ORIGINAL RESEARCH

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STABILITY INDICATING HPTLC METHOD FOR THE ESTIMATION OF EVOGLIPTIN TARTRATE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT:

A novel, simple and economic stability indicating high performance thin layer liquid chromatographic method was developed and validated as per the ICH guidelines for the quantitative estimation of Evogliptin Tartrate in pharmaceutical tablet dosage form. The mobile phase consisted of Chloroform: Acetone (6:4). The concentration of evogliptin tartrate was determined by densitometry at 254 nm.Thecalibration curve as linear over the concentration range of 1000-6000 ng/spot. Accuracy of method was determined through recovery studies which were found to be 94.29 –96.56 %. The LOD and LOQ were found to be 930.14 n g /spot and 2768.06 ng/spot respectively. Evogliptin tartrate was subjected to forced degradation like acid and alkali hydrolysis, oxidation, neutral, photo and thermal degradation. Validation studies demonstrated that the proposed RP-HPTLC method is simple, specific, rapid, reliable and reproducible. Hence the proposed method can be applied for the routine quality control analysis of Evogliptin Tartrate in bulk and Pharmaceutical tablet dosage forms.

KEYWORDS: Evogliptin Tartrate, HPTLC, Recovery studies, Stability, ICH guidelines

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INTRODUCTION:

Evogliptin tartrate is described chemically as (3R)-4-[(3R)-3-amino- 4-(2, 4, 5-trifluorophenyl) butanoyl]-3-[(2-methylpropan-2-yl) oxymethyl] piperazin-2-one ;(2R, 3R)-2, 3- dihydroxybutanedioic acid is shown as fig1. It has a molecular formula of $C_{23}H_{32}F_3N_3O_9$ and a molecular weight of 551.05 gm/mol. Evogliptin tartrate is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor for the treatment of typeII diabetes which improves glycaemic control by inhibiting DPP-4 inactivation of the incretin hormonesglucagon like peptide -1(GLP-1) and glucose – dependent insulinotropic polypeptide(GIP). Evogliptin tartrate is used to improve glycaemic control by stimulating glucose-mediated incretin secretion, which results in increased insulin secretion and decreased glucagon release with a lower risk of hypoglycemia. It also has a positive effect on metabolic abnormalities like obesity, hypertension, and dyslipidaemia, which are all linked to type 2 diabetes (non-insulin-dependent diabetes mellitus) It is Soluble in water and dilute methanol, acetonitrile while freely soluble in organic solvents like ethanol, acetone, chloroform and ethyl acetate.

According to the literature survey, only a few analytical methods, such as HPLC, UV and LC-MS, were reported for the estimation of evogliptin tartrate. Liquid chromatography with tandem MS10 or Orbitrap MS methods was reported for the determination of Evogliptin in biological fluids of humans. Although a literature survey reveals that no analytical method is reported related to the stability indicating high-performance thin-layer chromatographic (HPTLC) determination of evogliptin tartrate in bulk and tablet dosage forms. Therefore, the main objective of the proposed method was to develop a simple, new, accurate, precise, sensitive and robust HPTLC method for the estimation of Evogliptin Tartrate in bulk and tablet dosage form and validate it as per ICH guidelines.

MATERIALS AND METHODS:

Chemicals and reagents:

Active pharmaceutical ingredient (API) working standard of Evogliptin Tartrate was received as gift samples from Vivan Life sciences Pvt Ltd., Hyderabad, India respectively. The Pharmaceutical dosage form used in this study was Valera tablets manufactured by Alkem Laboratory Ltd. which were purchased from local market. Acetonitrile, Chloroform, and Acetone (all AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumental and Chromatographic Conditions:

Chromatographic separation of the drug was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm × 10 cm with 0.2mm layer thickness) from E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 500 µl sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 ×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Chloroform: Acetone (6:4: v/v) as mobile phase. The mobile phase was saturated in the chamber for 20 min. After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on a CAMAG thin layer chromatography scanner at 254 nm for all developments operated by WINCATS software. The source of radiation utilized was a deuterium lamp

emitting a continuous UV spectrum between 200 to 400 nm.

EXPERIMENTAL, RESULT AND DISCUSSION: SELECTION OF MOBILE PHASE AND CHROMATOGRAPHIC CONDITIONS:

On the working standard solution of Evogliptin tartrate 1000 µg/ml, chromatographic separation investigations were performed. Initially, studies were carried out on standard TLC plates using various solvents in varied amounts to acquire the appropriate R_F and shape for the drug peak. After a few attempts, the mobile phase was determined to be Chloroform: Acetone (6:4 v/v), which produced acceptable peak values. Other chromatographic conditions like chamber saturation time, run length, sample application volume was optimized.

Preparation of working standard solution:

Accurately weighed 10mg of Evogliptin tartrate working standard was taken in 10ml volumetric flask dissolved and diluted to volume with mobile phase and mixed.

