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Assessment of automated capillary electrophoresis for therapeutic and diagnostic drug monitoring: determination of bupivacaine in drain fluid and antipyrine in plasma

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ABSTRACT

In an effort to evaluate the use of electrokinetic capillary technology for therapeutic and diagnostic drug monitoring, samples were analysed batchwise with an automated, high-throughput capillary electrophoretic instrument coupled to an inexpensive PC data acquisition and evaluation system. Examples studied included the capillary electrophoretic (HPCE) determination of bupivacaine in drain fluid collected after pulmonary surgery and the micellar electrokinetic capillary chromatographic (MECC) determination of antipyrine in human plasma. Analyses for antipyrine could be accomplished without any sample pretreatment whereas bupivacaine required extraction prior to analysis. Antipyrine determination was effected through external calibration using either peak areas, relative peak areas or peak heights. The intraday and interday reproducibilities ($n = 15$) of the evaluated concentrations were 1.5–3% and 5–6%, respectively. For bupivacaine, determination based on internal and external calibration employing peak areas and peak heights was investigated. The intraday and interday reproducibilities ($n = 5$) of bupivacaine concentrations were about 1% and 2%, respectively, for internal calibration and both about 5% for external calibration. The electrokinetic capillary data compared well with data obtained by gas chromatography (bupivacaine) and high-performance liquid chromatography (antipyrine).

INTRODUCTION

With the more efficient therapeutic application of various drugs and the necessity for screening and confirmation of drugs in body fluids for diagnostic purposes, there has evolved a need for reliable analytical procedures. Currently used methods are based on the principles of spectrophotometry, immunoassays and chromatography [1–3]. All of these techniques have advantages and disadvantages. The reagents for many of the immunological assays are available in kit form, together with highly automated instrumentation. This permits such analyses to be performed easily and efficiently and with high sensitivity and precision. They provide the most rapid (high sample throughput) analytical procedures available to date. However, immunological techniques are prone to disturbances by molecules of similar structure (cross-reactivity) and the availability of antibodies is limited to the most frequently measured drugs. Owing to separation, the chromatographic assays provide specific results for multiple compounds but typically require extensive sample preparation and derivatization. The sample throughput is low because of sequential injection of the samples, and complete automation of chromatographic protocols is difficult.

Recently, instrumentation for electrokinetic separations in capillaries with very small I.D. (25–75 μm) has become available [4–8]. Both high-performance capillary electrophoresis

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(HPCE) and micellar electrokinetic capillary chromatography (MECC) have not yet been adopted in routine applications for drug monitoring. However, their feasibility for therapeutic and diagnostic drug determinations has been tested in various laboratories [8–17]. Compared with chromatographic assays, the advantages of electrokinetic capillary analyses are high resolution, efficiency and speed, automation, small sample size, rapid method development and the use of small amounts of inexpensive and non-polluting chemicals. Although little work has been reported on the ability of HPCE and MECC to provide quantitative analyses for drugs in biological matrices [11,13,17,18], this emerging technology has already been promoted for therapeutic drug monitoring [19].

In an effort to evaluate the use of automated electrokinetic capillary technology for therapeutic and diagnostic drug monitoring, several hundred patients' samples were analysed batchwise with a high-throughput capillary electrophoresis instrument coupled to a PC data acquisition and evaluation system. Examples studied included the MECC determination of antipyrine in human plasma and the HPCE determination of bupivacaine in drain fluid collected after pulmonary surgery, drugs which are typically measured by chromatographic techniques. Antipyrine levels are employed to determine microsomal enzyme activity of the liver [20,21] and bupivacaine monitoring is essential for optimized administration of this drug [22,23]. The aims of this work were to demonstrate the high-quality data obtained by HPCE and MECC, to elucidate the potential of employing this technology in a routine laboratory and to compare the electrokinetic data for antipyrine and bupivacaine with those obtained by high-performance liquid chromatography (HPLC) and gas chromatography (GC), respectively.

EXPERIMENTAL

Drugs and chemicals

The local anaesthetics, purchased as hydrochlorides, antipyrine and phenacetin were of European Pharmacopoeia quality. Bupivacaine was obtained from Sintetica (Mendrisio, Switzerland) and antipyrine, phenacetin, mepivacaine and lidocaine [supplied in vials as 2% (w/v) solutions] were supplied by the university hospital pharmacy (Berne, Switzerland). Hexane, methanol, 2-propanol, methylene chloride and ethyl acetate (all of HPLC grade) were obtained from Rathburn Chemicals (Walkerburn, UK), NaH_2PO_4, $Na_2B_4O_7$ and H_3PO_4 (85%) from Merck (Darmstadt, Germany) and sodium dodecyl sulphate (SDS) was from Sigma (St. Louis, MO, USA). Our own plasma, employed as a calibration matrix, was prepared by centrifugation [1350 *g* (3600 rpm) for 10 min] and stored at −20°C in aliquots of about 200 μl.

