Trade Science Inc.

December 2009 Volume 8 Issue 4

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 8(4) 2009 [594-599]

A new validated liquid chromatographic method for the determination of impurities in cinacalcet hydrochloride

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Received: 8th September, 2009 ; Accepted: 18th September, 2009

ABSTRACT

A reversed-phase liquid chromatographic method has been developed and subsequently validated for the determination of cinacalcet Hydrochloride and its process-related impurities. The separation was achieved on Ace C18 (250 x 4.6 mm, 5μ) column using a mobile phase consisting of potassium dihydrogen phosphate buffer mixed with 1.0 ml of triethylamine, adjusted to pH 6.0 with phosphoric acid and acetonitrile under gradient conditions. The flow rate was maintained at 1.2 mL min^{-1} and UV detection was performed at 223 nm. The method described is linear over the ranges of 0.028 to 0.68 μg mL^{-1} and 0.024 to 0.68 μg mL^{-1} for impurities A and B, respectively. The method is precise and accurate with % RSD value less than 1.0 % and the recovery of impurities were in the range of 96 – 102 %. The method is simple, selective and stability indicating and is useful in the quality control of bulk drug manufacturing.

INTRODUCTION

Cinacalcet Hydrochloride is a selective calcimimetic agent which acts on the calcium-sensing receptor of the parathyroid, the principal negative regulator of parathyroid hormone release, to increase its sensitivity to activation by extracellular calcium, thus decreasing parathyroid hormone. Cinacalcet is effective in the clinical setting, and is approved for the treatment of secondary hyperthyroidism in patients with chronic kidney disease on dialysis, and for the treatment of elevated calcium levels in patients with parathyroid carcinoma.

Primary hyperparathyroidism (HPT) is the leading cause of hypercalcemia in the outpatient setting, and it is treated primarily by parathyroidectomy. There are few nonsurgical treatment options for patients who do not wish to have surgery, who have failed surgery, or who have contraindications to surgery. Cinacalcet increases the sensitivity of parathyroid calcium-sensing receptors to extracellular calcium, thereby reducing serum calcium levels[1].

Persistent hyperparathyroidism is the most frequent cause of hypercalcemia after renal transplantation. This prospective study evaluated the effect of cinacalcet, a second-generation calcimimetic, on serum calcium and parathyroid hormone (PTH) blood levels among recipients with hypercalcemia due to persistent hyperparathyroidism. Cinacalcet corrected hypercalcemia and improved phosphatemia in patients with persistent hyperparathyroidism after transplantation with no negative effects on renal function[2].

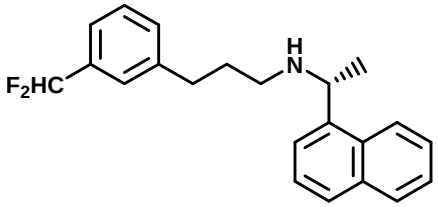
Clinical studies have shown that cinacalcet simultaneously lowers parathyroid hormone (PTH) serum phosphorous (P) and calcium (Ca) in dialysis patients with secondary hyperparathyroidism (SHPT). Evaluation of the Clinical use of Mimpara in Haemodialysis and Peritoneal Dialysis patients, an observational study (ECHO) is the first pan-European observational study to assess the use of cinacalcet in daily clinical practice[3-4].

So far to our knowledge, no analytical method for determination of process related impurities in Cinacalcet Hydrochloride API is reported in literature. So it is felt essential to develop a liquid chromatographic (LC) procedure, which will serve as a rapid and reliable method for the determination of process– related impurities in Cinacalcet Hydrochloride API. In the present method, all the process related impurities were well separated from the Cinacalcet peak. This method has been thoroughly validated as per the ICH guidelines[5]. The novelty of this work is, that the developed method can accurately quantify the Cinacalcet Hydrochloride and its related substances in bulk drugs.

