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ORIGINAL PAPER

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Capillary zone electrophoresis for simultaneous determination of seven nonsteroidal anti-inflammatory drugs in pharmaceuticals

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Abstract A simple capillary zone electrophoresis (CZE) method has been developed for analyzing seven nonsteroidal anti-inflammatory drugs (NSAIDs)—sulindac (SU), ketoprofen (KE), indomethacin (IN), piroxicam (PI), nimesulide (NI), ibuprofen (IB), and naproxen (NA). The separation was run using borate buffer (60 mmol L^{-1}, pH 8.5) containing 13% (*v*/*v*) methanol at 20 kV, and detected at 200 nm. Several conditions were studied, including concentration and pH of borate buffer, methanol percentage, and separation voltage. In method validation, the calibration plots were linear over the range 40.0–500.0 μmol L^{-1}. In intra-day and inter-day analysis, relative standard deviations (RSD) and relative errors (RE) were all less than 5%. The limits of detection were 10 μmol L^{-1} for SU, IN, PI, and 20 μmol L^{-1} for KE, NI, IB, NA (S/N = 3, sampling 6 s by pressure). All recoveries were greater than 95%. This method was applied to the quality control of six NSAIDs in pharmaceuticals using NI as internal standard (IS). The assay results were within the labeled amount required by USP 25.

Keywords NSAIDs · Pharmaceuticals · Capillary zone electrophoresis

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used as analgesics and, at higher doses, as anti-inflammatory drugs in treatment of rheumatic disease and other musculoskeletal disorders [1]. They are also pharmaceuticals available as over-the-counter preparations, not only by prescription [2]. The safety and efficacy of these drugs are critically related to whether their content conforms to labeled amounts. The analytical method for quality control is important for those commercial pharmaceuticals. Seven NSAIDs, structures and p K_a [3–5] as shown in Fig. 1, were chosen for this study—sulindac (SU), ketoprofen (KE), indomethacin (IN), piroxicam (PI), nimesulide (NI), ibuprofen (IB), and naproxen (NA). In US Pharmacopoeia 25, the assay methods include several HPLC systems for SU, PI, KE, IB, NA, and spectrophotometry for IN [6]. In drug analysis, speed, simplicity, selectivity, reproducibility, and the purpose of the research are most important considerations. Changing the instrument settings and running conditions from one test to another is a major burden in routine quality-control work. Our approach has been to investigate conditions suitable for a group of analytes, not only for a single drug. Thus several tests can be performed using the same capillary buffer, voltage, etc., with acceptable results. Therefore, we tried to develop a fast and selective CE method for the simultaneous determination of these NSAIDs.

A survey of recent CE methods for NSAIDs revealed micellar buffer systems contained sodium dodecyl sulfate (SDS) and/or cyclodextrin (CD) and/or organic solvent [7–11]. Guttman et al. [12] used gel and CD derivatives for chiral separation of naproxen. Isotachophoresis has been used for individual assay of NSAIDs in pharmaceuticals [13, 14]. The nonaqueous electrolytes ammonium acetate in methanol and/or acetonitrile have been used to examine the separation behavior of NSAIDs, but not in applications [15, 16]. Bechet et al. [17] tried glycine and triethanolamine buffer for the assay of

