Debating Resolution and Mass Accuracy

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A mass spectrometer's resolution and mass accuracy are the primary considerations for determining whether an instrument suits its intended purpose. Unlike UV and other chromatographic detectors that provide inferential data, mass spectrometers have traditionally provided chromatographers with qualitative information. Now that these instruments are becoming fairly commonplace in liquid chromatography (LC) systems, chromatographers expect them to provide not only mass data but also to possess all the usual performance attributes of traditional detectors, such as dynamic range and sensitivity.

My recent conversation with Roy Martin, a Waters (Milford, Massachusetts, USA) colleague and a well-recognized practitioner in proteomics work, underscores the confusion most practitioners face when they attempt to evaluate and employ a mass spectrometer's capabilities. He describes their almost daily frustration with the failure of well-intended attempts to replace good analytical practice with new technology.

Martin's succinct argument is: "A well-prepared sample, a well-defined analytical goal, the appropriate use of accurate mass, reproducible retention times and good instrument control generates unassailable data." To this he adds: "Make it run in triplicate, and [you] get real data from which to draw conclusions."

But there's an opposing argument, one brought by those who embrace the ever-increasing technological sophistication of these instruments and the software that operates them. It propounds the "one-time" phenomenon, where an operator requires only a single run to obtain a correct result. This argument champions an easy alternative to the daunting prospect of amassing enormous well-conceived data files, hinting seductively at something novel and more intriguing than the grunt work entailed in complex tasks such as characterizing facile changes in metabolic–host interactions in systems biology.

This month we take a quick look at what has become a confusing debate for many: When considering accurate mass and greater resolution, which mass spectrometer provides the information needed at the best purchase price and with the most accessible operational prospects? An industry report summarizing a number of interviews states:

It often seems that end-users are confused as to which types of LC–MS [instruments] are best for them. While in some cases, the performance requirements are so stringent that the choice is simple, more often than not, a potential customer must attempt to weigh the trade-offs of a particular type of LC–MS [instrument] for the application.[1]

When Klaus Biemann, renowned scientist and professor of chemistry at Massachusetts Institute of Technology (Cambridge, Massachusetts, USA), submitted in 1962 what would become a pivotal manuscript to the *Journal of the American Chemical Society*, an anecdote from that submission was later recalled in an autobiographical publication.[2] (The article, by the way, is well worth reading. It characterizes what researchers faced in the 1950s and 1960s, and in truth up through the past ten years or so, when mass spectrometry (MS) became so widely accepted, well characterized — if not completely understood — and accessible.) According to the anecdote, interest in characterizing plant-derived compounds was fuelled by the financial success of the antihypertensive derived from *Rauwolfia serpentina* (reserpine). CIBA (Basel, Switzerland) had marketed reserpine since the 1930s (though, interestingly, it was not characterized structurally until the 1940s). What we take as a simple infusion exercise today was an amazing and tedious adventure back then, made so by techniques such as zinc dust distillation, in which alkyl indoles and pyridines in complex mixtures were purified through crystallization as picrates and identified using melting-point and elemental analysis.

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But Biemann had relied on MS (what became known as the mass spectrometric shift technique) for his characterizations instead of then-conventional methods such as melting point and infrared spectroscopy. One of the reviewers, a well-versed scientist in his own right, questioned whether Biemann had supplied the appropriate level of what was then accepted as state-of-the-art characterization to substantiate his claims of having characterized a number of alkaloids of *Aspidosperma quebracho blanco*. Indeed, Biemann had worked extensively on the alkaloids, but the prospect of relying on an unknown technique was nevertheless seen as radical.

