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Development and Validation of HPTLC Method For Simultaneous Determination of Alogliptin and Metformin In Fixed Dose Combination Tablets

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ABSTRACT

A simple, rapid, accurate and precise high performance thin-layer chromatography (HPTLC) method was developed and validated for simultaneous estimation of alogliptin and metformin active pharmaceutical ingredients and fixed dose combination. Alogliptin and metformin densitograms were developed on silica gel 60 F_{254} HPTLC plates with chloroform: methanol: 0.5 % ammonium sulphate (4:4:2 %v/v) as mobile phase. Densitometric quantification was performed at 254 nm. For Alogliptin and Metformin R_f values were found as 0.66 and 0.44, respectively. The linearity curves of Alogliptin and Metformin were obtained in the concentration range of 100-500 ng/spot and 4000-20000 ng/spot by area with correlation co-efficient of 0.998 and 0.995 for Alogliptin and Metformin, respectively. Limit of detection was found to be 2 ng and 40 ng/spot for Alogliptin and Metformin, respectively; lowest possible quantity to be quantified by the proposed method was found to be 6 ng and 130 ng per spot for Alogliptin and Metformin, respectively. The method was validated for precision, accuracy, specificity and robustness. The developed method was validated and found to be selective, specific and suitable for application in pharmaceutical analysis of these drugs in bulk and fixed dose combination.

Keywords: Alogliptin, HPTLC, Metformin, Methanol.

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INTRODUCTION

Metformin (MET) is chemically, N, N- dimethyl imidocarbonimidic diamide monohydrochloride (Figure.1 A), is an anti-hyperglycemic agent that improves glucose tolerance in patient with type-II diabetes, lowering the both basal and postprandial plasma glucose. Metformin hydrochloride decrease hepatic glucose production, decrease intestinal absorption of glucose and improve insulin sensitivity by increasing peripheral glucose uptake and utilization (1).

Alogliptin (ALG) is a new anti-diabetic drug. It is chemically 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2, 4-dioxo-1, 2, 3,4-tetrahydropyrimidin-1-yl} methyl) benzonitrile benzoate (Figure.1 B). Alogliptin belongs to the class of Dipeptidyl peptidase -4 (DPP-4) inhibitor, a new class of anti-diabetic drugs which act by increasing glucose dependent insulin release. Therapeutically DPP-4 inhibitors are used to treat type 2 diabetes alone or combination with other drugs which increase the sensitivity of insulin at target site. DPP-4 inhibitors act by inhibiting the inactivation of enteroendocrine incretins such as glucagon – like peptide-1 (GLP-1) and glucose -dependent insulinotropic (GIP) polypeptide. The increased availability of incretins due to DPP-4 inhibitor results in glucose dependent insulin release and better glycemic control (2).Alogliptin benzoate is a new DPP-4 inhibitor quite effective alone or in combination with other anti-diabetic drugs. Takeda pharmaceuticals (Japan) received FDA approval for three new type 2 diabetes therapy in 2013 i.e. Nesina® (Alogliptin), Oseni® (Alogliptin and Pioglitazone) and Kazano® (Alogliptin and Metformin hydrochloride). Alogliptin is also approved for marketing in Europe as alone or combination with other anti-diabetics drugs. Alogliptin is a new drug and not official in any pharmacopeia. The literature survey reveals that there are analytical methods available for determination of Alogliptin and Metformin from biological matrices, bulk drug and dosage forms and for determination of Alogliptin and Metformin with combination of other drugs by RP-HPLC, LC-MS, HPTLC and UPLC(3-18). Literature surveys reveal only one HPTLC method Komal Sharma et al. [19] for simultaneous estimation of Alogliptin benzoate and Metformin hydrochloride in bulk drug only not in the fixed dose combination. To best of our knowledge none of the HPTLC methods are developed for the quantification of Metformin and Alogliptin in fixed dose combination. The present study was aimed to develop a simple, rapid and accurate HPTLC method for the simultaneous estimation of Alogliptin and Metformin in bulk and fixed dose combination for the routine analysis.

