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Development and validation of a capillary electrophoresis method for determining zolmitriptan enantiomers

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ABSTRACT

A cyclodextrin mediated capillary electrophoresis (CE) methods for determining the enantiomeric purity of Zolmitriptan in the presence of its potential process related impurities was developed with a systematic method development approach. The separation between Zolmitriptan enantiomers and potential impurities could be obtained in two background electrolyte (BGE) systems containing hydroxypropyl beta-cyclodextrin (HP-β-CD) and sulfobutyl ether-beta-cyclodextrin (SB-β-CD) respectively. The CE method containing SB-β-CD in the BGE system was validated as it provided higher efficiency and resolution between the enantiomers. The separation was carried out at 25°C in a 72 cm x 50 µm id bare fused silica "Extended light path" capillary with an applied voltage of 30kV, polarity set to negative. The background electrolyte (BGE) consisted of citrate buffer (pH 4.6, 50 mM) containing 20 mM sulfobutyl ether-beta-cyclodextrin (SB-β-CD) and the peaks were detected at 225 nm. The method was found to be specific, accurate and precise. The CE method can be conveniently applied for the analysis of bulk and formulation samples of Zolmitriptan.

KEYWORDS

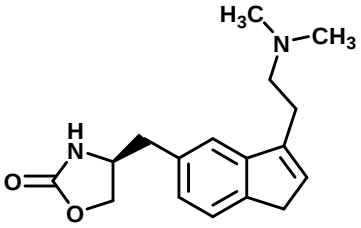
Zolmitriptan;
Chiral separation;
Capillary electrophoresis;
Cyclodextrin;
Bulk drug;
Formulation.

INTRODUCTION

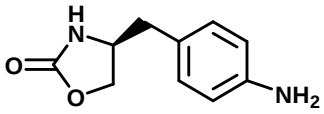
About 40% of the drugs in use are known to be chiral and it is well established that the pharmacological activity is mostly restricted to one of the enantiomers. In several cases unwanted side effects or even toxic effects can occur with the second enantiomers[1]. A good robust and accurate method for determining the enantiomeric purity of chiral drugs is therefore important. During the past several years, it has been demonstrated that capillary electrophoresis (CE) is a powerful technique for chiral separation[2,3] and in most cases, cyclodextrins (CDs) are used as chiral selectors.

Zolmitriptan (Zomig), a single enantiomer (4S)-4-[3-[2-dimethyl aminoethyl]-1*H*-5-indolyl-methyl]-1,3-oxazolan-2-one is a novel serotonin 5-hydroxytryptamine receptor agonist (Figure 1). This drug is highly effective in the acute oral treatment of migraine with or without aura[4,5]. It works by stimulating serotonin receptors in the brain. Serotonin is a natural substance in the brain that, among other things, causes blood vessels in the brain to narrow. Zolmitriptan mim-

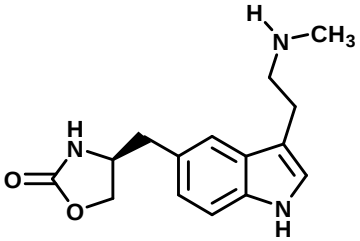
ics this action of serotonin by directly stimulating the serotonin receptors in the brain and it relieves the pain of migraines. In our earlier work we have reported the analysis of enantiomers of Zolmitriptan and its related impurities by HPLC[6]. Some methods for the quantification of Zolmitritpan in plasma samples by coulometric detection, liquid chromatography/tandem mass spectrometry and liquid chromatography with fluorescence detection have also been reported in literature[7-9].



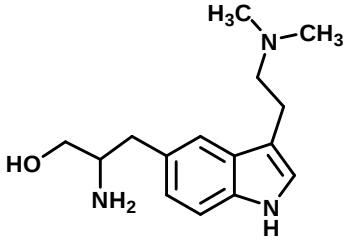
4(S)-4-[3-(2-Dimethyl aminoethyl)-1H-5-indolyl-methyl]-1,3-oxazolan-2-one (Zolmitriptan)



4(S)-4-(4-Aminobenzyl)-1,3-oxazolan-2-one (Imp-1)



4(S)-4-[3-(2-Methyl aminoethyl)-1H-5-indolyl-methyl]-1,3-oxazolan-2-one (Imp-2)



2(S)-2-Amino-3-[3-(2-dimethyl aminoethyl)-1H-5-indolyl]-propan-1-ol (Imp-3)

Figure 1 : Molecular structure of zolmitriptan, imp-1, imp-2 and imp-3.

