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Research Article

DEVELOPMENT AND VALIDATION OF DISSOLUTION METHOD FOR LINAGLIPTIN TABLETS

MEGHANA PANSARE, MRINALINI DAMLE*

Department of Quality Assurance, All India Shri Shivaji Memorial Society's College of Pharmacy, Kennedy Road, Near RTO, Pune, Maharashtra, India. Email: damle_mc@aissmscop.com

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ABSTRACT

Objective: Linagliptin is used to treat type-2 diabetes. It is available singly as 5 mg tablet. Since there is no official dissolution method in Indian pharmacopeia, there is a need to develop a method for testing dissolution of linagliptin immediate release tablet.

Methods: For establishing dissolution method, various media, volume of media, and speed of rotation were tried. Quantification of dissolution samples was carried out using HPTLC and UV method. TLC plate pre-coated with silica gel 60 F_{254} was used as stationary phase and mobile phase employed for the development of TLC plates was methanol: toluene in a ratio of 7:3 v/v. The mobile phase was allowed to travel a distance of 70 mm and saturation time set was 20 min. Detection wavelength set was 294 nm.

Results: The most suitable condition for dissolution of linagliptin tablet was found to be dissolution apparatus type II (paddle) using 900 ml 0.1 N HCl as medium at speed of 75 rpm. The optimized chromatographic condition resulted in compact band at Rf value of 0.76±0.02.

Conclusion: Both the chromatographic and spectroscopic methods which were used for quantification were found to be linear with r^2 value of 0.9929 and 0.9918, respectively, accurate, precise, and robust.

Keywords: HPTLC, UV spectrophotometry, Dissolution, Method development, Validation, Linagliptin.

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INTRODUCTION

Linagliptin is used in the treatment of non-insulin-dependent diabetes mellitus. It is a member of class "gliptins" which are orally active DPP-4 inhibitors that are dipeptidyl peptidase-4 inhibitors [1]. Linagliptin chemically is 8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl) methyl] purine-2,6-dione [2]. It is yellowish to white solid substance which is very slightly soluble in water, isopropanol, acetone, soluble in methanol, and sparingly soluble in ethanol. Structurally, it is (Fig. 1); The recommended dose for linagliptin is 5 mg once daily [3]. It is available as tablet dosage form with label claim of 5 mg per tablet.

The best way of assessing therapeutic efficacy of drug is *in vivo* determination of bioavailability which is done when a new formulation is introduced into market. However, to monitor batch to batch consistency, this method becomes costly, tedious, and time consuming; hence, *in vitro* dissolution test emerged as best quality control tool to quantitatively assure about biological availability of drug from its formulation [4]. *In vitro* test is useful in guiding formulation and development, monitor manufacturing process, assessing quality of batch, and may be useful to predict *in vivo* performance in terms of bioavailability. Dissolution test is also used to identify bioavailability problems and to assess the need for further bioequivalence (BE) studies relative to scale-up and post-approval changes (SUPAC) [5].

The literature survey revealed that there is one paper of dissolution method of linagliptin using HPLC and UV [6]. To the best of our knowledge, there is no report of dissolution method of linagliptin using HPTLC and UV comparison. Keeping in mind, high-throughput advantage of HPTLC, it was considered worthwhile to develop HPTLC method for quantification of linagliptin in dissolution test. The developed method was validated as per International Council for Harmonization (ICH) guidelines [7], based on parameters such as specificity, linearity, precision, accuracy, and robustness.

METHODS

Instruments

Dissolution samples were analyzed using Camag HPTLC system (winCATS 1.4.2) and Shimadzu UV 1780 UV–Visible spectrophotometer, Japan, and other equipment used were Shimadzu AY 120 Analytical Balance, Japan, Electrolab DI 08L Dissolution apparatus USP (type II) and Labtronics LT 11 Auto Digital pH meter.

Chemicals and reagents

Linagliptin reference standard was obtained as gift sample. The chemicals used were methanol and water of HPLC grade and toluene of AR grade. Methanol was purchased from Merck Lifesciences, Pvt. Ltd., Mumbai, India, and toluene was purchased from Loba Chemie, Pvt. Ltd., Mumbai, India. For dissolution purpose, marketed tablets of label claim 5 mg were purchased from local pharmacy.

Phosphate buffer of pH 4.5, citrate buffer of pH 3, and 0.1 N HCl were prepared freshly as per IP [8].

