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Application Note SI-01236

Accurate Analysis of Triterpene Glycosides in Black Cohosh by HPLC with ELSD

Stephen Bullock
Polymer Laboratories, now a part of Varian, Inc.

Introduction

The Varian evaporative light scattering detector is universal and its response is not dependent on the optical properties of the compound. Consequently, its good discriminating power and sensitivity is well suited to compounds such as triterpene glycosides that possess weak or no UV chromophores.

Triterpene glycosides (saponins) are molecules that have a four- or five-ring planar-base containing 30 carbon atoms (aglycone) with various attachments of sugar molecules such as glucose, galactose, glucuronic acid or xylose. The great complexity of triterpene glycoside structures arises from the variability of the aglycone structure, the nature of the side chains and the position of attachment of these sugar moieties. The isolation, analysis and structural determination of triterpene glycosides demands accurate and sophisticated techniques due to their unique chemical nature. The task of isolating these compounds from plant material is further complicated by the presence of many closely related substances in the plant tissue and by the fact that most of the saponins lack a chromophore.

HPLC is typically used as part of the purification process to isolate and identify triterpene glycosides in plant material, in order to achieve high purity extracts. Once the saponins have been purified, analytical techniques such as MS, NMR and infrared spectroscopy are used to elucidate their structure.

Triterpene glycosides occur in a number of plant species, such as black cohosh root (Cimicifuga racemosa (L.) Nutt.), which is taken as a dietary supplement in the belief that it relieves symptoms of the menopause and hot flushes. The primary active constituents of black cohosh root are the triterpene glycosides actein, 27 deoxyactein and cimifugoside, although biologically active substances including alkaloids, flavonoids and tannins are also thought to contribute to the herb's potency.

Instrumentation

Column: C18 5 µm, 150 x 4.6 mm
Detection: Varian ELSD (neb=30 °C, evap=50 °C, gas=1.4 SLM); UV-vis, 230 nm

Materials and Reagents

Eluent A: 0.1 % Formic acid in water
Eluent B: ACN

Sample Preparation

Sample: Black cohosh tablet

Conditions

Flow Rate: 1.0 mL/min
Injection Volume: 20 µL
Gradient: 30-40 % B in 30 min, 40-60 % B in 30 min, 60-30 % B in 10 min

Results and Discussion

Evaporative light scattering detection is a better alternative than UV detection for the analysis of triterpene glycosides because these compounds possess weak or no UV chromophores, thus limiting their sensitivity and the ability to run gradient elution on account of the need to analyze at short UV wavelengths (eg 230 nm).

This is highlighted in Figure 1, which shows the analysis of a black cohosh sample by UV and ELSD. To ensure the potency of black cohosh, commercially available extracts from manufacturer to manufacturer are standardized to contain 2.5 % triterpene glycosides.

However, the determination of two commercial, standardized tablets by ELSD, as shown in Figure 2, reveals differences in the composition and concentration of the active components.

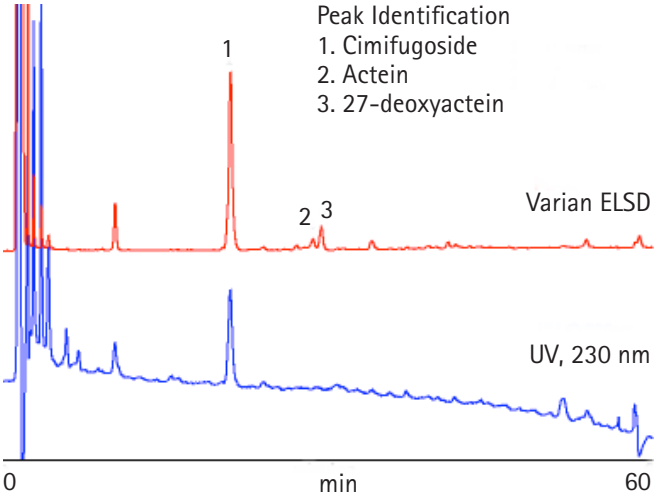

Figure 1. Separation of black cohosh tablets showing the superiority of the Varian evaporative light scattering detector over UV detection.

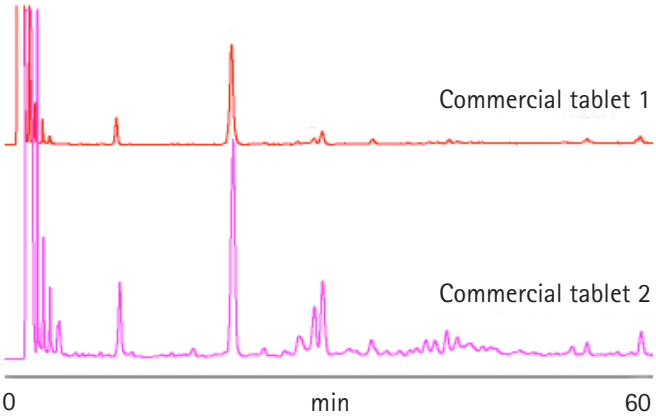

Figure 2. Highlighting the differences in active components in black cohosh tablets from different manufacturers using the Varian evaporative light scattering detector.

Conclusion

There is pressure to establish industry-wide standards to help ensure that dietary supplements are manufactured consistently with regard to their identity, purity, quality, strength and composition. Reliable and accurate analytical techniques are required to meet these objectives. HPLC with Varian evaporative light scattering detection are ideal for the elucidation of active ingredients in dietary supplements, providing important quality control data for industry, regulators and consumers.

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Application Note SI-01236 / TB1071

Analysis of Complex Triglycerides in Starflower Oil from Borage by HPLC using ELSD

Stephen Bullock
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Introduction

The Varian evaporative light scattering detector is universal and independent on the optical properties of the compound under analysis. Consequently, its good discriminating power and sensitivity is well suited to compounds, such as triglycerides, that possess weak or no UV chromophores.

Starflower oil (borage oil) is extracted from the annual herb, borage (Borago officianalis). The oil is rich in the fatty acid gamma linolenic acid (GLA), which is an important intermediary in the metabolic conversion of linolenic acid into prostaglandin E1 (which modulates the action of many hormones). It is claimed to be beneficial for a wide range of conditions, such as premenstrual syndrome, eczema, rheumatoid arthritis as well as reducing cholesterol and blood pressure. An optimal dose and length of treatment for borage oil has not been established, although manufacturers recommend a dose of approximately 3 g of borage oil per day, providing approximately 700 mg of GLA.

The majority of dietary supplements, such as borage oil, are not subject to review or approval by the US Food and Drug Administration (FDA). As a result, the amounts of active ingredients or contaminants which they contain, may vary between brands or between different batches of the same brand. In addition, few reliable studies into the safety and efficacy of borage oil have been done. Consequently, the side effects of long term exposure to the oil are unknown, and so there is a need for accurate, analytical methods that characterize the composition of borage oil supplements, in order to provide a means of standardizing the quality of the herbal product from batch to batch and between manufacturers.

Borage oil contains a complex mixture of triglycerides that can be analyzed using several methods. Gas chromatography (GC) of methyl ester derivatives and HPLC coupled with refractive index (RI) detection have been reasonably successful for triglyceride analysis.

However, derivatization in GC is problematic for complex mixtures, whereas refractive index lacks sensitivity and is not compatible with gradient elution. HPLC with UV detection is a better choice than refractive index, but due to the low UV wavelengths required to analyze triglycerides the choice of mobile phase solvents is limited, and baseline drift is common with gradient elution. Evaporative light scattering detection with Varian's ELSD is superior to both RI and UV detection.

Instrumentation

Column: C18 5 µm, 250 x 4.6 mm
Detection: Varian ELSD (neb=25 °C, evap=50 °C, gas=1.4 SLM)

Materials and Reagents

Eluent A: ACN
Eluent B: DCM

Sample Preparation

Sample: 2 mg borage oil/mL

Conditions

Flow Rate: 1.0 mL/min
Injection Volume: 20 µL
Gradient: 30-50 % B in 40 min, 50-90 % in 2 min, hold three min

Results and Discussion

The excellent baseline stability of the Varian ELSD is shown in Figure 1.

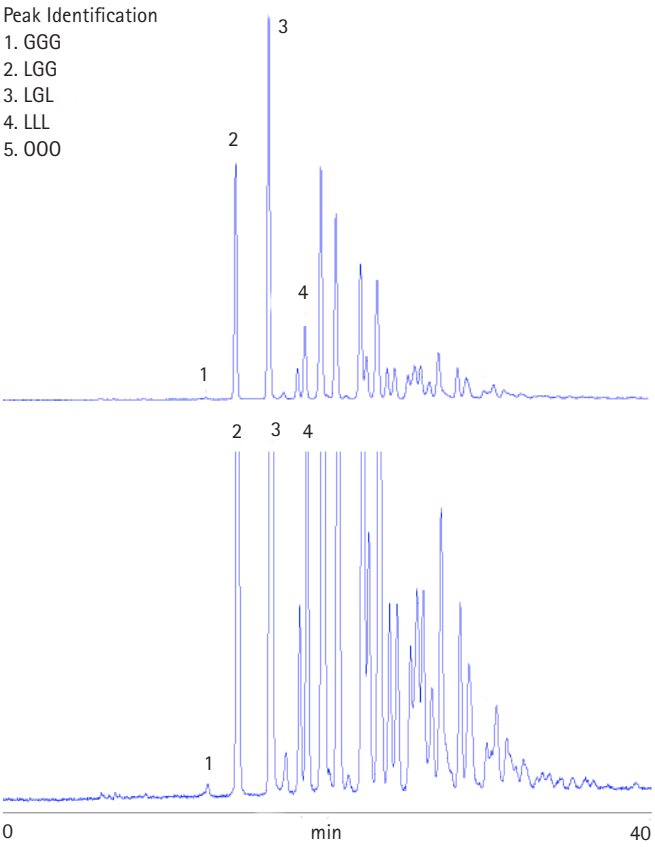

Figure 1. HPLC separation of borage oil using ELS detection (above) (with zoomed baseline (below)) showing the excellent baseline stability of the Varian ELSD.

Table 1. Abbreviations for fatty acids.

