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Purity control of carbamazepine by micellar electrokinetic chromatography

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Abstract

Carbamazepine is separated from 10,11-dihydrocarbamazepine and other impurities using a solution of sodium tetraborate decahydrate (12.5 mmol/l) and sodium dodecyl sulfate (50 mmol/l) in water–methanol (9:1, w/w; apparent pH 9.2). This method is optimised with regard to concentration of the organic modifier, temperature and voltage. The influence of the capillary type and the instrumentation has been studied in order to validate the robustness of this method.

Keywords: Pharmaceutical analysis; Carbamazepine; Dihydrocarbamazepine

1. Introduction

Carbamazepine is widely used in the therapy of epilepsy. Long term medications in high doses require reliable purity control by the selling companies. Impurities are usually detected using TLC and high-performance liquid chromatography (HPLC). However, it is difficult to separate carbamazepine and 10,11-dihydrocarbamazepine, due to their very similar polarity [1,2]. The performance of a purity control by HPLC requires a peak resolution of at least 1.5 [3].

Micellar electrokinetic chromatography (MEKC), which has been investigated by Terabe et al. [4,5] and Otsuka et al. [6,7], is an excellent method for analysing neutral compounds [8,9]. In order to build up a pseudo-stationary micellar phase, a charged surfactant (e.g. sodium dodecyl sulfate; SDS) is added to the background electrolyte system. Neutral substances can now be separated according to their hydrophobicity, aided by the distribution equilibrium between the aqueous (mobile) phase and the micelles. Substances can be characterised using the capacity factor k' [4,5].

Two applications of MEKC to analyse carbamazepine with other anti-epileptic drugs have been reported [10,11], but none to separate carbamazepine from its synthesis impurities.

Guidelines for capillary electrophoresis (CE) validation have been established during the last few years [12–14]. In order to test the transferability of the method to other laboratories, robustness was tested by using different instruments and especially capillaries. Only a few works have been published in this important field [12,15–17].

2. Experimental

2.1. Buffers

2.1.1. Cathode buffer

Borate buffer 50 mmol/l in 900 g water–100 g methanol mixture (9:1, w/w), apparent pH 9.2: 1.192

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Table 1
Capillaries

Batch	Company	Batch No./Lot No.	I.D. (μm)	O.D. (μm)	Total length (cm)	Effective length (cm)
1	Polymicro[a]	01-4DZ-001-11/LGF-11	52	360	42.1	34.9
2	Polymicro	01-5DT-005-14A/QQT-14A	48–50	360–361	42.0	34.8
3	Polymicro	01-5DJ-005-03A/QQT-03A	49–51	359–362	41.9	34.7
4	Polymicro	01-5DH-037-01/QNR-01	48–50	357–358	42.0	34.7
5	Polymicro	01-5DJ-003-06A/QPH-06A	47	360–361	42.0	34.8
6	Polymicro	01-5DJ-005-02A/QQT-02A	49–50	359–360	41.6	34.4
7	Polymicro	01-5CW-001-04A/QPM-04A	49	363	42.0	34.8
8	Scientific	P/N 062463	50	ca. 280	42.3	34.8
9	Müller (CS)	201956	50	360	42.0	34.9
10	CS	50/4-52/105180	50	360	42.1	34.9
11	MicroQuarz	–	48	283	42.1	34.9

I.D. = inner diameter; O.D. = outer diameter.
[a] Capillary for first method development

g (3.125 mmol) of disodium tetraborate decahydrate were made up to 250.0 ml with water–methanol (9:1, w/w). The solution had an apparent pH of 9.2.[1]

2.1.2. Running buffer

Cathode buffer containing SDS 50 mmol/l: 1.442 g (5 mmol) of SDS were made up to 100.0 ml with the cathode buffer described above.

2.2. Sample solution

2.2.1. Stock solution (A)

A 476.7 mg weight (1.25 mmol) of disodium tetraborate decahydrate and 1.442 g (5 mmol) SDS were made up to 100.0 ml with a water–methanol mixture (8:2, w/w).

2.2.2. Stock solution with markers (B)

Three drops dimethyl formamide (DMF) and Sudan III (saturated, 10 min ultrasonic bath) were mixed with 50 ml of the stock solution (A) described above.

2.2.3. Sample solution

A 10 mg weight of a carbamazepine sample was dissolved in 10.0 ml stock solution A and stock solution B, respectively (15 min ultrasonic bath).

[1] In order to avoid problems caused by tensides creeping on the outer capillary surface, the cathode buffer was prepared without adding SDS.

2.2.4. Reference substance solution

One crystal of the reference substance was dissolved in 2.0 ml stock solution A and 2.0 ml stock solution B respectively (15 min ultrasonic bath).

