

Original Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING SIMULTANEOUS ESTIMATION OF METFORMIN AND ALOGLIPTIN IN TABLETS BY HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Objective: A simple and stability-indicating high-performance thin-layer chromatographic method was developed and validated for the simultaneous estimation of metformin and alogliptin in tablets.

Methods: The method was developed in TLC aluminum plates pre-coated with silica gel 60F₂₅₄ as the stationary phase and the solvent system consists of methanol: chloroform: 0.5% ammonium sulphate [4:4:2, v/v/v]. The system was found to conferred a compact spot for metformin [Rf value of 0.44±0.02] and alogliptin [Rf value of 0.66 ± 0.22]. Densitometric analysis of metformin and alogliptin was carried out at the wavelength of 254 nm. Forced degradation studies were conducted to know the stability of the drug samples under various stress conditions like acid, base, peroxide, photolytic degradation according to the ICH guidelines.

Results: The developed method was found to be suitable for the excellent separation of the drug samples. Calibration curves were linear in the range of 40-200 ng/spot with a correlation coefficient of 0.996 for metformin and calibration curves were linear in the range of 1-5 ng/spot with a correlation co-efficient of 0.997 for alogliptin, respectively. Stability study shows that the chromatograms of samples degraded with acid, base, hydrogen peroxide, dry heat, and photolytic showed well-separated spots of pure metformin and alogliptin as well as some additional peaks at different Rf values. The method was successively applied to pharmaceutical formulation. No chromatographic interference from the tablet excipients was found.

Conclusion: The newly developed method can be applied for the identification and quantitative determination of metformin and alogliptin in the combined dosage form.

Keywords: Type 2 diabetes mellitus, Metformin, Alogliptin, High-performance thin-layer chromatography, Stability indicating, Method validation

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INTRODUCTION

Metformin hydrochloride is chemically N; N-Dimethylimido dicarbon imidic diamide. The chemical classification of metformin is a biguanide. The biguanide classes of anti-diabetic with an anti-hyperglycemic drugs are used in the treatment of type-2 diabetic patients. The classes of medications, particularly those which work via the incretin pathway, achieve glucose-lowering and minimizing risks ideally. The combination should be well-tolerated, convenient to take, should have few contraindications, a low risk of hypoglycemia, weight gain and be reasonably effective over both the short and long term such as the combination of metformin and alogliptin is a dipeptidyl peptidase-4 [DPP-4] inhibitor which is used in combination in the therapy of type 2 diabetes [1].

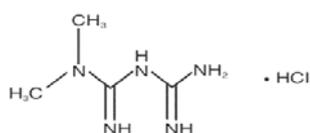


Fig. 1: Chemical structure of metformin

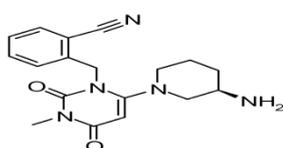


Fig. 2: Chemical structure of Alogliptin

Alogliptin is an orally administered antidiabetic drug in the dipeptidyl peptidase-4[DPP-4]. DPP-4 inhibitors represents a new therapeutic approach to the treatment of type 2 diabetes that functions to stimulate glucose-dependent insulin release and reduce glucagon's levels. This is done through inhibition of the inactivation of incretins, particularly glucagon-like peptide-1 [GLP-1] and gastric inhibitory polypeptide [GIP]. Alogliptin inhibits dipeptidyl peptidase 4[DPP-4], which normally degrades the incretin glucose-dependent insulin tropic polypeptide [GIP] and glucagon-like peptide1 [GLP-1], thereby improving glycemic control [2]. A combination of both drugs is recently launched in the market. The chemical structures of the drugs are shown in fig. 1, fig. 2, respectively. Literature survey reveals that spectrophotometric [3-5], High-Performance Liquid chromatography [HPLC] [6-8] and high-performance thin-layer chromatography [HPTLC] [9-11] methods for the estimation of metformin alone or in combination with other drugs from pharmaceutical formulation have been developed whereas spectrophotometric [12-14], RP-HPLC [15-17], HPTLC [18] and LC-MS/MS [19] methods for the estimation of alogliptin alone or in combination with other drugs from pharmaceutical formulation have been developed. However, no stability-indicating method has been reported so far simultaneous estimation of both drugs in combined pharmaceutical dosage form by HPTLC. The current method is the first method used so far for the simultaneous estimation of the alogliptin and metformin by HPTLC method. The advantages of HPTLC are, a large number of samples can be simultaneously analyzed in a shorter period. Unlike HPLC, this method utilizes fewer quantities of solvents, thus lowering the cost of analysis.