Origin of samples

Pleural drain fluid samples containing bupivacaine were received from the Department of Thoracic and Cardiovascular Surgery, University of Berne, and stemmed from patients undergoing thoracotomy. Plasma samples containing antipyrine stemmed from subjects who had been dosed with 1 g of antipyrine and blood samples drawn over a period of 48 h after administration.

Sample preparation for analysis of local anaesthetics

Aqueous standard solutions of bupivacaine (500 μg/ml), mepivacaine (300 μg/ml) and lidocaine (200 μg/ml) were prepared and stored at 4°C. For calibration, aqueous (HPCE) or plasma (GC) samples containing 0.5, 1, 2.5, 5, 10, 15 and 20 μg/ml of bupivacaine, each with 12 μg/ml of mepivacaine (internal standard), were employed. Independently prepared calibration samples were used as controls. Patients' samples were spiked by addition of known aliquots of the standard solutions to the drain fluid prior to sample extraction. Liquid–liquid extraction of the three local anaesthetics was achieved under basic conditions employing either ethyl acetate or hexane (modification of the procedure reported in ref. 22). Typically, 1 ml of tenfold diluted drain fluid (or 1 ml of a calibration or control solution), spiked with mepivacaine (40 μl of standard solution), 1 ml of 0.5 *M* NaOH and 6 ml of the organic solvent (ethyl

acetate or hexane) were added to an 11-ml screw-capped Sovirel test-tube. After vortex mixing for 30 s (HPCE) or vigorous shaking for 5 min (GC) and centrifugation at 1500 *g* (4000 rpm) for 5 min, the upper (organic) phase was transferred into a glass centrifuge tube with a short conical bottom and evaporated to dryness at 40°C. The residue was dissolved in a mixture of 50 μl of running buffer and 50 μl of 0.1 *M* HCl (for HPCE) or 50 μl of methanol (for GC) and vortex mixed for about 30 s. Using HPCE, the recoveries for bupivacaine and mepivacaine were determined to be 68 and 54% (hexane extraction) and 67 and 91% (ethyl acetate extraction), respectively.

Sample preparation for determination of antipyrine

Methanolic standard solutions of antipyrine (200 μg/ml) were prepared and stored at 4°C. Blank plasma (preparation of calibrator and 20 μg/ml control samples) was spiked by addition of known aliquots of these solutions to a test-tube and evaporation to dryness, followed by reconstitution with plasma prior to sample application (MECC) or extraction (HPLC). For MECC, patients' samples were vortex mixed for 30 s and filtered using 0.45-μm Nalgene (25-mm diameter) disposable syringe filters (Nalge, Rochester, NY, USA). Blank, calibration and control sera were defrosted and vortex mixed prior to application to the capillary (no filtration). For HPLC, antipyrine was extracted prior to sample analysis (see below).

HPCE of local anaesthetics

A Model 270A-HT capillary electrophoresis system (Applied Biosystems, San Jose, CA, USA) was employed. This apparatus features automated capillary rinsing, sampling and execution of the electrophoretic run. For our experiments it was equipped with a 75 μm I.D. fused-silica capillary of effective separation length *ca.* 72 cm. A PC Integration Pack (PCIP, version 3.0, Kontron Instruments, Zurich, Switzerland) together with a Mandax AT 286 computer system were used for data acquisition, raw data storage, peak integration and peak-height determination of the signals. The pack features automatic range switching and a dynamic sampling rate allowing sampling every 10 ms for rapidly changing signals. Before each run the capillary was rinsed sequentially with 0.1 *M* NaOH (2 min), water (1 min) and buffer (4 min). The running buffer was composed of 35 m*M* $Na_2B_4O_7$ and 45 m*M* NaH_2PO_4 (adjusted to pH 8.1 with 0.1 *M* phosphoric acid). Samples were injected via vacuum suction (typically 1 s). If not stated otherwise, a constant voltage of 19 kV (current 96–98 μA) was applied, the temperature was set at 30°C and detection was effected at 200 nm.

MECC of antipyrine

The Model 270A-HT capillary electrophoresis system was employed as described above. It was equipped with 75 μm I.D. fused-silica capillaries of effective separation length 40–50 cm. Before each run the capillary was rinsed with 0.1 *M* NaOH (2 min) and with buffer (4 min). The running buffer, if not stated otherwise, was composed of 50 m*M* SDS, 9 m*M* NaB_4O_7 and 15 m*M* NaH_2PO_4 and contained 2% (v/v) of 2-propanol (pH *ca.* 8.1). Samples were injected via vacuum suction (typically 1 s). If not stated otherwise, a constant voltage of 20 kV (current 70–80 μA, depending on the column length) was applied, the temperature was set at 35°C and detection was effected at 240 nm.

