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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ETELCALCETIDE IN BULK AND ITS DOSAGE FORM USING RP-HPLC

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ABSTRACT

A simple and precise RP-HPLC method was developed for the estimation of the Etecalcetide. Initially, various mobile phase compositions were tried to separate title ingredients. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates capacity or symmetry factor) run time and resolution. Finally The mobile phase containing mixture of 50% OPA buffer 50% methanol with the flow rate of 1.0 ml/ min. The optimum wavelength for detection was 235nm at which better detector response for drug was obtained. The Retention time for Etelcalcetide was found to be 4.653min respectively. To ascertain its effectiveness, system suitability test were carried out on fresh prepared stock solutions. The regression 0.999 respectively. The low value of % R.S.D indicate the method is precise and accurate.

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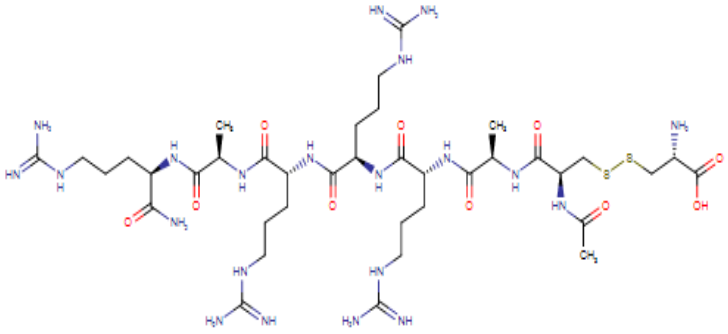
INTRODUCTION

Etelcalcetide is 2R)-2-amino-3-{[(2S)-2-{[(1R)-1-{[(1R)-4-carbamimidamido-{[(1R)-4-carbamimidamido-1-{[(1R)-4-carbamimidam- ido-1- {[(1R) -1 -{[(1R) -4- carbamimidamido-1 carbamoylbutyl] carbamoyl} ethyl] carbamoyl} butyl] carbamoyl} butyl] carbamoyl} butyl] carbamoyl}ethyl]carbamoyl}-2-acetamidoethyl]disulfonyl} propionic acid. It is a calcimimetic drug for the treatment of secondary hyperparathyroidism in patients undergoing hemodialysis. It is administered intravenously at the end of each dialysis session. Etelcalcetide functions by binding to and activating the calcium- sensing receptor in the parathyroid gland. It is medically used in the treatment of secondary hyperparathyroidism in patients with chronic kidney disease (CKD) on hemodialysis. The drug is of course contraindicated in people with blood serum calcium levels below the norm. Etelcalcetide is predominately bound to plasma albumin by reversible covalent binding. Non-covalent binding of etelcalcetide to plasma proteins is low with a fraction unbound ratio of 0.53. The ratio of blood-to-plasma [14C]- etelcalcetide concentrations is approximately 0.6. As the drug is new no stability indicating methods has been reported so far. Therefore the present work was aimed to develop a simple, rapid, precise, and economic reversed-phase stability indicating HPLC method for the Etelcalcetide was developed.

The present work describes the development of a simple, precise, accurate and reproducible RP-HPLC method for the Estimation of Etelcalcetide



Structure of Etelcalcetide.

MATERIALS

Etelcalcetide were received under the brand name of parsabiv. The HPLC system (LC water) consisted of UV detector (2487 Waters Dual λUV Detector) UV/VIS spectrophotometer (UV $3000^{+,}$ company (LABINDIA)) data was processed using Empower 2 software(waters). Water methanol, Acetonitrile, Ortho phosphoric Acid and Sodium hydroxide were used which were of HPLC grade.

EXPERIMENTAL WORK:

Chromatographic conditions

Isocratic elution of the mobile phase OPA buffer and Methanol in the ratio of 50:50v/v with the flow rate of 1ml/min. Separation was performed on waters (Inertsil, ODS 4.6x250mm ,5µm) analytical column and a pre column to protect the analytical Colum from strongly bonded material. Integration of the detector output was performed using the Water Empower Software to determine the peak area.

The flow rate of the mobile phase was optimized to 1ml/min. The run time was set at 8 min and the column temperature was maintained at Ambient(25^0C).The volume of injection was 20µl prior to injection of the analyt the column was equilibrated for 30-40 min with the mobile phase. The eluents were detected at 235nm. The development method was validated in terms of specify, linearity, accuracy, limit of detection(LOD), limit of quantification(LOQ), intra- day and inter day precision and robustness for the assay of Etelcalcetide as per ICH guidelines.

