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Optimization of sample stacking for the simultaneous determination of nonsteroidal anti-inflammatory drugs with a wall-coated histidine capillary column

A wall-coated histidine capillary column was developed for the on-line preconcentration of nonsteroidal anti-inflammatory drugs (NSAIDs) in capillary electrochromatography (CEC). A wide variety of experimental parameters, such as the sample buffer, background electrolyte (BGE) composition, concentration, sample plug lengths, water plug, and the effect of organic modifiers were studied. The relationship between peak height and injection times for the NSAIDs by variation of sample and BGE buffer concentration was investigated. On addition of sodium chloride (0.3–0.6%) to the sample zone, the stacking efficiency was increased. With acetate buffer (100 mM, pH 5.0)/ethanol (20% v/v) as BGE and sample solution in acetate buffer (0.2 mM, pH 5.0)/ethanol (20% v/v)/NaCl (0.3% w/v), NSAIDs could be determined at low μM levels without sample matrix removal. The detection limit was 0.096 μM for indoprofen, 0.110 μM for ketoprofen, 0.012 μM for naproxen, 0.023 μM for ibuprofen, 0.110 μM for fenoprofen, 0.140 μM for flurbiprofen, and 0.120 μM for suprofen. The method could be successfully applied to the simultaneous determination of NSAIDs in urine. The recoveries were better than 82% for all the analytes. The present method enables simple manipulation with UV detection for the determination of NSAIDs at low concentration levels in complex matrix samples.

Keywords: Capillary electrochromatography / Histidine / Nonsteroidal anti-inflammatory drugs / Sample stacking / Stationary phase DOI 10.1002/elps.200305780

1 Introduction

A number of high-performance liquid chromatographic (HPLC) methods for the determination of nonsteroidal anti-inflammatory drugs (NSAIDs) in biological samples have been described in the literature [1–5]. Due to its great resolution power and shorter separation time [6–9], capillary electrophoresis (CE) is rapidly developing as an alternative separation method to traditional HPLC. However, the sensitivity of CE is substantially lower than that of HPLC when common detectors are used, making it unsuitable for many practical applications. Different approaches for overcoming these limitations of CE have been studied, such as sample concentration [10–13] and the use of highly sensitive detection systems [14–18]. The three most widely used concentration techniques in CE are field-amplified sample stacking (FASS) [19–21], isotachophoresis (ITP) [22–24], and solid-phase extraction (SPE) [25–30].

The preconcentration technique of first choice in general seems to be SPE, because of its high concentration ability, diversification, relative simplicity, and clean-up capability. Moreover, it is not a convenient method for the on-line preconcentration in CE. FASS is one of the simplest sample concentration techniques. Theoretically, the amount of stacking is proportional to the field enhancement ratio for the sample buffer and the background electrolyte (BGE); the larger the difference in concentration, the narrower is the peak and the greater the amount of stacking. But longer sampleplug lengths augment the dispersive effects, resulting in additional broadening of the bands.

Capillary electrochromatography (CEC) represents one of the most recently developed microseparation methods, combining features of both HPLC and CE. In CEC, specific solute/stationary phase interactions can be obtained through the use of well-designed capillary chromato-

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Abbreviation: NSAID, nonsteroidal anti-inflammatory drug

graphic experiments, beside the electrophoretic mobility difference. In the CEC determination of anions, electroosmotic flow (EOF) reversal can be originated from the appropriate stationary phase, hence only a simple BGE can be used for the electrophoretic separation and the addition of EOF modifier is not required. Therefore, a lower detection limit is always obtained compared to that of bare fused-silica columns [31–33]. Electrophoretic and chromatographic methods for preconcentration can be used separately or consecutively for the enhancement of sensitivity of CE. Liquid-phase microextraction has been reported by Pedersen-Bjergaard and Rasmussen (34) for the analysis of naproxen in urine. Methods combining both a chromatographic and electrophoretic component simultaneously have the potential to provide even greater enhancements in sensitivity [15, 35–39].

Based on the above-mentioned property, a CEC stationary phase coupled with the FASS technique was expected to be an appropriate method for the determination of NSAIDs in biological samples. In a previous study, we have prepared an open-tubular column with the wall coated with histidine for the CEC separation of plant phenols [32]. The column also exhibited fine stability and good separation efficiency for NSAIDs [9]. The two major separation mechanisms, namely solute/bonded phase interactions and electrophoretic mobility, combined with the FASS technique will be investigated to demonstrate how the sensitivity enhancement and resolution of these mixtures can be affected by the appropriate choice of experimental variables.

