Comparative evaluation of dissolution profile of drug in its formulation by UV spectrophotometry

Rahul K. Chaudhari¹, Anvesha V. Ganorkar²*, Madhura P. Dixit³, Milind J Umekar⁴

¹Student, ²Professor, ³Assistant Professor, ⁴Principal, Dept. of Pharmaceutical Chemistry, Smt. Kishoritai Bhoyar College of Pharmacy, New Kamptee, Nagpur, Maharashtra, India

*Corresponding Author: Anvesha V. Ganorkar

Email: anveshaganorkar9999@gmail.com

Abstract

The aim of the research work was to develop a method for comparative evaluation of dissolution profile of two different brands of Teneligliptin hydrobromide hydrate drug in its formulations containing using UV Spectrophotometer. Simple, precise and accurate UV-spectrophotometric method was developed for Teneligliptin hydrobromide hydrate using optimized dissolution parameters like as 900mL of Phosphate buffer pH 6.8 as a dissolution medium and paddle (type II) apparatus at a stirring rate of 100 rpm. The drug release was evaluated by UV spectrophotometric method using 243.2nm as detection wavelength. Developed method obeyed Beer's-Lambert's law in the concentration range of 0.5-25 µg/mL, with correlation coefficient value less than 1. The percent drug amount released estimated by proposed method was nearly 100%, found to be in good agreement with label claim of marketed tablet formulation. The proposed method can be adopted for routine quality control test for estimation of drug in formulation. Also the statistical data analysis of percent drug release of brand 1 and 2 were compared with preexisting dissolution data of literature by using F-test and t-test.

Keywords: Teneligliptin hydrobromide, Spectrophotometric method.

Introduction

Teneligliptin (TEN) is chemically described as $\{(2S, 4S)-4-[4-(3-methyl-1phenyl 1H-pyrazol-5-yl) piperazin-1-yl]$ pyrrolidin-2-yl $\{(1, 3-thiazolidin-3-yl) methanone$ hemipentahydrobromide hydrate is a dipeptidyl peptidase inhibitor having a chemical formula (C₂₂H₃₀N₆OS)₂. 5HBr. XH₂O. (Fig. 1)



Fig. 1: Chemical structure of teneligliptin hydrobromide hydrate.

Teneligliptin acts by reducing inactivation of incretin hormones, thereby increasing bloodstream concentrations, reducing fasting and postprandial glucose concentrations in a glucose dependant manner in patients with type 2 diabetes mellitus. The inhibition of DPP 4 increases the amount of active plasma incretins which helps with glycemic control.¹ It slows inactivation of incretin hormones which is glucagonlike peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) helps for insulin secretion. DPP-4 inhibitors increase the levels of active GLP-1 and GIP by inhibiting DPP-4 enzymatic activity; thus, in patients with diabetes, these inhibitors improve hyperglycemia by maintaining serum insulin levels and decreasing serum glucagon levels. Therefore, DPP-4 inhibitors are widely used to maintain fluctuations in glucose level for diabetic patients.² As compared to disintegration, dissolution of the active pharmaceutical ingredient is a rate determining step in transformation of drug into solution for the absorbing membrane.³ Currently in pharmaceutical industry dissolution testing has become an essential critical parameter at various stages of development, manufacturing and marketing. various regulatory committees recommend similarity factors f2 for the comparison of dissolution profiles and dissolution profiles are considered similar if the calculated f2 value is between 50 and 100.⁴

Drug dissolution testing is routinely used to provide analytical in vitro drug release as a quality control scheme. In vivo drug release study will gives bioavailability and bioequivalence data.⁵

Literature survey reveals that there are various methods for Teneligliptin estimation such as RP-HPLC and identification of degradation products by UPLC tandem mass spectroscopy,⁶ UV spectrophotometric methods,⁷ RP-UFLC,⁸ and one dissolution method also developed for Teneligliptin by RP-HPLC and UV-Spectrophotometric Method.⁹ But none of the method yet developed on comparative evaluation of dissolution profile of pharmaceuticals drug formulations containing Teneligliptin. Hence comparative dissolution studies of two different commercially marketed Teneligliptine tablets 20mg were selected for our current research work.

