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Evaluation of a multiarray system for pharmaceutical analysis by microemulsion electrokinetic chromatography

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ABSTRACT

A multiplexed capillary electrophoresis (CE) system equipped with 96 channels was evaluated for high-throughput screening in drug discovery by microemulsion electrokinetic chromatography (MEEKC). Method transfer from a single channel to a multichannel CE system is described. Loss of efficiency and reduced migration times could be elucidated to the poor efficacy in Joule heat dissipation by forced air cooling in the multiarray system compared to liquid cooling in the single channel instrument. On the other hand, only 48 channels could actually be used because of the maximum total current of 3 mA. Precision data remained below 8% and 9% for migration times and peak areas, respectively. Some UV-detector cross-talk interference between neighboring capillary channels was noted. Impurities at 0.5% compared to the main peak (100%) could be detected with the multiplexed system which is 10 times lower compared to the single capillary system. Higher efficiency and improved figures of merit (absolute sensitivity and no cross-talk interferences) were obtained by using an array of only 24 capillaries.

1. Introduction

The versatility of capillary electrodriven separation techniques is reflected in the many modes that have developed over the last few decades. Because of this variety of operation modes, a solution to a separation problem for most mixtures can usually be found providing the analyst is highly skilled in capillary electrophoresis (CE) and has substantial amounts of time to choose and develop a suitable method. In pharmaceutical analysis of small molecules, routine application of CE is limited to chiral CE which is now considered as a niche application [1–5]. In drug discovery, mostly dealing with complex samples of hydrophilic and hydrophobic molecules, system requirements are high peak capacity and detectability at the μg/mL level using UV detection.

In this perspective microemulsion electrokinetic chromatography (MEEKC) offers promising prospects [6–8]. In the same way as in micellar electrokinetic chromatography (MEKC) a hydrophobic phase is created in a buffered aqueous medium by charged micelle formers such as sodium dodecyl sulfate (SDS). The modes differ in the addition of an immiscible solvent such as heptane or octane and of a cosurfactant such as *n*-butanol. The hydrophobic solvent is penetrating the micelles, creating large droplets in the aqueous solution, which are further stabilized by the co-surfactant. The hydrophobic charged droplets are transported at a different velocity than the EOF creating a separation window. Compounds will partition between the two phases and will be separated according to their affinity for the pseudo-stationary phase and the aqueous phase, respectively [9–14].

Therefore just as in MEKC, MEEKC allows for the simultaneous separation of neutral and charged species. A microemulsion has an enhanced solubilization capacity compared to micelles and is characterized by a low surface tension at the aqueous/hydrophobic interface which promotes fast partitioning which results in reduced peak broadening, enhanced separation efficiency, improved sensitivity and linear range compared to MEKC [15]. For these reasons MEEKC is promising as a generic approach for pharmaceutical analysis. Reports on the use of MEEKC have steadily increased, dealing with a wide variety of analytes, such as hop bitter acids [16,17], vitamins [18–20], analgesics [21], steroids [22], pesticides [23], polycyclic aromatic hydrocarbons (PAHs) [11], chiral compounds [24], coenzymes Q10 [25], adrenaline precursors [26] and paracetamol [27].

Broader acceptance of electrodriven separation methods for pharmaceutical analysis might also further rely on the possibility to perform simultaneous (parallel) analyses. Multicapillary, multiarray or multiplexed CE systems have been developed for the high analytical throughput required for DNA sequencing of the Human Genome Project [28,29]. The majority of commercial multicapillary instruments use laser induced fluorescence detection (LIF). This is very beneficial for sensitivity but limits the applicability

