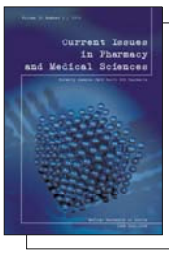

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Current Issues in Pharmacy and Medical Sciences

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The Effect of Mobile Phase Composition on Separation of Some Non-Steroidal Anti-Inflammatory Drugs of the 2-Arylpropanoic Acid Derivatives in System of Reversed-Phase Pressurized Planar Electrochromatography and High-Performance Thin-Layer Chromatography

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ABSTRACT

Separation of some Non-Steroidal Anti-Inflammatory Drugs, 2-arylpropanoic acid derivatives (naproxen, ketoprofen, fenbufen, indoprofen, fenprofen, flurbiprofen, ketorolac, carprofen) has been investigated with pressurized planar electrochromatography (PPEC) and high-performance thin-layer chromatography (HPTLC) in reversed-phase system.The mobile phase consisted of acetonitrile, and aqueous buffer. The influence of concentration of organic modifier in the mobile phase and the mobile phase buffer pH on migration distance (PPEC) and retardation factor (HPTLC) has been investigated and compared.

Keywords: pressurized planar electrochromatography, high-performance thin-layer chromatography, non-steroidal anti-inflammatory drugs

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAID's) form a broad, heterogeneous group of anti-inflammatory, analgesic and fever properties. Their pharmacological effects are demonstrated by inhibiting prostaglandin cyclooxygenase (COX). They are described as non-steroidal because of the structure, which is different from that of other drugs, which are also of anti-inflammatory properties – such as corticosteroids.

Thin-layer chromatography (TLC) is popular analytical technique, which is applied to determination of these drugs [8,9,14,15].

Pressurized planar electrochromatography (PPEC) is a relatively new mode of separation introduced by Nurok et al. [10]. Migration of the mobile phase, in this technique, is driven by the electric field (electroosmotic effect). The advantages such as high performance, short separation time, and separation selectivity different from liquid chromatography (LC), make this method very attractive for application in laboratory practice [11]. In addition, PPEC experiment proceeds in a closed system, so no vapor phase is present in the separating system [2,3,4,5]. Because of the features mentioned, PPEC also might be highly suitable for the pharmaceutical and biomedical analysis [1,6,7,12,13].

The use of PPEC method for separation of a series non-steroidal anti-inflammatory drugs (naproxen, ketoprofen, fenbufen, indoprofen, fenprofen, flurbiprofen, ketorolac, carprofen) is reported in this paper for the first time.

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MATERIALS AND METHODS

The mobile phase solution was prepared by mixing acetonitrile with appropriate buffer aqueous solutions. The organic modifier of the mobile phase was purchased from POCh (Gliwice, Poland). The buffer consisted of citric acid (Merck, Darmstadt, Germany) and disodium hydrogen phosphate (Standard, Lublin, Poland). The other organic solvents (acetone and methanol) were received from POCh (Gliwice, Poland).

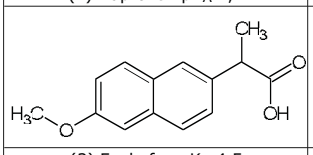
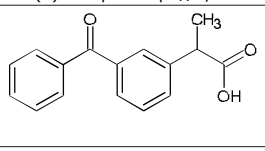
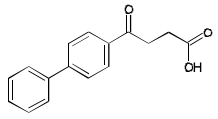
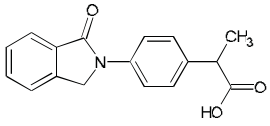
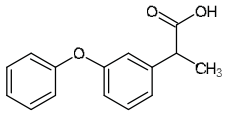
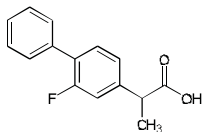
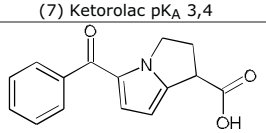
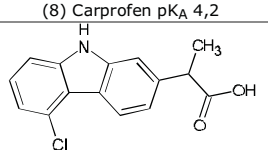
Silicone sealant solutions, Sarsil W and Sarsil H 50, were purchased from Zakłady Chemiczne Silikony Polskie (Nowa Sarzyna, Poland).

Standards of naproxen, ketoprofen, flurbiprofen, and ketorolac, were received from Sigma-Aldrich (St. Louis, Mo, USA), while fenbufen, indoprofen, fenprofen, carprofen from Fluka- Sigma-Aldrich (St. Louis, Mo, USA).

Sample solutions were prepared by dissolving 2 mg of investigated standards in 1 mL of acetone. Sample solutions have been freshly prepared.

Chemical structures of investigated solutes are presented in Table 1.