Material and Methods Chemicals and reagents

Teneligliptin in salt form was procured from Glenmark Pharmaceutical, Ltd, (Sinnar, India). The commercially formulation of Teneligliptin were purchased form Indian market. Chemicals include 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 was prepared as per the pharmacopoeia.

Instruments

Jasco-UV module version V-630 series prominence JASCO UV was used for spectral measurements. Analytical balance-CONTECH, CAS-44 was used for weighing, Magnetic stirrer REMI BCMS-364 was used for stirring. Paddle, Basket, cannula, Glass vessels, syringe. Electrola -Tablet Dissolution tester-TDT-06P used for dissolution testing.

Preparation of standard stock solution

An accurately weighted 10.0mg of Teneligliptin was transferred in 10.0 mL volumetric flask, dissolved in sufficient quantity of buffer solutions such as 0.1N HCL, Phosphate Buffer pH 6.8 and Acetate Buffer pH 4.5 to prepare a standard stock solution having concentration 1000 μ g/mL of Teneligliptin.

Working standard solution

A 1.0 mL of the standard stock solution was diluted up to 10.0 mL to prepare a solution having concentration 100 μ g/mL of Teneligliptin.

UV-visible spectophotometric analysis (Selection of wavelength)

For the selection of analytical wavelength Teneligliptin (10 μ g/mL) in various buffer solutions such as 0.1 N HCL, phosphate and acetate buffer solution were prepared and scanned in the range of 200-400 nm in 1.0 cm cell against solvent blank (buffer solution). Teneligliptin showed maximum absorbance in 0.1N HCl, phosphate buffer pH 6.8 and in acetate buffer at λ_{max} 243.0, 243.2 and 244.4 nm respectively. From the spectrum study, Teneligliptin shows maximum absorbance at 243.2nm in phosphate buffer. Therefore 243.2nm was considered as λ_{max} for further experimentation which was shown in Fig. 2A-C.



Fig. 2A: Spectrum in 0.1 N HCl



Fig. 2B: Spectrum in phosphate buffer



Fig. 2C: Spectrum in acetate buffer 4.5 pH

Preparation of calibration curve

Appropriate dilutions of standard stock solution were made to get final concentration in the range of 0.5-25 μ g/mL and absorbance of each was measured at above selected wavelengths. The calibration curves for 0.1 N HCL, phosphate and acetate buffer solution were plotted between concentration *vs* absorbance having correlation coefficient 0.986, 0.996 and 0.994 respectively.(Fig. 3AC)



3A): Plot in 0.1 N HCL



3B): Plot in phosphate buffer



3C): Plot in acetate buffer **Fig. 3:** Plot of beer's-lambert's law of teneligliptin in various buffers

Dissolution Method for Marketed Preparation of Teneligliptin Hydrobromide Hydrate Tablets Drug substance solubility study

The solubility of the drug for in vitro studies was performed using different dissolution condition according to USP Dissolution medium may be water an aqueous solution (typically pH 4.0 to 8.0 or a dilute acid solution (0.001 to 0.1 mol L-1 HCL) because drug is a hydrophilic therefore solutions such as surfactant and electrolyte were not added.

Dissolution of test samples: Selection of RPM

Weighed and dropped 1 tablet in each of the three vessels containing the 900ml dissolution medium for the respective drug under analysis at different RPM 50 and 100 RPM. After specified time interval 10-60 min, 10.0 mL of the aliquot was withdrawn. The solution was filtered with the whattman filter paper. Absorbance of each solution at each time point was measured respective wavelengths (0.1N HCl, phosphate buffer pH 6.8 and in acetate buffer at λ_{max} 243.0, 243.2 and 244.4 nm respectively) and % release were calculated for both brands. In dissolution test the temperature was maintained to 37°C and sink was maintained during the whole period of dissolution test.

For Brand 1

The study of drug release at 50and 100RPM data showed that drug was released faster at higher RPM speed in 0.1 N HCL and acetate buffer pH 4.5 media (Fig. 4A and 4C). But the release was found to be on a lower side as compared the release recommended as per IP and USP (85% drug release for conventional dosage form). In case of phosphate Buffer media (Fig. 4B) drug release data showed that drug was released earlier at higher RPM speed in but the release was found to be on upper side as compared the release recommended as per IP and USP. The release profile of brand 1 was found to be near to ideal dissolution curve at both the RPM speed in phosphate Buffer media.



