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Application of RP-HPLC and UV-Spectrophotometric Method in Dissolution Testing of Teneligliptin

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ABSTRACT

Characterization of drug product performance in the pharmaceutical field has become very essential; Dissolution testing has emerged as an important tool. Teneligliptin is mainly used for the treatment of type-II diabetes mellitus, not official in any of the monographs. The objective of the study was to develop and validate UV spectrophotometric and RP-HPLC methods for dissolution testing for the quality control of Teneligliptin tablets. In vitro dissolution tests of tablets were performed using different trial conditions. The satisfactory sink conditions (Test conditions) includes phosphate buffer of pH 7.5 (900 ml at 37 ± 0.5°C) as dissolution medium, USP Apparatus II (paddle method) with agitation speed of 50 rpm. The method was found to be linear with correlation coefficient (r2=0.999) in the concentration range of 10-60 µg/ml. The chromatographic separation was achieved on Shodex C18 column with isocratic mode using a mixture of methanol: phosphate buffer (pH 7.2) in the ratio of 70:30 v/v, flow rate of 1 ml/min and detection wavelength was 245.6 nm. The recovery of the drug at three levels was found to be nearly equal to 100%. The proposed dissolution test method is adequate and can be applied for the quality control of 20 mg Teneligliptin tablets.

Keywords: Teneligliptin (TENE), Validation, Dissolution testing, RP-HPLC

INTRODUCTION

Teneligliptin is a highly potent, competitive and long-lasting DPP-4 inhibitor mainly used for the treatment of Type-II Diabetes Mellitus (T2DM) acts by improving postprandial hyperglycemia and dyslipidemia.

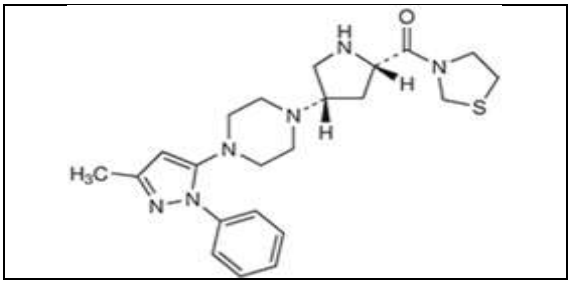

Figure 1: Structure of teneligliptin

Recent studies have suggested that Teneligliptin (TENE) exhibits multiple pharmacological effects that include vasoprotective and neuroprotective effects also. Literature reveals some analytical methods have been reported for the estimation of TENE in formulation and plasma [1-4]. The proposed work describes development of simple, precise and economic UV spectrophotometric and High Performance Liquid Chromatography (HPLC) method for estimation of TENE. In recent years, pharmaceutical industry and regulatory authorities are emphasizing on dissolution testing of formulations. To assure product quality and performance after the manufacturing process of a drug product, changes in the formulation can be assessed easily through dissolution tests from lot-to-lot during the development of new formulations [5]. TENE is not official drug in any of the Pharmacopoeia and also no dissolution test method was found in literature. Hence, objective of current study was to develop an accurate and precise dissolution test method for product performance.

MATERIAL AND METHODS

Chemicals and reagents

Teneligliptin in salt form (TEHH) was procured from Glenmark Pharmaceutical, Ltd, (Sinnar, India). The commercially formulation of Teneligliptin were purchased form Indian market. Chemicals include methanol and acetonitrile of HPLC grade, potassium dihydrogen phosphate; Ortho phosphoric acid, hydrogen chloride and sodium hydroxide of GR grade were used. 0.1 N HCl, acetate buffer pH 4.0, Phosphate Buffer pH 6.8 and phosphate buffer pH 7.5 were prepared as per the pharmacopoeia.

Instruments

Dissolution Apparatus: Electrolab, Tablet Dissolution tester (TDT) 06P, Lab India Ds1400, HPLC: Shimadzu HPLC series 1100, UV-Spectrophotometer: Jasco V-630, Shimadzu-1700 double beam, Sonicator: PCI Mumbai 3.5L 100H, Spectra lab. UCB-300, pH-meter: GLOBAL Model No. DPH-500, EI, Model No. 1102012 and Weighing Balance: Shimadzu AUX220, RADWAG PS1500

Development of UV-spectrophotometric method

Standard stock solution

An accurately weighed about 10.0 mg of TENE was transferred in a 10.0 ml volumetric flask, dissolved in methanol to prepare a stock standard solution of 1000 µg/ml of TENE.

