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Chiral and nonchiral determination of ketoprofen in pharmaceuticals by capillary zone electrophoresis

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Abstract

The new method for the enantiomeric resolution of various 2-arylpropionic acids by capillary zone electrophoresis (CZE) using heptakis-tri-O-methyl-β-cyclodextrin as chiral selector was applied to the determination of ketoprofen in different commercially-available pharmaceutical preparations. The analyte was determined under chiral and nonchiral conditions (viz. in the presence and absence of 50 m*M* heptakis-tri-O-methyl-β-cyclodextrin in the background electrolyte), with significantly similar results and relative standard deviations from 1.2 to 6.5% in both cases. The limits of detection and determination for the inactive enantiomer, *R*-(−)-ketoprofen, were calculated to be $7.0 \cdot 10^{-7}$ and $1.6 \cdot 10^{-6}$ *M*, respectively. The proposed method was successfully used to determine enantiomeric purity in the drugs studied, with results comparable to those provided by the chiral HPLC method. © 1998 Elsevier Science B.V.

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1. Introduction

Many substances of pharmacological interest possess one or more chiral groups. Also, many pharmaceuticals contain enantiomeric active principles that are administered as racemates; very often, however, one of the enantiomers is more active than its antipode, which may even be toxic. Typical examples include epinephrine, the (−) enantiomer of which is 10 times more powerful than its (+) isomer [1], and *S*-(−)-thalidomide, which exhibits a teratogenic effect not present in the *R*-(+) isomer [2]. This phenomenon has raised the need to obtain or use enantiomerically-pure compounds. A racemate can hardly be approved by health care authorities unless the safety and efficiency of both pure enantiomers have been established. The unwanted isomer is an impurity and as such its content in the final product should be reduced as far as possible; as a result, effective analytical methods for assessing enantiomeric products have become a strong need. Capillary electrophoresis (CE) has become an interesting alternative to classical chromatographic techniques (HPLC and GC) for detecting and quantifying optically-active impurities on account of their many advantages (e.g. analytical expeditiousness and high flexibility, efficiency and resolution). Thus, CE has been used in quantitative chiral analyses for various compounds including fluparoxan [3], epinephrine [4,5] and ibuprofen [6].

The antiinflammatory properties of profens are due to the *S*-(+) enantiomer; the *R*-(−) enantiomer can

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only be rendered pharmacologically active by bioinversion of the chiral centre. The extent in which this one-way process can take place depends on the particular profen and on the animal species; in man, for example, ibuprofen and fenoprofen are readily inverted but ketoprofen is not [7–9]. This maybe explains why no enantiomerically-pure ibuprofen or fenoprofen preparations are commercially available and why a drug containing *S*-(+)-ketoprofen as the active principle was recently introduced. Two papers [10,11] describe the use of heptakis-2,3,6-tri-O-methyl-β-cyclodextrin as chiral selector in the enantiomeric separation of profens, but they do not suggest any follow-up quantitative application. In a previous work [12], we developed a method of enantiomeric separation of ketoprofen, ibuprofen and fenoprofen with a view to its application in the quantification of these compounds in pharmaceuticals.

In this work, we focused on ketoprofen because the pharmaceuticals that contain it use it as their active principle, whether in racemic form or as the active isomer, *S*-(+). The active enantiomer of ketoprofen can be synthesized with excellent enantiomeric excesses by stereoselective hydrolysis of racemic ketoprofen ethyl ester using the enzyme esterase, which converts the *S*-(+) ester into the corresponding carboxylic acid. The aim of this work was to demonstrate the potential of capillary zone electrophoresis (CZE) for analytical control of ketoprofen in drugs in two different situations, viz. (a) the chiral and nonchiral determination (with and without a chiral selector, respectively, in the background electrolyte); and (b) the determination of enantiomer *R*-(−) as an impurity of drugs containing the active enantiomer, *S*-(+), alone—this latter requires using a chiral selector in the background electrolyte. For this purpose, we developed and determined several validation parameters for a new method for determining ketoprofen in commercially-available pharmaceuticals, as well as the enantiomeric excess in the pure product.

