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DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF LINAGLIPTIN BY RP-HPLC METHOD IN BULK FORM & MARKETED PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Linagliptin in bulk form and marketed formulation. Separation of Linagliptin was successfully achieved on a Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. column in an isocratic mode of separation utilizing Methanol : Phosphate buffer (0.02M, pH-3.6) in the ratio of 45:55% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 255nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 12-28mcg/mL for Linagliptin. The correlation coefficient was found to be 0.9995 for Linagliptin. The LOD and LOQ for Linagliptin were found to be 5.004µg/mL and 15.164µg/mL respectively. The proposed method was found to be good percentage recovery for Linagliptin, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

KEYWORDS: Linagliptin, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines.

INTRODUCTION
Linagliptin, sold under the brand name Tradjenta among others, is a medication used to treat diabetes mellitus type 2. It is generally less preferred than metformin and sulfonylureas as an initial treatment. It is used together with exercise and diet. It is not recommended in type 1 diabetes. It is taken by mouth. Linagliptin[1] is a dipeptidyl peptidase-4 (DPP-4) inhibitor which is used in combination with diet and exercise in the therapy of type 2 diabetes, either alone or in combination with other oral hypoglycemic agents. Linagliptin has been linked to rare instances of clinically apparent liver injury. Linagliptin[2] is a xanthine that is 7H-xanthine bearing (4-methylquinazolin-2-yl)methyl, methyl, but-2-yn-1-yl and 3-aminopiperidin-1-yl substituents at positions 1, 3, 7 and 8 respectively (the R-enantiomer). Used for treatment of type II diabetes. It has a role as an EC 3.4.14.5 (dipeptidyl-peptidase IV) inhibitor and a hypoglycemic agent. It is a member of quinazolines and an aminopiperidine. It derives from a 7H-xanthine. Linagliptin is a potent, orally bioavailable dihydropurinedione-based inhibitor of dipeptidyl peptidase 4 (DPP-4), with hypoglycemic activity. The inhibition of DPP-4 by Linagliptin appears to be longer lasting than that by some other DPP-4 inhibitors tested. The IUPAC Name of Linagliptin is 8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methyl quinazolin-2-yl) methyl] purine-2, 6-dione. The Chemical Structure[3] of Linagliptin is as follows.

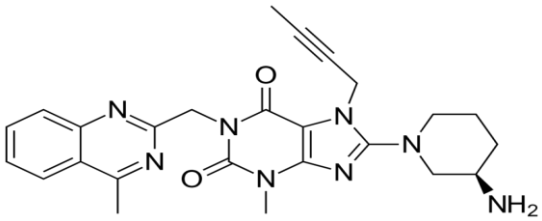

Fig-1: Chemical Structure of Linagliptin.

EXPERIMENTAL
Instruments: HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters), lambda max can be determined by T60-LAB INDIA UV – Vis spectrophotometer, Electronic Balance (SHIMADZU ATY224), Ultra Sonicator (Wensar wuc-2L), Thermal Oven, Symmetry' ODS RP C18,5µm, 15mm x 4.6mm

i.d. Column, PH Analyzer (ELICO) and Vacuum filtration kit (BOROSIL).

Chemicals/Reagents: Doubled distilled water, HPLC Grade Water, Acetonitrile, Methanol and Hydrochloric Acid, Sodium Hydroxide, Ethanol and Octanol all are 99.9% obtained from Sd fine-Chem ltd; Mumbai and Linagliptin was provided as a gift sample by Syncorp Clincare Technologies Pvt Ltd. Hyderabad.

Method Development

Wavelength Detection (Or) Selection of Wavelength

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm.

Preparation of phosphate buffer

1.36086 grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC grade water and pH was adjusted to 3.6 with Orthophosphoric acid solution.

Preparation of mobile phase

Mix a mixture of above buffer 550 mL (55%) and 450 mL of methanol HPLC grade (45%) and de gas in ultrasonic water bath for 15 minutes. Filter through 0.45 µ filter under vacuum filtration.

Standard solution preparation

10 mg of Linagliptin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution). Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents which gave 10 ppm Linagliptin working standard solution. The solution was mixed well and filtered through 0.45µm filter.

Sample Solution Preparation

Twenty tablets were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Linagliptin equivalent to 10mg were transferred to clean and dry 10 ml volumetric flask and 7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.1 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

Method Validation

The validation[4,5] of an analytical method confirms the characteristics of the method to fulfil the requirements of the application domain. The method was validated according to the ICH guidelines[6] for specificity, linearity, precision, recovery, and stability.

