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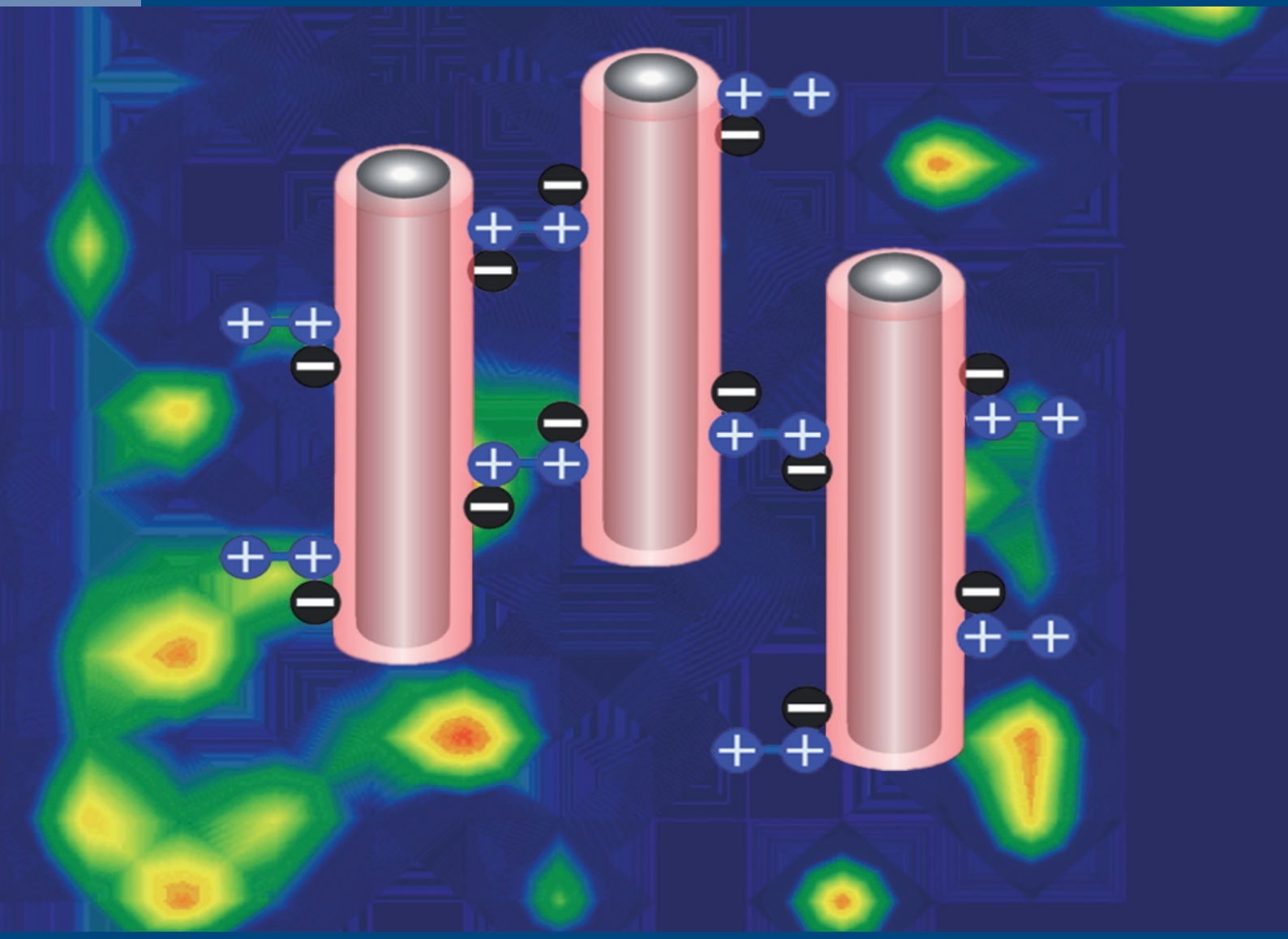

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RESEARCH ARTICLE

Submicellar liquid chromatography with fluorescence detection improves the analysis of naproxen in plasma and brain tissue

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Rapid, simple, and sensitive submicellar liquid chromatography with fluorescence detection was developed and validated to quantify naproxen in plasma and brain samples after oral administration of Naproxen formulations. The method used tramadol as an internal standard. Different submicellar mobile phases with organic phases ranging from 40 to 60% were studied to improve the native fluorescence of the Naproxen and decrease retention times. Separation was done in a Zorbax SB C8 column (250 × 4.6 mm, 5 μm) with a mobile phase containing acidic 0.007 M sodium dodecyl sulfate/acetonitrile (50:50, v/v) at a flow rate of 1 mL/min. Detection was performed with an excitation wavelength of 280 nm and emission of 310 nm and 360 nm for internal standard and Naproxen, respectively. The method was validated by International Conference of Harmonization standards. The method is specific, accurate, and precise (relative standard deviation <3%). Limits of detection and quantification were 0.08 and 0.25 μg/mL, respectively, for biological samples. This method was applied to analyze brain/plasma ratios in mice that had received oral administrations of Naproxen micellar formulations containing 10% w/w of sodium dodecyl sulfate, Cremophor RH 40, or Tween 80. The sodium dodecyl sulfate micelles were faster and more widely distributed in the mouse brains.

