Development and Validation of New RP-HPLC Method for the Simultaneous Estimation of Metformin Hydrochloride and Repaglinide in Pure and Pharmaceutical Formulations

Afroz Patan*, Syed Reeyaz Basha, Ravi Kumar Ketha, Binoy Vargheese Cheriyan,
Vijey Aanandhi Muthukumar

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Sciences, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai – 603117, Tamilnadu, India.
*Corresponding Author E-mail: **afroz.sps@velsuniv.ac.in**

ABSTRACT:
Objective: A simple, accurate, precise, new RP-HPLC method was developed and validated for the determination of Metformin hydrochloride and Repaglinide in bulk and its pharmaceutical formulations. **Method:** The literature survey reveals that good analytical methods are not available for simultaneous estimation of drugs like Metformin hydrochloride and Repaglinide, which suffer from major disadvantage such as lo w sensitivity, lack of selectivity and simplicity. The existing physicochemical methods are inadequate to meet the re quirements; hence a new RP-
HPLC method has been developed for simultaneous estimation of Metformin hydrochloride and Repaglinide in bulk and its pharmaceutical formulations. A XBridge C18 (4.6 x 150mm, 3.5 µm, Make: Waters) or equivalent in an isoc ratic mode with mobile phase Potassium dihyrogen ortho phosphate (2.2 pH): Acetonitrile (35:65%v/v) was used. T he flow rate was 0.6ml/ min and effluent was monitored at 240 nm. The retention time of Metformin hydrochloride a nd Repaglinide was 2.517 and 3.825 min. The linearity range was found to be 5-
50µg/ml. The proposed method was validated statistically. Tablets of Metformin hydrochloride and Repaglinide [50 0.0mg and 2.0mg] was formulated in the lab scale for estimation by the developed method. **Results:** The method de veloped was approved for various parameters like accuracy, specificity, precision, range, linearity, robustness, LOD, LOQ and system suitability according to ICH guidelines. The results got were according the acceptance criteria. **Co nclusion:** The sample recoveries in the formulation were in good agreement with their respective label claims and n o interference of formulation excipients in the estimation. The technique could be easily and conveniently adopted f or routine analysis of Zolpidem Tartrate in pure form and its dosage form and also for dissolution or similar studies.

KEYWORDS: Metformin hydrochloride, Repaglinide, RP – HPLC and Validation.

INTRODUCTION:

Metformin Hydrochloride is chemically 1,1- Dimethyl biguanide hydrochloride as shown in Fig-1. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.

Fig. 1: Structure of Metformin Hydrochloride

Repaglinide is chemically 2-ethoxy-4-({[(1S)-3-methyl-1-[2-(piperidin-1- yl) phenyl] butyl] carbamoyl} methyl) benzoic acid as shown in Fig-2. Repaglinide closes ATP- dependent potassium channels in the b-cell membrane by binding at characterizable sites. This potassium channel blockade depolarizes the b-cell, which leads to an opening

of calcium channels. The resulting increased calcium influx induces insulin secretion. The ion channel mechanism is highly tissue selective with low affinity for heart and skeletal muscle.

Fig. 2: Structure of Repaglinide

A survey of literature [1-14] reveals that good analytical methods are not available for simultaneous estimation of drugs like Metformin hydrochloride and Repaglinide, which suffer from major disadvantage such as low sensitivity, lack of selectivity and simplicity. The existing physicochemical methods are inadequate to meet the requirements; hence a new RP-HPLC method has been developed for simultaneous estimation of Metformin hydrochloride and Repaglinide in bulk and its pharmaceutical formulations adapting different available analytical techniques like RP-HPLC with a main objective to reduce the retention times of Metformin hydrochloride and Repaglinide. So according to ICH guidelines an attempt was made to create and validate a basic simple, accurate, precise and efficient RP-HPLC technique which could be easily and conveniently adopted for routine analysis of Metformin hydrochloride and Repaglinide in pure form and its dosage form.

MATERIALS AND METHODS:

Instrumentation:

HPLC (Make: WATERS, Model: Alliance 2695, Detector 2487 with Empower 2 software), UV Spectrophotometer (Make: Labindia, Model: UV-3000+ with UV win 5 software), Weighing Balance (Make: Ascoset, Model: ER200A), Sonicator (Make: Enertech, Model: SE60US), pH Meter (Make: ADWA, Model: AD102U), Filter Paper 0.45 microns (Make: Milli Pore)

Reagents and chemicals:

Potassium dihydrogen ortho phosphate, Ortho phosphoric Acid, HPLC Grade Methanol, HPLC Grade Acetonitrile, Double Distilled Water.

