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AUC, 1ST ORDER DERIVATIVE UV SPECTROPHOTOMETRY AND RP-HPLC ASSAY OF EVOGLIPTIN TARTRATE IN TABLET

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Abstract

Three simple, new, accurate and precise methods were developed and validated on the basis of ICH guidelines for estimation of Evogliptin tartrate in pure and its tablet. **Method 1:** AUC by UV spectrophotometry technique utilizing 262 to 266nm for measurement of analyte in tablet. **Method 2:** 1st Order derivative spectrophotometry utilizing 258nm for estimation of analyte in the tablet. The linearity and range were found to be 10 to 270µg/ml for method 1 & 2. The sample and standard solutions were prepared in the methanol. % RSD was found to be less than 2 in tablet and recovery study of both Method 1 & 2. **Method 3** comprises RP-HPLC method using Enable column C18 and mobile phase containing Methanol & water in the ratio of 70 and 30. The flow rate of 1 ml/min, with detection at 267nm, & temperature 25^0 C were used for analysis purposes. The retention time was found to be 2.806min. The linearity range was found to be 10-80µg/ml. The linear regression equation was found to be Y = 4006.6714 x + 713.6349 and Correlation coefficient (r^2) = 0.9999. % RSD is less than 2 in the precision, recovery and robustness studies of the proposed method. So the method was precise, accurate and robust in nature. Method-3 is more accurate & precise in comparison to method 1 & 2.

Keywords: Evogliptin Tartrate, Validation, AUC, Derivative, RP-HPLC.

INTRODUCTION

Diabetes mellitus (DM) affects about 422 million people around the world, or 8.8% of all adults. It is most common in middle-income and low-income countries.[1] When treating DM, the most important reason for polytherapy is to make drug control processes make more sense and make the drug work better. Metformin and Evogliptin have recently been cleared by the Food and Drug Administration to be used together to treat diabetes. [2] Patients with Type-2 DM (T2DM) are often first prescribed metformin, and then a DPP-4 inhibitor like Evogliptin. Metformin is a biguanide and an oral anti-diabetic medication. Increased insulin sensitivity, decreased body fat, a more favourable lipid profile, better glycemic management, and enhanced vascular function were also noted. The enzyme adenosine monophosphate-activated protein kinase in the liver, which regulates glucose and fatty acid metabolism, has its activity increased. Patients with type 2 diabetes are often given the DPP-4 inhibitor evogliptin to lower their blood sugar levels. Therapy was associated with an increase in glycated haemoglobin (HbA1c) levels, and subsequent treatment was associated with a decrease in these levels; however, DPP-4 have a moderate hypoglycemic effect, and so a response to therapy in terms of HbA1c parameter 7.0% is expected in patients with a moderate increase in glycated haemoglobin levels to begin with.

The chemical name of Evogliptin is **(**3R)-4-[(3R)-3-Amino-4-(2,4,5-trifluorophenyl) butanoyl]-3-{[(2-methyl-2-propanyl)oxy]methyl}-2-piperazinone.[3]

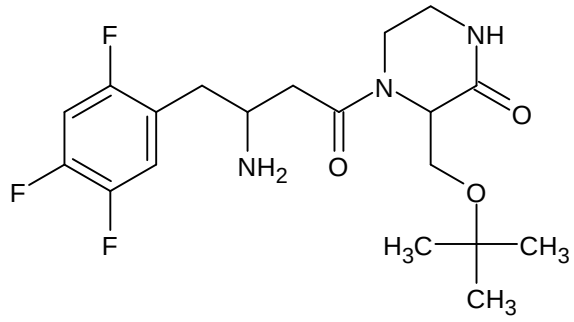

Fig 1: The chemical structure of Evogliptin

Previous work showed that few RP-HPLC methods have been developed for getting the Evogliptin content in dosage form (summarized in Table 1). In these methods different columns, mobile phase systems were taken. The retention time was also varied. The drug alone or in combination was also estimated by UV Spectrophotometry[11-13], by LC-MS[14] and nitrosoamine impurities along with the other drug were estimated by UHPLC-MS[15].

