The impact of resolution on accurate mass measurements of complex samples

Application Note

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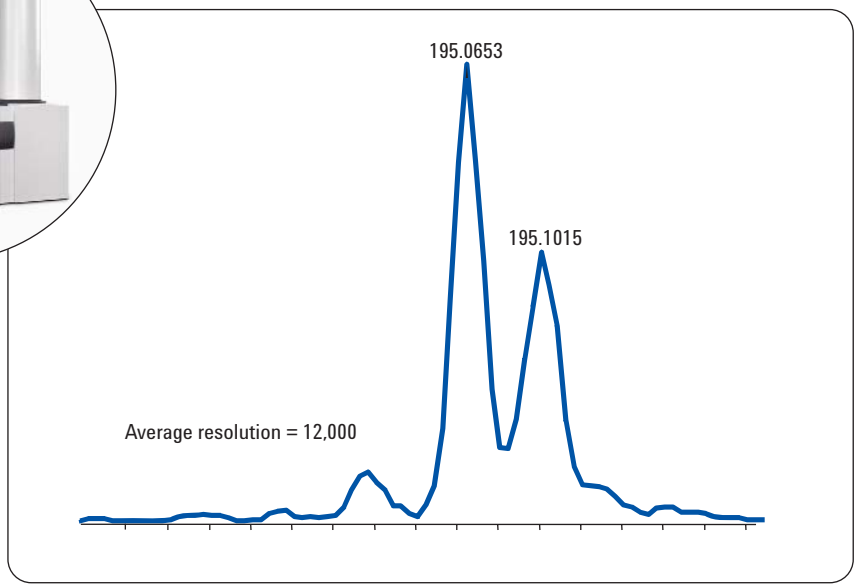

Abstract

This Application Note demonstrates:

- The performance of the Agilent 6520 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS and the Agilent 6220 Accurate-Mass TOF LC/MS.
- The increased dynamic range and mass resolution of the Agilent 6520 Accurate-Mass Q-TOF LC/MS and the Agilent 6220 Accurate-Mass TOF LC/MS.
- The highly accurate mass measurement and formula calculation for these systems.
- The influence of the increased performance on the results of sample measurements.

Agilent Equipment

- 1200 Series Rapid Resolution LC system
- 6520 Accurate-Mass Q-TOF LC/MS
- 6220 Accurate-Mass TOF LC/MS

Application Areas

- Pharmaceutical industry
- Forensic analysis
- Environmental analysis
- Food analysis

Agilent Technologies

Introduction

In mass spectrometry, there is no direct correlation of resolving power and dynamic range with mass accuracy. For example, when doing synthetic compound confirmation, Fourier transform (FT) mass spectrometers with resolving power of over 200,000 typically exhibit only a two-fold improvement in mass accuracy over time-of-flight (TOF) instruments with resolution in the range of 10,000. With the Agilent TOF and Q-TOF, recent two- to three-fold improvements in resolution showed no significant improvement in mass accuracy when analyzing pure compounds. The results were quite different, however, with complex mixtures. This study examines how improved resolution and dynamic range on TOF and Q-TOF systems impacts mass accuracy for analysis of complex mixtures where compounds produce ions that are very close together (<50 mDa separation).

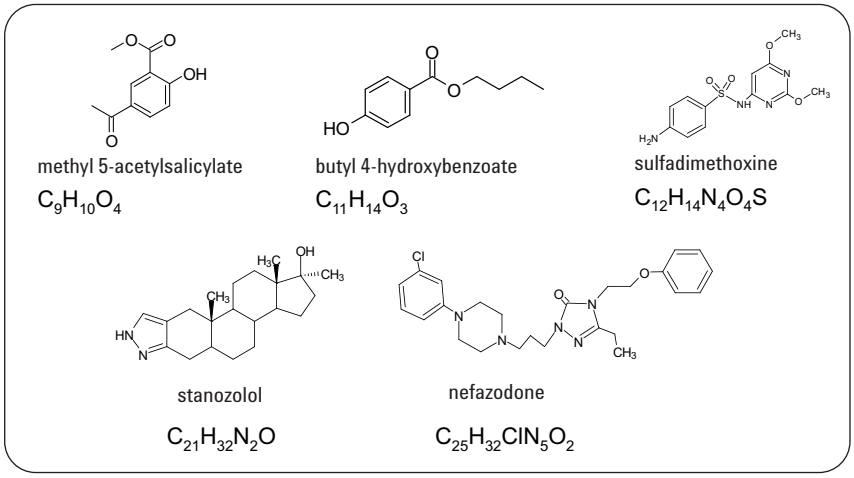

Figure 1
Structures and formulas of test compounds.

