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Liquid Chromatographic Determination of Pioglitazone in Pharmaceuticals, Serum and Urine Samples

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Abstract

A rapid and reliable analytical method based on high-performance liquid chromatography (HPLC) with UV detection (221nm) has been developed for the determination of the anti-hyperglycemic agent Pioglitazone in pharmaceutical formulations and biological fluids (serum and urine) after clean-up with solid-phase extraction. Chromatographic separation was achieved with a Chromolith® Performance RP-18e (100×4.6mm) column using mobile phase composition of acetonitrile: mixed phosphate buffer (pH 2.5; 10mM) (30:70, v/v) with a flow rate of 2.0mL/min. The total run time was 2 min. under optimized conditions. The calibration curve was found to be linear in the range of 1-10 μg mL^{-1} with regression coefficient of 0.9996, and the lower limit of detection 72 ng/20μL injection. The method has been validated for the system suitability, linearity, precision and accuracy, limits of detection, specificity, stability and robustness. The %recovery of Pioglitazone in pharmaceutical formulations was found to be 104.7%. The assay has been applied successfully to the pharmaceutical Tablet samples and biological fluids (serum and urine) of healthy volunteers.

Keywords: Pioglitazone; Monolith column; Pharmaceuticals; Biological fluids.

Introduction

Pioglitazone ([(±) – 5 - [[4-[2-(5-ethyl-2-yridinyl) ethoxy] phenyl]methyl]-2,4-] thiazolidinedione) hydrochloride is an oral anti-hyperglycemic agent that acts primarily by increasing insulin sensitivity in target tissues. It is used both as monotherapy and in combination with sulfonylurea or insulin in the management of type 2 diabetes mellitus (non-insulin dependent diabetes mellitus, NIDDM) [1-3]. Several liquid chromatographic methods have been described in the literature to determine pioglitazone and its metabolites in biological fluids. The already developed methods used high-performance liquid chromatography with ultraviolet detection (HPLC-UV) with run time of more than 20 min. [4-6], but the need of a fast and reliable method has always been the point of interest and is also increasing day by day [7-9]. Determination of pioglitazone in pharmaceuticals and biological fluids is also reported by using HPLC with MS detectors [10-14]. An excellent separation procedure is reported for simultaneous HPLC determination of six antidiabetic compounds using conventional particulate column [7]. Almost all the methods reported are optimized for specific separations, either for pharmaceutical or biological fluids, and use conventional particulate column hence there is need to develop a simple and faster HPLC procedure. Monolithic columns are new generation in HPLC stationary phases. Silica-based monoliths have small-sized skeletons and a bimodal pore size distribution with μm-sized through-pores and nm-sized mesopores. This gives silica-based monoliths favorable properties such as high-efficiency, fast separations,

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low-pressure drop across the column, fast mass transfer kinetics and a high binding capacity [15]. Many successful applications are reported in pharmaceutical and biological analysis [16-19]. The aim of this study was to develop a fast and reliable method for the determination of pioglitazone in pharmaceuticals and biological fluids using HPLC with monolithic column.

Experimental

Chemicals and reagents

Pioglitazone was obtained from Hilton Parma (Pvt.) Ltd. (Karachi, Pakistan). HPLC grade Methanol was purchased from TEDIA (Fairefield, USA). Potassium dihydrogen Phosphate and dipotassium hydrogen Phosphate were purchased from Sigma-Aldrich Chemi GmbH Germany. Millipore quality water was used for this study. Mobile phase components were degassed using Super Sonic X-3 Sonicator before use.

Instrumentation

Spectra SYSTEM P-2000 Pump with a UV6000LP diode array detector and SCM1000 Degasser (Thermo Electron Corporation, San Jose, California.) was used for the present study. The separation was achieved with an analytical column Chromolith® Performance RP-18e (100×4.6mm), by Merck KGaA (Darmstadt, Germany)) with an isocratic mobile phase of acetonitrile: mixed phosphate buffer (pH 2.5, 10 mM) (pH adjusted with phosphoric acid); (30:70, v/v) at a flow rate of 2 mL / min. Detection was achieved at 221 nm. ChromQuest software was used for data analysis.

Standard solutions of the pioglitazone

The stock solution of 1mg mL^{-1} of pioglitazone was prepared in methanol. The working standard solutions were prepared by diluting aliquots of each stock solution to obtain concentrations ranging from 1-10µg mL^{-1}. The calibration graph was constructed by plotting the peak areas obtained at wavelength 221 nm versus the corresponding injected concentrations.

