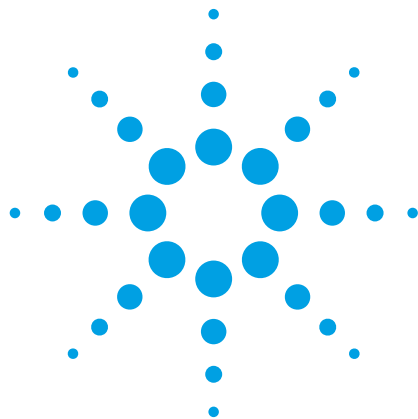




Superior resolution of Agilent 6540 UHD Q-TOF over Thermo LTQ Orbitrap XL for fast UHPLC applications

Technical Overview

Abstract

Mass spectral resolution is undeniably valuable and is an important decision criterion in the choice of an LC/MS system for accurate-mass analyses. Mass resolution allows the separate identification of near-isobaric components that coelute during an LC run. Ultrahigh resolution, previously the exclusive domain of complex and expensive Fourier transform-ion cyclotron resonance (FT-ICR) instruments, is now accessible by many labs through Orbitrap and the latest Agilent Q-TOF systems. In addition to resolution, speed is important. MS labs face time-pressure for sample turnaround or completion of studies. Many labs have discovered the tremendous productivity benefits of ultra high performance liquid chromatography (UHPLC) for improved chromatographic resolution and reduced runtimes. This technical overview demonstrates that when acquiring spectra at rates that are compatible with fast UHPLC analyses, the Agilent 6540 Ultra High Definition (UHD) Accurate-Mass Q-TOF LC/MS system delivers up to ten-fold better resolving power than the Thermo Scientific LTQ Orbitrap XL.

Introduction

In fields such as metabolomics, proteomics, and food safety, a combination of sample complexity and the need to increase throughput has many labs adopting UHPLC. As productivity demands continue to escalate, UHPLC becomes increasingly attractive because the technique enables better separations and shorter LC runs, allowing analysts to optimize throughput.

Fast, high-resolution separations offer the dual benefits of reduced ion suppression plus shorter method times and higher sample throughput. As sample complexity increases, near-isobaric analytes and interferences may overlap chromatographically. The accurate-mass MS must provide another dimension of resolution to ensure that unknown compounds can be properly identified and target compounds can be analyzed reliably—and it must do so at the spectral acquisition rates required by fast LC.



Agilent Technologies

This technical overview explores the resolving power of the Agilent 6540 Ultra High Definition (UHD) Accurate-Mass Q-TOF LC/MS system, when used at the high data rates that are required for fast LC analyses. It also compares the resolving power of the Agilent system with that of the Thermo Scientific LTQ Orbitrap XL, and proves the unequivocal superiority of the mass resolution on the Agilent system for this application.

Results and discussion

With high-resolution UHPLC separations, peak widths can be as small as 0.5 second. Very narrow peaks can help reduce ion suppression by resolving components that might interfere during the ionization process, but narrow peaks place new demands for speed on detection devices. As shown in **Figure 1**, the acquisition rate must be matched to the chromatographic peak width. MS systems that are used for UHPLC must be able to acquire spectra rapidly without loss of resolving power, mass accuracy, sensitivity, dynamic range, or isotopic fidelity. Unlike most competitive instruments, the robust Agilent time-of-flight (TOF) and quadrupole-TOF (Q-TOF) systems meet this challenge.

Agilent 6540 UHD Q-TOF maintains high resolution during high-speed spectral acquisition

To demonstrate the suitability of the Agilent 6540 UHD Q-TOF for rapid UHPLC analyses, **Figure 2** shows mass resolution as a function of acquisition rate. Note that resolution remains relatively constant at very high acquisition rates, and varies from just under 35,000 at low 368 m/z to greater than 40,000 for most masses studied.

