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Analysis of *cis–trans* isomers and enantiomers of sertraline by cyclodextrin-modified micellar electrokinetic chromatography

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Abstract

In this work development, optimization and validation of a cyclodextrin-modified micellar electrokinetic chromatography (CD-modified MEKC) method is proposed to resolve separation of the sertraline hydrochloride and synthesis-related substances. Sertraline hydrochloride, the *cis*-(1*S*,4*S*) enantiomer form, is used as an antidepressant therapeutic agent. A buffer concentration composed of 20 m*M* sodium borate, pH 9.0 with 50 m*M* sodium cholate, 15 m*M* sulfated β-cyclodextrin and 5 m*M* hydroxypropyl-β-cyclodextrin was found to be the most suitable background electrolyte. Quantitation of the impurities at levels of 0.1% in different samples of the bulk drug was determined. A comparison of the results with those obtained by HPLC methodology was also accomplished. The method proved appropriate for testing the purity of sertraline hydrochloride in bulk drug.

Keywords: Enantiomer separation; Sertraline; Cyclodextrins

1. Introduction

Capillary electrophoresis in different modes has become a competitive technique with traditional methods to resolve the separation of *cis–trans* isomers and enantiomeric forms of different chemical compounds.

This technique becomes of relevant importance in chiral analysis of drugs due to key features such as high resolution, easy development and optimization of the electrophoretic system, low consumption of expensive reagents and short time of analysis when it is compared to conventional methodologies [1–11].

The control analysis of drug impurities in pharmaceuticals assures therapeutic efficacy and safety avoiding that some unwanted pharmacologic and/or toxicologic side effects of the drugs may be produced.

Sertraline hydrochloride is a drug substance belonging to the group of selective serotonin (5-hydroxitriptamine, 5-HT) reuptake inhibitors (SSRIs) in the brain. The *cis*-(1*S*,4*S*) enantiomer form (*cis*-1*S*,4*S*-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine hydrochloride) is used as an antidepressant agent for oral administration [12] (Fig. 1).

Several methods have been reported for the determination of sertraline and its metabolites in biological fluids by GC, GC–MS [13–17] and HPLC

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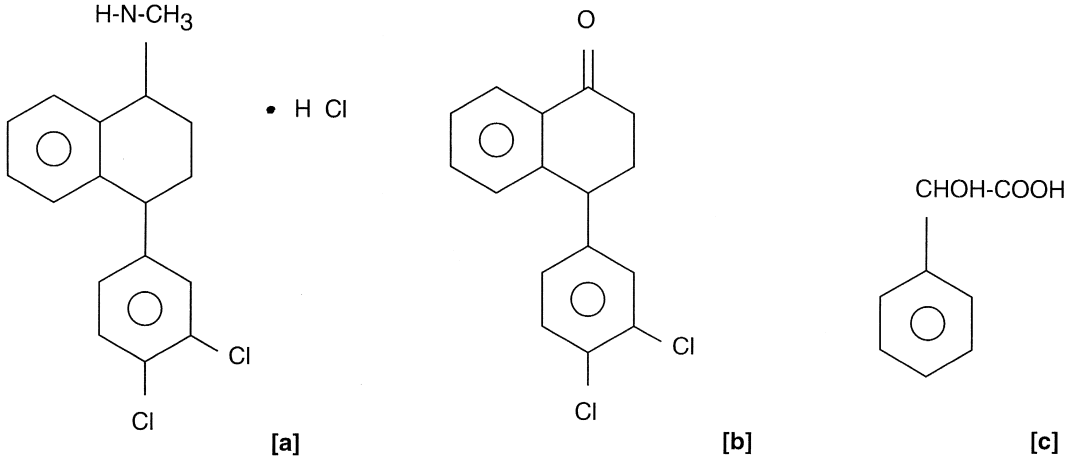

Fig. 1. Chemical structures of (a) sertraline hydrochloride (b) sertralone (c) mandelic acid.

