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Therapeutic drug monitoring of antiepileptics by capillary electrophoresis
Characterization of assays via analysis of quality control sera containing 14 analytes

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Abstract

Quality assurance is an important aspect in therapeutic drug monitoring (TDM). Capillary electrophoresis (CE) assays for determination of (i) ethosuximide via direct injection of serum or plasma, (ii) lamotrigine after protein precipitation by acetonitrile and analysis of an aliquot of the acidified supernatant, and (iii) carbamazepine and carbamazepine-10,11-epoxide after solute extraction followed by analysis of the reconstituted extract are characterized via analysis of a large number of commercial quality control sera containing up to 14 analytes (9 of them are anticonvulsants) in sub-therapeutic, therapeutic and toxicologic concentration levels. CE data obtained in single determinations are shown to compare well with the spike values and the mean of data determined in other laboratories using immunoassays and/or high-performance liquid chromatography, values that are reported by the external quality control scheme. Carbamazepine and ethosuximide drug levels are also shown to agree well with those determined in our departmental drug assay laboratory using automated immunoassays. The presented data reveal the effectiveness of assay assessment via analysis of quality control sera and confirm the robustness of the assays for TDM in a routine setting.

Keywords: Quality control; Pharmaceutical analysis; Antiepileptics; Carbamazepine; Lamotrigine; Ethosuximide

1. Introduction

It is common practice that pharmacotherapy with anticonvulsants, including carbamazepine (CBZ), ethosuximide (ETHO), phenytoin, valproic acid, phenobarbital and lamotrigine (LAMO), is accompanied by therapeutic drug monitoring (TDM). The determination of antiepileptic drugs in body fluids on a routine basis is important to clarify and control their therapeutic and toxic effects (optimization of pharmacotherapy) and to assess the patient's compliance to therapy [1–5]. Reported methods for analysis of antiepileptics in serum and plasma include immunological techniques, chromatographic approaches [mainly high-performance liquid chromatography (HPLC)] and techniques based upon capillary electrophoresis (CE). Immunoassays, including those based upon fluorescence polarization immunoassay (FPIA) and enzyme multiplied immunoassay technique (EMIT), may be utilized to rapidly

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monitor drug levels in blood. No multianalyte immunoassays for antiepileptics are commercially available. Furthermore, immunoassays for lamotrigine, carbamazepine-10,11-epoxide (CBZE) and others have not been developed. On the other hand, multiple antiepileptics can be determined simultaneously and selectively using chromatographic assays. This separation-based technology can, without much effort, be adapted to the analysis of new drugs while the development of new immunoassays is demanding and expensive. Thus, in the routine arena of e.g. clinics specializing in the treatment of epilepsy patients, anticonvulsants are often determined by HPLC [5,6].

Due to its high separation power, rapid analysis time and ease of operation, CE has enjoyed growing interest for drug monitoring during the last two decades [7–11]. Our laboratory as well as others have been engaged in the development of CE-based methods for the determination of antiepileptics in body fluids [8,12–27]. Assays for monitoring of serum and plasma levels of ETHO [8,14,18], phenobarbital [8,14,17,18,27], primidone [17,18], phenytoin [12,14,17], felbamate [21], LAMO [22], gabapentin [23], zonisamide [12,17], CBZ [14,16,17,26] and CBZE [16,26] have been reported. Micellar electrokinetic capillary chromatography (MECC), an approach that permits the analysis of uncharged and charged solutes, is the basis for most of these assays, whereas capillary zone electrophoresis (CZE) has been used for monitoring of LAMO and gabapentin.

The developed CE assays for antiepileptics are well characterized from an analytical point of view. However, very little information has thus far been published on quality assurance, a prerequisite for the adoption of drug assays into the routine arena. Preliminary data obtained with quality control samples have been reported for ETHO [18], phenobarbital and ETHO [8], CBZ and CBZE [26] and LAMO [10]. In this paper, the performance of three CE-based assays is assessed with data obtained from a large number of commercial quality control sera containing up to 14 different analytes in sub-therapeutic, therapeutic and toxic concentration levels. ETHO was analyzed by MECC via direct injection of serum, LAMO was determined by CZE after protein precipitation using acetonitrile and analysis of an aliquot of the acidified supernatant, and CBZ and CBZE were monitored by MECC after solute extraction followed by analysis of the reconstituted extract.

