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Development and validation of q-absorbance ratio method for simultaneous quantification of linagliptin and metformin HCl in synthetic mixture and combined tablets

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Abstract

The present study aims to develop and validate simple, cost effective Q-Absorbance Ratio Method for simultaneous quantification of Linagliptin (LIN) and Metformin HCl (MET). The proposed medium Acetonitrile: Methanol: Distilled Water at 1:1:1 (Solvent Blend) identified λmax 235nm for MET, 298nm for LIN and Isobestic point at 231.7nm for Q-Absorbance Ratio Method. The validation follows ICH Q2(R1) guidelines, demonstrating excellent linearity, accuracy, precision and sensitivity (LOD and LOQ) for LIN and MET. The developed method was successfully applied to a synthetic mixture and marketed formulation, showing % recovery within the acceptable limit with % RSD was less than 2.

Keywords: Linagliptin; Metformin HCl; Q-Absorbance Ratio Method; Validation; ICH

1. Introduction

Linagliptin (LIN)[1,2] appeared as white to pale coloured amorphous powder has melting point of 202°C, slightly soluble in DMSO, methanol and acetonitrile. Chemically LIN (figure 1) is 8-[(3R)-3 amino piperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1- [(4 methyl quinazolin-2-yl) methyl]-3, 7-dihydro-1H-purine 2, 6-dione. It is a Type 2 antidiabetic drug which acts by blocking the action of DPP-4, an enzyme that destroys the hormone GLP-1, which helps the body to provide more insulin when it is needed. It stimulates the release of insulin and inhibits the release of glucagon, resulting in the decrease levels of circulating glucose.

Metformin HCl (MET)[3,4] is a white to off-white crystalline powder, has melting point of 226 °C, soluble in water, methanol, acetonitrile and varied buffer pH, insoluble DMSO. Chemically MET (figure 1) is 1-carbamimidamido-N, N-dimethyl methanimidamide hydrochloride. MET is a biguanide antihyperglycemic agent and first-line pharmacotherapy used in the management of type II diabetes, which decreases blood glucose levels by decreasing hepatic glucose production, decreasing the intestinal absorption of glucose, and increasing insulin sensitivity by increasing peripheral glucose uptake and utilization. It is well established that MET inhibits mitochondrial complex I activity, and it has since been generally postulated that its potent antidiabetic effects occur through this mechanism.

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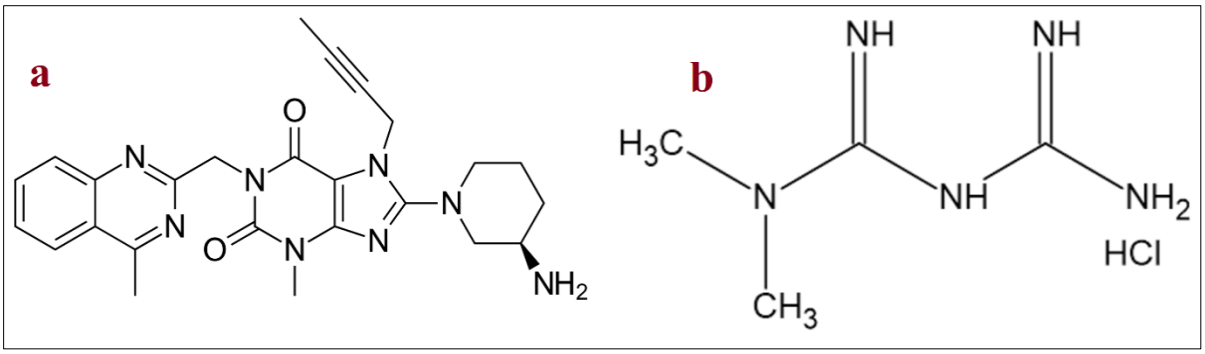

Figure 1 Chemical structure of a) LIN and b) MET

The literature shown various methods for the estimation of LIN[5-11] and MET[12-17] alone and in combination viz., UV[18], HPLC[19,20], HPTLC[21,22], RP-HPLC[23-37], LCMS[38]. These methods require sophisticated instrumentation, expensive solvents, and complex sample preparation. Moreover, the use of organic solvents in chromatographic techniques raises environmental concerns due to solvent waste and associated health hazards.

