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Development and validation of UV spectrophotometric method for simultaneous estimation of metformin hydrochloride and alogliptin benzoate in bulk drugs and combined dosage forms

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ABSTRACT

Two simple, precise and economical UV spectrophotometric methods have been developed for the simultaneous estimation of Alogliptin benzoate and Metformin hydrochloride in bulk and pharmaceutical dosage forms. Method A is simultaneous equation method (Vierodt's Method), which is based on measurement of absorption at 277nm and 232nm i.e.λ_{max} of Alogliptin benzoate and Metformin hydrochloride respectively. Method B is Absorbance ratio (Q-analysis method) which is based on measurement of absorption at wavelength of 250nm and 277nm i.e. iso-absorptive point of Alogliptin benzoate and Metformin hydrochloride and λ_{max} of Alogliptin benzoate respectively. Linearity was observed in the concentration range of 5-25µg/ml for Alogliptin benzoate and 1-10µg/ml for Metformin hydrochloride. The accuracy of methods was assessed by recovery studies and was found to be within range of 98-102% for both Alogliptin benzoate and Metformin hydrochloride. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The results were validated statistically as per ICH Q2 R1 guideline and were found to be satisfactory. Due to non-availability of product the condition of mixture was simulated by using Glucophase® tablets(Metformin hydrochloride 500mg) and API of Alogliptin benzoate was added to it. The proposed methods were successfully applied for the determination of Alogliptin benzoate and Metformin hydrochloride in the mixture.

Keywords: Alogliptin, Metformin, Simultaneous estimation, Q-analysis, UV spectrophotometry.

INTRODUCTION

Metformin hydrochloride: Metformin hydrochloride (MET) is chemically 1, 1- dimethylbiguanide hydrochloride and it belongs to biguanideclass of oral anti-diabeticdrugs (Fig. 1). MET acts as anti-hyperglycemic and lowers the blood glucose level by inhibiting hepatic glucose production, gluconeogenesis and increasing peripheral utilization of glucose [1]. Metformin hydrochloride is official in I.P., U.S.P., and B.P.[2-4]. I.P. recommends non aqueous titration method for raw materialand UV spectrophotometric method for tablets [2]. In literature a large number of methods for estimation of Metformin hydrochloride in drug products, either alone orin combination with other drugs have been reported [5-10]. Large number of specialized method for estimation of Metformin hydrochloride and its combination with other drugs in plasma, serum and urine are also reported in literature [11-14].

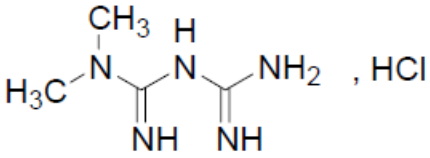

Fig. 1: Structure of Metformin hydrochloride

Alogliptin benzoate: Alogliptin benzoate (ALG) is a newanti-diabetic drug. Takeda pharmaceutical (Japan) received FDA approval for three new drug formulations for type 2 diabetes therapy in 2013 i.e. Nesina® (alogliptin), Oseni® (alogliptin and pioglitazone) and Kazano® (alogliptin and metformin HCL)[15]. Alogliptin is also approved for marketing in Europe as alone or in combination with other anti-diabetics drugs [16]. Alogliptin is new drug and not official in any pharmacopoeia. Alogliptin benzoate is chemically 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydropyrimidin-1-yl} methyl) benzonitrile benzoate (Fig.2). Alogliptin benzoate belongs to the class of dipeptidyl peptidase -4 (DPP-4) inhibitors, a new class of anti-diabetic drugs which act by increasing glucose dependent insulin release[17]. Therapeutically DPP-4 inhibitors are used to treat type 2 diabetes alone or combination with other drugs which increase the sensitivity of insulin at target site[18-21]. DPP-4 inhibitor acts by inhibiting the inactivation of enteroendocrineincretins such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic (GIP) polypeptide. The increased availability of incretins due to DPP-4 inhibitor results in glucose dependent insulin release and better glycemic control.