All solutions and buffers were filtered through a 0.22 μm filter (Optex obtained from Wepa, Höhr-Grenzhausen, Germany). Carbamazepine samples and reference substances were made available by Fluka (Neu-Ulm, Germany) and the Department of Pharmacy, Bonn, Germany. Comprehensive synthesis instructions could be obtained on request. All reagents were of analytical reagent grade. All were procured from Merck (Darmstadt, Germany). Water was of HPLC-grade (Milli-Q, Millipore, Darmstadt, Germany).

2.3. CE system

The method was performed on a SpectraPhoresis 1000 (TSP, Fremont, CA, USA) with the software CE-1000, Version 3.0.1 controlled by a personal computer featuring OS/2 Warp 3 operating system. Integration was done by a laboratory-built integration program (K.I.S.S., Würzburg, Germany). For specifications of the fused-silica capillaries see Table 1. The wavelengths of the UV detector were set to 210 and 254 nm with a rise time of 1.0 s. For UV spectra wavelengths of 200–300 nm (steps 5 nm) were used. The applied voltage was 25 kV resulting in a current of about 45 μA. Sample injection was done hydrodynamically (50 mbar) for 2 or 5 s. The temperature was held constant at 25°C.

Before the first use the capillary was conditioned with freshly prepared 1 *M* NaOH for 30 min at 30°C, 0.1 *M* NaOH for 30 min at 30°C, Millipore water for 30 min at 25°C and then equilibrated with running buffer for 90 min at 25°C. The capillary was rinsed between each run for 2 min with running buffer at 25°C.

The method was transferred on a HP^{3D}-CE System (Hewlett-Packard, Waldbronn, Germany) with the software HP-Chem Station Rev.A. 03.02, HP 1990-1995. The fused-silica capillary (Hewlett-Packard) had an I.D. of 50 μm, a total length of 50 cm and an effective length of 41.5 cm. The average current was 34 μA. The remaining features correspond to the method described above.

3. Results and discussion

3.1. MEKC conditions

An alkaline buffer solution was chosen because of better stability of carbamazepine and 10,11-dihydrocarbamazepine at high pH and in order to get a strong electroosmotic flow (EOF). Sodium tetraborate decahydrate was preferred to phosphate, because it has a good microbiological stability and shows nearly no interaction with the capillary walls. In order to dissolve and to separate uncharged substances, SDS as surfactant and methanol as organic modifier were added.

This MEKC method corresponds to a standard method [4,18–22]. Carbamazepine and its impurities were easily separated. Only a few parameters had to be optimised: the concentration of the organic modifier (methanol), temperature and voltage. The best selectivity was achieved using water–methanol mixture (9:1, w/w). However, in order to guarantee complete solubility of the samples, water–methanol mixture (8:2, w/w) was used for the sample solution.

3.2. Identification

In MEKC the capacity factor and the UV spectra can be used for peak identification. In order to determine the capacity factor, DMF and Sudan III were added to mark the EOF and the micelles. Through their migration times, k' was calculated.

UV spectra were recorded for highly concentrated substances (giving a S/N ratio above 20; e.g., carbamazepine and 10,11-dihydrocarbamazepine). Otherwise a peak-area ratio of two different detection wavelengths (210 nm/254 nm) was used to distinguish impurities. Due to the possibility of

Table 2
Systematic names of reference substances

Abbreviation	Chemical name
CA 2	5H-Dibenzo[*b*,*f*]azepine
CA 3	N-Chlorocarbonyl-5H-dibenzo[*b*,*f*]azepine
CA 4	N-Formyl-5H-dibenzo[*b*,*f*]azepine
CA 5	N-Acetyl-5H-dibenzo[*b*,*f*]azepine
CA 6	N-Ethyloxycarbonyl-5H-dibenzo[*b*,*f*]azepine
CA 8	10-Bromocarbamazepine
CA 13	N-Acetylcarbamazepine
CA 14	N-Trichloroacetyl-5H-dibenzo[*b*,*f*]azepine
DCA 1	10,11-Dihydrodibenzo[*b*,*f*]azepine
DCA 2	N-Chlorocarbonyl-10,11-dihydrodibenzo[*b*,*f*]azepine
DCA 3	N-Acetyl-10,11-dihydrodibenzo[*b*,*f*]azepine
DCA 4	10,11-Dihydrocarbamazepine
DCA 5	N-Chlorocarbonyl-10,11-dibromo-10,11-dihydrodibenzo[*b*,*f*]azepine
DCA 6	N-Chlorocarbonyl-10,11-epoxy-10,11-dihydrodibenzo[*b*,*f*]azepine
DCA 8	10,11-Epoxy-10,11-dihydrocarbamazepine
SO 2	1,2-Bis(2-nitrophenyl)ethene
SO 3	1,2-Bis(2-anilyl)ethene
SO 4	9-Methylacridine
SO 6	Acridone