Preparation of standard solution:

Accurately weigh and transfer 100 mg of Etelcalcetide working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicated to dissolve it completely and volume was made up to the mark with the same solvent. Further 0.6 ml of the above stock solutions was pipette into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution:

Accurately weigh and transfer equivalent to 100 mg of Etelcalcetide sample into a 10 ml clean dry volumetric flask about 7 mL of Diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent. Further 0.6 ml of the above stock solutions was pippeted into a 10ml volumetric flask and diluted up to the mark with diluent.

RESULTS AND DISCUSSION:

Method Development:

Number of mobile phase and their different proportions were tried and finally was selected as OPD buffer and methanol in the ratio of (50:50v/v) appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The results

of system suitability parameters were shown in table 1. The chromatogram of working standard solution is shown in fig 1.The summary of chromatographic condition was in table.

Table 1: Summary of Chromatographic conditions.

S. No	Parameter	Description/Value
1.	Stationary Phase	Inertsil ODS C_{18} Column (4.6mm x15mm)5µm.
2	Mobile Phase	0.1% Orth phosphoric acid buffer, methanol (50:50)
3	Flow rate	1ml/min
4	Detection Wavelength	250 nm
5	Detector	Photo diode array
6	Injection	Auto sampler -Waters, model 717 plus
7	RT	4.653Min
8	Injection volume	20 µl
9	Column Temperature	35 °C
10	Run time	8 mins
11	Diluent	Mobile Phase
12	Mode of separation	Isocratic mode

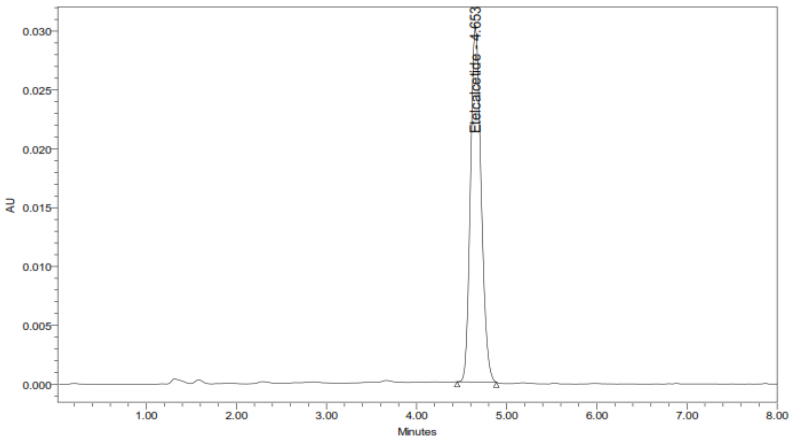


Fig.3 Typical Chromatogram of Etelcalcetide.

Table 2: System suitability parameters.

S. No	Parameter	Result
		Etelcalcetide
1	Retention Time	4.653min
2	Tailing	1.18
3	Theoretical Plates (n)	3828
4	Similarity Factor	1.25

Method Validation:

Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 50%, 100%and 150% to the pre analyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed method was accurate. The results were tabulated in Table 3.

Table 3: Results of Accuracy of Etelcalcetide.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	95505	50	49.74	99.47*	
100%	191399	100	99.67	99.67*	99.40**
150%	285309	150	148.58	99.05*	

*Mean % Recovery of 6 replicates; **Mean % Recovery of 3 replicates.

Precision

The intraday and interday precision of the proposed method was determined by analyzing Etelcalcetide, 3 times on the same day and on 3 different days. The results shown in table 4 were reported in terms of relative standard deviation.

Table 4: Results of Precision (%Assay).

Injection	Area
1	191345
2	191232
3	191671
4	191999
5	192898
6	194679
Average Assay	192304.0
STD	1308.1
% RSD	0.7

Linearity

Calibration graphs were constructed by plotting peak area vs concentration of Etelcalcetide and regression equations were calculated. The calibration graphs were plotted and over 5 different linear concentrations in the range of 5.

