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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETERMINATION OF ABALOPARATIDE TO ENSURE CLEANING OF MANUFACTURING EQUIPMENT

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ABSTRACT

This study describes the development and validation of HPLC method for determination of Abaloparatide. System suitability, specificity, LOD and LOQ, Linearity and Range, Precision at LOD and LOQ and Swab solution stability, Accuracy were studied to ensure cleaning of manufacturing equipment. The analytical method used for determination of traces for Abaloparatide Injection in Swab sample and rinse sample complies with the acceptance criteria set for the analytical parameters such as System Suitability, Specificity, LOO and LOQ, Linearity and range, Precision at LOD and LOQ, Accuracy (Recovery studies) and Swab Solution stability and solutions stable up to 48 hours. Hence, the method stands validated. The method can be used routinely for residual determination of Abaloparatide in the quality control department.

KEYWORDS

Abaloparatide, HPLC, Manufacturing equipment, LOD and LOQ.

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INTRODUCTION

Abaloparatide, a synthetic 34 amino acid peptide. Abaloparatide belongs to peptide family. Abaloparatide is analog of parathyroid hormone-related protein.

The purpose of this study is to develop and validate analytical method for determination of residue for Abaloparatide in Abaloparatide injection meets the requirements for its intended analytical application.

MATERIAL AND METHODS

Abaloparatide was available at Precise Biopharma Pvt Ltd. Trifluoroacetic acid, Acetonitrile, Glacial Acetic Acid and solvents were of analytical grade/IP/BP/USP equivalent grade available in the laboratory.

Preparation of Abaloparatide Standard Stock Solution:

Weigh accurately about 11.6mg of Abaloparatide working standard (equivalent to 10mg of Abaloparatide) into a 20ml volumetric flask, add about 15 ml of diluent and sonicate to dissolve the solid completely. Cool to room temperature & dilute up to the mark with diluent and mix well[1-3].

Method of Analysis by HPLC

Procedure

Chromatographic Condition

Buffer Preparation

Transfer 1ml Trifluoroacetic acid into 1000ml purified water. Mix well and filter through 0.45µm Nylon membrane filter.

Mobile Phase A: 100% Buffer

Mobile Phase B: Mix Buffer: Acetonitrile (25:75) (%v/v), Mix well and sonicate to avoid baseline disturbances.

Diluent: Accurately transfer 1ml Glacial Acetic Acid into 1000ml of purified water. Mix well and sonicate.

Column: Poroshell 120Å, Aq. C18, 4.6 x 150mm, 2.7µm (Make: Agilent, Part No.: 693975-742)

Flow Rate: 1.0ml/min

Injection Volume: 300µL

Column Temperature: 40ºC

Autosampler Temperature: 10ºC

Detector: PDA/UV

Wavelength: Abaloparatide: 210nm

Run time: 25 minutes

Retention Time of Abaloparatide: About 9.5 Minutes (For Information only)

Elution Mode: Gradient

Blank: Diluent

Important instructions

Always prepare solution freshly, preferably immediately before filling vial in compartment.

In lower concentration response may degraded faster.

Difference between 02 injections from same preparation/vial is expected significantly.

Good recovery response are observed for solution of 1ppm and above 1ppm.

Validation parameters

System Suitability

Preparation of Standard Solution

Transfer 4ml of the Abaloparatide standard stock solution into a 200ml volumetric flask. Dilute up to the mark with diluent and mix well. (Abaloparatide: 10ppm)

Acceptance criteria: The % RSD for Abaloparatide peak obtained from replicate injections of standard solution should not more than 10.0.

Specificity

Preparation of Standard Solution

Transfer 4ml of the Abaloparatide standard stock solution into a 200ml volumetric flask. Dilute up to the mark with diluent and mix well. (Abaloparatide: 10ppm)

Blank Swab

Dip 1 swab stick in test tube-containing specified volume of swabbing solvent 10ml of diluent. Sonicate for 3 min[4-7].

