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APPLICATION OF VALIDATED STABILITY INDICATING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR DETERMINATION OF LINAGLIPTIN IN TABLET DOSAGE FORM

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ABSTRACT
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 has been used for chromatographic separation of Linagliptin and was carried out by using precoated with silica gel 60 F254 aluminium plates as stationary phase and mixture consisting of Toluene: Methanol in the ratio of (8: 2, v/v) as optimum mobile phase. Densitometric detection of separated spot was carried out at 294 nm. The developed method has been successfully applied for the estimation of Linagliptin in tablet dosage form. Linagliptin was subjected to stress condition of hydrolysis (acid, base), oxidation, photolysis and thermal degradation and analyzed under optimized chromatographic conditions. Linagliptin was satisfactorily resolved with retention factor value of 0.38 ± 0.02. Linear response was observed in the concentration range of 100-600 ng band-1. The % assay (Mean ± S.D.) was found to be 100.61 ± 1.52. Linagliptin was found susceptible to hydrolysis, oxidative and thermal stress conditions. The developed method can be used for the analysis of Linagliptin without any interference from the excipients and can be successfully used to estimate the amount of drug in the formulations by easily available low cost materials.

KEYWORDS: Linagliptin; HPTLC; Forced degradation; Tablet dosage form.

INTRODUCTION
Linagliptin, chemically, 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-ynyl)-3-methyl-1-[(4-methylquinazolin-2-yl) methyl] purine-2, 6-dione is an inhibitor of dipeptidylpeptidase-4 (DPP-4) enzyme which degrades the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) and used to lower the increased blood sugar level.[1]

Extensive literature review revealed that analytical methods such as spectrophotometry[2,3] and High Performance Liquid Chromatography (HPLC)[4-18] has been reported for the estimation of Linagliptin as bulk and in tablet dosage form either as single drug or in combination with other drugs.

To best of our information, no reports were found for determination of Linagliptin in tablet dosage form by stability-indicating high performance thin layer chromatographic (HPTLC) method. The present work describes the development and validation of a simple, precise, accurate stability indicating HPTLC method for determination of Linagliptin as bulk drug and in tablet dosage form in accordance with ICH guidelines.[19,20]

MATERIALS AND METHODS
Reagents and chemicals
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. Toluene, Methanol (both AR grade) were obtained from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and optimized chromatographic conditions
Chromatographic studies were carried out on 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 separation was performed by linear ascending development in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using toluene: methanol (8: 2, v/v) as mobile phase. The chamber was saturated with mobile phase vapor 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 sec^{-1} was employed. After chromatographic development, plates were dried and densitometric evaluation was done on CAMAG thin layer chromatography scanner-3 at 294 nm for all developments operated by winCATS software version 1.4.2.

Preparation of standard stock solution
10 mg of Linagliptin was weighed accurately and dissolved in 10 mL of methanol to get concentration of 1 mg mL^{-1} which was further diluted to 10 mL with methanol to get solution of final concentration 100 ng μL^{-1}.

Development of optimum mobile phase
Different solvent systems containing various ratios of benzene, carbon tetrachloride, toluene, methanol, acetic acid, chloroform, and ethyl acetate were examined (data not shown) to separate and resolve spot of Linagliptin from its impurities and other excipients present in formulation. Finally, the mobile phase comprising of toluene: methanol (8: 2, v/v) was selected as optimal for obtaining well defined and resolved peak.

Estimation of the drug in tablet dosage form
Twenty tablets (labelled to contain 5 mg of Linagliptin) were weighed accurately and finely powdered. A quantity of powder equivalent to 5 mg was weighed and transferred to a 10 mL volumetric flask containing approximately 7 mL of methanol and sonicated for 10 min and filtered through Whatman filter paper No. 41 and volume was made with methanol. 1 mililitre 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 294 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.

