Catabolism of peptides using ion mobility enabled high resolution mass spectrometry coupled with Mass-MetaSite data processing

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INTRODUCTION

The pharmaceutical industry is increasingly exploring biotherapeutic molecules as an alternative to conventional small molecule drugs, with the number of biotherapeutic drugs approved each year continuing to increase. In response, a growing need exists to characterize and optimize the absorption, distribution, metabolism and excretion (ADME) properties of these diverse biotherapeutics. DMPK groups are looking to apply similar principles from their small molecule experience to the ADME of diverse biotherapeutics. The challenge is that few software packages are able to characterise the clearance and metabolic fate of biotherapeutics[1,2]. Here, we mine ion mobility high resolution mass spectrometric (DIA) data for the analysis of biotherapeutic drug metabolism using the Mass-MetaSite and WebMetabase software platform for processing.

Here we have taken two somatostatin analogues and investigated the catabolites formed after incubation in human serum:

Peptide 35: H-Ala-Gly-Cys-LOrn-Asn-Phe-Msa-DTrp-Lys-Thr-Phe-Thr-Ser-DCys-OH

Peptide 64: H-Ala-Gly-DCys-Lys-Asn-Phe-Msa-DTrp-Lys-Thr-Phe-Thr-Ser-DCys-OH

Both contain a disulphide bridge between Cys[3]—Cys[14] and the non natural amino acid Msa (Figure 1).

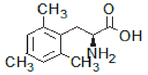
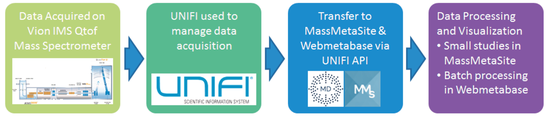


Figure 1.
Structure of Msa, 2,4,6-trimethyl-L-phenylalanine (Mesityl alanine)

METHODS

14-amino acid analogues of the natural hormone somatostatin were investigated. They 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). Data were collected on an ACQUITY IClass UPLC coupled to an IMS QTof using HDMS^E. (Data were processed in a prototype version of Mass-MetaSite 5.1.9 able to read data directly from UNIFI 1.9.2 using the built-in Application Programming Interface (API). The processed data were uploaded onto the server based application, WebMetabase 3.2.9, where all the samples from the same experiment were analysed and clustered. Samples were injected as supplied.



Analytical Method Details
- LC column: ACQUITY CSH C18 1.7 µm, 2.1 x 100 mm
- Mobile phase A and B: water and acetonitrile + 0.1 % formic acid
- Gradient: 2% to 30 % B over 7 min, to 100% at 8 min, hold until 10 min
- Flow rate: 0.4 mL/min
- Instrument: Vion IMS Qtof (positive ion mode, *m/z* 50-2000)
- Acquisition Mode: HDMS^E (using LE 6 and HE 25-50 eV ramp). 0.2 sec scan time

RESULTS

This study outlines and focuses on the benefits and ease of use of ion mobility for the application of catabolite identification in combination with Mass-MetaSite and WebMetabase for processing these complex data sets. A number of catabolites for the parent peptides were identified over the time course and could be attributed to hydrolysis of the peptide backbone, with the main catabolites identified attributed to hydrolysis of the non-cyclic portion of the peptides (Figure 3 and Table 1). Mass-MetaSite and WebMetabase were used to characterize metabolic fate of the somatostatin analogues showing that data can be collected routinely, processed and reviewed efficiently across time courses and treatments. A measure of the ratio of structurally matched to mismatched product ions found by Mass-Metasite provides confidence in catabolite assignment through data acquired by DIA (Figure 4). Substrate turnover varied depending on the peptide with approximately 99 % of the parent rapidly (<10 min) turned over for peptide 64 . Peptide 35 generated a key catabolite attributed to loss of the Ala residue. Other catabolites could be attributed to cleavage of Ala and Gly and ring opening of the cyclic component followed by downstream hydrolysis. The use of HDMS^E showed an improvement in data quality through alignment of precursor and fragment ions in both RT and DT (drift time) thus leading to improved spectral quality and an increase in identified fragment ions over the number of false positives for several peptides (Figure 5).

