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Solid-phase microextraction and chiral HPLC analysis of ibuprofen in urine

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Abstract

A simple and rapid solid-phase microextraction method was developed for the enantioselective analysis of ibuprofen in urine. The sampling was made with a polydimethylsiloxane-divinylbenzene coated fiber immersed in the liquid sample. After desorptioning from the fiber, ibuprofen enantiomers were analyzed by HPLC using a Chiralpak AD-RH column and UV detection. The mobile phase was made of methanol–pH 3.0 phosphoric acid solution (75:25, v/v), at a flow rate of 0.45 mL/min. The mean recoveries of SPME were 19.8 and 19.1% for (−)-*R*-ibuprofen and (+)-(*S*)-ibuprofen, respectively. The method was linear at the range of 0.25–25 μg/mL. Within-day and between-day assay precision and accuracy were below 15% for both ibuprofen enantiomers at concentrations of 0.75, 7.5 and 20 μg/mL. The method was tested with urine quality control samples and human urine fractions after administration of 200 mg *rac*-ibuprofen.

Keywords: Solid-phase microextraction; Ibuprofen enantiomers; Enantiomeric separation; Urine

1. Introduction

(*R*,*S*)-ibuprofen [(±)-(*R*,*S*)-2-(4-isobutylphenyl) propionic acid], a non-steroidal anti-inflammatory drug (NSAID) widely used for the treatment of pain and inflammation in rheumatic disease and other musculoskeletal disorders, is marketed, with the exception of Austria and Switzerland [1], as a racemate [2–6]. However, its anti-inflammatory action is mainly associated with the (+)-(*S*)-enantiomer [7–9].

Ibuprofen undergoes stereoselective metabolism, resulting in stereoselective pharmacokinetics parameters, with higher plasma and urinary concentrations for the (+)-(*S*)-isomer [2,3]. In addition, the disposition of the enantiomers of ibuprofen is particularly complex because (−)-(*R*)-ibuprofen undergoes biotransformation with inversion of configuration at the chiral center to yield (+)-(*S*)-enantiomer of the drug [10]. As a consequence, enantioselective methods are required for the analysis of ibuprofen in biological samples to evaluate the contributions of these stereoselective processes [11,12].

Several HPLC methods have been developed for the analysis of ibuprofen enantiomers in biological human samples [13–21], almost of them based on the use of chiral stationary phases [13,14,17,18,20]. In addition, these methods were developed using common methods of extraction, mainly liquid–liquid extraction (LLE) [13,14,16,17]. This traditional extraction technique has some disadvantage, such as the use of toxic and expensive solvents and being tedious and time consuming. These drawbacks can be avoided by the use of solid-phase microextraction (SPME), introduced by Arthur and Pawliszyn in 1990 [22]. This technique enables simultaneous extraction and pre-concentration of analytes from gaseous, aqueous, and solid matrices. SPME is based on the equilibrium of the analytes between the sample matrix and an organic polymeric phase usually coated on a fused-silica fiber; the amount of the analyte absorbed/adsorbed by the fiber is proportional to the initial concentration. It is also

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possible to obtain good extraction and reliable analysis under non-equilibrium conditions [23], if the extraction conditions are held constant. Selection of the fiber coating is mainly based on polarity of the analyte. Non-polar analytes have relatively high affinity for apolar phases, whereas polar fibers are the first choice for the extraction of polar analytes.

Most SPME methods developed until now are used in combination with gas chromatography (GC) with the fiber placed in the hot injector of the equipment, where the analytes are thermally desorbed. SPME and high-performance liquid chromatography (HPLC) were first coupled in 1995 [24] but in numerous applications, such as the analysis of drugs in biological samples, they have not been fully explored.

For SPME-HPLC coupling, the extraction procedure is similar to that used for GC analysis. The main difference between SPME-GC and SPME-HPLC is the second step, the desorption procedure. In HPLC analysis, an organic solvent or the mobile phase is used to desorb the analytes from the fiber. The desorption can be performed in a desorption chamber (on-line SPME-HPLC), or in a separate vial filled with the desorption solvent (off-line SPME-HPLC) [25,26].

