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Preconcentration of trace amounts of methadone in human urine, plasma, saliva and sweat samples using dispersive liquid–liquid microextraction followed by high performance liquid chromatography

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ABSTRACT

A simple, rapid and efficient method for the preconcentration of methadone was developed using dispersive liquid–liquid microextraction (DLLME) followed by high performance liquid chromatography with ultra violet detection (HPLC–UV). The extraction method is based on the fast injection of a mixture of extracting and disperser solvents into the aqueous solution to form a cloudy ternary component solvent (aqueous solution:extracting solvent:disperser solvent) system. The extraction parameters such as nature and volume of extracting and disperser solvents, pH of sample, and extraction time were studied for optimization. Under the optimal conditions (extracting solvent: chloroform, 250 μL; disperser solvent: methanol, 2.5 mL and pH of sample: 10.0) a linear calibration curve was obtained in the range of 0.5–5000 ng mL^{-1} with r^2 = 0.9995. To demonstrate analytical performance, figures of merits of the proposed method in four different biological matrices (urine, plasma, saliva and sweat) spiked with methadone were investigated. The limits of detection and quantification in these matrices were ranged from 4.90 to 24.85 ng mL^{-1} and 16.32 to 82.75 ng mL^{-1}, respectively. The extraction recoveries were above 97% and the preconcentration factors of methadone in distilled water, urine, plasma, saliva, and sweat samples were 196.52, 10.03, 9.93, 1.97 and 1.99, respectively. While the precision for inter-day was ≤6.43 (n = 5), it was ≤2.26 (n = 5) for intra-day assay. Finally, the method was successfully applied in the determination of methadone in the human urine, plasma, saliva and sweat samples.

1. Introduction

In many countries of the world, the selected treatment for opiates dependence is methadone maintenance therapy (MMT). Methadone (Fig. 1), also known as *Methadose*, *Dolophine*, *Amidone*, *Symoron*, *Physeptone*, *Heptadon* and many other names, is a synthetic analgesic drug which is commonly used to treat dependence on heroin and other opioids since the mid-1960s [1]. Because methadone treatment replaces a short-acting opioid (heroin) with a long-acting opioid (methadone), it has been controversial since its inception [2,3], particularly with regard to adequate dose levels.

According to clinicians and researchers, adequate methadone dosage should be based on an individualized clinical process using the best judgment of a physician trained to administer methadone [4,5]. In fact, due to the differences in the pharmacokinetics of methadone among different people, it is particularly important to develop analytical methods which can determine the total methadone concentration to individualize doses for achieving optimum treatment. Because of this, many analytical methods have been applied to the quantitation of methadone [6–18]. These include several analytical methods based on gas chromatography (GC) coupled with mass spectrometry (MS) [6,7] and flame ionization detection (FID) [8], liquid chromatography (LC) coupled with ultra violet (UV) [9], coulometric [10] and MS [11] detection, capillary electrophoresis [12] coupled with UV [13], MS [14] and electrochemiluminescence detection (ECL) [15], flow-injection analysis (FIA) [16], radioimmunoassay [17] and potentiometry with ion-selective electrode [18].

So far, analysis of methadone was performed in several biological samples such as serum [19], plasma [20], urine [21], hair [22], sweat [23] and saliva [24]. Due to the complex matrix of the real samples and the low concentration of methadone, making efforts to develop a simple and reliable method for preconcentration and determination of the methadone is the main challenge and a very important step for the analysis of it. The preconcentration methods, which are commonly used to monitor methadone in biological samples, are liquid–liquid extraction (LLE) and solid phase extraction (SPE). The SPE procedures used were based on several solid sorbents such as C8-SPE cartridge [25], Oasis HLB

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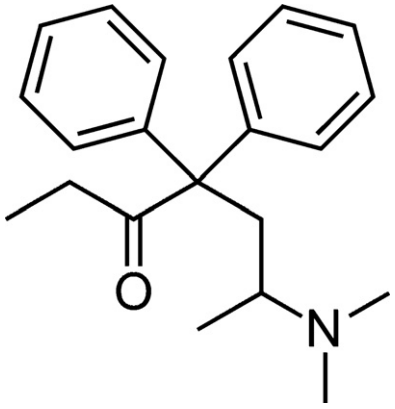

Fig. 1. Molecular structure of methadone.

96-well extraction plate [26], Bond Elut Certify cartridges [27] and Oasis cation-exchange cartridges (MCX) [28]. Recently, several solid sorbents comprise of mixed cationic exchange/lipophilic resin (BondElut Certify), hydrophilic/lipophilic balance cartridges (OASIS HLB), C8 cartridge and cyclohexyl (CH) were tested by Mercolini et al. [29], for the SPE, isolation and preconcentration of methadone and it was concluded that extraction using a C8-SPE cartridge provided a higher extraction yield with less interferences. Lucas et al. [30] employed solid-phase microextraction (SPME) as a rapid, solvent free and quicker procedure for the extraction of methadone from human hair. In 2002, Ho et al. [31] performed a comparison with liquid phase microextraction (LPME) and LLE for the extraction of methadone. LPME has been accomplished either by extraction into small water immiscible drops of organic solvents (two-phase LPME) or into small volumes of acceptor solution present inside the lumen of porous hollow fibers (three-phase LPME). Results confirmed that for the extraction of moderately or highly hydrophobic analytes, LPME provides higher analyte enrichment and superior selectivity as compared to LLE because the volumes of organic solvent used in both two- and three-phase LPME were very small.

