	UV6	Chrysin
20	Sinapic Acid	39	Carvacrol
21	Ferulic Acid	40	Thymol
22	Ellagic Acid Dihydrate	41	Carnosol
UV2	Coumarin	42	Xanthohumol
		43	Carnosic Acid

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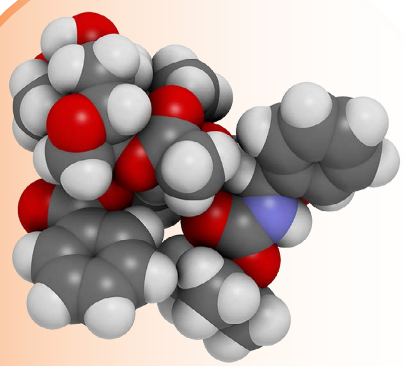

Molecular structure of Docetaxel, a taxane class drug.

Taxanes

Taxanes are diterpenes produced by the plants of the genus *Taxus* (yews), and are widely used as chemotherapy agents. Paclitaxel was approved as Taxol® by the National Cancer Institute (NCI) in 1991 for the treatment of ovarian cancer.

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Taxanes

An analysis of paclitaxel and related compounds—cephalomannine (related compound A),10-deacetyl-7-epipaclitaxel (related compound B), and 7-epipaclitaxel (related compound C) by reversed-phase (RP) HPLC was published by both the United States Pharmacopeia (USP) and the Chinese Pharmacopoeia (CP) but require more than 70 minutes to complete. There are several RP-HPLC assays for paclitaxel in the literature; however, all of them are also relatively long (15 to 35 min). Therefore, for researchers interested in the rapid separation of paclitaxel and related compounds developed a UHPLC method was developed. Use of an UltiMate 3000 RSLC system and an Acclaim RSLC C18 column packed with smaller particles is an easy way to increase speed and peak capacity. Therefore, this system was used to create an UHPLC method for the analysis of paclitaxel and related compounds including related compound B.

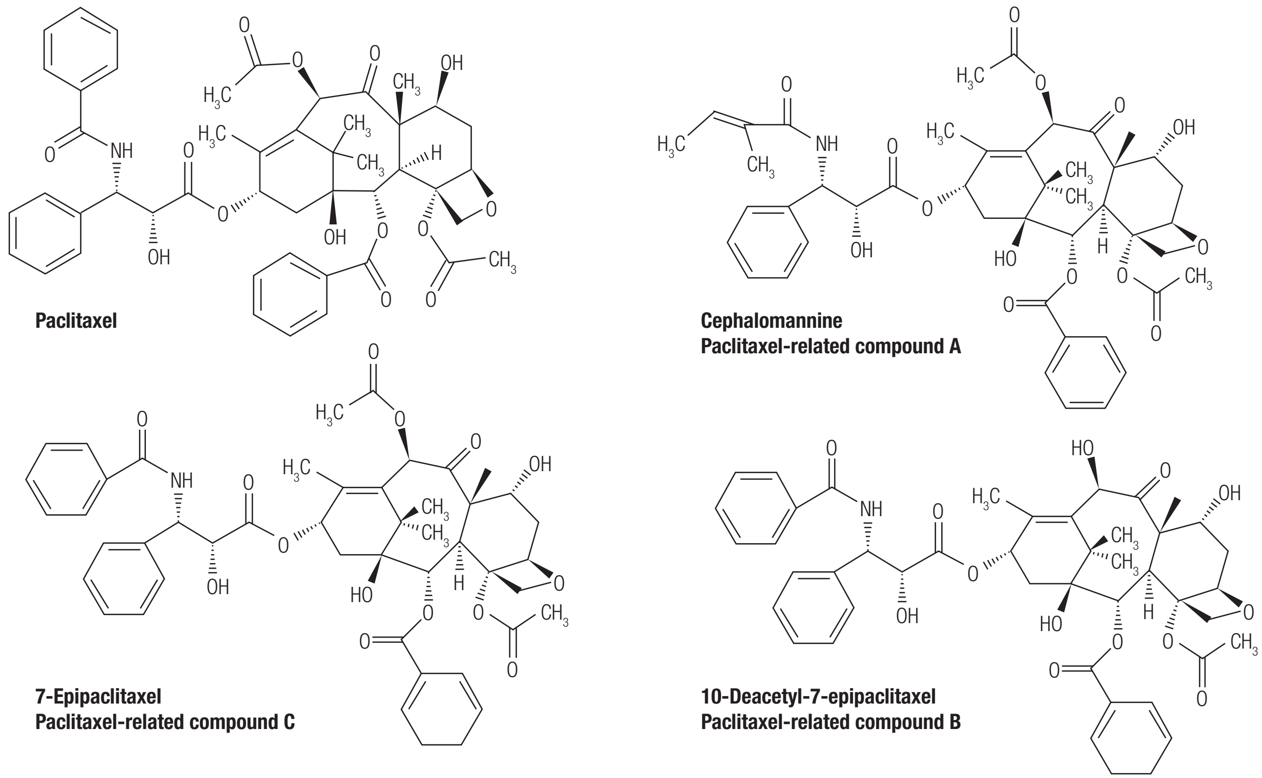

Figure 5-80. Chemical structures of paclitaxel and related compounds.

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Taxanes

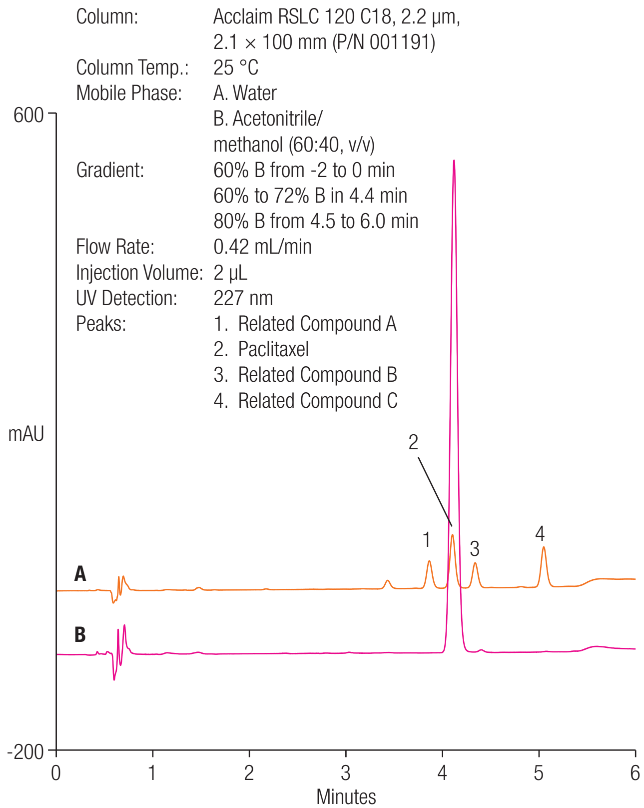

Figure 5-81. Overlay of HPLC-UV chromatograms of (A) mixture of paclitaxel and related compounds standards (5 µg/mL for each) and (B) Paclitaxel Injection sample.

HPLC with Charged Aerosol Detection
Column: C18, 4.6 × 150 mm SG300, 5 µm
Flow: 1.0 mL/min
Column Temp.: 25 °C
Injection Volume: 10 µL
Mobile Phase: A. Water
B. ACN
Gradient: 50% B to 75% B in 6 minutes
Peaks: 1. Cephalomannine
2. Paclitaxel

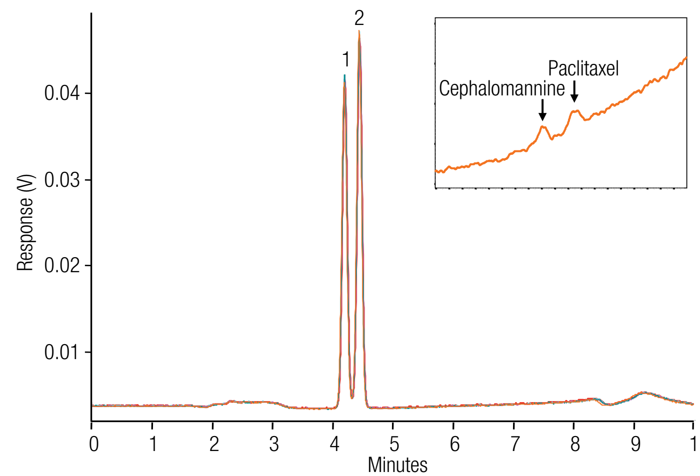

Figure 5-82. Taxane standards at 200 ng on column measured using HPLC-Charged Aerosol detection. Inset shown for 5 ng on column.

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Download Application Brief 119: Rapid Separation of Paclitaxel and Related Compounds in Paclitaxel Injection

Supplements

Ursolic acid is often found in plants and fruits such as apples

Ursolic Acid

Ursolic acid and related substances have a number of interesting pharmacological properties, and are starting materials for new drugs.

