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Research Article

Analytical Investigation and Validation of Sitagliptin and Metformin in Their Pure and Combined Dosage Form using Spectrophotometry

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ABSTRACT

The estimation of drug in their combined dosage form is crucial for assuring pharmacokinetic properties of formulation. All formulations are extensively investigated using various analytical techniques but they frequently encounter restricted sensitivity, specificity, and reproducibility. The need of development of simple, rapid, accurate, and cost-effective analytical method for their simultaneous estimation is essential for routine quality control. The present study aimed to develop and validate a UV-visible spectrophotometric method based on the simultaneous equation (Vierordt's) method for the quantitative estimation of Sitagliptin (STG) and Metformin (MFT) in bulk drugs and combined tablet dosage forms used for the treatment of metabolic disorder Diabeties Mellitus. Standard and sample solutions of STG and MFT were prepared using 0.1 N sodium hydroxide as the solvent and absorption maxima (λmax) of STG and MFT were determined as 234 nm and 269 nm, respectively. Quantitative estimation was carried out using the simultaneous equation method based on the absorptivity coefficients of both drugs at their selected wavelengths. The proposed analytical method was validated according to the International Council for Harmonisation (ICH Q2 (R1) guidelines with respect to linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and ruggedness. The developed method exhibited satisfactory linearity over the concentration range of 5–25 µg/mL for STG and 50–250 µg/mL for MFT. The validation parameters demonstrated acceptable precision, accuracy, and ruggedness, with percentage recoveries close to 100% and relative standard deviation values below the acceptable limit of 2%, indicating good reproducibility of the method. The proposed UV-visible spectrophotometric simultaneous equation method is simple, rapid, and precise for the simultaneous determination of both drugs in their bulk and pharmaceutical dosage forms. Owing to its satisfactory validation characteristics, the method is suitable for routine quality control analysis in pharmaceutical laboratories.

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INTRODUCTION

Diabetes mellitus is one of the most prevalent chronic metabolic disorders among worldwide individuals creating a challenge on healthcare facilities of any nation [1, 2]. Combination therapy has become the preferred treatment strategy for patients with Type 2 diabetes mellitus (T2DM), as it improves glycemic control through complementary mechanisms of action while reducing the risk of treatment failure . Sitagliptin (STG) chemically known as (3R)-3-purcino-l-[3-(trifiuoromethyl)-5,6-dihydro[1,2,4] triazolo [4,3-a]pyrazin-7 (8H)-yl] -4-(2,4,S-trifiuorophenyl) butan-lone phosphate monohydrate is a selective and orally active dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances endogenous incretin activity by preventing the degradation of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Owing to its favourable efficacy and safety profile, Sitagliptin is extensively used either as monotherapy or in combination with other oral antidiabetic agents [4, 5 6]. Metformin Hydrochloride (MFT), chemically known as N,N-dimethyl imidodicarbonimidic diamide hydrochloride, is a traditionally used oral antihyperglycaemic agent which primarily acts by suppressing hepatic gluconeogenesis, improving peripheral insulin sensitivity, and enhancing glucose uptake in skeletal muscle without causing significant hypoglycemia [7]. The complementary pharmacological actions of Sitagliptin and Metformin make their fixed-dose combination an effective therapeutic option for the long-term management of Type 2 diabetes mellitus [8]. A reliable analytical method is essential to ensure the quality, safety, and efficacy of pharmaceutical products throughout their development, manufacturing, and quality control processes. Among the available analytical techniques, UV-visible spectrophotometry remains one of the most widely employed methods because of its simplicity, rapidity, cost-effectiveness, and suitability for routine analysis in quality control laboratories[9].

The simultaneous equation (Vierordt's) method is particularly advantageous for the quantitative estimation of multicomponent formulations, as it enables the determination of individual drug concentrations without prior separation by utilizing the additive nature of absorbance at selected wavelengths[10]. An extensive review of the published literature indicates that several analytical methods, including high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS), have been reported for the estimation of STG and MFT in combination dosage form. Although a few spectrophotometric methods have been described for the simultaneous estimation of Sitagliptin and Metformin in combined dosage forms, hence there remains a need for a simple, economical, accurate, and validated analytical procedure that can be conveniently employed for routine quality control analysis without the requirement for sophisticated instrumentation.

