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Determination of the Enantiomers of Mianserin and its Metabolites in Plasma by Capillary Electrophoresis After Liquid–Liquid Extraction and On-Column Sample Preconcentration

Chin B. Eap*, Kerry Powell, and **Pierre Baumann**

Unité de Biochimie et Psychopharmacologie clinique, Département Universitaire de Psychiatrie Adulte, Hôpital de Cery, CH-1008 Prilly-Lausanne, Switzerland

Abstract

Capillary electrophoresis has drawn considerable attention in the past few years, particularly in the field of chiral separations because of its high separation efficiency. However, its routine use in therapeutic drug monitoring is hampered by its low sensitivity due to a short optical path. We have developed a capillary zone electrophoresis (CZE) method using 2mM of hydroxypropyl-β-cyclodextrin as a chiral selector, which allows base-to-base separation of the enantiomers of mianserin (MIA), desmethylmianserin (DMIA), and 8-hydroxymianserin (OHMIA). Through the use of an on-column sample concentration step after liquid–liquid extraction from plasma and through the presence of an internal standard, the quantitation limits were found to be 5 ng/mL for each enantiomer of MIA and DMIA and 15 ng/mL for each enantiomer of OHMIA. To our knowledge, this is the first published CE method that allows its use for therapeutic monitoring of antidepressants due to its sensitivity down to the low nanogram range. The variability of the assays, as assessed by the coefficients of variation (CV) measured at two concentrations for each substance, ranged from 2 to 14% for the intraday (eight replicates) and from 5 to 14% for the interday (eight replicates) experiments. The deviations from the theoretical concentrations, which represent the accuracy of the method, were all within 12.5%. A linear response was obtained for all compounds within the range of concentrations used for the calibration curves (10–150 ng/mL for each enantiomer of MIA and DMIA and 20–300 ng/mL for each enantiomer of OHMIA). Good correlations were calculated between [(*R*) + (*S*)]-MIA and DMIA concentrations measured in plasma samples of 20 patients by a nonchiral gas chromatography method and CZE, and between the (*R*)- and (*S*)-concentrations of MIA and DMIA measured in plasma samples of 37 patients by a previously described chiral high-performance liquid chromatography method and CZE. Finally, no interference was noted from more than 20 other psychotropic drugs. Thus, this method, which is both sensitive and selective, can be routinely used for therapeutic monitoring of the enantiomers of MIA and its metabolites. It could be very useful due to the demonstrated interindividual variability of the stereoselective metabolism of MIA.

* Author to whom correspondence should be addressed.

Introduction

Mianserin (MIA) is a tetracyclic antidepressant drug with less anticholinergic activity and cardiotoxicity than the tricyclic antidepressants (1). MIA's main metabolites are 8-hydroxymianserin (OHMIA), desmethylmianserin (DMIA), and mianserin-*N*-oxide (OXMIA) (see Figure 1). The latter is pharmacologically inactive, whereas OHMIA and DMIA probably contribute to the pharmacological activity of the parent drug (2). MIA is administered as a racemate, and (*S*)-MIA is considered the more potent antidepressant enantiomer (2).

In a previous study, we described a high-performance liquid chromatographic (HPLC) method with a cyclodextrin chiral column (Cyclobond I RSP) which allows the measurement of the enantiomers of MIA, DMIA, and OHMIA. This method was used for the analysis of plasma and urine of 10 MIA-treated patients (3). We demonstrated an enantioselectivity in the metabolism of MIA and a large interindividual variability in the *S*–*R* ratios of MIA and its metabolites, which could partially explain why, until now, no consistent relationship has been established between the therapeutic response and total ([*R*] + [*S*]) plasma levels of this antidepressant to our knowledge (3). However, with the above-mentioned method, it was not possible to obtain a base-to-base separation for MIA and DMIA. Furthermore, a nonlinear response was observed for OHMIA within the range of concentrations used for the calibration curves, which was probably due to the nature of the detection system used (fluorescence after photochemical reaction) and made the reliable quantitation of this metabolite difficult.

