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# High-performance liquid chromatography–tandem mass spectrometry with a new quadrupole/linear ion trap instrument

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**Abstract**

The use of a new hybrid quadrupole/linear ion trap known as the Q TRAP™ offers unique benefits as a LC–MS–MS detector for both small and large molecule analyses. The instrument combines the capabilities of a triple quadrupole mass spectrometer and ion trap technology on a single platform. Product ion scans are conducted in a hybrid fashion with the fragmentation step accomplished via acceleration into the collision cell followed by trapping and mass analysis in the Q3 linear ion trap. This results in triple quadrupole fragmentation patterns with no inherent low molecular mass cutoff. In-trap fragmentation is also possible in order to provide triple MS ($MS^3$) capabilities. There are also several scan modes that are not possible on conventional instruments that enable identification of analytes within complex biological matrixes for subsequent high sensitivity product ion scans. This report will describe the new hybrid instrument and the principles of operation, and also provide examples of the unique scan modes and capabilities of the Q TRAP™ for LC–MS–MS detection in metabolism identification.


*Keywords:* Mass spectrometry; Instrumentation; Buspirone

## 1. Introduction

Triple quadrupole and ion trap mass spectrometers provide complimentary information when performing MS–MS experiments [1]. Triple quadrupole instruments are “tandem-in-space” devices, meaning that each step of an MS–MS experiment is conducted at a spatially distinct location in the instrument. When both Q1 and Q3 are in resolving mode and at least one quadrupole is scanned, the overall instrument duty cycle is relatively poor since only one precursor-to-fragment pair is stable throughout the length of the instrument at any one time. Thus, most of the ions from the source are wasted. If, however, one is only interested in a single precursor-to-fragment transition, then the triple quadrupole is characterized by a very high duty cycle and associated sensitivity. This and the high dynamic range of triple quadrupoles are important reasons why these instruments have a prominent place in quantitation laboratories. Triple quadrupoles also have two very selective MS–MS scans, precursor ion scan and constant neutral loss scan, that are particularly useful in identification of

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analytes in complex matrices. Both of these scans involve simultaneous scanning of both Q1 and Q3, which makes the scans difficult to carry out on other instruments. Despite the relatively low duty cycles of the precursor ion and constant neutral loss scans, the extreme selectivity of these scans has made them useful in both small and large molecule experiments.

Conventional ion trap mass spectrometers perform MS–MS experiments in a "tandem-in-time" fashion rather than the "tandem-in-space" manner discussed above [1]. This means that, once the ions are introduced into the ion trap all of the different steps of ion manipulation occur within the same volume, but at different times. The advantage of this approach, relative to a tandem-in-space approach, is that a complete mass spectrum can be obtained for each pulse of ions introduced into the ion trap. This does lead to high instrument duty cycles and increased scanning sensitivity relative to the triple quadrupoles. However, conventional ion trap mass spectrometers are strictly scanning instruments and are consequently limited to product ion scans when operated in MS–MS mode. The very selective precursor ion scans and constant neutral loss MS–MS scans available on the triple quadrupoles are not, in general, possible with conventional ion traps.

The Q TRAP™ instrument combines the advantages of a triple quadrupole mass spectrometer with those of an ion trap mass spectrometer within the same platform. This is accomplished by operating the final quadrupole as either a standard radiofrequency (RF)/DC resolving quadrupole mass filter or as a linear ion trap mass spectrometer with axial ion ejection [2]. This unique linear ion trap MS allows it to be placed within a conventional triple quadrupole ion path and obtain all of the benefits of both tandem mass spectrometers in a single instrument without compromising the performance of either the triple quadrupole or the ion trap mass spectrometer. The linear ion trap (LIT) portion of the Q TRAP™ instrument has several advantages over conventional three-dimensional ion traps such as higher trapping efficiencies due to the negligible RF field along the longitudinal axis of the LIT along which ions are injected. The LIT also has higher ion capacity due to a considerably larger trapping volume. This results in a greater immunity to the deleterious effects of space charge in this new device [2].

