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RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF RELATED COMPOUNDS OF OCTREOTIDE ACETATE DEPOT SUSPENSION (MICROSPHERES)

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ABSTRACT

The analysis of Octreotide Acetate and related compounds Depot Suspension (Microspheres) By using RP-HPLC method using columm Waters X-Bridge C18 (250 X 4.6)mm, 5µm with Flow rate 1.0 mL/min, Injection volume 50 µL, Wavelength 210 nm, Run time 70 minutes, Elution using Gradient programm, the Rt for Octreotide was found to be 28.23, related compunds like N- Acetyl Octreotide was found to be 24.32 Des Threoninol Octreotide was found to be 34.24. The suitable was developed for the indetification of the related compounds present in the Octreotide Acetate formulation.

KEYWORDS: Octreotide Acetate, N-Acetyl Octreotide, Des Threoninol Octreotide, RP-HPLC, Microspheres, Depot Suspension.

INTRODCUTION

Octreotide[1] is the acetate salt of a cyclic octapeptide. It is a long-acting octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin.

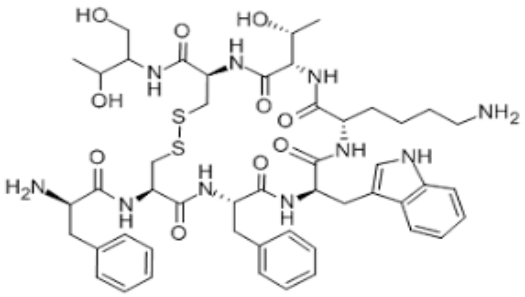


Fig. 1: Structure of Octreotide acetate.

The present study was Analytical method for Octreotide Acetate and related compounds Depot Suspension (Microspheres) By RP-HPLC. Based on the literature review found that still the less analytical methods were developed for the Octreotide Acetate and related compounds Depot Suspension.

Experimental work

Materials and methods

Octreotide Acetate, N- Acetyl Octreotide, Des Threoninol Octreotide were procured from the Celon R&D centre, Hyderabad and chemicals Acetonitrile HPLC Grade, Triethylamine, Dimethyl sulfoxide, Acetonitrile, Orthophosphoric acid, Glacial acetic acid, Sodium acetate trihydrate all are AR Garde purchased from the Merck Pvt.Ltd, Mumabai. All the instruments and glassware were used in this work well calibrated.

Preparation of 10% (v/v) Orthophosphoric acid solution

Transfer 10 mL of Orthophosphoric into a 100 mL volumetric flask and dilute with water, mix well.

Preparation of Mobile phase

Mobile phase A

Pipette 3 mL of triethylamine to one liter beaker containing 880 mL of water and mix well. Adjust the pH to 5.4 with 10% (v/v) orthophosphoric acid solution and add 100 mL of acetonitrile mix well and sonicate for 5 minutes.

Mobile phase B

Pipette 3 mL of triethylamine into one liter beaker containing 380 mL of water and mix well. Adjust the pH to 5.4 with 10% (v/v) orthophosphoric acid solution and add 600 mL of acetonitrile mix well and sonicate for 5 minutes.

Preparation of Diluent

Accurately Weigh and transfer 13.6 g of Sodium acetate trihydrate into 1000 mL of water mix well sonicate to dissolve the contents. Adjust the pH to 4.0 with glacial acetic acid mix well.

Prepration of Blank Solution

Transfer 4mL of Dimethyl sulfoxide (DMSO) into a 50 mL of volumetric flask, dilute to volume with diluent and mix well.

Chromatographic conditions

Column	:	Waters X-Bridge C18 (250 X 4.6)mm, 5µm.
Column temperature	:	55°C
Sampler temperature	:	5°C
Flow rate	:	1.0 mL/min
Injection volume	:	50 µL
Wavelength	:	210 nm
Run time	:	70 minutes
Elution	:	Gradient

Table 1: Gradient program.

Time in minutes	Mobile phase -A	Mobile phase -B
0.00	90	10
20.00	75	25
40.00	50	50
45.00	47	53
52.00	20	80
58.00	20	80
63.00	90	10
70.00	90	10

Preparation of peak identification solution

Prepare Octreotide 200µg/mL, N- Acetyl Octreotide-4µg/mL and Des Threoninol Octreotide impurity-4µg/mL.

