International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 14, Issue 2, 2022

Original Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF SITAGLIPTIN AND METFORMIN IN BULK AND COMBINED PHARMACEUTICAL FORMULATION

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Received: 05 Nov 2021, Revised and Accepted: 10 Jan 2022

ABSTRACT

Objective: To develop simple, accurate, precise UV Spectrophotometric method for the simultaneous estimation of Sitagliptin and Metformin in tablet dosage form.

Methodols: The method is based on the determination of Sitagliptin and Metformin in tablet using simultaneous equation method. Sitagliptin exhibits maximum absorbance at 267 and Metformin exhibits maximum absorbance at 237 nm using distilled water as diluents.

Results: The calibration curve was linear in the range of 10-300 µg/ml for Sitagliptin and 4-14µg/ml for Metformin. The %RSD were within the limit i.e., less than 2%. The % recovery of the proposed method was found to be 97.12-99.46% for Sitagliptin and 98.15-99.85% for Metformin. The LOD of the proposed method was 0.397µg/ml for Sitagliptin and 0.8952µg/ml for Metformin. The LOQ was 1.2951µg/ml for Sitagliptin and 2.7159µg/ml for Metformin.

Conclusion: A simple, accurate, precise UV Spectrophotometric method for the simultaneous estimation of Sitagliptin and Metformin in tablet dosage form.

Keywords: UV Spectrophotometric method, Sitagliptin, Metformin, simultaneous estimation

DOI: https://dx.doi.org/10.22159/ijcpr.2022v14i2.1963 Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

Sitagliptin is 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 [1]. It is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor used in conjunction with diet and exercise to improve glycemic control in patients with type 2 diabetes mellitus. Sitagliptin inhibits DPP-4 which leads to increased levels of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), decreased levels of glucagon, and a stronger insulin response to glucose. This enzyme splits the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal [2].

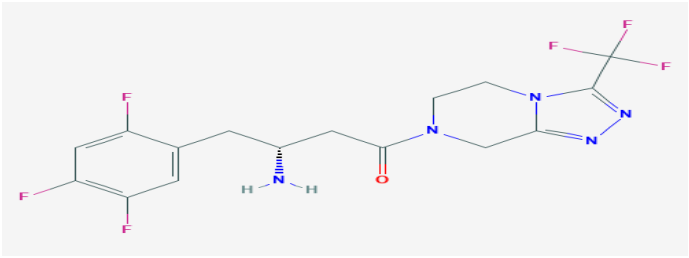

Fig. 1: Sitagliptin phosphate

Metformin is chemically, 1, 1-dimethyl biguanide hydrochloride [3]. Metformin is an antihyperglycemic agent of the biguanide class, used for the management of type II diabetes. It is a first line agent for the treatment of type 2 diabetes that can be used alone or in combination with sulfonylureas, thiazolidinediones, incretin-based drugs, sodium glucose cotransporter-2 inhibitors, or other hypoglycemic agents. Metformin is considered an antihyperglycemic drug because it lowers blood glucose concentrations in type II diabetes without causing hypoglycemia. Metformin is commonly described as an insulin sensitizer leading to a decrease in insulin resistance and a clinically significant reduction of plasma fasting insulin levels. Bio analytical, HPLC, HPTLC and UV-visible spectrophotometry methods have been reported for its individual determination of Metformin and in combination with other drugs [4-7].

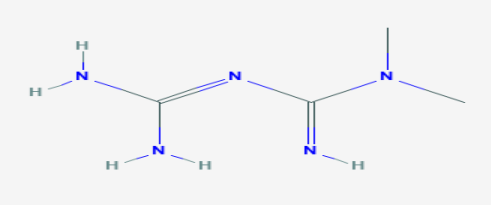

Fig. 2: Metformin hydrochloride

Sitagliptin Phosphate and Metformin Hydrochloride are available in combined dosage forms as film coated tablets (JANUMET). Each

tablet contains 50 mg of Sitagliptin Phosphate and 500 mg of Metformin Hydrochloride. The combination of Metformin and sitagliptin has been shown to be safe, effective and well-tolerated treatment for type II diabetes [8].

MATERIALS AND METHODS

Chemicals and reagents

Sitagliptin and Metformin hydrochloride API was gifted by pharma company, Hyderabad, Telangana, India. The marketed formulation, JANUMET 50 mg/500 mg was procured from the local market, distilled water.

Instruments

Double beam UV spectrophotometer; Model: SL 210; Make: ELICO. The output signal was checked and the acquisition and integration of data was performed using spectral treats Software on a computer. Contech electronic balance and labline sonicator was used during the experimentation.

