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Determination of indomethacin in plasma by micellar electrokinetic chromatography with UV detection for premature infants with patent ducts arteriosus

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Abstract

A simple and selective micellar electrokinetic chromatography (MEKC) is described for determination of indomethacin in plasma. Plasma proteins are precipitated by acetonitrile. An aliquot of supernatant was evaporated and reconstituted with Tris buffer for MEKC analysis. The separation of indomethacin was performed at 25 °C using a background electrolyte consisting of Tris buffer (30 mM; pH 8.0) with 100 mM sodium octanesulfonate (SOS) as an anionic surfactant. Under this condition, a good separation with high efficiency and short analysis time is achieved. Several parameters affecting the separation of indomethacin were studied, including pH and concentrations of the Tris buffer and SOS. The linear range of the method for the determination of indomethacin was over 0.3–10.0 μg/mL; the detection limit (signal-to-noise ratio = 3; injection 0.5 psi 5 s) was 0.1 μg/mL. The proposed method for determination of indomethacin in premature infants with patent ducts arteriosus has been demonstrated.

Keywords: Indomethacin; Micellar electrokinetic chromatography; Plasma; Patent ducts arteriosus

1. Introduction

The ductus arteriosus (DA) is a normal fetal vascular channel that allows communication between the right ventricle and the aorta in uterus. This patency is promoted by continual production of prostaglandin E_1 by the ductus. Spontaneous closure of the DA usually occurs within four days of birth in most premature and full-term infants. The incidence of patent ducts arteriosus (PDA) is related to birth weight in premature infants. PDA is one of the more common congenital heart defects. Depending on the size of the PDA, the gestational age of the neonate, and the pulmonary vascular resistance, a premature neonate may develop life-threatening pulmonary over-circulation in the first few days of life [1]. Management of symptomatic PDA included pharmacologic treatment and eventual surgical ligation. The premature neonate with a significant PDA is usually treated with indomethacin. This has been quite successful in most patients and the medication indicated for PDA closure is available in the United States. Indomethacin exerts its pharmacologic effect by inhibiting prostaglandin synthesis, which is responsible for maintaining ductal patency. Most neonatal intensive care units have adopted indomethacin as the standard therapy for the treatment of symptomatic PDA, thus replacing surgical ligation. The initial indomethacin dose is commonly prescribed 0.2 mg/kg per dose given in intravenous form for all neonates regardless of gestational age, postnatal age (PNA), or renal function. Subsequent dosing is dependent on the PNA of the infant at the time of therapy initiation because indomethacin clearance is directly proportional to PNA [2]. Shaffer et al. [3] have reported that the use of pharmacokinetic/pharmacodynamics to individualize indomethacin dosing allows clinically diagnosed PDA to be permanently closed in 91% of patients treated; this is higher than the current dosing standards that have a 60–70% of closure rate. Because of the characteristic inter-individual variability of indomethacin pharmacokinetics, it would be desirable to adjust individual indomethacin dosages according to the plasma

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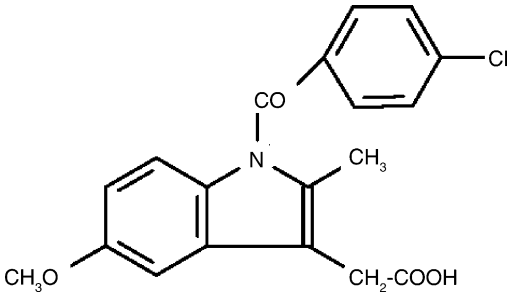

Fig. 1. Chemical structures of indomethacin.

indomethacin concentration after the first dose. In addition, it has been postulated that treatment failure in premature neonates could be attributed to an inability to maintain indomethacin at a therapeutic plasma concentration. For these reasons, reliable determination of the plasma concentrations of indomethacin for therapeutic efficacy and drug safety monitoring are very important.

Indomethacin, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid (Fig. 1) is an indole derivative, known as a nonsteroidal anti-inflammatory drug (NSAID) used in treatment of rheumatoid arthritis for antipyretic and analgesic effects. It is also the drug of choice for PDA in newborns [1].

