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Journal of Chromatography B, 712 (1998) 153–160

JOURNAL OF CHROMATOGRAPHY B

High-performance liquid chromatography with a column-switching system and capillary electrophoresis for the determination of ibuprofen in plasma

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Received 29 July 1997; received in revised form 6 February 1998; accepted 10 March 1998

Abstract

Quantitative aspects of high-performance liquid chromatography with a column-switching system (CSS–HPLC) and capillary electrophoresis (CE) were investigated for the determination of ibuprofen in plasma. For CSS–HPLC, 100 μl of plasma was directly injected onto the column system for the three separation steps: (1) deproteinization and fractionation of plasma samples with a polymer-coated mixed-function phase column, (2) concentration with an intermediate column and (3) final separation with a main column. For CE, a mixture of 50 μl of plasma and 1 ml of acetonitrile was centrifuged and the supernatant was introduced onto the capillary (66 cm×50 μm I.D.; 62 cm to detector) at 20°C. Run buffer was 250 m*M* sodium borate buffer (pH 8.5) and applied electric field was 379 V cm^{-1}. Linear dynamic ranges were 0.1–250 μg ml^{-1} in CSS–HPLC and 1–1000 μg ml^{-1} in CE. Intra-day and inter-day coefficients of variation were less than 5.6% and 6.5% for CSS–HPLC, 6.3% and 6.5% for CE, respectively. The limits of detection ($S/N=3$) for CSS–HPLC and CE were 25 ng ml^{-1} and 300 ng ml^{-1}, respectively. CSS–HPLC was superior in simplicity and sensitivity, while CE was better in efficiency, rapidity, and cost.

Keywords: Ibuprofen

1. Introduction

Ibuprofen is one of the nonsteroidal anti-inflammatory drugs. It has a chiral center (Fig. 1) and is commercially available as a racemic drug. In vivo and in vitro studies indicate that only the *l*-isomer of ibuprofen has clinical activity [1]. Current investigations on the analysis of ibuprofen seem to be focused on the separation of the two enantiomers [2–13]. However the *d*-isomer, which is considered clinically inactive, is slowly and incompletely (by about 60%) converted to the *l*-isomer in vivo [1]. Therefore, the development of a simple and reproducible method for the determination of total ibuprofen in vivo, in addition to the chiral separation, is important for the pharmacokinetics. Several approaches were developed for the determination of ibuprofen in various pharmaceutical forms [14] and serum [15] by high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE). HPLC analysis of ibuprofen in serum or plasma generally needs several pre-treatment processes, in-

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0378-4347/98/$19.00
PII: S0378-4347(98)00134-0

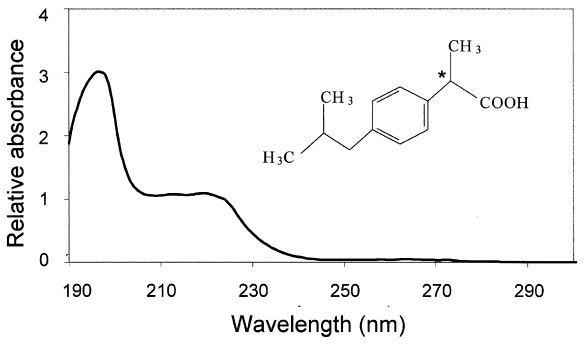

Fig. 1. Structure and UV absorption spectrum of ibuprofen. Concentration: 20 μg ml^{-1} in acetonitrile. Path length = 1 cm. *Indicates the chiral carbon atom.

cluding filtration, deproteinization, solvent extraction, and concentration [16–24]. Although pre-treatments are necessary to prevent column choking and to improve reproducibility and/or separation efficiency, they are time consuming and may contribute to experimental errors. CE methods are a little simpler and faster with respect to the deproteinization step and analysis time, but the reported detection limits of 1 μg ml^{-1} [14] and 8 μg ml^{-1} [15] are not sensitive enough for the pharmacokinetics studies of the drug in vivo.

