Altasciences CLINICAL RESEARCH ALGORITHME PHARMA

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Method Development of an Automated Hybrid LBA-LC/MS Assay for the Quantitative Bioanalysis of the Biotherapeutic Teriparatide in Human Plasma

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OVERVIEW

PURPOSE

To report the first application that couples immunocapture with LC-MS/MS for the bioanalysis of teriparatide at low pg/mL concentration.

METHOD

Teriparatide was extracted from human plasma by immunocapture using a biotinylated anti-hPTH mAb (mouse) conjugated to streptavidin-coated paramagnetic beads.

Sample processing was automated on the KingFisher™ Flex magnetic particle processor.

LC-MS/MS analysis was achieved on a C18 column eluted under gradient conditions coupled to a Sciex QTRAP 5500 operated in ESI(+).

RESULTS

Automation of sample processing on the KingFisher™ Flex platform improved throughput by 80% while improving assay reproducibility.

With a thorough and exhaustive optimization of all sample processing steps and LC-MS/MS parameters, reliable quantitation of teriparatide in human plasma was achieved with an LLOQ of 2.5 pg/mL.

INTRODUCTION

Bioanalysis of large peptide biotherapeutics by LC-MS/MS entails multiple challenges, with sensitivity and selectivity at the forefront. While sample preparation approaches such as SPE have proven effective for peptide quantitation in some instances, the technique often lacks the selectivity conducive for concentrating extracts, removing endogenous interferences and mitigating ion suppression. Many of these issues have recently been circumvented using a hybrid LBA-LC/MS approach, wherein a specific antibody is used to capture and extract the analyte of interest. The current investigation highlights the stages of method development requiring optimization in order to generate a robust and sensitive hybrid assay for the quantitation of intact teriparatide, a 4 kDa recombinant form of (1-34) human parathyroid hormone used for the treatment of osteoporosis.

METHODS

SAMPLE PROCESSING

- Teriparatide was spiked in human plasma from 2.50 to 500 pg/mL
- Teriparatide-SIL (D_8 Valine) was used as internal standard
- Immunocapture was performed for 60 minutes using a biotinylated anti-hPTH mAb (mouse) conjugated to streptavidin-coated paramagnetic beads
- Beads were washed with mild buffers and analytes were eluted using an aqueous solution of 5% HCO_2H and 20% acetonitrile
- Bead processing was performed on the KingFisher™ Flex

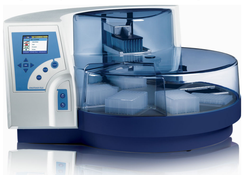

Figure 1. Thermo KingFisher™ Flex Magnetic Particle Processor

CHROMATOGRAPHY

- Agilent Technologies Series 1100 pumps and autosampler
- Reversed phase C18 column (50 x 2.0 mm, 5 µm)
- Gradient elution with 0.1% HCO_2H and ACN:MeOH (50:50% v/v) containing 0.25% DMSO

DETECTION

- Sciex QTRAP 5500 operated in MRM mode. Teriparatide and internal standard were detected as the $[M+7H]^{7+}$ ions with *m/z* 589.4 > 656.4 and *m/z* 590.6 > 657.7, respectively

RESULTS

LC-MS/MS OPTIMIZATION

Interrogation of the teriparatide mass spectrum revealed a wide charge state distribution from 4^+ to 8^+. Optimal sensitivity using MRM quantitation was obtained with the 7^+ charge state (*m/z* 589.3), leading to sensitive and specific $y_{32}{}^{+6}$ product ion at *m/z* 656.4 (Figure 2).

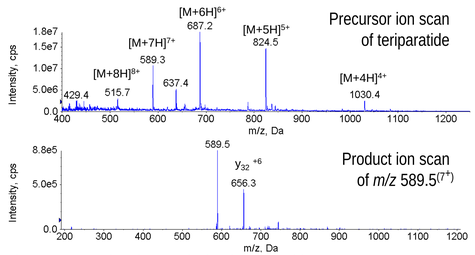

Figure 2. MS and MS/MS Analysis of Teriparatide

In order to shift the charge envelope towards higher charge states and therefore increase sensitivity, common superchargers were investigated (Figure 3). Notably, the abundance of the 7^+ charge state could be augmented 2-fold by supplementing the mobile phase with DMSO.

