Agilent 6540 Accurate-Mass Q-TOF: superior mass accuracy for high-confidence characterization of unknowns

Application Note

Author

David Weil
Agilent Technologies
Schaumburg, IL USA

Agilent Technologies

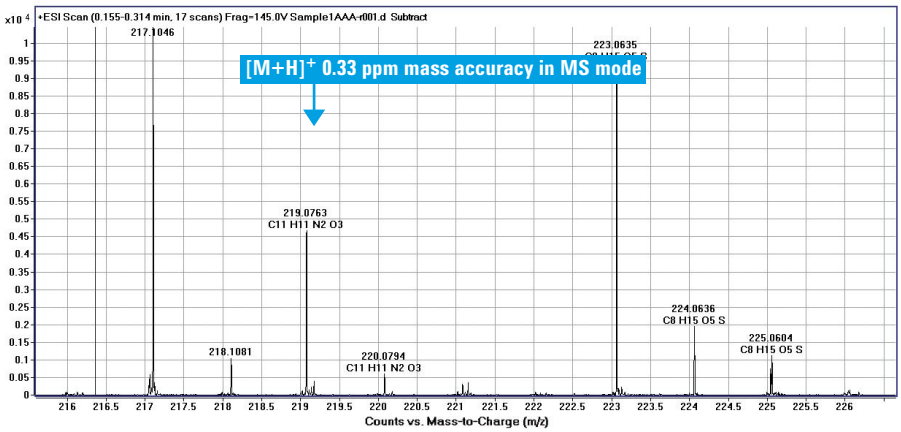
Introduction

Quadrupole time-of-flight (Q-TOF) instruments are invaluable for characterization of unknown compounds. They offer a combination of unbeatable separation speed and resolution as well as superior qualitative MS and MS/MS capabilities. However, even with their ability to give high resolution accurate mass data, it is common to get multiple candidate molecular formula for the $[M+H]^+$ ion, or several elemental compositions for a given ion in the MS/MS spectra. High quality Q-TOF data helps to eliminate unlikely and incorrect formula. The Agilent 6540 Accurate-Mass Q-TOF LC/MS System provides outstanding mass accuracy and isotopic fidelity, which reduces the number of formula for unknown samples. The system's high resolution can also resolve charge states or pinpoint small changes in structure.

Excellent MS mass accuracy limits number of possible formula

To test the capability of the instrument, a laboratory submitted a sample described as a compound with a nominal molecular weight 218. The test was to determine the correct molecular formula. The sample was analyzed using the Agilent 6540 Accurate-Mass Q-TOF.

Figure 1 shows the resulting MS spectrum, and analysis using Agilent Molecular Formula Generation (MFG) software,[1] part of Agilent MassHunter Qualitative Analysis software. The theoretical value for $[M+H]^+$ ion is *m/z* 219.0764. Because the mass error was only 0.33 ppm and the isotope abundance match was excellent, MFG was able to select the correct formula, i.e. $C_{11}H_{10}N_2O_3$, from among the many potential candidates.



m/z	Ion	Formula	Abundance
219.0763	(M+H)+	C11 H11 N2 O3	4902.3

Best	Formula (M)	Ion Formula	Score	Calc m/z	Diff (ppm)	Mass Match	Abund Matc	Spacing Match	DBE
☑	C11 H10 N2 O3	C11 H11 N2 O3	98.63	219.0764	0.33	99.95	99.27	95.22	8

Isotope	Abund Sum%	Calc Abund Sum%	m/z	Calc m/z	Diff (ppm)
1	88.42	87.53	219.0763	219.0764	0.33
2	10.9	11.26	220.0794	220.0795	0.41

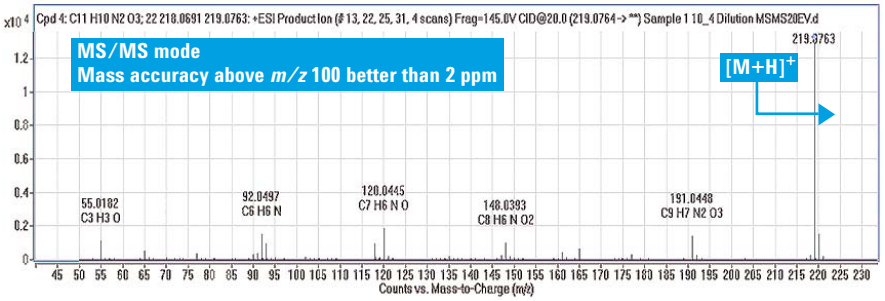
Figure 1. Superb MS mass accuracy leads to the correct formula.

