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Enantiomeric determination, validation and robustness studies of racemic citalopram in pharmaceutical formulations by capillary electrophoresis

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Abstract

A chiral capillary electrophoresis (CE) method has been developed allowing the enantiomeric separation of racemic citalopram (*R*-(−) and *S*-(+) citalopram) using as chiral selector carboxymethyl-γ-cyclodrextrin (CM-γ-CD). The influence of chemical and instrumental parameters on the separation such as cyclodextrin (CD) and buffer concentrations, buffer pH, voltage, injection pressure, . . ., was investigated. Good chiral separation of the racemic mixture was achieved in less than 4 min using a fused-silica capillary and as background electrolyte (BGE) a phosphate buffer solution (20 mM, pH 7) containing 0.15% (w/v) of CM-γ-CD as chiral selector. The separation was driven in normal polarity mode at 15 °C, 30 kV and hydrodynamic injection. In order to validate the method, the stability of the solutions, precision (repeatability, reproducibility and F-Snedecor test), linearity (Lack of Fit and ANOVA tests) accuracy (98–101%), detection and quantitation limits (0.06 and 0.2 mg L^{-1}, respectively), on a selected analytical placebo, were examined. Besides, a robustness test was performed using the Plackett–Burman fractional factorial experimental design using a matrix of 15 experiments for seven factors (internal parameters) with a statistical treatment suggested by Youden and Steinner. The proposed method is fast, sensitive, inexpensive and, besides, it has been evaluated by means of an extensive validation study and an exhaustive robustness test. The scope of this validated and robust method has been proved in the analysis of four pharmaceutical formulations; two of them (recently available in Spain), which just contained *S*-(+)-citalopram (escitalopram) as active principle. Recoveries between 101 and 103%, with regard to their nominal contents were obtained. In the other two pharmaceutical ones, the method provided the separation and quantification of both chiral isomers in the existing racemic mixture.

Keywords: Enantiomers/citalopram; Cyclodextrin; Chiral capillary electrophoresis; Validation; Robustness

1. Introduction

Citalopram (CIT) (Fig. 1) is a bicyclical phthalate derivative that belongs to the newest and meanest group of antidepressant drugs SSRIs (selective serotonin reuptake inhibitors). CIT seems to be active not only against major depression, but also against anxiety, panic and obsessive-compulsive disorder [1] and pathological laughing and crying [2]. Citalopram inhibits the reuptake of the neurotransmitter serotonin into nerve terminals of donor cells, so avoiding its accumulation in these cells and restoring its transport to the receiver ones.

Citalopram is a racemic compound (*S*-(+)-citalopram and *R*-(−)-citalopram), but just the (*S*-(+)-CIT) enantiomer seems to be therapeutically active showing the inhibitory effect already described [3].

Nowadays, there are several pharmaceutical preparations, commercially available in Spain, which contain citalopram in its racemic form, and there are also other ones (recently

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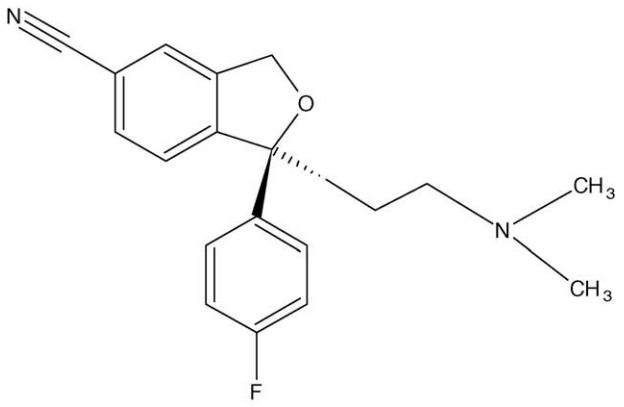

Fig. 1. Chemical structure and pK_a value of racemic citalopram.

introduced in countries like Spain) which just include the active *S*-(+)-CIT enantiomer named escitalopram (*S*-CIT). Therefore, scarce works about the stereoselective separation of racemic citalopram and its reliable application to pharmaceutical and biological samples have been published. In this way, ours work allows an enantioseparation using CM-γ-CD as chiral selector.

Several chiral analytical methods are available for the determination and separation of racemic citalopram by means of different separation techniques like liquid chromatography using modified columns with different chiral selectors as different β derivative cyclodextrins (e.g. acetylated and sulphated β-CD) [4–8] as stationary phase. An enantioselective analysis of citalopram and its metabolites in post-mortem cases has been also performed by means of a chiral HPLC method [9]. In the same way, an analytical method using liquid chromatography-electrospray ionization mass spectrometry has achieved the simultaneous determinations of 13 antidepressants including citalopram and escitalopram [10].