Table 1: Estimation of Evogliptin by RP-HPLC method

Sl. No.	Column	Mobile Phase, Temp & pH	Flow rate (ml/min)	Retention Time (in min)	Ref
1	Hypersil BDS C18 column (250mm × 4.6mm, 5µm)	Buffer: Methanol (45:55) v/v, 4.5	1	5.310	4
2	ACCLAIMED mix mode HILIC-1 (5µ, 150 X 4.6 mm. ID)	NH_4 Acetate: Acetonitrile (30:70) v/v	1	4.8	5
3	Waters XTerra RP-18 150X4.6 mm, 3.5µ column	Acetonitrile: KH_2PO_4: Methanol (50:40:10) v/v, ambient	1	4.468 (EVO) & 2.730 (MET)	6
4	ODS 5 µm (4.6 mm X 250 mm) C18 column	Methanol: Water: Acetonitrile (70:20:10)	1	3.6	7
5	Acclaimed Mix-Mode HILIC-1(5µ, 150 x 4.6 mm id)	15 Mm NH_4 Acetate B; AA-ACN (25:75% v/v)	1	4.86(EVO), 3.81(REM) & 5.88(MET)	8
6	Hypersil BDS C18 (250 mm x 4.6 mm 5 µ)	Methanol: Water: TFA mixture (70: 30: 0.1% v/v), ambient & 6.5	1	4.03	9
7	Water C18 column (250 mm × 4.6 mm, 5 µ)	Methanol and PO_4 buffer (pH 4.5) 60:40% v/v TFA mixture (70: 30: 0.1% v/v), ambient & 6.6	1	2.477	10

This paper describes the development of a simple, linear, accurate, robust, sensitive and reproducible RP-HPLC method which can be easily used for estimation of Evogliptin in bulk and tablet dosage forms.

EXPERIMENTAL

Apparatus:

Shimadzu UV-visible spectrophotometer (1900i) with 1cm matched quartz cells, UV-Visible spectrophotometer double beam (Elico SL-220), Contech precision balance (CAS-54), Liquid chromatography (LC-10 AT-VP, Shimadzu) {Analytical column used Enable column (C_{18}), Temperature: 22^0C., Elution: Isocratic, Diluent: Mobile Phase}, Hamilton Syringe (25µl), Manual injector & Single pump.

Reagents And Solutıons:

Standard Stock Solution (S1) for Method 1: Methanol was taken in a 100ml volumetric flask to which 10mg of Evogliptin tartrate was added. The drug was dissolved by shaking the flask for about 3minutes to produce 100ml solution (100µg/ml).

Standard Stock Solution (S2) for Method 2: Methanol was taken in a 100ml volumetric flask to which 20mg of Evogliptin tartrate was added. The drug was dissolved by shaking the flask for about 3minutes to produce 100ml solution (200µg/ml).

Standard Stock Solution (S3) for Method 3: Selected mobile phase was taken in a 100ml volumetric flask to which 10mg of Evogliptin tartrate was added. The drug was dissolved by shaking the flask for about 3minutes to produce 100ml solution (100µg/ml).

Working Stock Solutions (S4) for Method 1: Appropriate aliquots of S1 were taken in 9 vol. flasks (10ml) and diluted to get the concentration mentioned in Table 2.

Working Stock Solutions (S5) for Method 2: Appropriate aliquots of S2 were taken in 06 vol. flasks (10ml) and diluted to get the concentrations as mentioned in Table 3.

Assay Methods:

a) Method 1:

All the Working Stock Solutions (S3) was run in the UV-Visible spectrophotometer by using the range of wavelengths i.e. 200 to 400nm and area was prepared by using 262nm to 266nm to prepare the AUC spectrum. The UV Spectrum of the drug (60µg/ml) under AUC method is shown in Fig 2. The linearity curve of the method 1 is shown in Fig-3.

b) Method 2 (1st Order Derivative UV Spectrophotometry):

All the Working Stock Solutions (S4) were run in the UV-Visible spectrophotometer to generate zero order UV spectrum. Zero order UV spectrum was manipulated by derivative technique to prepare 1st order UV derivative UV spectrum. In the derivative spectrum 258nm was selected as maximum wavelengths. The zero Order UV Spectrum of Evogliptin is shown in Fig 4. The 1st Order UV Spectrum of Evogliptin is shown in Fig 5. The linearity data of the Method-2 is mention in Table 3. The linearity curve of UV Spectrophotometric derivative method is shown in Fig 6.

c) Method 3: Estimation of Evogliptin by RP-HPLC:

i) Optimized chromatographic condition:

The mobile phase consisting of methanol and water in 70:30 ratio was used to obtain clear peaks of Evogliptin (Fig 8). Then the mobile phase was degassed by the help of ultra sonicator to eliminate the dissolved gases.

