THE DEVELOPMENT OF AN ELISA ASSAY FOR THE DETERMINATION OF PTH (1–34) IN HUMAN PLASMA (EDTA) AND FOR THE DETERMINATION OF ANTI-PTH (1–34) ANTIBODIES IN HUMAN SERUM

Sarah K. Peters, MA, Senior Scientist
Richard Sukovaty, BS, Senior Scientist
Curtis Sheldon, BS, Associate Director, Bioanalytical Principal Investigator

Introduction

Parathyroid hormone (PTH) is an 84 amino acid polypeptide produced in the parathyroid gland. PTH plays a major role in maintaining serum calcium concentrations in the range required for metabolic and neuroregulatory functions. The N-terminal portion of the molecule is required for activity. PTH (1-34) is a 34 amino acid polypeptide consisting of the 34 N-terminal amino acids of PTH (1-84) which shows similar effects on serum calcium. PTH (1-34) is commercially available (teriparatide) as an injection for treatment of osteoporosis.

A quantitative method for PTH (1-34) has been validated for pharmacokinetic (PK) assessment of samples. A method for the detection of antibodies against (ADA) PTH (1-34) was also developed and validated.

Methods (PK)

Pre-treated samples and calibration samples in human plasma were pipetted into microtiter plates coated with the appropriate capture antibody. The wells were washed to remove the unbound sample material and enzyme-labeled antibody added. Unbound labeled antibody was removed and a chromogenic substrate added to the bound labeled antibody. The development of the colored reaction product was directly proportional to the amount of PTH (1-34) present in the sample and was detected using a colorimetric plate reader.

Results (PK)

PTH (1–34) uses a 4-parameter logistic regression weighted $1/Y^2$ over the analytical range 6.00 – 150 pg/mL. The concentrations of PTH (1-34) standards were back-calculated using the regression equation and the coefficient of variation (C.V.) was less than or equal to 8.5%.

Inter-batch precision (%CV) of PTH (1–34) quality control samples between 6.00 and 150 pg/mL was less than 9.7. Inter-batch accuracy (% Bias) of the same quality controls samples was between -8.3 and +12.2.

Table 1. PTH (1-34) Inter-Batch Precision and Accuracy

PTH (1-34)	LLOQ QC 6.00 pg/mL	Low QC 18.0 pg/mL	Mid QC 75.0 pg/mL	High QC 120 pg/mL	ULOQ QC 150 pg/mL
Inter-Batch Mean	6.73	16.5	77.6	125	162
Inter-Batch SD	0.651	0.774	2.26	4.17	8.04
Inter-Batch % CV	9.7	4.7	2.9	3.3	5.0
Inter-Batch % Bias	12.2	-8.3	3.5	4.2	8.0
n	17	18	18	21	18

Short-term stability in plasma was established for 13 hours at ambient temperature under white light. Freeze and Thaw stability was established for six Freeze (-20°C) and Thaw (ambient temperature) cycles.

Long term stability of matrix samples was established for 259 days at -20°C and -80°C. Sample collection and handling stability was established in whole blood for 2 hours at ambient temperature under white light.

Table 2. Stability of PTH (1-34) During Sample Collection and Handling from Human Whole Blood (EDTA) at Ambient Temperature Under White Light Conditions

PTH 1-34	SCHS Low (pg/mL)		SCHS High pg/mL	
	0 minutes	120 minutes	0 minutes	120 minutes
	33.3	46.9	219	212
	33.7	31.8	219	211
	34.7	25.9	222	215
	34.5	27.5	218	216
	34.8	25.1	223	214
	34.5	24.2	221	214
Mean	34.3	30.2	220	214
% CV	1.8	28.5	0.9	0.9
% of Control		88.0		97.3
n	6	6	6	6

An evaluation of dilution integrity demonstrated that a dilution factor of 150 can be applied to PTH (1-34) samples to dilute them into the quantifiable range.

The absence of a Hook Effect was demonstrated by assaying a sample with a concentration higher than the ULOQ, at a minimum of 3 dilution levels above the ULOQ. All samples back-calculated with concentrations greater than the ULOQ.