Nevertheless the most widely applied technique for enantiomeric separations is nowadays capillary electrophoresis (CE) for reasons concerning to its efficacy, speediness and cheapness with regard to liquid or gas chromatographic techniques. Most of chiral CE analyses at present were mainly achieved employing cyclodextrins (CDs) as the chiral selector [11,12] added to the background electrolyte (BGE). In this sense, several strategies have been published for the determination of *R* and *S* citalopram and its metabolites by CE in the reversed polarity mode using mixtures of negative charged and neutral CD with very low pH buffers [13,14] in order to reach a suitable selectivity and efficacy.

In this paper, we propose a valuable and easy alternative method for the enantiomeric separation of *R* and *S* citalopram using CM-γ-CD as chiral selector. Besides the reliability of the proposed electrophoretic method has been evaluated by means of an extensive and exhaustive validation procedure including different analytical tests and a statistical robustness study. Good results were found in this validation procedure such as low detection and quantitation limits, excellent precision with relative standard deviations (RSD) <3%, etc. From the whole study achieved in this manuscript it can be concluded that our method can be successfully and reliably applied to the quality control of pharmaceutical formulations containing *R* and *S* citalopram.

2. Materials and methods

2.1. Reagents

Milli-Q water was used throughout the study.

Racemic citalopram hydrobromide (containing *S*-(+) and *R*-(−) citalopram) and *S*-(+) citalopram oxalate (escitalopram) were kindly supplied by H. Lundbeck A/S (Copenhagen, DK).

Different chiral selectors were tried in the preliminary study to achieve the enantiomeric separation. The cyclodextrins γ-CD, β-CD, methyl-β-CD, dimethyl-β-CD, hydroxypropyl-β-CD, carboxymethyl-γ-CD (CM-γ-CD), bile salts, cholic, taurocholic and taurodeoxy-cholic acids and sodium dodecyl sulphate were purchased from Sigma (St. Louis, USA).

Several buffer solutions with different pH values were prepared using as reagents: sodium dihydrogenphosphate, disodium hydrogenphosphate, boric acid, sodium tetraborate, citric acid, tri-sodium citrate, ammonium acetate, sodium hydroxide, hydrochloric acid, acetic acid and ammonium hydroxide. All these products were obtained from Panreac (Barcelona, Spain).

Contained excipients in pharmaceutical formulations were obtained as a gift from Pfizer (Barcelona, Spain) or either purchased from Acofarma (Barcelona, Spain).

2.2. Instrumentation

A Beckman P/ACE System MDQ capillary electrophoresis equipment provided with a diode-array detection (DAD) system was used (Fullerton, CA, USA). A Beckman capillary electrophoresis software controlled this system. Separations were carried out in a 60 cm (50 cm effective length) × 75 μm inner diameter and 375 μm outer diameter fused-silica capillary housed in a cartridge with an 800 μm × 100 μm detection window.

The pH measurements were achieved in a Crison model 2002 pH meter with a combined glass electrode (Madrid, Spain).

2.3. Operating conditions

2.3.1. Solutions and sample preparations

2.3.1.1. Standard solutions. Standard stock solutions (200 mg L^{-1}) of racemic citalopram and *S*-(+)-citalopram were prepared in water (Milli-Q quality) and stored under refrigeration at 5 °C. Working standard solutions (20 mg L^{-1})

were daily prepared by diluting suitable aliquots of stock solutions with Milli-Q water.

2.3.1.2. Background electrolyte. Electrophoretic enantiomeric separation was driven using as BGE a pH 7, 20 mM NaH_2PO_4/Na_2HPO_4 buffer solution containing an 0.15% (w/v) of CM-γ-CD. The buffer solution was prepared by dissolving a suitable amount of sodium dihydrogenphosphate in deionizated water and adjusting the pH value to 7 with NaOH 0.5 M.

2.3.1.3. Analytical placebo. Both validation and robustness studies were developed on a selected and representative placebo solution. The analytical placebo stock solution was prepared containing all the components indicated in every pharmaceutical formulation except the active principle.

2.3.1.4. Pharmaceutical samples. Tablets of four pharmaceutical formulations: Almiral (Prodesfarma, Barcelona, Spain) and Seropram, Esertia and Cipralex (Lundbeck, Barcelona, Spain) were successfully analyzed using the following procedure: a tablet was weighed and ground in a mortar. The solid was transferred into a 100 mL volumetric flask, approximately 90 mL of water were added and this suspension was mechanically shaken for 10 min. The solution was diluted with water to the mark and it was left to sediment; an aliquot of the supernatant was taken and diluted (1/10) with water to give an expected final concentration close to 20 mg L^{-1} of citalopram or escitalopram.

