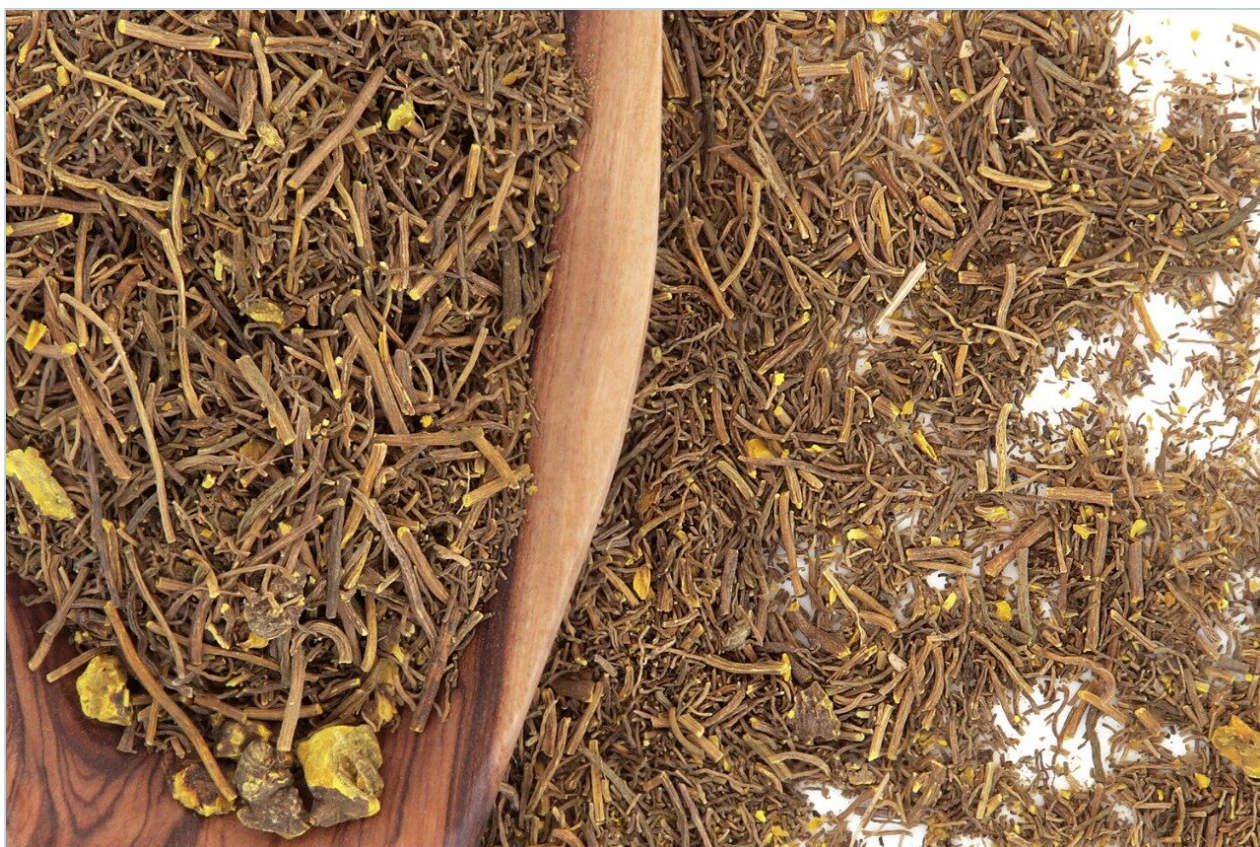


Nota de aplicación

Characterizing of the Natural Product Goldenseal Using CORTECS 2.7 μm Columns and ACQUITY QDa Detection

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Abstract

In this application note, a simple HPLC separation of five sources of Goldenseal was performed in five minutes, and seven compounds were identified.

Benefits

- Separation of complex sample matrices using CORTECS 2.7 μm Columns, allowing for accurate characterization of the sample
- Rapidly identify compounds by mass using the ACQUITY QDa Detector in 5 minutes

Introduction

Natural product herbal supplements are widely used as remedies for physical ailments. Depending on sample origin and processing, product composition can range widely in the number, type, and amount of natural product compounds present. As consumers are growing in concern to the side effects of chemicals used in cosmetics and personal care products, the use of herbal plants in cosmetics,¹ and personal care products,^{1,2} is growing in demand. Accurate sample characterization from numerous sources is useful to control supplement quality. However, the sample complexity and/or variability require highly efficient columns that do not sacrifice analysis speed. CORTECS 2.7 μm Columns contain solid-core particles which produce high peak capacity separations with reduced back pressure, making them ideal for use on traditional HPLC instruments. Using CORTECS 2.7 μm Columns, therefore, allows easier analysis of natural product mixtures.

