Bruker Daltonics

Technical Note # TN-35

amaZon Ion Trap: New Performance Levels Meeting Demanding Analytical Requirements

The amaZon mass spectrometer platform

The amaZon™ series represents a new generation of ion trap instrumentation equipped with a full array of novel technologies directly translating into user benefits. Designed for key applications in proteomics, metabolomics, screening and compound identification, the amaZon series is todays top performing trap.

Technology lead overview

- The novel, patented dual ion funnel technology opens the door to new sensitivity levels facilitating dramatically improved ion transfer from the ambient pressure ion source to the high-vacuum mass analyzer.
- The next-generation fast scanning ion trap sets new standards with regard to the combination of scan speed and mass resolution for any kind of application.
- Fast polarity switching: the new Zero Delay Alternating™ ion source interface extracts maximum information from screening samples.
- The state-of-the art ETD and PTR setup guarantees absolute benchmark robustness and sensitivity.

Optimized ion transfer

The key challenge in API (atmospheric pressure ionization) is still the transfer of ions formed at ambient pressure into the high vacuum mass analyzer. A pressure gradient of nine orders of magnitude has to be bridged by differential pumping. The first pumping stages in particular are a substantial challenge for ion transfer. The introduction of patented dual ion funnel in the Bruker orthogonal TOF instruments was a milestone in ion transmission technology and solved many of the problems caused by utilizing skimmers etc., thus raising Brukers ESI instruments to new heights of sensitivity. Applying this device to the amaZon ion trap platform also dramatically improves the ion transfer in the most critical initial pressure regions: a sensitivity gain of a full order of magnitude is achieved in comparison with the renowned HCTultra™. The scheme in Fig. 1 shows the basic principle of the dual ion funnel: Unlike any other ion guide it focuses ions from a large initial volume into the exit orifice. Due to the strong gas expansion at this stage, ions exit the capillary with considerable kinetic energy and a finite beam divergence. The funnel accepts ions from a wide solid angle and thermalizes their kinetic energy by collisional damping. An axial potential gradient drives the ions further down the funnel, where the geometric confinement independant of the m/z ratio (Fig.2) focusses the ions and delivers them through the small exit aperture to the next pumping stage. At the same time, the thin concentric plates of the funnel permit optimal pumping of the region.

Dual ion funnel

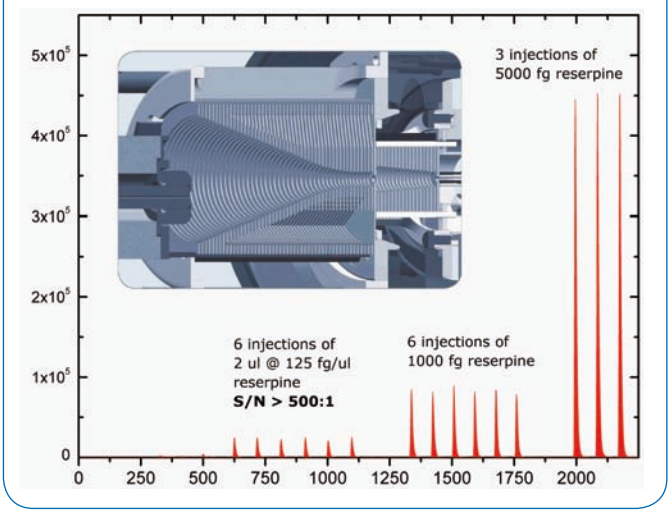


Fig. 1: Subsequent injections of different amounts of reserpine into the amaZon. The specification of S/N 500:1 for 2µLx125fg/µL reserpine on column proves that the amaZon is the most sensitive ion trap system on the market.

