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New Extractive Method Development of Sitagliptin Phosphate in API and Its Unit Dosage Forms by Spectrophotometry

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Abstract: *Two simple, accurate, sensitive and reproducible visible spectrophotometric methods (A & B) have been developed for the determination of Sitagliptin Phosphate (SGP) in bulk and also in pharmaceutical formulations. The proposed methods are based on complexation of the drug with Bromo Thymol Blue (BTB-Method A) & Bromo Cresol Green (BCG-Method B), extracted with chloroform, showing absorbance maxima at 412 nm and 419 nm respectively. Beer's law is obeyed over a concentration range of 25-125 µg/ml and 10-50 µg/ml respectively. Results of analysis for the two methods established, were validated statistically and also by recovery studies.*

All the variables were studied to optimize the reaction conditions. No interference was observed in the presence of common pharmaceutical excipients. The validity of the methods was tested by analyzing the drug in its pharmaceutical preparations. Good recoveries were obtained. The developed methods employed were successful for the determination of Sitagliptin Phosphate in various pharmaceutical preparations.

Keywords: *Visible spectrophotometric method, SGP, BTB, BCG & Molar Absorptivity*

I. Introduction

Sitagliptin Phosphate is chemically 7-[(3R)-3-Amino-1-oxo-4-(2,4,5 Trifluorophenyl) butyl]-5,6,7,8-Tetrahydo-3-(Trifluoromethyl)-1,2,4-Triazolo [4,3-a] pyrazine phosphate (1:1) monohydrate **(Figure 1)**. Sitagliptin Phosphate is the first and only prescription medication in a new class of oral antihyperglycemic agents, which enhance the body's own ability to lower blood glucose when it is elevated**.** The therapeutic combination in Type II is the use of the orally active Dipeptidyl Peptidase-4 (DPP - IV) inhibitors (1-3) like Sitagliptin Phosphate. It is an oral anti-diabetic drug (4-8) that helps to control blood sugar levels by regulating the levels of insulin in the body.

A survey of literature reveals that, the analytical methods reported for Sitagliptin Phosphate were based upon Spectrophotometry (9-12), HPLC (13,14) and other related analytical techniques like Tandem Mass Spectroscopy(15,16). As highlighted earlier, the use of the above drug has become, very wide spread. It is however, surprising to note that, not even a single method is available till now, for the extractive method development of the drug. The present article seeks to bridge this gap by developing a simple, sensitive, accurate, rapid and economical visible spectrophotometric method in the pure form and its tablet formulation as per ICH guidelines.

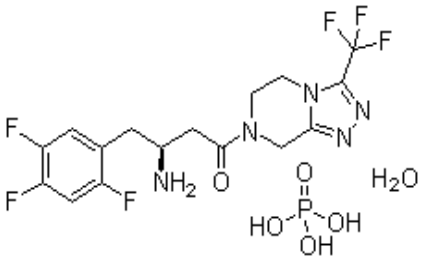

Figure 1: 7-[(3R)-3-Amino-1-oxo-4-(2, 4, 5 Trifluorophenyl) butyl]-5, 6, 7, 8-Tetrahydo-3-(Trifluoromethyl)-1, 2, 4-Triazolo [4, 3-a] pyrazine phosphate (1:1) monohydrate

II. Experimental

Instrument

ELICO Double Beam UV-visible Spectrophotometer SL-244 with 1 cm matched pair quartz cells was used for all the spectral measurements.

Reagents

All the chemicals used were of analytical reagent grade. All the solutions were freshly prepared.

1. Acid Phthalate Buffer pH 2.4
2. Bromo Cresol Green-BCG (0.1%)
3. Bromo Thymol Blue-BTB (0.1%)
4. Chloroform AR grade
5. Hydrochloric Acid (0.2M)
6. Methanol AR grade
7. Potassium Hydrogen Phthalate (0.2M)

III. Procedure

Preparation of Stock Solution

A standard stock solution containing 1 mg/ml was prepared by dissolving 100 mg of Sitagliptin Phosphate in 10 ml of methanol, shake well till it dissolves and make up to 100 ml with the same for both the methods A & B.

