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An improved hollow fiber solvent-stir bar microextraction for the preconcentration of anabolic steroids in biological matrix with determination by gas chromatography–mass spectrometry

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ABSTRACT

In this paper, a convenient and self-assembled hollow fiber solvent-stir bar microextraction (HF-SSBME) device was developed, which could stir by itself. In the extraction process, the proposed device made the solvent “bar” not floating at the sample solution and exposing to air while organic solvents outside hollow fiber always wrapped with donor phase solvent, which reduced the vaporization of organic solvents. This design could improve the precisions and recoveries of experiments. For evaluating the device, seven anabolic steroids (prasterone, 5α-androstane-3α, 17β-diol, methandriol, 19-norandrostenediol, androstenediol, methyltestosterone and methandienone) were used as model analytes and extraction conditions such as type and volume of organic solvents, agitation speed, extraction time, extraction temperature and salt addition were studied in detail. Under the optimum conditions (15 μL toluene, 40 °C, stirring at 750 rpm for 30 min with 1.5 g sodium chloride addition in 20.0 mL donor phase), the linear ranges of anabolic steroids were 0.25–200 ng mL^{-1} with gas chromatography–mass spectrometry. The limits of detection were lower than 0.10 ng mL^{-1}. The recoveries and precisions in spiked urine and hair samples were between 73.97–93.56% and 2.18–4.47% ($n = 5$). HF-SSBME method combined the intrinsical merits of hollow fiber with the superiority of the proposed self-stirring device which can be developed to two-phase, three-phase and in situ derivatization modes with wide prospect of application. Besides, the pedestal of this proposed device can be converted to fix stir bar in stir bar sorptive extraction (SBSE) method.

1. Introduction

Since liquid phase microextraction (LPME) was introduced as an amazing pretreatment method at 1996 [1,2], its vigorous developments and wide applications have been reported. Among them, membrane supported LPME mainly named hollow fiber-liquid phase microextraction (HF-LPME) was greatly concerned because of the inherent advantages of LPME such as combining extraction and enrichment, inexpensive, easy operation, nearly solvent-free, and the highlighted advantages of HF-LPME brought from porous hollow fibers such as efficient for sample clean-up, reducing or eliminating potential problems from matrix components. The modes of HF-LPME can be expanded such as two-phase, three-phase and in situ derivatization in hollow fiber, carrier mediated HF-LPME [3], surfactant enhanced HF-LPME [4] and so on. The extraction principles, historical development and major applications of it have been compiled, and recent forefront developments of HF-LPME have been discussed in reviews [5,6].HF-LPME devices have highly flexible formats. The first reported format is U-shaped HF-LPME in vial [7] which can also be attached with funnel-shaped injection guide consisting of stainless steel for semi-automated LPME [8]; besides, hollow fiber can be fixed in a pretreated pipet tip (used as needle guide and sealed by Teflon/PDMS septa) with for automated LPME [9,10]; the most common format is the hollow fiber held by the needle of conventional GC/HPLC syringe [11–13]. Review [14] has partly summarized the above LPME set-ups based on membrane supported.

In 2004, Jiang and Lee [15] proposed an alternative microextraction method derived from HF-LPME which named solvent bar microextraction (SBME). In this method, the organic extracting solvent (1-octanol) was confined within a short length of a hollow

