ROUTINE METABOLITE IDENTIFICATION FOR COMPLEX PEPTIDES BASED ON IMS ENABLED QTOF DIA DATA ACQUISITION AND MASS-METASITE DATA PROCESSING

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INTRODUCTION

In therapeutic peptide drug discovery and development, the inclusion of unnatural amino acids, backbone modifications, conjugations and cyclization are strategies employed to improve efficacy and ADME profiles. Metabolic fate of these potential drug candidates needs to be thoroughly and rapidly investigated to characterize their profiles. This requires information rich data acquisition modes coupled with tailored software tools that deal with the complexity of modified peptide LC-MS data effectively.

In this presentation, several complex cyclic peptides were screened for metabolism using an ion-mobility enabled HDMS^E acquisition workflow and processed using Mass-Metasite and WebMetabase macromolecule software packages.[1]

METHODS

Cyclic peptides, daptomycin, dalbavancin, oritavancin, anidulafungin, and lanreotide (figure 2) were incubated at 10 µM in simulated intestinal fluid (SIF) in the presence of 1000 µg/mL chymotrypsin using methodology previous described by Radchenko et al.[2] Incubates were sampled at 0 min, 5, 15, 120 and 300 min and quenched by adding 2 volumes of cold acetonitrile containing 1% formic acid.

Figure 1: Mass Metasite Batch Processor UNIFI Login Dialog and Browser

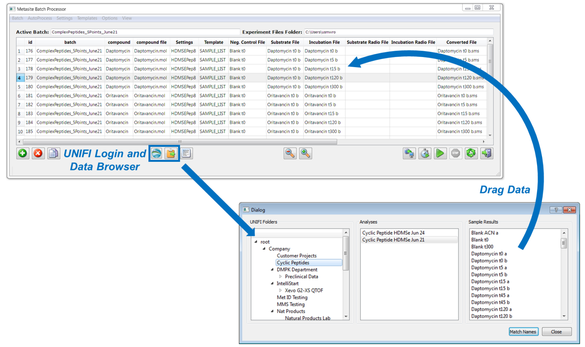
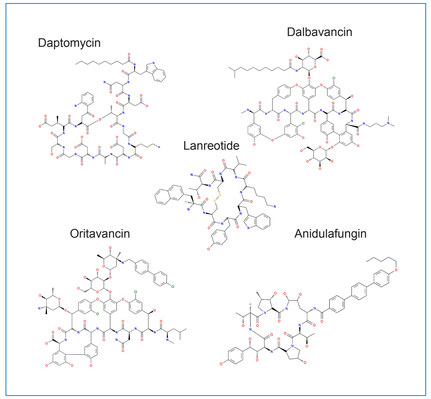


Figure 2: Structures of peptide drugs investigated



The LC system used was an ACQUITY H-Class UPLC equipped with an ACQUITY UPLC Peptide BEH C18 Column (300Å, 1.7 µm, 2.1 mm X 100 mm). A linear gradient from 5 to 40-70 B% in 8 minutes was used (mobile phase A: water + 0.1% formic acid, mobile phase B: acetonitrile + 0.1% formic acid (v/v)). Flow rate was 0.4 ml/min and column temperature was 60°C.

Data were acquired in UNIFI using HDMS^E (ion mobility enabled MS^E) on a Vion IMS QToF with intelligent data capture (IDC) enabled and data were processed using Mass-MetaSite and WebMetabase (Molecular Discovery Ltd). Figure 1 shows new links to UNIFI directly embedded in the Mass-Metasite browser (Version 3.4.2 x64 UNIFI 8 shown)

RESULTS AND DISCUSSION

Ion mobility enabled DIA acquisition generated CCS values and clean XICs for analysis. In addition, enabling IDC in data acquisition resulted in >70% reduced file size which streamlined data processing and the overall performance.[3] Extracted ion chromatograms (XIC) of parent peptides is shown in Figure 3. The benefit of IMS filtering is shown in Figure 4, where the –28 catabolite (2 x demethylation) of dalbavancin which has matrix and noise peaks automatically removed the automatic processing of HDMS^E data.

The turnover rate for peptides over 120 min were found to range from very low for anidulafungin, to medium for daptomycin. These results, along with CCS value ranges and the top three metabolites are summarized in Table 1. In almost all cases individual metabolites represented < 5% of parent, The turnover was generally found to be the result of many pathways.

Figure 3: Chromatography of 5 standards in SIF

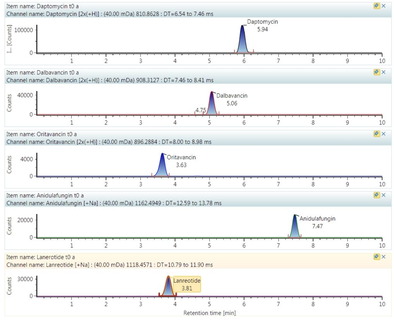
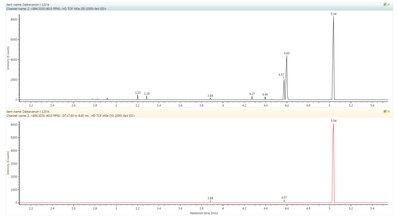


Figure 4: Dalbavancin Metabolite (-28 Da 2x demethylation) without (top) and with IMS (bottom) enabled data filtering



Daptomycin was further investigated in detail. WebMetabase cluster plots (response over time) and putative structures for the top 4 metabolites are shown in Figure 5. For substrate and the top 4 metabolites, RT, m/z, CCS ranges observed are also reported in figure 6. Mass error, MS area and area % are also summarized and provides multiple criteria to sort, filter and assess metabolites. Additional fragmentation information was used to produce scores and help rank proposed structures.

