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Application of Porous Membrane-Protected Micro-Solid-Phase Extraction Combined with HPLC for the Analysis of Acidic Drugs in Wastewater

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This report describes the use of a porous membrane-protected micro-solid-phase extraction (μ-SPE) procedure to extract acidic drugs from wastewater that are then determined by high-performance liquid chromatography with ultraviolet detection. The μ-SPE device consists of C_{18} sorbent held within a membrane envelope made of polypropylene. Ketoprofen and ibuprofen were selected as model compounds, and extraction parameters were optimized. Correlation coefficients of 0.9980 and 0.9953 were obtained for ketoprofen and ibuprofen, respectively, across a concentration range of 1−250 μg/L. Relative extraction recoveries were between 94 and 112%. The relative standard deviation of the analytical method ranged between 2 and 10%, respectively. The method detection limits for these target analytes in wastewater ranged from 0.03 to 0.08 μg/L. When compared to conventional solid-phase extraction (SPE), this new method showed better detection limits with good reproducibility. The results shows that this μ-SPE technique is a feasible alternative to multistep SPE for the extraction of analytes in complex samples.

The use of pharmaceutical products such as anti-inflammatories and lipid regulators is substantial in modern society, especially in developed countries.[1,2] Ibuprofen and ketoprofen are the most abundant drugs found in untreated wastewater samples.[3] The higher occurrence could be because of therapeutic doses of these drugs are excreted via urine in the form of free and conjugated metabolites.[4,5] Pharmaceutical residues in excreted waste reach sewage treatment plants (STPs) but may not be totally eliminated after STP processing.[6] Consequently, drug residues may contaminate drinking water produced from processed wastewater,[7] and acidic drugs are a major category of drug residues found in STP effluents.[8] The concentrations of various drug residues detected in the aquatic environment are relatively low.[9] Nevertheless, the impact of individual drug residues on nontarget organisms is still unknown and drug combinations may display synergistic effects.[10] Therefore, there is a need to develop a suitable analytical method to quantitatively evaluate the fate of pharmaceutical drugs in the aquatic environment for proper risk assessment.[9] Examples of drugs that have appeared in drinking water supplies are the acidic, nonsteroidal, anti-inflammatory drugs of the propionic acid group, ibuprofen and ketoprofen, both of which have analgesic and antipyretic functions.[11−13] The former is widely used in treatment of various forms of arthritis and as an antipyretic.[9] The latter is a pain-relieving agent for rheumatic and nonrheumatic inflammatory disorders, vascular headaches, and dysmenorrhea.[13]

Liquid−liquid extraction (LLE) and solid-phase extraction (SPE) are the most common techniques being used for sample preparation in drug analysis.[14] Both LLE and SPE entail multistep sample extraction and cleanup procedures that are tedious, time-consuming, and result in loss of analytes.[15,16] More recent sorbent-based sample preparation techniques, which minimize solvent consumption, include solid-phase microextraction (SPME)[17] and

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stir bar sorptive microextraction (SBSE).[18] The drawbacks associated with SPME are the high cost of the fiber, fiber fragility, loss of sorbent coating upon increased usage, and carryover effects.[19,20] In SBSE, only polydimethylsiloxilane (PDMS)-coated stirrer bars are commercially available hitherto. The nonpolar PDMS is more suitable for the extraction of nonpolar compounds, although extraction of polar compounds through SBSE has been reported.[21] Ketoprofen and ibuprofen are polar compounds and thus, require an additional derivatization procedure prior to their analysis by gas chromatography/mass spectrometry (GC/MS).[22,23] Derivatization reactions may introduce unwanted artifacts and analyte loss.[24] Capillary electrophoresis/ultraviolet detection,[25] liquid chromatography/mass spectrometry,[26] and high-performance liquid chromatography (HPLC)/UV[27,28] detection are more commonly used to analyze these drugs without derivatization

In this study, a novel extraction technique, micro-solid-phase microextraction (μ-SPE), involving the use of an sorbent wrapped in a porous membrane sheet was evaluated for the extraction of acidic drugs in wastewater samples. Due to the porosity of the membrane sheet, the analyte was able to diffuse through and be extracted by the sorbent. During extraction, the sample is stirred and tumbling of the μ-SPE device facilitated mass transfer. After extraction, desorption was carried out via ultrasonification with the extraction device immersed in a suitable organic solvent. The objective was to develop a procedure that could improve extraction, but was also accompanied by simultaneous cleanup for complex samples.