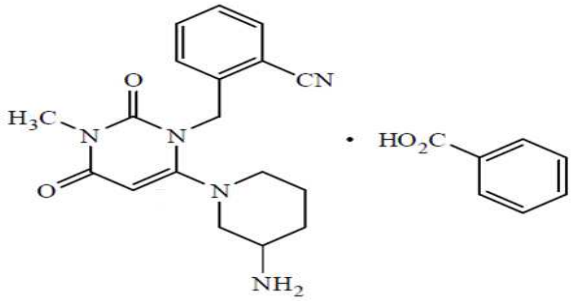

Fig. 2: Structure of Alogliptin benzoate

Literature survey indicates only one HPLC method by Ayoub B.M. et al. for estimation of Alogliptin in bulk drug as well as pharmaceutical preparation[22].There was no method reported in literature for simultaneous estimation of Alogliptin benzoate and Metformin hydrochloride.

The objective of present study was to develop and validate the UV spectrophotometric method for simultaneous estimation of Alogliptin benzoate and Metformin hydrochloride by Simultaneous equation (Method A) and Absorbance ratio (Method B)[23] methods. UV spectroscopy is simple, precise, accurate and economical technique for estimation of drugs. Using UV spectroscopy the two drugs can be estimated simultaneously without any prior separation.

MATERIALS AND METHODS

Instruments:
UV double beam spectrophotometer of Incarp Instruments Pvt. Ltd. (Hyderabad) Sican 2301with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm was used for analytical work along with matched quartz cell of length 1cm. The analysis was carried by using UV Solutions 2.2 software. All the weighing was carried out on the Sartorius weighing balance (BSA223S-CW). Sonication of samples wascarried out by Metrex sonicator.

Materials and reagents:
Alogliptin benzoate was purchased from Swapnroop Drugs and Pharmaceuticals, Aurangabad. Metformin hydrochloride was supplied as gift sample by Shreya life sciences. The analytical grade methanol was purchased from Loba Chemie Pvt. Ltd. (India). Glucophase® tablets(Metformin hydrochloride 500mg) were purchased from local pharmacy. The distilled water was used for analytical work and rinsing of clean glass wares.

Preparation of stock solution and selection of wavelength for analysis:
Standard stock solutionsof Alogliptin benzoate and Metformin hydrochloride were prepared separately by adding 50mgof drug to methanol taken in 50ml volumetric flasks and then sonicated for five minutes and the volume was made up with methanol. The resulting solutions contain 1mg/ml of the drug. The stock solutions of ALG and MET were further diluted with water to obtain the concentration of 20µg/ml. The resulting solutions were then scanned in UV spectrophotometer from 400 to 200nm. From the resulting spectraλ_{max} for ALG and MET were calculated separately (Fig. 3, 4). The overlay spectra of ALG and MET was also recorded.From the overlay spectra iso-absorptive point of ALG and MET was calculated (Fig. 5).

Method A: Simultaneous equation method (Vierodt's Method)
If a sample contains two drugs with reasonably dissimilar λ_{max}, each of which exhibits absorbance at the λ_{max} of other, then it is possible to determine the drugs by simultaneous equation method (Vierodt's Method). Two equations are constructed based on the fact that the absorbance at a particular λ_{max} is sum ofindividual absorbance of two components.

