Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 8, 2015

Short Communication

DETERMINATION OF ALOGLIPTIN BENZOATE AND METFORMIN HYDROCHLORIDE IN TABLET DOSAGE FORM BY SIMULTANEOUS EQUATION AND ABSORPTION RATIO METHOD

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Received: 20 May 2015 Revised and Accepted: 20 Jun 2015

ABSTRACT

Objective: Development and validation of two simple, rapid, accurate and sensitive UV-spectrophotometric methods for simultaneous estimation of alogliptin benzoate (ALO) and metformin hydrochloride (MET) in bulk and tablet dosage form.

Methods: First method (Method A) is simultaneous equation method, which is based on the measurement of absorption at 224 nm and 237 nm for both ALO and MET, respectively. Second method (Method B) is an absorption ratio method, which is based on the measurement of absorption at 251 nm i.e. Iso-absorptive point of ALO and MET and 224 nm which is λ_{max} of ALO.

Results: Both the drugs were found to be linear in the concentration range of 0.5-18 µg/ml and correlation co-efficient was found to be 0.9998 and 0.9992 for ALO and 0.9998 and 1 for MET at 224 nm and 237 nm, respectively for simultaneous equation method. For absorption ratio method, both the drugs were found to be linear in the concentration range of 0.5-18 µg/ml and correlation co-efficient was found to be 0.9998 and 0.9997 for ALO and 0.9998 and 0.9997 for MET at 224 nm and 251 nm, respectively. Recovery studies at 50, 100 and 150% levels were carried out to assess accuracy of the methods. Precision studies were also carried out and %RSD was found to be within the limit (% RSD<2). The percentage assay (Method A) was found to be 100.57±1.1367 and 101.24±1.0936 for ALO and MET, respectively. For Method B, percentage assay was found to be 101.46±0.7160 for ALO and 100.15±0.6953 for MET.

Conclusion: The developed methods were found to be simple, rapid, accurate and sensitive. Therefore, both the methods can be successfully applied for simultaneous determination of ALO and MET in tablet formulation.

Keywords: Alogliptin benzoate, Metformin hydrochloride, Simultaneous equation method, Absorption ratio method.

Alogliptin benzoate (ALO) which is chemically 2-({6-[(3*R*)-3-aminopiperidin-1-yl]-3-methyl-2, 4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl}methyl) benzonitrile is a dipeptidyl peptidase inhibitor, fig. 1. ALO slows the inactivation of incretin hormones, thereby increasing bloodstream concentrations and reducing fasting and postprandial glucose concentrations in a glucose-dependant manner in patients with type 2 diabetes mellitus. The inhibition of DPP-4 increases the amount of active plasma incretins which helps with glycemic control [1, 2].

Metformin hydrochloride (MET) is 1,1dimethyl biguanidine monohydrochloride is a biguanide antidiabetic (fig. 1). It is given orally in the treatment of type II diabetes mellitus and is the drug of first choice in overweight patients. They do not stimulate insulin release but require that some insulin be present in order to exert their antidiabetic effect. Possible mechanism of action includes the delay in the absorption of glucose from the GIT and increase in insulin sensitivity and glucose uptake in to cells and inhibition of hepatic gluconeogenesis [3-5].

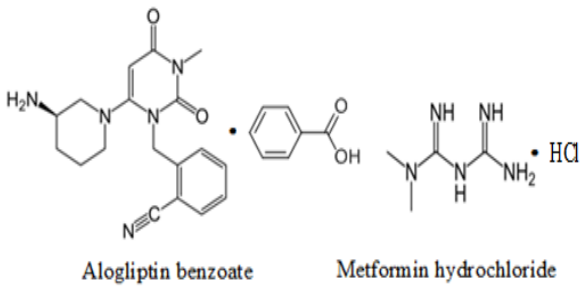

Fig. 1: Chemical structures of ALO (alogliptin benzoate) and MET (metformin hydrochloride)

