Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 471–476
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Determination of Ziprasidone in pharmaceutical formulations by capillary zone electrophoresis

Cecilia Farina [a,b], Leopold Kremser [b], Maria Augusta Raggi [a], Ernst Kenndler [b,*]

[a] *Department of Pharmaceutical Sciences, Faculty of Pharmacy, Alma Mater Studiorum-University of Bologna, Via Belmeloro 6, Bologna, Italy*
[b] *Institute of Analytical Chemistry, University of Vienna, Währingerstr. 38, Vienna, Austria*

Received 5 October 2007; received in revised form 7 November 2007; accepted 8 November 2007
Available online 17 November 2007

Abstract

The atypical antipsychotic drug Ziprasidone was determined by capillary zone electrophoresis in pharmaceutical formulations. Extraction of the drug from the formulation consisted in a simple dissolution step with methanol as solvent, and enables determination of the drug without any interference from the excipients. It was found that at pH of the background electrolyte above 5 the peak of the drug exhibited a tailing, at pH 6 or higher even a disappearance of the peak in the electropherogram was observed. This behaviour was related to the concomitant reduction of the solubility of the drug in the background electrolyte upon deprotonation at higher pH. As a consequence, analyses were carried out with formate buffer, pH 3.0, and enabled run times of about 3 min. The method was validated in terms of stability, specificity, precision, accuracy, linearity, quantitation limits, and robustness, and was applied to the analysis of different commercial capsules.

Keywords: Ziprasidone; Analysis; Determination; Capillary electrophoresis; Dosage forms; Validation

1. Introduction

Ziprasidone (5-[2-[4- (1,2-benzisothiazol-3-yl) -1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2*H*-indol-2-one) is an atypical antipsychotic drug, but it has potential application also as an anxiolytic and as an antidepressant. It acts both on serotonine and on dopamine receptors, but it shows much more affinity for serotonine receptors (5-HT_{1A} agonist and 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C} antagonist) than for dopamine receptors [1].

Ziprasidone was approved by the FDA and introduced onto the market in the USA during 2001 for the treatment of psychotic disorders. Subsequently, it was also approved for the treatment of manic or mixed episodes associated with bipolar disorder. In Europe, Ziprasidone is available in Sweden, Austria and in some other countries for both indications. It is usually administered at daily doses of 80–160 mg. Food can double its bioavailability and for this reason it should be taken during meals. Its elimination half-life after oral administration is 4 h and after parenteral administration is 2.9 h [2,3].

The main advantage with respect to the other atypical antipsychotics is that Ziprasidone was shown to have a very low liability for inducing weight gain [4]. The main adverse effects are dyspepsia, dizziness, somnolence, nausea and – rarely – extrapyramidal symptoms. Ziprasidone has been shown in many cases to prolong the cardiac corrected QT interval and for this reason it is not suitable in patients with cardiac problems [5].

The analytical determination of Ziprasidone has been carried out so far by spectrophotometry [6], but in the majority of the cases by HPLC. Janiszewski et al. [7] extracted the drug from biological fluids by solid-phase extraction with a weak cation-exchange phase, or by membrane solid-phase extraction [8] and quantified it after separation with a C18 reversed-phase column by UV absorbance or by MS detection. Reversed-phase HPLC with UV absorbance detection was carried out also by Li et al. [9,10] and Rani et al. [11]. El-Sherif et al. [12] used HPLC and TLC for the quantitation of the drug in pharmaceutical formulations. Suckow et al. [13] determined Ziprasidone in plasma by a one-step liquid–liquid extraction of alkalinized plasma, followed by a HPLC separation and detection by fluorescence at 320/410 nm. Sachse et al. [14] used HPLC with column switching for sample clean-up at the first column, and quantification of the drug by UV absorbance after separation at

* Corresponding author. Fax: +43 1 4277 9523.
E-mail address: ernst.kenndler@univie.ac.at (E. Kenndler).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.11.010

