Talanta 100 (2012) 153–161

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A novel extraction technique based on carbon nanotubes reinforced hollow fiber solid/liquid microextraction for the measurement of piroxicam and diclofenac combined with high performance liquid chromatography

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ARTICLE INFO

Article history:
Received 31 May 2012
Received in revised form
16 August 2012
Accepted 27 August 2012
Available online 3 September 2012

Keywords:
Multi-walled carbon nanotubes
Hollow fiber
Solid/liquid phase microextraction
Piroxicam
Diclofenac
Sol–gel technology

ABSTRACT

A novel design of carbon nanotubes reinforced hollow fiber solid/liquid phase microextraction (CNTs-HF-SLPME) was developed to determine piroxicam and diclofenac in different real water samples. Functionalized multi-walled carbon nanotubes (MWCNTs) were held in the pores of hollow fiber with sol–gel technology. The pores and lumen of carbon nanotubes reinforced hollow fiber were subsequently filled with a μL volume of organic solvent (1-octanol), and then the whole assembly was used for the extraction of the target analytes in direct immersion sampling mode. The target analytes were extracted from the sample by two extractants, one of which is organic solvent placed inside the pores and lumen of hollow fiber and the other one is CNTs held in the pores of hollow fiber. After extraction, the analytes were desorbed in acetonitrile and analyzed using high performance liquid chromatography. This novel extraction mode showed more excellent extraction performance in comparison with conventional hollow fiber liquid microextraction (without adding CNTs) and carbon nanotubes reinforced hollow fiber solid microextraction (CNTs held in the pores of hollow fiber, but no organic solvents placed inside the lumen of hollow fiber) under the respective optimum conditions. This method provided 47- and 184-fold enrichment factors for piroxicam and diclofenac, respectively, good inter-fiber repeatability and batch-to-batch reproducibility. Linearity was observed in the range of 20–960 μg L^{-1} for piroxicam, and 10–2560 μg L^{-1} for diclofenac, with correlation coefficients of 0.9985 and 0.9989, respectively. The limits of detection were 4.58 μg L^{-1} for piroxicam and 0.40 μg L^{-1} for diclofenac.

1. Introduction

Nowadays, people have paid more and more attention on poisonous substances in food, environment and drugs. However, most of them exist in complex sample matrices at trace levels. So appropriate sample preconcentration techniques are urgently needed to effectively monitor them. In recent years, miniaturized extraction techniques have arisen and developed continuously, such as solid phase microextraction (SPME) and liquid phase microextraction (LPME), and attracted increasing attention for their unparalleled advantages of low consumption of organic solvent, high enrichment efficiency and simple operation. As one of the operating modes of LPME, hollow fiber liquid phase microextraction (HF-LPME), applies porous hollow fiber to protect microdroplets of extract solvent, which effectively improves the stability and enables excellent clean-up. Although high enrichment factor, clean-up function and low solvent consumption are the major advantages of HF-LPME, low selectivity, limited kinds and easy loss of organic solvents are perhaps the major disadvantages of this method [1]. Recently, our group has applied HF-LPME combined with HPLC–UV to analyze trace ingredients in complex matrices such as food, biological and environmental sample and have attained satisfactory results [2–4].

The membrane is the key component in all membrane processes and determines both flux and selectivity. Thus the development of novel membrane architecture is of great importance to enhance the membrane's performance [5]. An interesting recent development is the mixed matrix membranes (MMM) which combine polymeric material with inorganic fillers such as zeolites, graphite, fullerenes, cyclodextrin, and metal oxide [6–10].

Abbreviations: MWCNTs, multi-walled carbon nanotubes; HPLC, high-performance liquid chromatography; HF-LPME, hollow fiber liquid phase microextraction; CNTs-HF-SPME, carbon nanotubes reinforced hollow fiber solid-phase microextraction; CNTs-HF-SLPME, carbon nanotubes reinforced hollow fiber solid/liquid phase microextraction

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http://dx.doi.org/10.1016/j.talanta.2012.08.042

Carbon nanotubes (CNTs), which are essentially grapheme sheets rolled into tubes as single-walled or multiple-walled structures exhibit excellent mechanical and thermal properties because of their unique geometric structure [11]. Moreover, by virtue of the large specific area and hydrophobic characteristic of the surface, CNTs have been regarded as a new type of sorbent and have been studied for adsorption of some inorganic [12] and organic compound classes [13–20]. The high adsorption capacity of CNTs and efficient extraction/desorption from it will increase the amount of analyte extracted and extraction efficiency [21].

Recently, our group has prepared a novel extract material, namely carbon nanotubes reinforced hollow fiber, and applied it to solid phase microextraction, in which the extractant is CNTs fixed in the porous wall of hollow fiber [22]. The satisfactory results demonstrated the effective extraction ability of carbon nanotubes. Considering the availability of organic solvents as extractant in conventional hollow fiber microextraction, the synergetic effect of carbon nanotubes and organic extract solvents would enhance the extraction efficiencies. The motivation for this research was to develop carbon nanotubes reinforced hollow fiber solid/liquid microextraction (CNTs-HF-SLPME) technique by fixing CNTs in the porous wall of hollow fiber. In this novel microextraction mode, the two extractants, organic solvent as liquid microextraction medium and CNTs as solid microextraction one will work together to increase the effective partition coefficient on the membrane, and lead to higher permeability of the analytes [23]. How CNTs are incorporated into the pores of hollow fiber without covering their active surface is a challenge. It determines whether the mixed matrix membrane can perform its unique properties or not. Sol–gel technology can efficiently incorporate inorganic compounds into organic polymeric structure in solution under mild conditions. In this technique, precursors are mixed at molecular level and multi-component materials could be formed at much lower temperature than the traditional processing method. Moreover, several inherent advantages of sol–gel technology such as their high thermal stability, porous structure, highly degree of flexibility in coating composition make it widely used for the preparation of materials and SPME fiber coatings [24].

Non-steroid anti-inflammatory drugs (NSAIDs) are a new class of emerging environmental pollutants that are widely used both in human and veterinary medicine. The principal cause of their presence in the environment is excreta and disposal of unused or expired products, but also the result of pharmaceutical industries waste [25–29]. Although piroxicam and diclofenac as two widely used NSAIDs have usual therapeutic use, their chronic abuse and accidental intoxications have also been described. Due to their biological activity, these drugs are of great concern if released into the environment [30].

In this work, an attempt was made to prepare CNTs reinforced hollow fiber by sol–gel technique and combine solid and liquid phase microextraction modes to preconcentrate piroxicam and diclofenac in different water samples.