Mass-Metasite and WebMetabase were able to parse the diverse set of peptides and propose both expected and unexpected metabolites. Hydrolysis was the most common metabolite, but demethylation and also loss of unusual monomers, such as the chlorobiphenyl moiety of oritavancin were also detected.

Figure 5: Daptomycin major metabolites - response vs time plots in WebMetabase

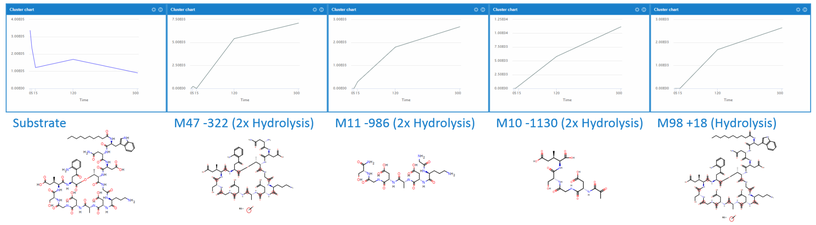


Figure 6: Daptomycin major metabolites - summaries in WebMetabase

Name	RT	m/z	CCS	Mass shift	m/z diff [ppm]	m/z diff [mDa]	MS Area	Area %	Ion formula	Max score	Uniqu…	Groups
Substrate/internal standard												
Substrate	5.93—5.95	810.8621—810.8641	404.544—407.72		-2.03—0.49	-1.64—0.39	3.37E05—9.03E04	67.75—24.92	$[C_{72}H_{92}N_{17}O_{26} + H]^{+2}$			
Known metabolites												
M47 -322 RT=5.79	5.79—5.80	649.7593—649.7615	355.115—358.106	-322.2045	-1.95—1.44	-1.27—0.93	2.34E02—7.11E03	0.07—1.96	$[C_{51}H_{70}N_{13}O_{20} + H]^{+2}$	0.0	Auto	
M11 -986 RT=2.29	2.29—2.31	634.2779—634.2808	236.127—236.805	-986.4386	-2.71—1.87	-1.72—1.19	2.91E02—2.66E03	0.16—0.74	$[C_{72}H_{89}N_{16}O_{23} + H]^{+}$	201.0	Auto	
M10 -1130 RT=2.23	2.23—2.25	491.1614—491.1638	197.218—197.998	-1,129.5556	-3.60—1.21	-1.77—0.60	4.89E02—1.12E04	0.27—3.09	$[C_{72}H_{89}N_{16}O_{23} + H]^{+}$	202.0	Auto	
M98 +18 RT=6.17	6.17—6.20	819.8661—819.8684	424.135—424.197	+18.0106	-0.83—2.01	-0.68—1.64	1.69E03—2.64E03	0.41—0.73	$[C_{72}H_{95}N_{17}O_{27} + H]^{+2}$	202.0	Auto	

Table 1: Summary of turnover, CCS of Substrate and major metabolites detected

Peptides	Turnover	Observed CCS	Major Metabolites Detected		
	t= 120 min	Range (Å^2)	1	2	3
Daptomycin	med	404.5-407.7	+18 (hydrolysis)	-332 (2x hydrolysis)	-986 (2x hydrolysis)
Dalbavancin	low	454.9-458.5	-14 (demethylation)	-14 (demethylation)	-28 (2x demethylation)
Oritavancin	low	488.9-491.3	-14 (demethylation)	+18 (hydrolysis)	-200 (-chlorobiphenyl)
Lanreotide	med	354.8-356.5	-179 (2x hydrolysis)	+18 (hydrolysis)	+18 (hydrolysis)
Anidulafungin	v.low	383.6-393.2	trace	trace	trace

Data were also acquired with new algorithms which further leverage HRMS and IMS to simultaneously denoise data and significantly decrease the file size.[3] File size reduction decreased data storage requirements, improved data quality and benefited processing, uploading and transfer times throughout the process.

CONCLUSIONS

Workflow was demonstrated using 5 FDA approved cyclic peptide drugs of varying non-natural structural complexity. Ion mobility afforded additional resolution that was shown to help further discriminate, characterize and resolve and track metabolites from matrix ions having similar *m/z* but significantly different ion mobility. Intelligent data compression, now directly implemented in raw data acquisition, afforded significant reduction of file sizes and denoising of the data.

Mass-Metasite or WebMetabase metabolite ID software was able to identify and elucidate key cyclic peptide metabolites using the macromolecule processing mode. Third party software is able to directly access HDMS^E data directly from the UNIFI software platform. Multiple sites of amide hydrolysis, demethylation or more complex cleavages could be determined using a single data processing software package which is also capable of analyzing small molecule drugs.

Daptomycin was further characterized to show the integrated response plots and proposed structure for the 4 major metabolites found. Proposed structures were consistent with known and expected pathways and provided a rapid workflow to find, review and plot the catabolism of peptides.

Comprehensive metabolite identification of complex cyclic peptides was achieved by combining IMS data with a powerful software solution tailored for macromolecules.

References

1. Wrona et al. *Somatostatin Analogue Catabolite Screening and Identification Using Vion IMS QTof with WebMetabase* 720006586EN
2. Radchenko et al. *Software-aided approach to investigate peptide structure and metabolic susceptibility of amide bonds in peptide drugs based on high resolution mass spectrometry*, PLOSOne 2017
3. Mortishire-Smith et al. *Intelligent Data Capture: Real-Time Noise Reduction for High Resolution Mass Spectrometry*. Waters White Paper LITR135023115

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