ORIGINAL ARTICLE

Simultaneous Assessment of Alogliptin Benzoate and Metformin Hydrochloride in Tablet Dosage Form by Reversed-phase High-performance Liquid Chromatography

Dhanya B. Sen* Ashim K. Sen, Aarti Zanwar, Kinjal P. Patel, Ramachandran Balaraman, Rajesh A. Maheshwari, Chintan Aundhia

Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Vadodara-391760, Gujarat, India

Abstract

Aim: A simple, accurate, precise, and sensitive reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous assessment of alogliptin benzoate (ALO) and metformin hydrochloride (MET) in combined tablet formulation. **Materials and Methods:** The proposed RP-HPLC method employed a reverse phase column (C_{18}) and mobile phase with a composition of 0.2% (v/v) triethylamine (pH 5.5 with orthophosphoric acid) and methanol (2:98, v/v) was utilized and a flow rate of 1 mL min^{-1} was employed. **Results and Discussion:** Quantification of effluents was monitored at 236 nm. Three symmetrical well resolved peaks of alogliptin, metformin, and benzoic acid (benzoic acid was separated from ALO) were obtained with retention time of 4.730 ± 0.0267, 6.109 ± 0.0301, and 2.281 ± 0.0324 min, respectively. Both, alogliptin and metformin, showed excellent linearity over the concentration range of 0.5–50 µg mL^{-1}. The developed method was, then, validated in accordance with ICH guidelines and applied in the determination of ALO and MET in the combined tablet dosage form. The formulation analysis revealed good agreement (98–100% w/w) with the label claim for both the analytes by RP-HPLC method. **Conclusion:** Developed method was simple, sensitive, and accurate which thus can be utilized for simultaneous determination of both the drugs in combined tablet dosage form.

Key words: Simultaneous assessment, Alogliptin benzoate, Metformin hydrochloride, Tablet dosage form, Reversed-phase high-performance liquid chromatography

INTRODUCTION

Alogliptin benzoate (ALO) is an oral antihyperglycemic drug which is a dipeptidyl peptidase inhibitor, as shown in Figure 1. ALO chemically is 2-({6-[(3*R*)-3-aminopiperidin-1-yl]-3-methyl-2, 4-dioxo-3, 4-dihydropyrimidin-1(2*H*)-yl} methyl) benzonitrile. It reduces fasting and postprandial glucose concentration in patients with type 2 diabetes mellitus by inactivating incretin hormones. ALO exert the main mechanism of action is due to the inhibition of DPP-4, thereby increasing the amount of active plasma concentration of incretins. ALO appears as crystalline powder which is white to off-white in color and it is soluble in dimethyl sulfoxide, sparingly soluble in methanol and water, marginally soluble in ethanol and very slightly soluble in isopropyl acetate and octanol.[1-3] Metformin hydrochloride (MET) is an oral antidiabetic drug, as shown in Figure 1. Chemically, MET is *N*, *N*-Dimethylimidodicarbonimidic diamide hydrochloride, a biguanide antidiabetic. It is an oral antihyperglycemic agent prescribed in the treatment of type II diabetes mellitus and considers the drug of first choice in overweight patients. It's mechanism of action to increase the insulin sensitivity to exert its antidiabetic effect and also possible mechanism of action includes the delay in the absorption of glucose from the GIT and glucose uptake into the cells and inhibition of hepatic gluconeogenesis. MET appears as white to off-white

Address for correspondence:
Dr. Dhanya B. Sen, Department of Pharmacy,
Sumandeep Vidyapeeth Deemed to be University,
Vadodara - 391 760, Gujarat, India.
Phone: +91-8140810531. E-mail: dhanyab1983@gmail.com

