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Research Article

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

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ABSTRACT

A simple, sensitive and reproducible spectrophotometric method was developed for the determination of sitagliptin phosphate in bulk and in pharmaceutical formulations. The proposed method is based on condensation of the primary amino group of sitagliptin phosphate with acetyl acetone and formaldehyde producing a yellow colored product, which is measured spectrophotometrically at 430nm. The color was stable for about 1 hour. Beer's law is obeyed over a concentration range of 5-25 µg/ml. The apparent molar absorptivity and Sandell sensitivity values are 1.067 x 10^4 $Lmol^{-1}cm^{-1}$ and 0.0471 μgcm^{-2} respectively. 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 method was tested by analyzing sitagliptin phosphate in its pharmaceutical preparations. Good recoveries were obtained. The developed method was successfully employed for the determination of sitagliptin phosphate in various pharmaceutical dosage forms.

Keywords: Acetyl acetone, Beer's Law, Sitagliptin phosphate, Sandell's sensitivity.

INTRODUCTION

Sitagliptin phosphate (STP)[1-4] is 1,2,4-triazolo[4,3-a]pyrazine,7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl), phosphate, whose structure is given in the Fig. 1. It is used in the treatment of diabetes. It is an oral antihyperglycemic (anti-diabetic) drug of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. This drug is not officially in any pharmacopoeia. Literature survey reveals that only LC-MS[5-8] methods were reported for the determination of sitagliptin phosphate in plasma and urine of humans, rats and dogs. So far, no assay procedure has been reported for the determination of this drug in its pharmaceutical formulations. Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity and low cost. This study presents new spectrophotometric method for the determination of sitagliptin phosphate in bulk and pharmaceutical formulations. The developed method is based on the reaction of sitagliptin phosphate with acetyl acetone and formaldehyde producing Hantzsch product.

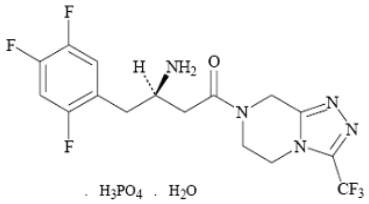

Fig. 1: Sitagliptin phosphate

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 5-25 µg/ml of STP was transferred into a series of serially numbered 10 ml 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 reagent as a blank. The amount of sitagliptin phosphate present in the sample was computed from the corresponding calibration curve.

The calibration graph 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.

Reference method

Different aliquots of working standard solutions containing 2-10 µg of STP was transferred into a series of serially numbered 10ml volumetric flasks. The flasks were diluted to 10 ml with distilled water. The absorbance of the solution was measured at 220 nm using water as a blank. The amount of sitagliptin phosphate present in the sample was computed from the corresponding calibration curve.

Assay procedure for pharmaceutical tablets

For the analysis of STP, three brands of commercially available tablets (20) were weighed and ground into a fine powder. An accurately weighed portion of the powder equivalent to 100 mg of STP was transferred into a 100 ml beaker containing small volume of water and the solution was shaken thoroughly for 10-15 minutes and filtered through a whatman filter paper no.1 to remove the insoluble matter. The filter paper was washed with water and the washings were added to the filtrate, the final volume (100 ml) was made with water. A suitable aliquot of this solution in the working range of STP was treated as per procedure described in the above determination of pure STP. The nominal content of STP in the tablets was calculated either from a previously plotted calibration graph or using the regression equation

RESULTS AND DISCUSSION

Determination of absorption maxima (λ_{max})

To determine the λ_{max} 10 µg/ml of the STP was added to 10 ml volumetric flask. Then 1 ml of 8.4% (*v/v*) acetyl acetone solution and 0.5 ml of 20% formaldehyde reagents were added. The contents were mixed well. The mixture was heated for 5 min, cooled and diluted to 10 ml with distilled water. The absorbance was measured against reagent blank in the range of 400-700 nm. λ_{max} for STP was found to be 430 nm. Absorption spectrum of the proposed method was shown in Fig. 2. Under the experimental conditions each reagent blank showed a negligible absorbance at the corresponding λ_{max}.

