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Short communication

Comparison between sodium dodecylsulphate and cetyltrimethylammonium bromide as mobile phases in the micellar liquid chromatography determination of non-steroidal anti-inflammatory drugs in pharmaceuticals

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Abstract

The retention behaviour of non-steroidal anti-inflammatory drugs (NSAIDs) using micellar mobile phases of sodium dodecylsulphate (SDS) is studied and compared with that observed with micellar mobile phases of cetyltrimethylammonium bromide (CTAB). A liquid chromatographic procedure for the determination of acemetacin, diclofenac, indomethacin, ketoprofen, naproxen and tolmetin in pharmaceutical preparations is described. The proposed system uses a Kromasil C_{18} analytical column and a solution of 0.15 M SDS at pH 3 with 10% 1-propanol as mobile phase. Under these conditions, the studied NSAIDs elute between 6 and 10 min at a 1 mL min^{-1} flow rate. Limits of detection (LOD) are lower than 0.5 μg mL^{-1}. Recoveries in the analysis of the pharmaceutical preparations are ranged between 91 and 104% respect to the nominal content declared by the manufacturers and the relative standard deviations are in general lower than 4%.

Keywords: Micellar liquid chromatography; Non-steroidal anti-inflammatory drugs; Pharmaceutical preparations; Sodium dodecylsuphate; Cetyltrimethylammonium bromide

1. Introduction

Non-steroidal anti-inflammatory drugs, commonly referred as NSAIDs, are some of the most commonly prescribed medications for the treatment of soft-tissue disorders associated with pain and inflammation. The common mechanism of action for all NSAIDs is the inhibition of the enzyme cyclooxygenase (COX). COX is necessary in the formation of prostaglandins, which cause swelling and pain [1]. NSAIDs can be administered orally, systemically, or as a localized injection. The most common side-effect of NSAIDs is the irritation of the stomach, nausea and vomiting.

Different procedures have been developed in order to determine NSAIDs in pharmaceuticals using several analytical techniques in static and flow injection schemes [2–17] such as potentiometric titrimetry [2,16], spectrophotometry [3–7,14,15] and photoluminescence techniques [8–13,16,17]. Most of these methods require a sample pre-treatment, mainly liquid-liquid extraction, to enhance selectivity. Several methods based on separation techniques, including capillary electrophoresis [18,19] thin-layer chromatography [20], supercritical-fluid chromatography [21] and mainly high performance liquid chromatography in reversed-phase mode (RP-HPLC) [3,22–26], have been also proposed. Because in conventional RP-HPLC NSAIDs are highly retained, high concentrations of organic solvents, elevated flow rates and/or gradient elution and in some cases high temperatures for their determination are required.

Micellar liquid chromatography (MLC) is an alternative mode to the conventional reversed-phase liquid chromatog-

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raphy, in which an aqueous solution of a surfactant above its critical micellar concentration is used as mobile phase. So the mobile phase is composed by surfactant micelles and monomers and the stationary phase remains constantly and reproducibly modified by the adsorption of surfactant monomers.

In MLC different kinds of interactions (electronic, hydrophobic and steric) solute-modified stationary phase and solute-micelle exist, which confer a high versatility to the technique and enable the simultaneous separation of compounds of different nature [27–29]. MLC analytical procedures to determine different kinds of drugs in pharmaceutical preparations have been reported [30–38].

In a previous paper [39], a MLC procedure for the determination of NSAIDs in pharmaceuticals that uses a C_{18} column and 0.06 M CTAB (pH 7) and 10% 1-butanol (v/v) solutions as micellar mobile phase was proposed. Under these conditions, analytes eluted between 11 and 23 min. It was not possible to reduce the retention times of compounds due to the risk of emulsion formation with 1-butanol.

In this paper, the retention behaviour of NSAIDs using mobile phases of SDS is studied and compared with that observed using mobile phases of CTAB. From the results, a new and rapid (<10 min) RP-HPLC procedure for determining six NSAIDs in various pharmaceutical preparations commercialized in Spain by using SDS micellar mobile phases and UV spectrophotometric detection is proposed.

