Better detection of trace components: in-spectrum dynamic range of Agilent 6540 UHD Q-TOF exceeds that of Thermo LTQ Orbitrap XL

Technical Overview

Abstract

When complex samples are analyzed by liquid chromatography/mass spectrometry (LC/MS), and they contain coeluting compounds that vary widely in concentration, in-spectrum dynamic range becomes critically important. Without sufficient in-spectrum dynamic range, the instrument may fail to detect low-level sample components when they overlap chromatographically with compounds at much higher concentrations. This technical overview describes a benchmarking study where the Agilent 6540 Ultra High Definition (UHD) Accurate-Mass Q-TOF LC/MS system demonstrated an in-spectrum dynamic range of 50,000 to 100,000, while that of the Thermo Scientific LTQ Orbitrap XL was 800. Because of its superior dynamic range, the Agilent system is better able to detect critical low-level sample components in complex sample mixtures.

Introduction

LC/MS samples in proteomics, metabolomics, food safety, and impurity analyses often contain mixtures of compounds that must be detected over a wide concentration range. When important low-level sample components, such as putative biomarkers, coelute with other compounds that are present in huge excess, the mass spectrometer (MS) must be able to reliably detect and measure the biomarkers. In-spectrum dynamic range (also called in-scan dynamic range) becomes critically important in this situation—this is the dynamic range within a single spectrum, which is defined as the ratio in signal abundance of the largest and smallest detectable mass peaks.

This technical overview compares the in-scan dynamic range of the Agilent 6540 UHD Q-TOF LC/MS system with that of the Thermo Scientific LTQ Orbitrap XL, and shows the clear superiority of the Agilent system.

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Results and discussion

Agilent 6540 UHD Q-TOF demonstrates wider dynamic range than Thermo LTQ Orbitrap XL

In a benchmarking study[1], dynamic range was evaluated for the Thermo Scientific LTQ Orbitrap XL and the Agilent 6540 UHD Q-TOF. **Figure 1** shows that the LTQ Orbitrap XL exhibited in-spectrum dynamic range of only 800. These results were obtained in the 15,000 resolution mode, with an acquisition rate of 3.3 spectra/sec. The bottom spectrum shows the most intense peak in the spectrum at full scale, while the others show zooms of the remaining peaks. Per definition, the dynamic range was calculated as the ratio of the maximum signal to the minimum signal.

At this limited dynamic range of 800, low-abundance peaks such as *m/z* 626 are completely absent in the spectrum, and even higher-abundance peaks such as *m/z* 625 occur in only one-third of the spectra. These data dropouts place serious limitations on detection of minor sample components such as low-abundance peptides in complex samples.

A similar display in **Figure 2** shows that the Agilent 6540 UHD Q-TOF has an in-scan dynamic range of 50,000. As shown later, the instrument has demonstrated five orders of magnitude dynamic range—a level that enables detection of trace-level compounds in the presence of high-abundance components such as those found in many sample matrices.

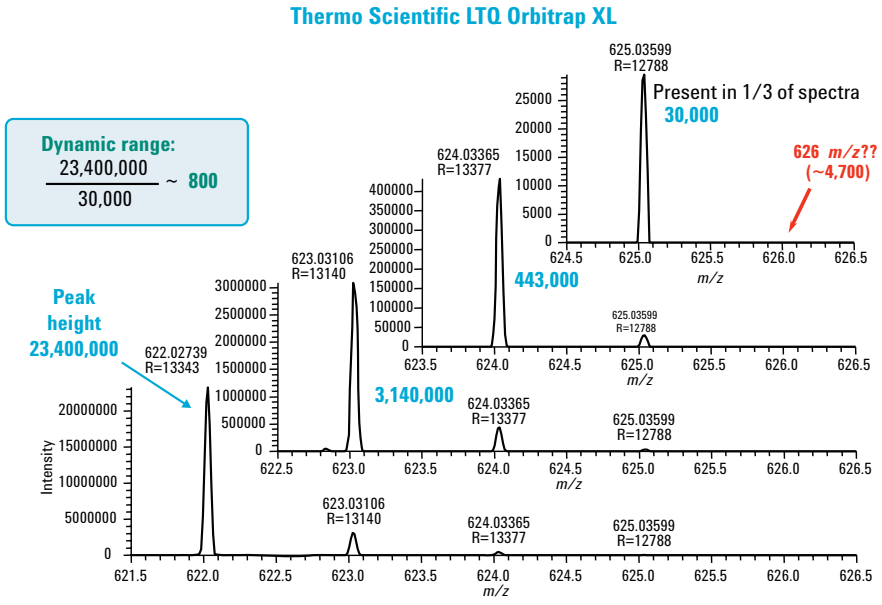

Figure 1. At 3.3 spectra/sec, dynamic range on the Thermo Scientific LTQ Orbitrap XL is only 800.

