International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 1, 2016

Original Article

STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ALOGLIPTIN BENZOATE AND METFORMIN HYDROCHLORIDE IN TABLET DOSAGE FORM

CHINNALALAIAH RUNJA*[1], P. RAVIKUMAR[2], SRINIVASA RAO AVANAPU[2]

***[1]Department of Pharmaceutical Chemistry, Joginpally B. R. Pharmacy College, Moinabad, Hyderabad 500075, Telangana, India, [2]Aizant Drug Research Solutions, Hyderabad 500014 Telangana, India, [2]Department of Pharmacology, Bhaskar Pharmacy College, Moinabad, Hyderabad 500075, Telangana, India**

Email: lalubpharm@gmail.com

Received: 31 Aug 2015 Revised and Accepted: 18 Nov 2015

ABSTRACT

Objective: A new simple, accurate, precise, economic and robust stability indicating RP-HPLC method has been developed and subsequently validated for the estimation of alogliptin and metformin in bulk and pharmaceutical dosage form.

Methods: The HPLC separation was carried out by using hypersil BDS, C18 (250 x 4.6 mm, 5µ.) column with a mobile phase comprising phosphate buffer and acetonitrile (48:52 % v/v) pH adjusted to 4.8 with orthophosphoric acid. The flow rate of the mobile phase was 1.0 ml/min and effluent was monitored at 210 nm using PDA detector. The retention time of alogliptin and metformin was 3.78 min and 2.78 min respectively. Forced degradation studies were conducted to know the stability of the drug samples under various stress conditions like acid, base, peroxide, and photolytic degradation according to ICH guidelines.

Results: The results of this study showed excellent separation of the drug samples using developed method. The percentage recoveries were found 99.91 to 101.01% for alogliptin and 99.78-100.87% for metformin which is in the limits of acceptance. The calibration curve was plotted and the method was found to be linear over a range of 3-18 µg/ml and 125–750µg/ml of alogliptin and metformin respectively and regression data for calibration curve showed good linear relationship with r^2= 0.9990 for the both alogliptin and metformin. In the study stability section, it was observed that there is no interference of the degradation products with drug samples.

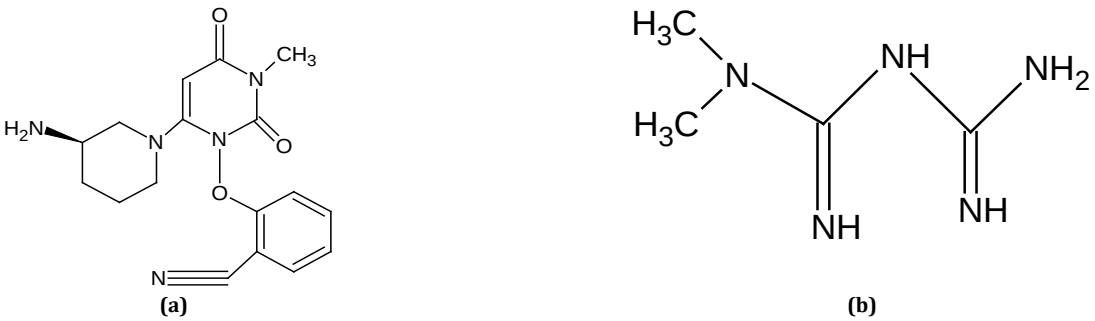
Conclusion: A new stability-indicating RP-HPLC method has been developed for estimation of alogliptin and metformin in bulk and pharmaceutical dosage form. The developed method was validated, and it was found to be simple, sensitive, precise, robust and it can be used for the routine analysis of alogliptin and metformin in both bulk and pharmaceutical dosage forms.

Keywords: Stability, Alogliptin benzoate, Validation, RP-HPLC.

INTRODUCTION

Alogliptin (ALO) is a novel hypoglycemic drug that belongs to dipeptidyl-peptidase-4 inhibitor class which stimulates glucose-dependent insulin release [1-2]. Chemically, alogliptin is prepared as a benzoate salt, which is identified as 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl} methyl) benzonitrile mono benzoate [fig. 1A]. It inhibits dipeptidyl peptidase 4 (DPP-4), which normally degrades the incretins glucose-dependent insulin tropic polypeptide (GIP) and glucagon-like peptide 1 [3-6]. Metformin is not chemically or pharmacologically related to any other classes of oral antihyperglycemic agents. Chemically, metformin hydrochloride [7-8] is N, N-dimethyl imido-carbonimidic diamide hydrochloride [fig. 1B]. It has been used as the first-line therapy in the treatment of type 2 diabetes mellitus patients. Mechanism of action of metformin [9-11] is differing from other classes of oral hypoglycemic agents; it decreases blood glucose levels by decreasing hepatic glucose production and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. Several analytical methods [12-17] were developed for alogliptin and metformin individually and also determining metformin simultaneously with other drug substances in combined dosage forms. From the thorough literature, it was thought of developing a new stability indicating assay method for alogliptin benzoate and metformin hydrochloride in combined dosage form.