Broad mass range

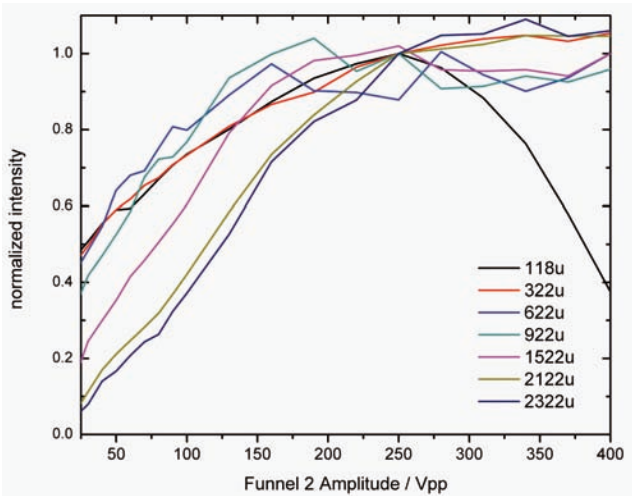


Fig. 2: Normalized ion intensities for a broad range of ion masses as a function of the radio frequency amplitude applied to the funnel device. The extremely broad mass range avoids any compromises such as RF stepping used by other ion guides in other trap devices.

Market-leading ETD/PTR performance

Bruker´s ETD setup is the industry standard for robustness, stability and unrivalled sensitivity. e.g., an injection of 5 fmol BSA digest on column, separated in a 10 min gradient on the EASY-nLC™ results in the identification of at least 18 peptides with a minimum MASCOT score of 20. In the negative chemical ionization ion source with methane as mediator gas, either ETD or PTR reagents can be formed from the same neutral compound via simple source settings. This setup provides an extremely fast switching behavior between ETD and PTR experiments under nLC conditions. Advanced applications like PTR/ETD are supported to tailor the charge envelope of large peptides or proteins before consecutive ETD fragmentation or ETD/PTR to reduce the complexity of highly charged ETD fragment spectra. All reagents are coupled into the standard ion transfer line. This most compact configuration avoids an additional vacuum pumping system. The compression of the analyte cations and ETD anions into the same spherical volume inside the high capacity ion trap generates a very efficient reaction cross section (Fig.7). Thus, effective ETD fragmentation, extremely short reaction times and a high duty-cycle are obtained. The intuitive GUI simplifies setting up of even advanced ETD/PTR experiments. User-experience of over three years of successful ETD/PTR market presence has streamlined method development. Workflows include neutral-loss (CID) triggered Auto-ETD experiments as well as data-dependent alternating ETD/CID. The smart decomposition technology (collisional post-activation) enables sequence information to be obtained even from low charge state ETD precursor ions as often observed in tryptic digests (Fig. 3).

Enhancing ETD fragmentation

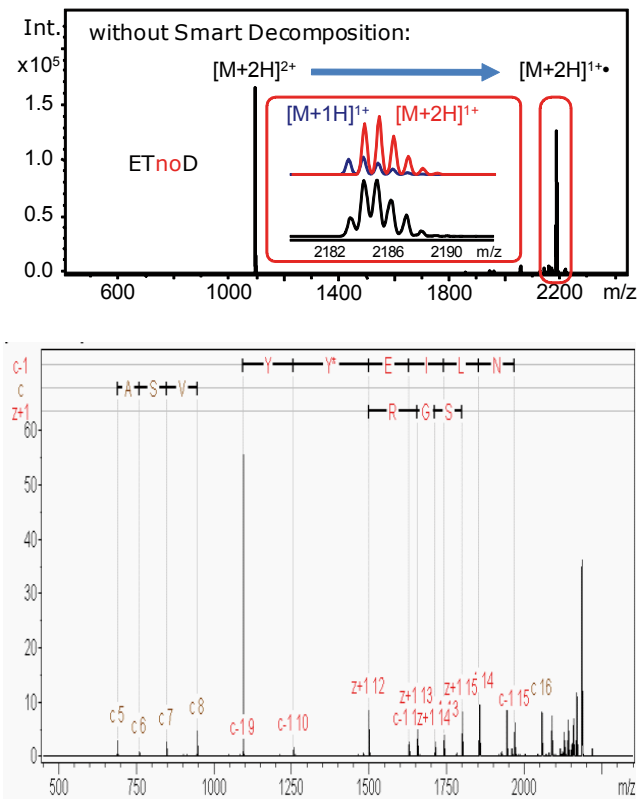


Fig. 3: Automatically enhancing the ETD fragmentation efficiency of the 2+ charge state of a Tyr-phosphorylated peptide by applying Smart Decomposition.