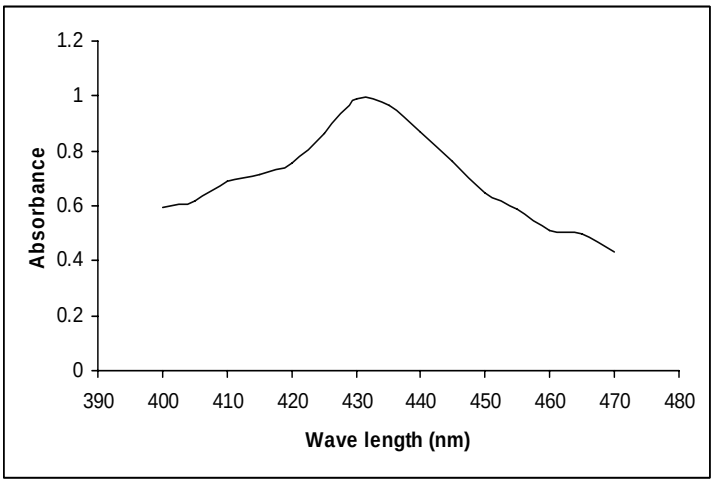

Fig. 2: Absorption spectra of the Hantzch product

Chemistry of the colored species

Hantzsch reaction is a known condensation reaction that was reported in the literatures as a useful pathway for pyrrole and pyridine synthesis[9]. In the same manner, acetylacetone together with formaldehyde react with aliphatic amines by Hantzsch reaction forming a yellow product that can be measured spectrophotometrically or spectrofluorimetrically. This reaction was applied for the determination of certain sulpha-Drug[10], kanamycin[11], lisinopril[12] and gabapentin and cefprozil[13].

The proposed method for determination of STP (primary amine compound) was based on Hantzsch condensation reaction using acetylacetone as β-diketone and formaldehyde as an aldehyde to form a colored condensation product. The formed yellow color showed maximum absorption at 430 nm (Fig. 2). The probable reaction mechanism was based on the reported method[14] (Fig. 3).

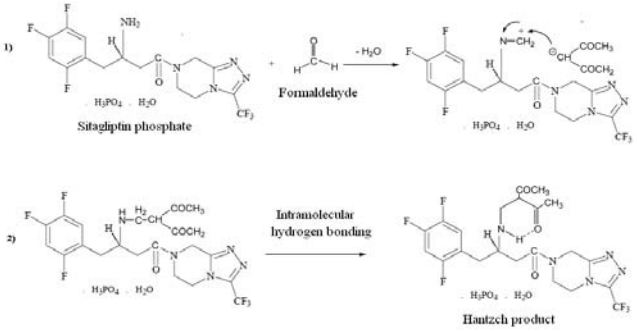

Fig. 3: Tentative reaction scheme for the formation of Hantzch product

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 ml 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 0.5-2.5 ml of 8.4% acetyl acetone solutions and 0.1 to 1.0 ml of 20% formaldehyde solutions were examined. The investigations showed that 1 ml of acetyl acetone and 0.5 ml of formaldehyde gave maximum absorbance. There is no change in the intensity of the color any further with the increasing amount of acetyl acetone and formaldehyde. So the 1 ml of acetyl acetone and 0.5 ml of formaldehyde were chosen for the procedure.

Effect of pH

Different acetate buffers with pH range of 3.0-7.0 were tried. Variations of the pH less than 4 and greater than 6 resulted in low absorbance values. So pH 5 was selected as pH of choice for the proposed method.

Effect of solvents

Different diluting solvents such as water, ethanol, methanol, acetonitrile and acetone, were used. Best color intensities were obtained using the first three solvents; water was used, being the most available solvent.

Interference studies

To study the potential interference from the commonly used excipients and other additives such as glucose, lactose, starch, sodium starch glycolate, cellulose, magnesium stearate and ascorbic acid, recovery studies were carried out. Under the experimental conditions employed, to a known amount of drug (sitagliptin phosphate 25 µg/ml), excipients in different concentrations were added and analyzed. Results of the recovery analysis are presented in Table 1. Excipients at the concentrations shown in Table 1 do not interfere with the assay. In addition recoveries in most cases were around 100% and the lower relative standard deviation (RSD) values indicate the good precision of the proposed method.

Table 1: Determination of STP in the presence of excipients

S. No.	Excipients (µg/ml)	Amount taken	Recovery (%)	RSD (%) (n=5)
1	Cellulose	300	99.8	0.26
2	Glucose	50	99.6	0.15
3	Lactose	300	99.4	0.36
4	Starch	200	100.2	0.42
5	Sodium starch glycolate	100	99.8	0.28
6	Magnesium stearate	50	99.5	0.37
7	Ascorbic acid	50	100.1	0.46

Validation of the method

Detection and Quantification limits

According to the Analytical Methods Committee[15] the detection limit (LOD) is the concentration of drug corresponding to a signal equal to the blank mean (Y_B) plus three times the standard deviation of the blank (S_B). Quantification limit (LOQ) is the concentration of drug corresponding to the blank mean plus ten times the standard deviation of the blank. The LOD and LOQ values for STP were found to be 1.947 and 2.90 µg/ml respectively.

