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RESEARCH ARTICLE

Dispersive liquid–liquid microextraction based on solidification of floating organic drop: Determination of nonsteroidal anti-inflammatory drugs in water

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Dispersive liquid–liquid microextraction based on solidification of floating organic drop method is a highly efficient method optimized for the first time in the determination of trace levels of ibuprofen, naproxen, diclofenac, and ketoprofen in water samples. Several important parameters affecting the dispersive liquid–liquid microextraction based on solidification of floating organic drop (extraction solvent, ionic strength, pH, dispersion solvent, vortex, and centrifugation times) were studied and optimized using a univariate optimization strategy. High-performance liquid chromatography coupled with a photo-diode array detector was used after the sample preparation. Limits of detection and quantification were in the range of 0.15–0.32 and 0.48–0.98 μg/L, respectively. Moreover, the linear dynamic range was up to 1000 μg/L and determination coefficient was higher than 0.9949. Recoveries in all water samples at two spiking levels (20 and 50 μg/L) were between 80.5 and 102.8% for all the pharmaceuticals. The intra- and interday ($n = 5$) precision were below 9.4%. Using dispersive liquid–liquid microextraction based on solidification of floating organic drop as a method for the extraction of pharmaceuticals from the aquatic environment is simple, rapid, environmentally friendly, and opens a new door for applications in this field where preconcentration is almost always required.

KEYWORDS
dispersive liquid–liquid microextraction, liquid chromatography, nonsteroidal anti-inflammatory drugs, solidification of floating organic drop, water

1 | INTRODUCTION

The presence of pharmaceutical residues in the aquatic environment has received considerable attention in recent years because they have been recognized as the most urgent emerging environmental pollutants [1]. Continuous discharge of pharmaceuticals into the environment; particularly, in water bodies causes a chronic threat to humans and wildlife [2]. These residues may enter aquatic systems through a variety of sources, including accidental spills, human and animal excretions, expired/unused pharmaceuticals, and manufacturing sites disposal [3].

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen (IBU), naproxen (NAP), diclofenac (DIC),

Abbreviations: DLLME-SFO, dispersive liquid–liquid microextraction based on solidification of floating organic drop

and ketoprofen (KET) are the most commonly prescribed NSAIDs used in human and veterinary applications [4–6] and they can be detected in receiving waters at the low micrograms per liter level [7, 8]. However, due to their low concentration and complex matrix in the aquatic environment, sample preparation involving extraction, clean-up, and preconcentration steps is still necessary to achieve adequate selectivity and sensitivity before instrumental analysis [9]. Traditional sample preparations like liquid-liquid extraction (LLE) and solid phase extraction (SPE) require multisteps and clean-up procedures that are laborious, time consuming, and require large amounts of organic solvents [10]. Some microextraction techniques, such as solid-phase microextraction (SPME) and liquid-phase microextraction (LPME) have been developed to avoid these drawbacks [11–13].

In recent years, a relatively new mode for LPME, dispersive liquid–liquid microextraction (DLLME) has received popular recognition for its rapidity, simplicity of operation, low cost, and high efficiency in comparison with classic sample preparation techniques [14]. In DLLME, the extraction is performed between the sample and a cloud of fine extractant drops formed when a dispersive solvent is added to the aqueous sample together with the extraction solvent using a syringe [15]. Then, the extraction solvent containing the target analytes can be isolated by centrifugation [16].

However, most of the available extraction solvents, mainly halogenated hydrocarbons, are highly toxic and environmentally unfriendly. In addition, the density of these solvents is higher than water, so they will sink to the bottom of the centrifugation tube that makes the separation step more challenging. To overcome these drawbacks, dispersive liquid–liquid microextraction based on solidification of floating organic drop (DLLME-SFOD) has been developed [17]. In this method, less toxic and low density organic solvent with a low melting point is used as the extraction solvent. When the sample solution is transferred into an ice bath where the temperature is below the melting point of the extraction solvent, the extractant turns into solid and floats on the surface of the sample solution, which makes the collecting procedure much simpler [18]. Up to now, DLLME-SFOD has been used for the extraction of polycyclic aromatic hydrocarbons [19] and some metal ions [20, 21]. However, to the best of our knowledge, the optimization of DLLME-SFOD for the extraction of pharmaceuticals in an aqueous environment is still scarce.