To address these challenges, conventional UV spectrophotometric methods, including the Q-Absorbance Ratio Method (QARM)[39-43] and the Simultaneous Equation Method (Vierordt's method), were selected for this study. These techniques offer a straightforward, cost-effective alternative to chromatographic methods, making them particularly suitable for laboratories with limited resources. Unlike chromatographic procedures, UV spectrophotometric methods do not require costly columns, organic solvents, or extensive sample preparation. Additionally, they provide a rapid and efficient means of quantification, especially for compounds with overlapping absorption spectra.

The QARM or Q-Absorption or Q-analysis method is one UV spectrophotometric technique used for the simultaneous quantification of two components in a mixture without prior separation. This method is based on the ratio of absorbances (Q-values) at two selected wavelengths viz., λ_1 (isoabsorptive point) where the wavelength at which both components have the same absorptivity and λ_2 is the λ max (absorption maxima) of one of the components. By measuring the absorbances at these two wavelengths, the concentrations of both components can be calculated using the absorptivity coefficients[44,45]. So far, Q- Absorption ratio spectrophotometric method was not reported for the estimation of LIN and MET in formulations.

Hence, the present research work was aimed to develop and validate Q-Absorbance Ratio UV spectrophotometric method for simultaneous quantification of LIN and MET in synthetic mixture and combined dosage forms.

2. Materials and methods

2.1. Chemicals and reagents

Drug sample of Linagliptin (LIN) and Metformin HCl (MET) were provided as a gift sample by Magnus Pharma Ltd, Nepal. Ondero Met 2.5/500 tablets, Trajenta Duo 2.5/500 tablets (Linagliptin/Metformin HCl) were purchased from local pharmacy store. Solvents like Acetonitrile, Methanol were purchased from SD Fine Chemicals, Mumbai. Distilled water was used throughout the study.

2.2. Apparatus

Instrument used was of Shimadzu UV-1900 series with a pair of 1cm path length matched quartz cells, software used was UV Probe. Anamed digital analytical balance was used for precise weighing. Validated glassware's were meticulously cleaned with distilled water and dried before use.

2.3. Preparation of standard stock solution

Stock solutions of LIN and MET were prepared separately by dissolving 25 mg of each drug in 25 mL solvent blend comprising Acetonitrile: Methanol: Distilled Water at 1:1:1 in a volumetric flask, resulting in a concentration of 1000 µg/mL. The solutions were sonicated for 10 mins to ensure complete dissolution. From each stock solution, 2.5 mL was withdrawn using a calibrated glass pipette and diluted to 25 mL with solvent blend to achieve a concentration of 100 µg/mL for both LIN and MET.

3. Methodology

3.1. Determination of absorption maxima and wavelength selection

Stock solutions of LIN and MET were made appropriate dilution separately with solvent blend to obtain final concentrations of 10 µg/mL for LIN and MET. These solutions were scanned individually in the ultraviolet range (400 nm to 200 nm) and determine their maximum absorbance wavelength (λ max). Overlay spectra of LIN and MET were generated and isoabsorptive point wavelength was selected.

3.2. Q-Absorbance Ratio Method (QARM)

The QARM which obeys Beer's law at all wavelength, the ratio of absorbance at any single wavelengths is constant value independent of concentration or path length. At Isobestic point nm solutions of both drugs of same concentration exhibit identical absorbance and consequently with zero difference. Such wavelengths of equal absorptivity of the two species are called isobestic or isoabsorptive points. QARM uses ratio of absorbance at two selected wavelengths, one which is an isoabsorptive point and other being the λ max of one of two components. From over lay spectra of two drugs, it was evident that LIN and MET have an isoabsorptive point at 231.17 nm (λ1). The second wavelength used was 235 nm of (λ max of MET) or 298 nm of (λ max of LIN). LIN and MET showed considerable absorbance at both wavelengths (figure 2). The concentration of two drugs of mixture in 1:1 ratio at 235 nm or 298 nm can be calculated using following equation,

$$C_x = \frac{Q_M - Q_y}{Q_x - Q_y} * \frac{A_1}{ax_1} \qquad C_y = \frac{Q_M - Q_x}{Q_y - Q_x} * \frac{A_2}{ay_1}$$

Where, A1 and A2 are absorbance of mixture solution at 231.7 nm and 235 nm

ax_1 = A (Absorptivity, 1 %, 1 cm) of LIN at 231.7 nm

ay_1 = A (Absorptivity, 1 %, 1 cm) of MET at 231.7 nm

ax_2 = A (Absorptivity, 1 %, 1 cm) of LIN at 235 nm

ay_2 = A (Absorptivity, 1 %, 1 cm) of MET at 235 nm

C_x and C_y are the unknown concentration of LIN and MET respectively in sample solution.