Numerous methods are available for the determination of indomethacin, including gas chromatography (GC) [4,5], GC–mass spectrometry [6], high performance liquid chromatographic (HPLC) methods [7–18], HPLC–mass spectrometry [19,20] and capillary electrophoresis (CE) [21–25]. Indomethacin has an active carboxyl group in the structure. It is commonly used as pre-column chemical derivatization with methylation reagent such as bis(trimethylsilyl)acetamide for increasing volatility and avoiding decomposition in GC under higher temperature. However, GC with chemical derivatization is usually time-consuming and a more complicated chromatogram is obtained. HPLC techniques coupled with solid-phase extraction or protein precipitation are the most widely used for the analysis of the NSAIDs in biological matrices. However, the HPLC separations generally need a large amount of solvent waste. Recently, CE has been increasingly viewed as an alternative technique to HPLC for determination of pharmaceutics due to its high separation efficiency, speed and minimal consumption of reagents. There are several reports for the separation of NSAIDs standard by capillary zone electrophoresis (CZE) or MEKC [23–25]. However, few determined these drugs in biological samples using CE methods [21,22]. Makino et al. [22] have already reported employing CE in the analysis of indomethacin in plasma. In this method, after precipitation of serum protein with acetonitrile, an aliquot of deproteinized samples was applied directly to CZE system and the determination range was 10–100 μg/mL. However, the therapeutic concentration of indomethacin for PDA is 0.5–2.5 μg/mL. Using solid-phase extraction coupled on-line to capillary electrophoresis has been reported for determination of low-level NSAIDs in biological samples. To determine indomethacin in the therapeutic range, however, a larger volume of biological sample (5–12 mL) is needed and this is difficult in premature infants [21].

In this paper, a sensitive, economic and small sample volume (100 μL) MEKC method was developed for the determination of indomethacin in premature infants with PDA after oral administration of indomethacin. Comparing the MEKC method with the HPLC method proved that the MEKC method is satisfactory for premature infants.

2. Materials and methods

2.1. Chemicals and reagents

Indomethacin was purchased from Fluka (Buchs, Switzerland); diclofenac, fluriprofen, ketorolac, meloxicam, mefenamic acid, piroxicam, sodium dodecylsulfate (SDS), sodium pentanesulfonate (SPS), sodium hexanesulfonate (SHS) and sodium octanesulfonate (SOS) were from Sigma (Sigma, St. Louis MO, USA). Sodium hydroxide (NaOH), tris(hydroxymethyl)-aminomethane (Tris) and phosphoric acid (H_3PO_4, 85%) were supplied by E. Merck (Darmstadt, Germany). Milli-Q (Millipore, Bedford, MA, USA) treated water was used for the preparation of buffer and related drugs. Solutions of various Tris buffers at different pHs were prepared by neutralizing the related Tris solution with H_3PO_4. Solutions of SOS as surfactant at various levels were obtained by dissolving different amounts of SOS in water and then diluting with Tris buffer.

2.2. CE conditions

The Beckman P/ACE MDQ system (Fullerton, CA, USA) equipped with a UV detector and a liquid-cooling device was used. MEKC was performed in an uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 31.2 cm (effective length 21 cm) × 75 μm I.D. Samples were injected by pressure (0.5 psi) for 5 s (about 9.57 nL) and the applied voltage for separation was 10 kV. Separations were performed at about 25 °C in Tris buffer (30 mM; pH 8.0) with SOS (100 mM). Detection was carried out by the on-column measurement of UV absorption at 254 nm (cathode at the detection side). Capillary conditioning before startup is as follows: methanol for 10 min, 1N HCl aqueous solution 10 min, deionized water 2 min, 1N NaOH aqueous solution for 10 min, deionized water for 2 min. The conditioning between runs was effected by rinsing with 0.5N NaOH (5 min), deionized water (5 min), and running buffer (3 min), under positive pressure applied at the injection end. A Beckman P/ACE MDQ Microsoft software system was used for data processing.

2.3. HPLC conditions

In a set of experiments, indomethacin was determined by HPLC with UV detection, using 2,4,5-trichloronitrobenzene as an internal standard (I.S.). A Waters 717 plus autosampler, a Model 486 UV–vis detector, a Beckman system Gold programmable solvent module 126 pump and sys-

tem Gold software were used. A Purospher® STAR RP-18e column (55 mm × 4 mm; 3 μm, Merck) and mobile phase water–acetonitrile–acetic acid (v/v/v, 45.2:54.2:0.6) at a flow-rate of 1.5 mL/min were used. The column eluate was monitored at 254 nm. The solvent was filtered (Millipore, HVLP, 0.45 μm) under vacuum for degassing before use.

2.4. Reference and sample preparations

A 450 μL aliquot of human plasma was pipetted into a 1.5 mL Eppendorf vial, and 50 μL indomethacin standard solution was added. The final concentration of each vial of indomethacin in plasma samples was over the range of 0.3–10 μg/mL. An aliquot of 100 μL of spiked indomethacin plasma or patient's plasma was taken to another 1.5 mL Eppendorf vial and 200 μL of acetonitrile was added and mixed by vortexing for 1 min. The vials were then centrifuged at 35,000 × *g* for 5 min and the supernatant further for CE and HPLC study. For CE analysis, a 0.2 mL of supernatant was evaporated to dryness by freeze evaporator (EYELA UT-80 and EYELA CVE-2000) and redissolved with 30 μL of 30 mM Tris buffer (pH 8.0) containing fluriprofen (I.S.) and it was transferred to a 0.2 mL mini-vial that could be placed into the autosampler for CE analyses. For HPLC analysis, the supernatant was used after centrifugation for HPLC analysis with an autosampler (sample size, 50 μL).

