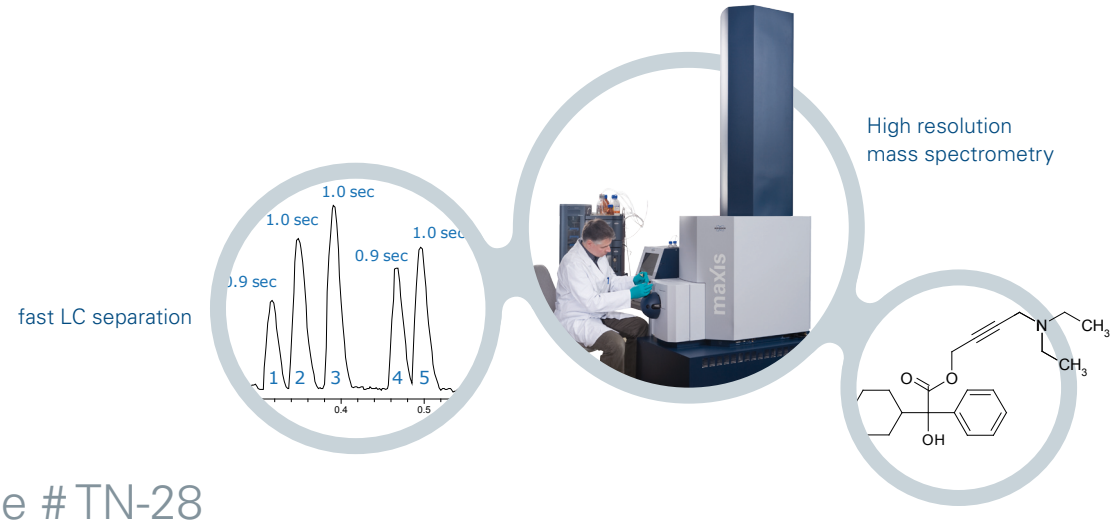
Bruker Daltonics



Technical Note # TN-28

maXis High Resolution LC/MS Makes the Most of Ultrafast LC Separations

Abstract

An ultrafast gradient LC separation method was developed to separate a 5-component drug mixture in 30 seconds, with peak widths of 1 second. maXis mass accuracy at sub-ppm levels and true isotopic pattern of the spectra from the peaks leads to a confident elemental formula assignment for each drug compound with the SmartFormula™ algorithm.

Introduction

Modern discovery applications demand definitive MS results on ever more complex samples. maXis is the only mass spectrometer able to deliver the maximum MS performance specification at the very highest speeds made available by modern ultrafast liquid chromatography. Here, a complete gradient LC separation of typical drug molecules is achieved in 30 seconds, with each component detected with sub-ppm mass accuracy.

Experimental

A drug mixture (norharmane, sulfamethazine, sulfamethoxazole, oxybutin and terfenadine) was separated with a Waters Acquity UPLC™ system with a 1x50mm Acuity BEH C18 column at using a flow rate of 0.5 mL/min and a gradient from 4% acetonitrile to 95% acetonitrile in 0.5mins. The maXis™ electrospray LC/MS dataset was acquired with an acquisition rate of 10Hz. The dataset was automatically recalibrated in the Compass Data Analysis™ 4.0 processing software with several masses of salt clusters resulting from a loop injection of lithium formate solution (10mM in water) at the end of the separation.

Chromatographic peak widths (FWHM) of one second or below

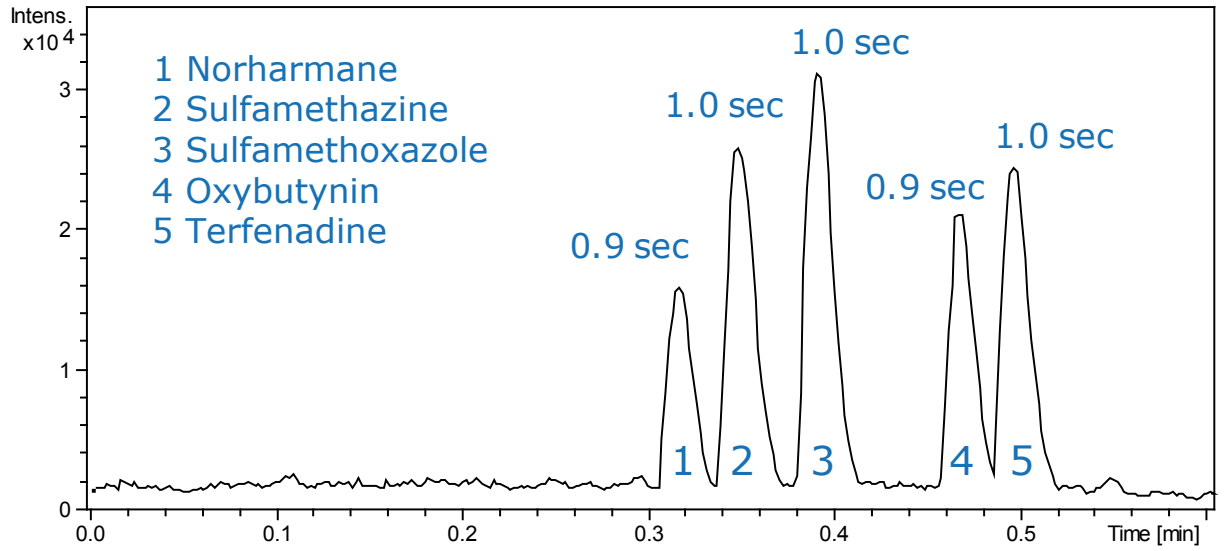


Fig. 1: Base peak chromatogram of the separation showing resolution of the 5 components in 30 seconds.

