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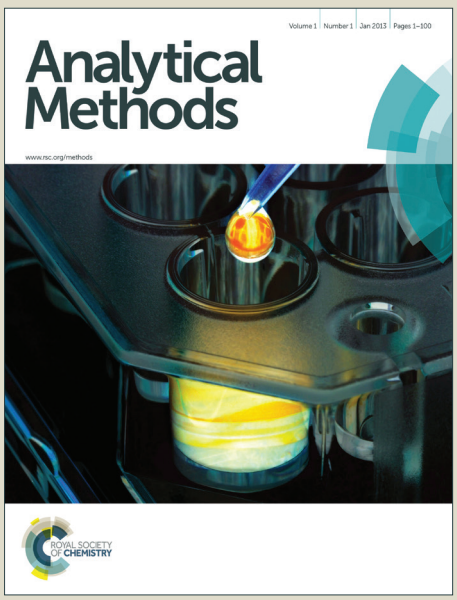

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Hollow fiber-protected liquid-phase microextraction followed by high performance liquid chromatography for simultaneously screening multiple trace level β-blockers in environmental water samples

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Abstract

A sensitive and reliable method using two-phase hollow fiber liquid-phase microextraction (HF-LPME) pretreatment technique was developed for simultaneously screening multiple β-blockers in the environmental samples. In the study, heptanol was chosen as the optimal extraction solvent, six β-blockers within a large range of polarity were extracted across the porous wall of hollow fiber and into the acceptor phase (AP). The fundamental parameters affecting the extraction efficiency (*EE*) of analytes including extraction time, temperature, pH of donor phase (DP) and AP, stirring speed, volume of the sample solution, ionic strength and the type of hollow fiber membranes were studied and optimized. Under the optimal conditions, extracts were analyzed by HPLC with ultraviolet detection (UV). Finally, satisfactory results were obtained, good linearity was observed for all β-blockers in the range of 0.16-200 ng mL^{-1}, limits of detections (LODs) were between 0.08 and 0.5 ng mL^{-1}, the intra and inter-day precision values of six β-blockers were 1.0–2.2% and 1.4–2.7%, respectively. The recovery with RSDs was less than 2.2% for the pretreatment method. The proposed technique was successfully applied for screening multiple β-blockers in complex aqueous samples with the best specificity and provided a simple and reliable new means for the self-checking of laboratory at basic level.

Keywords: β-blockers; Basic level; Hollow fiber liquid-phase microextraction (HF-LPME); Self-checking

1. Introduction

With increasing utilization of pharmaceuticals and continuous expansion of the pharmaceutical industry scale, more and more organic pollutants [1] were discharged into the environment from waste water effluents. Although long-term ecotoxicological effects remain unclear, most of them are biologically active compounds which inevitably contribute directly or indirectly harm to environment and humanity [2-4]. Therefore, the development and optimization of an analytical method for monitoring the concentrations of emerging pollutants in environment would be of greatly utility.

B-blockers are one among the most widely prescribed medicines worldwide. They are popularly used in the treatment of hypertension, angina, pectoris and arrhythmia [5-6] and are detected in aqueous environment frequently especially in developed countries [7-8]. It has been reported that the exposure to 531 μg/L of propranolol caused 85% of inhibition of photosynthesis after 24 h, and metoprolol was especially toxic for bacteria, the concentration of the 503 μg/L metoprolol caused bacterial mortality more than 50% [9]. Some investigations also indicated several β-blockers can cause acute and chronic hazard to aquatic organisms and human at levels close to the maximum value in wastewater [10].

Nevertheless, the common applied treatment processes of sewage treatment plants (STPs) can not fully eliminate the compounds and the pharmaceuticals were often released to environment. Hence, there is an urgent need to develop a highly specific detection method for quantitatively and simultaneously screening and monitoring the β-blockers discharged from STPs as well as industrials, hospitals and households for self-checking of labs at the grass-roots level.

Due to low concentrations of pharmaceuticals and high interferences in complicated sample matrices, in order to improve the selectivity and sensitivity of analytical method, sample pre concentration and clean up process are very necessary prior to assay for β-blockers. Generally, various extraction methods have been reported in the scientific literature for the pre-concentration of β-blockers in aqueous environment, including solid-phase microextraction (SPME) [11] liquid-liquid extraction (LLE) and (solid-phase extraction) SPE. However, these reported methods were mostly focus on the improvement of purification ability which always presented some drawbacks: traditional methods are usually time consuming, require large volumes of extraction solvent and involve complicated procedures. SPME is fiber fragile with a limited lifetime and sample carry-over [12] which always costs a lot for the analysis of low concentration analytes in complex matrix.

SPE [13-16] is the most versatile technique for removing interfering species in aqueous samples, but because it is able to extract substances within a wide spectrum [17]. Apart from the targets other components presented in the sample also might be extracted, leading to matrix effects, resulting in interference or suppressing of the analytes signals. Thus, the selectivity of a pretreatment method is an important parameter needed to be considered for analyzing pharmaceuticals at trace levels in samples with complex matrix. In view of these reasons, application of a highly specific and selective extractant, instead of the traditional pretreatment methods for these substances, would allow the β-blockers completely extraction.

Our study is driven by an increasing demand of developing more specific and environmentally benign procedures for the extraction of β-blockers. For complex samples, hollow fiber-protected LPME has been demonstrated to be effective [13]. Firstly, the method

needs a minimal of organic solvents for targets. Second, the hollow fiber as a filter could prevent other large molecules from the pore permeate into the lumen of hollow fiber. Third, the inexpensive device of HF-LPME is simple to operate and precludes carryover effects. Other advantage of the great utility of the method is heptanol as the extraction solvent which has a good specificity and inherent selectivity for β-blockers. Meanwhile, target compounds can be detected directly without several parallel pretreatment steps compared to previous methods. Therefore, the device has the best capability to concentrate β-blockers and attain the maximum extraction efficiency. Owing to these distinguishing characteristics, the method is considered to be the most robust and optimal especially suitable for self-inspection of β-blockers in basic level laboratory.

