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Optimization of solvent bar microextraction combined with gas chromatography for preconcentration and determination of methadone in human urine and plasma samples

Homeira Ebrahimzadeh*, Fatemeh Mirbabaei, Ali Akbar Asgharinezhad, Nafiseh Shekari, Narges Mollazadeh

Department of Chemistry, Shahid Beheshti University, G.C., Evin, Tehran, Iran

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ABSTRACT

In this study, solvent bar microextraction combined with gas chromatography–flame ionization detector (GC–FID) was used for preconcentration and determination of methadone in human body fluids. The target drug was extracted from an aqueous sample with pH 11.5 (source phase) into an organic extracting solvent (1-Undecanol) located inside the pores and lumen of a polypropylene hollow fiber as a receiving phase. To obtain high extraction efficiency, the effect of different variables on the extraction efficiency was studied using an experimental design. The variables of interest were the organic phase type, source phase pH, ionic strength, stirring rate, extraction time, concentration of Triton X-100, and extraction temperature, which were first investigated by Plackett–Burman design and subsequently by central composite design (CCD). So that the optimum experimental condition was obtained when the sodium chloride concentration was 5% (w/v); stirring rate, 700 rpm; extraction temperature, 20 °C; extraction time, 45 min and pH of the aqueous sample, 11.5. Under the optimized conditions, the preconcentration factors were between 275 and 300. The calibration curves were linear in the concentration range of 10–1500 μg L^{-1}. The limits of detection (LODs) were 2.7–7 and relative standard deviations (RSDs) of the proposed method were 5.9–7.3%. Ultimately, the applicability of the current method was evaluated by the extraction and determination of methadone in different biological samples.

1. Introduction

Methadone (6-(dimethylamino)-4,4-diphenylheptan-3-one, MDN) (Fig. 1) is a synthetic central acting analgesic agent with high affinity for μ-opioid receptors [1]. It is widely used in the prevention of opiate abstinence syndrome and maintenance treatment of opioid addicts [2]. It is considered as a vital public health strategy for HIV/AIDS risk reduction besides [3]. Also, MDN has been gradually used to treat the heroin addiction by counteracting the withdrawal syndrome and reducing drug craving. Moreover, athletes often take far high doses of MDN that have been given for therapeutic use or in clinical studies to excel in competition [4]. This drug has been barred to use by the International Olympic Committee and other sport organizations [5]. Due to the differences in the pharmacokinetics of MDN among different individuals, individualization of dose is necessary to achieve optimum treatment. Therefore, the extraction and determination of MDN from biological fluids are of utmost interest [6].

In plasma, therapeutic concentrations usually range between 50 and 1000 μg L^{-1}, with peak plasma levels at about 4 h after oral administration [8]. During MDN maintenance treatment, plasma concentrations considerably fluctuate day by day and the inter individual MDN half life varies significantly (15–25 h) [9,10]. In cases of patients who need special medical care, such as women in pregnancy or in postpartum period, there could be inter individual differences in MDN pharmacokinetics [11,12]. Hence determination of MDN and its metabolites in plasma is critical, in order to screen and maintain plasma concentrations of MDN within an effective range, to achieve maximum treatment efficacy, avoid toxicity, investigate the mechanisms involved in MDN metabolism [10,12,13] and define the relationship between the given dose and the resulting drug concentration in plasma [14].

Several analytical methods based on gas chromatography [11,12,14] and liquid chromatography [10] coupled with MS [7,10,11,14] and UV [15] detection, have been utilized for the determination of MDN and its metabolites in different biological matrices, such as urine [12,16,17], plasma or blood [10,11,16–19], hair [14,20], saliva [12,21], oral fluid [22], meconium [23] and

* Corresponding author. Tel.: +98 21 29902891; fax: +98 21 22403041.
E-mail address: h-ebrahim@sbu.ac.ir (H. Ebrahimzadeh).

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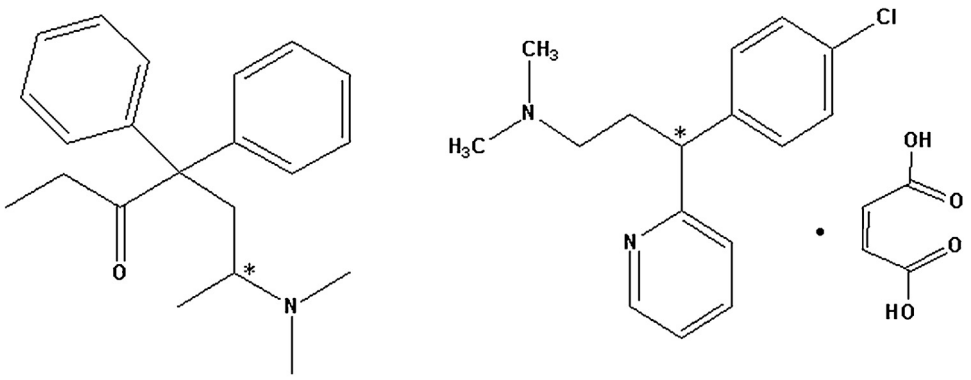

