Comparison of an Ion-Trap and a Quadrupole Mass Spectrometer using Diazepam as a Model Compound

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Abstract

Recent innovations in mass spectrometry (MS) have led to the development of instruments with increased capabilities, smaller footprints, and relatively low cost. The traditional MS in most toxicology laboratories is a quadrupole system equipped with electron impact ionization. Recently, an ion trap with electron impact, positive chemical ionization, negative chemical ionization, and tandem MS capabilities was introduced by Finnigan MAT. This paper compares the sensitivity and precision of ion-ratio measurements between a Finnigan GCQ ion-trap mass spectrometer (ITMS) and a Hewlett Packard quadrupole mass spectrometer (QMS) using electron impact ionization with diazepam as the model compound. Additionally, the sensitivity and precision of ion ratio measurements are evaluated for the ITMS using positive chemical ionization, negative chemical ionization and tandem MS modes of analysis. In the full scan mode (m/z 50–650, 1 Hz), the ITMS had an average signal-to-noise ratio (S/N) of 1400 for a 2-ng injection of diazepam (10 injections per day for 5 days), within-run ion ratio precision had coefficients of variation from 5 to 11%. Using similar full scan conditions, a 10-ng injection of diazepam on the QMS had an average S/N ratio of 160, and precision of ion ratio measurements varied from 5 to 13%. In the selected ion mode (SIM) of analysis (three ions, 2 Hz), the ITMS had an average S/N of 14,000 for a 2-ng injection and ion-ratio precision ranging from 6 to 15%. Using similar SIM conditions, a 2-ng injection in the QMS had an average S/N of 3000 with ion ratio standard deviations of 0.67 to 2.9%. Overall, the ITMS provided at greater S/N, equivalent precision in full scan, but was 5- to 10-fold less precise in measuring ion ratios in the SIM mode as compared with the QMS.

Introduction

Benchtop mass spectrometers (MS) have an essential role in identifying the presence and quantitating the amount of various xenobiotics in biological specimens. The two types of instruments most useful in toxicology laboratories that fit the generic definition of "benchtop" (e.g., low cost and small footprint) are continuously scanning quadrupole mass spectrometer (QMS) and ion-trap mass spectrometer (ITMS). Although there are many important distinctions between ion traps manufactured by various companies (and similarly for quadrupole mass analyzers), the larger difference is found when ion traps are compared with quadrupole mass analyzers. It is essential that the fundamental differences between these instruments are understood when planning for their expected routine use in the laboratory, that is, forensic urine drug testing confirmation versus general unknown identification.

Ion formation in a QMS takes place in the ion source which is physically located before the radio-frequency (RF) and direct-current (DC) rods that function as the mass analyzer. In the case of the ITMS, ions can be formed before the trap (external ionization) or within the trap itself (internal ionization). Again there are important differences between internally and externally ionized ITMS systems, but in the case of both QMS and ITMS, ions are formed through similar reactions. Once formed and separated according to mass-to-charge ratio (m/z), ions are typically detected in both QMS and ITMS by a continuous dynode electron multiplier. Again, the primary difference between these types of instruments, which is the focus of this article, is in the mass analysis stage. ITMS stores ions until the trap is full (millisecond time scale), whereas in the QMS, ions are continuously formed, accelerated into the quadrupole, and mass analyzed.

The ion storage capability of ITMS systems can give this system an inherent advantage in sensitivity as compared with the QMS. The ITMS stores ions by trapping them between two endcap electrodes and a ring electrode. An RF voltage is applied to the ring electrode (and sometimes a DC voltage relative to the endcap electrode), which causes a rapid change in field polarities. By fluctuating the electric field, ions are alternatively accelerated and decelerated in an oscillating manner, forming a stable trajectory within the trap. A mass spectrum is produced by varying the RF to eject ions out of the endcap (commercial instruments) at specific mass-to-charge ratio values where they are detected by the electron multiplier. Most commercially available ion traps have some sort of automatic gain...
control, a gating system to ensure that the trap is filled in an optimum manner. Time required to fill the trap typically varies between 0.1 and 25 ms (1). The ability of the ITMS to store a single mass range of interest is contrasted by the QMS where the majority of the ions never reach the detector. At any point in time during mass analysis using a QMS only one specific mass-to-charge value is reaching the multiplier.

