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Development and Validation of RP HPLC Method for the Simultaneous Estimation of repaglinide and Metformin HCL Tablet Formulation

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ABSTRACT: A new simple, rapid, precise and accurate assay method was developed for simultaneous estimation of Repaglinide and Metformin HCL in pure form and tablet form. The analytes were separated by RP HPLC on a RP-Purosnosphere C18 column (5 µm, 4.6mm* 250 mm). The mobile phase was Acetonitrile:methanol (60:40 v/v) at 1.1 ml/min flow rate satisfactorily resolves the tertiary mixture. The UV detector was operated at 214 nm for the determination of all the drugs. Linearity, accuracy and precision were found to be acceptable over the concentration ranges of 2-10 µg/ml for Repaglinide and 10-50 µg/ml for Metformin HCL with a R^2 0.9960 and 0.9974 values respectively. The optimized methods proved to be specific, robust and accurate for the quality control of drugs in bulk drug and pharmaceutical formulations.

KEY WORDS: Repaglinide, Metformin HCL sodium, ICH, Validation etc.

I. INTRODUCTION:

Repaglinide is a new carbomoxylmethyl benzoic acid derivative, also known as 2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl) phenyl] butyl amino]-2-oxoethyl] (Fig. 1a). It is a novel prandial glucose regulator for the treatment of type 2 diabetes mellitus [1,2]. It reduces the fasting glucose concentrations in patients with type 2 diabetes mellitus. It helps to control blood sugar by increasing the amount of insulin released by the pancreas. Repaglinide is rapidly absorbed from the gastrointestinal tract after oral administration. It differs from other antidiabetic agents in its structure, binding profile, duration of action and mode of excretion [3]. To date, several analytical methods are available for the determination of repaglinide in biological fluids, including liquid chromatography-tandem mass spectrometry (LC/MS/MS) [4,5].

Repaglinide (RGE), is an oral antidiabetic drug. Tablets containing 0.5, 1 and 2 mg of RGE are available for oral administration. The methods investigated for analysis of RGE include HPLC method with UV detector or electrochemical method by using carbon paste electrode and glassy carbon electrode. Liquid chromatography-tandem mass spectrometry (LC/MS/MS) and normal phase chiral HPLC methods for determination of RGE are also reported. The HPLC method reported by USP claims to be sensitive enough for the analysis of RGE. However, these procedures are expensive and inconvenient for routine analysis of RGE. Therefore, it was felt essential to develop a facile method suitable for routine analysis of RGE during early development phase of tablets/transdermal delivery systems. Further, the results of fluorimetric analysis were compared with those obtained by HPLC analysis.

The RP-HPLC method development was done by checking various parameters such as mobile phase composition, mobile phase ratio, column, column temperature, flow rate, etc., to obtain a good peak shape with less run time, less tailing, high theoretical plates, and resolution. In this study, various solvents were tried as the mobile phase like methanol, acetonitrile, and water containing 10 mM formic acid, glacial acetic acid, and sodium dihydrogen orthophosphate by changing the flow rates.

Metformin hydrochloride is chemically identified as (3-(diamino methylidene)-1, 1-dimethylguanidine; hydrochloride. Metformin has a molecular weight of 165.62 g mol^{-1} and has the molecular formula of $C_4H_{11}N_5$ [6]. Metformin belongs to biguanide a class of anti-hyperglycemic

