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Research Article

Optimisation and validation of a new CE method for the determination of lansoprazole enantiomers in pharmaceuticals

An analytical method based on CZE to determine lansoprazole enantiomers in pharmaceuticals was developed. The primary factors affecting its separation efficiency, which include chiral selector, pH, buffer concentration, capillary temperature and injection time, were optimised. The best results were obtained by using a background electrolyte consisting of 50 mM phosphate adjusted to pH 2.2, 12 mM β-CD and 5 mM sodium sulphite, in combination with hydrodynamic injection and a 15 kV separation voltage. Detection limits were calculated from baseline noise and found to be 0.64 mg L^{-1} for the *R* enantiomer and 0.72 mg L^{-1} for the *S* enantiomer. The proposed method was used to analyse three different pharmaceutical preparations with recoveries of 91–102% of the label content.

Keywords:
CD / Chiral separation / CZE / Lansoprazole DOI 10.1002/elps.200800810

1 Introduction

Lansoprazole 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]-sulphinyl]-1H-benzimidazole is a proton pump inhibitor that suppresses gastric acid secretion through interaction with (H^+/K^+)-ATPase in gastric parietal cells and has proved effective in the treatment of duodenal and gastric disorders [1]. Available evidence suggests that lansoprazole may be an effective alternative to omeoprazol, particularly by virtue of its potential for faster healing and symptom resolution [2]. This drug, which contains an asymmetric sulphur atom in its chemical structure, is clinically administered as a racemic mixture of *R*-(+) and *S*-(−) enantiomers (Fig. 1).

Several HPLC methods for the determination of lansoprazole and lansoprazole metabolites in biological fluids and pharmaceutical preparations, using UV [3–8] or mass spectrometry detection [9, 10],] have been reported. Also, the HPLC technique has been used for the chiral separation of lansoprazole [11, 14]. CE has recently emerged as a powerful analytical tool for separating and quantifying a large variety of substances including pharmaceutical compounds [15–18]. The CE technique has some advantages over HPLC including the use of smaller amounts of sample and reagents, an increased separation efficiency, short analysis times and easy conditioning of column. These advantages have promoted its use for the determination of various basic drugs including lansoprazole [19–21].

CE has proved as an attractive choice for chiral separations in the presence of various additives such as crown ethers [22], CD [23–28], bile salts [29], antibiotics [30] and proteins [31]. These chiral selectors are added to the background electrolyte in order to facilitate separation by formation of diastereomeric complexes with chiral analytes under various dynamic equilibria [32–34]. Thus, lansoprazole enantiomers and related sulphoxides (omeprazole and pantoprazole) were successfully resolved by using CZE with bovine serum albumin as chiral selector [35]; enantiomeric resolution was feasible over a narrow pH range (7–8) only, and each analysis took *ca*. 17 min.

The aim of this work was to develop and validate a straightforward, expeditious CZE method for the separation and quantitation of lansoprazole enantiomers in pharmaceutical formulations, using CDs as chiral selectors. The factors most markedly affecting the separation efficiency, which included the type of CD used and its concentration, and the buffer concentration, capillary temperature, applied voltage and injection time, were all optimised. The ensuing method was validated by establishing its precision, linearity, accuracy, robustness and detection and quantitation limits, and successfully applied to various pharmaceuticals formulations.

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Abbreviation: CPA, corrected peak area

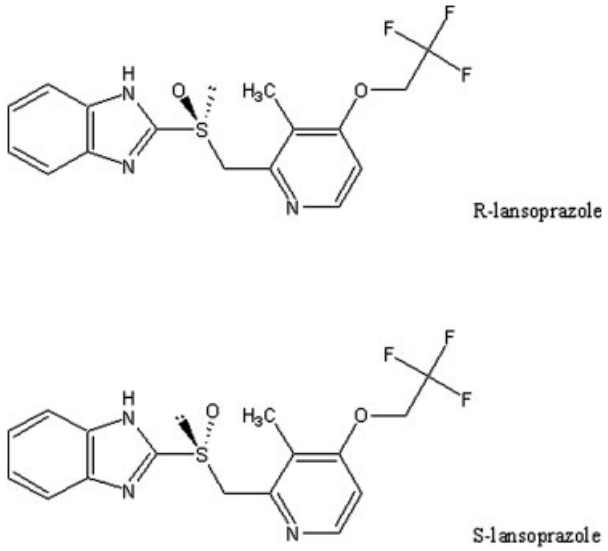

Figure 1. Structures of the lansoprazole enantiomers.

