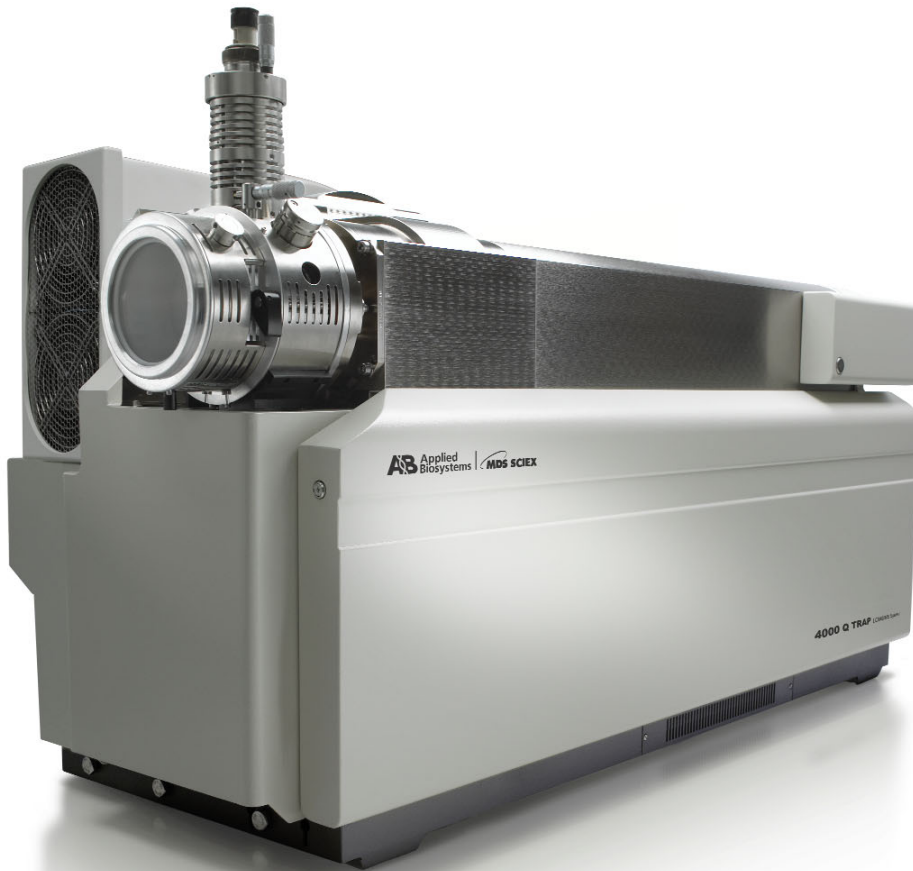


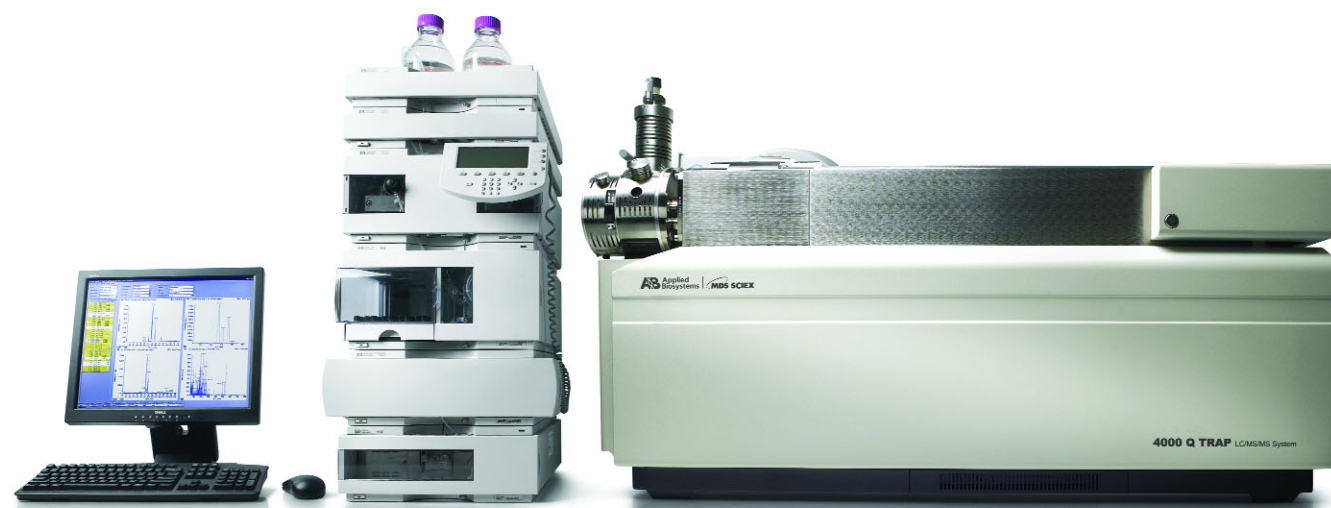
Advanced Linear Ion Trap  
technology at the highest  
level of sensitivity



4000 Q TRAP™  
LC/MS/MS System

# The highest performance ion trap and the highest sensitivity triple quad. All in one system.

Introducing the Applied Biosystems/MDS SCIEX 4000 Q TRAP™ LC/MS/MS System. Innovative Linear Ion Trap (LIT) technology from the leaders in mass spectrometry brings together fast, sensitive qualitative analysis with the proven, high sensitivity quantitation of the industry's premier triple quadrupole.



The innovative 4000 Q TRAP LC/MS/MS System provides an unmatched level of performance, while enabling fast, automated workflows that reduce analysis time and maximize the information you achieve from every run.