In this paper, we describe for the first time the development, validation and application of an SPME-HPLC method to the analysis of ibuprofen enantiomers in human urine. Three kinds of fiber coatings were compared: carbowax–templated resin (CW–TPR), polydimethylsiloxane–divinylbenzene (PDMS–DVB) and polyacrylate (PA). The extraction was carried out by direct immersion (SPME-DI) of the fiber into urine samples and off-line desorption was performed. The developed and validated method was applied to determine ibuprofen enantiomers in urine samples collected from a healthy volunteer after a single oral administration of 200 mg of *rac*-ibuprofen.

2. Experimental

2.1. Drugs and reagents

Rac-Ibuprofen (99.9%) was kindly supplied by Knoll Pharmaceuticals (Nottingham, England). Commercial *rac*-ibuprofen formulation (Advil, Whitehall) was obtained in a local drug store. (+)-(*S*)-ibuprofen (99%) was obtained from Sigma-Aldrich (St. Louis, MD, USA). Trifluoroacetic acid was supplied from Fluka (Buchs, Switzerland). Sodium chloride and sodium dihydrogen phosphate were obtained from Merck (Darmstadt, Germany). Sodium hydroxide was obtained from Nuclear (São Paulo, SP, Brazil) and hydrochloric acid from Chemco (Campinas, SP, Brazil), all of analytical grade. Methanol, hexane, ethanol, all of HPLC grade were purchased from EMD Omnsolv (Gibbstown, NJ, USA). The water used to prepare the solutions or mobile phase was purified in a Milli-Q-plus System (Millipore, Bedforte, MA, USA).

2.2. Instruments

The holder and the assembly of the SPME device for manual sampling were purchased from Supelco (Bellefonte, PA, USA). The microextraction fibers (Supelco) studied were coated with carbowax–templated resin (CW–TPR, 50 μm film thickness), polyacrylate (PA, 85 μm film thickness) and polydimethylsiloxane–divinylbenzene (PDMS–DVB, 60 μm film thickness).

The HPLC system consisted of an LC-AT VP solvent pump, an SPD-10A UV–vis detector, a Chromatopak CR6A integrator (all from Shimadzu, Kyoto, Japan) and a 7725 Rheodyne injector (Cotati, CA, USA) with a 50 μL loop.

2.3. Chromatographic condition

The chiral separation of ibuprofen enantiomers after SPME procedure was obtained by a slightly modified method previously described [13]. Separations were carried out at 23 ± 2 °C on a Chiralpak AD-RH column (150 mm × 4.6 mm i.d., 5 μm particle size, Chiral Technologies, Exton, PA, USA). A CN guard column (4 mm × 4 mm i.d., Merck, Darmstadt, Germany) was used to protect the analytical column. The mobile phase used for the analysis of ibuprofen consisted of methanol–pH 3.0 phosphoric acid solution (75:25, v/v), at a flow rate of 0.45 mL/min. The wavelength for detection was adjusted to 230 nm.

To evaluate the racemization of ibuprofen during sample preparation, we used urine samples spiked with pure ibuprofen enantiomers, previously obtained by semipreparative separation using a Chiralcel OJ column (250 mm × 4.6 mm, 10 μm particle size, Chiral Technologies, Exton, PA, USA) and a mixture of hexane-ethanol (98:2, v/v) and trifluoroacetic acid (0.1%) as mobile phase.

2.4. Standard solutions

Stock (2000 μg/mL) and working (10–1000 μg/mL) solutions were prepared in methanol, stored frozen at −20 °C and protected from direct light, keeping stable for at least 3 months.

2.5. Urine quality control

Urine quality controls (QC) were prepared by spiking drug-free urine samples with 0.75, 7.5, 20 μg/mL of both enantiomers and were used to measure the accuracy and the precision of the method. The drug-free urine samples were obtained from healthy volunteers, filtered through a 0.45 μm polyvinylidene fluoride filter (Millex-HV, Millipore) and stored frozen at −20 °C. Prior to use, the urines were allowed to thaw at room temperature and then mixed for 1 min.