There have been two reported methods of chiral purity analysis of Zolmitriptan using capillary electrophoresis as the separation technique[10,11]. In another article a study of the interaction of Zolmitriptan enantiomers with HP-β-CD by capillary electrophoresis[12] was reported. However this study was aimed towards estimating binding constants and the influence of pH on enantio-resolution was evaluated in a limited pH range of 2.0 - 4.5 using phosphate buffer. We have systematically explored the separation effects using different cylcodextrin systems in a wider pH range from pH 2.5 to pH 9.3 and found that the highest efficiency and resolution were achieved at a pH of 4.6 in citrate buffer. To the best of our knowledge there has been no literature reported for the determination of Zolmitriptan enantiomers in the presence of its potential process related impurities. In this paper, we describe a simple and convenient procedure for determining Zolmitriptan enantiomers in the presence of potential process related impurities and this method can be conveniently applied to bulk drugs and pharmaceutical formulations.

EXPERIMENTAL

Reagent and materials

Zolmig tablets (5 mg) were procured from Astra Zeneca, UK. Zolmitriptan enantiomers, bulk samples of Zolmitriptan API and the process related impurities were kindly gifted by Bulk Actives, Dr. Reddy's Laboratories Ltd., India. SB-beta-CD was purchased from Advasep® 4, Cydex Inc USA. 1.0 N and 0.1N sodium hydroxide (NaOH), 0.1 N phosphoric acid and HPCE Water, Heptakis (2,6-di-O-methyl)-beta-cyclodextrin (DM-β-CD) and Heptakis (2,3,6-tri-O-methyl)-beta-cyclodextrin (TM-β-CD), (2-Hydroxypropyl)-beta-cyclodextrin (HP-β-CD) and Gamma-cyclodextrin (γ-CD) were obtained from Agilent Technologies, Germany. Citric acid was procured from Aldrich, USA. 4-Aminobenzoic acid was obtained from Merck, Germany. Deoxycholic acid sodium salt (SDC) and Taurodeoxycholic acid sodium salt (STDC) Fluka, Germany. Acetonitrile (ACN), Methanol (MeOH), isopropyl alcohol (IPA) and etha-

nol (EtOH) were procured from Rankem, India.

Equipment and capillary electrophoretic conditions

Separations were performed on Agilent Capillary Electrophoresis system (Agilent Technologies, Germany) using a 72 cm x 50-µm id, bare fused silica capillary with extended light path (Agilent Technologies, Germany). A voltage of 30 kV with negative polarity was applied and the capillary temperature was maintained at 25°C. The peaks were monitored at 225 nm using a diode array UV detector. Samples were injected hydrodynamically by pressure at 50 mbars for 3 s followed by injection of a water plug by pressure at 50 mbars for 2 s.

Each new capillary was conditioned sequentially with 1.0 N NaOH (10 mins), 0.1N NaOH (10 mins) and HPCE water (10 mins) Prior to every use the capillary was conditioned with 0.1 N phosphoric acid (15 mins), HPCE water (2 mins) followed by BGE (20 mins). Between runs the capillary was conditioned with BGE for 3 mins. The BGE consisted of citrate buffer solution (50 mM) at pH 4.6 containing 20 mM SB-beta-CD in all the studies unless otherwise mentioned. All solutions were filtered through 0.2 µm nylon syringe filters.

PREPARATION OF SOLUTIONS

Preparation of standard solutions

The (R)-Zolmitriptan stock solution was prepared by dissolving 7.5±0.1 mg of (R)-Zolmitriptan standard in 25 mL diluent (10 mM citrate buffer, pH 4.6). Similarly a stock solution of the internal standard was obtained by dissolving 5.0±0.1 mg of imidazole in 25 mL diluent. About 200 mg of the standard sample was accurately weighed into a 100 mL volumetric flask and 1.0 mL each of the (R)-Zolmitriptan stock solution and internal standard stock solution were added to the same flask. The volume was made up to 100 mL mark with the diluent and sonicated to dissolve.

Preparation of sample solution

About 200 mg of the sample was accurately weighed into a 100 mL volumetric flask and 1.0 mL of the internal standard stock solution was added to the same flask. The volume was made up to 100 mL mark with the diluent and sonicated to dissolve.

METHOD VALIDATION

Specificity

Specificity was evaluated by injecting Zolmitriptan enantiomers individually and spiked with all known process related impurities (Imp-1, Imp-2 and Imp-3) at specification level. The electropherograms were examined for interferences of other analytes with the enantiomers of Zolmitriptan.