Chromatographic conditions

The quantification of dissolution samples was performed on Merck TLC plate pre-coated with silica gel 60 F_{254}. Samples were applied on plate in the form of 6 mm band using 100 µl sample syringe with the help of semiautomatic Linomat applicator IV. 20×10 twin trough chamber was used

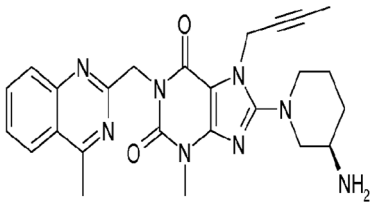

Fig. 1: Chemical structure of linagliptin

for ascending development with mobile phase methanol: toluene (7:3 v/v). The saturation time was 20 min, the plate was allowed to develop up to 70 mm of distance. The developed plates were allowed to dry and scanning was carried out at wavelength of 294 nm using Camag densitogram.

Method development

Solubility study

The solubility of linagliptin was checked in 0.1 N HCl, citrate buffer pH 3, and phosphate buffer 4.5.

Preparation of stock solution

Accurately weighed 10 mg of linagliptin was transferred to clean and calibrated 10 ml volumetric flask. It was dissolved in dissolution media, volume made up to the mark with respective dissolution medium. From the above stock solution, further dilution was made with dissolution medium to produce final concentration of 5 µg/ml.

Selection of wavelength

The prepared final dilution of 5 µg/ml was scanned over range of 200–400 nm in 1 cm quartz cell.

Selection of mobile phase

For quantification of dissolution sample by HPTLC, various mobile phases such as ethyl acetate: toluene (5:5 v/v), chloroform: methanol (5:5 v/v), and methanol: toluene (7:3 v/v) were tried. No peaks were observed in ethyl acetate: toluene (5:5 v/v) and no proper peak shape were observed in chloroform: methanol (5:5 v/v). Better results were obtained using methanol: toluene (7:3 v/v) as mobile phase.

Selection of dissolution condition

Various media such as citrate buffer pH 3, phosphate buffer pH 4.5, and 0.1 N HCl were tried. Dissolution test was tried by keeping the speed of apparatus 50 RPM and 75 RPM. Media volume of 500 ml and 900 ml was tried.

Filter compatibility

After samples are withdrawn from dissolution media, it is necessary to make it more suitable for analytical finish. The purpose of filtration is to remove undissolved material from withdrawn sample solution. Adsorption of drug substance by filter may occur, and hence, evaluation is needed [9]. Filter compatibility was checked using different filters that are 0.45 µm PTFE, 0.45 µm PVDF, 0.45 µm nylon filter, and Whatman filter. The standard solution of linagliptin in dissolution medium of concentration 5 µg/ml was filtered through these filters and both filtered and non-filtered solutions were analyzed.

Method validation

The above developed method was validated based on various parameters such as specificity, linearity, precision, accuracy, and robustness as per ICH guidelines.

Specificity

It is the ability of method to assess the analyte in presence of other components which can be present [7]. Specificity of method is assessed by analyzing the blank dissolution medium, placebo solution, standard solution, and dissolution sample. There should not be any peak at same Rf of linagliptin.

Linearity

Linearity of analytical procedure is ability to execute test response proportional to concentration of drug solution in given range [7]. The linearity was performed for concentration range of 25–125 ng/band for HPTLC method, and for UV, range set was 4–16 µg/ml. The calibration curve was plotted and regression coefficient was determined.

Accuracy

Accuracy of analytical method demonstrates the closeness of value between true value and value found. It is expressed in terms of % recovery [7]. The percent recovery of linagliptin was assessed by following standard addition with concentration range of 80%, 100%, and 120% to the dissolution sample.

Precision

Repeatability: It elicits the precision of method under same operating conditions over short period. It is performed by analyzing six different sample solutions at their 100% concentration and their % RSD was determined.

Intermediate precision: It was performed on different days on the 100% concentration of sample solution and the % RSD was determined [9].

Robustness

Robustness expresses the capacity of analytical procedure to remain unaffected by small deliberate variations in method parameters such as detection wavelength, saturation time in development chamber, plate scanning speed, and agitation speed of paddle.

Solution stability

To determine the solution stability in dissolution medium, the standard solution and sample solution were kept at temperature of 37°C for 2 h and then analyzed.

