Powerful scan modes of QTRAP® System Technology

Unique hybrid triple quadrupole linear ion trap technology provides powerful workflows to answer complex questions with no compromise

While there are many different types of mass spectrometers available today, the quality of the results will largely depend upon the type of mass analyzer used and the specific type of measurement or scan mode used. For targeted quantitative measurements such as multiple reaction monitoring (MRM), triple quadrupole (QqQ) instruments have long been considered the gold standard for the highest speed, sensitivity, dynamic range, and multiplexing capabilities. However, their lower duty cycle in full scan mode hinders their ability to obtain the highest performance in qualitative measurements. In contrast, QTOF or ion trap-based mass analyzers have a higher sensitivity and scan speed in full scan mode than a QqQ for qualitative measurements. The drawback however, is that these full scan-based instruments cannot typically not provide the same specifications in sensitivity, and dynamic range as a QqQ for targeted quantitative analysis.

The mass analyzer region of a QTRAP System is based on the conventional ion path of a QqQ mass spectrometer. However, in contrast to conventional QqQ systems, the third quadrupole (Q3) of a QTRAP System can also be operated as an LIT (Figure 1)[1]. The dual functionality of Q3 provides the QTRAP System **complete** functionality as a QqQ mass spectrometer but with additional powerful qualitative scan functions. These additional LIT scan functions greatly enhance the performance and flexibility for of the QTRAP System for applications such as screening, confirmation and identification applications. With a QTRAP System, all of the MRM sensitivity of a QqQ is combined with a multi-functional ion trap with no compromises. Not only does this afford the highest performance for quantitative and qualitative experiments on the same instrument, it also enables new and powerful TripleTrap™ Scanning workflows that combine QqQ functionality with LIT functionality within the same experiment.

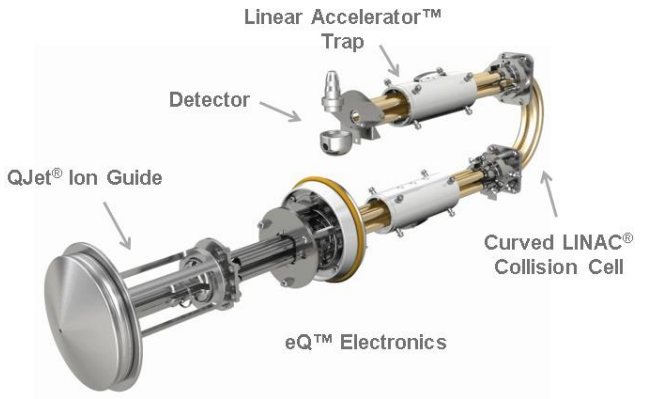

Figure 1. QTRAP System ion rail. The unique configuration of the QTRAP System consists of a triple quadruple-based ion rail, where the third quadrupole (Q3) can also operate as a linear ion trap (LIT). This configuration provides a broad range of traditional scan functions along with new and unique workflows that combine qualitative and quantitative data within one analysis without sacrificing performance.

Scan modes and unique workflows available on QTRAP® Systems

- **QqQ scan modes**
  - Multiple Reaction Monitoring (MRM) with *Scheduled* MRM™ Algorithm Pro
  - Precursor Ion Scan (PI)
  - Neutral Loss Scan (NL)
- **LIT scan modes**
  - Enhanced MS Scan (EMS)
  - Enhanced Resolution Scan (ER)
  - Enhanced Multiply Charged Scan (EMC)
- **Hybrid QqQ/LIT scan modes**
  - Enhanced Product Ion Scan (EPI)
  - MRM^3 Scan
  - MS^3 Scan
- **Unique and powerful workflows**
  - TripleTrap Scanning (multiple configurations)

Quantitative scans

There are two main types of quantitative scans on the QTRAP System, Multiple Reaction Monitoring and MRM^3.

Multiple Reaction Monitoring (MRM)

The triple quadrupole functionality of this instrument allows analyte quantitation to be performed at very high specificity and sensitivity using Multiple Reaction Monitoring (MRM).MRM (also known as Single Reaction Monitoring, SRM) is the scan type with the highest duty cycle and is used for monitoring one or more specific ion transition(s) at high sensitivity. Here, Q1 is set on the specific parent m/z (Q1 is not scanning), the collision energy is set to produce the optimal diagnostic charged fragment of that parent ion, and Q3 is set to the specific m/z of that fragment (Figure 2 and Figure 4, bottom). Only ions with this exact transition will be detected. Because of the speed of the LINAC® Collision Cell, many MRM scans can be looped together into one experiment to detect the presence of many specific ions in a complex mixture. Up to 4000 MRM transitions can be monitored within a single experiment and up to 500 MRMs can be monitored per second.

