

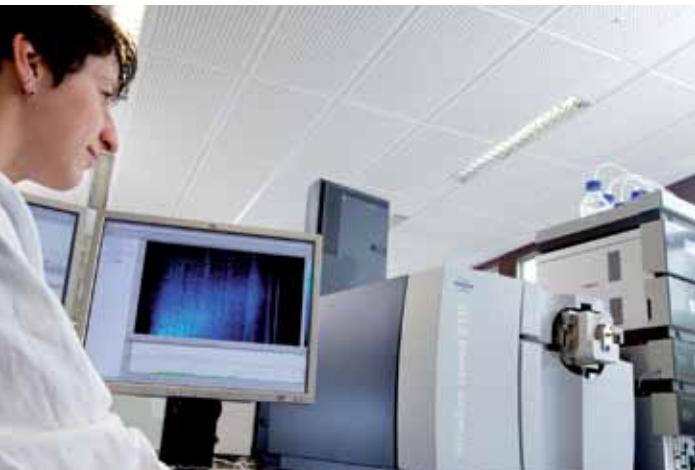
amazon speed

- Ion Trap Performance Beyond Imagination

Innovation with Integrity

Ion Trap MS

amaZon – Turning Speed into Solutions



The amaZon speed ion trap series sets new analytical standards for proteomics and small molecule applications. Design improvements to a well proven platform, offer dramatically enhanced speed, mass resolution, MS/MS efficiency and reproducible quantitation, delivering unsurpassed performance.

Unmatched ion trap performance through innovation

- Greatly increased MS/MS duty cycle for more productive data acquisition
- Further improved resolution on a market-leading system with an unrivalled value of 30,000 in full scan
- Highest ion trap mass accuracy through proprietary RF generator design
- Unrivalled ETD performance: most sensitive, robust and reliable technology
- Leading dual ion funnel technology for unparalleled sensitivity
- Zero Delay Alternating™ for polarity switching with 20 Hz MS acquisition speed
- New SMART isolation and fragmentation



● Accelerate your Success

Speed for proteomics

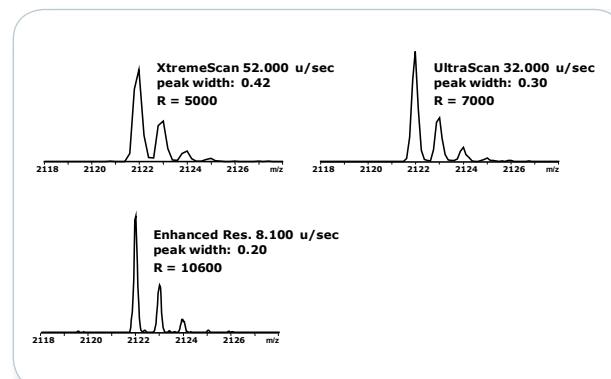
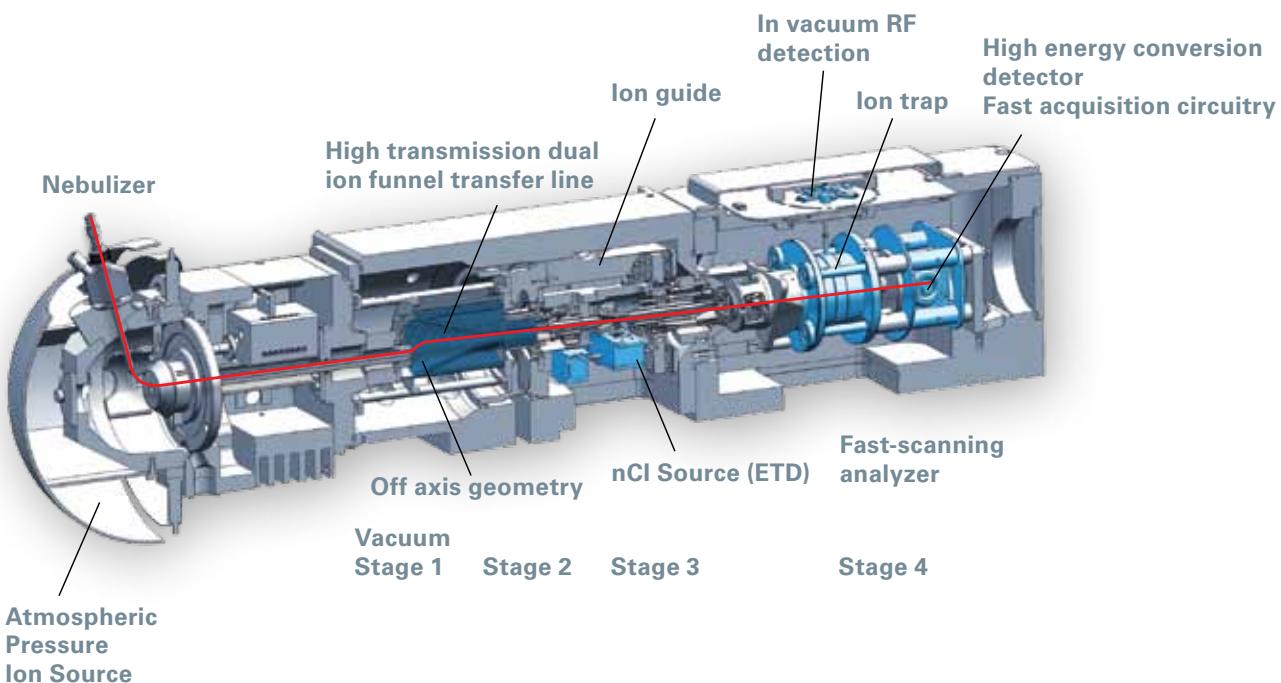
- Bottom-up proteomics identifying more low abundant proteins
- Outstanding ETP/PTR applications for full PTM analysis
- Top-down sequencing (TDS) for highest protein sequence coverage

