Altasciences CLINICAL RESEARCH ALGORITHME PHARMA

Algorithme Pharma, 575 Armand-Frappier, Laval (Montréal), Québec, Canada
algopharm.com | contact@algopharm.com

Elimination of Interference by Differential Mobility Spectrometry for Quantitation of the Biologic Rituximab in Human Plasma by LC-MS/MS

Jean-Nicholas Mess, Jeff Plomley, Kevork Mekhssian and Anahita Keyhani

OVERVIEW

PURPOSE

To demonstrate the applicability of the SelexION+® Differential Mobility System (DMS) for the elimination of interference in the quantitation of Rituximab by LC-MS/MS.

METHOD

Ritixumab was fortified in human plasma and extracted by pellet digestion. Two signature peptides from the variable domains were monitored: one from the light chain (LC) and one from the heavy chain (HC). Corresponding SIL-peptides were used as internal standards. Extracts were analyzed by LC-MS/MS and LC-DMS-MS/MS.

RESULTS

LOQ-limiting interference observed for the LC peptide by LC-MS/MS was eliminated using optimized DMS parameters. The targeted LOQ of 4.00 µg/mL was achieved for both surrogate peptides with intra-day precision <12% and accuracies between 96 and 110% for all QCs. DMS signal was notably stable over a 24-hour period (200 injections) with CVs of ca. 10% for both peptides.

INTRODUCTION

Quantitation of therapeutic proteins via bottom-up LC-MS approaches can be complicated by co-extracted interference when using non-selective sample preparation techniques. While high resolution/accurate mass spectrometry can mitigate such interference, it can be limited by poor duty cycle and sensitivity. Triple-stage quadrupole (QQQ) platforms can circumvent these limitations, at the expense of accurate mass filtering. Nonetheless, elimination of interference can still be achieved by coupling the QQQ with a Differential Mobility System (DMS), an orthogonal gas-phase separation technique separating ions based on their respective molecular cross-section. The applicability of this technology is herein demonstrated for the elimination of interference in the quantitation of the biologic Rituximab in human plasma.

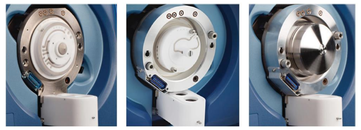

Figure 1. SelexION+ Interfaced to SCIEX Triple Quad System.

METHODS

SAMPLE PREPARATION

- Aliquot 50 µL of human plasma sample (4.00 – 400 µg/mL)
- Precipitate with MeOH (1:4)
- Centrifuge and discard supernatant
- Re-suspend protein pellet in buffer containing SIL-peptides
- Reduce (TCEP), alkylate (IAM), digest with trypsin for 2 hours
- Analyze by LC-MS/MS or LC-DMS-MS/MS

CHROMATOGRAPHY

- XBridge® Peptide BEH C18 (50 x 2.1 mm, 3.5 µm)
- Gradient elution with 0.2% acetic acid in water and ACN

DETECTION

- SCIEX Triple Quad 6500+ operated in (+)ESI-MRM mode
- SCIEX SelexION+® (Figures 1 and 2)
  - Separation voltage: 3.8 kV_{p-p}
  - DMS temperature: 300 ºC
  - Modifier : 2-propanol (1.5% mole ratio)
  - DMS resolution gas : N_2 at 25 psi

Table 1. Detection parameters for Rituximab peptides and SIL-peptides.

Peptide ID	Peptide Sequence	Transition (m/z)	CE (V)	COV (V)
HC	GLEWIGAIYPGNGDTSYNQK	1092.2 > 1180.5	45	10.5
SIL-HC	GLEWIGAIYPGNGDTSYNQ**K**($^{13}C_6$, $^{15}N_2$)	1096.2 > 1188.5	45	10.5
LC	pE(Q)IVLSQSPAILSASPGEK	904.5 > 1156.6	40	13.1
SIL-LC	pE(Q)IVLSQSPAILSASPGE**K**($^{13}C_6$, $^{15}N_2$)	908.5 > 1164.6	40	13.1

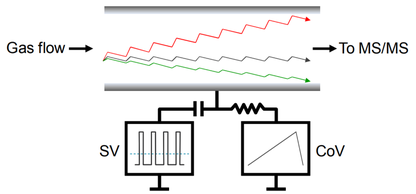

Figure 2. Differential mobility process in the DMS cell. Separation voltage (SV) is used to separate ions based on differential mobility between the high and low fields. Compensation voltage (COV) is used to correct the trajectory of the ion of interest.

RESULTS

RITUXIMAB QUANTITATION BY LC-MS/MS

Determination of the HC peptide from Rituximab was precise, accurate and linear from 4.00 – 400 µg/mL. However, significant interferences were observed for the LC peptide which prevented the targeted LLOQ of 4.00 µg/mL to be obtained; quantitation of the LC peptide was therefore only viable from 20.0 – 400 µg/mL (Figure 3 and Table 2).