2. Experimental

2.1. Chemicals and materials

Piroxicam and diclofenac (molecular structures shown in Fig. 1) were purchased from National Institutes for Food and Drug Control (Beijing, China). Chromatographic grade methanol was obtained from Merck Co. (Darmstadt, Germany). Other chemicals are of analytical grade and were purchased from Tianjin Chemical Reagent Co. (Tianjin, China). Ultrapure water gained by a water purification system (Shanghai Laikie Instrument Co., Ltd., Shanghai, China) was used to prepare mobile phase and sample solution. Accurel Q3/2 polypropylene hollow fiber membrane (200 μm wall thickness, 600 μm internal diameter, 0.2 μm average pore size) was purchased from Membrana GmbH (Wuppertal, Germany). Multi-walled carbon nanotubes (MWCNTs) with purity higher than 95%, length of 0.5–0.2 μm and mean diameter of 8–15 nm were purchased from Chengdu Organic Chemical Co. Ltd., Chinese Academy of Sciences (Chengdu, China).

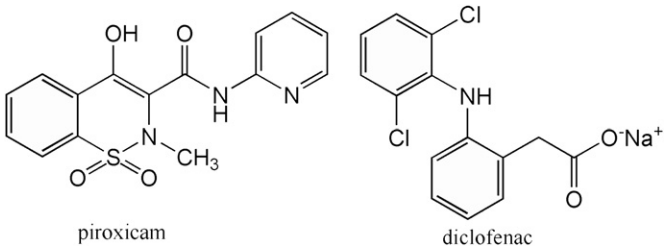

Fig. 1. The structures of the analytes.

2.2. Apparatus and chromatography

The HPLC system (Waters Corp., Milford, MA, USA) was made up by Waters quaternary pump (Mode Delta 600E), a photodiode array detector (Mode 2996), a manual injector, and Waters Millennium^{32} software for peak identification and integration. Chromatographic separation of the analytes was performed on a Kromasil C_{18} column (5 μm, 4.6 mm × 250 mm i.d.) (Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China). The mobile phase consisted of methanol–acetic acid/ammonium acetate buffer solution containing 0.1% acetic acid and 5 mM ammonium acetate (80:20, v/v) at a flow rate of 1.0 mL min^{-1}, and the wavelengths were set at 352 nm and 279 nm to detect piroxicam and diclofenac, respectively. The mobile phase was degassed with helium (He). The temperature of the column during analysis was maintained at 25 °C.

2.3. Oxidation of MWCNTs

Functionalization, by which carboxylic acid groups and hydroxyl groups could be added onto the surface of CNTs, is an effective process to help CNTs disperse in the sol–gel solution [31]. In the present study, 0.2 g of crude MWCNTs was immersed in 40 mL mixture of concentrated H_2SO_4/HNO_3 (3:1, v/v) and ultrasonicated in a water bath for 2 h at room temperature, then refluxed at 70 °C for 4 h. The mixture was cooled and washed with deionized water until the pH reached 7.0. Afterwards, the functionalized MWCNTs were dried at 70 °C.

2.4. Preparation of MWCNTs/silica composite-reinforced hollow fiber

The sol solution of MWCNTs/silica composite was prepared by acid-based catalyzed method [32]. First, 1 mL of tetraethylorthosilicate (TEOS) was added into the mixture of 1 mL of ethanol and 320 μL of water. Next, 30 μL of concentrated hydrochloric acid was added drop by drop and the solution was stirred to promote the hydrolysis and condensation reactions. After 20 min, 300 μL of polyethylene glycol 400 was added and stirring was performed for an additional 120 min. Finally, 40 mg of oxidized MWCNTs was added to the resulting mixture via stirring for 30 min. So the sol solution of MWCNTs/silica composite was formed.

The polypropylene hollow fiber was cut manually into small segments of 1 cm. Before use, the segments were ultrasonically cleaned in acetone for 10 min in order to remove any impurities and dried in air. The treated hollow fibers were entirely immersed into the above sol and ultrasonicated at room temperature for 120 min to make CNTs immobilized in the wall pores successfully.

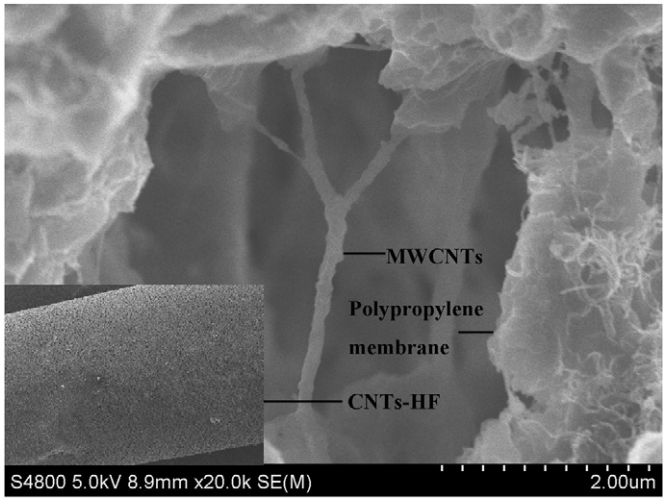

Fig. 2. Scanning electron microscope of carbon nanotubes-reinforced hollow fiber.

Afterwards, CNTs-reinforced hollow fibers (CNTs-HF) were washed with ultrapure water for several times to remove MWCNTs on the surface and the inner lumen, until no CNTs was observed in the cleaning solution. Finally the CNTs-HFs were dried at 80 °C for 1.5 h. Fig. 2 shows the scanning electron microscopy image of oxidized MWCNTs held in the wall pores of the hollow fiber.

2.5. Preparation of standard solutions

The stock solutions of piroxicam (0.218 mg mL^{-1}) and diclofenac (0.304 mg mL^{-1}) were separately prepared in methanol and stored at 4 °C before use. And working solutions at various concentrations were freshly prepared by appropriate dilution of the stock solution with deionized water.

2.6. Preparation of real samples

Four sets of water samples were separately obtained from the influent (raw water, WWR) and effluent (treated water after anaerobic digestion, WWT) of Yanerwan Wastewater Treatment Plant (WWTP) located in Lanzhou, Gansu, China, hospital drain water from the People's Hospital of Gansu Province and tap water from the laboratory. All samples were filtered through membrane filter of 0.45 μm and stored at 4 °C prior to extraction.

