Agilent 6300 Series Ion Trap LC/MS Systems

Flexible high-performance LC/MS^n

Agilent Technologies

Unprecedented flexibility in high-performance LC/MS^n

With four performance levels, a wide range of ionization choices, application-specific software, and a vast selection of LC technology, Agilent 6300 Series Ion Trap LC/MS systems can be tailored for the widest range of analytical challenges. Proven technology with Agilent's legendary reliability ensures that you achieve all the performance you need, all the time.

Performance levels to match your applications and budget

Whether you are searching for low-abundance proteins, drug metabolites in complex matrices, or pesticide residues in food products, there is a 6300 Series Ion Trap LC/MS system to meet your needs. And regardless of which 6300 Series ion trap you select, it will include high-speed, low-overhead data acquisition for compatability with modern high-resolution chromatography.

6310 makes high-performance MS^n economical

The 6310 is a great value, with outstanding sensitivity, flexibility, and reliability. It pairs fast polarity switching with data-dependant acquisition capabilities to expand the amount of data you can acquire in a single run.

6320 provides greater sensitivity, mass resolution, and scan speed

The 6320 Trap's high-capacity ion trap increases sensitivity and all-around performance. The 6320 can scan at 26,000 u/s with better than unit resolution, and offers a specialized peptide scan mode to improve identification of low-abundance proteins.

6330 offers ultimate sensitivity for low-abundance analytes

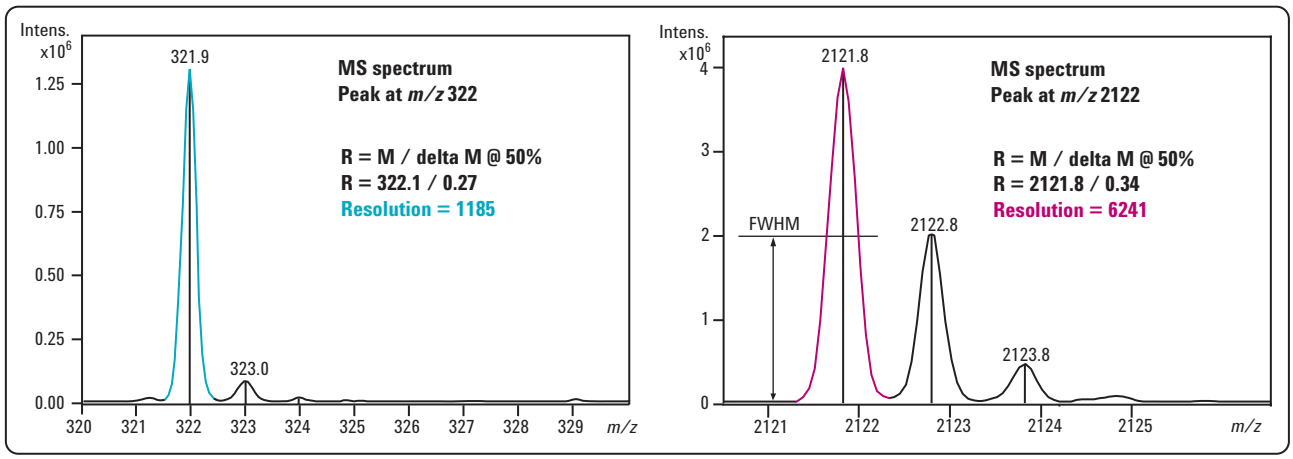
Thanks to a new high-efficiency detector, the 6330 Trap may be the most sensitive ion trap available for real-world applications. It achieves maximum sensitivity without the scan speed and resolution sacrifices required by two-dimensional ion traps.

6340 improves PTM characterization and protein identification

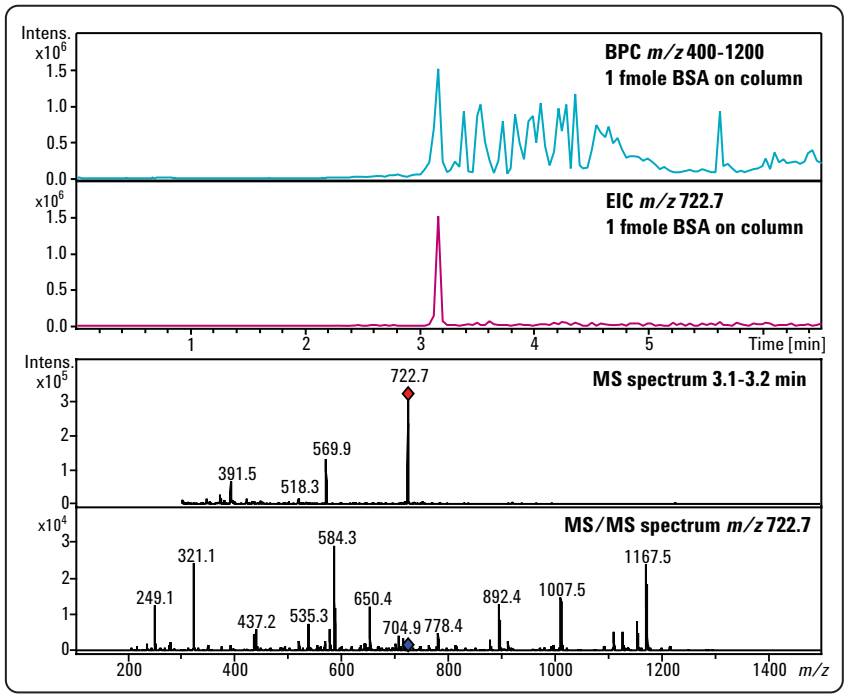
The 6340 starts with the superlative performance of the 6330 and adds electron transfer dissociation (ETD). ETD is an alternative fragmentation technique that provides a fuller picture of both peptide sequences and the locations of chemical modifications.