2. Experimental

2.1. Apparatus

Measurements were made on a Hewlett–Packard ^{3D}CE HPCE instrument (Waldbronn, Germany) equipped with a diode array detector, automatic injector and sampler, and a capillary thermostating unit precise to within ±0.1°C over a temperature range of 4–60°C. Hydrodynamic injection at the anode (obtained by applying a pressure of 50 mbar to the injection vial) was used throughout. All experiments were performed by using an HP fused-silica capillary 50 mm I.D. with a lightpath extended×3 and length of 64.5 cm (effective length 56 cm). An HP ^{3D}CE Chemstation was employed for instrumental control and data acquisition and processing.

The pH of the background electrolyte (BGE) was adjusted by addition of NaOH and measured by means of a Crison micropH 2001 pH meter (Alella, Barcelona).

2.2. Working conditions

The experimental conditions used in all experiments were as follows: a BGE consisting of 20 m*M* phosphate/20 m*M* triethanolamine, pH 5, a temperature of 35°C and a voltage of 20 kV. For the chiral determination of ketoprofen, heptakis-2,3,6-tri-O-methyl-β-cyclodextrin was added at a 50 m*M* concentration (unless otherwise noted) to the BGE.

The injection time used in each case is stated below.

2.3. Samples

The samples studied were Fastum, in gel and capsule forms (both contain racemic ketoprofen as the active principle), and Enantyum tablets, the active principle of which is *S*-(+)-ketoprofen.

2.4. Preparation of samples

About 0.8 g mass of gel was suspended in 30 ml of methanol and heated in a water bath at 50°C for 10 min. The suspension was then sonicated for 5 min and made up to 50 ml with ethanol, followed by centrifugation at 3000 rpm for 20 min. Finally, a volume of 10 ml of the resulting supernatant was made up to 50 ml with water. The solution was filtered prior to injection.

Several tablets or the contents of a few capsules were ground to a powder of which ca. 0.16 g was weighed and suspended in 30 ml of methanol. The

suspension was sonicated for 5 min, made up to 50 ml with methanol and centrifuged at 3000 rpm for 5 min. An aliquot of 5 ml (capsules) or 10 ml (tablets) of this solution was diluted to 50 ml with methanol and filtered.

3. Results

Ketoprofen in the pharmaceuticals studied was determined by quantitation in a nonchiral medium (i.e. a BGE containing no chiral selector) and a chiral medium (in the presence of cyclodextrin).

The corrected peak area (area/migration time) was found to be linearly related to the analyte concentration throughout the range studied (0.05–0.8 m*M*), both in the presence and in the absence of the chiral selector. Table 1 gives the figures of merit for the calibration curves obtained at different injection times in the absence of the chiral selector and in its presence at two different concentrations (25 and 50 m*M*). The injection time and hence the injected volume were altered as required for each determination. All the results were obtained by interpolating the corrected peak areas for the analyte in the calibration curves – the most appropriate curve for the type of sample concerned and separation efficiency required was chosen in each case.

Since one of the aims was to determine the amount of *R*-(−)-ketoprofen contained as an impurity in drugs with *S*-(+)-ketoprofen as the active principle, we determined the limit of detection (LOD) and limit of quantitation (LOQ) for enantiomer *R*-(−) in a chiral medium (BGE containing 25 m*M* heptakis-2.3.6-tri-O-methyl-β-cyclodextrin) from a calibration curve obtained at low concentrations (10^{-3}–10^{-1} m*M*). An intermediate injection time (5 s) was used. Table 1 gives the figures of merit for the calibration curve (curve 4). The LOD, defined as the minimum analyte concentration that produced an instrumental signal significantly different from that for the blank, was calculated in accordance with the IUPAC's criterion, i.e. as the analyte concentration giving a signal exceeding that of the blank (y_B) by 3 times its standard deviation (S_B). The LOQ, defined as the minimum analyte concentration needed to ensure precise quantitative measurements, was determined similarly, using 10 times the standard deviation for the blank instead. Thus, LOD and LOQ were calculated from the following expression:

$$\text{LOD (LOQ)} = \frac{y_B + 3(10)S_B}{b} \quad (1)$$

where b is the slope of the calibration curve. Specifically, we calculated the LOD as follows: time intervals on the baseline of the same width as the base peak and close to it in the electropherograms for the most dilute ketoprofen solution used (10^{-3} m*M*) were integrated and their mean and standard deviation calculated. The mean value was taken to be the blank signal (y_B) and the standard deviation that for the blank (S_B). Substitution of these values into Eq. (1) yielded LOD = $7.0 \cdot 10^{-7}$ *M* and LOQ = $1.6 \cdot 10^{-6}$ *M*.