Capillary electrophoresis (CE) is a method that has drawn considerable attention in the past few years, particularly in the field of chiral separations because of its high separation efficiency, its easy use, and its low cost (minimal use of reagents and cheap columns compared to expensive and usually short-lived chiral gas chromatography [GC] or HPLC columns). We have developed a capillary zone electrophoresis (CZE) method for the separations of MIA, DMIA, and OHMIA using hydroxypropyl-β-cyclodextrin (HPC) with resolution factors greater

than 1 and a linear response within the range of concentrations used for the calibration curves. Because plasma concentrations of MIA and its metabolites in MIA-treated patients are in the low nanogram per milliliter range, and given the poor sensitivity of the CE apparatus (due to a short optical path), an on-column sample preconcentration (4) after a liquid–liquid extraction from the plasma samples was necessary.

Experimental

Reagents

MIA–HCl, desmethylmianserin–HCl, 8-hydroxymianserin maleate, (+)-(*S*)-mianserin maleate, and (–)-(*R*)-mianserin maleate were kindly provided by Organon (Oss, The Netherlands). Propyl-norclozapine (PNC, internal standard) was synthesized as previously described (5). Hydroxypropyl-β-cyclodextrin (degree of substitution, 6–9; molecular weight calculated with seven substitutions, 1541.5) was from Aldrich (Fluka, Buchs, Switzerland).

The stock solution of buffer consisted of a 0.075M phosphoric acid aqueous solution adjusted to pH 3.0 with concentrated triethylamine. Unless otherwise stated, it contained 2mM of hydroxypropyl-β-cyclodextrin. Before use, it was filtered through a 0.22-µm pore size filter and degassed in an ultrasonic bath. Stock solutions of racemic MIA and DMIA and racemic OHMIA and PNC were made at 1 mg/mL in 0.1N HCl and in methanol, respectively. Working solutions were made at 1 or 10 ng/µL 0.1N HCl. All solutions were stored at –20°C until used. Water was obtained from a Milli Q-RG apparatus (Millipore, Le Mont-sur-Lausanne, Switzerland), and all other reagents were of analytical or HPLC grade. Injection vials were silanized before each use (with 4% dimethyl-dichlorosilane in toluene).

Instrumentation and CE conditions

An HP CE system with a diode-array detector (Hewlett-Packard, Geneva, Switzerland) was used with an untreated HP silica capillary column (total length, 64.5 cm; effective length, 56 cm; 50-µm i.d.; bubble factor, 3). Standard operating conditions, unless stated otherwise, were as follows: the voltage was 30 kV, and the temperature was 20°C. At the beginning of each run, the capillary was flushed with 0.1N NaOH for 2 min and with a running buffer for 3 min. Injection and on-column sample concentration were performed as follows: (*a*) pressure injection (50 mbar) of Milli-Q water for 3.7 s, (*b*) voltage injection (5 kV) of the sample for 10 s, and (*c*) pressure injection (50 mbar) of the running buffer for 3.7 s. The vials containing the anode and cathode buffer were emptied and refilled with running buffer before each analysis. The detector was set at 211 nm with a band width of 20 nm.

Resolution

The resolution was calculated with the following expression (6):

$$\text{Resolution} = 1.18\,(t_2 - t_1) / (W_{h/2})_1 + (W_{h/2})_2$$

where t_x is the migration time of the enantiomer (in minutes) and $(W_{h/2})_x$ is the peak width of the enantiomer at half its height (in minutes).

