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System Suitability Criteria and Variation of Parameters in Capillary Zone Electrophoresis

C. Büttner-Merz, U. Holzgrabe

1. INTRODUCTION

Capillary electrophoresis (CE) methods are customarily regarded as not being robust, but the influence of all parameters on the robustness has not been rigorously investigated. Moreover, the instructions for CE users given in the general chapter 2.2.47 of the European Pharmacopoeia (Ph. Eur.) are not sufficient [1]. This is in sharp contrast to the Ph. Eur. general method 2.2.46. Chromatographic separation techniques, where detailed information is given on the parameters which can be varied within certain limits in order to fulfil system suitability criteria. System suitability tests in turn guarantee reliable outcome and represent an integral part of every method described in the Ph. Eur. The following chromatographic separation parameters can be adjusted: the flow rate, temperature and composition, ionic strength and apparent pH of the mobile phase, as well as dimensions, stationary phase characteristics including type of chromatographic support, particle size, porosity and specific surface area of the column. However, the variation of the aforementioned parameters is limited because a fundamental modification of the method is not allowed. Corresponding detailed instructions are given for thin layer chromatography, isocratic and gradient elution liquid chromatography, gas and supercritical fluid chromatography [2]. However, for CE, no information is available on the possible variations of parameters neither according to the type nor to the extent of alteration. In order to assure the system suitability in a given monograph the parameters retention factor k (in case of MEKC), the number of theoretical plates, the symmetry factor and resolution should be applied.

Since parameters such as the pH value and molarity of a buffer govern the balance of electrophoretic and electroosmotic mobility sensitively, the possibility for variations of parameters for fulfilment of the system suitability is limited. Thus, the aim of this project is to systematically study the impact of the pH value alterations in the running buffer on migration times, migration order and resolution. Moreover, the often disregarded buffer depletion will be highlighted. For this purpose a validated capillary zone electrophoresis (CZE) method for the quantitative determination of a mixture of 4 local anaesthetics, namely lidocaine (Lido), prilocaine hydrochloride (Prilo), procaine (Pro) and tetracaine (Tetra), reported by Chik *et al*. [3], was employed (for structural formulae see Figure 1).

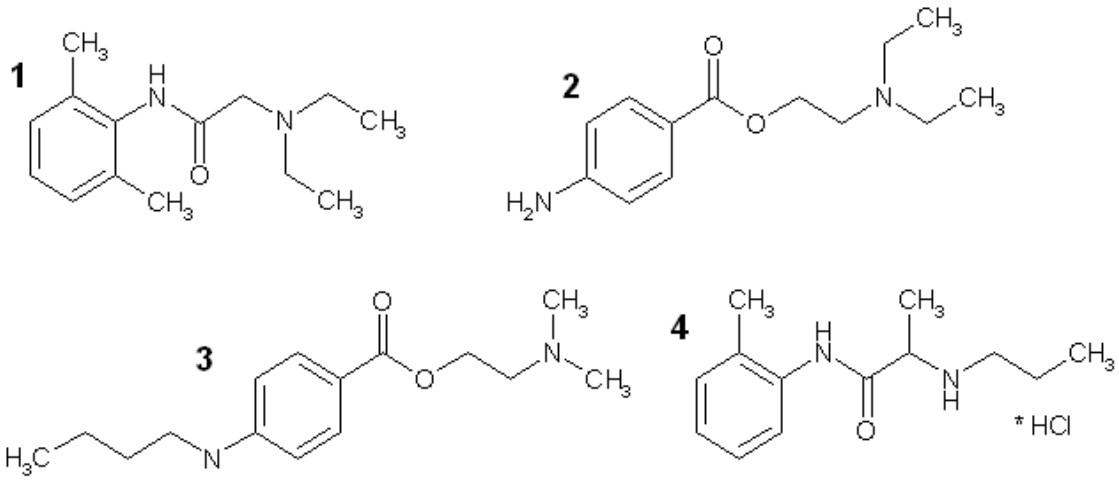

Figure 1 - *Structural formulae of the local anaesthetics studied; 1 lidocaine (Lido); 2 procaine (Pro); 3 tetracaine (Tetra); 4 prilocain hydrochloride (Prilo)*

