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Original Article

A NOVEL STABILITY-INDICATING REVERSE PHASE LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND TENELIGLIPTIN IN PURE AND PHARMACEUTICAL FORMULATIONS

A. SWETHA, B. RAMYA KUBER

Department of Pharmaceutical Analysis, Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam, Tirupathi, Andhra Pradesh, India
Email: Swetha.addanki06@gmail.com

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ABSTRACT

Objective: The present method was proposed to develop a simple, sensitive, rapid, accurate and stability-indicating reverse phase liquid chromatographic method for the simultaneous estimation of metformin and teneligliptin in pure and pharmaceutical formulations.

Methods: The chromatographic separation was done on Discovery [250 mm X 4.6 mm: 5 µm is particle size] using a mobile phase composed of 0.1% orthophosphoric acid buffer: acetonitrile [65:35, v/v], the flow rate is 1 ml/min and the detection was carried out at 260 nm.

Results: The retention time of metformin and teneligliptin were found to be 2.517 min and 3.687 min, respectively. Stability indicating studies were conducting under the guidelines of an international conference on harmonization [ICH] Q1A R2 and the developed method was validated as per guidelines of ICH Q2 RI. The linearity was found in the range of concentration of 125-750 µg/ml and 5-30 µg/ml for metformin and teneligliptin. The detection of limit and quantification of limit was found to be 0.02 µg/ml and 0.07 µg/ml for metformin and 0.19 µg/ml and 0.56 µg/ml for teneligliptin, respectively.

Conclusion: A novel stability-indicating reverse-phase liquid chromatographic method for the simultaneous estimation of metformin and teneligliptin. The proposed method was adopted for the routine estimation of metformin and teneligliptin in bulk and pharmaceutical dosage forms.

Keywords: Method validation, Estimation, Stability indicating, Metformin, Teneligliptin

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INTRODUCTION

Metformin (fig. 1) category is biguanide and chemically called as 3-[diaminomethylidene]-1, 1-dimethylguanidine. It is used for the treatment of type 2 diabetics; polycystic syndrome and limited use prevent the (CVD) and cancer complications of diabetes [1].

Teneligliptin (fig. 2) is potent, competitive and long-acting DPP-1V inhibitor and chemically called as {(2S, 4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl} (1, 3-thiazolidin-3-yl) methanone. It is used for the treatment of type-2 diabetic mellitus [2-3]. Literature survey revealed that very few analytical method have been reported for the estimation of metformin and teneligliptin by using ultraviolet spectroscopy [4-6], high-performance liquid chromatography [7-9] and liquid chromate-graphy-mass spectroscopy [10] by individually or simultaneously with other drugs. From the literature survey confirms that there is no method has been reported for the stability indicating a simultaneous estimation of metformin and teneligliptin in pure and pharmaceutical dosage form by using RP-HPLC.

The present method has so many advantages like simple standard preparation process, a large range of concentration with high sensitive, low-cost solvent are used in mobile phase preparation and all parameters must be validated as per ICH guidelines [11-12]. Hence, the developed method was used for the simultaneous determination of metformin and teneligliptin in pure and pharmaceutical dosage forms.

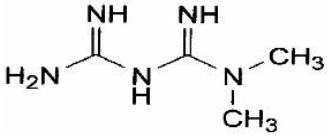

Fig. 1: Structure of metformin [1]

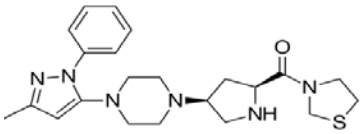

Fig. 2: Structure of teneligliptin [2]

MATERIALS AND METHODS

Chemicals and reagents

Metformin and Teneligliptin obtained from Spectrum Research Private limited, [Hyderabad, India]. Orthophosphoric acid purchased from Qualigens fine chemicals limited [Mumbai, India] and acetonitrile [HPLC grade] purchased from Merck chemicals private limited [Mumbai, India].

Instruments

The system composed Waters HPLC 2695 equipped with quaternary pumps with PDA detector. The chromatographic separation was done on Discovery [250 mm X 4.6 mm, 5 µm particle size] column. Empower 2 software was used for the data acquisition and integration purpose.