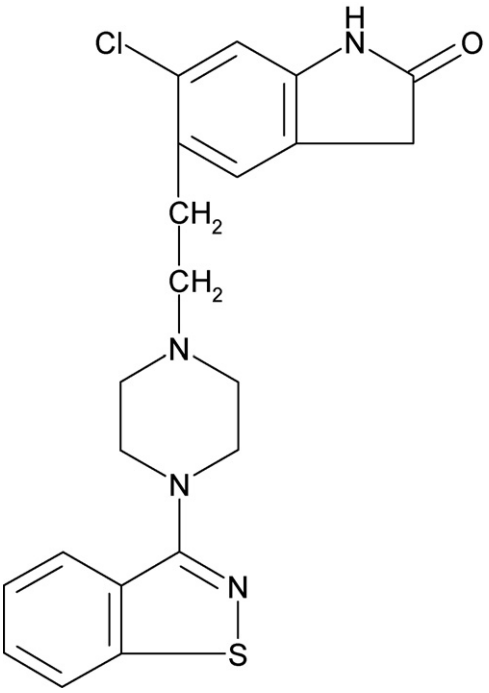

Fig. 1. Structural formula of Ziprasidone.

the second column. Al-Dirbashi et al. [15] extracted Ziprasidone from alkalinized plasma using *tert*-butyl methyl ether and quantified the drug by HPLC–MS/MS. Forty-eight antidepressants and antipsychotics including Ziprasidone could be determined in human serum by HPLC on a monolithic C18 column coupled with tandem MS with ESI [16]. After a one-step liquid–liquid extraction with 20% methylene dichloride in pentane Aravagiri et al. also determined Ziprasidone in the plasma of schizophrenia patients by HPLC–MS-MS [17]. Zhang et al. quantified Ziprasidone together with olanzapine, clozapine, haloperidol, risperidone, and its active metabolite 9-hydroxyrisperidone in rat plasma after a one-step extraction by HPLC with UV-detection [18] or by HPLC–MS/MS [19].

As Ziprasidone contains four nitrogen atoms in its molecule (see Fig. 1), and at least the tertiary amine linked to the aliphatic chain is capable for protonation at intermediate or low pH, capillary zone electrophoresis (CZE) seems to be an adequate analytical method for its determination due to its well-known advantages. We have thus used this method for the determination of the drug in dosage forms.

2. Experimental

2.1. Materials

Ziprasidone of 99.7% purity was from Sequoia Research Products (Pangbourne, Bershire, UK). Lidocain hydrochloride (2-diethylamino-*N*-(2,6-dimethylphenyl) acetamide hydrochloride) used as internal standard (IS) was supplied by Fluka (Buchs, Switzerland). Ziprasidone in pharmaceutical formulation and the excipients (magnesium stearate, maize starch, and lactose) were purchased from local pharmacies. Benzeneethanamine (phenethylamine), sodium acetate anhydrous and hydrochloric acid (25%) were from Fluka; formic acid was from Acros Organics (NJ, USA), boric acid, phosphoric acid and sodium hydroxide were from E. Merck (Darmstadt, Germany, all analytical grade). Methanol was from VWR (Vienna, HPLC gradient grade).

The following buffers (all at total concentration of 50 mM) were used as BGEs for the determination of the mobilities: pH 3.00, pH 3.50, pH 4.00: formate; pH 4.50, pH 5.00, pH 5.50: acetate; pH 6.00, pH 6.50: phosphate; pH 7.00, pH 7.50: Tris; pH 8.00, pH 8.50 borate. The buffers were sonicated for 10 min and filtered through 0.20 μm cellulose acetate filters (Minisart NML, Sartorius). Water for the preparation of the BGEs was double distilled before use. The stock solutions of lidocain, Ziprasidone and phenethylamine were prepared by dissolving the substance in methanol to the concentration of 1000 μg ml^{-1}, then filtered through 0.22 μm Corning® Spin-X® centrifuge tube filters and diluted with BGE prior to use.

2.2. Instrumentation

Electrophoretic measurements were carried out with a CE instrument (HP^{3D}, Agilent Technologies, Waldbronn, Germany) using uncoated fused-silica capillaries (total length 48.5 cm, length to detector 40.0 cm, 50 μm i.d.; Microsolv, Long Branch, NJ, USA). UV-absorbance was measured by the aid of a diode array detector (DAD) and recorded at 214 nm. Injection was at 120 mbar, separation voltage was 25 kV. The temperature of the capillary cassettes was set to 25 °C.