KEYWORDS
Cremophor RH 40, Naproxen, sodium dodecyl sulfate, submicellar mobile phases, Tween 80

1 | INTRODUCTION

Naproxen (NAP; Figure 1) is an anti-inflammatory drug with poor solubility in an aqueous medium. The incorporation of surfactants into NAP formulations has succeeded in increasing plasma concentrations [1,2]. According to Biopharmaceutics Classification System (BCS), NAP is considered to be a type II active molecule (poor solubility and good permeability). Therefore, the bioavailability of NAP is limited by its solubility. It is important to consider the low permeability of the brain to NAP. It is especially interesting to get high levels of NAP in the blood as soon as possible so that it can have an anti-migraine effect.

The native fluorescence of many raw materials is enhanced by the use of micelles of cationic, nonionic, and ionic surfactants [3].

These methods depend on surface active agents such as Tween 80 [4], Cremophor RH 40 [5,6], and SDS [3,7]. It is well known that micellar mobile phases in the absence of

Article Related Abbreviation: NAP, Naproxen; NAP:RH40, Naproxen:Cremophor RH 40 (90:10 w/w); NAP:SDS, Naproxen: Sodium dodecyl sulfate (90:10 w/w); NAP:T 80, Naproxen:Tween 80 (90:10 w/w); NAP-RM, Naproxen raw material; T 80, Tween 80.

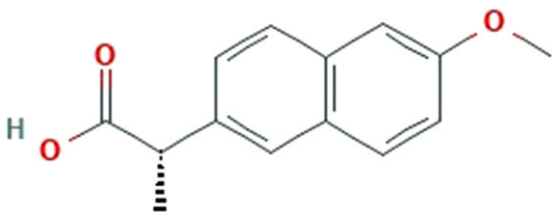

FIGURE 1 Chemical structure of NAP

organic solvent are weak and yield poor peak shape compared to conventional aqueous-organic mobile phases. The addition of organic solvent to the mobile phase avoids this problem. Organic solvent reduces the amount of surfactant absorbed in the stationary phase, with an effect that depends on the surfactant/organic solvent ratio [8,9]. However, an increase in the organic solvent concentration leads to a progressive reduction in the aggregation number and to the emergence of the submicellar LC mode [10,11]. In submicellar LC, only surfactant monomers exist in the mobile phase, which are dissolved in the hydro-organic medium, producing the surfactant coating in the stationary phase [10]. It has been found that in the presence of ≥60% acetonitrile, the partial surfactant adsorption does not occur [10,12]. Submicellar LC with acetonitrile proportions between 40 and 60% could have attractive advantages over other RP-LC modes in terms of efficiency, peak shape, selectivity, and analysis time [11]. It has been shown that the native fluorescence improved in the presence of micelles of surfactants. These micellar or submicellar systems are known to improve the intensity of compounds with weak fluorescence such as NAP.

The aim of this study was to develop a submicellar LC fluorescence method for the quantification of NAP brain samples validated by ICH guidelines, and to apply this analytical method to select a micellar system to obtain higher concentrations of cerebral NAP.

Micellar systems with nonionic surfactants such as Tween 80 (T 80) [4] or Cremophor RH 40 (RH 40) [5,6,13], and anionic surfactants such as SDS [1,5,22,23], improve the solubility of poorly soluble drugs.

Several methods have been reported to determine NAP in plasma samples [1,13–16] and brain tissue [2]. The native fluorescence of NAP can be used to quantify a low range of concentrations [17], which may be suitable for determining NAP in the brain. Submicellar LC with fluorescence detection could be a suitable method for the routine analysis of a large number of NAP samples. The addition of surfactants to the mobile phase could also increase the fluorescence intensity of the NAP [3]. The high selectivity and sensitivity of this submicellar LC fluorescence method allows the analysis of plasma and brain tissue without the previous extraction and concentration processes.

2 | MATERIALS AND METHODS

2.1 | Chemicals

NAP (98%), SDS (99%), Tween® 80 (T 80), Kolliphor® RH 40 (RH 40), were purchased from Sigma® (Munich, Germany). Acetic acid glacial for HPLC (purity >99.8%) was purchased from Panreac® (Barcelona, Spain). HPLC-grade acetonitrile was obtained from Scharlau® (Barcelona, Spain), Milli-Q water purification system (Millipore®, Madrid, Spain) was used to obtain ultra-pure water.

2.2 | Calibration standards

Stock solutions of NAP were prepared by mixing an appropriate amount of NAP with methanol to a final concentration of 0.2 mg/mL. An eight-point nonzero calibration standard, ranging from 2.7 to 16.3 μL/mL for plasma samples and 0.21 to 2.38 μg/g for brain samples, was prepared by spiking the drug-free mouse plasma and brain samples with an appropriate amount of NAP.

The calibration standards were prepared by adding 100.0 μL of mixed standard working solutions to 450 μL of acetonitrile and 100.0 μL of blank mouse plasma and brain samples. The QC samples were prepared by adding 100.0 μL of mixed standard working solutions to 100.0 μL of blank biological samples at three different concentrations (low, medium, and high quality control for plasma and brain samples). The QC samples were mixed with 450 μL of acetonitrile to verify the integrity of the method.

