WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 10, 1732-1746. Research Article ISSN 2277– 7105

A VALIDATED LCMS/MS METHOD FOR ESTIMATION OF CINACALCET IN HUMAN PLASMA

Srinivasa Reddy, Imran Ahmed. M, Licto Thomas, Nirmala Nayak, Arindam Mukhopadhyay* and Saral Thangam

Norwich Clinical Services Pvt. Ltd. 147/F, 8th Main, 3rd Block, Koramangala, Bangalore–560034.

Article Received on
7 Aug 2015,

Revised on 27 Aug 2015,
Accepted on 16 Sep 2015

***Correspondence for Author**
Dr. Arindam Mukhopadhyay
Norwich Clinical Services Pvt. Ltd. 147/F, 8th Main, 3rd Block, Koramangala, Bangalore–560034.

ABSTRACT

The calcium-sensing receptor (CaR) is responsible for controlling the synthesis and secretion of parathyroid hormone. Cinacalcet, an allosteric modulator of CaR signaling, is used for the treatment of secondary hyperthyroidism in patients with chronic kidney disease and for the treatment of elevated calcium levels in patients with parathyroid carcinoma. A highly sensitive LCMS/MS method for quantification of cinacalcet was developed and validated. An isocratic binary mobile phase containing 5mM Ammonium acetate (pH 4.0±0.2): Acetonitrile:: 40:60 (v/v) was used at a flow rate of 1.2 ml/min to achieve chromatographic separation of cinacalcet on SCX column (50 x 4.6 mm; 5 µm) maintained at 50 ^{0}C. It is estimated in API 4000 Mass spectrometer in the positive atmospheric pressure ionization mode by following multiple reactions monitoring (MRM) transitions for parent and daughter ions. A linear calibration plot was achieved for cinacalcet in the concentration ranges 0.207 to 120.507 ng/ml. The mean percentage of recovery was 108 with mean % CV of 2.10. It is a fully validated method and can successfully be applied for estimation of cinacalcet during bio-studies.

KEY WORDS: Cinacalcet, Liquid Chromatogrphy – mass spectrometry (LCMS/MS), Ion-exchange (SCX) column, Human Plasma, Calcium – sensing receptor, Parathyroid hormone.

INTRODUCTION

The calcium-sensing receptor (CaR), a seven-transmembrane domain receptor belonging to the G protein-coupled receptor family, is responsible for calcium-mediated signaling initiated

at the surface of parathyroid cells that controls the synthesis and secretion of parathyroid hormone (PTH). Cinacalcet [N-[1-(R)-(-)-(1-naphthyl) ethyl]-3-[3-(trifluoromethyl) phenyl]-1-aminopropane HCl] is a type II calcimimetic agent that acts as an allosteric modulator of CaR signaling. By increasing the sensitivity of the calcium sensing receptor to extracellular calcium it suppresses PTH secretion and regulates bone metabolism and thereby provides a novel approach for treatment of secondary hyperthyroidism in patients with chronic kidney disease.[1–3] Cinacalcet has recently been approved for the treatment of secondary hyperthyroidism in patients with chronic kidney disease placed on dialysis.[4] and for the treatment of elevated calcium levels in patients with parathyroid carcinoma.[5]

A simple, selective drug estimation method requiring small sample volume and rapid turnaround time is ideal for pharmacokinetic method. Several methods concerning the determination of cinacalcet in plasma samples have been reported.[6-11] However, these methods are having their own inherent problems which need to be addressed before it can be used as an accepted method for cinacalcet estimation for pharmacokinetic analysis involving human subjects. Wani et. al.[11] described recently a LCMS/MS method for cinacalcet estimation in human plasma. They used Liquid-liquid extraction procedure for sample preparation and abiraterone as an internal standard. The LCMS/MS method for cinacalcet estimation in plasma reported by Cangemi et.al.[6] has several advantages over the other methods which includes less plasma volume, precipitation technique for sample preparation, a deuterated labeled internal standard to minimize analytical and interpatient variation; however, the method has used gradient for reversed-phase chromatographic separation. Also, the total run time for one sample in this method was 7 minutes which may not be suitable for high-throughput analysis. We describe here a modified, validated and sensitive method for estimation of cinacalcet in human plasma which can be utilized for routine estimation of cinacalcet in human plasma.