Table 3
Average capacity factors and peak-area ratios of the reference substances with S.D. (n = 4–6) and UV maxima in the range of 200–300 nm

Substance	k'	S.D.	n	$A(210)/A(254)$	S.D.	UV (max) (nm)	Spectral properties (compound to carbamazepine)
CA 2	18.90	1.68	5	0.454	0.071	257	−
CA 3	49.61	5.12	4	5.117	0.582	216–223	+
CA 4	20.44	1.65	5	4.346	0.063	208	+
CA 5	20.54	1.31	5	4.946	0.228	214	+
CA 6	37.63	2.58	5	5.892	0.317	211	+ +
CA 8	25.40	1.52	4	3.427	0.002	214	+ +
CA 13a	1.34	0.005	3	4.530	0.085	207	+ +
CA 13b	22.33	0.092	3	3.743	0.031	207	+
CA 14	165.87	18.34	2	2.662	0.041	212, 223	○
DCA 1	29.73	5.44	4	6.300	0.030	206, 288	−
DCA 2	36.44	8.58	4	27.480	0.189	202	○
DCA 3	11.87	1.14	5	22.077	0.656	<200	−
DCA 4	6.57	0.51	4	28.937	1.168	<200	−
DCA 5	45.99	4.29	3	4.625	0.119	<200	−
DCA 6	15.19	1.20	5	46.398	8.044	210	−
DCA 8	4.54	0.27	5	24.091	1.701	210	−
SO 2	42.80	1.60	5	2.298	0.155	205	○
SO 3	4.80	0.11	5	13.249	2.968	<200	−
SO 4	30.52	0.81	5	0.108	0.324	251	−
SO 6	4.87	0.12	6	0.278	0.020	254	−

The last column compares the UV spectra of the reference substances with the UV spectra of carbamazepine: + + = very similar; + = similar; ○ = partly similar; − = different

achieving a mean for several runs, this measure was less influenced by baseline noise. In addition, the error of integration was minimised using of a precise integration program.

Nineteen described or probable (compare [3] and Section 2) synthesis by-products (reference substances, Table 2) were measured and characterised by the method described above. Table 3 shows the results. In addition to CA 13a (N-acetyl carbamazepine) its hydrolysis product CA 13b, probably N-carboxy-5H-dibenzo[b,f]azepine, is also formed.

A two-dimensional plot of the average peak-area

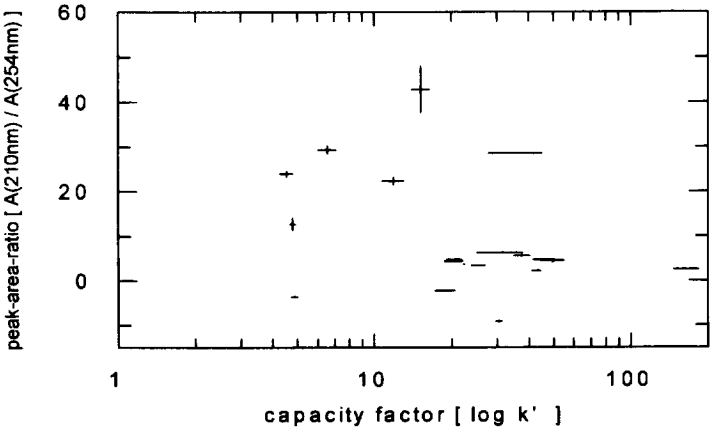


Fig. 1. Two-dimensional plot of the average peak-area ratio and the average logarithmic capacity factor (middle of the crosses) of the reference substances. The size of the crosses is built by ± standard deviation of the means. If data of an unknown substance fits to one of the depicted crosses, this compound can be identified.

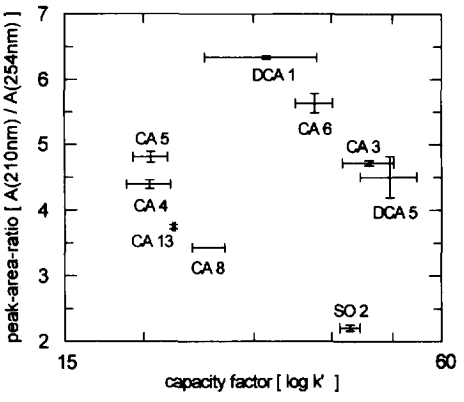


Fig. 2. Zoomed part of Fig. 1. The areas of CA 3 and DCA 5 are overlapping; for an unknown substance a distinction between the two is not possible. The other reference substances show significant distances to each other.

ratios and the average logarithmic capacity factors of all reference substances is shown in Figs. 1 and 2. It is evident that all depicted crosses of the by-products, except for CA 3 and DCA 5, show significant distances to each other. Unknown peaks could now be significantly identified, if their measured values are lying inside a depicted cross. Luckily, CA 3 and DCA 5 are irrelevant, because they were not found as impurities in the carbamazepine batches.