Separation buffer was formate adjusted to pH 3.00 (total concentration 50 mM) with NaOH (1 M). Before use, new capillaries were conditioned with 1 M NaOH for about 30 min and then washed with water and the running buffer, respectively, for another 30 min each. Before daily operation, the capillary was rinsed with 0.1 M NaOH for about 10 min, and then with water and the BGE for another 10 min each. Between each run, the capillary was rinsed with 0.1 M NaOH for 2 min, water for 2 min, and the buffer solution for 2 min.

2.3. Sample preparation

Sample aliquots were taken such to give a final concentration of 40.00 μg drug ml^{-1} of the sample solution. After addition of the IS (corresponding to 90.00 μg ml^{-1} final concentration), the samples were extracted with 1.00 ml methanol by vortexing at room temperature for 10 min. After extraction, the solution was filtered through 0.22 μm Corning® Spin-X® centrifuge tube filters at 5000 rpm for 10 min, and the solution was injected into the CE instrument.

3. Results and discussion

3.1. pH dependence of electrophoretic migration

From the structure of Ziprasidone it can be followed that the nitrogen atoms in the two 5-membered ring systems are very weakly basic, and will be not protonated in BGEs commonly

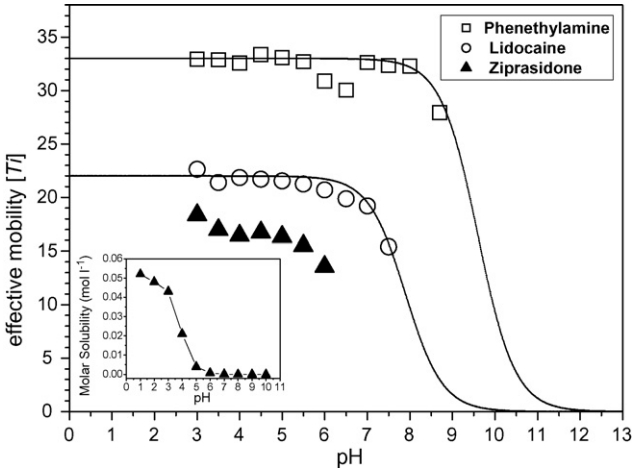

Fig. 2. Dependence of the effective mobility for Ziprasidone, lidocain used as IS, and phenethylamine on the pH of the BGE. *T*: 25 °C. Solid lines: mobility of phenethylamine and lidocain as function of pH according to the Henderson–Hasselbalch relation. The insert shows the calculated solubility of Ziprasidone depending on the pH (see text).