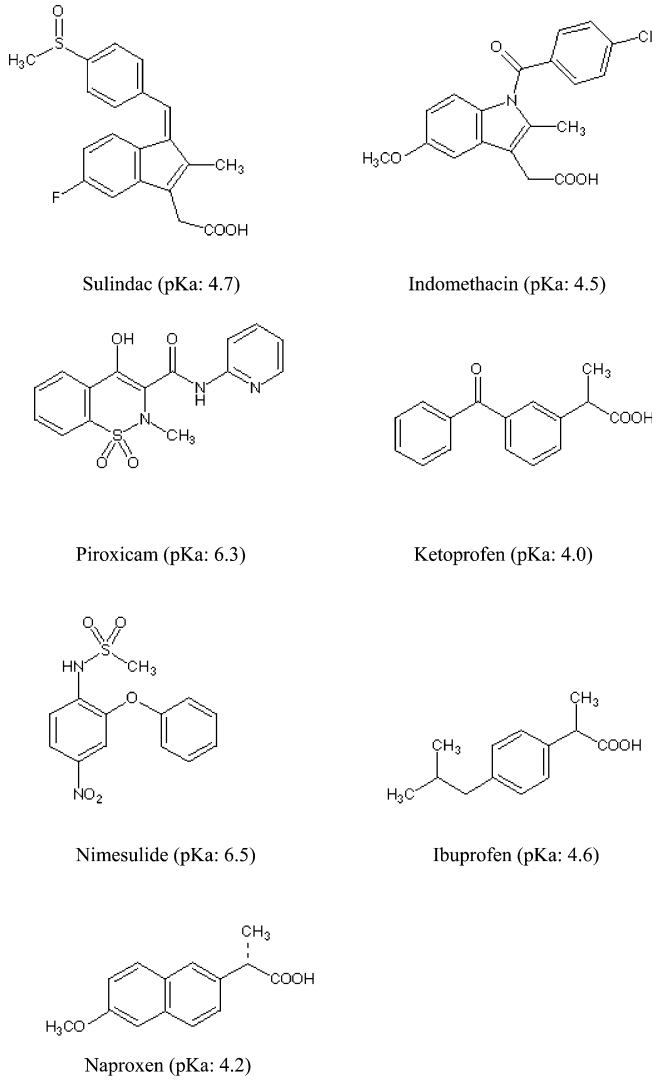

Fig. 1 Structures and p K_a of seven NSAIDs

NSAIDs. They report the use of efficient extraction methods and CE for analysis of NSAIDs in biological matrixes [18–21]. Desiderio et al. [22] used capillary electrochromatography and CE–MS for analysis of NSAIDs standards. In this study we developed a simpler CE method using borate–methanol buffer. Experiments were designed to evaluate the relative effects of several CE operating conditions on resolution and migration time. Method validation and application to pharmaceuticals were demonstrated and showed the good performance.

Materials and methods

Materials

All chemicals used were analytical grade. SU, KE (Sigma, St Louis, MO, USA), IN, PI, NI, IB, NA (Biomol, PA, USA), $Na_2B_4O_7.10H_2O$, and methanol (Merck, Darmstadt, Germany) were used without further treatment. Milli-Q water (Millipore, Bedford, MA, USA) was used for preparation of buffer and related aqueous solutions. Clinoril (SU, 200 mg/tablet), Indocid (IN, 25 mg/capsule), and Feldene (PI, 10 mg/capsule) (MSD, PA, USA), Motrin (IB, 400 mg/tablet) (Pharmacia, Michigan, USA), Proxen (NA, 375 mg/tablet) (Tanabe, Taipei, Taiwan), and Febin (KE, 25 mg/ampoule) (Tai - Yu, Hsinchu, Taiwan) were used for the applications.

CE system

A Prince CE system (PrinCE Technologies, Emmen, Netherlands) equipped with a HPLC spectrophotometer Lambda 1010 detector (Bischoff, Leonberg, Germany) was used. Capillary zone electrophoresis (CZE) was performed in uncoated fused-silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA) of 50 μm i.d. and 44.5 cm effective length (total length 70 cm). The detector was set at 200 nm. The running buffer was borate buffer (60 mmol L^{-1}, pH 8.5) containing 13% (*v*/*v*) methanol. The required pH was adjusted with 6 mol L^{-1} HCl. Sample loading was achieved by hydrodynamic injection (50 mbar, 6 s). Before start-up, the capillary was preconditioned with water, 0.1 mol L^{-1} HCl, water, 0.1 mol L^{-1} NaOH, and water, each for 10 min in regular sequence, and finally with running buffer for 5 min. Between runs, the capillary was rinsed with running buffer for 5 min. CE was carried out at 25°C and a potential of 20 kV (anode at injection end). The current gradually increased to about 67 μA during the first 15 s after power application. All operations and electropherogram acquisition were computer-controlled using DAx Data Acquisition and Analysis Software (Van Mierlo Software Consultancy, Eindhoven, Netherlands).

