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LC that takes your productivity to new heights

HPLC - UV-Vis absorption and charged aerosol detection

Dietary supplements and botanical natural products applications notebook
Complex samples, powerful chromatographic analysis

ThermoFisher SCIENTIFIC

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Reliable analysis of dietary supplements and botanical natural products is vital in ensuring the consistent quality and safety of raw materials and end products. However, the nature of these materials can make their analysis particularly demanding.

Botanical natural products, for example, are often complex mixtures. Their exact compositions may differ under the influence of variables such as genetics, cultivation conditions, adulteration, storage conditions, and processing methods.

Chromatographic analysis of both dietary supplements and botanical natural products presents specific challenges with respect to:

1. Sample preparation: the wide variety of sample matrices can affect the efficiency and reproducibility of extraction.
2. Separation: an exceedingly wide range of chemical structures will require different chromatographic approaches.
3. Detection: analytes lacking a chromophore are not detected by absorption detectors.
4. Quantitation: often there are no reference standards available.

This notebook offers a summary of applications and helpful information as to how high-performance liquid chromatography (HPLC) combined with charged aerosol detection, absorption detection or both can be used to meet the needs of scientists working with dietary supplements and botanical natural products.

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What is meant by dietary supplements and botanical natural products?

Dietary supplements

The US FDA defines dietary supplements, in part, as "products taken by mouth that contain a dietary ingredient. Dietary ingredients include vitamins, minerals, amino acids, and herbs or botanicals, as well as other substances that can be used to supplement the diet. Dietary supplements come in many forms, including tablets, capsules, powders, energy bars and liquids."

Natural products

The term natural product can be used in many ways. Some applications are specific, based on the FDA's definition of a chemical compound or substance produced by a living organism (bacteria, fungi, marine organisms or plants), that usually has a pharmacological or biological activity, and is of use in pharmaceutical drug discovery and drug design. Other definitions are broader, and natural product may be used as an umbrella term to describe complex plant-based preparations.

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Botanical natural products

Botanical natural products are referred to as supplements, herbal medicines, herbal drugs, herbs, botanical preparations, nutraceuticals, phytomedicines and botanical medicines. Botanical natural products are available to consumers in a variety of forms, including capsules containing raw or extracted material, extracts, teas (typically raw plant material that is extracted in hot water prior to use), tinctures (ethanolic extracts), and traditional formulations, such as powders used in traditional Chinese medicine or Ayurvedic practices.

Botanical drug products

The FDA defines botanical drug products as "…intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in humans…" and consisting of "…vegetable materials, which may include plant materials, algae, macroscopic fungi, or combinations thereof." Botanicals include drugs that are FDA-approved under the botanical drug pathway, while the category of plant-derived drugs includes both botanicals and other approved drugs that contain a mixture of natural plant-derived and synthetic or semi-synthetic substances.

Global market overview

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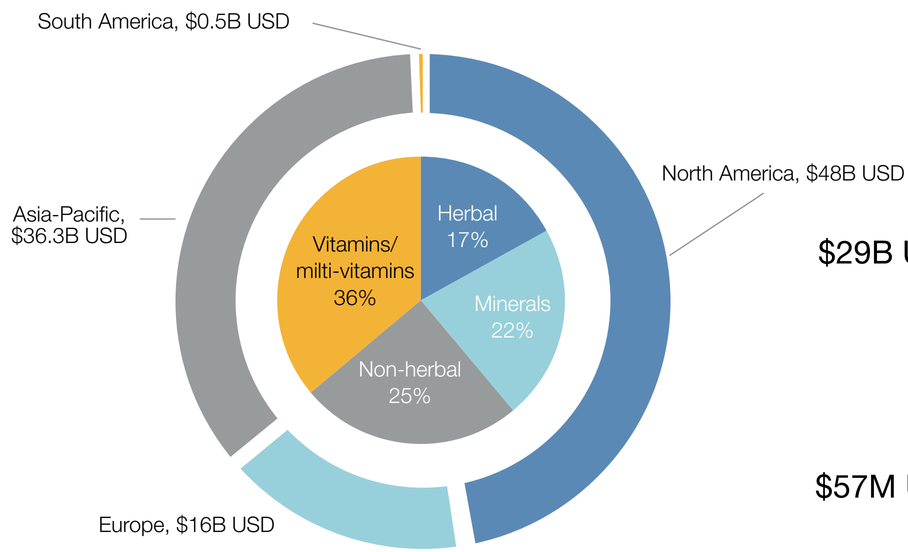
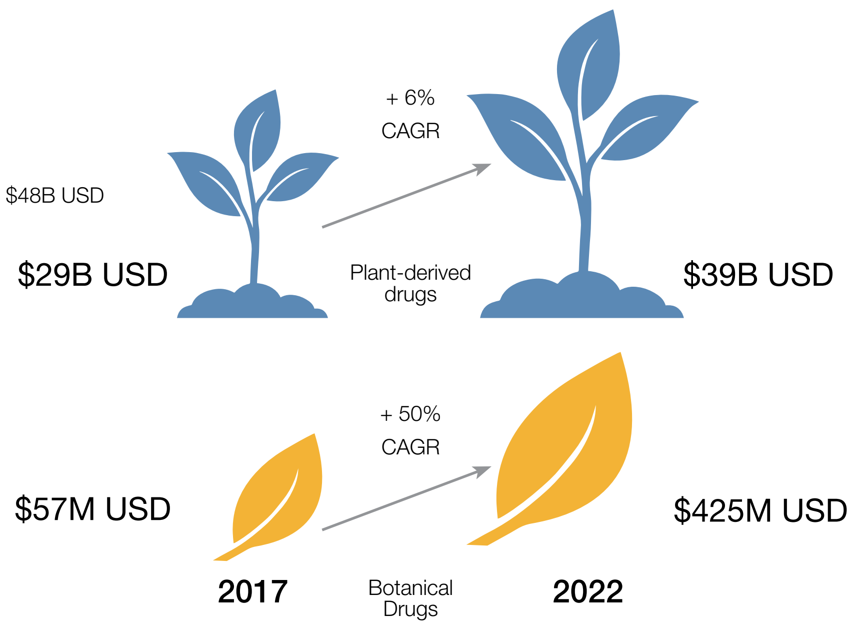
The increasing demand for dietary supplements is being driven by the growing health concerns of consumers, especially aging populations. Dietary supplements are one key segment of the nutraceuticals market, alongside functional foods and beverages. With respect to natural substances, a long history of use and a more favorable regulatory climate are key drivers in the market growth of botanical natural products.

The use of innovative technologies in production and enhanced quality monitoring by regulating bodies enable the growth of these markets and the delivery of safe drugs.



Outer circle: Dietary supplement market distribution by regions worth $100.8 Billions in total (est. for 2023)
Inner circle: Dietary supplement market distribution by ingredients (est. for 2023)

From bcc Research – Nutraceuticals: Global Markets to 2023. Report Code FOD013G



Estimated growth of plant-derived and botanical drugs markets. Compound annual growth rate (CAGR) shown for both.

From bcc Research – Botanical and Plant-derived Drugs: Global Market, Report Code BIO022H

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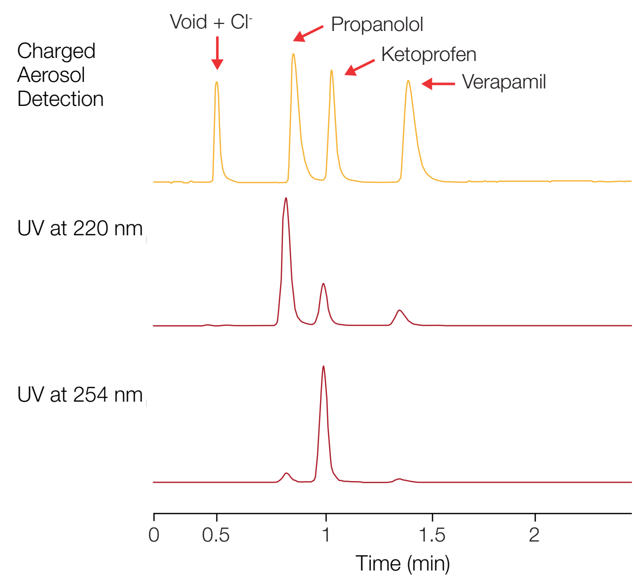
This Applications Notebook focuses on high performance liquid chromatography (HPLC) using two different HPLC/UHPLC detectors – UV-Vis Absorbance and Charged Aerosol Detection (CAD) – for the measurement of a wide variety of compounds in different sample matrices. There are differences in the performance of these detector technologies (shown in the table below), but they are also complementary. When used serially (UV-Vis then CAD) they can markedly extend the range of analytes measured in a sample.

	UV-Vis Absorbance	CAD
Type of Detector	Optical - selective*	Nebulizer/particle charging - universal
What Compounds are Measured?	Analytes must have a chromophore, but can be volatile or non-volatile	Analytes must be non- or semi-volatile but do not need to contain a chromophore
Detector Response	Dependent upon chromophore structure and absorption wavelength selected*	Uniform for all non-volatile compounds, independent of chemical structure
Calibration	Requires external standard	Single calibrant can be used for quantitation when individual standards are not available

*Low-wavelength UV detection is used for compounds that possess a weak chromophore. While this approach increases the range of analytes detected, it lacks sensitivity and results in more complex chromatograms with a greater chance for analyte coelution and interference.



CAD is a Universal Detector.

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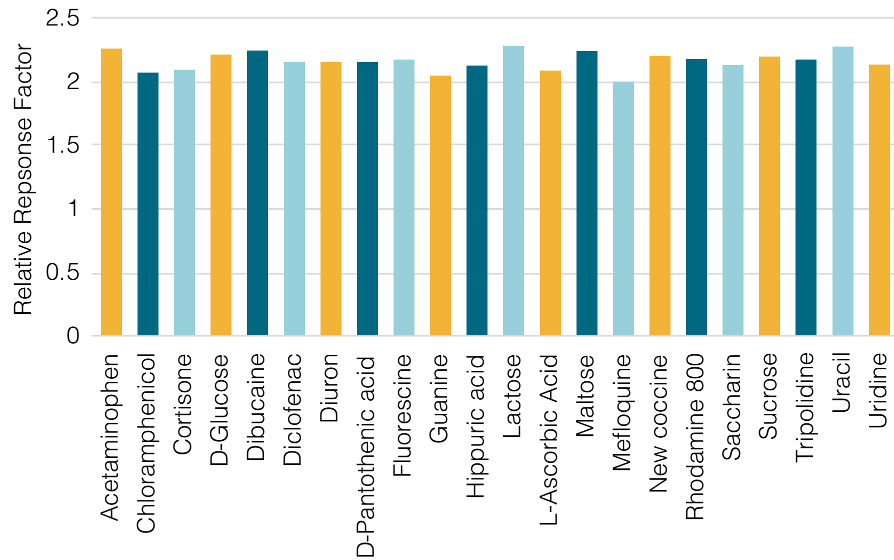
Using CAD for quantitation when standards are not available

A critical issue for the quantitation of analytes in many botanical natural products is the lack of authentic standards.

With UV-Vis absorbance, the analytes can be volatile or non-volatile, but the response of an analyte depends upon its chromophore structure and the absorption wavelength used for detection. One compound may show a strong response at a specific absorption wavelength, while others may respond only weakly or not at all. Using a single calibrant to quantify all analytes in a sample may, therefore, lead to erroneous quantitation.

In contrast, the CAD response for non-volatiles is independent of the analyte's chemical structure. Consequently, a single calibrant can be used for quantitation of all non-volatile analytes in the sample without the need for multiple authentic standards. For semi-volatile analytes smart-chosen mobile phase additives could support the single calibrant quantification in many cases.

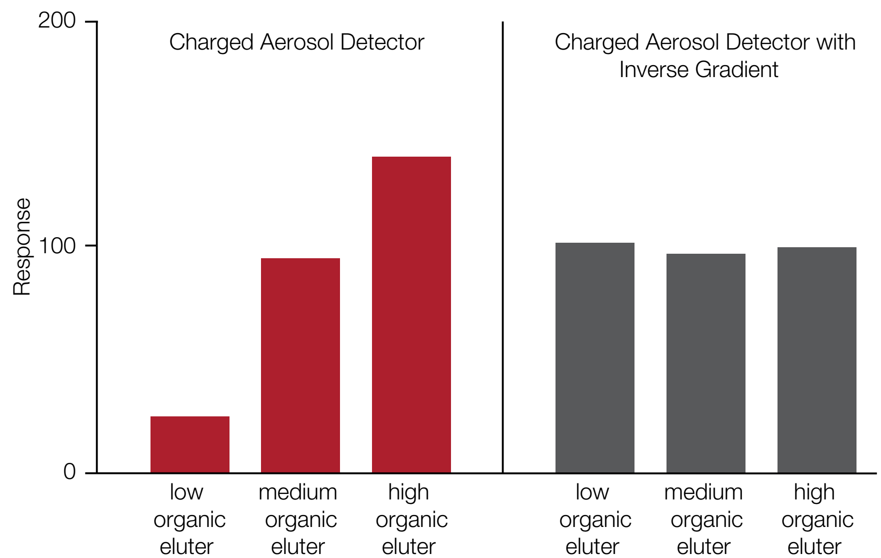


CAD shows uniform response.

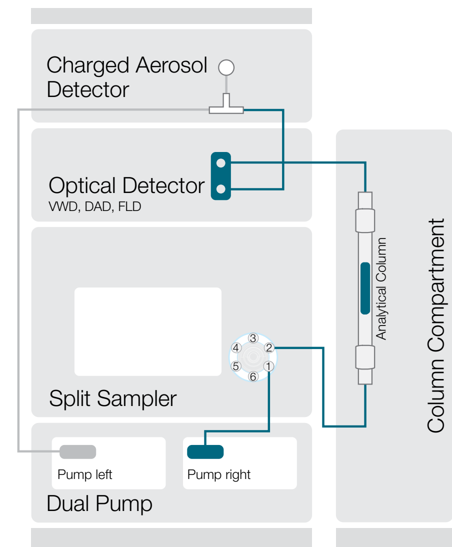
Using CAD for quantitation when standards are not available

CAD response is, however, dependent upon the organic content of the mobile phase. This means the uniformity of response will be affected when gradients are employed. In order to use single calibrant quantitation, changes in the detector response during gradient elution must be minimized. This is readily achieved using a secondary make up flow post column. Here the inverse gradient ensures that the detector "sees" a constant mobile phase composition, thereby ensuring uniform response. Such a set-up is easily implemented using the Thermo Scientific™ Vanquish™ Duo UHPLC System for Inverse Gradient LC Workflows.

CAD response for ionizable compounds may be affected (to some extent) by the composition of the mobile phase, as a result of salt formation. In general, this is a minor consideration when measuring the analyte composition of many dietary supplements and botanical natural products.



Response of the CAD is dependent upon mobile phase composition during a gradient (red bars). An inverse gradient overcomes the effect of the gradient (gray bars) on detector response.



An inverse gradient delivered by the left pump ensures that the composition of the mobile phase entering the detector remains constant thereby maintaining response uniformity

TN72806: Charged aerosol detection – factors affecting uniform response

TN73449: Why use Charged Aerosol Detection with Inverse Gradient?

SP73026-EN 0819C: Achieving standard free quantitation: Thermo Scientific Charged Aerosol Detectors

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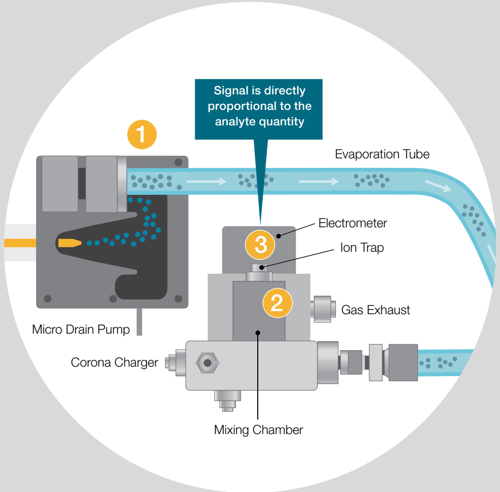
Collective power of chromatography

Instruments configured to meet your exact measurement needs

Sample Preparation

Separation

Detection



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Sample preparation

Sample preparation is a crucial step in any analytical workflow and the wide variety of sample matrices seen across dietary supplements and botanical natural products can make it especially challenging. However, modern sample preparation systems are designed to improve the speed, efficiency and reproducibility of this step, making it easy to extract even the most complex samples with confidence.

Automate sample preparation with an Accelerated Solvent Extractor system (ASE)

- Automates sample preparation for solid and semi-solid samples, using solvents at elevated temperatures.
- Operates above the boiling point of extraction solvents by using elevated pressure, thereby permitting fast liquid extractions at high temperature.
- Walk-away system that performs extraction and clean up on 24 samples unattended.
- Well-established and proven technique that is superior to Soxhlet and is approved for numerous regulatory methods.
- Ideal for the extraction of complex plant samples and dietary supplements prior to HPLC analysis.

Thermo Scientific™ Dionex™ ASE™ 150 and ASE™ 350 Accelerated Solvent Extractor

For more information about chromatography sample preparation and consumables click here

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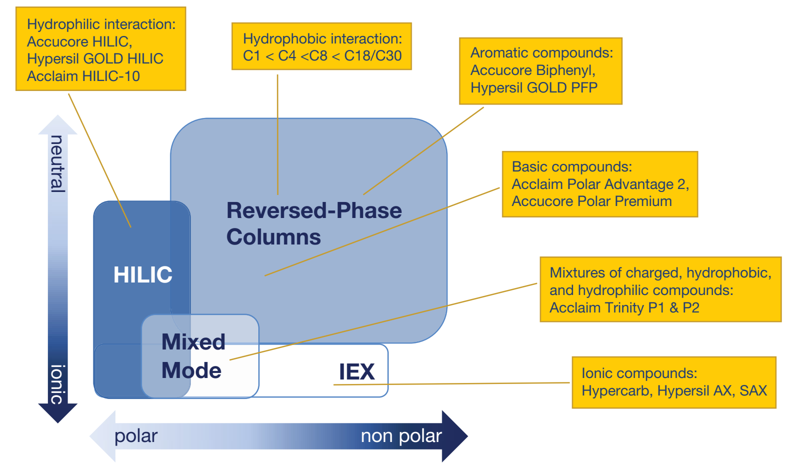
Choosing between general purpose and specialty HPLC columns

The choice of column used at the separation stage can significantly impact the quality of results obtained, so is an important element to consider.

Thermo Fisher Scientific offers columns with a wide range of bonded phases, particle sizes, particle morphologies and column dimensions. These comprehensive options are designed to meet any application need to deliver maximum analyte resolution. Some things to consider when choosing the most appropriate LC column for your requirements are:

- Fully porous particles, found in Thermo Scientific™ Hypersil™ GOLD columns and Thermo Scientific™ Acclaim™ columns, allow for large injection volumes and high resolution of complex sample matrices.
- Superficially porous (solid core) particles, found in Thermo Scientific™ Accucore™ columns, provide greater signal-to-noise characteristics with smaller injection volumes and/or when performing UHPLC separations without elevated system backpressure.
- A column's hydrophobic properties govern the separation of most analytes. While C18 is the most common phase selected in method development, consider hydrophobic retention trends with the carbon load on the column: C1 < C4 < C8 < C18 / C30.



Analyte	Suggested column(s)	Benefit
Lipids	• Thermo Scientific™ Acclaim™ C30 column • Thermo Scientific™ Accucore™ C30 column	Greater selectivity with shorter run times compared to a traditional C18
Large molecular weight (>4kDa)	• Thermo Scientific™ Acclaim 300 C18 column • Thermo Scientific™ Accucore 150 C18 column	Wider pore silica column minimizes sample carryover
Moderately polar analytes (including basic compounds)	• Thermo Scientific™ Acclaim™ PA2 column • Thermo Scientific™ Accucore™ Polar Premium column	Achieves greater retention by selecting a polar embedded column
Aromatic compounds	• Thermo Scientific™ Accucore™ Biphenyl column • Thermo Scientific™ Hypersil GOLD™ PFP column	Enhances steric selectivity and resolution with phenyl-based columns
Neutral and charged analytes	• Thermo Scientific™ Acclaim™ Trinity P1 column • Thermo Scientific™ Acclaim™ Trinity P2 column	Provides controlled ion exchange and reversed-phase (RP)/hydrophilic interaction liquid chromatography (HILIC) properties - the Trinity line works well for samples with a mix of ion exchange and hydrophobic/hydrophilic properties

For details of the complete range of HPLC/UHPLC columns click here

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The complexity and variety of potential analytes makes the analysis of dietary supplements and botanical natural products especially challenging.

Thermo Scientific Vanquish HPLC and UHPLC systems can solve even the toughest analytical challenges—they are dependable and consistently deliver high caliber results you can depend on.

Thermo Scientific™ Vanquish™ Core HPLC system
Absolute dependability to enable worry-free applications

Thermo Scientific™ Vanquish™ Flex UHPLC system
Complete flexibility for method development or fast and reliable UHPLC

Thermo Scientific™ Vanquish™ Horizon UHPLC system
Unrivaled performance and throughput for applications requiring high-end UHPLC

Thermo Scientific™ Vanquish™ Duo HPLC and UHPLC systems
increased productivity with two completely independent flow paths for higher throughput and improved sample characterization

Find out more about Thermo Scientific Vanquish HPLC and UHPLC systems

Detection: detector choice is key

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Choosing the most appropriate detection technology for your application is crucial in revealing all the components of interest in your sample. The Vanquish HPLC and UHPLC platforms offer a wide range of detection capabilities that can be easily integrated and combined to fit your analytical needs.

Although the focus here is on UV-Vis Absorbance and Charged Aerosol Detectors, a complete range of detectors is available for the analysis of dietary supplements and botanical natural products, including mass spectrometry solutions. These enable you to choose and configure the exact set-up for your specific requirements.

Industry-leading diode array detection

Thermo Scientific™ Vanquish™ Diode Array Detectors

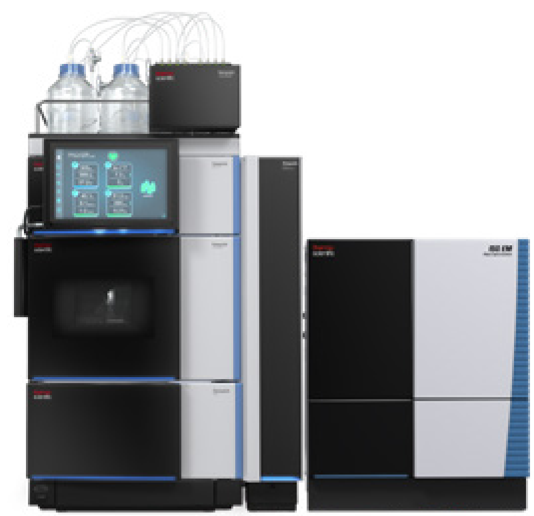
Cost-effective reliable UV-Vis detection

Thermo Scientific™ Vanquish™ Variable Wavelength Detectors

Unmatched all-round performance of CAD

Thermo Scientific™ Vanquish™ Charged Aerosol Detectors

Easily integrated mass detection



Thermo Scientific™ ISQ™ EC Single Quadrupole Mass Spectrometer or Thermo Scientific™ ISQ™ EM Single Quadrupole Mass Spectrometer

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Detection: Charged Aerosol Detectors

State-of-the-art instrumentation is essential for successful sample analysis using charged aerosol detection

Thermo Scientific HPLC and UHPLC systems combined with Thermo Scientific Charged Aerosol Detectors (CAD), advanced column technologies and proven analytical methods deliver the precise automation and advanced data handling that helps you to:

- Characterize many classes of compounds
- Analyze compounds in a broad range of samples types
- Profile or quantify analytes

Thermo Scientific Vanquish Charged Aerosol Detectors and Thermo Scientific™ Corona™ Veo™ Charged Aerosol Detectors provide:

- Simple, intuitive operation
- Wide linear and dynamic range
- Sub-nanogram sensitivity
- Method flexibility covering micro-flow HPLC and UHPLC applications with a single nebulizer
- Adjustable evaporation temperature to optimize signal-to-noise ratio

Vanquish Charged Aerosol Detector

Corona Veo Charged Aerosol Detector

Detection: UV-Vis

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Variable wavelength detectors offer sensitive, accurate UV-Vis detection

Ensure high sensitivity and accuracy during UV-Vis detection of your analytes with Thermo Scientific variable wavelength detectors (VWDs) for HPLC. The optical bench design of this detector type minimizes baseline drift and noise.

Diode array detectors combine flexibility and performance in UV-Vis detection

Thermo Scientific Vanquish Diode Array Detectors (DADs), supporting 3D-field detection, and Multiple Wavelength Detectors (MWDs) are designed for the highest performance and flexibility. A wide spectral range and several programmable detector optimization parameters meet challenging method development requirements and application demands.

Thermo Scientific UV-Vis detectors offer several advantages, including excellent noise, drift, and linearity performance to support a wide operating range and trace compound detection. These absorbance detectors are easy to operate and streamline sample analysis by covering both the UV and Vis ranges of light.

Product Specification Sheet: Vanquish Variable Wavelength Detectors
Product Specification Sheet: Vanquish Diode Array Detectors and Multiple Wavelength Detectors

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Authentication of supplements and raw materials

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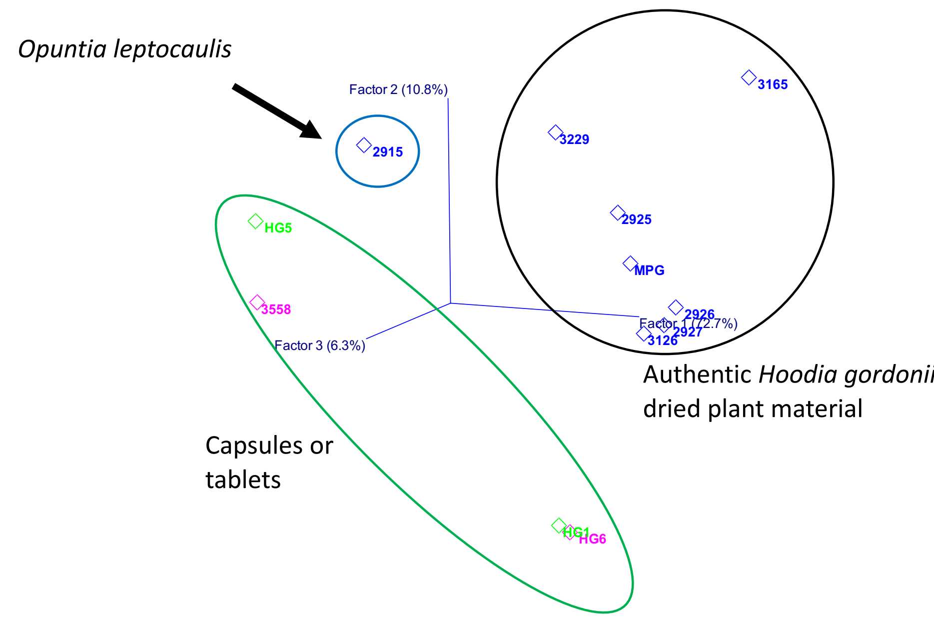
Safety, authenticity and sustainability are key issues when using plant-derived materials. Unintentional contamination or economically-motivated deliberate adulteration within the supply chain continue to be major problems affecting commercially available products. The need for authentication applies whether the sample is the source plant or a plant material such as a root, shoot or leaf.

As well as the analytical challenges already highlighted, authentication may be complicated by the use of poorly characterized material in previous studies. To address this, HPLC-based methods are used to evaluate botanical natural products including both untargeted and targeted metabolomics approaches. Presented here is an untargeted HPLC metabolomic method, which uses principal component analysis to distinguish between authentic samples and an adulterant, based on differences in their metabolite patterns.



Principal component analysis score plot of hoodia samples showing the ability to differentiate between authentic dried plant, capsules and tablets, and an adulterant *Opuntia leptocaulis* samples. See Poster Note 70540 for greater detail.

