Waters™
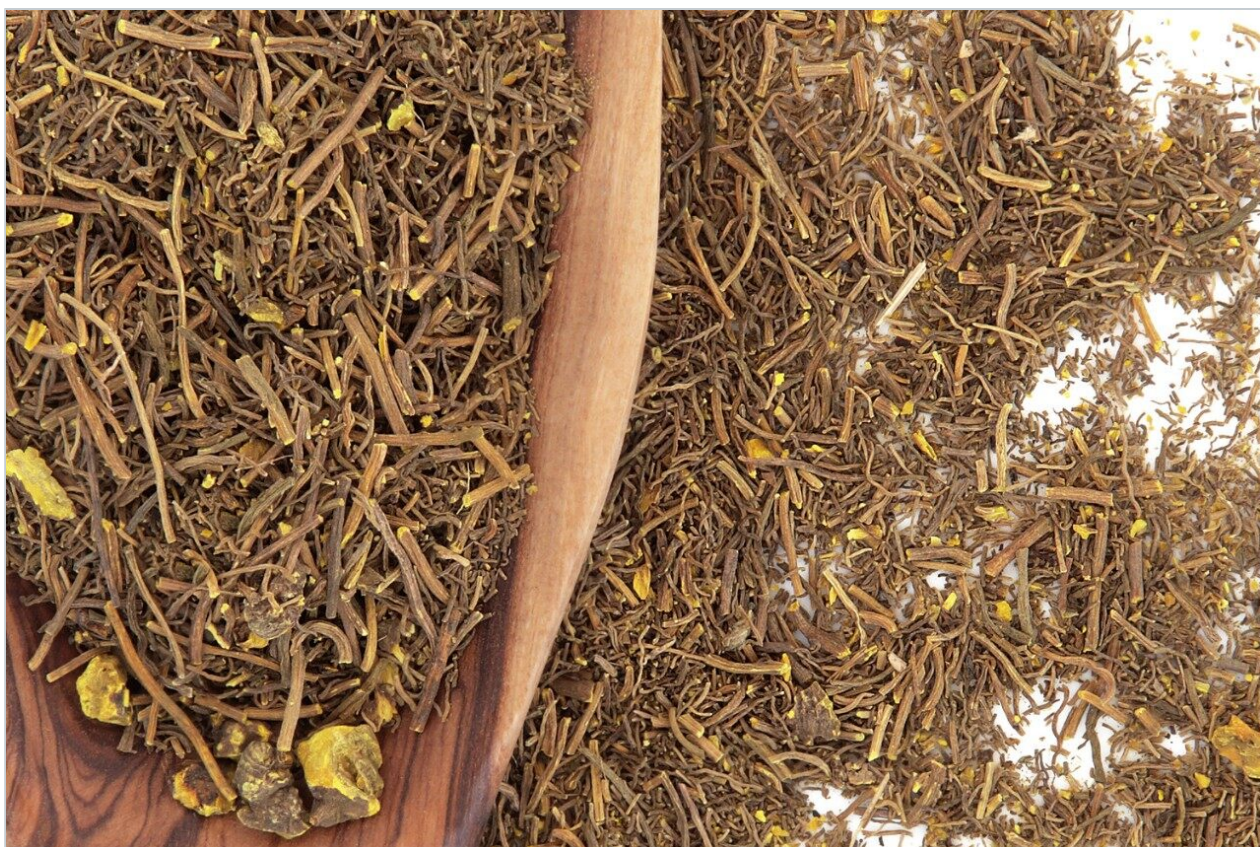
Improved Analysis of Goldenseal Root Extract Using Charged Surface Hybrid (CSH) Column Technology

Mia Summers, Kenneth J. Fountain

Waters Corporation

Abstract

This application note demonstrates the use of charged surface hybrid (CSH) column technology which greatly improves loadability of alkaloids found in Goldenseal extract, when compared to a high purity, fully-porous C_{18} and a superficially porous C_{18} column. Improved sample loading for basic compounds such as isoquinoline alkaloids in Goldenseal extract were observed using CSH technology.

