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Application of Accelerated Stability Studies on Linagliptin by HPTLC

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Linagliptin is an inhibitor of dipeptidylpeptidase-4 (DPP-4) enzyme that degrades the incretin hormones glucagon-like peptide-1 and glucose-dependent insulin tropic polypeptide and is used to lower the increased blood sugar level. High performance thin layer chromatography method was used for chromatographic separation of linagliptin. As stability testing is major step in the development of new drug as well as formulation, stress degradation studies were carried out according to ICH guidelines. Linagliptin was found susceptible to all the analyzed stress conditions. Chromatographic resolution of linagliptin and its degradation products was achieved by using precoated silica gel 60 F_{254} aluminium plates as stationary phase and chloroform:methanol (8.5:1.5, v/v) as optimum mobile phase. Densitometric detection was carried out at 242 nm. The developed method has been successfully applied for the estimation of linagliptin in tablet dosage form.

Keywords: Linagliptin, HPTLC, Stress degradation, Tablet dosage form.

INTRODUCTION

The objective of a pharmacist is to develop a drug and make it available to a common patient, in a form that is safe, stable and efficacious. Since safety of a drug is heavily dependent on its stability, it is necessary to monitor stability of a drug molecule individually as well as in a formulation using stability indicating method. Since the small amounts of impurities and the decomposition products can be resolved and quantified only by stability indicating method, rather than general assay methods, development of stability indicating methods is the need of the hour. Stability testing is a way to demonstrate that the pharmaceutical product, either a bulk drug or the formulation thereof, would meet the laid down specifications within the acceptance criteria throughout its lifetime. Moreover, the data generated during the stability testing is an important requirement for regulatory approval of any drug or formulation. Scientists have realized that the potential adverse effects of instability in pharmaceutical products can be any one or more of the following *viz.* decrease in the amount of active ingredient, alteration in bioavailability, loss of content uniformity, decline of microbiological status, loss of pharmaceutical elegance and patient acceptability, loss of package integrity *etc*. As per the guidelines issued by ICH and other regulatory bodies [1,2], stress degradation studies are require to be conducted under various conditions of pH, light, oxidation, dry heat *etc*.

Linagliptin (LING), chemically, 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-ynyl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]purine-2,6-dione (Fig. 1) is an inhibitor of dipeptidylpeptidase-4 (DPP-4) enzyme used to lower the increased blood sugar level [3].

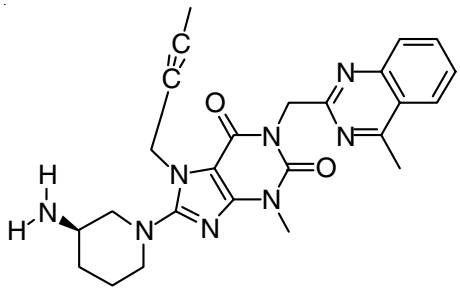

Fig. 1. Structure of linagliptin

Review of literature on studies of quantitative estimation of linagliptin revealed that several spectrophotometric [4-7], plasma extraction [8,9], ultra performance liquid chromatographic [10] and high performance liquid chromatographic [11-19] methods were reported either as single drug or in combination with other drugs. Stability indicating high performance liquid chromatographic methods [20-23] was also reported for the drug individually or in combination with other drugs, which involved the stress degradation studies of bulk drugs only.

One HPTLC method reported by Rajasekaran *et al*. [24] stating the stability study for simultaneous estimation of metformin HCl and linagliptin wherein both the drugs were exposed to acid and alkaline hydrolysis, oxidative stress condition and photolytic stress condition. Unfortunately, this study does not covered neutral hydrolytic and dry heat degradation which is recommended by ICH. The study also reported 100 % degradation of linagliptin, which is no longer recommended and resolution of linagliptin was poor with peak broadening. So there was need to develop suitable stability indicating densitometric method for determination of linagliptin. Based on this fact, we have developed a selective, precise, accurate and sensitive high performance thin layer chromatography method for the estimation of linagliptin in the presence of its degradation products by accelerated stability studies to overcome the defects of reported stability indicating method with well resolved peak from its degradation products and acceptable degradation. The drug was degraded by acid, alkali, oxide, dry heat and UV light to check the stability and to develop the stability indicating method.

