Utilizing HRAM Orbitrap MS to Quantify Therapeutic Monoclonal Antibodies (mAbs) in Human Serum for Clinical Research

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Background

In clinical testing, the presence of endogenous immunoglobulins with almost identical structures from patients' samples adds another challenge to the accurate quantitation of therapeutic mAbs. Accordingly, mass spectrometry has gained substantial popularity for therapeutic mAb monitoring in clinical laboratories due to its great versatility in detecting both tryptic peptides and intact light and heavy chains quantitatively.

Here we present the intact light chain quantitation approach for measuring concentrations of therapeutic mAbs in human serum using Orbitrap Exploris 240 MS.

Methods

Various therapeutic mAbs used in this study with different concentrations of standards ranging from 1 to 100 ug/mL. Thermo Scientific™ Pierce™ Protein L magnetic beads were used for purification followed by the disulfide bond reduction. LC-MS was performed by Thermo Scientific™ Vanquish™ HPLC system interfaced to Orbitrap Exploris™ 240 mass spectrometer (OE240).

Results

The workflow was optimized to provide optimum assay performance including minimum hands-on time and affordable reagents. Protein L magnetic beads were chosen among different purification techniques. The OE240 MS fully resolved isotopic clusters of different charge states of the light chains by operating at an orbitrap resolution > 120,000.

The isotope envelopes of subunits were deconvoluted to each intact molecular mass and accurately assigned with a mass accuracy of ≤ 10 ppm. Analytical performance evaluation resulted in LOQs between 1 to 5 ug/mL of the mAb concentration in human serum. Great linearity was observed with R^2 values higher than 0.99. Also, the variation of the detected retention time of two IS mAbs was determined to be less than ± 0.05 minutes and the % RSD of the peak areas was less than 15%. This supports the reproducibility of the entire process from sample preparation to LC-MS analysis. Through a quick column reproducibility evaluation, reproducible data were generated over two different column lots showing a 0.2-minute shift with less than 20% peak area differences.

Conclusions

The HRAM Orbitrap MS has been successfully applied to quantify mAbs. Currently, the application of FAIMS (high field asymmetric waveform ion mobility spectrometry) is in testing to further improve selectivity and sensitivity. Also, dried blood spots as alternative matrices are evaluated.

Key Words

TDM of mAbs, HRAM, LC-MS, light chain quantitation, magnetic beads, Orbitrap Exploris 240