Subsequently four carbamazepine batches were analysed and the peaks were identified comparing the k' values and peak-area ratios (electropherograms: Figs. 3–7; results: Tables 4 and 5). The identity was additionally confirmed by spiking with the corresponding substance. It becomes apparent that the batches CA I, II and IV are contaminated by impurities; batch CA III is pure. The results are listed

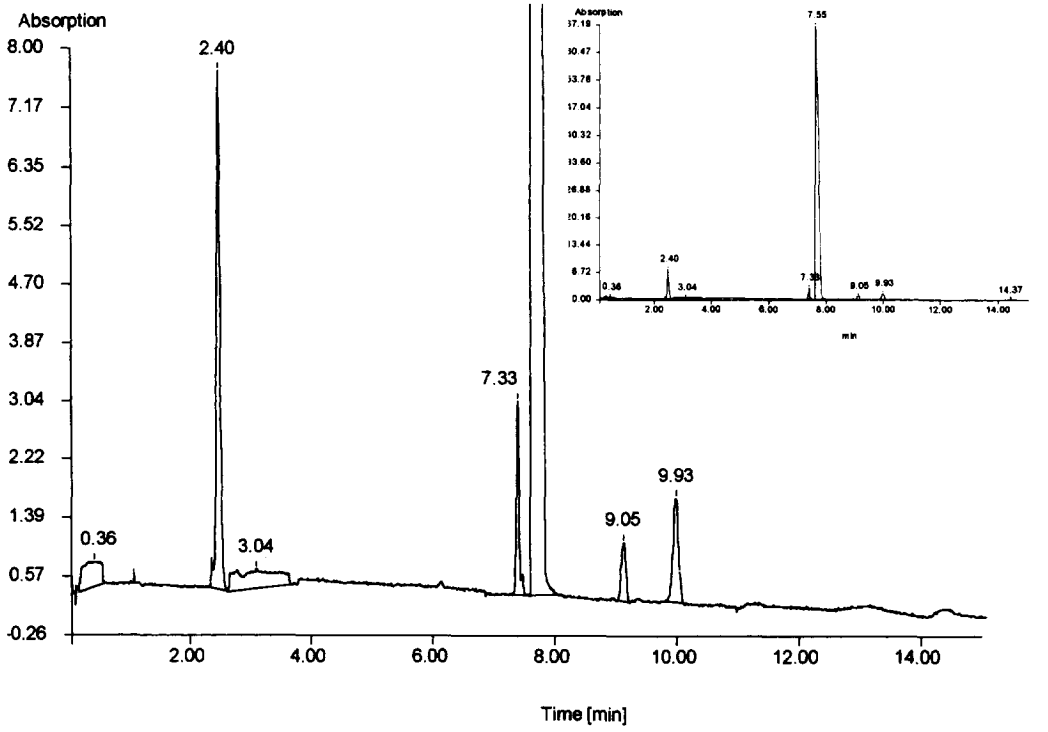


Fig. 3. Carbamazepine batch CA I with markers (λ=210 nm). Peak sequence: 2.4 min, DMF; 7.33 min, 10,11-dihydrocarbamazepine; 7.57 min, carbamazepine; 9.05 min, 10-bromocarbamazepine; 9.93 min, unknown substance + Sudan III. Separation was performed on a TSP SpectraPhoresis 1000 instrument; fused-silica capillary of Polymicro, 42.1 cm (effective length 34.9 cm)×50 μm I.D.; voltage 25 kV; hydrodynamic injection, pressure 50 mbar, injection time 5 s; detection wavelength λ=210 nm; temperature 25°C; buffers condition as reported in Section 2.