Symbol	Compound	No of Carbons
P	Palmitic acid	16
O	Oleic acid (cis-9)	18
L	Linoleic acid (cis, cis-9, 12)	18
Ln	α-Linolenic acid	18
G	γ-Linolenic acid (GLA)	18

Conclusion

The Varian ELSD revealed the true composition of triglycerides in borage oil, due to its sensitivity to compounds that possess weak or no UV chromophores. As the Varian ELSD is universal and independent of the optical properties of the compound, it detects any compound that is less volatile than the mobile phase, For samples, such as borage oil, that possess a complex triglycerides profile, gradient elution is required to provide optimum resolution and reduce run time. This is no problem for the Varian ELSD because it is compatible with a wide range of solvent gradients, displaying excellent baseline stability.

The Varian ELSD surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Varian ELSD's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100 % water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the Varian ELSD provides superior performance compared to competitors' detectors for the analysis of semi-volatile compounds.

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Single Run Analysis of St John's Wort Tablets by HPLC with ELSD

Stephen Bullock
Polymer Laboratories, now a part of Varian, Inc.

Introduction

St John's Wort *(Hypericum perforatum L.)* has been used extensively in Europe for the relief of mild neuralgia and to help reduce the effects of depression, mild anxiety and nervous tension. Although St John's Wort has been the focus of a number of studies, doubt remains as to its mode of action. The herb contains a complex mixture of chemicals, including hypericin and hyperforin, which are believed to be the two main active ingredients in St John's Wort. Hyperforin, in particular, has been noted as a potent uptake inhibitor of the neurotransmitters serotonin (5-HT), norepinephrine, dopamine and GABA, which are believed to be responsible for feelings of anxiety, depression and related conditions.

The US FDA classifies St John's Wort as a dietary supplement and, as a result, the herbal product can be sold without requiring studies on dosage, safety or effectiveness. However, work has shown that St John's Wort can interact with certain drugs, and these interactions can be dangerous depending on the dose of the herb. Therefore, the need to monitor and characterize herbal formulations is paramount, because the strength and quality of St John's Wort products are often unpredictable. Products can differ in content not only from brand to brand, but also from batch to batch.

While HPLC has proved an ideal method of separating the complex components within St John's Wort, detection methods such as fluorescence[1] are too specific to reveal all of the components within the formulation. UV[2] detection is less specific but the optical differences between compounds within St John's Wort require multiple wavelength analysis, which requires multiple chromatographic runs.

A better alternative for the detection of St John's Wort by HPLC is to use the Varian evaporative light scattering (ELS) detector, which is universal and not dependent on the optical properties of the compound. Consequently, the instrument detects all of the components within St John's Wort in a single chromatographic run, if the separation has been optimized to separate the individual compounds within the formulation.

The advantanges of the Varian ELS detector are highlighted in the isolation and quantification of hypercin and hyperforin in different herbal formulations.

Instrumentation

Column: C18 5 µm, 150 x 4.6 mm
Detection: Varian ELSD (neb=30 °C, evap=50 °C, gas=1.6 SLM)

Materials and Reagents

Eluent A: 0.1 % Ammonium formate, adjusted to pH 2.5
Eluent B: Acetonitrile

Sample Preparation

Sample: St John's Wort tablets

Conditions

Flow Rate: 1.0 mL/min
Injection Volume: 20 µL
Gradient: 50-95 % B in 10 min, hold 5 min

Results and Discussion

The chromatographic profile varies at different evaporator temperatures due to the loss of semi-volatile components within the St John's Wort formulation. For the analysis of the active ingredients, hypericin and hyperforin, 50 °C is the optimum temperature. However, some of the minor peaks show a reduction in response (Figure 1).

The Varian ELS detector can be used to compare the composition of St John's Wort tablets from different manufacturers, as shown in Figure 2.

These chromatograms show the differences in composition between standardized St John's Wort tablets from different manufacturers. The ratio of hypericin to hyperforin varies between the tablets, as well as the number and concentration of minor peaks. This shows how the Varian ELS detector can provide a fast, cost effective method for characterizing St John's Wort extract.

Peak Identification
1. Hypericin
2. Hyperforin
3. Adhyperforin

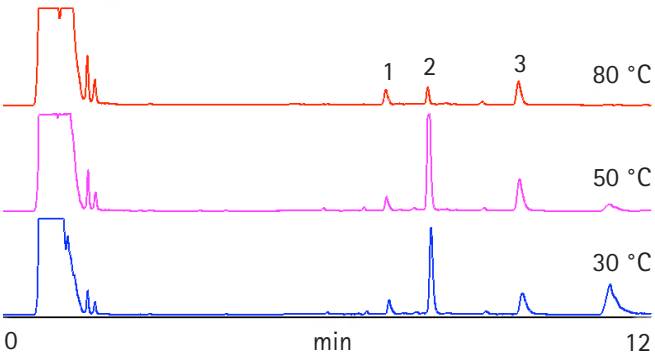

Figure 1. Chromatographic profile varies at different evaporator temperatures and some of the minor peaks show a reduction in response.

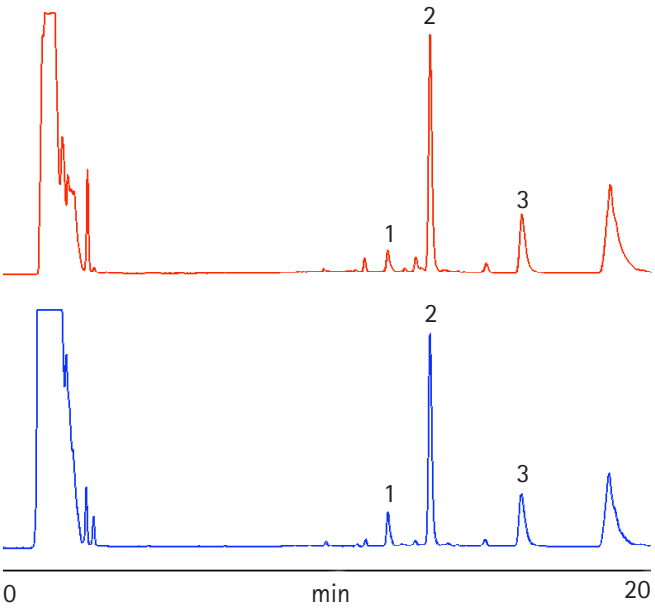

Figure 2. Differences in composition of St John's Wort tablets from two different manufacturers revealed by Varian ELSD.

Conclusion

ELSD is capable of detecting any compound that is less volatile than the mobile phase. The Varian ELS detector can evaporate highly aqueous eluents at 25 °C, facilitating the detection of semi-volatile compounds. For complex mixtures, such as St John's Wort, which contain compounds of differing volatilities, operating the Varian ELS detector at low temperature offers several advantages over standard detction methods.

References

[1]Draves, A. and Walker, S. 2000. Determination of hypericin and pseudohypericin in pharmaceutical preparations by HPLC with fluorescence detection. J. Chromatogr. B, 749: 57-66

[2]Ang, C.Y. et al. 2002. Determination of St John's Wort components in dietary supplements and functional foods by liquid chromatography. J. AOAC Int., 85: 1360-1369

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Application Note SI-01228 / TB1063

Excellent Separation of Ginseng Root Constituents by HPLC with ELSD

Stephen Bullock
Polymer Laboratories, now a part of Varian, Inc.

Introduction

The root of the ginseng plant (Panax quinquefolium L.) has been used in Chinese medicine for thousands of years, due to its many reported health benefits. Ginseng is said to help people with low and high blood pressure, energizes those suffering from fatigue and helps calm people prone to stress or nervousness. It also has a reputation for improving mental clarity and memory, enhancing physical stamina and bolstering the immune system. The biologically active constituents of ginseng are complex mixtures of triterpene saponins, known as ginsenosides. At least 30 ginsenosides have been isolated and characterized, with R_{g1}, R_{b1}, R_{b2}, R_c and R_d being the most important in ginseng root. Many different techniques have been employed to identify and quantify ginsenosides, such as TLC, colorimetry, GC and HPLC. HPLC-UV methods provide excellent separation of ginsenosides. However, ginsenosides possess poor UV chromophores, which limit their sensitivity and the ability to run gradient elution owing to the need to analyze at short wavelengths. The Varian evaporative light scattering (ELS) detector can recognize any compound less volatile than the mobile phase, regardless of its optical properties. It is for this reason that the ELS detector is often referred to as 'universal'.

Instrumentation

Column: C8 5 µm, 300 x 7.7 mm
Detection: Varian ELSD (neb=50 °C, evap=70 °C, gas=1.4 SLM)

Materials and Reagents

Eluent A: Water
Eluent B: Acetonitrile

Conditions

Gradient: 20-60 % B in 30 min
Flow Rate: 0.8 mL/min
Injection Volume: 20 µL

Results and Discussion

The benefits of evaporative light scattering detection with the Varian ELSD is apparent in the analysis of ginseng root powder (Figure 1) and two of its major ginsenosides, R_{g1} and R_{b1} (Figure 2). Since the Varian ELSD evaporates all mobile phase solvents before detection, it shows no response to change in solvent composition during gradient elution.

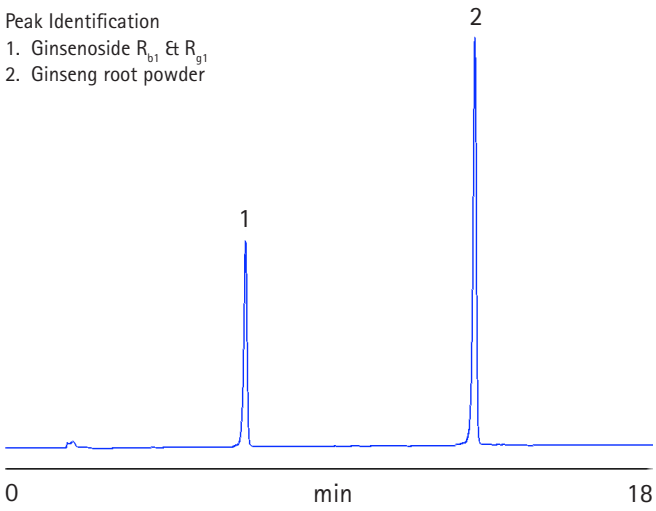

Figure 1. Ginseng root powder separation with the Varian ELSD.