GC of local anaesthetics after extraction with ethyl acetate

Aliquots of 5 μl were injected into a Model 3920 B gas chromatograph (Perkin-Elmer, Kuessnacht, Switzerland) equipped with a temperature programmer and a thermionic nitrogen–phosphorus-sensitive detector (Perkin-Elmer) operated in the nitrogen mode. A 1.8 m × 2 mm I.D. glass column packed with 3% SE-30 on Chromosorb W HP (80–100 mesh) (Supelco, Gland, Switzerland) was employed. The temperatures of the column, injector and interface were 200, 230 and 250°C, respectively. The carrier gas flow-rate and detector gas supply (nitrogen and hydrogen) were set at 45 ml/min and 0.8 kg/cm^2 respectively. Peak registration and integration were effected with an HP 3390A

integrator (Hewlett-Packard, Widen, Switzerland). Data evaluation was based on internal calibration using peak areas.

HPLC of antipyrine

HPLC analyses were performed according to the procedure of Eichelbaum and Spannbruckner [20] using an M45 solvent-delivery system, a WISP 712 autosampler (both from Waters, Milford, MA, USA). a reversed-phase C_{18} column (Hibar LiChrosorb RP-18, 7 μm, column dimensions 250-4) (Merck) and a Spectroflow 757 UV detector (Kratos Analytical, Ramsey, NJ, USA). Chromatograms were recorded and integrated with a Model 3390A integrator (Hewlett-Packard). The mobile phase consisted of a mixture of an aqueous phosphate buffer (10 m*M*, pH 8) and acetonitrile (80:20, v/v). Phenacetin (10 μg/ml in plasma) served as an internal standard, the flow-rate was 1.5 ml/min, the temperature was ambient and detection was effected at 254 nm. Liquid–liquid extraction of antipyrine and the internal standard (phenacetin) was achieved under basic conditions using methylene chloride. A volume of 0.1 ml of patient's plasma, calibrator plasma or control plasma, spiked with 10 μg/ml of internal standard, 0.1 ml of NaOH (0.1 *M*), 0.4 ml of water and 3 ml of methylene chloride were added to an 11-ml screw-capped Sovirel test-tube. After vigorous shaking for 15 min and centrifugation at 1500 *g* (4000 rpm) for 10 min, the upper (aqueous) phase was discarded and the organic phase was transferred into a centrifuge glass tube with a short conical bottom and evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was dissolved in 200 μl of mobile phase. For analysis, 40 μl were injected. Data evaluation was based on internal calibration employing peak areas.

RESULTS AND DISCUSSION

HPCE determination of bupivacaine in drain fluid

The assay conditions used were optimized for the resolution of three local anaesthetics, bupivacaine, lidocaine and mepivacaine (Fig. 1A). For the determination of bupivacaine, calibration and independent control samples

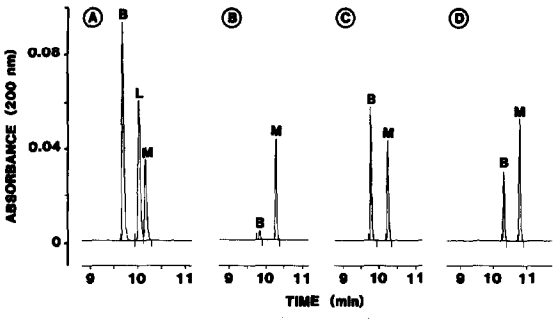

Fig. 1. HPCE of (A) an unextracted standard solution containing 200 μg/ml of bupivacaine (B), 100 μg/ml of lidocaine (L) and 50 μg/ml of mepivacaine (M), (B) a calibrator sample containing 1 μg/ml of bupivacaine, (C) a calibrator sample containing 15 μg/ml of bupivacaine and (D) a tenfold diluted patient's sample containing 64.3 μg/ml of bupivacaine. The applied voltages were (A) 20 kV and (B–D) 19 kV.

containing mepivacaine as internal standard were employed (Fig. 1B and C, respectively). The same internal standard was added to the patients' samples (Fig. 1D). It is important to note that all calibration, control and patients' samples were treated identically. Samples obtained after extraction with ethyl acetate were found to be unstable when stored for several days at 4°C. Decomposition of bupivacaine and mepivacaine was not observed when using the extraction procedure with hexane and storing the samples either for 6 days at 4°C or for 24 h at room temperature. Hence, the HPCE data discussed below were all obtained with hexane extraction.

For quantification, four different approaches were examined using the algorithms provided for automated evaluations with the PCIP. Quantification was studied based on external calibration using peak areas (referred to as HPCE-ext-a in the remainder of the text) or peak heights (HPCE-ext-h) of bupivacaine. The data were also evaluated based on internal calibration using the ratio of the peak areas (HPCE-int-a) or the peak heights (HPCE-int-h) of bupivacaine and mepivacaine (internal standard). Employing linear regression analysis, excellent linear calibration graphs were obtained for all four cases. Mean relative standard deviations (R.S.D.s) of the linear regression analyses (n = 8) for the four calibration principles were 3.25, 2.74, 1.35 and 1.36%, respectively. The corre-

sponding mean y-intercepts were 0.185, 0.055, 0.076 and −0.031 μg/ml. Not surprisingly, these data suggest that internal calibration, *i.e.*, the use of an internal standard, should provide more accurate data than external calibration.

