IMPROVING PEPTIDE CATABOLISM INTERPRETATION USING ION MOBILITY DATA AND SERVER-BASED DATA REVIEW WITH HELM INTEGRATION

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INTRODUCTION

A unique challenge in peptide-based drug metabolism for evaluating preclinical candidates is the need for processing and visualization software tools that accommodate both small molecule rules (such as oxidations, synthetic modifications), as well as large molecule modifications (such as amide hydrolysis and oxidative deamination).

As more peptide drugs with complex/unnatural modifications are developed, algorithms that enable thorough identification, rapid analysis and comparisons of candidate series are required. Herein we discuss a solution that provides: clearance and metabolic analysis of multiple peptide candidates involving directed substitution with non-natural based amino acid moieties; the addition of structural/tracking information via collision cross section (CCS) measurements for metabolites; and improvements to visualization of complex peptide analyses through integrated tools utilizing HELM notation (Pistoia Alliance).

METHODS

Experimental

Eight 14-amino acid analogues of somatostatin were studied (Figure 1). Somatostatin was systematically substituted using both D-amino acids and a non-natural amino acid, Msa (mesityl alanine). Analogues were incubated in human serum at eleven time points (0, 5 min, 10 min, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h, 30 h and 48 h) then evaluated.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Somatostatin	Ala	Gly	Cys	Lys	Asn	Phe	Phe	Trp	Lys	Thr	Phe	Tyr	Ser	Cys
Analogue 6	Ala	Gly	Cys	Lys	Asn	Phe	Msa	D-Trp	Lys	Thr	Phe	Tyr	Ser	D-Cys
Analogue 30	Ala	Gly	Cys	Orn	Asn	Phe	Msa	D-Trp	Lys	Thr	Phe	Tyr	Ser	Cys
Analogue 31	Ala	Gly	Cys	Lys	Asn	Phe	Msa	D-Trp	Lys	Thr	Phe	Tyr	Ser	Cys
Analogue 35	Ala	Gly	Cys	Orn	Asn	Phe	Msa	D-Trp	Lys	Thr	Phe	Tyr	Ser	D-Cys
Analogue 64	Ala	Gly	D-Cys	Lys	Asn	Phe	Msa	D-Trp	Lys	Thr	Phe	Tyr	Ser	D-Cys
Analogue 65	D-Ala	Gly	Cys	Lys	Asn	Phe	Msa	D-Trp	Lys	Thr	Phe	Tyr	Ser	D-Cys
Analogue 95	D-Ala	Gly	D-Cys	Lys	Asn	Phe	Msa	D-Trp	Lys	Thr	Phe	Tyr	Ser	D-Cys

Figure 1. Sequences of somatostatin and 7 modified analogues tested for stability in human serum. Msa = Mesityl alanine amino acid

Data Acquisition

Data were collected on an ACQUITY UPLC coupled to a benchtop IMS QTof (Vion IMS QTof MS, Waters) acquiring data independent IMS data (HDMS^E).

Data Processing and Upload

Data were batch processed using MassMeta-Site (Lead Molecular Design) via the built-in UNIFI Application Programming Interface (API). All data were uploaded onto WebMetabase version 4.0 (cloud/server based application, Lead Molecular Design) and analyzed.[1]

RESULTS

All analogues studied contained substitution of Phe[7] by Msa and Trp [8] by D-Trp. The remainder of changes were permutations of Ala[1], Cys[3], Cys[14] substitutions to their D-amino acid equivalents, as well as substitution of Lys[4] to ornithine. Figure 2 summarizes the location and nature of changes performed across the 8 analogues. Across all analogues, WebMetabase was used to monitor disappearance of the substrates, evaluate the modulation of key metabolite formation (particularly -Ala and -AlaGly), and monitor for other major metabolic pathways, including ring opening. As previously reported, metabolism of native somatostatin primarily showed the loss of Ala (-71 Da) and -AlaGly (-128 Da) from the tail portion of the molecule.[2,3]