The area under the MRM LC peak is used to quantitate the amount of the analyte present. In a typical quantitation assay, a standard concentration curve is generated for the analyte of interest. When the unknown sample is then run under identical conditions, the concentration for the analyte in the unknown sample can be determined using the peak area and the standard concentration curve. The *Scheduled* MRM Algorithm Pro enables many MRM transitions to be monitored in a single acquisition through time scheduling[2], with simplified, automatic method building using user supplied parameters.

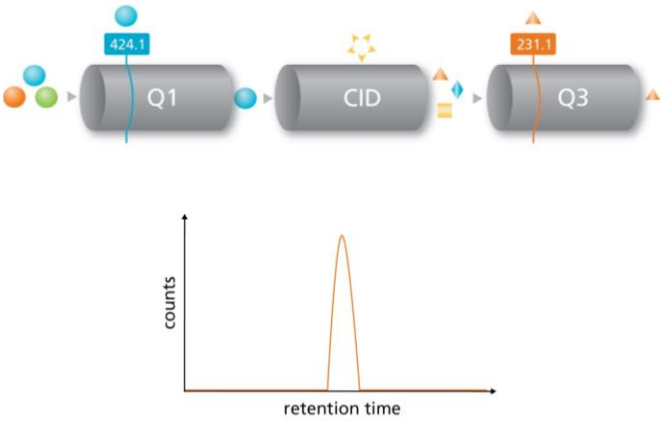

Figure 2. Multiple Reaction Monitoring (MRM). In this QqQ scan mode, a precursor ion is selected in Q1, it is then fragmented in the Q2 collision cell, and a specific product ion is monitored in Q3. The Q1 and Q3 quadrupoles are functioning in transmission only mode which means they are set to transmit a specific m/z only. This mode is what confers the high sensitivity of this scan as the instrument is fully optimized to transmit just that parent to fragment pair.

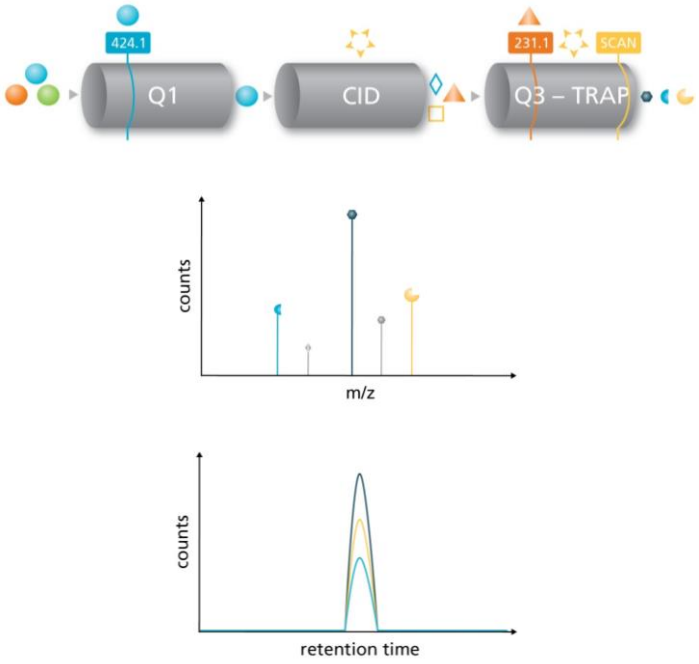

Figure 3. MRM^3 scan. The precursor ion is first selected in Q1, then fragmented in Q2. Fragment ions are trapped and then isolated in Q3 which is now acting as a linear ion trap. This is followed by excitation to perform the second fragmentation step. Second generation product ions are then scanned out to the detector.

