The Development of an IA-MS/MS Sensitive and High Throughput Method for the Determination of IgGx Therapeutic Drugs in Human Serum

Jamil Hantash, Laura Cojocaru, Roger Demers, Marta Mieczkowska and Erika Hess

Covance is the drug development business of Laboratory Corporation of America® Holdings (LabCorp®). Content of this material was developed by scientists who at the time were affiliated with LabCorp Clinical Trials or Tandem Labs, now part of Covance.

Purpose

Mass spectrometry is rapidly becoming a fundamental tool for biologists and biochemists in their large molecule therapeutic drug development. The ability of mass spectrometry for protein and peptide analysis lies in its ability to provide highly accurate molecular weight information on intact molecules and its ability to perform ultra sensitive, well established method to measure biological sample complexity by concentrating on the target protein of interest. However, this approach is labor intensive and its low output nature limits its feasibility compared to an ELISA platform. In this paper, we report the use of an immunological procedure, followed by an on and off-dissociation cleanup procedure and a protein digestion with MS detection— thereby providing a high throughput sample analysis.

Methods

The microplate was coated with recombinant human ligand capture standards, quality controls and sample IgG antibodies. In the ligand were then added to the microplate and coated for 2 hours. Upon equilibrium of the wells, the plate was coated with IgG antibodies for 4 hours. The plate was washed and blocked for 1 hour. After the wash step, the sample was added and incubated for 1 hour. The plate was again washed and blocked for 1 hour. After the wash step, the analyte was added and incubated for 1 hour. The plate was washed and blocked for 1 hour. After the wash step, the substrate was added and incubated for 1 hour. The plate was washed and blocked for 1 hour. After the wash step, the stop solution was added and the plate was read at 450 nm. The results were calculated using a standard curve.

Results

Typical LC-MS/MS work flow is presented in Figure 1.

Determination of Absolute Recovery under Increased Retention Time Conditions

To determine if an ion suppression from the matrix is responsible for some of the low recovery the slope of the gradient was decreased to permit more retention for the peptide in hopes to reduce ion suppression and signal recovery suppression. Both samples of the same sample set were injected after all of these gradients. The area counts for the control and the sample were compared. The results showed that ion suppression recovery increased from 20% to 40%. Refer to table 2.

Evaluation of Ion Suppression

Matrix was added to the PBS control by adding an aliquot of a blank serum digest. Then, PBS with and without matrix was added and compared. The results showed that the addition of matrix to the neat control resulted in an ~ 80% loss of signal. Therefore, ion suppression appeared to be a large part of signal loss.

Immunocapture Followed by Acid Dissociation Using Solid-phase Capture Coating

If the sample is cleaned from albumin proteins, then the sensitivity of the assay should increase. The results were promising, but not yet optimized. Refer to Figure 3. This question remained:
• Did the ELISA plate specificity cause an issue?
• Was the washing optimal?
• Did we capture what we wanted or did we capture what we didn’t want?

To determine if ion suppression from the matrix is responsible for some of the low recovery, the slope of the gradient was decreased to permit more retention for the peptide in hopes to reduce ion suppression and signal recovery suppression. Both samples of the same sample set were injected after all of these gradients. The area counts for the control and the sample were compared. The results showed that ion suppression recovery increased from 20% to 40%. Refer to table 2.

Immunocapture Followed by Acid Dissociation Using Solid-phase Capture Coating

If the sample is cleaned from albumin proteins, then the sensitivity of the assay should increase. The results were promising, but not yet optimized. Refer to Figure 3. This question remained:
• Did the ELISA plate specificity cause an issue?
• Was the washing optimal?
• Did we capture what we wanted or did we capture what we didn’t want?

To determine if ion suppression from the matrix is responsible for some of the low recovery, the slope of the gradient was decreased to permit more retention for the peptide in hopes to reduce ion suppression and signal recovery suppression. Both samples of the same sample set were injected after all of these gradients. The area counts for the control and the sample were compared. The results showed that ion suppression recovery increased from 20% to 40%. Refer to table 2.