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SIMULTANEOUS ESTIMATION OF ALOGLIPTIN BENZOATE AND METFORMIN HCL IN PHARMACEUTICAL DOSAGE FORM BY HPTLC

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ABSTRACT

Objective: A method for HPTLC was developed and validated for simultaneous estimation of Alogliptin benzoate and Metformin HCl in a pharmaceutical dosage form. **Method:** The Chromatographic separation was achieved on TLC aluminum plates precoated with silica gel G_{60} F_{254} as the stationary phase. The mobile phase consisted of Methanol: Tetrahydrofuran: Ammonium formate in the ratio of 3:5.5:2.5 v/v/v. **Results:** The method was found to be linear in the concentration range of 0.225-1.12µg/spot for ALG and 9-45 µg/spot for MET and the correlation coefficient were found to be 0.998 for ALG and 0.992 for MET. The % RSD values were found to be within the acceptance criteria, the low % RSD indicates that proposed method is precise as per ICH guidelines. The accuracy of the method was determined and the mean recovery of ALG was 99.25-101.35% and MET was 100.49-101.79%. LOD and LOQ values were found to be 0.002 µg/spot and 0.05 µg/spot for ALG, 0.04µg/spot and 0.1 µg/spot for MET. **Conclusion:** The method reported is accurate, precise, specific, robust and linear for the estimation of Alogliptin and Metformin in Pharmaceutical dosage form.

Keywords: Alogliptin, Metformin, HPTLC, Chromatography, ICH

INTRODUCTION

Alogliptin (ALG), from the class of gliptins, is a recent antihyperglycemic agent. Chemically it is 2-({6-[(3R)-3 amino piperidiny-1-yl]-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl}) methyl benzonitrile benzoate **[1]**. Basically, it is a dipeptidyl peptidase-4 (DPP-4) inhibitor, which acts by increasing glucose stimulated insulin release. These agents are of importance in treatment of type II diabetes when used alone/in combination with drugs to increase sensitivity of insulin at target site. Four inhibitors inhibit inactivation of entero endocrine incretins like glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic (GIP) polypeptide **[2]**. This mechanism allows increased incretins concentration which in turn results in glucose dependent insulin release leading to better glycemic control **[3]**. It is available in different formulations such as Nesina (ALG alone), Oseni (ALG with Pioglitazone) and Kazano (With MET). ALG being a new drug and not official in any Pharmacopoeia. There are few analytical methods available for its estimation in biological matrices, bulk drug and dosage forms. Metformin (MET) is N, N- dimethyl imidocarbonimidic diamide monochloride **[4]**. Generally known as Metformin (MET) is a widely used antidiabetic drug in type-II diabetic patients **[5, 6]**. This agent shows its clinical activity of improving glucose tolerance in type-II diabetic subjects by lowering both basal and post prandial plasma glucose levels **[7, 8]**. In addition, it decreases glucose production in liver, decrease glucose absorption from intestine and also improves insulin sensitivity by promoting peripheral glucose uptake and utilization **[9, 10]**. 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 **[11]** with other combination of drugs by RP-HPLC, LC-MS **[12]** HPTLC and UPLC **[13, 14]**. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. The present study was aimed to develop a simple, rapid and accurate HPTLC method for estimation of ALG and MET simultaneously in bulk as well as fixed dose combination which can be adopted for the day-to-day analysis. The structure of Alogliptin Benzoate and Metformin HCl were shown in **(Figure 1 & 2)**.

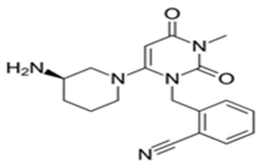

Figure 1: Structure of Alogliptin Benzoate

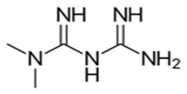

Figure 2: Structure of Metformin HCl

MATERIALS AND METHODS [15, 16]

Materials

Methanol, Tetrahydrofuran Ammonium formate were procured from Merck chemicals. Alogliptin and Metformin were procured from vivan life sciences, Bombay. Commercial tablets (Kazano®) containing Alogliptin 12.5mg and Metformin 500 mg were purchased from pharma save pharmacy, Canada. Kazano tablets (Label claim: Alogliptin 12.5mg and Metformin 500mg) were procured from Canada. Pre coated silica gel 60 F_{254} HPTLC plates was purchased from E-Merck.