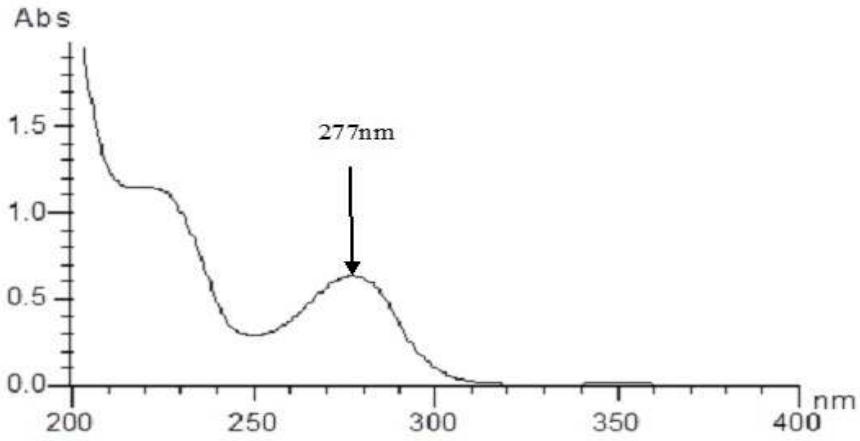

Fig. 3: UV spectra of ALG

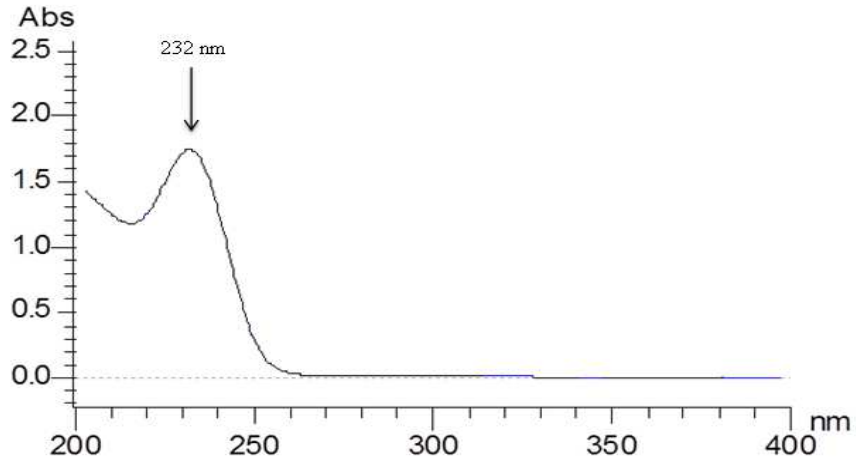

Fig. 4: UV spectra of MET

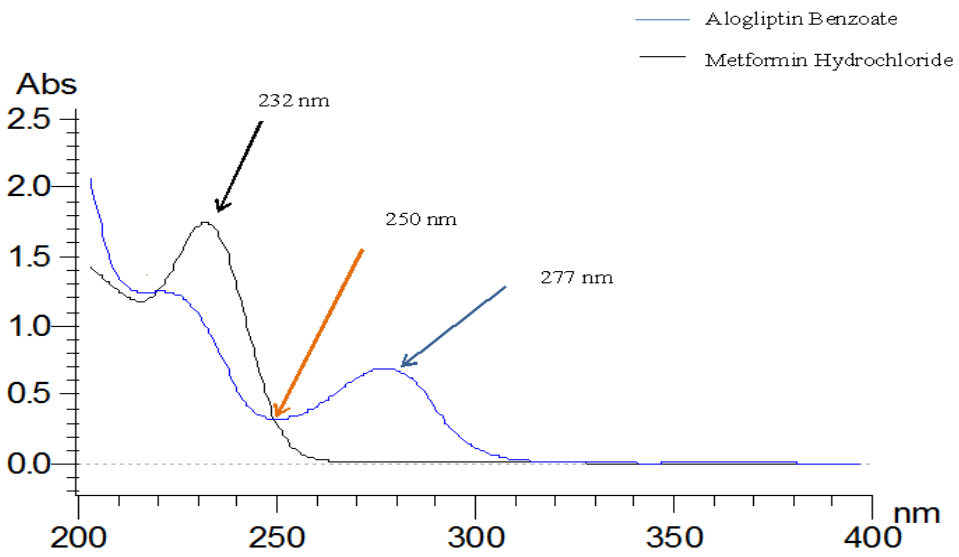

Fig.5: Overlay spectra of ALG and MET

The scanning spectra of 20µg/ml solution of ALG and MET show clear peaks at 277nm and 232nm respectively for ALG and MET (Fig.3, 4). The λ_{max} of each drug was selected for analysis.

The stock solution of ALG and MET was then diluted with water to 5-25µg/ml and 1-10µg/ml respectively.The absorbance of these solutions was measured at 277nm and 232nm respectively. Calibration curves were plotted to verify the Beer's law and the absorptivity values were calculated at the respective wavelengths for both the drugs. Two simultaneous equations as given below were formed using absorptivity values, A(1%, 1cm) (Table 1).

$$A_1 = ax_1C_x + ay_1C_y \quad (1)$$

$$A_2 = ax_2C_x + ay_2Cy \quad (2)$$

Where
ax_1 =A(1%, 1cm) of ALG at 277nm (334.05)
ay_1=A(1%, 1cm)of MET at 277nm (24.3)
ax_2 =A(1%, 1cm)of ALG at 232nm (497.65)
ay_2 = A(1%, 1cm)of MET at 277nm (860.25)