2. Materials and methods

2.1. Chemicals, quality control samples, blank matrices and preparation of calibrators and controls

All reagents were of analytical or research grade. Except for LAMO (Wellcome Foundation, London, UK) and CBZE (Alltech, PA, USA), the antiepileptics employed were of European pharmacopoeia quality and were obtained from the university hospital pharmacy (Berne, Switzerland). Thyramine chloride was from Fluka (Buchs, Switzerland), sodium acetate, acetic acid (100%), *p*-bromoacetanilide, NaOH, $Na_2B_4O_7$, Na_2HPO_4, 2-propanol and ethyl acetate were from Merck (Darmstadt, Germany), sodium dodecylsulfate (SDS) was from Bioprobe Systems Labs. (Montreuil-Sous-Bois, France) and acetonitrile was from Biosolve (Valkenswaard, Netherlands). Heath control UKNEQAS external quality control sera were purchased from Cardiff Bioanalytical Services (Cardiff, UK). Our own plasma or bovine plasma that was received from the local slaughter house were used for the preparation of calibration and control samples. Stock solutions of drugs (about 1 mg/ml) were prepared in methanol, stored at 5°C and mixed and diluted with water as required. For the preparation of calibrators and controls, aliquots of these solutions were added to bovine or human plasma to reach the desired concentrations. Calibration ranges employed for the various assays are presented in Table 1.

2.2. CE instrumentation

All CE analyses were performed in uncoated fused-silica capillaries of 50 μm I.D.×360 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA) using the BioFocus 3000 capillary electrophoresis system (Bio-Rad Labs., Hercules, CA, USA). The capillaries were mounted in a user-assembled cartridge (Bio-Rad). Injection of sample was effected by applying

Table 1
General assay specifications

Drug	Usual therapeutic range (μ*M*)	Method/sample preparation	Calibration range (μ*M*)	Number of calibrators	Calibration matrix	Calibration principle[a]	Range of regression coefficient *r* of calibration graphs	Detection limit (μ*M*)
CBZ	17–47	MECC/liquid/liquid extraction	0.84–84	5	Human or bovine plasma	Internal/rel. peak areas	0.9990–0.9995	0.40
CBZE	About 25% of CBZ	MECC/liquid/liquid extraction	0.40–40	5	Human or bovine plasma	Internal/rel. peak areas	0.9987–0.9991	0.30
ETHO	280–700	MECC/none	43.9–1770	5(4–6)	Human plasma	External/rel. peak areas	0.9989–0.9993	15
LAMO	8–24	CZE/protein precipitation	3.9–39	5	Bovine plasma	Internal/rel. peak areas	0.9986–0.9999	2.0

[a] Relative peak area refers to the peak area divided by the detection time of the peak maximum.

positive pressure and the temperatures of cartridge and carousel were maintained at specified values (see below). New capillaries were conditioned with 1 *M* NaOH (about 20 min) followed by 0.1 *M* NaOH (about 20 min). BioFocus Integration software (version 5.0, Bio-Rad) was employed for data conversion and evaluation.

2.3. MECC assay for CBZ and CBZE

The assay employed is based upon liquid/liquid extraction of the drugs and is essentially the same as described elsewhere [26]. Briefly, 0.25 ml of quality control, calibrator or control sample was combined with 25 μl of internal standard (IS) solution (0.5 mg/ml *p*-bromoacetanilide in methanol), 50 μl of 0.5 *M* sodium hydroxide solution and 1 ml of ethyl acetate in a 1.5 ml Eppendorf tube. After shaking the closed tube for 10 min and centrifugation at 9000 *g* for 3 min, the organic (upper) phase was transferred into a clean 1.5 ml Eppendorf tube and evaporated to dryness at 40°C employing a gentle stream of nitrogen. The residue was redissolved in 60 μl of 10-times diluted running buffer containing 5% (v/v) methanol instead of 2-propanol. Analyses were performed in capillaries of 50 cm total length (44.5 cm to the detector). The temperatures of cartridge and carousel were maintained at 25°C. Injection of sample was effected by applying positive pressure (6 p.s.i. s=41.37 kPa s), a voltage of 25 kV (current: about 58 μA) was employed and detection was effected at 210 nm. Before each experiment the capillary was rinsed with 0.1 *M* NaOH for 3 min, distilled water for 2 min and running buffer for 3 min. The running buffer was composed of 6 m*M* $Na_2B_4O_7$/10 m*M* Na_2HPO_4 (pH 9.2) containing 75 m*M* SDS and 5% (v/v) 2-propanol. The running buffer in the inlet vial was replenished after 6 runs. The assay is based upon five-point internal calibration in the ranges of 0.84–84 μ*M* (CBZ) and 0.4–40 μ*M* (CBZE) using relative peak areas (peak areas divided by detection time) for data evaluation (Table 1).