Outstanding MS/MS mass accuracy enables greater confidence in structure elucidation

Figure 2 shows the analysis of the same unknown in MS/MS mode. At the top is the MS/MS spectrum of the $[M+H]^+$ ion, *m/z* 219.0763. The inset table shows the MFG results. At *m/z* values greater than 100, MS/MS mass accuracy is better than 2 ppm. Even below 100, the mass accuracy is excellent, with values in the millidalton range.

Mass accuracy maintained even at the high data rates needed for UHPLC

The 6540 Accurate-Mass Q-TOF produces very accurate mass measurements for all MS acquisition rates, important when you want to use it with fast chromatography of complex mixtures of unknown compounds. With high resolution separations from ultra high performance liquid chromatography (UHPLC), peak widths can be as narrow as 0.5 second. Very narrow peaks reduce ion suppression by resolving components that might interfere during the ionization process. However, narrow peaks mean that mass spectrometers used for UHPLC must acquire spectra rapidly without loss of mass accuracy. Unlike some competing accurate-mass instruments, the Agilent Q-TOF systems meet this challenge by providing the speed needed to realize the benefits of today's fastest UHPLC separations.



m/z	Formula	Abund%	Diff (ppm)	Loss Mass	Loss Formula
191.0448	C9 H7 N2 O3	16.02	1.72	28.0313	C2 H4
148.0393	C8 H6 N O2	10.95	0.34	71.0371	C3 H5 N O
120.0445	C7 H6 N O	21.01	-0.52	99.032	C4 H5 N O2
118.0285	C7 H4 N O	10.74	1.94	101.0477	C4 H7 N O2
93.0337	C6 H5 O	10.81	-2.46	126.0429	C5 H6 N2 O2
92.0497	C6 H6 N	17.78	-2.25	127.0269	C5 H5 N O3
55.0182	C3 H3 O	12.69	-5.94	164.0586	C8 H8 N2 O2

Figure 2. This outstanding MS/MS mass accuracy provides more confident ion assignments, and a clearer picture of the substructures in an unknown compound.

For this test, the same unknown was run at various spectral acquisition rates in MS-only mode. **Table 1** shows the differences in ppm between calculated and measured masses. Regardless of spectral acquisition rate, the mass error was extremely small, ranging from –0.02 to 0.46 ppm, making the Agilent 6540 Accurate-Mass Q-TOF an ideal detector for fast chromatography.

Scan rate (Hz)	*m/z*	Mass error (ppm)
1	219.07642	-0.02
2	219.07641	0.05
4	219.07647	-0.24
6	219.07639	0.11
8	219.07632	0.46
10	219.07633	0.41

Table 1. Excellent mass accuracy across spectral acquisition rates makes the Agilent 6540 Accurate-Mass Q-TOF a perfect match for UHPLC analysis of unknown compounds.

Conclusion

The Agilent 6540 Accurate-Mass Q-TOF delivers clear and unambiguous information for unknown samples. Whether analyzing PTMs, profiling biomarkers, identifying metabolites, screening for pesticides, or characterizing intact proteins, Agilent Q-TOF solutions deliver the data quality demanded of the most critical science. The theoretical value for $[M+H]^+$ ion is *m/z* 219.0764. In this example, mass accuracy was better than 0.5 ppm in MS mode, and better than 2 ppm above *m/z* 100 in MS/MS mode. This outstanding performance, together with the unique Agilent Molecular Formula Generation software, produced the correct molecular formula for an unknown compound and its substructures. Mass accuracy was independent of spectral acquisition speeds, which means the instrument can be used for reliable identification of unknowns from UHPLC analyses. The Accurate-Mass Q-TOF platform provides sensitivity, accurate mass, dynamic range, and resolution—all fully compatible with ultra fast UHPLC separations.

Reference

1. E. Darland, D. McIntyre, D. Weil, F. Kuhlmann, and X. Li, "Superior Molecular Formula Generation from Accurate-Mass Data," Agilent publication number 5989-7409EN, **2008**.

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