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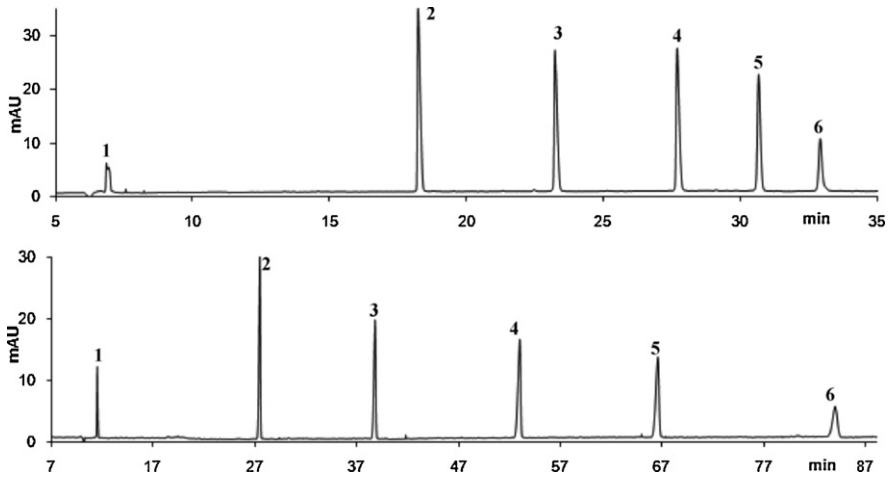

Fig. 1. Comparison of an MEKC (A) and MEEKC (B) analysis of a phenone mixture using the single capillary system. Peak identification: (1) thiourea (200 μg/mL), (2) acetophenone (200 μg/mL), (3) propiophenone (200 μg/mL), (4) butyrophenone (200 μg/mL), (5) valerophenone (200 μg/mL), (6) dodecanophenone (200 μg/mL). Capillary dimensions: 57 cm (46 cm effective, 50 μm ID), injection 1 s × 0.5 psi, detection at 254 nm. Applied voltage: 15 kV. MEKC buffer composition: 50 mM borate adjusted to pH 9.0 with NaOH and 120 mM of SDS. The MEEKC buffer consisted of 50 mM borate adjusted to pH 9.0 with NaOH, *n*-butanol 10% (v/v), *n*-heptane 1.2% (v/v) and 120 mM of SDS.

to samples which are fluorescent or which can be fluorescently derivatized [30]. In 1999, Gong and Yeung described a 96-capillary array instrument equipped with UV detection suitable for a wider range of applications [31]. The possibilities of this approach have been demonstrated in several applications such as CZE analysis of fluoresceins and MEKC analysis of PAHs [32], comprehensive peptide mapping [33], enantiomeric separations [34], enzyme activity measurements [35], organic reaction monitoring [36] and DNA sequencing [37]. Following these initial reports, a multiplexed CE instrument consisting of a 96-capillary array and equipped with UV absorbance detection became commercially available. The use of this instrument for pharmaceutical applications such as log *P* determinations [38,39], pK_a determinations [40,41], and enantioseparations [42,43] have been reported. MEEKC applications, to the best of our knowledge, have been restricted to log *P* determinations [30,38,44–46].

In this contribution, our experiences with a multiarray system for high throughput screening in drug discovery are presented. Particular attention is paid to the consequences of translating a single column MEEKC experiment to the multiplex system and to its figures of merit.

2. Materials and methods

2.1. Reagents and chemicals

Capillaries for the single column CE experiments were purchased from Polymicro Technologies (Phoenix, AZ, USA). Unless otherwise indicated all chemicals used were from Sigma–Aldrich (Steinheim, Germany). SDS was purchased from Acros (Geel, Belgium), dimethylsulfoxide from Janssen (Geel, Belgium), reserpine from Fluka (Buchs, Switzerland), and *o*-phosphoric acid from Merck (Darmstadt, Germany). Deionized water was obtained from a Milli-Q ultrapure water system (Millipore, Bedford, MA, USA). The stock solutions of analytes (10 mg/mL) were prepared in acetonitrile except for thiourea, which was dissolved in water, and phenylalanine and theophylline which were dissolved in 0.1 N NaOH. The pharmaceutical compounds and the solutes used in the precision experiments (Tables 2 and 3 and Fig. 5) were dissolved in DMSO. The final samples were prepared daily from the stock solutions by dilution with running buffer. The optimized MEEKC background electrolyte (BGE) in the multiplexed experiments consisted of 10 mM tetraborate pH 9.2, 60 mM SDS, 0.6% (v/v) *n*-heptane and 5% (v/v) *n*-butanol. The buffer was sonicated for 30 min after preparation to ensure a completely homogeneous and clear solution.