C_x and $C_y$are the concentrations of ALG and MET in gm/100ml respectively in sample solution. A_1andA_2 arethe absorbances of mixture at 277nm and 232nm respectively. Solving equation 1 and 2 C_x and $C_y$can be calculated as:

$$C_x = A_2\, ay_1 - A_1\, ay_2 / ax_2\, ay_1 - ax_1\, ay_2 \quad (3)$$

$$C_y = A_1\, ax_2 - A_2\, ax_1 / ax_2\, ay_1 - ax_1 ay_2 \quad (4)$$

Method B: Absorbance ratio method(Q-analysis method)
The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length e.g. two dilutions of the same substance give the same absorbance ratio A_1/A_2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in mixture by the absorbance ratio method, absorbance is measured at two wavelengths, one being the λ_{max} of one of the components (λ2) and the other being a wavelength of equal absorptivity of the two components (λ1), i.e., an iso-absorptive point. A series of standard solutions of ALG and MET in the concentration range of 5-25µg/ml and 1-10µg/ml respectively were prepared in water and the absorbance of these solutions were measured at 250nm (iso-absorptive point) and 277nm($\lambda_{max}$of ALG) (Fig. 3). Calibrationcurves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. The absorptivity values are reported in Table 1.

The concentration of two drugs in mixture was calculated by using the following equations:

$C_x = (Q_m - Q_y / Q_x - Q_y) \times (A_1 / ax1)$ (5)

$C_y = (Q_m - Q_x / Q_y - Q_x) \times A_1 / ay1)$ (6)

Where,
ax_1 = A (1%, 1cm) of ALG at 250nm (164.9)
ay_1= A (1%, 1cm) of MET at 250nm (169.2)
ax_2 = A (1%, 1cm) of ALG at 277nm (337.9)
ay_2 = A (1%, 1cm) of MET at 277nm (24.3)

A_1 and A_2 are the absorbances of mixture at 250nm and 277nm. C_x and $C_y$are the concentrations of ALG and MET in gm/100 ml respectively in sample solution

$Q_m = A_2 / A_1$, Q_x = ax2 / ax1 and Q_y= ay2 /ay1

Assay of tablets by method A and B:
Fixed dose combination of Alogliptin and Metformin is approved for marketing in USA (Kazano® Tablets) and Europe (Vipdomet® tablets). Kazano® Tablets contains Alogliptin/Metformin hydrochloride in the ratio of 12.5/500mg or 12.5/1000mg and Vipdomet® tablets contain Alogliptin/Metformin hydrochloride in the ratio of 12.5/850mg. Due to non-availability of product standard addition of Alogliptin benzoate API to Metformin tablets (Glucophase®500 mg) was used to simulate the condition of mixture. 20 Glucophase®tablets were weighed and triturated in a mortar pestle and powder equivalent to 50mg of Metformin hydrochloride was taken. To this powder100mg of Alogliptin benzoate was added, to make concentration of MET/ALG in ratio of 1:2. Since in actual product mixture contains 1.7mg ALG and 50 mg of MET, the quantity of ALG is quite less and linearity range (5-25µg/ml) is high as compared to MET (1-10 µg/ml). So excess of 98.3 mg of ALG is added to bring the concentration in the linearity range. The mixture is added to 50ml of volumetric flask containing 30ml methanol and sonicated for 10 minutes. Final volume was made up to 50ml with methanol and filtered through whatman filter paper (No. 41). 5ml of resulting solution was diluted with water to 50ml. 1ml of the resulting solution was again diluted with water to 10ml and absorbance was taken at 277nm and 232 nm for method A and at 277 nm and 250nm for method B. The amount of ALG and MET was calculated by equation 3, 4 for method A and equation 5, 6 for method B.

Method validation[24]:
The UV spectrophotometric method was validated as per ICH guidelines for method validation. The performance parameterslike lineariy, precision and accuarcy were evaluated.

Linearity:
Linearity was studied by diluting standard stock solution of ALG to 5-25µg/mland MET 1-10µg/ml concentrations (n=3). Calibration curves with concentration verses absorbance were plotted at their respective wavelengths and the obtained data was subjected to regression analysis using the least square method. The standard curves for ALG and MET are shown in Fig. 6, 7 respectively and data is presented in Table 2.

Precision:
Repeatability: A mixture containing 10µg/ml each of ALG and MET was prepared and analyzed both by method A and B (n=6). The data is represented in Table 3.