Injection volumes of 20µl for an each standard solution were injected into the column. The detection wavelength and chromatographic run times were selected at 267 nm and 6 min. respectively.

Analytical column used: Enable column: C_{18}, Temperature: 22^{0}C., Elution: Isocratic, Diluent: Mobile Phase.

ii) Selection of UV Wavelength

The UV Wavelength was selected by running the different concentration of drug by using the wavelength between 200 to 400nm. The maximum wavelength was found to be 267nm. The overlay of UV spectrum is shown in the Fig 7.

iii) Preparation of standard Calibration curve for RP-HPLC:

Appropriate aliquots of S3 were taken in six vol. flasks (10ml) and diluted with mobile phase to obtain the working solutions (Table 4). The solutions were injected using a Hamilton syringe (25µl) and chromatograms were recorded. The calibration curve was constructed by taking Area on the Y-axis and concentration on the X-axis. The representative chromatogram of Evogliptin is shown in Fig 8 & 9. The linearity curve of the Evogliptin tartrate is shown in Fig 10.

iv) Assay of Tablet: Ten tablets were weighed accurately and crushed into a fine powder with the help of motor and pestle. An amount of powder equivalent to 10mg of drug was weighed and transferred into a 100ml volumetric flask containing 40ml of mobile phase and sonicated thoroughly for about 5mins, the volume was made up to the mark with the mobile phase and filtered using 0.2µm filter paper. The first few milliliters of the filtrate were discarded. 3ml of filtrate was transferred into four volumetric flasks (10ml) and volume was made with the mobile phase. Sample solution (20µl) was injected into instrument by the help of Hamilton syringe and the chromatogram recorded. The concentration of the drug was calculated by employing the linear regression equation.

Assay of the tablet for Method 1 & 2 was also done by respective methods using methanol as solvent. The results of the assay of tablet by all three methods are recorded(Table 5).

Method Valıdatıon: [16]

The proposed method was validated as per ICH Q2B guidelines by following parameters.

(i) System suitability: Evogliptin solution (80µg/ml) was subjected to system suitability test and the results of parameters (Table 6) were obtained by five replicate injections.

(ii) Recovery study: Standard addition method was followed. Known amount of standard drug was added to pre-analysed tablet samples at a level of 50%, 100% and 150% and the drug content was determined by above three methods. The result of recovery studies is shown in Table 7.

(iii) Robustness: It was studied by manipulating some conditions/parameters of chromatography like **±** 2% change in volume of the mobile phase, ± 2 Temperature and ± 2% flow rate, & ± 2nm wavelength. Every parameter of robustness was found to be less than 1.5.

(iv) Specificity: The peak purity of Evogliptin tartrate was assessed by comparing the retention time (R_t) of standard and tablet sample containing Evogliptin tartrate.

(v) LOD & LOQ: The sensitivity of method was estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ).

LOD and LOQ of the newly proposed methods were calculated using the formula of ICH guideline;

$$\text{Limit of detection (LOD)} = 3.3 \times \sigma/S \quad \text{....Eq. 1}$$

$$\text{Limit of quantitation (LOQ)} = 10 \times \sigma/S \quad \text{....Eq. 2}$$

Where, “σ” is standard deviation of y intercepts of regression lines, “S” is Slope of calibration curve.

RESULTS & DISCUSSION

Three methods were developed and validated on the basis of ICH guidelines for estimation of Evogliptin tartrate in pure form and as tablet. Methanol was used as solvent for Method 1 & 2 and methanol & water(70:30) as mobile phase for Method 3.

Method 1: The linear regression equation was found to be Y = 0.0129 x – 0.0503 and $r^2 = 0.9996$. The linearity and range were found to be 10 to 90µg/ml. The % RSD was less than 2. The lower limit of detection and the limit of quantitation were found to be 12.45 and 34.67µg/ml respectively. It indicates that Method 1 is accurate and precise.