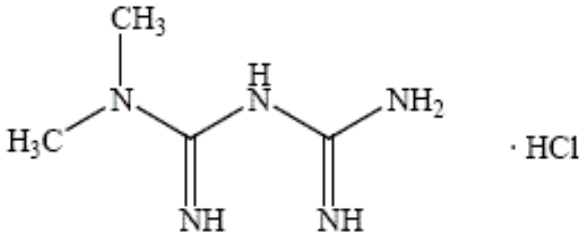

Figure1: (A) Structure of Metformin hydrochloride

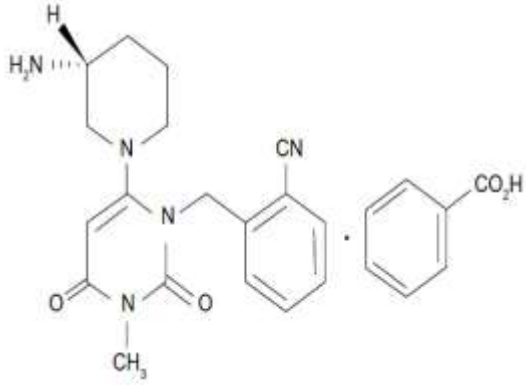

Figure 1: (B) Structure of Alogliptin benzoate

MATERIALS AND METHOD

Chemicals and reagents

Alogliptin and Metformin working standards were gift samples from Vivan life science Pvt. Ltd. (Mumbai). Commercial tablets (Kazano®, Takeda Ltd.) containing metformin 500 mg and alogliptin 12.5 mg were purchased from pharmasave pharmacy, Canada. Chloroform, methanol and 0.5 % ammonium sulphate of analytical grade were purchased from sigma-Aldrich Inc. Precoated silica gel 60 F_{254} HPTLC plates (Merck #5548) were purchased from E-Merck. Standard volumetric flasks were used for the preparation of all the dilutions. Millipore water and what man filter paper grade I were use in the whole experimental work.

Instrumentation and conditions

HPTLC plates pre-coated with silica gel GF aluminum TLC plate, (10cm-10 cm) were from Merck. Densitometry was carried out with a CAMAG TLC Scanner 3, fitted with winCATS 1.4.3 planar chromatography manager software. Samples were applied to the HPTLC plates using the spray-on technique of CAMAG LINOMAT V under nitrogen gas flow, and developed in a CAMAG 10cm-10 cm twin trough chambers.

Preparation of standard solution

Accurately weighed 10 mg quantity of Alogliptin and 400 mg of Metformin were taken and transferred into a 10 ml clean volumetric flask. The drugs were dissolved in methanol and made up

to the volume methanol to obtain 1000 µg/ml of Alogliptin and 40000 µg/ml of Metformin (stock solutions). Aliquots of standard solutions were applied in the concentration range of 100-500 ng/spot of Alogliptin and 4000-20000 ng/spot of Metformin by applying 0.1 -0.5 µl prepared standard solutions, which were used for calibration purpose.

Preparation of sample solution

For analysis of tablet dosage form, twenty tablets, each containing 12.5 mg of Alogliptin and 500mg Metformin, were weighed and their average weight was calculated. The tablets were finely powdered and powder equivalent to 10 mg Alogliptin and 400mg Metformin were accurately weighed and dissolved in 10 mL of methanol. The solution was sonicated for 30 min, filtered through the Whattman No. 41 filter paper and the residue was washed with methanol. This solution was further diluted with methanol to get the same concentration as that of the final standard solution.