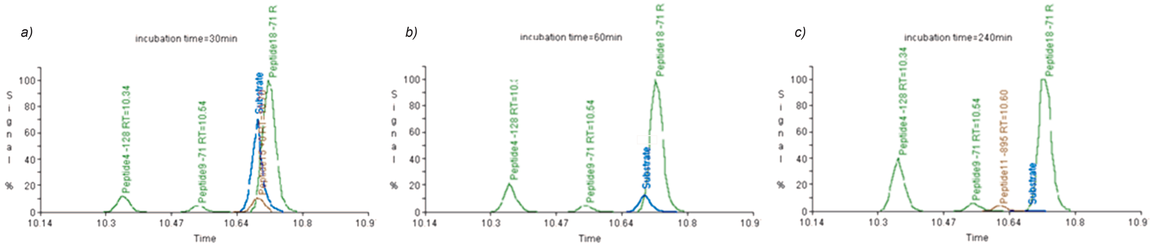
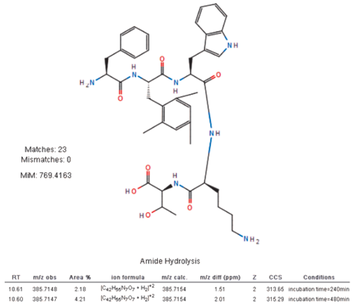


Figure 3. *Extracted ion chromatograms of the identified catabolites at a) 30 min b) 60 min and c) 240 min. Figure 3 c) Highlights the loss of substrate and the appearance of the catabolite at 10.60 min.*

Table 1. *Summary of the catabolites identified in Peptide 35. Green indicates a first generation catabolite and brown indicates greater than first generation catabolite.*

Peptide 35	Retention time (min)	Comment
Peptide -110 (3-14)	8.82	Loss of AG + Amide hydrolysis
Peptide -128 (3-14)	10.34	Loss of AG
Peptide -71 (2-14)	10.54	Loss of A
Peptide -895 (6-10)	10.60	NFXWKT Amide hydrolysis
Substrate	10.65	AGCKNFXWKTFTSC=
Peptide -71 (2-14)	10.71	Loss of A



RT	m/z obs	Area %	ion formula	m/z calc.	m/z diff (ppm)	Z	CCS	Conditions
10.61	385.7148	2.18	$[C_{42}H_{60}N_{10}O_7 + H_2]^{2+}$	385.7164	1.51	2	313.65	incubation time=240min
10.60	385.7147	4.21	$[C_{42}H_{60}N_{10}O_7 + H_2]^{2+}$	385.7164	2.01	2	315.29	incubation time=480min

Figure 4*. Proposed structure of the catabolite peptide-895 (6-10), with cleavage of the cyclic moiety of the peptide. A degree of the confidence in peptide identification is shown by the number of matches v mismatches using the high energy (HDMS^E) fragmentation data in addition to CCS measurements.*

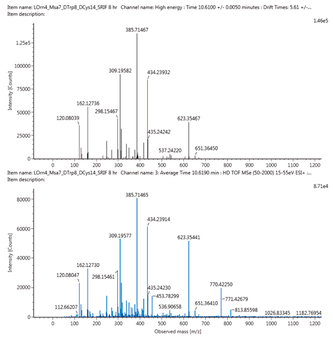


Figure 5*. Improvement in spectral quality using drift time and retention time alignment (a) compared to retention time (b) alone for the high energy spectrum of the peptide –895 (6-10) catabolite.*

CONCLUSIONS

- **Acquisition and automated processing of peptide catabolism data sets were successfully performed using the Vion IMS QToF and the UNIFI API Mass-MetaSite and WebMetabase.**
- **Mass-MetaSite and WebMetabase were able to process cyclic and non-native aa modified catabolism data sets and rationalize the detected hydrolysis products.**
- **The *in vitro* incubations of the synthetic peptides revealed a number of hydrolysis products, including residues 2-14, 3-14 in addition to the cleavage within the cyclic moiety (6-10) observed for peptide 35. For peptide 64 only cleavages of the non cyclic portion of the molecule were observed.**
- **Ion mobility data helps reduce the number of peaks that could be mis-assigned which minimized reporting of false positives and improved interpretation.**
- **A tightly integrated, UPLC HRMS informatics platform with high chromatographic performance, high mass accuracy and fast scanning capabilities afforded by the IMS QTof enabled rapid and confident detection of peptides and any corresponding catabolites.**

References

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2. Software-aided approach to investigate peptide structure and metabolic susceptibility of amide bonds in peptide drugs based on high resolution mass spectrometry. T. Radchenko *et al.*, PLOS

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