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Development and validation of a capillary electrophoresis method within a pharmaceutical quality control environment and comparison with high-performance liquid chromatography

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Abstract

Capillary zone electrophoresis (CZE) has been used for the quantitative determination of the drug substance, mirtazapine, and five structurally related substances in Remeron tablets. It is found that the choice of the extraction solvent, and, therefore, of the injection medium is of great significance for the repeatability of both migration times and peak areas. It is concluded that variation in current through the capillary during the run, due to the injection medium, can be correlated to variation in migration times. Furthermore, during method development the selectivity is optimized with respect to the electrolyte composition. For this purpose an experimental design is applied. As a result information is also obtained on the ruggedness of the method with respect to the selectivity. The optimized method is validated regarding selectivity, accuracy, precision, linearity and detection limits. For quantitative analysis of the main component the injection precision is rather poor (R.S.D.: 2–3%), which requires the use of an internal standard. In addition a comparison is made with a validated HPLC method. Validation data and batch analysis results of the CZE method were found to be comparable with results of the HPLC method.

Keywords: Mirtazapine; Pharmaceutical analysis; Chemometrics; Validation; Buffer composition

1. Introduction

Capillary electrophoresis (CE) has gained greatly in popularity during the last few years and is now routinely applied in pharmaceutical laboratories [1,2]. Separation in CE is based on differences in electrophoretic mobilities. Because of the differences between high-performance liquid chromatography (HPLC) and CE with respect to the selectivity process, these techniques are sometimes referred to as being complementary. Pharmaceutical substances show a variety in structure and, subsequently, in chemical properties such as polarity and acidity. Therefore, CE is a serious alternative for HPLC. Furthermore it is to be expected that the running costs of CE, e.g., caused by low solvent consumption and the use of cheap columns, are much lower than for HPLC. Personal computer-controlled and highly automated CE systems are now commercially available. Many improvements have been made during the last several years to assure sufficiently precise quantitative results. However, in general the reproducibility of CE is often not as good as that of HPLC. A combination of a constant capillary temperature, an appropriate electrolyte system, long

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injection times, the use of an internal standard and high sample concentrations were shown to improve the precision [3–6].

The selectivity is affected by the composition of the electrolyte system. Practical guidelines for pharmaceutical drug separations have been reported [7].

The selectivity can be optimized efficiently by using an experimental design. The factors of interest are varied simultaneously. Several standard designs are available which can be divided roughly in screening designs and designs for modelling response surfaces. A screening design like Plackett–Burman has been employed for optimizing a CE separation of testosterone esters [8]. Designs for response surface modelling like central composite [9] and Box–Behnken [10] designs require a larger number of experiments to be carried out, but give more information about possible curvature and interaction effects.

A trend is observed to implement ruggedness testing at the stage of method development [11,12], i.e. optimization to a rugged optimum and not afterwards during validation.

Application of a central composite design for the optimization of a separation of corticosteroids has been reported [13]. An advantage of this approach of optimizing is that information is obtained about the effect of individual factors. Therefore, a statement about the ruggedness of the method can be made.

In CE publications much emphasis is being placed on separation problems and less attention is being paid to validation aspects. Within a quality control environment validation aspects for HPLC and GC methods are clearly defined [14,15]. Generally results reported for validation of CE methods are similar [16,17] to those of the well established separation techniques. This means aspects like selectivity, linearity, accuracy, precision, ruggedness and, for related substances, limit of detection and limit of quantitation.

This article describes the development and validation of a CZE method for the assay of the antidepressant mirtazapine and five structurally related substances (structures are given in Fig. 1) in a tablet formulation. Validation data of the CE method are compared with data of the current HPLC release method.

2. Experimental

2.1. Materials

The active drug substance mirtazapine and the related substances were obtained from Diosynth (Oss, Netherlands). Both, Remeron (30 mg mirtazapine/tablet) and the internal standard substance ("Org 4428") were prepared at Organon (Oss, Netherlands). Analytical grade methanol, citric acid and phosphoric acid were obtained from Merck (Darmstadt, Germany). Sodium hydroxide was purchased from J.T. Baker (Deventer, Netherlands). Deionized water having a resistivity greater than 17 MΩ cm was produced with a Millipore Milli-Q reagent system (Bedford, MA, USA). Fused-silica capillary tubings (365 μm O.D.×50 μm I.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA) and Hewlett–Packard (Waldbronn, Germany). New (uncoated) capillaries (48.5 cm total length; 40 cm effective length) were flushed with 1 *M* NaOH under air pressure of 900 mbar for 30 min, followed by a 3-min rinse with deionized water before use.

2.2. Equipment

The electrophoresis equipment was an HP^{3D}-system from Hewlett–Packard, equipped with a diode array detector, a temperature controlled (air cooled) capillary compartment and an autosampler. Electrophoretic data were both acquired with the HP^{3D}-system and Multichrom data system (This, Weesp, Netherlands).

HPLC results were obtained with equipment consisting of an HP 1090 series M (PV5) liquid chromatograph from Hewlett–Packard with temperature controlled column compartment, automatic sample injector and an HP 1050 diode-array detector.