To date, several analytical methods such as liquid chromatography–tandem mass spectrometry (LC–MS) [1], LC/MS/MS [18], gas chromatography mass spectrometry (GC–MS) [19], and GC/MS/MS [20] .etc have become a favorite choice for the determination of trace amounts of β-blockers in sewages. Although the analytical methods have a high degree of sensitivity, while, owing to the complex samples and various kinds of sample types are pervasive in our daily life, such expensive instruments are actually not available for labs at basic level.

HPLC analytical method is simple and convenient to operate and lower requirement of experiment equipment. It has been demonstrated to be one of the best methods and is very suitable for the determination of β-blockers in the water samples at operation sites for local laboratories.

The paper developed a specific HF-LPME pretreatment method followed by HPLC-UV

for simultaneously screening and monitoring a wide range of polarity of β-blockers in the complex environmental samples. It carrys a significant improvement for monitoring β-blockers in waste waters compared to previously published methods and provides a simple and reliable new mean for self-checking of wastewater at the grass-roots level.

2. Experimental

2.1 Chemicals and materials

Metoprolol, oxprenolol, bevantolol references were all purchased from Sigma Chemical Co (St. Louis, MO, USA). Timolol, bisoprolol, propranolol were tablets purchased from Sangel Pharmaceutical Co (Beijing, China). Methanol of HPLC grade was purchased from DIKMA (Lake Forest, CA). All the solvents throughout the experiment were analytical-reagent grade and purchased from Tianjin Chemical Reagents Ltd. (Tianjin China). HPLC grade water was deionized water through a Milli-Q50 water purification system that was used for all the solutions. To prevent chromatographic column blockage, all buffers were filterd through 0.45μm filter membranes. The hollow fibers (Polyvinylidene fluoride with wall thickness was 200 μm，inner diameter was 800μm and pore size of 0.2 μm) were obtained from Taoxin Environment Science and Technology limited Company (Foshan, China). The polysulfone hollow fibers were purchased from Kaijie Membrane Separation Technology (Hangzhou, China) with an inner diameter of 1000 μm, wall thickness of 150 μm and wall pore size of 0.2 μm. The extraction water-bath was purchased from Qiqian Electronic Technology Co. Ltd (Shanghai, China).

2.2 Solution preparation

Stock solutions of metoprolol, oxprenolol, bevantolol with theoretical concentration of 1 mg

ml^{-1} were prepared by taking about 5 mg pure standards in 5 mL volumetric flask. The 5 mg mL^{-1} stock solution of timolol was obtained by grinding the tablets of timolol into fine powder and dissolving in methanol. 5 mg mL^{-1} stock solutions of bisoprolol and propranolol were also prepared from the tablets similar to timolol. Then 1 mL stock solution of timolol and propranolol with pipettes was transferred to 5 mL volumetric flask containing bisoprolol respectively. All the references dissolved in methanol to get the theoretical concentration of 1 mg mL^{-1}. The working standard solutions of six β-blockers were obtained by diluting the primary stock solutions with methanol. All the stock solutions and working solutions were stored at 4□.

2.3 Equipment and chromatographic system

The LC chromatographic separations were performed on a model 9001 chromatographic pump (Varian, Walnut Creek, USA) and a 785A UA-detector and a HW-2000 chromatograph workstation (Perkin Elmer USA). Separation process was accomplished on a C_{18} column (250mm×4.6mm, 5μm, Chromasil, China). The column was eluted with methanol and 0.02 mol L^{-1} phosphate buffer solution (containing sodium dihydrogen phosphate and isodium hydrogen phosphate with pH 6.8) at a flow rate of 1 ml min^{-1}. The elution step started with 50% methanol and then with a linear gradient from 50% to 60% over 10 min and to 70% over the next 5 min. Then the amount of methanol was up to 80% for 2 min and finally lowered to 50% in 1 min. Keep equilibration for 4 min before next injection.

2.4 HF-LPME device and pretreatment process

Firstly, hollow fibers were cleaned with pure water and methanol by ultrasonic for 15 min respectively to remove any contaminants that might interfere with the retention time of

β-blockers, and then dried in the air. Prior to use, 8 cm piece of hollow fiber was cut out and dipped in the heptanol solvent for 20 min to make it fill the pores of the hollow fibers. Finally, 25 μl of heptanol was injected to the hollow fiber lumen. 55 milliliter of sample solution containing six beta-blockers was transferred into the sample vial. 1.1g borax was into the vial and the pH of sample solution was adjusted to 11.5 with saturated solution of sodium hydroxide. Finally, the prepared hollow fiber immersed into the sample solution and fixed with syringe needle. The experiments were carried out under the optimal conditions. After the extraction was finished, the acceptor solution was collected and transferred into vial for further HPLC analysis.

2.5 Calculation of pre-concentration factor and extraction recovery of HF-LPME

The extraction efficiency of the method is expressed by pre-concentration factor *(PF*). Pre-concentration factor was defined as the ratio of the final concentration of analytes in the AP (C_f) to the initial concentration of analytes in the DP (C_i), the equation is described below [21]:

$$PF = \frac{C_f}{C_i} \quad (1)$$

Where C_f was calculated from the calibration graph obtained via direct injection of acceptor solutions of analytes in the linear range.

The relative extraction recovery (*Re*) for all analytes was defined as:

$$Re\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (2)$$

C_{found}, C_{real} and C_{added} are respectively the final concentrations of analytes after addition of known amount of standards into the real sample, the initial concentrations of analytes in real sample, and the concentrations of known amount of standards which were spiked into the

real sample.