2 Materials and methods

2.1 Apparatus

All experiments were carried out in a laboratory-built unit consisting of a ± 30 kV high-voltage power supply (Gamma High Voltage Research, Ormond Beach, FL, USA), a UV-visible detector (Model L-4200, Hitachi, Japan), and an integrator (Model D-2500, Hitachi, Japan). Fused-silica capillaries (75 μm ID; Polymicro Technologies, Phoenix, AZ, USA) were flushed with 1 M NaOH for 30 min.

2.2 Reagents and chemicals

Most chemicals were of analytical reagent grade from Merck (Darmstadt, Germany). Purified water (18 MΩ-cm) from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to prepare all solutions. Indoprofen, ketoprofen, naproxen, ibuprofen, fenprofen, flurbiprofen (calcium salt of the hydrate), suprofen, and histidine were obtained from Sigma (St. Louis, MO, USA). Acetic acid, hydrochloric acid (Merck), sodium acetate and sodium hydroxide (Wako, Japan), benzyl alcohol, acetone, methanol, 1-propanol and sodium chloride (Acros, Geel, Belgium), 3-chloropropyltrimethoxysilane (Aldrich, Milwaukee, WI, USA) and *m*-xylene (Janssen, Belgium) were purchased from the indicated sources. Stock solutions (0.01 M) of the drugs were prepared in methanol and diluted appropriately with methanol prior to use. All solvents and solutions for CEC analysis were filtered through a 0.45 μm PTFE (Millipore) or cellulose acetate membrane (Whatman).

2.3 Column preparation and CEC conditions

The detailed procedures for the preparation of the wall-coated histidine capillary column have been described previously [32]. Fused-silica capillaries (75 μm ID) were first flushed with 1 M NaOH (30 min), then pure water (15 min), 1 M HCl (30 min), and pure water (15 min). Before silanization, the capillaries were rinsed with methanol (5 min) and then dried in a gas chromatography (GC) oven at 110°C for 1 h under a nitrogen flow of 2.5 $kg \cdot cm^{-2}$. The silanol group of the capillary column was activated with 3-chlorotrimethoxysilane in *m*-xylene (10% v/v) and then functionalized with histidine in the GC oven at 110 and 150°C, respectively. The resulting column was flushed successively with acetone and pure water at ambient temperature. Before analysis, the coated capillaries were preconditioned with the running buffer. They were rinsed with methanol, pure water and buffer between runs at 1 or 2 min intervals. The samples were injected by siphoning at a height difference of 10 cm for 10 s. EOF was measured with benzyl alcohol. The samples were detected by UV light absorption measurement at 220 nm.

3 Results and discussion

A wall-coated histidine capillary column was developed for the on-line concentration of NSAIDs in CEC separation. The separation procedures were adapted from the work of Pai and Liu [9]. Using the prepared column, experiments were initially performed using a mixture of acetate buffer (20 mM, pH 5.0) and ethanol (20% v/v) but with much lower sample concentration (10 μM each, except naproxen, 5 μM) compared to that used in [9] (0.5 mM each, except naproxen, 0.25 mM). For improving the detection sensitivity, the duration of sample injection using the hydrostatic mode was increased from 10 to 120 s. After sample injection, a negative voltage, −20 kV,

was applied for both sample stacking and subsequent separation. The peak height increased as the injection time increased. However, an injection time 120 s (Fig. 1) resulted in poorer resolution and overloading.