[18–24]. However, only a paper has been reported where sertraline was included in a list of numerous drugs screened for toxicological purposes by a capillary zone electrophoretic method [25].

In this work a cyclodextrin-modified micellar electrokinetic chromatography (CD-modified MEKC) was developed employing a mixture of anionic and neutral cyclodextrins with a sodium cholate surfactant. Optimal separation of enantiomeric forms of racemic *cis–trans* stereoisomers, and other synthesis-related substances was performed. Quantitation of the impurities at levels of 0.1% was also achieved and different samples of the bulk drug were analyzed. The comparison of these results with those obtained by HPLC methodology showed to be in good agreement.

2. Experimental

2.1. Reagents

Sertraline hydrochloride (*cis*-1*S*,4*S*-N-methyl-4-(3, 4 - dichlorophenyl) - 1, 2, 3, 4 - tetrahydro - 1 - naphthalenamine hydrochloride) (*cis*-(1*S*,4*S*) enantiomer); *cis*-1*R*, 4*R*-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine hydrochloride (*cis*-(1*R*,4*R*) enantiomer); *trans*-(1*S*,4*R*) and (1*R*,4*S*) N-methyl-4-(3, 4-dichlorophenyl)-1, 2, 3, 4-tetrahydro-1-naphthalenamine hydrochloride (racemic *trans* isomer); N-methyl-4-(4-chlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine hydrochloride (impurity I) and (4*S*) and (4*R*) (3,4-dichlorophenyl)-3,4-dihydro-1(2H)-naphthalenone (racemic sertralone) were obtained by an in house procedure as described in Ref. [26]. Identification and characterization of the purified compounds were obtained by IR, ^{1}H-NMR and MS.

R-(−)-mandelic acid, sodium cholate, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), Heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-CD), Heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-CD) and β-cyclodextrin (β-CD) were purchased from Sigma (St. Louis, MO, USA). Sulfated-β-cyclodextrin was provided by Aldrich (Milwakee, WI, USA). Methanol, isopropanol and acetonitrile (HPLC grade), sodium borate, sodium dihydrogen phosphate and 85% phosphoric acid were supplied by Merck (Darmstadt, Germany). Ultrapure water was obtained from an EASYpure™ RF equipment (Barnstead, Dubuque, IO, USA).

Solutions and samples were filtered through a 0.45 μm nylon membrane (MSI, Westboro, MA, USA) and degassed before use.

2.2. Instrumentation

CD-modified MEKC was performed with a Capillary Ion Analyzer (Waters corp., Milford, MA, USA) and data were processed by MILLENNIUM software (Waters). An uncoated fused-silica capillary of 60

cm length (53 cm to detector) and 50 μm I.D. (Waters) was used.

HPLC analyses were carried out with a liquid chromatograph (Shimadzu, Kyoto, Japan) operating with a LC-10AS HPLC pump, a SIL-10AD auto-injector, a CTO-10AC vp oven, a SPD-10[a] UV detector, and a Class LC-10 for data acquisition. A chromatographic column LiChrospher 60 RP-select B (125 mm×4.0 mm I.D., 5 μm) (Merck) was employed.

2.3. CE system

In the analysis by CD-modified MEKC a background electrolyte (BGE) consisting of 20 m*M* sodium borate, pH 9.0, with 50 m*M* sodium cholate, 15 m*M* sulfated-β-CD and 5 m*M* HP-β-CD was employed. Hydrostatic injection (10 cm height) for 18 s, an operating voltage of 30 kV, a temperature of 10°C and UV detection at 214 nm (zinc lamp) were used.

At the beginning of each day the capillary was rinsed with 0.1 *M* potassium hydroxide for 3 min, washed with water for 10 min and then with BGE for 10 min. Between runs, the capillary was conditioned with BGE for 3 min. At the end of the day, the capillary was flushed with 0.1 *M* potassium hydroxide for 3 min and finally with water for 10 min.