Chromatographic conditions

Alogliptin and Metformin reference standard solutions were prepared using methanol as solvent. Solutions of 0.1-0.5 µL were applied to the HPTLC plates as spot bands of 6 mm using LINOMAT V. Application positions were at least 15 mm from the sides and 10 mm from the bottom of the plates. Mobile phase components were mixed prior to use and the development chamber was left for saturation with mobile phase vapor for 20 mins before each run. Development of the plate was carried out by the ascending technique to a migration distance of 7 cm. Then the plates were dried on a hot plate. All the analyses were carried out in a laboratory with temperature control (20–24ºC). Densitometry scanning was done in absorbance mode at 254 nm using a deuterium lamp. The slit dimensions were set at 6 mm-0.30 mm, the scanning speed of 10 mm/s, and the data resolution at100 µm/step. Single wavelength detection was performed since the main components were only analyzed. The UV spectrum and 3D Chromatogram of Alogliptin and Metformin are shown in Figure.2, Figure.3 and Figure.4, respectively.

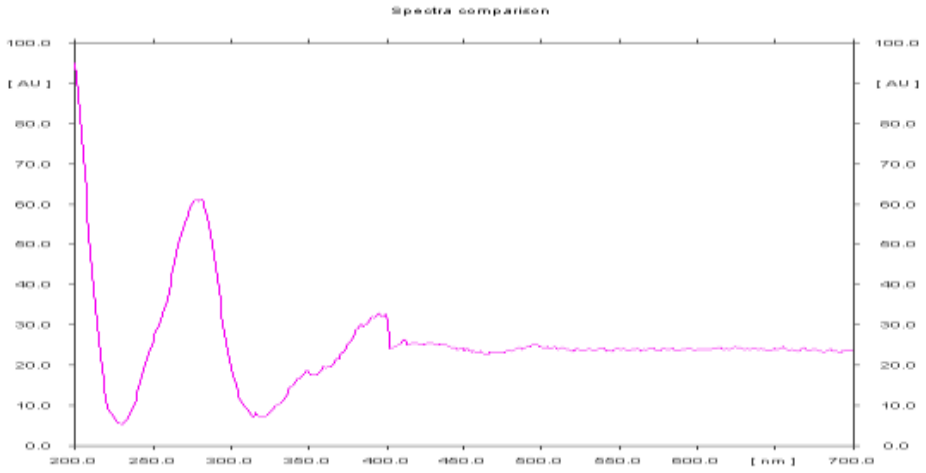

Figure 2: UV spectrum of Alogliptin

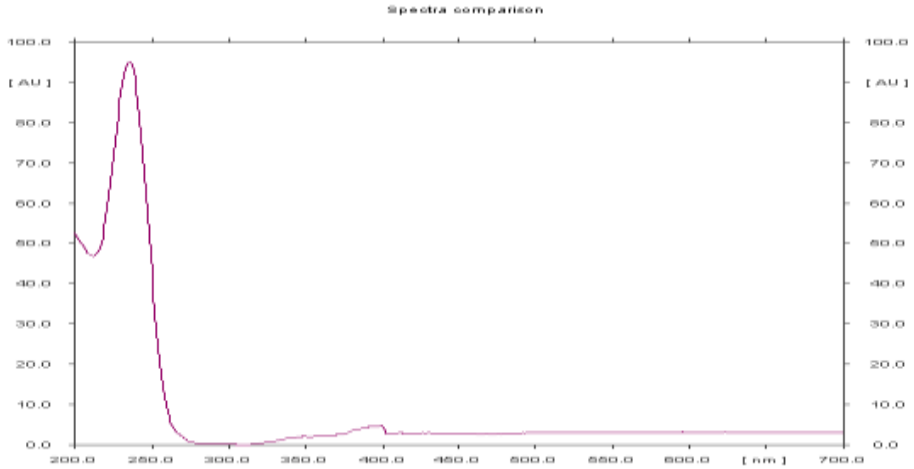

Figure 3: UV spectrum of Metformin

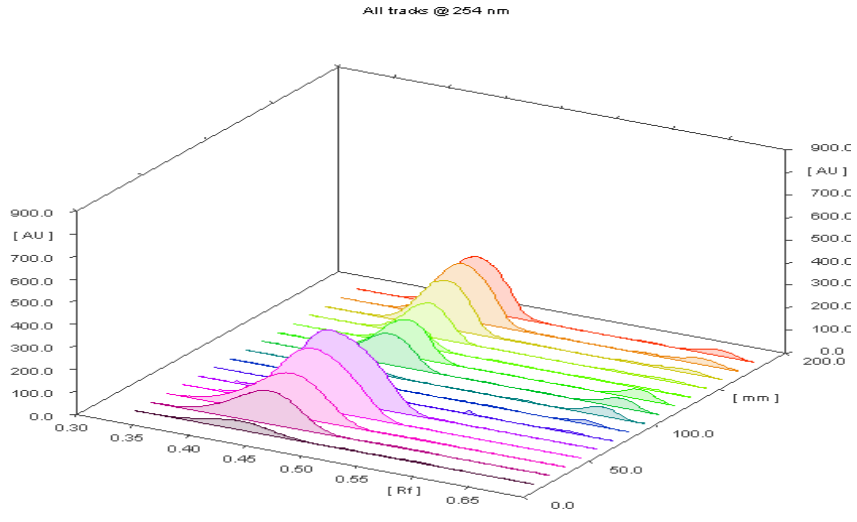