Statistical data analysis was performed with Matlab Plus for Windows from Mathworks (Natick, MA, USA), Unscrambler version 5.03 from Camo AS (Trondheim, Norway) and Statgraphics Plus for Windows 2.0 from Manugistics (Rockville, MD, USA).

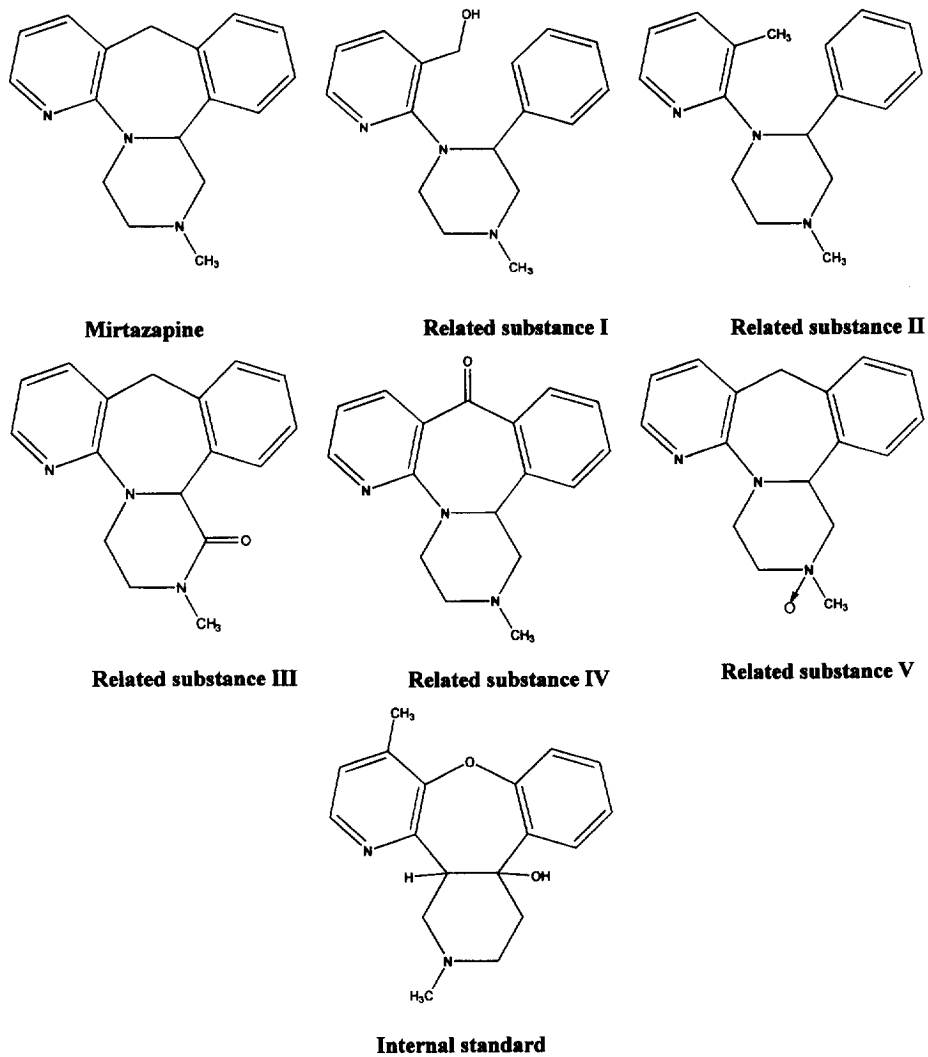

Fig. 1. Structural formulas for mirtazapine, related substances and internal standard.

2.3. Methods

In the development stage different running buffers were tested to search for a selective method. The method described below refers to the final validated method. Running buffer: 70 m*M* phosphate in water–methanol (75:25, v/v) with a pH of 2.0 was prepared by mixing: 4.05 g of phosphoric acid with 500 ml of a mixture of 75 volumes of water and 25 volumes of methanol. The pH was adjusted to a pH of 2.0 with 6 *M* sodium hydroxide. Extraction solvent: 20 m*M* phosphoric acid in water–methanol (65:35, v/v). Internal standard solution: a 0.75 mg/ml solution of internal standard in extraction solvent. Sample solution: 300 mg powdered sample was extracted with 30 ml of internal standard solution. The final concentration of mirtazapine amounts to 1 mg/ml. Reference solution for assay: mirtazapine in internal standard solution, 1 mg/ml. Reference solution related substances: 2 μg/ml related substance solution including mirtazapine (1 mg/ml) were prepared by dissolving all five related substances and mir-

tazapine in internal standard solution. The injection condition: 50 mbar for 5 s, followed by a 3 s, 50 mbar injection of running buffer. Co-injection of running buffer prevents the loss of sample due to expansion after applying voltage [4]. A gradual voltage ramp was applied during analysis. From 0 to 10 min the voltage was linearly increased from 0 to 25 kV followed by a constant voltage of 25 kV for 5 min. A gradual voltage ramp proved to reduce peak tailing as was shown for a peptide and protein analysis [18]. The capillary was thermostatted at 25°C. The components were detected at 210 nm.

