QUANTIFICATION OF AMYLOID BETA PEPTIDES WITH MALDI-TOF-MS

Waters

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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

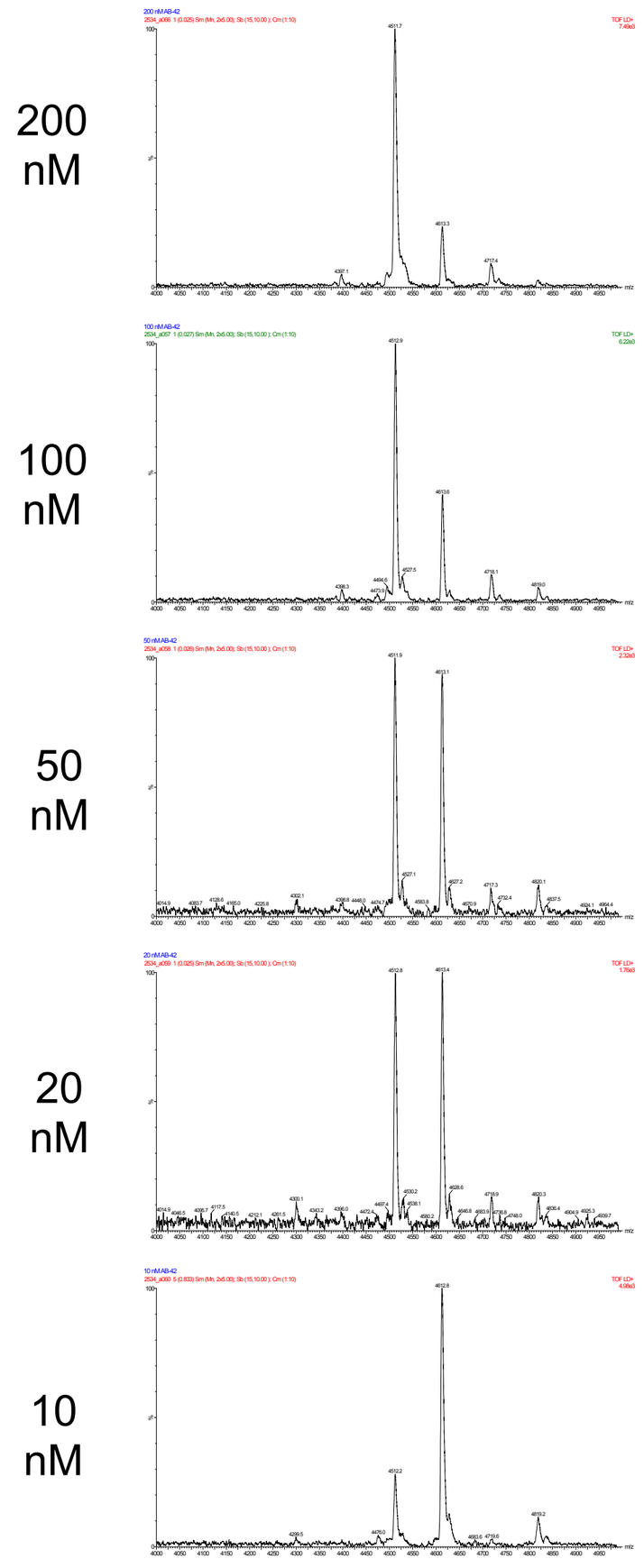


Figure 1: *MALDI-MS data obtained in linear mode of operation. Abeta 1-42 in different concentrations with Abeta 1-43 as internal standard (40 nM).*