Alternatively, LOD can be calculated from the calibration curve, run at low analyte concentrations, using the following expression:

$$\text{LOD (LOQ)} = \frac{3(10)S_a}{b} \quad (2)$$

where S_a is the standard deviation for the intercept. The LOD and LOQ values thus obtained were $7.7 \cdot 10^{-7}$ and $2.6 \cdot 10^{-6}$ *M*, respectively, both of which are consistent with the previous ones.

3.1. Nonchiral determinations

The racemic preparations (gel and capsules) and the nonracemic one (tablets) were analyzed by using the calibration curves obtained in the absence of chiral selector (curves 1, 2 and 3). The results are shown in Table 2. Electropherograms recorded on different days for both the samples and calibration standards (39 determinations) gave a mean migration time of 13.31 min and a relative standard deviation of 4.2%.

3.2. Chiral determinations

The above-mentioned preparations were also analyzed under chiral conditions, using some of the calibration curves given in Table 1. Each enantiomer was calibrated individually from the resolved peaks for racemic ketoprofen. The two enantiomers of ketoprofen in the real samples containing the racemate (gel and capsules) were quantified separately

Table 1
Figures of merit of the calibration curves used to quantify ketoprofen in the pharmaceutical preparations Fastum gel, Fastum capsules and Enantyum tablets, and to determine the LOD

Curve	Injection time (s)	Injection volume (nl)[c]	[tri-O-Me-β-CD] (m*M*)	Concentration range (m*M*)	Number of data points	Intercept	Slope	Standard error
Nonchiral determinations								
1	3	6.5	–	0.1–0.8	5	$8.47\cdot10^{-3}\pm5.49\cdot10^{-3}$	365.08±12.48	$6.51\cdot10^{-3}$
2	3	6.5	–	0.1–0.8	5	$5.59\cdot10^{-3}\pm1.16\cdot10^{-3}$	301.98±3.67	$1.94\cdot10^{-3}$
3	6	13	–	0.05–0.5	5	$1.15\cdot10^{-2}\pm1.52\cdot10^{-3}$	837.80±5.62	$1.77\cdot10^{-3}$
Chiral determinations								
4[a]	5	10.9	25	0.001–0.1	8	$3.92\cdot10^{-4}\pm1.72\cdot10^{-4}$	456.75±3.99	$3.61\cdot10^{-4}$
5[a]	3	6.5	25	0.05–0.25	4	$5.75\cdot10^{-4}\pm1.26\cdot10^{-4}$	228.06±7.98	$1.19\cdot10^{-3}$
6[b]	3	6.5	25	0.05–0.25	4	$5.50\cdot10^{-4}\pm1.12\cdot10^{-4}$	227.46±7.13	$1.06\cdot10^{-3}$
7[a]	6	13.0	50	0.05–0.3	4	$5.86\cdot10^{-4}\pm9.75\cdot10^{-4}$	432.13±5.49	$1.03\cdot10^{-3}$
8[b]	6	13.0	50	0.05–0.3	4	$7.98\cdot10^{-4}\pm5.49\cdot10^{-4}$	423.74±6.90	$1.30\cdot10^{-3}$
9[a]	6	13.0	50	0.05–0.3	5	$4.03\cdot10^{-3}\pm1.88\cdot10^{-3}$	384.11±9.40	$1.98\cdot10^{-3}$
10[b]	6	13.0	50	0.05–0.3	5	$3.53\cdot10^{-3}\pm1.09\cdot10^{-3}$	384.38±5.47	$1.16\cdot10^{-3}$
11[a]	12	26.1	50	0.003–0.012	4	$1.84\cdot10^{-3}\pm4.17\cdot10^{-4}$	1014.49±53.90	$3.61\cdot10^{-4}$
12[a]	7	15.2	50	0.0015–0.0045	4	$1.92\cdot10^{-3}\pm0.12\cdot10^{-3}$	556.72±43.15	$1.44\cdot10^{-4}$
13[a]	12	26.1	50	0.0015–0.0045	4	$3.96\cdot10^{-3}\pm0.08\cdot10^{-3}$	1765.64±30.26	$1.02\cdot10^{-4}$