Hence, the aim of this work is to develop and apply, for the first time, an efficient DLLME-SFOD procedure for the extraction of some frequently occurring pharmaceuticals in water for further analysis by HPLC with a photo-diode array detector (PDA).

2 | MATERIALS AND METHODS

2.1 | Chemicals

All the chemicals used in the present work were of analytical grade. Ibuprofen (IBU), naproxen (NAP), diclofenac (DIC), and ketoprofen (KET) were obtained from Sigma–Aldrich (St. Louis, USA). Stock standard solutions of all analytes were prepared in acetonitrile (ACN) at a concentration of 50 mg/L and stored at 4°C. Working solutions of 10 mg/L of all analytes were prepared daily by appropriate dilution of the corresponding stock solution with ultrapure water.

LC–MS grade water, methanol (MeOH), hydrochloric acid (HCl), sodium hydroxide (NaOH), acetone, and isopropanol were purchased from Scharlab (Barcelona, Spain). Sodium chloride (NaCl) was purchased from Sigma–Aldrich. 1-Decanol and decanoic acid were obtained from Merck (Darmstadt, Germany).

2.2 | Instruments

The HPLC instrument used was a Shimadzu Prominence LC-2030C 3D (Shimadzu Corporation, Japan), which consists of a binary pump, a thermostated column compartment, and a PDA detector. The analytical column employed was a hypersil ODS (C18) of 250 mm × 4.6 mm I.D. and 5 μm particle size (Thermo Scientific, USA). The mobile phase was filtered using a vacuum filtration system through 0.2 μm polyamide membrane filters (Sartorius Stedim Biotech GmbH, Göttingen, Germany). Samples were analyzed using a gradient separation of 30% ultrapure water (containing 0.1% acetic acid, v/v) and 70% ACN, as A and B solvents, respectively. The gradient program started as: 30% of B, constant for 0 min, then increased to 100% at $t = 5$ min and kept constant for 10 min. The detector was set at 275 nm, the flow rate was 1 mL/min, and the injection volume was 10 μL. An adequate separation is achieved in less than 10 min. Data analysis was carried out using Shimadzu Lab solutions software (Shimadzu Corporation).

2.3 | Sample collection

Tap water was sampled from our laboratory after allowing it to flow for 10 min. Different types of bottled drinking water (Jericho, Arwa, Aqwa Pure, and Ein Gedi) were randomly obtained from a local supermarket in Jenin-Palestine. In all cases, the sampling bottle was rinsed three times with water before it was filled up and stored at −20°C in amber glass vials until analysis.

2.4 | Dispersive liquid–liquid microextraction based on solidification of floating organic drop procedure

Ten milliliters of water sample adjusted to pH 4.0 was placed in a capped glass centrifuge tube with a conical bottom. NaCl (1.0 g) was added and dissolved to obtain a proper ionic strength. Two hundred microliters of 1-decanol as the extraction solvent and 300 μL of ACN as the dispersive solvent were pipetted into the solution that was subsequently vortexed for 2 min. A cloudy solution consisting of very fine droplets of 1-decanol dispersed into the aqueous sample was formed, and the analytes were extracted into the fine droplets of extraction solvent. After centrifugation at 5000 rpm for 6 min, a liquid organic droplet (1-decanol layer) floated on top of the tube due to its density that is lower than that of water and was incubated in an ice bath for 5 min in order to solidify the extractant. Then, the aqueous phase was rapidly removed by a syringe while the solidified organic drop was collected and melted at room temperature. Finally, 10 μL of the supernatant was injected into the HPLC-PDA system for further analysis.

2.5 | Performance parameters

The performance of the extraction was evaluated by calculating the enrichment factor (EF) and extraction recovery (ER) as follows:

$$EF = \frac{C_{final}}{C_{initial}} \quad (1)$$

$$ER = EF \times \frac{V_{final}}{V_{initial}} \times 100 \quad (2)$$

where $c_{initial}$ corresponds to the concentration of the analytes before DLLME-SFO, and c_{final} is the concentration of the analytes in the extracted phase, that is, the mixture of the droplet obtained by the DLLME-SFO method and the solvent in which it is dispersed. Likewise, $v_{initial}$ is the initial sample volume and v_{final} is the volume of the extracted phase.