MRM^3

There are some cases where the quantitation of specific analytes using MRM may be impaired due to co-eluting interferences or high background. Here, the use of MRM^3 can greatly improve signal-to-noise, reducing the need for exotic chromatography to separate interferences or extensive sample cleanup. MRM^3 is actually a hybrid QqQ/LIT workflow combining normal quadrupole functions with normal LIT functions to achieve an enhanced capability not possible with either analyzer alone[3]. In this scan mode (Figure 3), the precursor ion is selected in Q1 with a mass window of 1-4 amu and then fragmented in the Q2 collision cell. The fragment ions are then transferred to the ion trap where one of the resulting fragment ions is selectively isolated (isolation width of 1-5 amu). After isolation, the ion is excited using a single wavelength excitation frequency (~1 Da excitation width) to induce fragmentation (energy of excitation and excitation time are controlled by user). The resulting fragment ions are captured and then scanned out of the ion trap at one of the three scan speeds. Thus MRM^3 data is collected as full scan data. Then, post-acquisition, extracted ion chromatograms (XICs) are used to isolate the secondary product ion(s) of interest for quantitation.

Targeted Scans

Two triple quadrupole scan types, the Precursor Ion Scan and the Neutral Loss Scan, can be used for identifying analytes that share a common molecular substructure. These scans allow you to survey a complex mixture for specific components and obtain the masses of these components.

Precursor ion scan (PI)

When an ion loses a diagnostic fragment as a charged fragment, it can be detected by using a Precursor Ion scan. In this scan type, Q1 is scanned across a mass range, and ions are fragmented in the collision cell. Q3 is set to transmit only the mass of the diagnostic fragment ion to the detector. Therefore, only ions that are passed through Q1 that fragment to produce the diagnostic charged fragment ion will be detected (Figure 4, top).

An example of a precursor ion scan would be to monitor m/z 79 in negative ion mode in order to specifically detect phosphorylated peptides in proteomic samples. Another example would be monitoring m/z 272 in negative ion mode for the detection of glutathione (GSH) in order to detect low-level reactive metabolites in complex samples during drug discovery.

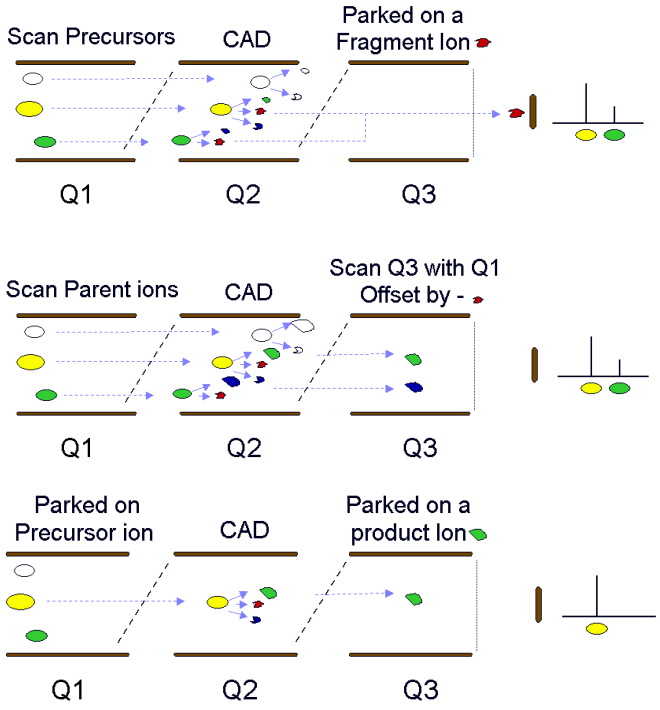

Figure 4. Triple Quadrupole Scan Types. Top – Precursor Ion scan. Middle – Neutral Loss scan. Bottom – MRM scan.

Neutral loss scan (NL)

When an ion loses a diagnostic fragment as a neutral fragment, it can be detected by using a Neutral Loss Scan (NL). In this scan mode, Q1 is scanned across a specific mass range. Ions are passed into the collision cell Q2 where they are fragmented. Q3 is scanned over a similar mass range, offset by the neutral mass of the diagnostic fragment. Therefore, any molecule being passed through Q1, which loses a neutral molecule of the defined mass will then be transmitted through Q3 and detected (Figure 4, middle).

Examples of NL scans include monitoring the neutral loss of 141 to detect phosphatidylethanolamines in lipidomics experiments or monitoring a neutral loss of 176 to detect glucuronide metabolites of opiates in urine samples.

Qualitative scans

There are two full scan LIT MS scan types available on QTRAP Systems, the Enhanced MS scan (EMS), and the Enhanced Multiply Charged scan (EMC) and one narrow range high resolution MS scan, the Enhanced Resolution scan (ER). Additionally, there are two MS/MS scan types available, the Enhanced Product Ion scan (EPI) and MS/MS/MS (MS^3).