2.4. HPLC conditions

HPLC analyses of the related substances, *R*-(−) mandelic acid, impurity I and racemic sertralone were performed using a mobile phase containing solvent A, 20 m*M* sodium dihydrogen phosphate, pH 2.5 (adjusted with 85% phosphoric acid, 1:5) and solvent B, methanol. A gradient system of 40 to 80% from 0.0 to 20.0 min of solvent B was employed. Detection was set at 225 nm, the flow-rate was 1.0 ml/min, temperature was 25°C and the injection volume was 20 μl. For the analysis of racemic *cis–trans* stereoisomers isocratic chromatographic conditions were used employing a 20 m*M* phosphate buffer, pH 4.5 (adjusted with 85% phosphoric acid, 1:5) with 10 m*M* β-CD and 10 m*M* HP-β-CD–acetonitrile (65:35, v/v). Detection was set at 225 nm, flow-rate was 1.0 ml/min, temperature was 15°C and the injection volume was 10 μl.

2.5. Stock solutions of impurities

Stock solutions of 0.5 mg/ml of *cis*-(1*R*,4*R*) enantiomer, racemic *trans* isomer, impurity I and *R*-(−)-mandelic acid were prepared in water. Racemic sertralone dissolved at 0.3 mg/ml in isopropanol was employed.

2.6. Standard solutions

The standard solutions were prepared as follows: 1.0 mg/ml of sertraline hydrochloride dissolved in water was spiked with stock solutions of *cis*-(1*R*,4*R*) enantiomer and racemic *trans* isomer in appropriate amounts. Another solution of 2.0 mg/ml of sertraline hydrochloride in water was spiked with stock solutions of *R*-(−)-mandelic acid, impurity I and racemic sertralone.

3. Results and discussion

3.1. Method development and optimization

In order to achieve the separation of the *cis* and *trans* diastereoisomers, their enantiomeric forms and other related substances, several operational variables were considered during the optimization of the CD-modified MEKC method. Factors affecting the resolution such as nature of the surfactant micelle, type and concentration of CDs, pH of the buffer, run voltage, temperature, and volume of sample were taken into account.

A first step was to evaluate a MEKC system with a buffer of 20 m*M* borate, pH 9.0 containing 50 m*M* SDS and different types of β-CDs. Neither native β-CD nor neutral derivatives such as DM-CD and TM-CD were effective to separate the enantiomers of *cis* and *trans* diastereoisomers. With sodium cholate used as surfactant instead of SDS and the same chiral additives mentioned above, enantiomeric separation of the racemic *cis* isomer was not achieved and only an incomplete separation of the racemic *trans* isomer was observed. Addition of HP-β-CD to the electrolyte system composed of 20 m*M* borate, pH 9.0 with 50 m*M* sodium cholate could resolve enantiomers of the *cis* isomer but not the *trans* enantiomers. On the other hand, an anionic sulfated-β-CD added

to the electrolyte was able to resolve *trans* but not *cis* enantiomers, resulting the best separation at 15 m*M* concentration. However, a combined BGE of sulfated-β-CD and HP-β-CD gave the most effective enantiomeric resolution of the *cis* and *trans* diastereoisomers. So, it was necessary to examine the influence of the concentration of HP-β-CD on the separation of *cis* enantiomers. The results are given in Fig. 2 and the optimal enantioseparation of the *cis* and *trans* diastereoisomers was achieved with 5 m*M* of the HP-β-CD (Fig. 3).

Finally, the BGE as described in Section 2 allowed the baseline resolution of all impurities derived from the synthesis process of sertraline hydrochloride: *cis*-(1*R*,4*R*) enantiomer, racemic *trans* isomer, racemic sertralone, impurity I and *R*-(−)-mandelic acid (Fig. 4). This latter compound is a resolution agent added in the process of sertraline synthesis to separate the racemic *cis* enantiomers.

