High Performance Routine Characterization of Proteins and Peptides by LC/MS

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ABSTRACT

All too often high performance combined with routine analysis methods result in a compromise in system performance. This work will illustrate how one can obtain high-performance in a routine manner without compromises. This LC/MS system and methods will be demonstrated with intact proteins and peptide maps from antibodies and recombinant proteins. The value to a work-flow of an optimized LC, columns, MS, and software for data analysis will also be presented.

INTODUCTION

Comprehensive characterization of biopolymers as drug candidates is a requirement for safety and regulatory agencies. LC/MS of proteins and peptides is a powerful method for characterization. The ability to reproducibly generate and interpret high quality LC/MS data in a timely manner is challenging. This is often hindered with the lack of tools addressing a complete work flow. This poster outlines a complete set of work flow tools for time efficient high performance LC/MS characterization of proteins and peptides.

Figure 1. Waters biopharmaceutical LC/MS system: ACQUITY UPLC® LC and Xevo™ QTof MS

METHODS

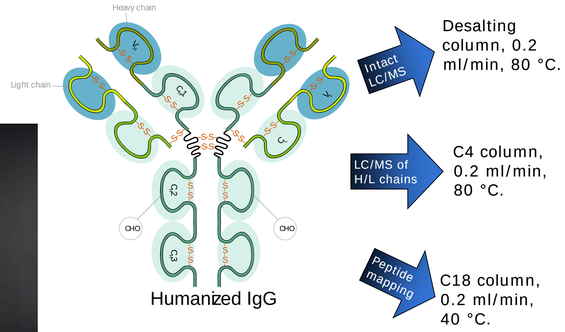
Samples:
–Humanized IgG (intact and reduced)
–RapiGest SF™ aided Trypsin digested IgG (Peptide mapping)

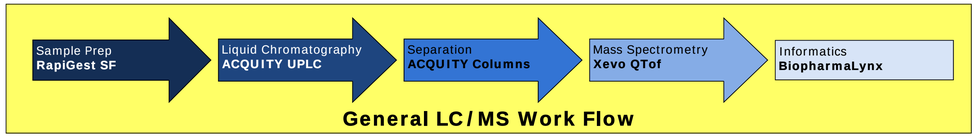
Instrumentation:
–Liquid chromatography, ACQUITY UPLC®
–Mass spectrometer, Xevo™ QTof

Columns:
–Intact IgG, MassPREP™ Micro desalting column polymeric 20µm particle size 1000Å pore size, 2.1×5 mm
–Separation of IgG heavy/light chains, Protein Separation Technology, ACQUITY UPLC® C4 BEH300, 1.7µm, 2.1×50 mm
–Peptide mapping, Peptide Separation Technology, ACQUITY UPLC® C18 BEH300, 1.7µm, 2.1×150 mm

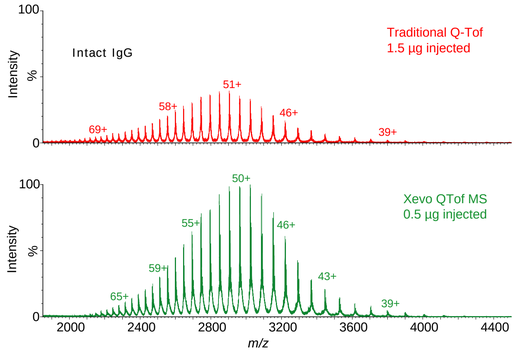
Informatics:
–BiopharmaLynx™ ver 1.2 application manager

