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

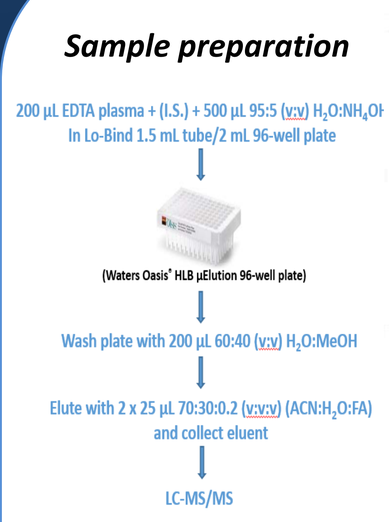
- Teriparatide [recombinant human PTH (1-34)] is an osteoanabolic agent for treatment of osteoporosis.
- Intermittent injection of low doses (20 µg) Teriparatide increases bone mineral density (BMD) and decreases the risk of vertebral and non-vertebral fractures in post-menopausal women with osteoporosis.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

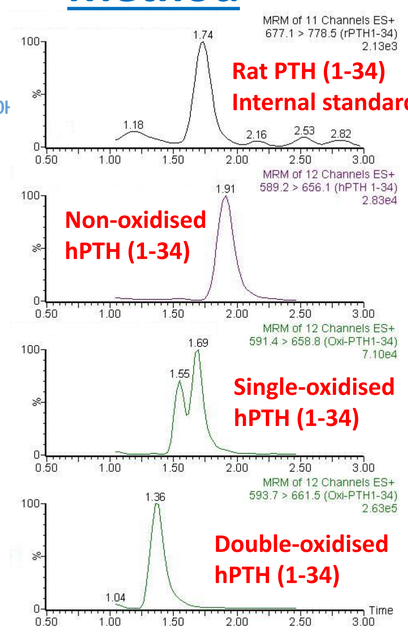
Aims and Objectives

- 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-iSYS 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 immunoassay methods.

Method

Sample preparation





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-hPTH (1-34) forms and rat PTH (1-34) (IS)

Assay Validation

- Linear calibration curve from 10 to 2000 pg/mL
- Typical linear regression analysis (r^2 = 0.999)
- Lower limit of Quantification (LLoQ): 10 pg/mL
- Lower limit of detection (LLoD): 2.1 pg/mL

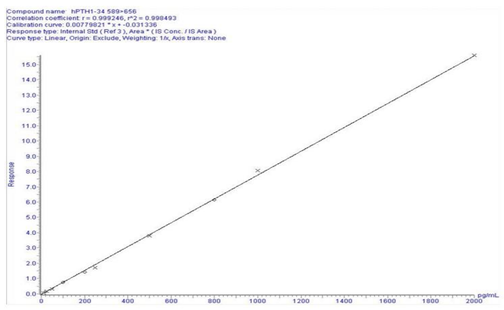


Figure (1). Typical calibration curve for hPTH (1-34) spiked into charcoal-stripped human EDTA plasma. R^2 value is 0.999.

Imprecision :

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

* %Accuracy = $[100 - (\frac{100}{n}\sum\frac{Actual - Measure}{Actual})]$

QC Level (pg/mL)	Inter-assay imprecision						Intra-assay imprecision				
	Mean	SD	SE	%CV	%Accuracy		Mean	SD	SE	%CV	%Accuracy
QC1 (20)	24.4	2.4	0.8	9.8	102.0		20.8	1.6	0.5	7.8	99.6
QC2 (100)	108.5	8.9	2.8	8.2	100.9		102.1	7.1	2.2	6.9	99.8
QC3 (200)	212.8	13.8	4.4	6.5	100.6		201.9	14.7	4.7	7.3	99.9
QC4 (800)	828.0	42.4	13.4	5.1	100.4		816.2	19.0	6.0	2.3	99.8

Recovery efficiency:

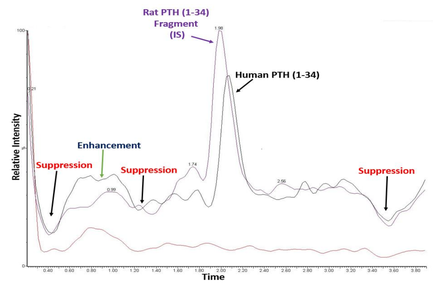
Endogenous PTH(1-34) (pg/mL)	Spiked (pg/mL)	Expected concentration (endogenous + spiked), pg/mL	Mean (±SD) measured hPTH (1-34) (pg/mL)	%Recovery mean (%CV)
8.4	20	28.4	28.0 (±1.0)	98.6 (3.6)
8.4	800	808.4	883.4 (±31.4)	109.3 (3.6)
77.5	20	97.5	101.9 (±2.0)	104.5 (1.9)
77.5	800	877.5	990.3 (±11.8)	112.9 (1.2)
602.7	20	622.7	665.0 (±14.8)	106.8 (2.2)
602.7	800	1402.7	1560.8 (±111.3)	111.3 (7.1)

Acknowledgement

The authors would like to acknowledge Norfolk and Norwich University Hospital Department of Laboratory Medicine for providing anonymised samples. We also acknowledge Entera Bio Ltd. for providing PTH (1-34) samples used for methods comparison.