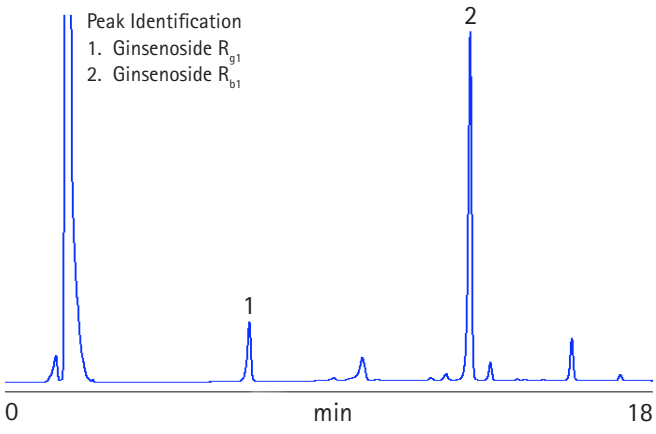

Figure 2. Good discrimination of R_{g1} and R_{b1} ginsenosides by the Varian ELSD.

Conclusion

Separation and identification of ginseng root constituents is straightforward using evaporative light scattering detection at the ambient temperatures available with the Varian ELSD. The Varian ELSD surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Varian ELSD's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100 % water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the Varian ELSD provides superior performance compared to detectors from other vendors for the analysis of semi-volatile compounds.

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Application Note SI-01219 / TB1053

Application Note SI-01215

Determination of the Limit of Detection for Caffeine by HPLC with ELSD

Stephen Bullock
Polymer Laboratories, now a part of Varian, Inc.

Introduction

Caffeine is widely used in the food and drink industry as well as being incorporated into some nutraceutical formulations. HPLC is often employed in assays for the quantification of caffeine. In this particular example, a gradient HPLC method was used to analyze caffeine at low concentrations to determine the on-column limit of detection (LOD). The Varian evaporative light scattering (ELS) detector was employed because of its superior performance, as the detection method is independent of the optical properties of the compound under consideration.

Instrumentation

Column: C8 3 µm, 50 x 4.6 mm
Detector: Varian ELSD (neb=50 °C, evap=50 °C, gas=1.4 SLM)

Materials and Reagents

Eluent A: 5 mM Ammonium acetate in Water
Eluent B: 5 mM Ammonium acetate in Acetonitrile

Conditions

Gradient: 10-100 % B in 5 min, 1 min hold at 100 % B
Flow Rate: 1.0 mL/min
Injection Volume: 10 µL

Results and Discussion

In this case, the LOD is defined as a peak height of greater than three times the baseline noise. The chromatograms in Figure 1 illustrate the signal to noise ratio (S/N) obtained for injections of 97 ng and 19 ng on column. The 19 ng loading gave a S/N = 15 and so, for this application, the LOD is well below 19 ng on-column.

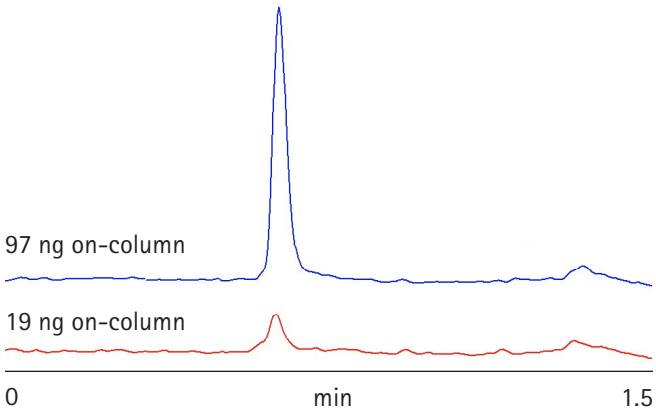

Figure 1. Very low signal to noise ratios achieved by Varian ELSD.

Conclusion

The Varian ELS detector successfully revealed an LOD for caffeine below 19 ng on-column. The Varian ELS detector surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Varian ELS detector's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100 % water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the Varian ELS detector provides superior performance compared to detectors from other vendors for the analysis of semi-volatile compounds.

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Fast analysis of fat soluble vitamins using the Agilent 1290 Infinity LC and ZORBAX RRHT and RRHD 1.8 µm columns

Application Note

Food Analysis

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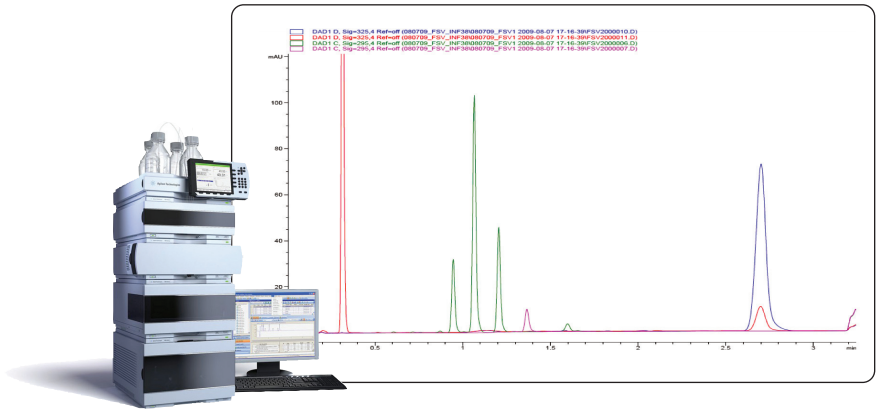

Abstract

The Agilent 1290 Infinity LC has significant capabilities for a wide range of HPLC and UHPLC applications. With a broader power range (that is, the combination of pressure and flow capabilities) than any other commercially available system, and the flexibility to operate a wide range of column dimensions and particle sizes, it is extremely useful for method transfer from any HPLC or UHPLC to the 1290 Infinity LC system. It allows the user to access capabilities not otherwise available.

Introduction

The speed and high resolution are demonstrated by a separation of fat-soluble vitamin isomers and esters, at a high pressure and flow rate. At 2 mL/min, utilizing a simple 1-min gradient and a 3.0 x 50 mm, 1.8 µm column, the analysis time is only 3 min including the late eluting retinyl palmitate component. The separation of the main components is shown in Figure 1.

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The speed, resolution and flexibility of the system are further demonstrated by a separation of vitamins D2 and D3. At 2 mL/min, utilizing a simple isocratic condition and a 3.0 mm × 150 mm, 1.8 µm column, the analysis time is only 3 min. The separation of the main components, at three column temperatures including sub-ambient, is shown in Figure 2. Sub-ambient column temperature control, a standard feature of the Agilent Thermostatted Column Compartment, has significant advantages for many difficult isomer separations, including enantiomeric separations, and for shape-selective separations such as polycyclic aromatic hydrocarbons.

Configuration

- G4220A 1290 Infinity Binary Pump with Integrated Vacuum Degasser
- G4226A 1290 Infinity Autosampler
- G1316C 1290 Infinity Thermostatted Column Compartment
- G4212A 1290 Infinity Diode Array Detector

Conclusion

Taking advantage of the combined high flow and high pressure capability of the system allows one to use high efficiency 3 mm id columns (having up to 40% higher efficiency than comparable 2.1 mm id columns) to produce rapid separations with remarkable resolution while conserving solvent over the use of 4.6 mm id columns.

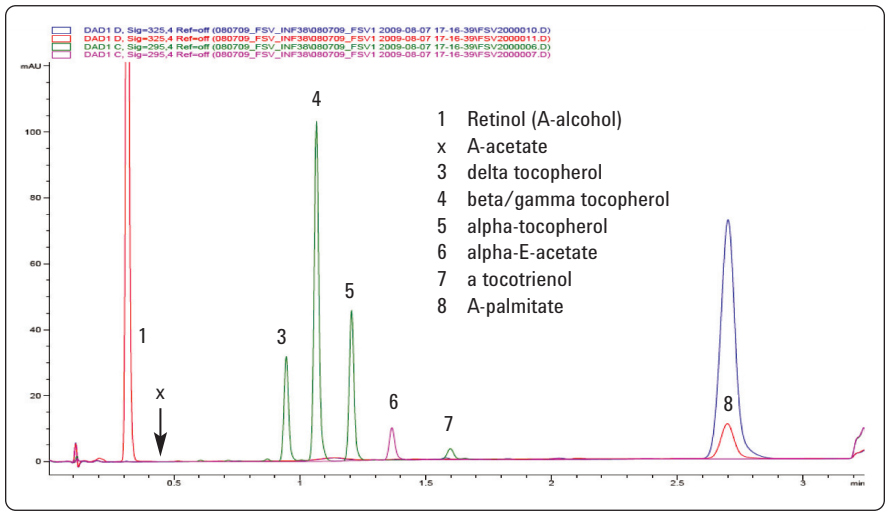

Figure 1
Analysis of important vitamins A and E components on the 1290 Infinity LC. Sample: solution of alcohols and esters of retinol and tocopherol. Conditions: 2.0 mL/min, 90% to 100% ACN at 1 min, hold to 3, run 4 min, ZORBAX RRHT StableBond C18, 3 mm × 50 mm, 1.8 µm, 45 °C.

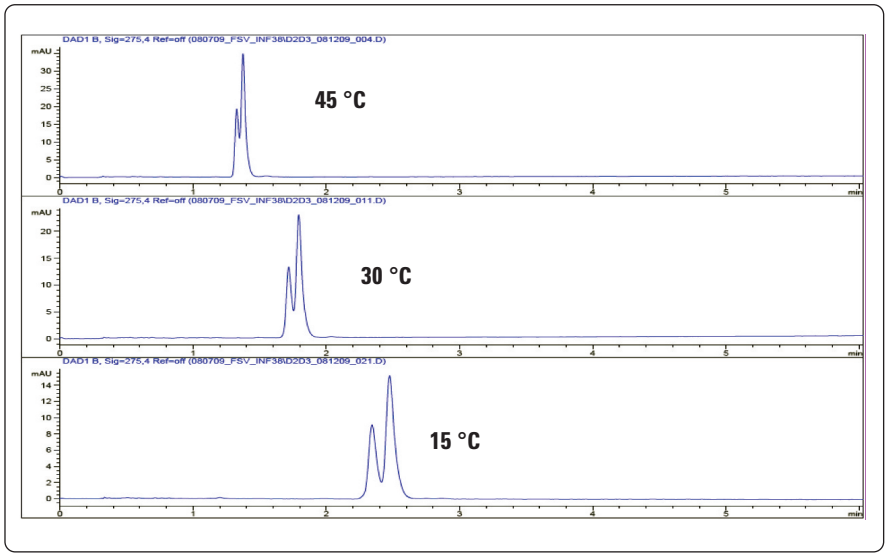

Figure 2
Analysis of vitamins D2 and D3 (order of elution) on the 1290 Infinity LC. Sample: standard mix (Sigma-Aldrich). Conditions: 2.0 mL/min, 75/25 ACN/MeOH isocratic, 280 nm UV ZORBAX RRHD StableBond C18, 3 mm × 150 mm, 1.8 µm, 45 °C, 30 °C and 15 °C.