Experimental

Equipment

Agilent 1200 Series Rapid Resolution LC system with:

- Agilent 1200 Series binary pump SL and degasser
- Agilent 1200 Series high performance autosampler SL (ALS SL) with thermostat
- Agilent 1200 Series thermostatted column compartment (TCC)
- Agilent 1200 Series diode-array detector SL (DAD SL)
- Agilent 6220 Accurate-Mass TOF LC/MS or Agilent 6520 Accurate-Mass Q-TOF LC/MS
- Column 1: Agilent ZORBAX SB-C18, 2.1 x 30 mm, 3.5 µm particle size
- Column 2: Agilent ZORBAX SB-C18, 2.1 x 150 mm, 1.8 µm particle size

Samples

The following test compounds were purchased from Sigma-Aldrich and were prepared as stock solutions of 1 mg/mL in methanol: methyl 5-acetylsalicylate (MAS), butyl 4-hydroxybenzoate (BP), sulfadimethoxine, and nefazodone. Stanozolol from Alltech was purchased in a 1 mg/mL methanol form. Samples were diluted to 10 to 30 ng/µL in water. For nefazodone, a dilution series was prepared from 2 ng/mL to 100 µg/mL. (The injection volume was 1 µL.) The structures are shown in figure 1.

LC TOF conditions

The HPLC was operated under the following conditions:

- Solvent A: water + 0.1 % formic acid (FA)
- Solvent B: methanol + 0.1 % FA
- Flow rate: 0.5 mL/min, isocratic at 90 %B for 1 min
- TCC temperature: 45 °C

The Agilent 6220 Accurate-Mass TOF LC/MS was used with the following acquisition parameters:

- Source: Electrospray (ESI) in positive mode with dual spray for reference mass solution
- Drying gas: 10 L/min at 300 °C
- Nebulizer: 45 psi
- Mass range: 100 to 1000
- Scanning: 3 spectra/sec
- Fragmentor: 125 V
- Skimmer: 60 V
- Capillary: 4000 V
- Instrument state: 1700 *m/z* at 1, 2, and 4 GHz

LC Q-TOF conditions

The HPLC was operated under the following conditions:

- Solvent A: water + 0.1 % FA
- Solvent B: acetonitrile + 0.1 % FA
- Flow rate: 0.5 mL/min
- Gradient 1: from 20 %B to 60 %B in 4 minutes with column 1 and TCC at 45 °C (These conditions were used for the stanozolol experiment.)
- Gradient 2: from 5 %B to 75 %B in 15 minutes with column 2 and TCC at 60 °C (These conditions were used in another experiment to identify the metabolites of nefazodone.[2])

The Agilent 6520 Accurate-Mass Q-TOF LC/MS was used with the following acquisition parameters:

- Source: ESI in positive mode with dual spray for reference mass solution
- Drying gas: 10 L/min at 300 °C
- Nebulizer: 45 psi
- Mass range 80 to 1000 (MS and MS/MS)
- Scanning: 3 spectra/sec in both modes
- Fragmentor: 200 V
- Skimmer: 60 V
- Capillary: 4000 V
- Collision energy: 35 V (sulfadimethoxine) or 55 V (stanozolol)
- Operated in targeted MS/MS mode with isolation width set to medium (4 *m/z*)
- Instrument state: 1700 *m/z* at 1, 2, and 4 GHz

Results and discussion

Instrument resolution

The resolution observed in TOF instruments is a function of both the actual resolving power of the analyzer and the ability of the detector circuitry to respond to the signal and measure the output at the needed speed. Compared with earlier models that used 1 GHz digitization, the Agilent 6220 Accurate-Mass TOF LC/MS and the Agilent 6520 Accurate-Mass Q-TOF LC/MS both utilize a faster-responding amplification circuit, as well as the ability to digitize the detector output at either 1, 2, or 4 GHz. This new signal processing has been described earlier.[1]

At 4 GHz, these analyzers typically demonstrate a resolution of at least 10,000 at *m/z* 118 and at least 20,000 at *m/z* 1522. The impact of digitization rate on observed resolution is shown in table 1.

