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Spectrophotometric and Chromatographic Estimation of Linagliptin in Bulk and Tablet Dosage Form

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Abstract: Objective: An accurate, precise, rapid & economical Zero order derivative spectrophotometric and RP-HPLC method have been developed for thee estimation of Linagliptin as per International Conference on Harmonization (ICH) guideline in pharmaceutical dosage form using ultraviolet detector (UV).
Methods: The zero order derivative spectrophotometric method was used for the determination of LNG in the range of 1-11µg/ml by measuring the absorbance at 227nm. Besides, a reversed-phase liquid chromatographic (RP-LC) method is described for the simultaneous determination of LIN. Chromatographic separation was achieved on a Pronto SIL-C8 column (250mm×4.6mm, 5µm). Gradient elution was carried out using a mobile phase consisting of Phosphate Buffer (pH 3) and Acetonitrile (35:65 v/v) and the flow rate was set 1ml/min at 227 nm, retention time for Linagliptin was found to be 2.41 min.
Results: The zero order derivative spectrophotometric method was found to be linear in the concentration range of 1-11µg/ml, in the linearity study regression equation was found to be y=0.1213x-0.0572 and correlation coefficient was found to be 0.9982.Whereas, chromatographic method was found to be linear in the concentration range of 5-100µg/ml, in the linearity study regression equation was found to be y=448404x + 10568 and correlation coefficient was found to be 0.9998. This method was Rugged and Robust in different testing criteria, LOD and LOQ were found to be 2.6×10^{-07} and 7.9×10^{-07}respectively. Accuracy study was done in 3 different concentration level i.e. 80%, 100%, 120% and % recovery of the method was found to be 103%, 101% and 104% respectively in 3 different levels and mean recovery was 103%-105%, so method was accurate.
Conclusion: Results of all validation parameters were within the limits as per ICH guidelines.
Keywords : UV, HPLC, Validation, Method Development, Linagliptin, Accuracy, Precision.

Introduction

Linagliptin (LNG), 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl) methyl]-3, 7-dihydro-1H-purine-2, 6-Dione] (Fig. 1) is a novel hypoglycemic drug that belongs to dipeptidyl-peptidase-4 inhibitor class [1, 2]. DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes that functions to stimulate glucose- dependent insulin release and reduce glucagon

levels. This is done through inhibition of the inactivation of incretins, particularly glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby improving glycaemic control [(3)].Recently, DPP-4 inhibitors have been recommended in the treatment of diabetes mellitus to improve glycaemic control [(4)]and it is effective in controlling the metabolic syndrome and resulted in significant weight loss, a reversal of insulin resistance, islet and adipocyte hypertrophy, and alleviated hepatic steatosis[(5)].In summary, LIN reduces blood glucose levels by inhibiting DPP-4 and increasing the levels of GLP-1 and GIP. Linagliptin was approved by the FDA in May 2011 [(6, 7)].

Figure 1: Structure of Linagliptin

Linagliptin may be taken with or without food. The recommended dose is 5mg/day. The most common side effects of Linagliptin are stuffy or runny nose and sore throat. Hypoglycaemia may occur when Linagliptin is combined with insulin or a sulfonylurea-type drug. Allergic reactions and muscle pain also may occur. Pancreatitis is also has been reported.

Rifampicin decreases the blood concentration of Linagliptin by stimulating break down of Linagliptin by CYP3A4 liver enzymes. Other drugs that increase activity CYP3A4 may also reduce the blood concentration of Linagliptin.

Very few methods have been developed for the estimation of Linagliptin in pharmaceutical dosage forms by HPLC and UV [(8)].The aim of present work was the development and validation, following ICH guidelines [(9-16)] of a HPLC method for the determination of Linagliptin in pharmaceutical formulations.

Experimental

Instrumentation

A Shimadzu UV-visible spectrophotometer (SHIMADZU UV-1800 Spectrophotometer) with a pair of matched quartz cells was used for all absorbance measurements. The HPLC system consisted of Agilent Infinity using Pronto SIL-C8 column (250mm×4.6mm, 5µm) (Germany).The system was equipped with a UV-visible detector and a autosampler.