Extraction of plasma samples

For the determination of free MIA and DMIA, 300 ng of the internal standard, 0.5 mL 1M carbonate buffer (pH 9.4), and 6 mL *n*-heptane-ethyl acetate (80:20, v/v) were added to a 1-mL aliquot of heparinized plasma. The extraction was then performed on a shaker for 20 min. After centrifugation (6 min, 3400 *g*, 8°C), the organic layer was transferred to another tube containing 1.2 mL 0.1N HCl. After 15 min of shaking and 6 min of centrifugation, the organic phase was discarded, and the aqueous phase was transferred to another tube containing 150 µL toluene–isoamyl alcohol (85:15, v/v) and 1 mL 1M carbonate buffer (pH 9.4). After 20 min of shaking and 6 min of centrifugation, the organic phase was transferred to a home-made microtube (length, 40 mm; internal diameter, 3 mm) which allowed a good visualization of the two phases. The organic phase was then pipetted with care to avoid contamination by the aqueous phase and was gently transferred to the injection vial above a layer containing 100 µL of 0.0001% diethylamine (DEA). The organic phase was then gently dried under N_2 at 40°C until the final volume was approximately 50 µL water containing DEA. The vial was then left uncapped at 40°C without N_2 for 30 min to allow any remaining toluene to evaporate. The vial was then thoroughly vortexed for 15 s, and the aquous contents were injected into the CE.

For the determination of total (free + conjugated) MIA, DMIA, and OHMIA, 1 mL of plasma was incubated with 1 mL of 0.2M acetate buffer (pH 5.0), 50 µL 4% sodium azide, 300 ng of the internal standard, and 200 µL of a solution of *H. pomatia* β-glucuronidase (25,000 units/mL of 0.2% NaCl) (Sigma, St. Louis, MO) at 37°C overnight. The mixture was then treated as previously described.

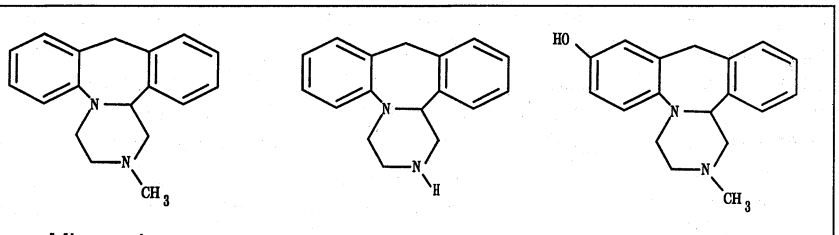

Figure 1. Chemical structures of MIA and its principal human metabolites.

Calibration curve, sensitivity, and reproducibility

For calculation, corrected areas were used (area divided by migration times). Calibration curves were prepared by adding known amounts of (*S*)- and (*R*)-enantiomers of MIA and its metabolites

(10–150 ng/mL for each enantiomer of MIA and DMIA and 20–300 ng/mL for each enantiomer of OHMIA) and PNC (300 ng/mL) to a 1-mL drug-free plasma. Within-day and interday replicates were calculated with plasma containing 20 or 100 ng/mL of each enantiomer of MIA and DMIA and 50 or 200 ng/mL of each enantiomer of OHMIA. For each enantiomer of each compound, quantitation limits were set at the minimal plasma concentration, which produced, after extraction and injection, a signal-to-noise ratio of at least four, a coefficient of variation (CV) for replicate determination (nine replicates) of 20% or less, and a deviation of the mean value less than 20% of the actual value.

Identification of the enantiomers of MIA and its metabolites on the chromatogram

Pure enantiomers were only available for MIA ([*S*]-MIA and [*R*]-MIA). The order of elution of the (*S*)- and (*R*)-forms of DMIA and OHMIA was determined by analyzing a patient's plasma sample that had been previously analyzed by HPLC (3).

Results and Discussion

Optimization of the achiral and chiral separation parameters

The achiral separation of MIA from DMIA and OHMIA was first tested with three buffers (H_3PO_4–KH_2PO_4, H_3PO_4–triethylamine [TEA], and H_3PO_4–triethanolamine [TENA]) at two concentrations (100 and 50mM) and at pH 3.0 without cyclodextrin (6). A complete separation of the three substances was obtained with the three buffers at the two concentrations, but the best resolution was obtained with TEA (TEA > TENA > KH_2PO_4) and at 100mM (data not shown). A shorter migration time was obtained with 50mM buffers and with TENA (TENA < KH_2PO_4 < TEA). Finally, the best peak shapes (symmetric peaks) were obtained with TEA (data not shown). An H_3PO_4–TEA 75mM buffer was chosen for subsequent experiments.

