

## **A rapid analytical method using an LC-MS/MS for quantitation of serum tocilizumab and its clinical application**

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### Background

Tocilizumab, a humanized anti-IL-6 receptor monoclonal antibody, is commonly used for the treatment of rheumatoid arthritis (RA). In RA patients, serum tocilizumab level is reported to be associated with clinical responses. Some proteomics-based quantitative methods using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) for serum specimen need time-consuming pretreatment processes including sample cleanup and tryptic digestion. The aim of this study is to develop a rapid quantitative LC-MS/MS method of serum tocilizumab and to apply it to clinical samples.

### Methods

Candidate signature peptides derived from digested tocilizumab were identified by an orbitrap mass spectrometer. An immobilized trypsin rapidly digested reduced-alkylated serum tocilizumab without protein purification for 30 minutes. The selected signature peptide and its stable isotope labeled peptide as an internal standard (IS) were quantified by a triple quadrupole mass spectrometer and its total run time was 15 minutes. This method was applied to the quantitation of serum samples in 22 RA patients treated with intravenous or subcutaneous tocilizumab.

### Results

The peptide including complementary determining region of tocilizumab L-chain was selected as signature peptide for the quantitation of serum tocilizumab. The chromatographic peaks of signature peptide and IS were separated from serum derived-peaks using a core-shell octadecyl silyl microparticulate column. The calibration curve of serum tocilizumab was liner within a range of 2-200 µg/mL and its lower limit of quantification was 2 µg/mL. The intra- and inter-assay accuracy and imprecision were 90.7-109.4% and less than 8.5%, respectively. Tocilizumab was stable in human serum under following storage conditions: 24 hours at room temperature and 4°C, 1 month at -80°C, and three freeze-thaw cycles. Serum tocilizumab levels in RA patients receiving an intravenous injection and subcutaneous administration were 5.8-28.9 and 2.4-63.5 µg/mL, respectively.

### Conclusions

The present method with rapid sample pretreatment processes and acceptable analytical performance can be helpful for the quantitation of serum tocilizumab in clinical settings.

### Key words

tocilizumab, pharmacokinetics, LC-MS/MS, proteomics, human serum, rheumatoid arthritis