Today, the *Journal of the American Society for Mass Spectrometry* requires authors to consider the "acceptable uncertainty" of the method used to determine exact mass data to support structural determinations. For example, where nominal parent masses encompassing $C_{0–100}$, $H_{3–74}$, $O_{0–4}$ and $N_{0–4}$ display a mass measurement of 118 *m/z*, there must be no alternative formulas within 34 ppm before such a claim is made. Increase the nominal parent mass measurement to 750 *m/z* (where $C_{0–100}$, $H_{25–110}$, $O_{0–15}$ and $N_{0–15}$), and there are 626 alternative formulas within only 5 ppm. The error measurement acceptable at 118 *m/z* must now be 0.018 ppm to eliminate all alternative formulas.[2]

As practitioners have become more experienced and their tools more sophisticated, the differences in the techniques and instruments employed are becoming less pronounced. Mass accuracy was never a disputable commodity, just a difficult, expensive prospect. But to what degree have today's time of flight (TOF) instruments and deconvolution software mitigated the difficulty and expense? How much mass accuracy is enough for a given task? For answers, we must ask: How much of an increase over unit mass accuracy provided by low-resolution quadrupoles and ion traps is needed and actually attainable? How do we evaluate the resolution required to make use of any increased mass accuracy? What are the real costs?

Defining Resolution

First, we must look at resolution and mass accuracy. The ability to "image" a detected ion in a mass spectrometer and differentiate it from any other depends on the characteristics of the detection device — a photomultiplier or electron multiplier in lower resolution instruments or a multichannel plate in higher resolution instruments such as TOF mass spectrometers.

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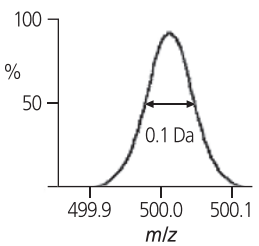
In either instance, the output is similar. When displayed, a change in ion current (using the instrument's tuning software or an oscilloscope) looks like a chromatographic peak except instead of time, the apparent width determines the resolution.

Figure 1 shows a widely accepted comparison based upon measuring the width at half-height (full-width half-height maximum or FWHM) for the response of an instrument to a given ion. Low-resolution (unit mass) instruments typically achieve 0.6 amu widths at any given ion or mass-to-charge ratio in its operating range, yielding figures such as "3000" if, for instance, the mass-to-charge ratio being measured is 1800 (1800 *m/z* divided by 0.6 amu). This comparison method works for a wide variety of instruments. Higher resolution instruments such as TOF mass spectrometers use 0.1 Da (or amu) criteria.

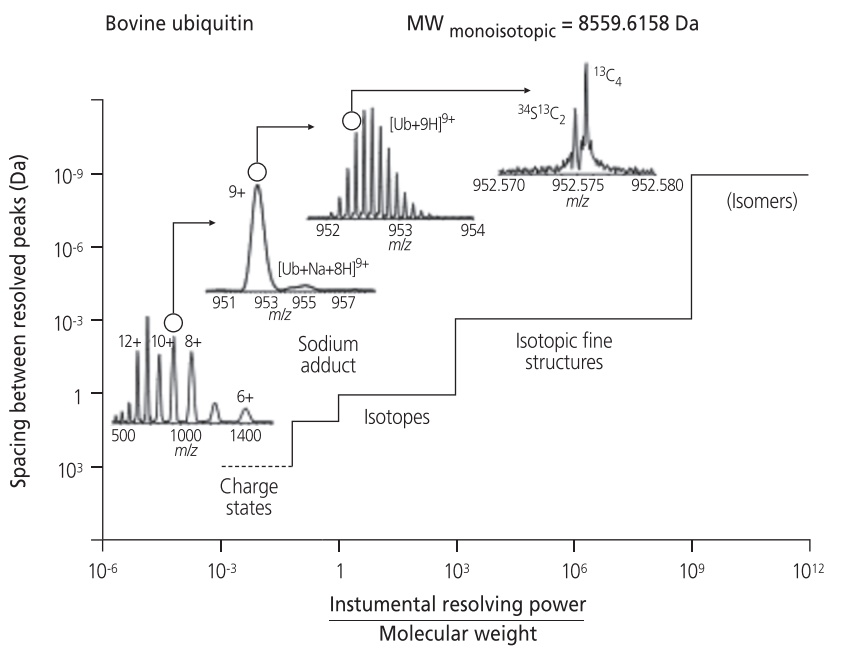
It should be noted that the "10% valley"

Figure 1: Mass accuracy determination and the FWHM method for determining resolution for a mass spectrometer measured at a given ion.