EXPERIMENTAL

Chemical and Reagents

Cinacalcet and Cinacalcet D3 used as internal standard were procured from Vivan Life Sciences, India.

Acetonitrile (HPLC-grade) and ammonium acetate were procured from Merck, India. Others chemicals required for the study were of analytical grade and purchased locally.

Milli Q purified water (Millipore, Milford, MA) was used to prepare the aqueous solutions. Blood was collected from adult, healthy volunteers in a vacutainer containing K_2 EDTA as an anticoagulant. Plasma was separated by centrifugation at 3000 g for 10 min, and kept frozen at -70^{o}C until analysis.

Sample Preparation

After vortexing 200 µl of thawed sample was transferred to a new RIA vial. 50 µl of internal standard (IS; Cinacalcet D3) was then added and mixed. 1mL of acetonitrile was added to the vial and kept in a vibramax at 2500 rpm for 15 min. It was then centrifuged at 11000 rpm for 5 min at 4°C. 500µl of supernatant was transferred to new RIA vial and 300 µl of Milli-Q water was then added. After vortexing the sample was transferred to labeled HPLC vials to load into auto sampler.

Chromatography & Mass Spectrometry

An isocratic method for separation with the mobile phase consisting of 5mM Ammonium acetate (pH 4.0±0.2): Acetonitrile:: 40:60 (v/v) was used. Flow rate was fixed at 1.2 mL/min with a splitter. 20 µl of sample was injected to an ion-exchange column, Bio-basic SCX, 50 X 4.6mm, 5µm (Thermo Scientific) for separation. The column oven temperature was maintained at 50 °C. Run time was 3.5 min.

Mass spectrophotometric detection was performed on LCMS/MS API 4000 (AB Scientific Applied Biosystems) in positive mass ionization mode with multiple reaction monitoring (MRM) using an electrospray interface (ESI). Cinacalcet and IS were detected using selective reaction monitoring of the specific transitions m/Z 358.2 > 155.1 and 361.1 > 158.1, respectively. The collision energy and cone voltage were optimized for each analyte to maximize the signal corresponding to the major transition observed in the MS/MS spectra, following the fragmentation of the $[M+H]^+$ ions corresponding to the selected compounds. The ion source parameters were the following: CUR 30 psi; GS1 45 psi; GS2 55 psi; CAD 8 psi; ion spray voltage (ISV) 5500 V; and temperature 450^{0}C. The other parameters for both cinacalcet and the IS were: declustering potential (DP): 60 V, entrance potential (EP): 10 V, collision energy (CE): 21 V and cell exit potential (CXP): 10 V.

Quality Control Samples and Calibration Standards

Cinacalcet and IS standard stock solutions were prepared in methanol (final concentration 200µg/ml). This final concentration was further corrected accounting for its potency and

actual amount weighed. Standard stock solution of cinacalcet was serially diluted to prepare working solutions in the required concentration range with diluent methanol–water (50:50, v/v). The calibration standards were prepared by spiking with working solutions yielding concentration range from 0.207 to 120.507 ng/ml for cinacalcet. Cinacalcet QC stock solutions have been prepared separately in methanol–water (50:50, v/v). QC samples at four different levels: 0.2 ng/ml lower limit of quantitation (LLOQ), 0.558 ng/ml low quality control (LQC) (LQC, within three times of the LLOQ), 37.168 ng/ml middle quality control (MQC) and 92.921 ng/ml high quality control (HQC) were prepared similarly as calibration standards. Internal standard working solution was prepared by diluting the IS stock solution in methanol–water (50:50, v/v) to get 50 ng/ml.

