Development of an Immunoprecipitation and LC-MS/MS Based Method for Quantifying the 105 kDa Recombinant Protein SXN101959 in Plasma

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The quantitation of proteins in blood based matrices using LC-MS/MS as the detection mechanism is challenging due to the high background protein content. Chemical extraction methods, such as solid phase extraction or organic solvent precipitation, can achieve lower limits of quantitation (LLOQ) in the low ng/mL range, and work best for small proteins and peptides. However, in order to quantify large proteins to the sub ng/mL level, a more targeted approach is required. Immunoprecipitation (IP) extraction methods exploit the highly specific nature of the antibody-antigen interaction, enabling a highly targeted enrichment of the protein of interest. The binding of drug specific antibodies to paramagnetic beads facilitates the extraction and concentration of the protein from plasma or related matrices.

SXN101959

SXN101959 is a recombinantly produced 105 kDa Targeted Secretion Inhibitor (TSI). TSI are based on botulinum toxin, a di-peptide protein – comprising light and heavy chains *(Figure 1)*. SXN101959 is designed to specifically target the GHRH receptor on somatotrophs, which allows internalisation and delivery of the endopeptidase (light chain) leading to inhibition of vesicular secretion of GH.

The therapeutic aim of SXN101959 was to help treat acromegaly, through the reduction of GH release from the pituitary [2,3].

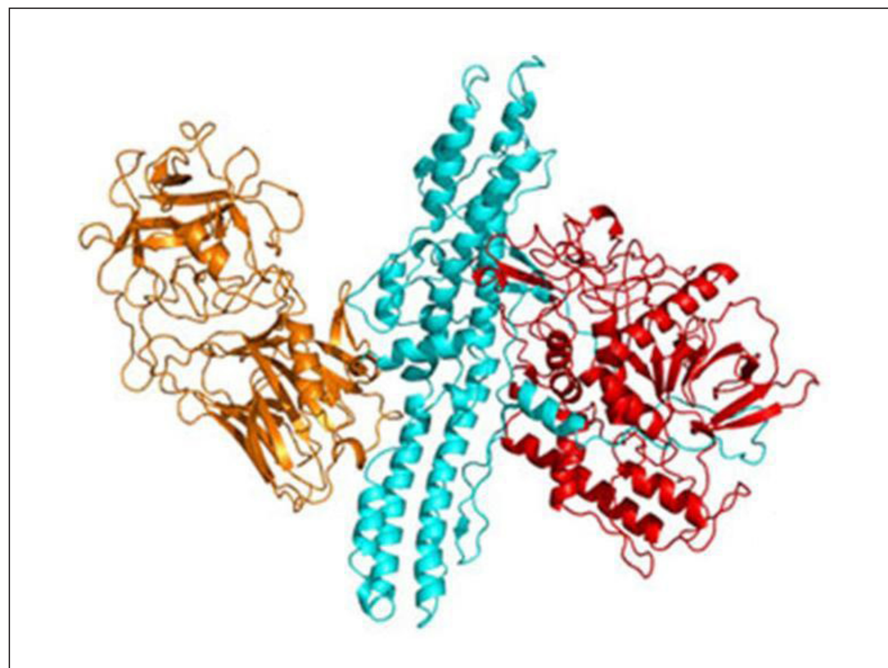
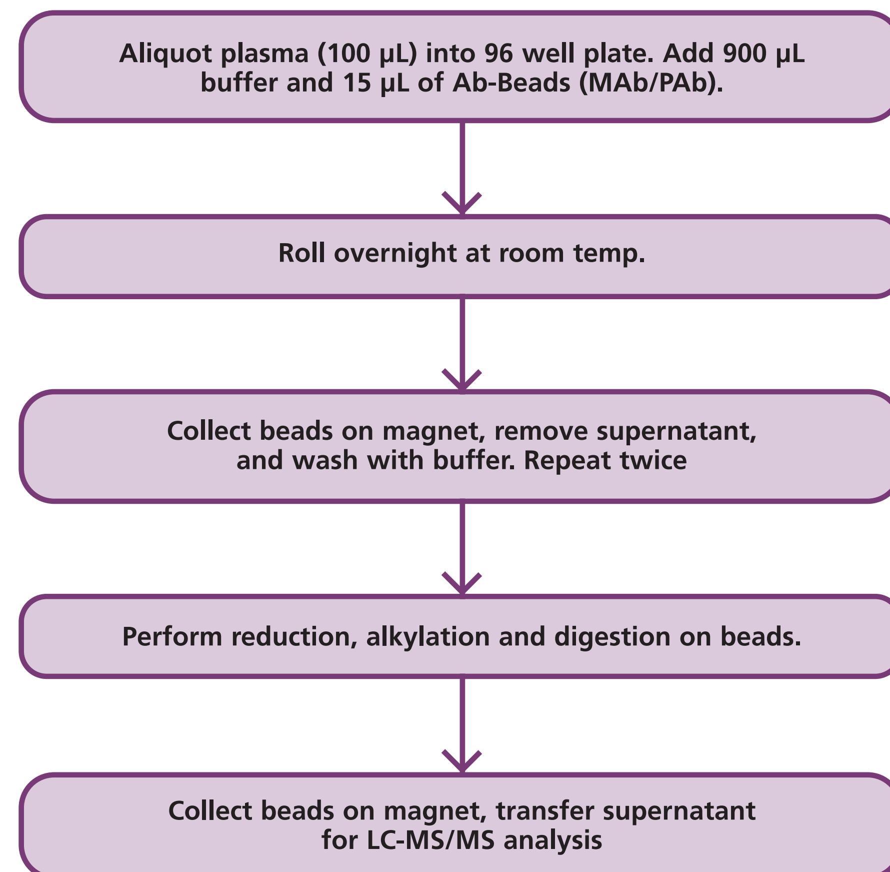


