Stability Indicating RP-HPLC Method for Determination of Pioglitazone from Tablets

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A stability indicating simple, rapid and precise reversed-phase HPLC method has been developed for the quantitation of pioglitazone in tablet on a Hypersil C-8 (250x4.6 mm) column using a mobile phase consisting acetonitrile:0.15% v/v triethylamine (40:60 v/v) adjusted to pH 4.6 with orthophosphoric acid at a flow rate of 1.5 ml/min and detection at 220 nm. The retention time of pioglitazone have been found to be 7.6 min and recoveries were between 99-101%. Validation of the proposed method also been done.

Pioglitazone is a thiozolidinedione class of antidiabetic agents. It is selective agonists for nuclear peroxide proliferator-actived receptor-gamma. It also reduces the insulin resistance in periphery and in the liver of patients. It increases glucose transport into muscle and adipose tissue by enhancing the synthesis and translocation of specific forms of the glucose transporter proteins. Pioglitazone is not official in any pharmacopoeia. Literature survey revealed only one HPLC[1] method for its determination in pharmaceuticals and two in human serum[2,3]. The present work describes a simple, precise and accurate reversed-phase HPLC method for the estimation of pioglitazone in tablet dosage form.

All chemicals/solvents used were of AR/HPLC grade. Standard pioglitazone was provided by Sun Pharmaceutical Industries Ltd. Vadodara. A Shimadzu HPLC (LC-10AT VP) system was used for the analysis. The method was carried out on a Hypersil C-8 (250x4.6 mm) column as a stationary phase and acetonitrile:0.15% v/v triethylamine (40:60 v/v) adjusted to pH 4.6 with orthophosphoric acid as a mobile phase at a flow rate of 1.5 ml/min. A rheodyne injector with a 20-μl loop was used for the injection of samples. Detection was done at 210 nm with sensitivity 0.010 AUFS. The mobile phase was filtered through a 0.45 μ membrane filter (Millipore) and degassed. The analysis was carried out at room temperature (about 20°).

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Standard stock solution was prepared containing 1 mg/ml of pioglitazone in methanol. Subsequently dilutions were made to get the concentration about 40 μg/ml in mobile phase. Sample solution was prepared in 50 ml volumetric flask by shaking tablet powder equivalent to 25 mg of pioglitazone in methanol. This solution was filtered (Whatman No.1) and further dilution was made with mobile phase. A steady baseline was recorded with optimised chromatographic conditions. Chromatographs of standard solution (six replicates) and sample solution (two replicates of each) were recorded (one of which is depicted in fig.1). The retention time of pioglitazone was found to be 7.6 min. The concentrations of pioglitazone in sample solution were obtained by comparing with the standard solution.
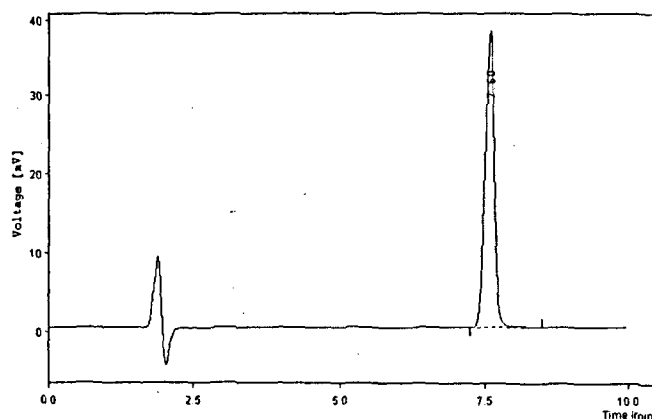

Fig. 1: Chromatogram of pioglitazone

Accuracy of the method was studied by recovery experiments. Reference standard drug at the level of 25, 50, 75 and 100% of the label claim was added to the tablet powder equivalent to 25 mg of pioglitazone. These were analysed by injecting three replicates each of sample solution and the percent recovery was calculated. Precision of the method was demonstrated by reproducibility studies. This was done analysing six samples prepared from a homogeneous sample. Specificity was carried out by exposing the samples to different stress conditions for 24 h such as acidic (0.1N HCl, 40°), basic (0.1N NaOH, 40°), oxidation (3% v/v H_2O_2, 40°), heat (60°), UV light (254 nm), and humidity (75% RH, 40°), before analysing using the proposed method and chromatograms were recorded up to 20 min for each sample. Linearity and range of the method was determined by analysing standard solutions containing 10 to 80 mg/ml (25 to 200% of targeted level of the assay concentration). The calibration curve was plotted using area under curve Vs concentration of the standard solution. Ruggedness of the method was evaluated by carrying out the experiment by different analysts and on different days. Stability of standard and sample solution was ascertained by analysing it periodically. Robustness of the method was demonstrated by variation in composition of mobile phase (±5%), concentration of buffer (±5%) and pH of mobile phase (0.1).

