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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE DETERMINATION OF ALOGLIPTIN BENZOATE AND METFORMIN HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

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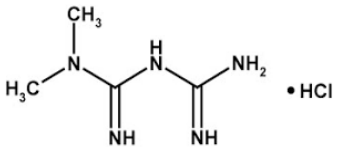
ABSTRACT

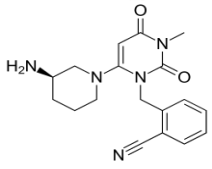
A simple, accurate and precise UV spectroscopic method has been developed for the simultaneous estimation of Alogliptin benzoate and metformin hydrochloride in bulk and tablet dosage forms. Both the drugs are used as anti-diabetic drugs. The combination is estimated by simultaneous equation method, which is based on measurement of absorption of alogliptin benzoate at λ_{max} 275nm and metformin hydrochloride at 235nm respectively. Linearity was observed in the concentration range of 2.5-12.5µg/ml for alogliptin benzoate and 2-10µg/ml for metformin hydrochloride. The accuracy of the methods was assessed by recovery studies and was found to be within range of 98-101% for both Alogliptin benzoate and metformin hydrochloride respectively. The developed methods were validated with respect to accuracy, precision and linearity. The results were validated statistically as per ICH guidelines and were found to be satisfactory. Due to the non-availability of combination product the tablet was prepared in laboratory and used to simulate the condition of actual product. This development was successfully applied for the simultaneous determination for both the drugs in bulk and commercial tablet preparation.

INTRODUCTION

Alogliptin (trade name Nesina and Vipidia) is a new anti-diabetic drug in the DPP-4 inhibitor (gliptin) class used for the treatment of Type-2 diabetes[1]. Alogliptin exhibits relatively little risk of hypoglycemia and has relatively modest glucose-lowering activity. Alogliptin and other gliptins are commonly used in combination with metformin in people whose diabetes cannot adequately be controlled with metformin alone[2]. It was developed by Syrrx, a company which was acquired by Takeda Pharmaceutical Company in 2005.Chemically, alogliptin is prepared as a benzoate salt and exists predominantly as the R enantiomer (>99%). It undergoes little or no chiral conversion *in vivo* to the (S) enantiomer. Metformin hydrochloride is an oral antihyperglycemic drug (*N, N dimethyl imido dicarbonimidicdiamide hydrochloride),* marketed under the trade name Glucophage, is the first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It is also used in the treatment of polycystic ovary syndrome.



Metformin Hydrochloride



Alogliptin

EXPERIMENTAL PROCEDURE:

Chemicals:

Alogliptin benzoate was procured from Ranbaxy labs, Hyderabad and Metformin hydrochloride from Hetero labs, Hyderabad.

All the weighings were carried out on the Sartorius weighing balance (BSA223S-CW). For assay due to the non-availability of the commercial product of alogliptin benzoate and metformin hydrochloride, tablets were prepared in the laboratory.

Instrumentation:

T60 a high performance compact Split Beam Spectrophotometer is used.

Preparation of Stock & Standard Solutions:

Standard stock solutions of alogliptin benzoate and metformin hydrochloride were prepared separately by adding 100mg of drug to 10ml of distilled water taken in 100ml volumetric flasks and then sonicated for ten minutes and volume was made upto 100ml with distilled water. The resulting solutions contain 1000µg/ml (1mg/ml) of the drug. The stock solutions of ALO and MET were further diluted by pipetting out 10ml from above stock solution into a volumetric flask and make up the volume into 100ml by using distilled water. The drugs were appropriately diluted to give 2-10µg/ml of metformin and 2.5-12.5µg/ml of alogliptin respectively.

Determination of λmax:

Standard solution containing 10µg/ml each of ALO and MET was scanned using distilled water as blank in the range of 200-400 nm to determine the wavelength of maximum absorption (λmax) of the drugs. MET showed absorbance maxima at 235 nm and ALO showed the absorbance maxima at 275 nm.

Determination of iso-absorptive Point and wavelength of Maximum Absorbance (λmax): Solutions of 10µg/ml of both drugs were prepared from working stock solution and scanned in the range of 200 nm to 400 nm against distilled water as blank. The iso-absorptive point was found to be 250 nm.