Acceptance criteria: No interference should be observed from Diluent, mobile phase & Blank Swab at the retention time of Abaloparatide.

Limit of Detection and Limit of Quantitation

Procedure based on standard deviation of the response and slope

Preparation of Standard Solution

Transfer 4ml of the Abaloparatide standard stock solution into a 200ml volumetric flask. Dilute up to the mark with diluent and mix well. (Abaloparatide: 10ppm)

Transfer 2ml) of the standard solution into 20 volumetric flask. Dilute up to the mark with diluent and mix well. (Abaloparatide: 1ppm). (Standard solution-1).

Prepare a series of solutions by quantitative dilutions of the to obtain solutions of suitable concentrations from 10 % to 50 % of specification

limit 0.1 ppm (Concentration limit). 0.01ppm [10%], 0.03ppm [30%], 0.05ppm [50%])[8-10].

Different concentrations Preparation

Standard Preparation: (0.01ppm)

Take 1ml of standard solution - 1 and dilute it up to 100.0ml of diluent to prepare 0.01ppm solution[11].

Standard Preparation: (0.03ppm)

Take 3ml of standard solution - 1 and dilute it up to 100.0ml of diluent to prepare 0.03ppm solution[12].

Standard Preparation: (0.05ppm)

Take 5ml of standard solution - 1 and dilute it up to 100.0ml) of diluent to prepare 0.05ppm solution[13].

Inject each solution into duplicate and record the chromatogram.

Determine the slope and RSD (Relative Standard Deviation) for each standard using the corrected peak areas and concentration (ppm). Calculate the value of limit of detection and Limit of Quantitation using the following formula[14].

Calculation

$$LOD = \frac{3.3 \times STEYX}{S} \qquad LOQ = \frac{10 \times STEYX}{S}$$

Where,

STEYX = Standard error of predicted y-value

S = The Slope of the calibration curve

LOD = Limit of Detection

LOQ = Limit of Quantitation

Linearity and Range

Preparation of Standard Solution

Transfer 4ml of the Abaloparatide standard stock solution into a 200ml volumetric flask. Dilute up to the mark with diluent and mix well. (Abaloparatide: 10ppm).

Procedure

Inject each level in duplicate and plot the graph of peak area response versus concentration and check correlation co-efficient of average peak response. (15-18)

Report y-intercept (at 100%), slope of regression line and residual sum of squares

Calculation

Y-intercept value at 100% level

$$= \frac{\text{Y Intercept}}{\text{Area of 100\% level}} \times 100$$

Acceptance criteria

Correlation co-efficient should not be less than 0.98. (Being Peptide)

Precision at LOD and LOQ

Preparation of Standard Solution

Transfer 4ml of the Abaloparatide standard stock solution into a 200ml volumetric flask. Dilute up to the mark with diluent and mix well. (Abaloparatide: 10ppm)

Acceptance criteria

Peak should be detected for LOO level. (Being Peptide)

The %RSD of the six replicates should not be more than 25.0 for LOQ level. (Being Peptide) (19-20)

Accuracy

Preparation of Standard Solution:

Transfer 4ml of the Abaloparatide standard stock solution into a 200ml volumetric flask. Dilute up to the mark with diluent and mix well. (Abaloparatide: 10ppm)

Note

In case of the expected result not observed, do not dry the solution on water bath. Spike on SS plate and use as such

Acceptance criteria: Recovery should be not less than 70.0%

Swab Solution Stability

Preparation of Standard Solution

Transfer 4 ml of the Abaloparatide standard stock solution into a 200ml volumetric flask. Dilute up to the mark with diluent and mix well. (Abaloparatide: 10ppm)

Procedure

Hold the swabbing solvent (water) for 0 hours, 4 hours, 8 hours, 12 hours, 24 hours and 48 hours. Inject standard solution and each hold swabbing solvent solution and check interference with principal peak RT.

Acceptance criteria: No interference with principal peak RT' due to swabbing solvent[21-22].