$Q_M = A_2/A_1$, $Q_X = ax_2/ax_1$ and $Q_Y = ay_2/ay_1$

3.3. Determination of linearity curve

Stock solutions of LIN and MET were made appropriate dilution with solvent blend in a series of sets of 10 ml volumetric flask to obtain 2, 4, 6, 8 and 10 µg/mL solutions separately for LIN and MET. The absorbance of both solutions was taken at their respective λ max viz., 298 nm for LIN and 235 nm for MET and at isoabsorptive point 231.7 nm. The linearity curves were constructed by plotting concentration against absorbance where each reading was an average of six determinations.

3.4. Preparation of test solution for assay

LIN (2.5 mg to 5 mg) and MET (500 mg to 2000 mg) alone and in combination LIN: MET in the ratio of 1:200 were used in the treatment of diabetes. Practicability of such ration make difficult for analytical estimation, to make simulation for the analytical study appropriate ratios of LIN: MET (2.5 mg: 12.5 mg; 2.5 mg: 25 mg) were chosen. Mix appropriately the stock solutions of LIN and MET to get simulated solutions viz., 1:5 and 1:10 and were considered as test solutions. Similarly sample test solution for marketed combined tablets were prepared. In each case 10 tablets were weighed and crushed into powder. The powder equivalent to 10 mg of LIN and 500 mg MET were extracted with solvent blend under the study for 2 hr, followed by sonication for 30 min. The content were filtered and the filtrate was collected and stored for further analysis.

4. Method validation

The proposed method was validated as per ICH guidelines Q2 (R1).

4.1. Precision

The precision of the proposed method was assessed as repeatability, intraday precision and interday precision. Repeatability was performed by applying six replicates of sample solution. For intermediate precision, Intraday and Interday precision was performed by determining corresponding responses of six replicates on same and different days for test solution containing LIN (10 µg/mL) and MET (10 µg/mL) alone and in mixture of LIN and MET. The results were reported in terms of % RSD.

4.2. Accuracy

Recovery studies were carried out by standard addition method. A known amount of standard LIN and MET viz., 50 %,100 % and150 % of the label claim were spiked to test solution of LIN (10 and 16 µg/mL) and MET (10 and 16 µg/mL). The results were reported in terms of % RSD.

4.3. LOD and LOQ

The LOD and LOQ of the developed method were calculated from the linearity curve using following equations,

$$\mathbf{LOD = 3.3\ X\ \sigma/S;} \qquad \mathbf{LOQ = 10\ X\ \sigma/S.}$$

Where,

σ = Standard deviation (y-intercepts of calibration curves) S= Slopes of linearity curves

5. Result and discussion

5.1. Absorption maxima and Isobestic point

The absorption maxima were found to be 235 nm, 298 nm respectively for MET and LIN and Isobestic point at 231.7 nm with characteristic peak as shown in figure 2.

5.2. Linearity

The linearity curve was constructed in the concentration range 2-10 µg/mL respectively for LIN and MET at their respective absorption maxima and at their Isobestic point nm, data was given table 1-3, linearity curves in figure 3 and overlay spectra in figure 4. The linear relationship between concentration and absorbance was studied by method of least square regression analysis, and relevant statistical and linearity curve data were given in table 4. The results suggest linear relationship with a correlation coefficient R^2 was 0.9899 to 0.9988 for LIN and MET.