High Performance next-generation trap

The basis for the superior combination of mass resolution and scan speed is Bruker's patented phase-coupled ion ejection using nonlinear resonances introduced into the ion trap (Fig. 4). As shown in Fig. 5, ions to be ejected are first excited to higher orbits, thus effectively decoupling them mutually and from the remaining ion cloud. Careful selection of the excitation phase optimizes the dwell time of ions in this state before the higher-order resonance executes ejection. This exclusive technology allows for absoluted benchmark values for ion capacity, scan rate and resolution in a matchless combination. Further improvements in the trap control electronics and the data aquisition setup lead to the next performance level. An additional pre-trap module has been added to the analyzer to decouple the ion transfer from the ion injection and to optimize injection. In connection with new ultra-stable, fast acting PID gas controllers, the pre-trap module provides highly defined pressure conditions inside the ion trap.

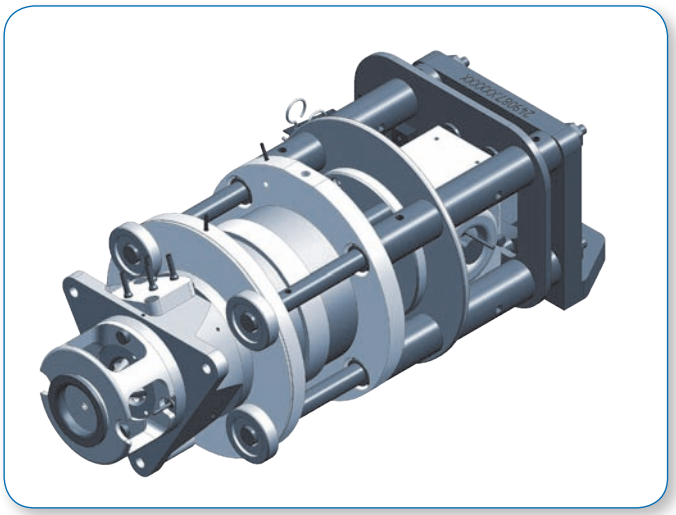


Fig. 4: The new fast scanning, high performance ion trap.

Scan modes

High resolution with a peak width at < 0.1u FWHM is achieved for protein and peptide identification at an LC compatible scan speed of 4,600 u/s. Even at the fastest scan speed of 52,000 u/s, a truly superior peak width of 0.58 u is can be realized.

XtremeScan:	52,000 u/s @ 0.58 u FWHM
UltraScan:	32,000 u/s @ 0.5 u FWHM
Enhanced Resolution:	8,100 u/s @ 0.3 u FWHM
Maximum Resolution:	4,600 u/s @ 0.1 u FWHM for multiply charged peptides
Extended Scan:	27,000 u/s for 200-6000 u