PN70540: Profiling Hoodia Extracts by HPLC with Charged Aerosol Detection, Elelctrochemical Array Detection, and Principal Component Analysis

For more background on the use of CAD to determine product authentication and adulteration see:

AN7317: Determination of olive oil purity based on triacylglycerols profiling by UHPLC-CAD and Principal Component Analysis

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Application Examples

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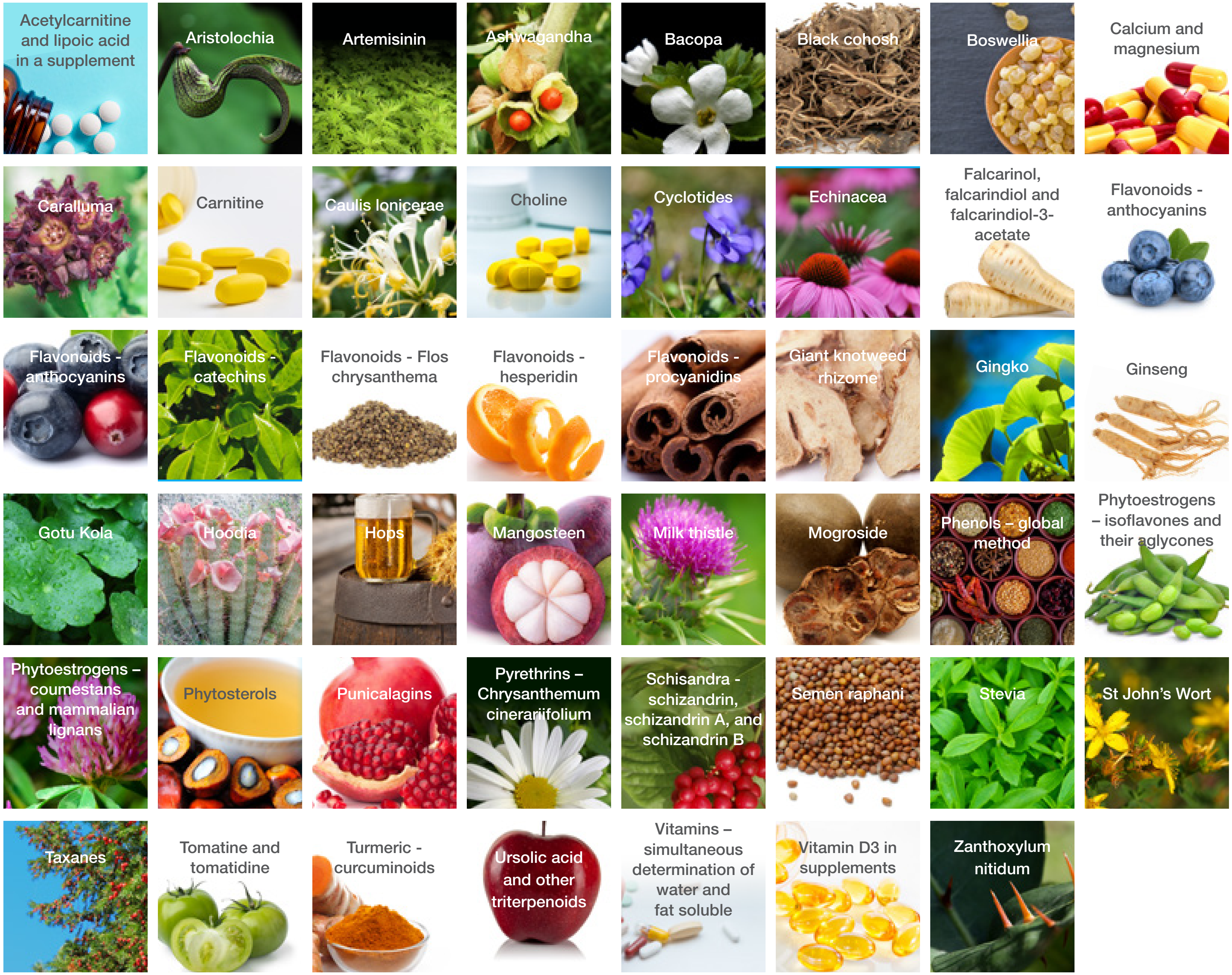
In the following pages, the analysis of a selection of dietary supplements and botanical natural products is described.



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Acetylcarnitine and lipoic acid in a supplement

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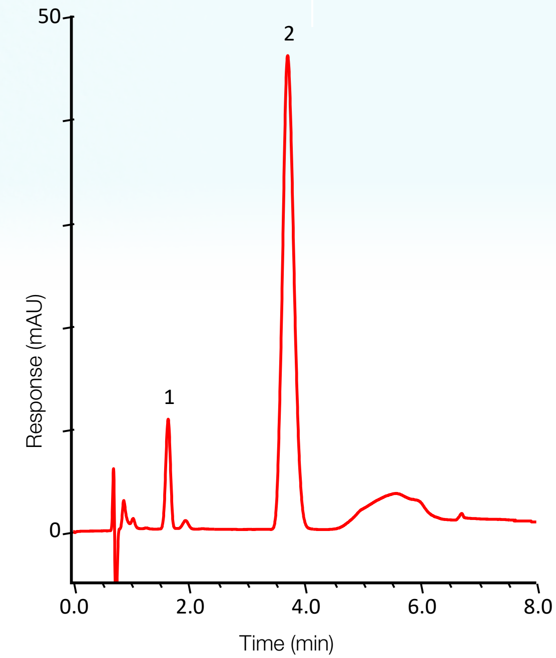
Acetylcarnitine is produced naturally by the body and is also taken as a dietary supplement. It is broken down in the blood by plasma esterases to carnitine, which functions as part of the system that transports fatty acids into the mitochondria for energy metabolism.

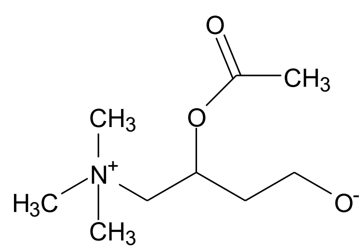
Lipoic acid is an essential cofactor for several enzyme complexes involved in aerobic metabolism. As well as occurring naturally in many foods, lipoic acid is also formed in the body and may additionally be taken as a supplement.

Both acetylcarnitine and lipoic acid are highly polar compounds and they are not well-retained on RP-HPLC columns. Presented here is an HPLC-UV method using an Acclaim Trinity P1 column for the simultaneous measurement of acetylcarnitine and lipoic acid in a dietary supplement. The cation-exchange, anion-exchange and reversed-phase retention mechanisms of the Acclaim Trinity P1 make it an ideal column for this application.

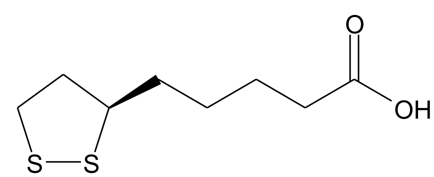
Column:	Thermo Scientific Acclaim Trinity P1, 3 µm, 3.0 x 50 mm
Mobile phase A:	3.00 g Monobasic sodium phosphate (25 mmol), 22 mg Tetrasodium pyrophosphate decahydrate (0.5 mmol) + 270 µL 85% Phosphoric acid (4 mmol) + 196 g Acetonitrile + 750 g DI Water
Mobile Phase B:	196 g Acetonitrile + 750 g DI Water
Detector:	Absorbance, UV 210 nm



HPLC-UV analysis of dietary supplement.
1 – Acetylcarnitine;
2 – Lipoic acid.



Acetylcarnitine



Lipoic acid

AppsLab 762: Rapid determination of Acetylcarnitine and Lipoic Acid in a nutritional supplement using a Thermo Scientific Acclaim Trinity P1 HPLC column

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Aristolochia

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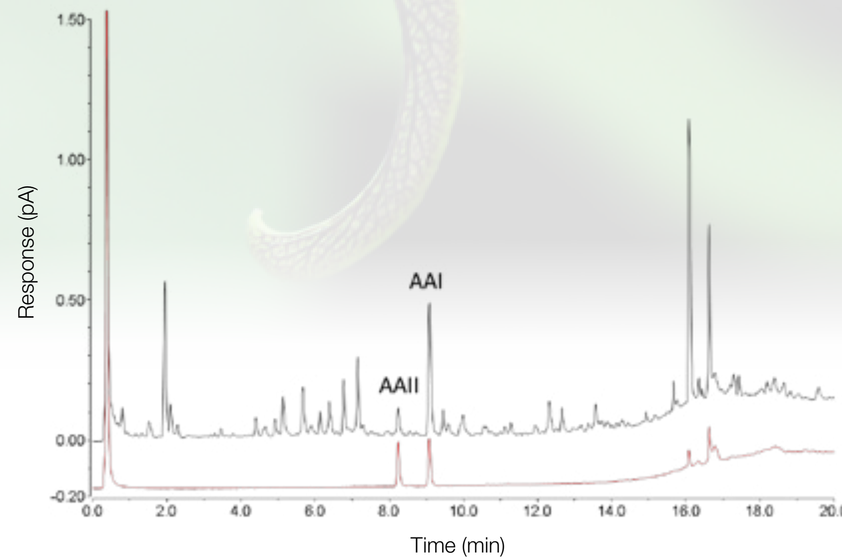
The genus *Aristolochia* includes several plants known as birthwort or Dutchman's pipe. One species, *Aristolochia clematitis*, was used as a medicinal plant by the ancient Egyptians, Greeks and Romans, to treat asthma, hiccups, spasms, pains, and expulsion of afterbirth. In traditional Chinese medicine *Aristolochia* species are used to treat arthritis and edema. *Aristolochia*-containing supplements are also promoted to help with weight loss.

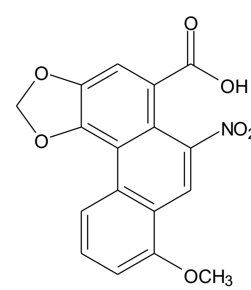
Aristolochia species are now considered to be toxic. There are reports of people suffering nephritis and rapid kidney failure after consuming certain weight loss supplements from China. The toxins responsible are most likely the aristolochic acids (AA I and AA II). These are potent carcinogens that can cause liver and urothelial cancer. Consequently, the FDA has issued warnings against consumption of AA-containing supplements.

Presented here is an HPLC-CAD method for the determination of AA1 and AA2 in supplements.

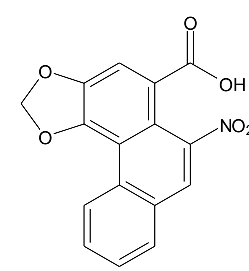
HPLC Column:	Thermo Scientific Accucore C18, 2.6 µm, 2.1 x 150 mm
Mobile Phase A:	0.1 % Formic acid in 10 mM Ammonium formate
Mobile Phase B:	0.1 % Formic acid in Acetonitrile
Detector:	Charged Aerosol



HPLC-CAD analysis of a methanolic extract of *Aristolochia fangchi* root (black) and standards (red).



Aristolochic acid I (AAI)



Aristolochic acid II (AAII)

Further information on Thermo Scientific AppsLab Library of Analytical Applications

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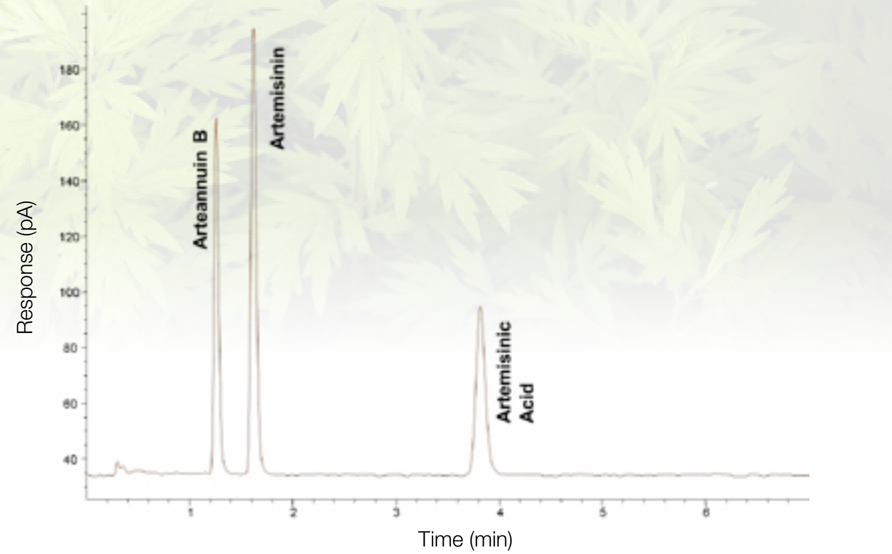
Artemisinin

Sweet wormwood (*Artemisia annua*), a plant used in traditional Chinese medicine, is the source of the drug artemisinin. Artemisinin and its semisynthetic derivatives are used in the treatment of malaria and parasitic worm infections.

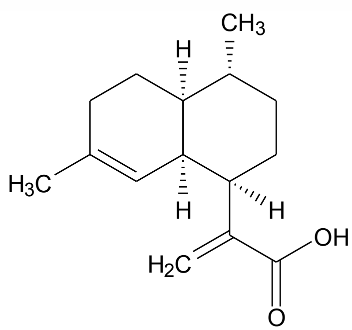
Artemisinin does not contain a chromophore. Low-wavelength UV absorbance detection lacks sensitivity and is prone to chromatographic interferences. Artemisinin is an ideal candidate for analysis by HPLC-CAD.

Presented here is an HPLC-CAD method that can be used for the analysis of artemisinin and related compounds.

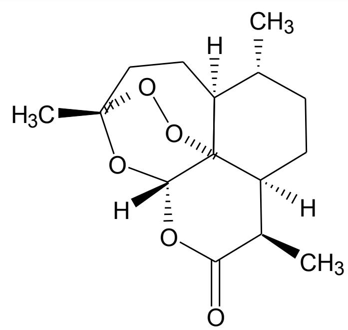
HPLC Column:	C18, 3 µm, 4.6 x 75 mm
Mobile Phase:	60% Acetonitrile, pH 3 with Trifluoroacetic acid
Detector:	Charged Aerosol



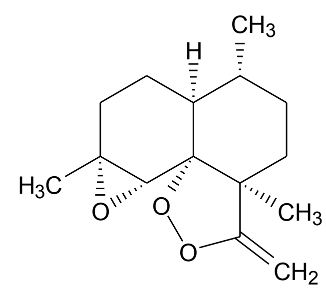
Analysis of artemisinin and related compounds (3.3 µg each on column).



Artemisinic acid



Artemisinin



Arteannuin B

For HPLC-UV methods see:

Separation of artemether and its impurities using reversed-phase HPLC-UV

Rapid analysis of artesunate and dihydroartemisinin using a Thermo Scientific Accucore RP-MS HPLC column

Fast analysis of artesunate and dihydroartemisinin using a Thermo Scientific Syncronis C18 HPLC column

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Ashwagandha

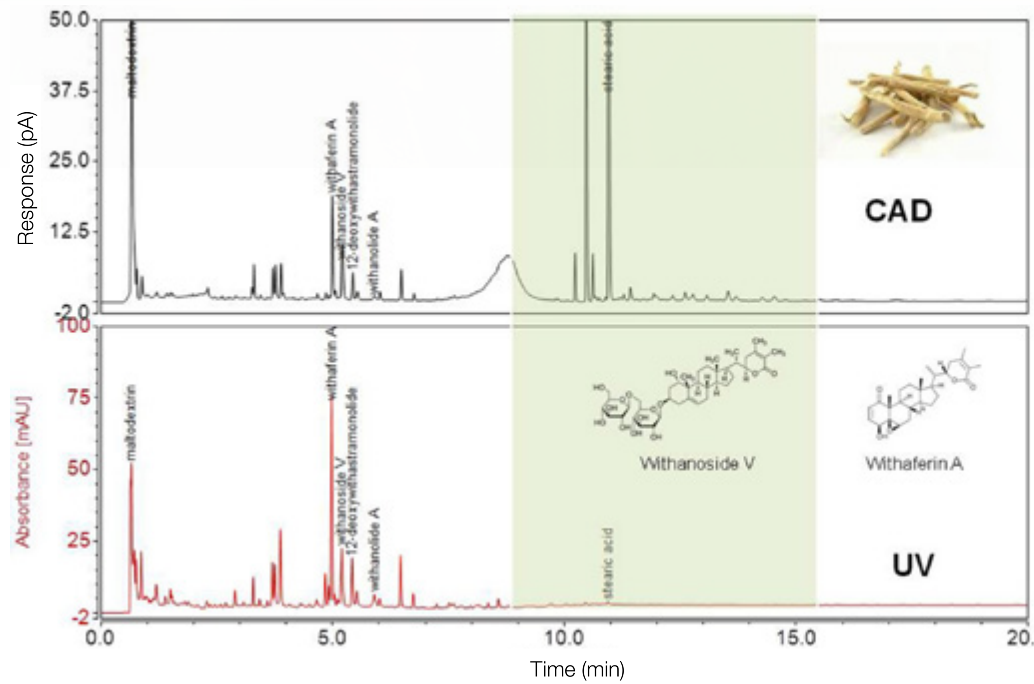
Withania somnifera, a member of the nightshade family, is commonly called Indian winter cherry, poison gooseberry or Indian ginseng. Known as ashwagandha, it is used in Ayurvedic medicine to treat stress, improve concentration and increase energy.

Ashwagandha is taken as a strengthening tonic, sexual tonic, memory enhancer, stress reliever and to cool the body. Purported active compounds include the steroidal lactones (e.g., withanolides and withaferins), alkaloids (anaferine, anahygrine, cuseohygrine, and isopelletierine) and saponins. The withanolides are structurally similar to the ginsenosides of *Panax ginseng*.

Ashwagandha extracts can be analyzed by HPLC-UV detection, but this approach is limited as many compounds possess only weak chromophores. HPLC-CAD provides a more comprehensive analysis.

As shown here, HPLC-UV absorbance detection fails to measure many compounds found in an ashwagandha extract.

Column:	Thermo Scientific Accucore C8, 2.6 µm, 4.6 x 150 mm
Mobile Phase A:	DI Water
Mobile Phase B:	Acetonitrile, Optima LCMS
Detector:	Charged Aerosol and Absorbance, UV 230 nm



Comparison between CAD and UV absorbance detection for HPLC separation of ashwagandha extract. The shaded area shows that many components are missed by the HPLC-UV approach.

PN70543: Novel, Universal Approach for the Measurement of Natural Products in a Variety of Botanicals and Supplements, Part 2

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Bacopa

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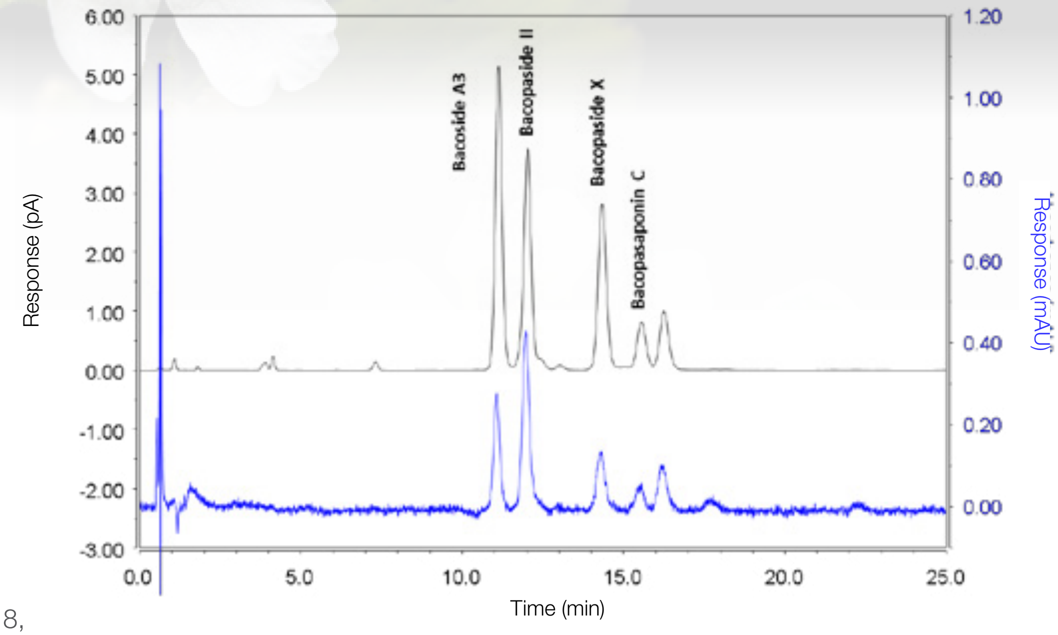
Bacopa monnieri, commonly called brahmi, water hyssop, herb of grace or Indian pennywort, is used in Ayurvedic medicine to enhance mind power (the Medhya effect) and improve all aspects of mental functioning, including memory and comprehension. Among the purported bioactive compounds found in *Bacopa monnieri* are dammarane-type triterpenoid saponins, known as bacosides.

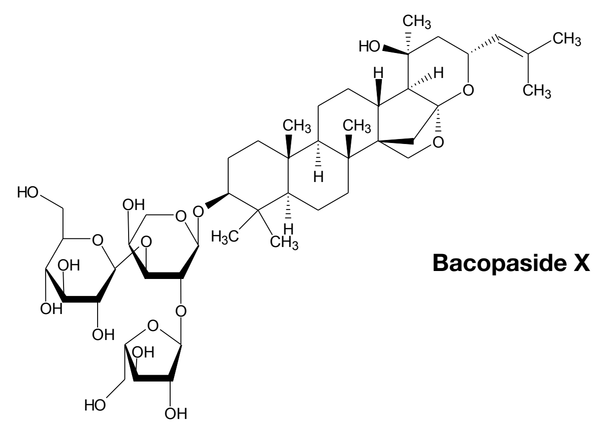
Although extracts can be analyzed by HPLC-UV detection, this approach is limited because many compounds possess weak chromophores. HPLC-CAD enables a more comprehensive and sensitive analysis.

Presented here is a comparison of HPLC with either CAD or UV detection of a *Bacopa monnieri* extract.

Column:	Thermo Scientific Accucore C18, 2.6 µm, 2.1 x 150 mm
Mobile Phase A:	DI Water
Mobile Phase B:	Acetonitrile, Optima LCMS
Detector:	Charged Aerosol and Absorbance, UV 210 nm



Comparison of HPLC with either CAD or UV detection of a *Bacopa monnieri* extract. Note UV detection misses early eluting analytes.



Bacopaside X

PN70543: Improved Universal Approach to Measure Natural Products in a Variety of Natural Products and Supplements

thermo scientific

Black cohosh

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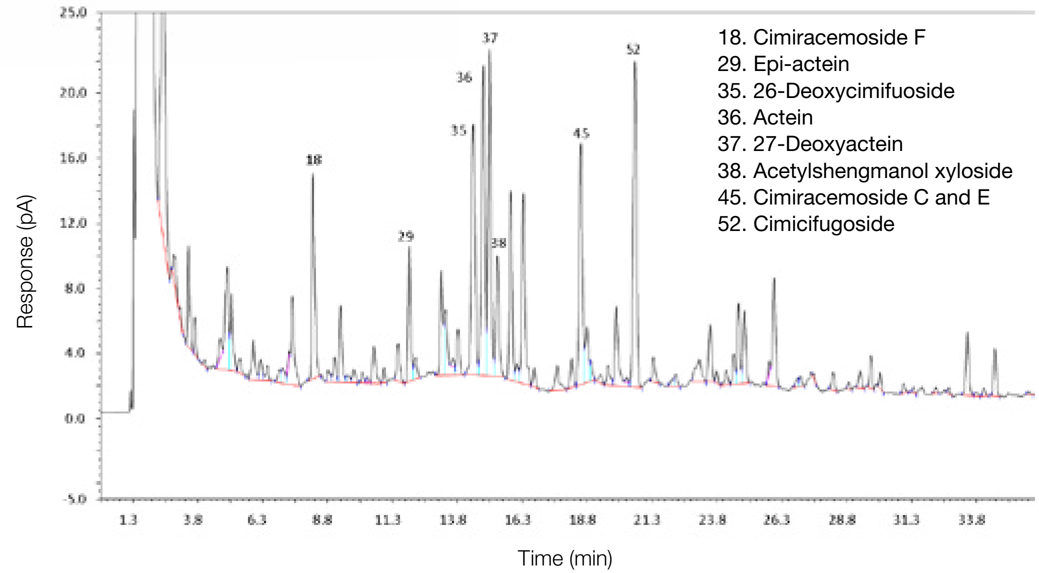
Actaea racemosa (synonym *Cimicifuga racemose*), also called black cohosh, black bugbane, black snakeroot or fairy candle, is a species of flowering plant native to eastern North America. Black cohosh was used by Native Americans to treat gynecological and other disorders, while European settlers used it to treat snakebite, inflamed lungs and pain from childbirth. Today, it is taken as a supplement to address gynecological issues.

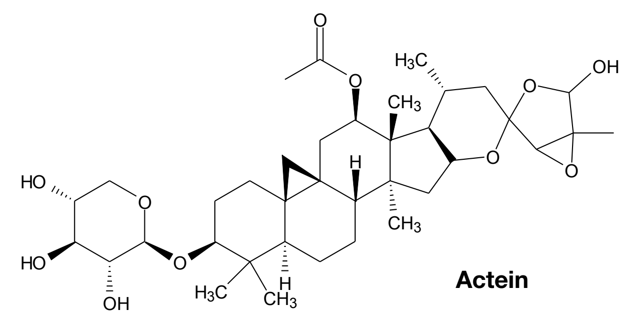
The active ingredients are believed to be the triterpene glycosides, including actein, cimifugoside and related compounds. Extracts can be analyzed by HPLC-UV detection, but this approach is limited as the triterpene glycosides contain weak chromophores. A more comprehensive and sensitive analysis is obtained using HPLC-CAD.

Presented here is a method for HPLC-CAD analysis of a black cohosh extract.



HPLC-CAD analysis of black cohosh extract.

Column:	Thermo Scientific Accucore C18, 2.6 µm, 2.1 x 150 mm
Mobile Phase A:	DI Water
Mobile Phase B:	Acetonitrile, Optima LCMS
Detector:	Charged Aerosol

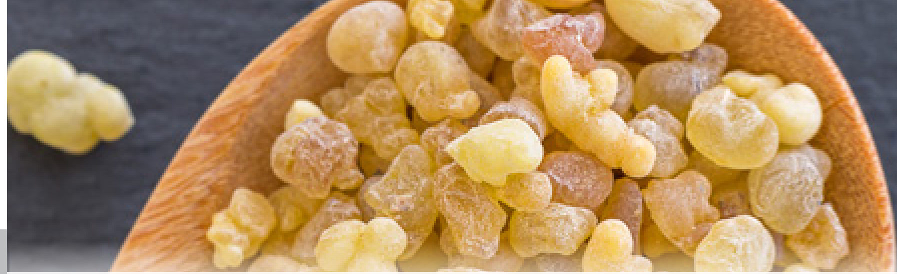


Actein

CAN113: Determination of Triterpene Glycosides in Cimicifuga racemosa (Black Cohosh) by HPLC-CAD

PN70543: Novel, Universal Approach for the Measurement of Natural Products in a Variety of Botanicals and Supplement, Part 2

PN70153: Can High Peak Capacity and Universal Detection Solve the Challenges in LC Characterization of Botanicals and Natural Products



Boswellia

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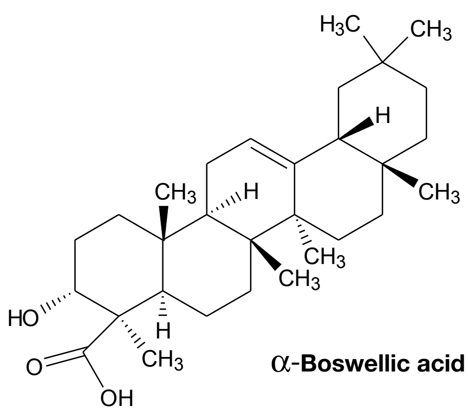
Boswellia is a genus of tree known for its fragrant resin. A well-known extract is frankincense, which comes from the resin of *Boswellia sacra*. More generally, *Boswellia* extracts have been used for centuries in Asian and African folk medicine to treat chronic inflammatory illnesses and various other health conditions.

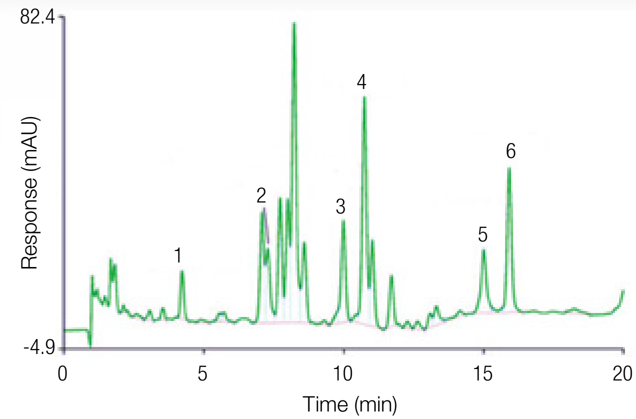
The active ingredients are believed to be the pentacyclic triterpene boswellic acids. These compounds contain weak chromophores so are often measured using HPLC with low-wavelength UV detection.

Presented here is method for analyzing boswellic acid and related compounds in a *Boswellia* extract using HPLC-UV.