Therefore, the present study was undertaken to develop and validate a simple UV-visible spectrophotometric method based on the simultaneous equation approach for the quantitative estimation of Sitagliptin and Metformin in bulk drugs and combined tablet dosage forms. The proposed method was validated in accordance with the International Council for Harmonisation (ICH Q2(R1)) guidelines by evaluating its linearity, accuracy, precision, ruggedness, limit of detection, and limit of quantification. The validated method is intended to provide a reliable, rapid, and cost-effective analytical tool for routine pharmaceutical quality control.

MATERIALS AND METHODS

Materials and Method: The relation between the concentration of analyte and the amount of light absorbed based on Beer–Lambert law is the basis used here[11]. All spectrophotometric tracing were performed using double beam Uv-Visible spectrophotometer (Shimadzu UV-1900i, Kyoto, Japan), equipped with matched 1cm quartz cells. Precise masses of all the chemicals were weighed by using electronic balance (Kerro BL3003KE) with precision upto 1mg, while all the glasswares employed were calibrated before the study. Chemicals and reagents used are of analytical grade. Pharmaceutical grade Sitagliptin Phosphate (purity: 99.99%) and Metformin Hydrochloride (purity: 99.99%) were procured from Dhamtech Pharma Supplies, Mumbai, India, and were used as reference standards without further purification. Commercially available tablet formulation containing Sitagliptin Phosphate equivalent to 50 mg of Sitagliptin and Metformin Hydrochloride 500 mg per tablet (Batch No. 0426580) was purchased from the local market for analytical evaluation. Freshly prepared 0.1 N sodium hydroxide (NaOH) was employed as the solvent throughout the investigation.

Preparation of Standard Stock Solutions: Standard stock solutions of Sitagliptin and Metformin were prepared separately by accurately weighing 100 mg of each drug and diluting with 100 mL of 0.1 N NaOH in volumetric flasks. Further working standard solutions (100 µg/mL) were prepared by titering 10 mL of the respective stock solution with separate 100 mL 0.1 N NaOH.

Determination of Maximum Absorption Wavelength (λmax): Aliquots of the working standard solutions were appropriately diluted with 0.1 N NaOH to obtain concentrations ranging from 5–25 µg/mL for Sitagliptin and Metformin. Each solution was scanned over the wavelength range of 200–400 nm against 0.1 N NaOH as the blank. The wavelength corresponding to the maximum absorbance (λmax) was selected for quantitative estimation. Sitagliptin exhibited maximum absorbance at 234 nm, whereas Metformin showed maximum absorbance at 269 nm.

Preparation of Sample Solution: Twenty tablets of the marketed formulation weighed individually, and average tablet weight was calculated. The tablets were finely powdered, and powder equivalent to 100 mg of Sitagliptin and in-situ of Metformin was diluted upto 100ml with 0.1 N NaOH and sonicated for 20 minutes to ensure complete dilution. An appropriate aliquot was diluted with 0.1 N NaOH to obtain the required working concentration for analysis.

Simultaneous Estimation: The absorbance of both standard and sample solutions was measured at 234 nm and 269 nm. The concentrations of Sitagliptin and Metformin present in the sample solution were calculated using the simultaneous equation (Vierordt's). The concentrations were determined by applying Cramer's rule to the simultaneous equations [12, 13].

$$\text{Concentration of Drug (Cx)} = \frac{(A2 \text{ x } ay1) - (A1 \text{ x } ay2)}{(ax2 \text{ x } ay1) - (ax1 \text{ x } ay2)}$$

$$\text{Concentration of Drug (Cy)} = \frac{(A1 \text{ x } ax2) - (A2 \text{ x } ay1)}{(ax2 \text{ x } ay1) - (ax1 \text{ x } ay2)}$$

Method Validation: The proposed analytical method was validated in accordance with the International Council for Harmonisation (ICH Q2(R1)) guideline for analytical method validation.[14, 15]

Linearity and range: Linearity was evaluated by analyzing a series of standard solutions at

concentration range of 5–25 µg/mL for Sitagliptin and 50–250 µg/mL for Metformin. Calibration curves were constructed by plotting absorbance against concentration, and the corresponding regression equations and correlation coefficients were determined.