Working standard solution

1.0 ml of the standard stock solution was diluted to 10.0 ml (100 µg/ml of TENE); from this solution further 3.0 ml was diluted to 10.0 ml to prepare working standard solution having concentration about 30 µg/ml of TENE.

Selection of wavelength

The working standard solution prepared above of TENE (30 µg/ml) was scanned in the UV range of 200-400 nm in 1.0 cm cell against solvent blank (methanol) and spectrum recorded. The recorded spectrum of TENE showed peak maxima at 245.6 nm and 280 nm. The wavelength of 245.6 nm was considered as detection wavelength for further experimentation.

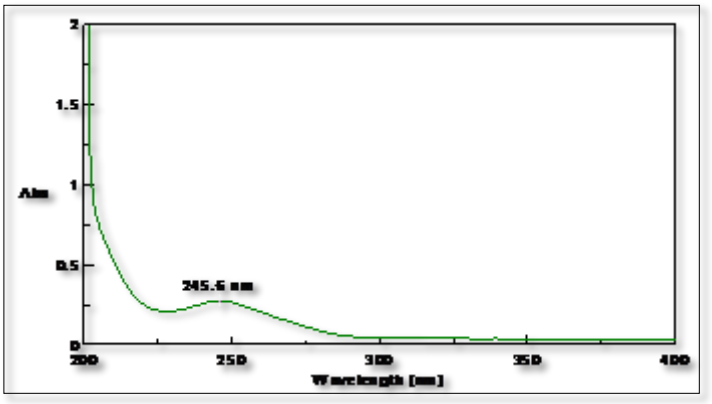

Figure 2: UV-Spectrum of TENE

Development of RP- HPLC method

Standard stock solution

Transfer an accurately weighed quantity of TEHH is equivalent to TENE (~10.0 mg) was in a 10.0 ml volumetric flask, dissolved and volume make up with diluents to prepare standard stock solution of 1000 µg/ml of TENE.

Working standard solution

The working stock solution was further appropriately diluted with mobile phase to get the final concentration of 30 µg/ml of TENE.

Phosphate buffer (pH 7.2)

Transfer 50.0 ml of 0.2 M potassium dihydrogen phosphate in 200.0 ml volumetric flask, to its 34.7 ml of 0.2 N NaOH was added and volume make up with water, sonicated and filtered through 0.4 µm membrane filter paper.

Development of dissolution test method

Determination of solubility and sink conditions

Solubility profile was used as the basis for the selection of a dissolution medium for TENE. Drug solubility was determined at 25°C in different media and expressed as mg/ml. Sink conditions were determined in different media.

Mechanical calibration of dissolution apparatus

Conventionally, for oral solid dosage forms, dissolution Apparatus I or II is suggested by FDA guideline but to satisfy with cGMP requirements mechanical calibration for Apparatus I and II should be carried out.

Optimization of dissolution test

The dissolution studies were performed using a six-station dissolution apparatus by subjecting six commercial formulation in each dissolution

medium containing 900 ml of dissolution media using both a paddle and basket dissolution apparatus and stirring speeds of 50, 75 and 100 rpm at temperature 37 ± 0.5°C were tried [6-8]. Aliquots of 10 ml were withdrawn manually at intervals of 5, 10, 15, 20 and 25 min. The same volume of fresh medium at 37 ± 0.5°C was replaced to maintain the constant volume. The sample was filtered through Whatman filter paper and analyzed by UV and RP-HPLC method.

Validation of dissolution method

The proposed method was validated for its specificity, linearity, accuracy, precision and robustness to demonstrate reproducibility and reliability [9-10].

Linearity

Aliquots of TENE stock solution (100 µg/ml) were diluted with phosphate buffer pH 7.5 to give concentrations of 10-60 µg/ml. Each solution was read in triplicate. Calibration curve was plotted as absorbance/AUC Vs concentration.

Precision

Using the optimized dissolution parameters, test solutions were obtained and the test solution was withdrawn at 5 min time interval upto 25 min. The absorbance and area under curve was noted to estimate the amount of drug release at each time interval using proposed methods. Thus, repeatability was evaluated at the 100% level and the Relative Standard Deviation (RSD) of the data was calculated. The evaluation of intermediate precision was performed by analysing the sample on different days by different analyst, and the %RSD values were calculated.

