Quantification of Linagliptin by Chemical Derivatization with Appliance of Chromogenic Reagents

Panikumar Durga Anumolu, Nanduri Satyakala Rani Sowndarya, Rajeshwari Galennagari, Radhagayathri Achanta, Sunitha Gurrala

Gokaraju Rangaraju College of Pharmacy, Osmania University, Hyderabad, Telangana, India

Abstract

Two simple, specific, accurate, precise, sensitive and cost effective spectrophotometric methods have been developed and validated for quantification of linagliptin in pure form and pharmaceutical formulations. Method A is established on the computation of absorbance of purple coloured chromogen complex at 463 nm which is formed by the condensation reaction of the primary amine group of linagliptin with vanillin (Schiff base formation). Method B is established on computation of absorbance of orange coloured chromogen at 454 nm which is formed by the condensation reaction of the primary amino group of linagliptin with NQS (1,2-naphtho quinine 4- sulphonic acid sodium salt) reagent. Two methods executed linearity in the concentration range of 2.5-20 μg/ml and 10-90 μg/ml for method A and B respectively. Linear relationship with good correlation coefficients of 0.998 and 0.995 were monitored between absorbance and corresponding concentrations of linagliptin in vanillin and NQS respectively. The limit of detection, limit of quantification, molar absorptivity, sandell's sensitivity and ring born concentration

values were determined for the two spectrophotometric methods. The contemplated methods were validated statistically as per ICH guidelines. The reliability of both the methods is further ascertained by performing recovery tests by standard addition method. No significant interference was inspected from the excipients commonly used as pharmaceutical aids with the assay procedure. The contemplated methods were simple, sensitive, specific and can be successfully employed in routine analysis of linagliptin in pharmaceutical dosage forms.

**Keywords:** *Linagliptin, vanillin, NQS, validation.*

Corresponding author: *Dr. Panikumar D Anumolu, Gokaraju Rangaraju College of Pharmacy, Department of Pharmaceutical Analysis, Hyderabad, Telanganas-500090. Email: panindrapharma@yahoo.co.in. Cell: 919010014734.*

Introduction

Diabetes is a chronic disease that occurs either when the pancreas does not fruitage enough insulin or when the body cannot adequately utilize the insulin it produces, which regulates the blood sugar. Hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes and it leads to serious damage to many of the body's systems, especially the nerves and blood vessels. Linagliptin is a DPP-4 inhibitor, chemically known as 8-[(3*R*)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin -2-yl) methyl]-3,7-dihydro-1*H*-purine-2,6-dione and class of organic compounds known as alkaloid derivatives for the treatment of type II diabetes (Figure-1). It works by increasing the amount of insulin produced by human body, which controls the level of sugar in human blood [1].
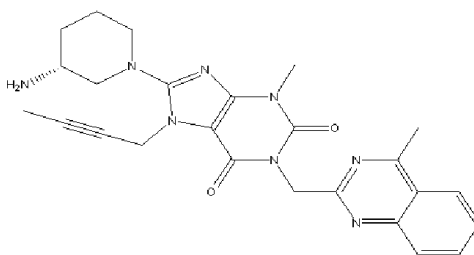
Figure 1. Chemical structure of linagliptin.

Literature survey revealed that few analytical methods such as spectrofluorimetry, spectrophotometry and HPLC [2-7] were available for quantification of linagliptin but, most of the proclaimed procedures are not simple for routine analysis and require expensive or sophisticated instruments. Hence it is always required to develop simple, fast, inexpensive analytical methods that can be readily adopted for routine analysis at a relatively low-cost to the different requirements of analytical problems.

Visible spectrophotometry, because of its simple and cost effectiveness, sensitivity and selectivity, fair accuracy, precision and easy access in most quality control laboratories, has remained competitive in the area of chromatographic techniques for pharmaceutical analysis [8-11]. Compared to HPLC the results acquisition through visible spectrophotometry is relatively faster and the preparation process for samples is simpler and time saving. Hence keeping this point into consideration, the present investigation was undertaken with objective to develop simple specific, and

extraction-free sensitive visible spectrophotometric methods using chemical derivatization technique, employing chromogenic reagents for the determination of linagliptin in bulk drug and pharmaceuticals dosage forms.Highlights are that the sensitivity of the contemplated methods were indicated by sandell's sensitivity, molar absorptivity, ring born optimum concentrations. The development of chemical derivatization method for quantification of linagliptin using chromogenic reagents was of interest as no such method has been reported.

Experimental

Double beam 1800 UV-Visible spectrophotometer (Shimadzu, Japan), analytical balance (Shimadzu AUX 220, Japan) and ultrasonic cleaner (Sonica) were used for the study. Linagliptin obtained as a gift sample from Dr. Reddy's Laboratories Limited, Hyderabad, India. Methanol, sulphuric acid, sodium hydroxide and chromogenic reagents (vanillin and NQS) were purchased from SD Fine-Chem Limited, Mumbai; Double distilled water was used throughout the study. Linagliptin tablet formulations – Tradjenta were purchased from local market.