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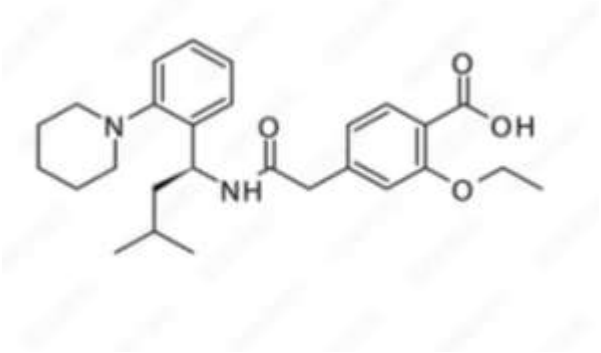
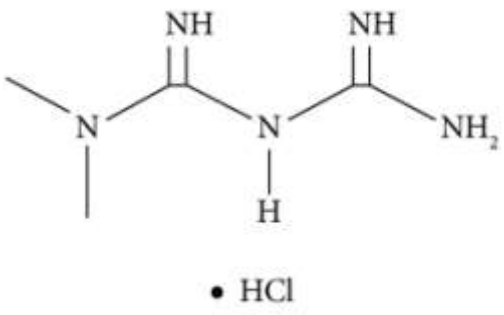
agents and is used for the management of type 2 DM. It is useful in treating type 2 DM, as it decreases the intestinal absorption of glucose, and hepatic glucose production, and improves insulin sensitivity by increasing utilization and uptake of peripheral glucose. All of these properties were initiated by the primary stimulation of AMP-activated protein kinase, which is a liver enzyme and plays an important part in insulin signaling, metabolism of fats and glucose and whole body energy balance [6]

The mechanism of action involves binding of the a-polar biguanide hydrocarbon side-chain to membrane phospholipids, evoking a change in the electrostatic surface potential (Hermann, 1979) [7]. Subsequently, various metabolic effects are elicited, depending on the target cell, tissue, organ, species (Bailey, 1985) and metabolic regulation (Hermann, 1981) [8].

To the best of our knowledge no method is reported in literature for simultaneous determination of MET and REPA by high performance liquid chromatography (HPLC). Several HPLC methods have been reported in the literature for quantitative determination of MET alone [9] or in combination with other drugs in tablets [10-12], human serum and other biological fluids [13-14]. Similarly, a few HPLC methods have been reported for the quantitative determination of REPA in tablets alone or in combination with other drugs, and in human serum and other biological fluids [15]. Only one UV spectroscopic method has been reported for the simultaneous determination of these compounds, which, however, lacks stability indicating nature [16]. The objective of the present work was to develop and validate a simple, economic, rapid, precise, isocratic, and accurate stability-indicating method with good sensitivity for simultaneous determination of MET and REPA in accordance with ICH guidelines.[17] The proposed method was successfully applied to a synthetic mixture of MET and REPA and analyzed in presence of commonly used tablet excipients. This method can also be employed for quality control during manufacture of drug product [18].



Structure of repaglinide



Structure of metformin HCL

II. MATERIALS AND METHODS

Chemicals and reagents

Repaglinide (1mg) and Metformin HCL (500mg) drugs were obtained as a gift sample fromAstrazeneca Pharmaceuticals India. The combined formulation EUREPA MF2

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(2mg/500mg) ofthe two drugs purchased from Vikram Pharmacy Jalgaon. Analytical grade methanol purchased from Merck Chemicals Pvt. Ltd. Mumbai. Acetonitrile, methanol and water used were of HPLC grade (Qualigens Fine Chemicals, Mumbai, India). Ammonium Acetate Buffer was AR grade (Qualigens Fine Chemicals, Mumbai, India). A 0.2 µm nylon filter (Pall life Sciences, Mumbai, India) was used. All other chemicals and reagents used were analytical grade unless otherwise indicated.

Apparatus

The chromatographic system (Systronics Corporation, India) consisted of LC 8600aprominence solvent delivery module, a manual injector with a 20 µL fixed loop and a UV-visible detector. The separation was performed on a Hibar® (Merk, Germany) RP-Presnosphere Star C18 column (5 µm, 4.6mm* 250 mm) at an ambient temperature. Chromatographic data were recorded and processed using Chemitochrom 2000 software. A Fast clean ultrasonicate cleaner (India) was used for degassing the mobile phase. Shimadzu UV 1800 double beam UV visible spectrophotometer and Sansui-vibra DJ-150S-S electronic balance were used for Spectrophotometric and weighing purposes respectively.