Accuracy

The accuracy of proposed method was carried out by performing recovery study for TENE; standard drug substance was added to the dissolution vessels in known amounts at the 80%, 100% and 120% levels. Accordingly, 11.2, 14 and 16.8 mg of standard drug was added along with 20.0 mg tablet. Dissolution test was carried out for 25 min using 900.0 ml of phosphate buffer pH 7.5 as dissolution medium in paddle type-II apparatus at 50 rpm (Temp. 37 ± 0.5°C). Aliquots of 10.0 ml were withdrawn at appropriate interval and filtered through Whatman filter paper and analysed by UV and RP-HPLC.

Robustness of the test method

The robustness of analytical method is the ability to remain unaffected by small but deliberate variations in method parameters and provide an indication of its repeatability during normal uses. The robustness study was performed for change in flow rate and wavelength.

RESULTS AND DISCUSSION

Teneligliptin was found to highly soluble and stable in methanol, distilled water and mixture of methanol: phosphate buffer (pH 7.2). Using these solvents, working standard solutions were prepared of desired concentration for UV and HPLC estimation of TENE.

Optimised chromatographic conditions

In order to achieve the optimized chromatographic condition, one or two parameter modified at each trial and chromatograms were recorded with all specified chromatographic conditions. The various mobile phases were tried to select the most suitable one by changing flow rate, buffer and its pH (Table 1).

Table 1: Optimized chromatographic conditions

HPLC System	Shimadzu HPLC series 1100
Column	Shodex C-18-4E (5 µm), 250 × 4.6 mm
Mobile phase	Methanol: Phosphate buffer pH 7.2 (70:30% v/v)
Detection wavelength	245.6 nm
Flow rate	1.0 ml/min
pH	7.2
Injection volume	20 µl

The chromatographic conditions were set as per final chromatographic conditions; mobile phase was allowed to equilibrate with stationary phase as indicated by steady baseline. The mobile phase containing Methanol: Phosphate buffer, pH 7.2 (70:30% v/v) gave well resolved peak and reasonable retention time as shown in Figure 3.

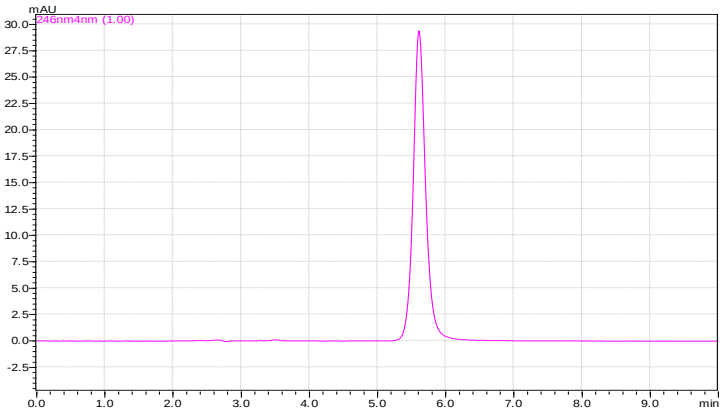

Figure 3: Chromatogram of standard TENE

System suitability parameters

The system suitability study was performed by injecting six replicate injection of standard TENE (30 µg/ml) were injected and chromatographed. The results obtained indicate that proposed method was suitable for further experimentation (Table 2).

Table 2: Results of system suitability parameters

Sr. No.	Wt. of Std. drug taken (mg)	Area (µV)
1	10.03 mg	760561
2		769738
3		779503
4		768035
5		771992
6		775843
Mean		770945.33
± SD		6573.86
% RSD		0.85
Retention time		5.615
Tailing factor (Asymmetry)		0.956
Theoretical plate		16720.50

Drug substance solubility study

Solubility study of TENE was carried out by using different dissolution media. The solution was sonicated, filtered and analysed by UV spectroscopy to determine the solubility of the drug in respective dissolution media. The graph was plotted between pH of dissolution media and observed solubility.

