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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF DPP-4 INHIBITOR, SITAGLIPTIN, IN ITS PHARMACEUTICAL PREPARATIONS

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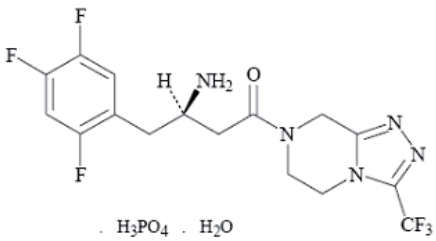
Fig 1. Sitagliptin phosphate



EXPERIMENTAL

Apparatus

- Spectral and absorbance measurements were carried out by using Systronics UV – Visible Double beam spectrophotometer model 2201.
- Systronics digital pH meter was used to adjust and determine the hydrogen ion concentration (pH) of the buffer solution.

Materials and Reagents: All the chemicals used were of analytical grade. All the solutions were freshly prepared in distilled water.

- Acetylacetone: 8.4% v/v solution was freshly prepared by mixing 2.1 ml of acetyl acetone with 10 ml of acetate buffer (pH 5) and diluted to 25 ml with distilled water.
- Formaldehyde (34 - 40%): Twenty percent solution was prepared by mixing 5 ml of formaldehyde with 10ml of acetate buffer (pH 5) and diluted to 25 ml with distilled water.
- Acetate buffer (pH 5): Prepared by dissolving 13.6 g of sodium acetate and 6 ml of glacial acetic acid in sufficient water to produce 1000 ml.
- Pharmaceutical grade Sitagliptin phosphate, certified to be 99.8% pure was procured from local pharmaceutical industry and was used as received.
- Januvia 100mg, 50mg and 25mg (labeled to contain 100mg, 50mg and 25mg of sitagliptin phosphate per tablet) were obtained from the local pharmacy.

Standard drug solution

Stock solution of STP (1 mg/ml) was prepared by dissolving 100 mg of STP in distilled water and making the volume to 100 ml in a standard volumetric flask. Working solution of lower concentration (100 µg/ml) was prepared by further dilution of the above standard stock solution with water.

General procedure for the determination of Sitagliptin phosphate

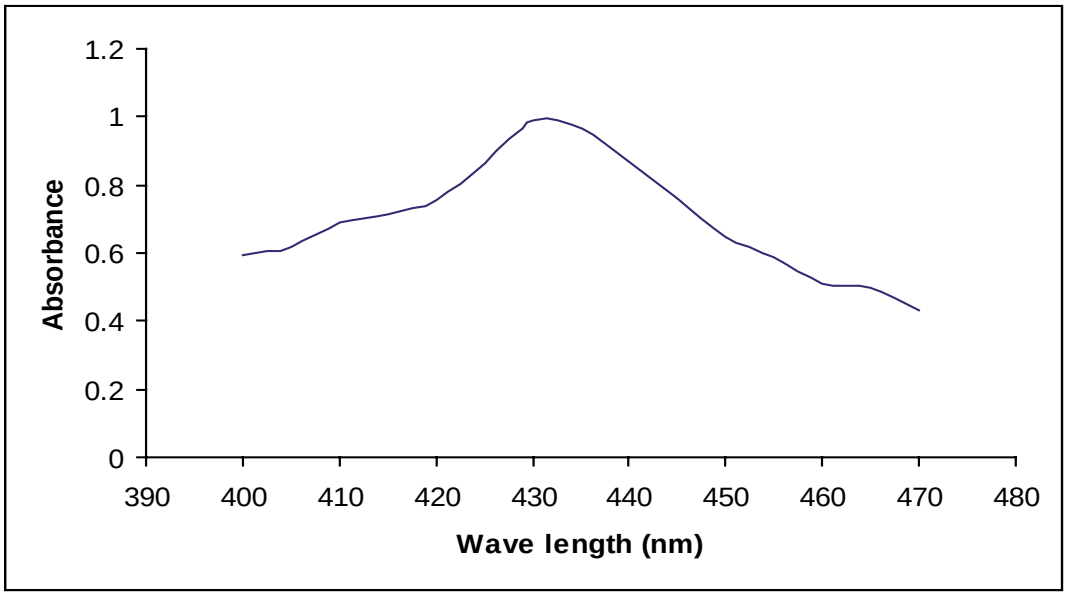
Different aliquots of working standard solutions containing 50-250µg of STP was transferred into a series of serially numbered 10ml volumetric flasks. To each flask 1 ml of 8.4% v/v acetyl acetone solution and 0.5 ml of 20% formaldehyde reagents were added. The flasks were stoppered, contents were mixed well. The mixture was heated for 5 min, cooled and diluted to 10 ml with distilled water. The absorbance of the yellow color solution was measured at 430 nm using the experiment as a blank. The amount of sitagliptin phosphate present in the sample was computed from the corresponding calibration curve.

The calibration graph was prepared was prepared by plotting absorbance versus concentration of drug and the concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data. The calibration graph was then prepared by plotting the absorbance versus concentration of the drug

Assay procedure for pharmaceutical tablets

For the analysis of STP, three brands of commercially available tablets (20) were weighed

Fig 3. Absorption spectra of the Hantzch product



Investigation of Assay Parameters

Optimum reagent concentrations required for the formation of sensitive and quantitative colored products were determined by varying one reagent concentration and fixing the concentrations of other reagents and its effect on absorbance was measured at 430nm.