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2. EXPERIMENTAL

2.1 Chemicals

Lidocaine was purchased from Astra Chemical (Southerton, Zimbabwe), prilocaine hydrochloride from Fagron (Barsbüttel, Germany), procaine and tetracaine from Sanofi-Aventis (Frankfurt, Germany). All drug substances were of pharmacopoeia quality. All buffer substances, reagents for rinsing solutions for capillaries and solvents were purchased from Sigma-Aldrich Company (Poole, UK) and were of analytical purity. Buffer and sample substances were dissolved in ultra pure Milli-Q water (Millipore, Milford, MA, USA). All solutions were filtered through a 0.22 µm filter (Carl Roth, Karlsruhe, Germany) before injecting into the capillary.

2.2 CE-Instrumentation and capillaries

Measurements were carried out on a Beckman P/ACE System MDQ equipped with a UV detector (Beckman, Fullerton, CA, USA). The detection wavelength was set to 200 nm. A fused-silica capillary (BGB Analytik, Schloßböckelheim, Germany) with a total length of 50.2 cm, a detection length of 40.0 cm and an internal diameter of 50 µm was employed. Samples were injected at the anodic end of capillary by pressure (0.7 psi; 12 s) and separated at 25.0 °C using a constant voltage of 25.0 kV. New capillaries were conditioned by rinsing with 0.1 M NaOH for 20 min, water for 10 min, 0.1 M HCl for 20 min and water for 10 min at 25.0 °C using a pressure of 20 psi. Before each run the capillaries were washed with 0.1 M HCl for 3 min and with running buffer for 2 min using a pressure of 20 psi.

2.3 Running buffer

The separations were performed in tris(hydroxymethyl) aminomethane (tris) solution with phosphoric acid. The molarity of tris was varied in the range of 75 mM to 100 mM. The pH values of the running buffer were altered between 2.5 and 4.4 ; 100 mM H_3PO_4 were employed throughout. For pH adjustment, 0.1 M NaOH or 0.1 M HCl solutions were used.

2.4 Sample solutions

The mixtures of sample substances were dissolved in 5 mL of methanol and diluted to 100.0 mL with water. The concentration of sample substances was 40 µg/mL for each substance.

3. RESULTS AND DISCUSSION

Based on the aforementioned CZE method, the influence of pH and molarity alterations of the running buffer on the migration time and consequently the resolution between the signals of the 4 local anaesthetics was investigated. The differences in migration times can not be constituted by changes in the charge-to-size ratio of the substances, because the pK_a values of the basic drugs used for this investigations are all higher than 7.5. Thus, all drug substances are single positively charged in the pH range studied. Furthermore, the impact of buffer depletion in case of this CZE method was investigated.

Figure 2 displays an electropherogram of a sample consisting of a mixture of the local anaesthetic drugs, obtained with the optimal conditions described in [3].

