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Spectrophotometric estimation of linagliptin using ion-pair complexation and oxidative coupling reactions – A green approach

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ABSTRACT

Background: Two specific spectrophotometric methods (visible region) have been established using water as a solvent and validated for the analysis of linagliptin in API and pharmaceutical dosage forms. **Materials and Methods:** Method-A is established on the computation of the absorbance of green-colored chromogen complex at 660 nm, which is formed by the oxidative coupling reaction of linagliptin with 3-methyl-2-benzothiazoline hydrazine in the presence of ferric chloride. Method-B is established on the computation of the absorbance of an orange-colored ion-pair chromogen at 490 nm, which is formed by the charge transfer reaction of the primary amino group of linagliptin with picric acid. **Results:** Beer's law is obeyed in the drug concentration range of 2–12 µg/mL and 1–25 µg/mL for methods A and B, respectively, with a correlation coefficient of 0.999. The contemplated methods are validated statistically according to ICH Q2(R1) guidelines and results are found to be within the acceptable limits. The labeled amount of linagliptin in a marketed formulation (TRAJENTA®) is determined without interference owed to excipients. **Conclusion:** The contemplated green approached spectrophotometric methods can prolifically be employed for analysis of linagliptin because of its easy access in most quality control laboratories.

Keywords: Linagliptin, spectrophotometric, 3-methyl-2-benzothiazoline hydrazine, picric acid

INTRODUCTION

Linagliptin is a competitive and reversible dipeptidyl peptidase (DPP)-4 inhibitor that slows the breakdown of insulinotropic hormone glucagon-like peptide (GLP)-1 for better glycemic control in diabetes patients. Chemically, it is known as 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.

All-embracing literature survey revealed that few analytical methods such as spectrophotometric,[1-7] spectrofluorimetric,[8] and high-performance liquid chromatography[9,10] were reported for the determination of linagliptin. One colorimetric method using NQS and vanillin as chromogenic reagents was reported.[11] Colorimetry is found to be more sensitive and specific than spectrophotometry (UV region). The advantage of using chromogenic reagent in drug analysis is its selective chemical reaction with an analyte to form a colored derivative.[12-16] Visible spectrophotometric methods are relatively inexpensive, simple, and faster (in terms of sample preparation) than chromatographic techniques and can prolifically be adopted for drug analysis due to easy access in most quality control laboratories.

The present investigation aims to develop economic, sensitive, and extraction-free visible spectrophotometric methods for the estimation of the linagliptin subjugating chemical derivatization technique. Two chromogenic reagents (3-methyl-2-benzothiazoline hydrazine [MBTH] and picric acid) are employed for chemical derivatization of linagliptin, which were not reported earlier. The proposed methods have high sensitivity and rely on the use of distilled water as a solvent (eco-friendly) which dwindles the cost of the experiment.

MATERIALS AND METHODS

Instrumentation and Chemicals

Double beam 1800 UV-Visible spectrophotometer (Shimadzu, Japan), analytical balance (Shimadzu AUX 220, Japan), and ultrasonic cleaner (Sonica) were used for the study. Linagliptin standard gift sample was provided by Dr. Reddy's Laboratories Pvt. Ltd., Hyderabad, India. Methanol was purchased from Qualigens, Mumbai. Ferric chloride, sodium hydroxide, and chromogenic reagents (MBTH and picric) were purchased from SD Fine-Chem Ltd., Mumbai. Double distilled water was used throughout the study. Marketed dosage form (TRADJENTA®) of linagliptin was procured from the local pharmacy.

Preparation of Solutions

The standard stock solution (1000 µg/mL) of linagliptin was prepared by solubilizing accurately weighed 10 mg of linagliptin in 10 mL of methanol. The solution was further diluted with distilled water to get the required concentration of linagliptin. Solutions of ferric chloride (3% w/v), MBTH reagent (0.9% w/v), picric acid (0.4% w/v), and sodium hydroxide (2M) were prepared in distilled water.

General Analytical Procedures

Method-A

Determination of linagliptin based on measurement of oxidative-coupling derivative. Linagliptin standard solution (100 µg/mL) of 1 mL was transferred into a volumetric flask of 10 mL capacity. To this 2 mL of MBTH (0.9% w/v) reagent, 2 mL of ferric chloride (3% w/v) was added, shaken vigorously and kept aside for 10 min for color development. The volume was contrived up to the mark with distilled water and absorbance of green colored chromogen was measured at 660 nm against corresponding reagent blank.