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Ursolic Acid

Ursolic acid and related substances (oleanolic, moronic, and betulinic acids) are secondary metabolites produced in a variety of plants including apples and the Chinese herb *Oldenlandia Diusa.*

LC System: UltiMate 3000 RSLC quaternary pump
Column: Acclaim C30, 3 µm, 3.0 × 150 mm
Temperature: 20 °C
Injection Volume: 5 µL
Mobile Phases: A. 2 mM formic acid + 0.4 mM ammonium formate in water
B. Methanol

Gradient Times (min):	-10	0	7	8	9	10	20
%A	10	10	10	0	0	0	0
%B	90	90	90	100	100	100	100
Flow Rate (mL/min):	0.5	0.5	0.5	0.5	0.5	1.0	1.0

Detection: Diode array, UV at 210 nm
Chromatograms: A. Fuji Apple
B. Red Delicious Apple
C. Standards, 100 µg/mL
Peaks: 1. Oleanolic acid
2. Ursolic acid
Sample Preparation: Peel one small apple (~170 g). Macerate peel with diatomaceous earth. Extract with three 15-mL portions of cold acetone; combine filtered extracts into a graduated cylinder. Evaporate a 1.0 mL aliquot, reconstitute in 0.5 mL of methanol, and filter.

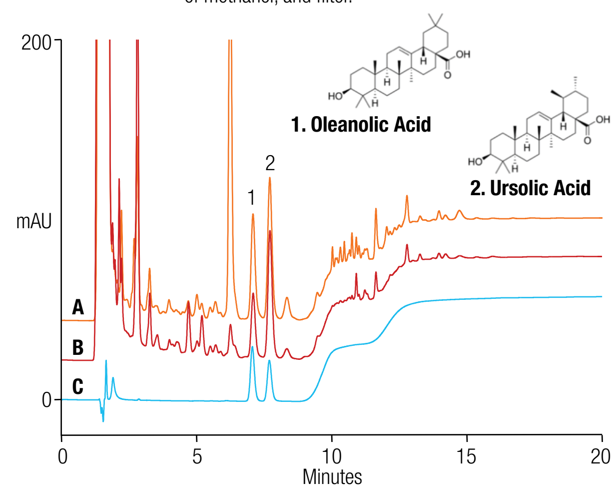

Figure 5-83. Ursolic acid in apple peel using an Acclaim C30 column and UV detection.

Download the Poster Note: Sensitive HPLC Method for Triterpenoid Analysis Using Charged Aerosol Detection with Improved Resolution

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Ursolic Acid

HPLC System:	UltiMate 3000 SD
HPLC Column:	Acclaim C30, 5 µm, 250 × 4.6 mm
Flow:	1.0 mL/min
Column Temperature:	30 °C
Injection Volume:	5.0 µL
Mobile Phase A:	1 w/v% Ammonium acetate in water
Mobile Phase B:	Acetonitrile/methanol (750 : 250)
Detector:	Corona *ultra* RS
Nebulizer Temperature:	15 °C
Filter Setting:	4
Sample Temperature:	Ambient

Gradient:

Time (min)	%A	%B	Curve
0.0	92	8	—
20.0	20	80	1
20.0	92	8	5
25.0	92	8	5

Peaks:
1. Glycyrehetinic Acid
2. Betulinic Acid
3. Oleanolic Acid
4. Ursolic Acid

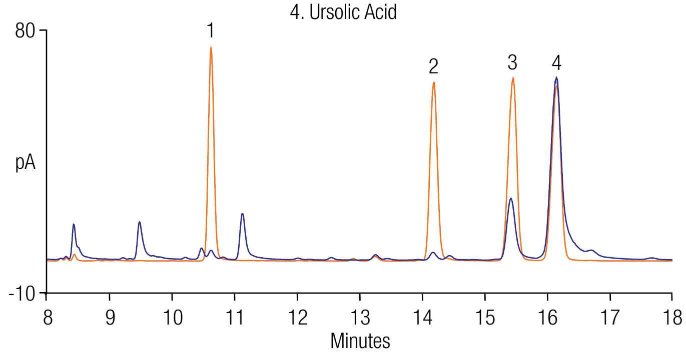

Figure 5-84. HPLC-Charged Aerosol detection chromatogram of apple peel extract (purple) and 500 ng on column triterpenoid standard solution (orange).

HPLC System:	UltiMate 3000 SD
HPLC Column:	Acclaim C30, 5 µm, 250 × 4.6 mm
Flow:	1.0 mL/min
Column Temperature:	30 °C
Injection Volume:	5.0 µL
Mobile Phase A:	1 w/v% Ammonium acetate in water
Mobile Phase B:	Acetonitrile/methanol (750 : 250)
Detector:	Corona *ultra* RS
Nebulizer Temperature:	15 °C
Filter Setting:	4
Sample Temperature:	Ambient

Gradient:

Time (min)	%A	%B	Curve
0.0	92	8	—
20.0	20	80	1
20.0	92	8	5
25.0	92	8	5

Peaks:
1. Glycyrehetinic Acid
2. Betulinic Acid
3. Oleanolic Acid
4. Ursolic Acid

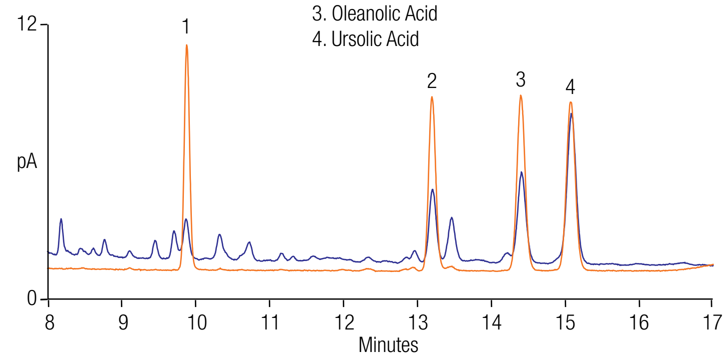

Figure 5-85. HPLC-Charged Aerosol detection chromatogram of basil extract (purple) and 32 ng on-column triterpenoid standard solution (orange).

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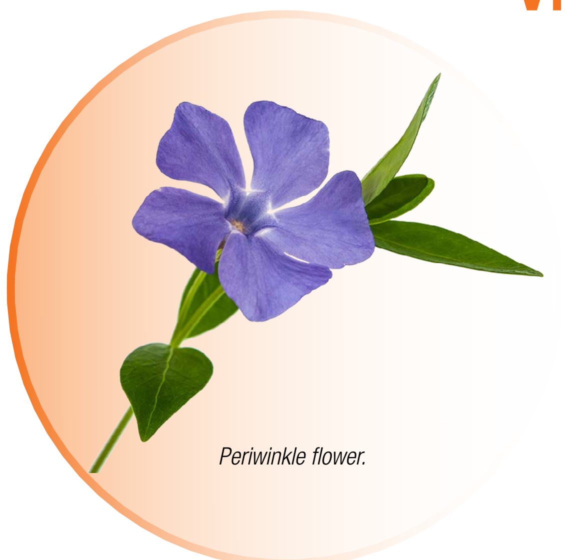
Vinca and Yohimbine



Periwinkle flower.

The vinca alkaloids consist of the bis-indole derivatives, vinblastine and vincristine isolated from the periwinkle plant, Vinca rosea Linn and the monomeric indole derivative vincamine, which is the major alkaloid of *Vinca minor* Linn. The vinca alkaloids are used as chemotherapeutic agents to treat a variety of neoplastic diseases including Hodgkin's disease, choriocarcinoma, acute and chronic leukemias, lymphosarcomas and a variety of other cancers.

Yohimbine is an indolealkylamine alkoloid with chemical similarity to reserpine. Yohimbine has been isolated from Cornanthe johimbe, Rubiaceae, and related trees, as well as Rauwolfia serpentina. Both yohimbine and a diastereoisomer, corynanthine, are 2-adrenoceptor antagonists of limited duration

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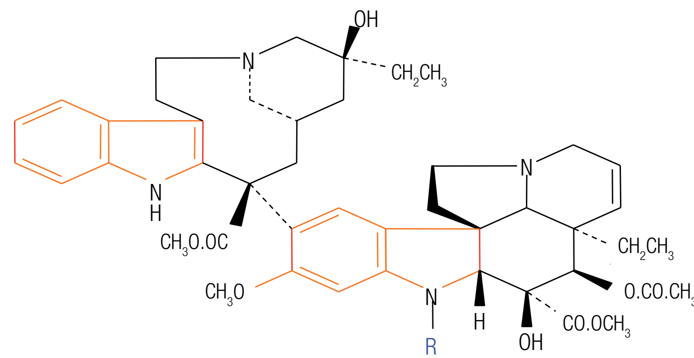
Vinca

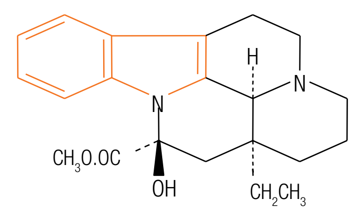
The vinca alkaloids' mechanism of action involves binding to tubulin with disruption of mitotic spindle formation eventually leading to inhibtion of mitosis, metaphase arrest, and cell death.

Vinca alkaloids have been measured using a variety of techniques. In general these approaches suffer from either a lack of sensitivity (HPLC techniques) or selectivity (radioimunoassay and HPLC techniques). Coulometric electrode array detection when coupled to gradient HPLC possesses both the selectivity and sensitivity required to measure the vinca alkaloids in natural products (e.g. periwinkle leaf extracts) and for the study of their pharmacokinetics and metabolism in vivo. Furthermore, this method is readily adaptable for the analysis of the ever broadening variety of synthetic analogs.



