Simultaneous Estimation of Glimepiride, Rosiglitazone and Pioglitazone Hydrochloride in the Pharmaceutical Dosage Form

FREDDY H. HAVALDAR* and DHARMENDRA L. VAIRAL

Nadkarny-Sacasa Research Laboratory, Department of Chemistry,
St.Xavier's College, Mumbai - 400 001, India.

vairal21@yahoo.com

Received 5 February 2010; Accepted 2 April 2010

Abstract: A simple, specific, accurate and economical gradient reversed phase liquid chromatographic (RP-HPLC) method was developed and subsequently validated for the determination of glimepiride, rosiglitazone and pioglitazone hydrochloride. Separation was achieved with a nucleodur C-18 column having 250X4.6 mm i.d. with 5 μ m particle size and water HPLC grade adjusted to pH 3.0 using diluted orthophosphoric acid and acetonitrile (80:20 v/v) with gradient program as eluent at a constant flow rate of 0.8 ml per min. UV detection was performed at 215 nm. The retention time of glimepiride, rosiglitazone and pioglitazone hydrochloride were about 17.9 min, 6.31 min and 8.24 min respectively. This method is simple, rapid and selective and can be used for routine analysis of antidiabetic drugs in pharmaceutical preparation. The proposed method was validated and successfully used for estimation of glimepiride, rosiglitazone and pioglitazone hydrochloride in the pharmaceutical dosage form.

Keywords: Validation, RP-HPLC, Glimepiride (GLI), Rosiglitazone (ROSE), Pioglitazone hydrochloride (PIO).

Introduction

The purpose of this method is to develop the reversed phase liquid chromatographic technique (RP-HPLC) method for simultaneous determination of antidiabetic drugs - glimepiride, rosiglitazone and pioglitazone hydrochloride in pharmaceutical formulations. It is essential to have a validated, stability indicated or specific analytical method of analysis for the drug for which the drug delivery system is to be designed. If the suitable analytical method is not available then it is essential to develop a simple, specific, accurate and economical analytical method for the selected drug to aid during various steps of formulation design. Reversed phase liquid chromatographic technique (RP-HPLC) is one of the latest and most widely applied techniques for drug estimation. This method is simple, rapid and selective. The proposed method was validated and successfully used for determination of

glimipiride, rosiglitazone and pioglitazone hydrochloride in the pharmaceutical dosage form. A literature survey regarding quantitative analysis of these drugs revealed that attempts were made to develop analytical methods for glimepiride, rosiglitazone and pioglitazone hydrochloride using spectrophotometry¹⁻³, high performance liquid chromatography (HPLC)⁴⁻²⁰, have been reported for the determination of glimepiride, rosiglitazone and pioglitazone hydrochloride. LC-MS/MS²¹ and LC - electrospray tandem mass spectrometry²²⁻²⁴ have been reported for determination of glimepiride, rosiglitazone and pioglitazone hydrochloride. Glimepiride is official in pharmacopoeia and high performance liquid chromatography (HPLC) method had been reported for its estimation. This paper describes a new reversed phase high performance liquid chromatography (RP-HPLC) method for the estimation of glimepiride, rosiglitazone and pioglitazone hydrochloride combination in mixture using simple mobile phase.

Experimental

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 Nucleodur C-18 column with 250x4.6 mm i.d. and 5 μ m particle size. The Mettler Telleo electronic balance (AX 105) was used for weighing purpose. Analytically pure Glimepiride, Rosiglitazone and Pioglitazone hydrochloride were obtained as gift samples from my colleague Mr. Haresh Gurav (Asst. Manager QA/RA Dept. - Nicholas Piramal India Ltd. Mumbai, India). Acetonitrile, methanol (E. Merck, Mumbai, India), water (TKA water purification system - Germany) were of HPLC grade and ortho phosphoric acid (S.D. Fine Chemicals, Mumbai, India) were of analytical grade used for the preparation of mobile phase. Three commercial formulations each of Glimepiride (Glimetop-1 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

Water HPLC grade. Finally the pH 3.0 was adjusted with diluted ortho phosphoric acid. The mobile phase solution was sonicated for about 10 minutes and filtered through 0.45 μ filter paper.

