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RESEARCH ARTICLE

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DEVELOPMENT AND VALIDATION OF A REVERSED-PHASE HPLC METHOD FOR ASSAY OF THE OCTAPEPTIDE OCTREOTIDE IN RAW MATERIAL AND PHARMACEUTICAL DOSAGE FORM

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Abstract

The objective of this study was to develop and validate reverse phase high-performance liquid chromatography (RP-HPLC) method for the quantification of octreotide, a somatostatin analogue, in raw material and injectable dosage form by using a UV detector. Octreotide drug molecules are used for carcinoid tumor and acromegaly. RP-HPLC quantification was carried out by using mobile phase comprising acetonitrile, water and tetrabutylammonium hydroxide through an octadecylsilyl (C18) end-capped column (250 mm × 4.6 mm, 5 µm). The flow rate for this assay was 1 ml/min and detection was performed at 210 nm. This method was validated according to the guidelines of the International Council for Harmonization (ICH). The assay was linear in concentration range 0.2-1.8 µg/ml with relative standard deviation less than 2. The performed assay was sensitive with a limit of detection (LOD) 0.025 µg/mL and a limit of quantification (LOQ) 0.084 µg/ml. Similarly, the percentage recovery was 97.02-99.25% that showed the accuracy of the developed method. Results of this study confirmed that method was sensitive, specific, accurate and precise for quantification of

octreotide in raw material and injectable dosage forms by using common UV detector.

Keywords: Octreotide, RP-HPLC Assay, UV detection, Injectable dosage form, Raw material Mobile Phase

Introduction

Octreotide (a synthetic, long-acting, cyclic octapeptide) is a somatostatin analogue that has prolonged pharmacological effect (Chanson et al., 1993) and selectively blocks hormone release compared to somatostatin (Banerjee et al., 2013). The first synthetic somatostatin analogue was Octreotide. Initially, it was used for the treatment of gastrointestinal symptoms. These symptoms were associated with functional carcinoid tumors. Further research on octreotide pharmacokinetics, has led to long-acting repeatable octreotide acetate. It is a long-acting version that provides greater administration feasibility (Yau et al., 2017). Octreotide long-acting repeatable (LAR) is commonly used for the treatment of functional or metastatic neuroendocrine neoplasms (NENs). The pharmacological effect of octreotide to control carcinoid symptoms are due to its binding with somatostatin receptors. These receptors are on the tumor and metastasis. These receptors are responsible for regulation of growth hormones and ultimately, regulate the cell growth. Notably, somatostatin receptors are also expressed on Tregs at different levels (Claudia et al., 2020).

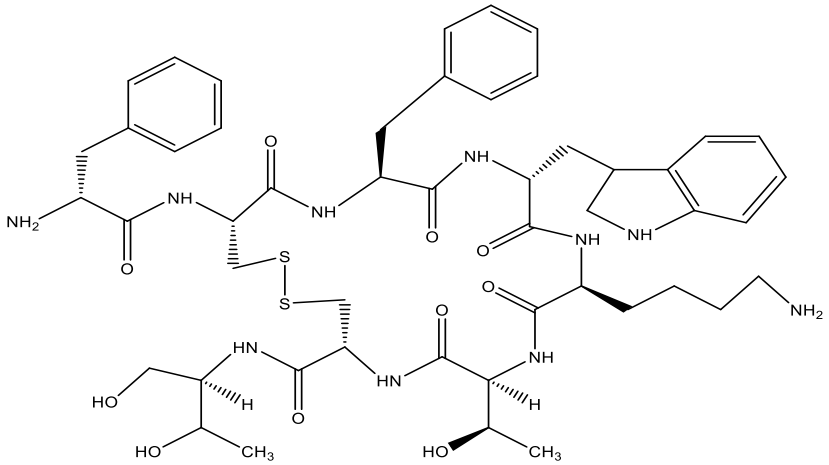

Figure 1. Structure of Octreotide

Octreotide which is marketed with the name of Sandostatin by Novartis pharmaceuticals, it is a more potent inhibitor of growth hormone (GH), than natural hormones (Fery et al., 2005). Octreotide is absorbed quickly and completely after subcutaneous administration (Zhang et al., 2006). The elimination half-life of octreotide is approximately 1.5 h after intravenous or subcutaneous administration in humans (Frank et al., 2017). This injection formulation is approved for the treatment of acromegaly, gigantism, thyrotropinoma, flushing episodes, carcinoid symptoms and in diarrheal conditions for patients with vasoactive intestinal peptide-secreting tumors (Haris et al., 2020).

