



Sensitive, High Resolution Mass Spec Analysis of Complex Pharmaceutical Mixtures after UHPLC Separation

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In the pharmaceutical industry the increased efficiency brought about by new fast chromatography methods needs to be matched by commensurate mass spec capabilities. maXis UHR-TOF is the instrument of choice to keep up with fastest chromatography.

Many high performance accurate mass and orbital trapping MS systems are unable to provide sufficient high resolution data at fast chromatography speeds. Fast separation technology can significantly reduce the time required to analyze a given sample. Chromatography methods can be reduced sometimes by an order of magnitude. These short run times produce chromatograms with peak widths of less than a few seconds wide. The new maXis system is the only high performance MS system capable of operating with those fast run times without sacrificing resolution or sensitivity. The maXis UHR-TOF is the only mass spec capable of providing high resolution, highly accurate results with UHPLC.

Experimental

UHPLC System:

- Waters Acquity UPLC with DAD detector (210–400 nm)
- Acquity BEH C18 1.7 μm 2.1 \times 50 mm
- Column Temperature: 40 $^{\circ}\text{C}$

Mobile phase:

- 0.1% Formic Acid in Water
- 0.1% Formic Acid in ACN

Gradient:

- 20%–40% B in 60 s
- 40%–100% B in 5 s
- 100% B for 172 s *followed by column equilibration

Flow Rate: 0.6 mL/min

Injection volume: 2 μL

Technology:

- UHR-TOF
- Dual Ion Funnel
- Ion Cooler Technology
- Dual State Reflector

Results

In this study, the maXis was challenged to identify a series of unknown drug compounds that had been separated by UHPLC. Using a typical reverse phase C18 gradient separation method, 7 of the 10 compounds were base line separated by the UHPLC

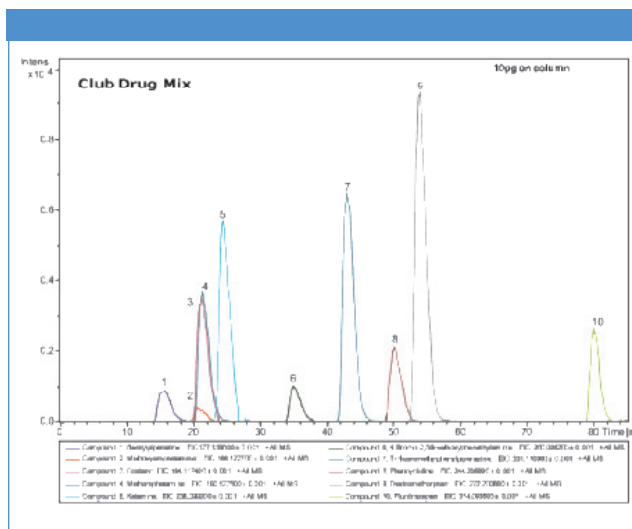


Figure 1: Extracted ion chromatograms of $\pm 1\text{mDa}$ at acquisition speed of 10 spectra per s analyzed under 85 s.