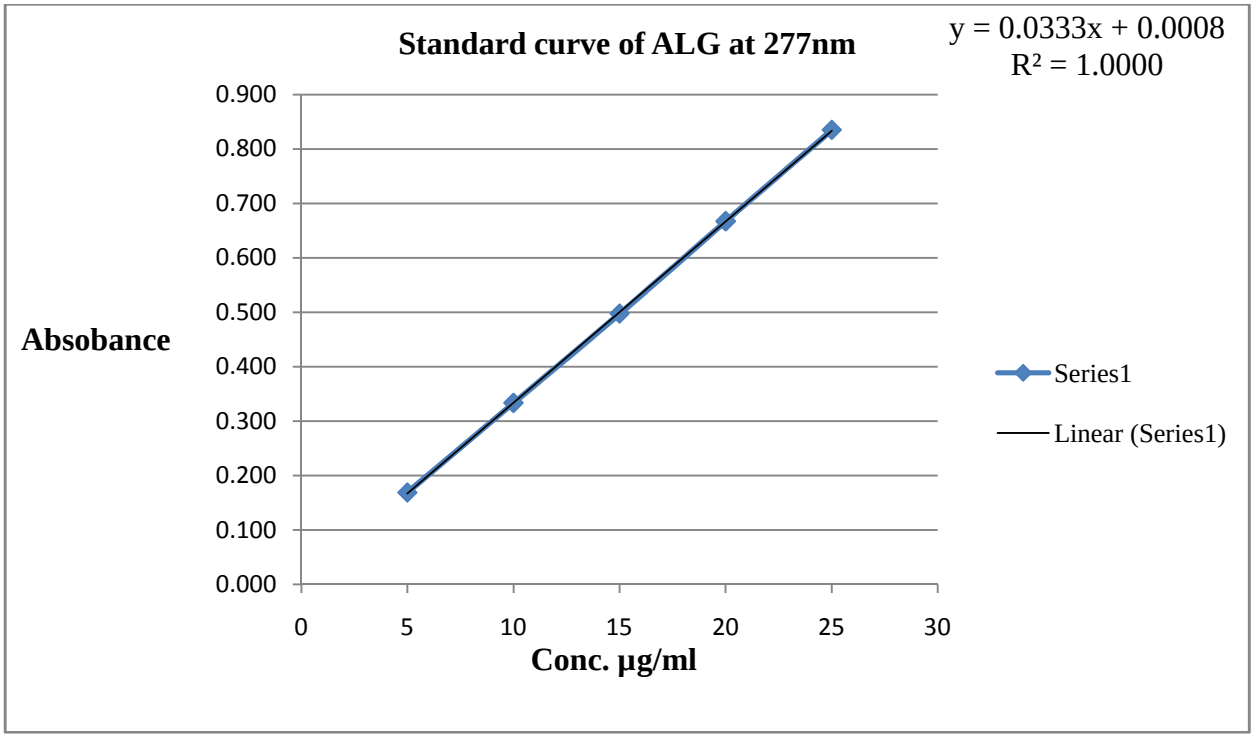

Fig. 6: Standard curve of ALG at 277 nm

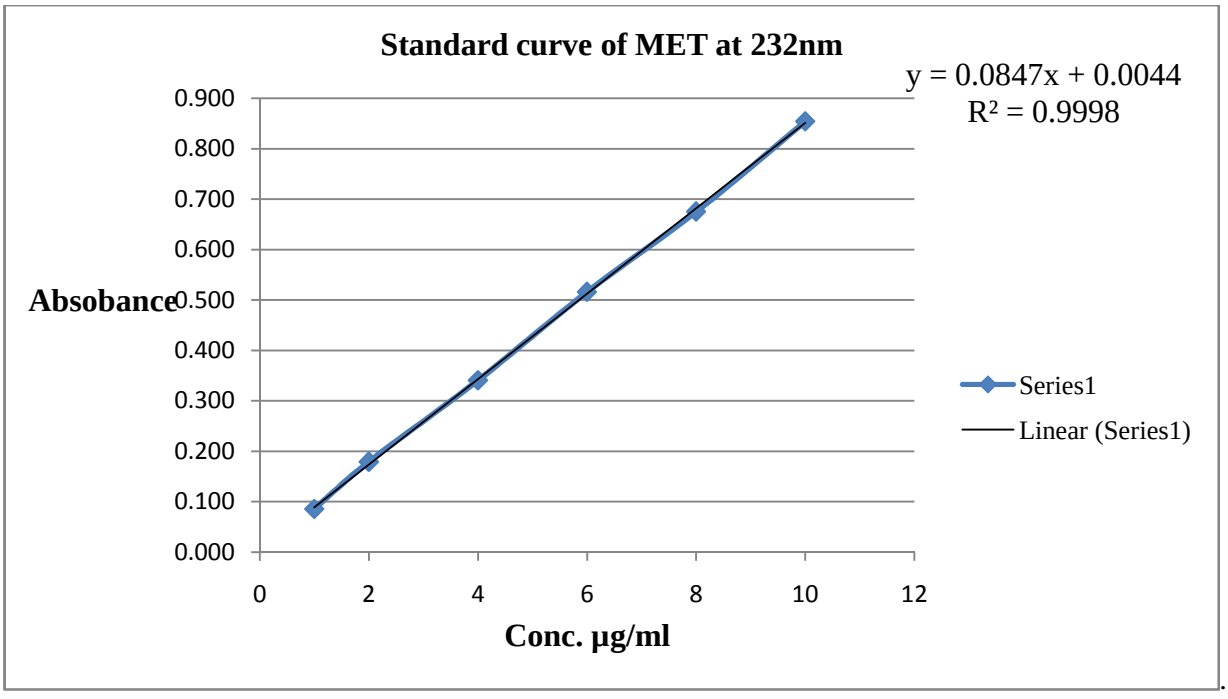

Fig. 7: Standard curve of MET at 232 nm

Intermediate precision: intermediate precision is studied in terms of intraday and inter-day precision. Three concentrations of ALG and MET was selected in a mixture and analyzed by method A and B (n=3). For intraday, the analysis was carried out at different intervals on the same day and for inter day, the analysis was carried on different days. Table 4 and 5 give the results for intraday and inter-day studies respectively.

Accuracy:

To check the accuracy of the developed methods and to study interference of formulation additives, analytical recovery experiments were carried out by using standard addition method. Reference standard solution of each drug

was added to tablet samples at three different concentrations level (50,100 and 150%). At each level, samples were prepared in triplicate and the mean percentage recoveries and % RSD value were calculated. Table 6 shows the result for accuracy of the method.

Table 1: Absoptivity values (A1%,1cm) of Alogliptin benzoate (ALG) and Metformin hydrochloride (MET) for method A and B

