Development of a method for parathyroid hormone fragments quantitation using liquid chromatography coupled with tandem mass spectrometry

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Introduction

- Parathyroid hormone (PTH) is a 84 amino acids peptide synthetized by the parathyroid glands (PTGs). Intact PTH (PTH 1-84) plays an important role in the calcium phosphate metabolism.
- PTH 1-84 is routinely quantified for the diagnosis of primary and secondary hyperparathyroidism.
- In addition to PTH 1-84, various fragments can be detected in plasma samples and can represent up to 80% of circulating PTH.
- The exact role of these fragments is still unclear but these species are known to be high in abundance in the case of chronic kidney disease (CKD).
- The goal of this study is to develop a LC-MS method using solid phase extraction (SPE) coupled with LC-MS/MS detection.

Material and Methods

1) Mass spectrometry parameters (quantification ions)

Fragment name	MS Q1 (Da)	MS Q3 (Da)	Transitions	CE (V)	DP (V)	EP (V)	CXP (V)
PTH 34-84	609.2	721.3	PTH 34-84 : 9+ -y47 7+	24	70	10	31
PTH 37-84	516.6	547.1	PTH 37-84 : 10+ -y45 9+	21	50	10	23
PTH 38-84	561.3	615.2	PTH 38-84 : 9+ -y45 8+	21	50	10	26
PTH 38-77	613.3	594.8	PTH 38-77 : 6+ -b39 5+	30	100	10	34
PTH 45-84	626.7	700.1	PTH 45-84 : 6+ -y38 7+	29	75	10	28
PTH 28-84	562.9	615.3	PTH 28-84 11+ - y45 8+	23	70	10	26
PTH 34-77	674.6	745.9	PTH 34-77 7+ - y42 6+	24	80	10	32
PTH 37-77	629.3	714.7	PTH 37-77 7+ - b40 6+	26	70	10	29

- Analyses were performed on a QTRAP® 6500 LC-MS/MS System from AB SCIEX coupled with of HPLC from Shimadzu.

Curtain gas (CUR)	25
Collision	High
IonSpray Voltage	5500
Temperature	350°C
GS 1	40
GS 2	60

- In addition to these peptides, internal standard previously used in a HRMS study were used (1).