Flexibility for general chemistry

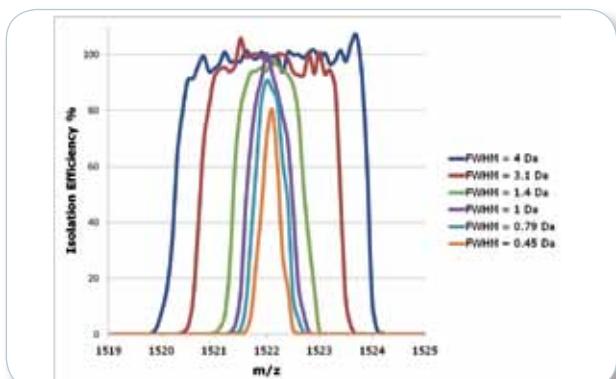
- Walk-up OpenAccess push-button software
 - Fast MSⁿ library search solutions
- Most flexible API sources including the new CaptiveSpray™ for proteomics, APCI, and DIP for solid samples

Speed for drug research

- Drug quantification by UHPLC driven LC-MS methods
- Metabolite identification and prediction by advanced software tools



Different scan rates and respective resolution. Even in the fastest XtremeScan a peak width well below 0.5 u can be achieved opening up the capability to resolve 2 + ions.



New fast isolation of 1522 m/z ion with isotopic isolation showing no comprise in efficiency. Ions with a peak width of 0.45 Da are isolated with 80 % efficiency.

Translating Speed into Proteomic Information

Bottom-up analysis with a maximum number of identified proteins

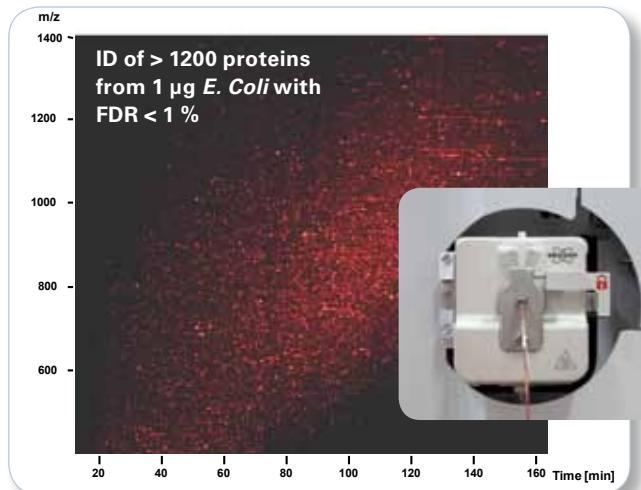
The complexity of the proteome still challenges analytical technologies. The high dynamic range and complexity due to posttranslational modifications require maximum speed and sensitivity to capture as many peptides as possible. The amaZon speed ion trap sets new standards in protein identification due to its enhanced MS/MS speed, new faster isolation and SMART fragmentation. This results in the identification of > 1200 unambiguously identified proteins in a single LC-MS/MS run from 1 µg *E. coli* digest.

Excellent dynamic range

The UPS-2 standard is a mixture of 48 proteins spanning a concentration range of 6 decades in total. The amaZon speed allows protein identifications over 5 concentration levels in a single run.