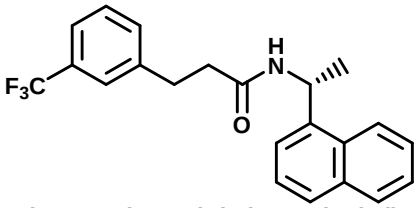
EXPERIMENTAL

Chemicals

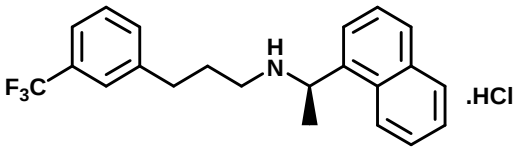
High pure (99 %) samples of cinacalcet hydrochloride and its two related substances were received from process development laboratory of Dr. Reddy's Laboratories Ltd., IPDO, Hyderabad, India. The structures and chemical names of process-related impurities and cinacalcet hydrochloride are shown in Figure 1. HPLC grade acetonitrile was purchased from Merck, Germany, 'while' analytical reagent grade potassium di hydrogen phosphate was purchased from Rankem, India. High pure water was prepared by using Millipore Milli Q plus purification system (USA).



Impurity-A : [3-(3-Difluoromethyl-phenyl)-propyl]-(1-naphthalen-1-yl-ethyl)-amine



Impurity-B : N-(1-Naphthalen-1-yl-ethyl)-3-(3-trifuoromethyl-phenyl)-propionamide



Cinacalcet Hydrochloride: N-[(1R)-1-naphthalen-1-yl-ethyl]-3-[3-(trifluoromethyl)phenyl] propan-1-amine Hydrochloride

Figure 1 : Chemical structures of Impurity-A, Impurity-B and Cinacalcet hydrochloride

Instrumentation

Waters LC system (USA) with diode array detection was used for method development, validation and forced degradation studies. The out put signal was monitored and processed using waters empower software on Pentium computer (Digital Equipment Co).

Chromatographic conditions

The chromatographic column used was Ace C18 (250 x 4.6 mm, 5 micron). The mobile phase 'A' contained a mixture of buffer and acetonitrile in the ratio of 800:200 (*v/v*). Buffer consisted of 20 mM potassium dihydrogen phosphate mixed with 1.0 ml of triethylamine, pH adjusted to 6.0 using phosphoric acid. The mobile phase B consisted of acetonitrile and buffer in the ratio of 800:200 (*v/v*). The flow rate of the mobile phase was 1.2 mL min^{-1}. The LC gradient program was set as: time (min)/% solution B: 0/30, 5/30, 20/85, 50/85, 52/30 and 60/30. The column temperature was maintained at 30 ° C and the detection was monitored at a wavelength of 223 nm. The injection volume was 10 µL. Buffer and acetonitrile in the ratio of 4 : 6 (*v/v*) was used as diluent.

Preparation of standard solutions

A stock solution of cinacalcet (2 mg mL^{-1}) was prepared by dissolving appropriate amount in the diluent. Working solutions of 300 µg mL^{-1}prepared from above stock solution for related substances determination. A stock solution of impurity (mixture of Imp-A and Imp-B) at 300 g mL^{-1} was also prepared in diluent.

Forced degradation samples for specificity study

Cinacalcet hydrochloride was heated with aqueous 1 N hydrochloric acid solution at 60°C for 3 hours and separately with aqueous 1 N sodium hydroxide at 60 °C for 4 hours to study the formation of degradation products under acidic and basic conditions, respectively. Cinacalcet hydrochloride sample was heated with 3 % hydrogen peroxide solution at 60 ° C for 30 minutes to study formation of degradation products under oxida-

tive condition. To study degradation products under photolytic and thermal degradations, cinacalcet hydrochloride sample was exposed to ultraviolet light (254 nm) for 48 hours and another sample was kept at 105 °C temperature for 48 hours respectively.