Preparation of stock solution

The standard stock solution (1000 µg/ml) of linagliptin was prepared by solubilizing accurately weighed 10 mg of linagliptin in 10 ml of methanol. From this 1 ml solution was diluted to 10 ml with methanol to obtain standard solution of linagliptin having final concentration of 100 µg/ml.

Preparation of 0.1% Vanillin reagent solution

Accurately weighed 0.1 g of vanillin reagent was dissolved in sufficient distilled water to produce 100 ml.

Preparation of 0.5% NQS reagent solution

Accurately weighed 0.5 g of NQS reagent was dissolved in sufficient distilled water to produce 100 ml.

Procedure for calibration curve (Method-A)

Aliquots of standard drug solution of linagliptin ranging from 0.25-2.0 ml were taken into a series of 10 ml volumetric flasks. To this 1 ml of 0.1% vanillin solution and 1ml of conc. H_2SO_4 were added and shaken vigorously. The volume was made upto the mark with water to prepare a series of standard solutions containing 2.5-20 μg/ml. Then the absorbance of the colored chromogen was measured at 463 nm against corresponding reagent blank. The amount of linagliptin was computed from the calibration plot.

Procedure for calibration curve (Method-B)

Aliquots of standard drug solution of linagliptin ranging from 0.1-0.9 ml were taken into a series of 10 ml volumetric flasks. To this 0.5 ml of 0.5% NQS reagent solution and 2 ml of 20% NaOH was added, kept aside for 15 min and shaken vigorously. The volume was then made upto the mark with water to prepare a series of standard solutions containing 10-90 μg/ml. Then the absorbance of the coloured chromogen was measured at 454 nm against corresponding reagent blank. The amount of linagliptin was computed from the calibration plot.

Determination of linagliptin in capsule dosage forms (assay):

Twenty tablets were weighed and powdered. The quantity equivalent to 10 mg of active ingredient was dissolved in methanol and the volume was made upto 10 ml with methanol and filtered using whatmann's filter paper. Subsequent dilution of this solution was made.1 ml of 0.1% vanillin solution and 1 ml of conc. H_2SO_4/ 0.5 ml of 0.5% NQS reagent solution and 2 ml of 20% NaOH were added for method-A and B respectively. The solutions were kept aside for 15 min, shaken vigorously and made up to the mark with water. The absorbance of the coloured chromogen was measured at 463/454 nm against the corresponding reagent blank. Drug content in each brand of tablet were calculated and their results were statistically validated.

Validation of methods

The methods were validated for accuracy, precision, linearity, LOD and LOQ by the following procedures [12].

Accuracy

The accuracy of the methods was determined by calculating recoveries of linagliptin by the method of standard addition. Known amount of standard solutions of linagliptin were added at 80, 100 and 120% levels to pre-quantified sample solutions of linagliptin. Each sample was prepared in triplicate at each level. The amount of linagliptin was estimated by applying obtained values to regression equation.

Precision

The intra-day and inter-day precision of the proposed colorimetric method was determined by estimating the corresponding response three times on the same day and 3 different days over a period of 1 week for three different concentrations of linagliptin. The results are reported in terms of relative standard deviation (% RSD).

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) of the linagliptin was derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) as per International Conference on Harmonization (ICH) guidelines.

**Results and discussions**

Principle- Method-A

In this method, linagliptin undergoes condensation reaction with vanillin giving purple coloured schiff base product. Linagliptin contains primary amine group which reacts with an active carbonyl group in vanillin forming Schiff bases [compounds containing an imine or azomethine group (-RCH=N-)] of stable purple colour exhibiting absorption maxima at 463 nm [13-14]. (Scheme-1)
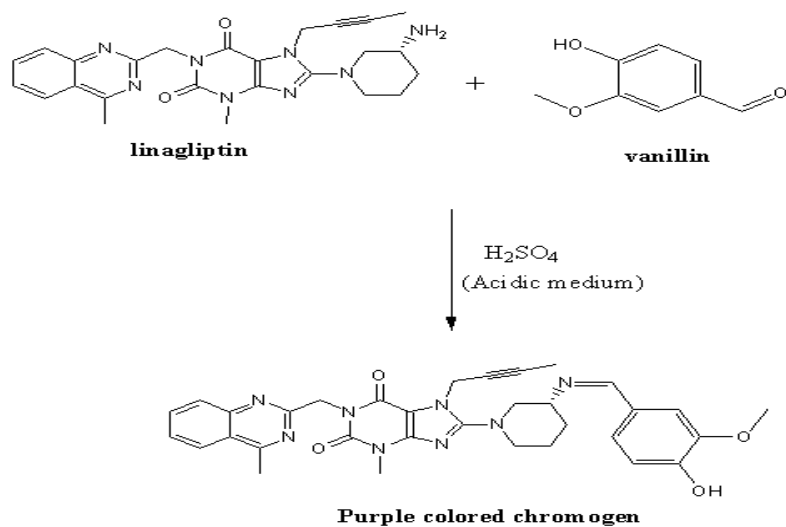
Scheme 1. Chemical reaction between linagliptin and vanillin.