2.5. Validation for CE method

Calibration curves were prepared by indomethacin at five different concentrations and a fixed concentration of flurbiprofen (I.S.) in acetonitrile–water (v/v, 50:50) to make the final concentrations in human plasma of 10 μg/mL for flurbiprofen and 0.3, 0.5, 1.0, 2.0, 5.0 and 10 μg/mL for indomethacin. The calibration graphs were established with the peak area ratio of indomethacin to I.S. as ordinate (y) versus the concentration of indomethacin in μg/mL as abscissa (x). Intra-day precision and accuracy were based on four replicate analyses for three concentration levels at 0.3, 2.0 and 10.0 μg/mL for indomethacin. Inter-day precision and accuracy were calculated from six consecutive days for these concentration levels of indomethacin.

2.6. Application

There is a limited availability of intravenous indomethacin dosage in Taiwan. An alternative would be orally administered indomethacin (0.2 mg/kg) at 12 h intervals up to three times. In a previous report, the frequency of closures and transient closures of the ductus arteriosus was similar for both routes of administrations [26]. Three premature infants with PDA were administered indomethacin by enteral route at an initial dose of 0.2 mg/kg at the Department of Pediatrics intensive care ward. Venous blood sample was withdrawn and plasma fraction was separated immediately at 8 h after first dosing. The plasma sample was stored frozen at −40 °C until analysis.

3. Results and discussion

The simple CZE method had been reported for determination of indomethacin in plasma and its detection limit is 3 μg/mL [26]. The therapeutic level of indomethacin, however, is 0.5–2.5 μg/mL. Thus, this method cannot effectively assay indomethacin in PDA neonates. Several sample pretreatment methods were examined for achieving simple and sensitive determination of indomethacin in plasma. Finally, evaporating the supernatant after precipitating the plasma protein by acetonitrile procedure was the choice. The time of supernatant evaporation needed is about 20 min. After drying, the residue was redissolved in Tris buffer and CE analysis was performed. In a preliminary test of indomethacin in human plasma at a low level by CZE under above simple sample pretreatment, an interference peak was observed in our study as shown as Fig. 2A. MEKC is the most general approach because it permits the simultaneous determination of acidic, neutral and basic drugs. Moreover, chemical substances can be separated easily from serum albumin in the MEKC analysis. The drug should be eluted in front of the solubilized serum proteins. In MEKC, two distinct phases – the aqueous and micellar phases – are used. These two phases are established by employing buffer containing surfactants that are added above their critical micellar concentrations. The very commonly used anionic surfactant, SDS, as a micellar source with Tris buffer was used to study the resolution of the analyte. The SDS at various concentrations in Tris buffer in the pH range 7.0–9.5 was tested to separate the analyte in plasma. Although indomethacin can resolve from sample matrix in Tris buffer with SDS as surfactant, a fronting peak of indomethacin with lower theoretic plate number and poor peak asymmetric factor ($A_s = 2.1$) was observed in the tested conditions. Organic solvents miscible with water are widely used as mobile phase modifiers to adjust the capacity factor or the alteration of selectivity. In general, the addition of organic solvents reduces the electroosmotic velocity, influences partitioning of analytes into micelles and expands the migration time window. Therefore, a concentration of 10% of organic modifier, including methanol and ethanol, added to Tris buffer in SDS condition was tried in this study. The organic solvents added to Tris buffer in SDS MEKC condition can make the analyte slow down the migration rate but do not promote the separation efficiency of indomethacin from matrix. Therefore, compounds with a carbon number lower than that of the SDS such as sodium octanesulfonate (SOS), sodium hexanesulfonate (SHS) and sodium pentanesulfonate (SPS) were investigated. The results are shown in Fig. 2B–E. Different anionic surfactants in Tris buffer with respect to separation efficiency, according to the plate number, were calculated. The calculation of separation efficiency was based on $N = 16(t_R/w)^2$, where N is the number of theoretical plate, t_R the migration time of the compound and w the peak width. Comparing the surfactants used for separation efficiency of indomethacin in plasma, the theoretical plates of about 6.12×10^4, 2.05×10^4 and 1.32×10^4 for SOS, SHS and SPS, respectively, were obtained in Tris 30 mM (pH 8) with 100 mM of each surfactant. The SOS has better separation efficiency as a micelle than the other surfac-

