Development and Validation of a LC-MS/MS Assay for Quantification of Parathyroid Hormone (PTH 1-34) in Human Plasma

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Introduction

- Teriparatide [recombinant human PTH (1-34)] is an osteoanabolic agent for treatment of osteoporosis.
- Intermittent injection of low doses (20 µg) Teriparatide increases bone mineral density (BMD) and decreases the risk of vertebral and non-vertebral fractures in post-menopausal women with osteoporosis.
- Measurement of PTH (1-34) is valuable in assessing treatment response and concordance with therapy.

Aims and Objectives

- To develop and validate a method for quantification of PTH (1-34) using liquid-chromatography tandem mass spectrometry (LC-MS/MS).
- To perform method comparison with IDS-9YSY PTH (1-34) immunoassay kit (IDS, Boldon Tyne and Wear, UK).
- To highlight factors/interferences that may contribute to the difference in PTH (1-34) results on both LC-MS/MS and immunossay methods.

Method

Sample preparation

- Rat PTH (1-34) (Inter-Standard)
- HPLC Column
  - Waters (UK) AQUITY UPLC® Peptide CSH® C18 column 130 Å (1.7 μm, 2.1 x 50 mm).
  - Flow Rate: 0.4 ml/min
- LC/MS/MS system
  - Micromass Quattro Ultima triple quadrupole tandem mass spectrometer.

Chromatograms showing elution time of non-oxidised PTH (1-34) forms and rat PTH (1-34) (15)

Assay Validation

- Linear calibration curve from 10 to 2000 pg/mL.
- Typical linear regression analysis (r² = 0.998).
- Lower limit of quantification (LLOQ): 10 pg/mL.
- Lower limit of detection (LOD): 2.1 pg/mL.

Imprecision:

Stock of PTH (1-34) calibrators and controls were prepared in our laboratory by spiking high purity (>98.0%) recombinant PTH (1-34) (PROSPEC, USA) in charcoal-stripped human EDTA plasma. Intra-impriemion profile was generated by running all QC samples 10 times within a single run, while inter-impriemion profile was generated by repeated measurements (n=10) of all QCs over a period of 1 month.

%Accuracy = \[ \left( \frac{\text{Actual} - \text{Measured}}{\text{Actual}} \right) \times 100 \]

Recovery efficiency:

Endogenous PTH(1-34) pg/mL Spiked pg/mL Expected concentration recovery spiked pg/mL Mean (SD) measured PTH(1-34) pg/mL Silhouette mean (NCV)

<table>
<thead>
<tr>
<th>Endogenous PTH(1-34) pg/mL</th>
<th>Spiked pg/mL</th>
<th>Expected concentration recovery spiked pg/mL</th>
<th>Mean (SD) measured PTH(1-34) pg/mL</th>
<th>Silhouette mean (NCV)</th>
</tr>
</thead>
<tbody>
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<td>4.8</td>
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<td>622.7</td>
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<tr>
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</tr>
</tbody>
</table>

Conclusion

- Our LC-MS/MS method showed good reproducibility, selectivity, sensitivity and recovery.
- LC-MS/MS method showed high correlation with commercial immunoassay with concentration-dependent, negative bias of 35.5% across the range of 0-800 pg/mL.
- Matrix effect, cross-reactivity of immunoassay kit to other PTH fragments, and interference from oxidised forms of PTH (1-34) are likely to be the major contributors to the difference in results between the two methods.
- LC-MS/MS result reflects the true status of biologically active form of PTH therapy and will help in better patient management.
- Capability of being able to measure oxidised forms can help with drug development and increase the potency of the drug.
- Due to the lack of reference standards and external proficiency scheme available for PTH (1-34), the true accuracy to the actual endogenous PTH (1-34) concentration in human plasma can not be assessed at present.