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

Peak intensities (height)

	m/z Abeta 1-42		m/z Abeta 1-43		Ratio
200 nM	4514.9280	8100	4615.7640	1848	4.38
100 nM	4515.0860	2990	4615.8900	1177	2.54
50 nM	4512.6820	1500	4613.6170	1359	1.10
20 nM	4512.8800	1941	4613.8100	1897	1.02
10 nM	4515.9090	759.7	4616.9290	2572	0.30
200 nM	4512.1350	7426	4613.2090	1837	4.04
100 nM	4513.8030	6244	4614.8940	2588	2.41
50 nM	4512.2580	2292	4613.1540	2214	1.04
20 nM	4512.7940	1732	4613.5680	1748	0.99
10 nM	4514.3930	1396	4615.0270	4973	0.28
200 nM	4512.2910	3095	4613.4200	702	4.41
100 nM	4510.8530	4641	4611.7890	1943	2.39
50 nM	4514.3940	2158	4615.1960	2071	1.04
20 nM	4512.7390	1723	4613.6760	1512	1.14
10 nM	4514.6480	1904	4615.4130	7019	0.27
200 nM	4513.9650	7483	4615.2130	1749	4.28
100 nM	4511.2360	5057	4612.2460	2022	2.50
50 nM	4511.6190	2350	4612.6160	2221	1.06
20 nM	4513.4520	1684	4614.3170	1556	1.08
10 nM	4514.1040	1311	4614.9500	4992	0.26
200 nM	4515.2140	2335	4616.3770	564	4.14
100 nM	4513.3140	2953	4614.1870	1089	2.71
50 nM	4513.7810	1832	4614.7720	1694	1.08
20 nM	4513.6390	1899	4614.4630	1855	1.02
10 nM	4514.5400	290.8	4614.9720	1027	0.28
200 nM	4512.3250	7447	4613.2860	1809	4.12
100 nM	4511.7470	5063	4612.7030	2266	2.23
50 nM	4512.1310	1639	4613.0710	1516	1.08
20 nM	4513.7240	2691	4614.5640	2681	1.00
10 nM	4514.4650	1205	4615.3350	4197	0.29
200 nM	4516.9490	2363	4615.6530	560.9	4.21
100 nM	4511.7090	3606	4612.5940	1433	2.52
50 nM	4512.3390	1887	4613.1500	1862	1.01
20 nM	4514.8290	1545	4615.9420	1455	1.06
10 nM	4514.9660	1109	4615.7410	4021	0.28
200 nM	4512.9580	9231	4614.0300	2175	4.24
100 nM	4511.7830	2239	4612.9880	912.4	2.45
50 nM	4514.5120	2236	4615.3420	1959	1.14
20 nM	4515.8370	1379	4616.7270	1367	1.01
10 nM	4514.7750	1135	4615.6090	4292	0.26

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

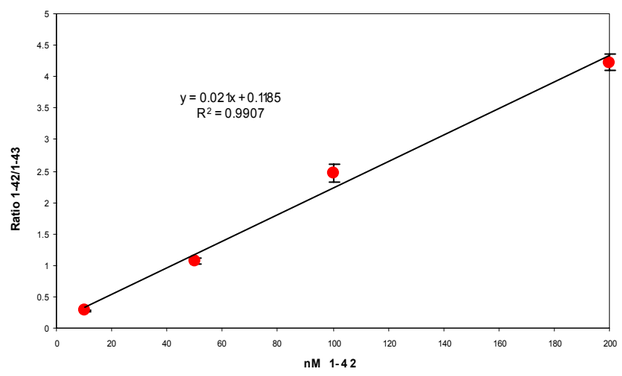


Figure 2: Linear regression between 20 and 200 nM of 1-42 (20 fmol and 200 fmol on target respectively; n = 8).

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

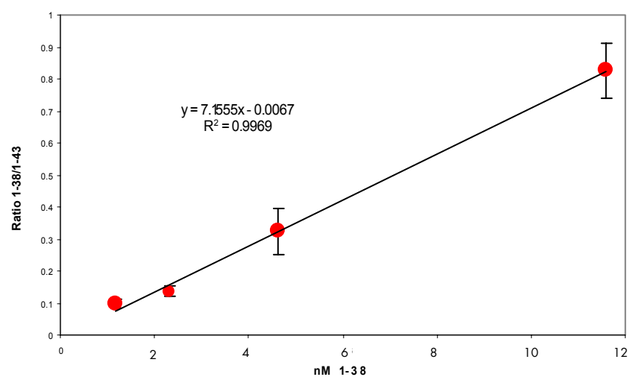


Figure 3: *Linear regression between 1.2 and 12nM of 1-38 (1.2 to12fmol on target respectively; n = 6).*