Reproducibility data are summarized in Table I. First, a drain sample containing about 100 μg/ml of bupivacaine was extracted once and analysed with five consecutive injections (intraday data with one extraction). For that case, R.S.D.s of retention time, peak areas and signal ratios were all found to be smaller than 1%. For peak heights, R.S.D.s between 0.9 and 1.4% were obtained. The intraday data listed in the central columns of Table I were obtained with the same sample extracted separately and analysed on the same day, whereas the interday data represent those which were generated on five different days. While the R.S.D.s of peak areas and heights of these intraday and interday data are about the same (5–6%), the variations of the ratios and the detection times were smaller for the intraday runs. Hence concentration values determined with external calibration are of lower accuracy than those obtained with internal calibration. This is clearly seen with the concentration data given in Table I. Using internal calibration, the R.S.D.s for intraday and interday evaluations were found to be 1 and 2.3%, respectively, a result which is excellent in comparison with such data reported for immunoassays and chromatographic procedures [1,2].

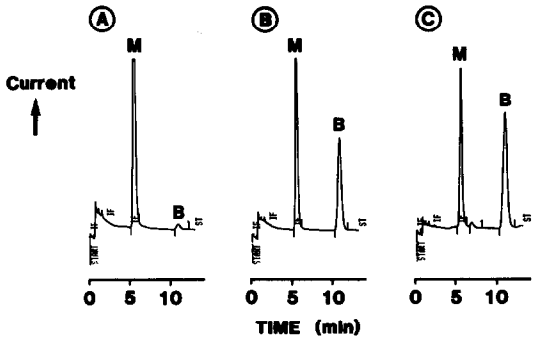

Fig. 2. GC of (A) a calibrator sample containing 0.5 μg/ml of bupivacaine, (B) a calibrator sample containing 10 μg/ml of bupivacaine and (C) a twentyfold diluted patient's sample containing 374 μg/ml of bupivacaine. B = bupivacaine; M = mepivacaine (internal standard).

Over 80 drain samples containing bupivacaine levels up to 500 μg/ml were analysed by HPCE

TABLE I

HPCE REPRODUCIBILITY DATA FOR BUPIVACAINE DETERMINATIONS ($n = 5$)

Intraday refers to evaluations which were made via five consecutive injections of the same extract, and with five extracts of the same sample analysed on the same day. Interday data were obtained through analysis of the same sample on five different days.

Property	Intraday (one extraction)		Intraday (five extractions)		Interday (five extractions)	
	Mean	R.S.D. (%)	Mean	R.S.D. (%)	Mean	R.S.D. (%)
Detection time (min)	10.58	0.09	10.34	0.30	10.44	2.53
Peak area (mV min)	1.868	0.66	2.160	5.55	1.895	5.18
Peak area I.S.[a] (mV min)	2.191	0.82	2.684	6.07	2.275	5.32
Peak-area ratio	85.15	0.54	80.83	0.98	83.39	1.91
Peak height (mV)	39.87	1.34	47.75	5.00	41.13	4.98
Peak height I.S.[a] (mV)	43.58	0.94	54.75	4.97	46.00	4.46
Peak-height ratio	91.50	0.47	87.29	0.98	89.43	2.08
Concentration (int-a) (μg/ml)	104.3	0.55	100.4	0.96	104.1	2.32
Concentration (int-h) (μg/ml)	105.5	0.48	103.6	0.98	105.9	2.31
Concentration (ext-a) (μg/ml)	101.7	0.67	102.5	5.36	101.7	4.46
Concentration (ext-h) (μg/ml)	103.6	1.33	106.8	4.87	104.9	4.70

[a] Internal standard.

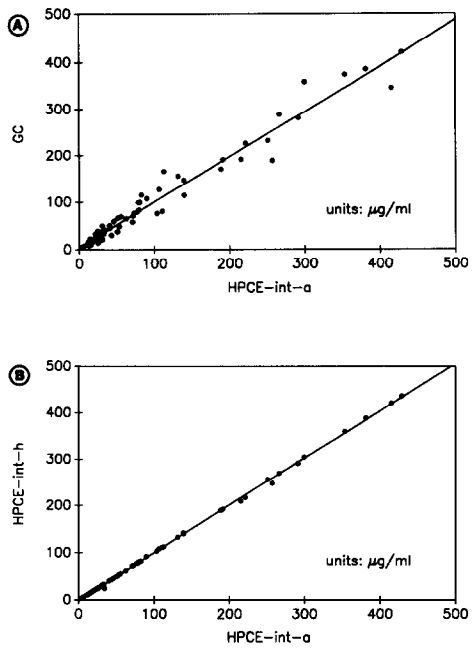

Fig. 3. Comparative analysis of bupivacaine levels in 82 patients' samples (A) monitored by GC and HPCE with internal calibration and using peak areas and (B) monitored by HPCE with internal calibration based on peak areas and peak heights. The data represent those given in sections 1 (line 1) and 2 (line 3), respectively, of Table II.