VINBLASTINE (R = CH_3)

VINCRISTINE (R = CHO)



VINCAMINE

Figure 5-86. Chemical structures of Vinca alkaloids.

Yohimbine

Yohimbine has been reported to produce central excitation (e.g., elevated blood pressure, increased heart rate, increased motor activity, tremor, irritability, vomiting, nausea, and sweating) and to act as an antidiuretic (through its ability to release antidiuretic hormone). Some evidence also suggests that yohimbine can act as an aphrodisiac although this effect may be limited to individuals with vascular diseases. A variety of techniques have been used to measure yohimbines including: GC-MS and HPLC with UV, fluorescence, or amperometric detection. In general these approaches suffer from a lack of sensitivity, selectivity, or require extensive sample preparation and derivatization before analysis.

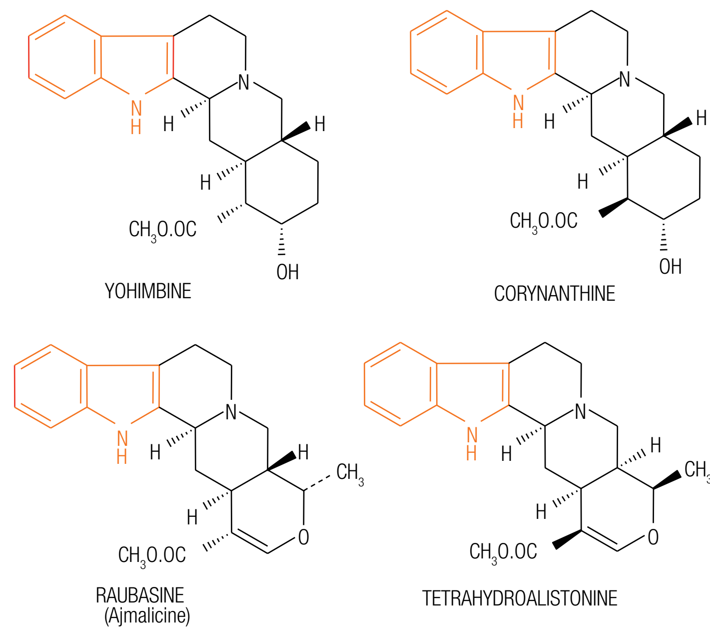


Figure 5-87. Chemical structure of yohimbine and related alkoloids

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Vinca and Yohimbine

The gradient HPLC coulometric array method shown here is capable of measuring yohimbine, related alkoloids, and the vinca alkoloids simultaneously. This approach offers low picogram sensitivity and uses voltammetric resolution to correctly identify each analyte and check for possible coelutions. The gradient analytical system consisted of two pumps, an autosampler, and a twelve-channel CoulArray detector. Higher levels of analytes were also verified using an UV absorbance detector placed after the array.

Column:	Base deactivated (4.6 × 150 mm; 5 µm)
Flow:	1.0 mL/min
Temperature:	Ambient
Injection Volume:	20 µL
Mobile Phase A:	100 mM Sodium acetate; acetonitrile; methanol (85:10: 5 v/v/v), final pH 6.2 with phosphoric acid
Mobile Phase B:	100 mM Sodium acetate; acetontrile; methanol (50:30:20 v/v/v), final pH 6.2 with phosphoric acid
Gradient Conditions:	Isocratic, 25% B until 1.1 minutes. Linear increase of phase B from 25% to 100% over 19 min. Hold for 18 min. Isocratic, 25% B for 3 min
Applied Potentials:	+200 to + 950 mV vs. Pd. in 75mV absorbance
Wavelength:	274 nm
Peaks:	1. Corynanthine 2. Yohimbine 3. Vincamine 4. Ajmalicine 5. Vincristine 6. Vinblastine 7. Tetrahydro-alistonine

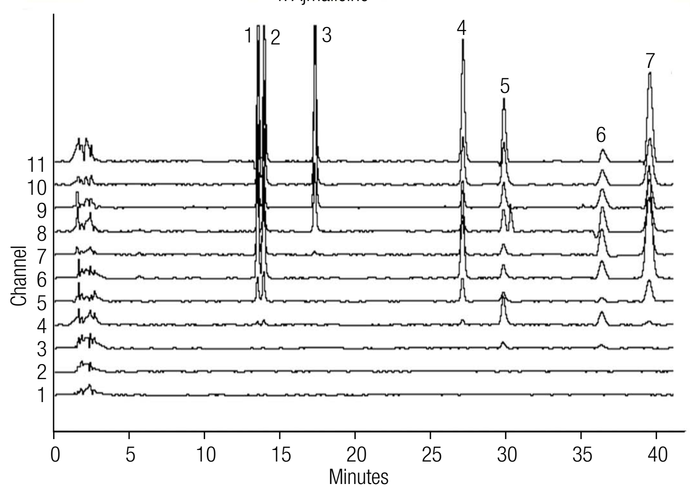

Figure 5-88. Chromatogram showing separation of vinca and other alkaloids. Analytes were separated using the gradient CoulArray method. The applied potentials were channel 1 to channel 11, +200 to +950 mV in +75 mV increments. The chromatogram is presented at a gain of 500 nA.

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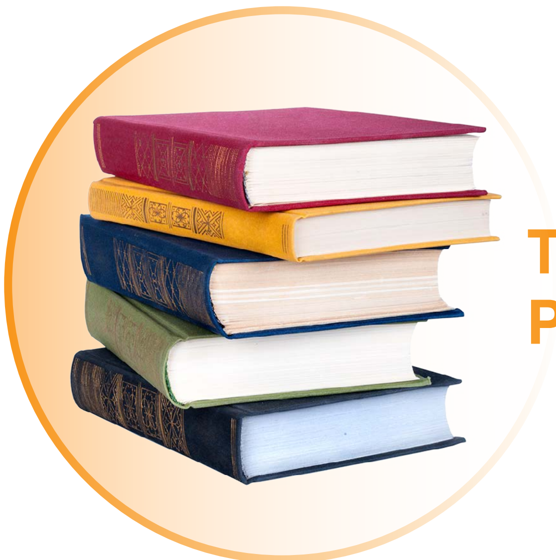

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Pharmacokinetic study of caffeic and rosmarinic acids in rats after oral administration	Konishi, Y.; Hitomi, Y.; Yoshida, M.; Yoshioka, E.	*J. Agric. Food Chem. 53 (12),* 4740–4746	2005 Jun 15
Intestinal absorption of *p*-coumaric and gallic acids in rats after oral administration	Konishi, Y.; Hitomi, Y.; Yoshioka, E.	*J. Agric. Food Chem. 52 (9),* 2527–2532	2004 May 5
Microbial metabolites of ingested caffeic acid are absorbed by the monocarboxylic acid transporter (MCT) in intestinal Caco-2 cell monolayers	Konishi, Y.; Kobayashi, S.	*J. Agric. Food Chem. 52 (21),* 6418–6424	2004 Oct 20
Transepithelial transport of rosmarinic acid in intestinal Caco-2 cell monolayers	Konishi, Y.; Kobayashi, S.	*Biosci., Biotechnol., Biochem. 69 (3),* 583–591	2005 Mar
Effects of various doses of selenite on stinging nettle (*Urtica dioica L.*)	Krystofova, O.; Adam, V.; Babula, P.; Zehnalek, J.; Beklova, M.; Havel, L.; Kizek, R.	*Int. J. Environ. Res. Public Health 7 (10),* 3804–3815	2010 Oct
Biofortified cassava increases β-carotene and vitamin A concentrations in the TAG-rich plasma layer of American women	La Frano, M. R.; Woodhouse, L. R.; Burnett, D. J.; Burri, B. J.	*Br. J. Nutr. 110 (2),* 310–320	2013 Jul 28
Chlorogenic acid is absorbed in its intact form in the stomach of rats	Lafay, S.; Gil-Izquierdo, A.; Manach, C.; Morand, C.; Besson, C.; Scalbert, A.	*J. Nutr. 136 (5),* 1192–1197	2006 May
Determination of 4-ethylcatechol in wine by high-performance liquid chromatography-coulometric electrochemical array detection	Larcher, R.; Nicolini, G.; Bertoldi, D.; Nardin, T.	*Anal. Chim. Acta 609 (2),* 235–240	2008 Feb 25
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.	*Anal. Chim. Acta 582 (1),* 55–60	2007 Jan 16