Table 3. PTH (1-34) Validation Summary

Validation Summary	
Analyte	PTH (1-34)
Method Description	Sample pre-treatment and direct analysis using enzyme linked immunosorbent assay (ELISA)
Limit of Quantitation (pg/mL)	6.00 pg/mL
Standard Curve Concentrations (pg/mL)	3.00 (anchor point), 6.00, 10.0, 25.0, 40.0, 60.0, 75.0, 100, 130, and 150 pg/mL
QC Concentrations (pg/mL)	LLOQ QC, 18.0, 75.0, 120, and ULOQ QC pg/mL
QC Intra-Batch Precision Range (% CV)	0.3 to 7.4%
QC Intra-Batch Accuracy Range (% Bias)	-16.1 to 21.7%
QC Inter-Batch Precision Range (% CV)	2.9 to 9.7%
QC Inter-Batch Accuracy Range (% Bias)	-8.3 to 12.2%
Bench-Top Stability (Hrs)	Short-Term Stability: 13 hours in polypropylene tubes at ambient temperature under white light
Stock Stability (Days)	Long-Term Stability for Stock Solutions (Stock): 131 days at 1.00 mg/mL in water in a polypropylene container at -20°C
Freeze-Thaw Stability (Cycles)	6 freeze (-20°C)-thaw (ambient temperature) cycles in polypropylene tubes under white light (LLOQ and ULOQ QCs), 5 freeze (-20°C)-thaw (ambient temperature) cycles in polypropylene tubes under white light (QC D)
Long-Term Storage Stability (Days)	Long-Term Stability: 284 days (QC A and ULOQ QC) and 259 days (QC D) in polypropylene tubes at -20°C and -80°C
Dilution Integrity	Samples diluted up to 150-fold can be quantified
Selectivity	No quantitation greater than the LLOQ of PTH 1-34 was observed from endogenous components in any of the 10 human plasma (EDTA) lots screened
Assay Volume Required	0.600 mL
Regression Type	4PL, $1/Y^2$
Known Metabolite	PTH 1-84 100, 150, and 500 pg/mL
Matrix Effect	No significant matrix effect was observed in 8 of the 9 human plasma (EDTA) lots that were fortified near the concentration of the LLOQ (6.00 pg/mL) and in 8 of the 10 human plasma (EDTA) lots that were fortified near the concentration of the high QC (120 pg/mL) sample
Long-Term Stability for Stock Solutions (Substock)	285 days at 10,000 ng/mL in assay dilution buffer in a polypropylene container at -20°C 99 days at 5000 ng/mL in human plasma (EDTA) in a polypropylene container at -20°C 285 days at 5.00 ng/mL in assay dilution buffer in a polypropylene container at -20°C
Short-Term Stability for Stock Solutions (Substock)	8 hours at 1.00 mg/mL in water in a polypropylene container at ambient temperature under white light
Short-Term Stability for Stock Solutions (Substock)	8 hours at 5.00 ng/mL in assay dilution buffer in a polypropylene container at ambient temperature under white light
Stability of Analyte During Sample Collections and Handling	up to 120 minutes in human whole blood (EDTA) in polypropylene tubes at ambient temperature under white light
Batch Size	1, 96 well plate

Conclusion (PK)

The validated methods allow for rapid, selective, accurate and reproducible quantitation of PTH (1-34) in human plasma samples for PK evaluation and detection of antibodies against PTH (1-34).

Methods (ADA)

Antibodies against PTH (1-34) were detected using a bridging ELISA method. Pre-treated samples were added to a plate coated with PTH (1-34) and anti-PTH (1-34) antibodies present detected by the addition of biotinylated PTH (1-34), followed by subsequent washing and addition of HRP-streptavidin and a chromogenic substrate. The development of the colored reaction product was directly proportional to the amount of anti PTH (1-34) antibodes present in the sample and was detected using a colorimetric plate reader.

Conclusions (ADA)

The validated methods allows for detection of antibodies against PTH (1-34).

Table 4. Anti PTH (1-34) Antibodies Validation Summary

Validation Summary	
Analyte	Anti-PTH (1-34) Antibodies
Method Description	Bridging enzyme linked immunosorbent assay (ELISA)
Limit of Quantitation	206 ng/mL (Sensitivity at 99%)
Bench-Top Stability (Hrs)	Short-Term Stability: 24 hours in polypropylene tubes at ambient temperature under white light
Stock Stability (Days)	Long-Term Stability for Stock Solutions (PTH 1-34 Stock): 131 days at 1.00 mg/mL in water in a polypropylene container at -20°C Long-Term Stability for Stock Solutions [PTH (1-84) Stock]: 132 days at approximately 100 µg/mL in phosphate-buffered saline/0.1% bovine serum albumin in polypropylene tubes at -20°C
Freeze-Thaw Stability (Cycles)	6 freeze (-20°C)-thaw (ambient temperature) cycles in polypropylene tubes under white light
Assay Volume Required	0.0200 mL
Inhibition Compound Evaluation	PTH (1-34) at 500 ng/mL, PTH (1-84) at 500 ng/mL
Minimum Required Dilution	1:20
Validation Cut Point (vCP)	0.092
Specificity Cut Point	24% (for PTH 1-34), 20% (for PTH 1-84)
Correction Factor	0.020
Batch Size	1, 96 well plate