HPC is probably the most used cyclodextrin for chiral separation in CZE. It was the first that we tested, and because an excellent separation was obtained, no attempt to try other cyclodextrins was made. Figure 2 shows a plot of resolutions between each pair of enantiomers of MIA, DMIA, and OHMIA as a function of HPC concentrations. Excellent separations were obtained over a wide range of HPC concentrations (1.25–45mM); the maximum was about 15mM. However, between 5 and 45mM HPC, it was not possible to separate the six isomers due to comigration of the enantiomer of one substance with the enantiomer of another substance. The complete separation of the six isomers was only possible around 2.5mM, a separation which was again lost at 1.25mM. Some additional control experiments allowed us to determine that the best separation was obtained at a concentration of 2mM HPC diluted in H_3PO_4–TEA. Finally, using different pH values between 2.0 and 5.0 and different temperatures (15, 20, and 30°C), it was found that the best separation, without excessive increase in migration time, was obtained at pH 3.0 and 20°C. Figure 3 shows the electrophoretic separation of the enantiomers of MIA and its metabolites after extraction of a blank plasma sample spiked with various amounts of substances (see figure caption) and injection into the CE apparatus. It is worth noting that, with the highest concentrations of the calibration curve, distorted peak shapes were produced due to overloading, but the calibration curve was still linear within that range.

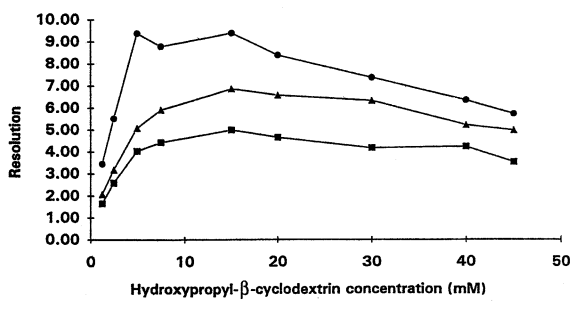

Figure 2. Effect of hydroxypropyl-β-cyclodextrin concentrations on resolution of racemic MIA (▲), DMIA (■), and OHMIA (●).

Validation of enantiomer determination in plasma

It is well-known that a weak point of CE is its poor sensitivity due to a short optical path. A sensitive method down to a low nanogram concentration is needed, so a field-amplified sample injection for positive ion injection method (4) was attempted and found to give excellent results: an approximately 50–100-fold concentration of the sample was obtained. In summary, the method is based on the following sequence and principles. First, a small plug of diluted buffer (in our case, deionized water) was introduced into the column before sample injection. This resulted in an electric field at the injection point that was much stronger than that in the column. The analytes prepared in the diluted buffer were then electrically injected into the column and concentrated at the beginning of the column buffer. Because stacking and broadening work against each other, there is an optimal length of water plug that can be introduced into the column that still achieves high resolution (4). Several lengths of water plug (pressure injection of 50 mbar from 1.8 to 7.4 s) and several times (5–40 s) or voltage (5–10 kV) for the electroinjection were tested. The sequence of injection as described in the Experimental section was found to be optimal.

When developing the method, a large variability in the measured concentrations of MIA and its metabolites, OHMIA in particular, was found. At this time, the organic phase was completely dried in the last step of the extraction method, and the dry residue was reconstituted in water before injection. An adsorption of the hydroxy-metabolites to the walls of the injection vials was thus suspected. The procedure (described in the Experimental section) consisted of transferring the organic phase to the injection vial on the top of a layer of 100 µL 0.0001% DEA and drying the mixture until the final volume was approximately 50 µL DEA-containing water; this was found to solve the problem. However, it is worth noting that, although toluene is very volatile, its presence, even in trace amounts, resulted in small peaks of MIA, as measured by CE (data not shown). An additional 30-min drying step at 40°C without N_2 was added to ensure a complete evaporation of

toluene. We also decided to silanize all injection vials and use very dilute concentrations of DEA in the last step to further minimize adsorption (a control experiment showed that this very dilute concentration of DEA did not decrease the efficacy of the on-column sample preconcentration).