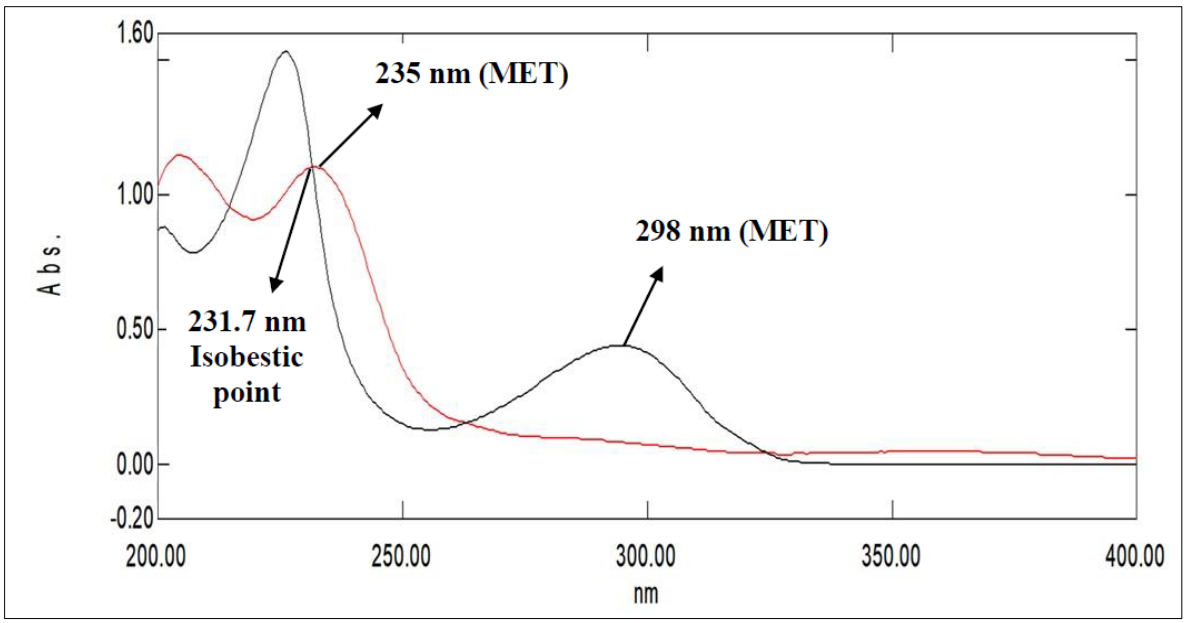

Figure 2 Overlay absorption maxima spectra of LIN, MET and Isobestic point spectra in Solvent blend

Table 1 Linearity data of MET and LIN at 231.5 nm

MET at λ1 n=6			**LIN at λ1 n=6**		
Concentration (µg/mL)	**Absorbance ± SD**	**% RSD**	**Concentration (µg/mL)**	**Absorbance ± SD**	**% RSD**
2	0.11± 0.001528	1.384	2	0.305±0.00058	0.1891
4	0.22± 0.001000	0.455	4	0.608±0.00058	0.0949
5	0.330±0.001732	0.525	6	0.911±0.00100	0.1098
8	0.440±0.000577	0.131	8	1.216±0.00153	0.1257
10	0.550±0.001528	0.277	10	1.519±0.00173	0.1140

Table 2 Linearity data of MET and LIN at 235 nm

MET at λ2 n=6			**LIN at λ2 n=6**		
Concentration (µg/mL)	**Absorbance ± SD**	**% RSD**	**Concentration (µg/mL)**	**Absorbance ± SD**	**% RSD**
2	0.068±0.00153	0.742	2	0.084±0.00305	1.566
4	0.136±0.00057	0.325	4	0.165±0.00208	1.172
6	0.204±0.00057	0.219	6	0.242±0.00352	1.447
8	0.273±0.00153	0.434	8	0.324±0.00404	1.246
10	0.330±0.00153	0.346	10	0.404±0.00557	1.427

Table 3 Linearity data of MET and LIN at 298 nm

MET at λ2 n=6			**LIN at λ2 n=6**		
Concentration (µg/mL)	**Absorbance ± SD**	**% RSD**	**Concentration (µg/mL)**	**Absorbance ± SD**	**% RSD**
2	0.090 ± 0.00153	1.685	2	0.068 ±0.00200	0.941
4	0.176 ± 0.01097	1.209	4	0.136 ±0.00115	0.844
6	0.271 ± 0.0010	0.370	6	0.204 ±0.00116	0.565
8	0.284 ± 0.00153	0.537	8	0.273 ±0.00159	0.565
10	0.358 ±0.00153	0.426	10	0.339 ±0.00100	0.295