This report presents some important characteristics of the hybrid Q TRAP™ instrument and the way in which these capabilities can be brought to bear on often complex LC–MS–MS challenges.

## 2. Experimental

All experiments were carried out on a standard Q TRAP™ (AB|MDS Sciex, Concord, Canada) tandem mass spectrometer. The instrument is based on the ion path of a triple quadrupole MS system as is shown schematically in Fig. 1. Q0 is an RF-only quadrupole ion guide maintained at approximately $6{\cdot}10^{-3}$ Torr (1 Torr = 133.322 Pa) and is used to collisionally cool the incoming ion beam. Q1 is a conventional RF/DC quadrupole mass filter. The collision cell (Q2) is an enclosed LINAC quadrupole array pressurized with nitrogen gas to between $2–8{\cdot}10^{-3}$ Torr.

The Q3 quadrupole rod array is mechanically identical to Q1, but can be operated as either a conventional RF/DC quadrupole mass filter or as a linear ion trap mass spectrometer with axial ion ejection. A center-tapped toroidal transformer is located within the vacuum chamber to support the LIT mode of operation. This transformer supplies the auxiliary a.c. voltage used for the resonance excitation steps for fragmentation and mass scanning. The ability to operate Q3 as a LIT does not compromise its performance as a conventional quadrupole. Changes between conventional quadrupole and ion trap modes of operation occur in less than 1 ms. Importantly, the same pulse counting ion detector is used for both modes of operation.

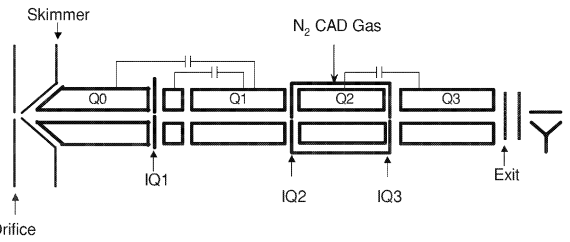


Fig. 1. A schematic of the Q TRAP™ instrument. The final quadrupole Q3 can be operated as either a conventional RF/DC quadrupole mass filter or as a linear ion trap mass spectrometer with axial ion ejection.

Rat liver microsomes were incubated with buspirone to generate metabolites. Samples were injected on an Hypersil-Betasil $C_{18}$ column ($100 \times 1$ mm). Samples were eluted using Shimadzu 10 AD pumps with a gradient of water–methanol, from 10 to 90% methanol over 10 min at a flow-rate of 70 μl/min. Direct injection (5 μl) on column was performed using a PE Series 200 autosampler. Turboionspray operated at 350 °C was used to introduce the LC eluent into the MS instrument.

## 3. Results and discussion

Most Q TRAP™ product ion mass spectra are generated in a truly hybrid manner. Q1 is used as a resolving RF/DC transmission quadrupole to select the precursor ion of interest. The precursor ion is then accelerated into the pressurized collision cell inducing fragmentation and the resulting fragment and residual precursor ions are transmitted into the Q3 LIT where they are mass selectively scanned out toward the detector. While the Q3 LIT is performing the mass scan ions can be accumulated in Q0 further enhancing instrument duty cycle. This scan is referred to as an “enhanced product ion” (EPI) scan.

