# High Resolution on a Quadrupole Ion Trap Mass Spectrometer

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By using a modified ion trap mass spectrometer, resolution in excess of 30,000 (FWHM) at $m/z$ 502 is demonstrated. The method of increasing resolution in the ion trap mass spectrometer operated in the mass-selective instability mode depends on decreasing the rate of scanning the primary radio frequency amplitude as well as using resonance ejection at the appropriate frequency and amplitude. A theoretical basis for the method is introduced. *(J Am Soc Mass Spectrom 1991, 2, 198–204)*

The capabilities of quadrupole ion traps have continued to expand since the development of the mass-selective instability mode of operation and its subsequent commercial availability [1]. The versatility of these relatively simple mass spectrometers has been demonstrated by their high sensitivity in both electron impact [2] and chemical [3] ionization and their ability to serve as gas-phase ion/molecule reactors [4]. Successful injection of ions into these devices has also allowed techniques such as laser desorption [5], cesium ion desorption [6, 7], and, most recently, electrospray ionization [8, 9] to be used for the study of biomolecules. The ion storage ability of the quadrupole ion trap makes possible tandem mass spectrometry (MS/MS) involving many stages of mass analysis with efficient dissociation of ions [10]. Even parent MS/MS scans have been reported, with the possibility of neutral loss scans to follow [11]. Remarkably, extension of the usable mass range of these devices has also been demonstrated to be beyond 45,000 u [12].

Despite this list of capabilities, one arguable limitation of a quadrupole ion trap mass spectrometer (QITMS) with respect to other types of instruments, such as sector (including three- and four-sector instruments) and Fourier transform-ion cyclotron resonance instruments, is the constraint of always operating at relatively low resolution. In this article, achievement of resolution beyond 30,000 FWHM (full width at half maximum) by using a QITMS is reported.

## Experimental

The QITMS used for this work was designed for biotechnology research and is similar to instruments that have been described previously [5] for ion injection experiments. The instrument included an external ionization region in which electron ionization, chemical ionization, cesium ion desorption, laser desorption, as well as electrospray ionization, can be implemented. The source region is differentially pumped with respect to the analyzer region, and the ions are injected axially into the ion trap. To aid in the detection of high mass ions, a 20-kV off-axis dynode with a continuous dynode electron multiplier was used [13]. The electrospray source is of the Fenn-Whitehouse design as described in the literature [14]. Theoretically, external ionization, as well as the other modifications mentioned, are not required for implementation of the method for obtaining high resolution, although at this point the hardware modifications necessary to achieve high resolution have not been performed on a standard system using internal ionization.

The combination of mass-selective instability with resonance ejection is required for achieving high resolution on the ion trap. Thus, an auxiliary frequency synthesizer is connected between the trap end-cap electrodes, allowing dipolar excitation and ejection of trapped ions. A frequency synthesizer that can deliver a maximum amplitude of 6.0 volts peak-to-peak between end-cap electrodes is standard on an ITMS$^{TM}$. A radio-frequency (rf) amplifier with a gain of approximately 10 dB was used in these experiments to achieve higher auxiliary voltages.

A relatively simple modification of the standard ITMS$^{TM}$ electronics for slowing down the rate of rf scanning is described by Kaiser et al. [12], and this method is used for the work reported here, although facilities for even slower scan speeds were implemented. However, a limitation of this method for decreased scan speeds is that the slower the scan speed, the narrower the mass window that can be scanned. Nonetheless, this mass window can be positioned anywhere within the mass range. Accessible scan rates ranged from the normal rate of 5550 u $s^{-1}$ down to 27.8 u $s^{-1}$.

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## Discussion

A theoretical basis for obtaining high resolution on a QITMS may be extrapolated from arguments made by Fischer in 1959 [15]. In that article, Fischer considers resolution with respect to the mass-selective detection mode of operating an ion trap and the effect of the auxiliary field amplitude. Fischer argues and provides a mathematical model asserting that the resolution using this detection method is proportional to the number of resonance field periods that an ion experiences before being lost. Fischer uses these arguments to conclude that to increase resolution in the ion trap, the amplitude of the auxiliary field should be minimized in order to maximize the number of ion oscillations. However, varying scan speed (in his case the rate of change of the dc potential) was not considered.