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October 1, 2009
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Agilent SampliQ OPT Solid Phase Extraction Sorbent in the Clean-up of Alkaloids in Goldenseal by HPLC-DAD

Application Note

Pharmaceutical

Authors

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Abstract

Sample clean-up of alkaloids of Goldenseal commercial products (hydrastine and berberine) was achieved by solid phase extraction employing Agilent's SampliQ Optimized Polymer Technology (OPT) sorbents. Separation of the products with a 0.1% phosphoric acid/methanol mobile phase was carried out on an Agilent 1200 series HPLC coupled with a diode array detector (DAD) on an Agilent ZORBAX Eclipse Plus C18 column (4.6 mm × 75 mm × 3.5 µm) using gradient elution with a 6 min total run time. The recovery for hydrastine ranged from 101% to 106% (n = 8) while that of berberine ranged from 71% to 82%, (n = 8), each with % relative standard deviation (RSD) of less than 1. The limits of detection and quantification for hydrastine were 0.50 and 1.65 µg/mL respectively while those of berberine were 0.47 and 1.55 µg/mL, respectively. Goldenseal sample from Willow contained 17 µg/mL hydrastine and 35 µg/mL berberine while Goldenseal sample from Solga contained 6 µg/mL hydrastine and 12 µg/mL berberine.

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Introduction

Goldenseal (*Hydrastis canadensis* L.) is a perennial herb in the Ranunculaceae family native to southeastern Canada and northeastern United States of America (USA) and is amongst the oldest herbal medicinal plants, most commonly employed in the Traditional Chinese Medicines (TCM) [1, 2]. The biological activities of Goldenseal are associated with the isoquinoline alkaloids hydrastine and berberine (see structures in Figure 1) even though the plant also contains other alkaloids including hydrastinine, tetrahydroberberine and canadine [2]. Goldenseal has been used for the treatment of infections, inflammation and as an immune system booster [3]. However, due to the imminence of new directives and legislation intended to regulate both the herbal and nutraceutical industries, there is a growing need for robust and highly sensitive analytical methods involving sample handling, which includes sampling, clean up and pre-concentration.

Sample handling is considered to be a fundamental step in the analytical procedure because it helps to achieve the low detection limits set by regulatory authorities [4]. Solid phase extraction (SPE) is one of the most popular sample clean up techniques used in sample handling prior to analysis of environmental, food, pharmaceutical, and biological samples by high-performance liquid chromatography (HPLC) or gas chromatography (GC). SPE has many advantages over traditional liquid-liquid extraction, such as the use of minimal amounts of organic solvent, ease of automation, lower cost, and reduced volumes of toxic residues [5]. In recent years, many reports have described the development of new SPE materials, for example mixed-mode sorbents as well as restricted access sorbents, immunoaffinity extraction sorbents, molecularly imprinted polymers, and conductive polymers [6, 7, 8].

This application note presents a method that has been optimized for SPE of hydrastine and berberine in Goldenseal employing Agilent SampliQ Optimized Polymer Technology (OPT) cartridges, which utilize polymeric sorbents, with significant reduction of matrix interferences, resulting in improved analysis.

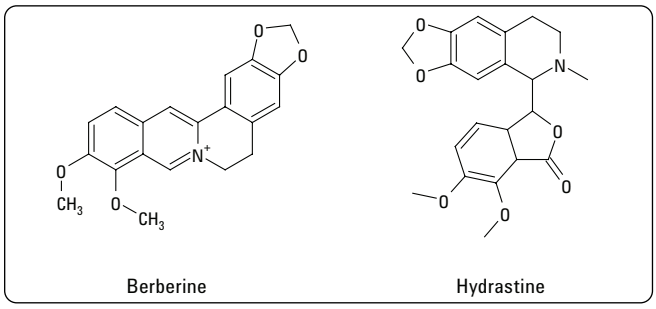

Figure 1. Structures of hydrastine and berberine.

Experimental

Materials and Chemicals

Berberine hydrochloride and hydrastine hydrochloride standards were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Phosphoric acid and potassium hydroxide were purchased from Merck Chemicals (Gauteng, South Africa) while HPLC grade methanol was purchased from Merck KGaA (Darmstadt, Germany). Potassium dihydrogen phosphate was from Saarchem Analytic (Krugersdorp, South Africa). Goldenseal capsules (Golden Seal Capsules, Willow Products, Port Elizabeth, South Africa and Solga Full Potency Herbs, Solgar Corporation, Leonia, NJ, USA) were purchased from a local herbal store in Grahamstown, South Africa. SPE cartridges were Agilent SampliQ OPT, 1 mL/30 mg tubes. Analysis was performed on an Agilent 1200 series gradient HPLC coupled with a diode array detector (DAD). The analytical column was an Agilent ZORBAX Eclipse Plus C18 column (4.6 mm × 75 mm × 3.5 µm).

Preparation of Stock and Working Solutions

The stock solution of hydrastine and berberine, 1000 µg/mL each, were prepared in methanol and stored at 4 °C when not used. All other standard solutions were prepared from the stock solution as required.

Sample Preparation

The contents of the capsules were first homogenized. Then, 200 mg of the homogenized sample were mixed with 50 mL of methanol and then stirred with a magnetic stirrer for 1 h, resulting in a suspension with undissolved particulates floating in it. The extracts were then filtered using a hydrophobic polyvinlyidene fluoride (PVDF) 0.45 µm Millipore Millex – HV membrane filter (Billerica, MA, USA). The methanolic extracts were diluted 1:3 with water and the pH adjusted to ~ 7 with 0.01 M potassium hydroxide.

Separation

A 5 µL aliquot of a hydrastine-berberine mixed standard (50 µg/mL of each) was injected into the HPLC column to optimize their separation. The separation of both alkaloids was monitored at 242 nm while wavelengths 294 and 350 nm were used for quantifying berberine and hydrastine, respectively. The HPLC conditions are as outlined in Table 1 below:

Table 1. HPLC Conditions

Column	Agilent ZORBAX Eclipse Rapid Resolution Plus C18, column, 4.6 mm × 75 mm, 3.5 µm (p/n 959933-902)			
Flow rate	1 mL/min			
Injection volume	5 µL			
Column temperature	35 °C			
Mobile phase	A: 0.1% Phosphoric acid B: Methanol			
Run time	6 min			
Post time	2 min			
Detection	UV 294 nm (berberine), 350 nm (hydrastine), 242 nm (for monitoring both)			
Gradient	Time	0	0.5	5
	%B	20	20	50

SPE Procedure

A systematic study of a series of conditioning, loading, washing and elution solvents was performed. The procedure was optimized by evaluating the isolation of hydrastine and berberine from a standard solution. Figure 2 shows the results of the optimization process.

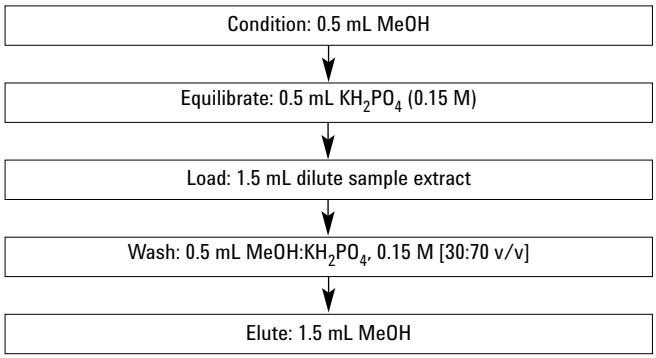

Figure 2. SPE procedure for cleaning alkaloids in Goldenseal using SampliQ OPT sorbent.

Results and Discussion

Separation and SPE Clean Up

Although the hydrastine and berberine standards could be separated isocratically, the initial analysis time was rather long (15 min) so gradient elution was essential to reduce the run time. Using the optimized HPLC conditions outlined in Table 1, Figure 3 shows the well-separated symmetrical peaks for the hydrastine and berberine standards in just over 4.5 minutes. A blank carried through the entire procedure showed no discernible peaks in the baseline. Next, a simple filtered extract from the Willow Goldenseal sample was injected prior to (Figure 4a) and after sample cleanup using the SPE procedure (Figure 4b). Note the decrease in the number of small peaks in Figure 4b indicating that the SPE treatment removed a number of potentially interfering species. A second sample of Goldenseal from Willow was spiked and treated in a similar manner to confirm the peak assignments. The results in Figure 5a and 5b showed that the peak intensity was increased and interfering peaks were significantly reduced.

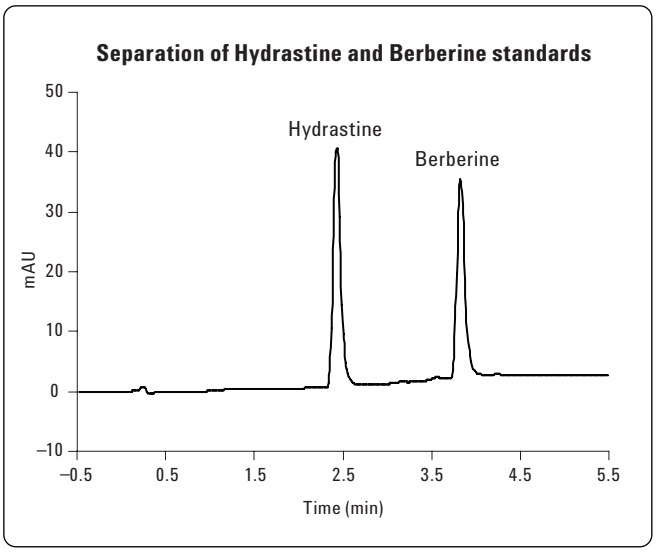

Figure 3. Chromatogram of hydrastine (120 µg/mL) and berberine (100 µg/mL) standard mix.