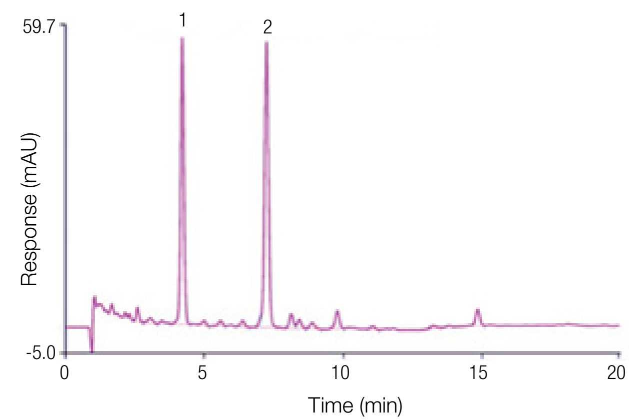
Column:	C18, 5 µm, 4.6 × 150 mm
Mobile phase A:	Acetonitrile/ DI Water/ Phosphoric acid (85%) 80:20:0.1 v/v/v
Mobile phase B:	Acetonitrile
Detector:	Absorbance, UV 210 or 250 nm



α-Boswellic acid



Determination of *boswellic* acids in *boswellia* extracts, UV at 210 nm.
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



Determination of *boswellic* acids in *boswellia* extracts, UV at 250 nm.
1. 11-keto-α-Boswellic acid
2. 3-acetyl-11-keto-α-Boswellic acid

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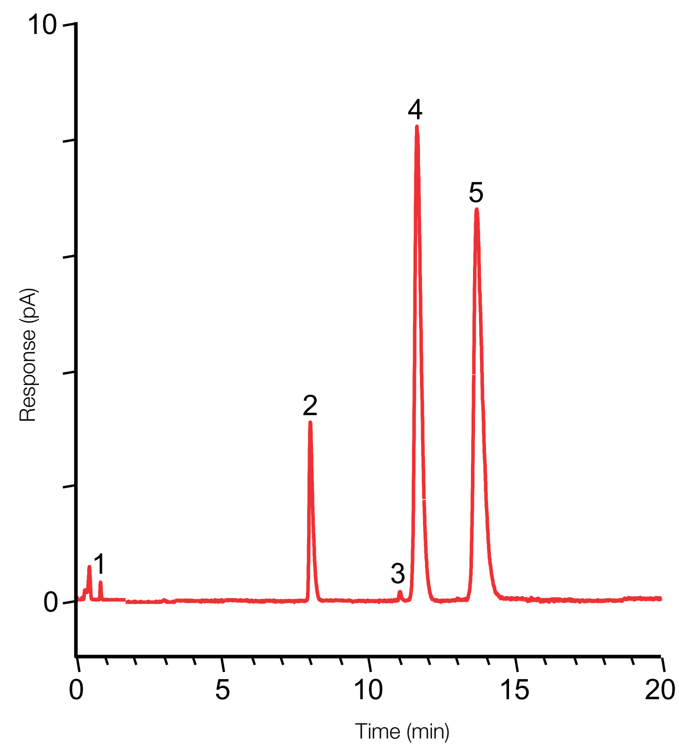
Calcium and magnesium

Calcium and magnesium are essential nutrients and are commonly taken as dietary supplements. The type of calcium or magnesium salt present can affect the rate of absorption, so a variety of counterions may be included in the formulation. The analysis of mineral supplements provides an interesting analytical challenge in that both the anions and cations are functional ingredients that require determination. In the past, separate assays for anions and cations were used. However, advances in multi-mode chromatography now permit resolution of both anions and cations in one run using a single HPLC column.

Presented here is an HPLC-CAD method using an Acclaim Trinity P2 column for separation and detection of various cations and anions in a mineral supplement.

Column:	Thermo Scientific Acclaim Trinity P2, 3 µm, 3 x 50 mm
Mobile phase A:	DI Water
Mobile phase B:	100 mM Ammonium formate, pH 3.65
Detector:	Charged Aerosol



Analysis of a mineral supplement.
1. Aspartate
2. Citrate
3. Unknown
4. Magnesium
5. Calcium

AppsLab 656: Simple gradient method for the analysis of calcium and magnesium in a dietary mineral supplement using HPLC-CAD

AN20871: Separation of Calcium, Magnesium and Counterions in a Dietary Supplement Using Multi-mode Liquid Chromatography with Charged Aerosol Detection

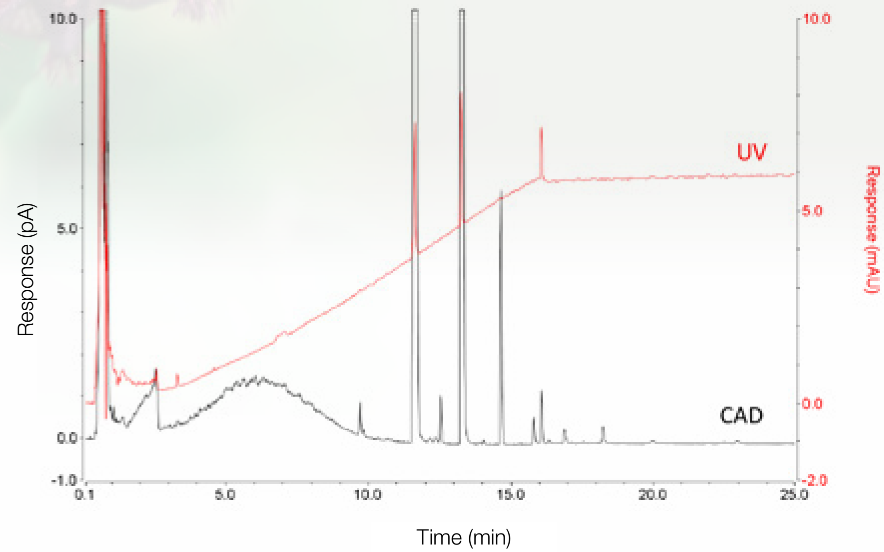
Caralluma

The edible cactus *Caralluma fimbriata* has been used in India for thousands of years as part of the diet and as an appetite suppressant, typically during long hunts and in times of famine. Today, *Caralluma* supplements are taken to suppress hunger and to enhance endurance.

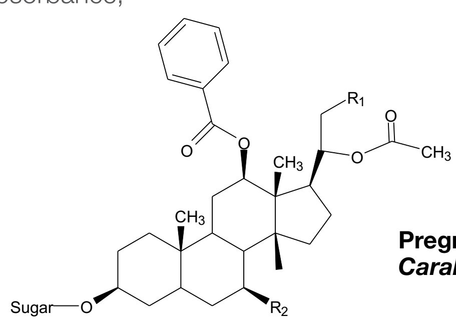
The purported active components contained in *Caralluma* supplements are the oxypregnane glycosides. These compounds contain weak chromophores so are often measured by HPLC with low-wavelength UV detection. A more comprehensive and sensitive analysis is obtained using HPLC-CAD.

Presented here is a method for the analysis of oxypregnane glycosides in *Caralluma* using HPLC with both CAD and UV detection.

Column:	Thermo Scientific Accucore Vanquish C8, 2.6 µm, 4.6 × 150 mm
Mobile phase A:	DI Water
Mobile phase B:	Acetonitrile, Optima LCMS
Detector:	Charged Aerosol and Absorbance, UV 210 nm



Analysis of oxypregnane glycosides in a *Caralluma* supplement extract by HPLC with both CAD and UV detection. Note that many analytes are not detected by HPLC-UV detection and that this approach suffers from baseline perturbation throughout the gradient.



Pregnane glycoside from *Caralluma fimbriata*

PN70543-EN: Improved Universal Approach to Measure Natural Products in a Variety of Botanical and Supplements

thermo scientific

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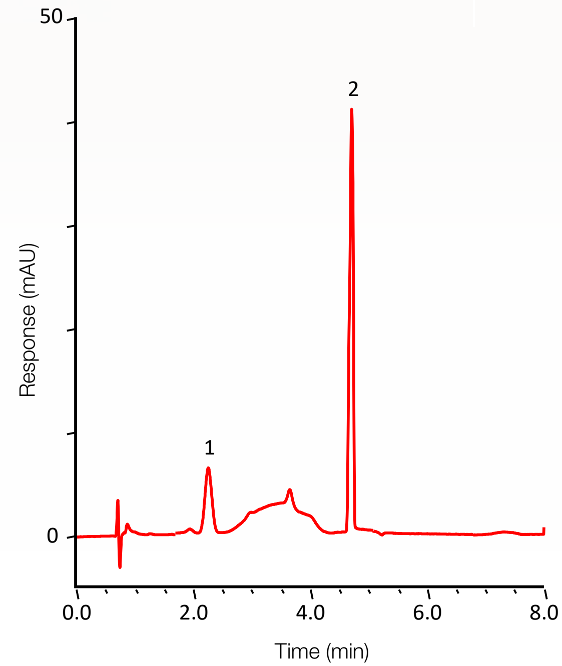
Carnitine

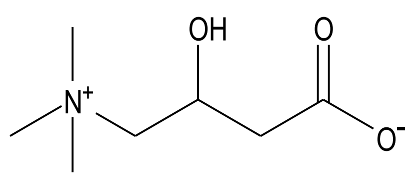
Carnitine functions as part of the system that transports fatty acids into the mitochondria for energy metabolism. Carnitine is produced by the liver, but hepatic synthesis may not always meet the body's needs, so it is sometimes taken as a dietary supplement. Carnitine is highly polar and not well retained on RP-HPLC columns. The Acclaim Trinity P1 column provides cation-exchange, anion-exchange and reversed-phase retention mechanisms, and is found to be ideal for carnitine analysis.

Presented here is an HPLC-UV method using an Acclaim Trinity P1 column for the measurement of carnitine tartrate in a dietary supplement.

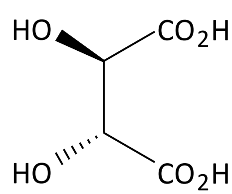
Column:	Thermo Scientific Acclaim Trinity P1, 3 µm, 3.0 x 50 mm
Mobile phase A:	3.00 g Monobasic sodium phosphate (25 mmol), 22 mg Tetrasodium pyrophosphate decahydrate (0.5 mmol) + 270 µL 85% Phosphoric acid (4 mmol) + 196 g Acetonitrile + 750 g DI Water
Mobile Phase B:	196 g Acetonitrile + 750 g DI Water
Detector:	Absorbance, UV 210 nm



HPLC-UV analysis of dietary supplement.
1. Carnitine
2. Tartrate



Carnitine



Tartaric acid

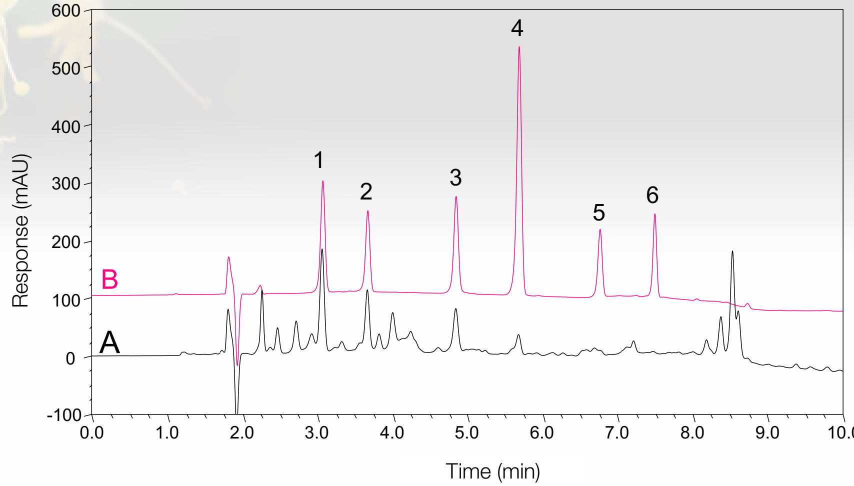
AppsLab 764: Rapid Determination of Carnitine in a Nutritional Supplement using a Thermo Scientific Acclaim Trinity P1 HPLC Column

Caulis lonicerae

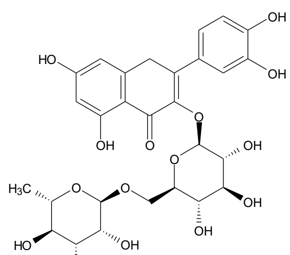
Lonicera japonica, known as Japanese honeysuckle and golden-and-silver honeysuckle, is a twining vine native to eastern Asia. In traditional Chinese medicine *Lonicera japonica* is called ren dong téng or jin yín hua. The dried rattan (caulis lonicerae) leaves and flowers (flos lonicerae) are used to treat fever, headache, cough, thirst, sore throat and epidermal diseases.

Presented here is an HPLC-UV method that can simultaneously measure the six main active components (caffeic acid, chlorogenic acid, galuteolin, loganin, rutin and sweroside) of caulis lonicerae, in under 10 minutes. It is a viable alternative to the two HPLC methods required by The Pharmacopeia of the People's Republic of China for the regulation of this herbal material.

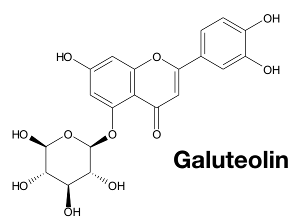
Column:	Thermo Scientific Acclaim Phenyl-1, 3 µm, 4.6 x 150 mm
Mobile phase:	Acetonitrile, 0.4% Formic acid
Detector:	Absorbance, UV 236 nm



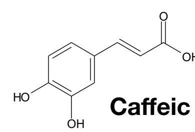
Analysis of a A) caulis lonicerae sample and B) mixture of standards by HPLC-UV.
1. Loganin
2. Sweroside
3. Chlorogenic acid
4. Caffeic acid
5. Rutin
6. Galuteolin



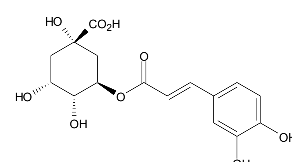
Rutin



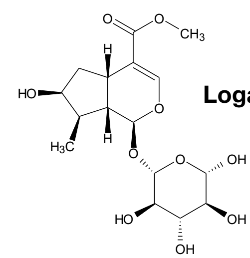
Galuteolin



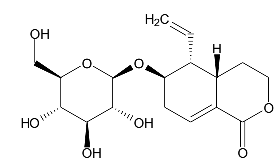
Caffeic acid



Chlorogenic acid



Loganin



Sweroside

AN299: HPLC Analysis of Six Active Components of Caulis Lonicerae Using a Phenyl-1 Column

thermoscientific

Choline

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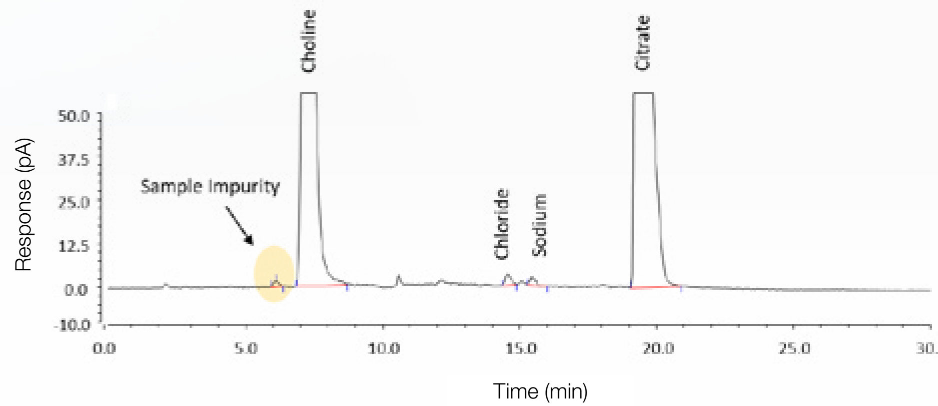
Choline is an essential nutrient involved in cell membrane integrity, cell membrane signaling, lipid transport and metabolism, neurotransmission, fetal brain development, and modulation of gene expression. Although choline can be produced endogenously in the liver, this synthesis is insufficient for human needs. Choline is naturally present in some foods, especially animal-based products (meat, poultry, fish, dairy products and eggs), and in cruciferous vegetables, certain beans, nuts, seeds and whole grains. However, most people in the United States consume less than the adequate intake for choline. Choline deficiency can lead to muscle damage, liver damage and nonalcoholic fatty liver disease. Choline supplements, including choline bitartrate, choline chloride, choline citrate, phosphatidylcholine and lecithin, are used to address this problem.

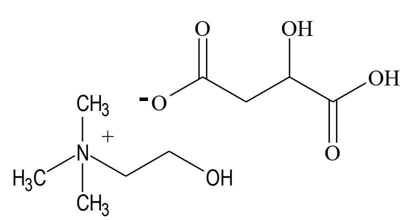
Quality testing is needed to ensure the safety and effectiveness of choline dietary supplements. Choline and its salts possess weak chromophores, and so are not suitable candidates for detection by UV absorbance.

Presented here is a mixed mode (zwitterionic operated in HILIC mode) chromatographic method using CAD to measure choline and its salts. The method is compatible with LC-MS, which was used to identify the impurity O-(2-hydroxyethyl)choline, in choline supplements.

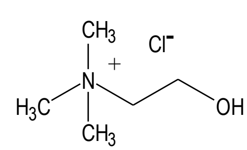


HPLC-CAD measurement of sodium, chloride and sample impurities in a 2 mg/mL choline citrate sample. The sample impurity (RT~6 min) was studied using a flow split to Thermo Scientific Vanquish ISQ EM Single Quadrupole Mass Spectrometer.

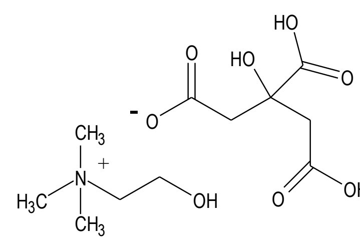
Column:	Zwitterionic HILIC
Mobile phase A:	Ammonium acetate
Mobile Phase B:	Acetonitrile
Detector:	Charged Aerosol



Choline bitartrate



Choline chloride



Choline citrate

PP73732: Quality Control of Choline as a Dietary Supplement by Liquid Chromatography Coupled to a Charged Aerosol Detector

thermoscientific

Cyclotides

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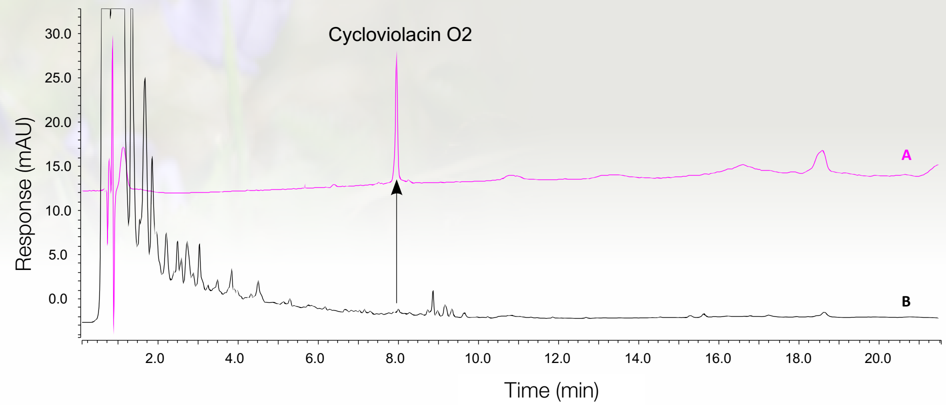
Cyclotides are bioactive peptides that typically contain 28-37 amino acid residues, forming a cyclotide peptide with a structure that is locked in place by three disulfide bonds. More than 100 cyclotides have so far been identified in plants. Cyclotides contain a conserved core of amino acids and a series of hypervariable loops. This suggests they may play an important role in nature, for example protecting the plant from predators and pathogens. Cyclotides are reported to have a wide range of biological activities including insecticidal, antimicrobial, antitumor, anti-HIV, protease inhibitory, hormone-like, cytotoxic and uterotonic properties. Recent research suggests that cyclotides are also novel candidates as scaffolds for peptide drug delivery.

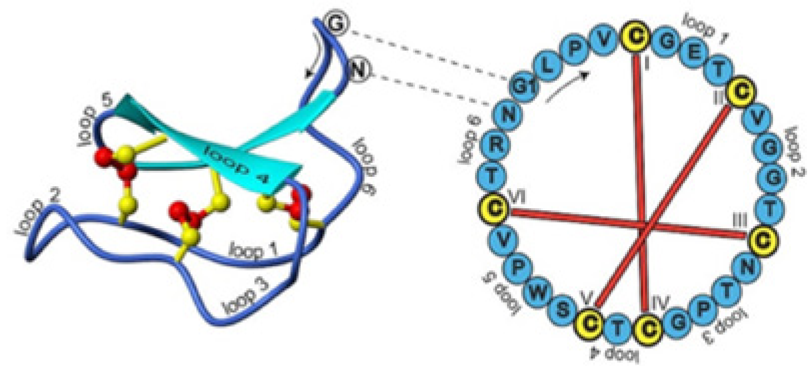
Cyclotides possess strong chromophores so are usually measured using HPLC-UV detection.

Presented here is an HPLC-UV method for the measurement of the cyclotide cycloviolacin O2 in a plant extract.

Column:	Thermo Scientific Acclaim C18, 2.2 µm, 3 x 75 mm
Mobile Phase A:	50 mM Ammonium formate, pH 4.4,
Mobile Phase B:	65% acetonitrile, 15% DI Water, 20% 100mM Ammonium formate pH 4.4
Detectors:	Absorbance, UV 280 nm



HPLC-UV analysis of A) cycloviolacin O2 standard (100 µg/mL) and B) a *Viola odorata* plant extract



PN71010: Cystine, an Essential Determinant of Protein Tertiary Structure, Is Also a Target for Electrochemical Manipulation

thermoscientific

Echinacea

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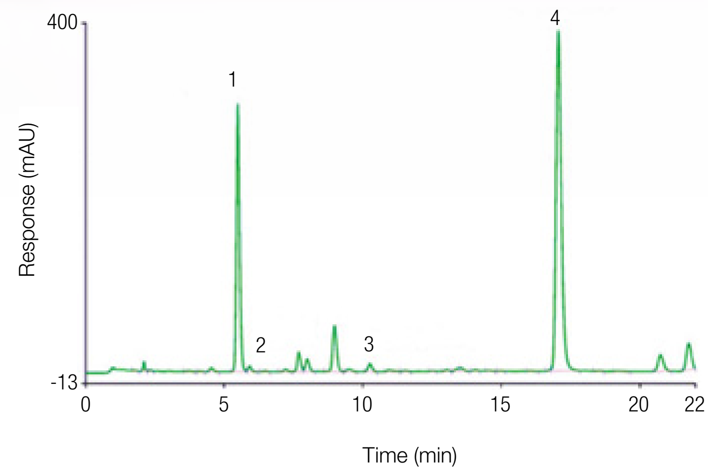
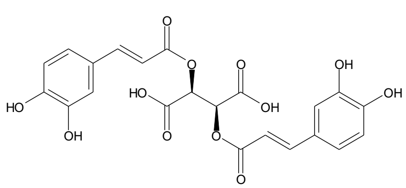
Echinacea is a group of herbaceous flowering plants in the daisy family, commonly called the coneflower. Three species of Echinacea are used as herbal remedies, including *Echinacea angustifolia, Echinacea pallida* and *Echinacea purpurea. Echinacea angustifolia* was widely used by the North American Plains Indians for its supposed medicinal qualities. Today, as an herbal supplement, echinacea is consumed in teas, liquid extracts, as a dried herb, or capsules or pills to reduce many of the symptoms of the common cold, flu and some other illnesses, infections and conditions. However, studies to date have not reported any benefit of echinacea on common cold prevention or duration.

Presented here is an HPLC-UV absorbance method for measuring key phenolic compounds, derivatives of caffeic acid, in *echinacea* extracts.

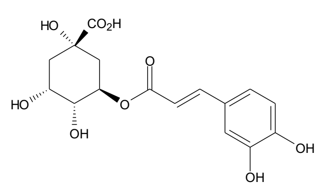
Column:	C18, 3 µm, 4.6 x 150 mm
Mobile Phase A:	Acetonitrile/DI Water (90:10 v/v)
Mobile Phase B:	Acetonitrile/DI Water/Phosphoric acid (27:75:0.1 v/v/v)
Column Temp.:	25°C
Flow rate:	1.5 mL/min
Inj. volume:	25 µL
Gradient:	100 % A to 100 % B 20 min; hold 100% B 2 min
Detector:	Absorbance, UV 330 nm



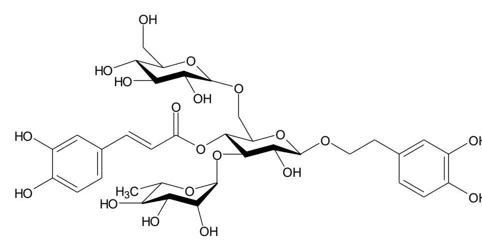
Determination of phenolic compounds in Echinacea extracts.
1. Cattaric acid
2. Chlorogenic acid
3. Echinacoside
4. Cichonic acid



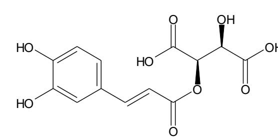
Cichoric acid



Chlorogenic acid



Echinacoside



Cattaric acid

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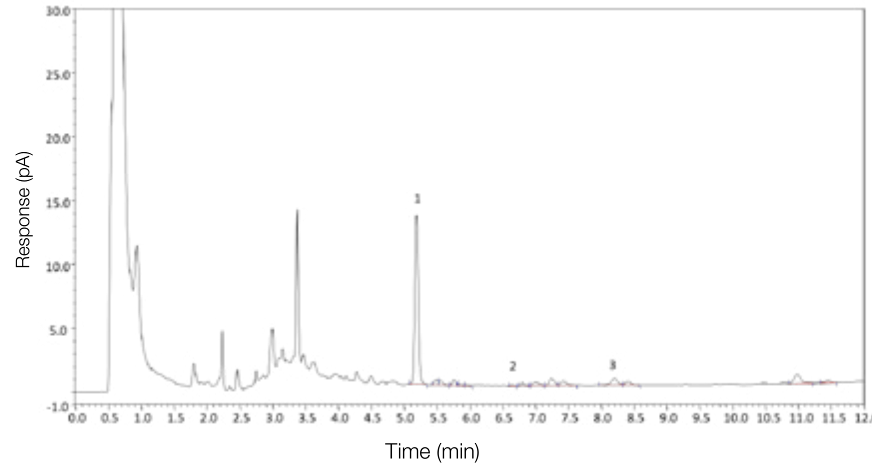
Falcarinol, falcarindiol and falcarindiol-3-acetate

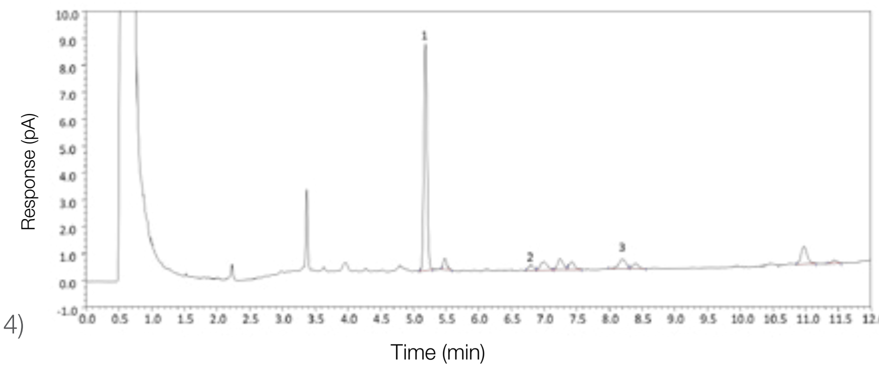
Some plants in the *Apiaceae* (or *Umbelliferae*) family (e.g., carrots, parsnips, parsley and celery) produce a group of bioactive C17-polyacetylene compounds, the polyacetylenic oxylipins, in response to pathogens. Three such compounds - falcarinol, falcarindiol and falcarindiol-3-acetate - are natural pesticides and highly toxic towards bacteria and fungi. They also 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).

Falcarinol and related compounds contain weak chromophores so are usually measured using low-wavelength UV absorbance (205 nm), but sample chromatograms tend to be complex due to the presence of many other compounds absorbing at this wavelength. HPLC-CAD overcomes limitations of HPLC-UV and is much more sensitive.

Presented here is an HPLC-CAD method for the analysis falcarinol and related compounds showing results from parsnip skin and parsnip core extracts.

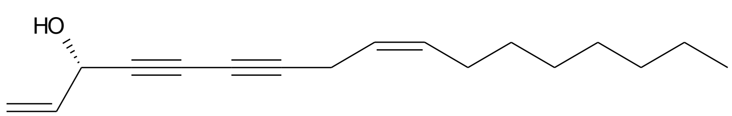
Column:	Thermo Scientific Acclaim RSLC 120 C18, 2.2 µm, 2.1 x 150 mm
Mobile phase A:	Methanol: DI Water: Acetic acid (500 : 500 : 4)
Mobile phase B:	Acetone: Methanol: Tetrahydrofuran: Acetic acid (500 : 375 : 125 : 4)
Detector:	Charged Aerosol





HPLC-CAD analysis of A) parsnip skin extract and B) parsnip core extract.

1. Falcarindiol
2. Falcarindiol-3-acetate
3. Falcarinol



Falcarinol

PN-2023: Simple and Direct Analysis of Falcarinol and other Polyacetylenic Oxylipins in Carrots by Reverse Phase HPLC and Charged Aerosol Detection

Flavonoids - anthocyanins

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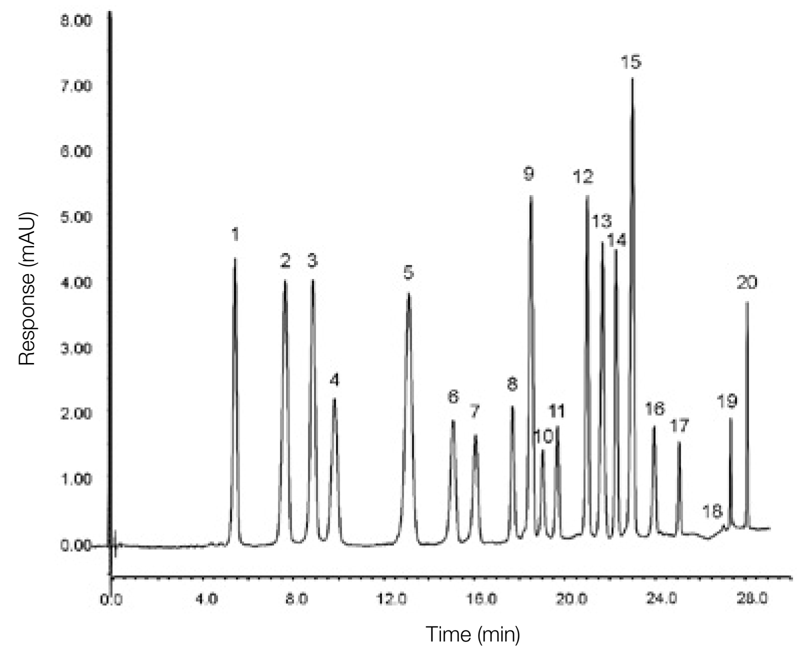
Anthocyanins and their aglycones, the anthocyanidins, are flavonoid plant pigments. Anthocyanins are particularly abundant in both the European bilberry (*Vaccinium myrtillus* L.) and North American blueberry (*Vaccinium corymbosum*). However, fresh bilberries contain four times more anthocyanin than fresh blueberries. 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.

