SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE, ROSIGLITAZONE AND PIOGLITAZONE HYDROCHLORIDE IN THE TABLETS DOSAGE FORM

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ABSTRACT: A simple, specific, accurate and isocratic reversed phase liquid chromatographic method was developed and subsequently validated for the determination of metformin hydrochloride, rosiglitazone and pioglitazone hydrochloride. Separation was achieved with a Zorbax C8 column of 150×4.6 mm i.d. with 5 µm particle size and ammonium dihydrogen phosphate buffer adjusted to pH 3.0 using diluted ortho phosphoric acid and acetonitrile (65:35 v/v) as eluent at a constant flow rate of 0.7 ml per min. UV detection was performed at 215 nm. The retention time of metformin hydrochloride, rosiglitazone and pioglitazone hydrochloride were about 1.9, 3.4 and 6.7 min, respectively. This method is simple, rapid and selective and can be used for routine analysis of antidiabetic drugs in pharmaceutical preparation. It is a convenient method for separation and simultaneous determination of metformin hydrochloride, rosiglitazone and pioglitazone hydrochloride in pharmaceutical formulations.

Key words: Validation, RP-HPLC, metformin hydrochloride, rosiglitazone and pioglitazone hydrochloride.

INTRODUCTION:

Metformin hydrochloride (MET) is chemically 1,1-dimethylbiguanide hydrochloride which acts by decreasing intestinal absorption of glucose reducing hepatic glucose production and increasing sensitivity. It is official in IP[1], BP[2] and USP[3]. All the pharmacopoeias describe HPLC method for estimation of MET. A literature survey revealed spectrophotometry[4], HPLC[5-6], LC-MS/MS[7] and LC–electrospray tandem mass spectrometry[8-12] methods for simultaneous estimation of MET in pharmaceutical formulation. Rosiglitazone (ROSE) is chemically 5-(4-(2-(methyl (pyridin-2-yl) amino)ethoxy)benzyl) thiazolidine -2,4-dione. It is a thiazolidine-dione antidiabetic agent which targets insulin resistance by lowering blood-glucose level without increasing pancreatic insulin secretion. Its action is dependent on the presence of insulin. It is official in IP[13]. Literature survey indicated difference spectrophotometry[14], HPLC[15-17] , LC-MS/MS[18] and LC–electrospray tandem mass spectrometry[19] methods for ROSE in pharmaceutical formulation with other drugs. Pioglitazone hydrochloride (PIO) is chemically [(5-[[4-[2-(5-ethyl-2-yridinyl) ethoxy] phenyl] methyl]-2,4-]thiazolidine-dione mono-hydrochloride. It is a potent and highly selective agonist for the peroxisome proliferators activated receptor gamma (PPAR). Activation of these receptors promotes the production of gene products involved in lipid and glucose metabolism. It also improves insulin response to target cells without increasing pancreatic insulin secretion. Literature survey revealed HPLC[20-21] and LC–electrospray tandem mass spectrometry[22] methods for simultaneous estimation of PIO in pharmaceutical formulation with other drugs.

MATERIALS AND METHOD

The liquid chromatographic system consists of the following components: Agilent HPLC model (1100 series) containing quaternary pump, sample thermostat, column thermostat, thermostated autosampler and variable wavelength programmable detector. Chromatographic analysis was performed using Chemstation software on a Zorbax C8 column with 150 × 4.6 mm i.d. and 5 µm particle size.

The Metter Telledo electronic balance (AX 105) was used for weighing purpose. Analytically pure MET, ROSE and PIO were obtained as gift samples from my colleagues.
Acetonitrile, methanol (E. Merck, Mumbai, India), water (TKA water purification system) were of HPLC grade, while ortho phosphoric acid and ammonium dihydrogen phosphate (S.D. Fine Chemicals, Mumbai, India) were of Analytical grade used for the preparation of mobile phase. Three commercial formulations each of metformin hydrochloride (GLYCOMET TABLETS, USV Limited. Mumbai, India), rosiglitazone (REGLIT TABLETS, Dr.Reddy's Laboratories Limited. Hyderabad, India) and pioglitazone hydrochloride (DAIVISTA TABLETS, Dr.Reddy's Laboratories Limited. Hyderabad, India) were selected from local market on random basis.