RESULTS

Solubility

Linagliptin was found to be freely soluble in 0.1 N HCl, citrate buffer pH 3, and phosphate buffer pH 4.5. It is reported that linagliptin is very slightly soluble in water; hence, dissolution test was not tried with distilled water as medium.

Selection of wavelength

The stock solution of concentration 5 µg/ml was scanned over the range of 200–400 nm and from recorded spectra the wavelength selected was 294 nm (Fig. 2).

Dissolution condition selection

Media selection

The various media tried were 0.1 N HCl, citrate buffer pH 3, and phosphate buffer pH 4.5. Linagliptin showed degradation in citrate

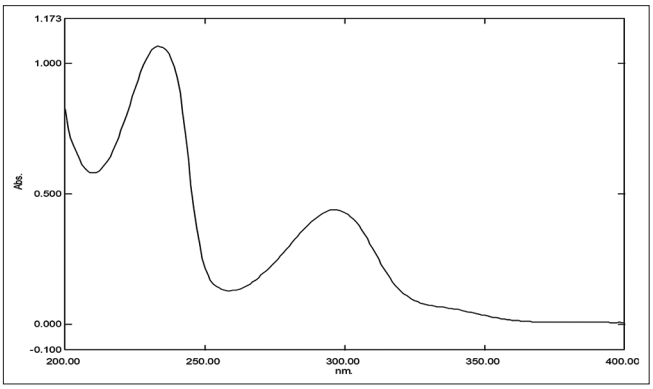

Fig. 2: UV spectrum of linagliptin

Table 1: Filter compatibility

S. No.	Filter type	Amount spotted	Area
1	Nylon 0.45 µm	50 ng/band	1045.3
2	PVDF 0.45 µm	50 ng/band	1033.4
3	PTFE 0.45 µm	50 ng/band	1032.1
4	Whatman filter	50 ng/band	1038.6
5	Unfiltered	50 ng/band	1041.3

buffer and in the phosphate buffer pH 4.5, the acceptance criteria for dissolution of immediate release solid dosage form that is Q = 80% in 30 min failed [10]. Hence, the medium selected for dissolution of linagliptin tablet was 0.1 N HCl.

Volume and speed

Dissolution of tablet was checked with volume of 500 ml and 900 ml and at the speed of 50 rpm and 75 rpm. From this, 900 ml and 75 rpm condition were selected depending on the drug release.

Filter compatibility

Both the unfiltered and filtered standard solutions from various types of filters led to following results. Because of easy availability and lesser cost, Whatman filter paper was preferred (Table 1).

Validation parameters

Specificity

The method was found to be specific as no other peaks from blank and placebo were observed at Rf of linagliptin from standard and dissolution sample solution (Figs. 3-6).