Benefits

· Improved loadability and peak shape of basic compounds

· Fast identification of components in complex mixtures using UPLC-ToF-MS

· Streamlined method transfer from analytical to preparative scale using scalable column chemistries alleviates the need for method re-development

Introduction

Goldenseal (*Hydrastis canadensis*) is a plant native to Northeastern US and Canada that has long been used as a traditional medicine across many cultures. Goldenseal root extract contains several isoquinoline alkaloids including berberine and hydrastine, which have been studied extensively for their medicinal properties.[1] These alkaloids typically show poor peak shape on traditional reversed-phase columns in low-ionic strength mobile phases due to their basic nature and limited sample loadability.

This application note demonstrates the use of charged surface hybrid (CSH) column technology which greatly improves loadability of alkaloids found in Goldenseal extract, when compared to a high purity, fully-porous C_{18} and a superficially porous C_{18} column. By improving sample loadability, the peak shape, resolution and sensitivity of detection of the alkaloids by UV is enhanced, facilitating identification of these components using a time-of-flight mass spectrometer (ToF-MS). The Goldenseal separation is then seamlessly transferred from UPLC to preparative HPLC using scalable CSH chemistries, alleviating the need to fully re-develop a preparative HPLC method for target compound purification. Using the same CSH stationary phase on the preparative scale, the selectivity of the Goldenseal separation and improved peak shape of the alkaloids is maintained.

Experimental

ACQUITY UPLC Conditions

Column: ACQUITY UPLC CSH C_{18} 2.1 x 100 mm, 1.7 µm, part number: 186005297

Flow Rate: 0.4 mL/min

Inj. Volume: 1 µL

Gradient: 0 to 40% B in 6 minutes, hold for 2 minutes, re-equilibrate at 0% B to 10 min.

All instruments (unless otherwise noted)

Mobile Phase: A: 0.1% formic acid in water

                           B: 0.1% formic acid in acetonitrile

Needle Wash: 10:90 water:acetonitrile

Sample Purge: 90:10 water:acetonitrile

Seal Wash: 90:10 water:acetonitrile

Column Temp.:   30 °C

Detection: UV at 254nm

Auto-Purification Preparative HPLC Conditions

Column: XSelect CSH C_{18} OBD 19 x 100 mm, 5 µm, part number 186005421

Flow Rate:	17 mL/min
Inj. Volume:	164 µL
Gradient:	0 to 40% B in 11.6 minutes, hold to 15.40 minutes, re-equilibrate to 0% B

LCT-Premier ToF-MS Conditions

Mass Scan Range:	150-500 amu
Mode:	ESI, V+ mode
Capillary Voltage:	3000 V
Cone Voltage:	10 V
Desolvation Temp.:	350 L/hr
Desolvation Gas:	700 L/hr
Reference Standard:	200 pg/mL leucine enkephalin in 50:50 water:acetonitrile
Data Management:	Empower 2 CDS (UPLC and HPLC) MassLynx (UPLC-ToF-MS and Preparative HPLC)

Sample Preparation

Goldenseal Extract:	1605 mg lyophilized goldenseal root powder was extracted at room temperature in 10 mL of ethyl acetate. The sample was stirred for one hour and filtered through a 0.2 µm nylon filter.

Results and Discussion

Major secondary metabolites of Goldenseal include isoquinoline alkaloids such as berberine and hydrastine. These basic compounds typically have poor peak shape and limited loading capacity on reversed-phase columns using low ionic strength mobile phases.[2] When analyzing complex mixtures, poor peak tailing and resolution due to overloading can easily mask minor components that may be of interest. Charged Surface Hybrid (CSH) column technology applies a low-level charge to the surface of a hybrid particle, which provides superior peak shape and loading of basic compounds in mobile phases containing additives such as formic acid.