System Suitability: A standard solution of Linagliptin working standard was prepared as per procedure and injected 6 times into the HPLC system.[7] Then, the system suitability parameters were evaluated from standard chromatograms obtained. The % relative standard deviations (RSD) of retention time, tailing factor, theoretical plates, and peak areas from six replicate injections was within range and results were shown in Table 6.

Linearity: To demonstrate linearity[8] of the assay method, five standard solutions with concentrations of about 12-28 ppm (Table-4) of Linagliptin was injected. Then, a graph was plotted between concentrations and peak area. Linearity plot and table was shown in Fig. 4 and 4.

Accuracy: Three concentrations of 80%, 100%, and 120% were injected in a triplicate manner then % recovery[9] and % RSD were calculated and shown in Table 1.

Precision: Precision[10] was estimated by studying repeatability, intra- and interday tests by injecting 10 ppm concentration of Linagliptin. The results were calculated as standard deviation, relative standard deviation and shown in Table 2.

Limit of detection (LOD): LOD[11] is the lowest level of concentration of analyte in the sample that can be detected, though not necessarily quantitated. It can be calculated from the below formula.

$LOD = 3.3\ \sigma/S$

Where, σ = Standard deviation of the response,

S = Slope of calibration curve.

Limit of quantitation (LOQ): LOQ[12] is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied. It can be calculated from the below formula.

$LOQ = 10\ \sigma/S$

Where, σ = Standard deviation of the response,

S = Slope of calibration curve.[13]

Robustness: It is the capacity of the method to remain unaffected by small but deliberate variations in method parameters. The analysis was performed by slightly changing the temperature, mobile phase composition and flow rate.[14] The results were calculated as % RSD and were given in Table 5.

Assay: – Assay refers to chromatography based purity

assay where a compound of unknown activity or purity is compared to a reference standard with precisely determined bioactivity or purity. The Assay[15] results were shown in table-7.

$$\text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Average weight} = \text{mg/tab}$$

Where:
AT = Test Preparation Peak Area
AS = Standard preparation Peak Area
WS = Working standard weight taken in mg
WT = Sample weight taken in mg
DS = Standard solution dilution
DT = Sample solution dilution
P = Working standard percentage purity

Forced Degradation Studies

The API (Linagliptin) was subjected to keep in some stress conditions in various ways to observe the rate and extent of degradation[16,17] that is likely to occur in the course of storage and/or after administration to body. It is one type of accelerated stability studies of the drugs that is used to help us to determining the total fate of the drug that is likely to happen after long time storage, within a very short time as compare to the real time or long term stability testing. The different types of forced degradation pathways/studies are studied here are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation.

RESULTS AND DISCUSSION

Method Development

Selection of Wavelength

The UV spectrum of Linagliptin was obtained and the Linagliptin showed absorbance's maxima at 255nm. The UV spectra of drug are follows.

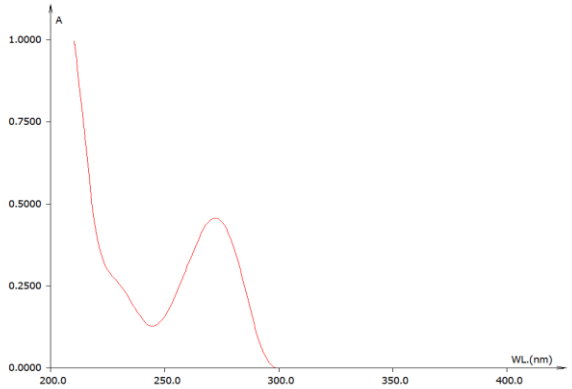

Fig-2: UV Spectrum of Linagliptin.

While scanning the Linagliptin solution we observed the maxima at 255nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

Optimized Chromatographic Method

Mobile phase: Methanol: Phosphate buffer (0.02M, pH-3.6) = 45:55
Column: Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. Column
Temperature: Ambient
Detection Wavelength : 255 nm
Flow rate: 1.0 ml/ min.
Run time: 07 min.
Temperature of Auto sampler: Ambient
Diluent : Mobile Phase
Injection Volume: 20µl
Type of Elution: Isocratic

