Waters

Development and Evaluation of a LC/UV/MS Peptide Mapping System

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Introduction

Characterization and quality control of biotherapeutics relies heavily on the use of peptide mapping. Peptide mapping is used to determine small chemical variations in proteins that can be the difference between active and inactive protein therapeutics. Historically, this technique has been based on UV detection and TFA mobile phase modifiers. Recently, peptide mapping has implemented mass spectrometry to complement traditional UV detection. In order to improve performance of mass spectrometry, it is highly desirable to replace TFA with formic acid as the mobile phase modifier. Waters has therefore developed coumns specific to improve resolution with formic acid modified mobile phases. In addition, we developed highly robust, reproducible peptide mapping systems that are designed both for UV and mass spectrometry detection. Data presented here will show the reproducibility of these peptide mapping systems as well as their capabilities in detecting modifications of IgG1 such as oxidation, deamidation and lysine variants.

Methods and Materials

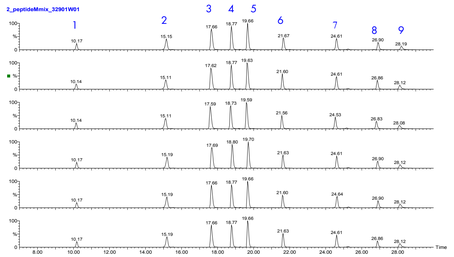
BioSuite™ Peptide Mapping System
BioSuite™ Peptide Mapping MS System
BioSuite™ Peptide Mapping MS/MS System
MassPREP™ peptide standard

Column:	BioSuite™ PA-A C_{18} 3µm 2.1 x 150 mm unless specified	
Column temperature:	40.0°C, unless specified	
Flow rate:	0.2 mL/min	
Mobile phase:	A: 0.1% formic acid unless specified	
	B: 0.1%formic acid/MeCN unless specified	
Gradient elution	see figure legends	
MS conditions:	Mode:	ESI +
	Capillary:	3300 V
	Cone:	30 V
	Desolvation gas flow:	500 L/hr
	Cone gas flow:	50 L/hr
	Source temperature:	150 °C
	Desolvation temperature:	350 °C
MS/MS conditions:	Mode:	ESI +
	Capillary:	3300 V
	Cone:	35 V
	Desolvation gas flow:	500 L/hr
	Cone gas flow:	50 L/hr
	Source temperature:	150 °C
	Desolvation temperature:	350 °C
	Collision cell:	27eV

Sample preparation:
IgG1 was obtained from mouse acities and purified using a protein G column (Shodex). The purified IgG1 was concentrated by TCA precipitation and resuspended in ammonium bicarbonate buffer, pH 7.8, and digested overnight with trypsin (Promega) at 37.0°C

MassPREP peptide was dissolved in 150µL of Mobile phase A and mixed well

Figure 1: BioSuite peptide mapping system consisting of the 2796 Bioseparations module, MassLynx™ software, Waters 2487 dual wavelength detector, BioSuite PA columns and MassPrep standards



	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9
Avg	10.16	15.16	17.65	18.77	19.65	21.62	24.60	26.88	28.13
%RSD	0.15	0.26	0.20	0.12	0.19	0.17	0.15	0.11	0.13

Figure 2: Separation reproducibility of MassPREP peptide standard on BioSuite Peptide Mapping System. Mobile phase A: 0.02%TFA, mobile phase B 0.018%TFAin MeCN Tg= 0-50%B/30 min

Figure 3: BioSuite peptide mapping MS system consisting of the 2796 Bioseparations module, MassLynx™ software, Waters 2487 dual wavelength detector, the Waters Micromass® ZQ mass spectrometer , BioSuite PA columns and MassPrep standards

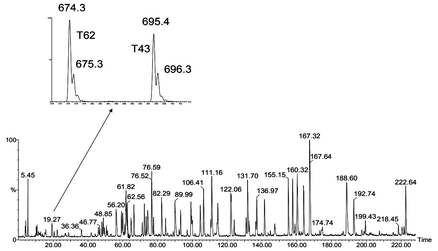
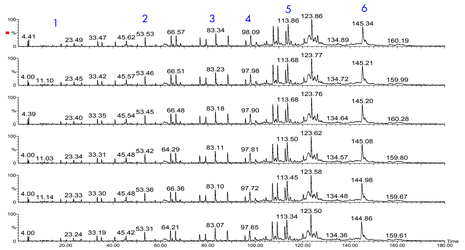


