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Development and validation for Simultaneous Estimation of Alogliptin and Metformin in combined dosage form by UV Method

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ABSTRACT

A simple, sensitive, accurate and precise simultaneous UV spectrophotometric method has been developed for the estimation of alogliptin and metformin in tablet dosage form. The absorption maxima of the drugs were found to be 225 and 237 nm for alogliptin and metformin, respectively, in methanol, using a Shimadzu UV- visible spectrophotometer (model UV-1650). Alogliptin and metformin in obeyed beer's law in the range of 0.05-0.25 µg/ml and 2-10 µg/ml, respectively. The correlation co-efficient value for metformin was found to be 0.99963 and 0.99936 at 225 nm and 237 nm, respectively. The correlation co-efficient value for alogliptin was found to be 0.99968 and 0.9333 at 225 nm and 237 nm, respectively. The method was validated for various parameters according to ICH guidelines. The low relative standard deviation values indicate good precision and high recovery values indicate accuracy of the proposed method.

Keywords: Alogliptin, Metformin, Methanol, UV spectrophotometric method, simultaneous equation method.

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INTRODUCTION

Metformin (MET) is chemically, N, N- dimethyl imidocarbonimidic diamide monohydrochloride (Figure.1 A), is an anti-hyperglycemic agent that improves glucose tolerance in patient with type-II diabetes, lowering the both basal and postprandial plasma glucose. Metformin hydrochloride decrease hepatic glucose production, decrease intestinal absorption of glucose and improve insulin sensitivity by increasing peripheral glucose uptake and utilization (1).

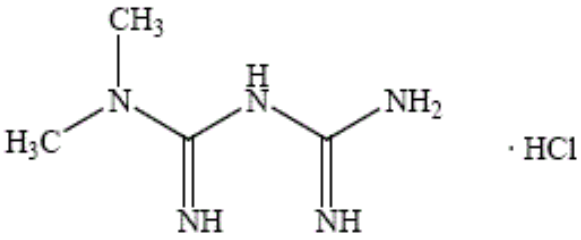

Figure1: (A) Structure of Metformin hydrochloride

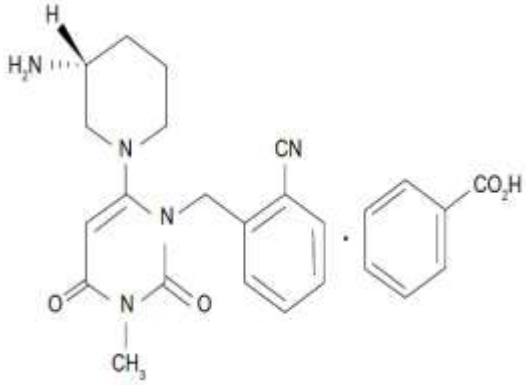

Figure 1: (B) Structure of Alogliptin benzoate

Alogliptin (ALG) is a new anti-diabetic drug. It is chemically 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2, 4-dioxo-1, 2, 3,4-tetrahydropyrimidin-1-yl} methyl) benzonitrile benzoate (Figure.1 B). Alogliptin belongs to the class of Dipeptidyl peptidase -4 (DPP-4) inhibitor, a new class of anti-diabetic drugs which act by increasing glucose dependent insulin release. 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. DPP-4 inhibitors act by inhibiting the inactivation of enteroendocrine incretins 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 (2).Alogliptin benzoate is a new DPP-4 inhibitor quite effective alone or in combination with other anti-diabetic drugs. Takeda pharmaceuticals (Japan) received FDA approval for three new type 2 diabetes therapy in 2013 i.e. Nesina® (Alogliptin), Oseni® (Alogliptin and

Pioglitazone) and Kazano® (Alogliptin and Metformin hydrochloride). Alogliptin is also approved for marketing in Europe as alone or combination with other anti-diabetics drugs. Alogliptin is a new drug and not official in any pharmacopeia. The literature survey reveals that there are analytical methods available for determination of Alogliptin and Metformin from biological matrices, bulk drug and dosage forms and for determination of Alogliptin and Metformin with combination of other drugs by RP-HPLC, LC-MS, HPTLC and UPLC(3-19).