2.7. Extraction procedure

2.7.1. CNTs-HF-SLPME procedure

A 10 mL aliquot of sample solution was adjusted to a pH value of 3.0 by hydrochloric acid and placed in a 12-mL sample vial. The sample vial was clamped to fix its position above the magnetic stirrer. 1 cm of CNTs-HF supported by a 50-μL microsyringe was impregnated in 1-octanol for 1.5 min and the microsyringe was withdrawn to make the lumen full of 1-octanol. After that, one end of the CNTs-HF was flame-sealed and the surface was washed with ultrapure water to remove superfluous membrane liquid. The prepared extraction device was introduced into the sample solution at 950 rpm of agitation rate. After extracting for 60 min, the CNTs–HF was put into an end-sealed pipette tip with 15 μL acetonitrile for desorption via ultrasonic-assisted effect for 25 min. Then 10 μL of the desorbed solution was injected for HPLC analysis. Considering the relatively low cost, a fresh CNTs-HF was used in each experiment to eliminate the possible carry-over effect.

2.7.2. CNTs-HF-SPME procedure

To compare with the performance of CNTs-HF-SLPME, CNTs-HF reinforced solid phase microextraction (CNTs-HF-SPME) was used to enrich the analytes. In this mode, only CNTs, serving as sorbents, performed the extraction. For this reason, micro-syringe was inserted into the lumen of hollow fiber during the whole extraction process without the same withdrawal step as in CNTs-HF-SLPME procedure, so no organic solvent was filled in the lumen. After extraction, the CNTs-HF was cleaned with ultrapure water, dried with filter paper to eliminate 1-octanol in the pores, and then desorbed in acetonitrile for 25 min. The other extraction procedures were achieved according to the CNTs-HF-SLPME process.

2.7.3. HF-LPME procedure

The extraction procedure was carried out as described in Section 2.7.1. In this microextraction mode, conventional hollow fiber was used instead of MWCNTs immobilized one. The extraction process was performed for 40 min at 850 rpm stirring rate. To guarantee the consistency of the three microextraction modes and eliminate the possible errors, ultrasonic-assisted desorption was also used to obtain the extracted compounds in this mode. So after extraction, the hollow fiber was removed and plunged into 15 μL of acetonitrile in an end-sealed pipette tip, and the analytes were desorbed from the fiber with ultrasonication for 15 min.

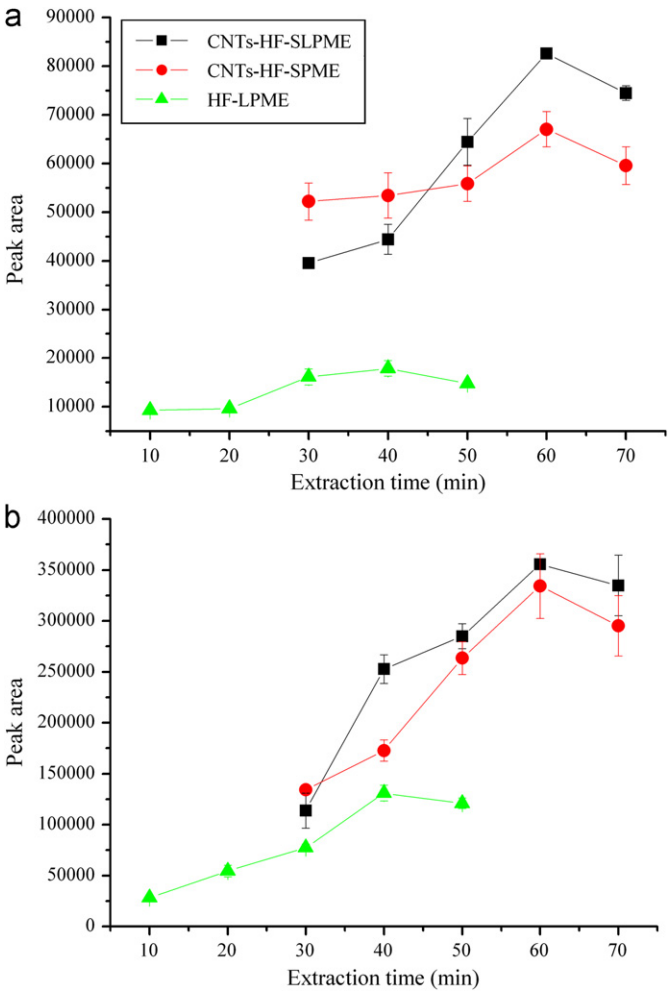

Fig. 3. Effect of extraction time on the peak areas of piroxicam (a) and diclofenac (b) extracted with the three modes. Extraction conditions: organic solvent, 1-octanol; sample pH, 3.0; stirring rate, 950 rpm for SLPME and SPME, 850 rpm for LPME; desorption solvent, acetonitrile; desorption time, 25 min for SLPME and SPME, 10 min for LPME.

3. Results and discussion

3.1. Optimization of extraction procedure

In order to obtain high extraction efficiency and enrichment factor, the main parameters were optimized not only for newly developed CNTs-HF-SLPME, but also for CNTs-HF-SPME and conventional HF-LPME. In optimization, 10 mL of sample solution containing 100 μg L^{-1} of piroxicam and diclofenac was used and five parallel experiments were performed for each experimental condition.

3.1.1. Extraction solvent

CNTs-HF-SLPME operated in direct immersion sampling mode is a two-phase LPME mode consisting of a sample solution, organic solvent/nano sorbent held in the wall pores, and organic solvent placed inside the lumen. The type of organic solvent immobilized in the pores and lumen of the hollow fiber is a critical factor in this mode. On the one hand, it serves as an extracting solvent, so it should be able to provide high solubility for the target analytes, and should be compatible with the fiber, immiscible in water, and stable enough over the extraction time [33]. On the other hand, it also serves as a wetting agent. CNTs and polypropylene membrane are hydrophobic in nature, and low wettability was observed when CNTs-HF was directly exposed to a sample solution [34]. So the wettability of the CNTs-HF needed to be enhanced. In previous reports, 1-octanol served as a perfect organic solvent which can not only wet the surface of CNTs-HF well but also extract two analytes effectively [22,30]. So in the present case, 1-octanol was selected for subsequent experiments.