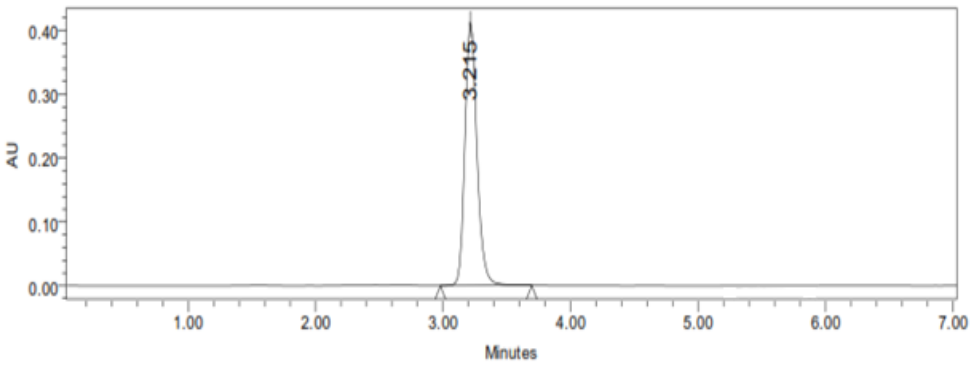

Fig-3: Optimized Chromatographic Condition of Linagliptin.

Method Validation

Accuracy

Recovery Study: To decide the exactness of the proposed strategy, recuperation contemplates were completed by including diverse sums (80%, 100%, and 120%) of unadulterated medication of LINAGLIPTIN were taken and added to the pre-examined plan of fixation 10µg/ml. From that rate recuperation esteems were figured. The outcomes were appeared in table-1.

Table-1: Accuracy results of Linagliptin.

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	8.064107	458679	99.867	Mean= 100.4113% S.D. = 0.473694346 % R.S.D.= 0.471753
S_2 : 80 %	8	7.843532	446485	100.637	
S_3 : 80 %	8	8.19449	465887	100.73	
S_4 : 100 %	10	9.892661	559767	99.41	Mean= 100.6646667% S.D. = 1.166369295 R.S.D.= 1.158667
S_5 : 100 %	10	9.978655	564521	100.868	
S_6 : 100 %	10	10.19623	576549	101.716	
S_7 : 120 %	12	11.85907	668476	99.878	Mean= 100.4637% S.D. = 0.51154309 % R.S.D. = 0.509181
S_8 : 120 %	12	12.16785	685546	100.69	
S_9 : 120 %	12	12.18644	686574	100.823	

Precision

Repeatability: The accuracy of every technique was found out independently from the pinnacle regions and maintenance times gotten by real assurance of six recreates of a fixed amount of drug Linagliptin (API). The percent relative standard deviation[18] was calculated for Linagliptin are presented in the table-2.

Table-2: Repeatability Results of Linagliptin.

HPLC Injection Replicates	AUC for Linagliptin
Replicate – 1	285479
Replicate – 2	284571
Replicate – 3	286954
Replicate – 4	283261
Replicate – 5	285964
Replicate – 6	284259
Average	**285081.3**
Standard Deviation	**1318.666**
% RSD	**0.462558**

Intermediate Precision

Intra-assay & inter-assay: The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Linagliptin revealed that the proposed method[19] is precise.

Table 3: Ruggedness Results for Linagliptin.

Conc. of Linagliptin (API) (µg/ml)	Observed Conc. of Linagliptin (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
8	8.21	0.76	8.23	0.46
10	10.37	0.33	10.36	0.57
12	12.56	0.23	12.56	0.75

Linearity & Range

The calibration curve showed good linearity in the range of 0 – 28 µg/ml, for Linagliptin (API) with correlation coefficient[20] (r^2) of 0.9995 (Fig-4). A typical calibration curve has the regression equation[21] of y = 55283x + 12871 for Linagliptin.

Plotting of Calibration Graphs: The resultant areas of linearity peaks are plotted against Concentration.

Table 4: Linearity Readings for Linagliptin.

CONC. (µg/ml)	MEAN AUC (n=6)
0	0
12	690316
16	910621
20	1121057
24	1328903
28	1554666