2.3.2. Electrophoretic procedure

Before its first use, the capillary was consecutively conditioned by flushing 0.5 M NaOH for 12 min at 20 p.s.i. (1 p.s.i. = 6894.76 Pa), water for 12 min at 20 p.s.i. and finally it was electroconditioned with the BGE solution for 6 min at 20 p.s.i.

Between consecutive separations the capillary was rinsed with 0.1 M NaOH, water and the running buffer in this order during pumping periods of 1 min at 20 p.s.i. for every one of these flushing reagents.

Different vials of electrolytes were used for rinsing and separating operations in order to keep constant the electrolyte level on the anodic side. The set of separation vials was changed after eight separations runs.

The running electrolyte was a pH 7, 20 mM phosphate buffer solution containing 0.15% (w/v) CM-γ-CD. Samples were injected by hydrodynamic mode for 9 s at 0.5 p.s.i. Both injection and separation stages were driven in the normal polarity mode. The separation voltage was 30 kV (with a voltage ramp of 2.9 kV s^{-1}), which resulted in an electrophoretic current of 71 μA at a constant temperature of 15 °C. Triplicate injections of the solutions were performed and average corrected peak area (peak area/migration time) were used for quantitation. Electropherograms were recorded at 205 nm.

3. Results and discussion

3.1. Preliminary studies

As it is well known the pH parameter has got a strong influence in the ionization of the compounds, according to their pK_a values. Racemic citalopram presents a pK_a value of 9.5, thus for pH values lower than this one, both enantiomers could move like cations in an electrophoretic system. As a consequence of this behaviour, the selected electrolyte for preliminary experiments could be a phosphate buffer solution, 50 mM, adjusted to pH 7, being performed the electrophoretic separation at 30 kV (0.17 min), 15 °C and injecting the samples for 5 s at 0.5 p.s.i.

Different single chiral selectors, several mixtures of them or either combinations of these ones with surfactant agents and organic modifiers were tried in the buffer solution to reach the enantiomeric separation of racemic mixture.

Cyclodextrins are widely used as chiral selectors in capillary electrophoresis [15,16], for this reason different kinds of these ones have been assayed in the preliminary experiments. Neutral CDs and their derivative compounds such as β-CD, methyl-β-CD, dimethyl-β-CD, hydroxypropyl-β-CD and γ-CD were tried giving achiral separations in all the experiences, even when these CDs were combined with anionic surfactants like SDS. Different mixtures between neutral CDs, organic modifiers and other chiral selectors like bile salts were assayed to reach the enantiomeric resolution (R_S). On the other hand, assays using carboxymethyl-γ-CD (CM-γ-CD) (anionic cyclodextrin) produced very good results in the enantiomeric separation.

As a result of these preliminary experiments we decided to use as a first background electrolyte to begin the optimization process, the following one: a phosphate buffer solution (50 mM and pH 7) containing 0.1% (w/v) of CM-γ-CD in the instrumental conditions above indicated.

3.2. Optimization of the separation electrophoretic procedure

3.2.1. Effect of pH and composition of the electrolyte

The pH of the running electrolyte has got a high contribution on the ionization of the silanol groups of the capillary wall and on the migration times (t_m) of the studied compounds. Based on the chemical structure (Fig. 1) and pK_a value of racemic citalopram (9.5), the pH of the BGE was studied in the 2–8.5 range with different buffers (phosphate, borate, citrate and acetate), since in these pH conditions it can be supposed that both enantiomeric forms are moving as cations with electrophoretic mobilities in the same sense as EOF and therefore they display t_m lower than those of EOF.

In order to evaluate the influence of pH and composition of electrolyte on the separation, resolution and migration times of *R* and *S* citalopram, several 50 mM buffers solutions at different pH (0.1% (w/v) CM-γ-CD all of them) were prepared: phosphate buffer solutions, whose pH was adjusted

in the range 2–8.5, borate buffer solution at pH 8.5, citrate buffers solutions adjusted between 2 and 7 pH values and acetate buffers solutions that were adjusted between 4 and 6 pH units. Electropherograms of the racemic CIT were obtained in these conditions and from their study we can do the following remarks:

(a) As it is expected and for any buffers solutions, when the pH values decreased the migration times increased.
(b) With regard to electrolyte composition, it is noticeable to comment that acetate buffers (4–6 pH values range) presented migration times shorter than phosphate or citrate in the same pH range, but those acetate buffer conditions do not provide the separation of enantiomers.
(c) The electropherograms obtained with phosphate buffers (2–8.5 pH range) showed migration times and baseline levels smaller than those obtained for borate (8.5) or citrate (2–7 pH values) buffers.
(d) When phosphate buffer is used, the best enantiomeric separation was achieved for pH values between 4 and 7.