After comparing several chromatographic techniques, a reverse phase HPLC was selected for developing stability indicating method based on the physical properties of both drugs. Therefore, the present investigating was aimed to develop a new RP-HPLC method and subsequently stability studies [18-19] were conducted to know the degradation products of alogliptin and metformin.



(a) (b)

Fig. 1: Chemical structure of Alogliptin (a) and Metformin (b)

MATERIALS AND METHODS

Reagents and instruments

Alogliptin and metformin were obtained as a gift sample from spectrum Pharma research laboratory in the hyderabad and marketed formulation (KAZANO, 12.5 mg alogliptine, and 500 mg metformin) were obtained local market. Acetonitrile, water were obtained from Merck. Mumbai and potassium dihydrogen orthophosphate, orthophosphoric acid obtained from RANKEM Mumbai. All solvents used in this work are HPLC grade. RP-HPLC waters 2695 separation module equipped with 2996 Photodiode Array Detector were employed in this method**.** Empower 2 software was used for LC peak integration along with data acquisition and data processing. The column used for the separation of analytes is Hypersil BDS C18 (250 x 4.6 mm, 5µ). A UV Crosslinker with a series of 234100 model UV chamber equipped with UV fluorescence lamp was used for the photolytic stability studies.

Preparation of alogliptin (125 µg/ml) and metformin (5000µg/ml) standard solution

Accurately weighed and transferred 6.25 mg of alogliptin and 50 mg of metformin working standards into a separate 50 ml & 10 ml clean dry volumetric flasks, add 7 ml of diluent to each flask then sonicated for 5 min and make up to the final volume with diluent. From the above stock solutions, 1 ml of alogliptin and metformin was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluent.

Preparation of sample solution in tablet dosage form (Kazano tablets)

20 tablets each containing alogliptin 12.5 mg and metformin 500 mg were powdered and calculated the average weight of each tablet. The tablet powder equivalent to 20 tablets was weighed and transferred into 50 ml volumetric flask. Add 30 ml of the diluent to dissolve the powder and sonicate it for about 25 min and further the volume was made up with diluent. From the prepared solution, 1 ml was pipetted out and transferred into 10 ml of volumetric flask and make up the volume with diluent. From the prepared solutions, 10 µl of the sample solution was injected into HPLC system and peak area response was compared with standard values and the % assay was calculated. The % assay was found to be 99.88%for alogliptin and 99.64% for metformin.

Stress degradation studies

Forced degradation studies were performed to know the degradation products and to establish degradation pathway for alogliptin and metformin. The study involves acid hydrolysis (1 ml HCl heated for 30 min at 60 °) alkali hydrolysis (2N NaOH heated for 30 min at 60 °C), oxidative degradation (20% H_2O_2 heated at 60 °C for 30 min) and thermal degradation (samples placed in oven at 105 °C for 6 h). For photolytic stress studies, samples were exposed to UV light by keeping in UV chamber for 7days.

RESULTS AND DISCUSSION

Optimized chromatographic conditions

The main objective of the present work is to develop and validate a stability indicating assay method for simultaneous estimation of alogliptin benzoate and metformin hydrochloride by reverse phase high-performance liquid chromatography. During the mobile phase selection, it was found that the acidic nature of the buffer could help in separating four drugs with good resolution. Then it was thought of changing pH of the buffer at different ranges like 2.5, 3.5, 4.5 & 6 to separate the samples. The best results were achieved with Hypersil BDS C18, (250 x 4.6 mm, 5µ). C18 asymmetry column with a mobile phase consisting of Phosphate Buffer: Acetonitrile in the ration of 48:52 %v/v at a flow rate of 1 ml/min. Samples were analyzed at 210 nm at an injection volume of 10 µL. The proposed method was optimized to give a sharp peak with minimum tailing (fig: 2).