Figure 4: Peptide map of human serum albumin demonstrating the BioSuite Peptide Mapping MS System's ability to resolve co-eluting peptide. Tg= 0-40% B/225 min



Peak#	1	2	3	4	5	6
Avg	17.91	53.42	83.17	97.86	123.68	145.11
%RSD	0.42	0.13	0.11	0.15	0.10	0.11

Figure 5: Separation reproducibility of MAb tryptic digest on BioSuite Peptide Mapping MS Sytem. Tg= 0-40%B/180 min on BioSuite PA-B C_{18} 3.5µm 2.1 x 250mm mm

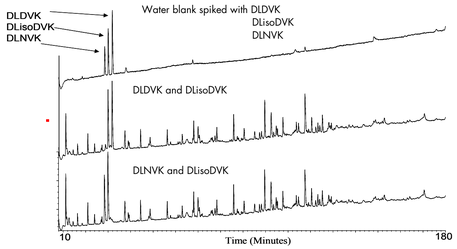


Figure 6: Analysis and identification of spiked deamidation products in a peptide map of IgG1 on BioSuite Peptide Mapping MS System. Tg=0-40%B/180 min

Figure 6. BioSuite peptide mapping system consisting of the 2796 Bioseparations module, MassLynx™ software, Waters 2487 dual wavelength detector, BioSuite™ PA columns and MassPrep™ standards

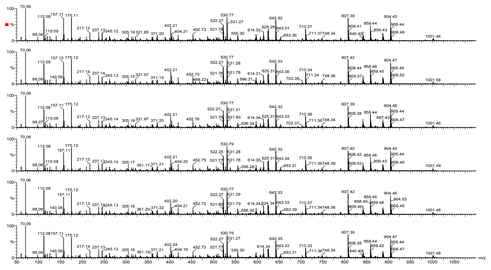


Figure 7: Fragmentation reproducibility of Bradykinin (doubly charged parent ion 530.79) on BioSuite Peptide Mapping MS/MS System.

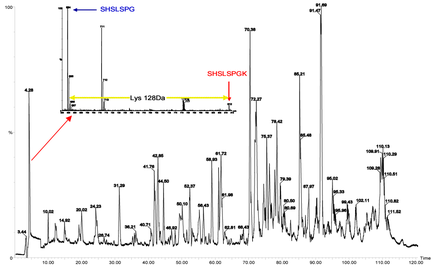


Figure 8: Identification of an early eluting peptide sequence corresponding to a lysine variant. Tg = 0-40%B/120 min

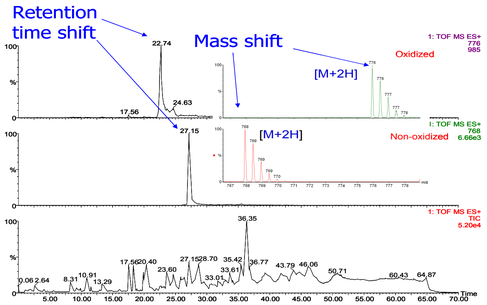


Figure 9: Separation and identification of oxidized T19 of IgG1

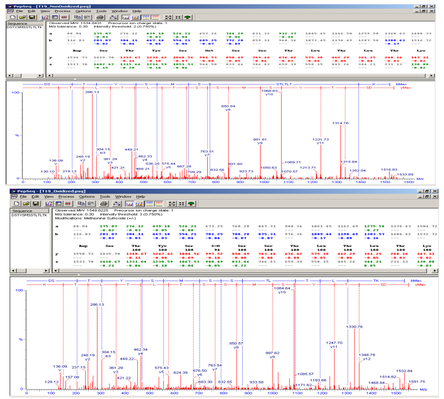


Figure 10: Identification and de novo sequencing of T19 and oxidized T19 of IgG1 demonstrating peak identification and modification location by MS/MS sequencing.

Conclusions

- BioSuite™ Peptide mapping systems and BioSuite™ columns showed excellent reproducibility in separating peptides of a IgG1 tryptic digest.
- Detection and identification of post translational modifications was accomplished by LC/MS.
- Site specific modification of amino acids was determined by Biolynx™ software following fragmentation experiments.