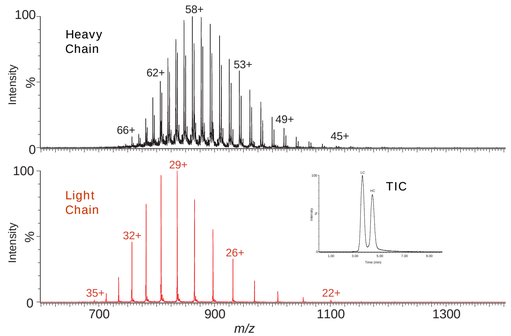
EXPERIMENTAL



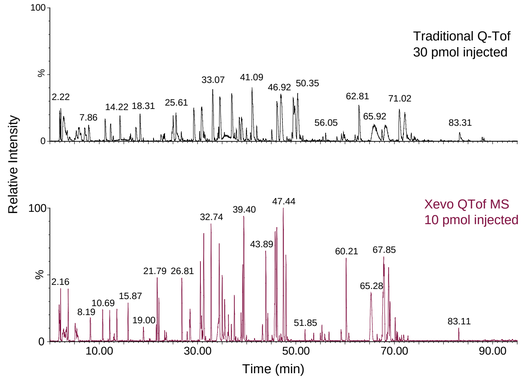


INTACT LC/MS of IgG





PEPTIDE MAP



Protein	Peptide	Fragment Number	Modifiers	Control b/y Found	Control b/y List
humanized mab	DIQMTQSPSSLSASV	1:T001		28	1/b2;1/b3;1/b4;1/b5;1/b6;1/b7;1/b9;1/b10;1/b11;1/b12;1/b13;1/b14;1/b15
humanized mab	DIQMTQSPSSLSASV	1:T001*	decom of carbamid	9	1/b2;1/b7*;1/b10*;1/y3;1/y4;1/y5;1/y6;1/y7;1/y8
humanized mab	DIQMTQSPSSLSASV	1:T001-002*	Carbamidomethyl C(1)		
humanized mab	VTITCR	1:T002*	Carbamidomethyl C	4	1/b2;1/y1;1/y2*;1/y3*
humanized mab	VTITCRASQDVNTAV	1:T002-003*	Carbamidomethyl C(1)		
humanized mab	ASQDVNTAVAWYQQ	1:T003		22	1/b2;1/b3;1/b4;1/b5;1/b18;1/y1;1/y2;1/y3;1/y4;1/y5;1/y6;1/y7;1/y8;1/y9;
humanized mab	ASQDVNTAVAWYQQ	1:T003*	Deamidation N(1)	14	1/b2;1/b4;1/b18*;1/y2;1/y3;1/y4;1/y6;1/y7;1/y8;1/y9;1/y13*;1/y14*;1/y15
humanized mab	ASQDVNTAVAWYQQ	1:T003-004			
humanized mab	APK	1:T004			
humanized mab	APKLLIYSASFLYSG	1:T004-005			
humanized mab	LLIYSASFLYSGVPSF	1:T005		14	1/b2;1/b3;1/b9;1/b13;1/y3;1/y5;1/y6;1/y7;1/y8;1/y9;1/y10;1/y11;1/y12;1
humanized mab	LLIYSASFLYSGVPSF	1:T005*	carbamidomethy Y(	0	
humanized mab	LLIYSASFLYSGVPSF	1:T005-006			
humanized mab	FSGSR	1:T006		4	1/b5;1/y1;1/y3;1/y4
humanized mab	FSGSRSGTDFTLTISS	1:T006-007*	Carbamidomethyl C(1)		
humanized mab	SGSR	1:T006/y4		2	1/y1;1/y3
humanized mab	SGTDFTLTISSLQPEC	1:T007*	Carbamidomethyl C	24	1/b4;1/b5;1/b6;1/b7;1/b8;1/b9;1/b10;1/b11;1/b12;1/b13;1/b28*;1/b37*;1/
humanized mab	SGTDFTLTISSLQPEC	1:T007-008*	Carbamidomethyl C(1)		
humanized mab	VEIK	1:T008		4	1/b2;1/y1;1/y2;1/y3
humanized mab	VEIKR	1:T008-009			
humanized mab	R	1:T009			
humanized mab	RTVAAPSVFIFPPSDE	1:T009-010			
humanized mab	TVAAPSVFIFPPSDE	1:T010		16	1/b2;1/b3;1/b4;1/b6;1/b8;1/b9;1/b10;1/y7;1/y8;1/y9;1/y10;1/y11;1/y12;1
humanized mab	TVAAPSVFIFPPSDE	1:T010-011*	Carbamidomethyl C(1)		
humanized mab	TVAAPSVFIF	1:T010/b10		6	1/b2;1/b3;1/b4;1/b6;1/b8;1/b9
humanized mab	PPSDEQLK	1:T010/y8		1	1/y7
humanized mab	SGTASVVCLLNNFYP	1:T011*	Carbamidomethyl C	25	1/b2;1/b3;1/b4;1/b5;1/b6;1/b7;1/b8*;1/b9*;1/b10*;1/b11*;1/b12*;1/b13*;1
humanized mab	SGTASVVCLLNNFYP	1:T011*	Deamidation N(1),C	10	1/b14*;1/y2;1/y3;1/y4;1/y5;1/y6*;1/y7*;1/y8*;1/y9*;1/y10*
humanized mab	SGTASVVCLLNNFYP	1:T011*/b16*	Carbamidomethyl C	13	1/b2;1/b3;1/b4;1/b5;1/b6;1/b7;1/b8*;1/b9*;1/b10*;1/b11*;1/b12*;1/b13*;1

Table 1
Results of BiopharmaLynx processed Xevo QTof data posing MS and MS/MS (MS^E) data. Fragment ions (b/y ions) are identified and listed in the Peak Match Data Table.

CONCLUSION

–High performance routine characterization of intact proteins and peptide maps
–No compromise LC/MS performance
–*Featuring*
  –Waters ACQUITY UPLC® LC System
  –ACQUITY UPLC Columns (1.7µm particles)
  –Xevo™ QTof Mass Spectrometer
  –BiopharmaLynx™ informatics
–Ideal for characterization of:
  –Intact Proteins
  –Peptide Mapping
  –Oligonucleotides
–Advance characterization of peptides with MS^E enabled with BiopharmaLynx
–Complete solution (work flow) tightly integrated

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