Pharmaceutical tablets

Ten tablets of pioglitazone (15mg) were weighed and finely ground. Weight equal to average of one tablet was taken in 100mL volumetric flask and was made-up with methanol. From that 1.66mL was diluted to 50mL so as to obtain the concentration of 5µg mL^{-1} of pioglitazone as working concentration. The sample was filtered through 0.45µm filter paper and 20µL was injected into HPLC for further analysis.

Biological sample collection

Blood samples were collected from five healthy volunteers who were directed not to take any other prescription for one week before collecting the blood sample. Blank serum samples were collected half an hour before taking the single dose oral administration of pioglitazone tablet. After two hours of single dose oral administration of pioglitazone tablet containing 15mg pioglitazone, again blood samples were collected from volunteers and separated the serum.

Urine samples from same healthy volunteers were cleaned-up using solid-phase extraction procedure and run as blank. Same procedure was used for spiked samples of urine. Pharmaceutical tablet samples of Pioglitazone were purchased from Karachi, Pakistan,

Blood serum sample

The serum was separated from whole blood and was kept at − 4 °C till further analysis. Pioglitazone was determined in serum samples obtained from healthy volunteers using solid phase extraction technique. Lichrolut® RP-18 E (200 mg 3 mL) SPE cartridge from Merck KGaA (Darmstadt, Germany) was conditioned by passing 2 times 3 mL methanol followed by 2 times 3 mL water, 1 mL of serum sample was passed through cartridge, hydrophilic endogenous material was washed with 5mL of 10% methanol and drug was eluted with 2 mL of methanol. The eluent was filtered through 0.45µm filter paper, evaporated to bring 200µL volume and 20µL was injected into HPLC for further analysis.

Urine sample

Pioglitazone was also determined in urine samples. Urine samples obtained from healthy volunteers were spiked with pioglitazone standard and analyzed using solid phase extraction

technique. 1mL of urine sample and 1-mL acetonitrile was taken in the 5mL vial and centrifuged for 5 min. Lichrolut® RP-18 E (200mg 3mL) SPE cartridge from Merck KGaA (Darmstadt, Germany) was conditioned as described above and 1mL of already treated urine sample was passed through cartridge. After that 5mL of 10% Methanol was passed from cartridge to remove the hydrophilic endogenous material and further treated as for blood samples.

Results and Discussion

For mobile phase optimization different tests were performed which include the solvent selection, pH of phosphate buffer and the ratio of organic solvent to buffer. For the HPLC determination of pioglitazone, methanol and acetonitrile were tested as organic solvents, acetonitrile was able to elute pioglitazone in shorter time and produced better peak shape as compared to methanol. So, further optimization studies were carried out using acetonitrile. Monolith can better tolerate pH effect as compared to particulate reverse-phase columns. Pioglitazone is administered as base (pioglitazone HCl), at lower pH it may be available in protonated form while at basic pH in deprotonated form, so mobile phase is one of the important parameter that may affect its retention on reverse-phase packing materials. Fig. 1 shows the effect of pH on the retention of pioglitazone on reverse-phase monolithic column. At pH 2.5 the drug eluted in shortest time while on increasing pH the retention time increases and peak broadens which may be due to increasing deprotonation and reduced solubility of drug in acetonitrile in neutral form.

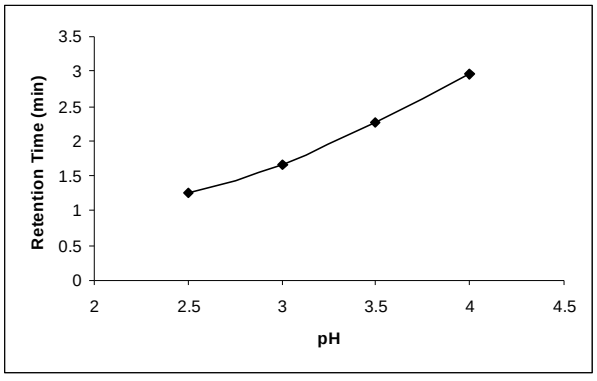

Figure 1. **Effect of mobile phase pH on retention time of pioglitazone.**

N. A. Al-Arfaj *et al:* in report for voltametric determination has shown well-defined voltametric curve, which can be attributed to pH dependant forms of pioglitazone [20]. In our study, we selected pH 2.5 for further studies.