The Goldenseal extract separation on a CSH C_{18} column was compared to a BEH C_{18} column as well as a superficially-porous C_{18} column on an ACQUITY UPLC system. The resulting chromatograms are shown in Figure 1. Both the BEH C_{18} column and the superficially-porous C_{18} column display peak shape that is indicative of mass overloading for the two major peaks. By comparison, the CSH C_{18} column shows much sharper peak shape and increased sensitivity by UV for metabolites in the Goldenseal extract.
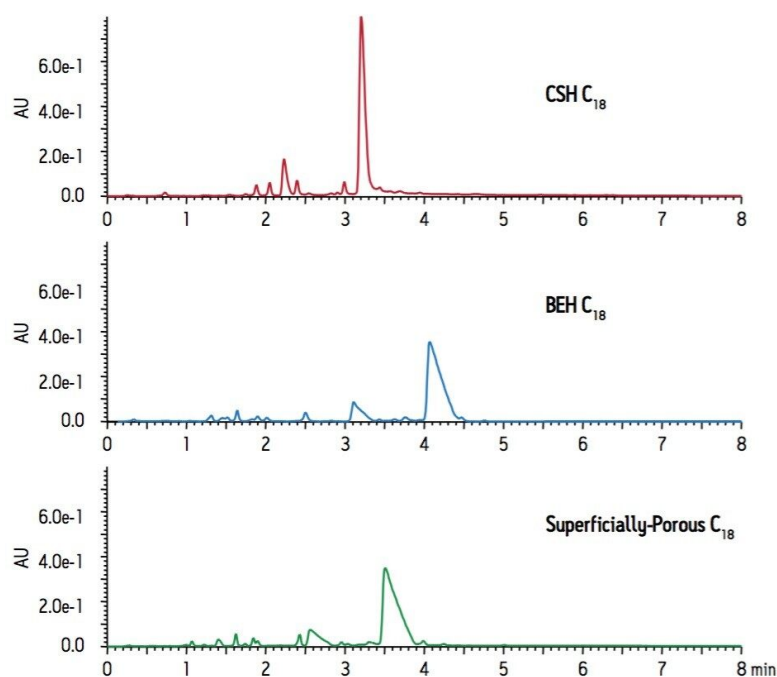
Figure 1. Comparison of Goldenseal extract on CSH C$_{18}$, BEH C$_{18}$ and superficially-porous C$_{18}$, keeping column dimensions and method conditions constant.

Target Compound Identification

In complex separations, peak tailing and poor resolution can interfere with the identification of components of interest. Using CSH technology in combination with UPLC-ToF MS, a rapid one-step identification of target compounds from complex mixtures can be performed. In this case, Goldenseal extract was analyzed using the CSH C$_{18}$ column on an ACQUITY UPLC coupled to an LCT Premier ToF mass spectrometer to identify secondary metabolites. The masses of unknown major and minor peaks in the total ion chromatogram were evaluated and compared to known secondary metabolites of Goldenseal. The identity of each component was confirmed by accurate mass analysis (Figure 2) using the Elemental Composition Calculator in MassLynx software, which provides a formula that best fits the experimental mass based on isotope fit and mass error, compared to the theoretical calculated mass.

Component	Experimental Mass (M+H+)	Calculated Mass (M+H+)	Error (PPM)	Formula (M+H+)
Berberine	336.1236	336.1236	0.0	$C_{20}H_{18}NO_4$
Hydrastine	384.1520	384.1447	0.8	$C_{21}H_{22}NO_6$
Canadine	340.1549	340.1549	0.0	$C_{20}H_{22}NO_4$
Berberastine	352.1182	352.1185	0.6	$C_{20}H_{18}NO_5$
Canadaline	370.1648	170.1654	1.6	$C_{21}H_{24}NO_5$

Figure 2. Elemental composition analysis of Goldenseal components.

