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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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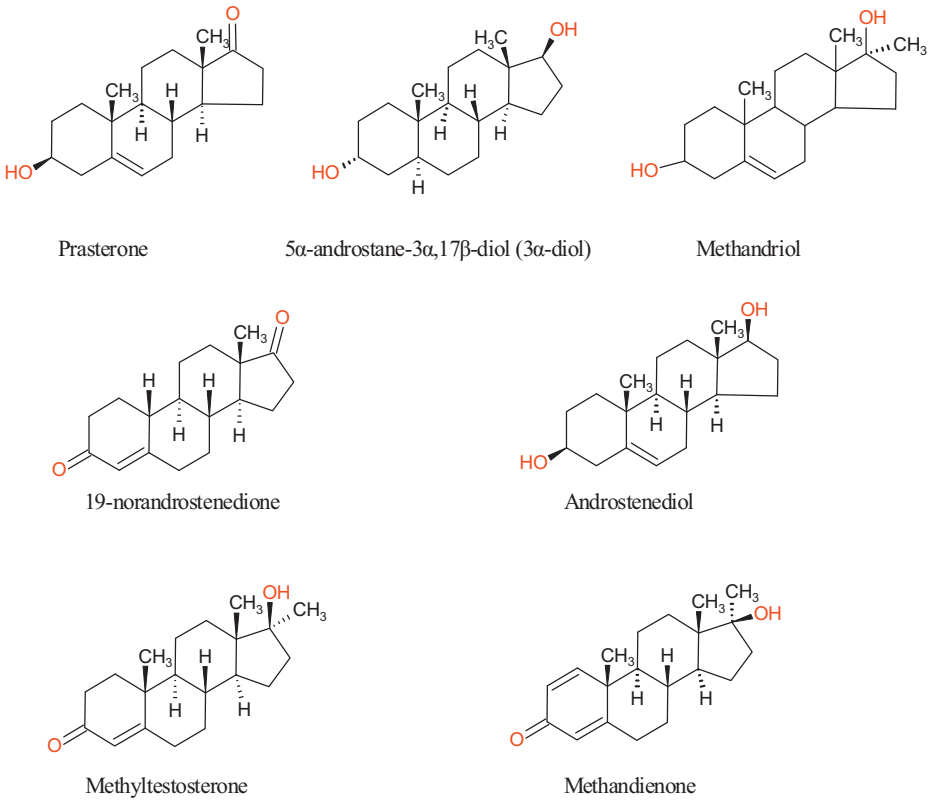

Fig. 1. Structures of the studied molecules.

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 (DSS-BME), 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

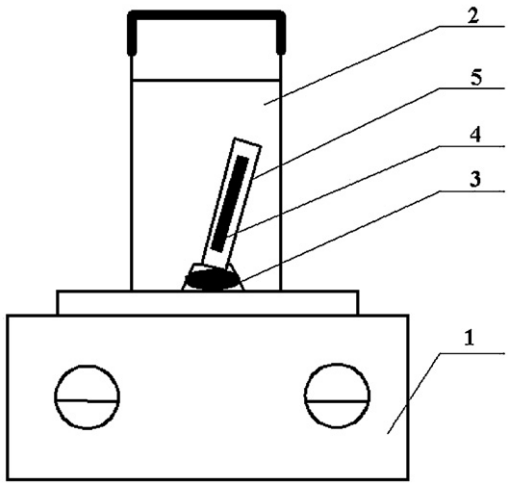

Fig. 2. Setup of HF-SSBME: 1. magnetic stirrer; 2. aqueous phase; 3. stirring bar pedestal; 4. acceptor phase; 5. hollow fiber membrane (sealed at both ends).

calibration curves were prepared by appropriate dilution of the stock standard solutions with ultrapure water.

2.2. Instrumentation

All gas chromatographic separations were performed on an Agilent 6890N-5973I GC/MSD (Hewlett-Packard, Palo Alto, CA, USA) and split-splitless injector. A DB-5MS GC column (0.25 μm film thickness, 0.25 mm × 30 m, Agilent Technologies) was used for separation. Milli-Q ultrapure water (Millipore, Bedford, USA) was used throughout the experiments.