Principle-Method-B

In this method, primary amine of linagliptin exhibit nucleophillic character in alkali medium attributable to the presence of the lone pair of electrons on the nitrogen atom and reacts with electron deficient center of sulfonic group in NQS reagent produced orange coloured chromogen complex. The absorbance maximum was observed at 454 nm [15-21] (Scheme-2).
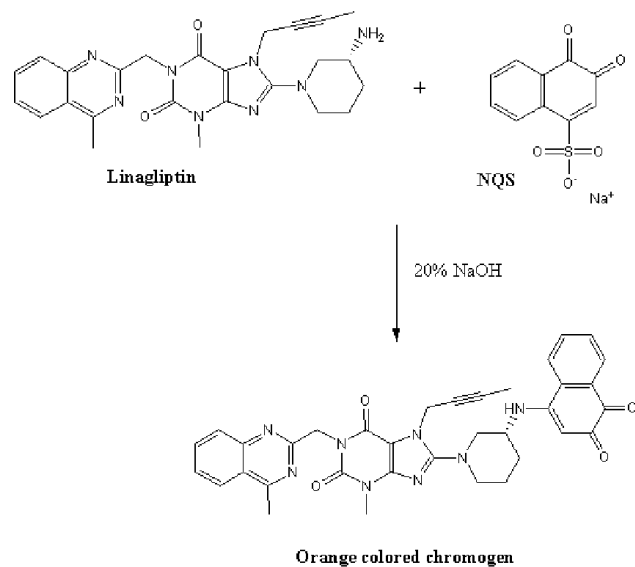
**Scheme 2.** Chemical reaction between linagliptin and NQS.

Validation of proposed methods

The proposed methods were statistically validated as per ICH guidelines. The optimized conditions of proposed method mentioned in Table-1 and follows Beer's Law in the concentration range of 2.5-20 and 10-90 µg/ml for linagliptin by method A and B respectively with correlation co-efficient 0.995. The linearity spectra and calibration plots are shown in figures 2, 3 and 4. The reproducibility of proposed methods was evidenced by there was no significant difference between intra and inter-day precision values and % RSD values were less than 2. The % recoveries of linagliptin were found to be in the range of 98.33-104.9 and 98.74-101.42 for method- A and B respectively. The sensitivity of the contemplated methods was indicated by sandell's sensitivity, molar absorptivity, ring born optimum concentrations, LOD and LOQ values, reported in Table-1.

Table 1. Optimized characteristics of linagliptin.

