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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF REMOGLIFLOZIN ETABONATE AND METFORMIN HYDROCHLORIDE

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Type 2 diabetes mellitus (T2DM) is an emerging epidemic in Asian countries, especially in India. With the advent of the SGLT2 inhibitor class of drugs demonstrating benefits beyond glycemic control, viz. weight loss, blood pressure reduction, and cardiovascular and renal protection, the management of T2DM has taken a quantum leap. Remogliflozin etabonate (REM) is the latest addition to the SGLT2 inhibitor class of drugs and Metformin Hydrochloride(MET), a dimethylbiguanide, is an effective oral antihyperglycemic agent, and this combination have been recently approved in India for the management of T2DM.A RP-HPLC method for simultaneous determination of Remogliflozin etabonate and Metformin Hydrochloride in bulk and formulation has been developed and validated.A RP-HPLC method was performed on Phenomenex Hyperclone 5µm BDS C18 250 ×4.6 mm using PHP(pH 4.5 maintained using 1% OPA) and Acetonitrile, in the ratio of 60:40 as mobile phase at a flow rate of 1ml/min and analytes were monitored at 229 nm. The retention time for Metformin Hydrochloride and Remogliflozin etabonate was found to be 4.239 and 5.657 min respectively with resolution was more than 1.5. The peaks obtained were symmetrical with tailing factor less than 2 and theoretical plates more than 2000.The linearity was found in the concentration range of 0.1-500µg/mL for Remogliflozin etabonate and 0.01-100µg/mL for Metformin Hydrochloride. LOD and LOQ were found to be 0.024 and 0.075µg/mL for Remogliflozin etabonate 0.008 and 0.0096µg/mL Metformin Hydrochloride respectively. The percentage Mean recovery at three different levels (80%,100% and 120%) for Remogliflozin etabonate and Metformin Hydrochloride was found to be 98.40-100.87%w/w and 98.33-102.53%w/w respectively. The % Assay of Remogliflozin etabonate and Metformin Hydrochloride in tablets was found to be 98.66%w/w and 97.54%w/w respectively.

The method was validated in accordance with ICH guidelines and was found to be specific, accurate, precise and robust and can be successfully applied for routine analysis of Remogliflozin etabonate and Metformin Hydrochloride in bulk and formulation (tablet).

Key words: Remogliflozin etabonate (REM), Metformin Hydrochloride (MET), RP- HPLC.

1. Introduction

Market is flooded with fixed dose combination products now a days for the treatment of various diseases. FDC therapies/products have been shown to improve adherence by reducing cost, pill burden and complexity of treatment regimen and complimentary mechanisms are used to maximize the efficacy, decrease toxicity and reduce development of drug resistance of combination therapy. A new marketed combination product **REMO®-ZEN M**[1] in the form of tablets, consisting of Remogliflozin etabonate as SGLT2 inhibitor and Metformin hydrochloride as Oral hypoglycemic agent in a Single pill is available in market for the treatment of diabetes mellitus.

Remogliflozin etabonate[2] is an orally bio available prodrug of remogliflozin hypoglycemic agent, which acts by inhibiting SGLT2, an enzyme accountable for reabsorption of sugar in the kidneys, thereby; increasing the elimination of excess sugars in the urine. Apart from glycemic control, SGLT2

inhibitors posses many beneficial effects that include lowering of body weight, reduction of systolic blood pressure, and lowering hemoglobin A1c levels. Hence, Remogliflozin etabonate is more helpful when co-administered with metformin, particularly in patients with cardiac and renal diseases, who require further reduction of heamoglobinA1c level.

Metformin hydrochloride[3] is an orally available biguanide derivative antidiabetic agent, which acts by decreasing intestinal absorption of glucose, reducing hepatic glucose production and increasing insulin sensitivity. Metformin activates adenosine monophosphate activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling whole body energy balance and metabolism of glucose and fats.

The drug Metformin hydrochloride (MET) is official in Indian Pharmacopoeia[4], British Pharmacopoeia[5], European Pharmacopoeia[6], United State Pharmacopoeia[7].

There are several methods HPTLC[8], HPLC[9][10], UPLC[11] methods reported in the literature for determination of Metformin hydrochloride and Remogliflozin etabonate individually and in combination with other drugs some of the other methods available recently are Green second- derivative spectroscopic methods, Green triple-derivative spectroscopic methods

The present study is aiming to develop and validate, a single, sensitive, rapid, economic and isocratic RP-HPLC method for simultaneous determination of Metformin hydrochloride and Remogliflozin etabonate in bulk and in formulation (tablets).

2. OBJECTIVES

Pharmaceutical industries rely upon quantitative and qualification analysis to ensure that the raw materials used and the final products obtained meet the required specifications.

