Estimation and Validation of Sitagilptin by First Order Derivative Spectroscopy Method

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Abstract*: A method has been successfully developed for the simultaneous estimation of Sitagilptin in both its bulk drug and tablet dosage form. The drug exhibits absorbance maxima at 228 nm in a 0.1N NaOH solution, and conforms to Beer's law within a concentration range of 10-50 µg/ml. The mean recovery rate for both the bulk drug and tablet form was determined to be 100%, indicating the method's efficacy in accurately retrieving the expected quantities of the drugs. Furthermore, the method demonstrated linearity close to 1, suggesting a strong correlation between concentration and absorbance, which is crucial for precise quantification. Assessment of precision through various parameters including intraday, interday, and intermediate precision, as well as robustness, yielded consistent results. The percentage recovery fell within the acceptable range of 82-138% for both drugs, further affirming the method's accuracy and reproducibility. Importantly, all validation parameters were found to be in compliance with the guidelines set forth by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), ensuring the method's adherence to industry standards. In conclusion, these findings collectively underscore the accuracy, precision, and simplicity of the developed method for the simultaneous estimation of Sitagilptin bulk drug and its tablet dosage form.*

Keywords: Sitagilptin, UV Spectroscopy, First derivative spectrophotometric, Spectrophotometric determination

I. INTRODUCTION

Sitagilptin, classified as an oral anti-hyperglycemic agent within the new DPP-4 inhibitor class, plays a pivotal role in managing type 2 diabetes mellitus. Its chemical composition, represented by (S)-1-[2-(3-Hydroxyadamantan-1-ylamino) acetyl] pyrrolidine-2(S)-carbonitrile, underscores its targeted approach in ameliorating hyperglycemia. Functioning as a reversible, selective, and competitive inhibitor of DPP4, Sitagilptin forms complexes with this enzyme, thereby hindering its activity. DPP4, extensively distributed across various tissues including the pancreas, liver, kidneys, and intestines, exerts control over the inactivation of multiple molecules such as chemokines, neuropeptides, cytokines, and gastrointestinal hormones. Notably, DPP4 targets key hormones like glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), essential for maintaining glucose homeostasis. Inhibition of DPP4 results in elevated levels of active GLP-1, ultimately proving efficacious in managing type 2 diabetes mellitus.

Within the realm of pharmaceutical analysis, ultraviolet-visible (UV-VIS) spectrophotometry emerges as a vital tool. This method revolves around measuring the absorption of ultraviolet (190–380 mm) or visible (380–800 mm) radiation by substances dissolved in solution. UV-visible spectrophotometers analyze the intensity ratio or function thereof, of two light beams in the UV-visible region, facilitating the determination of substance concentrations. The absorption of light in both UV and visible regions occurs when the energy aligns with the requisite threshold to induce electronic, vibrational, and rotational transitions within molecules. Given these fundamental principles, UV and visible spectrophotometry techniques are often amalgamated, offering comprehensive insights into pharmaceutical analysis.
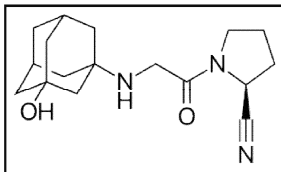
Fig 1: Structure of Sitagilptin

II. DERIVATIVE METHOD [3&4*]

Derivative spectroscopy is a method primarily employed in UV-Visible absorption spectrometry to distinguish spectra. The derivative techniques in analytical chemistry serve three key objectives:

- Spectral differentiation: This involves discerning unique features within spectra, aiding in the identification of individual components and the separation of overlapping signals.
- Spectral resolution enhancement: By improving the clarity and sharpness of spectral peaks, derivative methods enhance resolution, particularly useful when dealing with closely spaced peaks or background noise.
- Quantitative analysis: Derivative spectroscopy enables precise quantitative analysis by enhancing sensitivity and specificity. It allows for accurate determination of analyte concentrations within complex mixtures, contributing to reliable quantitative measurements.