Literature survey revealed various analytical methods for the determination of ALO along with MET and other drugs in their pharmaceutical formulation using HPLC [6-10], spectrophotometry [11-13] and other techniques. It was observed that most of the reported methods did not describe about procurement of tablet formulation and remaining methods did not use the tablet formulation for assay. All these observations were taken in to consideration and it was thought to prepare the tablet formulation in laboratory using all the excipients as per the marketed formulation. Therefore, the aim of present work is to develop and validate simple, rapid, accurate and sensitive simultaneous equation and absorption ratio method for the determination of ALO and MET in tablet dosage form. The advantages of these proposed methods are as follows: both the methods describe the assay procedure developed using laboratory made tablet formulation, describes standard and sample preparation procedure based on the form of analytes under investigation, i.e. alogliptin benzoate (13.60 mg of alogliptin benzoate is equivalent to 10 mg of alogliptin).

ALO was purchased from Swapnroop Drugs and Pharmaceuticals, Aurangabad, Maharashtra, India and MET was supplied by Alpha Drugs, Vadodara, Gujarat, India. Methanol (AR Grade) was purchased from SD Fine Chemicals, Mumbai, India.

Shimadzu double beam UV-visible spectrophotometer (UV-1800, UV Probe, Shimadzu Corporation, Kyoto, Japan) with matched quartz cell of 1 cm path length was used for the analysis. All weighing was performed on high sensitive Adventurer-Pro, AVG264C electronic balance, Ohaus Corporation, Pine Brook, NJ, USA.

Stock solution of ALO and MET were prepared by weighing accurately 13.60 mg of ALO (13.60 mg of alogliptin benzoate is equivalent to 10 mg of alogliptin) and 10 mg of MET standard drug which was then transferred to a 10 ml volumetric flask separately and diluted to 10 ml with methanol to get the concentration of the drugs, 1000 µg/ml. From the stock solution 6 µg/ml solutions of ALO and MET were prepared and scanned in the range of 200 to 400 nm.

ALO and MET tablets (alogliptin benzoate 17 mg equivalent to 12.5 mg of alogliptin and metformin hydrochloride 500 mg) were prepared using all the excipients shown in table 1.

Table 1: Formula of laboratory made tablet formulation

S. No.	Ingredients used	Quantity (mg)
1	Alogliptin benzoate	17
2	Metformin hydrochloride	500
4	Microcrystalline cellulose	50
5	Povidone	20
6	Crospovidone	12
7	Magnesium stearate	6
8	Distilled water	q. s.
Total weight		605

Twenty laboratory made tablets (12.5 mg of ALO and 500 mg of MET) were accurately weighed and average weight was calculated. All the tablets were crushed to fine powder and the quantity equivalent to 5 mg of ALO and 200 mg of MET were weighed and transferred to 50 ml volumetric flask. Flasks were vortexed after adding 30 ml of methanol and shaken for 10 minutes and volume was made up to the mark with methanol and filtered through whatman filter paper. From the above mentioned solution, 1 ml solution was transferred to 10 ml volumetric flask and 3.9 mg equivalents of ALO was also added and the volume was made up to the mark with methanol to maintain the same concentration for both the drugs. Suitable aliquots were prepared to get desired concentrations (ALO 6 µg/ml and MET 6 µg/ml).

Method A (Simultaneous equation method): After scanning the drug solutions between 200 to 400 nm, two wavelengths were selected from the overlain spectra of ALO and MET for simultaneous equation method. Using the below mentioned simultaneous equation formula, concentration of drugs in sample solutions were determined.

$$Cx = \frac{A2\ ay1 - A1ay2}{ax2\ ay1 - ax1ay2}$$

$$Cx = \frac{A1\ ax2 - A2\ ax1}{ax2\ ay1 - ax1ay2}$$

Where, Cx and Cy are the concentrations of ALO and MET, ax1 and ax2 are absorptivities of ALO at 224 and 237 nm, respectively. ay1 and ay2 are absorptivities of MET at 224 nm and 237 nm, respectively. A1 and A2 are absorbances of mixture at 224 and 237 nm, respectively [13].