Results

The separation of the 5 drug mix gave baseline resolution of each compound within 30 seconds (Figure 1). For each peak more than 15 spectral measurements were made across each peak, to give reproducible peak areas suitable for quantitative analysis (Figure 2).

In the mass spectrum, the mass accuracy for each peak of the isotopes is below 1 ppm, with a resolution of 43,700 - acquired at an acquisition rate of 10 Hz (Figure 3). SmartFormula analysis is automated in the Compass™ 1.3 data processing software. SmartFormula™ automatically determines the possible elemental compositions of each compound LC peak, using a mass accuracy of < 2 ppm error with allowed chemical rules and elements ($C_nH_nN_nO_nS_n$). Formulae are ranked according to the closest match factor between the measured isotopic pattern and the theoretical pattern for a given formula – the Sigma value, which should be close to zero. For these drug compounds, a unique SmartFormula result was produced within the allowed mass accuracy and Sigma values (Figure 4). Peak 4, Oxybutynin (Figure 5) was unequivocally confirmed in this experiment.

Multiple mass spectra from each peak

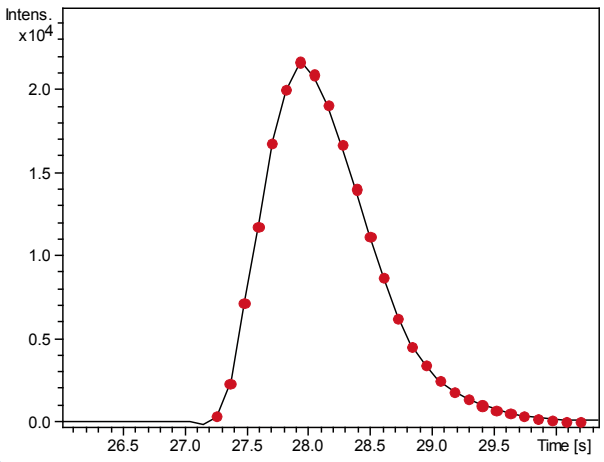


Fig. 2: More than 15 spectral datapoints were obtained for each LC peak, this example is peak #4 from figure 1.

Mass accuracy and resolution at speed

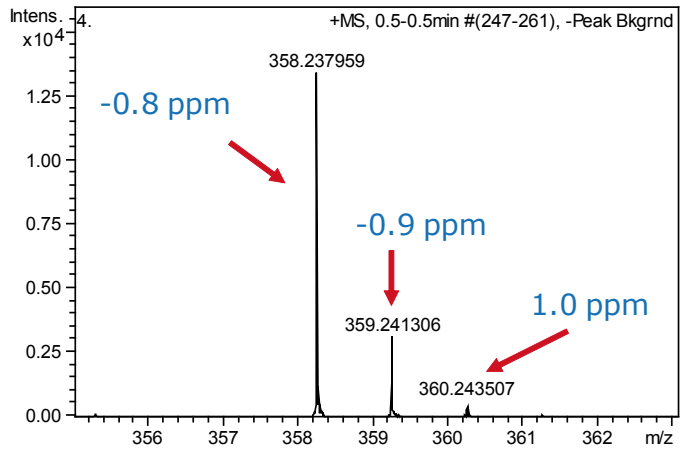


Fig. 3: Sub-ppm mass accuracy was obtained for each isotope of the m/z peak for compound #4 (oxybutynin) together with >40K resolution.

SmartFormula provides one single formula candidate

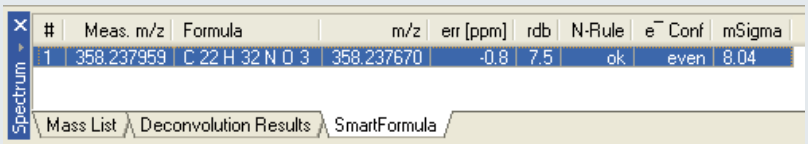


Fig. 4: SmartFormula result for peak #4 with a 2 ppm mass window, only one candidate formula meets the mass accuracy and isotopic pattern tolerance.

Conclusion

The maxis is an ideal instrument for maximum MS performance at the very highest speeds delivered by modern ultrafast chromatography. Sub-ppm mass accuracy is shown for small molecule drugs, together with a resolution in excess of 40,000. Thus the instrument allows extraction of full quantitative information from high speed chromatography. Supported by SmartFormula, the maXis provides unequivocal molecular formula determination capabilities. For compounds with higher molecular weight (up to 1000 Da) SmartFormula 3D™ is using MS/MS fragment data for certainty in small molecule identifications [1].

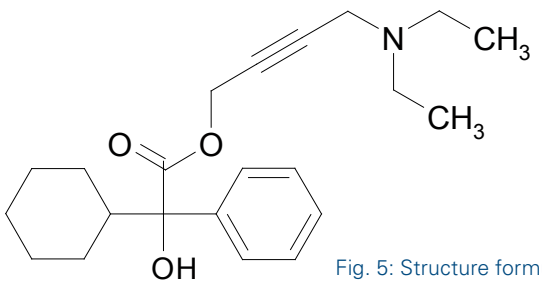


Fig. 5: Structure formula of Oxybutynin ($C_{22}H_{31}NO_3$) with a $M_{[M+H]}$=358.23767

Acknowledgement

SmartFormula 3D was developed from an original concept by Don Richards, Pfizer.

References

[1] Bruker Daltonics Technical Note-23. Certainty in Small Molecule Identification by Applying SmartFormula 3D on a UHR-TOF Mass Spectrometer.

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Keywords
ultra performance liquid chromatography
UHR-TOF
ab initio formula determination

Instrumentation & Software
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