Effect of heating time:

To study the effect of heating time for the development of maximum color, the contents of the mixture were heated for up to 30 min at 100 ± 1°C. The intensity of the color developed was measured at room temperature (25 ± 1°C) after dilution to 10.0ml with double distilled water. It is apparent from this investigation that the maximum intensity of color was obtained after 5 min of heating and remained constant up to 30 min. Therefore, the optimum heating time was fixed at 5 min.

Effect of reagent concentration:

The effect of acetyl acetone and formaldehyde concentration on the absorbance was studied; volumes from 0.5-2.5ml of 8.4% acetyl acetone solutions and 0.1 to 1.0ml of 20% formaldehyde solutions were examined. The investigations showed that 1ml of acetyl acetone and 0.5ml of formaldehyde gave maximum absorbance. So the same volumes of both the reagents were chosen for the procedure.

Effect of pH:

Different acetate buffers with pH range of 3.0 - 7.0 were tried and pH 5 was the pH of choice for the proposed method.

Effect of Solvents:

Different diluting solvents were used, such as water, ethanol, methanol, acetonitrile and ace-

Fig 4. Linearity of absorbance to concentration of Hantzch product

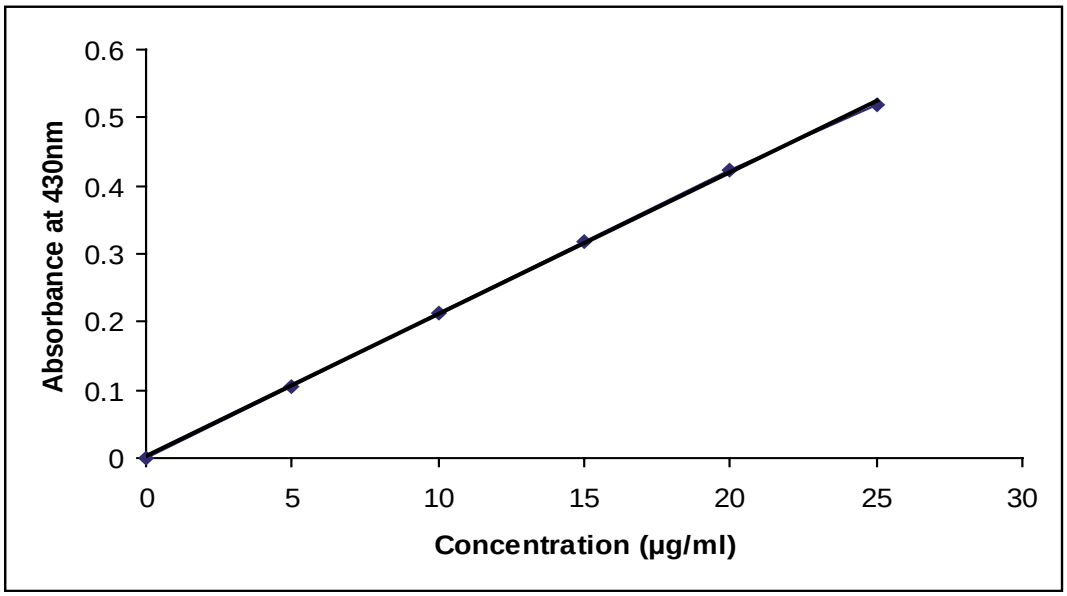


Table 2. Optical and Regression characteristics, Precision and Accuracy of the proposed method

Parameters	Method B
λ_{max} (nm)	430
Beer's law limit (µg/ ml)	5 - 25
Sandell's Sensitivity (µg/cm^2/0.001 abs. unit)	0.0471
Molar absorptivity(Litre.mole^{-1}.cm^{-1})	1.067 x 10^4
Stability of Color (hours)	1
Regression equation (Y)*	
Intercept (c)	0.0106
Slope(b)	0.0020
Correlation coefficient	0.9998
% Relative standard deviation**	1.13
% Range of errors	
0.05% level	0.951
0.01% level	1.397
Limit of detection (µg/ ml)	1.947
Limit of quantification (µg/ ml)	5.90

* Y= c + bx, where Y is the absorbance and x is the concentration of Sitagliptin phosphate in µg/ml
** Average of six determinants

Table 5. Results of analysis of tablet formulations containing STP

Formulations	Labeled amount(mg)	% Found** ± S.D			
		Reference method*	Proposed method	t-test	F-test
Tablet I	100	99.83 ± 0.24	99.94	2.01	5.01
Tablet II	50	100.16 ± 0.56	98.82	1.58	4.25
Tablet III	25	99.83±0.24	99.93	1.56	3.45

** Recovery amount was the average of five determinants
* UV method developed in the laboratory
Tabulated t-value at 95% confidence level is 2.306
Tabulated F-value at 95% confidence level is 6.39

CONCLUSIONS

The proposed method was quite simple and do not require any pretreatment of the drug and tedious extraction procedure. The methods have wider linear range with good accuracy and precision. Hence, the data presented in the manuscript "Development and validation of a spectrophotometric method for the determination of a DPP-4 inhibitor, sitagliptin, in its pharmaceutical preparations" demonstrate that the proposed method was accurate, precise, linear, selective and offer advantages of reagent availability and stability, less time consumption and high sensitivity. Thus it can be extended for routine analysis of STP in pharmaceutical industries, hospitals and research laboratories. Unlike the LC/MS procedure and high performance liquid chromatography procedures, the UV-visible spectrophotometer instrument is simple and not of high cost, on the other hand in terms of simplicity and expense, the method could be considered superior in comparison with the previously reported methods. Moreover the methods are free from interferences by common additives and excipients.

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