Solidified floating organic drop microextraction (SFODME) for the simultaneous analysis of three non-steroidal anti-inflammatory drugs in aqueous samples by HPLC

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ABSTRACT

In this work, a liquid-liquid microextraction methodology using solidified floating organic drop (SFODME) was combined with liquid chromatography and UV/Vis detector to determine non-steroidal anti-inflammatory drugs (NSAIDs), naproxen (NPX), diclofenac (DCF) and mefenamic acid (MFN) in tap water, surface water and seawater samples. Parameters that can influence the efficiency of the process were evaluated, such as the type and volume of the extractor and dispersive solvents, effect of pH, agitation type and ionic strength. The optimized method showed low detection limits (0.09 to 0.25 μg L^{-1}), satisfactory recoveries rates (90 % to 116 %), and enrichment factor in the range between 149 and 199. SFODME showed simplicity, low cost, speed and high concentration capacity of the analytes under study. Its use in real samples did not demonstrate a matrix effect that would compromise the effectiveness of the method, being possible to apply it successfully in water samples with different characteristics.

Keywords: Floating drop; Microextraction; Non-steroidal anti-inflammatory; Water sample.

1. INTRODUCTION

Pharmaceuticals present in aquatic environments are among the contaminants that have most attracted attention of the scientific community. Among these substances are non-steroidal anti-inflammatory (NSAIDs), widely recommended by healthcare professionals worldwide [1]. These pharmaceuticals have great applicability in human and animal medicine due to the analgesic, antipyretic and anti-inflammatory effect [2]. Because of their extensive use, the compound itself, in their unaltered form, or even their metabolites can reach the environment in several ways. Inappropriate disposal of unused pharmaceuticals, domestic sewage discharge, animal excretion and their use in soil fertilization [2-4] can be considered the main sources of environmental contamination. The presence of NSAIDs in ecosystems, even at trace levels, is potentially dangerous due to their toxicity and, in some cases, bioaccumulation capacity [5,6]. This class of pharmaceuticals includes diclofenac (DCF), naproxen (NPX), and mefenamic acid (MFN), among others. NSAIDs are often found in the environment at concentrations in the range of ng L^{-1} to μg L^{-1} [5,7]. Although there are some reports of high concentrations [8-10] in aquatic environment, generally DCF, NPX and MFN are present at concentrations that reach a few μg L^{-1}. Moreover, various studies have observed toxic effects in aquatic organisms even at these low concentrations [11-14]. Therefore, the determination of these substances in aquatic environments is extremely important [7,15].

Since, generally the concentrations of pharmaceuticals in environmental matrices are very low, it is necessary to incorporate pre-concentration and *clean-up* procedures before analysis by chromatographic methods [16]. Solid-phase extraction (SPE) and liquid-liquid extraction (LLE) are widely used to prepare samples from different matrices [17-19], but the disadvantages are the high use of organic solvents,

large amount of waste generated and multi-stage analytical procedures with consequent high time-consuming and labor intense. Therefore, new extraction methods have been proposed, such as solid-phase microextraction (SPME), which compared to LLE and SPE requires less time and solvent consumption but has the disadvantage of the high-cost [3,16,20-22]. As alternative, other microextraction techniques have experienced great development lately, such as single drop microextraction (SDME) [23,24], hollow fiber liquid-phase microextraction (HF-LPME) [25,26] and dispersive liquid-liquid microextraction (DLLME) using different approaches [27].

DLLME has numerous advantages such as simplicity and low-cost, but has the disadvantage of using toxic solvents, as well as the difficulty of the removal of the solvent drop, accumulated at the bottom of the tube [28]. The DLLME technique with floating organic droplet solidification (SFODME) [29], in addition to having the advantages of the conventional DLLME, makes use of less toxic solvents and allows easy removal of the extracting solvent, since it is completely solidified as a drop on the sample surface [30,31]. Succinctly, a few microliters of an organic solvent (extracting solvent), with low miscibility and with lower density than water, together with a dispersive solvent, are quickly injected into the aqueous sample, producing high turbulence cloud. This turbulence causes the formation of organic droplets, which are dispersed throughout the aqueous sample. After the formation of a cloudy solution, the equilibrium state is reached, the mixture is vortexed and centrifuged. After this step, the extracting solvent is completely concentrated on the sample surface. Then, the system is placed in contact with an ice bath for a few minutes and the frozen drop of organic solvent that remains at the top of the aqueous sample is collected into a vial for HPLC analysis. Based on this methodology, this work aims to develop an accurate, selective and sensitive SFODME procedure followed by HPLC–UV/Vis analytical method for the simultaneous

88 determination of three NSAIDs in aqueous samples. The parameters that can influence
89 the efficiency of the extraction process were evaluated, such as the type and volume of
90 the extracting and dispersive solvents, the effect of pH, agitation mode and ionic strength.
91 The proposed method has been validated and satisfactorily tested in different aqueous
92 samples, such as tap water, surface water and seawater.