## Exciting new application possibilities.

Delivering maximum sensitivity quantitative and qualitative performance in one instrument, the new 4000 Q TRAP™ LC/MS/MS System opens up a whole new class of application workflows for proteomics, drug discovery, and drug development. By combining true triple quadrupole scan modes with sensitive ion trap scans in a single LC/MS/MS run, you can achieve results that previously required multiple analyses on multiple MS platforms. And in many cases, you can acquire data that is not easily obtainable by any other means.

## A complete, integrated system.

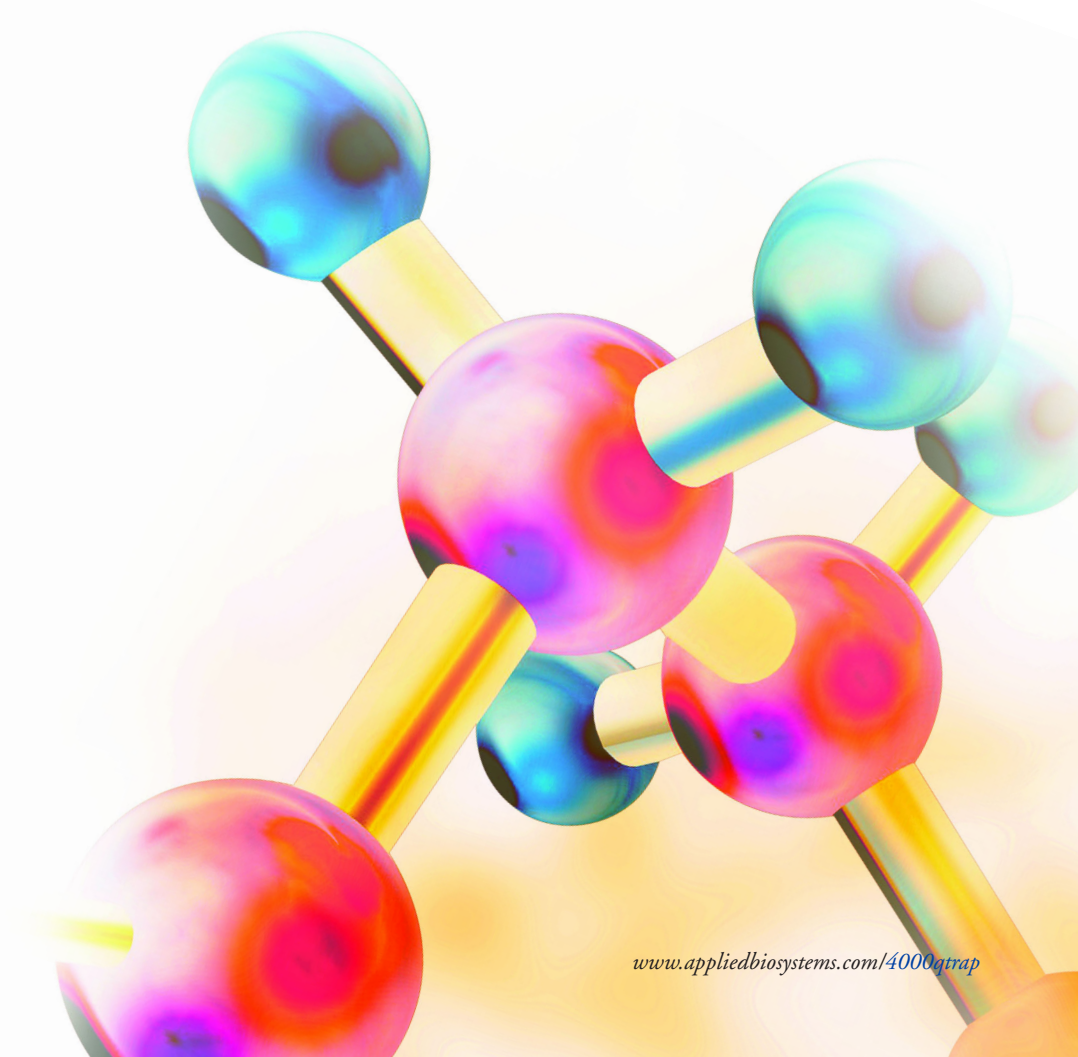
The rugged, robust 4000 Q TRAP system sets a new standard of dependability for the high throughput laboratory. With a full complement of automation features, it fits seamlessly into your lab's workflow and boosts your discovery productivity. The system includes intuitive application-specific software with all the controls required for 21 CFR Part 11 compliance; we'll also help with any validation support you may need. In addition to the mass spectrometer, Applied Biosystems/MDS SCIEX can also supply the chromatography front end through our LC partners.

## Powerful, industry-leading software.

Powerful Analyst® and BioAnalyst™ software puts all of the 4000 Q TRAP system's sophisticated performance features right at your fingertips, and simplifies every aspect of methods development, data acquisition and processing. Advanced, built-in automation capabilities make it easy to get meaningful results, and the flexible control software supports most popular LC platforms.

## A single solution for discovery and development.

The combination of the highest sensitivity triple quad with the most sensitive linear ion trap technology, coupled with versatile Analyst® software, offers a total system solution that's a perfect fit for any busy laboratory. The extended productivity features of the application-specific software—Metabolite ID, Pro ID, and Pro ICAT with links to Celera Discovery System™ (CDS) software—complement the 4000 Q TRAP system's superior sensitivity and performance, to give you more useful information per sample than any other single system.