Reagents and reference samples

Pharmaceutical grade LNG, certified to contain 99.80% and Ondero tablets nominally containing 5mg of LIN were supplied from Medical store (Boehringerlngelheim Pharmaceuticals, Inc.). HPLC grade methanol and acetonitrile was purchased from Merck. Potassium dihydrogen phosphate was purchased from VWR Chemicals (Pool, England). Distilled water was supplied by AP Enterprises. Membrane filters Nylon 6, 6membrane 0.45µmfrom Pall corporation were used. All other chemicals and reagents used were of analytical grade unless indicated otherwise. Standard stock solution of 100µg/ml was prepared in 100ml volumetric flask by dissolving 10mg of the drug in 5ml methanol and made upto the mark by distill water for Spectrophotometric method. Then required concentrations were prepared by serial dilutions with distill water of these stock solutions. Whereas for Chromatographic method standard stock solution of 1000 µg/ml was

prepared in 5ml volumetric flask by dissolving 5mg of the drug in methanol in a 5ml volumetric flask and then completed to volume with methanol. Then required concentrations were prepared by serial dilutions methanol of these stock solutions.

Chromatographic conditions

Chromatographic separation was achieved on a Pronto SIL-C8 column (250mm×4.6mm, 5µm). Gradient elution using a mobile phase consisting of potassium dihydrogen phosphate buffer pH (3) -acetonitrile (35:75v/v) with UV detection at 227nm was performed. The buffer solution was filtered through 0.45µm membrane filter and degassed for 30min in an ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1ml/min. Analysis was performed at ambient temperature and the injection volume was 3µl.

Samples preparation

Ten tablets were weighed accurately and their average weight was determined and crushed to powder. Then weigh accurately about 18.53mg of powder sample (equivalent to 10mg of Linagliptin) into a clean and dry 100ml volumetric flask. Dilute with 5ml of methanol and the flask was shaken ultrasonically for 10 minutes. Then the volume was made up to 100ml using water and mix well. Filter the solution through Whatman No. 42 filter paper. After filtration an aliquot of this solution was transferred into a clean 10ml volumetric flask and volume was adjusted up to the mark with water. Absorbance of this solution at 227nm was recorded.

Procedures

Zero order derivative spectrophotometric method:-

Aliquots from LNG standard stock solution equivalent to 1-11µg were accurately measured and transferred into sets of 10ml volumetric flasks and then completed to volume with distil water. The zero order absorption of each solution was recorded against water as a blank at 227nm, then plotted against its corresponding concentration and the regression parameters were computed.

Liquid chromatography linearity and repeatability:-

Accurately measured aliquots of working standard solutions equivalent to 5-100µg LNG were separately transferred to 10ml volumetric flasks and then completed to volume with Phosphate buffer pH(3). A volume 3µl of each solution was injected into the chromatograph. The chromatographic conditions mentioned under [Chromatographic conditions], including the mobile phase at a flow rate 1ml/min, detection at 227nm and run time program for 10 min were adjusted. A calibration curve was obtained by plotting area under the peak (AUP) against concentration (C). The repeatability of the method was assessed by analysing solution containing 5µg of LIN (n=6). The precision (%RSD) for each compound was calculated.

Results and Discussion

Method development

Spectrophotometric methods:-

LNG could have been determined using zero order derivative spectrophotometry and good results were obtained (Table 1). Direct UV-absorbance measurement and derivative spectrophotometry are well established techniques for the assay of drugs in mixtures and in pharmaceutical dosage forms enhancing the resolution of overlapping bands. It can be applied for the determination of a drug in the presence of another by selecting a wavelength where contribution of one compound is almost zero while the compound to be determined has a reasonable value, so it has been used in the determination of many drugs[17-23].