True mass	=	400.0000
Measured mass	=	400.0020
Difference	=	0.0020 or 2 mmu
Error	=	$\frac{0.002}{400} \times 10^6 = 5$ ppm
Mass	=	500
Peak width (@ 50%)	=	0.1
Resolution (FWHM)		$\frac{500}{0.1} = 5000$

Figure 2: The need for informational spacing to increase (instrumental resolving power) between ion peaks and associated isotopes as informational demand increases. (Reprinted with permission from reference 7.)



method is sometimes used but is more appropriate when adjacent peaks of equal height are introduced (as when calibrating magnetic sector instruments but not when comparing a peak and its isotope). Applying this method, one could also measure the width at 5% of the peak height and divide the result into the peak mass. However, such a measurement would typically suppose a 5% contribution from each adjacent peak tail, making up the 10% valley.

FT–MS Ion Cyclotrons and TOF Mass Spectrometers

No matter how high the capability, there are clearly experimental consequences of insufficient resolution, where mass interferences will complicate the outcome. Yet in many applications, this is not a major concern. Arguably, the highest order of resolution is provided by the ion cyclotron resonance (ICR) instrument, which recently took on a higher public profile when Thermo (Waltham, Massachusetts, USA) introduced the ion cyclotron addition to their product line. Bruker (Billerica, Massachusetts, USA) and others have been the market leaders for some time, but their instruments were primarily known only to those well-versed in the art. The cost of the ion cyclotron and the upkeep of a high-powered Tesla magnet, along with the ability to exercise Fourier transform (FT)-based imaging of the output, is not within everyone's reach. In some instances, neither are the instruments' prices.

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Nevertheless, these instruments provide an excellent starting point. We must acknowledge Alan G. Marshall, whose work as director of the National High Field Fourier Transform Ion Cyclotron Resonance Center (Tallahassee, Florida, USA) has contributed to much of what we see in use today. I found one of his figures of particular value in considering the benefits of increasing resolution (Figure 2). We can clearly deduce multiply charged ions with limited resolution (unit mass). But we can do so only up to a point (perhaps where for m/z, $z = 2$ or 3), which is good enough for peptides and the majority of the high-sensitivity work that quadrupole instruments are noted for. Note, however, that need influences one's choice of instrument, varying as the importance of accurately determining mass and rendering careful evaluations of spectral character increases.

The resolution method used can affect the apparent value by roughly twofold if you apply the 10% valley method instead of the FWHM method (see Figure 3). When the analytical need is met by low resolution with low mass accuracy, the higher resolution instruments provide little more than what can be obtained with the readily available triple- or single-quadrupole instruments. Another consideration as resolution increases into the thousands is the fact that sensitivity typically decreases. The importance of improved resolution does not become a persuasive argument until the molecular weight becomes significant (Figure 4).

MS has evinced a rapid evolution of fairly well-known technologies in need of enterprising solutions to make them practical. Electrospray was well publicized in the 1980s but thought impractical except by those willing to work at very low flow-rates. This belief continued until the 1990s, which brought commercial development of heated nebulizers. And although the ion cyclotron can be traced to

Figure 3: Comparison of resolution values and the consequent ability to discern spectral features using (a) the 10% valley method and (b) the FWHM method. Analyte: bradykinin dimer [$C_{100}H_{144}N_{30}O_{21}$ with a nominal mass of 2100]. (Adapted from reference 8.)