Preparation of Working Standard Solution

From the above stock solution, working standard solution was prepared from 25-125 µg/ml for method A (100 µg/ml) and 10-50 µg/ml (50 µg/ml) for method B respectively.

IV. Assay Procedure

METHOD A

Aliquots of standard drug solution of Sitagliptin Phosphate containing 0.5-2.5 ml (25-125 µg/ml) were taken and transferred into series of graduated test tubes. To each test tube 2 ml of Bromo Thymol Blue, 2 ml of Phthalate buffer pH 2.4 and 5 ml of Chloroform were added. The solutions were shaken for 2 to 3 minutes and kept aside for the formation of colored complex. The absorbance of the yellow colored chromogen was measured at 412 nm against reagent blank and a calibration curve was constructed as depicted in **Figure 2 & 3**. The absorbance of the sample solution was measured, and the amount of the drug was determined by referring to the calibration curve or computed from the regression equation.

METHOD B

Aliquots of standard drug solution of Sitagliptin Phosphate containing 0.5-1.0 ml (10-50 µg/ml) were taken and transferred into series of graduated test tubes. To each test tube 1 ml of Bromo Cresol Green, 2 ml of Phthalate buffer pH 2.4 and 5 ml of Chloroform were added. The solutions were shaken for 2 to 3 minutes and kept aside for the formation of colored complex. The absorbance of the yellow colored chromogen was measured at 419 nm against reagent blank and a calibration curve was constructed as shown in **Figure 4 & 5**. The absorbance of the sample solution was measured, and the amount of the drug was determined by referring to the calibration curve or computed from the regression equation.

V. Preparation Of The Sample Solution

Ten tablets of Sitagliptin Phosphate were accurately weighed and powdered. Tablet powder equivalent to 100 mg of Sitagliptin Phosphate was dissolved in 50 ml of methanol, sonicated for 15 mins and filtered. The filtrate is combined and the final volume was made to 100 ml with methanol for the above method. The solution was suitably diluted and analyzed as given under the assay procedure for bulk sample. The analysis procedure was repeated three times with Tablet formulations and the results of analysis for both the methods A & B were shown in **Table: 1.**

VI. Recovery Studies

To ensure the accuracy and reproducibility of the results obtained, known amounts of the pure drug was added to the previously analyzed formulates samples and these samples were reanalyzed by the proposed method and also preformed recovery studies. The percentage recoveries, thus obtained for methods A & B were given in **Table: 1.**

VII. Results And Discussion

The optimum conditions were established by varying one parameter at a time and keeping the others fixed and observing the effect on absorbance of chromogen. In the present work, methods A & B have been developed for the estimation of Sitagliptin Phosphate from tablet formulation. The developed methods A & B are based on formation of chloroform extractable colored complexes with Bromo Thymol Blue & Bromo Cresol Green respectively. The conditions required for the formation of colored complexes to form colored species were optimized.

Statistical analysis was carried out and the results were found to be satisfactory. Relative standard deviation values were low indicating the reproducibility of the proposed methods. Recovery studies were close to 100% that indicates the accuracy and precision of the proposed methods. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity, Sandell's sensitivity and other parameters are presented in **Table: 2**

VIII. Conclusion

The new procedure for the spectroscopic determination of Sitagliptin Phosphate described in this work is simple, rapid and cost-effective with high accuracy and precision when compared with previously reported procedures. It could find application as a convenient technique for the in-process control analysis of Sitagliptin Phosphate in bulk and its pharmaceutical formulations.