The chromatographic parameters were validated by system suitability studies[4] and peak asymmetry and column efficiency were determined (Table 1). The precision data shows that reproducibility of the assay procedure is satisfactory and %RSD was found to be 0.4 (Table 2). Accuracy studies indicated that the mean percent recovery of the added standard was found to be 100.2% (Table 2). The results of specificity studies indicated no interference from excipients, impurities and degradation products due to various stress conditions and assured that the peak response was due to a single component only. In basic stress condition about 25% degradation was found but there were no interference from degradation peaks. A linear relationship was obtained in the concentration range of 10-80 µg/ml with the equation 10.084X-2.2066 and correlation coefficient 0.9999. Ruggedness study signified the reproducibility of the method for different analysts and different days. The method was found to be robust with respect to theoretical plates and retention time. Limit of detection and limit of quantitation was found to be 0.6 µg/ml and 2.0 µg/ml, respectively. The proposed HPLC method was found to be simple, accurate, precise, linear, rugged and rapid. Hence this method can be applied for the routine quality control of tablet formulations.

TABLE 1: SYSTEM SUITABILITY STUDIES

Parameters	Results
Mean Peak Area	411.6
(% RSD)	(0.82)
Capacity factor	7.98
Tailing Factor	1.00
Theoretical Plates (per column)	11187

TABLE 2: ANALYSIS OF FORMULATIONS AND RECOVERY STUDIES

Brand Name (Manufacturer)	Label Claim (mg/tablet)	Estimated*		%Recovery**
		mg/tablet	%Label Claim	
Piozone (Nicholas)	15	14.98	99.9 (0.4)	99.4 (0.5)
	30	30.02	100.7 (0.3)	100.0 (0.6)
Glito	15	15.05	100.3 (0.6)	100.6 (0.5)
(Medley)	30	30.06	100.2 (0.3)	100.7 (0.4)
		Mean	100.3 (0.4)	100.2 (0.5)

*Mean (%RSD) of six observations, **Mean (%RSD) of three observations. Assay and precision was studied by analysing six sample solutions (two replicate of each) prepared from homogenous sample. Accuracy of the method was studied by recovery experiments by adding standard drug at the level of 25, 50, 75 and 100% of the label claim to the tablet powder and analysed by injecting three replicate of each sample solution.

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Simultaneous Spectrophotometric Estimation of Nalidixic Acid and Metronidazole in Combined Dosage Forms.

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Nalidixic acid and metronidazole in combination are routinely used as antidiarrhoeal. The present investigation attempts to develop estimation method for these two drugs in combined dosage forms. It was found that both the drugs follow the Beer's Law from 2.0-10.0 µg/ml and 2.0-12.0 µg/ml at 257 and 277 nm, respectively. The absorptivity (1%, 1 cm), values at the specified wavelengths for nalidixic acid was found to be 1232.84 and 211.05, respectively and for metronidazole 263.65 and 376.18, respectively. Thus, the present method is simple, rapid and accurate and is based on the principle of simultaneous UV spectrophotometric determination of binary mixture.

The combination of nalidixic acid and metronidazole is marketed as tablet and suspension formulations and is used as antidiarrhoeal. Chemically, nalidixic acid is 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid and official in IP[1]. It is an antibacterial agent active against enterobacteriaceae and is given orally to treat urinary tract infections[2]. Chemically, metronidazole is 2-(2-methyl-5-nitro-1H-imidazol-1-yl)-ethanol and is also official in IP[3]. It is an antimicrobial drug, active against obligate anaerobic microorganisms both bacteria and protozoa[4]. Though both the drugs have overlapping UV absorption spectra in acidic media, their absorption maxima are quite well separated from each other. In this attempt, the Vierodt's method, one of the standard methods for simultaneous estimation of binary mixture was employed [5].

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Nalidixic acid was procured from M/s Ranbaxy Laboratories Ltd., Devas and metronidazole from M/s Oscar Laboratories Pvt. Ltd, New Delhi as gift samples. The tablets (referred as T₁ and T₂) and suspension (referred as S) of the said combination were purchased from a local pharmacy. (The label claim for both T₁ and T₂ contained 300 mg nalidixic acid and 200 mg of metronidazole and each 5 ml of S contained 150 mg of nalidixic acid and 100 mg of metronidazole). All the chemicals and solvents used were of AR/GR grade. The instrument, LKB Ultrospec 4050 Spectrophotometer and a Shimadzu 150 UV/Vis Spectrophotometer were used for spectrophotometric readings.

To establish the suitability of the proposed method for determination of nalidixic acid and metronidazole in the pharmaceutical formulation, the method was first tried for the estimation of components in standard laboratory mixture of two drugs.