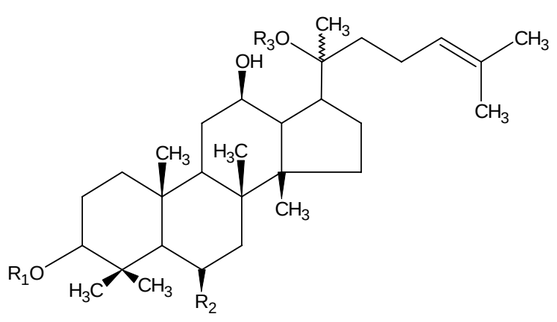
PURIFICATION OF GINSENG EXTRACT USING REVERSED-PHASE LIQUID CHROMATOGRAPHY

Jie Y. Cavanaugh, Fang Xia, Diane M. Diehl and Jeffrey R. Mazzeo
Waters Corporation, Milford, MA 01757, USA
(Send correspondence to jie_cavanaugh@waters.com)

OVERVIEW

Ginseng has a long history of medicinal use in Asia. The active ingredients from Ginseng, ginsenosides, are reported to reduce mental impairment and cell loss from brain degeneration. Many researchers are interested in isolating these active ingredients from the Ginseng root. In our study, we first screened several columns using high and low pH as well as methanol and acetonitrile to develop an optimized methodology. We then extracted the ginsenosides from the roots of commercially grown American Ginseng and then used reversed-phase liquid chromatography (RPLC) to separate and isolate the components. We know that more than 30 components have been found in Ginseng roots, and we identified the most significant components using both HPLC-mass spectrometry (MS) and HPLC-evaporative light scattering detection (ELSD). The analytical method was scaled up to preparative chromatography and a fraction was collected. These results indicate that effective method development, multiple detection methods and chromatographic instrumentation are all important parameters in obtaining an optimized purification process.

GINSENOSIDE STRUCTURES



Ginsenoside	R_1	R_2	R_3
Rb_1	Glc-Glc-	H	Glc-Glc-
Rb_2	Glc-Glc-	H	Ara(p)-Glc-
Rc	Glc-Glc-	H	Ara(f)-Glc-
Rd	Glc-Glc-	H	Glc-
Rg_1	H	Glc-O-	Glc-

SCOUTING CONDITIONS

Columns: Atlantis® dC_{18}, 4.6 x 150 mm, 5 µm
SunFire™ C_{18}, 4.6 x 150 mm, 5 µm
XBridge™ C_{18}, 4.6 x 150 mm, 5 µm
XBridge™ Shield RP_{18}, 4.6 x 150 mm, 5 µm
XBridge™ Phenyl, 4.6 x 150 mm, 5 µm
Mobile Phase A1: Water with 0.1% NH_4OH (High pH)
Mobile Phase A1: Water with 0.1% HCOOH (Low pH)
Mobile Phase B1: MeOH
Mobile Phase B2: MeCN
Flow Rate: 1.0 mL/min
Gradient: 20-min gradient from 5% to 100% B, then 10-min hold at 100% B
Sample Concentration: 60 µg/mL each
Sample Diluent: H_2O
Injection Volume: 5 µL
Temperature: Ambient
Detection: ELSD and MS (ESI+ scan 200-1500 m/z)
Instrument: Waters AutoPurification™ system with Waters 2420 ELS detector and Waters Micromass® ZQ™

ELSD Conditions:
Data Ratio: 10 points/sec Gain: 50
Time Constant: 1 sec Gas pressure: 25 psi
Heater Level: 59% Drift Tube Temperature: 49 °C