# One system not only does it all. It does it better.

## Unequalled triple quad and ion trap sensitivity

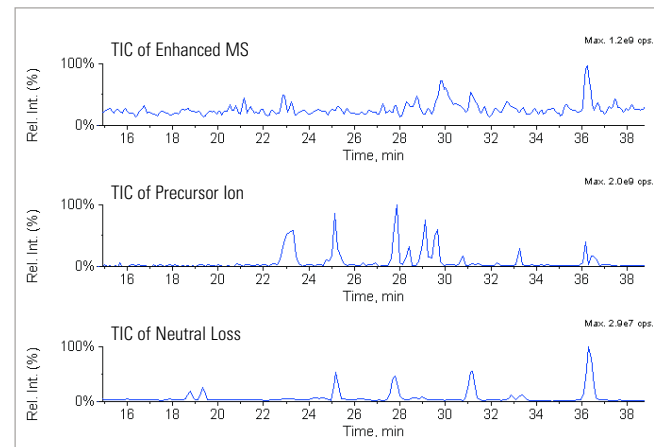
Superior triple quad performance, plus patented collisional focusing and linear ion trap technologies combine to maximize full scan MS and MS/MS sensitivity, enabling you to identify more low abundance metabolites, proteins and post-translational modifications with a high degree of confidence.

## Highest sensitivity MRM

The 4000 Q TRAP™ system provides true triple quadrupole multiple reaction monitoring (MRM) at the highest level of sensitivity, as well as extended dynamic range, ensuring superior quantitation performance for both small molecules and peptides.

## MS<sup>3</sup> capability

Advanced MS<sup>3</sup> functionality, together with triple quadrupole fragmentation patterns, gives you more useful information in fewer experiments—including detailed structural information and insight into metabolic pathways.

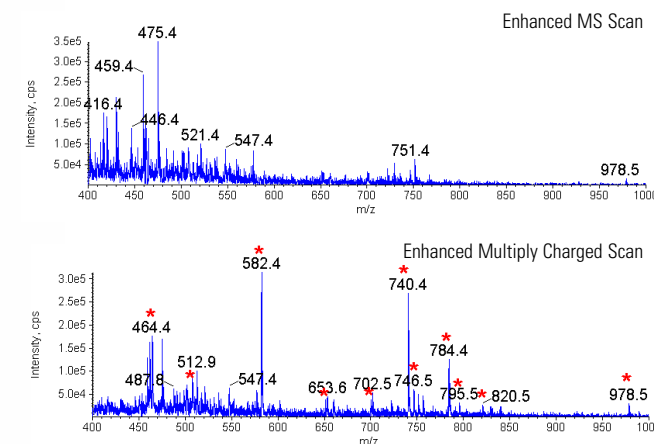


Highly specific precursor ion and neutral loss scans extract more meaningful information from any given run.

## Advanced scanning capabilities

The highest sensitivity neutral loss and precursor ion scans and enhanced multiply charged scan can be used in flexible combinations to enable information-rich, high throughput workflows.

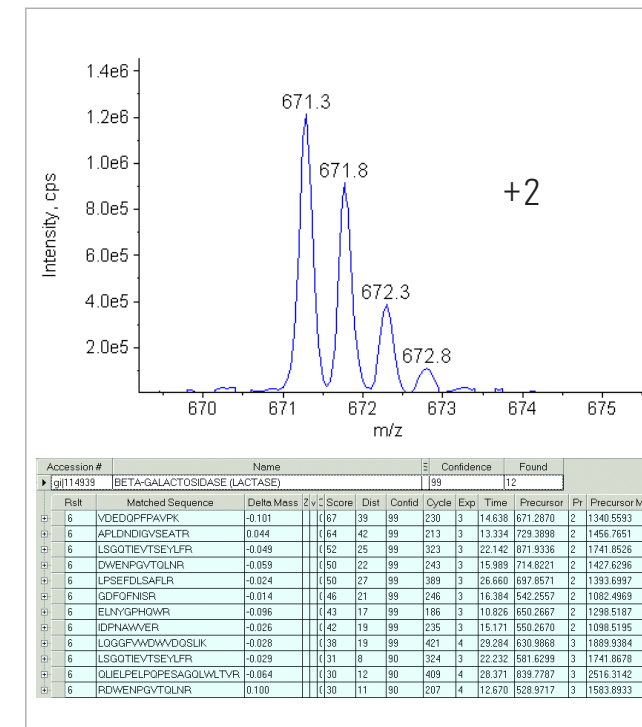
Automated LC/MS/MS workflows using Information Dependent Acquisition (IDA) provide the framework for deriving maximum information from every experiment. When used with the 4000 Q TRAP system's powerful mixed scan modes, IDA lets you focus on specific ions of interest for increased productivity.



Enhanced multiply charged scan preferentially reduces singly charged ions and highlights the peptide ions of interest.

## Higher resolution, improved mass accuracy

Next generation linear ion trap technology provides enhanced resolution for reliable real-time charge state and isotope pattern determination, plus superior mass accuracy across the entire mass range.



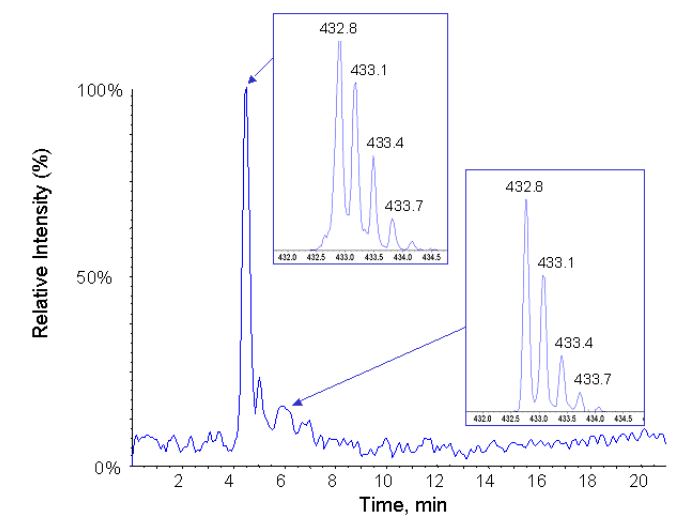
Superior resolution and higher mass accuracy greatly increase the specificity and speed of database searches to give you better confidence in all identification results.