Preparation of reagent and solution

Ammonium dihydrogen phosphate $(NH_4)H_2PO_4$ was weighed (2.30 g) and dissolved in 1000 ml water. Finally the pH 3.0 was adjusted with diluted ortho phosphoric acid. The buffer solution was sonicated for about 10 minutes and filtered using 0.45 µ filter paper.
Mobile phase composition: 65 volume of buffer and 35 volume of acetonitrile were mixed. MET, ROSE and PIO were weighed (50 mg of each) and transferred to three separate 50 ml of volumetric flasks and dissolved in water: acetonitrile (1:1) which gives 1000 µg/ml each of MET, ROSE and PIO respectively. These stock solutions were further diluted with diluent to obtain final concentration of 100 µg/ml.

Optimization of experimental condition

An isocratic reversed phase C8 column equilibrated with mobile phase. Mobile phase flow rate was maintained at 0.7 ml per min. and effluents were monitored at 215 nm. The sample was injected using a 10 µl fixed loop and the total run time was 10 min. Appropriate aliquots of MET, ROSE and PIO stock solutions were taken in 10 ml volumetric flasks and diluted up to the mark with diluent to obtain final concentration of 50, 75, 100, 125, 150 µg/ml of MET, ROSE and PIO respectively. The solutions were injected using 10 µl fixed loop system and chromatograms were recorded.

Limit of detection and limit of quantification

A calibration curve was prepared using concentrations in the range of 0.05-0.150 mg/ml for MET, ROSE and PIO. The standard deviations of y-intercepts of regression lines were determined and kept in the following equation for the determination of detection limit and quantitation limit. Detection limit = 3.3σ /s; quantitation limit = 10σ /s; where in σ is standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve. Detection limit and quantitation limit could also be estimated using signal to noise and relative standard deviation method.

Precision and accuracy of method (recovery studies)

The content of twenty tablets were taken and weighed. Powder equivalent to 50 mg of MET, ROSE and PIO respectively were accurately weighed and transferred to three separate 50 ml volumetric flasks and 30 ml of diluent was added to the same and flasks were sonicated for 5.0 min. The flasks were shaken and the volume was diluted up to the mark with the same mixture. The above solutions were filtered using Whatman filter paper (No. 1).Appropriate volume of the aliquots of MET, ROSE and PIO stock solutions were taken in different 50 ml volumetric flasks and the final volume was made up to the mark with diluent to obtain 120,100 and 80 µg/ml of MET, ROSE and PIO respectively. The accuracy of the method was determined by calculating recoveries of MET, ROSE and PIO using standard solutions.

RESULTS AND DISCUSSION

Optimization of mobile phase or chromatographic condition was performed based on resolution, tailing factor, symmetric factor and peak areas obtained for MET, ROSE and PIO are shown in Table 1.

The mobile phase 0.02M ammonium dihydrogen phosphate adjusted to pH 3.0 using diluted ortho phosphoric acid and acetonitrile in the composition (65:35) was found to be satisfactory. This mobile phase composition gave symmetric and well-resolved peaks for MET, ROSE and PIO. The resolution between the MET and ROSE was found to be 9.99 and resolution between ROSE and PIO was found 13.14, which indicates good separation of these three compounds. The retention time for MET, ROSE and PIO were about 1.9 min, 3.4 min and 6.7 min respectively (figure 1). The symmetric factors for MET, ROSE and PIO were 0.64, 0.57 and 0.78, respectively. Overlain UV spectra of MET, ROSE and PIO showed that these drugs absorbs appreciably at 215 nm. Hence 215 nm was selected as the detection wavelength in the liquid chromatography. The data of regression analysis of the calibration curves are shown in Table 2. The detection limit for MET, ROSE and PIO were 0.19 µg/ml. The quantification limit for MET, ROSE and PIO were 0.58 µg/ml, which suggest that these compounds can be estimated accurately. The system suitability parameters are summarized in Table 3. The linearity or calibration curves for MET, ROSE and PIO were obtained by plotting the peak areas of MET, ROSE and PIO versus concentrations over a range of 50, 75, 100, 125, 150 µg / ml of MET, ROSE and PIO respectively are shown in Table 4. MET was found to be linear with correlation coefficient (r) = 0.999. Similarly the calibration curves for ROSE and PIO were found to be linear with correlation coefficient (r) = 0.999 and 0.999 respectively (figures 2, 3 and 4).