SCOUTING RESULTS

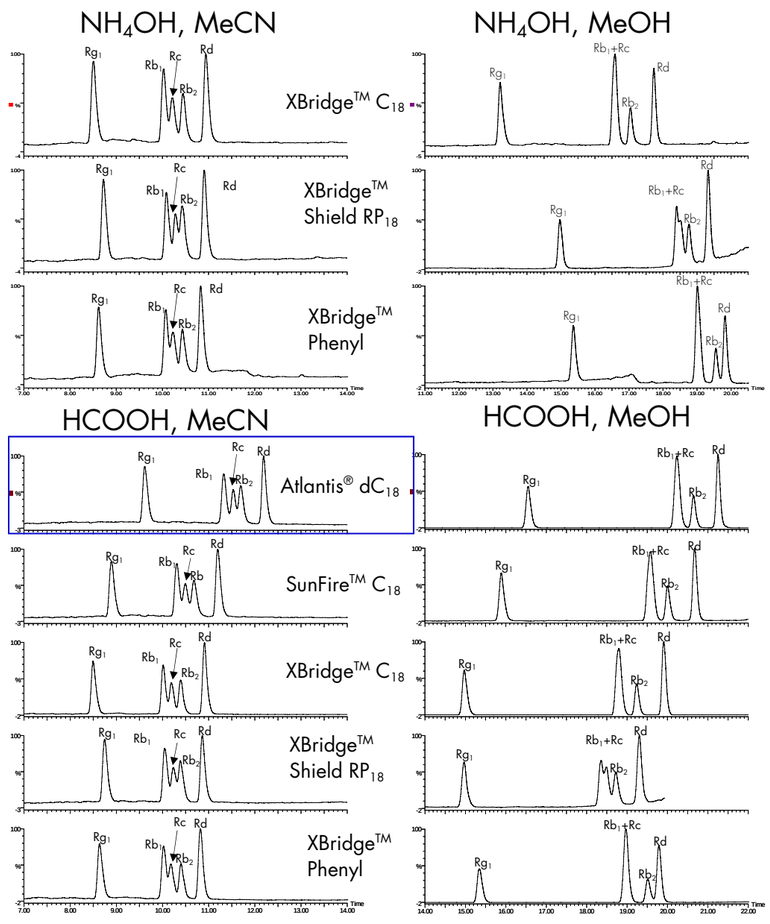


Figure 1. Scouting results on ELSD.

The five ginsenosides exhibit different selectivities under various chromatographic conditions. Atlantis® dC18 with HCOOH, MeCN offers the best set of starting conditions to separate the five ginsenosides.

GINSENOSIDES EXTRACT

- Take Hsu's American Ginseng powder 200 mg
- Add 1 mL 80% MeOH, sonicate for 5 min
- Centrifuge at 10,000 rpm for 5min
- Take the supernatant
- Repeat extraction two more times
- Pool the extracts, mix well

OPTIMIZED GRADIENT

Columns: Atlantis® dC_{18}, 4.6 x 100 mm, 5 µm
Mobile Phase A: Water with 0.1% HCOOH
Mobile Phase B: MeCN with 0.1% HCOOH
Flow Rate: 1mL/min
Gradient:

Time (min)	A%	B%
0	75	25
1	70	30
3	65	35
20	55	45
25	55	45

Sample: Undiluted Ginseng extract
Injection Volume: 5 µL
Detection: MS (ESI+ scan 200-1500 m/z)
Instrument: Waters AutoPurification™ system with Waters 2420 ELS detector and Waters Micromass® ZQ™

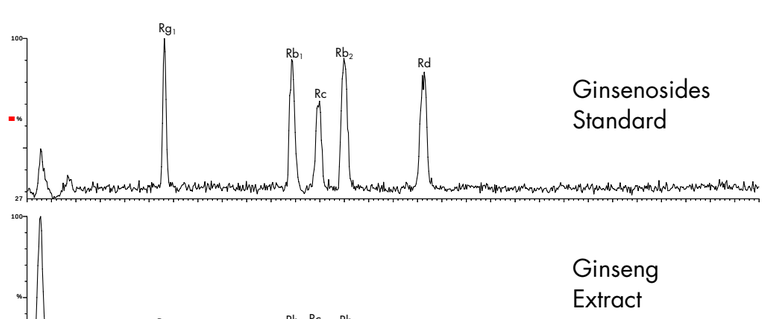


Figure 2. Gradient optimization for ginsenosides standard mixture and ginseng extract on MS.

To shorten the run time, we used a 4.6 x 100 mm Atlantis® dC_{18} column. We can get the best resolution for our target peak: ginsenoside Rb_1.

The ELSD is saturated when the extract was analyzed. Therefore, we used the MS for optimization and purification.