Based on the LC-MS analysis, the two main peaks in the UV chromatogram were identified as hydrastine and berberine (Figure 3). The improved loadability and peak shape using CSH technology allowed for closely eluting minor components canadine, canadaline and berberastine to be resolved and identified.
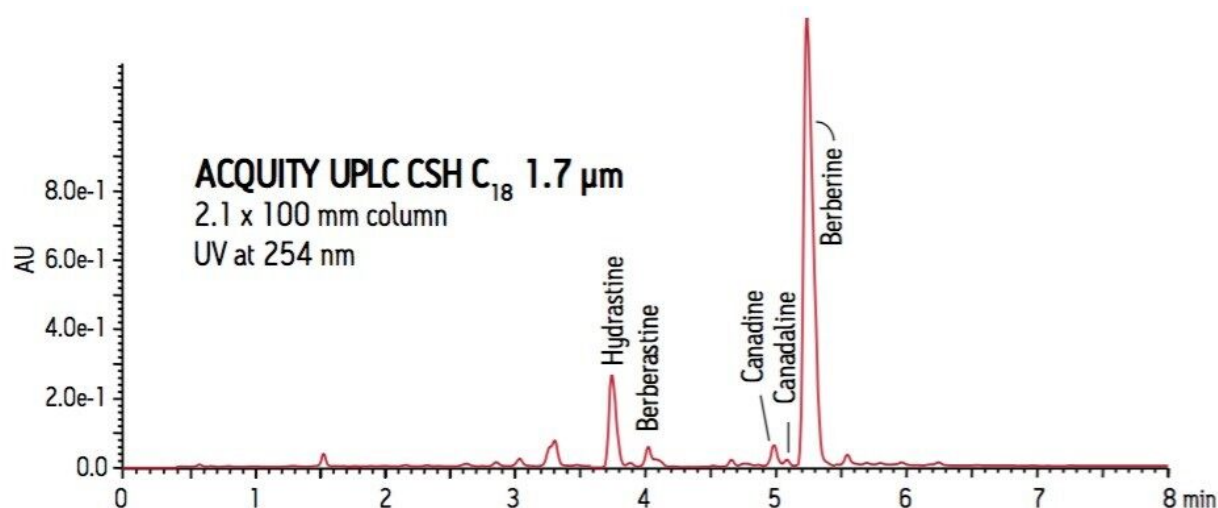

Figure 3. Secondary metabolites of Goldenseal are resolved and identified using CSH technology and UPLC-ToF MS.

Method Transfer

Analytical methods (i.e., UPLC or HPLC) are often used in screening natural product extracts for target compounds. Method with compounds of interest are often further scaled to preparative HPLC for isolation of desired components. The use of different column stationary phases for the analytical and preparative separations can affect selectivity and peak identification, necessitating time-consuming re-development of the method on the preparative scale. The availability of CSH stationary phases in analytical and preparative

sizes allows for seamless scaling of separations, maintaining peak elution order. In addition, the increased sample loading capacity of CSH columns compared to BEH or superficially-porous columns is highly advantageous for preparative-scale separations, where improved peak tailing can result in cleaner fractions and fewer iterative purification steps.