Figure 1. Crystal structure of botulinum toxin [1]. Heavy chain (orange and blue). Light chain (red).

Antibody-Bead Preparation

Monoclonal (MAb) and polyclonal (PAb) antibodies raised against SXN101959 were attached to tosyl-activated dynabeads. Attachment was confirmed by tryptically digesting the beads and monitoring antibody specific tryptic peptides by LC-MS/MS.

SXN101959 Extraction Process



LC-MS/MS Analysis

Following tryptic digestion, peptides were separated by uHPLC-MS/MS on a Waters UPLC system. A Waters T3 HSS 100 x 2.1mm column was used, with a flow rate of 0.7mL/minute. The method had a total run time of 5.5 minutes including a column flush.

Two peptides from SXN101959 were monitored using SRM detection, one each from the heavy and light chain. Tryptic peptides were also monitored from the monoclonal and polyclonal antibodies.

Sensitivity and Linearity of IP and LC-MS/MS Method

A calibration line of SXN101959 was generated from 0.5 ng/mL to 1000 ng/mL in both dog and human plasma. Similar sensitivity was achieved for both plasmas (using both MAb and PAb beads) and in both cases, a linear response was achieved. *Figure 2* demonstrates an example calibration line of 0.5 ng/mL to 1000 ng/mL.

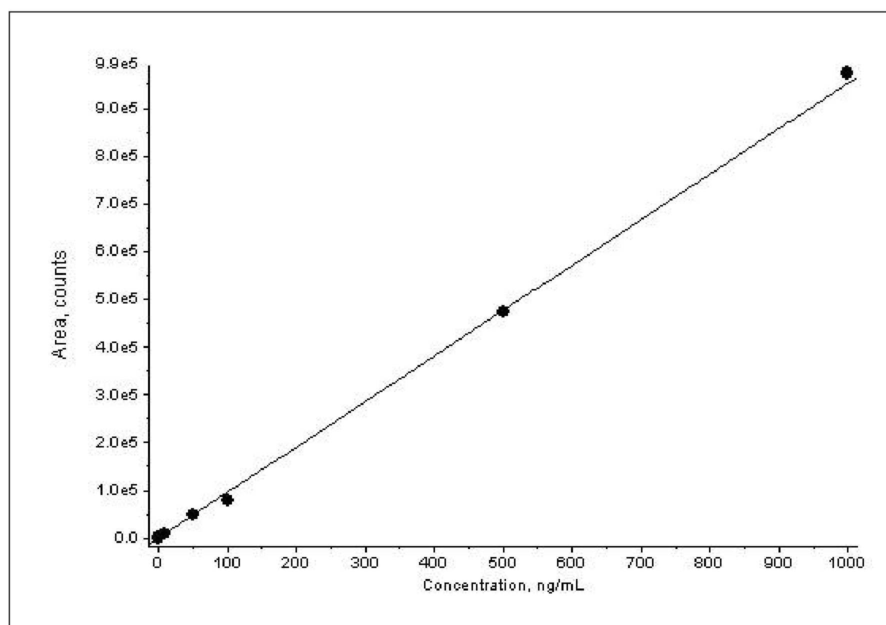


Figure 2. Calibration line of SXN101959 in extracted human plasma.

Rat PK Sample Analysis

An administration of SXN101959 to rats had previously been performed and samples analysed using an ELISA [4]. Some of these samples were analysed using an LC-MS/MS, approach with MAb beads. The concentrations of SXN101959 were compared in two ways:

1. LC-MS/MS Inter-Peptide Analysis

The concentration of SXN101959 assigned to 39 unknown rat plasma samples using peptides from the light and heavy chain demonstrated high agreement (*Figure 3*). The assigned values were also very close – with only 2 of the 39 values being greater than ± 20% different.