Different compositions of mobile phase were also checked and the best performance was found to be at acetonitrile: mixed phosphate buffer (pH = 2.5) in the ratio of (30: 70 v/v).

The system performance was calculated by the reproducibility tests of the retention time, number of theoretical plates, capacity factor, resolution and the relative retention of pioglitazone (Table 1). All these parameters showed that efficiency of method is comparable with commercially used particle packed columns and the retention time for pioglitazone was very short (1.2 ± 0.05min), due to that sample throughput can be increased many folds. The linearity and calibration of pioglitazone was determined in the range of 1 to 10µg mL^{-1} with regression coefficient, slope and intercept of 0.9996, 1.135 and 0.02 respectively. The detection limit was calculated by the classical method of 3σ and was found to be 72ng/20µL injection.

Table 1. **System Performance for Pioglitazone (n=5) using monolithic and 5µm Hypersil Gold (250×4.6mm) column using the method.**

	Flow rate	t_R ± SD (min)	Pressure psi	N	K'	Rs
Monolithic Column	2mL/min	1.2±0.05	670	866	0.893	3.2
Particulate Column	1mL/min	10.58±0.03	1260	3120	6.25	9.9
	2mL/mn	5.448±0.05	2450	2036	5.28	8.5

For reproducibility, inter and intra-day precision analysis was carried out by analyzing the standard of known concentration five times a day for five consecutive days (Fig. 2a). A 5µg mL^{-1} concentration of pioglitazone was used to check the variation in determination. Response for both inter-day & intra-day analysis was calculated and both showed a very good agreement in terms of reproducibility and recovery. The coefficient of variance was 1.09 for intra-day and 1.1 for inter-day precision analysis of pioglitazone (Table 2).

Table 2. **Inter-day and Intra-day Reproducibility of determinations.**

	Actual µg mL^{-1}	Observed µg mL^{-1}	Accuracy (%)**	C.V (%)*
Intraday Analysis				
Pioglitazone	5.0	4.97±0.05	99.6	1.09
Interday Analysis				
Pioglitazone	5.0	4.94±0.05	98.8	1.1

* Coefficient of Variance (%) = S.D x 100/mean
** Accuracy (%) = observed concentration x 100/used concentration

The robustness of the method was determined by calculating the slight variations in analytical conditions. Flow rate of mobile phase did not show any significant change in the resolution of the peak but minor variations were noted in the retention times. Where as slight variations of the acetonitrile content in the mobile phase were determined to be very sensitive for retention time so the acetonitrile content in the mobile phase is to be controlled carefully to attain the reproducible retention. Wavelength was not found much sensitive as all the recoveries were in the range of ± 5%.

The validated method was applied to determine pioglitazone in pharmaceutical and biological samples.

Applications

Pharmaceutical tablet samples

Pharmaceutical tablet samples were prepared as above mentioned procedure and then were analyzed using the currently developed method. The results showed a good agreement of percent recovery and label claim from the manufacturer, which was determined to be 104% with the coefficient of variance of 0.84 (Fig. 2b and Table 3).