93 **2. MATERIAL AND METHODS**

94 **2.1 Reagents and samples**

95 Analytical standards NPX (≥ 98.0 %), DCF (≥ 98.0 %), MFN (≥ 98.0 %) and the
96 extracting solvents 1-undecanol (≥ 98.0 %) and 1-dodecanol (≥ 98.0 %) were provided
97 by Sigma-Aldrich (United States). All of the HPLC grade organic solvents (≥ 99.9 %)
98 used, methanol (MET), acetonitrile (ACN) and ethanol (ETN) were provided by Merck
99 (Germany) and all other standard analytical grade reagents employed phosphoric acid (≥
100 85%), sodium hydroxide (≥ 99%) and sodium chloride (≥ 99%) were from Isofar (Brazil).
101 The ultrapure water (18.2MΩ-cm) used was obtained through Milli-Q systems from
102 Merck Millipore (Darmstadt, Germany).

103 The individual stock solutions of NPX, DCF and MFN were prepared in
104 methanol at the concentration of 100 mg L^{-1}. Successive dilutions were prepared in
105 methanol.

106 **2.2 Instrumentation**

107 A Shimadzu® liquid chromatograph (LC-20AT Prominence) equipped with a
108 UV/Vis detector SPD-20A with a slit of 8nm was used. In the first 7 minutes of analysis,
109 the 256 nm wavelength was used in the UV/Vis (for NPX) and in the last 8 minutes (for

DCF and MFN), the 234 nm wavelength. The separation of the compounds was performed on a Luna C-18 reverse phase column (Phenomenex®) (250 mm x 4.6 mm, 5 µm), maintained at 25 °C, using a mobile phase of ACN: acidified water with H_3PO_4 (pH 2.24) 60/40 (*v/v*), with a flow of 1.2 mL min^{-1}. The mobile phase was filtered through 0.45 µm nylon membrane filters (Millipore). To control the equipment and obtain the data, a microcomputer and LC solution® software version 1.24 SP1 of Shimadzu were used.

An MX-S mini vortex and a USC-1400A ultrasound were used for the extraction process.

2.3 SFODME procedure

SFODME method was performed by injecting a mixture containing 30 µL of 1-dodecanol (extracting solvent) and 150 µL of ACN (dispersive solvent) into 5 mL of water sample with pH adjusted to 2 with H_3PO_4 85 % (*v/v*) and containing 2.5 % (*w/v*) of NaCl. Due to the presence of salt in the seawater sample, NaCl was not added. The choice of using 1-dodecanol (water insoluble, melting point of 24 °C) as extracting solvent was made based on preliminary experiences reported in detail in supplementary material (SM). After addition of the extraction mixture, the tube containing the sample was vortexed for 20 s and then centrifuged at 5000 *rpm* for 4 minutes [32]. After this step, the extractor was completely concentrated as a drop on the sample surface. To cause this drop to solidify, the system was placed in contact with an ice bath for a few minutes. After complete solidification of the extracting solvent, it was easily removed in an interval lower than 1 min with a spatula and placed in a vial at room temperature. 20µL of liquid drop were then analysed by HPLC-UV/Vis. For each condition tested, five extraction replicates were performed, which were analysed once by HPLC.

2.4 Optimization of extraction conditions

2.4.1 Effect of dispersive solvent type and volume

The dispersive solvent must be miscible in both the organic and aqueous phases and must cause adequate dispersion of the extracting solvent throughout aqueous sample when the extraction mixture is added. It favors the contact between the extracting solvent and the sample containing the analytes, in order to improve the extraction capacity and reduce the time needed to achieve the equilibrium state. However, high volumes of dispersive solvent can also increase the solubility of the analytes in the aqueous phase [31,33].

In this work, three dispersive solvents (MET, ACN and ETN) using three different volumes (50, 150 and 200 µL) were tested in order to evaluate the extraction capacity of the NSAIDs with each combination. The protocol consisted in the addition of 30 µL of extracting solvent (1-dodecanol) with the different volumes of each dispersive solvent tested (MET, ACN and ETN in 50 µL, 150 µL and 200 µL) to 5 mL of ultrapure water at pH 2 (adjusted with H_3PO_4 85 % (*v/v*)) fortified with 1.2 µg L^{-1} of each NSAIDs.

