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Der Pharmacia Lettre, 2015, 7 (7):321-328
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ISSN 0975-5071
USA CODEN: DPLEB4

Development and validation of HPTLC method for simultaneous estimation of metformin hydrochloride and alogliptin benzoate in bulk drugs and combined dosage forms

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ABSTRACT

A new, simple, precise, accurate and selective high performance thin-layer chromatographic (HPTLC) method has been developed and validated for the simultaneous estimation of Metformin hydrochloride (MET) and Alogliptin benzoate (ALG) in a tablet dosage form. Chromatographic separation was carried out on Merck HPTLC aluminium sheets of silica gel 60F254 using Acetonitrile: 1% ammonium acetate in Methanol (4.5:5.5 v/v) as mobile phase followed by densitometry analysis at 253 nm. The reliability of the method was assessed by evaluation of linearity (100-2500 ng/spot for Metformin hydrochloride as well as for Alogliptin benzoate). The accuracy of methods was assessed by recovery studies and was found to be within range of 98-102% for both Alogliptin benzoate and Metformin hydrochloride. The developed method was validated with respect to linearity, accuracy (recovery), and precision. The results were validated statistically as per ICH Q2 R1 guidelines and were found to be satisfactory. Due to non-availability of product, the simulation was done by using Glycomet® tablets (Metformin hydrochloride 250mg) and API of Alogliptin benzoate. The proposed method was successfully applied for the determination of Alogliptin benzoate and Metformin hydrochloride in the mixture.

Keywords: Metformin hydrochloride, Alogliptin benzoate, HPTLC, simultaneous determination, validation.

INTRODUCTION

Metformin hydrochloride:
Metformin hydrochloride (MET) is chemically 1,1- dimethyl biguanide hydrochloride and it belongs to biguanide class of oral anti-diabetic drugs (Fig. 1). MET acts as anti-hyperglycaemic and lowers the blood glucose level by inhibiting hepatic glucose production, gluconeogenesis and increasing peripheral utilization of glucose [1]. Metformin hydrochloride is official in I.P., U.S.P., and B.P. [2-4]. I.P. recommends non aqueous titration method for raw material and UV spectrophotometric method for tablets [2]. In literature a large number of methods for estimation of Metformin hydrochloride in drug products, either alone or in combination with other drugs have been reported [5-11]. Large number of specialized methods for estimation of Metformin hydrochloride and its combinations with other drugs in plasma, serum and urine are also reported in literature [11-14]

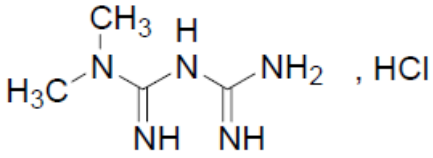

Fig. 1: Structure of Metformin hydrochloride

Alogliptin benzoate:
Alogliptin benzoate (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 (Fig.2).

Alogliptin benzoate belongs to the class of Dipeptidyl peptidase-4 (DPP-4) inhibitors, a new class of anti-diabetic drugs which act by increasing glucose dependent insulin release[16]. 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 [17-20]. 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. 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 HCL) [21]. Alogliptin is also approved for marketing in Europe as alone or combination with other anti-diabetics drugs. Alogliptin is new drug and not official in any pharmacopoeia. Literature survey does not reveal any HPTLC method for simultaneous estimation of Alogliptin benzoate and Metformin hydrochloride in bulk drug as well as pharmaceutical preparation. [22]

The objective of present study was to develop and validate the HPTLC method for simultaneous estimation of Alogliptin benzoate and Metformin hydrochloride. The developed method is new, simple, precise, accurate and selective for simultaneous determination of both drugs in their tablet dosage form as per International Conference on Harmonisation (ICH) guidelines. [23]

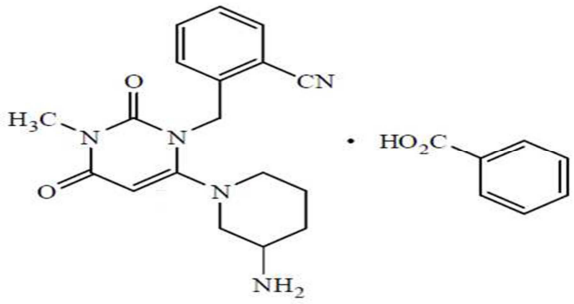

Fig. 2: Structure of Alogliptin benzoate

MATERIALS AND METHODS

3.1 Material and reagents

Metformin hydrochloride was kindly supplied by Aarti Drugs Ltd, Gujarat, India as a gift sample with Certificate of analysis (COA) indicating its authenticity. Alogliptin benzoate was purchased from Swapnroop Drugs and Pharmaceuticals, Aurangabad, Maharashtra, India with Certificate of analysis (COA) indicating its authenticity Acetonitrile, Ammonium acetate and methanol were used as solvents to prepare the mobile phase.