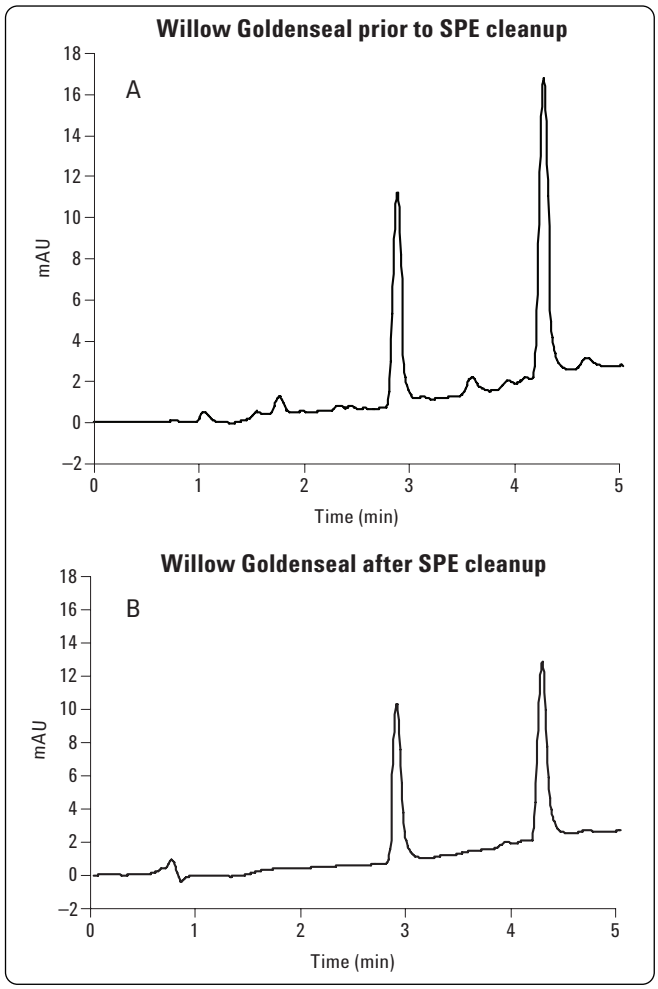

Figure 4. Chromatograms of Goldenseal samples before (a) and after (b) clean up.

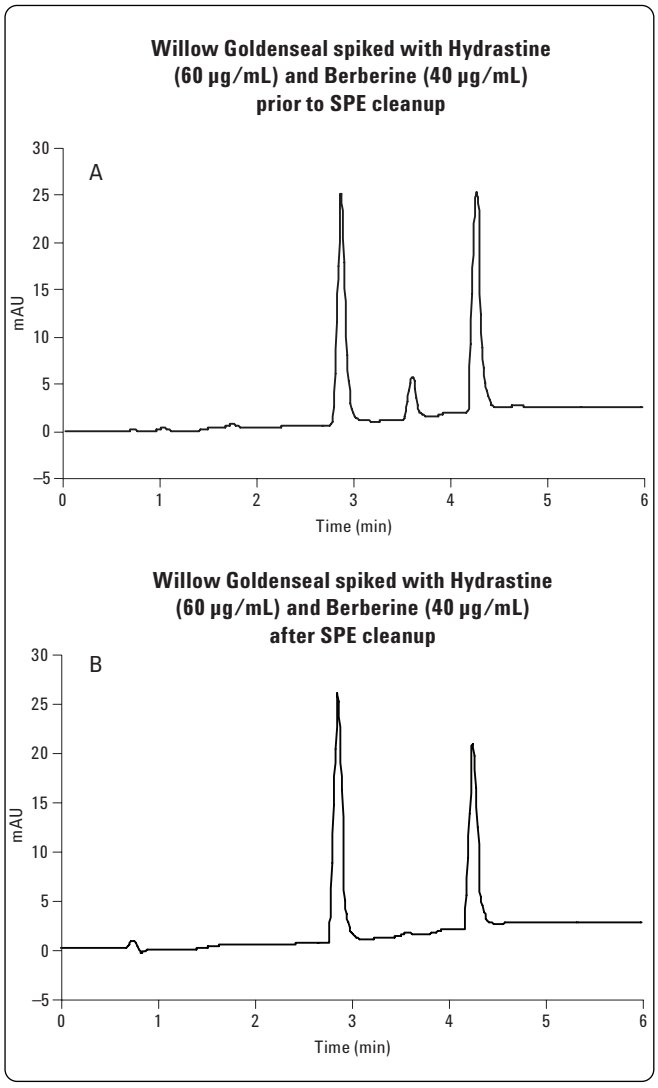

Figure 5. Chromatograms of Goldenseal Willow spiked samples (hydrastine and berberine, 60 and 40 µg/mL, respectively) before (a) and after (b) clean-up with SampliQ OPT sorbent.

Recovery and Reproducibility

The recoveries and reproducibility of berberine and hydrastine were evaluated by analyzing eight replicates of the commercial sample (Goldenseal Willow capsules) that were spiked at three different concentration levels of hydrastine and berberine within a day and then introduced to the SPE procedure. The background level of the spiked samples was determined for each concentration before clean up. Recovery was calculated by comparison of peak areas of unclean to those of the cleaned extracts. The recovery and reproducibility values for berberine and hydrastine are as outlined in the Table 2 below. The % RSDs were all less than 1, quite acceptable for an SPE cleanup and HPLC analysis procedure.

Table 2. Recovery and Reproducibility Data for the Two Alkaloids Standards

Compound	Level spiked (µg/mL) n=8	% Recovery	% RSD
Hydrastine	40	101	0.16
	60	102	0.56
	100	106	0.75
Berberine	10	71	0.17
	40	71	0.40
	80	82	0.32

Calibration Curves

The calibration curves were determined by preparing appropriate concentrations in methanol from berberine and hydrastine stock solutions and injecting directly into the HPLC column without SPE procedure. The method was found to be linear in the concentration ranges of 0–120 µg/mL for hydrastine and 0–100 µg/mL of berberine each with r^2 of 0.9994.

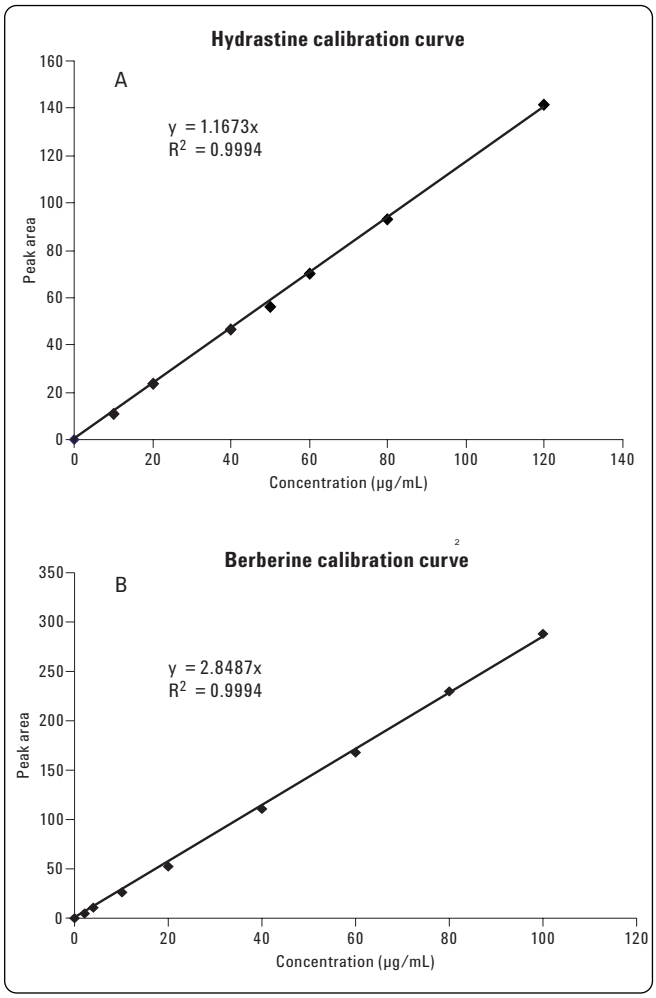

Figure 6. Calibration curves of (a) hydrastine and (b) berberine.

Linearity of the SPE Method

Linearity was studied on the SampliQ OPT sorbent by spiking sample extracts with increasing concentrations of hydrastine and berberine followed by SPE clean-up. At concentrations higher than 200 µg/mL for berberine, linearity was no longer observed (Figure 7). This is due to the fact that the SPE sorbent was overloaded and could no longer retain the alkaloid berberine. Most of the sample was lost at the washing step while some was lost even at the loading stage. For hydrastine, SampliQ OPT sorbent showed linearity for up to 500 µg/mL.

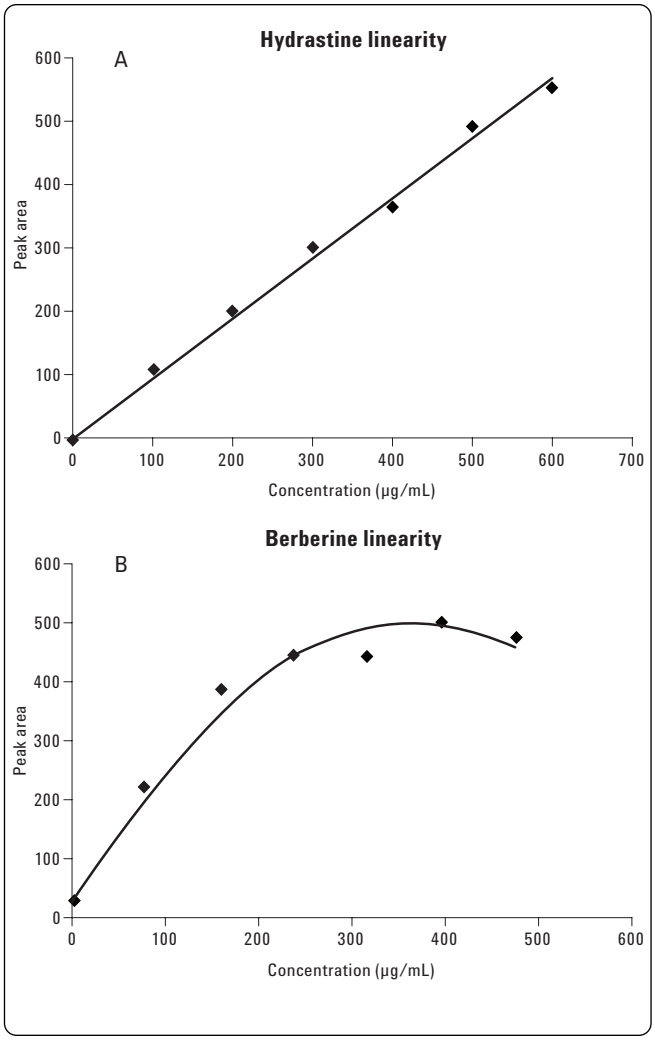

Figure 7. Linearity of (a) hydrastine and (b) berberine at higher concentrations.