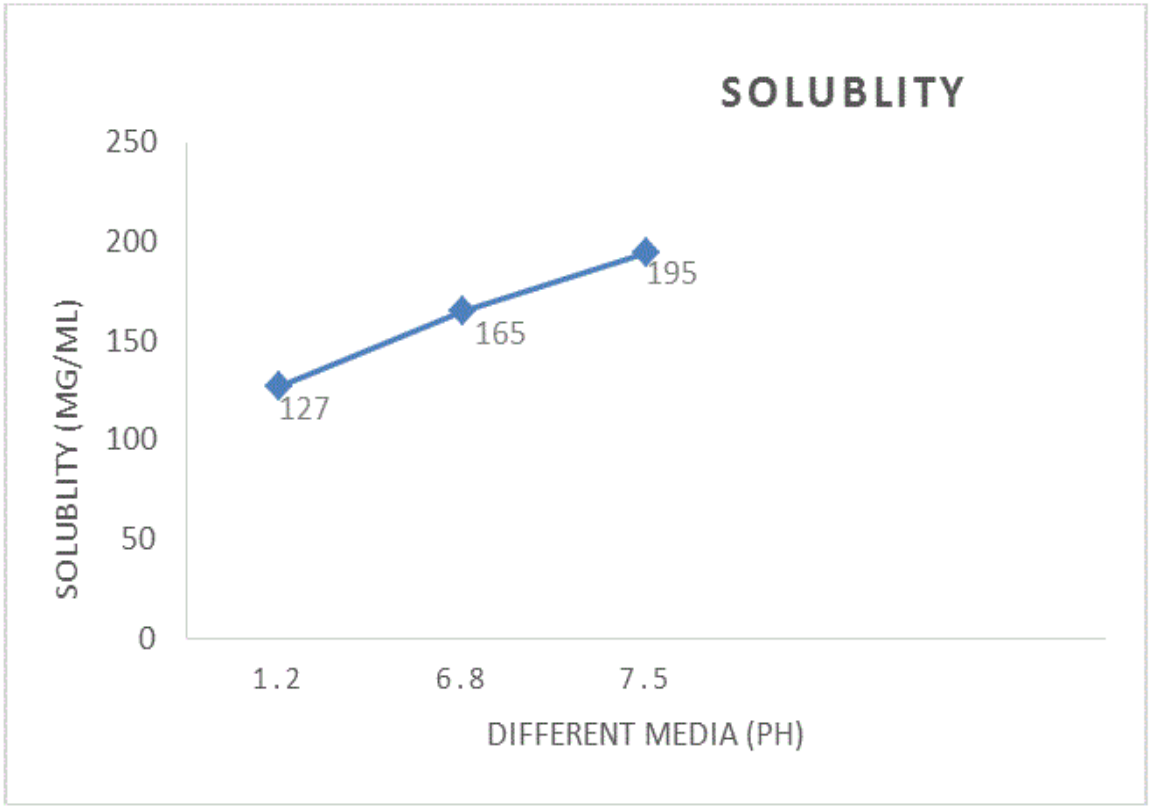

Figure 4: Graph showing solubility at different pH

Optimization of dissolution parameters

Various dissolutions were performed to optimized dissolution parameter. For maximum percent release of drug, trials were taken by using USP Apparatus I and II, i.e., Basket and paddle type at different rpm 50, 75 and 100. Based on the solubility of TENE, phosphate buffer pH 7.5 was selected as suitable buffer media as compared to Acetate buffer pH 4.0 and Phosphate buffer pH 6.8 (Tables 3 and 4).

Table 3: Results showing % release of drug using USP type-I basket apparatus

Phosphate Buffer pH 7.5 as dissolution media		Time points (min) % release				
		5	10	15	20	25
Type of apparatus	USP type-I	19.28	28.97	42.57	66.77	73.20
Speed of rotation	100 RPM					

Table 4: Results showing % release of drug using USP type-II paddle apparatus

Phosphate Buffer pH 7.5 as dissolution media		Time points (min) % release				
		5	10	15	20	25
Type of apparatus	USP type-II	6.59	21.59	33.43	56.13	72.80
Speed of rotation	50 RPM					

It was observed that the release of drug in USP type-I (Basket) apparatus, shows maximum release at first time point. Using type-II apparatus, proper profiling for drug release was observed (Table 5).