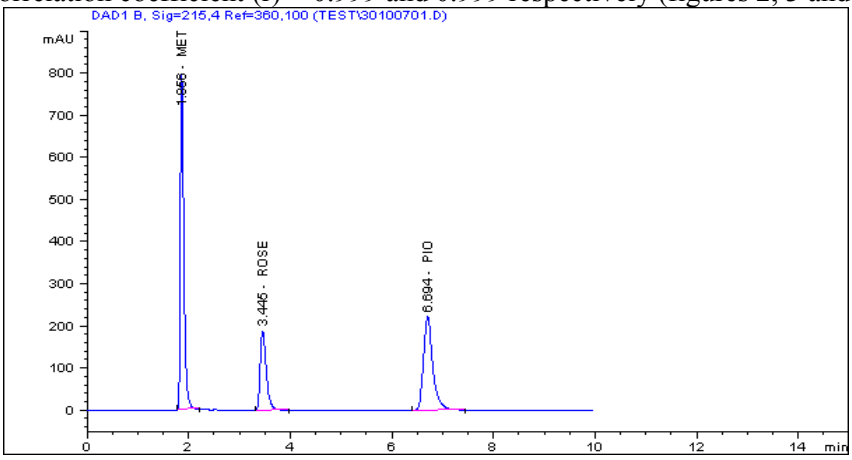

Figure 1: Typical HPLC chromatogram of MET, ROSE and PIO

Chromatogram showing well resolved peaks of MET is metformin Hydrochloride, ROSE is rosiglitazone and PIO is pioglitazone Hydrochloride.

TABLE 1: OPTIMIZATION OF EXPERIMETNTAL CONDITIONS

Parameters	Optimized condition
Chromatograph	Agilent HPLC
Column	Zorbax C-8 , 150 x 4.6 mm , 5µm
Mobile phase	20 mM $(NH_4)H_2PO_4$ pH-3.0 : Acetonitrile (65:35 v/v)
Flow rate	0.7 ml per min.
Detection	UV at 215 nm
Injection volume	10 µl
Temperature	Ambient
Run Time	10.0 min.
Retention time – MET	About 1.9 min.
Retention time – ROSE	About 3.4 min.
Retention time – PIO	About 6.7 min.

* Buffer filtered through a 0.45µ membrane filter (Millipore), degassed and sonicated.
MET is Metformin Hydrochloride, ROSE is Rosiglitazone and PIO is Pioglitazone Hydrochloride.

TABLE 2: REGRESSION ANALYSIS OF THE CALIBRATION CURVES FOR THE PROPOSED METHOD

Parameters	MET	ROSE	PIO
Linearity range (µg/ml)	50 – 150	50 – 150	50 – 150
Slope	37885.7	18846.0	27796.8
Intercept	850.03	426.0	618.7
Standard deviation of slope	2187.1	1089.3	1602.2
Regression (R^2)	0.999	0.999	0.999
Correlation coefficient (r)	0.999	0.999	0.999

MET is metformin hydrochloride, ROSE is rosiglitazone and PIO is pioglitazone hydrochloride.