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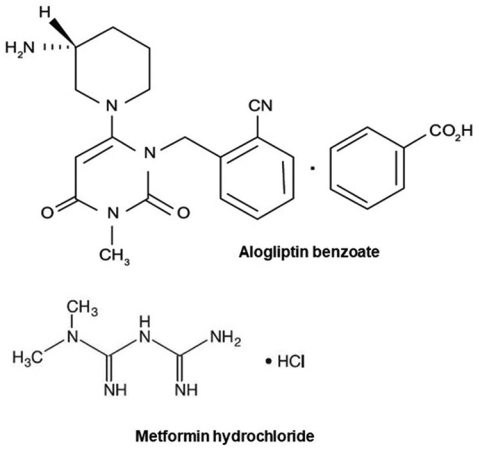

Figure 1: Chemical structures of alogliptin benzoate and metformin hydrochloride

crystalline powder, freely soluble in methanol, water, and practically insoluble in chloroform, ether and acetone.[4-8]

There are several reported methods for the quantification of ALO and MET individually and in combined tablet dosage form by UV spectrophotometry,[3,9-11] HPLC,[12-18] HPTLC,[19] and LC-MS.[20] Most of the reported method suffers from lower sensitivity, tedious sample and mobile phase preparation techniques or longer run time. Hence, it was thought to develop a new, simple, accurate, and sensitive alternative methods for simultaneous assessment of both the drugs in combined tablet formulation. Moreover, proposed method is the only method which describes the estimation of alogliptin rather than ALO unlike other methods. None of the methods have separated BA from ALO. Projected work explains a more sensitive and accurate HPLC method than the reported methods for the quantification of ALO and MET in tablet formulation.

MATERIALS AND METHODS

Materials and pharmaceutical formulations

Reference standard of ALO was bought from Swapnroop Drugs and Pharmaceuticals, Aurangabad, Maharashtra, India and MET was kindly supplied by IPCA laboratories, Mumbai, Maharashtra, India. In-house laboratory manufactured tablet formulation containing 12.5 mg of ALO and 500 mg of MET was utilized for the research work.

Chemicals and reagents

Merck Specialties Pvt. Ltd. and LobaChemie Pvt. Ltd., Mumbai, India were chosen as supplier for HPLC grade solvents such as methanol, orthophosphoric acid (OPA), and triethylamine (TEA). For all the analyses, HPLC grade water (Millipore Direct Q3, Millipore India, Bangalore, India) was used. Tablet excipients such as microcrystalline cellulose, magnesium stearate, povidone, and crospovidone used in the research work were of AR grade for preparing placebo solution which was used for specificity study.

Instruments used and chromatographic conditions

All weighing was achieved on highly sensitive Adventurer-Pro, AVG264C electronic balance, Ohaus Corporation, Pine Brook, NJ, USA. Proposed method utilized UFLC Prominence (Shimadzu, Kyoto, Japan) outfitted with LC-20AD binary pump, SPD-M20A PDA detector, and LC solution as software for the investigation. An Enable C_{18} column (250 × 4.6 mm i.d., 5 µm particle size, 120 Å) was employed for separation with 20 µL injector volume. A guard column (C_{18}, 120 Å) also used to protect the analytical column which was utilized for the separation. The chromatography was performed using a mobile phase comprising 0.2% TEA in water (pH 5.5 adjusted with 5 %v/v OPA) and methanol (2:98 %v/v) with 1 mL min^{-1} as flow rate (F/R). The effluent was monitored at 236 nm using PDA detector and peak area was recorded.

Preparation of Solutions

Reference stock solutions of ALO and MET

Utilizing highly sensitive and precise electronic balance quantity equivalent to13.60 mg of ALO (13.60 mg of ALO is comparable to 10 mg of alogliptin) and 10 mg of MET were shifted into a standard flask (10 mL) separately and volume was completed up to the mark using mobile phase to attain the concentration 1000 µg mL^{-1}.

Mixed working standard solutions of ALO and MET

Standard solutions were mixed together by transferring 1 mL of each stock solution into a 10 mL standard flask and level of the solution was completed up to the mark using mobile phase to get mixed solution containing ALO and MET (100 µg mL^{-1}).