The ideal stability-indicating chromatographic method should estimate the drug and also be able to resolve the drug from its

degradation products. Hence an attempt has been made to develop an accurate, rapid and reproducible method for the determination of metformin and alogliptin in presence of their degradation products for their content analysis in pharmaceutical dosage forms, containing this combination as per ICH guidelines [20].

MATERIALS AND METHODS

Metformin Hcl was received from Cadila pharmaceutical, Ahmedabad; Gujarat. Alogliptin was kindly supplied by Vivian Life sciences, Thane, Maharashtra. All other chemicals like methanol, ammonium sulphate and chloroform were used for AR grade.

HPTLC method

Instrumentation

Camag HPTLC system equipped with Linomat V sample applicator operated under a gentle stream of nitrogen, coupled with 100 μ l HAMILTON syringe and CAMAG TLC scanner 3 controlled by WINCATS software was used for the application and detection of spots respectively. The Chromatographic separations of drugs were performed using pre-coated silica gel TLC aluminum plate 60 F₂₅₄ [10 cm x10 cm] with 250 μ m thickness and a CAMAG twin trough chamber was used for the chromatographic development.

Selection of mobile phase and optimization of condition

Initially, chloroform: methanol in ratio 4:6 [v/v] was tried for both drugs simultaneously. The spots were not developed properly and dragging was observed. Then chloroform: methanol in the ratio 6: 4 [v/v] was tried. The developed spots were diffused to the above mobile phase; 2 ml of 0.5 % ammonium sulphate was added. Both the peaks were symmetrical and no tailing was observed. Finally, the mobile phase consisting of chloroform: methanol: 0.5 % ammonium sulphate [4:4:2, v/v] gave good resolution. By optimizing chromatographic conditions such as having chamber saturation for 20 min, using 10 ml of the mobile phase and activating the plates before spotting, we obtain a good resolution as well as a sharp and symmetrical peak with Rf value of 0.44 \pm 0.02 for metformin and 0.66 \pm 0.02 for alogliptin.

Chromatographic condition

For chamber saturation, 20 ml of the mobile phase was transferred into the development tank with the lid closed. The assembly was aside for 20 min under room temperature. The Plates were prewashed with methanol and activated at 110 $^{\circ}$ C for 5 min before chromatography. The mobile phase consists of methanol: chloroform: 0.5% ammonium sulphate [4:4:2, v/v/v]. The plate was air-dried using an air dryer after the application of spots and the development but before densitometric scanning. The source of radiation utilized was a deuterium lamp. The evaluation was performed using peak areas with concentration.

Preparation of standard solutions

A combined standard stock solution containing 4000 μ g/ml of metformin and 100 μ g/ml of alogliptin was prepared in methanol. The application of standards was done by Hamilton syringe with the help of automatic sample applicator Linomat V on the TLC plate that gave a concentration, 40-200 ng/spot of metformin and 1-5 ng/spot of alogliptin, respectively. Each concentration was spotted five times on the TLC plates. The plates were developed using the previously described mobile phase. The calibration graph was plotted as peak areas versus corresponding concentrations.