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Acute, quercetin-induced reductions in blood pressure in hypertensive individuals are not secondary to lower plasma angiotensin-converting enzyme activity or endothelin-1: nitric oxide	Larson, A.; Witman, M. A. H.; Guo, Y.; Ives, S.; Richardson, R. S.; Bruno, R. S.; Jalili, T.; Symons, J. D.	*Nutr. Res. (N. Y., NY, U.S.) 32 (8),* 557–564	2012 Aug
High-performance liquid chromatography method for the determination of folic acid in fortified food products	Lebiedzińska, A.; Dąbrowska, M.; Szefer, P.; Marszałł M.	*Toxicol. Mech. Methods 18 (6),* 463–467	2008 Jul
Reversed-phase high-performance liquid chromatography method with coulometric electrochemical and ultraviolet detection for the quantification of vitamins B(1) (thiamine), B(6) (pyridoxamine, pyridoxal and pyridoxine) and B(12) in animal and plant foods	Lebiedzińska, A.; Marszałł, M. L.; Kuta, J.; Szefer, P.	*J. Chromatogr., A 1173 (1–2),* 71–80	2007 Nov 30
An improved method for the determination of green and black tea polyphenols in biomatrices by high-performance liquid chromatography with coulometric array detection	Lee, M. J.; Prabhu, S.; Meng, X.; Li, C.; Yang, C. S.	*Anal. Biochem. 279 (2),* 164–169	2000 Mar 15
Characterisation, extraction efficiency, stability and antioxidant activity of phytonutrients in *Angelica keiskei*	Li, L.; Aldini, G.; Carini, M.; Chen, C. Y. O.; Chun, H.; Cho, S.; Park, K.; Correa, C. R.; Russell, R. M.; Blumberg, J. B.; Yeum, K.	*Food Chem.* 115 (1), 227–232	2009 Jul
Vitamin A equivalence of the β-carotene in β-carotene-biofortified maize porridge consumed by women	Li, S.; Nugroho, A.; Rocheford, T.; White, W. S.	*Am. J. Clin. Nutr. 92 (5),* 1105–1112	2010 Nov
Phase IIa chemoprevention trial of green tea polyphenols in high-risk individuals of liver cancer: modulation of urinary excretion of green tea polyphenols and 8-hydroxydeoxyguanosine	Luo, H.; Tang, L.; Tang, M.; Billam, M.; Huang, T.; Yu, J.; Wei, Z.; Liang, Y.; Wang, K.; Zhang, Z. Q.; Zhang, L.; Wang, J. S.	*Carcinogenesis 27 (2),* 262–268	2006 Feb
Determination of four water-soluble compounds in *Salvia miltiorrhiza Bunge* by high-performance liquid chromatography with a coulometric electrode array system	Ma, L.; Zhang, X.; Guo, H.; Gan, Y.	*J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 833* (2), 260–263	2006 Apr 3
Effect of green tea powder (*Camellia sinensis L.* cv. Benifuuki) particle size on O-methylated EGCG absorption in rats, The Kakegawa Study	Maeda-Yamamoto, M.; Ema, K.; Tokuda, Y.; Monobe, M.; Tachibana, H.; Sameshima, Y.; Kuriyama, S.	*Cytotechnology 63 (2),* 171–179	2011 Mar
Supplementation of a γ-tocopherol-rich mixture of tocopherols in healthy men protects against vascular endothelial dysfunction induced by postprandial hyperglycemia	Mah, E.; Noh, S. K.; Ballard, K. D.; Park, H. J.; Volek, J. S.; Bruno, R. S.	*J. Nutr. Biochem. 24 (1),* 196–203	2013 Jan

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Mediterranean diet reduces endothelial damage and improves the regenerative capacity of endothelium	Marin, C.; Ramirez, R.; Delgado-Lista, J.; Yubero-Serrano, E. M.; Perez-Martinez, P.; Carracedo, J.; Garcia-Rios, A.; Rodriguez. F.; Gutierrez-Mariscal, F. M.; Gomez, P.; Perez-Jimenez, F.; Lopez-Miranda, J.	*Am. J. Clin. Nutr. 93 (2),* 267–274	2011 Feb
Photodiode array (PDA) and other detection methods in HPLC of plant metabolites	Markowski, W.; Waksmundzka-Hajnos, M.	Chapter 13 in *High Performance Liquid Chromatography in Phytochemical Analysis,* Chromatographic Science Series, Markowski, W., Sherma, J., Eds.; Taylor & Francis Group, LLC: Boca Raton, FL; 331–350	2010 Nov
Determination of water-soluble vitamins in infant milk and dietary supplement using a liquid chromatography on-line coupled to a corona-charged aerosol detector	Márquez-Sillero, I.; Cárdenas, S.; Valcárcel, M.	*J. Chromatogr., A. 1313C,* 253–258	2013 Oct 25
Sensitive high-performance liquid chromatographic method using coulometric electrode array detection for measurement of phytoestrogens in dried blood spots	Melby, M. K.; Watanabe, S.; Whitten, P. L.; Worthman, C. M.	*J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 826 (1–2),* 81–90	2005 Nov 5
Phenolic acids from beer are absorbed and extensively metabolized in humans	Nardini, M.; Natella, F.; Scaccini, C.; Ghiselli, A.	*J. Nutr. Biochem. 17 (1),* 14–22	2006 Jan
High-performance liquid chromatography analysis of plant saponins: An update 2005-2010	Negi, J. S.; Singh, P.; Pant, G. J.; Rawat, M. S.	*Pharmacogn. Rev. 5 (10),* 155–158	2011 Jul
Physicochemical effect of pH and antioxidants on mono- and triglutamate forms of 5-methyltetrahydrofolate, and evaluation of vitamin stability in human gastric juice: Implications for folate bioavailability	Ng, X.; Lucock, M.; Veysey, M.	*Food Chem. 106 (1),* 200–210	2008 Jan
Practical preparation of lacto-*N*-biose I, a candidate for the bifidus factor in human milk	Nishimoto, M.; Kitaoka, M.	*Biosci., Biotechnol., Biochem. 71 (8),* 2101-2104	2007 Aug
Hydrophilic interaction liquid chromatography—charged aerosol detection as a straightforward solution for simultaneous analysis of ascorbic acid and dehydroascorbic acid	Nováková, L.; Solichová, D.; Solich, P.	*J. Chromatogr., A. 1216 (21),* 4574–4581	2009 May 22

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No effect on adenoma formation in Min mice after moderate amount of flaxseed	Oikarinen, S.; Heinonen, S. M.; Nurmi, T.; Adlercreutz, H.; Mutanen, M.	*Eur. J. Nutr. 44 (5),* 273–280	2005 Aug
Measurement of isoflavones using liquid chromatography with multi-channel coulometric electrochemical detection	Ouchi, K.; Gamache, P.; Acworth, I.; Watanabe, S.	*BioFactors. 22 (1–4),* 353–356	2004
Quantitation of clovamide-type phenylpropenoic acid amides in cells and plasma using high-performance liquid chromatography with a coulometric electrochemical detector	Park, J. B.	*J. Agric. Food Chem. 53 (21),* 8135–8140	2005 Oct 19
Synthesis, HPLC measurement and bioavailability of the phenolic amide amkamide	Park, J. B.	*J. Chromatogr. Sci.* [Epub ahead of print]	2013 May 27
Synthesis of safflomide and its HPLC measurement in mouse plasma after oral administration	Park, J. B.; Chen, P.	*J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* 852 (1–2), 398–402	2007 Jun 1
Determination of lignans in human plasma by liquid chromatography with coulometric electrode array detection	Peñalvo, J. L.; Nurmi, T.; Haajanen, K.; Al-Maharik, N.; Botting, N.; Adlercreutz, H.	*Anal. Biochem. 332 (2),* 384–393	2004 Sep 15
Supercritical antisolvent fractionation of lignans from the ethanol extract of flaxseed	Perretti, G.; Virgili, C.; Troilo, A.; Marconi, O.; Regnicoli, G. F.; Fantozzi, P.	*J. Supercrit. Fluids 75,* 94–100	2013 Mar
Analysis of flavonoids in honey by HPLC coupled with coulometric electrode array detection and electrospray ionization mass spectrometry	Petrus, K.; Schwartz, H.; Sontag, G.	*Anal. Bioanal. Chem. 400 (8),* 2555–2563	2011 Jun
High-dose supplementation with natural α-tocopherol does neither alter the pharmacodynamics of atorvastatin nor its phase I metabolism in guinea pigs	Podszun, M. C.; Grebenstein, N.; Hofmann, U.; Frank, J.	*Toxicol. Appl. Pharmacol. 266 (3),* 452–458	2013 Feb 1
Application of high-performance liquid chromatography with charged aerosol detection for universal quantitation of undeclared phosphodiesterase-5 inhibitors in herbal dietary supplements	Poplawska, M.; Blazewicz, A.; Bukowinska, K.; Fijalek, Z.	*J. Pharm. Biomed. Anal. 84,* 232–243	2013 Oct
Isolation and analysis of ginseng: advances and challenges	Qi, L.; Wang, C.; Yuan, C.	*Nat. Prod. Rep. 28 (3),* 467–495	2011 Mar
Folate analysis in complex food matrices: Use of a recombinant Arabidopsis γ-glutamyl hydrolase for folate deglutamylation	Ramos-Parra, P. A.; Urrea-López, R.; Díaz de la Garza, R. I.	*Food Res. Int. 54 (1),* 177–185	2013 Nov
Optimisation of gradient HPLC analysis of phenolic compounds and flavonoids in beer using a coularray detector	Rehová, L.; Skeríková, V.; Jandera, P.	*J. Sep. Sci. 27 (15–16),* 1345–1359	2004 Nov
Chiral separation of (+)/(-)-catechin from sulfated and glucuronidated metabolites in human plasma after cocoa consumption	Ritter, C.; Zimmermann, B. F.; Galensa, R.	*Anal. Bioanal. Chem. 397 (2),* 723–730	2010 May