2.6. Solid-phase microextraction procedure

To prepare the calibration curves, drug-free urine aliquots of 0.5 mL were transferred to 2 mL glass extraction vials, spiked with 25 μL ibuprofen working solutions and mixed for 1 min. Next, the hydrolysis of the acyl glucuronic acid conjugates was carried out by adding 100 μL 1 M NaOH [14]. The hydrolysis reaction was left to proceed for 1.5 h at room temperature, and the hydrolyzed urine samples were then neutralized with 200 μL 1 M HCl and supplemented with 1 mL 1 M phosphate buffer, pH 3.8, and 10% NaCl (w/v). The final pH was about 2.5–3.0. The pH was kept acid to prevent ibuprofen protonation since its pK_a is 3.8 and an alkaline pH could decrease the extraction efficiency due to the increase of the more hydrophilic form. The extraction was carried out at room temperature ($23 \pm 2\,^{\circ}C$) by direct immersion of the fiber for 30 min under magnetic stirring using a cylindrical-shaped stirring bar (10 mm × 4 mm). The desorption was carried out in 200 μL mobile phase during 5 min and 50 μL was chromatographed.

Urine quality controls or urine samples obtained from a medicated volunteer (item 2.8) were analyzed using the same procedure.

2.7. Method validation

Linearity was evaluated by analyzing human urine samples (0.5 mL, $n = 2$, for each concentration) spiked with 25 μL ibuprofen standard solutions in the range of 0.25–25 μg for each enantiomer/mL of urine. The results were plotted on a graph of peak area versus urine concentration and the best relationship was obtained by linear least-squares regression analysis. No internal standard was used.

The quantification limit was assayed by analyzing aliquots of human urine (0.5 mL, $n = 5$) spiked with 0.25 μg/mL of each enantiomer.

The absolute recoveries were determined by comparing the concentration of spiked urine samples at concentrations of 0.5, 1, 5, 10 and 25 μg/mL of each enantiomer ($n = 3$ for each concentration) calculated based on a calibration curve constructed by direct analysis of standard solutions of ibuprofen in the mobile phase. Recovery was expressed as percentage of the amount extracted.

Precision was expressed as relative standard deviation (R.S.D.%) and accuracy as percent of deviation between the true and the measured value. To assess within-day precision and accuracy, replicate analyses ($n = 10$) of 0.5 mL of urine spiked at concentrations of 0.75, 7.5 and 20 μg/mL of each enantiomer were performed. For between-day assays, triplicate urine samples of each concentration were analyzed for four consecutive days ($n = 4$).

The selectivity of the method was assured by analyzing 25 μL standard solutions of several drugs (Table 2) at the concentration of 1 mg/mL. Chiral drugs evaluated as interferents were analyzed as racemates to check the interference of both enantiomers. For drugs exhibiting retention times similar to ibuprofen enantiomers, we also evaluated the efficiency of the SPME procedure in avoiding this interference: aliquots of 0.5 mL blank urine were spiked with the drug at its maximum therapeutic concentration and the sample was submitted to the SPME process and chromatographic analysis. Drug-free urine samples were also analyzed to assess the capacity of sample pretreatment to eliminate endogenous interferents.

Freeze–thaw cycle stability and short-term room temperature stability were determined. To perform the freeze–thaw cycle stability test, three aliquots at the low (0.75 μg/mL) and high concentration (20 μg/mL) of the quality control samples were stored at $-20\,^{\circ}C$ for 24 h and thawed at room temperature. When completely thawed, the samples were refrozen for 12 h under the same conditions. The freeze–thaw cycle was repeated two more times, and then the samples were analyzed on the third cycle. For the determination of short-term room temperature stability, three aliquots of each quality control sample (at the same concentrations as described above) were prepared and kept at room temperature ($23 \pm 2\,^{\circ}C$) for 12 h. After this period, the samples were analyzed. The peak area obtained from both stability tests was compared with the peak area obtained with freshly prepared samples. Student's t test was applied, with the level of significance set at $p \leq 0.05$.

Inversion of the configuration of chiral compounds may occur during the extraction procedure. In order to verify the occurrence of racemization, ibuprofen enantiomers were separated under normal phase conditions as described in 2.3 and collected in the chromatographic system. After mobile phase evaporation, the residues were dissolved in methanol. Next, 0.5 mL urine samples ($n = 3$) were spiked with 50 μL of each enantiomer solution and submitted to the SPME process and subsequent chromatographic analysis.

2.8. Method application

To assess the applicability of the validated method, ibuprofen enantiomers were determined in urine samples collected from a healthy volunteer after a single oral administration of 200 mg of *rac*-ibuprofen. Sequential urine samples were collected both pre-dose and at 2-h intervals up to 12 h post-dosing. All urine samples were filtered through a 0.45 μm Millex-HV type filter (Millipore) and stored frozen ($-20\,^{\circ}C$) until required for analysis. The concentration of ibuprofen enantiomers in these samples was determined by using a calibration curve (0.25–25 μg/mL of each enantiomer) submitted to the extraction procedure and chromatographic analysis.