Instrumentation

A Camag HPTLC system (Switzerland) with Camag Linomat V sample applicator, Camag TLC Scanner 3, Camag Plate heater, Camag twin-trough glass chamber, Pre coated plates (20cm X 20cm) with 200µm layer thickness, UV lamp (190-400nm), Camag winCATS software, Hamilton syringe100µl and Sartorius Analytical balance were used for the study.

Chromatographic conditions

The experiment was performed on silica gel 60F 254 aluminium sheets (20 x 20 cm) as stationary phase, using mobile phase comprised Methanol: Tetrahydrofuran: Ammonium formate of 3:5.5:2.5 v/v/v. The solutions were applied on TLC plate in the form of bands of 6 mm width under a stream of nitrogen gas using a Camag Linomat V automatic sample applicator, space between two bands were10 mm. Ascending development to 80 mm was performed in 10 cm x 10 cm Camag twin trough glass chamber saturated with the mobile phase for 30 min at room temperature. The developed TLC plate was air dried and then scanned between 200 to 400 nm using Camag TLC scanner 3 using WinCATS software. Both components showed good response at 254nm. The spectra was shown in **(Figure 3)**.

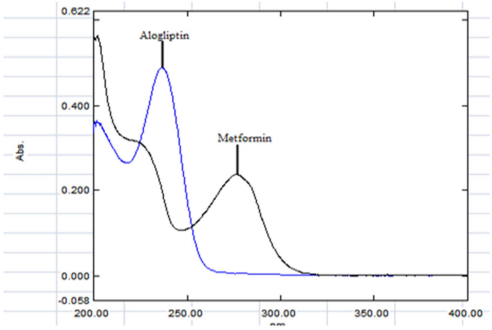

Figure 3: Overlay UV spectra of Alogliptin (244nm) and Metformin (291nm)

Preparation of standard solution

ALG (12.5mg) and MET (500mg) were weighed accurately, transferred to 10ml volumetric flask, dissolved and diluted with methanol to get 1250µg/ml and 50000µg/ml. From the above stock pipette, out 3ml and transferred to 10ml flask, and diluted with methanol to get 375µg/ml (0.375µg/µl) and 15,000µg/ml (15µg/µl). Different volumes of mixed standard

solution (0.6µl, 1.2µl, 1.8µl, 2.4µl and 3.0µl) were spotted on the TLC plate to obtain the concentrations of 0.225µg, 0.45µg, 0.675µg, 0.9µg and 1.125µg/spot for ALG and 9µg, 18µg, 27µg, 36µg and 45µg/spot for MET respectively.

Preparation of sample solution

For analysis of formulation, twenty tablets, each containing 12.5mg ALG and 500mg MET, were weighed and their average weight was calculated. The tablets were powdered and weighed equivalent to 10mg and transferred to a 10ml volumetric flask and the volume was adjusted up to the mark with methanol. 1.0µl of the sample solution was spotted on the TLC plate to obtain the concentrations of 0.375µg/spot for ALG and 15µg/spot for MET.

Validation of the proposed method

The Proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.

Linearity (Calibration curve)

Calibration curves were plotted over the concentration range of 0.225-1.125µg/spot for ALG and 9-45µg/spot for MET, respectively. Accurately measured mixed standard solutions of ALG and MET were applied to the TLC plate. The TLC plate was developed photometrically analysed as described under chromatographic separation. The R_f values of 0.78 and 0.59 for ALG and MET and the chromatogram was shown in **(Figure 4)**.

The calibration curve was prepared by plotting peak area versus concentration (µg/spot) corresponding to each spot. Each reading was an average of five determinations. The results were shown in **(Figure 5 & 6 and Table 1)**.