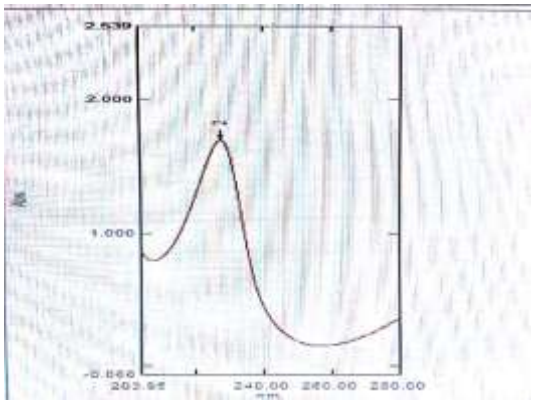

Figure 2.Zero order spectra of Linagliptin 11µg ml^{-1}.

Table 1: Optical characteristic and linearity data

Parameter	**UV-ZOD**
Absorption maximum (nm)	227
Beer's law limit (µg/ml)	1-11
Correlation coefficient	0.9984
Regression equation Y=mx+c	0.1213x - 0.0572
Intercept	0.0572
Slope (m)	0.1213

HPLC method:-

HPLC greatly reduces the analysis time and allows for the determination of many individual components in a mixture using one single procedure [(23)].

Various reversed-phase columns, gradient mobile phase systems were attempted. Gradient elution based on potassium dihydrogen phosphate buffer pH (3)-Acetonitrile (35:65 v/v) was found optimum for the resolution and peak shapes. Minimum retention times were obtained at a flow rate 1ml min^{-1}. The UV detector was operated at 227nm where good detector sensitivity was achieved. The retention time was 2.41min for LIN; as presented in Figure 2.

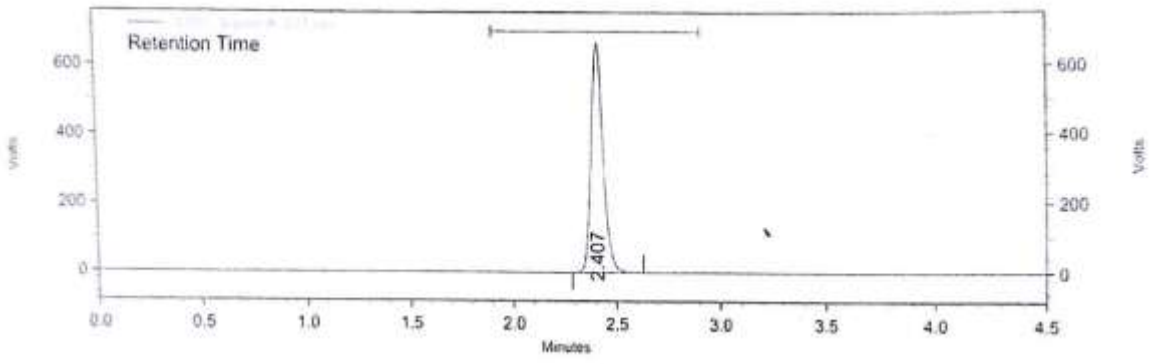

Figure 3. A typical LC chromatogram of 3µL injector of Linagliptin sample solution.

Table 2: Optimized chromatographic conditions

Parameters	**Specifications**
Mobile phase	Phosphate buffer: Acetonitrile (35:65 v/v)
Flow rate	1ml/min

Column	ProntoSIL-C8
Detector wavelength	227
Column temperature	25^0C
Injection volume	3µL
Run time	2.41
Diluent	Methanol
Detector	UV detector

Table 3: Optical characteristic and linearity data

Parameter	**RP-HPLC**
Absorption maximum (nm)	227
Linearity range (µg/ml)	5-100
Correlation coefficient	0.9998
Regression equation Y=mx+c	448404x + 10568
Intercept	10568
Slope (m)	448404

Analytical Method Development

The developed spectrophotometric and chromatographic method was validated for system suitability, specificity, precision, sensitivity, linearity, accuracy and robustness studies.

System suitability:-

This test ensures that the analytical system is working properly and can give accurate and precise results. Working standard was injected and parameters like theoretical plates per column, tailing factor, retention time were calculated from the chromatogram and the results obtained were within the acceptance criteria. The results are recorded in table 4.