2.4. MECC assay for ethosuximide

Ethosuximide was determined as reported previously [18]. Serum and plasma samples were injected directly and did not include any internal standard (Table 1). A phosphate–tetraborate buffer (6 m*M* $Na_2B_4O_7$ and 10 m*M* Na_2HPO_4, pH 9.2) containing 75 m*M* SDS was used. Analyses were performed in capillaries of 75 cm total length (69.5 cm to the detector). The temperatures of cartridge and carousel were maintained at 35 and 30°C, respectively. Injection of sample was effected by applying positive pressure (6 p.s.i. s=41.37 kPa s), a voltage of 20 kV was applied (current: about 30.5 μA) and detection was effected at 220 nm. Before each experiment, capillaries were sequentially rinsed with 0.1 *M* NaOH (6 min), water (1 min) and buffer (6.7 min). The running buffer in the inlet vial was replenished

after 7–8 runs. The assay is based upon 4–6-point external calibration in the range of 43.9–1770 μ*M* using relative peak areas (peak areas divided by detection time) for data evaluation (Table 1).

2.5. CZE assay for lamotrigine

Lamotrigine was determined using the CZE approach described by Shihabi and Oles [22]. This assay is based upon deproteination with acetonitrile and injection of the acidified supernatant. Briefly, 50 μl of serum or plasma (quality control, calibrator or control sample) and 100 μ1 of acetonitrile containing 30 μg/ml thyramine (internal standard) were vortex mixed for about 15 s and centrifuged at 10 000 *g* for 3 min. Then, the clear supernatant was decanted and combined with 100 μ1 of 0.9 *M* acetic acid and an aliquot of this mixture was injected. Analyses were carried out with fused-silica capillaries of 34 cm (28.5 cm effective) length, an applied voltage of 12 kV (current: about 62 μA), solute detection at 210 nm and hydrodynamic sample injection at 34.47 kPa s (5 p.s.i. s). The temperatures of cartridge and carousel were maintained at 27 and 30°C, respectively. Before each experiment the capillary was rinsed with 0.2 *M* NaOH for 1 min and running buffer for 1 min. The running buffer was composed of 130 m*M* sodium acetate that was adjusted with acetic acid to pH 4.5. The running buffer in the inlet vial was replenished after about 15 runs. The assay is based upon five-point internal calibration in the range of 3.9–39 μ*M* using relative peak areas (peak areas divided by detection time) for data evaluation (Table 1).

2.6. Reference routine assays

CBZ serum levels were either determined by EMIT (Syva, Palo Alto, CA, USA) on a Cobas Fara centrifugal analyzer (F. Hoffmann-La Roche, Diagnostica, Basel, Switzerland) or by FPIA on the TDxFLx analyzer (Abbott Labs. Irving, Texas, USA). ETHO levels were determined by FPIA on the TDxFLx analyzer (Abbott Labs). The immunoassays were performed according to the manufacturers instructions using their reagent kits. Internal quality control of CBZ assessed by FPIA over one year revealed inter-day imprecisions of 6.9%, 8.0% and 4.1% for reported drug levels of 12.8 μ*M* (mean: 12.95 μ*M*; $n=83$), 25.6 μ*M* (mean: 26.45 μ*M*; $n=82$) and 68.4 μ*M* (mean: 66.21 μ*M*; $n=79$), respectively, values that were found to be comparable to those obtained with EMIT. Values for ETHO determined by FPIA were 5.0%, 3.7% and 4.4% for reported drug levels of 247.8 μ*M* (mean: 238.13 μ*M*; $n=26$), 495.6 μ*M* (mean: 476.78 μ*M*; $n=27$) and 849.6 μ*M* (mean: 816.67 μ*M*; $n=22$), respectively.