Conditions for the HPLC method were as follows. Column: 250×4.6 mm Hypersil ODS (5 μm particles). Mobile phase: a mixture of 65 volumes of a solution of 18.0 g of tetramethylammonium hydroxide pentahydrate in 1000 ml of water adjusted to a pH of 7.4 with phosphoric acid 85% and 35 volumes of a mixture of methanol–acetonitrile–tetrahydrofurane, (36.2:42.5:21.3, v/v/v). Flow rate: 1.5 ml/min. Extraction solvent: a mixture of deionized water and acetonitrile (50:50, v/v). Sample solutions: for the assay 0.3 g of powdered sample was extracted with 100 ml of extraction solvent, for the related substances 0.3 g was extracted with 20 ml of the extraction solvent. The final concentration of the sample solution of mirtazapine for the assay amounts to 0.3 mg/ml, for related substances to 1.5 mg/ml. Reference solution for the assay: mirtazapine in extraction solvent, 0.3 mg/ml. Reference solution related substances: 3 mg of each related substance/liter of extraction solvent. Injection volume: 10 μl. The column was thermostatted at 40°C. The components were detected at 210 nm wavelength.

2.4. Development

The composition of the buffer was optimized with respect to selectivity and analysis time. The concentration of phosphate, amount of methanol and pH of the running buffer were varied using a 3-factor Box–Behnken design [10]. Box–Behnken designs in general require three levels for each factor to be tested. The levels are given in Table 1. This scheme consists of 16 experiments including three centre points. These centre points are included for a better error estimation. Different buffer systems were prepared and tested in random order. The experiments were carried out automatically without intervention of the analyst. Two samples containing mirtazapine (1 mg/ml) and five related substances (0.01 mg/ml) were injected at each condition. The results were statistically evaluated.

Individual factor effects were calculated by performing regression analysis. Functional relationships of migration times and peak widths of the components formed the base for deducing the optimal buffer system.

2.5. Validation

The method was validated with respect to aspects routinely used for validation of chromatographic methods.

2.5.1. Selectivity

Separation of mirtazapine and its known synthetic and degradative related substances was assessed by analysing spiked test solutions. To verify separation with unknown related substances test solutions of stressed samples were analysed. Samples were stressed by exposing the tablets to a temperature of 75°C for one week (both at ambient and 100% relative humidity) and to artificial light of 100 kLux for 2 h. To show that the analyte electrophoretic signal is a result of not more than one component, peak purity tests of the main component were performed. This test is based on similarity of UV

Table 1
Levels of phosphate concentration, percentage methanol and pH used for the optimization experiments

Factor	Level			Inaccuracy of the settings
	−1	0	+1	
Phosphate (m*M*)	60	70	80	68–72
MeOH (%, v/v)	15	25	35	24–26
pH	2.0	2.5	3.0	1.95–2.05

spectra at different positions within the peak. The similarity is expressed as a purity factor [19].

2.5.2. Linearity

Solutions of mirtazapine were prepared at levels of 1, 25, 50, 75, 100 and 125% with respect to the expected concentration of mirtazapine in the sample solution; the concentration of the internal reference standard was held constant. Each solution was injected twice. Linearity of the internal standard was tested; thereby mirtazapine acting as internal standard.

For each of the related substances relative response–concentration curves were made. Levels: 0.25, 0.5, 1.0, 1.5 and 2.0% with respect to the expected amount of mirtazapine in the sample solution. Each solution was injected twice. Response–concentration curves were made.

2.5.3. Accuracy

In six-fold and at 100% level, mirtazapine was added to a mixture of placebo ingredients according to the composition of the tablets. From the results the recoveries were calculated with respect to the amount of mirtazapine added.

For the related substances accuracy was assessed by analysis of spiked ground tablets at a level of 0.2%. Results were expressed as a percentage of the amount added. A correction was made for the amount of related substances already present in the tablets.

2.5.4. Precision

For the active compound the system precision (R.S.D. of injection) was calculated from a six-fold injection of a 100% reference solution. For the related substances this precision was calculated from the (area) results of repeated injections of reference solutions at 0.2% level. Relative standard deviations of responses were calculated with and without the use of internal standard.

For the assay as well as for the related substances the repeatability of the method was deduced from accuracy results. The solutions were regarded as resulting from one homogeneous sample.

2.6. Limit of detection and quantitation

Generally, limit of detection (LOD) and limit of quantitation (LOQ) are being defined as 3 times and 10 times the signal-to-noise ratio [14], respectively. The noise is defined as the signal height divided by half the peak-to-peak distance of the noise [15]. Hence, the LOD and LOQ are defined as 9 times the standard deviation and 30 times the standard deviation, respectively. The LOD and LOQ were derived from the responses of the 0.25% reference solution of the related substances used for linearity experiments.

3. Results and discussion

3.1. Influence injection solvent

Initially citrate buffers were used. These buffers were mixed with different amounts of methanol and adjusted to different pH-values. Although this resulted in a good selectivity the analysis time of 35 min was relatively long.

