Altasciences, 575 Armand-Frappier, Laval, Québec, H7V 4B3, Canada
Altasciences.com | contact@altasciences.com

Quantitation of the Monoclonal Antibody Rituximab Using Volumetric Absorptive Microsampling, Impact-Assisted Extraction, Trypsin Digestion and LC-MRM

Jean-Nicholas Mess, Kevork Mekhssian, Nikolay Youhnovsky, Milton Furtado, and Anahita Keyhani
Altasciences Clinical Research, Laval, Québec, Canada

INTRODUCTION

VAMS has emerged as an alternative approach for blood sampling during clinical and nonclinical studies. It enables precise and accurate collection of a determined blood volume, therefore reducing the hematocrit effect associated with the dried blood spot (DBS) technique. Nevertheless, the sample hematocrit level can still bias drug measurements by affecting the desorption of the analytes from the VAMS device. The recently established IAE approach has proven to overcome such bias for small molecules and peptides, but its applicability to biotherapeutic proteins quantitation has yet to be verified. The usefulness of this approach for the bottom-up LC-MRM quantitation of the monoclonal antibody Rituximab in human blood is herein demonstrated.

Figure 1. Neoteryx Mitra® volumetric absorptive microsampling (VAMS)

METHODS

SAMPLE PREPARATION

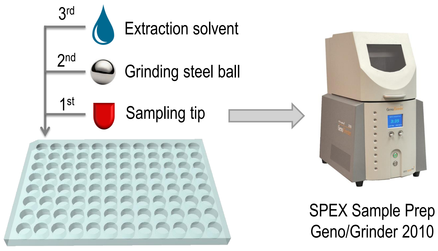

Figure 2. Impact Assisted Extraction (IAE)

Load Mitra® sampler into 96 well plate
Add grinding steel ball and extraction buffer
Seal plate and mix aggressively
Reduce (TCEP) and alkylate (IAM)
Digest with Trypsin (2 hours, 37°C)
Quench with formic acid
Analyze by LC-MRM on SCIEX Triple Quad 6500+

Figure 3. Rituximab Sample Processing

RESULTS

RITUXIMAB SAMPLE PROCESSING

Optimization of the extraction conditions included screening of multiple solvent mixtures to achieve optimal desorption of Rituximab from the Mitra® substrate. Protein solubility and compatibility with tryptic digestion were the primary drivers in the selection of extraction solvent. Optimal Rituximab recovery was obtained using a mixture of ammonium bicarbonate containing 20% acetonitrile and 0.25% octyl beta-glucopyranoside (**Figure 4**).

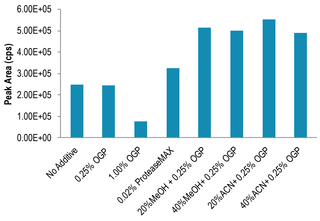

Figure 4. Extraction of Rituximab from VAMS using various extraction buffers. All buffers tested included 50mM ammonium bicarbonate supplemented with additives. OGP: octyl β-glucopyranoside.

The rate of desorption for Rituximab from the Mitra® substrate was found to be much slower than that observed for typical small molecules applications. However, extraction of Rituximab was greatly improved when the Mitra® substrate remained in solution during the entire sample processing procedure, including reduction with TCEP, alkylation with iodoacetamide and trypsin digestion (**Figure 5**).

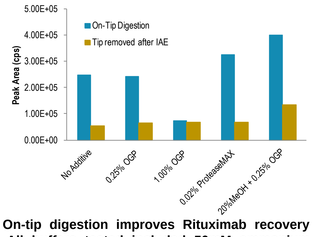

Figure 5. On-tip digestion improves Rituximab recovery from Mitra® substrate. All buffers tested included 50mM ammonium bicarbonate supplemented with additives. OGP: octyl β-glucopyranoside.

HEMATOCRIT EFFECT

Rituximab recovery using IAE was independent of blood HCT as indicated in Table 6 with yields ranging from 95.6 to 104% across all levels (**Figure 6**).

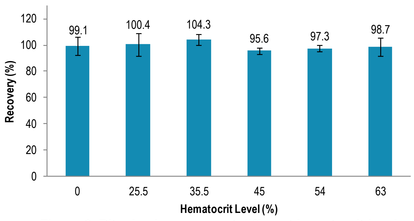

Figure 6. Rituximab recovery at different hematocrit levels

LINEARITY, PRECISION AND ACCURACY

A precision and accuracy analytical batch was assayed using the optimal conditions described above. The assay was linear (weighted $1/x^2$) from 1.00 to 400.00 µg/mL with an LLOQ S:N > 10 (**Figure 7** and **Figure 8**). Intra-day precision of the assay was < 5.3% with accuracies between 96.8 and 103.6% for all QCs.

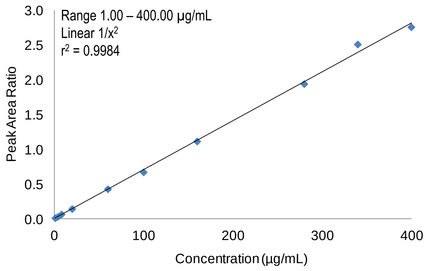

Figure 7. Calibration curve of Rituximab extracted from Mitra®

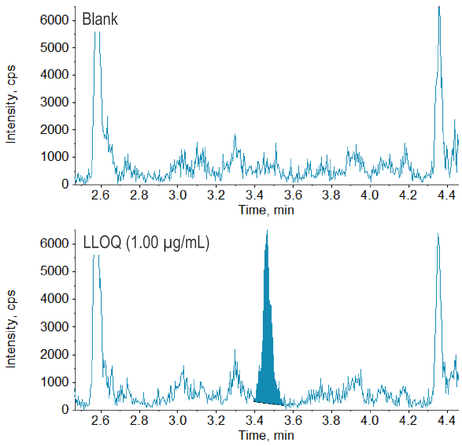

Figure 8. Representative chromatograms of Rituximab blank and LLOQ extracted from Mitra® (surrogate peptide monitored)

Table 1. Precision and accuracy of Rituximab extracted from Mitra®

	LOQ QC 1.00 µg/mL	Low QC 4.00 µg/mL	Mid QC 125.00 µg/mL	High QC 300.00 µg/mL
% Nominal	102.6	96.8	103.6	99.8
% CV	2.7	3.3	5.3	4.8

Table 2. Stability of Rituximab in VAMs dried blood samples at 22ºC for 371 days

	Low QC 4.00 µg/mL		High QC 300.00 µg/mL	
	Comparison	Stability	Comparison	Stability
Mean (n=6)	3.66	4.50	284.04	324.80
% Nominal	91.6	112.4	94.7	108.3
% CV	9.2	9.3	2.4	8.3

CONCLUSION

The applicability of VAMS combined with IAE was illustrated for the quantitation of the monoclonal antibody Rituximab using a bottom-up LC-MRM approach. The developed assay demonstrated excellent linearity, precision and accuracy whilst negating hematocrit effect.

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