Mobile phase composition: 80 volume of water and 20 volume of acetonitrile. Using linear gradient program. Glimepiride, rosiglitazone and pioglitazone hydrochloride 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 μ g/mL of glimepiride (GLI), rosiglitazone (ROSE) and pioglitazone (PIO) hydrochloride respectively. These stock solutions were further diluted with water: acetonitrile (1:1) to obtain final concentration of 100 μ g/mL.

Optimization of experimental condition

A linear gradient reversed phase C-18 column equilibrated with mobile phase water-acetonitrile (80:20) was used with gradient program initial 80:20 at 20 min. 20:80 at 21 min. 80:20 and stable up to 30 min. with 80:20 ratio. Mobile phase flow rate was maintained at 0.8 mL per min. and effluents were monitored at 215 nm. The sample was injected using a 10 μ L fixed loop and the total run time was 30 min. Appropriate aliquots of glimepiride, rosiglitazone and pioglitazone hydrochloride 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 μ g/mL of glimepiride, rosiglitazone and pioglitazone hydrochloride respectively. The solutions were injected using 10 μ L fixed loop system and chromatograms

were recorded. Calibration curves were constructed by plotting average peaks *versus* concentrations. Correlation coefficients were computed for glimepiride, rosiglitazone and pioglitazone hydrochloride.

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 glimepiride, rosiglitazone and pioglitazone hydrochloride. 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 = $3.3\sigma/s$; quantitation limit = $10\sigma/s$; 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 can also be estimated using signal to noise and relative standard deviation method.

Linearity studies

The content of twenty tablets were taken and weighed. Powder equivalent to 50 mg of glimepiride, rosiglitazone and pioglitazone hydrochloride respectively were accurately weighed and transferred to three separate 100 mL volumetric flasks and 50 mL of diluent was added to the same and the flasks were sonicated for 15.0 min. The flasks were shaken and the volume was diluted up to the mark with the same mixture. The above solution was filtered using Whatman filter paper (No.1). Appropriate volume of the aliquots of glimepiride, rosiglitazone and pioglitazone hydrochloride stock solutions were taken in different 10 mL volumetric flasks and the volume was made up to the mark with diluent to obtain final concentration of 50, 75, 100, 125, 150 $\mu\text{g/mL}$ of glimepiride, rosiglitazone and pioglitazone hydrochloride respectively. The solutions were sonicated for 5.0 min. The solutions were injected at above chromatographic conditions and peak areas were measured. Calibration curves were constructed by plotting the average peak areas *versus* concentrations.

Precision and accuracy of method (Recovery studies)

Glimepiride

The content of twenty tablets of glimepiride were taken and weighed. Powder equivalent to 50 mg of glimepiride was accurately weighed and transferred to 50 mL volumetric flask and 30 mL of diluent was added to the same and flask was sonicated for 15.0 min. The flask was shaken and the volume was diluted up to the mark with the same mixture. The above solution was filtered using Whatman filter paper (No.1). Appropriate volume of the aliquot of glimepiride stock solution was taken in different 100 mL volumetric flasks and the final volume was made up to the mark with diluent to obtain 120, 100 and 80 $\mu\text{g/mL}$ of glimepiride. The drug content per tablets of the above brand of glimepiride was calculated from the absorbance values obtained (taking average of three determinations).

Rosiglitazone

The content of twenty tablets of REGLIT were taken and weighed. Powder equivalent to 50 mg of rosiglitazone was accurately weighed and transferred to 50 mL volumetric flask and 30 mL of diluent was added to the same and flask was sonicated for 15.0 min. The flask was shaken and the volume was diluted up to the mark with the same mixture. The above solution was filtered using Whatman filter paper (No.1). Appropriate volume of the aliquot of rosiglitazone stock solution was taken in different 100 mL volumetric flasks and the final volume was made up to the mark with diluent to obtain 120, 100 and 80 $\mu\text{g/mL}$ of rosiglitazone. The drug content per tablets of the above brand of rosiglitazone was calculated from the absorbance values obtained (taking average of three determinations).

