AUTOMATIZATION OF STRUCTURAL ELUCIDATION WORKFLOW FOR DETECTING DEGRADATION IMPURITIES IN PEPTIDES

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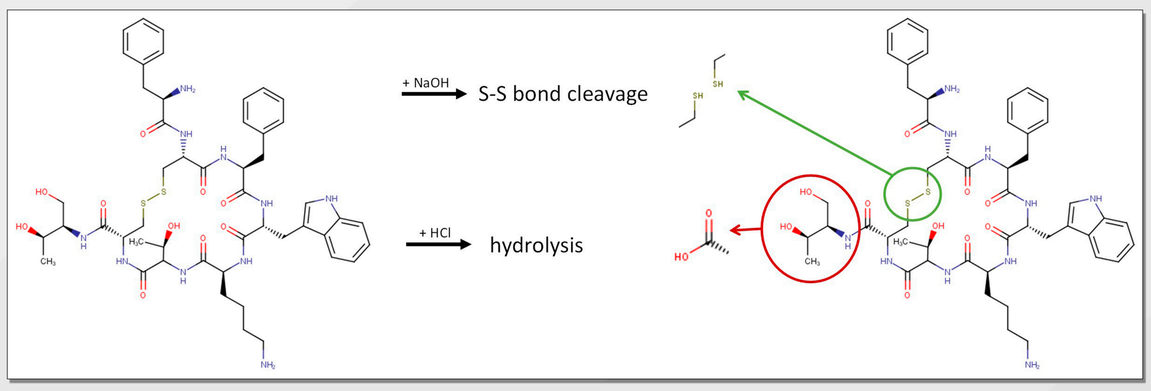
INTRODUCTION

Detection and identification of drug degradation impurities in drug products is important to the development of formulated drugs. Structures and formation mechanisms of degradation impurities need to be identified once the degradants exceed certain specified levels, as required for the regulatory guidelines. A rapid structure elucidation of those drug substance related impurities is essential to have a clear understanding of the quality of the new drug.

For this purpose, liquid chromatography-mass spectrometry (LC-MS) techniques are the most frequently used. However, the processing and rationalization of MS/MS data can be quite time consuming, especially in peptide studies due to their size and multiple charge. In this poster we present a fully automatic workflow for structural elucidation of degradation impurities in peptides implemented in **MassChemsite** (Molecular Discovery, Ltd., UK) program.



METHODS

Forced degradation studies

Forced degradation studies were conducted by mixing drug substance solution with force reagent solution of HCl and NaOH. Reaction mixture was incubated for a period of time at room temperature and 50ºC.

UPLC-HR-MS/MS

UPLC: Chromatographic analysis was conducted using an Acquity UPLC system. Mobile phases: 0.1% formic acid in water (solvent A) and acetonitrile (solvent B).

HR-MS/MS: experiments were performed on a Waters Synapt G1 qTOF HDMS mass spectrometer. MSe method was developed with low collision energy of 5eV as a channel one to detect intact molecules and ramping high collision energy of 20-40eV as channel two to obtain pseudo MS/MS data.

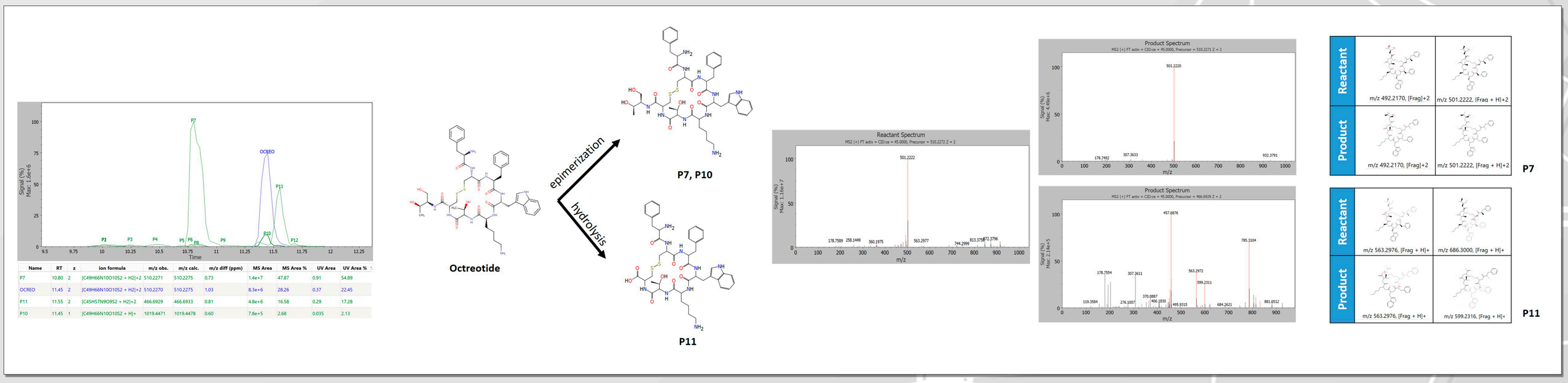
Data processing

Raw files from Synapt G1 instrument were automatically processed using MassChemsite 2.0 (Late Stage Derivatization workflow). Expected degradation reactions were taken into account.