Method development

Preparation of sitagliptin standard stock solution

10 mg of Sitagliptin was accurately weighed and transferred into 10 ml of volumetric flask and the volume was made up to the mark with distilled water to obtain the concentration of 1000µg/ml.

Preparation of metformin hydrochloride standard stock solution

10 mg of Metformin hydrochloride was accurately weighed and transferred into 10 ml of volumetric flask and the volume was made up to the mark with distilled water to obtain the concentration of 1000µg/ml.

Determination of λ_{max}

10µg/ml solution of Sitagliptin and Metformin hydrochloride were prepared from the standard stock solution and scanned in the range of 200-400 nm. Sitagliptin and Metformin hydrochloride showed maximum absorbance at 267 nm and 237 nm respectively.

RESULTS AND DISCUSSION

Validation parameters

Validation is the process of "establishing documented evidence" which provides high degree of assurance that a specific activity will consistently produce desired results or product meeting its predetermined specifications and quality specifications.

Linearity

From the standard stock solution of Sitagliptin pipette out 0.1-3µg/ml to obtain the concentration of 10-300µg/ml.

From the standard stock solution of Metformin pipette out 0.04-0.14µg/ml to obtain the concentration of 4-14µg/ml.

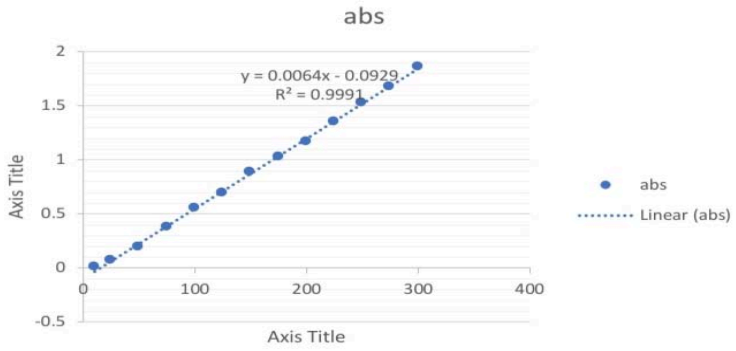

Fig. 4: Calibration curve of sitagliptin

Table 1: Results of quantitative determination of sitagliptin

S. No.	Parameters	Results
1.	Absorbance maximum (nm)	267
2.	Linearity and range (µg/ml)	10-300µg/ml
3.	Slope	0.0064
4.	Correlation coefficient	0.9991
5.	Y-intercept	0.0929

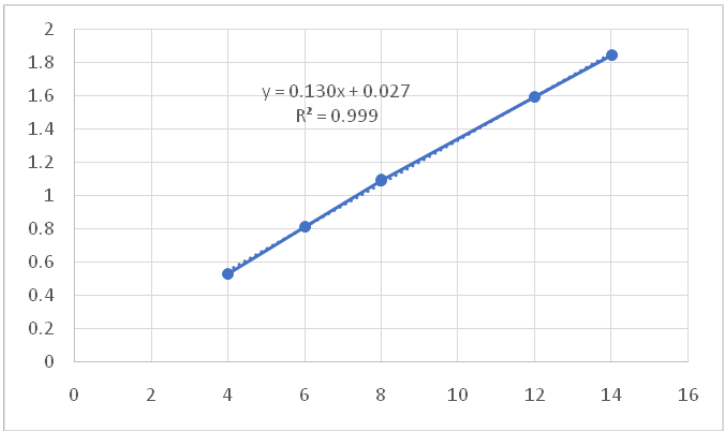

Fig. 5: Calibration curve of metformin

Table 2: Results of quantitative determination of metformin

S. No.	Parameters	Results
1.	Absorbance maximum (nm)	237
2.	Linearity and range (µg/ml)	4-14µg/ml
3.	Slope	0.1308
4.	Correlation coefficient	0.9992
5.	Y-intercept	0.0271

Precision

The precision of the proposed method was estimated in terms of inter-day and intra-day precision wherein the standard solution was observed for 6 times respectively. The results shown below indicating %RSD of less than 2% each level clearly indicate that the proposed method was precise enough for the analysis of drug.

% RSD = (SD of measurement/mean value of measurement) x 100

Table 3: Results of sitagliptin precision studies

Concentration	Intra-day precision (%RSD)	Inter-day precision (%RSD) Day 1 D 2
100µg/ml	0.065859	0.5935. 0.2165.

Table 3a: Results of metformin precision studies

Concentration	Intra-day precision (%RSD)	Inter-day precision (%RSD) Day-1. Day-2
10µg/ml	0.0773	0.1698. 0.3576

Accuracy

The accuracy of the method was determined by performing recovery studies by spiking standard solution to that of sample solution at three different levels i.e., 50%, 100%, 150% was injected.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Ruggedness

The ruggedness of the proposed method was evaluated by varying conditions different analyst and different instrument ("ELICO SL 210" and "SYSTRONIC 2203"). The %RSD was calculated. The low values of %RSD obtained by changing the conditions indicates that the method was rugged and the results were presented below.