RESULTS AND DISCUSSION

Optimisation of chromatographic conditions

In order to develop a suitable and robust LC method for the determination of cinacalcet hydrochloride and its process-related impurities, different mobilephases and columns were employed to achieve the best separation and resolution. Potassium dihydrogen phosphate buffer (0.02 M) with pH 2.5 and methanol (80:20, v/v) is used as mobilephase-A and pure methanol as mobilephase-B, gradient mode was chosen for initial trial on C-18 stationary phase (zorbax SB-C18) with a 25m length, 4.6 mm ID and 5 micron particle size. Flow rate was kept as 1.0 mL min-1. When cinacalcet hydrochloride sample spiked with the two impurities (system suitability solution) was injected the resolution between the impurities and cinacalcet was good (>2.0) but the peak tailing was observed to be high (about 2.1). Similar results were obtained even with a 25m length, 4.6 mm ID and 5 micron particle size C-8 column. When acetonitrile was used instead of methanol in the above mentioned conditions no improvement observed in peak symmetry. To improve the resolution and peak symmetry further trials were made by varying the phosphate buffer pH between 2.5 and 6.8 . When phosphate buffer is used at pH 6.0 resolutions were improved (>4.0) and slightly improved peak symmetry (peak tailing about 1.4) are achieved. Different C18 stationary phases like Inertsil ODS 3V, 25m length, 4.6 mm ID with 5 micron particle size, Hypersil gold,15m length, 4.6 mm ID with 3 micron particle size and Ace –C18, 25m length, 4.6 mm ID with 5 micron particle size were further employed to study cinacalcet peak symmetry. Different buffers like sodium dihydrogen phosphate, phosphoric acid and dipotassium hydrogen phosphate were also tried during the development. Finally, the required separation and peak symmetry was achieved on Ace C18 (250 x 4.6 mm, 5) column using buffer, 0.02 M potassium dihydrogen phosphate mixed with 1.0 ml of triethylamine, adjusted to pH 6.0 with phosphoric acid and acetonitrile in the ratio of 80 : 20 (v / v) as mobilephase-A and the mobilephase-B consisted of acetonitrile and buffer in the ratio of 80 : 20 (v/v) with gradient conditions as time (min) / % solution B: 0/30, 5/30, 20/85, 50/85, 52/30 and 60/30 . The flow rate was maintained at 1.2 mL min^{-1} and UV detection was performed at 223 nm.

Quantification of process-related impurities

The relative response factors (RRF) of impurities A&B with respect to Cinacalcet hydrochloride were found to be 0.94 and 0.92. The weight percentage of the impurity present in cinacalcet hydrochloride sample was calculated using its RRF value and peak response.

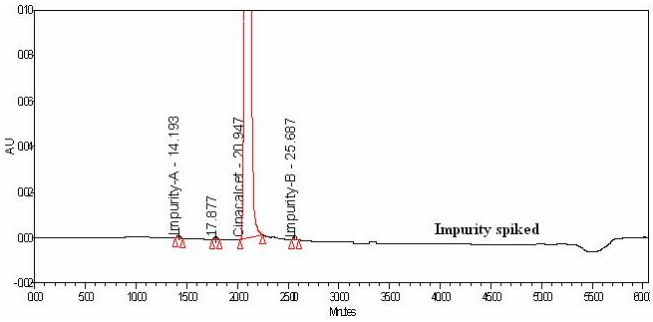
Method validation

The LC method developed has been extensively validated for the process-related impurities of cinacalcet hydrochloride using the following parameters.