It must be emphasized that in order to decrease adsorption and avoid toluene in the final phase, all steps, as described above, were essential for the reproducibility of the method. Another important point is that no carbonate buffer, even as a small contamination, was transferred with the organic phase into the injection vial at the last extraction step (for this, we used a homemade microtube to allow a clear visualization of the two phases; see Experimental section). Otherwise, this would raise the ionic concentration of the sample and prevent the stacking process from occurring, resulting in very small or undetectable peaks, including that of the internal standard. However, in such cases, it was possible to re-extract (last extraction step) and reinject the sample. MIA and its metabolites were stable in the dilute solution of DEA for at least four days at room temperature and for at least two months in plasma at –20°C (data not shown). Finally, it should be mentioned that the on-column sample concentration of the injection step affected, to a minor extent, the total content of the drug in the injection vial, which allowed several injections from the same vial.

Table I shows a summary of the statistical data from the analysis of the MIA, DMIA, and OHMIA enantiomers. To summarize, the mean correlation coefficients for the calibration curves (five concentrations, from 10 to 150 ng/mL for MIA and DMIA and from 20 to 300 ng/mL for OHMIA) obtained from four separate experiments ranged from 0.992 to 0.999. Recovery was found to be satisfactory for all compounds, ranging from 72 to 90%. However, it is worth noting that very large coefficients of variation were calculated (up to 58%), which was probably due to the variable efficacy of the injection method rather than that of the extraction method. This variability was not detrimental to the method, given the presence of an internal standard. Indeed, the variability of the assays, as assessed by the CVs measured at two concentrations for each substance, was always less than 14% for both the intraday (eight replicates) and interday (eight replicates) experiments. The deviations from the theoretical concentrations, which represent the accuracy of the method, were all within 12.5%. The quantitation limits were found to be 5 ng/mL for each enantiomer of MIA and DMIA and 15 ng/mL for each enantiomer of OHMIA.

Migration time reproducibilty was acceptable; the CVs of 10 successive injections of the same sample were less than 1.5%. Furthermore, the CVs calculated for the relative migration times of one enantiomer compared with the internal standard or calculated for the (*S*)-enantiomer compared with the (*R*)-enantiomer did not exceed 1.35 and 0.2%, respectively. The specificity of the assay was also controlled; 500 ng of each of the following substances were dried, reconstituted in 100 µL of 0.0001% DEA, and injected into the CE with the on-column sample concentration method: amitriptyline, nortriptyline, citalopram, norcitalopram, clozapine, norclozapine, clomipramine, norclomipramine, fluvoxamine, fluoxetine, norfluoxetine,