Variations of the buffer pH were also investigated and an optimum selectivity was found at pH 9.0. Temperature was another important parameter taken into account during the optimization of the method because selectivity was enhanced by decreasing the operating temperature. A run at 10°C proved to be the most convenient condition at the voltage applied. The influence of the volume injection in peak shapes and resolution was studied resulting to be 18 s corresponding to 4.5 nl the optimal condition for the best separation. Calculated values of resolution for sertraline and its related impurities were higher than 1.4.

3.2. Validation of the method

Selectivity was demonstrated by spiking known amounts of all the impurities evaluated to a 25 μg/ml of sertraline hydrochloride solution.

Repeatibility and intermediate precision of the CE system was studied and Table 1 gives the RSD values of migration times obtained by spiking impurities at the 0.5% (m/m) concentration level to the sertraline hydrochloride solution. Normalized areas for inter-day assay were obtained with RSD values between 1.7 and 2.4% for 0.5% (m/m) level, 1.5 and 2.0% for 1.0% (m/m) level and 1.3 and 1.9% for 1.5% (m/m) level.

Calibration curves for the sertraline hydrochloride solution were prepared with standard solutions at six different concentrations and each solution was injected twice. For the *cis*/*trans* isomers, enantiomeric forms and other related impurities linear range of the

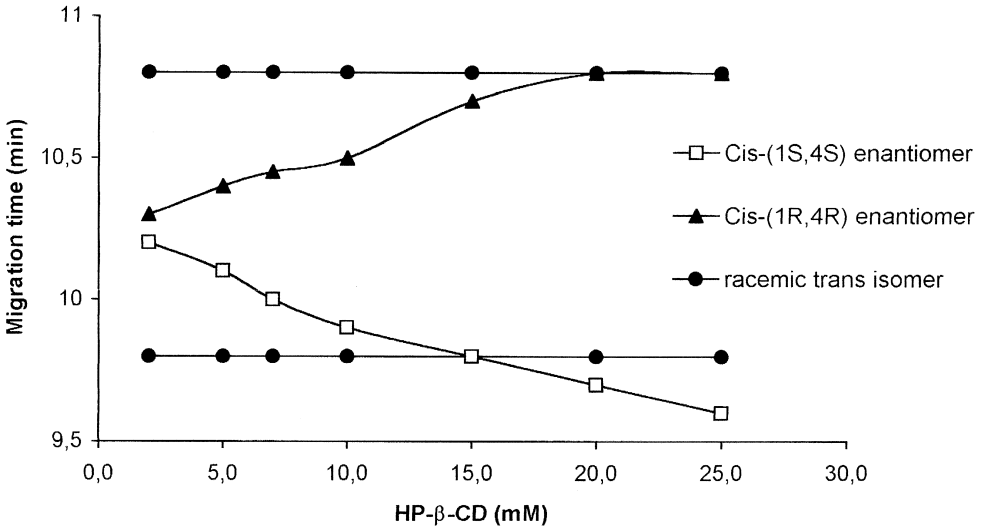

Fig. 2. Effect of concentration of HP-β-CD on migration times. Experimental conditions are described in the text.