Sample solution preparation

Laboratory prepared 20 tablets (12.5 mg ALO and 500 mg of MET) were correctly weighed and transformed into powder. Equivalent quantity of the powder (5 mg of ALO and 200 mg of MET) was moved to a standard flask of 50 mL volume. Subsequently, 30 mL of methanol was added in the flask, vortexed, and later sonicated for 10 min. Volume was completed adding methanol and filtration was carried out using 0.45 µm membrane filter. From this 1 mL solution was transferred to a 10 mL volumetric flask and 3.9 mg equivalent of pure ALO was also added and volume was made up to the mark with methanol to maintain the same concentration for both the drugs (400 µg mL^{-1} ALO and MET). From this 2.5 mL was pipetted out into a 10 mL volumetric flask and

volume was made up with methanol to get 100 µg mL^{-1} of ALO and MET. Suitable aliquots were prepared to get desired concentrations (10 µg mL^{-1} of ALO and MET).

Validation of the method

Projected method was validated in accordance with the official protocol described in "International Conference on Harmonization guidelines" for validation of analytical procedures.[21-23]

Specificity

Method specificity was studied to check how accurately and specifically the drugs under study are assessed in presence of expected components present in a formulation. Placebo, mixed working standard, and sample solutions were injected simultaneously and compared the peak position with that of standard drug to find out any interference exist between excipients and drug peaks in the proposed reversed-phase high-performance liquid chromatography (RP-HPLC) method.

Linearity and range

Previously prepared mixed working reference solutions comprising of ALO and MET (0.5–50 µg mL^{-1}) were made and analyzed 6 times repeatedly. Peak areas of each concentration were recorded to judge the linearity of the projected RP-HPLC method. A 0.45 µm membrane filter was employed for the filtration of the mobile phase and degassing was done in an ultra-sonic bath to get rid of any particulate materials and dissolved gases. The column was equilibrated for 15 min with the optimized mobile phase before injecting the mixed reference solutions. Calibration graphs were built by means of peak areas and corresponding concentrations.

Precision

Appropriately diluted mixed reference solution (ALO and MET: 5 and 10 µg mL^{-1}) was investigated 6 times and %RSD was evaluated to assess the repeatability of the method. Whereas, in case of intraday precision, analysis was completed in triplicate at two different concentration levels (ALO and MET: 5 and 10 µg mL^{-1}) with in a day at a predetermined time interval within the linearity range and % RSD was calculated. Interday precision was evaluated by correlating the outcome of 3 consecutive days, where triplicate injections at two different concentration levels (ALO and MET: 5 and 10 µg mL^{-1}) were studied within the linearity range and % RSD was calculated.

Accuracy

Analyte recovery was estimated to assess the accuracy of the projected method by adding standard analyte to pre-analyzed sample solution (Formulation, ALO and MET: 5, 10 and 15 µg mL^{-1}) at different % levels (50, 100 and 150%). The resulting combined solutions were reanalyzed for calculation of % recovery. Method accuracy was judged on the basis of standard ALO and MET recovered in terms of percentage from the pre-analyzed sample solution by applying following formula:

% Recovery=(Amount of drug found after addition of standard drug-Amount of drug found before addition of standard drug)/(Amount of standard drug added)×100

Limit of detection (LOD) and limit of quantification (LOQ)

With the help of calibration curve, LOD and LOQ of ALO and MET were evaluated by utilizing the formula as mentioned in ICH guidelines.

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

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

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

Robustness

It is the ability of an analytical method to stay unaffected after slight and intentional modification in method parameter. The method should be able to restrict its influential parameters within the tolerable limit to achieve its goal to be used in quality control laboratory. Method robustness was assessed after slight alteration in parameters such as buffer strength (±0.1%), buffer pH (±0.2 units), and flow rate (±0.1 mL min^{-1}).

Solution stability

Variations in response against newly prepared solutions, were noted after keeping at room temperature and analyzed at a predetermined time interval.

Formulation analysis

Sample formulations were extracted and diluted with the help of mobile phase as per the described procedure. Diluted sample solutions were analyzed and responses were documented. Recorded peak area of analytes was utilized to calculate the percentage purity of the formulation.