Recently, a new silicone polymer-coated mixed-functional (PCMF) silica packing material was developed by Kanda et al. for a direct determination of drugs contained in serum [25]. A silicone polymer-coated (PC) silica had advantages such as a good separating efficiency and a strong alkali resistance, and a mixed-functional (MF) stationary silica phase was designed so that large protein molecules were quickly eluted [26,27]. It was shown that the combination of the MF stationary phase and the silicone PC silicas improved recovery, column efficiency and durability of the PCMF column by reducing the undesirable interactions with the silica surface [25]. Shirota et al. also reported an increased concentration sensitivity for the drug molecules such as diazepam, carbamazepine, and phenobarbital, without any loss in the chromatographic efficiency when analyzed by semi-microcolumn LC with a PCMF precolumn [28].

In this study, we utilized high-performance liquid chromatography with a column-switching system (CSS–HPLC) equipped with a PCMF column for a simple and sensitive determination of ibuprofen in plasma. The CSS–HPLC method directly used an intact plasma sample without any pretreatment. A CE method with a rapid one-step deproteinization by using acetonitrile was also attempted for a fast and sensitive determination of ibuprofen. Both methods sucessfully analyzed ibuprofen in plasma with high sensitivity (25 ng ml^{-1} for CSS–HPLC, and 300 ng ml^{-1} for CE). We also applied the two methods for the pharmacokinetics of the drug in vivo.

2. Experimental

2.1. Chemicals

Ibuprofen was obtained from Sigma (St. Louis, MO, USA). Oxaprozin was obtained from Cilag (Schaffhausen, Switzerland). ACS reagent grade boric acid was from Tedia (Ohio, USA) and HPLC-grade acetonitrile was from Merck (Darmstadt, FR, Germany). Water was purified with a Milli-Q TM/Milli-RO® Water System (Bedford, MA, USA) and a 0.22 μm-Millipak® 40 (Bedford, MA, USA). All other chemicals were reagent grade.

2.2. Sample preparation

Ibuprofen (10 mg kg^{-1}) was administrated orally to rats (male Sprague–Dawley, 250±20 g). 250 μl of blood was collected from jugular vein at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12 and 24 h after the administration. Collected blood was immediately transferred into 0.25 ml polypropylene tubes containing 10 μl of heparin (250 I.U.), and centrifuged at 7000 *g* for 5 min to obtain plasma. For CSS–HPLC, the plasma was filtrated through a 0.2-μm membrane filter (Millex-GV4, Millipore, MA, USA) and 100 μl was directly injected onto the CSS–HPLC without any other pre-treatment. For CE, 50 μl of plasma was mixed vigorously for 30 s with 1 ml of acetonitrile containing 30 μg of oxaprozin as an internal standard. The mixture was centrifuged at 14 000 *g* for 1 min and the supernatant was introduced into the CE system.

2.3. CSS–HPLC

The schematic diagram of CSS–HPLC is described in Fig. 2. CSS–HPLC consisted of a Nanospace SI-1 pump (Shiseido, Tokyo, Japan) indicated as pump 1, a Hitachi L-6000 pump (Hitachi, Tokyo, Japan) as pump 2, a column oven (Waters, MA, USA), a Hitachi L-40000 UV detector, and a Nanospace SI-1 column-switching system (Shiseido, Tokyo, Japan). The column-switching system was based on the following processes. At the valve state *a* (Fig. 2A), the plasma was deproteinized and fractionated through the PCMF column (CAPCELL PAK® MF, 150 mm×4.6 mm I.D., 5 μm particle size, Shiseido, Tokyo, Japan), and concentrated in the intermediate column (CAPCELL PAK® C_{18}, 35 mm×4.6 mm I.D., 5 μm particle size, Shiseido, Tokyo, Japan). At the state *b* (Fig. 2B), the concentrated ibuprofen was transferred to the main column (CAPCELL PAK® C_{18}, 250 mm×4.6 mm I.D., 5 μm particle size, Shiseido, Tokyo, Japan) and separated at 40°C. The mobile phase 1 used for the PCMF column was a mixture of 50 m*M* sodium phosphate buffer (pH 7.0) and acetonitrile (95:5, v/v), and the mobile phase 2 used for the intermediate column and the main column was a mixture of 50 m*M* sodium phosphate buffer (pH 7.0) and acetonitrile (73:27, v/v). The flow-rate was 1.0 ml min^{-1} and the detection was performed by monitoring the absorbance at 223 nm. Signals were collected and analyzed by Autochro–Win Chromatography data system (Young Lin Instrument Co., Korea).