Therefore we decided to use phosphate buffer solution of pH 7 along the separation procedure because it provides the maximal R_S between peaks, the smallest migration times and a suitable baseline.

3.2.2. Effect of the ionic strength

The influence of phosphate buffer concentrations on the migration times and on the R_S between compounds was studied in the range 5–70 mM. For concentrations of buffer higher than 20 mM the R_S between enantiomers decreased below 1.1 whereas for concentrations smaller than 20 mM, although the resolution was quite good ($R_S \geq 1.3$), distorted and asymmetric peaks were observed. Thus, a concentration of 20 mM of phosphate buffer (pH 7) was selected as optimal one since it maintains a good peak shape, a low current (30 μA), short migration times and R_S values higher than 1.2 between enantiomeric peaks.

3.2.3. Influence of cyclodextrin concentration (CM-γ-CD)

The effect of the cyclodextrin concentration (CM-γ-CD) on the resolution between the peaks of the enantiomeric isomers and on their migration times was studied. A range between 0.05 and 0.4% (w/v) of a previously selected anionic CD (CM-γ-CD) was taken for this study. It was observed that as higher the CD percentage as longer the migration times of *S*- and *R*-CIT. The R_S values were almost maximal and constant in the range between 0.1 and 0.2% (w/v) whereas outside this range it decreased. So, a CD concentration of 0.15% (w/v) was chosen as a compromise between a good resolution between enantiomeric peaks and migration times providing also good peaks shape and low noise in the baseline.

3.2.4. Effect of voltage and ramp applied

The effect of the applied voltage was studied in the range 5–30 kV. A potential of 30 kV yielded the best response in terms of run time and efficiency (N) of separation.

Once selected the working voltage of our electrophoretic procedure, the influence of the applied ramp on the separation was investigated in the interval between 0.15 min (3.33 kV s^{-1}) and 1 min (0.5 kV s^{-1}), being selected from this study a 0.17 min (2.9 kV s^{-1}) value as a compromise solution.

3.2.5. Effect of the temperature on the electrophoretic system

Changes of the capillary temperature can produce variations in the N, R_S, t_m, detector response and current. The effect of the temperature on the enantiomeric separation was tested in the range from 15 to 30 °C. An increase of the capillary temperature resulted in a decrease of migration times for both compounds due to smaller electrolyte viscosity, but it also produced a R_S value lower than 1, being therefore very affected the selectivity of the separation. On the other hand, a current decrease was observed for lower temperatures. For these reasons, a value of 15 °C was selected as optimal one providing the best resolution ($R_S \cong 1.4$).

3.2.6. Optimization of the injection parameters (time and pressure)

In order to improve the sensitivity the amount of injected sample was varied, attending to the time (3–11 s) and pressure (0.3–1.0 p.s.i.) of the injection stage. For values higher than 9 s and 0.5 p.s.i., a loss of resolution and distorted peaks were observed. So, 9 s and 0.5 p.s.i. were selected as optimal injection time and pressure, respectively.

Fig. 2 shows an electropherogram corresponding to a standard solution of 20 mg L^{-1} of racemic citalopram under selected conditions above optimized. The *S* isomer was identified from a 10 mg L^{-1} isolated *S*-CIT standard solution that was checked under the electrophoretic conditions already established.

3.3. Validation of selected chiral CE procedure

In order to assess the reliability of our CE method, its analytical performance characteristics were evaluated on an analytical placebo made up of the following excipients: maize starch, microcrystalline cellulose, lactose, stearate of magnesium, hydroxypropyl methylcellulose, polyethylene glycol and titanium dioxide.

3.3.1. Stability of solutions

Although this test is often considered part of the robustness of the procedure, it should be carried out at the beginning of the validation process because it determines the validity of data of the subsequent tests.

The stability of the stock and diluted standard solutions of racemic CIT and *S*-CIT was determined by comparing the response factors (concentration/average corrected peak area) of triplicate solutions stored at 5 °C and in darkness with those

