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INTRODUCTION

The ACQUITY UPLC® BEH Amide column utilizes an amide phase that is trifunctionally bonded to an ethylene bridged hybrid (BEH) particle. This facilitates the use of a wide range of mobile phase pH in order to retain polar analytes using hydrophilic-interaction chromatography (HILIC). In this application, simple and complex carbohydrates, which are often analyzed by HILIC, are separated on the BEH Amide column and then directly detected with electrospray ionization mass spectrometry (ESI-MS) with no need for derivatization, post-column addition, or metal complexing agents.

EXPERIMENTAL

System: Waters ACQUITY UPLC System with ACQUITY® SQD
Column: ACQUITY UPLC BEH Amide, 1.7 mm, 2.1 x 50 mm
Part Number: [186004800](#)
Mobile Phase A: 70/30 H₂O/MeCN with 0.1% NH₄OH
Mobile Phase B: 20/80 H₂O/MeCN with 0.1% NH₄OH
Gradient: 0 to 60% A in 5 min
Flow Rate: 0.17 mL/min
Injection: 0.7 µL (PLNO mode)
Temperature: 35 °C
Sample: Each saccharide is present at 10 µg/mL in 50/50 MeCN/H₂O
Ionization Mode: ES⁻, selected ion recording (SIR)
Capillary: 2.8 kV
Cone: 25 V
Dwell time: 80 ms

RESULTS AND DISCUSSION

Figure 1 shows the separation of 9 different simple and complex carbohydrates that are detected by MS. The use of 0.1% NH₄OH in the mobile phase is crucial for collapsing the anomers of the reducing sugars and simplifying the separation. This method is useful for the sensitive detection of saccharides in commercial food products, and for monitoring fermentation procedures in process development and

production. The limit of detection (LOD) for saccharides using MS is 1 to 5 µg/mL (ppm), which is approximately 10-fold better than evaporative light scattering (ELS) and 100-fold better than refractive index (RI) detection.

CONCLUSIONS

The superior chemical stability of the ACQUITY UPLC BEH Amide column enables direct ESI-MS detection of simple and complex carbohydrates at high pH, thus allowing direct identification of these analytes in commercial food and beverage products and other difficult matrices.

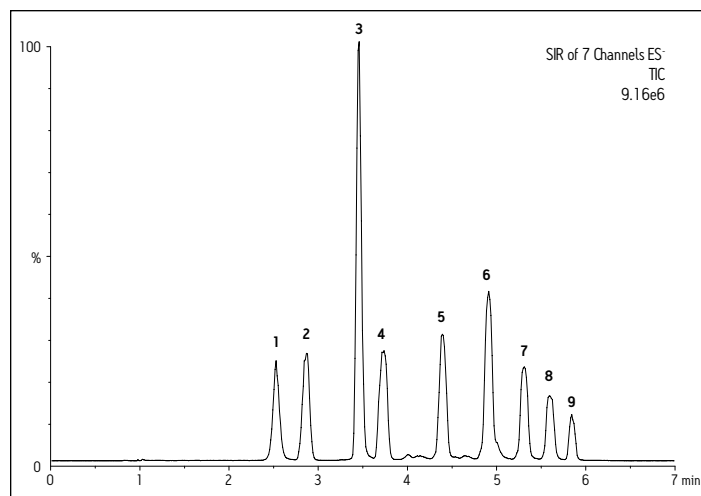


Figure 1: UPLC-MS separation of mono-, di-, and oligosaccharides. Peaks: (1) fructose, (2) glucose, (3) sucrose, (4) maltose, (5) maltotriose, (6) maltotetraose, (7) maltopentaose, (8) maltohexahose, (9) maltoheptaose. SIR *m/z* 179.2 (fructose, glucose), 341.3 (sucrose, maltose), 503.4, 665.5, 827.6, 989.7, 1151.8 (maltooligosaccharides; *n* = 1 to 5).

ORDERING INFORMATION

Products Used in this Application	Part Number
ACQUITY UPLC BEH Amide, 1.7 mm, 2.1 x 50 mm	186004800

For the complete UPLC application note, visit www.waters.com and search for: [WA64081](#)