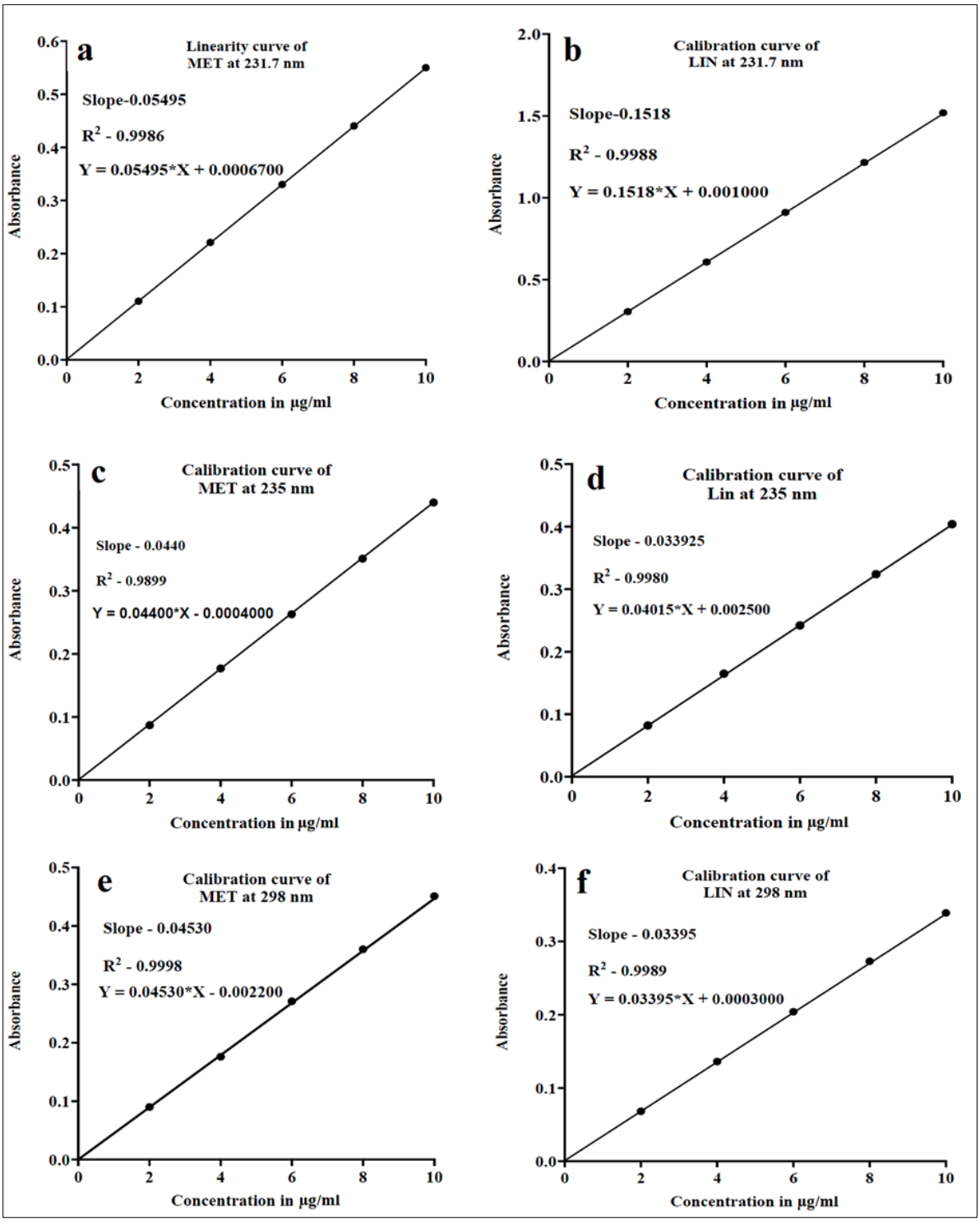

Figure 3 Linearity curves a) MET at 231.7nm, b) LIN at 231.7 nm, c) MET at 235 nm, d) LIN at 235 nm e) MET at 298 nm f) LIN at 298 nm