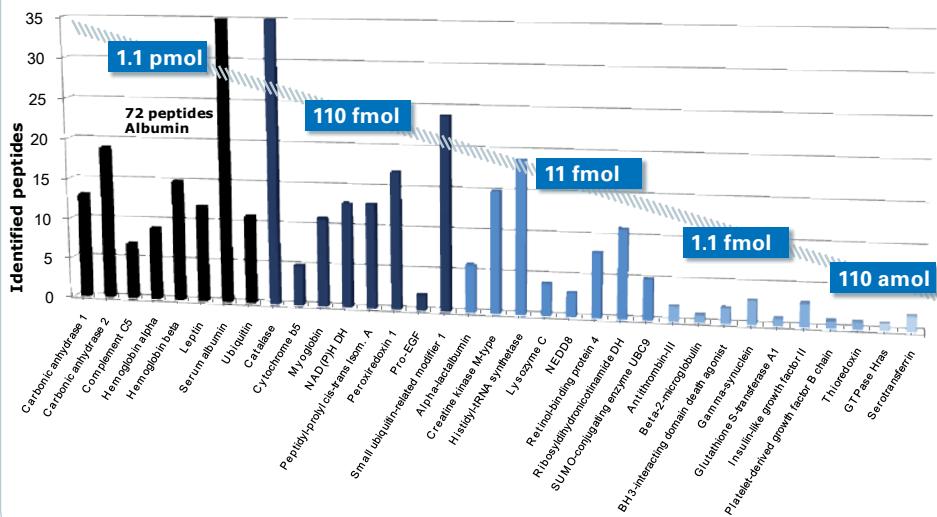
Productivity, robustness and high sequence coverage by CaptiveSpray™

The amaZon speed is equipped with the high-transmission, robust CaptiveSpray source for highest performance and reproducibility in Bottom-up studies.

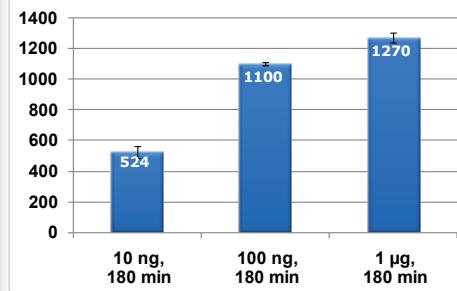


Survey view of an *E. coli* digest (1 µg) separated by a nano UHPLC using a 25 cm column and a 3 h LC gradient. MS detection was performed using the amaZon speed ETD system equipped with a CaptiveSpray ion source.

