Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

ANALYSIS OF HERBAL MEDICINE ON AN ACQUITY UPLC BEH AMIDE COLUMN

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UPLC CONDITIONS

Column:	ACQUITY UPLC® BEH Amide, 1.7 µm, 2.1 x 100 mm
Part Number:	186004801
Mobile Phase:	80:20 MeCN:H_2O
Isocratic Flow Rate:	0.6 mL/min
Column Temp.:	60 °C
Sample Temp.:	10 °C
Injection Vol.:	1.7 µL; PLNO on 10 µL loop
Strong & Weak Needle Wash:	95:5 MeCN:H_2O
Seal Wash:	10:90 MeOH:H_2O
UV:	203 nm
Sampling Rate:	20 Hz
Filter Time Constant:	0.2 sec
Total Run Time:	2.5 min
Instrument:	ACQUITY UPLC with ACQUITY UPLC PDA

PRETREATMENT

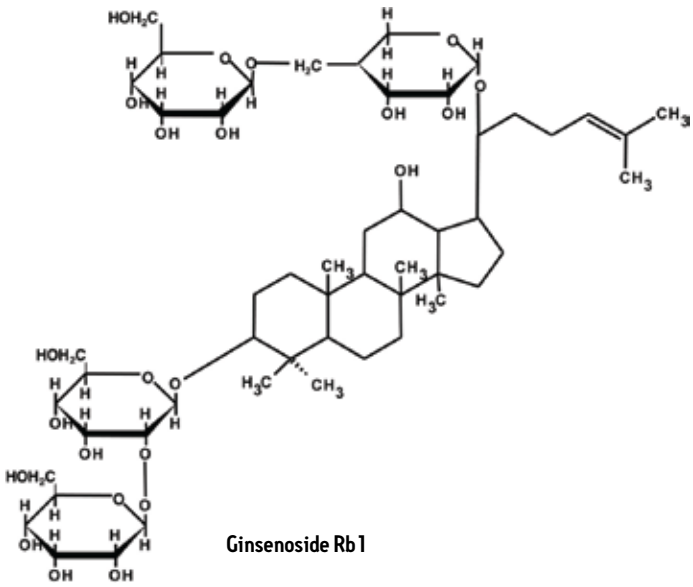
1. Weigh 2 g of herbal medicine powder into a centrifuge tube.
2. Add 30 mL of 60% MeOH/40% H_2O.
3. Shake for 15 min.
4. Centrifuge at 4,000 rpm for 10 min.
5. Obtain the supernatant.
6. Repeat steps 2-5 with the residue using 15 mL of 60% MeOH/40% H_2O.
7. Combine the supernatant, and make exactly 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 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).

COMPOUND



Ginsenoside Rb1