2.2. Instrumentation

A Beckman MDQ (Beckman Coulter, Fullerton, CA, USA) equipped with UV detector and controlled by 32karat 7.0 software was used for the single capillary experiments. Fused silica capillaries of 50 μm ID and 57 and 40 cm length were used. Detection was performed at 11 cm from the capillary end and at 214 or 254 nm. In between analyses, the capillary was flushed with 0.1 N NaOH (1 min), 10 mM sodium tetraborate pH 9.2 (1 min) and with running buffer (5 min) at 40 psi. Hydrodynamic injection was performed at 0.5 psi (injection time specified in text). The applied voltage during the analyses was 10 kV.

A CEpro 9600 system (Advanced Analytical Technologies, Ames, IA, USA) equipped with a capillary cooling unit driven by a Peltier element, a UV detector and controlled by a CEpro manager software was used for the multiplexed experiments. Two capillary arrays were used: the first containing 96-capillaries of 50 μm ID and 55 cm length (33 cm effective length) and the second containing a bundle of 24-capillaries of 75 μm ID and 80 cm length (55 cm effective length). Voltages of 13.75 and 16 kV were applied on the 96 and 24 capillary arrays, respectively. In between runs, the capillaries were flushed with 0.1 N NaOH and 10 mM sodium tetraborate pH 9.2 for 1 min at 60 psi each followed by a vacuum assisted rinse step with the running buffer for 5 min at −3 psi. The common outlet reservoir contained a 10 mM sodium tetraborate solution (pH 9.2). Because the capillaries are glued tightly in the capillary array, the unused capillaries are also always in contact with the electrodes and therefore experiencing a voltage drop and contributing to the current which is generated. In order to be able to control and compare the current and heat being generated during the experiments the solution in the common outlet reservoir was also used to fill the unused capillaries. Detection was performed at 214 nm. The data were treated with the software from the respective systems.

3. Results and discussion

The features of MEEKC for pharmaceutical analysis have been thoroughly presented in the literature [11,19,47] and will not be discussed in detail in this paper. Typical analyses by MEEKC and

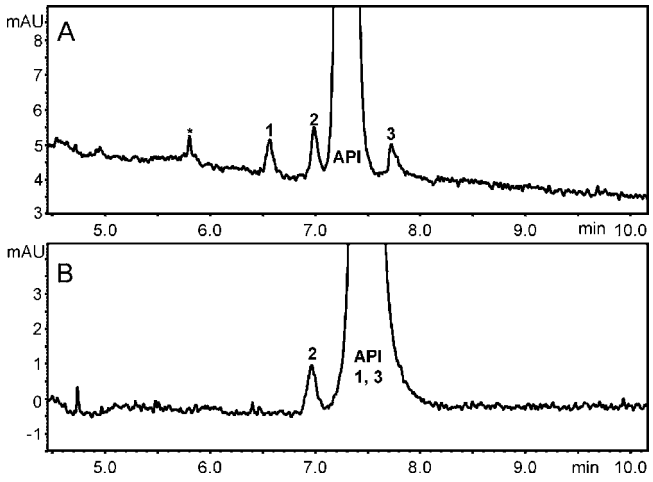

Fig. 2. Example of the MEEKC (A) and the MEKC (B) analysis of a pharmaceutical sample with impurities at the 0.05% level. Peak identity; API: active pharmaceutical ingredient (1000 μg/mL); 1,2,3: impurities. Capillary dimensions: 40 cm (29 cm effective, 50 μm ID). Injection 10 s × 0.5 psi, detection at 214 nm. Running voltage 15 kV. Buffer compositions as in Fig. 1.