Pioglitazone hydrochloride

The content of twenty tablets of DAIVISTA were taken and weighed. Powder equivalent to 50 mg of pioglitazone hydrochloride was accurately weighed and transferred to 50 mL volumetric flask and 30 mL of diluent was added to the same and flask was sonicated for 15.0 min. The flask was shaken and the volume was diluted up to the mark with the same mixture. The above solution was filtered using Whatman filter paper (No.1). Appropriate volume of the aliquot of pioglitazone hydrochloride stock solution was taken in different 100 mL volumetric flasks and the final volume was made up to the mark with diluent to obtain 120, 100 and 80 µg/mL of pioglitazone hydrochloride. The drug content per tablets of the above brand of pioglitazone hydrochloride was calculated from the absorbance values obtained (taking average of three determinations). The accuracy of the method was determined by calculating recoveries of glimepiride, rosiglitazone and pioglitazone hydrochloride 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 glimepiride, rosiglitazone and pioglitazone hydrochloride are shown in Table 1. The data of regression analysis of the glimepiride was found to be linear with correlation coefficient (r) = 1.000. Similarly the calibration curves for rosiglitazone and pioglitazone hydrochloride were found to be linear with correlation coefficient (r) = 0.998 and 0.999 respectively are shown in Table 2. The mobile phase water HPLC grade adjusted to pH 3.0 using diluted orthophosphoric acid and acetonitrile in the composition (80:20) with linear gradient was found to be satisfactory. These mobile phase composition gave symmetric and well-resolved peaks for glimepiride, rosiglitazone and pioglitazone hydrochloride. The resolution between the rosiglitazone and pioglitazone hydrochloride was found 5.58 and resolution between pioglitazone hydrochloride and glimepiride was found 38.4, which indicates good separation of these three compounds.

Table 1. Optimization of experimental conditions

Parameters	Optimized condition
Chromatograph	Agilent HPLC
Column	Nucleodur C-18 , 250 x 4.6 mm , 5 µm
Mobile phase	Water HPLC grade pH-3.0: Acetonitrile (80:20 v/v) with linear gradient program.
Flow rate	0.8 mL per min.
Detection	UV at 215 nm
Injection volume	10 µL
Temperature	Ambient
Run Time	30.0 min.
Retention time – GLI	17.9 min.
Retention time – ROSE	6.31 min.
Retention time – PIO	8.24 min.

* Water HPLC grade filtered through a 0.45 µ membrane filter (Millipore), degassed and sonicated

Table 2. Regression analysis of the calibration curves for the proposed method

Parameters	GLI	ROSE	PIO
Linearity range, µg/mL	50 - 150	50 - 150	50 - 150
Slope	27896.8	18014.8	27213.2
Intercept	644.94	715.45	650.57
Standard deviation of slope	1619.1	1188.5	1589.5
Regression (R^2)	0.9999	0.9961	0.9998
Correlation coefficient (r)	1.000	0.998	0.999

The retention time for glimepiride, rosiglitazone and pioglitazone hydrochloride were about 17.9 min, 6.31 min and 8.24 min respectively (Figure 1). The symmetric factors for glimepiride, rosiglitazone and pioglitazone hydrochloride were 0.91, 0.43 and 0.53 respectively. Overlain UV spectra of glimepiride, rosiglitazone and pioglitazone hydrochloride showed that these drugs absorb appreciably at 215 nm. Hence 215 nm was selected as the detection wavelength in the liquid chromatography (Figures 2 and 3).