Accuracy: The accuracy of the proposed method was evaluated by the standard addition technique at three concentration levels (80%, 100%, and 120%). Known quantities of standard drug were added to pre-analysed sample solutions, and the percentage recovery of each analyte was calculated.

Precision: Method precision was assessed by analyzing replicate measurements (n = 3) of homogeneous sample solutions under identical experimental conditions. The results were expressed as mean, standard deviation (SD), and percentage relative standard deviation (%RSD).

Limit of Detection and Limit of Quantification: The limit of detection (LOD) and limit of quantification (LOQ) were calculated according the signal-to-noise ratio (S/N, i.e.,3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3\sigma/S$$

$$LOQ = 10\sigma/S$$

where σ represents the standard deviation of the response and S represents the slope of the calibration curve.

Ruggedness: Ruggedness of the developed method was evaluated by performing intra-day and inter-day precision studies. The results were expressed as percentage relative standard deviation (%RSD), and values below 2% were considered indicative of acceptable ruggedness and reproducibility.

RESULT AND DISCUSSION

The UV-Visible spectrophotometer was employed easily to assess the concentration of component drugs in combined dosage form using a simultaneously equation method. In this method, the diluted solution of STG and MFT was scanned from 200-400nm. Two wavelengths 234 nm and 269nm are selected for STG and MTZ respectively as λmax as shown in spectrum figure.

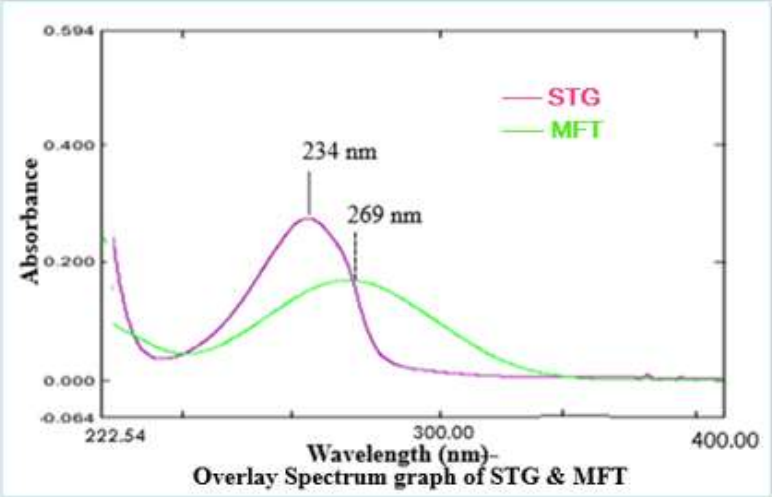


Figure 1: Calibration curve of STG and MFT.

Table 5: Showing result of Combined Evaluation of Pure drug and in their Dosage form

Drug Name	Concentration (µg/ml) (c)	Absorbance of STG at 234 nm (abs)	Absorbance of STG at 269 nm(abs)	Absorptivity at 234 nm (abs/c)	Absorptivity at 269 nm (abs/c)
STG (x)	5	0.050	0.097	0.0100	0.0194
	10	0.026	0.132	0.0026	0.0132
	15	0.046	0.147	0.0030	0.0098
	20	0.037	0.175	0.0018	0.0087
	25	0.031	0.189	0.0012	0.0075
Mean	--	**0.038**	**0.148**	**0.0037 (ax1)**	**0.01172 (ax2)**
MFT **(y)**	5	0.532	0.077	0.1064	0.0154
	10	0.084	0.093	0.0084	0.0093
	15	1.186	0.086	0.0790	0.0057
	20	1.901	0.065	0.0950	0.0032
	25	2.444	0.073	0.0977	0.0029
Mean	--	**1.2294**	**0.0788**	**0.0773(ay1)**	**0.0073(ay2)**
Dosage Form		**2.651 (A1)**	**3.214 (A2)**	--	--