Superior ion trap technology yields superior performance

In ion traps, mass accuracy, mass resolution, and scan speed depend greatly on the speed and timing of ion ejection. The patented three-dimensional, multipole ion trap used in the 6300 Series creates nonlinear higher-order resonance. This nonlinear resonance very efficiently transfers energy to the ions for fast, precise ejection from the trap. The result is a superior combination of mass range, mass resolution, and scan speed. Patented phase locking precisely controls the timing of ion ejection, producing high scan-to-scan reproducibility and increasing confidence in your results.



With three-dimensional ion traps, you do not have to sacrifice scan speed for resolution. This analysis of perfluorophosphozines over a wide *m/z* 200 – 2200 mass range, performed with a 6320 Trap, demonstrates excellent resolution at a very fast 8,100 u/s scan speed.



Analysis of 1 femtomole of BSA digest on column by the 6330 Trap shows outstanding sensitivity

A better way to increase trap capacity and sensitivity

Trap capacity has a direct impact on sensitivity. Some manufacturers have adopted a two-dimensional ion trap geometry as an easy way to increase trap capacity. However, two-dimensional ion traps compromise other important operating parameters such as scan speed and mass resolution. They also require two detectors for optimum sensitivity. Dual detectors can decrease reliability and increase maintenance requirements.

Agilent has increased the capacity of the three-dimensional ion traps in the 6300 Series through careful adjustment of device geometry, materials, precision fabrication, and operating parameters. The result is high capacity with the inherent scan speed and resolution advantages of three-dimensional ion traps.

Electron transfer dissociation improves peptide sequence coverage and location of PTMs

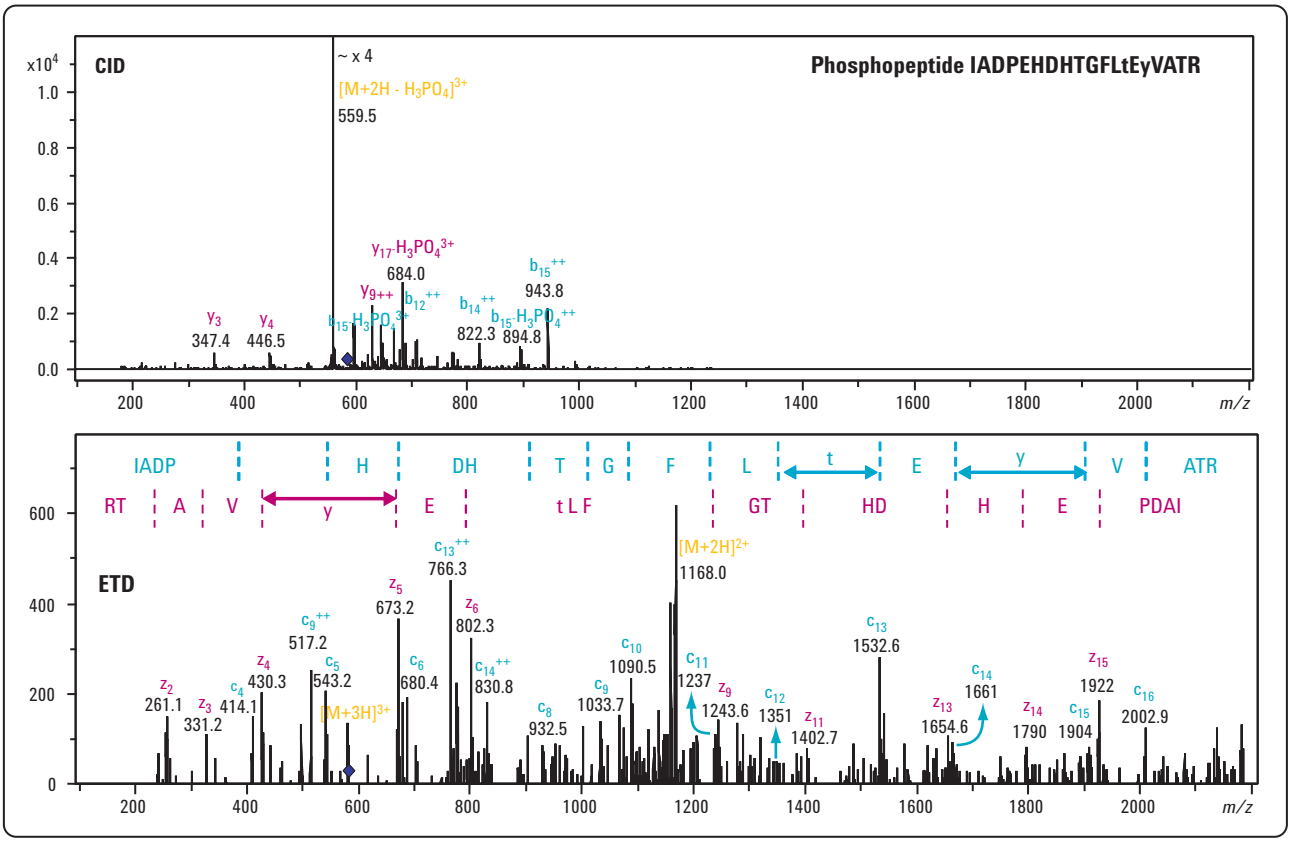
The Agilent 6340 Ion Trap LC/MS features not only standard CID fragmentation, but also an alternate form of fragmentation called electron transfer dissociation (ETD) that can enhance proteomic analyses. ETD produces primarily c- and z-series ions. ETD typically provides more even fragmentation across a peptide than CID, and hence greater sequence coverage. This greater sequence coverage enhances protein identification by either database searching or *de novo* sequencing.