This investigation was approved by the Ethics Committee of The Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (process number 29-CEP/FCFRP).

3. Results and discussion

3.1. Optimization of the extraction procedure

These experiments were performed using 0.5 mL drug-free urine samples spiked with 1.25 μg/mL of each ibuprofen enantiomer. Before immersion of the fiber for extraction, urine samples were submitted to alcaline hydrolysis as detailed in 2.6. Because ibuprofen is a weak acid ($pK_a = 3.8$), the pH of the solutions must be adjusted to 2.5– 3, to keep it mainly in the undissociated form, that will be extracted by the fiber. The sample pH was adjusted by neutralizing the NaOH previously added during the hydrolysis step using 200 μL 1 M HCl solution and adding 1 mL 1 M phosphate buffer, pH 3.8. Since the extraction mixture pH was controlled by a buffer, as soon as the undissociated form of the drug is extracted by the fiber, the equilibrium is displaced toward the undissociated form, favoring the extraction by the fiber [27].

3.2. Fiber evaluation

A preliminary assay was performed in order to evaluate the available fibers, CW–TPR (50 μm, polar), PDMS–DVB (60 μm, semipolar) and PA (85 μm, polar). The relative extraction efficiencies (obtained by comparing the peak areas under the same experimental conditions) of the ibuprofen enantiomers are shown in Fig. 1. Moeder et al. [28] used SPME for the extraction of ibuprofen and other pharmaceuticals from water samples. Since ibuprofen has a high water solubility (octanol–water partition coefficient, 3.97), the most polar fiber (PA, 85 μm film thickness) showed the best extraction efficiency. The different behavior observed in the present, i.e., the PA fiber exhibiting a low extraction efficiency could be explained by the difference in the matrix analyzed (urine or water).

The efficiency of polar fibers in the extraction of ibuprofen was comproved by the use of the polar CW–TPR fiber. However, this fiber was not used because it is not suitable for use at low pH [26,29]. Therefore, PDMS–DVB was used for further investigation.

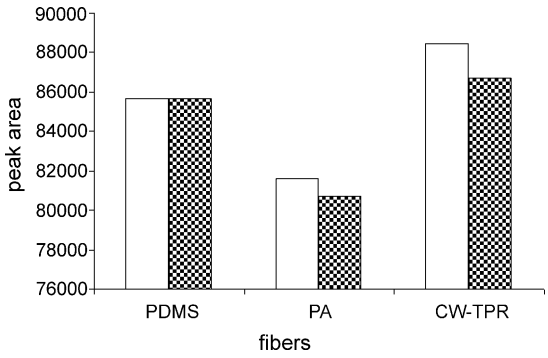

Fig. 1. Extraction efficiencies (measured by the peak area) for ibuprofen enantiomers with some fiber coatings. Extraction time, 30 min; desorption time, 5 min; extraction temperature, 25 °C; desorption solvent, mobile phase; (−)-(*R*)-ibuprofen (□); (+)-(*S*)-ibuprofen (⊠).

3.3. Extraction time

SPME is not an exhaustive process but an equilibrium process, in which analytes are partitioned between the sample matrix and the coating. The recovery of the analytes increases with extraction time until it reaches equilibrium. Fig. 2A shows the time profile of extraction for ibuprofen enantiomers, $n = 2$ for each extraction time. Equilibrium was not reached for both enantiomers for the period studied (60 min). An extraction period of 30 min was chosen for subsequent experiments since this time is a reasonable compromise between recovery and acceptable analysis time. Factors that influence the equilibration period were investigated by Arthur and Pawliszyn [22]. The equilibration rate is limited by the mass transfer rate of the analytes through a thin