Fig. 4: Percent drug release for brand 1 in 4A): 0.1 N HCL, 4B): Phosphate and 4C): Acetate buffer solution

Brand 2

The study of % drug release at 50 and 100 rpm data showed that drug was released faster at higher RPM speed in 0.1 N HCL and acetate buffer pH 4.5 media (Fig. 5A and 5C). But the release was found to be on a lower side as compared the release recommended as per IP and USP. In case of phosphate buffer media (Fig. 5B) drug release data showed that drug was released earlier at higher rpm speed in but the release was found to be on upper side as compared the release recommended as per IP and USP. The release was found to be near to ideal dissolution curve at both the RPM speed in phosphate Buffer media.



Fig. 5: % drug release for brand 2 in 5A): 0.1 N HCL, 5B): Phosphate and 5C): Acetate buffer solution

Dissolution of test samples: For selection dissolution media

Same procedure is followed which is given in dissolution of test sample: for selection of RPM and from the observations 100RPM selected for the dissolution study. The %drug released calculated and displayed in Table 1.

S. No.	Brand	Parameter	Acetate buffer	0.1 N HCL	Phosphate buffer
1	1 Brand 1	Absorbance	0.379	0.541	0.317
1		Drug release (%)	61.05	79.9	99.89
2	Buond 2	Absorbance	0.389	0.417	0.461
2	Brand 2	Drug release (%)	61.05	76.21	97.25

 Table 1: Results of dissolution test for Brand 1 and 2 in all buffer solutions in different buffers at 100 rpm.

Finalized dissolution parameter for further study

From the above observation of % drug release of two different brands, dissolution parameters finalized were 900mL of Phosphate buffer pH 6.8 as a dissolution medium and dissolution apparatus paddle (type II) at a stirring rate of 100 rpm were selected for dissolution study.

Dissolution assay of teneligliptin tablet

Weighed and dropped each brand tablet to dissolution apparatus and dissolution study was performed under above finalized parameters. At the end of 60 min sample was withdrawn and dilute up to 10 ml with phosphate buffer solution. Absorbance of sample was measured at 243.2 nm and % drug release was calculated. (Table 2).

Table 2: Dissolution assay for brand 1 and 2

S. No	Tablet	Absorbance	Drug release (%)
1	Brand 1	0.496	99.79
2	Brand 2	0.398	95.85

Evaluation of similarity factor

The in-vitro drug release profiles of the hydrophilic matrix tablets were compared with the drug release profile of competitor brand for selected final phosphate buffer media by determining the similarity factor (f) (Table 3). The similarity factor (f2) is a logarithmic transformation of the sum-of-squared error of differences between the test and the reference products Rt over all time points.

S. No. Time		Avg % Release		f2	MDT (T) /	AUC (T) /
		Reference	Test		MDT (R)	AUC (R)
1	0	0.00	0.00	0.00	0.000	0.000
2	10	0.35	0.27	99.97	1.000	0.768
3	20	0.44	0.35	99.95	1.039	0.779
4	30	0.42	0.42	99.96	1.674	0.830
5	40	0.42	0.44	99.97	1.690	3.019
6	50	0.48	0.50	99.97	1.438	0.945
7	<u>60</u>	0.52	0.65	<u>99.95</u>	1.786	0.987

Table 3: Similarity factor determination

f2= 99.95

Dissolution method validation:¹⁰

The proposed method was validated as per ICH guidelines.

Precision

The precision of the analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The test solutions were obtained by performing the dissolution of the respective drug using optimized dissolution parameters. The six replicate of the test solutions of each drug so obtained were dissolution. The % drug release was calculated. (Table 4).

Table 4	1 • 1	Result	of	precision	for	tenel	iol	in	ntin
Lanc -	r. 1	Nesun	or	precision	101	unu	igi	ıμ	[/] um

S. No	% Dissolution			
Replicate test	Brand 1	Brand 2		
1	99.10	94.80		
2	99.25	95.25		
3	99.36	94.92		
4	99.29	94.98		
5	99.15	95.15		
6	99.28	95.20		

Accuracy

The accuracy of proposed method was ascertained on the basis of recovery studies. Weighed the pre-analyzed tablet powder equivalent to 2.5 mg; a known amounts of standard drug was added at different levels 50-150 %. The resultant solutions were then re-analyzed by the developed methods. At each concentration, each sample was analyzed thrice at

each level to check repeatability and from the data it was analyzed that the methods were found to accurate (Table 5).

