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The determination of non-steroidal antiinflammatory drugs in pharmaceuticals by capillary zone electrophoresis and micellar electrokinetic capillary chromatography*

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Abstract: Ibuprofen, indomethacin, ketoprofen, piroxicam and diclofenac have been quantified in dragees, suspension, suppositories, capsules, injection solutions and tablets by capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC). The experiments were performed without specific sample pretreatment. The reproducibility of the method was investigated. Good quantitation was obtained in short analysis times. CE and MEKC are found to offer a good alternative to conventional HPLC methods.

Keywords: *NSAIDs; pharmaceutical formulations; capillary electrophoresis; MEKC.*

Introduction

Capillary electrophoresis, with its two major modes, capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC), is gaining acceptance in the pharmaceutical field for the determination of drugs in formulations [1–12].

Among the non-steroidal antiinflammatory drugs (NSAIDs), sulindac, naproxen and ibuprofen have been determined in a tablet dosage form [13]; the sample preparation involved a single tablet and no internal standard was used.

This paper deals with the quantitative analysis of the most common NSAIDs in different pharmaceutical forms. The commercial products used as samples were dissolved directly in the running buffer and the internal standard (I.S.) method was used for assays with respect to standard solutions. The experiments were carried out in triplicate for each formulation within the same day and on three different days, the results obtained indicating a satisfactory precision for the method.

Experimental

Chemicals

Boric acid and formamide were obtained from UCB (Belgium), monosodium dihydrogen phosphate, disodium hydrogen phosphate and tetraborate from E. Merck (Germany), sodium dodecyl sulphate (SDS) from Sigma (Germany) and sodium hydroxide from Janssen Chimica (Belgium).

Flurbiprofen was obtained from Boots Pharmaceuticals, piroxicam from Pfizer, acemetacin and ketoprofen from Rhone-Poulenc Rorer, sodium diclofenac from Ciba–Geigy and niflumic acid from Upsa.

Capillary electrophoresis

Instruments. The experiments were performed on a Waters Quanta 4000 CE instrument (Millipore, Waters). The separation column used was a 75 μm capillary (60 cm long, 52.5 cm to the detector). Hydrodynamic injections were performed by lifting the sample vial approximately 10 cm above the height of the buffer vial for 5 s; detection was by means

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of an on-line fixed-wavelength UV detector with a zinc discharge lamp and a 214 nm-filter; the output range selected was 0.002 AUFS; the running voltage was 15 kV.

The data were collected on a Hewlett–Packard Integrator (HP 3396 Series II), processing both the areas and the heights of the peaks.

Reagents. Running buffers consisting of 40 mM SDS in 50 mM borate buffer pH 9.0 (2.94 ml of 200 mM boric acid solution, 6.37 ml of 75 mM tetraborate solution, 10.0 ml of 200 mM SDS solution and water to a final volume of 50.0 ml) and of 30 mM phosphate buffer, pH 8.0 (0.57 ml of 200 mM monosodium dihydrogen phosphate solution and 6.93 ml of 200 mM disodium hydrogen phosphate solution and water to a final volume of 50.0 ml) were freshly prepared each day.

Operating conditions. The capillary was stored overnight in water; at the beginning of each day, it was rinsed with 0.5 N NaOH, followed by water, then running buffer. Prior to each injection, an automated purge of 2 min with buffer was applied. Formamide was added as t_o marker.

Standard solutions

Standard solutions were prepared by weighing accurately the active compounds and the corresponding internal standards, dissolving and diluting them in the running buffer.

Sample preparation for ibuprofen dragees

Ten dragees were weighed and ground. An amount of the powder, equivalent to one average dragee, was transferred to a flask and mixed with the SDS–borate buffer and the I.S. (flurbiprofen) solution. The sample was filtered through paper and appropriate dilutions were made with buffer to a final concentration of 20 μg ml^{-1} for ibuprofen and 10 μg ml^{-1} for flurbiprofen.

Sample preparation for ibuprofen suspension

An amount of suspension, equivalent to 1 ml, was weighed and mixed with the SDS–borate buffer and the I.S. (flurbiprofen) solution was added. The sample was centrifuged at 3000 rpm for 10 min and dilutions were made with buffer to a final concentration of 50 μg ml^{-1} for ibuprofen and 25 μg ml^{-1} for flurbiprofen.

Sample preparation for piroxicam injection solution

The content of three vials was mixed and 1.0 ml of the resulting solution was transferred to a flask, and then diluted with the phosphate buffer and the I.S. (acemetacin) solution. Appropriate dilutions were made with buffer to have a final concentration of 20 μg ml^{-1} for both piroxicam and acemetacin.

