Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Teneligliptin and Metformin

Rajani Vetapalem, Rajendra Prasad Yejella, Lakshmana Rao Atmakuri

¹Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, AP, India

²Department of Pharmaceutical Chemistry, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, AP, India

³Department of Pharmaceutical Analysis, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, AP, India

INTRODUCTION: The main objective of the present work was to develop a simple, precise, specific & stability indicating RP-HPLC method for simultaneous estimation of Teneligliptin and Metformin in bulk and tablet dosage form.

METHODS: The analysis has been performed using Kromasil C18 column (250 x 4.6 mm, 5 μ) at 30°C using buffer: acetonitrile: methanol (65: 25: 10, v/v/v) as mobile phase. The detection was carried out at 254 nm with a flow rate of 1.0 ml/min.

RESULTS: The retention time of Teneligliptin and Metformin was found to be 2.842 min & 2.017 min respectively. The linearity range was 5-30 μ g/ml for Teneligliptin and 125-750 μ g/ml for Metformin respectively. The forced degradation studies were performed as per the guidelines of ICH under acidic, alkaline, oxidative, thermal, photo stability & neutral conditions.

DISCUSSION AND CONCLUSION: The developed method was successfully validated for all the parameters and was found to be within the limits. The developed method could be successfully employed for the simultaneous estimation of Teneligliptin and Metformin in bulk and tablet dosage form.

Keywords: Teneligliptin, Metformin, RP-HPLC, Validation, Stability studies.

INTRODUCTION

Teneligliptin (TEN) (Fig. 1) is chemically [(2S,4S)-4-[4-(5-methyl-2-phenylpyrazol-3-yl)piperazin-1-yl]pyrrolidin-2-yl]-(1,3-thiazolidin-3-yl)methanone. It is highly effective in lowering blood glucose levels. This drug inhibits the enzyme dipeptidyl peptidase-4 (DPP4) which degrades incretin, a hormone adjusting blood glucose control. It is effectively used to treat type-II diabetes mellitus¹⁻².

Metformin (MET) (Fig. 2) is chemically 1-carbamimidamido-N,N-dimethylmethanimidamide. It belongs to biguanide class of anti diabetic drugs. It is the first line drug of choice for the treatment of type-II diabetes. It activates AMP-activated protein kinase (AMPK), a liver enzyme

that plays an important role in insulin signaling, whole body energy balance and metabolism of glucose and fats³⁻⁵.

Literature survey reveals good number of analytical methods for the estimation of TEN and MET individually or in combination with other drugs using UV spectrophotometry⁶⁻⁸, HPLC⁹⁻¹⁹, HPTLC²⁰ and LC-MS/MS²¹. Moreover, few methods were reported for the estimation of the selected drugs in their combinations using UV spectrophotometry²²⁻²⁴, HPLC²⁵⁻²⁹. As per the knowledge of the authors, no stability indicating RP-HPLC method was reported so far for the simultaneous estimation of the Teneligliptin and Metformin. Hence, we tried to develop a simple stability indicating HPLC method for the estimation of selected drugs. The developed method has been validated as per the guidelines of ICH³⁰. To establish stability indicating nature of the method forced degradation studies were planned for the proposed method under acidic, alkaline, oxidative, thermal, photo stability and neutral conditions³¹.

EXPERIMENTAL

Materials & reagents

Reference standards of Teneligliptin and Metformin were provided as gift samples by Spectrum Labs, Hyderabad. Commercially available tablet formulation Tendia M tablets for the assay studies were purchased from local pharmacy. HPLC grade methanol, HPLC grade acetonitrile, analytical grade ortho phosphoric acid and HPLC grade water were purchased from Merck specialties, Mumbai, India.

Instrumentation

Development and validation of the method was performed on waters HPLC 2695 system equipped with quaternary pumps, auto sampler & photo diode array detector. Empower 2 software was applied for data collection and processing.

Methodology

Preparation of standard stock solutions

The standard stock solutions of 200 µg/ml for TEN and 5000 µg/ml for MET were prepared by accurately weighing and transferring 2 mg of TEN and 50 mg of MET into 10 ml volumetric flasks. Added about three fourth of the volume with diluent, sonicated for 10 minutes. Finally, flasks were made up to the mark with diluent to obtain the mentioned concentrations. One ml of the above solution was pipetted out and transferred into a 10 ml volumetric flask and diluted up to the mark with diluent to obtain a concentration of 20 µg/ml for TEN and 500 µg/ml for MET.