When using the mass-selective instability mode of operation with resonance ejection, the same relationship will exist between resolution and the interaction time of the ion with the auxiliary field. Using this mode of operation (and Fischer's mode), one can use not only the auxiliary field amplitude but also the scan speed (in this case the rate of change in the primary rf field amplitude) to control the interaction time, and therefore the resolution.

The amplitude of the auxiliary field is, indeed, found to be critical in obtaining high resolution; however, its ability to enhance resolution is bounded by the primary parameter of scan speed. That is, high resolution is ultimately obtained by using low scan speeds in conjunction with an appropriately low amplitude auxiliary field (at the appropriate frequency). The amplitude required for a particular experiment is complicated by the fact that it can be dependent on the number of ions in the trap as well as the mass of the ions being ejected. Thus, for all data shown, the amplitude of the auxiliary field was kept at the minimum level, which still caused efficient and favorable ejection of ions.

The frequency of the auxiliary field is also found to be an important parameter. This frequency determines the $q_z$ (and $\beta_z$) value at which ions are ejected. It therefore determines the mass range and also the scan rate in u $s^{-1}$ of the instrument [12]. For a fixed rate of change of the rf amplitude, the lower the $q_{z\text{-eject}}$, the higher the mass range and the higher the scan rate in terms of u $s^{-1}$. In these studies, significant enhancement in resolution has been obtained when using $q_{z\text{-eject}}$ values between ca. 0.1 and 0.85. Thus far, the highest demonstrated resolutions were obtained with $q_{z\text{-eject}}$ values in the range between 0.75 and 0.85. This may be accounted for, in part, by the fact that it was possible to achieve lower scan rates (u $s^{-1}$) when using the higher $q_{z\text{-eject}}$ values. As previously mentioned, substantial mass range extension is realized when $q_{z\text{-eject}}$ is dropped below ca. 0.75. In this case, one effectively trades resolution for increases in the mass range. With resonant ejection near the stability limit of 0.908, reduction in the scan rate much below the standard scan rate of 5550 u $s^{-1}$ does not result in significant improvement in resolution. A detailed description of the relationships between the trapping field frequency, the auxiliary field frequency, the auxiliary amplitude, and scan speed are still under investigation and will be the subject of a future publication.

Increased resolution has been previously reported when the effective scan speed was reduced, and a resolution of approximately 3500 (FWHM) at $m/z$ 2850 was achieved [12, 16]. However, a theoretical basis for the observation was not discussed, and experimental limitations did not allow further exploitation of the process for obtaining higher resolution.

## Results

The first observations of increased resolution in this study were made while performing electrospray/ion trap experiments [8, 9]. In these early experiments, the mass range had been extended by a factor of 10 by using resonance ejection [6] at a frequency of 35371 Hz ($q_z$ = 0.0908) and an amplitude of 2.9 volts (all auxiliary field amplitudes are given in peak-to-peak, endcap-to-endcap values). This factor of 10 increase in mass range results in a scan speed of 55500 u $s^{-1}$, which is ten times the normal scan speed of 5550 u $s^{-1}$. As is shown in Figure 1, an increase in resolution was observed for the electrospray ion trap mass spectrum of horse heart myoglobin when slowing the scan down from 55500 u $s^{-1}$ (Figure 1a) back to the normal scan speed of 5550 u $s^{-1}$ (Figure 1b).

It is difficult to calculate directly the resolution of these spectra due to the intrinsic peak-width attributed to a particular charge state by the normal presence of isotope peaks. That is, for myoglobin, the molecular ion isotope distribution at mass 16,950 has a width at half-height of approximately 10 u. For the +20 charge state, the calculated intrinsic peak-width is, therefore, 10/20, or 0.5 u. An approximate resolution may then be calculated by subtracting the observed peak-width from the theoretical peak-width (0.5 u at FWHM) and using this for $\Delta m$. For the myoglobin mass spectra shown, the amended value for $\Delta m$ yields estimated resolutions of 360 and 5000, at $m/z$ 1000, for the faster (55500 u $s^{-1}$) and slower (5550 u $s^{-1}$) scans, respectively. Other experimental conditions are indicated in Figure 1a and b.