Figure 4: 3D chromatogram of Alogliptin and Metformin

Method validation

The developed method was validated as per the International Conference on Harmonization (ICH) [20, 21] guidelines with respect to linearity and range, specificity, precision, accuracy, limit of detection and limit of quantification.

RESULTS AND DISCUSSION

Linearity and range

A stock standard solution was prepared for both Alogliptin and Metformin they were serially diluted to yield five standard solutions. A volume of 0.1-0.5 µL of each solution was applied to the HPTLC plate to deliver 100, 200,300, 400 and 500 ng/spot of Alogliptin, 4000, 8000, 12000, 16000 and 20000 ng of Metformin /spot. The calibration plots revealed good linear relationships between area and concentration over the range of 100-500 ng/spot for Alogliptin and 4000-20000 ng/spot for Metformin. The standard curves for Alogliptin and Metformin are shown in Figure.5 and Figure.6**,** respectively and data for both Alogliptin and Metformin is presented in Table 1.

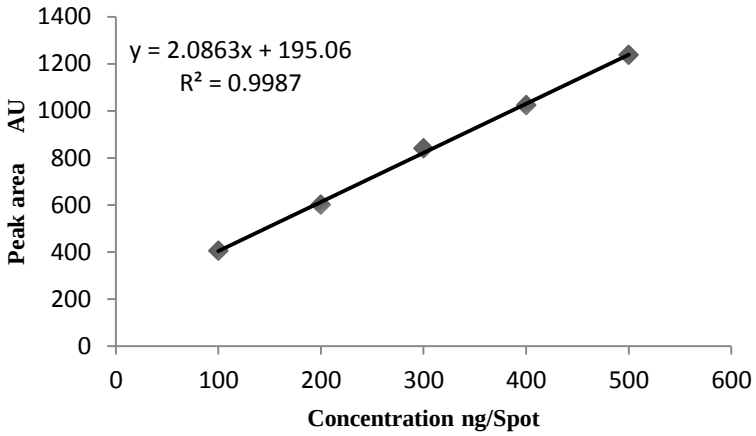

Figure 5: Linearity curve of Alogliptin benzoate

Table 1 Linearity results

Components	Concentration range (ng/spot)	Equation for regression line	r^2
Alogliptin	100-500	y = 2.086x + 195.0	0.998
Metformin	4000-20000	y = 1.134x - 2814	0.988