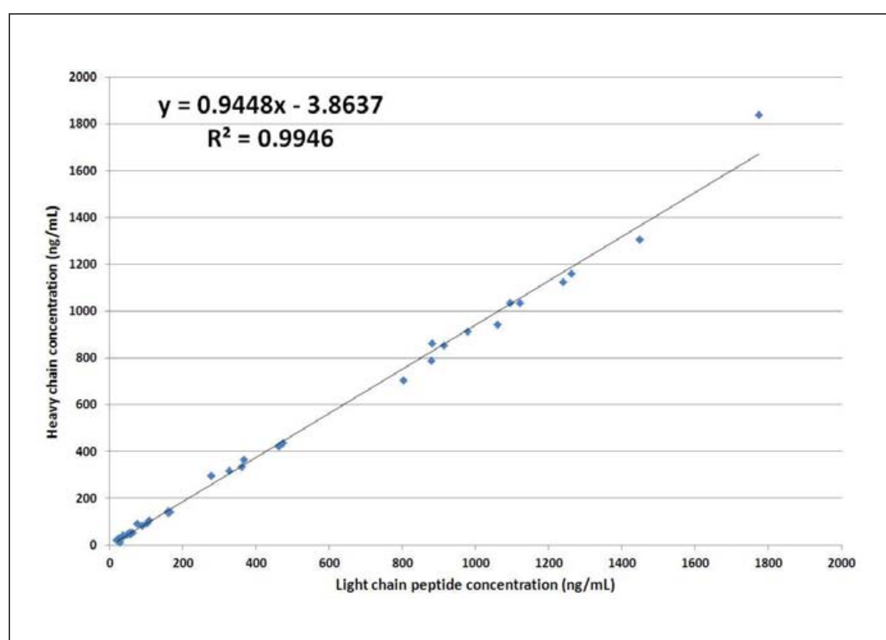


Figure 3. Light Vs. heavy chain peptide concentrations for SXN101959 in 39 rat samples.

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2. ELISA Vs.LC-MS/MS

The comparison of the LC-MS/MS and ELISA values demonstrated good agreement and correlation for the two approaches (R>0.91 for both peptides). *Figure 4* shows the correlation of the LC-MS/MS and ELISA values for the 39 rat plasma samples.

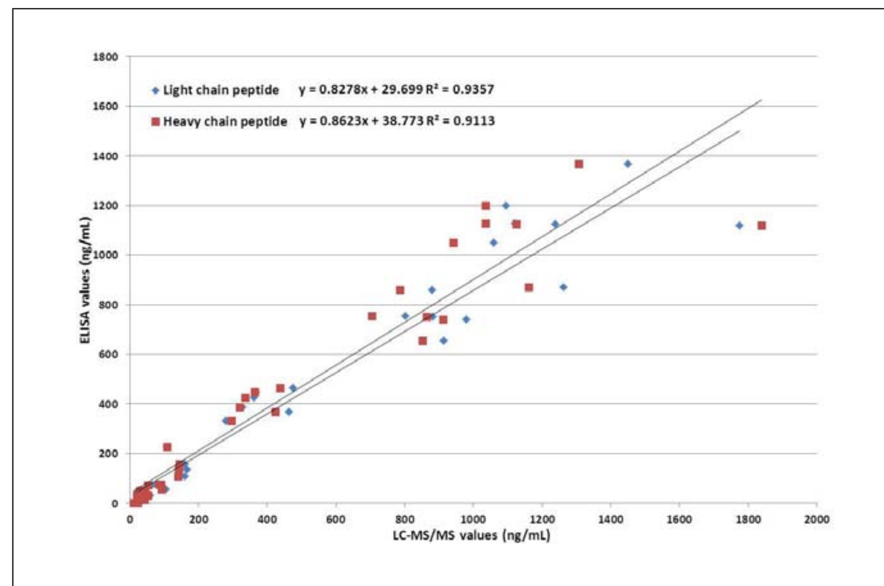

Figure 4. Comparison of LC-MS/MS and ELISA values for 39 rat plasma samples.

Conclusion

The developed IP and LC-MS/MS methodology showed good sensitivity and selectivity for SXN101959 in dog and human plasma. The analysis of 39 rat plasma samples by both ELISA and LC-MS/MS demonstrated good agreement in the assigned values. This demonstrates that IP based LC-MS/MS methods can be sensitive for high molecular weight proteins, and can also generate complimentary data to established ELISA based approaches.

References

1. Lacy, DB et al, 1998, Nature Structural Biol, 5, 898-902

2. Somm et al. (2012) A botulinum toxin-derived targeted secretion inhibitor down regulates the GH/IGF1 axis. Journal of Clinical Investigation. Aug 1. pii: 63232. doi: 10.1172/JCI63232.

3. Harper et al. (2012) The targeted secretion inhibitor (TSI) SXN101959 produces dose-dependent inhibition of pulsatile growth hormone secretion in male rats after intravenous administration. Endocr Rev 2012 33: Sun-669

4. Martinez et al. (2012) PK model approach to understanding the efficacy of SXN101959 in rats. Endocr Rev 33, Sat-741

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