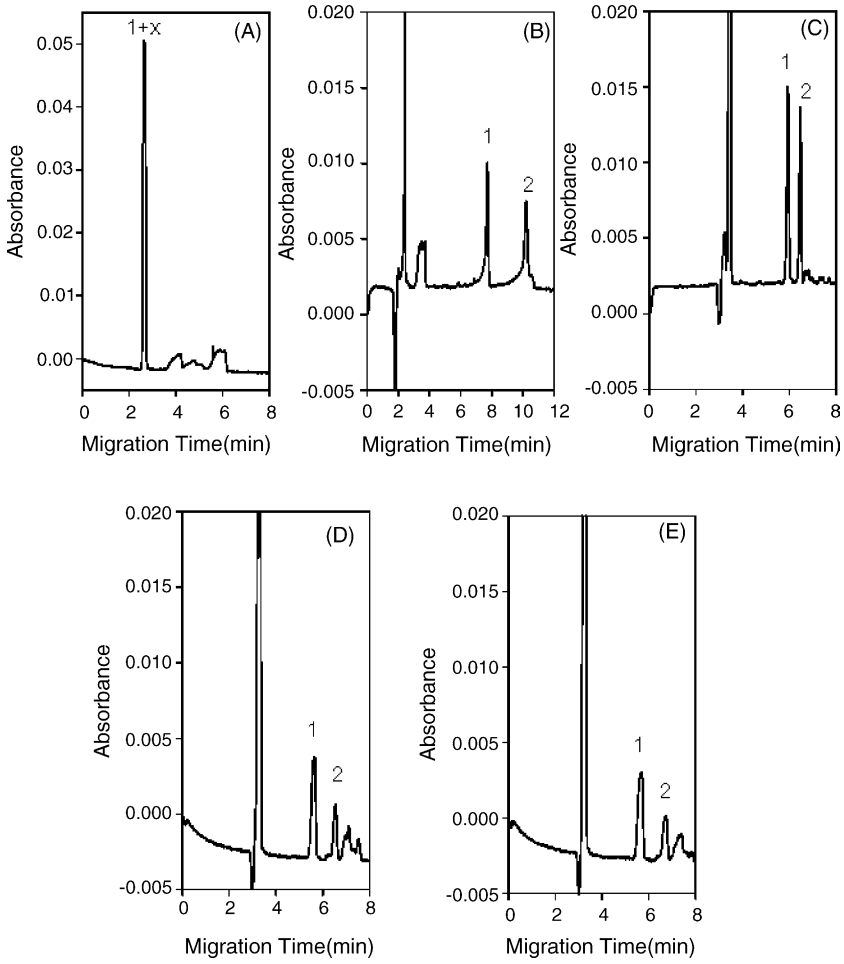

Fig. 2. Electropherograms of analyses of indomethacin in spiked plasma by (A) CZE (100 mM, pH 8 Tris buffer); (B) MEKC (Tris buffer + 100 mM SDS); (C) MEKC (Tris buffer + 100 mM SOS); (D) MEKC (Tris buffer + 100 mM SHS); (E) MEKC (Tris buffer + 100 mM SPS). Peaks 1 and 2 for indomethacin and flurbiprofen (I.S.) each at 10 μg/mL, respectively. CE conditions: applied voltage, 10 kV (detector at cathode side); uncoated fused-silica capillary, 31.2 cm (effective length 21 cm) × 75 μm I.D; sample size, 0.5 psi, 5 s; wavelength, 254 nm.

tants tested. The surfactant micelles in MEKC were interacted with indomethacin and a lot of human protein in biological sample. Human protein in biological sample strongly interacted with hydrophobic surfactant. This phenomenon made the protein highly soluble in micellar phase and retained it better than the test drug in capillary. The carbon chain length of SDS is more than that of the other surfactants tested. Indomethacin has a high lipophilic property that may strongly react with SDS resulting an asymmetric peak observed. SHS and SPS might be less interacting with protein and indomethacin, so a minor interference with a broader peak of indomethacin was observed in electropherograms. The carbon octyl of SOS effectively interacts with indomethacin and human protein. As a consequence, simple parameters affecting the MEKC using SOS as anionic surfactant for indomethacin in plasma were studied, including concentrations of the buffer, pH and SOS. Comparing different voltages (8, 10 and 12 kV were tested), 10 kV can provide suitable separation with the shortest migration time and the best resolution. After MEKC separation of indomethacin in Tris buffer, the eluted compounds were monitored at 254 nm.