From the table, it is apparent that digitizing at 1 GHz will result in undersampling the data at low m/z values and reporting a resolution much less than the instrument capability. The sampling rate of 1 GHz corresponds to taking a sample every 1 nanosecond, and therefore getting 1 sample in a peak at 118 *m/z* that has a peak-width of 1 nanosecond. In fact, at 1 GHz the observed resolution for *m/z* 118 is around 4000.

This two- to three-fold improvement in resolution achieved by changing from 1 GHz to 4 GHz digitization did not result in any significant improvement in mass accuracy when pure samples were analyzed. Typical mass accuracy for compounds in the molecular weight range of 100 to 800 was between 1 and 2 ppm. On examining samples that displayed mass errors of 10 ppm or more, it was apparent that some nominally isobaric ion from another compound was impacting the measurement. Improved resolution should therefore provide better accuracy for mass measurements in complex samples, and consequently a better job of generating correct molecular formulas for both MS and MS/MS data.

Resolution and accuracy in TOF mode

A test was done to compare mass error in TOF mode for 1 GHz versus 4 GHz digitization. MAS and BP differ by 36 mDa in molecular weight. The two compounds were mixed together in a ratio that varied from 128:1 to 1:1, and the mobile phase composition was adjusted until the compounds were no longer resolved chromatographically. For 1 GHz data, the mass error for the MAS was calculated for each sample and is shown in table 2.

Dilution	Mass error (ppm)
128:1	2.89
64:1	3.25
32:1	5.09
16:1	10.13
8:1	17.13
4:1	33.17
2:1	64.06
1:1	120.51

Table 2
MAS mass error as a function of MAS/BP ratio, at 1 GHz digitization rate.

m/z	Arrival time (nsec)	ΔM (mDa)	Δt (nsec)	Samples across FWHM at 1 GHz	Samples across FWHM at 4 GHz
118	20,000	12	1	1	4
1522	69,211	76	1.7	1.7	6.8

Table 1
Delta mass and time values for example *m/z* values, as well as number of samples as a function of sampling rate of the analog-to-digital converter (ADC). FWHM = full width at half maximum of the mass peak.

At 4 GHz, the observed $[M+H]^+$ ions were completely resolved and the mass accuracy was better than 1 ppm for both compounds. When the Agilent molecular formula generator was applied to each ion, the correct formula was the best match in each case. Furthermore, extracting the ion chromatograms over a narrow *m/z* window actually revealed that the two compounds elute 0.5 seconds apart. These results are shown in figures 2 and 3.

Instrument dynamic range

Another important factor in the acquisition of a mass spectrum is the dynamic range of the mass spectrometer, both between scans and within a scan. With an expanded dynamic range, potential low-abundance compounds can be detected in the presence of other high-abundance matrix components.

As an example of between-scans dynamic range, a series of concentrations of the pharmaceutical drug nefazodone was measured on the Agilent 6520 Accurate-Mass Q-TOF LC/MS.[2] The detection began at the limit of detection (LOD) of 2 pg on-column and

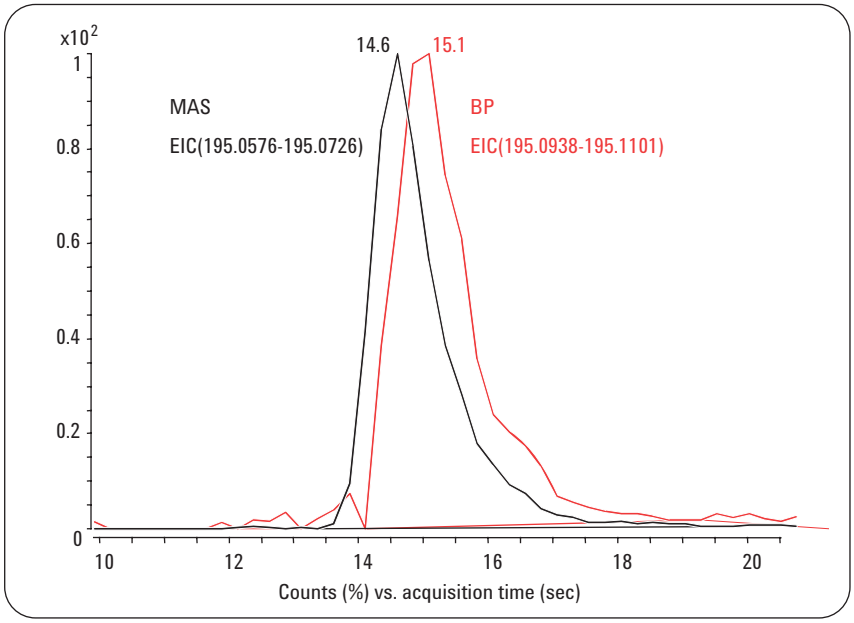

Figure 2
Extracted ion chromatograms (EICs) of MAS and BP acquired at 4 GHz digitization rate.