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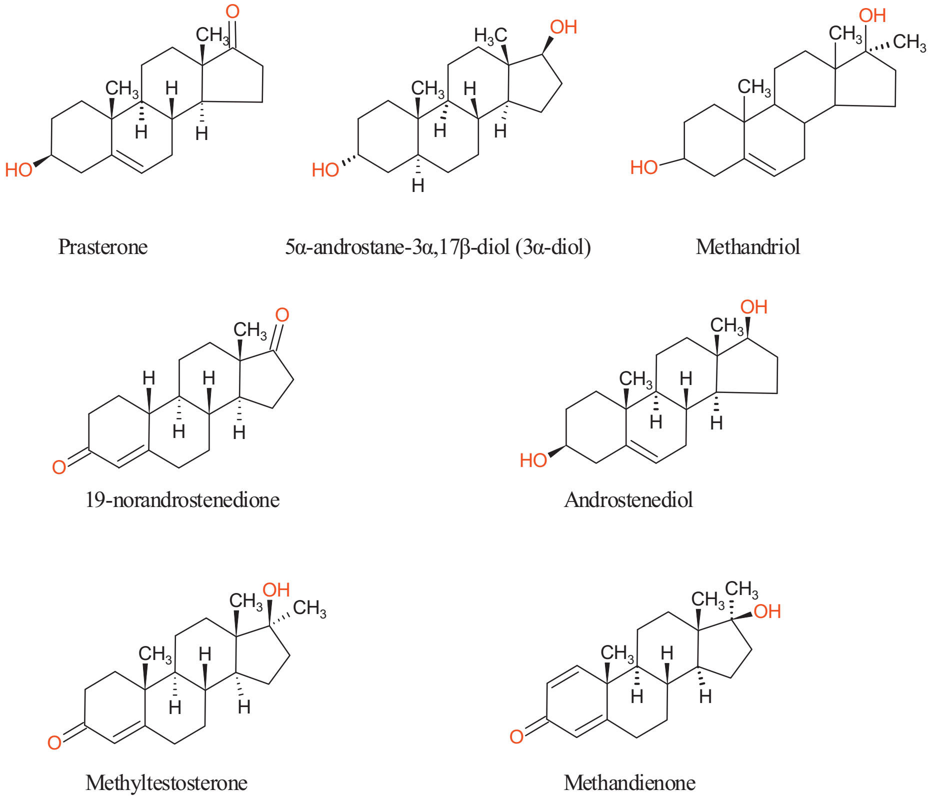

Fig. 1. Structures of the studied molecules.

fiber membrane (sealed at both ends) that was placed in a stirred aqueous sample solution. The extraction device was tumbled in sample solution which facilitated extraction and improved extraction efficiency. From then on, this method has been applied for organochlorine pesticides in wine [16], clenbuterol in human urine with three-phase mode [17], plasma protein binding of bisoprolol with three-phase mode [18], some ionizable organic compounds in river waters [19], some aliphatic amines in waste water samples [20] and for the speciation of As(III) and As(V) in water samples [21]. Besides, Ionic liquid was also introduced into SBME as the intermediary solvent for three-phase SBME [22].

Yu et al. proposed dual solvent-stir bars microextraction (DSSBME), in which hollow fibers were fixed in a stainless-steel wire and could stir by itself [23]. Xu [24] used a silica monolith instead of common hollow fiber as the extractant phase holder for extraction since the silica monolith was of high porosity to hold the extractant solvent in the pores. In 2009, Valcárcel proposed a new sample treatment technique called stir membrane extraction (SME) in which a membrane sealed cartridge was driven by an iron bar to do a plane rotation [25]. They also extended its application to coupling with infrared spectroscopy [26] and derived the extraction device to stir membrane liquid–liquid microextraction (SM-LLME) [27] and stir membrane liquid–liquid–liquid microextraction (SM-LLLME) [28].

Anabolic steroids are a kind of neutral growth promoters and have been on the list of prohibited substances published by the World Anti-Doping Agency (WADA) [29]. The standard method for anabolic steroids is gas chromatography–mass spectrometry conducted on a urine sample. But hair analysis has been proposed for identifying drug abusers in contest because the specimens could be easily collected without embarrassment and could not be evaded. Unlike urine, hair analysis has a wide window of detection, ranging from weeks to months, depending on the length of the hair shaft, and provides information concerning the pattern of an individual's drug abuse. Hair analysis has been accepted in most courts of Justice [30,31] although it is not yet adopted as the standard method by the International Olympic Committee. And the comparison of anabolic steroids in hair analysis and urinalysis with SPE and gas chromatography triple quadrupole mass spectrum has been reported [32].

In this paper, a new hollow fiber solvent-stir bar microextraction (HF-SSBME) method was developed by using a pipet tip, a magnetic rotor and polypropylene hollow fiber. It is cheaply manufactured and easily assembled, can self-stirring and solve the problem of solvent bar floating up on the water because specific gravities of most commonly used organic solvents in liquid phase microextraction are less than 1.0 and hollow fiber wall is multiporous. The developed pretreatment method has been applied to the analysis of anabolic steroids in human urine and hair samples using gas chromatography/mass spectrometry as instrumental technique and the results have been compared with reported LPME-related methods for anabolic steroids.

2. Experimental procedures

2.1. Chemicals and reagents

In this paper, seven anabolic steroids prasterone, 5α-androstane-3α, 17β-diol (3α-diol), methandriol, 19-norandrostenediol, androstenediol, methyltestosterone and methandienone (structures see Fig. 1) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Toluene, methanol and other organic solvent (HPLC grade) were from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Stock standard solutions of seven AAS (1.0 mg mL^{-1}) were prepared in methanol and stored at 4 °C in dark. Working solutions for optimization experiments and