Precision

The repeatability of the method was evaluated by injecting replicate preparations (n = 6) of a spiked solution of (S)-Zolmitriptan sample containing the internal standard and (R)-Zolmitriptan spiked at LOQ at 0.15% levels. To evaluate intermediate precision, six replicate preparations of (S)-Zolmitriptan sample containing the internal standard and (R)-Zolmitriptan were prepared and injected every day, on three different days. The %RSD for migration times, peak area and peak area ratios were evaluated.

Sensitivity

Sensitivity of the method was determined by establishing the limit of detection (LOD) and limit of quantitation (LOQ) for (R)-Zolmitriptan. The detector response was obtained for a series of dilute solutions with known concentrations of (R)-Zolmitriptan. Concentrations resulting in signal-to-noise ratios of about 3:1 and 10:1 were considered as detection limits and quantitation limits respectively.

Linearity and range

The linearity solutions were prepared from stock solution (R)-Zolmitriptan at six concentration levels – LOQ to 1.5% of analyte concentration, each in triplicate. The data was subjected to linear regression analysis with the least squares method.

Accuracy

Samples of Zomitriptan were spiked with (R)-Zolmitriptan at LOQ, 80, 100 and 120% of the nominal analyte concentration. The spiking was performed in triplicate at each level and the spiked samples were analyzed as per the method. Recoveries for (R)-Zolmitriptan were calculated against freshly prepared standard.

RESULTS AND DISCUSSION

Method development and optimization

(a) Effect of buffer pH and ionic strength

Selecting buffer pH is a key strategy for optimizing the separation of ionizable analytes in CE because buffer pH determines the extent of the ionization of each analyte and the magnitude of the electroosmotic flow. The effect of buffer pH on enantioseparation and peak symmetry was investigated using various buffers at constant molar strength.

TABLE 1 shows the affects of pH on resolution (Rs), selectivity (α) and efficiency (N) with the use of SB-β-CD and HP-β-CD as chiral selectors. Zolmitriptan has a pKa of 9.6 and is ionized completely at all experimental pH values except at pH 9.3. At pH 9.3 (50 mM borate buffer) the enantiomers failed to resolve and only a single peak could be obtained for the racemate. This can be attributed to the unionized nature of Zolmitriptan at higher pH and thus failing to be discriminated by the chiral selectors. The resolution was maximum at pH 4.6 with the use of either SB-β-CD or HP-β-CD and therefore the pH of running electrolyte was optimized to 4.6 using citrate buffer.

TABLE 1 : Optimization of buffer pH and cyclodextrin

Cyclodextrin (20 mM)	Parameters	Buffer (50mM) pH 2.5 (Phosphate)	pH 4.6 (Citrate)	pH 7.0 (Phosphate)	pH 9.3 (Borate)
SB-β-CD	R_s	1.94	2.77	1.73	No resolution
	α	1.02	1.02	1.01	
	N*	192853	224333	65527	
HP-β-CD	R_s	1.80	1.96	1.29	No resolution
	α	1.05	1.05	1.01	
	N*	25817	25916	145358	

The buffer concentration was varied to study the affects on resolution. No significant change in migration time or resolution was observed when the separation was performed at 10, 30, 50 and 100 mM citrate buffer. As higher buffer concentrations can lead to generation of higher current which result in joule heating, the buffer concentration was maintained at 50 mM for further studies.

(b) Effect of chiral selector type and concentration

A variety of cyclodextrins like γ-CD, DM-β-CD, TM-β-CD, HP-β-CD, SB-β-CD and chiral surfactants SDC and STDC were investigated to exploit the enantioselectivity of Zolmitriptan. The enantioresolution was achieved with both SB-β-CD and HP-β-CD whereas other chiral selectors could not provide any separation. Attempts were made to improve the resolution by using combination of cyclodextrins. Two chiral selectors in their mixture may interact at two different levels – at first at the level of molecular recognition and secondly at the level of transformation of the enantioselectivity of their 'independent' molecular recognition in an overall mobility difference between enantiomers[13]. But the use of cyclodextrin mixtures could not provide any enhanced resolution when compared to the individual cyclodextrins itself. Further the chiral selectivity of SB-β-CD was found to be better than HP-β-CD as shown in TABLE 1. The highest efficiency in separation was achieved with SB-β-CD at pH 4.6 using citrate buffer.

Figure 3a and Figure 3b show the dependence of migration time and resolution on SB-β-CD concentration. The resolution was found to increase with increase in concentration from 5 mM to 20 mM and then decrease at higher concentrations. The cyclodextrin concentration was therefore optimized at 20 mM.