Although sensitivity is an important component of any mass spectrometer system, precision of ion-ratio measurements is equally important in many toxicology settings. Many ITMS procedures have been developed for the analysis of drugs of abuse (2-11). Unlike QMS methods, which are often based on selected ion monitoring (SIM), most ITMS procedures scan a single mass range of interest. One paper specifically compared an ITMS with a QMS for NIDA urine drug testing and demonstrated increased sensitivity of the ITMS for this purpose (12). However, there are very few data comparing the precision of ion ratio measurements between these two types of instruments. Here we compare the sensitivity and precision of ion ratio measurements between a QMS and an ITMS using diazepam as a model compound. Additionally, the sensitivity and ion ratio stability of the ITMS were evaluated in the positive chemical ionization (PCI), electron capture negative chemical ionization (NCI), and tandem mass spectrometry (MS-MS) modes of analysis.

Materials and Methods

Diazepam was obtained from Sigma Chemical (St. Louis, MO). High-purity grade methanol was from Burdick and Jackson (Muskegon, MI). High-purity helium (99.99%, Airgas, Radnor, PA) was the carrier gas used for all experiments. The reagent gas for chemical ionization was methane (research grade, 99.99%, Airgas).

The ITMS was a Finnigan MAT (San José, CA) GCQ equipped with external ionization, MS-MS capabilities, electron impact ionization, positive chemical ionization and negative chemical ionization. The QMS was a Hewlett Packard (Palo Alto, CA) HP 5971A equipped with a 5890 gas chromatograph (GC).

For ITMS experiments, a crosslinked dimethyl polysiloxane (DB-1, 15 m x 0.32 mm, 0.25-µm film thickness, J&W Scientific, Folsom, CA) column was used. A similar column was used on the QMS (DB-1, 12 m x 0.2 mm x 0.33 µm, J&W Scientific). The same temperature program was used by both the ITMS and the QMS and consisted of an initial column temperature of 120°C for 1 min followed by a 20°C/min ramp to 260°C for a total run time of 8 min. Splitless injections (1 µL) were performed on both system at an injection port temperature of 280°C. The transfer lines of both instruments were set at 275°C. The ITMS source was set to 180°C, and the QMS source was set to 172°C.

Full scan EI analysis on the ITMS and QMS were performed from m/z 50 to 650 at 2 Hz (with three microscans per data point for the ITMS). SIM ions for EI on the ITMS were m/z 221, 256, and 283 with 1 AMU windows at 2 Hz and two microscans per data point. For the QMS, the same three ions were scanned with dwell times of 100 ms. Two nanograms of diazepam was injected for all ITMS experiments. Ten nanograms of diazepam was injected for all full scan QMS experiments, and 2 ng was injected for all SIM QMS experiments. For comparison of the ITMS and the QMS, 10 injections were made each day in two batches (N = 5 per batch) for a total of five days (50 injections).

Sensitivity for the ITMS in positive chemical ionization was optimized by monitoring signal-to-noise (S/N) ratios for diazepam injections at analyzer pressures from 1 x 10^-4 to 4 x 10^-4 torr with the latter being optimum. The same full scan and SIM parameters described previously for EI were used for PCI.

Sensitivity for the ITMS in the negative chemical ionization mode was optimized by monitoring peak shape and S/N of diazepam injections and varying the source temperature (130-180°C), source pressure (0.5 x 10^-4 to 4 x 10^-4 torr), and offset voltage (0 to 10 V). Optimum conditions used were offset 3 V, source 180°C, and source pressure 4 x 10^-4 torr.

