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Analytical Method Development and Validation Studies of Anti Diabetic Drugs by HPLC.

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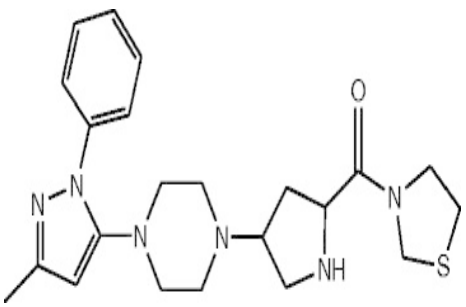
ABSTRACT:

A simple, sensitive and high performance liquid chromatographic method was developed for the estimation of anti-diabetic drugs Metformin Hydrochloride (MET) and Teneligliptin (TEN) in pure and in pharmaceutical dosage forms. A Gemini C18 column (150x4.6mm, 5µ) was used with a mobile phase contain a mixture of Ammonium Acetate Buffer (pH-3) and Acetonitrile taking ratio of 42: 58. Set the flow rate was 0.3ml/min and effluents were monitored at 255nm and eluted at 5.18min (MET) and 8.1min (TEN). Calibration curve was plotted within range from 0.5-51 µg/ml for MET and 0.3-31 µg/ml for TEN. This assay was validated for the parameters i.e. accuracy, precision, robustness and all system suitability parameter. The method can be useful routine analysis for the determination on metformin and teneligliptin in pharmaceutical dosage forms.

KEYWORDS: - Metformin hydrochloride, Teneligliptin, HPLC, Pharmaceutical dosage forms.

INTRODUCTION:

Metformin (I, *N*, *N*-dimethyldiguanide) and Teneligliptin,1-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-4-[(3S,5S)-5-(1-3-thiazolidine-3-carbonyl) pyrrolidin-3-yl) piperazine are used in the treatment of type 2 diabetes. Structurally, Teneligliptin (I) and Metformin hydrochloride (II)



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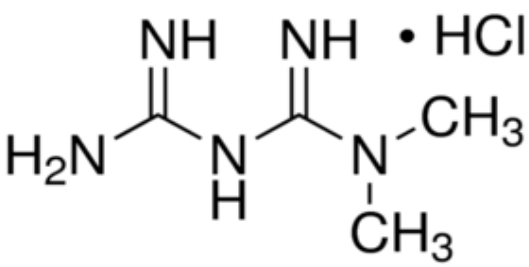

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Figure 1 – Structure of Teneligliptin and Metformin

Metformin improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis whereas Teneligliptin has been shown to affect abnormal glucose and lipid metabolism associated with the insulin resistance by enhancing insulin action on body peripheral tissues. Many people suffering from type 2 diabetes require treatment with more than one antidiabetic drug to achieve glycemic control. Some methods available for study of metformin and teneligliptin i.e. UV, HPLC, Stability studies and Potentiometry. For metformin hydrochloride and teneligliptin have been reported is not cost-effective in concerns of run time and consumption of solvent.

Thus, the current study was performed. The goal of current investigation was HPLC for validation analysis of teneligliptin and metformin hydrochloride.

MATERIALS AND METHODS:

Reagents-

Metformin and Teneligliptin were obtained from Macleoids Pharmaceuticals Ltd., Mumbai, India. Acetonitrile and Methanol (HPLC grade, MERCK), water (Milli Q). Other reagents were of AR grade

Instrumentation-

The HPLC system consisted of a Shimadzu Class LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-Visible detector. The data acquisition was performed by Spinco Winchrome software.

Chromatographic conditions-

The HPLC system consisted of Shimadzu Class LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV detector. The data acquisition was performed by Spincotech software. Analysis was carried out at 255nm using a phenomenex C18 reverse phase column of 150x 4.6mm, 5µm dimensions at ambient temperature. The mobile phase consists of Ammonium acetate buffer (pH 3): Acetonitrile in the ration of (42: 58, v/v) that was set at a flow rate 0.3ml/min.

Preparation of stock and sample solutions-

The standard stock solutions were prepared by using methanol make concentration of 1000 µg/ml. The working standard solutions of MET and TEN were prepared by taking suitable aliquots of drug solution from the standard solutions and the volume was made up to 10 ml with mobile phase to get concentrations of 0.5-51 µg/ml for MET and 0.3-31 µg/ml for TEN. A mixed standard solution was prepared by 0.2 ml of each from the stock (1000 µg/ml) into 10 ml volumetric flask and made up the volume with mobile phase to get 20 µg/ml each solution. For analysis of pharmaceutical dosage forms, 10 tablets were weighed and powdered. A quantity equivalent to one tablet containing 500 mg of Metformin HCl and 30 mg of Teneligliptin was transferred into extraction flask. Then suitable amount of methanol was added and mixture was subjected to sonicators for 5-10 min for complete extraction of drugs, and then centrifuged at 5000 rpm for 20 min (Remi R8C laboratory centrifuge). Supernatant was collected from each prepare set and diluted with mobile phase and injected to HPLC system for the analysis.

