WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 8, 1280-1291. Research Article ISSN 2277– 7105

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN AND METFORMIN BY USING RP HPLC

R. Vasanthi*[1], Nazia Begum[1], M. Alagar Raja[1], K. Rajeswar Dutt[1], K. N. V. Rao[1], H. Ramana[2] and G. Koteshwar Rao[1]

[1]Nalanda College of Pharmacy, Charlapally, Nalgonda, T.S.

[2]Venkateshwara Institute of Pharmaceutical Sciences, Charlapally, Nalgonda, T.S.

Article Received on
29 May 2017,

Revised on 19 June 2017,
Accepted on 10 July 2017

DOI: 10.20959/wjpr20178-8977

***Corresponding Author**
Dr. R. Vasanthi
Nalanda College of Pharmacy, Charlapally, Nalgonda, T.S.

ABSTRACT

A Rapid and Precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Teneligliptin and Metformin, in its pure form as well as in tablet dosage form. Chromatography was carried out on X-Terra C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 243 nm. The retention time of the Teneligliptin and Metformin was 2.090, 5.289±0.02min respectively. The method produce linear responses in the concentration range of 5-25mg/ml of Teneligliptin and 45-225mg/ml of Metformin. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Teneligliptin, Metformin, RP-HPLC, validation.

INTRODUCTION

Teneligliptin (Figure No.1) is the inhibitors of dipeptidyl peptidase-4, gliptins, are a class of oral hypoglycemic drugs that block DPP-4. They are used for diabetes mellitus type-2. Glucagon increases blood glucose levels and DPP-4 inhibitor which reduces glucagon and blood glucose levels. The mechanism of action of DPP-4 inhibitors is to increase incretin levels (GLP-1 & GIP), which inhibit glucagon release, which in turn increases insulin secretion, decreases gastric emptying and decreases blood glucose levels.[1] Metformin

(Figure No.2) is a biguanide anti hyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, glucose absorption and increasing insulin-mediated glucose uptake. This may induce weight loss and drug of choice for obese NIDDM patients. Metformin hydrochloride (MET) is chemically N, N dimethylimidodicarbonimidic diamide hydrochloride (1, 1-Dimethylbiguanide hydrochloride) which acts by decreasing intestinal absorption of glucose reducing hepatic glucose production and increasing sensitivity.[2] Teneligliptin shows effective control of blood sugar when combined with Metformin. Literature survey reveals that few HPLC and UV spectrophotometric methods have been reported for determination of Metformin, Teneligliptin individually and combined with various gliptins tablet form.[3-9] A successful study is done for estimation of by. However, the development of simultaneous estimation of Teneligliptin and Metformin in combined dosage form by RP-HPLC method has not yet been reported. Hence, this manuscript is the first to describe the development and validation of some simpler, sensitive, precise, accurate and cost effective UV spectroscopic methods for the simultaneous determination of Teneligliptin and Metformin in combined tablet formulation.

MATERIALS AND METHODS

Chemicals and Reagents

Acetonitrile, Water and Methanol of HPLC grade were obtained from LICHROSOLV (MERCK) Mumbai, India. Pharmaceutical grade Teneligliptin and Metformin were obtained from, Hyderabad, India.

Instruments

The analysis was performed by using the weighing machine, pH meter (Lab India). The HPLC used is of WATERS Alliance 2695seperation module with 996 PDA detector. The output signal was monitored and integrated using Empower 2 software. A Symmetry C18, X-bridge column, Xterra. Phenomenex Luna C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

REAGENTS AND SOLUTIONS

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Triethylamine (TEA) buffer (pH-4.5)

Dissolve 1.5ml of Triethyl amine in 250 ml HPLC water and adjust the p^H 4.5. Fliter and sonicate the solution by vaccum filtration and ultra sonication.

Preparation of Mobile phase

Accurately weigh and transfer 10 mg of Teneligliptin and Metformin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Preparation of Standard stock Solution

Accurately weigh and transfer 10 mg of Teneligliptin and 10mg of Metformin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.15ml of the above Teneligliptin and 1.35ml of Metformin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution

Take average weight of tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Teneligliptin and Metformin sample into a 10ml clean dry volumetric flask and add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1.35ml of sample stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Assay

10µL of the standard solution was injected five times into the chromatographic system, chromatograms were recorded and peak areas were measured. 10µL of the sample solution was injected in five times into the chromatographic system, chromatograms were recorded and peak areas were measured.

METHODS DEVELOPMENT[10-13]

The developed method was fully validated for the parameters as per ICH guidelines.

