



## Application Note # ET-15

# Analysis of Complex Pharmaceutical Mixtures by maXis with Fast Chromatography

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 peaks 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. Here we demonstrate the typical results that can be achieved by the combination of fast chromatography with the maXis to analyze a complex pharmaceutical sample.

### Experimental

UHPLC System:

- Waters Acquity UPLC with DAD detector (210-400 nm)
- Acquity BEH C18 1.7  $\mu$ m 2.1x50 mm
- Column Temperature: 40  $^{\circ}$ C

Mobile phase:

- A) 0.1% Formic Acid in Water
- B) 0.1% Formic Acid in ACN

Gradient:

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

Flow Rate: 0.6 mL/min

Injection volume: 2  $\mu$ L

## High resolution extracted ion chromatograms of the sample mixture

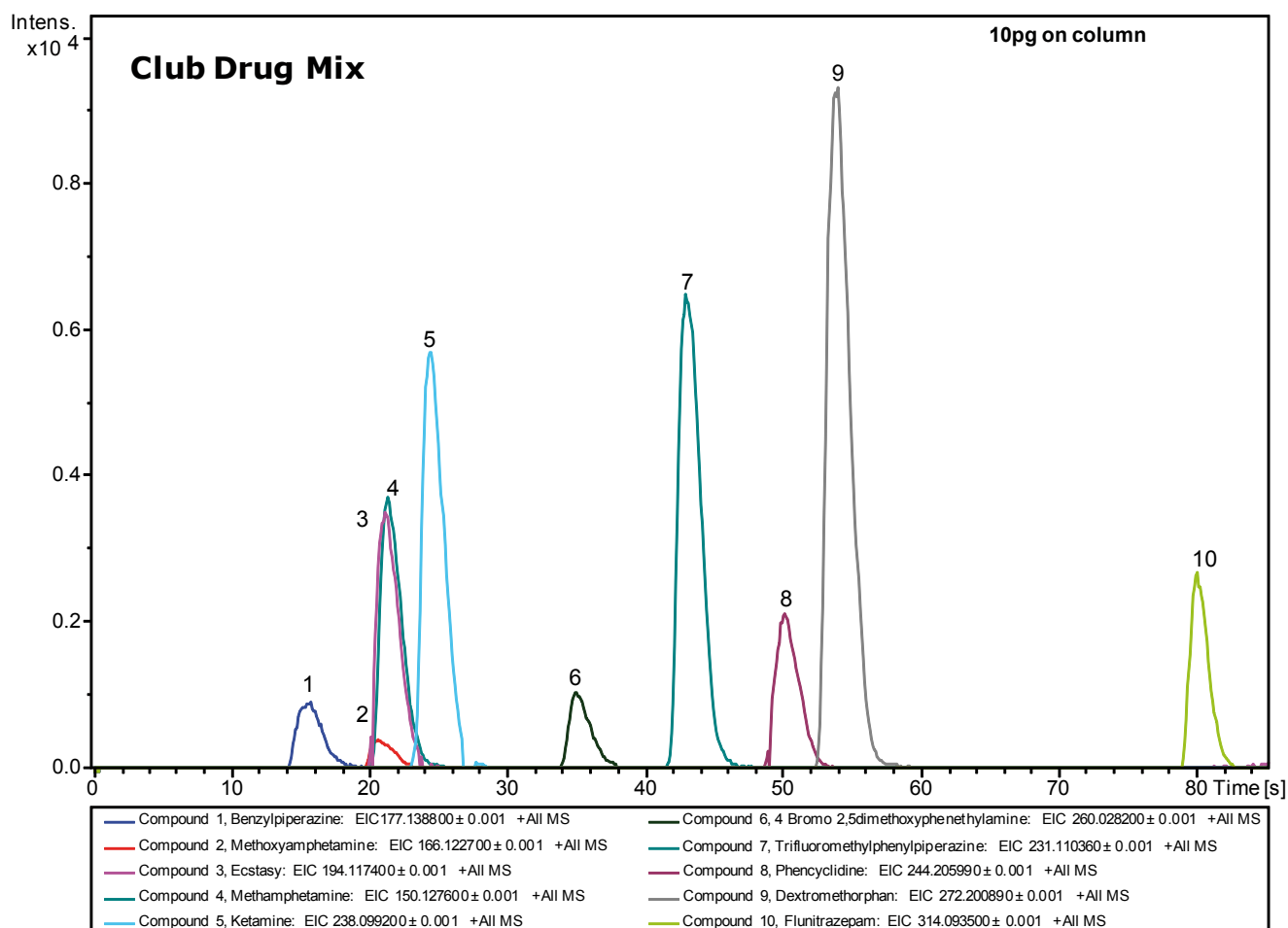


Fig. 1: Extracted ion chromatograms of  $\pm 1$  mDa at acquisition speed of 10 spectra per second analyzed in less than 85 seconds.