RESULTS AND DISCUSSION

Preparation of stock solution for Metformin hydrochloride

An accurately weighed quantity of MET HCl (10mg) was transferred in to 100ml volumetric flask, 10-15ml of distilled water was added and sonicated for 5 min and diluted up to the mark 100ml with distilled water.

Preparation of stock solution for Teneligliptin

An accurately weighed quantity of TENE (10mg) was transferred in to 100ml volumetric flask, 10-15ml of distilled water was added and sonicated for 5 min and diluted up to the mark 100ml with distilled water.

Preparation of working standard solution for Metformin hydrochloride

From standard stock solution(100µg/ml) of MET HCl (0.6, 0.8, 1.0, 1.2, 1.4, 1.6) were taken and transferred in 10ml volumetric flask and make up the volume with distilled water, which gives (6, 8, 10, 12, 14, 16) µg/ml. Further absorbance of above prepared solutions were measured.

Preparation of working standard solution for Teneligliptin hydro bromide hydrate

From standard stock solution (100µg/ml) of TENE (0.6, 0.8, 1.0, 1.2, 1.4, 1.6) were taken and transferred in 10ml volumetric flask and make up the volume with distilled water, which gives (6, 8, 10, 12, 14, 16) µg/ml. Further absorbances of above prepared solutions were measured

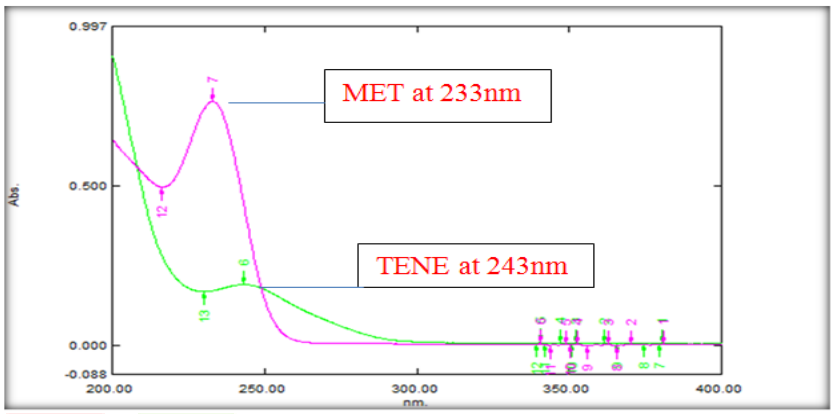

Figure. 2: A Typical Absorption showing the peaks of Metformin and Teneligliptin

A reversed-phase column procedure was is suitable method for the simultaneous determination of metformin and teneligliptin in combined pharmaceutical dosage form. The chromatographic situation was obtained by changing the mobile phase composition, different pH, and buffers used in the mobile phase. A mixture of Acetonitrile and Ammonium Acetate buffer (pH-3) in the ratio of 42:58 was used. A typical chromatogram obtained by using the a for composition mobile phase from 20 µL of the assay preparation is given in Fig. 2. The retention times of MET and TEN.