Chromatographic conditions:

Parameters	Method	
Column (Stationary Phase)	XBridge C18 (4.6 x 150mm, 3.5 µm, Make: Waters) or equivalent	
Mobile Phase	Potassium dihydrogen ortho phosphate (2.2 p^H): Acetonitrile (35:65%v/v)	
Flow rate (ml/min)	0.6	
Run time (min)	7	
Column temperature (°C)	Ambient	
Volume of injection loop (µl)	10	
Detection wavelength (nm)	240	
Drug RT (min)	Metformin HCl	Repaglinide
	2.517	3.825

Method Development and Optimisation:

The detection wavelength was ascertained at 240nm from the UV spectrum of Metformin hydrochloride and Repaglinide (20µg/ml) in the mobile phase against reagent blank.

To develop a suitable and robust method for the determination of Metformin hydrochloride and Repaglinide, different mobile phases like Potassium dihydrogen ortho phosphate-Methanol, Potassium dihydrogen ortho phosphate- Acetonitrile solution in various ratios (60:40, 50:50, 40:60, 35:65) with different pH (3.0, 2.5, 2.3 and 2.2) were tried at flow rate (0.8, 0.7 and 0.6ml/min). Potassium dihydrogen ortho phosphate-Acetonitrile solution in the ratio 35:65 at a flow rate of 0.6ml/min was selected of all since it gave the best result. The peak was symmetric and the drug Metformin hydrochloride and Repaglinide was eluted at 2.517 and 3.825 minutes. The run time was thus fixed at 7 minutes.

Method:

Preparation of mobile phase:

Weigh 7.0grams of Potassium dihyrogen ortho phosphate in to a 1000ml beaker, dissolve and diluted to 1000ml with HPLC water. Adjusted the pH to 2.2 with ortho- phosphoric acid. Mix a mixture of above buffer 350mL (35%)

and 650mL of Methanol HPLC (65%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of drug solutions:
10mg of Metformin hydrochloride and Repaglinide pure drug was weighed and dissolved in the mobile phase in a 10ml volumetric flask to get the stock solution (1000 µg/ml). From this further aliquots of 0.05-0.5ml from the stock solution were taken and diluted with the mobile phase to get solutions in the concentration range of 5µg to 50µg/ml.

Preparation of sample drug solution from pharmaceutical dosage form:
Twenty tablets containing the drug were taken and powdered. The powder equivalent to 10mg of the Repaglinide was accurately weighed and taken in a 100ml volumetric flask and mobile phase was added to make up to volume. The volumetric flask was sonicated for 45 minutes to effect complete dissolution of drug and the solution was made up to volume with mobile phase and filtered through Whatman filter paper (0.45µm) made up of cellulose nitrate. Aliquots solutions were prepared by taking 2.0ml of the filtered solution into 10ml volumetric flasks, separately and made up to volume with mobile phase to yield concentrations of Repaglinide in range of linearity previously described. From the above solution 2.0ml was transferred to 10ml volumetric flask and made up to volume with mobile phase. From the above solution 0.2ml was transferred to 10ml volumetric flask, separately and made up to volume with mobile phase to yield concentrations of Metformin Hydrochloride in range of linearity previously described.

Procedure for calibration curve:
The contents of the mobile phase were filtered before use through Whatman filter paper (0.45µm) made up of cellulose nitrate, and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug solutions, the column was equilibrated with the mobile phase flowing through the systems. The chromatographic separation was achieved using a mobile phase consisting of Patassium dihydrogen ortho phosphate- Acetonitrile solution in the ratio (35:65v/v) at a flow rate of 0.6ml/min. The eluent was monitored using UV detection at a wavelength of 240 nm. The column was maintained an ambient temperature (25°C) and an injection volume of 10µl of each of standard and sample solutions were injected into the HPLC system to get the chromatograms. The retention time and peak areas of the drug were recorded. A graph was plotted by taking concentration of the drug on x-axis and peak area on y-axis. The linearity range was found to be in between 5-50µg/ml for Metformin hydrochloride and Repaglinide. The linearity table and calibration curve are as shown in Table 1 and Figure 4 and 5 respectively.