Analysis of marketed formulation

To determine the content of metformin and alogliptin simultaneously in conventional tablets, [label claim containing 500 mg metformin and 12.5 mg alogliptin] twenty tablets were accurately weighed. The average weight was calculated and ground to a fine powder. A quantity of powder equivalent to 400 mg metformin and 10 mg alogliptin was transferred into a 100 ml volumetric flask containing 50 ml methanol was sonicated for 30 min and diluted to mark with the same solvent. The resulting solution was filtered using 0.45 μ m Millifilter. The aliquots solutions are applied on the TLC plate. The analysis was repeated for six times. Metformin and alogliptin gave sharp and well-defined peaks at Rf 0.44 \pm 0.02 and 0.66 \pm 0.02 respectively when scanned at 254 nm [fig. 3].

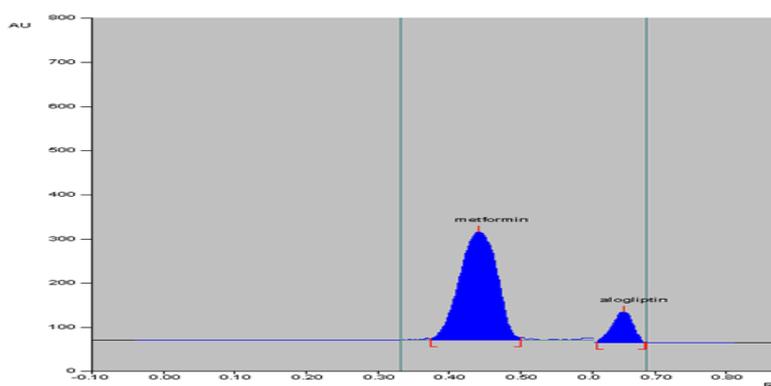


Fig. 3: Densitogram of standard metformin and alogliptin

Validation of HPTLC method

Accuracy

Accuracy of the method was determined by replicates [n=3] analysis and was 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 the found value [9, 10].

Precision

Intra-day precision

Intra-day precision was found out by carrying out the analysis of the standard drug at three different concentrations of 80,120 and 160 ng/spot for metformin; 2, 3 and 4 ng/spot for alogliptin were

selected from linearity range. The intraday analysis was carried on the same day in three replicates. Each concentration was applied in duplicate and % RSD was calculated [11, 12].

Inter-day precision

Inter-day precision was found out by carrying out the analysis of the standard drug at three different concentrations of 80,120 and 160 ng/spot for metformin; 2, 3, and 4 ng/spot for alogliptin were selected from linearity range. The interday analysis was carried on three different days in three replicates. Each concentration was applied in duplicate and % RSD was calculated [11, 12].

Repeatability

Repeatability was determined by applying the corresponding μ l of a standard solution containing 120 ng/spot of metformin and 3

ng/spot of alogliptin in six replicates and the respective areas were calculated. The % RSD was calculated [11, 12].

Forced degradation studies

Forced degradation of each drug substance was carried out under acid, base, hydrolytic, oxidation, photolytic and thermal stress conditions. A thermal and photodegradation study was carried out in a solid-state. Solutions were prepared by dissolving drug substances in a small volume of methanol and later diluted with hydrochloric acid, 0.1N sodium hydroxide or hydrogen peroxide to achieve a concentration of 100 µg/ml of each metformin and alogliptin [16].

Preparation of acid-induced degradation product

The 40 mg of metformin and 1 mg alogliptin in 10 ml volumetric flask to add 1 ml of 0.1M hydrochloric acid and make up the volume with mobile phase then refluxed at 40 °C for 30 min. After completion of 30 min, about 1 ml of the above solution was taken and diluted up to 10 ml with methanol. The resultant solution was applied to TLC plates in triplicates. The chromatograms run as described in section [16].

Preparation of base induced degradation product

The 40 mg of metformin and 1 mg of alogliptin in 10 ml volumetric flask to add 1 ml of 0.1M sodium hydroxide and make up the volume with mobile phase then refluxed at 40 °C for 30 min. After completion of 30 min, about 1 ml of the above solution was taken and diluted up to 10 ml with methanol. The resultant solution was applied to TLC plates in triplicates [17].