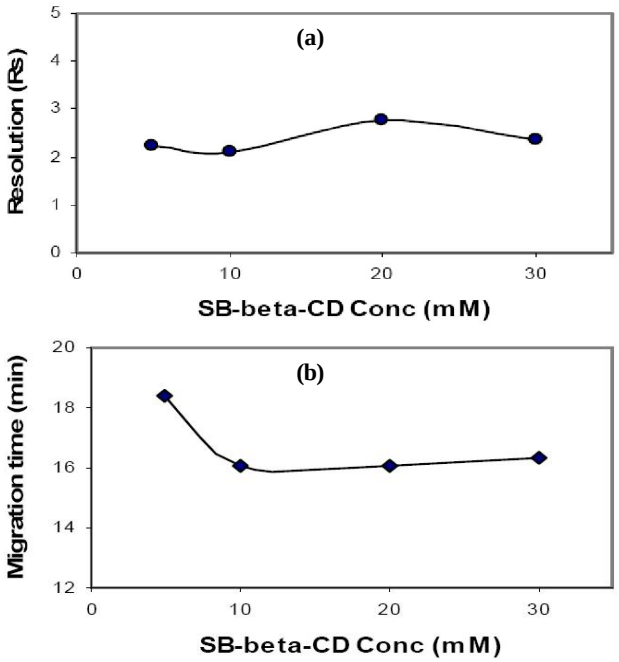


Figure 3 : Effect of (a) SB-β-CD concentration on resolution and (b) SB-β-CD concentration on migration time

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(c) Effect of organic additives

The addition of an organic additive to the buffer affects several variables, including enantioselectivity, viscosity, dielectric constant and zeta potential. Acetonitrile, methanol, isopropyl alcohol and ethanol were added to the BGE at 10% (v/v) concentration. The addition of organic solvents reduces the binding capacity of the analyte within the cyclodextrin cavity due to the less polarity of the solvent compared to the buffer electrolyte[14]. The formation of cyclodextrin analyte complex becomes less favored unless the working cyclodextrin concentrations are much higher than the optimum concentrations. Migration times were found to increase and the resolution decreased with the introduction of organic solvents (Figure 4a and Figure 4b). The decrease in resolution indicates that the trials were attempted at the optimum concentration of the cyclodextrin and the addition of the organic solvents only decreased the equilibrium constants for the two enantiomers. Therefore no organic solvent was included in the optimized method.

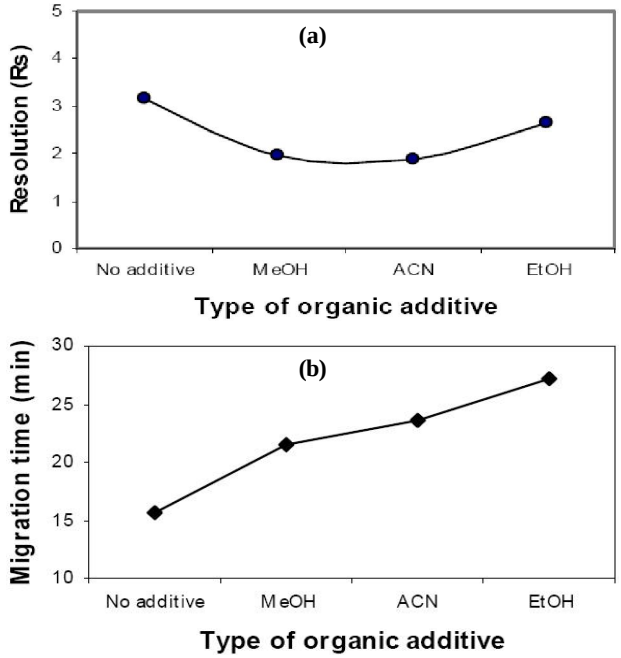

Figure 4 : Effect of (a) Organic additives on resolution and (b) Organic additives on migration time.

(d) Effect of temperature

There was a decrease in migration times with increase in temperature from 15 to 30 °C due to a decrease in viscosity of the BGE. But no significant change in the resolution was observed. As elevated temperature may cause zone spreading, sample decomposition or boiling buffer, the working temperature was optimized to 25 °C. A typical electropherogram of Zolmitriptan enantiomers spiked with its potential impurities and internal standard under optimized separation conditions is shown in Figure 2.