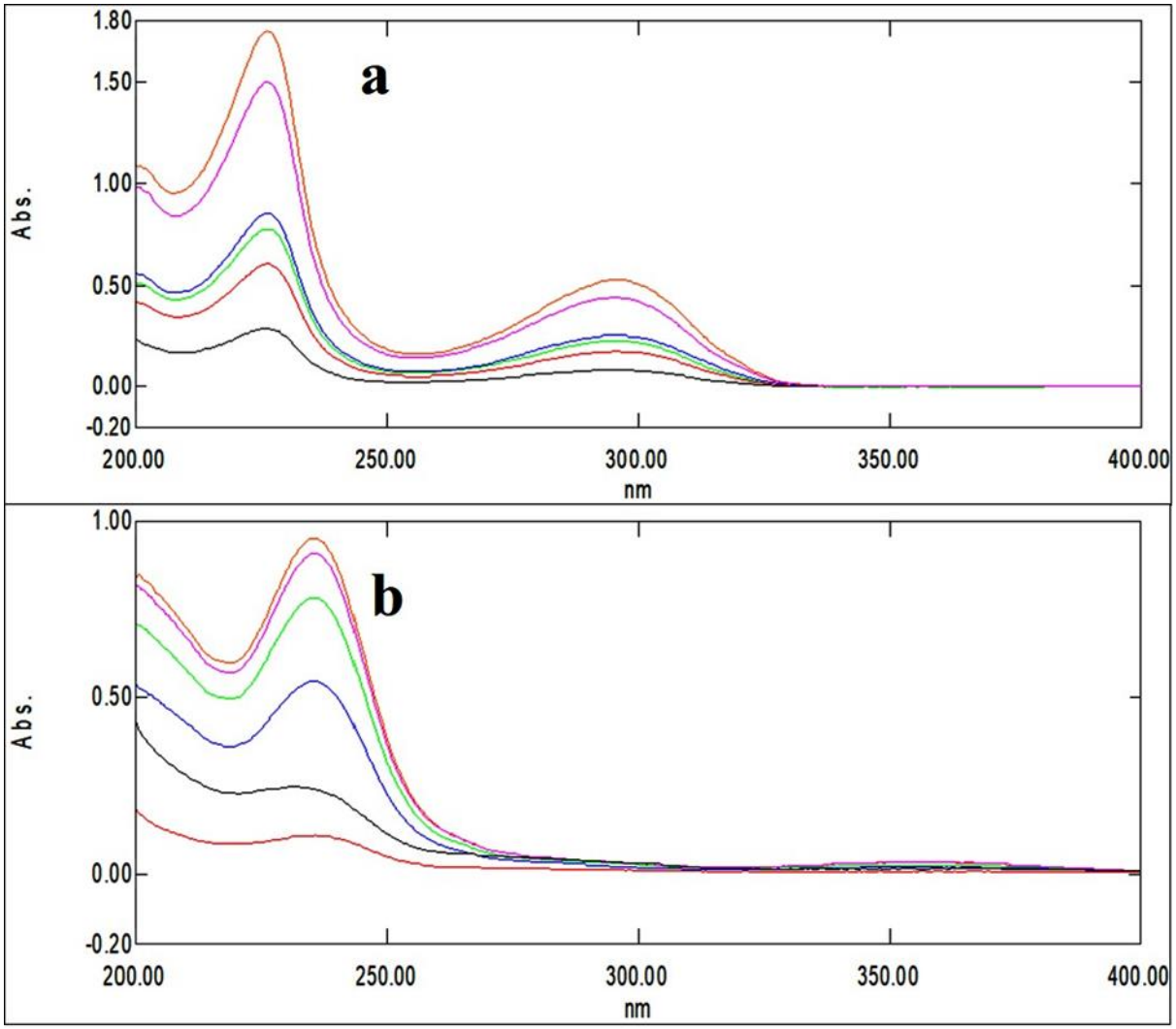

Figure 4 Overlay Linearity spectra of a) LIN 2-12 µg/mL and b) MET 2-12 µg/mL

5.3. Precision

The Repeatability, Intraday and Interday precision data for test solution containing LIN 6 µg/mL and MET 10 µg/mL was given in table 4. The % RSD for repeatability was in the range of 0.5553 to 1.430, for Intraday precision 0.5075 to 1.326 and Interday precision 0.5453 to 1.332. The results suggest the adapted method was reproducible, reliable and precise for the simultaneous estimation of LIN and MET.