It was reported that octreotide usage lead to growth hormone reduction in 90% of patients and in 40-50% lead to reduction of growth hormone to less than 10 μg/L (Vance and Harris, 1999). Another study reported that the therapeutic effect of Sandostatin LAR injection was equal at 4 and 6 weeks intervals (Biemazsz et al., 2003). This mechanism is responsible for prolonged intestinal transit time and decreased gallbladder contractility (Singh et al., 2007).

Octreotide causes gastrointestinal disorders (34% to 61%). These disorders may be mild or moderately in severity. These include diarrheal symptoms, feeling of nausea, abdominal cramps, abnormalities of gallbladder, like cholelithiasis and microlithiasis, biliary sediment and sludge due to varying of fat absorption and possibly by decreasing motility of the gallbladder (McKeage et al., 2003).

The reversed phase HPLC procedure is used for analysis of proteins and peptide. The column used in these procedures is normally n alkyl silica based and mobile phases used are organic solvents like acetonitrile having ionic modifier e.g, trifluoroacetic acid (Mant and Hodges, 2019). Octreotide has been assayed by different HPLC methods using fluorimetric techniques, mass spectroscopy, and UV-detection for quantification in plasma (Jiang et al., 2007; Akhlaghi et al., 2015; Park et al., 2009). Fluorimetric techniques and mass spectrometry are not commonly available in all laboratories (Gharia et al., 2013).

Although, mass spectrometry has the highest sensitivity, but the process of determination is complex (Tamvakopoulos, 2007). The chromatographic behavior of peptide is different from simple organic molecules. Small organic molecules chromatographic behavior is described by a defined partitioning equilibrium between the stationary phase and the mobile phase. Peptides reveal an adsorption in which the peptides are adsorbed onto the stationary phase and elutes when the solvent strength of the mobile phase is quite enough to compete with the hydrophobic forces (Carr, 2002; Colin et al., 2007). In addition, to the best of our knowledge, no official report for the assay of octreotide in bulk material and parenteral formulations has ever been published in any pharmacopeia. Thus, it would be of particular interest for the development and validation of simple, precise, accurate, specific and stable RP-HPLC method with UV detector. This developed and validated method should be according to ICH guidelines for the determination and routine analysis of octreotide in pure and injectable formulations (ICH, 1996).

Materials and methods

Chemicals and solvents

An analytically pure sample of octreotide standard was obtained from Novartis pharma Pakistan manufactured by Novartis, Switzerland batch number H4252. HPLC-grade acetonitrile, tetramethylammonium hydroxide and tetrabutylammonium hydroxide were purchased from Merck Private Limited, India. Injections of sandostatin containing octreotide with labeled amount of 0.05 mg/ml and 0.1 mg/ml per injection were procured from local pharmacy.

Instruments

HPLC analysis was performed on a waters Alliance e 2695 quaternary gradient liquid chromatography system comprising a pump, DAD and UV-visible detectors. HPLC was available with "empower" data acquisition software and ODS C18 column (25 cm x 4.6 mm, 5 µm). An auto-sampler injector with a 20 µL sample loop was utilized.

Development of reversed phase HPLC method

Preparation of mobile phase

Water for HPLC distilled and filtered was mixed with acetonitrile and tetrabutylammonium hydroxide at a ratio (45:55:3 v/v/v). A 0.45 µm nylon membrane filter was used to filter the pre-mixed mobile phase and then sonicated for degassing. The pH was adjusted to 4.6, using 0.1N o-phosphoric acid.

Preparation of sample

The samples were taken with concentrations of octreotide 0.1 mg/mL and 0.05 mg/mL as such as available in the market without dilution for analysis.

Preparation of standard

Standard stock solution of octreotide was prepared by taking 10 mg of octreotide and dissolving it 100 ml of HPLC grade water to prepare 0.1 mg/ml stock solution.

Working solutions were prepared by diluting the stock solution with HPLC grade water to obtain a range of 0.2-1.8 µg/ml. Then, 0.45 µm polytetrafluoroethylene (PTFE) syringe filters were used to filter all solutions.