System suitability assessment

System suitability assessment was done to assure that the system was in suitable condition for the assessment to be completed. Standard solution was injected (6 times) and

chromatographic response was recorded to evaluate the appropriateness of the chromatograph using parameters such as peak area reproducibility, no of theoretical plates, tailing factor, retention time, and resolution.

RESULTS AND DISCUSSION

Chromatography is one of the most extensively used separation techniques applied for quantitative estimation and quality control of drugs. The challenge of proposed method was effective separation of ALO, MET, and BA using RP-HPLC. Initially, various solvents such as methanol, acetonitrile, and buffers were tried as mobile phase for separation of ALO, MET, and BA. However, desired separation could not be achieved. Different chromatographic conditions such as detection wave length, strength of buffer, flow rate, mobile phase composition, and pH were varied to get optimum chromatographic conditions. Enable C_{18} column with a mobile phase 0.2% TEA (pH 5.5 was adjusted with OPA) and methanol (2:98 %v/v) at 1 mL min^{-1} flow rate was employed for the separation of components and PDA detection at 236 nm was employed. Finally, under optimized conditions, the retention time of ALO, MET, and BA was found to be 4.730 ± 0.0267, 6.109 ± 0.0301, and 2.281 ± 0.0324 min, respectively [Figure 2].

Projected method was validated in accordance with ICH guidelines. The projected method was found to be specific as no interfering peaks were found within the specified run time. Standard graphs were established using chromatographic response (peak areas) of standard drugs against concentration for establishing linearity and range of the method. ALO and MET were found to be linear in the specified range 0.50–50 µg mL^{-1}, [Figures 3 and 4].

Outcome of linearity was utilized in regression analysis by means of least squares technique for evaluating correlation coefficient, slope, and intercept [Table 1]. The method preciseness was assessed through parameters such as repeatability, intraday, and interday precision. Results of precision studies expressed in %RSD follows ICH guideline acceptable limits, which indicates good repeatability and low interday variability [Table 1]. The results of recovery studies ranged from 98 to 102% for both the drugs showing the accuracy of the method, which reveals that there is no interference from tablet excipients. LOD and LOQ of the projected method were found to be 0.1253 and 0.3797 µg $mL^{-1}$for ALO, 0.1092 and 0.3308 µg mL^{-1} for MET, respectively [Table 1].

The proposed scheme was checked through all the parameters described earlier under robustness studies. However, there were no considerable variations in the chromatographic pattern after introducing small changes in experimental condition, which indicates that the developed method is robust [Table 2].

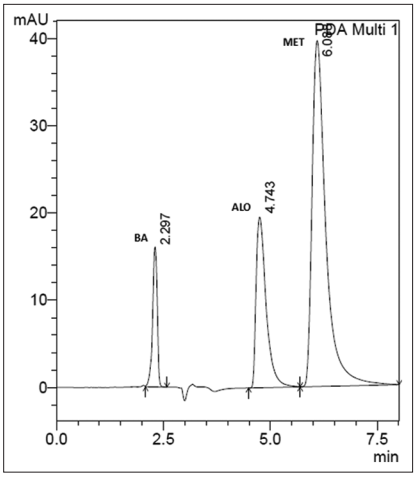

Figure 2: Standard reversed-phase high-performance liquid chromatography chromatogram of alogliptin benzoate and metformin hydrochloride (10 µg mL^{-1})

Table 1: Summarized data of validation parameters for the reversed-phase high-performance liquid chromatography method

Parameters	ALO	MET
Linearity range (µg mL^{-1})	0.50–50	
Correlation coefficient	0.9999	0.9997
Regression equation	y=32708x–892.76	y=91926x–3100.4
LOD[a] (µg mL^{-1})	0.1253	0.1092
LOQ[b] (µg mL^{-1})	0.3797	0.3308
Precision (%RSD[c])		
Repeatability (*n*=6)	0.6789	0.4458
Intraday (*n*=3)	0.8977	1.3546
Interday (*n*=3)	1.3558	1.5195
Accuracy[d]		
% Recovery (*n*=3)	100.1598±1.6324	99.1032±1.1182
% RSD (*n*=3)	1.6298	1.1283
Specificity	No interference	

[a]LOD: Limit of detection, [b]LOQ: Limit of quantitation, [c]% RSD (Relative standard deviation) = SD/mean × 100; *n*: no. of determinations. [d]Mean±SD

Solution stability was performed at room temperature and it was learnt to be unchanged up to 2 days. The projected method was successfully used for the quantitative assessment of ALO and MET in tablet formulation (12.5 mg ALO and 500 mg of MET). System suitability tests were performed and results revealed that the parameters tested were within the tolerable limit as per the ICH guidelines indicating the proposed assessment method is suitable for the analysis intended [Table 3].