3.1. Optimization of the experimental conditions

Ionic strength or concentration of buffer has significant effect on solute mobility and separation efficiency. The retention behavior of indomethacin in Tris buffer (pH 8.0) at a concentration range of 10–50 mM with 100 mM SOS as an anionic surfactant was studied. MEKC of indomethacin in Tris buffer (pH 8.0) in the concentration range of 10–50 mM causes no interference from sample matrix but flurbiprofen (I.S.) will be affected by endogenous components in Tris buffer ≤10 mM, as shown as Fig. 3. High ionic strength buffers have been used to enhance efficiencies in separation. Higher separation efficiency between analytes and plasma endogenous components was obtained in ≥30 mM. To prevent the generation of too much Joule heating resulting in a decrease of theoretical plate *N*, 30 mM of Tris buffer was chosen. The 30 mM Tris buffers with SOS (100 mM) at different pH levels (7.0, 7.5, 8.0, 8.5 and 9.0) were studied. Based on the pK_a of indomethacin at 4.5, a carboxyl group in structure can be fully dissociated as COO^- in the pH tested, and its migration velocity is only affected by electroosmotic flow (EOF). The resolution of the tested drugs

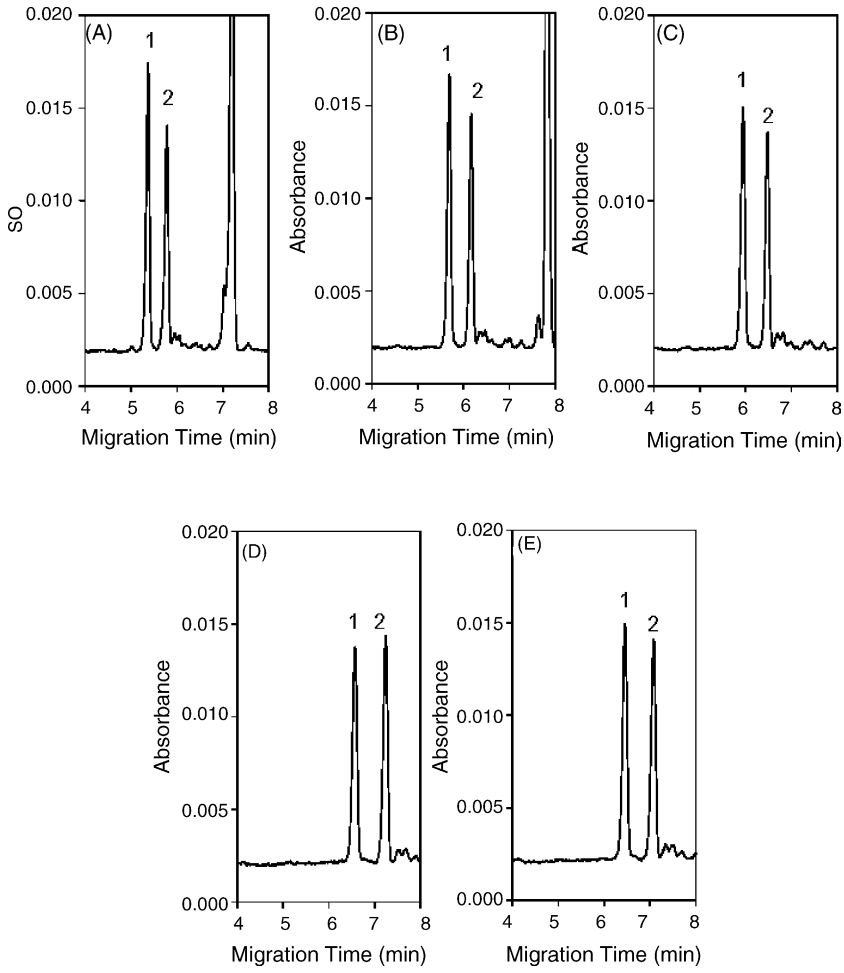

Fig. 3. Effect of concentration of Tris buffer (pH 8.0) with 100 mM SOS on the migration of indomethacin and flurbiprofen (I.S.) each at 10 μg/mL in human plasma: (A) 10 mM; (B) 20 mM; (C) 30 mM; (D) 40 mM; (E) 50 mM. Peaks 1 and 2 for indomethacin and flurbiprofen, respectively. For other CE conditions see Fig. 2.