An electron ionization source was then used for (external) ionization, and the spectrum of xenon with all its isotopes acquired under normal operating conditions, including a scan speed of 5550 u $s^{-1}$, mass range of 650 u, and resonance ejection at 520000 Hz with 6.0 volts amplitude, is displayed in Figure 2a. These conditions yield typical peak widths of 0.33 u at FWHM and, therefore, a resolution of approximately 400 at $m/z$ 132. Figure 2b shows the full isotope cluster of xenon at a scan speed of 1/20 the normal

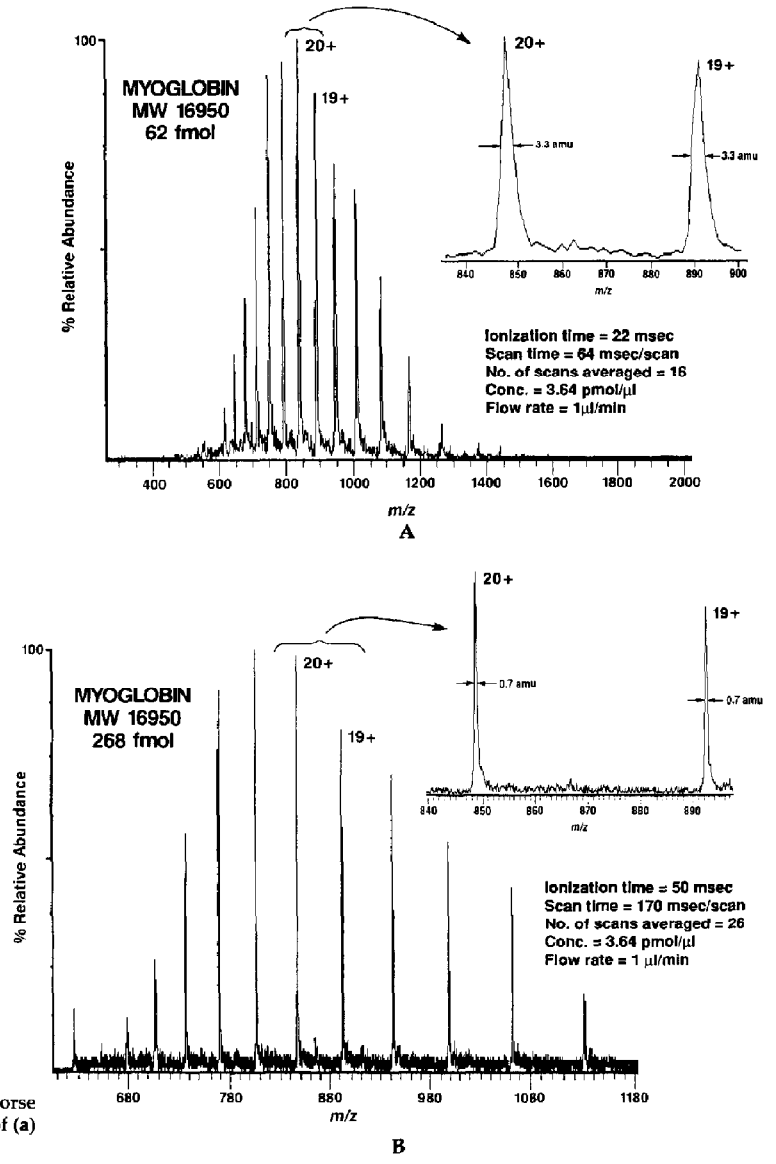


**Figure 1.** Electrospray ion trap spectrum of horse heart myoglobin (MW 16900) at a scan speed of (**a**) 55500 u $s^{-1}$ and (**b**) 5550 u $s^{-1}$.

scan speed, i.e., 278 u $s^{-1}$, using resonance ejection at 403017 Hz and an amplitude of 4.5 volts. The peak-width of $m/z$ 132 at FWHM is approximately 0.073 u, thus a resolution of roughly 1800 is achieved. Figure 2c shows a portion of the xenon isotope spectrum including the abundant isotopes of $m/z$ 131 and 132 under conditions of 1/100 the scan speed (55.5 u $s^{-1}$) using a resonance frequency of 363543 Hz and an amplitude of 4.6 volts. The peak-width of the $m/z$ 132 peak at FWHM is shown to be approximately 0.035 u, giving a resolution of approximately 3800 at this mass.