2.3 | Solubility studies in submicellar mobile phases

Classical approaches for measuring solubility were based on the saturation shake-flask method. Excessive amounts of NAP were placed in 5 mL vials containing different submicellar mobile phases. The vials were sealed and stored at a constant temperature (25°C), and shaken for 24 h until equilibrium was evident. After centrifugation of the incubated suspensions at 15 000 rpm for 15 min, the concentrations of NAP in the supernatant solutions were determined after dilution with acetonitrile and determined by an HPLC procedure. Comparative statistical studies of NAP concentrations were performed by paired Student's *t*-test. *p*-Values < 0.05 were considered significant.

2.4 | Chromatographic conditions

An Agilent® 1100 liquid chromatographic system (Madrid, Spain) equipped with an Agilent® G1321A multi λ fluorescence detector was used for the HPLC analysis of brain samples. The fluorescence detector excitation wavelength was set at 280 nm and the emission wavelengths were set at 310 nm

(IS) and 360 nm (NAP), respectively. A 250 × 4.6 mm, 5 μm Zorbax® SB-C8 column (Agilent Technologies, Madrid, Spain) was used for the separation processes, after which a 12.5 × 4.6 mm guard column was filled with the same packing material (Agilent Technologies, Madrid, Spain). The mobile phases of the hydro-organic mode consisted of three solutions of 2% acetic acid/acetonitrile (40:60, 50:50, and 60:40, v/v). The submicellar mode comprised nine submicellar mobile phases with 0.007 M SDS, 0.0008 M RH 40, and 0.0015 M T 80, which were obtained by weighing 0.2% (w/v) of each surfactant and dissolving in aqueous solution (2% acid acetic) with 40–60% acetonitrile. The analysis started with a flow rate of 1.0 mL/min for 10 min. Once the analysis was complete, the chromatographic system was rinsed using a linear gradient of 2% acetic acid/ACN.

2.5 | Method validation

2.5.1 | Specificity

The specificity of a bioanalytical method is generally defined as the lack of interfering peaks at the retention times of the assayed drug in the chromatograms. The specificity of the assay was investigated by processing and analysing blanks prepared from six independent batches of plasma and brain sample controls, according to ICH Q2 (R1) guidelines [18].

2.5.2 | Linearity, detection limit, and quantification limit

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample. The linearity of the bioanalytical assay for the test compounds was evaluated by an eight-point calibration curve with plasma or brain prepared and assayed on 3 consecutive days. Linear least-squares regression analysis with a weighting factor of $1/x^2$ was performed to assess linearity and generate the standard calibration equations. The lower LOD and the lower LOQ were determined by applying ICH Q2 (R1), which can be quantitatively determined with a precision of 15% [19].

2.5.3 | Accuracy and precision

Intra-batch accuracy and precision were determined by analysing six replicates of QC samples at three concentrations (9.23, 12.30, and 15.37 μL/mL for plasma samples, and 0.54, 0.72, and 0.90 μg/g for brain samples) on 3 consecutive days. Accuracy and precision were expressed by relative error and RSD, respectively [18].

2.5.4 | Recovery

Recovery was calculated by comparing the concentration obtained from an extracted sample (recovered %) with the concentration obtained after the direct injection of a solution of the same drug concentration diluted in the mobile phase (100%). The percentage of recovery was determined by comparing the concentrations of three QC samples ($n = 6$, 9.23, 12.30, and 15.37 μL/mL for plasma samples and 0.54, 0.72, and 0.90 μg/g for brain samples) with unextracted reference standards containing the same amount of analyte.

2.6 | Surfactant formulations for "in vivo" studies

The surfactant formulations contain NAP/surfactant proportions 90:10 w/w (NAP:SDS, NAP:RH40, and NAP:T80). The surfactant solutions were made by mixing the corresponding amount of surfactants to an aqueous solution by stirring for 2 min. Wet granulation was achieved by mixing 750 mg of NAP with 75 mg of disintegrant, then, 600 μL of the surfactant solution was added. After mixing, the mixture was dried for 4 h at 60°C and passed through a 0.840 mm sieve. The granules were dried in an oven at 60°C for 48 h. The final product was sieved to isolate the 0.297–0.840 mm fraction [20].

2.7 | Plasma and brain preparation

Twenty-four adult male BALB/c mice (18–24 g body weight, 8 weeks of age) were purchased from Harlan, Barcelona, Spain. This animal experiment was approved by the Animal Ethics Committee at the Alcala University, Madrid, Spain (ES28005000 1165). An NAP raw material was used as a reference formulation (NAP-RM). Micellar systems with three different surfactants were studied: NAP:T 80, NAP:RH 40, and NAP:SDS. The amount of each surfactant was 10% w/w referred to NAP. All the formulations were administered orally via bucogastric tube. Each freshly prepared reconstituted formulation was administered to six mice at a dose of 5 mg/kg dissolved in 0.5% w/v of sodium carboxymethyl cellulose. Six mice per formulation were euthanized by 15% urethane solution at 1 h. Whole blood (approximately 1.5 mL) was collected by cardiac puncture into heparinized vials and centrifuged at 9000 rpm for 15 min to obtain plasma. For the brain samples, the brains were excised after the euthanasia of the mice, washed with 0.5 mL of saline, and homogenized for 1 min. The plasma and tissue homogenates were stored at −20 ± 5°C until extraction [20]. For quantification in plasma aliquots, 200 μL of plasma were thawed. Then, 50 μL of SDS solution (0.75% w/v) was added with Tramadol as IS and mixed with 450 μL of acetonitrile in a vortex mixer for 2 min, then centrifuged at 5000× *g* for 5 min. The supernatant phase was filtered through a 45 μm Millipore® HVLP filter (Millipore Iberica, Madrid, Spain) and the NAP plasma samples were determined by the HPLC-fluorescence method. To quantify the brain samples, 200 μL of the homogenate tissue were prepared using the same procedure. The second and third extractions of the tissue were done in a similar way to the first

extraction. The sample from each extraction was concentrated at 50°C for 2 h and resuspended in 200 µL of mobile phase. The NAP was determined by the HPLC-fluorescence method. The concentrations in the third extracts of NAP were always lower than the relevant LOQ. Comparative statistical studies of different formulations were done using paired Student's *t*-test. A p-value < 0.05 was considered significant.