Simultaneous Equation in Multi-Component Samples[3,4]:

Simultaneous Equation Method:

If a sample contains two absorbing drugs X and Y, each of which absorbs at the wavelength maximum of other, it may be possible to determine both drugs by the technique of simultaneous equations. Concentrations of several compounds present in the same mixture can be determined by solving a set of simultaneous equations even if their spectra overlap. Consider a multi-component system consisting of 2 components X and Y each of which absorbs at the λ max of the other, λ_1 being the wavelength of maximum absorbance of X(λ_{max}) and λ_2 being the wavelength of maximum absorbance of Y(λ_{max}). In such cases it can be possible to determine both drugs by the components of simultaneous equation method. The information required is the absorptivities of x at λ_1 and λ_2, ax1 and ax2 respectively. The absorptivities of y at λ_1 andλ_2, ay1 and ay2 respectively The absorbance of the diluted samples at λ_1 and λ_2, A1 and A2 respectively

Let Cx and Cy be the concentration of x and y respectively in the diluted samples. Thus the absorbance of the mixture at λ_1 and λ_2 may be expressed as follows:

A1=ax1bcx+ay1bcy……..1

0.904=(0.0689)1cx+(0.0191)1cy

A2=ax2bcx+ay2bcy

……2

0.160=

(-0.0020)1cx+(0.0436)1cy

For measurements in 1 cm cells, b =1cm.

.'. rearrangement of eq……2

$$Cy=\frac{A2-ax2cx}{ay2}$$

……. 3

Substituting for Cy ie eq. (1) and rearranging gives

$$Cx=\frac{A2ay1-A1ay2}{ax2ay1-ax1ay2}$$

……. 4

$$Cx=\frac{0.160(0.019)-0.904(0.0388)}{0.002(0.0191)-0.0689(0.0436)}$$

Cx=12.3

……5

$$\mathbf{Cy=\frac{A1ax2-A2ax1}{ax2ay1-ax1ay2}}$$

…… 6

$$Cy=\frac{0.904(-0.0020)-0.160(0.0689)}{-0020(0.0191)-0.0689(0.0436)}$$

Cy=4.2

…….7

The concentration of each componenet can be calculated by mathematical equation:

Cx=(Qm-Qy/Qx-Qy) A/a1

Cy=(Qm-Qx/Qy-Qx) A/a2

Where,

Cx and Cy = concentration of X and Y respectively,

A=absorbance of the sample at isoabsorptive wavelength,

a1 and a2 = absorptivities of x and y respectively at isoabsorptive wavelength,

Qm

=

$$\frac{\text{absorbance of sample solution at } \lambda \text{ max of one of the components}(\lambda 2)}{\text{absorbance of sample solution at isoabsorptive wavelength}}$$

Qx=

$$\frac{\text{absorptivity of } x \text{ at} \lambda \text{ max of one of the components } (\lambda 2)}{\text{absorptivity of } x \text{ at isoabsorptivity wavelength}}$$

Qy=

$$\frac{\text{absorptivity of } y \text{ at} \lambda \text{ max of one of the components}(\lambda 2)}{\text{absorptivity of } y \text{ at isoabsorptivity wavelength}}$$

.

Method Validation[5]:

The UV-Spectrophotometric method was validated as per ICH guidelines for method validation.

The performance parameters like; Accuracy, precision and linearity were evaluated.

Accuracy: Accuracy was determined by standard addition method. Three different levels (80%, 100% and 120%) of standards were spiked to commercial sample in triplicate. The mean of percentage recoveries and the %RSD was calculated. The mean % recoveries were found to be 100.50% and 100.62%, for MET and ALO respectively.

Precision: The repeatability of the method was determined by assaying five standard solutions of ALO and MET by using the concentration 10µg/ml. The reproducibility of the proposed method was determined by performing tablet assay at different time intervals (2 hour interval) on same day (Intra-day precision) and on three different days (Inter-day precision). Results of Intra-

day and Inter-day precision is expressed in %RSD.

Linearity: Linearity was studied by diluting standard stock solutions of ALO in the range of 2.5-12.5µg/ml and MET in the range of 2-10µg/ml concentrations (n=3). Calibration curves with conc vs. absorbance were plotted at their respective wavelengths and the data was subjected to regression analysis using the least square method.