ID over 5 levels of protein concentration - UPS-2 standard



Number of identified proteins

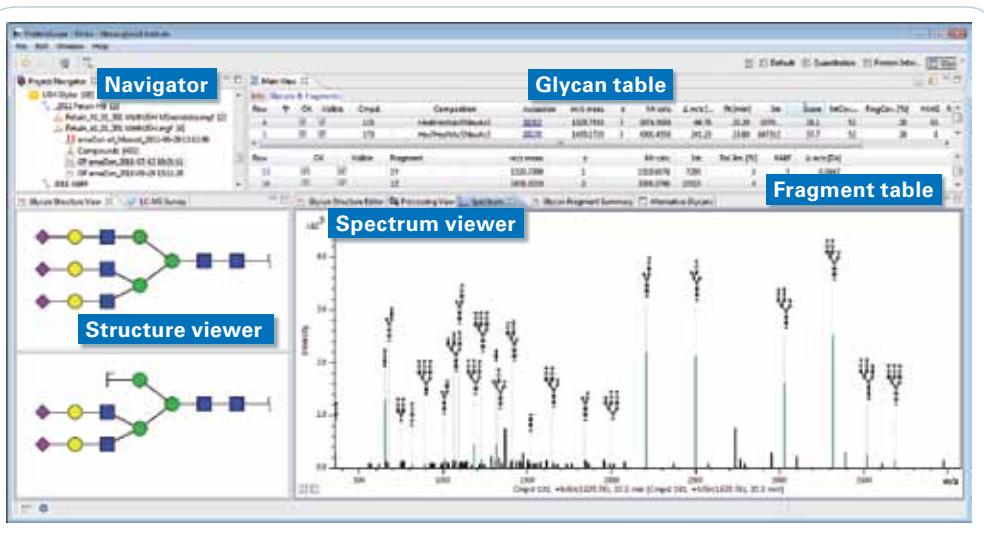
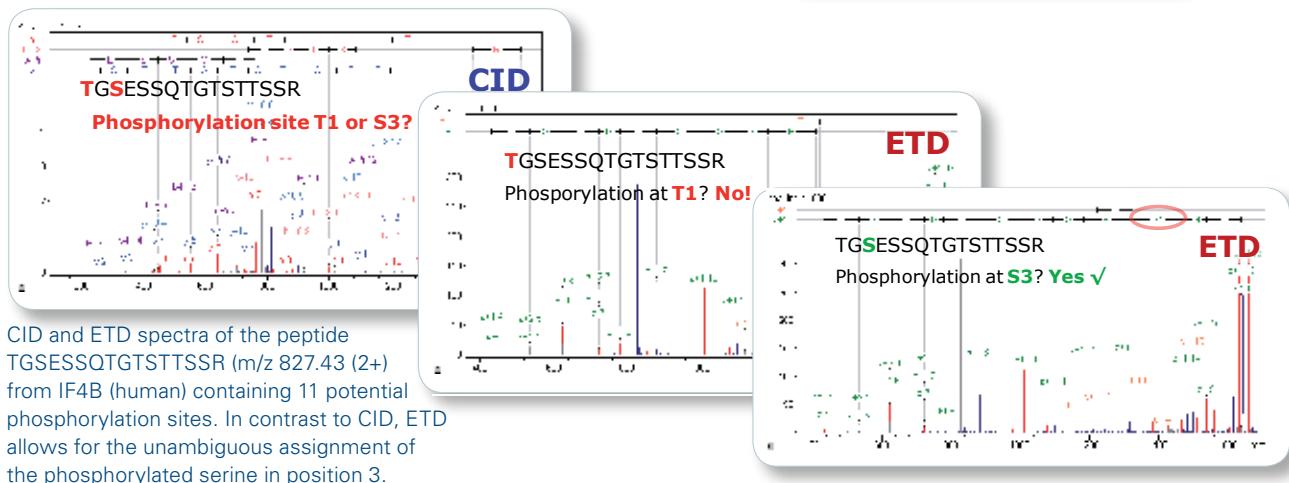
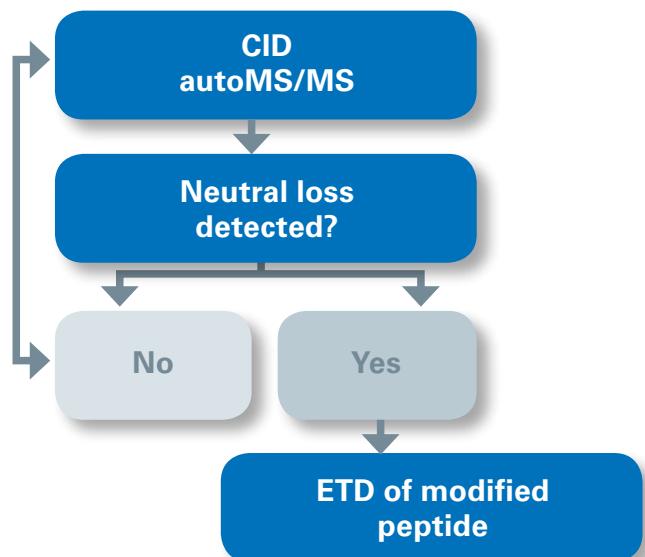


Number of identified proteins from *E. coli* digests (average of three technical replicates for different protein loadings).

● Get the Full Picture: PTM Discovery

Complete PTM characterization

The regulatory function of many proteins is controlled by reversible posttranslational modifications (PTMs) such as phosphorylation. In PTM analysis, both characterization and localization of the modification site are of major importance. ETD is the method of choice for analyzing biologically important PTMs. In contrast to CID, ETD preserves modifications of the peptide backbone, enabling unambiguous assignment of the modification site. Market-proven ETD technology makes the amaZon speed the most sensitive and reliable instrument available.



Glycan analysis

The analysis of glycans is one of the most demanding tasks in modern mass spectrometry. Bruker's novel proprietary GlycoQuest™ glycan DB search engine – supplied as part of the bioinformatic software package ProteinScape – retrieves the glycan structure and released glycans are easily identified by the amaZon ion trap.

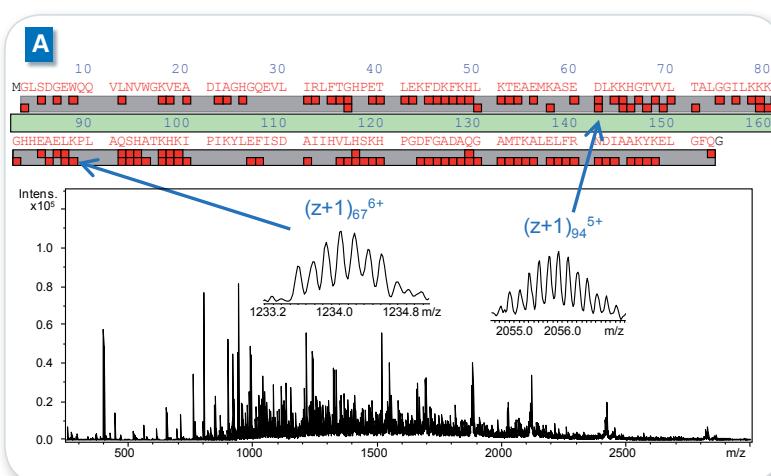
Top-down Protein Characterization by ETD/PTR