3 | RESULTS AND DISCUSSION

3.1 | Surfactant adsorption to the stationary phase

Surfactants are added to the hydro-organic mobile phases to improve NAP native fluorescence with a reduced retention time. Submicellar mobile phases have been studied with ACN ratios of 40–60%. [12]. Figure 2 shows the different fluorescence scans of NAP in the hydro-organic mobile phase (2% acetic acid/ACN [50:50] v/v), and NAP in the submicellar mobile phases with nonionic surfactants: T 80 or RH 40 and anionic surfactant (SDS). The hydro-organic mobile phase 2% acetic acid/ACN (50:50) v/v (Scan 1) presents a maximum peak at 360 nm corresponding to the native fluorescence of NAP. The submicellar mobile phase 0.0015 M T 80/ACN 50:50 v/v (Scan 2) shows a slight change in the NAP peak (335 nm) with an improvement in intensity values (4.3-fold). The nonionic surfactant T 80 probably produces submicellar structures with a slight increase in the fluorescence intensity of NAP [4]. The use of RH 40 in the mobile phase (0.0008 M RH 40/ACN (50:50) v/v (Scan 3) has a maximum peak at 325 nm, with a sharp increase in the fluorescence intensity of NAP (9.9-fold). Similar fluorescence increases with RH 40 compared to T 80 have been previously described with different drugs [6]. The mobile phase with 0.007 M SDS/ACN (50:50) v/v (Scan 4), produced a 10.8-fold increase in the maximum emission fluorescence (335 nm) compared to the same mobile phase without SDS.

A similar increase in the fluorescence signal by adding SDS to the mobile phase has been previously observed with other raw materials with native fluorescence [7].

Table 1 shows the solubility of the NAP in the hydro-organic and submicellar mobile phases (T 80/ACN, RH 40/ACN and SDS/ACN) containing different proportions of 2% acetic acid/ACN (40:60, 50:50, and 60:40). The mobile phases with high proportions of ACN (40:60) showed high solubility values, and no differences ($p > 0.05$) were observed between the hydro-organic mobile phase and the different submicellar systems. These results indicate that submicellar systems are interrupted at a concentration of 60% ACN v/v in the absence of submicellar systems in the mobile phases; increased NAP solubility is related to the high proportion of ACN in the hydro-organic phase. However, all intermediate

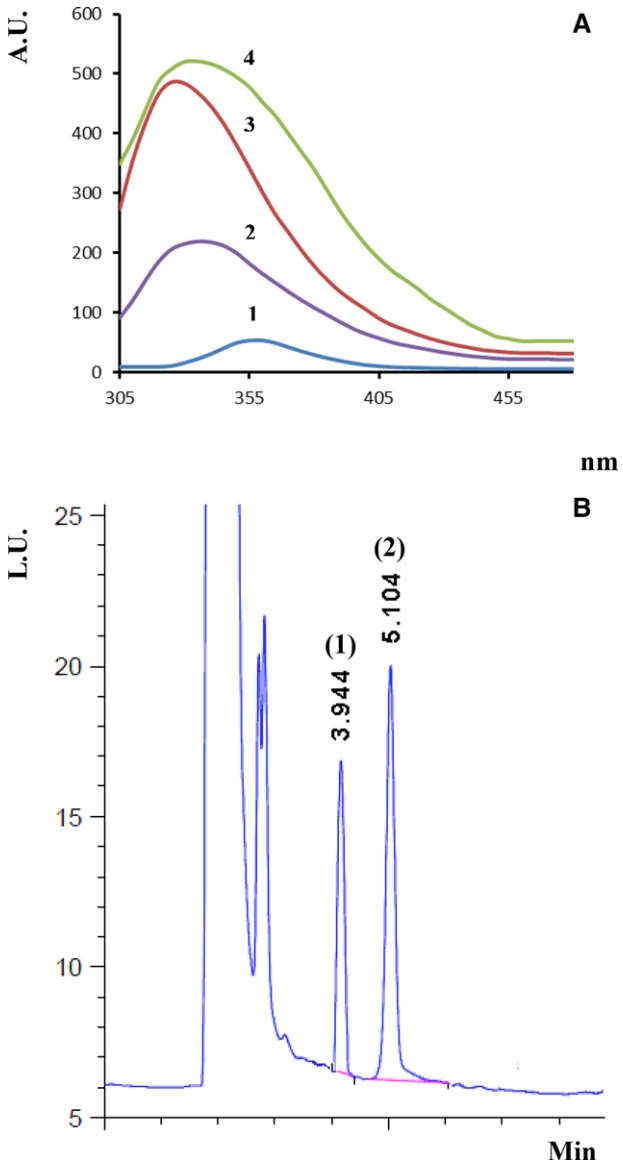

FIGURE 2 Fluorescence spectra and representative chromatogram of NAP samples. (A) Fluorescence spectra of NAP in different mobile phases: (1) hydro-organic phase (acetic acid/ACN 50:50 v/v), (2) submicellar mobile phase consisting of 0.0.0015 M T 80/ACN (50:50) v/v, (3) submicellar mobile phase with 0.0008 M RH 40/ACN (50:50) v/v and (4) submicellar mobile phase with 0.007 M SDS/ACN (50:50) v/v. (B) Brain sample chromatogram of 1 h after oral administration of 7.5 mg/kg of NAP in a submicellar mobile phase (0.007 M SDS/ACN 50:50 v/v). Compounds: (1) IS, (2) NAP