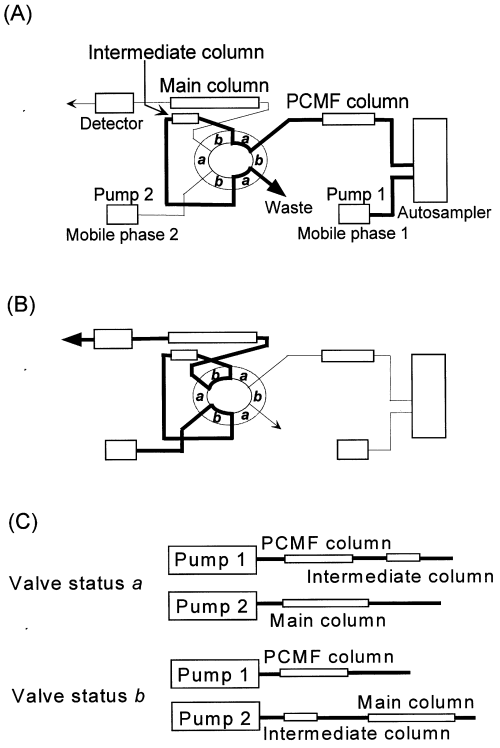

Fig. 2. Schematic diagram and separation processes of the CSS–HPLC. (A) Deproteinzation and concentration step. (B) Separation step. (C) Connections of pumps and columns for each valve status.

2.4. CE

All CE analyses were carried out using a BioFocus® 3000 system (Bio–Rad Laboratories, CA, USA) equipped with an UV/Vis detector monitoring the absorbance at 200 nm. A bare fused-silica capillary (Polymicro Technologies, AZ, USA) with an inlet to detector length of 62 cm and a total length of 66 cm (50 μm I.D.) was kept at 20°C. Run buffer was 250 m*M* boric acid (pH 8.5 adjusted with 1 *M* NaOH) and the applied voltage was 25 kV. The sample was introduced with low pressure (5 psi) for 0.2–2 s at the anodic end of capillary. After each run, the capillary was rinsed by the following sequence at 100 psi: water, 0.1 *M* NaOH, water for 1 min each, and run buffer for 3 min. BioFocus CE system software was used for system control, data collection, and data analysis.

2.5. Calibration curves

Calibration curves were obtained by measuring the blank plasma samples spiked with ibuprofen in the concentration range of 0.1–250 μg ml^{-1} for CSS–HPLC and 1–1000 μg ml^{-1} for CE. In the case of CSS–HPLC, a plot of concentration versus peak area was made, and its linear regression line was used for the determination of sample concentrations. For CE, the peak area was divided by the migration time (see Section 3.3), and its linear regression line was used for the determination of sample concentrations.

2.6. Precision and accuracy

Precision and accuracy were determined by analyzing spiked plasma samples at nine different concentrations (0.1–250 $\mu g\,ml^{-1}$ for CSS–HPLC and 1–1000 $\mu g\,ml^{-1}$ for CE) with respect to the calibration curve prepared each day. The precision of each method was expressed as the intra- and inter-day coefficient of variation (C.V.). The intra-day precision ($n=5$) was determined by analyzing the spiked plasma samples prepared in a day. The inter-day precision ($n=6$) was determined by analyzing the spiked plasma samples prepared at six different days. The accuracy of this method was shown as the % deviation of all the nine different concentrations.