Also, with ETD, post-translational modifications such as phosphorylation or glycosylation generally remain attached to the amino acid during fragmentation. This allows the specific location of the modification to be more easily determined.

The 6340 can acquire CID spectra or ETD spectra, depending on the requirements of your application. The 6340 can also alternate between CID and ETD fragmentation on a scan-by-scan basis for the fullest possible picture of both protein identity and the precise locations of chemical modifications. A special data-dependent acquisition mode is available to trigger reaccumulation and ETD when user-specified neutral losses are detected.

More fragmentation alternatives

Fast excitation dissociation is a new mode of CID operation. It eliminates the "1/3 cutoff" associated with traditional ion trap CID spectra. This enables the trapping of low-mass product ions needed for applications such as iTRAQ isotope labeling. Agilent plans to introduce fast excitation dissociation in the 6300 Series in 2007.



CID and ETD MS/MS spectra of the phosphopeptide IADPEHDHTGFLtEyVATR from recombinant human extracellular signal-regulated kinase (ERK1). The CID spectrum shows some fragmentation, but the dominant ion is from loss of phosphoric acid. The ETD spectrum shows a nearly complete c- and z-series. In the ETD spectrum, the locations of phosphorylations can be identified directly from the spectrum.

Ion source choices expand ion trap versatility

The versatility of Agilent 6300 Series Ion Trap LC/MS systems is enhanced by a wide selection of industry-leading ion sources. Available sources include:

- Electrospray (ESI) at standard, capillary (microliter), and nanoliter flow rates
- Atmospheric pressure chemical ionization (APCI)
- Multimode with true simultaneous ESI and APCI
- Atmospheric pressure photoionization (APPI)
- Atmospheric pressure MALDI with pulsed dynamic focusing

The LC/MS sources all include Agilent's patented orthogonal nebulization technology that eliminates adjustments and keeps the capillary and ion optics cleaner. All sources also include a high-capacity, counter-flow drying gas system that improves spectral quality, sensitivity, and reproducibility by reducing solvent clusters and mobile-phase adducts. Together, orthogonal spray and high-capacity drying gas make Agilent's ion sources highly tolerant of non-volatile components.

Reliable electrospray source with orthogonal nebulization and high-capacity, counter-flow drying gas

Multimode source can double throughput

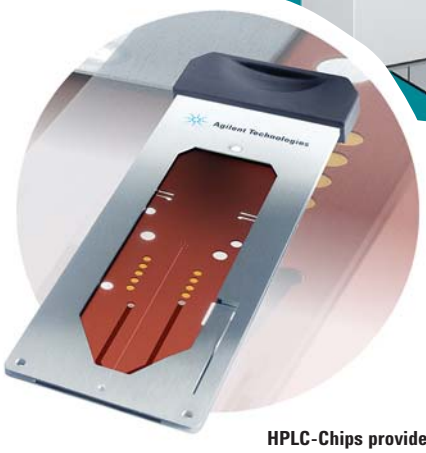
MALDI source with pulsed dynamic focusing for improved sensitivity and consistency

Easy, reproducible nanoflow separations

Agilent's revolutionary HPLC-Chips seamlessly integrate the sample enrichment and separation columns of a nanoflow LC system with the intricate connections and spray tip used in electrospray mass spectrometry. The result is a chip only slightly larger than a microscope slide that provides exceptional chromatographic resolution and enhances MS sensitivity. HPLC-Chips are much more reliable and easier to use than conventional nanocolumns. HPLC-Chips are available for both large-molecule and small-molecule applications.

The HPLC-Chip Cube MS interface incorporates chip-handling mechanisms and elements of an electrospray ion source. It completely automates chip handling, positioning, and connections to ensure maximum performance with minimum effort.

HPLC-Chip Cube MS interface maximizes sensitivity and convenience



HPLC-Chips provide superb chromatographic performance and ease of use

Intelligent data acquisition ensures high-quality data

The ability to collect multiple levels of MS data in a single run is one of the many advantages of an ion trap. But the challenge is not just to acquire data, but to do so intelligently, acquiring the best, most-informative data from each scan. 6300 Series Ion Trap LC/MS systems feature a wide array of intelligent data acquisition features to optimize the data collected from every sample.

Fully automated, data-dependent acquisition

All 6300 Series Ion Trap LC/MS instruments can perform up to 5 stages (MS^5) of fully automated, data-dependent MS/MS. The sophisticated software of the 6300 Series includes a wide array of static and active data-dependent acquisition features that help you maximize the amount of useful data acquired. For simplicity and convenience, all MS/MS data acquired from a run are stored in a single file.

Data-dependent feature	Benefit
N most abundant precursors*	Increases the number of unique precursor ions from which data are acquired. Especially helpful for coeluting compounds.
Isotopic exclusion	Prevents acquisition of unnecessary MS/MS data from isotopes (^{13}C etc.)
Static include list	Ensures data is acquired from target ions, even if they are present at low abundance
Static exclude list	Eliminates acquisition of MS/MS data from solvent ions and other known background components
Static preferred list	Ensures data is acquired from target ions, if present, but allows acquisition of data from other ions if target ions are not present
Preferred charge-state selection	Ensures selection of doubly charged peptide ions for higher quality CID MS/MS data or greater-than-doubly charged peptide ions for higher quality ETD MS/MS data
Active exclusion – repeat count	Increases the amount of unique data acquired by preventing acquisition of MS/MS data from the same ion more than a user-specified number of times
Active exclusion – exclude time	Removes ions from the active exclusion list after a user-specified time to ensure reacquisition if an ion appears in more than one chromatographic peak
Threshold – absolute*	Ensures data is acquired only from ions of user-specified abundance
Threshold – relative*	Ensures data is acquired only from ions of user-specified abundance relative to the most abundant ion in an MS scan
Neutral-loss-dependent AutoMS^3 †	Improves identification of PTMs by performing MS^3 on the N most abundant product ions that show user-specified neutral losses from their precursors
Neutral-loss-dependent pseudo-MS^n †	Improves identification of PTMs by fragmenting the N most abundant product ions that correspond to user-specified neutral losses from their precursors
Stable isotope labeling experiments (SILE)†	Provides detection and fragmentation of SILE pairs in protein quantification experiments involving ICAT, SILAC, $^{16}O/^{18}O$, ICPL, and other isotopic labeling strategies
Neutral-loss-dependent ETD‡	Improves site determination of PTMs by reaccumulating the precursor and performing ETD fragmentation when user-specified neutral losses are detected