2.3. GC/MS analysis

The injector temperature was maintained at 280 °C. Splitless injection was employed and the injection volume was 2.0 μL. The oven temperature was set at 180 °C and increased to 240 °C at 20 °C min^{-1} and held for 2 min, then increased to 250 °C at 5 °C min^{-1} and held for 5 min, finally increased to 310 °C at 30 °C min^{-1} and held for 3 min. Carrier gas was helium (purity > 99.999%) and the flow rate was set at 0.9 mL min^{-1}. The electron impact (EI) ion source, quadrupole mass analyzer, and the interface temperature were maintained at 230 °C, 150 °C and 280 °C, respectively. Electron impact ionization (70 eV) was utilized. EM voltage was 1635 mV and solvent delay was 8.0 min. Selected ion monitoring (SIM) mode was employed. Ions for quantification (labelled as underlined) and identification of those analytes were the ion fraction groups of prasterone (288, 255 and 270), 3α-diol (215, 292 and 233), methandriol (253, 213 and 271), 19-norandrostenediol (272, 186 and 110), androstenediol (286, 107 and 246), methyltestosterone (302, 124 and 229) and methandienone (122, 91 and 147) respectively.

2.4. HF-SSBME

In this paper, a simple and improved HF-SSBME device was designed (Fig. 2). Briefly, the top of a pipet tip (100–1000 μL, VWR, Mississauga, ON, Canada) was cut off, and the remaining part (~1.8 cm long) was used to trap a magnetic rotor. After that, it was drilled a gap at the periphery of the pipet for preparing to fix hollow fiber solvent bar. This part was used as the pedestal of the self-stir SBME device.

Accurel S6/2 polypropylene hollow fiber with a wall thickness of 200 μm (0.2 μm pore size) and an i.d. 1800 μm was purchased from Membrana (Wuppertal, Germany) and employed for the experiments. The hollow fiber was ultrasonically cleaned in acetone. After the fiber was dried, it was then cut into 1.2 cm lengths. One end of fiber was squeezed and sealed by a hot tweezers; then this part would be cooled as a hard piece and could be inserted into the gap expediently.

After moistening in toluene for immobilization organic solution in the fiber pores, a 25 μL syringe was depressed to fill the hollow fiber with 15 μL toluene as acceptor phase solvent. Another end of fiber was sealed by heat and the entire set-up was put into the sample solution. The practical length of the fiber, after sealing is about 1.0 cm.

After extraction, the set-up was put out by a tweezers, one end of fiber was opened by scissors, and the analyte-enriched solvent was withdrawn into the syringe and the hollow fiber was discarded. Usually, 10 μL toluene was collected after extraction because the evaporation and dissolution of organic phase during operation and extraction process could not be avoided. Then two microliter of solvent was injected into the GC/MS.

2.5. Sample preparation

Negative human urine and head hair samples were submitted to initial procedure of decontamination. The blank urine samples were prepared by adding NaCl to make the final concentration of NaCl to be 0.075 g mL^{-1}, then centrifuged at 4500 rpm for 15 min, filtered through a 0.22 μm membrane filter and stored in the refrigerator at −20 °C. The spiked urine samples were prepared by adding standard solutions in 20.0 mL blank urine sample and adjusted to optimum conditions.

The blank hair samples were prepared as below description. 50 mg hair samples were cut into small pieces of about 2 mm, and then washed three times for 5 min in 5.0 mL of deionized water, petroleum ether and dichloromethane by vortex-mixing. After drying, the samples were digested with 2.0 mL of 1.0 mol L^{-1} NaOH for 12 h at 60 °C and then centrifuged at 2500 rpm for 15 min. Afterwards, the clear supernatant was diluted with 20.0 mL water. After adjusting pH and ion strength, it was stored in the refrigerator at −20 °C. The spiked hair samples were prepared by adding standard solutions in 20.0 mL blank hair sample.