MEKC on a single channel instrument using generic conditions for both CE modes are shown in Figs. 1 and 2. In Fig. 1 the increased elution window and peak capacity of MEEKC compared to MEKC are demonstrated for the analyses of 5 phenones. In Fig. 2 the impurities spiked in an active pharmaceutical ingredient (API) at the 0.05% level are completely separated in MEEKC while peaks 1 and 3 co-elute with the API in MEKC. The background electrolyte in the MEEKC analysis namely 50 mM borate pH 9, 120 mM SDS, 1.2% (v/v) *n*-heptane and 10% (v/v) *n*-butanol could not be applied on the multiplexed CE instrument because of the maximum total current achievable of 3 mA. Each individual channel should not produce a current above 31 μA for a given applied voltage (when all the channels are used simultaneously). After several trial and error experiments, the best approach seemed to use of a microemulsion composed of 10 mM tetraborate pH 9.2, 60 mM SDS, 0.6% (v/v) *n*-heptane, 5% (v/v) *n*-butanol. The buffer still generated more than 31 μA per channel at 250 V/cm and it was therefore decided to reduce the number of capillaries used from 96 to 48. The test mixture was analyzed on the single channel system at 250 V/cm (40 cm at 10 kV) and at a temperature of 15 °C using this electrolyte and the corresponding electropherogram is shown in Fig. 3A. The same conditions were then applied to run 48 channels on the multiplexed CE system and this electropherogram is shown in Fig. 3B. A loss of resolution and a reduction in analysis time are immediately apparent.

The difference in efficacy of Joule heat dissipation between both systems is the main reason for the observed disparities. Both systems were operated at 15 °C. The single capillary system was equipped with a liquid cooling system while the multicapillary system was thermostated by forced air cooling. The deleterious influence of inefficient heat removal in electrodriven separation techniques is well known [48] and has also been mentioned in MEEKC [11]. The advantages of liquid assisted cooling compared to forced air cooling have been demonstrated. In order to get an idea of the actual temperature under which the MEEKC analyses take place in the multiplexed system, a series of experiments was performed with the test mixture under the above described conditions on the single capillary system at temperatures between 15 and 50 °C. The electropherogram obtained at 45 °C on the single capillary system compared very well to the profile obtained on the multiplexed system suggesting a 30 °C mismatch between the actual capillary temperature and the set temperature in the multiplexed CE system. It should be mentioned that method transfer

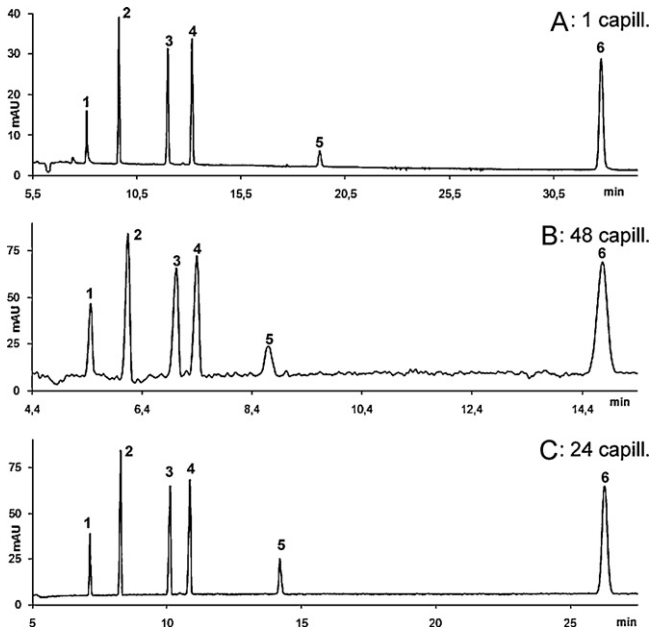

Fig. 3. Electropherograms obtained for the analysis of the same test mix on (A) a single capillary system, (B) on a multiplexed system with 96 parallel capillaries (of which 48 used) and on a multiplexed system with 24 parallel capillaries. Sample composition: (1) phenylalanine (200 μg/mL), (2) theophylline (200 μg/mL), (3) bumetanide (200 μg/mL), (4) fenoprofen (200 μg/mL), (5) cortisone (400 μg/mL), (6) reserpine (200 μg/mL). Capillary total length: 40 cm (29 cm effective, 50 μm ID), 55 cm (33 cm effective, 50 μm ID) and 80 cm (55 cm effective, 75 μm ID) for the single, 96 and 24 capillary array, respectively. Injection volume: 5, 11 and 16 nL, detection at 214 nm. Applied voltage: 10, 13.75 and 16 kV respectively (field strength: 250, 250 and 200 V/cm), operating temperature: 15 °C. MEEKC buffer composition: 10 mM sodium tetraborate pH 9.2, 60 mM SDS, 5% *n*-butanol, 0.6% *n*-heptane.

from CZE and MEKC methods from the single to the multi-capillary system showed less pronounced temperature effects compared to MEEKC because of the lower current and therefore Joule heating generated in these modes.