TABLE 3: SYSTEM SUITABILITY PARAMETER

Parameters	MET	ROSE	PIO
Linearity range (µg/ml)	50 – 150	50 – 150	50 – 150
Correlation coefficient (r)	0.999	0.999	0.999
Theoretical plates (meter)	14750	17260	28897
Resolution	---	9.99	13.14
Tailing factor	1.4	1.5	1.3
Symmetry factor	0.64	0.57	0.78
Detection limit (µg/ml)	0.19	0.19	0.19
Quantification limit (µg/ml)	0.58	0.58	0.58

MET is metformin hydrochloride, ROSE is rosiglitazone and PIO is pioglitazone hydrochloride.

TABLE 4: LINEARITY OR CALIBRATION CURVES

Concentration ppm	MET	ROSE	PIO
	Average .Area	Average .Area	Average .Area
50	1843.2	910.67	1327.24
75	2719.68	1349.53	1979.93
100	3671.44	1834.48	2692.54
125	4568.47	2322.7	3451.84
150	5654.52	2779.83	4065.88

MET is Metformin Hydrochloride, ROSE is Rosiglitazone and PIO is Pioglitazone Hydrochloride.

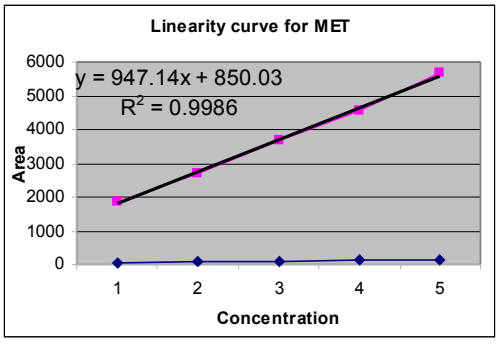

Figure 2: Linearity curve for MET

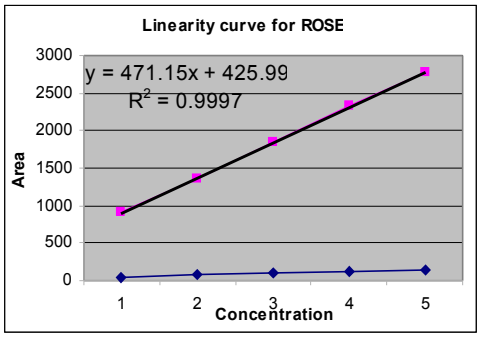

Figure 3: Linearity curve for ROSE

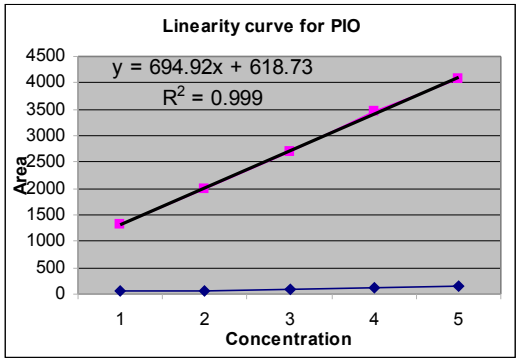

Figure 4: Linearity curve for PIO

The recovery studies of MET, ROSE and PIO were found in the range of 99.7 to 99.8, 99.6 to 99.9 and 99.6 to 100.5 % respectively. The recovery of MET was found in tablets in the range of 498.5 mg to 499.0 mg. The recovery of ROSE was found in the range of 3.984 mg to 3.996 mg and PIO was found in the range of 29.90 mg to 30.15 mg. The chromatographic method was applied to the determination of MET, ROSE and PIO in their tablets dosage form. The results for MET, ROSE and PIO were comparable with their corresponding labeled amounts are shown in Table 5.

Proposed study describes a new RP-HPLC method for the estimation of MET, ROSE and PIO combination in mixture using simple mobile phase with low buffer concentration compared to the reported method. The method gives good resolution between these three compounds with a short analysis time (less than 10 min.). The method was validated and found to be simple, sensitive, accurate and precise. Percentage of assay recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of MET, ROSE and PIO in their tablets dosage form.