shows no significant changes at the various pH values tested. Therefore, 30 mM Tris buffer at pH 8.0 was chosen as optimal buffer concentration and pH for determination of indomethacin in plasma. The effects of SOS at the concentration range of 50–130 mM in Tris buffer (30 mM; pH 8.0) on the separation are shown in Fig. 4. At a concentration of SOS ≥80 mM, a baseline resolution electropherograms of tested drug was observed. However, a significant peak shape improvement is obtainable by increasing the concentration of SOS, leading to a sharper and higher plate number. The optimization of the MEKC mode of CE conditions for analysis of indomethacin in plasma was set at 30 mM Tris buffer (pH 8.0) with 100 mM SOS as running buffer and the analytes were monitored at 254 nm. Under 10 kV as a separation voltage, the current (μA) produced about 115 μA in this background electrolyte. Fig. 5A–C presents the typical electropherograms of the MEKC separation of plasma blank, 10 μg/mL of indomethacin and 0.3 μg/mL of indomethacin (LOQ) with 10 μg/mL of flurbiprofen (I.S.) as reference standard spiked in plasma, respectively. Peaks 1 and 2 represent indomethacin and flurbiprofen, respectively. The migration times of indomethacin, flurbiprofen and EOF were 5.94, 6.43 and 3.04 min, respectively. The apparent mobility (μ_A) was calculated according to the equation: $\mu_A = \mu_E + \mu_{EOF} = (lL/tV)$ where l is the length along the capillary (cm) to detector, V the voltage, t the migration time (s) and L is the total length (cm) of the capillary [27]. In optimized CE conditions, the apparent mobility values of indomethacin, flurbiprofen and EOF are 1.84×10^{-4}, 1.69×10^{-4} and 3.60×10^{-4} cm^2 V^{-1} s^{-1}, respectively. According to the equation, $\mu_A = \mu_E + \mu_{EOF}$, the electrophoretic mobility values (μ_E) of indomethacin and flurbiprofen are -1.76×10^{-4} and -1.91×10^{-4} cm^2 V^{-1} s^{-1}, respectively. The electropherogram of an extracted plasma sample obtained from a patient receiving 0.2 mg/kg of indomethacin did not show interference peaks (Fig. 5D, indomethacin concentration: 1.69 μg/mL).

3.2. Analytical calibration

For evaluating the quantitative applicability of the method, five different concentrations of indomethacin over the range of 0.3–10 μg/mL in plasma were analyzed using flurbiprofen as an I.S. The linearity between the peak-area ratios (y) of the analyte to I.S. and the concentration (x, μg/mL) of the analyte was investigated. The linear regression equations for indomethacin assay were obtained as follows: $y = (0.272 \pm 0.007)x + (0.026 \pm 0.001)$ for intra-day ($n = 4$,

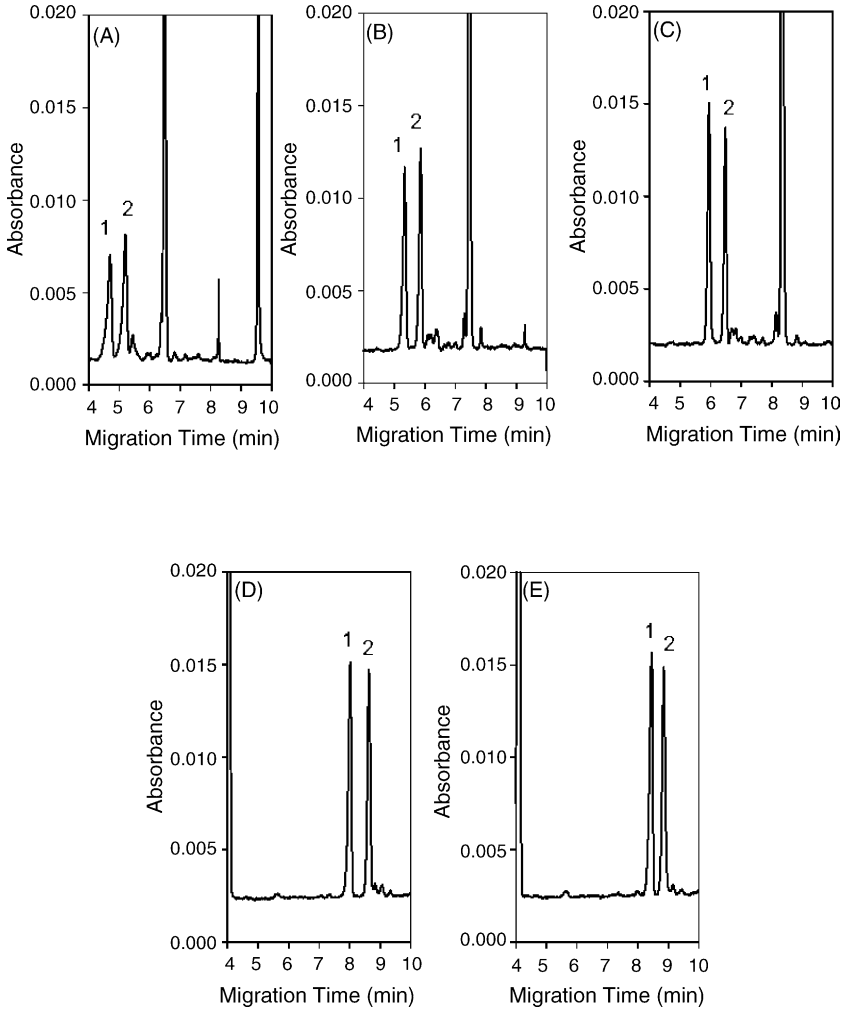

Fig. 4. Effect of concentration SOS with 30 mM Tris buffer (pH 8) on the migration of indomethacin and flurbiprofen (I.S.) each at 10 μg/mL in human plasma: (A) 50 mM; (B) 80 mM; (C) 100 mM; (D) 120 mM; (E) 130 mM. Peaks 1 and 2 for indomethacin and flurbiprofen, respectively. For other CE conditions see Fig. 2.