Preparation of hydrogen peroxide-induced degradation product

The 40 mg of metformin and 1 mg of alogliptin in 10 ml volumetric flask to add 1 ml of 0.1M 30% hydrogen peroxide and make up the volume with mobile phase then refluxed at 40 °C for 30 min. After completion of 30 min, about 1 ml of the above solution was taken, neutralized and diluted up to 10 ml with methanol. The resultant solutions were applied to TLC plates in triplicates [17].

Preparation of light heat degradation products

The 40 mg of metformin and 1 mg of alogliptin was transferred into a clean and dry Petridis. The Petridis was placed in direct sunlight for 5 d. In this study, the drug substance was exposed to direct sunlight for 5 d to determine the effect of irradiation on the stability of the drugs in solid-state. Afterward, the drug was transferred into a 10 ml volumetric flask and make up the volume with the mobile phase. 1 ml of the above solution was transferred into a 10 ml volumetric flask and diluted with 10 ml using the mobile phase. The resultant solution was applied to the TLC plate in triplicate [17].

Preparation of dry heat degradation products

Accurately weighed and transferred 40 mg of metformin and 1 mg of alogliptin was transferred into a clean and dry Petridis. The Petridis was placed in an oven at 50 °C for 3h. The drug was transferred into a 10 ml volumetric flask and make up the volume with mobile phase. 1 ml of the above solution was transferred into a 10 ml volumetric flask and diluted with 10 ml using the mobile phase. The resultant solution was applied to the TLC plate in triplicate [17].

RESULTS AND DISCUSSION

A novel, simple and precise HPTLC method coupled with densitometer was developed for the estimation of alogliptin and metformin present in the marketed formulation. Several analytical methods have been reported for the determination of metformin with alogliptin and combined with other drugs in pure and pharmaceutical dosage forms using spectrophotometry [3-5, 14], HPLC [6-8, 12, 15-17] Colorimetry [13]. Few methods like LC-MS/MS for determination of alogliptin and voglibose in human plasma [19] are found in the literature but these methods are not preferred for routine analysis of alogliptin and metformin in bulk and formulation studies because of the high cost of analytical technique and the skilled requirement for sample treatment. There are some HPTLC methods [9-11, 18] reported in the literature but

they have certain restrictions like requiring a large quantity of samples and organic solvents or sensitive to microgram concentration. In this work, new stability-indicating HPTLC method has been developed for the simultaneous estimation of the alogliptin and metformin. Initially, chloroform: methanol in ratio 4:6 [v/v] was tried for both drugs simultaneously; the spots were not developed properly and dragging was observed. Then, chloroform: methanol in the ratio 6: 4 [v/v] was tried. The developed spots were diffused to the above mobile phase; 2 ml of 0.5 % ammonium sulphate was added. Both the peaks were symmetrical and no tailing was observed. Finally, the mobile phase consisting of chloroform: methanol: 0.5 % ammonium sulphate [4:4:2, v/v] gave good resolution. By optimizing chromatographic conditions such as having chamber saturation for 20 min, using 10 ml of the mobile phase and activating the plates before spotting and obtain a good resolution as well as a sharp and symmetrical peak with Rf value of 0.44 ±0.02 for metformin and 0.66 ±0.02 for alogliptin. The method ensures minimal use of the mobile phase with minimal run time compared to other reported analytical methods. The ideal stability-indicating chromatographic method should estimate the drug and also be able to resolve the drug from its degradation products. Accuracy results displayed good reproducibility with % RSD values 2. This was found to be accurate as the percent recovery observed was high i.e. within the range of 98.84-100.4. The LOD of the developed HPTLC method was found to be 40 ng/spot for metformin and 1 ng/spot for alogliptin, respectively. The LOQ of the developed HPTLC method was found to be 130 ng/spot for metformin and 60 ng/spot for alogliptin, respectively. It confirms the method is sensitive. Besides, the estimation of the marketed preparation of alogliptin and metformin with the validated methods showed that the drug contents separated with no interfering peaks were generated by the excipients in the marketed formulation as shown in the fig. 5. The method is versatile and simple for the analysis of alogliptin and metformin in pure and pharmaceutical formulations. The method confirms minimal use of the mobile phase with a short run time compared to other reported analytical methods. The method was found to obey all the validation parameters as per ICH guidelines [20].