3. Results and discussion

3.1. CSS–HPLC

The PCMF column was used to remove serum proteins and fractionate an analyte-containing zone under its limited chromatographic efficiency (column length=15 cm). It was necessary to know the retention behavior of ibuprofen on the PCMF column in order to determine the appropriate time for column-switching. In this study, the time for column-switching was 6.0–7.5 min. The fraction from the first separation was focused into an intermediate column regardless of the diameter of the main column (Fig. 2A). The substances trapped in the intermediate column were transferred to the main column when the valve was switched to the *b* position (Fig. 2B). Fig. 3A shows a typical chromatogram of ibuprofen (5 $\mu g\,ml^{-1}$ in acetonitrile) after the final separation using the main column. Ibuprofen was eluted with the baseline separation and an efficiency of $N=23\,000$ within 30 min at the selected CSS–HPLC conditions. Two unknown peaks (UNK) not affecting the resolution or the area of the ibuprofen peak appeared at the left side of ibuprofen peak (Fig. 3A). We assume that these peaks may be system peaks produced from the column-switching processing and identifications of the chemical nature of these peaks are beyond the scope of this study. The simpilicity was excellent since the PCMF column eliminated the lengthy sample preparation and the intermediate column concentrated ibuprofen from plasma. An additional advantage of this CSS–HPLC comes from the fact that the PCMF column is not directly connected to the main column. Therefore, by the column-switching process, the PCMF column does not have to endure high pressure, which contributes to a longer lifetime of the column.

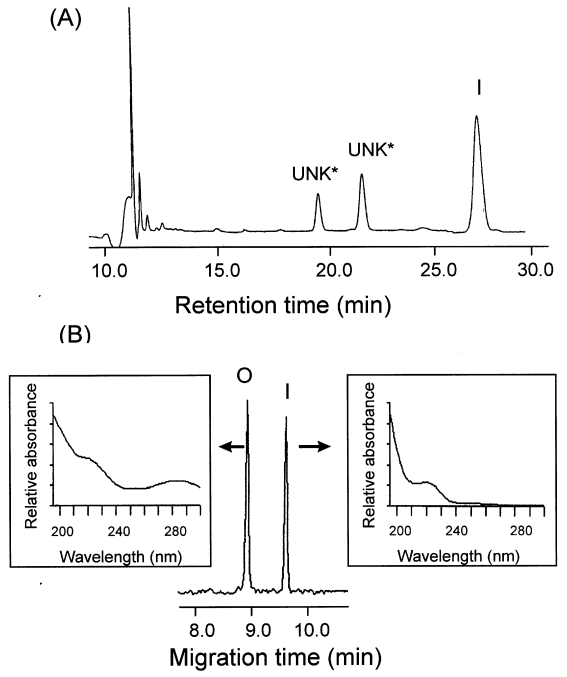

Fig. 3. Typical (A) CSS–HPLC chromatogram of the standard ibuprofen in acetonitrile (5 $\mu g\,ml^{-1}$). CSS–HPLC condition: Valve status *a*, PCMF and C_{18} intermediate column; valve status *b*, C_{18} intermediate column and main column; mobile phase 1 and 2, 50 m*M* sodium phosphate buffer (pH 7.0) – acetonitrile=95:5 and 73:27 (v/v), respectively; flow-rate, 1.0 $ml\,min^{-1}$; detection wavelength, 223 nm (see also section 2.3 for details). *UNK=Unknown peak. (B) CE electropherogram of the blank plasma spiked with 30 $\mu g\,ml^{-1}$ ibuprofen and the internal standard (oxaprozin). *Insets are UV absorption spectra of the corresponding peaks. I=Ibuprofen. O=Oxaprozin. CE condition: Run buffer, 250 m*M* boric acid (pH 8.5); capillary, 66 cm×50 μm I.D. (62 cm to detector); hydrodynamic injection for 0.6 s at 5 psi; detection, 200 nm; applied voltage, 25 kV at 20°C.

3.2. CE

A relatively high ionic strength buffer was necessary to separate ibuprofen from endogenous com-

ponents in plasma. Using 250 m*M* boric acid (pH 8.5 adjusted with 1 *M* NaOH), we could determine ibuprofen with baseline separation within 10 min, and also avoid adsorption of remaining proteins after the deproteinization process to the capillary inner wall. Fig. 3B shows the electropherogram of a blank plasma spiked with ibuprofen (30 $\mu g\,ml^{-1}$) and the internal standard (oxaprozin, 30 $\mu g\,ml^{-1}$) under the selected CE condition. The ibuprofen peak shows perfect baseline separation with a high efficiency of $N=149\,000$. In addition to generating electropherograms, UV absorption spectra could be generated when the detector was in the fast scanning mode. The produced UV absorption spectra were useful for the qualitative identification of ibuprofen in plasma samples (insets in Fig. 3B and Fig. 5B).

