Waters

OVERVIEW

Purpose: The objective of this study was to develop a fast and selective, quantitative assay, for a potential Alzheimer’s disease (AD) marker using MALDI-TOF MS.

Method: An axial MALDI-TOF mass spectrometer was used as a screening instrument utilizing stainless steel target plates and custom made MALDI-Quan software.

Results: Good correlation was obtained for amyloid beta peptides 1-42 and 1-38 applying appropriate internal standard concentrations.

INTRODUCTION

Amyloid beta peptides are found in an aggregated, poorly soluble form in senile plaques deposited in the brain of individuals affected by Alzheimer’s disease (AD). In addition, soluble amyloid beta peptides are endogenous to human body fluids. There has been increasing interest in using MALDI-TOF instrumentation for not just qualitative, but also quantitative serum profiling. Amyloid beta peptides are of interest as changing serum levels are considered to influence the onset of AD. To evaluate the viability of studies based on MALDI-TOF MS data, different dilution series of two amyloid beta peptide fragments 1-38 and 1-42 were prepared, with amyloid beta peptide fragment 1-43 applied as internal standard. The samples were prepared in different matrix preparations and were analysed to determine the best conditions for the analysis of these peptides. The method of choice was found to be a specific sinapinic acid preparation, resulting in homogenous matrix-analyte layers, facilitating automatic data acquisition. This method was used due to its reproducibility, despite a DHB sample preparation procedure providing slightly better sensitivity.

RESULTS AND DISCUSSION – PART 1

Quantification of 1-42 using 1-43 as internal standard

Quantification of 1-42 with 1-43 as internal standard

Quantification of 1-38 with 1-43 as internal standard

CONCLUSIONS

- The use of MALDI MS provide good linearity between 20 to 200 nM for 1-42 and 1 nM to 20 nM for Abeta 1-38.
- In addition, good correlation over a greater dynamic range was achieved by applying 4 parameter logistic.
- The limit of detection for the beta amyloid peptides by MALDI MS was shown to be in the low femtogram range.
- This technique provides a rapid, versatile screening tool.

FUTURE PROSPECTS

- The use of alternative MALDI targets should allow improved limit of detection and limit of quantification for neuropeptides.
- To extend the dynamic range of the assay, different concentrations of analyte & internal standards will be required, on separate target spots.
- The specificity of the experiment could be increased by combining affinity purifications with MALDITOF MS.

Acknowledgement: We thank Christian Boxer (Waters, Switzerland) for his kind support.

QUANTIFICATION OF AMYLOID BETA PEPTIDES WITH MALDI-TOF-MS

Table 1: MALDI quantification results for 1-42 (n=8). Table displays the software output.

<table>
<thead>
<tr>
<th>Peak intensities (height)</th>
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<tbody>
<tr>
<td>200 nM</td>
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<tr>
<td>4510.60</td>
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<td>4510.60</td>
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<td>4510.60</td>
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<td>4510.60</td>
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</table>

Table 2: Quantification of 1-42 (data acquired in linear mode and automatically processed).

<table>
<thead>
<tr>
<th>Concentration (nM)</th>
<th>std 1</th>
<th>std 2</th>
<th>std 3</th>
<th>std 4</th>
<th>std 5</th>
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<tbody>
<tr>
<td>100 nM</td>
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<tr>
<td>50 nM</td>
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<tr>
<td>20 nM</td>
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<td>10 nM</td>
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<td>5 nM</td>
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Figure 1: MALDI/MS data obtained in linear mode of operation. Abeta 1-42 in different concentrations with Abeta 1-43 in internal standard (40 nM).

Figure 2: Linear regression between 20 and 200 nM of 1-42 (20 fmol and 200 fmol on target respectively). n = 8.

Figure 3: Linear regression between 1.2 and 12 nM of 1-38 (1.2 to 12 fmol on target respectively; n = 8).

Figure 4: Spectrum of a mixture of 1-38, 1-42 and 1-43 acquired in positive ion, linear-mode MALDI. The amount of each peptide, on-target, was 0.02, 0.25 and 0.2ng respectively. The data shows that MALDI can easily distinguish a dynamic range of greater than 10:1 within a single spectrum. As a full TOF spectrum is acquired in this analysis, quantitation can be performed on multiple analytes of interest from a single acquisition.

PART 2

Quantification with Bovine Insulin as internal standard

Calibration curve 4-parameter logistic – amyloid beta 1-42 in sinapinic acid

PART 3

Amyloid beta peptide mixture (1-38, 1-42 and 1-43)

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