Presented here is HPLC method using absorbance detection for the analysis of anthocyanins and anthocyanidins.

HPLC Column:	Thermo Scientific Acclaim 120 C18, 2.2 µm, 2.1 x 150 mm
Mobile Phase A:	10 % Formic acid
Mobile Phase B:	10 % Formic acid, 22.5% Methanol, 22.5% Acetonitrile
Detector:	Absorbance, Vis 520 nm

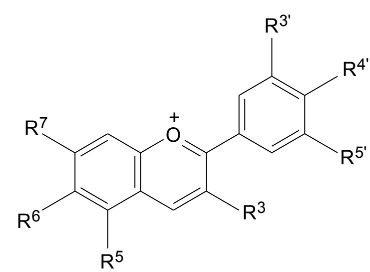
Anthocyanidin	R3	R5	R6	R7	R3'	R4'	R5'
Pelargonidin	OH	OH	H	OH	H	OH	H
Cyanidin	OH	OH	H	OH	OH	OH	H
Delphinidin	OH	OH	H	OH	OH	OH	OH
Petunidin	OH	OH	H	OH	OCH_3	OH	OH
Peonidin	OH	OH	H	OH	OCH_3	OH	OH
Malvidin	OH	OH	H	OH	OCH_3	OH	OCH_3



Separation of a fifteen anthocyanin standard and five anthocyanidins on the solid core Acclaim RSLC C18 column.

1. Dp3Gal
2. Dp3Glu
3. Cy3Gal
4. Dp3Ara
5. Cy3Glu
6. Pet3Gal
7. Cy3Ara
8. Delphinidin
9. Pet3Glu
10. Peo3Gal
11. Pet3Ara
12. Peo3Glu
13. Mal3Gal
14. Peo3Ara
15. Cyanidin
16. Mal3Glu
17. Mal3Ara
18. Petunidin
19. Peonidin
20. Malvidin

Abbreviations are defined in AN281



AN1042: Rapid Separation of Anthocyanins in Cranberry and Bilberry Extracts Using a Core-Shell Particle Column

AN281: Rapid and Sensitive Determination of Anthocyanins in Bilberries Using UHPLC

AN264: Fast Determination of Anthocyanins in Pomegranate Juice

thermo scientific

Flavonoids - anthocyanins

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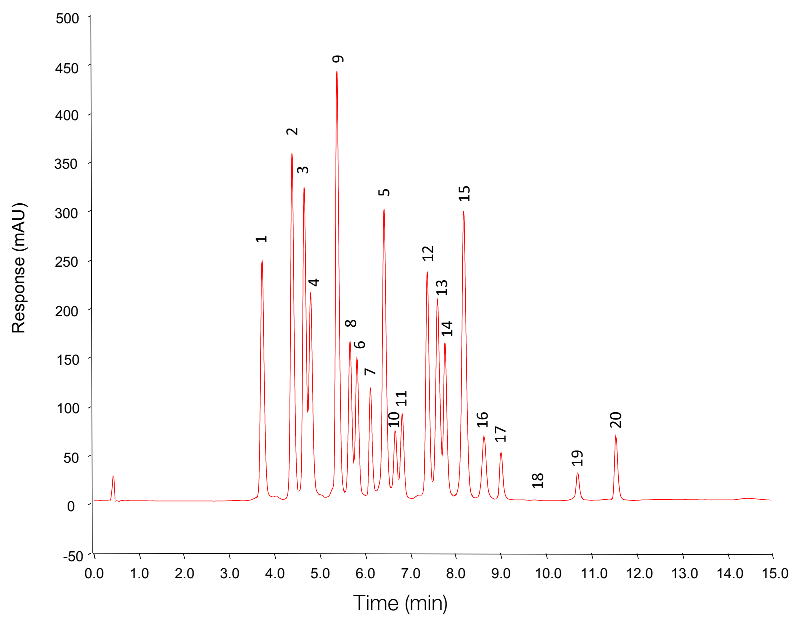
HPLC columns with fully porous 2.2µm and solid core-porous shell particles are two of the newer developments in separation science. Both the fully porous Thermo Scientific Accucore C18 HPLC Column and solid core Acclaim Rapid Separation LC (RSLC) 120, C18 Analytical Column can be used for high-speed, high-resolution separations without excessive backpressure.

Presented here is a simple and rapid HPLC-UV method for the simultaneous determination of anthocyanins and anthocyanidins in natural products.

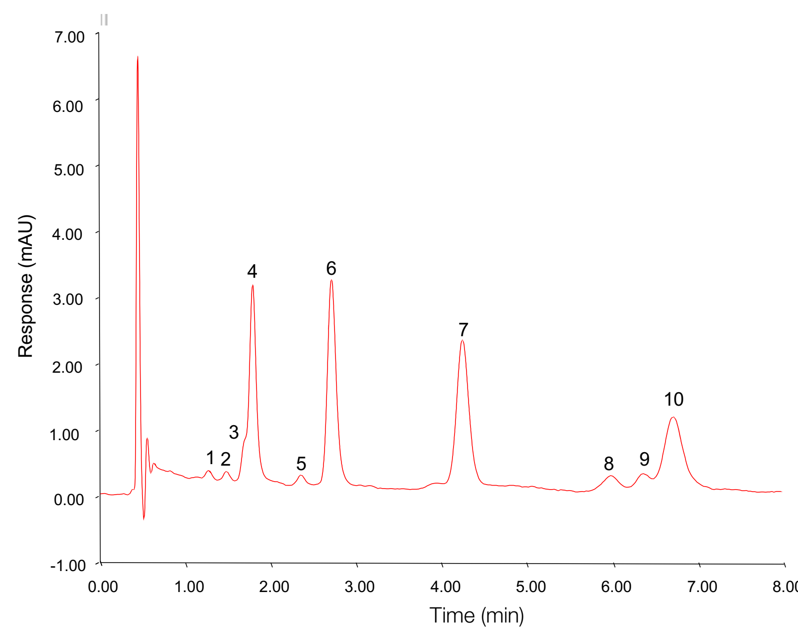
HPLC Column:	Thermo Scientific Accucore C18, 2.6 µm, 2.1 × 150 mm
Mobile Phase A:	10 % Formic acid
Mobile Phase B:	10 % Formic acid, 22.5% Methanol, 22.5% Acetonitrile
Detector:	Absorbance, Vis 520 nm



Separation of 15 anthocyanins and 5 anthocyanidins standards using a fully porous Accucore C18 column using conditions shown to left.

1. Dp3Gal
2. Dp3Glu
3. Cy3Gal
4. Dp3Ara
5. Cy3Glu
6. Pet3Gal
7. Cy3Ara
8. Delphinidin
9. Pet3Glu
10. Peo3Gal
11. Pet3Ara
12. Peo3Glu
13. Mal3Gal
14. Peo3Ara
15. Cyanidin
16. Mal3Glu
17. Mal3Ara
18. Petunidin
19. Peonidin
20. Malvidin

Abbreviations are defined in AN281.



Separation of anthocyanins in cranberry extract using a fully porous Accucore C18 column. See AN1042 for modifications to flow rate, temperature and gradient conditions.

1. Dp3Gal
2. Dp3Glu
3. Unknown
4. Cy3Gal
5. Cy3Glu
6. Cy3Ara
7. Peo3Gal
8. Peo3Glu
9. Peo3Ara
10. Mal3Gal

Abbreviations are defined in AN281.

AN1042: Rapid Separation of Anthocyanins in Cranberry and Bilberry Extracts Using a Core-Shell Particle Column

AN281: Rapid and Sensitive Determination of Anthocyanins in Bilberries Using UHPLC.

AN264: Fast Determination of Anthocyanins in Pomegranate Juice

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Flavonoids - catechins

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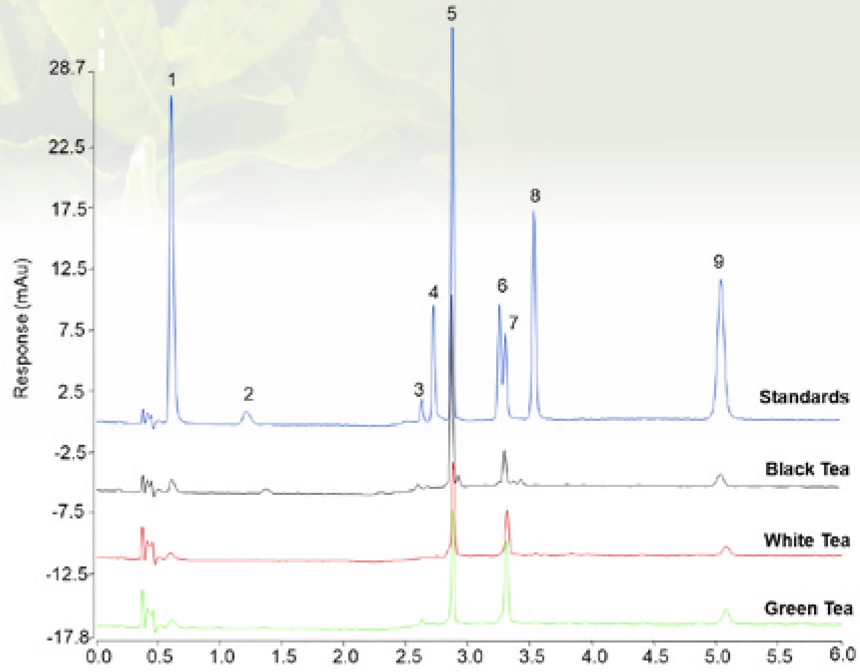
Catechins are flavonoids found primarily in green tea, and in smaller amounts, in grapes, black tea, chocolate and wine. Catechins are potent antioxidants *in vitro* and some suggest they provide protection against certain diseases, such as cardiovascular disease and cancer. In North America, the consumption of green tea products increased due to reported health benefits. However, commercially available teas show a high variability in catechin content, so simple and rapid methods are needed to evaluate product quality.

Presented here is a study that evaluates a Thermo Scientific Accucore C18 High-Performance LC (HPLC) column to rapidly (<6 min) determine catechins in three different types of tea.

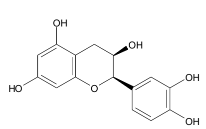
Column:	Thermo Scientific Accucore C18, 2.6 µm, 2.1 × 150 mm
Mobile phase A:	2.5 % aq Acetonitrile
Mobile phase B:	0.1 % THF in Acetonitrile
Detector:	Absorbance, UV 280 nm



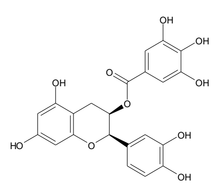
Analysis of catechins and caffeine in tea samples.

1. Gallic acid
2. Gallocatechin
3. Epigallocatechin
4. Catechin
5. Caffeine
6. Epicatechin
7. Epigallocatechin gallate
8. Gallocatechin gallate
9. Epicatechin gallate

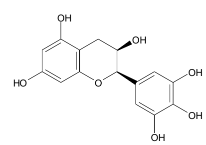
Structures of catechins from *Camellia sinensis*



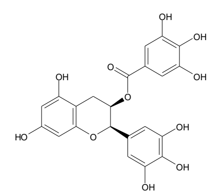
Epicatechin



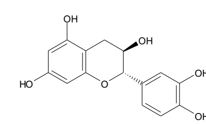
Epicatechin gallate



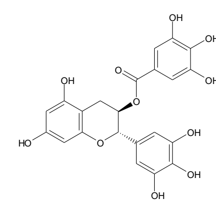
Epigallocatechin



Epigallocatechin gallate



Gallocatechin



Gallocatechin gallate

AB150: Rapid separation of catechins in tea using core-shell columns

AN275: Sensitive determination of catechins in tea by HPLC

AN20536: Analysis of Catechins Using an Accucore XL C8 4 µm HPLC Column

AN20583: Determination of Catechins and Phenolic Acids in Red Wine by Solid Phase Extraction and HPLC

Flavonoids - Flos chrysanthemi indici

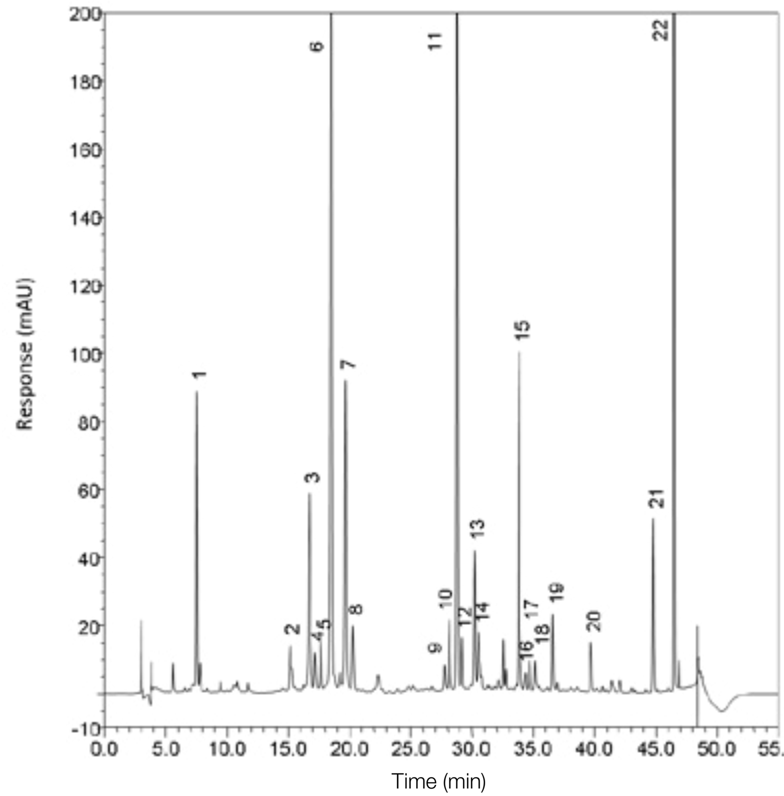
Table of contents

Flos chrysanthemi indici, *Dendranthema indicum* L. (D. *indicum*), is a common medicinal plant known in China as wild chrysanthemum (ye ju hua). The Chinese Pharmacopeia (CP) Edition 2005 regulates its use as a traditional Chinese medicine (TCM) It is used with the belief that it improves eyesight and cures fever, swelling, erysipelas (a bacterial infection of the skin), sore throat, and headache.

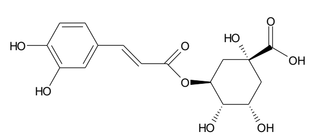
Unfortunately, another plant, *Dendranthema lavandulaefolium* (Fish) Mak, in the same genus as D. *indicum* and growing in the same types of environments, appears similar to *Flos chrysanthemi indici*. It is not approved for use as a TCM and often mislabeled as *Flos chrysanthemi indici* when the plant is harvested. HPLC methods described in CP 2005 (see AN207) are inadequate and inaccurate and are not suitable for use in quality control of *Flos chrysanthemi indici.*

Presented here is an HPLC-UV method for fingerprinting *Flos chrysanthemi indici* based on measurement of discriminating peaks, including chlorogenic acid and flavonoids (luteolin-7-o-glucoside, linarin, luteolin and apigenin). Accelerated Solvent Extraction (ASE®) was used for isolation of the target components from the herbal samples.

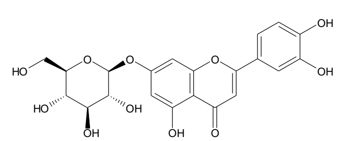
Column:	Thermo Scientific Acclaim C18, 5 µm, 4.6 x 250 mm
Mobile Phase A:	Acetonitrile
Mobile Phase B:	0.1 % Acetic acid
Detectors:	Absorbance, UV 326 nm



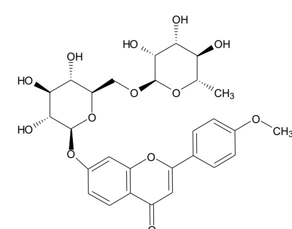
Chromatogram of a *Flos chrysanthemi indici* sample (purchased from Tongrentang, Beijing).
1. Chlorogenic acid
3. Luteolin-7-O-glucoside
6. Apigenin
11. Linarin
12. Luteolin-1



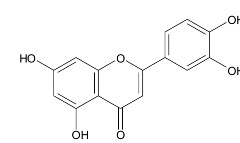
Chlorogenic acid



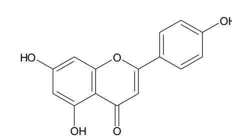
Luteolin-7-O-glucoside



Linarin



Luteolin



Apigenin

AN207: Chromatographic fingerprinting of chrysanthemi indici using HPLC

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Flavonoids - hesperidin

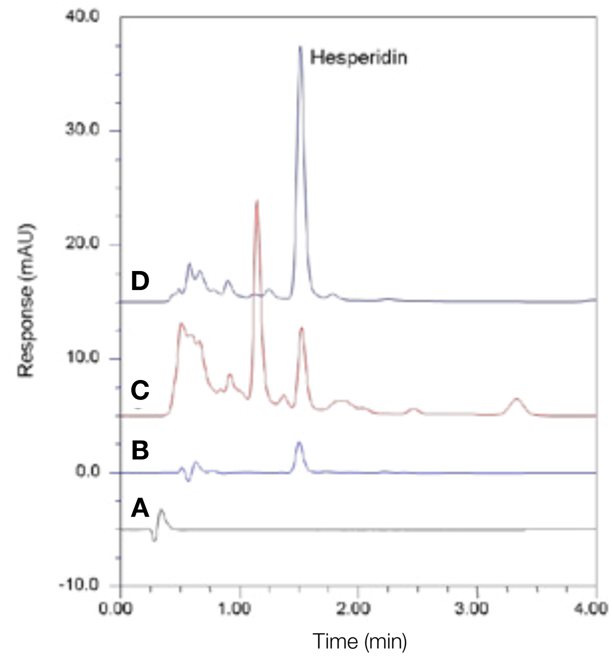
Table of contents

Hesperidin, a flavanone glycoside, is the predominant flavonoid in orange peel and other citrus fruits. Hesperidin is an antioxidant suggested to enhance the action of vitamin C to lower cholesterol levels. It is reported to have pharmacological action as an anti-inflammatory, antihistaminic and antiviral agent.

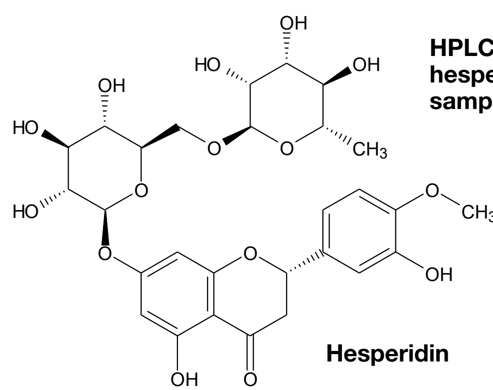
The Pharmacopeia of the People's Republic of China (PPRC) 2010 recommends its extraction from fruits with a Soxhlet extraction method using ligarine and methanol. This method is both time- and solvent-consuming, requiring ≥5 hours and >200 mL of ligarine and methanol for each sample. The PPRC 2010 also recommends determination with a 12-minute RP-HPLC method (See AB142).

Presented here is a more efficient and cost-effective HPLC-UV absorbance method to determine hesperidin extracted from orange peel and other citrus fruits using Accelerated Solvent Extraction (ASE®).

Column:	Thermo Scientific Accucore C18, 2.6 µm, 4.6 × 150 mm
Mobile Phase A:	DI Water
Mobile Phase B:	Acetonitrile, Optima LCMS
Detector:	Absorbance, UV 210 nm



HPLC-UV chromatograms of A) mobile phase; B) hesperidin standard (2 µg/mL); C) orange peel sample; D) lemon peel sample (50-fold dilution).



Hesperidin

AB142: Rapid determination of hesperidin in orange peel using accelerated solvent extraction and UHPLC

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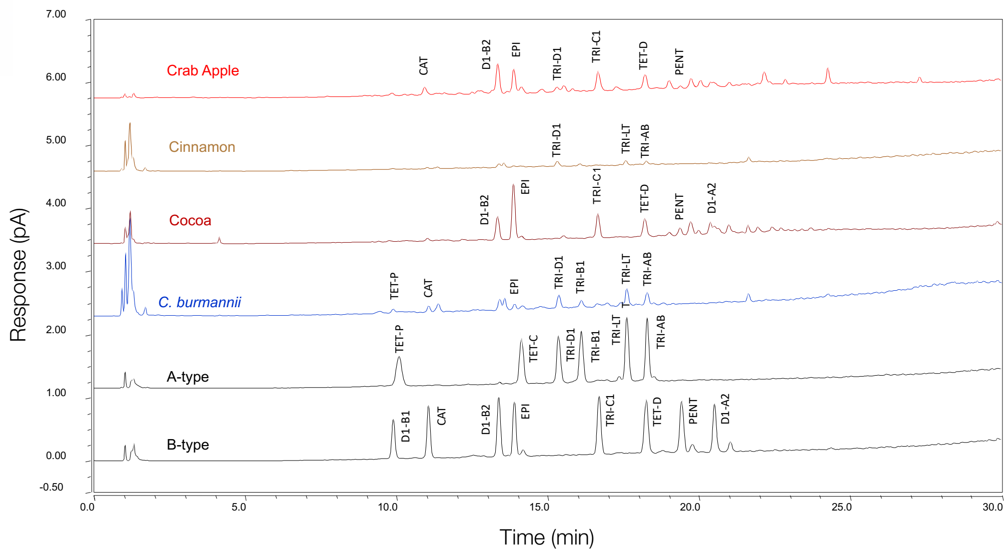
Flavonoids - procyanidins

Table of contents

Procyanidins are members of the proanthocyanidin class of flavonoids. They are polyphenols consisting of polymerized subunits of epicatechin or catechin and are structurally highly diverse because of the many possible combinations of subunits, type of bonding and branching. Procyanidins, after lignans, are the second most common class of natural phenolic substances found in nature. They are abundant in many foods, with apples and cocoa being most prominent in the Western diet. Procyanidins are purported to have many health benefits, including anti-inflammatory, hypoglycemic, insulin activation, antioxidant, hypocholesterolemic and anti-allergic properties. Particularly important may be a connection between procyanidin consumption and the lowering of risk of cardiovascular disease. To correlate dietary intake of procyanidins with an impact on disease prevention and amelioration, there is a need to develop new, as well as improved, analytical methodologies for pharmacological studies and the standardization of foods and dietary supplements.

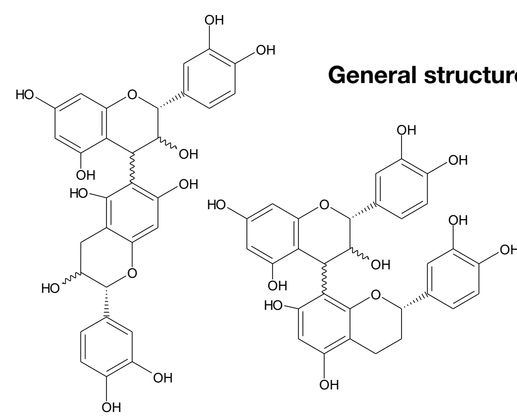
Presented here is an HPLC-CAD method for the determination of individual procyanidins in various sample matrices, including extracts of crab apple, cocoa and cinnamon.

Column:	Thermo Scientific Acclaim 120 C18, 3 µm, 3 × 150 mm,
Mobile Phase A:	0.05% Formic acid, 3% Acetonitrile, 0.2% Tetrahydrofuran
Mobile Phase B:	0.05% Formic acid, 50% Acetonitrile, 10% Tetrahydrofuran
Mobile Phase C:	90% Methanol
Detector:	Charged Aerosol



HPLC-CAD analysis of A-type and B-type procyanidins in plant extracts. CAT – catechin; EPI – epicatechin.

General structures of B-Type procyanidins



B5: EC-(4β⟶6)-EC
B7: EC-(4β⟶6)-C
B8: C-(4α⟶6)-EC
B6: C-(4α⟶6)-C

B2: EC-(4β⟶8)-EC
B1: EC-(4β⟶8)-C
B4: C-(4α⟶8)-EC
B3: C-(4α⟶8)-C

PN71527: Determination of A-Type and B-Type Procyanidins in Apple, Cocoa and Cinnamon Extracts

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Giant knotweed rhizome

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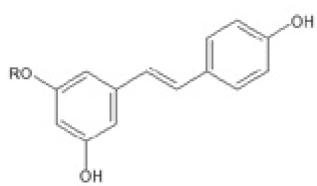
Giant knotweed rhizome, the dried rhizome and root of Polygonum *cuspidatum* Sieb. et Zucc. is a common medicinal plant in China. Chinese Pharmacopeia Edition 2005 regulates its use as an herbal medicine. It is used to treat angiocardiopathy, skin inflammations and liver diseases, reduce fever, and relieve arthritis.

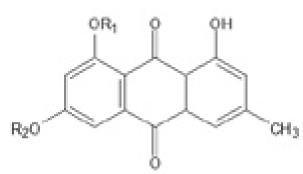
Purported active components, include anthraquinones (e.g., anthraglycoside A, anthraglycoside B, emodin, physcion, rhein, and chrysophanol) and stilbenes (e.g., resveratrol and polydatin).

Presented here is a method that uses an ASE® 200 Accelerated Solvent Extractor for efficient and reproducible sample preparation, and an HPLC-DAD method for simultaneous measurement of all key analytes.

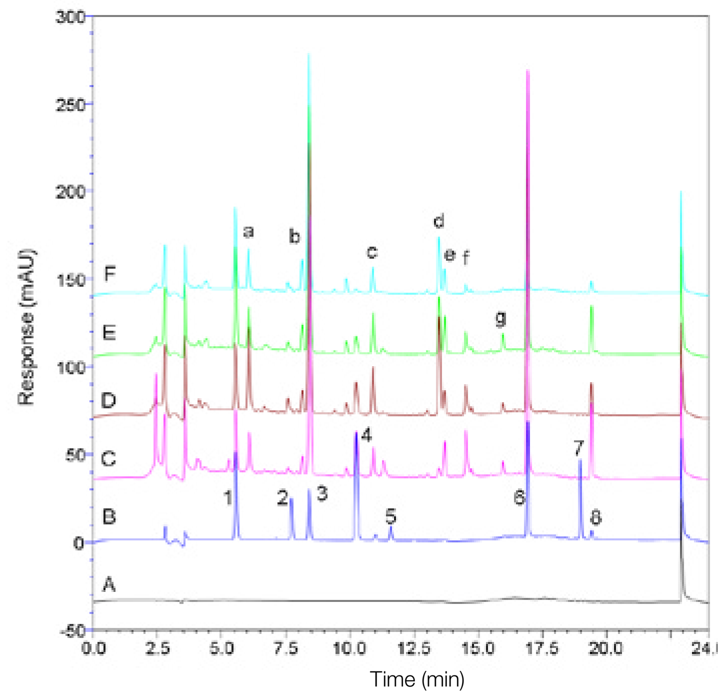
HPLC Column:	Thermo Scientific Acclaim 120 C18, 5 µm, 4.6 x 250 mm
Mobile Phase A:	Acetonitrile
Mobile Phase B:	20 mM Ammonium acetate
Detector:	Absorbance, Vis 254 nm



	R
Resveratrol	H
Polydatin	Glucose

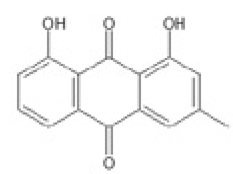


	R_1	R_2
Anthraglycoside A	Glucose	Me
Anthraglycoside B	Glucose	H
Emodin	H	H
Physcion	H	Me

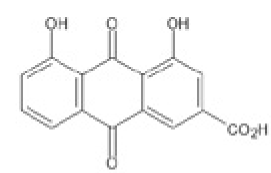


Overlay of chromatograms of A) water, B) the mixed standard, and C–F) samples.