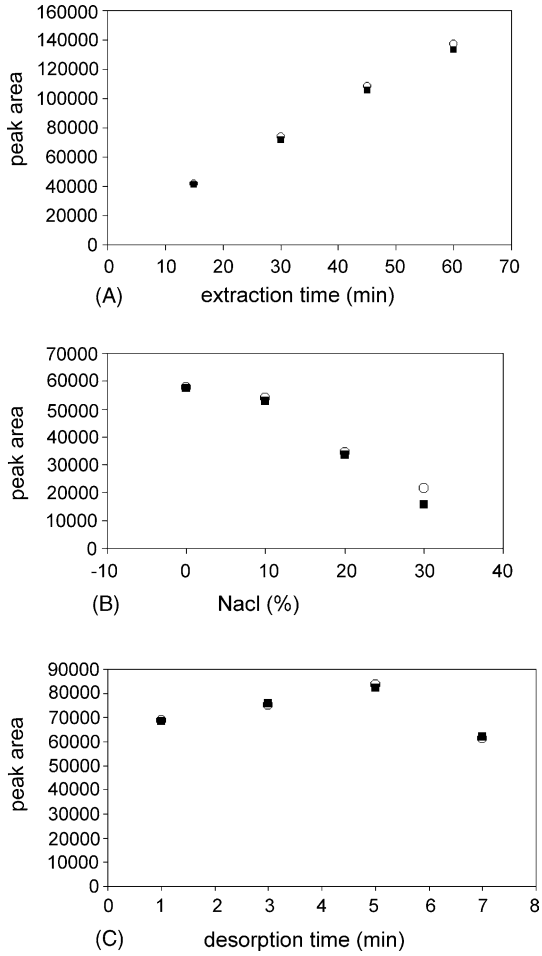

Fig. 2. Extraction (A and B) and desorption time (C) optimization. Fiber, PDMS–DVB; desorption solvent, mobile phase (methanol–pH 3.0 phosphoric acid solution, 75:25, v/v); (−)-(*R*)-ibuprofen (○); (+)-(*S*)-ibuprofen (■). (A) Extraction time (10% NaCl added; extraction temperature, 25 °C; desorption time, 5 min); (B) ionic strength (extraction time, 30 min; extraction temperature, 25 °C; desorption time, 5 min); (C) desorption time (extraction time, 30 min; extraction temperature, 25 °C; 10% NaCl added).

static aqueous layer at the fiber–solution interface. The period increases with increasing thickness of the fiber coating. It is not necessary to reach equilibrium, and shorter times can be used as long as the extraction is timed carefully and the mixing conditions remain constant [22]. The longer times required to achieve extraction equilibrium with PDMS–DVB coating has previously been reported for other types of analytes [30,31]. The presence of a porous polymeric material, such as DVB not only provided larger surface area, but also lengthened the distance the analyte to diffuse through [32].

3.4. Effect of ionic strength

The effect of ionic strength on the absorption of ibuprofen enantiomers into the fiber coating was studied by preparing spiked urine samples supplemented with NaCl in the range of 0–30% (w/v). It was found that the increase in ionic strength had a negative effect on the extraction of the enantiomers (Fig. 2B). The NaCl added in the sample could affect the equilibrium between the dissociated and undissociated form of ibuprofen, due to electrostatic interactions with the dissociated form [27]. The peaks area started to decrease when 20% of NaCl was added. The ionic strength was set at 10% of NaCl for the subsequent experiments in order to prevent salt variations that could happen in human urine samples [27].

3.5. Desorption period and carryover

The effects of the desorption period, i.e., the period when the fiber is exposed to the desorption solvent, was studied (Fig. 2C) When the desorption time was increased, the recoveries remained approximately constant, showing that the analytes desorbed rapidly from the fiber. However, when the carryover was studied at the same time, little or no carryover (1.2% or less for both enantiomers) was observed only after 5 min desorption time.

3.6. Optimized SPME conditions and method validation

Fig. 3A shows the chromatogram of a urine sample spiked with 1.25 μg/mL of each ibuprofen enantiomers and submitted to SPME extraction as described in 2.6. Analysis of pure (+)-(*S*)-ibuprofen demonstrated that, under the chromatographic conditions used, the first peak analyzed corresponds to the (−)-(*R*)-ibuprofen and the second to the (+)-(*S*)-ibuprofen.

Under the optimized conditions, SPME recoveries were 19.8 and 19.1 % for (−)-(*R*)- and (+)-(*S*)- ibuprofen, respectively with R.S.D.% values lower than 11.5 for both enantiomers. Although these recoveries are considered low for the traditional extraction methods, they are considered high for SPME, due to the microscale characteristic of the technique [27]. In addition, the analysis of urine samples spiked with pure ibuprofen enantiomers did not demonstrate any racemization during the extraction procedure (Fig. 3B and C).