Stress degradation studies
The stress degradation studies were performed by subjecting the bulk drug to the different stress conditions as recommended by ICH and stability was accessed. The stability studies were carried out at concentration of 1000 µg μL^{-1}. The hydrolytic studies were carried out by treatment of stock solution of drug separately with 0.05N HCl and 0.05 N NaOH at room temperature for 30 min. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 500 ng $band^{-1}$. The oxidative degradation was carried out in 3% H_2O_2 at room temperature for 30 min and sample was diluted with methanol to obtain 500 ng $band^{-1}$ solution. Thermal stress degradation was performed by keeping drug in oven at 80ºC for period of 3 h. Photolytic degradation studies were carried out by exposure of drug to UV light and fluorescence light for 4 d.

RESULTS AND DISCUSSION
Resolution of drug
The aim of present research work was to develop suitable stability indicating HPLTC method which would be capable to give the satisfactory resolution between Linagliptin and its degradation products. The chromatographic separation was accomplished by linear ascending development in 10 cm × 10 cm twin trough glass chamber using toluene: methanol (8: 2, v/v) as mobile phase. Densitometric detection was performed at 294 nm. The retention factor (Rf) was found to be 0.38 ± 0.02. Representative densitogram of standard solution of Linagliptin is represented in Figure 1.

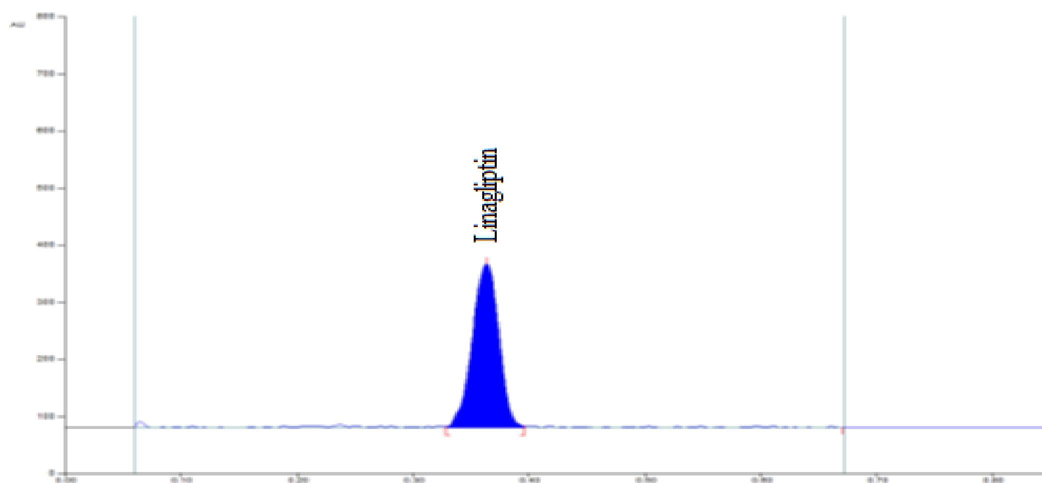

Figure 1: Representative densitogram of standard solution of Linagliptin (300 ng $band^{-1}$, Rf= 0.38 ± 0.02).

Stress degradation studies

The stress degradation results after exposure of drug to different stress conditions indicated susceptibility of drug to hydrolytic, oxidative and thermal stress conditions and stability of the drug under photolytic stress condition. Figures 2 and 3 represents the densitograms of acid and alkali hydrolytic degradation, while Figures 4 and 5 show the densitograms of oxidative degradation and thermal degradation, respectively.