2 Materials and methods

2.1 Reagents

Lansoprazole was obtained from Sigma (Madrid, Spain). Standard solutions containing a 800 mg L^{-1} concentration of the pharmaceutical in ethanol were prepared and stored in the dark. Working strength standards in Milli-Q deionised water were prepared on a daily basis by dilution of the stock standard solutions. Electrophoretic separation was done by using a buffering solution consisting of 50 mM phosphate at pH 2.2, 12 mM β-CD and 5 mM sodium sulphide. The electrolyte solution was prepared fresh on a daily basis.

2.2 Apparatus

CE analyses were performed with a Beckman (Fullerton, CA, USA) P/ACE System MDQ equipped with a diode-array detector and controlled by Beckman CE software. Separations were done on a 31 cm long (21 cm to the detector) × 75 μm id fused-silica capillary housed in a cartridge with a 800 μm × 100 μm detector window.

HPLC analysis were performed in a Shimadzu (Tokyo, Japan) LC, model LC-10A, with a diode-array detector (model SPD-M10A). A Rheodyne (Cotati, CA, USA) Model 7725 injector with a 20 μL sample loop was used, and a Silicon 486/33 computer fitted with CLASS–LC 10 software was used for all the measures and data treatments. The analytical column was a CHIRAL-AGP (100 mm long, 4.0 mm id, particle size 5 μm).

2.3 Procedure

2.3.1 Sample preparation from commercial formulations

Following mixing and powdering of the contents of ten capsules of the studied pharmaceutical, an amount of solid corresponding to the weight of one capsule was dissolved in 100 mL of ethanol with sonication. The resulting solution was filtered by using a Swinnex polypropylene disc filter holder 13 mm in diameter furnished with a 0.5 mm FH Fluoropore (PTFE) membrane. This provided a solution containing 20 mg L^{-1} lansoprazole ready for CE analysis.

2.3.2 Operating conditions

CE: Before first use, the capillary was conditioned by rinsing with 0.1 M NaOH for 20 min, water for 10 min and separation electrolyte for 10 min. In order to avoid adsorption-related problems and ensure consistent EOF, the capillary was rinsed with 1:4 H_2O_2 for 2 min, water for 0.5 min, 0.1 M NaOH for 1 min and separation electrolyte for 2 min between successive injections. Vials were refrigerated at 16°C and separations done at the same temperature, using a voltage of 15 kV for 10 min. Electropherograms were monitored at 285 nm. All the samples were injected in triplicate and corrected peak areas (CPA) (area/migration time) used for quantitative analysis.

HPLC: Separation was achieved with a mobile phase containing 6% v/v 2-propanol in 1 mM sodium phosphate buffer pH 7.0. The flow-rate was 0.9 mL/min and the detection was performed at a wavelength of 283 nm.

3 Results and discussion

3.1 Optimisation of the separation conditions

The pH of the running buffer exhibited a strong effect on the ionisation of the studied compounds and the magnitude of EOF, through which it influenced both resolution and the migration time. Lansoprazole enantiomers and its complexes migrate to the CE cathode. A low pH can be used to reduce adsorption of cationic analytes on the fused-silica surface of the capillary; also, because EOF can be reduced by a low pH and it can, in theory, be more suitable for resolving lansoprazole isomers as it will allow the analytes to interact with CDs over longer periods. The type of solvent used and the buffer pH affect the stability of lansoprazole aqueous solutions, which can be degraded within only a few hours under especially unfavourable conditions. Thus, lansoprazole is unstable in acid media, which facilitate its oxidation by air; this led us to add sodium sulphide to the solution in order to avoid degradation of the analyte.

The effect of the buffer pH was examined over the range 1.8–4 by using a 40 mM phosphate buffer concentration, 10 mM β-CD as chiral selector and 5 mM sodium sulphide. By decreasing the buffer pH, a general decrease of EOF and a raise of migration times were observed. As can be seen from Fig. 2, the best resolution between peaks for the lansoprazole enantiomers was obtained at pH 2.2.