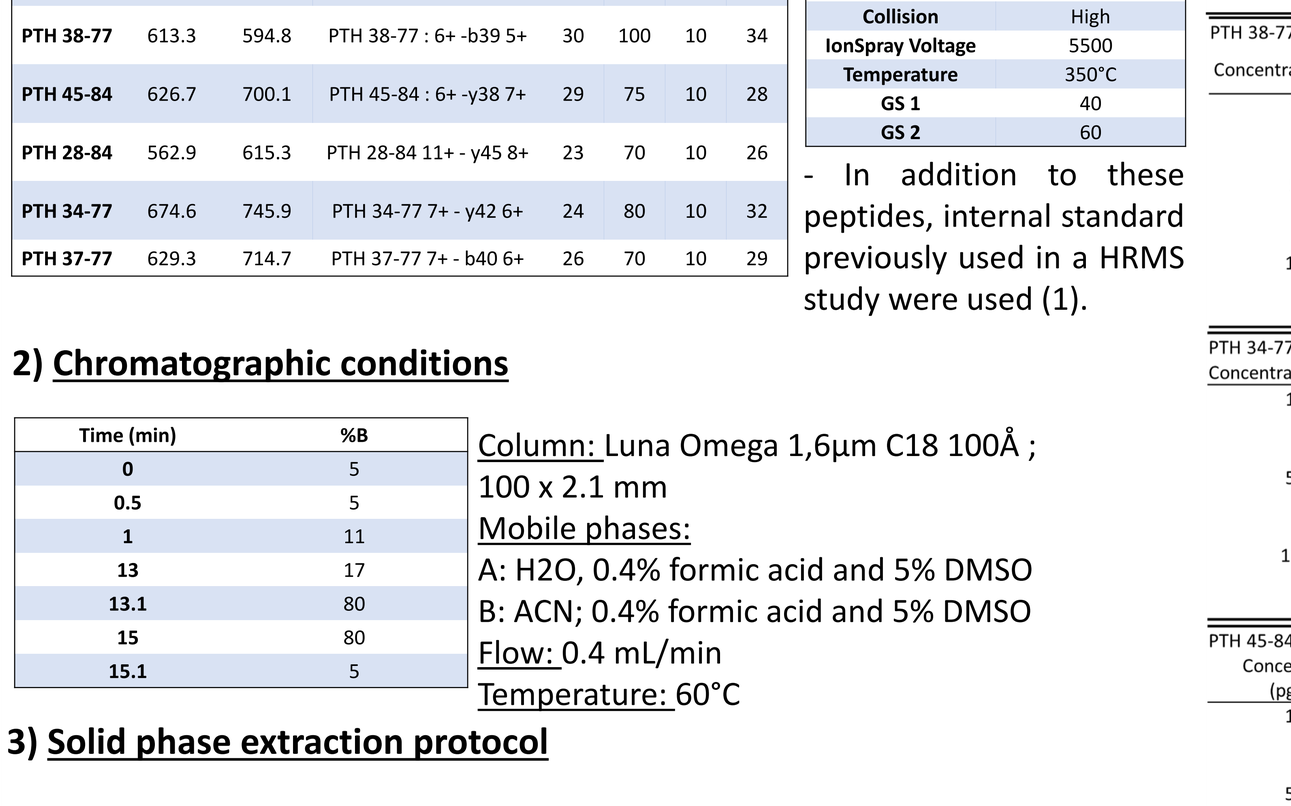
2) Chromatographic conditions

Time (min)	%B
0	5
0.5	5
1	11
13	17
13.1	80
15	80
15.1	5

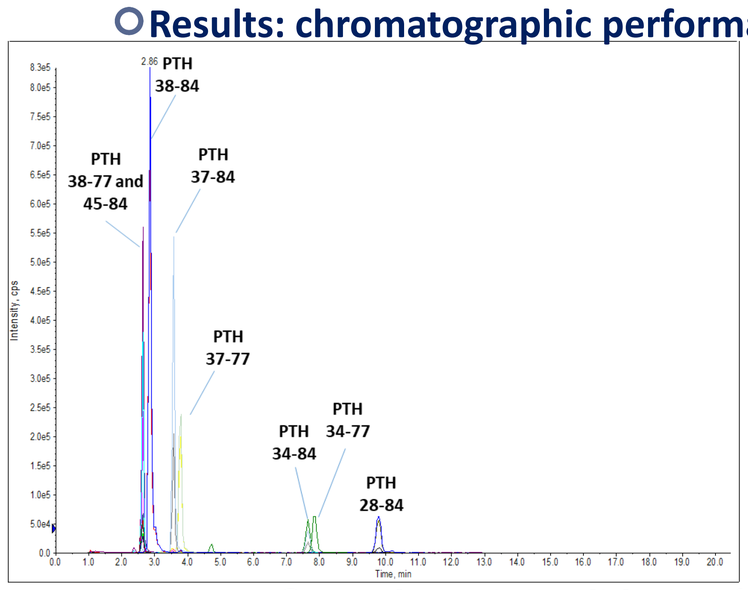
Column: Luna Omega 1,6µm C18 100Å ; 100 x 2.1 mm
Mobile phases:
A: H2O, 0.4% formic acid and 5% DMSO
B: ACN; 0.4% formic acid and 5% DMSO
Flow: 0.4 mL/min
Temperature: 60°C

3) Solid phase extraction protocol

Oasis MAX (Waters, UK)



Results: chromatographic performances and calibration points



- 10 calibration points: 25, 50, 100, 200, 300, 400, 500, 750, 1000 and 1500 pg/mL
- All calibration curves with an $r^2 > 0.99$

Results: mini-validation (n = 3 on 2 or 3 days)

PTH 38-84 - 9+ - y45 8+

Concentration (pg/ml)	Day	Mean (pg/ml)	Recovery (%)	Intraday (CV, %)	Interday (CV, %)
150	1	136.5	91.0	11.6	
	2	149.8	99.9	5.0	13.3
	3	176.9	118	4.7	
500	1	468.2	93.6	6.2	
	2	475.3	95.1	7.6	/
	/	/	/	/	
1000	1	894.4	89.4	2.0	
	2	1007.2	100.7	4.6	12.9
	3	1157.4	115.7	15.4	

PTH 34-84 - 9+ - y47 7+

Concentration (pg/ml)	Day	Mean (pg/ml)	Recovery (%)	Intraday (CV, %)	Interday (CV, %)
150	1	149.5	99.6	3.7	
	2	134.3	89.5	7.0	8.2
	3	158.2	105.4	9.1	
500	1	472.0	94.4	2.6	
	2	436.4	87.3	3.8	10.3
	3	534.4	106.9	1.8	
1000	1	932.8	93.3	3.8	
	2	970.9	97.1	5.6	2.1
	3	944.3	94.4	1.0	

PTH 38-77 7+ - y38 6+

Concentration (pg/ml)	Day	Mean (pg/ml)	Recovery (%)	Intraday (CV, %)
150	1	177.9	111.5	4.2
	2	151.0	100.6	8.9
500	1	543.1	108.6	5.8
	2	516.3	103.3	6.6
1000	1	1052.5	105.3	5.2
	2	987.0	98.7	2.2