submicellar mobile phases (surfactant/ACN 50:50) showed an increased solubility of NAP (see Table 1) compared to the hydro-organic raw material ($p < 0.05$). These solubility results confirm the presence of submicellar structures in these hydro-organic mobile phases (50:50). Finally, the lower proportions of ACN in the mobile phase (60:40) allowed the formation of stable submicellar structures. Anionic surfactants in the submicellar mobile phase (0.007 M SDS/ACN 60:40) increased NAP solubility (119.11 ± 0.8.67 µg/mL)

TABLE 1 Solubility of NAP (μg/mL) in three organic mobile phases with ranges 40–60% ACN and nine different submicellar mobile phases obtained by adding of surfactant: 0.007 M SDS, 0.0008 M RH 40 or 0.0015 M T 80 to the different hydro-organic mobile phases

Mobile phases	2%acetic acid/ACN 60 : 40	2% acetic acid/ACN 50:50	2% acetic acid/ACN 40 : 60
Without surfactant	80.77 ± 4.65	299.75 ± 6.08	475.74 ± 5.19
SDS	121.71 ± 4.47* ($p = 0.0003$)	345.61 ± 8.22* ($p = 0.0021$)	473.29 ± 7.94
RH 40	101.61 ± 2.95* ($p = 0.0060$)	344.40 ± 9.76* ($p = 0.0029$)	466.27 ± 7.00
T 80	89.33 ± 2.95	324.29 ± 9.68* (p = 0.0082)	450.85 ± 9.49

*Compared to mobile phase without surfactant ($p < 0.05$).

compared to nonionic surfactants such as 0.0015 M T 80/ACN 60:40 (85.24 ± 10.01 μg/mL) or the hydro-organic mobile phase (80.77 ± 4.65 μg/mL, $p < 0.05$). Probably, the low molar ratio (0.0015 M T 80) in this mobile phase probably produced a decrease in the submicellar structure, which reduced the solubility of NAP [4]. The increased solubility of NAP in mobile phases with surfactant solution/ACN ratios (50:50 and 60:40) was related to the formation of submicellar mobile phases [12]. Under these conditions, organic phases with 0.007 M SDS/ACN 50:50 v/v showed a submicellar structure with SDS similar to other studies [11].

Table 2 shows the retention times, theoretical plates, and width and peak symmetry in the submicellar mobile phases (2% acetic acid/ACN 50:50 v/v) with anionic (SDS) and nonionic surfactants (T 80 or RH 40). A decrease was observed in retention times in all submicellar mobile phases (SDS/ACN, RH 40/ACN, and T 80/ACN). SDS submicellar mobile phases showed the shortest retention times (5.10 min), while RH 40 and T 80 had intermediate retention times (6.86 and 6.98 min) compared to the mobile phase without surfactant (7.28 min). Differences were observed between the theoretical plate values and width peaks in the different submicellar mobile phases. Nonionic submicellar mobile phases (0.0008 M RH 40/ACN and 0.0015 M T 80/ACN) showed greater retention peaks (6.86 and 6.98, respectively) and large peak widths (0.197 and 0.187, respectively). These nonionic surfactants RH 40 and T 80 produced fewer interactions between the submicellar monomers with ionized silanols on the conventional silica-based stationary phase, leading to less efficiency. This could be due to the fact that a significant portion of the surfactant layer in the stationary phase reduces the masking effect on the silanol groups [12]. However, SDS monomers reveal a greater desorption of the surfactant in the mobile phase (0.007 M SDS/ACN). Thus the SDS mobile phase had a lower retention peak (5.10 min) and peak width (0137 min), but increased height (46.64 U.A.) and efficiency (values of 7693 N) compared to the other submicellar mobile phases. These results indicate that this mobile phase improves the penetration of the solutes and has a minor interaction with the silanol chains, which may be due to a greater association between the ionic monomer surfactant and the alkyl bonded to the silica stationary phase [11]. The surfactant monolayer was thus partially coated in the stationary phase, resulting in short retention times [12,21–23]. In these conditions, the submicellar mobile phase of SDS (0.007 M SDS/ACN 50:50 v/v) was selected to analyze low concentrations of NAP in tissues such as the brain.

3.2 | Specificity

The method used fluorescence detection due to its high sensitivity and specificity in plasma and brain tissue. From the fluorescence scans of IS (tramadol) and NAP, emission wavelengths of 310 nm (IS) and 360 nm (NAP) were selected for the same excitation wavelength of 280 nm.