2.4.2 Effect of ionic strength

The increase in the ionic strength of the sample results in a decrease of analyte solubility and generally enhances the extraction efficiency, due to the *salting-out* effect. This effect is caused by changing the partition coefficients of the analytes between the aqueous and organic phases [29,31,34]. In order to evaluate the *salting-out* effect on the extraction efficiency, four NaCl concentrations in the range of 0 and 10 % (*w/v*) were tested. The procedure included the introduction of a mixture of 30 µL of the extracting solvent (1-dodecanol) and 150 µL of the dispersive solvent (ACN) in 5 mL of ultrapure

157 water, with pH 2 (adjusted with H_3PO_4 85 % (*v/v*)), fortified with 1.2 µg L^{-1} of the
158 NSAIDs and containing the different amounts of NaCl (0 %, 2.5 %, 5 %, and 10 % (*w/v*)).

159 *2.4.3 Effect of pH*

160 The pH of the aqueous solution can have a large effect on the microextraction
161 capacity since it can influence the charge of the target compound and the amount
162 extracted will depend on ionization. At low pH, the NSAIDs will be in neutral form (pKa
163 NPX=4.15; pKa DCF=4.15; pKa MFN=4.2), which will facilitate their transfer to the
164 extractor solvent [3,31]. Initially, pH 2 was the condition used according to Beldean-
165 Galea et al [3]. However, the reference used did not evaluate the extraction of MFN. Thus,
166 in this work, the extraction efficiency of the three NSAIDs under study, using three
167 different values (1.0, 2.0 and 4.0), was evaluated. The protocol included the introduction
168 of a mixture containing 30 µL of the extracting solvent (1-dodecanol) and 150 µL of the
169 dispersive solvent (ACN) in 5 mL of ultrapure water at different pH (adjusted with H_3PO_4
170 85 %), with NaCl 2.5 % (*w/v*) and fortified with 1.2 µg L^{-1} of the NSAIDs.
171
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173 *2.4.4 Effect of extracting solvent volume*
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175 The volume of extracting solvent can have a great influence on the efficiency of
176 SFODME and it can affect the enrichment factor (ratio between the analyte
177 concentrations in the organic (C_o) and aqueous (C_{aq}) phases) [31]. To verify the effect of
178 the extracting volume, three volumes were tested: 30 µL, 40 µL and 50 µL. The procedure
179 involved the addition of the corresponding volume of the extracting solvent (1-dodecanol)
180 and 150 µL of dispersive solvent (ACN) in 5 mL of ultrapure water pH 2 (adjusted with

H_3PO_4 85% *(v/v)*), containing NaCl 2.5 % (*w/v*) and fortified with 1.2 µg L^{-1} of the NSAIDs.

2.4.5 Stirring type effect

To evaluate the influence of the stirring type effect of vortex, ultrasound and manual agitation in the extraction efficiency, several experiments were performed. The protocol consisted in the mixture of 30 µL of extractor (1-dodecanol) and 150 µL of disperser (ACN) in 5 mL of ultrapure water pH 2 (adjusted with H_3PO_4 85% (*v/v*)), fortified with 1.2 µg L^{-1} of the NSAIDs and NaCl 2.5 % (*w/v*). The samples were submitted to the three mixing models separately, followed by centrifugation for 4 minutes at 5000 *rpm*.

2.5 Determination of NSAIDs in water samples

Finally, to evaluate the applicability of the proposed SFODME, tap water, surface water and seawater were collected in glass containers and subjected to the optimized method described previously. Samples were collected in October 2019 in the city of São Luis, Brazil. The 1 L amber flasks used in the collection were previously washed and the samples were transferred, stored and refrigerated at approximately 4 °C until use. Samples were filtered through 0.45µm nylon membrane filters (Millipore), previously to the extraction procedure described in section 2.3 and submitted to HPLC-UV/Vis analysis. The evaluation of the water matrix influence on the extraction was performed by spiking known amounts of NPX, DCF and MFN simultaneously on the water samples and subjecting them to the previously optimized extraction procedure. Spiked concentrations used were 1.2 µg L^{-1}, 3 µg L^{-1} and 5 µg L^{-1}. Five replicates of extraction were performed for each of the three levels of fortification studied. The

recovery (%) was calculated as the ratio between the experimentally determined average concentration and the corresponding expected concentration.

3. RESULTS AND DISCUSSION

3.1 Optimization of extraction conditions

3.1.1 Effect of dispersive solvent type and volume

The recovery (%) (calculated as described in section 2.3) obtained for the selection of the type and volume of the dispersive solvent are shown in Fig. 1.