EXPERIMENTAL

Data was acquired on a Waters® Micromass® MALDI micro MX™ mass spectrometer (Waters, Manchester, UK) operating in the positive ion, linear-mode.
Analyte samples were mixed 1:1 with a 10 mg/mL sinapinic acid matrix solution (50/50, acetonitrile/0.1%TFA) and 1 µL deposited on to a MALDI target pre-coated with a sinapinic acid thin layer (10 mg/mL in acetone).
Amyloid beta peptide fragments 1-38, 1-42, 1-43 and Bovine Insulin were from Sigma-Aldrich and dilution series were prepared in 0.1% TFA from 1 mg/mL stock solutions. 100 laser shots per spot were combined, background subtracted and smoothed. Processing was done with a custom developed software tool (Maldi-Quan-Tool).

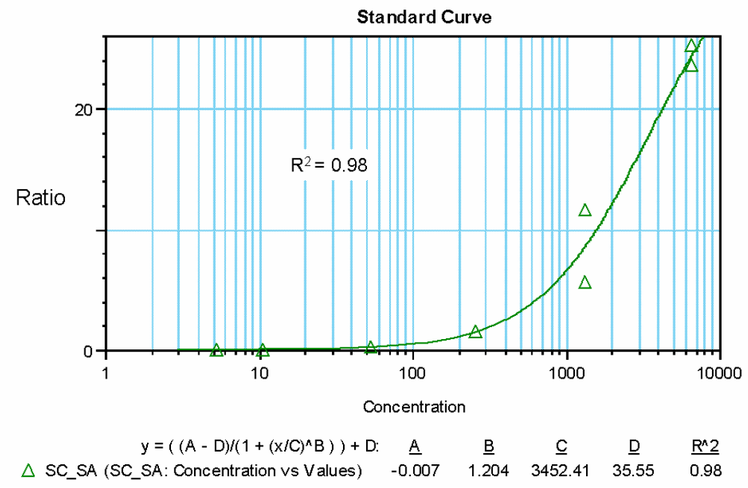
PART 2

Quantification with Bovine Insulin as internal standard

concentration fmol/spot	ratio	mean	STDV	CV%	Acc%
6640	25.29 23.57	24.43	882.12	13.2	100.6
1330	11.55 5.56	8.555	727.7	53.24	102.8
260	1.48 1.53	1.505	5.27	2.03	99.9
53.2	0.21 0.25	0.23	5.39	9.98	101.6
10.6	0.02 0.02	0.02	0	0	84.1
5.3	0.01 0.01	0.01	0	0	115

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

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)

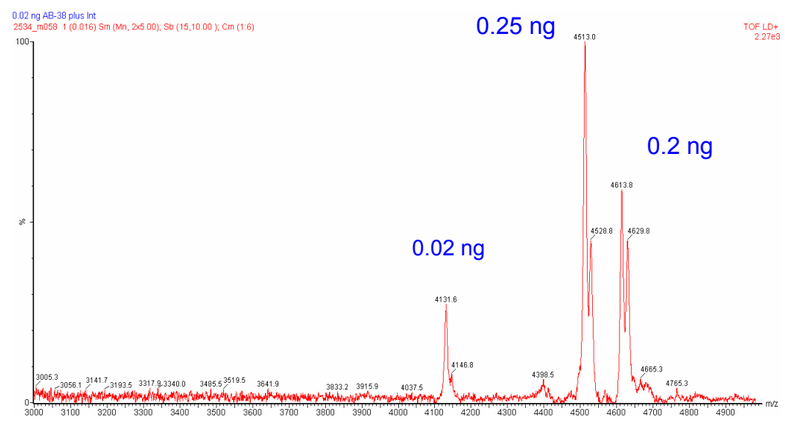


Figure 4: Spectrum of a mixture of 1-38, 1-42 and 1-43 acquired in positive ion, linear-mode MALDI. The amount of each peptide, on-target, was 0.02, 0.25 and 0.2ng respectively. The data shows that MALDI can easily distinguish a dynamic range of greater than 10:1 within a single spectrum. As a full TOF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

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 femtomole range.
- This technique provides a rapid, versatile screening tool.

FUTURE PROSPECTS

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

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