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Analytical Method Development of Saxagliptin HCl by RP-HPLC

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ABSTRACT

Reversed-phase chromatography is the mainly used in chromatographic mode, it is used to separate neutral molecules in solution based on their hydrophobicity. This technique is the reverse of normal-phase chromatography in the intelligence that it involves the employ of a polar mobile phase and a non-polar stationary phase. A sensitive, accurate, rapid, cost effective and robust HPLC method was developed for the quantification of Saxagliptin Hydrochloride (SGH) with UV detector. In this method, a reversed-phase Grace C18 (250mm x 4.6ID, Particle size: 5 micron) column with a mobile phase of methanol: water (80:20; v/v) at 0.8ml/min flow rate was used to separate SGH with a detection of 212nm.The volume injected was 20 µL. The retention time of SGH was obtained as 4.196 min. Hence it can be applied for routine analysis of Saxagliptin Hydrochloride (SGH) in bulk drug.

Keywords: Saxagliptin Hydrochloride (SGH), RP-HPLC, Assay, Method validation.

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1. INTRODUCTION

Analytical chemistry termed as science of determining the components of materials in terms of the elements or compound contained. The techniques of this science are used to identify the substances which may be present in a material and to determine the exact amounts of the identified substances. Analytical chemistry is important in nearly all aspects of chemistry, e.g. clinical, agricultural, environmental, forensic, manufacturing metallurgical and pharmaceutical chemistry. Analytical techniques proved in assuring and maintaining the quality of substance and are critical components of QA and QC[1, 2].

Analytical method should be,

1. Most productive, economical and convenient,
2. As accurate and precise as required,
3. As simple as possible,
4. Most specific

Should be fully optimized before transfer for validation of its characteristics such as precision, accuracy, sensitivity etc.[2]

According to USP, system suitability tests are integral part of chromatographic methods. These tests are used to verify that the reproducibility and resolution of the system are adequate for the analysis to be performed. Parameters such as plate count, tailing factor, resolution and reproducibility (%RSD retention time and area for 6 repetitions) are determined and compared against the specifications set for the method.[3, 4]

Now a day reversed-phase chromatography is the most commonly used separation technique in HPLC due to its broad application range. It is estimated that over 65% (possibly up to 90%) of all HPLC separations are carried out in the reversed phase mode. The reasons for this include the simplicity, versatility, and scope of the reversed-phase method as it is able to handle compounds of a diverse polarity and molecular mass[5-7].

Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character can be separated by reversed phase chromatography with excellent recovery and resolution[8]. The separation mechanism in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilised hydrophobic ligand, i.e. the stationary phase. The actual nature of the hydrophobic binding interaction itself is a matter of heated debate[9] but the conventional wisdom assumes the binding interaction to be the result of a favourable entropy effect.

2. AIM AND OBJECTIVE

Aim:

Analytical method development and validation of Saxagliptin HCL by RP-HPLC method.

Objectives:

1. To develop a simple validated stability indicating method for the estimation for Saxagliptin HCL by RP-HPLC.

2. To validate methods as per ICH guidelines.

3. MATERIALS AND METHOD

3.1. UV Spectroscopic Method

Selection of Wavelength: Weighed accurately about 10mg of working standard of Saxagliptin HCl and transferred in 10mL of volumetric flask. Added about 5mL of diluent, Sonicated to dissolve and volume was made up the mark with diluent.

Further diluted 0.2mL of above solution to 10mL and volume was made up to the mark with diluent.

Determination of λmax [10-11]

Initially the Ultra violet (U.V) spectrum of Saxagliptin HCl was produced using appropriate U.V spectrophotometer, so as to determine the absorbance maxima or Lambda max (λ max). This is essential since HPLC detection is basically UV based, thus a 20ppm solution of Saxagliptin HCl in water was used to get the following spectra.

3.2 High Performance Liquid Chromatography Method Development by RP-HPLC

Preparation of Saxagliptin HCl Standard:[12-17]

Weighed accurately about 10mg of Saxagliptin HCl standard and transffered into 10mL of volumetric flask, added about 5mL of diluent, shaked to dissolved and volume was made up to the mark with diluent. (concentration of Saxagliptin HCl 1000ppm).

