Validation of an LCMS Hybrid Assay with EVOSEP cleanup for the quantitation of Islet Amyloid polypeptide in human plasma

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The authors declare no competing financial interest.

Introduction

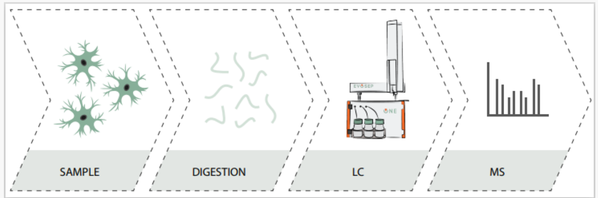
Islet Amyloid polypeptide (IAPP) is a peptide hormone produced by the pancreas' beta cells and plays a role in regulating blood glucose. Research on IAPP and its role in diabetes is ongoing, and there is a need for a reliable method to accurately detect this hormone at clinically relevant levels and possibly the different forms of the peptide. We set out to validate a hybrid LC/MS assay for this biomarker that could be validated to an appropriate level to support clinical studies.

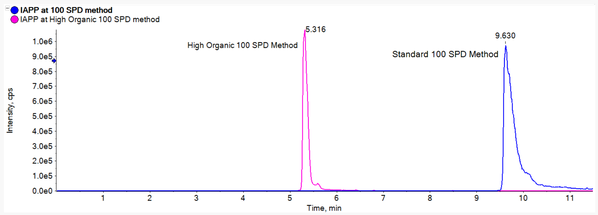
The method captures IAPP (for instance, the active amidated form with 37 amino acids) from plasma samples which are washed to remove the non-captured matrix. The IAPP is released from the antibody, and the entirety of the sample is then loaded onto an Evosep tip. The solid phase of the Evotip is then washed to remove any remaining impurities from the capture process and analyzed on a Sciex 7600 system. The quantitation range is 50 to 4000 pg/mL.

IAPP is known to aggregate to form islet amyloid. Special care and conditions were developed to prevent aggregation during analysis.

Evosep One Overview and Methodology

The Evosep One is a high-throughput liquid chromatographic system that uses individual disposable trap columns built into a pipette tip format. Evosep One is designed to use preconfigured LC gradient methods utilizing µL/min flow rates. For this work, a High Organic 100-sample-a-day method utilizing an 8 cm x 100 µm column with a 1.9 µm particle size was used (EVOSEP P/N EV1064).





Antibody Characteristics

The antibodies selected for this study have a predicted epitope in the C-terminal of the peptide, away from the disulfide bond.

Two antibodies developed by Mercodia were used to test the pull-down and release conditions:

Antibody 1: 5.47E-12M
Antibody 2: 7.03E-13M

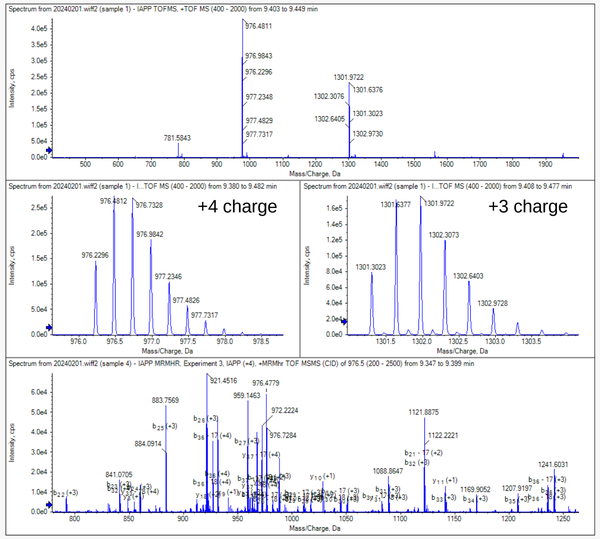
Due to the predicted epitope, this antibody (over more selective ELISA kits) would be able to identify various forms of IAPP, such as the fully deamidated (954.94 m/z, +4) form.

MS Development

We quickly determined that digestion would not be required and would limit the availability of TOFMS data for monitoring other post-translational modifications or bioactive variants of IAPP.

Optimization came into play in selecting the fragments once the chromatography was selected.