Method-B

Determination of linagliptin based on the measurement of ion-pair complex. Linagliptin standard solution (100 µg/mL) of 1 mL was transferred into a volumetric flask of 10 mL capacity. To this 2 mL of picric acid (0.4% w/v) reagent, 2 mL of sodium hydroxide (2 M) was added, shaken vigorously and kept aside for 20 min for color development. The volume was contrived up to the mark with distilled water and absorbance of orange-colored chromogen was measured at 490 nm against corresponding reagent blank.

Evidence of Chemical Derivatization

The thin-layer chromatography (TLC) technique was used with ethyl acetate:acetonitrile (8:2) as a mobile phase on pre-coated TLC plates. Plates were spotted separately for method-A and B with a freshly prepared solution of linagliptin, reagent blank solution, and chromogen produced by that method. Plates were developed in saturated chromatographic tanks and spots were visualized in the UV chamber at 254 nm.

Validation of Methods

The proposed methods were validated for linearity, accuracy, precision, LOD, and LOQ as per the ICH guidelines.[17]

Linearity studies

Method-A

Aliquots of standard drug solution of linagliptin (100 µg/mL) ranging from 0.2 to 1 mL were taken into a series of 10 mL volumetric flasks. To these flasks, 2 mL of MBTH (0.9% w/v) reagent, 2 mL of ferric chloride (3% w/v) were added, shaken vigorously and kept aside for 10 min for color development. The volume was contrived up to the mark with distilled water to get a series of standard solutions containing 2, 4, 6, 8, 10, and 12 µg/mL of linagliptin. The absorbance of the green-colored chromogen was measured at 660 nm against the corresponding reagent blank.

Method-B: Aliquots of standard drug solution of linagliptin (100 µg/mL) ranging from 0.1 to 2.5 mL were taken into a series of 10 mL volumetric flasks. To these flasks, 2 mL of picric acid (0.4% w/v) and 2 mL of sodium hydroxide (2 M) were added, shaken vigorously and kept aside for 20 min for color development. The volume was contrived up to the mark with distilled water to get a series of standard solutions containing 1, 5, 10, 15, 20, and 25 µg/mL of linagliptin. The absorbance of the orange-colored chromogen was measured at 490 nm against the corresponding reagent blank.

Precision

The intra-day and inter-day precision of the proposed colorimetric methods were established with three different concentrations of linagliptin within the linearity range. These solutions were prepared in triplicate on the same day and 3 consecutive days over a period of 1 week. The percent relative standard deviation (% RSD) values were calculated.

Accuracy

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

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

The sensitivity of the proposed methods was determined concerning LOD and LOQ. These were separately determined based on standard calibration curve using 3.3 σ/s and 10 σ/s, formulae, respectively, where s is the slope of the calibration curve and σ is the standard deviation of the y-intercept of the regression equation.

Assay of Linagliptin Marketed Dosage Forms

Twenty tablets of linagliptin (TRADJENTA®) were weighed and powdered. The powder quantity equivalent to 10 mg of linagliptin was dissolved in 10 mL methanol and filtered using Whatman's filter paper. Filtrate (0.1 mL) was transferred into a 10 mL volumetric flask, 2 mL of MBTH and 2 mL of FeCl3 (method-A)/2 mL of picric acid and 2 mL of NaOH (method-B) were added, shaken vigorously and kept aside for 10 min/20 min. The volume was made up to the mark with water. The absorbance

of the colored chromogen was measured at 660/490 nm against the corresponding reagent blank. The amount of linagliptin was computed from the Beer–Lambert's plot.

RESULTS AND DISCUSSION

Basis of Chemical Derivatization

In method-A, linagliptin undergoes an oxidative coupling reaction with MBTH giving green colored chromogen as a product in the presence of oxidizing agent (ferric chloride). Oxidation (loss of two electrons and one proton) of MBTH by ferric chloride produces an electrophilic intermediate, which coupled at the most nucleophilic site of linagliptin and forms a green colored chromogen [Figure 1], which absorbs visible light maximally at a wavelength of 660 nm in a spectrophotometer.