Estimation of Metformin hydrochloride and Repaglinide in the pharmaceutical dosage forms:
The amount of drug present in the In house formulation was calculated by using the regression equation of the linearity plot. The result obtained is as shown in table 24. A typical chromatogram of Metformin hydrochloride and Repaglinide (20µg/ml) (In House Formulation) is as shown in Figure 3.

Fig. 3: Typical chromatogram of pure drug (20µg/ml)

Method validation parameters:
Linearity:
For all methods, 6-point calibration curve were prepared on single day. The results obtained were used to calculate the equation of the line by using linear regression by the least square method. The linearity table and calibration curve are as shown in Table 1 and Figure 4 and 5 respectively.

Table No. 1: Linearity range of proposed RP-HPLC method

S. No.	Linearity Level	Concentration	Area of Metformin Hcl	Area of Repaglinide
1	I	5µg/ml	362120	120239
2	II	10µg/ml	726426	242570
3	III	20µg/ml	1492439	498282
4	IV	30µg/ml	2183380	749949
5	V	40µg/ml	2951238	988812
6	V	50µg/ml	3650958	1240721
Slope			73266	24865

Intercept	1485	1922
Correlation Coefficient	0.999	0.999

Figure 4: Linearity plot of Metformin hydrochloride **Figure 5: Linearity plot of Repaglinide**

Precision: (System precision)

The repeatability of the method was ascertained by precision (intra-day and inter-day) of the method by estimation of one replicates at single intermediate level (20µg/ml) on the same day and next day, respectively. The results along with the statistical calculations are as presented in the Table 2.

Method precision:

The repeatability of the method was ascertained by precision (intra-day and inter-day) of the method by estimation of one replicates at single intermediate level (20µg/ml) (Formulation) on the same day and next day, respectively. The results along with the statistical calculations are as presented in the Table 3.

Table No. 2: System Precision (Intra-day and Inter-day)

System Precision (Intra-day)		
Injection	**Area of Metformin Hcl**	**Area of Repaglinide**
Injection-1	1489301	494736
Injection-2	1492080	497000
Injection-3	1489889	496238
Injection-4	1493862	497617
Injection-5	1484376	494658
Average	1489901	496949
Standard Deviation	3582.5	1328.3
%RSD	0.24	0.26
System Precision (Inter-day)		
Injection	**Area of Metformin Hcl**	**Area of Repaglinide**
Injection-1	1497177	498303
Injection-2	1501852	499086
Injection-3	1496466	497665
Injection-4	1494920	496151
Injection-5	1501919	499217
Average	1498466	498084
Standard Deviation	3225.8	1249.7
%RSD	0.21	0.25

Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of bulk samples of Metformin hydrochloride and Repaglinide to pre-analyzed amount of formulation of concentration 20µg/ml. From this percentage recovery values were calculated. The results were shown in Table 4.

Table No. 3: Method Precision (Intra-day and Inter-day)

Method Precision (Intra-day)		
Injection	**Area of Metformin**	**Area of**

	Hcl	Repaglinide
Injection-1	1466304	486236
Injection-2	1472460	486345
Injection-3	1468940	486289
Injection-4	1470674	486446
Injection-5	1476102	486498
Injection-6	1480496	486309
Average	1472496	486353
Standard Deviation	5124.6	99.45
%RSD	0.34	0.02
Method Precision (Inter-day)		
Injection	**Area of Metformin Hcl**	**Area of Repaglinide**
Injection-1	1475716	486513
Injection-2	1477408	486329
Injection-3	1480323	486390
Injection-4	1486710	486358
Injection-5	1477798	486574
Injection-6	1477554	486299
Average	1479251	486410
Standard Deviation	3941.3	109
%RSD	0.26	0.02

Robustness:

Robustness of the method reflects the reliability of an analysis with respect to deliberate variations in the method parameters. Here, the flow rate and mobile phase composition were slightly changed to lower and higher sides of the actual values to find if the change in the peak area and retention time were within limits. The results obtained with changes in the parameters on a 20µg/ml solution are as shown in Table 5 and Table 6 respectively.