References and sample solutions

Stock solutions of seven NSAIDs at 2 mmol L^{-1} were prepared in methanol and suitably diluted as reference solutions. Sample solutions were prepared as follows: 20 capsules of Indocid, Feldene, or Proxen, or 20 tablets of Clinoril or Motrin were weighed and finely powdered. Ten ampoules of Febin were well mixed. An accurately weighed amount of the powder or solution of each analyte, equivalent to about 2 mmol L^{-1} of the analyte being assayed, was transferred to a 10-mL volumetric flask, dissolved by methanol with the aid of sonication for 10 min, and centrifuged at 1000*g* for 10 min. The supernatant was suitably diluted with methanol. After mixing with IS, the solution was analyzed by CZE.

Results and discussion

Experiments were performed to determine the optimum conditions. We tried not to add SDS, CD, or other additives to achieve baseline resolution. After

investigation, it was found necessary to add a suitable amount of organic modifier for separation. The conditions studied included detection wavelength, buffer system (concentration and pH of borate, amount of methanol), and analytical potential.

Wavelength for detection

The UV spectra of these seven NSAIDs in borate buffer (60 mmol L^{-1}, pH 8.5, containing 13% methanol) were scanned by means of a Beckman spectrophotometer (model DU 640B). The wavelength of maximum absorbance for all analytes was 200 nm except for NA, which was 230 nm. For simultaneous analysis, we operated at 200 nm for detection.

Buffer system

The effects of borate buffer concentration (20–80 mmol L^{-1}), buffer pH (8.0–9.5), and amount of methanol on the resolution of the seven NSAIDs are shown in Fig. 2. Separation conditions were varied and investigated simultaneously. Baseline separation and rapid migration were used for evaluation. From the results shown in Fig. 2 the CE conditions selected were: borate buffer (60 mmol L^{-1}, pH 8.5) containing 13% (*v*/*v*) methanol; voltage, 20 kV (except where specified otherwise). CZE of the drugs in borate buffer of concentration 60–80 mmol L^{-1} containing 13% (*v*/*v*) methanol achieved good resolution. To shorten the migration time and to prevent the generation of too much of Joule heat, 60 mmol L^{-1} borate buffer was chosen.

The NSAIDs are acidic compounds with p K_a of 4.2–6.5 [3–5]. For deprotonation of the compounds, borate buffer (60 mmol L^{-1}) of different pH (8.0, 8.5, 9.0 and 9.5) was studied. The results indicate that complete resolution was achieved at pH 8.5. At other pH, analytes co-eluted and separation capacity was insufficient. By changing the zeta potential and reducing the EOF, methanol could increase the separation efficiency. The effect of different amounts of methanol in the borate buffer (0, 10, 13, and 15%, *v*/*v*) on the separation of the