Shorter analysis times, of around 13 min, were obtained using phosphate buffer mixed with methanol. However, as for the citrate buffers, a very poor repeatability of migration times and peak areas was observed. For both, relative standard deviations up to 10% were obtained frequently. Typical electropherograms of consecutive injections of one single sample solution are shown in Fig. 2. Additionally, the monitored electrical current profiles of consecutive injections showed a poor repeatability, as illustrated in Fig. 3. In earlier studies [20,21] it was concluded that the fluctuations in electrical current originate from temperature fluctuations at the outer surface of the capillary wall by non-efficient cooling. In our study the composition of the extraction/injection medium proved to be of great significance. An acetonitrile–water mixture (50:50, v/v), currently used for the validated release HPLC was used as extraction/injection solvent. However, this extraction medium did not match the running buffer used for the CE experiments and resulted in current fluctuations and poor repeatability of the migration times and peak areas. Experiments showed that the fluctua-

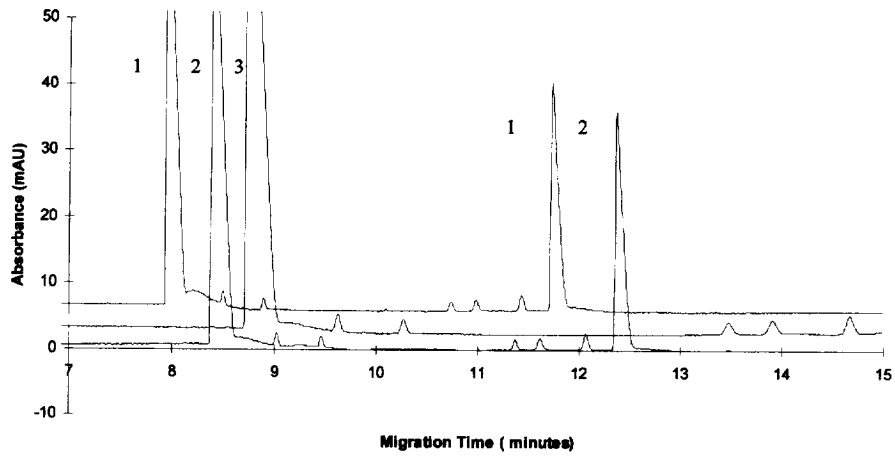

Fig. 2. Electropherograms of consecutive injections showing the poor repeatability of the migration times. The internal standard peak of the third injection is larger than 15 min. Conditions as described in Section 2.3.

tions were caused by thermal effects in the sample zone and the running buffer during the analysis.

A model describing the relation between the current profiles and both migration times and peak areas was studied, in such a way that from a change in current profile the change in migration time could be predicted. Each current profile consists of 1500 measuring points, which can be regarded as variables in the model. However, since the variables are interdependent, multiple linear regression (MLR) cannot be used.

Because of the complex relation a multivariate modelling technique, viz. partial least squares (PLS) was used. PLS is a term for technically related multivariate modelling methods [22]. In its basic form it models the relation between two blocks of variables, X and Y. In our study the matrix X represents the current profiles and Y, single variables, representing the corresponding migration times and peak areas of a peak in the electropherogram.

A series of 24 repeated injections of samples extracted with the acetonitrile–water mixture was

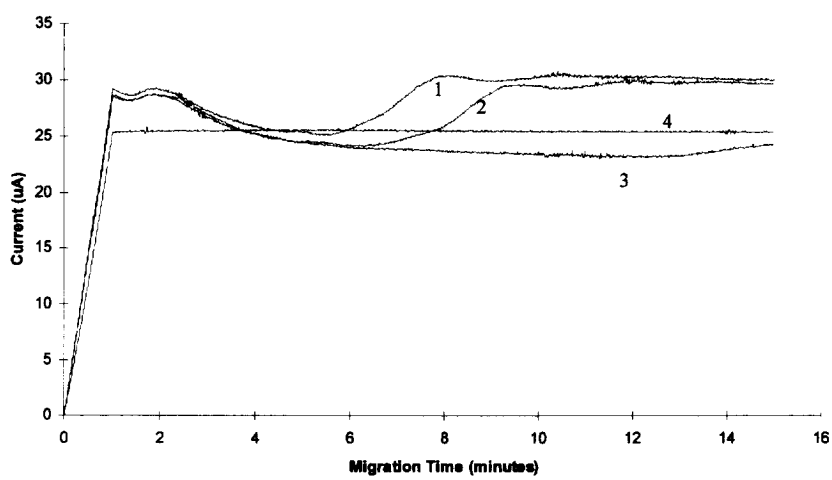

Fig. 3. Monitored current profiles of consecutive injections. The corresponding time range 0–15 min. The linear increase of the current (μA) during the first minute is a result of the linear voltage ramp applied. The numbers correspond to the electropherograms given in Fig. 2. Profile indicated with number 4 is obtained using the optimized injection solvent. Conditions as described in Fig. 2.

used for the set-up of the model. An optimal PLS-model with three factors was obtained by means of cross validation. Cross validation is an internal validation method wich results in the optimal number of PLS-factors and gives information about the prediction ability of the model. Fig. 4 shows the measured migration time of mirtazapine versus the predicted migration times according to the used model. It is clear that the migration time of a component is highly correlated to the current going through the capillary during the analysis. The correlation coefficient and the standard error of prediction (SEP) are 0.995 and 0.033, respectively.

Similar results were produced for the related substances as well. From the PLS factors themselves no chemical information could be derived.

Relationships between peak area and electrical current profiles could not be deduced. The reason is that peak area depends on the velocity at the moment of detector passage. In contrast the migration time is the result of all combined velocities from zero time to the time of detector passage. Therefore it can be concluded that, in the case of fluctuating currents, correction of peak areas by the migration time is not possible.