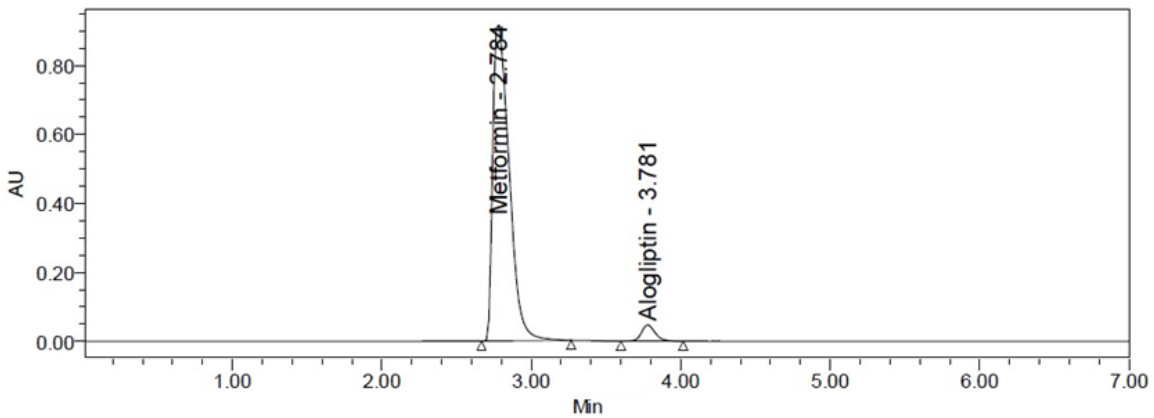


Fig. 2: A typical standard chromatogram of alogliptin benzoate and metformin hydrochloride

Table 1: Percentage recovery studies of alogliptin and metformin

Spiked level	% Recovery		Mean % recovery		% RSD	
	ALO	MET	ALO	MET	ALO	MET
50%	100.01	100.34				
	99.79	100.37	99.90	100.16	0.016	0.326
	99.91	99.78				
100%	100.42	100.84				
	101.11	100.87	100.51	100.65	0.557	0.346
	100.00	100.25				
150%	100.68	100.54				
	100.24	100.18	100.41	100.31	0.235	0.194
	100.31	100.23				

ALO-Alogliptin MET-Metformin % RSD-Percentage relative standard deviation

Validation

Accuracy

The accuracy of the proposed method was established by determining the percentage recoveries of both the drug substances. Accuracy was performed for three concentrations 50%, 100% and 150% and percentage recovery was found to be 99.79-101.11 % for alogliptin and 99.78-100.87 % for metformin (table 1).

Precision

For evaluation of precision, intraday and interday precision was performed at 100% concentration of alogliptin and metformin by injecting each three replicates. Mean average, standard deviation, percentage relative standard deviation were calculated and found within the acceptance limits (table 2).

Linearity

In linearity studies were performed in the range of 25-150% by analyzing six concentrations (n=6) of each three replicates and a calibration curve was plotted between concentration vs area of the six concentrations.

The linearity range was determined as 3.12-18.75 µg/ml of alogliptin and 125-750 µg/ml of metformin and the regression value was calculated as 0.9990. Limit of detection (LOD) and Limit of quantification (LOQ) have been established by evaluating the minimum level at which the analyte could be readily detected and quantified accurately. The LOD & LOQ values was found to be 0.0172µg/ml and 0.0521µg/ml for alogliptin and 1.218µg/ml and 3.693µg/ml for metformin. Linearity data were given in table 3.

Robustness

Robustness of the proposed method was performed by changing conditions of the parameters such as temperature (±5 °C), mobile phase (±5%) and flow rate (±0.2 ml). In the robustness, no drastic change was observed in system suitability parameters when the small deliberate changes in above-mentioned parameters. Robustness results of the method were placed in table 4.

Forced degradation studies

Forced degradation studies were performed to demonstrate the stability of the sample. Degradation studies were carried out under conditions of hydrolysis, dry heat, oxidation, UV light, and photolysis. Acid hydrolysis was performed by treating the drug with 2N HCl at room temperature for 24 h and it was showed little degradation of alogliptin and metformin with degraded products peak at retention time 2.362. For alkali degradation, the drug was treated with 2N NaOH. A chromatogram of base hydrolysis showed degradation of the sample with degraded product peak at retention time 2.295 and 3.383. Degradation studies under oxidative conditions were performed by heating the drug sample with 30% H_2O_2at 60 °C and degraded product peaks were observed.