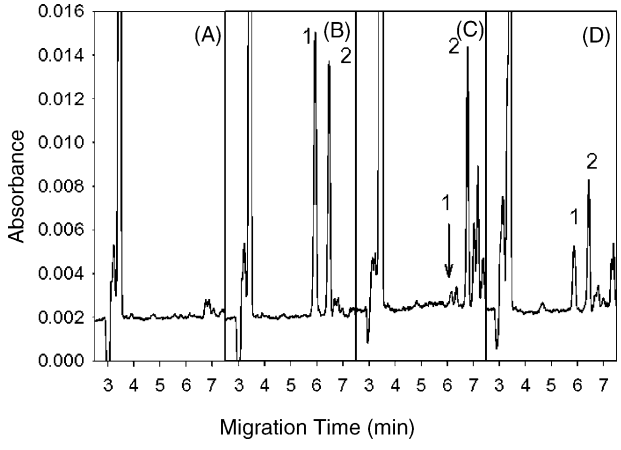

Fig. 5. Electropherograms of indomethacin and flurbiprofen (I.S.) in human plasma: (A) plasma blank; (B) plasma spiked with indomethacin at 10 μg/mL; (C) plasma spiked with indomethacin at 0.3 μg/mL; (D) plasma from a patient with PDA patient treated with 0.2 mg/kg of indomethacin. Peaks 1 and 2 for indomethacin and flurbiprofen, respectively. Calculated concentration was 1.69 μg/mL for indomethacin. For other CE conditions see Fig. 2.

$r = 0.999$) and $y = (0.269 \pm 0.013)x + (0.002 \pm 0.001)$ for inter-day ($n = 6$, $r = 0.999$). The data indicate good linearity of the proposed method. The limit of quantitation (LOQ) is the suitable injected amount that gives precise measurements. The LOQ in plasma was defined as the sample concentrations generating a peak height ten times the level of the baseline noise. The limits of detection (LOD) were calculated on the basis of the baseline noise, which was defined as the sample concentration generating a peak of height three times the level of the baseline noise (signal-to-noise ratio of 3). The LOQ and LOD for indomethacin were 0.3 and 0.1 μg/mL, respectively. The reproducibility and reliability of the proposed method were assessed at three different concentrations of indomethacin and evaluated for relative standard deviation (R.S.D.) and relative error (R.E.). As shown in Table 1, the precision of the method for indomethacin for both intra- and inter-day analyses at three concentrations are all less than 9.7% for R.S.D. and 3.3% for R.E. The selectivity of the proposed method was briefly tested on the separation of indomethacin and flubiprofen with other NSAIDs including piroxicam, diclofenac, ketorolac, meloxicam and mefenamic acid. Under present MEKC conditions, a complete separa-

Table 1
Precision and accuracy for the analyses of indomethacin in plasma

Concentration known (μg/mL)	Concentration found (μg/mL)	R.S.D. (%)	R.E. (%)
Intra-day[a] ($n = 4$)			
0.3	0.31 ± 0.02	6.4	3.3
2.0	2.00 ± 0.07	3.5	0.0
10.0	9.99 ± 0.53	5.3	0.0
Inter-day[a] ($n = 6$)			
0.3	0.31 ± 0.03	9.7	3.3
2.0	1.96 ± 0.09	4.6	−2.0
10.0	9.96 ± 0.37	3.7	−0.4

[a] Intra-day data were based on four replicate analyses and inter-day were from six consecutive days.

tion of indomethacin with other commonly used NSAIDs was obtained.

3.3. HPLC chromatogram

A HPLC chromatograms of plasma blank, plasma blank spiked with indomethacin and one plasma of a patient with PDA who took 0.2 mg/kg of indomethacin are shown in Fig. 6A–C, respectively. Peaks 1 and 2 represent indomethacin and 2,4,5-trichloronitrobenzene (I.S.), respectively. These figures illustrate the specificity of the assay, with no evidence of interference from metabolites or endogenous components. Unfortunately, when we tried two other plasma samples of PDA patients, one showed serious interference with indomethacin determination.