| Parameters	Value	
	Vanillin	**NQS**
Absorption Wavelength(nm)	463	454
Beers law range (µg/ml)	2.5-20	10-90
Ring born optimum concentration (µg/ml)	5-15	30-70
Molar absorptivity (L/ mol cm)	2.06×10^4	5.8×10^3
Sandell's sensitivity (µg/cm)	0.02	0.08
Limit of Detection (µg/ml)	0.0249	2.86
Limit of Quantification (µg/ml)	0.754	8.66
Correlation coefficient(r^2)	0.998	0.995
Slope(m)	0.053	0.009
Intercept(c)	0.021	0.016
Regression equation (y)	Y=0.053x-0.021	Y=0.009x+0.016
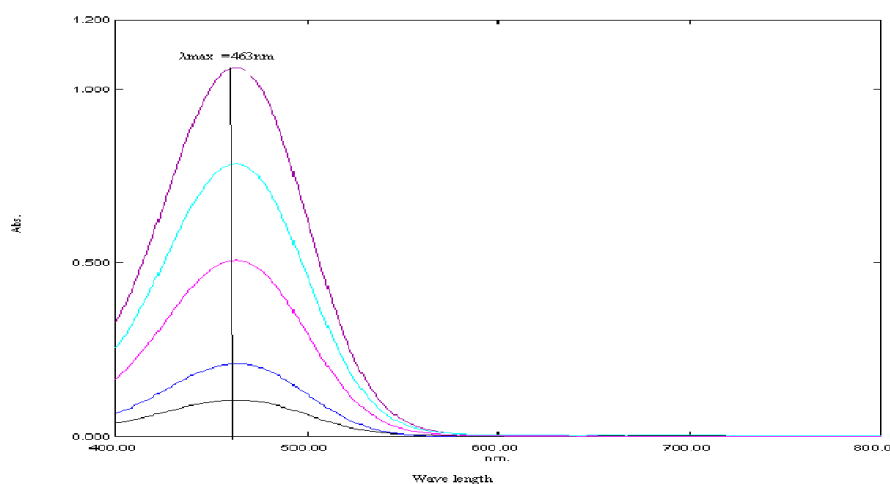
Figure 2. Linearity spectra of linagliptin (2.5-20 µg/ml) with vanillin.
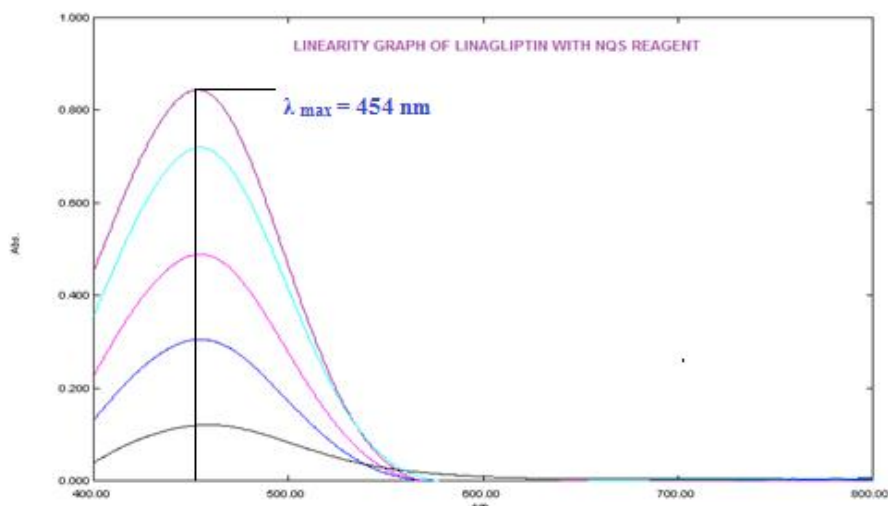
Figure 3. Linearity spectrum of linagliptin (10-90 µg/ml) with NQS.

Assay of marketed tablet formulation

The contemplated methods were applied to the assay of marketed tablets containing 5 mg of linagliptin. The results obtained for assay values were compared with the labeled claim and reported in Table-2. The % assay of linagliptin in marketed tablets found to be 104 and 98 by method –A and B respectively. The % RSD values were less than 2.
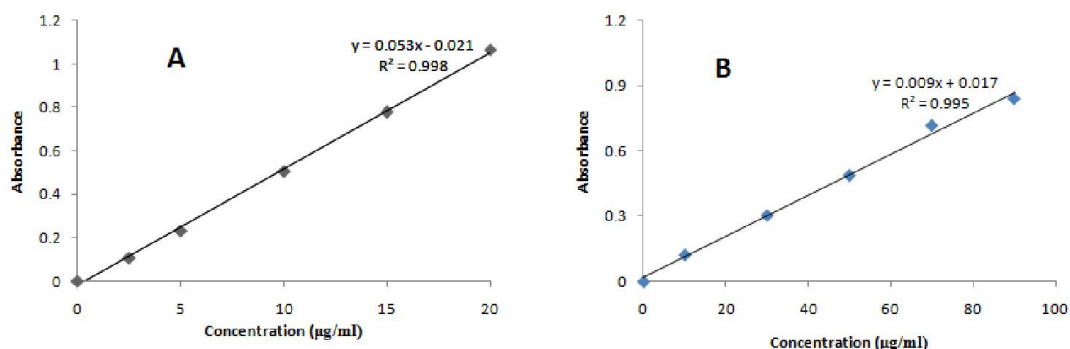
Figure 4. Calibration plot for linagliptin with vanillin (A) and NQS (B).

Table 2. Data for assay studies.

Reagent name	Label claim (mg)	Amount found (AM ±SD)	% RSD
Vanillin	5	5.24±0.004	0.076
NQS	5	4.88 ± 0.078	1.598

Conclusion

The present investigation was undertaken with an objective to develop and validate two simple and sensitive extraction-free spectrophotometric methods using chemical derivatization technique utilizing functional group reagents vanillin and NQS reagent for the determination of linagliptin in bulk drug and pharmaceuticals dosage forms.The assay values were in good agreement with their respective label claim which suggested no interference of formulation excipients in the estimation and obtained results from validation evidenced that the proposed methods were scientifically sound. The sensitivity of the proposed method was evidenced by Sandell's sensitivity, LOD and LOQ values. These advantages encourage that the proposed method can be routinely employed in the quality control for analysis of linagliptin in the pharmaceutical dosage forms.

Acknowledgement

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