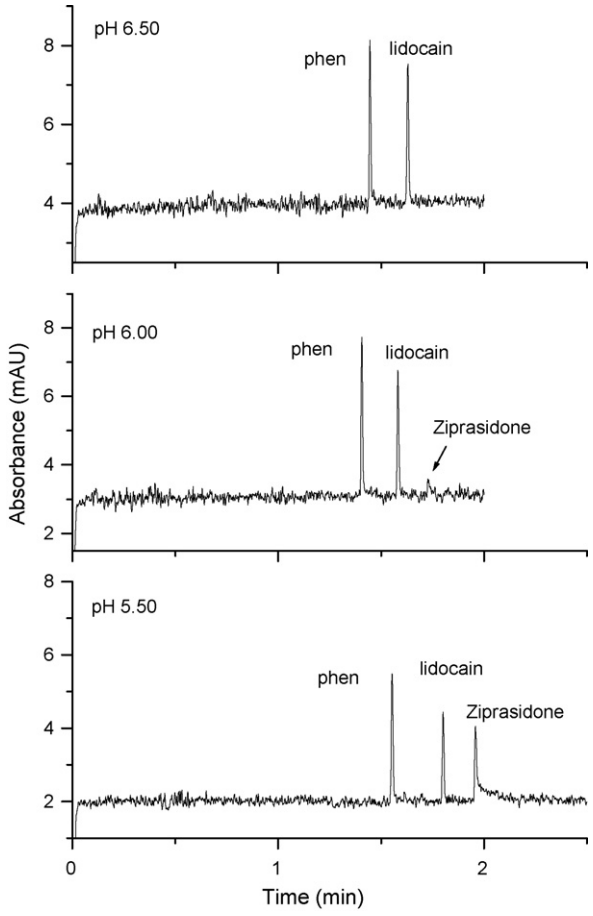

Fig. 3. Electropherograms of Ziprasidone, lidocain and phenethylamine (phen) at different pH of the BGE. Experimental conditions—uncoated fused-silica capillary: 48.5 cm total length; 40.0 cm effective length; 50 μm i.d.; voltage: 25 kV; *T*: 25 °C; Inj.: 120 mbar·s; detection at 214 nm. Concentrations: Ziprasidone, 30.00 μg ml^{-1}; lidocain, 90.00 μg ml^{-1}.

used in CE. The exact pK_a value of the piperazine nitrogen connected to the benzisothiazol system is not available, but the most basic nitrogen is certainly that of the piperazine connected to the aliphatic chain. This tertiary amino group has a calculated pK_a of 8.41 ± 0.50 (according to Advanced Chemistry Development – ACD/Labs – Software V8.19 for Solaris (© 1994–2007 ACD/Labs); data taken from Scifinder Scholar). It is thus evident that at intermediate or lower pH the drug is protonated and thus positively charged, and will migrate electrophoretically under the influence of an electric field. We have measured the effective mobility of the analyte as function of the pH and obtained the data depicted in Fig. 2. This plot includes the mobility dependence of lidocain used as IS, and of phenethylamine which turned out to possess an electrophoretic behaviour much better predictable than the more complex one of the drug insofar as it is better soluble in water (see below). Moreover, it had a much lower tendency to wall adsorption, which could be the cause of deviations of the mobility from the expected one [20]. Indeed it can be seen that the mobility vs. pH curve for phenethylamine follows the prediction in accordance to the Henderson–Hasselbalch relation adapted to the electrophoretic mobility. For this curve we take an actual mobility of 33 Ti (a *Tiselius* mobility unit has the dimension of 10^{-9} m^2 V^{-1} s^{-1}) and a pK_a of 9.8 [21]. Small deviations from this curve are explainable by the different ionic strength of the buffers at the particular pH values.

The measurement of the mobility of Ziprasidone (and lidocain) resulted in the unfeasibility to record the Ziprasidone peak at pH values above 6. This can clearly be seen from the electropherograms measured in this critical pH region (between 5.5 and 6.5) as shown in Fig. 3. At these pH values not only phenethylamine, but also lidocain gives a symmetrical peak. The peak of Ziprasidone, in contrast, exhibits a severe tailing at pH 5.5, at pH 6.0 its size is strongly reduced, and at pH 6.5 no peak can be detected anymore. We interpret this behaviour as caused by the varying solubility of Ziprasidone in the aqueous BGE, depending on the pH. At low pH the drug is present in its ionic form, which is sufficiently soluble for detection. The plot of the calculated molar solubilities of Ziprasidone as function of the pH (according to Advanced Chemistry Development – ACD/Labs – Software V8.19 for Solaris (© 1994–2007 ACD/Labs); data taken from Scifinder Scholar) is depicted as insert of Fig. 2. It can be seen that with increasing pH the solubility is strongly reduced, the consequence of its loss of the electric charge. A steep decrease in solubility is seen up to a pH around 5, where the solubility is only 4×10^{-3} M. At pH 6.5 the solubility falls off by one-order of magnitude to the 10^{-4} M range and decreases further with increasing pH.

We relate the irregular electrophoretic behaviour of Ziprasidone to the change in solubility in this critical pH range. The tailing of the Ziprasidone peak at pH 5.5 is then not caused by wall adsorption, but by the mutual conversion of the dissolved and the not soluble form of the drug in the electrokinetically driven sample zone [22]. At pH 6 the low solubility of the drug causes the drastic reduction of the peak size; note that this small peak does not remarkably tail, which indicates the