RESULTS AND DISCUSSION

In system suitability parameter; The % RSD for Abaloparatide peak obtained from replicate injections of standard solution obtained 2.28%. It

was concluded system was suitable for analysis if Abaloparatide.

In specificity parameter; No interference pf diluent, mobile phase and black swab observed at the retention time of Abaloparatide. It was concluded method was specific for Abaloparatide.

LOD and LOQ was calculated 0.02 and 0.06ppm respectively.

Limit of Detection and Limit of Quantitation: Correlation coefficient was found 0.9972%.

Precision for LOD and LOQ: Peak was detected for LOO level. The %RSD of the six replicates was obtained 21.20%.

In Accuracy parameter, Recovery was obtained 86.9% and 76.5% for Rinse sample and Swab sample respectably.

In Swab Solution Stability Parameter; No interference observed at principal peak RT due to swabbing solvent.

Table No.1: Gradient Programme

S.No	Time (Minute)	MP A (%)	MP B (%)
1	0.01	75	25
2	20.0	25	75
3	21.0	75	25
4	25.0	75	25

Table No.2: Injection Sequences

S.No	Solution Name	No. of Injections
1	Blank (Diluent)	1
2	Standard Solution	6

Table No.3: Injection Sequences

S.No	Solution Name	No. of Injections
1	Blank (Diluent)	1
2	Mobile Phase A	1
3	Mobile Phase B	1
4	Swab Blank	1
5	Standard Solution 10ppm	1

Table No.4: Injection Sequences

S.No	Solution Name	No. of Injections
1	Blank (Diluent)	1
2	Standard Preparation: 0.01ppm	2
3	Standard Preparation: 0.03ppm	2
4	Standard Preparation: 0.05ppm	2

Table No.5: Preparation linearity level

Level	% of Linearity Level	volume of Standard Solution in ml to be added	Diluted to volume (ml) with Diluent	Cone. of Abaloparatide (ppm)
1	LOQ**	0.6	100	0.06
2	50	1.0	20	0.5
3	80	1.6	20	0.8
4	100	2.0	20	1.0
5	120	2.4	20	1.2
6	150	3.0	20	1.5

** As per LOD/LOQ section

Table No.6: Injection Sequences

S.No	Solution Name	No. of Injections
1	Blank (Diluent)	1
2	Linearity Level (LOQ)	2
3	Linearity Level (50%)	2
4	Linearity Level (80%)	2
5	Linearity Level (100%)	2
6	Linearity Level (120%)	2
7	Linearity Level (150%)	2

Table No.7: Injection Sequences

S.No	Solution Name	No. of Injections
1	Blank (Diluent)	1
2	LOD Level Preparation	3
3	LOQ Level Preparation	6

Table No.8: Sample Preparation: (Rinse sample)

S.No	Accuracy Level	Volume of Standard Solution in ml to be spiked on SS plate	Volume of solvent in ml with diluent	Cone. of Abaloparatide (ppm)
1	LOQ**	0.6	100	0.06
2	50%	1.0	20	0.5
3	100%	2.0	20	1.0
4	150%	3.0	20	1.5

** **As per LOD/LOQ chapter**

Table No.9: Injection Sequences

S.No	Solution Name	No. of Injections
1	Blank (Diluent)	1
2	Standard Solution-1	5
3	Accuracy Level (LOQ)	2
4	Accuracy Level (50%)	2
5	Accuracy Level (100%)	2
6	Standard Solution - 1 (BKT)	1
7	Accuracy Level (150%)	2
8	Standard Solution - 1 (BKT)	1

Table No.10: Injection Sequences

S.No	Solution Name	No. of Injections
1	Blank	1
2	Diluent	1
3	Swabbing solvent water _0 hr	1
4	Standard Solution (0 hr)	1
5	Swabbing solvent water 4 hr	1
6	Standard Solution (4 hr)	1
7	Swabbing solvent water 8 hr	1
8	Standard Solution (8 hr)	1
9	Swabbing solvent water 12 hr	1
10	Standard Solution (12 hr)	1
11	Swabbing solvent water 24 hr	1
12	Standard Solution (24 hr)	1
13	Swabbing solvent water 48 hr	1
14	Standard Solution (48 hr)	1