For the thermal degradation, the powdered drug was exposed to heat at 105 °C for 6 h. UV degradation studies were carried out for drug solutions by exposing solution in UV chamber at 100 watts for 7 d. In all the conditions, the purity of angle was found less than that of purity of threshold which indicates that the All the degradation samples were analyzed using PDA detector to monitor the purity of the peaks and the purity of the angle was found to be less than purity of threshold. Forced degradation studies were given in table 5.

Table 2: Percentage assay of intraday and interday precision data

Sample No	% Assay of intraday precision		% Assay of interday precision	
	ALO	MET	ALO	MET
1	100.64	99.90	99.46	99.29
2	100.65	99.55	101.93	99.55
3	99.49	99.41	99.74	99.75
4	99.81	99.97	99.79	99.56
5	100.75	99.78	99.51	99.42
6	99.50	99.22	99.78	98.53

ALO-Alogliptin MET-Metformin

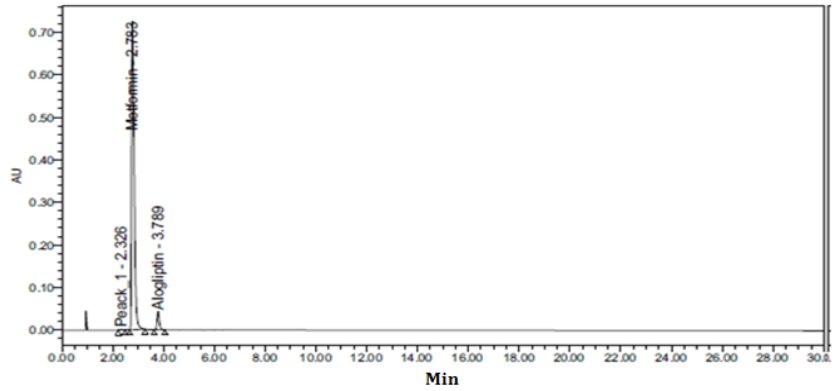
Table 3: Linearity data of alogliptin and metformin

Drug name	Concentration (µg/ml)	Area	Drug name	Concentration (µg/ml)	Area
Alogliptin	3.12	70934	**Metformin**	125	1595665
	6.25	142300		250	3005621
	9.37	212895		375	4488605
	12.5	283908		500	6109256
	15.62	350176		625	7537189
	18.75	424958		750	9039883
	Correlation coefficient (r^2)	0.9990		Correlation coefficient (r^2)	0.9990
	Regression Equation	Y=22571x+563.7		Regression Equation	Y=12030x+28067

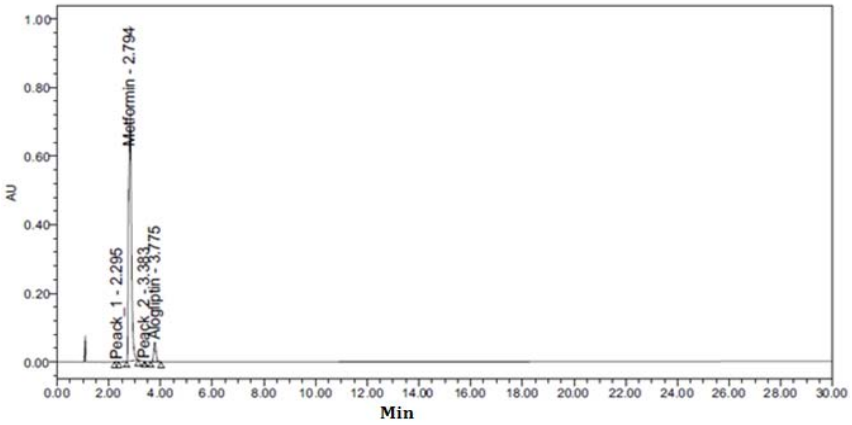
Table 4: Robustness data of alogliptin and metformin

Parameter	Alogliptin			Metformin		
	RT(min)	USPC	TF	RT(min)	USPC	TF
Flow rate-0.8 ml	4.20	9100	1.20	3.09	3046	1.80
Flow rate-1.2 ml	3.43	8709	1.20	2.54	3021	1.80
Temperature-25 °C	3.78	9031	1.17	2.79	3799	1.64
Temparature-35 °C	3.77	8791	1.21	2.78	3132	1.74
Mobile Phase (-5%)	3.74	8882	1.20	2.80	3088	1.78
Mobile Phase (+5%)	3.83	8897	1.18	2.79	3142	1.72