Preparation of standard solution

Prepare 5µg/mL Octreotide acetate.

Preparation of sample solution: (200 ppm)

Take 5 vials, tear flip-off seal and note down the weights of 5 vials individually, (W1).

Empty the vials and rinse with acetone allow to dryness and take the empty vial weights individually, (W2).

Calculate the average fill weight by using the following formula:

$$\text{Average fill weight (mg)} = \frac{W1 - W2}{5}$$

Mix the contents of five sample vials, then accurately weigh and transfer the sample (Equivalent to 10 mg of Octreotide) into a 50 mL volumetric flask add 4 mL of DMSO and sonicate to dissolve the material completely with occasional shaking. Make up the volume with diluent slowly and mix well for 5 min. Filter the solution by using 0.45 µm Nylon filter.

Note

The retention time of the Octreotide peak is about 28 minutes (For information purpose only). Disregard the peaks due to blank and placebo in the sample chromatogram. The RRT of known impurities calculated against Octreotide peak as tabulated below,

Calculation the percentage of known impurity by the following formula

$$= \frac{R_K}{R_S} \times \frac{W_S}{50} \times \frac{5}{50} \times \frac{4}{50} \times \frac{50}{S_{wt}} \times \frac{Avg.Wt}{LC} \times \frac{1}{RRF} \times \frac{P}{100} \times 100$$

Calculation the percentage of Un known impurity by the following formula

$$= \frac{R_U}{R_S} \times \frac{W_S}{50} \times \frac{5}{50} \times \frac{4}{50} \times \frac{50}{S_{wt}} \times \frac{Avg.Wt}{LC} \times \frac{P}{100} \times 100$$

Where

R_K = Area of known impurity peak from the sample solution

R_U = Area for unknown impurity peak from the sample solution

R_S = Mean area of three replicate injections of Octreotide in standard solution

W_S = Weight of Octreotide acetate standard in mg

S_{wt} = Weight of sample taken for sample preparation in mg.

Avg.Wt = Average fill weight of Octreotide per vial in mg

LC = Labeled amount of Octreotide per vial in mg

P = % Potency of Standard on as is basis.