However, each of these procedures has its own disadvantages; for instance, LLE and SPE methods are expensive, time-consuming and labor-intensive. The main drawback of two-phase LPME is the instability of the drop at high stirring rates or temperatures [32]. Three-phase LPME procedure suffers from manipulation of the hollow fiber at the time of placing it at the tip of the needle of the microsyringe before the microextraction process, because manipulation could be a source of contamination [32]. Drawbacks of SPME are mainly related to the polymeric extractant phase nature and the desorption process; in fact, the use of a polymer as extractant phase includes disadvantages such as batch-to-batch variation, artifact formation and low repeatability [32].

Despite the widespread usage of dispersive liquid–liquid microextraction (DLLME) in trace analysis of numerous materials in the variety samples [33–35], there is not any report about the extraction of methadone from the urine, plasma, saliva and sweat samples using DLLME. This method was introduced by Assadi and co-workers [33]. DLLME is based on a ternary component solvent system like homogeneous liquid–liquid extraction [36] and cloud point extraction [37] which the appropriate mixture of extracting solvent and disperser solvent is injected rapidly into a conical test tube containing aqueous solution; therefore, a cloudy solution is formed. At this time the analyte in the aqueous solution is extracted into fine droplets of extracting solvent. After centrifugation, the enriched analyte in the sedimented phase is withdrawn and is determined by chromatography or spectrometry methods.

Although, urine and plasma analysis is a commonly used method to detect drug abuse, it is impractical to collect urine or plasma samples under particular situations, such as in the monitoring of drivers, monitoring individuals in safety-related work, and surveying of drug use in the general population. For the first time, extraction of methadone was developed using DLLME–HPLC–UV which showed sufficient specificity and simplicity of operation for the measurement of trace amounts of methadone in urine, plasma, saliva and sweat.

2. Experimental

2.1. Chemicals

Methadone hydrochloride was purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol, acetonitrile, acetone (HPLC-grade), dichloromethane, chloroform, carbon tetrachloride and three flouroacetic acid (TFA) were purchased from Merck (Darmstadt, Germany). The water used for mobile phase was double distilled deionized which was produced by a Milli-Q system (Millipore, Bedford, MA, USA). A stock standard solution of methadone (100 mg L^{-1}) was prepared in methanol. The working solutions were prepared by appropriate dilution of the stock solution with double distilled/deionized water.

2.2. Instrumentation and operating condition

The chromatographic analysis was performed on an HPLC system equipped with a series 10 LC pumps, UV detector model LC-95 set at 205 nm, and model 7125i manual injector with a 20 μL sample loop all from Perkin-Elmer (Norwalk, CT, USA). Separation was done by an isocratic elution on a C_{18} (250 mm × 4.6 mm, 10 μm) column from Dr. Maisch GmbH (Beim Brueckle, Germany). Mobile phase was a mixture of 0.1% TFA in methanol:water (60:40, v/v) with flow rate of 1.0 mL min^{-1}. Adjustment of pH was done by model 3030 Jenway pH meter (Leeds, UK). A Denley bench centrifuge model BS400 (Denley Instruments Ltd., Billingshurst, UK) was used to accelerate the phase separation.

2.3. Dispersive liquid–liquid microextraction procedure

For DLLME, 10.0 mL aliquot of water sample containing 100 ng mL^{-1} of methadone was placed in a 15 mL conical glass test tube fitted with a plastic cap. A mixture of 2.5 mL of methanol (as disperser solvent) and 250 μL of chloroform (as extracting solvent) was injected into a sample solution using 5.0 mL syringe rapidly, so that a cloudy solution was formed. The cloudy solution was centrifuged for 3 min at 3000 rpm. After centrifuging, the sedimented phase was completely transferred into another test tube and was evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 50 μL of HPLC grade methanol and injected into the HPLC using a 20 μL sample loop. All the experiments were performed in triplicates and average of the results was reported.

2.4. Sample collection and preparation

Blank urine and plasma samples were provided by healthy volunteer in our lab. According to the method of Shamsipur and Fattahi [38], for the sedimentation of undesirable compounds in the bottom of the conical test tube, these samples were kept frozen at −20 °C before extraction process. The frozen urine and plasma samples were thawed at room temperature and centrifuged for 10 min at 5000 rpm. Then, supernatants were decanted into clean glass tube and filtered through a 0.45 μm filter. 500 μL of filteration products were diluted to 10.0 mL and applied for extraction process as it was described in Section 2.3.