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

Application Note

Food

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Abstract

Sample cleanup of alkaloids of Goldenseal commercial products (hydrastine and berberine) was achieved by solid phase extraction (SPE) employing Agilent SampliQ Optimized Polymer Technology (OPT) sorbents. Separation of the products with a 0.1% phosphoric acid/methanol mobile phase was carried out using an Agilent 1200 Series LC coupled with a diode array detector (DAD) on an Agilent ZORBAX Eclipse Plus C18 column (4.5 mm × 75 mm × 3.5 µm) using gradient elution with a 6 minute 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 among 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 the structures in Figure 1) even though the plant also contains other alkaloids including hydrastinine, tetrahydroberberine, and canadine [2]. There is a growing need for robust and highly sensitive analytical methods involving sample handling, which includes sampling, clean up, and preconcentration.

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 [3]. 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 [4]. 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 [5, 6, 7].

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

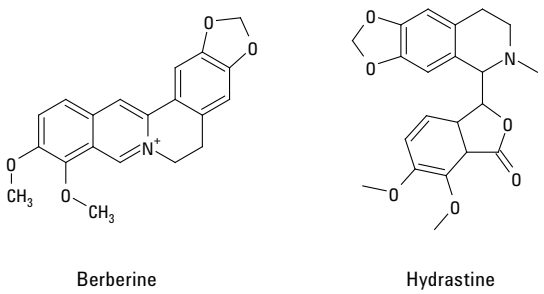

Figure1. Structures of hydrastine and berberine.

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 LC coupled with a DAD. The analytical column was an Eclipse Plus C18 column (4.5 mm × 75 mm × 3.5 µm).

Preparation of stock and working solutions

The stock solution of hydrastine and berberine, 1,000 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 stirred with a magnetic stirrer for 1 hour, 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 approximately 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. Table 1 outlines the HPLC conditions.

Table 1. HPLC conditions.

Column	Agilent ZORBAX Eclipse Rapid Resolution Plus C18, 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			
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.

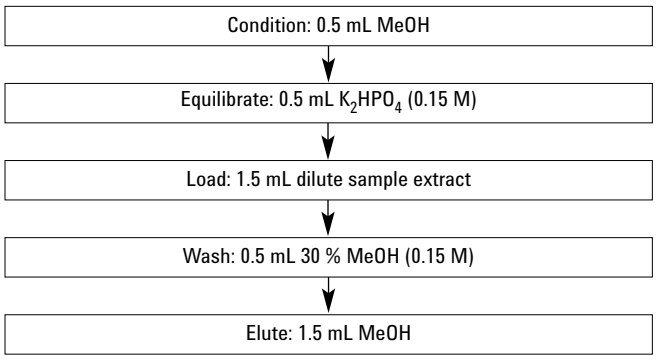

Figure 2. SPE procedure for cleaning alkaloids in Goldenseal using Agilent SampliQ OPT sorbent.

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 minutes), 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 show that the peak intensity was increased and interfering peaks were significantly reduced.

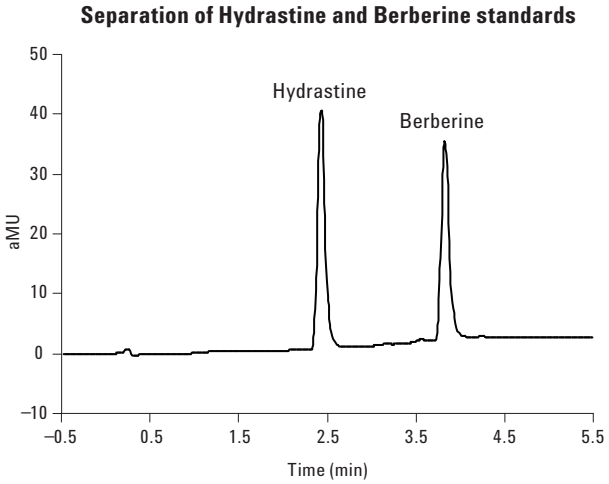

Figure 3. Chromatogram of hydrastine (120 µg/mL) and berberine (100 µg/mL) standard mix.