MATERIALS AND METHOD

Procurement of drug samples and formulation

Alogliptin and Metformin working standards were gift samples from Vivan life science Pvt. Ltd. (Mumbai). Commercial tablets (Kazano®, Takeda Ltd.) containing metformin 500 mg and alogliptin 12.5 mg were purchased from pharmasave pharmacy, Canada.

Reagents and chemicals used

Doubled distilled water used throughout the study. Methanol analytical grade were purchased from sigma-Aldrich Inc.

Instruments used

An UV- visible double beam spectrophotometer (UV probe, UV1650PC) with 10 mm matched quartz cells was used. All weighing were done on electronic balance (A&D, HR-200). Ultrasonicator (ultrasonic) was used for sample solution preparation.

Selection of solvent and wavelength

Solution of metformin hydrochloride (40 µg) and alogliptin (1µg) were prepared in Methanol, 0.05 N Sodium hydroxide and Acetonitrile. Absorbances of both the drugs were higher and gave good sharp peak in methanol, so it was decided to prepare drug solution in methanol for further studies. The λmax of alogliptin and metformin were found to be 225 nm and 237 nm respectively. The overlain spectra of alogliptin and metformin were shown in Figure 2.

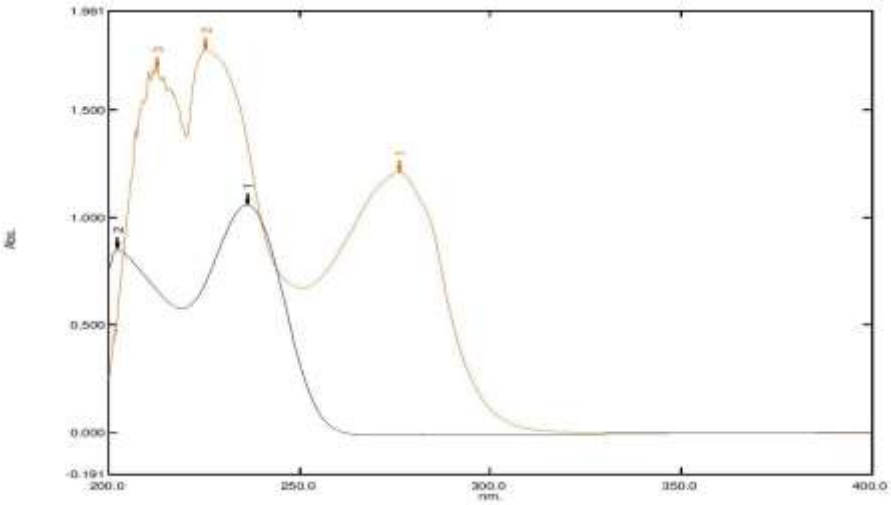

Figure 2: Overlain spectra of alogliptin and metformin

Preparation of standard stock solution

Stock solutions of metformin (400 µg/ml) and alogliptin (10 µg/ml) are prepared in methanol. A solution of metformin (40 µg/ml) and alogliptin (1 µg/ml) and mixture of metformin and alogliptin (40 µg/ml and 1 µg/ml) were prepared in methanol.

Application of simultaneous equation method

In quantitative estimation of two components by simultaneous equation method, two wavelengths i.e., Alogliptin and metformin were selected as their respective λ_{max} from the overlain spectrum, at which two drugs have maximum absorbance. The concentration of two drugs in the mixture can be calculated using the following equations.

$$c_{metformin} = \frac{A_2\, ay_1 - A_1\, ay_2}{ax_2\, ay_1 - ax_1\, ay_2}$$

and

$$c_{alogliptin} = \frac{A_1\, ax_2 - A_2\, ax_1}{ax_2\, ay_1 - ax_1\, ay_2}$$

Where,

A_1 and A_2 are absorbances of formulation at 237 and 225 respectively,

c_x and c_y are the concentration of metformin and alogliptin respectively,

ax_1 and $ax_2$are absorptivities of metformin at 237 and 225 respectively,

ay_1 and ay_2 are absorptivities of alogliptin at 237 and 225 respectively.

Analysis of formulation

20 tablet each containing 500 mg of metformin and 12.5 mg of alogliptin were weighed and quantity equivalent to 40 mg of metformin and 1 mg of alogliptin were dissolved in Methanol. Sonicated for 20 mins and make upto the mark by using same. The solution was filtered and further diluted to get a concentration 40 µg/ml and 1µg/ml of metformin and alogliptin respectively. The absorbances of the above solution were noted at 237 nm and 225 nm. The concentration of metformin and alogliptin were calculated using simultaneous equation method.