Table 5: Result of accuracy for tenelig	gliptin
---	---------

Accuracy level	% Recovery			
	Brand 1	Brand 2		
50%	99.32	98.56		
100%	100.26	100.54		
150%	99.52	99.65		
Mean	99.70	99.58		
±SD	259.964	218.939		

Statistical comparision with literature data

In these we utilized available precision data of Teneligliptin marketed formulation denoted as Brand-A.⁹ The precision data interday day precision data of brand-A compared with precision data of brand 1 and 2 in % drug release. The t-test and F-test is applied to their dissolution data to check significant difference in drug released at different pH (Brand-A at 7.5 and Brand 1 and 2 at 6.8 pH of phosphate buffer.

Table 6: Precision data of brand-A, brand 1 and 2 in % drug release

Brand-A	Brand-1	Brand-2
71.05	99.1	94.8
71.54	99.2	95.25
72.67	99.36	94.92
	99.29	94.98
	99.15	95.15
	99.28	95.2

Table 7: Statistical data of F-test for precision of drug in marketed formulation

	Va	riance			E ouitical on a	
Sample	Brand-A or	Brand 1	F-value	P(F<=f)one tail	r critical one	
	Brand-1	Or Brand 2			ıdli	
Brand-A v/s Brand-1	0.63845	0.00583	109.511	0.000471	7.7086	
Brand-A v/s Brand-2	0.63845	0.02045	31.2200	0.00503223	7.70864	
Brand-1 v/s Brand-2	0.00583	0.02045	0.285086	0.1258059	0.156538	

Table 8: Statistical data of t-test for precision of drug in marketed formulation

Sample	t Stat	P(T<=t) one-tail	t Critical one-	P(T<=t) two-	t Critical two-
			tail	tail	tail
Brand-A v/s Brand-1	47.99	0.0066	6.313	0.0132	12.706
Brand-A v/s Brand-2	-40.441	0.0079	6.314	0.0157	12.706
Brand-1 v/s Brand-2	57.463	9.3294	1.9432	1.8659	2.4469

From the observations of above statistical data of F-test and t-test, it was concluded that:

F-test: The variance of Brand-A is different from brand-1 and brand 2.whereas the variance value between brand 1 and 2 nearly equal.

T-test: The value of P of one tail and two tails is lower than the value (Generally it was taken as 0.05). Hence the null hypothesis rejected i.e. there is a significance differences in drug release of Brand-A compared with Brand 1 and 2 drug release by using different pH(Phosphate buffer pH 6.8). Whereas the drug release of brand 1 and 2 was significantly same.¹¹

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ values for Teneligliptin were found to be $25.02 \ \mu g$ and $76.02 \ \mu g$, respectively. The low LOD and LOQ values for Teneligliptin indicate the sensitivity of the method.

Discussion

Teneligliptin was found to be water soluble so hydrophilic solvents were selected, for study of beers Lambert law, Teneligliptin showed maximum absorbance in 0.1N HCl, phosphate buffer pH 6.8 and in acetate buffer at λ_{max} 243.0, 243.2 and 244.4 nm respectively, hence was selected as the wavelengths for dissolution study. The solubility was considered on the recommendations of the USP for hydrophilic drugs. Hence following dissolution media were studied 0.1N HCl, Phosphate buffer 6.8 and Acetate buffer 4.5 pH. Various dissolution test parameters were evaluated such as dissolution media, pH of buffers and rpm for dissolution study. From the above observation of % drug release of two different brands dissolution parameters such 900mL of Phosphate buffer pH 6.8 as a dissolution medium and paddle (type II) apparatus at a stirring rate of 100 rpm were selected. From the data obtained above the release profile was plotted as percent drug release Vs time points are shown below for both the brands. The solubility of Teneligliptin was in buffer and hence it was selected as solvent for the estimation of Teneligliptin. The selected wavelength for dissolution test was found to be 243.2 nm from UV spectrum (Figure No. 2 B) Beer-Lambert's law was obeyed in concentration range of 0.5 to 25 µg/mL for the using phosphate buffer (pH 6.8).