3. Results and discussion

3.1. Selection of organic solvent

It is important to choose a suitable extracting organic solvent in all LPME method, and some universalistic requirements should be contented. Those organic solvents should be easily immobilized on the spongy hollow fiber and immiscible with water, have low vapor pressure to prevent loss during agitation and can withstand high stirring speed without leakage. And first of all, those selected solvents should ensure high enrichment for those tested analytes. Considering the above aspects, the most common extracting organic solvents such as, acetidin, octanol, cyclohexane and toluene were selected for investigating the extraction efficiencies. As shown in Fig. 3, with this usage of toluene as extraction solvent, high extraction efficiency was obtained for the target analytes by this HF-SSBME method. Thus, toluene was selected for subsequent experiments, and appeared to perform satisfactorily.

3.2. Volume of organic solvent

The volume of sample solution (donor phase) and organic solvent (acceptor phase) would affect enrichment factor, which depends directly on the volume of the donor and the acceptor phases [27]. In the extraction process, the sample volume was hold

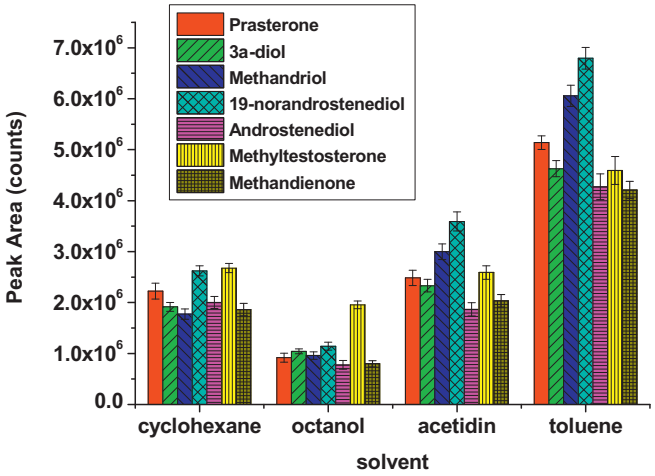

Fig. 3. Selection of extraction solvent HF-SSBME conditions: extraction time, 30 min; extraction temperature, 40 °C; NaCl 0.075 g mL^{-1}; stirring speed, 750 rpm. The concentrations of each compound were 25 ng mL^{-1}, respectively.

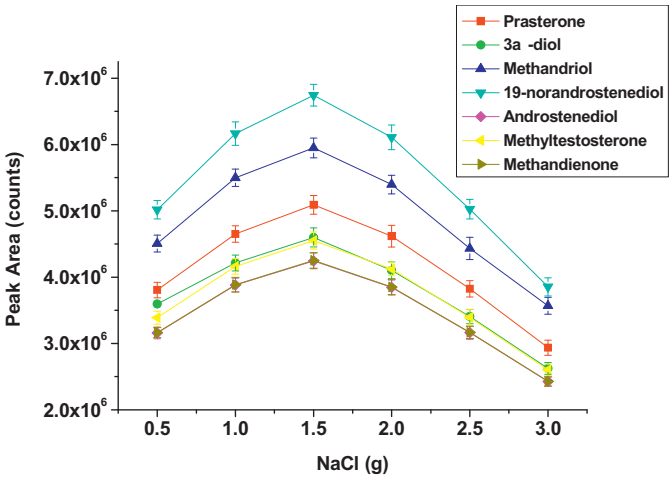

Fig. 5. Optimization of concentration of NaCl. HF-SSBME conditions: 15 μL toluene; extraction time, 30 min; extraction temperature, 40 °C; stirring speed, 750 rpm. The concentrations of each compound were 25 ng mL^{-1}, respectively.

at 20 mL while organic solvent toluene was increased in the range from 15 to 30 μL. It was found that the enrichment factors were increased when lower volumes of toluene were used. Therefore, 1.2 cm hollow fiber with effective length of about 1.0 cm was used and the volume of the organic solvent toluene was 15 μL.