Method 2: The linear regression equation was found to be Y = 0.003 x + 0.002 and $r^2 = 0.999$. The linearity and range were found to be 20 to 200µg/ml. The % RSD was less than 2. The lower limit of detection and the limit of quantization were found to be 11.05 and 33.67µg/ml respectively. It indicates that Method 2 is accurate and precise.

Method 3: Several trials were done with various proportions of phosphate buffer and methanol i.e. 40:60, 25:75 and at different pH values i.e. 2, 3.45, 4.48 and also various proportions of acetonitrile, methanol and water in the ratio of 20 + 30 + 50, 50 + 40 + 10 to select the mobile phase. A mobile phase consisting of methanol and water in the ratio of 70: 30 was selected to achieve best chromatographic peak and sensitivity.

The modalities adopted in experimentation were successfully validated as per analytical procedures laid down in routine. The proposed method was validated by preliminary analysis of standard sample and by recovery studies. The detection wavelength and chromatographic run times were selected at 267 nm and 6 min. respectively. Linear regression equation was found to be Y = 4006.6714 x + 713.6349 and Correlation coefficient (r^2) = 0.9999. The % of recoveries was obtained in the range of 99 to 102. The results of Student‘t’ test is within the acceptable limit in the tablet assay and recovery study of this proposed method 3. In Robustness study the normal result was unaffected by small changes like **±** 2% change in volume of the mobile phase, ± 2 ^{o}C in Temperature and ± 2% in flow rate, & ± 2nm of wavelength. Every parameter of robustness was found to be less than 1.5. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. The lower limit of detection and the limit of quantization were found to be 10.25 and 29.67µg/ml respectively. Good correlations were found between the retention time of standard and tablet sample of drugs.

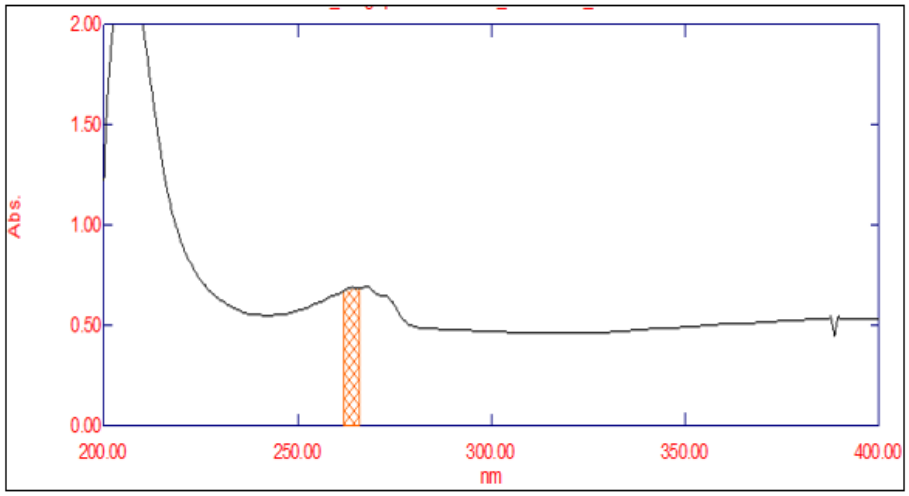

Fig 2: UV Spectrum of Evogliptin (AUC Method) (60 µg/ml)

Table 2: The linearity data of Evogliptin for AUC method (Method 1)

Sl. No.	Conc. (µg/ml)	Area
1	0	0
2	10	0.101
3	20	0.206
4	30	0.319
5	40	0.421
6	50	0.57
7	60	0.714
8	70	0.842
9	80	0.981
10	90	1.104