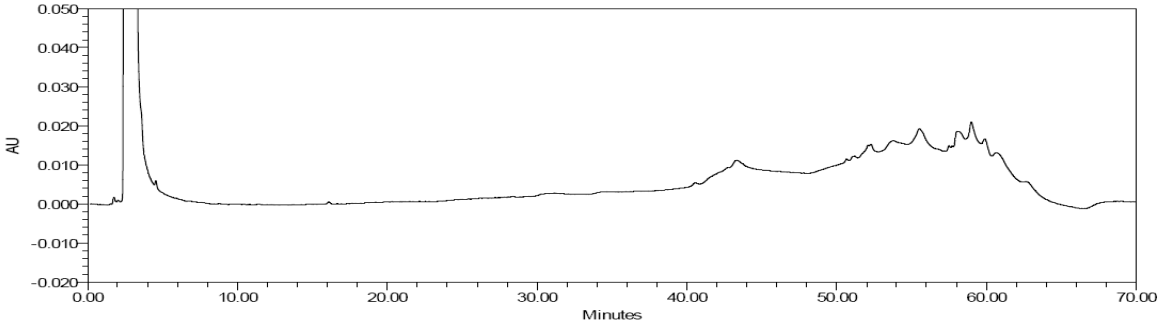
RRF = Relative response factor

System suitability requirements

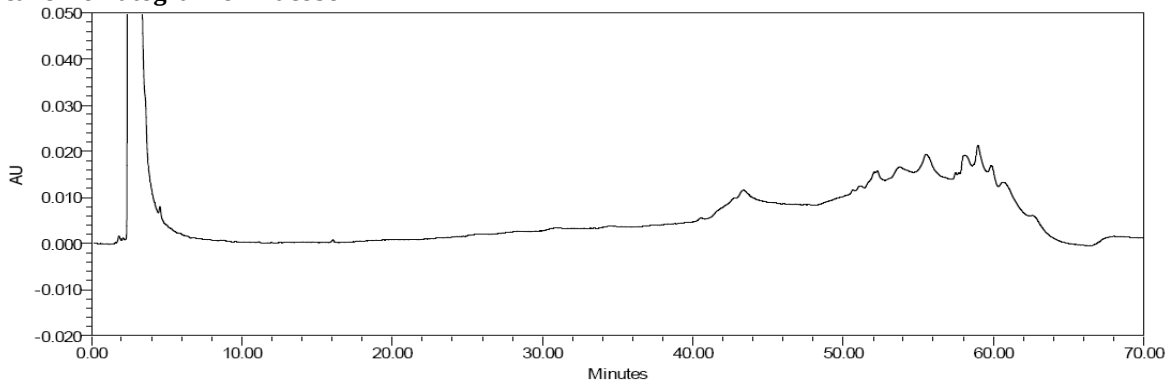
1. The tailing factor of Octreotide from standard solution should be NMT 2.0.
2. The theoretical plates of Octreotide from standard solution should be NLT 5000.
3. The % RSD for three replicate injections of standard solution should be NMT 5.0.

Typical chromatograms

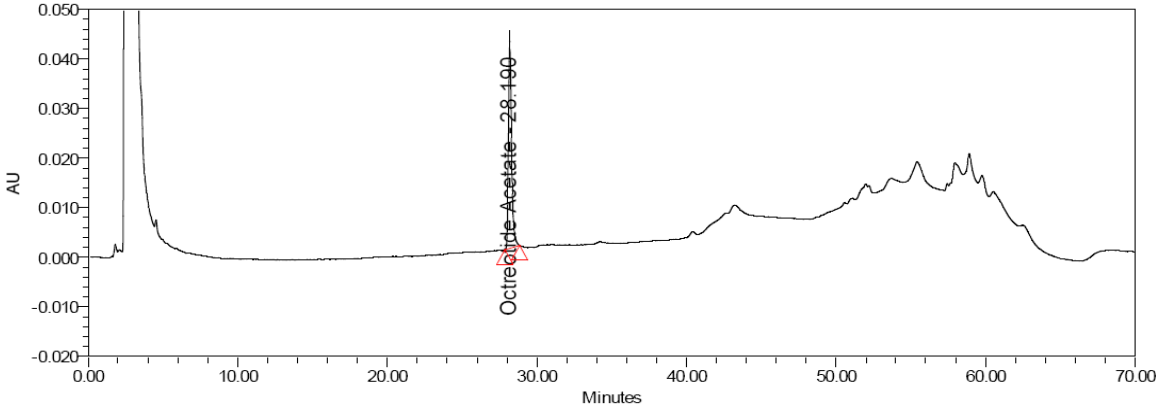
Typical chromatogram of Blank



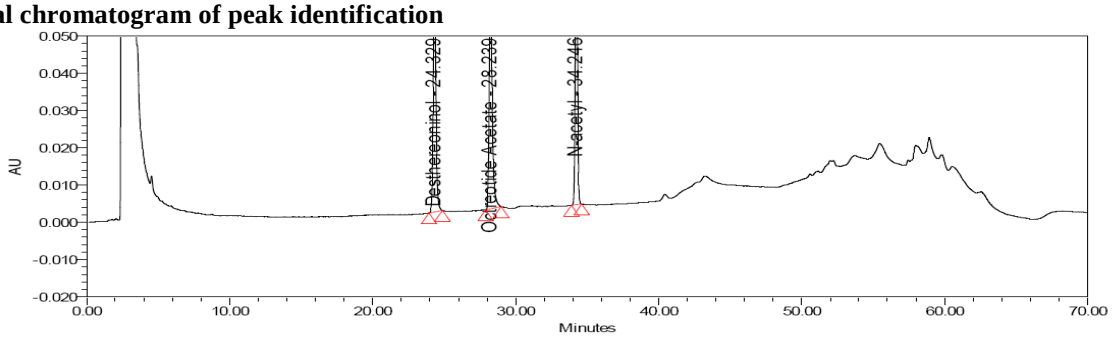
Typical chromatogram of Placebo



Typical chromatogram of Standard



Typical chromatogram of peak identification



CONCLUSION

The present study emerged with suitable method for quantification of octreotide and its related compounds. thus, related compounds method was designed by taking adequate care to separate process related and degradation impurities each other from octreotide. This method also used for routine analysis of production samples and check stability.

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