All reagents used were of analytical reagent grade (Merck India Ltd. and Loba Chemie Pvt. Ltd.).The tablet samples of Glycomet® 250mg were obtained from local market.

3.2 Instrumentation and chromatographic conditions

The HPTLC system (CAMAG, Switzerland) consisted of Linomat V auto sprayer connected to a nitrogen cylinder, a twin trough chamber (20 × 10cm), a derivatization chamber, and a plate heater. Pre-coated silica gel $60F_{254}$ HPTLC plates (20 × 10 cm, layer thickness 0.2 mm (E. Merck KGaA, Darmstadt, Germany) was used as stationary phase. HPTLC plates were pre-washed twice with 10 mL of methanol and activated at 80°C for 5 min, prior to sample application. The standard and formulation samples of MET and ALG in mixture were spotted on pre-coated HPTLC plates in the form of narrow bands of lengths 6 mm. Samples were applied under continuous drying stream of nitrogen gas and application positions were at least 8 mm from the sides and 10 mm from the bottom of the plates at constant application rate of 150 nL/s. The mobile phase consists of Acetonitrile: 1% ammonium acetate in Methanol (4.5:5.5 v/v). Linear ascending development was carried out in twin trough chamber (20 x 10 cm). The optimized chamber saturation time for mobile phase was 30min, at 25°C ± 2; the length of chromatogram run was 7cm and HPTLC plates were air dried. Densitometric scanning was performed on CAMAG TLC scanner III in absorbance mode and operated by winCATS version 1.4.4. The source of radiation utilized was deuterium lamp. The spots were analysed at a wavelength of 253 nm. The slit dimensions used in the analysis were having length and width of 5 mm and 0.45mm, respectively, with a scanning rate of 20mm/s. Evaluation was performed using linear regression analysis via peak areas.

3.3 Preparation of standard solutions and calibration curves

An accurately weighed quantity of Alogliptin benzoate (25mg) was transferred to 25ml volumetric flask, dissolved and diluted up to the mark with methanol to give 1000µg/ml. Similarly an accurately weighed quantity of Metformin hydrochloride (25mg) was transferred to 25ml volumetric flask, dissolved and diluted up to the mark with methanol to give 1000µg/ml. Both the solutions were mixed together in ratio of 1:1. Calibration was done by applying mixture of standard solutions ranging from 0.2–5.0 µL using 100µL Hamilton syringe with the help of Linomat V autosprayer on HPTLC plate that gave concentration of 100-2500 ng/spot for both MET and ALG . The plates were developed and analysed at 253nm by TLC Scanner 3. Six different sets of calibration curves were prepared in the same way. The area report thus obtained was recorded and plot of concentration verses area under curve was prepared. The Equation of the standard curve, regression coefficient, correlation coefficient, slope and intercept were reported.

3.4. Assay of tablets:

Fixed dose combination of alogliptin and metformin is approved for marketing in USA (Kazano® tablets) and Europe (Vipdomet® tablets). Kazano® tablets contain alogliptin/metformin hydrochloride in the ratio of either 12.5:500mg or 12.5:1000mg and Vipdomet® tablets contain alogliptin/metformin hydrochloride in the ratio of 12.5:850mg. Due to non-availability of product, standard addition of Alogliptin benzoate API to Metformin tablets (Glycomet®250mg) was used to simulate the condition of mixture. Twenty Glycomet® tablets were weighed and triturated in a mortar pestle and 170mg of Alogliptin benzoate was added to the mixture. Then, powder mixture equivalent to 10mg of Alogliptin benzoate containing 294.11mg of Metformin hydrochloride was taken. In order to keep concentration of Metformin hydrochloride /Alogliptin benzoate in ratio of 1:1 and to bring the concentration in the linearity range 280mg of alogliptin benzoate was added. The mixture was added to 10ml volumetric flask containing 5ml methanol and sonicated for 10 minutes. Final volume was made up to 10ml with methanol and filtered through whatman filter paper (No. 41). 1ml of this solution was diluted with water to 10ml. 1µl of the resulting solution was applied in replicates of 6 on the HPTLC plate. The plate was developed and scanned at 253nm using TLC Scanner 3.