Achievement of considerably higher resolution on a QITMS is demonstrated in Figure 3, which shows the $m/z$ 502 and 503 peaks of the mass spectrum of perfluorotributylamine (FC-43) ionized by using an external electron ionization source. Figure 3a shows the mass spectrum that was acquired by using a normal scan speed of 5550 u $s^{-1}$ and resonance ejection at 520000 Hz and an amplitude of 6.0 volts, indicating typical peak-widths and resolution ($M/\Delta M$ = 1700) under standard operating conditions. Figure 3b shows the same mass spectrum at a scan speed of 55.5 u $s^{-1}$ with a tickle frequency of 468623 Hz and an amplitude of 1.1 volts. The inset shows that by increasing the gain and the number of scans averaged, $m/z$ 504 may also be observed. The peak-width of $m/z$ 502 is approximately 0.030 u (FWHM), and thus the resolution is approximately 17000. This peak-width

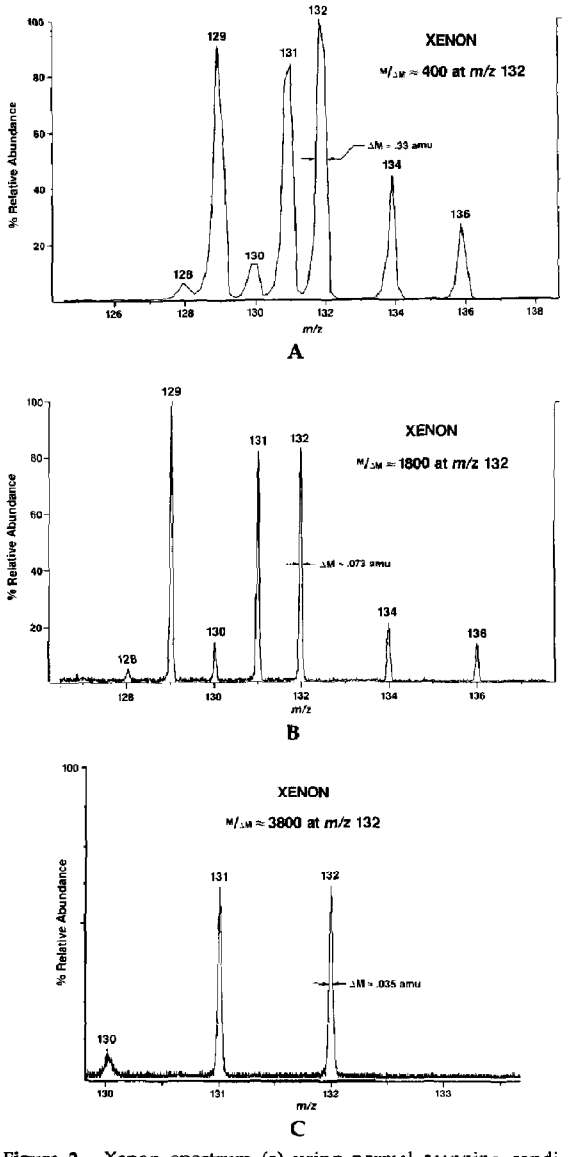


**Figure 2.** Xenon spectrum (**a**) using normal scanning conditions of 5550 u $s^{-1}$; (**b**) using 278 u $s^{-1}$ scan speed; (**c**) $m/z$ 131 and 132 scanned at a speed of 55.5 u $s^{-1}$.