The hybrid approach to product ion generation decouples the steps of precursor ion selection and fragmentation from the ion trap and enables the analyst to use the entire LIT trapping volume for the ions of interest, namely fragments and residual precursors. This also means that there is no inherent low mass cutoff in the product ion spectra since the fragmentation step is spatially separated from the LIT. Furthermore, the use of Q1 to select the precursor ion eliminates the problem seen with conventional ion traps where the act of isolating the precursor ion from the rest of the ions admitted into the ion trap leads to activation and loss of the precursor ion itself [3]. Finally the EPI spectra generated with the Q TRAP™ yield rich triple quadrupole fragmentation patterns since the fragmentation generation step is the same as that used in a triple quadrupole instrument

Fig. 2 displays a comparison of product ion mass spectra of reserpine using the Q TRAP™ operated as a classical triple quadrupole (top spectrum) and when Q3 is operated in LIT mode (bottom spectrum). As expected the fragmentation patterns of these two spectra are nearly identical, but when Q3 is operated as an LIT a 200× sensitivity enhancement is observed as can be seen in the EPI spectrum. The differences in spectral quality are more clearly illustrated in Fig. 3,

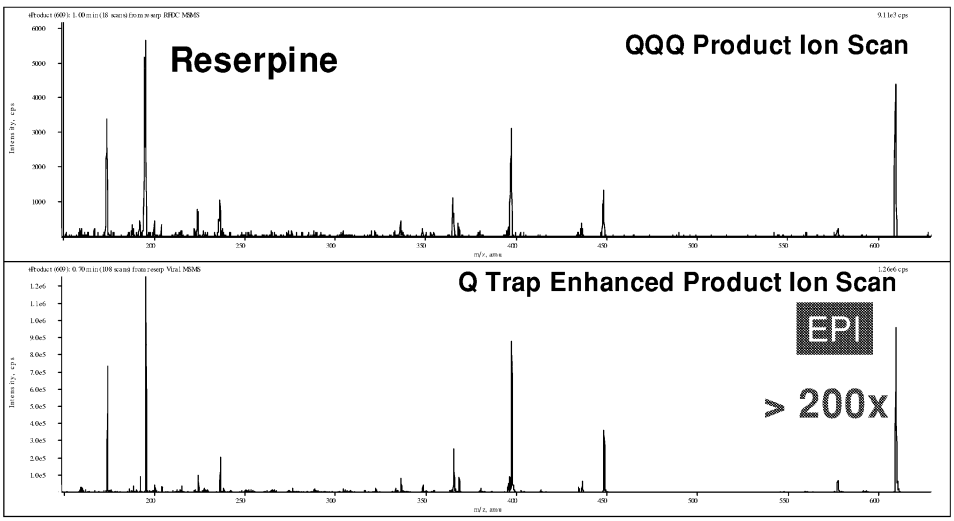


Fig. 2. Product ion spectra of reserpine obtained with the Q TRAP™ instrument. The upper trace was recorded with the instrument operating as a conventional triple quadrupole mass spectrometer. The lower trace was recorded in “enhanced product ion” mode, in which the Q3 linear ion trap performed the analytical mass scan, which results in intensity enhancements of >200×.