Methods

Chromatographic conditions

The method development for separation of metformin and teneligliptin by using different solvents finally the separation was achieved with a mobile phase 0.1% orthophosphoric acid buffer: acetonitrile [65:35, v/v], pumped at a flow rate is 1 ml/min. The eluent detection was carried out at 260 nm by the observing of PDA detector. The mobile phase was vacuum filtered through a 0.45 µm membrane filter.

Preparation of solutions

Preparation of mobile of buffer

The buffer solution was prepared by dissolving 1 ml orthophosphoric acid in 1000 ml of water.

Preparation of standard solutions

The powder of 50 mg of metformin and 2 mg of teneligliptin were weighed and transferred into a 100 ml of calibrated volumetric flasks, 70 ml of diluent was added and sonicated for 25 min and makeup to the final volume with diluents. 1 ml was pipetted out from above stock solution and trance vertex into 10 ml volumetric flask and made up to 10 ml with diluent.

Preparation of sample solutions

20 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 70 ml of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered.

From the filtered solution 1 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluent.

Method validation

The developed method was validated for system suitability, linearity, precision, limit of detection [LOD], limit of quantitation [LOQ] and accuracy under the guideline of ICH Q2R1.

System suitability

Verifying the system suitability parameters like theoretical plate count, tailing factor, percentage relative standard deviation of the peak and retention time.

Linearity

The range of linearity was evaluated between 125-750 µg/ml for metformin and 5-30 µg/ml and for teneligliptin. The calibration curve was a plot between concentration against corresponding peak area and linearity was estimated by least square method.

Precision

The precision of the developed method was carried out for same concentration level, six determinations were established, both intra-day and inter-day precision were conveyed in terms of percent relative standard deviation [% RSD].

LOD and LOQ

Determination value of the limit of detection and quantification by using the following formulas:

Limit of detection= 3.3 α/S

Limit of quantitation= 10 α/S

Where α is the standard deviation of the y-intercept and S is the slope from linearity plot

Accuracy

The accuracy was estimated by using standard addition method at 50 %, 100 % and 150 % levels. The percentage recovery and percentage relative standard deviations [% RSD] were taken into consideration for examine the accuracy.

Stability indicating studies

Acid hydrolysis

1 ml stock solution of metformin and teneligliptin, 1 ml of 2N hydrochloric acid was added and refluxed for 30 min at 60 °C. The solution was diluted to obtain 500 µg/ml for metformin and 20 µg/ml for teneligliptin. 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Base hydrolysis

1 ml stock solution of metformin and teneligliptin, 1 ml of 2N sodium hydroxide was added and refluxed for 30 min at 60 °C. The solution was diluted to obtain 500 µg/ml for metformin and 20 µg/ml for teneligliptin. 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Peroxide hydrolysis

1 ml stock solution of metformin and teneligliptin, 1 ml of 20% hydrogen peroxide was added and refluxed for 30 min at 60 °C. The solution was diluted to obtain 500 µg/ml for metformin and 20 µg/ml for teneligliptin. 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Thermal hydrolysis

1 ml stock solution of metformin and teneligliptin, placed in an oven at 105 °C for 6 h. The solution was diluted to obtain 500 µg/ml for metformin and 20 µg/ml for teneligliptin. 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo hydrolysis

Exposing the 5000 µg/ml for metformin and 200 µg/ml for teneligliptin solution to UV Light by keeping the beaker in UV chamber for 7 d or 200 Watt-hours/m^2in photostability chamber. The resultant solution was diluted to obtain 500 µg/ml for metformin and 20 µg/ml for teneligliptin solutions. 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Neutral hydrolysis

Refluxing the drug solutions in water for 6 h at a temperature at 60 °C. The solution was diluted to obtain 500 µg/ml for metformin and 20 µg/ml for teneligliptin. 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

Method development

Optimization of mobile phase

Method development for the simultaneous estimation of metformin and teneligliptin was begins with a different combination of solvents with different ratios like [35:65, 45:55, and 50:50]. Although, finally a combination of 0.1% orthophosphoric acid buffer: acetonitrile [65:35, v/v] has appeared good resolution for metformin and teneligliptin.

