Development and Validation of a Simple RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride and Rosiglitazone in Pharmaceutical Dosage Forms

Md. Akteruzzaman[1], Asma Rahman[2], Md. Zakir Sultan[2], Farhana Islam[3], Md. Abdus Salam[1] and Mohammad A. Rashid[3]

[1]Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh.
[2]Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka-1000, Bangladesh.
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

ABSTRACT: The objective of this study was to develop a simple, efficient, precise and accurate reversed phase HPLC (RP-HPLC) method for simultaneous determination of metformin in combination with rosiglitazone in newly formulated tablets. The combination of these drugs are commonly used and prescribed as anti-diabetic drugs in Bangladesh. The method has been developed by using the mobile phase comprising of sodium dihydrogen phosphate (NaH_2PO_4) buffer (pH 3.5) and acetonitrile (60:40, v/v) at a flow rate of 0.7 ml/min over C_{18} bonded silica column (ODS) (250 x 4.6 mm, 5 µ) at ambient temperature. The effluents were monitored at 230 nm and retention times were found to be 3.35 min and 11.95 min for metformin and rosiglitazone, respectively. Calibration curves were determined with a range from 0.03125 to 0.50 µmole/ml of standards and the regression coefficients (r^2) were found as 0.999 for metformin and 1.0 for rosiglitazone. The assay was validated for the accuracy, precision etc. according to ICH, USP and FDA guidelines. The proposed method can be useful in routine analysis for quantitative determination of metformin hydrochloride and rosiglitazone in pharmaceutical dosage forms.

Key words: HPLC, metformin hydrochloride, rosiglitazone, formulated tablet.

INTRODUCTION

Metformin (MET) is a drug of biguanide group. Chemically it is (N,*N*-dimethyl imidodicarbonimidic diamide). MET is also used in the treatment of type 2 diabetes mellitus. It improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis[1-6]. Rosiglitazone (ROSI) is used as an anti-diabetic drug. Chemically it is 5-[4-(2-[methyl(pyridin-2-yl)amino]ethoxy)benzyl]-thia-zolidine-2,4-dione. It acts by activation of the intracellular receptor class of the peroxisome activated receptors (PPARS)[1,7-10]. Many patients suffering from type 2 diabetes require treatment with more than one anti-hyperglycemic drug to achieve optimal glycemic control. Therefore, both of these drugs are frequently prescribed for concomitant use.[11,12] For this reason, it is necessary to have a rapid and easy method for simultaneous analysis and quantitative evaluation of these drugs in pharmaceutical dosage forms. A number of methods for individual determination of MET and ROSI have been reported in literature.[13-23] A very few papers described the simultaneous analysis of MET and ROSI.[24] However, none of these methods are free from limitations. Most of the methods used for individual analysis of MET or ROSI were complex and expensive. Therefore, development of a simple HPLC method for the estimation of MET and ROSI is highly desirable. The aim of the present study was to develop a new rapid RP-HPLC method for simultaneous quantification of combination drug MET and ROSI in pharmaceutical preparations and to validate that method according to ICH, USP and FDA guidelines.[25-31]

Correspondence to: Mohammad A. Rashid
Tel.: +88-02-9661900-73, Extn. - 8137
Fax: +88-02-8615583;
E-mail: rashidma@du.ac.bd

MATERIALS AND METHODS

Drugs and reagents. Working standards of MET and ROSI with a potency of 99.29 and 99.65%, respectively were collected from ACI Pharmaceuticals Ltd., Dhaka, Bangladesh. Sodium dihydrogen phosphate buffer (NaH_2PO_4), HPLC grade acetonitrile and methanol were purchased from Active Fine Chemicals Ltd., Dhaka, Bangladesh.

Instrumentation. High Performance Liquid Chromatographic system (Shimadzu-UFLC Prominence), equipped with an auto sampler (Model-SIL 20AC HT) and UV-Visible detector (Model-SPD 20A) was used for the analysis. The data was recorded using LC-solutions software. Phenomenex C_{18} (4.6 mm x 250 mm; 5 µm) column was used for the analysis.

Preparation of mobile phase. To prepare buffer solution of pH 3.5, sodium dihydrogen phosphate (NaH_2PO_4) (195.5 mg) was taken in a 1000 ml volumetric flask. About 500 ml of double distilled water was added into the flask, dissolved the salt and finally water was added up to the mark. Then pH was adjusted to 3.5 by adding dilute phosphoric acid. The mixture was sonicated for 10 minutes and then filtered through a 0.22 µm millipore filter. HPLC grade actonitrile was also filtered and degassed before use into the HPLC system.