Table 4 Repeatability Intraday and Interday precision of MET and LIN

Precision	**Concentration of test solution (µg/mL)**	**Absorbance ± SD n=6**		**% RSD**	
		λ_1 231.7 nm	**λ_2 235 nm**	**λ_1 231.7 nm**	**λ_2 235 nm**
LIN					
Repeatability	6	0.9063±0.00503	0.2457±0.00351	0.5553	1.430
Intraday	6	0.9030±0.00458	0.2423±0.00321	0.5075	1.326
Interday	6	0.9047±0.00493	0.2413±0.00321	0.5453	1.332

MET					
Repeatability	10	0.5527±0.00550	0.3343±0.00451	0.9965	1.349
Intraday	10	0.5507±0.00153	0.3337±0.00416	0.5075	1.326
Interday	10	0.5463±0.00379	0.3317±0.00252	0.6930	0.7588

5.4. Accuracy

The accuracy data for LIN and MET form marketed sample solutions at three levels (50 %, 100 % and 150 %) of standard addition method was given in table 5. The % recovery for LIN was in the range of 99.00±0.577 (50 % level); 99.40±0.577 (100 % level; 99.26±0.171 (150 % level) with % RSD less than 2. The % recovery of MET was in the range of 99.40±0.231 (50 % level); 99.50±0.289 (100 % level);99.33±0.156 (150 % level) with % RSD less than 2, the results suggest the method was accurate and can be used for assay and recovery study.

Table 5 Accuracy data of LIN and MET

Drug	Prequantified sample (µg)	% of pure drug added	Pure drug added (µg)	Amount Recovery µg/ml	Mean % Recovery± SD	% RSD
LIN	10	50	5	4.95	99.00±0.577	0.5852
	10	100	10	9.94	99.40±0.577	0.5852
	10	150	15	14.89	99.26±0.171	0.1722
MET	10	50	5	4.97	99.40±0.231	0.2326
	10	100	10	9.95	99.50±0.289	0.2896
	10	150	15	14.90	99.33±0.156	0.1571

5.5. LOD & LOQ

The LOD and LOQ was calculated by standard formula as given in ICH guide lines and was given in table 6. The LOD was 0.0433 µg/mL and 0.1313 µg/ mL for LIN at λ1 and λ2; LOQ was 0.0521 µg/ mL and 0.3123 µg/ mL for LIN at λ1 and λ2. The LOD was 0.0512 µg/ mL and 0.1292 µg/ mL for MET at λ1 and λ2; LOQ was 0.0511 µg/ mL and 0.2143 µg/ mL for MET at λ1 and λ2.

5.6. Analysis of LIN and MET in test solution

The developed QARM was applied to sample mixture solution and marketed tablet solution and summary of the statistical and Q-absorbance data was given in table 6 and spectra of marketed sample solution was given in figure 5. The % Assay of LIN and MET synthetic mixture solution was 100.11±0.6311, 100.31±0.3345, 100.21±0.5811 and 100.43±0.1394 respectively of the labelled claim. The % Assay of LIN and MET marketed sample solution was 100.98±0.8721, 100.14±0.6689, 99.98±0.9213 and 99.78±0.8821respectively of the labelled claim.