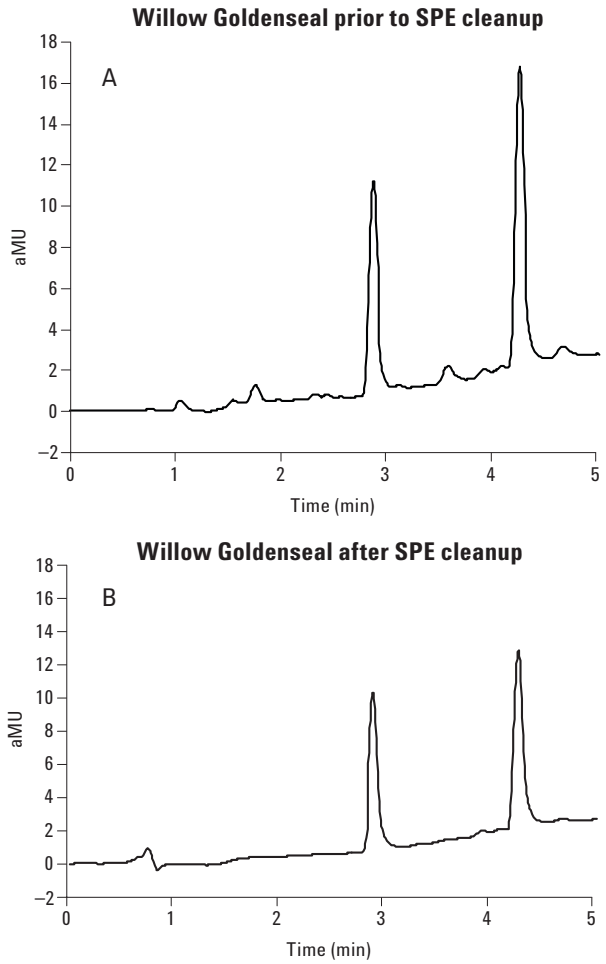

Figure 4. Chromatograms of Goldenseal samples before (A) and after (B) clean up.

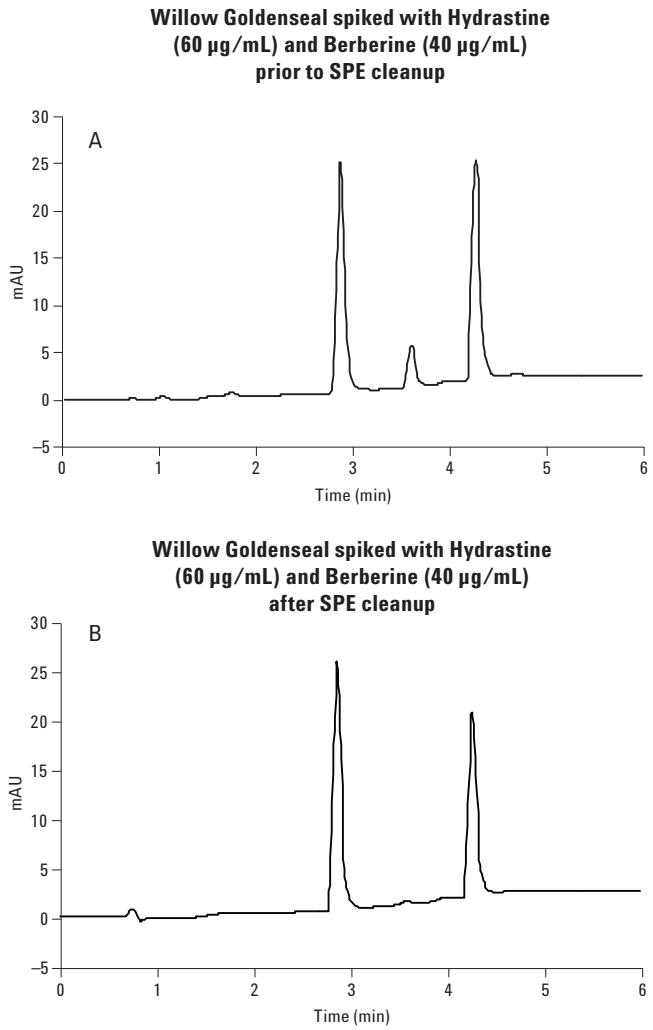

Figure 5. Chromatograms of Goldenseal Willow spiked samples (hydrastine and berberine, 60 and 40 µg/mL, respectively) before (A) and after (B) clean-up with Agilent SampliQ OPT sorbent.

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. Table 2 outlines the recovery and reproducibility values for berberine and hydrastine. 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.

Linearity of the SPE method

Linearity was studied on the Agilent 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, Agilent SampliQ OPT sorbent showed linearity for up to 500 µg/mL.