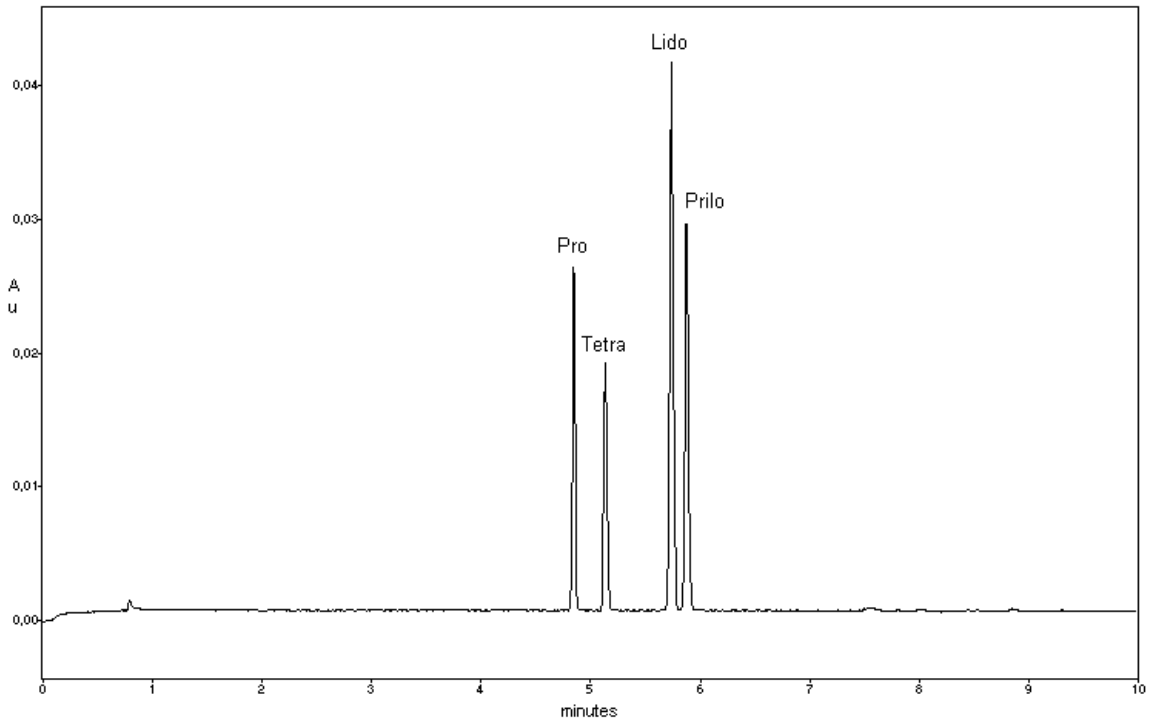

Figure 2 - *Electropherogram of a mixture of Pro, Tetra, Lido and Prilo in a 40 µg/mL concentration of each drug substance dissolved in 5 % methanol; Conditions: running buffers 70 mM tris, 100 mM H_3PO_4; pH 2.5; Temperature: 25.0 °C; Voltage: + 25.0 kV*

3.1 Influence of pH value and molarity variations on migration times and order

In order to meet the system suitability criteria the pH variations are normally performed in small steps, because even small variations in pH or molarity of running buffer can cause significant changes in the electropherograms achieved.

First, the influence of pH variation of running buffer was determined with regard to migration times and order of the 4 substances of interest. Buffer pH was altered in the range of 2.5 to 4.4 in 0.2 log unit steps by adjusting the running buffer containing 75 mM tris and 100 mM H_3PO_4 with 0.1 M sodium hydroxide solution. Figure 3 shows the influence of the pH alteration on the migration time and order. It can be seen that small variations of the buffer pH does not only influence the migration time and resolution, even the migration order is changing, e. g. the tetracaine peak is "migrating" through the lidocaine peak by increasing the pH.