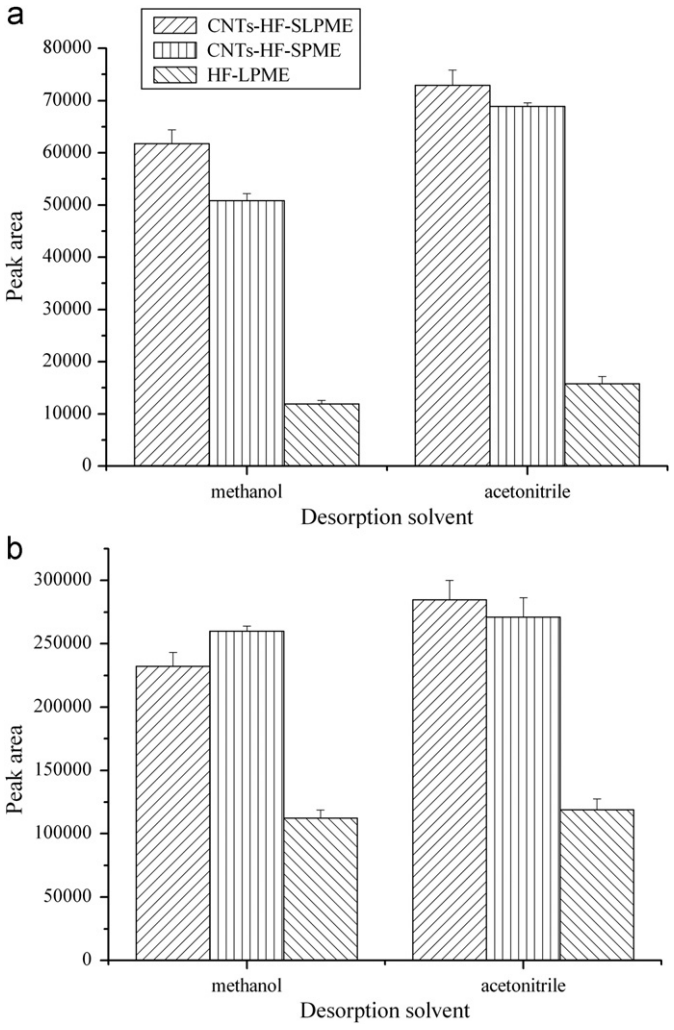

Fig. 4. Effect of desorption solvent on the peak areas of piroxicam (a) and diclofenac (b) extracted with the three modes. Extraction conditions: extraction time, 60 min for SLPME and SPME, 40 min for LPME, and the others were the same as Fig. 3.

3.1.2. Selection of extraction time

In the three microextraction modes, extraction efficiency depends on the mass transfer of analytes from the sample solution to the extractants [34]. Since mass transfer is a time-dependent process, it is important to establish extraction-time profiles of target compounds. A series of exposure times was tested and the corresponding results were provided in Fig. 3. The extraction efficiencies of the three modes all increased with an increase in extraction time, and this increase was followed by an apparent decrease. But the respective time points at which the highest peaks of the analytes were obtained were different. 1-octanol was served as conditioning solvent in the three microextraction modes, and also served as extraction solvent in LPME and SLPME. Prolongation of the extraction time would cause the loss of 1-octanol. On one hand, this will hinder the contact between the analytes and MWCNTs. On the other hand, this will decrease the extraction efficiency of 1-octanol itself. MWCNTs were a porous layer, in which mass transfer was a process of diffusion through the pores. Therefore, the porosity of MWCNTs will have a strong influence on the extraction dynamics. The nanometer-sized pores on the surface of the MWCNTs might lead to longer equilibrium time for the extraction of the analytes compared with conventional HF-LPME mode [31]. It was also proved by this experiment. In both

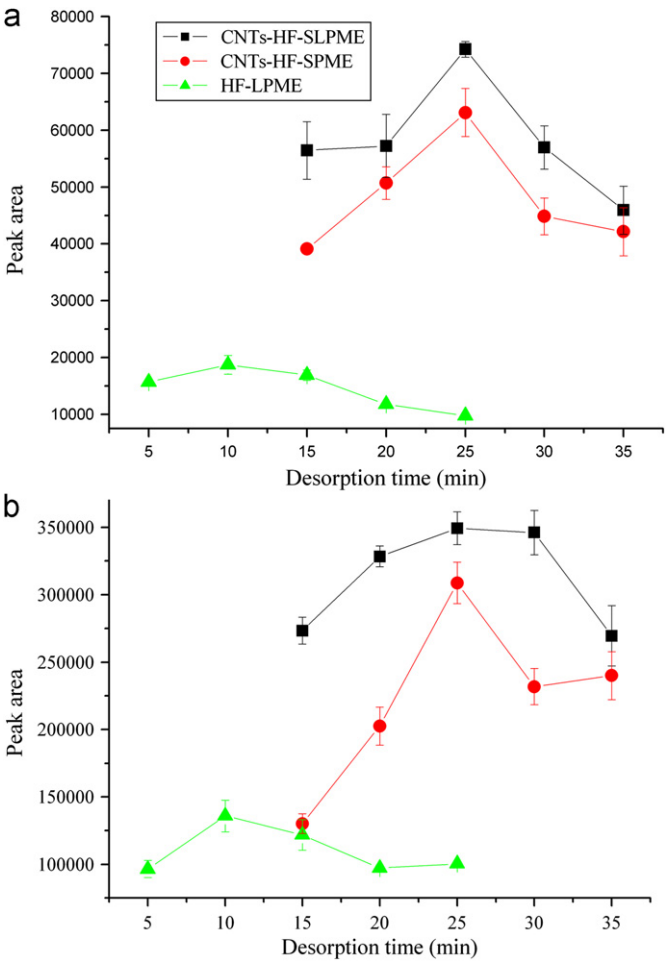

Fig. 5. Effect of desorption time on the peak areas of piroxicam (a) and diclofenac (b) extracted with the three modes. Extraction conditions were the same as Fig. 4.

CNTs-HF-SLPME and CNTs-HF-SPME modes, the highest peaks of the analytes were obtained at the extraction time of 60 min; however, for HF-LPME, this time point was 40 min.

3.1.3. Desorption condition

In the three microextraction procedures, the compounds extracted were desorbed ultrasonically under the appropriate amount of time and analyzed. So both desorption time and desorption solvent have great influence on the peak areas of the extracted analytes and need to be optimized. Due to their absence of interferential peaks, acetonitrile and methanol are the common organic solvents used in HPLC. Moreover, they cannot dissolve polypropylene membrane and MWCNTs [34]. In this research, acetonitrile and methanol were evaluated as desorption solvents. As shown in Fig. 4, acetonitrile gave higher peak area response than methanol. So in the following experiment, acetonitrile was chosen as the desorption solvent. A series of desorption time was also investigated. Fig. 5 indicates that the highest peak area was obtained at 25 min both in CNTs-HF-SLPME and CNTs-HF-SPME. Desorption was incomplete when shorter times were used as expected, while a decrease in peak area was observed above 25 min. This might be accounted for the fact that the desorbed analytes would be absorbed by CNTs again. In addition, the desorbed analytes would diffuse to the pores or lumen of hollow fiber with the help of concentration difference and ultrasonication. Owing to the strong adsorption ability of CNTs, longer desorption time (25 min) was needed in extraction modes relative to CNTs, compared with that in HF-LPME mode (10 min).