Table 5: Results showing effect of change in speed of rotation on TENE

Phosphate buffer pH 7.5 as dissolution media		Time points (min) % release				
		5	10	15	20	25
Type of Apparatus	USP type-II	14.27	22.81	35.39	69.98	72.84
Speed of rotation	75 RPM					

The release of drug at first time point was more when the speed of rotation was changed from 50 rpm to 75 rpm i.e. very fast release was observed at 75 rpm as compared to 50 rpm using type-II apparatus. The following dissolution parameters have been finalized for the estimation of TENE as depicted in Table 6.

Table 6: Final optimized dissolution conditions

Drug	Dissolution media	Media volume	USP apparatus	RPM
TENE	Phosphate buffer pH 7.5	900.0 ml	Type-II paddle	50

Linearity

Pipette out 1.0-6.0 ml from the working stock solution (100 µg/ml) and diluted with the buffer upto 10.0 ml in separate volumetric flasks. Absorbance of each solution and peak area was noted. Calibration curve was plotted as absorbance/AUC Vs concentration as shown in Figure 5. The obtained result shows linearity between concentration and absorbance/AUC.

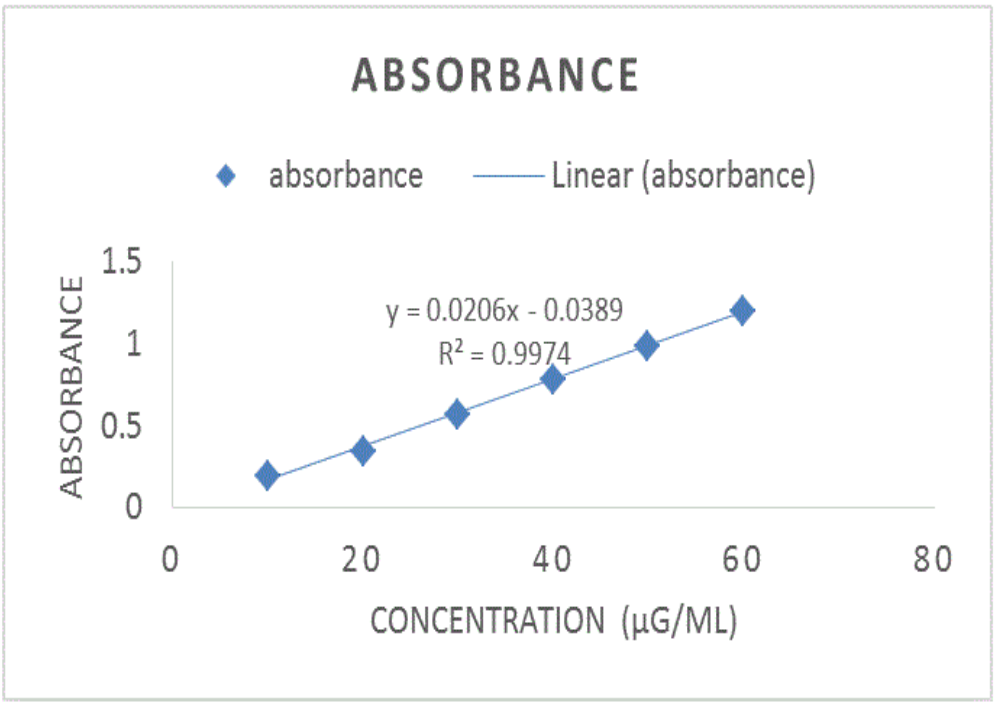

Figure 5: Linearity plot of TENE

Percent release of Teneligliptin

The test solution was obtained by performing the dissolution of drug under finalised dissolution parameters. The six replicates of test solution of drug so obtained were chromatographed and %RSD of drug was calculated and recorded in Table 7. The proposed RP-HPLC method was found to be precise with %RSD 1.30.