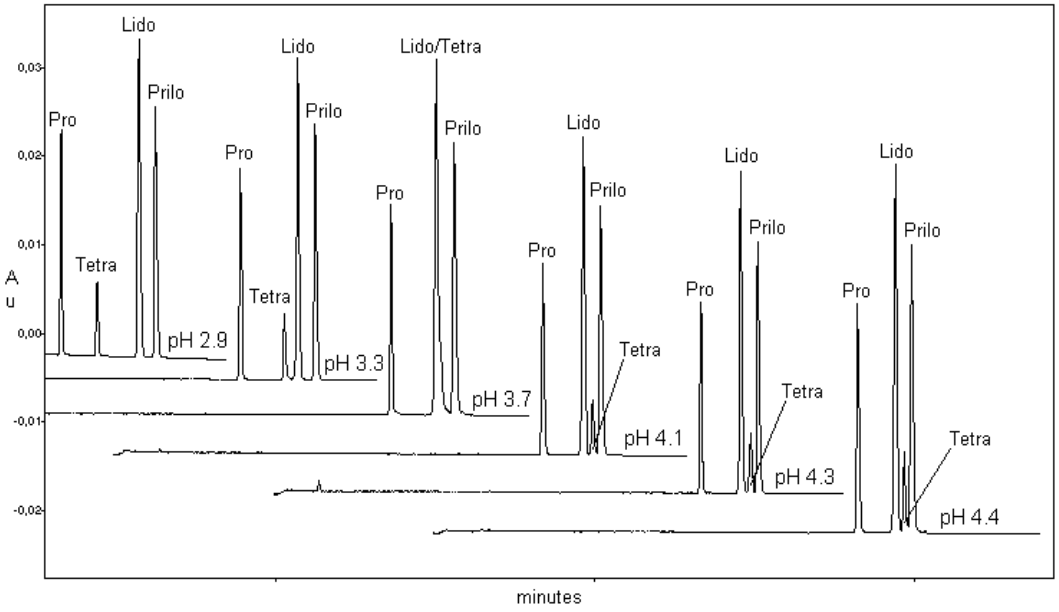

Figure 3 - *Electropherograms of a mixture of Pro, Tetra, Lido and Prilo in a 40 µg/mL concentration of each drug substance dissolved in 5 % methanol. Conditions: running buffer 75 mM tris, 100 mM H_3PO_4; for pH see Figure 3; other conditions see Figure 1*

The influence of changes in molarity of running buffer without varying the pH value is pictured in Figure 4. In this case the pH was kept constant at 2.5 by adding an appropriate quantity of 0.1 M hydrochloride acid (before filling up buffer flasks). Three running buffers of 90, 95 and 100 mM tris in 100 mM H_3PO_4 were employed. The comparison of the 3 electropherograms reveals hardly a difference.

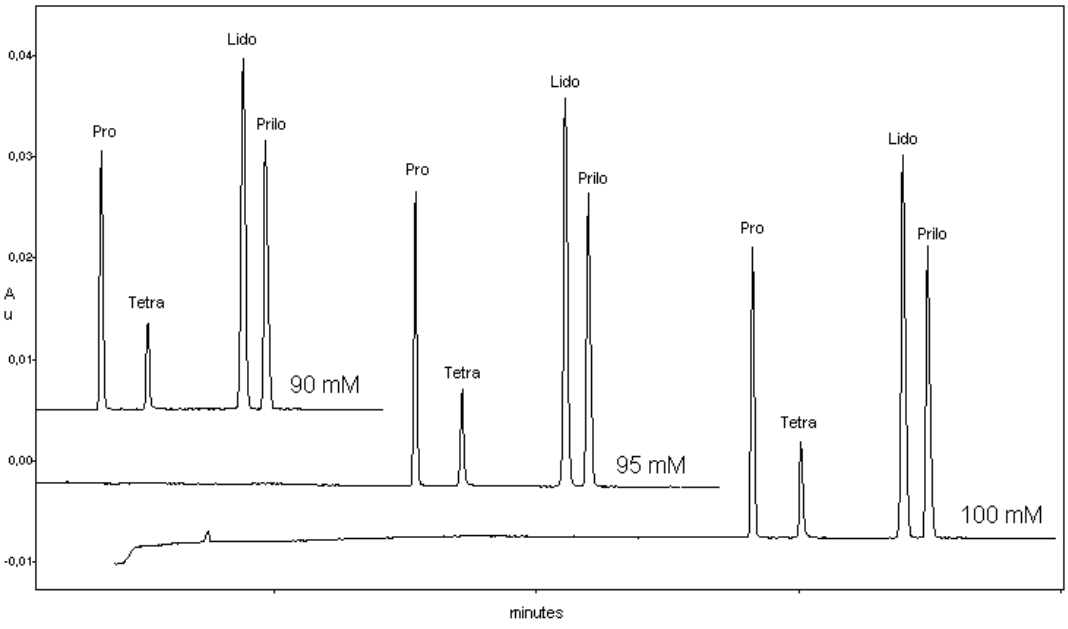

Figure 4 - *Electropherograms of a mixture of Pro, Tetra, Lido and Prilo in a concentration of 40 µg/mL of each drug substance soluted in 5 % methanol. Conditions: for running buffer molarity see Figure 4, 100 mM H_3PO_4; pH 2.5; other conditions see Figure 1*