3.5. Analytical Method validation

The developed method was validated as per ICH Q2A and Q2B guidelines and different parameters evaluated were linearity, precision, accuracy, specificity, quantification limits and robustness.

Table1. Regression analysis data for both MET and ALG (n=6)

Parameter	Metformin hydrochloride	Alogliptin benzoate
Linearity range(ng/band)	100-2500	100-2500
Regression coefficient(r^2)	0.991	0.998
Correlation coefficient (r)	0.995	0.999
Slope	2.921	3.534
Intercept	396.6	54.04

a. Linearity: Linear regression data from the calibration plots revealed good linear relationships between area and concentration over the range of 100-2500 ng/spot for MET and ALG. The standard curves for ALG and MET are shown in Fig3 and Fig 4 respectively and data for both MET and ALG is presented in Table 1.

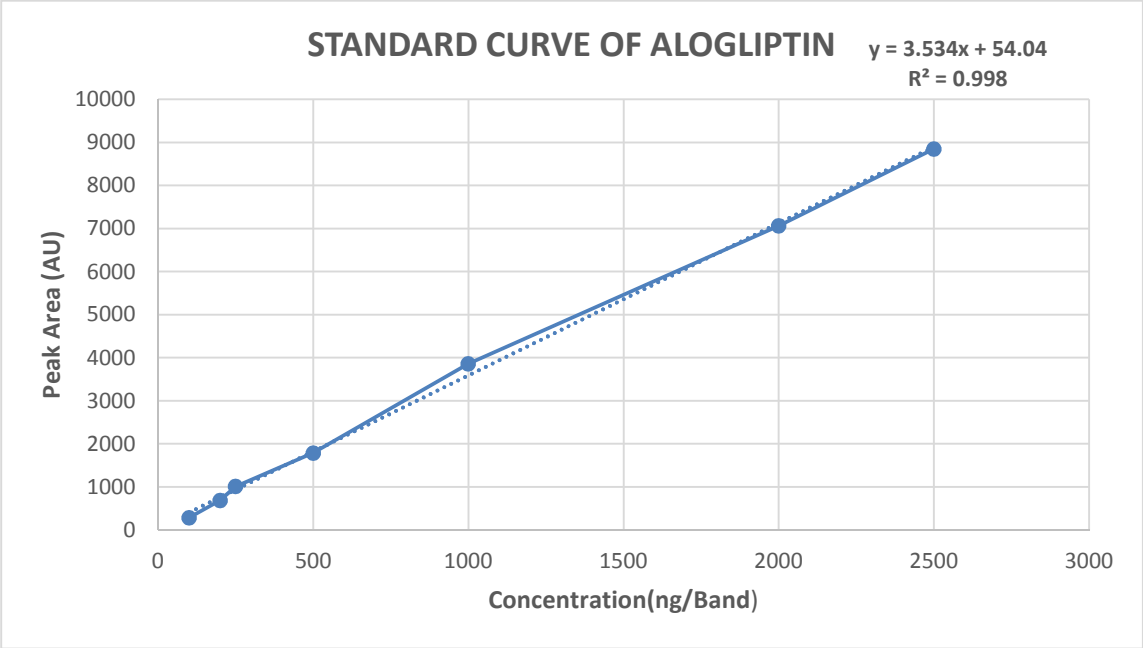

Fig 3. Standard Curve of Alogliptin benzoate

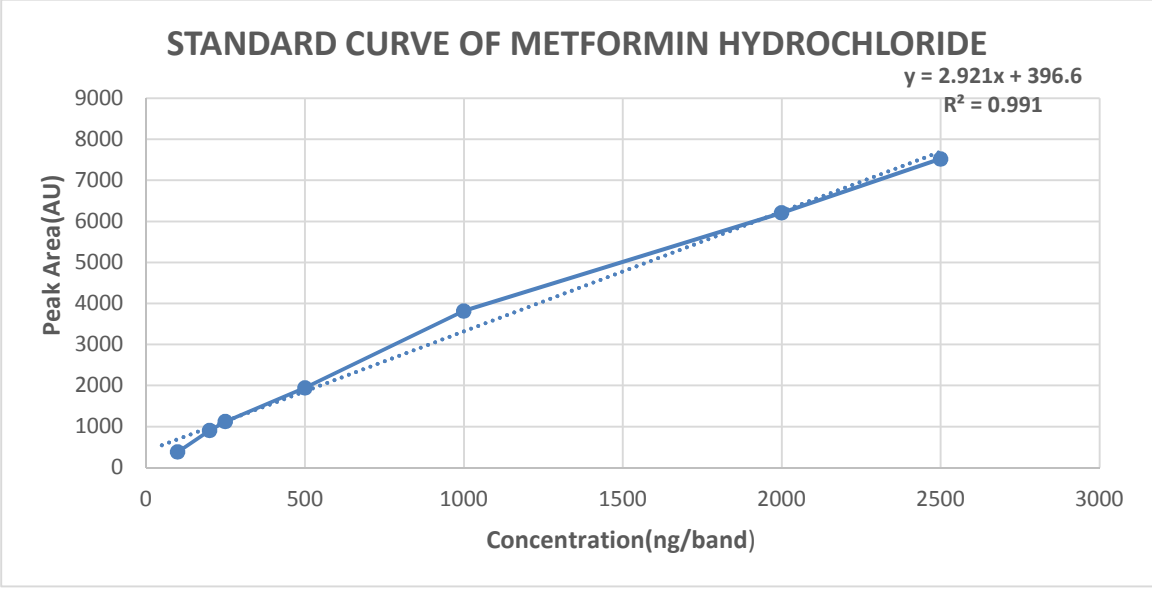

Fig 3. Standard Curve of Metformin hydrochloride

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

Table 2. Recovery study data for ALG and MET (n=3)

DRUG	PRE ANALYSED (ng)	CONC. ADDED(ng)	CONC. FOUND (ng)	AMOUNT RECOVERED(ng)	%RECOVERY (MEAN±SD)	%RSD
ALG	500	-	499.50	-	-	0.43
		250	752.40	252.90	101.16±0.54	0.54
		500	997.08	497.58	99.52±0.62	0.62
		750	1251.65	752.14	100.29±0.76	0.75
MET	500	-	500.66	-	-	0.30
		250	753.76	253.09	101.23±1.87	1.85
		500	1002.57	501.90	100.38±0.25	0.25
		750	1256.13	755.46	100.73±0.52	0.52

c. **Precision:**