2. Experimental

2.1. Reagents and standards

The following surfactants were used to prepare the different mobile phases assayed: sodium dodecylsulphate (SDS, 99%, Merck, Darmstadt, Germany) and cetyltrimethylammonium bromide (CTAB, 99%, Acros Organics, Geel, Belgium), anionic and cationic surfactants, respectively. Surfactants were dissolved in 0.05 M aqueous solutions of phosphate buffer, prepared with sodium dihydrogen phosphate (analytical reagent, Panreac, Barcelona, Spain) and the appropriate amount of 2 M solutions of sodium hydroxide (for analysis, Panreac) or hydrochloric acid (for analysis, Merck) to adjust the pH of the micellar eluent. After that, an adequate amount of 1-propanol or 1-butanol (both HPLC grade, Scharlab, Barcelona, Spain) was added to the micellar eluent to obtain the working concentration (v/v).

Non-steroidal anti-inflammatory drugs were kindly donated by several pharmaceutical laboratories: acemetacin (Laboratorios Fher, Barcelona, Spain), diclofenac (Novatis, Barcelona, Spain), indomethacin (Laboratorios Llorens, Barcelona, Spain), ketoprofen (Rhône-Poulenc Rorer, Madrid, Spain), naproxen (Syntex Latino, Madrid, Spain), piketoprofen (Laboratorios Farmacéuticos Almirall, Barcelona, Spain) and tolmetin (Laboratorio Estedi, Barcelona, Spain).

Stock standard solutions of NSAIDs were prepared by dissolving the compound in 0.1 M SDS or 0.04 M CTAB solutions, depending on the surfactant used in the mobile phase. Working solutions were prepared by dilution of the stock standard solutions in the mobile phase solution used. The solutions were stored in the refrigerator at 4 °C and they were stable at least for 15 days. Indomethacin solutions were prepared every 3 days because a decomposition peak appeared in the chromatograms.

Barnstead E-pure, deionized water (Sybron, Boston, MA) was used throughout. The mobile phases and the solutions injected into the chromatograph were vacuum-filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA, USA).

2.2. Instrumental and measurement

A Hewlett-Packard HP-1100 series chromatograph with an isocratic pump, and an UV–visible detector was used (Palo Alto, CA, USA). Data acquisition and processing were performed on an HP Vectra XM computer (Amsterdam, The Netherlands) equipped with HP-ChemStation software from Hewlett-Packard (1996 version, Waldbronn, Germany).

The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA), with a 20 μL loop. A Spherisorb ODS-2 C_{18} column (5 μm, 250 × 4.6 mm i.d.) (Scharlab, Barcelona, Spain) and a Kromasil

Table 1
Retention factors obtained in different SDS mobile phases

Compound	$\lambda_{detection}$ (nm); log K; log P	Modifier (%), SDS (M), pH; k			
		1-Propanol (10%), 0.1, 3	1-Propanol (10%), 0.15, 3	1-Butanol (5%), 0.15, 3	1-Propanol (10%), 0.15, 7
Acemetacin	250; 4; 4	3.81	2.01	2.27	1.24
Diclofenac	284; 4.5; 4.77	6.41	3.92	4.02	1.25
Indomethacin	234; 4.5; 4.23	5.44	3.06	3.23	1.38
Ketoprofen	250; 4.6; 2.79	3.88	2.38	2.50	0.62
Naproxen	234; 4.2; 2.82	4.19	2.40	2.58	0.57
Piketoprofen	250; n.a.; 4.24	43.3	28.0	26.8	18.8
Tolmetin	322; 3.5; 2.79	3.31	2.05	2.18	0.71

n.a.: Non-available data.

C_8 column (5 μm, 150 × 4.6 mm i.d.) (Análisis Vínicos, Tomelloso, Spain) were used. The mobile phase flow rate was 1 mL min^{-1}. The detection was performed in UV at the wavelengths depicted in Table 1. All the assays were carried out at room temperature. In order to avoid the CTAB precipitation inside the chromatographic system, a thermostatic bath (J.P. Selecta, S.A. Barcelona, Spain) was used when room temperature was lower than 25 °C.

2.3. Sample preparation

The pharmaceuticals of NSAIDs are commercialized in different presentations: tablets, capsules, gels, ointments and suppositories. For the analysis of tablets, 10 tablets were weighed, ground in a mortar and an adequate amount of the solid was weighed and dissolved without difficulty in 0.1 M SDS solution. For capsules, an entire capsule was taken and was dissolved in 0.1 M SDS solution by immersion in an ultrasonic bath (1 h).