Figure 2 shows the typical chromatograms of IS (3.94 min) and NAP (5.10 min) for brain samples using the submicellar mobile phase (0.007 M SDS/ACN (50:50) v/v). The selectivity of our method was evaluated by comparing the plasma and brain chromatograms in the control group with those prepared by spiking the standard solutions into the corresponding blank samples [18]. This submicellar mobile phase with fluorescence detection was selective for both plasma and brain methods. No interfering peaks were observed from endogenous

TABLE 2 Retention Time (RT), Height, Peak Symmetry (Symm) Width, Theoretical Plates (Plates) by USP methods, in hydro-organic mobile phase (50:50) and three different submicellar mobile phases: 0.007 M SDS/ACN(50:50), 0.0008 M RH 40/ACN (50:50) and 0.0.0015 M T 80/ACN (50:50)

	Acetic acid/ACN	SDS/ACN (50:50)	T 80/ACN(50:50)	RH 40/ACN(50:50)
TR (min)	7.283	5.101	6.980	6.859
Height (LU)	42.52	46.64	32.96	13.16
Symm	0.92	0.90	0.95	0.92
Width (min)	0.151	0.1368	0.1872	0.1421
Plates	12866	7693	7695	6735

plasma or brain components at or near the NAP retention time, indicating the high selectivity of this method (data not shown). This fluorescence detection technique by HPLC with the submicellar mobile phase increases the native fluorescence of NAP [16,17] and was selective in plasma and brain samples.

3.3 | Linearity, detection limit, and quantification limit

The parameters of the linear calibration curves were derived from the statistical analysis of three independently prepared eight-point calibration curves in plasma and brain samples. The mean regression equation was $y = 151.9x$ (μg/g) + 12.50 for plasma and brain samples ($r^2 = 0.9975$).

The assays were linear between 0.25 and 2.5 μg/g. Although there are different methods for determining NAP in plasma samples [14], only a few studies have determined NAP in the brain [2,24]. The LOD of 0.08 μg/g and the LOQ of 0.25 μg/g were adequate to determine NAP concentrations in brain samples and their RSDs were always >15% [19].

3.4 | Precision and accuracy

To determine precision and accuracy, three replicate analyses were performed at each of the three concentrations. The methods of quantifying NAP with quality control samples in plasma and brain tissue are summarized in Table 3. The mean intra- and interday precision values for all plasma samples were within 0.74–2.96 and 0.24–3.05 RSD (%), respectively, (these values were similar to those obtained by other authors in plasma samples [1,25]). In brain samples, the mean intra- and interday precision values were between 0.17–2.26 and 0.80–2.75 RSD (%), respectively. These results agree with previously published data in the brain [2,25]. Both methods showed similar accuracy, with relative error % ranging from 0.40 to 2.86% in plasma and 0.80 to 2.75% in brain samples (see Table 3).

3.5 | Recovery

These values ranged from 93.33 to 96.64 and 95.06 to 96.25% for plasma and brain samples, respectively (Table 3). The RSD% obtained in brain samples was between 0.31 and 4.05% in all experiments. The recovery results in brain samples were similar to other studies [1,8]. These data suggest that this method has excellent accuracy and reproducibility and can be considered adequate to quantify NAP in plasma samples and brain tissues.

3.6 | Analysis of plasma and brain samples in mice

The submicellar LC fluorescence method validated in this study was successfully applied to determine plasma and brain

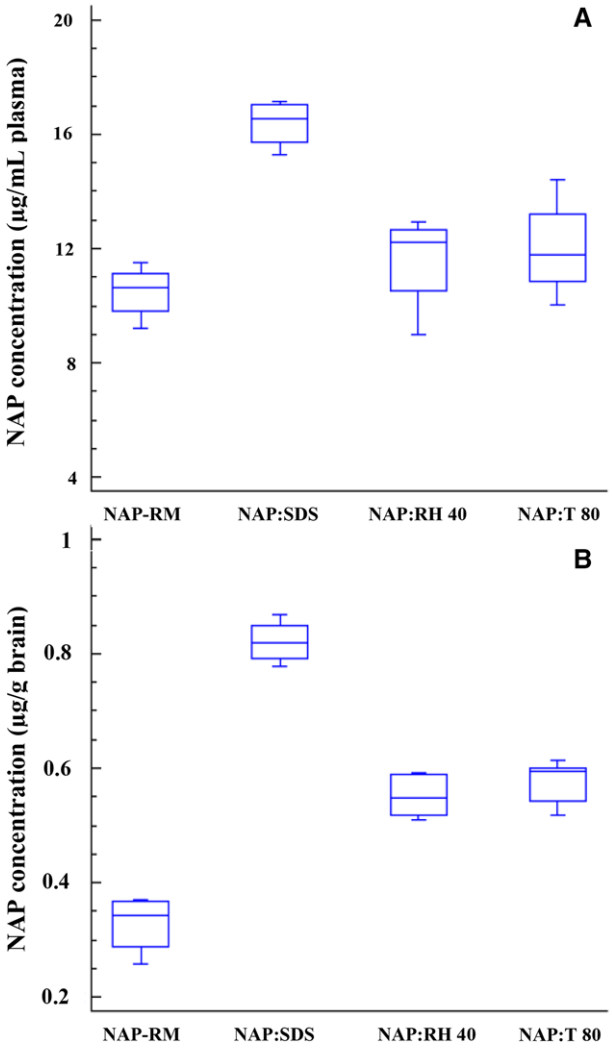

FIGURE 3 Concentrations of NAP in plasma (A) and brain (B) samples after oral administration (equivalent to 7.5 mg/kg of NAP) of the different formulations: NAP-RM, NAP:T 80, NAP:RH 40, and NAP:SDS. Data show boxplots indicating the upper/lower quartile and the median with whiskers from min to max values