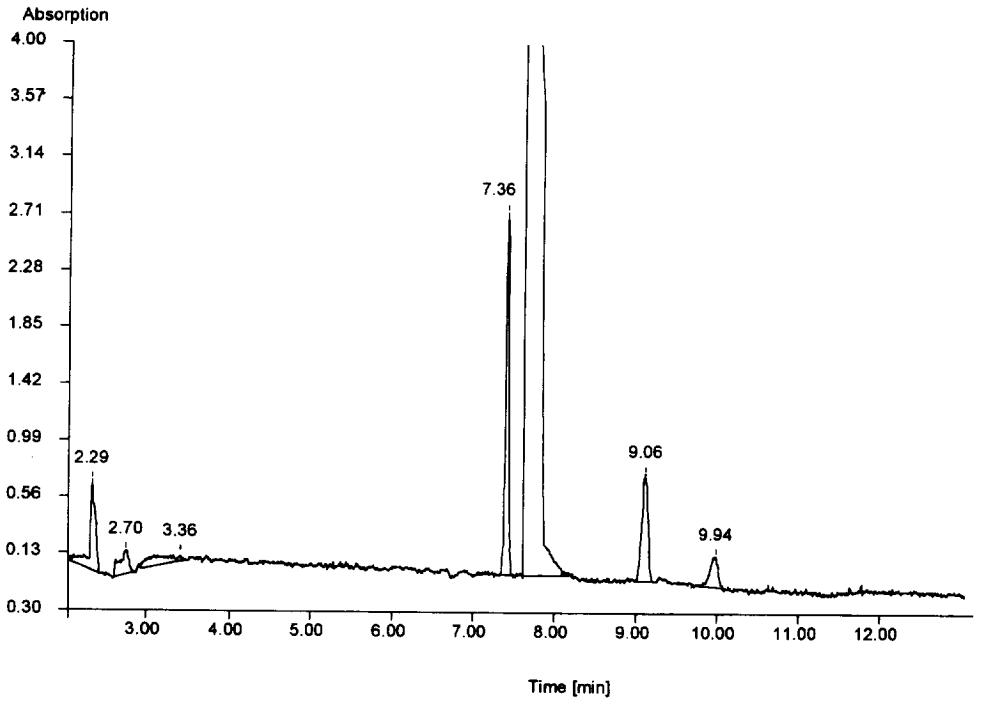


Fig. 4. Carbamazepine batch CA I (λ=210 nm). Peak sequence: 7.36 min, 10,11-dihydrocarbamazepine; 7.57 min, carbamazepine; 9.06 min, A_{210}=56.5, 10-bromocarbamazepine; 9.94 min, unknown substance. Separation conditions as reported in Fig. 1.

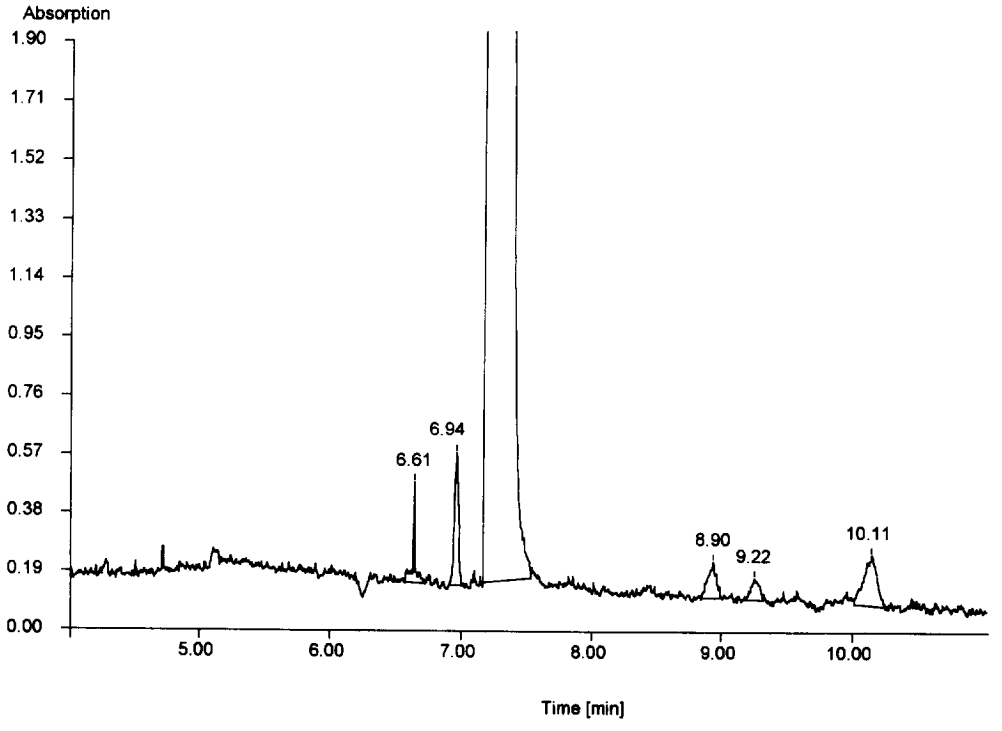


Fig. 5. Carbamazepine batch CA II (λ=210 nm). Peak sequence: 6.61 min, spike (not reproducible); 6.94 min, 10,11-dihydrocarbamazepine; 7.16 min, carbamazepine; 8.9 min, A_{210}=56.9, 10-bromocarbamazepine; 9.22 min, N-ethyloxycarbonyl-5H-dibenzo[*b*,*f*]azepine; 10.11 min, unknown substance. Separation conditions as reported in Fig. 1.

