Waters™

Application Note

Analysis of Herbal Medicine on an XBridge HPLC Amide Column

Waters Corporation

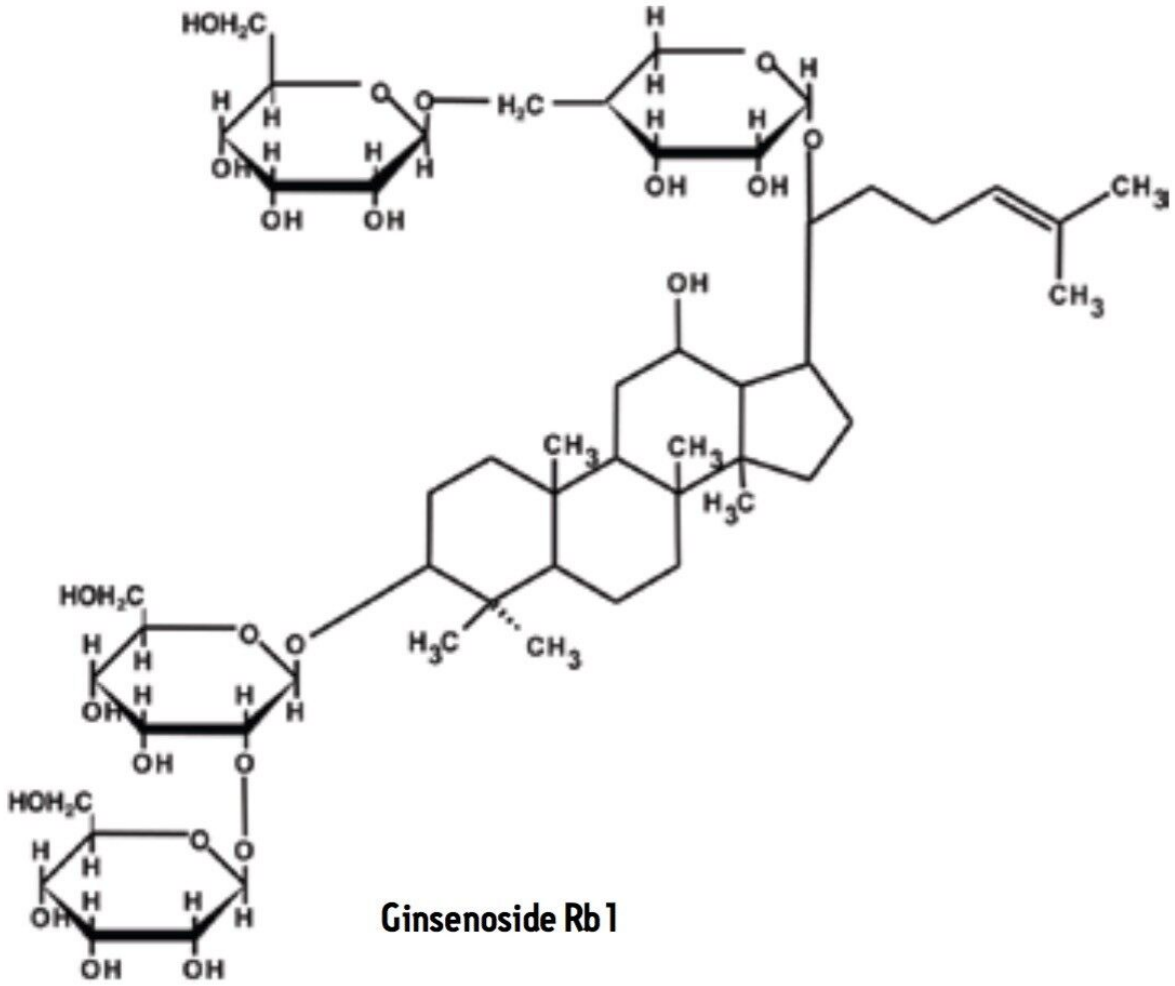
This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates analysis of herbal medicine on an XBridge HPLC amide column.

Introduction

Compound



Experimental

HPLC Conditions

Column:	XBridge Amide, 3.5 µm, 4.6 x 250 mm
Part Number:	186004870
Mobile Phase:	80:20 MeCN:H_2O
Isocratic Flow Rate:	0.8 mL/min
Column Temp.:	60 °C
Sample Temp.:	10 °C
Injection Vol.:	20 µL
Needle Wash:	95:5 MeCN:H_2O
Seal Wash:	10:90 MeOH:H_2O
UV:	203 nm
Sampling Rate:	20 Hz
Filter Time Constant:	0.1 sec
Total Run Time:	18 min
Instrument:	Alliance 2695 and 2998 PDA

Pretreatment

1. Weigh 2 g of herbal medicine powder into a centrifuge tube.
2. Add 30 mL of 60% MeOH/40% H_2O.

3. Shake for 15 min.
4. Centrifuge at 4,000 rpm for 10 min.
5. Obtain the supernatant.
6. Repeat steps 2-5 with the residue using 15 mL of 60% MeOH/40% H_2O.
7. Combine the supernatant, and make exactly 50 mL by adding 60% MeOH/40% H_2O.
8. Take 10 mL of this solution and add 3 mL of NaOH test solution (1 mol/L).
9. Let stand for 30 min.
10. Add 3 mL of HCl test solution (1 mol/L).
11. Add 60% MeOH/40% H_2O to make exactly 20 mL.

Solid-Phase Extraction

SPE Device:	Sep-Pak Plus C_{18} cartridge 360 mg (55-105 µm)
Part Number:	WAT020515

1. Condition with 2 mL MeOH.
2. Equilibrate with 2 mL of 30% MeOH/70% H_2O just before loading.
3. Load 5 mL of the solution from step 11 in the pretreatment stage.
4. Wash with 2 mL of 30% MeOH/70% H_2O.
5. Wash with 1 mL of Na_2CO_3 test solution (1 mol/L).
6. Wash with 10 mL of 30% MeOH/70% H_2O.
7. Elute with 5 mL MeOH (this is the injection solution).

Featured Products

Alliance HPLC System <https://www.waters.com/534293>

2998 Photodiode Array (PDA) Detector <https://www.waters.com/1001362>

WA64084, February 2010