Quantitation of monoclonal antibody infliximab in human plasma by LC-MS/MS using Fab-selective limited proteolysis nSMOL technology

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Overview

● Infliximab was quantified from plasma by a novel enrichment technique using protein A/G bound resin beads from a commercial sample preparation kit.
● Nano-surface and molecular-orientation limited (nSMOL) proteolysis was performed using immobilized trypsin FG beads. This approach resulted in a selective digestion of the Fab region, which consists of peptides from the CDR region of monoclonal antibody (mAB).
● Three proteotypic peptides were selected for quantitation by LC-MS/MS with a detection limit of 0.25 ng/mL with a fast 8 min gradient using the Shimpack GISS column.

1. Introduction

Infliximab, commercially known as Remicade, is a chimeric IgG1 kappa monoclonal antibody (mAb), that targets tumor necrosis factor-alpha (TNF). However, a number of studies have identified a substantial proportion of patients (between 30-40%) who fail to respond to anti-TNF therapy. Current methods for monitoring infliximab are almost exclusively immunoassay based. In this study, we describe the use of nSMOL proteolysis, which selectively targets the Fab CDR region of infliximab, and LC-MS/MS quantitation.

2. Methods and Materials

Plasma samples were prepared using nSMOL kit (Shimadzu Corporation) and proteotypic peptides of infliximab quantified by LC-MS/MS MRM (LCMS-8060, Shimadzu Corporation).

2-1. nano-Surface and Molecular-Orientation Limited (nSMOL) proteolysis

Infliximab Capture

Collecting monoclonal antibodies from blood or other biological samples using immunoglobulin collection resin Protein A/G bound within 100 nm well resin beads specifically binds the Fc region of infliximab to the resin. As the Fc region is directly bound, the Fab region is then in the optimum geometry for tryptic digestion.

Selective Digestion

FG bead trypsin DARTTM: ferrite particles coated with poly-GMA (glycidyl methacrylate)

The nSMOL enables collection of IgG fractions in plasma via Fc regions, and selective proteolysis on Fv of antibody drugs using trypsin immobilized on the surface of nanoparticles. This reaction field allows selection of quantitation peptides that reflect the structural characteristics of antibodies. Antibodies have three CDRs respectively on each heavy and light chain, and the collected peptides using the nSMOL are mainly peptides including CDRs.

MRM detection of CDR peptides

Minimizing sample complexity for LC-MS/MS analysis CDR peptides were enriched by centrifugal filtration then measured by LC-MS/MS with high specificity and sensitivity.

Table 1. LC-MS/MS method for the analysis of infliximab in plasma.

<table>
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<tr>
<th>Step</th>
<th>Description</th>
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<td>1.</td>
<td>Agitate for 15 minutes at room temperature and centrifuge. Add nSMOL kit: Wash solution 1 and reaction solution. Reaction solution + ISTD F14H (10 femtomol).</td>
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Figure 1. Schematic structure of infliximab.

Figure 2. Schematic workflow for the selective proteolysis of the Fab region of infliximab via trypsin-immobilized nanoparticles.
3. Results

3-1. Peptide selection
Skyline software (MacCoss Lab, University of Washington) was used to perform in-silico protein digestion and predict candidate peptides and MRM transitions. Six proteotypic candidate peptides were evaluated and three selected for quantitation: SINSATHYAESVK, YASEMSGIPSR and DILLTQPSAILSVSPGER (Figure 3) from Fab heavy and light chains respectively.

![Figure 3. Amino acid sequence of infliximab Fab heavy and light chains. Green regions represent conserved peptide sequence, orange - unique sequence, blue – point of interaction of CDR with TNFα. Red boxes represent peptide sequences selected for quantitation.](image)

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3-2. Infliximab quantitation

![Figure 4. Skyline in-silico predicted proteotypic peptides of infliximab in plasma. SINSATHYAESVK, YASEMSGIPSR and DILLTQPSAILSVSPGER were selected based on response, linearity and accuracy. Other peptides and quantifier/reference ions were evaluated but finally not selected due to either interference or insufficient sensitivity at the lowest calibration level.](image)

Figure 4. Skyline in-silico predicted proteotypic peptides of infliximab in plasma. SINSATHYAESVK, YASEMSGIPSR and DILLTQPSAILSVSPGER were selected based on response, linearity and accuracy. Other peptides and quantifier/reference ions were evaluated but finally not selected due to either interference or insufficient sensitivity at the lowest calibration level.

![Figure 5. MRM chromatograms for 3 tryptic peptides for infliximab (1 ug/mL [Red trace] and 0.25 ug/mL [Blue trace] in plasma) and internal standard peptide P14R. *P14R internal standard (10 pmol/mL) XIC scaled to x0.05.*](image)

Figure 5. MRM chromatograms for 3 tryptic peptides for infliximab (1 ug/mL [Red trace] and 0.25 ug/mL [Blue trace] in plasma) and internal standard peptide P14R. *P14R internal standard (10 pmol/mL) XIC scaled to x0.05.*

4. Conclusions
- To quantify infliximab in plasma samples nSMOL (nano-surface and molecular-orientation limited) was used to selectively digest the Fab region, which consists of peptides from the CDR region of monoclonal antibody (mAB).
- This approach results in higher selectivity as the technique is focused on specific signature peptides from the CDR region.
- In this work, SINSATHYAESVK was selected as the peptide for quantitation as a result of lower limits of detection and higher selectivity in patient samples.

5. References