Table No. 4: Accuracy

% of pure drug spiked	Pure drug		Formulation		MET		REP	
	MET	REP	MET	REP	% recovery	Statistical analysis	% recovery	Statistical analysis
50%	10	10	20	20	98.96	**Mean**=99.10 **SD** = 0.135 **%RSD** =0.13	98.10	**Mean** = 98.05 **SD** = 0.058 **%RSD** = 0.05
50%	10	10	20	20	99.23		97.99	
50%	10	10	20	20	99.11		98.08	
100%	20	20	20	20	97.57	**Mean** =97.58 **SD** = 0.032 **%RSD** =0.03	98.04	**Mean** = 98.03 **SD** = 0.045 **%RSD** = 0.04
100%	20	20	20	20	97.63		97.98	
100%	20	20	20	20	97.58		98.07	
150%	30	30	20	20	98.81	**Mean** =98.80 **SD** = 0.005 **%RSD** = 0.005	98.02	**Mean** = 98.03 **SD** = 0.015 **%RSD** = 0.01
150%	30	30	20	20	98.80		98.05	
150%	30	30	20	20	98.81		98.04	

Table No. 5: Robustness (Metformin Hydrochloride)

Sl. No.	Parameter	Condition	Peak area	Statistical analysis	Retention time	Statistical analysis
1	**Flow rate (ml/min)**	0.5	1521762	**Mean**=1493710 **SD**= 26354 **%RSD**= 1.76	2.58	**Mean**=2.53 **SD**= 0.045 **%RSD**=1.77
		0.6	**1489901**		**2.52**	
		0.7	1469468		2.49	
2	**Mobile phase ratio**	37:63	1448116	**Mean**=1476634 **SD**= 24717 **%RSD**=1.673	2.52	**Mean**= 2.523 **SD**=0.005 **%RSD**=0.198
		35:65	**1489901**		**2.52**	
		33:67	1491885		2.53	

Table No. 6: Robustness (Repaglinide)

Sl. No.	Parameter	Condition	Peak area	Statistical analysis	Retention time	Statistical analysis
1	**Flow rate (ml/min)**	0.5	512147	**Mean**=500551 **SD**= 10125 **%RSD**=2.02	3.91	**Mean**= 3.83 **SD**= 0.075 **%RSD**=1.958
		0.6	496049		3.82	
		0.7	493457		3.76	
2	**Mobile phase ratio**	37:63	509006	**Mean**=505633 **SD**= 8421 **%RSD**=1.665	3.93	**Mean**=3.84 **SD**=0.073 **%RSD**=1.901
		35:65	496049		3.82	
		33:67	511846		3.79	

System suitability parameters:

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation has been completed. The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Asymmetry (A), LOD (µg/ml) and LOQ (µg/ml) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of Metformin hydrochloride and Repaglinide in pharmaceutical formulations was validated or not. The results obtained are as shown in table 7.

Table No.7: System Suitability Parameters

Parameters	Obtained Values	
	MET	REP
Theoretical plates (N)	2133.6	2694.7
Asymmetry (A_s)	1.7	1.4
LOD (µg/ml)	0.018	0.05
LOQ (µg/ml)	0.06	0.19

RESULTS AND DISCUSSION:

From the above experiment it was found that Metformin hydrochloride and Repaglinide can effectively be analysed by the HPLC method with Potassium dihyrogen ortho phosphate (2.2pH): Acetonitrile as the mobile phase in the ratio 35:65 at a flow rate of 0.6ml/minute and detection wavelength of 240nm. The retention time of Metformin hydrochloride and Repaglinide was 2.517 and 3.825 minutes. The linearity range was found to be 5-50µg/ml. The regression equation obtained for Metformin Hydrochloride and Repaglinide from the linearity plot was y = 73266x + 1485 and y = 24865x - 1922 with correlation coefficient 0.999 and 0.999. In the System precision study, %RSD for Metformin hydrochloride and Repaglinide was found to be less than 2% intraday (0.24 and 0.21) inter day (0.26 and 0.25) and the Method precision study, %RSD for Metformin hydrochloride and Repaglinide was found to be less than 2% intraday (0.34 and 0.26) inter day (0.02 and 0.02) which indicates that the method has good reproducibility. The accuracy studies showed % recovery for Metformin hydrochloride and Repaglinide in the range 97.58 – 99.10% (% RSD = 0.005– 0.13) and 98.03 – 98.05% (% RSD = 0.01– 0.05) which indicates that the method was accurate and also revealed that the commonly used excipients present in the pharmaceutical formulations do not interfere in the proposed method. Robustness studies reveal that the method was reliable. The system suitability parameters were found to be within the specified limits for the proposed method.

CONCLUSION:

The proposed method was found to be simple, precise, accurate and rapid for determination of Metformin Hydrochloride and Repaglinide from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Metformin Hydrochloride and Repaglinide in pure form and its dosage form and also can be used for dissolution or similar studies.

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