Sample dissolved in running buffer resulted in a good precision of migration times and peak areas; a stable electrical current profile was obtained.

The shape of the relatively broad skewed peaks could be improved by optimization of the composition of the extraction/injection solvent. For this a central composite design was applied. It resulted in an optimal solvent consisting of 20 m*M* phosphoric acid in methanol–water (35:65, v/v).

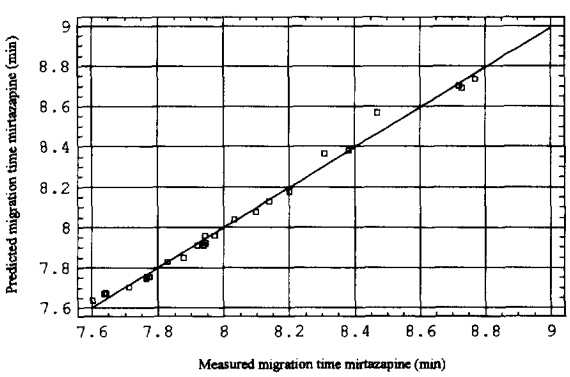

Fig. 4. Relation between predicted migration times and migration times measured. The migration times were predicted according to the current profiles measured during the analysis. The underlying model is a PLS model based on 3 factors; standard error of prediction (SEP): 0.033; correlation coefficient: 0.995.

3.2. Buffer optimization

The electrophoretic separation was optimized with respect to selectivity and time of analysis. To that end, the resolution of the most critical separated component couple in each experiment was used as a parameter for the selectivity. This resolution is further referred to as minimal resolution. The minimal resolution observed at each condition is given in Fig. 5.

The buffer composition of 80 m*M* phosphate in water–methanol (75:25, v/v) with a pH of 2 showed the highest resolution (approx. 2).

The migration times and peak widths for all six components were measured for each experiment. Functional relationships between these parameters and the buffer characteristics, concentration of phosphate, methanol content and pH value, were calculated by performing MLR. This analysis resulted in main, quadratic and two factor interaction effects. The significance of these effects was achieved by means of an analysis of variance (ANOVA). The calculated effects corresponding to the migration time of mirtazapine are shown in Fig. 6. The migration time of mirtazapine as well as of the five related substances are mainly affected by methanol and pH (first order effects). With respect to separation of the compounds the functional relationships for the migration times and peak widths formed the base for reconstructing electropherograms. In Fig. 7 reconstructed electropherograms corresponding to three optimal buffer compositions (minimal resolution >1.5) are given.

Experiments were carried out at the buffer conditions corresponding to Fig. 7a and Fig. 7b to confirm the predicted results. The optimal separation conditions were achieved using a buffer composition of 70 m*M* phosphate buffer in methanol–water (25:75, v/v) at a pH of 2.0. The corresponding electropherogram is given in Fig. 8.

From the relationships of the electrolyte composition and the separation of the various components, information of the ruggedness can be ob-

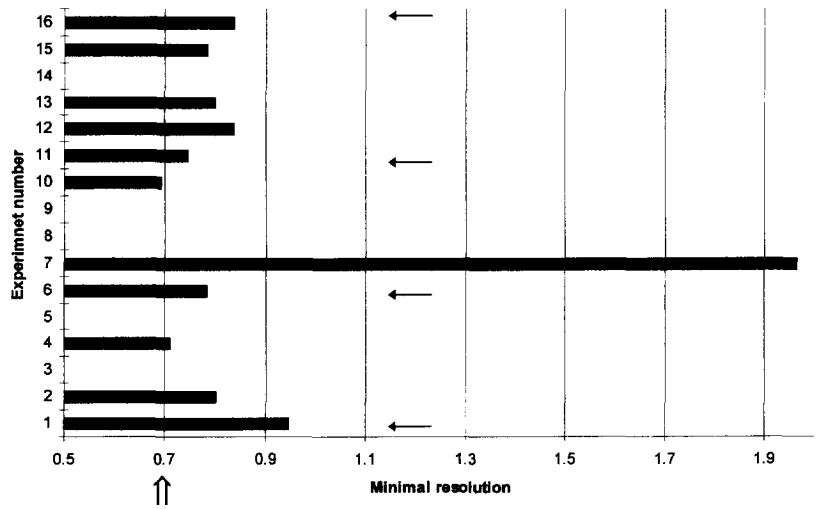

Fig. 5. The minimal resolution observed at each of the 13 different electrolyte compositions. The bars indicated by an arrow are replicates of the electrolyte corresponding to the center point of the experimental scheme. Resolutions <0.7 were regarded as not separated.

tained. For this, practical boundaries for each of the three factors were defined (Table 1). The boundaries are based on limitations of the accuracy of the equipment and practical experience. Calculations showed that the lowest resolution factor within these boundaries will not be smaller than 1.0. Therefore it can be concluded that the method is rugged with respect to small changes in the composition of the electrolyte system.