RESULTS AND DISCUSSION

The analytical method was validated with respect to parameter according to ICH guidelines [20, 21] such as Linearity, Precision, accuracy and stability.

Linearity and range

Preparation of calibration curve and linearity studies

Metformin was found to be linear at the concentration range of 2-10 µg/ml. Individual standard solutions were scanned using methanol as blank.T he absorbance of this solution was noted at the wavelength 237 nm and 225 nm and calibration curve were plotted using concentration Vs absorbance, the calibration graph was shown in Figure.3, 4. Alogliptin was found to be linear at the concentration range of 0.05-0.25 µg/ml. The absorbance of this solution was noted at the wavelength 237 nm and 225 nm and calibration curve were plotted using concentration Vs absorbance, the calibration graph was shown in Figure.5, 6. The values are shown in Table 1 and 2.

Table 1: Absorbance of metformin at selected wavelength

S. No	Concentration (µg/ml)	Absorbance at 237nm	Absorbance at 225nm
1	2	0.217	0.181
2	4	0.420	0.298
3	6	0.640	0.433
4	8	0.823	0.554
5	10	1.028	0.683

Table 2: Absorbance of alogliptin at selected wavelength.

S. No	Concentration (µg/ml)	Absorbance at 237nm	Absorbance at 225nm
1	0.05	0.009	0.028
2	0.1	0.010	0.033
3	0.15	0.019	0.039
4	0.2	0.023	0.043
5	0.25	0.035	0.048