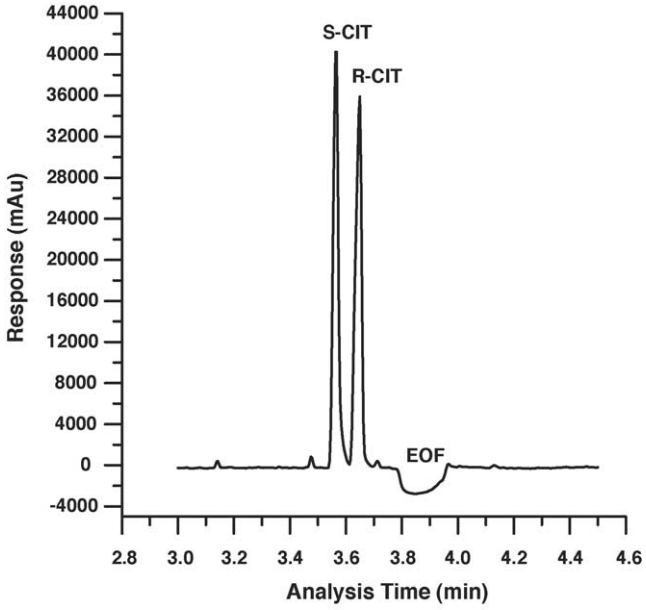

Fig. 2. Electropherogram of a 20 mg L^{-1} racemic citalopram standard solution. Operating conditions: injection: 9 s and 0.5 p.s.i.; separation (normal polarity mode), BGE: phosphate buffer (pH: 7, 20 mM), 0.15% (w/v) CM-γ-CD, 30 kV (0.17 min), 15 °C; detection: 205 nm.

ones of freshly prepared by triplicate. The difference between the concentrations of freshly prepared solutions and those aged for 15 days was lower than 0.2% for CIT and *S*-CIT. The absorption spectra of the solutions were also checked and they were found to be unchanged within this period. These solutions can therefore be used during this interval of time without the results being affected.

The stability of spiked placebo solutions containing racemic CIT and *S*-CIT were also evaluated and it was found that working placebo solutions were stable for at least 12 h.

3.3.2. Precision

The precision of the proposed method has been expressed in terms of relative standard deviation.

In order to check the precision of the optimized method, different placebo solutions containing 20 mg L^{-1} of CIT (test amount) were prepared and analyzed on three different days using the related method. Upon ICH criteria [17], the experimental conditions under this analytical performance characteristic was studied involved evaluation of repeatability and intermediate precision.

Repeatability was studied by performing sequentially a series of 10 injections of spiked standard placebo solutions and the obtained results ($n = 10$) for *S* and *R*-CIT were respectively 0.24 and 0.25% for their t_m, 1.2 and 1.4% for their corrected peak areas, 2.3% for their resolution between peaks and 1.7% for the ratio peak areas of enantiomers (*R*/*S*).

Intermediate precision was studied by performing 10 injections of freshly prepared mixtures, 24 and 48 h later than the first series under the same experimental and instrumental conditions, but by different analysts. In this case, the obtained data ($n = 30$) for *S* and *R* enantiomers were respectively 1.0 and 1.1% for their migration times, 1.6 and 2.0% for their corrected peaks areas, 3.7% for R_S between compounds and 1.3% for the ratio peak areas of enantiomers (*R*/*S*).

Table 1
Statistical parameters for precision study

	S-Citalopram				*R*-Citalopram			
	μ	SD	RSD	F_{exp}	μ	SD	RSD	F_{exp}
t_m								
1	3.81	0.01	0.24	2.27	3.90	0.01	0.25	2.27
2	3.78	0.01	0.37		3.87	0.01	0.37	
CPA								
1	4265	49	1.2	1.43	4214	59	1.4	1.43
2	4319	59	1.4		4306	57	1.3	
R_S								
1	1.31	0.03	2.3	2.03				
2	1.33	0.04	3.2					
RPA								
1	1.01	0.02	1.7	2.84				
2	1.00	0.01	1.0					

$F_{exp} = (SD_1)^2/(SD_2)^2 \geq 1$; $F_{(9,9)th} = 4.026$; t_m: migration time; CPA: corrected peaks area; R_S: resolution; RPA: ratio peak area of enantiomers (*S*/*R*).

With regard to this intermediate precision study, the comparison between two sets of data (first and third) to detect random errors was achieved by means of the Snedecor *F*-test; not significant differences were found in any case at a confidence level of 95% and $n - 1$ degrees of freedom, since the obtained experimental values of *F* were lower than their respective theoretical ones. The Table 1 presents experimental and theoretical values of *F* with regard to migration times, corrected peak areas, resolution and ratio peak areas for *S* and *R* citalopram.

3.3.3. Limits of detection (LODs) and quantitation (LOQs)

Limits of detection (LODs) and quantitation (LOQs) were calculated by measuring six placebo blanks, using the maximal sensitivity allowed by the system and calculating the standard deviation (SD) of this response. LOD and LOQ were estimated multiplying the SD by a factor of 3 and 10 times, respectively and dividing by the slope of the calibration graph.

The LODs and LOQs estimated in this way were 0.06 and 0.2 mg L^{-1}, respectively for both studied enantiomers; LOQs were subsequently validated by the analysis of five standard solutions prepared at 0.4 mg L^{-1} for racemic citalopram in presence of the suitable excipients.

3.3.4. Linearity

All the results concerning to this analytical performance were obtained by using corrected peak areas [18] for calculations.