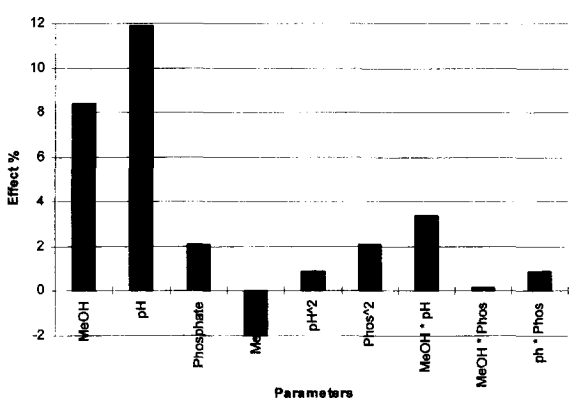

Fig. 6. Effects on the migration time of mirtazapine expressed as percentages of the average migration time (11.1 min). From left to right: three main effects, three quadratic effects and three two-factor interactions. The main effects and the interaction between methanol and pH were statistically significant.

3.3. Validation

3.3.1. Selectivity

The electropherogram in Fig. 8 shows the separation of mirtazapine, the 5 related substances and the internal standard. All components are baseline separated at relevant concentration levels (1%). The stress-study showed no increase of unknown related substances. Purity factors of the mirtazapine peaks indicated the absence of coeluting components.

3.3.2. Linearity

Calibration graphs for mirtazapine as well as for the internal standard are rectilinear within the studied range of 1–125% of the nominal target concentration (corresponding with 10–1250 μg/ml). The same holds true for the calibration graphs of the related substances within the studied range of 0.25–2.0% of the nominal mirtazapine concentration (corresponding with 2.5–20 μg/ml). Response concentration curves proved to be of first order; confidence intervals for the intercept included zero. For the calibration graph of both mirtazapine and internal standard a correlation coefficient of >0.9999 was found and for the related substances >0.995. A linearity and residual plot are given in Fig. 9a and Fig. 9b.

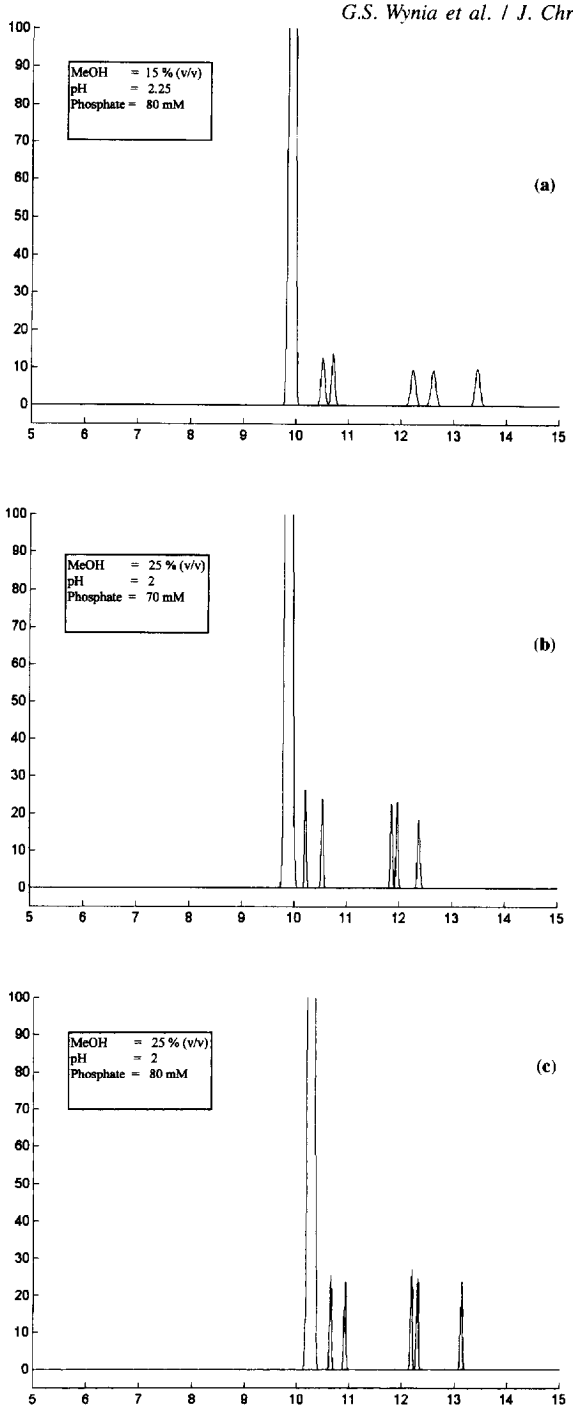

Fig. 7. Optimal separations derived from mathematical relations between both peak width and migration time on the one side and electrolyte composition on the other. Corresponding buffer compositions: (a) 80 m*M* phosphate in water–methanol (85:15, v/v), pH=2.25; (b) 70 m*M* phosphate in water–methanol (75:25, v/v), pH=2.0; (c) 80 m*M* phosphate in water–methanol (75:25, v/v), pH=2.0.

3.3.3. Accuracy

Fig. 10 demonstrates the results achieved for the accuracy experiment for the active ingredient mirtazapine and the related substances. Accuracies (recoveries in %) for the main compound and for the related substances comply with requirements of 99-101 and 90-110, respectively.