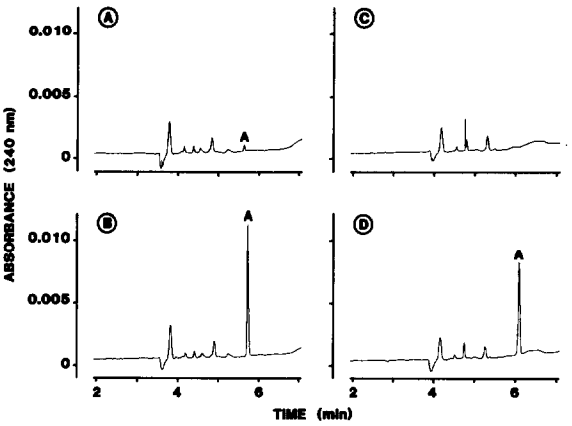

Fig. 4. MECC of (A) a calibrator plasma sample spiked with 1 μg/ml of antipyrine (A), (B) a calibrator plasma sample containing 20 μg/ml of antipyrine, (C) a patient's plasma sample drawn prior to drug intake and (D) a patient's plasma sample containing 16.9 μg/ml of antipyrine. Capillaries of effective length *ca.* 43 and 46 cm were used for the runs shown in (A) and (B), and in (C) and (D), respectively.

and GC (Fig. 2). The comparative results are shown in Fig. 3A and regression data are given in section 1 of Table II. When internal calibration was employed, good agreement between the data from these two methods was obtained, the calculated regression lines showing only small deviations from the line of equality. For HPCE with external calibration, the correlation was found to be in poorer agreement (correlation coefficients of about 0.976 compared to 0.984). It was interesting that according to these correlations HPCE peak areas and peak heights can be recommended for quantification. This is further demonstrated with the correlation data shown in Fig. 3B. Comparison of the data evaluated with the four different approaches (discussed above; for data see section 2 in Table 2) reveal the superiority of data obtained with internal calibration over those evaluated without the inclusion of the internal standard. The mean bupivacaine

TABLE II

LINEAR REGRESSION ANALYSIS DATA FOR COMPARATIVE BUPIVACAINE LEVELS

Section No.	Assay 1 (*x*-axis)	Assay 2 (*y*-axis)	*n*	Slope	*y*-Intercept (μg/ml)	*r*
1	HPCE-int-a	GC	82	0.967	4.02	0.984
	HPCE-ext-a	GC	82	0.899	7.09	0.976
	HPCE-int-h	GC	82	0.956	4.55	0.985
	HPCE-ext-h	GC	82	0.847	8.78	0.977
2	HPCE-int-a	HPCE-ext-a	82	1.060	−2.16	0.994
	HPCE-int-h	HPCE-ext-h	82	1.113	−3.73	0.995
	HPCE-int-a	HPCE-int-h	82	1.013	−0.624	1.000
	HPCE-ext-a	HPCE-ext-h	82	1.062	−2.07	1.000

levels ($n = 82$) obtained with GC, HPCE-ext-a, HPCE-ext-h, HPCE-int-a and HPCE-int-h were 81.2, 82.4, 85.4, 79.8 and 80.1 μg/ml, respectively. Together with the intraday and interday reproducibilities reported in Table I, these data suggest that bupivacaine can be reliably determined using HPCE with internal calibration based on peak areas or peak heights.

MECC determination of antipyrine using direct sample injection

Typical electropherograms obtained with direct injection of plasma blank spiked with 1 and 20 μg/ml of antipyrine are presented in Fig. 4A and B, respectively. Fig. 4C and D depict data obtained with plasma drawn from a patient prior to and after antipyrine administration, respectively. Antipyrine is shown to form a sharp peak that is well separated from endogenous compounds. Using HPLC (Fig. 5) the plasma sample was found to have an antipyrine level of 16.9 μg/ml. Hence MECC with direct injection appears to have the potential to determine this drug at clinically interesting concentration levels. Employing the external standard approach, calibration graphs between 1 and 40 μg/ml (five data points) were constructed and data evaluation was based on peak areas (referred to as MECC-ext-a), relative peak areas (MECC-ext-ra), which represent areas divided by detection times, and peak heights (MECC-ext-h). All graphs showed excellent linearity with R.S.D.s ranging from 1 to 3%. The y-intercepts for peak area, relative peak area and peak height calibrations were all considerably smaller than 1 μg/ml.