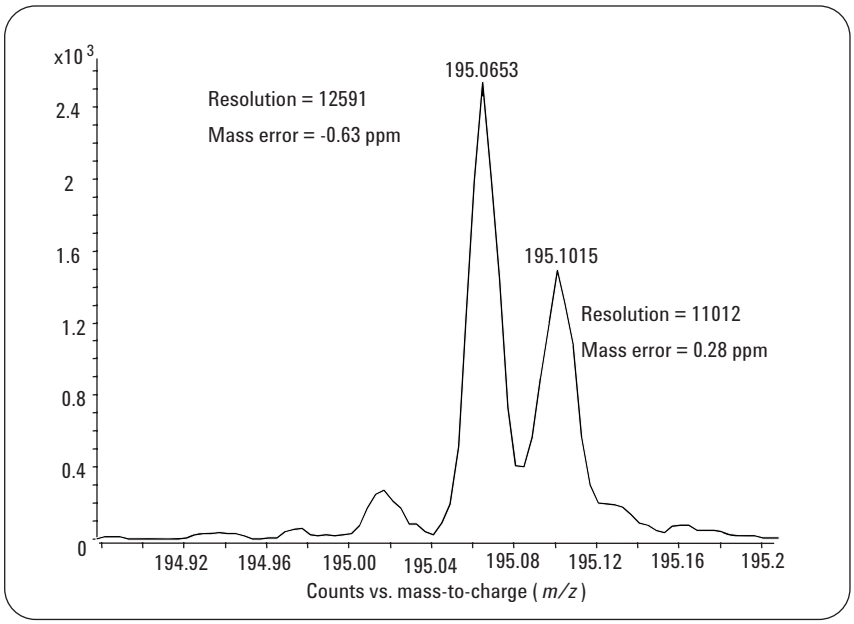

Figure 3
$[M+H]^+$ ions for MAS and BP at 4 GHz digitization rate, showing resolution and mass error.

finished when the detector began to saturate at a concentration of 100 ng on-column (figure 4). For all concentrations, the formula was calculated from the measured mass with a relatively low mass error of less than 2 ppm.

As an example for the in-scan dynamic range, erythromycin at a concentration of 500 fg/µL was measured in the presence of an excess of niacinamide at a concentration of 10 ng/µL (figure 5). The measurement clearly demonstrates an in-scan dynamic range of 4.36 orders of magnitude, together with an accurate mass measurement of less than 1 ppm for both compounds.

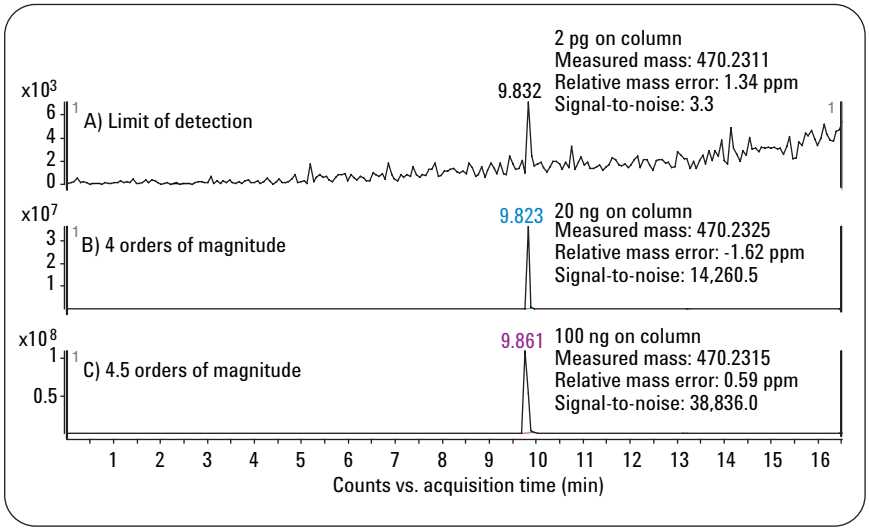

Figure 4
Dynamic range of nefazodone by Q-TOF, demonstrated at 4.5 orders of magnitude with mass errors less than 2 ppm. Nefazodone formula $[M+H]^+ = C_{25}H_{33}N_5O_2Cl$. Calculated mass *m/z* = 470.2317. The separation used gradient 2.

