# Determination of Alkaloids in Goldenseal Using Agilent Bond Elut Plexa Solid Phase Extraction Sorbent for Cleanup and HPLC-DAD Analysis

Application Note

Dietary Supplement


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## Abstract

The alkaloids (hydrastine and berberine) in goldenseal were successfully cleaned up using the Bond Elut Plexa solid phase extraction sorbent. Their HPLC separation was achieved in less than 6 minutes employing an Agilent RRHD ZORBAX Eclipse Plus C18 column (4.6 x 75 mm, 3.5 µm) at 35 °C with gradient elution of 0.1% phosphoric acid/methanol mobile phase. The calibration curves of both alkaloids gave good correlation coefficient of 0.9994. The recoveries of hydrastine ranged from 76–102% while those of berberine ranged from 99–104% with a relative standard deviation (RSD) % of less than 5.



Agilent Technologies

## Introduction

Goldenseal is a small perennial plant indigenous to the hardwood forest of eastern United States of America and Canada [1]. It has been used by Native Americans to treat wounds, ulcers, digestive disorders, skin and eye ailments [2]. The extracts of the tree have been made available as dietary supplements as they are believed to possess antimicrobial, antiparasitic, and anti-viral properties [3]. These properties are believed to be due to the plant's alkaloid content. The main alkaloids found in Goldenseal are berberine and hydrastine (see structures in Figure 1) [4]. The wide usage of herbal supplements and alternative medicines has led to new directives and legislation aimed at regulating both the herbal and nutraceutical industries [5]. Therefore, there is a need for simple, reproducible, accurate, and easy to use test methods for detection of these compounds.

Sample pretreatment is a very important step in many analytical procedures, especially with low concentration analytes in complex matrices [6]. Solid-phase extraction (SPE) is the most popular sample clean up technique employed prior to analysis of environmental, food, pharmaceutical, and biological samples by high-performance liquid chromatography (HPLC) [7]. It possesses a wide range of sorbent chemistries based on inorganic oxides, bonded phases of polymers, silica, and carbon, as well as analyte or group selective materials (ion exchange, mixed mode, restricted access, immunoaffinity, and molecularly imprinted polymers). There has been increasing demand for SPE material with improved analyte recoveries, sorptive capacity, selectivity, mechanical, and chemical stability [8].

This application note presents an Agilent Bond Elut Plexa solid phase extraction method, that uses a polymeric sorbent for the clean-up and preconcentration of alkaloids in goldenseal. It has been designed for simplicity, improved analytical performance, and ease of use [9]. To achieve these qualities, the proposed SPE mechanism suggests that the hydrophobic core strongly retains the analyte of interest while the hydrophilic exterior surface keeps the matrix out of the pore structure. During the washing stage, the matrix components are easily washed off while the analytes are still retained strongly in the hydrophobic core. This results in cleaner extracts with good recoveries and reproducible results. The uniform and narrow size distribution helps in avoiding blockage of the cartridge, thus improving the flow rate as well as cartridge to cartridge reproducibility [10].

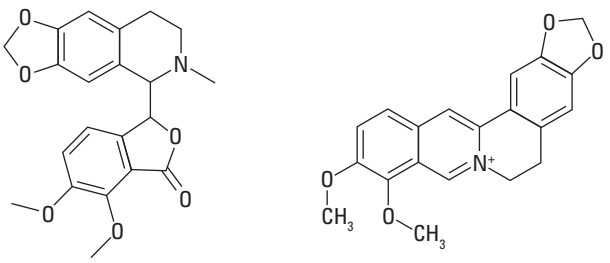


*Figure 1. Hydrastine and berberine chemical structures.*

## Experimental

### Materials and chemicals

Berberine hydrochloride and hydrastine hydrochloride standards were purchased from Sigma-Aldrich (Saint Louis, USA). Phosphoric and hydrochloric acids were purchased from Merck Chemicals (Gauteng, South Africa) while HPLC grade methanol was purchased from Merck KGaA (Darmstadt, Germany). Ammonia solution (25%) was from Saarchem Analytic (Krugersdorp, South Africa). Goldenseal roots were purchased from a local herbal store in Grahamstown, South Africa. SPE cartridges were Agilent Bond Elut Plexa, 1 mL/30 mg tubes, p/n 12109301. Analysis was performed on an Agilent 1200 series HPLC coupled with a diode array detector (DAD). The analytical column was an Agilent ZORBAX Eclipse Plus C18 (4.6 × 75 mm, 3.5 µm, p/n 959933-902).