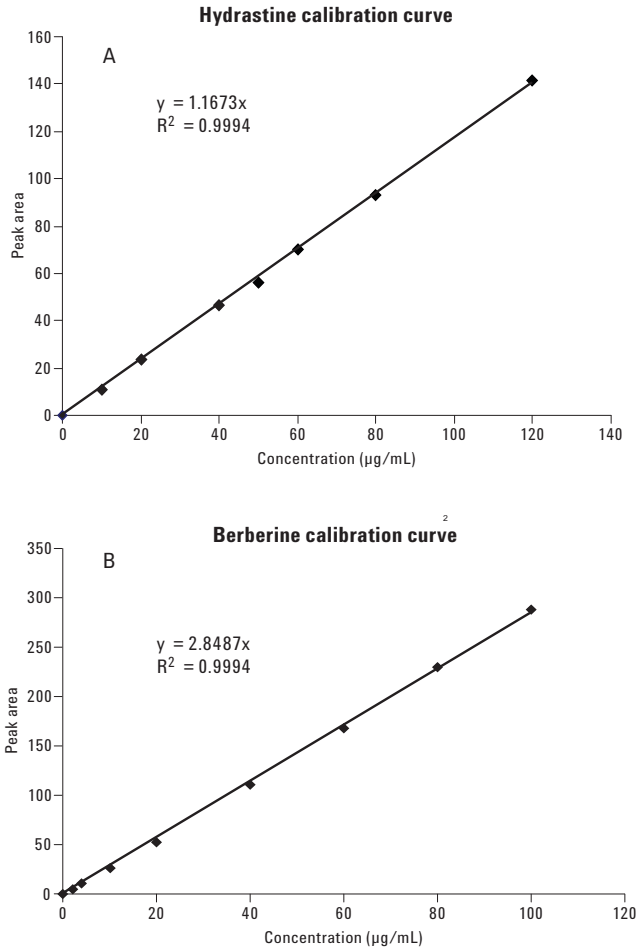

Figure 6. Calibration curves of (A) hydrastine and (B) berberine.

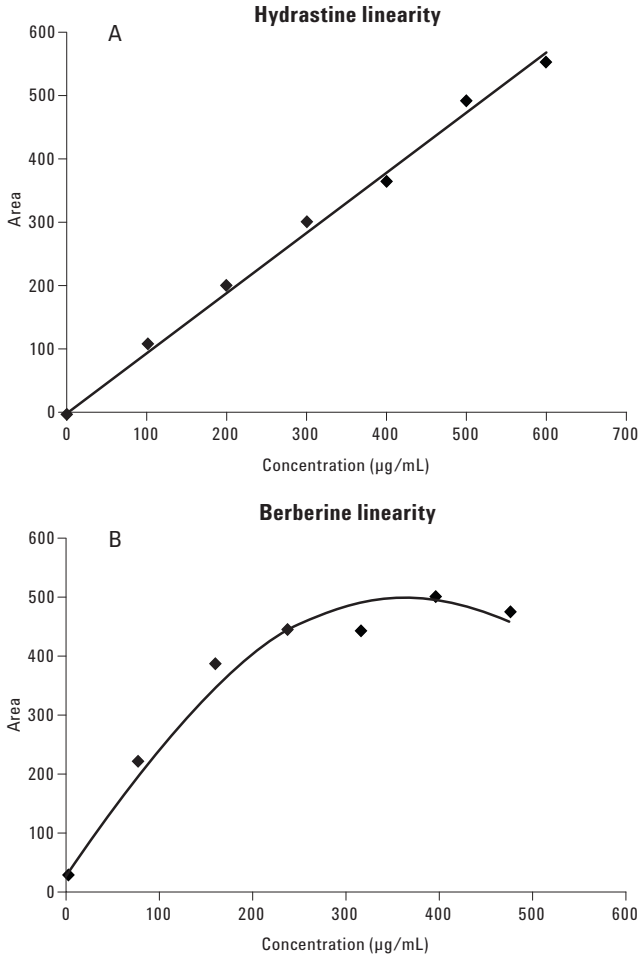

Figure 7. Linearity of (A) hydrastine and (B) berberine at higher concentrations.

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 (LOD) and limit of quantification (LOQ)

The limits of detection were calculated using the intercept, y_B, and the standard error of the regression line, s_B, at three times the standard error, and LOD values were calculated using equations 1 and 2 [8,9].

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

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

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).

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

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

The LOD and LOQ 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 LOD and LOQ 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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