Chromatographic conditions

The analytical conditions were selected, keeping in mind the chemical nature of metformin and teneligliptin. The development trails were taken using different conditions. The column selection has been done on the basis of back pressure, peak shape and theoretical plates. After evaluating all these factors, the chromatographic separation was carried out on Discovery column [250 mm X 4.6 mm; 5 µm is particle size] using a mobile phase consisting 0.1% orthophosphoric acid buffer: acetonitrile [65:35 v/v], the flow rate 1 ml/min and the injection volume were 10 µl, the detection was carried out at 260 nm. The peak retention time of metformin and teneligliptin were found to be 2.517 min and 3.687 min respectively. Hence this method was finalised as an optimized method for the simultaneous estimation of metformin and teneligliptin. The optimised chromatographic condition was shown in table 1 and the typical HPLC chromatogram of standard and sample were shown in fig. 3 and 4.

Method validation

System suitability

The developed method has produced theoretical plate above 2000 for metformin and teneligliptin with tailing factor less than 2. Similarly, the percent relative standard deviation [% RSD] of metformin and teneligliptin were less than 2, which ensure the

suitability of the developed method. The results of the system suitability study were summarised in table 2.

Acceptance criteria

1. The relative standard deviation of six replicate injections for peak area should not be more than 2.0%.

2. The tailing factor should not be more than 2.

3. The theoretical plates should not be less than 2000.

Linearity

For linearity of six point's calibration curve were obtained in concentration ranges from 125-750 µg/ml for metformin and 5-30 µg/ml for teneligliptin.

The response of the drug was found to be linear in the selected concentration range the correlation coefficient for metformin and teneligliptin were 0.9993 and 0.9991 respectively. The results of linearity of v metformin and teneligliptin were summarised in table 3.

Table 1: Optimised chromatographic condition for the estimation of metformin and teneligliptin

Parameter	Condition
Mobile phase	0.1 % ortho phosphoric acid buffer: acetonitrile (65:35, v/v)
Diluent	Water: acetonitrile
Column	Discovery (250 mm X 4.6 mm, 5µm is particle size)
Column temperature	30 °C
Detection wavelength	260 nm
Injection volume	10 µl
Flow rate	1 ml/min
Run time	6 min

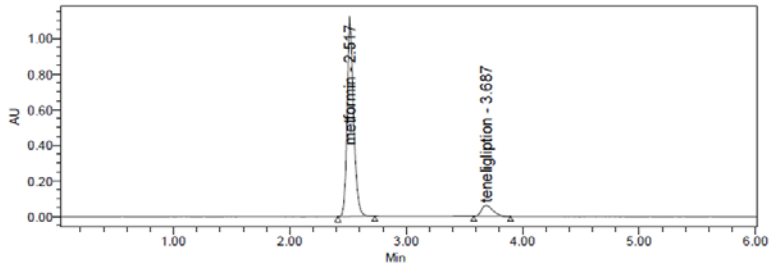

Fig. 3: Typical HPLC chromatogram of standard

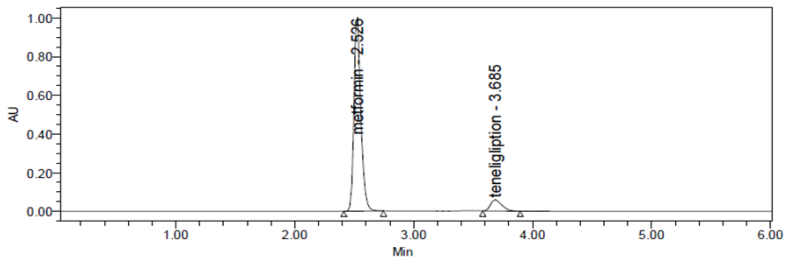

Fig. 4: Typical HPLC chromatogram of a sample

Table 2: System suitability of the developed method

Parameters	Metformin	Teneligliptin	Acceptance criteria
Retention time	2.517	3.687	…….
Theoretical plates	9788	6734	>2000
Tailing factor	1.22	1.40	<2
Asymmetry factor	1.68	1.75	>1<10
Resolution	8.0	8.0	>2

Table 3: Linearity and range of the developed method

S. NO	Metformin		Teneligliptin	
	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
1	125	1092284	5	106601
2	250	2040782	10	205080
3	375	3006306	15	294146
4	500	4217649	20	380439
5	625	5222174	25	473114
6	750	6198935	30	569050
Slope		8295.3		18628
Y-intercept		434.31		9539.8
Correlation coefficient		0.9993		0.9991

n is the number of experiments (n=6)

Precision

The developed method has shown percent relative standard deviation [% RSD] less than 2 for both intra-day and inter-day precision study, which ensures precision of the developed method. The results of the precision study were summarised in table 4 and 5.