2.7. Statistical and graphical data analysis

Comparative drug levels were analyzed by linear regression analysis and by bias analysis defined as the mean and standard deviation of the differences of each data pair [28]. Comparative sets of data were statistically compared using the Mann–Whitney rank sum test (comparison of two groups) or by analysis of variance (ANOVA, comparison of three groups) and each set of data was subjected to the normality test. Statistical evaluations were performed with SigmaStat for Windows version 1.0 (Jandel, Corte Madera, CA, USA) and graphical presentations were made with Microsoft Excel 97 (Microsoft, Redmond, WA, USA).

3. Results and discussion

3.1. General comments to the assays and the quality control samples

As the assays employed for the determination of LAMO [22], ETHO [18], CBZ [26] and CBZE [26] were essentially those described elsewhere, no detailed descriptions are presented here. The general specs and typical imprecision data are presented in Tables 1 and 2, respectively. It is important to mention that human or bovine plasma can be employed as calibration matrices for assays based upon solute extraction and protein precipitation. However, for drug analysis via direct injection of sample, bovine plasma should not be used as interferences were observed. The purchased quality control samples referred to as UKNEQAS therapeutic drugs mixture are prepared from human serum containing 14 analytes, namely nine antiepileptics (CBZ, CBZE, ETHO, LAMO, phenytoin, primidone, valproic acid,

Table 2
Typical intra-day and inter-day imprecision data

Drug	*n*	Intra-day (same day)			Inter-day (different days)		
		Drug level (μM)	Mean (μM)	RSD (%)	Drug level (μM)	Mean (μM)	RSD (%)
CBZ	5	21.0	19.9	2.98	21.0	19.9	6.40
CBZE	5	9.9	10.7	2.55	9.9	11.1	7.48
ETHO	5	498.1	499.8	0.58	741.7	733.5	2.39
LAMO	5	9.75	9.56	1.54	9.75	9.28	5.27

clonazepam and phenobarbital), theophylline, caffeine, gentamicin, digoxin and lithium. These analytes are present in sub-therapeutic, therapeutic and toxic concentration levels. Typical electropherograms obtained with calibrator and quality control samples are presented in Figs. 1–3 and typical assay imprecision data are summarized in Table 2. Intra-day and inter-day reproducibilities are shown to be ≤8% for all cases studied. These data compare favorably with the specs of drug assays based on immunoassays, HPLC or GC. Throughout this work, samples were analyzed only once. Calibration graphs were found to be linear ($r>0.998$, Table 1) and *y*-intercepts were determined to be considerably smaller than the concentration value of the lowest calibrator (data not shown).

The data of Fig. 1B are those for the 3TD0399 control serum that is reported to have been spiked with 17.2 μM CBZ and 7.9 μM CBZE. The CBZ value determined with our MECC assay was 17.3

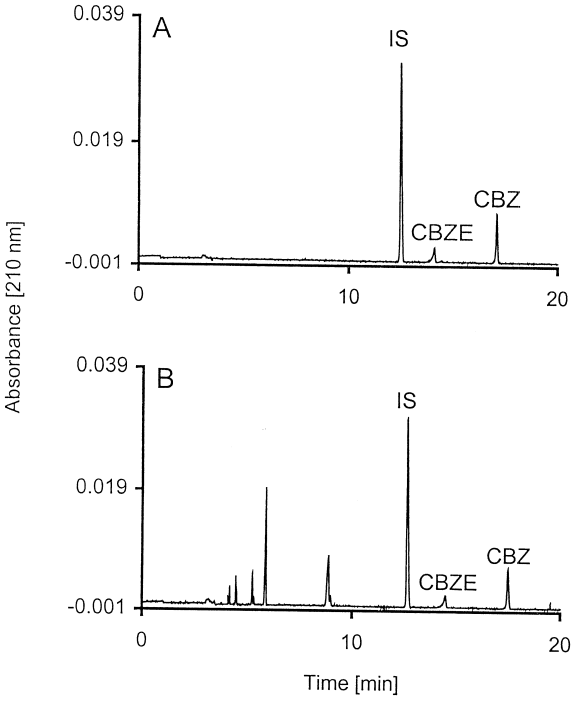

Fig. 1. MECC electropherograms of extracts prepared (A) from a calibrator serum containing 21 μM CBZ and 9.9 μM CBZE and (B) a quality control serum that was determined to contain 17.3 μM CBZ and 8.1 μM CBZE. Experimental conditions as described in Section 2.3.

