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 (RPIC) 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

<table>
<thead>
<tr>
<th>GINSENOside</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rb1</td>
<td>Glc-Glc</td>
<td>H</td>
<td>Glc-Glc</td>
</tr>
<tr>
<td>Rb2</td>
<td>Glc-Glc</td>
<td>H</td>
<td>Ara(p)-Glc</td>
</tr>
<tr>
<td>Rc</td>
<td>Glc-Glc</td>
<td>H</td>
<td>Ara(f)-Glc</td>
</tr>
<tr>
<td>Rd</td>
<td>Glc-Glc</td>
<td>H</td>
<td>Glc</td>
</tr>
<tr>
<td>Rg1</td>
<td>H</td>
<td>Glc-O</td>
<td>Glc</td>
</tr>
</tbody>
</table>

SCOUTING CONDITIONS

Columns: Atlantis® dC18, 4.6 x 150 mm, 5 μm
XBridge™ Shield RP 18, 4.6 x 150 mm, 5 μm
XBridge™ C18, 4.6 x 150 mm, 5 μm
SunFire™ C18, 4.6 x 150 mm, 5 μm
Instrument: Waters AutoPurification™ system with Waters 2420 ELS detector and Waters Micromass® ZQTM
Flow Rate: 1mL/min for analytical and 17 mL/min for prep
Mobile Phase B: MeCN with 0.1% HCOOH
Sample: Undiluted Ginseng extract
Injection Volume: 10 μL for analytical and 170 μL for preparative
Detection: MS (ESI+ scan 200-1500 m/z)
Source Temperature: 100 °C
Desolvation Gas Flow: 500 L/hr
Drift Tube Temperature: 49 °C
Multiplier: 650
Drift Energy: 1.0
Multiplier: 650

MS Conditions (ESI+ scan 200-1500 m/z)
Capillary Voltage: 3.5 kV
Source Temperature: 100 °C
Desolvation Gas Flow: 500 L/hr
Drift Tube Temperature: 49 °C

ANALYTICAL LOADING STUDY AND PREPARATIVE FRACTION COLLECTION

Columns: Atlantis® dC18, 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) | Prep Anal  | A% | B%
--- | --- | --- | ---
0 | 75 | 25 |
1 | 70 | 30 |
2 | 65 | 35 |
3 | 60 | 40 |
4 | 55 | 45 |
5 | 55 | 45 |

Sample: Undiluted Ginseng extract
Injection Volume: 5 μL
Detection: MS (ESI+ scan 200-1500 m/z)
Instruments: Waters AutoPurification™ system with Waters 2420 ELS detector and Waters Micromass® ZQTM
ION ENERGY: 1.0
MULTIPLIER: 650

CONCLUSIONS

• Five ginsenosides from Ginseng extract were identified and separated. Ginsenoside Rb1 was collected from the Atlantis® dC18 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.

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