MS Conditions (ESI+ scan 200-1500 m/z)**:**
Capillary Voltage: 3.5 kV Cone Voltage: 200 V
Extractor Voltage: 3 V RF Lens: 0.3 V
Source Temperature: 100 °C
Desolvation Temperature: 250 °C
Desolvation Gas Flow: 500 L/hr
Cone Gas Flow: 50 L/hr
LM Resolution: 15.0 HM Resolution: 15.0
Ion Energy: 1.0 Multiplier: 650

ANALYTICAL LOADING STUDY AND PREPARATIVE FRACTION COLLECTION

Columns: Atlantis® dC_{18}, 4.6 x 100 mm, 5 µm
Atlantis® Prep OBD™ dC_{18}, 19 x 100 mm, 5 µm
Mobile Phase A: Water with 0.1% HCOOH
Mobile Phase B: MeCN with 0.1% HCOOH
Flow Rate: 1mL/min for analytical and 17 mL/min for prep
Gradient:

Anal Time (min)	Prep Time (min)	A%	B%
0	0	75	25
1	2.65	70	30
3	4.65	65	35
20	21.65	55	45
25	26.65	55	45

Sample: Undiluted Ginseng extract
Injection Volume: 10 µL for analytical and 170 µL for preparative
Detection: MS (ESI+ scan 200-1500 m/z)
Instruments: Waters AutoPurification™ system with Waters 2420 ELS detector and Waters Micromass® ZQ™

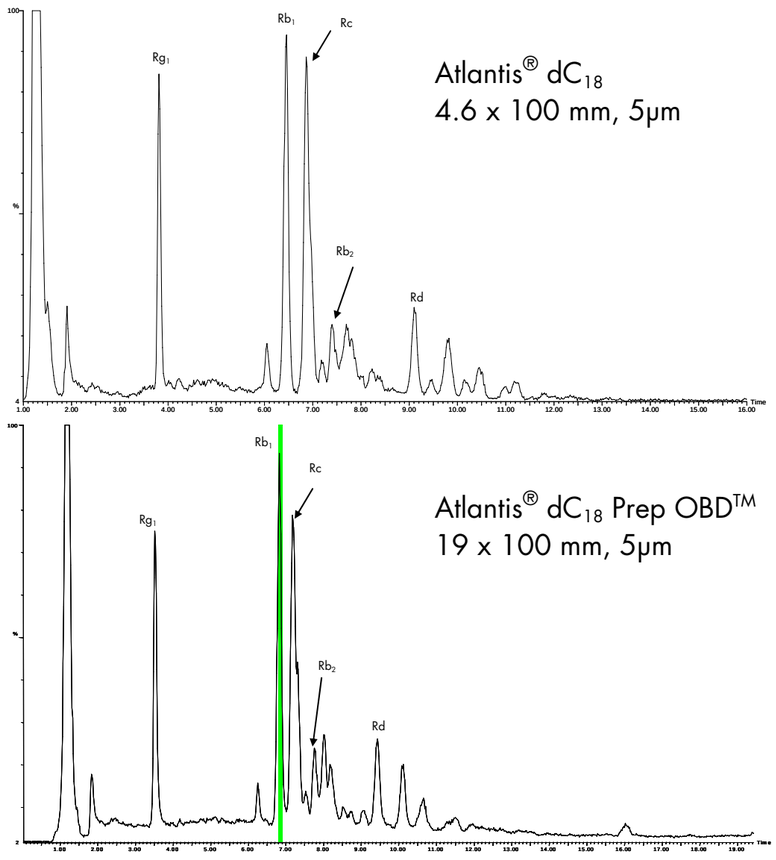


Figure 3. Analytical and preparative chromatography of Ginseng extract and fraction collection of ginsenoside Rb_1.

CONCLUSIONS

- Five ginsenosides from Ginseng extract were identified and separated. Ginsenoside Rb_1 was collected from the Atlantis® dC_{18} column using MS directed fraction collection.
- An approach to a successful separation was developed.
  - Run scouting study to select column chemistry and mobile phase conditions
  - Optimize the chromatographic conditions to get best separation on analytical column
  - Perform a loading study on analytical column
  - Scale from analytical to preparative columns
- The MS directed fraction collection system enables compound specified fraction collection.

TO DOWNLOAD A COPY OF THIS POSTER VISIT WWW.WATERS.COM/POSTERS