Validation of the Bioanalytical Method

The method was validated as per US-FDA guideline in human plasma.[12] The method was evaluated for linearity of response, selectivity, precision, accuracy, recovery, stability of analytes and dilution integrity during both long-term storage and short-term sample processing.

RESULT AND DISCUSSION

Estimation of plasma cinacalcet by LCMS/MS using solid phase sample extraction procedure has been described.[8] Also, a LCMS/MS method for estimation of cinacalcet in plasma using liquid – liquid extraction method is also available.[10] Clean samples are important to minimize ion -suppression and matrix effect. Sample preparation using liquid –liquid extraction (LLE) or solid phase extraction technique can be helpful in producing the clean samples devoid of any endogenous substance to interfere with the analysis and functioning of LC and MS/MS systems. However, these extraction techniques are time consuming and costly. This method involves a more cost effective precipitation method which gave a cleaner sample with relatively less matrix effect. Moreover, use of atmospheric pressure ionization technique along with SCX column further reduces the matrix effect drastically. One of the main advantages of ion exchange is that there is only one interaction involved in the separation: the analytical species interacting with the stationary phase. As a result, ion-exchange chromatography may have more matrix tolerance.

Matrix Effect

The assessment of matrix effect (co-eluting, undetected endogenous matrix compounds that may influence the analyte ionization) was evaluated for eight lots of blank matrix including

one heamolysed and one lipemic lot from different sources. 200 µL of blank plasma from each lot was mixed with 50 µL IS (Cinacalcet D3) and 1 mL of acetonitrile and kept in a vibramax at 2500 rpm for 15 min for complete precipitation of protein. Aqueous solution of cinacalcet either at LQC or HQC level was added to each of the supernatant obtained after centrifugation at 11000 rpm for 5 min at 4°C. These samples were considered as post extracted samples (presence of matrix).

Similarly, the aqueous solutions of cinacalcet either at LQC or HQC level were mixed with precipitating agent (containing 50 µL IS) and considered as aqueous samples (absence of matrix). Six replicates each of aqueous samples was injected along with post extracted samples of LQC and HQC.

Individual analyte area response and IS area response of each post extracted sample were compared with the mean analyte area response and mean IS area response of the aqueous sample respectively. The matrix effect was calculated via the formula: Matrix effect (%) = A_2/ A_1 x 100 (%), Where A_1= response of aqueous concentrations and A_2 is response of post-spiked concentrations.

From the calculations, it was observed that cinacalcet showed an average (n=6) matrix factor of 95.959% at LQC level with a CV of 3.55 % and 102.90 at HQC level with a CV of 1.26% which is within the accepted limit (% CV ≤15) (Table- 1).

Table -1: Matrix Effect for Estimation of Cinacalcet.

Matrix ID	LQC analyte area in absence of matrix	LQC analyte area in presence of matrix	LQC matrix factor for analyte	HQC analyte area in absence of matrix	HQC analyte area in presence of matrix	HQC matrix factor for analyte
PL_579	10159	9193	89.87	1670372	1623774	100.60
PL_586	10456	9623	94.07	1637797	1648547	102.13
PL_587	10216	10158	99.30	1601486	1675502	103.80
PL_588	9824	9916	96.94	1584115	1667130	103.28
PL_589	10437	10109	98.82	1599491	1684138	104.34
PL_590	10285	9588	93.73	1591395	1682633	104.25
LPL_593		10203	99.74		1661347	102.93
HPL_573		9728	95.10		1644747	101.90
Average	10229.500	9814.750	95.946	1614109.333	1660977.250	102.904
SD	231.094	347.989	3.402	33212.162	20895.547	1.295
%CV	2.26	3.55	3.55	2.06	1.26	1.26

Selectivity

Human blank plasma lots were assessed for the selectivity of the method and batches showing negligible or no interference at the retention time of internal standards and analytes were selected. Eight different lots of blank matrix including one haemolysed and one lipemic lot (used for specificity) were spiked with LLOQ and intended concentration (50 ng/ml) of internal standard and processed. Interferences at the retention times of analytes and IS were evaluated by comparing peak area response with that of blank plasma. Signal to noise ratio for all lots was more than 5.0 indicating the method is selective for cinacalcet (Table - 2).