Analysis of the Commercial Product

The method described was successfully applied to the analysis of the commercial products, Goldenseal capsules from Willow and Solga. Both products contained low but quantifiable amounts of the alkaloids as indicated in Table 3.

Table 3. Concentrations of hydrastine and berberine in Commercial Samples

	Concentration (µg/mL)	
	Hydrastine	Berberine
Willow	17	34
Solga	6	12

Limit of Detection and Limit of Quantification

The limits of detection were calculated using the intercept, y_B, and the standard error of the regression line, s_B at 3 times the standard error and LOD values were calculated using equations 1 and 2 [9,10].

$$y_{LOD} = y_B + 3S_B \quad \text{(Eqn 1)}$$

$$LOD = (y_{LOD} - y_B)/m \quad \text{where m = gradient} \quad \text{(Eqn 2)}$$

LOQ values were calculated using the same method as in equations 1 and 2, but using 10 times the standard error of regression line, (equations 3 and 4).

$$y_{LOQ} = y_B + 10S_B \quad \text{(Eqn 3)}$$

$$LOQ = (y_{LOQ} - y_B)/m \quad \text{(Eqn 4)}$$

The limit of detection and quantification for hydrastine were found to be 0.50 and 1.65 µg/mL, respectively, while that of berberine was 0.47 and 1.55 µg/mL, respectively.

Conclusions

Agilent SampliQ OPT cartridges achieved effective sample clean up of the TCM Goldenseal for the separation and analysis of hydrastine and berberine. The results demonstrated that the method was reproducible and reliable with good recoveries (101% to106% for hydrastine and 71% to 86% for berberine) at n = 8 and RSD less than 1%. The limits of detection and quantification for hydrastine were 0.50 and 1.65 µg/mL respectively while those of berberine were 0.47 and 1.55 µg/mL respectively. Goldenseal sample from Willow contained 17 µg/mL hydrastine and 35 µg/mL berberine while Goldenseal sample from Solga contained 6 µg/mL hydrastine and 12 µg/mL berberine.

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Agilent Technologies

Extraction and HPLC Analysis of Alkaloids in Goldenseal

Application

Consumer Products and Drug Manufacturing/QA/QC

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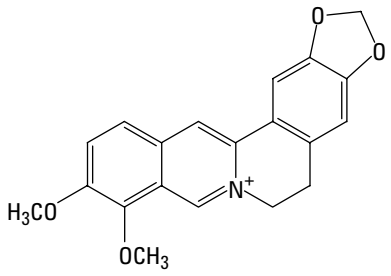
Abstract

An ambient extraction of goldenseal root powder followed by HPLC analysis of the alkaloids on a Zorbax Rapid Resolution Eclipse XDB-C18 column provides an accurate method for the determination of key alkaloids in goldenseal, including berberine and hydrastine. The extraction and HPLC analysis can be applied to several other alkaloids, including canadine, hydrastinine, and palmatine, and may be applicable to other berberine-containing plant roots. The Rapid Resolution Eclipse XDB-C18 column is used for an isocratic separation with high resolution of all components in under 15 minutes.

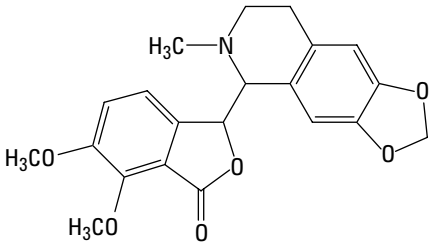
Introduction

Goldenseal, *Hydrastis canadensis* L., is one of the oldest herbal medicinal plants and is of current interest as a natural medicine. There are two alkaloids present in goldenseal that are the expected active components, berberine and hydrastine. Canadine, hydrastinine, berberastine, and canadaline are minor alkaoloids. Palmatine, which is closely related to berberine, is not found in *H. canadensis*, but is found in *Coptis*, another berberine-containing plant [1].

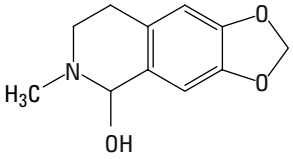
Goldenseal has been used as an anti-inflammatory and antibiotic. It has also been used to treat nasal congestion, cold, flu, and a variety of intestinal disorders. The whole root of the plant is used and is currently available in bulk (dried or powdered roots), tablets, capsules, and tinctures. Goldenseal plants have been overharvested and many are now grown on farms for use as herbal supplements. Inconsistent quantities of alkaloids are present in the products sold as herbal supplements. A simple process for extracting and analyzing the alkaloids is highly desirable to evaluate product quality. Figure 1 shows the structures of the alkaloids in goldenseal.



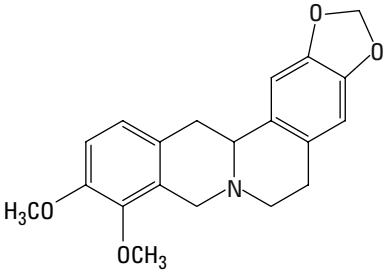
Berberine



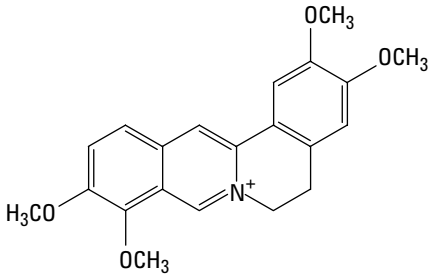
Hydrastine



Hydrastinine



Canadine



Palmatine

Figure 1. Structures of key alkaloids in goldenseal and related plants.

Experimental

Extraction Procedure

Literature reports by Betz and Anderson [1] and Burney [2] indicate that ambient extraction of alkaloids from *H. canadensis* is possible. This is followed by an HPLC determination for accurate quantitation of the alkaloid extracts. The optimized ambient extraction conditions used for the analysis of neat goldenseal root powder are summarized in the steps below.

1. Weigh ~ 0.5 g of root powder
2. Mix with 100 mL of acetonitrile:water:H_3PO_4 (70:30:0.1, v/v/v)
3. Sonicate 5 min, shake (wrist-action shaker) 10 min, centrifuge 5 min
4. Dilute extracts 1/5
5. Direct HPLC analysis of diluted extracts

HPLC Analysis

The HPLC analysis of alkaloids present in goldenseal needs to resolve the major alkaloids. In addition, it is desirable to resolve palmatine, because it is present in other berberine-containing plants. An HPLC method was developed to resolve all of these components. Optimum resolution and peak shape were obtained using the Zorbax Eclipse XDB-C18 column with an ammonium acetate buffer and acetonitrile. The separation is shown in Figure 2 with a complete list of the optimized conditions used. For consistent retention times, temperature control was required at 30°C [3].

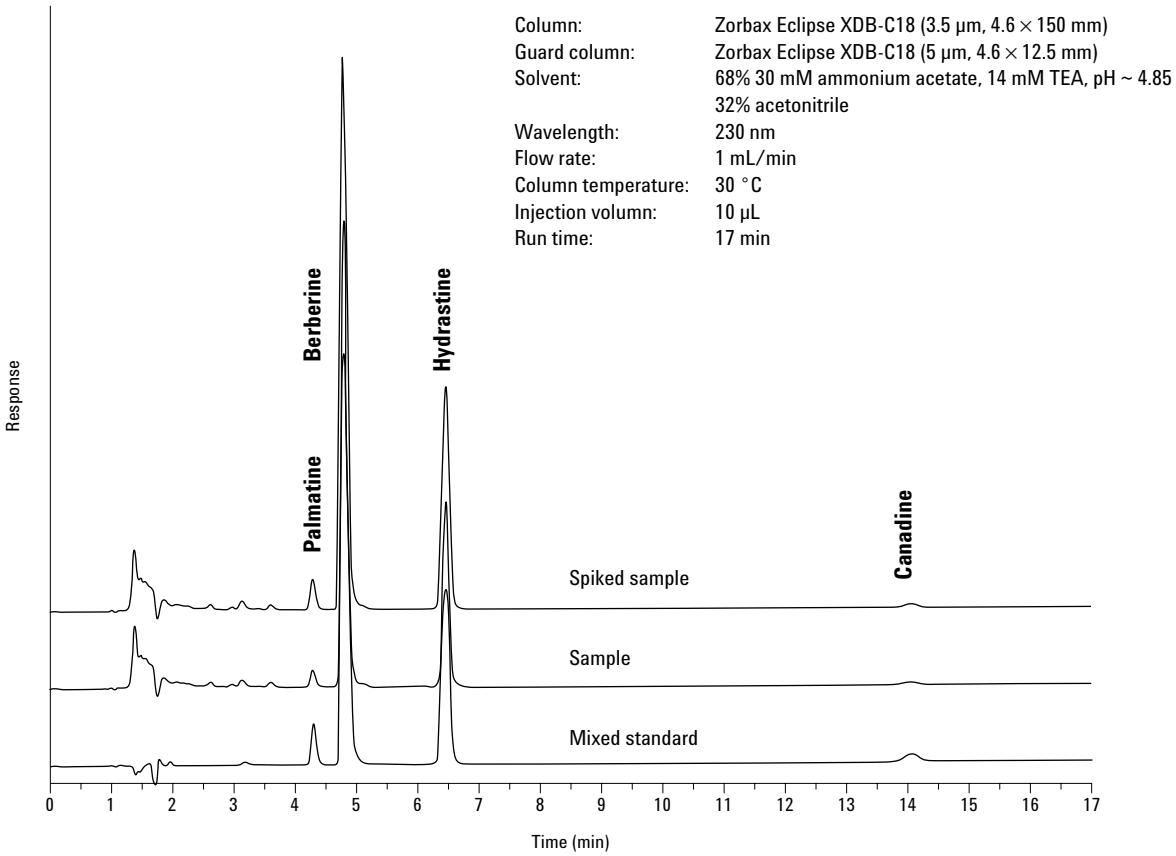


Figure 2. HPLC separation of goldenseal extract on Eclipse XDB-C18 column.