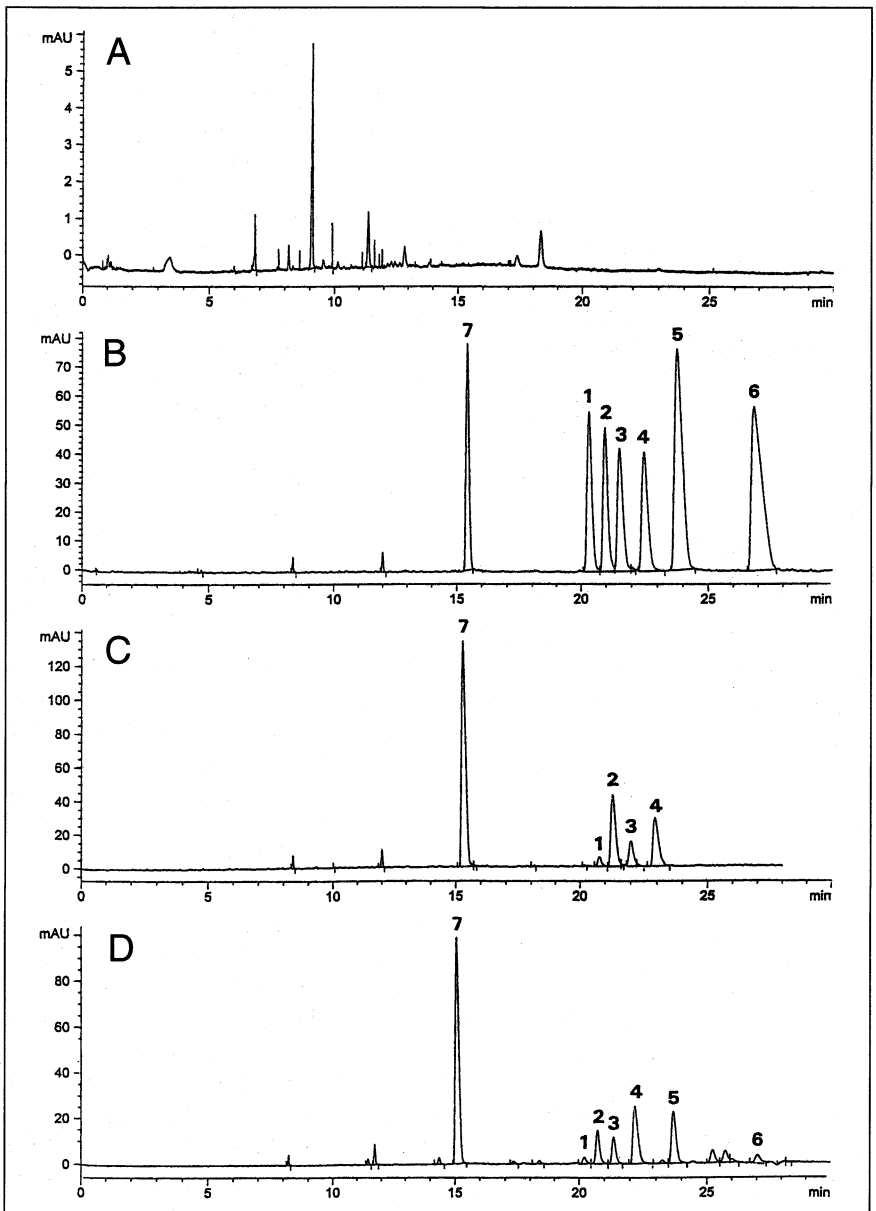

Figure 3. Electropherogram of (A) a blank plasma, (B) a blank plasma spiked with 100 ng/mL of each enantiomer of MIA and DMIA and with 200 ng/mL of each enantiomer of OHMIA, and (C) a nonhydrolyzed and (D) hydrolyzed plasma from a patient treated with 30 mg of racemic MIA for 50 days. Typical retention times were 20.3, 20.9, 21.5, 22.5, 23.7, 26.8, and 15.4 min for (*S*)-DMIA (peak 1), (*S*)-MIA (peak 2), (*R*)-DMIA (peak 3), (*R*)-MIA (peak 4), (*S*)-OHMIA (peak 5), (*R*)-OHMIA (peak 6), and propylnorclozapine (internal standard, peak 7).

imipramine, desipramine, paroxetine, maprotiline, methadone, sertraline, norsertraline, thioridazine, trimipramine, and nortrimipramine. No peaks were detected for up to 30 min, except for clozapine, norclozapine, and thioridazine, which migrated before PNC, the internal standard (relative migration compared to PNC: 0.87, 0.76, and 0.61, respectively). It should be mentioned that clozapine and norclozapine could also probably be used as internal standards. However, PNC, which was synthesized in our laboratory for the analysis of clozapine, had the advantage that it could not be found in any patients.

