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DEVELOPMENT AND VALIDATION OF A UV-VISIBLE SPECTROPHOTOMETER METHOD FOR DETERMINATION OF PRAMLINTIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Background: The objective of my research work is to develop and validate the UV-spectrophotometric method for the estimation of Pramlintide in bulk and pharmaceutical formulations as per ICH guidelines **Materials and Methods**: A UV-visible spectrophotometric method has been developed using distilled water as solvent. Pramlintide standard solutions (5–25 µg/mL) were prepared and scanned between 200 and 400 nm. Calibration curve, precision, accuracy, Limit of Detection (LOD) and Limit of Quantification (LOQ) were evaluated as per ICH Q2(R2) guidelines. **Results:** The λ_{max} of Pramlintide in distilled water was found to be 230 nm. The drug follows linearity in the concentration range 5-25 µg/ml with a correlation coefficient value of 0.9847. The precision of the method was studied for an intraday and interday variation. The proposed method was applied to pharmaceutical formulation and percentage amount of drug estimated between 98.66 to 101.5% and was found to be in good agreement with the label claim. The recovery was found to be in the range of 100.68 to 102.13%. The low values of %RSD are indicative of the accuracy and reproducibility of the method. Robustness was evaluated by applying small deliberate changes in wavelength to observe the method's consistency. Ruggedness assessment across different analysts and days yielded %RSD values below 2, affirming the stability of the method. LOD and LOQ were found to be 1.58 and 5.22 µg/ ml respectively, demonstrating high sensitivity. **Conclusion:** The above method was a rapid tool for routine analysis of Pramlintide in the bulk and in the pharmaceutical dosage form.

KEYWORDS: Quantitative determination, Pramlintide, UV Spectroscopic method, Validation.

INTRODUCTION

Pramlintide, Proline-25,28,29-triphenylalanine, human amylin (1-37) [Figure 1], is a new potent antidiabetic agent of amylin class that works by slowing gastric emptying, which delays glucose absorption and suppressing postprandial glucagon secretion, reducing hepatic glucose output and promoting satiety, helps to reduce caloric intake. Pramlintide is not yet official in any pharmacopeia, where, only few analytical methods have been reported for its determination in pharmaceutical formulations and biological fluids. Such methods include RP-HPLC[1[1,2]], HPLC[2,3], SCX-HPLC[2][2], LC-MS[3], SPE LC-MS/MS.[4]

Among the various methods available for the determination of Pramlintide, spectrophotometry continues to be very popular, because of its simplicity, specificity, and low cost. This study presents a new spectrophotometric method for the determination of Pramlintide in bulk and pharmaceutical formulations. Accordingly, the objective of this study was to develop and validate the UV-spectrophotometric method for the estimation of Pramlintide in bulk and pharmaceutical formulations as per ICH guidelines.

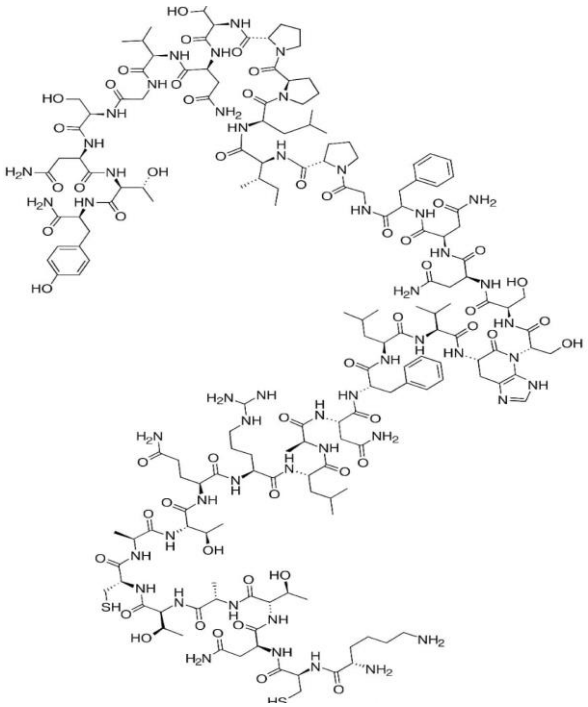

Figure 1: Chemical structure of Pramlintide.

DRUG PROFILE

Pramlintide is an antidiabetic drug classified as an amylinomimetic. It appears as a white solid. The molecular formula and molecular weight of the compound is $C_{171}H_{267}N_{51}O_{53}S_2$ and 3949 g/mol respectively. It is soluble in distilled water and practically insoluble in most organic solvents such as ethanol, methanol, acetone, and chloroform. The strongest acidic pKa value is 2.31 and the strongest basic pKa value is 11.88. The compound has a melting point of 175 °C and should be stored under refrigerated conditions at 2–8 °C. Pramlintide is administered parenterally, typically by subcutaneous injection. Its bioavailability is about 30–40%. Pramlintide shows 40% protein binding, leaving a significant fraction free and active. Its biological half-life is 48 minutes, indicating rapid clearance from the body.