According to Shihabi et al. [15,30], the treatment with acetonitrile almost eliminated proteins and allowed a larger sample volume to be injected into the capillary which resulted in a better sensitivity. However, the increased injection volume can cause a substantial loss of resolution, accuracy and precision. The volume of the sample introduced into the capillary during the hydrodynamic injection can be calculated from the following equation [29]:

$$V_i = \pi \Delta P t r^4 / 8 L \eta$$

where V_i is the volume injected, ΔP is the pressure across the capillary, t is the introduction time, r is the inner capillary radius, L is the capillary length, and η is the buffer viscosity. In this study, we found the optimal injection volume was about 5.0 nl which was the amount introduced for 0.6 s at 5 psi (using $\mu=1$ centipoise at 20°C). The optimal detection wavelength was 200 nm. Under these CE conditions, ibuprofen could be easily determined with high efficiency and rapidity.

3.3. Linearity and limit of detection (LOD)

The calibration curves for ibuprofen were linear over the concentration range 0.1–250 $\mu g\,ml^{-1}$ for

Table 1
Precision and accuracy of ibuprofen determined by the CSS–HPLC

Known concentration ($\mu g\,ml^{-1}$)	Concentration found[a] ($\mu g\,ml^{-1}$)	C.V.[b] (%)	Accuracy[c] (% deviation)
Intra-day (n=5)			
0.10	0.104±0.006	4.8	4.0
0.50	0.482±0.034	5.6	−3.6
1.00	1.03±0.05	3.9	3.0
2.50	2.48±0.10	3.2	−0.8
5.00	4.87±0.16	2.7	−2.6
10.0	10.9±0.4	2.8	9.0
50.0	48.9±2.0	3.3	−2.2
100.0	104.3±3.0	2.3	4.3
250.0	245.7±9.8	3.2	−1.72
Inter-day (n=6)			
0.10	0.107±0.007	6.5	7.0
0.50	0.482±0.020	4.1	−3.6
1.00	1.07±0.04	3.7	7.0
2.50	2.62±0.08	3.1	4.8
5.00	4.97±0.15	2.8	−0.6
10.0	10.7±0.4	3.7	7.0
50.0	53.4±2.4	4.3	6.8
100.0	95.3±2.9	2.9	−4.7
250.0	247.9±8.8	3.4	−0.8

[a]95% confidence interval = mean ± (Student's $t \times$ (S.D.*)/$n^{1/2}$.
[b]C.V.: Coefficient of variation (%) = (S.D./mean) × 100.
[c]Accuracy: % deviation = [(concentration found − concentration added)/concentration added] × 100.
*S.D. = Standard deviation.

CSS–HPLC and 1–1000 μg ml^{-1} for CE. The regression equations of the curves were calculated as $y=2212.9x-574.21$ (correlation coefficient, $r=0.9999$) for CSS–HPLC, and $y=3079x+4043$ ($r=0.9998$) for CE, respectively. Here, y is the peak area (CSS–HPLC) or the peak area divided by the migration time (CE), and x is the concentration of ibuprofen. The results showed an excellent linearity ($r>0.999$) between the peak area or the peak area/migration time versus the concentration. In HPLC, all solutes move through the column and the detector at the same velocity which is equal to that of the mobile phase. However, in CE, the peak area is proportional to the migration time because later eluting compounds move through the detector slower than earlier eluting compounds. Thus, any variation in peak areas caused by changes in solute velocity can be compensated by using the peak areas divided by the migration time instead of the peak area or peak height [31]. In CE, oxaprozin was used as an internal standard in order to identify ibuprofen in plasma by comparing migration times.