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Analysis of alkylresorcinols in cereal grains and products using ultrahigh-pressure liquid chromatography with fluorescence, ultraviolet, and CoulArray electrochemical detection	Ross, A. B.	*J. Agric. Food Chem. 60 (36),* 8954–8962	2012 Sep 12
Rapid and sensitive analysis of alkylresorcinols from cereal grains and products using HPLC-CoulArray-based electrochemical detection	Ross, A. B.; Kochhar, S.	*J. Agric. Food Chem. 57 (12),* 5187–5193	2009 Jun 24
Analysis of soy isoflavone plasma levels using HPLC with coulometric detection in postmenopausal women	Saracino, M. A.; Raggi, M. A.	*J. Pharm. Biomed. Anal. 53 (3),* 682–687	2010 Nov 2
A biosynthetic pathway for BE-7585A, a 2-thiosugar-containing angucycline-type natural product	Sasaki, E.; Ogasawara, Y.; Liu, H. W.	*J. Am. Chem. Soc. 132 (21),* 7405–7417	2010 Jun 2
The senescence-accelerated mouse-prone 8 is not a suitable model for the investigation of cardiac inflammation and oxidative stress and their modulation by dietary phytochemicals	Schiborr, C.; Schwamm, D.; Kocher, A.; Rimbach, G.; Eckert, G. P.; Frank, J.	*Pharmacol. Res. 74,* 113–120	2013 Aug
Comprehensive impurity profiling of nutritional infusion solutions by multidimensional off-line reversed-phase liquid chromatography × hydrophilic interaction chromatography-ion trap mass-spectrometry and charged aerosol detection with universal calibration	Schiesel, S.; Lämmerhofer, M.; Lindner, W.	*J. Chromatogr., A. 1259,* 100–10	2012 Oct 12
The effect of α-tocopherol supplementation on training-induced elevation of S100B protein in sera of basketball players	Schulpis, K. H.; Moukas, M.; Parthimos, T.; Tsakiris, T.; Parthimos, N.; Tsakiris, S.	*Clin. Biochem. 40 (12),* 900–906	2007 Aug
Determination of secoisolariciresinol, lariciresinol and isolariciresinol in plant foods by high performance liquid chromatography coupled with coulometric electrode array detection	Schwartz, H.; Sontag, G.	*J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* 838 (2), 78–85	2006 Jul 11
Assessment of probiotic strains ability to reduce the bioaccessibility of aflatoxin M 1 in artificially contaminated milk using an in vitro digestive model	Serrano-Niño, J. C.; Cavazos-Garduño, A.; Hernandez-Mendoza, A.; Applegate, B.; Ferruzzi, M. G.; San Martin-González, M. F.; García, H. S.	*Food Control 31 (1),* 202–207	2013 May
Intestinal uptake of quercetin-3-glucoside in rats involves hydrolysis by lactase phlorizin hydrolase	Sesink, A. L.; Arts, I. C.; Faassen-Peters, M.; Hollman, P. C.	*J. Nutr. 133 (3),* 773–776	2003 Mar
Quercetin glucuronides but not glucosides are present in human plasma after consumption of quercetin-3-glucoside or quercetin-4'-glucoside	Sesink, A. L.; O'Leary, K. A.; Hollman, P. C.	*J. Nutr. 131 (7),* 1938–1941	2001 Jul
Co-administration of quercetin and catechin in rats alters their absorption but not their metabolism	Silberberg, M.; Morand, C.; Manach, C.; Scalbert, A.; Remesy, C.	*Life Sci. 77 (25),* 3156–3167	2005 Nov 4
Nutritional status is altered in the self-neglecting elderly	Smith, S. M.; Mathews Oliver, S. A.; Zwart, S. R.; Kala, G.; Kelly, P. A.; Goodwin, J. S.; Dyer, C. B.	*J. Nutr. 136 (10),* 2534–2541	2006 Oct

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Binding of heterocyclic aromatic amines by lactic acid bacteria: results of a comprehensive screening trial	Stidl, R.; Sontag, G.; Koller, V.; Knasmüller, S.	*Mol. Nutr. Food Res. 52 (3),* 322–329	2008 Mar
Direct separation and detection of biogenic amines by ion-pair liquid chromatography with chemiluminescent nitrogen detector	Sun, J.; Guo, H. X.; Semin, D.; Cheetham, J.	*J. Chromatogr., A. 1218 (29),* 4689–4697	2011 Jul 22
Rapid purification method for fumonisin B1 using centrifugal partition chromatography	Szekeres, A.; Lorántfy, L.; Bencsik, O.; Kecskeméti, A.; Szécsi, Á.; Mesterházy, Á.; Vágvölgyi, C.	*Food Addit. Contam. 30 (1),* 147–155	2013
Determination of coenzyme Q10 in over-the-counter dietary supplements by high-performance liquid chromatography with coulometric detection	Tang, P. H.	*J. AOAC Int. 89 (1),* 35–39	2006 Jan–Feb
α-Tocopherol supplementation restores the reduction of erythrocyte glucose-6-phosphate dehydrogenase activity induced by forced training	Tsakiris, S.; Reclos, G. J.; Parthimos, T.; Tsakiris, T.; Parthimos, N.; Schulpis, K. H.	*Pharmacol. Res. 54 (5),* 373–379	2006 Nov
Tissue distribution of isoflavones in ewes after consumption of red clover silage	Urpi-Sarda, M.; Morand, C.; Besson, C.; Kraft, G.; Viala, D.; Scalbert, A.; Besle, J. M.; Manach, C.	*Arch. Biochem. Biophys. 476 (2),* 205–210	2008 Aug 15
Performance evaluation of charged aerosol and evaporative light scattering detection for the determination of ginsenosides by LC	Wang, L.; He, W. S.; Yan, H. X.; Jiang, Y.; Bi, K. S.; Tu, P. F.	*Chromatographia 70 (3–4),* 603–608	2009 Aug
Catechins are bioavailable in men and women drinking black tea throughout the day	Warden, B. A.; Smith, L. S.; Beecher, G. R.; Balentine, D. A.; Clevidence, B. A.	*J. Nutr. 131 (6),* 1731–1737	2001 Jun
Identification and quantification of polyphenol phytoestrogens in foods and human biological fluids	Wilkinson, A. P.; Wähälä, K.; Williamson, G.	*J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 777 (1–2),* 93–109	2002 Sep 25
Bioavailability and pharmacokinetics of caffeoylquinic acids and flavonoids after oral administration of Artichoke leaf extracts in humans	Wittemer, S. M.; Ploch, M.; Windeck, T.; Müller, S. C.; Drewelow, B.; Derendorf, H.; Veit, M.	*Phytomedicine 12 (1–2),* 28–38	2005 Jan
Validated method for the determination of six metabolites derived from artichoke leaf extract in human plasma by high-performance liquid chromatography-coulometric-array detection	Wittemer, S. M.; Veit, M.	*J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 793 (2),* 367–375	2003 Aug 15
HPLC in natural product analysis: The detection issue	Wolfender, J. L.	*Planta Med. 75 (07),* 719–734	2009 Jun
Simultaneous determination of isoflavones and bisphenol A in rat serum by high-performance liquid chromatography coupled with coulometric array detection	Yasuda, S.; Wu, P. S.; Hattori, E.; Tachibana, H.; Yamada, K.	*Biosci., Biotechnol., Biochem. 68 (1),* 51–58	2004 Jan
Impurities from polypropylene microcentrifuge tubes as a potential source of interference in simultaneous analysis of multiple lipid-soluble antioxidants by HPLC with electrochemical detection	Yen, H. C.; Hsu, Y. T.	*Clin. Chem. Lab. Med. 42 (4),* 390–395	2004 Apr

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Simultaneous determination of triterpenoid saponins from *pulsatilla koreana* using high performance liquid chromatography coupled with a charged aerosol detector (HPLC-CAD)	Yeom, H.; Suh, J. H.; Youm, J. R.; Han, S. B.	*Bull. Korean Chem. Soc. 31 (5),* 1159–1164	2010
DPPH radical scavenging activities of 31 flavonoids and phenolic acids and 10 extracts of Chinese materia medica	Yuan, Y.; Chen, C.; Yang, B.; Kusu, F.; Kotani, A.	*Zhongguo Zhongyao Zazhi 34 (13),* 1695–1700	2009 Jul
Determination of residual clenbuterol in pork meat and liver by HPLC with electrochemical detection	Zhang, X. Z.; Gan, Y. R.; Zhao, F. N.	*Yaoxue Xuebao 39 (4),* 276–280	2004 Apr
Identification of equol producers in a Japanese population by high-performance liquid chromatography with coulometric array for determining serum isoflavones	Zhao, J. H.; Sun, S. J.; Arao, Y.; Oguma, E.; Yamada, K.; Horiguchi, H.; Kayama, F.	*Phytomedicine 13 (5),* 304–309	2006 May
Simultaneous sampling of volatile and non-volatile analytes in beer for fast fingerprinting by extractive electrospray ionization mass spectrometry	Zhu, L.; Hu, Z.; Gamez, G.; Law, W. S.; Chen, H.; Yang, S.; Chingin, K.; Balabin, R. M.; Wang, R.; Zhang, T.; Zenobi, R.	*Anal. Bioanal. Chem. 398 (1),* 405–413	2010 Sep
Comparison of various easy-to-use procedures for extraction of phenols from apricot fruits	Zitka, O.; Sochor, J.; Rop, O.; Skalickova, S.; Sobrova, P.; Zehnalek, J.; Beklova, M.; Krska, B.; Adam, V.; Kizek, R.	*Molecules 16 (4),* 2914–2936	2011 Apr 4