Table 2: Specificity and Selectivity of Cinacalcet.

	Specificity		Selectivity					
Blank matrix ID	Analyte response	IS response	Analyte response	IS response	Area ratio	% Interference for analyte	% Interference for IS	Signal to noise
PL_579	0	0	2523	162513	0.015	0.00	0.00	558.362
PL_586	0	0	2575	170181	0.015	0.00	0.00	281.092
PL_587	0	0	2903	169711	0.017	0.00	0.00	172.216
PL_588	0	0	2882	173437	0.016	0.00	0.00	136.398
PL_589	0	0	2820	172537	0.016	0.00	0.00	510.142
PL_590	0	0	3006	173719	0.017	0.00	0.00	133.251
LPL_593	0	0	3054	174707	0.017	0.00	0.00	264.266
HPL_573	0	0	2776	172708	0.016	0.00	0.00	485.401

Carry Over Effect

Carryover effect was evaluated to ensure that the rinsing solution used to clean the injection needle and port was able to avoid any carry forward of injected sample in subsequent runs.

The design of the experiment comprised LLOQ, blank plasma, upper limit of quantitation (ULOQ) followed by blank plasma to check for any possible interference due to carryover. There was no carry over observed.

Chromatography

Retention times for both cinacalcet and cinacalcet D3 were 1.66 min and 1.64 min (Fig.1 a & b). As per FDA guidelines, standards should be preferably identical to the analyte (19) and hence deuterated standards were used as the reference compounds. No interfering peaks were observed in the blank at the retention times corresponding to both analytes and IS indicating that the procedure is specific to cinacalcet.

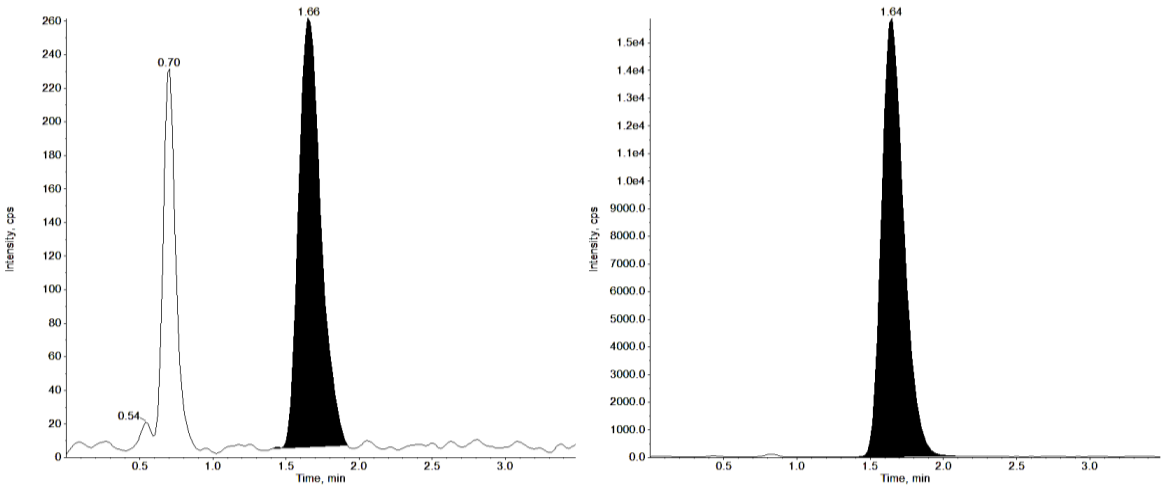

Figure 1 a & b: Chromatograms of Cinacalcet and IS.