PTH 37-84 10+ - y45 9+

Concentration (pg/ml)	Day	Mean (pg/ml)	Recovery (%)	Intraday (CV, %)
150	1	144.0	96.0	9.4
	2	132.0	88.0	12.2
500	1	420.8	85.3	6.8
	2	470.1	94.0	6.5
1000	1	968.0	96.8	4.5
	2	898.9	89.9	8.5

PTH 34-77 7+ - b43 7+

Concentration (pg/ml)	Day	Mean (pg/ml)	Recovery (%)	Intraday (CV, %)
150	1	167.6	111.7	10.4
	2	175.3	116.9	3.8
500	1	525.8	105.2	11.6
	2	512.5	102.5	2.7
1000	1	950.2	95.0	5.6
	2	916.9	91.7	3.5

PTH 37-77 7+ - b40 6+

Concentration (pg/ml)	Day	Mean (pg/ml)	Recovery (%)	Intraday (CV, %)
150	1	181.8	**121.2**	4.0
	2	169.6	113.1	6.5
500	1	558.7	111.7	2.7
	2	501.1	100.2	3.6
1000	1	1009.8	101.0	3.5
	2	957.0	95.7	2.6

PTH 45-84 7+ - y38 7+

Concentration (pg/ml)	Day	Mean (pg/ml)	Recovery (%)	Intraday (CV, %)
150	1	149.7	99.8	14.8
	2	161.1	107.4	7.5
500	1	506.7	101.3	9.9
	2	**522.2**	**104.4**	**23.7**
1000	1	962.8	96.3	12.7
	2	979.1	97.9	13.8

PTH 28-84 10+ - y45 8+

Concentration (pg/ml)	Day	Mean (pg/ml)	Recovery (%)	Intraday (CV, %)
150	1	155.7	103.8	8.6
	2	143.1	95.4	8.0
500	1	526.4	105.3	10.5
	2	**546.5**	**109.3**	**30.5**
1000	1	988.8	98.9	9.2
	2	**1200.5**	**120.0**	**23.1**

- Results on PTH 28-84 and 45-84 to be improved.

Results on CKD patients

- 40 samples:
  * 10 with GFR < 15
  * 10 between 15 and 30
  * 10 between 30 and 45
  * 10 between 45 and 60
- Similar trends as other previously reported article (1)

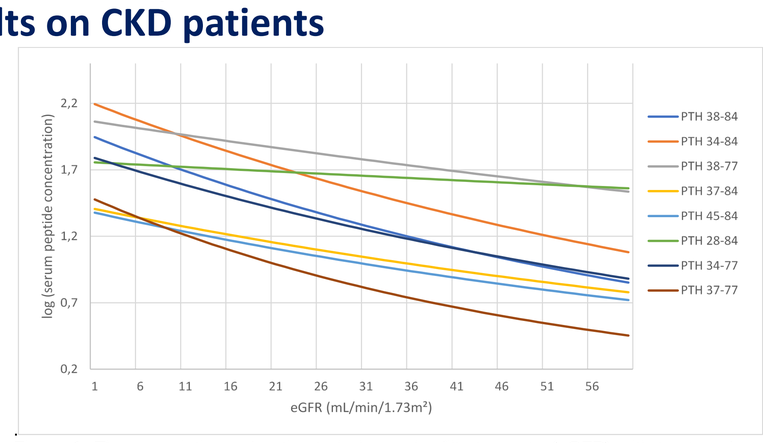


Figure 1: The associations of estimated glomerular filtration rate (eGFR) with log-transformed concentrations of eight PTH-related peptides. Results were obtained with 40 patient samples (10 with one eGFR between 0 and 15; 10 with one eGFR between 15 and 30; 10 with one eGFR between 30 and 45 and 10 with one eGFR between 45 and 60). For a better vizualisation, only exponential trend curves are presented. PTH 38-84 : $y = 1.9729e^{-0.014x}$; PTH 38-77: $y = 2.0726e^{-0.005x}$; PTH 34-84 : $y = 2.2204e^{-0.012x}$; PTH 37-84 : $y = 1.4198e^{-0.01x}$; PTH 48-84 : $y = 1.3928e^{-0.011x}$; PTH 28-84 : $y = 1.7596e^{-0.002x}$; PTH 34-77 : $y = 1.8105e^{-0.012x}$; PTH 37-77 : $y = 1.4775e^{-0.02x}$.

Conclusions and prospects

- One LC-MS/MS method has been developped for the quantitation of 8 PTH-related peptides in human serum. This method can easily be used in clinical routine.
- Results can be improved for PTH 28-84 and PTH 45-84 peptides.
- Results obtained with CKD-patients are consistent with previously reported data.

Bibliography

(1) Kritmetapak, K. *et al.* Chemical Characterization and Quantification of Circulating Intact PTH and PTH Fragments by High-Resolution Mass Spectrometry in Chronic Renal Failure. *Clinical Chemistry* **67**, 843–853 (2021)