Precision of the method was ascertained in the terms of repeatability, intraday and interday precision. Repeatability was determined by applying 1µl of standard solution containing 1000ng/band ALG and MET in six replicates and respective areas were calculated. For intra-day and inter-day variation, three concentrations of AlG and MET 500, 1000 and 2000ng 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 3, 4, 5 shows the precision data for the method.

Table 3. Repeatability data for ALG and MET (n=6)

DRUG	CONC. TAKEN	CONC. FOUND (ng)	% AMOUNT FOUND (MEAN±SD)	%RSD
ALG	1000	962.87	96.28±1.07	1.11
MET	1000	981.37	98.13±1.11	1.13

Table 4. Interday precision data for ALG and MET (n=3)

DRUG	CONC. TAKEN	CONC. FOUND (ng)	% AMOUNT FOUND (MEAN±SD)	%RSD
ALOGLIPTIN BENZOATE	250	248.43	99.37±1.41	1.42
	500	496.65	99.33±0.52	0.52
	1000	1008.4	100.84±0.16	0.16
METFORMIN HYDROCHLORIDE	250	248.03	99.21±0.38	0.38
	500	504.27	100.85±1.009	1.00
	1000	1004.24	100.42±0.5073	0.50

Table 5. Intraday precision data for ALG and MET (n=3)

DRUG	CONC. TAKEN	CONC. FOUND (ng)	% AMOUNT FOUND (MEAN±SD)	%RSD
ALG	250	252.72	101.08±0.4976	0.49
	500	497.85	99.57±1.105	1.11
	1000	1007.12	100.71±0.6823	0.67
MET	250	247.85	99.14±1.227	1.23
	500	497.47	99.49±0.3803	0.38
	1000	1006.60	100.66±1.031	1.02

d. **Specificity:**

The specificity of the proposed HPTLC method was determined by complete separation of peaks of both tablet and API. The spots of MET and ALG in the sample were confirmed by seeing the R_f and spectra of the standard spots. Fig 5 and Fig 6 shows the spectra of standard and sample respectively. The peak purity of ALG and MET was assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e. r (s, m) = 1 and r (m, e) = 1 for ALG and r (s, m) = 1 and r (m, e) = 1 for MET. The method was therefore considered to be specific.