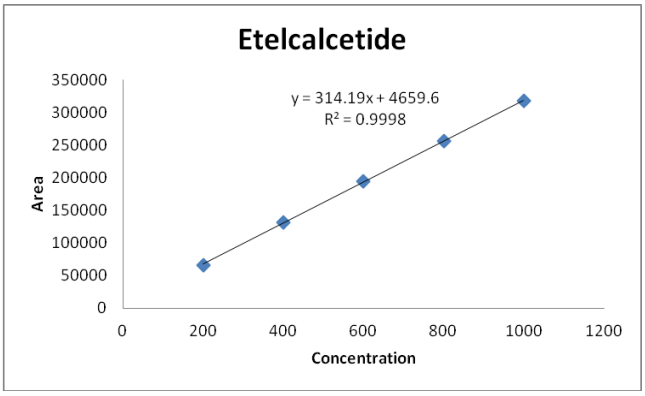


Figure 4: Calibration graph for Etelcalcetide

Limit of detection (LOD) and limit of quantitation (LOQ):

The limit of detection (LOD) and limit of quantitation (LOD) were determined by calculating the signal-to-noise(S/N) ratio 3.00µg/ml. Respectively according to International conference on Harmonization guidelines LOD value for Etelcalcetide were found to be 9.98µg/ml.

Assay of the tablet dosage form

The proposed validated method was successfully applied to determine etelcalcetide in dosage form. The results obtained is comparable with corresponding label amount. The results were tabulated in the table.

DEGRADATION STUDIES:

As per the ICH guidelines force degradation studies are conducted to show the degradation mechanisms under the various stress conditions like hydrolysis (i.e., acid, base, and water), oxidation, heat, and photolytic degradation was carried out. As well as these are simple, precise, economic and rapid to perform.

Preparation of stock:

Accurately Transferred equivalent solution of 100 mg of Etelcalcetide into a 10 ml dry VF added few ml of Diluent and made to dissolved completely and volume is made with a diluent. Then is Filtered through 0.44 micron Injection filter. (Stock solution)

Hydrolytic degradation under acidic condition

Pipette 0.6 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the VF was kept at 60^{o}C for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent.

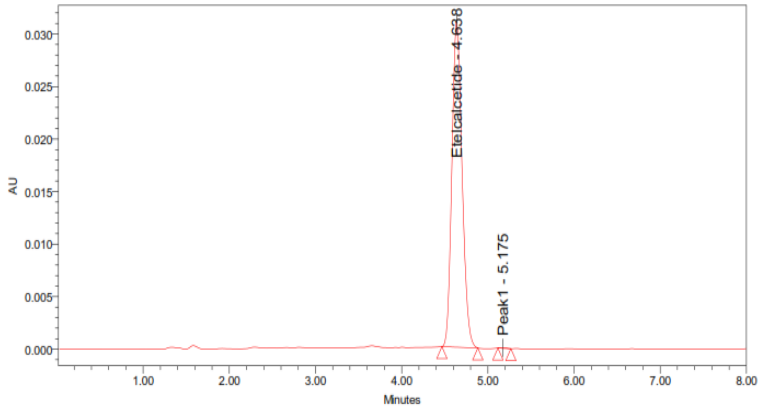


Figure 5: Chromatogram showing Acid degradation.

Hydrolytic degradation under alkaline condition

Pipette 0.6 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60oC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent.

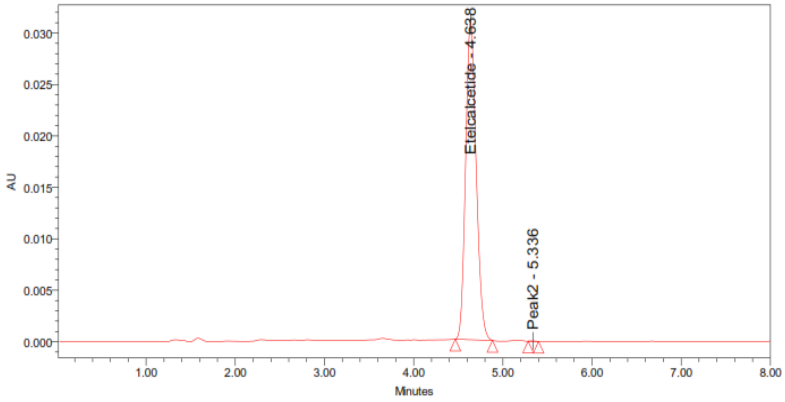


Figure 6: Chromatogram showing Alkaline degradation.