Table 2: Results of robustness study for reversed-phase high-performance liquid chromatography method

Parameters modified	ALO (20 µg mL^{-1})				MET (20 µg mL^{-1})			
	R_t±SD[a]	% RSD[b]	Peak area±SD[a]	% RSD[b]	R_t±SD[a]	% RSD[b]	Peak area±SD[a]	% RSD[b]
Strength of buffer (0.2±0.1%v/v)	4.758±0.0688	1.4453	654111±3484.44	0.5327	6.211±0.0791	1.2728	1808929±3974.47	0.2197
Effect of pH (5.5±0.2 unit)	4.780±0.0767	1.6056	651940±7462.73	1.1447	6.184±0.0562	0.9091	1809670±5004.16	0.2765
Effect of flow rate (1±0.1 mL min^{-1})	4.778±0.0753	1.5820	655763±3650.92	0.5567	6.2050±0.0618	0.9965	1818106±10459.21	0.5753

[a]Mean±SD, (n=3) number of determinations, SD: Standard deviation. [b]% RSD (Relative standard deviation)=SD/mean×100

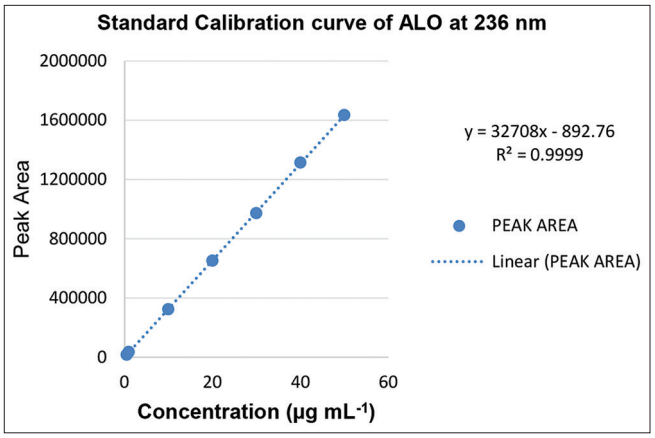

Figure 3: Standard calibration curve of alogliptin benzoate (0.5–50 µg mL^{-1}) at 236 nm

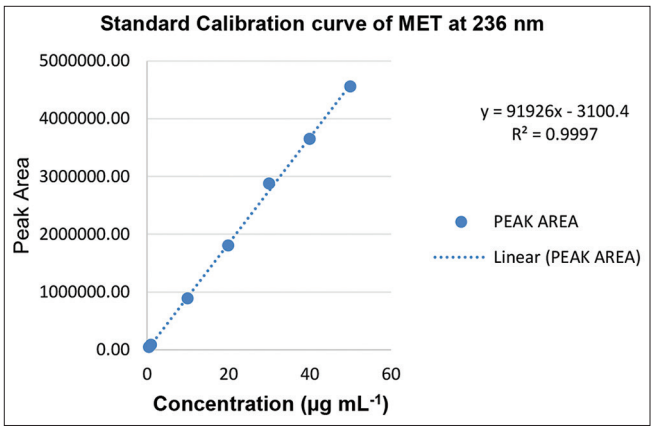

Figure 4: Standard calibration curve of metformin hydrochloride (0.5–50 µg mL^{-1}) at 236 nm