Preparation of standard stock solution. Standard solutions of the pure drugs were prepared separately by dissolving 16.68 mg metformin HCl (MW of metformin HCl = 165.63, potency = 99.29%) powder equivalent to 100 µmole MET and 35.868 mg rosiglitazone (MW 357.428, potency = 99.65%) powder equivalent to 100 µmole ROSI in each 100 mL volumetric flask. The drugs were dissolved in aqueous methanol (50:50, v/v) and final volume was made up to the mark of each of the volumetric flask with the same solvent to get the concentration of 1.0 µmole/mL.

Preparation of sample solution. Ten tablets were weighed and powdered. The powders equivalent to 25.61 mg of MET and 0.133 mg of ROSI were taken in a 100 ml volumetric flask. The drugs were dissolved in about 50 ml aqueous methanol (50**:**50

v/v) and the volume was made up to the mark with same solvent. 5 ml of this solution was further diluted to 25 ml with same solvent to get the concentration of 1.0 µmole/ml and 0.003707 µmole/ml for MET and ROSI, respectively. Appropriate volumes of these solutions were further diluted with mobile phase to get different concentrations.

Chromatographic conditions. For simultaneous determination of MET and ROSI by RP-HPLC method, the mobile phase was comprised of sodium dihydrogen phosphate buffer (pH 3.5) and acetonitrile in the ratio of 60:40 (v/v) at a flow rate of 0.7 ml/min. The injection volume was 20 µl for both standard and samples. The run time was set for 15 min.

Before analysis, every standard and sample was filtered through 0.45 µm filter tips. The mobile phase was also filtered, sonicated and degassed before use. The column eluate was monitored with a UV detector at 230 nm. All analyses were done at ambient temperature under isocratic condition.

Method Validation

Specificity. The specificity of the LC method was evaluated to ensure that there was no interference from the drugs or excipients present in the pharmaceutical product. The specificity was studied by injecting the excipients and standard solutions of MET and ROSI.

Linearity. Five different solutions were prepared having concentrations of 0.03125 µmole/ml, 0.0625 µmole/ml, 0.125 µmole/ml, 0.25 µmole/ml and 0.5 µmole/ml for standard by dissolving in aqueous methanol (50:50, v/v). Then 20 µl from each solution was injected using auto-sampler and the analyses were monitored at 230 nm and repeated thrice times. The average peak areas were plotted against respective concentration. The linearity of the proposed method was evaluated by using calibration curves to calculate coefficient of correlation and intercept values.

Accuracy. The accuracy of the method is the nearness of the result obtained to the true value. The accuracy is expressed by calculating the percent recovery (R%) of analyte recovered by the assay of

spiked samples. To evaluate the accuracy of the proposed method, successive analysis (n = 3) for three different concentrations (0.0125 µmole/ml, 0.025 µmole/ml and 0.05 µmole/ml) of standard solutions of MET and ROSI were carried out by using the proposed method.

Precision. Precision of the assay was assessed with respect to both repeatability and reproducibility. The precision of an analytical method is the degree of agreement among individual test results where the method is applied repeatedly to multiple samplings. It was checked by intra and inter-day repeatability of responses after replicate injections and expressed as RSD % amongst responses using the formula [RSD (%) = (Standard deviation/Mean) x 100 %]. In the current method development and validation protocol, precision was determined by using single concentration of 0.0125 µmole/ml , 0.025 µmole/ml and 0.05 µmole/ml for both standard solutions of MET and ROSI.

Sample analysis. To quantify the active drugs in finished pharmaceutical formulation, successive analysis (n = 3) of the samples of MET and ROSI were analyzed by the proposed method and the contents were determined by using the calibration curves of the standards MET and ROSI, and the formula, y = mx + c.

Where, y = peak area of the analyzed sample, m = slope of the calibration curve, c = intercept of the calibration curve, x = concentration of the analyzed sample.

RESULTS AND DISCUSSION

HPLC is of one the most accurate analytical techniques used for qualitative and quantitative determinations of bulk and finished pharmaceutical products. A RP-HPLC method was developed and validated as per ICH, USP and FDA guidelines for determination of combined drugs (MET and ROSI) in pharmaceutical raw materials and finished products by using the mobile phase comprising sodium dihydrogen phosphate buffer (pH 3.5) and acetonitrile in the ratio of 60:40 (v/v) at a flow rate of 0.7 ml/min. The injection volume was 20 µl for both

standard and samples. The two anti-diabetic drugs (MET and ROSI) were detected at 230 nm and at ambient temperature. The retention times of MET and ROSI were found to be 3.35 ± 0.1 min and 11.95 ± 0.1, respectively (Figure 1). The specificity of the method was monitored by analyzing the placebo (containing all the ingredients of the formulation except the analyte) and a mixture solution of standards MET and ROSI. No other peak was detected close to the retention times of MET and ROSI which proved the high degree of specificity of the method (Figure 1).