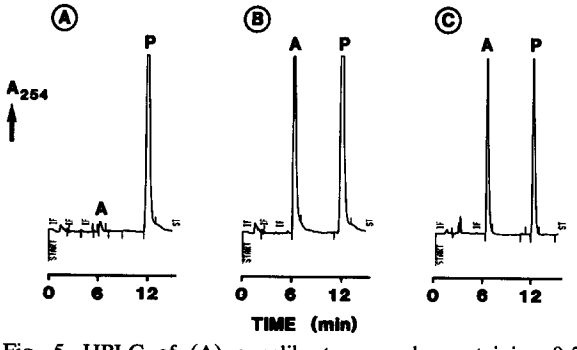

Fig. 5. HPLC of (A) a calibrator sample containing 0.5 μg/ml of antipyrine, (B) a calibrator sample containing 10 μg/ml of antipyrine and (C) a patient's sample containing 16.8 μg/ml of antipyrine. A = antipyrine; P = phenacetin (internal standard). Note that the attenuation for registration of the data in (C) was different to that in (A) and (B).

Not only the excellent calibration graphs but also the reproducibility data summarized in Table III suggest that external calibration should be sufficiently reliable for MECC with direct sample injection. For fifteen consecutive injections of the same sample, the R.S.D.s for retention times, peak areas, relative peak areas and peak heights were 0.20, 3.11 and 2.51 and 1.45%, respectively (first set of intraday data in Table III). With the exception of retention times, lower values were obtained with the implementation of buffer renewal in the anodic electrode compartment after every fifth run (second set of intraday data in Table III). The R.S.D.s of the intraday concentration levels were in the range 1.3–3.1%, with the lowest values being observed for quantification based on peak heights. The data further suggest that quantification based on relative peak areas should provide better accuracy than that based on peak areas. Peak areas, relative peak areas and peak heights were employed for the calculation of the MECC data discussed below. Interday reproducibilities were determined to be about 5.5% for all three quantitation schemes (third set of data in Table III). The determined mean levels were found to be very close to the expected spike values of 10 and 20 μg/ml for intraday and interday data, respectively.

The results of the MECC determination of antipyrine in 72 plasma samples in comparison with the values obtained by HPLC are depicted in Fig. 6A. Correlation data based on linear regression analysis are presented in Table IV. Irrespective of the basis for quantification in MECC, there was good agreement between the data obtained by these two methods, the calculated regression lines showing only small deviations from the line of equality.

The mean plasma levels evaluated with HPLC, MECC-ext-a, MECC-ext-ra and MECC-ext-h were 12.33, 12.37, 12.04 and 10.73 μg/ml, respectively. Although the MECC data based on peak-height calibration deviate the most from both the HPLC data and the MECC data ob-

TABLE III

MECC REPRODUCIBILITY DATA FOR ANTIPYRINE

Buffer change refers to a change of the buffer in the anodic electrode compartment after every fifth run. Intraday evaluations were made via fifteen consecutive injections of a sample and interday data were obtained through analysis of a control sample on fifteen different days. Relative peak areas are peak areas divided by the detection time. NA = not applicable.

Type	Property	Buffer change	*n*	Mean	R.S.D. (%)
Intraday	Detection time (min)	No	15	6.04	0.20
	Peak area (mV min)	No	15	0.219	3.11
	Relative peak area (mV)	No	15	0.0363	2.51
	Peak height (mV)	No	15	4.75	1.45
	Concentration (ext-a) (μg/ml)	No	15	9.51	3.12
	Concentration (ext-h) (μg/ml)	No	15	9.38	1.56
	Concentration (ext-ra) (μg/ml)	No	15	9.88	2.25
Intraday	Detection time (min)	Yes	15	6.42	1.77
	Peak area (mV min)	Yes	15	0.242	2.13
	Relative peak area (mV)	Yes	15	0.0376	1.86
	Peak height (mV)	Yes	15	5.07	1.24
	Concentration (ext-a) (μg/ml)	Yes	15	10.5	2.70
	Concentration (ext-h) (μg/ml)	Yes	15	10.1	1.32
	Concentration (ext-ra) (μg/ml)	Yes	15	10.2	1.67
Interday	Concentration (ext-a) (μg/ml)	NA	15	20.46	5.36
	Concentration (ext-h) (μg/ml)	NA	15	21.68	5.46
	Concentration (ext-ra) (μg/ml)	NA	15	20.40	5.71

tained with peak-area calibration (Fig. 6B), all three evaluation principles seem to provide MECC data of clinical relevance. The excellent agreement between the MECC-ext-a and MECC-ext-ra data (Fig. 6C) indicates that there is no need to use calibrations based on relative peak areas. Together with the intraday and interday reproducibilities reported in Table III, these data suggest that antipyrine can be reliably determined using MECC peak areas obtained with direct plasma injection.