Unrivalled performance in Top-down MS/MS by ETD/PTR

Top-down sequence elucidation of intact proteins requires highly efficient MS/MS. The powerful combination of ETD and PTR generates multiply charged fragments controlling the product ion charge state on demand. Using the amaZon speed in Maximum Resolution mode enables sequencing of proteins of > 16 kDa from both termini, as shown for

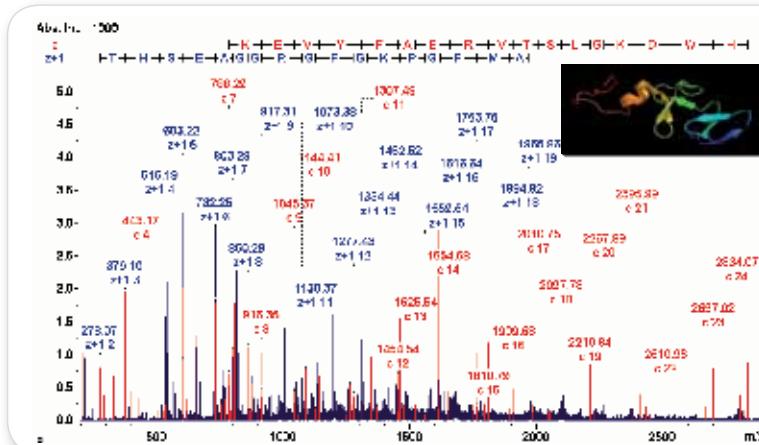
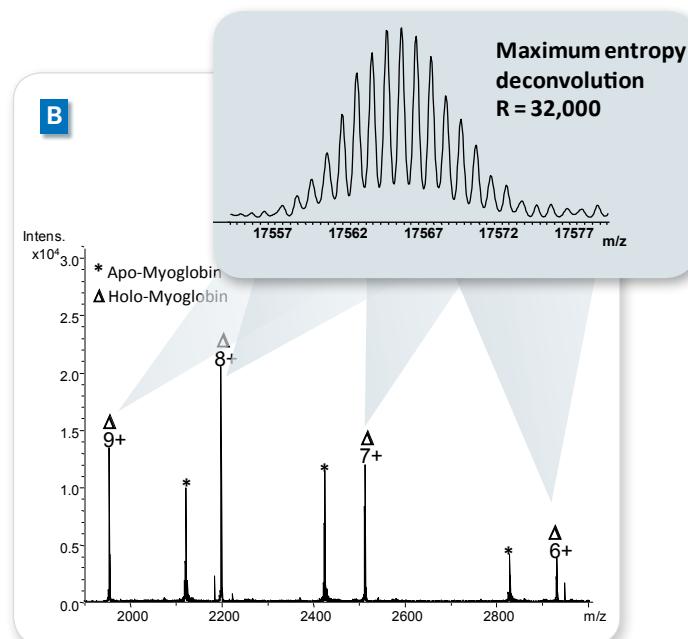
intact myoglobin (sequence coverage of 98 % by a Mascot TDS database search).

The unrivaled performance of the new Maximum Resolution mode is demonstrated by the deconvoluted data of intact Holo-Myoglobin (charge state 6+ to 9+) with a resolution = 32 000.



(A) Sequence annotation from Mascot TDS database search. Top-down ETD MS/MS followed by PTR of Myoglobin (horse heart) with the insets showing the excellent resolution of ETD fragments.

(B) The charge state of intact Holo-Myoglobin was shifted by proton transfer reaction (PTR) prior to mass analysis. In Maximum Resolution mode $[M+8H]^{8+}$ $[M+9H]^{9+}$ and can be clearly resolved.



Top-down identification of MALDI Imaging biomarkers

Bruker provides biomarker discovery and identification in an integrated Top-down strategy. Starting with tissue imaging by MALDI, statistical tools pull out differential proteins. These proteins are identified by ETD/PTR in the amaZon ion trap. Shown is the identification of CRIP 1 as a biomarker co-localizing with HER2 positive breast cancer tissue.

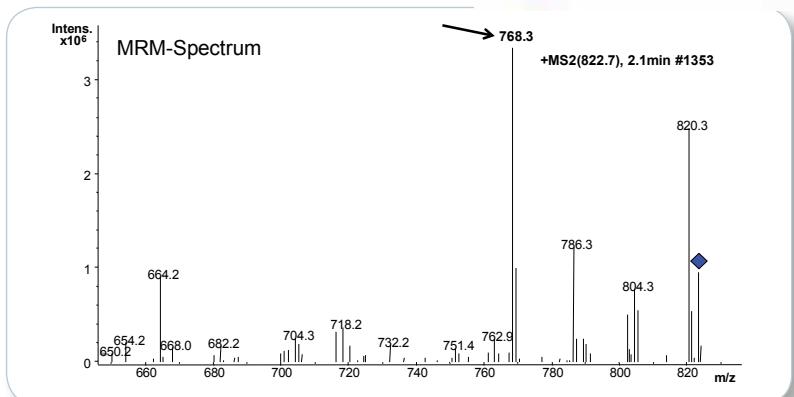


Quantification Built on Speed

Enhancing productivity for Therapeutic Drug Monitoring (TDM)