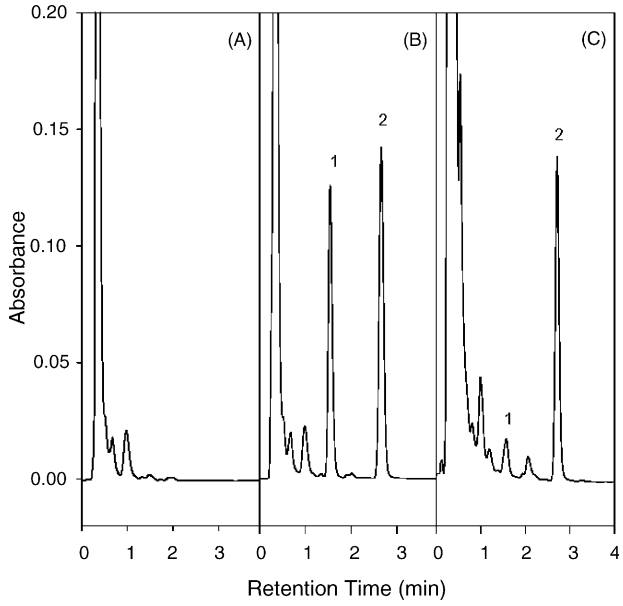

Fig. 6. HPLC chromatograms of indomethacin at 10 μg/mL and 2,4,5-trichloronitrobenzene (I.S.) in human plasma: (A) plasma blank; (B) plasma spiked with indomethacin; (C) plasma of a PDA patient who took 0.2 mg/kg of indomethacin. Peaks 1 and 2 for indomethacin and 2,4,5-trichloronitrobenzene (I.S.), respectively. HPLC conditions: LiChroCART Rp-C_{18} column (55 mm × 4 mm I.D.; 3 μm); mobile phase, water–acetonitrile–acetic acid (v/v/v, 45.2:54.2:0.6) at a flow-rate of 1.5 mL/min; detection, 254 nm. Calculated concentration was 0.93 μg/mL for indomethacin.

3.4. Application

The plasmas of three premature infants with PDA were compared 8 h after receiving 0.2 mg/kg of indomethacin from oral administration, determined by the proposed MEKC method and HPLC method described above. Electropherograms obtained from these three patients show no interference in the MEKC condition (Fig. 7A). However, one patient's plasma showed obvious interference in the assay of indomethacin by HPLC as shown in Fig. 7B. From the results, the proposed MEKC method presents

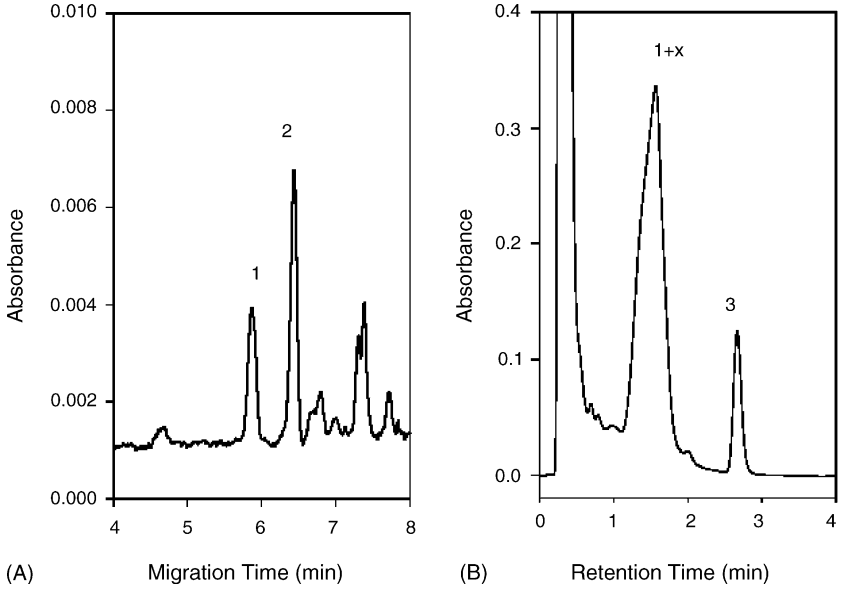

Fig. 7. Compared the electropherogram, (A) and HPLC chromatogram, (B) of one premature infant with PDA treated 0.2 mg/kg of indomethacin. Peaks: 1, 2, 3 and x for indomethacin, flurbiprofen (I.S.), 2,4,5-trichloronitrobenzene (I.S.) and unknown components in plasma, respectively. Calculated concentration was 1.69 μg/mL for indomethacin by CE method.

less interference than the HPLC method after a simple sample pretreatment, such as protein precipitation by acetonitrile. The proposed MEKC method was carried out on three PDA patients after dosing 8 h later and the values of 3.70, 1.69 and 0.91 μg/mL were obtained.

4. Conclusions

We demonstrated a simple, speedy and accurate MEKC method for determination of indomethacin in plasma for PDA patients. We used CE method based on anionic surfactant SOS as a micelle to differentiate the tested drug from endogenous components and detection was achieved at 254 nm. Validation of the methods for quantitation of indomethacin in plasma showed that the methods have high sensitivity and accuracy.

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