## Plug-and-play sources

Rugged, reliable ion sources are easily interchanged for a wide range of applications and flow rates to suit your lab's needs. Choices include the exclusive Turbo V™ source with TurboIonSpray® probe and APCI probe, the new DuoSpray™ ion source—a combined software-selectable ESI/APCI ionization source—and a new NanoSpray™ source and interface for nanoflow applications.

## Dynamic Fill Time (DFT)

The system dynamically calculates the time required to fill the linear ion trap. For abundant compounds, a short fill time reduces the space charge effects by limiting the number of ions in the ion trap, while a longer fill time increases weak signals by allowing ions to accumulate.



Dynamic Fill Time capability ensures high quality data over a wide dynamic range.

# Taking Linear Ion Trap technology to new levels of performance and sensitivity.

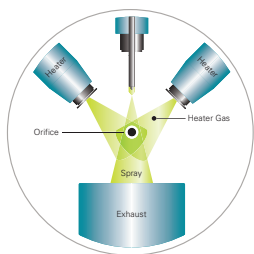
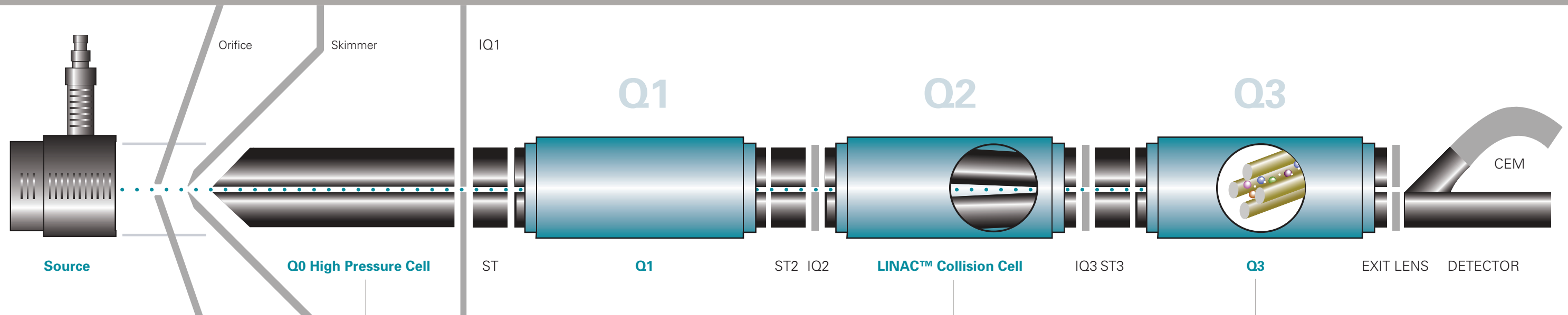
The 4000 Q TRAP™ LC/MS/MS System takes advantage of a number of mass spectrometry innovations to deliver unmatched quantitative and qualitative performance within a single system. The instrument combines the advanced features of Applied Biosystems/MDS SCIEX Linear Ion Trap (LIT) technology—including significantly higher injection and trapping efficiencies, greater ion capacity, and higher duty cycle—with the unequalled sensitivity of the leading triple quadrupole system for drug development.



“The 4000 Q TRAP™ system is really more than just a hybrid instrument. It gives you all the capabilities of the world’s most sensitive triple quad and the world’s largest capacity linear ion trap, without sacrificing performance on either side.”

Dr. Jim Hager, Senior Research Scientist, MDS SCIEX

LINEAR ION TRAP



## Innovative Turbo V™ ion source

Embedded ceramic heater technology and improved gas dynamics give you the lowest detection limits available, enabling the highest sensitivity quantitation over the wide range of flow rates used in drug metabolism and DMPK analyses. Quick-change APCI and TurbolonSpray® probes let you switch between ionization modes in seconds.



## New NanoSpray™ Source and Interface

A new NanoSpray source gives you the versatility of discrete nanospray, nanoflow or microflow capabilities. There’s also a new interface for more rugged and efficient transfer of ions from the NanoSpray source into the system, increasing robustness and sensitivity.

## Patented Q0 High Pressure Cell

**Q0 collisional focusing.** Unique, high-pressure collisional focusing technology maximizes transmission of ions for superior sensitivity.

**Q0 trapping.** Ions can be accumulated in the Q0 region of the system while the Q3 trap is scanning ions during MS/MS and MS<sup>n</sup> scans. This results in greatly improved duty cycle, as well as improved sensitivity.

## Patented LINAC™ collision cell technology

The patented LINAC high-pressure collision cell accelerates ions through the collision quadrupole, providing increased sensitivity at greatly reduced dwell times.

## Patented Q3 linear ion trap

Use of a quadrupole as a linear ion trap significantly enhances ion trap performance while maintaining complete triple quadrupole functionality:

**Greater ion capacity.** The larger, linear ion trap can accommodate up to 70X more ions than a 3D ion trap, providing greater sensitivity before the onset of space charge effects.

**Improved injection and trapping efficiencies.** With an ion path 30X longer than a 3D ion trap, ions have more time to lose energy, promoting capture and further enhancing sensitivity.