The Goldenseal separation was transferred from the ACQUITY UPLC CSH C_{18} column on a UPLC system to the equivalent chemistry (XSelect CSH C_{18} 19 x 100 mm, 5 µm) on a preparative HPLC system. The method was first transferred to HPLC using the ACQUITY UPLC Columns Calculator[3] and subsequently scaled to preparative HPLC using the Waters Preparative Columns Calculator. Properly scaling the 10-minute UPLC method to HPLC, while maintaining resolution by keeping the column length to particle size (L/d_p) ratio constant, would require a 4.6 x 250 mm 5 µm column and result in a method that is approximately 75-minutes long. Rather than run this long HPLC method, the calculated HPLC method was transferred directly to preparative HPLC, disregarding particle size and scaling for constant linear velocity. Scaling for constant linear velocity by adjusting flow rate, injection volume and gradient time resulted in a very short preparative run time, sacrificing only a small amount of resolution. Using the same column chemistry from analytical to preparative scale, the increased loading capacity, improved peak shape and separation using CSH technology that was observed by UPLC is maintained on the preparative HPLC system (Figure 4).
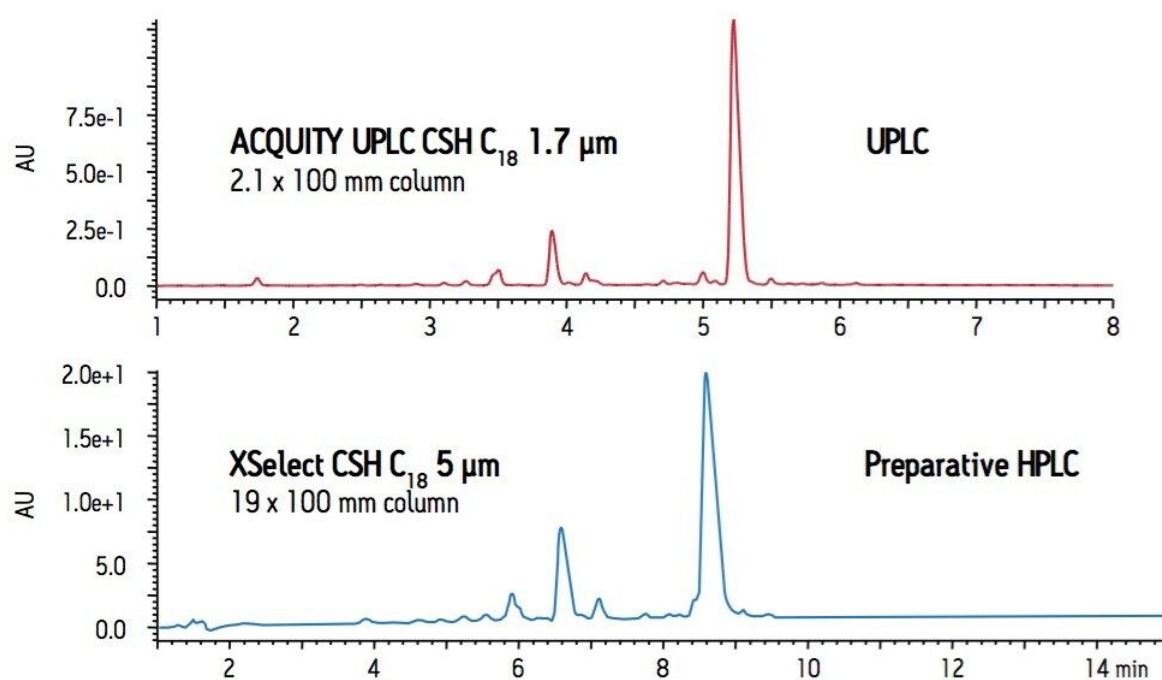

Figure 4. UPLC and preparative HPLC separation of Goldenseal extract at 254 nm.

Conclusion

- Improved sample loading for basic compounds such as isoquinoline alkaloids in Goldenseal extract were observed using CSH technology. Reduced peak tailing and higher sensitivity of detection using the CSH column resulted in an overall better quality separation, compared to a more traditional fully-porous or superficially-porous column.

- With the improved peak shape and resolution of the Goldenseal separation using CSH technology, major and minor secondary metabolites were identified more effectively using accurate mass analysis performed on an ACQUITY UPLC coupled to an LCT-Premier ToF-MS.

- Method transfer from UPLC to preparative HPLC on the Goldenseal separation was performed using the same CSH column chemistry at each scale. The resulting preparative separation maintained selectivity and peak shape without requiring a full re-development of the method, demonstrating a faster, more efficient method transfer from analytical to preparative LC.

References

1. Weber, H.A. et al. *J. Agric. Food Chem*. 51: 7352 [2003].

2. McCalley, D. V. *Anal. Chem*. 78: 2532 [2006].

3. Jones MD, Alden P, Fountain KJ, Aubin A. *Implementation of Methods Translation between Liquid Chromatography Instrumentation*, Waters Application Note [2010], Part Number 720003721EN.

Featured Products

ACQUITY UPLC System <https://www.waters.com/514207>

Empower 3 Chromatography Data Software <https://www.waters.com/513188>

MassLynx MS Software <https://www.waters.com/513662>