3 | RESULT AND DISCUSSION

3.1 | Optimization assays

The optimization of the parameters that affect the efficiency of the DLLME-SFO technique was carried out using a univariate strategy using 10 mL of ultrapure water spiked with the target compounds at 50 μg/L. Extraction recovery (ER) was used during the optimization of parameters. All experiments were carried out in triplicate and average data was calculated. The initial standard conditions were 100 μL of 1-decanol as extraction solvent with pH adjusted to 2.0 and 250 μL of ACN as dispersive solvent under 10 min of centrifugation.

3.1.1 | Extraction solvent volume

The selection of an extraction solvent is a very important parameter in the DLLME- SFO procedure. Generally, the extractant must have a density lower than water and its coagulation can float on the top of the solution. Therefore, 1-decanol was chosen as an extractant that can be collected easily at temperatures lower than its melting point. A sufficient volume of extraction solvent ensures complete extraction of analytes with good recovery. In this experiment, the effect of extractant volume on the recovery was investigated from 100 μL to 350 μL (data not shown). It can be found that all the analytes roughly reach their extraction plateaus with 200 μL of 1-decanol; therefore, this volume was selected.

3.1.2 | Ionic strength

The addition of salt usually decreases the solubility of the target compounds in an aqueous solution. To investigate the effect of adding NaCl on the recovery of pharmaceuticals, a series of experiments have investigated the effect of different amounts of NaCl in the range of 0–2.5 g. Figure 1A shows an increase in the extraction efficiency of target analytes when the amount of NaCl was increased from 0 to 1.0 g. Increasing the amount of NaCl can affect the analytes' diffusion between the sample solution and the extraction solvent, decreasing the solubility of the analytes in the sample solution and therefore, increasing extraction efficiency. On the other hand, the recovery of pharmaceuticals decreased slightly as the amount of salt increased from 1.0 to 2.5 g. The process of adding excessive amount of salt led to an increase in solution viscosity that in turn caused the partitioning and diffusion rate of the analytes to decrease. Therefore, 1.0 g NaCl was selected for all subsequent experiments.

3.1.3 | pH

Sample pH plays an important role in the extraction efficiency [22]. To obtain the optimum pH conditions for maximum recovery, a set of experiments were conducted with various pH ranging from 1 to 8 (adjusted with 0.5 M HCl