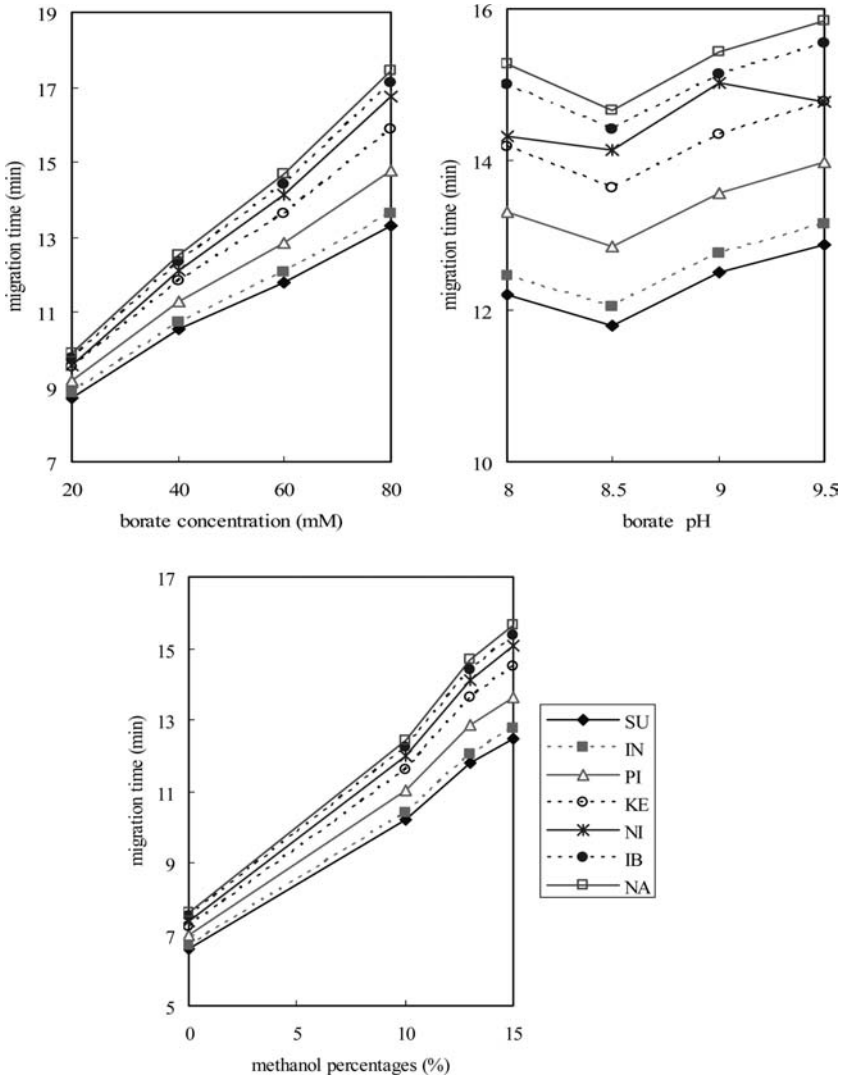

Fig. 2 Effects of borate concentration, buffer pH, and amount of methanol on the migration of seven NSAIDs, each at 500 μmol L^{-1}. CE conditions were: borate buffer (60 mmol L^{-1}, pH 8.5) containing 13% (*v*/*v*) methanol; voltage, 20 kV; uncoated fused-silica capillary, 44.5 cm (effective length)×50 μm i.d.; sample size 6 s by pressure; wavelength, 200 nm

Table 1 Regression analysis for determination of six NSAIDs in intra-day and inter-day analysis

Concentration range (μmol L^{-1})	Regression equation	Coefficient of correlation (r)
40.0–500.0		
SU	[a] $Y=(0.0062\pm0.0000)X+(0.0626\pm0.0257)$	0.9993
	[b] $Y=(0.0061\pm0.0001)X+(0.0681\pm0.0234)$	0.9996
IN	[a] $Y=(0.0069\pm0.0000)X+(0.0718\pm0.0248)$	0.9993
	[b] $Y=(0.0068\pm0.0001)X+(0.0782\pm0.0240)$	0.9993
PI	[a] $Y=(0.0063\pm0.0002)X+(0.0934\pm0.0213)$	0.9987
	[b] $Y=(0.0061\pm0.0000)X+(0.0908\pm0.0070)$	0.9985
60.0–500.0		
KE	[a] $Y=(0.0044\pm0.0000)X+(0.0672\pm0.0167)$	0.9992
	[b] $Y=(0.0044\pm0.0000)X+(0.0632\pm0.0141)$	0.9998
IB	[a] $Y=(0.0025\pm0.0000)X+(0.0274\pm0.0045)$	0.9996
	[b] $Y=(0.0025\pm0.0000)X+(0.0284\pm0.0040)$	0.9993
NA	[b] $Y=(0.0025\pm0.0000)X+(0.0209\pm0.0084)$	0.9993
	[b] $Y=(0.0025\pm0.0000)X+(0.0402\pm0.0266)$	0.9999

[a]The regression equations for intra-day analysis were calculated from the assay values of prepared standards on a single day ($n=3$)
[b]The regression equations for inter-day analysis were calculated from the assay values of prepared standards on five different days ($n=5$)

drugs was studied. Addition of methanol made separation possible. After detailed checking, baseline resolution of the analytes was achieved by use of buffer containing 13% (v/v) methanol. The running buffer used was therefore borate buffer (60 mmol L^{-1}, pH 8.5) containing 13% methanol, shown as Fig. 2.