1. Polydatin
2. Resveratrol
3. Anthraglycoside B
4, Anrthaglycoside A
5. Rhein
6. Emodin
7. Chrysophenol
8. Physcion



Chrysophanol



Rhein

AN232: Determination of Anthraquinones and Stilbenes in Giant Knotweed Rhizome by HPLC with UV detection.

thermoscientific

Gingko

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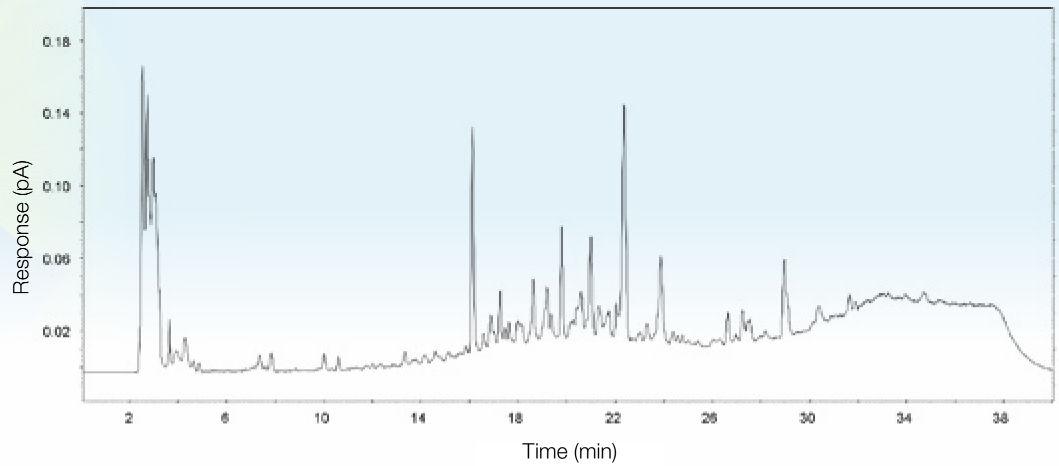
Ginkgo biloba (known as ginkgo) is an ancient species and the only member of the Ginkgophyta. Its leaf is used in traditional medicine, while ginkgo nuts are used as food. Extracts of Ginkgo biloba leaf sold as a dietary supplement are marketed as being beneficial for cognitive function (improving memory and enhancing concentration), and in treating a number of other health issues, including high blood pressure, peripheral arterial disease, macular degeneration, tinnitus and altitude sickness. However, there is no conclusive evidence that ginkgo is helpful for any health condition.

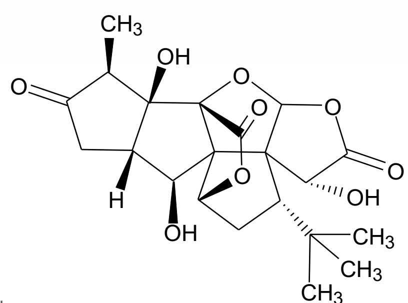
The purported biologically active compounds present in gingko extracts are the sesquiterpenoid bilobalide and numerous diterpenoid gingkolides. These compounds contain weak chromophores so measurement by HPLC with low-wavelength UV is limited, lacking sensitivity and generating complex chromatograms.

Presented here is a method for more comprehensive and sensitive analysis using HPLC-CAD.

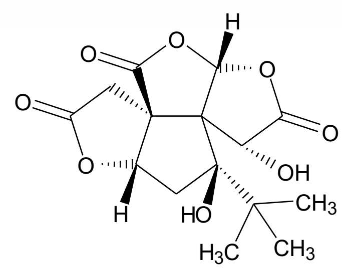
Column:	C18, 5 µm, 4.6 x 250 mm
Mobile phase A:	5 % Acetonitrile in 0.1 % Trifluoroacetic acid
Mobile phase B:	70 % Acetonitrile in 0.1 % Trifluoroacetic acid
Detector:	Charged Aerosol



The use of HPLC-CAD to profile a ginkgo supplement extract.



Gingkolide



Bilobalide

Apps Lab 2366: AN1048: Novel, Universal Approach for the Measurement of Natural Products in a Variety of Botanicals and Supplements

USP Monograph: Content of flavonol glycosides in ginkgo tablets using a C18 HPLC column

thermo scientific

Ginseng

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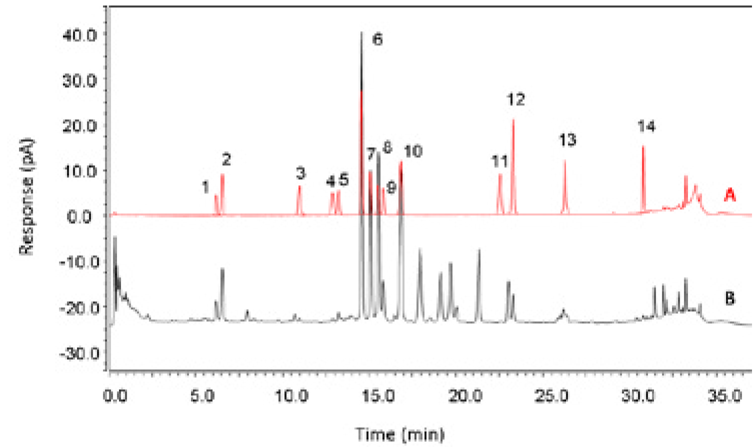
The name ginseng applies to various herbs that have been used in traditional medicines for many centuries. Asian ginseng (*Panax ginseng*), also known as red ginseng or Korean ginseng, is one of several types of true ginseng. Another is American ginseng, *Panax quinquefolius*, which is traditionally used by Native Americans. However, Siberian ginseng (*Eleutherococcus senticosus*) is not a true ginseng. Ginseng is used to improve memory, fatigue, menopause symptoms and insulin response in people with mild diabetes. Ginseng-infused teas and energy drinks are consumed daily in China and neighboring countries as a tonic for vitality. The dried root is also used in foods.

The main bioactive compounds contained in *Panax ginseng* are the ginsenoside triterpene saponins, including the protopanazatriols (Rg1, Re and Rf) and protopaxadiols (RB1, Rc, Rb2 and Rd). *Panax quinquefolius* contains the same suite of ginsenosides except for Rf. Gradient RP-HPLC with low-wavelength UV detection is typically used to measure ginsenosides as they lack strong chromophores. However, this approach typically results in strongly sloping baselines that complicate peak integration, and interferences from minor components that have stronger UV chromophores than the ginsenosides. HPLC-CAD does not suffer these drawbacks.

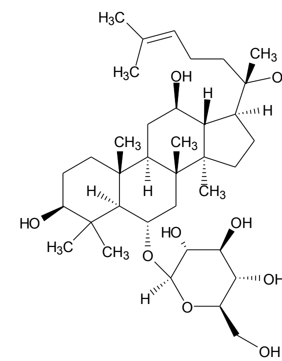
Presented here us an HPLC-CAD method that can be used for the routine measurement of ginsenosides in ginseng samples.

Column:	Thermo Scientific Acclaim RSLC PA, 2.2 µm, 2.1 x 100 mm
Mobile phase A:	DI Water
Mobile phase B:	Acetonitrile
Detector:	Charged Aerosol



HPLC-CAD analysis of A) ginsenoside standards and B) ginseng extract.

1. Rg1	4. Rh1	7. Rc	10. RD	13. Rh2
2. Re	5. Rg2	8. Rb2	11. SRg3	14. PPD
3. Rf	6. Rb1	9. Rb3	12. PPT	



Ginsenoside Rg_1

AN1048: Novel, Universal Approach for the Measurement of Natural Products in a Variety of Botanicals and Supplements

CAN112: Determination of ginsenosides in Panax ginseng by HPLC-CAD

PN70153: Can High Peak Capacity and Universal Detection Solve the Challenges in LC Characterization of Botanicals and Natural Products

AN192: Rapid analysis of ginseng using accelerated solvent extraction and HPLC

thermo scientific

Gotu Kola

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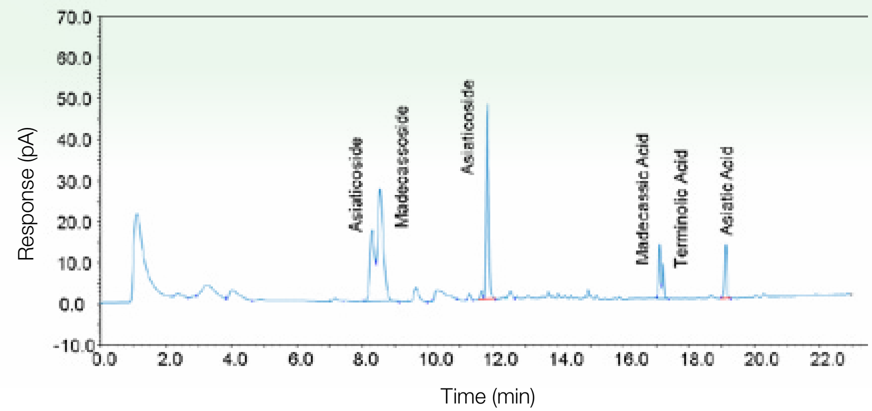
Centella asiatica (commonly called gotu kola) is a small herbaceous annual plant native to India, Sri Lanka, other parts of Asia, Northern Australia and the Western Pacific. It is employed as a medicinal herb in Ayurvedic medicine and traditional Chinese medicine to treat a wide variety of conditions. These include its use for improving memory and blood flow, as an agent for wound-healing, an anti-epileptic and an antidepressant, and for topical application in skin conditions such as ulcers, wounds and eczema. The chemical compounds of interest in gotu kola are usually considered to be the ursane- and oleanane-type triterpenes and the triterpene glycosides.

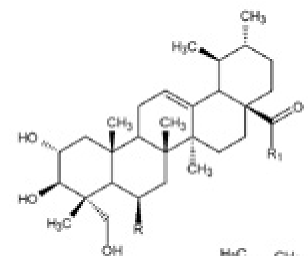
Gradient RP-HPLC with low-wavelength UV detection is typically used to measure total triterpenes as they lack strong chromophores. However, this approach often results in strongly sloping baselines that complicate peak integration, and interferences from minor components that have stronger UV chromophores than the triterpenes.

Presented here is an HPLC-CAD method that does not suffer these drawbacks and can be used for the routine measurement of total triterpenes in gotu kola samples.

Column:	Fused-Core C18, 2.7 µm, 3.0 × 100 mm,
Mobile Phase A:	0.1 % Formic acid in DI Water
Mobile Phase B:	Acetonitrile
Detector:	Charged Aerosol

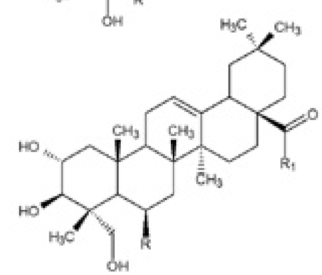


Analysis of a gotu kola extract by HPLC-CAD.



Ursane-type triterpenes

Asiatic acid: R = H, R_1 = OH
Madecassic acid: R = OH, R_1 = OH
Asiaticoside: R = H; R_1 = O-Glu(6-1)-Glu(4-1)-Rham
Madecassoside: R = OH, R_1 = O-Glu(6-1)-Glu(4-1)-Rham

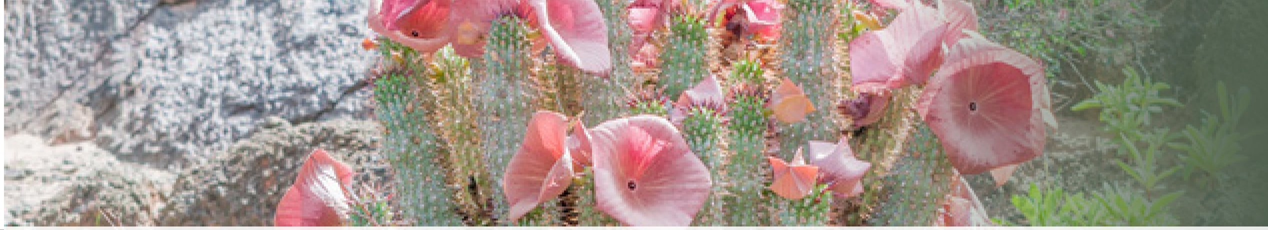


Oleanane-type triterpenes

Terminoloc acid: R = OH, R1 = OH
Asiaticoside B: R = OH, R1 = O-Glu(6-1)-Glu(4-1)-Rham

AN1048: Novel, Universal Approach for the Measurement of Natural Products in a Variety of Botanicals and Supplements

CAN111: Determination of Triterpenes in Centella asiatica (Gotu Kola) by HPLC-CAD

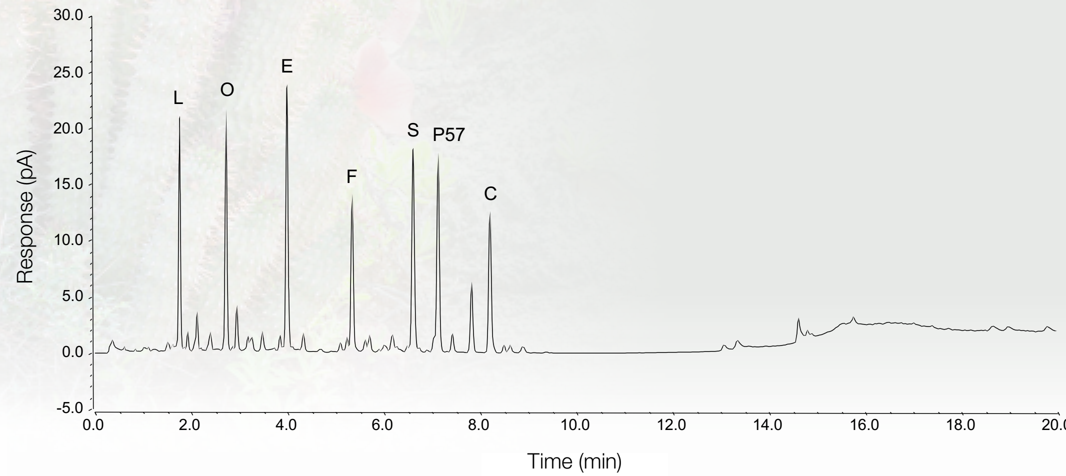


Hoodia

Hoodia, scientific name *Hoodia gordonii*, is a flowering succulent that grows in the Kalahari Desert in Africa. Historically, the San Bushmen used hoodia to suppress hunger during long hunts. Although dietary supplements are used as an appetite suppressant for weight loss, published clinical evidence that hoodia aids weight loss is currently lacking.

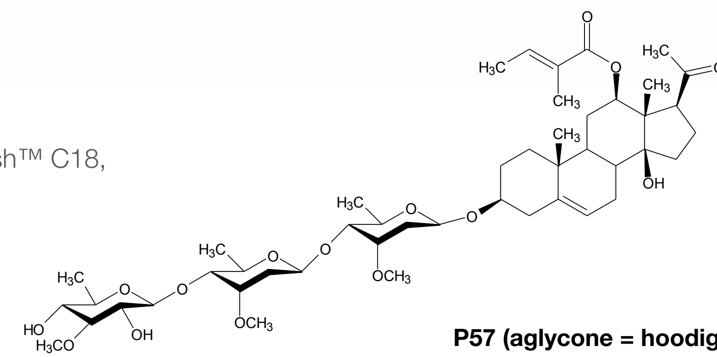
The alleged biological effects of hoodia may be due to several oxypregnane steroidal glycosides, the hoodigosides, that are abundant in *Hoodia gordonii*. One, P57, may be responsible for its putative appetite-suppressant effect. Measuring hoodigoside content is essential in determining whether commercial products actually contain *Hoodia gordonii*. As these compounds contain weak chromophores, measurement by HPLC with low- wavelength UV is limited, lacks sensitivity and generates complex chromatograms.

Presented here is a sensitive HPLC-CAD method capable of profiling numerous hoodigosides in supplements and plant extracts.



Analysis of hoodigosides C, E, F, L, O, S and P57 standards by UHPLC-CAD.

Column:	Thermo Scientific™ Accucore™ Vanquish™ C18, 1.5 µm, 2.1 ×100 mm
Mobile phase A:	DI Water
Mobile phase B:	Acetonitrile, Optima LCMS
Detector:	Charged Aerosol



P57 (aglycone = hoodigogenin)

PN70543: Improved universal approach to measure natural products in a variety of botanicals and supplements

thermo scientific

Hops

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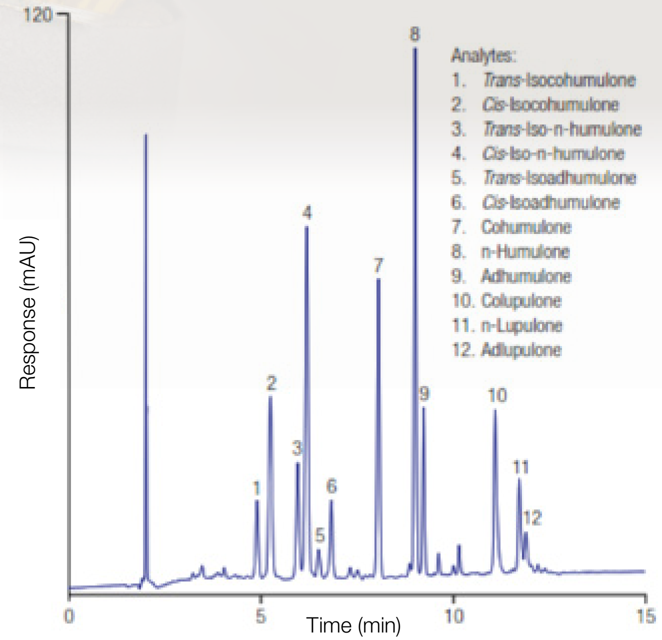
Since ancient times, hops (*Humulus lupulus* L.) have been a main ingredient of beer, used not only to provide bitterness, but also for their purported human health benefits (antioxidant, anti-inflammatory, immunosuppressive and chemopreventitive properties). Hop supplements are used as a natural alternative to traditional hormone therapy to relieve menopausal symptoms in older women.

The chemistry of hops during beer production is complex. Isohumulones (iso-α-acids), formed from humulones (α-acids), are the essential bitter constituents of hop resin. The poorly water-soluble α-acids are isomerized to the water-soluble iso-α-acids during wort-boiling. Their antimicrobial effect leads to a sterile beverage, their tensioactive character stabilizes the foam, and they have a major influence on the general flavor, smell and smoothness of beer. The three major iso-α-acid variants, differing in their acyl side chain, include the iso-n-humulones, iso-cohumulones and iso-adhumulones. Due to the stereochemistry of iso-α-acids, they all occur as cis- and trans-isomers. The lifetimes of cis- and trans-isomers significantly differ from each other. Degradation products of iso-α-acids sensitively impact the important beer attributes mentioned above and avoidance of less stable iso-α-acid variants is beneficial.

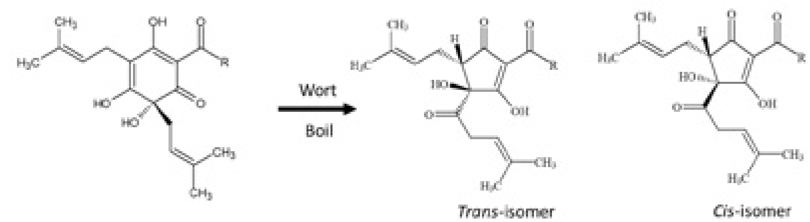
Monitoring the isomerization progress, as well as the general content of iso-α-acids in beer during and after the brewing process, is mandatory in order to control important beer properties.

Presented here is an application that uses online solid phase extraction (SPE) with UHPLC-UV absorbance detection, to rapidly profile all key analytes in untreated beer samples.

Column:	Thermo Scientific Hypersil GOLD C18, 1.9 µm, 2.1 × 100 mm
Mobile Phase A:	1% aq Formic acid containing 100 mg/L EDTA
Mobile Phase B:	Acetonitrile, Optima LCMS
Detector:	Absorbance, UV 270 nm



Online SPE, UHPLC-UV analysis of a German Pilsner beer sample.



Cohumulone	R = $CH(CH_3)_2$	Iso-cohumulone
N-Humulone	R = $CH_2CH(CH_3$	Iso-n humulone
Adhumulone	R = $CH(CH_3)CH$	Iso-adhumulone

AB153: Savor the flavor – robust iso-a-acids assaying in beer within ten minutes
AB155: Monitor the brewing process with LC – transformation of hop a-acids into beer iso-a-acids
AB156: The everlasting paradigm – keep beer tradition or prevent beer from a skunky off-flavor?

Mangosteen

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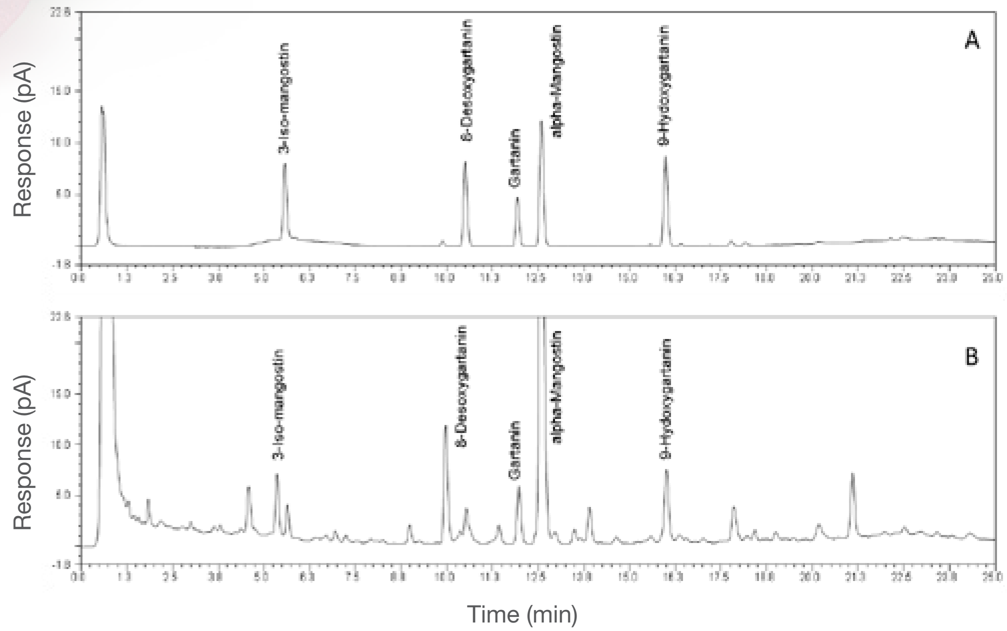
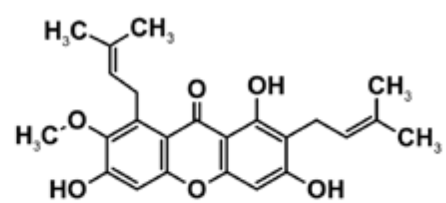
Mangosteen (*Garcinia mangostana L*) is a tropical fruit indigenous to Southeast Asia. It is used broadly in Ayurvedic medicine to treat abdominal pain, cholera, diarrhea, dysentery, inflammation, wound infection, suppuration and chronic ulcers. Recently, mangosteen pericarp was proposed as an adjunctive therapy for bipolar disorder and schizophrenia. Such therapeutic benefits have been mostly attributed to a unique family of compounds, the xanthones, that are most abundant in the pericarp of the fruit.

Presented here is a UHPLC-CAD method capable of analyzing several xanthones in mangosteen pericarp. Conventional extraction methods, such as Soxhlet, are time consuming, labor-intensive and often lack reproducibility. To overcome these issues Accelerated Solvent Extraction (ASE®), an automated extraction technique that rapidly performs solvent extractions using high temperature and pressure, was used.

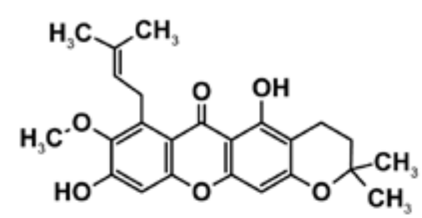


UHPLC-CAD analysis of mangosteenxanthones. A) Standards and B) ASE extraction of mangosteen pericarp powder.

Column:	Thermo Scientific Acclaim 120 C18, 2.2 µm, 2.1 ×100 mm,
Mobile Phase A:	DI Water
Mobile Phase B:	Acetonitrile
Detector:	Charged Aerosol



α-Mangostin



3-Isomangostin

PN70991: Fast Analysis of Selected Xanthones in Mangosteen Pericarp Using Accelerated Solvent Extraction and Ultra High Performance Liquid Chromatography.

AB172: The Vanquish Platform: Major Improvement in Throughput and Resolution of Xanthones in Mangosteen Pericarp

thermoscientific

Milk thistle

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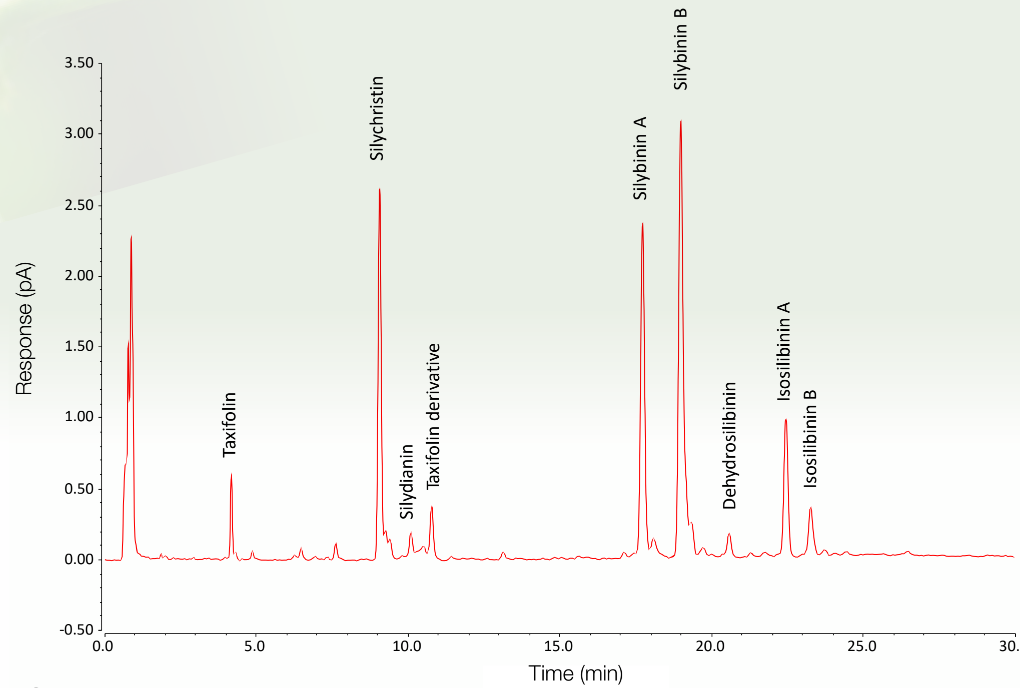
The milk thistle plant, *Silybum marianum*, is native to Mediterranean Europe and has long been used as an herbal remedy to promote liver health and treat liver disorders, such as hepatitis and cirrhosis, and gallbladder problems. Recent research suggests that it may be beneficial for diabetes and dyspepsia.

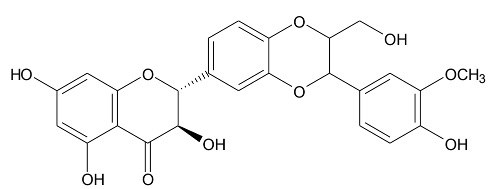
The purported active ingredient in milk thistle is silymarin, which is particularly abundant in the plant's seeds. Silymarin consists of a mixture of different chemical species including a flavonoid (taxifolin) and several flavonolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin and silydianin).

Presented here is a high-resolution UHPLC-CAD method that measures not only the major silymarin components, but also numerous minor constituents that may be missed by typical HPLC-UV approaches.

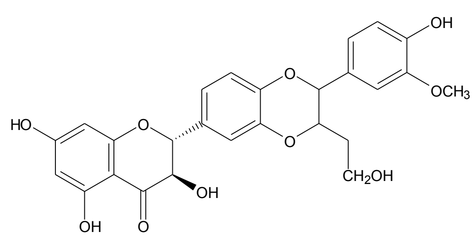


High resolution analysis of milk thistle supplement extract by UHPLC-CAD.

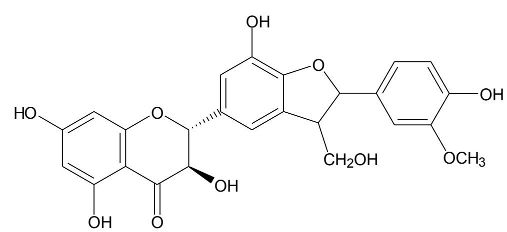
Column:	Thermo Scientific Acclaim Vanquish C18, 2.2 µm, 2.1 ×250 mm
Mobile phase A:	0.1 % Formic acid in DI Water
Mobile phase B:	0.1 % Formic acid in Methanol
Detector:	Charged Aerosol



Sylibin



Isosylibin



Silychristin

AN1048: Novel, universal approach for the measurement of natural products in a variety of botanicals and supplements

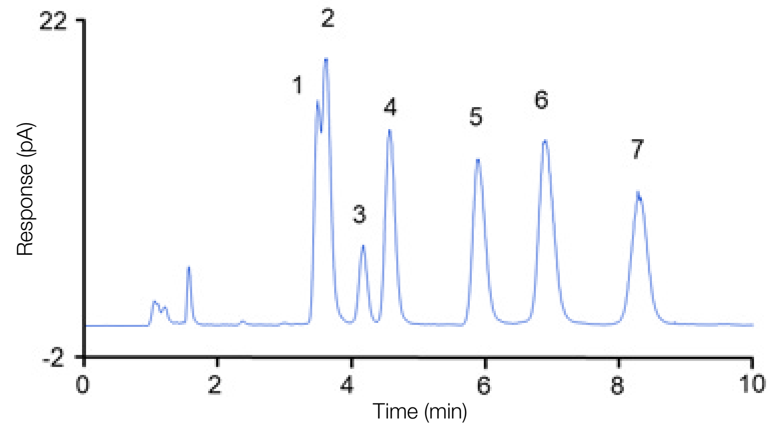
PN70153: Can High Peak Capacity and Universal Detection Solve the Challenges in LC Characterization of Botanicals and Natural Products

Mogroside

Luo han kuo fruit (*Siraitia grosvenorii* Swingle), also called monk fruit, has long been used in traditional Asian medicine to treat coughs, sore throats and fatigue. Cucurbitane-type and other triterpenes isolated from the fruit were investigated for numerous potential health benefits, such as possible anti-cancer and antihyperglycemic effects. Many of these compounds are intensely sweet and, therefore, are also used as sugar substitutes and flavor enhancers. Extracts of luo han kuo fruit used as sweeteners are acknowledged as Generally Recognized as Safe (GRAS) based on a **GRAS submission** to the FDA in January of 2010.