**New Dynamic Fill Time (DFT).** Ensures high quality data for a wide range of analyte concentrations.

**Higher duty cycle.** Faster scan time provides more information in less time for any given experiment; more scans over a given chromatographic peak result in more thorough investigation of your complex samples.

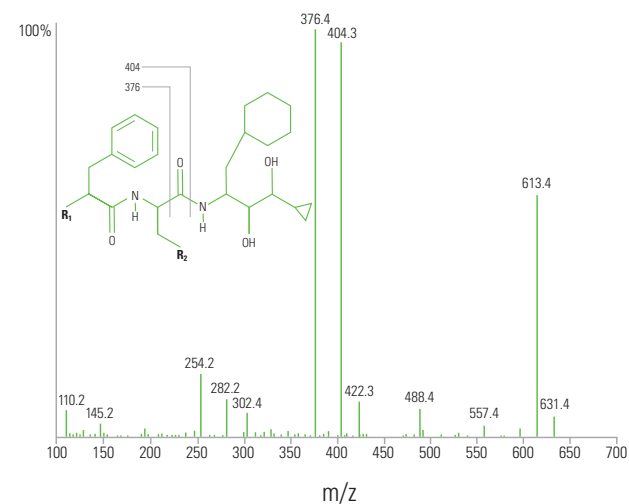
**No low mass cut-off.** The fragmentation step (LINAC collision cell) and the trapping step (Q3) are spatially separated, making capture and analysis of lower mass ions possible.

# Automated metabolite identification and confirmation.

The 4000 Q TRAP™ system's highest quantitative (MRM) sensitivity and highly specific scan functions, along with the ability to trigger MS/MS and MS<sup>3</sup> from MRM transitions, enables rapid, single-run identification, characterization and confirmation of Phase I and II metabolites.

In complex biological matrices, the added selectivity of triple quadrupole scan functions such as precursor ion (PI) and neutral loss (NL) scans can identify expected as well as unexpected metabolites based on basic knowledge of the drug and its MS/MS fragments. The superior triple quadrupole performance—with the highest sensitivity PI and NL scans available—assures detection of even low abundance metabolites; the highest sensitivity ion trap guarantees superb product ion spectra for confirmation, all in the same run.

## Parent Drug MS/MS



Product ion spectrum of the drug compound (m/z 631) results in two prominent fragment ions (m/z 404 and 376).

For targeted Phase I and II metabolite analysis, MRM provides an extremely sensitive and selective approach. In this example, potential Phase I metabolites were screened based on a list of 26 theoretical MRM transitions automatically compiled from the mass shifts of six potential common biotransformations of the parent drug, together with two fragment ion masses determined from the parent drug MS/MS fragmentation pattern.

With the LINAC™ collision cell, up to 100 MRM transitions can be monitored in a single experiment. In order to eliminate erroneous metabolite identifications from the MRM transitions, the system has the ability to trigger MS/MS and MS<sup>3</sup> to generate a spectrum that can be used to confirm the presence and structure of the metabolite.

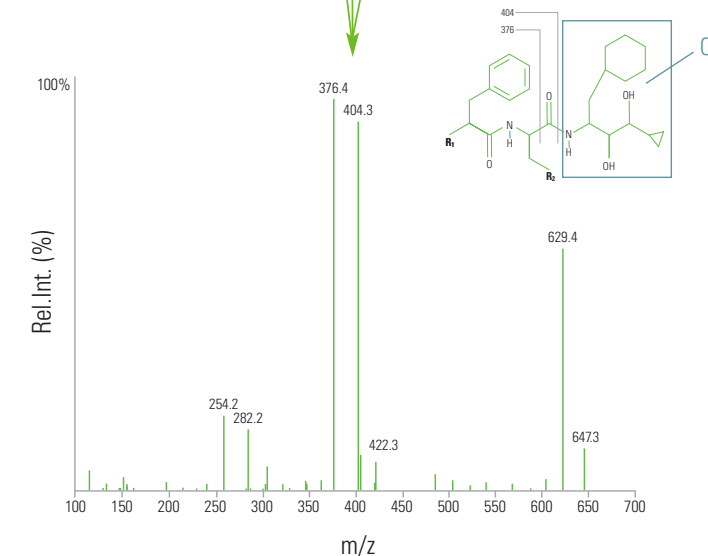
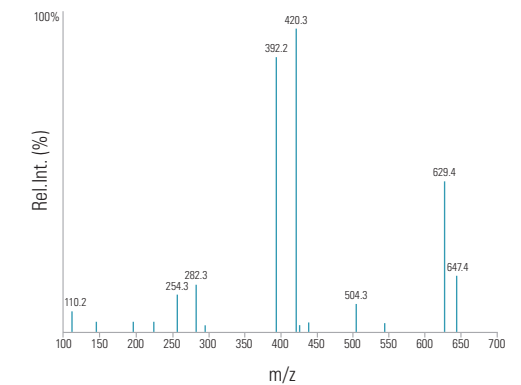
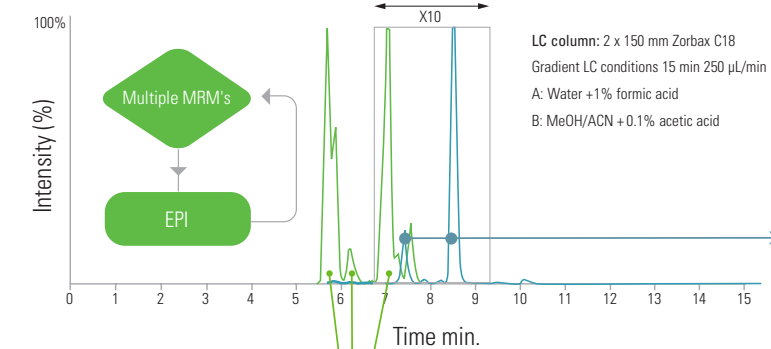
## Six Phase I transformations yield 26 MRM transitions

