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DEVELOMENT AND VALIDATION BY UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF SITAGLIPTIN IN BULK AND ITS FORMULATION

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ABSTRACT

Back ground: Validation is a documented program that provides a high degree of assurance that a facility or operation will consistently produce product meeting a predetermined specification.Sitagliptin is the oral hypoglycemic drug. *Objective:* The main objective is to develop and validate the UV-spectrophotometric method for the estimation of sitagliptin in bulk and pharmaceutical formulations as per ICH guidelines. *Method:* The estimation was carried out using HPLC grade methanol as solvent and quantitation was achieved using double beam UV spectrophotometer at 265 nm. *Results:* The λ_{max} of sitagliptin in methanol was found to be 265 nm. The drug follows linearity in the concentration range 1-5 µg/ml with correlation coefficient value 0.9998. The results of analysis were validated by recovery studies. The recovery was found to be 99.87 to 100.18%. The relative standard deviation was found to be <2.0 % in all cases. *Conclusion:* The above method was a rapid and cost-effective quality control tool for routine analysis of sitagliptin in bulk and in pharmaceutical dosage form. The method can be useful for the day-to-day routine analysis in the quality control departments of bulk and pharmaceutical formulations industries.

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INTRODUCTION

Type 2 diabetes which is also referred to as non-insulin-dependent diabetes, accounts for more than 90% of patients with diabetes. The treatment guidelines for management of type 2 diabetes recommend addition of second-line agents like DPP-4 inhibitors to metformin (first line agents) for patients with insufficient control of hyperglycemia.Sitagliptin phosphate monohydrateis chemically((R)-4-oxo-4-[3(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo [4,3-a]pyrazin-7-(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine)phosphate hydrate (Mol. Formula is $C_{16}H_{15}F_6N_5O$; Mol. wt. is 407.314 g/mol). The molecular structure was illustrated in Figure 1. It is oral hypoglycemic drug of the dipeptidyl peptidase (DPP) inhibitor class. DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes that functions to stimulate glucose-dependent insulin release and reduce glucagon levels. This is done through inhibition of the inactivation of in cretins, particularly glucagon-like peptide- 1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby improving glycemic control.Inhibition of DPP-4 reduces the breakdown of GLP-1 and increases insulin secretion; this suppresses the release of glucagon from the pancreas and drives down blood sugar levels[1,2]. Several analytical methods based on UV[2-4], spectroflourimetry[5], RP-HPLC[6,7],LC-MS/MS[8-10]was reported for the determination of sitagliptin phosphate in plasma and urine of humans, rats and dogs. Sitagliptin (STG) is used as a single therapy or in combination with metformin. Metformin is a biguanide drug effective in patients who lack functioning islet cells as it acts by simulations of glycolysis in peripheral tissues[11,12].
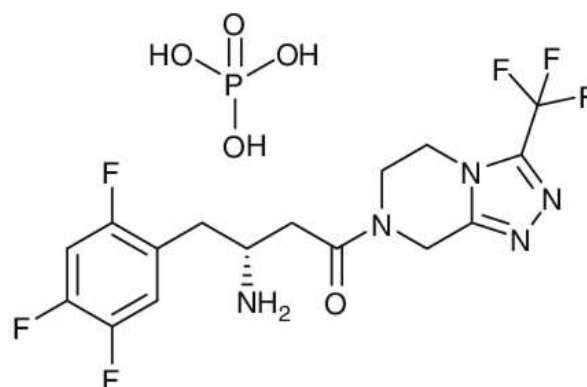
Figure 1. Molecular structure of sitagliptin phosphate

Spectrophotometry continues to be very popular amongst the various methods available for the determination of drugs, because of their simplicity, specificity and low cost. In present study, for the first time, a simple new spectrophotometric method for quantification of sitagliptin has been developed and validated according to the International Conference on Harmonization (ICH) guidelinesand successfully applied for assay of pharmaceutical formulations.

MATERIALS AND METHODS

Instruments used: UV-Visible spectrophotometer with UV Win software. Weighing balances and matching quartz cells with a 1 cm cell path length were utilized along with the mentioned equipment, which had automatic wavelength accuracy of 0.1 nm.

Chemicals and reagents: Pharmaceutical grade sitagliptin(API) was procured as gift sample from Hetero Drugs Ltd., Hyderabad, Telangana, India. The marketed pharmaceutical dosage form of sitagliptin tablets (Januvia50 mg) was purchased from local Pharmacy, Hyderabad, Telangana, India. All chemicals and reagents were of analytical grade.

Solvent selection: A number of trails were done to find out the ideal solvent for dissolving the drug. The solvents such as double distilled water, methanol and acetonitrile were tried based on the solubility of the drug.