Preparation of sample solution:

A tablet containing 10 mg of Evogliptin tartrate was weighed and powdered. Powder equivalent to 10 mg of drug was transferred to 10 ml volumetric flask and volume was made up with acetonitrile to get concentration (1000 µg/ml) and was sonicated for 10 min. The solution was filtered and 3µl of the resultant solution was applied on a TLC plate to get a concentration of 3000 ng/band.

Selection of Analytical Wavelength:

From the standard stock solution further dilutions were made using acetonitrile and scanned over the range of 200 – 400 nm and the spectra were obtained. It was observed that the drug showed considerable absorbance at 254 nm.

Method validation:

Accuracy: Accuracy of a method is defined as the closeness of a measured value to the true value. To carry out accuracy study of proposed method, the recovery studies were performed by spiking the previously analyzed sample of Evogliptin tartrate with the known amounts of pure drug at different concentration levels. The spiked levels were 50%, 100% and 150%. The basic concentration of the sample chosen was 1000 ng/spot. The % recovery was calculated three times at each level and the average % recovery was calculated. The % recovery was determined from the linearity equation. The results obtained are shown in **Table1**.

Linearity: Linearity is the ability of the method to respond proportionally to the changes in the concentration of the analyte in a sample. From the standard stock solution (1000 µg/ml) of Evogliptin, Six replicates per concentration were spotted. The linearity (the relationship between peak area and concentration) was determined by analyzing six concentrations over the concentration range of 1000-6000 ng/spot for Evogliptin. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve as shown in **Fig 2**. The results found to be linear with the regression equation of y=2.471x+1086. with $R^2 = 0.991$.

Limit of detection and Limit of quantitation: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but necessarily quantitated as an exact value. The quantification limit of an individual analytical procedure is the lowest amount of analyte

in a standard which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ were experimentally verified by the known concentration of Evogliptin tartrate until the average response approximately 3 or 10 times the standard deviation of the responses for five replicate determinations. The results obtained are shown in **Table 3**.

LOD and LOQ are calculated from the formula: -

LOD = 3.3 σ / S LOQ = 10 σ / S

Where, σ = standard deviation of Y-intercept S = slope of the calibration curve.

Precision: The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day studies, 3 replicates of 3 concentrations were analyzed on the same day and percentage RSD was calculated. For the inter-day variation studies, 3 replicates of 3 concentrations were analyzed on 3 consecutive days and percentage RSD was calculated. For intraday precision and inter-day precision results obtained are shown in **Table 4.**

Repeatability: Repeatability of measurement of the peak area was determined by spotting 3000(ng/spot) Evogliptin tartrate of drug solution on a pre-coated TLC plate. The separated spots were scanned six times without changing the position of the plate and the percentage RSD was calculated. The results obtained are shown in Table 5.

Ruggedness: It expresses the precision within laboratory variations like different days, different analyst and different equipments. Ruggedness of the method was assessed by spiking the standard concentration of Evogliptin tartrate 3000(ng/spot) 6 times in two different days with different analyst. The results obtained are shown in **Table 6**

Robustness: Robustness of the method was determined by carrying out the analysis under conditions during which detection wavelength, chamber saturation time were altered, Time was also changed from spotting to development and development to scanning and the effects on the area were noted. It was found that the method is robust. The results obtained are shown in **Table 7**.

Assay: Valera 10 mg tablet formulation analysis was carried out as mentioned under the preparation of sample solution. The procedure was repeated six times. The sample solution was injected and the area was recorded. Concentration and % recovery were determined from linear equation **Table 8.**

Table 1: Results for Accuracy

Drug	Label Claim mg/tab	Estimated amount (mg/ ml)	Spike Level (%)	Amount of drug added (%)	Percentage Recovery (%)	%RSD*
Evogliptin tartrate	5mg	4.57	50	50	94.29	1.194
			100	100	95.70	0.767
			150	150	96.56	1.217

Table 2: Data for linear graph

Sr.No	Concentration	Peak area
1.	1000	3100.55
2.	2000	5402.71
3.	3000	8124.78
4.	4000	10468.08
5.	5000	12258.71
6.	6000	14196.29

Table 3: Results for LOD &LOQ

Sample	LOD	LOQ
Evogliptin tartrate	930.14 ng/spot	2768.06 ng/spot

*mean of six observations

Table 4: Results for Intraday precision and Interday precision

Drug	Concentration (ng/spot)	Intraday			Interday		
		Peak area*	SD	% RSD*	Peak area*	SD	% RSD*
Evogliptin tartrate	2000	5398.01	19.24	0.356	5408.02	17.27	0.319
	3000	8107.87	23.34	0.287	8112.52	29.54	0.364
	4000	10464.19	17.03	0.162	10459.34	18.48	0.176

*mean of six observations

Table 5: Results for Repeatability

Concentration (ng/spot)	Peak area*	%RSD*
3000	8110.19	0.302

*mean of six observations

Table 6: Result for Ruggedness

Drug	Concentration (ng/spot)	Mean Peak area*	%RSD*
Evogliptin tartrate	Day I, Analyst I		
	3000	8122.75	0.379
	Day II, Analyst II		
	3000	8140.98	0.426