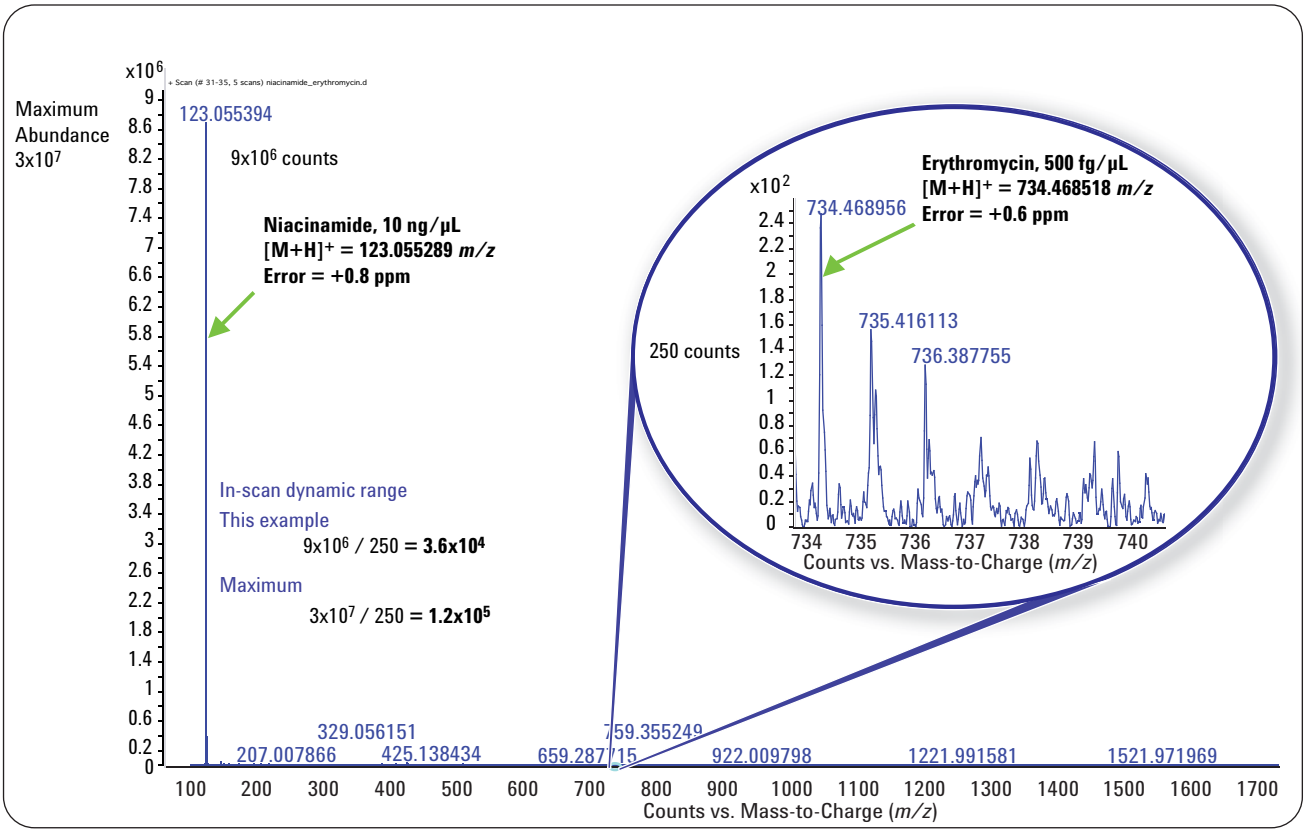

Figure 5
Up to five orders of magnitude in-scan dynamic range with accurate mass measurement.

Resolution and mass accuracy in MS/MS mode

The Agilent 6520 Accurate-Mass Q-TOF LC/MS can provide MS/MS data with mass accuracies of 5 ppm or better. This can be very useful in identifying the structures of impurities and metabolites.[2] Resolution can be important when it is necessary to distinguish between fragment ions that are nominally isobaric but have different formulas. Both sulfadimethoxine and stanozolol produce such fragments.