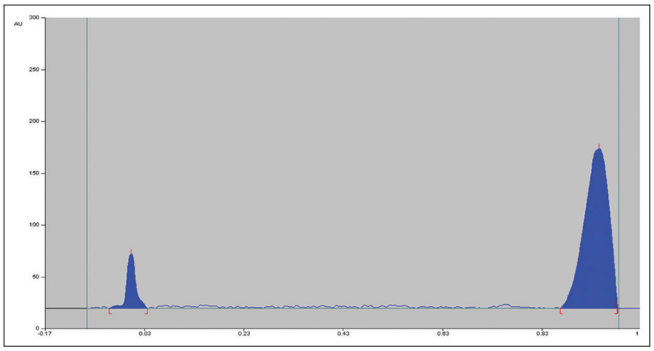

Fig. 3: Blank solution

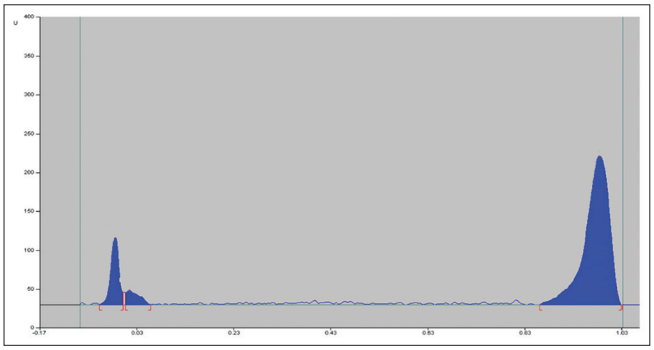

Fig. 4: Placebo solution

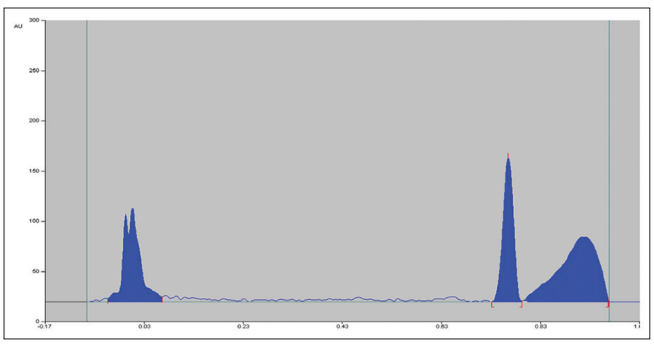

Fig. 5: Linagliptin standard solution (Rf = 0.76)

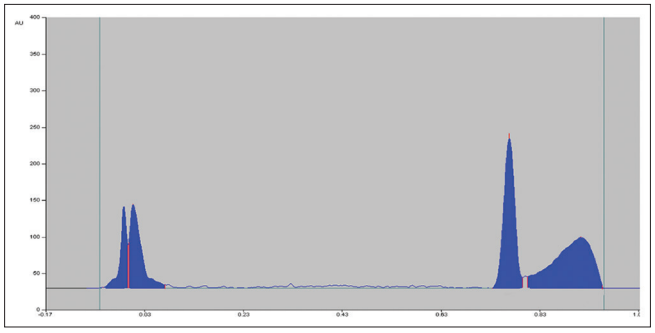

Fig. 6: Linagliptin dissolution sample (Rf = 0.76)

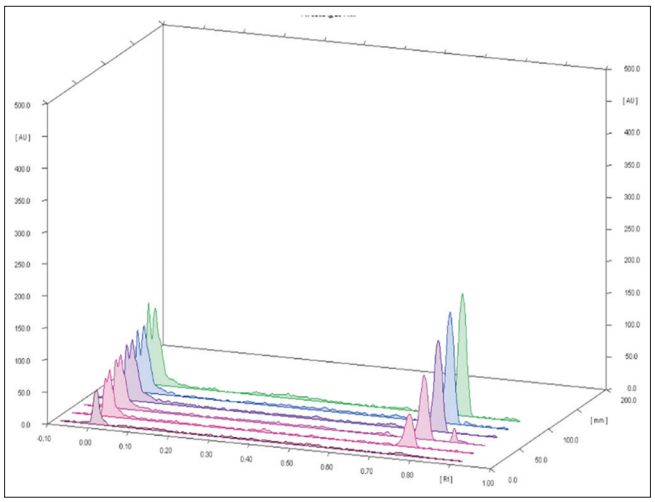

Fig. 7: Linearity of linagliptin standard 25–125 ng/band

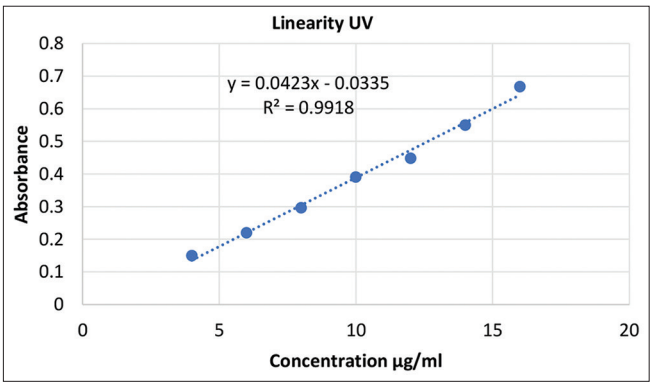

Fig. 8: Calibration curve of linagliptin conc. 4–16 µg/ml

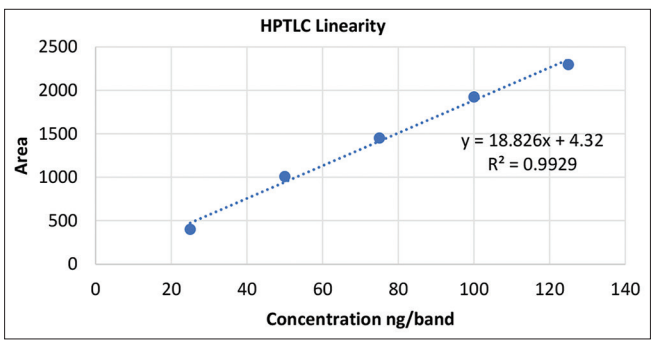

Fig. 9: Calibration curve of linagliptin conc. 25–125 ng/band

Linearity

The linearity was obtained over the concentration range of 25–125 ng/band as well as 4–16 µg/ml. The value of r^2 was found to be 0.9929 and 0.9918, respectively (Figs. 7-9).