*mean of six observations

Table7: Results for Robustness

Parameters	Modification	Percentage Recovery (%)
Mobile Phase Ratio	3:7	99.56
	7:3	98.56
Development Distance	9 mm	98.92
Detection Wavelength(nm)	264 nm	98.82
Slit Dimension	5.00 x .30m micro	98.76

*mean of six observations

Table8: Assay of Marketed Formulation

Drug	Peak Area	Amount Recovered (µg/ml)	% Recovery	± %RSD
Evogliptin tartrate	5410.7	1931.53	96.57	0.90
	5380.2	1917.91	95.89	
	5450.5	1949.30	97.46	
	5438.3	1943.85	97.19	
	5467.6	1956.94	97.84	
	5489.5	1966.72	98.33	

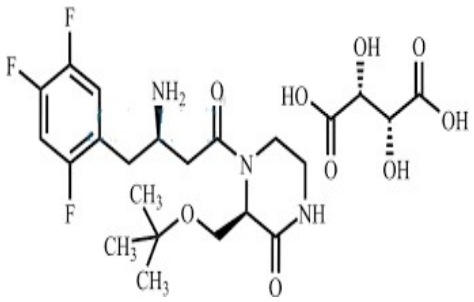

Fig 1: Structure of Evogliptin tartrate

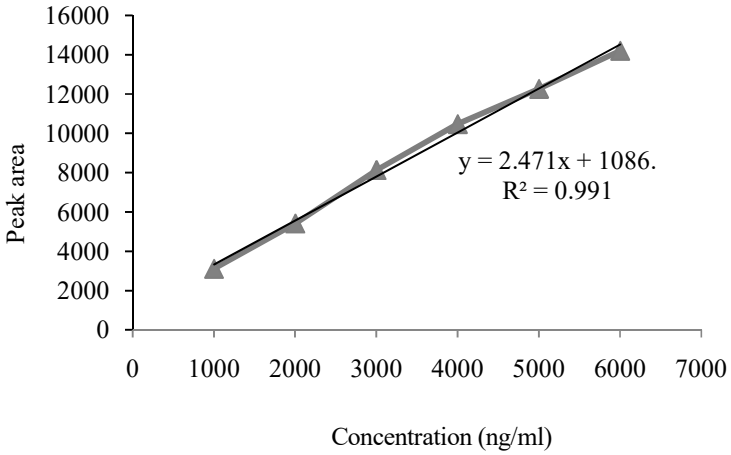

Fig 2: Calibration curve of Evogliptin tartrate

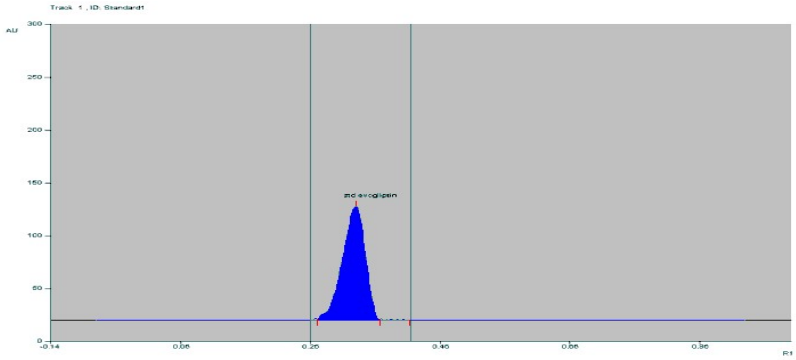

Fig 3: Chromatogram of Standard Evogliptin tartrate (1000 ng/band)

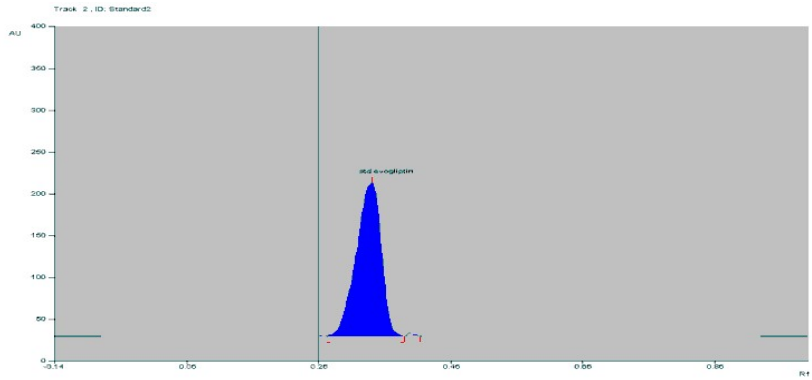

Fig 4: Chromatogram of Standard Evogliptin tartrate (2000 ng/band)