Sample preparation for indomethacin suppositories

Four suppositories were dissolved in the SDS–borate buffer on a water bath at 36°C. An aliquot of the solution, equivalent to one suppository, was transferred into a flask, the I.S. (acemetacin) solution was added and dilutions were made with buffer to a final concentration of 20 μg ml^{-1} for both indomethacin and acemetacin.

Sample preparation for ketoprofen capsules

The content of 10 capsules was mixed. An amount of powder equivalent to one average capsule was transferred to a flask and dissolved with the SDS–borate buffer and the I.S. (flurbiprofen) solution. Appropriate dilutions were made with buffer to a final concentration of 20 μg ml^{-1} for both ketoprofen and flurbiprofen.

Sample preparation for diclofenac tablets

Ten tablets were weighed and ground. An amount of the powder, equivalent to one average tablet, was transferred to a flask and mixed with the SDS–borate buffer and the I.S. (niflumic acid) solution. The sample was filtered through paper and appropriate dilutions were made with buffer to a final concentration of 25 μg ml^{-1} for diclofenac and 50 μg ml^{-1} for niflumic acid.

Results and Discussion

The structures of the NSAIDs determined in the pharmaceutical products and those of the corresponding internal standards are shown in Fig. 1.

All the experiments were carried out by MEKC, because of the better separation achieved for the drugs and the internal standards, except for piroxicam, which displayed peak tailing in MEKC.

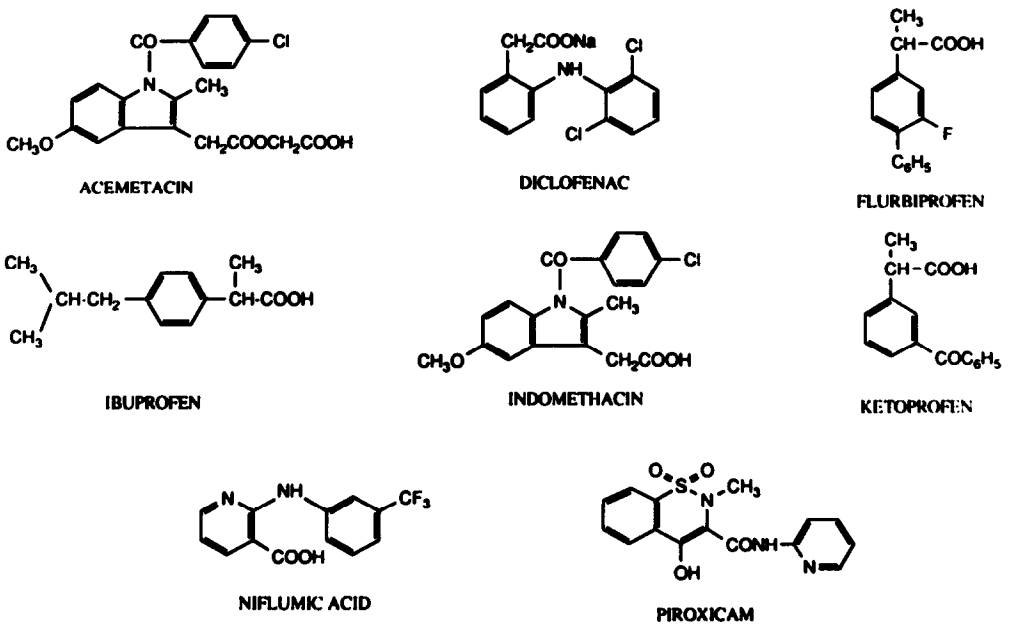

Figure 1
Structures of the NSAIDs studied.

Table 1
Regression data for the calibration curves of the NSAIDs studied measured as peak area

NSAIDs	Range of linearity	Regression data	
		Line	r
Ibuprofen (flurbiprofen)*	5–50 μg ml^{-1}	$y = 0.025x + 0.022$	0.9997
Piroxicam (acemetacin)	5–50 μg ml^{-1}	$y = 0.048x + 0.022$	0.9998
Indomethacin (acemetacin)	5–45 μg ml^{-1}	$y = 0.049x + 0.035$	0.9985
Ketoprofen (flurbiprofen)	5–50 μg ml^{-1}	$y = 0.047x - 0.024$	0.9990
Diclofenac (niflumic acid)	5–45 μg ml^{-1}	$y = 0.083x + 0.054$	0.9993

*Internal standard in brackets.
Experimental conditions as given in the text.