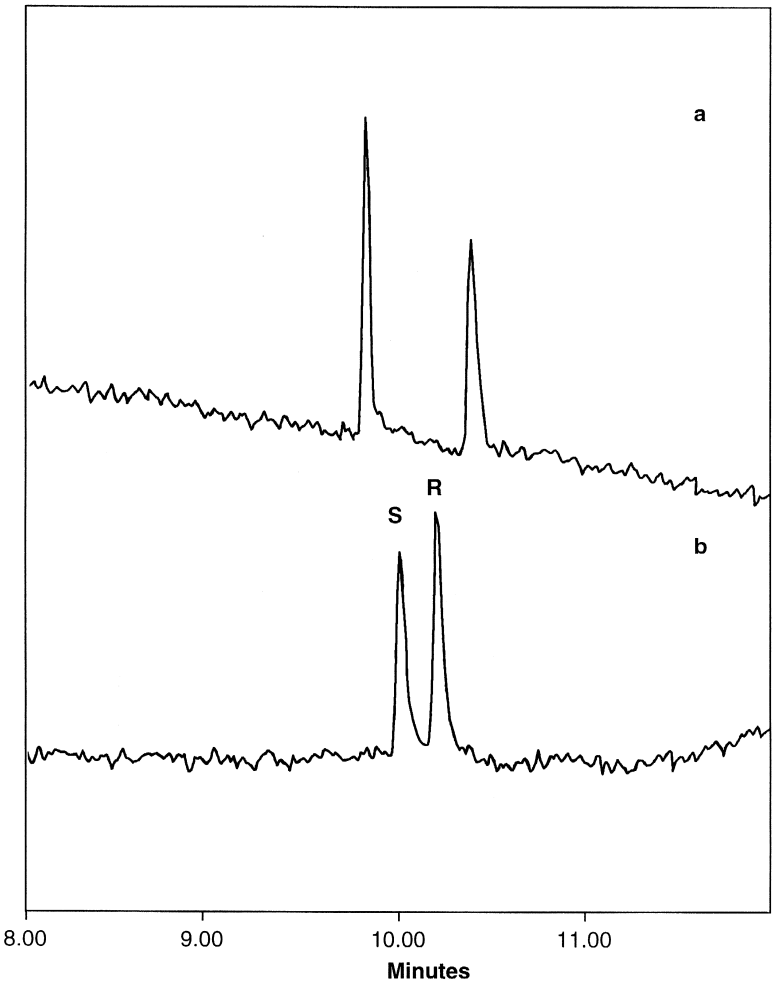

Fig. 3. Electropherogram showing the enantioseparation of (a) racemic *trans* isomer (50.0 μg/ml) (b) *cis*-(1*S*,4*S*) enantiomer (sertraline hydrochloride) (25.0 μg/ml) and *cis*-(1*R*,4*R*) enantiomer (30.0 μg/ml).

detector was demonstrated within the range of 0.1 to 5.0% (m/m) respect to the main compound. Regression data are shown in Table 2. LODs and LOQs of the substances evaluated were determined at S/N ratios of 3 and 10, respectively. LOD of sertraline hydrochloride was 0.2 μg/ml and LOQ was 0.7 μg/ml ($n=6$). LODs for the *cis*-(1*R*,4*R*) enantiomer, racemic *trans* isomer and other impurities were between 0.04 and 0.1% (m/m) and LOQs were between 0.1 and 0.4% (m/m) respect to sertraline hydrochloride.

Robustness was investigated by modification of the composition of the BGE with variations of ±5% in the concentration of chiral selectors. Variations of ±5% in the voltage and ±1.0°C in temperature were also tested and no changes in resolution were observed.