Method-B involves recceing the charge transfer reaction of linagliptin with picric acid. The hydroxy group of picric acid (Lewis acid) transfers a proton to the amine group of linagliptin (Lewis base) leads to the formation of an ion-pair complex. This proton transfer reaction between linagliptin and picric acid produces orange-colored complex [Figure 2] exhibiting absorption maxima at 490 nm in a spectrophotometer.

Evidence of Chemical Derivatization

Evidence of linagliptin chemical derivatization with proposed reagents was ascertained by the TLC analysis of reaction mixture. Three spots were observed with different R_f values on both plates (method-A and method-B), indicate the presence of three different compounds. Higher retardation factor values were observed for derivatives [Table 1], denote the formation of a new compound by the proposed reaction mechanism.

Optimization of Reaction Conditions

Optimization of reagent concentration, diluting solvent and time for color development were established to accomplish maximum absorbance and stability. The influence of variables on absorption values of colored species was studied by varying one parameter at a time and keeping others at constant.

Effect of reagent concentration

The effect of MBTH and picric acid concentration on their reaction with linagliptin was studied by adding 2 mL of various concentrations of MBTH (0.1, 0.3, 0.6, 0.9, 1.2, and 1.5 %w/v) in method-A/picric acid (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 %w/v) in method-B to a fixed concentration of linagliptin (10 µg/mL). It was observed that absorbance values were increased with increasing reagent concentration up to a certain level, thereafter decreased absorbance may due to the saturated level of reagent. In method-A, 0.9% MBTH and in method-B, 0.4% picric acid was optimized, these levels were adequate for reproducible and maximum color development.

Table 1: TLC analysis of linagliptin and reaction products

Sample	Method-A	Method-B
	R_f value	R_f value
Linagliptin standard	0.05±0.002	0.06±0.003
Reagent blank	0.42±0.01	0.53±0.02
Linagliptin-chemical derivative	0.68±0.03	0.77±0.02

TLC: Thin-layer chromatography

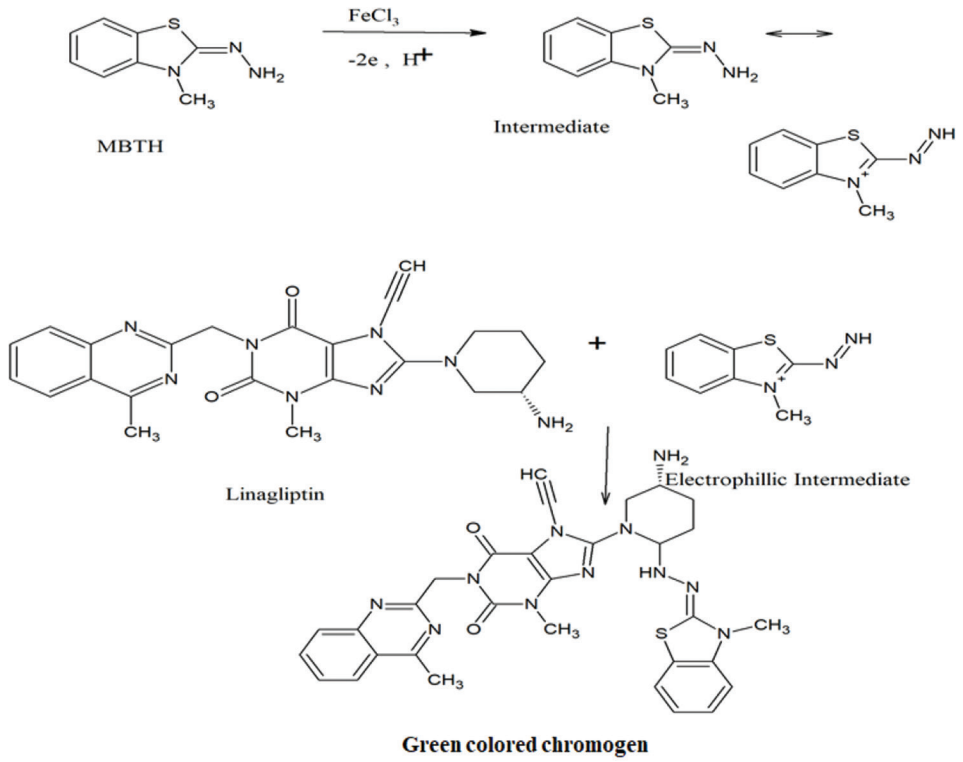

Figure 1: Oxidative coupling reaction between linagliptin and 3-methyl-2-benzothiazoline hydrazine