Limit of detection and limit of quantification

Limit of detection [LOD] and limit of quantification [LOQ] was estimated from the standard deviation of the y-intercepts and slope of the calibration curve of metformin and teneligliptin. The LOD and LOQ were found to be 0.02 and 0.07 µg/ml for metformin and 0.19 and 0.56 µg/ml for teneligliptin. This showed that the developed method can detect and quantify at lower concentration was highly sensitive whereas other methods is less sensitive.

Accuracy

The percentage recovery of the spiked sample was within 99±2% which ensures the accuracy of the developed method. The results of recovery studies were summarised in table 6 and 7.

Table 4: Intra-day and inter-day precision of the developed method for metformin

S. No.	Intra-day precision	Inter-day precision
Assay-1	4262777	4202650
Assay-2	4201714	4180943
Assay-3	4223667	4207508
Assay-4	4241537	4219171
Assay-5	4280186	4204943
Assay-6	4283314	4204943
Mean	4248866	4203360
S. D	32475	12451.8
%RSD	0.8	0.3

SD: standard deviation, RSD%: relative standard deviation, n = number of experiments.

Table 5: Intra-day and inter-day precision of the developed method for teneligliptin

S. No.	Intra-day precision	Inter-day precision
Assay-1	389369	333029
Assay-2	384696	334982
Assay-3	384297	335332
Assay-4	389597	336546
Assay-5	389035	335049
Assay-6	384171	334049
Mean	386861	334998
S. D	2720	1130.5
%RSD	0.7	0.3

SD: standard deviation, RSD%: relative standard deviation, n = number of experiments.

Table 6: Accuracy of the developed method for met for min

Drug name	Level of addition (%)	Amount added (mg)	Drug found (mg/ml)	% recovery	Average % recovery
Metformin	50	250	249.83	98.73	
	50	250	245.96	98.38	
	50	250	245.98	98.39	
	100	500	491.39	98.28	98.78±0.9
	100	500	497.25	99.45	
	100	500	490.65	98.13	
	150	750	736.86	98.25	
	150	750	744.38	99.25	
	150	750	750.78	100.10	

Table 7: Accuracy of the developed method for teneligliptin

Drug name	Level of addition (%)	Amount added (mg)	Drug found (mg/ml)	% recovery	Average % recovery
Teneligliptin	50	10	9.94	99.45	
	50	10	9.87	98.73	
	50	10	9.91	99.15	
	100	20	19.75	98.79	99.46±0.89
	100	20	20.10	100.51	
	100	20	20.04	100.20	
	150	30	29.82	99.42	
	150	30	29.42	98.09	
	150	30	30.22	10077	

Stability-indicating studies

Stability indicating studies were carried under a condition of acid/base/neutral hydrolysis, oxidation, dry heat and photolysis. For each study, samples were prepared. The blank subjected to stress in the same manner for the drug solution, working standard solution of metformin and teneligliptin subjected to stress degradation. Dry heat and photolytic degradation were carried out in a solid state.

The concentration of degrading reagent and time of exposure was optimised to degradation within the range of 10%. During optimisation of degradation conditions, if the higher percentage of degradation was observed, milder conditions were used for the lesser duration of exposure. Although percent assay reduced under all conditions; the separate peak for degradation product was observed only under acid and alkali conditions fig 5 and 6. Summary of stress degradation results is given in table 9 and 10.

In order to develop a suitable RP-HPLC method for the estimation of metformin and teneligliptin, different buffer ratios at different flow rate were applied. Some of the reported methods were costly due to the use of expensive solvents and it was replaced by buffer and acetonitrile in this study. The LOD and LOQ were found to be 0.02 and 0.07 µg/ml for metformin and 0.19 and 0.56 µg/ml for teneligliptin which indicates that the method was sensitive, and can detect and quantify at lower levels metformin and teneligliptin. Linearity range was from 125-750 µg/ml for metformin and 5-30 µg/ml for teneligliptin. The response of the drug was found to be linear in the selected concentration range the correlation coefficient for metformin.