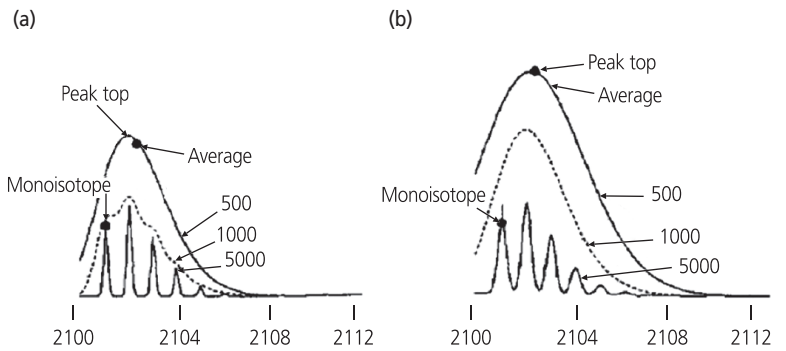
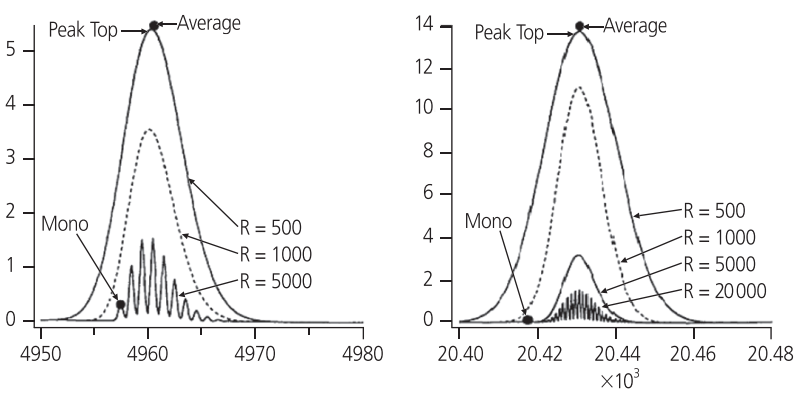


Figure 4: Although spectral features are discerned only as mass increases when the resolution is sufficient, the "composite" envelope (and hence the difference between the peak top and the average mass value) is less pronounced. (Adapted from reference 8.)



Nominal mass: 4957
Monoiso. mass: 4957.486
Avg. mass: 4960.638
Peak top: 4960.2
Gastric inhib. polypeptide $C_{225}H_{341}N_{59}O_{66}S$

Nominal mass: 20417
Monoiso. mass: 20417.578
Avg. mass: 20431.055
Peak top: 20430.9
Pulmonary surfactant $C_{917}H_{1473}N_{235}O_{268}S_{11}$

the work of Hipple, Sommer and Thomas[4] in the late 1940s, it was not until Cooley and Tukey[4] developed the fast FT algorithm in 1965 that widespread work, notably by Marshall and Comisarow,[4] began to appear. (See an excellent timeline of notable publications at http://masspec.scripps.edu.) The first TOF instruments came about in the1940s. Commercialized by the Bendix Corporation, the reflectron, which corrects for effects of kinetic energy distribution of the ions and makes today's resolution in a small instrument possible, actually came from work in 1974 by Boris Mamyrin.[3] Of course, much-improved high-speed electronics were necessary many years later, before TOF became a practical tool.

Although factors such as ease-of-use, service reputation and other issues would be considered when purchasing an instrument, here we are simply evaluating the best tool for the job. Quadrupoles might have lower resolution values, whereas TOF instruments boast resolution up to 10 000. But when mass accuracy is added to the equation, or when the utility of fragmentation experiments is considered as well as sensitivity, a performance-for-the-dollar appraisal emerges.

Defining Mass Accuracy

Simply stated, mass accuracy is the ability to measure or calibrate the instrument response against a known entity. Usually expressed in parts per million (ppm), the measurement indicates the deviation of the instrument response from a known monoisotopic calculated mass. More critical measurements are demanded today as such work becomes more commonplace. Although simply adding a known calibrant to the sample or introducing the calibrant postcolumn sometimes suffices, a recurring lock mass emulates the atmospheric ionization phenomena, reducing anomalies to ensure stabilization against changes in the instrument's environment. Whereas a few years ago some instruments were operated without calibration, most critical operation today recommends continuous calibration.