Conc. (µg/ml)	Method A				Method B			
	ALG		MET		ALG		MET	
	277 nm	232 nm	277 nm	232 nm	277 nm	250 nm	277 nm	250 nm
1	-	-	50.00	856.67	-	-	50.0	170.0
2	-	-	45.00	895.00	-	-	45.0	185.0
4	-	-	16.67	851.67	-	-	16.7	165.0
5	338.00	502.00	-	-	346.0	178.0	-	-
6	-	-	15.56	860.00	-	-	15.6	166.7
8	-	-	9.58	844.17	-	-	9.6	166.3
10	333.33	496.00	9.00	854.00	338.0	166.0	9.0	163.0
15	331.56	494.22	-	-	332.0	160.7	-	-
20	333.50	498.83	-	-	336.5	159.1	-	-
25	333.87	497.20	-	-	337.2	160.8	-	-
Mean	334.05	497.65	24.30	860.25	337.9	164.9	24.3	169.2

Table 2 : Regression analysis of calibration curves and summary of validation parameter for method A and B

Sr. No.	Parameter	Drug	Method A		Method B	
			277 nm	232 nm	277 nm	250 nm
1.	Beer's law limit (µg/ml)	ALG	5-25			
		MET	1-10			
2.	Molar absorptivity (L mol^{-1} $cm^{-1)}$	ALG	15416	22967	15594	7610
		MET	4024	14248	4024	2802
3.	Sandell's Sensitivity (µg/cm^2/0.001)	ALG	0.030	0.020	0.030	0.060
		MET	0.041	0.011	0.041	0.059
4.	Intercept	ALG	0.0008	0.0001	0.0025	0.0098
		MET	0.0064	0.0044	0.0064	0.0028
5	Slope	ALG	0.0333	0.0497	0.0335	0.0156
		MET	0.0003	0.0847	0.0003	0.0161
6.	Correlation cofficient	ALG	1	0.9999	0.9998	0.9996
		MET	0.2888	0.9998	0.2888	0.9991