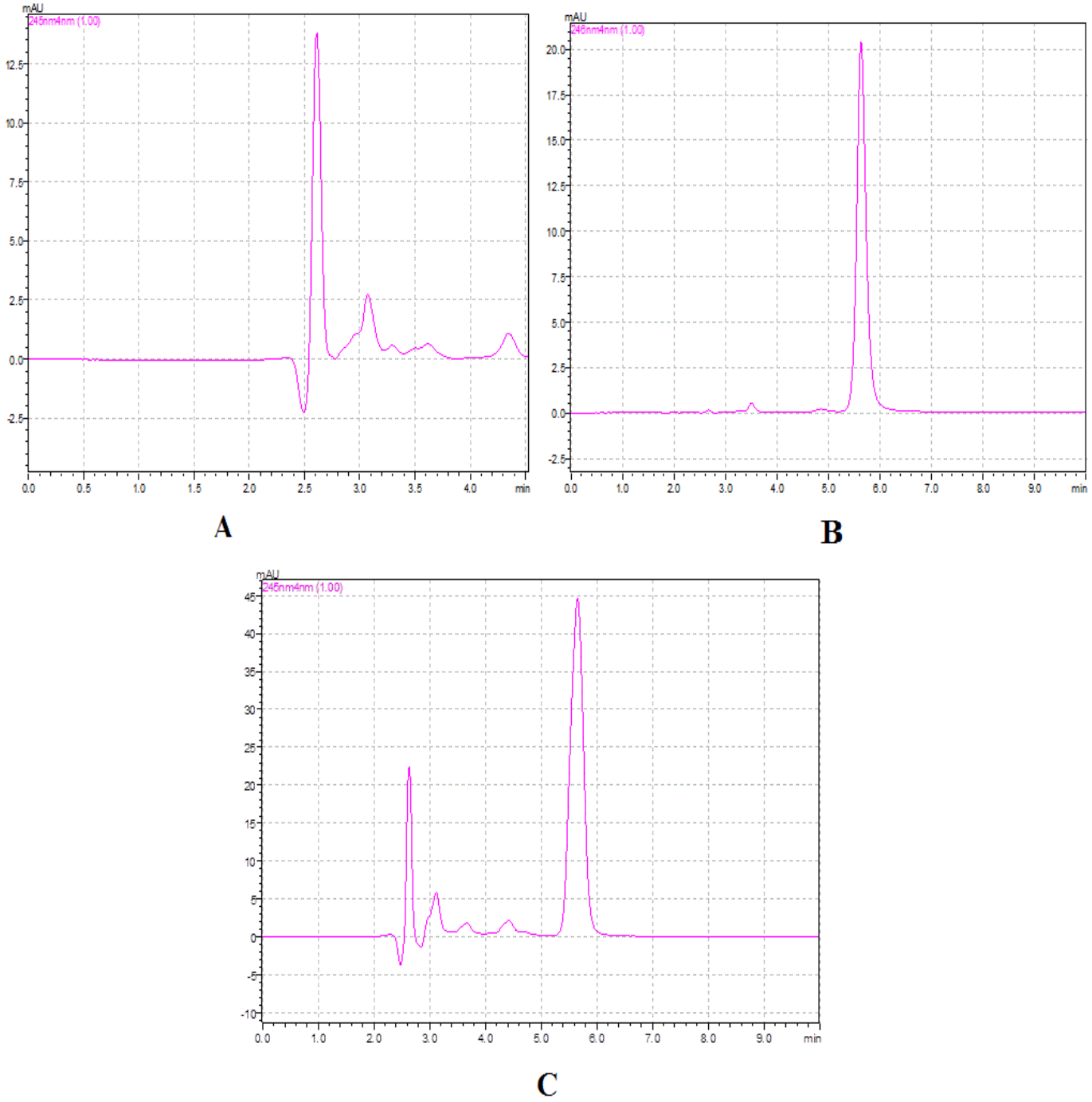

Figure 6: (a) Chromatogram for blank, (b): Chromatogram of Std., (c) Chromatogram of sample (25 min)

Table 7: Observations and results of precision study

Test sample	Peak area (µV)	% Dissolution of TENE at 25 min
1	1043682	70.33
2	1047465	70.58
3	1068373	71.99
4	1072983	72.23
5	1076389	72.53
6	1059784	72.09
Mean	10611446	71.625
± SD	13551.15	0.9277
%RSD	1.28	1.30

Intermediate precision

Estimation of TENE in marked formulation analyzed by proposed methods had yield quit concurrent results, standard deviation and %RSD of series of measurement were found to be within limit (Not more than 2%) (Tables 8 and 9).