Figures 3C and 3D show the electrophoretic separations of MIA and its metabolites after liquid–liquid extraction of nonhydrolyzed and hydrolyzed plasma from a patient treated with 30 mg of racemic MIA per day for 50 days. The measured concentrations of (*S*)-MIA, (*R*)-MIA, (*S*)-DMIA, and (*R*)-DMIA in nonhydrolyzed plasma (free drug concentrations) were 41, 22, 7, and 22 ng/mL, respectively. The measured concentrations of (*S*)-MIA, (*R*)-MIA, (*S*)-DMIA, (*R*)-DMIA, (*S*)-OHMIA, and (*R*)-OHMIA in hydrolyzed plasma (total drug concentrations) were 64, 80, 16, 31, 123, and 47, respectively. This confirmed the stereoselectivity in the metabolism of MIA as previously described (3). A set of 20 plasma samples from MIA-treated patients, which were sent to our laboratory for therapeutic drug monitoring of MIA and DMIA (nonstereospecific GC assay) (7), were reanalyzed by CE. Figure 4 shows the good correlations between the concentrations of MIA obtained with the two methods (the [*R*]- and [*S*]-concentrations obtained by CE were added before comparison with the concentrations obtained by GC). Similar results were obtained for DMIA (correlation coefficient = 0.98, 20 samples, slope = 0.861, intercept = 12.1). Finally, for a clinical study, plasma was collected from 37 patients receiving various doses of racemic MIA for at least one week (C.B. Eap, C.A. de Mendonça Lima, F. Macciardi, B. Woggon et al., submitted to *Therapeutic Drug Monitoring*, 1997), and free concentrations of MIA and DMIA (nonhydrolyzed plasma samples) were measured by CZE and HPLC (3). Figure 5 shows the good correlations between the concentrations of (*S*)-MIA obtained with the two methods.

Table I. Statistical Data from the Analysis of MIA, DMIA, and OHMIA Enantiomers

	(*S*)-MIA	(*R*)-MIA	(*S*)-DMIA	(*R*)-DMIA	(*S*)-OHMIA	(*R*)-OHMIA
Calibration (four replicates)						
Range (ng/mL)	10–150	10–150	10–150	10–150	20–300	20–300
Slope: mean ± SD (CV)	1.88 ± 0.08 (4)	1.81 ± 0.04 (2)	2.12 ± 0.06 (3)	1.82 ± 0.11 (6)	1.82 ± 0.16 (9)	1.70 ± 0.13 (8)
Intercept: mean (range)	–0.0053 (–0.0169, 0.0048)	–0.009 (–0.0105, –0.0071)	–0.019 (–0.0289, –0.0103)	–0.0178 (–0.0265, –0.0095)	–0.0916 (–0.163, –0.046)	–0.0851 (–0.155, –0.05)
Correlation coefficient: mean (range)	0.998 (0.997–0.999)	0.996 (0.993–0.998)	0.998 (0.998–0.999)	0.996 (0.994–0.997)	0.996 (0.992–0.998)	0.997 (0.996–0.998)
Retention times						
CV for 10 replicate injections	1.29	1.33	1.31	1.33	1.35	1.48
Recovery (10 replicates)						
Concentration used (ng/mL)	20	20	20	20	50	50
Recovery (%): mean ± SD (CV)	86 ± 48 (55)	90 ± 49 (54)	80 ± 45 (56)	72 ± 42 (58)	87 ± 46 (52)	84 ± 45 (53)
Within-day variation (eight replicates)						
Theoretical values (ng/mL)	20	20	20	20	50	50
Measured values (ng/mL): mean ± SD (CV)	19.6 ± 0.3 (2)	21.7 ± 0.6 (3)	20.2 ± 0.9 (5)	22.5 ± 1.1 (5)	52.6 ± 5.2 (10)	51.2 ± 5.6 (11)
Percentage of theory	98	108.5	101	112.5	105.2	102.4
Theoretical values (ng/mL)	100	100	100	100	200	200
Measured values (ng/mL): mean ± SD (CV)	99.9 ± 5.8 (6)	100.3 ± 5.6 (6)	98.2 ± 10.9 (11)	98.9 ± 10.2 (10)	191.3 ± 26.4 (14)	191.8 ± 25.7 (13)
Percentage of theory	99.9	100.3	98.2	98.9	95.7	95.9
Day-to-day variation (eight replicates)						
Theoretical values (ng/mL)	20	20	20	20	50	50
Measured values (ng/mL): mean ± SD (CV)	20.7 ± 1.6 (8)	20.1 ± 1.7 (9)	19.6 ± 1.7 (9)	19.7 ± 1.9 (10)	45.6 ± 3.7 (8)	46.0 ± 3.6 (8)
Percentage of theory	103.5	100.5	98	98.5	91.2	92
Theoretical values (ng/mL)	100	100	100	100	200	200
Measured values (ng/mL): mean ± SD (CV)	95.5 ± 12.2 (13)	94.2 ± 12.8 (14)	98.4 ± 8.0 (8)	95.4 ± 7.3 (8)	206.5 ± 10.5 (5)	208.3 ± 11.6 (6)
Percentage of theory	95.5	94.2	98.4	95.4	103.3	104.2
Quantitation limit (nine replicates)						
Theoretical values (ng/mL)	5	5	5	5	15	15
Measured values (ng/mL): mean ± SD (CV)	5.1 ± 0.5 (10)	4.9 ± 0.7 (15)	4.8 ± 0.7 (15)	5.2 ± 0.4 (8)	16.3 ± 3.2 (19.7)	16.3 ± 3.1 (19)
Percentage of theory	102	98	96	104	108.7	108.7