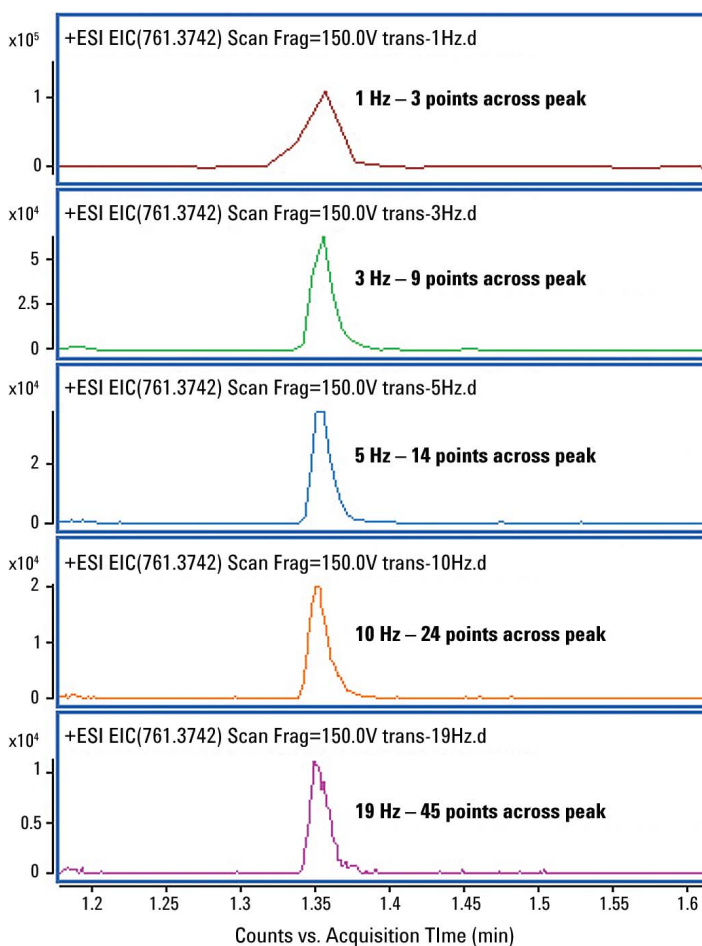


Figure 1. Faster acquisition rates provide better definition for this peptide peak from a UHPLC /MS run. The minimum number of data points that a chromatographer would accept is 10 to 12.

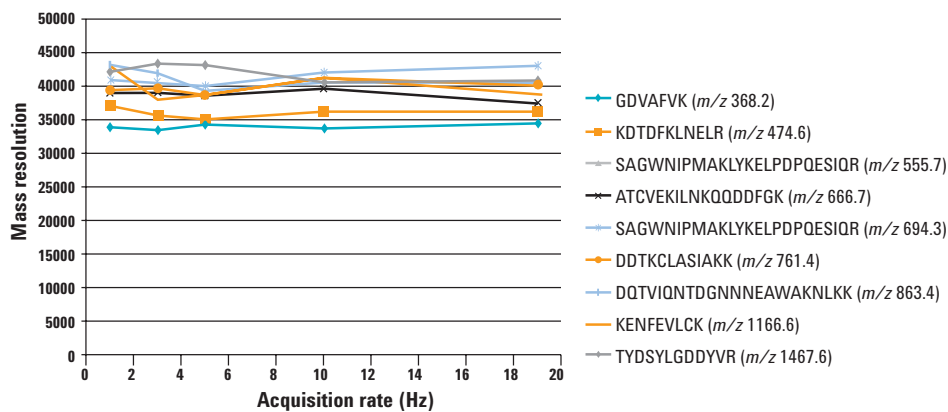


Figure 2. Mass resolution on the Agilent 6540 UHD Q-TOF remains constant as acquisition rates increase.