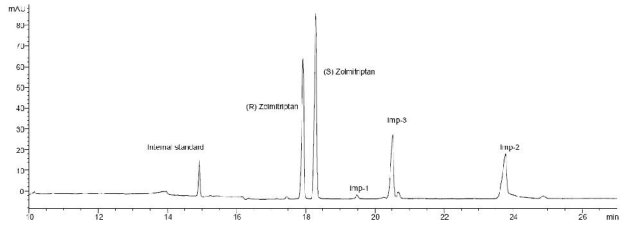

Figure 2a : Representative electropherogram of zolmitriptan enantiomers spiked with impurities and internal standard

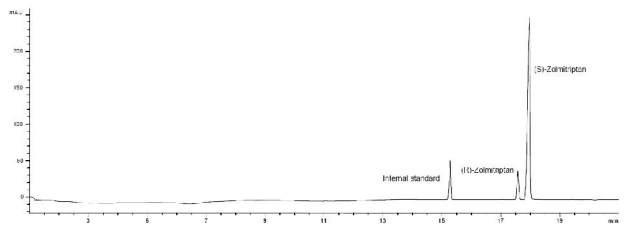

Figure 2b : Electropherogram of zolmitriptan sample spiked with internal standard and (R)-enantiomer

Method validation

(a) Precision, linearity and detection limits

Repeatability was determined by performing replicate injections (n=6) of a solution containing the unwanted isomer spiked at LOQ and 0.15% levels in standard drug using 4-Aminobenzoic acid as internal standard. The precision was improved with the use of internal standard[15]. Peak area ratios were obtained by dividing the peak area of analyte peak by the internal standard peak area. Intraday precision was assessed

TABLE 2 : Precision for (R)-zolmitriptan

Parameter	Migration time (%RSD)	Peak Area (%RSD)	Peak Area Ratio (%RSD)
Repeatability at LOQ level (n=6)*	3.61	8.89	2.14
Repeatability at 0.15% level (n=6)**	3.15	3.23	0.80
Intra-day precision at 0.15% level**	1.53	2.12	1.05
Inter-day precision at 0.15% level**	3.85	5.57	1.68

***Sample concentration: 2.0 mg/mL sample spiked with 1.0 µg/mL of (R)-zolmitriptan and 2.0 µg/mL of internal standard**
****Sample concentration:2.0 mg/mL sample spiked with 3.0 µg/mL of (R)-zolmitriptan and 2.0 µg/mL of internal standard**

by injecting independently prepared solutions (n=6) at 0.15% concentration. To evaluate interday precision, each solution was injected six times every day, for three consecutive days. The results shown in TABLE 2 indicate that this method is precise and suitable for analyses of real samples.

Detector response linearity was assessed with six calibration solutions in the range of 1.0 µg/mL to 31.02 µg/mL (LOQ to 1.5% of analyte concentration). Each calibration solution was spiked with the internal standard and injected in triplicate. The straight line equation for (R)-Zolmitriptan was y=0.1023x-0.0294 with a coefficient of regression (R^2) of 0.9995. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 1.0 µg/mL and 0.3 µg/mL respectively.

(b) Sample analysis, recovery and stability

The optimized conditions were applied to determine the chiral purity of bulk drug samples and tablets. The results obtained show very good precision in quantification of (R)-Zolmitriptan. The results obtained by CE were compared with the results obtained using our previously reported Chiral HPLC method and both were found be in very good agreement (TABLE 3).

TABLE 3 : Chiral analysis of zolmitriptan bulk drug and formulation batches

Sample	Batch	% (R)-Zolmitriptan*	
		By CE	By HPLC
Bulk Drug	I	0.07±0.002	0.06±0.004
	II	0.06±0.005	0.06±0.002
	III	0.12±0.002	0.11±0.001
Formulation	I	0.05±0.001	0.05±0.005
	II	0.04±0.003	0.03±0.001

***n= 3 determinations**

Recovery experiments were performed by spiking the standard drug with the (R)-Zolmitriptan (distomer) at LOQ, 80, 100 and 120% of specification concentration. The percentage recoveries were 95.0% at LOQ and ranged from to 99.7 to 101.3 % for (R)-Zolmitriptan at other concentrations (TABLE 4). There was no change in the enantiomeric purity when the samples were reanalyzed after 24 hours confirming that the sample solutions were stable at bench top for at least 24 hours.

TABLE 4 : Accuracy data for zolmitriptan

Accuracy Level	Amount spiked* (ng/mL)	Amount recovered* (ng/mL)	Percentage recovery
LOQ	1012	961	95.0
80%	2443	2448	100.2
100%	3008	2999	99.7
120%	3622	3669	101.3

CONCLUSION

A CE was developed for determining the chiral purity of Zolmitriptan in the presence of its potential process related impurities. The effect of various parameters like the pH of running electrolyte, the type and concentration of cyclodextrin, type of organic modifier and temperature were studied during method optimization process and the finest separation conditions were selected. Analysis results were found be in good agreement with the results obtained by using our previously reported chiral HPLC method. This method is specific, precise and accurate and is suitable for performing analysis of bulk drug and formulations of Zolmitriptan.

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