3. Results and discussions

3.1 Optimization of the extraction conditions

In order to obtain the optimal *EE*, all the relevant parameters affecting the extraction performance were investigated such as extraction solvent, time, temperature, pH of the DP, the stirring speed , volume of sample solution, ionic strength and the type of hollow fiber membrane.

3.1.1 Selection for extraction solvent

The selectivity of suitable extraction solvent is highly critical for successful HF-LPME pretreatment process. The extraction solvent directly affects the *EE* and selectivity of targets. So the extraction solvent of the method should have the following advantages: firstly, according to the similarity principle of compatibility, solvent extraction should have similar structure to drugs and be immiscible with water samples; Secondly, the organic solvent should be stable in the hollow fiber pores and non-volatile to avoid loss of extraction solvent during extraction. Based on above considerations, in the study, octanol, heptanol, toluene, hexane were tested for extraction of β-blockers. As can be seen in Fig.1 (a), toluene, hexane were impregnated in hollow fibers, the *EE*s for all analytes were lower even had no extraction, the phenomenon was well explained that the main interaction between toluene, hexane and β-blockers were van der waals' force which is weaker than strong hydrogen bond force that formed by octanol, heptanol and β-blockers, plus the fact that toluene, hexane were partly loss during extraction process. So in present study, heptanol showed higher *EE* for β-blockers. Table 1 listed the structures of analytes and some of their properties. Consequently, heptanol

was chosen for further experiments.

3.1.2 Effect of pH in DP

The pH of the DP is another major factor affecting the *EE*, the range between 9.0 and 12.0 was investigated to select the optimum pH for the extraction of β-blockers. The extraction results were shown in Fig.1 (b). Results showed 11.5 would be more effective which are quite considering pK_a values of the drugs. As was expected, the significant increase in *EE* as the pH of DP is increased. It is because an increasing of pH value declines the solubility of analytes in water and increase the solubility in organic solvents. The pK_a values of drugs in the study are in the range of 9.01-9.64. Therefore, the *EE* increased with higher pH value. Nevertheless, when the pH value increased continuously, *EE* of β-blockers changes is not obvious. It is probably when pH was up to a certain value, the number of molecules in the form of undissociated remains constant. So, 11.5 were selected as the optimal pH for the subsequent experiments.

3.1.3 Effect of extraction time

Extraction is a continuous mass transfer processes, extraction time is a parameter that measures the maximum *EE* at the equilibrium state [22]. In the study, *EE* was assessed when extraction time was 40, 50, 60, 70, 80 min respectively, Fig.2 (c) showed that the relative peak areas of the drugs increase quickly within 60 min. when the extraction time was 60 min or longer, the recoveries slightly decreased. It might be the loss of the extraction solvent from pores of hollow fibers and finally dissolved in water. Therefore, 60 min was selected for subsequent experiments.

3.1.4 Evaluation of extraction temperature

The temperature has a great effect on the *EE*, and it is directly related to the thermodynamics and kinetics of the extraction process. In general, higher temperature is benefit to the mass transfer efficiency and the *EE* was studied when temperature was 20, 30, 40, 50, 60□. Fig.2 (d) showed that the highest *EE* was obtained when the temperature is 60□. No matter temperature was above or lower 60□, the extraction amount was declined. It is due to higher temperature may produce high vapor pressure of extraction solvent. The vapor was out from the top of hollow fibers connected with needle and dissolved into water samples [23]. While lower temperature made mass transfer process ineffective. Therefore, 60□ was chosen as the suitable temperature.

3.1.5 Investigation of stirring speed

In present study, the influence of the agitation speed from 200 to 1000 rpm on *EE* was investigated. With the increase of agitation speed from 200 to 800 rpm, the analytical signal increased, when the stirring speed above 800 rpm, the peak areas for analytes had no significant increase or decreased a little.

In LPME process, stirring speed is a parameter to accelerate the mass transfer of analytes from the sample solution to organic solvent and reduce equilibrium time [24], especially for large volume sample solution. Moreover, appropriate agitation speed favors the extraction process [25] by decreasing the thickness of boundary layer between sample solution and supported liquid membrane (SLM). However, when agitation speed over 800 rpm, bubbles could be observed in the sample solution which hindered the transfer process of analytes from sample solution to lumen of hollow fibers. On the other hand, the loss of organic solvent immobilized in SLM may potentially occur, and therefore reduced the *EE* and precision of the

method. As described above, an agitation speed of 800 rpm was seemed to be the optimal and the result was shown in Fig. 3 (e).

3.1.6 Effect of the sample volume

In HF-LPME pretreatment method, analytes can be extracted by the principle of passive diffusion, increasing the volume of sample phase would increase the amount of analytes to some extent. Therefore, the volume of sample solution was an important factor affecting the extraction efficiency [27]. In this paper, different sample volumes (10-110 mL) were examined. Fig. 3 (f) was the effect of sample volume on the extraction efficiency. The results indicated that about 55 mL sample solution was sufficient, and no significant influence was found when the sample volume from 55 to 110 mL. The reason is probably attributed to extraction solvent reached saturation and could not continue to extract analytes. As a result, a sample volume of 55 mL was selected for subsequent experiments.

3.1.7 Effect of ionic strength

In general, the ionic strength of sample solution could affect the extraction efficiency and result in enhancement or suppression the extraction of analytes. This is due to salts added to aqueous sample solutions potentially increase ionic strength and decrease the solubility of analytes in aqueous solutions through such a salting-out effect [28]. In order to investigate the effect of ionic strength on the extraction, various amount of sodium chloride ranging from 0-30% (w/v) was added into the sample solution. According to the results in Fig. 3 (g), as the concentration of NaCl increased, the *EE*s of the analytes decreased. This phenomenon can be explained by the theories: addition of NaCl to sample solutions increased the viscosity of the DP which impedes the mass transfer. Thus, in view of the result, no NaCl was selected for

subsequent studies.

