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Research Article

RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride and Gliclazide as API and in Synthetic Mixture

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ABSTRACT
A new, simple, accurate, precise and selective reverse phase-high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide as API and in synthetic mixture is developed. The determination was carried out on a ODS, (250 X 4.6 mm, 5 µm) column using a mobile phase of buffer solution: acetonitrile (55:45 % v/v, pH 5.0). The flow rate was 1.0 ml/min with detection at 230 nm. The retention time for Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide were 2.11 min, 8.6 min and 10.49 min respectively. Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazidel showed a linear response in the concentration range of 50-350 µg/ml, 1.5- 10.5 µg/ml and 6- 42 µg/ml respectively. The results of analysis have been validated statistically and by recovery studies. The mean recoveries found for Metformin hydrochloride was 99.05%, Pioglitazone hydrochloride was 99.91% and Gliclazide was 99.26%. Developed method was found to be simple, accurate, precise and selective for simultaneous estimation of Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide as API and in synthetic mixture.

Keywords: Simultaneous estimation, RP-HPLC method, Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide.

INTRODUCTION

Metformin hydrochloride (MET) is chemically 1-(3-azabicyclo [3.3.0] oct-3-yl)-3-pmethylpheylsulphonylurea (Fig. 1). Metformin Hydrochloride is an anti-diabetic drug[1]. Metformin is considered a cornerstone in the treatment of diabetes and is the most frequently prescribed first line therapy for individuals with type 2 diabetes[2]. In addition, it is one of a few antihyperglycaemic agents associated with improvements in cardiovascular morbidity and mortality[3,4], which is a major cause of death in patients with type 2 diabetes[5]. Several analytical method have been reported for quantitative determination of Metformin hydrochloride by UV[6], TLC[7], HPLC[8], RP-HPLC[9]. Pioglitazone hydrochloride (PIO) is chemically [(±)-5-[[4-[2-[5-ethyl -2- pyridinyl) ethoxy] phenyl]- methyl]-2,4-] thiazolidinedione mono hydrochloride(Fig. 1). Pioglitazone is a Thiazolidine Dione derivative and it is an anti-diabetic preparation[10-13]. Pioglitazone selectively stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) and to a lesser extent PPAR-α[14,15]. Gliclazide (GLE) is chemically 1-(3-azabicyclo [3.3.0] oct-3-yl)-3-pmethylpheylsulphonylurea and act as an oral hypoglycemic (anti-diabetic drug) and is classified as a Sulfonylurea[16,17] (Fig. 1). Gliclazide was proven to protect human pancreatic beta-cells from hyperglycemia-induced apoptosis[18]. It was also shown to have an antiatherogenic effect (preventing accumulation of fat in arteries) in type 2 diabetes. MET, PIO and GLE official in IP[19], BP[20] and USP[21]. Although many methods have been reported in the literature for the estimation of Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide individually like by titrimetric, UV, reverse phase-high performance liquid chromatography (RP-HPLC). But there is no single method reported for simultaneous estimation of these drugs in combined dosage form. Hence, in the present research a new, simple, rapid, accurate, precise and specific RP-HPLC method is developed and validated for simultaneous estimation of MET, PIO and GLE as API and in synthetic mixture.

Chemicals and reagents

Reference standards of MET, PIO, and GLI were obtained as a gift sample from Amol Pharmaceuticals, Jaipur, India. The solvents used were of HPLC grade.

Instrumentation

Liquid chromatographic Shimadzu (LC-2010C $_{HT}$) system was manufactured by Waters Ltd, Japan, and is equipped with auto-sampler, UV detector, and Rheodyne injector with 20 µl loop volume. UV-1800 spectrophotometer manufactured by Shimadzu, corp. Japan. Weighing was done on a Digital Micro Balance an AY- 220 analytical balance, and pH of buffer was maintained by DPH-115 PM pH analyzer.

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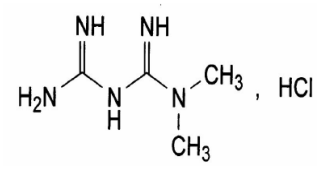
Table 1: system sutability parameters.