Enhanced MS scan (EMS)

The Enhanced MS scan is the standard ion trap MS scan. Ions are transmitted from the source through the quadrupoles (quadrupoles are in RF only mode) into the ion trap. After the ion trap is filled, the ions are scanned out axially to the detector. This provides a full scan analysis of all the analytes entering that QTRAP System at that point in time.

Enhanced multiply charged scan (EMC)

The Enhanced Multiply Charged Scan is a standard ion trap MS scan that can be used to improve the signal/noise ratio on multiply charged ions. Ions are transmitted from the source through the quadrupoles (quadrupoles are in RF only mode) into the ion trap. Once the ion trap is filled, the singly charged ions are emptied from the ion trap (~100ms), leaving predominantly the multiply charged ions behind. Ions are then scanned out axially to the detector.

High resolution scan – enhanced resolution scan (ER)

Obtaining an accurate molecular weight of a biomolecule requires both the determination of a correct charge state and an accurate m/z. This information is important when trying to determine structural information, perform database or library searches, de novo sequence peptides, etc. This scan mode allows for high resolution MS to be obtained for an ion of interest. The first mass scanning quadrupole (Q1) is set to transmit a mass window of ~6 amu around the ion of interest. This ion is stored in the ion trap, then scanned out at 250 amu/sec over a narrow mass range (~30 amu). Peak widths of ~0.15 – 0.30 amu (width at half of intensity maximum, FWHM) can be achieved in this scan mode (Figure 5). This scan is very fast (200 ms) and can be easily used in Information Dependent Acquisition (IDA) on the LC time scale for 'on the fly' charge state determination and is used to set the precursor m/z for MS/MS.

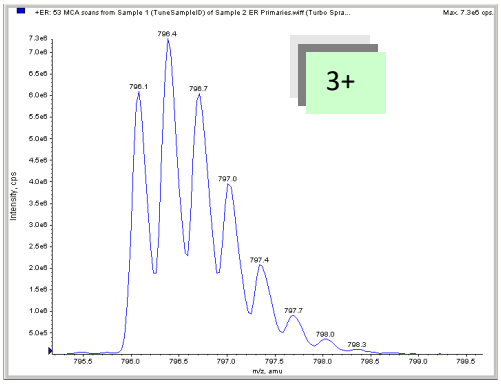

Figure 5. Enhanced resolution scan (ER). A high resolution scan can be obtained on any ion of interest. This can be used to determine the charge state of an ion and is typically used on the fly during data dependent acquisition experiments.

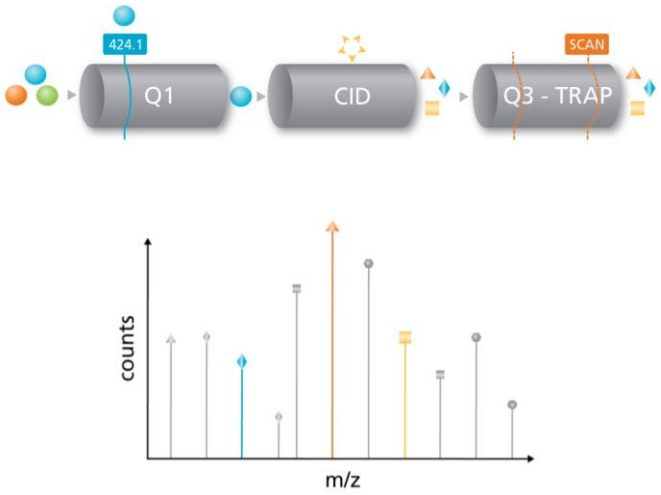

Figure 6. Enhanced product ion scan (EPI). Precursor ions are selected in Q1, fragmented in Q2 using CAD, and then detected in the LIT. This unique QTRAP System scan type will provide a high sensitivity full scan MS/MS spectrum of any precursor ion without the low mass cut-off typically observed with conventional ion trap instruments.