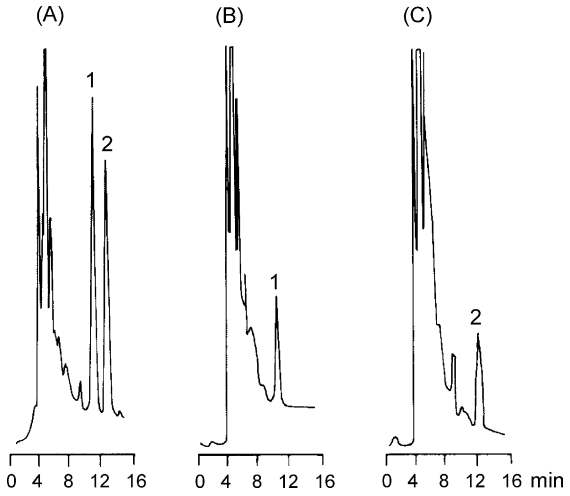

Fig. 3. Chromatograms for the analysis of ibuprofen enantiomers in urine after SPME extraction. (A) *rac*-Ibuprofen; (B) (−)-(*R*)-ibuprofen; (C) (+)-(*S*)-ibuprofen. Fiber, PDMS–DVB; experimental conditions: extraction time, 30 min; extraction temperature, 25 °C; NaCl 10%; desorption time, 5 min; desorption solvent (methanol–pH 3.0 phosphoric acid solution, 75:25, v/v).

The method proved to be linear over the concentration range of 0.25–25 μg/mL, with typical calibration curve equations determined as $Y = 630.34x + 56.33$ and $Y = 758.08x + 96.69$ for the (+)-(*S*)- and (−)-(*R*)- enantiomers of ibuprofen, respectively, and a determination coefficient (r^2) ≥ 0.99.

The precision and accuracy of the method were assessed for both within-day (10 spiked urine samples for each concentration on the same day) and between-day (three spiked urine samples for each concentration on four consecutive days) determinations. Table 1 shows the results achieved with three concentrations in the evaluation of the precision and accuracy of the method; neither R.S.D.% nor relative errors exceeded a value of 15%, in agreement with literature recommendations [33,34]. The lowest concentration quantified by the validated method (LOQs) was 0.25 μg/mL (Table 1).

The method developed here proved to be selective since the retention times for drugs analyzed under the established chromatographic conditions were not similar to those obtained for ibuprofen enantiomers (Table 2). In addition, the enantiomers of the two major ibuprofen metabolites found in urine, 2-hydroxyibuprofen and carboxyibuprofen, were also adequately resolved from ibuprofen enantiomers.

The stability test showed no statistically significant difference between freeze–thaw cycles and short-term room temperature stability studies. However, the low concentration samples in the short-term room temperature study were partially degraded, as observed by a statistically significant reduction in the areas of ibuprofen peaks in these samples. These samples became stable only after the addition of 1 mL of 1 M phosphate buffer, pH 3.8 (Table 3). The degradation

Table 1
Precision, accuracy and quantification limit for the analysis of ibuprofen enantiomers in urine

Nominal concentration (μg/mL)	Analyzed concentration (μg/mL)		Accuracy[a]		Precision[b]	
	(−)-(*R*)-	(+)-(*S*)-	(−)-(*R*)-	(+)-(*S*)-	(−)-(*R*)-	(+)-(*S*)-
Within-day ($n = 10$)[c]						
0.25[e]	0.26	0.24	5.4	−2.8	9.5	12.3
0.75	0.70	0.70	−5.8	−6.3	10.6	11.2
7.5	7.0	7.2	−6.6	−3.5	6.6	6.6
20	20.7	21.2	3.9	6.1	8.2	8.5
Between-day ($n = 4$)[d]						
0.75	0.75	0.74	1.1	−1.3	11.2	12.1
7.5	7.1	7.3	−4.3	−1.4	10.7	10.2
20	19.1	20.1	−4.4	0.6	12.6	12.7

[a] Expressed as deviation from theoretical values.
[b] Expressed as relative standard deviation.
[c] Number of samples.
[d] Number of days.
[e] Quantification limit, $n = 5$.