Chromatography Conditions

Chromatographic separations of active (REPA andMET)substances were obtained by using Systronics LC-138 RP-Presnosphere Star C18 column (5 µm, 4.6mm* 250 mm),Mobile phase Acetonitrile:methanol (60:40 v/v)) (pH 4.4 was adjusted with o-phosphoric acid Buffer) was prepared, filtered through a 0.2 µm nylon filter and degassed for 5 min in an ultrasonicator. The mobile phase was pumped through the column at flow rate of 1.1 ml/min^{-1}. Analyses were carried out at ambient temperature with detection at 214 nm. The injection volume was 20 µL and each analysis required 12 min.

Standard Solutions

Stock standard solutions of REPA 100 µg/ml and MET 1 mg/ml were prepared by dissolving 1 mg REPA standard and 10 mg MET standard in 10 ml methanol. Working standard solutions of REPA2µg/ml and MET 10µg/ml were prepared by diluting suitable aliquots of corresponding stock solutions with mobile phase.

Sample Solution

EUREPA MF2 20 Tablets containing Repaglinide (2mg) and Metformin HCL(500 mg) wereweighed and ground to fine powder. A quantity of sample equivalent to Repaglinide (2 mg) and Metformin (500 mg) was transferred into 100 mL volumetric flask containing methanol (60 mL), sonicated for 15 min and the volume was made up to the mark and filtered through Whatman filter paper (No. 45). This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved andvolume was adjusted to the mark.The response of solution was measured at 214 nm and quantification of REPA and MET was done by using present HPLC method. Typical chromatogram of final resultant formulation solution was shown in (Fig. 1)

Validation of Proposed Method

Calibration curve (linearity)

Accurately measured aliquots of working standard solutions equivalent to 2-10 µg/ml REPA, and 10-50µg/ml MET were transferred to series of 10 ml volumetric flasks and the contents of the flasks were diluted to volume with mobile phase. A 20 µL aliquot of each solution was injected in triplicate into the liquid chromatography. The conditions including the flow rate of mobile phase at 1.1 ml/min, detection at 214 nm and run time program for 8 min, were adjusted. A calibration curve for each drug was obtained by plotting area under the peak versus concentration. The graphs of area vs concentration were recorded for all the drugs and are shown in (Fig. 2 and 3).

Accuracy (% recovery)

Recovery studies were carried out by adding a known amount of pure drugs REPA and MET to a pre analyzed sample solution. These studies were carried out by spiking 80%, 100% and 120% respective drug. The recovery studies showed that the results were within acceptable limits, above 99% and below 101%. The results are given in table no. 2

Method Precision (repeatability)

The precision of the developed method was assessed in terms of repeatability, intraday and inter-day precision by analyzing six replicate standard samples. The % R.S.D. values of the results corresponding to the peak area and retention time were expressed for intra-day precision and on 3 days for inter-day precision.

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Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method were determined by estimating the corresponding responses 5 times on the same day and on 5 different days for present method. The results are reported in terms of relative standard deviation (RSD).

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ of the drug were calculated using the equations according to International Conference on Harmonization (ICH) guidelines.

Robustness

Robustness of the method was determined by making slight changes in chromatographic conditions. Effect of % of methanol (59, 60 and 61%) in mobile phase on the retention time and slight changes in flow rate were applied as variable parameters. Flow rate varied at three levels (-1, 0, 1). One factor at the time was changed to estimate the effect. Thus, standard solution at varied pH (pH4.2, 4.3 and 4.4) three pH levels was performed.

Specificity

Specificity is the ability of the analytical method to measure analyzed response in presence of interferences including degradation products and related substances. Specificity was checked by determining REPA and MET in laboratory prepared binary mixture and in binary mixture containing different degradation products.

System suitability Test

In the system suitability test tertiary solution of 40µg/ml of REPA and 20µg/ml of MET (n=6) was prepared and injected. Then the system suitability parameters like retention time, theoretical plates, tailing factor and resolution were calculated from the chromatogram.

III. RESULTS AND DISCUSSION

The absorption spectra of REPA and MET greatly overlap; so conventional determination of these compounds in mixture is not possible. To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for REPA and MET were obtained with a mobile phase consisting of Acetonitrile: Methanol (60:40v/v), pH 4.4 adjusted using o-phosphoric acidBuffer. Quantification of the drugs was performed at 214 nm. Resolution of the components with clear baseline separation was obtained.