Literature survey cites many method for determination of Remogliflozin etabonate and Metformin Hydrochloride as individual drug or in combination with other drugs in pharmaceutical formulations (tablets), but no method has been reported so far, for simultaneous estimation of REM and MET in formulation. Hence there is need to develop RP-HPLC method for simultaneous estimation of Remogliflozin etabonate and Metformin Hydrochloride in pharmaceutical formulation (tablets).

Objectives of the present work are:

- To develop Reverse Phase High Performance Liquid Chromatography method for simultaneous estimation of Remogliflozin etabonate and Metformin Hydrochloride in bulk and in pharmaceutical formulation (tablets).
- To validate the developed method for specificity, linearity, range, accuracy, precision, LOD, LOQ and robustness as per ICH guidelines[16].
- To apply the developed and validated RP-HPLC method for simultaneous determination of Remogliflozin etabonate and Metformin Hydrochloride in marketed formulation(tablets).

METHODOLOGY:

A RP-HPLC method was developed and validated for simultaneous estimation of Remogliflozin etabonate (REM) and Metformin Hydrochloride (MET) in bulk and in formulation.

PRELIMINARY ANALYSIS AND ASSAY OF REM AND MET.

The drug Remogliflozin Etabonate (REM) is not official in Any Pharmacopoeia. ,The drug Metformin

hydrochloride (MET) is official in Indian Pharmacopoeia[4], British Pharmacopoeia[5], European Pharmacopoeia[6], United State Pharmacopoeia[7].

Hence, Preliminary analysis of MET were performed according to IP 2007.

REMOGLIFLOZIN ETABONATE

Description

The sample of REM was observed for its colour and texture. It is white crystalline powder.

Solubility

The sample of REM was taken in test tubes and observed for solubility in various solvents like Water, Methanol and Acetonitrile.

Identification Test:

Remogliflozin etabonate was identified by comparing HPLC Chromatogram of REM with an another formulation containing REMOGLIFLOZIN ETABONATE and chromatogram

METFORMIN HYDROCHLORIDE

Description

The sample of MET was observed for its colour and texture. It is White ,Crystalline powder and Hygroscopic.

Solubility

The sample of MET was taken in test tubes and observed for solubility in various solvents like Water, Methanol, and Acetonitrile.

METHOD: REVERSE PHASE HPLC METHOD

I. Instrument Used

» Electronic Weighing Balance (Sartorius – TE 214 S)
» Ultrasonicator (RC Systems – MU 1700)
» UV–Visible Spectrophotometer (Shimadzu – 1700, Software Version - UVProb 2.34)
» Digital pH Meter (Digisun Electronics – 7007)
» Vacuum Pump (Servewell Instruments Pvt. Ltd.)
» Supor 200 Membrane Filter, 0.2 µm (Pall India Pvt. Ltd.)
» Cellulose Acetate Filter, 0.45 µm (Sartorius AG)
» ***HPLC System***

Liquid Chromatograph: Shimadzu LC–10AT

UV–Visible Detector: Shimadzu SPD–10A

Analytical Column: Phenomenon C18 (250 mm × 4.6 mm, 5µm) **Data Processor**: Spinchrome CFR Software, Version 2.1.4.93 **Injector**: Rheodyne – 7725i (Fixed Capacity Loop of 20 µl) **Syringe**: Hamilton, 50 µl

II. Chemicals and Reagents Used

1. Acetonitrile (HPLC grade)
2. Methanol (HPLC grade)
3. Phosphate Buffer(Potassium di hydrogen ortho phosphate Mono basic)

4. HPLC grade water
5. Remogliflozin Etabonate (standard)
6. Metformin Hydrochloride (standard)
7. Tablets

SELECTION OF ANALYTICAL WAVELENGTH.

The standard solutions of REM (10µg/mL) and MET (10µg/mL) in mobile phase were scanned separately in the UV region of 200 to 400 nm using shimadzu 1700 UV-Visible Spectrophotometer. The UV spectrum was observed and a overlain spectrum was prepared and optimum wavelength at which REM and MET shows good absorbance was selected, and the spectrum

A RP-HPLC method was successfully developed and validated for Simultaneous estimationof Remogliflozin Etabonate and Metformin Hydrochloride in bulk and formulation (tablets).

Preliminary analysis and assay:

Description, solubility were performed for Remogliflozin Etabonate and Metformin Hydrochloride as per USP 2007 and results were found to comply with USP standards.

Reverse Phase High Performance Liquid Chromatographic Method.