3.1.8 Effect of the type of hollow fiber

The type of hollow fiber is directly related to the amount of analytes in AP, the paper investigated two types of hollow fiber including polyvinylidene fluoride and polysulfone hollow fibers. Finally, polyvinylidene fluoride hollow fiber was chosen for further experiments. There are two main reasons, on the one hand, the wall of polysulfone hollow fiber is much thicker than polyvinylidene fluoride hollow fiber which potentially increases of extraction time, and reduces the rate constant. More importantly, the materials of polysulfone were dissolved in toluene which further limited its application.

3.2 Quantification and method validation

To evaluate the practical applicability of the HF-LPME pretreatment technique, the validation procedures were carried out under all the optimized extraction conditions. As provided below, the method had satisfactory results.

3.2.1 Linearity, limit of quantitation, limit of detection

To establish linearity, a series of concentrations of six β-blockers were prepared in triplicate of each point in water samples. The linearity of timolol, oxprenolol, propranolol was evaluated over the concentration range between 0.400-200 ng mL^{-1}, the linearity of bisoprolol and metoprolol was evaluated over the concentration range of 1.00-200 ng mL^{-1}, the linearity of bevantolol was 0.16-200 ng mL^{-1}. Then, the analytical peak areas were plotted against the corresponding concentrations of analytes. Linearity relationship of all analytes was determined by the correlation coefficient (R^2). LOD was lower ng/ml level calculated as the peak of the analyte concentration is 3 times the baseline noise. All the experimental results

were summarized in Table 2. It is apparent that this method provided good linearity with R^2 values between 0.9992 and 0.9997. The LOD and limit of quantification (LOQ) calculated based on the response signal of drugs versus noise ratio (S/N) was equal to 3 and 10 were in the range of 0.08-0.50, 0.16-1.00 ng ml^{-1} respectively. The *PF*s of the analytes were in the range of 81-294 which could effectively analytes from complex samples. Relatively higher purification, enrichment capacity and lower LOD allow the presented method determine and quantify multiple trace amounts of drugs in complicated matrices.

3.2.2 Accuracy and precision

Inter-day precision and accuracy of the method were evaluated by injecting validation samples six times over six different days. The intra-day precision and accuracy were obtained by running validation samples six times on the same day, each validation samples consisted of five replicates of spiked samples at low, medium and high concentration. The accuracy and precision were expressed by percentage relative standard deviations (RSD%). The mean RSD% value of accuracy should be lower than 15% except at LLOQ and the precision determined for all analytes should also not exceed 15% at three concentration level, except for the LLOQ. As listed in Table 3, the intra-day and inter-day precision of the experiment were less than 2.7%, which provided a satisfactory reproducibility.

3.2.3 Recovery

Aliquots of 55 μL of standard solutions of six beta-blockers at low, medium, high three different concentrations were added to 55 mL real environmental samples in which analytes were not detected. The extraction recovery of the analytes in water samples was evaluated by comparing the detector response values of processed spiked samples at the same theoretical

concentrations to the response values of the same concentration of standard solutions (metoprolol, bisoprolol to obtain on-column concentrations of 1.00, 8.00, 200ng mL^{-1}, the column concentrations of timolol, oxprenolol, propranolol were 0.400, 8.00, 200 ng mL^{-1} and the concentrations of bevantolol were 0.160, 8.00, 200). The spiked samples were subjected to the extraction procedure previously described (Section 2.4) and finally analyzed by HPLC system. The extraction recovery of the proposed method for all beta-blockers in water samples was also provided in Table 3. As can be seen, more than 95% all analytes were obtained with the RSD less than 2.1% which revealed an acceptable precision and recovery.

3.2.4 Specificity

The method specificity was evaluated by injecting sample solutions to quantify the analytes in the presence of other endogenous components in the samples. It was investigated by determination five lots of blank samples, standard solutions, and spiked with the known concentration of analytes at 8 μg mL^{-1}. The resulting chromatograms were checked to examine the interference, no interfering peak was higher than the peak of analytes corresponding to the LOD. The chromatograms of blank and spiked sample solution and standard solution were shown in Fig. 4.

3.2.5 Stability

The stability of stock solutions of the analytes was tested by comparing the chromatographic peak area of 8 μg mL^{-1} a standard solution from the stock solution stored for 30 days at 2-8℃ with those obtained from the fresh stock solutions (n = 3).

Long term stability of sample solutions was investigated by three aliquots of sample solutions with the same concentration of 8 ng mL^{-1} were kept for 1 month at -20±5℃, the

sample solutions were processed according to the section 2.4. Then, the AP solutions were analyzed, the chromatographic peak signal compared with the actual value of the extracted sample solutions acquired under normal conditions. Three aliquots sample solutions of same concentration were kept at ambient temperature for 8 h to determine the short term stability of analytes. After extraction, the measured concentrations were compared to those of sample solutions of the same concentrations which extracted and immediately analyzed. All RSD values for stability were evaluated below 3.8%, showing that the stability of analytes was acceptable.

3.2.6 Comparison with other methods

The comparison of the proposed method with previously reported methods for analyzing β-blockers in water samples was provided. As shown in Table.4, the figure for merit of the proposed method is between 0.08 and 0.5 ng mL^{-1}, which is significantly better than the reported in the literatures including HPLC-DAD [28], CE [29], MEKC [30]. Although several detectors such as MS, tandem MS/MS, GC–MS. etc have higher sensitivity, the method established in our study also has a level of sensitivity owing to the high ability of extraction and enrichment. If the pretreatment method combined with more sensitive instrumental techniques such as MS, the resulting merit figures could be further improved and even better.