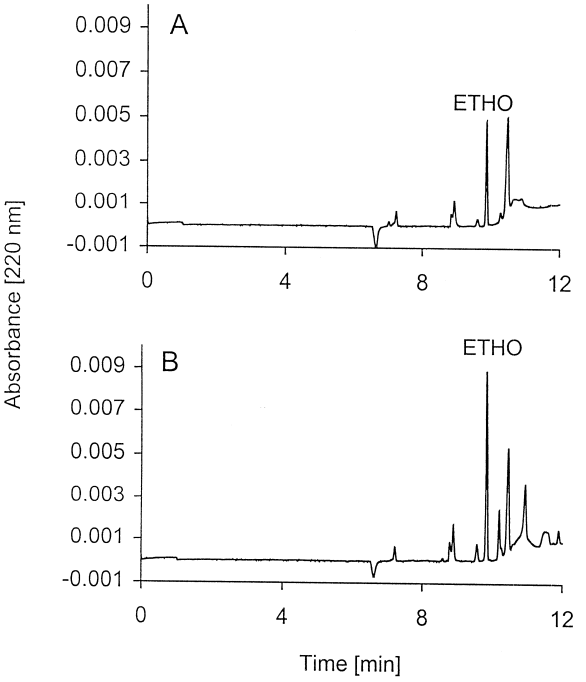

Fig. 2. MECC electropherograms of directly injected sera of (A) a calibrator containing 311.5 μM ETHO and (B) a quality control sample that was determined to contain 611.7 μM ETHO. Experimental conditions as described in Section 2.4.

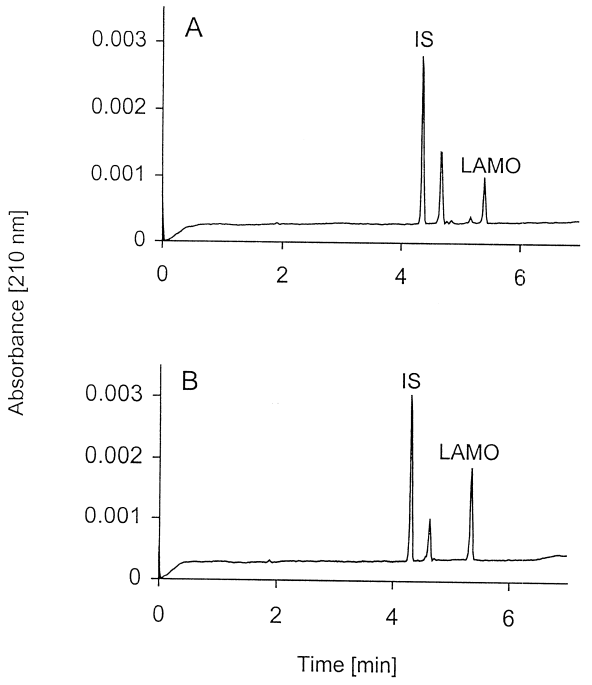

Fig. 3. CZE electropherograms of acidified deproteinized supernatants of (A) a calibrator plasma containing 9.75 μ*M* LAMO and (B) a quality control serum that was determined to contain 19.83 μ*M* LAMO. Experimental conditions as described in Section 2.5.