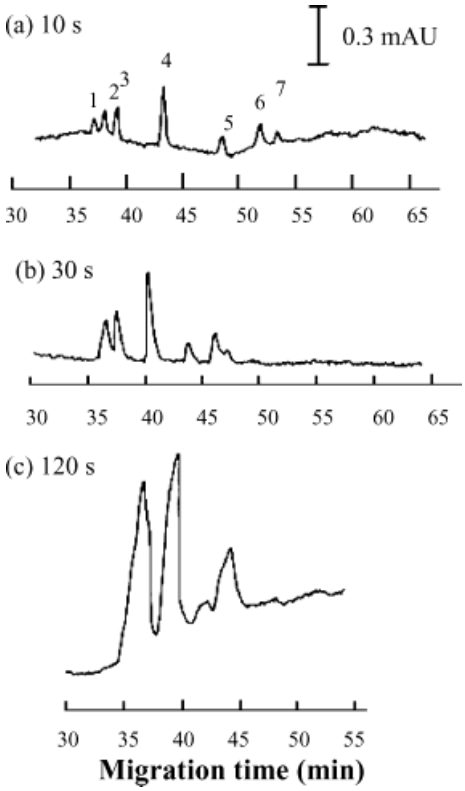

Figure 1. Effect of sample loading on separation efficiency. Column: histidine-bonded capillary, 90 cm (70 cm to the detector) × 75 μm ID; BGE, acetate buffer (20 mM, pH 5.0)/ethanol (20% v/v); sample solution in methanol, 10 μM each, except naproxen 5 μM; applied voltage, −20 kV; detection, UV at 220 nm; hydrodynamic injection (10 cm): (a) 10 s, (b) 30 s, (c) 120 s. Peak identification: (1) indoprofen, (2) ketoprofen, (3) naproxen, (4) ibuprofen, (5) fenoprofen, (6) flurbiprofen, (7) suprofen.

3.1 Optimization of the experimental conditions for FASS

To optimize the sample loading, the buffer concentrations in the analyte and the background as well as the resolution of the separation were considered. Since a higher ionic strength of the electrolyte buffer in BGE as compared to that in the sample zone is required for enhanced efficiency *via* sample stacking, the acetate buffer concentration for the BGE was varied from 100 mM to 250 mM and that for the sample from 0.2 mM to 0.8 mM, resulting in an increase of the peak area of the analytes, except the combination of 150 mM in BGE with the sample zone less than 0.2 mM as well as that of 200 mM in BGE with sample zone less than 0.6 mM, due to great discontinuity in EOFs, and the acetate buffer of 250 mM in BGE due to excessive Joule heating.

The measured relationships between peak height and injection times for some of the data were summarized in Fig. 2, illustrating the fact that increasing the sample volume causes an increase in peak height. However, this increase deviates slightly from linearity at greater loadings because of a slight loss of plate numbers, and it differs from one analyte to another. Meanwhile, a resolution less than 1.5 for flurbiprofen and suprofen was found in most cases. The decrease in peak height in some cases might be due to the significant increase in peak width caused by band broadening. It was also observed that the more early the analytes eluted, the greater concentration factor was achieved.

Considering the column performance and stacking efficiency, acetate buffer (100 mM, pH 5.0)/ethanol (20% v/v) in the electrolyte zone and acetate buffer (0.2 mM, pH 5.0)/ethanol (20% v/v) in the sample zone with an injection time of 210 s was chosen as the optimum condition (Table 1), although a worse resolution for the pair of flurbiprofen and suprofen was shown compared to that for an injection time less than 150 s. In this case, the injection volume was 23.8% of the capillary column.

3.2 Effect of the organic modifier on the resolution

To investigate the effect of the organic modifier on the stacking, the fraction of ethanol in the sample solution was increased from 0 to 30% v/v, resulting in lower conductivity and higher viscosity of the sample solution. The ionic mobility of the analyte is higher in a large dielectric constant of the medium, which is inversely proportional to the ethanol content of the solution. Therefore, the addition of ethanol would lead to a large mobility difference of analyte in the sample zone and BGE. So a better stacking effect was observed in high concentrations of ethanol (Table 2). However, when the ethanol content exceeded 30% v/v, the resolution for ketoprofen and naproxen decreased to 1.0. Meanwhile, the peak height of ketoprofen decreased significantly. Hence, 20% v/v ethanol was chosen for further work. By comparison with conventional injection, a factor of ∼ 20 in signal enhancement for NSAIDs was demonstrated.

3.3 Effect of water plug on the stacking

It is well known in CE that sample enhancement can be achieved by preparing the sample in highly diluted buffer or water. Due to the poor water solubility of the analyte,