3.3.4. Precision

There was no need to correct the peak areas for their corresponding migration times; R.S.D. observed for the migration times was <0.5%. The R.S.D. of the relative area responses (internal standard was used throughout the whole validation) of 6 consecutive injections of mirtazapine reference solution was 0.5%. R.S.D. values of the relative responses for the related substances at a level of 0.2% are up to 6.0%. Repeatability (R.S.D.) for the determination of the the main component was 1.7%. For the related substances at a level of 0.2% this varies between 12 and 28%.

3.3.5. Limit of detection and quantitation

Results for the limit of detection (LOD) for the related substances are 0.02–0.04% with respect to the main component; which implicates limits of quantitation between 0.06 and 0.13%.

3.3.6. Ruggedness

The method proved to be rugged with respect to small changes in electrolyte composition as was derived during method development. The use of different lots of fused silica capillaries (three were tested) obtained from the same supplier was not significant regarding the separation (resolution) and peak shapes. It is therefore expected that routine use of this method on this type of CE instrument is not attended with problems.

3.3.7. System suitability testing

Generally suitability testing is an integral part of the analytical procedure. The tests are based on the concept that equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Data obtained from ruggedness test results were used to deduce system suitability criteria for the method as given in Table 2.

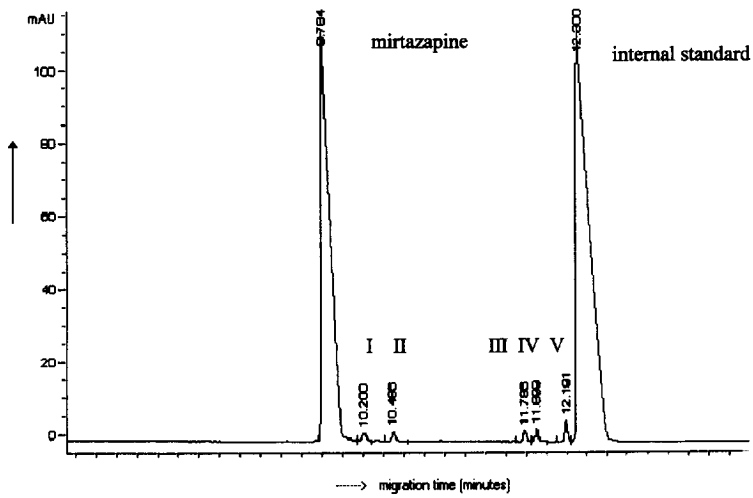

Fig. 8. Electropherogram at optimal electrolyte composition: 70 m*M* phosphate in methanol–water (25:75, v/v), pH=2.0, detection 210 nm. Sample (1 mg/ml) spiked with 1% of the related substances. Elution order: mirtazapine, related substance V, related substance II, related substance I, related substance IV, related substance III and internal standard.

3.4. Comparison of CE and HPLC

Table 3 summarizes the validation results obtained with the CE and the current validated HPLC method. For these parameters also, requirements are given which are used within quality control environment. For the assay validation data are comparable to those obtained with the current release HPLC method. For the determination of related substances CE results for injection precision (with internal standard), repeatability and LOD/LOQ are less favourable. The use of a so called "bubble cell detector" (150 μm internal diameter of the detector cell, Hewlett–Packard) improves the LOD/LOQ by a factor 2–3. In contrast with HPLC, for CE, injecting of more concentrated solutions will not simply lead to an improvement of LOD/LOQ. In CE, increment of the ionic strength can seriously suppress the stacking effects. This results in non-linearity of the peak height versus the amount injected.

All CE validation results fully comply with the requirements valid within our quality control department. In Fig. 11 assay results of CE versus results of HPLC analysis are compared to each other. The results were obtained from analyses of several batches of mirtazapine granulate of different strengths. Statistical evaluation showed a first order relationship with an acceptable correlation. No bias

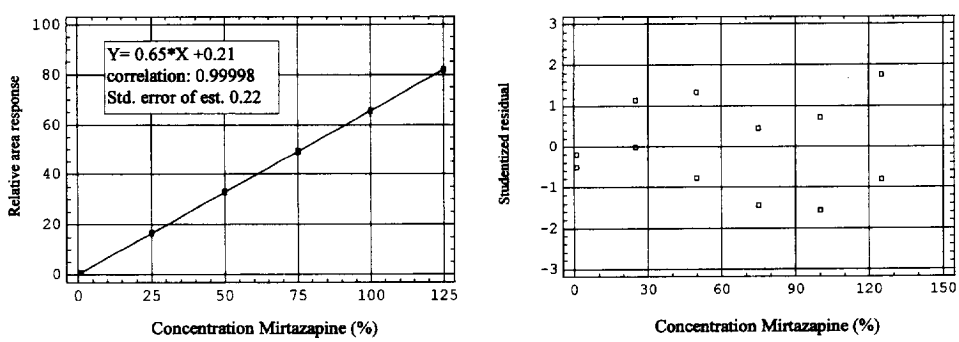

Fig. 9. (a, left) Linearity plot of the main component mirtazapine. (b, right) Residual plot illustrating the deviation between the measured and predicted response values.