Specificity

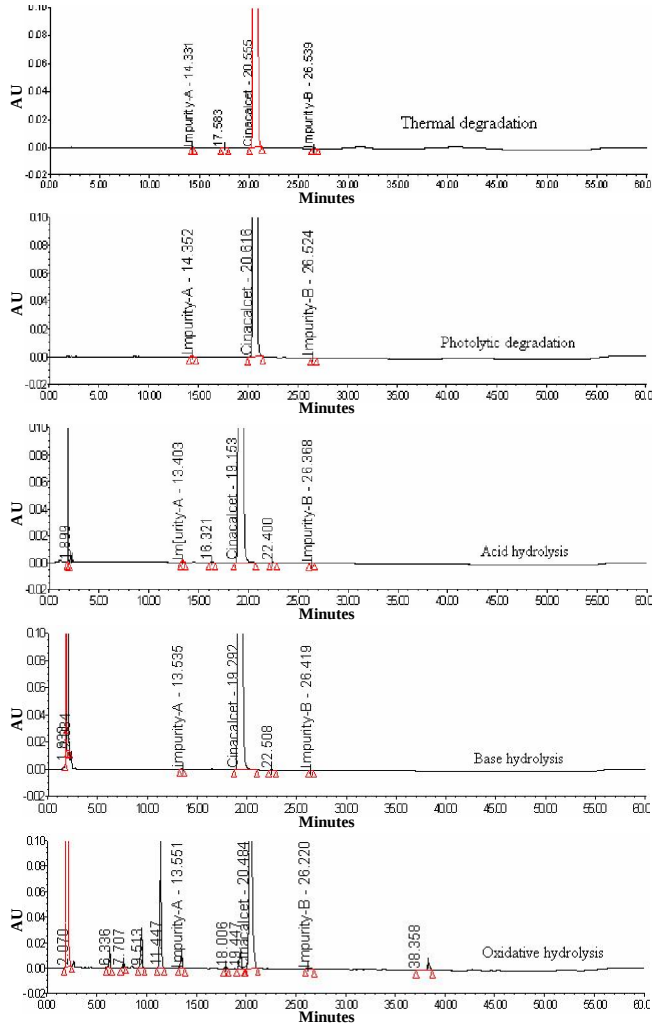
The specificity of the method was demonstrated by analyzing the two possible process-related impurities, discussed above, to pure cinacalcet hydrochloride sample. Forced degradation studies were performed to demonstrate the validity of the method. The sample exposed to thermal and UV light do not lead to any traceable degradation. But the sample heated with 1 N sodium hydroxide, 1 N HCl and 3 % H_2O_2 were mostly converted to degraded products and these are well separated from the cinacalcet peak. Photodiode array detection was also used as evidence of the specificity of the method, and to evaluate the homogeneity of the peak. The sample exposed to acidic, basic, oxidative, thermal and UV stress conditions were subjected to photodiode array analysis for peak purity of cinacalcet. The plots with flat tops in all instances showed that cinacalcet peak had no detectable impurity peaks embedded in and are free from co eluting degradant peaks. From the above results, it is clear that the method is specific and able to resolve all the process-related impurities and degradation products and can be used for determining the stability of cinacalcet hydrochloride in

bulk. A typical chromatogram of the same sample spiked with impurities and the degradation samples are shown in Figure 2 and Figure 3



X: Axis : retention time in minutes ; Y-axis : Absorbance in mAU

Figure 2 : Reference chromatograms of spiked sample



X: Axis : retention time in minutes; Y-axis : Absorbance in mAU

Figure 3 : Reference chromatograms of forced degradation studies

Linearity

Standard solutions at nine different concentration levels ranging from LOQ to 0.68 μg mL^{-1} (150 % of specification limit) were prepared and analyzed in duplicate in order to demonstrate the linearity for all the impurities. Linearity regression analysis demonstrated acceptability of the method for quantitative determination range of LOQ to 150 % of specification limit. The coefficient of correlation was found to be more than 0.999. The values of slope, intercept and coefficient correlation for each impurity are shown in TABLE 2.

TABLE 1 : System suitability report

Compound	USP resolution (Rs)	USP tailing factor	No. of theoretical plates USP tangent method (*N*)
Impurity-A	-	1.08	10,578
Cinacalcet	11.0	1.19	13.373
Impurity-B	7.8	1.06	39,967

TABLE 2 : Linearity results of the two impurities and the cinacalcet hydrochloride

	Impurity-A	Impurity-B	Cinacalcet
Trend line equation	y = 324.1 *x* – 374	y = 391.7 *x* + 863	y = 401.0 *x* + 1086
Linearity range	6.0 – 200 %	5.3 – 200 %	5.3 – 200 %
Regression coefficient	0.99993	0.99907	0.99929
Slope	324	392	401
Intercept	-374	863	1086
% Intercept	-1.17	2.19	2.67

Accuracy

Accuracy of the method was demonstrated at four different concentration levels in triplicate. The analysis carried out at 25 %, 50 % 100 % and 150 % of specification limit. The mean recoveries of all the impurities were found to be in the range of 96-102 % as shown in TABLE 3.