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Evaporative light scattering and charged aerosol detector.	Chaminade, P.	Chapter 5. In *Hyphenated and Alternative Methods of Detection in Chromatography,* Chromatographic Science Series; Shalliker, A., Ed.; Taylor & Francis Group, LLC: Boca Raton, FL.; 145–160	2012
Simple and efficient profiling of phospholipids in phospholipase D-modified soy lecithin by HPLC with charged aerosol detection	Damnjanovic´, J.; Nakano, H.; Iwasaki, Y.	*J. Am. Oil Chem. Soc. 90 (7),* 951–957	2013 Jul
Discriminating olive and non-olive oils using HPLC-CAD and chemometrics	de la Mata-Espinosa, P.; Bosque-Sendra, J. M.; Bro, R.; Cuadros-Rodríguez, L.	*Anal. Bioanal. Chem. 399 (6),* 2083–2092	2011 Feb
Olive oil quantification of edible vegetable oil blends using triacylglycerols chromatographic fingerprints and chemometric tools	de la Mata-Espinosa, P.; Bosque-Sendra, J. M.; Bro, R.; Cuadros-Rodríguez, L.	*Talanta 85 (1),* 177–182	2011 Jul 15
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Quantification of pegylated phospholipids decorating polymeric microcapsules of perfluorooctyl bromide by reverse phase HPLC with a charged aerosol detector	Díaz-López, R.; Libong, D.; Tsapis, N.; Fattal, E.; Chaminade, P.	*J. Pharm. Biomed. Anal. 48 (3),* 702–707	2008 Nov 4
Squalene emulsions for parenteral vaccine and drug delivery	Fox, C. B.	*Molecules 14 (9),* 3286–3312	2009 Sep 1
Interactions between parenteral lipid emulsions and container surfaces	Gonyon, T.; Tomaso, A.; Kotha, P.; Owen, H.; Patel, D.; Carter, P.; Cronin, J.; Green, J.	*PDA J. Pharm. Sci. and Tech. 67 (3),* 247–254	2013 May–Jun
Composition analysis of positional isomers of phosphatidylinositol by high-performance liquid chromatography	Iwasaki, Y.; Masayama, A.; Mori, A.; Ikeda, C.; Nakano, H.	*J. Chromatogr., A 1216 (32),* 6077–6080	2009 Aug 7
Determination of phospholipid and its degradation products in liposomes for injection by HPLC-charged aerosol detection (CAD)	Jiang, Q.; Yang, R.; Mei, X.	*Chinese Pharmaceutical Journal (Zhongguo Yaoxue Zazhi, Beijing, China) 42 (23),* 1794–1796	2007

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A new liquid chromatography method with charge aerosol detector (CAD) for the determination of phospholipid classes. Application to milk phospholipids	Kieibowicz, G.; Micek, P.; Wawrzenczyk, C.	*Talanta 105,* 28–33	2013 Feb 15
An LC method for the analysis of phosphatidylcholine hydrolysis products and its application to the monitoring of the acyl migration process	Kiełbowicz, G.; Smuga, D.; Gładkowski, W.; Chojnacka, A.; Wawrzeńczyk, C.	*Talanta 94,* 22–29	2012 May 30
Separation of acylglycerols, FAME and FFA in biodiesel by size exclusion chromatography	Kittirattanapiboon, K.; Krisnangkurá, K.	*Eur. J. Lipid Sci. Technol. 110 (5),* 422–427	2008 Mar 17
Quantitation of triacylglycerols from plant oils using charged aerosol detection with gradient compensation	Lísa, M.; Lynen, F.; Holčapek, M.; Sandra, P.	*J. Chromatogr., A. 1176 (1–2),* 135–142	2007 Dec 28
Quantitative study of the stratum corneum lipid classes by normal phase liquid chromatography: comparison between two universal detectors	Merle, C.; Laugel, C.; Chaminade, P.; Baillet-Guffroy, A.	*J. Liq. Chromatogr. Relat. Technol. 33,* 629–644	2010 Mar
The analysis of lipids via HPLC with a charged aerosol detector	Moreau, R. A.	*Lipids 41 (7),* 727–34	2006 Jul
Lipid analysis via HPLC with a charged aerosol detector	Moreau, R. A.	*Lipid Technol. 21* (8–9), 191–194	2009 Oct 23
Extraction and analysis of food lipids	Moreau, R. A.; Winkler-Moser, J. K.	Chapter 6 in *Methods of Analysis of Food Components and Additives,* Second Edition; Ötles, S., Ed.; Taylor & Francis Group, LLC: Boca Raton, FL.; 115–134	2011 Nov
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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.	*J. Biol. Chem. 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.	*J. Lipid Res. 48 (1),* 242–251	2007 Jan

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The use of charged aerosol detection with HPLC for the measurement of lipids	Plante, M.; Bailey, B.; Acworth, I.	*Methods Mol. Biol.* (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.	*J. Chromatogr., A. 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.	*Talanta. 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.	*J. Chromatogr., A. 1216 (5),* 781–786	2009 Jan 30
Determination of intralumenal individual bile acids by HPLC with charged aerosol detection	Vertzoni, M.; Archontaki, H.; Reppas, C.	*J. Lipid Res. 49 (12),* 2690–2695	2008 Dec
Neurolipids and the use of a charged aerosol detector	Waraska, J.; Acworth, I.	*Am. Biotechnol. Lab. 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 Skunky 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 *Flos Chrysanthema indici* 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 Detections
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 *Caulis lonicerae* Using a Phenyl-1 Column
AN 1008	UV	Determination of Nitidine Chloride, Toddalolactone, and Chelerythrine Chloride by HPLC

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Technical Collateral: HPLC and UHPLC Methods

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 Detections
CAN 106	UV	Determination of the Punicalagins Found in Pomegranate by High Performance Liquid Chromatography
CAN 111	CAD	Determination of Triterpenes in *Centella asiatica* (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 *Hoodia* 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 Postcol-umn 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-µg/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-μg/L Bromate in Municipal Waters Using Preconcentration with Two-Dimensional Ion Chromatography and Suppressed Conductivity Detection
AN1 88	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.	*J. Food Sci. 79 (2),* C138–46	2014 Feb
Multiresidue 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.	*J. Agric. Food Chem. 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.	*J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 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.	*J. Agric. Food Chem. 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.;	*J. Chromatogr., A. 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.	*Nat. Prod. Commun. 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.	*J. Food Protection 65 (1),* 161–166	2002
Pressurized liquid extraction of medicinal plants	Benthin, B.; Danz, H.; Hamburger, M.	*J. Chromatogr., A. 837 (1-2),* 211–9	1999 Apr
Comparison of the chemical composition of extracts from *Scutellaria lateriflora* 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.	*J. Agric. Food Chem. 53 (8),* 3076–80	2005 Apr
Polybrominated diphenyl ethers (PBDEs) in Mediterranean mussels (*Mytilus galloprovincialis*) from selected Apulia coastal sites evaluated by GC-HRMS	Bianco, G.; Novario, G.; Anzilotta, G.; Palma, A.; Mangone, A.; Cataldi, T. R.	*J. Mass Spectrom. 45 (9),* 1046–55	2010 Sep
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Pressurized liquid extraction of lipids for the determination of oxysterols in egg-containing food	Boselli, E.; Velazco, V.; Caboni, M. F.; Lercker, G.	*J. Chromatogr., A. 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.	*J. Sep. Sci. 24 (10-11),* 865–873	2001 Nov