Derivative spectroscopy is a technique used to convert a standard spectrum, also known as a fundamental or zero-order spectrum, into its first, second, or higher derivative spectra by differentiating the absorbance of the sample with respect to wavelength (λ). This method offers several benefits, including the separation of overlapped signals, the elimination of background interference caused by the presence of other compounds in a sample, and the enhancement of resolution in mixtures. By improving the detectability of mirror spectral features, derivative spectroscopy contributes to a more characteristic profile compared to the parent spectrum, with the appearance of new maxima, minima, and points where derivative spectra intersect the X-axis. Moreover, derivative spectroscopy maintains adherence to the laws of classical spectrophotometry, such as the dependence of derivative values on analyte concentration and the additives law. These features enable the determination of multiple components in a mixture by measuring the amplitude of the derivative spectrum at various wavelengths. Notably, computer software facilitates this entire process, allowing for efficient analysis and interpretation of spectral data.

First order derivative spectrum [5*]

Spectra obtained by derivatizing zero order spectrum once. It is a plot of change of absorbance with wavelength against wavelength i.e. rate of change of the absorbance with wavelength,

$dA/d\lambda = f'(\lambda)$

Even if in derivatized form it is more complex than zero order spectrum. First order spectra passes through zero as λ max of the absorbance band. Absorbance band of first order derivative shows certain positive and negative band with maxima and minima. By scanning the spectrum with a minimum and constant difference between two wavelengths, dual-wavelength spectrophotometer obtains first-derivative spectra.

Drug Analysis [6*]

Analytical chemistry is a branch of science which includes the nature and identity of the components and its composition. It is pharmaceutical science, which includes quality of the product and separation of the components. It gives both qualitative and quantitative analysis data for the product.

III. MATERIALS AND METHODS [7 to 9*]

Steps Involved in Spectroscopic Method
Chemicals and reagents
Sitagilptin , water, sodium hydroxide used are of analytical grade.

Instruments & glassware

UV-Visible Spectrophotometer, Digital balance, volumetric flask(100,500ml), beaker, tripod stand, funnel were used in analytical work.

Marketed formulation

Sitagilptin tablet containing 50mg of Sitagilptin manufactured by Mankind pharmaceuticals Ltd. were purchased from local market.

Selection of Solvent

To ensure accurate determinations, it's crucial to select solvents where the solute dissolves easily without interaction. Additionally, solvents with minimal absorption in the UV range should be chosen. High-purity materials are necessary to prevent impurities from affecting absorption at specific wavelengths. Sitagilptin, being more soluble in 0.1N NaOH solution, is dissolved in this solvent for the process.

Standard solutions

Standard stock solutions of Sitagilptin (100μg/ml) were prepared in 0.1 N NaOH: drug:Naoh (w/v)

Spectral characteristics and wavelength selection

The absorption spectra of 10 μg/ml of Sitagilptin and the diluent were recorded by scanning in the UV wave length range 200-400 nm and overlay zero order (D0) spectrum is shown in Figure 2. Zero order spectrums (D0) were converted to first derivative spectra (D1) using computer software. Overlay first order derivative spectrum was shown in Figure 3.

Selection of analytical concentration ranges

Calibration standards at five levels were prepared by diluting the standard stock solution in the concentration range of 10-50 μg/ml for Sitagilptin. The absorbance of these solutions was measured at their zero crossing point wavelengths.

Method validation [10 to 13*]

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Validation is documented evidence, which provides a high degree of assurance for a specific method.

Validation Parameters

- Linearity
- LOD and LOQ
- Robustness
- Repeatability
- Precision
- Accuracy

Linearity

The linearity of analytical method is ability to obtain test results that are directly proportional to the concentration of analyte in the sample. The range of analytical method is the interval between upper and lower concentration (amounts) of analyte that have been demonstrated with suitable level of precision, linearity and accuracy. Series of calibration standards were prepared in the concentration range 10-50 μg/ml. The absorbance of these solutions was measured at 246 nm and 276 nm against solvent blank respectively and D1 (first order derivative) absorbance values were recorded. A graph of concentration versus absorbance was plotted and correlation coefficient (r2) was reported.

Limit of detection (LOD) and limit of quantification (LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. Quantification limit of an individual analytical procedure is the lowest amount of analyze in a sample which can be quantitatively determined with suitable precision and accuracy. The sensitivity of proposed method for measurement of atorvastatin and aspirin was estimated in terms of LOD & LOQ determined using the standard deviation of the response and slope method.