High scan rate and resolution due to phase-coupled ion ejection

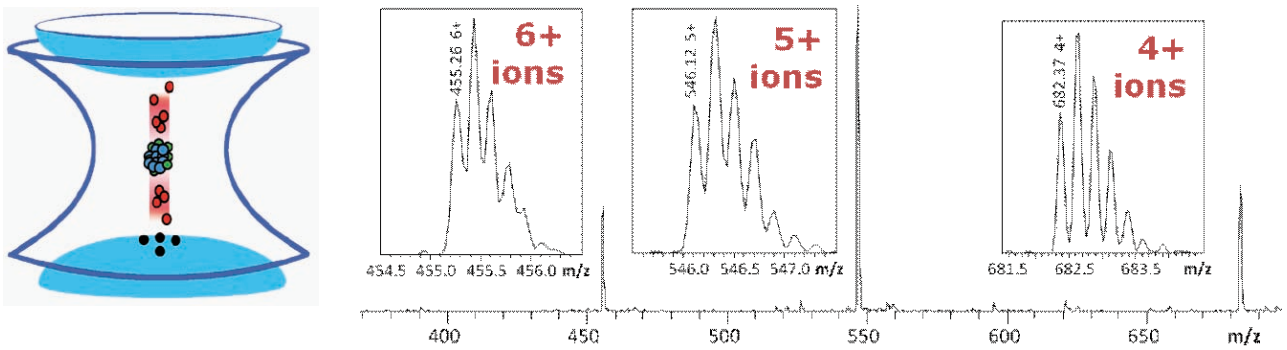


Fig. 5: Left: Scheme of phase-coupled ion ejection using nonlinear resonances. Right: Example of the new Maximum Resolution Scan mode at 4600 m/z /s. In a seamless scan using LC compatible conditions, up to 6+ of thymosin beta are isotopically resolved.

Compound ID

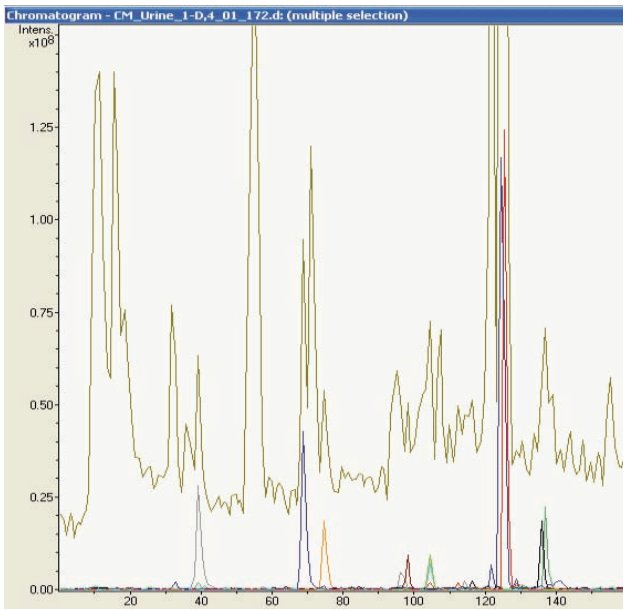


Fig. 6: Urine spiked with 10 toxins, drugs and antibiotics (50 ng/ml each) and separated on a fast UPLC gradient of 180 sec. Auto-MS/MS was performed in both polarities and the MS/MS spectra searched against a spectral library. All 10 components were successfully identified.

Fast polarity switching for compound screening

The amaZon series ion traps generate a high level of information for MSn screening. The fast switching electronics and a modified ion transfer capillary enable extremely fast polarity switching and high speed data acquisition: Zero Delay Alternating. Bruker's high transmission glass capillary based inlet technology offers strong advantages for source interfacing as the sprayer is operated at ground potential. A highly-resistive coating of the interior glass channel achieves the additional optimization for fast switching. Characteristics, that make the amaZon series best suited for applications like target screening as well as data-dependent auto-MS^n of unknowns. Supported by spectral libraries, compounds can be confidently identified (Fig. 6). Bruker's Compass OpenAccess™ software module supports push-button experiments for any kind of routine screening applications. Using MS/MS in addition to polarity switching, at least 3 cycles of full ±MS and ±MS/MS can be performed on typical UHPLC peaks of ~ 1 sec FWHM i.e. a total of 12 MS and MSMS spectra. A spectral rate of 7 Hz for consecutive MSMS spectra can be achieved.

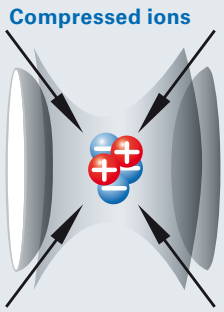
Forging ideas into analytical success

The outstanding perfomance of the amaZon ion trap mass spectrometers is based on and safeguarded by a series of exclusive technologies. The most relevant patents include:

Patent	Benefit	Current amaZon-related patents
Ion Funnel Source	Mass-independent ion transfer and sensitivity boost	DE 195 23 859 C2; GB 2 302 985 B;US 5,572,035 A; US 7,064,321 B2
High Transmission Glass Capillary	Fast polarity switching, grounded API source setup	DE 195 15 271 C2 ;GB 2 300 295 B ;US 5,736,740 A
Non-linear Excitation for Ion Ejection	Ultrafast scan rates with unmatched resolution	EP 0 383 961 B1;US 4,975,577 A ;DE 41 42 870 C2 ;GB 2 263 192 B
Smart Charge Control	Optimization of the charge density inside the ion trap without the need for a time-consuming prescan	DE 43 26 549 C1 ;GB 2 280 781 B ;US 5,559,325 A ;DE 197 09 086 B4 ;GB 2 322 961 B ;US 5,936,241 A
Smart CID. Smart ETD Smart CID/ETD (pending)	Optimized fragmetation strategies to obtain maximum MSMS information	US 6,410,913 B1; DE 10 2005 004 324 B4; DE 10 2005 061 425
Easy Access to ETD and PTR reagent	Robust ETD and PTR operation and direct toggling using a single neutral compound	DE 10 2006 049 241 A1 GB 2 443 066 A US 2008/093546 A (all pending)
High Precision Ion Guide	Optimal transfer of sample ions	DE 10 2004 037 511 B4; GB 2 415 088 B;US 7,351,963 B2
Temperature Stabilized Trap	Ultimate stability in trap performance	DE 197 33 834 C1; GB 2 328 076 B; US 6,133,568 A

Spherical ion traps are best for ETD/PTR

- Direct ETD reaction as soon as anions enter the trap
- Cations and anions are compressed into the same globular volume in the center of the trap: Maximum interaction for ion-ion reactions gives highly efficient ETD fragmentation



Hardware Features

Instrument

- 4-stage, differentially pumped vacuum-system evacuated by a dedicated split-flow turbomolecular pump to 10^{-6} mbar to minimize contamination
- Autonomous vacuum-controller for highest system integrity and up-time
- Automatic calibration procedures for the instrument
- Patented dual-funnel ion concentration and transfer unit
- Proprietory wire-eroded multipole ion guides – ultimate precision for ultimate ion transfer performance
- Wide range of source options: APCI, APPI, Multimode (ESI/APCI), Online-/Offline-Nanospray
- Grounded needle allows easy CE/MS interfacing
- Combined ion optics cartridge for easy maintenance
- Single instrument controlled rough-pump unit

Ion trap mass spectrometer

- High capacity quadrupolar ion trap with non-linear ion ejection for highest scan speed and mass resolution
- Bipolar high-energy conversion dynode detector for optimal sensitivity and reduced mass bias
- Automatic detector gain calibration for quantitative and reproducable intensity scales
- Detector gate for increased multiplier lifetime and stability
- Low-noise preamplifier for optimal sensitivity
- Monoisotopic Isolation in the mass range 50-3000 m/z
- fully integrated negative CI source for ETD / PTR work, ideally suited to top-down proteomics
- Workflow including data processing with Snap II™ allows the database ID of intact proteins
- Fast Zero Delay Alternating for polarity switching

Operation modes

- Peptide Scan: mixed enhanced rapid identification mode
- Neutral Loss Scan: on the fly identification of e.g. phosphopetides
- SILE Scan: for protein identification and quantification
- Data-dependent Auto MSMS and AutoMS^n for n upto 5
- PANorama fragmentation overcomes 1/3 cutoff in e.g. iTRAQ™ applications
- Selected Reaction Monitoring up to MS^3 for 10 different channels

Integrated syringe pump

- Automated sample infusion under full software control
- Low-pulsation syringe-pump module

Integrated divert valve options

- Software controlled 2-position divert valve
- User exchangable valve head for advanced workflows (6-port/10-port, analytical or nano scale)

Advanced external interfaces

- I/O channels for remote start/stop
- Two analog input channels (0-1 V or 0-10 V), one analog output channel
- User-definable trigger to reflect different stages in measuring cycle; fast accumulation trigger
- Advanced system bus for the integration of options
- Serial communication interfaces
- Instrument-controlled power-socket for external devices

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Instrumentation & Software
amaZon series
EASY-nLC
TrapControl
Compass

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