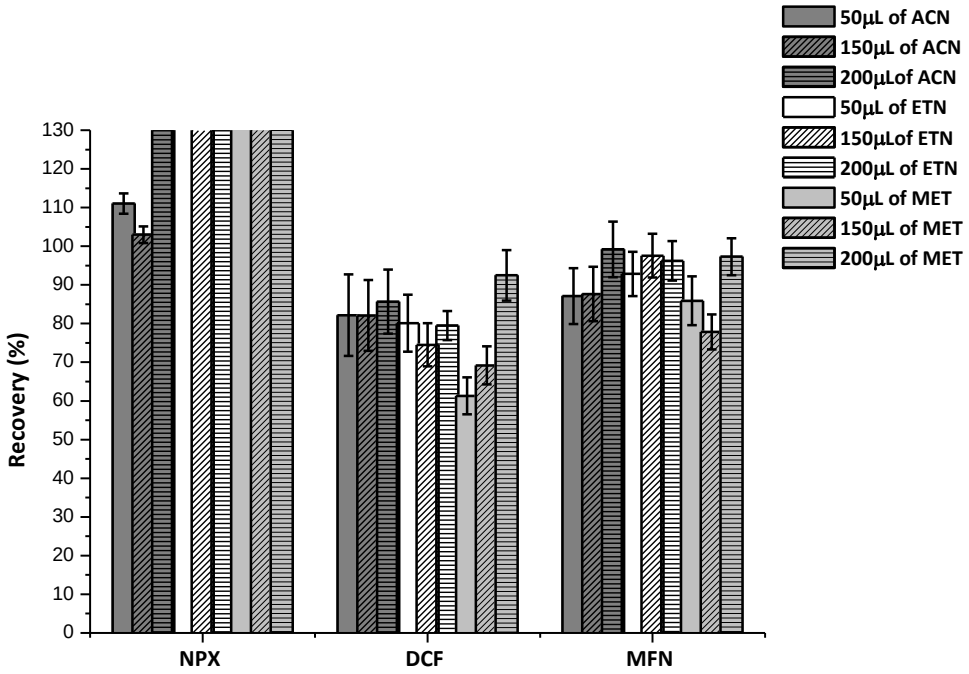

Fig. 1 Effect of the type and volume of the dispersive solvent. Extraction conditions: 5 mL of ultrapure water, pH adjusted to 2, fortified with NSAIDs (1.2 µg L^{-1}), different volumes of dispersive solvents, and 30 µL of 1-dodecanol. The y-axis was adjusted to 130% to allow a better visualization of DCF and MFN data. (n=5)

Similar results, for all NSAIDs, were obtained using 50 and 150 µL of ACN. Although the solvents ETN and MET could provide a better extraction efficiency of DCF and MFN, they did not provide satisfactory recoveries for the NPX (with values in the range between 177% and 434%, resulting in an overestimation of NPX). This overestimation might be associated with the interference of substances in 1-dodecanol that appears at the same retention time of NPX when ETN and MET were used as dispersive solvents. Given this scenario and considering that ACN exhibited good results, it was selected as dispersive solvent. Recoveries, for NPX, using 50 µL of ACN were slightly higher than 100 %, while using 150 µL the extraction recovery of the three analytes ranged from 82 % to 103 %. For these reasons, volume of 150 µL of ACN was selected for the following experiments.