Peptide 31 stability and metabolite review in WebMetabase is shown here in more detail. Figure 3 shows the relative stability versus somatostatin. Figure 4 shows the 60 min timepoint and selected major metabolites, -Ala, -AlaGly, and minor peptide fragments NF-Msa-dWKT and F-Msa-dWKT. It includes all respective measured properties (and ranges across all samples including RT, response and measured CCS (Collision Cross Section) values as part of the table. Figure 5 shows the predicted structures of the metabolites reported in Figure 4. Only a representative selection of major metabolites are reported here, deamidation and other minor cleavages were also observed for most peptides.

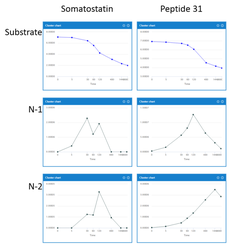

Figure 3: Stability plots (WebMetabase) for somatostatin (left) and peptide 31 (right) and major metabolites, -Ala and –AlaGly (48 hours shown)

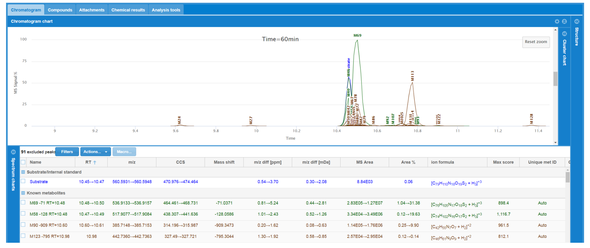

Figure 4. Webmetabase summary of peptide 31, substrate, and 4 metabolites, -Ala (-71 Da), -AlaGly (-128 Da), NF-Msa-dWKT (-909 Da), and F-Msa-dWKT (-795 Da)

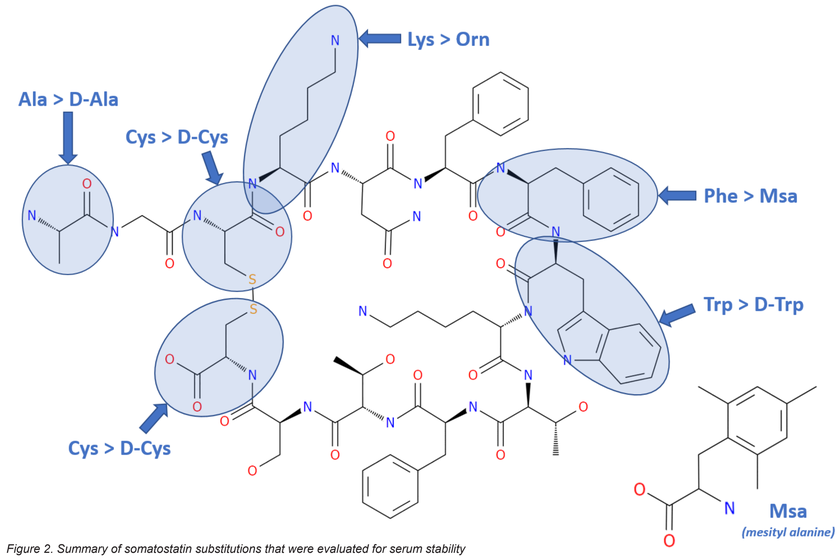

Figure 2. Summary of somatostatin substitutions that were evaluated for serum stability