EXPERIMENTAL SECTION

Chemicals. HPLC-grade organic solvents were obtained from Merck (Darmstadt, Germany). Sodium dihydrogen phosphate monohydrate (98% purity), hydrochloric acid, and sodium hydroxide were obtained from J.T. Baker (Philipsburg, NJ), and orthophosphoric acid (85% purity) was purchased from Carlo Erba (Milan, Italy). Ultrapure water was prepared on a Milli-Q (Milford, MA) system. Ketoprofen and ibuprofen were purchased from Sigma-Aldrich (Milwaukee, WI). Both analytes were dissolved in methanol to prepare 1 mg/mL stock solutions. Working methanolic standard (5 μg/mL of each analyte) mixtures was prepared from the stock solution. Solutions were stored at 4 °C. For calibration purposes, a wide range of calibration standards (1−250 μg/L) was spiked to the acidified (pH 2) ultrapure water sample (40 mL) solutions. Extractions were carried out on these samples. Wastewater was collected from a local drainage canal.

Materials. Q3/2 Accurel 2E HF (R/P) polypropylene sheet membranes (157-μm thickness, 0.2-μm pore size and microporous PES polysulfone sheet membrane with 0.2 μm pore size) were purchased from Membrana (Wuppertal, Germany). GV Durapore sheet membrane 0.2-μm pore size was purchased from Millipore (Billerica, MA). Polycarbonate sheet membrane (0.2-μm pore size) was obtained from Sartorius (Goettingen, Germany). Various sorbents including C_{18}, C_2, Carbograph, HayeSep A, and HayeSep B were bought from Alltech (Deerfield, IL). Multiwalled carbon nanotubes (MWCNTs) were obtained from Honeywell Private Limited (Singapore). Plastic crimper vials of 0.2-mL capacity from Bioplastics (Landgraaf, The Netherlands) were used for solvent desorption by ultrasonication. Oasis-HLB SPE cartridges (200 mg) were obtained from Waters (Milford, MA).

Wastewater Samples. Wastewater samples were collected from local domestic drainage canals in glass bottles precleaned with acetone. The bottles were covered with aluminum foil, transported under cool conditions to the laboratory, and stored in the dark at −20 °C until analysis. Blank analysis of wastewater using μ-SPE and SPE showed no contamination of ketoprofen and ibuprofen. Therefore, the samples were used for method evaluation. Samples were not extracted immediately after spiking. A portion of the wastewater sample (500 mL) was spiked with standard ketoprofen and ibuprofen. The sample pH was adjusted and then carefully homogenized. The sample was then allowed to stand overnight before being extracted.

Instruments. The HPLC system consisted of a Waters 600E quaternary pump and M486 UV detector. Data collection and integration were accomplished with Waters Empower software. Isocratic separation was carried out using a reversed-phase Novopak C_{18} column (3.9 mm × 300 mm i.d., 4-μm particle size) from Waters (25 ± 1 °C) and a mobile-phase flow rate of 1.0 mL/min, with UV detection at 210 nm. The mobile phase used consisted of 70% acetonitrile in water and 0.01 M sodium dihydrogen phosphate solution acidified to pH 3.5 with orthophosphoric acid (30%). A 200-μL sample injection loop on a Rheodyne (Cotati, CA) injector was used.

Preparation of μ-SPE Device. The μ-SPE device consists of sorbent materials enclosed within a polypropylene sheet membrane envelope. Several sorbents were evaluated. To prepare the device, the longer edge of a polypropylene sheet was folded over to a width of ~1 cm. The edge of the fold-over flap was then heat-sealed using a electrical sealer to the main sheet. The fold-over section was then trimmed off from the main membrane sheet. The former was then cut (at ~1.5-cm intervals) into individual (1 cm × 1.5 cm) pieces. One of the two open ends of each piece was then heat-sealed. A glass Pasteur pipet and a glass funnel were used to introduce sorbent (20 mg) into the resulting membrane envelope via the remaining open end that was then heat-sealed to secure the contents.