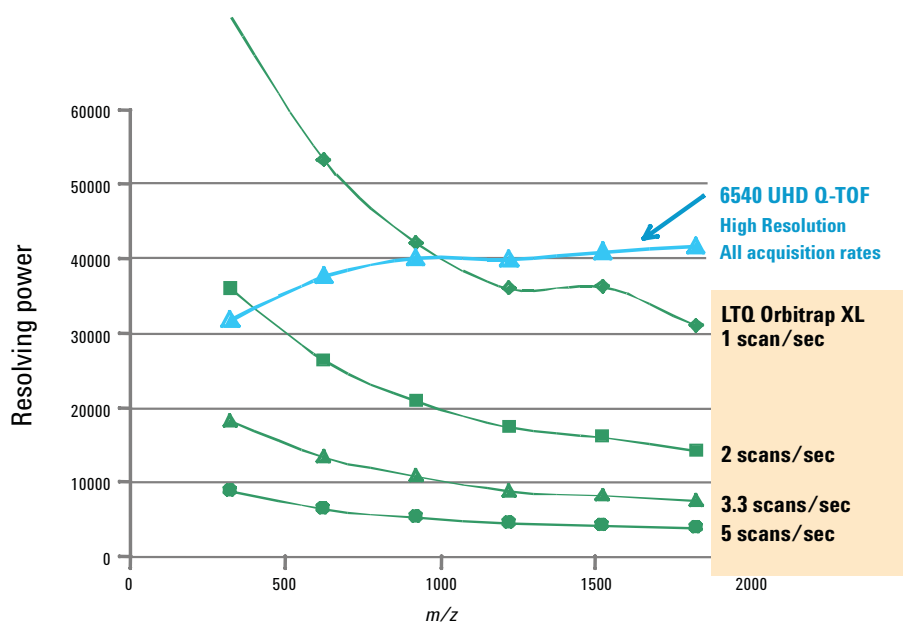


Figure 3. Graphical comparison of resolution measurements for a nine-compound mixture infused into an Agilent 6540 UHD Q-TOF and a Thermo LTQ Orbitrap XL. Resolution was measured at acquisition rates of 1, 2, 3.3, and 5 spectra/second.

Agilent 6540 UHD Q-TOF shows better resolution across acquisition rates and mass

As described in defining papers on Orbitrap mass analyzers, it was observed that Orbitrap resolution is proportional to scan time and inversely proportional to the square root of m/z .¹⁻³ Thus, we expect Orbitrap resolution to be highest at low m/z and to decline as spectral acquisition rates increase.

In Figure 3, it is very easy to see that the resolution of the Agilent 6540 UHD Q-TOF exceeds the resolution of the Thermo LTQ Orbitrap XL at almost all masses and acquisition rates studied, except at the slowest acquisition rates and lowest masses. In addition, the results show that resolution drops off precipitously at higher acquisition speeds with the Thermo LTQ Orbitrap XL, and at 5 spectra/second is below 10,000 over the entire mass range that was examined. Resolving power on the Thermo LTQ Orbitrap XL also diminishes with mass, and at 5 spectra/second is only about 4000 at m/z values around 1800. This reduction in resolution with mass makes it more difficult to distinguish higher-mass analytes from coeluting sample components.

Conclusions

Theory predicts that Orbitrap mass analyzers will lose resolution at higher m/z and at higher data acquisition rates. This has been demonstrated in this study. Many other studies have confirmed this result. Orbitrap mass analyzers are capable of high-resolution mass measurements—if one is willing to sacrifice speed and work in a very limited mass range.

The Orbitrap introduced the scientific community to the value of routine access to high-resolution, accurate-mass data. The Agilent 6540 UHD Accurate-Mass Q-TOF now extends this capability to samples and analyses that require higher acquisition rates. At acquisition rates above 1 spectrum/second, Thermo LTQ Orbitrap XL resolution is typically lower than Agilent 6540 UHD Q-TOF resolution. At rates of only 5 spectra/second (the minimum conceivable acquisition rate for UHPLC), the Agilent 6540 UHD Q-TOF has demonstrably better resolution than the Thermo LTQ Orbitrap XL. The 6540 UHD Q-TOF also maintains sensitivity, dynamic range, mass accuracy and isotopic fidelity at this acquisition rate.

References

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Printed in the U.S.A. August 25, 2009
5990-4507EN



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