Figure 1. Typical HPLC chromatogram of GLI, ROSE and PIO Chromatogram showing well resolved peaks of GLI, ROSE and PIO

Figure 2. UV overlay spectra of GLI and ROSE

Figure 3. UV overlain spectra of GLI and PIO

The detection limits for glimepiride, rosiglitazone and pioglitazone hydrochloride were 0.19 μg per mL, 0.22 μg per mL and 0.19 μg per mL respectively. The quantification limit for glimepiride, rosiglitazone and pioglitazone hydrochloride were 0.58 μg per mL, 0.66 μg per mL and 0.58 μg per mL respectively, which suggest that these compounds can be estimated accurately. The system suitability parameters are summarized in Table 3. The calibration curves for glimepiride, rosiglitazone and pioglitazone hydrochloride were obtained by plotting the peak areas of glimepiride, rosiglitazone and pioglitazone hydrochloride versus concentrations over a range of 50, 75, 100, 125 and 150 $\mu\text{g}/\text{mL}$ of glimepiride, rosiglitazone and pioglitazone hydrochloride respectively are shown in Table 4.

Table 3. System suitability parameter

Parameters	GLI	ROSE	PIO
Linearity range, $\mu\text{g}/\text{mL}$	50 - 150	50 - 150	50 - 150
Theoretical plates (meter)	394761	15055	50785
Resolution	38.4	---	5.58
Tailing factor	1.09	1.97	1.79
Symmetry factor	0.91	0.43	0.53
Detection limit, $\mu\text{g}/\text{mL}$	0.19	0.22	0.19
Quantification limit, $\mu\text{g}/\text{mL}$	0.58	0.66	0.58

Table 4. Linearity or calibration curves

GLI		ROSE		PIO	
Con.ppm	Av.Area	Con.ppm	Av.Area	Con.ppm	Av.Area
50	1348.9	50	1132.0	50	1329.2
75	2041.9	75	1611.3	75	2024.3
100	2719.5	100	2129.8	100	2668.2
125	3437.5	125	2540.4	125	3386.2
150	4138.2	150	2919.3	150	4049.9

Glimepiride was found to be linear with regression (R^2) = 1.000. Similarly the calibration curves for rosiglitazone and pioglitazone hydrochloride were found to be linear with regression (R^2) = 0.996 and 0.999 respectively shown in (Figure 4, 5 and 6). The recovery studies of glimepiride, rosiglitazone and pioglitazone hydrochloride were found in the range of 99.9 to 100.1, 100.0 to 100.2 and 100.2 to 100.3% respectively. The recovery of glimepiride was found in tablets in the range of 0.999 mg to 1.000 mg.

Figure 4. Linearity of GLI is Glimepiride

Figure 5. Linearity of ROSE

Figure 6. Linearity of PIO

The recovery of rosiglitazone was found in the range of 4.00 mg to 4.01 mg and pioglitazone hydrochloride was found in the range of 30.06 mg to 30.09 mg. The chromatographic method was applied to the determination of glimepiride, rosiglitazone and pioglitazone hydrochloride in their pharmaceutical dosage form. The results for glimepiride, rosiglitazone and pioglitazone hydrochloride were comparable with their corresponding labeled amounts are shown in Table 5.

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
GLI	I	100.1	1.00	1.0
	II	99.9	0.999	
	III	100.1	1.00	
ROSE	I	100.2	4.01	4.0
	II	100.0	4.00	
	III	100.0	4.00	
PIO	I	100.2	30.06	30.0
	II	100.2	30.06	
	III	100.3	30.09	

Conclusion

We have successfully developed a new RP-HPLC method for the estimation of glimepiride, rosiglitazone and pioglitazone hydrochloride were found to be simple, economical and useful with high accuracy, precision and low detection limit and quantitation limit. Rapidity and capability of quantifying very low concentration of respective drugs, made them useful for variety of analyses, including pure drug analysis, assay of formulations and stability studies analysis. The proposed methods did not utilize any extraction step for recovering the

drug from the formulation excipient matrixes and their by decreased the degree of error, time in estimation of drugs and the overall cost of the analysis. The solvent system used was simple mobile phase compared to the reported method. The method gives good resolution between glimepiride, rosiglitazone and pioglitazone hydrochloride. The method was validated and found to be simple, sensitive, accurate, precise and economical. 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 glimepiride, rosiglitazone and pioglitazone hydrochloride in their pharmaceutical dosage form.