Method Validation

This HPLC method was applied in the validation of the ambient extraction method. Linearity, precision, and alkaloid recovery were investigated. Table 1 shows these results. The precision of the method is excellent, and recoveries of the different alkaloids ranged from 92%–102%, excellent for a quantitative method. The linearity was very good (Figure 3) and sensitivity was good. From the standard data, the calculated LOD (limit of detection) for berberine was 0.50 mg/mL and the LOQ (limit of quantitation) was 1.65 mg/mL, so that accurate quantitation down to low levels is possible, making it easy to test the quality of different goldenseal products.

Table 1. Validation Results of the Ambient Extraction Method

	Palmatine	Berberine	Hydrastine	Canadine
Precision (n = 10)	0.18 ±0.002(s)%	3.06 ±0.05(s)%	2.04 ±0.01(s)%	0.08 ±0.001(s)%
Alkaloid recovery (~ 0.3–2 g of GS) (~ 0.6–1 mg/mL) (n = 12)	0.18 ±0.003(s)	3.10 ±0.06(s)%	2.05 ±0.02(s)%	0.08 ±0.001(s)%
Spike and recovery (n = 3)	Spike level = ~ 0.15% 92.2 ±5.5(s)%	Spike level = ~ 2.0% 101.5 ±0.2(s)%	Spike level = ~ 2.0% 101.9 ±0.2(s)%	Spike level = ~ 0.10% 101.9 ±7.9(s)%

(s) = standard deviation

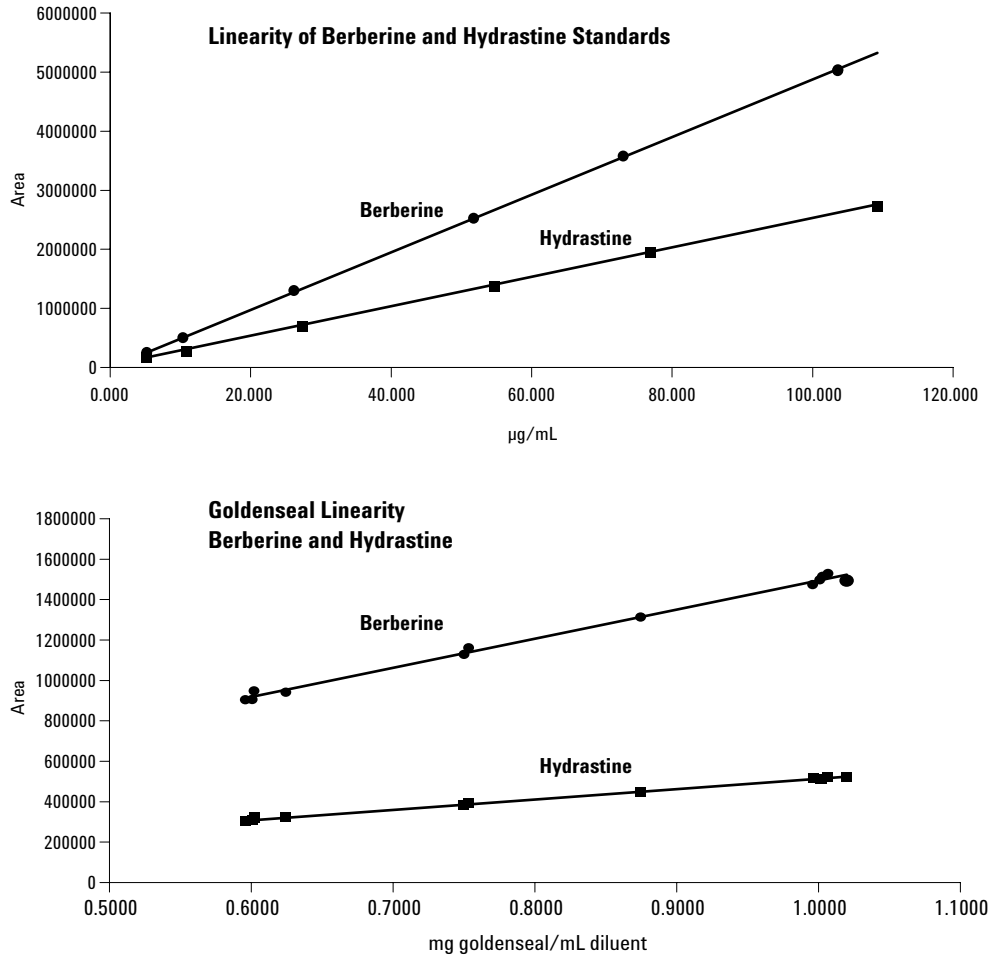


Figure 3. Linearity of berberine and hydrastine as standards and from goldenseal extracts.

Results

Goldenseal Testing

The ambient extraction and HPLC analysis method were applied to six lots of goldenseal root powder from three different vendors to determine the alkaloid content. The results are summarized in Table 2. These results demonstrate variability among vendors and the reason a good quantitative HPLC method is desirable.

Table 2. Results of Testing Goldenseal Root Powder from Multiple Lots and Multiple Vendors with the Ambient Extraction Method and HPLC

Vendor	Lot number	% Palmatine	% Berberine	% Hydrastine	% Canadine	Total weight % of known alkaloids
1	A	nd	3.27	2.36	0.09	5.94
	B	nd	3.29	2.40	0.07	5.98
2	C	0.19	3.01	1.99	0.09	5.53
	D	0.18	3.06	2.04	0.08	5.36
3	E	nd	4.60	4.06	0.12	8.99
	F	nd	3.93	2.67	0.20	6.93

Conclusions

This ambient extraction method of goldenseal is simple and reliable. This is followed by an isocratic HPLC analysis with a Zorbax Rapid Resolution Eclipse XDB-C18 column, which provides high resolution and excellent peak shape of six alkaloids in 15 minutes. This analysis provides reliable quantitative results of the alkaloids in goldenseal, including berberine and hydrastine. The method was applied to goldenseal from three different vendors and may be applicable to other berberine-containing plants.

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Separation of Hypericin in St. John's Wort

Application

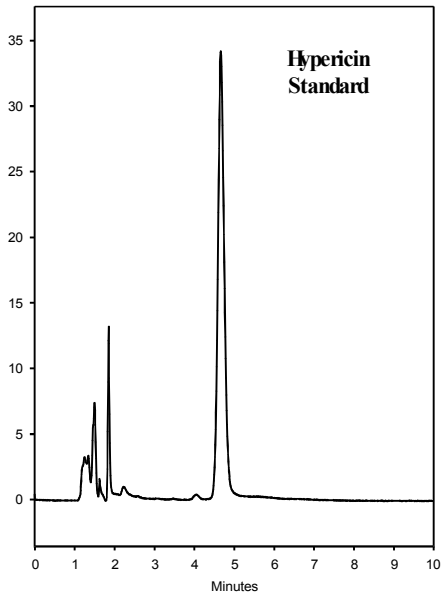
Pharmaceutical

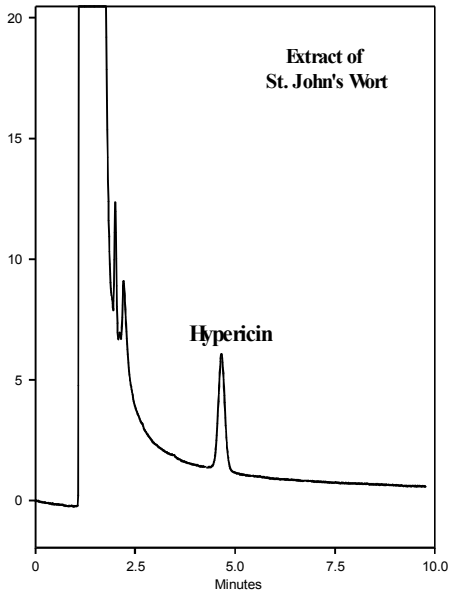
Robert Ricker

Hypericin is a major component in the herbal extract of Hypericum Perforatum, otherwise known as St. John's Wort. St. John's Wort has been used to treat depression but has also been recommended as an appetite suppressant. Due to the increasing interest in natural product therapies and the growing concern regarding government regulation of these manufacturers, a method was developed for hypericin and an herbal product of St. John's Wort.

Highlights

- *Good peak shape for hypericin at neutral pH on an Agilent ZORBAX Eclipse XDB-C8 column.*
- *Double endcapped Eclipse XDB-C8 yields longer column lifetime with neutral-pH mobile phases.*
- *Good retention of the hypericin allows adequate separation from other UV-absorbing compounds in the actual extract.*





Conditions:
Column: ZORBAX XDB-C8 (4.6 x 150 mm) (Agilent P/N: 993967-906)
Mobile Phase: 23% 25 mM Na_2HPO_4, Dibasic (pH 7.0 with H_3PO_4): 77% Methanol
Inj. Vol.: 2µL
Flow Rate: 1 mL/min
Temp.: 35°C
Detect.: UV (254 nm)

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Agilent Technologies

Multi-Element Determination of Heavy Metals in Dietary Supplements Using Collision/Reaction Cell ICP-MS

Application

Foods

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Abstract

Determination of 11 metals (including arsenic, chromium, mercury, iron, copper, nickel, zinc, selenium, lead, cadmium, and thallium) in UK-consumed dietary supplements was carried out using ICP-MS. The instrument used was the Agilent 7500ce, which is equipped with a collision/reaction cell (Octopole Reaction System), and was operated in no-gas, helium, and hydrogen modes, all acquired within a single method. Samples were microwave digested with nitric acid/hydrogen peroxide/hydrofluoric acid and quantified using external calibration. The method was assessed by the analysis of two certified reference materials (LGC7160 and SRM1577b), and recoveries were 100 ± 15% of the certified value for all elements.

Introduction

Heavy metals are natural components of the earth's crust and are widely used in agricultural, construction, manufacturing, and food/material processing industries. As trace elements, some heavy metals are metabolically essential to humans at low levels, but at higher concentrations they can become toxic. Heavy metal toxicity can result from high ambient air concentrations near emission sources, drinking-water contamination, or intake via the food chain.

Conversely, as our lifestyles have become increasingly busy, leaving less time to prepare and eat well-balanced meals, our diets are at an increased risk of becoming nutrient deficient. Dietary supplements, sold in capsule, tablet, or liquid form offer a simple and convenient way to supplement our diets and reduce the risk of nutrient deficiency.