Using the mixture of Fig. 3 the performance of both systems could be compared as is shown in Table 1. The injection time was thereby adjusted to obtain the same injected volumes to facilitate comparison of the sensitivity. Ca. 25 times lower sensitivity and a threefold loss in resolution were measured for the multiplexed system when the same sample volume was injected. Emphasis is hereby set on relative sensitivity as absolute sensitivities can often be easily enhanced on both systems by increasing injection volumes and sample concentration.

This affects, for example, the capability of the system to detect impurities at the 0.05% level compared to the main pharmaceu-

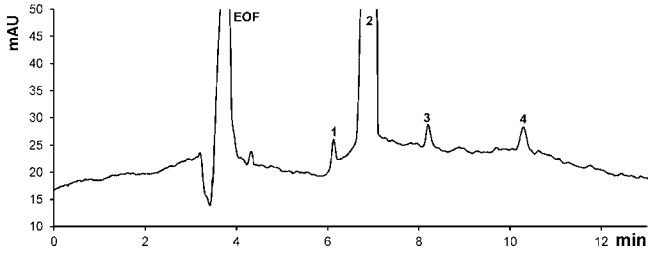

Fig. 4. Electropherogram obtained for the analysis of 3 parabens at the 0.5% level relative to the main peak (ethylparaben) on the multiplexed system. Peak identification: (EOF) thiourea (200 μg/mL), (1) methylparaben (5 μg/mL), (2) ethylparaben (1000 μg/mL), (3) propylparaben (A: 5 μg/mL), (4) butylparaben (A: 5 μg/mL). Injection: 30 s × 0.5 psi. Detection at 214 nm. Capillary dimensions: 55 cm (33 cm effective, 50 μm ID), applied voltage: 13.75 kV, buffer as in Fig. 3.

Table 1
Comparison between a Beckman MDQ system and a Combisep cePRO 9600 equipped with a 96 capillary array (of which 48 were used) and a 24 capillary array.

	Single capillary system	Multiplex system (96/48 array)	Multiplex system (24 array)
Capillary dimensions	40 cm total/29 cm effective/50 μm ID	55 cm total/33 cm effective/50 μm ID	80 cm total/55 cm effective/75 μm ID
Applied voltage	10 kV (250 V/cm)	13.75 kV (250 V/cm)	16 kV (200 V/cm)
Temperature	15 °C	Set-at 15 °C	Set-at 15 °C
LOD[a] (relative, μg/mL)	4.0	99	18
Injection volume (nL)[b]	5.0	5.0	5.0
LOD[a] (absolute, pg)	20	495	90
Resolution (peaks 3,4)	4.3	1.5	4.0

[a] Defined as the peak height equivalent to 3 times the noise (represented for fenoprofen).
[b] The injected volume was calculated from flow rate and injection time by means of the experimentally obtained elution time of methylparaben when pressurized through the system at 0.5 psi.

Table 2
Intraday and interday reproducibility data on a 96-channel capillary array cartridge (CAC). Capillary dimensions: 50 μm i.d. and 33 cm effective length (55 cm total). Column-to-column reproducibility was calculated for the complete set of channels (values in bold). Column-by-column reproducibility was calculated for every capillary on the array (of which the minimum and maximum values are represented between brackets). Conditions as in Fig. 3. Theophylline was used as internal standard. n_t: total number of analyses over the (used) section of the array, n: number of repetitive analyses per capillary.