Further diluted 0.5mL of above stock solution to 10mL of volumetric flask and volume was made up to the mark with diluent (concentration of Saxagliptin HCl 50ppm).

Trial 1

Table 3.1: Chromatographic conditions for Trial 1

Column	Grace C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	Methanol : Buffer (70:30)
Pump mode	Isocratic
Flow rate	0.8ml/min
Detector	UV- Detector
Injection volume	20µL
Column Temperature	25°C
Wavelength	212nm
Run time	7.71min

Preparation of Buffer: 0.136gm of Potassium dihydrogen phosphate dissolved in 100mL of distilled water, gives 10mM solution.

Trial 2

Table 3.2: Chromatographic conditions for Trial 2

Column	Grace C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	Methanol : Buffer (80:20)
Pump mode	Isocratic
Flow rate	0.8ml/min
Detector	UV- Detector
Injection volume	20µL
Column Temperature	25°C
Wavelength	212nm
Run time	6.88min

Trial 3

Table 3.3: Chromatographic conditions for Trial 3

Column	Grace C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	Methanol : Water (80:20)
Pump mode	Isocratic
Flow rate	0.8ml/min
Detector	UV- Detector
Injection volume	20µL
Column Temperature	25°C
Wavelength	212nm
Run time	9.21min

Trial 4

Table 3.4: Chromatographic conditions for Trial 4

Column	Grace C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	Methanol : Water (80:20)
Pump mode	Isocratic
Flow rate	0.8ml/min
Detector	UV- Detector
Injection volume	20µL
Column Temperature	25°C
Wavelength	212nm
Run time	7.15min

TABLE 4.1: FINAL METHOD FOR ASSAY

PARAMETER	CONDITIONS
Stationary Phase (Column)	Grace C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	Methanol: Water (80:20)
Diluent	Methanol : Water (200:100)
Flow rate	0.8ml/min
Injection volume	20 µL
Pump mode	Isocratic
Detector	PDA
Wavelength	212nm
Column Temperature	25°C
Run Time	7.15 min
Retention Time	4.195

Conclusion:

The peak obtained in Trail 4 is sharper. Shape is better. The retention time of the Trial 4 was less i.e. 4.165’