Compound	(M+H) <sup>+</sup>	Frag. Shift	No Shift	Frag. Shift 2	No Shift 2
Drug	631	404		376	
Hydroxy	647	420	404	392	376
Methylation	645	418	404	390	376
Dehydroxylation	614	387	404	359	376
Demethylation	617	390	404	362	376
Methylation + Hydroxylation	661	434	404	406	376
Dehydrox + Methylation	629	402	404	374	376

Use?	Gain/Loss	Formula	Mass Offset	Name
1	✓	-1 OCH2	-30.011	Hydroxy-Methylation
2	✓	-1 H2	-2.016	Dehydroxy-Methylation
3	✓	-1 CH2	-14.016	Demethylation
4	✓	-1 OH	-17.003	Dehydroxylation
5	✓	1 CH2	14.016	Methylation
6	✓	1 O	15.995	Hydroxy
7	✓	1 SO3	79.957	sulfonation
8		2 CH2	28.032	di-methylation
9		1 O609H8	176.034	glucuronidation
10		2 O609H8	352.068	bis-glucuronide
11		1 O609HSO3	255.991	gluc-sulphate
12		1 O609H8O	192.029	gluc-oxidation
13		1 C10H15N3O6S	305.071	GSH
14		1 O6H10O5	162.055	glucose
15		2 O	31.990	di-oxidation
16		3 O	47.985	tri-oxidation
17		1 H2O	18.011	gain of H2O
18		1 H2	2.016	hydrogenation

The precursor and product ion information from the product ion spectrum, along with the six biotransformations (user-defined or from the Metabolite ID software) can be used to automatically generate an MRM acquisition method that contains 26 theoretical transitions.

## Hydroxylation metabolite mass chromatograms



Extracted ion chromatograms (XIC) for two MRM transitions, 647→ 404 and 647→ 420 of the parent drug in rat hepatocytes are displayed above. Five hydroxylation metabolites were confirmed with MS/MS. For three metabolites, shown in the green 647→ 404 XIC, the MS/MS spectrum clearly indicates the site of hydroxylation is present in the blue box.

## Comparison of EMS vs. MRM

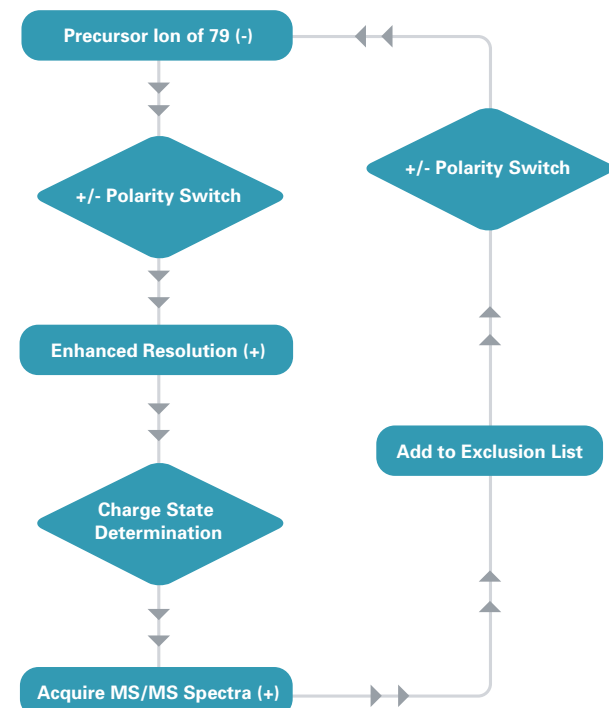
Metabolite	(M+H) <sup>+</sup>	EMS	MRM
Drug	(631)	1	1
Hydroxylation	(647)	2	5
Methylation	(645)	1	3
Dehydroxylation	(614)	0	1
Demethylation	(617)	0	1
Methylation + Hydroxylation	(661)	0	1
Dehydroxylation + Methylation	(629)	0	2
<b>Total</b>		<b>4</b>	<b>14</b>

In a single 15-minute run, targeted MRM analysis significantly increased the number of identified and confirmed metabolites compared to traditional full scan enhanced MS analysis, a standard 3D ion trap scan mode.

Now extremely sensitive metabolite identification can be performed with theoretical MRM transitions and verified with IDA triggered MS/MS to confirm the identity of each metabolite in a single, quick experiment.

# Fast, automated workflow lets you quickly identify PTMs in complex protein samples.

A novel single-run 4000 Q TRAP™ system workflow enables the automated investigation of post-translational modifications. By taking advantage of the system's sensitive, highly specific precursor ion and neutral loss scan functions, and linking them to the highest sensitivity ion trap MS/MS scans, in a single experiment you can achieve fast, definitive results that previously required multiple runs on multiple instruments.