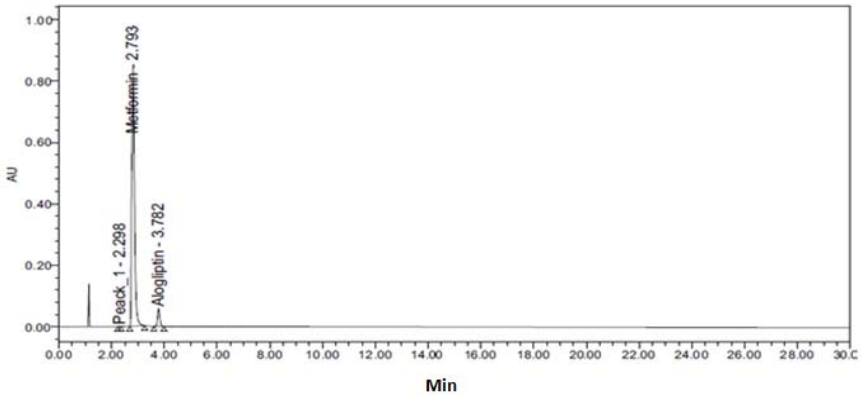
RT-Retention time USPC-US Plate count TF-Tailing Factor


Peack_1 - 2.326
Metformin - 2.783
Alogliptin - 3.789
AU
Min

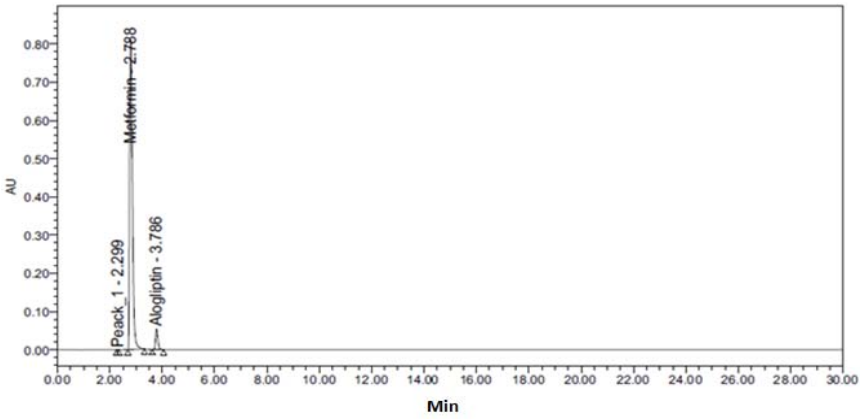
A)


Peack_1 - 2.295
Metformin - 2.794
Peack_2 - 3.383
Alogliptin - 3.775
AU
Min

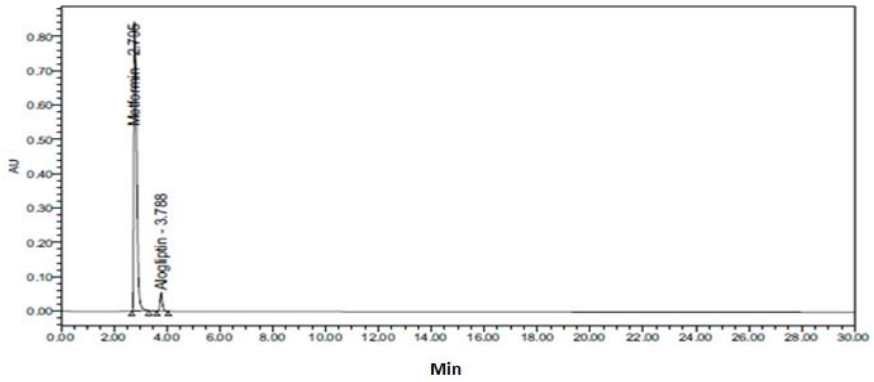
B)


Peack_1 - 2.298
Metformin - 2.793
Alogliptin - 3.782
AU
Min

C)


Peack_1 - 2.299
Metformin - 2.788
Alogliptin - 3.786
AU
Min

D)



E)

Fig. 3: Representative chromatograms of Acid (A), Base (B), Peroxide (C), Thermal (D), Photolytic (E) degradation of alogliptin and metformin

Table 5: Forced degradation data

Degradation	Stress conditions	% Degradation	
		Alogliptin	Metformin
Acid hydrolysis	2N HCl heated at 60 °C for 30 min	7.92	7.73
Alkali hydrolysis	2N NaOH heated at 60 °C for 30 min	6.24	6.47
Oxidation	20% H2O2 Heated 60 °C for 30 min	5.74	5.73
Photolytic	UV chamber for 7 d	1.42	1.07
Thermal	Oven at 105 °C for 6 h	4.52	4.47