Robustness

Sitagilptin (10µg/ml) were prepared and analyzed by different wavelengths. The solution of Sitagilptin were analyzed at 215nm, 217nm, 219nm, absorbance at each wavelength were measured.

Repeatability

Repeatability was evaluated by the analysis six replicates of 10 µg/ml of Sitagilptin for checking the variation of results on the same day.

Precision

The precision of an analytical procedure defines the degree of closeness of agreement between a series of measurements obtained from multiple samplings of homogenous sample under prescribed conditions. Precision of the method was reported as RSD% at different levels repeatability, Intra-day precision and Inter-day precision.

Accuracy

Recovery studies were carried out by using standard addition method at three levels, 80%, 100% and 120% for Sitagilptin (10µg/ml). At each level, the determination was done in triplicate and the amount of drug recovered was calculated and reported.

IV. RESULT AND DISCUSSION

Developing simple and accurate UV-spectrophotometric methods provides a cost-effective and time-saving alternative to HPLC for routine quantitative analysis of samples. UV spectroscopic method in derivative mode has been developed and optimized for simultaneous analysis of Sitagilptin tab. Derivatization of spectral data obtained in zero-order spectra was performed to enhance the resolution of the broad band peaks of Sitagilptin. This approach not only ensures efficient utilization of resources but also expedites the analysis process, making it suitable for routine applications.
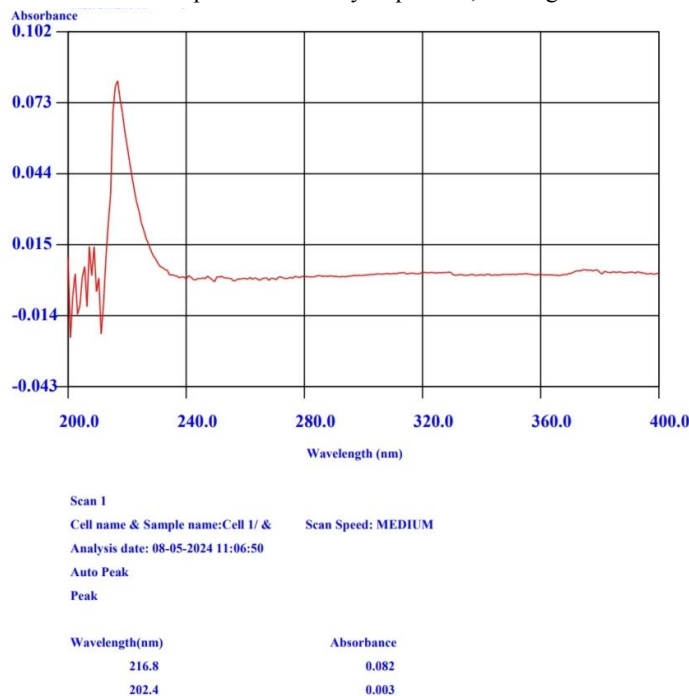

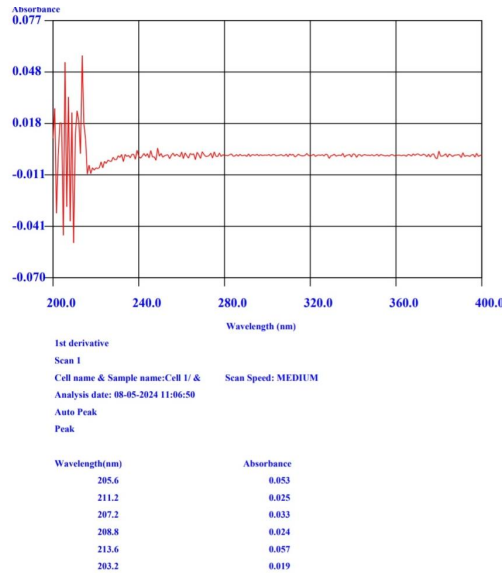Fig 2: Zero derivative of Sitagilptin

Fig 3: First derivative of Sitagilptin

Linearity

Linearity of the method was established from calibration curves constructed with the standard solutions of Sitagilptin in the concentration range 10-50 μg/ml (Table 1). The absorbance of the drug was plotted against the corresponding concentration. Linearity was proved by using linear regression analysis and calibration curves generated were found to be linear over the selected range, with coefficient of determination values ($r2$) 0.999. The concentration of drugs in the formulation samples were calculated from the resulting regression equations.