Validation of the Proposed Method

Linearity

Linear correlation was obtained between peak areas and concentrations of REPA and MET in range of 2-10 and 10–50µg/ml respectively. The linearity of calibration curves was found to be acceptable over the concentration ranges of 20-120 µg/ml for REPA while 5-30 µg/ml for MET with a R^2 0.9994 and 0.9995 values respectively.
(Table- 1, Fig- 2 and 3). The results show that good correlation existed between the peak area and concentration of the analysts.

Table 1: Regression analysis of the calibration curves for Repaglinideand Metformin in the proposed HPLC Method

Parameter	Repaglinide	Metformin
Linearity Range (µg/ml)	2-10	10-50
Detection Wavelength (nm)	214	
Slope ± SD	52.99	9.93
Correlation coefficient	0.9994	0.9995

(n= mean of three determinations)

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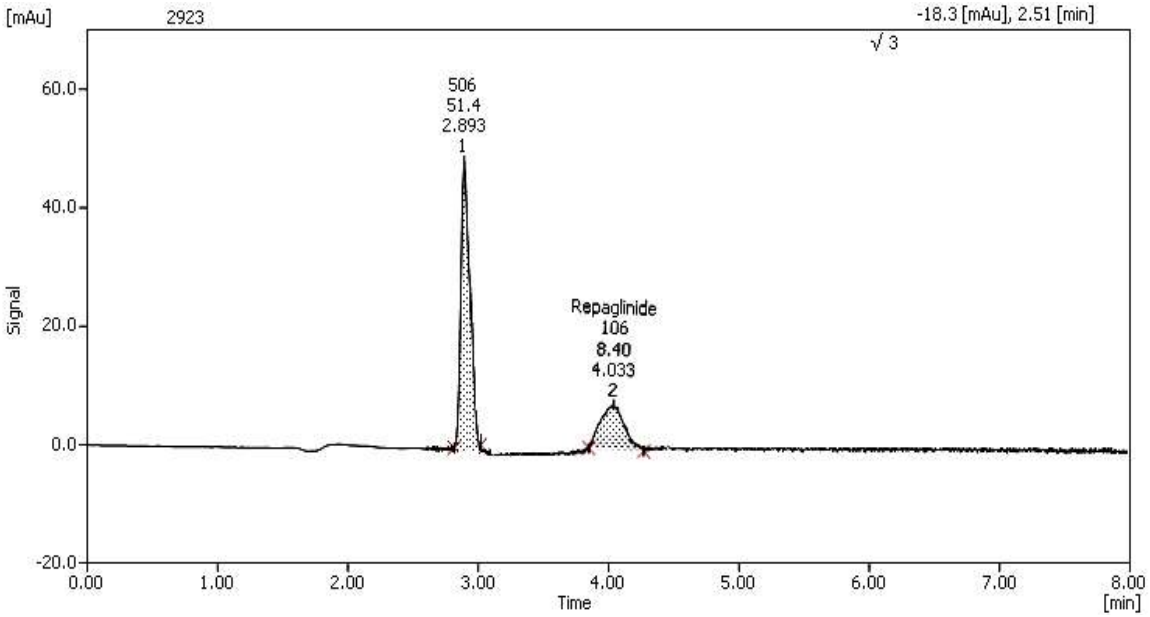

Figure 1: Typical liquid chromatogram obtained for a 20 µL injection of tablet formulation of REPA and MET