Selection of detection wavelength

A Standard solution of octreotide having strength 0.1 mg/mL was prepared in HPLC-grade water. The UV spectra of solutions with various concentrations of the sample and reference standard of octreotide in mobile phase/ HPLC grade water were recorded using a diode array detector (DAD). Since octreotide is a protein-based drug, it showed maximum absorbance at 210 nm.

Validation

The Developed method was validated with respect to ICH parameters such as system suitability, linearity, accuracy (recovery interday and intraday), sensitivity, limit of detection (LOD) and limit of quantification (LOQ) and specificity (ICH, 1996).

RESULTS AND DISCUSSION

Development and validation of HPLC method

The objective of this study was to develop an HPLC method for octreotide acetate (a protein molecule) analysis. In the first stage of method development, the spectrum was obtained using DAD to determine the maximum absorbance of octreotide. The resultant spectrum showed absorbance at 210 nm. A representative spectrum is shown in Figure 2.

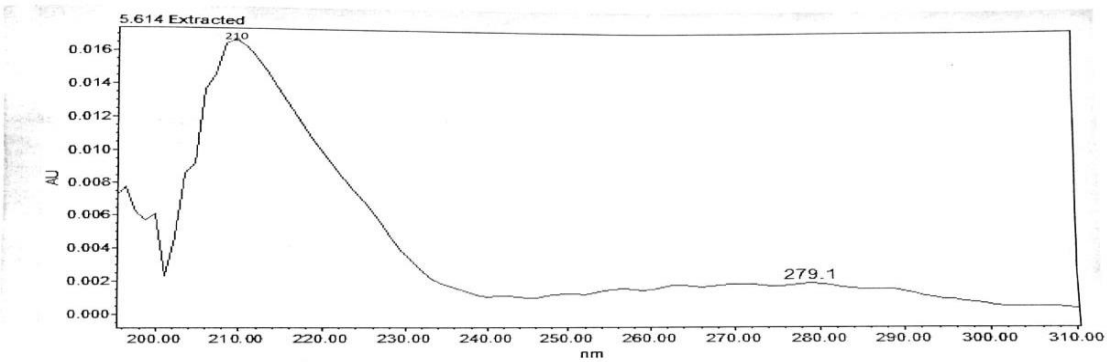

Figure 2: UV Spectrum of octreotide obtained by DAD showing the maximum absorbance at 210 nm

Initially, the following two mobile phases were selected as the most commonly employed for the determination of protein molecules.

A: Acetonitrile: Water: Tetramethylammonium Hydroxide (100:880:20)

B: Acetonitrile: Water: Tetramethylammonium Hydroxide (600:380:20)

The Detection wavelength was 210 nm and the injection volume was 100 µL. The maximum absorbance/response was at 210 nm. Chromatograms revealed a very early peak (at 0.82 min), which was less than the void volume time of the column (1.3 min). The Secondary peaks are not well resolved from the principle peak. In order to develop a more suitable and easy method, gradient flow was changed to isocratic initially as acetonitrile-water (50:50), having flow rate of 1 ml/min, Column used was the same as above method, sample temperature 5^{o}C and column temperature ambient, detector wavelength was set at 210 nm. As both solvents were neutral to slightly basic in nature, it was proposed that the acidified solution could improve the separation. Water was replaced with a 0.1% formic acid solution and the same method was repeated. The resultant chromatogram revealed that peaks were better separated and shifted forward, but the peaks were not base to base as an ideal chromatogram. Peaks still had a co-extrusion issue. The method was repeated using an End-capped column to ensure that there was no change in the response due to residual silanols. Further, the ion-

pairing agent tetrabutylammonium hydroxide 2% was used instead of water. The purpose was to increase the retention of the analyte in the column. Tetrabutylammonium hydroxide was used as it is more powerful (non-polar) as compared to tetramethylammonium hydroxide (less nonpolar) as used at initial stage in the method. The results are satisfactory. As the column was short (50 mm), peaks were observed early. The peak at 3.2 min corresponds to octreotide, as seen by the UV spectrum obtained by the DAD detector. The peak was shifted to approximately 8.367 min when column of 250 mm length was used. The Same procedure was repeated with octreotide injection (octreotide, 0.1 mg/mL). A Peak was observed at 275 and 210 nm. At 275 nm, tailing appeared which was evident to be interference in the chromatogram at 210 nm.