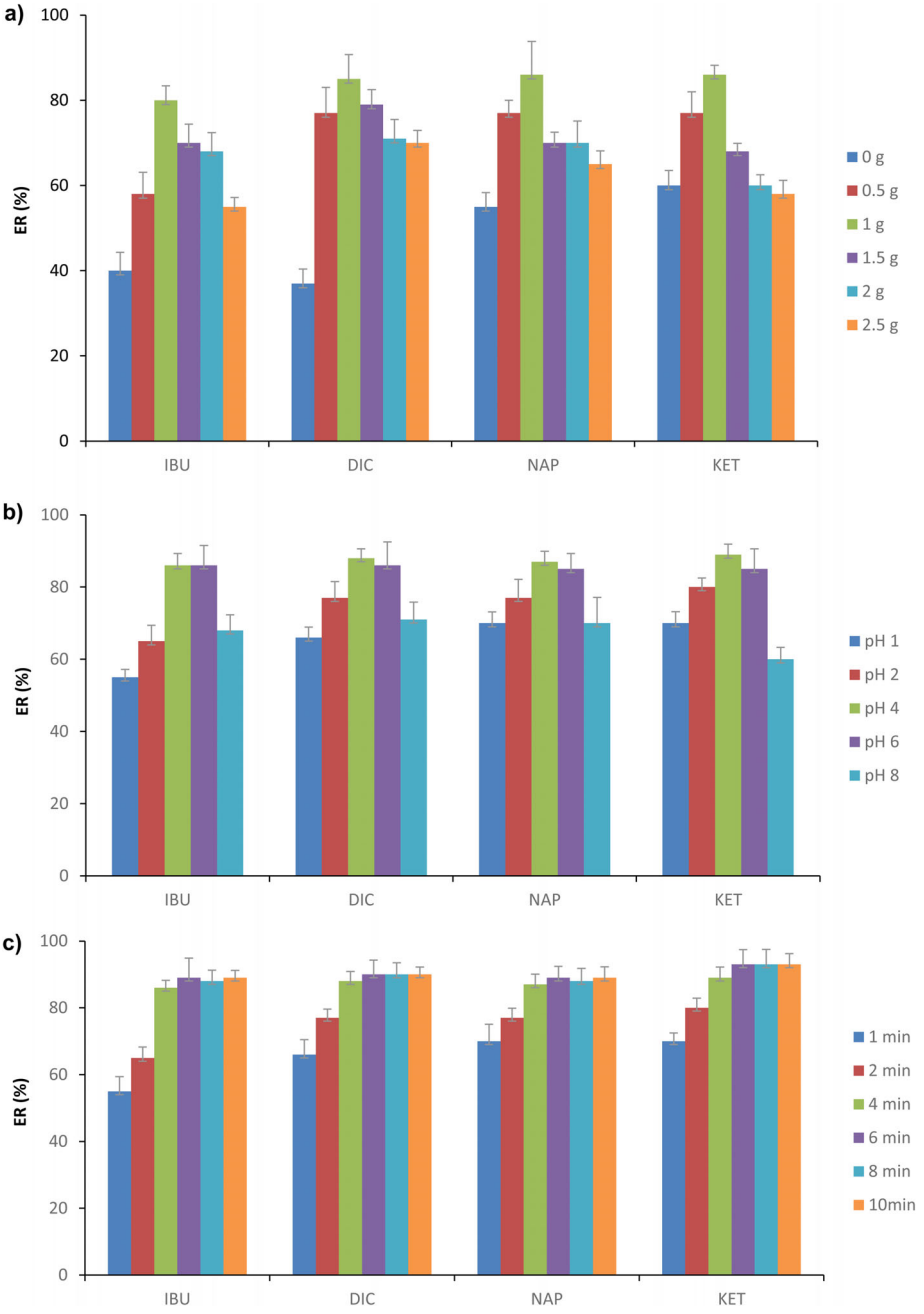

FIGURE 1 Influence of the (A) ionic strength, (B) pH, and (C) centrifugation time on extraction recovery of a standard solution of 50 μg/L of all analytes. Standard deviations ($n = 3$) are plotted as error bars

and 0.5 M NaOH). From Figure 1b, it is clear that the maximum recovery of pharmaceuticals was achieved when the pH of the sample was maintained at 4.0, which indicates that the ionization of the analytes at pH 4.0 was crucial to the extraction procedure and selected as the optimum pH.

3.1.4 | Dispersion solvent

The selected dispersion solvent must have great miscibility in aqueous sample and extractant, low toxicity, and low cost. In this study, several dispersion solvents, such as MeOH, acetone, and ACN were tested. According to the results obtained (data not shown), the recovery of pharmaceuticals using different dispersants is higher when ACN was used as the dispersant compared to acetone and methanol. In addition, ACN was also used as the mobile phase in chromatography analysis, so it is the best choice for dispersant.

The volume of dispersion solvent was investigated because the variation in the volume of ACN affects the amount of floating phase; consequently, affecting extrac-

TABLE 1 List of the variables studied, including the initial and optimum values as well as the interval tested in the DLLME-SFO

Variable	Initial	Interval studied	Optimum
NaCl (g)	0	0–2.5	1
pH	2	1–8	4
Dispersive solvent	ACN	ACN, MeOH, acetone	ACN
Volume of dispersive solvent (μL)	250	100–1000	300
Volume of extraction solvent (μL)	100	100–350	200
Vortex time (min)	Not adjusted	0.5–5	2
Centrifugation time (min)	10	1–10	5