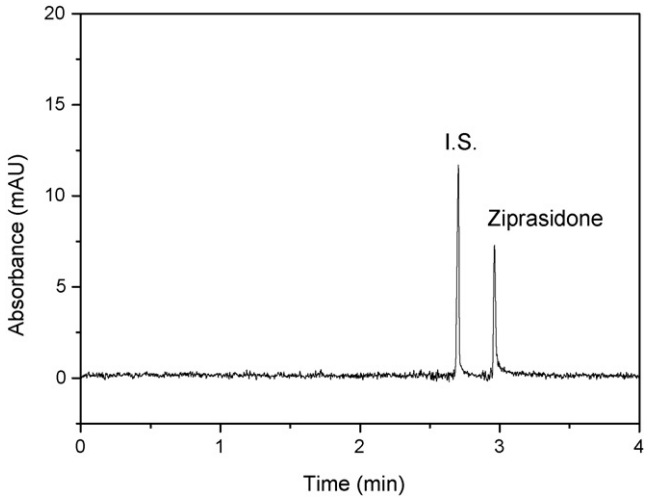

Fig. 4. Electropherogram of Ziprasidone and lidocain (IS). Conditions—BGE: formate buffer; pH 3.0; total concentration 50 mM. CE conditions as in Fig. 3. Concentrations: Ziprasidone, 30.00 μg ml^{-1}; lidocain, 90.00 μg ml^{-1}.

absence of wall adsorption. At pH 6.5 the solubility is finally lower than needed for detection. This behaviour of Ziprasidone has another cause than the irregular behaviour we have observed with Clomipramine, a basic tricyclic antidepressant, and its metabolites. These compounds also showed strong peak distortion at higher pH (phenethylamine also exhibited a regular peak shape under these conditions) [20]. We could relate this distortion to the adsorption of the cationic drug at the negatively charged surface of the silica wall. It leads to an increased tailing of the electrophoretic peak with increasing pH of the BGE, but to a concomitant reduction of the electrophoretic mobility due to the adsorptive retention of the analyte; this differs from the effect observed for Ziprasidone.

Lidocain shows a similar effect as Ziprasidone (reduction of peak size), whereas at higher pH. This difference can be explained by the better solubility of lidocain, as it consists of only one aromatic ring and has no Cl atom in its molecule.

It follows that a relatively low pH is favourable for the analysis of Ziprasidone; therefore pH 3.0 was chosen. The resulting electropherogram is depicted in Fig. 4. The EOF directed towards the cathode, which is still present at this pH (its mobility is about 22 Ti), together with the electrophoretic mobility of about 18 Ti of the analyte results in an analysis time of about 3 min; this is acceptably short, even for routine analysis.

3.2. Method validation

The method was validated according to the International Conference for Harmonization (ICH) guidelines Q2A (http://www.ich.org). For this purpose the calibration curve and its linearity were determined, the reproducibility of the measurements was characterised by the inter-day and intra-day precision, and the accuracy was measured according to recovery determinations; specificity and robustness of the developed method were determined as well. All these parameters are described in detail in the following.

3.2.1. Stability of solutions

BGE and drug solutions stability under refrigerated storage condition at 4 °C for a period of 1 month was evaluated. The measured concentrations of freshly made solutions and those tested after 1 month period varied only within 0.6%.

3.2.2. Specificity

The specificity of the method was proved by adding a known amount of standard drug to the capsule placebo. This placebo sample was prepared (without drug) by mixing the capsule excipients (without the materials the capsule shell is composed of): magnesium stearate, maize starch, and lactose. To this mixture the drug was added in proportions according to the composition of the formulations. Both samples were extracted as described and the electropherograms recorded. They are shown in Fig. 5A. It can be seen that no peak is detected in the migration range of the analyte and the IS in the placebo. Moreover, the sample of the excipients spiked with the drug gives an elec-