Quantification

The optical characteristics such as Beer's law limits, Sandell's sensitivity and molar absorptivity were calculated for the proposed method and the results are summarized in Table 2. Regression analysis of the Beer's law plot at their λ_{max} revealed a good correlation. Graphs of absorbance *versus* concentration showed zero intercept and are described by the regression equation Y = bx + a (where Y is the absorbance, b is the slope, x is the concentration of drug in µg /ml and a is the intercept) obtained by least squares method. The results were summarized in Table 2.

Table 2: Optical and Regression characteristics, precision and accuracy of the proposed method

S. No.	Parameters	Value
1	λ_{max}	430
2	Beer's law limit (µg/ ml)	5 - 25
3	Sandell's Sensitivity (µg/cm^2/0.001 abs. unit)	0.0471
4	Molar absorptivity(Litre.mole^{-1}.cm^{-1})	1.067 x 10^4
5	Stability of Color (hours)	1
6	Regression equation (Y)*	
	Intercept (c)	0.0106
	Slope(b)	0.0020
7	Correlation coefficient	0.9998
8	% Relative standard deviation**	1.13
9	% Range of errors	
	0.05% level	0.951
	0.01% level	1.397
10	Limit of detection (µg/ ml)	1.947
11	Limit of quantification (µg/ ml)	2.90

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

** Average of six determinants

Accuracy precision and recovery studies

The accuracy and precision of the proposed method was evaluated by performing five replicate determinations of STP in pure form at three different concentrations (5, 10 and 15 µg/ml) by short term (intra day) and daily (inter day) precisions (Table 3). The standard analytical errors, relative standard deviations (RSD) and recoveries obtained in the intra day and inter day analysis for the proposed method was found to be acceptable. Thus the proposed method is effective for the determination of STP.

Table 3: Evaluation of the accuracy and precision of the proposed method by Intra day and Inter day assay

Observed concentration of STP (µg/ml)								
Concentration of STP (µg/ml)	Intra-day				Inter-day			
	Mean*	Error (%)	RSD (%)	Recovery (%)	Mean*	Error (%)	RSD (%)	Recovery (%)
5	4.97	0.60	0.56	99.40	5.02	0.40	0.48	100.40
10	10.10	1.00	0.39	101.00	9.98	0.20	0.19	99.80
15	15.06	0.40	0.43	100.40	14.97	0.20	0.73	99.80

* For five determinants

The accuracy of the proposed method was further checked by performing recovery experiments through standard addition technique. For this purpose, a known amount of pure STP was added to pre-analyzed dosage forms and then determined by the recommended procedure. The results (Table 4) shown that the mean recovery and relative standard deviation (RSD) were in the range of 99.94–100.12 and 0.26–0.54% indicating the reproducibility of the method. No interference from the common excipients was observed.

Table 4: Determination of STP in pharmaceutical formulations by Standard addition technique

Amount of drug added (µg)	Theoretical amount (µg)	Mean amount (µg) recovered (n=5)	Mean % of recovery (n=5)	RSD (%)
50	50	100.12	100.12	0.26
100	100	199.89	99.94	0.54

Robustness and Ruggedness

For the evaluation of the method robustness, an important experimental variable, pH was slightly varied deliberately. The analysis was performed at ±0.5 pH of the optimum pH by taking three different concentrations of STP and found to remain unaffected as shown by the RSD values in the range of 1.05 to 1.65%. Method ruggedness was expressed as the RSD of the same procedure applied by three different analysts as well as using two different instruments. The inter-analysts RSD were within 0.76% whereas the inter-instruments RSD for the same STP concentrations ranged from 0.97 to 1.32% suggesting that the developed method was rugged. The results are shown in Table 5.

Table 5: Results of method robustness and ruggedness (all values in %RSD) studies

Amount of drug added (µg/ml)	pH (n=4)	Different analysts (n=3)	Different instruments (n=2)
5	1.65	0.36	1.32
10	1.24	0.76	0.97
15	1.05	0.49	1.16

APPLICABILITY OF THE METHOD

The proposed methods were applied to the analysis of STP in pharmaceutical dosage forms and the results were statistically compared with reference UV method by calculating the Student's t- and F-values. The evaluated t- and F-values were less than the tabulated values at the 95% confidence level for eight degrees of freedom, as revealed by the results complied in Table 6. This actually suggests that the proposed methods are accurate and precise as the UV method.