The effect of the phosphate concentration in the running buffer was examined over the range 20–80 mM at

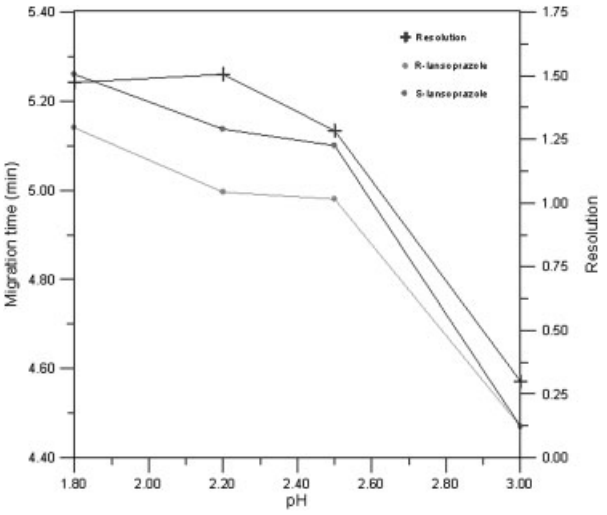

Figure 2. Effect of pH on the resolution of lansoprazole enantiomers with 40 mM phosphate buffer containing 10 mM β-CD and 5 mM sodium sulphide at 16°C. Applied voltage = 15 kV.

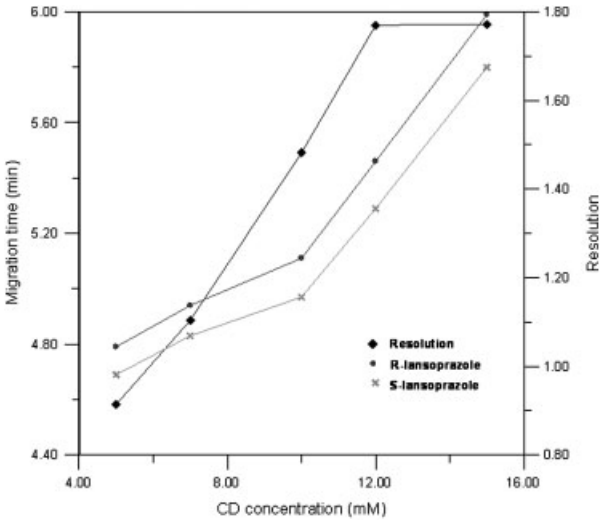

Figure 3. Effect of the CD concentration on the resolution of lansoprazole enantiomers with 50 mM phosphate buffer at pH 2.2 containing 5 mM sodium sulphide at 16°C. Applied voltage = 15 kV.

pH 2.2, using 10 mM β-CD and 5 mM sodium sulphide as in previous tests. Raising the phosphate concentration increased the migration time and resulted in improved resolution. A 50 mM buffer concentration was chosen in order to ensure good resolution, peak shape and efficiency.

CDs are the most widely used chiral selectors in CE, resolution increasing with increase in their concentration. In order to use the best possible chiral selector to quantify the analyte, various uncharged CDs including β-CD, methyl-β-CD, dimethyl-β-CD and hydroxypropyl-β-CD were compared. β-CD and methyl-β-CD were found to provide similar resolution and surpass dimethyl-β-CD and hydroxypropyl-β-CD in this respect. Based on these results, and on the low cost of β-CD relative to methyl-β-CD, we chose to use the former for the chiral separation of lansoprazole enantiomers.

After β-CD was selected as a chiral selector, the effect of its concentration was investigated. Increasing its concentration was found to result in increased resolution and migration times (Fig. 3). The best results were obtained with 12 mM β-CD. In any case, concentrations above 15 mM resulted in incomplete dissolution of the CD in water.

The addition of organic modifiers to the running buffer was considered on account of their effects on various properties including viscosity, dielectric constant, zeta potential, migration time, peak symmetry and resolution [25]. Tests with methanol and acetonitrile at concentrations over the 5–20% range revealed that neither additive was effective in increasing enantiomer resolution with lansoprazole.

The influence of the applied voltage was studied over the range 5–20 kV. Raising the voltage led to shorter analysis times and sharper peaks. However, increased voltages also resulted in higher currents, increased Joule heating and degraded resolution. As can be seen from Fig. 4, the maximum possible resolution was obtained at 15 kV.

Controlling the capillary temperature in CE is important in order to avoid unwanted changes in EOF, efficiency, viscosity, electrophoretic mobility and migration time. The effect of temperature was studied over the range 16–30°C. Increasing the capillary temperature resulted in decreased migration times and also in poorer resolution, probably by effect of a restricted solute – CD interaction. The temperature providing the best compromise between resolution and run time, 16°C, was adopted for further testing.