Goldenseal is a plant native to southeastern Canada and the northeastern United States. Traditionally, it has been used to support digestion, mucous membranes, bile secretions, and many other bodily functions, as well as a topical treatment. In this application note, five different sources of Goldenseal which span four different manufacturers, two different parts of the plant, and two different formulation matrices are analyzed. This variety of sources was tested to determine the differences between each sample and if there were any compounds common to all the tested sources. Each sample was analyzed using a CORTECS C₁₈+, 2.7 μm , 3.0 x 50 mm Column on an Alliance HPLC System with both UV and an ACQUITY QDa Detector in order to get fast and reliable mass data for peaks present in the sample, providing additional and crucial information

for the characterization of the natural products.

Experimental

Capsule samples³

20 mg of powdered sample was removed and placed into a 10 mL centrifuge tube. 2.5 mL of 90:10 methanol:water with 0.1% acetic acid was added. Samples were sonicated for 15 minutes, and centrifuged at 4000 rpm for 5 minutes. The supernatant was then removed and placed into a separate vial. Extraction was performed three additional times. Extracted liquid filtered through a 0.1 µm nylon filter prior to injection.

Liquid sample

Two drops of liquid Goldenseal was added to 10 mL of 90:10 methanol:water with 0.1% acetic acid. Sample filtered through a 0.1 µm nylon filter prior to injection.

LC conditions

System:	Alliance HPLC
Column:	CORTECS C ₁₈ +, 2.7 µm, 3.0 x 50 mm (p/n 186007400)
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Gradient:	7–30% B in 5.0 minutes, return to 7% B in 0.1 minutes, hold for 1.0 minutes
Flow rate:	1.0 mL/min
Column temp.:	30 °C
Detection (UV):	300 nm

ACQUITY QDa setting:	ESI+ mode, full scan from 150–1250 amu
Injection volume:	1.0 μ L
Sample vials:	LC-MS Certified Max Recovery Vials (p/n 600000670CV)
Data management:	Empower 3 CDS

Results and Discussion

Five commercial sources of Goldenseal were acquired for characterization. Table 1 outlines the source of each sample as well as details regarding the part of the plant used and the sample format.

Name	Source	Format
Goldenseal liquid	Root	Liquid
Goldenseal herb	Stem, flower, leaf	Capsule
Goldenseal root	Root	Capsule
Goldenseal extract	Root	Capsule
Goldenseal	Rhizome/root	Capsule

Table 1. Summary of the five sources of Goldenseal obtained.

After sample preparation, the samples were injected and both UV and mass data were collected. Figure 1 shows the full scale separation of the five samples.