Table 6: Results of analysis of tablet formulations containing STP

		% Found** ± S.D			
Formulations	Labelled amount (mg)	Reference method*	Proposed method	T-test	F-test
Tablet I	100	99.83 ± 0.24	99.94 ± 0.51	2.01	3.01
Tablet II	50	100.16 ± 0.56	98.82 ± 0.26	1.58	2.25
Tablet III	25	99.83 ± 0.24	99.93 ± 0.34	1.56	2.45

** Recovery amount was the average of five determinants,s * 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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REFERENCES

1. Herman G, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M, et al. Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. Clin Pharmacol Ther 2005; 78: 675-688.
2. Januvia Side Effects & Drug Interactions. RxList.com, 2007.
3. U.S. Food and Drug Administration. FDA Approves New Treatment for Diabetes. 2006.
4. Herman G, Bergman A, Liu F, Stevens C, Wang A, Zeng W, et al. Pharmacokinetics and pharmacodynamic effects of the oral DPP-4 inhibitor sitagliptin in middle-aged obese subjects. J Clin Pharmacol 2006; 46: 876-886.
5. Stella Vincent H, James Reed R, Bergman AJ, Elmore CS, et al. Metabolism and excretion of dipeptidase 4 inhibitor (^{14}C) sitagliptin in humans. Drug Metab Dispos 2007; 35: 533-538.
6. Wei Zeng, Donald G Musson, Alison L Fisher, Li Chen, Michael S Schwartz, Eric J Woolf & Amy Qiu Wang. Determination of sitagliptin in human urine and hemodialysate using turbulent flow online extraction and tendem mass spectrophotometry. J Pharmaceut Biomed 2008; 46: 534-542.
7. Ramakrishna Nirogi, Vishwottam Kandikere, Koteshwara Mudigonda, Prashanth Komarneni, Raghupathi Aleti & Rajeshkumar Boggavarapu. Sensitive liquid chromatography tandem mass spectrometry method for the quantification of sitagliptin, a DPP-4 inhibitor, in human plasma using liquid-liquid extraction. Biomed Chromatogr 2008; 22: 214-222.
8. Beconi MG, Reed JR, Yohannes Teffera, Yuan-Qing Xia, Kochansky CJ, Liu D Q, et al. Disposition of the Dipeptidyl Peptidase 4 Inhibitor Sitagliptin in Rats and Dogs. Drug Metab Dispos 2007; 35: 525-532 .
9. Derker Bratlon FRS and David Olis W. "Comprehensive Organic Chemistry", The Synthesis and Reactions of Organic Compounds 4. London: Pergamon Press; 1979.
10. Amin AS and Zareh MM. Acetylacetone-formaldehyde reagent for the spectrophotometric determination of some sulfa drugs in pure and dosage forms. Microchim Acta 1996; 124: 227-233.
11. Ahmad AS, Hoda NMD, Ahmad M, Islam F and Qureshi SZ. A simple and selective kinetic spectrophotometric method for the determination of kanamycin using acetylacetone-formaldehyde reagent in N,N′-dimethylformamide medium. J Anal Chem 2006; 61: 870-874 .
12. Fawzy El-Yazbi A, Abdine HH and Shaalan RA. Spectrophotometric and spectrofluorometric methods for the assay of lisinopril in single and multicomponent pharmaceutical dosage forms. J Pharmaceut Biomed 1999; 19: 819–827.
13. Ayad MM, El-Henawee MM, Abdellatef HE and El-Sayed HM. Spectrophotometric and Spectrofluorimetric Determination of Gabapentin and Cefprozil Monohydrate Using Acetylacetone and Formaldehyde. Alex J Pharm Sci 2005; 9: 157-161.
14. Khalifa Abulghasem El-Aroud, *Adel* Mohamed Abushoffa and Hisham Ezzat Abdellatef. Spectrophotometric and Spectrofluorimetric Methods for the Determination of Tranexamic Acid in Pharmaceutical Formulation. Chem Pharm Bull 2007; 55: 364-367.
15. Analytical Methods Committee, 1987, Recommendations for the definition, estimation and use of the detection limit. Analyst, 1987.