μ*M* and the reported consensus mean evaluated from the data of 284 laboratories was 16.2 μ*M*. FPIA revealed a CBZ level of 16.0 μ*M*. Similarly, this serum was found to contain 8.1 μ*M* CBZE and the mean of the data of 26 laboratories was 7.6 μ*M*. MECC with liquid/liquid extraction is demonstrated to provide results that are well within the ±10% range of the spike values and that are close to the means which were calculated with data from other laboratories. The other antiepileptics present in this serum, namely phenytoin (spike value: 10.0 μ*M*), phenobarbital (228.8 μ*M*), valproic acid (199.0 μ*M*), primidone (49.8 μ*M*), LAMO (36.83 μ*M*), clonazepam (142.6 μ*M*) and ETHO (447.5 μ*M*), as well as theophylline (54.3 μ*M*) and caffeine (4.9 μ*M*), do not interfere with CBZ and CBZE under the chosen experimental conditions. As a matter of fact, with the exception of valproic acid, the same or slightly modified assay could be employed for the simultaneous monitoring of all antiepileptics [14,17,26].

The assay for ETHO is very simple as serum and plasma can be injected directly and thus without elaborate sample preparation. The data of Fig. 2B are those for the 4TD1097 control serum that is reported to have been spiked with 662.4 μ*M* ETHO. The value determined with our MECC assay was 611.7 μ*M* and the reported consensus mean evaluated from the data of 72 laboratories was 652.4 μ*M*. Analysis by FPIA in our departmental drug assay laboratory revealed a value of 643.7 μ*M*. MECC with direct sample injection is demonstrated to provide a result that is within the ±10% range of the spike value and the consensus mean of values that were mainly produced by various immunoassays. The other antiepileptics present in this serum, namely phenytoin (spike value: 49.5 μ*M*), phenobarbital (117.9 μ*M*), CBZ (7.5 μ*M*), CBZE (2.0 μ*M*), valproic acid (100.2 μ*M*), primidone (34.8 μ*M*), clonazepam (151 μ*M*) and LAMO (34.07 μ*M*), as well as theophylline (99.6 μ*M*) and caffeine (5.0 μ*M*), do not interfere with ETHO under the chosen experimental conditions. This assay format has also been shown to be applicable to the determination of felbamate [21] and to multiple antiepileptics (phenobarbital, primidone and ETHO) in one run [18].

With the assay for LAMO, very simple electropherograms are obtained (Fig. 3). Based upon the acetonitrile content of the injected sample, analytes are being stacked, this providing the required sensitivity for this drug [22]. The data of Fig. 3B are those for the 4TD0799 control serum that is reported to have been spiked with 21.97 μ*M* LAMO. The value determined with our CZE assay was 19.83 μ*M* and the reported consensus mean evaluated from the data of 56 laboratories was 20.20 μ*M*. CZE with acetonitrile deproteination is demonstrated to provide a result that is within the ±10% range of the spike value and that is close to the consensus mean of values that were mainly produced by HPLC. The other antiepileptics present in this serum, namely phenytoin (spike value: 40.1 μ*M*), phenobarbital (99.1 μ*M*), CBZ (57.7 μ*M*), CBZE (7.5 μ*M*), valproic acid (441.6 μ*M*), primidone (27.4 μ*M*), clonazepam (203.7 μ*M*) and ETHO (454.2 μ*M*), as well as theophylline (118.3 μ*M*) and caffeine (15 μ*M*), do not interfere with LAMO under the chosen experimental conditions. Thus, this assay appears to be selective for LAMO.

3.2. Assay characterization via external quality assessment

Using the three selected CE assays, a large number of quality control samples purchased between 1997 and 1999 was analyzed and the obtained data were compared graphically (Figs. 4 and 5) and statistically (Table 3). Drug concentrations in these sera are covering ranges that go beyond the usual therapeutic ranges but were within the calibration ranges of the assays (Table 1). The CE data were compared with the reported spike values and with the mean of the data obtained in other laboratories (denoted as consensus mean, data that were monitored with different techniques). For all four compounds, mean and median values were found to be very similar. For the comparison of the CE and spike values, statistical analysis using the Mann–Whitney rank sum test revealed the absence of a statistically significant difference between each pair of input groups ($P>0.44$). Except for CBZE, the same was found to be true for the comparison of the CE and consensus mean values ($P>0.30$; for CBZE $P=0.035$). Using the Kruskal–Wallis one-way analysis of variance by ranks for the comparison of all three input groups revealed the absence of a statistically significant difference ($P=0.307$, 0.0873, 0.662 and 0.729 for CBZ, CBZE, ETHO and LAMO, respec-