Limit of detection

To find out the detection limit stock solution was diluted to 0.104ng/ml (A/2) and 0.052ng/ml (A/4) which were close to the detection limit (based on LLOQ area response). The above diluted solutions were spiked into matrix in triplicates, and processed along with LLOQ sample in triplicates. As indicated in the Table – 3 limit of detection in this method was 0.052ng/ml as signal to noise was more than 7.

Table – 3: Limit of detection of Cinacalcet.

Sample ID	Nominal conc. (ng/ml)	Analyte area counts	Signal to noise
STD_A	0.207	3238	54.483
STD_A	0.207	3245	78.467
STD_A	0.207	3058	72.099
STD_A/2	0.104	1366	50.610
STD_A/2	0.104	1275	27.705
STD_A/2	0.104	1526	18.305
STD_A/4	0.052	597	10.030
STD_A/4	0.052	600	20.177
STD_A/4	0.052	583	7.046

Sensitivity

To determine sensitivity of the method six samples of LLOQ (STD A) were processed and were injected along with the PA. This method for quantification of cinacalcet is found to be very sensitive even for very low concentration (LLOQ) of 0.207 ng/ml with precision of 6.27% (acceptable limit $\leq$ 20%) and accuracy of 93.48% (acceptable limit ±20%).

Precision and Accuracy

Precision and accuracy were determined by injecting a set of calibration curve samples and quality control samples at four different concentrations – LLOQC (lower limit of quantification), LQC (low QC sample; within 3x the LLOQC), MQC (middle QC) and HQC (high QC; one near the upper boundary of the standard curve) samples. 5 precision and accuracy batches were run during the course of 3 days. In each of the precision and accuracy batches, eight calibration standards and six replicates at each quality control level were analyzed. Precision and accuracy were calculated in terms coefficient of variation (%CV). At each concentration level a deviation within ± 15.0% from the nominal concentration was acceptable except LLOQ, for which it should be within ± 20.0%. Minimum 67% (4 out of 6) of the quality control samples at each level should meet the acceptance criteria. Table – 4 (a & b) showed the mean accuracy and precision for intra- (within run) & inter-day (between) batches.

Table – 4a: Precision & Accuracy of method for cinacalcet analysis for calibration standards.

Sample ID	Mean conc. (ng/ml)	SD	Mean accuracy	%CV
STD A	0.209	0.004	100.77	1.75
STD B	0.408	0.014	98.31	3.52
STD C	6.526	0.166	100.72	2.54
STD D	18.063	0.225	100.36	1.24
STD E	35.838	0.334	99.56	0.93
STD F	59.990	0.610	99.99	1.02
STD G	96.427	1.035	99.65	1.07
STD H	121.685	1.723	100.61	1.42

Table – 4b: Precision & Accuracy of method for cinacalcet analysis for quality control samples.

Analyte	LOQQC	LQC	MQC	HQC
Within run accuracy	100.08% to 113.94%	96.29% to 114.39%	100.39% to 104.44%	97.98% to 101.68%
Within run precision	2.79% to 18.60%	1.11% to 13.85%	0.34% to 0.97%	0.79% to 3.48%
Between batch accuracy	106.28%	103.69%	101.97%	99.95%
Between batch precision	10.43%	8.74%	1.62%	2.10%

As per FDA guidelines the within run and between run percent accuracy should be within ±15% of the actual concentration for each quality control sample except for LOQQC. For LOQQC, it should be within ±20%.The within run and between run precision (%CV) should be less than or equal to 15% for each QC except for LOQQC for which it should be less than or equal to 20%.

Thus the result given in Table - 4 (a & b) showed that the method was precise and accurate.