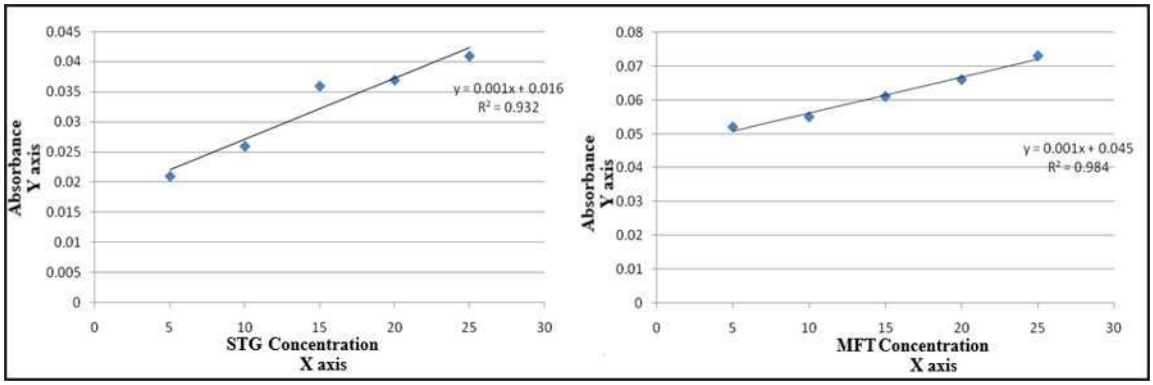
Discussion: The final resultant concentration was 20µg/ml for STG and the final resultant concentration 200 µg/ml of MFT in solution. As the concentration of STG obtained is 20.71 µg/ml referrers to 103.55 % and for MFT was 207.15 µg/ml referrers to 103.57 % yielding a optimal concentration as claimed in the label as obtained from Crammer's rule.

b) Linearity and Range:

Table showing results of Linearity for STG and MFT:

Sr. No	Volume taken in ml	Diluted upto	Concentration STG µg/ml	Concentration MFT µg/ml	Abs of STG at λmax 234 nm	Abs of MFT at λmax 269 nm	STG Obtained Concentration µg/ml	MFT Obtained Concentration µg/ml
1	0.5	10	5	50	0.021	0.484	5	49.7
2	01	10	10	100	0.026	0.633	10	99.3
3	1.5	10	15	150	0.036	0.785	20	150.0
4	2.0	10	20	200	0.037	0.932	21	199.0
5	2.5	10	25	250	0.041	1.084	25	249.7

Discussion: The linearity range for STG is 05–25 µg/ml and MFT is 50–250 µg/ml at respective selected wavelengths. The coefficient of correlation for STG at 234 nm and for MFT at 269 nm is 0.9932 and 0.9984, respectively. Both drugs showed good regression values at their respective wavelengths, and the results of a recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed methods. Percentage estimation of STG and MFT from the tablet dosage form by the said method is 103.55 % and 103.57 % with standard deviation less than 2.



c) LOD & LOQ:

Table showing results of LOD & LOQ determinations.

Sr. No	Volume taken in ml	Diluted upto	Concentration µg/ml		Abs of STG at λmax 234 nm	Abs of MFT at λmax 269 nm
			STG	MFT		
F	0.1	10	1	10	0.159	0.014
2	0.2	10	2	20	0.018	0.017
3	0.3	10	3	30	0.022	0.018
4	0.4	10	4	40	0.006	0.022
5	0.5	10	5	50	0.007	0.025
Standard Deviation					0.065546	0.004324
Slope					0.316	0.027
LOD					0.6845	0.528532
LOQ					1.07425	1.601611

Discussion: The LOD and LOQ value for STG is 0.6845 µg/ml and 1.07425 µg/ml and for MFT were 0.528532 µg/ml and 1.601611 µg/ml respectively. Low values of LOD and LOQ indicates good sensitivity of proposed methods.

d) Accuracy:

Table showing results of accuracy studies.