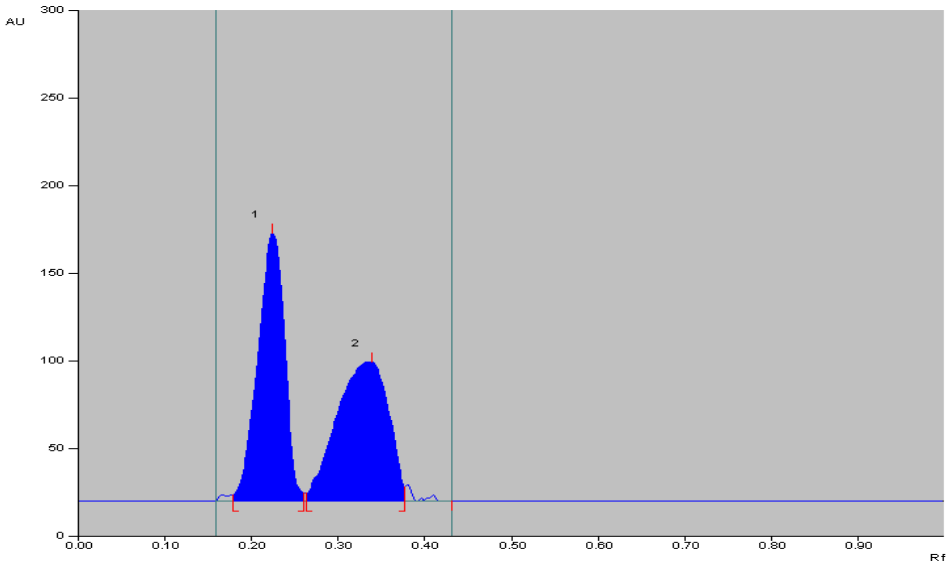

Fig 5. The spectra of Metformin and Alogliptin in bulk at 253nm

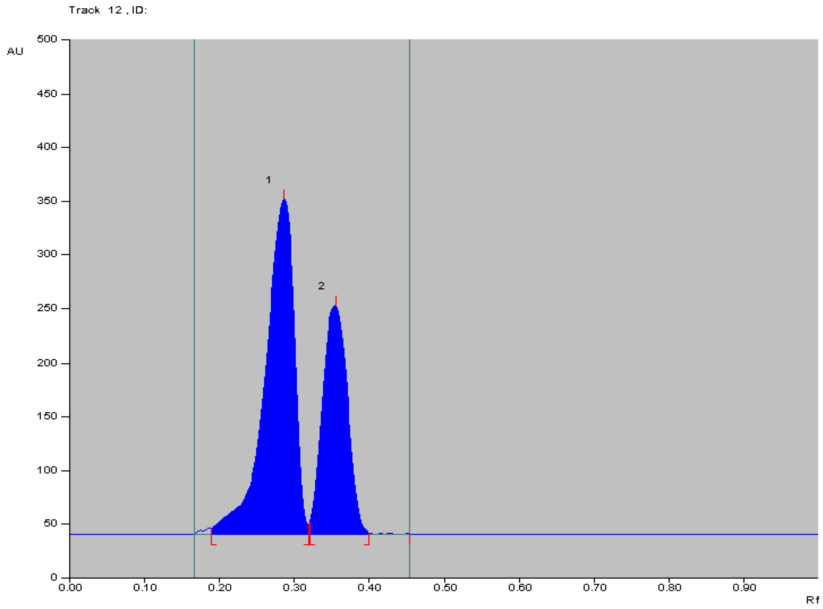

Fig 6. The spectra of Metformin and Alogliptin in Tablet dosage form at 253nm

e. **Robustness:**

To evaluate the robustness of the method, the chromatographic conditions were deliberately altered .The chromatographic conditions selected were: difference in saturation time (30±5min), change in the mobile phase composition (Acetonitrile: 1% ammonium acetate in methanol:: 5:5) . During robustness testing, each condition was varied separately, all other conditions being held constant at the optimized values. No significant change was observed in the peak area and R_f values. The results indicate that the method is robust and is capable of generating the data of acceptable quality.