4. RESULT AND DISCUSSION

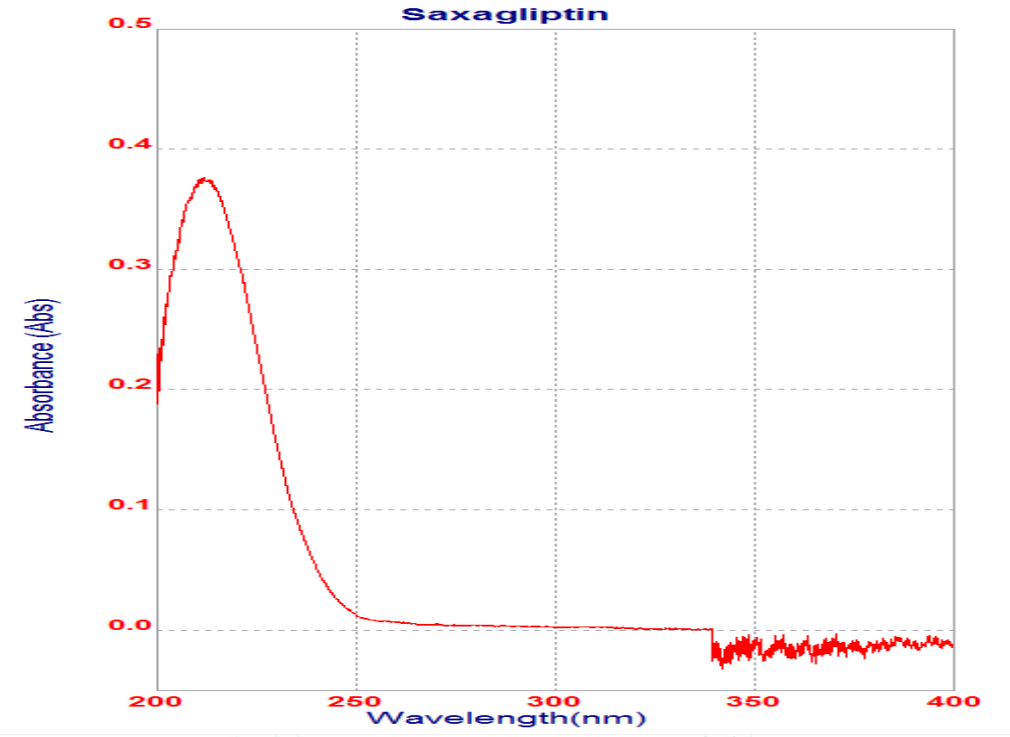

Figure 1.2: UV spectra of Saxagliptin HCl

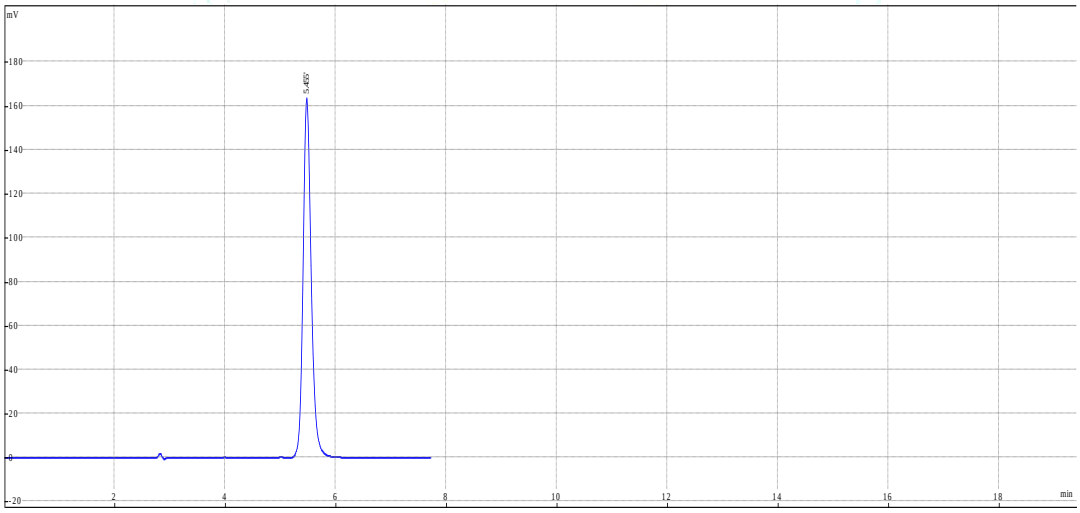

Figure 3.2: Observed chromatogram of Trial 1

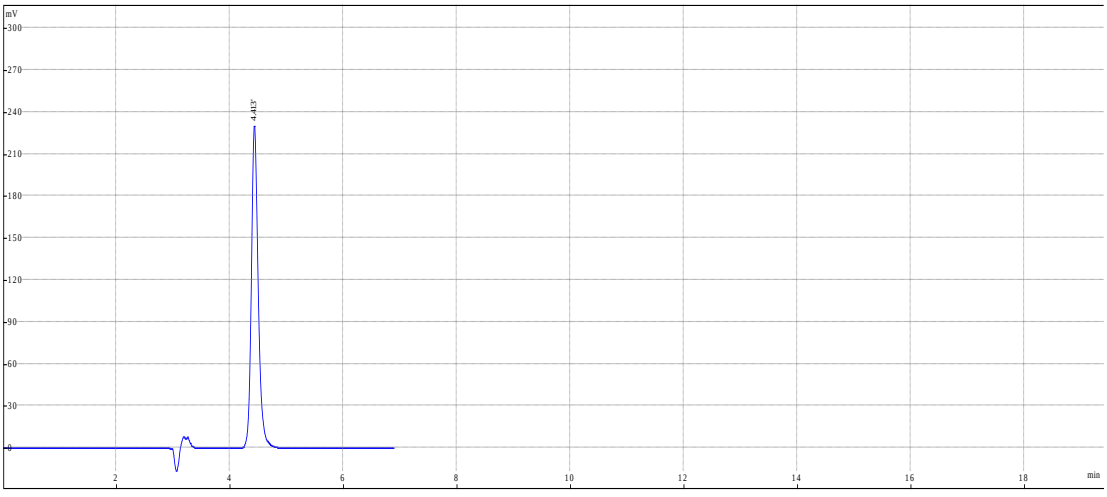

Figure 3.3: Observed chromatogram of Trial 2

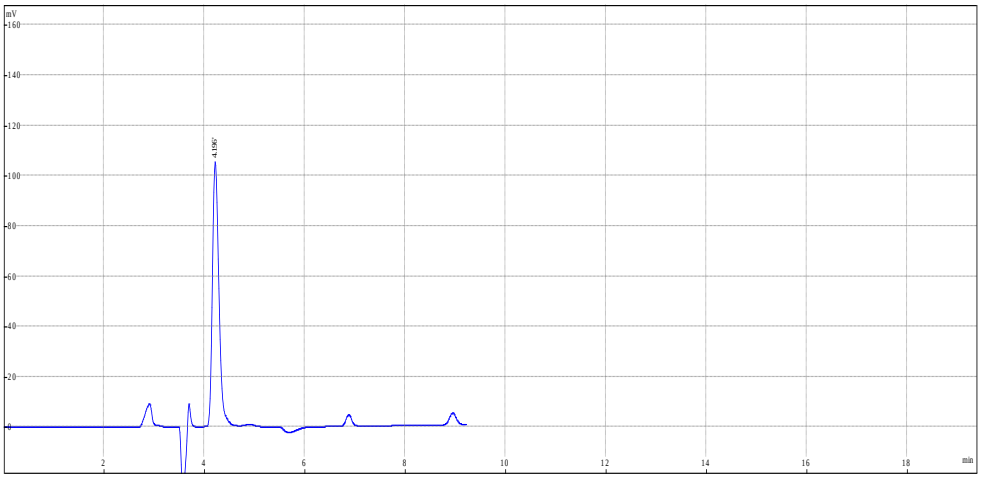

Figure 3.4: Observed Chromatogram of Trial 3

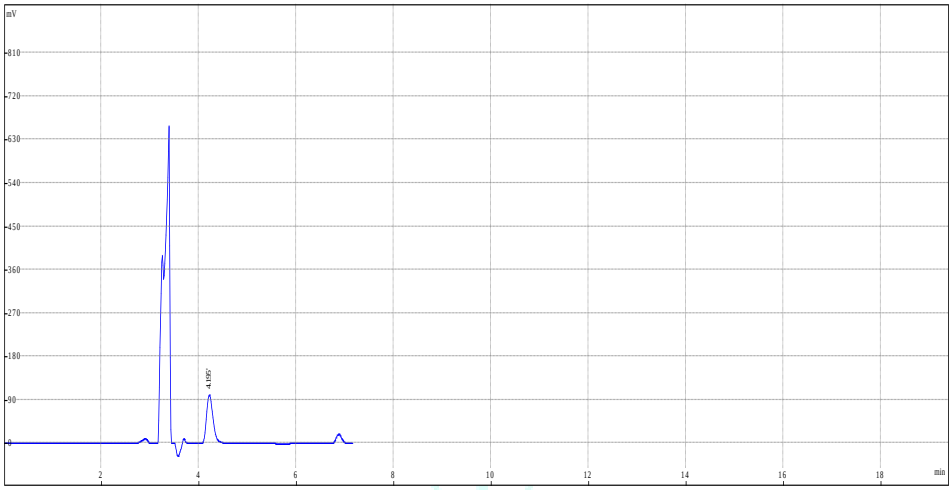

Figure 3.5: Observed Chromatogram of Trial 4

5. CONCLUSION

The present investigation is an attempt to develop method for estimation of saxagliptin HCl in bulk by RP-HPLC. The developed method is less costly with lower retention time than the method reported so far. The developed methods will be useful in the estimation of Saxagliptin HCl in bulk in future.

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