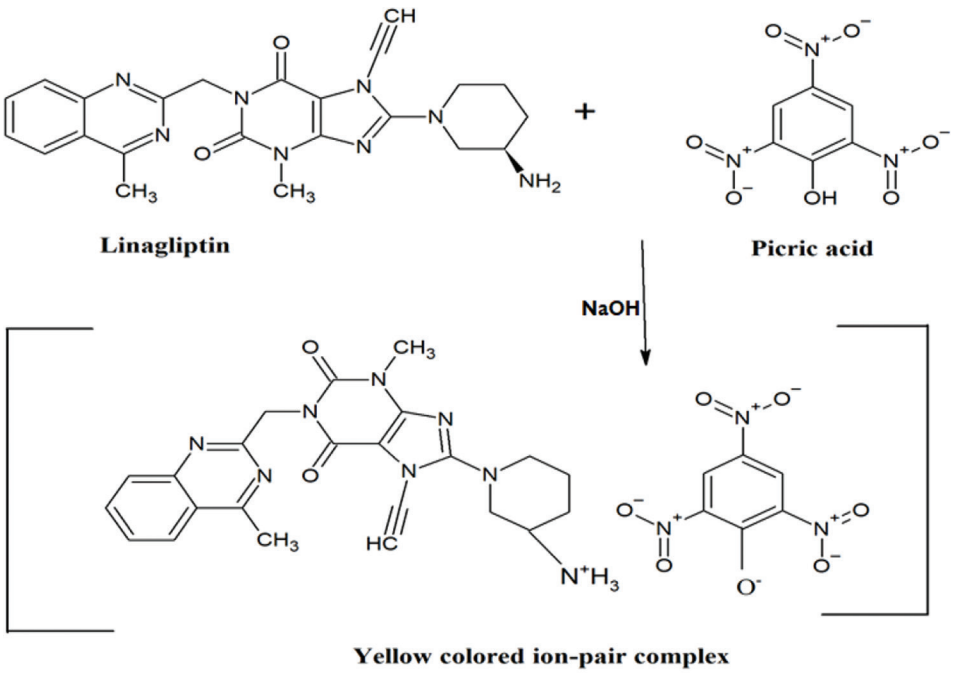

Figure 2: Ion-pair complexation between linagliptin and picric acid

Effect of solvents

Diluting solvent plays an important role in the stability of the colored complex. The effect of different diluting solvents such as acetone, acetonitrile, ethanol, methanol, and distilled water have been studied [Figure 3] for measurement under optimized time and reagent concentration. The best sensitivity, maximum UV absorption, and product stability were attained when water was used as a solvent for both the methods. Both reagents were freely soluble in water. Hence, distilled water was selected as a diluting solvent for proposed methods that dwindle the cost of experiment and considered as a green approach for spectrophotometric method development.

Effect of oxidizing agent/alkalinity

In method-A, ferric chloride was utilized as an oxidizing agent for coupling reaction between linagliptin and MBTH reagent. It was found that reagent and an oxidizing agent (3% of ferric chloride) at a 1:1 ratio were adequate for the formation of an electrophilic intermediate. In method-B, sodium hydroxide was employed as an alkaline media for ion-pair complexation between linagliptin and picric acid. The rate of reaction increases with an increase in sodium hydroxide concentration (first-order rate kinetic reaction). There was also a decrease in absorbance with an increase in hydroxide concentration (more than 2 M) due to a backward reaction. Hence, 2M sodium hydroxide was optimized and 1:1 ratio of picric acid:sodium hydroxide was adequate for the formation of orange-colored chromogen with linagliptin.

Optimization of reaction time

The optimum reaction time was determined by monitoring the color development at different time intervals. Maximum absorbance values were obtained at 10 min for method-A and 20 min for method-B. The color developed for linagliptin derivatives was stable up to 6 h with both reagents under optimized conditions.