3.1.2 Effect of ionic strength

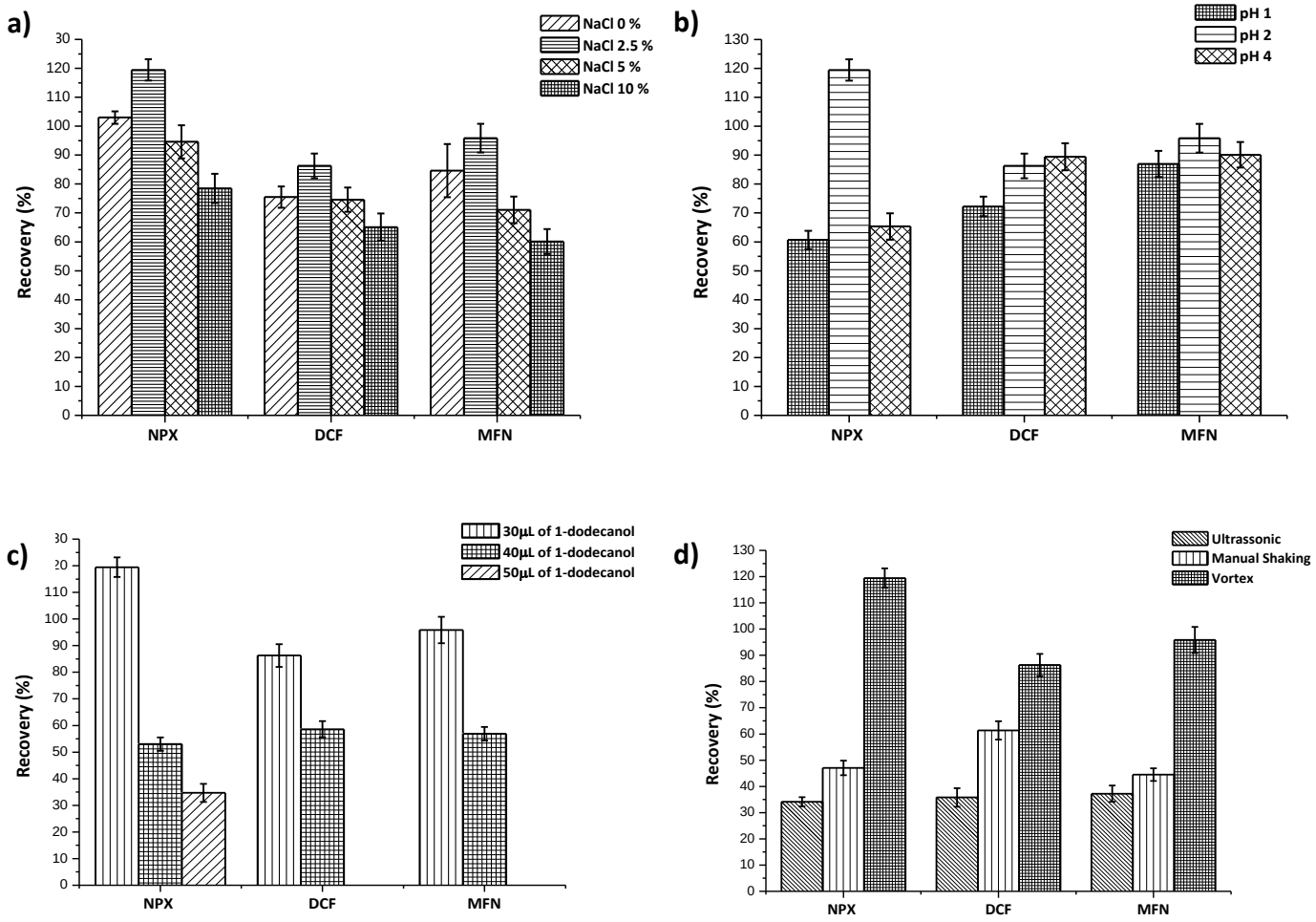

Fig. 2 Effect of (a) NaCl (b) pH, (c) extracting solvent volume and (d) stirring type on the extraction efficiency of NSAIDs using 5 mL of ultrapure water fortified with 1.2 µg L^{-1} of each compound. (n=5)

For all the NSAIDs tested, the extraction capacity improved by the addition of 2.5 % (*w/v*) of NaCl to the aqueous sample. This is explained by the *salting-out* effect, in which the solubility of compounds in water is reduced due to the change in their partition coefficients [31,34], thus increasing its affinity to the organic solvent. However, when

NaCl concentrations increases too much (5 % and 10 % *(w/v)*), a decrease in the extraction recovery was obtained. This is in agreement with the Khalili Zanjani et al [29], who attribute this reduction to restrictions on the diffusion of analytes to the organic phase due to the increase in the viscosity of the sample. Moreover, this study allows to conclude that this method should be successfully applied to seawater samples, since salinity generally is around 3.5 %.

3.1.3 Effect of pH

In order to evaluate the influence of aqueous pH in the extraction procedure, aqueous samples were adjusted to different pH. The chosen pH values are accordingly to pK_a of the NSAIDs under study. Results obtained are shown in Fig. 2 (b).

Recovery rates for DCF increases with increasing pH from 1 to 4. In the case of NPX and MFN, there is an increase in recovery results from pH 1 to 2 and a decrease when pH 4 is used. Considering the standard deviations, results obtained for DCF and MFN at pH 2 and 4 cannot be considered different. Thus, the condition that allows a better extraction of all the NSAIDs under study and chosen for the further tests was pH 2, in which recovery rates obtained, for the three NSAIDs studied, varied between 86 % and 119 %. This is in agreement with Beldean-Galea et al [3] and Shukri et al. [32] who also demonstrated in their work that pH 2 would be the best to be used for NSAIDs. In fact, the NSAIDs under study present a pK_a around 4, which means that at pH lower than their pK_a values the compounds are in its neutral form, thus facilitating their transfer to the extracting solvent [31]. At higher pH values, the analytes are mostly in ionized form, which negatively influences the ability to be extracted.

3.1.4 Effect of extracting solvent volume

Fig. 2 (c) shows the results of the variation of the recovery with the increase of the extracting volume. For all the compounds it was possible to verify that the increase in the extracting solvent volume caused a decrease in the extraction capacity. In fact, for DCF and MFN, using 50 µL of extracting solvent, no peak was detected. The increase of extracting solvent volume originates the dilution of the analyte, thus a reduction in the concentration of the compound in the organic phase, which impacts the enrichment factor and consequently the efficiency of the process. From the three volumes tested, the one that provided the best results was 30 µL, being the volume chosen for the next experiments.