Sensitivity of the ITMS in the EI tandem MS mode was optimized by fragmenting the most abundant high-mass ion at m/z 283 (M-I)^+ and monitoring the ion intensity of daughter ions as a function of collision energy. The optimum collision energy used was 1.0 V. Sensitivity and ion-ratio precision was evaluated in the scan mode (daughter ions from m/z 230 to 275) and in the selected reaction monitoring mode (daughters m/z 238, 248, and 268 with a 3 AMU window).

The electron multiplier of the ITMS was maintained at 1100 V for all of the studies. The electron multiplier of the QMS was set to 1500 V for all studies.

The S/N ratios were calculated by dividing the integrated diazepam peak area (signal) by an estimated noise area. Noise was estimated by averaging the peak area of several background peaks near the diazepam peak.

Results and Discussion

Diazepam was chosen as the model compound for these studies because it exhibits good chromatographic peak shape, does not require derivatization, and contains an electron capturing halogenated aromatic functionality. Because one of the goals of these studies was to compare the sensitivity of a QMS with an ITMS and these instruments had different GCs, we tried to eliminate any effect that the GC had on sensitivity. The GC columns in both instruments were less than 1-month old at the time these experiments were begun and were performing well on routine quality control samples. The same type of GC columns were used along with the same temperature programs. The similarities of the GCs combined with the chromatographic performance of diazepam helped reduce the effect of the inlet systems on sensitivity. We chose to inject unextracted samples of diazepam to eliminate the possibility that the matrix was confounding our results because we wanted to compare these systems in an ideal setting.

When ion traps first became commercially available in 1984, these instruments produced electron impact spectra that varied
A major concern when evaluating an ITMS is whether the mass spectra obtained are concentration dependent and whether they can be searched against conventional libraries such as the Wiley Registry. Comparing a spectrum at the initial part of a GC peak for diazepam on the ITMS with a spectrum at the apex showed that the spectrum was consistent across the peak. Figure 1 shows the spectra of diazepam obtained on the ITMS and on the QMS. As can be seen from this figure, both instruments produce very similar spectra in the EI mode of analysis for diazepam.

As can be seen from Table I, a 2-ng injection of diazepam produced an average S/N ratio of 1400 and precision of ion ratio measurements of about 7% for the ITMS when scanning from m/z 50 to 650. Initially, 2 ng of diazepam was also injected on the QMS but there was insufficient signal in the full scan mode, consequently 10 ng was injected in all subsequent full scan experiments. Table I shows that a 10 ng injection on the QMS resulted in an average S/N of 160 with ion ratio precision ranging from 8 to 10.5%. This table clearly shows that the ITMS is at least an order of magnitude more sensitive than the QMS for depending on the concentration of the sample introduced. The distorted spectra were caused by the combination of space charging and charge exchange. Space charging occurs when there are sufficient amounts of ions in the trap to affect the electric fields applied to the ions of interest. Charge exchange and other ion molecule reactions occur when high concentrations of ions are stored in the trap for several milliseconds, allowing them to collide with other molecules, which produces chemical ionization. Space charging and charge exchange led to spectra that were not consistent with those obtained on classical instruments such as the QMS. In order to eliminate these effects, ITMS now use automatic gain control, which is a prescan of approximately 200 μs to determine how many ions are being formed. The analytical scan follows with the ionization time adjusted to optimize filling of the trap based on the prescan. After the trap is emptied, ion abundance, as detected by the multiplier, is then normalized to account for the ionization time. Consequently, the ion abundance recorded is the value measured by the detector multiplied by the correction factor for ionization time (1).