Detector response in terms of corrected peak areas was linearly dependent with sample concentration over the range 0.25–25.00 mg L^{-1} for the two enantiomeric forms. The linearity was determined from triplicate injections at eight dif-

ferent concentration levels for each compound over two different days. An analysis of variance (ANOVA test) was performed to compare the two different obtained regression lines in order to determine if the data could be combined allowing an estimation of the appropriate quantities by the use of a whole regression line [19,20]. Experimental values of F, 6.89 and 7.07 for S- and R-CIT were lower than the theoretical ones (6.90 and 7.08, respectively); so it can be concluded there were not significant differences between the compared regression lines and therefore the data could be combined for a suitable estimation of quantities by the reliable use of a comprehensive regression line. So, the suitable regression lines equations obtained were:

$$y = (403.165 \pm 5.123)C_S - (130.800 \pm 59.250) \qquad r^2 = 0.9995$$

$$y = (401.050 \pm 5.074)C_R - (125.086 \pm 58.620) \qquad r^2 = 0.9995$$

y is the relative peak area; C_S and C_R the concentration for S- and R-CIT in mg L^{-1}.

Upon AMC (Analytical Methods Committee) [21], a value of regression coefficient close to unity is not necessarily the outcome of a linear relationship and in consequence the test for the Lack of Fit should be checked. In order to achieve this test, residuals (distances of the experimental points from the fitted regression lines) are plotted against concentration. If there is no lack of fit (calibration inherently linear) the plot will look like a random sample from a normal distribution with zero mean. The result to apply this test to our calibration graphs allows us to prove the existence of linearity.

3.3.5. Accuracy

In order to check the accuracy of the proposed method, several synthetic mixtures containing spiked racemic citalopram in variable amounts (0.61–51.0 mg L^{-1}) on the analytical placebo were prepared and analyzed using the described electrophoretic procedure.

The obtained results using corrected peaks area are summarized in Table 2. As it can be observed, recoveries between 98.2 and 101.4% were reached in all cases.

3.3.6. Robustness

Robustness evaluates the influence of changes in the internal factors of the established analytical procedure, like variations in flow range, pH, solvents, etc. providing an indication of its reliability during normal usage [17,22]. The robustness test is considered as a part of the validation method related to the precision (reproducibility) of this one; its purpose is to identify possible sources of error when changes occur in the method specified internal conditions [23–27].

In this paper we have tested the influence of small variations on internal parameters of method like pH and ionic strength of buffer, concentration of chiral selector, voltage, capillary temperature, etc. whose influence has been studied at different levels.

Plackett–Burman fractional factorial model based on balanced incomplete blocks has been employed for this evaluation. For statistical reasons concerning to effects interpretation, designs with less than 8 experiments are not used, while those ones with more than 24 experiments are considered unpractical [28].

The Plackett–Burman design for 7 factors and 15 experiments ($N = 15$) is presented in Table 3. The choice of variables (factors) and the levels at which to test them is very important for a reliable robustness test. Variables must be significant in practice, and levels must reflect the variation that may be usually observed. The chosen factors and the selected levels in our case are summarized in Table 4, where the level (0) is the optimal value in the procedure, whereas levels (+1) and (−1) are respectively upper and lower values with regard to the selected one (0).

The robustness was determined in our case from triplicate injections of standard solutions containing 20 mg L^{-1} of spiked racemic citalopram on placebo solutions.

The mean effect of each variable is the average difference between observations made at the extreme levels and those made at the optimal one. Mean effects and standard errors (DA, DB, DC, . . .) were calculated using the procedures described by Youden and Steiner [29].

Results of the levels variation effects were checked on the most critical electrophoretic responses of method like resolution between enantiomers (R_S), efficacy of each compound (N-SCIT and N-RCIT) and corrected peak areas for each analyte (CPA_S and CPA_R). The proposed method has proved

Table 2
S and *R* enantiomers accuracy for racemic citalopram

Racemic samples	Added (mg L^{-1})	*S*-(+) Citalopram		*S*-(−) Citalopram	
		Found (mg L^{-1})[a]	%Recovered[a]	Found (mg L^{-1})[a]	%Recovered[a]
1	0.61	0.31 ± 0.01	101.4 ± 0.5	0.30 ± 0.02	99.7 ± 0.6
2	1.20	0.50 ± 0.05	98.5 ± 1.1	0.50 ± 0.01	98.2 ± 0.3
3	3.06	1.50 ± 0.02	98.6 ± 1.6	1.51 ± 0.02	98.4 ± 1.2
4	6.18	3.06 ± 0.03	100.0 ± 1.1	3.09 ± 0.03	101.1 ± 0.9
5	10.2	5.15 ± 0.03	100.9 ± 0.5	5.14 ± 0.10	99.6 ± 1.1
6	20.0	10.06 ± 0.04	100.7 ± 0.4	10.0 ± 0.1	99.6 ± 1.2
7	34.7	17.5 ± 0.4	100.8 ± 2.0	17.2 ± 0.1	99.3 ± 0.7
8	51.0	25.6 ± 0.4	100.4 ± 1.6	25.3 ± 0.1	99.2 ± 0.9

[a]Mean value ± SD for $n = 3$.