3.1.5 Effect of stirring type

Stirring is very important to guarantee that the organic solvent drops are totally dispersed through the aqueous sample, improving the contact between the analyte to be extracted and the extracting solution. Three types of stirring were tested, and results obtained are shown in Fig. 2 (d).

The type of stirring that provided the best results for all the analysed compounds was the vortex, being therefore chosen to be used in this study. Guiñez et al. [35] demonstrated in their work with nitro-PAHs that the use of vortex also generated higher percentages of recovery compared to manual mixing and ultrasound. The vortex allows a better and more uniform dispersion of the system, favouring the contact between the aqueous and organic phases and thus significantly improving the extraction capacity associated to the method, when compared to manual agitation and the ultrasound assisted extraction.

3.2 Analytical Performance SFODME

Six standards were prepared in ultrapure water in concentrations between 0.6 to 5 μg L^{-1} for NPX and DCF and 1.2 to 5 μg L^{-1} for MFN. The standards were subjected to the optimized extraction method described in section 2.3 in order to determine the corresponding calibration curves. The analytical performance of the proposed method, presented on Table 1, was performed by assessing linearity (Lin (%) = 100-*RSD*, where *RSD* is the slope's relative standard deviation), determination coefficients (r^2), detection limits (LOD) and quantification limits (LOQ), using the conditions of optimized SFODME. The LOD and LOQ were determined from the equations 3*(*s*/*S*) and 10*(*s*/*S*), respectively, where *s* is the estimated standard deviation of the regression equation and *S* is the slope of the calibration curve [36]. Recovery (%) was calculated as the ratio between the experimental average concentration obtained from HPLC analysis and the corresponding expected concentration. Enrichment factor (EF) was determined by the ratio between the analyte concentration determined using the developed method and the initial analyte concentration added.

Table 1. Quantitative parameters for analytical curves obtained by SFODME–HPLC–UV/Vis for NPX, DCF and MFN.

Analyte	Linear range (μg L^{-1})	r^2	Linearity (%)	LOD (μg L^{-1})	LOQ (μg L^{-1})	Extraction Recovery[a] (%)	Enrichment factor[a]
NPX	0.6 – 5	0.9964	93.04	0.09	0.29	116±11	199±18
DCF	0.6 – 5	0.9956	91.68	0.25	0.82	90±4	149±6
MFN	1.2 – 5	0.9940	94.49	0.15	0.51	109±5	182±9

[a] Mean value ± standard deviation (n=5) obtained for a concentration of 1.2 μg L^{-1} for NPX, DCF and MFN. Extraction conditions: 5 mL of standard NPX, DCF and MFN with pH adjusted to 2; 30 μL of 1-dodecanol as extracting solvent; 150 μL of acetonitrile as dispersive solvent and NaCl 2.5 % (*w/v*).

309 The SFODME-HPLC-UV/Vis resulted in good linearity with determination
310 coefficients greater than 0.99, showing low dispersion between their experimental
311 analysis. The low LODs (between 0.09 and 0.25 μg L^{-1}) and LOQs (between 0.29 and
312 0.82 μg L^{-1}) confirm the applicability to detect and quantify the NSAIDs in environmental
313 water samples. Also, the NPX showed superior EF and ER when compared to the other
314 analytes under study, but all the NSAIDs presented good recoveries, between 90 and
315 116%, and good EF values, between 149 and 199. For the optimized conditions, the
316 SFODME method presents satisfactory parameters, thus an evalution of the applicability
317 in complex natural water matrices should be performed.

318 **3.3 Analysis of environmental water samples**

319 Spikes of 1.2, 3 and 5 μg L^{-1} of NPX, DCF and MFN were performed into three
320 different types of samples: seawater, surface water and tap water. All samples were
321 submitted to the optimized SFODME method described in section 2.3 and analysed by
322 HPLC-UV/Vis. Fig. 3 shows the chromatograms obtained from the analysis of the real
323 samples with and without spiked of anti-inflammatories. Water samples chromatograms
324 allow us to verify that the compounds under study were not present in the samples before
325 the standard addition. On the other hand, some studies reported the occurrence of the
326 same NSAIDs in natural waters. Sodré and Sampaio [37] quantified them in drinking
327 waters from Brazilian Federal District (Brazil) DCF in the range of 0.0042 μg L^{-1} to
328 0.006 μg L^{-1}, and MFN in the range of 0.0016 μg L^{-1} to 0.0083 μg L^{-1}, but NPX was not
329 detected in that samples. Ide et al. [38] verified the presence of NPX in Iguaçu River
330 (Brazil) at maximum concentration of 0.34 μg L^{-1}. Pereira et al. [39] detected DCF in a
331 seawater sample from Santos Bay (Brazil) at concentration of 0.0194 μg L^{-1} and Chaves

et al [40] found concentrations of DCF in a range of 0.105 to 0.463 µg L^{-1} in surface waters from Anil and Bacanga Rivers (Brazil).