Table 8: Dissolution test precision (Intraday) results

Average % release ± SD (n=3)				
S. No.	**Time (min)**	**10:00 am**	**01.00 pm**	**04.00 pm**
1.	5	7.29 ± 0.004	6.83 ± 0.0062	8.68 ± 0.00264
2.	10	24.02 ± 0.0198	23.17 ± 0.01358	26.76 ± 0.0125
3.	15	59.09 ± 0.0160	57.26 ± 0.01058	61.06 ± 0.00513
4.	20	66.95 ± 0.00321	66.34 ± 0.00643	67.63 ± 0.00814
5.	25	71.17 ± 0.00513	71.49 ± 0.004	73.49 ± 0.00458
Average at 25 min		72.05 ± 0.00457		
%RSD		1.66		

Table 9: Dissolution test precision (Interday) results

Average % release ± SD (n=3)				
Sr. No.	**Time (min)**	**1st day**	**2nd day**	**3rd day**
1.	5	4.88 ± 0.00551	4.51 ± 0.00321	8.57 ± 0.00321
2.	10	28.52 ± 0.007	28.51 ± 0.00361	34.33 ± 0.00651
3.	15	47.60 ± 0.00551	48.45 ± 0.00115	54.57 ± 0.0052
4.	20	60.92 ± 0.00351	60.78 ± 0.003	68.97 ± 0.00379
5.	25	71.05 ± 0.00351	71.54 ± 0.00251	72.67 ± 0.0153
Average at 25 min		71.75 ± 0.007106		
%RSD		1.56		

The %RSD for dissolution of the test sample of TENE was found to be 1.66 and 1.56 which is within the acceptance limit.

Accuracy of test method

The accuracy study for Teneligliptin was demonstrated by adding standard drug substance to the dissolution vessel in known amounts at the 80%, 100% and 120% levels. Accordingly about 11.2 mg, 14.0 mg and 16.8 mg of reference drug was added along with 20.0 mg tablet. Dissolution test was performed for 25 min using 900.0 ml of phosphate buffer pH 7.5 as a dissolution medium in a paddle type at 50 rpm. Aliquots of 10.0 ml were withdrawn and filtered through Whatman filter paper and analyzed by proposed methods (Table 10).

Table 10: Results of recovery study

S. No.	**Accuracy level**	**% Recovery***	
		UV	**RP-HPLC**
1.	80%	97.08	99.45
2.	100%	98.84	100.70
3.	120%	100.26	101.42
Mean		98.72	100.52
± SD		1.59	0.99
%RSD		1.61	1.01

*Each value is mean of three observations

The data indicated that %RSD values and the mean % recovery found against added amount was under acceptance criteria.

Robustness of test method

The robustness of the proposed method was evaluated by change in analyst and instrument using optimized dissolution parameters (Table 11).

Table 11: Results of robustness study

Avg. % release ± SD (n=3)					
S. No.	**Time (min)**	**Analyst**		**Instrument**	
		I	**II**	**I**	**II**
1.	5	8.92 ± 0.00656	9.54 ± 0.00666	6.08 ± 0.002193	7.52 ± 0.0231
2.	10	20.05 ± 0.005	18.33 ± 0.01217	27.67 ± 0.00231	25.09 ± 0.00755
3.	15	41.27 ± 0.01413	41.18 ± 0.00379	55.67 ± 0.00651	58.20 ± 0.00701
4.	20	49.25 ± 0.00866	49.93 ± 0.01044	69.19 ± 0.01015	70.27 ± 0.0164
5.	25	74.07 ± 0.00503	74.29 ± 0.00208	74.92 ± 0.00751	75.22 ± 0.00346
Avg. % release at 25 min			74.18 ± 0.003555	75.065 ± 0.005485	
% RSD			1.15	1.77	

The %RSD of dissolution study for TENE was found to 1.15 and 1.77, which is within the limit of acceptance limit. Also robustness study by RP-HPLC method was carried out via change in flow rate (± 0.2 ml/min.) and detection wavelength (± 5 nm). The overall %RSD for the deliberate variations was found within the range.

CONCLUSION

The proposed dissolution test method for Teneligliptin by using UV spectrophotometer and RP-HPLC was developed and validated as per the ICH guidelines. The result obtained by proposed method was found to be reliable, accurate and precise. Hence, the developed methods can be employed for routine dissolution analysis of Teneligliptin tablets.

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