Selection of detection wavelength: Appropriate volume 1 ml of standard stock solution of sitagliptin was transferred into a 10 ml volumetric flask, diluted to a mark with methanol to give concentration of 10 μg/ml. The resulting solution was scanned in the UV range (200-400 nm).

Preparation of stock solution: A precisely weighed, 10 mg of sitagliptin hydrate monophosphate was transferred to 10 ml volumetric flask (clean and dry). Then few ml of methanol was added and dissolved the drug by vigorous shaking. The volume was then made up to the mark with methanol to obtain the stock solution of 1000 μg/ml.

Preparation of working standard solution: From stock solution 1 ml was pipetted out further diluted to 10 ml with methanol to get the solution having the concentration of 100μg/ml.

Preparation of calibration curve: From the working standard solution, pipetted out 0.1 ml, 0.2ml, 0.3 ml, 0.4ml, and 0.5 ml and diluted to 10 ml using methanolto produce1μ g/ml, 2μg/ml, 3 μg/ml, 4μg/ml and 5μg/ml solutions, respectively. The absorbance of the solutions at the λ_{max} of 265 nm using methanol as blank was measured. The calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis. The curve shows linearity in the concentration range of 1 to 5 μg/ml. The correlation co-efficient (r^2) was found to be 0.9998.

Procedure for assay of pharmaceutical formulation: 20 Tablets of sitagliptin marketed formulations were weighed and powdered. A quantity of tablet powder equivalent to 50mg of sitagliptin was transferred to 50 ml volumetric flask and ultrasonicated for 20 minand volume was made up to the mark with methanol. The solution was then filtered through a Whatman filter paper grade1. The filtrate was appropriately diluted further. The absorbance of the resulting solution was measured at 265 nm and the amount of SITA was computed from its calibration plot.

Method development and validation: These current validation characteristics describe the validation parameters stated by the International Conference on Harmonization [ICH] guidelines Q2 (R1)[13-15].

Linearity: The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity can be assessed by performing single measurements at several analyte concentrations. A linearity correlation coefficient above 0.998 is acceptable for most methods, especially for major components in assay methods. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. Precision was determined by intra-day and inter-day study. The repeatability of the method was evaluated by carrying out the assay 3 times on the same day and intermediate precision was evaluated by carrying out the assay on 3 consecutive days for the sample solution. The percent relative standard deviation (%RSD) was calculated.

Accuracy (Recovery studies): The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. Accuracy studies were performed at three different levels (80%, 100% and 120%) by standard addition method and the samples were analyzed in triplicate by the proposed method. Known amount of standard sitagliptin at 80%, 100% and 120% of predetermined sample was added to a prequantified tablet sample.

Ruggedness: Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

Robustness: The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters". The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. The absorbance was measured and assay was calculated for six times.

LOD and 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. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOD = 3.3 × σ/S
LOQ = 10 × σ/S

Where,
    σ = Standard deviation of the response, and
    S = Slope of the calibration curve.

RESULTS AND DISCUSSION

New spectrophotometric methods were developed for the determination of Sitagliptin in bulk and pharmaceutical dosage form.The absorption spectra were recorded in the wavelength region of 200-400 nm by UV.

Determination of absorbance maxima: From the above working standard solution, 1 ml was pipetted out into a 10 ml volumetric flask and the volume was made up to the mark with methanol to prepare a concentration of 10 μg/ml. The sample was then scanned in UV/Vis-spectrophotometer in the range 200-400nm using methanol as blank and the wavelength corresponding to maximum absorbance was found to be 265 nm.It was observed that the drug showed maximum absorbance at 265 nm which was selected as the wavelength for detection. The absorption maxima curve was shown in Figure 2.The proposed method obeyed Beer's law in the concentration range of 1-5 μg/ml with good correlation coefficient of r^2 =0.9998. Calibration data was represented in Table 1.Beer's law range was confirmed by

the linearity of the calibration curve of sitagliptin was shown in Figure 3. The optical characteristics and the data concerning to the proposed method is represented in Table 2. Precision of the method was reported in terms of relative standard deviation and it should be evaluated by using a minimum of 3 determinations over which shows % RSD less than 2 indicates that the method was precise and the results are presented in Table 3. Recovery studies were carried out for the developed method by addition of known amount of standard drug solution of sitagliptin to pre-analyzed tablet sample solution at three different concentration levels. The resulting solutions were analyzed by the proposed methods. The recovery (Table 4) was in the range of 99.87 to 100.18percentages.
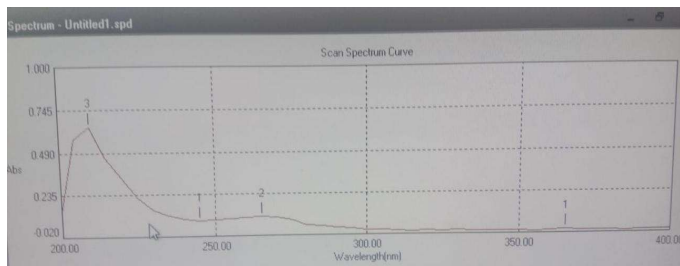