Table 4: System Suitability Parameters for the proposed method.

Sr.No.	**Name**	**Retention time**	**Area**	**USP Plate Count**	**USP tailing**
1.	Linagliptin	2.41	45013279	8472	1.15

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of compounds that might be expected to be present such as impurities and matrix components. Placebo and diluents showed no interference with the drug peaks and thus the proposed method is specific. Figure 4 shows the typical chromatogram for the drug matrix (mixture of the drug-excipients) showed almost no peaks.

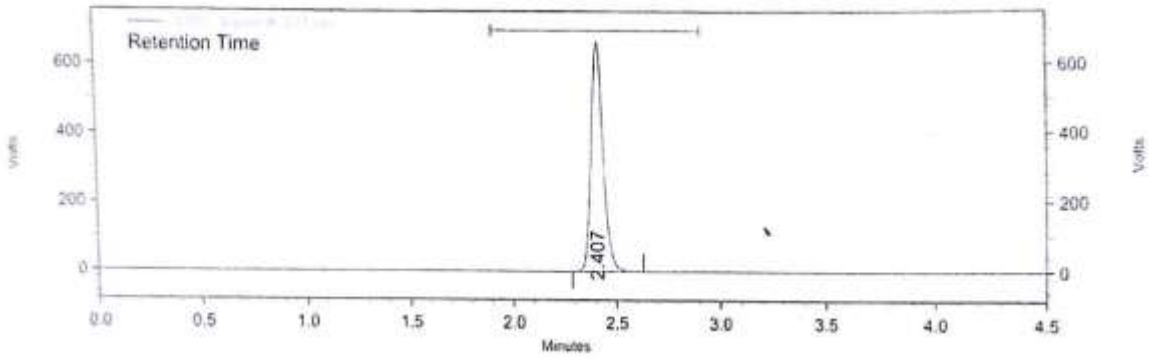

Figure 4: Standard chromatogram showing well separated peak of Linagliptin without the Interference of excipients.

Quantitative Aspects:-

Linearity:-

The study was performed by preparing range of 1-11µg/ml for spectrophotometric method and 5-100 µg/ml of analyte concentration from the working standard solution. The coefficient of determination, equation of regression line obtained from the linear regression graph is shown in the table 1 and 3. The calibration plots of Zero order spectrometric method and chromatographic method for Linagliptin are recorded in figure 5and 6 respectively.

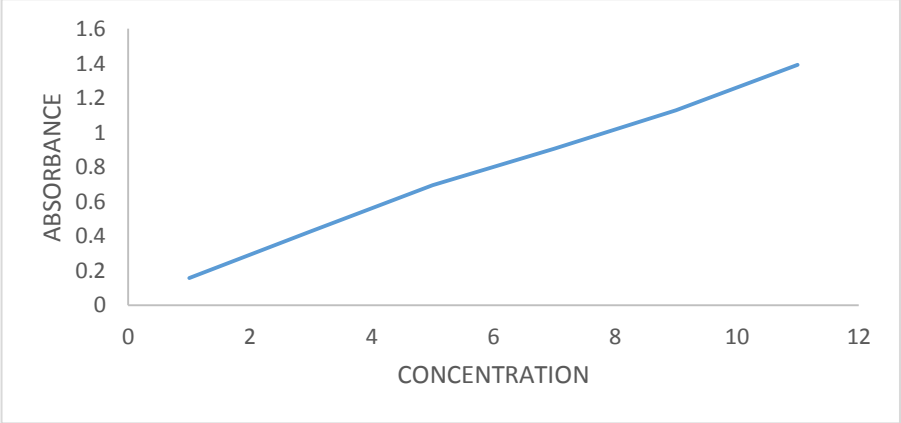

Figure 5: Calibration plot of Zero order spectrophotometric method for Linagliptin.