3.3. Effection of stirring speed

Agitation is one of significant parameters in the kinetics of extraction. With agitation, a new and fresh interface between aqueous phase and organic phase can be provided continuously, and mass transfer of target compounds through the organic solvent in the pores of the fibre can be improved, thereby the extraction efficiency is increased. But too high a stirring rate (A) can cause air bubbles to attach to the fibre surface and limits the mass transfer of analytes; (B) may improve the diffusion/dissolution of analytes from organic liquid on membrane into sample solution and caused the decrease of extraction efficiency; (C) can also lead to loss of organic liquid impregnated in the membrane. Those reasons made high stirring speed decrease the extraction ability.

In this method, the hollow fiber was settled on the device with stirrer and the whole device could stir by itself during the extraction program in HF-SSBME. In stirring speed experiment (Fig. 4), it was tested from 500 to 1100 rpm, and 750 rpm was selected for HF-SSBME in future work.

3.4. Effection of ion strength

For ionizable organic molecules, there are some important critical parameters which need to be optimized in hollow fiber extraction technique and have been reviewed in Ref. [33]. Anabolic steroids are a kind of neutral materials, so pH of donor phase does not affect the extraction and need not to be adjusted ion strength of the sample solution was optimized by preparing standard solutions of the analytes together with a series of NaCl at concentrations from 0.5 to 3.0 g (in 20.0 mL donor phase). As shown in Fig. 5, the responses of the anabolic steroids increased with the increasing of NaCl concentration until 1.5 g (that's 0.075 g mL^{-1}) and then declined. It's possible because the proper concentrations of NaCl could reduce the affinity of anabolic steroids in donor phase and increase extraction efficiency. But the excess of the ionic strength affect diffusion of the analytes into the organic phase because of the electrostatic interaction of salt ions and analytes in solution. Considering the overall responses of the target

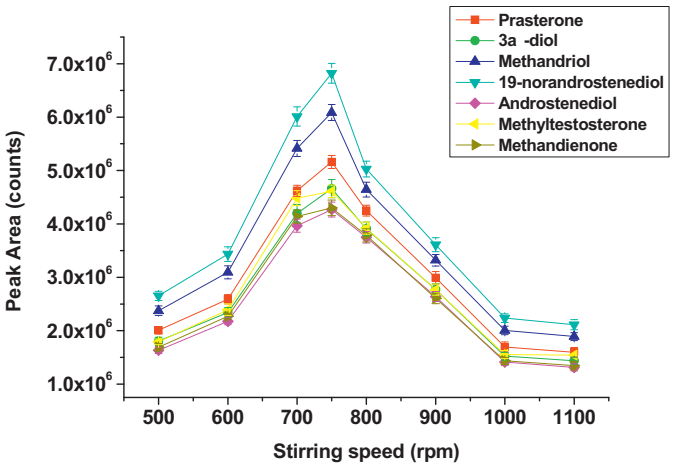

Fig. 4. Optimization of the stirring speed. HF-SSBME conditions: 15 μL toluene; extraction time, 30 min; extraction temperature, 40 °C; NaCl 0.075 g mL^{-1}. The concentrations of each compound were 25 ng mL^{-1}, respectively.

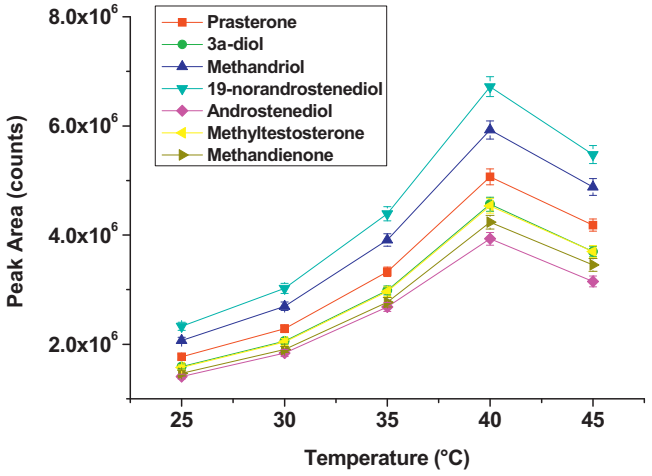

Fig. 6. Optimization of extraction temperature. HF-SSBME conditions: 15 μL toluene; extraction time, 30 min; NaCl 0.075 g mL^{-1}; stirring speed, 750 rpm. The concentrations of each compound were 25 ng mL^{-1}, respectively.