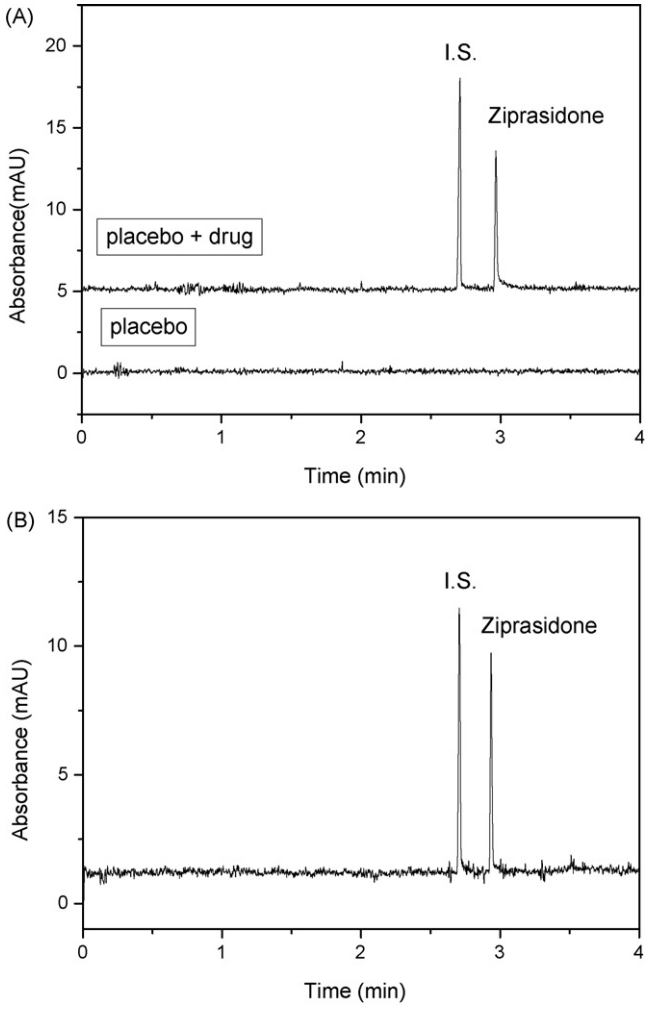

Fig. 5. Electropherograms of Ziprasidone and IS. (A) From extracts of placebo and from excipients with drug added (Concentrations: Ziprasidone, 30.00 μg ml^{-1}; lidocain, 90.00 μg ml^{-1}). (B) From a commercial formulation (sample 4 in Table 4). BGE: formate buffer; pH 3.0; total concentration 50 mM. CE conditions as in Fig. 3.

tropherogram which is identical with that of the pure standards (compare Fig. 5A with Fig. 4).

3.2.3. Calibration curve and linearity

The calibration line was measured in the concentration range of 10.00–100.0 μg ml^{-1} Ziprasidone with the IS at a constant concentration of 90.00 μg ml^{-1}. The peak area of the drug was determined at five different concentration levels with three measurements each, and was related to that of the IS. The calibration line was expressed by the equation $y = ax + d$, where y is the dimensionless ratio of the area of drug to internal standard (the relative peak area, RPA), and x is the drug concentration in μg ml^{-1}. The calibration line was calculated based on the mean values of the RPAs at 10.00, 30.00, 50.00, 75.00 and 100.0 μg drug per ml. As a result of linear regression analysis the slope, a, of the curve – the sensitivity of the method – is 0.02438 ml μg^{-1}, with an S.D. of 0.00049 ml μg^{-1}. The intercept, d, is −0.075 (S.D. 0.030). The linearity of the curve, given by the linear correlation coefficient, r, is 0.9994.

3.2.4. Precision

The repeatability (the intra-day precision) was determined by measuring the RPAs with five injections each of three sample solutions in the low, middle, and high linearity range with a constant amount of IS. Repeatability is expressed by the relative standard deviation (R.S.D.%). The R.S.D.% values for the migration time and peak area ratios are given in Table 1; they are between 0.8% and 2%, which is below 3% as demanded by the International Guidelines. Migration times were reproducible within less than 0.4%, which is a satisfying value taking into account the presence of the EOF and its potential variation.

The intermediate precision (the inter-day precision) was determined at concentrations at the low, middle, and high linearity range at seven consecutive days (with five consecutive measurements each). The according R.S.D.% values are given in Table 1 as well. The RPAs vary between 1.1% and 1.7%, which is in the same range as the intra-day data. The same holds for the migration time reproducibility.

3.2.5. Accuracy

The accuracy of the method was determined from the recovery upon addition of drug standard at 50%, 100%, and 150% of the nominal concentration to the pharmaceutical preparation. In detail we have taken an aliquot of a capsule corresponding to a concentration of 40.00 μg ml^{-1} Ziprasidone, and added 20.00, 40.00 and 60.00 μg ml^{-1}, respectively, of drug standard.