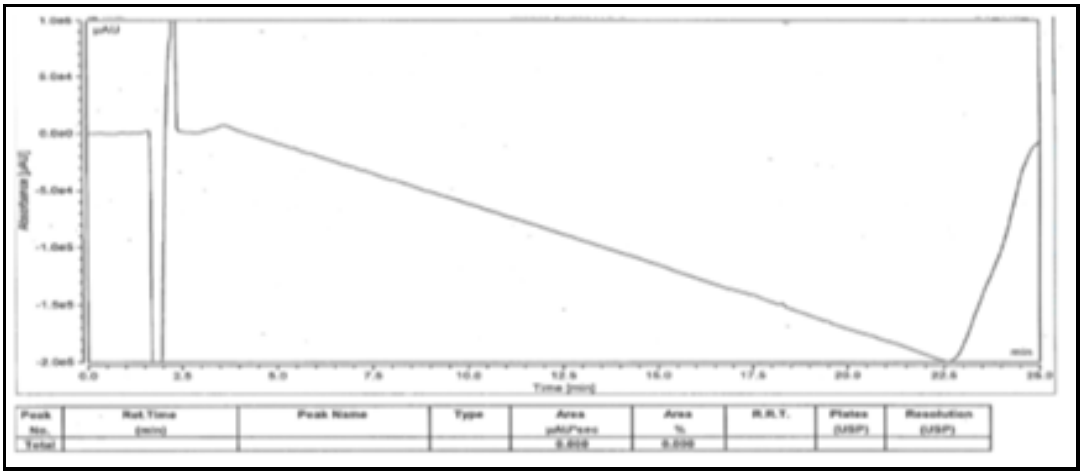

Figure No.1: Chromatogram of system suitability parameter

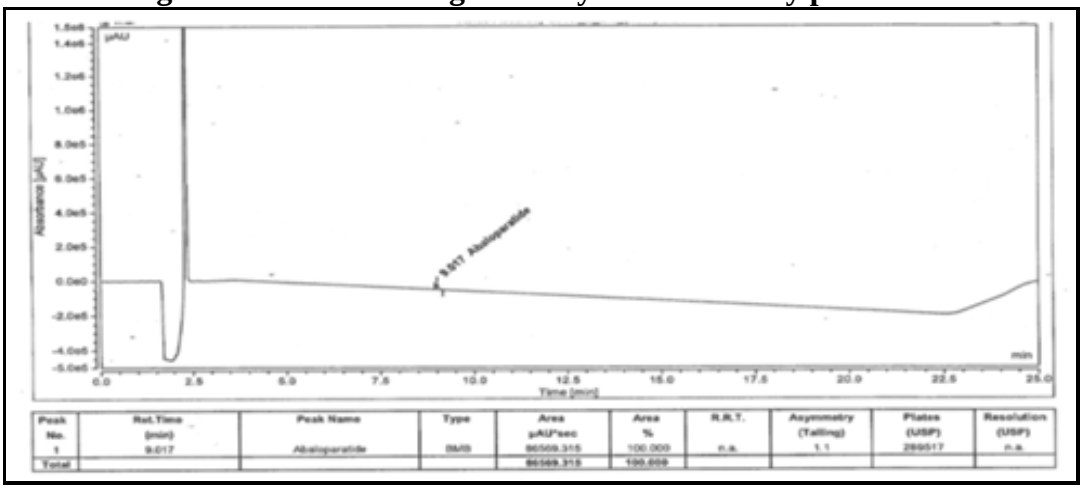

Figure No.2: Chromatogram of Accuracy parameter

CONCLUSION

From the results obtained by applying the suggested procedures, it is obvious that the proposed methods are accurate, precise, simple, sensitive, rugged, robust and rapid and can be applied successfully in routine analysis for the estimation of Abaloparatide.

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CONFLICT OF INTEREST

I declare that I have no conflict of interest.

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