The combination of pH and molarity variations is displayed in Figure 5. The variations were obtained by preparation of a buffer of increasing molarity without adjustment of the pH with hydrochloride acid and sodium hydroxide solution, respectively. Again, huge variations of migration time and order of the components which are even higher than in case of the single variation of pH are observed. However, the strong influence of especially the pH on the separation of compounds has been often observed [4]. From our findings it can be concluded that only very small changes of buffer pH values should be allowed, e.g. 0.1 pH units, for meeting the system suitability criteria of a given method in the Ph. Eur. For molarity of the buffer, variations of ± 5 mM can be allowed.

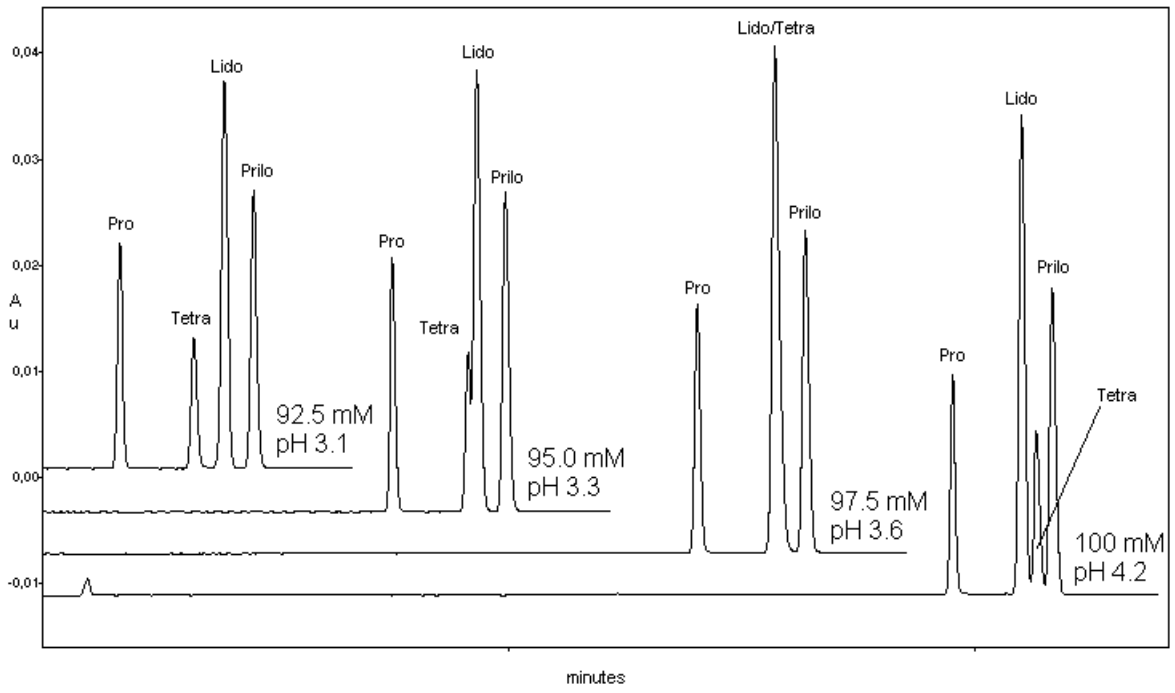

Figure 5 - *Electropherograms of a mixture of Pro, Tetra, Lido and Prilo in a 40 µg/mL concentration of each drug substance dissolved in 5 % methanol. Conditions: running buffer 92.5 mM tris, 100 mM H_3PO_4, pH 3.1; 95.0 mM tris, 100 mM H_3PO_4, pH 3.3; 97.5 mM tris, 100 mM H_3PO_4, pH 3.6; 100 mM tris, 100 mM H_3PO_4, pH 4.2; other conditions see Figure 1*

3.2 Influence of buffer depletion on resolution of 2 critically separated peaks

Buffer depletion occurs because of the electrolysis of water at the electrodes, migration of buffer ions and elution of analytes and solvents into the destination vial. The extent of buffer depletion depends on a multitude of factors, such as molarity of buffer, applied voltage, analysis time and volume of the buffer reservoirs, respectively [5]. Buffer depletion causes a change of the buffer composition and, thus, of the electric field strength in the vials and in the capillary [6]. By electrolysis of water, soluble protons are produced at the anode and hydroxide ions at the cathode. This circumstance causes differences in pH values of buffer in the source and in the destination vial leading to negative influences on reproducibility and precision in CE [7].