	Migration times (relative to the I.S.)		Time corrected areas (relative to the I.S.)	
	Intraday RSD $n_t = 480$ ($n = 5$)	Interday RSD $n_t = 960$ ($n = 10$)	Intraday RSD $n_t = 480$ ($n = 5$)	Interday RSD $n_t = 960$ ($n = 10$)
Phenylalanine	**1.5**% (0.1–0.8%)	**1.5**% (0.1–0.8%)	**6.1**% (1.3–15%)	**6.6**% (2.4–12%)
Bumetanide	**2.0**% (0.3–1.1%)	**2.0**% (0.6–1.9%)	**3.9**% (1.0–9.3%)	**4.0**% (1.2–8.0%)
Fenoprofen	**2.6**% (0.2–3.6%)	**3.1**% (0.6–2.5%)	**4.1**% (0.7–9.5%)	**4.0**% (1.3–7.9%)
Cortisone	**6.3**% (0.5–3.4%)	**6.4**% (0.7–3.9%)	**8.3**% (0.6–15%)	**8.7**% (2.6–16%)
Reserpine	**7.0**% (1.3–8.7%)	**7.5**% (1.6–14%)	**7.3**% (1.5–17%)	**7.5**% (2.4–15%)

tical compound. This was measured by performing experiments under the same conditions on the single and multiplex CE systems whereby ethylparaben was considered as a main pharmaceutical compound (100%) and methyl-, propyl- and butylparaben were treated as impurities at a 1, 0.5, 0.25, 0.1 and 0.05% concentration level (data not shown). Various injection volumes were evaluated (from 6 to 70 nL injection volume). In Fig. 4, the analysis at the 0.5% impurity level versus the main peak is illustrated for a 30 s injection of the sample at 0.5 psi on the multiplexed system. Impurities at a 0.25% level could still be detected by increasing the injection time, but lower concentrations were below the LOD as this required the injection of much larger volumes or concentrations to make the impurities visible. This inevitably resulted in overloading of the system (by the main peak) and peak overlap as a consequence. On the other hand, as illustrated before, 0.05% impurities could easily be detected on the single channel system (Fig. 2).

To further evaluate the system, precision measurements of multiplexed MEEKC were performed. Two types of precision were measured: column-by-column precision, which refers to the (inter- and intraday) repeatability achieved in a single capillary in the array, and column-to-column precision which refers to the (inter- and intraday) repeatability achieved among all the capillaries of a given array set [32]. Column-to-column and run-to-run precision data below 1% and 4% (after correcting with an internal standard) have been reported for this instrument for CZE analyses for migration times and peak areas, respectively [44]. The test mixture from Fig. 3 was used for the precision experiments. Because of the current limitation only 48 of the 96 channels could be used simultaneously for the multiplexed analyses. To assess the precision of the entire array both halves were therefore analyzed consecutively ($n = 5$). Table 2 summarizes the obtained results. Precision data remained below 8% and 9% for migration times and peak areas, respectively. An example of analyses on 3 neighboring capillaries on the 96 capillary array is shown in Fig. 5 (capillary 1–3). Interestingly the appearance of an increasing or decreasing baseline seemed to be dependent on the capillary number.

Although the precision data are acceptable, because of the problems described with excessive current generation, limited efficiency and peak capacity were achieved with the 96 array system. A 24-channel array was therefore evaluated under conditions which were as similar as possible to the 96 array. Due to the different dimensions (75 μm ID, 55 cm effective length and 80 cm total length) the heat generation was reduced in comparison with the former array. Therefore, the complete set of capillaries could be used at the same time while operating them at the maximum potential available from the instrumentation (16 kV). Therefore the electric field generated was 200 V/cm, which when combined with a longer capillary configuration yielded longer analysis time, increased peak efficiencies and better resolution.

The use of the 24 array (75 μm ID) capillaries resulted in much improved results compared to the 96/48 array and resembling the obtained electropherograms on the single capillary system (with

Table 3
Intraday and interday reproducibility data on a 24-channel capillary array cartridge. CAC dimensions are 75 μm i.d. 55 cm effective length (77 cm total). Column-to-column reproducibility was calculated for the complete set of channels (values in bold). Column-by-column reproducibility was calculated for every capillary on the array (of which the minimum and maximum values are represented between brackets). Conditions as in Fig. 3. Theophylline was selected as internal standard. n_t: total number of analyses over the (used) section of the array, n: number of repetitive analyses per capillary.