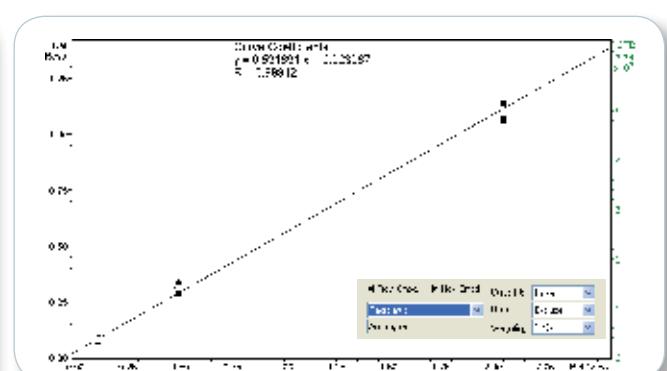
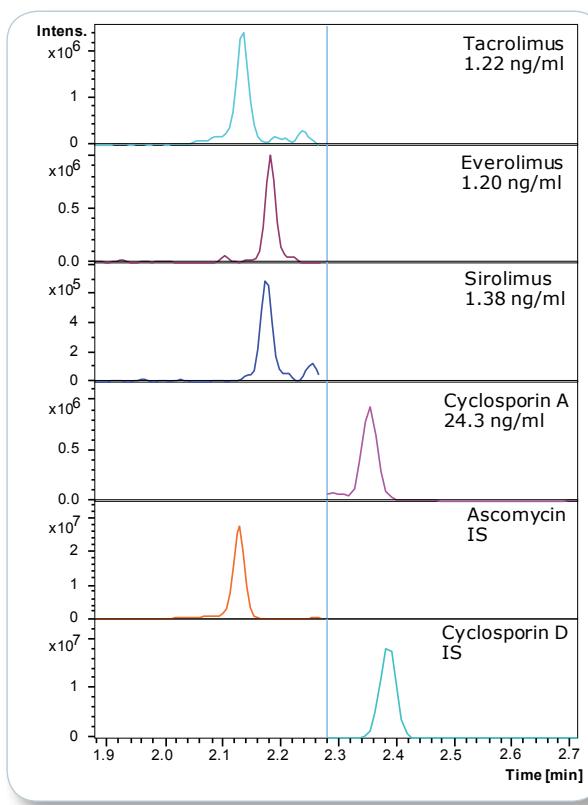
Reliable and routine quantification is a task so far reserved for triple quadrupole instruments. The enhanced speed and reproducibility of the amaZon speed in MRM experiments enables the reliable quantification of target compounds in diverse matrices.

Shown here is the quantitation of four immunosuppressants in a multiplexed MRM assay. The requirements for this TDM assay were adopted directly from a clinic and transferred to the amaZon speed together with the respective calibrators, controls and patient samples. The SmartFrag™ routine of the amaZon ensures a highly reproducible MS/MS data quality. The averaged CV of control samples was below 10 % at excellent sensitivity. The analysis was done at high turn around: data acquisition rate was 14 Hz at a UHPLC gradient of 3,5 min only.



Full scan MS/MS spectrum from the lowest level calibrator (1,2 ng/ml) of Tacrolimus. The fragment with m/z 768 was used for quantification. Unlike with triple quad instruments multiple fragments can be combined for quantification.

| Clinical requirements for TDM assay | |
|-------------------------------------|--|
| Targets | Tacrolimus, Sirolimus, Everolimus, Cyclosporin A |
| Matrix and Preparation | Whole blood, protein precipitation with MeOH/ZnSO ₄ |
| Required LOQ | 1 ng/ml (Tacrolimus, Sirolimus, Everolimus) 24 ng/ml (Cyclosporin A) |
| Required linearity | 1-22 ng/ml (Tacrolimus, Sirolimus, Everolimus) 24-472 ng/ml (Cyclosporin A) |
| Time | Gradient: 3,5 min Whole cycle: 4,5 min |



Calibration curve of Tacrolimus covering the clinical required therapeutic range for routine quantification as described above. The linearity was $R=0.9991$ in the required quantification area.