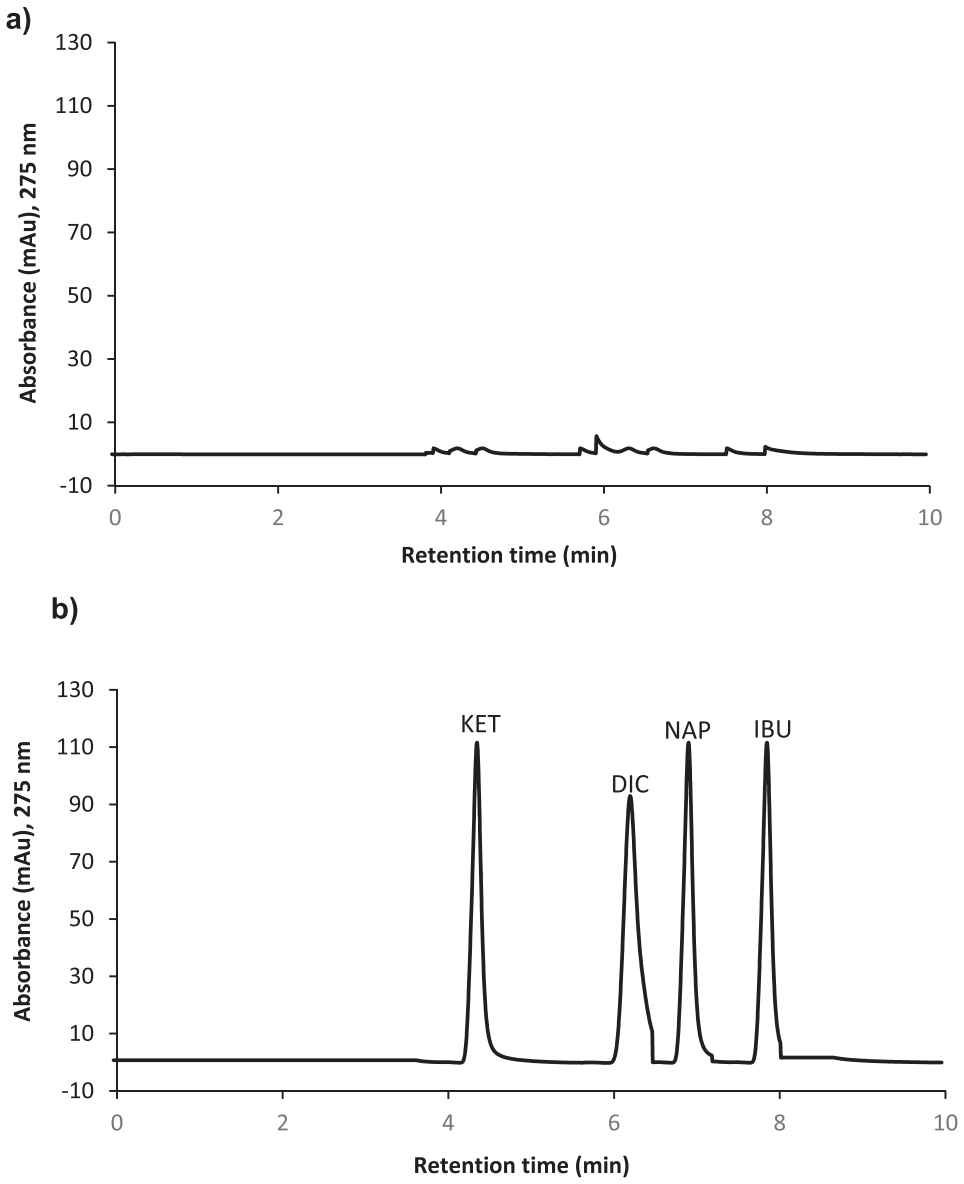

FIGURE 2 Sample chromatograms after the DLLME-SFO/HPLC-PDA process, under optimized experimental conditions (λ = 275 nm) of (A) a non-spiked and (B) spiked at 50 μg/L

TABLE 2 Analytical figures of merit

	LOD/LOQ (μg/L)	R^2	Precision[a] (%RSD) Intraday	Interday
IBU	0.15/0.48	0.9949	2.1	8.8
DIC	0.18/0.55	0.9988	3.4	9.4
NAP	0.21/0.65	0.9985	3.7	6.6
KET	0.32/0.98	0.9955	4.2	6.5

[a]Precision is calculated for $n = 5$.

tion recovery [23]. The volume of dispersion solvent was tested in the range of 100–1000 μL. The results show (data not shown) that when the volume of ACN was increased to 300 μL, the extraction efficiency of the four analytes increased. On the other hand, when the volume of ACN was increased from 300 to 1000 μL, the recovery of pharmaceuticals decreased slightly. This could be simply explained that a large amount of ACN increased the solubility of pharmaceuticals in the aqueous phase. Therefore, 300 μL of ACN was chosen as the optimum dispersion solvent volume.

