Journal of Chromatography B, 877 (2009) 1923–1929

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Extraction of trace amounts of pioglitazone as an anti-diabetic drug with hollow fiber liquid phase microextraction and determination by high-performance liquid chromatography-ultraviolet detection in biological fluids

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ARTICLE INFO

Article history:
Received 6 April 2009
Accepted 16 May 2009
Available online 21 May 2009

Keywords:
HF-LPME
Orthogonal array design
Pioglitazone
Biological samples
High-performance liquid chromatography

ABSTRACT

The applicability of hollow fiber liquid phase microextraction (HF-LPME) for extraction and preconcentration of trace amounts of pioglitazone (PGL) as an anti-diabetic drug in biological fluids, prior to determination by high-performance liquid chromatography (HPLC), was evaluated. In this technique, the target drug was extracted into di-*n*-hexyl ether immobilized in the wall pores of a porous hollow fiber from 10 mL of the aqueous sample (source phase, SP) with pH 8.0, and then back extracted into the receiving phase (RP) with pH 2.2 located in the lumen of the hollow fiber. The extraction occurred due to a pH gradient between the two sides of the hollow fiber. After extracting for a prescribed time, 24 μL of the RP solution was taken back into the syringe and injected directly into a HPLC instrument for quantification. The Taguchi orthogonal array (OAD) experimental design with an OA_{16} (4^5) matrix was employed to optimize the HF-LPME conditions. Different factors affecting the HF-LPME efficiency such as the nature of organic solvent used to impregnate the membrane, pH of the SP and RP, stirring speed, extraction time and ionic strength were studied and optimized. Under the optimum conditions (di-*n*-hexyl ether as membrane impregnation solvent, pHs of the SP and RP equal to 8.0 and 2.2, respectively, extraction time of 30 min, stirring speed of 500 rpm and 10% (w/v) NaCl for adjusting the ionic strength), preconcentration factor of 180, linear dynamic range (LDR) of 2.5–250 $\mu g\,L^{-1}$ with good correlation of determination ($r^2 > 0.998$) and limit of detection (LOD) of 1.0 $\mu g\,L^{-1}$ were obtained for the target drug. The percent relative intra-day and inter-day standard deviations (RSDs%) based on five replicate determinations were 4.7 and 15%, respectively. Once LPME was optimized, the performance of the proposed technique was evaluated for the determination of PGL in different types of biological fluids such as plasma and urine samples. The results showed that the proposed HF-LPME method could be successfully applied to determine trace amounts of PGL in biological samples.

1. Introduction

Pioglitazone ([(±)-5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy] phenyl]methyl]-2,4-] thiazolidinedione) hydrochloride (PGL) (Fig. 1) 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]. Pharmacological studies indicate that PGL improves sensitivity to insulin in muscle and adipose tissues and inhibits hepatic gluconeogenesis. Therapeutic concentration range of PGL in plasma is 34–2000 $\mu g\,L^{-1}$ [4]. Several liquid chromatography and capillary electrophoresis methods have been reported in the literature for quantitative determination of PGL and its metabolites in biological fluids [5–13]. In the reported methods, the sample preparation techniques are based on either liquid–liquid extraction (LLE) or solid-phase extraction (SPE) [5–13]. Although these techniques provide adequate analyte enrichment, high reproducibility and high sample capacity [14], they have many disadvantages as they are tedious, labor intensive and time consuming. LLE, in particular, requires the use of large amounts of high-purity solvents, which are often hazardous and result in the production of toxic laboratory waste. Both LLE and SPE require solvent evaporation in order to preconcentrate the analytes. During the evaporation step, loss and/or deterioration of target analytes has been reported [15]. In response to the problems with traditional sample preparation techniques, solid-phase microextraction (SPME) as a solvent-free

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doi:10.1016/j.jchromb.2009.05.033

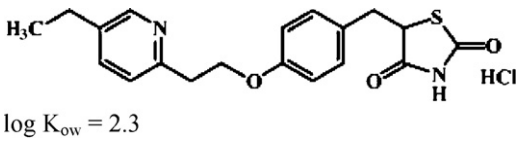

Fig. 1. Chemical structure and log K_{ow} value of pioglitazone.

process was developed based on partition equilibrium of the analytes in an aqueous samples and a polymer coating on a fused-silica fiber that has been successfully used for extracting different compounds [16–18]. However, SPME fibers are fragile and relatively expensive. They tend to degrade with multiple usages. To facilitate automation and effective reduction of the consumption of organic solvents in sample preparation, the miniaturized LLE or liquid phase microextraction (LPME) was introduced in 1996 [19]. LPME can be classified as two phase and three phase techniques [20–28] and may be performed as hollow fiber or droplet based modes. In two-phase LPME, the analytes are extracted from an aqueous sample matrix into an organic acceptor phase and, after the extraction, the extracted organic phase is directly injected into a gas chromatograph (GC) for analysis. Three-phase LPME was previously developed to extract ionizable and charged compounds from aqueous samples [28–30]. In single drop based three-phase LPME, the analytes are first extracted from an aqueous sample matrix into an organic phase immiscible with water and located on top of the aqueous phase, followed by back-extraction into a separate microdrop of aqueous phase suspended from the tip of a microsyringe and penetrating into the organic phase [24]. In recent years, an alternative concept of LPME has been introduced based on the use of single, low-cost, disposable and porous hollow fibers made of polypropylene to support the organic phase in the pores of the wall while holding the second aqueous phase in the lumen of the fiber [31–38]. This mode of hollow fiber liquid phase microextraction (HF-LPME) is limited to the basic or acidic analytes with ionizable functionalities. After extraction, the acceptor solution may be directly injected into high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) without further treatment. Due to the high ratio of the source phase (SP) to the receiving phase (RP) volume, very high preconcentration factors can be obtained. An important advantage of three-phase LPME is an excellent clean-up that enables the extraction of drugs and metabolites from biological matrixes and pollutants from the environmental samples with simultaneous clean-up of the extracts [31–43].