IX. Acknowledgements

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Figure 2: Absorption Spectrum of Sitagliptin Phosphate with Bromo Thymol Blue (Method A)

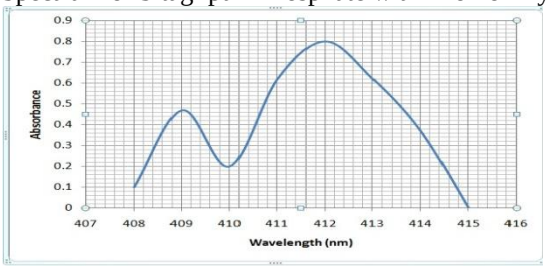

Figure 3: Linearity calibration curve of Sitagliptin Phosphate with Bromo Thymol Blue (Method A)

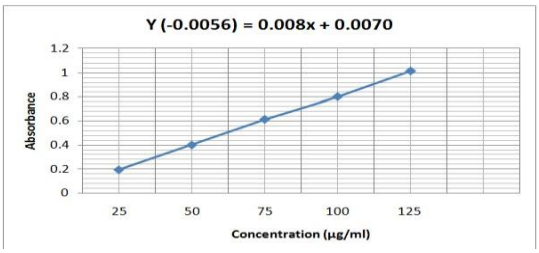

Figure 4: Absorption Spectrum of Sitagliptin Phosphate with Bromo Cresol Green (Method B)

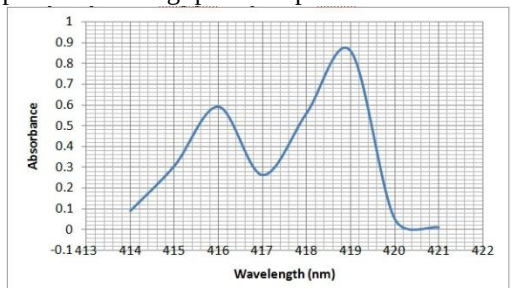

Figure 5: Linearity calibration curve of Sitagliptin Phosphate with Bromo Cresol Green (Method B)

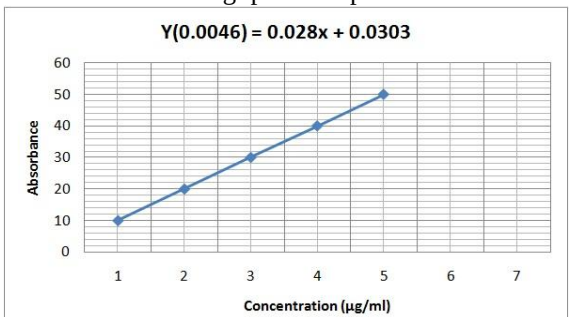

Table 1: Assay of Sitagliptin Phosphate in Tablet Formulation by Methods A & B

Tablet Formulation	**Amount claim (mg/tablet)**	***amount obtained (mg) by the proposed methods**		****** **% Recovery by the proposed methods**	
		Method A	Method B	Method A	Method B
1	100	99.31	98.77	102.28	98.73
2	100	98.27	99.43	101.19	99.45
3	100	98.74	101.24	100.83	101.89

* Average of Three determinations ** After spiking the sample

Table 2: Optical characteristics and precision data parameters of Methods A & B for Sitagliptin Phosphate

Parameter	**Method A**	**Method B**
Measured λ_{max} (nm)	412	419
Beers law limit (µg/ml)	25-125	10-50
Moloar absorptivity (micrograms/cm^2/0.001 absorbance unit)	1.028×10^4	1.086×10^4
Optimum photometric range (µg/ml)	30-100	10-40
Regression equation (Y= mx + c)	Y(-0.0056) = 0.008x + 0.0070	Y(0.0046) = 0.028 + 0.0303
Intercept (c)	-0.0056	0.0046
Slope (m)	0.008	0.028
Standard error of estimate	0.0070	0.0303
Correlation coefficient (r)	1	0.998
% RSD	0.3217	0.445
Color stability (hours)	1	1.30
Confidence intervals (upper limit = 1)	0.963-0.985	0.927-0.969

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