IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2016 Vol.:6, Issue:1

Spectrophotometric Estimation of Glibenclamide and Alogliptin in Synthetic Mixture by Area under Curve Method

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Rana Anjali V*, Patel Paresh U.

Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Ganpat Vidyanagar – 384012, Mehsana, Gujarat, India.

Submission: 4 April 2016
Accepted: 9 April 2016
Published: 25 April 2016

Keywords: Alogliptin, Glibenclamide, Area under Curve method, Validation

ABSTRACT

A simple, accurate, rapid and precise spectrophotometric method has been developed for simultaneous estimation of Glibenclamide and Alogliptin in bulk and synthetic mixture. This method was based on UV spectrophotometric determination of two drugs, using Area under Curve method. It involves measurement of area under curve in the range of 224-234nm (For Glibenclamide) and 270-280nm (For Alogliptin) for the analysis in methanol. The linearity was observed in the concentration range of 2-16µg/ml for Glibenclamide and 1-16µg/ml for Alogliptin. The method showed good reproducibility and recovery with % RSD less than 2. Method was found to be rapid, specific, precise and accurate, can be successfully applied for the routine analysis of Glibenclamide and Alogliptin in bulk, and combined dosage form without any interference by the excipients. The method was validated according to ICH guidelines.

INTRODUCTION

Glibenclamide (GLI) is chemically N-p-[2-(5-Chloro-2-methoxybenzamido)ethyl]benzenesulfonyl-N'-cyclohexylurea.[1] The drug works by binding to and activating the ATP-sensitive potassium channels (K_{ATP}) inhibitory regulatory subunit sulfonylurea receptor 1 (SUR1) in pancreatic beta cells, used in the treatment of Diabetes mellitus. It is official in Indian Pharmacopoeia (IP) [2], British Pharmacopoeia (BP) [3]. Various methods like UV Spectrophotometry [4], RP-HPLC [5], and HPTLC [6] method for estimation of GLI in tablet, bulk, and spectrophotometric method for simultaneous determination of GLI with other drug [7] and RP-HPLC method for simultaneous determination of GLI with other drug [8] are reported in literature for estimation of GLI in pharmaceutical dosage forms, pure, as well as in biological fluid. Alogliptin (ALO) chemically 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydropyrimidin-1-yl} methyl) benzonitrile [9] is a dipeptidyl peptidase 4 inhibitor (DPP-4 Inhibitor), used in the treatment of Antidiabetic. ALO is not official in any pharmacopoeia. Various methods like LC [10], spectrophotometric[11] and HPLC[12] method for simultaneous estimation of ALO with other drug, RP-HPLC method for simultaneous estimation of ALO with other drug [13] and stability-indicating RP-HPLC method [14]for the determination of ALO are reported in literature for estimation of ALO in pharmaceutical dosage forms as well as in biological fluids. Literature survey does not reveal any simple spectroscopic method for determination of GLI and ALO in synthetic mixture. The present manuscript describes simple, sensitive, accurate, precise, rapid and economic spectrophotometric method based on simultaneous equations for simultaneous estimation of ALO and GLI in tablet dosage form.

MATERIALS AND METHODS

A Shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2nm, wavelength accuracy of 0.5nm and a pair of 10mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

ALO and GLI bulk powder were kindly gifted by Cadila Pharma, Ahmedabad, India. Methanol AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India. Whatman filter paper no. 41 (Millipore, USA) was also used in the study.

Preparation of standard stock solutions

An accurately weighed quantity of ALO (10mg) and GLI (10mg) were transferred to a separate 100ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of ALO (100µg/ml) and GLI (100µg/ml). For GLI preparation amber colored volumetric flask was used.

Methodology

The working standard solutions of GLI and ALO were prepared separately in methanol having concentration of 10µg/ml. They were scanned in the wavelength range of 200-400nm against methanol as blank. Area was measured at 224-234nm and 270-280nm for GLI and ALO, respectively. These two wavelengths ranges can be employed for the determination of GLI and ALO without any interference from other components in their synthetic formulations.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.[18]

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-16µg/ml for GLI and 1-14µg/ml for ALO. Accurately measured standard solutions of GLI (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6ml) and ALO (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4ml) were transferred to a series of 10ml of volumetric flasks and diluted to the mark with methanol. The area under curve of the solutions was measured at 224-234 and 270-280nm against methanol as blank. The calibration curves were constructed by plotting area under curve versus concentrations and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for GLI and ALO (10µg/ml for GLI and 8µg/ml for ALO) without changing the parameter of the proposed spectrophotometry method.