RESULTS

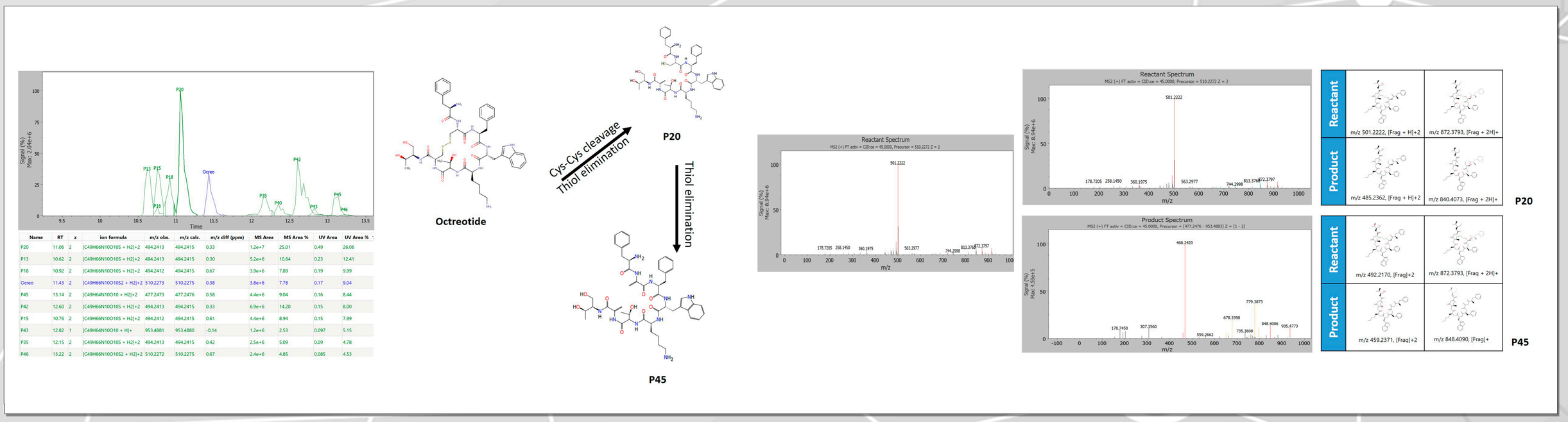
The workflow has been tested in a set of small peptides. As an example here we present Octreotide acid and basic forced degradation.

Acid degradation: there are two main degradants present in acid media: epimerization products (P7 and P10) and hydrolysis at the C-terminus (P11).



Name	RT	z	ion formula	m/z obs.	m/z calc.	m/z diff (ppm)	MS Area	MS Area %	UV Area	UV Area %
P7	10.80	2	[C49H66N10O10S2 + H2]+2	510.2271	510.2275	0.73	1.4e+7	47.87	0.91	54.89
OCREO	11.45	2	[C49H66N10O10S2 + H2]+2	510.2270	510.2275	1.03	8.3e+6	28.26	0.37	22.45
P11	11.55	2	[C45H57N9O9S2 + H2]+2	466.6929	466.6933	0.81	4.8e+6	16.58	0.29	17.28
P10	11.45	1	[C49H66N10O10S2 + H]+	1019.4471	1019.4478	0.60	7.8e+5	2.68	0.035	2.13

Basic degradation: the Cys-Cys bond was cleaved. Multiple products related to the disulphide hydrolysis and the elimination of SH groups were found.



Name	RT	z	ion formula	m/z obs.	m/z calc.	m/z diff (ppm)	MS Area	MS Area %	UV Area	UV Area %
P20	11.06	2	[C49H66N10O10S + H2]+2	494.2413	494.2415	0.33	1.2e+7	25.01	0.49	26.06
P13	10.62	2	[C49H66N10O10S + H2]+2	494.2413	494.2415	0.30	5.2e+6	10.64	0.23	12.41
P18	10.92	2	[C49H66N10O10S + H2]+2	494.2412	494.2415	0.67	3.9e+6	7.89	0.19	9.99
Ocreo	11.43	2	[C49H66N10O10S2 + H2]+2	510.2273	510.2275	0.38	3.8e+6	7.78	0.17	9.04
P45	13.14	2	[C49H64N10O10 + H2]+2	477.2473	477.2476	0.58	4.4e+6	9.04	0.16	8.44
P42	12.60	2	[C49H66N10O10S + H2]+2	494.2413	494.2415	0.33	6.9e+6	14.20	0.15	8.00
P15	10.76	2	[C49H66N10O10S + H2]+2	494.2412	494.2415	0.61	4.4e+6	8.94	0.15	7.99
P43	12.82	1	[C49H64N10O10 + H]+	953.4881	953.4880	-0.14	1.2e+6	2.53	0.097	5.15
P35	12.15	2	[C49H66N10O10S + H2]+2	494.2413	494.2415	0.42	2.5e+6	5.09	0.09	4.78
P46	13.22	2	[C49H66N10O10S2 + H2]+2	510.2272	510.2275	0.67	2.4e+6	4.85	0.085	4.53

CONCLUSIONS

In this poster we presented an example of the usability of MassChemsite for peptide degradant detection and identification.

The utility of MassChemsite to improve the efficiency of structural elucidation of small peptide degradants has been demonstrated in two ways: an initial detection of the LC degradant peaks and a later mass/mass spectra extraction to perform the final structure assignment.