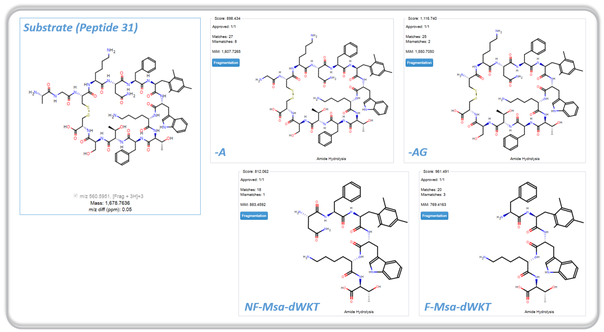

Figure 5. Predicted metabolites and scores provided in WebMetabase

DISCUSSION

WebMetabase was used to review and visualize all 8 analogues, which enabled thorough interrogations of key metabolic processes. Catabolism occurred primarily at the terminal amino acids outside the disulfide linked ring portion of somatostatin (as reported previously). Modifications and their effects on reducing somatostatin degradation where identified and linked to specific cleavages that were enhanced or reduced through substitution. It was therefore important to review multiple hot spots in the context of the overall stability plot to ensure that multiple substitutions had the desired effect of stabilizing the original substrate in a holistic manner, as often single point changes did not improve stability by themselves. Concerted modifications were required to produce peptide 95, which had significantly improved stability.

Ion mobility derived collision cross section (CCS) values were reported for all species (Figure 4). CCS provided a routine mechanism to keep track of and differentiate a number of (highly related with numerous isobaric) analogues and their substitutions across multiple timepoints (repeat measurements at varying concentrations where all found to be <1%, not shown). The +3 charge states of -Ala metabolite were compared for CCS changes. -Ala variants across peptides produced a difference of over 3% across analogues, indicating that these modifications impacted gross molecular structure and are measurable.

Ion mobility data processed using the web-based package provided an efficient method to review complex data in a straightforward manner. WebMetabase further enabled offloading of key processing time/burden from local hardware installations, allowing data insights to be routinely viewed, shared and discussed between several researchers at different sites (Figure 6).

HELM integration with WebMetabase is now possible and enables visualization at the peptide level (rather than .mol or .SDF). Examples of HELM notation are shown in Figure 7. Figure 8 summarizes the stability of all modified peptides. Peptide 95 showed the greatest stability improvement versus somatostatin, which was confirmed by the relative low abundance of metabolites detected in the sample.

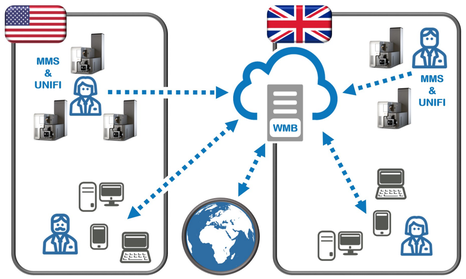

Figure 6. Representation of UNIFI MS system network utilizing WMB server located in the UK. HTML/Web based nature of software facilitated access from any internet enabled system via browser, worldwide

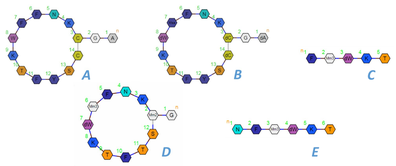

Figure 7. HELM representations of key metabolites in WebMetabase

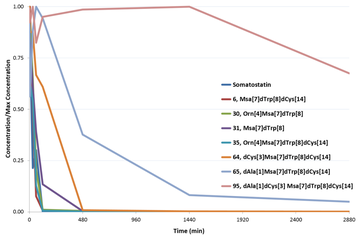

Figure 8. Overall serum stability of all 8 analogues over 48 hours

CONCLUSION

- **MS Data were processed in an automated fashion and uploaded to a centralized server.**
- **Assays could be analyzed and visualized from any internet enabled location.**
- **Peptide analogues were assessed for stability primarily quickly and effectively. Hydrolyzation, ring cleavages, deamidation, disulfide reduction were screened for rapidly and efficiently.**
- **Key metabolites for peptide 31 were reported in detail including CCS values. These values could be used to track the metabolites across samples and also measure and compare the size of the metabolites and substrates.**
- **Peptides, metabolites could be visualized using traditional (mol based) or HELM based formats.**

References

1 J Kirk et al. Using Mass-MetaSite and WebMetabase to Process HDMSE data acquired on the Vion IMS QTof Mass Spectrometer. Waters Technical Brief 720006362EN, www.waters.com.

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