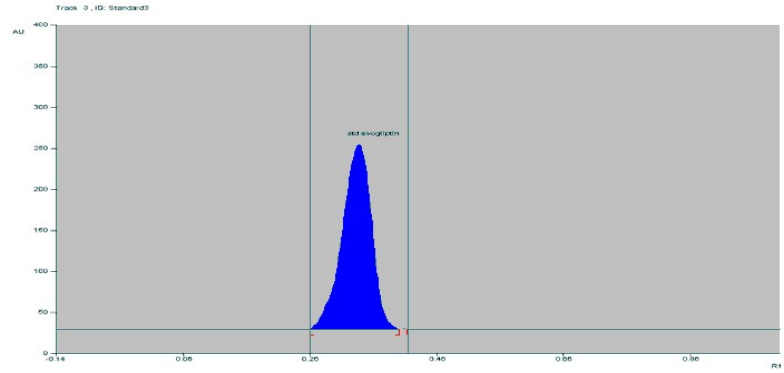

Fig 5: Chromatogram of Standard Evogliptin tartrate (3000 ng/band)

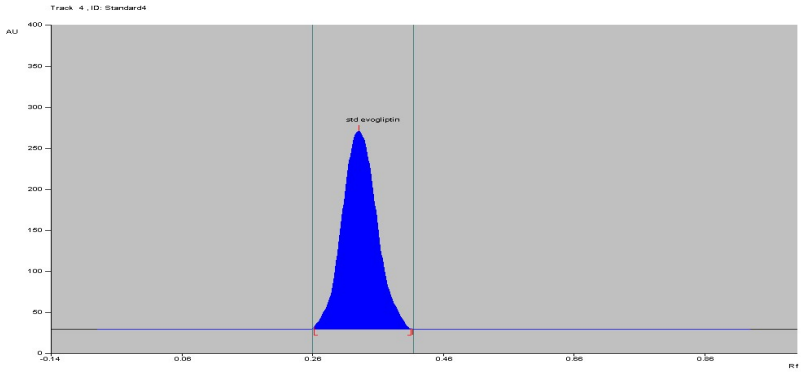

Fig 6: Chromatogram of Standard Evogliptin tartrate (4000 ng/band)

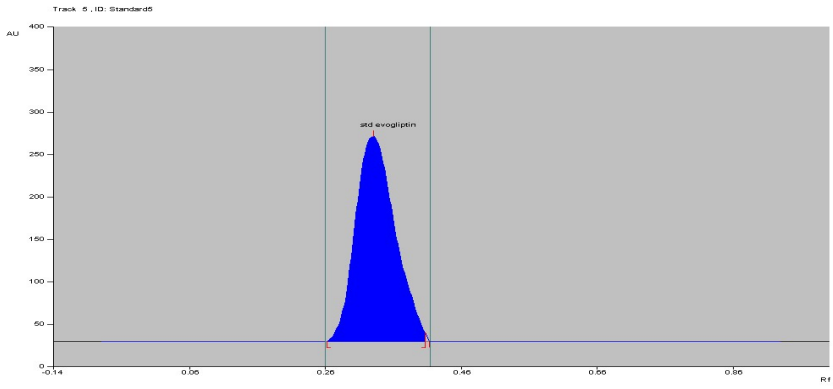

Fig 7: Chromatogram of Standard Evogliptin tartrate (5000 ng/band)

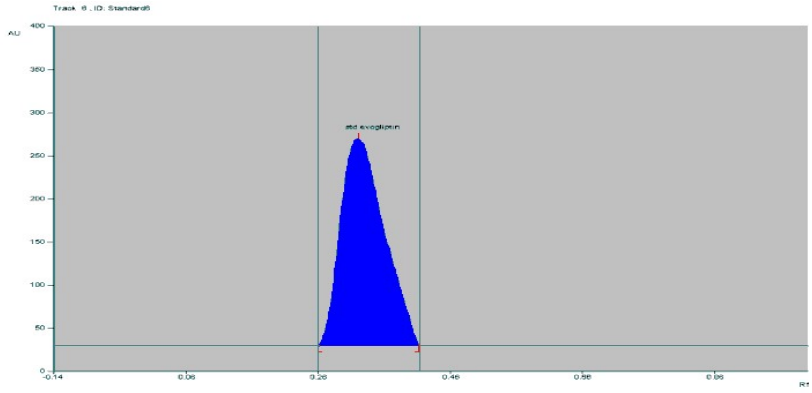

Fig 8: Chromatogram of Standard Evogliptin tartrate (6000 ng/band)

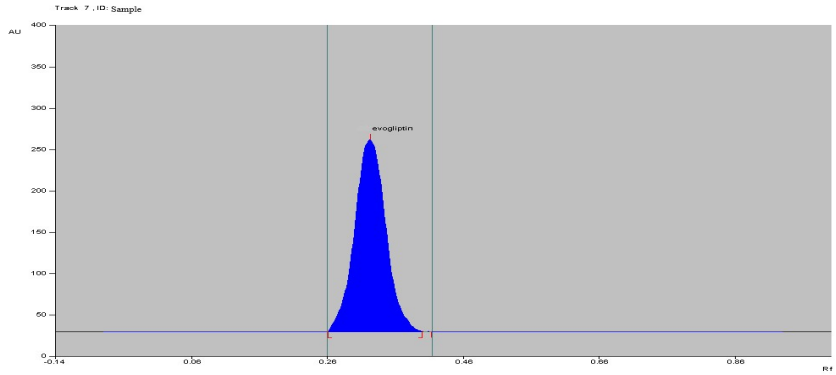

Fig 9: Chromatogram of Sample Evogliptin tartrate (3000 ng/band)

