

# Absolute Quantitation of Peptide Biomarkers by means of Immuno-MALDI-TOF MS

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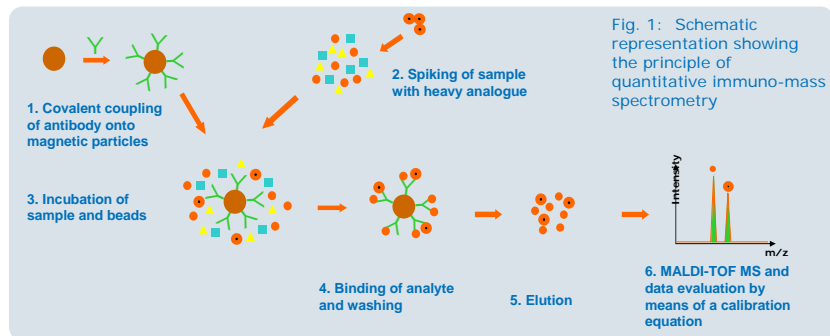
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## Introduction

Clinical routine diagnosis of biomarkers is mostly based on immunological techniques e.g. ELISA for quantitation. These methods are often not applicable for small antigens or antigen isoforms. MALDI-TOF MS is a sensitive and rapid method for the detection of peptides and protein/peptide isoforms. Hence, quantitative mass spectrometry is a desirable diagnostic tool. Des-Ala-Fibrinopeptide A (Des-Ala-FPA) as model system was used to develop a technical platform based on ClinProt<sup>TM</sup> immunoparticles and heavy internal peptide standard.

## Methods

Pre-activated magnetic micro-particles were covalently functionalized with specific rabbit antibody against Des-Ala-FPA. A calibration equation was generated by mixing Des-Ala-FPA and its heavy analogue at different concentration ratios and subsequent isolation of both peptides with immuno-particles. After spectra acquisition the calculated ratio of peak areas were plotted against the concentration of the light peptide. 30 µl serum were spiked with heavy analogue (10 ng/µl) and precipitated with ethanol. The supernatant after centrifugation was dried in a SpeedVac and the pellet was redissolved in 25 µl H<sub>2</sub>O. 5 µl of the reconstituted serum were incubated with 5 µl PBS and 5 µl immuno-beads for 1 h. Beads were directly eluted with HCCA matrix. The concentration of Des-Ala-FPA was calculated from the MALDI-TOF spectra according to the calibration equation.



## Results

The anti-Des-Ala-FPA modified particles allowed for the selective isolation of Des-Ala-FPA and its next two degradation products from human serum (Fig. 4A). Compared to the HIC C8 purification only Des-Ala-FPA derived peaks were detectable after immuno-affinity purification. Acquisition of a calibration equation revealed a linear working range from 2 to 25 ng/µl Des-Ala-FPA when working with a concentration of 10 ng/µl heavy analogue. The use of a heavy internal analogue facilitated the quantitation of Des-Ala-FPA from unknown samples according to the calibration equation. The concentration of Des-Ala-FPA of

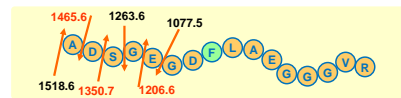


Fig. 2: Sequence of Des-Ala-FPA. Red labeled masses indicate degradation products detectable after immuno-affinity isolation (see Fig. 3A). The green highlighted phenylalanine is substituted in the heavy analogue by an isotope labeled amino acid (+ 10 Da).

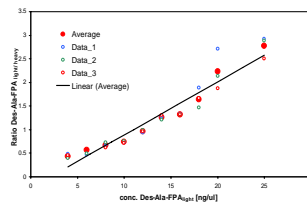


Fig. 3: Calibration equation acquired after immuno-capturing and used for the immuno-MS quantitation of Des-Ala-FPA of unknown samples.

fresh serum samples varies around 10 ng/µl. To investigate the stability of Des-Ala-FPA, serum was incubated either at room temperature or on ice for different times (Fig. 5A). The quantitation of Des-Ala-FPA revealed a clear degradation of the peptide at RT after 3 h, but a nearly constant concentration when the serum was kept on ice. Further, the influence of repeated freeze/thaw cycles was analyzed (Fig. 5B). When thawing of the serum samples was performed on ice degradation was observed not till the 5. freeze/thaw cycle. In contrast, thawing at RT already led to a degradation of Des-Ala-FPA after the first cycle.

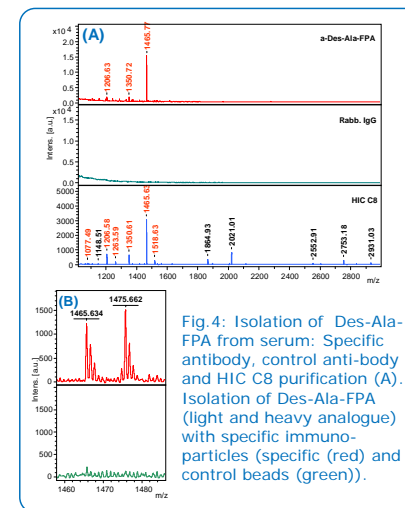


Fig. 4: Isolation of Des-Ala-FPA from serum: Specific antibody, control anti-body and HIC C8 purification (A). Isolation of Des-Ala-FPA (light and heavy analogue) with specific immuno-particles (specific red) and control beads (green).

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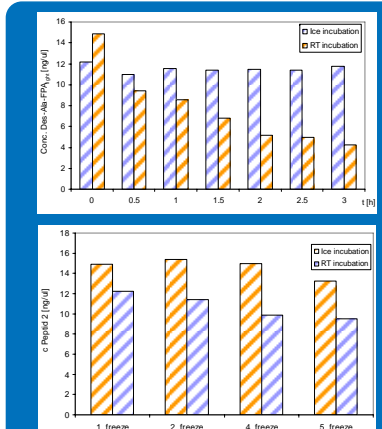


Fig. 5: (A) Time and temperature dependent degradation of Des-Ala-FPA. (B) Degradation of Des-Ala-FPA during repeated freeze/thaw cycles

## Conclusions

- This proof-of-concept study employing specific immuno-affinity particles and heavy internal standard demonstrates the absolute quantitation of biomarkers by MALDI-TOF in a clinical setting.
- Des-Ala-FPA and a corresponding specific antibody were successfully used to determine the concentration of the peptide from human serum.
- A kinetic analysis revealed the influence of time and temperature on the stability of Des-Ala-FPA.