levels of NAP for pharmacokinetic studies [20]. Plasma and brain analyses were performed 1 h after oral administration of NAP (7.5 mg/kg) under these conditions. Figure 3 shows the plasma and brain tissue concentrations after NAP-RM and in micellar systems with three different surfactants. Concentrations for the NAP-RM formulation were 10.49 ± 0.77 μg/mL and 0.33 ± 0.039 μg/g for plasma and brain tissue, respectively. These values were in agreement with the previously published values of T_{max} and C_{max} [1,2]. The correlation between brain/plasma ratios (B/P ratios) for NAP-RM showed a low value (0.031) [14]. The use of micellar systems with different surfactants produced an increase in NAP values in plasma and brain tissues compared to NAP-RM. The addition of surfactants in the micellar systems, therefore, increased the concentrations of NAP to 1 h in plasma and brain in the following order: NAP-RM < < NAP:RH 40 < NAP:T

TABLE 3 Intra- and inter-day precision (RSD%), accuracy (RE%), and recovery of NAP determined by plasma and brain at three levels ($n = 6$)

	Concentration	Precision RSD[a] (%)		Accuracy RE[b] (%)		Recovery	
		Intraday	Interday	Intraday	Interday	Mean (%)	RSD[a] (%)
	(μg/mL)						
Plasma	0.93	2.96	1.46	1.75	0.70	95.09	0.56
	1.23	1.45	3.05	−1.00	2.86	93.33	1.83
	1.54	0.74	0.24	−1.67	0.40	96.64	0.48
	(μg/g)						
Brain	0.54	1.64	2.75	2.75	−2.09	95.06	4.05
	0.72	2.26	2.38	2.38	1.79	95.55	2.81
	0.90	0.17	0.80	0.80	−1.84	96.25	0.31

[a]RSD, relative standard deviation.
[b]RE, relative error.

80 < NAP:SDS. The presence of nonionic surfactants (RH 40 and T 80) showed a B/P ratio of 0.047 for both formulations. The highest concentrations were obtained with NAP:SDS compared to NAP-RM. One hour after administration, the plasma and brain concentrations of NAP:SDS compared with NAP-RM were 1.45 and 2.52 times, respectively. In this case, the correlation between the B/P ratios for NAP:SDS presented the highest value in comparison with the other surfactants (B/P ratio of 0.055). A recent study reports that surfactants such as T 80 were rapidly transferred from the plasma circulation to other tissues, rather than to the brain [26]. However, the intestinal absorption of SDS micelles was related to a greater accumulation in the brain [27]. However, further studies will be required to determine how the different proportions of SDS in micellar systems could modify the B/P ratio.

4 | CONCLUDING REMARKS

A quantitative submicellar LC fluorescence method was developed and validated to determine NAP levels in mouse plasma and brain samples. This method was sensitive, robust, and suitable for routine clinical analysis involving a large number of samples. It was used to investigate the brain/plasma distribution of NAP for formulations containing three different surfactants. All micellar systems had higher concentrations of NAP in plasma and brain tissue compared to NAP raw material. SDS micelles showed a higher brain distribution in mice than RH 40 or T 80 micelles. The information obtained from this study allows us to improve the plasma and brain concentrations in NAP formulations.

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REFERENCES

1. Yilmaz, B., Asci, A., Erdem, A. F., HPLC method for naproxen determination in human plasma and its application to a pharmacokinetic study in Turkey. *J. Chromatogr. Sci*. 2014, *52*, 584–589.
2. Sheha, M., Pharmacokinetic and ulcerogenic studies of naproxen prodrugs designed for specific brain delivery. *Arch. Pharm. Res*. 2012, *35*, 523–530.
3. Silva, R. A., Wang, C. C., Fernández, L. P., Masi, A. N., Flow injection spectrofluorimetric determination of carvedilol mediated by micelles. *Talanta* 2008, *76*, 166–171.
4. Mohamed, A. I., Omar, M. A., Hammad, M. A., Mohamed, A. A., Development and validation of highly sensitive stability indicating spectrofluorimetric method for determination of Amlodipine in pharmaceutical preparations and human plasma. *J. Fluoresc*. 2016, *26*, 2141–2149.
5. Zidan, D. W., Elmasry, M. S., Hassan, W. S., Shalaby, A. A. Flow injection spectrofluorimetric determination of carvendilol mediated by micelles. *Luminescence* 2018. *33*, 232–242.
6. Zidan, D. W., Elmasry, M. S., Hassan, W. S., Shalaby, A. A. Flow injection spectrofluorimetric determination of carvendilol mediated by micelles. *Luminescence* 2018. *33*, 326–335.
7. Mostafa, A. M., Rageh, A.H., Ali, M. F. B., Mohamed, F. A., Micelle and inclusion complex enhanced spectrofluorimetric methods for determination of Retugabine: Application in pharmaceutical and biological analysis. *Spectrochim Acta A Mol. Biomol. Spectrosc*. 2019, *15*, 251–261.
8. Ruiz-Angel, M. J., Carda-Broch, S., Torres-Lapasió, J. R., García-Alvarez-Coque, M. C., Retention mechanisms in micellar liquid chromatography. *J. Chromatogr A*. 2009,*1216*, 1798–1814.
9. Belal, F., Ibrahim, F., Sheribah, Z. A., Alaa, H. Micellar HPLC-UV method for the simultaneous determination of levodopa, car-

bidopa and entacapone in pharmaceuticals and human plasma. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2018, *1091*:36–45.