Drug	Accuracy Level (%)	Actual Amount (µg/mL)	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean ± SD	% RSD
STG	80 %	20	16	35.94	99.83	99.87333333 ± 0.066	0.066667
	100%	20	20	39.96	99.95		
	120%	20	24	43.93	99.84		
MFT	80 %	200	160	359. 93	99.98	99.97667 ± 0.015	0.015279
	100%	200	200	399.95	99.99		
	120%	200	240	439.86	99.96		

Discussion: Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for STG was 99.87 % and for MFT was found to be in range of 99.97%. In these results, recovery greater than 95 % with minimum SD and coefficient of variance is even minimum, i.e. less than 0.5 % for both the drugs, signifies the accuracy of the method.

e) Precision: Precision of an analytical method are expressed in S.D. or R.S.D. using a series of measurements to align the degree of agreement among the individual results.

Table showing determination of Precision for STG and MFT:

Sr. No	Volume taken in ml	Diluted upto	Conc. µg/ml	STG		MFT	
				Abs of 234 nm	Obtained Conc µg/ml	Abs of 269 nm	Obtained Conc µg/ml
1	1	10	10	0.0259	9.9	0.0548	9.8
2	1	10	10	0.0261	10.1	0.0549	9.9
3	1	10	10	0.0262	10.2	0.0549	9.9
4	1	10	10	0.0261	10.1	0.0551	10.1
5	1	10	10	0.0262	10.2	0.0549	9.9
Mean Concentration					10.10		9.9
Standard Deviation					0.1225		0.1100
Relative Standard Deviation					1.212619		1.104279

Discussion: The precision of the simultaneous equation method was evaluated through replicate measurements of homogeneous samples (10µg/ml) in compliance with ICH guidelines. Both analytes exhibited a high degree of scatter agreement and repeatability, with low standard deviations (0.1225 for STG and 0.1100 for MFT) and close mean recoveries. The similarity in their calculated relative standard deviations (%RSD = 1.21 % for STG and 1.10 % for MFT) falls comfortably below the acceptable threshold of 2.0%, confirming that the proposed method is uniformly precise and reproducible for both drugs.

f) Ruggedness: Inter-day and Intra-day precision of the formulation was conducted three times a day and three different consecutive day with the same concentration and verified the method's repeatability.

Table No: Results and statistical data of Intraday Study:

Sr. No	Intraday Condition	Conc. µg/ml	Absorbance		Obtained Concentration µg/ml	
			STG λmax 234 nm	MFT λmax 269 nm	STG	MFT
1	Morning	10	0.0256	0.0548	9.60	9.80
2	Afternoon	10	0.0259	0.0547	9.90	9.70
3	Evening	10	0.0257	0.0549	9.70	9.90
Mean					9.733	9.800
Standard Deviation					0.153	0.100
Relative Standard Deviation					1.574	1.032

Table No: Results and statistical data of Inter-day Study:

Sr. No	Intraday Condition	Conc. µg/ml	Absorbance		Obtained Concentration µg/ml	
			STG λmax 234 nm	MFT λmax 269 nm	STG	MFT
1	Day 1	10	0.0256	0.0547	9.62	9.82
2	Day 2	10	0.0259	0.0546	9.93	9.74
3	Day 3	10	0.0257	0.0549	9.76	9.97
Mean					9.743	9.86
Standard Deviation					0.1543	0.1013
Relative Standard Deviation					1.564	1.012

Discussion: The RSD as a percentage was verified using intra- and inter-day analysis. The findings of precision studies conducted within day showed % RSD 1.574 for STG and 1.032 for MFT and between days showed % RSD 1.564 for STG and 1.012 for MFT are presented.

CONCLUSION

The UV-Spectrophotometric method was developed and validated as per ICH guidelines using simultaneous equation method for the estimation of STG and MFT using 0.1 N NaOH as the solvent system. The developed method was found to be more simple, accurate, precise, and economic, thus can be used routinely for the analysis of Sitagliptin and Metformin in the bulk and combined dosage forms. The proposed Vierodt's method offers a sensitive, accurate, and precise framework for simultaneous drug analysis, provided the linearity parameters are optimized.

LIST OF SYMBOLS:

λ- Lanbda

µg- Microgram

LIST OF ABBREVIATIONS:

ICH- International Council for Harmonisation

N –Normality

NaOH-Sodium Hydroxide

nm- Nanometer

RSD- Relative Standard Deviation

S.D. - Standard Deviation

Λmax- Absorption maxima

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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