### Preparation of stock and working standards

The stock solution of hydrastine and berberine, 1,000 µg/mL each, were prepared in methanol and stored at 4 °C when in use. All other standard solutions were prepared from the stock solution as required.

### Sample preparation

A 200-mg portion of the goldenseal root was ground and homogenized, then mixed with 200 mL deionized water. The mixture was refluxed for 1 hour with continuous stirring then cooled to room temperature. The extracts were filtered using a Whatman filter paper (125 mm diameter) and diluted to 1:3 *(v/v)* with 2% ammonia solution. The pH was adjusted to approximately 7 with 0.01 M hydrochloric acid, and then analysis was conducted by HPLC-DAD.

## Optimization of parameters

### Separation and clean-up

A 5 µL of 80 µg/mL diluted stock solution was injected into the HPLC and the separation of the peaks optimized. Berberine and hydrastine were both monitored at 242 nm but, due to their stronger spectrophotometric absorption at these wavelengths, were quantified at 294 and 350 nm, respectively. The HPLC conditions are as summarized in Table 1, modified and adopted from a previous application note [11].

*Table 1. HPLC Conditions*

| Parameter | Condition |
|---|---|
| Column | Agilent Zorbax Eclipse Plus C18<br>4.6 × 75 mm, 3.5 µm (p/n 959933-902) |
| Flow rate | 1.00 mL/min |
| Injection volume | 5 µL |
| Column temperature | 35 °C |
| Mobile phase | A: 0.1% phosphoric acid<br>B: methanol |
| Run time | 6 min |
| Gradient | Time 0 0.5 3<br>%B 25 25 50 |

## SPE optimization

A series of SPE conditions for conditioning, loading, washing and elution were evaluated using the standard solution. The optimal conditions are outlined in Figure 2.

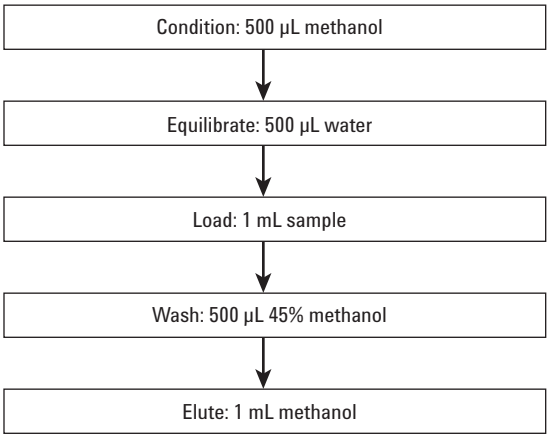


*Figure 2. SPE procedure for cleaning alkaloids using Bond Elut Plexa Sorbent.*

# Results and Discussion

## Separation of peaks and SPE clean up

Good peak separation was achieved for the standard mixture for hydrastine and berberine using HPLC conditions outlined in Table 1 (see Figure 3). A goldenseal root sample extract was run with similar HPLC conditions before and after SPE. As was shown by the decrease in the number of small peaks and an increase in the intensity of the peak height (see Figure 4), results showed a cleaner chromatogram for SPE extract, an indication that SPE procedure was able to remove potential interferences.