Table 2
Regression data for the calibration curves of the NSAIDs studied measured as peak height

NSAIDs	Range of linearity	Regression data	
		Line	r
Ibuprofen (flurbiprofen)*	5–50 μg ml^{-1}	$y = 0.025x + 0.025$	0.9998
Piroxicam (acemetacin)	5–50 μg ml^{-1}	$y = 0.042x + 0.032$	0.9996
Indomethacin (acemetacin)	5–45 μg ml^{-1}	$y = 0.058x + 0.041$	0.9996
Ketoprofen (flurbiprofen)	5–50 μg ml^{-1}	$y = 0.048x + 0.005$	0.9995
Diclofenac (niflumic acid)	5–45 μg ml^{-1}	$y = 0.079x + 0.040$	0.9994

*Internal standard in brackets.
Experimental conditions as given in the text.

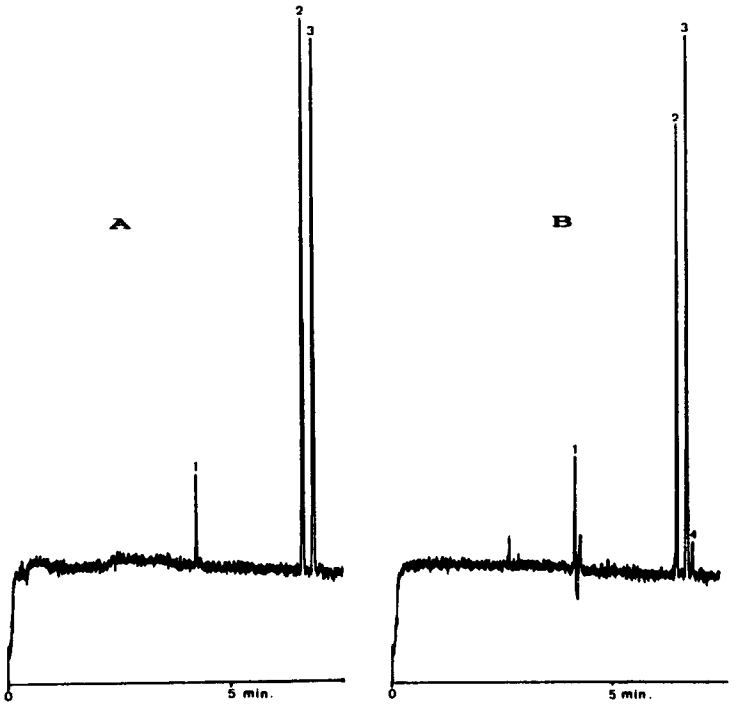

Figure 2
Chromatogram of standard solution (A) and sample solution (B) for ibuprofen suspension. Buffer: 40 mM SDS in 50 mM borate pH 9.0. 1, Formamide (A), formamide + glycerol (B); 2, ibuprofen; 3, flurbiprofen (I.S.); 4, benzoate.