** Can be set separately for MS >2*
† *Available only with the 6330 and 6340 models*
‡ *Available only with the 6340 model*

Maximum-resolution mode for unique, quality data

Using data-dependent acquisition criteria, the 6300 Series ion traps can scan at maximum resolution over a narrow mass window around each selected ion. The result is the best of all worlds: very high quality data—peaks are often baseline resolved—from more unique ions. You can use maximum-resolution mode for both MS and MS/MS analyses.

Peptide scan mode for better protein ID

Peptide scan mode uses the higher-resolution enhanced scan mode during MS^1 for more accurate determination of +1, +2, and +3 charge states of peptide ions, and uses the faster ultrascan mode in MS^2 and beyond to shorten cycle times. The net result is that you identify more proteins in complex peptide digests.

Fast polarity switching for increased throughput

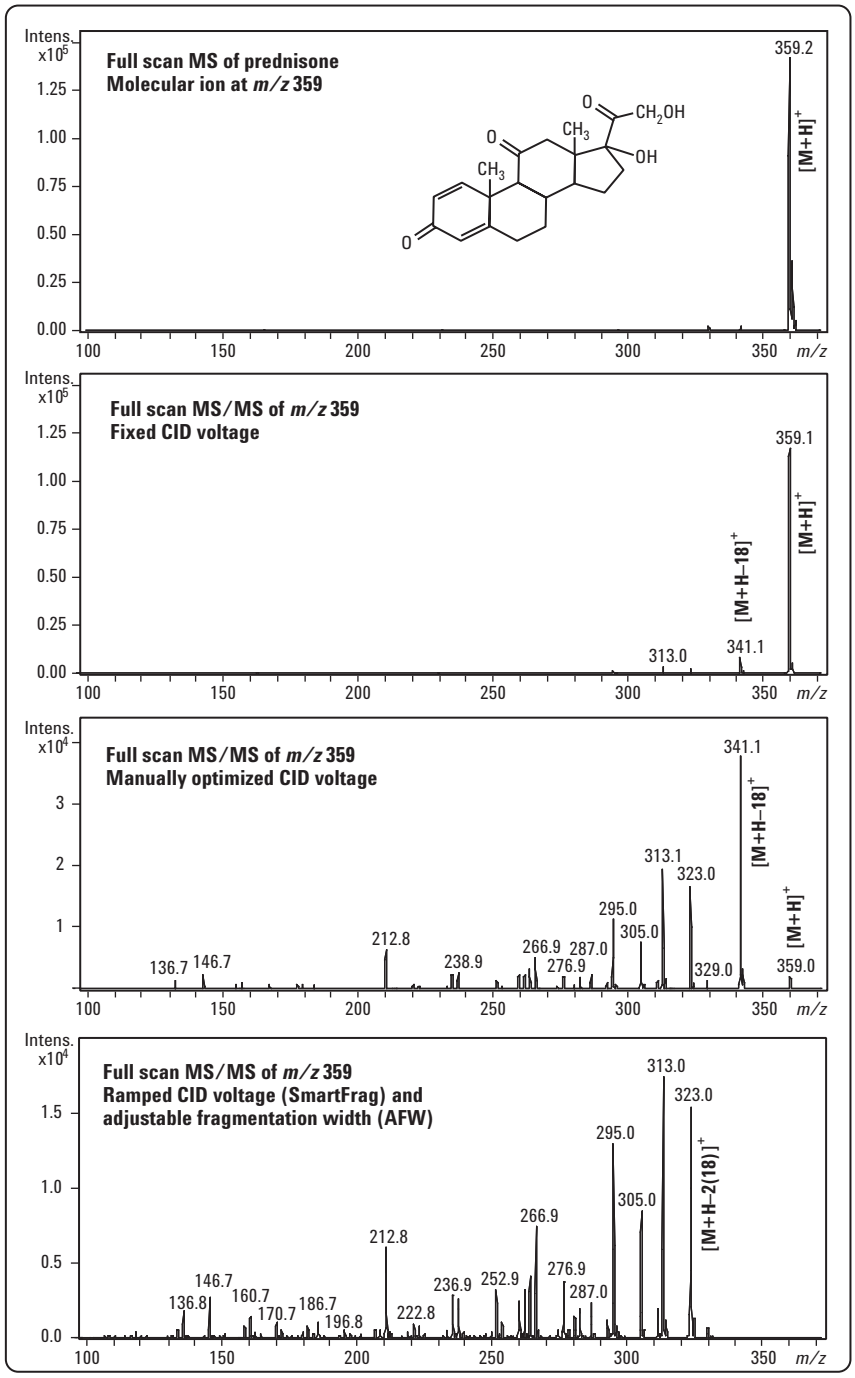
The ability to collect complementary positive- and negative-ionization data in a single run can save you precious time and sample. The fast polarity switching of the 6300 Series allows you to collect more scans over a peak for better data quality and sensitivity.