3.3. Analysis of real samples

Different lots of sertraline in bulk drug and

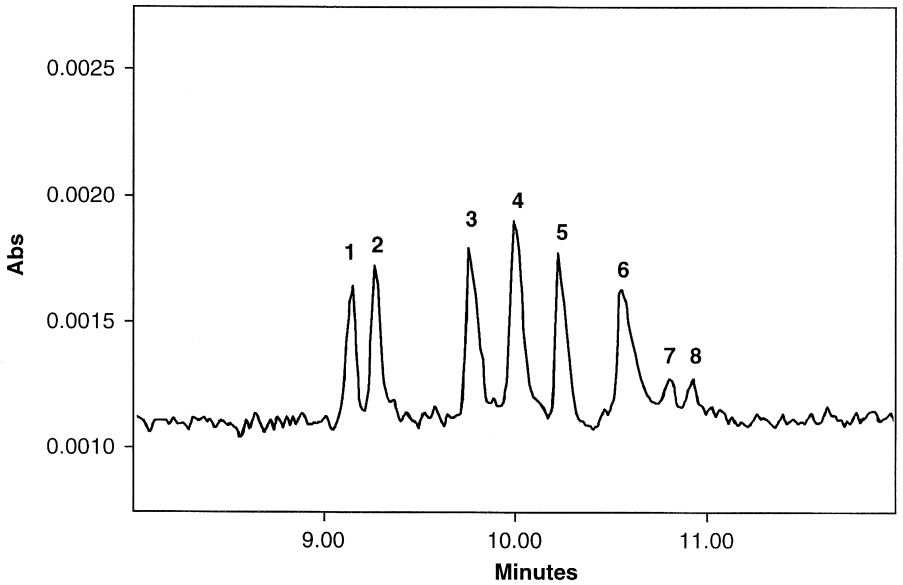

Fig. 4. Electropherogram showing the separation of sertraline hydrochloride and its impurities: 1=*R*-(−)-mandelic acid (40 μg/ml); 2=impurity I (20.0 μg/ml); 3 and 6=racemic *trans* isomer (28.0 μg/ml); 4=sertraline hydrochloride (*cis*-(1*S*,4*S*) enantiomer) (30.0 μg/ml); 5=*cis*-(1*R*,4*R*) enantiomer (30.0 μg/ml); 7 and 8=racemic sertralone (12.0 μg/ml). Conditions are described in Section 2.

intermediate synthetic products were analyzed by CE (Figs. 5 and 6) and HPLC (Fig. 7). A comparison of the results obtained by both methods was performed and good agreement in values of % (m/m) of the impurities with respect to the main compound was obtained (Tables 3 and 4).

4. Conclusions

The method described is suitable for testing the impurities of sertraline hydrochloride in bulk drug from the synthesis process promising a great potential application in the pharmaceutical laboratory. Advantages such as simplicity of performance and lower cost of analysis with respect to HPLC are demonstrated.

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Table 1
Repeatibility and intermediate precision of the migration times of electrophoretic system[a]

Compound	Sertraline	*cis*-(1*R*,4*R*) enantiomer	Racemic *trans* isomer	Impurity I	Racemic sertralone	*R*-(−)-mandelic acid
Intra-day assay (n=6)	0.5	1.3	1.5 1.6	1.2	1.7 1.8	1.0
Inter-day assay[b] (n=18)	0.9	1.6	1.8 1.9	1.7	2.0 2.1	1.4

[a] RSD values are the mean of six replicate injections. Level 0.5% (m/m) of impurities.
[b] Mean values of RSD obtained on 3 different days.

Table 2
Linearity and limits of detection and quantitation of sertraline and synthetic related impurities[a]

Main compound/ impurity	LOD (μg/ml)	LOQ (μg/ml)	Linear range (μg/ml)
Sertraline	0.2	0.7	0.7–400.0 μg/ml $y = 0.32x + 0.29$; slope: SE, 0.01 intercept: SE, 0.005; $r = 0.9999$
Cis-(1*R*,4*R*) enantiomer	0.4 (0.04%)	1.5 (0.1%)	1.5–50.0 μg/ml $y = 0.21x + 0.85$; slope: SE, 0.06 intercept: SE, 0.11; $r = 0.9996$
Racemic *trans* isomer	0.6/0.6 (0.06%)	2.0/2.0 (0.2%)	2.0–50.0 μg/ml $y = 0.89x + 0.04$; slope: SE, 0.08 intercept: SE, 0.04; $r = 0.9995$ 2.0–50.0 μg/ml $y = 0.94x + 0.02$; slope: SE, 0.04 intercept: SE, 0.009; $r = 0.9994$
Impurity I	0.9 (0.04%)	3.0 (0.1%)	3.0–150.0 μg/ml $y = 0.14x + 0.35$; slope: SE, 0.01 intercept: SE, 0.02; $r = 0.9993$
Racemic sertralone	1.0/1.0 (0.05%)	3.3/3.3 (0.1%)	3.3–150.0 μg/ml $y = 0.25x + 0.21$; slope: SE, 0.01 intercept: SE, 0.01; $r = 0.9992$ 3.3–150.0 μg/ml $y = 0.28x + 0.20$; slope: SE, 0.01 intercept: SE, 0.009; $r = 0.9991$
R-(−)-mandelic acid	2.5 (0.1%)	8.3 (0.4%)	8.3–200.0 μg/ml $y = 0.10x - 0.89$; slope: SE, 0.06 intercept: SE, 0.007; $r = 0.9999$

