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Hollow fiber liquid phase microextraction with *in situ* derivatization for the determination of trace amounts of metformin hydrochloride (anti-diabetic drug) in biological fluids

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ABSTRACT

A three phase hollow fiber liquid-phase microextraction with *in situ* derivatization (*in situ* HF-LPME) followed by high-performance liquid chromatography-ultraviolet detection (HPLC-UV) method was developed for the trace determination of metformin hydrochloride (MH) in biological fluids. A new derivatization agent pentafluorobenzoyl chloride (PFBC) was used. Several parameters that affect the derivatization and extraction efficiency were studied and optimized (*i.e.*, type of organic solvent, volume of NaOH (4 M) and derivatization agent in the donor phase, acceptor phase (HCl) concentration, stirring speed, temperature, time and salt addition). Under the optimum conditions (organic solvent, dihexyl ether; volume of NaOH (4 M) and derivatization agent (10 mg PFBC in 1 mL acetonitrile) in the donor phase, 600 and100 μL, respectively; acceptor phase, 100 mM HCl (10 μL); stirring speed, 300 rpm; extraction time, 30 min; derivatization temperature, 70 °C; without addition of salt) an enrichment factor of 210-fold was achieved. Good linearity was observed over the range of 1–1000 ng mL^{-1} (r^2 = 0.9998). The limits of detection and quantitation were 0.56 and 1.68 ng mL^{-1}, respectively. The proposed method has been applied for the determination of MH in biological fluids (plasma and urine) and water samples. Prior to the microextraction treatment of plasma samples, deproteinization step using acetonitrile was conducted. The proposed method is simple, rapid, sensitive and suitable for the determination of MH in a variety of samples.

1. Introduction

Metformin hydrochloride (MH), N,N-dimethylimidodicarbonimidic diamide hydrochloride (Fig. 1), is a biguanide antidiabetic agent that improves peripheral glucose uptake and reduces hepatic glucose in patients with type II diabetes mellitus (non-insulin-dependent diabetes) [1]. It is a high dose drug that is generally used as the first-line oral antihyperglycemic drug. The chemical and pharmacological properties of MH are different from other oral antihyperglycemic agents [2]. MH is normally formulated in two forms, either as immediate release (500, 850 and 1000 mg) or extended release (500 and 750 mg). In the Bioavailability Classification System, MH is classified as class III drug due to its high solubility in water [3]. The oral bioavailability was reported to be 50% to 60% (500 mg dose). Maximum plasma concentration is reached after 1–3 and 4–8 h for immediate and extended release formulations, respectively [2]. MH has negligible plasma protein binding and it is excreted through the urinary tract without any changes [4–6].

The determination of MH concentration in biological fluids is important in order to study its pharmacokinetics, for therapeutic drug monitoring, determination of patient adherence with prescribed treatment in diabetic patients and bioequivalence assays [7,8]. Limited information is available about the disposition of MH in different biological fluids. This is due to the difficulty in the analysis of MH in complex matrices due to its high polarity (log P = −1.43) [9] and strong basic properties (pKa = 12.4) [2].

Several analytical methods for the determination of MH in biological fluids [1,4–23] and pharmaceutical formulations [20,24–27] have been reported. Most of these methods were conducted using high performance liquid chromatography with ultraviolet detection (HPLC-UV) [5–8,11–20]. However, these methods suffered from several drawbacks, such as the lack of sensitivity [4,8,12,19,20,25]. In order to overcome this problem, high injection

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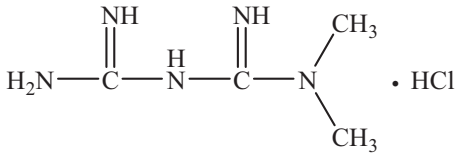

Fig. 1. Chemical structure of metformin hydrochloride (MH).

volumes (50–150 μL) were used [7,11,13,17], but this option is not recommended as it affects the efficiency of the column and shortening its lifetime. Alternatively, derivatization of MH using *p*-nitrobenzoyl chloride, desyl chloride, benzoin or anthraquinone-2-sulfonyl chloride have been attempted [10,14–16]. Although derivatization resulted in improvement in the detection, the derivatization procedure itself was rather long (60–120 min), using toxic organic solvents (*e.g.*, CH_2Cl_2), producing unstable derivatives with many side products and multi-extraction steps were involved [10,14–16]. Despite the derivatization, large injection volume was still needed [15]. The use of liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the analysis of MH in biological fluids were also reported [1,4,22,23,26]. Although LC–MS/MS can provide better sensitivity compared to HPLC-UV, but the analysis is costly.

Different approaches have been reported for the extraction of MH in biological fluids. Protein precipitation is the most common technique used [4–6,9]. This technique is simple, fast and minimum amounts of organic solvents are consumed. However, this method is subjected to interferences and long analysis time [6]. The high solubility of MH in water prevents the use of many extraction techniques. Liquid–liquid extraction (LLE) can only be used after the derivatization of MH. Solid-phase extraction (SPE) is the most common sample preparation method used [1,7,11,17,21,26]. SPE and LLE techniques can provide clean extracts and satisfactory enrichment of the analyte. However, they present some drawbacks such as being time-consuming, using large amounts of organic solvents and frequent analyte-loss due to the multi-extraction steps and involve evaporation step prior to the analysis [7,15–17]. In order to overcome these problems, a simple extraction method based on dried blood spot has been reported [8]. The blood (10 μL) was placed on a filter paper to form dried spot which was then extracted using small amounts of mobile phase (0.2 mL) and subsequently injected into the HPLC unit [8]. This extraction method is simple and minimized the use of organic solvents. However, it lacks sensitivity (LOQ 150 ng mL^{-1}) and time consuming (drying the blood spot overnight) [8]. Therefore, methods that are sensitive, reliable, rapid that allows good clean-up for the accurate measurement of low concentrations of MH in biological fluids are required.