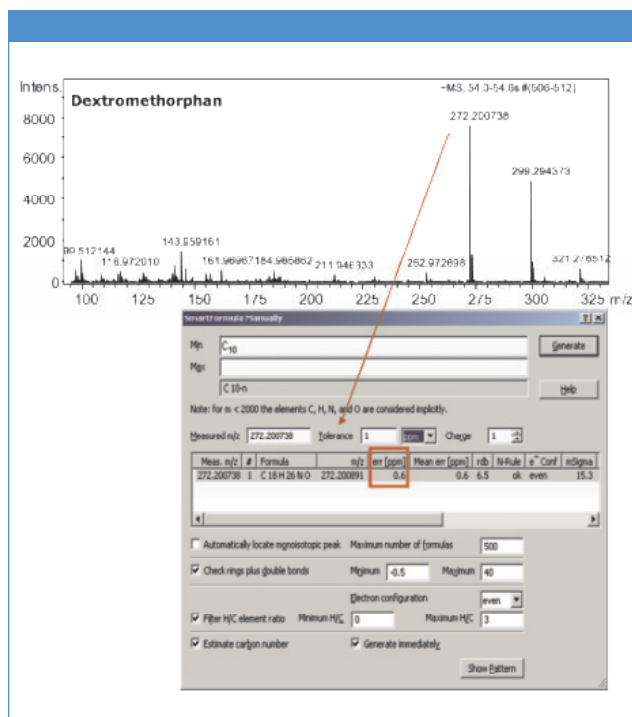


Figure 2: maXis™ and SmartFormula™ routinely deliver confidence in molecular identification with unsurpassed sub-ppm accuracy.

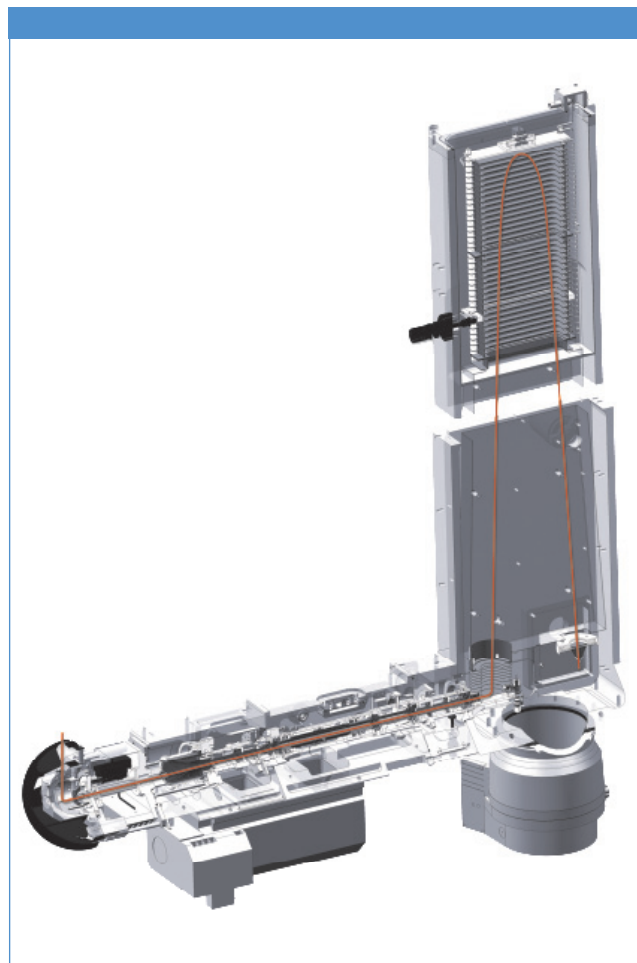


Table I: MS system: Bruker Daltonics maXis™

Spectral Acquisition	10 full spectra per s
Dry Heater	180 °C
Nebulizer	2 Bar
Capillary	4500 V
Dry Gas	6 L/min
ESI	Positive polarity
Range	100–800 m/z
Calibrant	Lithium Formate (16 mM)
LockMass Calibration	methylstearate

in 85 s at 10 Hz sampling rate. The maXis was then able to discern the presence of 10 compounds in the sample, despite the lack of chromatographic separation of 3 of the compounds, even at levels as low as 10 pg on column. Furthermore, the excellent mass accuracy (<1 ppm) and resolution (Avg ~50 K) of the results generated by the maXis, when used in conjunction with the SmartFormula 3D software, were able to utilize the data to correctly identify all 10 compounds and unequivocally determine their molecular formula. This is exceptional performance from a qTOF, especially with a UHPLC speed separation.

Conclusion

Bruker Daltonics has developed a new state of the art mass spectrometer, the maXis, capable of keeping pace with the current demands of high-throughput analysis without sacrificing accuracy, sensitivity, or resolution. In this study, the maXis was capable of delivering:

- Mass Accuracy of 1ppm or less for typical pharmaceutical compounds
- Extracted Ion Chromatograms with $\pm 1\text{mDa}$ window
- 20 Spectra per s
- 40,000 to 60,000 Resolution

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