CONCLUSIONS

It has been shown that high-quality HPCE and MECC data with clinical relevance can be generated using an automated capillary electrophoresis system equipped with untreated fused-silica capillaries and an inexpensive (*ca.* US$ 3500) chromatographic PCIP data station. Automated data evaluation can be based on peak areas or peak heights employing either external or internal calibration. For the two examples discussed, the determination of bupivacaine in drain fluid and antipyrine in plasma, series of up to 30 samples each could easily be run overnight. Based on linear regression analysis and considering the correlation coefficient *r* between two measurement methods, the HPCE and MECC data agree well with those obtained by conventional chromatographic methods (GC and HPLC; Figs. 3A and 6A, respectively). However, as was pointed out by Bland and Altman [24], such a comparison could be misleading. Therefore, comparative data were further evaluated graphically by plotting the difference against the average of the corresponding drug levels (Fig. 7). For antipyrine (Fig. 7A), the mean difference ± standard deviation ($n = 72$) between the HPLC and MECC-ext-a data was found to be -0.046 ± 1.981 μg/ml, indicating that the two methods provide very comparative levels. Sixty-eight of the 72 data points are within the region defined by the mean

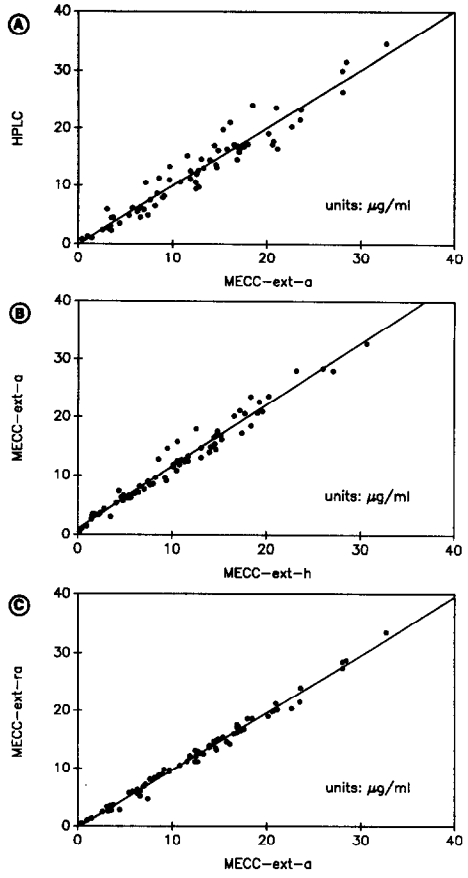

Fig. 6. Comparative analysis of antipyrine levels in 72 patients' samples monitored by (A) HPLC and MECC with external calibration and using peak areas, (B) MECC with external calibration based on peak areas and peak heights and (C) MECC with external calibration based on peak areas and relative peak areas. The data represent those given in lines 1, 4 and 5, respectively, in Table IV.

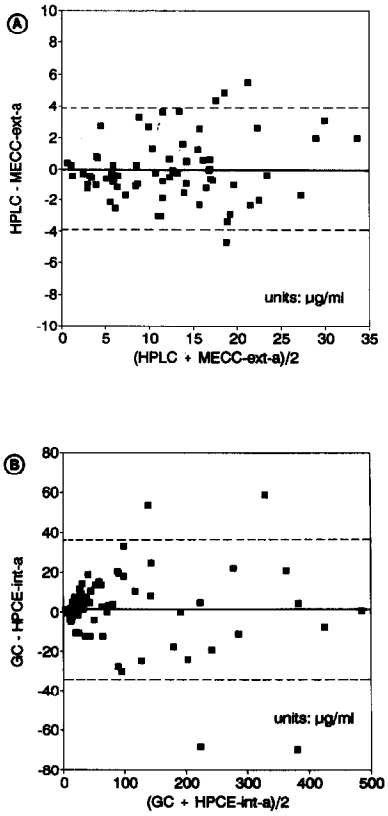

Fig. 7. Difference *versus* mean for comparative (A) antipyrine ($n = 72$) and (B) bupivacaine ($n = 82$) data. The solid line represents the mean of the differences and the broken lines this mean ± two standard deviations.

difference ± two standard deviations. The differences are clinically not important. Hence the two methods can be employed interchangeably. The mean difference ± standard deviation ($n = 72$) between the MECC-ext-a and MECC-ext-h data was calculated to be 1.582 ± 1.313 μg/ml, in-

TABLE IV

LINEAR REGRESSION ANALYSIS DATA FOR COMPARATIVE ANTIPYRINE LEVELS

Assay 1 (*x*-axis)	Assay 2 (*y*-axis)	*n*	Slope	*y*-Intercept (μg/ml)	*r*
MECC-ext-a	HPLC	72	1.009	−0.151	0.965
MECC-ext-h	HPLC	72	1.094	0.584	0.972
MECC-ext-ra	HPLC	72	1.013	0.124	0.968
MECC-ext-h	MECC-ext-a	72	1.059	1.008	0.983
MECC-ext-a	MECC-ext-ra	72	0.994	−0.249	0.995

dicating that data evaluation based on peak heights tends to give a lower antipyrine level. For bupivacaine, a different picture was obtained (Fig. 7B). The mean difference ± standard deviation ($n = 82$) between the GC and HPCE-int-a data was found to be 1.243 ± 17.84 μg/ml. Again, most of the differences fall within the limits of agreement (mean ± two standard deviations). The scatter of the differences increases with increasing bupivacaine level.

