Altasciences CLINICAL RESEARCH ALGORITHME PHARMA

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Quantitative Bioanalysis of Rituximab and Reditux for Biosimilarity Assessment: Comparing Triple Quadrupole and Hybrid Time-of-Flight Platforms

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OVERVIEW

PURPOSE

Comparison of triple quadrupole (QQQ) and hybrid time-of-flight (Q-TOF) platforms for quantitative biosimilarity assessment.

METHOD

Ritixumab and Reditux were spiked in human plasma and extracted using the pellet digestion approach.

Two signature peptides were used for quantitation, one from the light chain (LC) and one from the heavy chain (HC). Corresponding SIL-peptides were used as internal standards.

Extracts were analyzed with QQQ and Q-TOF mass spectrometers.

RESULTS

Determination of the HC peptide from Rituximab and Reditux was precise and accurate on both platforms from 1.00 – 400 µg/mL.

For the LC peptide, quantitation on the Q-TOF was also possible from 1.00 – 400 µg/mL but only from 20.0 – 400 µg/mL on the QQQ due to significant interference.

In all cases, variations in the calibration curves' slope between Rituximab and Reditux were observed, suggesting differences in their respective protein content.

INTRODUCTION

Biosimilars are rarely the exact duplicate of innovator biotherapeutics and thus require extensive characterization prior to regulatory approval. The determination of pharmacokinetic equivalence is central to this process and it is crucial that the bioanalytical assay remains unbiased by small variations between the biosimilar and the reference product. Consequently, the orthogonal selectivity and specificity offered by LC-MS has increasingly become the technique of choice for reliably quantifying these analytes, and is illustrated in the current investigation for the quantitation of Rituximab and its biosimilar Reditux using a dual-peptide approach. Furthermore, the performance characteristics of the triple-stage quadrupole (QQQ) and quadrupole time-of-flight (Q-TOF) platforms are compared and the advantages of accurate mass filtering highlighted.

METHODS

SAMPLE PROCESSING

- Aliquot 50 µL of human plasma sample (1.00 – 400 µg/mL)
- Precipitate with MeOH (1:4)
- Centrifuge and discard supernatant
- Resuspend protein pellet buffer containing SIL-peptides
- Reduce (TCEP) and alkylate (IAM)
- Digest with trypsin for 2 hours
- Analyze extracts by LC-MS/MS or LC-HRMS

CHROMATOGRAPHY

- Agilent Technologies Series 1100 pumps and autosampler
- Xbridge BEH300 C18 (50 x 2.1 mm, 3.5 µm)
- Gradient elution with 0.2% acetic acid and ACN

DETECTION

API 5000 operated in MRM mode
- HC peptide: *m/z* 1092.2 > 1180.5
- LC peptide: *m/z* 904.5 > 1069.6

TripleTOF 6600 operated in MRM^{HS} mode (XIC 25 mDa)
- HC peptide:
  - *m/z* 1092.2 > 1180.5047, 1181.5077, 1343.5697, 1344.5739
- LC peptide:
  - *m/z* 904.5 > 1069.5728, 1070.5755, 1156.6066, 1157.6086

RESULTS

GENERAL CONSIDERATIONS

Rituximab and its biosimilar Reditux were quantified in human plasma, using a dual-peptide approach. Samples were prepared by the pellet digestion method which does not necessitate the usage of high affinity reagents.

As opposed to a hybrid LBA-LC/MS assay where the capture antibody could potentially show different affinity for the biosimilar and the reference product, it is anticipated that the pellet digestion will be insensitive to these differences.

The tryptic peptides GLEWIGAIYPGNGDTSYNQK and pE(Q)IVLSQSPAILSASPGEK were chosen as surrogates for quantitation.

TRIPLE QUADRUPOLE MS (QQQ)

Determination of the HC peptide from both Rituximab and Reditux was precise, accurate and linear from 1.00 – 400 µg/mL. However, for the LC peptide, quantitation was only achieved from 20 – 400 µg/mL due to significant interferences (Tables 1-2, Figure 1).

Table 1. Precision and Accuracy of Rituximab on a QQQ

	LOQ QC 1.00 µg/mL	Low QC 4.00 µg/mL	Mid QC(a) 12.00 µg/mL	Mid QC(b) 125.00 µg/mL	High QC 300.00 µg/mL
HC Peptide - GLEWIGAIYPGNGDTSYNQK					
% Nominal	97.5	97.8	97.6	95.0	89.5
% CV	11.2	4.6	4.3	3.1	6.0
LC Peptide - pE(Q)IVLSQSPAILSASPGEK					
% Nominal	Below limit of detection			100.0	89.0
% CV				7.1	4.3