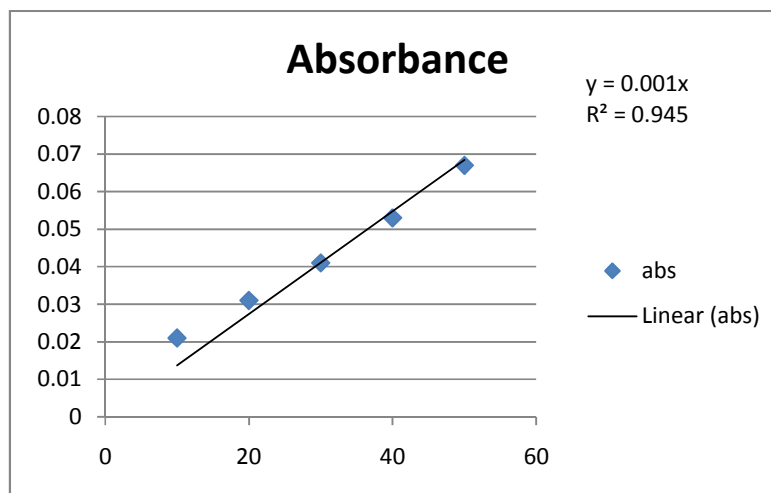| Sr.no	Concentraion	Wavelength	Absorbance
1	10 μg/ml	216nm	0.021
2	20 μg/ml	216nm	0.031
3	30 μg/ml	216nm	0.041
4	40 μg/ml	216nm	0.051
5	50 μg/ml	216nm	0.062

Fig 4: Standard calibration curve of Sitagilptin

LOD and LOQ

The calculated LOD and LOQ values of Sitagilptin were 5.50μg/ml and 16.60μg/ml respectively, indicate the sensitivity of the method

Robustness

Sample	Wavelength	Absorbance
10μg/ml	215	0.212
10μg/ml	217	0.214
10μg/ml	219	0.211

Table 1: change in wavelength and their absorbance

Sample	Temperature	Absorbance
10μg/ml	33^0C	0.211
10μg/ml	35^0C	0.212
10μg/ml	36^0C	0.214

Table 2: change in temperature and their absorbance

Repeatability

Sr.no	Sample	Wavelength	Absorbance
1	10μg/ml	217	0.103
2	10μg/ml	217	0.104
3	10μg/ml	217	0.105
4	10μg/ml	217	0.104
5	10μg/ml	217	0.103
6	10μg/ml	217	0.104

Precision

Sr no	Time	Wavelength	Absorbance
1	11:00	217	0.104
2	12:00	217	0.103
3	1:00	217	0.104

Accuracy

Prepare the standard stock solution (Solution B).

Weight 100mg of drug. Dissolve in 100ml of 0.1M Naoh solution (Solution C).

Pippete out 10ml of solution C dilute up to 100ml 0f 0.1M Naoh (Solution D).

Prepare dilution as per table , make upto 25ml and measure at 217nm.

Calculate % Recovery.

Level	Volume of solution D	Volume of solution B	Absorbance	%Recovery
80%	1.5	1.2	0.299	82.5
	1.5	1.2		
	1.5	1.2		
100%	1.5	1.5	0.358	98.83
	1.5	1.5		
	1.5	1.5		
120	1.5	1.8	0.480	138.83
	1.5	1.8		
	1.5	1.8		

V. CONCLUSION

Increased resolution and differentiation of spectra was observed in derivative mode. The developed derivative spectrophotometric method can be employed for routine analysis of Sitagilptin marketed formulations. Fixed dose tablet were analyzed using the developed method and assay results were within the limits for Sitagilptin. Spectrophotometry could be a suitable alternative analytical method for time consuming.

ACKNOWLEDGEMENT

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