Confidence intervals are given as standard deviations. Correlation coefficients were higuer than 0.99 in all cases. Conditions: BGE, 20 m*M* phosphate–20 m*M* triethanolamine, pH 5.0; temperature, 35°C; applied voltage, 20 kV, 7.1–8.5 μA.

[a] *R*-(−)-ketoprofen.

[b] *S*-(+)-ketoprofen.

[c] Injection volume calculated using the Hagen–Poiseuille equation.

Table 2
Quantitation of Fastum gel, Fastum capsules and Enantyum tablets

Concentration			
Pharmaceutical preparation	Nonchiral	Chiral	Nominal value
Fastum gel (mg ketoprofen/g gel)	24.05±1.04[a] (1)	24.49±1.60[b] (5,6)	25
Fastum capsules (mg ketoprofen per capsule)	53.21±2.49[a] (2)	54.85±0.65[c] (7,8)	50
Enantyum batch 1 (mg ketoprofen per tablet)	23.94±0.57[c] (3)	23.41±0.41[a] (9,10)	25
Enantyum batch 2 (mg ketoprofen per tablet)	23.82±0.31[c] (2)	24.15±0.61[c] (7,8)	25

Confidence intervals are given as standard deviations. The curve used to determine ketoprofen in each case is given in brackets.
[a] The obtained value is the average of 2 aliquots, each one injected in triplicate.
[b] The obtained value is the average of 3 aliquots, each one injected in triplicate.
[c] The obtained value is the average of 2 aliquots, each one injected in duplicate.

from their corresponding calibration curves and their contents added up to obtain the overall amount of ketoprofen.

The results obtained in the chiral and nonchiral determinations were compared via a *t*-test for means (Table 3). Means were found to be not significantly different at a confidence level of 95%. The same procedure revealed that the mean values for the two batches of Enantyum tablets analyzed, did not differ significantly in their ketoprofen contents and hence that uniformity between manufactured batches was quite good.

Only the corrected area for the main peak was used in analysing the nonracemic preparation (tablets) because the peak for *R*-(−)-ketoprofen was imperceptible under the working conditions used owing to the low concentration of the enantiomer. The mean migration times obtained from the electropherograms for the calibration standards and the samples (24 determinations) for the *R*-(−) isomer using 25 and 50 m*M* cyclodextrin in the BGE were 11.35 and 14.90 min (standard deviation 1.2 and 2.1%), respectively. The mean *R*-(−)/*S*-(+) corrected area ratio was 1.008±0.008 for the capsules and 1.022±0.009 for the gel – neither confidence interval includes unity, a slight difference that can be ascribed to potential errors made in integrating the peaks.

3.2.1. Determination of enantiomeric purity in the pharmaceutical preparations

Prior to determining enantiomeric excess in the nonracemic drug, tablets were used to detect the *R*-(−)-ketoprofen as an impurity introduced during

Table 3
t-Test for comparison of means obtained in the quantitation of ketoprofen in a chiral and a nonchiral medium

Pharmaceutical preparation	Degrees of freedom	Calculated *t*	Tabulated *t*
Chiral/nonchiral comparison			
Gel	13	0.59	2.16
capsules	8	1.26	2.31
Tablets (batch 1)	8	1.72	2.31
Tablets (batch 2)	6	0.84	2.45
Comparison between batches of tablets			
Batch 1/batch 2	14	1.30	2.14