System Suitability

System suitability was done by replicate analysis injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Linearity

Linearity is determined by a series of three to five injections of five or more standards. Plot a graph of peak area (or heights) of the calibration standards are usually plotted in the Y-axis against the nominal standard concentration, and the linearity of the plotted curve is evaluated through the value of the co-relation coefficient (r2).The methods were linear in the range of 5ppm- 25ppm of Teneligliptin, 45-225ppm for metformin and inject each level into the chromatographic system and measure the peak area.

Accuracy

Accuracy of the method was determined by inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the amount found and amount added for Metformin and Teneligliptin and calculate the individual recovery and mean recovery values.

Precision

To determine the precision, intra-day and inter-day analysis was performed. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.Solutions corresponding to each concentration level were injected in duplicate. The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample.

Ruggedness

To evaluate the ruggedness of the method, precision was performed on different days by maintaining same conditions. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method.

Robustness

Robustness of the method was performed in different conditions to find the variability of test results.The sample was analyzed at 0.8 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were

recorded.Their effects on the retention time (TR), tailing factor (T), theoretical plate numbers (N) and repeatability of peak areas (n = 6) were studied.

Limit of detection and Limit of quantification

The limit of detection and quantification were calculated using signal to noise ratio. The LOD for Teneligliptin and Metformin were tested at specific level i.e 0.9µg/ml and 9.7µg/ml The LOQ for Teneligliptin and Metformin were tested at specific level i.e2.0µg/ml and 29.4µg/ml.

RESULTS AND DISCUSSION

The goal of this study was to develop a new RPHPLC method, several mobile phase compositions were tried for separation and quantification of Teneligliptin and Metformin in bulk and pharmaceutical dosage forms. To develop an effective method for the analysis of the drugs preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection wavelength, mobile phase composition and pH, mobile phase comprising of Acetonitrile: Water in 70: 30v/v at a flow rate 0.8 ml/min to get a better reproducibility and repeatability. Quantification was achieved with UV detection at 243 nm and the retention time for Teneligliptin and Metformin were found to be 7.255 and 9.643 mins respectively. Typical chromatogram of Teneligliptin and Metformin is shown in Figure No.3, 4. The optimized method was validated as per ICH guidelines.

System suitability

System suitability tests were carried out on freshly prepared standard solutions and the parameters are summarized in Table.No.1.

Linearity

The correlation coefficient for linear curve obtained between concentration vs. area for standard preparations of Teneligliptin and Metformin (Figure No.5 & 6) is 0.999and 0.999 respectively. It shows that the good correlation exist between the drug and response. The results are summarized in the Table No.2, 3.

Accuracy

The % Recovery for each level obtained for Teneligliptin was found to be within the limits (98-102.0 %).The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate. The %Recovery for each level obtained for Metformin was found

to be within the limits (98-102%) as per the ICH guidelines the results were within the limit. The results are shown in Table No.4, 5.

Precision

The % RSD of 6 determinations of Teneligliptin and Metformin for System precision intraday and inter day was found to be within the acceptance criteria of not more than 2.0%. The results are tabulated in Table No. 6, 7, 8, 9.

Limit of Detection and Limit of Quantification

Limit of detection result for Teneligliptin and Metformin was found to be 0.6 and 9.7 respectively and were within the limits. S/N ratio for Teneligliptin and Metformin were found to be within the limits. Results are summarized in Table No.10, 11.

Robustness

The analysis was performed in different conditions to fine the variability of test results. The conditions are checked for variation of results. Results are summarized in Table No. 12, 13.

Table No.1: System Suitability of Proposed Method

S.No	Parameters	Teneligliptin	Metformin
1.	Theoretical plates	5463	5786
2.	Resolution	9.7	---
3.	Tailing Factor	1.44	1.40
4.	Retention Time(min)	2.090	5.289

Table No.2: Linearity data of Teneligliptin

S.No	Linearity level	Concentration	Area
1.	I	5ppm	134436
2.	II	10ppm	245571
3.	III	15ppm	371548
4.	IV	20ppm	499024
5.	V	25ppm	619830
Correlation Coefficient (r^2)			0.999

Table No.3: Linearity data of Metformin

S. No	Linearity level	Concentration	Area
1.	I	45ppm	1330054
2.	II	90ppm	2728974
3.	III	135ppm	3917063
4.	IV	180ppm	5300022
5.	V	225ppm	6412695
Correlation Coefficient (r^2)			0.999

Table No.4: Accuracy results for Metformin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2001752	67.5	67.3	99.7	99.7%
100%	3927797	135	134.8	99.8	
150%	5858665	202.5	202.1	99.8	