Table 2
Accuracy of the method determined by the recovery upon addition of drug standard solutions at 50%, 100%, and 150% to a capsule ($n = 5$)

Concentration added (μg ml^{-1})	Recovery (%)	R.S.D. (%)
20.00	99.8	2.1
40.00	99.9	1.2
60.00	99.8	0.42

Ziprasidone content of the capsule was 40.00 μg ml^{-1}.

Table 3
Robustness of the method for the quantitation of Ziprasidone: dependence of the peak area ratio on variations of pH and concentration (in mM) of the BGE

pH	RPA	S.D.
2.8	1.087	0.014
2.9	1.084	0.019
3.0	1.084	0.012
3.1	1.094	0.030
3.2	1.083	0.005
Concentration		
45	1.084	0.012
50	1.085	0.005
55	1.084	0.003

Peak area ratios are mean values from four measurements each.

For each concentration level the recovery was derived from five replicates. The resulting yields are given in Table 2. It can be seen that the accuracy is 99.8% or better at each concentration level, with a relative standard deviation between 0.42% and 2.1%.

3.2.6. Quantitation limits: LOQ and LOD

The LOD and the LOQ were determined from the height of the peak of Ziprasidone related to the multiple, z, of the standard deviation, σ_0, of the noise of the background signal according to $z\sigma_0/a$, where a is the sensitivity of the method as described in 3.2.3. The LOD is for $z = 3.3$, the LOQ for $z = 10$. For the present method the LOD is 1.6 μg ml^{-1}, the LOQ is 5.0 μg ml^{-1}.

3.2.7. Robustness of the method

The robustness of the analytical conditions was investigated by variations of the pH around the value (3.0) at which CE analyses were carried out. Therefore, four pH values were selected, two below and two above the chosen one. For the same reason the concentration of the BGE was varied between 45 and 55 mM (Table 3). Measurements were carried out in quadruplicate. The RPAs (1.084 at pH 3.0 and 45 mM BGE concentration) vary

Table 1
Repeatability (intra-day precision) and reproducibility (inter-day precision) of relative peak area, RPA (related to the IS) and migration time of Ziprasidone, expressed by the relative standard deviation (R.S.D.%)

Ziprasidone concentration (μg ml^{-1})	Intra-day R.S.D. (%) ($n = 5$)		Inter-day R.S.D. (%) ($n = 7$)	
	RPA	Time	RPA	Time
30.00	2.0	0.39	1.7	0.23
50.00	1.1	0.10	1.1	0.18
100.0	0.82	0.08	1.4	0.10

IS was added to the samples at a constant concentration of 90.00 μg ml^{-1}.

Table 4
Results of the determination of Ziprasidone in commercial pharmaceutical formulations

Sample	Dosage form	Labelled claim (mg)	Amount found (mg)	Recovery (%)	R.S.D. (%) ($n=5$)
1	Capsule	20.00	19.4	96.7	1.9
2	Capsule	20.00	20.0	99.8	1.4
3	Capsule	20.00	20.0	100.2	2.0
4	Capsule	20.00	19.8	99.1	1.8
5	Capsule	20.00	20.0	100.2	1.8
6	Capsule	40.00	39.8	99.4	1.6
7	Capsule	40.00	39.3	98.4	0.96

between 1.083 and 1.094 (S.D. between 0.005 and 0.030) for the pH variation and between 1.084 and 1.085 (S.D. 0.003–0.012) for varying concentration; this is within the repeatability of the method.

3.3. Analysis of dosage forms

Seven different commercial formulations were analysed (all were capsules). They were treated according to the International Guidelines, and aliquots of their powdered content were subjected to analysis as described. A typical electropherogram is shown in Fig. 5B. The peak of Ziprasidone and of the internal standard is not interfered by any of the matrix compounds of the formulations. The quantitative results of the determination of Ziprasidone are given in Table 4. It can be seen that in all cases between 96.7 and 100.2 of the nominal content was found, with a R.S.D.% of about 2% for $n=5$. This means that the variation of the content in all preparations is in accordance to the requirements of the European and American Pharmacopoeia.