3.1.5 | Vortex and centrifugation times

Vortex agitation is used to transfer the target analytes from the aqueous phase to the extraction phase. The effect of the vortex time on extraction recovery was investigated in the range of 0.5–5.0 min while maintaining all other parameters fixed. The results showed that the recovery of the analytes increased slightly with increasing vortex time and reached maximum at 2 min. Likewise, the effect of centrifugation time was studied from 1 to 10 min. As shown in Figure 1c, the minimum time that provided the highest recovery was 6 min; therefore, this time was selected.

In order to summarize the optimization process, the initial, studied, and selected values are listed in Table 1. Figure 2 depicts two chromatograms obtained from assays performed in Aqwa Pure water samples spiked with 50.0 μg/L (solid line) and without spiking (dotted line) by DLLME-SFO/HPLC-PDA, under optimized experimental conditions ($\lambda = 275$ nm).

3.2 | Analytical figures of merit

To demonstrate the performance and practical ability of the proposed methodology (DLLME-SFO/HPLC-PDA), assays were done on real samples. Under the optimal experimental conditions mentioned above, a series of experiments were designed for obtaining linear ranges, precision, detection limits, and other characteristics of the method. The limits of detection (LOD) were calculated from a chromatogram corresponding to a standard of 50 μg/L after the DLLME-SFO process. LOD was calculated based on the concentration corresponding to S/N of 3, and the LOQ was calculated by S/N of 10. The values of LOD and LOQ were in the range of 0.15–0.32 and 0.48–0.98 μg/L, respectively. Linearity was evaluated by measuring standard solutions in the range from 20 to 1000 μg/L after being submitted to the DLLME-SFO treatment. The determination coefficients (R^2) ranged from 0.9949 to 0.9988.

Intraday and interday precision were carried out at a concentration of 20 μg/L for each analyte by three replicate experiments on the same day and on three different days, respectively. The relative standard deviations (RSDs) were in the range of 2.1–4.2% for intraday precision and of 6.5–9.4% for interday precision.

3.3 | Analysis of water samples

To investigate the practicability of the developed DLLME-SFO method, four kinds of water samples as explained in Section 2.3 were subjected to DLLME-SFO followed by HPLC-PDA detection, and the results are shown in Table 2 ($n = 5$). The results indicated that there were no pharmaceuticals found in the samples. These samples were then spiked with the target analytes at a concentration of 20 and 50 μg/L to investigate the effect of sample matrices. As can

TABLE 3 Extraction recovery (ER), expressed as average ± SD ($n = 3$), in water samples spiked with the analytes at 20 and 50 μg/L

	ER (%) at 20 μg/L ($n = 3$)					ER (%) at 50 μg/L ($n = 3$)				
	Tap	Jericho	Arwa	Aqwa Pure	Ein Gedi	Tap	Jericho	Arwa	Aqwa Pure	Ein Gedi
IBU	80.5 ± 9.0	85.6 ± 5.5	86.8 ± 9.0	84.8 ± 9.0	88.8 ± 9.0	82.8 ± 9.0	87.6 ± 5.5	100.8 ± 9.0	89.8 ± 9.0	99.8 ± 9.0
DIC	91.3 ± 5.7	82.6 ± 6.7	92.5 ± 5.7	90.5 ± 5.7	93.5 ± 5.7	90.5 ± 5.7	82.7 ± 6.7	95.5 ± 5.7	92.5 ± 5.7	95.5 ± 5.7
NAP	92.0 ± 3.7	90.1 ± 6.8	95.0 ± 3.7	90.0 ± 3.7	91.0 ± 3.7	90.0 ± 3.7	90.9 ± 6.8	100.0 ± 3.7	102.8 ± 3.7	101.8 ± 3.7
KET	87.8 ± 8.7	82.9 ± 5.4	87.8 ± 8.7	87.8 ± 8.7	83.8 ± 8.7	87.8 ± 8.7	89.9 ± 5.4	88.8 ± 8.7	89.8 ± 8.7	87.8 ± 8.7

be seen from Table 3, the spiked recoveries were satisfied in the range of 80.5–102.8%.

4 | CONCLUDING REMARKS

A simple and efficient method based on the DLLME-SFO procedure coupled with HPLC-PDA was developed for the first time for the extraction of IBU, NAP, DIC, and KET in water. In this study, the process of DLLME-SFO provided a method to extract pharmaceuticals in water samples without complicated procedures. The developed method showed short extraction time, reliable recovery, simplicity in operation and high extraction efficiency.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data support the findings of this study are available from the corresponding author upon reasonable request.

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