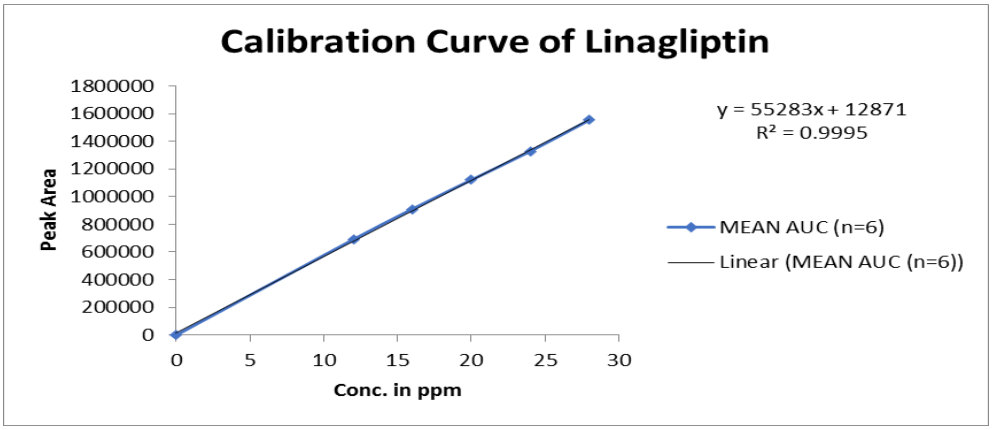

Fig 4: Standard curve for Linagliptin.

Linearity range was found to be 0-28µg/ml for Linagliptin. The correlation coefficient[22] was found to be 0.9995, the slope was found to be 55283 and intercept was found to be 12871 for Linagliptin.

Method Robustness

Impact of little changes in chromatographic conditions, for example, change in Flow rate (± 0.1ml/min), Wavelength of location (±2nm) and organic phase content in mobile phase[23] (±5%) concentrated to decide the Robustness[24] of the technique are additionally for (Table-5, % RSD < 2%) the created RP-HPLC[25] strategy for the examination of Linagliptin (API).

Table-5: Result of Method Robustness Test for Linagliptin.

Change in parameter	% RSD
Flow (0.8 ml/min)	0.554
Flow (1.2 ml/min)	0.867
More Organic	0.886
Less Organic	0.817
Wavelength of Detection (257 nm)	0.813
Wavelength of detection (253 nm)	0.794

LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 5.004 & 15.164µg/ml respectively.

System Suitability Parameter

Framework appropriateness testing is a necessary piece of numerous explanatory methodologies. The tests depend on the idea that the gear, hardware, logical tasks and tests to be examined comprise a necessary framework that can be assessed thusly. Following framework reasonableness test parameters were built up. The information is appeared in Table-6.

Table 6: System suitability results for Linagliptin (Flow rate).

S.No.	Parameter	Limit	Result
1	Asymmetry	T ≤ 2	Linagliptin = 0.12
2	Theoretical plate	N > 2000	Linagliptin = 7258
3	Tailing Factor	(Tf) < 2	Linagliptin = 1.25

7. Estimation of Linagliptin in Pharmaceutical Dosage Form

Each tablet contains: 5mg.

Twenty pharmaceutical dosage forms were taken and the I.P. technique was taken after to decide the normal weight. Above measured tablets were at long last powdered and triturated well. An amount of powder comparable to 25 mg of medications were exchanged to 25 ml volumetric jar, make and arrangement was sonicated for 15 minutes, there after volume was made up to 25 ml with same dissolvable. At that point 10 ml of the above arrangement was weakened to 100 ml with mobile phase. The arrangement was separated through a film channel (0.45 µm) and sonicated to degas. The arrangement arranged was infused in five repeats into the HPLC framework and the perceptions were recorded.

A copy infusion of the standard arrangement was likewise infused into the HPLC framework and the pinnacle zones were recorded. The information is appeared in Table-40.

ASSAY

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where

AT = Peak Area of medication acquired with test readiness

AS = Peak Area of medication acquired with standard readiness

WS = Weight of working standard taken in mg

WT = Weight of test taken in mg

DS = Dilution of Standard arrangement

DT = Dilution of test arrangement

P = Percentage virtue of working standard

Table-7: Recovery Data for estimation Linagliptin in Ondero Tablet

Brand name of Linagliptin	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Ondero (Lupin Pharmaceuticals Pvt Ltd)	5mg	4.823 (± 0.368)	99.698 (± 0.476)

Forced Degradation Studies

Table-8: Results of Force Degradation Studies of Linagliptin.

Stress Condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	91.326	8.674	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	83.215	16.785	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	90.311	9.689	100.00
UV (254nm)	24Hrs.	81.322	18.678	100.00
3% Hydrogen Peroxide	24Hrs.	73.514	26.486	100.00

CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Linagliptin, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, water, 0.1N NaOH, 0.1NHCl).

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Linagliptin it is evident that most of the HPLC work can be accomplished in the wavelength range of 255 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20µl were found to be the best analysis.