Here it is demonstrated that the influence of buffer depletion on separation is serious. In this case the attention is turned to the resolution (R_S) of 2 critically separated peaks, namely the signals of lidocaine and tetracaine. The electropherograms displayed in Figure 6 show a sequence of 12 consecutive measurements in each case. Figure 6A displays a sequence of runs using a fresh buffer for each run and Figure 6B displays the same sequence of runs using the buffer from the same vial. Comparing sequences A and B reveals that buffer depletion decreases the separating capacity rather quickly. With the 4th run of the sequence a baseline separation is no longer achieved between lidocaine and tetracaine. In the 10th run lidocaine and tetracaine are migrating together. Figure 7 shows the decreasing separation capacity expressed in R_S values. Interestingly this issue can be noticed just in case of divergent conditions. The running buffer used for this investigation is composed of 98.5 mM tris and 100 mM H_3PO_4, which leads to a pH value of 3.9. Using the conditions of the reported method [3] (75 mM tris, 100 mM H_3PO_4, pH 2.5) no decreasing resolution can be observed within a sequence of 12 measurements (data not shown). However, the results clearly demonstrate that buffer depletion may occur and that investigations within the context of method development are necessary which provide information about the number of runs possible without a loss of separating capacity. If necessary the monograph has to contain the advice, that only fresh buffer has to be used for each run.