Interlocking factors make the task of effectively evaluating an instrument difficult because, as already noted, they depend upon the intended application. The data provided in the SDI (Los Angeles, California, USA) report[1] do provide a compelling argument, however, when we consider mass range, cost, resolution and what we have seen of obtainable mass accuracy (see Figure 5 and Table 1). The report states:

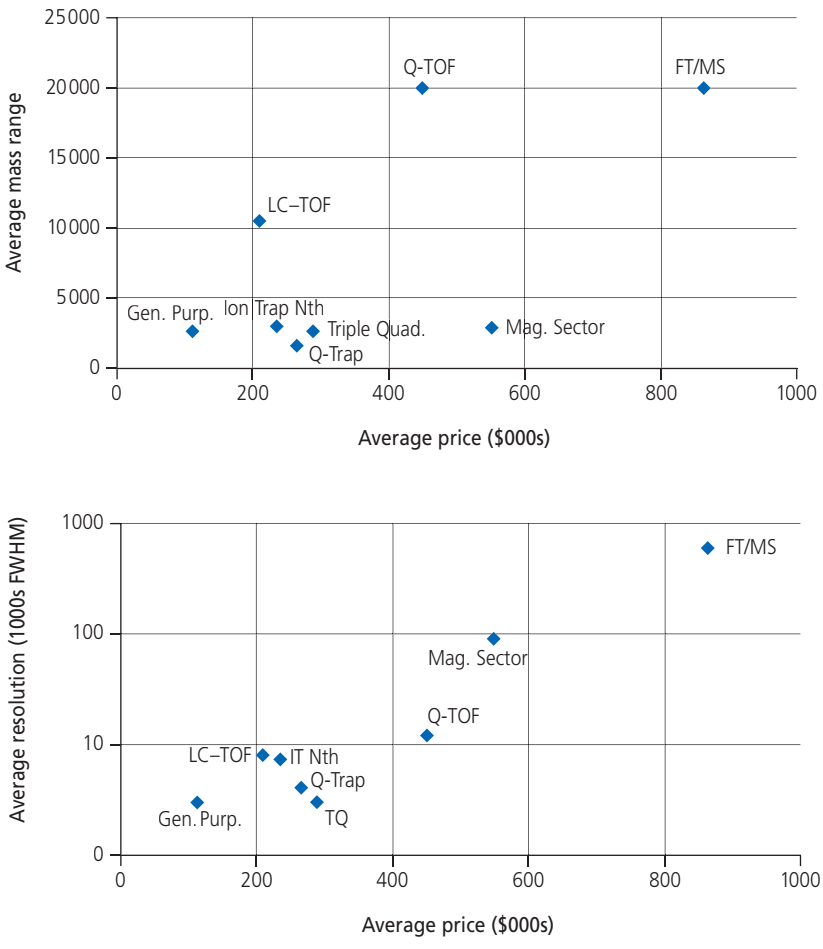
When mass range is compared to price, the obvious strengths of various instruments become very apparent. Time-of-flight (LC-TOF and Q-TOF) clearly have the best mass range per dollar. High resolution systems are all approximately even with general purpose (low resolution) instruments in base mass range, although their MS/MS capabilities can extend (their utility) somewhat.[1]

Accepting the argument that the magnetic sectors and FT–MS are not indicated except for specific applications, leaves the TOF-based instruments when sensitivity and other factors are applied.

Metabolism studies following the administration of a novel drug candidate can give rise to various outcomes. Much like Biemann's experience before the common availability of high-quality acquisitions, the approach to such an experiment was quite a different exercise. Consider the differentiation of a sulphide from an aldehyde for rabeprazole. At low resolution, the practitioner obtains retention times but would be unable to make a *de novo* determination. However, the ability to discriminate at 2 or 3 ppm facilitates the task, as shown in Figure 6 and Table 2.