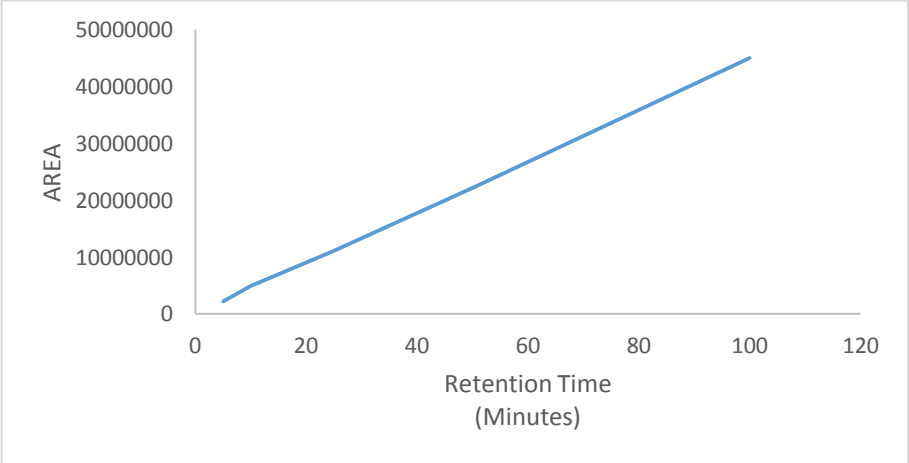

Figure 6: Calibration plot of Chromatographic method for Linagliptin.

Sensitivity:

The LOD and LOQ values were calculated mathematically based on the standard deviation of the response and the slope of the calibration plot. Results are summarized in table 5.

Table 5: Summary of results of LOD and LOQ for Linagliptin.

Sr. No.	Methods	LOD (µg/ml)	LOQ(µg/ml)
1	UV (ZOD)	0.1080	0.3297
2	RP-HPLC	2.6×10^{-07}	7.9×10^{-07}

Precision

The precision of the developed method was assessed for intraday and interday . The %RSD for Linagliptin was calculated, which is found to be within the acceptance limits (RSD < 2) and presented in table 6 and table 7.

Table 6: Precision-Intra Day data for UV-ZOD and RP-HPLC method.

Method	Concentration (µg/ml)	% Found	% RSD
UV-ZOD	3.0	99.59±0.0040	0.84%
UV-ZOD	7.0	99.11±0.0010	0.10%
UV-ZOD	11	99.05±0.0010	0.07%
RP-HPLC	10	98.87±0.035	0.10%
RP-HPLC	50	100.99±0.003	0.01%
RP-HPLC	100	99.07±0.001	0.003%

Table 7: Precision-Inter Day data for UV-ZOD and RP-HPLC method.

Method	Concentration (µg/ml)	% Found	% RSD
UV-ZOD	3.0	99.99±0.0022	0.46%
UV-ZOD	7.0	99.59±0.0026	0.28%
UV-ZOD	11	99.07±0.0015	0.10%
RP-HPLC	10	105.41±0.032	0.09%
RP-HPLC	50	105.72±0.0004	0.001%
RP-HPLC	100	103.80±0.0004	0.001%

Repeatability

It was identified by performing nine determinations of the same batch of product. Table 8 shows the recorded values.

Table 8: Repeatability study data for Linagliptin (n=9)

Method	Concentration taken (µg/ml)	% Found	% RSD
UV-ZOD	11	100.01±0.0030	0.21%
RP-HPLC	10	98.13±0.019	0.17%

Accuracy

Known amounts of reference solution for Linagliptin equivalent to 80%, 100% and 120% of the label claim were added to the tablet solution of Linagliptin. These results are summarized in Table 9.

Table 9: Accuracy data of Linagliptin.

Level of % recovery	Methods	Amount Recovered (µg/ml)	Recovery (%)
80%	UV-ZOD	4.4	102.01
80%	RP-HPLC	22.5	103.34
100%	UV-ZOD	5.0	99.23
100%	RP-HPLC	25	101.10
120%	UV-ZOD	5.3	101.10
120%	RP-HPLC	27.5	104.02

Robustness

The robustness study was performed as per USP guidelines under a variety of conditions including change in temperature and change in wavelength for RP-HPLC and UV (ZOD) method respectively. The %RSD for Linagliptin was calculated, which is found to be within the acceptance limits (RSD < 2).