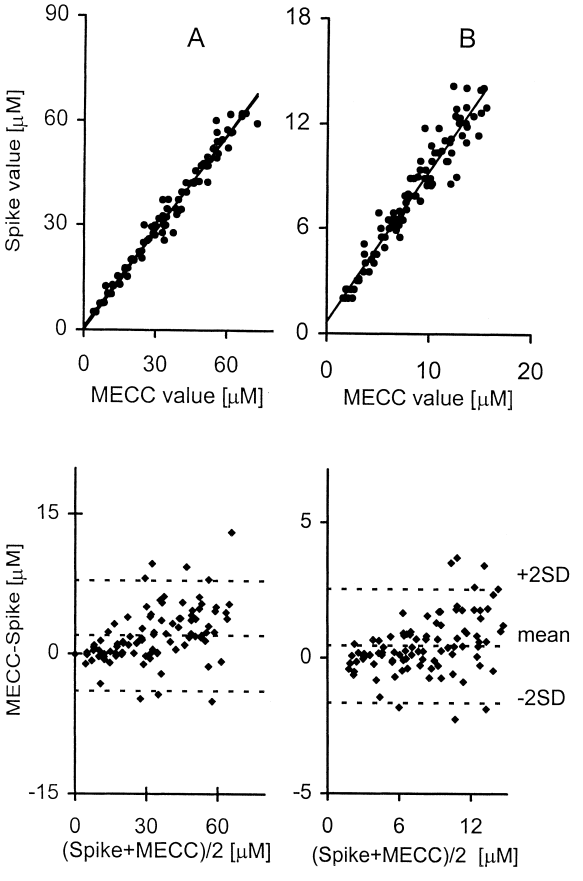

Fig. 4. Comparative drug levels for (A) CBZ and (B) CBZE of 96 quality control sera. The solid lines in the upper graphs represent correlation graphs determined by linear regression analysis and the broken lines in the lower graphs are mean and ±2 SD of the differences of the data pairs (for statistical data see Table 3).

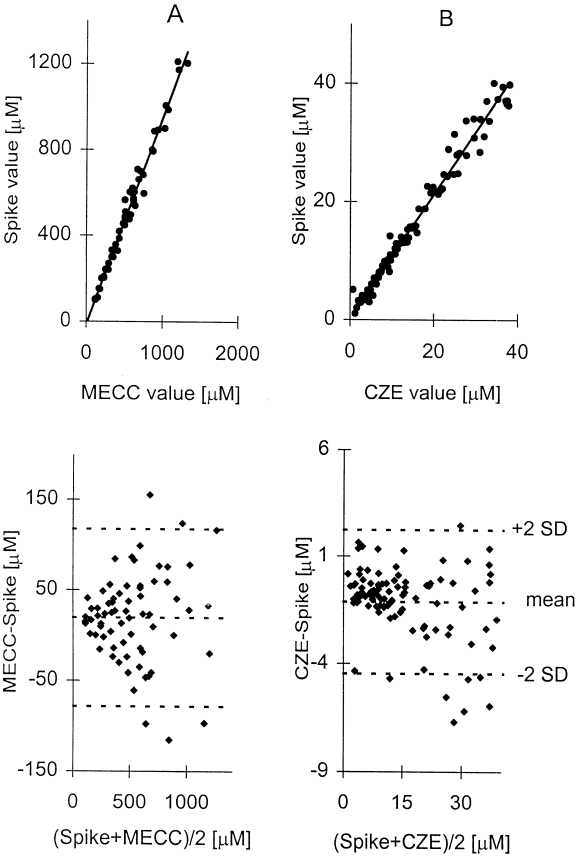

Fig. 5. Comparative drug levels for (A) ETHO ($n=69$) and (B) LAMO ($n=100$) of quality control sera. The solid lines in the upper graphs represent correlation graphs determined by linear regression analysis and the broken lines in the lower graphs are mean and ±2 SD of the differences of the data pairs (for statistical data see Table 3).