Limit of Detection (LOD): The LOD of MET and ALO by the proposed methods were determined. The value of LOD for MET and ALO were found to be 0.098, 0.183 µg/ml respectively.

$$\textbf{LOD} = \frac{3.3\times\sigma}{slope}$$

Limit of Quantitation (LOQ): The LOQ of MET and ALO by the proposed methods were determined using calibration standards. The value of LOQ for MET and ALO were found to be 0.297 and 0.555 µg/ml respectively.

$$\textbf{LOQ} = \frac{10\times\sigma}{slope}$$

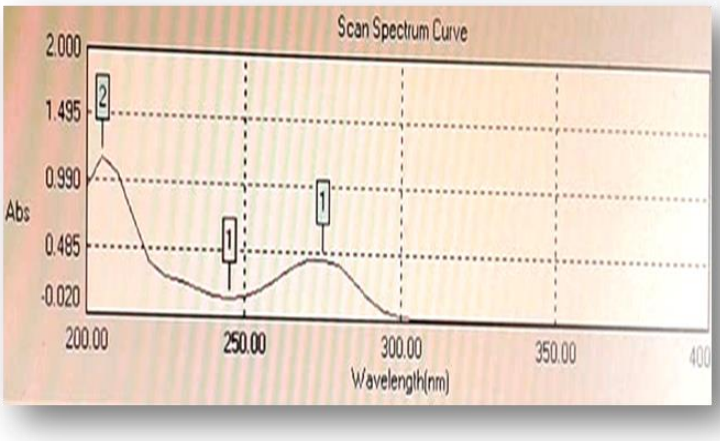
Along with these parameters we have also studied and observed correlation co-efficient, Molar absorptivity, slope, intercept, coefficient of variation etc.

Ruggedness: Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slots by different analysts using similar operational conditions.

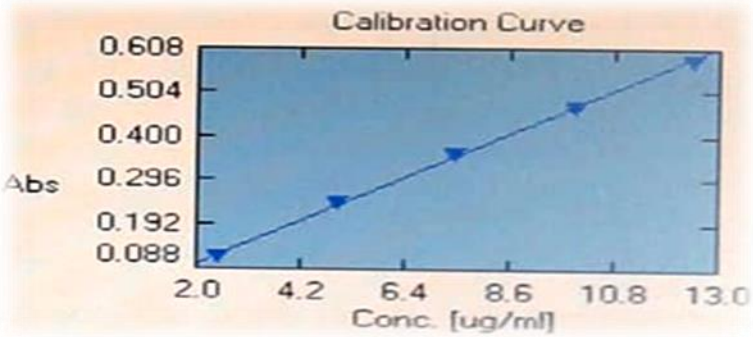
Assay: Fixed dose combination of Alogliptin and Metformin is approved for marketing in USA in the brand names of (*Kazano Tablets*) and Europe (*Vipdomet Tablets*)[6]. The ratio is maintained 12.5/500mg or 12.5/1000mg in tablet for ALO & MET concentrations respectively. Due to the non-avalaibility of the product, by standard addition of alogliptin benzoate and metformin hydrochloride in the ratio of 8.5-250mg tablets were prepared in the laboratory. To the above prepared tablets 91.1mg of alogliptin benzoate was extra added to make concentration of MET/ALO in ratio of 1:2. Ten tablets prepared were accurately weighed and average weight was calculated. These tablets are triturated in motar and pestle. Then from that mixture 300mg was weighed and added to 100ml of distilled water and sonicated it for 10mins.

RESULTS AND DISCUSSION:

The methods discussed in the present work provide a convenient, precise and accurate way for the simultaneous analysis of Alogliptin benzoate and Metformin hydrochloride in bulk and its pharmaceutical dosage form compared to the previous methods[7,8,9]. Absorbance maxima of ALO–275nm and Absorbance maxima of MET-235nm were selected for the analysis. Regression analysis shows linearity over the concentration range of 2.5-12.5µg/ml for ALG and 2.0-10.0µg/ml for MET. 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 method satisfactorily. 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 results are expressed as % recovery. Due to the non-availability of the combination product the tablet is prepared in laboratory to stimulate the condition of actual product. The assay values of MET and ALG were found to be 100.50 ± 0.37 and 100.21± 0.59 respectively.