EXPERIMENTAL

Pharmaceutical grade working standard linagliptin was obtained from Getz Pharma Research Pvt. Ltd. (Thane, India) used as such without further purification. Pharmaceutical dosage form used in this study was Tradjenta tablets (Avanscure Life Sciences Pvt. Ltd., Haryana, India) labeled to contain 5 mg of linagliptin was procured from the local market. Chloroform, methanol (all AR grade) were obtained from Sisco Research Laboratories (Mumbai, India).

CAMAG HPTLC system equipped with Camag Linomat V sample applicator, Hamilton syringe (100 µL), Camag TLC scanner-3 with winCATS software version 1.4.2 and Camag twin-trough chamber (10 × 10 cm), silica gel 60 F_{254} TLC plates (20 × 20 cm, layer thickness 0.2 mm, E. Merck, Germany) were used for the present study.

The chromatographic resolution of drug was performed by linear ascending development in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using chloroform:methanol (8.5:1.5, v/v) as mobile phase. The chamber was saturated with mobile phase vapour for 15 min. The development distance was 9 cm and the development time approximately 15 min. The slit dimensions 6 mm × 0.45 mm and scanning speed of 20 mm s^{-1} was employed. After chromatographic development, plates were dried and densitometric evaluation was done on CAMAG thin layer chromatography scanner-3 at 242 nm for all developments operated by winCATS software version 1.4.2.

Selection of detection wavelength: Stock solution of the drug was prepared in 10 mL of methanol and UV spectra was taken and it was found that linagliptin showed maximum absorbance at 242 nm and was selected as wavelength for the detection.

Preparation of standard stock solution: Accurately weighed 10 mg of linagliptin was dissolved in 10 mL of methanol to get concentration of 1 mg mL^{-1}. From the above solution, 1 mL was further diluted to 10 mL with methanol to get solution of final concentration100 ng μL^{-1}.

Tablet formulation analysis: For tablet formulation analysis, 20 tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 5 mg of linagliptin was weighed and transferred to a 10 mL volumetric flask containing approximately 7 mL of methanol. The contents were sonicated for 10 min and volume was made with the methanol. The resulting solution was filtered through Whatman filter paper No. 41 and 1 mL of filtrate was further diluted to 10 mL with methanol. Four micro-liter volume of this solution was applied to a TLC plate to furnish final concentration of 200 ng $band^{-1}$. After chromatographic development the peak areas of the bands were measured at 242 nm and the amount of drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Forced degradation study: The stability studies were examined by subjecting the bulk drug as well as formulation to the physical stress and stability was accessed. The hydrolytic studies were carried out by refluxing the stock solution of drug (50 ng μL^{-1}) with 5 N HCl at 45 °C for 0.5 h and 1 N NaOH at 80 °C for 1 h, respectively. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 400 ng $band^{-1}$. Neutral hydrolysis study was performed by refluxing the drug with water for 72 h. The oxidative degradation was carried out in 30 % H_2O_2 at 80 °C for 0.5 h and sample was diluted with methanol to obtain 400 ng $band^{-1}$ solution. Thermal stress degradation was performed by keeping drug in oven at 105 °C for period of 72 h. Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt h square $meter^{-1}$ for 7 days. Thermal and photolytic samples were diluted with methanol to get concentration of 400 ng $band^{-1}$.

RESULTS AND DISCUSSION

Method optimization: The aim of present research work was to develop stability indicating HPTLC method, which would be capable to give the satisfactory resolution between linagliptin and its degradation products. Different solvent systems containing various ratios of toluene, methanol, acetic acid, chloroform and ethyl acetate were examined (data not shown) were examined to separate and resolve spot of linagliptin from its impurities and other excipients present in formulation. Finally, the mobile phase comprising of chloroform: methanol (8.5: 1.5, v/v) was selected as optimal for obtaining well defined and resolved peak. Densitometric detection was performed at 242 nm. The retention factor (R_f) was found to be 0.28 ± 0.004 (Fig. 2).

The stress degradation results indicated that drug was found to be susceptible to acid and base catalyzed hydrolysis, oxidation, thermal stress as well as photolysis and found to be stable in neutral hydrolytic condition. Figs. 3 and 4 show the densitograms of acid and alkali hydrolytic degradation, while Figs. 5-7 show the densitograms of oxidative degradation, thermal degradation and photolytic degradation, respectively. Marked degradation in the densitograms was observed but the degraded products were well resolved from the drug indicating specificity of the method. The findings of degradation studies are represented in Table-1.