Preparation of sample solution

Twenty tablets were weighed and average weight was calculated. Then they were powdered using mortar and pestle and the powder equivalent to 20 mg of TEN and 500 mg of MET was accurately weighed and transferred in to a 100 ml volumetric flask. 50 ml of diluent was added and sonicated for 25 min. Further the volume was made up with diluent to obtain a concentration of 200 µg/ml for TEN and 5000 µg/ml for MET. Filters of 0.45 micron size were employed for filtration purpose in the mentioned procedure. One ml of the above solution was pipette out and transferred into a 10 ml volumetric flask and diluted up to the mark with diluents to obtain a concentration of 20 µg/ml for TEN and 500 µg/ml for MET.

Preparation of buffer

One ml of ortho phosphoric acid was diluted to 100 ml with HPLC grade water to obtain 0.1% ortho phosphoric acid buffer.

Mobile phase

Buffer, acetonitrile and methanol were taken in the ratio of 65:25:10, v/v/v and was used as mobile phase.

Method Validation

Method validation was done as per the guidelines of ICH^{30, 31}.

System suitability

System performance parameters like retention time, number of theoretical plates, tailing factor, resolution were calculated by injecting standard solutions for six times. The resultant results were compared with the standard limits as per guidelines.

Specificity

It is the ability of a method to discriminate between the analyte of interest and other components that are present in the sample. These studies are to check the interferences in the optimized method. To assess the method specificity, blank and placebo were injected into HPLC system under optimized conditions. There should not be any interfering peak in the blank or placebo chromatograms at the retention times of the selected drugs.

Linearity

The linearity of the method was obtained by preparation of the calibration standards of six different concentrations in 6 replicates. The calibration curve plots for TEN & MET were obtained by plotting the peaks areas on y-axis and concentrations on x-axis over the concentration ranges of 5-30 µg/ml for TEN and 125-750 µg/ml for MET. The correlation coefficient should be greater than 0.99.

Accuracy

The accuracy of the method was assessed by recovery experiments by adding a known quantity of pure standard drug into the sample solution and recovering the same in terms of its peak areas. The sample was spiked with standard at levels of 50%, 100% and 150% of test concentrations.

The resultant spiked sample was assayed in triplicate. The %recovery for each level should be in between 98%-102%.

Precision

It is the degree of closeness of agreement between the series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. It is expressed in terms of standard deviation (SD) or relative standard deviation (RSD). Precision may be measure of either degree of repeatability or reproducibility of the analytical method.

Method precision: Sample solutions were injected under optimized conditions for six times on six different days and their peak areas were recorded. %RSD for the peak areas of 6 standard injection results should not be greater than 2.

Intermediate precision: Six replicates of sample solutions were injected under optimized conditions on the same day and their peak areas were recorded. %RSD for the peak areas of 6 replicate injection results should not be greater than 2.

Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments, by different operators and using different columns of similar types.

Robustness

The robustness of the method was determined by making small deliberate changes in the method like flow rate, mobile phase ratio & temperature. But, one should not find remarkable change in the results and the obtained results should be within range as per ICH guidelines.

Effect of variation of flow: Sample was analyzed at 0.9 ml/min & 1.1 ml/min flow rate instead of 1.0 ml/min, remaining conditions are kept constant.

Effect of variation of temperature: Temperature of 25°C and 35°C was maintained instead of 30°C. Samples were injected in triplicate & chromatograms were recorded.

LOD & LOQ

LOD is the smallest concentration that can be detected but not necessarily be quantified as an exact value. It is calculated using formula

$$\text{LOD} = 3.3 \sigma/S; \text{ where, } \sigma = \text{S.D}; S = \text{Slope}$$

LOQ is the lowest amount of analyte in the sample that can be quantitatively determined with precision & accuracy.

$$\text{LOQ} = 10 \sigma/S; \text{ where, } \sigma = \text{S.D}; S = \text{Slope}$$

Forced Degradation Studies

TEN & MET standard samples were subjected to degradation under different stress conditions like acidic, alkali, oxidative, thermal, photo stability and neutral conditions.