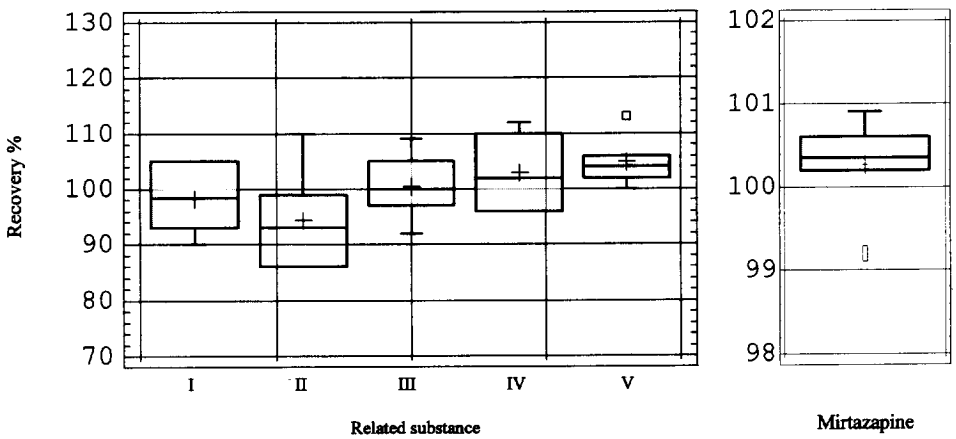

Fig. 10. Box–Whisker plot of accuracy (% recovery) values for mirtazapine and the five related substances. The + symbol represents the average value of the six replicates and the □ symbol is used for suspect outliers.

Table 2
System suitability criteria for the CE method for mirtazapine and related substances

Parameter	Criterion
Migration time (t_M) mirtazapine (min)	$9.6<t_M<10.0$
Number of theoretical plates (N) mirtazapine	$N>40\,000$
Resolution factor (R_s) between related substance I and related substance IV	$R_s>1.5$

in results was observed. As already concluded during validation, also now repeatability of the CE method was slightly less favourable than with HPLC.

The CE and HPLC methods were also used for cross contamination studies as part of the validation of cleaning procedures. In order to achieve sufficient sensitivity, relatively high concentrations of material were used. It was found that the CE method was less

Table 3
Validation data of the CE and HPLC method and quality control (QC) requirements

	CE	HPLC	QC requirement
Mirtazapine			
Accuracy (%)	100.3	100.2	99–100
System precision (R.S.D. of injection, %)	0.5 (I.S.)	0.3 (E.S.)	<1.5
Repeatability (R.S.D. %)	0.6	0.2%	<2
Linearity range tested (μg/ml) and results	10–1400: complies	4–800: complies	
Related imp's (0.2%)			
Accuracy (%)	95–105	90–105	90–110
System precision(%) (R.S.D. of injection, %)	2–7 (I.S.)	1–3 (E.S.)	<10
Repeatability (R.S.D. %)	4–10	1–8	<10
LOD (%)	0.02–0.4	0.01–0.04[a]	
LOQ (%)	0.07–0.13	0.02–0.12[a]	
Linearity range tested (μg/ml) and results	2.5–20: complies	0.8–20: complies	
Run time (min)	15 (incl. pre-cond. steps)	27	

[a] Data based on equal sample concentration. This was achieved by recalculating LOD/LOQ values of the HPLC results to a concentration of 1 mg/ml.

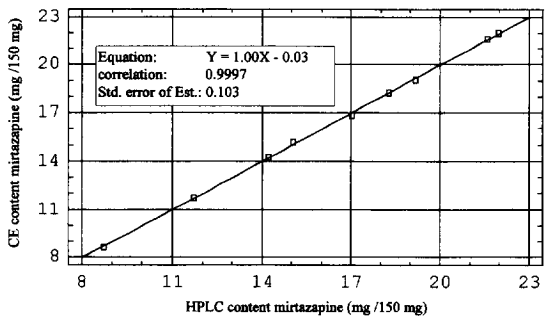

Fig. 11. Comparison of average CE and HPLC results for the content of mirtazapine in granulates of different strengths. CE and HPLC conditions as described in Section 2.3.

rugged in comparison to the HPLC method, considering the concentration of the matrix in the extraction medium. A poor peak shape, viz. peak splitting and non-symmetry and a resulting decrease in resolution was observed at higher concentrations. Moreover, the slope of the calibration curve for peak height response and concentration showed a decreasing sensitivity at higher concentrations. Therefore, it can be concluded that the dynamic range for CE is less than for HPLC and that CE is more sensitive to interfering matrix components in the injection liquid.

4. Conclusions

For the CE method, developed for the determination of mirtazapine and related substances, the choice of the injection solvent proved to be of great significance in order to obtain a good repeatability of both migration times and peak areas. In capillary electrophoresis, correcting peak area for the migration time does not always improve the precision. It can therefore be concluded that the injection solvent is not only of importance to obtain a good peak shape and stacking effect but also to obtain repeatable migration times and quantitative results. After some initial experiments, the selectivity could be optimized efficiently by using an experimental scheme. The optimization was facilitated by the use of an automated CE system. From these experiments it can be concluded that the method is rugged with respect to buffer concentration, percentage of modifier and pH. Validation data showed that the CE method allows a selective quantitative determination of the content of mirtazapine and related substances. The method can therefore be used for release as well as for stability investigations. In general, validation data and batch analysis results are similar to those obtained with a validated HPLC method used for release of mirtazapine tablets.

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