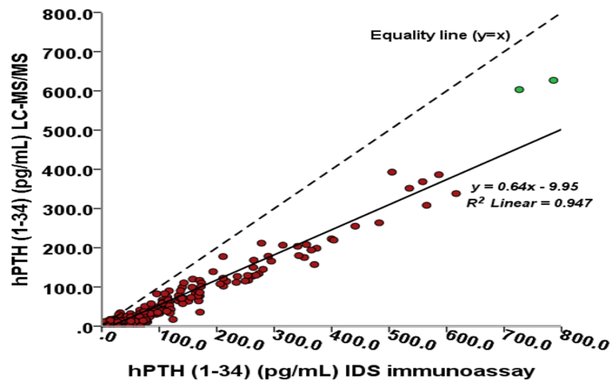
Ion Suppression

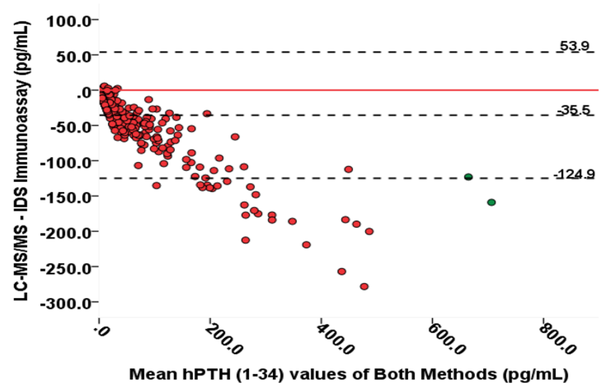
- Suppression in baseline signals was observed during co-injection of extracted human plasma sample and post column infusion of PTH (1-34).
- hPTH (1-34) and the IS eluted away from suppression and enhancement areas.



Method comparison

Comparison of the LC-MS/MS method with IDS-iSYS immunoassay for PTH (1-34) (n=390).





- The two methods showed high correlation (R^2 =0.95).
- LC-MS/MS assay showed an average negative bias of -35.5 pg/mL (p<0.0001).
- The negative bias is proportional/concentration-dependent across the concentration range (0-800 pg/mL).
- Violated dots (in green) more likely indicates hook-effect on immunoassay.

Interference/Cross-reactivity study

- **Cross-reactivity of LC-MS/MS and IDS immunoassay to human PTH (1-84), rat PTH (1-34), human PTH (13-34), human PTHrP (1-86), and PTHrP (1-36) was studied.**
- **The immunoassay showed a 7% cross-reactivity to human PTH (1-86) and 44% to rat PTH (1-34), whilst no interference was observed in the LC-MS/MS method.**

Oxidised PTH (1-34)

Figure (5). LC-MS/MS spectrum of plasma collected from a rat that has been given oral PTH (1-34). Sample was not treated with H_2O_2. Note the presence of oxidised PTH (1-34) peaks.

Figure (6). LC-MS/MS spectrum of plasma collected from a rat that has been given oral PTH (1-34). Sample was incubated with 0.1 M H_2O_2 for 60 min. Note the change in peak intensity of oxidised/non oxidised PTH (1-34) forms.

Cross-reactivity of IDS immunoassay to oxidised PTH (1-34) forms

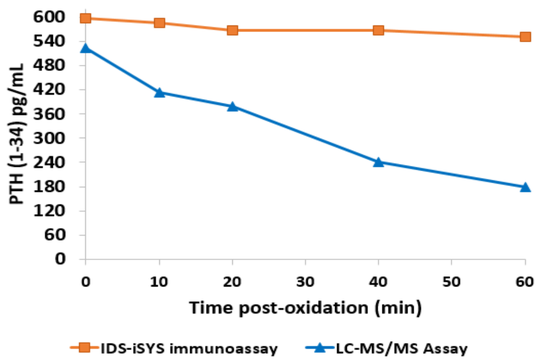


Figure (7). Time-point profile of change in hPTH (1-34) (non-oxidised form) concentration after treatment of human plasma spiked with 500 pg/mL hPTH (1-34) with 1 mM H_2O_2. A decline in non-oxidised PTH (1-34) by time was observed with LC-MS/MS, whereas no change was observe with IDS immunoassay. This indicates that immunoassay detects both oxidised and non-oxidised forms of PTH (1-34).

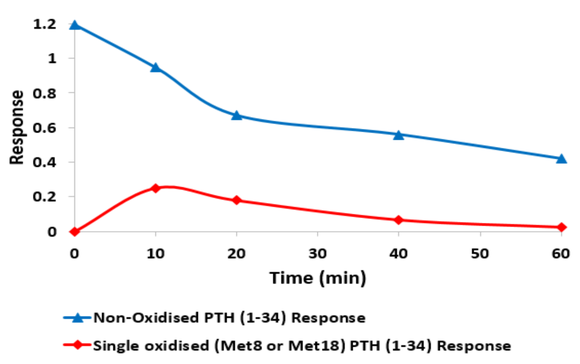


Figure (8). Illustration of time-point change in LC-MS/MS response of single oxidised and non-oxidised forms of hPTH (1-34) when human plasma spiked with 500 pg/mL hPTH (1-34) treated with 1 mM H_2O_2. The decline in non-oxidised PTH (1-34) level associated with a concurrent increase in oxidised PTH (1-34) forms level.

Conclusions

- **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 standard materials 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.**