Pharmacologically, Pramlintide mimics the effect of amylin, a hormone co-secreted with insulin by pancreatic beta cells. It slows gastric emptying, suppresses postprandial glucagon secretion, and promotes satiety, thereby reducing food intake. It is used as an adjunct therapy in type 1 and type 2 diabetes mellitus to improve glycaemic control.

MATERIALS AND METHODS

Materials

Pramlintide drug was received as a gift sample from an Industrial Estate, Hyderabad, India. Pramlintide acetate injection containing 0.6 mg/ml of Pramlintide were obtained from local pharmacies.

Preparation of standard stock solution

Accurately weighed 100 mg of Pramlintide was transferred to a 100 ml volumetric flask, dissolved with 50 ml distilled water by shaking manually for 10 min. The volume was made up to the mark with distilled water to get the final concentration of 1mg/ml. From this stock solution, 10 mL was transferred into a 100 mL volumetric flask and diluted to the mark with distilled water to obtain a final concentration of 100 µg/mL, which served as the working standard solution.

Selection of wavelength for analysis of Pramlintide

10 ml of standard stock solution of Pramlintide was transferred into a 100 ml volumetric flask, diluted to a mark with distilled water to get a concentration of 10 µg/ml. The resulting solution was scanned in the UV range of 200-400 nm.

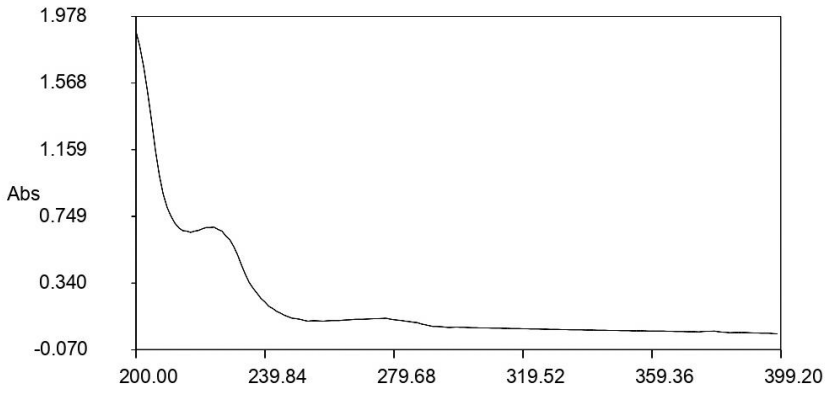

Figure 2: UV Spectrum of Pramlintide.

Validation of the method

The method was validated in terms of linearity and range, precision, accuracy, robustness, ruggedness, limit of detection, and limit of quantification.

Linearity and Range

Different aliquots of Pramlintide in the range 5-25 ml were transferred into series of 100 ml volumetric flasks, and the volume was made up to the mark with distilled

water to get concentrations of 5, 10, 15, 20, and 25 µg/ml, respectively. The solutions were scanned on a spectrophotometer in the UV range 200-400 nm. The absorbance maxima was found at 230 nm. The calibration plot was constructed as concentration vs. amplitude [Figure 3].

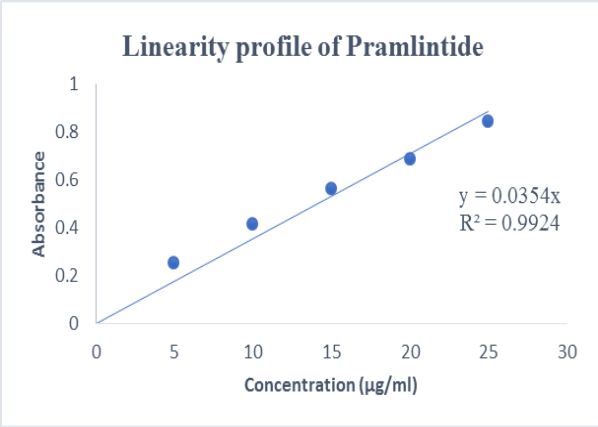

Figure 3: Calibration curve of Pramlintide.

Precision

A quantity equivalent to 1.2mg (2ml) of Pramlintide withdrawn from the marketed sample (vial) was transferred into a 100 mL volumetric flask and made up to the mark with distilled water. The prepared solution was filtered through Whatman filter paper to obtain a concentration of 12 µg/ml. The absorbance of six replicate samples was measured at 230 nm on the same day under identical conditions.

Accuracy

The accuracy of the method was evaluated by standard addition technique. A known quantity of pure pramlintide was spiked into the pre analysed sample at three concentration levels: 50%, 100%, and 150%. For each level, a quantity equivalent to 1.2mg(2ml) of Pramlintide was transferred to a 100 mL volumetric flask. Then, standard Pramlintide solution was added [0.6 mL for 50% (600 µg), 1.2 mL for 100% (1.2 mg), and 1.8 mL for 150% (1.8 mg)] and the volume was made up with distilled water. Each level was prepared in triplicate.

Robustness

Robustness was evaluated by making small but deliberate variations in analytical parameter such as detection wavelength (±2 nm).