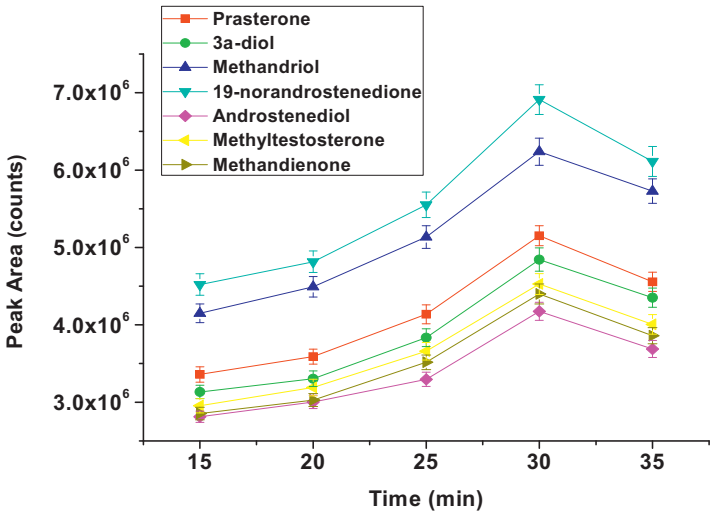

Fig. 7. Optimization of extraction time. HF-SSBME conditions: 15 μL toluene; extraction temperature, 40 °C; NaCl 0.075 g mL^{-1}; stirring speed, 750 rpm. The concentrations of each compound were 25 ng mL^{-1}, respectively.

compounds, 1.5 g NaCl (0.075 g mL^{-1}) was selected in further experiments.

3.5. Selection of temperature

Temperature plays a major role in the extraction process for it influences the rates of mass transfer and the partition coefficients of the analytes. A series of extraction temperatures was studied by extracting aqueous solution containing 25 ng mL^{-1} of each analyte at 750 rpm stirring speed. It was found that the analytical signals increased quickly within 40 °C of extraction temperatures. After 40 °C, the plots were declined. This result can be explained by the fact that high temperature would make the organic phase into aqueous, and thus would make the partition coefficients between the organic and aqueous phase decreased more than increasing of the mass transfer rates of analytes. Therefore, the extraction temperature was held at 40 °C in further experiments (see Fig. 6).

3.6. Selection of extraction time

Extraction time decided extraction efficiency. Hence, the tendency of peak responses of analytes with extraction time was studied. As shown in Fig. 7, the peak areas of analytes were increased obviously with increasing extraction time from 15 to 30 min and then the plot appeared a little descent. As discussed previously in Ref. [15], SBME is not a process dependent on exhaustive extraction but an equilibrium process that analytes partitioned between the aqueous phase and the organic phase, and the practical equilibrium to be established was from prolong extraction time with stable extraction efficiencies. However, the longer the extraction time made the greater the potential of solvent loss into the sample solution. Therefore, an extraction period of 30 min was chosen for further experiments.