Table No.5: Accuracy results for Teneligliptin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	192446.6	7.5	7.4	98.6	98.7%
100%	374222	15	14.8	98.66	
150%	555891.3	22.5	22.3	99.1	

Table No.6: Results of Intra-day precision for Teneligliptin

S.No	Injection	Area
1.	Injection- 1	362266
2.	Injection-2	364902
3.	Injection-3	366870
4.	Injection-4	367273
5.	Injection-5	368101
	Mean	365882.4
	Std.dev	2338.4
	%RSD	0.6

Table No.7: Results of Intra-day precision for Metformin

S.No	Injection	Area
1.	Injection- 1	3903548
2.	Injection-2	3905819
3.	Injection-3	3916120
4.	Injection-4	3916542
5.	Injection-5	3920943
	Mean	3912594.4
	Std.dev	7507.6
	%RSD	0.2

Table No.8: Results of Intermediate precision for Metformin

S. No	Injection	Area
1.	Injection- 1	3743003
2.	Injection-2	3845359
3.	Injection-3	3885014
4.	Injection-4	3743003
5.	Injection-5	3722513
6.	Injection-6	3728789
	Mean	3777947
	Std.dev	69194.4
	%RSD	1.8

Table No.9: Results of Intermediate precision for Teneligliptin

S. No	Injection	Area
1.	Injection- 1	369246
2.	Injection-2	370766
3.	Injection-3	370840
4.	Injection-4	370840
5.	Injection-5	371041
6.	Injection-6	371386
	Mean	370686.5
	Std.dev	740.7369
	%RSD	0.19

Table No.10: Limit of Detection Chromatogram of Teneligliptin and Metformin

S.No	Name	Retention time	Area	s/n
1.	Teneligliptin	2.090	192446.6	0.6
2.	Metformin	3.202	2001752	9.7

Table No.11: Limit of Quantification Chromatogram of Teneligliptin and Metformin

S.No	Name	Retention time	Area	s/n
1.	Teneligliptin	1.966	24536	2.0
2.	Metformin	2.264	53443	29.4

Table No. 12: Results for Robustness

Metformin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	3864998	5.289	5698	1.77
Less Flow rate of 0.9mL/min	3546737	6.746	5546	1.88
More Flow rate of 1.1mL/min	3857216	4.032	5124	1.91
Less organic phase	3810347	6.746	5034	1.88
More organic phase	3875642	4.032	5612	1.91

Table No. 13: Results for Robustness

Teneligliptin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	372126	2.090	5587	1.70
Less Flow rate of 0.9mL/min	356765	2.736	5432	1.82
More Flow rate of 1.1mL/min	342356	1.673	5644	1.91
Less organic phase	312434	2.736	5098	1.82
More organic phase	305623	1.673	5123	1.91

Table No.14: Peak results for Assay standard

Metformin

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount
1	Metformin	5.289	3864998	231194	1.77	5628
2	Metformin	5.338	3881443	3231044	1.83	5688
3	Metformin	5.327	3896952	231969	1.86	5712

Teneligliptin

S.No	Name	RT	Area	Height	USPPlateCount	USP Tailing
1	Teneligliptin	2.090	348126	39690	5587	1.70
2	Teneligliptin	2.089	352564	39990	5571	1.66
3	Teneligliptin	2.089	357976	40396	5530	1.68

Table No. 15: Peak results for Assay sample

Metformin

S.No	Name	RT	Area	Height	USPTTTTailingTTTailing	USPPlateCount
1.	Metformin	5.276	3883794	231354	1.89	5677
2.	Metformin	5.268	3896493	234961	1.91	5804
3.	Metformin	5.262	3900103	233541	1.95	5790

Teneligliptin

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount
1.	Teneligliptin	2.088	352290	40269	1.69	5516
2.	Teneligliptin	2.087	356547	41157	1.72	5557
3.	Teneligliptin	2.085	358914	40963	1.75	5489

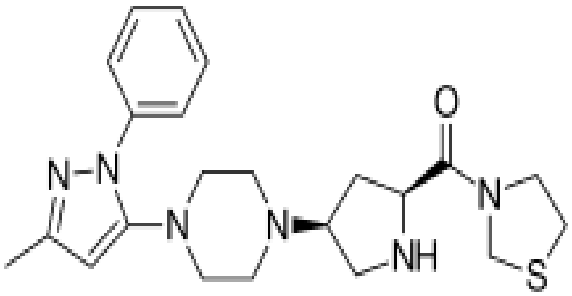
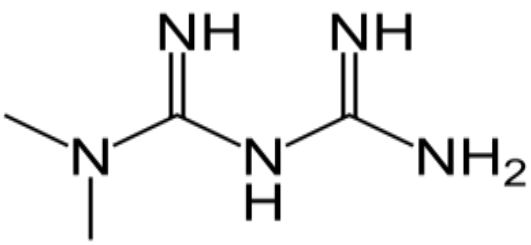