Validation of the developed method

Linearity

Metformin and alogliptin showed linearity in the range of 40-200 ng/spot and 1-5 ng/spot respectively. The slope, intercept and correlation coefficient values for metformin were found to be 4.3725, 501.5 and 0.997 respectively. The slope, intercept and correlation coefficient values for alogliptin were found to be 31.7, 205.02 and 0.999 respectively.

Precision

Intra-day and Inter-day was found out by carrying out the analysis of the standard drug at three different concentrations of 80,120 and 160 ng/spot for metformin 2,3 and 4 ng/spot for alogliptin were selected from linearity range. Repeatability was determined by applying the corresponding µl of a standard solution containing 120 ng/spot of metformin and 3 ng/spot of alogliptin in six replicates. The % RSD was found to be less than 2%.

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 the found value. Results are presented in the term of % recovery of the active pharmaceutical ingredient and data for both metformin and alogliptin. The summary of validation parameters was listed in table 1.

Analysis of marketed formulation

The chromatograms of the drugs are extracted from commercial formulation, exhibited two peaks at Rf value of 0.44 and 0.66 for metformin and alogliptin, respectively. The results of the analysis of the marketed formulation are given in table 2.

Table 1: Summary of validation parameters

Parameters	Metformin	Alogliptin
Linearity range [ng/spot]	40-200	1-5
Correlation coefficient [R ²]	0.996	0.997
Slope	4.3725	31.7
Intercept	501.58	205.02
Limit of detection[ng/spot]	40	1
Limit of quantification [ng/spot]	130	6
% Recovery [n=3]	100.4	98.87
Precision[%RSD], [mean ± S. D]		
Repeatability of application [n=6]	1.18,1197±14.2	2.210,801.3 ± 17.6
Intra-day [n=3]	0.58,1008.7±5.9	2.02,875.8±17.7
Inter-day [n=3]	1.45,1221.9±	1.64,779.6±12.8

% RSD =Relative standard deviation, SD=Standard deviation, n= number of injections

Table 2: Analysis of marketed formulation

Component	Amount [mg]		% Label claim	% RSD*
	Labeled	Found		
Metformin	500	498	99.6	0.32
Alogliptin	12.5	12	96.0	0.85

*Relative standard deviation; [n=5]

Forced degradation results

Peaks obtained from samples degraded by treatment with acid, base, hydrogen peroxide, dry heat treatment and photolytic contained well-separated spots of the pure drugs and same additional peaks at

different R_f values. It is apparent from [fig. 4-8] the spots of degradation products were well resolved from those of the drugs. The peaks of the metformin and alogliptin were not significantly shifted in the presence of the degradation peaks, which indicated the stability-indicating the nature of the method. The results are shown in table 3.