Linearity of the Calibration Plot

Calibration curve of cinacalcet indicated a linear graph for cinacalcet in the concentration ranges of 0.207 to 120.507 ng/ml (Fig. 2). Residual sum of squares were obtained to check the large linear ranges used for this drug. The concentration of unknown was calculated from the equation y= mx +c using regression analysis of spiked plasma calibration standards with reciprocal of the square of the drug concentration ($1/X^2$).

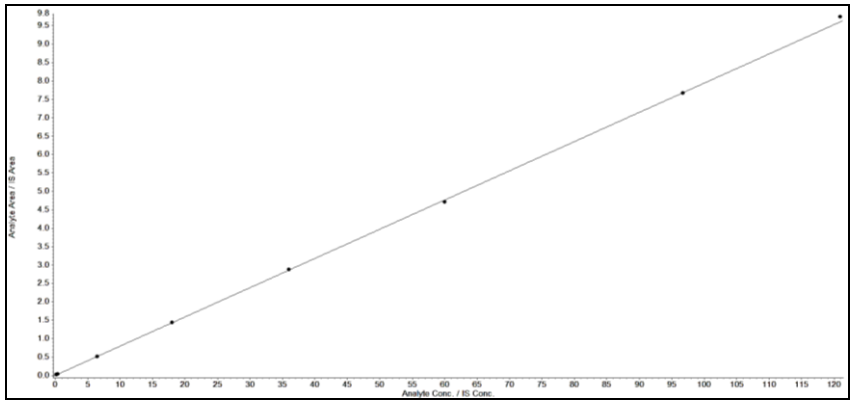

Fig. – 2: Calibration curve for Cinacalcet.

Recovery

Absolute recovery percentage was determined by comparing the mean peak area of analyte obtained by injecting 6 extracted samples of LQC, MQC and HQC with the mean peak area obtained by injection of respective aqueous standard solutions. In this method overall recovery was 108% with a %CV of 2.10 which is well within 15% (Table 5).

Table – 5: Recovery of Cinacalcet from Biological Matrix.

LQC (0.557 ng/mL)			MQC (37.151 ng/mL)			HQC (92.878 ng/mL)		
Unextracted area (n=6)	Extracted area (n=6)	Mean percentage recovery	Unextracted area (n=6)	Extracted area (n=6)	Mean percentage recovery	Unextracted area (n=6)	Extracted area (n=6)	Mean percentage recovery
7337.500	7870.500	107.26	486905.167	539721.167	110.85	1258750.833	1342350.500	106.64

Stability studies

Short – Term/bench -top stability (STSS)

To determine the short term stability, stock solutions of analyte was prepared separately and kept at room temperature. MQC conc. of analyte was then prepared by stock dilution and stored at 25^0C for 24 hrs. Six replicate injections were given for MQC sample. No significant differences were noticed when these results were compared with those obtained from the freshly prepared MQC samples indicating that cinacalcet was stable at room temperature (Table 6). Accepted criteria for the ratio of mean response for stability samples should be between 90-110%.

Long term stock solution stability (LTSS)

LTSS is performed to ensure that the analyte is stable at 2-8^{0}C. Aqueous MQC sample was prepared from stability stock stored at 2-8 ^{0}C for 10 days and injected. Mean area response of MQC of stored stock solution was then compared against MQC from freshly prepared stock solution. Mean percent stability was 104.75 which was well within accepted limit (90 – 110%) indicating the stability of cinacalcet solution for 10 days at 2-8 ^{0}C (Table 6).

Table – 6: Stability Studies of Aqueous Stock Solutions of Cinacalcet.