[a] In brackets % (m/m) with respect to the main compound.

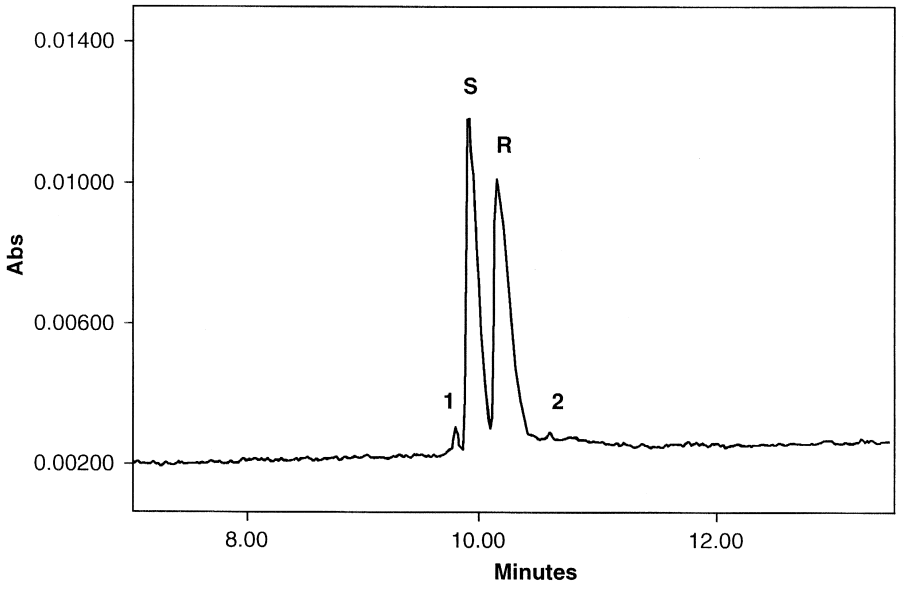

Fig. 5. Electropherogram of sertraline hydrochloride in an intermediate product of the synthesis: 1 and 2 = racemic *trans* isomer (1.7% m/m) and *cis*-(1*S*,4*S*) and (1*R*,4*R*) enantiomers.