Sulfadimethoxine, as well as many of the sulfa drugs, exhibits a fragment at *m/z* 156 when analyzed by MS/MS. When analyzed by the Agilent 6520 Accurate-Mass Q-TOF LC/MS, close examination of the 156 fragment reveals that there are actually two different fragment ions separated by 65.4 mDa. Even at 1 GHz digitization rate, these two fragment ions are resolved sufficiently so that accurate mass measurement is possible on both fragments. Figure 6 shows the expanded MS/MS spectrum for the ion at *m/z* 156 acquired at 1, 2, and 4 GHz.

Stanozolol fragments to produce a several pairs of ions (for instance, at *m/z* 95, 119, 135, 147, and 161) that differ by 25 mDa in each case (figure 7). With these small differences, 4 GHz digitization is required to adequately measure

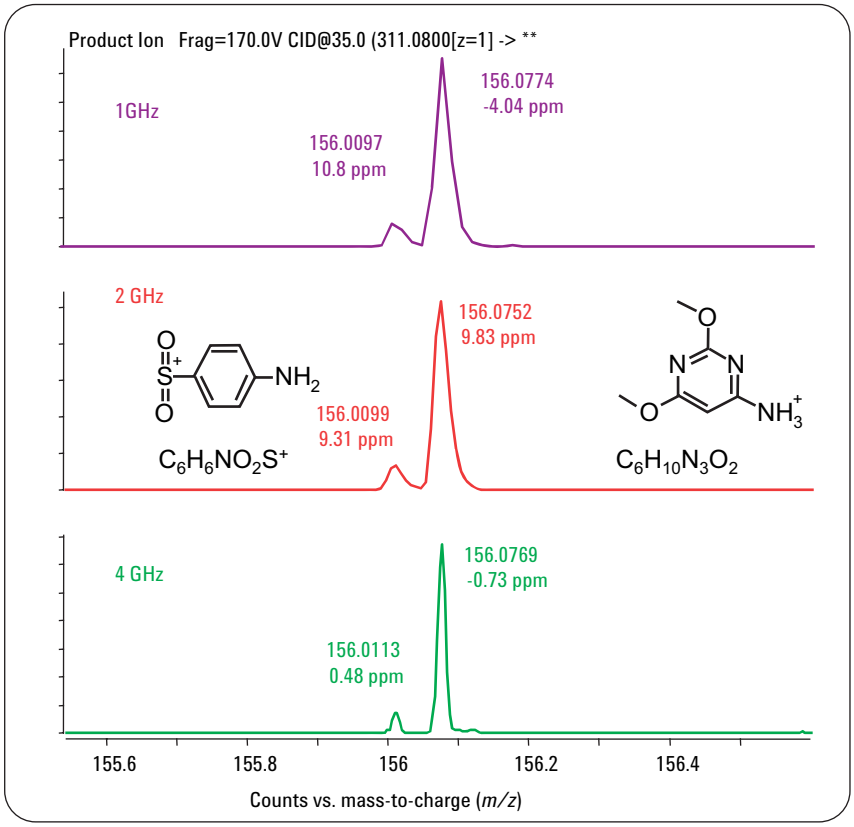

Figure 6
Mass error as a function of digitization rate for the sulfadimethoxine fragments at *m/z* 156.

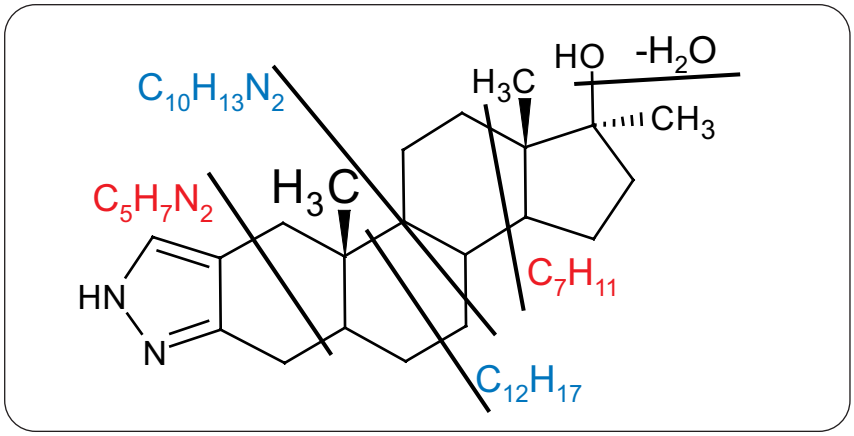

Figure 7
Fragmentation of stanozolol for the ions at *m/z* 95 (red) and at *m/z* 161 (blue).

the mass values. Figure 8 shows the results for the fragment at *m/z* 95. Application of the Agilent molecular formula generator to the MS/MS spectrum acquired at 4 GHz with a typical resolution around 15,000 generated the formulas shown in table 3. These formulas are highly plausible because they are within an absolute mass error window of typically less than 1 mDa and are consistent with the formula for the precursor ion.