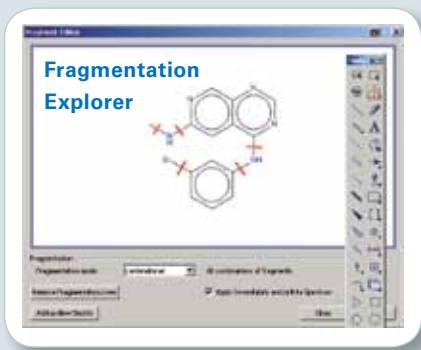
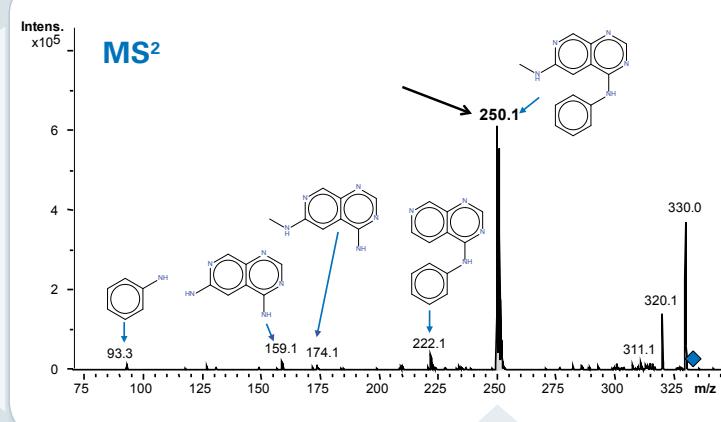
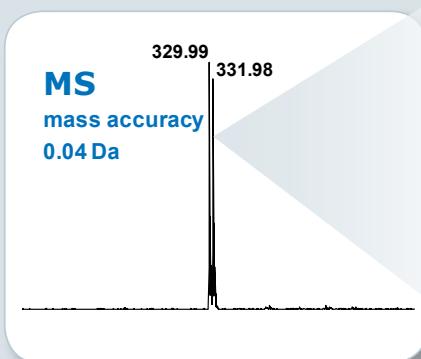
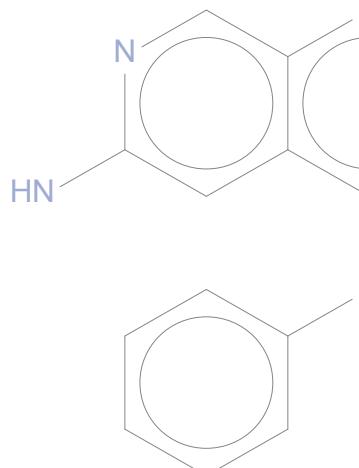
Extracted ion chromatograms (EICs) of the required lowest level calibrators for the four immunosuppressive drugs. Even for the lowest therapeutically required concentrations (e.g. 1.2 ng/ml for Tacrolimus) an excellent S/N > 360 was achieved. Thus, the LLOQ is expected to be much lower than that, enabling a detection of immunosuppressants at a level much below the therapeutic concentration. Blood samples were precipitated and supernatants directly injected into an UHPLC system. Two internal standards - Ascomycin and Cyclosporin D - were used for quantification.

Structure Verification and Metabolite Identification

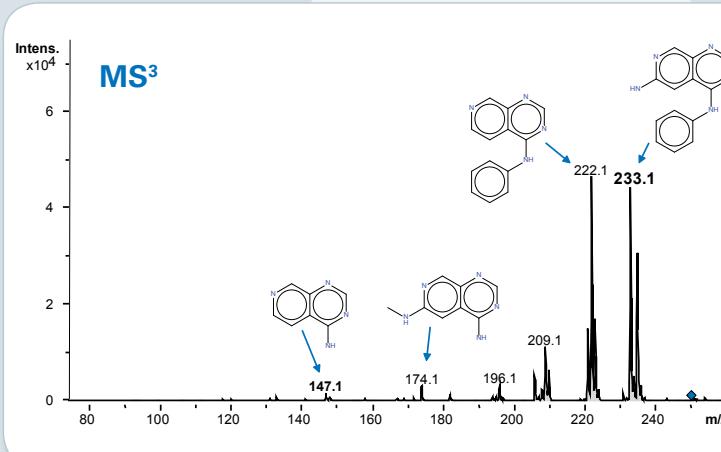
**MSⁿ for structure analysis,
supported by powerful data
interpretation software**

The identification of drug metabolites and their structure verification are a vital part in pharma discovery and development. This is also true for synthesis control in organic chemistry in general. Software features including the FragmentExplorer™ are the ideal tools for

structure confirmation taking advantage of the fast and sensitive stepwise MSⁿ fragmentation of the amaZon speed ion trap. Bruker's MetaboliteTools™ software suite provides confident metabolite prediction as well as detection of new compounds.



Structural confirmation by stepwise MSⁿ. Data interpretation by FragmentExplorer software confirms the expected structure of the compound and leads to a complete interpretation of the data.