3.1.4. Effect of pH value of the sample solution

A suitable pH value of the sample solution can improve the extraction efficiency and reduce matrix interferences [31]. The main interactions between the MWCNTs and analytes were hydrophobic and π–π interactions, so a majority of analytes should remain in molecular form to enhance extraction efficiency by adjusting the pH value. Since piroxicam and diclofenac are acid compounds with *pKa* of 6.3 and 4.0, respectively, the sample solution should be acidized to deionize the analytes. However, when a lower pH was employed, relatively poorer extraction efficiency was also observed. It is possible that ionized species were formed as the acidic analytes accepted extra protons at low pH, thus reducing the distribution ratios. Therefore, the influence of sample pH in the range of 1.0–5.0 was investigated. Fig. 6 shows that the peak areas of piroxicam and diclofenac increased with pH increasing from 1.0 to 3.0, and reached the maximum at pH 3.0, and declined gradually with a further increase in pH. Thus, the optimum pH value of the sample solution was selected as 3.0.

3.1.5. Effect of donor phase volume

Generally, extraction factor (EF) can be improved by increasing the volume ratio of donor phase to acceptor phase. However, a larger sample volume can be disadvantageous to extraction

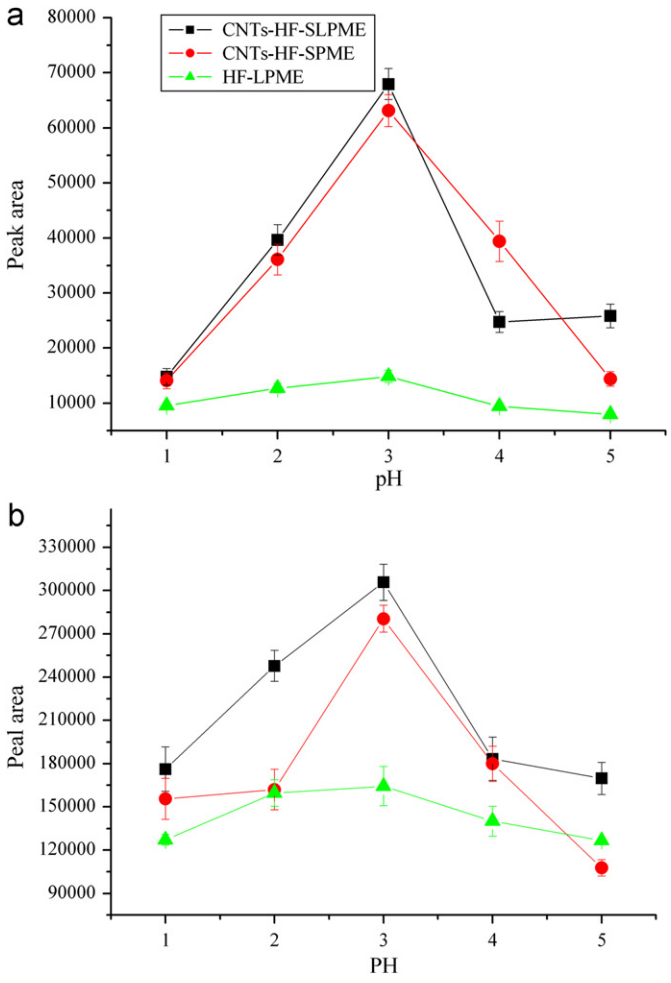

Fig. 6. Effect of sample pH on the peak areas of piroxicam (a) and diclofenac (b) extracted with the three modes. Extraction conditions were the same as Fig. 4.

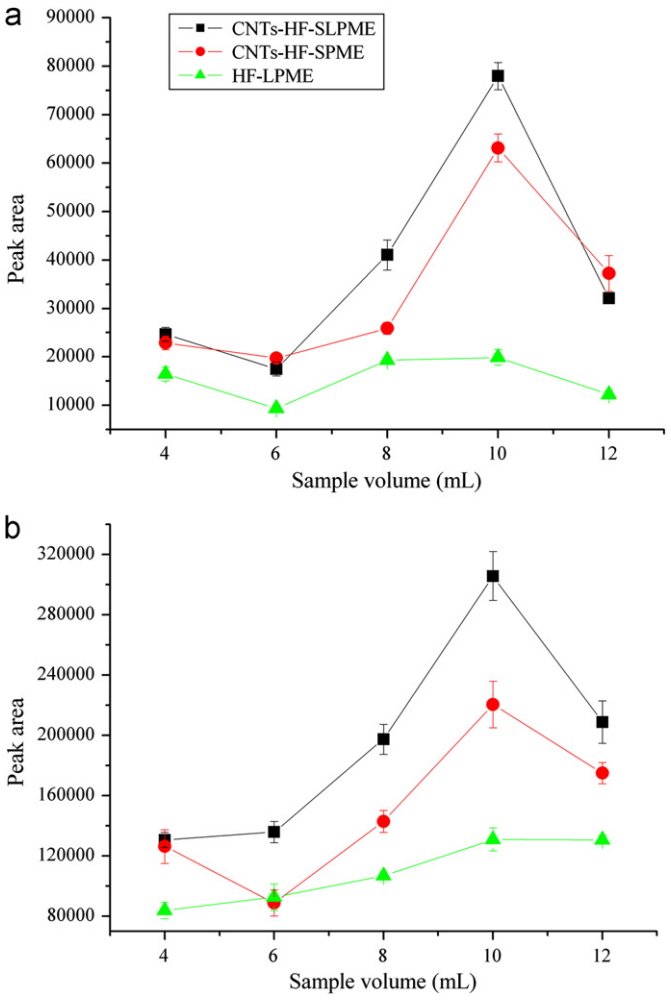

Fig. 7. Effect of sample volume on the peak areas of piroxicam (a) and diclofenac (b) extracted with the three modes. Extraction conditions were the same as Fig. 4.

efficiency owing to poorer mass-transfer kinetics [31]. So in this experiment, the volume of donor phase containing a constant concentration of the analytes from 4 to 12 mL at the interval of 2 mL was investigated. The results shown in Fig. 7 indicated that the peak areas of the two target analytes increased with sample volume increasing from 4 to 10 mL, but decreased with a further increase. This phenomenon might be due to the saturation of the MWCNTs capacity for a large sample volume [35].