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Influence of extraction methodologies on the analysis of five major volatile aromatic compounds of citronella grass (*Cymbopogon nardus*) and lemongrass (*Cymbopogon citratus*) grown in Thailand	Chanthai, S.; Prachakoll, S.; Ruangviriyachai, C.; Luthria, D. L.	*J. AOAC Int. 95 (3),* 763–72	2012 May-Jun
Accelerated solvent extraction of vitamin K_1 in medical foods in conjunction with matrix solid-phase dispersion	Chase, G. W.; Thompson, B.	*J. AOAC Int. 83 (2),* 407–10	2000
Development of a liquid chromatography-tandem mass spectrometry with pressurized liquid extraction method for the determination of benzimidazole residues in edible tissues	Chen, D.; Tao, Y.; Zhang, H.; Pan, Y.; Liu, Z.; Huang, L.; Wang, Y.; Peng, D.; Wang, X.; Dai, M; Yuan, Z.	*J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 879 (19),* 1659–67	2011 Jun
Determination of 88 pesticide residues in tea using gas chromatography-tandem mass spectrometry	Chen, H.; Liu, X.; Wang, Q.; Jiang, Y.	*Se Pu. 29 (5),* 409–16	2011 May
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Evaluation of analytical methods for determining pesticides in baby foods and adult duplicate-diet samples	Chuang, J. C.; Hart, K.; Chang, J. S.; Boman, L. E.; Van Emon, J. M.; Reed, A. W.	*Anal. Chim. Acta. 444 (1),* 87–95	2001 Oct
Comparison of extraction techniques and modeling of accelerated solvent extraction for the authentication of natural vanilla flavors	Cicchetti, E.; Chaintreau, A..	*J. Sep. Sci. 32 (11),* 1957–64	2009 Jun
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Development and comparison of two multiresidue methods for the analysis of 17 mycotoxins in cereals by liquid chromatography electrospray ionization tandem mass spectrometry	Desmarchelier, A.; Oberson, J. M.; Tella, P.; Gremaud, E.; Seefelder, W.; Mottier, P.	*J. Agric. Food Chem. 58 (13),* 7510–9	2010 Jul
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Pressurized liquid extraction-capillary electrophoresis-mass spectrometry for the analysis of polar antioxidants in rosemary extracts	Herrero, M.; Arráez-Román, D.; Segura A.; Kenndler, E.; Gius, B.; Raggid, M. A.; Ibáñez, E.; Cifuentes, A.	*J. Chromatogr., A 1084 (1-2),* 54–62.	2005 Aug
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Application of response surface methodology to optimize pressurized liquid extraction of antioxidant compounds from sage (*Salvia officinalis* L.), basil (*Ocimum basilicum* L.) and thyme (*Thymus vulgaris* L.)	Hossain, M. B.; Brunton, N. P.; Martin-Diana, A. B.; Barry-Ryan, C.	*Food Funct. 1(3),* 269–77	2010 Dec
A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants	Huie, C. W.	*Anal. Bioanal. Chem. 373 (1-2),* 23–30.	2002 May
Polychlorinated dioxins, furans, and biphenyls, and polybrominated diphenyl ethers in a U.S. meat market basket and estimates of dietary intake	Huwe, J. K.; Larsen, G. L.	*Environ. Sci. Technol. 39 (15),* 5606–5611	2005
Study of the effect of sample preparation and cooking on the selenium speciation of selenized potatoes by HPLC with ICP-MS and electrospray ionization MS/MS	Infante, H. G.; Borrego, A. A.; Peachey, E.; Hearn, R.; O'Connor, G.; Barrera, T G.; Ariza, J. L.	*J. Agric. Food Chem. 57(1),* 38–45.	2009 Jan
Pentacyclic triterpene distribution in various plants – rich sources for a new group of multi-potent plant extracts	Jäger, S.; Trojan, H.; Kopp, T.; Laszczyk, M. N.; Scheffler, A.	*Molecules. 14 (6),* 2016–31.	2009 Jun
Comprehensive multiresidue method for the simultaneous determination of 74 pesticides and metabolites in traditional Chinese herbal medicines by accelerated solvent extraction with high-performance liquid chromatography/tandem mass spectrometry	Jia, Z.; Mao, X.; Chen, K.; Wang, K.; Ji S.	*J. AOAC Int.; 93(5),* 1570–88.	2010 Sep-Oct
Gas chromatography-mass spectrometry (GC-MS) method for the determination of 16 European priority polycyclic aromatic hydrocarbons in smoked meat products and edible oils	Jira, W.; Ziegenhals, K.; Speer, K.	*Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 25 (6),* 704–13.	2008 Jun
Assessing pressurized liquid extraction for the high-throughput extraction of marine-sponge-derived natural products	Johnson, T. A.; Morgan, M. V.; Aratow, N. A.; Estee, S. A.; Sashidhara, K. V.; Loveridge, S. T.; Segraves, N L.; Crews, P.	*J. Nat. Prod. 73 (3),* 359–64.	2010 Mar
Lipophilic stinging nettle extracts possess potent anti-inflammatory activity, are not cytotoxic and may be superior to traditional tinctures for treating inflammatory disorders	Johnson, T. A.; Sohn, J.; Inman, W. D.; Bjeldanes, L. F.; Rayburn, K.	*Phytomedicine 20(2),* 143–7.	2013 Jan
Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin	Ju Z. Y.; Howard, L. R.	*J. Agric. Food Chem. 51 (18),* 5207–13.	2003 Aug

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Accelerated solvent extraction of ochratoxin A from rice samples	Juan, C.; González, L.; Soriano, J. M.; Moltó, J. C.; Mañes, J.	*J. Agric. Food Chem. 53 (24),* 9348–9351	2005
Accelerated solvent extraction of paclitaxel and related compounds from the bark of *Taxus cuspidate*	Kawamura, F.; Kikuchi, Y.; Ohira, T.; Yatagai, M.	*J. Nat. Prod. 62 (2),* 244–7.	1999 Feb
Determination of polybromodiphenyl ethers (PBDEs) in milk cream by gas chromatography-mass spectrometry	Kinani, S.; Bouchonnet, S.; Abjean, J.; Campargue, C.	*Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 25 (8),* 1007–14	2008 Aug
Determination of isoflavones in soy bits by fast column high-performance liquid chromatography coupled with UV–visible diode-array detection	Klejdus, B.; Mikelová, R.; Petrlová, J.; Potešil, D.; Adam, V.; Stiborová, J.; Hodek, P.; Vacek, J.; Kizek, R.; Kubán, V.	*J. Chromatogr., A. 1084 (1–2), 19,* 71–79	2005 Aug
Accelerated solvent extraction of lignin from *Aleurites moluccana* (candlenut) nutshells	Klein, A. P.; Beach, E. S.; Emerson, J. W.; Zimmerman, J. B.	*J. Agric. Food Chem. 58 (18),* 10045–8	2010 Sep
Application of TLC method with video scanning in estimation of daily dietary intake of specific flavonoids – preliminary studies	Koch, W.; Kukuła-Koch, W.; Marzec, Z.; Marc, D.	*Acta Pol. Pharm. 70 (4),* 611–20	2013 Jul-Aug
Evaluation of a fibrous cellulose drying agent in supercritical fluid extraction and pressurized liquid extraction of diverse pesticides	Lehotay, S. J.; Lee, C. H.	*J. Chromatogr., A.785 (1-2),* 313–27	1997 Oct
Application of accelerated solvent extraction to the investigation of saikosaponins from the roots of *Bupleurum falcatum*	Li, W.; Liu, Z.; Wang, Z.; Chen, L.; Sun, Y.; Hou, J.; Zheng, Y.	*J. Sep. Sci. 33 (12),* 1870–6	2010 Jun
Applicability of accelerated solvent extraction for synthetic colorants analysis in meat products with ultrahigh performance liquid chromatography-photodiode array detection	Liao, Q. G.; Li ,W. H.; Luo, LG.	*Anal. Chim. Acta. 716,* 128–32	2012 Feb
Extraction, isolation, and purification of analytes from samples of marine origin – a multivariate task	Liguori, L.; Bjørsvik, H. R.	*J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 910,* 46–53	2012 Dec
Investigation on levels of polybrominated diphenyl ethers in retail fish and egg products in Shenzhen	Liu, B.; Zhang, L. S.; Zhang, J. Q.; Jiang, Y. S.; Zhou, J.; Huang, H. Y.	*Zhonghua Yu Fang Yi Xue Za Zhi. 45 (12),* 1068–72	2011 Dec
Characterization of secondary volatile profiles in *Nigella sativa* seeds from two different origins using accelerated solvent extraction and gas chromatography-mass spectrometry	Liu, X.; Abd El-Aty, A. M.; Cho, S. K.; Yang, A.; Park, J. H.; Shim, J. H.	*Biomed. Chromatogr. 26 (10),* 1157–62	2012 Oct
Accelerated solvent extraction of monacolin K from red yeast rice and purification by high-speed counter-current chromatography	Liu, Y.; Guo, X.; Duan, W.; Wang, X.; Du, J.	*J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 878 (28),* 2881–5	2010 Oct
Multiresidue determination of organophosphorus pesticides in ginkgo leaves by accelerated solvent extraction and gas chromatography with flame photometric detection	Lu, Y.; Yi, X.	*J. AOAC Int. 88 (3),* 729–735	2005

