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Validation of an LCMS Hybrid Assay with EVOSEP Cleanup for the Quantitation of Islet Amyloid Polypeptide in Human Plasma

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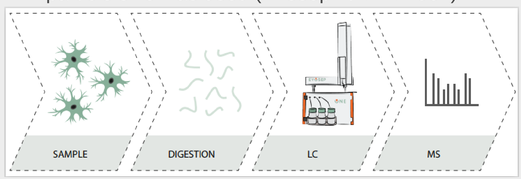
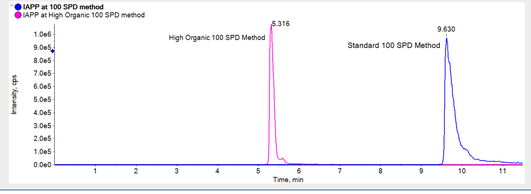
PURPOSE

Islet Amyloid Polypeptide (IAPP) is a peptide hormone produced by the pancreas' beta cells that regulates 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 LCMS assay for this biomarker that could be validated to an appropriate level to support clinical studies.

EVOSEP ONE OVERVIEW

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: KM = 5.47E-12M
Antibody 2: KM = 7.03E-13M

Due to the predicted epitope, these antibodies (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.

EVOSEP TIP OPTIMIZATION

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 coated on a 300 µL well plate, requiring 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.

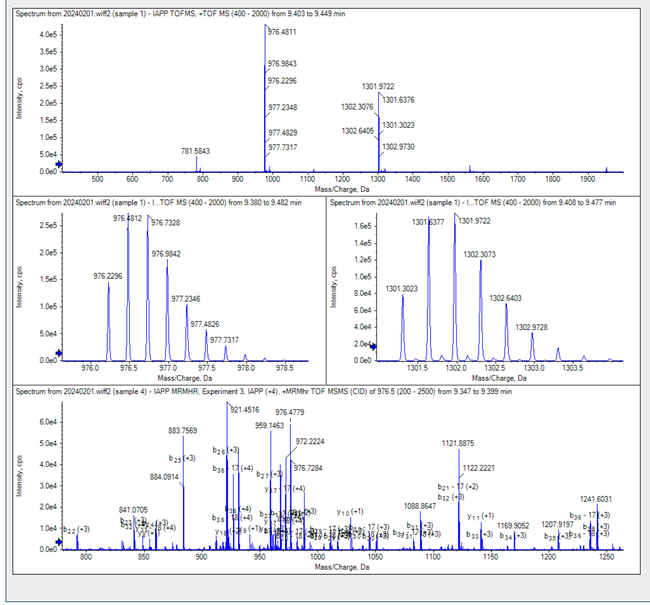
MS DEVELOPMENT

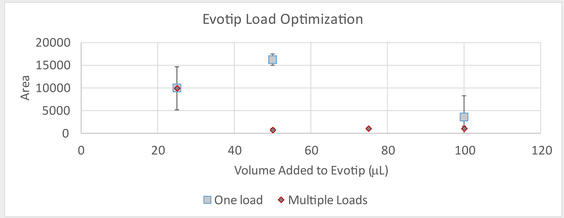
We quickly determined that digestion would not be required and would limit the availability of TOFMS data for monitoring other post-translational modifications or bioactive variants of IAPP.

Optimization came into play in determining 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.

Sub 100 µL/min flow rates require optimization of the source parameters which were done in the order ISV, GS1, TEM, GS2.



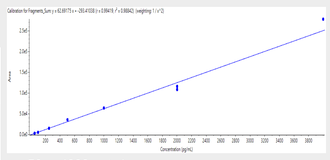


Optimization Order	Parameter	Range	Ideal Value	%CV
1	ISV	3400-3700	3500	82.1%
2	GS1	10-25	10	25.1%
3	TEM	150-225	225	13.7%
4	GS2	45-60	50	5.3%

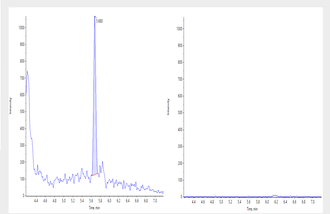
METHOD

The method captures 100 µL aliquots of IAPP from plasma samples which are washed several times to remove the non-captured matrix. The IAPP is released from the antibody using 50 µL of 100 mM acetic acid, and the entirety of the sample is then loaded onto an Evosep tip. The solid phase of the Evotip is then washed with 20 µL of 0.1% formic acid in water 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. The sample is injected using the Evosep system on an EV1064 column with the 100 Sample Per Day High Organic Method. Quantification was conducted by summing multiple product ions, ensuring each product ion had a signal at the LLOQ.

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}	1026.46	0.2



50 to 4000 pg/mL



Std 1 (50.0 pg/mL) Equivalent Blank

RESULTS

By coupling Evosep One cleanup to an HRMS system and using microflow liquid chromatographic conditions, the detection limits are comparable with those of traditional immunocapture methodologies. We optimized the Evosep SPE and Mass Spectrometer conditions separately and then combined them into a fit-for-purpose method. Quantitation was performed on a SCIEX 7600 ZenoTOF, where multiple transitions collected using MRM-HR, including the B26, B32, B25, and B27 ions, were summed.

We determined the optimal parameters of the 7600 ZenoTOF system for IAPP under a high organic micro-flow condition to maintain a stable spray. The Evosep tip loading and preparation were optimized away from the published recommended level to prevent over-pressure of the column while maintaining the signal of the analyte of interest. The use of individual trap columns (Evotips) eliminated carryover in the assay, which is often present in traditional LC assays.

CONCLUSION

A method of analysis for Islet Amyloid Polypeptide in human plasma was developed fit-for-purpose to support clinical studies. This method will be validated following the principles outlined in the FDA M10 bioanalytical method validation guidance and from the AAPS “Biomarker Assay Validation by Mass Spectrometry” white paper. Included in the validation will be experiments to measure accuracy and precision in an authentic and surrogate matrix, parallelism, and stability experiments.

ABBREVIATIONS

TOFMS = time-of-flight mass spec; MRM^{HR} = multiple reactant monitoring high resolution; TEM= source temperature LC = liquid chromatograph; HFIP = hexafluoro-2-propanol; SPE = solid phase extraction; ISV = ion spray voltage UPLC = ultra performance liquid chromatograph; CE = collision energy; KM = Michaelis constant GS1 = ion source gas 1; GS2 = ion source gas 2

Aliri BIOANALYSIS Mercodia