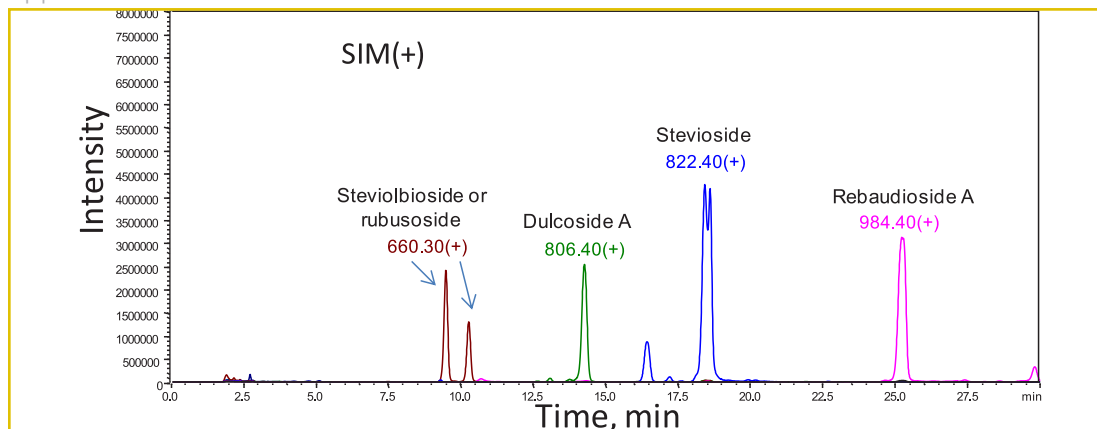




LC-MS Analysis of Stevia Extract on HALO® Penta-HILIC, 5 µm

Application Note 124-F



Stevia is a natural sweetener and is used as a substitute for sugar. LC/MS analysis of Stevia glycosides from a Stevia extract is easily accomplished using a HALO® Penta-HILIC, 5 µm column due to its unique bonded phase containing five OH groups and the high efficiency of the 5-micron Fused-Core® particles.

TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 5 µm,
3.0 x 250 mm
Part Number: 95813-905
Mobile Phase:
A: 50/50 water/acetonitrile with 5 mM ammonium formate, pH 3.0
B: 5/95 water/acetonitrile with 5 mM ammonium formate, pH 3.0
Gradient: 90% B to 67% B in 30 min
Flow Rate: 0.5 mL/min
Pressure: 60 bar
Temperature: Ambient
Injection Volume: 5.0 µL
Sample Solvent: 80/20 acetonitrile/water
LC System: Shimadzu Nexera
MS: Shimadzu LCMS 2020 (single quadrupole)
ESI: +4.5 kV
Scan Range: 200-1200 m/z
Scan Rate: 2 pps
Capillary: 250 °C
Heat Block: 350 °C
Nebulizing Gas Flow: 1.5 L/min
Drying Gas Flow: 15 L/min

EXTRACTION PROCEDURE:

1. Weigh 400 mg of Stevia rebaudiana leaves (Sigma S5381)
2. Crush leaves with mortar and pestle and transfer to vial
3. Add 8.0 mL of 50/50 (v/v) acetonitrile/water
4. Sonicate vial contents for 15 minutes
5. Filter sample using 25 mm syringe filter having 0.2 µm PTFE membrane (VWR 28145-495)
6. Centrifuge @ 10K rpm (5 min) and collect supernate
7. Dilute 400 µL of extract in 600 µL of acetonitrile for overall concentration of 80/20 acetonitrile/water
8. Centrifuge diluted sample @ 10K (5 min.) rpm and inject the supernate