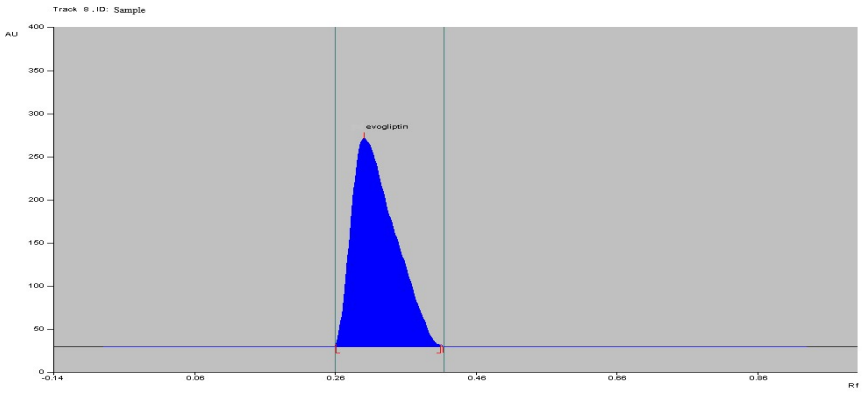

Fig 10: Chromatogram of Sample Evogliptin tartrate (5000 ng/band)

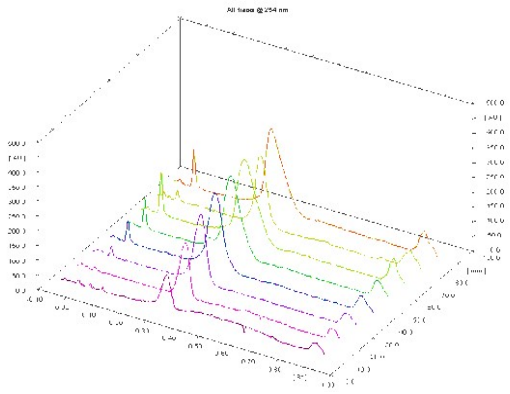

Fig 11: Densitogram of Linearity of Evogliptin tartrate (1000-6000 ng/band)

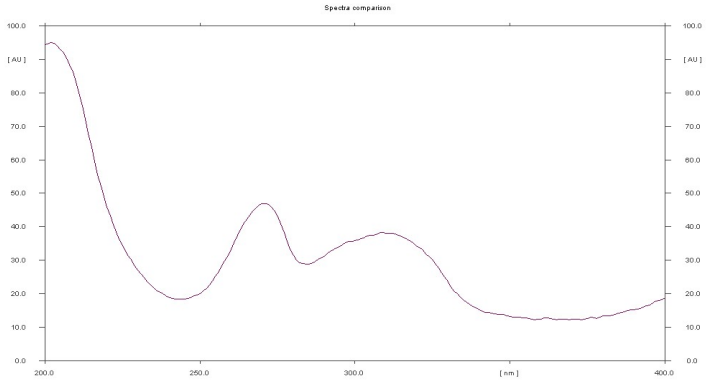

Fig 12: Spectrum of Standard Evogliptin tartrate at 254 nm

Table 9: Summary of Validation Parameters

Sl.No	Parameters	Evogliptin tartrate
1	Linearity	y=2.471. x+1086.
2	Range	1000-6000 ng/spot
3	Precision Intraday Interday	%RSD 0.162 - 0.356 0.176 - 0.364
4	Assay	97.2%
5	Accuracy 50% 100% 150%	%Recovery(Mean) 94.29 95.70 96.56
6	LOD	930.14ng/spot
7	LOQ	2768.06ng/spot
8	Robustness	Robust

Forced degradation studies:

In forced degradation studies, Evogliptin was subjected to acidic, alkaline, neutral, oxidative, photolytic and thermal stress conditions. The percentage assay and percentage degradation compared with the control sample and is given in **table10.** In acidic, alkaline, neutral, oxidative, photolytic and thermal the % degradation was found to be 6.24%, 8.61%, 13.71%, 9.46%, 17.92% and 8.41% respectively. For each study, sample solution of Evogliptin subjected to stress condition. Dry heat and photolytic degradation were carried out in solid state. The drug was found to be more degradable under oxidative degradation as compared to other stress conditions. The resultant chromatograms of various stress conditions are shown in respective figures **(fig-13- 18).**

Degradation under Acid-Catalyzed Hydrolytic Condition:

To 10 ml of sample solution of Evogliptin, 10 ml of 1 N HCl was added. The above solution was kept for 5 hours at room temperature at 60ºC. Sample solution of evogliptin was spotted on TLC plate of size 10x10cm and the plate was run with mobile phase consisting of Chloroform: Acetone in the ratio of 6:4 v/v/. Average 94.18 % Evogliptin was recovered with no peak of degradant. Representative densitogram is shown in **Fig. 13.**