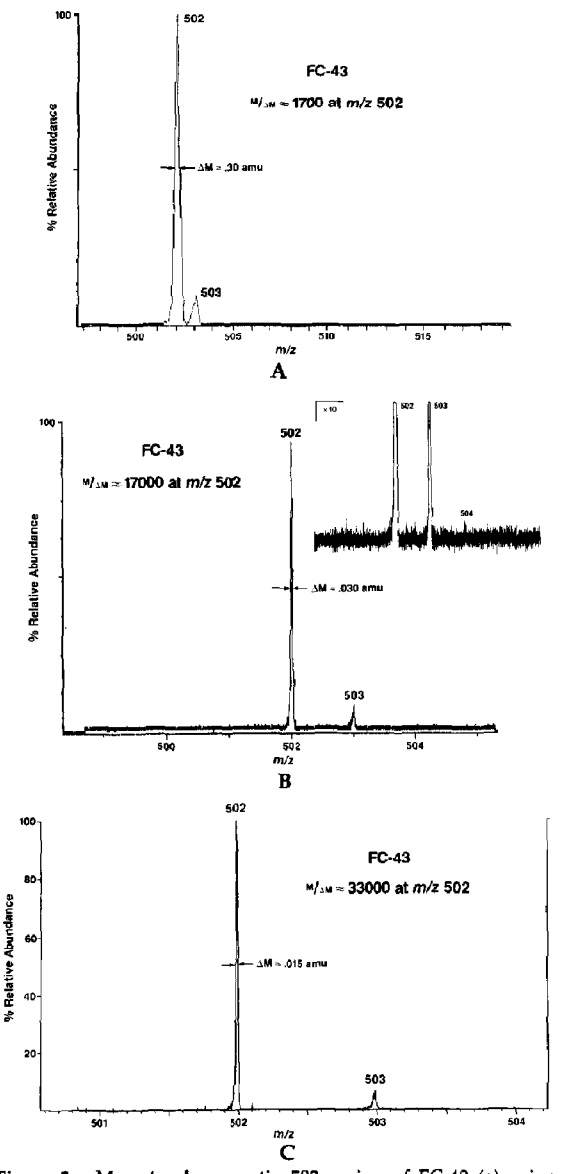


**Figure 3.** Mass to charge ratio 502 region of FC-43 (**a**) using normal conditions of 5550 u $s^{-1}$; (**b**) at 55.5 u $s^{-1}$ scan speed. Inset shows that, at approximately ten times the gain, the presence of $m/z$ 504 is detected (**c**) using 27.8 u $s^{-1}$ scan speed.

is comparable to the one observed in Figure 2c and indicates the property of the trap to provide constant peak-width throughout the mass range and, therefore, increasing resolution with increasing mass. In Figure 3c, the scan rate has been attenuated by a factor of 200 to 27.8 u $s^{-1}$, with a tickle frequency of 466623 Hz and an amplitude of 0.58 volts. The peak-width at FWHM of mass 502 is approximately 0.015 u wide, thus the resolution (M/ΔM) is 33000.

One difficulty observed in these experiments is that, at the very low scan speeds (55.5 u $s^{-1}$ or 27.8 u $s^{-1}$), a slight shifting of the peak position occurs with each scan (and most likely within a single scan). The result is that, when averaging many scans, the observed peak-width is actually broadened. This drift is most probably due to rf instability, which can only be observed at these low scan rates and narrow peak-widths. It is possible that with appropriate hardware modifications this problem can be rectified, thereby allowing even greater resolution. For these experi-

ments, however, this difficulty set the fundamental limit for achieving greater resolution.