S. No.	System suitability parameter	Metformin Hydrochloride	Gliclazide	Pioglitazone Hydrochloride	ICH limits
1.	Retention time	2.119	8.603	10.494	-
2.	Resolution	-	11.349	2.605616	>2
3.	Asymmetry factor	1.02	1.54	1.50	< 2
4.	Tailing factor	1.02	1.54	1.50	< 2
5.	Capacity factor	1.1194	7.6028	9.4940	1-10
6.	Plate count	910.72	2559.2	3531.11	>2000



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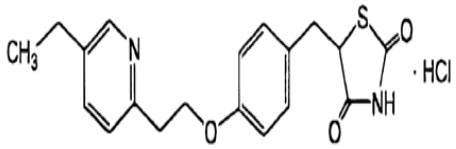

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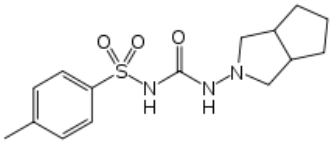

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Figure 1: Chemical structures of Metformin hydrochloride(I), Pioglitazone hydrochloride(II) and Gliclazide(III).

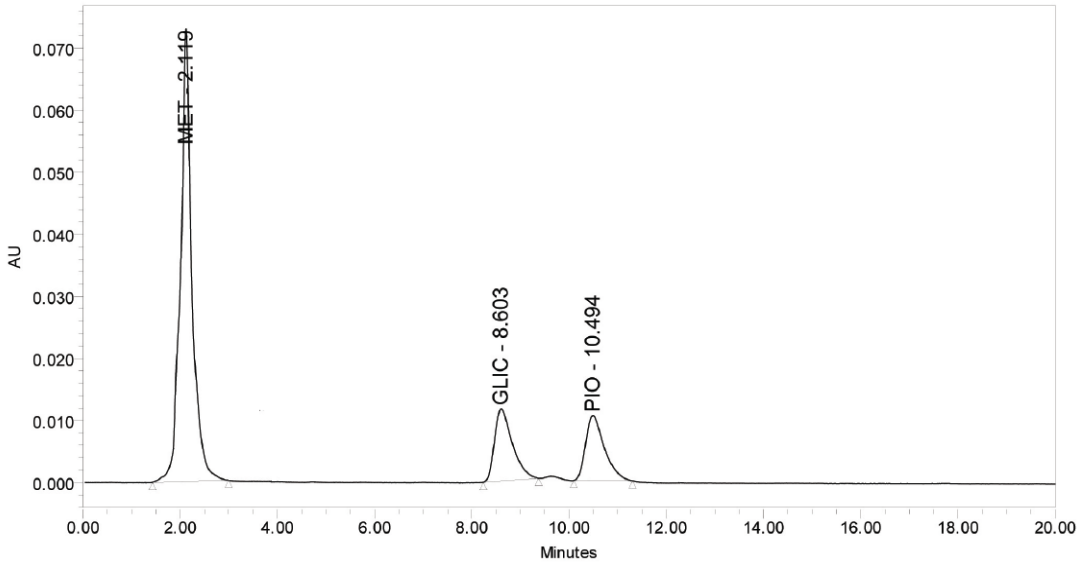

Figure 2: Typical chromatogram of MET, GLE and PIO.
Chromatogram showing retention time 2.11, 8.8 and 10.49 for Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide respectively.

Chromatographic conditions

The determination was carried out on a ODS, (250 X 4.6 mm, 5 µm) column using a mobile phase of buffer solution: acetonitrile (55:45 % v/v, pH 5.0). The flow rate was 1.0 ml/min with detection at 230 nm. By considering the chromatographic parameter, sensitivity, and selectivity of the method for each of three drugs, 230 nm was selected as the detection wavelength for UV- detector. The HPLC system was operated at a room temperature of 25°C.

Standard stock solution

The UV- Spectra of three drugs were taken by dissolving 10mg of pure drug into methanol in 10 ml to yield a stock solution of 1000 µg /ml. From this 1 ml of stock solution was taken and diluted to 10 ml with the same solvent to yield standard dilution of 100 µg/ml. From this 1 ml of stock solution was taken and diluted to 10 ml with the same solvent to yield standard dilution of 10 µg/ml.