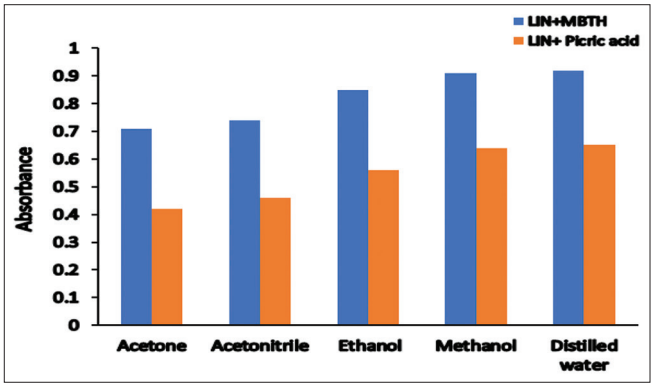

Figure 3: Effect of solvent on linagliptin reaction with 3-methyl-2-benzothiazoline hydrazine and picric acid

Stoichiometry of reaction

Stoichiometry of reaction in method-A and method-B was studied by continuous variation method. Equimolar solutions (2.11×10^{-5} M) of linagliptin and reagents (MBTH and picric acid) were prepared in distilled water. The drug and reagent (MBTH- method A/Picric acid-method B) were mixed in various proportions to produce different mole ratio values (0, 0.2, 0.4, 0.5, 0.6, 0.8, and 1). The stoichiometric relationship exhibited in Figure 4. A mole ratio of 0.5 gave the highest absorbance value for both methods. This indicates that linagliptin has one center (primary amino group) available for chromogenic reaction with reagents at their optimum wavelengths.

Validation of Proposed Methods

The proposed methods were statistically validated as per the ICH guidelines and results are depicted in Table 2. Linear regression analysis was performed for the Beer's Law data and

calibration plots were drawn (correlation coefficient 0.999). A linear increase in the absorbance was found with an increase in linagliptin concentration at a range of 2–12 and 1–25 µg/ml of linagliptin by methods A and B, respectively. Overlaid UV-Visible spectra of linagliptin in the linearity range are shown in Figures 5 and 6. The reproducibility of proposed methods was evinced by precision studies [Table 3], where no significant difference between intra- and inter-day precision values was observed and % RSD values were less than 2. The results of accuracy studies are demonstrated in Table 4. The % recoveries of linagliptin denote the fair accuracy of proposed methods with no interference of tablet excipients. A high value of molar absorptivity and low values of Sandell's sensitivity, LOD, and LOQ signposts the good sensitivity of proposed methods.

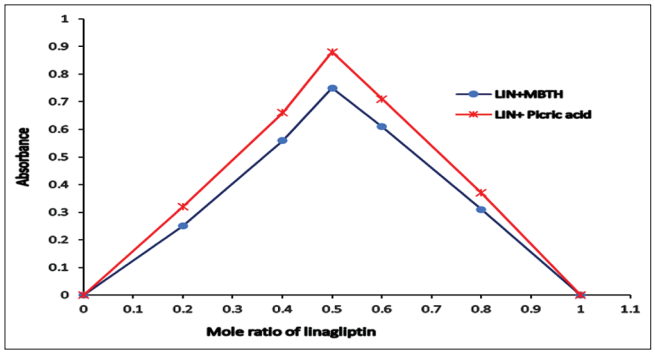

Figure 4: Job's continuous variation plot for method-A and method-B

Table 2: Optimized characteristics of linagliptin

Parameters	Value	
	MBTH	Picric acid
Absorption wavelength (nm)	660	490
Beers law range (µg/mL)	2–12	1–25
Regression equation (y)	Y=0.102x+0.011	Y=0.036x–0.002
Correlation coefficient(r^2)	0.999	0.999
Limit of Detection (µg/ml)	0.35	0.091
Limit of Quantification (µg/ml)	1.17	0.27
Molar Absorptivity (L $mole^{-1}cm^{-1}$)	0.393×10^5	0.189×10^5
Sandell's sensitivity (µg cm^{-2})	1.2×10^{-2}	2.5×10^{-2}
Stability of colored species	6 h	7 h

MBTH: 3-methyl-2-benzothiazoline hydrazine

Assay of Linagliptin Marketed Dosage Forms

The proposed methods were applied for the assay of marketed dosage forms containing linagliptin (label claim 5 mg). The % assay of linagliptin found to be 99.8 and 100.3 by method-A