For acidic & alkali degradation samples were refluxed with 2N HCl & 2N NaOH at 60°C for 30 min. For oxidative degradation 20% v/v, H₂O₂ was used and the same was refluxed at 60°C for 30 min. For thermal degradation, sample was placed in oven at 105°C for 6 hr; and for photo stability degradation, drug was exposed to UV light by keeping the sample in UV chamber for 7 days or 200 watt hours/m² in photo stability chamber; for neutral degradation, the drugs were refluxed in water for 6 hours at a temperature of 60°C. All the samples were diluted to obtain a final concentration of 20 µg/ml of TEN & 500 µg/ml of MET. Ten micro liters of the samples were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Solution Stability

The stability of the drug solution was determined for the short term stability, auto sampler stability. Short term stability was carried out by keeping the samples, at room temp (25°C) for 24 hrs. Auto sampler stability was determined by storing the samples for 24 hrs in the auto sampler. Each sample was injected 6 times into HPLC & the results obtained were compared with nominal values of QC samples.

RESULTS

The results of optimized chromatographic conditions were shown in Table 1. System suitability parameters (tailing factor, retention time & theoretical plates) were found to be within acceptance criteria. Summary of system suitability parameters were tabulated in Table 2. We did not find any interfering peaks at the retention times of TEN & MET (Fig. 3), which declares that the method is specific. The quantification was linear in the concentration range of 5-30 µg/ml for TEN with a correlation coefficient 0.999 (Fig. 4); 125-750 µg/ml for MET with a correlation coefficient 0.999 (Fig. 5) respectively. The results of linearity were tabulated in Table 3. The recoveries of TEN and MET were found to be in the range of 99.35-99.94% and 99.80-100.61% respectively. The results were compared with the guidelines & expressed as percentages and the results were given in Table 4. The precision of the method is satisfactory as %RSD is NMT 2%. The ruggedness was performed by different analyst & on different days. The results were given in Table 5. No remarkable changes in the results were notice in robustness studies and hence the method is robust. The results were tabulated in Table 6. The assay results were compared with the labeled claim of TEN & MET marketed formulation and the results were tabulated in Table 7. The LOD & LOQ values were calculated using slope and standard deviation values and the same were tabulated in Table 8.

Forced degradation study

The standard solutions were subjected to different stress conditions as mentioned in the procedure. Under acidic conditions, drugs showed degradation of about 3.66% for TEN & 3.14% for MET and we have noticed about 3 degradation peaks (Fig. 6). Under alkali conditions, drugs showed degradation of about 2.75% for TEN & 2.67% for MET and 2 degradation peaks were noticed (Fig. 7). Under oxidative conditions, drugs showed degradation of about 1.01% for TEN & 1.62% for MET and 1 degradation peak was noticed (Fig. 8). Under remaining conditions, thermal, photo stability and neutral conditions, the degradation was less than 1% for both the drugs and no degradant peak was noticed (Fig. 9-11). Result of forced degradation studies are tabulated in Table 9.

Stability studies

The drug solutions were found to be stable for 24 hrs at 25°C for short term stability and 24 hrs for auto sampler stability.

DISCUSSION

For method optimization different ratios of acetonitrile & buffer were tried but, resolution of peak was not achieved. Hence, methanol was used in mobile phase. Different ratios of ortho phosphoric acid buffer, methanol were tried i.e., 65:15:20, v/v/v, 60:20:20, v/v/v, 65:20:15, v/v/v. Finally, it was found that buffer:acetonitrile:methanol in the ratio of 65:25:10, v/v/v, gave good peaks and hence fixed as mobile phase. Kromasil C18 (250 X 4.6 mm, 5 µm) column, 1 ml/min flow rate, 10 µl injection volume, 30°C column oven temperature, 254 nm wave length were fixed as optimized conditions which were found to be suitable for the separation of peaks. These optimized conditions gave a retention time of 2.842 min and 2.017 min for TEN & MET. All the validation results were as per the limits of ICH and hence, found to be a reliable and economical method for the estimation of drugs. The effectiveness of the method to separate the

degraded peaks from analyte shows its stability indicating nature. The degradation on the lower side i.e., the degradation percent under all conditions is in the range of 0.05% to 3.66% shows the stability of the selected drugs. The %RSD values were found to be less than 2.

CONCLUSION

The developed method possesses all the qualities to be a reliable, rapid, sensitive, specific and economical method from the above discussed results and data. The study showed stability indicating nature of the method with the possible short runtime. Hence, the developed method could be conveniently and effectively used for routine simultaneous estimation of Tenilgliptin and Metformin in bulk and pharmaceutical dosage form.