3.1.6. *Effect of stirring rate*

An appropriately high agitation can increase extraction rate by increasing the mass-transfer rate of analyte to the membrane and reducing the thickness of boundary layer at the outer membrane surface [31]. So stirring rate is an important parameter that requires to be optimized. As shown in Fig. 8, agitation of the sample greatly enhanced extraction. Furthermore, the introduction of CNTs made the mechanical stress of fiber to increase, so the two extraction modes relative to CNTs could endure higher stirring rate while keeping the best extraction efficiency. Fig. 8 demonstrates that the optimum stirring rate in CNTs-HF-SLPME and CNTs-HF-SPME was 950 rpm, whereas 850 rpm in HF-LPME.

3.1.7. *The effect of salt*

The presence of salt in sample solution often increases the ionic strength of aqueous solution, which will affect the solubility of extracted compounds further. This effect leads to varying the partition coefficient of analytes between sorbent and solution; hence the extraction efficiency may be changed [24]. To evaluate the effect of salt in this work, NaCl with the concentration ranging from 0% to 20% (w/v) was added into the sample solution. The results in Fig. 9 revealed that in the three microextraction modes, addition of salt restricted extraction of target analytes especially to diclofenac. This phenomenon may be caused by salting-in effect in which the salt dissolved in the aqueous solution may change the physical properties of the Nernst diffusion film and reduce the rate of diffusion of analytes into the organic solvent [36]. So extraction was conducted without addition of salt in this work to guarantee extraction efficiency.

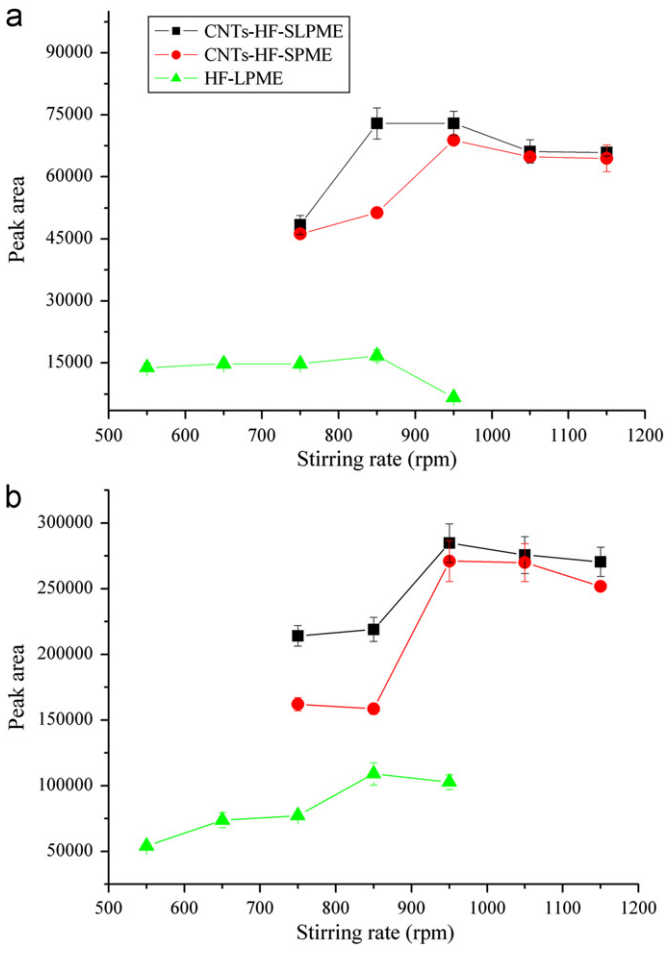

Fig. 8. Effect of stirring rate on the peak areas of piroxicam (a) and diclofenac (b) extracted with the three modes. Extraction conditions were the same as Fig. 4.

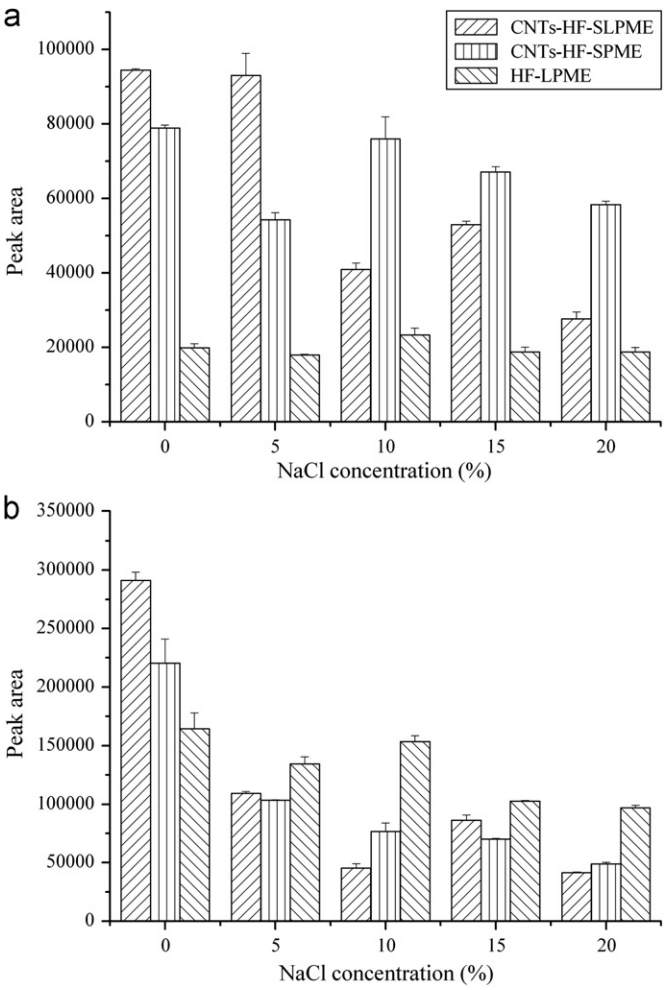

Fig. 9. Effect of salt concentration on the peak areas of piroxicam (a) and diclofenac (b) extracted with the three modes. Extraction conditions were the same as Fig. 4.

Table 1
Performance parameters of the three microextraction procedures.

