# Simultaneous determination of linagliptin and metformin by reverse phase-high performance liquid chromatography method: An application in quantitative analysis of pharmaceutical dosage forms

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## Abstract

To enhance patient compliance toward treatment in diseases like diabetes, usually a combination of drugs is prescribed. Therefore, an anti-diabetic fixed-dose combination of 2.5 mg of linagliptin 500 mg of metformin was taken for simultaneous estimation of both the drugs by reverse phase-high performance liquid chromatography (RP-HPLC) method. The present study aimed to develop a simple and sensitive RP-HPLC method for the simultaneous determination of linagliptin and metformin in pharmaceutical dosage forms. The chromatographic separation was designed and evaluated by using linagliptin and metformin working standard and sample solutions in the linearity range. Chromatographic separation was performed on a $C_{18}$ column using a mobile phase of 70:30 (v/v) mixture of methanol and 0.05 M potassium dihydrogen orthophosphate (pH adjusted to 4.6 with orthophosphoric acid) delivered at a flow rate of 0.6 mL/min and UV detection at 267 nm. Linagliptin and metformin shown linearity in the range of 2–12 μg/mL and 400–2400 μg/mL respectively with correlation co-efficient of 0.9996 and 0.9989. The resultant findings analyzed for standard deviation (SD) and relative standard deviation to validate the developed method. The retention time of linagliptin and metformin was found to be 6.3 and 4.6 min and separation was complete in <10 min. The method was validated for linearity, accuracy and precision were found to be acceptable over the linearity range of the linagliptin and metformin. The method was found suitable for the routine quantitative analysis of linagliptin and metformin in pharmaceutical dosage forms.

**Key words:** Linagliptin, metformin, reverse phase-high performance liquid chromatography

## INTRODUCTION

Despite the various advances in the management of diabetes, it remains to be the major cause of disability

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and morbidity, including blindness, amputation, heart disease, peripheral neuropathy, and kidney disease.[1] Various studies recommended combination therapy in the treatment of diabetes mellitus to improve glycemic control among which combination of linagliptin with first line of drugs such as metformin was proved to be effective in controlling the metabolic syndrome and resulted in significant reversal of insulin resistance, islet and adipocyte hypertrophy and achieved hepatic steatosis.[2-4] Linagliptin, 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-ethylquinazolin-2-yl) methyl]-3,7-dihydro-1H-purine-2,6-dione] is a novel dipeptidyl peptidase-4 inhibitor representing a new therapeutic approach by stimulating glucose-dependent insulin release and reduction of glucagon levels. It acts through inhibiting the inactivation of incretins particularly glucagon like peptide-1 and gastric inhibitory polypeptide. Metformin, N, N-dimethylimidodicarbonimidic diamide

is a biguanide hypoglycemic agent which stimulates glycolysis in peripheral tissues and is considered to be a vital component in mixed therapies of oral hypoglycemics.[5,6]

These drugs are generally prescribed in multi-component dosage forms, which are available in the market. In view of this, a simple, precise and accurate method for the simultaneous estimation of metformin and linagliptin in pharmaceutical dosage forms by reverse phase-high performance liquid chromatography (HPLC) has been developed and the developed method was validated as per International Conference on Harmonization guidelines.

## MATERIALS AND METHODS

### Chemicals and reagents
Linagliptin and metformin were obtained as a gift samples from MSN Pharmachem Pvt. Ltd. and Aurbindo Pharmaceuticals Ltd. respectively. HPLC-grade methanol and acetonitrile were obtained from Sigma-Aldrich (Bangalore, India). HPLC-grade water was produced with Millipore-DI system coupled with Synergy 185 system by Millipore (Billerica, MA, USA). All the other chemicals used were of analytical grade purchased from Merck Chemicals, Mumbai, India. Fixed-dose combination tablets (Brand name: Jentadueto) containing 2.5 mg of linagliptin 500 mg of metformin manufactured by Eli-lilly., USA, were procured from USA market.

### Instrumentation and chromatographic conditions
The HPLC system consisted of a Shimadzu LC-10AT pump, a Rheodyne 7725i sample injector with a 20 μL loop and a Shimadzu SPD-M10Avp diode array detector. The data acquisition was performed by processing software "LC Solution" (Shimadzu Corp, Kyoto, Japan). The method was developed on a LiChrosphere 100 RP 18e (125 mm × 4.0 mm i.d, 5 μm) column maintained at ambient temperature.

The mobile phase was 70:30 (v/v) mixture of methanol and 0.05 M potassium dihydrogen orthophosphate (pH adjusted to 4.6 with orthophosphoric acid) delivered at a flow rate of 0.6 mL/min. The column was maintained at 25°C and the absorption of the elution was measured at 267 nm. The injection volume was 20 μL. The developed method with a simple mobile phase comprising of two components found advantageous over the already reported methods by Swamy and Baba[7] and Kavitha *et al.*,[8] where a mobile phase composed of more than two solvents.

### Preparation of standard and sample solutions
#### Working standard solution
The stock solution of linagliptin and metformin was prepared by dissolving accurately weighed amount of drugs in 25 mL methanol, followed by sonication for 5 min to obtain a final concentration of 100 μg/mL linagliptin

and 20,000 μg/mL metformin and further dilutions were prepared in methanol to obtain working standards of different concentrations.