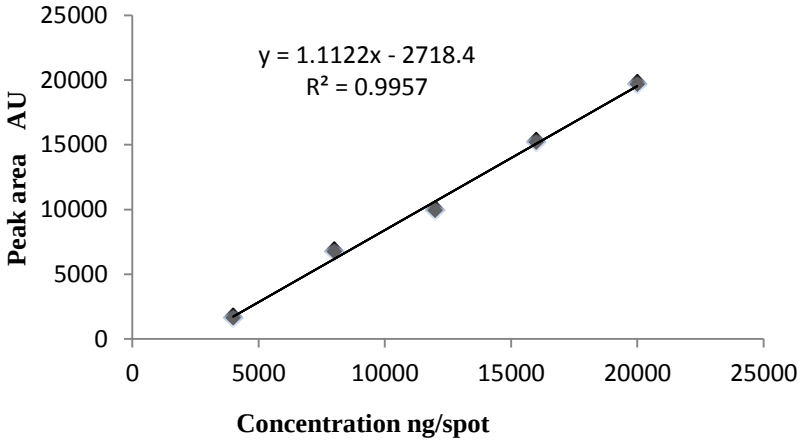

Figure 6: Linearity curve of Metformin hydrochloride

Precision

Precision of the method was ascertained in the terms of repeatability, intraday and interday precision. Repeatability was determined by applying 0.4 µL of standard solution containing 400 ng/spot of Alogliptin and 16000 ng/spot of Metformin in six replicates and respective areas were calculated. For intra-day and inter-day variation, three concentration of 100,200and 300 ng for Alogliptin, 4000, 8000 and 12000 ng for Metformin were selected from linearity range. Intraday analysis was carried on same day in three replicates. Interday analysis was carried on three different days in three replicates. The respective peak areas for a set of drug solutions were calculated. Results are expressed in the term of % RSD. Table 2, 3, 4 shows the precision data for the method.

Table 2 Repeatability data for Alogliptin and Metformin (n=6)

Concentration	Injection	Peak area		%RSD	
		ALG	MET	ALG	MET
	1	1458.5	12745.1		
Alogliptin(400ng/ml)	2	1443.0	12470.1		
	3	1456.4	12365.4	0.82	1.03
Metformin (16000ng/ml)	4	1435.6	12569.5		
	5	1436.2	12458.9		
	6	1428.1	12487.8		

RSD Relative standard deviation

Table 3 Interday precision data for Alogliptin and Metformin (n=3)

Day	Concentration (ng/ml)		Peak area		%RSD	
	ALG	MET	ALG	MET	ALG	MET
1			550	7142.5		
2	200	8000	553	7033.2	0.66	0.91
3			546.2	7028.1		
1			600.5	10091.5		

2	300	12000	596.3	10188	0.35	0.59
3			598	10076		
1			750.3	15996.3		
2	400	16000	746	15953.2	0.70	0.30
3			756.5	15900.2		

RSD Relative standard deviation

Table 4 Intraday precision data for Alogliptin and Metformin (n=3)

Level	Concentration (ng/ml)		Peak area		%RSD	
	ALG	MET	ALG	MET	ALG	MET
			585.3	12349		
I	200	8000	582.1	12566	0.43	1.05
			580.3	12329		
			600.2	12449		
II	300	12000	598	12226.5	0.61	1.03
			593	12229.5		
			500.8	12925.4		
III	400	16000	496.2	12966.6	1.04	0.88
			490.5	12751.7		

RSD Relative standard deviation

Accuracy

Accuracy of the method was determined by replicates (n=3) analysis, carried out using three solutions prepared by standard addition of pure active pharmaceutical ingredient at three different concentration levels 80%,100% and 120%. Accuracy was calculated by comparing the difference between the spiked value (theoretical value) and that actual found value. Results are presented in the tem of %recovery of the active pharmaceutical ingredient.

Limits of detection and quantification

The limit of detection was found to be 2 ng/spot and 40 ng/spot for Alogliptin and Metformin, respectively. The limit of quantification was found to be 6 ng/spot and 130 ng/spot for Alogliptin and Metformin, respectively, which was lower than that reported earlier.

Specificity

The chromatogram of the solution, which was not spiked with Alogliptin and Metformin, did not show any spot, while the chromatogram of the solution of the tablet matrix spiked with Alogliptin and Metformin showed clear, compact and well separated peaks of Alogliptin and Metformin Figure. 7. Moreover, from Figure.7, it can be seen that no other peaks were eluted besides the two active compounds. The method was therefore considered to be specific.