Smart parameter settings provide expert results for non-experts

To simplify operation, the ion trap software includes a "smart" mode. Based on simple information such as target mass, compound stability, and expected ion distribution, and using a real-time spectral-data-evaluation feedback loop, the software can automatically determine the optimum ion trap settings. You still have full, independent control over ion source settings for complete compatibility with LC methods.

Better MS/MS data through collision-energy ramping

Achieving optimum CID fragmentation generally requires tedious, sample-specific manual optimization. The unique SmartFrag collision-energy ramping, and adjustable fragmentation width (AFW), ensure that every precursor ion receives exactly the energy it needs for optimum fragmentation—automatically. The result is greater product ion generation and more structural information with less effort.



Automated SmartFrag CID voltage ramping and adjustable fragmentation width (AFW) provide richer MS/MS spectra and eliminate the need for time-consuming manual CID voltage optimization

Software tools make the most of your data

Sensitive, repeatable instrument performance and high-quality data are necessary, but not sufficient, to achieve your analytical objectives. It takes the right software tools to turn good data into useful information. Agilent offers a wide array of powerful software tools to help you turn your MS^n data into answers.

Simplified results navigation

Automated MS^n analyses can generate massive amounts of multigenerational MS data. The 6300 Series Ion Trap LC/MS software simplifies this by storing all data from a run in a single file. A familiar, hierarchical tree structure makes for easy navigation and location of exactly the data you need. Special filters help you extract specific subsets of data quickly.

Find compounds automatically

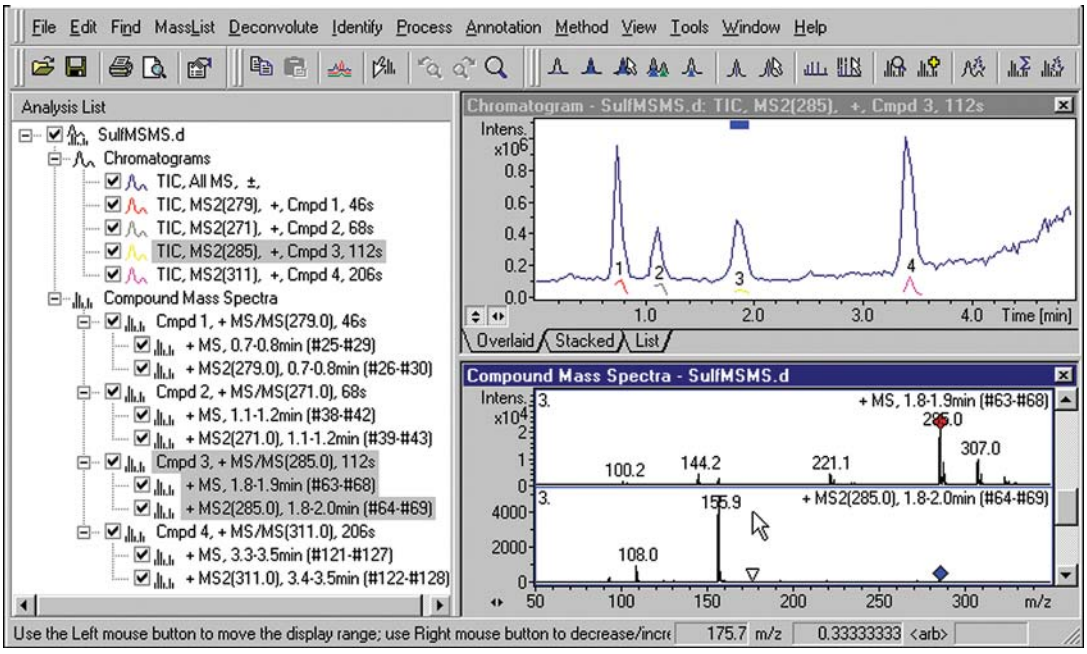
The Find Compounds functions provide powerful, compound-based data mining and data browsing for complex LC/MS^n experiments. Find Compounds will

- Locate all unique MS^n experiments
- Generate compound entries and MS^2 total ion chromatograms for each unique MS precursor ion
- Extract, average, and hierarchically organize all related MS and MS^n spectra by compound entry

Functions are also available to automatically generate averaged mass spectra for all integrated chromatographic peaks in MS-only or manual MS^n experiments.

Results can be reviewed, printed, and saved for archiving or further processing.

