Enabling a Multi-Attribute Method Comparison of Infliximab

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Introduction

The pace of biosimilar development and pressures for rapid commercialization have led to the widespread adoption of mass spectrometry to support or disprove similarity to an innovator product. Analysts routinely use a wide array of biophysical and biochemical methods to characterize protein samples. As mass spectrometry development has progressed, the ability to have "multi-attribute methods" to efficiently report the quality attributes of a well-characterized biotherapeutic product has become more realistic. It is only possible, however, with deep data mining of peptide maps and other data. In order to maximize the amount of information available and report it automatically, human intervention is at a premium. Although the development of mass spectrometer based methods are rapid, the tools needed to process the explosion of data are in short supply. Total and efficient analysis with the Protein Metrics suite of software reduces the human burden.

Methods

Four commercial lots of Remicade (Janssen) and Remsima (Celltrion) were sourced from the University of Michigan Hospital Pharmacy and South Korea, respectively.
Samples were denatured, reduced, alkylated and digested with Promega's low pH typsin kit (Promega, CAS # CS1895A01). Digested samples were analyzed with an Orbitrap Q Exactive mass spectrometer (Thermo Fisher).

Raw MS/MS data were analyzed with Byonic™, Byologic® and , Intact Mass™, software packages (Protein Metrics). Samples were quantitatively compared with pivot summary reports specifically formatted for MAM.

Data analysis workflow:

LC-MS/MS data

Sequence FASTA file

Byonic™ Protein Metrics Inc.

Search engine
Comprehensive identifications

MS2 Identifications

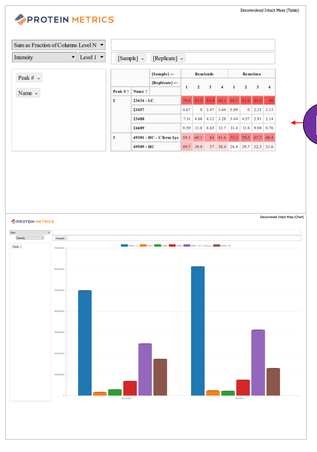
Byologic® Protein Metrics Inc.

Inspection and quantification
Peptide-Centric sensitive PTM analysis

Report

Data Analysis & Reporting – Intact & Sub-unit Level

LC-MS/MS data file(s) on reduced samples



D

[Sample] [Replicate]

Peak #	Name	[Sample]	Remicade				Remsima			
		[Replicate]	1	2	3	4	1	2	3	4
3	49381 - HC - CTerm Lys		50.3	60.1	63	61.6	73.1	70.3	67.7	68.4
	49509 - HC		49.7	39.9	37	38.4	26.9	29.7	32.3	31.6

Observed increase in C-term Lys truncation in Remsima

Remicade 59±6%

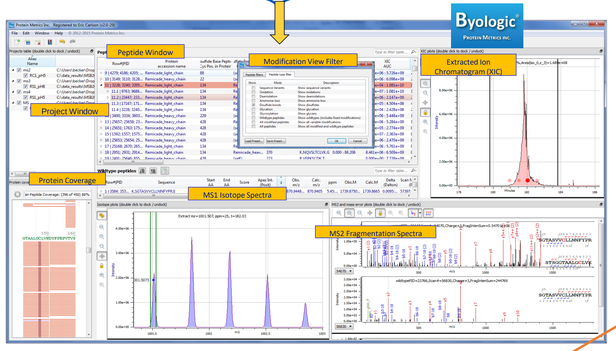
vs

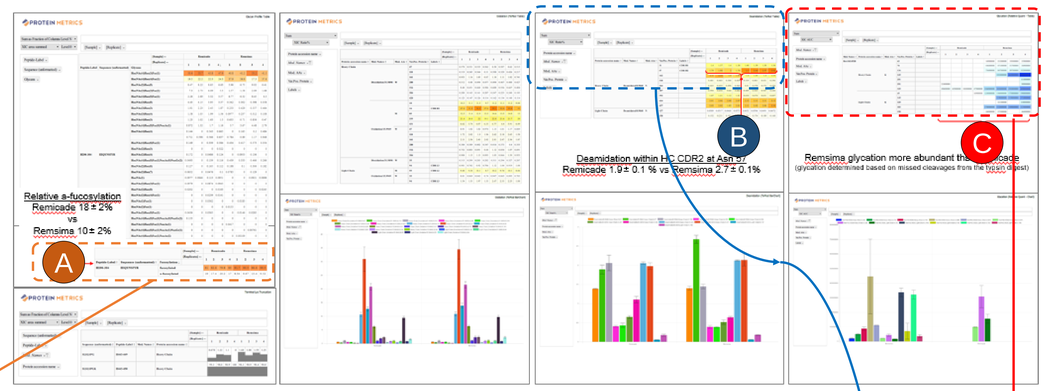
Remsima 70±2%

Data Analysis & Reporting – Peptide Level

Sequence file(s)