Method B (Absorbance ratio method): In absorbance ratio method, ratios of absorption at two selected wavelengths were taken. One is at iso-absorptive point and other at λ_{max} of one of the component. The concentration of two drugs in mixture was calculated by using the following equations:

$$Cx = \frac{Qm - Qy}{Qx - Qy} \times \frac{A1}{ax1}$$

$$Cy = \frac{Qm - Qx}{Qy - Qx} \times \frac{A1}{ay1}$$

Where, ax_1 and ax_2 are the absorptivities of ALO at 224 nm and 251 nm. ay_1 and ay_2 are absorptivities of MET at 224 and 251 nm, respectively.

A1 and A2 are the absorbances of mixture at 224 and 251 nm, respectively. Cx and Cy are the concentrations of ALO and MET, respectively in sample solution [14].

$$Qm = \frac{A2}{A1} Qx = \frac{ax2}{ax1} Qy = \frac{ay2}{ay1}$$

Both the developed methods were validated according to ICH guidelines. In order to determine the specificity of the method, a placebo solution (In-house mixture of all the tablet excipients) was prepared and analyzed to evaluate the interference among excipients and drugs spectra.

Linearity and range of the method was checked by analyzing all the standard solutions separately, containing both the drugs ALO and MET (0.5–18 µg/ml) and absorbances were measured. Calibration graphs were plotted using absorbances of standard drug solutions versus concentration. Results were subjected to regression analysis by the least squares method to calculate the values of slope, intercept and correlation coefficient.

Repeatability was checked by taking two concentrations from the linearity range and analyzing repeatedly (n=6). Intra-day and inter-day precision studies were performed by repeated measurements of absorbance of standard solutions three times on the same day and three different days.

Recovery studies were performed by standard addition method. Known amount of standard solutions were added to pre-analyzed sample solutions at 50, 100 and 150% levels.

The limit of detection and limit of quantification of ALO and MET was calculated using the following equation as per ICH guidelines.

$$LOD = 3.3 \times \frac{\sigma}{S}$$

$$LOQ = 10 \times \frac{\sigma}{S}$$

Where, σ = Standard deviation of the response, S = Slope of the calibration curve.

The robustness of an analytical procedure is refers to its ability to remain unaffected by small and deliberate variations in the method parameters. The method should be robust enough with respect to all critical parameters so as to allow routine laboratory use. Robustness of the method was checked on the basis of slight alteration in the wave length of detection (±1 nm).

Stability of the solutions were checked by observing any changes in the spectral pattern compared with freshly prepared solutions by keeping the solutions at room temperature and analyzing at frequent interval.

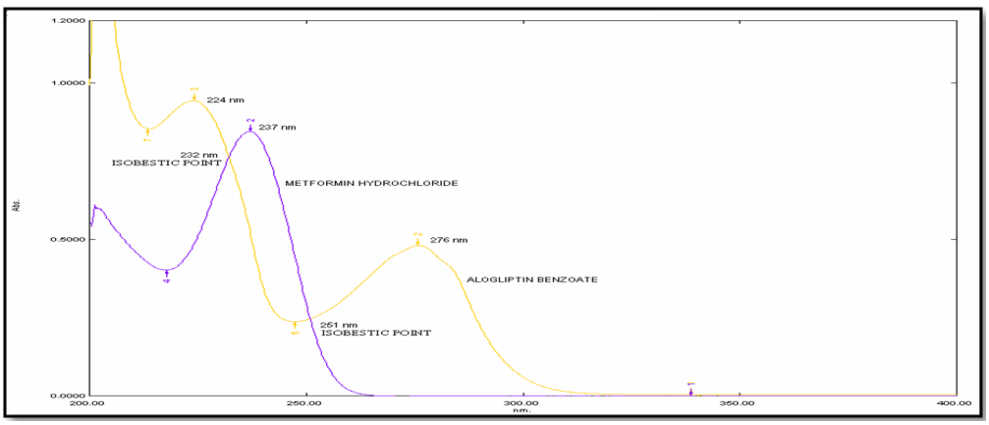

Fig. 2: Overlain UV–spectra of ALO and MET (6 µg/ml)

Sample solution was scanned between 200–400 nm and absorbances were measured at 224 and 237 nm for Method A and 224 and 251 nm for Method B. Percentage assay was calculated solving simultaneous equation for Method A and absorbance ratio for Method B.