Ruggedness

Ruggedness of the proposed method is determined for 12 µg/ml concentration of Pramlintide by analysis of aliquots from a homogenous slot by two analysts using same operational and environmental conditions.

Limit of Detection and Limit of Quantification

The sensitivity of measurements of Pramlintide by the use of the proposed method was estimated in terms of the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were calculated using equation LOD=3.3×σ/S and LOQ=10×σ/S, where σ is standard deviation and S is the slope of the corresponding calibration curve.

Stability of the sample solution

Stability of the sample solution was carried out to determine the holding time of the Pramlintide sample solution at a room temperature while analyzing in UV-Visible Spectrophotometer. The sample solution with a concentration of 15 µg/mL, prepared for the linearity study, was used for the analysis. The sample solution was analysed at 230 nm for a period of 3 hours at different time intervals of 0, 10, 20, 30, 60, 90, 120 and 180 minutes.

RESULTS AND DISCUSSION

Method validation

The proposed method was validated as per ICH guidelines. The solution of the drug was prepared and evaluated as per the adopted procedure given in the experiment. UV- spectra of Pramlintide was scanned between 200 and 400 nm. The spectra show λ_{max} at 230 nm which was shown in Figure 2.

Linearity studies

The linear regression data for the calibration curves showed good linear relationship over the concentration range 5-25 µg/ml for Pramlintide [Figure 3]. Linear regression equation was found to be Y = 0.0323X with the correlation coefficient of R^2=0.9924. The calibration data is given in Table 1.

Table 1: Linearity study of Pramlintide.

S. No	Concentration (µg/ml)	Absorbance
1	5	0.255
2	10	0.415
3	15	0.565
4	20	0.685
5	25	0.844

Precision

The Precision data was found in the range of 98.66 – 101.5 %. The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). The result shows its reproducibility of the assay. The % RSD values found to be less than 2 indicates that the developed method is more precise for the determination of both the drugs in formulation [Table 2]

Table 2: Precision studies for Pramlintide.

S. No	Absorbance	Amount found (mg)	Content (%)
1	0.448	610.54	101.5
2	0.441	595.25	99.16
3	0.442	596.54	99.41
4	0.441	595.53	99.16
5	0.439	592.65	98.66
6	0.445	601.51	100.25
Mean± SD = 99.71±1.085			
%RSD = 1.088			

Accuracy

The solutions were carried out for the recovery studies using the proposed method. Results of recovery studies were presented in Table 3, which showed that the percentage recovery was between 100.68% and 102.13% with % RSD of <2.

Table 3: Recovery studies for Pramlintide.

Level Added (%)	Amount Added (ml)	Absorbance	Amount Found (mg)	Amount Recovered (mg)	Mean with Standard Deviation
50	0.6ml	0.454	607.5	101.66	101.33±0.5288
	0.6ml	0.453	604.5	100.22	
	0.6ml	0.455	609.6	101.61	
100	1.2ml	0.688	1228.24	102.35	102.13±0.22
	1.2ml	0.686	1222.93	101.91	
	1.2ml	0.687	1225.59	102.13	
150	1.8ml	0.907	1809.66	100.53	100.68±0.15
	1.8ml	0.909	1814.97	100.83	
	1.8ml	0.908	1812.31	100.68	
Mean ±SD = 101.38±0.726					
%RSD = 0.716					

Robustness

The robustness data shows the reliability and consistency of the method while partial change in wavelength. The %RSD values obtained were less than 2, indicating that the method is robust and unaffected by minor experimental changes.

Ruggedness

Ruggedness was determined by analysing samples under similar conditions by two different analysts. The %RSD values obtained were less than 2, indicating that the developed method is rugged and reproducible under variable conditions.

Limit of Detection and Limit of Quantification

The LOD and LOQ for Pramlintide was found to be 1.58 µg /ml and 5.22 µg/ ml respectively.

Stability of the sample solution

The Sample solution remained stable up to 2 hours, as there was no significant change in absorbance was observed [Table4]. After 2 hours, a significant decrease in absorbance was observed, showing that the sample started to lose its stability. This indicates that the sample solution can be analysed with a buffer time of 2 hours at room temperature.

Table 4: Stability of the sample solution.

Time (min)	Absorbance
0	0.565
10	0.564
20	0.560
30	0.556
60	0.546
90	0.543
120	0.541
180	0.530

CONCLUSION

This UV-spectrophotometric technique is quite simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for quantification of Pramlintide in Pharmaceutical formulation. The method showed good linearity over the range of 5- 25 µg/ ml (R^2=0.9847), high precise (97.71% ±1.085), high recovery (100.68-102.13%) with low %RSD values (<2), demonstrating its reliability. The obtained LOD (1.58 µg/mL) and LOQ (5.22 µg/mL) values indicate the method's adequate sensitivity. Moreover, the robustness and ruggedness results confirm its reproducibility under varied analytical conditions. The validation procedure confirms that it is an appropriate method for the quantification of Pramlintide in the formulation.

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