Table 1. Full Scan (m/z 50 to 650) Comparison of Signal-to-Noise and Ion Ratio Precision (N = 10 each day) for the Ion Trap Mass Spectrometer (ITMS) and the Quadrupole Mass Spectrometer (QMS)*

<table>
<thead>
<tr>
<th>Day</th>
<th>ITMS S/N</th>
<th>QMS S/N</th>
<th>ITMS CV m/z 221/283</th>
<th>QMS CV m/z 221/283</th>
<th>ITMS CV m/z 256/283</th>
<th>QMS CV m/z 256/283</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1500</td>
<td>250</td>
<td>5.3%</td>
<td>9.7%</td>
<td>6.3%</td>
<td>9.1%</td>
</tr>
<tr>
<td>2</td>
<td>1600</td>
<td>130</td>
<td>7.0%</td>
<td>8.2%</td>
<td>4.8%</td>
<td>6.9%</td>
</tr>
<tr>
<td>3</td>
<td>1400</td>
<td>140</td>
<td>8.8%</td>
<td>10.6%</td>
<td>8.0%</td>
<td>11%</td>
</tr>
<tr>
<td>4</td>
<td>1300</td>
<td>160</td>
<td>7.7%</td>
<td>12.5%</td>
<td>5.1%</td>
<td>8.0%</td>
</tr>
<tr>
<td>5</td>
<td>1300</td>
<td>120</td>
<td>7.7%</td>
<td>11.5%</td>
<td>10.9%</td>
<td>4.8%</td>
</tr>
<tr>
<td>Mean</td>
<td>1400</td>
<td>160</td>
<td>7.3%</td>
<td>10.5%</td>
<td>7.0%</td>
<td>8.0%</td>
</tr>
</tbody>
</table>

* For the ITMS, 2 ng of diazepam was injected; for the QMS, 10 ng of diazepam was injected.

Abbreviations: CV, coefficient of variation; S/N, signal-to-noise ratio.
this experiment. The precision of measuring ion ratios on both instruments in the full scan mode was similar, with the ITMS being slightly better.

Selected ion monitoring results shown in Table II demonstrate an order of magnitude increase in sensitivity as compared with full scan analysis for both the ITMS and the QMS. The most important difference noted with SIM experiments is that the QMS is approximately 10-fold more precise in measuring ion ratios. Although the ITMS can be scanned at a fast rate (11,000 amu/sec) in full scan, the implementation of SIM reduces the duty cycle of the ITMS to less than that used by the QMS. In ITMS SIM, ions are injected into the trap and then a single mass-to-charge ratio is isolated and scanned out for detection. In a serial manner, ions of different intensities are mass filtered. The steps of ion injection, isolation and detection for each mass reduce the usable duty cycle of the ITMS to no greater than 5 Hz per mass (5 masses per second). The slower duty cycle helps explain why the ITMS is less precise in measuring ion ratios as compared with the QMS, where more dwell time can be spent measuring the ions of interest. In most situations, SIM on the ITMS does not lead to increased sensitivity as is usually observed with QMS instruments. When performing ITMS SIM experiments on biological extracts, the peak width of the isolation waveform can allow interfering ions to enter the source, causing a decrease in ionization time (the trap fills up with a combination of desired and interfering ions), leading to a decrease in signal (13).

The precision of SIM ion ratio measurements has important implications for forensic testing, where ion ratios typically must be within ±20% of the calibrator for a specimen to be confirmed positive. Under the ideal conditions of no matrix effects and at exactly the same concentration, the ITMS had ion ratios with coefficients of variation (CV) in the 10% range for the SIM mode of analysis. Although more experiments are required, it is logical that, with real samples spanning a range of concentrations, it will be difficult for the ITMS, in its present configuration, to meet the ±20% criterion generally accepted in confirmation of identity for legal purposes. Because of the increased sensitivity of the ITMS, this instrument is better suited to confirm chemical identity based on full scan analysis. Although this is problematic when employing deuterated analogues of the target compound, full scan analysis is ideal in many situations, such as a postmortem toxicology lab, where hundreds of different drugs must be identified. While the lack of precision of measuring SIM ion ratios is a drawback of the ITMS instrument we evaluated, ion-trap mass spectrometry has made many advances in recent years and, with further improvements, future instruments may be more precise in measuring ion ratios.