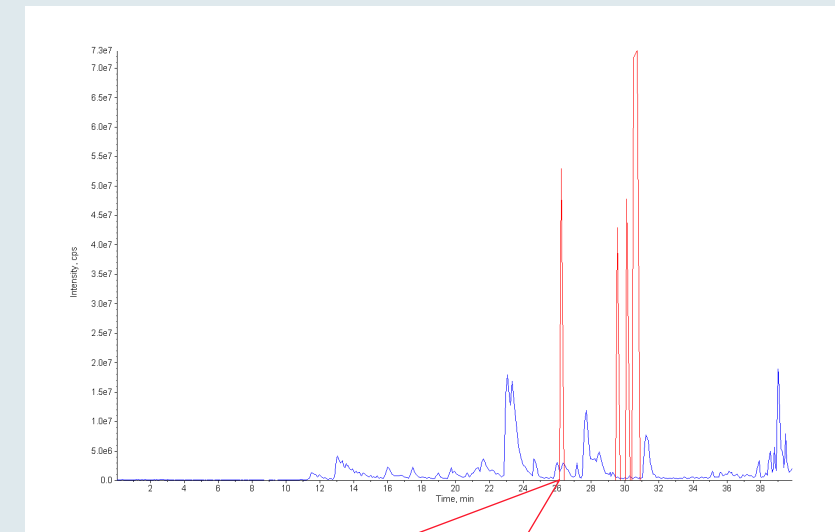


**Automated PTM discovery workflow using Information Dependent Acquisition (IDA)**

- Negative ion precursor ion (PI) scan for m/z 79 as a survey scan
- Switch polarity to positive ion
- Positive ion enhanced resolution (ER) scan for charge state determination and accurate mass
- Positive ion enhanced product ion (EPI) scan
- Database search using Pro ID software or de novo sequencing to identify protein and phosphorylation site

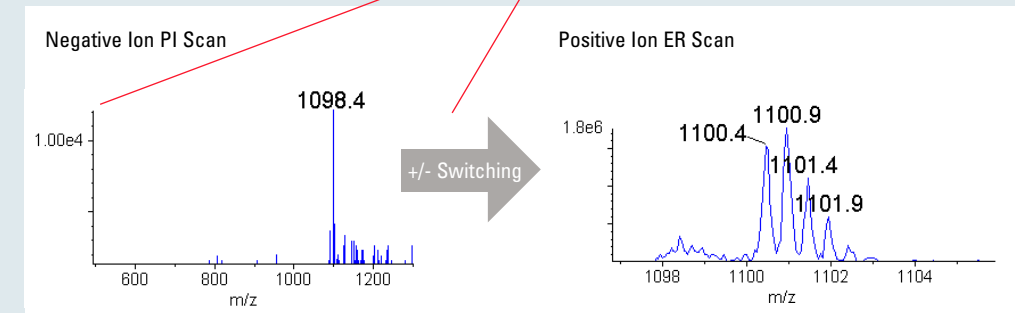
In the automated PTM workflow, Information Dependent Acquisition (IDA) links advanced scan functions to identify phosphopeptides and determine specific phosphorylation sites.

A highly sensitive and specific negative ion precursor ion scan for m/z 79 determines which peptide ions are releasing a phosphate ion (PO<sub>3</sub><sup>-</sup>). The most intense ions from the negative ion survey scan are automatically selected for a positive ion, Enhanced Resolution (ER) scan to determine charge state and assign an accurate monoisotopic mass. Subsequently, these ions are subjected to a high sensitivity, Enhanced Product Ion (EPI) scan to acquire fragmentation data. This fragmentation data is used to determine the sequence and phosphorylation site on the phosphopeptide.

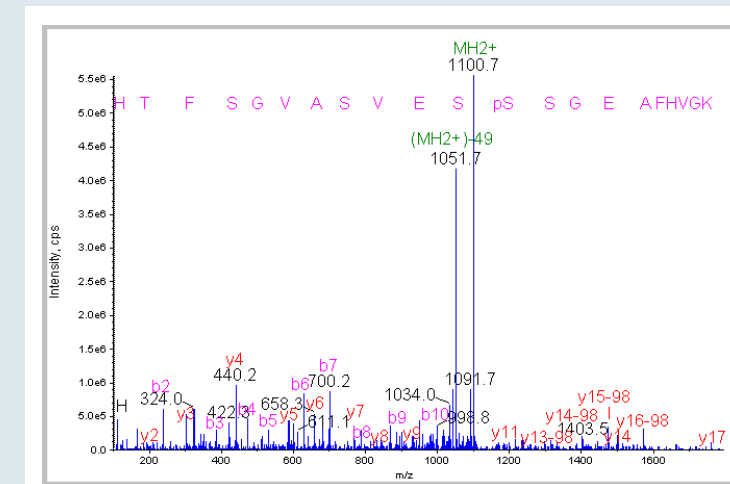


◀ A tryptic digest of reduced and alkylated standard glycoprotein, fetuin, was analyzed using the automated PTM discovery workflow on the 4000 Q TRAP™ LC/MS/MS System.

An overlay of the negative ion precursor ion (PI) scan (red trace) with a corresponding positive ion full scan (blue trace) demonstrates the specificity of the precursor ion experiment and the ability to identify the elution time of the phosphopeptides in an LC/MS experiment.



◀ The most intense negative ion PI peaks are automatically selected for an ER scan. The IDA software automatically adjusts the mass for the polarity switch from negative ion to positive ion, and assigns a more accurate monoisotopic mass. In this case, the negatively charged precursor ion at 1098.4 was assigned a more accurate positive ion m/z value of 1100.4 and a charge state of 2.



◀ Each of the ions identified by the PI scan is subjected to a high sensitivity EPI scan to acquire fragmentation data. This fragmentation data is used by BioAnalyst™ and Pro ID software to identify the protein source of the phosphopeptide, as well as the site of phosphorylation on the phosphopeptide.