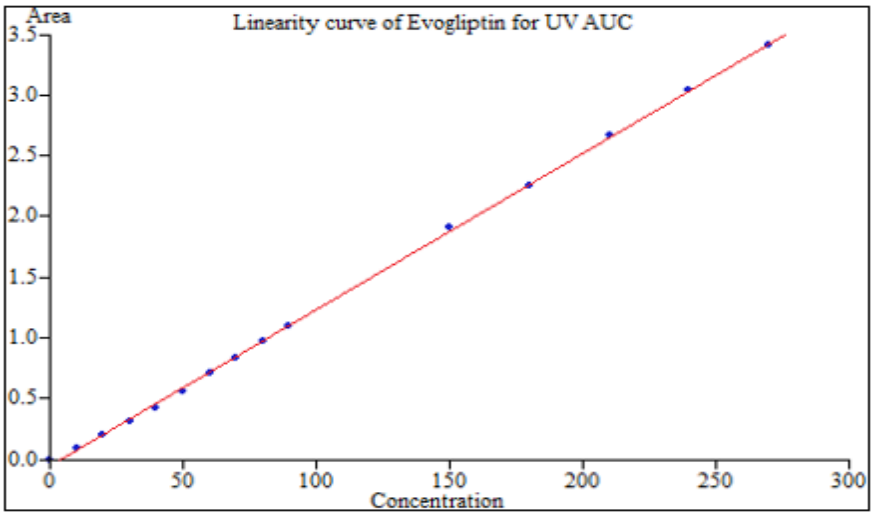

Fig 3: The linearity curve of Evogliptin for AUC method (Method 1)

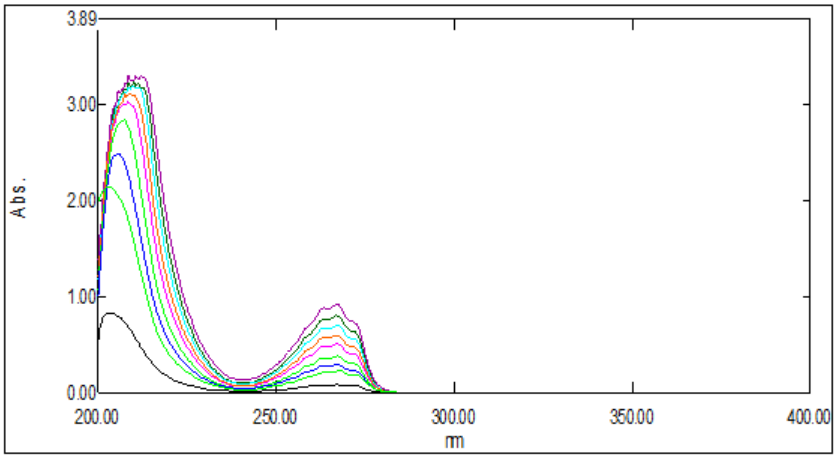

Fig 4: Zero Order UV Spectrum of Evogliptin

Table 3: Linearity data for UV Spectrophotometric Derivative method (Method 2)

Sl. No.	Conc.	Absorbance
1	0	0
2	20	0.072
3	40	0.147
4	80	0.277
5	120	0.426
6	160	0.564
7	200	0.697