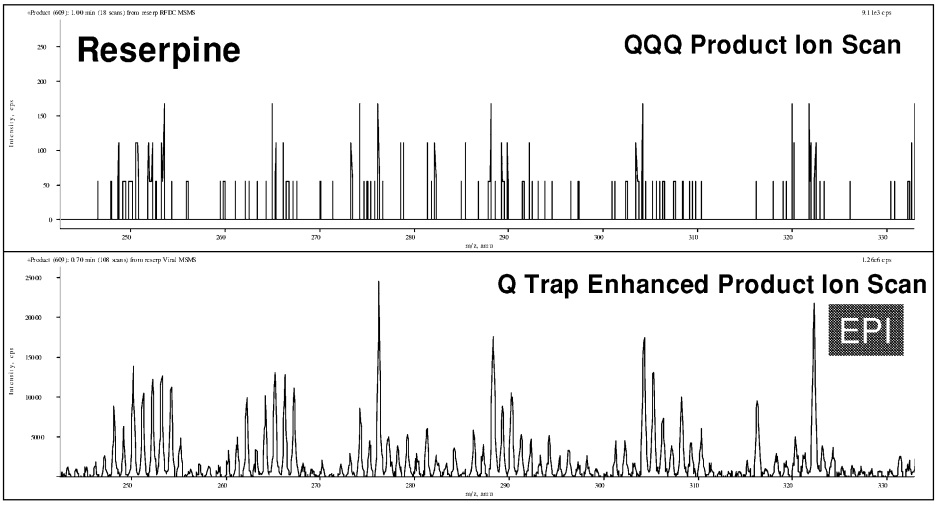


Fig. 3. A scale expanded version of the two product ion spectra shown in Fig. 2 presented to demonstrate the superior signal-to-noise characteristics of the EPI scan using the Q3 linear ion trap mass spectrometer.

which is a magnified portion of the same two reserpine product ion spectra. The high spectral quality of the EPI spectrum is maintained throughout the entire mass range of the instrument.

Single MS survey scans can also be generated using Q3 as an LIT. Here, Q1 is switched to RF-only operation in order to transmit ions from the source into the Q3 LIT. The LIT mass spectrometer is then scanned to yield a single MS scan with enhanced sensitivity compared with what would be obtained using the Q1 quadrupole mass filter. This scan is referred to as the "enhanced MS" (EMS) scan. However, this LIT survey scan offers no additional selectivity with respect to a conventional quadrupole survey scan. Both are often dominated by complex matrix ion signals that can completely obscure analytes of interest. Often the selective classical triple quadrupole precursor ion or constant neutral loss survey scans can provide the needed selectivity.

A frequently used strategy for metabolism identification is to rely on the quadrupole specificity for the identification of metabolites and confirm these predictions on 3-D ion trap [4]. This approach has proven to be useful in the identification of unique unexpected metabolites. However, multiple instruments and LC set-ups are required, in addition to familiarity of various vendor software. With the Q TRAP™ system, this approach can be combined into a single instrument platform using information-dependent acquisition (IDA); the metabolites are identified with precursor ion scan and neutral loss as a survey scan, and confirmed with an EPI scan as a dependent scan. The survey and dependent scans are performed sequentially, and repeated for the entire duration of the LC analysis, thus providing maximum information from a single injection. As a case study, metabolites of buspirone generated by rat liver microsomal incubation were analyzed using the IDA mode [5]. Fig. 4 shows the EPI spectrum of buspirone with interpretation of some of the major fragment ions observed. For the precursor ion scan as survey, 122 and 168 were selected to identify metabolism that will occur on the B-side and A-side of the molecule, respectively. In addition, a precursor ion scan of 138 was also used to monitor metabolites that would undergo hydroxylation on the pyrimidine moiety. These specific and less sensitive precursor scans were compared to the less specific but much more sensitive EMS survey scan for the identification and confirmation of buspirone metabolites in IDA mode. Fig. 5 displays a comparison of the total ion chromatogram (TIC) between the EMS survey scan and the precursor ion scan (precursors of $m/z$ 122) for the LC separation of in vitro buspirone metabolites. For this complicated sample the additional ion trap sensitivity provided by the EMS scan does not help identify the metabolites,