Working standard solution

1 ml of stock solution was taken and diluted to 10 ml with the mobile phase to yield standard dilution of 100 µg/ml. From this 1 ml of stock solution was taken and diluted to 10 ml with the mobile phase to yield standard dilution of 10 µg/ml.

Preparation of sample solution

From the stock solution **(**1000 µg/ml) of all three drugs, 5ml Metformin hydrochloride, 0.15 ml Pioglitazone

Table 2: recovery studies with sample solution.

Drug	Amount addeed (µg/ml)	Recovery (%)	Mean±SD
Metformin	0.8	99.10	99.73± 0.555
	1.0	99.08	99.05± .020
	1.2	99.22	99.24± .025
Pioglitazone	0.8	100.49	100.70 ±0.210
	1.0	99.91	99.91± 0.045
	1.2	100.72	100.72± 0.201
Gliclazide	0.8	100.81	100.8 ± 0.105
	1.0	99.34	99.26± 0.087
	1.2	100.61	99.92±0.829941

SD is standard deviation for n=6 observations

Table 3: Results of Formulation Analysis.

Name of product	Metformin hydrochloride	Pioglitazone hydrochloride	Gliclazide
Claim Amount	500 mg	15 mg	60mg
Found Amount	497.957	14.180	58.834
% Purity	98.37	98.67	98.40
SD	0.146363	0.075830	0.0805536
RSD	0.148778	0.076885	0.0818579

SD is standard deviation for n=6 observations.
RSD is relative standard deviation for n=6 observations.

hydrochloride and 0.6 ml Gliclazide was taken and transferred to the 10 ml volumetric flask and volume was made upto the mark by same mobile phase yield 500 µg/ml, 15 µg/ml and 60 µg/ml, then it was filtered through 0.45 micron whatman filter paper.

Ten microlitre solution of the each drug was injected separately and chromatograms were recorded. A representative chromatogram is shown in fig 2. The retention time for Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide were 2.11 min, 8.6 min and 10.49 min respectively. The peak shapes of all the drugs were symmetrical and asymmetry factor was less than 2. The proposed method was validated as per the standard analytical procedure. Each sample was repeated 6 times and the same retention time was observed in all the cases. Linearity experiments performed by giving solution for these drugs and response was found to be linear in the range of 50-350 µg/ml for Metformin hydrochloride, 1.5-10.5 µg/ml for Pioglitazone hydrochloride and 6- 42 µg/ml for Gliclazide. Each standard solution (10 µl) was injected into the column after filtration using 0.45 µm membrane constructed by plotting filter. The calibration curve was constructed by plotting the peak area versus the corresponding drug concentration. The slope and correlation coefficient were determined, which were found to be 0.99995 for MET, 0.9992 for PIO and 0.99997 for GLE. In precision studies, the injection repeatability shows a RSD of 0.164%, 0.720% 0.450%for MET, PIO and GLE. The intra day RSD shows 0.140% for MET, 0.169% for PIO and 0.864% for GLE and inter day showed RSD of 0.235% for MET, 0.437% for PIO and 0.575% for GLE. These results indicate good precision of the sample analyzed. Accuracy was calculated by recovery studies (n=3) at five levels. Standard drug solutions containing drugs in the concentration range 5 µg/ml for all three days. The mean recoveries found for Metformin hydrochloride was 99.05%, for Pioglitazone hydrochloride was 99.91% and for Gliclazide was 99.26%. The data of result of marketed formulation analysis is shown in table no.3. The result of the study indicates the proposed HPLC method was simple, precise, accurate, and selective.

CONCLUSION

The developed RP-HPLC method is new, simple, sensitive, accurate, precise and appears to be suitable for routine analysis of MET, PIO and GLE in combined dosage form.