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of standard solutions of GLI and ALO (8, 10, 12µg/ml for both GLI and ALO).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of GLI and ALO by the standard addition method. Known amounts of standard solutions of GLI and ALO were added at 80, 100 and 120% level to prequantified sample solutions of GLI and ALO 1µg/ml and 5µg/ml respectively. The amounts of GLI and ALO were estimated by applying obtained values to the respective regression line equations.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of TOL and DIC from synthetic mixture

Glibenclamide (5mg) and Alogliptin (25mg) standard drug powder were accurately weighed and then mixed with commonly used formulation excipients like starch, lactose, magnesium stearate and talc. The synthetic mixture was then transferred to 100ml volumetric flask containing 50ml

methanol and sonicated for 20min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. The above solution was suitably diluted with methanol to get a final concentration of 10µg/ml of GLI and 10µg/ml of ALO. The area under curve of the solution i.e. A_1 and A_2 were recorded at 224-234nm and 270-280nm and ratios of area under curve were calculated, i.e. A_2/A_1. Relative concentration of two drugs in the sample solution was calculated using respective simultaneous equations generated by using absorptivity coefficients and absorbance values of GLI and ALO at these wavelengths.

$$Cx = (A2\ aY1 – A1\ aY2) / (aY1\ aX2 - aY2\ aX1)$$

$$Cy = (A1\ aX2 - A2\ aX1) / (aY1\ aX2 - aY2\ aX1)$$

Where,

Cx = Concentrations of TGLI,

Cy = Concentrations of ALO,

A_1= Area at 224-234nm,

A_2= Area at 270-280nm,

aX_1 and $aY_1$are AUC constants of GLI and ALO respectively at 224-234nm,

aX_2 and $aY_2$are AUC constants of GLI and ALO respectively at 270-280nm.

AUC constant = Area/ concentration in gm/l.

RESULTS AND DISCUSSION

The present work provides an accurate, reproducible, sensitive method for the simultaneous analysis of GLI & ALO in bulk and synthetic mixture. Linear relationships between drug concentrations were obtained over the range of 2-16µg/ml & 1-14µg/ml for GLI and ALO respectively. Under experimental conditions described assay, linearity, accuracy studies and precision, LOD and LOQ were estimated. Correlation coefficient was found to be > 0.995. The results are presented in Table 1. The % assay was found to be 102.5% for GLI and 99.1% for ALO, and S.D. and R.S.D. for six determinations of sample, by this method, was found to be less

than 2.0 indicating the precision of this method. No interference was observed from the pharmaceutical adjuvants /excipients.

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of GLI and ALO in synthetic mixture. The method utilizes easily available and cheap solvent for analysis of GLI and ALO. Hence, the method was economical for estimation of GLI and ALO from synthetic mixture.

ACKNOWLEDGEMENT

The authors are thankful to Cadila Zydus Ltd., Ahmedabad, Gujarat, India for providing gift sample of GLI and ALO for research. The authors are highly thankful to Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Ganpat Vidyanagar, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

REFERENCES

1) O'Neil MJ. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 14th ed. New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station: Glibenclamide; 2006; 4478.

2) British Pharmacopoeia, The Department of Health, Social Services and Public Safety, London Her Majesty's. Stationary office, 6th ed., Volume 1, 2010; 976,977.

3) Indian Pharmacopoeia, Government of India, Ministry of health and family welfare Ghaziabad, Indian Pharmacopoeia Commission, 7th ed.,Volume 2,2010; 1414-1415.

4) Dhole Seema M., Khedekar Pramod B., Amnerkar Nikhil D., UV Spectrophotometric Absorption Correction Method for the Simultaneous Estimation of Glibenclamide in tablet dosage form, Int. J of Ana. And Bio. Chem., 2013; 3(1): 18-22.