Table 3
Statistical, linear regression analysis and bias analysis data of comparative drug levels in external quality control samples

Drug	Number of samples	CE data		Reported data of quality assurance[a]			Linear regression analysis data[b]			Bias analysis data[c]	
		Mean (μ*M*)	Median (μ*M*)		Mean (μ*M*)	Median (μ*M*)	Slope	*y*-Intercept (μ*M*)	*r*	Mean (μ*M*)	SD (μ*M*)
CBZ	96	34.2	33.8	Spiked value	32.2	31.9	0.915	0.96	0.989	1.96	2.96
				Consensus mean	30.1	29.5	0.827	1.86	0.989	4.05	3.89
				EMIT/FPIA value[d]	30.2	28.0	0.832	1.74	0.984	4.00	4.08
CBZE	96	8.1	7.9	Spiked value	7.7	7.8	0.863	0.66	0.967	0.45	1.05
				Consensus mean	6.9	6.9	0.770	0.64	0.973	1.22	1.16
ETHO	69	536.1	505.5	Spiked value	516.4	486.4	0.975	−6.41	0.985	19.45	48.89
				Consensus mean	496.0	463.7	0.939	−7.44	0.988	39.72	45.75
				FPIA value[e]	489.9	451.3	0.930	−8.87	0.987	46.38	47.47
LAMO	100	14.9	11.5	Spiked value	16.0	12.9	1.041	0.50	0.989	−1.12	1.67
				Consensus mean	14.9	12.0	0.970	0.47	0.989	−0.024	1.57

[a] The consensus mean represents the mean of all data reported by 260–290 laboratories for CBZ, 23–28 laboratories for CBZE, 70–75 laboratories for ETHO and 40–50 laboratories for LAMO (independent of analytical method used).

[b] CE data and reported or immunoassay data were taken as *x*-axis and *y*-axis, respectively.

[c] To analyze the bias, the mean and SD of the difference of each data pair (CE value — reported or immunoassay value) was calculated.

[d] These data were generated in the departmental drug assay laboratory using EMIT ($n=24$) and FPIA ($n=40$).

[e] The FPIA data ($n=69$) were generated in the departmental drug assay laboratory.

tively). Furthermore, linear regression analysis of comparative data pairs revealed linear relationships ($r \geq 0.967$) with relatively small *y*-intercepts and slopes that were reasonably close to unity (Table 3). Graphs comparing CE data with spike values are presented in Figs. 4 and 5. Other graphs were found to be very similar and are thus not depicted. Furthermore, the bias expressed by the mean of the differences of the data pairs was found to be small, this particularly in relation to the concentration range monitored. CBZ and ETHO drug levels were found to compare well with those determined in our departmental drug assay laboratory using commercial immunoassays (Table 3, for assay specs refer to Section 2.6).

For the three assays, analysis of quality control sera containing up to 14 drugs is shown to reveal data that compare well with those obtained by other methods (immunoassays, HPLC) and the spike concentrations. Interferences originating from the other drugs were not observed. This is very assuring and is an important aspect for the adoption of CE technology in TDM. Thus, the presented work further broadens the confidence in CE technology and assay characterization via analysis of quality control samples is highly recommended. The data presented in this paper were generated via analysis of samples that were available in our departmental drug assay laboratory. Except for some LAMO results, the CE data were not reported to the external quality control scheme and were compared to the reported values by ourselves. For the case of LAMO, experiences with the long-time use of CE in the routine arena, including the analysis of four monthly external quality control samples whose data are directly reported to the quality control scheme, are currently being gained in our drug assay laboratory and will be reported in the not too distant future.

4. Conclusions

In this paper, the performance of three simple, laboratory developed CE assays for monitoring of antiepileptics in serum and plasma are characterized with data obtained by analysis of a large number of external quality control sera that contain up to 14 analytes (including 9 antiepileptics) in sub-therapeutic, therapeutic and toxic concentration levels. The assays are based upon single-step liquid/liquid extraction (for CBZ and CBZE), direct injection (for ETHO) and protein precipitation with acetonitrile

(for LAMO). The described methods, in which each sample is analyzed only once, are demonstrated to be suitable and sufficiently robust for TDM. CE data were found to compare well with the spike values and the consensus mean of drug levels generated in other laboratories using immunoassays and/or HPLC. Compared to HPLC, CE technology is attractive for several reasons, namely the high degree of automation, the consumption of small amounts of chemicals and organic solvents, the requirement of small amounts of sample and the low cost of capillary columns. Analysis of a large number of quality control sera as reported here is recommended for assay validation prior to adoption of an assay into the routine arena.

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