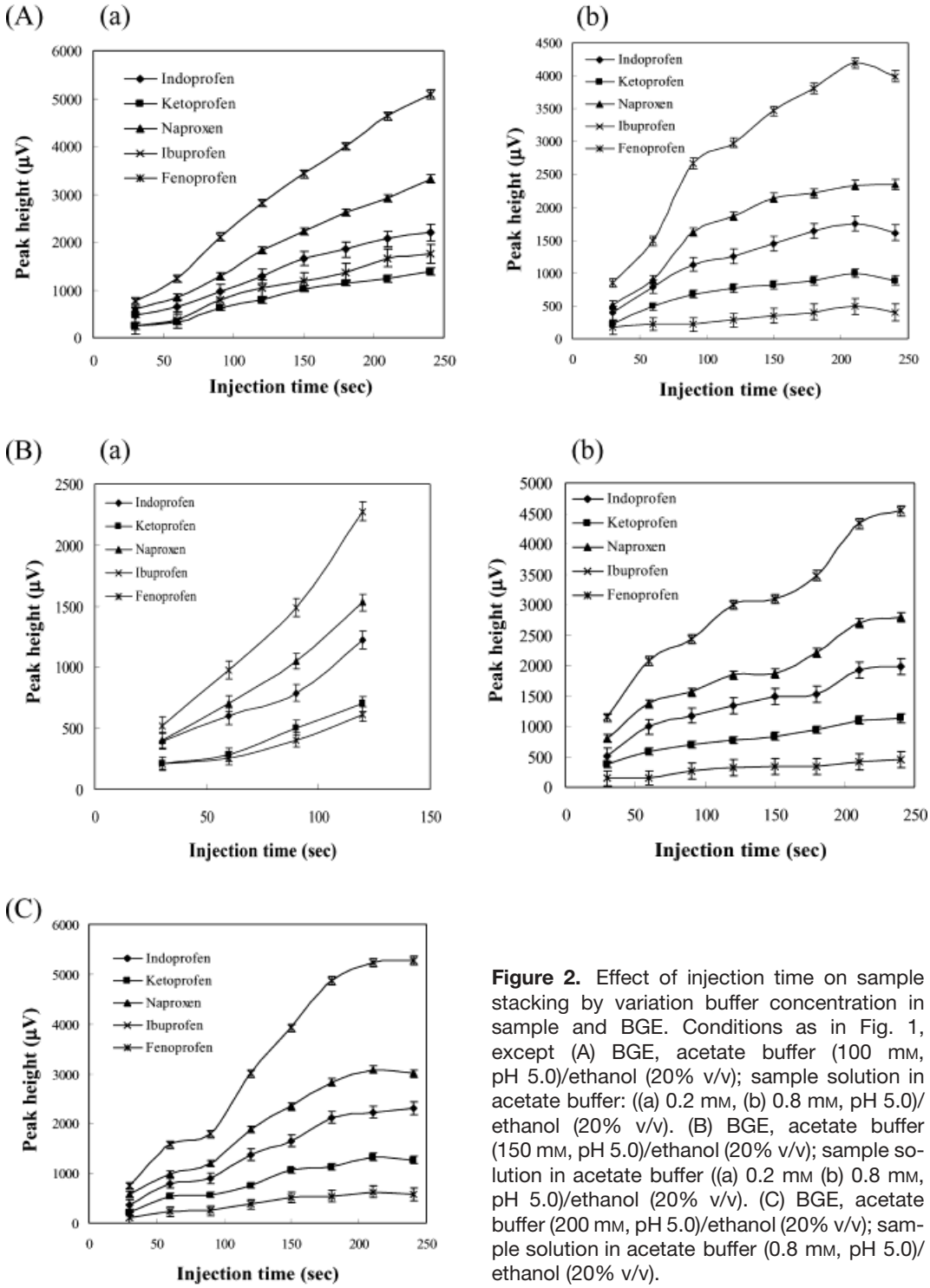

Figure 2. Effect of injection time on sample stacking by variation buffer concentration in sample and BGE. Conditions as in Fig. 1, except (A) BGE, acetate buffer (100 mM, pH 5.0)/ethanol (20% v/v); sample solution in acetate buffer: ((a) 0.2 mM, (b) 0.8 mM, pH 5.0)/ethanol (20% v/v). (B) BGE, acetate buffer (150 mM, pH 5.0)/ethanol (20% v/v); sample solution in acetate buffer ((a) 0.2 mM (b) 0.8 mM, pH 5.0)/ethanol (20% v/v). (C) BGE, acetate buffer (200 mM, pH 5.0)/ethanol (20% v/v); sample solution in acetate buffer (0.8 mM, pH 5.0)/ethanol (20% v/v).

a plug of water was introduced into the column first by raising the water reservoir up to 10 cm for 2 s, 4 s and 6 s, respectively. After the water plug was injected, the inlet end of the column was then switched to the reservoir containing the sample dissolved in acetate buffer (0.2 mM, pH 5.0)/ethanol (20% v/v). After sample injection, the column was then switched back to the high-concentration buffer and the negative separation voltage was applied. The effect of water plug on sample stacking is demonstrated in Fig. 3. Increasing the duration of sample