STABILITY CHECK	SAMPLES	AREA FOR STORED SOLUTION	AREA FOR FRESH SOLUTION		% STABILITY
STSS (24hrs)	MQC (n=6)	748482.833	750083.667		101.69
LTSS (10 days)	MQC (n=6)	631179.000	604753.833		104.75

Stability in biological matrix

Bench-top stability

To check whether the sample is stable during analysis, six aliquots of LQC & HQC samples were thawed and kept at room temperature (25^0C) for 6 hrs 30 min, which has been decided based on the time required for analysis. The samples were then processed and analyzed as mentioned above. The % stability was 97.56 for LQC and 98.76 for HQC (well within the accepted limit of 85-115%) indicating that cinacalcet was stable at room temperature (Table – 7).

Auto-sampler stability

The stability of the processed samples in the auto sampler during analysis was determined by using six aliquots of LQC and HQC samples. The stability of cinacalcet was assessed for 22 hours and 30 mins, the expected run time for batches of validation samples. The result was

then compared with those obtained from fresh QC samples. For IS in-injector stability, the IS/analyte area ratio of MQC stored in auto sampler for 23 hours was compared against freshly prepared MQC samples. No significant difference in the results indicated that the analytes and IS were stable for at least 22.5 hour in the auto sampler (Table – 7)

Freeze thaw stability

Analyte stability was determined after four freeze thaw cycles for six aliquots of each of the LQC and HQC. The samples were stored below – 70 ^{0}C for 24h and then allowed to thaw at room temperature. After complete thawing, the samples were again stored at same temperature (– 70 ^{0}C) for 12h. The freeze thaw cycle was repeated another three times before analyzing the samples. No differences were noticed when the results were compared with the fresh QC samples indicating the stabilities of cinacalcet in K_2EDTA human plasma for four freeze thaw cycles (Table - 7).

Wet Extract stability

To check whether the sample is stable after processing, six aliquots of LQC & HQC samples were processed and kept at room temperature for 7 hours. The samples were then analyzed as mentioned above. No significant differences were noticed when these results were compared with those obtained from the fresh QC samples indicating that processed samples of cinacalcet were stable at room temperature (Table 7)

Table – 7: Stability Studies of Cinacalcet.

STABILITY CHECK	SAMPLES	NOMINAL CONC. (ng/ml)	OBSERVED CONC. (ng/ml)	%CV	% STABILITY
Bench Top for (6.5hrs)	LQC (n=6)	0.557	0.567	2.94	97.56
	HQC(n=6)	92.878	89.091	1.10	98.76
Freeze Thaw (4 cycles)	LQC (n=6)	0.557	0.566	2.87	97.36
	HQC(n=6)	92.878	90.441	1.23	100.25
In-Auto-sampler(22.5hr)	LQC (n=6)	0.557	0.574	2.63	98.82
	HQC(n=6)	92.878	90.277	0.60	100.07
Wet Extract (4.5 hr)	LQC (n=6)	0.557	0.572	4.67	98.48
	HQC(n=6)	92.878	88.206	0.99	97.77

Dilution Integrity

In order to validate the dilution test, dilution integrity experiment was carried out on higher analyte concentrations (above ULOQ), which may be encountered during real subject

samples analysis. Stock dilution at twice the concentration of HQC was spiked into plasma and aliquots were prepared with the same and stored at about -70°C until analysis.

Dilution integrity samples were prepared by diluting 2 fold and 4 fold with interference free biological matrix. Six replicates of these samples were processed and analyzed against a set of freshly spiked calibration standards. If % nominal was within ± 15 % (±20% in case of LLOQ) of nominal values and % CVs ± 15 % at both diluted levels, the integrity of the samples were considered to be maintained.

For 2 fold (2T) and 4 fold (4T) dilutions, accuracy were 94.17% & 96.54% whereas precision were 1.36% and 1.24%, respectively (data not shown).

CONCLUSION

A simple, accurate, precise, sensitive and reproducible LCMS/MS method has been developed and validated for the determination of Cinacalcet. The sample extraction method described here is protein precipitation method which is not only a simpler method compared to other available extraction methods but also produces cleaner samples with no matrix effect. Also, this method employs the simpler isocratic method for chromatographic separation. Run time is half of the previous reported one[6] and therefore making it suitable for high throughput analysis. Moreover, use of ion-exchange (SCX) column has made this method further advantageous in respect to matrix tolerance over the previously described methods using reversed-phase ion-pairing chromatography. This method is suitable for industrial use for routine cinacalcet estimation for therapeutic drug monitoring as well as pharmacokinetic analysis.