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Influence of sample preparation on assay of phenolic acids from eggplant	Luthria, DL.; Mukhopadhyay, S.	*J. Agric. Food Chem. 54 (1),* 41–47	2006
Pressurised solvent extraction for organotin speciation in vegetable matrices	Marcic, C.; Lespe,s G.; Potin-Gautier, M.	*Anal. Bioanal. Chem. 382 (7),* 1574–83	2005 Aug
Comparison of different methods for the determination of the oil content in oilseeds	Matthäus, B.; Brühl, L.	*J. AOCS 78* 95–102.	2001 Jan
A comparison of automated and traditional methods for the extraction of arsenicals from fish	McKiernan, J. W.; Creed, J. T.; Brockhoff, C. A.; Caruso, J. A.; Lorenzana, R. M.	*J. Anal. At. Spectrom. 14,* 607–613	1999
Subcritical solvent extraction of anthocyanins from dried red grape pomace	Monrad, J. K.; Howard, L. R.; King, J.;, Srinivas, K.; Mauromoustakos, A.	*J. Agric. Food Chem. 58 (5),* 2862–8	2010 Mar
Subcritical solvent extraction of procyanidins from dried red grape pomace	Monrad, J. K.; Howard, L. R.; King, J. W.; Srinivas, K.; Mauromoustakos, A.	*J. Agric. Food Chem.58 (7),* 4014–21	2010 Apr
Pressurized liquid extraction of polar and nonpolar lipids in corn and oats with hexane, methylene chloride, isopropanol, and ethanol	Moreau, R. A.; Powell, M. J.; Singh, V.	*J. Oil Fat Industr. 80 (11),* 1063–1067	2003 Jan
Accelerated solvent extraction for natural products isolation	Mottaleb, M. A.; Sarker, S. D.	*Methods Mol. Biol. 864,* 75–87	2012
Optimization of extraction process for phenolic acids from black cohosh (*Cimicifuga racemosa*) by pressurized liquid extraction	Mukhopadhyay, S,.;Luthria, D. L,.;Robbins, R. J.	*J. Sci. Food Agric. 86 (1),* 156–162, 15	2006 Jan
Anxiolytic activity of a supercritical carbon dioxide extract of *Souroubea sympetala* (Marcgraviaceae)	Mullally, M.; Kramp, K.; Cayer, C.; Saleem, A.; Ahmed, F, McRae, C.; Baker, J.; Goulah, A.; Otorola, M.; Sanchez, P.; Garcia, M.; Poveda, L.; Merali, Z.; Durst, T.; Trudeau, V. L.; Arnason, J. T.	*Phytother. Res. 25 (2),* 264–70	2011 Feb
On-line clean-up of pressurized liquid extracts for the determination of polychlorinated biphenyls in feedingstuffs and food matrices using gas chromatography–mass spectrometry	Müller, A.; Björklund, E.; von Holst, C.	*J. Chromatogr., A. 925 (1–2),* 197–205	2001 Aug
Analysis of multiple herbicides in soybeans using pressurized liquid extraction and capillary electrophoresis	Nemoto, S.; Lehotay, S. J.	*J. Agric. Food Chem.; 46 (6),* 2190–2199	1998
Comparison of sample preparation methods, validation of an UPLC-MS/MS procedure for the quantification of tetrodotoxin present in marine gastropods and analysis of pufferfish	Nzoughet, J. K.; Campbell, K.; Barnes, P.; Cooper, K. M.; Chevallier, O. P, Elliott, C. T.	*Food Chem. 15; 136 (3-4),* 1584–9	2013 Feb
Multiresidue analysis of pesticides in vegetables and fruits using two-layered column with graphitized carbon and water absorbent polymer	Obana, H.; Akutsu, K.; Okihashi, M.; Hori, S.	*The Analyst 123,* 711–714	1998
Analysis of 2-alkylcyclobutanones with accelerated solvent extraction to detect irradiated meat and fish	Obana, H.; Furuta, M.; Tanaka, Y.	*J. Agric. Food Chem. 53 (17),* 6603–8	2005 Aug

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Title	Authors	Publication	Publication Date
Determination of organophosphorus pesticides in foods using an accelerated solvent extraction system	Obana, H.; Kikuchi, K.; Okihashi, M.; Hori, S.	*Analyst 122 (3),* 217–20	1997 Mar
Pressurized hot water extraction of berberine, baicalein and glycyrrhizin in medicinal plants	Ong, E. S.; Shea Mei, L.	*Anal. Chim. Acta. 482 (1),* 81–89	2003 Apr
Pressurized liquid extraction of berberine and aristolochic acids in medicinal plants	Ong E. S.; Woo S. O.; Yong, Y. K.	*J. Chromatogr., A. 904 (1),* 57–6422	2000 Dec
Rapid determination of pesticide multiresidues in vegetables and fruits by accelerated solvent extraction coupled with online gel permeation chromatography-gas chromatography-mass spectrometry	Ouyang, Y.; Tang, H.; Wu, Y.; Li, G.	*Se Pu. 30(7),* 654–9	2012 Jul
Determination of zearalenone from wheat and corn by pressurized liquid extraction and liquid chromatography-electrospray mass spectrometry	Pallaroni, L.; von Holst, C.	*J. Chromatogr., A. 993,* 39–45	2003
Development of an extraction method for the determination of zearalenone in corn using less organic solvents	Pallaroni, L.; von Holst, C.	*J. Chromatogr., A. 5 1055 (1-2),* 247–9	2004 Nov
Stability of phenolic compounds during extraction with superheated solvents	Palma, M.; Piñeiro, Z.; Barroso, C. G.	*J. Chromatogr., A. 6 921 (2),* 169–74	2001 Jul
Extraction and analysis of trace amounts of cyclonite (RDX) and its nitroso-metabolites in animal liver tissue using gas chromatography with electron capture detection (GC-ECD)	Pan, X.; Zhang, B.; Cobb, G. P.	*Talanta 67 (4),* 816–23	2005 Oct
Simultaneous determination of 405 pesticide residues in grain by accelerated solvent extraction then gas chromatography-mass spectrometry or liquid chromatography-tandem mass spectrometry	Pang, G.; Liu, Y.; Fan, C.; Zhang, J.; Cao, Y.; Li, X.; Li, Z.; Wu, Y.; Guo, T.	*Anal. Bioanal. Chem. 384,* 1366–1408	2006 Mar
Automated sample preparation by pressurized liquid extraction-solid-phase extraction for the liquid chromatographic-mass spectrometric investigation of polyphenols in the brewing process	Papagiannopoulos, M.; Mellenthin, A.	*J. Chromatogr., A. 8 976 (1-2),* 345–8	2002 Nov
Online coupling of pressurized liquid extraction, solid-phase extraction and high-performance liquid chromatography for automated analysis of proanthocyanidins in malt	Papagiannopoulos, M.; Zimmermann, B.; Mellenthin, A.; Krappe, M.; Maio, G.; Galensa, R.	*J. Chromatogr., A. 7 958 (1-2),* 9–16	2002 Jun
Simultaneous determination of 13 quinolones from feeds using accelerated solvent extraction and liquid chromatography	Pecorelli, I.; Galarini, R.; Bibi, R.; Floridi, A. I.; Casciarri, E.; Floridi, A.	*Anal. Chim. Acta. 483 (1-2),* 81–89	2003 April
Comparison of soxhlet, ultrasound-assisted and pressurized liquid extraction of terpenes, fatty acids and Vitamin E from *Piper gaudichaudianum* Kunth	Péres, V. F.; Saffi, J.; Melecchi, M. I.; Abad, F. C.; de Assis Jacques, R.; Martinez, M. M.; Oliveira, E. C.; Caramão, E. B.	*J. Chromatogr., A. 1105 (1-2),* 115–8	2006 Feb
Pressurised fluid extraction (PFE) as an alternative general method for the determination of pesticide residues in rape seed	Pihlström, T.; Isaac, G.; Waldebäck, M.; Osterdahl, B. G.; Markides, K. E.	*Analyst 127 (4),* 554–9	2002 Apr
Determination of catechins by means of extraction with pressurized liquids	Piñeiro, Z.; Palma, M.; Barroso C. G.	*J. Chromatogr., A. 13 1026 (1-2),* 19–23.	2004 Feb

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An improved clean-up strategy for simultaneous analysis of polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and polychlorinated biphenyls (PCB) in fatty food samples	Pirard, C.; Focant, J. F.; De, P. E.	*Anal. Bioanal. Chem. 372 (2),* 373–81.	2002 Jan
Extraction of polar and hydrophobic pollutants using accelerated solvent extraction (ASE)	Pörschmann, J., Plugge, J.	*Fresen. J. Anal. Chem. 364 (7),* 643–645	1999
Quantification of the total amount of artemisinin in leaf samples by thin layer chromatography	Quennoz, M.; Bastian, C.; Simonnet, X.; Grogg, A. F.	*Chimia (Aarau) 64 (10),* 755–7.	2010
Determination of fat in dairy products using pressurized solvent extraction	Richardson, R. K.	*J. AOAC Int. 84 (5),* 1522–1533	2001
Influence of altitudinal variation on the content of phenolic compounds in wild populations of *Calluna vulgaris*, Sambucus nigra, and *Vaccinium myrtillus*	Rieger, G.; Müller, M.; Guttenberger, H.; Bucar, F.	*J. Agric. Food Chem. 56 (19),* 9080–6.	2008 Oct
Pressurized liquid extraction of isoflavones from soybeans	Rostagno, M. A.; Palma, M.; Barroso, C. G.	*Anal. Chim. Acta. 522 (2),* 169–177.	2004 Sep
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.	*J. Environ. Sci. Health, B 44 (7),* 706–16.	2009 Sep
Accelerated solvent extraction of lipids for determining the fatty acid composition of biological material	Schäfer, K.	*Anal. Chim. Acta. 358 (1),* 69–77	1998 Jan
HPLC analysis of kaempherol and quercetin derivatives isolated by different extraction techniques from plant matrix	Skalicka-Wozniak, K.; Szypowski, J.; Głowniak, K.	*J. AOAC Int. 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.	*Anal. Chim. Acta. 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.	*J. Chromatogr., A. 1237,* 1–23.	2012 May
Development of an accelerated solvent extraction, ultrasonic derivatisation 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.	*Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 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.	*Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 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.	*J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 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.	*Se Pu. 30 (1),* 14–20.	2012 Jan

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A consecutive preparation method based upon accelerated solvent extraction and high-speed counter-current chromatography for isolation of aesculin from *Cortex fraxinus*	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 *Sophora flavescens* 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 *Curcuma* 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 *Radix glycyrrhizae* 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.; Ishsan, 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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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.	*J. Chromatogr. Sci.*	2013 May
Response surface modeling and optimization of accelerated solvent extraction of four lignans from *fructus schisandrae*	Zhao, L. C.; He, Y, Deng.; X, Yang, G. L.; Li, W.; Liang, J.; Tang, Q. L.	*Molecules. 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.	*J. Sep. Sci. 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 *Hypericum perforatum* L	Zhang, Y.; Liu, C.; Yu, M.; Zhang, Z.; Qi, Y.; Wang, J.; Wu, G.; Li, S.; Yu, J.; Hu, Y.	*J. Chromatogr., A. 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.	*Se Pu. 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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