Analytical voltage

Both the electroosmotic and electrophoretic velocities are directly proportional to the field strength, so use of highest voltage possible will result in the shortest separation time. This will result in the highest efficiency, because diffusion is the most important feature contributing to band broadening. The limiting factor here is Joule heat. Four voltages (10, 15, 20, and 25 kV) were studied. The separation voltage was set at 20 kV, which affords short migration time and acceptable current generation.

Method validation

To evaluate the quantitative applicability of this method, five different concentrations of six NSAID drugs, in the range 60–500 μmol L^{-1} for KE, IB, and NA, and 40–500 μmol L^{-1} for SU, IN, and PI, were analyzed using NI (200 μmol L^{-1}) as IS. Depending on the LOD of each analyte, we set different calibration ranges. The linearity of the dependence on analyte concentration (X, μmol L^{-1}) of the normalized peak-area ratios (Y) of analyte to IS was investigated. As shown in Table 1, the linear regression equation results ($n=5$) were indicative of high linearity ($r\geq0.9987$) between Y and X over the range studied. At the wavelength 200 nm the detection limits ($S/N=3$, injection 6 s) were 10 μmol L^{-1} for SU, IN, and PI and 20 μmol L^{-1} for KE, NI, IB, and NA. To evaluate the precision and accuracy of the method for intra- and inter-day assay, the relative standard deviation (RSD) and relative error (RE) were studied, using the peak-area ratio for replicate determinations ($n=5$) of each analyte at three levels. The RSD and RE are shown in Table 2; all were below 5.0%. We tried more than five capillaries; each led to similar results. Extraction recoveries of the six NSAIDs from pharmaceutical products spiked at three levels (40.0, 80.0, and

Table 2 Precision and accuracy for the determination of six NSAIDs in intra-day and inter-day analysis

Intra-day	Concentration known (μmol L^{-1})	Concentration found (μmol L^{-1})	RSD (%)	RE (%)
$n=3$				
SU	60	58 ± 1	2.00	−3.96
	250	251 ± 3	1.24	0.51
	400	400 ± 3	0.70	−0.10
IN	60	58 ± 0	0.36	−3.91
	250	250 ± 3	1.02	1.25
	400	401 ± 5	1.24	0.63
PI	60	57 ± 0	0.32	−4.60
	250	248 ± 3	1.19	−0.75
	400	398 ± 4	0.92	−0.51
KE	80	78 ± 2	2.35	−0.31
	250	252 ± 3	1.23	0.64
	400	402 ± 6	1.50	0.58
IB	80	79 ± 3	3.60	−1.29
	250	251 ± 3	1.10	0.45
	400	401 ± 3	0.84	0.48
NA	80	80 ± 2	2.87	−0.31
	250	252 ± 3	1.24	0.69
	400	401 ± 3	0.73	0.18
$n=5$				
SU	60	60 ± 2	2.95	0.72
	250	251 ± 3	1.07	0.33
	400	401 ± 4	1.09	0.14
IN	60	59 ± 1	1.80	−2.42
	250	250 ± 3	1.10	−0.04
	400	401 ± 5	1.19	0.20
PI	60	58 ± 1	2.11	−2.88
	250	251 ± 6	2.46	0.30
	400	403 ± 4	0.89	0.87
KE	80	80 ± 1	1.39	0.04
	250	253 ± 2	0.88	1.37
	400	398 ± 4	0.97	−0.54
IB	80	80 ± 2	2.60	0.39
	250	253 ± 1	0.40	1.14
	400	401 ± 2	0.48	0.21
NA	80	80 ± 1	1.15	0.61
	250	250 ± 3	1.13	1.26
	400	401 ± 3	0.64	0.33