Figure 2. Absorption maxima of sitagliptin

The results were reported to be within the limits. In fact, there was no difference in mean assay results of the method obtained from two instruments of different manufacturers.

Table 1. Linearity of sitagliptin

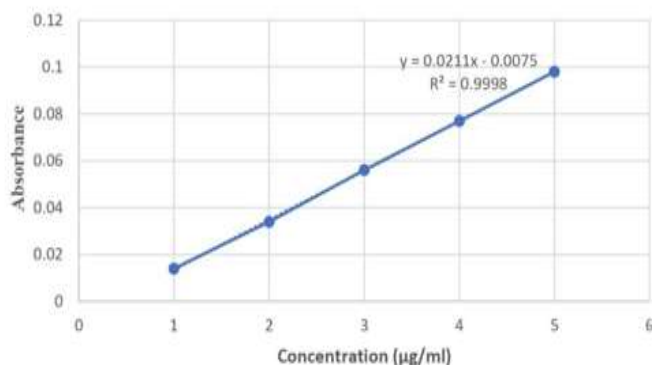| Concentration(µg/ml)	Absorbance
1	0.015
2	0.024
3	0.040
4	0.061
5	0.114

Figure 3. Linearity of sitagliptin

Table 2. Optical characteristics of sitagliptin phosphate monohydrate

Parameter	Result
λ_{max}	265 nm
Slope	0.0211
Intercept	-0.0075
Linearity	1-5µg/ml
Correlation coefficient	0.9998

For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influences of the variables were determined. The absorbance was measured and assay was calculated for six times. The results of robustness wererepresented in Table 5. The results are within the specified limits which states that this method is robust.The results of sitagliptin ruggednesswere reported in Table 6. The developed method was applied to the analysis of tabletformulations

found to be within the proposed limits and the mean %assay value was found to be 98.36%. The assay results are given in Table 7. The limit of detection and limit of quantitation for estimation of sitagliptin were 0.2269 µg/ml, 0.6875 µg/ml respectively, and illustrated in Table 8. The developed method has good linearity, accuracy and precision results indicates that the high quality of the method.

Table 3. Results for precision study

S. No.	Concentration (µg/ml)	Intra-day absorbance (Day-1)	Inter-day absorbance (Day-2)
1	3.0	0.040	0.039
2	3.0	0.042	0.045
3	3.0	0.041	0.040
4	3.0	0.043	0.041
5	3.0	0.039	0.032
6	3.0	0.046	0.040
Mean		0.04183	0.0395
Std. Dev.		0.004231	0.004231
%RSD		10.71099	10.71099

Table 4. Results of accuracy study

S.No.	Level of addition (%)	Amount added(µg/ml)	Amount recovered (µg/ml)	Average
1	80	8	8.15	100.18±1.56
2	100	10	9.97	99.09±1.38
3	120	12	11.88	99.87±1.93

Table 5. Results for robustness study

S.No.	Wavelength (nm)	Absorbance
1	263	0.042
2	265	0.098
3	267	0.087

Table 6. Results of sitagliptin ruggedness

S. No.	Analyst	%RSD
1	Analyst 1	0.0453
2	Analyst 2	0.0274

Table 7. Assay results

Brand name	Drug	Labelled amount	Mean% ± SD	%Assay	%RSD
Januvia	Sitagliptin	50 mg	49.18 ± 0.8	98.36	1.626

Table 8. Results of LOD and LOQ

Drug name	LOD	LOQ
Sitagliptin	0.2269 µg/ml	0.6875 µg/ml

CONCLUSION

Developed selective, accurate, precise, sensitive and robust UV spectroscopic method for the first time, for concurrent quantification of sitagliptinin pure form and pharmaceutical tablets formulations with low cost. A good recovery of method showed that there is no interference of excipients used in the formulations at analyte. The outcome of the validation study showed that the developed UV spectrophotometric method for sitagliptinwill serve as a standard protocol for routine analysis of sitagliptinin bulk, pharmaceutical formulation and would be of great help to pharmaceutical industries in the future.

Conflict of interest: The authors declare no conflict of interest.

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