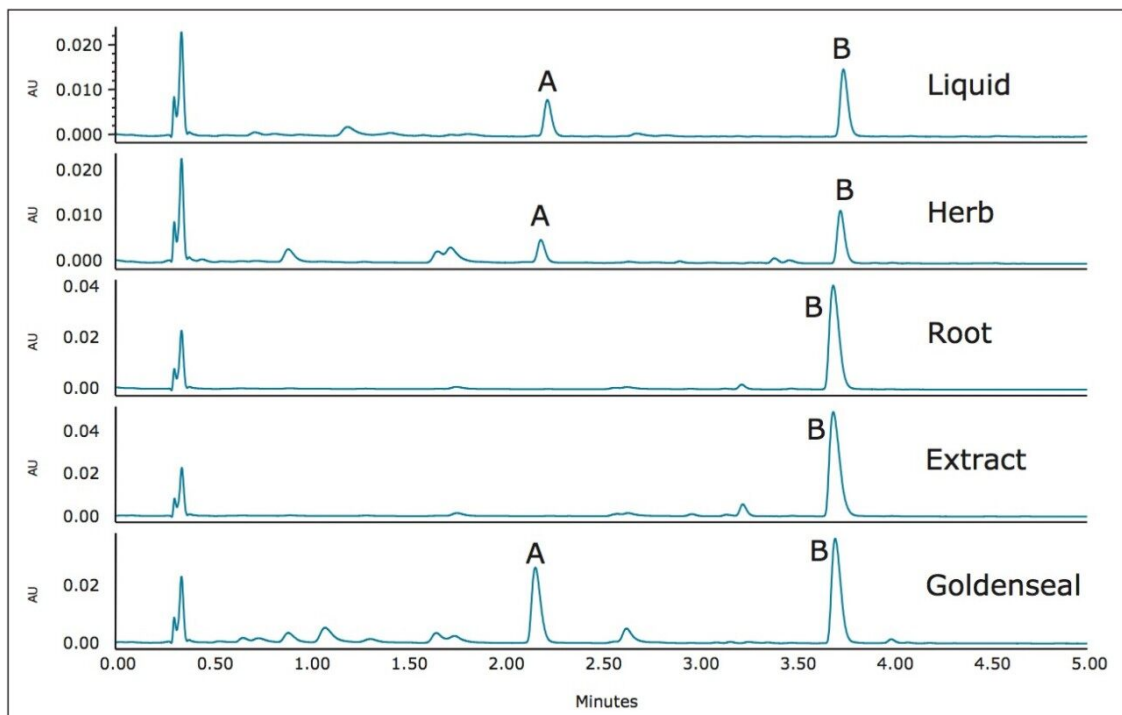


Figure 1. Separation of five different sources of Goldenseal on a CORTECS $C_{18}+$, $2.7 \mu\text{m}$, $3.0 \times 50 \text{ mm}$ Column at 300 nm . Main components A and B are indicated. Maximum pressure for the separation was 2600 psi .

The chromatograms were then scaled to show the low level constituents that exist in the samples. Figure 2 shows the zoomed in chromatograms of the five samples.

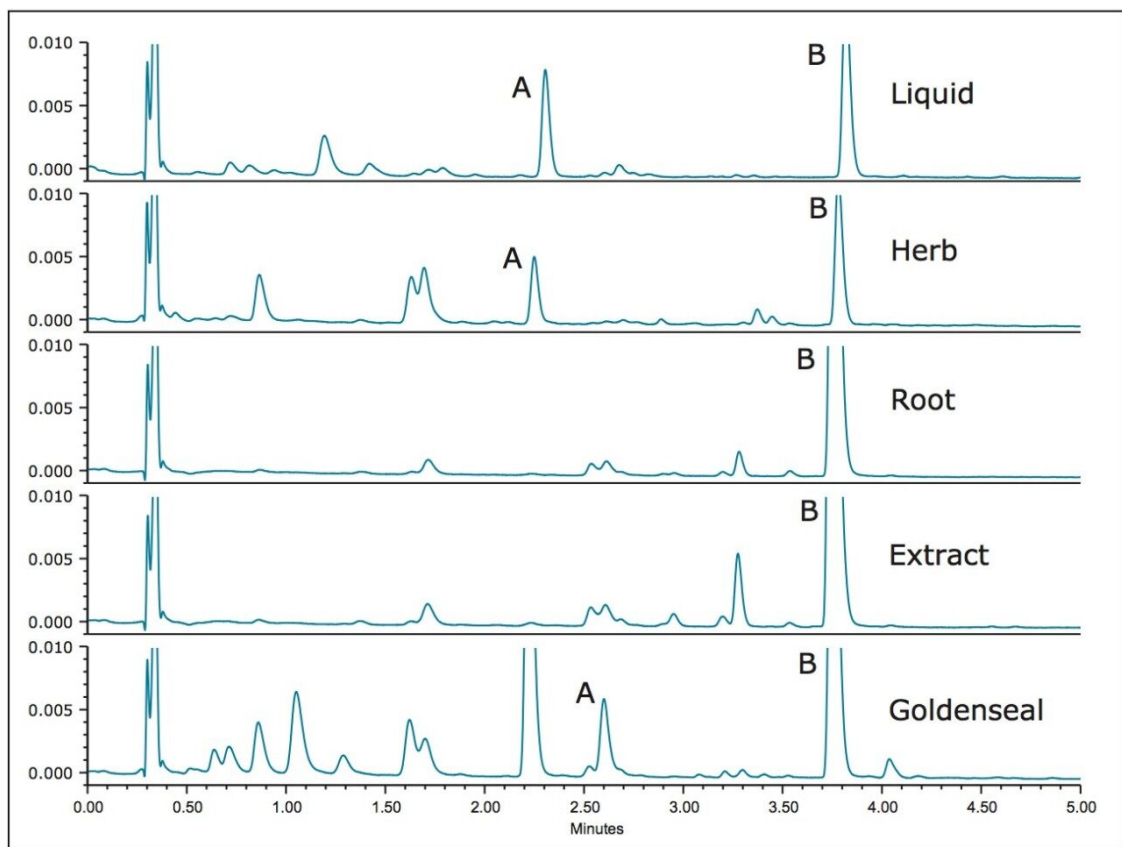


Figure 2. Zoomed in UV chromatograms of the five samples from Figure 1.