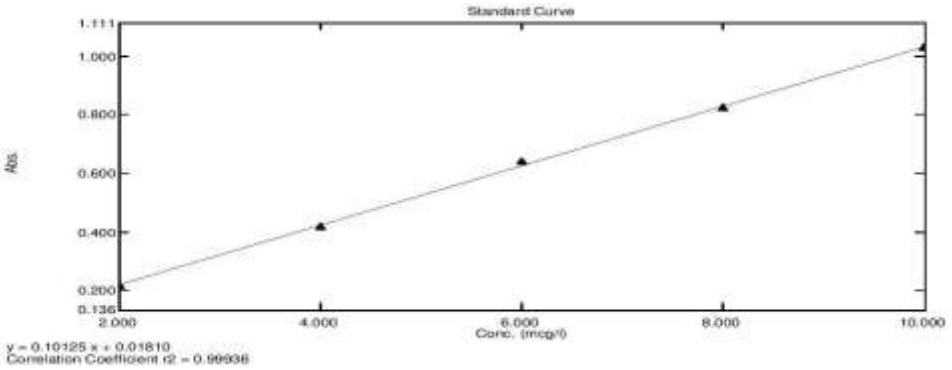

Figure 3: calibration graph of metformin at 237 nm

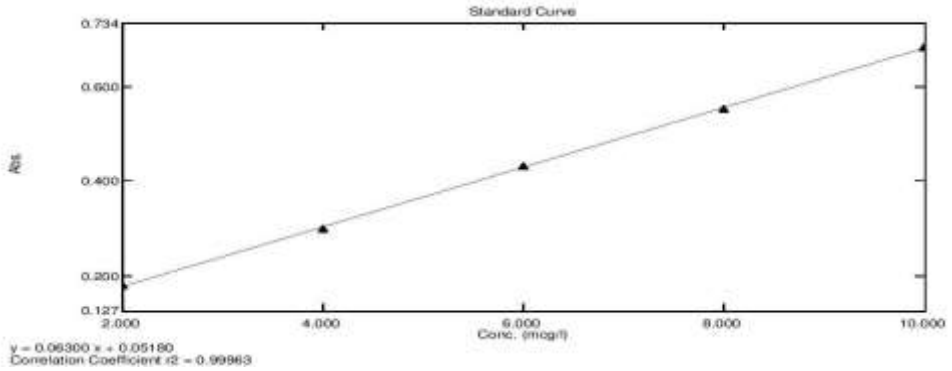

Figure 4: Calibration graph of Metformin at 225 nm

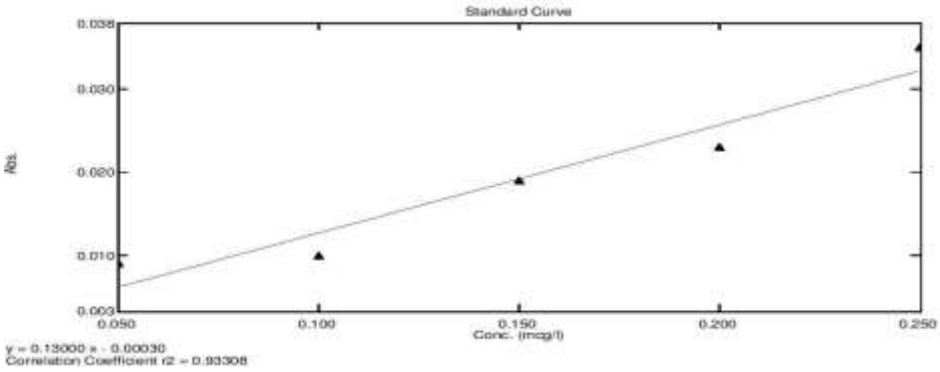

Figure 5: Calibration graph of Alogliptin at 237 nm

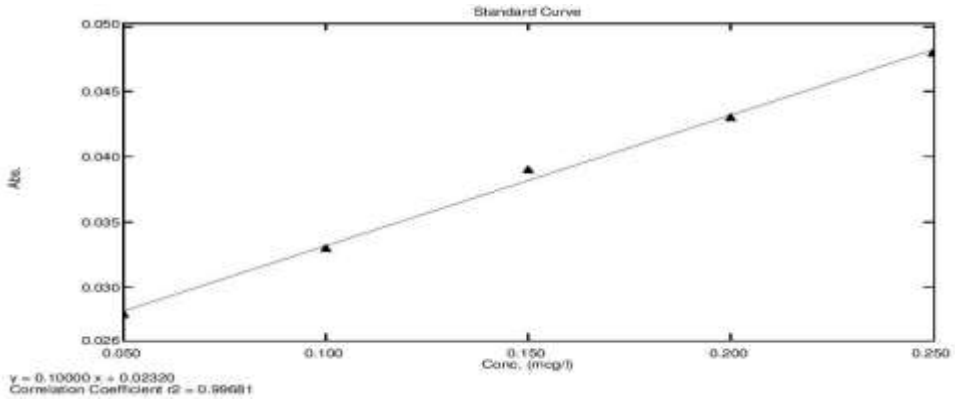

Figure 6: Calibration graph of Alogliptin at 225 nm

Precision

Precision of the method was demonstrated by,

1. Intraday precision
2. Interday precision

Intraday precision

Intraday precision was found out by carrying out analysis of standard drug solution at three different concentrations in the linearity range for three times on the same day and % RSD was calculated.

Interday precision

Interday precision was found out by carrying out analysis of standard drug solution at three different concentrations in the linearity range for three day over a period of one week and % RSD was calculated.

Accuracy

The results of recovery studies at various levels shows that the recovery is between 98.60 to 101.50 % (Ideally should be between 98-102 %). It indicates that there is no interference in the analysis of the drug from the excipients in the tablet formulation. The results of recovery studies of the marketed formulation are shown in the following Table 3.

Table 3: Recovery studies

S.No	Level	% Recovery		% RSD*	
		MET	ALO	MET	ALO
1	80%	99.82	98.60	0.201	0.260
2	120%	98.80	101.50	0.106	0.424

*RSD of five observations

Stability

The sample solution was subjected to stability studies under room condition. Stability was studied looking for any change in absorbance and peak shape when compared to UV spectra of freshly prepared solution. The solution store under room temperature was stable upto 3 hours.

Assay

The tablets were analysed and the results were obtained in the range of 96-99.6 % compared to the label claim. The results of analysis of marketed formulation are shown in Table 4.

Table 4: Analysis of formulation

S.No	Drug	Amount(mg)		%Label claim	%RSD*
		Labelled	Found		
1	**MET**	500	498	99.6	0.32
2	**ALO**	12.5	12	96.0	0.85

*RSD of five observations

CONCLUSION

The most noteworthy feature of this method is its simplicity and rapidity and non-requiring time consuming sample preparation such as extraction of solvents. This method is novel and can be employed for routine analysis in quality control analysis. The described method is giving accurate and precise results for the determination of alogliptin and metformin in mixture in marketed formulation.

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