Acknowledgments

I thank my friend Mr. Haresh Gurav (M/s Nicholas Piramal India Limited, Mumbai, India) for providing me the gift samples of glimepiride, rosiglitazone and pioglitazone hydrochloride.

References

1. Gomes P and Steppe M, *J AOAC International*, 2006, **89**(5), 1296-1299.
2. Altinoz S and Tekeli D, *J Pharma Biomed Anal.*, 2001, **24**, 507-515.
3. Patrica Gomes and Martin Steppe, *J AOAC Int.*, 2006, **89**, 5.
4. Radhakrishna T, Satyanarayana J and Satyanarayana A, *J Pharma Biomed Anal.*, 2002, **29**, 873.
5. Kolte B L, Raut B B, Deo A A, Bagool M A and Shinde D B, *J Chromatogr Sci.*, 2004, **42**, 27.
6. Venkatesh P, Harisudhan T, Hira Choudhury, Ramesh Mullangi, Nuggehally and Srinivas R, *Biomed Chromatogr.*, 2006.
7. Zhong W Z and Lakings D B, *J Chromatogr.*, 1989, **490**, 377.
8. Yamashita K, Murakami H, Okuda T and Motohashi M, *J Chromatogr B Biomed Appl.*, 1996, **677**, 144-146.
9. AbuRuz, S Millership J and McElnay J, *J Chromatogr B.*, 2005, **817**, 277.
10. Pattana Sripalakit, Penorn Neamhom and Aurasorn Saraphanchotiwitthaya, *J Chromatogr B.*, 2006, **843**, 164.
11. Ceren Yardimera, Nuran Ozaltma and Alper Gurlek, *Talanta*, 2007, **72**, 1416-1422.
12. Jing Yao, Ya-Qin Shi, Zhuo-Rong Li and Shao-Hong Jin, *J Chromatogr B.*, 2007, **853**, 254.
13. Wanjari D B and Gaikwad N J, *Indian J Pharm Sci.*, 2005, **67**, 253.
14. Wanjari D B and Gaikwad N J, *Indian J Pharm Sci.*, 2005, **67**, 256.
15. Khan M A, Sinha S, Vartak S, Bhartiya A and Kumar S, *J Pharma Biomed Anal.*, 2005, **39**, 928.
16. El Deeb S, Schepers U and Watzig H, *J Sep Sci.*, 2006, **29**, 1571.
17. Jedlicka A, Klimes J and Grafnetterova T, *Pharmazie*, 2004, **59**, 178.
18. Collin F, Khoury H, Bonnefont Rousselot D, Therond P, Legrand A, Jore D, Gardes M, Albert, *Rapid Commun Mass Spectrom.*, 2004, **39**, 890.
19. Osadebe P O, Akabogu I C, *Oll Chim Farm*, 2004, **143**, 170.
20. Matthew W Hruska and Reginald F Frye, *J Chromatogr B.*, 2004, **803**, 317-320.
21. He J, Hu Y F, Duan L F, Tan Z R, Wang L S, Wang D, Zhang W, Li Z, Liu J, Tu J H, Yao Y M and Hong-Hao Zhou, *J Pharma Biomed Anal.*, 2007, **43**.
22. Lu Zhang, Yuan Tian, Zunjian Zhang and Yun Chen, *J Chromatogr B*, 2007, **854**, 91.
23. Emmie N M Ho, Kenneth C H Yiu, Terence S M Wan, Brian D Stewart and Keith L Watkins, *J Chromatogr B.*, 2004, **811**, 65.
24. Yannis Dotsikas, Constantinos Kousoulos, Georgia Tsatsou and Yannis L Loukas, *Rapid Commun Mass Spectrom.*, 2005, **19**, 2055.