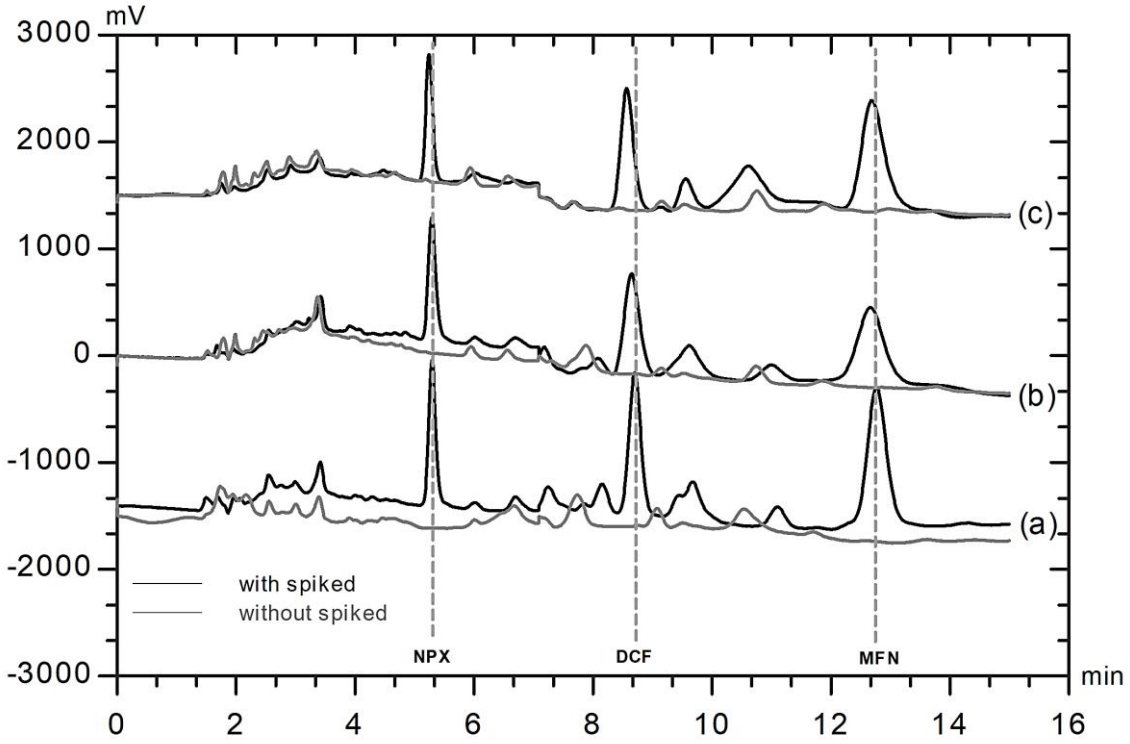

Fig. 3 Chromatograms of different water samples with and without of 5 µg L^{-1} of NSAIDs (a) seawater, (b) tap water and (c) surface water. Extraction conditions: 5 mL of sample with pH adjusted to 2, NaCl 2.5% (*w/v*) (except to seawater sample, which no salt was added), 150 µL of ACN and 30 µL of 1-dodecanol. (n=5).

It is possible to observe the increase in the peaks attributed to the NSAIDs in the fortified samples that were absent in the aqueous samples. The results obtained were considered satisfactory because there were no interferences at the retention time of the compounds under study.

The recovery results obtained for the optimized method applied to the different water samples are presented in Table 2.

Table 2. Recovery (%) results obtained for the three NSAIDs spiked into the aqueous samples with different characteristics.

Analyte	Recovery (%)[a] from Seawater			Recovery (%)[a] from Tap water			Recovery (%)[a] from Surface water		
	Spiking level (µg L^{-1})			Spiking level (µg L^{-1})			Spiking level (µg L^{-1})		
	1.2	3	5	1.2	3	5	1.2	3	5
NPX	110±6	118±1	119±1	76±2	85±3	95±5	107±3	113±1	118±3
DCF	111±5	113±2	119±4	107±5	102±5	104±5	95±6	92±5	107±5
MFN	114±13	118±5	120±3	80±6	97±2	107±2	84±9	116±3	120±1

[a] Mean value ± standard deviation (n=5), which corresponds to the number of extractions performed. Extraction conditions: 5 mL fortified water samples, with pH adjusted to 2, NaCl 2.5 % (*w/v*) (except to seawater sample, which no salt was added), 150 µL of ACN and 30 µL of 1-dodecanol.