μ-SPE. The schematic of a typical μ-SPE device is shown in Figure 1. Each μ-SPE device was conditioned by ultrasonication in ultrapure water and methanol for 2 min. For extraction, the μ-SPE device was placed in a pH-adjusted sample and stirred at ~105 rad/s (1000 rpm; 1 rpm = 0.1047 rad/s). The device tumbled freely within the sample during extraction. After extraction (50 min), the device was removed, rinsed in ultrapure water, dried with lint-free tissue, and placed in a 200-μL crimper vial. Tweezers

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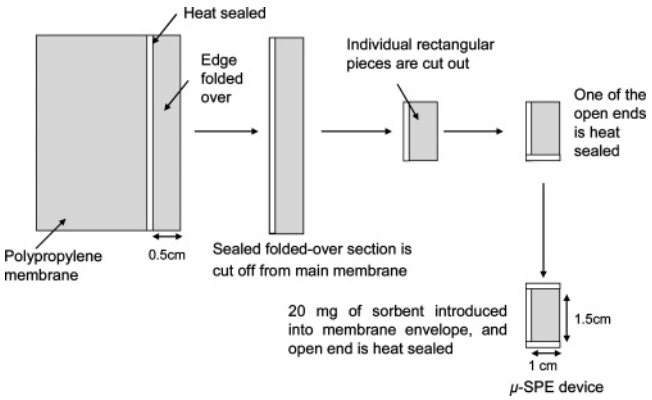

Figure 1. Schematic of preparation of a μ-SPE device

were used to handle the device. The analytes were desorbed by ultrasonication in acetonitrile (150 μL), out of which 100 μL was used for HPLC analysis. The μ-SPE device could be reused after careful rinsing with methanol.

SPE. SPE of water samples was performed as previously reported.[29,30] We considered that the extraction conditions were optimized. SPE cartridges (Waters Oasis HLB-SPE) were conditioned with 3 mL of methanol and 3 mL of ultrapure water. Extraction of 500-mL filtered wastewater samples, acidified to pH 2 with HCl, was carried out under vacuum at a flow rate of ~15 mL/min. Subsequently, the cartridge was washed with 1 mL of methanol−water (10:90, v/v). Analytes were eluted with 2 mL of methanol. Eluates were evaporated to dryness by a stream of nitrogen gas, and the residue was reconstituted with 150 μL of acetonitrile. A volume of 100 μL was injected into the HPLC.

Recovery Calculations. The absolute extraction recoveries were calculated based on our previous μ-SPE study.[31] The amount of analyte extracted and the extraction efficiency of μ-SPE may be evaluated by the following equation

$$n_A = FA = (m/A_d)A(V_d/V_i) \quad (1)$$

where n_A is the amount (mass) of analyte extracted by μ-SPE, F is the detector response factor, which can be calculated by comparing the amount of analyte (m) injected to the area counts (A_d) obtained by liquid injection, and A is the response obtained by μ-SPE. n_A can be easily obtained from experimental measurements with the above equation. Since, in μ-SPE and SPE, analytes were desorbed with an organic solvent, the volume of desorption solvent V_d and volume of solvent injected (V_i) into the HPLC were used to calculate the response factor F.

The absolute extraction efficiency (%E) was calculated as follows

$$\%E = n_{A/}C_i \quad (2)$$

where C_i is the initial concentration. The respective extraction efficiencies of μ-SPE and SPE for clean water and wastewater samples were calculated and are listed in Table 2. The relative recovery is defined as the ratio of the peak areas of the analytes in wastewater and ultrapure water sample extracts, with both samples spiked at the same concentration levels of ketoprofen and ibuprofen.

Method Validation. The objective of the optimization procedure was to obtain maximum analyte recovery. The parameters investigated were types of sorbent material, envelope membrane type, extraction time, sample pH, addition of sodium chloride, desorption solvent, desorption time, sample volume, and number of μ-SPE devices used for each extraction. Optimization experiments were performed in triplicate.