5) Haq Nazrul, Alanazi Fars Kaed., Abdullah Ibrahim, Shakeel Faiyaz., Rapid Analysis of Glibenclamide Using an Environmentally Benign Stability-Indicating RP-HPLC Method, Iranian journal of pharm. Res.,2014;13(3):863-872.

6) Havele shweta s.,Dhaneshwar Sunil R., Determination of Glibenclamide in tablets by Donsitometic HPTLC, Scholars Research Library,2010;2(4):440-446.

7) Patil Sudarshan, S. Bonde, C. G.,Development and Validation of analytical method for Simultaneous Estimation of Glibenclamide and Metformin HCl in Bulk and Tablets using UV - visible spectroscopy, 2009; 1(4): 905.

8) EdlaSubashini and SyamaSundhar B., New Analytical Method Development and Validation for the Simultaneous Estimation Of Metformin And Glibenclamide in bulk and tablet dosage form using RP-HPLC , Rasayan journal chem.,2014;7(1):55-63

9) http://www.drugbank.ca/drugs/DB06203.

10) Ramzia I., Bagary El, Elkady F. Ehab, Ayoub M. Bassam., Liquid Chromatographic determination of Alogliptin in Bulk and in its Pharmaceutical Preparation, Int. jof Bio. Sci., 2012; 8(3): 215-218.

11) Raval Kashyap, Srinivasa U., First Order Derivative and dual wavelength Spectrophotometry methods development and validation for simultaneous estimation of Alogliptin and Pioglitazone in bulk and dosage form, Int. J of Pharmacy and Pharm. Sci., 2014; 6(2): 730-738.
12) Thangabalan B., Parvathareddy Sri Sowmya,Babu S.Manohar, Method Development And Validation For Metformin Hydrochloride And Alogliptin In Bulk And Pharmaceutical Formulation By RP-HPLC Method, Int. J of Innovative Pharm. Sci. and Res., 2014; 2(7): 1451-1464.
13) A. Praveen Kumar, G. Aruna, Rajasekar K., ReddyP. Jayachandra, Analytical method development and validation of Alogliptin and Metformin hydrochloride tablet dosage form by RP-HPLC method, International Bulletin of Drug Research, 2013; 3(5): 58-68.
14) G. Satya Sri, S. Ashutosh Kumar, J. Saravanan, ManidipaDebnath, V. Greeshma et.al, A new stability indicating RP-HPLC method Development For Simultaneous Estimation Of Metformin And Alogliptin in bulk as well as in pharmaceutical formulation by using PDA detector, Indo American Journal of Pharmaceutical Research, 2013; 3(11).
15) ICH, Q2 (R1) Validation of Analytical Procedure: Text and Methodology, International Conference on Harmonization, 2005.
16) Beckett A.H., Stenlake J.B. Practice Pharmaceutical Chemistry, 4th ed. Part II, CBS Publisher, New Delhi: 1997. p. 285.

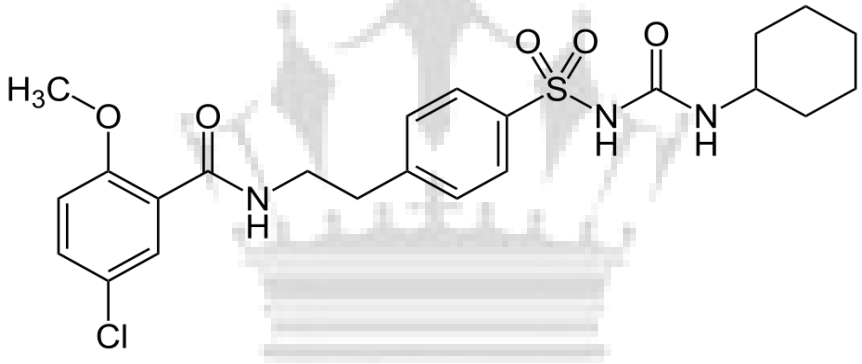

Figure 1: Chemical structure of Glibenclamide (GLI)