To sum up, the optimum conditions of extraction were as follows: in 20 mL spiked water solution including 0.075 g mL^{-1} NaCl, the HF-SSBME was extracted analytes for 30 min with 750 rpm

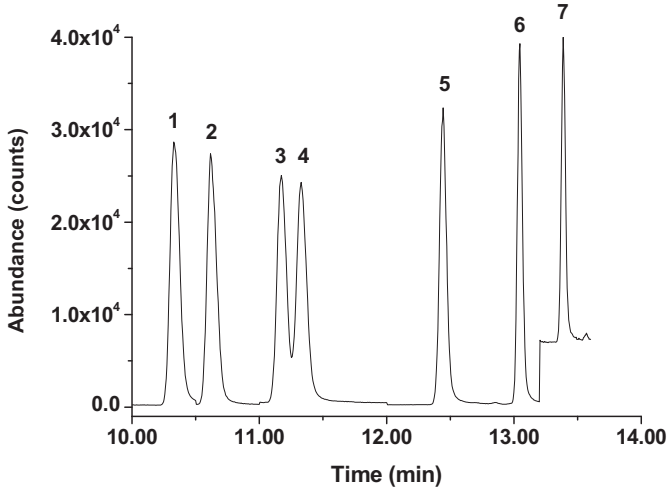

Fig. 8. Typical ion chromatograms of analytes after HF-SSBME. 1, prasterone; 2, 3α-diol; 3, methandriol; 4, 19-norandrostenediol; 5, androstenediol; 6, methyltestosterone; 7, methandienone; The concentrations of each compound were 10 ng mL^{-1}, respectively; Conditions: TIC in SIM mode. 15 μL toluene; extraction time, 30 min; extraction temperature, 40 °C; NaCl 0.075 g mL^{-1}; stirring speed, 750 rpm.

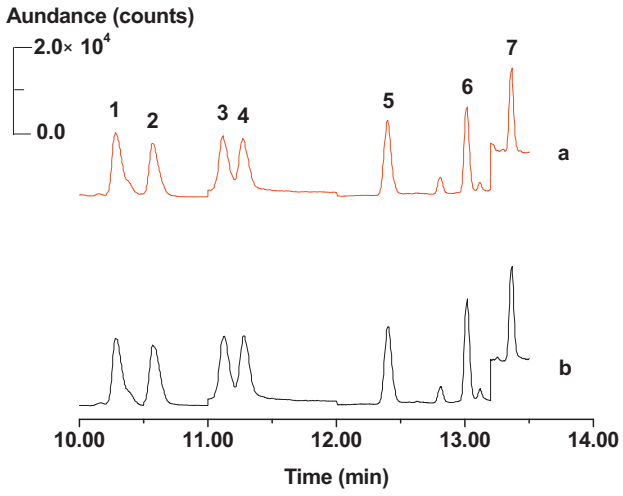

Fig. 9. Typical ion chromatograms of spiked urine and spiked hair samples. 1, prasterone; 2, 3α-diol; 3, methandriol; 4, 19-norandrostenediol; 5, androstenediol; 6, methyltestosterone; 7, methandienone; the concentrations of each compound were 2.5 ng mL^{-1}, respectively; Conditions: TIC in SIM mode. (a) in spiked urine sample, (b) in spiked hair sample.

Table 1
Regression equations, linear ranges, correlation coefficients, limits of detection and intraday deviation.

Analyte[a]	Extraction	Regression equation[b]	R^2	Linear range ($ng\,mL^{-1}$)	LOD[c] ($ng\,mL^{-1}$)	Intraday deviation[d] %RSD ($n=7$)
Prasterone	After	$y=7.9\times10^4x+5.1\times10^3$	0.9938	0.25–200	0.10	2.15
	Before	$y=1.4\times10^2x-1.7\times10^4$	0.9970	200–50,000	100	
3α-Diol	After	$y=6.9\times10^4x+1.0\times10^4$	0.9907	0.25–200	0.10	3.87
	Before	$y=1.4\times10^2x-1.3\times10^4$	0.9981	200–50,000	100	
Methandriol	After	$y=9.2\times10^4x+4.3\times10^3$	0.9965	0.25–200	0.10	3.40
	Before	$y=1.6\times10^2x-0.56\times10^4$	0.9999	200–50,000	100	
19-Norandrostenediol	After	$y=9.3\times10^4x-5.1\times10^3$	0.9995	0.25–200	0.10	2.84
	Before	$y=2.5\times10^2x-3.3\times10^4$	0.9966	200–50,000	100	
Androstenediol	After	$y=6.2\times10^4x-7.0\times10^2$	0.9988	0.25–200	0.10	3.61
	Before	$y=1.6\times10^2-2.6\times10^4$	0.9944	200–50,000	100	
Methyltestosterone	After	$y=6.6\times10^4x-1.0\times10^3$	0.9974	0.25–200	0.10	2.29
	Before	$y=1.8\times10^2x-2.6\times10^4$	0.9962	200–50,000	100	
Methandienone	After	$y=4.8\times10^4x-1.1\times10^3$	0.9942	0.25–200	0.10	4.69
	Before	$y=2.3\times10^2x-4.0\times10^4$	0.9935	200–50,000	100	