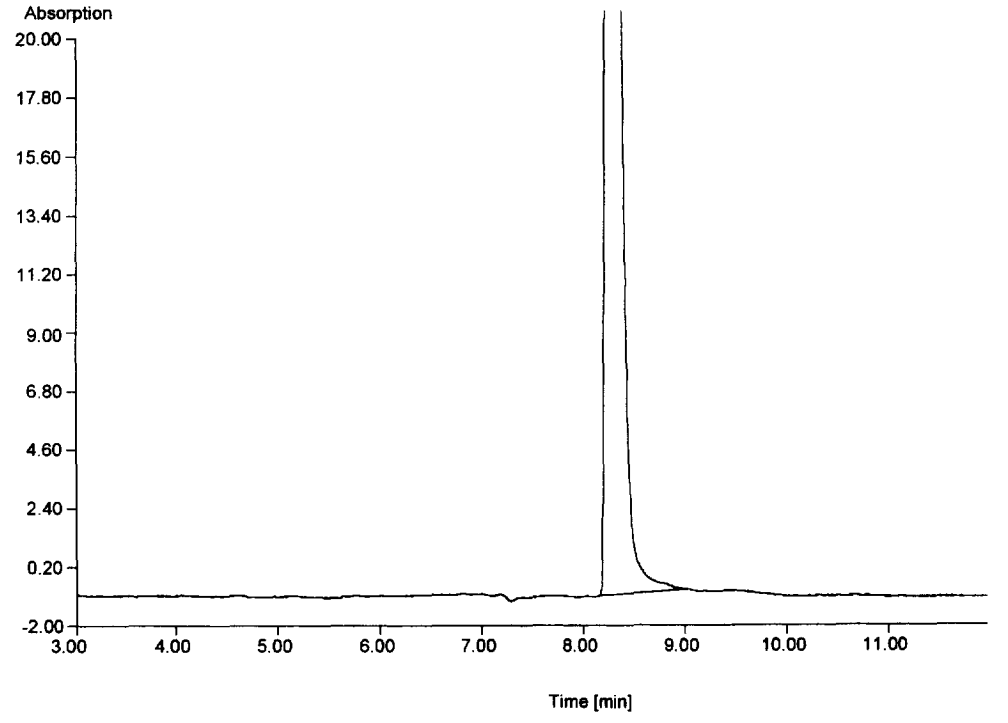


Fig. 6. Carbamazepine batch CA III (λ=210 nm). Peak: 8.65 min, carbamazepine, A_{210}=59.2. Separation conditions as reported in Fig. 1.