Table 1. Effect of injection time on intensity and resolution[a)]

Analyte	Peak height (μV) (Resolution)[b)]					
	30 s	60 s	120 s	180 s	210 s	240 s
Indoprofen	467	635	1279	1842	2072	2192
Ketoprofen	237 (2.0)	329 (2.7)	784 (2.4)	1141 (1.7)	1237 (1.7)	1392 (0.9)
Naproxen	582 (2.0)	851 (1.7)	1826 (1.6)	2620 (1.5)	2923 (1.5)	3319 (0.6)
Ibuprofen	766 (4.6)	1231 (4.1)	2808 (4.0)	4004 (2.6)	4641 (2.6)	5088 (1.9)
Fenoprofen	240 (3.8)	380 (3.0)	1033 (2.9)	1369 (2.0)	1663 (1.9)	1746 (1.7)
Flurbiprofen	241 (4.0)	379 (3.8)	790 (3.7)	1240 (1.9)	1778 (2.0)	1829 (1.9)
Suprofen	122 (1.1)	359 (0.7)	765 (1.0)	896 (0.3)	1778 (–)[c)]	1829 (–)

a) Column: histidine-bonded capillary, 90 cm (70 cm to the detector) × 75 μm ID; BGE: acetate buffer (100 mM, pH 5.0)/ethanol (20% v/v); sample solution in acetate buffer (0.2 mM, pH 5.0)/ethanol (20% v/v); sample concentration, 10 μM each, except naproxen 5 μM; applied voltage, −20 kV; detection, UV at 220 nm
b) $Rs = 2\,(t_{R2} - t_{R1})/(W_1 + W_2)$
c) Unresolved

Table 2. Effect of ethanol content in sample buffer solution on intensity and resolution[a)]

Analyte	Peak height (μV)[b)]				Resolution[c)]			
	0%[d)]	10%	20%	30%	0%	10%	20%	30%
Indoprofen	2010	2186	2172	2619	–	–	–	–
Ketoprofen	718	786	1237	1046	3.1	3.5	1.7	1.6
Naproxen	2286	2477	2923	3359	1.3	2.4	1.5	1.0
Ibuprofen	3775	4200	4641	5518	4.0	3.1	2.6	2.1
Fenoprofen	911	1463	1663	1968	2.6	2.0	1.9	1.5
Flurbiprofen	1125	1263	1778	1880	3.7	2.7	2.0	2.0
Suprofen	1125	1263	1778	1880	–	–	–	–

a) Conditions as in Table 1
b) Absorbance
c) $R_s = 2\,(t_2 - t_1)/(w_1 + w_2)$
d) Volume fraction of ethanol in sample buffer solution

injection from 60 s, 90 s to 210 s led to an improved peak shape. Meanwhile, the duration for the analysis was reduced for the longer water plug. When the injection time for the water plug was increased to 6 s and sample injection was 10 cm, 210 s, both the pairs of indoprofen and ketoprofen as well as flurbiprofen and suprofen were coeluted. Hence, only five signals could be seen for the seven analytes. It is worth mentioning that sometimes air bubbles may be generated following these in-out processes of injection of water plug, sample and buffer successively into the column, resulting in a current decreasing abruptly to zero and difficult sample injection. Meanwhile, due to no matrix removal during the process, a much longer water plug is not advantageous for the stacking since resolution would decrease in a highly diluted sample.

3.4 Salt effect on the stacking

Peptide stacking by acetonitrile-salt mixtures for CZE has been reported by Shihabi [40]. FASS is highly desirable in CZE because it offers a simple means for concentrating compounds directly on the capillary. Moreover, it is not useful for the analysis of low levels of compounds present in biological samples. For studying the feasibility of the