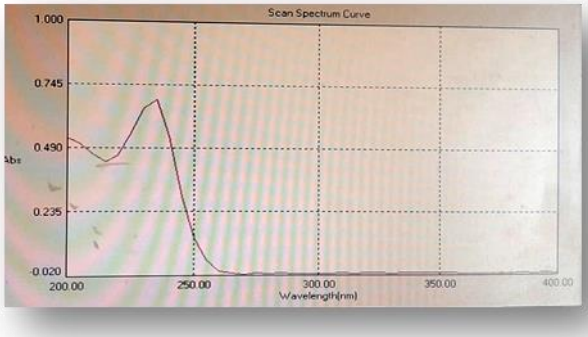


Determination of λ_{max} of alogliptin 275nm

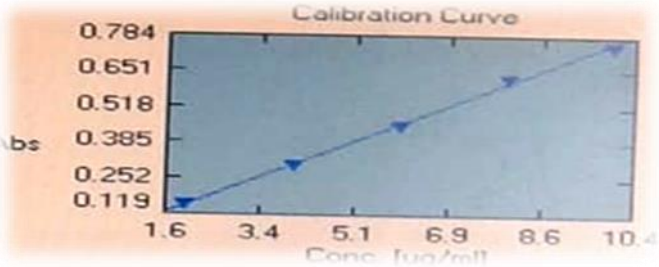


Calibration curve of Alogliptin

UV-SPECTRA OF METFORMIN



Determination of λ_{max} of metformin – 235nm



Calibration curve of Metformin

Linearity Data:

Table 1: Linearity data of Alogliptin

Concentration (µg/ml)	Absorbance
2.5	O.111
5.0	0.243
7.5	0.360
10.5	0.473
12.5	0.584
Regression equation	Y=0.011x+0.002
R^2	0.999

Table 2: Linearity data of Metformin

Concentration(µg/ml)	Absorbance
2	0.149
4	0.297
6	0.149
8	0.624
10	0.753
Regression equation	Y=0.011x+0.002
R^2	0.999

Table 3: Formula of laboratory made tablet formulation

S. No	Ingredients	Quantity (mg)
1	Alogliptin benzoate	8.5
2	Metformin hydrochloride	250
3	Microcrystalline cellulose	25
4	Crospovidone	12
5	Magnesium stearate	3
6	Starch	10mg/10ml
7	Distilled water	q.s
8	Total weight	305 mg

EVALUATION OF PARAMETERS:

Table 4: Regression Analysis of Calibration Curves and Validation Parameters

S. No	Parameter	Drug	Values
1	Linearity [µg/ml]	ALG	2.5-12.5
		MET	2-10
2	Molar Absorptivity ($L\ Mol^{-1}\ Cm^{-1}$]	ALO	4.2
		MET	12.1
3	Correlation Co-efficient	ALO	0.9994
		MET	0.9994
4	Intercept	ALO	0.009
		MET	0.076
5	Slope	ALO	0.0468
		MET	0.0875
6	LOD (µg/ml)	ALO	0.183
		MET	0.0980
7	LOQ(µg/ml)	ALO	0.555
		MET	0.297

Table 5: Inter-day Precision Data for ALO & MET

Drug	Concentration (µg/ml)	Absorbance	%RSD
ALO	7.5	0.315	0.56
	10	0.473	0.50
	12.5	0.492	0.30
MET	6	0.336	0.92
	8	0.586	0.83
	10	0.753	0.31

Table 6: Intraday Precision Data for ALO & MET

Drug	Conc (µg/ml)	1st day	2nd day	3rd day	Standard Deviation
ALO	7.5	0.360	0.294	0.357	0.0357
	10	0.473	0.436	0.391	0.0187
	12.5	0.584	0.492	0.543	0.0460
MET	6	0.449	0.336	0.421	0.0588
	8	0.624	0.607	0.586	0.0190
	10	0.753	0.689	0.692	0.0361

Table No 7: Assay Formulation (n=6)

Drug	Pre-analysed Concentration µg/ml	Drug Added µg/ml	%Recovery	% RSD
ALG	12.3	5	99.87±0.66	0.66
		10	100.21±0.59	0.58
		15	101.15±0.87	0.87
MET	4.2	2	100.77±0.58	0.57
		4	100.50±1.15	0.14
		6	100.28±0.42	0.42

CONCLUSION:

Based on the results obtained, it was found that the developed and validated UV- Spectrophotometric technique is quite simple accurate, precise, reproducible, sensitive and economical for simultaneous estimation of bulk drugs and tablet dosage form. It can become effective analytical tool in routine quality control of alogliptin benzoate and metformin hydrochloride in bulk drug combination and its combined pharmaceutical dosage form without any prior separation of components.

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