INNOVATOR AND BIOSIMILAR INFLIXIMAB: COMPARABILITY ASSESSMENT OF THE HOST CELL PROTEINS AND PROTEIN HIGHER ORDER STRUCTURE

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INTRODUCTION

- **HCP** and **HOS** comparability analysis between an innovator and a biosimilar mAbs (infliximab and inflectra).
- Both studies were carried out using a micro flow ACQUITY UPLC system interfaced to a high resolution mass spectrometry (Synapt HDMS).
- Two HCPs (epidermal growth-factor like protein 8 and WD repeat containing protein 37) were found in both the innovator and the biosimilar mAbs using our HCP platform.
- Protein tertiary structure analysis was conducted by Hydrogen Deuterium Exchange Mass Spectrometry (HDX MS). Results showed the conformation for both mAbs are highly comparable except a minute difference in CH2 domain.

Host Cell Proteins Analysis

METHODS

Sample preparation:
Two high-purity therapeutic monoclonal antibodies (mAbs), expressed in murine cell cultures, an innovator (**Infliximab**, 21 mg/mL) and its biosimilar (**Inflectra**, 10 mg/mL) were analyzed using a recently developed 2D-LC/HDMS^E assay.

2D-LC configuration

An M-class ACQUITY^{TM} UPLC® system with 2D technology was used for peptide separations. A reversed-phase/reversed-phase (RP/RP) 2DLC method, using the pH of the mobile phases to change the selectivity of peptide separations in two separate dimensions, was developed:

- **First Dimension (1D) pH=10**: 1.0 mm x 50 mm XBridge C_{18} column (5 µm particles), 10 µL/min flow. Mobile phase: 20 mM ammonium formate in water (Solvent A) and ACN (Solvent B).
- **Online dilution (**1:10) of the eluent from 1D before analyte trapping onto the trap column.
- **Trap column**: 0.3 x50 mm packed with 5-µm Symmetry C_{18} particles.
- **Second Dimension (2D) pH=2.4**: 0.3 mm x 150 mm analytical column CSH C_{18} 1.7 µm, kept at 60 °C and operated at 10 µL/min. Fractions were eluted in ten steps (Each step was mixed in a 1:10 ratio with 0.1% TFA in water (pH=2.1) before trapping.

MS and data processing

Data independent, alternate scanning 2D-LC/HDMS^E experiments were performed on a SYNAPT G2-S mass spectrometer:

- Acquisition time was 0.5 sec, m/z range: 100-1,990 amu.
- Fixed CE at 5 V for low-energy MS scans; drift-time specific CEs were applied for the high-energy scans
- For the IMS separations a fixed wave velocity (650 m/s) and a fixed wave height (40 V) were employed
- Data Processing: ProteinLynx Global Server (PLGS) 3.0.2.

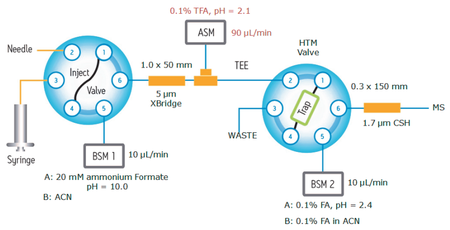

Figure 1. Fluidic configuration for the two-dimensional high pH/ low pH RP/RP chromatographic setup employing on-line dilution. Fractions were eluted in ten steps (1: 10.8% , 2: 12.4%, 3: 14.0%, 4: 15.4%, 5: 16.7%, 6: 18.6%, 7: 20.4%, 8: 25.0%, 9: 30.0%, 10: 50.0% Eluent B). Each step was mixed in a 1:10 ratio with 0.1% TFA in water (pH=2.1) before trapping. Low pH separations in the second chromatographic dimension used a 40 min gradient from 3 to 40% acetonitrile (0.1% FA-formic acid).