f. **Detection limit and Quantitation Limit**:

A limit of detection (LOD) and a limit of quantification (LOQ) were established based on the calibration curve parameters, according to the formula:

LOD = 3.3 σ/s

LOQ = 10 σ/s

Where, 'σ' is the standard deviation of 'y' intercept of regression line and 's' is the slope of the calibration curve.

g. **Assay of the tablets:**

Table 6. Assay of formulation (n=6)

Drug	%Assay	%RSD
ALG	91.88±1.36	1.48
MET	99.92±0.66	0.67

RESULTS AND DISCUSSION

The method discussed in the present work provides a convenient, precise and accurate way for simultaneous analysis of Alogliptin benzoate and Metformin hydrochloride in its bulk and pharmaceutical dosage form. The TLC procedure was optimized for simultaneous determination of MET and ALG. The mobile phase Acetonitrile: 1% ammonium acetate in Methanol (4.5:5.5 v/v) resulted good resolution, sharp and symmetrical peaks at Rf 0.24 for MET and 0.34 for ALG. It was observed that pre- washing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 30 min (optimum chamber saturation time) ensured good reproducibility and peak shape of both drugs. The developed method was validated with respect to linearity, accuracy (recovery), and precision, LOD, LOQ, robustness and specificity. Regression analysis shows linearity over the concentration range of 100-2500ng/band for ALG as well as MET, with respective regression coefficients of 0.998 and 0.991 respectively. The % RSD for repeatability (n=6), intraday and interday (n=3) precision was found to be less than 2% indicating the precision of method. Accuracy of proposed method was ascertained by recovery studies and the results are expressed as % recovery. Percentage recovery for ALG and MET was found within the range of 98% and 102%. Values of standard deviation and % RSD were satisfactorily low, indicating the accuracy of the method. The LOD values for MET and ALG were found to be 1.26ng/band and 0.91ng/band respectively. The LOQ values for MET and ALG were found to be 3.82ng/band and 3.03ng/band respectively. Due to non-availability of combination product the Glycomet® tablets (250mg) with standard addition of Alogliptin benzoate is used to simulate the condition of actual product. The assay for MET and ALG was found to be 99.92±0.66 and 91.88±1.36 respectively. The % RSD value for both ALG and MET was found to be less than 2%, indicating the suitability of the method for routine analysis of MET and ALG in tablet dosage form. The % RSD values for all the parameters in robustness study are found to be less than 2% indicating method to be robust. To confirm the specificity of the proposed method, the solution of formulation was spotted on the HPTLC plates, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the sample peak. The regression analysis and summary of validation parameters of MET and ALG is given in Table 7.

Table7. Regression analysis and Summary of Validation Parameters of MET and ALG

Parameters	MET	ALG
Linearity range (ng/band)	100-2500	100-2500
Regression equation(y=mx+c)	y = 2.9216x + 396.69	y = 3.5348x + 54.042
Regression coefficient (r^2)	0.991	0.998
Correlation coefficient (r)	0.995	0.999
Slope	2.921	3.534
Intercept	396.6	54.04
R_f value	0.24	0.34
Recovery (%)	100.5	100.3
Repeatability (%RSD)	1.13	1.11
Intra-day Precision (n=3) (%RSD)	0.75	0.87
Inter-day Precision (n=3) (%RSD)	0.62	0.7
LOD	1.26	0.91
LOQ	3.82	3.03
Robustness	Robust	Robust
Specificity	Specific	Specific
Selectivity	Selective	Selective

CONCLUSION

Based on the results obtained, it is found that the developed HPTLC technique is quite simple, accurate, precise, reproducible, sensitive, robust, specific and economical. It can become effective analytical tool for routine quality control of Alogliptin benzoate and Metformin hydrochloride in bulk drug combinations and its combined pharmaceutical dosage forms without any prior separation of components.

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