The PCI spectra of diazepam is shown in Figure 2. Under CI conditions, older ITMS produced spectra containing a mixture of EI and CI that was concentration dependent (14). This was caused by reactive radical ions and was corrected by additional waveforms applied to the endcaps to eject unwanted reagent gas ions during the reaction phase (15). The PCI diazepam spectra obtained on the ITMS is consistent across the peak (not concentration dependent) and is similar to a diazepam PCI spectra obtained in the authors' lab on a conventional quadrupole system. The major diagnostic ions consistent with methane positive chemical ionization are m/z 285 (M + H)+, m/z 313 (M + C2H5)+, and m/z 325 (M+ C3H4)+. After optimizing the signal by evaluating source pressures from 0.5 x 10^-4 to 4 x 10^-4 torr (upper end recommended by manufacturer), the latter was chosen as the optimum because it had the largest S/N. In the full scan mode, a 2-ng

<table>
<thead>
<tr>
<th>Day</th>
<th>ITMS S/N</th>
<th>QMS S/N</th>
<th>ITMS CV</th>
<th>QMS CV</th>
<th>ITMS CV</th>
<th>QMS CV</th>
<th>ITMS CV</th>
<th>QMS CV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m/z 221/283</td>
<td>m/z 211/283</td>
<td>m/z 256/283</td>
<td>m/z 256/283</td>
<td>m/z 256/283</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>14000</td>
<td>4200</td>
<td>15%</td>
<td>1.3%</td>
<td>9.8%</td>
<td>0.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13000</td>
<td>2800</td>
<td>8.5%</td>
<td>2.9%</td>
<td>7.9%</td>
<td>0.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17000</td>
<td>2800</td>
<td>14%</td>
<td>1.8%</td>
<td>7.8%</td>
<td>2.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11000</td>
<td>1900</td>
<td>8.8%</td>
<td>1.7%</td>
<td>8.5%</td>
<td>0.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>16000</td>
<td>3100</td>
<td>9.9%</td>
<td>0.8%</td>
<td>5.9%</td>
<td>1.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>14000</td>
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<td>11.2%</td>
<td>1.7%</td>
<td>8.0%</td>
<td>1.1%</td>
<td></td>
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</tr>
</tbody>
</table>

* For both instruments, 2 ng of diazepam was injected.
† Abbreviations: CV, coefficient of variation; S/N, signal-to-noise ratio.

Figure 2. Positive chemical ionization mass spectra of diazepam obtained on the ITMS.
injection of diazepam had an average S/N of 64 ($N = 10$), and CVs for ion ratios ranged from 15% ($m/z$ 313/$m/z$ 285) to 5% ($m/z$ 287/$m/z$ 285). Using SIM conditions monitoring $m/z$ 285, 313, and 287 (1 AMU windows), the average S/N was 1775, and CVs for ion ratio measurements were 3% ($m/z$ 313/$m/z$ 285) and 3% ($m/z$ 287/$m/z$ 285).

The mass spectrum obtained from scanning diazepam under NCI conditions is shown in Figure 3. This spectrum is similar to that obtained in our lab on a conventional quadrupole system, with the exception of a prominent (M + 15) adduct ion and more fragmentation observed in the ITMS. Previously, we obtained a single molecular ion cluster for diazepam using a quadrupole MS but ionization conditions were different, notably a lower source temperature (100°C and lower source pressure (1 x 10^-5 torr).