Table 3 Assay results for six NSAID pharmaceuticals

Sample	Amount found (mg)	Percentage of claimed content
SU		
1	199.3 ± 1.3	99.5
2	204.6 ± 2.0	102.3
3	204.3 ± 1.9	102.1
4	203.0 ± 0.9	101.5
Mean		101.4
SD		1.3
IN		
1	25.1 ± 0.5	100.4
2	25.9 ± 0.9	103.6
3	25.5 ± 0.3	102.0
4	25.3 ± 0.2	101.2
Mean		101.8
SD		1.4
PI		
1	10.0 ± 0.1	100.0
2	10.0 ± 0.0	100.0
3	10.0 ± 0.0	100.0
4	10.0 ± 0.1	100.0
Mean		100.0
SD		0.0
KE		
1	25.1 ± 0.3	100.4
2	25.1 ± 0.3	100.4
3	25.2 ± 0.2	100.8
4	25.1 ± 0.2	100.4
Mean		100.5
SD		0.2
IB		
1	395.0 ± 7.8	98.7
2	383.0 ± 2.1	95.7
3	392.5 ± 5.6	98.1
4	387.3 ± 2.0	96.8
Mean		97.3
SD		1.3
NA		
1	375.7 ± 0.7	100.2
2	381.8 ± 4.6	101.8
3	385.7 ± 8.0	102.6
4	396.0 ± 2.8	105.6
Mean		102.6
SD		2.3

120.0 μmol L^{-1}) were studied. All recoveries were >95%. To assess interference from degradation products, we investigated the stability of analytes during 24 h. We compared the peak-area ratios of the analytes after 1, 3, 6, 12, and 24 h, calculated the RSD, and monitored additional peaks in electropherogram. The RSD were less than 5%, and no observable peaks were noted.

Applications

Application of the method to the quality control of NSAID pharmaceuticals was investigated. The results are shown in Table 3. All of the assay results fell between 97 and 103%. Conformation with the claimed content required by USP25 is 90–110% for Clinoril, Indocid, Motrin, and Proxen and 92.5–107.5% for Feldene and Febin [6]. A typical electropherogram obtained from analysis of pharmaceuticals is shown in Fig. 3. We have established a simple and selective CZE method for assay of NSAIDs. Further studies in biological samples are under investigation.

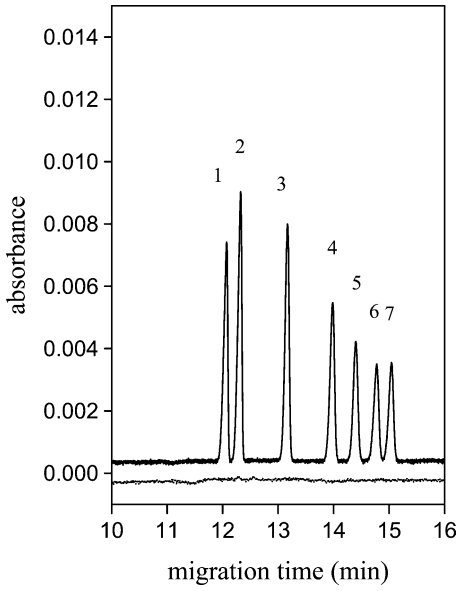

Fig. 3 Electropherograms obtained from blank (*dotted line*) and six NSAIDs pharmaceuticals (*solid line*). CE conditions: borate buffer (60 mmol L^{-1}, pH 8.5) containing 13% (*v*/*v*) methanol; applied potential, 20 kV (detector at cathode side) ; uncoated fused-silica capillary, 44.5 cm (effective length)×50 μm i.d.; sample size 6 s by pressure; wavelength, 200 nm. Peaks:*1* SU; *2* IN; *3* PI; *4* KE; *5* NI (I.S.); *6* IB; *7* NA

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