Hierarchical tree structure makes it easy to find exactly the data you need

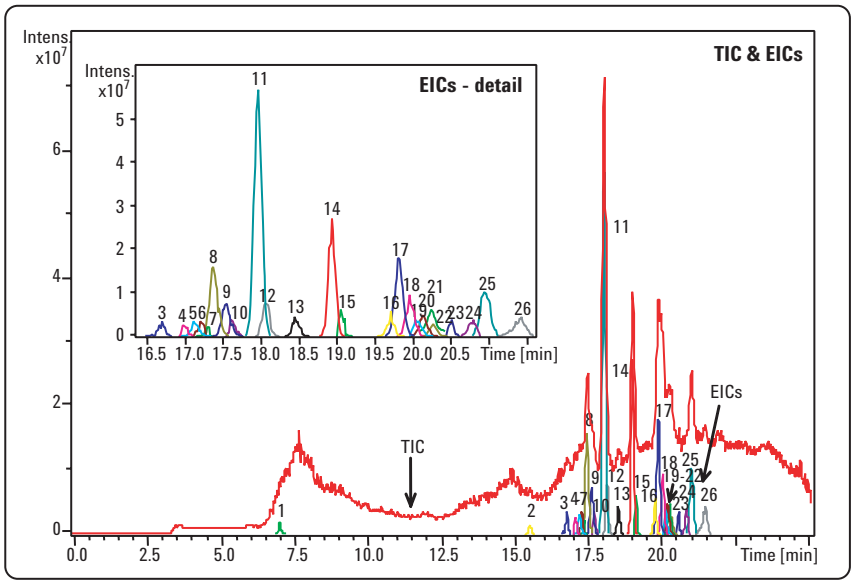


Automated compound identification speeds analyses and increases productivity

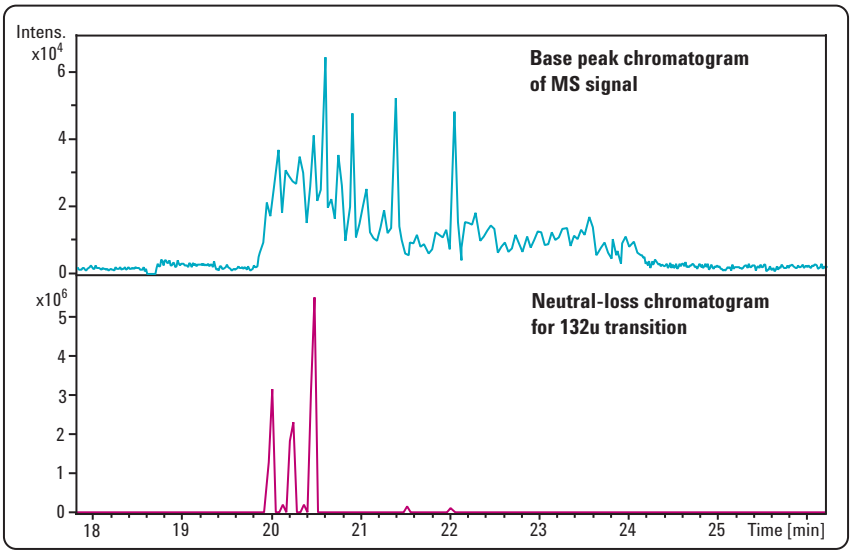
Dissect automates identification of minor components in complex samples

Trace-level components in biological samples are difficult to detect in LC/MS total ion chromatograms (TICs) because endogenous interferences produce significant background signals. Extraction of trace-level signals often requires time-consuming data analysis, and is complicated by overlapping elutions, adduct formation, and matrix interferences.

The optional Dissect program automates the location of minor components in complex MS-only data. It first generates and integrates extracted ion chromatograms (EICs) for each mass, and decides—based on symmetry, broadness, and other factors—which are valid chromatographic peaks. The program then applies fuzzy logic and multiple criteria to eliminate noise spikes and group related peaks from the same compound. Finally, Dissect obtains clean mass spectra for each compound.



In an analysis of rat liver, Dissect software located 26 metabolites of an antagonist drug in less than a minute. Results compared favorably with those obtained by an hour's worth of tedious manual identification. Even though there were many coeluting compounds, the mass spectra reconstructed by the Dissect software were relatively pure.



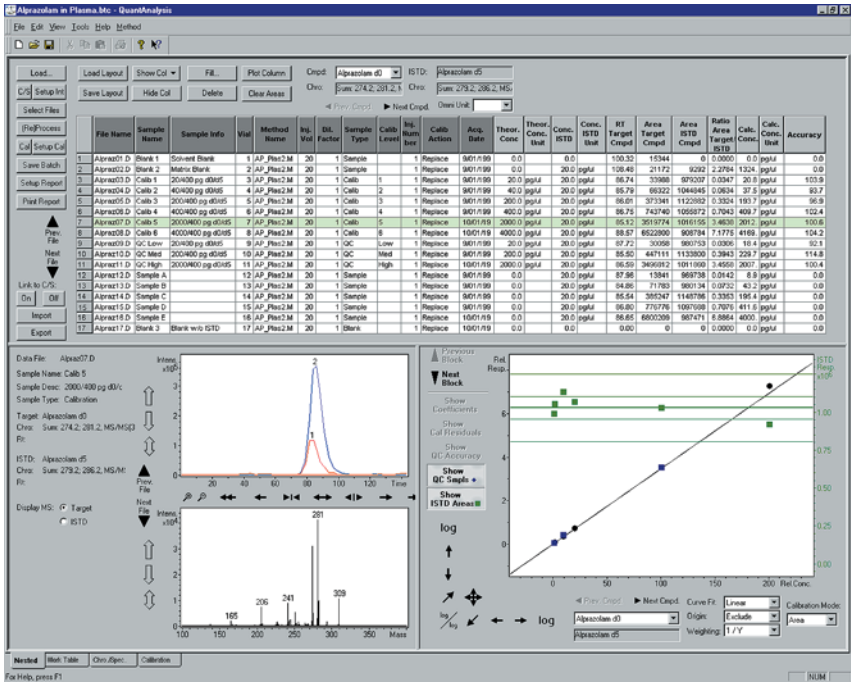
Full-scan MS and subsequent neutral-loss analysis identify anthocyanins that are O-glycosylated with arabinose

Neutral-loss analysis identifies common elements in multiple analytes

Neutral-loss analysis is a powerful tool for identifying common structural elements in multiple analytes. An inherent advantage of ion trap MS/MS is full-scan data that allows you to perform neutral-loss analysis post acquisition; you do not have to predict neutral losses in advance. The 6300 Series Ion Trap LC/MS software includes tools to make neutral-loss analysis easy. Related features such as collision-energy ramping ensure optimum fragmentation so there are plenty of product ions to analyze.