- The standard solutions of **REM**(10µg/mL) and **MET**(10µg/mL) in mobile phase were scanned in the UV region of 200 to 400 nm, both the drugs showed good absorption at 229nm, Hence the isobestic wavelength of 229nm was selected.
- Several attempts were made to select optimum mobile phase to get good resolution peak of **REM** and **MET**. The mobile phase of PHP buffer(10mM adjusted using 1% OPA) and Acetonitrile in the ratio of 60:40 was selected at a flow rate of 1 mL/min was found to give optimum resolution.
- The other major criteria considered was the pka values of two drugs. Thus pH of PHP between 4 and 4.5 were tried and **pH 4.5** showed better resolution and was selected.
- RP-HPLC method was developed for simultaneous estimation of **Remogliflozin Etabonate and Metformin Hydrochloride** on Phenomenex Hyperclone BDS C18 250 X 4.6 X5µm column as stationary phase and mobile phase comprised of Phosphate buffer (10Mm, pH adjusted to 4.5 with 1% 0PA):Acetonitrile 60:40 v/v at a flow rate of 1 mL/min and the detection of analytes was done at 229nm.
- The retention time was found to be **4.239** and **5.657** for **MET** and **REM**.The developed method was validated in accordance with ICH guidelines for specificity, accuracy, precision, linearity & range, LOD, LOQ and Robustness.

➢ For specificity studies, no extra peak was observed in the chromatogram for excipient near the retention time of Remogliflozin etabonate and Metformin Hydrochloride.It canbe concluded that there was no interference in the retention time of analyte hence the method was found to be specific.

➢ To determine the linearity and range, RP-HPLC analysis was performed on series of concentration range for **REM** and **MET**. The graph was plotted by AUC vs Concentration and the range was determined from the linear part of the graph and was found to be in the concentration ranges of **0.1-500µg/ml for REM** and **0.01-100µg/ml for MET** with correlation coefficients of **0.999** for both the drugs.

➢ LOD and LOQ of **REM** were found to be **0.024 and 0.075µg/mL** and for **MET** were found to be

0.008 and 0.096µg/mL respectively.

- Accuracy was determined by standard addition method at three different levels. (80%,100% and 120%) by calculating mean percentage recovery. The mean % recovery at three different levels was found to be **98.40-100.87%w/w and 98.33-102.53%w/w**for **Remogliflozin etabonate** and **Metformin Hydrochloride** respectively and was well within the range of 95-105%, hence the method was found to be accurate.

- Precision of the method was determined by % RSD found among intra-day precision, inter-day precision, repeatability and reproducibility studies.

- The concentration of **REM** (10µg/mL) and **MET** (10µg/mL) were selected for Intra - day and Inter - day precision. The %RSD for AUC of the study was found to be less than 2% indicating that the method was stable during Inter and Intraday studies.

- From the data obtained for Repeatability studies of **REM**(10µg/mL) and **MET**(10 µg/mL) the %RSD for AUC was calculated and was found to NMT 2%.

- Reproducibility was determined for the mixed standard solutions of **REM**(10µg/mL) and **MET** (10µg/mL) by Analyst 1 and Analyst 2, separately. The percentage RSD was found to be less than 2% indicating that the method is reproducible.

- From Robustness studies, the effects of small changes (±3%) in the pH of mobile phase, flow rate and organic phase ratios on the retention time were studied. Mixed standard solutions of **REM** (10µg/mL) and **MET** (10µg/mL) were prepared and analysed at different pH (4.36, **4.5**, 4.63) of the mobile phase, at different flow rate (0.97, **1.0**, 1.03ml/min) and organic phase ratios (61.8:38.2, **60:40** and 58.2:41.8). The results obtained for all the system suitability parameters like TP, TF & Resolution were well within the acceptance criteria indicating that the method is robust.

- System suitability studies were performed to confirm that the system was appropriate for the analysis to be performed. The study was carried out by injecting six replicate injections of standard solution containing **REM** (10µg/mL) and **MET** (10µg/mL)and analyzing each solute for their peak area, theoretical plates, resolution and tailing factor. The results of all system suitability parameters were well within the acceptance limits

indicating that the system was suitable for analysis of REM & MET by this method.

- The developed and validated method was then applied for determination of **REM** and **MET** in formulation (tablets). The percentage assay for **REM** and **MET** was found to be **97.24-100.48%w/w** and **97.44-97.65%w/w** respectively which is well within the acceptance criteria of 95-105% w/w.

Hence the developed and validated method was found to be specific, accurate, precise, linear and robust and thus can be routinely applied for simultaneous estimation of Remogliflozin Etabonate and Metformin Hydrochloride in bulk and formulation (Tablets).

A RP-HPLC Method for simultaneous estimation of Remogliflozin Etabonate and Metformin Hydrochloride in bulk and formulation was developed and validated successfully.

The Chromatograph used was LC 10AT Shimadzu-SPD 10A detector with Rheodyne injector 20µL and the column Phenomenex BDS C18 (250 x 4.6mm, 5 µm).

The mobile phase comprising of Acetonitrile and Phosphate buffer (10mM) pH 4.5 adjusted with 1% OPA in

the ratio of 40:60 v/v with a flow rate of 1.0ml/min and detection at 229 nm.The peaks produced for a Metformin Hydrochloride and Remogliflozin Etabonate were well resolved with retention time of 4.239 and 5.657min respectively.