Table 3
Experimental design for the robustness test using the Plackett–Burman model

Factor[a]	N[b]														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	1	1	1	0	1	0	0	0	−1	−1	−1	0	−1	0	0
B	0	1	1	1	0	1	0	0	0	−1	−1	−1	0	−1	0
C	0	0	1	1	1	0	1	0	0	0	−1	−1	−1	0	−1
D	1	0	0	1	1	1	0	0	−1	0	0	−1	−1	−1	0
E	0	1	0	0	1	1	1	0	0	−1	0	0	−1	−1	−1
F	1	0	1	0	0	1	1	0	−1	0	−1	0	0	−1	−1
G	1	1	0	1	0	0	1	0	−1	−1	0	−1	0	0	−1

−1, 0, +1: levels for the factors.
[a] A–G: selected factors.
[b] N (1–15): number of experiments $= 2n + 1$, being n = number of factors.

Table 4
Variables selected as factors and values chosen as levels

Factor	Units	Limits	Level (−1)[a]	Level (+1)[b]	Nominal (0)[c]
(A) pH phosphate buffer		±0.3	6.7	7.3	7.0
(B) Buffer concentration	mM	±3	17	23	20
(C) Cyclodextrin concentration	% (w/v)	±0.05	0.10	0.20	0.15
(D) Voltage ramp	kV min^{-1}	±0.2	0.15	0.19	0.17
(E) Injection time	s	±1	8	10	9
(F) Injection pressure	p.s.i.	±0.1	0.4	0.6	0.5
(G) Time of rinsed with electrolyte	min	±0.25	0.75	1.25	1.00

[a] Level −1: lower values.
[b] Level +1: upper values.
[c] Level 0: optimal values.

to be robust for all the variations tested in this study, since in all cases for the operating factors (A–G) on every studied electrophoretic parameter, the following rule is observed:

$$\sqrt{2}S > |\mathrm{DA}| \quad \text{being}$$

$$S = \sqrt{\frac{2}{7}(\mathrm{DA}^2 + \mathrm{DB}^2 + \mathrm{DC}^2 + \cdots + \mathrm{DG}^2)}$$

For example, in case of the factor A (pH phosphate buffer) on a specific electrophoretic response like resolution between enantiomers (R_S).

Fig. 3 shows the effect of (−1) and (+1) levels for the seven selected factors on R_S between both enantiomers, where it can be seen the most critical factors in our developed electrophoretic procedure could be the decrease of CD concentration below its optimal value and the variation of voltage ramp and injection pressure too.

3.4. Analysis of pharmaceutical formulations

The usefulness of our electrophoretic procedure has been tested by means of the quantitative and qualitative (peak purity) analysis of citalopram in all their pharmaceutical formulations commercially available in Spain, which contain racemic citalopram (Prisdal and Seropram) or either *S*-CIT (Esertia and Cipralex) in presence of other components (excipients).

The preparation of pharmaceutical samples has been already described in Section 2.3.

Determination of the amount of each antidepressant in the pharmaceutical formulations was performed from triplicate injections of three tablets for every pharmaceutical preparation.

As it is summarized in Table 5, precise results (RSD lower than 1.6%) with recoveries between 101.0 and 102.7% of the values declared by the manufacturer were obtained. As an

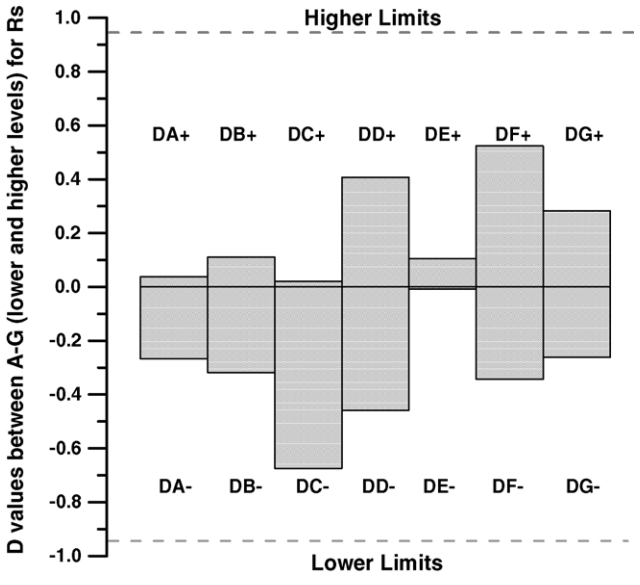

Fig. 3. Variations effects of the levels (−1), and (+1) for the seven selected operating factors (A–G) over resolution between peaks (R_S).