4. Conclusions

A method has been developed for the determination of Ziprasidone in pharmaceutical formulations based on CZE. Electrophoretic migration of the basic analyte at intermediate pH of the BGE is hampered by the low solubility of the free drug, which differs by orders of magnitude from the solubility of the cationic form at low pH. For this reason at pH higher than 6 the analyte cannot be recorded in the electropherogram. At a pH of 3.0 of the BGE the peak shape of the analyte is symmetrical, the migration times are reproducible, and the high specificity of the method results in no interferences with excipients of the dosage forms. For sample pre-treatment a simple extraction step with methanol is sufficient to enable quantitative recovery of the drug. All parameters of method validation are fulfilled according to the International Guidelines.

Acknowledgement

The Authors thank the Faculty of Pharmacy, University of Bologna, for the financial support for C. F.

References

[1] E. Richelson, T. Souder, Life Sci. 68 (2000) 29–38.
[2] K.D. Wilner, T.G. Tensfeldt, B. Baris, T.A. Smolarek, R.Z. Turncliff, W.A. Colburn, R.A. Hansen, Br. J. Clin. Pharm. 49 (2000) 15S–20S.
[3] K.D. Wilner, S.B. Demattos, R.J. Anziano, G. Apseloff, N. Gerber, Br. J. Clin. Pharm. 49 (2000) 43S–47S.
[4] D.G. Daniel, D.L. Zimbroff, S.G. Potkin, K.R. Reeves, E.P. Harrigan, M. Lakshminarayanan, Neuropsychopharmacology 20 (1999) 491–505.
[5] M.A. Raggi, R. Mandrioli, C. Sabbioni, V. Pucci, Curr. Med. Chem. 11 (2004) 279–296.
[6] G. Srinubabu, B.S. Rani, J.V.L.N.S. Rao, E-J.Chem. 3 (2006) 9.
[7] J.S. Janiszewski, H.G. Fouda, R.O. Cole, J. Chromatogr. B 668 (1995) 133–139.
[8] J. Janiszewski, P. Schneider, K. Hoffmaster, M. Swyden, D. Wells, H. Fouda, Rapid Commun. Mass Spectrom. 11 (1997) 1033–1037.
[9] N. Li, S. Shen, Y. Zhong, Z. Wang, Y. Yu, Fudan Xuebao, Yixueban 31 (2004) 656.
[10] N. Li, Y. Yu, S. Shen, Fenxi Huaxue 32 (2004) 916.
[11] B.S. Rani, P.V. Reddy, E-J. Chem. 3 (2006) 169.
[12] Z.A. El-Sherif, B. El-Zeany, O.M. El-Houssini, M.S. Rashed, H.Y. Aboul-Enein, Biomed. Chromatogr. 18 (2004) 143–149.
[13] R.F. Suckow, M. Fein, C.U. Correll, T.B. Cooper, J. Chromatogr. B 799 (2004) 201–208.
[14] J. Sachse, S. Hartter, C. Hiemke, Ther. Drug Monit. 27 (2005) 158–162.
[15] O.Y. Al-Dirbashi, H.Y. Aboul-Enein, A. Al-Odaib, M. Jacob, M.S. Rashed, Biomed. Chromatogr. 20 (2006) 365–368.
[16] H. Kirchherr, W.N. Kuhn-Velten, J. Chromatogr. B 843 (2006) 100–113.
[17] M. Aravagiri, S.R. Marder, B. Pollock, J. Chromatogr. B 847 (2007) 237–244.
[18] G. Zhang, A.V. Terry Jr., M.G. Bartlett, J. Chromatogr. B 856 (2007) 20–28.
[19] G. Zhang, A.V. Terry Jr., M.G. Bartlett, Rapid Commun. Mass Spectrom. 21 (2007) 920–928.
[20] R. Martello, V. Kolivoška, M.A. Raggi, E. Kenndler, Electrophoresis 28 (2007) 3650.
[21] A. Albert, E.P. Serjeant, The Determination of Ionization Constants—A Laboratory Manual, Chapman and Hall, London, 1984.
[22] R. Vespalec, M. Vlckova, V. Kuban, Electrophoresis 26 (2005) 3265.