#### Sample solutions
Twenty tablets, Jentadueto (Eli-lilly., USA) containing 2.5 mg of linagliptin 500 mg of metformin were weighed and finely powdered. A quantity of powder equivalent to 2.5 mg of linagliptin and 500 mg of metformin was weighed and transferred to a standard flask. The drug was diluted using methanol to get a final concentration of 10 μg/mL of linagliptin and 200 μg/mL of metformin.

## RESULTS AND DISCUSSION

### Validation procedures
The validation experiments were performed according "guidance to industry-bioanalytical method validation," recommended by US Food and Drug administration (US Food and Drug administration, 2001).[9]

#### Specificity and linearity
The chromatograms of the linagliptin and metformin in standard and sample were recorded. In the chromatograms of the formulations, some additional peaks were observed, which however did not interfere with the standard peaks demonstrating the specificity of the study method. The response for the detector was determined to be linear over the range 2–12 μg/mL for linagliptin and 400–2400 μg/mL for metformin. Each of the concentration was injected in duplicate to get reproducible response. The linearity range of the method was found more accurate and precise over the reported methods.[7,8] The calibration curve was plotted as concentration of the respective drug versus the mean of the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study and were represented by a linear regression equation [Figures 1-3]. The observed retention times are 4.6 and 6.3 min for metformin and linagliptin respectively, and these retention times were found shorter than the reported methods.[7,8]

#### Limits of quantification and detection
Limit of detection was obtained by injecting each standard sample solution at different concentrations to get the signal to noise ratio ≥3 which was found to be 0.07591 μg/mL and 0.0414 μg/mL for linagliptin and metformin respectively. Further, limit of quantification, with a signal to noise ratio ≥10 was found to be 0.2300 μg/mL and 0.1255 μg/mL for linagliptin and metformin respectively.

#### Precision and accuracy
The intra-day precision expressed as relative standard deviation (RSD %) and accuracy expressed as standard deviation were calculated by analyzing six different

standard samples (*n* = 6) at each of the low, medium and high concentrations on the same day. The inter-day precision

and accuracy was evaluated by analyzing six batches of all standard samples on three different days [Tables 1 and 2].
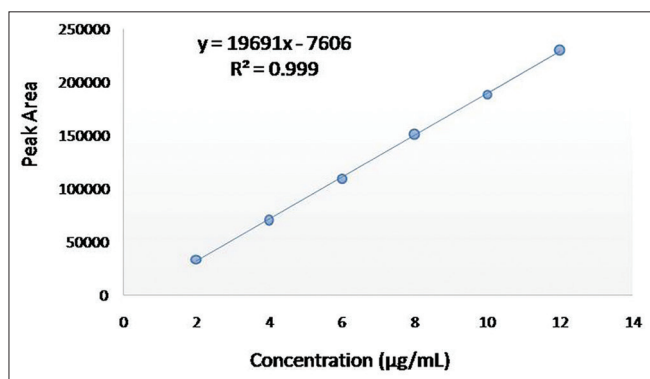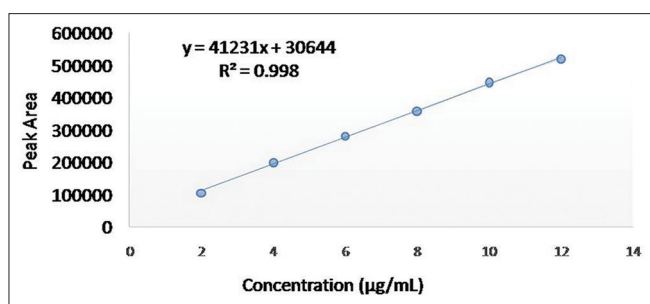


**Figure 1:** Calibration curve for linagliptin


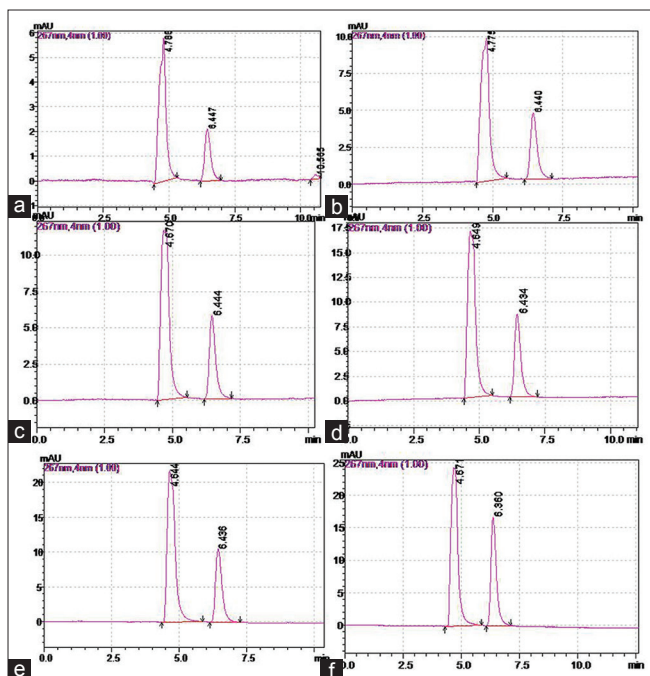
**Figure 2:** Calibration curve for metformin