RESULTS

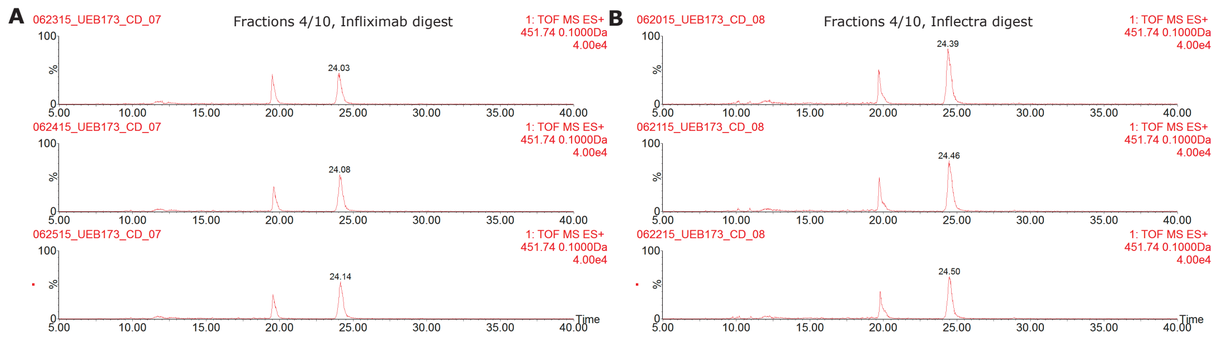

Figure 2. Reproducibility of the 2D-LC chromatographic system: (A,B) Extracted mass chromatograms generated for the monoisotopic peak of a low-abundance HCP peptide detected in both mAbs: peptide WEVAELR (m/z = 451.74, +2) from epidermal growth-factor like protein 8, eluting in Fraction 4/10.

INFLIXIMAB

No crt	Accesssion Number	Protein Description	Sequence Coverage (%)	Average MW (kDa)	Amount on column fmoles	Amount on column ng	Concentration ng/mL	Concentration ppm	RSD (%)
1	P00489	Glycogen phosphorylase rabbit (PHO) - 1000 fmoles	41.2	97.1	1000	97	1942	92	0.0
2	P00330	Alcohol dehydrogenase yeast (ADH) - 5000 fmoles	44.6	36.7	1981	73	1454	69	3.2
3	P02769	Bovine serum albumin (BSA) - 250 fmoles	21.9	66.3	296	20	392	19	10.3
4	Q6GUQ1	Epidermal growth factor-like protein 8	8.2	33.3	792	26	527	25	14.2
5	Q8CBE3	WD repeat-containing protein 37	4.8	55.1	288	16	317	15	23.1
6	P00924	Enolase 1 yeast (ENL) - 50 fmoles	15.7	46.6	102	5	95	5	10.8
					Total	ng/mL	845		
					Total	ppm		40	
					mAb	purity		99.996%	

INFLECTRA

No crt	Accesssion Number	Protein Description	Sequence Coverage (%)	Average MW (kDa)	Amount on column fmoles	Amount on column ng	Concentration ng/mL	Concentration ppm	RSD (%)
1	P00489	Glycogen phosphorylase rabbit (PHO) - 1000 fmoles	35.7	97.1	1000	97	3884	388	0.0
2	P00330	Alcohol dehydrogenase yeast (ADH) - 5000 fmoles	40.7	36.7	2073	76	3043	304	4.5
3	Q6GUQ1	Epidermal growth factor-like protein 8	8.2	33.3	687	23	915	92	18.9
4	P02769	Bovine serum albumin (BSA) - 250 fmoles	19.1	66.3	294	19	780	78	11.1
5	Q8CBE3	WD repeat-containing protein 37	4.8	55.1	142	8	313	31	35.0
6	P00924	Enolase 1 yeast (ENL) - 50 fmoles	18.3	46.6	58	3	108	11	26.5
					Total	ng/mL	1228		
					Total	ppm		123	
					mAb	purity		99.988%	

Table 1. Comparison of HCPs identified and quantified in Infliximab and Inflectra. The same 2 HCPs were detected in both samples: epidermal growth-factor like protein 8 and WD repeat containing protein 37.

HOS analysis by HDX MS

METHODS

Sample Preparation

Both innovator (3 biological lots) and biosimilar (1 biological lot) were treated equally during all analysis. The labeling reaction was initialized by adding 15-fold of phosphate buffer (pH 6.8) to protein stock (~14 µM). After variable reaction times (30 sec, 1 min, 10 min, 60 min and 240 min), the labeling reaction was quenched by adding pre-chilled quenching buffer with TCEP and GdnHCl. All the sample preparation were operated by HDX-2 Automation utilizing PAL RTC Robotics and timing was scheduled by Chronos (LEAP).

LC condition

Quenched solution flew through Enzymate^{TM} column (Waters, 2.1 x 30 mm, 130 Å, 5 µm) to complete online digestion and then went to cold chamber inside of HDX manager for separation. Analytical column was ACQUITY UPLC BEH C18 column, 1.7 µm 1.0 x 100 mm. The trap column was an ACQUITY VanGuard column, BEH 18 1.7 µm 2.1 x 5 mm.

MS and data processing

- Data were collected by Waters Synapt G2S HDMS instrument.
- Undeuterated control was processed using PLGS 3.0.2 for peptide identification. DynamX 3.0 was used to measure the deuterium uptake of each peptides and generate all visualization graphs.