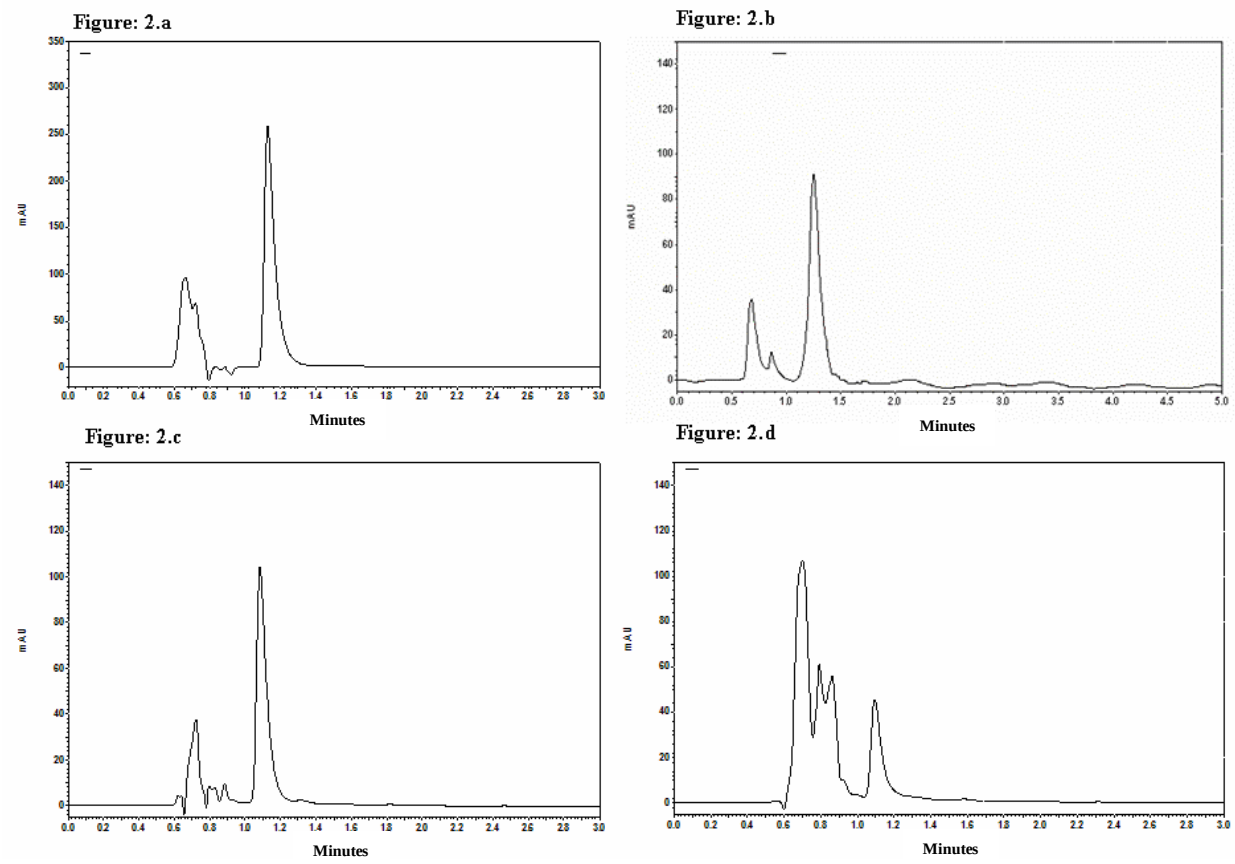

Figure 2. **HPLC chromatogram of 5µg mL^{-1} standard pioglitazone (a); pioglitazone in pharmaceutical tablets (b); spiked urine sample chromatogram of pioglitazone (c); real blood sample chromatogram of pioglitazone after oral administration of drug (d)- conditions as in-text.**

Table 3. **Assay values of Pioglitazone from Pharmaceutical Tablets (n=3).**

Amount Taken (mg)	Amount Recovered (mg)	Mean Recovery (%)
0.0375	0.039±0.003	104.0
0.0499	0.052±0.005	104.9
0.0749	0.078±0.002	103.7
0.1498	0.157±0.008	104.7
0.1501	0.159±0.001	106.0

Serum samples

For the analysis of serum samples, solid phase extraction technique is preferred on Liquid – Liquid extraction because the retention time for pioglitazone is very short (1.20±0.05min) and with Liquid – Liquid extraction many interfering compounds were found in that range of retention time which can be avoided using SPE technique. Serum samples were analyzed successfully and the %recovery for spiked serum samples was found to be 99%. The real serum samples (1 mL) were also analyzed and amount of pioglitazone in serum samples was found to be in the range of 0.30 to 0.55µg mL^{-1} after two hours of single oral dose administration of pioglitazone tablet containing 15mg pioglitazone (Fig. 2c).

Urine samples

The spiked urine samples were analyzed by above mentioned procedure, which shows the clean chromatogram with baseline separation from endogenous compounds and pioglitazone is completely resolved (Fig. 2d). Percent recovery for the spiked samples of urine ranges from 99% - 102%.

Conclusion

A fast chromatographic method for the determination of pioglitazone is developed using monolithic column. The proposed HPLC method ensures a precise, accurate and fast determination of pioglitazone in pharmaceutical tablets and biological fluids. Solid phase extraction technique is found good in terms of recovery and time saving specially in biological fluids. Monolithic column has good potential to be introduced as standard C-18 column for faster separation.

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