While the effect of slowing the rf scan rate on sensitivity is not yet completely characterized, a reduction in signal-to-noise (S/N) ratio is generally observed with a decrease in scan rate. For xenon, a large decrease in S/N ratio is observed that is roughly proportional to the scan speed. However, much of this reduction in S/N ratio is attributed to charge exchange with background neutrals such as oxygen. This is evidenced by the increase in abundance of the oxygen peak as overall scan time increases. The FC-43 data suggest a much smaller decrease in S/N ratio with scan speed that is roughly one tenth that observed for the xenon data. By neglecting loss of ions due to sources such as charge exchange and collision induced dissociation, the mass peak area (i.e., the number of ions) should remain constant with a decrease in scan speed. Although the peak-width decreases when measured in units of mass, it actually increases when measured in units of time. Hence, peak height will decrease proportionally. The resultant S/N ratio will vary inversely with peak-width (measured in units of time). The FC-43 data are consistent with this analysis. With appropriate filtering, the S/N ratio can be made to vary inversely with the

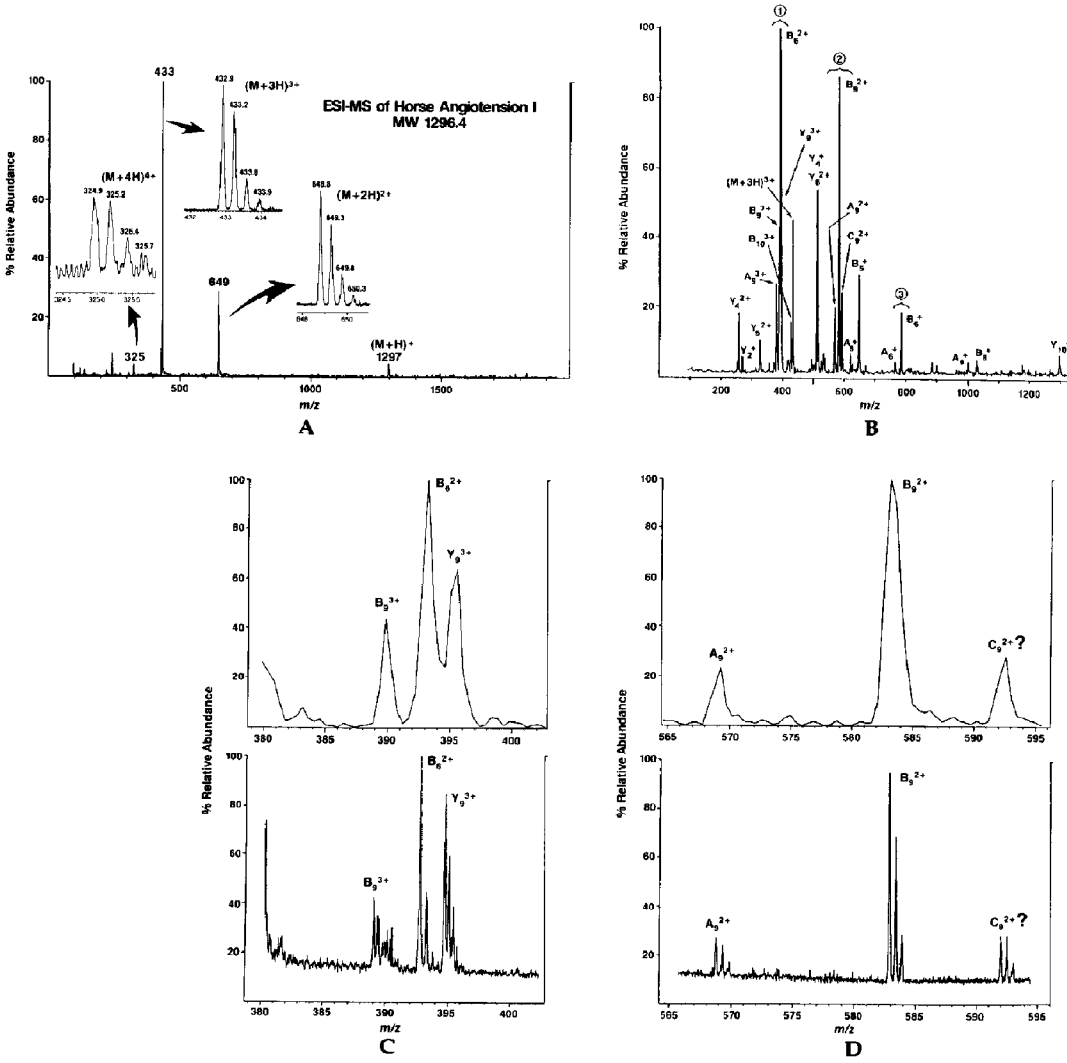


**Figure 4.** Electrospray ion trap data of horse angiotensin I (a) mass spectrum at 16650 u $s^{-1}$. Insets show pseudomolecular ions scanned using 278 u $s^{-1}$ scan rate. (b) Tandem mass spectrometry daughter ion spectrum of the $[M + 3H]^{3+}$ ion at $m/z$ 433. Regions scanned using higher resolution are indicated. (c) Region 1 scanned at 5550 u $s^{-1}$ and using 278 u $s^{-1}$. (d) Region 2 scanned at 5550 u $s^{-1}$ and using 278 u $s^{-1}$. (e) Region 3 scanned at 5550 u $s^{-1}$ and using 278 u $s^{-1}$.

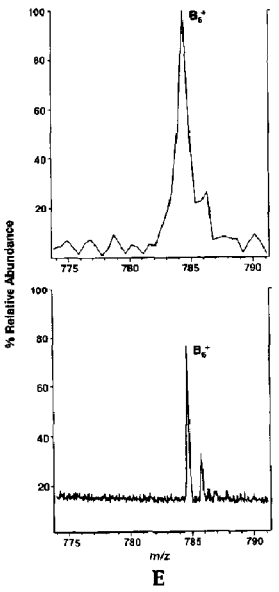


Figure 4. (continued).

*square root* of the peak-width (measured in time), thus minimizing the penalty of decreased S/N ratio when reducing scan rates in order to achieve higher resolution.

Another basic problem of the ion trap used for high resolution work involves mass accuracy. The mass assignment of ions produced by the quadrupole ion trap is sensitive to many parameters including auxiliary field frequency, auxiliary field amplitude, helium pressure, and the number of ions in the trap. Although each of these factors can be characterized and compensated for, the utility of high resolution on the ion trap for accurate mass measurements is still to be determined.