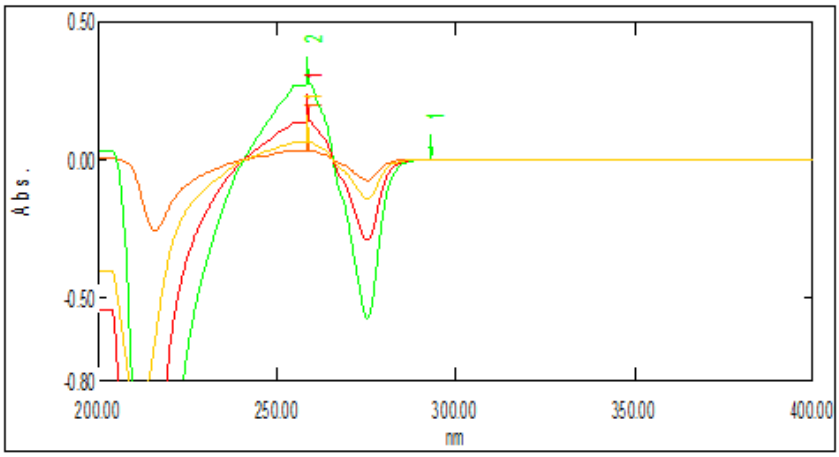

Fig 5: 1st order Derivative UV spectrum of Evogliptin

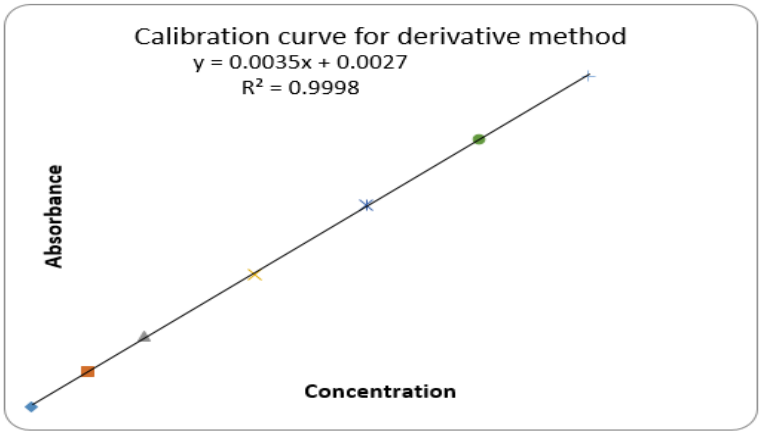

Fig 6: The linearity curve of UV Spectrophotometric derivative method

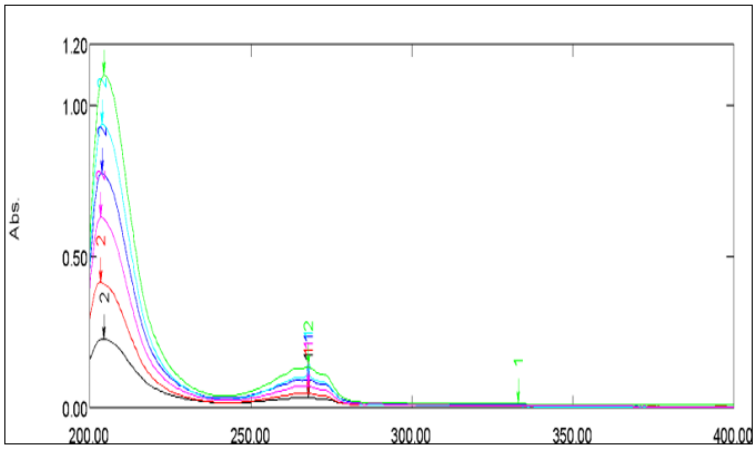

Fig 7: UV absorption spectrum of the drug by RP-HPLC method

Table 4: Linearity data of Evogliptin for RP-HPLC method

Sl. No.	Conc.(µg/ml)	Area
1	0	0
2	30	120029
3	40	163961
4	50	219293
5	60	248022
6	70	284838
7	80	343054