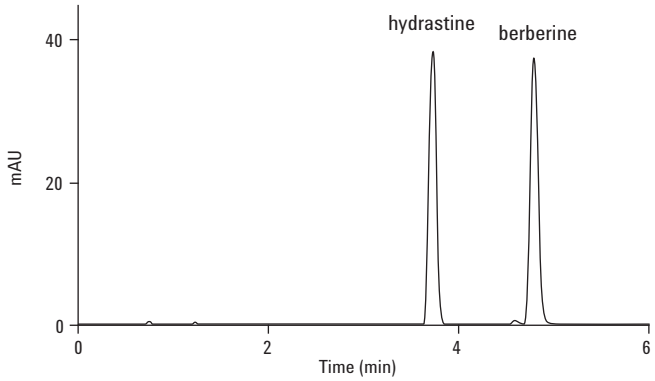


*Figure 3. Chromatograms of hydrastine and berberine standards.*

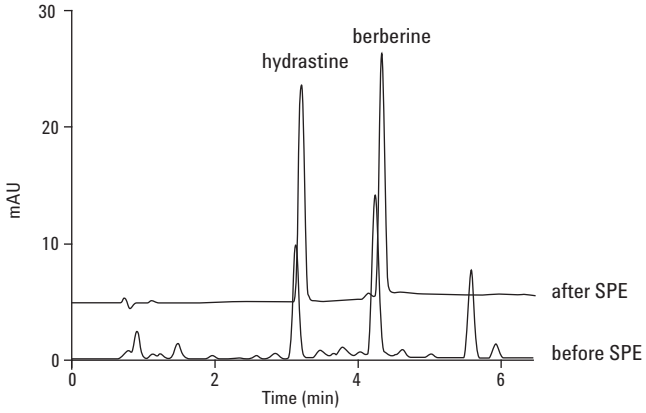


*Figure 4. HPLC-DAD chromatograms of hydrastine and berberine from goldenseal roots extract before and after SPE.*

## Recovery and reproducibility

The recovery and reproducibility of the method were studied by spiking six replicates of the commercial goldenseal roots at three different levels for hydrastine, and two levels for berberine. The spiked sample extracts were cleaned up using the optimized SPE procedure. Recovery was obtained by calculating the difference of concentrations between the spiked and the unspiked sample after SPE. The results obtained are shown in Table 2. The % RSDs were all less than 5.

*Table 2. Recovery and Reproducibility Data for Hydrastine and Berberine (n = 6)*

| Alakloid | Spiking level (µg/mL) | % Recovery | %R.S.D. |
|---|---|---|---|
| Hydrastine | 10 | 76 | 3.94 |
| | 50 | 83 | 4.98 |
| | 100 | 102 | 2.40 |
| Berberine | 5 | 99 | 4.68 |
| | 75 | 104 | 3.12 |