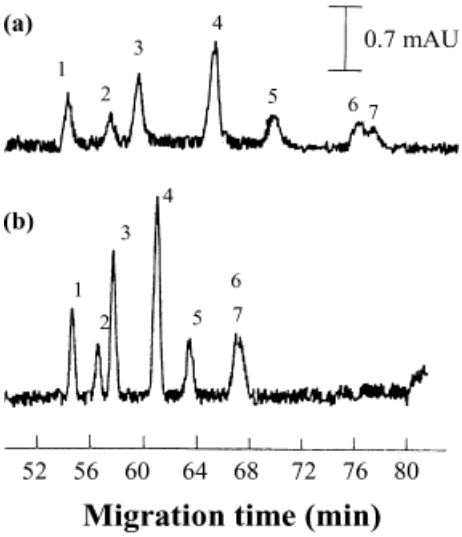

Figure 3. Effect of water plug on sample stacking. Conditions as in Fig. 2 but with sample injection (hydrodynamic mode, 10 cm, 60 s); BGE, acetate buffer (100 mM, pH 5.0)/ethanol (20% v/v) and sample solution in acetate buffer (0.2 mM, pH 5.0)/ethanol (20% v/v). (a) Without water plug injection, (b) with water plug injection (2 s) prior to sample injection. Peak identification: (1) indoprofen, (2) ketoprofen, (3) naproxen, (4) ibuprofen, (5) fenoprofen, (6) flurbiprofen, (7) suprofen.

established method applied to the analysis of complex matrix samples, sodium chloride was spiked into the sample zone. A 10- to 45-fold sample enrichment was observed in the presence of sodium chloride over the concentration range of 0.3%–0.6%. A probable reason is that, during voltage application, chloride ions move faster towards the anode and sodium ions move faster towards the cathode. The phenomena resulted in a narrow sample zone in the ethanol region. Therefore, a sharper peak was observed compared to that without sodium chloride in the sample solution. Increasing the amount of sodium chloride to 1.0% led to an obviously poorer resolution.

3.5 Stacking efficiency

Different buffer compositions were shown to amplify the stacking effects to varying degrees for the analytes (Fig. 2). The resolutions obviously decreased as a result of a larger injection volume, although they were still > 1.5 in most cases. One of the reasons might be due to the difference in field strength between sample and BGE zone affecting the local pH inside the capillary [41]. In a typical stacking experiment, protons will migrate much more quickly through the sample zone than the BGE. Therefore, the number of protons leaving the sample zone will be greater than the number of protons entering, and the pH will increase. This results in more highly dissociated analytes. Hence, some loss in resolution might be seen for the analytes of similar mobility or retention on the column. By further considering the separation efficiency after stacking, a significant increase in the number of theoretical plates for most of the analytes was shown (Table 3).

Table 3. Stacking efficiency for the simultaneous determination of several NSAIDs under different conditions[a)]

Analyte	Peak height (μV)[b)]			No. of theoretical plates (m^{-1})[c)] (Resolution)		
	Nonstacking[d)]	Stacking (A)[e)]	Stacking (B)[f)]	Nonstacking	Stacking (A)	Stacking (B)
Indoprofen	81	2 072	3 726	–	20 300	28 400
Ketoprofen	121	1 237	1 240	(1.6)	21 900 (1.7)	31 800 (1.7)
Naproxen	122	2 923	4 510	(1.7)	23 500 (1.5)	26 600 (1.5)
Ibuprofen	405	4 641	6 216	(6.0)	10 300 (2.6)	17 700 (2.0)
Fenoprofen	83	1 663	2 101	(6.0)	17 900 (1.9)	27 100 (2.3)
Flurbiprofen	85	1 778[g)]	1 245[h)]	(3.5)	9 500 (2.0)[g)]	30 000 (3.5)[h)]
Suprofen	76	–	1 166[i)]	(2.0)	–	31 400 (4.4)[i)]

a) Conditions as in Table 1
b) Absorbance
c) $N = 16\,(t_R/w)^2$
d) Sample solution in methanol; BGE: acetate buffer (20 mM, pH 5.0)/ethanol (20% v/v)
e) Sample solution in acetate buffer (0.2 mM, pH 5.0)/ethanol (20% v/v); BGE: acetate buffer (100 mM, pH 5.0)/ethanol (20% v/v)
f) Sample solution in acetate buffer (0.2 mM, pH 5.0)/ethanol (20% v/v)/NaCl (0.3% v/v), BGE: acetate buffer (100 mM, pH 5.0)/ethanol (20% v/v)
g) No resolution for the mixture of flurbiprofen and suprofen
h) Sample mixture as before but without suprofen
i) Sample mixture as before but without flurbiprofen

3.6 Calibration curves, detection limits, and reproducibilities

Under the optimum conditions, the simultaneous determination of NSAIDs was examined using a reference standard. The calibration curves displayed good linearity in the range of 1–30 μM, where y (10^3μV·s) = 8.82x (μM) − 0.06, r^2 = 0.9975 for ibuprofen; y = 2.92x − 0.23, r^2 = 0.9998 for indoprofen; y = 4.83x + 0.54, r^2 = 0.9980 for naproxen; y = 2.51x − 0.11, r^2 = 0.9991 for fenoprofen; y = 1.81x + 0.28, r^2 = 0.9990 for ketoprofen; y = 2.39x − 0.33, r^2 = 0.9995 for flurbiprofen; and y = 2.56x − 0.10, r^2 = 0.9992 for suprofen. Good RSDs ranging from 2.3 to 3.1% could be obtained for most of the concentrations (Table 4). The detection limits defined by three times signal-to-noise ratio ranged from 0.012 to 0.140 μM or 2.76 to 57.48 μg/L. Compared to the simultaneous analysis of several NSAIDs in biological samples by HPLC [3, 5] and by CZE [42] with UV detection, the results were satisfactory.