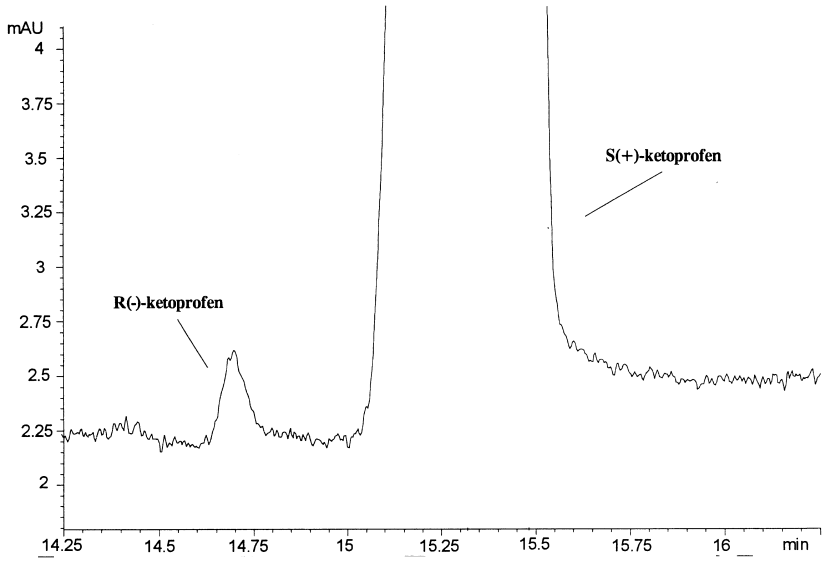

Fig. 1. Detection of the *R* (−)-ketoprofen impurity contained in Enantyum tablets by use of a 20 m*M* phosphate–20 m*M* triethanolamine BGE at pH 5.0 containing 50 m*M* of tri-O-Me-β-CD. Other conditions: temperature, 35°C; applied voltage, 20 kV, 8.1 mA.

the stereoselective synthesis of *S*-(+)-ketoprofen. The determination was performed under the typical conditions described in Section 2.2, using a BGE containing 25 m*M* heptakis-2,3,6-tri-O-methyl-β-cyclodextrin. In order to facilitate detection of *R*-(−) impurities, the injection time was extended to 7 s (ca. 15 nl) and the concentration of the injected solution increased to 1.5 m*M*. While these conditions allowed the *R*-(−) enantiomer to be detected, the insertion of a larger amount of analyte broadened the main peak and diminished resolution – peaks overlapped to an extent that prevented correct quantitation of the impurity. Increasing the tri-O-Me-β-CD concentration to 50 m*M* ensured adequate resolution and avoided peak overlap (Fig. 1).

Once detected, the *R*-(−)-ketoprofen impurity contained in a pure *S*-(+)-ketoprofen batch was determined by using the standard-addition method under the same experimental conditions as for detection. To this end, *S*-(+)-ketoprofen solutions were spiked with variable amounts of *R*-(−)- ketoprofen and the results extrapolated in order to calculate the enantiomeric excess (curve 12 in Table 1). A value of 99.54% (not significantly different from the 99.6% provided by HPLC) was obtained.

The enantiomeric excess in the pharmaceutical (Enantyum batch 2) was determined from a calibration curve run at a low analyte concentration (curve 11 in Table 1), using the *R*-(−) enantiomer as standard, and from the standard-addition method using, in both cases, an extended injection time of 12 min intended to improve peak integration and increase the sensitivity. Interpolation of the corrected peak area obtained for *R*-(−)-ketoprofen in the drug into the calibration graph provided an enantiomeric excess of 99.87%; the standard-addition method (curve 13 en Table 1), used similarly as with the *S*-(+) standard, gave 99.71% and the HPLC technique 99.5%. As can be seen, the results obtained with the two methods were not significantly different from each other, nor with the HPLC value.

4. Conclusions

Capillary electrophoresis is an effective choice for drug quality control in both chiral and nonchiral determinations. It provides results comparable to those of HPLC, with substantial advantages not only in expeditiousness but also in ease of operation – in fact, both types of determination can be accomplished simply by changing the composition of the

background electrolyte. The results provided by both methods are similarly accurate and precise. The high resolution achieved under chiral conditions allows one to adjust the sensitivity (by altering the injected sample volume) in order to determine enantiomeric excess.

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