The source temperature of the ITMS in the NCI mode had an effect on the apparent chromatographic peak shape. Obviously the source temperature does not affect GC conditions, but at an analyzer temperature of 130°C, the diazepam peak was 57 scans wide, and at 180°C, it was 17 scans wide. The probable mechanism explaining this observation is that the diazepam vapor effluent from the GC column is temporarily condensing on the relatively cold source. This condensation is followed by slow revaporization, ionization, and detection, which lead to broad chromatographic peaks. The obvious solution to this problem is to maintain the source temperature at high enough temperatures to prevent analyte condensation.

Using NCI conditions, the scan function had an unexpected effect on relative abundances of ions. For example as shown in Figure 3 for a full scan analysis, the base peak is the molecular ion at $m/z$ 284 with other diagnostic ions at $m/z$ 227, 249, and 299 at with relative abundances of 10% or less. When using 1 AMU SIM conditions ($m/z$ 227, 284, and 299), the most abundant ion was $m/z$ 299 with $m/z$ 227 having a relative abundance of 90% and $m/z$ 284 of 15%. Monitoring the same ions with a 2 AMU window changed the appearance of the spectra again, with $m/z$ 226 as the base peak, $m/z$ 299 with a relative abundance of 35%, and $m/z$ 284 at 3%. The most likely explanation for these results is that the negative ion fragments of diazepam are not stable in the ITMS when the SIM waveform is applied.

The ability to perform multiple MS experiments is particularly attractive feature of any GC–MS system. On a QMS instrument, three physically distinct stages of mass analysis are required to perform tandem MS, adding substantially to the initial instrument cost. ITMSs avoid the costs associated with extra hardware by performing multiple MS experiments within the trap. The ITMS we evaluated performs tandem MS experiments by isolating and storing the parent ion, colliding the parent with helium buffer gas resident in the trap, and selectively ejecting the daughter ions out at specific mass-to-charge ratios.

We optimized MS–MS conditions in the trap by plotting the signal intensity of characteristic daughter ions as a function of collision energy as shown in Figure 4. As expected, with increasing excitation energy the total ion current and percent of the parent ion ($m/z$ 283) decreases. At about 1 V of collision energy, the $m/z$ 248 daughter ion reaches an optimum. This figure illustrates an important point of any MS–MS experiment. The sensitivity of MS–MS is dependent the efficiency in which daughter ions are created relative to the reduction in noise. In this experiment with no collision energy, the parent ion has $8 \times 10^6$ area counts and at 1 V of collision energy has about $4 \times 10^5$ area counts. The most intense daughter ion only has $4 \times 10^5$ area counts at the optimum collision energy of 1 V. Because the signal of the daughter ion has decreased by a factor of 10 as compared with the parent ion, in order for S/N
to increase in this experiment, the noise level would have to be reduced by a factor of at least 10. We were evaluating the ITMS with pure diazepam, and consequently did not assess the ability of tandem MS to perform where it would be most beneficial, in a dirty sample matrix. Previous studies have addressed this point by showing that an ITMS in the SRM mode provided increased sensitivity, specificity, and accuracy as compared with a QMS for the analysis of tebufelone in a complex matrix (16).

When m/z 283 was chosen as the parent ion and daughters were scanned from m/z 230 to 275, a 2-ng injection of diazepam had an average S/N ratio of about 5500. Stability of daughter ion ratios was 6–7%. When selected reaction monitoring was employed with m/z 283 as the parent and daughters of m/z 268, 248, and 238, the total S/N was 3700 with ion ratio precision in the 4–7% range.

**Conclusion**

The experiments described in this manuscript were designed to be a simple comparison of a new ion-trap instrument with a conventional benchtop QMS using a clean model system free of matrix effects. This is an important first step in the evaluation of new instrumentation but more experiments are needed to extend the findings to drugs extracted from a biological matrix. The results demonstrate some important differences between the two systems which can be generalized as increased sensitivity for the ITMS and better ion-ratio precision with the QMS in SIM. The relatively low cost of the additional PCI, NCI, and MS–MS capabilities of the ITMS appear to be useful in a variety of settings.

**References**