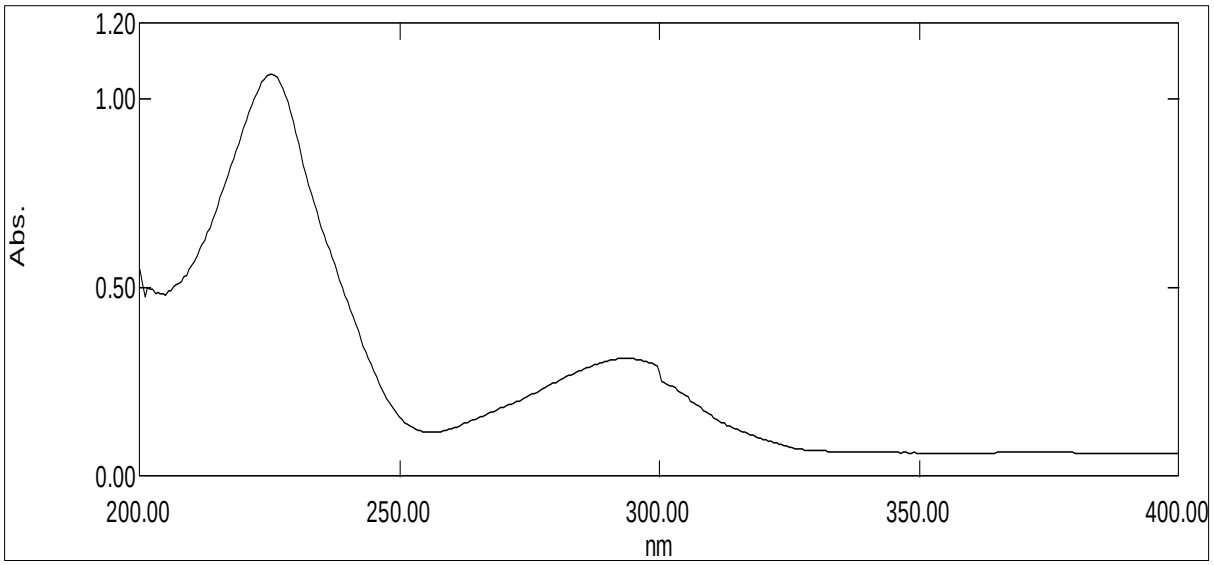

Figure 5 Spectra of test solution of LIN and MET

Table 6 Q absorption method and Validation parameters

MET		
Parameters	λ1 (231.7)	λ2 (235)
Specificity	Specific	Specific
Linearity Range	2-10 µg/ml	2-10 µg/ml
Regression Equation	Y = 0.05495*X+ 0.000670	Y = 0.04400*X- 0.0004
Molar Absorptivity	ay_1 = 0.055 L mol^{-1} cm^{-1}	ay_2 = 0.043 L mol^{-1} cm^{-1}
Absorbance of mixture	A1 = 0.9063±0.0050	A2 = 0.2457±0.00351
Analysis of mixture (LIN+MET)	100.11±0.6311	100.31±0.3345
Analysis of tablet (Mktd)	100.98±0.8721	100.14±0.6689
Sandell Sensitivity	0.021 µg/cm^2	0.024 µg/cm^2
Correlation Coefficient (R^2)	0.9986	0.9899
Repeatability (% RSD)	0.5553	1.430
Intraday (% RSD)	0.5075	1.326
Interday (% RSD)	0.5453	1.332
Accuracy % Recovery	99.23 ± 0.278	99.53 ± 0.193
LOD	0.0512 µg/mL	0.1292 µg/mL
LOQ	0.0511 µg/mL	0.2143 µg/mL
LIN		
Parameters	λ1 (231.7)	λ2 (235)
Specificity	Specific	Specific
Linearity Range	2-10 µg/ml	2-10 µg/ml
Regression Equation	Y = 0.1518*X+ 0.001	Y = 0.03925*X+ 0.0109

Molar Absorptivity	ax_1 = 0.151 L mol^{-1} cm^{-1}	ax_2 = =0.041 L mol^{-1} cm^{-1}
Absorbance of mixture	A1 = 0.5527±0.00550	A2 = 0.3343±0.00451
Analysis of mixture (LIN+MET)	100.21±0.5811	100.43±0.1394
Analysis of tablet (Mktd)	99.98±0.9213	99.78±0.8821
Sandell Sensitivity	0.027 µg/cm^2	0.023 µg/cm^2
Correlation Coefficient (R^2)	0.9988	0.9980
Repeatability % RSD	0.5553	1.430
Intraday % RSD	0.5075	1.326
Interday % RSD	0.5453	1.332
Accuracy % Recovery	99.22±0.5728	99.43±0.1982
LOD	0.0433 µg/mL	0.1313 µg/mL
LOQ	0.0521 µg/mL	0.3123 µg/mL

6. Conclusion

For the simultaneous quantification of Linagliptin and Metformin HCl a new technique, such as Q-Absorbance ratio approaches, represent an enormous advancement in analytical chemistry. This approach is innovative because it uses extremely precise and repeatable UV spectrophotometric techniques. The proposed method concludes that Q-Absorbance Raton Method can be successfully used for simultaneous estimation of Linagliptin and Metformin HCl both drugs in presence of each other.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Authors contribution

The collaborative efforts of all authors have successfully led to the development of a Q-Absorbance Ratio Method for simultaneous quantification of Linagliptin and Metformin HCl in commercially available combined tablets. Vasavi Yadav and Rishika Vaishnav, under the mentorship of N. Srinivasulu, conducted the development and validation of the technique. Y. Anand Kumar provided guidance in crafting the manuscript and structuring the research paper in accordance with the journal's guidelines.

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