Enhanced product ion scan (EPI)

The Enhanced Product Ion Scan is the standard method for performing MS/MS on the QTRAP System. It is actually a hybrid QqQ/LIT scan because it combines the capabilities of the QqQ with the LIT to achieve a performance level not possible with either analyzer alone. The fragmentation is done in the collision cell and thus provides the information rich MS/MS spectrum typical of collisionally activated dissociation (CAD) fragmentation seen on QqTOF and triple quadrupole instruments[4]. In this scan mode, the precursor ion to be fragmented is first selected in Q1 with a mass window of 1-4 amu wide, filtering out all other ions. The precursor ion is fragmented by CAD in the Q2 collision cell. The fragment ions generated are captured in the ion trap; then scanned out at one of three scan speeds, depending on the required fragment ion resolution (Figure 6). This provides extremely high sensitivity, high quality MS/MS data because the fragment ions are captured and analyzed in the LIT. However, it is important to note that because the mass isolation step is performed in Q1 and ***not*** within the ion trap, a complete spectrum is obtained covering the full mass range. This is in contrast to when isolation and fragmentation are performed within the ion trap itself where masses from the lower 1/3 of the parent ion will be "cut off" and unobservable due to the energetics required for the experiment. Thus an EPI scan does not suffer from the low mass cut-off problem typical of other ion trap mass analyzers (Figure 7).

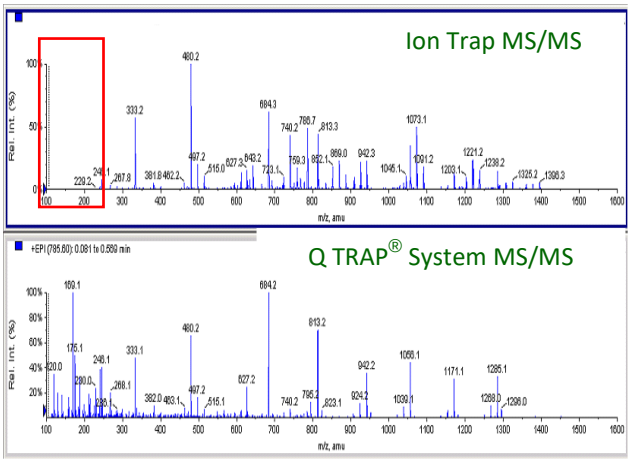

Figure 7. Ion trap sensitivity enables qualitative workflows on a quantitative platform. The full scan sensitivity when using the QTRAP Systems in linear ion trap mode is typically >50-100x more sensitive than the equivalent scan in quadrupole mode and without the low mass cut-off typically observed with conventional trap-based instruments. This hybrid functionality makes the QTRAP System the only triple quadrupole based instrument that can do full quantitative and qualitative workflows ***and*** hybrid quantitative / qualitative workflows.

MS/MS/MS (MS^3)

The MS/MS/MS Scan is a very powerful tool for obtaining further structural information on a compound of interest. This scan is in practice performed similar to the MRM^3 scan. Q1 selects a precursor of interest. Q2 fragments the precursor by CAD. Fragment ions are passed to the LIT where one is selectively isolated to induce further fragmentation. The resulting secondary fragment ions are then scanned to the detector. The full scan collected on the secondary fragment ion can be used to further distinguish the structure of the target analyte.

Unique and powerful workflows

The unique configuration of the QTRAP Systems also enables powerful workflows that combine quantitative and qualitative capabilities within the same experiment. Here QqQ and LIT scans can be linked in a cyclic manner to provide unmatched power and flexibility for a multitude of applications.

TripleTrap™ Scanning

TripleTrap Scanning refers to any workflow that engages both a QqQ scan and LIT scan within the same experiment. Typically TripleTrap Scanning is employed during information dependent acquisition (IDA) experiments in which a QqQ scan is followed by an LIT scan. Some examples of TripleTrap Scanning workflows include:

- MRM > ER > EPI. This workflow is for targeted quantitation with qualitative confirmation (also known as the MIDAS™ Workflow when used for peptides analysis).
- EMS > ER > EPI. This is an untargeted screening workflow for identifying analytes in a complex mixture.
- NL or PI > EPI. This workflow is for targeted detection of specific compounds classes with qualitative identification.

Performance enhancement features

Sensitivity enhancement feature – Q0 Trapping

Q0 trapping can be employed to increase the sensitivity of any LIT or hybrid scan. When Q0 trapping is turned on, ions produced at the source are accumulated in Q0 during the scanning of the LIT. When the ion trap is ready to be filled again, the ions from Q0 are released (Figure 8). Often, ~5-10x increase in signal intensity is observed in MS/MS mode when Q0 trapping is activated.