When peak areas were plotted against concentration levels of 0.03125 µmole/ml, 0.0625 µmole/ml, 0.125 µmole/ml, 0.25 µmole/ml and 0.5 µmole/ml of each drug, good correlation coefficients (r^2) were obtained as 0.999 for MET and 1.0 for ROSI which were within the accepted range of guidelines and represented a good linear relationship of the newly developed method. The slopes (m) and intercepts (c) of the calibration curves were found to be as 12063935 and 80476 for MET (Figure 2A) and 2923829 and -23470 for ROSI (Figure 2B), respectively.

The accuracy was evaluated at three different concentrations with spikes which were conducted in successive analysis (n = 3) using the proposed method and the value was expressed as percentage of recovery between the mean concentrations of found and added concentration for both of these drugs. The average recoveries were found to be as 96.40%, 95.84 and 97.93% for MET for the concentration levels of 0.125 µmole/ml, 0.25 µmole/ml and 0.5 µmole/ml, respectively (Table 1); and 99.84%, 100.47 and 102.75% for ROSI for the concentration levels of 0.1255 µmole/ml, 0.2509 µmole/ml and 0.50185 µmole/ml, respectively (Table 1).

The precision of the proposed method was checked by intraday and interday repeatability of responses after replicate injections of standard solutions of different concentrations (0.0125, 0.025, 0.05 µmole/ml) three times each day for three days where RSD % amongst responses were found as ≤ 2 (Table 2 and 3).

All experimental results were wihin the range of the acceptability for precision and accuracy, which indicated that the developed method was sensitive enough and accurate for qualitative, quantitative and simultaneous analysis of MET and ROSI.

The method was applied for quantitative and simultaneous analysis of combined pharmaceutical preparation of MET and ROSI formulated by local manufacturer. The quantity of active drugs was determined for three marketed preparations (sample-1, sample-2 and sample-3) for each drug and the potency of MET was found to be 96.47, 97.24 and 97.54%, respectively; where as 103.08, 102.44 and 99.68% for ROSI, respectively (Table 4).
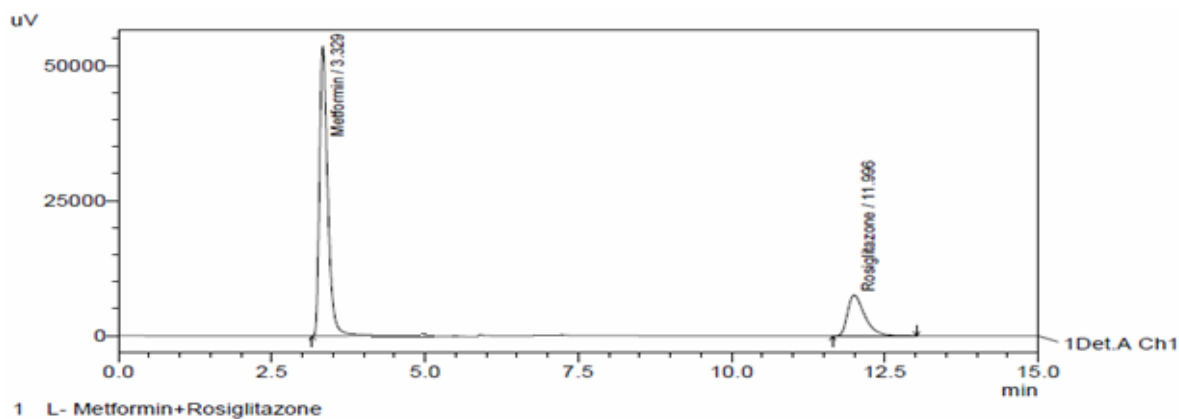