Extraction mode	Analyte	EF[a]	LOD[b] (μg L^{-1})	LOQ[c] (μg L^{-1})
CNTs-HF-SLPME	piroxicam	47.49	4.48	11.99
	diclofenac	184.65	0.40	3.61
CNTs-HF-SPME	piroxicam	36.88	8.72	16.35
	diclofenac	129.72	1.63	6.02
HF-LPME	piroxicam	23.73	34.88	65.40
	diclofenac	63.76	2.01	8.03

[a] EF was the concentration ratio of analyte presented in the desorption solvent to that originally presented in the sample.
[b] LOD was defined as the concentration for which the signal-to-noise ratio was 3.
[c] LOQ was defined as the concentration for which the signal-to-noise ratio was 10.

Table 2
Recovery of the method (n=4).

Wastewater	Spiked amount ($\mu g\ L^{-1}$)	diclofenac		piroxicam	
		Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)
WWR	0	Detected but unquantified	Undetected	Undetected	
	80	99.94	7.12	72.10	3.36
	100	117.26	4.52	84.98	3.18
	120	91.42	5.17	104.64	4.10
WWT	0	Detected but unquantified		Undetected	
	80	114.07	4.16	110.98	6.24
	100	77.78	7.11	89.44	6.17
	120	73.00	5.23	93.11	4.72
Tap water	0	Undetected		Undetected	
	80	71.10	6.12	89.12	4.32
	100	89.14	7.12	70.02	8.10
	120	113.48	6.36	79.43	3.35
Hospital drain water	0	Undetected		Undetected	
	80	78.64	3.79	83.56	7.19
	100	83.41	6.30	70.22	7.19
	120	77.48	8.17	86.78	5.72

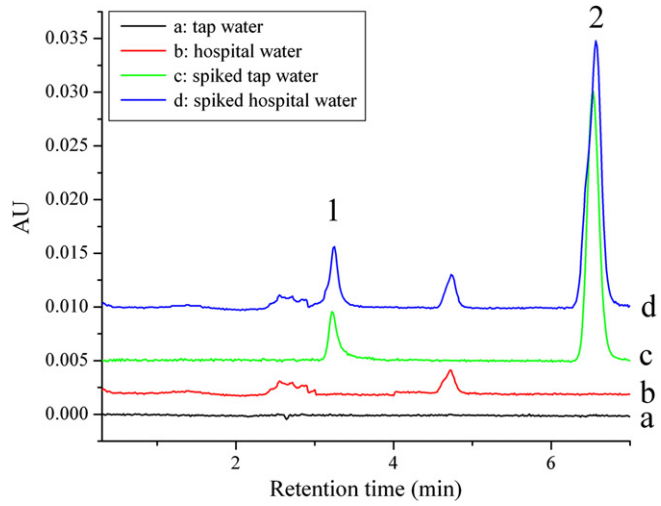

Fig. 10. Chromatograms of (a) tap water, (b) hospital drain water, (c) tap water spiked with 220 $\mu g\ L^{-1}$ of analytes and (d) hospital water spiked with 220 $\mu g\ L^{-1}$ of analytes extracted by CNTs-HF-SLPME. Peak 1: piroxicam; Peak 2: diclofenac; Mobile phase: methanol–acetic acid/ammonium acetate buffer solution (80: 20, v/v); flow rate: 1.0 mL min^{-1}; detection wavelength: 299 nm.

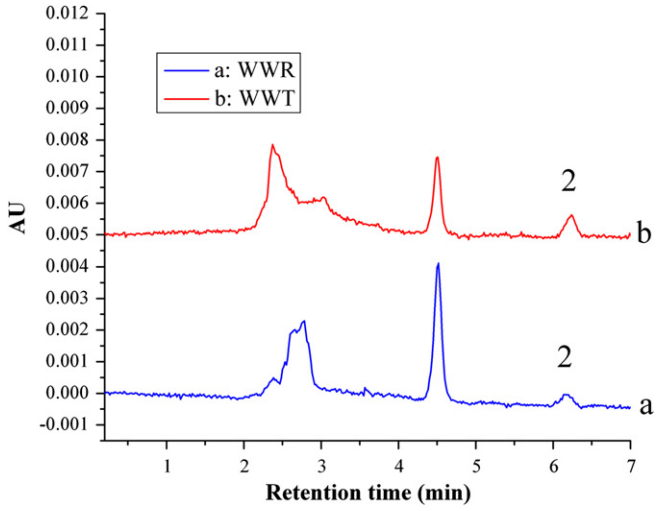

Fig. 11. Chromatograms of (a) WWT (b) WWR extracted by CNTs-HF-SLPME. Peak 2: diclofenac; Mobile phase: methanol–acetic acid/ ammonium acetate buffer solution (80: 20, v/v); flow rate: 1.0 mL min^{-1}; detection wavelength: 299 nm.

3.1.8. *Effect of MWCNTs concentration in sol solution*

Usually, an increase of the CNTs doping level will lead to an improvement in extraction efficiency [24]. The more the CNTs dispersing in the sol solution, the higher the extraction efficiency gained. In the sol–gel process, we discovered that when the amount of CNTs exceeded 40 mg in the above of sol solution, the CNTs would not disperse well. So the CNTs doping level of 40 mg was chosen as the most suitable value.

3.2. *Method evaluation*

To effectively evaluate the extraction performance of this novel microextraction mode, enrichment factors (EFs), limits of detection (LODs) and quantification (LOQs) were studied in comparison with CNTs-HF-SPME and HF-LPME under their individual optimized conditions. As shown in Table 1, nanotube mediated extraction, i.e. CNTs-HF-SLPME and CNTs-HF-SPME, yielded much higher EFs than conventional polypropylene fiber supported extraction. This phenomenon might be attributed to the presence of CNTs which increased the effective surface area and the overall partition coefficient [23]. The π–π interaction between the benzene rings of the two analytes and the graphitic ring structure of CNT sorbents contributes greatly to the strong sorption properties. In addition, the polar functional groups such as –COOH and –OH on the surface of oxidized MWCNTs may form hydrogen bonds with –NH and –OH on the structure of the two analytes, which would strengthen the interaction between the sorbent and the analytes further. A little higher EF was obtained in CNTs-HF-SLPME than in CNTS-HF-SPME which demonstrated that 1-octanol placed in the pores and lumen of hollow fiber also displayed extractability. As expected, CNTs-HF-SLPME had the lowest LOD, 4.58 $\mu g\ L^{-1}$ for piroxicam and 0.40 $\mu g\ L^{-1}$ for diclofenac, and the lowest LOQ, 11.99 $\mu g\ L^{-1}$ for piroxicam and 3.61 $\mu g\ L^{-1}$ for diclofenac, compared with the other two modes.