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Table 1: Optimized chromatographic conditions

Parameter	Condition
Column	Kromasil C18 (250 X 4.6 mm, 5 μ)
Mobile phase	Buffer:acetonitrile:methanol (65:25:10, v/v/v)
Diluent	Acetonitrile:water (50:50, v/v)
Column temperature	30°C
Wavelength	254 nm
Flow rate	1 ml/min
Run time	6 min
Injection volume	10 μ l

Table 2: Summary of system suitability parameters

Parameter	TEN	MET	Acceptance criteria
Tailing factor	1.30	1.06	≤ 2
Retention time	2.842	2.017	≥ 2
Theoretical plates	4463	6783	≥ 2000
%RSD of area	0.72	1.08	≤ 2

Table 3: Linearity values of TEN and MET

Parameter	TEN	MET
Linearity range (μ g/ml)	5-30	125-750
Regression coefficient $\pm$ SD	0.999 $\pm$ 0.0003	0.999 $\pm$ 0.0005
slope $\pm$ SD	8891 $\pm$ 4.358	4665 $\pm$ 8.386
Intercept $\pm$ SD	1773 $\pm$ 58.66	35915 $\pm$ 2654.363

Table 4: Recovery values of TEN and MET

Drug	Level	Analyte	Recovery	Mean %	%RSD
		amount (mg)	amount (mg)	recovery	
TEN	50%	10	9.94	99.43	0.20
	100%	20	19.98	99.91	0.60
	150%	30	29.97	99.92	0.67
MET	50%	250	249.50	99.80	0.90
	100%	500	503.07	100.61	0.40
	150%	750	752.82	100.37	0.50

Table 5: Ruggedness values of TEN and MET

Drug	Analysts-1	Analyst-2	SD	%RSD
	(Peak area)*	(Peak area)*		
TEN	172792	173167	1248	0.72
MET	2363854	2320575	12026	0.50

*Average of six readings

Table 6: Robustness values of TEN and MET

Condition	TEN			MET		
	Retention	Area	Tailing	Retention	Area	Tailing
	time		factor	time		factor
Initial conditions	2.837	268209	1.14	2.027	3521349	0.98
More flow(+0.1 ml/min)	2.827	269207	1.13	2.012	3540846	0.95
Less flow(-0.1 ml/min)	2.851	267902	1.10	2.041	3536801	1.00
At 35°C	2.841	267189	1.13	2.028	3520932	0.99
At 25°C	2.840	269218	1.09	2.029	3519867	1.11

Table 7: Assay results of marketed formulation (Tendia M tablets)

Drug	Label claim	Amount found	%Assay
TEN	20 mg	19.98 mg	99.90
MET	500 mg	498.85 mg	99.77

Table 8: LOD & LOQ values of TEN & MET

Drug	LOD (µg/ml)	LOQ (µg/ml)
TEN	0.02	0.07
MET	1.88	5.69

Table 9: Forced degradation data*($\pm$ SD) of the method

Stress condition	Amount of TEN degraded (%)	Amount of TEN recovered (%)	Amount of MET degraded (%)	Amount of MET recovered (%)
Acidic	3.67 $\pm$ 0.89	96.33 $\pm$ 3.12	3.15 $\pm$ 0.62	96.85 $\pm$ 2.19
Alkali	2.76 $\pm$ 0.58	97.24 $\pm$ 2.98	2.68 $\pm$ 0.85	97.32 $\pm$ 2.81
Oxidative	1.02 $\pm$ 0.69	98.98 $\pm$ 1.98	1.63 $\pm$ 0.98	98.37 $\pm$ 2.06
Thermal	0.75 $\pm$ 0.08	99.25 $\pm$ 1.87	0.62 $\pm$ 0.10	99.38 $\pm$ 0.91
Photo stability	0.63 $\pm$ 0.06	99.37 $\pm$ 1.39	0.59 $\pm$ 0.09	99.41 $\pm$ 1.92
Neutral	0.53 $\pm$ 0.08	99.47 $\pm$ 2.01	0.05 $\pm$ 0.08	99.95 $\pm$ 1.79

*Average of three determinations (each condition); SD;Standard deviation

Figure 1: Chemical structure of TEN

Figure 2: Chemical structure of MET

Figure 3: Chromatogram showing resolved peaks of TEN and MET

Figure 4: Linearity plot of TEN

Figure 5: Linearity plot of MET

Figure 6: Chromatogram showing degraded peaks under acidic conditions

Figure 7: Chromatogram showing degraded peaks under alkali conditions

Figure 8: Chromatogram showing degraded peaks under oxidative conditions

Figure 9: Chromatogram showing degraded peaks under thermal conditions

Figure 10: Chromatogram showing degraded peaks under photo stability conditions

Figure 11: Chromatogram showing degraded peaks under neutral conditions