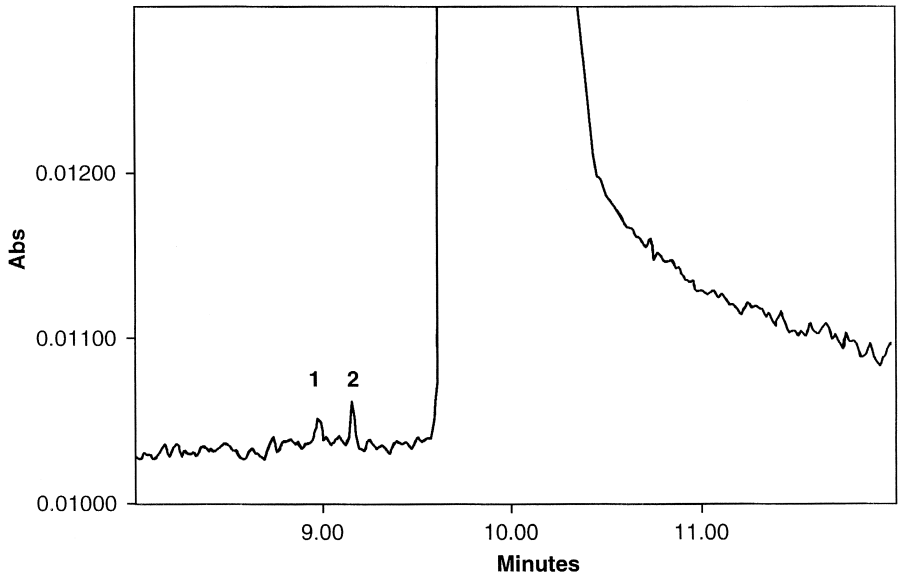

Fig. 6. Electropherogram of sertraline hydrochloride in bulk drug containing 2 mg/ml and its impurities: 1=*R*-(−)-mandelic acid (0.1%, m/m); 2=impurity I (0.08%, m/m). Conditions are described in the text.

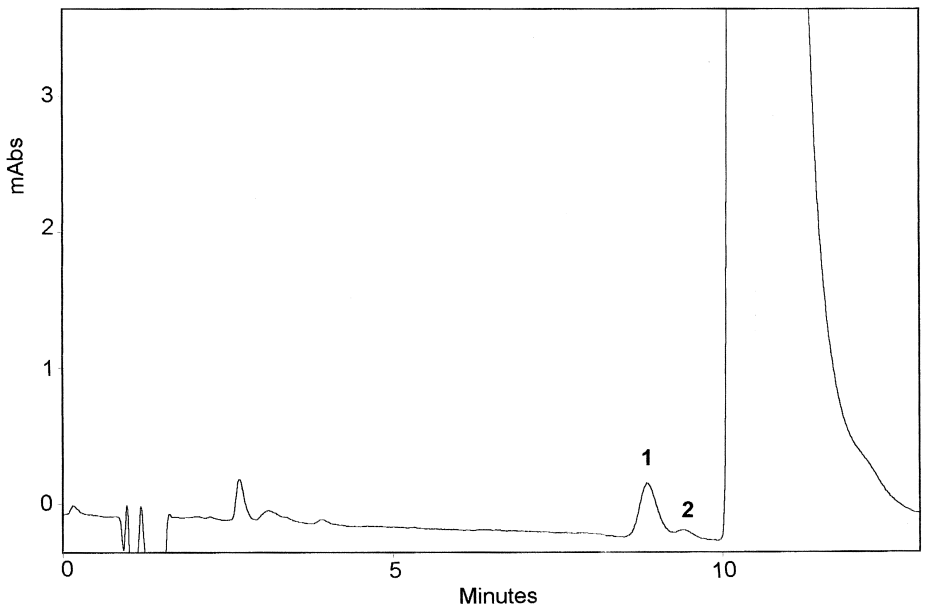

Fig. 7. Chromatographic separation of sertraline hydrochloride and its impurities in an intermediate product of the synthesis: 1 and 2=racemic *trans* isomers (1.5%, m/m). Conditions are described in the text.

Table 3
Analysis of an intermediate synthetic product of sertraline hydrochloride[a]

Impurity	CE	HPLC
Racemic *trans* isomer	1.7	1.5
Impurity I	ND	ND
Racemic sertralone	ND	ND

[a] Mean values % (m/m) of impurities respect to the main compound obtained by triplicate analyses; ND, not detected.

Table 4
Analysis of sertraline hydrochloride in bulk drug[a]

Impurity	CE Lot 1	HPLC Lot 1
cis-(1*R*,4*R*) Enantiomer	ND	ND
Racemic *trans* isomer	ND	ND
Impurity I	0.08	0.09
Racemic sertralone	ND	ND
R-(−)-mandelic acid	0.1	0.05

[a] Mean values % (m/m) of impurities respect to the main compound obtained by triplicate analyses; ND, not detected.

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