In the UK, most products described as dietary supplements are regulated as foods and are subject to the general provisions of The Food Safety Act 1990 and secondary legislation on safety and labeling (Food Labeling Regulations 1996 [as amended] and the Food Supplements Directive 2002/46/EC). Past investigations have shown that some dietary supplements can contain elevated concentrations of metals and other elements. While some metals, such as selenium, iron, copper, chromium, and zinc [1], are essential at low concentrations, others, such as arsenic, cadmium, lead, and mercury, are toxic [2]. In the UK, arsenic is the only element for which there is legislation on permitted levels in foods. Arsenic is regulated under the "Arsenic in Food (as amended) Regulations 1959," which states a limit of 1 mg/kg in "general" food [3]. Cadmium, lead, mercury, and tin in specific foods are regulated under Commission Regulation 1881/2006 [4].

It is important that up-to-date information on the levels of metals and other elements in dietary supplements consumed in the UK is obtained, in order to assess whether there is any risk to consumers. The purpose of this study was to provide the Food Standards Agency (FSA) with up-to-date and accurate information on the concentration of a suite of metals contained within dietary supplements consumed in the UK.

To do this, microwave acid digestion was used for sample preparation, followed by multi-element determination by collision/reaction cell inductively coupled plasma mass spectrometer (CRC-ICP-MS) using He and H_2 collision gases to remove spectral interferences. The suite of metals included arsenic (As), chromium (Cr), iron (Fe), copper (Cu), nickel (Ni), zinc (Zn), selenium (Se), lead (Pb), mercury (Hg), cadmium (Cd), and thallium (Tl). Most elements were measured in standard mode (no gas) since multi-isotope ICP-MS data obtained for the food samples suggested the absence of significant polyatomic interferences. However, the ICP-MS detection of three of these elements, two essential (Fe and Se) and one toxic (As), was found to be strongly hampered by polyatomic ions. The purpose of this application is to demonstrate the ability of the Agilent 7500ce using the Octopole Reaction System (ORS) to eliminate these interferences (Table 1), providing accurate determination of these three elements in food supplements.

Experimental

Samples

Two hundred different dietary supplements (either tablet, capsule, liquid, or powder form) commercially available in the UK were sourced. The average weight of each tablet/capsule was determined using an electronic balance.

Sample Preparation

Tablets were crushed with a pestle and mortar. Crushed tablets, liquids, and powders were subsampled after thorough mixing. Oil capsules were digested whole. Approximately 0.7 g of sample was accurately weighed and microwave digested with 7 + 3 + 0.2 mL of nitric acid + hydrogen peroxide + hydrofluoric acid, respectively. The microwave program consisted of heating the samples to 180 °C over 20 min and holding for a further 10 min. Once cool, the digests were made up to 100 g using deionized water, and the resultant solutions were subjected to element determination by ICP-MS. Approximately 10% of the samples were digested in duplicate. A blank and QC material were included in each digestion run, containing a maximum of 12 samples.

QC Materials

Two certified reference materials were analyzed to assess the accuracy of the methodology. These were bovine liver SRM 1577b (NIST, Gaithersburg, USA) containing 0.73 ± 0.06 mg/kg Se and 184 ± 15 mg/kg Fe and crab paste LGC7160 (LGC, Teddington, UK) containing 11 ± 1 mg/kg As.

Instrumentation

Sample digestion was undertaken in a Mars 5 microwave (CEM, Buckingham, UK). Elemental measurements were performed using an Agilent 7500ce CRC-ICP-MS operating in hydrogen mode for Se and Fe, and helium mode for As to remove spectral interferences (Table 1). All other elements were measured in standard (no gas) mode within the same method. Typical operating conditions are illustrated in Table 1. The Integrated Sample Introduction System (ISIS) was used with a pump speed set at 0.1 rps during the analysis and washout in order to minimize the amount of matrix onto the interface and optimize sample throughput.

Table 1. Instrumental Conditions for 7500ce ORS Collision/Reaction Cell Mode

Parameter	ORS Cell Mode	
	He	H_2
Elements measured	As	Se, Fe
Spectral interferences removed by ORS gas	$^{40}Ar^{35}Cl^+$ on $^{75}As^+$ $^{40}Ca^{35}Cl^+$ on $^{75}As^+$	$^{38}Ar^{40}Ar^+$ on $^{78}Se^+$ $^{40}Ar^{37}Cl^+$ on $^{77}Se^+$ $^{40}Ca^{37}Cl^+$ on $^{77}Se^+$ $^{40}Ar^{16}O^+$ on $^{56}Fe^+$ $^{40}Ca^{16}O^+$ on $^{56}Fe^+$
RF power (W)	1520	
Carrier gas (L/min)	0.9	
Make-up gas (L/min)	0.26	
Nebulizer	Glass concentric, MicroMist	
Spray chamber	Quartz cooled to 2 °C	
Interface cones	Ni	
Cell gas	He	H_2
Cell gas flow rate (mL/min)	2.5	2.2
Points per peak	3	
Repetitions	10	
Integration time per mass (sec)	0.3	

Measurement

Five-point external calibrations with standards traceable to the National Institute of Standards and Technology (NIST, Gaithersburg, USA) were used to quantify the elements in the digests. Rhodium (Rh) was used as an internal standard and added on-line (1:1 with samples). The internal standard solution also contained 4% propanol to compensate for enhancement of As and Se signal from any residual carbon in the samples. Water

Research Council's (WRC) Aquacheck solutions (used in a proficiency testing scheme) with known concentrations of the analytes (660.3 ng/g Fe, 12.6 ng/g As, and 13.27 ng/g Se) were also analyzed as independent checks on the accuracy and precision of each ICP-MS run.

Results and Discussion

All sample results were checked against legislative limits and/or limits agreed with the FSA. The action limits used were 1 mg/kg arsenic, lead, and cadmium and 0.5 mg/kg for mercury. Samples that exceeded these limits were reanalyzed to confirm the results.

As illustrated in Table 2, five samples were confirmed to contain As concentrations greater than the 1 mg/kg limit recommended in The Arsenic in Food (as amended) Regulations 1959 [3]. A further two samples were found to contain As concentrations between 0.75 mg/kg and 1 mg/kg. It should be noted that several of these supplements were derived from marine animals, and the form of the As in such materials is likely to be nontoxic arsenobetaine. The majority of supplements (> 75%) were found to contain < 0.1 mg/kg As and Se, and > 20 mg/kg Fe.

Table 2. Samples with Arsenic Concentrations Above Recommended Limit

Product	Form	As in sample (mg/kg)
Product 1	Tablet	3.3 ± 0.7
Product 2	Capsule	2.5 ± 0.6
Product 3	Capsule	20.5 ± 4.8
Product 4	Capsule	1.5 ± 0.4
Product 5	Capsule	7.3 ± 1.7

Legislative limit = 1 mg/kg [3]
The uncertainty quoted is the expanded uncertainty calculated using a coverage factor of 2, which gives a level of confidence of approximately 95%. The uncertainty was calculated based upon the principles of the Eurachem Guide [5].

None of the samples was found to contain an elevated concentration of Cd, and only one sample was close to the limit for Hg. Ten samples were confirmed to contain concentrations of Pb above 1 mg/kg.

Results in mg/kg were calculated back to mg/tablet in order to allow comparison with label claims. For a number of supplements, differences were found between the measured concentrations of Se, Fe, Zn, Cu, Cr, or Ni and the values stated on the label. This makes it difficult for the FSA to accurately assess the dietary intake of these elements from such supplements based on label claim alone and demonstrates the necessity of this survey.

The limits of detection (LOD) and quantification (LOQ) of the described procedure, calculated according to International Union of Pure and Applied Chemistry (IUPAC) guidelines [6], are presented in Table 3. For 25% of the dietary supplements tested the Se concentration was found to be less than the LOD.

Table 3. Limits of Detection and Quantification of Se, Fe, and As

	Concentration (mg/kg)[1]		
	Se	Fe	As
LOD	0.009	0.072	0.006
LOQ	0.029	0.240	0.022

[1] Values shown are based on the average weight of dietary supplement tablet/liquid/capsule digested (0.67 g).

Recovery results for the QC materials and Aquacheck solutions were very good, with results for all elements falling within 100 ± 15% (n = > 9) of the certified/expected value. The recoveries obtained for the reference materials analyzed are illustrated in Figure 1. A number of sample and blank solutions were also spiked with Se, Fe, and As prior to microwave digestion. The resultant recoveries fell within 100 ± 10% (n = > 5) of the expected value. As a check on the repeatability of

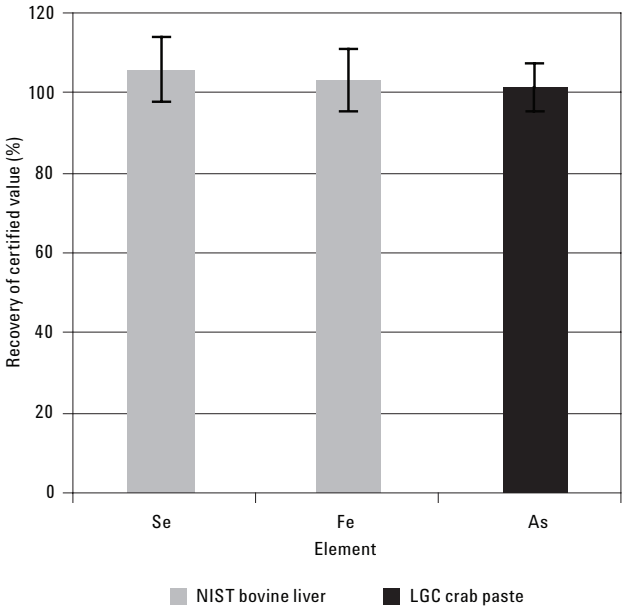

Figure 1. Recovery of Se, Fe, and As from the CRMs analyzed.

the method, approximately 10% of the samples were also independently digested and analyzed in duplicate. For these, coefficient of variation values of < 5% were found for all three elements measured in collision/reaction mode on the 7500ce ICP-MS.

Conclusions

Microwave digestion followed by analysis by ICP-ORS-MS has been shown to be a simple, reliable method for the multi-element determination of trace metals in nutritional supplements and foodstuffs. A number of supplements were found to contain Se, Fe, Zn, Cu, Cr, and Ni at concentrations that deviated from the label claim. Five samples for As and 10 samples for Pb were found to contain elevated concentrations above the recommended 1 mg/kg limit. The data generated in this survey has provided the FSA with up-to-date concentrations of these metals in a range of dietary supplements. The results have enabled the risk of metal toxicity from the consumption of dietary supplements to be assessed and published in a Food Standard Agency's Food Surveillance Information sheet [7].

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