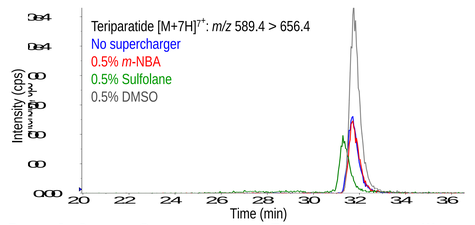

Figure 3. Overlaid Chromatograms of Teriparatide Analyzed Without Supercharger or With *m*-NBA, Sulfolane or DMSO

IMMUNOCAPTURE DEVELOPMENT

Extensive fine-tuning of the immunocapture process involved antibody screening, determination of paramagnetic bead type and binding, washing and elution condition optimization.

More specifically, the choice of streptavidin-coated paramagnetic beads was particularly challenging since different bead types demonstrated variable levels of non-specific binding and co-extraction of endogenous interference (Figure 4).

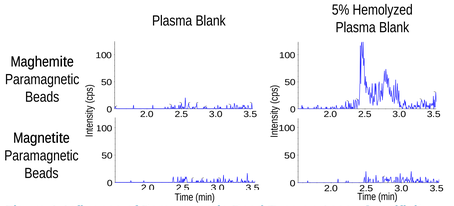

Figure 4. Influence of Paramagnetic Bead Type on Assay Specificity

Additionally, the magnetite-based beads used in this method were found to sediment rapidly, making them difficult to handle when processing multiple samples. To overcome the rapid sedimentation observed in PBS, beads were re-suspended in 25% glycerol (Figure 5). Not only were beads maintained in suspension for longer periods, but handling and reproducibility were notably improved without impact on recovery.

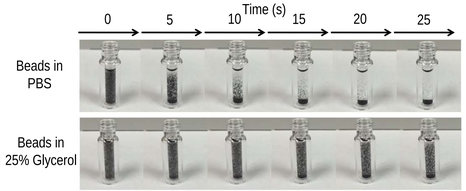

Figure 5. Addition of Glycerol (25%) to Paramagnetic Beads Suspension to Facilitate Handling and Improve Reproducibility

The extraction procedure was automated using a KingFisher™ Flex, resulting in an 80% increase in sample throughput while concomitantly improving assay reproducibility (Figure 6).

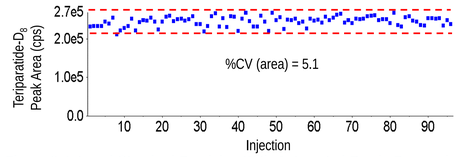

Figure 6. Internal Standard Precision With Automated Beads Processing Using KingFisher™ Flex

METHOD VALIDATION

Table 1. Summary of Method Validation

Evaluation	Results
Precision and Accuracy (inter-day)	LLOQ QC: 98.3%, CV = 12.2% QC (4 levels): 100.4 - 107.1%, CV = 6.4 - 8.3%
Percent Extraction Yield	62% to 67% through QC levels
Matrix Factor	Acceptable for 8 lots including hemolyzed (5%) and lipemic plasma
Selectivity	Acceptable for 8 lots including hemolyzed (5%) and lipemic plasma
Dilution Integrity (10x Dilution)	2x ULOQ QC: 104.6%, CV = 4.3%
Processed Samples Stability	74 hours at 4°C
Short-Term Stability (ice-water bath)	24 hours
Long-Term Stability (-80°C)	12 days
Freeze Thaw	3 cycles
Whole Blood Stability	2.0 hours

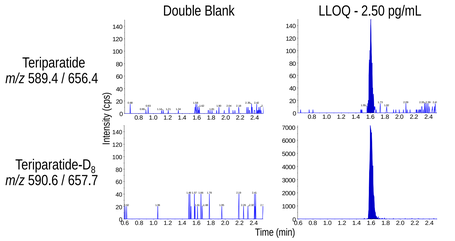

Figure 7. Teriparatide Double Blank and LLOQ (2.50 pg/mL) Analyzed by a Hybrid LBA-LC/MS Assay

CONCLUSION

This LBA-LC/MS assay for teriparatide demonstrated adherence to the strictest of industry standards for specificity, sensitivity, linearity, precision and accuracy. This work highlighted that the combination of selective affinity purification with tandem mass spectrometry offers a viable approach to highly sensitive peptide quantitation.

ACKNOWLEDGMENTS

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