The adoption of microextraction techniques for drug analysis is increasing. In particular, hollow-fiber liquid phase microextraction (HF-LPME) has attracted lots of attention. This is due to its simplicity, fast analysis, inexpensive and the use of negligible volumes of water-immiscible organic solvents (in μL or even sub-μL range). Moreover, HF-LPME can provide clean extract and high enrichments due to the high sample-to-acceptor ratio [28–30]. The use of HF-LPME for the extraction of anti-diabetic drugs (pioglitazone, rosiglitazone and mitiglinide) has been previously reported [31–33]. The adaptation of HF-LPME for the extraction of these anti-diabetic drugs (except MH) is rather straightforward due to their affinity toward the organic solvents (high log *P* values). As MH is highly soluble in water, its mass transfer to the organic solvents is difficult. Thus, it is rationalized that the extraction of MH using HF-LPME can only be feasible by a pre-derivatization step in order to reduce its polarity. The way forward is to combine the derivatization step with extraction in the HF-LPME format. In this *in situ* HF-LPME approach, the derivatization reaction takes place in the sample solution (donor phase) and the derivatized-product is extracted at the same time into the acceptor phase [34–36]. This technique helps to improve the recovery and sensitivity of analysis. Moreover, it is simple, convenient, rapid, and easy to operate [37,38].

This study, for the first time, aims to develop an HF-LPME with *in situ* derivatization method coupled with HPLC-UV for the determination of MH in biological fluids. Different derivatization agents will be examined. The experimental conditions and chromatographic parameters will be studied and optimized.

2. Experimental

2.1. Chemicals and reagents

Metformin HCl (MH) was kindly donated by Hikma Pharmaceuticals (Amman, Jordan). HPLC-grade methanol (≥99.96%), hydrochloric acid (37%, w/w) were purchased from Merck (Darmstadt, Germany). HPLC-grade acetonitrile (99.99%) was purchased from Fisher Scientific (Milwaukee, WI, USA). 3-fluorobenzoyl chloride (99%), 2-fluorobenzoylchloride (99%), 1-heptanol (≥99.9%) and 1-octanol (≥99.5%) were purchased from Fluka (Buchs, Switzerland). *n*-decane (99.0%) and *n*-tridecane (99.0%) were from Acros Organics (Geel, Belgium). Pentafluorobenzoyl chloride (PFBC, 99.0%), sodium hydroxide (≥98.0%), dihexyl ether (97.0%), *n*-heptane (99.0%), *n*-hexadecane (99.0%) and nitrobenzene (≥99.0%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphoric acid (85%) was purchased from Univar (Ingleburn, Australia). Ultrapure water (resistivity, 18.2 MΩ cm^{-1}) was produced by a Milli-Q system (Millipore, MA, USA) and was used throughout for the preparation of solutions. Derivatization solution was prepared by diluting 10 mg of PFBC in 1 mL acetonitrile and stored at 4 °C until used. 4 M NaOH was prepared in water.

2.2. Instrumentation

Separation and determination of the derivatized MH was performed using a Hitachi LC-6200 intelligent pump (Tokyo, Japan) equipped with a Hewlett-Packard 1050 UV detector (Waldbronn, Germany). Sample injection was performed via a Rheodyne 7125 injection valve (Cotati, CA, USA) with a 5 μL loop. A PowerChrom data acquisition was obtained from eDAQ (Denistone East, Australia) and performed with PowerChrom software (version 2.6.11) for the recording and analysis of the chromatographic data. The separation was obtained using a ODS Hypersil C18 column (250 × 4.6 mm, 5 μm particle size). UV detector was set at 230 nm. The mobile phase composition was a mixture of acetonitrile and 10 mM sodium phosphate buffer (pH 4) (60:40, v/v). The elution was performed under isocratic mode at a flow rate of 1.0 mL min^{-1}. Prior to the analysis, the mobile phase was filtered using nylon membrane filter (0.45 μm) from Agilent Technologies (Waldbronn, Germany) and degassed in an ultrasonic bath for 15 min.

For UV-scanning (190–600 nm), a Waters HPLC system (Cambridge, MA, USA), consists of Alliance (model 2695) as solvent and sample manager equipped with photodiode array detector (model 2998) and operated with a licensed empower software (version 2), were used. A multi hotplate stirrer from DAIHAN Scientific (Seoul, South Korea) was used for the stirring during the extraction process. The extraction was performed using a 25 μL microsyringe with a blunt needle tip (model 702SNR) that was purchased from Hamilton (Reno, NV, USA).

2.3. Preparation of standard and sample solutions

Stock standard solution of MH (1000 μg mL^{-1}) was prepared by dissolving the desired amount in water and stored at 4 °C until the