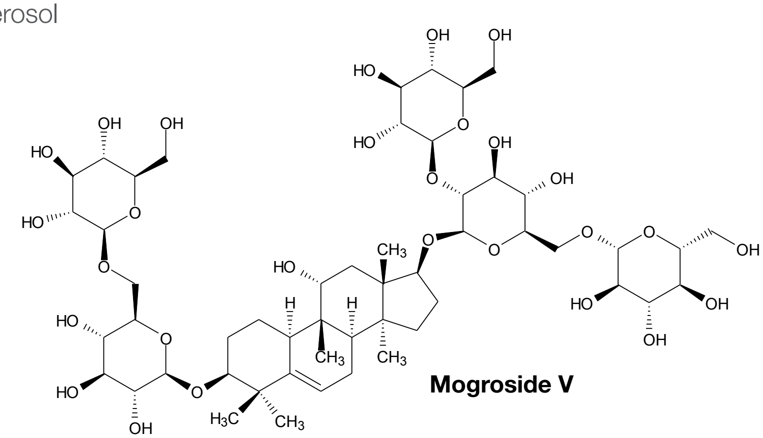
Presented here is a method that separates analytes on an Acclaim Trinity P1 column using HILIC conditions. CAD rather than UV absorbance was used to quantify these compounds as they lack a strong chromophore.

Column:	Thermo Scientific Acclaim Trinity P1, 3 µm, 2.1 × 10 mm
Mobile phase:	81:19 Acetonitrile/ 10 mM Ammonium formate, pH = 3
Detector:	Charged Aerosol



HPLC-CAD analysis of steviol glycoside and mogroside V standards standards.

1. Dulcoside A
2. Stevioside
3. Rebaudioside C
4. Rebaudioside A
5. Steviolbioside
6. Rebaudioside B
7. Mogroside V



Mogroside V

AU184: Mogroside V determination by HPLC with Charged Aerosol and UV detections

Phenols – global method

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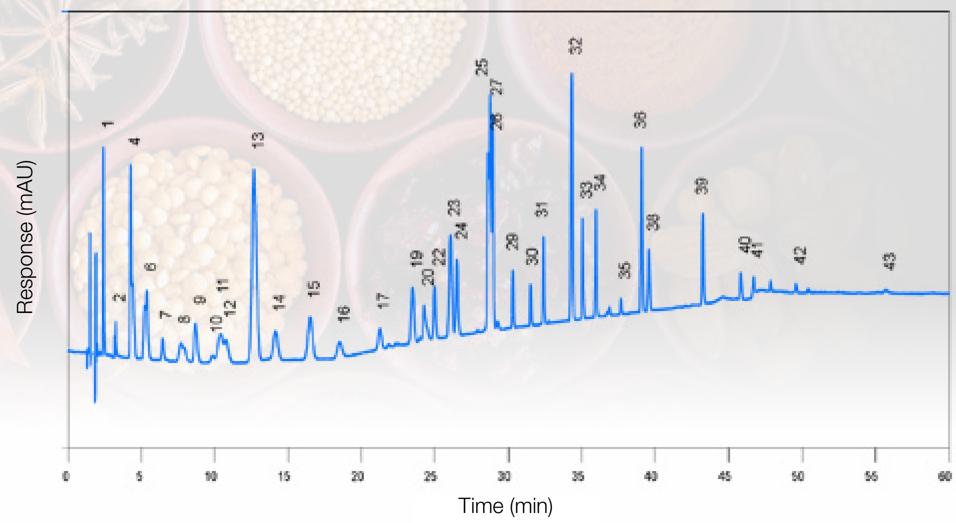
A phenol is a compound that contains a hydroxyl group attached to an aromatic hydrocarbon group. Many phenolic phytochemicals are found in plants and classified as either **simple phenols** or **polyphenols**, based on the number of phenol hydroxyl groups and aromatic rings in the molecule. Representative **simple phenols**, include: carvacrol (a phenol), salicylic acid (a phenolic acid), resveratrol (a stilbenoid), caffeic acid (a hydroxycinnamic acid), tyrosol (a phenylethanoid), and flavonoids. **Polyphenols** include condensed tannins (e.g., proanthocyanidins); hydrolyzable tannins (e.g., ellagitannins) and phlorotannins. However, terminology and classification can be complex with the term polyphenol erroneously used to refer to all compounds with more than one phenol group.

Phenols have strong chromophores so are typically measured using HPLC with UV absorbance detection. Presented here is a general gradient HPLC-UV method for the measurement of multiple phenols and other compounds in a wide variety of botanicals.

Column:	Thermo Scientific Acclaim 120 C18, 3 µm, 3 x 150 mm
Mobile phase A:	20 mM Monobasic sodium phosphate, 3 % Acetonitrile, 0.2 % Tetrahydrofuran, pH 3.35
Mobile phase B:	20 mM Monobasic sodium phosphate, 50 % Acetonitrile, 10 % Tetrahydrofuran, pH 3.45
Mobile Phase C:	90 % Methanol
Detector:	Absorbance, UV 275 nm



HPLC-UV analysis of multiple phenols.

1. Gallic acid
2. 4-Hydroxybenzyl alcohol
3. p-Aminobenzoic acid
4. 3,4-Dihydroxybenzoic acid
5. Gentisic acid
6. 2-Hydroxybenzyl alcohol
7. 4-Hydroxybenzoic acid
8. Chlorogenic acid
9. p-Hydroxyphenylacetic acid
10. Catechin
11. Vanillic acid
12. 4-Hydroxybenzaldehyde
13. Syringic acid
14. Caffeic acid
15. Vanillin
16. Syringaldehyde
17. Umbelliferone
18. p-Coumaric acid
19. Salicylic acid
20. Sinapic acid
21. Ferulic acid
22. Ellagic acid
23. Rutin
24. Ethyl-vanillin
25. 4-Hydroxycoumarin
26. Hesperidin
27. Naringin
28. Rosemarinic acid
29. Fisetin
30. Myricetin
31. Trans-resveratrol
32. Luteolin
33. Cis-resveratrol
34. Quercetin
35. Kaempferol
36. Isorhamnetin
37. Eugenol
38. Isoxanthohumol
39. Cavacrol
40. Thymol
41. Carnisol
42. Xanthohumol
43. Carnosic acid

AN1063: Targeted Analyses of Secondary Metabolites in Herbs, Spices, and Beverages Using a Novel Spectro-Electro Array Platform

PN70019: The Spectro-Electro Array: A Novel Platform for the Measurement of Secondary Metabolites in Botanicals, Supplements, Foods and Beverages - Part 1: Theory and Concepts

AN1077: Determination of phenolic compounds in apple orchard soil

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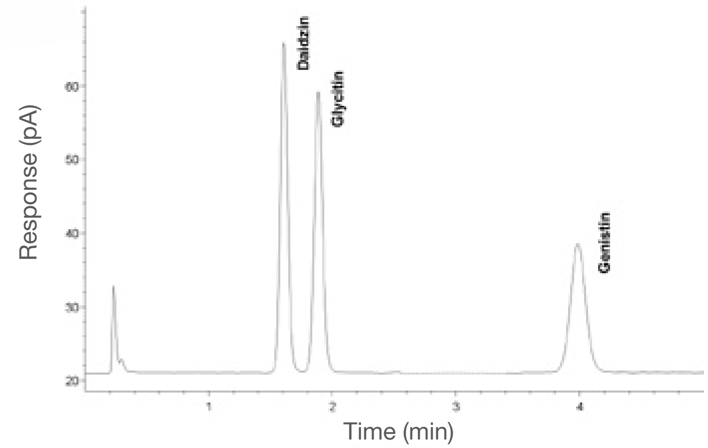
Phytoestrogens – isoflavones and their aglycones

A phytoestrogen, also called a dietary estrogen, is a xenoestrogen obtained from a number of dietary plants. Phytoestrogens are a diverse group of naturally occurring nonsteroidal phytochemicals that, because of their structural similarity with estradiol (17-β-estradiol), cause mild estrogenic and/or antiestrogenic effects. Phytoestrogens include flavonoids, (e.g., the isoflavone daidzein), coumestans (e.g., coumestrol), prenylflavonoids and mammalian lignans (enterodiol and enterolactone).

Presented here is a rapid HPLC-CAD method for routine determination of isoflavones and aglycones.

Isoflavone Analysis

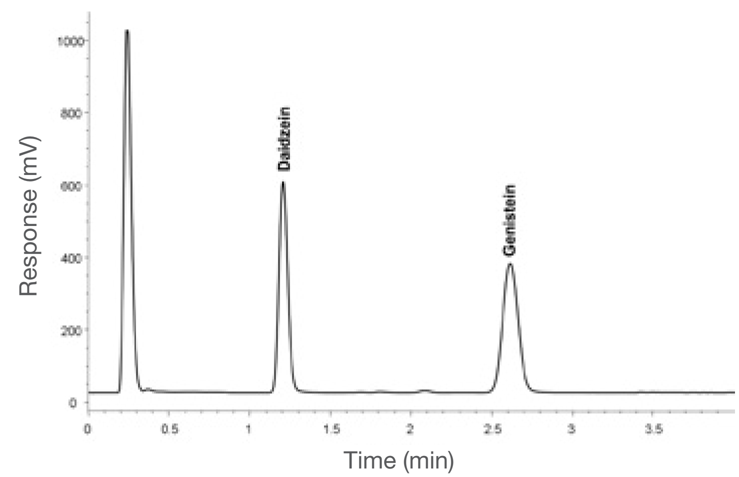
Column:	C18, 3 µm, 4.6 × 35 mm
Mobile phase :	15% acetonitrile in 0.1% Acetic acid
Detector:	Charged Aerosol



HPLC-CAD analysis of soy isoflavones.

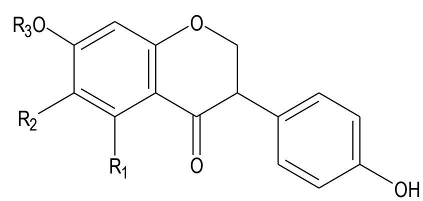
Isoflavone Aglycone Analysis

Column:	C18, 3 µm, 4.6 ×35 mm
Mobile phase :	30% acetonitrile in 0.1% Acetic acid
Detector:	Charged Aerosol



HPLC-CAD analysis of soy isoflavones and aglycones.

	R_1	R_2	R_3
Daidzein	H	H	H
Daidzin	H	H	Glucose
Genistein	OH	H	H
Genistin	OH	H	Glucose
Glycitein	H	OCH_3	H
Glycitin	H	OCH_3	Glucose



LPN2930: Determination of the composition of natural products by HPLC with charged aerosol detection

For an HPLC-UV method see: An improved separation of isoflavones in red clover using a Thermo Scientific Acclaim 120 C18 HPLC column

Phytoestrogens – coumestans and mammalian lignans

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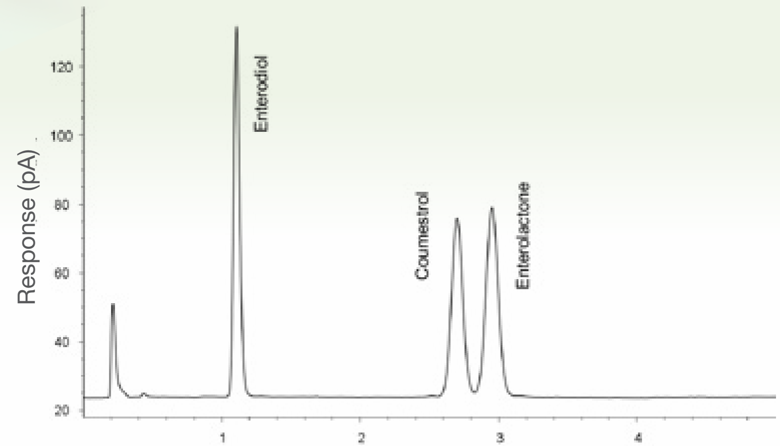
Foods with the highest relative phytoestrogen content include nuts and oilseeds, soy products, cereals and breads, legumes, vegetables, and fruits. Their consumption may offer a range of health benefits with positive impacts on the cardiovascular, metabolic and central nervous systems, reduction in the risk of cancer, and by improving post-menopausal symptoms. However, phytoestrogens may also act as endocrine disruptors, adversely affecting health. Based on currently available evidence, it is not clear whether any potential health benefits of phytoestrogens outweigh their risks.

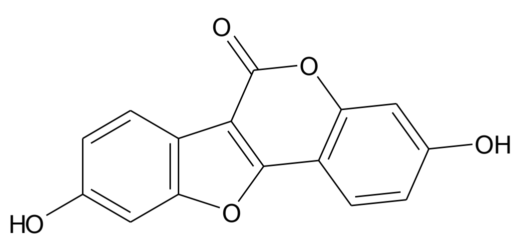
Presented here is an HPLC-CAD method for analysis of coumestans and mammalian lignans.

Isoflavone Aglycone Analysis

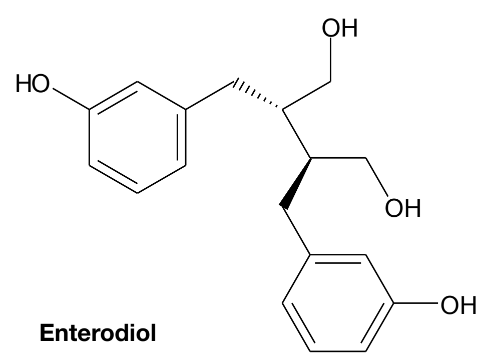
Column:	C18, 3 µm, 4.6 ×35 mm
Mobile phase :	30% acetonitrile in 0.1% Acetic acid
Detector:	Charged Aerosol



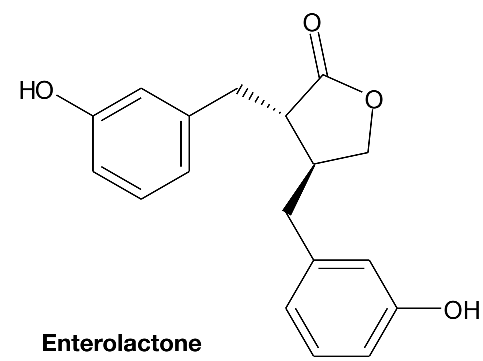
HPLC-CAD analysis of coumestans and mammalian lignans.



Coumestrol



Enterodiol



Enterolactone

LPN2930: Determination of the composition of natural products by HPLC with charged aerosol detection

For a UHPLC-UV-CAD-MS approach see: Profiling and quantitating the constituents of red clover extracts using UHPLC/UV/CAD/HRMS: A component of the safety assessment process

thermoscientific

Phytosterols

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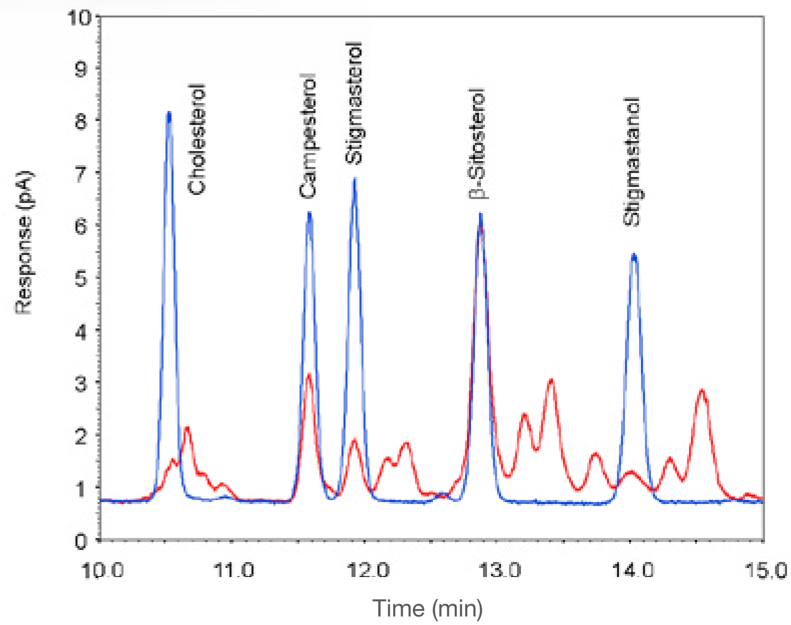
Phytosterols (sterols and stanols) are steroid alcohols that are found in plants. They are key structural components of plant cell membranes, assuming the role that cholesterol plays in mammalian cells. In foods, phytosterols exist in the free form, as esters with fatty acids, and as glycolipids. Good food sources for phytosterols include unrefined vegetable oils, whole grains, nuts, seeds and legumes. Phytosterols are purported to have health benefits, such as lowering cholesterol and positively impacting cardiovascular disease. Consequently, foods and beverages supplemented with phytosterols are available in many countries. However, consumption of phytosterol-enriched foods may have undesirable effects too, such as reduced plasma carotenoid concentrations.

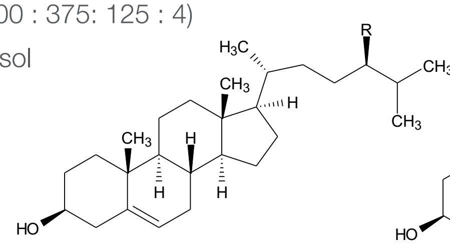
Phytosterols lack a good chromophore, so UV detection below 210 nm is typically used for their analysis. Gas chromatographic approaches are sometimes used, but require extensive sample preparation (hydrolysis and derivatization).

Presented here is a direct, simple and sensitive HPLC-CAD approach for the measurement of several phytosterols in red palm oil extracts.

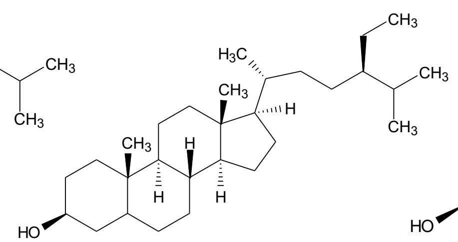


HPLC-CAD analysis of standards (blue) and red palm oil extract (red).

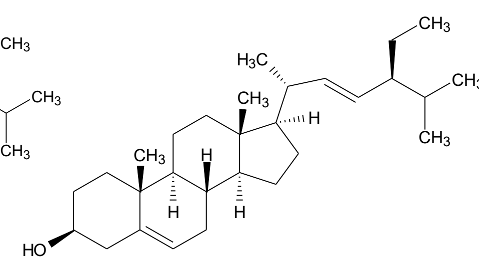
Column:	Porous Shell C8, 2.7 µm, 4.6 ×150 mm,
Mobile Phase A:	Methanol/Water/Acetic acid (750 : 250 : 4)
Mobile Phase B:	Acetone/Methanol/Tetrahydrofuran/ Acetic acid (500 : 375: 125 : 4)
Detector:	Charged Aerosol



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



Stigmastanol



Stigmasterol

AN1041: Simple and Direct Analysis of Phytosterols by Reversed Phase HPLC and Charged Aerosol Detection

thermoscientific

Punicalagins

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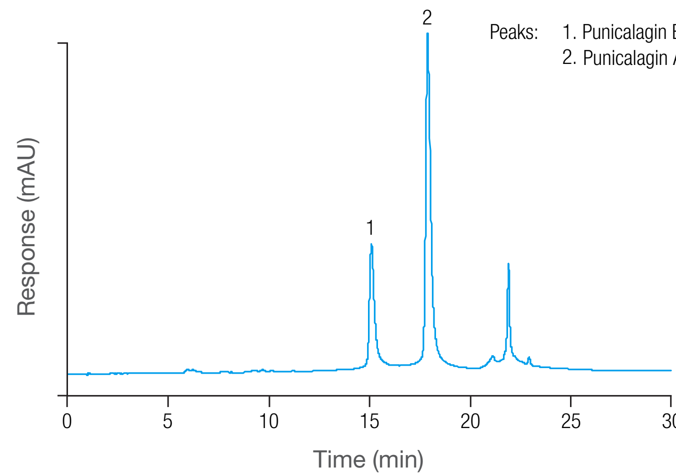
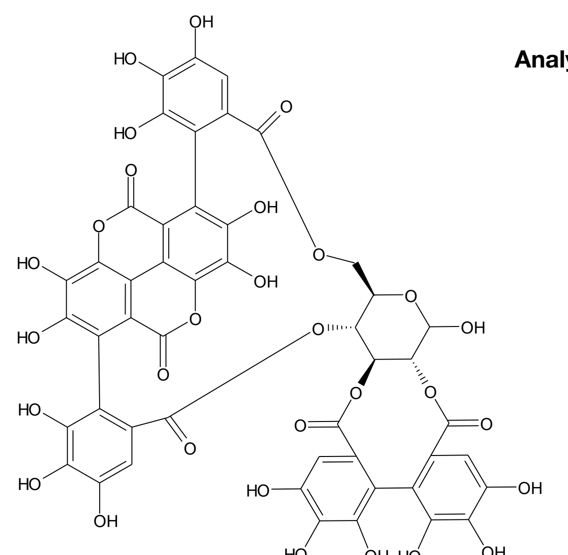
Pomegranate (*Punica granatum*) fruit has been used since ancient times for medicinal purposes. Today, consumption of pomegranate juice is purported to have several health benefits (e.g., positively impacting arthritis, cancer and cardiovascular disease), possibly due to its high antioxidant content. Ellagitannins (hydrolysable tannins) are a family of water-soluble bioactive polyphenols particularly abundant in pomegranates. Amongst them, the punicalagins are reported to be responsible for more than half the potent antioxidant activity of the juice.

Presented here is an HPLC-UV absorbance method for the determination of punicalagin A and B.

Column:	Thermo Scientific Acclaim Polar Advantage PA2, 3 µm, 3 × 150 mm,
Mobile Phase A:	1% Formic acid
Mobile Phase B:	Acetonitrile
Detector:	Absorbance, UV 260 nm



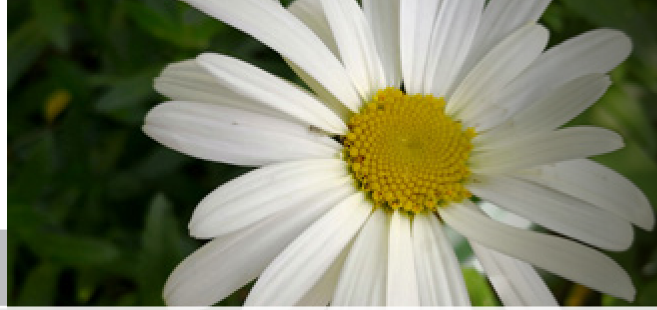
Analysis of punicalagin standards.



Punicalagin

CAN106: Determination of the Punicalagins Found in Pomegranate by High Performance Liquid Chromatography

thermoscientific



Pyrethrins – *Chrysanthemum cinerariifolium*

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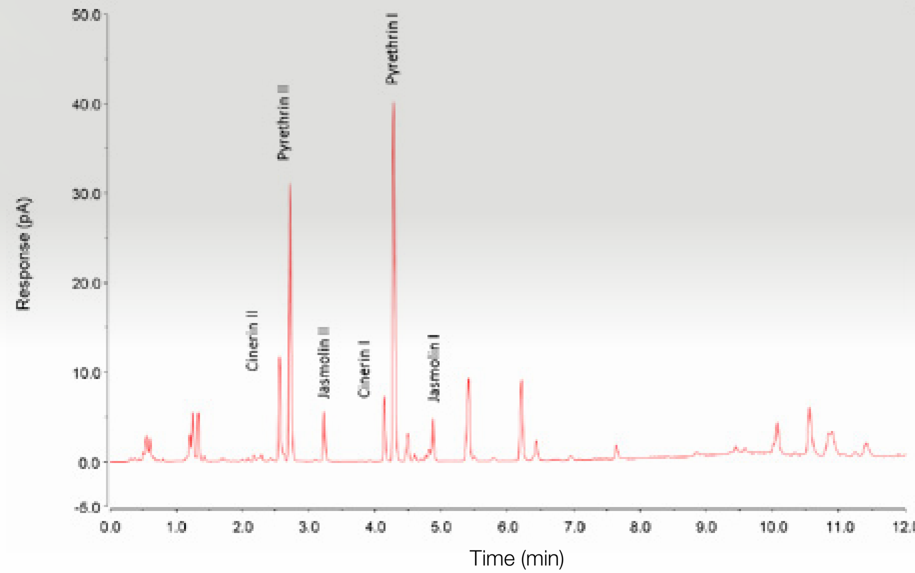
Pyrethrins are terpenoid esters derived from the flowers of *Chrysanthemum cinerariifolium*. The pyrethrin family includes six similar compounds that contain a cyclopropane core (see below). Pyrethrins both repel and kill insects by delaying the closure of voltage-gated sodium ion channels in their nerve cells. The insecticidal and insect repellent properties of these compounds have been known for millennia and chrysanthemum species have long been cultivated for this purpose. Interest in using pyrethrin-based insecticides is growing because of their low toxicity to humans (allowing home use) and favorable, fast biodegradability. However, they are also toxic to bees, with fatal doses as low as 0.02 µg, thus requiring very cautious application. Increasing pyrethrin use in agricultural and consumer products means there is a need for improved analytical techniques, both to assure product quality and to monitor the fate of pyrethrins in the environment.

Pyrethrins contain weak chromophores, and are often measured using insensitive low-wavelength UV detection. Furthermore, published methods typically require excessively long run times to fully resolve closely eluting components.

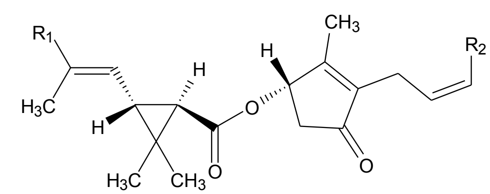
Presented here is a comprehensive UHPLC-UV-CAD method that enables the resolution and detection of more compounds in pyrethrum oil in less time than previously possible.

Column:	Thermo Scientific Acclaim Vanquish C18, 1.5 µm, 2.1 × 100 mm
Mobile phase A:	DI Water
Mobile phase B:	Acetonitrile, Optima LCMS
Detectors:	Charged Aerosol and Absorbance, UV 220 nm



UHPLC-CAD analysis of pyrethrum oil extract.

	R_1	R_2
Cinerin I	CH_3	CH_3
Cinerin II	CO_2CH_3	CH_3
Jasmolin I	CH_3	CH_2CH_3
Jasmolin II	CO_2CH_3	CH_2CH_3
Pyrethrin I	CH_3	$CH=CH_2$
Pyrethrin II	CO_2CH_3	$CH=CH_2$



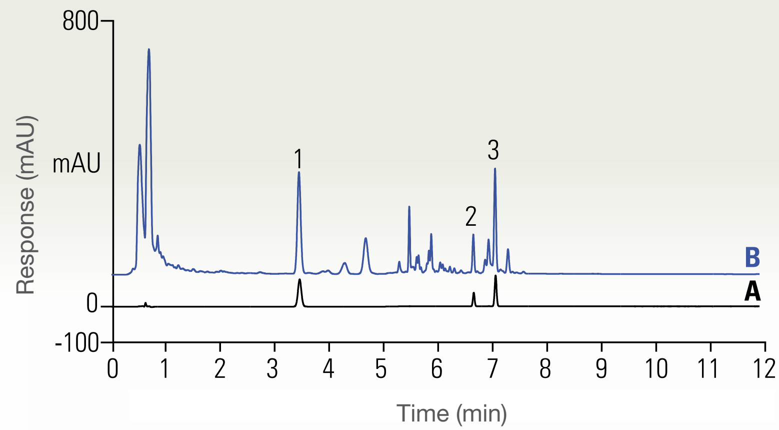
PN21431: Determination of Pyrethrins in Pyrethrum Oil Extracts by UHPLC with Charged Aerosol Detection

Schisandra – schizandrin, schizandrin A, and schizandrin B

Schisandra chinensis (Turcz.) Baill, (commonly known as Chinese magnolia-vine or schisandra) produces a fruit called magnolia berry or five-flavor-fruit (wû wèi zi). It is used both as a food and in traditional Chinese medicine, where it is considered one of the 50 fundamental herbs. Schisandra has many proposed health benefits. It is promoted as an "adaptogen" for increasing resistance to disease and stress, boosting energy, and improving physical performance. It is also used for treating liver disease (hepatitis) and protecting the liver from poisons.

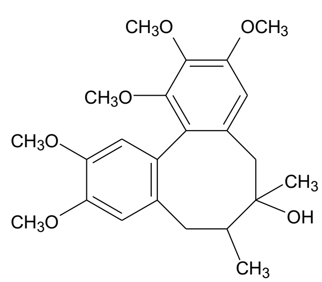
The major active components found in Schisandra are the lignanoids - schizandrin, schizandrin A and schizandrin B. The Pharmacopeia of the People's Republic of China 2010 regulates its quality control using a UHPLC method for determining levels of these three analytes (see AB139).

Presented here is an efficient UHPLC method that can be used to measure schizandrin, schizandrin A and schizandrin B, for the quality control of Hugan tablets, a traditional Chinese medicine used for treating nonalcoholic fatty liver disease.

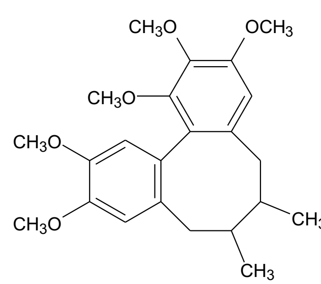


HPLC-UV analysis of schizandrin, schizandrin A and schizandrin B. A - standards; B - "Hugan" capsule extract.

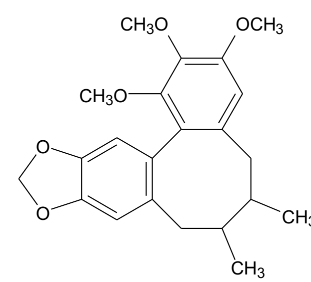
Column:	Thermo Scientific Accucore C18, 2.6 µm, 4.6 × 150 mm
Mobile Phase A:	DI Water
Mobile Phase B:	Acetonitrile, Optima LCMS
Detector:	Absorbance, UV 210 nm



Schizandrin



Schizandrin A



Schizandrin B

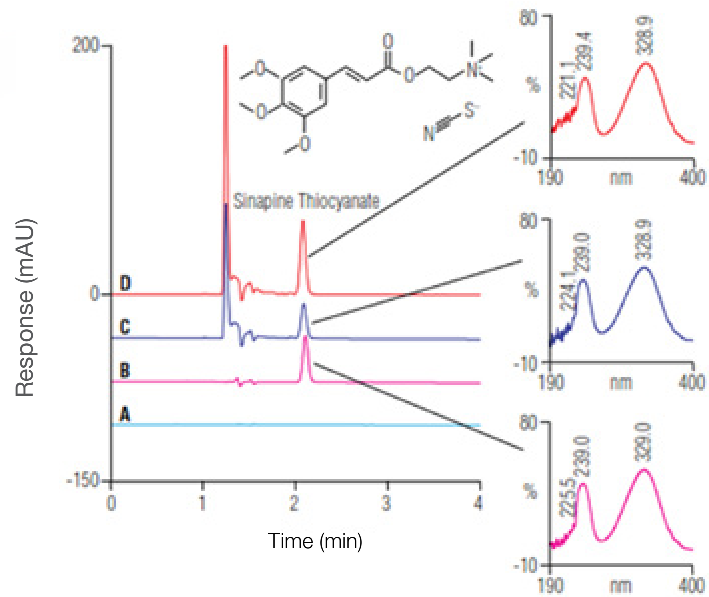
AB139: Separation of Schizandrin, Schizandrin A, and Schizandrin B in a Tablet Sample

Semen raphani

Semen raphani, the seed of *Raphanus sativus* L., is a Chinese medicinal plant commonly used for treatment of dyspepsia and hypertension. Its anti-hypertension properties are attributed to the presence of sinapine thiocyanate. The Pharmacopeia of the People's Republic of China 2010 monitors the quality control of semen raphani with a RP-HPLC method for the determination of sinapine thiocyanate. The method specifies a stationary phase with phenyl groups bonded to silica (see AB126).

Presented here is an HPLC-UV method for the determination of sinapine thiocyanate in semen raphanin seeds using an Acclaim Phenyl-1 column and UV absorbance detection.

Column:	Thermo Scientific Acclaim Phenyl-1, 3.0 µm, 4.6 ×150 mm
Mobile phase:	Acetonitrile/3% Acetic acid, 10/90 (v/v)
Detector:	Absorbance, 326 nm



Separation of sinapine thiocyanate on the Acclaim Phenyl-1 column following the Chinese Pharmacopeial method. A) mobile phase, B) sinapine thiocyanate standard (10 µg/mL), C) semen raphani sample, and D) semen raphani sample spiked with sinapine thiocyanate (5 µg/mL).

AB126: Separation of Sinapine Thiocyanate in Semen Raphani Using an Acclaim Phenyl-1 Column

thermoscientific

Stevia

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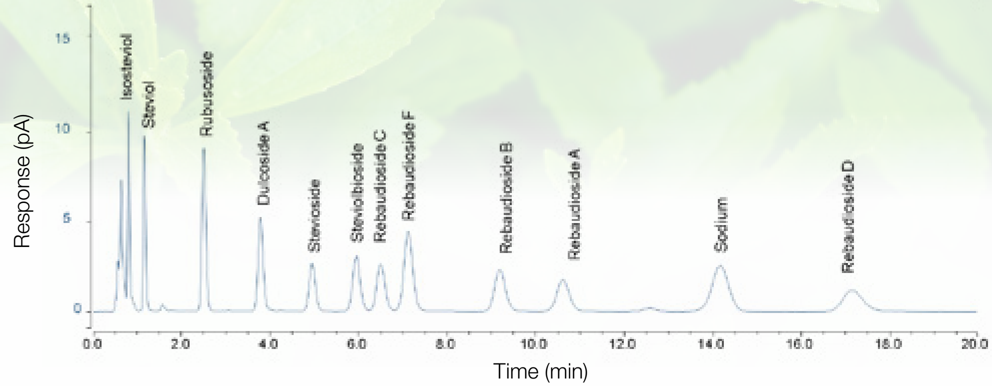
Stevia plant and extracts from stevia leaves have long been used as sweeteners in Asia and Latin America. Since the FDA approved the steviol glycoside rebaudioside A, purified from *Stevia rebaudiana* (Bertoni), as Generally Recognized as Safe for use as a sugar substitute, stevia products have become common table-top and beverage sweeteners.

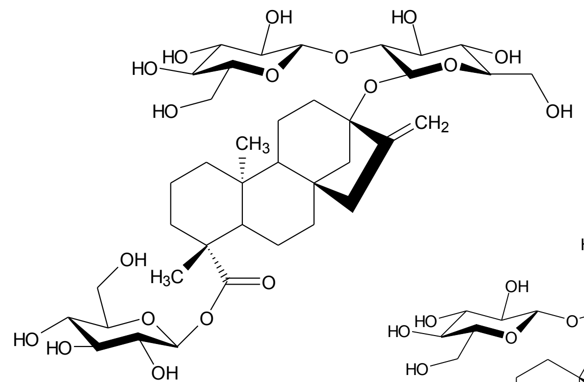
Stevia plants contain many terpene glycosides that have different flavor profiles with both sweet and unpleasant bitter flavors. Two steviol glycosides, stevioside and rebaudioside A, are largely responsible for the desired sweet flavor of the leaves, with rebaudioside A preferred for sweeteners.

Steviol glycoside determination is challenging for many reasons. The glycoside structures are quite similar, differing only in small changes in glycosylation which makes chromatographic separation difficult. Furthermore, they do not absorb strongly in the UV spectrum, and typical detection wavelengths for steviol glycosides, such as 210 nm, are nonspecific and lack sensitivity. CAD can be used together with UV detection to improve steviol glycoside quantification. In addition, CAD has the advantage of measuring additional components in the sample that are not UV absorbing.

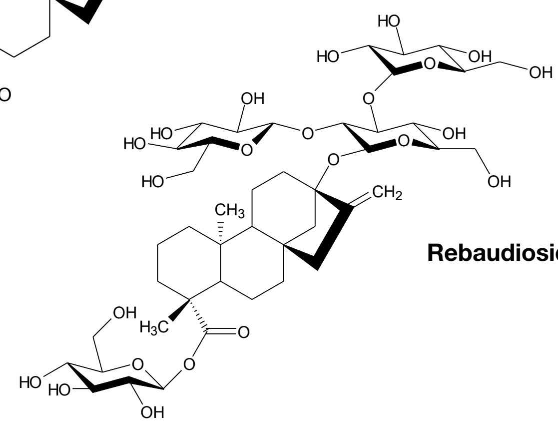
Presented here is a HPLC-CAD method for measurement of steviol glycoside and related compounds in botanicals and stevia products.



Analysis of stevia glycosides by HPLC-CAD.



Stevioside



Rebaudioside A

Column:	Thermo Scientific Acclaim Trinity P1, 3 µm, 2.1 × 150 mm
Mobile Phase:	88:12 (v/v) Acetonitrile:10 mM Ammonium formate, pH 3
Detection:	Charged Aerosol

AN70278: Analysis of Commercially Available Products Containing Stevia

AN293: Steviol Glycoside Determination by HPLC with Charged Aerosol and UV Detections Using the Acclaim Trinity P1 Column

AN1048: Novel, Universal Approach for the Measurement of Natural Products in a Variety of Botanicals and Supplements

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St. John's Wort

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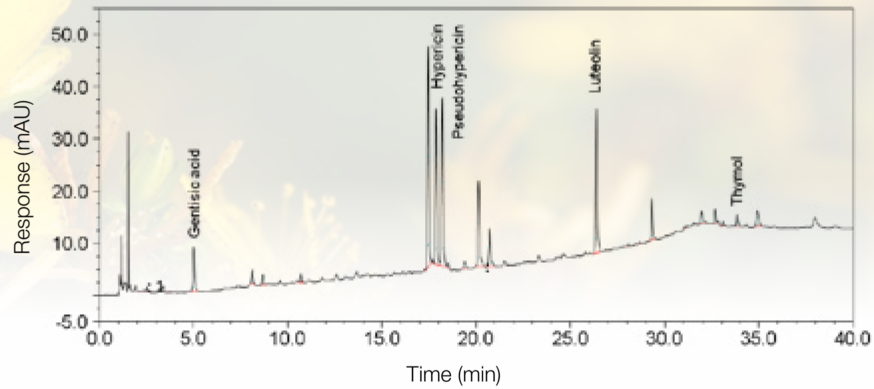
St. John's Wort (*Hypericum perforatum*) is used in folk medicine to treat a variety of conditions, including depression, kidney and lung ailments, and insomnia, and to aid wound healing. Currently, St. John's Wort is most often used as a dietary supplement to treat mild depression. However, studies have shown that it is not consistently effective. Unfortunately, not only can it cause photosensitivity it may also interact with a numerous medications, causing major health issues.

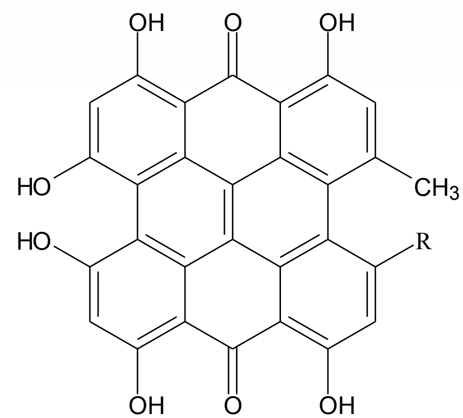
Many biologically active compounds have been isolated from the extracts of St. John's wort, but the most potent appear to be the phloroglucinol-derivatives hyperforin and adhyperforin, and the naphthodianthrones hypericin and pseudohypericin.

Presented here is an HPLC-UV method capable of profiling key components in St. John's Wort supplements.



HPLC-UV analysis of a St. John's Wort extract.

Column:	Thermo Scientific Acclaim 120 C18, 3 µm, 3 x 150 mm
Mobile phase A:	20 mM Monobasic sodium phosphate, 3 % Acetonitrile, 0.2 % Tetrahydrofuran, pH 3.35
Mobile phase B:	20 mM Monobasic sodium phosphate, 50 % Acetonitrile, 10 % Tetrahydrofuran, pH 3.45
Mobile Phase C:	90 % Methanol
Detector:	Absorbance, UV 254 nm



Hypericin **R = CH_3**
Pseudohypericin **R = CH_2OH**

AN 1063: Targeted Analyses of Secondary Metabolites in Herbs, Spices, and Beverages Using a Novel Spectro-Electro Array Platform

AN335: Accelerated Solvent Extraction (ASE) of Active Ingredients From Natural Products

AN346: Totally Automated Sample Preparation Using Accelerated Solvent Extraction (ASE) Coupled with Gilson ASPEC: The Determination of Dianthrones in St. John's Wort

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Taxanes

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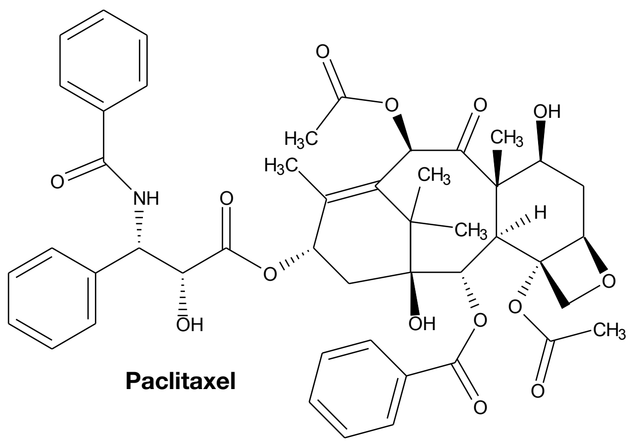
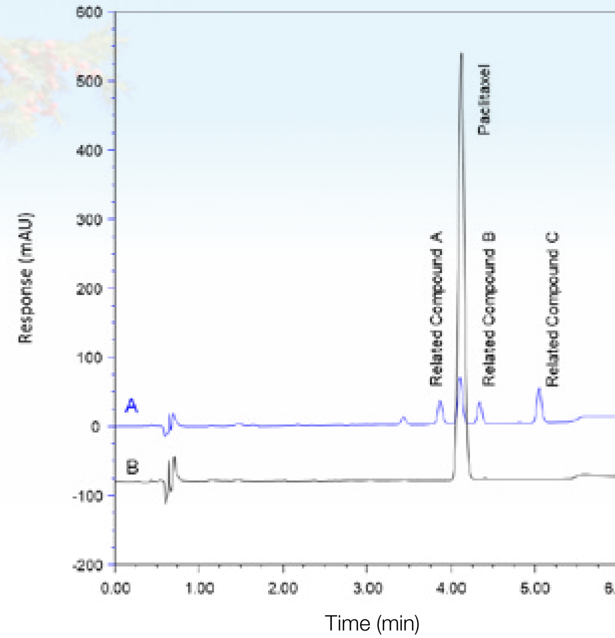
Paclitaxel , first isolated from the Pacific yew *(Taxus brevifolia)*, was approved as Taxol® by the National Cancer Institute (NCI) in 1992 for the treatment of ovarian cancer. An analysis of paclitaxel and related compounds, including cephalomannine (related compound A), 10-deacetyl-7-epipaclitaxel (related compound B) and 7-epipaclitaxel (related compound C) by RP-HPLC was published by both the United States Pharmacopeia 2009 and Chinese Pharmacopeia 2010 (see AB119). These methods each required longer than 70 minutes.

Presented here is a UHPLC-UV method using an Acclaim RSLC C18. This method is capable of resolving all key analytes in under 6 minutes and was developed to address the demand for a more rapid analysis.

Column:	Thermo Scientific Acclaim 120 C18, 2.2 µm, 2.1 x 100 mm
Mobile phase A:	Water
Mobile phase B:	Acetonitrile/Methanol 60:40 v/v
Detector:	Absorbance, UV 227 nm



Paclitaxel



Overlay of chromatograms of A) mixture of paclitaxel and related compounds standards (5 µg/mL for each) and B) paclitaxel injection sample.

AB119: Rapid Separation of Paclitaxel and Related Compounds in Paclitaxel Injection

Tomatine and tomatidine

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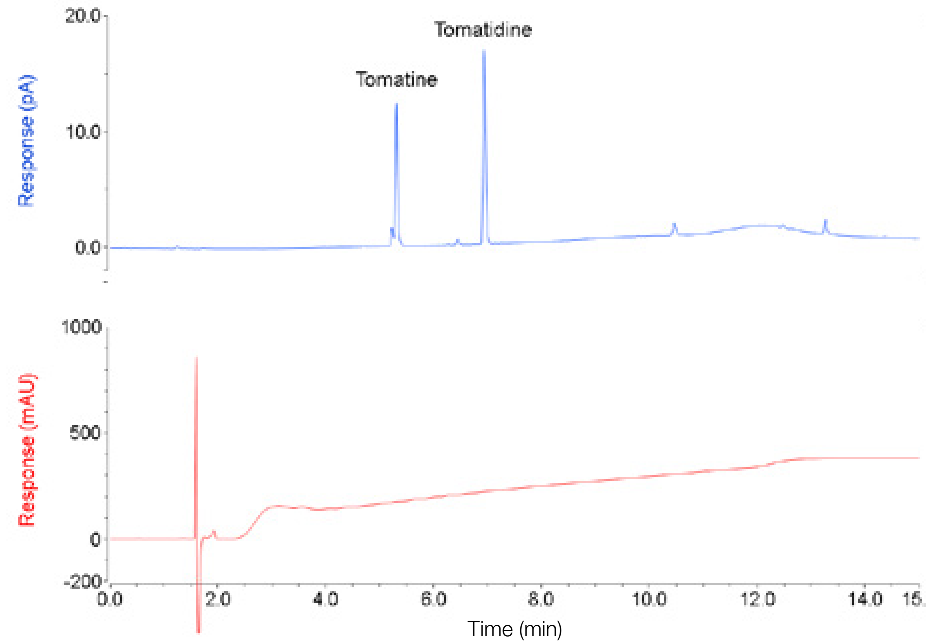
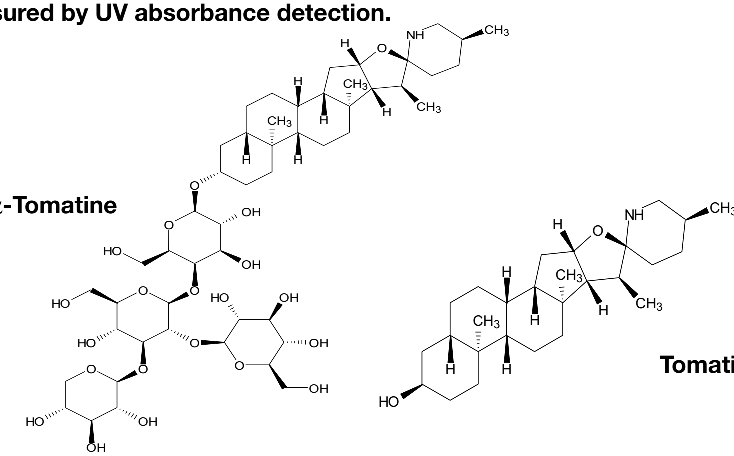
Tomatine (or lycopersicin) is a steroidal glycoalkaloid particularly abundant in the stems and leaves of tomato plants where it acts as a natural fungicide and insecticide. Tomatine and its aglycone tomatidine are reported to have health benefits, including possessing antibiotic and anticarcinogenic properties, and having a positive impact on cardiovascular disease.

Tomatine and tomatidine possess weak chromophores making analysis by HPLC-UV difficult. Presented here is an HPLC-CAD method that overcomes such analytical limitations.

HPLC Column:	Thermo Scientific Accucore C8, 2.6 µm, 2.1 ×100 mm
Mobile Phase A:	0.2 % Formic acid in Deionized water
Mobile Phase B:	0.2 % Formic acid in Acetonitrile
Column Temp.:	35 °C
Flow Rate:	1.0 mL/min
Injection Volume:	0.2 µL
Gradient:	5-95% B in 10 min
Detectors:	Charged Aerosol and Absorbance, UV 227 nm



HPLC-CAD can be used to measure tomatine and tomatidine along with a number of impurities in commercially available standards. (Each standard - 0.25 mg/mL in methanol containing 0.2% formic acid). Compounds cannot be measured by UV absorbance detection.



α-Tomatine

Tomatidine

For an LC-MS approach see: Plant Metabolomics: Tomato Metabolite Profiling and Identification Employing High-Resolution LC-MS Strategies

Turmeric – curcuminoids

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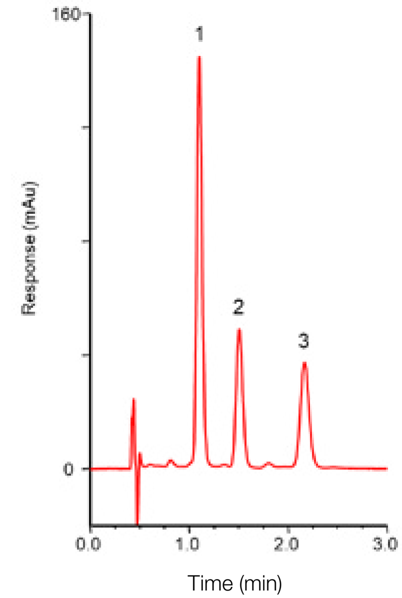
Turmeric is a popular spice and dye made from the powdered dried root of *Curcuma longa*. It is widely used as a culinary additive to impart a distinctive yellow-orange color to Pakistani, Indian and Thai cuisines. It has been used in Asia for thousands of years, and is a major part of Ayurvedic, Siddha medicine, traditional Chinese medicine and Unani medicine. In Ayurvedic medicine, it is called haridra and used as an anti-inflammatory, and in the treatment of arthritis, cancer, gastric ulcers, neurodegenerative diseases and allergies. Its purported bioactive components are the brilliant yellow pigments curcumin, desmethoxycurcumin and bis-desmethoxycurcumin, together with other minor curcuminoids.

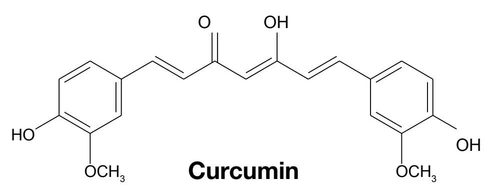
HPLC analysis with a C18 column is typically used for measurement of curcuminoids. While C18 may be satisfactory for some applications, the selectivity of polar-embedded stationary phases, such as the Accucore Polar Premium phase provides a superior solution, capable of completely resolving the curcuminoids in under 3 minutes.

Presented here is a rapid HPLC-UV method for the measurement of three curcuminoids in spice extracts.

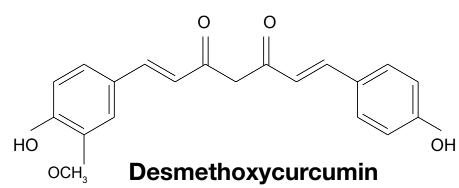
Column:	Thermo Scientific Accucore Polar Premium, 2.6 µm, 3.0 x 100 mm
Mobile phase:	Methanol: 10 mM Phosphoric acid (80:20 v/v)
Detector:	Absorbance, Vis 428 nm



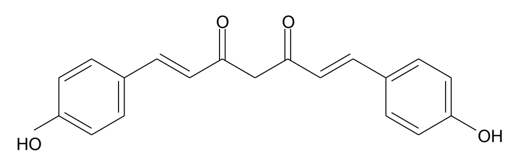
Analysis of ethanolic turmeric extract analyzed using HPLC with Vis absorbance.
1. Curcumin
2. Desmethoxycurcumin
3. Bis-desmethoxycurcumin



Curcumin



Desmethoxycurcumin



Bis-desmethoxycucurmin

AN20853: Separation of Curcuminoids from Turmeric – Comparison of Polar Embedded and C18 Solid HPLC Core Columns

PN70677: The Quantitative Analysis of Curcuminoids in Food and Food Additives Using Rapid HPLC With Electrochemical, UV, or Fluorescence Detection

AppsLab: Rapid analysis of pigments in turmeric on a Thermo Scientific Acclaim PolarAdvantage II (PA2) column

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Ursolic acid and other triterpenoids

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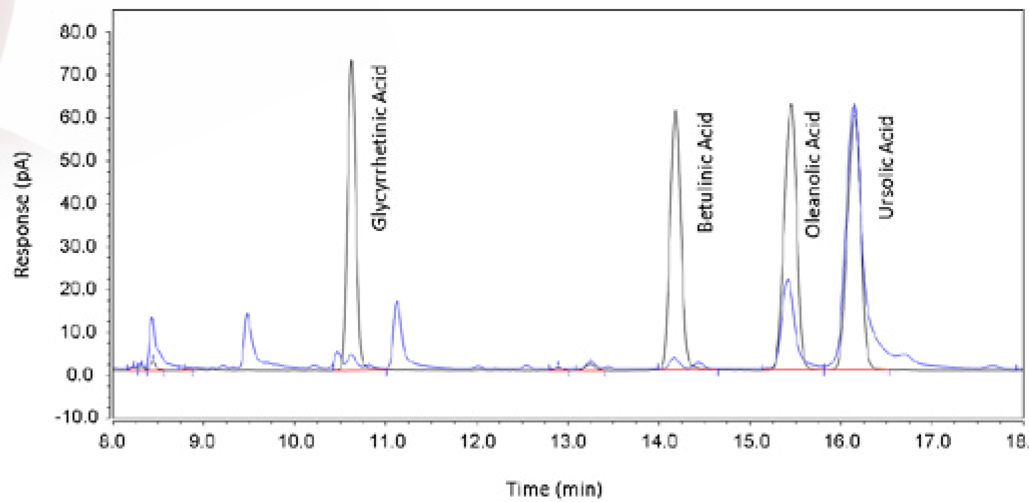
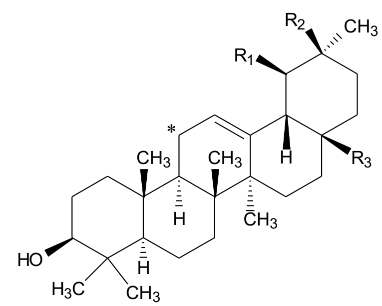
Ursolic acid is a pentacyclic triterpenoid found in the epicuticular waxes and peels of fruits and herbs, such as rosemary and thyme, but is particularly abundant in apple peel. There is a growing interest in ursolic acid because of its purported health benefits, which include antioxidant, anticarcinogenic and anti-inflammatory effects.

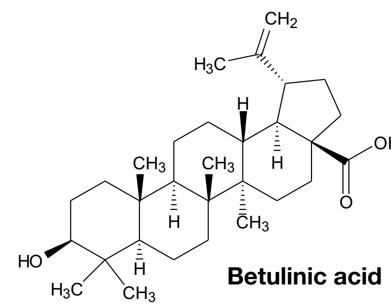
Ursolic acid and related compounds do not have a strong chromophore, so HPLC-UV methods lack sensitivity. Presented here is a sensitive HPLC-CAD method using a C30 column that easily overcomes the poor resolution between ursolic acid and the structurally similar triterpenoid oleanolic acid that is often seen with other HPLC methods.

HPLC Column:	Thermo Scientific Acclaim C30, 5 µm, 4.6 × 250 mm
Mobile Phase A:	1% aq Ammonium acetate
Mobile Phase B:	Acetonitrile/Methanol 3:1 v/v
Detectors:	Charged Aerosol



Measurement of four triterpenoids by HPLC-CAD. Black – standards (500 ng each on column); Blue – apple peel extract.





Betulinic acid

Ursolic acid	R_1= CH_3	R_2= H	R_3= CO_2H
Oleanolic acid	R_1= H	R_2= CH_3	R_3= CO_2H
Glycyrrhetinic acid	R_1= H	R_2= CO_2H	R_3= H Ketone at (*)

PN70037: Sensitive HPLC Method for Triterpenoid Analysis Using Charged Aerosol Detection with Improved Resolution

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Vitamins – simultaneous determination of water and fat soluble

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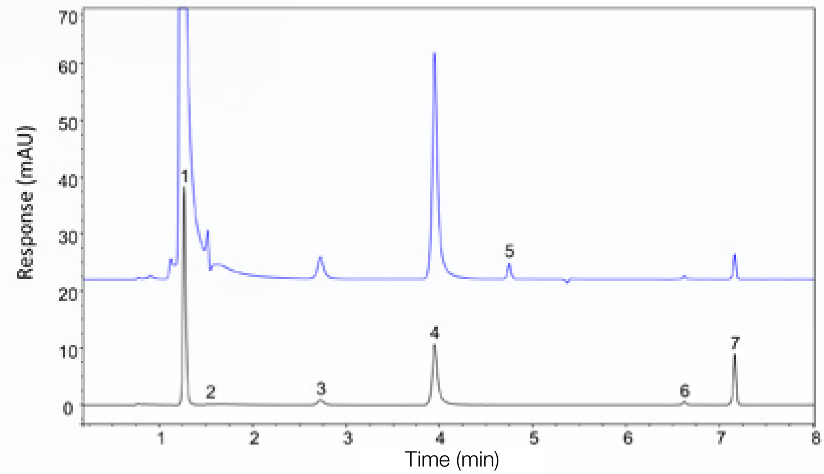
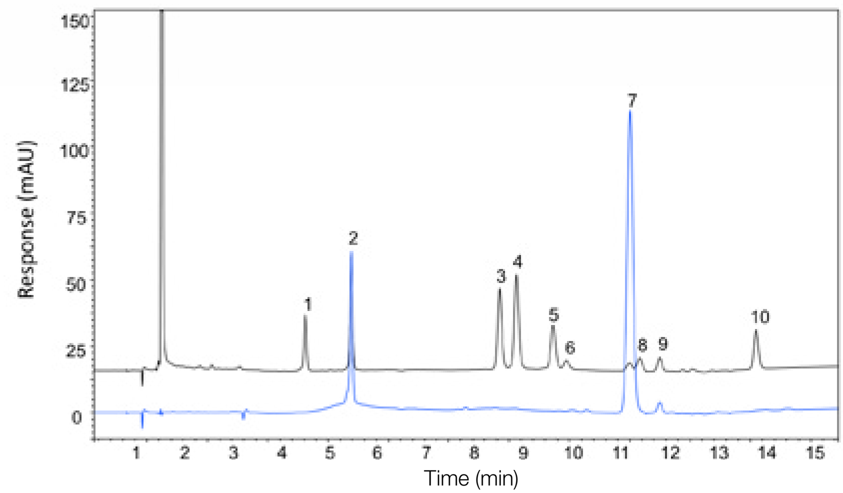
Vitamins are essential nutrients found in various natural foods and food supplements. Vitamins can be classified as water-soluble vitamins (WSV) or fat-soluble vitamins (FSV), based on their hydrophobicity. RP-HPLC is widely used to determine vitamins in food, supplements and beverages. The dramatically different hydrophobicity of WSV and FSV makes simultaneous liquid chromatography analysis with the same method difficult, so typically, separate methods are used for each class. To address this, a method for the separation of FSV and WSV was developed with two columns operated sequentially on one system. However, this required a complex hardware setup, and even more complex chromatography data system programming. Additionally, since the columns were run sequentially, sample throughput was low.

The work presented here shows an improved method capable of the simultaneous analysis of WSV and FSV in the same sample. The workflow is based on a novel Vanquish Flex Duo system for Dual LC. The system enables the independent and simultaneous use of two different columns and methods. Compared to the previous solution, this approach is remarkably simple to implement, allows the use of better optimized methods, and increases throughput thanks to the simultaneous use of two columns with two methods, and faster analysis cycles.



Separation of water-soluble vitamins in a supplement tablet. Blue trace. 210 nm Black trace. 270 nm.

1. Ascorbic acid
2. Thiamine
3. Pyridoxin
4. Nicotinamide
5. Pantothenic acid
6. Folic acid
7. Riboflavin.



Comparison of the standard mixture (black) with 100 µg/mL and the vitamin tablet (blue) at a wavelength of 280 nm.

1. Retinol
2. Retinyl acetate
3. Ergocalciferol
4. Cholecalciferol
5. Menaquinone
6. δ-Tocopherol
7. α-Tocopheryl acetate
8. γ-Tocopherol
9. α-Tocopherol
10. Phyllochinone

AN72592: Simultaneous Determination of Water- and Fat-Soluble Vitamins in Tablets and Energy Drinks by Using a Novel Vanquish Flex Duo System for Dual LC

AN72877: Accelerated Method Development for the Separation of Water-soluble Vitamins by RP HPLC with UV Detection using an Empirical Approach to Predict Separation

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Vitamin D3 in supplements

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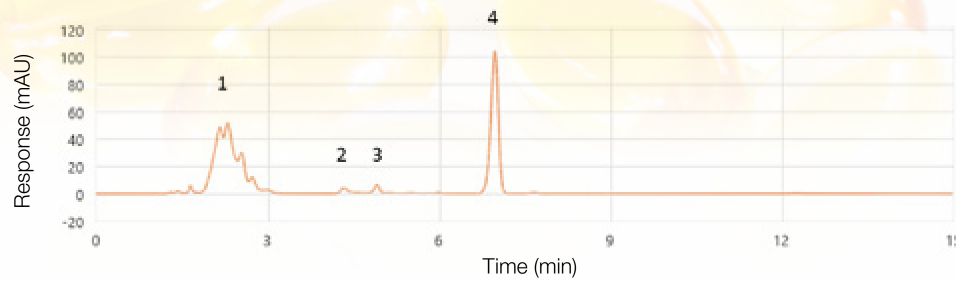
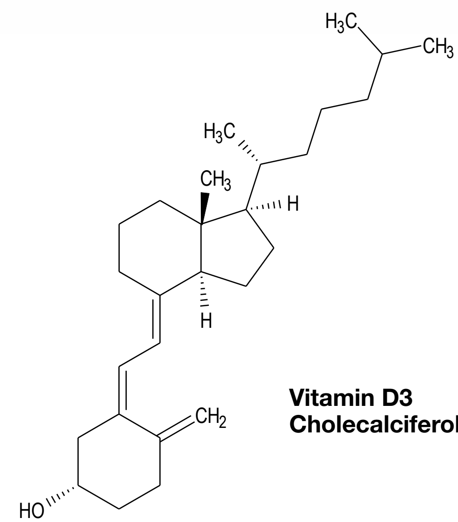
Vitamin D, a fat-soluble vitamin, occurs naturally in a few foods (e.g., fatty fish and fish oils), is added to others and can also be consumed as a dietary supplement. It is also produced endogenously when skin is exposed to sunlight, triggering synthesis. Vitamin D is biologically inert and must undergo biochemical modification in the body for activation. The first reaction occurs in the liver, converting vitamin D to calcidiol (25-hydroxyvitamin D). The second reaction occurs primarily in the kidney and forms the physiologically active Vitamin D3 – calcitriol (1,25-dihydroxyvitamin D). Vitamin D is important for maintaining calcium levels and promoting bone health, and has other roles in the body, including modulation of cell growth, neuromuscular and immune function, and reduction of inflammation.

Presented here is an HPLC method using HILIC separation and UV absorbance detection to measure Vitamin D3 in an oil-based supplement.



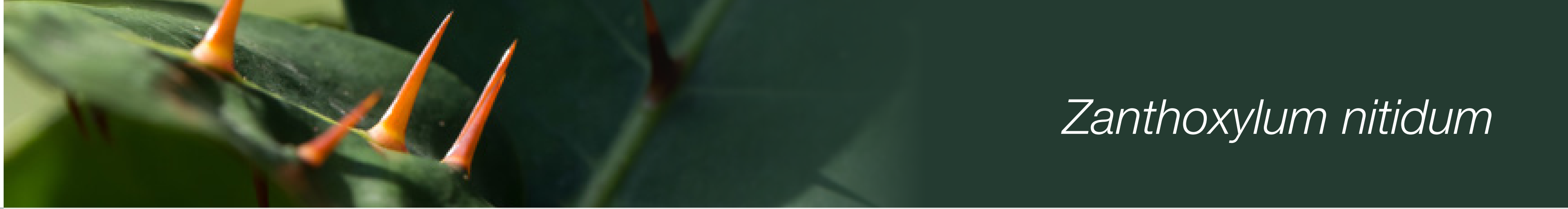
Analysis of a vitamin D3 in an oil-based supplement.
1. Excipient
2. Excipient
3. Excipient
4. Vitamin D3



Vitamin D3
Cholecalciferol

HPLC Column:	Thermo Scientific Acclaim HILIC-10, 5 µm, 4.6 ×150 mm
Mobile Phase:	Heptane/Acetonitrile/Propan-2-ol/Acetic acid (98.4:1:0.5:0.1 v/v/v/v)
Detectors:	Absorbance, UV 265 nm

AppsLab 693: Rapid analysis of vitamin D3 in supplements using the Thermo Scientific Acclaim HILIC-10 HPLC column



Zanthoxylum nitidum

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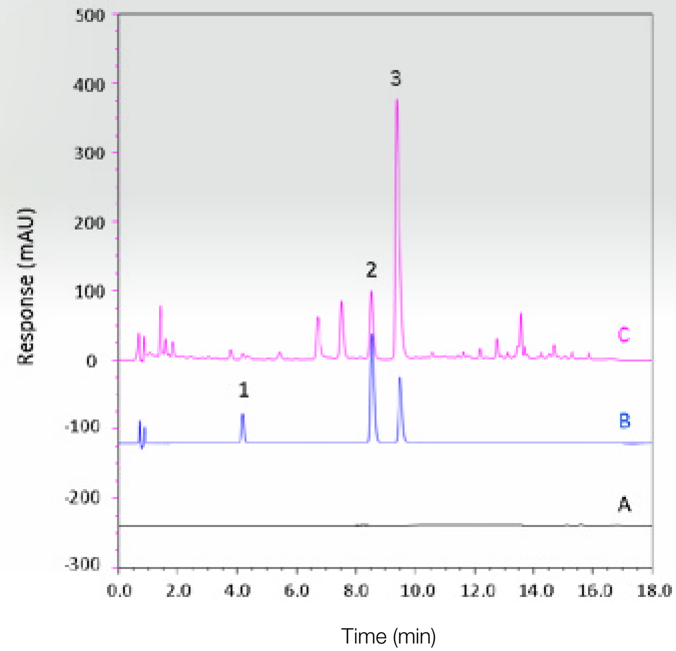
Zanthoxylum nitidum (Roxb.) DC (ZN) (liang mian zhen) is an important traditional Chinese medicine. The Pharmacopeia of the People's Republic of China (PPRC) 2010 regulates this dried root as an herbal medicine (see AN 1008). ZN *var. fastuosum* (ZNF) is another plant in the same genus as ZN. Although ZNF is not regulated in the PPRC, its dried root is still used in Chinese folk medicine because some of its reported medical benefits are the same as those reported for ZN.

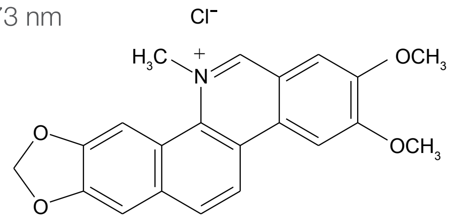
The major active components of ZN and ZNF are alkaloids. Nitidine and toddalolactone are the specific active components of ZN and ZNF, respectively. The PPRC 2010 method regulates the quality control of ZN using a HPLC method for determination of nitidine chloride but uses a thin-layer chromatography method for detection of chelerythrine chloride and toddalolactone. The presence of toddalolactone in ZN is not permitted.

The HPLC-UV method presented here is an efficient and comprehensive method for the quality control analysis of ZN through quantification of the main active components: nitidine chloride, chelerythrine chloride and toddalolactone.

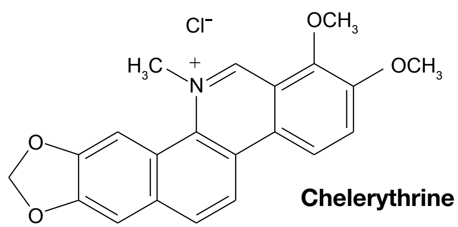
Column:	Thermo Scientific Acclaim LC PA, 3 µm, 2.1 ×150 mm
Mobile phase A:	25 mM Ammonium acetate (pH 4.5)
Mobile Phase B:	Acetonitrile
Detector:	Absorbance, UV 273 nm



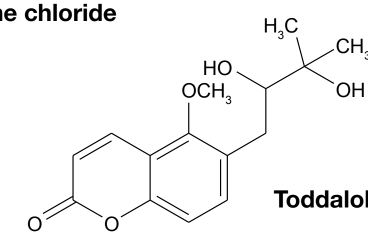
Analysis of a *Zanthoxylum nitidum* (Roxb.) DC sample.
A. Blank
B. Standards (20 µg/mL each) and
C. Zanthoxylum Nitidum (Roxb.) sample
1. Toddalolactone
2. Nitidine chloride
3. Chelerythrine chloride.



Nitidine chloride



Chelerythrine chloride



Toddalolactone

AN1008: Determination of Nitidinechloride, Toddalolactone, and Chelerythrinechloride by HPLC

Title	Authors	Publication
Why use Charged Aerosol Detection with Inverse Gradient?	Grosse, S.; Muellner, T.; Lovejoy, K.; Acworth, I.; Gamache, P.	TN73449, 2020
Achieving standard free quantitation: Thermo Scientific Charged Aerosol Detectors	Anon	SP73026, 2019
Determination of olive oil purity based on triacylglycerols profiling by UHPLC-CAD and Principal Component Analysis	Green, H.; Li, Q.; De Pra, M.; Acworth, I.; Wang, S.	AN73174, 2019
Simultaneous determination of water- and fat-soluble vitamins in tablets and energy drinks by using a novel Vanquish Flex Duo system for Dual LC	Grosse, S.; De Pra, M.; Steiner, F.	AN72592, 2018
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Accelerated Solvent Extraction: A Technique for Sample Preparation	Richter, B. E.; Jones, B. A.; Ezzell, J. L.; Porter, N. L.; Avdalovic, N.; Pohl, C.	Anal. Chem. **1996**, 68, 1033-1039.

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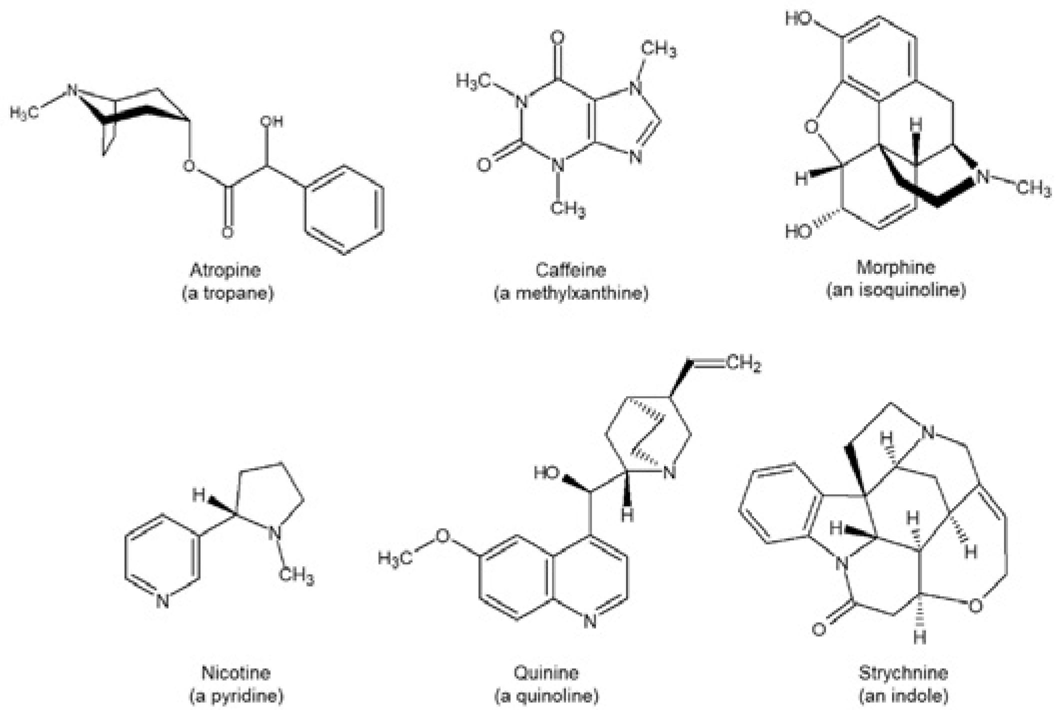
Aglycone

The compound that is left when the carbohydrate is removed from a glycoside. Examples include anthocyanidins (from anthocyanins), genistein (from genistin), and hesperetin (from hesperidin).

Alkaloid

Alkaloids are a large group of structurally diverse, nitrogen-containing, secondary metabolites found in numerous flowering plants and to a lesser extent in animals (e.g., insects, amphibians, reptiles and mammals), fungi and bacteria. Their exact role in plants remains elusive but they may act as protectants preventing damage by herbivores and other pests. Many alkaloids are used in medicine; others are used as recreational drugs. Examples of alkaloids include atropine, caffeine, morphine, nicotine, quinine and strychnine.



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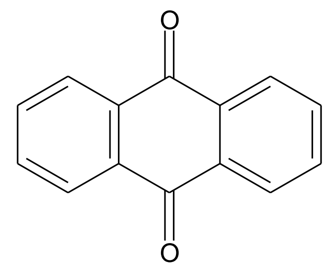
Anthraquinone

Anthraquinone is a polycyclic aromatic compound with a 9,10-dioxoanthracene core:

Numerous anthraquinone derivatives are found in plants including cascarin, catenarin, chrysophanol, emodin, physcion, and rhein. Naturally occurring anthraquinones and their derivatives are reported to possess anticancer, anti-inflammatory, antimicrobial and antimalarial properties.



Anthraquinone

Ayurvedic Medicine

Ayurvedic medicine is a traditional approach used in India for thousands of years. It is a holistic in nature and uses herbs, diet, exercise, meditation, breathing exercises and other methods to treat illness by attempting to restore the balance between mind, body and spirit.

Botanicals

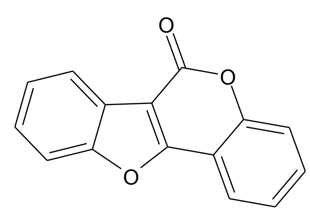
Derived from plants. Includes fresh or dried parts of plants (e.g., roots, stems, leaves, flowers, fruits, and seeds) and their extracts.

Chromophore

A chromophore is a chemical structure that can absorb light at a particular wavelength. The presence of a chromophore in a molecule is essential for it to be measured by UV/Vis absorbance detection.

Coumestans

Coumestan (1-Benzoxolo[3,2-c]chromen-6-one) is the polycyclic aromatic backbone of a group of plant secondary metabolites called the coumestans. One such compound, coumestrol, is a phytoestrogen and can bind to mammalian estrogen receptors, although its activity is much weaker than the endogenous ligand, estradiol.



Coumestan

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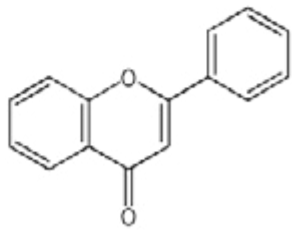
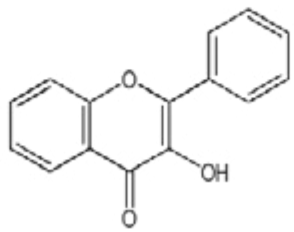
Flavonoids

Flavonoids, sometimes referred to as bioflavonoids are a class of plant secondary metabolites. Originally, the term flavonoid was used to describe only ketone containing compounds, but it now includes many non-ketone polyhydroxy-polyphenolic compounds (e.g., the flavanoids). It has been estimated that several thousand flavonoids occur naturally, and these can be subdivided based upon their chemical structure, as shown below.

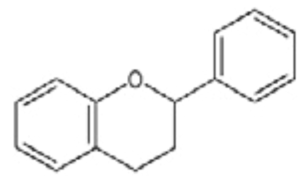
Although flavonoids are reported to possess a wide range of biological and pharmacological activities (e.g., anti-allergic, anti-inflammatory, antioxidant, anti-microbial and anti-cancer) in *in vitro* studies, their potential health benefits *in vivo* are far less clear.



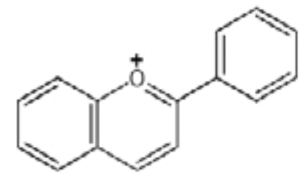
Flavone
(Anthoxanthins - apigenin, luteolin, tangeritin)



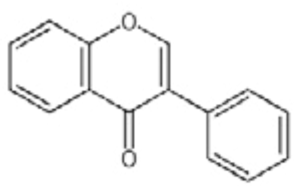
Flavonol
(Anthoxanthins - kaempferol, myricetin, quercetin)



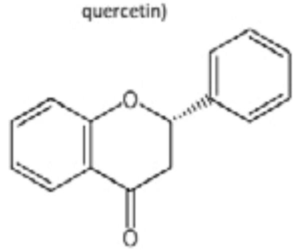
Flavan
(Flavan-3-ol: catechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, proanthocyanidins, theaflavins, thearubigins)



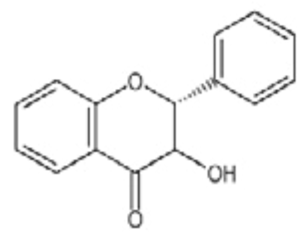
Flavylium
(Anthocyanins and their aglycone anthocyanidins - cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin)



Isoflavone
(Phytoestrogens such as daidzein and genistein and their glucosides daidzin and genistin)



Flavanone
(Hesperetin, naringenin)



Flavanonol
(Taxifolin)

Flos

Flower blossoms.

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Glycoside

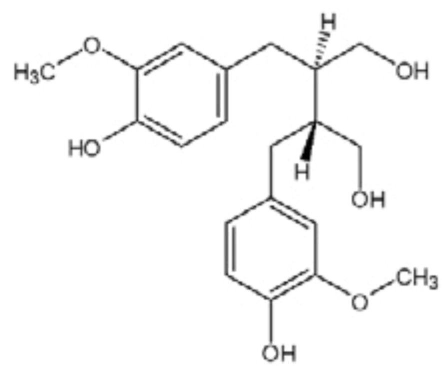
A glycoside is a secondary metabolite formed when a carbohydrate molecule is linked to another molecule (an aglycone) via a glycosidic bond. A wide variety of glycosides are found in plants – resulting from differences in the aglycone moiety, carbohydrate structure and type of glycosidic bond present.

Herbal Medicine

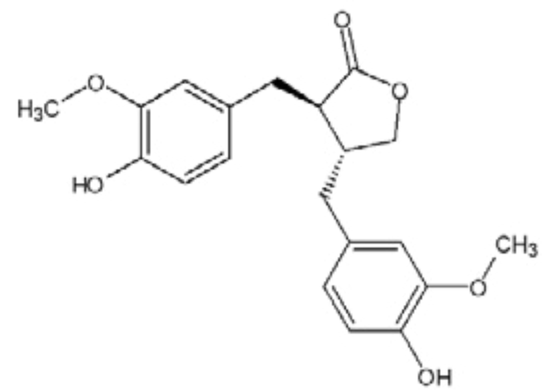
As defined by The World Health Organization, herbal medicines include "herbs, herbal materials, herbal preparations and finished herbal products, that contain as active ingredients parts of plants, or other plant materials, or combinations".

Mammalian Lignans

Mammalian lignans are phytoestrogens formed by the action of gut bacteria on inactive precursors contained within foods. For example, secoisolariciresinol and matairesinol, abundant in flax seed and whole grains, respectively, are converted in the intestine to the mammalian lignans, enterolactone and enterodiol. Flavonolignans are molecules composed of both a flavonoid and a lignan, for example, silibinin from milk thistle.



Secoisolariciresinol



Matairesinol

Mass Spectrometry (MS)

LC-MS is a powerful analytical approach often used for the identification and quantification of analytes in botanical natural products. Additionally, LC-MS with metabolomic workflows can be used for authentication and adulteration studies.

ThermoFisher Scientific offers a complete range of MS detectors including single quadrupole, triple quadrupole, ion trap and orbitrap mass spectrometers.

To learn more about LC-MS see the ThermoFisher Scientific Mass Spectrometry Learning Center.

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Medhya effect

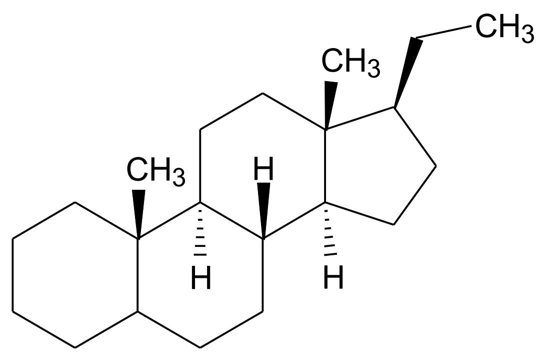
In Ayurvedic medicine the term Medhya Rasayanas refers to a group of several medicinal plants that can be used, singly or in combination, to improve memory and intellect – the medhya effect. *Bacopa monnieri*, called brahmi, is one of these plants.

Metabolomics

Metabolomic approaches can be either untargeted or targeted. Untargeted metabolomics can be defined as the comprehensive measurement of all low-molecular-weight metabolites in a biological specimen. By contrast, targeted metabolomics focuses on the identification and quantitation of a defined subset of metabolites. Analytes are identified and quantified using a wide range of analytical technologies e.g., UHPLC-MS, UHPLC-absorbance detection, and UHPLC-CAD. Multivariate statistical methods can then be used for data interpretation and extraction of information (see PCA). Metabolomic approaches can be used, for example, to authenticate botanical supplements or to help identify possible adulteration.

Oxypregnane Glycosides

These glycated steroidal secondary plant metabolites contain a pregnane backbone and are particularly abundant in Caralluma and Hoodia species. Oxypregnane glycosides are proposed to be the compounds responsible for the purported biological activities of Caralluma and Hoodia supplements.



Pregnane

Pharmacopeia

A pharmacopeia is a book containing detailed methods (monographs) for the determination and quality control of drugs, supplements, traditional medicines, food components etc. It is usually authorized by governments or an appropriate scientific society. Examples include the United States Pharmacopeia, The European Pharmacopeia and The Pharmacopeia of the People's Republic of China.

Principal Component Analysis (PCA)

PCA is a is a mathematical approach used to look for patterns in large data sets. PCA software increases the ability to interpret complex data but without information loss. See "Principal component analysis explained simply" for greater details. PCA has a wide range of applications e.g., authentication of botanicals, supplements and foods.

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Phytoestrogen

Phytoestrogens, or dietary estrogens, are a group of plant-derived secondary metabolites that mimic the effects of endogenous estrogen. These xenoestrogens are structurally diverse and include coumestans, isoflavones, and mammalian lignans.

Rattan

The dried stem of some climbing plants (e.g., honeysuckle and climbing palms).

Saponins

Saponins are a group of terpene and steroid glycosides that are widely distributed in plants. They are characterized by their ability to form emulsions, produce a soapy lather, and acting as detergents.

Secondary Metabolites

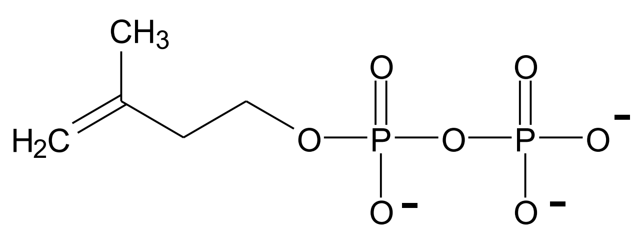
Primary metabolites consist of a wide range of biochemicals that are involved in normal growth and development of an organism. Secondary metabolites are compounds that are not essential for life but for example in plants, may play an adaptive role produced during periods of environmental stress or by acting as defense molecules formed in response to damage by pathogens or herbivores. Plant secondary metabolites include alkaloids, glycosides, phenolics and terpenes.

Standards

The quantification of key analytes in a botanical natural product can be challenging. Method development may be hampered by the availability of authentic standards. Some standards are commercially available or can be obtained through collaboration. However, in many instances, the researcher may be required to isolate, purify, identify and quantify analytes, themselves. The CAD is an extremely useful tool for analyte quantification as its response is independent of chemical structure, and so can be used to measure the amount of an analyte even though authentic standards are unavailable.

Terpenes

Terpenes are a large group of structurally diverse plant secondary metabolites formed from units of isopentenyl pyrophosphate:



Isopentenyl Pyrophosphate

Terpenes include many compounds discussed in this document and include diterpenes e.g., taxane, triterpenes found in bacopa, Boswellia, black cohosh and ginseng, and the sesquiterpene, ursolic acid found in ginkgo. For a more in-depth discussion of the classification of the various terpenes found in plants, see: Terpenes and Terpenoids by S. Perveen.

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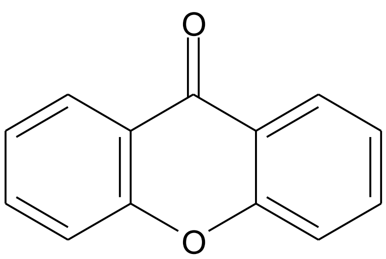
Traditional Medicine

Traditional medicine (TM) (sometimes called indigenous medicine) can be defined as "the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses". TM is used by many cultures to treat sickness, prevent illness and maintain good health. It typically integrates herbal, animal and mineral medicines, along with spiritual therapies and exercises. Examples of TM include Ayurveda, Siddha medicine, Traditional African medicine and Traditional Chinese medicine.

Xanthones

Xanthone (9H-Xanthen-9-one) is the central core of many secondary metabolite phytochemicals. Examples include mangostin, a xanthone found in the pericarp of the mangosteen fruit, and mangiferin found in mangoes.



Xanthone

Xenoestrogens

The xenoestrogens are a group of compounds, sometimes called endocrine disruptors, that can mimic the effects of endogenous estrogen. These "foreign" compounds include natural compounds (e.g., phytoestrogens), as well as synthetic chemicals (e.g., bisphenols).

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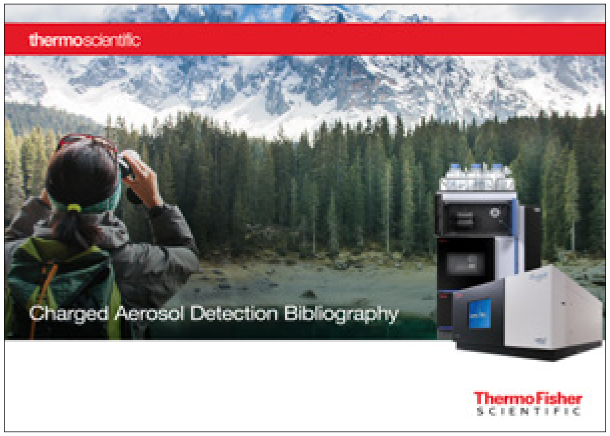

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