TABLE 5: ASSAY OF COMBINED DOSAGE FORM AND RECOVERY STUDIES

Drugs	Level	Average assay recovery (%)	Amount obtained mg per tablets	Labeled amount mg per tablets
MET	I	99.8	499.0	500.0
	II	99.7	498.5	
	III	99.8	499.0	
ROSE	I	99.6	3.984	4.0
	II	99.9	3.996	
	III	99.8	3.992	
PIO	I	99.6	29.90	30.0
	II	100.5	30.15	
	III	99.7	29.91	

MET is Metformin Hydrochloride, ROSE is Rosiglitazone and PIO is Pioglitazone Hydrochloride.

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REFERENCES

1. Indian Pharmacopoeia, Government of India, Ghaziabad: The Indian Pharmacopoeia Commission: (2007). Vol.2; 1358.
2. British Pharmacopoeia, Her. Majesty's Stationary Office. London: UK. (2009). Vol.I and II; 3813.
3. The United States Pharmacopoeia, 31st Revision. US Pharmacopoeial convention. Inc. Rockville, MD. (2008); 2640.
4. Jain AK, and Agrawal R K (2002). Indian J. Pharm. Sci., Vol.64 (1) 88-91.
5. S. AbuRuz, J.Millership and J.McElnay (2005). Journal of Chromatography B. Vol.817 (2) 277-286.
6. Hossein Amini, Abolhassan Ahmadiani and Parisa Gazerani.(2005) J Chromatogr B. Vol. 824(1-2) 319-322.
7. Cristina Georgita, Florin Albu, Victor David and Andrei Medvedovici.(2007). J Chromatogr B. Vol. 854 (1-2) 211-218.
8. Osadebe PO, Akabogu IC.(2004). Boll Chim Farm. Vol. 143 (4) 170-3.
9. Marques MA, Soares Ade S, Pinto OW, Barroso PT, Pinto DP, Ferreira-Filho M, et al. (2007). J Chromatogr B. Vol. 852 (1-2) 308-316.
10. Mistria HN, Jangid AG.(2007). J Pharm Biomed Anal.Vol.45 (1) 97-106.
11. Wang Y, Tang Y, Gu J, Fawcett JP, Bai X.(2004). J Chromatogr B. Vol. 808 (2) 215-219.
12. Chen X, Gu Q, Qiu F, Zhong D (2004). J Chromatogr B Vol. 802 (2) 377-381.
13. Indian Pharmacopoeia,Government of India, Ghaziabad: The Indian Pharmacopoeia Commission:(2007) Vol. 3 ;1674.
14. Gomes P, Steppe M. (2006). Journal of AOAC. Vol. 89 (5) 1296-9.
15. Radhakrishna T, Satyanarayana J, Satyanarayana A. (2002). J Pharm Biomed Anal. Vol. 29 (5) 873-800.
16. Venkatesh P, Harisudhan T, Choudhury H, Mullangi R, Srinivas NR.(2006). Biomed Chromatogr. Vol. 20 (10)1043-1048.
17. Hruska MW, Frye RF (2004). J Chromatogr B Vol. 803 (2) 317-320.
18. He J, Hu YF, Duan LF, Tan ZR, Wang LS, Wang D, et al.(2007). J Pharm Biomed Anal Vol. 43 (2) 580-585.
19. Zhang L, Tian Y, Zhang Z, Chen Y.(2007). J Chromatogr B. Vol. 854 (1-2) 91-98.
20. Kolte BL, Raut BB, Deo AA, Bagool MA, Shinde DB.(2004). J Chromatogr Sci. Vol.42 (1) 27-31.
21. Sripalakit P, Neamhom P, Saraphanchotiwitthaya A. (2006). J Chromatogr B.Vol. 843 (2) 164-69.
22. Ho EN, Yiu KC, Wan TS, Stewart BD, Watkins KL.(2004). J Chromatogr B. Vol. 811(1) 65-73.

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