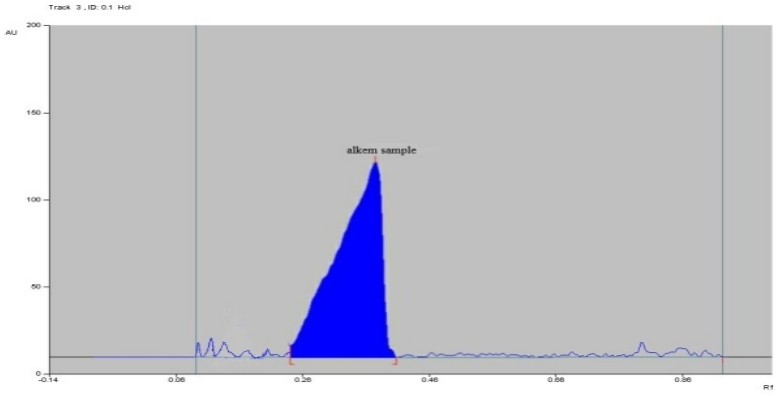

Fig 13: Chromatogram of Acid Induced degradation of Evogliptin tartrate

Degradation under Alkali-Catalyzed Hydrolytic Condition:

To 10 ml of sample solution of Evogliptin, 10 ml of 1N NaOH was added. The above solution was kept for 5 hours at room temperature at 60ºC. Sample solution of evogliptin was spotted on TLC plate of size 10x10cm and the plate was run with mobile phase consisting of Chloroform: Acetone in the ratio of 6:4 v/v/. Average 91.39 % Evogliptin was recovered with three peaks of degradant. Representative densitogram is shown in **Fig. 14.**

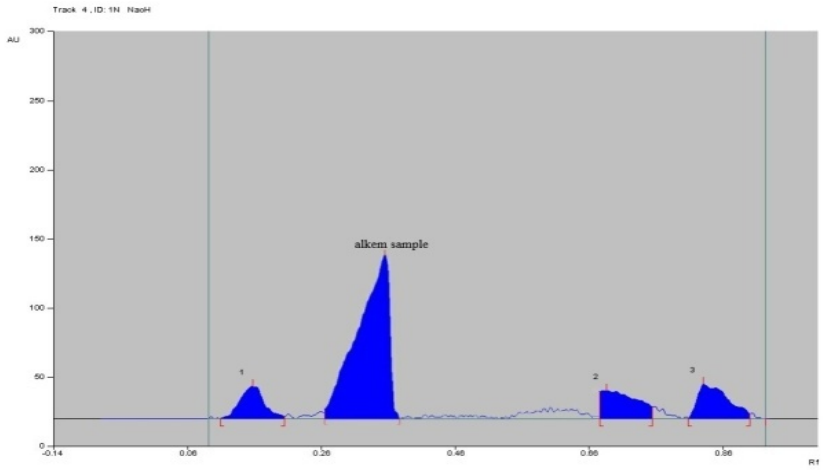

Fig 14: Chromatogram of Alkali Induced degradation of Evogliptin tartrate

Degradation Under Neutral Hydrolytic Condition:

To 10 ml of sample solution of Evogliptin, 10 ml of distilled water was added. The above solution was kept for 5 hours at room temperature at 60ºC. Sample solution of evogliptin was spotted on TLC plate of size 10x10cm and the plate was run with mobile phase consisting of Chloroform: Acetone in the ratio of 6:4 v/v/. Average 86.29% of Evogliptin was recovered with no peak of degradant. Representative densitogram is shown in **Fig. 15.**

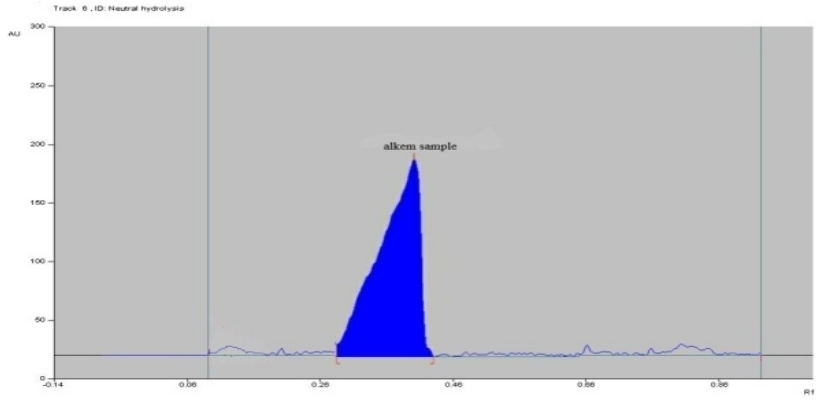

Fig 15: Chromatogram of Neutral degradation of Evogliptin tartrate

Degradation Under Oxidative Condition:

To 10 ml of sample solution of Evogliptin, 10 ml of 3% H_2O_2 was added. The above solution was kept for 5 hours at room temperature at 60ºC. Sample solution of evogliptin was spotted on TLC plate of size 10x10cm and the plate was run with mobile phase consisting of Chloroform: Acetone in the ratio of 6:4 v/v/. Average 90.54% of Evogliptin was recovered with two peaks of degradant. Representative densitogram is shown in **Fig. 16.**