In order to perform the analysis of gels, an adequate amount of the sample was weighed and dissolved in 0.15 M SDS solution using a magnetic stirrer with gentle heating. For the analysis of suppository pharmaceutical presentations, a suppository was taken and dissolved in 1-propanol by means of agitation and heating with a magnetic stirrer.

In all cases appropriate dilutions were performed using the mobile phase solution (0.15 M SDS (pH 3) + 10% 1-propanol). In the gel and suppositories samples containing diclofenac (Voltarén®) and ketoprofen (Orudis®), after dilution a precipitate appeared, which was separated by centrifugation and the supernatant solution was injected into the chromatograph.

Solutions were injected into the chromatographic system through 0.45 μm membrane. In all cases, three sample solutions were independently prepared and for each one triplicate determinations were performed (nine injections per sample).

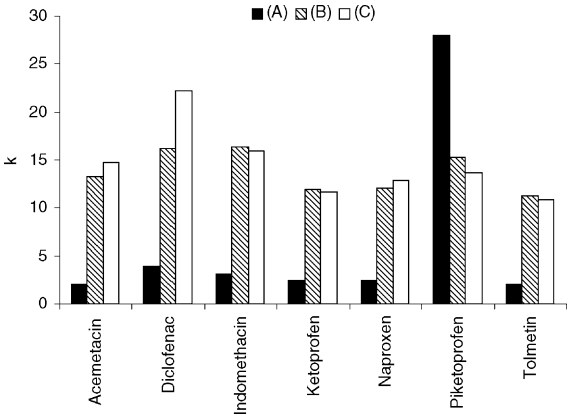

Fig. 1. Comparison between the retention data of NSAIDs obtained using the following mobile/stationary phases: (A) 0.15 M SDS (pH 3) + 10% 1-propanol/C_{18}; (B) 0.06 M CTAB (pH 7) + 10% 1-butanol/C_8 and (C) 0.06 M CTAB (pH 7) + 10% 1-butanol/C_{18} [39].

Table 2
Regression statistics for the NSAIDs calibration curves, relative standard deviation (R.S.D.) and limits of detection (LOD) using peak areas and peaks heights data

NSAID	Peak area							Peak height						
	$b_1 \pm ts_{b1}$	$b_0 \pm ts_{b0}$	r	S.E.	R.S.D.[a](%)	R.S.D.[b](%)	LOD[c] (μg mL^{-1})	$b_1 \pm ts_{b1}$	$b_0 \pm ts_{b0}$	r	S.E.	R.S.D.[a](%)	R.S.D.[b](%)	LOD[c] (μg mL^{-1})
Acemetacin	0.56 ± 0.01	0.4 ± 0.4[d]	0.99990	0.19	5.43	1.25	0.2	0.73 ± 0.05	−0.2 ± 1.7[d]	0.9990	0.8	2.95	1.88	0.11
Diclofenac	0.48 ± 0.02	−0.0 ± 0.6[d]	0.9996	0.3	3.71	2.94	0.5	0.55 ± 0.02	−0.2 ± 0.5[d]	0.9997	0.2	2.96	0.76	0.4
Indomethacin	0.70 ± 0.03	0.0 ± 0.8[d]	0.9994	0.6	4.32	2.23	0.09	0.81 ± 0.02	0.1 ± 0.6[d]	0.9997	0.4	7.54	1.7	0.15
Ketoprofen	0.85 ± 0.04	0.9 ± 1.2[d]	0.9990	0.8	2.29	2.18	0.08	1.33 ± 0.06	0.8 ± 1.7[d]	0.9992	1.1	0.85	0.97	0.03
Naproxen	3.41 ± 0.05	−0.2 ± 0.7[d]	0.99995	0.4	7.45	4.21	0.01	5.67 ± 0.10	0.6 ± 1.6[d]	0.99990	0.9	7.24	3.37	0.008
Tolmetin	0.79 ± 0.09	0 ± 3[d]	0.997	1.3	2.47	0.92	0.4	1.22 ± 0.05	−0.5 ± 1.5[d]	0.9996	0.7	3.13	1.12	0.5

r: Correlation coefficient; S.E.: standard error; b_1: slope; b_0: intercept; ts_b: confidence interval at the 95% level.