The LOD and LOQ were found to be 0.02 and 0.07 µg/ml for metformin and 0.19 and 0.56 µg/ml were 0.9993 and 0.9991 for teneligliptin, respectively. Which indicates that at this concentration range both were highly linear. Present assay the amount of both the drugs recovered was found to be 98.78% for metformin and 99.46% for teneligliptin. The developed RP-HPLC stability indicating assay method was found to be appropriate for the analysis of drug in their pharmaceutical dosage form.

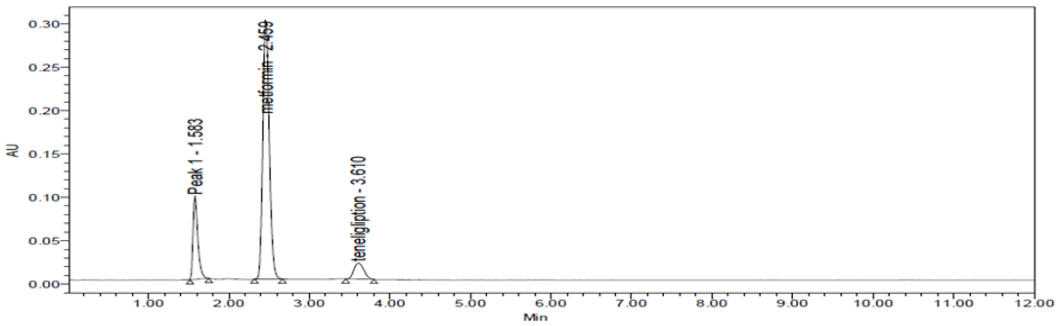

Fig. 5: Chromatogram of acid hydrolysis

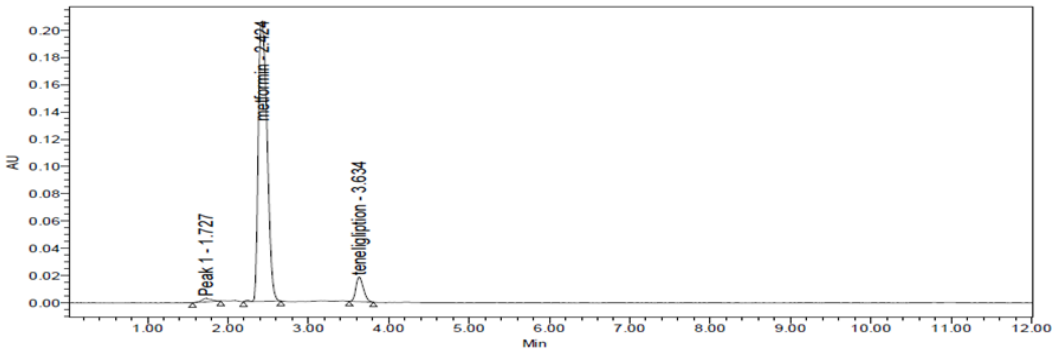

Fig. 6: Chromatogram of alkali hydrolysis

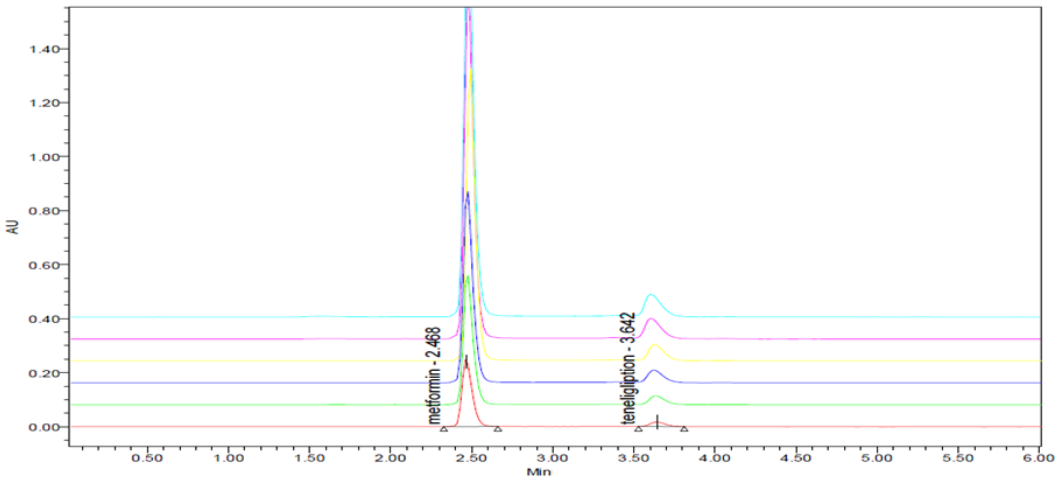

Fig. 7: Overlay of linearity chromatograms

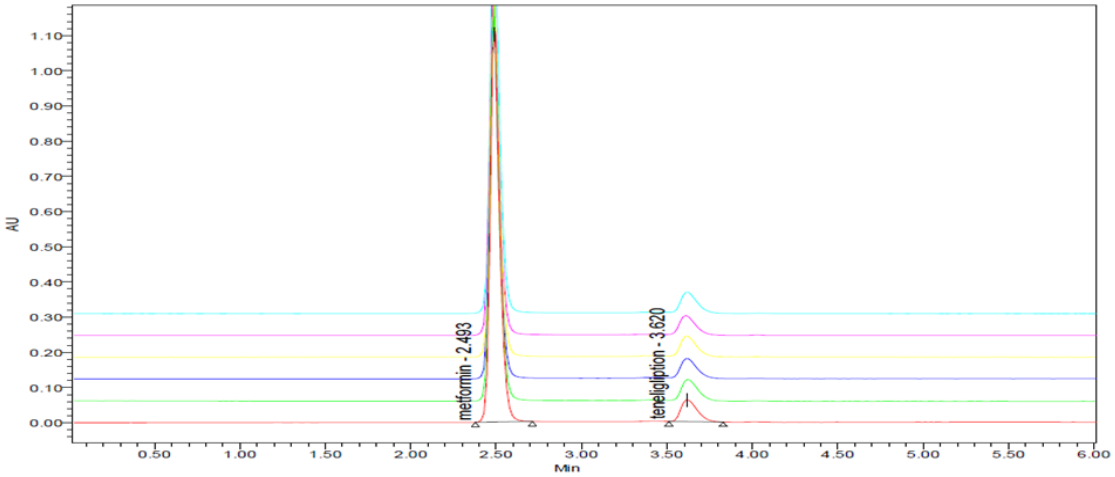

Fig. 8: Overlay of precision chromatograms

Table 9: Stability-indicating data of met for min

Degradation parameter	Peak area of sample	Peak area of standard	% recovery	% degradation
Acid degradation	4059092	4267272	95.03	4.97
Alkali degradation	4155479	4267272	97.28	2.72
Oxidative degradation	4191118	4267272	98.12	1.88
Dry heat degradation	4248957	4267272	99.47	0.53
Photo stability degradation	4247378	4267272	99.43	0.57
Neutral degradation	4236053	4267272	99.90	0.10

Table 10: Stability-indicating data of teneligliptin

Degradation Parameter	Peak area of sample	Peak area of standard	% recovery	% degradation
Acid degradation	366712	385565	95.02	4.98
Alkali degradation	375116	385565	97.19	2.81
Oxidative degradation	379152	385565	98.24	1.76
Dry heat degradation	382367	365565	99.07	0.93
Photo stability degradation	383633	385565	99.40	0.60
Neutral degradation	382781	385565	99.18	0.82

CONCLUSION

In pharmaceutical industry settings, recent studies that quantify two drugs reported a simple, sensitive and more precise spectrophotometric method with UV-Visible [4-6]. Another study quantify more than on drug, used PDA as analyte detector [7-9]. In this study, we used PDA detector to prove the selectivity of the method. Another study was reported for teneligliptin in human plasma and its application to a pharmacokinetic study by LC-MS-MS [10]. In this method to identify a wide range of linearity, recovery, rapid extraction and shorter run time. It concludes that the present method can be useful for pharmacokinetic/bioequivalence studies with desired precision and accuracy. The newly developed RP-HPLC method for simultaneous determination of metformin and teneligliptin in pure and in the pharmaceutical formulation was found to be simple, sensitive, rapid, precise and accurate. The proposed method was completely validated as per ICH guidelines. The method validation data showing satisfactory results for all the method parameters tested. The stability-indicating nature of the proposed method was established by performing forced degradation, which provided degradation behaviour of metformin and teneligliptin under various conditions. Hence the developed RP-HPLC method is stability-indicating and can be used for routine analysis of production samples and also to check the stability of bulk samples of metformin and teneligliptin.

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CONFLICT OF INTERESTS

Declared none

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