The precision of the instrument was evaluated by performing intra-day and inter-day assays by replicate injection of a standard solution. Intra-assay precision was measured for five continuous injections during the same day, and relative standard deviation (RSD) values of peak area obtained was 4.27% for piroxicam and 2.79% for diclofenac, while inter-assay precision was measured on three consecutive days with RSD values 2.01% for piroxicam and 4.83% for diclofenac.

Table 3
Comparison of some methods used for determination of piroxicam and diclofenac.

No.	Matrix	Target compounds	Extraction method	Detection	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	Recovery (%)	Ref.
1	WWR,WW1,WWT	diclofenac	HF-LPME	CE–DAD	0.43	–	88.9 – 93.2	[37]
2	WWR,WW1,WW2,WWT	diclofenac	HF-LPME	HPLC–MS	0.1	–	70.8 – 72.9	[38]
3	Urine	diclofenac	HF-LPME	HPLC–DAD	52.9	–	52.9	[39]
4	WWR,WWT	diclofenac	HF-LPME	LC–MS–MS	0.025	–	111	[40]
		piroxicam	HF-LPME	LC–MS–MS	0.033	–	106	[40]
5	Wastewater, bovine milk, urine, plasma	diclofenac	EME	HPLC–UV	2.7 – 5.0	–	44-95	[41]
6	Liquid formulations	diclofenac	SBSE	HPLC–UV	16.06	48.68	70	[42]
7	Wastewater, river, sea	diclofenac	SBSE–PDMS SBSE–PU	HPLC–DAD	1.6	5.4	34.6	[43]
					0.7	2.4	77.7	
8	Urine	piroxicam	SDME	CE	17.64	19.04	94.8	[44]
9	Serum	piroxicam	Semi–micro column	HPLC–UV	4.2	–	–	[45]
	Plasma				4.7	–	–	
10	WWR,WWT, hospital drain,	diclofenac	CNTs-HF-SLPME	HPLC–DAD	0.40	3.61	71.1 – 114.1	This one
	tap water	piroxicam			4.48	11.99	70.0 – 111.0	

WWR: samples of the influent of wastewater plant, raw water; WW1: samples after the primary sedimentation tank of wastewater plant; WW2: samples after aeration tank of wastewater plant; WWT: samples of the effluent of wastewater plant, treated water after anaerobic digestion; HF-LPME: Hollow fiber liquid phase microextraction; EME: Electro-membrane extraction; SBSE: Stir bar sorptive extraction; PDMS: Polydimethylsiloxane; PU: Polyurethane; SDME: Single-drop microextraction.

The calibration standard working solutions at six concentration levels in the range of 20–960 $\mu g\ L^{-1}$ for piroxicam and 10–2560 $\mu g\ L^{-1}$ for diclofenac were extracted with CNTs-HF-SLPME procedure established above, then analyzed by HPLC. Each set of concentrations was repeated five times. The linear regression equations for piroxicam and diclofenac were $y=271.84\ x+3793.6$ and $y=1748.9.0\ x+29763.0$, with correlation coefficients of 0.9985 and 0.9989, respectively.

Repeatability was studied by extracting tap water spiked with 100 $\mu g\ L^{-1}$ of piroxicam and diclofenac with the same batch produced fiber under the optimum conditions. The RSD values ($n=5$) were 5.49% for piroxicam and 6.74% for diclofenac.

The batch-to-batch reproducibility of CNTs-HF preparation was evaluated by extracting tap water spiked with 100 $\mu g\ L^{-1}$ of piroxicam and diclofenac with four different batch fibers prepared in the same procedure. The RSD values ($n=4$) were 2.37% and 7.35% for piroxicam and diclofenac, respectively.

3.3. Analysis of real water samples using CNTs-HF-SLPME

3.3.1. Matrix effect

Matrix effect on recoveries of the target compounds was investigated by extracting four different water samples under optimum extraction conditions with three spiked concentration levels. 100 $\mu g\ L^{-1}$ of piroxicam and diclofenac each was added to the real sample as original amount, then three different quantities, i.e. 80% (low), 100% (medium), and 120% (high) of above concentration of the analytes, were added to the original sample. Afterwards, the three sets of spiked samples were extracted and analyzed in four parallel experiments. As can be seen in Table 2, the recoveries from all the real samples varied in the range of 70.02–110.98% with RSDs of 3.18–8.10%, demonstrating that this novel mode can be applied in relatively complicated matrices. So this novel solid/liquid microextraction combines strong adsorption capacity of CNTs with clean-up function of hollow fiber leading to high enrichment factors and applicability in relatively complicated matrices.

3.3.2. Determination of analytes in real samples

The proposed CNTs-HF-SLPME procedure was applied to the four real water samples. As shown in Table 2, piroxicam was not found in all the analyzed samples, and diclofenac was detected but could not be quantified in WWR and WWT sample. This demonstrated the wide application of diclofenac. Fig. 10 shows the typical chromatograms of the tap and hospital drain water samples and samples spiked with 220 $\mu g\ L^{-1}$ of piroxicam and diclofenac. Representative chromatograms obtained from the wastewater samples (WWR and WWT) were shown in Fig. 11. The well-defined peak of the analytes demonstrated that CNTs-HF-SLPME is an adequate extraction and clean-up procedure for the analysis of real water samples.

3.3.3. Method comparison

A review of selected methods used in the determination of piroxicam and diclofenac in several matrices is shown in Table 3. Compared to other microextraction modes, the developed method has the merits of improved simplicity, sensitivity and relatively lower LOD.

4. Conclusion

A novel procedure based on carbon nanotubes reinforced hollow fiber solid/liquid phase microextraction (CNTs-HF-SLPME) combined with HPLC has been developed to determine trace piroxicam and diclofenac in different water samples. In this mode, functionalized MWCNTs were held in the pores of hollow fiber with sol–gel technology acting as solid-phase sorbent, and 1-octanol was placed inside the pores and lumen of hollow fiber acting as liquid-phase extractant. The overall effect of the two extractants is to increase the extraction efficiency. In conclusion, the mix matrix membrane based on CNTs immobilized hollow fiber has several valuable advantages such as reproducibility, absence of sample carryover (due to the disposable nature of the membranes), high enrichment performance, low cost and conversion into green analytical techniques.

Acknowledgments

The work was supported by the National Nature Science Foundation of China (Nos. 21105106 and 21075127) and Natural Science Foundation of Gansu Province (No. 1107RJZA146)

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