Similarity factor was determined for the drug release in Phosphate buffer as it was found that the drug released almost 100% in the said media. The drug release was compared for brand 1 and 2 and found to be f2-95.95. The similarity factor value should be above 50 indicating good comparison between the brands. The results of similarity factor indicate that the drug excipients in both brands might be almost similar. Validation was performed to assure the reliability of the proposed method and was carried out as per ICH guideline for the following parameter.

Conclusion

The UV-Spectrophotometric method was developed for the determination of Teneligliptin is based on Calibration curve method. The comparative results obtained by UV method for dissolution test were reliable, accurate and precise. The drug release was compared for brand 1 and 2 and found to be f2-95.95. The similarity factor value were more than 50 indicating good comparison between the brands. There was no intra variation and inter variation between the two different brands of Tanegliptin. The statistical data of F-test and T-test concluded that there is a significance difference in drug release at different pH of phosphate buffer. Hence, the developed comparative study of teneligliptin marketed formulation using dissolution profile can be employed for routine dissolution analysis of Teneligliptin hydrobromide hydrate tablet.

Acknowledgement

Authors are thankful to the Principal SKB College of Pharmacy, Kamptee for providing necessary facilities for carrying out the work in the laboratory.

Source of Funding

None.

Conflict of Interest

None.

References

- Patil D, Ahmad S, Shastry VM, Mujawar T, Thakare L. Analytical Method Development and Validation for the Simultaneous Estimation of Metformin and Teneligliptin by RP-HPLC in Bulk and Tablet Dosage Forms. *J Pharm Res.* 2017;11(6):676-81.
- Kishimoto M. Teneligliptin. A DPP-4 inhibitor for the treatment of type 2 diabetes. *Diabetes, Metab Syndr Obes*. 2013;6:187.
- Nagabandi V, Kumar MS, Prasad G, Someshwar K, Varaprasad A. Comparative Dissolution Studies of Marketed Preparations and Treatment of Data by Using ANOVA. *Int J Adv Pharm Sci.* 2010;1(2).
- Hassouna ME, Issa YM, Zayed AG. A Comparative Study of the In-vitro Dissolution Profiles of Paracetamol and Caffeine Combination in Different Formulations Using HPLC. *Jf Appl Pharm Sci.* 2012;2(5):52.
- 5. Nayak S, Rakshita AS, Shwetha S, Kamath K. Study of post compression parameters of various marketed paracetamol tablets in India. *Pharma Tutor.* 2019;7(2):35-42.
- Kumar TG, Vidyadhara S, Narkhede NA, Silpa YS, Lakshmi MR. Method Development, Validation, and Stability Studies of Teneligliptin by RP-HPLC and Identification of Degradation Products by UPLC Tandem Mass Spectroscopy. J Anal Sci Technol. 2016;7(1):27.
- Yadav N, Goyal A. Method Development and Validation of Teneligliptin in Pharmaceutical Dosage form by UV Spectrophotometric Methods. *Int J Pharm Chem Anal.* 2017;4(3):54-8.
- 8. Maruthi R, Chandan RS, Tengli AK. Method Development, Validation and Stability Indicating Assay for Teneligliptin Hydrobromide by RP-UFLC. *IJPSR* 2019;10(2):728-35.
- Hemke AT, Ghuge YG, Gupta KR. Application of RP-HPLC and UV-Spectrophotometric Method in Dissolution Testing of Teneligliptin. *Der Pharma Chemica*. 2018;10(1):103-10
- Vaghela B, Kayastha R, Bhatt N, Pathak N, Rathod D. Development and Validation of Dissolution Procedures. J Appl Pharm Sci. 2011;1(03):50-6.
- Mendham J, Denney RC, Bares JD, Thomas M and Sivasankar B. Statistical Treatment of Analytical Data and Introduction to Chemometrics. In Vogel's Textbook of Quantitative Chemical Analysis.6th edition.2000:104-156.

How to cite: Chaudhari RK, Ganorkar AV, Dixit MP, Umekar MJ. Comparative evaluation of dissolution profile of drug in its formulation by UV spectrophotometry. *Int J Pharm Chem Anal.* 2020;7(1):26-31.

International Journal of Pharmaceutical Chemistry and Analysis, January-March, 2020;7(1):26-31