Second, owing to the complex environmental matrix and a wide variety of unknown pharmaceuticals are pervasive in daily monitoring works. So, a wide application, high purification and separation efficiency method required to design for analyzing various kinds of compounds in the field of labs at basic level. Traditional sample preparation methods, such as LLE and SPE are time consuming and waste high amounts of organic solvent. SPME [11]

is a new technique, based and developed from SPE. It has obvious advantages including solvent-free, simplicity and compatibility. While, it is considered expensive, fiber fragile with limited life time and always need high cost for the determination of low concentration of β-blockers in complex matrix. Electromembrane extraction (EME) [33] as an alternative method provides faster extractions. For the complex environmental sample, the existence of high levels of ionic substances results in a dramatic increase of ion balance value [33] in the solution, which in turn decreases the *EE* of analytes. Thus, the EME method is not suitable for the pre-treatment of samples with complicated matrix.

In present study, HF-LPME method was developed and it can integrate different steps (for example: extraction, separation and purification) in single devices which potentially saves considerable time and energy. As shown in Figure 4-B, heptanol as the optimal extraction solvent presents higher specificity and selectivity for the special structure of β-blockers, it greatly improves the efficiency of separation, clean-up and allowed better extraction for multiple β-blockers.

In addition, HF-LPME-UV method is particularly the most effective technique with simple equipment and more easy operation, it is more suitable to be used in any general laboratory for routine detecting of β-blockers on spot compared with high sensitive detection methods which are always used in specialized laboratory and need professional personnel to operate. The LC system is more universal and is of great practical value with more accurate and reliable results. All in all, based on the advantages above mentioned, the method could be of great utility for laboratory at basic level to solve the problem of routine screening trace amounts of β-blockers on-spot.

3.3 Application to real samples

To investigate the applicability of the proposed two-phase LPME pretreatment method, final experiments were carried out on determination of target analytes in waste water from WWTPs in He bei. The samples were directly used to analyze and pH was adjusted to 11.5 by dropwise addition of a saturated solution of sodium hydroxide. After extraction, nearly 30 μL AP was collected and analyzed by HPLC system. To measure the accuracy of the proposed technique, all analytes were spiked into the samples, the relative recoveries and RSD% (n=3) were calculated, the RSD% values were within the range 2.7–6.4% and the relative recoveries for spiked samples were between 96% and 108%. Therefore, the linearity range could directly calculate the amounts of analytes in the samples. The chromatograms obtained from real sample and spiked samples with 8 ng mL^{-1} of the analytes were depicted in Fig. 5. According to the chromatogram, propranolol can be detected in water sample and it is found the concentration is higher than LOD in the studied WWTPs. So the chemical analysis of the method for β-blockers is significant which reveals the formation of complex environmental matrix and confirms the finding of contaminants. In addition, other lipophilic β-blockers can also be identified by the method.

4. Concluding remarks

The paper described a pretreatment method with high specificity as well as good selectivity for screening multiple trace amounts of β-blockers. The method applied a two phase HF-LPME principle and heptanol was as the extraction solvent that has a highly sensitive, good specificity, inherent selectivity and stronger enrichment ability over the wide polarity range of β-blockers. Furthermore, the proposed method is expected to have higher sensitivity

when combined with more sensitive detector. Finally, the technique is simple to operate without professional personnels and provides a reliable and effective mean for self-checking of β-blockers in wastewater at operation sites for local laboratories.

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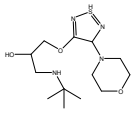
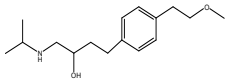
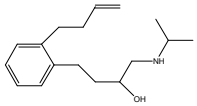
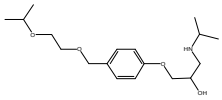
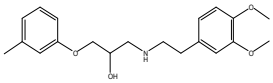
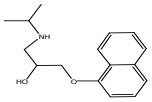
Table 1 Structure and physiochemical properties for targets

Compounds	Structures	pk_a[a]	log$P_{o/w}$[a]
Metoprolol		9.53	1.91
Timolol		9.45	1.95
Oxprenolol		9.44	2.16
Bisoprolol		9.42	2.2
Bevantolol		9.01	2.65
Propranolol		9.09	3.65

a. pk_a (the acid dissociation constant), log$P_{o/w}$（the logarithm of the octanol/water partition coefficient of analytes) which all were calculated by Advanced Chemistry Development Software V11.02.

Table 2 Figures of merit of HF-HPME method

Analytes	Regression equation	Correlation Coefficent (R^2)	Linearity (ng mL^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	PF[a]
Metoprolol	A=7.00×10^{-3}C+7.9×10^{-3}	0.9997	1.000-200.0	0.500	1.00	81
Timolol	A=38.7×10^{-3}C-43.4×10^{-3}	0.9992	0.400-200.0	0.200	0.400	115
Oxprenolol	A=25.6×10^{-3}C+0.5×10^{-3}	0.9996	0.400-200.0	0.200	0.400	158
Bisoprolol	A=8.5×10^{-3}C-5.8×10^{-3}	0.9995	1.000-200.0	0.200	1.00	132
Bevantolol	A=70.3×10^{-3}C-41.3×10^{-3}	0.9997	0.160-200.0	0.080	0.160	224
Propranolol	A=63.6×10^{-3}C-37.6×10^{-3}	0.9997	0.400-200.0	0.200	0.400	294

a. PF was calculated at concentration of 200 ng mL^{-1} for each drug.