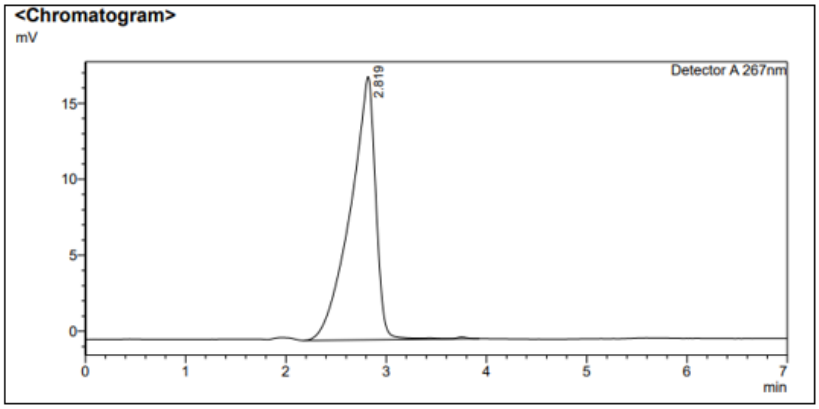

Fig 8: Representative Chromatogram of standard sample 80µg/ml

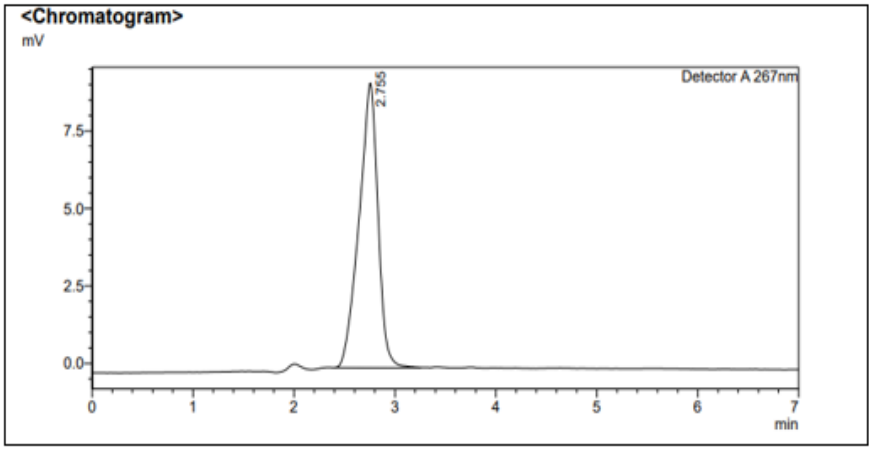

Fig 9: Representative chromatogram of tablet sample 30µg/ml

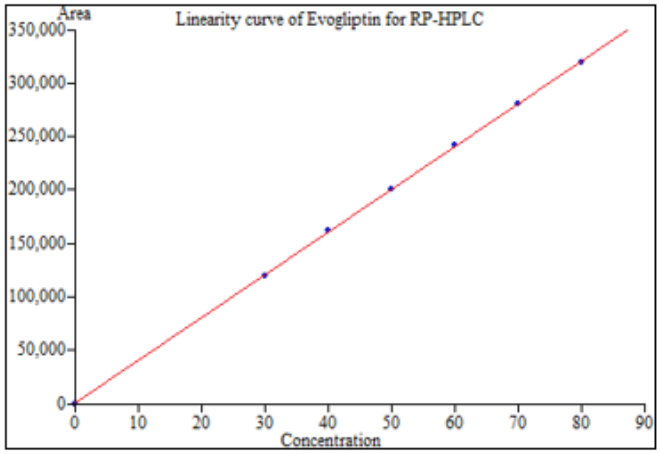

Fig 10: Linearity curve of Evogliptin for RP-HPLC

Table 5: The results of tablet analysis (Valera 5mg) (Alkem)

Method	Formulation (µg/ml)	Label claim (mg/tab)	Found conc. (mg/tab)	C.I.	% RSD	SE	t
Method 1	30	5	5.45	100.6± 2.262	0.603	0.3	0.418
Method 2	30	5	5.71	99.6± 2.062	0.643	0.32	0.588
Method 3	30	5	5.21	99.6± 2.162	0.703	0.35	0.328

SD: Standard deviation, SE: Standard error, C.I.: Confidence Interval within which true value may be found at 95% confidence level = R ± ts/√n, R: Mean percent result of analysis of Recovery study (n = 4). Theoretical 't' values at 95% confidence level for n-1 degrees of freedom t (0.05, 3) = 3.182.

Table 6: Results of System suitability parameter

Parameter	Results of Evogliptin	Limits
Asymmetry factor	1.1	NMT 1.5
Retention Time (mins)	1.81	____
Theoretical plates	3704.693	(NLT 3000)
Repeatability (% RSD)	0.791	< 1.5

Table 7: Results of recovery study

Method	% Level of recovery	Formulation (µg/ml)	Pure drug added (µg/ml)	Amount of drug found (µg/ml)	C.I.	% RSD	SE	t
Method 1	50	30	15	46.032	101.072 ±2.471	1.577	0.732	0.076
	100	30	30	60.016	100.016 ±2.515	1.613	0.816	1.09
	150	30	45	75.856	101.523±1.453	1.36	0.686	1.248
Method 2	50	30	15	45.257	100.572±1.328	0.856	0.430	1.328
	100	30	30	60.965	101.608 ±2.52	1.559	0.79	2.02
	150	30	45	75.217	100.290 ±1.89	1.188	0.596	0.468
Method 3	50	30	15	45.032	100.072 ±2.671	1.677	0.839	0.086
	100	30	30	61.965	101.708 ±2.52	1.659	0.89	2.12
	150	30	45	75.856	101.13 ±1.453	1.36	0.686	1.248

CONCLUSION

In conclusion, the presented RP-HPLC method is new, simple, linear, accurate, robust, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage forms. Method 3 is the more accurate, reliable, precise, reproducibility, robust in comparison to the Method 1 & 2.

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Conflict of Interest: None.

Author Contributions: All authors equally participated.

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