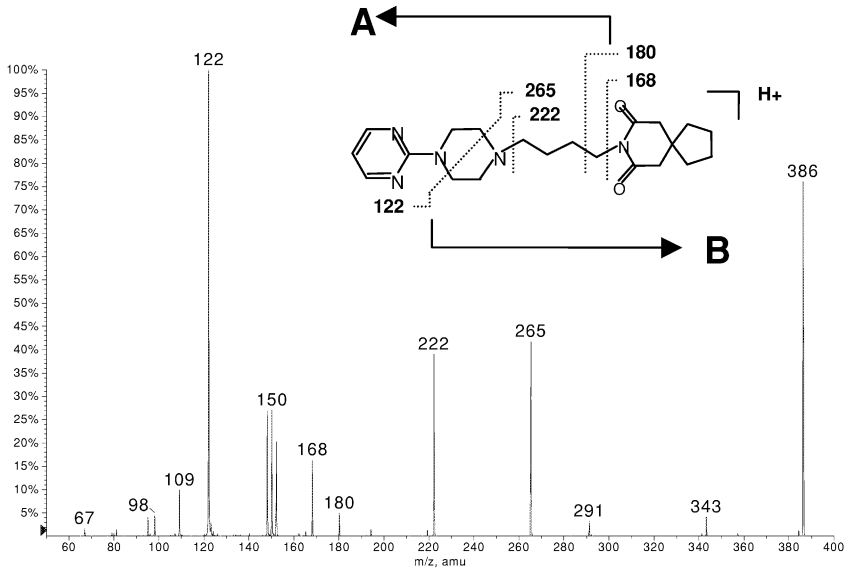


Fig. 4. Enhanced product ion (EPI) spectrum and structure of buspirone. Major fragment ions observed are indicated on the structure. The A-side and B-side refer to the text description for the sites of metabolism with respect to fragment ion observed.

because the selectivity of this scan is low. However, even though the sensitivity of the triple quadrupole precursor ion scan is considerably lower than that of the EMS scan, the added selectivity makes for rapid and automated identification of metabolites from survey scans using IDA. Fig. 6 shows the EPI scan of the peak at 6.86 min, which can be readily identified as a hydroxylated metabolite of the parent drug.

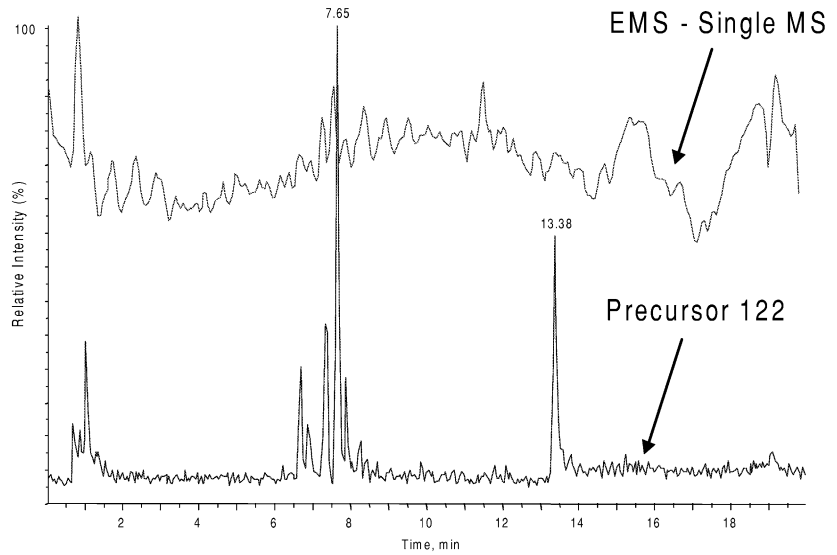


Fig. 5. Comparative total ion chromatograms for the LC–MS–MS analysis of buspirone metabolites. The enhanced MS (EMS) trace was generated using the Q3 linear ion trap. The well resolved total ion chromatogram was recorded using the precursors of $m/z$ 122 triple quadrupole scan mode.