Table 4: Results of sitagliptin accuracy studies

Level	Amount of standard added (µg/ml)	Pre-analysed sample (µg/ml)	% Recovery
50%	50	100	97.12%
100%	100	100	98.75%
150%	150	100	99.46%

Table 4a: Results of Metformin accuracy studies

Level	Amount of standard Added (µg/ml)	Pre-analysed sample (µg/ml)	% Recovery
50%	5	10	98.15%
100%	10	10	99.38%
150%	15	10	99.85%

Table 5: Results of sitagliptin robustness

Concentration	Wavelength	%RSD
100µg/ml	266	0.4580%
	268	0.1967%

Table 5a: Results of metformin robustness

Concentration	Wavelength	%RSD
10µg/ml	236	0.2925%
	238	0.1672%

Table 6: Results of sitagliptin ruggedness

Concentration	Analyst	%RSD	Instruments	%RSD
100µg/ml	Analyst 1	0.04523%	Instrument-1(ELICO)	0.0358%
	Analyst 2	0.02735%	Instrument-2(SYSTRONIC)	0.04626%

Table 6a: Results of metformin ruggedness

Concentration	Analyst	%RSD	Instruments	%RSD
10µg/ml	Analyst 1	0.0574%	Instrument-1(ELICO)	0.0596%
	Analyst 2	0.0215%	Instrument-2(SYSTRONIC)	0.0453%

Limit of detection: DL= 3.3*σ/S

Where σ = the standard deviation of the response S = the slope of the calibration curves the slope S may be estimated from the calibration curve of the analyte.

Table 7: Results of LOD

Drug name	LOD
Sitagliptin	0.397µg/ml
Metformin	0.8952µg/ml

Limit of quantification

L. O. Q=10 σ/S

Where σ = the standard deviation of the response

S = the slope of the calibration curves the slope S may be estimated from the calibration curve of the analyte.

Table 8: Results of LOQ

Drug name	LOQ
Sitagliptin	1.2951µg/ml
Metformin	2.7159µg/ml

Assay

Five tablets were accurately weighed and the average weight was determined. Then the tablets were grounded to a fine powder using clean and dry pestle and mortar. A quantity equivalent to 5 mgof Sitagliptin and 50 mg of Metformin was transferred to a 500 ml volumetric flask and dissolved in insufficient water. The contents were Ultra-Sonicated for 15 min and made up to the volume to obtain the concentration of 100µg/ml and filtered through Whatmann filter paper. Absorbance of these solutions was measured at 267 nm and 237 nm, and concentration of Sitagliptin and Metformin in the sample solution was determined using following equation:

Cx = A2ay2-A1ay2/ax2ay1-ax1ay2

Cy= A1ax2-A2ax1/ax2ay1-ax1ay2

Where CX and Cy are the concentration of Sitagliptin and Metformin respectively, ax1 and ax2 are the absorptivity values of Sitagliptin at 267 and 237 nm respectively, ay1 and ay2 are the absorptivity values of Metformin at 267 and 237 nm respectively. A1 and A2 are the absorbances of diluted sample at 267 and 237 nm respectively.

Cx = A2ay2-A1ay2/ax2ay1-ax1ay2

= (0.5020*0.00249)-(0.5060*0.00446)/(0.00227*0.00249)-(0.1389*0.00446)

= 1.6476 µg/ml

Cy = A1ax2-A2ax1/ax2ay1-ax1ay2

= (0.5060*0.00227)-(0.5020*0.1389)/(0.00227*0.00249)-(0.1389*0.00446)

= 9.9184 µg/ml

Table 9: Simultaneous data sitagliptin and metformin

	Absorbance maxima λ 1 (267)	Absorbance maxima λ (237)
Janumet 50 mg/500 mg	0.5060(A1)	0.5020(A2)
Absorbance of sitagliptin	1.389	0.0227
Absorbance of metformin HCl	0.0446	0.2491
Absorptivity of sitagliptin	0.1389(ax1)	0.00227(ax2)
Absorptivity of metformin	0.00249(ay1)	0.00446(ay2)

CONCLUSION

A simple and selective Spectrophotometric method was developed for the simultaneous estimation of Sitagliptin and Metformin in pharmaceutical dosage form. The developed method was validated as per ICH guidelines.

ACKNOWLEDGMENT

I want to acknowledge our beloved principal Prof. M. Sumakanth RBVRR women's college of pharmacy for giving me the opportunity for performing the research work.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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