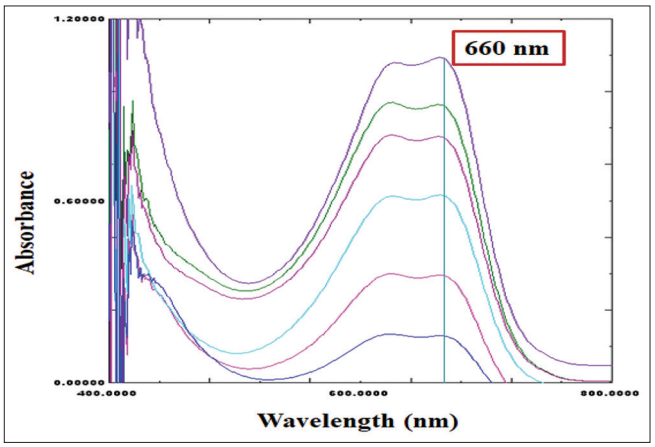

Figure 5: Overlaid UV-Visible spectra of linagliptin (2–12 µg/mL) with 3-methyl-2-benzothiazoline hydrazine

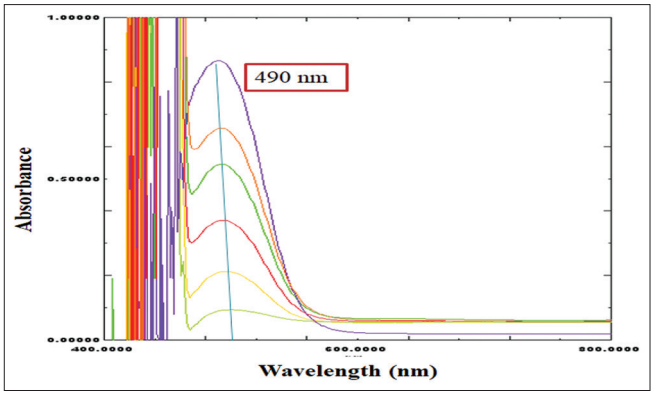

Figure 6: Overlaid UV-Visible spectra of linagliptin (1–15 µg/mL) with picric acid

Table 3: Results of precision studies using proposed methods

Method	Concentration linagliptin (µg/mL)	Intra-day analysis		Inter-day analysis	
		Amount found (AM ±SD), *n*=3	% RSD	Amount found (AM ±SD), *n*=3	% RSD
Method-A	4	3.96±0.011	0.27	4.01±0.012	0.299
	6	6.1±0.023	0.377	5.96±0.021	0.352
	8	8.1±0.023	0.283	8.27±0.025	0.302
Method-B	5	5.06±0.004	0.079	5.2±0.002	0.038
	10	9.92±0.021	0.211	10.2±0.01	0.09
	15	14.9±0.019	0.127	14.8±0.02	0.135

Table 4: Results of accuracy studies of proposed methods with tablets

Method	Level of recovery (%)	Conc. test sample (μg/mL)	Conc. standard spiked (μg/mL)	Total amount (μg/mL)	Amount recovery (AM±SD), n=3 (μg/mL)	% recovery	% RSD
Method-A	80	4	3.2	7.2	7.15±0.06	99.8	0.83
	100	4	4	8	7.92±0.04	99.9	0.505
	120	4	4.8	8.8	8.75±0.03	99.4	0.342
Method-B	80	10	8	18	17.99±0.08	99.44	0.446
	100	10	10	20	19.92±0.06	99.5	0.030
	120	10	12	22	21.9±0.05	99.81	0.227

RSD: Relative standard deviation

and method-B, respectively. There was no interference of formulation excipients during the estimation of linagliptin in tablets. The assay values were found to be within the limits and % RSD was <2.

CONCLUSION

In this study, two spectrophotometric methods were developed which evaded the use of organic solvents for analysis of linagliptin in bulk and pharmaceutical dosage form. Chemical derivatization mechanisms for linagliptin with MBTH and picric acid were proposed. Proposed methods were passes through validation parameters as per the ICH guidelines. The assay values were in good agreement with the label claim and suggested that no interference of formulation excipients during the estimation of the drug. Contemplated methods are more sensitive, less chemical perilous, and versatile over reported methods. These have broad linearity range, high precision, and accuracy. Hence, the proposed eco-friendly and economical methods can be routinely employed in the quality control for analysis of linagliptin in the pharmaceutical dosage forms.

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