C_{18}, C_2, Carbograph, HayeSep A, HayeSep B, and MWCNT sorbents were evaluated. As shown in Figure 2, C_{18} had the highest extraction efficiency for both drugs. C_{18} sorbent possesses a silica surface covered with linear octylsilyl chains, akin to bristles of a brush, thereby providing a large surface and higher electrostatic interaction with target analytes. Unreacted silanol groups of the silica phase also serve as hydrogen-bonding motifs, which can interact with the carboxylic acid groups of the analytes. The HayeSep-A and HayeSep-B materials possess no hydrogen-bonding capability and their compact structures and bulky aromatic rings probably also hinder bonding interactions between analyte and sorbent, thus leading to lower partition affinities. C_2 sorbent showed slightly lower extraction efficiency than C_{18} for both analytes. Therefore, C_{18} was selected as the sorbent material for μ-SPE procedure. The electrostatic interaction between MWCNTs and target analytes was apparently not sufficient to achieve higher extraction efficiency compared to C_{18}.

Polypropylene, polysulfone, GV Durapore, and polycarbonate membranes with the same pore size (0.2 μm) were evaluated as envelope materials. For the same sorbent, the polypropylene membrane gave highest analyte enrichment. This may be due to better compatibility of polypropylene with organic solvents or greater matrix interference protection afforded by this material.

Like SBSE and SPME, the μ-SPE procedure is an equilibrium-based rather than exhaustive extraction procedure. The extraction efficiency of the μ-SPE device depends on partitioning of analyte to sorbent. Extraction duration from 10 to 60 min was investigated to determine equilibrium time (Figure 3). Initial partitioning was rapid, followed by a more gradual and protracted uptake; eventually a plateau was reached at ~50 min. Fifty minutes was selected as the optimal extraction time.

The influence of sample pH on extraction efficiency was investigated. Acidic sample pH values were effected via acidification with 1 M HCl while alkaline pH values were obtained using 0.1 M NaOH. The extraction efficiency was higher with lower sample pH, with maximum extraction efficiency observed at pH 2. At pH values of 6, 8, and 10, the corresponding peak for ibuprofen was not detected. pK_a values of both ketoprofen and ibuprofen are above 4. In a sample solution with a pH value lower than the pK_a values of the drugs, the acid−base equilibrium of the analyte would be shifted toward the neutral form and its partitioning into the sorbent phase would be increased. Thus, highest extraction efficiency was observed at a pH value of 2, and this condition was selected as the optimum.

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Table 1. Linearity Range, Limits of Detection, Limits of Quantification, and Precision of μ-SPE

analyte	linearity range (μg/L)	correlation coefficient (r)	calibration curve	RSD (%, $n = 3$)	LOD (μg/L)	LOQ (μg/L)
ketoprofen	1−250	0.9980	$y = 20065x + 8978.1$	6	0.03	0.08
ibuprofen	1−250	0.9953	$y = 7551x - 13299$	8	0.08	0.2

Table 2. Extraction Relative Recoveries Obtained by μ-SPE on Spiked Wastewater Samples ($n = 3$)

	μ-SPE (%) %, relative recoveries, (% RSD)[a]	
analyte	analytes spiked at 5 μg/L	analytes spiked at 25 μg/L
ketoprofen	112 (4)	102 (8)
ibuprofen	103 (5)	94 (6)

[a] RSDs are given in parentheses.

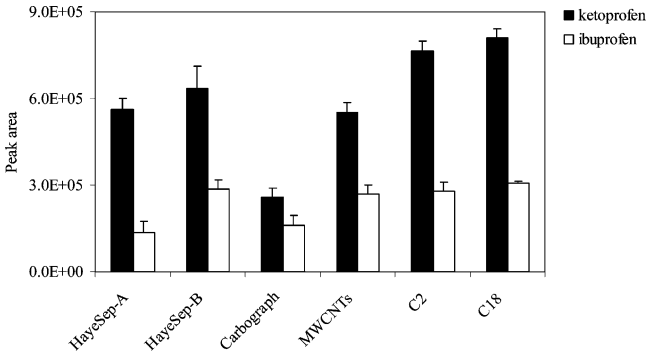

Figure 2. Effect of sorbent type on extraction efficiency of μ-SPE. Extraction conditions: extraction time 30 min, desorption time 30 min with acetonitrile as desorption solvent and sample volume 20 mL. No adjustment of sample pH and salt.