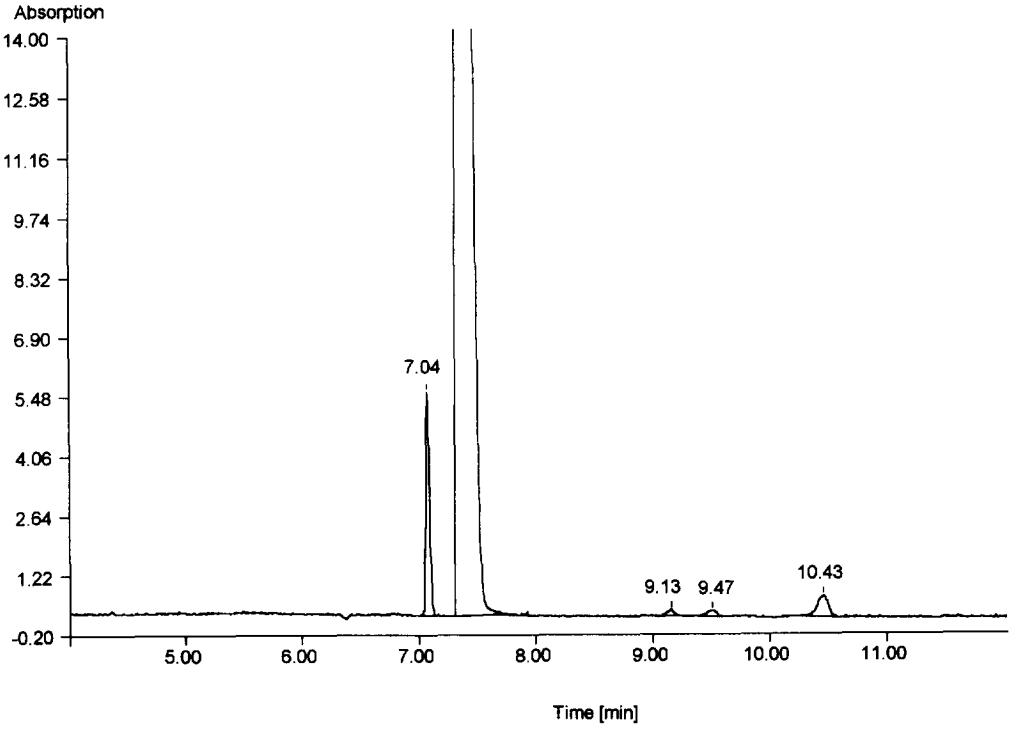


Fig. 7. Carbamazepine batch CA IV (λ=210 nm). Peak sequence: 7.04 min, 10,11-dihydrocarbamazepine; 7.29 min, carbamazepine; 9.13 min, A_{210}=55.9, 10-bromocarbamazepine; 9.47 min, N-ethyloxycarbonyl-5H-dibenzo[*b*,*f*]azepine ?; 10.4 min, unknown substance. Separation conditions as reported in Fig. 1.

Table 4
Identification of the impurities found in the batches CA I–IV

	t (s)	t (s)	k'	Area ratio	t (s)	k'	Area ratio	t (s)	k'	Area ratio	t (s)	k'	Area ratio	t (s)[a]	Area ratio
Ref. substance		10,11-dihydrocarbamazepine (DCA 4)						10-Bromocarbamazepine (CA 8)			N-Ethyloxycarbonyl-5H-dibenzo[*b*,*f*]azepine (CA 6)				
Mean		359.8	6.57	28.94				443.1	25.4	3.43	459.19	37.63	5.89		
S.D.		2.23	0.51	1.168				5.85	1.52	0.002	7.76	2.58	0.317		
CA I	EOF	Impurity 1 (I1)			Carbamazepine (CBZ)			Impurity 2 (I2)			Impurity 3 (I3)			Impurity 4 (I4)[b]	
Mean	131.35	383.01	7.07	29.39	394.21	8.01	3.54	475.23	27.31	3.41	Not detected			525.83	1.46
S.D.	2.03	14.64	0.13	2.13	16.46	0.24	0.17	22.47	0.63	0.17				28.20	0.06
CA II														Impurity 4 (I4)[b]	
Mean	129.81	374.01	6.61	25.10	383.11	7.42	3.48	465.53	25.72	3.44	478.26	35.67	5.28	504.69	1.66
S.D.	4.05	4.74	0.27	0.24	4.60	0.22	0.06	7.54	1.27	0.32	7.80	1.67	0.52	17.99	0.10
CA III														Not detected	
Mean	128.34	Not detected			378.98	7.47	3.46	Not detected			Not detected			509.50[c]	
S.D.	4.26				3.47	0.19	0.06							6.96	
CA IV														Impurity 5 (I5)[b]	
Mean	129.96	369.28	6.80	29.08	378.83	7.61	3.52	458.48	26.18	3.23	470.73	36.92	3.41	507.00	2.37
S.D.	2.60	6.04	0.15	1.72	7.19	0.21	0.10	7.96	0.65	0.45	8.58	1.17	0.67	9.01	0.15

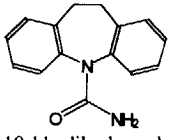
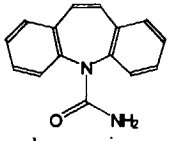
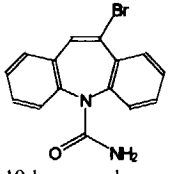
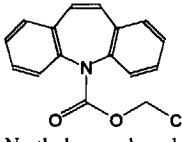
For each impurity migration time, capacity factor and peak-area ratio (210 nm/254 nm) is listed. In the upper table measured data of the corresponding references are shown (compare Table 3). The impurities found in the batches CA I–IV were identified by comparing their k' and the peak-area ratio with the data of the reference substances, and, in addition, by spiking.

[a] t (s) = t_{mc}; k' cannot be sensibly calculated.

[b] Impurity 4 and 5 migrate with Sudan III.

[c] Migration time for Sudan III.