Orthogonal array design (OAD) is a type of fractional factorial design [44–46] that has proved to be a cost-effective optimization strategy. OAD is used to assign factors to a series of experimental combinations, whose results can then be analyzed using a common mathematical procedure. The theory and methodology of OAD, as a chemometric method for the optimization of analytical procedures, has been described in detail elsewhere [47–50]. In this way, it allows for the identification of key factors that are highly effective on the performance of the characteristic value. Analysis of variance (ANOVA) is employed for estimating the main significant factors and two-way interaction factors after the OAD procedure has been conducted [47–59].

Based on our knowledge, no LPME experiences have been published previously on the extraction of PGL from biological samples. Therefore, in the present study, HF-LPME combination with HPLC/UV was applied for the extraction and preconcentration of PGL in aqueous samples. Moreover, mixed-level OAD procedure with OA_{16} (4^5) matrix (Taguchi Method) was applied to study the factors influencing HF-LPME efficiency. Analysis of variance (ANOVA) was employed for estimating the main significant factors and their percentage contributions. The optimized conditions were then applied for the analysis of PGL in different aqueous and biological samples.

2. Experimental

2.1. Chemicals

Pioglitazone was kindly donated by the Department of Medical Sciences of Tehran University (Tehran, Iran). HPLC-grade acetonitrile and methanol were purchased from Aldrich (Milwaukee, WI, USA). Di-*n*-hexyl ether was purchased from the International Laboratory (USA). The ultrapure water used was purified on Aqua Max-Ultra Youngling ultrapure water purification system (Dongangu, South Korea). All the other chemicals used were of reagent grade or the highest purity available. The plastic and glassware used for the experiments were previously soaked in nitric acid (0.1 M) for 24 h and rinsed carefully with the ultrapure water.

2.2. Apparatus

Chromatographic separations were carried out on a Varian HPLC equipped with a 9012 HPLC pump (Mulgrave, Victoria, Australia), a six-port Valco HPLC valve (Houston, USA) equipped with a 20 μL sample loop and a Varian 9050 UV–vis detector. Chromatographic data were recorded and analyzed using Chromana software (version 3.6.4). The separations were carried out on an ODS-3 column (150 mm × 4.0 mm, with 3 μm particle size) from MZ-Analysentechnik GmbH (Mainz, Germany). A mixture of 50 mM ammonium acetate (pH 4.6) and acetonitrile (20/80, v/v) with a flow rate of 0.7 mL min^{-1} was used as the mobile phase. The injection volume was 20 μL for all the standards and the samples, and the detection was performed at the wavelength of 270 nm. All of the pH measurements were performed with a WTW Inolab pH meter (Weilheim, Germany). All of the extractions were carried out using a Q3/2 Accurel polypropylene hollow fiber membrane from Membrana (Wuppertal, Germany) with a 0.2 μm pore size, 600 μm internal diameter and 200 μm wall thickness.

2.3. Standard solutions and real samples

The stock standard solution of PGL (400 μg mL^{-1}) was prepared by dissolving its hydrochloride salt in acetonitrile. All of the working standard solutions were freshly prepared by proper dilution of the stock standard solution with acetonitrile or ultrapure water to the required concentration. The concentration of the drug in the preliminary experiments was 500 μg L^{-1}. All of the standard solutions were stored at 4 °C and re-prepared every month. Tap water sample was collected freshly from our laboratory (Tarbiat Modares University, Tehran, Iran) and the human urine sample was obtained from a healthy volunteer (Tehran, Iran). The drug free plasma sample was obtained from the Iranian Blood Transfusion Organization (Tehran, Iran). The pH of the real samples was adjusted at 8.0 by dropwise addition of 0.1 M NaOH solution. Before extraction of the drug, the plasma and urine samples were diluted to 1:4 and 1:1 with ultrapure water, respectively. The working standards for real sample analysis were prepared by spiking the target drug in the water and biological samples.

2.4. Extraction procedure

The extraction was performed according to the following steps: (1) before use, the hollow fiber was ultrasonically cleaned in acetone for several minutes in order to remove any contaminants and then the solvent was allowed to evaporate completely; (2) a 10 mL aliquot of the sample solution was added to a 12 mL sample vial containing a 4 mm × 14 mm magnetic stirrer bar; (3) the sample