Table 3 The results of precision and recovery of six beta-blockers at three sample concentration levels

Analystes	Sample concentration (ng/mL)	Inter-day RSD (%) (n=5)	Intra-day RSD (%) (n=5)	Recovery (%)	RSD (%)
Metoprolol	1.00	2.3	1.9	100.5	2.1
	8.00	2.1	1.7	100.2	
	200	1.9	1.5	96.7	
Timolol	0.400	2.4	1.8	101.1	2.2
	8.00	1.6	1.6	98.5	
	200	1.4	1.3	96.8	
Oxprenolol	0.400	2.7	1.8	99.3	0.81
	8.00	2.1	1.3	97.7	
	200	1.7	1.4	98.3	
Bisoprolol	1.00	2.5	2.2	100.4	1.54
	8.00	2.1	1.8	99.5	
	200	1.8	1.2	97.4	
Bevantolol	0.160	1.9	1.7	100.2	0.59
	8.00	1.6	1.5	99.1	
	200	1.8	1.0	99.3	
Propranolol	0.400	1.6	1.6	100.1	1.47
	8.00	1.5	1.4	98.2	
	200	1.8	1.1	97.2	

Table 4. Comparison of the proposed method with other analytical techniques for determination of beta-blockers in environmental samples

Analytical method	Analytes	Sample preparation	Sample type	Organic solvent volume(μL)	LOD[a]	Linear range[a]	Recovery (%)	Ref.
LC-MS–MS	Metoprolol	SPE	Wastewaters	6000	0.042	25–1000	104±54	31
	Propranolol				0.017		106±24	
LC–MS–MS	Propranolol	SPE	Wastewaters	-	0.008	0.2–6.5	-	32
LC–MS/MS	Bisoprolol	SPE	Sewage samples	6000	0.008	0.2-2	88±6	19
	Propranolol				0.010		91±7	
	Timolol				0.007		89±5	
HPLC–DAD	Metoprolol	SPE	naturalwater	5000	0.008	125-50000	91±5	28
	Propranolol				0.4		98±8	
Liquid chromatography quadrupolelinear ion trap mass spectrometry (LC–QqLIT MS)	Metoprolol	SPE	Wastewater	7000	0.0005	0.1–200	75±6	7
	Propranolol						80±5	
	Timolol				0.0004		93±4	
Precolumn switching (PC-LC–DAD)	Metoprolol	-	Watewater	-	0.13	0.5–10.0	-	8
	Timolol				0.14			
	Bisoprolol				0.11	0.2–10.0		
	Propanolol				0.15			
GC–MS	Alprenolol	EME	Wastewater	54	0.18	1-200	18	33
	Propanolol				0.0081		53	
CE	Alprenolol	polymer-coated-HFME	Wastewater		0.9	25-500	91	29
Sweeping micellar electrokinetic chromatography (MEKC)	Propranolol	LLE	Wastewater		7	40-1200	77–113	30
	Alprenolol				14			
HPLC	Metoprolol	HF-LPME	Environmeal samples	30	0.5	1.00-200		This work
	Timolol				0.2	0.400-200		
	Oxprenolol				0.2	0.400-200		
	Bisoprolol				0.5	1.00-200		
	Bevantolol				0.08	0.160-200		
	Propranolol				0.5	0.400-200		

a. Concentration is based on ng mL^{-1}

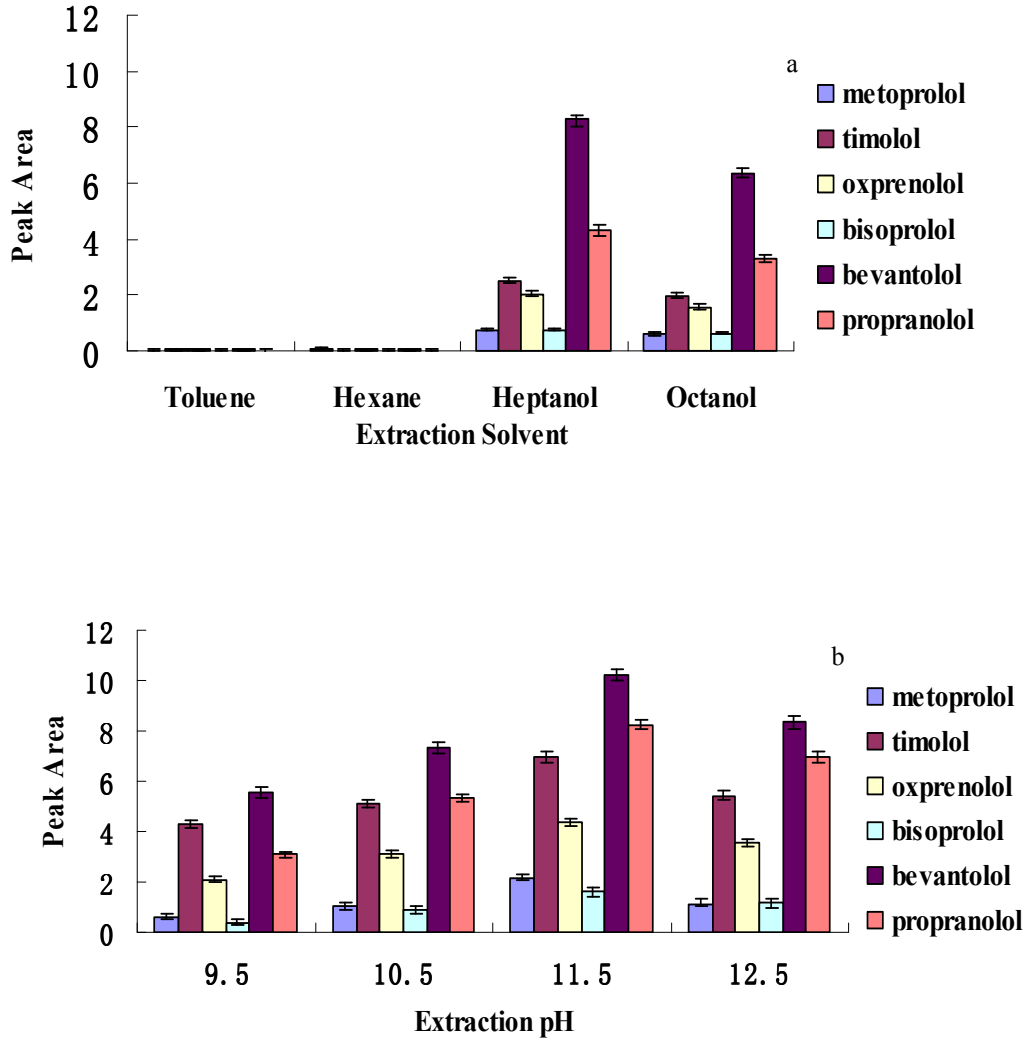


Fig. 1 The influence of different factors on the extraction efficiency (a) type of extraction solvent; (b) sample solution pH