● OpenAccess

Walk-up solutions for a multi-user environment

The Compass OpenAccess OA/QC software allows users with little to no training to generate valuable LC-MS/MS data on the fly. This is a web-based client server system with push-button

solutions i.e. for quality control (QC), recombinant protein MW verification and automatic library searches. The intuitive workflow-oriented GUI in combination with the flexibility and speed of the amaZon is an ideal solution for multi-user industrial, clinical or teaching labs.

The screenshot shows the 'Compass openaccess QC' web interface. On the left, a sidebar menu includes: Home, Submit Sample, Submit Sequence, Import Sample File, Job Status, Approved Jobs, Search Job, Search Acquisition, Station Status, Logout, Help, and a status bar indicating 'Logged in as Demo User Version 1.6.0 build 962'. The main area is titled 'Welcome Demo User' and features four options with icons: 'Submit a single sample', 'Submit a sequence of samples', 'Import samples from file', and 'View job status and results'.



The screenshot shows a 'Compound Verification Report (ITMS)' for Sample-ID A3. It displays two chromatograms: one for 'A3_000_043_01_007.D' and another for 'A3_000_043_01_007.D'. A red arrow points to the first chromatogram, which shows a peak at approximately 1.8 minutes. A yellow box highlights the text 'Automated check of a synthesis compound - 80% purity detected.' Below the chromatograms, there is a 'Compound Verification Results' section with a table:

| # | mass.mz | theo.mz | err.(ppm) | Rel. Intensity | Formula | Modification | Purity(FC%) |
|---|---------|---------|-----------|----------------|-----------------|--------------|-------------|
| 1 | 470.21 | 471.24 | 0.03 | 2.99 | C20 H22 O10 C3 | 301.09 | 8.1 |
| 2 | 462.00 | 463.20 | 0.07 | 2.08 | C20 H21 O11 N+O | 301.09 | 9.7 |
| 3 | 462.01 | 463.20 | 0.07 | 1.02 | C20 H21 O11 N+O | 301.09 | 1.0 |
| 4 | 462.21 | 463.20 | 0.10 | 1.02 | C20 H21 O12 N+O | 301.09 | 0.3 |
| 5 | 462.24 | 463.20 | 0.02 | 1.02 | C20 H20 O11 C3 | 301.09 | 0.0 |

The screenshot shows a 'Job Results' page. It includes a table for 'Job Information' and a grid visualization. The grid has 10 columns and 10 rows, with each cell containing a colored circle representing purity levels. A legend on the right defines the colors: green for 80-100%, yellow for 50-80%, red for <50%, and black for 'Compound not detected'. Below the grid are buttons for 'Load Job History', 'New Job History', and 'Load All Data'.

Compass OpenAccess guides the user through all necessary steps to acquire high-quality data in an intuitive, automated workflow. Results, for purity checks of synthetic compounds, are visualized in MTP formats. PDF reports with all needed data information are provided in a customized fashion and sent automatically after sample analysis by email.

Technical Specifications

Technology driven solutions

- SmartFrag™ for reproducible MS/MS spectra generation and confident library searches
- Zero Delay Alternating™ polarity for up to 20 Hz MS acquisition speed supporting UHPLC applications
- Dual ion funnel for maximum sensitivity over a broad m/z range
- Patented SmartICCTM for optimal ion storage
- SMART isolation and SMART fragmentation
- Panorama fragmentation (PAN) enabling CID fragmentation without 1/3 cutoff

MS/MS modes

- Data-dependent auto MSⁿ
- Preferred mass lists for automated feedback experiments from MetaboliteTools and ProteinScape
- Scheduled precursor lists supporting MS², MS³ in positive and negative mode
- Neutral loss scans for analysis of target compounds and posttranslational modifications (by ETD)
- Multiple reaction monitoring (MRM) for drug quantification
- Manual MSⁿ up to MS¹¹ in all scan modes for structural elucidation
- Highly efficient and sensitive ETD/PTR capabilities for PTM detection and Top-down proteomics

Source options

- ESI, APCI, APPI
- DIP source for direct analysis of solids and liquids
- CaptiveSpray: robust and reliable plug-and-play nano/cap ESI source for flow rates from 50-5000 nl/min
- Advion Triversa NanoMate
- Smart CE-MS coupling with grounded spray needle



Software solutions

- ProteinScape including GlycoQuest glycan DB search engine
- RapiDeNovo for de-novo sequencing of proteins
- MetaboliteTools for metabolite prediction and identification
- ProfileAnalysis for statistical comparison of metabolite and proteomic profiles
- Compass OpenAccess client server based system for walk-up users
- Compass Security Pack for work in regulated environments
- ACD/MS Manager for structural interpretation and classification

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