**Figure 3:** High performance liquid chromatography chromatograms of linagliptin and metformin at different concentrations in the linearity range. (a) 2 µg/mL linagliptin and 400 µg/mL metformin; (b) 4 µg/mL linagliptin and 800 µg/mL metformin; (c) 6 µg/mL linagliptin and 1200 µg/mL metformin; (d) 8 µg/mL linagliptin and 1600 µg/mL metformin; (e) 10 µg/mL linagliptin and 2000 µg/mL metformin; (e) 12 µg/mL linagliptin and 2400 µg/mL metformin

**Table 1: Intra-day precision data for linagliptin and metformin**

| Concentration (µg/mL) | Peak area | Mean of peak area | SD | % RSD |
|---|---|---|---|---|
| Linagliptin | | | | |
| 8 | 154,368 | 155,193.7 | 1213.53 | 0.78 |
| | 154,626 | | | |
| | 156,587 | | | |
| 10 | 183,145 | 182,178 | 850.33 | 0.466 |
| | 181,842 | | | |
| | 181,847 | | | |
| 12 | 286,065 | 2,845,665.7 | 1477.98 | 0.519 |
| | 283,110 | | | |
| | 284,522 | | | |
| Metformin | | | | |
| 1600 | 362,340 | 36,321.33 | 3166.92 | 0.8718 |
| | 366,774 | | | |
| | 360,640 | | | |
| 2000 | 423,498 | 424,539 | 1184.632 | 0.279 |
| | 425,828 | | | |
| | 424,291 | | | |
| 2400 | 570,114 | 573,508 | 3756.379 | 0.654 |
| | 577,544 | | | |
| | 572,866 | | | |

RSD: Relative standard deviation, SD: Standard deviation

**Table 2: Inter-day precision data for linagliptin and metformin**

| Day | Concentration (µg/ml) | Peak area | Mean of peak area | SD | % RSD |
|---|---|---|---|---|---|
| Linagliptin | | | | | |
| 1 | 8 | 154,478 | 1,543,907 | 885.2836 | 0.5733 |
| | | 155,239 | | | |
| | | 153,474 | | | |
| 2 | 10 | 183,473 | 183,967.7 | 1253.477 | 0.681 |
| | | 183,037 | | | |
| | | 185,393 | | | |
| 3 | 12 | 286,120 | 285,963.3 | 669.8838 | 0.234 |
| | | 286,541 | | | |
| | | 285,229 | | | |
| Metformin | | | | | |
| 1 | 1600 | 365,505 | 36,637.65 | 1062.36 | 0.289 |
| | | 367,560 | | | |
| | | 366,065 | | | |
| 2 | 2000 | 420,518 | 420,641 | 517.694 | 0.123 |
| | | 420,197 | | | |
| | | 421,210 | | | |
| 3 | 2400 | 571,147 | 573,107 | 1856.33 | 0.323 |
| | | 573,335 | | | |
| | | 574,839 | | | |

RSD: Relative standard deviation, SD: Standard deviation

### Recovery

The extraction recoveries of linagliptin and metformin were determined by comparing the peak areas of the samples added to standard solutions (*n* = 6) with those obtained from the direct injection of the standard solutions without any preparations at same concentrations. The recoveries of linagliptin and metformin were determined at low medium and high concentrations with the recoveries ranging from 98% to 102% at different analyte concentrations [Table 3].

### Robustness

The robustness of the method was studied by deliberate changes in the method like alteration in, percentage organic content, pH of the mobile phase and changes in the wavelength. It was observed that there were no distinct changes in the chromatograms demonstrating that the robustness of the developed HPLC method.

### Stability

The amount of linagliptin and metformin recovered over a period of 30 days in samples stored at −20°C did not differ from the initial concentrations. The stability of these solutions was studied by performing an experiment and looking for changes in separation, retention, and asymmetry of the peaks that were then compared with the pattern of the chromatogram of freshly prepared solutions.

## CONCLUSION

The developed HPLC method is simple, accurate, precise and reliable for the simultaneous estimation of linagliptin and metformin in combined dosage form. The RSD for all

**Table 3: Recovery data for linagliptin and metformin**

| Analyte | Concentration added | Extraction recovery (%) | RSD (%) |
|---|---|---|---|
| Linagliptin (µg/mL) | 15 | 100.1 | 0.79 |
| | 20 | 100.13 | 0.4 |
| | 25 | 100.17 | 0.94 |
| Metformin (mg/mL) | 3 | 98.97 | 0.36 |
| | 4 | 100.03 | 0.3 |
| | 5 | 99.46 | 0.81 |

RSD: Relative standard deviation

parameters was found to be within the prescribed limits. Further, the noninterference of tablet excipients makes the method suitable for routine quantitative simultaneous estimation of both the drugs in multi-component pharmaceutical preparation.

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