BIBLIOGRAPHY

1. https://go.drugbank.com/drugs/DB08882.
2. https://pubchem.ncbi.nlm.nih.gov/compound/Linagliptin.
3. https://en.wikipedia.org/wiki/Linagliptin.
4. R. Snyder, J. Kirkland, L. Glajch, Practical HPLC Method Development, john Wiley and sons International publication, II Edn, 2011.
5. S. Ashutoshkar, Pharmaceutical Drug Analysis 2nd Edn, New Age International Private Limited Publishers, 2005; 452-474.
6. H. Beckett and J.B. Stenlake, Practical Pharmaceutical Chemistry, 4th End. C.B.S. Publishers and Distributors', New Delhi. 1-9, 157-167.
7. H.H. Williard, L.L. Merit, F.A. Dean, F.A. Settle, Instrumental Methods Of Analysis, 6th Edn, C.B.S. Publishers and Distributors, New Delhi, 430-440, 495-504, 529-545.
8. B.K. Sharma, Instrumental Methods of Chemical Analysis. GOEL Publishing House, Meerut, 286-300.
9. Instant notes on analytical chemistry by D. Kealey and P.J. Haines, © BIOS Scientific Publishers Limited, UK, 2002; 6-7.
10. Gurdeep R. Chatwal, Sham K. Anand, Instrumental methods of Chemical Analysis,5th edition, Himalaya Publishing House(Mumbai), 2005; P-2. 566.
11. M. E. Swartz, Journal of liquid chromatography, 2005; 28(7/8): 1253-1263.
12. Journal of Chromatography. B, Analytical Technologies in the Biomedical and life Sciences, 2008 March 1; 863(2): 258-265. Published on Jan 18 2008.
13. International Conference on Harmonization, Harmonized Tripartite Guideline. Validation of Analytical Procedures. Text and Methodology. Q2 (R1), November 2005.
14. International Conference on Harmonization (ICH). Validation of Analytical Methods: Definitions and Terminology. ICH Q2A. 1994.
15. J. M. Green, a practical guide to analytical method validation, anal. Chem. News & features, 1 May 1996; 305a–309a.
16. P. A. Winslow and r. F. Meyer, defining a master plan for the validation of analytical methods, j. Validation technology, 1997; 361–367.
17. H.H. Williard, L.L. Merit, F.A. Dean and F.A. Settle, Instrumental methods of analysis, 7th edition, C.B.S. Publishers, New Delhi, 2002.
18. GN Menon, LB White, Department of Analytical Research, Abbott Laboratories, (pub med-index for MEDLINE).
19. Food and Drug Administration (FDA), "Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation;" Federal Register (Notices), 2000; 65(169): 52776–52777.
20. Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4): 22-23.
21. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc, 2006; 88-92.
22. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
23. Development and validation of HPLC method - A Review, Vibha Gupta et al, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4): 17-25.

24. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC chromacademy.
25. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
26. Anjali Bakshi*, A. Mounika, Shweta Bhutada and Dr. M. Bhagvan Raju, Simultaneous Estimation Of Empagliflozin And Linagliptin by RP-HPLC Method, World Journal of Pharmacy and Pharmaceutical Sciences, 7(8): 1062-1071.
27. Ragaa El Sheikh1, Wafaa S Hassan2, Emanh Youssef1, Abdulrahman Y Hamdi3, Naif Ahmed Badahdah3, Muneer Esa Alzuhiri3, Ayman Abou Elfetouh Gouda1,4*, Development and Validation of Rapid Stability-Indicating High-Performance Liquid Chromatography Method for the Determination of Linagliptin and Empagliflozin In Pure and Dosage Forms, Asian J Pharm Clin Res, 2020; 13(4): 172-177.
28. S. Naazneen1* and A. Sridevi2, Development and validation of stability indicating RP-HPLC method for simultaneous estimation of Empagliflozin and Linagliptin in tablet formulation, Scholars Research Library, Der Pharmacia Lettre, 2016; 8(17): 57-65.
29. V. Sruthi*1, B.Poornima2, Analytical Method Development and Validation for Simultaneous Estimation of Linagliptin and Empagliflozin in Bulk and Pharmaceutical Formulation by RP-HPLC, International journal of Pharmacy and Pharmaceutical Research, September 2019 Vol.:16, Issue:2, Ijppr. Human, 2019; 16(2): 555-568.
30. Shyamala*, M. Soumika, E. Sangeetha, L. Mahender, Method Development and Validation of Empagliflozin By Rp-Hplc In Bulk and Pharmaceutical Dosage Form, An International Journal of Advances in Pharmaceutical Sciences, January-February 2016; 7(1): 3040-3042.