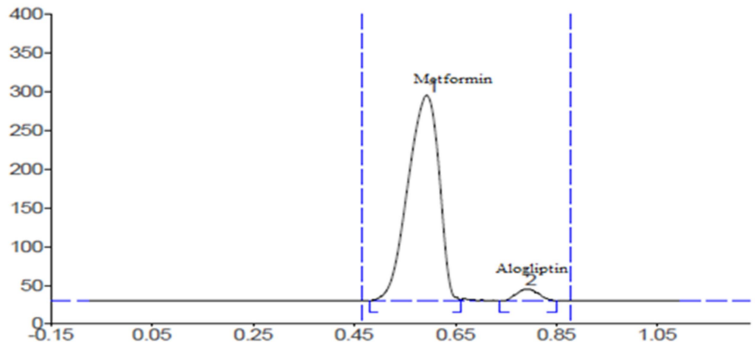

Figure 4: Chromatogram of ALG and MET with corresponding Rf values at 254 nm. Stationary phase: 10 X 10 cm HPTLC silica gel 60F254 aluminium plates, Mobile phase: Methanol: Tetrahydrofuran: Ammonium formate of 3:5.5:2.5 v/v/v Detection: UV at 254nm

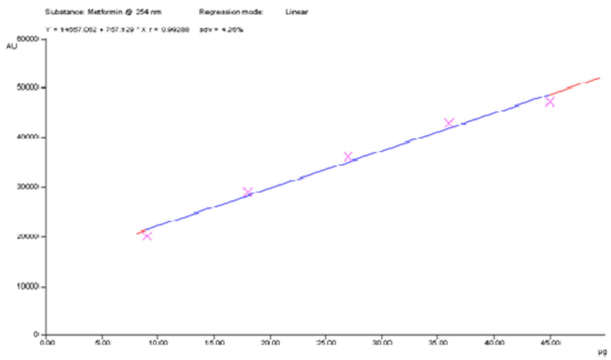

Figure 5: Calibration graph of Metformin HCl

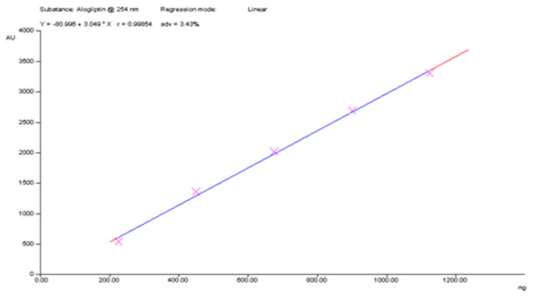

Figure 6: Calibration graph of Alogliptin Benzoate

Table 1: Linearity of ALG and MET

Sl. No	Concentration of ALG(µg/spot)	Concentration of MET(µg/spot)	ALG	MET
1	0.225	9	480.2	20046.5
2	0.45	18	1185.0	28978.5
3	0.675	27	1777.9	36129.5
4	0.90	36	2382.1	43016.8
5	1.125	45	3037.1	47465.0

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of ALG and MET by the standard addition method. Known amounts of standard solutions of ALG and MET was added at 80, 100 and 120 % level to pre-quantified sample solution of ALG and MET respectively. The amount of ALG and MET was estimated by applying obtained values to the respective regression line equations. The results are tabulated in **(Table 2)**.

Method Precision (% Repeatability)

The precision of the instrument was checked by repeatedly injecting (n= 6) solutions of ALG and MET without changing the parameters of the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Intermediate Precision (Reproducibility)

The intraday and inter-day precision of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for different concentration of standard solution of ALG and MET for the proposed method. The results were reported in terms of relative standard deviation (% RSD) and shown in **(Table 3 & 4)**.

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

LOD and the LOQ of the drug were calculated using the following equations as per international Conference on Harmonization (ICH) guidelines.

LOD = 3.3 X σ/S

LOQ = 10 X σ/S

Where = Standard deviation of the response

S = Slope of calibration curve

Specificity

The specificity of the method was ascertained by analysing standard drugs and the sample. The spots for ALG and MET in the samples were confirmed by comparing the R_f and spectra of the spots with that of the standards.