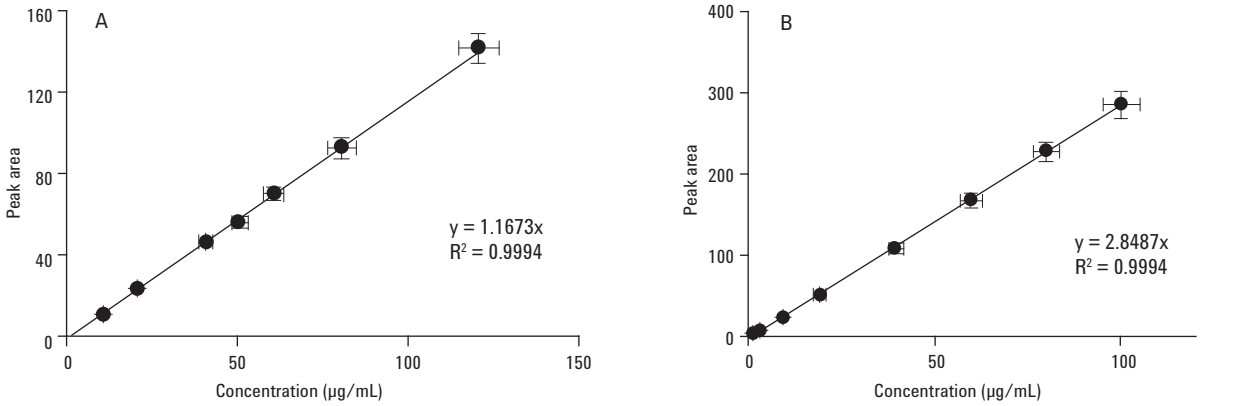


*Figure 5. Calibration curves of (A) hydrastine and (B) berberine.*

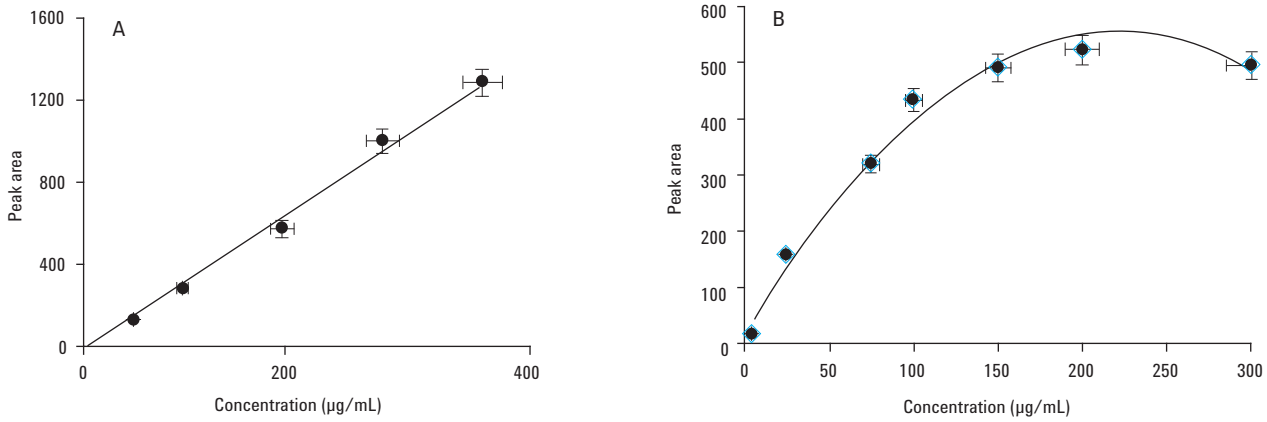


*Figure 6. Loading capacity of Bond Elute SPE sorbents on (A) hydrastine and (B) berberine.*

## Analytical parameters

### Calibration curves

A 7-point calibration curve was prepared using a concentration range of 0–120 µg/mL for hydrastine and an 8-point calibration curve for berberine was prepared using a concentration range of 0–100 µg/mL. The different concentration levels were achieved by diluting the 1,000 µg/mL stock standard solutions into the required concentrations. The calibration curves were linear each with correlation coefficient of 0.9994 (see Figure 5).

### Linearity of the SPE method

The loading capacity of the Bond Elut Plexa SPE was studied by spiking the goldenseal root extracts with increasing concentrations of the standard mixture followed by SPE clean up. Linearity of hydrastine was still observed at 400 µg/mL spiking level while for berberine it was no longer observed at concentrations higher than 150 µg/mL. At concentrations above 150 µg/mL, the analyte was lost even at the loading stage, indicating that the sorbent was overloaded and could not hold any more sample (see Figure 6).

## Limit of detection and limit of quantification

The limits of detection (LOD) were calculated using the intercept, $y_B$, and the standard error of the regression line, $S_B$ at 3 times standard error. The LOD values were calculated using equations 1 and 2.

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

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

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

$$y_{LOQ} = y_B + 10S_B \qquad \text{Equation 3}$$

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

The LOD and LOQ for hydrastine are 0.50 and 1.65 µg/mL, respectively, while that of berberine is 0.47 and 1.55 µg/mL, respectively.

## Application to real sample

Goldenseal root extracts were analyzed using the developed method. The roots contained 22.24 and 68.13 µg/mL hydrastine and berberine, respectively.

# Conclusion

Bond Elut Plexa solid phase extraction achieved effective sample clean up by yielding cleaner chromatograms. The results showed that the method is accurate and reproducible with recovery ranges of 76–102% for hydrastine and 99–104% for berberine with R.S.D less than 5. The LOD and LOQ for hydrastine were 0.50 and 1.65 µg/mL, respectively, while that of berberine is 0.47 and 1.55 µg/mL, respectively. Goldenseal extracts contained 22.24 and 68.13 µg/mL hydrastine and berberine, respectively.

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