The developed method was validated as per ICH (Q2R1) guidelines. The results obtained for all validation parameters, were found within the acceptance criteria.

Thus the proposed method was found to be specific, accurate, linear, precise, robust and can be successfully applied for routine analysis of Remogliflozin Etabonate and Metformin Hydrochloride in pharmaceutical formulation (tablets). An attempt was made to develop A RP-HPLC Method for simultaneous estimation of Remogliflozin Etabonate and Metformin Hydrochloride and to validate the developed method according to ICH guidelines. A RP-HPLC Method development was started with preliminary studies of the drugs according to USP 2008. REM is soluble in Water,Methanol and Acetonitrile and MET is soluble in Water,Methanol and Acetonitrile. REM shows maximum absorbance at 226 nm and MET shows maximum absorbance at 230 nm. The overlaid spectrum of REM and MET was prepared and isobestic wavelength of 229 nm was selected.The quantification was carried out by using PhenomenexC18 column (250 mm × 4.6 mm, 5 µm) as stationary phase and Phosphate Buffer(10mM):Acetonitrile 60:40 as mobile phase where pH was adjusted to 4.5 using 1% OPA. Mobile phase was maintained at a flow rate of mL/min. 20µL solution was injected by Rheodyne injector &the UV detector was operated at 229 nm. A RP-HPLC Method was validated as per ICH guidelines and the results were found to be within the acceptance limit. The assay results conformed to the label claim of the formulation.

The summarized results for REM and MET by RP-HPLC Method are tabulated below TF-Tailingfactor,TP-Theoreticalplates,Rs-Resolution

TABLE 1. Summary of results for RP-HPLC method

Parameters	**REM**	**MET**
Retention time(min)	5.657	4.239
Linearity(µg/ml)	0.1-500	0.01-100
Regression equation(y=mx+c)	y = 17831x + 119391	y = 124507x + 128386
Correlation coefficient (r^2)	0.9992	0.9991
LOD (µg/ml)	0.024	0.008
LOQ (µg/ml)	0.075	0.096
Accuracy (Recovery at three levels) 80%	100.87	98.33
Recovery at 100%	98.40	102.53

Recovery at 120%	100.58	98.88
Intra-day precision (%RSD)	0.883	0.785
Inter- day precision (%RSD)	0.664	0.384
Repeatability(%RSD)	0.095	0.473
Robustness	TF, TP, Rs were found to be within acceptance criteria	
Analysis of Tablets (%assay)w/w	98.66	97.54

References:

1. Glenmark Receives Approval for combination of Remogliflozin etabonate and Metformin hydrochloride for adults with type 2 diabetes in India [media release].19 Aug,2019.

2. Markham, A. Remogliflozin Etabonate: First Global Approval. *Drugs 2019,79,1151-1161.*

3. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017 Sep;60(9):1577-1585.

4. Indian Parmacopoeia, Government of India, Ghaziabad. The Indian Pharmacopoeia Comission 2007; 2:1358

5. British Pharmacopoeia, Her Majesty's Stationary Office.London, UK 2009;1 and 2:3813.

6. European Pharmacopoeia. Council of Europe, France. 3rd edition.1997;

7. The United States Pharmacopoeia. US Pharmacopoeial Convention,Inc.:Rockville, MD. 31ST Revision. 2008;2640.8.

8. Ankita B Tayade, Amod S Patil, Atul A Shirkhedkar. Development and Validation of Zero Order UV-Spectrophotometric Method by Area Under Curve Technique and High Performance Thin Layer Chromatography for the Estimation of Remogliflozin Etabonate inBulk and In-House Tablets. Invention Rapid: Pharm Analysis & Quality Assurance, 2019(3):1

9. Itigimatha N, Chadchan Ks, YallurBc, Hadagali Md, Hadagali Md. Simple And Sensitive RP-HPLC And UV Spectroscopic Methods For The Determination Of Remogliflozin Etabonate In Pure And Pharmaceutical Formulations Remogliflozin.

10. Tammisetty MO, Challa BR, Puttagunta SB. A novel analytical method for the simultaneous estimation of remogliflozin and metformin hydrochloride by UPLC/PDA in bulk and formulation. Application to the estimation of product traces. Turk. J. Pharm. Sci. 2020 Jun 8;39699.

11. Shah DA, Gondalia II, Patel VB, Mahajan A, Chhalotiya UK. Stability indicating liquid chromatographic method for the estimation of remogliflozin etabonate. Journal of Chemical Metrology. 2020 Jul 1;14(2):132.

12. Ahmed Gedawy, Hani Al-Salami and Crispin R Dass. Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide. Journal of Food and Drug Analysis .2019; 27(1), 315-322.