3.7 Analytical application

In order to demonstrate the applicability of the established method, blank urine samples of a healthy volunteer spiked with four different concentrations of indoprofen, ketoprofen, naproxen, ibuprofen, fenoprofen, flurbiprofen, and suprofen, respectively, were analyzed by three measurements. Since flurbiprofen and suprofen were not baseline-separated, they were not injected simultaneously. Figure 4 and Table 4 illustrate the separation of NSAIDs spiked in the urine diluted tenfold with water. The calibration curves for the determination of NSAIDs in urine were y(10^3μV·s) = 1.05x (μM) + 1.08, r^2 = 0.9975 for indoprofen; y = 1.00x + 2.26, r^2 = 0.9973 for ketoprofen; y = 1.01x − 0.12, r^2 = 0.9981 for naproxen; y = 1.07x + 0.62, r^2 = 0.9918 for ibuprofen; y = 1.06x − 1.12, r^2 = 0.9991 for fenoprofen; y = 0.96x + 3.56, r^2 = 0.9774 for flurbiprofen; and y = 1.14x + 1.38, r^2 = 0.9911 for suprofen. The migration times for NSAIDs in the urine samples were obviously longer than those in standard samples. The results were rational since the urine sample had a higher ionic strength. By further comparison of the slopes of the calibration curves to those of the standard NSAIDs, the average recoveries of three measurements for each spiked NSAIDs in urine are shown in Table 5. Most results were satisfactory.

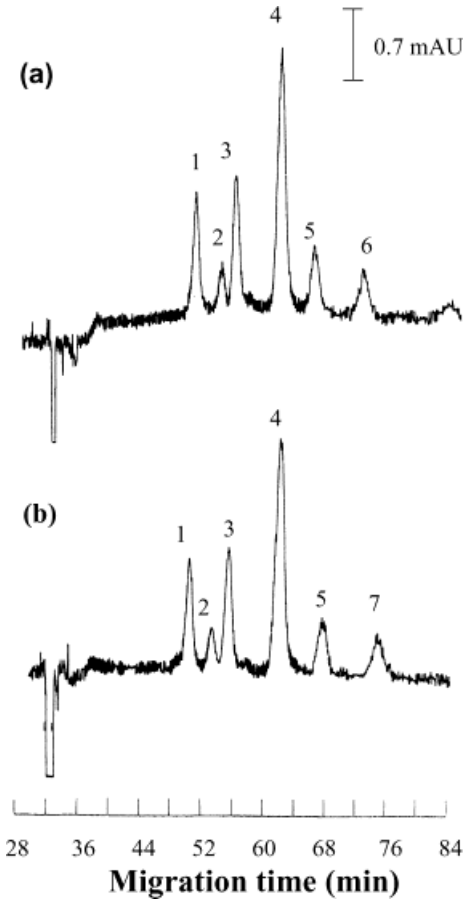

Figure 4. Electrochromatograms of blank human urine spiked with NSAIDs. Conditions as in Fig. 3, except sample solution in acetate buffer (0.2 mM, pH 5.0)/ethanol (20% v/v)/NaCl (0.3% w/v) and BGE, acetate buffer (100 mM, pH 5.0)/ethanol (20% v/v); hydrodynamic injection (10 cm, 210 s). (a) Sample mixture without suprofen; (b) sample mixture without flurbiprofen. Peak identification: (1) indoprofen, (2) ketoprofen, (3) naproxen, (4) ibuprofen, (5) fenoprofen, (6) flurbiprofen, (7) suprofen.