	Migration times (relative to the I.S.)		Time corrected areas (relative to the I.S.)	
	Intraday RSD $n_t = 120$ ($n = 5$)	Interday RSD $n_t = 240$ ($n = 10$)	Intraday RSD $n_t = 120$ ($n = 5$)	Interday RSD $n_t = 240$ ($n = 10$)
Phenylalanine	**1.1**% (0.4–1.2%)	**1.2**% (0.4–1.4%)	**3.0**% (0.6–4.2%)	**3.3**% (0.7–4.1%)
Bumetanide	**1.8**% (1.4–2.4%)	**1.8**% (1.5–2.8%)	**2.7**% (0.8–5.5%)	**2.8**% (1.1–4.5%)
Fenoprofen	**2.5**% (1.3–3.3%)	**2.5**% (1.8–3.1%)	**2.9**% (1.4–6.3%)	**3.2**% (1.9–5.3%)
Cortisone	**6.7**% (3.9–10%)	**6.9**% (4.6–7.0%)	**6.1**% (1.2–12%)	**6.3**% (1.7–9.2%)
Reserpine	**4.4**% (1.3–6.9%)	**4.8**% (1.8–4.8%)	**5.8**% (2.7–12%)	**7.2**% (2.5–12%)

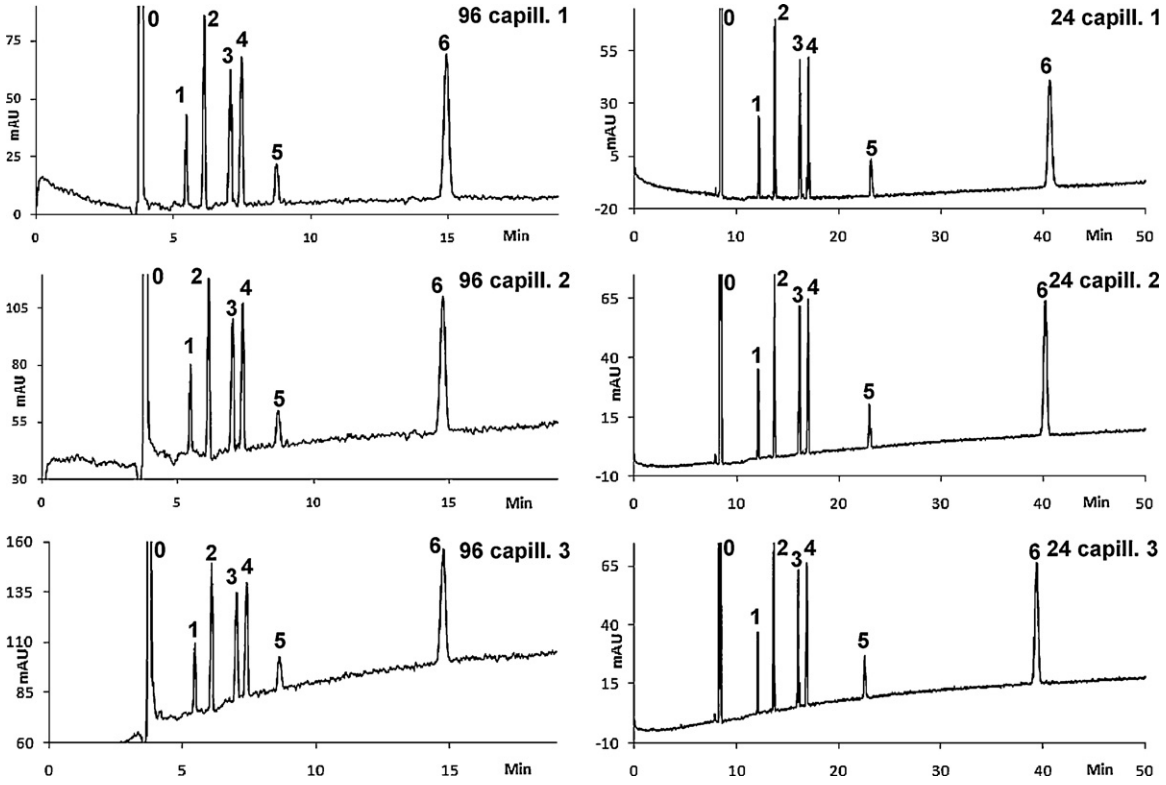

Fig. 5. Electropherogram obtained for the test mix on 3 neighboring capillaries on the 96 and on the 24 capillary array. Sample composition and conditions as in Fig. 3. The stock solutions were dissolved in DMSO, leading to "peak 0" eluting with the EOF.