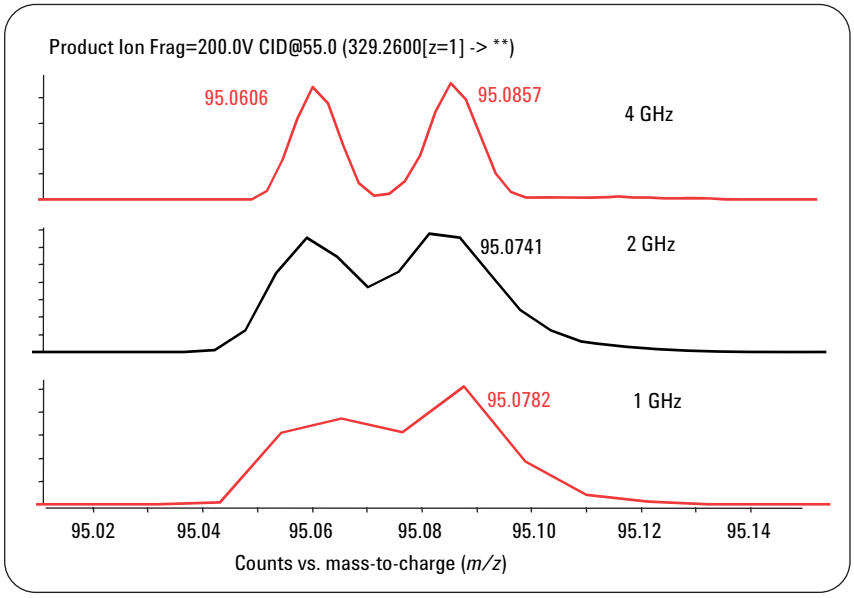

Figure 8
Product ions at *m/z* 95 for stanozolol.

Measured *m/z*	Calculated *m/z*	Difference (mDA)	Difference (ppm)	Formula	Loss mass	Loss formula	Abundance
95.06151	95.06037	-1.13	-11.93	$C_5H_7N_2$	234.19837	$C_{16}H_{26}O$	3495
95.08624	95.08553	-0.72	-7.53	C_7H_{11}	234.17321	$C_{14}H_{22}N_2O$	3337
119.06040	119.06037	-0.02	-0.18	$C_7H_7N_2$	210.19837	$C_{14}H_{26}O$	357
119.08578	119.08553	-0.26	-2.15	C_9H_{11}	210.17321	$C_{12}H_{22}N_2O$	1990
135.09166	135.09167	0.02	0.11	$C_8H_{11}N_2$	194.16707	$C_{13}H_{22}O$	628
135.11642	135.11683	0.41	3.05	$C_{10}H_{15}$	194.14191	$C_{11}H_{18}N_2O$	389
147.09201	147.09167	-0.33	-2.28	$C_9H_{11}N_2$	182.16707	$C_{12}H_{22}O$	376
147.11785	147.11683	-1.02	-6.96	$C_{11}H_{15}$	182.14191	$C_{10}H_{18}N_2O$	301
161.10823	161.10732	-0.90	-5.60	$C_{10}H_{13}N_2$	168.15142	$C_{11}H_{20}O$	112
161.13242	161.13248	0.05	0.32	$C_{12}H_{17}$	168.12626	$C_9H_{16}N_2O$	190

Table 3
Proposed formulas for the fragment ions of stanozolol at *m/z* 95, 119, 135, 147, and 161.

Conclusion

This work shows that:

- Improving the resolving power in TOF mass spectrometry improves mass accuracy when dealing with nominally isobaric impurities.
- Improved dynamic range allows the accurate mass measurement for high- and low-abundance compounds between scans and in-scan.
- Improved resolution of MS/MS data provides more confidence in assigning accurate mass and formula information, leading to easier structure identification.

References

1.
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2.
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