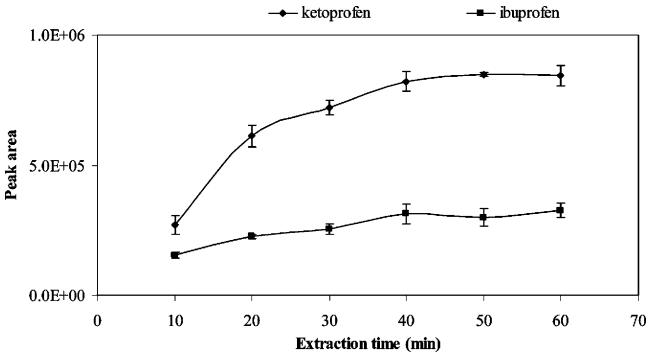

Figure 3. Effect of extraction time on extraction efficiency of μ-SPE. Extraction conditions: C_{18} as sorbent, desorption time 30 min with acetonitrile as desorption solvent and sample volume 20 mL. No adjustment of sample pH and salt.

Addition of sodium chloride was expected to enhance extraction efficiency of μ-SPE since analyte solubility in aqueous solutions generally decreases with increasing ionic strength (salting-out effect).[32] Hence, the effect of sodium chloride addition, ranging from 5 to 30% (w/v) in the sample solution, was studied. Addition of sodium chloride did not result in an increase in extraction efficiency. This may be due to the increase in viscosity

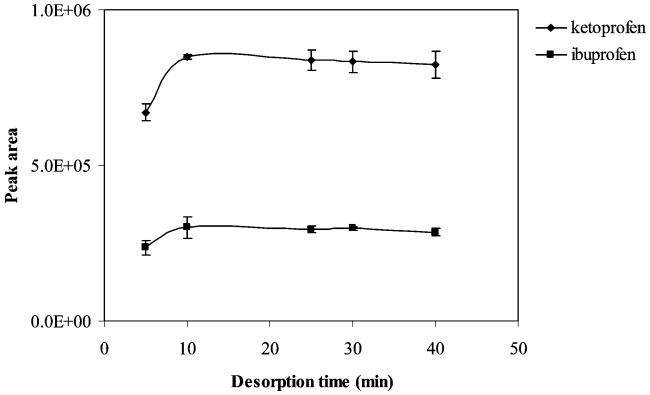

Figure 4. Effect of desorption time on extraction efficiency of μ-SPE. Extraction conditions: C_{18} as sorbent, extraction time 50 min with acetonitrile as desorption solvent and sample volume 20 mL with sample pH of 2.

of the aqueous sample following addition of large amounts of sodium chloride,[33] thereby impeding the mass-transfer process. The rate of mass transfer of analyte from aqueous phase to solid sorbent decreased, and the amount of time required to attain equilibrium increased. This is not an unusual observation for microextraction procedures.[34]

Selection of a suitable desorption solvent was assessed based on solubilization capability. Acetone, *tert*-methyl butyl ether, 1-octanol, tetrahydrofuran, methanol, acetonitrile, and acetonitrile−phosphate buffer (1:1 ratio, pH 3) were considered. Acetonitrile, methanol, and a mixture of acetonitrile−phosphate buffer showed the best results, with acetonitrile affording the highest desorption efficiency. No analyte carryover was observed after the first desorption (data not shown).

The most suitable volume of acetonitrile used for desorption was also determined. Volumes ranging from 100 to 300 μL (the μ-SPE device had to be completely immersed in the desorption solvent) were studied. A small volume (<100 μL) of desorption solvent led to higher peak areas of the analytes albeit with poor reproducibility, while larger volumes (>150 μL) gave low peak areas due to dilution. A volume of 150 μL of acetonitrile was found to be sufficient to completely desorb the analytes. Ultrasonication was also varied from 10 to 40 min. Figure 4 depicts the desorption profile of ketoprofen and ibuprofen (spiked at 20 μg/L) showing that 10-min desorption time was sufficient. No analyte carryover effect was observed with the above conditions.