The distinct differences in the UV chromatograms of the five different samples were evident due to the high efficiency of the CORTECS Column. The intensity of peak B varied from 0.04 to 0.02 AU, indicating that different samples had different amounts of that particular compound. Further sample characterization required additional information. To obtain this, ACQUITY QDa mass spectral data was examined and compared with Goldenseal data from the literature.⁴ A total of seven alkaloids were identified (see Table 2 and Figure 3).

Compound	Mass (M+H)
Dihydro Berberine	338.1
Canadine	340.3
Berberine	336.1
Isocorypalmine	370.1
Methyl Hydrastine	398.4
Hydrastine	384.1
Palmatine	352.2

Table 2. Summary of alkaloids identified and their masses as described in the literature.⁴

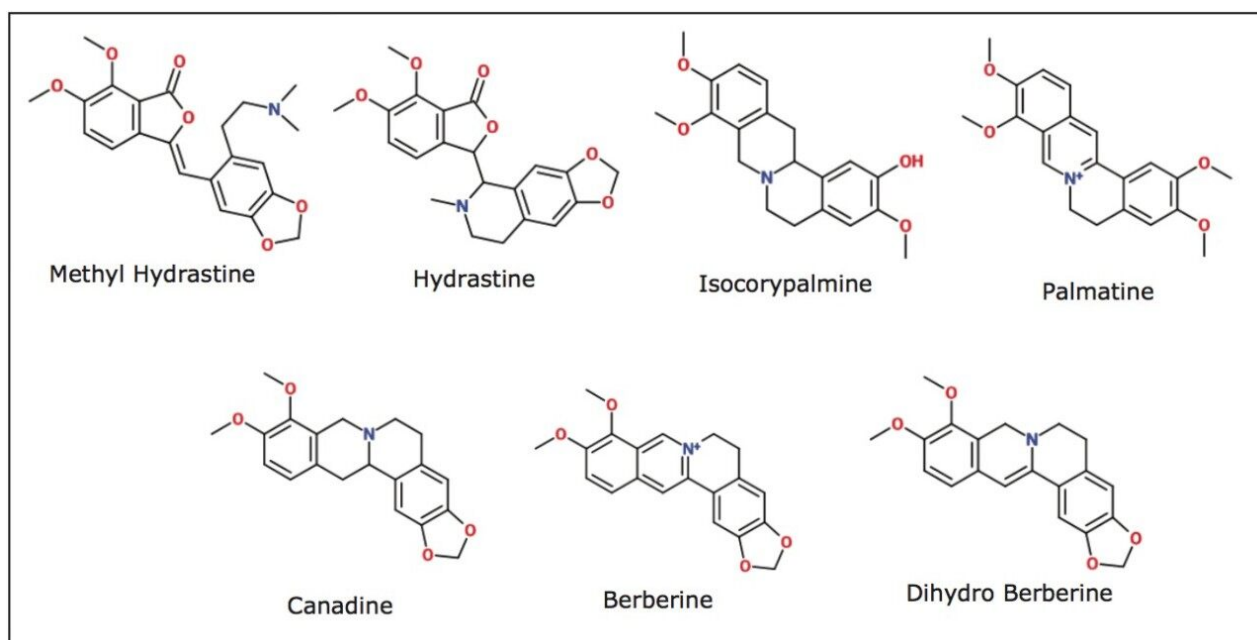


Figure 3. Structures of identified alkaloids.

Using the ACQUITY QDa Detector, some of the peaks observed in the UV chromatograms were identified. The liquid sample had the most identifiable peaks, showing the presence of all seven compounds listed in Table 2. The remaining samples each exhibited two or more identifiable peaks. Berberine was the most abundant component and the only compound present in all samples. Figure 4 shows the identification of peaks in the liquid sample using extracted ion chromatograms (EIC).