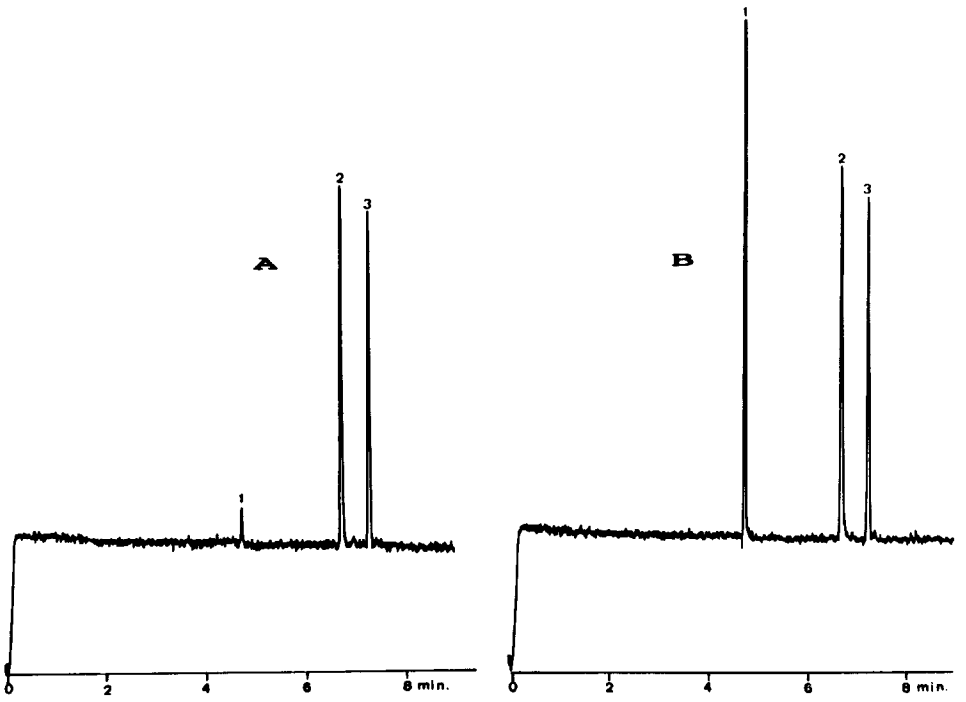

Figure 3
Electropherogram of standard solution (A) and sample solution (B) for piroxicam injection solution. Buffer: 30 mM phosphate pH 8.0. 1, Formamide (A), nicotinamide + benzyl alcohol (B); 2, acemetacin (I.S.); 3, piroxicam.

Table 3
Inter- and intra-day precision of the quantitative determination of NSAIDs in commercial products over 3 days according to calculations based on peak area

Commercial product	Amount found (% mean ± % RSD, n = 3)			
	Day 1	Day 2	Day 3	Mean
Ibuprofen dragees 200 mg	99.95 ± 3.46	95.79 ± 3.73	97.37 ± 1.75	97.70 ± 2.15
Ibuprofen suspension 20 mg ml^{-1}	95.58 ± 2.63	98.21 ± 1.14	97.61 ± 1.92	97.13 ± 1.42
Piroxicam injection solution 20 mg	98.74 ± 1.68	99.07 ± 1.08	101.59 ± 0.74	99.80 ± 1.56
Indomethacin suppositories 100 mg	96.87 ± 1.63	96.83 ± 1.74	98.33 ± 1.84	97.34 ± 0.88
Ketoprofen capsules 100 mg	101.87 ± 0.69	101.31 ± 1.19	102.43 ± 0.98	101.87 ± 0.55
Diclofenac tablets 25 mg	97.60 ± 0.66	98.62 ± 0.84	99.15 ± 1.44	98.46 ± 0.80

Table 4
Inter- and intra-day precision of the quantitative determination of NSAIDs in commercial products over 3 days according to calculations based on peak height

Commercial product	Amount found (% mean ± % RSD, n = 3)			
	Day 1	Day 2	Day 3	Mean
Ibuprofen dragees 200 mg	97.73 ± 1.29	97.52 ± 1.22	97.68 ± 0.41	97.64 ± 0.11
Ibuprofen suspension 20 mg ml^{-1}	99.71 ± 1.98	100.32 ± 0.20	100.42 ± 0.62	100.15 ± 0.38
Piroxicam injection solution 20 mg	97.50 ± 1.76	98.46 ± 0.22	102.96 ± 0.83	99.64 ± 2.92
Indomethacin suppositories 100 mg	97.44 ± 0.40	97.11 ± 0.20	97.62 ± 0.60	97.39 ± 0.27
Ketoprofen capsules 100 mg	101.85 ± 0.55	101.88 ± 0.66	102.92 ± 1.34	102.22 ± 0.60
Diclofenac tablets 25 mg	98.60 ± 0.33	99.38 ± 1.09	99.69 ± 1.67	99.22 ± 0.57

Calibration curves

Tables 1 and 2 report the regression analysis data for some of the calibration curves after calculations of area and height, respectively. The curves proved more linear with respect to the peak height, except for piroxicam, although the difference between area and height is usually not too significant. The limit of detection (considered as the amount of drug exhibiting a response twice the baseline noise) was 1 $\mu g\ ml^{-1}$ for each drug examined.

Precision

The RSD for 10 consecutive injections of the same sample was 1.5% for peak area and 0.8% for peak height.

Figure 2 shows some chromatograms obtained for the determination of an ibuprofen suspension. The baseline disturbance of the sample chromatogram is due to glycerol which is present in the formulation and the small peak eluting after the internal standard is due to the benzoate presence in the suspension.

Figure 3 shows typical electropherograms for the determination of piroxicam in an injection solution. The highest peak of the sample is due to neutral solutes present in the formulation, like nicotinamide and benzyl alcohol.

Tables 3 and 4 show the amounts of drugs determined in commercial products using peak area and peak height, respectively.

Although it is documented that peak height is non-linear at high sample concentration [14], the amount of NSAID found in the formulations according to calculations based on peak area are almost always found to coincide with the results given by peak height measurements, apart from ibuprofen suspension. In some cases, the RSD is higher after calculating the peak area, which can be attributed to partial peak asymmetry.

MEKC and CZE have proved to be good

methods for the quantitative analysis of NSAIDs in pharmaceutical formulations. Sample preparation involving dissolution and dilution of the formulations in the running buffer is perfectly adequate.

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