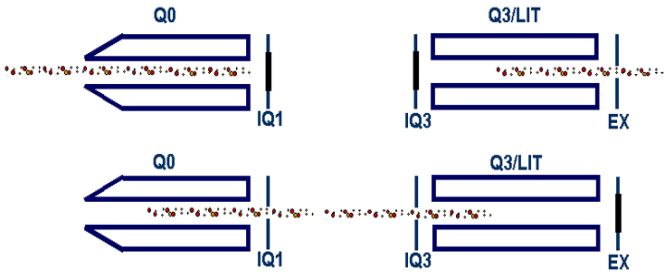

Figure 8. Q0 trapping. Ions can be captured and stored in Q0 during trap scanning, reducing ion losses. When the trap is ready to be filled for the next scan, ions are released from Q0 and passed back to Q3. This can provide significant gains in sensitivity in EPI mode (MS/MS).

Optimized dynamic range – dynamic fill time

Dynamic Fill Time (DFT) is a feature specifically designed to optimize the data obtained in every spectrum for the linear ion trap scans. DFT will automatically adjust the fill time used to fill the ion trap based on the ion flux coming from the source. A very quick quadrupole scan is performed using the same mass range to be measured and determines the number of ions reaching the detector. For more intense ions, the fill time will be automatically reduced to ensure the trap is not overfilled with ions. For less intense ions, the fill time will be automatically increased, ensuring that good ion statistics are obtained in the spectrum.

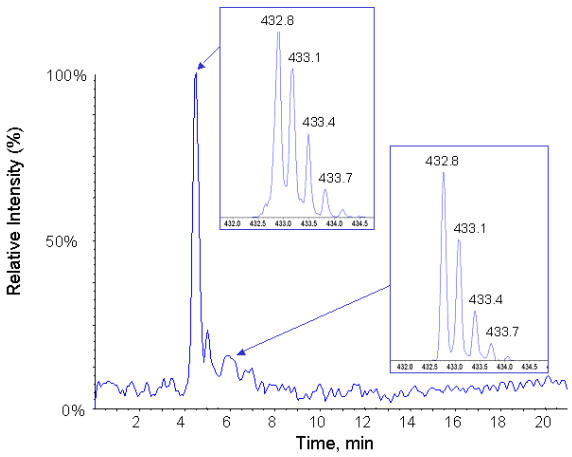

Figure 9. Dynamic fill time (DFT). Dynamic Fill Time carefully controls the number of ions that fill the linear ion trap so that optimum sensitivity and good resolution can be achieved across the LC-MS peak. Shown here is good resolution achieved at the LC peak apex where DFT automatically reduces the trap filling to reduce the chance of saturation (top) and good sensitivity obtained low in the peak tail where the trap is filled for longer (right).

Improving MS/MS through collision energy spread

Collision Energy Spread is a feature designed to improve the fragmentation quality of the MS/MS data collected 'on the fly' during Information Dependent Acquisition (IDA). The collision energy used during the filling of the ion trap is stepped across a range of energies preset in the software, ensuring that an information rich spectrum is obtained during every MS/MS.

Conclusions

The unique combination of a QqQ and LIT analyzer within the QTRAP System enables both quantitative and qualitative experiments on the same platform with the same performance one would expect from two separate high quality, high performance instruments. High sensitivity quantitation experiments can be performed in MRM mode with no compromise in performance. When required, the unique configuration can be leveraged to obtain qualitative information on samples. In fact, the unique configuration of the two analyzers together actually enables *higher* sensitivity and *higher* quality MS/MS scanning beyond what is possible with traditional triple quadrupole and linear ion trap instruments alone. Additionally, the QTRAP System configuration enables valuable new workflows that can link QqQ scans with LIT scans within automated acquisition strategies to provide the ultimate flexibility for a wealth of different analytes and applications.

References

1. Hager JW, (2002) A new linear ion trap mass spectrometer, *Rapid Commun. Mass Spectrom**16***, 512-526.
2. The *Scheduled* MRM™ Algorithm Pro. SCIEX Technical Note RUO-MKT-02-8539-A.
3. MRM^3 Quantitation for Highest Selectivity in Complex Matrices. SCIEX Technical Note RUO-MKT-02-2739-A.
4. Hager JW and Le Blanc JCY, (2003) Product ion scanning using a Q-q-Q linear ion trap (Q TRAP) mass spectrometer, *Rapid Commun. Mass Spectrom*. **17**, 1056-64.

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices