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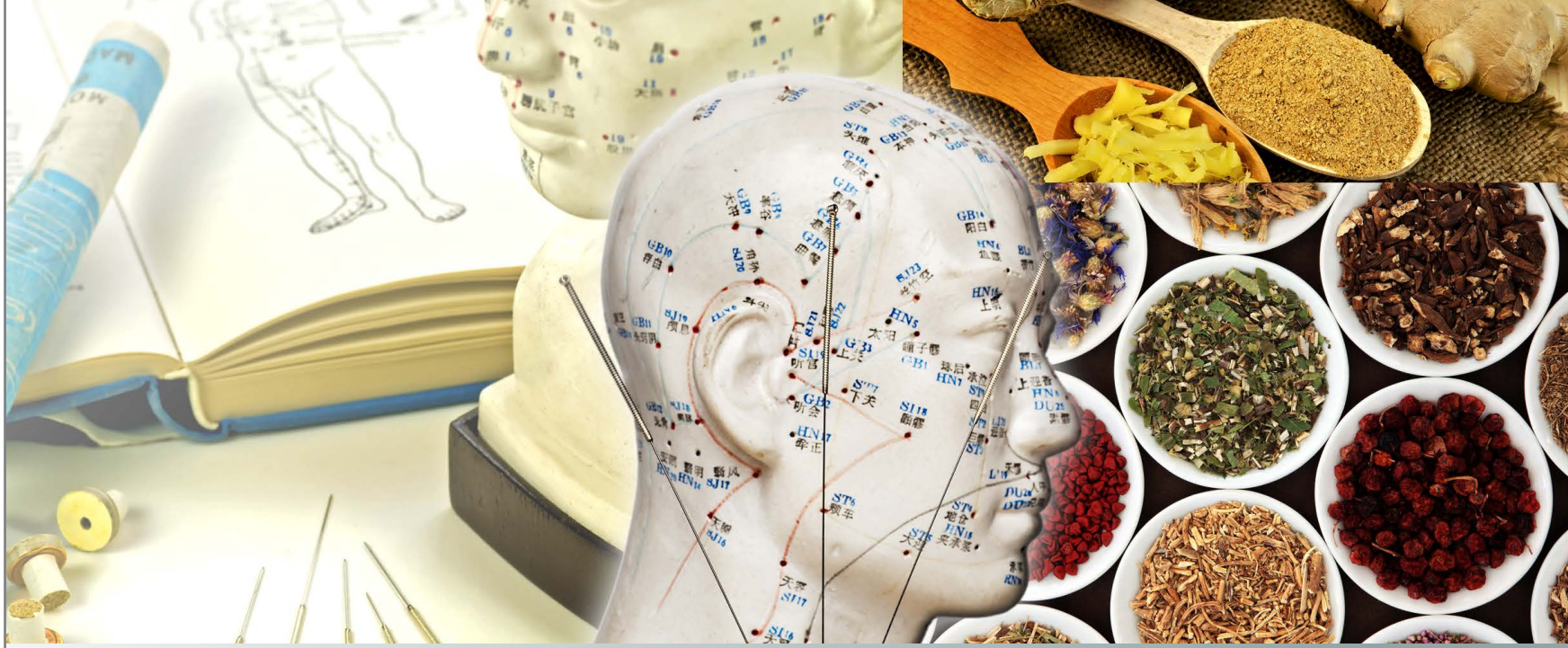

Traditional Chinese Medicine HPLC Applications Notebook

Time-Honored Remedies, Innovative Analysis

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The Liquid Chromatography System

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Traditional Chinese Medicine HPLC Analysis

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Traditional Chinese Medicines (TCM) is a group of treatments that have been regularly employed over many hundreds of years and are still in use today. These medicines have been shown to alleviate illness, improve physical appearances as well as increase overall individual health. Strong tradition and culture has helped to maintain the popularity of TCM and Chinese herbal medicines but in recent years concerns have grown that they have been affected by environmental and external factors. Thermo Scientific LC Systems offer distinct benefits to help ensure the efficacy, purity and safety of these traditional remedies.

Using our UHPLC-ready systems and our highly sensitive and selective detectors, state of the art column technologies, along with proven analytical methods, precise automation, and advanced data handling will help you to:

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- Perform reliable separations to ensure confidence in product quality control
- Determine and identify a wide variety of compounds with hard to detect analytes

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Aristolochia species (e.g., Virginia snakeroot, guaco) are common ingredients in traditional Chinese herbal remedies and herbal dietary supplements. Unfortunately, over the last ten years consumption of herbals containing Aristolochia has resulted in numerous cases of late stage renal failure often associated with urothelial tract carcinoma. Initially termed "Chinese herbs neuropathy" the disease has now been renamed "aristolochic acid nephropathy" in recognition of the active toxin(s) present and the fact that Aristolochia species are used in many non-Chinese herbal supplements. In 2000, the FDA released a warning to health care professionals warning of the consequences of Aristolochia consumption. The principal nephratoxin present is aristolochic acid (AA). AA is composed of a group of several nitrophenanthrene carboxylic acids – aristolochic acid I (AA1) and aristolochic acid II (AA2) being the most abundant. This example below shows a highly selective and sensitive method for measurement of AA found in both plant and animal tissues.

Conditions	
Flow:	1.0 mL/min
Temperature:	Ambient
Column:	C18, 5 µm, 4.6 × 150 mm
Injection Volume:	50 µL
Mobile Phase:	Acetonitrile – water, 70:10 (v/v) containing 100 mg/L sodium dodecyl sulfate; final pH 2.0 with phosphoric acid
Detection:	Electrochemical, Model 5600A, CoulArray, UV
Detector Wavelength:	252 nm

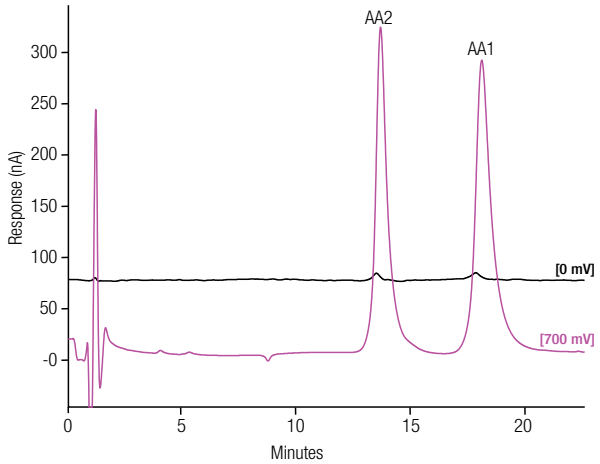

Figure 1. Comparison of electrochemical (purple trace) and UV (black trace) responses.

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Caulis lonicerae, the dried rattan of Lonicerajaponica (Caprifoliaceae or honeysuckle family), is an important traditional Chinese medicine used for the treatment of such ailments as acute fever, headache, respiratory infection, and epidermic diseases. The major active components in Caulis lonicerae are loganin, sweroside, chlorogenic acid, caffeic acid, rutin, and galuteolin. This example shows an efficient and comprehensive HPLC QC method to separate the six main active components found in Caulis lonicerae.

Conditions	
Flow:	1.0 mL/min
Temperature:	30 °C
Column:	Acclaim Phenyl-1, 4.6 × 150 mm, 3 µm
Injection Volume:	5 µL
Mobile Phase:	Acetonitrile 0.4%; Formic Acid Aqueous (v/v)
Gradient:	Acetonitrile, -2—0 min, 17%; 0—4min, 17—30%; 4—6 min, 30—45%; 6—10 min, 45%
Detection:	UV at 236 nm

Analyte	Detected (mg/g)	Added (mg/g)	Found (mg/g)	Recovery (%)
Loganin	2.73	2.50	2.18	87
Sweroside	2.79	2.50	2.23	89
Chlorogenic acid	2.77	2.50	2.33	93
Caffeic acid	0.40	0.50	0.45	90
Rutin	Not Found	0.50	0.425	85
Galuteolin	Not Found	0.50	0.495	99

Table 1. Analytical results of the active components of Caulis lonicerae.