Precision

The precision of the method for the determination of impurities related to cinacalcet was studied for repeatability and intermediate precision. Repeatability was demonstrated by analyzing cinacalcet hydrochloride sample six times. Intermediate precision was demon-

strated by analyzing same sample of cinacalcet by two different chemists on two different days. Intra-day variations of impurities of cinacalcet are expressed in terms of % R.S.D. values. Repeatability and intermediate precision for the process-related impurities in cinacalcet hydrochloride were found to be <10 % R.S.D. The results of repeatability, intraday and intermediate precision data are shown in TABLE 4(a) and TABLE 4(b).

TABLE 3 : Parameters of recoveries of impurities of cinacalcet hydrochloride

Impurity Name	Spike level (%)	Added (μg) (n = 3)	Recovered (μg)	% Recovery	% RSD
Impurity-A	25	11.25	11.32	100.6	2.5
	50	22.50	22.61	100.48	2.3
	100	45.0	45.24	100.53	2.1
	150	67.50	67.43	99.89	1.9
Impurity-B	25	11.30	11.46	101.41	2.5
	50	22.50	22.63	100.57	2.4
	100	45.0	45.32	100.71	2.6
	150	67.50	67.44	99.91	2.0

n=3, Number of determinations

Table 4(a) : Repeatability and Intra-day precision data

	% RSD
Repeatability of cinacalcet spiked at 0.45 μg mL^{-1} level (n= 6)	
Impurity-A	2.5
Impurity-B	2.4
Intra-day precision of cinacalcet spiked at 0.45 μg mL^{-1} level (n= 6)	
Impurity-A	2.5
Impurity-B	2.4

n = 6 determinations

Table 4(b) : Intermediate precision data

S. No.	Parameter	Variation	%RSD for related substances
1	Different system:	(a) Waters 2695 Alliance system	< 2.5
		(b) Agilent 1100 series VWD system	<2.5
2	Different column:	Column No. (a) #001	< 2.5
		(b) #002	< 2.5
3	Different analyst:	(a) Analyst-1	< 2.5
		(b) Analyst-2	< 2.5

n = 6 determinations

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection for impurities A and B were calculated from the linearity data using residual standard deviation of the response and slope of the calibration curve for each impurity. The limit of detection of a compound is defined as the lowest concentration that can be detected. LOD values were found to be 0.007 and 0.006 μg mL^{-1} for impurity-A and B respectively. The limit of quantification is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy. LOQ values were found to be 0.028 and 0.024 μg mL^{-1} for impurity-A and B respectively.

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in the chromatographic conditions, viz, change in flow rate by $\pm$0.2 ml/min, change in pH of the buffer $\pm$0.2 unit and changing the organic phase in the mobilephase from 100% to 90% and 110%. The method was demonstrated to be robust over an acceptable working range of its HPLC operational parameters.

CONCLUSION

The present paper describes the development of a new HPLC method for the determination of process-related impurities in cinacalcet hydrochloride API and its validation. The method was found to be selective, sensitive, precise and accurate for the determination of process-related impurities and degradation products. This method can be used for the routine determinations in pharmaceutical quality control.

ACKNOWLEDGEMENTS

The authors wish to thank the management of Dr. Reddy's Laboratories Ltd, for supporting this work. Authors wish to acknowledge the process research group for providing the samples for our research. Authors would also like to thank colleagues in separation science division of Analytical Research and Development department of Dr. Reddys Laboratories Limited, IPDO.

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