LC-MS/MS data file(s) on digest samples





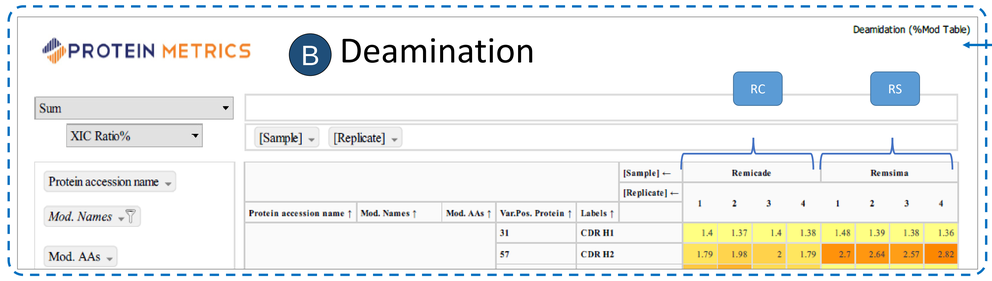
Observed Differences

A Glycosylation

Peptide-Label	Sequence (unformatted)	Fucosylation	[Sample]	Remicade				Remsima			
			[Replicate]	1	2	3	4	1	2	3	4
H296-304	EEQYNSTYR	fucosylated		81	82.6	79.8	83	91.7	90.3	86.6	90.5
		a-fucosylated		19	17.4	20.2	17	8.34	9.67	13.4	9.52

Relative a-fucosylation
Remicade 18±2% vs *Remsima* 10±2%

B Deamination

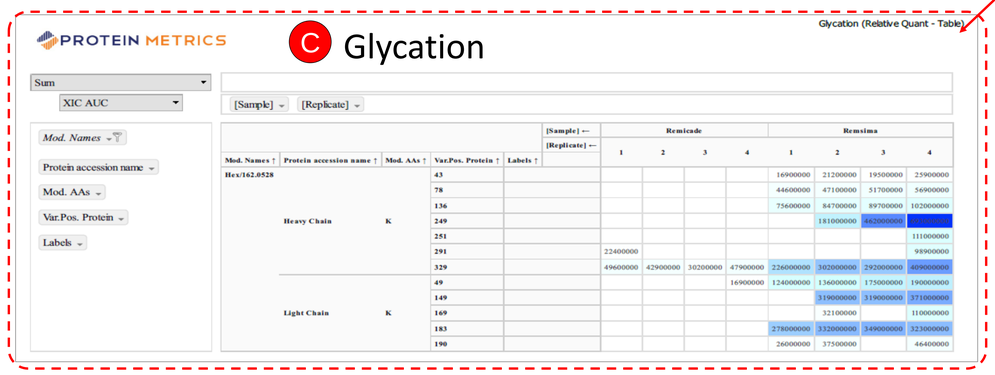


Protein accession name	Mod. Names	Mod. AAs	Var.Pos. Protein	Labels	[Sample]	Remicade				Remsima			
					[Replicate]	1	2	3	4	1	2	3	4
			31	CDR H1		1.4	1.37	1.4	1.38	1.48	1.39	1.38	1.36
			57	CDR H2		1.79	1.98	2	1.79	2.7	2.64	2.57	2.82

Deamidation within HC CDR2 at Asn 57

Remicade 1.9±0.1 % vs Remsima 2.7±0.1%

C Glycation



Mod. Names	Protein accession name	Mod. AAs	Var.Pos. Protein	Labels	[Sample]	Remicade				Remsima			
					[Replicate]	1	2	3	4	1	2	3	4
Hex/162.0528			43							16900000	21200000	19500000	25900000
			78							44600000	47100000	51700000	56900000
			136							75600000	84700000	89700000	102000000
	Heavy Chain	K	249								181000000	462000000	503000000
			251										111000000
			291			22400000							98900000
			329			49600000	42900000	30200000	47900000	226000000	302000000	292000000	409000000
			49						16900000	124000000	136000000	175000000	190000000
	Light Chain	K	149								319000000	319000000	371000000
			169								32100000		110000000
			183							278000000	332000000	349000000	323000000
			190							26000000	37500000		46400000

Remsima glycation more abundant that Remicade
(glycation determined based on missed cleavages from the typsin digest)

Published Differences

Property	Remicade	Remsima
Sequence	Identical	Identical
TNF-a binding	Identical	Identical
FcgR-IIIa binding	Strong	Lower
ADCC assay	Strong	Lower
N-Glycan (Asn 300)	G0F, G1F, G2F, Man5	G0F, G1F, G2F
C-terminal lysine		More truncation

Discussion and Conclusions

The commercial lots of Remicade (originator) and Remsima (biosimilar) show substantial similarity when compared for a list of variants, PTMs, and overall intact mass of major species.

This multi-attribute comparison does reveal some analytical differences between the samples. Using this multi-attribute method (MAM), we were able to efficiently highlight the differences in deamidation in the HC CDR2 region, relative glycation, C-terminal Lys truncation, and relative a-fucosylation of the glycan profile. (See notes **A → D**.)

As interest in MAM increases, it is essential that the historically separate strands of information are pulled together. Here, we present a method that copes not only with complex data from peptide mapping, which has been a focus of MAM, but also with the intact mass information, which provides identity and reports on major isoforms.

Reporting data, whether for MAM methods, or for process samples, or for assay development, relies on bringing together data sets from different sources and with varying skill sets. To scale this for consistent business processes, this requires a consistent platform, like the suite of software shown here. The Protein Metrics Biopharma Software Suite facilitates multi-attribute methods analysis with a platform able to compare across samples and produce reports that summarize PTM and peptide level variants and as well as Intact Mass reporting.

Acknowledgment

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