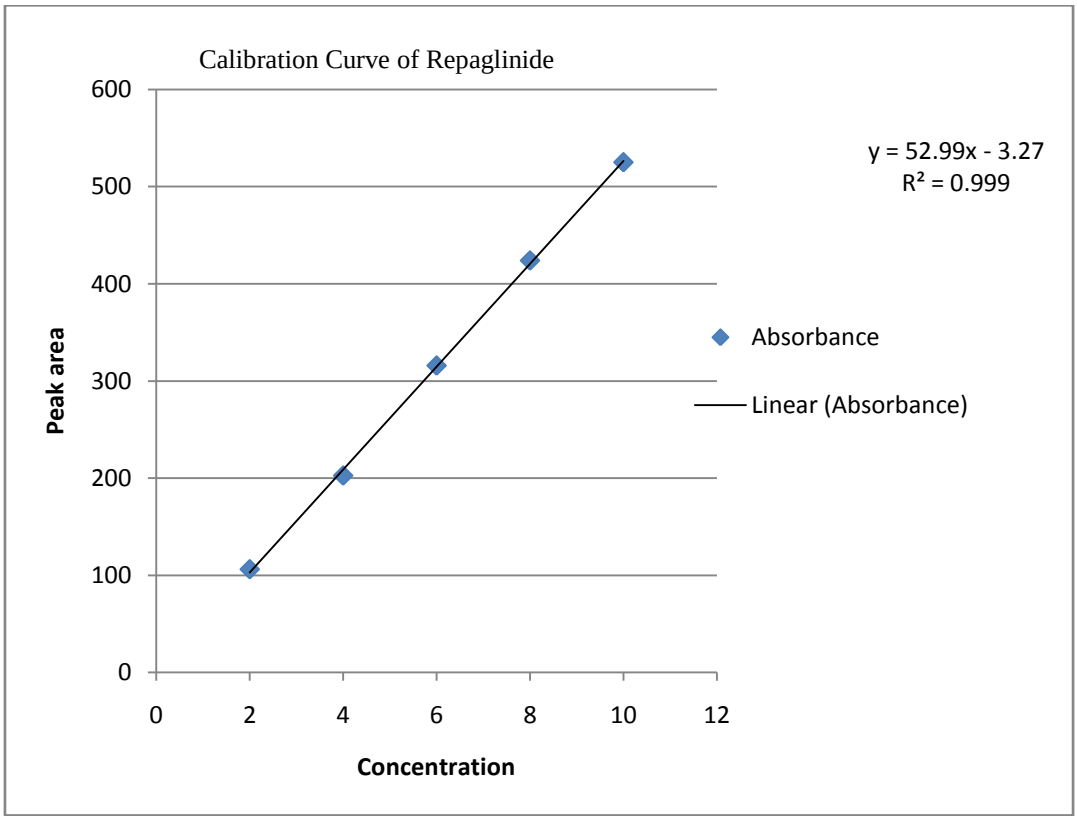

Figure 2: Calibration Curve for Repaglinide

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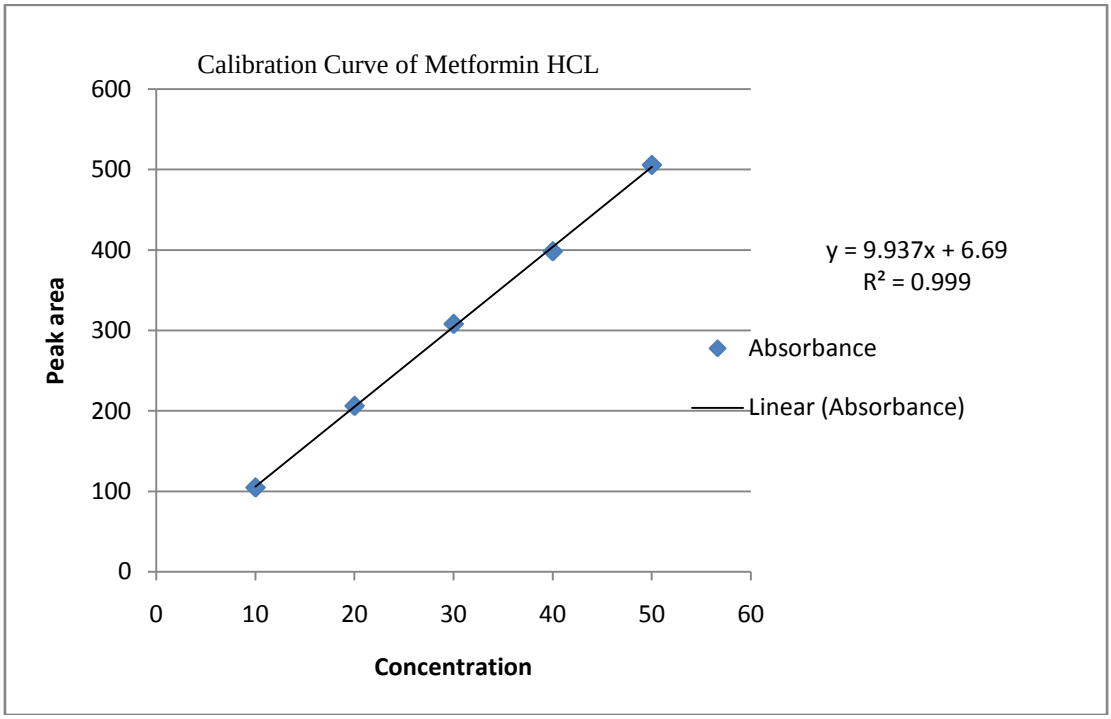