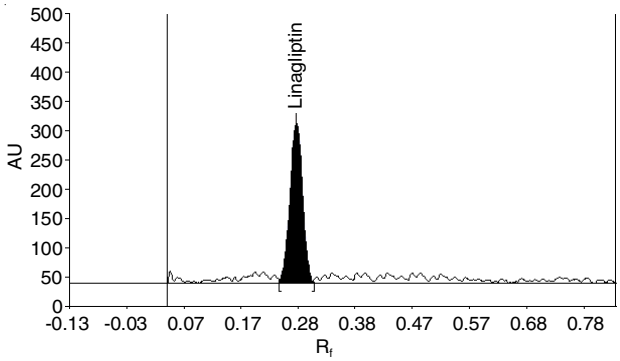

Fig. 2. Representative densitogram of standard solution of linagliptin (300 ng band^{-1}, R_f = 0.28 ± 0.004)

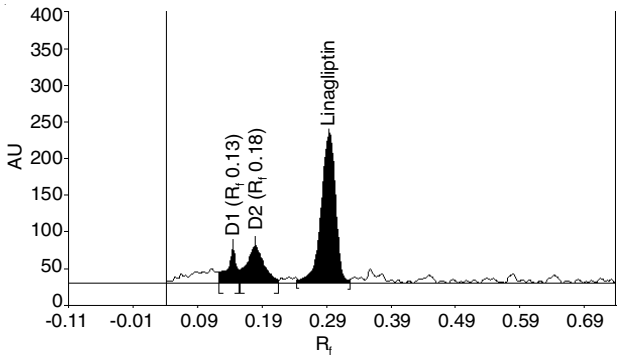

Fig. 3. HPTLC densitogram after acid hydrolysis at 242 nm with 5 N HCl at 45 °C for 0.5 h

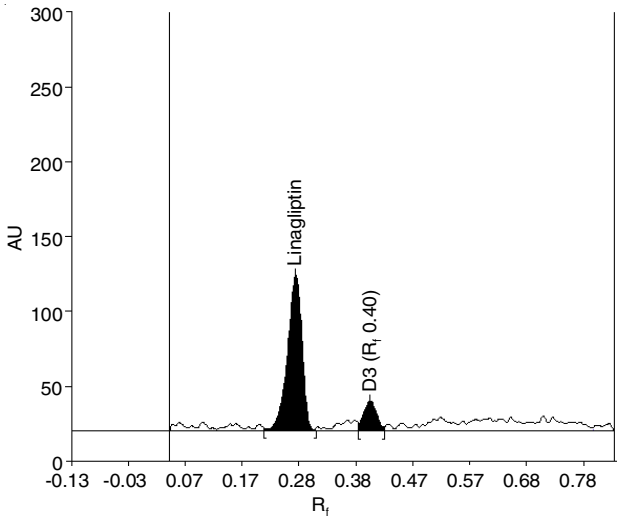

Fig. 4. Densitogram after alkali hydrolysis at 242 nm with 1 N NaOH at 80 °C for 1 h

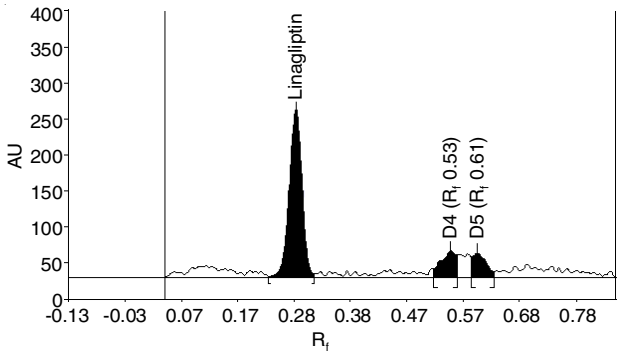

Fig. 5. Densitogram after oxidative degradation by using 30 % H_2O_2 at 80 °C for 0.5 h