Faster, easier quantitation

Powerful QuantAnalysis software makes quantitation faster and easier. The software is comprised of three simple views—work table, chromatogram/spectrum, and calibration curve—that can be displayed in a single window or tabbed. The work table is a single spreadsheet where you both set up quantitation and view results. You can customize it to show only essential data, and these custom layouts can be saved for future use. All three views are dynamically linked; a change made in one is reflected in the others. For example, selecting a different curve fit for the calibration curve automatically recalculates the concentrations.



The work table (top), chromatogram/spectrum (bottom left), and calibration curve (bottom right) are dynamically linked—a change in one view is automatically reflected in the others

Extract the most information from every sample

ACD/SpecManager software, available from Advanced Chemistry Development, can help you extract the maximum information from your MS data:

- Component detection algorithm to reduce random noise and background
- ChemSketch, the industry-standard tool for drawing chemical structures
- Tools to simplify the addition and subtraction of spectra
- Tools to store processed chromatograms and spectra, and generate reports
- Correlation tools to assist with MS interpretation by correlating structures and MS spectra
- A direct link for easy transfer of mass spectra from the ion trap software to ACD/SpecManager

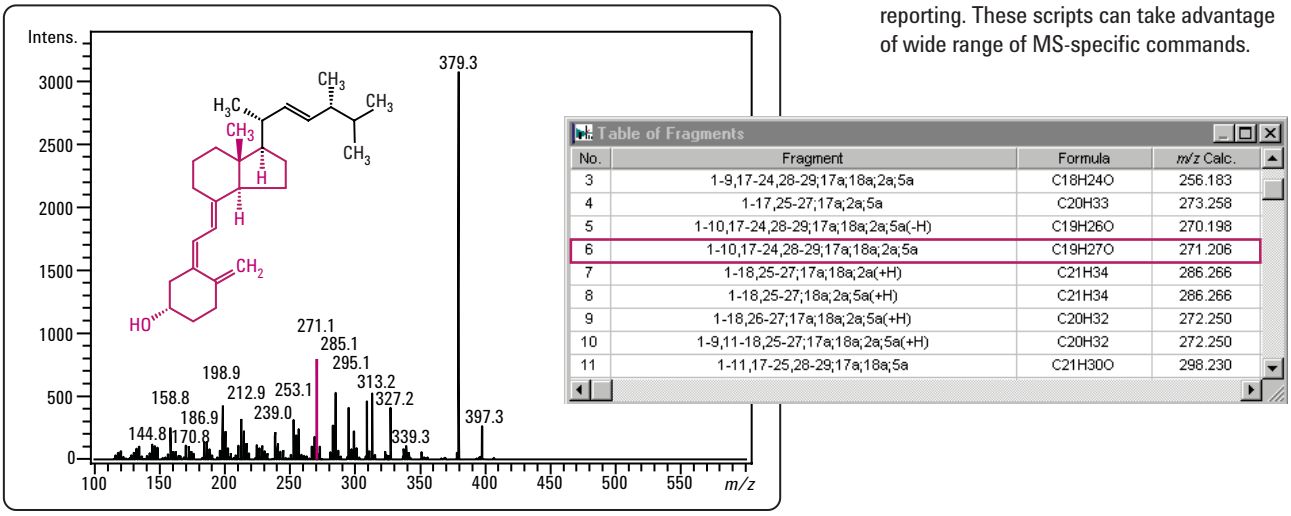
The SpecManager software also provides support for cross-technique, as well as cross-vendor-platform, multispectral data integration including NMR, MS, UV-Vis and IR.

Custom reports editor

A custom reports editor makes it faster and easier to create reports in exactly the format you need.

User-designed automation

Visual Basic scripting provides almost unlimited opportunities for user-designed automation. Your methods can call custom scripts to automate data analysis and reporting. These scripts can take advantage of wide range of MS-specific commands.



No.	Fragment	Formula	m/z Calc.
3	1-9,17-24,28-29;17a;18a;2a;5a	C18H24O	256.183
4	1-17,25-27;17a;2a;5a	C20H33	273.258
5	1-10,17-24,28-29;17a;18a;2a;5a(-H)	C19H26O	270.198
6	1-10,17-24,28-29;17a;18a;2a;5a	C19H27O	271.206
7	1-18,25-27;17a;18a;2a(+H)	C21H34	286.266
8	1-18,25-27;18a;2a;5a(+H)	C21H34	286.266
9	1-18,26-27;17a;18a;2a;5a(+H)	C20H32	272.250
10	1-9,11-18,25-27;17a;18a;2a;5a(+H)	C20H32	272.250
11	1-11,17-25,28-29;17a;18a;5a	C21H30O	298.230

With ACD/SpecManager software, you can build databases of correlations between structures and your MS data. Search these correlation tables against unknown spectra.

Spectrum Mill software facilitates high-throughput proteomics

Spectrum Mill software helps you identify proteins from MS and MS/MS data through database searching or *de novo* sequencing.

Intelligent spectral extraction speeds protein identification

Spectrum Mill software identifies and excludes noise spectra and poor-quality spectra before database searching, so search speed is greatly increased. This also reduces the number of false positives.

Multiple search options for protein identification add flexibility

The MS/MS search program includes both identity mode for unmodified peptides and variable mode for modifications.
The peptide mass fingerprinting (PMF) program does an outstanding job of identifying proteins from MS spectra.

Support for ETD data

Spectrum Mill software supports both standard CID spectra and spectra generated by ETD.

Automatic and manual match validation increases confidence

Spectrum Mill software quickly and automatically validates matches. You can also interactively review proposed matches, comparing them to the actual MS/MS spectra. Unvalidated spectra can be re-searched using alternate parameters or databases.

De novo spectral interpretation

The *de novo* sequencing algorithm generates a ranked list of potential peptide sequences. It discards unrealistic solutions and compensates for common spectral difficulties such as noise and incomplete fragmentation.