The effect of sample volume (from 5 to 50 mL) on extraction efficiency was investigated. Larger extraction efficiencies were observed as sample volumes were increased. This phenomenon is due to increasing analyte enrichment with increasing volume

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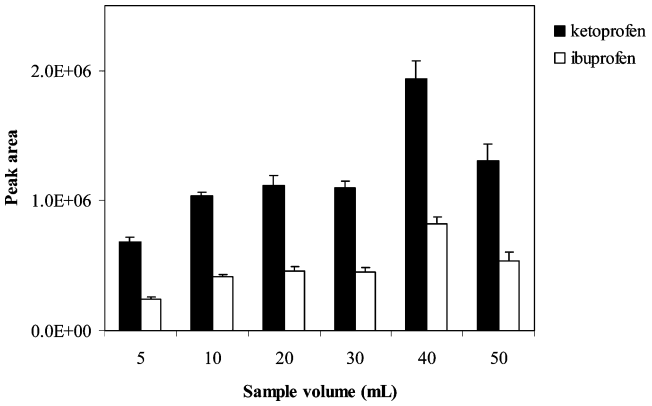

Figure 5. Effect of sample volume on extraction efficiency of μ-SPE. Extraction conditions: C_{18} as sorbent, extraction time 50 min with acetonitrile as desorption solvent for 10-min desorption with sample pH of 2.

of the sample. A limit to this enrichment is reached when the adsorption sites of the sorbent are fully saturated with analyte.[35] As shown in Figure 5, the extraction efficiency was reached at a maximum at 40 mL of sample. Hence, 40 mL was selected as the optimal sample volume.

The extraction efficiency based on the use of multiple μ-SPE devices (1−4) was evaluated with sample solutions spiked at 20 μg/L analytes. As expected, as the number of μ-SPE devices was increased, higher extraction efficiency was observed. However, when more than two μ-SPE devices were used, no additional enhancement was observed. At the concentration of the analytes in the sample solution considered, two μ-SPE devices exhibited optimum extraction, and additional devices provided an overcapacity that was not necessary (thus, the extraction did not show any additional enhancement). However, for less concentrated samples, more than two μ-SPE devices may be useful to increase extraction. No attempt was made to use more than two μ-SPE devices in the experiments because the limits of detection (LODs) for this number of devices were sufficient to detect these drugs in environmental samples (present at parts per billion to parts per trillion concentration range).

DISCUSSION

The extraction mechanism of μ-SPE is similar to that of SPME and SBSE. Preliminary studies were conducted to investigate the most appropriate C_{18} sorbent amounts (10−30 mg/device for extracting 20 μg/L spiked drugs) used for extraction for 10 mL of sample solution. The μ-SPE with 20 mg/device gave the best results (data not shown). Other parameters that influence the extraction efficiency of the procedure were investigated prior to using it in the determination of ketoprofen and ibuprofen from wastewater samples.

Summarizing, on the basis of the foregoing, the optimized μ-SPE conditions were as follows: extraction time of 50 min; use of two μ-SPE devices each containing 20 mg of sorbent packed in polypropylene; 40-mL sample solution acidified to pH 2; stirring speed maintained at 105 rad/s; 10 min of desorption time using 150 μL of acetonitrile; and finally, an injection volume of 100 μL for HPLC analysis. The individual μ-SPE device would be reused

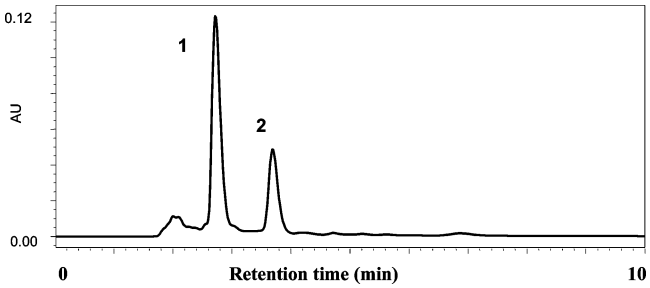

Figure 6. Liquid chromatogram of drug-spiked wastewater extract (20 μg/L, spiking level) after μ-SPE with C_{18} as sorbent. Peaks: (1) ketoprofen and (2) ibruprofen

for up to 30 analyses without any carryover effects or deterioration in extraction capability.

Optimized extraction conditions were utilized to measure linearity, precision, LOD, and limit of quantification (LOQ). Calibration curves were linear for a wide range of concentrations (1, 5, 10, 25, 50, 100, and 250 μg/L). Although, in real-world samples, the levels of ketoprofen and ibuprofen are generally in the lower range of the calibration standards used here, the upper limits (100 and 250 μg/L) were included to demonstrate the wide linearity range that could be achieved by the μ-SPE procedure. Correlation coefficients for ketoprofen and ibuprofen were 0.9980 and 0.9953, respectively. The LODs and LOQs were calculated according to IUPAC critera, i.e., as analyte concentrations giving signals exceeding that of the blank (y_B) by 3 times standard deviation (S_B). The LOQs, defined as the minimum analyte concentration required to ensure precise quantitative measurements, was determined similarly, using 10 times the standard deviation for the blank instead. LODs and LOQs were calculated from the following equation:

$$\text{LOD (LOQ)} = \frac{y_B + 3\ (10)\ S_B}{b}$$

where b is the slope of the calibration curve.