Finally, the sample injection time was also optimised. The injection time affects peak width and height. In order to improve sensitivity and decrease detection limits, samples were hydrodynamically injected at 0.5 psi for 3–9 s. Peak area was found to increase with increasing injection time; however, times longer than 7 s led to distorted peaks and decreased resolution as a result, so 7 s was selected as the best value.

Table 1 summarizes the optimum conditions for separating lansoprazole enantiomer. Figure 5 shows the electropherogram for a standard solution containing 20 mg L^{-1} lansoprazole as obtained under such conditions, where the migration times for the *R* and *S* isomers were 4.92 ± 0.04 and 5.33 ± 0.03 min, respectively.

The identification of *R*- and *S*-lansoprazole was accomplished by using an HPLC method reported by Borner *et al.* [4] to collect each fraction separately. Thus, the enantiomers were separated on a CHIRAL-AGP column containing covalently bound acid α-glycoprotein as chiral selector. Two aliquots of 20 mg L^{-1} lansoprazole were spiked separately with each fraction collected in the HPLC electrophoretic separation and analysed under the above-described optimum conditions. The results obtained show that the electrophoretic elution order was *R*-lansoprazole followed by *S*-lansoprazole. This selectivity was similar to that found for omeprazole enantiomers using CDs as chiral selectors [36].

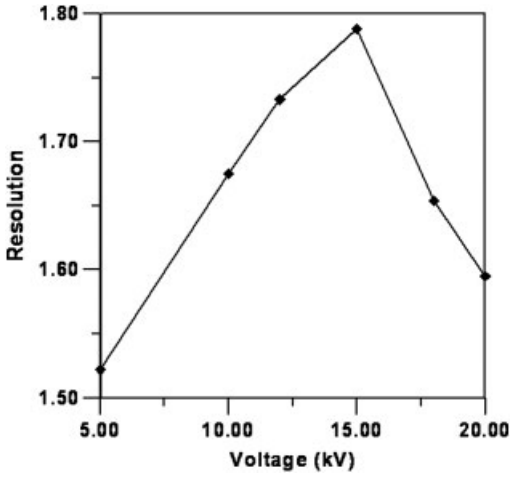

Figure 4. Effect of the applied voltage on the resolution of lansoprazole enantiomers with 50 mM phosphate buffer at pH 2.2 containing 12 mM β-CD and 5 mM sodium sulphide at 16°C.

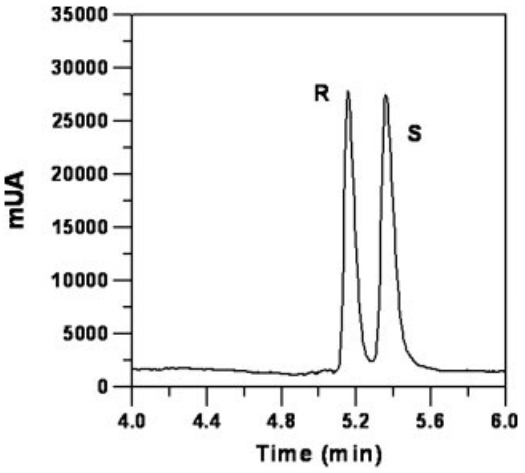

Figure 5. Electropherogram for lansoprazole enantiomers at a 20 mg L^{-1} concentration each. Conditions: CD concentration 12 mM, pH 2.2, buffer concentration 50 mM, sodium sulphide concentration 5 mM, applied voltage 15 kV and temperature 16°C.

Table 1. Optimum separation conditions

Separation electrolyte	40 mM phosphate buffer (pH 2.2) 12 mM β-CD 5 mM sodium sulphide
Sample injection	Hydrodynamic, 7 s, 0.5 psi
Voltage	15 kV, voltage ramp 88 kV min^{-1} in 17 min
Silica-fused capillary	31 cm total length (21 cm effective length) × 75 μm id
Temperature	16°C
Detection wavelength	285 nm

3.2 Method validation

Validation tests were conducted with a view to confirming the suitability of the proposed method for its intended use. To this end, the stability of the solutions, and specificity, precision, linearity, accuracy, detection and quantitation limits and robustness of the method were assessed.

3.2.1 Stability of the solutions

Lansoprazole solutions are stable above pH 7.0; on the other hand, they are considerably degraded by an acid medium or sunlight [37]. Lansoprazole standard solutions prepared in ethanol were found to remain stable for at least one month; however, similar aqueous solutions were degraded within a few hours, so they required preparation on a daily basis. The solutions were supplied with 5 mM sodium sulphide in order to avoid oxidation during electrophoretic separation.