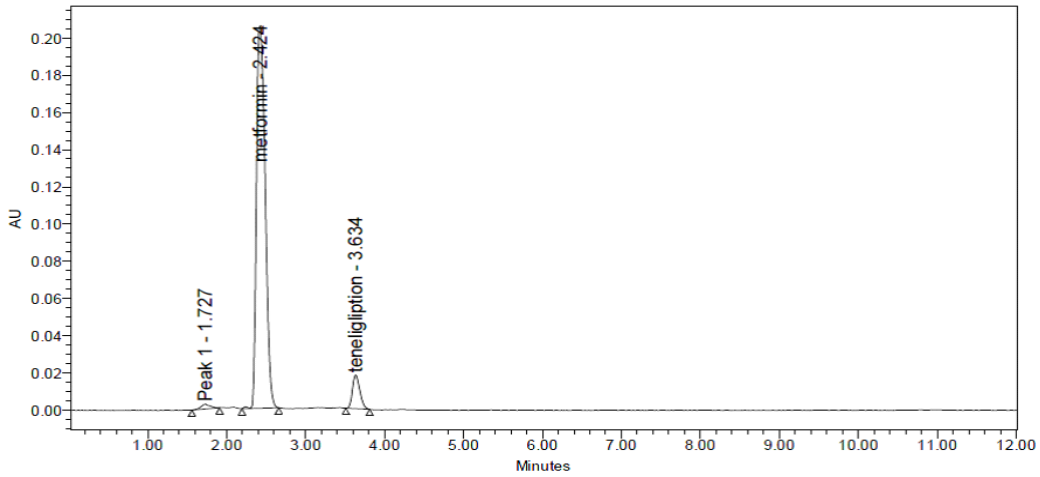

Fig. 2. The retention times of MET and TEN

The linearity method was tested from 0.5- 51 µg/ml for MET and 0.3- 31 µg/ml for TEN. Linearity solutions were injected in triplicate and the calibration graphs were plotted as peak area of the analytic against the concentration of the drug in µg/ml. In the simultaneous determination, the calibration the analytic in the mentioned concentrations and the correlation coefficients for the regression line were 0.9968 and 0.9986 for MET and TEN respectively. The accuracy of this method was studied by using recovery experiments. The recovery experiments were performed by using known amounts of the drugs to the placebo nature. The recovery was determined different three level i.e. 80%, 100%, and 120% of the selected concentrations.

	Pure Drug		Formulation		% Recovery	
Sample ID	**MET**	**TEN**	**MET**	**TEN**	**MET**	**TEN**
80%	40	2.4	50	3	99.22	97.87
100%	50	3.0	50	3	98.25	100.11
120%	60	3.6	50	3	97.80	99.73

Table 1: Recovery of MET and TEN n=3)

Three different samples were prepared for each recovery level. The recovery values of MET and TEN ranged from 98.42-102% and 99.23-103%, respectively (Table 1). The precision (i.e. repeatability and intermediate precision) of the method was determined from one lot of combined pharmaceutical dosage form. The Intra and Inter day studies were performed by taking the six replicates of different three concentrations. The results are shown in (Table 2). The limit of detection (LOD) and limit of quantitation (LOQ) for MET, TEN was 0.003 µg/ml, 0.0061 µg/ml and 0.01 µg/ml, 0.02 µg/ml, respectively. To determine the robustness of the developed method experimental conditions were purposely altered and RSD of the peak areas of MET and TEN were found not greater than 2.0 illustrate the robustness of the method.

Nominal concentrations (µg/ml)		Mean ± S.D, %RSD	
MET	**TEN**	**MET**	**TEN**
25	1.5	24.67± 0.42, 1.75	1.46± 0.019, 1.30
50	3	48.76± 0.95, 1.95	2.89± 0.051, 1.76
100	6	98.35± 1.89, 1.92	5.86± 0.113, 1.93

Each mean value is the result of triplicate analysis for three times a day
% R.S.D= (S.D/mean) x

Table 2: Precision data for MET and TEN

Application of the method to pharmaceutical dosage forms:

The method is sensitive and specific for the quantitative determination of MET and PIO and also subjected to validation for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. Tablets from two different manufacturers (Zita-Met Plus MET 500 mg and TEN 30 mg, Glenmark Pharmaceuticals Ltd. and Teneza M MET 500 mg and TEN 15 mg, Torrent Pharmaceuticals Ltd) were evaluated for the amount of MET and TEN present in the formulations. Each sample was analyzing in triplicate from after extracting the drug as given above in experimental section. The amount of metformin and pioglitazone was found to be within the range of 95%-105%. The none tablet excipients were found to interfere with the analyte peak and the results express shown in Table 3.

	Labeled amount (mg)	Amount (µg) Taken	Found ±S.D	Assay %RSD	%w/w
Zita-Met Plus					
MET	500	500	491.76±6.75	1.37	98.3
TET	30	30	29.79±0.45	1.51	99.2
Teneza M					
MET	500	500	487.32±5.46	1.12	97.5
TET	15	15	28.67±0.31	1.08	95.7

Table 3: Results of the determination of metformin and teneligliptin in Tablets (n=6)

CONCLUSION:

The performed method was found to be easy, precise, accurate and quick for simultaneous determination of Metformin and Teneligliptin from pure and in pharmaceutical dosage forms. The mobile phase preparation is very simple and economical. The drug sample recoveries in experiments formulations were in good agreement with their respectively label claims and they suggested non-interference with formulation different excipients in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Metformin and Teneligliptin in combined dosage forms and can also be used for dissolution or similar studies.

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