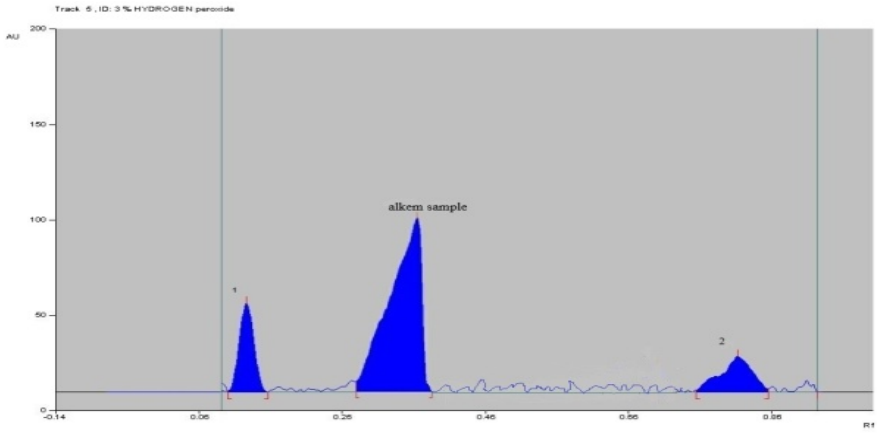

Fig 16: Chromatogram of Oxidative degradation of Evogliptin tartrate

Photo-Degradation Studies:

The photo degradation study of the drug was studied by exposing the drug to sunlight for 24 hours at 7 days. After exposure, the sample was withdrawn, dissolved in acetonitrile and the resultant solution was then applied at TLC plate of size 10x10cm and the plate was run with mobile phase consisting of Chloroform: Acetone in the ratio of 6:4 v/v.Average 82.08% of Evogliptin was recovered with no peak of degradant. Representative densitogram is shown in **Fig. 17.**

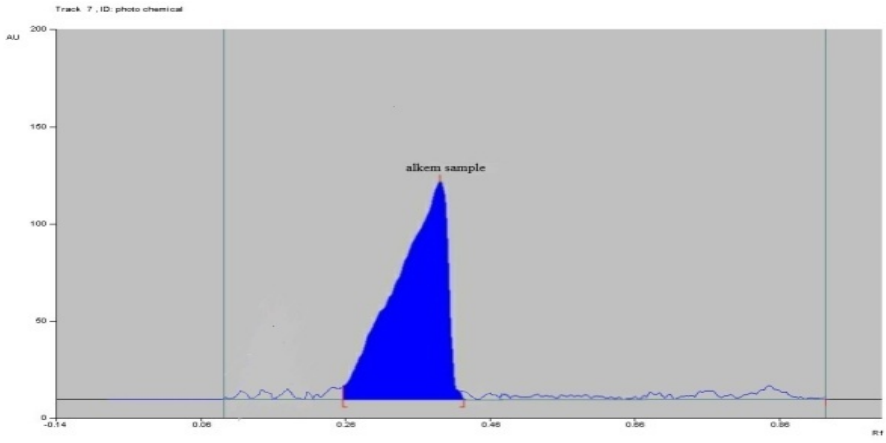

Fig 17: Chromatogram of Photolytic degradation of Evogliptin tartrate

Degradation Under Dry Heat:

Dry heat studies were performed by keeping drug sample in the oven (120 ºC) for a period of 2 hours at 7 days. The sample was withdrawn, dissolved in acetonitrile and diluted to get 1000 µg/ml.The evogliptin solution was applied on TLC plate and the plate was run with mobile phase consisting of Chloroform: Acetone 6:4v/v. Average 91.59% Evogliptin was recovered with no peak of degradant. Representative densitogram is shown in **Fig. 18.**