Figure No: 1 Teneligliptin



No.2: Metformin

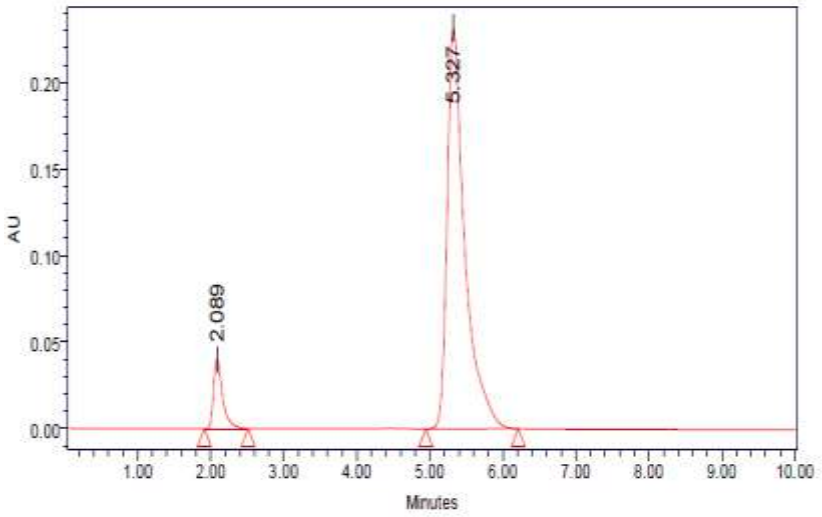


Figure No.3: Standard Chromatograms of Teneligliptin and Metformin

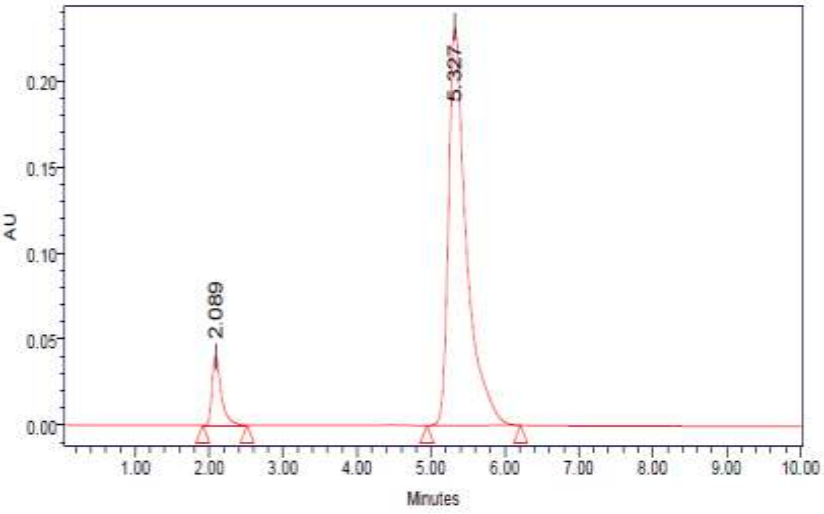


Figure No.4: Sample Chromatograms of Teneligliptin and Metformin

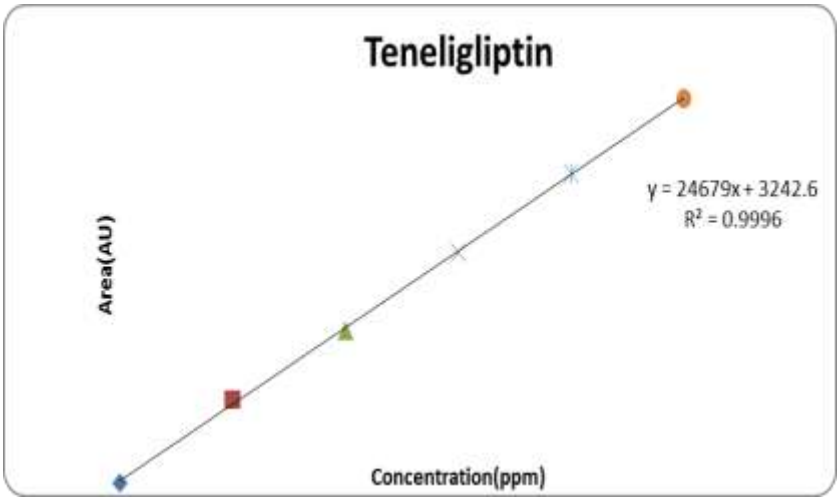


Figure No.5: Linearity study of Teneligliptin

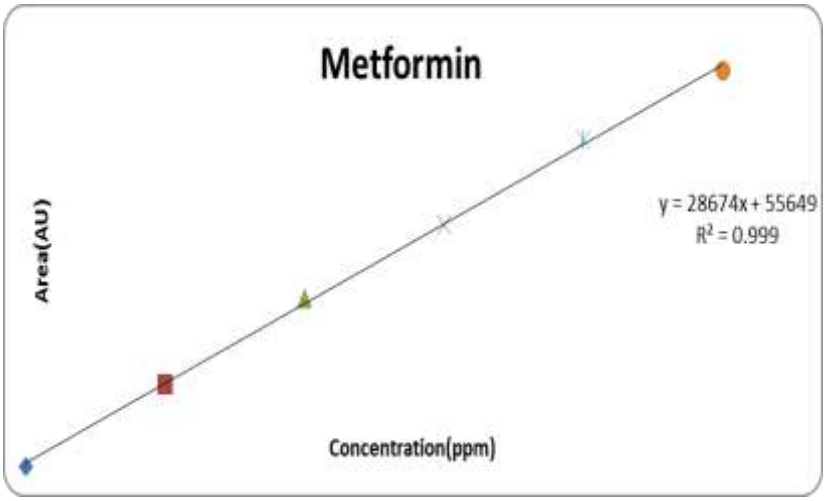


Figure No.6: Linearity study of Metformin

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Teneligliptin and Metformin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Teneligliptin and Metformin was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Teneligliptin and Metformin in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The authors are grateful to the Management of Nalanda College of Pharmacy, Nalgonda for providing the facilities carry out the present research our work.

BIBLIOGRAPHY

1. https://en.wikipedia.org/wiki/Teneligliptin.
2. https://en.wikipedia.org/wiki/Metformin.
3. Sohan S. Chitlange, Diptee G. Rawat, SnehaChandani. Estimation of Anti-Diabetic Teneligliptin Hydrobromide Hydrate By RP-HPLC and Derivative Spectroscopic Method .Indo American Journal of Pharmaceutical Research, 2016; 6(7).

4. Chandrabatla Varaprasad, Asif Md, Ramakrishna K. RP-HPLC Method for Simultaneous Estimation of Metformin and Linagliptin in Tablet Dosage form, 2015; 8(4): 426–432.
5. Chandana M,. PrasadRao M, Samrajyam B, Sireesha K.S.K.D, Nagapremi V.V. Analytical Method Development And Validation of Teneligliptin In Pharmaceutical Dosage Form By RP-HPLC Method.IJRDO-Journal of Health Sciences and Nursing, 2016; 1(12): 1-12.
