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Diamond Analytics Application Note: DA1016-A

FLARE C18 Mixed-Mode Column: Separation of Ginsenosides Re and Rd by LC-MS

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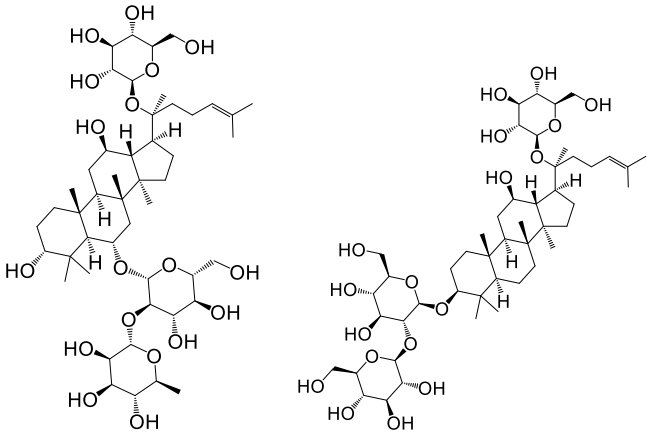
Introduction

Ginseng is a commonly used herb in Asia. In 2010, approximate 80,000 tons of it were produced in Asia (China and South Korea) and North America (Canada and the United States).[1] The worldwide market for ginseng is approximately 2 billion dollars per year. Ginsenosides or panaxosides are the active compounds in ginseng. Liquid chromatography is commonly used to assay the amounts of ginsenosides in ginseng. In this application note, ginsenoside Re and ginsenoside Rd were separated by LC-MS using the FLARE mixed-mode column.

Analytes:



1. Ginsenoside Re (MW: 947.15)
2. Ginsenoside Rd (MW: 947.15)

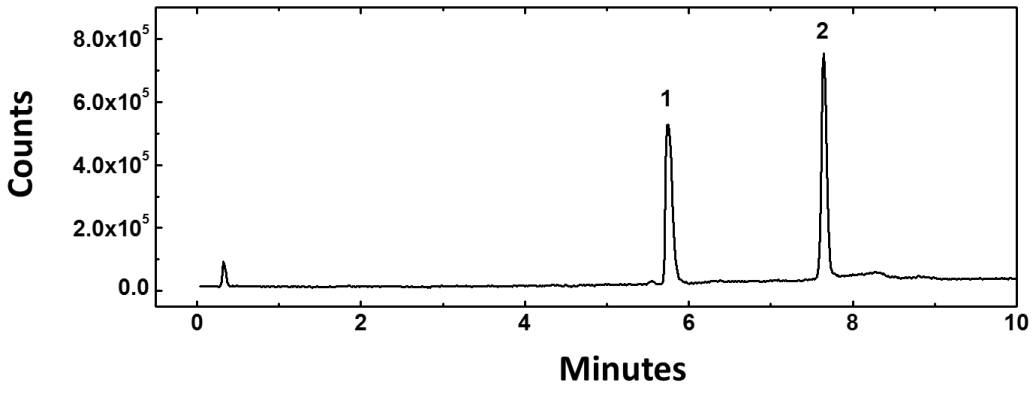


Figure 1. Separations of ginsenoside Re (1) and ginsenoside Rd (2).

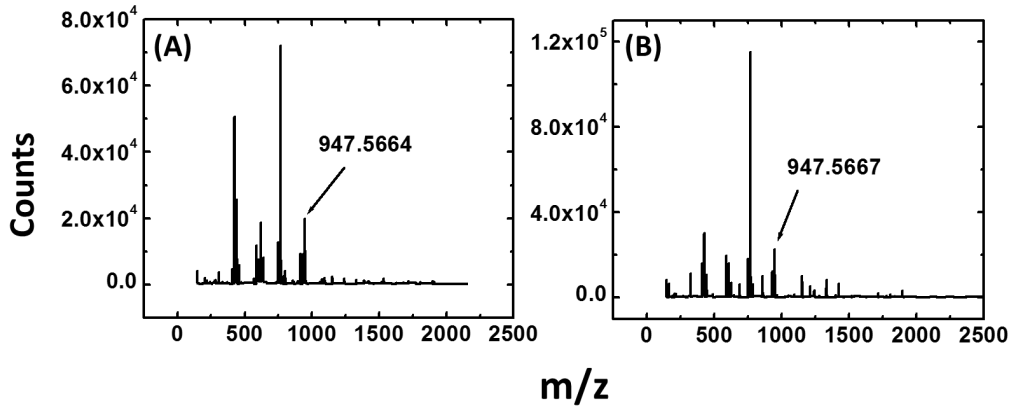


Figure 2. Mass spectra of ginsenoside Re (A) and ginsenoside Rd (B) from the corresponding peaks (1 and 2) in the chromatogram in Figure 1.

The particles in the FLARE C18 mixed-mode (MM) core shell column are made of carbon, nanodiamond, and an amine-based polymer, and are stable at elevated temperature and pH.[2-5] The amine-based polymer in the stationary phase provides it with mixed-mode properties, allowing it to retain analytes via anionic and hydrophobic interactions with a retention mechanism that varies with pH.[2] In this application note, a 2.1 x 50 mm mixed-mode FLARE C18 column was used to separate two ginsenosides using TFA as an additive.

Experimental

HPLC system: Agilent 1260 Infinity coupled with an Agilent 6230 TOF LC-MS in positive ion mode

Analytes: Ginsenoside Re and Rd (ca. 0.5 mg/mL in MeOH)

LC Conditions:

Column dimensions: 2.1 x 50 mm (id: 16598.16-3)

Injection volume: 6.0 µL

Flow rate: 0.4 mL/min

Temperature: 35 °C

A: 1000 mL H_2O + 1 mL TFA

B: 1000 mL ACN + 0.875 mL TFA

Gradient:

0 min	100% A	0% B
15 min	0% A	100% B
15.1 min	100% A	0% B
20 min	End	

MS conditions:

Gas temperature: 300 °C

Drying gas: 5 L/min

Nebuilizer: 35 psig

Capillary voltage: 3500 V

Fragmentor: 170 V

Skimmer: 65 V

OCT: 1Rf

Vpp:750V

References

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[3] Hung C-H, Wiest LA, Singh B, Diwan A, Valentim MJC, Christensen JM, et al. Improved Efficiency of Reversed-Phase Carbon/Nanodiamond/Polymer Core-Shell Particles for HPLC Using Carbonized Poly(divinylbenzene) Microspheres as the Core Materials. Journal of Separation Science. 2013;36(24):3821-9.

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