Table 5
Overview of all identified substances of the CA batches and their relative concentrations (%; $\lambda = 210$ nm)

Batch CA	 10,11- dihydrocarbamazepine DCA 4	 carbamazepine	 10-bromocarbamazepine CA 8	 N-ethyloxycarbonyl-5H-dibenzo[*b*,*f*]azepine CA 6		
I	I1	Carbamazepine	I2	–	I4	
($n=15$)	1.60±0.075%	97.54±0.178%	0.70±0.081%	–	0.23±0.017%[a]	–
II	I1	Carbamazepine	I2	I3	I4	
($n=11$)	0.25±0.017%	99.34±0.05%	0.09±0.007%	0.10±0.011%	0.21±0.014%[b]	–
III	–	Carbamazepine	–	–	–	–
(n=11)		100%				
IV	I1	Carbamazepine	I2	I3?	–	I5
($n=15$)	3.11±0.123%	95.80±0.288%	0.12±0.012%	0.17±0.012%	–	0.66±0.058%[b]

The number of runs is reduced to [a] $n=5$; [b] $n=4$. The identification of I3 in batch CA IV as CA 6 is not certain.

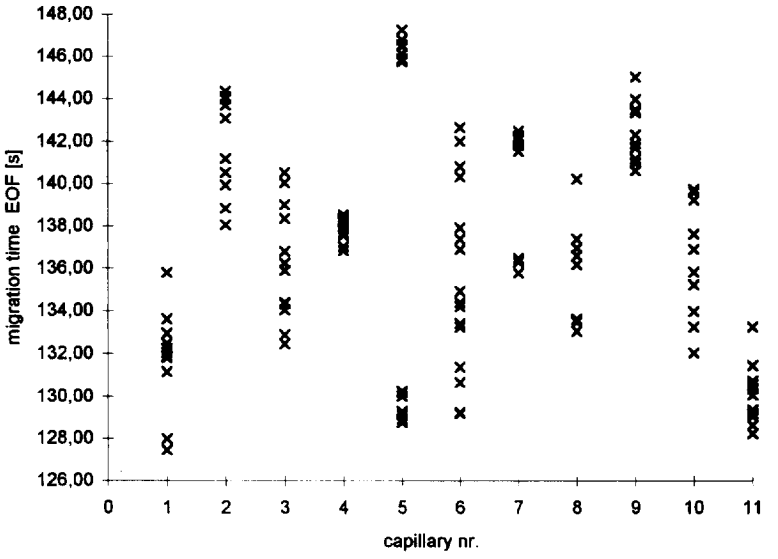


Fig. 8. EOF migration times obtained on all investigated capillaries. Most series shows a big aberration, and consequently a big standard deviation. A significant difference between capillaries is not obvious.

in Table 5. However, a few impurities are still unknown. I4 and I5 are very hydrophobic impurities. They migrate with Sudan III (compare Figs. 3 and 4). Suitable reference substances were not available.

10,11-Dihydrocarbamazepine was baseline-separated from carbamazepine (Figs. 3, 4, 6 and 7). The resolution (R_S) was calculated to approximately 4 using Eq. (1). If the sample buffer concentration were reduced a resolution of 10 could be achieved. This is a remarkable advantage compared to HPLC applications.

$$R_S = \frac{2(t_2 - t_1)}{w_{h1} + w_{h2}} \quad (1)$$

where t = migration time, w_h = peak width at half height (in time), 1 and 2 refer to the two solutes.

3.3. Quantitation

Quantitation was done using corrected peak-areas at the wavelength of 210 nm. The R.S.D. was between 4 and 10% for the impurities and between 0.05 and 0.3% for carbamazepine (Table 5).

3.4. Robustness

The method was successfully transferred to another CE instrument, the HP^{3D}-CE System (Hewlett-Packard). However, due to a lower detector sensitivity, resulting in a higher limit of detection, some peaks could not be detected any more. Application of a bubble cell did not improve the sensitivity.

Using the standard method described in Section 2, capillaries of various lots and different manufacturers were tested. Fig. 8 shows the distribution of the EOF migration times over all runs of a sequence and over all tested capillaries. Analysis of the data has shown, that the R.S.D. (0.4–3%) of the EOF migration times (n = 9–16) of a single capillary batch was only insignificantly smaller than the R.S.D. (3.22%) of the average EOF migration times of all capillaries. A difference between capillaries from various lots or companies was not evident. This result is in accordance with a preceding statement by Altria and Rudd [12].

4. Conclusions

A robust MEKC method to control the purity of carbamazepines was developed and validated. Carbamazepine and 10,11-dihydrocarbamazepine were baseline-separated. The achieved resolution of 4 was considerably better than the resolution obtained by HPLC would have been [1,2].

By transferring the method to another instrument and different capillaries the robustness was confirmed.

Acknowledgments

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