### MS System: Bruker Daltonics maXis™

Spectral Acquisition	10 full spectra per second
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 (16mM)
LockMass Calibration	methylstearate

### Technology

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

## High confidence molecular formula determination

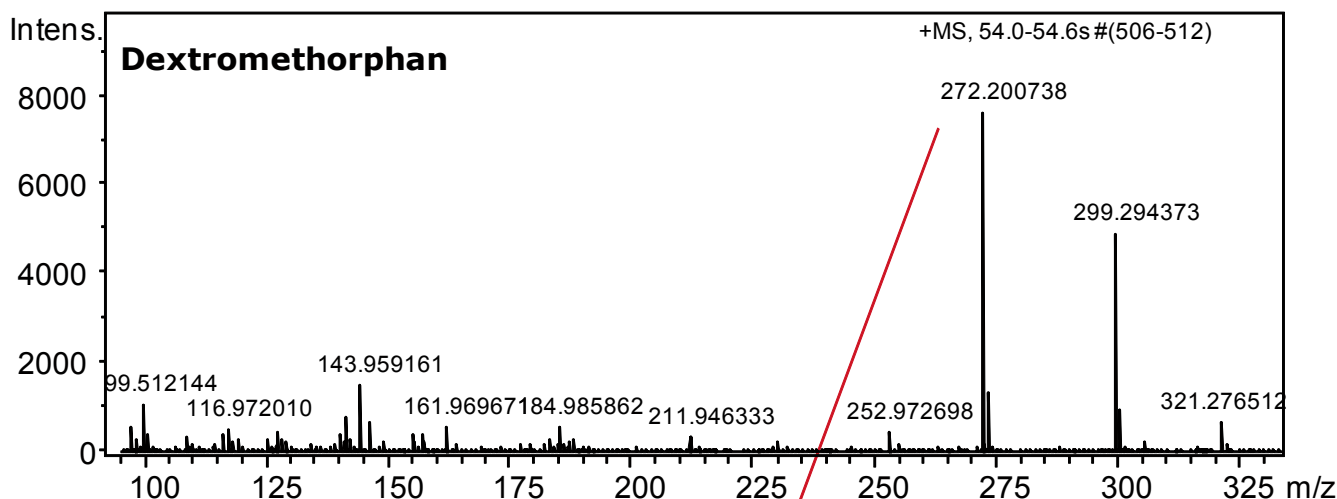


Fig. 2: maXis™ and SmartFormula™ routinely deliver confidence in molecular identification with unsurpassed sub-ppm accuracy.

**SmartFormula Manually**

Min:  Max:

Note: for m < 2000 the elements C, H, N, and O are considered implicitly.

Measured m/z:  Tolerance:  ppm Charge:

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdB	N-Rule	e <sup>-</sup> Conf	mSigma
272.200738	1	C <sub>18</sub> H <sub>26</sub> N <sub>2</sub> O	272.200891	0.6	0.6	6.5	ok	even	15.3

☐ Automatically locate monoisotopic peak Maximum number of formulas:

☒ Check rings plus double bonds Minimum:  Maximum:

Electron configuration:

☒ Filter H/C element ratio Minimum H/C:  Maximum H/C:

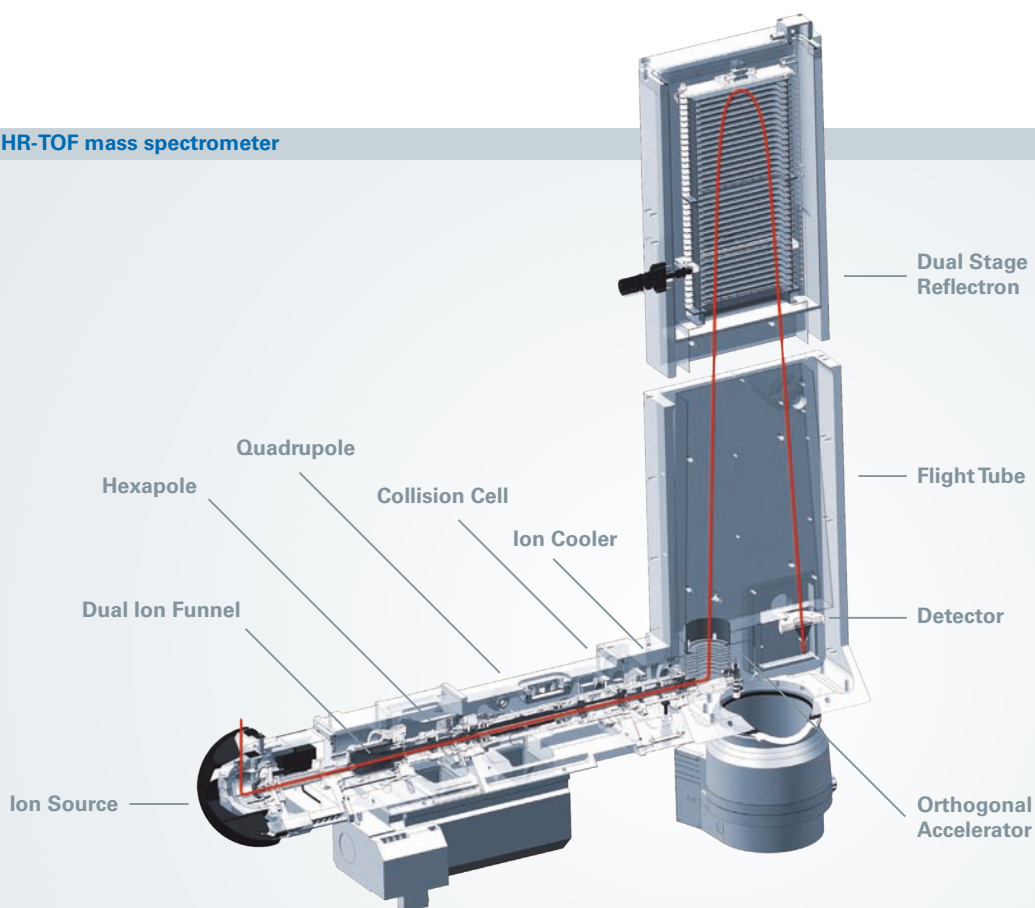
☒ Estimate carbon number ☒ Generate immediately

## 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 in 85 seconds 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 three 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 Q-TOF, especially with a UHPLC speed separation.

## maXis UHR-TOF mass spectrometer



## 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 second
- 40,000 to 60,000 Resolution

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molecular formula  
determination

### Instrumentation & Software

maXis  
SmartFormula

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