The LODs ($S/N=3$) for CSS–HPLC and CE were 25 ng ml^{-1} and 300 ng ml^{-1}, respectively. These results lead to the direct submicromolar determination in plasma with no pre-treatment (CSS–HPLC) or a one-step pre-treatment (CE).

3.4. Precision and accuracy

For CSS–HPLC (Table 1), intra-day percent C.V.s were less than 5.6% in plasma samples and inter-day percent C.V.s were less than 6.5% over the nine different concentrations (0.1–250 μg ml^{-1}). For CE (Table 2), both intra-day and inter-day percent C.V.s were in the range of 1.5%–6.5% over the concentrations (1–1000 μg ml^{-1}). The accuracies of the assay are also shown in Tables 1 and 2. The absolute % deviations for all concentrations ranged from

Table 2
Precision and accuracy of ibuprofen determined by the CE method

Known concentration (μg ml^{-1})	Concentration found[a] (μg ml^{-1})	C.V.[b] (%)	Accuracy[c] (% deviation)
Intra-day (n=5)			
1.0	0.95±0.07	6.3	−5.0
2.5	2.39±0.17	5.9	−4.4
5.0	4.82±0.21	3.5	−3.6
10.0	9.7±0.4	3.1	−3.0
25.0	26.1±1.1	3.4	4.4
100.0	97.8±1.9	1.5	−2.2
250.0	254.7±9.1	2.9	1.9
500.0	493.7±14.4	2.3	−1.3
1000.0	1014.1±37.4	3.0	1.4
Inter-day (n=6)			
1.0	1.07±0.07	6.5	7.0
2.5	2.32±0.15	6.0	−7.2
5.0	4.73±0.26	5.3	−5.4
10.0	10.3±0.7	6.8	3.0
25.0	24.3±0.7	2.8	−2.8
100.0	97.2±2.0	2.0	−2.8
250.0	257.3±8.1	3.0	2.9
500.0	493.2±19.6	3.8	−1.4
1000.0	1012.0±42.5	4.0	1.2

[a]95% confidence interval = mean (Student's t (S.D.*)/$n^{1/2}$.
[b]C.V.: Coefficient of variation (%) = (S.D./mean) × 100.
[c]Accuracy: % deviation = [(concentration found − concentration added)/concentration added] × 100.
*S.D. = Standard deviation.

0.6% to 9.0% for CSS–HPLC and 1.2% to 7.2% for CE, respectively.

3.5. Applications

A typical chromatogram and electropherogram obtained from the rat plasma taken at 2 h (CSS–HPLC) and 12 h (CE) after an oral administration are shown in Fig. 4 and Fig. 5, respectively. The retention time of ibuprofen was 27.2 min in CSS–HPLC and the migration time was 9.6 min in CE. Fig. 4A and Fig. 5A show a CSS–HPLC chromatogram and a CE electropherogram obtained by a drug-free plasma. Fig. 6 shows the temporal changes of the ibuprofen concentration in the rat plasma after an oral administration of 10 mg kg^{-1} ibuprofen determined by CSS–HPLC (solid line) and CE (dashed line). The *t*-test showed that the two methods are not significantly different at the 95% confidence level [32].

In conclusion, the presented CSS–HPLC and CE methods appear to be suitable for analyzing ibuoprofen in a large number of biological samples such as

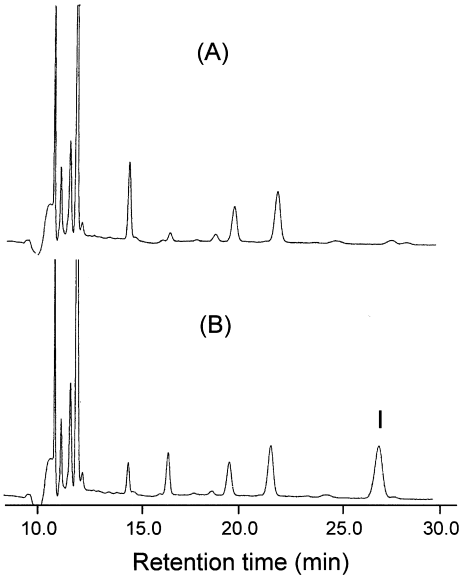

Fig. 4. CSS–HPLC chromatograms obtained by (A) a drug-free plasma and (B) a rat plasma sample which was taken at 2 h after the oral administration. CSS–HPLC condition was the same as Fig. 3(A). I: Ibuprofen.