Table 2. Precision and Accuracy of Reditux on a QQQ

	LOQ QC 1.00 µg/mL	Low QC 4.00 µg/mL	Mid QC(a) 12.00 µg/mL	Mid QC(b) 125.00 µg/mL	High QC 300.00 µg/mL
HC Peptide - GLEWIGAIYPGNGDTSYNQK					
% Nominal	104.2	103.4	99.6	94.1	93.5
% CV	9.7	4.1	3.4	4.0	4.3
LC Peptide - pE(Q)IVLSQSPAILSASPGEK					
% Nominal	Below limit of detection			94.0	92.3
% CV				7.1	7.3

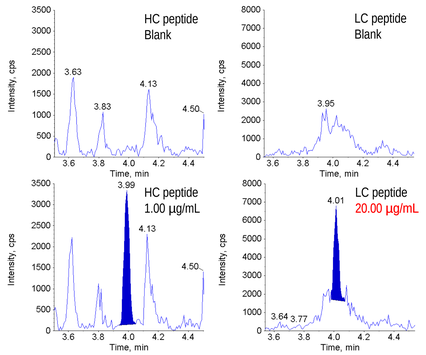

Figure 1. Representative Chromatograms of the HC and LC peptides Analyzed on a QQQ

HYBRID TIME-OF-FLIGHT MS (Q-TOF)

The interferences observed in the QQQ for the LC peptide could be discriminated by leveraging the increased selectivity of the Q-TOF via accurate mass filtering. As a result, quantitation of both peptides was possible from 1.00 – 400 µg/mL. (Tables 3-4, Figure 2).

Table 3. Precision and Accuracy of Rituximab on a Q-TOF

	LOQ QC 1.00 µg/mL	Low QC 4.00 µg/mL	Mid QC(a) 12.00 µg/mL	Mid QC(b) 125.00 µg/mL	High QC 300.00 µg/mL
HC Peptide - GLEWIGAIYPGNGDTSYNQK					
% Nominal	96.1	88.3	91.3	103.2	104.3
% CV	6.5	4.5	6.5	4.9	5.5
LC Peptide - pE(Q)IVLSQSPAILSASPGEK					
% Nominal	92.9	80.4	84.6	93.2	95.3
% CV	7.3	4.5	3.6	6.3	6.4

Table 4. Precision and Accuracy of Reditux on a Q-TOF

	LOQ QC 1.00 µg/mL	Low QC 4.00 µg/mL	Mid QC(a) 12.00 µg/mL	Mid QC(b) 125.00 µg/mL	High QC 300.00 µg/mL
HC Peptide - GLEWIGAIYPGNGDTSYNQK					
% Nominal	96.6	88.6	89.1	95.7	99.2
% CV	12.8	7.0	3.7	3.4	6.8
LC Peptide - pE(Q)IVLSQSPAILSASPGEK					
% Nominal	88.6	86.4	84.8	97.9	85.3
% CV	19.2	7.4	3.3	5.6	8.1

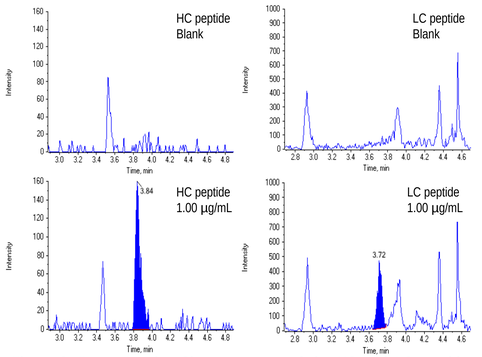

Figure 2. Representative Chromatograms of the HC and LC peptides Analyzed on a Q-TOF

RITUXIMAB AND REDITUX COMPARISON

For both the HC and LC peptides, a difference in calibration curve slopes of -14% and -19% was observed between Rituximab and Reditux, respectively (Figure 3). These results suggest that the protein content of Rituximab and Reditux formulations may differ.

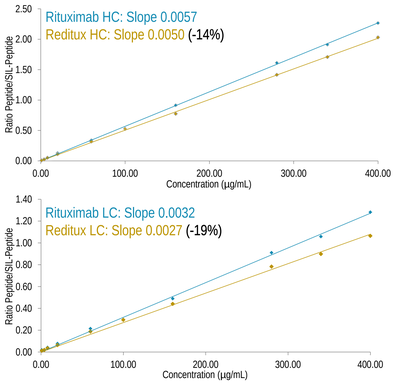

Figure 3. Calibration Curves of Rituximab and Reditux HC Peptide (Top) and LC Peptide (Bottom)

CONCLUSION

This work highlights the benefits of the SCIEX TripleTOF6600 high resolution mass spectrometer for the precise and accurate quantitation of therapeutic proteins in complex matrices.

The culmination of results using a dual-peptide approach coupled with tandem mass spectrometric quantitation comparing nominal mass (QQQ) and accurate mass (QOF) platforms suggests the protein content of Rituximab and Reditux formulations may in fact differ.

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