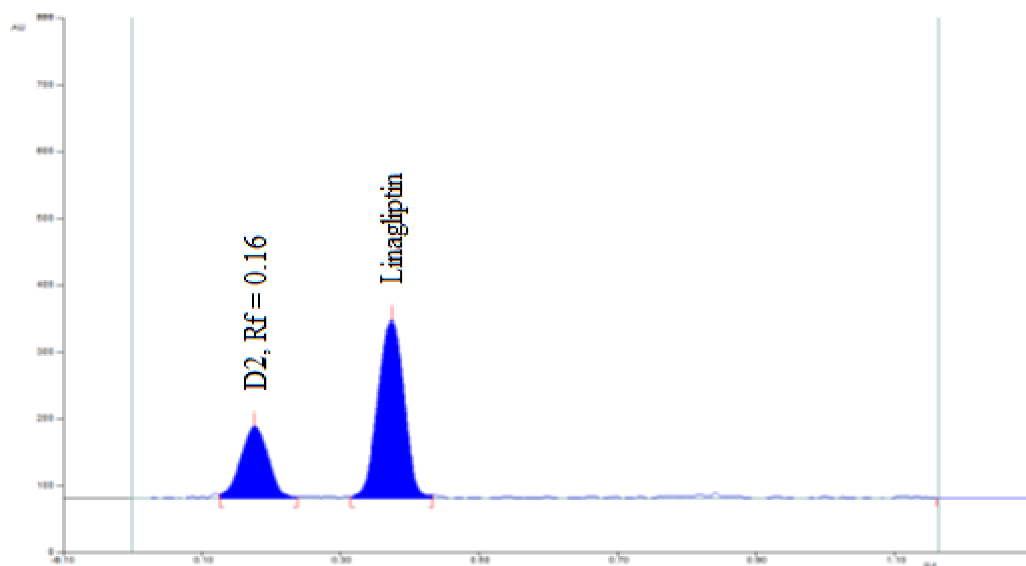

Figure 2: Representative densitogram after acid hydrolysis with degradation product (D1, Rf = 0.16).

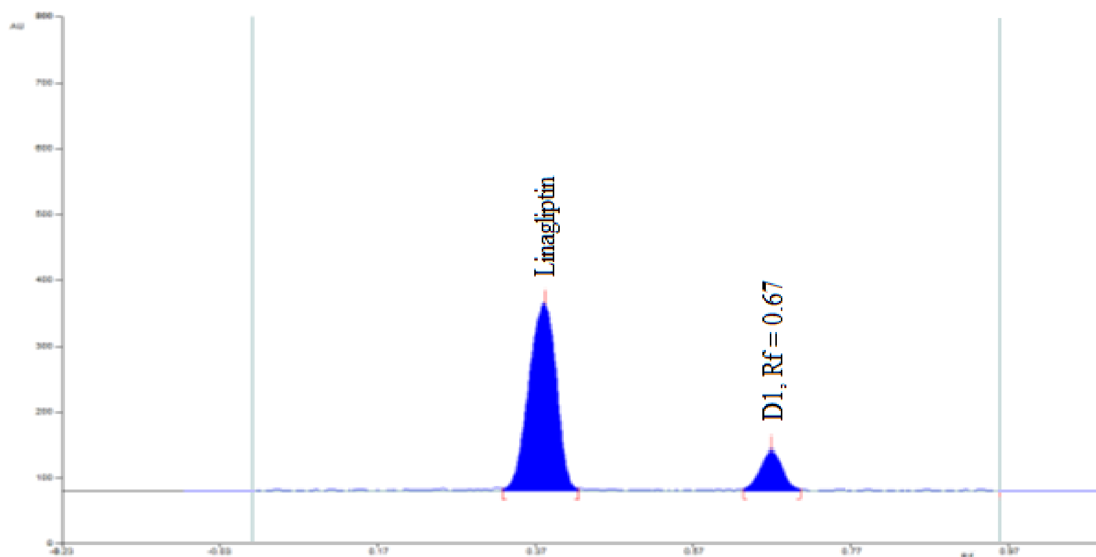

Figure 3: Densitogram for alkali induced degradation with degradation peak (D2, Rf = 0.67).

