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Hollow fiber liquid-phase microextraction combined with high performance liquid chromatography for the determination of trace mitiglinide in biological fluids

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ABSTRACT

A hollow fiber liquid phase microextraction (HF-LPME) in conjunction with reversed phase HPLC–UV method was developed for the extraction and determination of trace amounts of the antidiabetic drug, mitiglinide (MIT) in biological fluids. The drug was extracted from 10 mL aqueous sample (donor phase (DP)) into an organic phase impregnated in the pores of hollow fiber, followed by the back extraction into a second aqueous solution (acceptor phase (AP)) located in the lumen of the hollow fiber. Parameters influencing the extraction efficiency including the kind of organic solvent, composition of DP and AP, extraction time, stirring rate and salt addition were investigated and optimized. Under the optimized extraction conditions, high enrichment factors (210-fold), good linearity (5–1000 ng mL^{-1}) and detection limit lower than 1.38 ng mL^{-1} were achieved. Recoveries of spiked samples were in the range (88.3–96.3%) and (92.0–99.3%) for urine and plasma samples, respectively. The percent relative standard deviation ($n = 9$) for the extraction and determination of three concentration levels (100, 400 and 800 ng mL^{-1}) of MIT were less than 10.6% and 13.6% for urine and plasma samples, respectively. The developed method is simple, sensitive and has been successfully applied to the analysis of MIT in biological fluids.

1. Introduction

It is estimated that currently there are about 285 million diabetics worldwide, and the number is expected to increase to 439 million by 2030 [1,2]. Due to the alarming increase, the World Health Organization has declared diabetes mellitus as a global epidemic and 14 November as World Diabetes Day [3]. Diabetes is incurable but manageable. Many new drugs are currently in the developmental stage for the treatment of diabetes. One such drug is mitiglinide (MIT), (2S)-benzyl-4-(cisperhydroisoindol-2-yl) butyric acid (Fig. 1). It is a novel glinide class compound with fast-onset of action as insulinotropic agent [4]. It has been used in clinical studies as calcium hydrate form. Similar to other glinide group members, MIT acts by stimulating the secretion of insulin from pancreatic beta cells by closing the ATP-sensitive K^+ [K(ATP)] channels [5]. MIT and the other glinides such as, repaglinide and the phenylalanine derivative were found to share similar pharmacokinetic and pharmacodynamic properties [6], and it has been used either singly or in combination with metformin for the treatment of type II diabetes mellitus. The daily intake dosage of MIT ranges between 5 and 20 mg (three times) [7]. However, the pharmacokinetic properties of MIT are still not well understood. Therefore, the determination of MIT at physiological levels in biological fluids using suitable methods of analysis is of much interest.

High-performance liquid chromatography (HPLC) employing either ultraviolet (UV) [8] or mass spectrometry (MS) [9–11] detections and ultra high pressurized liquid chromatography with MS detection [12] methods have previously been reported for the determination of MIT in biological fluids [8,10,12] and to establish the pharmacokinetic properties of MIT [9,11]. These methods either lack sensitivity to monitor at the therapeutic levels (lower limit of quantification (LLOQ) (0.1 μg mL^{-1})) [8] or use expensive mass spectrometers [9–12]. Moreover, the long separation time involved (14 min) [8,10], is not suitable for the analysis of large batches of biological samples.

The drive for "green" methods to overcome the inherent problems of the conventional liquid–liquid extraction (LLE) has led to the development of numerous of solventless and microextraction techniques. Of these, the hollow-fiber liquid phase microextraction (HF-LPME) technique, originally proposed by Pedersen-Bjergaard and Rasmussen in 1999 [13] has gained prominence. The heart of method lies in the use of a porous polypropylene hollow fiber (HF) where an organic solvent (~4 μL) is usually used to fill its pores before the initiation of the extraction procedure. Experimental

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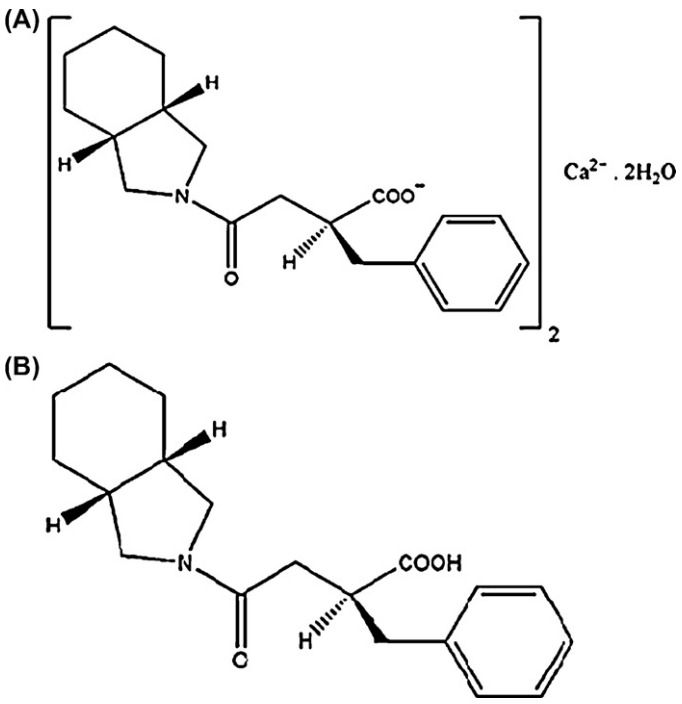

Fig. 1. Chemical structures of mitiglinide (A) the state of administration, (B) the state of detection; log P = 2.73.

conditions are adjusted so that the analyte is transferred through the organic phase of the HF pores to the acceptor solution which is situated within the lumen of the HF. In cases, where a large number of samples need to be analyzed, HF-LPME method could be more economical compared to the solid phase extraction for the same equivalent number of samples [14]. The technique has found numerous applications, especially for pharmaceutical and environmental samples [15].