45 of 147 matched - 31%

Residue	Mass	Immonium	a	a-NH3	b	b-H3PO4	y	y-H3PO4
1 H, His	137.0589	<b>110.0713</b>	93.0447	138.0662	40.0893	2199.9707	2101.9938	
2 T, Thr	101.0477	74.0600	211.1190	194.0924	<b>239.1139</b>	141.1370	2062.9117	1964.9348
3 F, Phe	147.0684	120.0808	358.1874	341.1608	<b>386.1923</b>	288.2054	1961.8641	1863.8872
4 S, Ser	87.0320	60.0444	445.2194	428.1928	<b>473.2143</b>	375.2374	1814.7956	1716.8188
5 G, Gly	57.0215	30.0338	502.2409	485.2143	<b>530.2358</b>	432.2589	1727.7636	1629.7867
6 V, Val	99.0684	72.0808	<b>601.3093</b>	584.2827	<b>629.3042</b>	<b>531.3273</b>	1670.7422	<b>1572.7653</b>
7 A, Ala	71.0371	44.0495	672.3464	655.3198	<b>700.3413</b>	<b>602.3644</b>	<b>1571.6737</b>	<b>1473.6968</b>
8 S, Ser	87.0320	60.0444	759.3784	742.3519	<b>787.3733</b>	689.3964	<b>1500.6366</b>	<b>1402.6597</b>
9 V, Val	99.0684	72.0808	858.4468	841.4203	<b>886.4417</b>	<b>788.4648</b>	<b>1413.6046</b>	<b>1315.6277</b>
10 E, Glu	129.0426	102.0550	<b>987.4894</b>	970.4629	<b>1015.4843</b>	917.5074	<b>1374.5362</b>	<b>1276.5593</b>
11 S, Ser	87.0320	60.0444	1074.5214	1057.4949	1102.5164	1004.5395	<b>1185.4936</b>	<b>1087.5167</b>
12 J, PhS	166.9984	140.0107	1241.5198	1224.4933	1269.5147	<b>1171.5378</b>	1098.4616	<b>1000.4847</b>
13 S, Ser	87.0320	60.0444	1328.5518	1311.5253	1356.5468	1258.5699	<b>931.4632</b>	833.4863
14 G, Gly	57.0215	30.0338	1385.5733	1368.5468	<b>1413.5682</b>	<b>1315.5913</b>	<b>844.4312</b>	746.4543
15 E, Glu	129.0426	102.0550	<b>1514.6159</b>	1497.5893	<b>1542.6108</b>	<b>1444.6339</b>	<b>787.4097</b>	689.4328
16 A, Ala	71.0371	44.0495	1585.6530	1568.6265	1613.6479	<b>1515.6710</b>	<b>658.3671</b>	560.3902
17 F, Phe	147.0684	120.0808	1732.7214	1715.6949	1760.7163	1662.7394	<b>587.3300</b>	489.3531
18 H, His	137.0589	<b>110.0713</b>	1869.7803	1852.7538	1897.7752	1799.7984	<b>440.2616</b>	342.2847
19 V, Val	99.0684	72.0808	1968.8487	1951.8222	1996.8437	1898.8668	<b>303.2027</b>	<b>205.2258</b>
20 G, Gly	57.0215	30.0338	2025.8702	2008.8437	2053.8651	1955.8882	<b>204.1343</b>	106.1574
21 K, Lys	128.0950	101.1073	2153.9652	2136.9386	2181.9601	2083.9832	147.1128	49.1359

◀ Using the ion matching features in BioAnalyst software, the theoretical fragment ion coverage matched to the experimental fragment ions in the spectrum can be displayed, including the phosphorylation-specific ions where phosphoric acid is lost from the b and y ions.

# Explore the advantages of new Linear Ion Trap technology.

If your research involves proteomics, drug discovery, or drug development studies, Applied Biosystems/MDS SCIEX 4000 Q TRAP™ LC/MS/MS system can make your efforts more productive by giving you a powerful, single-system solution for a wide range of applications. For more information, call the Applied Biosystems sales office nearest you, or visit <http://www.appliedbiosystems.com/4000qtrap>



**iScience.** To better understand the complex interaction of biological systems, life scientists are developing revolutionary approaches to discovery that unite technology, informatics, and traditional laboratory research. In partnership with our customers, Applied Biosystems provides the innovative products, services and knowledge resources that make this new, **Integrated Science** possible.

## Worldwide Sales Offices

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For international office locations, please call the division headquarters or refer to our Web site at [www.appliedbiosystems.com](http://www.appliedbiosystems.com).

Applera is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses. Applied Biosystems/MDS SCIEX is a joint venture between Applera Corporation and MDS Inc.

## Headquarters

850 Lincoln Centre Drive  
Foster City, CA 94404 USA  
Phone: 650.638.5800  
Toll Free: 800.345.5224  
Fax: 650.638.5884

For Research Use Only.  
Not for use in diagnostic procedures.

4000 Q TRAP system products are covered by U.S. patents 4,861,988; 4,963,736; 5,248,875; 5,381,008; 5,412,208; 5,565,679; 5,847,386; 6,111,250; 6,166,378; 6,177,668 and 6,340,814.

AB (Design), Applera, iScience, iScience (Design), BioAnalyst, Celera Discovery System, NanoSpray, and Turbo V are trademarks, and Applied Biosystems, Analyst, and TurbolonSpray are registered trademarks of Applera Corporation or its subsidiaries in the US and/or certain other countries. Q TRAP and DuoSpray are trademarks of Applied Biosystems/MDS SCIEX. LINAC, MDS, and SCIEX are trademarks of MDS Inc.

Agilent is a registered trademark of Agilent Technologies, Inc.

©2003 Applied Biosystems. All rights reserved.

Printed in the USA, 07/03, P+s

Publication 114BR12-01