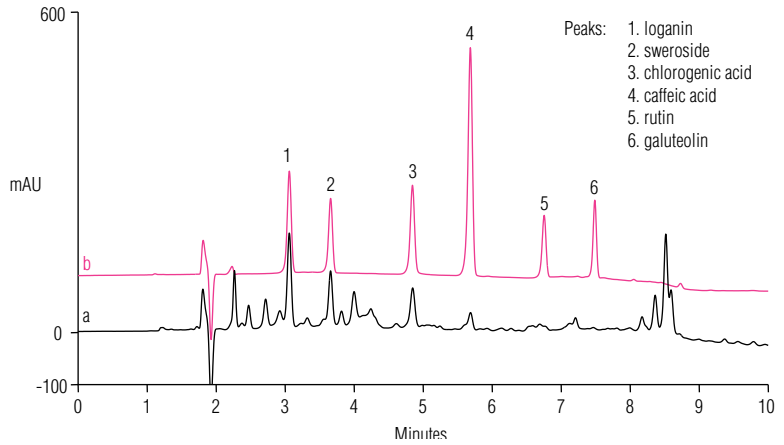

Figure 2. Separation of a Caulis lonicerae sample (a) and mixture of standards (b).

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Ginkgo biloba has been used in Traditional Chinese Medicines for thousands of years, and is thought to possess nootropic activity. It is taken to combat memory loss, enhance concentration and improve blood circulation, particularly in the brain. Sesquiterpenoid bilobalide and numerous diterpenoid ginkgolides are believed to be the active ingredients. As shown in the example below, charged aerosol detection is able to detect numerous non-volatile compounds in a *Ginko biloba* extract.

Conditions	
Flow:	1.0 mL/min
Temperature:	Ambient
Column:	C18, 4.6 × 250 mm; 5 µm
Injection Volume:	10 µL
Mobile Phase:	A: 5% Acetonitrile in 0.1% trifluoroacetic acid; B: 70% acetonitrile in 0.1% trifluoroacetic acid
Gradient:	% A: 0 min, 100; 30 min, 25; 35 min, 25; 40 min, 100
Detection:	Charged Aerosol

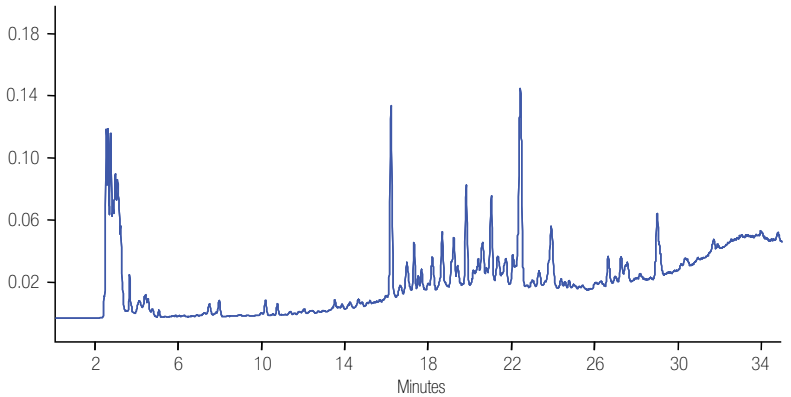

Figure 3. Analysis of *Ginkgo biloba* extract.

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

Asian Ginseng (Panax ginseng) has traditionally been used as a tonic to reduce the effects of stress, counteract fatigue, and increase stamina. The main bioactive ingredients found in Panax ginseng, and a related species *Panax quinquefolius* (American ginseng) are triterpene saponins, commonly referred to as ginsenosides. There are 7 major ginsenosides present in Panax ginseng: the protopanaxatriols (Rg1, Re and Rf), and protopanaxadiols (Rb1, Rc, Rb2 and Rd). HPLC with low-wavelength UV detection (203 nm) is most commonly used but suffers from poor sensitivity. As shown in the example below, HPLC with charged aerosol detection not only improves the baseline slope seen with gradient elution, but also offers improved sensitivity.

Conditions	
Flow:	0.67 mL/min
Temperature:	32 °C
Column:	Fused-Core C18 HPLC Column, 3.0 x 100 mm, 2.7 µm
Injection Volume:	20 µL
Mobile Phase:	A—Water; B—Acetonitrile
Gradient:	15% B to 35% B in 30 minutes
Detection:	Charged Aerosol

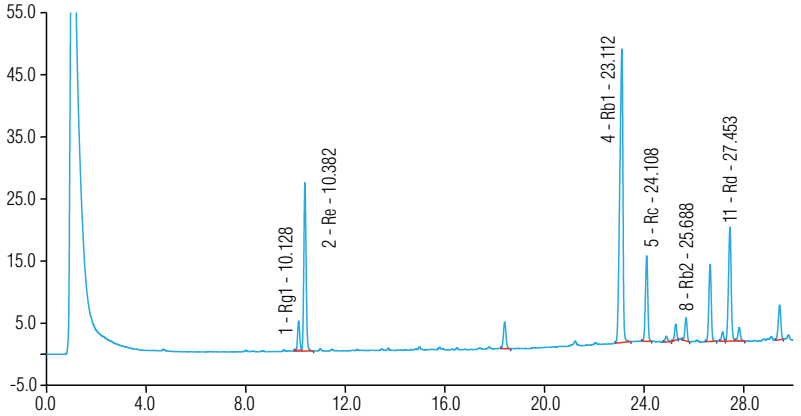

Figure 4. Analysis of ginseng extract.

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

Centella asiatica (commonly called gotu kola) is a small herbaceous annual plant native to India, Sri Lanka, Northern Australia, other parts of Asia, and the Western Pacific. It is used as a medicinal herb in Ayurvedic medicine and in traditional Chinese medicine to treat a wide variety of conditions, such as improving memory, blood flow, as a wound-healing agent, and as a topical application for skin conditions such as ulcers, wounds, and eczema. The chemical compounds of interest in gotu kola are usually considered to be the ursane- and oleanane-type triterpenes and triterpene glycosides. Although low-wavelength UV can be used to measure these compounds, it suffers from sensitivity and baseline issues – these can be readily overcome by using charged aerosol detection, as seen in the example below.

Conditions	
Flow:	0.64 mL/min
Temperature:	35 °C
Column:	Fused-Core C18 HPLC Column, 3.0 x 100 mm, 2.7 µm
Injection Volume:	5 µL
Mobile Phase:	A—0.1% Formic Acid in Water; B—Acetonitrile
Gradient:	18% B to 22% B in 8 min; 22% B to 45% B from 8 min to 17 min; 45% B to 80% B form 17 to 23 min
Detection:	Charged Aerosol

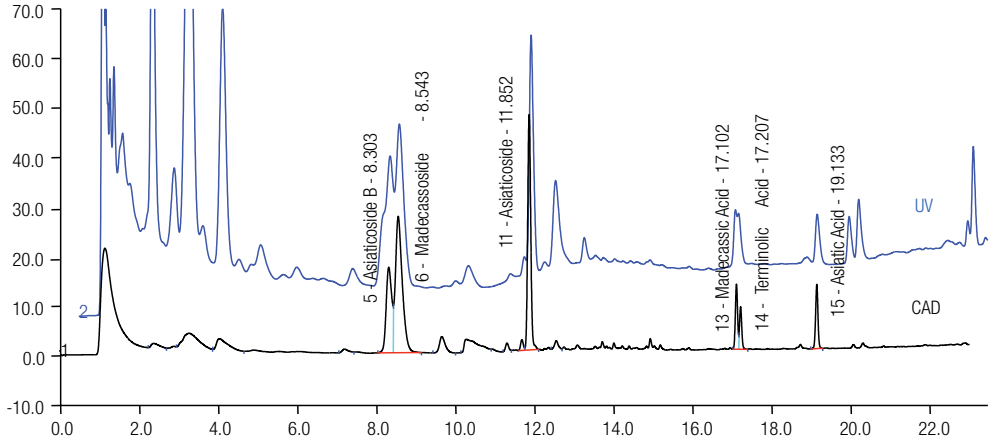

Figure 5. Comparison of UV and charged aerosol detection response for gotu kola extract.

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Hesperidin, a polyphenolic bioflavonoid, is the predominant flavonoid in orange peel and other citrus fruits. Hesperidin is an antioxidant that enhances the action of vitamin C to lower cholesterol levels. It is also known to have pharmacological action as an anti-inflammatory, antihistaminic, and antiviral agent. The Pharmacopoeia of the People's Republic of China (PPRC) 2010 recommends its extraction from fruits with a Soxhlet extraction method using ligarine and methanol, which is both time and solvent consuming. In the example below, we demonstrate a more efficient and cost-effective method to determine hesperidin in orange peel and other citrus fruits. Additionally, a gradient HPLC spectro-electro array platform can be used to resolve and quantify specific polyphenols and related flavonoids for product authentication and evaluating adulteration in product quality control.

Extraction Method	Soxhlet Extraction	Dionex ASE 350
Sample Amount (g)	1	1
Solvent Amount (mL)	200	40
Time (min)	300	35
Detected Amount of Hesperidin (%)	5.2	6.3
RSD	7.4	3.1

Table 2. Comparison of extraction methods using Solhlet extraction and the Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor System.

Conditions	
Flow:	0.50 mL/min
Temperature:	40 °C
Column:	Thermo Scientific™ Acclaim™ RSLC 120 C18, 2.2 µm, 3 x 30 mm
Injection Volume:	1 µL
Mobile Phase:	Methanol/Water/Acetic Acid, 31/65/4 (v/v)
Detection:	UV at 283 nm

Chromatograms: a. Mobile phase
b. Hesperidin standard (2 µg/mL)
c. Orange peel sample 1
d. Orange peel sample 2 (100-fold dilution)
e. Lemon peel sample (50-fold dilution)

Peak: 1. Hesperidin

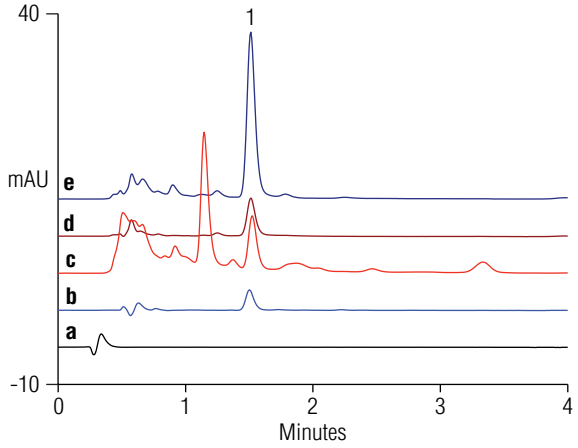

Figure 6. Chromatograms of hesperidin in orange and lemon peel samples

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Download Application Brief 142: Rapid Determination of Hesperidin in Orange Peel Using Accelerated Solvent Extraction and UHPLC

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

Mogroside

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Luo han kuo fruit (*Siraitia grosvenori* Swingle) has long been used in traditional Asian medicine. Recently cucurbitane-type and other triterpene glycosides have been isolated from the fruit and investigated for numerous potential health benefits such as antioxidant activity, anticancer effects, and antihyperglycemic effects. In this work, mogroside V is determined in a luo han kuo beverage by both charged aerosol and UV detections. This method uses HILIC conditions suitable for the trimode column, allowing separation of multiple terpene glycosides, and has also been used to separate steviol glycosides. The volatile mobile phase makes charged aerosol detection possible, which adds further method flexibility and improved detection sensitivity.

Conditions	
Flow:	0.30 mL/min
Temperature:	20 °C
Column:	Thermo Scientific™ Acclaim™ Trinity™ P1, 3 µm analytical, 2.1 x 100 mm and guard
Injection Volume:	5 µL
Mobile Phase:	81/19 Acetonitrile/10mM, Ammonium Formate, pH = 3.00
Detection:	A) Charged Aerosol, Nebulizer temp. 10 °C B) UV at 210 nm

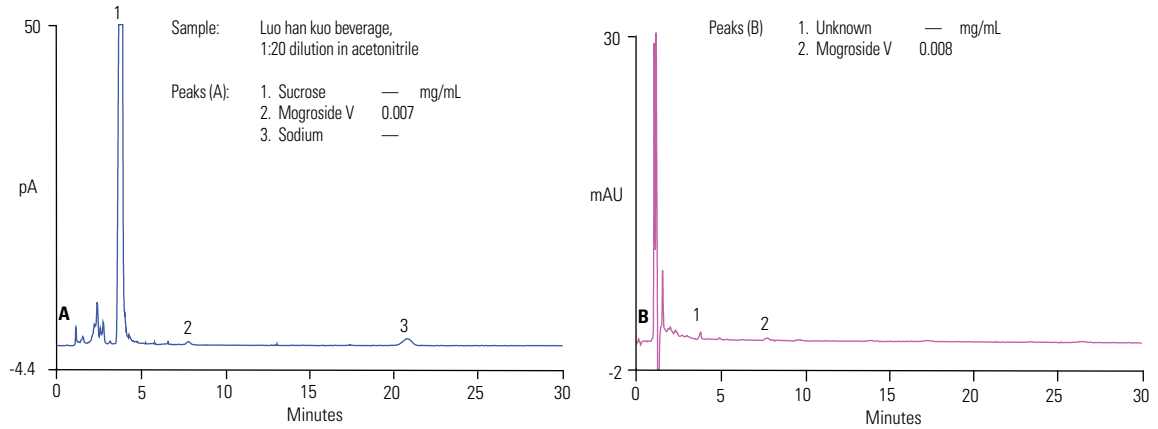

Figure 6. Separation of mogroside V in a luo han kuo beverage as detected by A) charged aerosol detection and B) UV, 210 nm. Note the good separation between sucrose and mogroside V in chromatogram A.

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Download Application Update 184: Mongroside V Determination by HPLC with Charged Aerosol and UV Detectors

Zanthoxylum nitidum (Roxb.) DC is an important traditional Chinese medicine. The Pharmacopeia of the People's Republic of China (PPRC) 2010 regulates this dried root as an herbal medicine. Nitidine is the specific active component in the dried root version of *Zanthoxylum nitidum* (Roxb.) DC, and nitidine chloride is reported to be beneficial for both killing and constraining the growth of cancerous cells. *Zanthoxylum nitidum* var. fastuosum is another plant in the same genus as *Zanthoxylum nitidum* (Roxb.) DC. Although *Zanthoxylum nitidum* var. fastuosum is not regulated in the PPRC, its dried root is still used in Chinese folk medicine because some of its reported medical benefits, such as promotion of blood circulation, pain relief, treatment of gastric ulcers, and reduction of inflammation, are the same as those reported for *Zanthoxylum nitidum* (Roxb.) DC. Nitidine and toddalolactone are the specific active components in *Zanthoxylum nitidum* (Roxb).

Analyte	Retention Time RSD	Peak Area RSD
Toddalolactone	0.045	0.865
Nitidine chloride	0.048	1.433
Chelerythrine chloride	0.043	1.320

Table 5. Reproducibility for peak retention time and area.

Conditions	
Flow:	0.60 mL/min
Temperature:	30 °C
Column:	Acclaim LC PA, 3 µm, Analytical, 2.1 x 150 mm
Injection Volume:	5 µL
Mobile Phase:	25 mM Ammonium Acetate (pH 4.5 with Acetic Acid)/Acetonitrile
Gradient:	Acetonitrile: 0 min, 20%; 8 min, 30%; 15 min, 70%, curve 4; 15.5—18 min, 20%
Detection:	UV at 273 nm

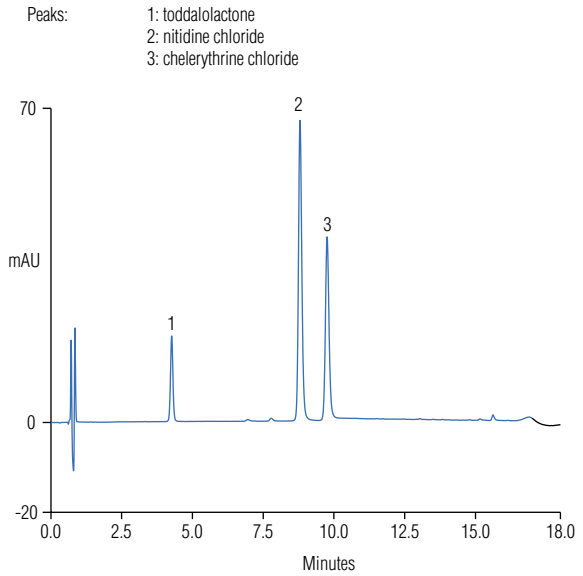

Figure 8. Chromatogram of a mix of nitidine chloride, chelerythrine chloride, and toddalolactone standards (10 µg/mL each)

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

Schizandrin

Schisandra chinensis (Turcz.) Baill is an important traditional Chinese medicine believed to be an anticarcinogen and provide hepatoprotection, among other attributes. Its major active components are lignanoids, and the three major lignanoids are schizandrin, schizandrin A, and schizandrin B. Baill, are a traditional Chinese medicine for hepatoprotection. The Pharmacopoeia of the People's Republic of China (PPRC) 2010 regulates its quality control with a UHPLC method for the determination of schizandrin, schizandrin A and schizandrin B. The work shown here describes an efficient UHPLC method to determine these compounds in Hugan tablets for product quality control, using an Acclaim RSLC column.

Conditions	
Flow:	0.40 mL/min
Temperature:	40 °C
Column:	Acclaim RSLC 120 C18, 2.1 x 100 mm, 2.1 µm
Injection Volume:	2 µL
Mobile Phase:	Acetonitrile/Water in gradient
Gradient:	Acetonitrile: 0—3 min, 45%; 3—5 min, 45—80%; 15.1 min, 80—100%; 12 min, 100%
Detection	UV at 250 nm

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

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

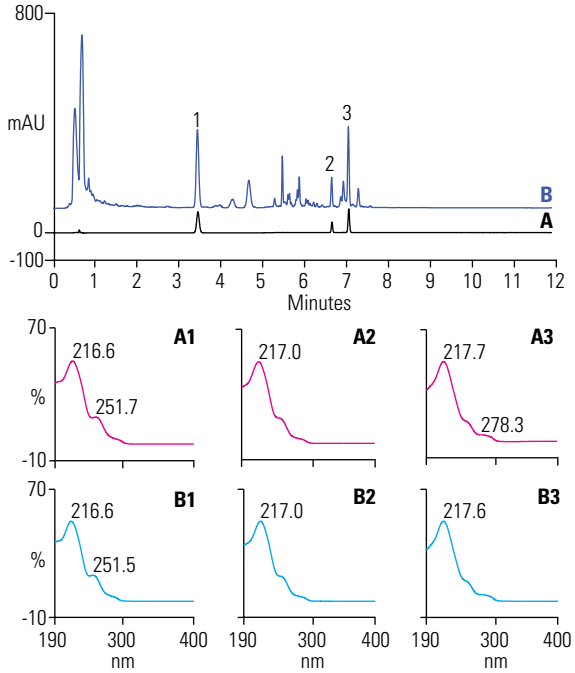

Figure 9. Chromatograms A) standards and B) tablet samples of a schizandrin, schizandrin A, and schizandrin B mixed standard and a Hugan tablet sample.

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Charged Aerosol Detection Bibliography

This bibliography is designed to readily show the analytical capabilities of charged aerosol detection and highlights the breadth and scope of the different analytical methods found within the literature.

See why charged aerosol detection is the industry preferred universal LC detector!

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