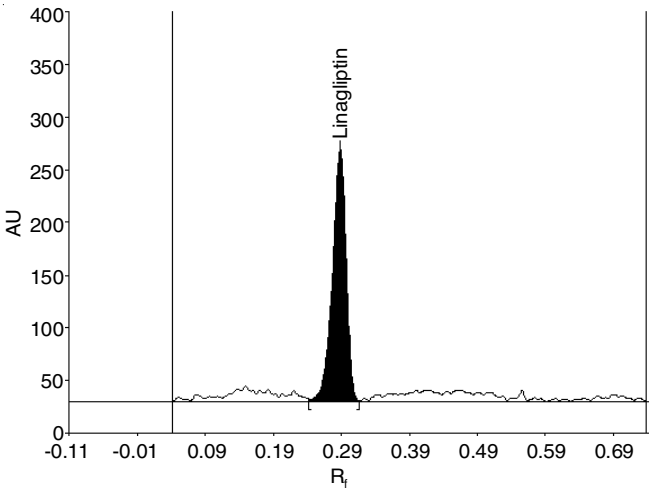

Fig. 6. Densitogram of linagliptin after dry heating at 105 °C for 72 h

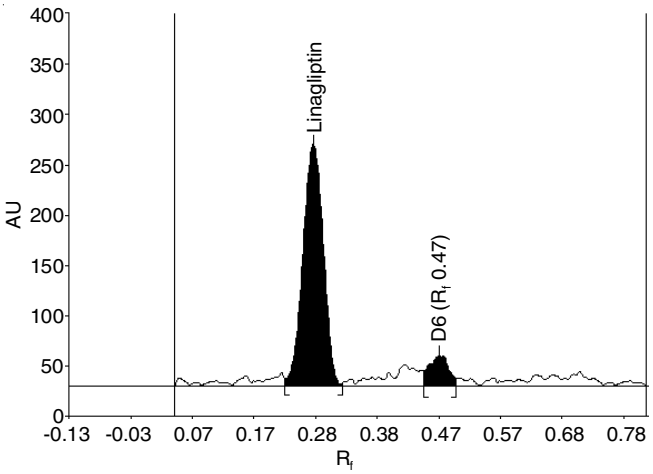

Fig. 7. Densitogram after exposure to UV light

TABLE-1
RESULTS OF FORCED DEGRADATION STUDIES

Stress conditions/duration	Assay of active substance (%)	R_f values of degraded products
Acid/5 N HCl/Refluxed at 45 °C for 0.5 h	75.48	0.13, 0.18
Alkali/1 N NaOH/Refluxed at 80 °C for 1 h	79.87	0.40
Neutral/H_2O/Reflux for 72 h	100.02	–
Oxidation/30 % H_2O_2/Refluxed at 80 °C for 0.5 h	83.60	0.53, 0.61
Photolysis: UV light 200 watt h square meter^{-1}	89.83	0.47
Dry heat/105 °C/72 h	93.18	–

Accelerated stability study of marketed formulation: Marketed formulation (Tradjenta tablets labeled to contain 5 mg of linagliptin) was initially assayed by the developed method and then subjected to accelerated stability testing by exposing the formulation to various conditions such as 30 °C, 75 % RH, 30 °C, 75 % RH and 40 °C, 75 % RH for a period of 6 months. The sample concentration chosen was 200 ng band^{-1}. The results demonstrated that assay of marketed formulation was retaining and was found to be within limits after the analysis at the end of six months accelerated stability studies (Table-2).

TABLE-2
RESULTS OF ASSAY OF FORMULATION EXPOSED FOR 6 MONTHS

Standard/Sample	Area	Average area	Amount found (ng band^{-1})	Assay (%)
Standard	3961 3928	3945	201.09	100.54
Sample (6 M at 30 °C, 65 % RH)	3937 3876	3907	198.23	99.11
Sample (6 M at 30 °C, 75 % RH)	3948 3847	3898	197.55	98.77
Sample (6 M at 40 °C, 75 % RH)	3835 3739	3787	189.23	94.61

Analytical method validation: The developed method was validated with respect to linearity, accuracy, precision, robustness, limit of quantization (LOQ), limit of detection (LOD) to ensure the reliability of results of analysis as per International Conference on Harmonisation (ICH) guidelines for validation of analytical procedures: text and methodology Q2 (R1) [25]. The linearity of was determined by application of aliquots of 1, 2, 3, 4, 5 and 6 µL of standard solution of linagliptin (100 ng µL^{-1}) on TLC plate. The plate was developed and scanned under above established chromatographic conditions. Each standard in six replicates (n = 6) was analyzed and peak areas were recorded. The linearity was observed in the range of 100-600 ng band^{-1} with linear regression equation y = 13.28x + 1274 and correlation coefficient 0.996. The calibration curve obtained for linagliptin is shown in Fig. 8.