[a] The same experimental conditions as in Fig. 9.
[b] The parameters, x and y, refer to the concentration of the target compound ($ng\,mL^{-1}$) and the corresponding peak area.
[c] S/N = 3.
[d] $n=2.5\,ng\,mL^{-1}$ spiked in water solutions, respectively.

Table 2
Recoveries of spiked urine samples and hair samples by HF-SSBME.[a]

Analyte	%Recovery rate in spiked urine samples (±%RSD, $n=5$)			%Recovery rate in spiked hair samples (±%RSD, $n=5$)		
	200.0 ($ng\,mL^{-1}$)	20.0 ($ng\,mL^{-1}$)	2.50 ($ng\,mL^{-1}$)	200.0 ($ng\,mL^{-1}$)	20.0 ($ng\,mL^{-1}$)	2.50 ($ng\,mL^{-1}$)
Prasterone	82.21% (±2.39%)	81.33% (±2.18%)	78.47% (±3.82%)	90.22% (±2.21%)	92.82% (±3.26%)	82.26% (±3.76%)
3α-Diol	86.53% (±2.62%)	86.97% (±2.47%)	81.93% (±3.78%)	93.48% (±3.04%)	90.83% (±3.35%)	74.63%(±3.64%)
Methandriol	92.37% (±2.98%)	90.91% (±2.75%)	78.28% (±3.33%)	89.08% (±2.20%)	93.00% (±3.98%)	79.58% (±4.45%)
19-Norandrostenediol	90.69% (±2.77%)	91.18% (±3.64%)	82.34%(±2.45%)	93.56%(±2.86%)	93.25% (±2.97%)	87.31% (±3.79%)
Androstenediol	88.70% (±3.03%)	88.32% (±3.41%)	75.65% (±4.17%)	90.04% (±3.65%)	91.51% (±3.42%)	73.97%(±4.30%)
Methyltestosterone	85.48%(±3.27%)	87.52% (±2.23%)	84.28% (±2.64%)	84.79% (±2.28%)	92.29% (±2.75%)	81.12%(±3.21%)
Methandienone	85.39%(±2.84%)	92.04% (±3.50%)	86.99%(±3.59%)	92.96% (±2.82%)	89.31% (±3.66%)	85.05%(±4.47%)

[a] The same experimental conditions as in Fig. 9.

Table 3
Compared three kinds of LPME methods for anabolic steroids in urine.

LPME methods	Determination techniques	LODs	Intra-day RSD %	References
U-LPME	LC–MS/MS	2–20 $ng\,mL^{-1}$	2–10% (100 $ng\,mL^{-1}$, $n=6$)	[36]
In-fiber silylation LPME	GC–MS	2 $ng\,mL^{-1}$	2.7% (100 $ng\,mL^{-1}$, $n=12$)	[37]
This method[a]	GC–MS	0.1 $ng\,mL^{-1}$	2.45–4.17% (2.5 $ng\,mL^{-1}$, $n=5$)	–

[a] The same experimental conditions as in Fig. 9.

stirring rate in 40 °C. The typical chromatograms of spiked water sample by HF-SSBME were shown in Fig. 8.