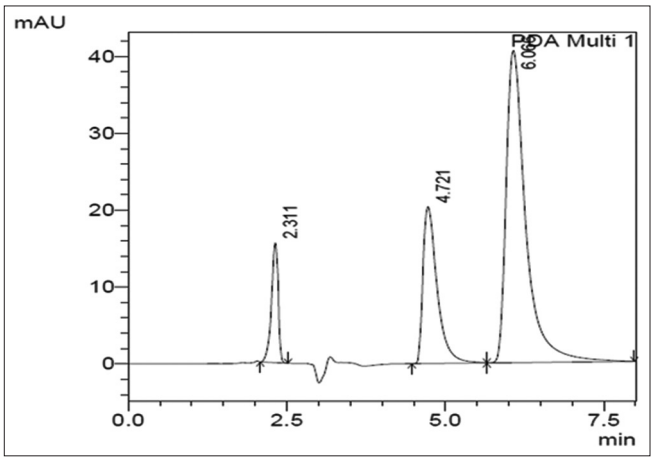

Figure 5: Chromatogram of formulation containing alogliptin benzoate and metformin hydrochloride (10 µg mL^{-1})

Six replicate determinations were carried out to assess the formulation [Figure 5] and average experimental values were found to be 99.0123 and 99.1963 %w/w for ALO and MET, respectively [Table 4]. Hence, the developed method can be utilized for the simultaneous assessment of ALO and MET in tablet formulation.

Table 3: System suitability studies for reversed-phase high-performance liquid chromatography method

Parameters	Drugs[a]		%RSD[b]	
	ALO	MET	ALO	MET
Peak area reproducibility (5 µg mL^{-1})	164662±641.49	446581±1468.36	0.3896	0.3288
Retention time (R_t) min	4.730±0.0267	6.109±0.0301	0.5654	0.4931
Resolution (R_s)	7.63±0.1025	3.01±0.0549	1.3433	1.8253
Theoretical plate (N)	2656±39.2042	2258.43±33.0252	1.4761	1.4623
Tailing factor (T)	1.87±0.0311	1.75±0.0308	1.6586	1.7500

[a]Mean±SD, (n=6) number of determinations; SD: Standard deviation. [b]% RSD (relative standard deviation)=SD/mean×100

Table 4: Assay of formulation by proposed reversed-phase high-performance liquid chromatography method

Drugs	Labeled Amount (mg/tab)	Amount found (mg/tab)	Amount found (%)[a]	RSD (%)[b]
ALO	12.5	12.38	99.0123±0.9374	0.9468
MET	500	495.98	99.1963±0.7314	0.7373

[a]Mean±SD, (n=6) number of determinations; SD: Standard deviation. [b]% RSD (relative standard deviation)=SD/mean×100

CONCLUSION

The projected RP-HPLC method for simultaneous assessment of ALO and MET is simple, accurate, precise, and sensitive. The developed method was validated in accordance with ICH guidelines and applied for the determination of ALO and MET in combined tablet formulation. The formulation analysis revealed good agreement (98–100% w/w) with the label claim for both the analytes. Moreover, wider linearity range, lower LOD and LOQ values, lower standard deviation, acceptable % RSD, and shorter run time make the method versatile and valuable. Hence, the method can be utilized for simultaneous determination of both the drugs in combined tablet formulation.