[a] R.S.D. values estimated from 10 replicated injections of solutions containing the analytes at the lowest concentration levels studied: 1 μg mL^{-1} for acemetacin and ketoprofen; 5 μg mL^{-1} for diclofenac and tolmetin; 0.5 μg mL^{-1} for naproxen and indomethacin.

[b] R.S.D. values estimated from 10 replicated injections of solutions containing the analytes at the following concentration levels: 50 μg mL^{-1} for acemetacin, ketoprofen, diclofenac and tolmetin; 20 μg mL^{-1} for naproxen and indomethacin.

[c] LOD values calculated using the 3 s criterion, from the standard deviation of the peak areas or heights corresponding to 10 replicated injections of solutions containing concentrations of analytes next to the LOD (the lowest concentrations used for R.S.D. calculation).

[d] Statistically non-significant.

3. Results and discussion

As can be observed in Table 1, NSAIDs are hydrophobic compounds with logarithm of the 1-octanol/water partition coefficients ($\log P$) for the non-ionized forms ranged between 2.79 and 4.77. They are acidic compounds that present in their molecules carboxylic groups with logarithms of the protonation constants ($\log K$) values close to 4 in aqueous medium. Piketoprofen is a basic compound with a pyridine ring (estimated $\log K$ value 2–4).

3.1. Retention behaviour of NSAIDs with SDS micellar mobile phases

In order to optimise the retention of NSAIDs, a C_{18} column and SDS micellar mobile phases were used. The use of an anionic surfactant can difficult the retention of the anionic form of NSAIDs due to the existence of repulsive electrostatic interactions between the solutes and the modified stationary phase by surfactant adsorption.

The effect of the mobile phase pH, the surfactant concentration and the nature and concentration of modifier on the retention of compounds was studied (See Table 1). At pH 3 in the presence of a fixed amount of 1-propanol (10%), the increase of SDS concentration from 0.1 to 0.15 M produced, as expected, a decrease in the retention of compounds. This effect was more evident for highly hydrophobic NSAIDs ($\log P \geq 4$). The effect of the mobile phase pH on the retention of compounds was studied using 0.15 M SDS + 10% 1-propanol mobile phases prepared at pH 3 and 7. Due to repulsive electrostatic interactions between analytes and the modified stationary phase, NSAIDs were less retained at pH 7 than at pH 3. However, in some cases the retention factors at pH 7 (ketoprofen, naproxen and tolmetin) were lower than the unity. The addition of a 5% 1-butanol to the 0.15 M SDS (pH 3) mobile phase instead of 10% 1-propanol, scarcely modified the retention of compounds.

A comparative study of retention behaviour of NSAIDs with SDS and CTAB micellar mobile phases and C_{18} and C_8 stationary phases was performed (see Fig. 1 for details). As can be observed, except for piketoprofen, the retention of NSAIDs in SDS micellar mobile phases was very much lower than in CTAB micellar eluents. On the other hand, using the same CTAB mobile phase, only a slight decrease on the retention of compounds was observed when a C_8 column was used instead of a C_{18} column. This fact indicates that electrostatic interactions between solute and the modified stationary phases mainly determine retention of compounds. Using the CTAB mobile phase with highest elution strength, 0.06 M CTAB buffered at pH 7 containing 10% 1-butanol and a C_8 column, the retention factors of compounds were ranged between 11.3 (tolmetin) and 16.3 (indomethacin).

According to the results obtained, a C_{18} column and a micellar mobile phase containing 0.15 M SDS (pH 3) + 10% 1-propanol was selected for the determination of the NSAIDs studied in pharmaceuticals preparations. Under these conditions, NSAIDs, except piketoprofen, eluted between 6 and 10 min ($2.01 \leq k \leq 3.92$). The higher retention of piketoprofen in these conditions can be explained taking into account the attractive electrostatic interactions in addition to the hydrophobic solute-stationary phase. For piketoprofen, CTAB mobile phases and the chromatographic conditions previously reported [39] are recommended.

