

LECTURE

MULTIPLE HYPERNATION OF LC WITH ESI MS, MALDI MS AND SALD ICP MS – THE DETECTION PLATFORM FOR METALLOPROTEOMIC STUDIES

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Summary

The combination of the molecular and elemental detectors for a single separation by utilizing on-line and off-line approach is presented. This arrangement is demonstrated on analysis of a metallothionein mixture.

1 Introduction

We present a new methodology for coupling conventional on-line LC-ESI MS with two off-line detection techniques: matrix-assisted laser desorption/ionization (MALDI) and substrate-assisted laser desorption inductively coupled plasma (SALD ICP) mass spectrometry (MS) analyses. The Off-line approach is facilitated using a commercial MALDI target made of conductive plastics, which is suitable for the both MALDI MS and SALD ICP MS. Due to this arrangement, two types of analysis, molecular and elemental determination of metallothionein 1 (MT-1) were performed.

2 Experimental

LC separations were performed using a Vydac C8 (150 x 4.6 mm x 5 μ m) column. For the separation of MT-1 isoforms (MT-1a, MT-2d, MT-2e), the effluent coming out of the HPLC column and passing via a UV detector was split with a MicroTee. One part of the flow was introduced on-line to ESI oTOF MS detector, the second part of the effluent was deposited on MALDI plastic targets (Prespotted AnchorChip⁹⁶) as 1.6- μ L fractions using a tapered fused silica capillary (I.D. 30 μ m). After drying at room temperature, dry fractions were overlaid with 0.5 μ L of saturated CHCA solution in 20% ACN and 1% TFA (v/v) with In as an internal standard. Off-line MALDI TOF and SALD ICP quadrupole MS detection were performed from the same target.

3 Results and Discussion

Simultaneous detection of the MT-1 isoforms (complexes with metals), apoforms (forms without metals) and metal quantification were recorded using off-line and on-line approach. The MT-1 complexes were separated at the neutral pH (7.0) and detected with ESI MS to obtain information about metal complexes and determine their metal-complex stoichiometry. Spotted fractions overlaid with acidified MALDI matrix were measured with MALDI MS for apoform determination. Subsequently, the total metal content was quantified by SALD ICP MS from the same deposited fractions. On-line ESI MS provided information about the MT-1 complexes and their metal stoichiometry. MALDI MS offered additional information about protein apoforms, thus confirming ESI MS identification. SALD ICP MS supplied quantitative data on metals in the MT-1 complexes.

3 Conclusions

A new combination of on-line and off-line MS detection techniques, ESI MS, MALDI MS and SALD ICP MS, was demonstrated in a single separation run. A commercial MALDI plastic target was found to be applicable for both MALDI and SALD ICP MS analysis. Using this approach both, molecular and elemental determination of measured sample was obtained. In addition, two complementary soft ionization techniques, ESI and MALDI MS were compared.

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References

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