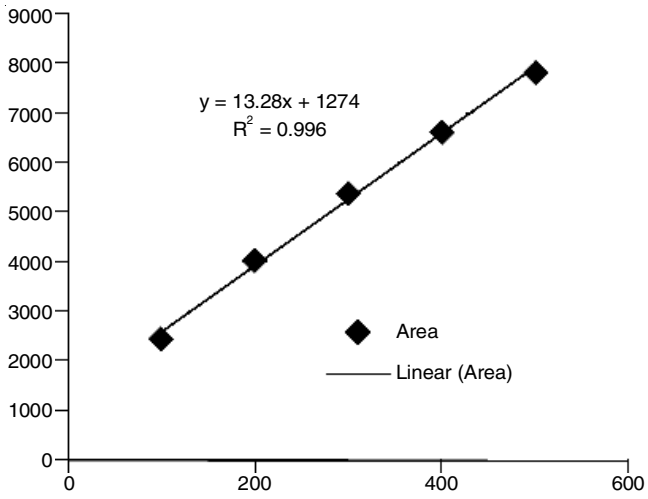

Fig. 8. Calibration curve for linagliptin

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated as signal-to-noise ratio of 3:1 and 10:1 and were found to be 19.92 and 60.36 ng band^{-1} respectively. The repeatability of separation was accessed by intra-day and inter-day precision studies with concentrations of 300, 400 and 500 ng band^{-1} and % RSD was not more than 2. The results of precision studied are represented in Table-3. Recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80, 100 and 120 %. At each level of the amount, three determinations were carried out. The method was found to be accurate and precise, as indicated by recovery studies the recoveries were close to 100 % and % RSD not more than 2 (Table-4). Robustness of the method (n = 3) was examined at a concentration level of 600 ng band^{-1} under the influence of small, deliberate variations of the analytical parameters. Parameters varied were mobile phase composition (± 1 % methanol), chamber saturation time (± 5 %). The areas of peaks of interest remained unaffected by small changes of the operational parameters and % RSD was within the limit (< 2 %) indicating the robustness of the developed method (Table-5).

TABLE-3
PERCENTAGE RECOVERY RESULTS FOR INTRA-DAY AND INTER-DAY VARIATION STUDIES

Concentration (ng/band)	Recovery (%)	
	Intra-day	Inter-day
300	101.43	100.82
300	100.70	101.20
300	99.59	99.97
400	100.09	99.92
400	99.84	99.69
400	100.32	100.79
500	98.81	98.99
500	98.97	99.96
500	97.89	98.07
Mean (% Recovery)	99.73	99.93
% RSD	0.58	0.71

TABLE-5
RELATIVE STANDARD DEVIATION OF PEAK AREA OBTAINED UNDER ROBUSTNESS STUDY

Parameters	% RSD*
Mobile phase composition (± 1 % methanol)	0.74
Chamber saturation time (± 5 min)	0.53

*Average of three determinations.

Conclusion

Stability indicating HPTLC method has been developed and validated for the determination of linagliptin as bulk drug and in tablet dosage form. The developed method is simple, precise, accurate and selective and can be used for quantitative analysis of linagliptin in pharmaceutical dosage form as well as for routine analysis in quality control laboratories. Marketed formulation was found to retain assay of linagliptin within limits even after the analysis at the end of six months of accelerated testing.

TABLE-4
RECOVERY STUDIES OF LINAGLIPTIN

Level of recovery (%)	Amount of analyte taken (ng band^{-1})	Amount added (ng band^{-1})	Mean amount recovered (ng band^{-1})	Mean recovery (%)	RSD (%)
80	200	160	358.91	99.69	0.85
100	200	200	400.72	100.08	0.43
120	200	240	439.05	99.78	0.31

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