ACKNOWLEDGEMENT

The authors thank the management of Norwich Clinical Services for providing the opportunity to complete the project.

Competing Interests

All authors hereby declare that no conflict of interest is associated with the publication of this manuscript.

REFERENCES

1. Mizobuchi M, Ogata H, Hatamura I, Saji F, Koiwa F, et al. Activation of calcium-sensing receptor accelerates apoptosis in hyperplastic parathyroid cells. Biochem Biophys Res Commun, 2007; 362(1): 11– 6.
2. Mentaverri R, Yano S, Chattopadhyay N, Petit L, Kifor O, et al. The calcium sensing receptor is directly involved in both osteoclast differentiation and apoptosis. FASEB J, 2006; 20(14): 2562– 4.
3. Riccardi D and Martin D. The Role of the Calcium-Sensing Receptor in the Pathophysiology of Secondary Hyperparathyroidism -, NDT Plus, 2008; 1(1): i7–i11.
4. Amgen (2009) Sensipar for Parathyroid Carcinoma. [http://www.sensipar.com/Sensipar_for_HPC.html].
5. Block GA, Martin KJ, de Francisco AL, Turner SA, Avram MM, et al. Cinacalcet for secondary hyperparathyroidism in patients receiving hemodialysis. N Engl J Med, 2004; 350(15): 1516-25.
6. Cangemi G, Barco S, Verrina EE, Scurati S, Melioli G, Alberighi ODC. Micromethod for quantification of Cinacalcet in human plasma by liquid chromatography – tandem mass spectrometry using a stable isotope labeled internal standard. Ther Drug Monit, 2013; 35(1): 112 – 7.
7. Padhi D, Harris RZ, Salfi M, Noveck RJ, Sullivan JT. Pharmacokinetics and pharmacodynamics of cinacalcet in hepatic impairment: phase I, open-label, parallel-group, single-dose, single- centre study. Clin Drug Investig**,** 2008; 28(10): 635-43.
8. Yang F, Wang H, Zhao Q, Liu H, Determination of cinacalcet hydrochloride in human plasma by liquid chromatography- tandem mass spectrometry. J Pharm Biomed Anal, 2012; 61: 237-41.
9. Lee S, Gu N, Kim BH, Lim KS, Shin SG, Jang IJ, Yu KS. Pharmacokinetic and Pharmacodynamic Properties of the Calcimimetic Agent Cinacalcet (KRN1493) in Healthy Male Korean Subjects: A Randomized, Open-Label, Single Ascending–Dose, Parallel-Group Study. Clin Thera, 2012; 34(5): 1160 – 9.
10. Nirogi R, Kandikere V, Komarneni P, Aleti R, Padala N, Kalaikadiban I. Quantification of cinacalcet by LC-MS/MS using liquid-liquid extraction from 50 □L of plasma. J Pharm Biomed Anal**,** 2011; 56(2): 373-81.
11. Wani TA, Khalil NY, Darwish I, Iqbal M and Bakheit AH. Highly Sensitive and Simple Validated Ultra-performance Liquid Chromatography/Tandem Mass Spectrometry

Method for the Determination of Cinacalcet in Human Plasma. Curr Pharm Anal, 2014; 10: 51-7.

12. US Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for industry, Bioanalytical Method Validation. 2001 http://www.fda.gov/downloads/Drugs/.../ Guidances/ ucm070107. pdf.
13. Neubauer K. Advantages and Disadvantages of Different Column Types for Speciation Analysis by LC-ICP-MS. Spectroscopy, 2009; 24: 01.