The optimum separation was achieved using a mobile phase consisting of a mixture of acetonitrile: water: tetrabutylammonium hydroxide (45:55:3v/v/v) in isocratic mode, resulting in peaks of standard and injection octreotide at retention times of 4.901 and 4.896, respectively. The chromatograms of the standard and sample are shown in Figure 3 and Figure 4, respectively.

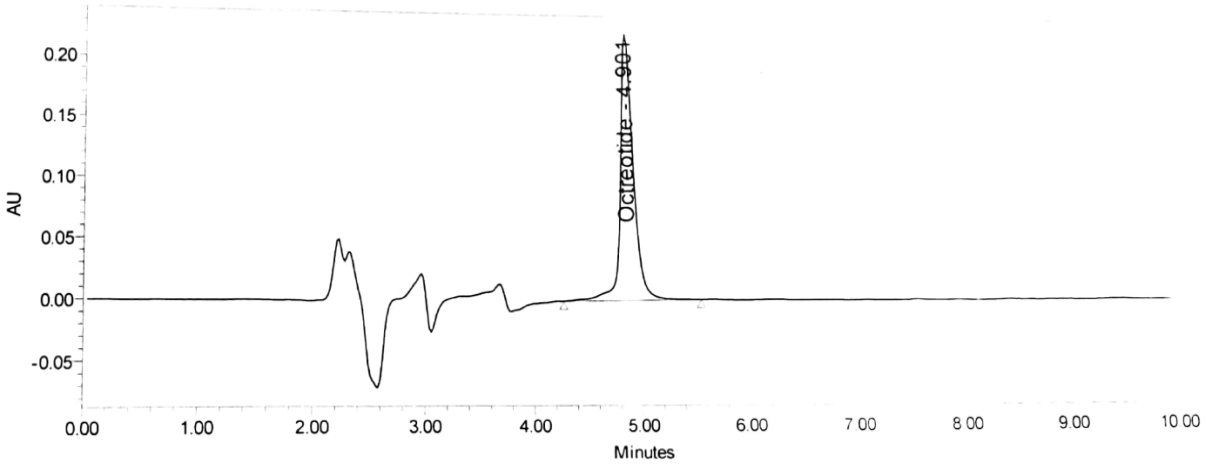

Figure 3: Chromatogram of octreotide showing gaussian peak of standard solution of octreotide

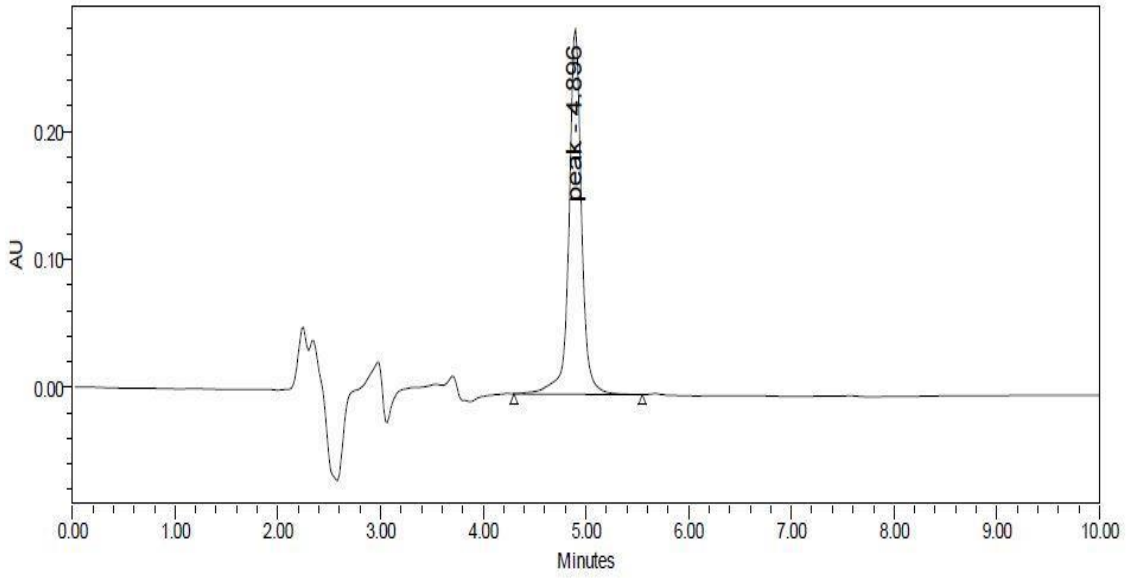

Figure 4: Chromatogram of octreotide showing gaussian peak of sample solution of octreotide