Thermal induced degradation

Etelcalcetide sample was taken in Petri dish and kept in Hot air oven at 1100 C for 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

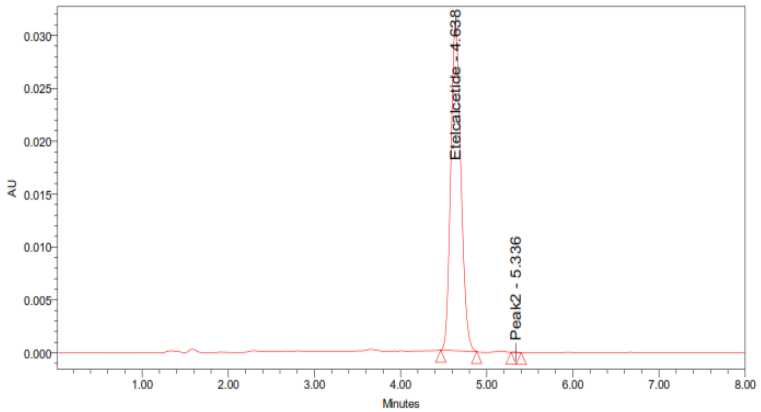


Figure 7: Chromatogram showing Thermal degradation.

Oxidative degradation

Pipette 0.6 ml above stock solution into a 10ml volumetric flask and 1ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent.

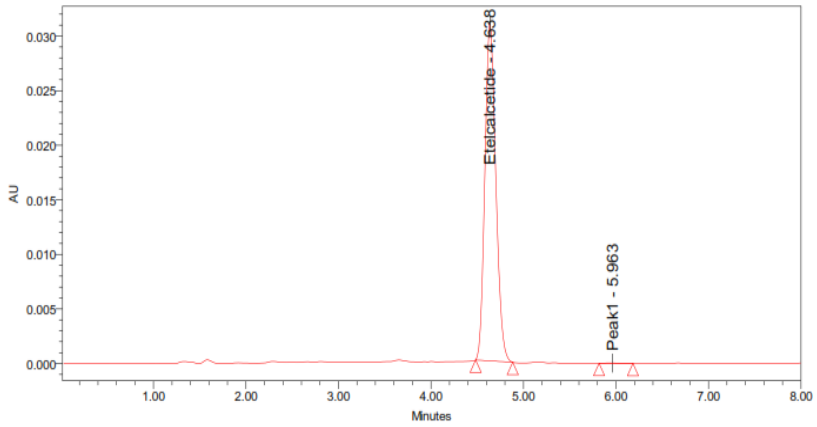


Figure 8: Chromatogram showing Peroxide degradation.

Photo degradation

Pipette 0.6 ml above stock solution into a 10ml volumetric flask and exposed to sunlight for 24hrs and the volume was made up to the mark with diluents

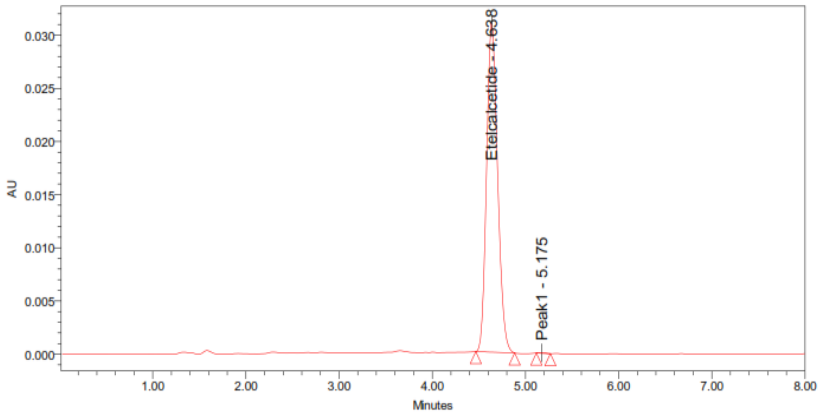


Figure 9: Chromatogram showing Photo degradation.

Table 05: Degradation results for Etelcalcetide.

Sample Name	Etelcalcetide	
	Area	% Degraded
Standard	191642	
Acid	183252	4.38
Base	183532	4.23
Peroxide	183253	4.38
Thermal	187552	2.13
Photo	186452	2.71

CONCLUSION

The proposed method has advantage of simplicity and convenience for the separation and quantitation of Etelcalcetide which can be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedure. The method is accurate, precise, rapid and selective for estimation of Etelcalcetide. Hence it can be conveniently adopted.

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ABBREVIATIONS:

RP-HPLC : Reverse phase high performance/pressure liquid chromatography
CKD : Chronic kidney disease
VF : Volumetric flask
LOD : limit of detection
LOQ : limit of quantitation
OPA : Ortho phosphoric acid

CONFLICT OF INTEREST:

There is no conflict of interest.

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