An MRM-HR experiment was used for analysis, and the top nine product ions were summed for quantitation. Two precursor ions were qualitatively monitored.

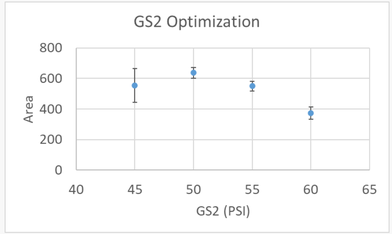


Optimization of MS Parameters for Evosep

The source parameters for sub-100 µL/min flow rates must be optimized more rigorously than standard flow LC. The high organic gradient adds another layer of instability in the spray conditions compared to standard methods. As such, we fully optimized the source parameters.

Three replicates were used to optimize the source parameters looking for the highest signal with the lowest variability. Only the +4 precursor ion was monitored as fragmentation is not source dependent.

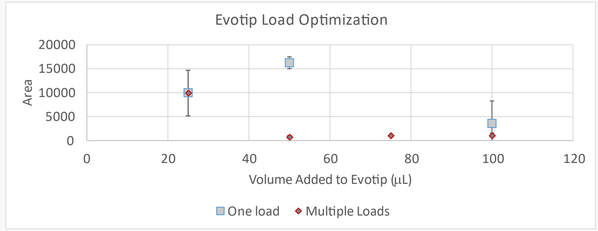
Optimization Order	Parameter	Range	Ideal Value	%CV
1	ISV	3400-3700	3500	82.1%
2	GS1	10-25	10	25.1%
3	TEM	150-225	225	13.7%
4	GS2	45-60	50	5.3%



An example of GS2 optimization showing the largest value also had the lowest variability

Optimization of Evosep Tip preparation parameters

Given the high propensity for IAPP to form aggregates, HFIP was used for stock solutions while acids were explored for the EVOSEP loading diluent. Antibodies are provided in a standard 300 µL well which requires more than the Evosep-recommended aliquot to elute the antibody. As such, the load volume and conditions needed to be optimized. We settled on 50 µL of 100 mM acetic acid to elute from antibody 1.



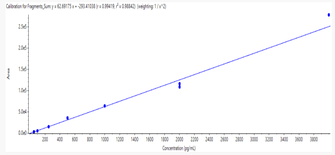
Evosep Advantages

Parameter	EVOSEP ONE	SPE with UPLC
Analyte mass	All mass of analyte injected	~ 1:5 of analyte in total extract
Absorbents	Limited to C18	C18, WCX, AX, etc.
Chromatographic columns	Limited to C18	C18, HILIC, ion exchange, etc.
Flow rates	Micro and nanoflow (5-1 µL/min; <1 µL/min)	Analytical (>100 µL/min)
LC Development	Fixed methods	Ability to modify

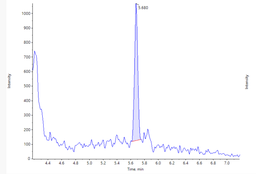
Quantitation Method

The quantitation summed the top nine fragmentation ions after optimizing the CE so minimal precursor was observed. Only fragments that were observable in the low standard were included in the sum.

Ion	Fragment	XIC Width
B26^{+3}	921.782	0.2
B32^{+3}	1121.888	0.2
B25^{+3}	884.088	0.2
B27^{+3}	959.479	0.2
B22^{+3}	792.0174	0.2
B36^{+3}	1241.603	0.2
B28^{+3}	988.1573	0.2
Y11^{+1}	1141.55	0.2
Y10^{+1}	1028.46	0.2



Std 1 (50.0 pg/mL)



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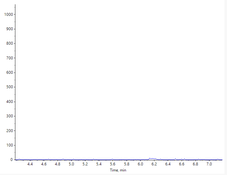

Conclusions

We have developed a method for the detection of IAPP using an antibody pulldown followed by Evosep on-tip C18 micro-flow chromatography and detection by a SCIEX 7600 system. The development required fine-tuning of solvation parameters to prevent aggregation of IAPP, maximizing signal at micro-flow conditions, and minimizing variability of the electrospray. Linearity was established from 50 to 5000 pg/mL. This biomarker method is validated for the appropriate context of use and could be expanded to other isoforms of IAPP.