The leading edge of instrumental art, at least for instruments in common use, is now 2–5 ppm, which many instruments achieve with an external calibration or lock mass. Clearly, the closer the result of an unknown is to the actual value, the better the results. Metabonomics, the name given by Jeremy Nicholson (Imperial College, London, UK) to the study of the metabolic interactions within a living organism or the quantitative and qualitative analysis of proteins expressed in cells in response to

Figure 5: Differentiated by overall cost (and complexity), the combination of quadrupole technology and TOF appears to deliver the best capability, especially in terms of performance (fragmentation for structural characterization, quantitative capabilities and sensitivity). (Reprinted with permission from reference 1.)



varying conditions, offers good examples. A protein can display various functions, each coming about in cellular pathways via interactions with various components such as other proteins, small molecules and DNA. Determining a protein's function requires, first and foremost, knowledge of its structure.

Small-molecule work shares the same need for increased mass accuracy for reasons similar to those that benefit large-molecule work. The benefit might be that mass accuracy is easier to obtain at lower molecular weights with greater opportunities (e.g., fragmentation experiments) for structural characterization. Metabolite determination based on the M + 4 isotope is an excellent example of extended-resolution requirements in practice with small molecules.[4]

Similarly, in complex analyses, such accuracy and precision greatly aids finding unknowns, as in an effort to track the dispersion of pharmaceuticals in the environment. Accuracy and precision, along with the "well-prepared sample and well-defined analytical goal" concept, the improved dynamic range, and other performance facets of properly applied modern software and hardware, meet the needs. Although the capabilities of FT–ICR MS are undeniable, it might prove otherwise in practical application. An excerpt from an article I recommend (because the authors made a considerable effort to provide illustrations of mass accuracy in practice) illustrates this conclusion:

FT–ICR MS provides unequalled resolving power and mass accuracy as long as sufficient time is available to acquire the necessary degree of information . . . the problem is (the) required time is too long to allow sufficient spectra to be obtained across a high-resolution (chromatographic) peak for the peak to be properly delineated.[6]

The necessity we often find to ensure "the correct tentative identification" is to use, as these authors suggest, "chemical intuition to discard unlikely compositions… making extensive literature searches and making limiting assumptions concerning the elements present."

The quadrupole TOF therefore appears to be a good choice. Its price is decreasing and its software ease-of-use is increasing through experience of years in the field. Thus, it follows the pattern established by the Thermo LTQ ion trap, whose improvements now make it amenable to quantitative applications when not so long ago, an ion trap would not have warranted consideration. Increasingly, TOF instruments are used in walk-up or open-access applications in which the sample submitter is not an experienced MS operator.

A recent conversation with Luke Miller, Head of Analytical Research at the GlaxoSmithKline RTP Facility (Reasearch Triangle Park, North Carolina, USA), argues for this paradigm. A critically important effort in drug-discovery support is to reduce the burden of characterizing hundreds of submitted samples by increasing run throughput. Thus, runs that formerly required minutes to return an answer to the submitter would be measured in mere seconds. GlaxoSmithKline wants to develop a very high-speed acquisition system for which the TOF mass spectrometer is so far the most likely candidate. This conclusion is not because of superior resolution and mass

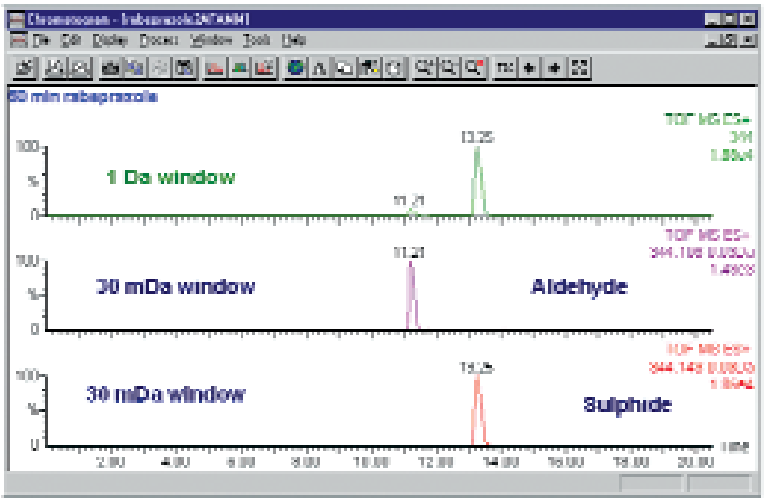
Table 1: A comparison of leading higher resolution mass spectrometers (adapted from reference 1).