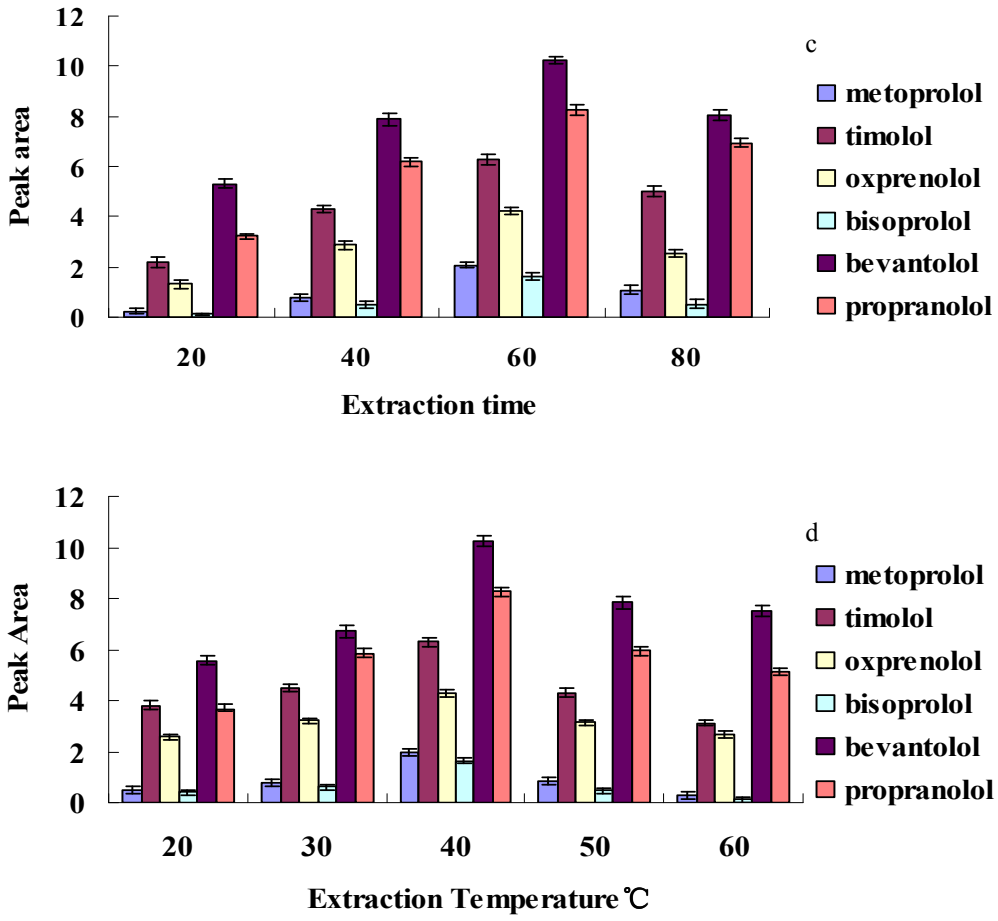
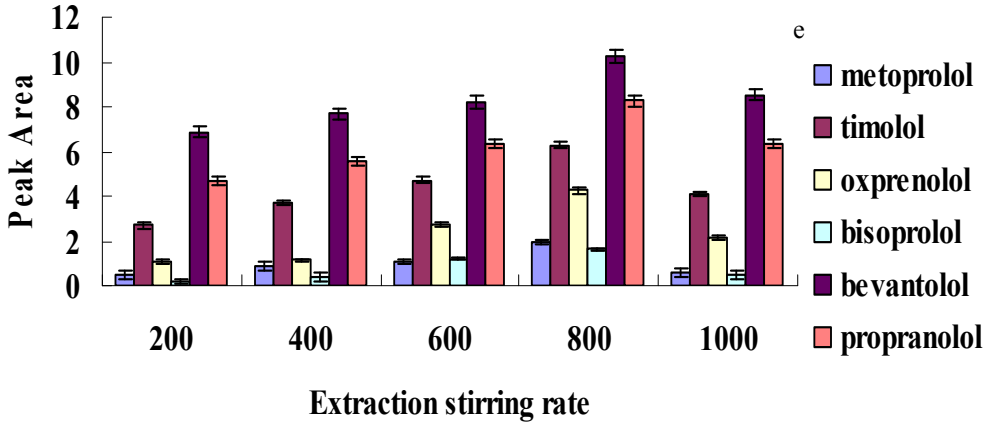
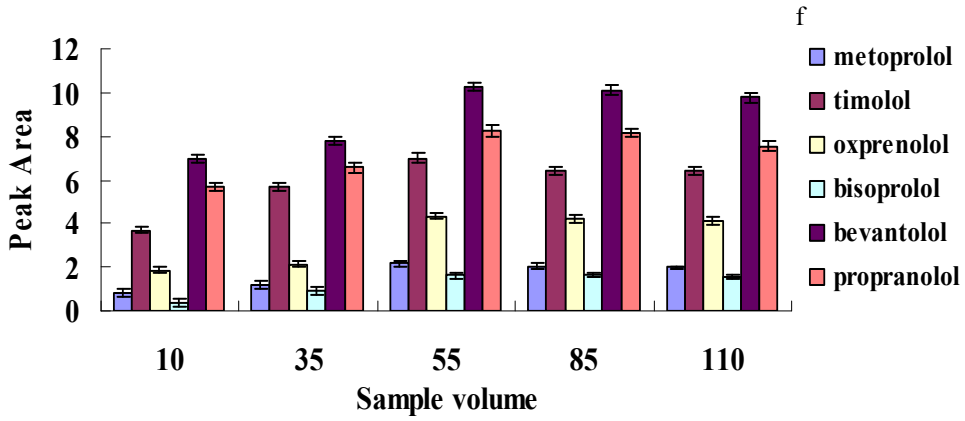