3.2. Analytical data

In the selected chromatographic conditions the calibration curves, limits of detection (LOD) and reproducibility of NSAIDs were obtained. The calibration curves of the compounds obtained in the range 1–50 $\mu g\,mL^{-1}$ using peak

Table 3
Composition of the pharmaceutical preparations containing NSAIDs and recoveries obtained

Pharmaceutical (presentation) manufacturer	Composition	Recovery[a] ± s (%)	Recovery[b] ± s (%)
Oldan® (caspules) Europharma S.A.Madrid, Spain	Acemetacin (60 mg) Excipients	104.15 ± 0.05	97.9 ± 0.3
Voltarén® (tablets) Novartis Farmacéutica S.A. Barcelona, Spain	Diclofenac sodium (50 mg) Excipients	99.0 ± 1.0	104 ± 2
Voltarén® (suppositories) Novartis Farmacéutica S.A. Barcelona, Spain	Diclofenac sodium (100 mg) Excipients	102 ± 3	105 ± 2
Voltarén® Emulgel® (gel) Novartis Farmacéutica S.A. Barcelona, Spain	Diclofenac sodium (10 mg g^{-1}) Excipients	106 ± 9	111 ± 9
Inacid® (capsules) Merck Sharp & Dohme de España S.A. Madrid, Spain	Indomethacin (25 mg) Excipients	103 ± 2	110 ± 3
Inacid® (suppositories) Merck Sharp & Dohme de España S.A. Madrid, Spain	Indomethacin (50 mg) Excipients	94 ± 3	103 ± 3
Inacid® (gel) Merck Sharp & Dohme de España S.A. Madrid, Spain	Indomethacin (10 mg g^{-1}) Excipients	91.4 ± 1.4	102.2 ± 0.4
Oridus® (capsules) Rhône-Poulenc Rorer S.A. Madrid, Spain	Ketoprofen (50 mg) Excipients	99.0 ± 1.0	107.1 ± 1.3
Oridus® (suppositories) Rhône-Poulenc Rorer S.A. Madrid, Spain	Ketoprofen (100 mg) Excipients	96.1 ± 0.5	103.6 ± 0.6
Oridus® (gel) Rhône-Poulenc Rorer S.A. Madrid, Spain	Ketoprofen (25 mg g^{-1}) Excipients	98.0 ± 1.0	106.6 ± 0.6
Naprosyn® (tablets) Syntex Latino S.A. Madrid, Spain	Naproxen (500 mg) Excipients	101 ± 2	100 ± 3
Calmatel® (oinment) Almirall Prodesfarma S.A. Barcelona, Spain	Piketoprofen hydrochloride (18 mg g^{-1}) Base O/W, surfactants, cationic biguanide, water	–	117 ± 4
Artrocaptin (tablets) Estedi S.L. Barcelona, Spain	Tolmetin (400 mg) Excipients	93 ± 3	106 ± 3

[a] Calculated using peak areas as dependent variable.
[b] Calculated using peak height as dependent variable.