Quantitative as well as qualitative information

Spectrum Mill software automatically determines differences in relative abundances of the proteins found. It also supports ICAT, iTRAQ, and other stable-isotope labeling technologies.

Complex data made accessible

Spectrum Mill software summarizes and correlates results in ways that make the information accessible even for non-expert MS users. You can compare large data sets across multiple experiments and summarize the results at the protein level.

Compatibility with data from multiple vendors

Add-on modules are available that allow the Spectrum Mill software to process non-Agilent data formats:

- .RAW (Thermo Finnigan)
- .wiff (SCIEX LC/MS)
- .pkl (Waters and others)

MASCAT simplifies Mascot protein database searches

For protein researchers who have standardized on the Mascot database search program, the software for the 6300 Series includes the MASCAT program. MASCAT concatenates Mascot generic format (*.mgf) files, providing a means to consolidate results from multiple two-dimensional LC/MS/MS analyses into a single protein database search.

Run #	Run Name	Group (#)	Spectra (#)	Distinct Peptides (#)	Distinct Summed MS/MS Search Score	% AA Coverage	Mean Peptide Spectral Intensity	Database Accession #	Protein Name
1	ERK_CIDETD_45MIN	1	39	20	289.22	53	1.84e+006	P27361	Mitogen-activated protein kinase 3 (EC 2.7.1.-) (E

#	Frag Mode	Score	SPI (%)	Spectrum Intensity	Sequence	Modifications	m/z Measured (Da)	MH+ Matched (Da)
1	ETD	27.71	88.7	4.39e+005	(R)IADPEHDHTGFLtEyVATR(W)	t:Phosphorylated T y:Phosphorylated Y	584.24	2172.036
2	ETD	25.14	89.5	5.10e+005	(R)LKELIFQETAR(F)		450.04	1347.763
3	ETD	23.68	82.6	4.86e+005	(K)SDSKALDLLDR(M)		411.98	1232.648
4	CID	21.70	97.0	5.65e+005	(R)DVYIVQDLMETDLYK(L)		923.03	1844.899
5	CID	18.94	93.1	1.68e+006	(R)RTEGVGPGVPGEVEMVKGQPFDVGPR(Y)		899.39	2694.367
6	CID	18.89	96.9	3.38e+006	(R)DLKPSNLLINTTCDLK(I)	C:Carbamidomethylation	923.44	1844.979
7	CID	18.85	96.5	1.37e+006	(R)LKELIFQETAR(F)		674.38	1347.763
8	CID	17.13	91.8	7.22e+006	(R)YTQLQYIGEGAYGMVSSAYDHVR(K)		870.42	2608.214
9	ETD	16.70	65.2	1.37e+006	(R)LKELIFQETAR(F)		674.38	1347.763
10	CID	15.57	91.5	4.86e+005	(K)SDSKALDLLDR(M)		411.98	1232.648
11	CID	14.94	93.2	2.40e+006	(K)ELIFQETAR(F)		554.01	1106.584
12	CID	14.90	85.0	1.06e+006	(R)DLKPSNLLINTTCDLK(I)	C:Carbamidomethylation	923.07	1844.979
13	CID	14.70	87.0	3.68e+005	(R)YTQLQYIGEGAYGMVSSAYDHVRK(T)		912.49	2736.309
14	CID	14.26	90.8	5.52e+005	(K)SDSKALDLLDR(M)		616.84	1232.648

Spectrum Mill software simplifies and accelerates the identification of proteins and location of post-translational modifications

Specifications

Mass accuracy (All)

± 0.2 u within the calibrated standard mass range at normal resolution in full scan mode, with proper calibration, ICC target and ion statistics, and thermal equilibrium of electronics and ion source.

Mass axis stability (All)

Within ± 0.2 u of the observed calibrated value over 8 hours in standard mass range at normal resolution in full scan mode, with proper ICC target and ion statistics, and thermal equilibrium of electronics and ion source and ambient temperature of 21°C ± 3°C (70°F ± 6°F)

Monoisotopic precursor selection (All)

Monoisotopic precursor selection is possible throughout the standard mass range (*m/z* 50 – 2200) at ambient temperature of 21°C ± 3°C (70°F ± 6°F).

Mass range, mass resolution, and scan speed

	6310		6320, 6330, and 6340	
Mass Range *m/z*	Resolution FWHM (u)	Scan Speed (u/s)	Resolution FWHM (u)	Scan Speed (u/s)
50 – 2200	≤ 0.6	13,000	≤ 0.6	26,000
	≤ 0.45	5,500	≤ 0.35	8,100
	≤ 0.35	1,650	≤ 0.25	800
200 – 4000	≅ 3 – 4	27,000	≤ 3	27,000

Polarity switching

Scan-to-scan polarity switching with 1 positive spectrum and 1 negative spectrum in approximately 1 second for all models

Sensitivity

Conditions

Column – ZORBAX Rapid Resolution SB-C18 2.1x30 mm 3.5 micron
Mobile phase – 25% water 75% methanol 5 mM ammonium acetate
Flow rate – 400 µL/min
Mode – full scan MS/MS
MS/MS – transition of the protonated molecular ion (*m/z* 609) to the sum of the two most abundant product ions
Product ion scan range – *m/z* 175 – 650
Mass range – standard (*m/z* 50 – 2200)
Resolution – standard (0.6 u)

	6310	6320	6330	6340
Quantity (reserpine - on column)	5 pg	1 pg	250 fg	250 fg
Signal-to-Noise Ratio (full scan MS/MS)	≥ 50:1	≥ 50:1	≥ 50:1	≥ 50:1

For more information

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