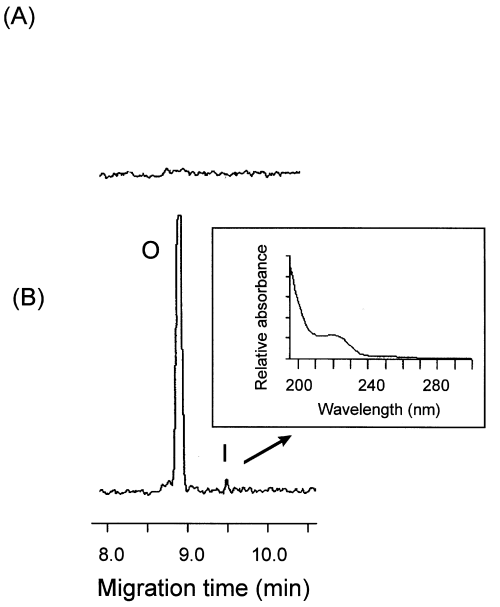

Fig. 5. CE electropherograms obtained by (A) a drug-free plasma and (B) a rat plasma sample which was taken at 12 h after the oral administration. CE condition was same as Fig. 3(B). *Inset is the UV absorption spectum of the peak labelled as I. I: Ibuprofen. O: Oxaprozin (internal standard).

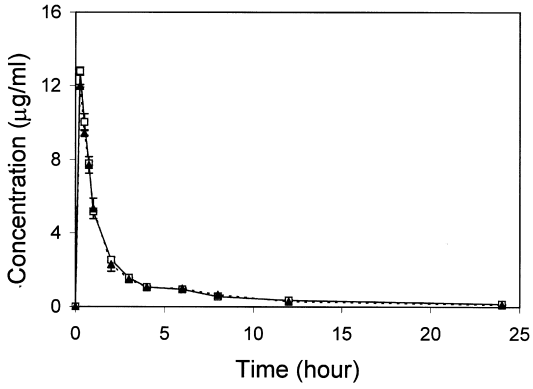

Fig. 6. Ibuprofen concentration in rat plasma versus time curves following the oral administration of 10 mg kg^{-1} ibuprofen. Ibuprofen concentration was determined by both CSS–HPLC (solid line) and CE (dashed line) methods. The vertical bars represent the standard deviations of the respective means ($n=3$).

plasma and urine. Most previous methods for the determination of ibuprofen in plasma by HPLC required deproteinization and extraction [9–24], and LOD's have been reported as 0.2 $\mu g\,ml^{-1}$–1 $\mu g\,ml^{-1}$ [12,22,23]. In this study, CSS–HPLC method was capable of directly determining ibuprofen in intact plasma samples with an increased sensitivity (LOD = 25 $ng\,ml^{-1}$) and baseline separation by using an intermediate column. We believe that this is the first report on the CSS–HPLC separation of ibuprofen in intact plasma without any pre-treatment.

Previously reported HPLC methods also required a large amount of organic solvents in the mobile phase or for the pre-treatment steps, which are expensive and environmentally hazardous. In contrast, the presented CE method could determine ibuprofen in plasma with one-step deproteinization by using 1 ml of acetonitrile, and a little amount of run buffer (<100 μl) in 10 min. In addition, we showed that both CSS–HPLC and CE methods could be applied successfully to the pharmacokinetics of ibuprofen in vivo with an excellent sensitivity. When comparing the presented CSS–HPLC and CE methods, CSS–HPLC is superior to CE in simplicity and sensitivity, while CE was better in efficiency, rapidity and cost.

Acknowledgements

This study was partly supported by Ministry of Trade Industry and Energy, Korea (1996) and the Basic Sciences Research Institute grant (BSRI-96-3418). We thank Dr. M. Suh for her support and helpful discussion.

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