## Waters™



# Separation of Organic Acids with Mixed-Mode LC Column and Mass Detector

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This is an Application Brief and does not contain a detailed Experimental section.

#### Abstract

This application brief demonstrates the capability of Waters Atlantis PREMIER BEH C<sub>18</sub> AX Column and the ACQUITY QDa Mass Detector for the analysis of organic acids in juices.

#### **Benefits**

Waters Atlantis PREMIER BEH C<sub>18</sub> AX Column and ACQUITY QDa Mass Detector provide fast analysis of organic acids.

## Introduction

Organic acids (OA) are an important group of compounds that are often tested for the quality and authenticity of beverages, including fruit juice, wine, and beer, and for patient health as diagnostic biomarkers. The analysis of OA is mainly carried out by LC with anion-exchange, reversed-phase  $C_{18}$ , or mixed-mode columns. The anion-exchange chromatography of OA has an excellent separation but run time is often long (about 40 min). The  $C_{18}$ 

column has limited retention for OA, and the separation efficiency is low. The mixed-mode LC column has a better retention and good separation efficiency for OA, and the run time is shorter than the anion-exchange columns. The nonselective detectors that are often used in the OA analysis, such as the UV/Vis detector for the mixed-mode and the reversed-phase columns, and the conductivity detector for anion-exchange columns, are prone to interference from sample matrix. A better solution is needed for the analysis of OA.

## Results and Discussion

Waters Atlantis PREMIER BEH C<sub>18</sub> AX Columns and Waters ACQUITY QDa Mass Detector offer an excellent solution for the OA routine analysis. The Atlantis PREMIER BEH C<sub>18</sub> AX Column is a mixed-mode column that offers good retention of polar compounds like OA. ACQUITY QDa Mass Detector offers a highly selective detection of ions. Analytes with different nominal masses can be detected in different selected ion recording (SIR) channels with no interference from other compounds. This makes the integration and quantitation of analytes easy and reliable. Closely eluting OA, such as quinic acid and tartaric acid, are no longer required to be baseline separated for accurate and reliable quantitation. Figure 1 shows chromatograms of 11 SIR channels that detect 15 OAs. Some of the OAs are in the same SIR channels but are baseline separated in the chromatograms. Total run time is 7 min, which is about one sixth of a typical 40-min run time by an anion-exchange column. Figure 2 shows the 11 SIR chromatograms of a 100% orange juice. Due to the complexity of the sample, some minor peaks in Figure 2 have not been identified yet.

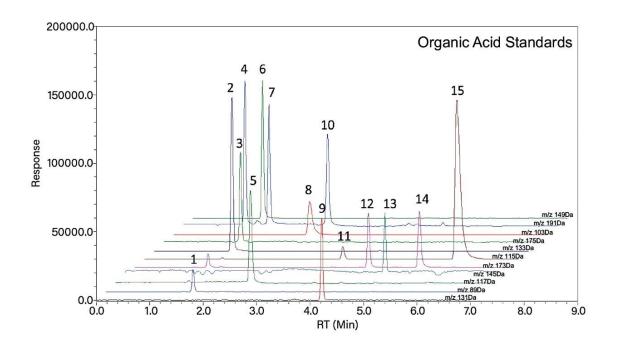


Figure 1. Chromatograms of 15 organic acid standards in 11 SIR channels. Organic acid concentration: 20 ppm. Column: Atlantis PREMIER BEH C<sub>18</sub> AX, 1.7 μm, 2.1 x 100 mm. Peaks ID: 1. Lactic acid; 2. Malic acid; 3. Ascorbic acid; 4. Quinic acid; 5. Succinic acid; 6. Tartaric acid; 7. Isocitric acid; 8. Malonic acid; 9. Glutaric acid; 10. Citric acid; 11. Fumaric acid; 12. cis-Aconitic acid; 13. Adipic acid; 14. Shikimic acid; 15. Maleic acid.

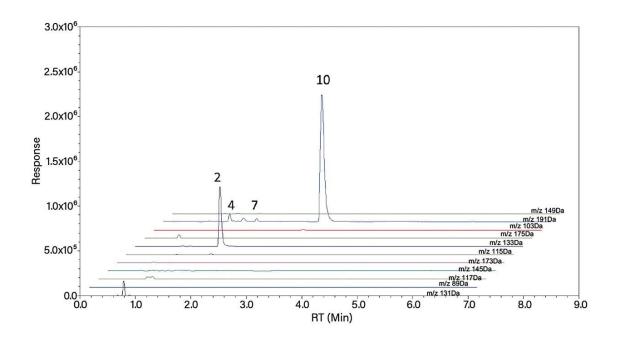


Figure 2. SIR chromatogram overlay of a 100% orange juice. The 100% orange juice was obtained from a local grocery store. The juice was filtered and diluted 100 times with deionized water before it was analyzed. Peak IDs are the same as in Figure 1. Peak: 2. Malic acid; 4. Quinic acid; 7. Isocitric acid; 10. Citric acid.

### Conclusion

Waters Atlantis PREMIER BEH  $C_{18}$  AX Column and ACQUITY QDa Mass Detector provide a fast analysis of organic acids in juices. Atlantis PREMIER BEH  $C_{18}$  AX Column has good retention and separation efficiency for organic acids, and the ACQUITY QDa offers highly selective detection of similar and closely eluting organic acids. Due to less interference from other compounds in the SIR chromatogram, the peak integration and quantitation are more accurate than the non-selective detectors, such as UV/Vis detector or RI detector. This solution offers a fast analysis of organic acids in complex sample matrices.

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