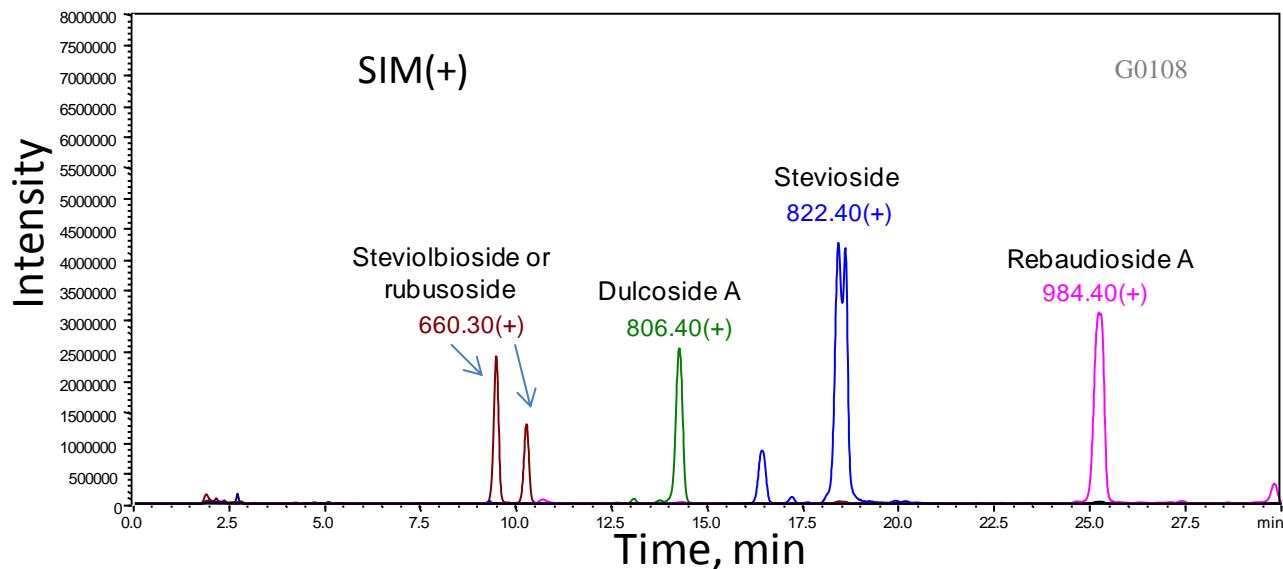


## LC-MS Analysis of Stevia Extract on HALO-5 Penta-HILIC



### TEST CONDITIONS:

Column: 3.0 x 250 mm, HALO-5 Penta-HILIC  
 Part Number: 95813-905  
 Mobile Phase:  
 A= 50/50 Water/acetonitrile with 5 mM Ammonium formate, pH 3  
 B= 5/95 Water/acetonitrile with 5 mM Ammonium formate, pH 3  
 Gradient : 90% B to 67% B over 30 min  
 Flow Rate: 0.5 mL/min.  
 Pressure: 60 bar  
 Temperature: Ambient  
 Injection Volume: 5 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 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

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 HALO-5 Penta-HILIC column due to its unique bonded phase containing five OH groups and the high efficiency of the 5-micron Fused-Core particles.