Similar results were obtained for (*R*)-MIA, (*S*)-DMIA, and (*R*)-DMIA (correlation coefficient = 0.91, 35 samples, slope = 0.893, intercept = 2; correlation coefficient = 0.93, 14 samples, slope = 0.683, intercept = 4.3; and correlation coefficient = 0.95, 33 samples, slope = 0.738, intercept = 5.7, respectively [an unequal number of values for each isomer were included because certain concentrations were below the quantitation limits]).

Conclusion

To summarize, this CZE method is a good alternative to a previously described HPLC method (3). Indeed, from a general point of view, CE has well-known advantages over HPLC, such as its simplicity of use, the low cost of reagents, and the low cost of columns (particularly important when considering chiral columns that are expensive and often short-lived). Furthermore, the high separation efficiency of CE with the present method allows a base-to-base separation for all enantiomers. Until now, the inherent lack of sensitivity of CE prevented its use when a sensitivity down to the low nanogram level was needed. Using an on-column sample preconcentration step with the presence of an internal standard resulted in a quantitation limit that allows the use of this method for therapeutic monitoring of antidepressants. To our knowledge, this is the first time that this has been demonstrated. Using the same development scheme, a method has now been introduced in our laboratory for the determination of the enantiomers of trimipramine and metabolites (Eap et al., manuscript in preparation). Finally, we believe that CE with an on-column sample preconcentration step could also be useful for the determination of nonchiral antidepressants or other drugs.

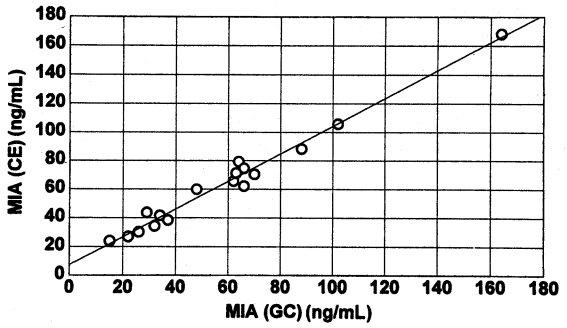

Figure 4. Spearman correlations between ([*R*] + [*S*])-MIA concentrations as measured by GC and CE. $y = 7.4 + 0.969x$, 20 replicates, correlation coefficient = 0.95.

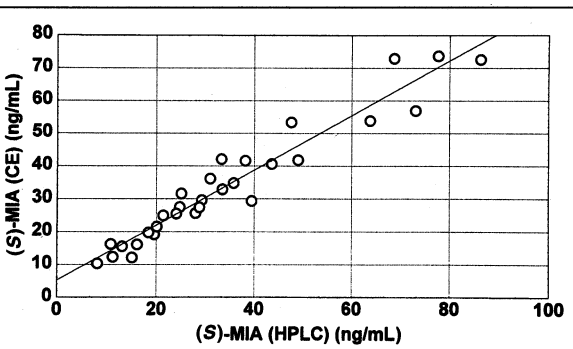

Figure 5. Spearman correlation between (*S*)-MIA concentrations measured by HPLC and CE. $y = 5.3 + 0.837x$, 36 replicates, correlation coefficient = 0.97.

Acknowledgments

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