Conclusion:

The proposed UV and HPLC method was found to be simple, precise, accurate, rapid and sensitive for the simultaneous estimation of Linagliptin in pure and pharmaceutical dosage forms by UV and RP-HPLC. The values obtained for various validation parameters were within the acceptable limits as per ICH guidelines. Hence the proposed method can be easily and conveniently adopted for routine quality control analysis in pure and their dosage forms.

Abbreviation

ZOD-Zero order derivative, LIN-Linagliptin, ICH-International Conference on harmonization, HPLC-High performance liquid chromatography, LC-MS-Liquid chromatography-mass spectrophotometer, µg-microgram(s), mg-milligram(s), nm-nanometre(s), %RSD-Percentage Relative Standard Deviation, SD-Standard deviation, LOD-Limit of detection, LOQ- Limit of Quantitation.

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References

1. Kirby M, Yu D, Conor S, *et al, Clinical Sci.* 2010;118:31.
2. Sekaran B, Rani P.*Int.J. Pharm. Pharm. Sci.* 2010; 2:4.
3. Niorgi R, Kandikere V, Mudigonda K, *et al. Biomed. Chromatogr*.2008; 22: 214.
4. Salsali A, Pratley R, *Nat. Rev. Endocrin.*2007; 3:450.
5. Mello S, Gregorio B, Fernando S, *et al. Clin. Sci.* 2010; 119: 239.
6. H. Spreitzer (September 1, 2008).” NeueWirkstoffe- BI-1356” (in German). *Osterreichische Apothekerzeitung*(18/2008): 918.
7. Wang Y, Serradell N, Rosa, E Castaner, R BI-1356. *Drugs of the Future* 2008; 33(6): 473-477.
8. Lakshmi B, Reddy TV, A novel RP-HPLC method for the Quantification of Linagliptin in Formulations, *J Atoms and Molecules* 2012; 2(2): 155-164.
9. Krause S.O, Analytical method validation for biopharmaceuticals: a practical guide, Guide to Validation, *Supp to Bio Pharm Int.* 2005 Mar; 26-34.

10. Kanarek A.D, Method validation guidelines. Guide to Bio analytical Advances. *Supp to Bio Pharm Int.* 2005 Sep; 28-33.
11. US FDA, Guideline for industry: text on validation of analytical procedures: ICH Q2A. Rockville, MD: Mar 1995.
12. RodbardD.Statistical estimation of the minimal detectable concentration (sensitivity) for radio ligand assays. Analytical 1978; 90:1-12.
13. Hauck W.W, Capen R.C, Callahan J.D, De Muth J.E, Hsu H, Lansky D, et al.Assessin parallelism prior to determining relative potency. *PDA J PharmaSciTechnol*2005; 59(2): 127-37.
14. ICH Q5C: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (July 1996).
15. ICH Q6A: Specifications: Test Procedures and Acceptance Criteria for New Drug Substances.
16. ICH Q6B: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products (March 1999).
17. Bebawy L, Abbas S, Fattah L, Refaat H. *IL Farmaco*. 2005; 60: 859.
18. Toral MI, Pope S, Quintanilla S, *et al. Interl J. Pharm.* 2002; 249: 117.
19. Abbaspour A, Mirzajani R. *J. Pharm. Biomed. Anal.*2005; 38: 420.
20. Satana E, Altmay S, Goger N, *et al. J. Pharm. Biomed. Anal.*2001; 25: 1009.
21. Gadila S, Batchu S, Lade S. *Chem. Pharm. Bull.*2006; 54: 819.
22. Bebawy LI. *J. Pharm. Biomed. Anal.*2002; 27: 737.
23. Altinoz S, Toptan S. *J. Fd. Comp. Anal.*2003; 16: 517.

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