System suitability

The analysis method was used to determine the content of commercially available products. System suitability is used for verification of the chromatographic system and to confirm if it works properly, as it is not a source of error in the overall analysis. The results of the number of theoretical plates,

resolution, and tailing factors are shown in Table 1. The mean tailing factor is 1.5419 which is less than 2 and resolution values for all peaks were greater than 2.

Table 1: Results of system suitability (relative standard deviation, tailing factor and plate count) of octreotide standard

Injections	Retention time	Area	Tailing factor	Plate count
1	4.869	2154265	1.548712	6789.09
2	4.871	2154265	1.547711	6770.09
3	4.873	2157726	1.53016	6761.62
4	4.869	2157611	1.54061	6775.26
5	4.872	2155623	1.54231	6788.29
Mean	**4.8708**	**2155898**	**1.5419**	**6776.87**
Std.	**0.0016**	**1528.72**		

Linearity

The linearity of different concentrations of standard drugs and mixtures was studied in the range of 0.2-1.8 µg/ml for octreotide. All standard solutions were analyzed in triplicate using methanol as a blank to assess linearity. The calibration curve plotted between concentrations versus the peak height is shown in Figure 5. The linear regression equation for octreotide was determined to be Y= 2195942.583X - 31168.25

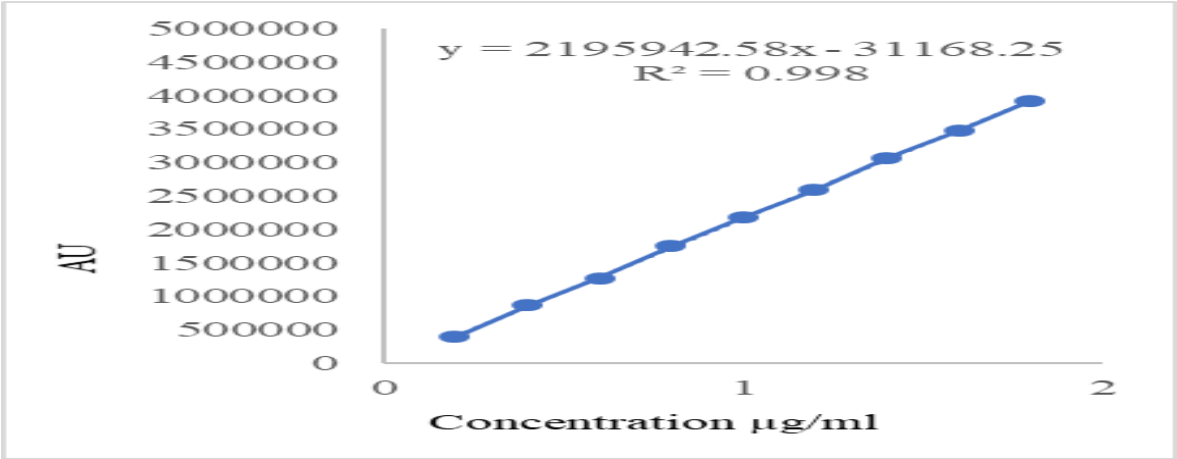

Figure 5: Linearity curve obtained by plotting concentration (x-axis) against the area (Y- axis)

Sensitivity

The results shown in Table 2 indicate the calibration data, LOD and LOQ values. The method was found to be linear over the range of investigation, with a correlation coefficient of 0.996 to 0.999 and a standard deviation of less than 5%. The LOD value was 0.025 µg/ml. The LOQ value was taken as 0.084 µg/ml at a signal-to-noise ratio of 10:1.