6. Shrikrishna B. Baokar , Sugandha V. Mulgund , Nisharani S. Ranpise Development and Validation of RP-HPLC Method for Simultaneous Estimation of Vildagliptin and Metformin. J. Pharma. Dosage Forms and Tech., 2013; 5(2): 95-98.
7. Madhukar. A, Prince A, Vijaykumar, Sanjeeva R, Jagadeeshwar Y, Raghupratap KD. Simple And Sensitive Analytical Method Development And Validation Of Metformin Hydrochloride By Rp-Hplc.International Journal of Pharmacy and Pharmaceutical Sciences., 2011; 3(3): 117-120.
8. Ganesh Kumar T. N. V, Vidyadhara S , Niteen Ashok Narkhede, SaiSilpa Y, Rajya Lakshmi M. Method development, validation, and stability studies of teneligliptin by RP-HPLC and identification of degradation products by UPLC tandem mass spectroscopy.Journal of Analytical Science and Technology, 2016; 7: DOI 10.1186/s40543-016-0099-0.
9. AshimKumar Sen , Denish N. Hinsu, Dhanya B. Sen, Aarti S. Zanwar, Rajesh A. Maheshwari, Vikas R. Chandrakar, Analytical method development and validation for simultaneous estimation of Teneligliptin hydrobromide hydrate and Metformin hydrochloride from it's pharmaceutical dosage form by three different UV spectrophotometric methods, Journal of Applied Pharmaceutical Science, 2016; 6(09): 157-165.
10. Validation of Analytical Procedures: Methodology, ICH Tripartite Guidelines 1996.
11. Validation of Analytical Procedures, ICH Harmonized Tripartite Guidelines, 1994.
12. ICH, Q2A Text on validation of analytical procedures, Oct, 1994.
13. ICH, Q3B Validation of analytical procedures: methodology, Nov, 1996.