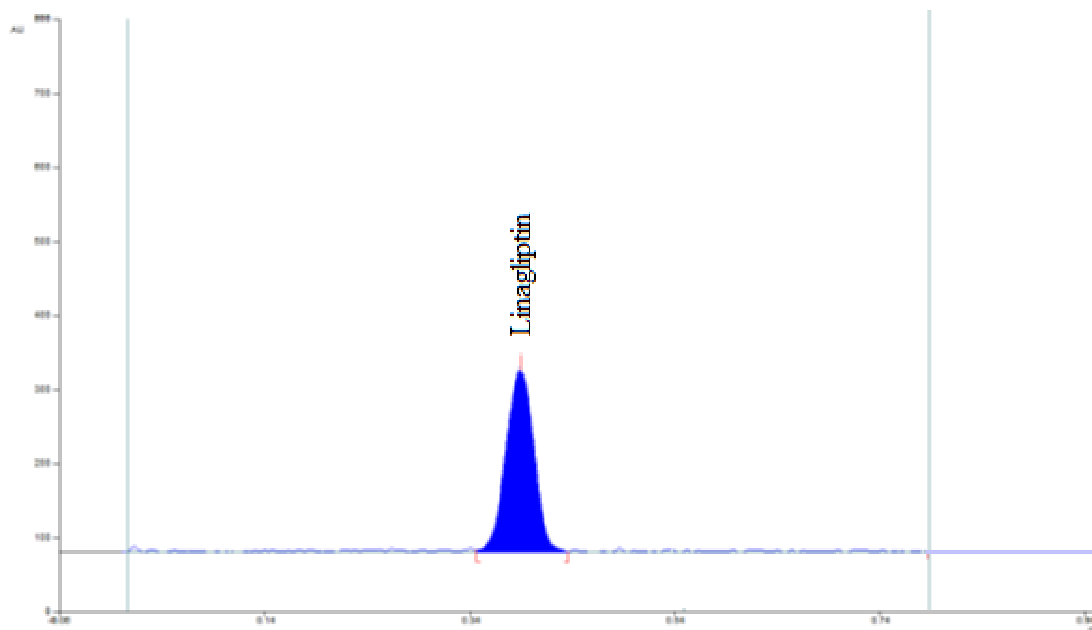

Figure 4: Densitogram of peroxide induced degradation.

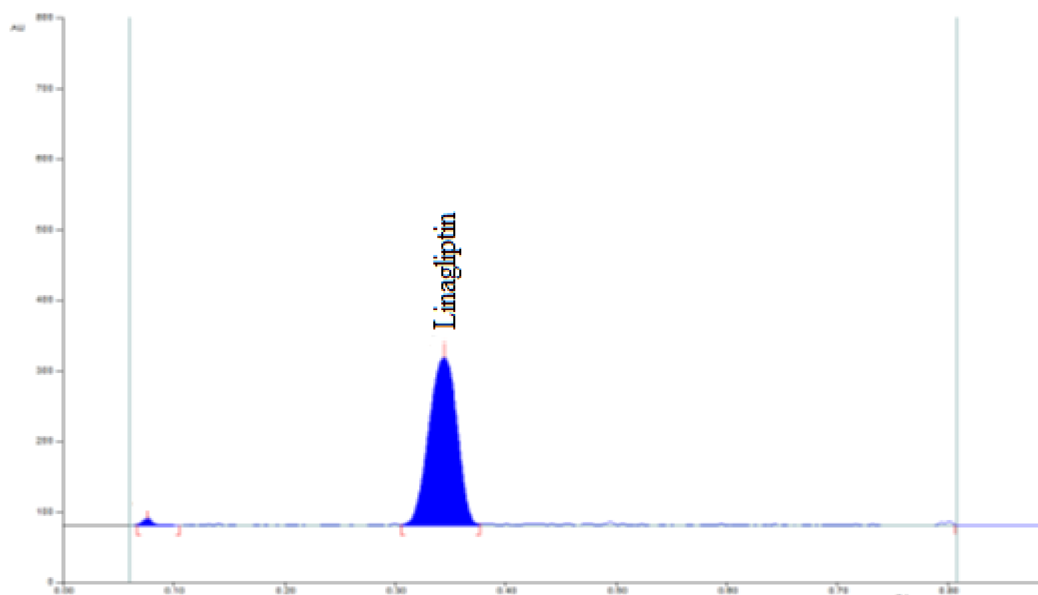

Figure 5: Densitogram obtained after dry heat degradation at 80ºC for 3 h.

The degradation products formed during stress degradation were not interfering with the active drug indicating the specificity of the method. The results of degradation studies are represented in Table 1.