3.7. Comparison of HF-SSBME and SBME

The HF-SSBME method was compared with SBME method in the same 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), but none of toluene could be collected in the SBME method after 30 min extraction. Perhaps, it is because when the solvent bar is floating at the sample solution and exposing to air, toluene is vaporized continuously during extraction process. In fact, we found other reports about SBME were contrasted and adopted the extraction solvents that boiling point are higher than toluene such as 1-octanol [15,17,18], nonanol [20], a 1:1 mixture of 1-octanol:dihexylether [19], n-tetradecane [16], nitrobenzene [21], ionic liquid [22] and so on. Although, toluene is one of the most common used extraction solvent in the LPME methods.

3.8. Method evaluation

Under the optimized HF-SSBME conditions, calibration curve were drawn using spiked deionized water samples and using the direct injection method, respectively. In spiked water solutions, calibration standards were prepared by concentration of 0.25, 0.5, 1, 5, 10, 25, 100 and 200 $ng\,mL^{-1}$. The proposed method was evaluated for linear range, limits of detection (LODs) which were demonstrated in Table 1. Compared with the direct injection method, the LODs of analytes with HF-SSBME method are dropped 1000 times (from 100 to 0.1 $ng\,mL^{-1}$) with good linear range (0.25–200 $ng\,mL^{-1}$) and satisfactory correlation coefficients ($R^2 \geq 0.9907$). The intra-day standard deviations of this new preparation method were assessed with seven spiked water solutions (2.5 $ng\,mL^{-1}$) on the same day and they were 2.15% to 4.69% for the analytes.

3.9. Applications

In order to validate the feasibility of the proposed method, the competition of hair analysis and urinary analysis was performed with the preferred HF-SSBME–GC/MS method for anabolic steroids. And the recoveries were assessed by spiking urine and digested hair solutions with trace mixed standards solutions (2.5, 20 and 200 $ng\,mL^{-1}$, respectively) and calculating with the regression equation of analytes in water matrix.

The typical chromatograms of spiked human urine samples and hair digestion samples by HF-SSBME were shown in Fig. 9. It could be seen that no significant interference of impurities in determination of the seven anabolic steroids. The method recoveries and deviations of anabolic steroids in different matrices were consulted in Table 2. In all, although the determination of drug abuse in the human urine and the hair is difficult because of complex sample matrix and low analyte concentrations, HF-SSBME provides a good alternative extraction technique for anabolic steroids in human urine and hair and offers analyte pre-concentration and sample clean-up at the same time.

Finally, the method was compared with U-shaped liquid phase microextraction (U-LPME) and in-fiber silylation derivatization LPME for anabolic steroids in human urine (see Table 3). Among three HF-LPME methods, the porous hollow fibers were confirmed remarkably straight-forward and fast; at the same time, it eliminated risk of sample contamination with good precisions. Besides, the solvent-stir bar device is confirmed effectively improving sensitivity in experiments. So, this proposed HF-SSBME method is rapid, sensitive, robust and reliable for the quantitative determination of the drug abuse such as anabolic steroids in urine and hair samples.

4. Conclusions

The described HF-SSBME mode using 15 μL of organic solvent protected by a 1.0 cm length of porous hollow fiber with sealed two ends and settled in a magnetic base. This experimental equipment is extremely simple and can be facilely assembled. It has been used to extract anabolic steroids in those complicated matrix such as human urine and hair samples with acceptable accuracy and precision.

This method made clean-up and preenrichment into one-step, not only isolated acceptor phase from water-soluble impurities and macromolecular material but also increased precisions because of always submerging in the donor phase liquids. This self-stirring device can be conveniently assembled and widely applied in two-phase, three-phase and in situ derivatization modes. Furthermore, we think that the pedestal of this proposed device can be converted to fix stir bar in stir bar sorptive extraction (SBSE) method [34] which is based on the same principle with solid phase microextraction and exhibits a considerable high sensitivity. It can avoid the direct contact of the stir bar coating with the bottom of vessel and avoid the abrasion from friction during the SBSE extraction process [35].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2012.01.064.

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