The results obtained for the anti-inflammatory's recovery tests were satisfactory, ranging from 110 % and 120 % for seawater, 76 % and 107 % for tap water and 84 % and 120 % for surface water, showing that the proposed method can be used to extract NSAIDs from aqueous samples with different characteristics. For the EF, values between 142 and 196 were reached, indicating the good ability of SFODME to concentrate the evaluated compounds.

3.4 Comparison with other methods

Table 3 shows a comparison of the methodology developed in this work with others used in the determination of NSAIDs.

364 **Table 3.** Comparison of SFODME-HPLC-UV/Vis with other methods used for the
365 quantification of NSAIDs in water samples.

Method[a]	**Analyte**	**Recovery (%)**	**LOD (μg L^{-1})**	**Sample volume (mL)**	**Reference**
IL-DLLME-HPLC-DAD-FLD	DCF	89	95	5	[1]
MSPE-HPLC-UV	NPX, DCF	75, 83	0.06, 0.05	50	[2]
SPE-HPLC-DAD	NPX, DCF	90, 84	5.8, 23.3	1000	[41]
SPE-SUPRAS-HPLC-UV	DCF, MFN	*n.a*	0.4, 1.0	30	[42]
MIP-SPE-HPLC-DAD	NPX, DCF	*n.a*	300, 400	50	[43]
MIP-SPE-UHPLC-MS/MS	NPX, DCF	*n.a*	0.3, 0.7	50	[43]
US-IL-DLLME-UHPSFC-PDA	NPX, DCF	81, 100	0.31, 2.26	10	[44]
SFODME-HPLC-UV	NPX, DCF, MFN	116, 90, 109	0.09, 0.25, 0.15	5	**This work**

366 [a] SUPRAS: supramolecular solvent; MIP: molecular imprinted polymer extraction; IL: ionic liquids;
367 MSPE: magnetic solid-phase extraction; US-IL: ultrasound-assisted ionic liquid; UHPSFC-PDA: ultra-
368 high performance supercritical fluid chromatography coupled to photo-diode array detector; *n.a:* not
369 applied.

370 Comparing the hereby developed extraction method with other methods reported
371 in literature, for the three analytes under study, and presented in Table 3 it is possible to
372 identify several advantages. Comparing with IL-DLLME-HPLC-DAD-FLD [1], our
373 study obtained similar recoveries, but a LOD 380 times lower. Moreover, the reported
374 method was only used for DCF. Alinezhad et al. [2] used a larger volume of sample, a
375 factor that can influence the LOD values, but even so the detection limit obtained in this
376 study for NPX was similar. Comparing with SPE-HPLC-DAD [41], the method presented
377 in this study showed similar efficiency in terms of recovery results, however lower LOD
378 were obtained. Comparing the SPE-SUPRAS-HPLC-UV [42] and the results obtained by
379 Martinez-Sena, et al. [39] using MIP-SPE-HPLC-DAD, SFODME developed in this
380 work obtained lower detection limits, using a lower sample volume. When comparing
381 with results obtained by Martinez-Sena, et al. [43] but using a MS detector, lower

detection limits were obtained, even using a less expensive detection instrument. Comparing with US-IL-DLLME-UHPSFC-PDA [44], our study presented the following advantages: lower LOD and the use of lower sample volume.

Concluding, the method developed in this work showed a significant ability to detect low concentrations of NSAIDs in water, using low sample volume and a cheap, simple, efficient and fast technique. In general, SFODME proves to be quite advantageous concerning other techniques due to its simplicity, because it makes use of a small amount of organic solvents, has a low cost, is fast and still provides satisfactory results, being an excellent tool for the determination of anti-inflammatory drugs in aqueous samples with different characteristics.

4. CONCLUSIONS

A method using SFODME combined with HPLC and UV/Vis detector was developed and validated, allowing the determination of NPX, DCF and MFN anti-inflammatories, in aqueous samples. The use of 1-dodecanol as extracting solvent in the optimized methodology was chosen due to low cost and low toxicity compared to other organic solvents. Its capacity to melt at room temperature avoids subsequent separation step, reducing the analysis time. The high enrichment factors obtained allowed to determine NSAIDs in different matrix of natural waters, at trace levels. The LOD and LOQ reached compare with the best methods published in the scientific literature. Besides this, the method did not demonstrate a matrix effect that would compromise its effectiveness, being possible to apply it successfully in samples of seawater, surface water and tap water.

Declarations

Conflicts of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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