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REFERENCES

1. Christopher R, Karim A. Clinical pharmacology of alogliptin, a dipeptidyl peptidase-4 inhibitor, for the treatment of type 2 diabetes. Expert Rev Clin Pharmacol 2009;2:589-600.
2. White JR. Alogliptin for the treatment of type 2 diabetes. Drugs Today 2011;47:99-107.
3. Sen DB, Sen AK, Zanwar A, Balaraman R, Seth A. Determination of alogliptin benzoate and metformin hydrochloride in tablet dosage form by simultaneous equation and absorption ratio method. Int J Pharm Pharm Sci 2015;7:380-3.
4. The Merck Index, An Encyclopedia of Chemicals, Drug, and Biological. 13th ed. White House Station, NJ: Merck & Co., Inc.; 2001 pp. 1061.
5. USP NF. The Official Compendia of Standards. Vol. 3. Rockville, MD: The United States Pharmacopoeial Convention; 2008. p. 2641.
6. British Pharmacopoeia. Vol. 1. London, UK, Stationary Office, MHRA; 2008. p. 2410-1.
7. Indian Pharmacopoeia. Government of India, Ministry of Health and Family Welfare, Vol. 2. Ghaziabad: Indian Pharmacopoeia Commission; 2007. p. 1358-60.
8. Martindale: The Complete Drug Reference. 36th ed., Vol. 1. London, UK: Pharmaceutical Press (An Imprint of RPS Publishing); 2009. p. 438, 453, 457, 462.
9. Parle CA. Development and validation of UV spectrophotometric method for simultaneous estimation of metformin hydrochloride and alogliptin benzoate in bulk drugs and combined dosage forms. Pharma Chem 2014;6:303-11.
10. Patel BP, Mashru RC. Sensitive and selective approaches for real time estimation of alogliptin benzoate and metformin hydrochloride in synthetic mixture. Int Bull Drug Res 2014;4:148-59.
11. Pavan KH, Manzoor A, Satishkumar SA, Aradhya VK. UV-visible spectrophotometric method for simultaneous estimation of alogliptin benzoate and metformin hydrochloride in combined tablet dosage form. Int J Univers Pharm Bio Sci 2014;3:336-49.
12. Kumar AP, Aruna G, Rajasekar K, Reddy PJ. 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.
13. Sri SG, Kumar AS, Saravanan J. A new RP-HPLC method development for simultaneous estimation of metformin and alogliptin in bulk as well as in pharmaceutical formulation by using PDA detector. World J Pharm Pharm Sci 2013;2:6720-24.
14. Thangabalan B, Parvathareddy SS, Manohar BS.

Method development and validation for metformin hydrochloride and alogliptin in bulk and pharmaceutical formulation by RP-HPLC method. Int J Innov Pharm Sci Res 2014;2:1451-64.
15. Sri GS, Kumar SA, Saravanan J, Debnath M, Greeshma V, Krishna NS. 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 Am J Pharm Res 2013;3:9222-41.
16. Runja C, Ravikumar P, Avanapu SR. Stability indicating RP-HPLC method for simultaneous estimation of alogliptin benzoate and metformin hydrochloride in tablet dosage form. Int JPharm Pharm Sci 2016;8:116-20.
17. Ashutosh KS, Manidipa D, Seshagiri RJ, Gowri SD. New validated stability indicating RP-HPLC method for simultaneous estimation of metformin and alogliptin in human plasma. J Chromatogr Sep Tech 2015;6:1-6.
18. Mahrouse MA, Lamie NT. Experimental design methodology for optimization and robustness determination in ion pair RP-HPLC method development: Application for the simultaneous determination of metformin hydrochloride, alogliptin benzoate and repaglinide in tablets. Microchem J 2019;147:691-706.
19. Sharma K, Parle A, Ahmad S. Development and validation of HPTLC method for simultaneous estimation of metformin hydrochloride and alogliptin benzoate in bulk drugs and combined dosage forms. Pharm Lett 2015;7:321-8.
20. Mowaka S, Elkady EF, Elmazar MM, Ayoub BM. Enhanced LC-MS/MS determination of alogliptin and metformin in plasma: Application to a pharmacokinetic study. Microchem J 2017;130:360-5.
21. International Conference on Harmonization. Validation of Analytical Procedures: Text and Methodology Q2(R1). Geneva: International Conference on Harmonization; 2005.
22. Sen AK, Sen DB, Maheshwari RA, Balaraman R, Seth A. Quantitative simultaneous determination of aliskiren hemifumarate and hydrochlorothiazide in combined tablet formulation by RP-HPLC. Int J Pharm Pharm Sci 2015;7:142-5.
23. Sen AK, Hinsu D, Sen D, Zanwar A, Maheshwari R, Chandrakar V. Analytical method development and validation for simultaneous estimation of Teneligliptin hydrobromide hydrate and Metformin hydrochloride from it's pharmaceutical dosage form by three different UV spectrophotometric methods. J Appl Pharm Sci 2016;6:157-65.

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