Table 1: Summary of stress degradation studies.

Stress conditions/ duration		% Recovered	% Degradation
Acidic / 0.05 N HCl/ Kept at RT for 30 min		78.95	21.04
Alkaline /0.05 N NaOH/ Kept at RT for 30 min		83.16	16.83
Oxidative /3 % H_2O_2 / Kept at RT for 30 min		88.50	11.49
Dry heat/ 80ºC/ 3 h		91.73	08.26
Photolysis	UV light 200 watt h square meter^{-1} 4 d	98.46	----
	Fluorescence light 4 d	98.63	----

Analytical method validation

The developed method was validated in terms of linearity, accuracy, precision, robustness, limit of quantization (LOQ), limit of detection (LOD) to confirm the consistency of results of analysis.

Linearity

The linearity of was determined by application of volumes 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 method was found to be linear in the concentration range 100-600 ng $band^{-1}$ with good correlation coefficient. The linear regression equation was found to be y = 15.45x + 487.69 with correlation coefficient 0.990. The calibration curve obtained by plotting concentration vs peak area is represented in Figure 6.

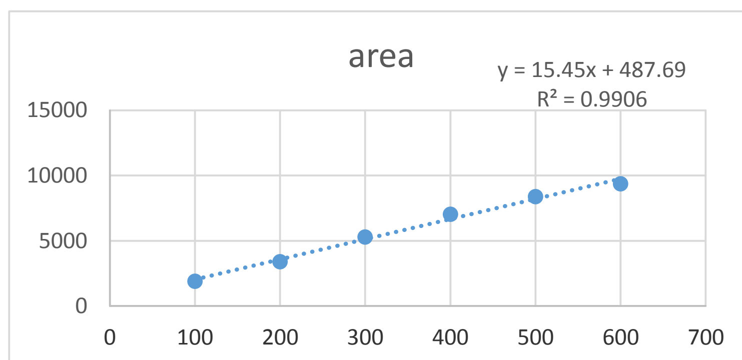

Figure 6: Calibration curve.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 5.80 ng $band^{-1}$ and 17.60 ng $band^{-1}$, respectively.

Precision

Set of three different concentrations in three replicates of standard solutions of Linagliptin were prepared. All the solutions were analyzed on the same day in order to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.62 to 1.28. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.54 to 1.53. The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

Recovery studies

Recovery studies were carried out by standard addition method which involved addition of standard drug to pre-analysed sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 200 ng $band^{-1}$ from tablet solution. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies showed the accuracy of method for determination of drug in tablet dosage form. The results obtained are depicted in Table 2.

Table 2: Recovery studies.

Drug	Amount taken (ng $band^{-1}$)	Amount added (ng $band^{-1}$)	Amount found (ng $band^{-1}$)	% Recovery±R.S.D.
Linagliptin	200	160	356.74	99.09±0.77
	200	200	401.84	100.46±1.04
	200	240	443.06	100.69±0.61

*Average of three determinations.

Robustness

Robustness of the method was determined by making deliberate variations in method parameters during which mobile phase composition (± 2% methanol), wavelength (± 1 nm) was altered and the effect on the area of drug was noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that 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.

CONCLUSION

A rapid, simple, precise and accurate stability-demonstrating HPTLC method with no interference from the excipients or from degradation products has been developed and validated for the determination of Linagliptin in tablet dosage form. The developed procedure can be used for quantitative analysis of drug in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic.