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REFRENCES

1. Tripathi, KD. Essentials of Medicinal Pharmacology, 5th ed.; Jaypee Brothers Medicinal Publication (P) Ltd: New Delhi, 2004, 235-253.
2. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin : an update. Ann Intern Med 2002; 137:25-33.
3. UK Prospective Diabetes Study (UKPDS) Group. Effe ct of intensive blood-glucose control with metformin o n complications in overweight patients with type 2 dia betes (UKPDS 34). Lancet 1998; 352:854-65.
4. Kooy A, de Jager J, Lehert P, Bets D, Wulffele MG, D onker AJM, et al. Long-term effects of metformin on metabolism and microvascular and macrovascular dise ase in patients with type 2 diabetes mellitus. Arch Inte rn Med 2009; 169:616-25.
5. Pyorala K, Laakso M, Uusitupa M. Diabetes and ather osclerosis: an epidemiologic view. Diabetes Metab Re v 1987; 3:463-524.
6. Patil, SS and Bonde CG. Development and Validation of analytical method for Simultaneous Estimation of G libenclamide and Metformin HCl in Bulk and Tablets using UV – visible spectroscopy. International Journal of Chem Tech Research. 2009, 1, 905-909.
7. Dhabale, PN and Seervi, CR. Simultaneous UV Spectr ophotometric Method for Estimation of Gliclazide and Metformine Hydrochloride in Tablet Dosage Form. In ternational Journal of Chem Tech Research. 2002, 2, 8 13-817.
8. Lakshmi, KS, Rajesh, T and Sharma S. Simultaneous d etermination of Metformin and Pioglitazone by Revers e Phase HPLC in pharmaceutical Dosage Forms. Inter national Journal of Pharmacy and Pharmaceutical Scie nce. 2009, 1, 162-166.
9. Jain, HK and Agrawal, RK. Simultaneous estimation o f Gliclazide and Metformin hydrochloride in combine d dosage form. Indian Journal of Pharmaceutical Scie nces. 2002, 88- 91.

10. Chaturvedi, PK and Sharma, R. Development and Vali dation of an RP-HPLC Method for Simultaneous Anal ysis of a Three-Component Tablet Formulation Contai ning Metformin Hydrochloride, Pioglitazone Hydroch loride, and Glibenclamide. Acta Chromatographica. 20 08, 3, 451–461.
11. Srinivasulu, D, Sastry, BS and Omprakash, G. Develo pment and Validation of New RP-HPLC Method for D etermination of Pioglitazone HCl in Pharmaceutical D osage Forms. International Journal of Chemistry. 2010 , 1, 1820.
12. Praveenkumar, RB, Boopathy, D, Bibin, M, Prakash, M and Perumal P. Method Development and Validati on of Simultaneous Determination of Pioglitazone and Glimepiride in Pharmaceutical Dosage Form by RP-H PLC. International Journal of Chemtech Research. 201 0, 2, 50-53.
13. Karthik, A, Subramanian, G, Mallikarjuna, RC, Krishn amurthy, B, Ranjit KA, Musmade, P, *et al.* Simultaneo us Determination of Pioglitazone and Glimepiride in B ulk Drug and Pharmaceutical Dosage Form by RP-HP LC Method. Pakistan Journal of Pharmaceutical Scien ce. 2008, 21, 4, 421-425.
14. Smith U. Pioglitazone: mechanism of action. Int J Clin Pract Suppl, 2001, (121): 13–8.
15. Colca, JR, McDonald, WG, Waldon, DJ, Leone, JW, L ull, JM, Bannow, CA, *et al.* Identification of a novel m itochondrial protein ("mitoNEET") cross-linked specif ically by a thiazolidinedione photoprobe. Am. J. Physi ol. Endocrinol. Metab., 2003, 286 (2): E252–60.
16. Rathinavel, G, Umanath, U, Valarmathy, J, Samueljos hua, L, Selvin, TC, Ganesh, M, *et al.* RP-HPLC Metho d for the Simultaneous Estimation of Rosiglitazone an d Gliclazide in Tablets. E-Journal of Chemistry. 2009 , 6(4), 1188-1192.
17. Gillies, PS and Dunn, CJ. Pioglitazone. Drugs. 2000, 6 0 (2): 333–43; p.no.344–5.
18. Smith U. Pioglitazone: mechanism of action. Int J Clin Pract Suppl. 2001, (121): 13–8.
19. Indian Pharmacopoeia, 2007, Published by Ministry of Health and Family Welfare, Government of India, Vol ume II & III, 1416, 1657, 1916.
20. British Pharmacopoeia, Vol. I, H.M. Stationary Office , London, 2007, 638.
21. United State Pharmacopoeia, 2007, Published by Boar d of trustees, United State Pharmacopoeial Convention INC, Rockhill, MD, 2595.