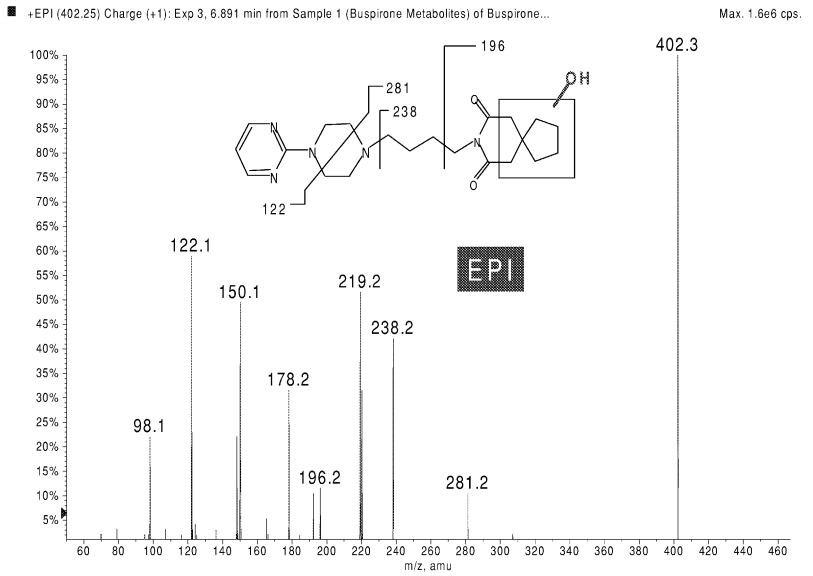


Fig. 6. The enhanced product ion (EPI) scan of the $m/z$ 402 precursor identified at a retention time of 6.87 min in Fig. 4.

Using a combination of precursor ion and constant neutral loss survey scans considerably more buspirone metabolites could be identified than when the conventional single MS ion trap survey scan was used. This is shown in Table 1. When dealing with complex biological samples such as this one, increased instrument sensitivity without selectivity does not aid in analyte identification. Table 2 illustrates that the number of useful product ion spectra generated when using the precursor of $m/z$ 122 survey scan is considerably greater than when employing a simple enhanced MS survey scan. More spectra are generated with the EMS survey scan, but ∼99% of these were not found to be useful. The more selective survey scan

Table 1
Summary of buspirone metabolites identified and confirmed when using different survey scans. The metabolites were considered to be confirmed only if their EPI spectra could be interpreted. The precursor ion and constant neutral loss survey scans resulted in detection of more metabolites than did the use of the enhanced MS survey scan

| Metabolite ($MH^+$) | Enhanced MS | Precursor 122 | Precursor 168 | Precursor 138 | Precursor 176 |
|---|---|---|---|---|---|
| Hydroxy-buspirone glucuronide (594) | 0 | 0 | 0 | 0 | 2 |
| Buspirone glucuronide (578) | 0 | 0 | 0 | 0 | 1 |
| Dihydroxy-buspirone (418) | 1 | 4 | 2 | 2 | 0 |
| Hydroxy-buspirone (402) | 3 | 5 | 3 | 2 | 0 |
| Buspirone (386) | 1 | 1 | 1 | 0 | 0 |

Table 2
Comparison of the success rates for the enhanced MS survey scan and precursor of $m/z$ 122 survey scan. Many more interpretable product ion spectra were obtained using the precursors of $m/z$ 122 survey scan

| | Enhanced MS | Precursor 122 |
|---|---|---|
| Number of cycles | 321 | 403 |
| Number of MS–MS | 606 | 260 |
| Number of useful MS–MS spectra | 5 | 33 |
| Success rate (%) | 0.8 | 12.7 |
| Number of species identified (including drug) | 5 | 10 |

resulted in fewer product ion scans being recorded, but more of these spectra were useful and the success rate was $\sim$10$\times$ greater.

## 4. Conclusions

The Q TRAP$^{TM}$ instrument combines all of the functionality of a classical triple quadrupole mass spectrometer with the capabilities of a very high sensitivity linear ion trap mass spectrometer. The ability to decouple many of the ion processing steps in the generation of a product ion mass spectrum means that many of the limitations of a conventional ion trap MS system, such as the presence of a low mass cut-off, are eliminated. Furthermore, this hybrid approach to scanning means that the entire capacity of the linear ion trap can be used for storage of analyte precursor and fragment ions increasing instrumental dynamic range over all other ion trap instruments

The combination of highly selective triple quadrupole MS–MS scans and high sensitivity ion trap product scans on the same instrument platform provides rapid identification and confirmation of metabolites even in the presence of complex endogenous biological compounds.

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