Table 2: Recovery study from binary mixture

Sl. No	Level	*% Recovery		%RSD	
		ALG	MET	ALG	MET
1	80%	99.25	100.49	0.181	0.66
2	100%	99.89	101.39	0.04	0.39
3	120%	101.35	101.79	0.49	0.24

*Average of three determinations

Table 3: Intraday precision

Replicate	Concentration (µg/spot)		Peak Area		% RSD	
	ALG	MET	ALG	MET	ALG	MET
1	0.45	18	1185.0	28898.5	0.08	0.130
2	0.45	18	1187.0	28955.6		
3	0.45	18	1185.5	28969.8		
1	0.675	27	1777.9	36128.4	0.38	0.027
2	0.675	27	1788.6	36147.8		
3	0.675	27	1775.7	36135.7		

% RSD-Relative Standard Deviation

Table 4: Inter day precision

Replicate	Concentration (µg/spot)		Peak Area		% RSD	
	ALG	MET	ALG	MET	ALG	MET
1	0.45	18	1181.0	28751.2	0.06	0.015
2	0.45	18	1183.0	28864.3		
3	0.45	18	1180.5	28806.4		
1	0.675	27	1767.4	36148.2	0.08	0.005
2	0.675	27	1785.1	36136.4		
3	0.675	27	1791.0	36147.2		

% RSD-Relative Standard Deviation

RESULTS AND DISCUSSION

The TLC procedure was optimized with a view to develop an assay method for the simultaneous estimation of ALG and MET. The standard solutions of both the drugs were spotted on the TLC plates and run-in different solvent systems. The mobile phase consisting of methanol: tetrahydrofuran: ammonium formate 3:5.5:2.5 v/v/v gave sharp and symmetrical peaks with the R_f values of 0.78 and 0.59 for ALG and MET, respectively. Well defined spots were obtained when the chamber was saturated with mobile phase for 30 min at room temperature (27 ±30ºC). A combined densitogram of mixed standards and 3-D chromatogram showing peaks of ALG and MET in different concentrations at 254 nm. The proposed HPTLC method was validated in terms of linearity, precision, accuracy, LOD, LOQ and specificity. The calibration plot was found to be linear over the concentration range 0.225-1.125µg/spot for ALG and 9-45µg/spot for MET, respectively with a correlation coefficient of 0.998 for ALG and 0.992 for MET, respectively. LOD for ALG and MET were found to be 0.002µg/spot and 0.04µg/spot, respectively. LOQ for ALG and MET were found to be 0.005µg/spot and 0.1µg/spot, respectively indicate the sensitivity of the method. The low % RSD values of intraday and inter day precision reveals that the proposed method is precise. To study the accuracy of the method, recovery studies were performed. The percent average recoveries obtained were 99.25-101.35% and 100.49-101.79 for ALG and MET, respectively indicating that the proposed HPTLC method is highly accurate. The proposed validated method was successfully applied to determine ALG and MET in tablet dosage forms. The percent average assay was found to be 99.76 ± 0.48 and be 99.98 ± 0.17 for ALG and MET respectively was shown in **(Table 5)**.

The low values of standard deviation indicate the suitability of this method for routine analysis of ALG and MET in pharmaceutical dosage forms. To confirm the specificity of the proposed method, the solution of formulation was spotted on TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the sample peak. The validation parameters are presented in (**Table 6)**.

Table 5: Assay of Alogliptin and Metformin

Drug	Label Claim (mg)		Amount found (mg)		*%Label Claim ±S.D.	
	ALG	MET	ALG	MET	ALG	MET
Kazano	12.5	500	12.42	498.25	99.60 ± 0.38	99.65 ± 0.26

*Average of three determinations

Table 6: Summary of Validation parameters

Validation Parameters	Alogliptin Benzoate	Metformin HCl
Linearity	0.225-1.125µg/spot	9-45µg/spot
Correlation Coefficient	0.998	0.992
Accuracy	99.25 to 101.35%	100.49 to 101.79%
Precision(%RSD)	Less than 2%	Less than 2%
LOD	0.002 µg/spot	0.04 µg/spot
LOQ	0.05 µg/spot	0.1 µg/spot
Specificity	Specific	Specific

CONCLUSION

The proposed HPTLC method for simultaneous estimation of ALG and MET in combined dosage forms was validated and found to be suitable for the routine quantitative analysis. The result of linearity, precision, accuracy and specificity were proved to be within the limits. The method provides selective quantitative of ALG and MET. Therefore, this method can be employed for the routine analysis for simultaneous estimation of ALG and MET in quality control of formulation.

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