Based on the above equation, LODs for ketoprofen and ibuprofen were found to be 0.03 and 0.08 μg/L, respectively. The LOQs were 0.08 μg/L for ketoprofen and 0.2 μg/L for ibuprofen. These values were lower than values previously reported for SPE-GC/MS and LLE-GC/MS methods[22,23] and higher than those for SPE-GC/MS with large volume injection[36] and comparable with SPME-GC/MS.[15] Repeatability was evaluated by performing triplicate analysis at the various analyte concentrations in the linearity range. The RSDs were calculated to be 6% for ketoprofen and 8% for ibuprofen.

Wastewater Analysis. Several samples of wastewater taken from drainage canals were extracted using conventional SPE and μ-SPE procedures. However, no target analytes were detected. Thus, to assess matrix effects, wastewater samples were spiked and extraction performance was evaluated. Figure 6 shows the liquid chromatogram of ketoprofen and ibuprofen in wastewater extracts after μ-SPE; the original samples were spiked at 20 μg/L of the analytes. Comparison of the extraction efficiency of μ-SPE

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Table 3. Extraction Recoveries Obtained by μ-SPE and SPE on Spiked Wastewater and Clean Water Samples (n = 3)

	absolute recoveries					
	spiked wastewater samples (25 μg/L analytes), recovery (%) (RSD)		spiked wastewater samples (50 μg/L analytes), recovery (%) (RSD)		spiked clean water samples (250 μg/L analytes), recovery (%) (RSD)	
analyte	μ-SPE	SPE	μ-SPE	SPE	μ-SPE	SPE
ketoprofen	68 (3)	72 (10)	69 (4)	89 (12)	68 (11)	93 (8)
ibuprofen	55 (6)	67 (12)	56 (5)	79 (9)	54 (8)	116 (11)

with conventional SPE using the Oasis-HLB cartridges was carried out for spiked wastewater (analyte concentrations of 5 and 25 μg/L). Results are shown in Table 2; for μ-SPE, the relative recoveries ranged between 94 and 112%. The absolute extraction recoveries were also calculated. They ranged from 55 to 69%, with RSD values between 3 and 6% for wastewater samples and from 54 to 68% for clean water samples (Table 3). The conventional SPE procedure based on the conditions published previously,[29,30] on the other hand, gave recoveries ranging between 67 and 89% with RSD values between 9 and 12% for wastewater samples and between 93 and 116% with RSD values between 8 and 11% for clean water samples (Table 3). The extraction recovery of any method is dependent on the sample matrix. From this table, it is clearly seen that SPE has higher extraction recoveries for clean water samples but lower recoveries for wastewater extracts. On the other hand, for μ-SPE, there are no significant differences in the recoveries for both types of samples. This indicates that matrix effects on μ-SPE are negligible.

CONCLUSION

Porous membrane-protected extraction termed micro-solid-phase extraction, with C_{18} sorbent, in conjunction with HPLC/UV, is a simple and cost-effective method for extracting and determining drug residues in wastewater and can also serve as an effective screening procedure for aqueous contaminants. The procedure can achieve limits of detection ranging from 0.03 and 0.08 μg/L for ketoprofen and ibuprofen, respectively. With the protection afforded by the porous membrane, the technique could be used to extract drugs from "dirty or challenging" matrixes. The comparison between the present technique and conventional SPE indicates that μ-SPE is more precise in extracting drugs from wastewater samples, is simpler, and needs shorter extraction time. Both methods showed similar absolute recoveries of the analytes for the wastewater sample. For the clean water sample, no additional improvement in absolute recoveries by μ-SPE was observed when compared to conventional SPE. On the basis of these considerations, we can conclude that porous membrane-protected μ-SPE is an effective preconcentration technique that can be particularly applied as a cleanup procedure for "dirty" samples.

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