Table 1. Chemical structures and the pK_A values of investigated compounds

(1) Naproxen pK_A 4,2	(2) Ketoprofen pK_A 4,5
	
(3) Fenbufen pK_A 4,5	(4) Indoprofen pK_A 4,41
	
(5) Fenoprofen pK_A 3,81	(6) Flurbiprofen pK_A 4,2
	
(7) Ketorolac pK_A 3,4	(8) Carprofen pK_A 4,2
	

HPTLC mode was performed on 10 cm × 10 cm RP-18 W $F254_S$ HPTLC plates from Merck, (Darmstadt, Germany). The plates were washed before use by immersion in methanol for 1 min. After solvent evaporation the plates were activated in an oven at 105-110°C for 15 min. Sample solutions (0.7 μL) were applied onto the plate with aerosol applicator (Automatic TLC Sampler 4, Camag, Muttenz, Switzerland). Chromatograms were developed in the Horizontal DS-II-10×10 Chamber (Chromdes, Lublin, Poland) after 15 min saturation with mobile phase vapor. The distance migration of the mobile phase was 45 mm (from the origin of sample application). After the separation process the plates were dried in air. The solute zones were registered under UV lamp with TLC SCANNER 4 (Camag, Muttenz, Switzerland).

PPEC experiments were performed with the device composed of PPEC chamber, high – voltage power supply (Consort, Turnhout, Belgium) and hydraulic press (Współpraca, Lublin, Poland). The cover in the PPEC device was pressed to the adsorbent layer of the chromatographic plate under pressure of 25 bar. Conceptual view of the equipment with longitudinal cross-section of the PPEC chamber was previously published [12].The adsorbent layer of the chromatographic plates was immersed in methanol for 1 min then the plates were dried in air. After methanol evaporation the plates were activated in the oven (105 °C) for 15 min. Then a margin 4 mm wide around the whole periphery of the plate was produced. The method used has been reported elsewhere [3]. Subsequently the plates were placed in the oven at 105-110°C for 1 h to polymerize the sealant then left in a desiccator for use within 1 day. Sample solutions (0.7 μL) were applied onto the plates with aerosol applicator (Automatic TLC Sampler 4, Camag, Muttenz, Switzerland).

RESULTS AND DISCUSSION

The relationships retardation factor, R_F, of the investigated solutes vs. concentration of acetonitrile in the range 40-70 % [v/v] in the mobile phase buffer pH 3.0 (1,21 mM citric acid, 1,84 mM disodium phosphate) for HPTLC mode is presented in Fig. 1.

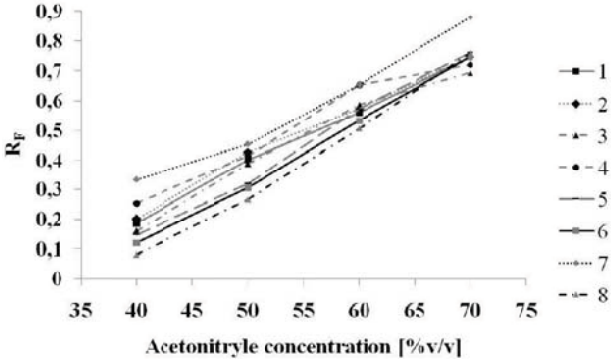


Fig. 1. Relationship R_F of solutes (non-steroidal anti-inflammatory drugs) vs. acetonitrile concentration in the mobile phase, buffer pH 3.0 (1.21 mM citric acid, 1.84 mM disodium phosphate) for HPTLC system.
Solutes identification: naproxen (1), ketoprofen (2), fenbufen (3), indoprofen (4), fenprofen (5), flurbiprofen (6), ketorolac (7), carprofen (8).

The increase of acetonitrile concentration in the mobile phase leads to decrease of solute retention. This is in accordance with elution strength rise of the mobile phase. The order of retardation factor increase in the system with 50% [v/v] acetonitrile is as follows: carprofen, flurbiprofen, fenprofen, fenbufen, naproxen, indoprofen, ketoprofen, ketorolac. The relationships solute migration distances vs. acetonitrile concentration in the range 40-70% [v/v] in the mobile phase buffer pH 3.0 (1,21 mM citric acid, 1,84 mM disodium phosphate) for PPEC mode is presented in Fig. 2. Similarly to HPTLC system, the solute migration distances increase when acetonitrile concentration rises. Contrary to HPTLC system in the PPEC one, the rise of elution strength and electroosmotic mobility of the mobile phase is responsible for this phenomenon. The best separation selectivity was achieved in

PPEC system with 60% [v/v] acetonitrile in the mobile phase. In this system, the order of increase of solute migration distances has been observed as follows: carprofen, ketoprofen, flurbiprofen, naproxen, fenbufen, fenprofen, ketorolac, indoprofen. It is noticeable that the solute migration distance order in HPTLC system differs from that of PPEC one. This indicates that electrophoretic effect was involved in change of solute selectivity in PPEC system relative to HPTLC one. These results confirm the above-mentioned assertion that separation selectivity in planar electrochromatography is different from that in thin-layer chromatography.