Fig. 1. Molecular structure and physiochemical properties of MDN and internal standard [7,8].

breast milk [7,15]. Liquid–liquid extraction (LLE) [10,16,17] and solid-phase extraction (SPE) [11] techniques, without an initial preparative step of protein precipitation, have been applied for the isolation of MDN and/or its major metabolites from plasma or blood samples before any chromatographic analysis. LLE and SPE are time consuming, generally labor intensive and requires large quantities of expensive, toxic and environmentally unfriendly organic solvents. Microextraction techniques, usually represented by solid-phase microextraction (SPME) and liquid-phase microextraction (LPME), have the most important advantages that they can integrate sampling, extraction, concentration and sample introduction into one step [24,25]. Drawbacks of SPME are mainly related to the nature of polymeric extractant phase nature and the desorption process; in fact, the use of polymer as an extractant phase includes batch-to-batch variation and low repeatability [26]. LPME is an emerging technique developed from LLE, in which a small amount of solvent is employed to extract analytes. Ho et al. [27] performed a comparison between liquid phase microextraction (LPME) and LLE for the extraction of methadone. LPME provided higher analyte enrichment and superior selectivity as compared to LLE. Compared with SPME, LPME is more simple, fast, efficient, inexpensive since there is no need for special coating material and reduces the extracting solvent volume to microliters level. Solvent bar microextraction (SBME) without using microsyringe was proposed by Jian and Lee [28], while the basic HF–LPME system uses microsyringes for the introduction and collection of the acceptor phase. The free movement of the solvent bar in an aqueous sample solution greatly increases the transfer of analytes from the aqueous sample to the extraction solvent. Furthermore, since the ratio of surface area to volume is large the column like configuration increases the solvent's surface area and this makes the extraction more stable and tolerable during the stirring of sample. Simplicity of operation, high recovery and high preconcentration factor are the main advantages of SBME [29].

In this study, SBME followed by gas chromatography (GC) with FID detection was applied for extraction and determination of methadone in different biological samples. The experimental variables such as the extraction solvent, source phase pH, extraction temperature, ionic strength, stirring rate and extraction time were optimized. For this purpose, a multivariate strategy based on an experimental design methodology using a Plackett–Burman design (PBD) was applied to screen and subsequently a central-composite design (CCD) was exploited to optimize the significant factors. Finally, the optimized procedure was applied to determine methadone concentration in plasma and urine samples, satisfactorily.

2. Experimental

2.1. Chemicals and reagents

MDN (>99.5%) was obtained from Dr. Abidi Laboratories (Tehran, Iran). 1-Undecanol, *n*-nonanol, *n*-octanol, decanol, dodecanol, cyclohexanol, tetradecan, acetone, sodium hydroxide, triflouroacetic acid and hydrochloric acid were purchased from Merck (Darmstadt, Germany). HPLC-grade methanol was purchased from Caledon (Georgetown, Ontario, Canada). NaCl and NaOH were obtained from Merck (Darmstradt, Germany) and used to evaluate the effect of salt content of the sample and pH on the extraction of MDN, respectively. Triton X-100 was purchased from Sigma-Aldrich (Milwaukee, WI, USA). Chlorpheniramine maleate, as an internal standard (IS), was kindly donated by Sobhan Daro (Tehran, Iran) and used without further purification. Ultrapure water was prepared using a Milli-Q system from Millipore (Bedford, MA, USA). The Accurel Q3/2 polypropylene hollow fiber membrane (200 μm wall thickness, 600 μm I.D. and 0.2 μm pore size) was purchased from Membrana Company (Wuppertal, Germany) and used for all experiments.

2.2. Preparation of standard solutions and real samples

Stock standard solution of MDN (1000 mg L^{-1}) was prepared in HPLC-grade methanol. It was stored in a refrigerator at 4 °C and brought to ambient temperature just prior to use. The working solutions were prepared daily by diluting the standard solutions prior to use. Plasma sample was obtained from the Clinic of Taleghani Hospital (Tehran, Iran) and urine samples were obtained from Taleghani Hospital and Dr. Bagherzadeh Addiction Treatment Center (Tehran, Iran). The plasma and urine samples were collected into test tubes and stored at −20 °C prior to use. Sample preparation step for plasma and urine samples follows two distinct process; for plasma samples: (1) 4 mL spiked plasma sample is mixed with 100 μL hydrochloric acid (37%) and 100 μL triflouroacetic acid in order to precipitate proteins, (2) the obtained solution was vortexed and centrifuged for 3 min at 3000 rpm, (3) the supernatant was removed and diluted at the ratio of 2:3 with ultrapure water. For urine samples: 10 mL urine samples were used without any dilution.