10. Ruiz-Ángel, M. J., Torres-Lapasió, J. R., Carda-Broch, S., García-Álvarez-Coque, M. C., Retention mechanisms in micellar liquid chromatography. *J. Chromatogr. A*. 2010,*1217*, 7090–7099.
11. Hadjmohammadi, M. R., Nazari, S. J. S. S., Separation optimization of aniline and seven 4-substituted anilines in high-submicellar liquid chromatography using empirical retention modeling and Derringer's desirability function. *J. Sep. Sci*. 2013, *36*, 3667–3672.
12. Ortiz-Bolsico, C., Ruiz-Angel, M. J., García-Alvarez-Coque, M. C., Adsorption of the anionic surfactant sodium dodecyl sulfate on a C18 column under micellar and high submicellar conditions in reversed-phase liquid chromatography. *J. Sep. Sci*. 2015 *38*, 550–555.
13. Tiong, N., Elkordy, A. A., Effects of liquisolid formulations on dissolution of naproxen. *Eur. J. Pharm. Biopharm.* 2009, *73*, 373–384.
14. Della Rocca, G., Di Salvo, A., Cagnardi, P., Marchesi, M. C., Conti, M. B., Naproxen in the horse: pharmacokinetics and side effects in the elderly. *Res. Vet. Sci*. 2014, *96*, 147–152.
15. Sakaguchi, Y., Yoshida, H., Hayama, T., Yoshitake, M., Itoyama, M., Todoroki, K., Yamaguchi, M., Nohta, H., Fluorous derivatization and fluorous-phase separation for fluorometric determination of naproxen and felbinac in human plasma. *J. Pharm. Biomed. Anal.* 2011, *55*, 176–180.
16. Rezk, M. R., Michael, A. M., Lotfy, H. M., El-Kadi, A. O., Shehata, M. A., A rapid and sensitive HPLC assay of some concomitant anti-migraine drugs. *J. Chromatogr. Sci.* 2014, *52*, 704–706.
17. Gallo, P., Fabbrocino, S., Dowling, G., Salini, M., Fiori, M., Perretta, G., Serpe, L., Confirmatory analysis of non-steroidal anti-inflammatory drugs in bovine milk by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. A* 2010, *1217*, 2832–2839.
18. ICH Harmonized Tripartite Guideline. Validation of analytical procedures: text and methodology, Q2 (R1), Geneva, 2005. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf
19. Martins, S. M., Wendling, T., Gonçalves, V. M., Sarmento, B., Ferreira, D. C., Development and validation of a simple reversed-phase HPLC method for the determination of camptothecin in animal organs following administration in solid lipid nanoparticles. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*. 2012, *880*, 100–107.
20. García-Herrero, V., Torrado, C., García-Rodríguez, J. J., López-Sánchez, A., Torrado, S., Torrado-Santiago, S., Improvement of the surface hydrophilic properties of naproxen particles with addition of hydroxypropylmethyl cellulose and sodium dodecyl sulphate: in vitro and in vivo studies. *Int. J. Pharm*. 2017, *529*, 381–390.
21. Richardson, A. E., McPherson, S. D., Fasciano, J. M., Pauls, R. E., Danielson, N. D., Corrigendum to "Micellar liquid chromatography of terephthalic acid impurities". *J. Chromatogr A*. 2017, *1491*, 67–74.
22. Eid, M., El-Shabrawy, Y., El-Shaheny, R., Green micellar_HPLC analysis of three angiotensin-converting enzyme inhibitors in their mixtures with hydrochlorothiazide and modeling of their retention behavior by fitting to Foley's model. *J. Sep. Sci*. 2017, *40*, 3646–3654.
23. Petr, J., Rapid determination of the critical micelle concentration by Taylor dispersion analysis in capillaries using both direct and indirect detection. *J. Sep. Sci*. 2017, *40*, 1421–1426.
24. Andersen, C. A., Perfetti, P., Nibbio, M., Bellini, M., Angelini, R., Fornasier, M., Andersen., Brain penetration assessment in vivo: a reliable and simple method in anesthetized rats at steady state. *J. Neurosci. Methods*. 2017, *232*, 199–206.
25. Yehia, A. M., Essam, H. M., Development and validation of a generic high-performance liquid chromatography for the simultaneous separation and determination of six cough ingredients: robustness study on core-shell particles. *J. Sep. Sci*. 2016, *39*, 3357–3367.
26. Miyazawa, T., Nakagawa, K., Harigae, T., Onuma, R., Kimura, F., Fujii, T., Miyazawa, T., Distribution of β-carotene-encapsulated polysorbate 80-coated poly(D, L-lactide-co-glycolide) nanoparticles in rodent tissues following intravenous administration. *Int. J. Nanomedicine*. 2015, *10*, 7223–7230.
27. Wang, G, Wang, J. J., Li, F., To, S. S., Development and evaluation of a novel drug delivery: pluronics/SDS mixed micelle loaded with myricetin in vitro and in vivo. *J. Pharm. Sci*. 2016, *105*, 1535–1543.

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