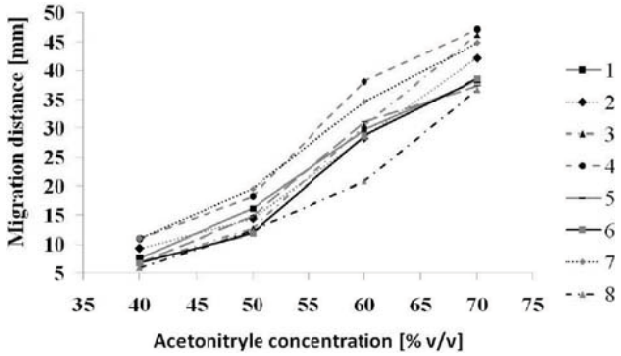


Fig. 2. Relationships the migration distances of solutes (non-steroidal anti-inflammatory drugs) vs. acetonitrile concentration in the mobile phase, buffer pH 3.0 (1.21 mM citric acid, 1.84 mM disodium phosphate) for PPEC, potential 1.00 kV and the experiment time 10 min. The solute legend as in Fig. 1

The relationship between retardation factor of investigated solutes and pH of the buffer in the mobile phase of HPTLC system is presented in Fig. 3. The mobile phase consisted of 60% [v/v] acetonitrile and buffer solution in the pH range 3.0-5.0. Concentration of the buffer constituents in the mobile phase was in the range 1,21-2,51 mM and 1.84-1.18 mM of disodium phosphate and citric acid, respectively. The best separation selectivity of the solutes has been obtained when buffer pH was equal to 3.2.

The relationship migration distances of the solutes versus buffer pH of the mobile phase of PPEC system is presented in Figure 4. In PPEC system, migration of sol-

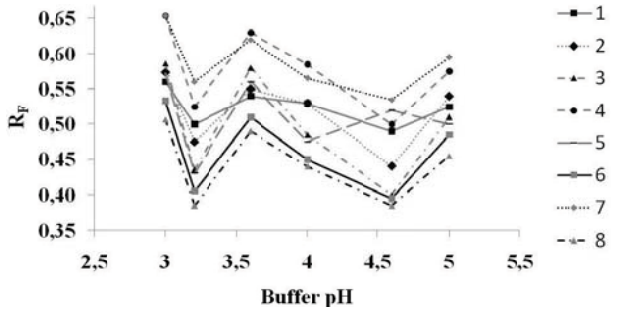


Fig. 3. R_F of solutes vs. buffer pH of organic–aqueous mobile phase (60 [% v/v]) acetonitrile + 40 [% v/v]) aqueous buffer (citric acid and disodium phosphate), HPTLC. Mobile phase migration distance 45 mm. The solute legend as in Fig. 1

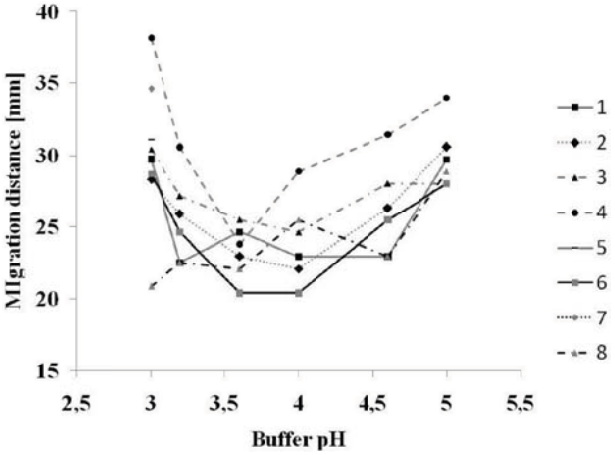


Fig. 4. Migration distance of investigated solutes vs. buffer pH of the organic–aqueous mobile phase (60 [% v/v]) acetonitrile + 40 [% v/v]) aqueous buffer (citric acid and disodium phosphate), PPEC, potential 1.00 kV, experiment time 10 min. The solute legend as in Fig. 1

utes, which undergo dissociation, is highly dependent both on their partition between mobile and stationary phases and on electrophoretic mobility.

Migration distances of non – steroidal anti-inflammatory drugs at different buffer pH are considerably different. Figure 4 shows a concave shape of majority of the relationships migration distance vs. pH for PPEC system what is contrary to the analogous relationships for HPTLC demonstrated in Figure 3. This effect is advantageous with regard to optimization of solute separation. It means PPEC technique, beside contemporary established separation techniques, can be applied as additional tool to optimization of separation of sample mixtures based on different separation selectivity relative to liquid chromatography.

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