Two simple, sensitive and precise simultaneous equation and absorbance ratio methods (Q analysis) were developed and validated for the simultaneous estimation of ALO and MET in pure and pharmaceutical dosage form. Moreover, the developed methods were applied to laboratory made tablet formulation. In simultaneous equation method 224 and 237 nm and in absorbance ratio method 224 and 251 nm was selected as the wavelength of analysis fig. 2.

The developed method was validated according to ICH guidelines. Both the drugs were found to be linear in the concentration range of 0.5-18 µg/ml. Results were subjected to regression analysis by the least squares method to calculate the values of slope, intercept and correlation coefficient. Precision studies were also carried out for both the methods and % RSD was found to be within the limits (%RSD<2), which indicates the developed methods have good repeatability and low Inter–day variability. Hence, both the methods were found to have good precision (table 2). Recovery studies at 50, 100 and 150% levels were carried out to assess accuracy of the methods (table 3). The results of recovery studies indicate that there is no interference from tablet excipients. The values of LOD and LOQ were found to be low, which proves the sensitivity of the methods.

Table 2: Summary of validation parameters for proposed methods

Parameters	Drugs	Method A		Method B	
		224 nm	237 nm	224 nm	251 nm
Beer's law limit (µg/ml)	ALO	0.5-18 µg/ml			
	MET				
Correlation coefficient	ALO	0.9998	0.9992	0.9998	0.9997
	MET	0.9998	1	0.9998	0.9997
Slope	ALO	0.0779	0.0435	0.0779	0.0204
	MET	0.0399	0.0696	0.0399	0.0204
Intercept	ALO	0.0042	0.0042	0.0042	0.0014
	MET	0.0008	0.0022	0.0008	0.0014
Intra-day precision (%RSD) (n=3)	ALO	1.2603	0.8606	1.2603	1.0874
	MET	0.9860	0.7311	0.9860	1.0874
Inter-day precision(%RSD) (n=3)	ALO	1.4978	1.2548	1.4978	1.6721
	MET	1.5776	1.0381	1.5776	1.6721
LOD (µg/ml)	ALO	0.0695	0.0933	0.0695	0.1412
	MET	0.1095	0.0885	0.1045	0.1412
LOQ (µg/ml)	ALO	0.2107	0.2826	0.2107	0.4279
	MET	0.3317	0.2682	0.3166	0.4279

n = Number of determination

Table 3: Recovery study data for ALO and MET by method A and B

Drug	Level (%)	Method A		Method B	
		% Recovery*	% RSD	% Recovery*	% RSD*
	50	99.91	1.8062	101.71	0.2033
ALO	100	100.41	0.4676	101.71	0.6291
	150	100.07	0.8278	97.77	0.5289
	50	101.13	0.5864	102.13	0.3400
MET	100	100.23	0.4503	100.85	1.1122
	150	100.54	0.6629	96.97	0.9511

*(n=6), n = Number of determination

Table 4: Analysis of formulation

Methods	Drug	Labeled amount (mg/tablet)	Amount found (%)*	RSD (%)*
Method A	ALO	12.5	100.57±1.1367	1.1303
	MET	500	101.24±1.0936	1.0802
Method B	ALO	12.5	101.46±0.7160	0.7057
	MET	500	100.15±0.6953	0.6942

*mean±SD (n=6), n = Number of determination

Proposed methods were successfully used for the quantitative determination of ALO and MET in tablet formulation. Six replicate determinations were carried out and experimental values are presented in table 4. The results of percentage assay were found to be between 98 and 102. Therefore, the proposed methods can be successfully applied for the quantitative analysis of ALO and MET in tablet formulation.

The developed UV-spectrophotometric methods were found to be simple, accurate, precise and economical. These methods are also found to be more sensitive than the existing methods. Therefore, the developed methods can be applied in routine quality control of ALO and MET in their tablet dosage form.

CONFLICT OF INTERESTS

Declared None

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