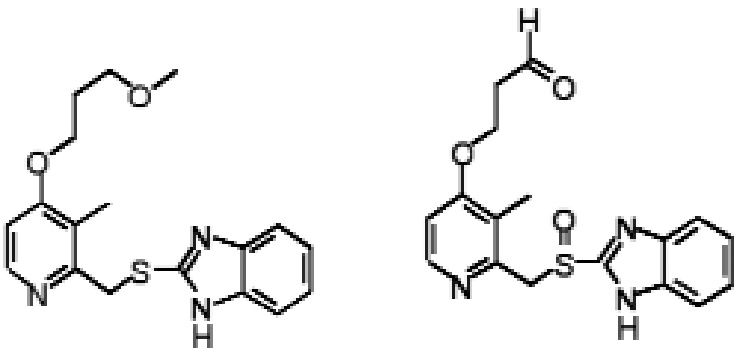
	Resolution (FWHM)	Mass range (*m/z*)	Mass accuracy (ppm)	Price ($, typical range comparison)
TOF			2–5 typical	200–250K
Waters LCT Premier	10000	18000		
Agilent LC/MSD TOF	10000	7000		
Bruker MicroTOF	10000	3000		
Q-TOF			Same as TOF	300–700K (450K typical)
Waters QTOF Ultima	17500	32000		
Waters QTOF Micro	5000	20000		
MDS Sciex Qstar XL	10000	40000 (TOF) 20000 (Q)		
Bruker BioTOF	20000	10000 (TOF)		
ICR (FT–MS)				500K–1.4M
Bruker Apex IV	100000	66000	<1	
Ion Spec	1300000	18000	1	
Thermo LTQ FT	500000	2000	2	

Table 2: Sulphide and aldehyde metabolites that can be differentiated only by high mass accuracy (Courtesy of Waters Corporation, Milford, Massachusetts, USA).

Component	Calculated (*m/z*)	Measured (*m/z*)	± (mDa)	± (ppm)
Parent	360.1382	360.1366	1.6	4.4
Sulphide	344.1433	344.1424	0.9	2.6
Sulphone	376.1331	376.1330	0.1	0.3
Desmethyl	346.1225	346.1218	0.7	2.0
S-Desmethyl	330.1276	330.1265	1.1	3.3
Aldehyde	344.1069	344.1074	0.5	1.5
S-Pyridone	272.0858	272.0867	0.9	3.3

Figure 6: Two metabolites of rabeprozole: (bottom left) a sulphide and (bottom right) an aldehyde, measured as differing by only 0.035 amu (or mDa). This result illustrates the ability of high mass accuracy instruments compared with (top) low-resolution (unit mass) instrument responses (shown in the upper frame), which is capable of only 1 Da (or mass unit) resolution. (Courtesy of Waters Corporation, Milford, Massachusetts, USA.)





Sulphide
m/z 344.1433 (2.3 ppm)

Aldehyde
m/z 344.1069 (1.5 ppm)

accuracy but rather is because of its potential for acquisition speed when chromatographic peaks have been reduced to a width of 1 s.

A final note to those interested in current practice and the near future of small-molecule science: You might wish to visit the "Conference on Small Molecule Science" website (www.COSMOS2004.org). The meeting will be held 8–14 August 2004 on the shores of Narragansett Bay at Roger Williams University, Bristol, Rhode Island, USA.

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