

HPLC Coupling with ESI, MALDI and SALD ICP MS – the Detection Platform for Metallothionein Characterization

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We demonstrate the use of a detection platform that complements conventional LC-ESI MS with two detection techniques: matrix-assisted laser desorption/ionization (MALDI) and substrate-assisted laser desorption inductively coupled plasma (SALD ICP) mass spectrometry (MS) analyses. As a model system, metallothionein 1, a low molecular protein enabling binding wide range of heavy metals (Zn, Cu, Cd, Hg) was chosen.

Presentujeme použití detekční platformy která spojuje konvenční LC-ESI MS se dvěma detekčními technikami: matrix-assisted laser desorption/ionization (MALDI) a substrate-assisted laser desorption inductively coupled plasma (SALD ICP) mass spectrometry (MS). Jako modelový systém byl zvolen metallothionein 1, nízkomolekulární protein umožňující vazbu širokého množství kovů (Zn, Cu, Cd, Hg).

Methods

LC separations were performed using a Vydac C8 (150 x 4.6 mm x 5 μm) column. For the separation of MT1 isoforms (MT1a, MT2d, MT2e), an effluent coming out of the HPLC column was split. One part of the flow was introduced on-line for ESI oTOF MS detection, second part of the effluent was deposited on MALDI plastic targets (Prespotted AnchorChip 96). Dry fractions were overlaid with a 0.5 μL of saturated solution of CHCA in 20% ACN and 1% TFA with In as internal standard. Off-line MALDI TOF and SALD ICP quadrupole MS detection was performed from the same target.

LC separace byla provedena použitím Vydac C8 (150 x 4.6 mm x 5 μm) kolony. Pro separaci MT1 isoformy (MT1a, MT2d, MT2e), effluent vycházející z HPLC kolony byl rozdělen. Jedna část toku byla vedena přímo on-line do ESI MS oTOF MS detekce, druhá část eluátu byla nanesena na MALDI plastovou destičku (Prespotted AnchorChip 96). Suché frakce byly převrstveny 0.5 μl nasyceného roztoku CHCA v 20% ACN a 1% TFA s

Results

Simultaneous detection of the MT1 isoforms (complexes with metals), apoforms (forms without metals) and metal quantification were obtained using off-line and on-line approach. On-line ESI MS provides information about the MT1 complexes and their metal stoichiometry. MALDI MS provides additional information about apoforms and thus confirms ESI MS identification. SALD ICP MS provides quantitative data on metals in MT1 complexes.

Innovative aspects

- HPLC separation coupled with on-line ESI MS and off-line MALDI MS and ICP MS detection techniques
- The multiple detection platform for MALDI and SALD ICP MS allows both qualitative and quantitative analysis of MT1 complexes
- Direct comparison of ESI and MALDI analysis of MT1 isoforms

References

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