There are many advantages in this method development over the earlier reported method [19]. One is solvent system, lower proportion of methanol was used for the method development of Alogliptin and Metformin instead of a higher proportion of Acetonitrile: 1% ammonium acetate in

methanol. Second is, the assay is carried out by using the tablet kazano®. But in earlier reported method they added Alogliptin benzoate API to Metformin tablets to simulate the condition of mixture. Then the standard solutions are prepared in the ratio of 1:1. But in tablet the Alogliptin and Metformin are in the ratio of 1:40. In our method the standard solutions are prepared as per the label claim ratio. Both the drugs are well resolved from each other not like earlier reported method. The R_f value of Alogliptin and Metformin was 0.66 and 0.44, respectively.

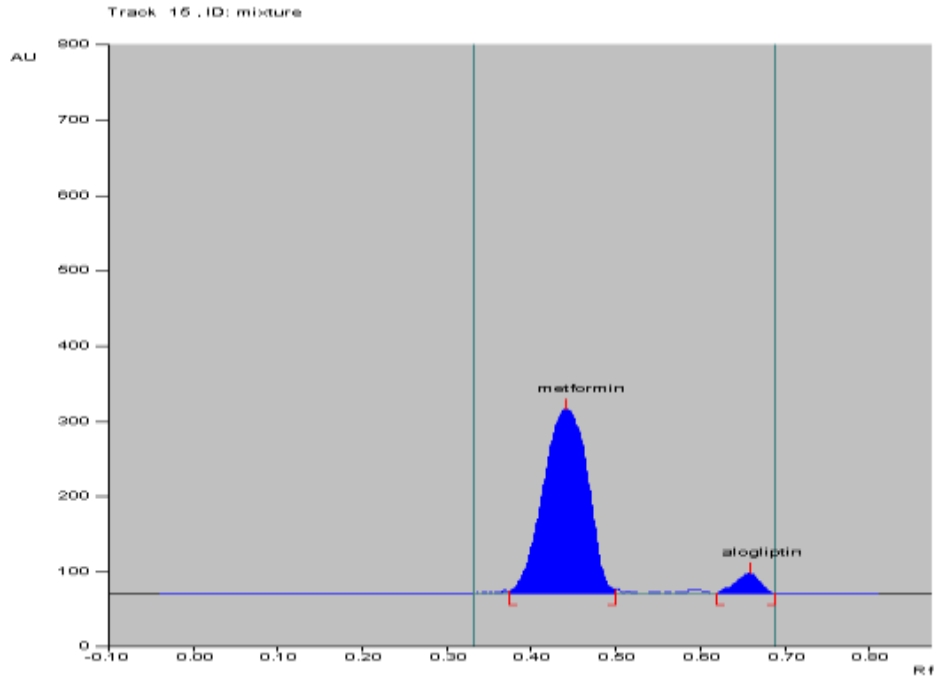

Figure 7: Chromatogram of fixed-dose tablet solution of Alogliptin and Metformin. Mobile phase – Chloroform: Methanol: 0.5 %Ammonium sulphate (4:4:2), detection at 254nm

Table 5 Accuracy for Alogliptin and Metformin

Level	% Recovery		% RSD	
	ALG	MET	ALG	MET
80 %	100	102.5	0.349	0.484
100 %	98.87	100.24	0.375	0.440
120 %	101.75	100.30	0.472	0.308

RSD Relative standard deviation

Robustness

The robustness of an analytical method evaluates the method capacity to remain unaffected by minor but purposeful variations in the method parameter and provides an indication of its reliability during normal usage. Robustness of the developed method was evaluated by the analysis of sample solution after making small changes in the mobile phase volume and mobile phase

saturation time. The low value of % RSD shows that the method is robust and that a slight change in mobile phase volume and mobile phase saturation time does not affect the results.

CONCLUSION

The developed method was simple, precise and accurate for the determination of alogliptin and metformin in bulk drug and pharmaceutical preparations. The method was validated for precision, accuracy, specificity and robustness. Therefore, the method can be applied for routine analysis of Metformin and Alogliptin in active pharmaceutical ingredients and in fixed dose combinations.

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