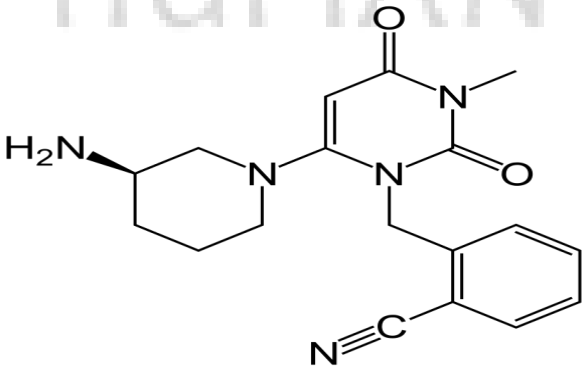

Figure 2: Chemical structure of Alogliptin (ALO)

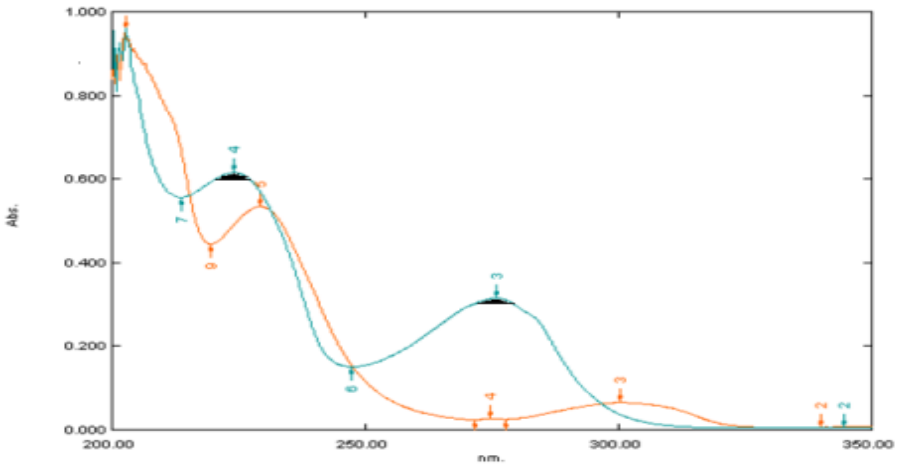

Fig 3: Overlain AUC spectra of GLI (10µg/ml) and ALO (10µg/ml) in methnol

TABLE 1: RECOVERY DATA FOR PROPOSED METHOD

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
GLI	I	1	80	99.9 ± 1.25
	II	1	100	101.3 ± 1.15
	III	1	120	100.5 ± 0.92
ALO	I	5	80	101.9 ± 0.40
	II	5	100	101.2 ± 0.46
	III	5	120	101.8 ± 0.28

TABLE 2: ANALYSIS OF GLI AND ALO BY PROPOSED METHOD

Synthetic mixture	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 5)	
	GLI	ALO	GLI	ALO	GLI	ALO
I	5	25	5.025	25.025	100.1 ± 0.97	100.0 ± 0.57

TABLE 3: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED METHOD

PARAMETERS	GLI		ALO	
Wavelength range (nm)	224-234	270-280	224-234	270-280
Beer's law limit (µg/ml)	2-16	1-14	2-16	1-14
Regression equation (y = mx± c) Slope (b) Intercept (a)	y = 0.0321x-0.0029 0.0321 0.0029	y = 0.0260x-0.0041 0.0260 0.0041	y = 0.0005x+0.0006 0.0005 0.0006	y = 0.0126x+0.0009 0.0126 0.0009
Correlation Coefficient (r^2)	0.9992	0.995	0.9976	0.9994
Method precision (Repeatability) (% RSD, n = 6)	1.90	1.68	0.66	0.48
Intraday (n = 3) (% RSD)	0.54-0.55	0.03-1.27	0.56-0.88	0.31-1.06
Interday (n = 3) (%RSD)	2.91-0.15	0.03-1.25	0.56-0.53	0.31-1.04
LOD (µg/ml)	0.10	0.13	0.16	0.24
LOQ (µg/ml)	0.32	0.42	0.50	0.74
Accuracy (Mean % Recovery ± S.D) (n = 3)	100 ± 0.97		101.09± 0.49	
% Assay ± S.D. (n = 5)	100.1±0.97		100.0± 0.57	

LOD = Limit of Detection. LOQ = Limit of Quantification. S.D = Standard Deviation. RSD = Relative Standard Deviation.