Table 5
Analysis results for the pharmaceutical formulations containing racemic citalopram or escitalopram

Preparation (mg)	Active principle	Obtained (mg L^{-1})[a]	Recoveries (%)[a]
Seropram (20 mg)	Escitalopram	10.25 ± 0.12	102.5 ± 1.2
	R-Citalopram	10.27 ± 0.09	102.7 ± 0.9
Prisdal (20 mg)	Escitalopram	10.22 ± 0.16	102.2 ± 1.6
	R-Citalopram	10.17 ± 0.11	101.7 ± 1.1
Esertia (20 mg)	Escitalopram	20.24 ± 0.11	101.0 ± 0.5
Cipralex (20 mg)	Escitalopram	20.17 ± 0.21	101.7 ± 1.3

[a] Mean value ± SD (n = 6).

example Fig. 4 shows the electropherograms of two pharmaceutical preparations, one of them containing racemic citalopram (Prisdal) and the other one (Cipralex) just including *S*-CIT as active principle. Besides it was proved the absence of *R*-isomer in Esertia and Cipralex formulations.

3.4.1. *Specificity: peak purity*

Co-migration of peaks is also possible in capillary zone electrophoresis (CZE), as in any other separation technique. It is, therefore, useful to investigate the homogeneity of separated peaks.

The techniques used to asses the peak purity of *S* and *R* citalopram in their pharmaceutical formulations were [30]:

- Normalization and comparison of spectra from different peak sections.
- Comparison of absorbance at two wavelengths.

Both techniques proved the purity of *R* and *S* Citalopram peaks, so, not interferences from the excipients of formulations were found.

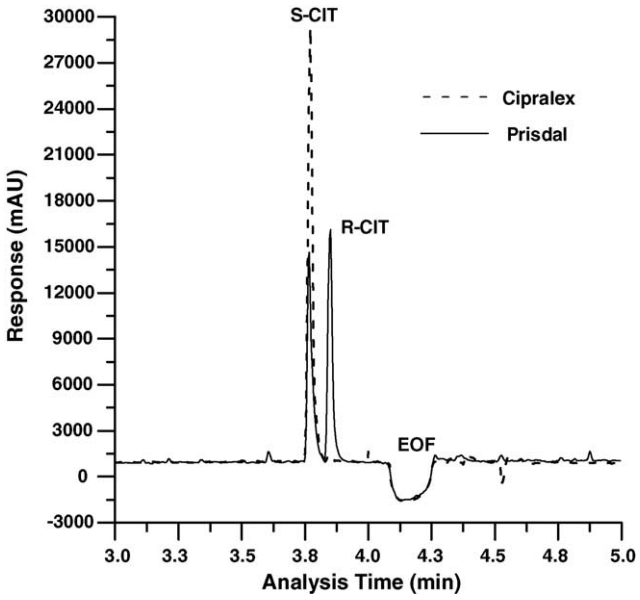

Fig. 4. Electropherograms of two analyzed pharmaceutical preparations (Prisdal and Cipralex).

4. Concluding remarks

An easy, cheap and rapid method with a simple background electrolyte and normal polarity mode have been evolved and proposed for the enantioselective separation of the SSRI antidepressant citalopram (racemic compound), which presents as pharmacologically active enantiomer the *S*-isomer (escitalopram). For the first time, carboxymethyl-γ-cyclodextrin has been used as single chiral selector for the separation of *S* and *R* enantiomers, whereas the only previous option involved the use of complex mixtures of several CDs and other reagents. Thus this method could be a valuable stereoselective alternative to other ones, much more expensive and unapproachable options that allow enantiomeric determinations upon optical activity behaviour like circular dichroism detection. Besides, nowadays this is the first article where it is achieved the analysis of *S*-CIT in their new pharmaceutical formulations (Esertia and Cipralex), proving so the usefulness of our method.

This paper has intended to propose a basic but exhaustive analytical experimental design including several statistical tests (Lack of Fit, ANOVA, etc.) to validate and to evaluate the robustness of a simple electrophoretic procedure. It has been shown that experimental results concerning to stability of solutions, precision, detection and quantitation limits, linearity, accuracy and specificity of the test validation and robustness on placebo prove the reliability of our procedure in order to use it for a suitable quality control of formulations containing racemic CIT or either *S*-CIT.

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