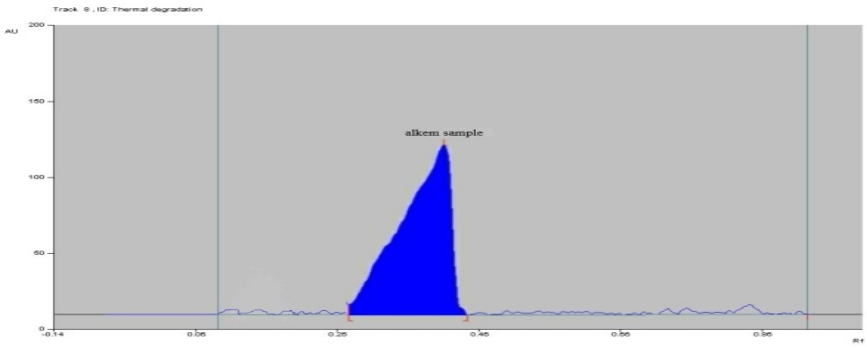

Fig 18: Chromatogram of Dry Heat degradation of Evogliptin tartrate

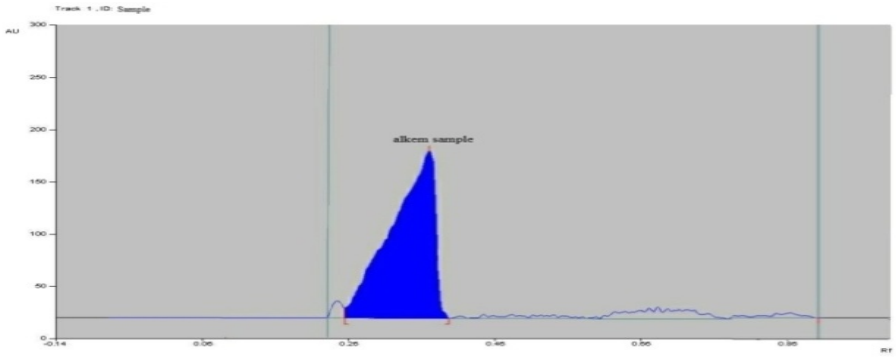

Fig 19: Chromatogram of sample drug subjected to degradation (Evogliptin tartrate R_F = 0.32)

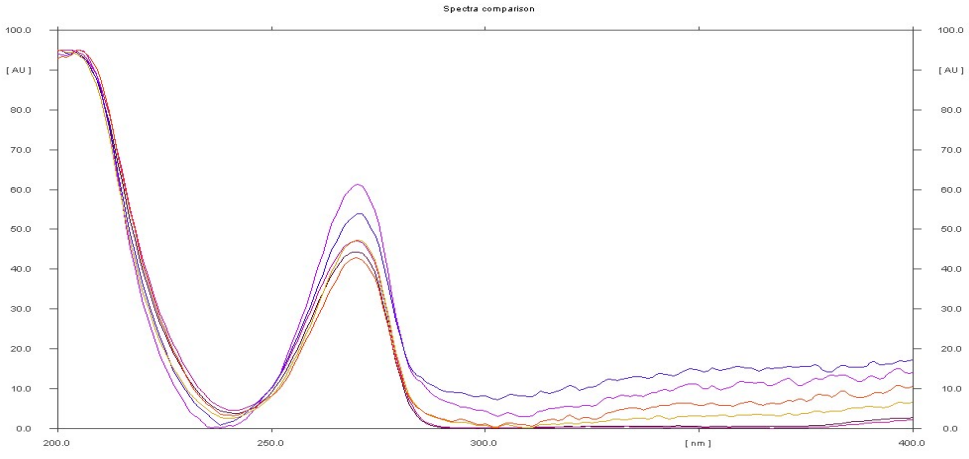

Fig 20: Overlay spectrum of Forced degradation studies

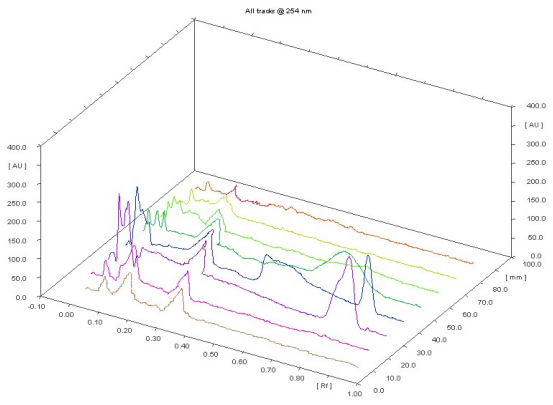

Fig 21: Over all 3D diagram for sample Evogliptin tartrate

Table10: Forced Degradation Studies

Sr. No.	Stressed conditions	Percentage Assay	Rf values of degraded products	Degradation (%)
1	Control sample	97.2%	--	--
2	Acidic/ 0.1 N HCl/ at room temperature 5 h rs	94.18%	--	6.24%
3	Alkaline/ 1 N NaOH /at room temperature 5 hrs	91.39%	0.19,0.68,0.83	8.21%
4	Neutral / H2O / at room /temperature 5 hrs	86.29%	--	13.71%
5	Oxidative/ 3 % H2O2 / at room temperature 5 hrs	90.54%	0.12,0.81	9.46%
6	Photolysis/Sunlight/at 24 hrs/7 days	82.08%	--	17.92%
7	Thermal/Hot air oven/120º C at 2 hrs/7days	91.59%	--	8.41%

CONCLUSION:

A stability indicating validated HPTLC method was developed for the determination of evogliptin tartrate in accordance with the requirements of ICH guidelines. The developed method was found to be simple, accurate, precise, linear, specific, sensitive and cost effective for the determination of Evogliptin tartrate. Moreover, cost per sample analysis is relatively low in comparison with HPLC method. The statistical analysis proves that method is reproducible and selective for the determination of Evogliptin tartrate indicating non-interference of excipients in the estimation. Forced degradation study showed that all degradation products were well separated from evogliptin tartrate under various conditions, thus confirming the method as stability-indicating analytical .This method does not require an internal standard. The outcome of the validation study showed that the developed HPTLC method for the Evogliptin tartrate will serve as a standard protocol for routine analysis in bulk and Pharmaceutical formulations .The proposed method could be applied for routine analysis in quality control laboratory. This method offered several other

advantages including simplicity, rapid, less solvent used, less time of analysis then compared to HPLC method.

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