50 μm ID capillaries), as can be seen from Fig. 3C. The resolution of the critical pair is almost the same as on the single capillary system and a fivefold improvement in absolute sensitivity was measured compared to the 96 capillary array as shown in Table 1. The better performance of the 24-channel array was also reflected in a slightly decreased variability of migration time and peak areas as summarized in Table 3. Both the variability of the migration time and of the peak areas remained around or below 7%. Analyses on 3 neighboring capillaries on the 24 capillary array are shown in Fig. 5 (capillary 1–3). In general improved peak shape and more stable baselines were observed while impurities at the 0.1% level could be measured.

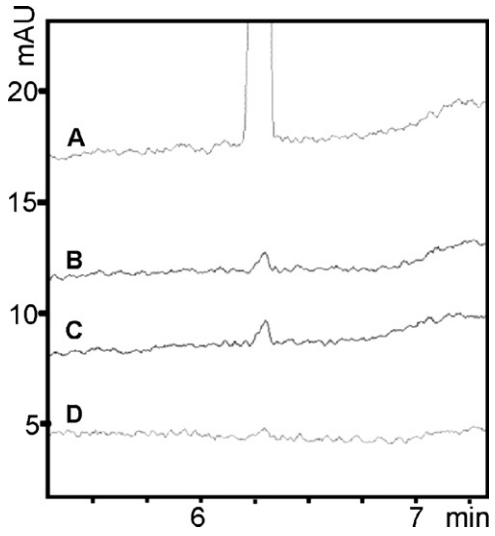

Fig. 6. Example of the "cross talk" problem observed with the 96 capillary array for the analysis of 200 μg/mL theophylline on one capillary (A) and the resulting, false positive, detection in the neighboring capillaries (B, C, and D) where no sample was injected. Conditions as in Fig. 3.

Finally a particular problem, intrinsic to many multicapillary systems, is the so-called "cross talk" phenomenon, which is interference of the signals between neighboring capillaries in the UV detector. A solute migrating in one capillary can therefore generate a small signal in the next capillary. The area of the false positive peak detected in the capillary adjacent to the one in which the analysis was performed was about 1% compared to the actual signal. In Fig. 6 an example is shown for the analysis of theophylline in one capillary and the influence this has on the neighboring channels. The phenomenon seems to be directly related to the proximity of the capillaries in the detector as this was only observed in the 96/48 array system and no cross-talk was observed when the 24 capillary array was used.

4. Conclusion

A multiplexed CE system was evaluated and compared to a single capillary system. Two capillary arrays were used in the multiplexed system: one consisting of 96 parallel, 50 μm ID capillaries and one containing 24, 75 ID μm capillaries. All comparisons were made in the MEEKC mode because the increased separation window and peak capacity of this mode makes it ideally suitable for generic pharmaceutical analysis. MEEKC methods on the single capillary system were successfully transferred to the multiplexed format. Only 48 of the 96 capillaries could be used simultaneously because of the high current which is generated in MEEKC. 25 times less sensitivity and a threefold loss in resolution of the critical pair were observed when comparing both systems. The multiplexed instrumentation equipped with the 96/48 capillary array could be used for the detection of

pharmaceutical impurities down to the 0.5% level. Precision data below 8% and 9% were measured for the migration times and peak areas, respectively. With an array containing 24 parallel capillaries (75 μm ID) much improved sensitivities and separations could be obtained opening up the possibility of faster, multiplexed analysis of the composition of samples from drug discovery or the determination of impurities in pharmaceutical substances.

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