Accuracy

The accuracy estimated by determining the % recovery at three levels 80, 100, and 120%. The % recovery at each level was calculated and is shown in Tables 2 and 3.

Table 2: Accuracy (UV)

S. No.	Level	Method	Concentration	Concentration	% recovery
1	80%	UV	5 µg/ml	9 µg/ml	98.70
2	100%		5 µg/ml	10 µg/ml	99.66
3	120%		5 µg/ml	11 µg/ml	97.95

Table 3: Accuracy (HPTLC)

S. No.	Level	Method	Amount spotted	Amount spotted	% recovery
1	80%	HPTLC	50 ng/band	90 ng/band	99.25
2	100%		50 ng/band	100 ng/band	98.61
3	120%		50 ng/band	110 ng/band	98.73

Precision

The precision of method was expressed by repeatability and intermediate precision which was found to be as follows (Tables 4 and 5):

Robustness

The method was found to be robust as % RSD obtained from calculation of average of peak area for HPTLC and absorbance for UV was found to be within limit. Hence, method was proven to be robust (Table 6).

Solution stability

The samples were found to be stable and the results found were as follows (Table 7):

DISCUSSION

Here, the dissolution condition was the medium 0.1 N HCl, speed of apparatus 75 rpm, and volume of 900 ml was different from the condition given in Engel *et al*. [6]. Furthermore, the same condition with volume of 500 ml gave satisfactory result but better release observed with volume of 900 ml. The linagliptin was found to be stable at condition of 0.1 N HCl, 37°C. It has been reported to degrade by about 4.5% at stress condition of 0.1 N HCl and temperature of 60–80°C [11,12]. Thus, 0.1 N HCl at 37°C is suitable as dissolution medium since solution stability has been confirmed for 2 h under this condition.

CONCLUSION

The dissolution method was developed and optimized. Quantification was carried out using HPTLC and UV. Validation of method was performed as per ICH guidelines. The selected dissolution medium for

Table 4: Repeatability

S. No.	Method	Amount spotted	Average area	Standard deviation	% RSD
1	HPTLC	50 ng/band	1052.28	20.73	1.17
		Concentration	**Average absorbance**		
2	UV	6 µg/ml	0.201	0.0024	1.19

Table 5: Intermediate precision

S. No.	Method	Condition	Amount spotted	Average area	Standard deviation	% RSD
1	HPTLC	Intermediate precision	50 ng/band	1016.93	6.73	0.66
			50 ng/band	1068.98	27.43	1.56
			Concentration	**Average absorbance**		
2	UV	Intermediate precision	6 µg/ml	0.202	0.0023	1.17
			6 µg/ml	0.200	0.0022	1.12

Table 6: Robustness

S. No.	Parameters		%RSD HPTLC	% RSDUV
1.	Mobile phase composition (±0.2)	Methanol: toluene (7.2:2.8v/v)	1.57	-
		Methanol: toluene (6.8:3.2v/v)	1.61	-
2.	Saturation time (±2)	18 min	1.13	-
		22 min	1.20	-
3.	Wavelength (±2)	292 nm	0.97	0.65
		296 nm	1.05	1.14
4.	Speed (±2)	73 mm/s	0.49	1.29
		77 mm/s	0.32	0.55

Table 7: Solution stability

S. No.	Condition	Amount spotted	Freshly prepared	After 2 h	% stability
1.	Sample solution	50 ng/band	1015.5	940.6	99.49
2.	Standard solution	50 ng/band	1009.4	931.8	98.56

5 mg linagliptin tablet was 0.1 N HCl and the apparatus used was paddle (type 1). The optimum speed and volume selected were 75 rpm and 900 ml, respectively. The proposed method was validated with respect to parameters such as specificity, linearity, accuracy, precision, and robustness. The method was found to be specific, linear, and robust.

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AUTHORS' CONTRIBUTIONS

Meghana Pansare studied, carried out the research work, and drafted the manuscript under the guidance of Mrinalini Damle. The manuscript was checked and approved by Mrinalini Damle.

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

AUTHORS' FUNDING

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