Among the many possible applications, mass spectrometry for biochemical applications should particularly benefit from high resolution on the ion trap. As mentioned earlier, electrospray ionization has recently been coupled with the ion trap [8, 9], and notable uses of high resolution will be in the identification of charge states of multiply charged ions by using isotope separation and in the identification of heterogeneities in protein samples, such as those that occur with multiplicities of glycoforms. In addition, obtaining high resolution daughter ion mass spectra to help with the interpretation of peptide and protein sequencing data is also possible, potentially at even higher resolution than obtainable on four-sector magnetic or Fourier-transform–ion cyclotron resonance instruments. Some preliminary results demonstrating applications in these areas are described in the following figures. The electrospray ionization mass spectrum of horse angiotensin I is shown in Figure 4a. This spectrum was obtained by extending the mass range by a factor of 3 and using resonance ejection at 119936 Hz ($q_{z\text{-eject}}$ = .30), resulting in a scan speed of 16650 u $s^{-1}$ scan. The singly, doubly, triply, and quadruply protonated ions are indicated. Scanning more slowly across the multiply charged ions using a scan rate of 278 u $s^{-1}$ and a resonance ejection frequency of 464371 Hz provides isotopic separation of these species, as displayed in the inset windows. This scan speed yields a peak-width (approximately 0.087 u FWHM) that readily allows identification of the charge state for these pseudomolecular ions. The electrospray ionization MS/MS daughter ion mass spectrum of the $[M + 3H]^{3+}$ ion at $m/z$ 433 after its isolation using a normal 5550 u $s^{-1}$ scan is shown in Figure 4b. Figure 4c–e, shows small sections of the daughter spectrum (indicated in Figure 4b) that have been obtained utilizing a scan speed of 278 u $s^{-1}$. Again, the resolution achieved readily allows the identification of charge states for these daughter ions by using the mass separation of the isotopes and, therefore, simplifies sequence ion assignments in the daughter ion mass spectrum.

## Conclusions

High resolution (> 30000 FWHM) using a QITMS has been demonstrated. These preliminary results show considerable promise in advancing the applications of the already versatile QITMS into more diverse areas of research including biochemistry and biotechnology.

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