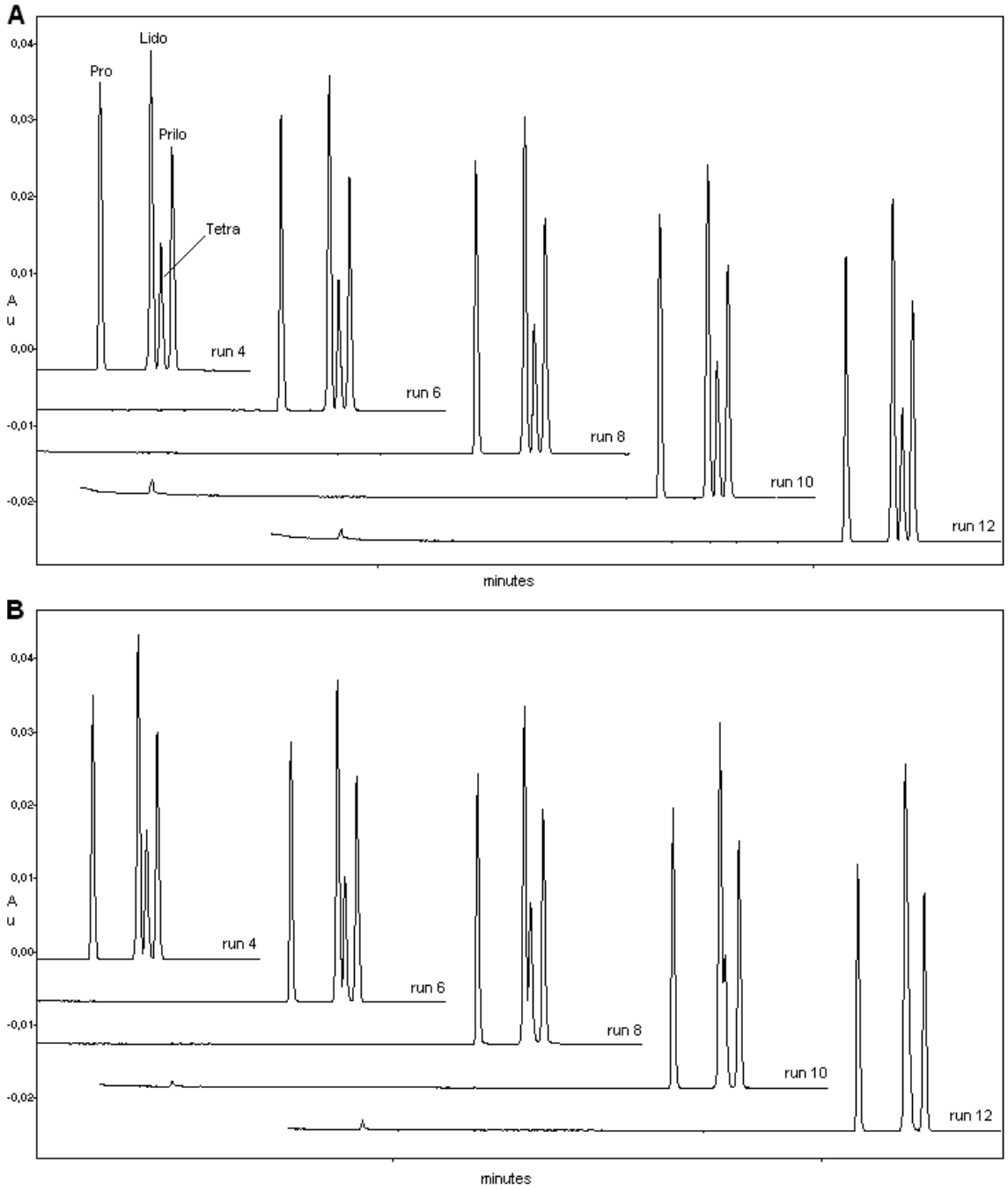

Figure 6 - *Electropherograms of a mixture of Tetra, Lido, Prilo in a 40 µg/mL concentration of each drug substance and of Pro in a concentration of 80 µg/mL dissolved in 5 % methanol. A: fresh buffer for every measurement (runs 4, 6, 8, 10 and 12) of a sequence of 12 consecutive runs; B: same buffer for all measurements (runs 4, 6, 8, 10 and 12) of a sequence of 12 runs. Conditions: running buffer 98.5 mM tris, 100 mM H_3PO_4; other conditions see Figure 1*

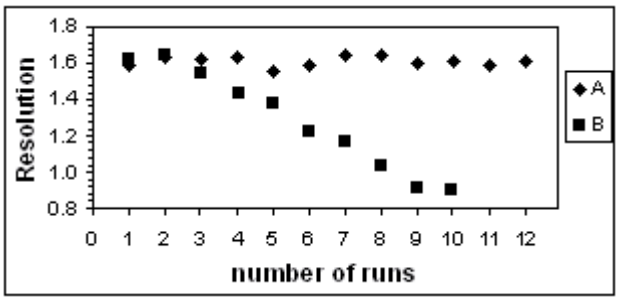

Figure 7 - *Change of resolution between lidocaine and tetracaine-signal in a sequence of 12 measurements. Conditions: see Figure 5*

4. CONCLUSION

Since CE is often superior to HPLC with regard to efficiency and peak capacity, it should be used more often in the Ph. Eur. [8]. However, there is a great demand for a revision of the general chapter 2.2.47 on Capillary electrophoresis to fill the gap of information on system suitability. With regard to pH alteration for meeting the system suitability criteria, a variation of ± 0.1 can be allowed only and with regard to the buffer molarity a variation of ± 5 mM is possible. However, the robustness against both parameters has to be tested during method development. Additionally the buffer depletion has to be investigated, which may result in corresponding advices in the monograph. Beside a revision of chapter 2.2.47 the Technical guide for the elaboration of monographs for the Ph. Eur. has to be complemented accordingly. Our investigations are supported by a recommendation of Altria K. [9] who describes the use and advantage of pre-made buffers (purchased by certain suppliers) which are devoid of accidental changes.

5. REFERENCES

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