ACQUITY UPLC® M-Class System with HDX Technology and HDX-2 Automation

DynamX 3.0: Industry leading HDX MS informatics for automated processing of global (Intact), local (peptide), and residue (AA, ETD) levels of HDX MS data.

RESULTS

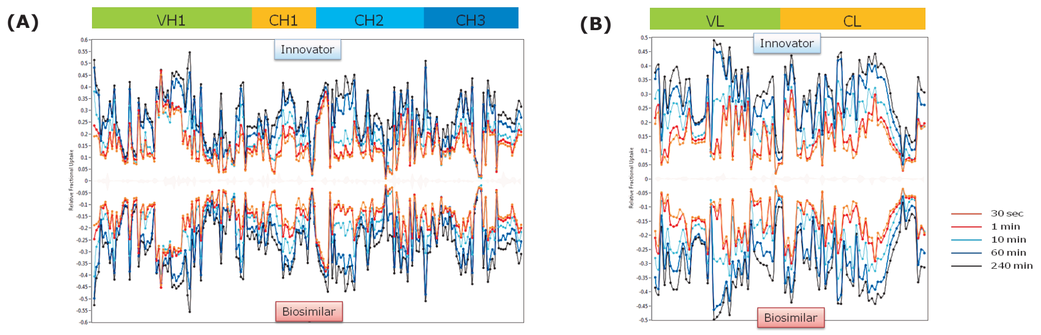

Figure 3. Butterfly plot of (A) heavy chain and (B) light chain.
One batch of the innovator sample was randomly chosen and compared with biosimilar sample in relatively fractional uptake. Each point represents a peptide. No back-exchange correction was applied.

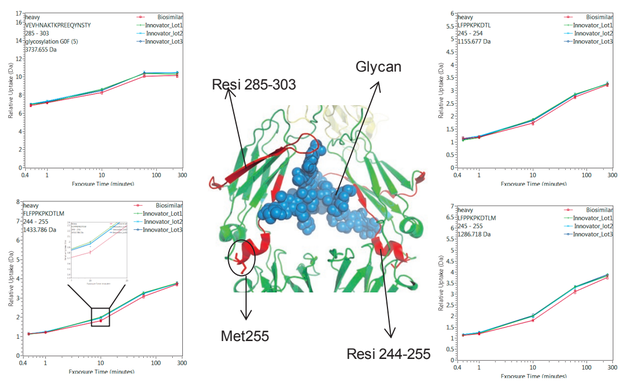

Figure 4. Comparison of deuterium incorporation of innovator and biosimilar samples. Representative deuterium incorporation profiles of regions (residues 244-255, 245-254, 245-255, 285-303) in CH2 domains show minute difference. The red line represents the data from biosimilar product, the green, cyan and blue lines represent the data from the three batches of innovator samples. The experiments have been repeated in triplicate runs. The location of the region displayed minor difference among biosimilar and innovator sample is colored in red in the model structure of IgG1 (PDB: 1HZH). Glycosylation is shown in blue. Met255 is circled and shown in stick.

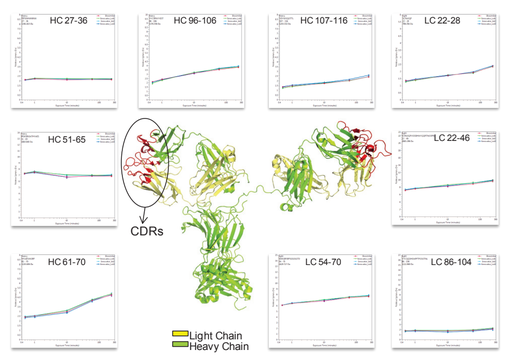

Figure 5. Representative peptides covered the complementarily determining regions (CDRs) of inflixmimab displayed identical conformation and dynamics. The heavy chain and light chain structures are colored in the 3D model of IgG1 (PDB: 1HZH) in green and yellow, respectively. The three light and heavy chain CDRs are colored in red. The deuterium incorporation curves of the sample peptides, which covered all the CDRs, are showed.

CONCLUSION

- HCP study showed that the biosimilar mAb has higher level of HCP compare to the innovator (2-4 fold higher). Two HCPs (epidermal growth-factor like protein 8 and WD repeat containing protein 37) were found in both the innovator and the biosimilar.
- HDX MS study showed the conformation for both mAbs are highly comparable except a minute difference on CH2 domain.
- Our study shows great promise in adapting these analytical capabilities into biosimilar drug development process.

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