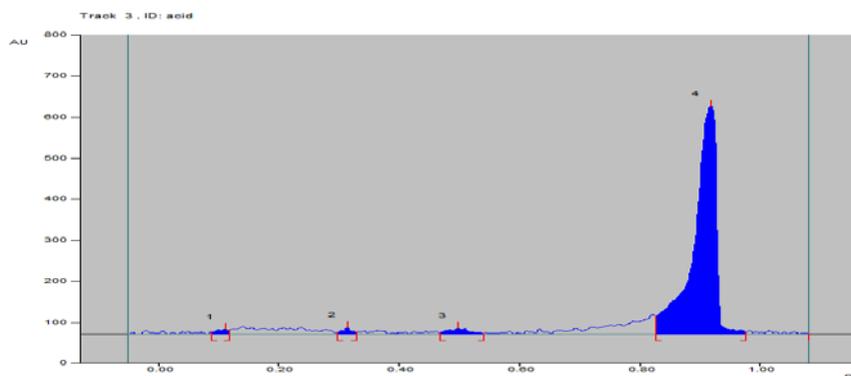


Fig. 4: Densitogram of acid-treated metformin and alogliptin

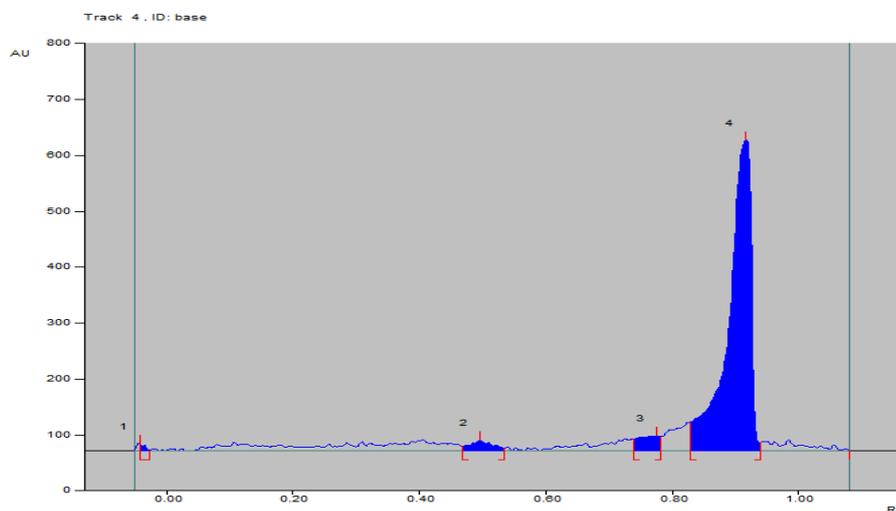


Fig. 5: Densitogram of base treated metformin and alogliptin

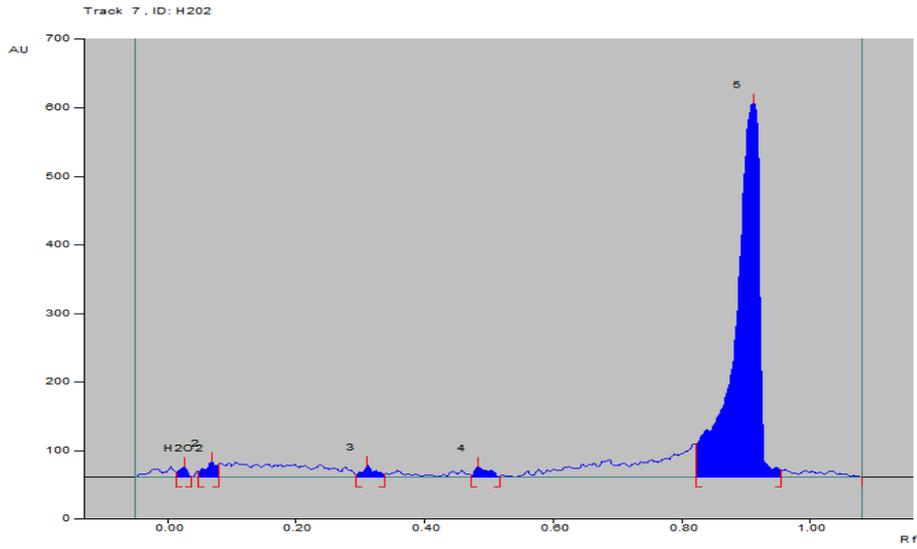


Fig. 6: Densitogram of hydrogen peroxide-treated metformin and alogliptin

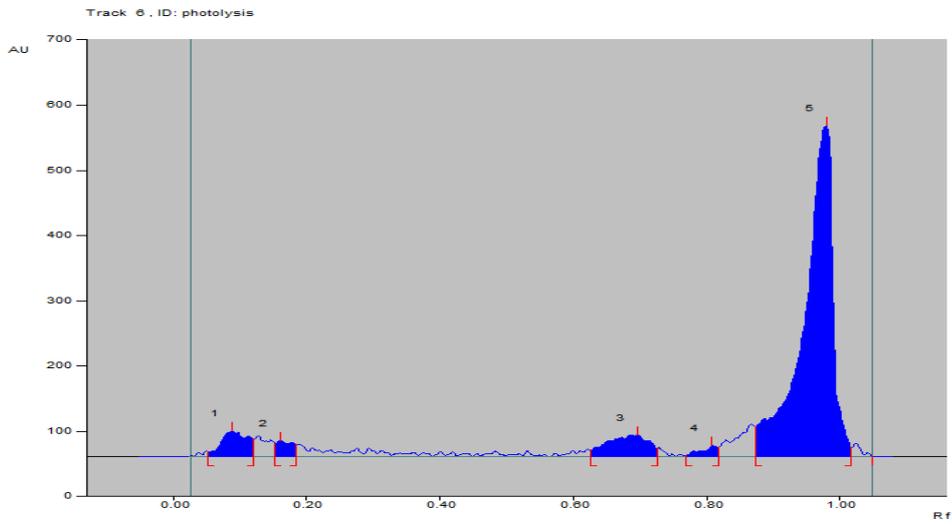


Fig. 7: Densitogram of dry heat-treated metformin and alogliptin

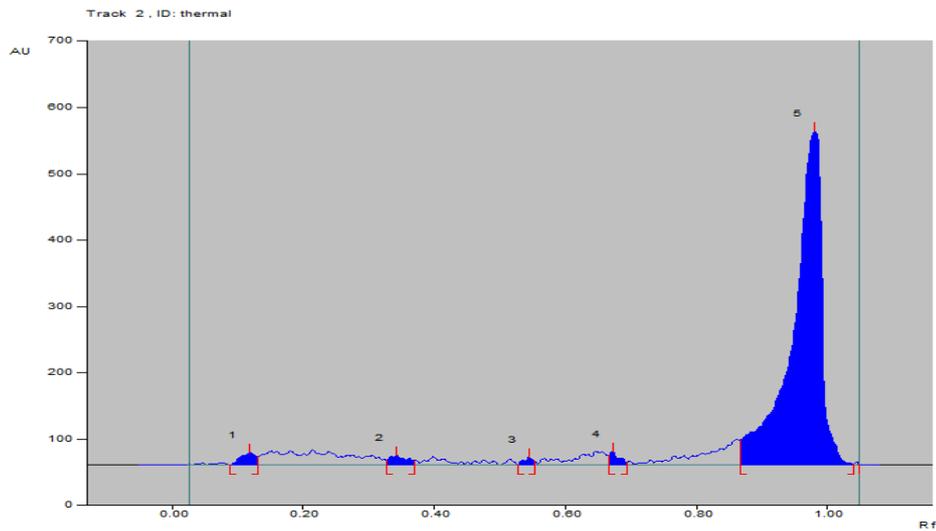


Fig. 8: Densitogram of light heat-treated metformin and alogliptin

CONCLUSION

The present study represents an accurate, simple, specific, precise and stability-indicating HPTLC method has been developed for the quantitative determination of metformin and alogliptin in bulk drug and tablet formulation. The developed method was validated based on ICH guidelines. Non-requirement of skilled personnel to operate the instruments involved is an added advantage of this method. Therefore, the method can be applied for routine quality control analysis of metformin and alogliptin in active pharmaceutical ingredients and the combined dosage form.

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Nil

AUTHORS CONTRIBUTIONS

Both authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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