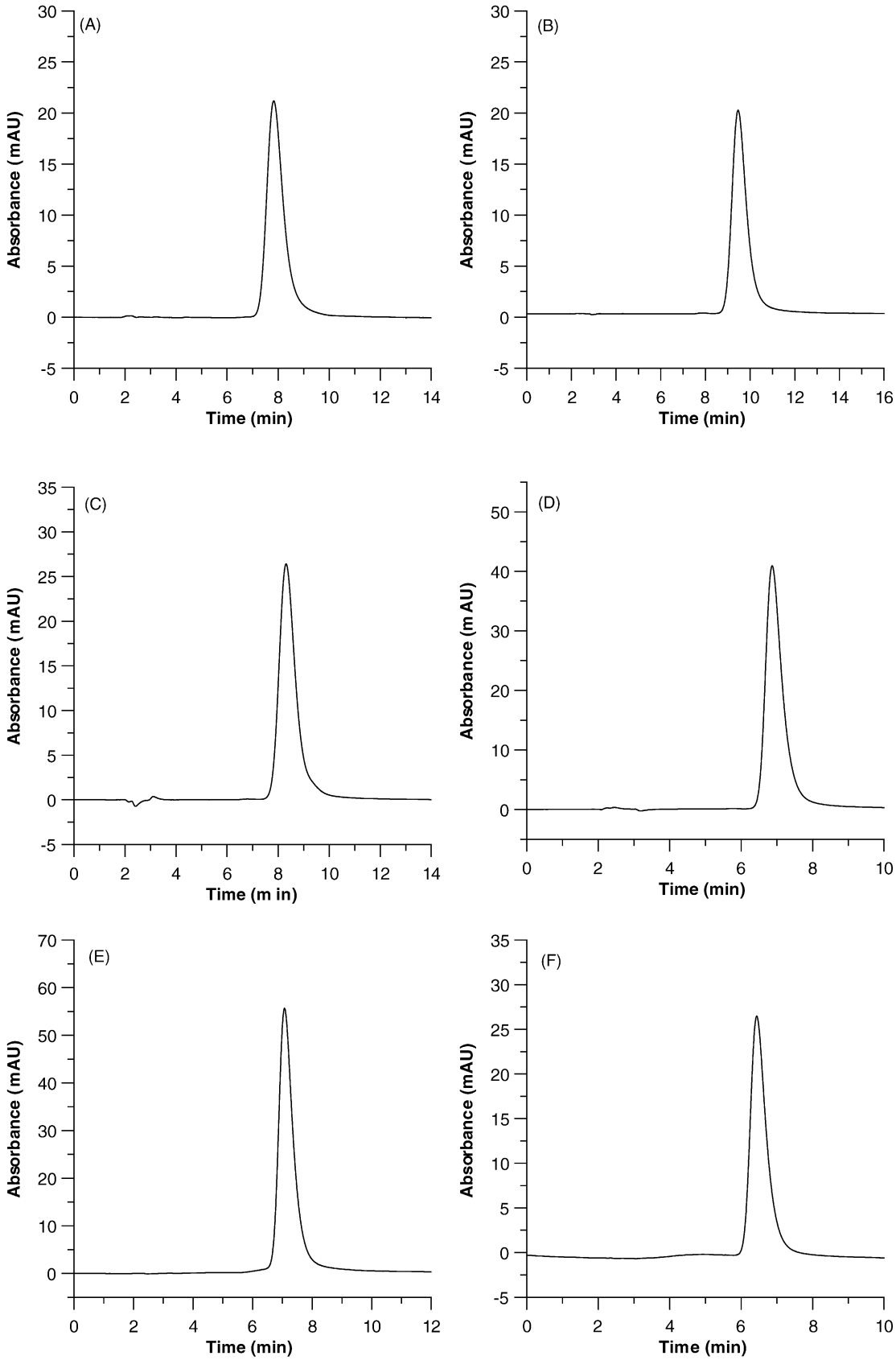

Fig. 2. Chromatograms of some pharmaceutical preparations: (A) Oldan® (capsules); (B) Voltarén® (gel); (C) Inacid® (capsules); (D) Orudis® (suppositories); (E) Naprosyn® (tablets); (F) Artrocaptin® (tablets).

heights and areas as dependent variables (Table 2) showed adequate linearity ($r > 0.999$). The intercepts were statistically equal to zero indicating the absence of systematic errors.

The repeatability of the method, expressed as the relative standard deviation, R.S.D. (%), was evaluated at two different concentration levels. The R.S.D. values were adequate (<5% and 7.5% for the highest and the lowest concentration levels, see Table 2). The limits of detection, calculated by applying the 3 s criterion, were lower than 0.5 $\mu g\,mL^{-1}$ using both, peak areas and heights (Table 2).

3.3. Analysis of pharmaceuticals containing NSAIDs

Table 3 shows the commercial names of pharmaceuticals analyzed together with their corresponding dosage forms, manufacturer laboratories and declared compositions. All samples showed very clean chromatograms and no interferences were observed (Fig. 2).

As can be observed in Table 3, the recovery values obtained respect to the nominal contents declared by the manufacturers were ranged between 91.4 and 110%. These results are included within the pharmacopoeia tolerances [3] and the R.S.D. were, in general, lower than 4%.

4. Conclusions

In this paper, a new micellar liquid chromatography procedure for the determination of six non-steroidal anti-inflammatory drugs (acemetacin, diclofenac, indomethacin, ketoprofen, naproxen and tolmetin) in pharmaceutical preparations is proposed. In spite of NSAIDs are highly hydrophobic compounds, the use of SDS hybrid micellar mobile phases allow the rapid elution of analytes using a C_{18} column due to the existence of repulsive electrostatic interactions between analytes and the modified stationary phase.

The proposed procedures for the determination of NSAIDs in pharmaceuticals are rapid (between 6 and 10 min per sample), reliable and free from interferences, and sample preparation is simple.

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