Fig. 2 The influence of different factors on the extraction efficiency (c) extraction time; (d) extraction temperature





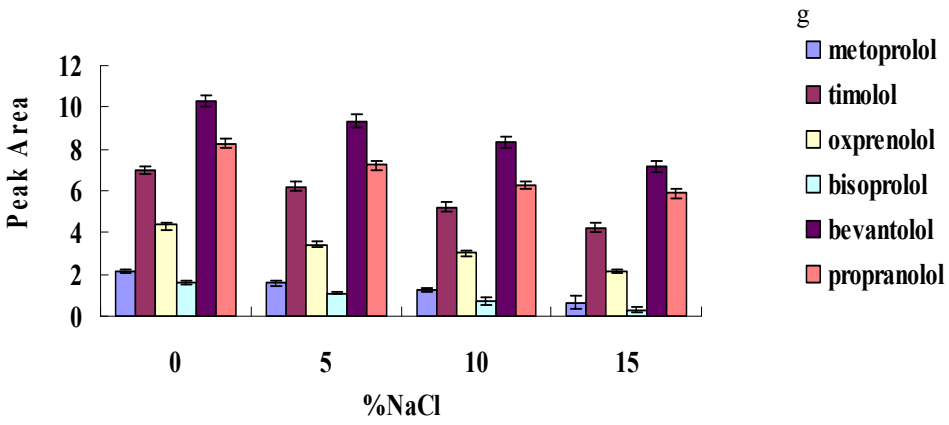


Fig. 3 Optimization of (e) extraction stirring rate; (f) the sample volume; (g) ionic strength

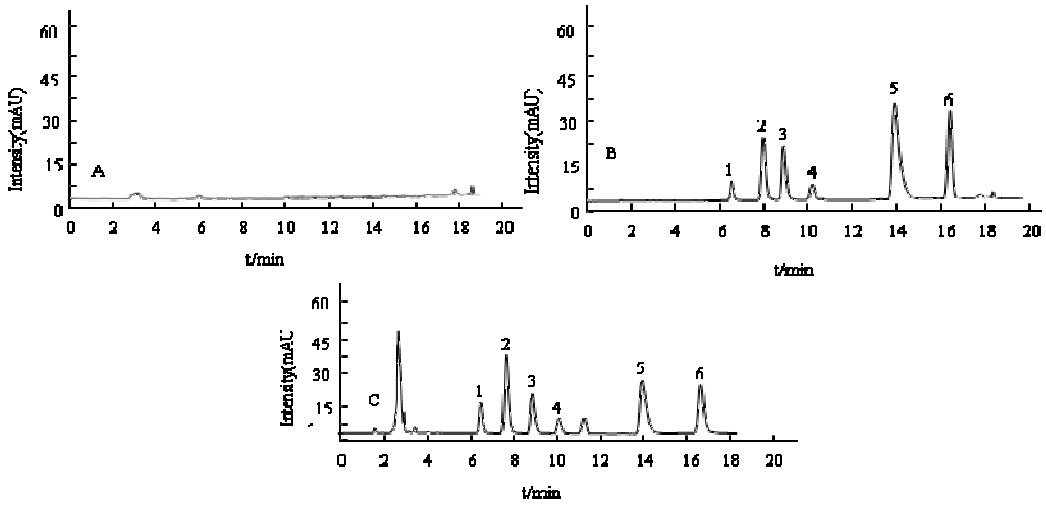


Fig. 4 Chromatograms of six β-blockers: A. Blank sample solution, B. Spiked sample solution (8 ng mL^{-1}), C. Standard solution (at concentration of 8 μg mL^{-1})

1. Metoprolol 2. Timolol 3. Oxprenolol 4. Bisoprolol 5. Bevantolol 6. Propranolol

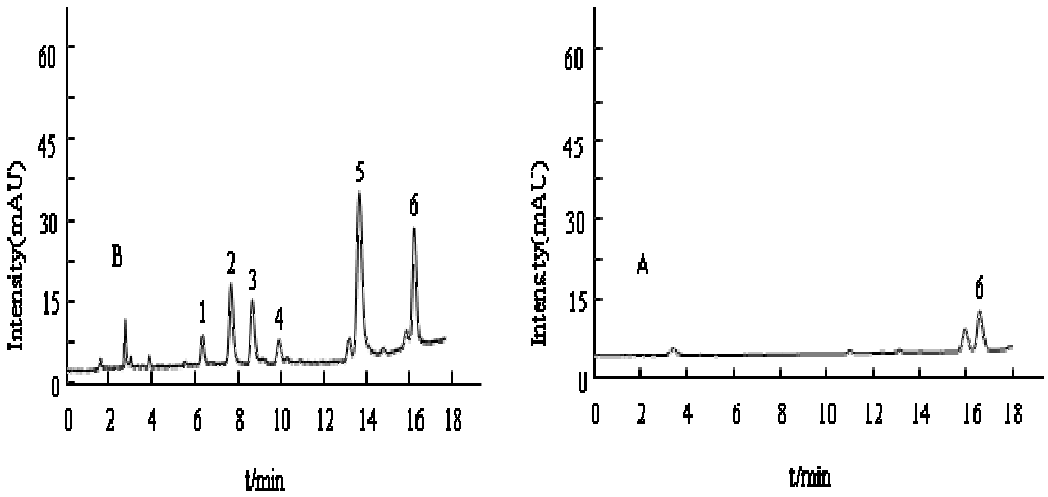


Fig. 5 Chromatograms of, A. Real water sample; B. Spiked water sample (8 ng mL^{-1})