Table 3.Repeatabilty study data for mixture of ALG and MET (n=6)

Drug	Conc. Taken	Method A		Method B	
		% Found	%RSD	% Found	%RSD
ALG	10	99.12 ± 0.88	0.88	98.83± 0.76	0.77
MET	10	99.45 ± 0.69	0.69	99.40± 1.14	1.15

Table 4: Intraday precision data for mixture of ALG and MET. (n=3)

Drug	Conc. Taken	Method A		Method B	
		% Found	%RSD	% Found	%RSD
ALG	8	101.46 ± 0.38	0.38	100.46 ± 0.59	0.59
	12	101.53 ± 0.42	0.41	100.28 ±0.38	0.37
	20	101.23 ± 0.28	0.28	100.27 ± 0.24	0.24
MET	4	101.33 ± .52	0.51	100.17± 1.70	1.70
	6	100.39 ± 1.21	1.20	100.67± 1.32	1.31
	10	99.50 ± 0.62	0.63	99.07± 0.85	0.86

Table 5: Interday precision data for mixture of ALG and MET. (n=3)

Drug	Conc. Taken	Method A		Method B	
		% Found	%RSD	% Found	%RSD
ALG	8	101.29±0.59	0.58	100.75± 0.75	0.74
	12	101.44± 0.55	0.55	100.00± 0.38	0.38
	20	101.03± 0.49	0.48	100.07± 0.34	0.34
MET	4	101.58± 0.14	0.14	100.17± 1.01	1.01
	6	100.22± 0.92	0.92	101.17± 0.50	0.49
	10	99.73± 0.31	0.31	100.87± 1.02	1.01

Table 6: Recovery study data for ALGand MET by method A and B (n=3)

Drug	Pre-analyzed conc.	Drug added	Method A		Method B	
			% Recovery	% RSD	% Recovery	% RSD
ALG	8	-	-	0.38	-	0.50
		4	101.25± 0.87	0.86	100.08 ± 1.76	1.75
		8	101.21± 0.59	0.58	100.08 ± 0.56	0.56
		12	100.33± 0.66	0.66	99.89 ± 0.46	0.46
MET	4	-	-	0.51	-	1.13
		2	100.83± 0.58	0.57	101.17 ± 1.04	1.03
		4	100.50± 1.15	1.14	99.91 ± 1.19	1.19
		6	100.28± 0.42	0.42	99.06 ± 0.63	0.64

Table 7: Assay of formulation (n=6)

Drug	Method A		Method B	
	% Assay	% RSD	% Assay	% RSD
ALG	100.77± 0.58	0.57	100.08± 0.30	0.30
MET	98.63± 0.37	0.37	100.45± 1.24	1.24

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient, precise and accurateway for simultaneous analysis of Alogliptin benzoate and Metformin hydrochloride in its bulk and pharmaceutical dosage form. Absorbance maxima of ALG at 277nm and MET at 232nm were selected for the analysis. Regression analysis shows linearity over the concentration range of 5-25µg/ml for ALG and 1-10µg/ml for MET with respective correlation coefficients of 1 and 0.9998 respectively. The % RSD for repeatability (n=6), intraday and interday (n=3) precision was found to be less than 2% indicating the precision of method.Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. % recovery for ALG and METwas found within the range of 98 % and 102%. Values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of both the methods.Due to non-availability of combination product the Glucophase tablets with standard addition of Alogliptin benzoate is used to simulate the condition of actual product. The assay for MET were found to be 98.63± 0.37 and 100.45± 1.24 for method A and B respectively. The assays for ALG were found to be 100.77± 0.58 and 100.08± 0.30 for method A and B respectively. The % RSD value for both ALG and MET was found to be less than 2%.In this study the simultaneous estimation of Alogliptin benzoate and Metformin hydrochloride was carried out by simultaneous equation and absorbance ratio methods satisfactorily.

CONCLUSION

Based on the results obtained, it is found that the developed UV-spectrophotometric technique is quite simple, accurate, precise, reproducible, sensitive and economical. They can become effective analytical tool for routine quality control of Alogliptin benzoate and Metformin hydrochloridein bulk drug combination and its combined pharmaceutical dosage form without any prior separation of components.

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