Figure 3: Calibration Curve for Metformin HCL

Accuracy

The recovery experiments were performed by the standard addition method. The recoveries obtained were 99.34% and 99.92% for REPA and MET respectively (Table 2). The high values indicate that the method was accurate.

Method precision

Precision study was carried out using parameter like method repeatability study which showed that results were within acceptable limit 1.265and 0.115 i.e. % RSD below 2.0 indicating that the method is reproducible.

Intermediate precision

The intraday RSD values for REPA and MET were 0.5894-0.7344 % and 0.4419-1.1590 %, respectively. The interday RSD values for REPA and MET were 0.4378-0.8105 %, and 0.8254–0.9479%, respectively. The % RSD (< 2%) values indicate that the method was sufficiently precise (Table 2).

LOD and LOQ

LOD values for REPA and MET found to be 0.6963µg/ml and 0.3291µg /ml, respectively. LOQ values for REPA and MET were found to be 2.1101µg /ml and 0.9975µg /ml, respectively (Table2). These data showed that the method was sensitive enough for the determination of REPA and MET.

Table 2: Summary of the validation parameters for the proposed HPLC method

Parameter	Repaglinide	Metformin
LOD	0.6963µg /ml	0.3291µg /ml
LOQ	2.1101µg /ml	0.9975µg /ml
Accuracy, %	99.88 ± 0.65	99.85 ± 0.62
Repeatability (%RSD, n = 3)	1.265	0.115
Interday, n = 3	0.522	0.143
Intraday, n = 3	0.606	0.189

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LOD = Limit of detection
LOQ = Limit of quantification
RSD = Relative standard deviation.

Robustness

The method was found to be robust with no significant changes on test result upon change of analytical conditions like different flow rate, % methanol in mobile phase and pH of mobile phase with the standard deviation was found to be below 1 and % RSD is less than 2 for all results. It was found that under small deliberate changes of chromatographic factors, there was no considerable change in under study parameters.

System Suitability Test

A tertiary solution of 40 µg/ml of REPA and 20µg/ml of MET(n=5) was prepared and same was injected, then the system suitability parameters were calculated from the chromatogram. The parameters, retention times, resolution factor, tailing factor and theoretical plates were evaluated. The results (Table) obtained from system suitability tests are in agreement with the official requirements.

Table. 3: System suitability test parameters for REPA and MET for the proposed HPLC method

System Suitability Parameters	Proposed Method	
	REPA	MET
Retention Time (t_R)	4.033	2.893
Area	106.2	505.8
Theoretical Plate Number (N)	2111	11662
Asymmetry factor	1.298	0.219
Resolution Factor (R)	4.392	

IV. CONCLUSIONS

The proposed LC method presented in this paper has advantages of simplicity, accuracy, precision and convenience for separation and quantitation of REPA and MET in combination and can be used for the assay of their respective dosage form. Moreover, the proposed LC method is a stability indicating assay method that can determine REPA and MET in presence of their degradation products. Thus, the proposed LC method can be used for the quality control of REPA and MET in typical laboratories.

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