In this study, a HF-LPME combined with HPLC–UV was applied for the extraction and determination of MIT in biological samples. As MIT is acidic (pK_a = 4.37) [16] so a three-phase HF-LPME method was developed using an acidic donor phase (DP) and a basic solution as acceptor phase (AP) which can be directly injected into the HPLC system. All the HF-LPME parameters have been optimized in order to propose a sensitive and simple method for the determination of MIT in biological fluids.

2. Experimental

2.1. Chemicals and reagents

Mitiglinide calcium hydrate reference standard (100.0% purity) was kindly donated by Hikma Pharmaceuticals (Amman, Jordan). HPLC-grade methanol (≥99.96%) was purchased from Fisher Scientific (Milwaukee, USA). Potassium dihydrogen phosphate (98–102%), orthophosphoric acid (37%) and hydrochloric acid (37%) were purchased from Merck (Darmstadt, Germany). 1-Heptanol (≥99.9%) and 1-octanol (≥99.5%) were purchased from Fluka (Buchs, Switzerland). *n*-Decane (99.0%) and *n*-octane (97.0%) were from Acros Organics (Geel, Belgium). Sodium hydroxide (≥98.0%), dihexyl ether (97.0%), *n*-tridecane (99.8%), 2-hexanol (≥99.0%), *n*-hexadecane (99.0%), 1-nonanol (99%) and diethylether (98%) were purchased from Sigma–Aldrich (St. Louis, USA). Ultrapure water (resistivity, 18.2 MΩ cm^{-1}) was produced by a Milli-Q system (Millipore, USA), and was used throughout for the preparation of solutions.

2.2. Materials

Q3/2 Accurel polypropylene HF membrane (600 μm inner diameter, 200 μm wall thickness and 0.2 μm pore size) was purchased from Membrana GmbH (Wuppertal, Germany). 6 cm of the HF was cut. The HF was discarded after single use. A 25 μL Hamilton microsyringe (model 702SNR) with a blunt needle tip was used to introduce the AP and support the HF. The syringe with the attached HF was clamped to a retort stand during the extraction. A hot plate stirrer (model GLHPS-G) purchased from Global Lab (South Korea) was used for stirring during the extraction. An Orion pH meter model EA 940 (Cambridge, USA) was used for pH measurements. Centrifuge (model 2100) was purchased from Kubota (Tokyo, Japan).

2.3. Instrumentation

A Hitachi LC-6200 intelligent pump (Tokyo, Japan) was used to deliver the mobile phase to the analytical column (GraceSmart RP 18 Column 150 mm × 4.6 mm, 5 μm) and was purchased from Grace Davison Discovery Sciences (IL, USA). Sample injection was performed via a Rheodyne 7125 injection value (Cotati, California, USA) with a 5 μL loop. Detection was achieved by a Hewlett-Packand 1050 UV detector (Waldbronn, Germany) at 210 nm. A powerchrom data acquisition was purchased from eDAQ (Denistone East, Australia) and was performed with powerchrom V2.6.7 software.

2.4. Standard solutions and real samples

A stock solution of MIT (1000 μg mL^{-1} as mitiglinide calcium hydrate) was prepared by dissolving the proper amount in methanol and stored at 4 °C. Working standard solutions were prepared daily by appropriate dilution of the stock solution with water to the desired concentration. Human urine sample was obtained from a healthy student volunteer. Drug free plasma sample was obtained from the Centre for Drug Research, Universiti Sains Malaysia, Penang, Malaysia. The pH of the real samples was adjusted to 1.5 using 1.0 M HCl solution.

2.5. Preparation of hollow fiber and extraction procedure

The HF was cut into segments (6 cm) which approximately accommodated 15 μL of the AP. The HF segments were washed with acetone to remove any contaminants and it was allowed to evaporate completely. A 10 mL of the sample solution (pH 1.5, adjusted with 1.0 M HCl) containing 500 ng mL^{-1} of MIT was placed in a 12 mL vial. A magnetic stir bar (5 mm × 15 mm) was placed in the solution. A Hamilton micro syringe (25 μL) was used to introduce the AP into the HF. 15 μL of the basic receiving phase (0.1 M NaOH) was withdrawn into the micro syringe and was inserted into the lumen of the HF. The fiber was placed in the organic phase (1-octanol) for 10 s and any excess of the organic phase was carefully removed by washing the outside of the HF with ultrapure water for 5 s. Subsequently, 15 μL of the receiving phase was injected into the lumen of the HF and tip needles were used to connect the HF ends.

The fiber was formed as U-shape and it was immersed into the sample solution. The extraction was performed at room temperature and the sample was stirred at 300 rpm during the extraction (45 min). After the extraction, the HF was removed from the sample vial, and one end of the HF was opened. A 5 μL of AP was retracted into the micro syringe and finally injected into the HPLC system for subsequent analysis.