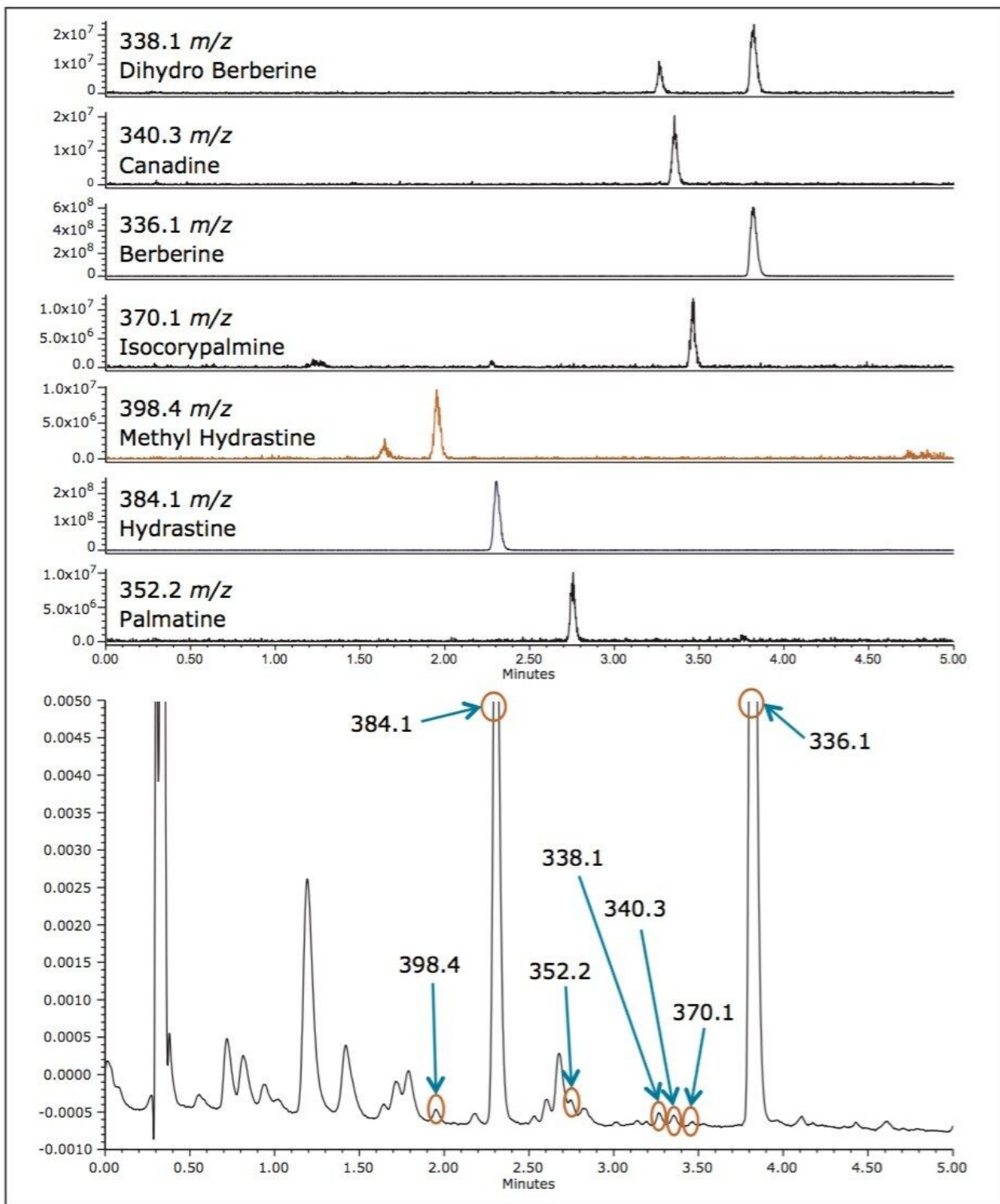


Figure 4. Identification of UV peaks in the liquid sample of Goldenseal by m/z value.

By using the ACQUITY QDa Detector, the two main component peaks (A and B in Figures 1 and 2) were

identified as hydrastine and berberine, respectively. An additional five compounds were also identified and linked to peaks in the UV chromatogram. No two samples show exactly the same compounds at the same concentrations. These traces represent a type of “fingerprint” that is characteristic of each Goldenseal sample. Such fingerprints can be useful in comparing Goldenseal from different manufacturers as well as different sources of the plant. The rapid separation of a complex sample such as Goldenseal is possible due to high efficiency of CORTECS 2.7 μm Columns. By combining UV data with the mass data obtained with an ACQUITY QDa Detector, a full characterization of each sample can be made giving an analyst valuable information with minimal effort.

Conclusion

Natural product analysis and characterization can be a difficult process due to the complex nature of the sample. Gathering fast and reliable data is essential for characterization of complex samples such as Goldenseal in this application. Using a CORTECS C₁₈+ 2.7 μm Column, a complex separation can be performed more easily. CORTECS 2.7 μm Columns offer high efficiency while operating within the pressure limits of an HPLC system. By combining the newest column technology with the newest technology in mass detection (ACQUITY QDa), a simple separation of five sources of Goldenseal was performed in five minutes and seven compounds were identified.

References

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