CONCLUSION

The proposed study, a new stability-indicating RP-HPLC method has been developed for estimation of alogliptin benzoate and metformin hydrochloride in bulk and pharmaceutical dosage form. The developed method was validated and it was found to be simple, sensitive, precise, robust and it can be used for the routine analysis of alogliptin benzoate and metformin hydrochloride in both bulk and pharmaceutical dosage forms. The forced degradation studies were carried out in accordance with ICH guidelines, and the results revealed suitability of the method to study the stability of alogliptin benzoate and metformin hydrochloride under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations. Finally, it was concluded that the method is simple, sensitive and has the ability to separate the drug from degradation products and excipients found in the dosage form.

ACKNOWLEDGEMENT

The authors wish to thank spectrum pharma research laboratory for providing gift samples.

CONFLICT OF INTERESTS

Authors have no conflict of interest.

REFERENCES

1. Uche AN, Okoli O, Erowele G. Alogliptin: a new dipeptidyl peptidase-4 inhibitor for the management of type 2 diabetes mellitus. Am J Health-Syst Pharm 2014;71:103-9.
2. Radha A, Andjela D, Marc R. Algliptin: a new addition to the class of DPP-4 inhibitors. J Diabetes, Metab Syndrome Obesity 2009;2:117-26.
3. Baetta R, Corsisni A. Pharmacology of dipetidy peptidase-4 inhibitors, similarities, and differences. Drugs 2011;71:1441-67.
4. Deacon CF. Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. Diabetes Obesity Metab 2011;13:7-18.
5. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology 2007;132:2131-57.
6. Gautier JF, Fetita S, Sobngwi E, Salaun-Martin C. Biological actions of incretins GIP and GLP-1 and therapeutic perceptive in patients with type 2 diabetes. Diabetes Metab 2005;31:233-42.
7. Indian Pharmacopoeia. The government of India, Ministry of health and Family Welfare. The Indian Pharmacopoeia Commission, Ghaziabad 2005;2-3:1657, 2186.
8. Martindale. The Complete Drug Reference: Pharmaceutical Press; 2011. p. 411.
9. Marc F, Bruno G, Bertrand L, Michael P, Benoit V. Metformin: from the mechanism of action to therapies. Cell Metab 2014;20:953-66.
10. Graham R, Ewan RP, Kei S. Molecular mechanism of action of metformin: old or new insights. Diabetologia 2013;56:1898-906.
11. Lilian BR, Marilia B. Metformin: an old but still the best treatment for type 2 diabetes. Diabetol Metab Syndr 2013;5:1-15.
12. Sadhana BT, Mohite SK, Snehal M, Sucheta R. Development and validation of UV spectrophotometric methods for simultaneous estimation of voglibose and metformin hydrochloride in bulk and tablet dosage form. Indo Am J Pharm Res 2013;3:7018-24.
13. Murthy TK, Geethanjali J. Development of a validated RP-HPLC method for simultaneous estimation of metformin hydrochloride and rosuvastatin calcium in bulk and in-house formulation. J Chromatogr Sep-Tech 2014;5:1-7.
14. G Srinivasa R, Mallesh K, Vijay Kumar G, Surekha CH, Venugopala Rao B. A validated chiral HPLC method for the enantiomeric purity of alogliptin benzoate. Der Pharm Chem 2014;6:234-9.
15. Chirag, Amrita P. Development and validation of UV spectrophotometric method for simultaneous estimation of metformin hydrochloride and alogliptin benzoate in bulk drugs and combined dosage forms. Der Pharm Chem 2014;6:303-11.
16. Ramzia I, Ehab FE, Bassam MA. Liquid chromatographic determination of alogliptin in bulk and in its pharmaceutical. Int J Biomed Sci 2012;8:215–8.
17. Praveen kumar A, Aruna G. Analytical method development, and validation of alogliptin and metformin hydrochloride tablet dosage form by RP-HPLC method. Int Bull Drug Res 2013;3:58-68.
18. ICH, Q2A Text on Validation of Analytical Procedures, International Conference On Harmonization, Geneva; 1994. p. 1-5.
19. ICH, Stability Testing of New Drug Substances and Products (Q1AR2), International Conference on Harmonization, IFPMA, Geneva; 2003.