Table 2
Evaluation of the interference of some drugs with the analysis of ibuprofen enantiomers

Drug	t_R	Drug	t_R
(−)-(*R*)-ibuprofen	11.8	Fenproporex	ND
(+)-(*S*)-ibuprofen	13.6	Fluoxetine	5.8
2-Hidroxyibuprofen	7.3	Haloperidol	7.2
Carboxyibuprofen	6.4/7.1/8.9/14.5	Imipramine	6.9
Atenolol	6.0	Phenylbutazone	ND
Amitriptyline	7.0	Phenacetine	8.7
Bromazepam	11.1	Phenylephrine	5.4
Carbamazepine	9.0	Salbutamol	5.3
Chlordiazepoxide	10.1	Salicylic acid	8.7
Cimetidine	5.6	Thioridazine	8.3
Diazepam	22.9	Trimethoprim	5.9
Dipyrone	ND	Valproic acid	ND
Ethosuximide	ND		

t_R, retention time in minutes; ND, not detected by the chromatographic method up to 30 min of analysis.

of ibuprofen observed at low concentration may be caused by the pH variation in urine samples that occurs at room temperature [35] and by oxidative process that can occur in ibuprofen molecules [36].

It is important to mention that all validation procedure was carried out using a single fiber, without any damage or need of cleaning, showing its durability and reproducibility.

Table 3
Stability test for the analysis of ibuprofen enantiomers in urine

Nominal concentration (μg/mL)	*p*-Value	
	(−)-(*R*)-	(+)-(*S*)-
Freeze–thaw cycles		
0.75	0.5984	0.7341
20	0.1250	0.1235
Short-term room temperature		
0.75	0.0018	0.0029
20	0.6504	0.8265
0.75[a]	0.7934	0.7556

[a] Samples with 1 mL of 1 M phosphate buffer (pH 3.8).

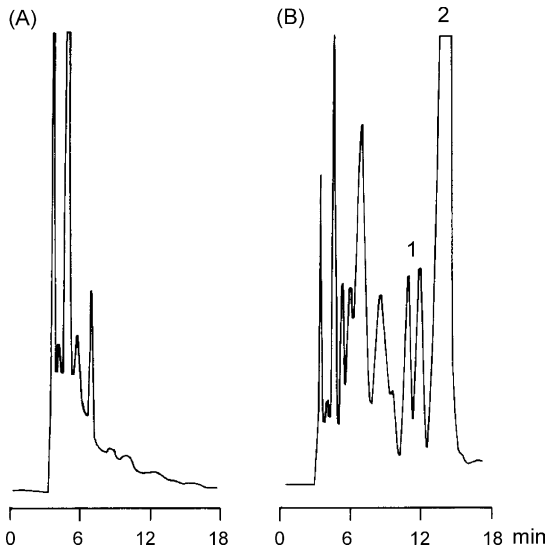

Fig. 4. SPME-HPLC chromatograms obtained from (A) a blank urine sample and (B) urine collected 4 h after oral administration of the racemic drug; (−)-(*R*)-ibuprofen (1) and (+)-(*S*)-ibuprofen (2). Experimental conditions as in Fig. 3. Non-identified peaks correspond to ibuprofen metabolites.

3.7. Analysis of ibuprofen enantiomers in human urine fractions after administration of rac-ibuprofen

The results of the analysis of samples collected from one volunteer after oral administration of *rac*-ibuprofen (Fig. 4)

Table 4
Ibuprofen concentrations in volunteer urine samples after oral administration of 200 mg *rac*-ibuprofen

Collection time intervals (h)	Urine concentrations (μg/mL)	
	(−)-(*R*)-ibuprofen	(+)-(*S*)-ibuprofen
0–2	1.56	11.18
2–4	2.76	23.98
4–6	1.74	22.12
6–8	1.02	13.06
8–10	0.3	4.87
10–12	0.26	2.24

are present in Table 4. The profile obtained in this study is in accordance with the studies conducted by Tan et al. [12] showing the chiral metabolic inversion that occurs in ibuprofen metabolism.

4. Conclusion

This paper describes for the first time the use of SPME-DI for the determination of ibuprofen enantiomers in human urine sample. The method is simple, highly sensitive and solvent-free. A single fiber was able to perform more than 100 extractions, showing that the desorption mode employed is reproductible. The validated method allows the determination of ibuprofen in the 0.25–25 μg/mL range with a quantification limit of 0.25 μg/mL for both enantiomers.

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