4 Concluding remarks

Generally, it is almost impossible that one patient would be prescribed for more than one kind of NSAIDs at the same time. Nevertheless, methods for the simultaneous determination of these widely used NSAIDs have several advantages for routine clinical and pharmaceutical analysis, for accurate determination of the analyte, and for drug-drug interaction study, as stated in [5]. In extremely large-volume sample stacking, the electrophoretic mobility of the ions must be in the direction opposite to that of the EOF. Moreover, in this work EOF reversal was indicated and the analytes were co-eluted with the EOF with the histidine-coated column. Burgi and Chien [43] reported that injections occupying a volume greater than 10 times the diffusion-limited peak width of the sample would result in a reduction in separation efficiency due to laminar flow arising from different EOFs in the sample and electrolyte zones. Too large a portion of the capillary used for stacking might cause an insufficient length left

Table 4. Analytical performance and reproducibility of the stacking method compared to the conventional injection[a)]

Analyte	Conventional injection[b)]		Stacking (standard sample)[c)]		Stacking (urine as matrix)
	t_R (min) (RSD%)[d)]	LOD (μM)[e)]	t_R (min) (RSD%)	LOD (μM, μg·L^{-1})	t_R (min) (RSD%)
Indoprofen	33.2 (1.7)	1.3	42.2 (2.9)	0.096, 27.00	51.8 (4.2)
Ketoprofen	35.5 (1.7)	1.2	44.6 (3.1)	0.110, 27.92	54.5 (4.3)
Naproxen	37.0 (0.9)	0.5	45.9 (2.3)	0.012, 2.76	57.0 (3.5)
Ibuprofen	39.9 (1.2)	0.7	49.9 (2.5)	0.023, 4.74	63.6 (3.8)
Fenoprofen	43.6 (1.3)	1.2	51.5 (2.6)	0.110, 57.48	67.1 (4.3)
Flurbiprofen	45.9 (1.4)	1.1	54.2 (2.7)	0.140, 34.20	73.5 (3.9)
Suprofen	47.1 (1.7)	1.2	55.4 (2.6)	0.120, 31.23	75.4 (4.5)

a) Column: histidine-bonded phase capillary, 90 cm (70 cm to the detector) × 75 μm ID; applied voltage, −20 kV; detection, UV at 220 nm
b) BGE: acetate buffer (20 mM, pH 5.0)/ethanol (20% v/v); injection: hydrodynamic (10 s, 10 cm); sample concentration (in methanol), 0.5 mM each, except naproxen 0.25 mM
c) BGE: acetate buffer (100 mM, pH 5.0)/ethanol (20% v/v); injection: hydrodynamic (210 s, 10 cm); sample solution in acetate buffer (0.2 mM, pH 5.0)/ethanol (20% v/v)/NaCl (0.3% w/v); concentration, 10 μM each, except naproxen 5 μM
d) Average of five measurements between run to run
e) Signal-to-noise ratio 3

Table 5. Recovery (%) for the simultaneous determination of NSAIDs in urine[a)]

Analyte	Spiked concentration (μM)			
	5	15	20	30
Indoprofen	105.6 (9.0%)[b)]	107.7 (7.6%)	105.3 (9.1%)	100.6 (8.4%)
Ketoprofen	126.8 (7.4%)	111.4 (8.9%)	109.4 (6.4%)	104.7 (3.5%)
Naproxen	93.4 (10.2%)	99.3 (9.3%)	99.0 (7.4%)	99.7 (2.5%)
Ibuprofen	106.6 (8.2%)	103.8 (8.3%)	101.0 (7.5%)	101.0 (7.5%)
Fenoprofen	82.2 (9.4%)	99.3 (6.5%)	96.6 (5.8%)	96.6 (5.8%)
Flurbiprofen	115.8 (8.1%)	107.2 (7.9%)	109.7 (7.7%)	105.5 (5.0%)
Suprofen	112.0 (9.7%)	106.5 (8.4%)	103.6 (7.3%)	103.2 (7.0%)

a) Column: histidine-bonded phase capillary, 90 cm (70 cm to the detector) × 75 μm ID; hydrodynamic injection (210 s, 10 cm); sample solution in the mixture of acetate buffer (0.2 mM, pH 5.0)/ethanol (20% v/v)/NaCl (0.3% w/v), BGE: acetate buffer (100 mM, pH 5.0)/ethanol (20% v/v); applied voltage, −20 kV; detection, UV at 220 nm
b) RSD for the recovery of three measurements in 2 mL of urine, tenfold diluted prior to use

for the separation. In this work, an injection volume of ~ one-fourth of the capillary column could be successfully applied for the analysis of NSAIDs in urine or other high-electrolyte matrix samples. This might be due to the high affinity, high selectivity, and high separation efficiency of the prepared column.

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