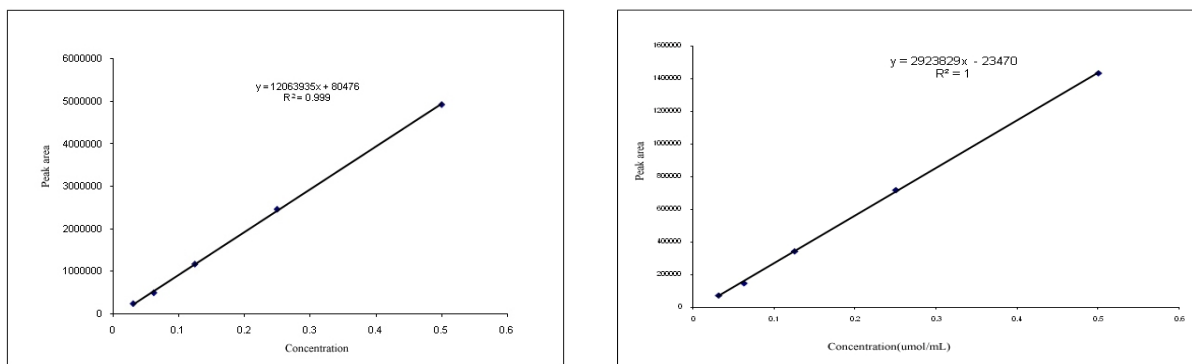Figure 1. Chromatogram of MET and ROSI.

Figure 2. Calibration curves of MET and ROSI (A = MET and B = ROSI).

Table 1. Accuracy of MET and ROSI.

Drugs	Injected concentration Std + Spike (µmole/ml)	Average Recovered (µmole/ml)	% Recovery = (Recovered conc./ injected conc.)×100
MET	0.125+ 0.0	0.1205	96.40
	0.25+ 0.0	0.23961	95.84
	0.50+ 0.0	0.48964	97.93
ROSI	0.125+ 0.0005	0.1253	99.84
	0.25+ 0.0009	0.25209	100.47
	0.50+ 0.00185	0.51567	102.75

Table 2. Intraday precision of MET and ROSI.

Drug	Day	Injected conc. μmole/ml	Mean area	SD	Intraday %RSD
MET	Day-1	0.0125	232181	1071.974	0.461698
		0.025	381772.5	1129.25	0.295791
		0.05	703665.5	1573.313	0.223588
	Day-2	0.0125	232990	1316.633	0.565103
		0.025	382640.5	2024.447	0.529073
		0.05	703073	1779.081	0.253044
	Day-3	0.0125	226313.5	1704.835	0.75330656
		0.025	370640.5	1819.38575	0.490876
		0.05	705576	8851.56269	1.25451584
ROSI	Day-1	0.0125	13443	192.333	1.43073
		0.025	53156.5	113.8442	0.214168
		0.05	121034.5	1033.083	0.853544
	Day-2	0.0125	13037.5	210.0107	1.61082
		0.025	51790.5	915.7033	1.768091
		0.05	124372.5	1956.564	1.573149
	Day-3	0.0125	13496.5	226.98127	1.68178
		0.025	51258.5	317.4909	0.619392
		0.05	122838	1043.69	0.849647

Table 3: Interday precision of MET and ROSI.

Drug	Day	Injected conc. μmole/ml	Intraday mean area	Mean	SD	Inter-day %RSD
MET	Day-1	0.0125	232181	230494.8333	3643.663	1.5808
	Day-2		232990			
	Day-3		226313.5			
	Day-1	0.025	381772.5	378351.1667	6691.722	1.768654
	Day-2		382640.5			
	Day-3		370640.5			
	Day-1	0.05	703665.5	704104.8333	1308.057	0.185776
	Day-2		703073			
	Day-3		705576			
ROSI	Day-1	0.0125	13443	13325.67	250.9892	1.883502
	Day-2		13037.5			
	Day-3		13496.5			
	Day-1	0.025	53156.5	52068.5	979.0628	1.880336
	Day-2		51790.5			
	Day-3		51258.5			
	Day-1	0.05	121034.5	122748.3	1670.806	1.361164
	Day-2		124372.5			
	Day-3		122838			

Table 4. Sample analysis.

Drug	Sample Code	Injected conc. (μmole/mL)	Mean recovered (μmole/ml)	Average % Recovery = (Mean recovered conc./ injected conc.) ×100
MET	Sample-1	0.25	0.24117	96.468
	Sample-2	0.5	0.48619	97.238
	Sample-3	1.0	0.975365	97.5365
ROSI	Sample-1	0.0125	0.012885	103.08
	Sample-2	0.025	0.02561	102.44
	Sample-3	0.05	0.04984	99.68

CONCLUSION

The validation study showed that the developed method was accurate, rapid, precise, reproducible and inexpensive with acceptable correlation co-efficient and standard deviations which make it versatile and valuable for simultaneous determination of metformin hydrochloride and rosiglitazone from pharmaceutical dosage forms, especially combined formulations. The advantages included simplicity of sample preparation and the low costs of reagents used. The proposed method was simple and did not involve laborious time-consuming sample preparation. So this HPLC method can be used in the quality control department.

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