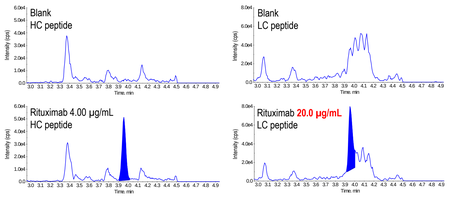

Figure 3. Representative chromatograms of Rituximab HC and LC peptides analyzed by LC-MS/MS.

Table 2. Precision and accuracy of Rituximab HC and LC peptides analyzed by LC-MS/MS.

	LOQ QC 4.00 µg/mL	Low QC 12.00 µg/mL	Mid QC 125.00 µg/mL	High QC 300.00 µg/mL
HC Peptide - GLEWIGAIYPGNGDTSYNQK				
% Nominal	97.5	94.5	100.3	97.5
% CV	5.1	9.4	7.1	4.1
LC Peptide - pE(Q)IVLSQSPAILSASPGEK				
% Nominal	Presence of Interference		90.4	99.3
% CV			9.4	6.6

OPTIMIZATION OF DMS PARAMETERS

The potential of DMS to resolve the LC peptide from the LOQ-limiting interference was investigated through the optimization of both the separation voltage (SV) and compensation voltage (COV) as a function of transport gas temperature and pressure. Isopropanol was added to the carrier gas stream (1.5% mole ratio) to enhance the separation process via clustering/declustering during the application of low and high field RF, respectively. Exemplary ionograms of the Rituximab LC peptide are shown in Figures 4 and 5.

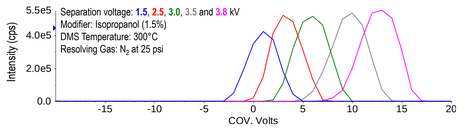

Figure 4. Overlaid ionograms of increasing separation voltage (SV) for the optimization of the LC peptide of rituximab indicating optimal COV at which the parent ion is transmitted through the DMS cell.

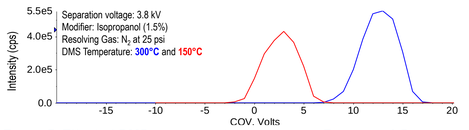

Figure 5. Effect of DMS carrier gas temperature on Rituximab LC peptide compensation voltage (COV).

RITUXIMAB QUANTITATION BY LC-DMS-MS/MS

As shown in Figure 6, DMS greatly improved the selectivity for both peptides, albeit at the cost of peak intensity. The LC peptide co-eluting interference was eliminated and the targeted LOQ of 4.00 µg/mL was achieved (Figure 7).

Intra-day precision for both HC and LC peptides was <12% with accuracies between 96 and 110% for all QCs (Table 3). DMS signal was notably stable over a 24-hour period (injection of 200 processed samples) with CVs of ca. 10% for both peptides (Figure 8).

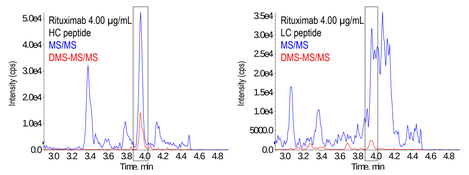

Figure 6. Overlaid chromatograms of Rituximab HC and LC peptides analyzed by LC-MS/MS (blue trace) and LC-DMS-MS/MS (red trace).

Table 3. Precision and accuracy of Rituximab HC and LC peptides analyzed by LC-DMS-MS/MS.

	LOQ QC 4.00 µg/mL	Low QC 12.00 µg/mL	Mid QC 125.00 µg/mL	High QC 300.00 µg/mL
HC Peptide - GLEWIGAIYPGNGDTSYNQK				
% Nominal	103.1	102.6	99.9	95.9
% CV	9.5	3.9	2.2	4.5
LC Peptide - pE(Q)IVLSQSPAILSASPGEK				
% Nominal	109.7	104.6	104.0	99.9
% CV	11.6	7.1	5.5	3.4

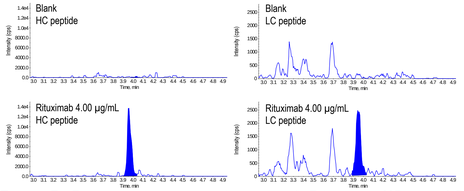

Figure 7. Representative chromatograms of Rituximab HC and LC peptides analyzed by LC-DMS-MS/MS.

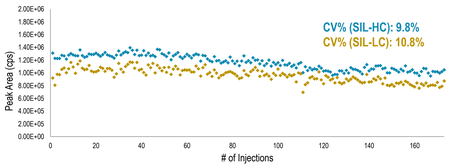

Figure 8. Extracted Rituximab SIL-peptide signal intensity over 24-hour by LC-DMS-MS/MS.

CONCLUSION

The dual peptide quantitation approach increases reliability for the structural integrity of Rituximab and therefore, enhances confidence in reported data. Without the additional stage of gas-phase selectivity provided by SelexION+® DMS technology, this dual peptide approach would not have been feasible at the required LOQ of the assay as a result of significant interference co-eluting with the LC peptide.

ACKNOWLEDGEMENTS

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