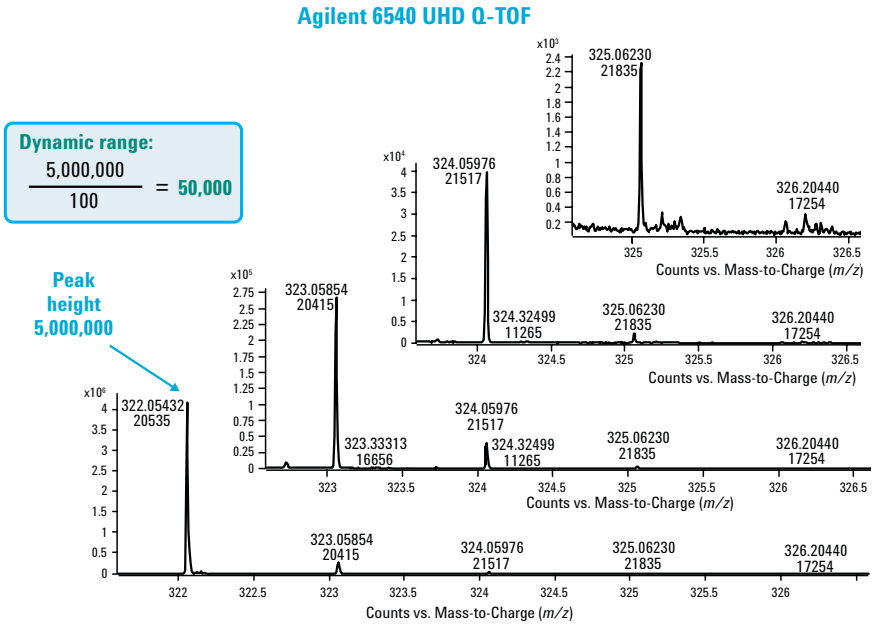

Figure 2. The Agilent 6540 UHD Q-TOF demonstrates a dynamic range of 50,000.

With the Thermo LTQ Orbitrap XL, the lower end of the dynamic range is compromised because the image current detector requires a minimum of 20 to 50 ions to detect an ion signal. In contrast, the Agilent detector requires only one ion. This difference has large implications for trace-level analyses.

Furthermore, the Thermo instrument, like other instruments that trap ions in a confined space, suffers from space-charge effects when too many ions enter the trap. To compensate, the instrument control software limits the number of ions in the trap, which reduces sensitivity for trace-level components when they coelute with compounds at much greater concentration. The Agilent 6540 UHD Q-TOF is not a trapping instrument, and so does not have this limitation.

Application proof: Agilent 6540 UHD Q-TOF detects trace metabolites

One of the most challenging analyses is the detection of trace metabolites that coelute with the parent drug. **Figure 3** shows that, with five full decades of dynamic range, this can be done with the Agilent 6540 UHD Q-TOF. This figure shows a *single* spectrum, taken in 330 msec, of a sample resulting from an S9 liver microsomal incubation of verapamil. The mass peak due for verapamil $[M+H]^+$ is at an abundance of 2.68 million counts, while that of a minor metabolite peak in the spectrum is only 25 counts, demonstrating a dynamic range of more than 100,000.

The Agilent Molecular Formula Generator (MFG) provides the correct result for the parent drug, with a mass error of 0.25 ppm and a superb match score of 99.43. By zooming in on the spectrum, one can see that minor metabolites are present. Buried close to the baseline is the signal from a dihydroxy metabolite. Selecting 487.2 *m/z* and running MFG gives a formula for the dihydroxy metabolite with a mass error of 1.03 ppm. Significantly, the A+2 isotope is present and recognized by MFG with an abundance of around 25 counts. This example clearly shows the benefit of wide dynamic range for detection of trace components on the Agilent system.

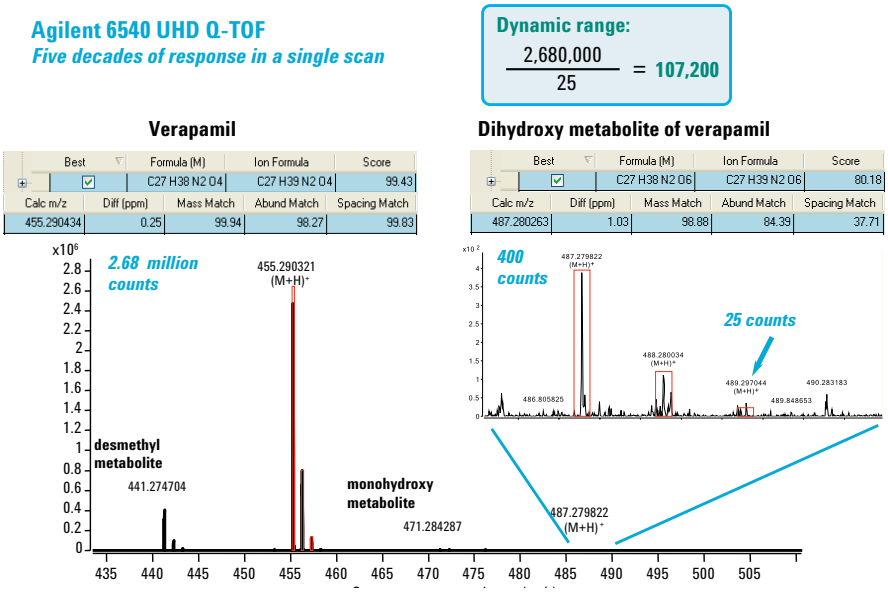

Figure 3. This analysis of a trace metabolite that coelutes with the parent drug shows a dynamic range of five decades on the Agilent 6540 UHD Q-TOF. Shown is a single spectrum from data obtained at 3.3 spectra/sec.

Conclusions

Mass spectrometers that have a wide dynamic range are better able to analyze "real-life" samples that include compounds across a wide range of concentrations. The Agilent 6540 UHD Q-TOF has a much wider dynamic range than the Thermo LTQ Orbitrap XL, meaning the Agilent instrument can better detect trace-level sample components even when they overlap chromatographically with those at much higher concentration. In complex samples where concentrations span many orders of magnitude and components coelute, the Agilent 6540 UHD Q-TOF is much more likely to detect important low-level compounds.

Reference

1. "Superior resolution of Agilent 6540 UHD Q-TOF over Thermo LTQ Orbitrap XL for fast UHPLC applications," Agilent publication number 5990-4507EN, **2009**.

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