

Analysis of peptide mixtures using AP-MALDI UHR on an LTQ/Orbitrap instrument

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MassTech's AP MALDI Source

The AP MALDI UHR ion source (MassTech, Inc., USA) is used for the herein presented experiments and results. Distance between sample plate and heated capillary in the MS-interface is adapted to a distance of about 1 mm. A Nd:YAG laser beam at 355 nm (pulse duration 3-5 ns) is tightly focused down to a spot size of 15 x 20 μm^2 . A spiral motion across the selected spot on the plate while scanning is checked in MassTech's Target Software (Instrument Control Software of AP MALDI source). The laser repetition rate can be varied between 100 and 10000 Hz in MassTech's Target software. For most of the experiments repetition rate is set to 50 or 200 Hz.



- MALDI imaging capabilities down to 10 micron lateral resolution
- Switch from the AP-MALDI configuration to LCMS configuration within 2 minutes.
- Provides MALDI analysis and imaging capabilities to high-end LCMS instruments
- Laser focus can be adjusted from 10 to 30 microns

LTQ/Orbitrap Elite HR MS Instrumentation

All experiments are performed on the Thermo LTQ/Orbitrap Elite mass spectrometer, using an AP MALDI UHR ion source.

The mass spectrometer is operated in Full Scan MS and MS/MS mode settings using resolving power of up to 240,000 at m/z 400 (FWHM) and with mass range up to m/z 4000.

Calibration mixture

Samples and Sample Preparation

Sigma-Aldrich's ProteoMass™ MALDI Calibration Kit for LTQ XL and LTQ Hybrids "MSCAL4" (MALDI Calibration mixtures) is prepared according to the protocol provided with the kit.

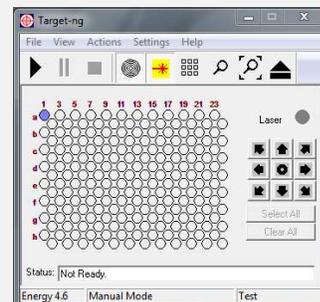
Alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix is used to prepare the MALDI calibration solution.

The peptide mixtures were prepared according to product information available at (<https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/428/025/mscal4dat.pdf>).

The spotted volume was reduced from 1.5 to 0.5 μl on an HST microfocus plate. The amount of spotted peptide was in the range 0.38-1.05 pmol on target for the Normal mass range calmix, and 0.57-2.94 pmol on target for the High mass range.

Available modes of operation

- Sequential MALDI analysis of multiple spot analysis using spiral or raster motion (AB Sciex OptiTOF 192, HST μFocus) with tunable parameters
- MALDI imaging using Pixel-Map, with tunable in-pixel motion for multiplexed analysis (HRMS, SRM...).
- MALDI imaging using Constant Speed Raster (CSR) modes for increased pixel rate (MS dependent)



Auto-sequence acquisition:

AP-MALDI UHR control software allows for sequence acquisition with either internal or external timing. Consequently, the user can select a mode of operation where:

- each MALDI spot is considered as a different injection in an LCMS sequence (with adjustable MS or MSMS methods for each irradiated spot), resulting of one raw file per irradiated spot
- all selected spot will be irradiated and measured in one single raw file

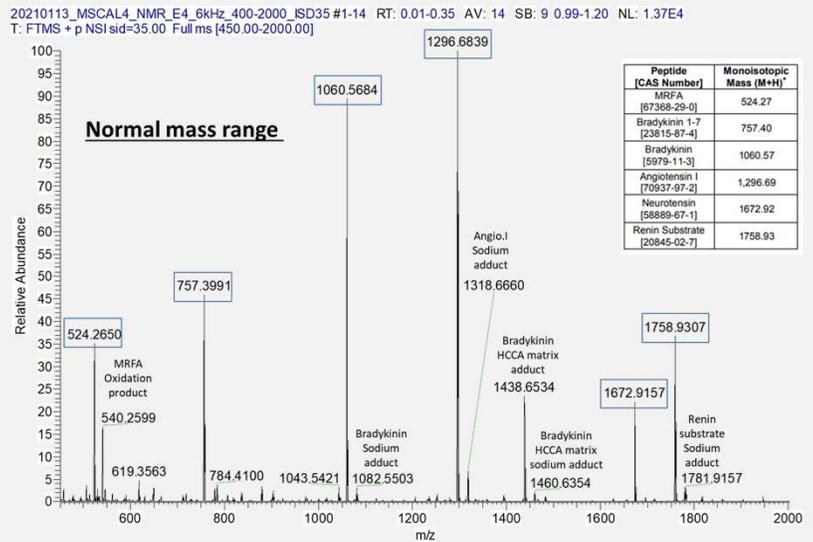


Fig.1: Average mass spectrum of the MSCAL4 peptide mixture in the Normal Mass Range

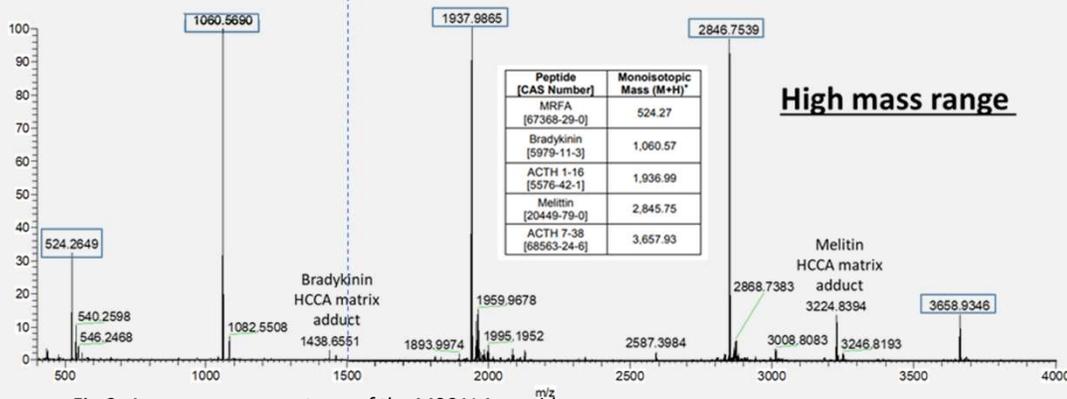


Fig.2: Average mass spectrum of the MSCAL4 peptide mixture in the High Mass Range

Conclusion:

MassTech AP-MALDI UHR provides analysis and imaging capabilities to existing LCMS instruments such as Thermo HRMS equipment. APMALDI/ESI swap is done within minutes.

AP-MALDI HRMS data can be handled by Thermo Xcalibur or alternative mzML-based software.

MassTech offers a range of analytical ionization sources, accessories and complete instruments for advanced analytical platforms. The AP-MALDI UHR is available exclusively from MassTech.

To request further information, please contact:

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MassTech selected LIST as European Application Lab. www.list.lu