The data presented clearly suggest that automated capillary electrophoresis (HPCE and MECC) is well suited for therapeutic and diagnostic drug monitoring. Its superiority over chromatographic methods is based on several important facts, including the feasibility of directly injecting proteinaceous samples (such as plasma or serum, as is illustrated with the antipyrine example), the high degree of efficiency and automation, the intraday and interday reproducibility data being at the 1–3% and 5% levels, respectively, the small sample size, no requirement for large amounts of organic solvents and the rapidity of analysis. All data obtained so far are very encouraging and demonstrate the high potential of HPCE and MECC. However, instrumental problems associated with the reliability of autosampling and capillary fouling will have to be solved prior to the adoption of these techniques as routine methodologies in a drug assay laboratory. For example, when sampling plasma (or serum) for the MECC determination of antipyrine, the proportion of failures (drop outs, runs without a driving current and therefore no data) was observed to be 12% ($n = 457$) or even higher in other assays (data not shown). On dilution (threefold) of the antipyrine samples with saline this number was reduced to 5% ($n = 119$) and no such failures were obtained in either of the assays described when aqueous samples were applied. The exact origin of this sampling problem could not be identified, but employment of another instrument from the same manufacturer did provide significantly better data (failure rate *ca.* 2% with direct serum injection). Another problem associated with this kind of technology is occasional plugging of the capillary on the sampling side.

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REFERENCES

1 W.J. Taylor and M.H. Diers Caviness, *A Textbook for the Clinical Application of TDM*, Abbott Laboratories, Irving, CA, 1986.
2 K. Aziz, *Am. Lab.*, April (1988) 78.
3 R. DeCresce, A. Mazura, M. Lifshitz and J. Tilson, *Drug Testing in the Workplace*, ASCP Press, Chicago, 1989.
4 J.W. Jorgenson and K.D. Lukacs, *Science*, 222 (1983) 266.
5 B.L. Karger, A.S. Cohen and A. Guttman, *J. Chromatogr.*, 492 (1989) 585.
6 N.A. Guzman, L. Hernandez and B.G. Hoebel, *BioPharm*, 2 (1989) 22.
7 Z. Deyl and R. Struzinsky, *J. Chromatogr.*, 569 (1991) 63.
8 S. Terabe, *Trends Anal. Chem.*, 8 (1989) 129.
9 T. Nakagawa, Y. Oda, A. Shibukawa, H. Fukuda and H. Tanaka, *Chem. Pharm. Bull.*, 37 (1989) 707.
10 H. Nishi, T. Fukuyama and M. Matsuo, *J. Chromatogr.*, 515 (1990) 245.
11 D.K. Lloyd, A.M. Cypess and I.W. Wainer, *J. Chromatogr.*, 568 (1991) 117.
12 W. Thormann, P. Meier, C. Marcolli and F. Binder, *J. Chromatogr.*, 545 (1991) 445.
13 W. Thormann, A. Minger, S. Molteni, J. Caslavska and P. Gebauer, *J. Chromatogr.*, 593 (1992) 275.
14 P. Wernly and W. Thormann, *Anal. Chem.*, 63 (1991) 2878.
15 D.K. Lloyd, K. Fried and I.W. Wainer, *J. Chromatogr.*, 578 (1992) 283.
16 H. Nishi and S. Terabe, *Electrophoresis*, 11 (1990) 691.
17 P. Meier and W. Thormann, *J. Chromatogr.*, 559 (1991) 505.
18 M. Miyake, A. Shibukawa and T. Nakagawa, *J. High Resolut. Chromatogr.*, 14 (1991) 181.
19 M.A. Evenson and J.E. Wiktorowicz, *Clin. Chem.*, 38 (1992) 1847.
20 M. Eichelbaum and N. Spannbruckner, *J. Chromatogr.*, 140 (1977) 288.
21 A. El-Yazigi, D.A. Raines, H. Ali, J. Sieck, P. Ernst and M. Dossing, *Pharm. Res.*, 8 (1991) 269.
22 M.E. Kruczek, *J. Pharmacol. Methods*, 5 (1981) 137.
23 B.J. Clark, A. Hamdi, R.G. Berrisford, S. Sabanathan and A.J. Mearns, *J. Chromatogr.*, 553 (1991) 383.
24 J.M. Bland and D.G. Altman, *Lancet*, 1 (1986) 307.