REFERENCES

1. https://en.wikipedia.org/wiki/Linagliptin (accessed on 04/12/2019)
2. Sekhar CK, Sudhakar P, Mohan Rao T, Vijaya Babu P, Manikanta KA. A new UV method for determination of linagliptin in bulk and pharmaceutical dosage form. Int. J. Universal Pharm. Bio. Sci., 2013; 2: 1-6.
3. El-Bagary R, Elkady EF, Ayoub BM. Spectrophotometric methods for the determination of linagliptin in binary mixture with metformin hydrochloride and simultaneous determination of linagliptin and metformin hydrochloride using high performance liquid chromatography. Int. J. Biomed. Sci., 2013; 9: 41-47.
4. Ferreira RBH et al. Biological safety studies and simultaneous determination of linagliptin and synthetic impurities by LC-PDA. Journal of Analytical Methods in Chemistry, 2019; 1-10.
5. Rajbangshi, J Alam, MM, Hossain, MS, Islam MS, Rouf, AS. Development and validation of a RP-HPLC method for quantitative analysis of linagliptin in bulk and dosage forms. Dhaka University Journal of Pharmaceutical Sciences, 2018; 17(2): 175-82.
6. Mourad SS, El-Kimary EI, Hamdy DA, Barary MA. Stability-Indicating HPLC-DAD method for the determination of linagliptin in tablet dosage form: application to degradation kinetics. J. Chromatogr. Sci., 2016; 54(9): 1560-66.
7. Jadhav SB, Reddy PS, Narayanan KL, Bhosale PN. Development of RP-HPLC stability indicating method for degradation products of linagliptin in presence of metformin HCl by applying 2 level factorial design; and identification of impurity-VII, VIII and IX and synthesis of impurity-VII. Sci. Pharm., 2017; 85(25): 1-17.
8. Lakshman RB et al. A validated RP-HPLC method for the determination of linagliptin. Am. J. Pharm Tech. Res., 2012; 2: 463-70.
9. Sujatha K, Seshagiri Rao JVLN. A new RP-HPLC method for the estimation of linagliptin in tablet dosage forms. Indo Am. J. Pharma Res., 3: 8376-81.
10. Reddy TV. A novel RP-HPLC method for the quantification of linagliptin formulations. J. Atoms and Molecules, 2012; 2: 155-64.
11. Archana M, Sriram N, Gayasuddin MD. Method development and validation of RP-HPLC method for determination of new antidiabetic agent linagliptin in bulk and in pharmaceutical formulation. Int. J. Med. Chem. Anal., 2013; 3: 1-5.
12. Janardhan Swamy A, Harinadha Baba K. Analytical method development and method validation for the simultaneous estimation of metformin HCL and linagliptin in bulk and tablet dosage form by RP-HPLC method. Int. J. Pharm., 2013; 3: 594-600.
13. Moncy S, Rohini Reddy G, Chaitanya PS, Priyanka G, Hima Bindu E. Simultaneous determination of metformin hydrochloride and linagliptin by RP-HPLC in bulk and pharmaceutical formulations. IAJPR, 2014; 4: 4047-53.
14. Shirisha S, Akiful Haque M, Sireesha D, Bakshi V, Harshini S. Development and validation of RP-HPLC method for simultaneous estimation of metformin and linagliptin in combined pharmaceutical dosage form. Int. J. Pharma Res. Health Sci., 2014; 2: 491-95.
15. Sekhar Reddy BRC, Vijaya Bhaskar Rao N, Saraswathi K. A validated stability indicating HPLC assay method for linagliptin. Der Pharmacia Sinica, 2014; 5: 131-37.
16. Kavitha KY, Geetha G, Hariprasad R, Kaviarasu M, Venkatnarayanan R. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of linagliptin and metformin in pure and pharmaceutical dosage form. J. Chem. Pharm. Res., 2013; 5: 230-35.
17. Ravi Varma A, Shanmukhakumar JV, Mutta Reddy S. Stability indicating liquid chromatographic method for the simultaneous assay of anti-diabetic drugs, linagliptin and metformin, in pure and in their commercial tablet dosage form. Int. J. Innov Tech Res., 2014; 2: 1131-38.
18. Mallikarjuna Rao N, Gowri Sankar D. RP-HPLC method for simultaneous estimation and stability indicating study of metformin and linagliptin in pure and pharmaceutical dosage forms. Int. J. Pharm. Pharm. Sci., 2015; 7: 191-97.
19. ICH (2005) Q2 (R1): Validation of Analytical Procedures: Text and Methodology, Geneva.
20. ICH (2003) Q1A (R2): Stability testing of new drug substances and products, Geneva.