Table 2: Showing peak area corresponding to increased concentration of octreotide

Concentration (µg/ml)	Linear regression equation	Intercept	Slope	R^2
0.2 - 1.8	y = 2195942.583x - 31168.25	31168.25	2195942.58	R^2 = 0.999
0.2 - 1.8	y = 2143472.833x - 3648.94	3648.94	2143472.83	R^2 = 0.996
0.2 - 1.8	y = 2219560.5x - 34479.83	34479.83	2219560.50	R^2 = 0.998
0.2 - 1.8	y = 2214649.67x - 61769.67	61769.67	2214649.66	R^2 = 0.998

0.2 - 1.8	y = 2198849.33x - 26917.67	26917.67	2198849.33	R^2 = 0.999
Mean Slope= 2194494.98				
Intercept Standard Deviation= 18557.34				

LOD= 3 SD/S 0.025 where "SD" is Standard Deviation and "S" is slope
LOQ= 10 SD/S 0.084 where "SD" is Standard Deviation and "S" is slope

Recovery, repeatability and reproducibility

The results of recovery, intraday and inter-day accuracy and precision are shown in Table 3. The Average recovery was 97.106 with an RSD of less than 5%, indicating that the method is reliable. Intra-and inter-day accuracy values were 97.8 to 101.4% and 98.9 to 100.2, respectively with RSD less than 5%.

Table 3: Recovery, repeatability and reproducibility of HPLC analysis for determination of octreotide

Concentration mg/mL	**Recovery (n=3) %**	**SD**	**Intraday accuracy and precision (n=6) Accuracy**	**RSD**	**Inter-day accuracy and precision (n=6) Accuracy**	**RSD**
0.15	97.02	0.39	98.88	0.41	101.39	1.42
0.2	95.04	0.32	99.93	0.76	98.97	0.28
0.25	99.25	0.31	100.18	1.56	97.81	0.46

Selectivity

Selectivity was evaluated by running the test solutions on HPLC by scanning through a range of wavelengths (200-400 nm). The resultant 3D orientation of the peak was used to confirm that the peak was of sufficient purity. The value of the purity angle was 4.83 for the octreotide peak, which was less than the threshold value i.e. 7.083 determined by the software for the specific peak. The 3D orientation of the peak and purity plot is shown in Figure 6.

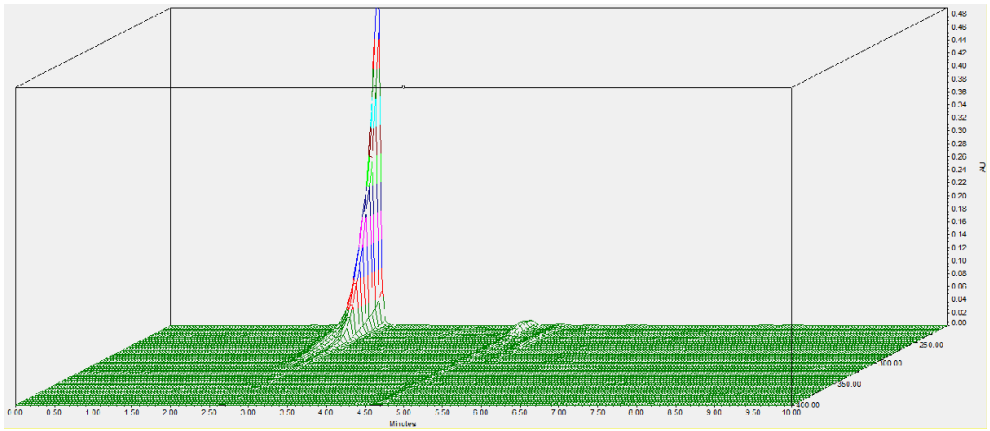

Figure 6. 3D orientation of the peak

Table 4. Purity data of 3D oriented peak

Purity angle	Purity threshold	Purity flag
4.837	7.083	3.017

CONCLUSION

A RP-HPLC equipped with a UV-visible detector method has been successfully developed and validated for routine quality control analysis of octreotide in dosage form and bulk material. Octreotide, a highly expensive thermolabile protein-based drug, requires a specific and reliable method for accurate determination. The reported method is convenient for routine quality control

analysis and requires less consumption of organic solvents with excellent and valuable results in terms of linearity, sensitivity, recovery (repeatability/ reproducibility) and specificity. As the HPLC method is not available in international pharmacopeia for octreotide in dosage form, the reported study can be used in quality control laboratories of pharmaceutical industries as well as in drug testing laboratories for qualitative and quantitative analysis of octreotide in dosage forms.

Conflict of interest

All the authors declare that there are no conflicts of interest.

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