3.2.2 Specificity

Specificity can be determined *via* peak uniformity. Because the different detection modes available from a DAD are not equally effective to detect impurities in an electrophoretic peak, the simultaneous use of several techniques for this purpose is recommended. In this work, we validated the purity of the peaks for *R*- and *S*-omeprazole in their respective formulations by (i) normalizing and comparing spectra from several peak sections; and (ii) comparing the absorbance at two different wavelengths. Both procedures showed the peaks for the *R* and *S* enantiomer in each formulation to be highly pure, and hence subject to no interference from the excipients.

3.2.3 Precision

The precision of the electrophoretic method was assessed in terms of repeatability and intermediate precision. Repeatability was determined from ten consecutive injections of a 20 mg L^{-1} standard solution of the pharmaceutical, which were used to calculate the RSD of migration times, peak heights and CPA for the enantiomers. RSD was less than 2.3% for migration times and peak heights, and less than 2.5% for corrected peaks areas. Intermediate precision was assessed by analysing a standard solution containing 20 mg L^{-1} lansoprazole over a period of 3 wk. The RSD values thus obtained were less than 2.6% for migration times and peak heights, and less than 2.7% for CPA. The proposed method is therefore highly repeatable and reproducible for the determination of lansoprazole enantiomers.

3.2.4 Linearity

Under optimal conditions, linearity was examined by analysing solutions containing 2–25 mg L^{-1} lansoprazole under the above-described optimum operating conditions. The total corrected area was thus found to be linearly related

to the lansoprazole concentration. The relationship conformed to the equation

$$Y = (506.51 \pm 6.33)X + (283.00 \pm 78.34)\ (r = 0.999)$$

for the *R* enantiomer and to

$$Y = (507.37 \pm 11.87)X + (506.34 \pm 146.96)\ (r = 0.998)$$

for the *S* enantiomer, *Y* being the CPA and *X* the analyte concentration in $mg\,L^{-1}$.

3.2.5 Accuracy

The accuracy of a method expresses the closeness of agreement between the value found and that which is accepted as a reference value [38–40]. The accuracy of the proposed method was assessed by analysing real samples consisting of pharmaceutical formulations spiked with a known amount of lansoprazole. An additional sample was used unspiked in order to check for the presence of lansoprazole in the formulations. All samples were analysed in triplicate with the proposed method. The recoveries thus obtained are summarised in Table 2.

3.2.6 LOD and LOQ

LODs were calculated from baseline noise. LOD is defined as the sample concentration giving a peak three times as high as baseline noise [41]. The LODs were found to be $0.64\,mg\,L^{-1}$ for *R*-lansoprazole and $0.72\,mg\,L^{-1}$ for *S*-lansoprazole, and the respective LOQs were 2.13 and $2.40\,mg\,L^{-1}$.

3.2.7 Robustness

The robustness of the proposed method was assessed as described elsewhere [42–44]. The choice of variables and the levels at which to test them are very important if the robustness test is to be of value. Variables must be those that are likely to be significant in practice and the levels must reflect the variations, which are usually observed. To this end, we examined the effect of small changes in the major operating variables, namely: (i) CD concentration ($15\,mM_{(+1)}$, $12\,mM_{(0)}$, $10\,mM_{(-1)}$), (ii) electrolyte ionic strength ($55\,mM_{(+1)}$, $50\,mM_{(0)}$, $45\,mM_{(-1)}$), (iii) pH ($2.7_{(+1)}$, $2.2_{(0)}$, $1.7_{(-1)}$), (iv) applied voltage ($18\,kV_{(+1)}$, $15\,kV_{(0)}$, $12\,kV_{(-1)}$), (v) separation temperature ($17^{\circ}C_{(+1)}$, $16^{\circ}C_{(0)}$, $15^{\circ}C_{(-1)}$), (vi) injection time ($9\,s_{(+1)}$, $7\,s_{(0)}$, $5\,s_{(-1)}$), and (vii) detection wavelength ($289\,nm_{(+1)}$, $285\,nm_{(0)}$, $281\,nm_{(-1)}$). Robustness was carried out from triplicate injections of a solution and only one parameter at a time was changed in each experiment. The effects of each factor level on resolution, efficacy, corrected peaks areas and peak height were determined. The main effect of each variable is the average difference between observations at the extreme level and those at the method level.

The main interaction effects are produced by the ionic strength of the electrolyte over the CPA and by the pH of the electrolyte over resolution. However, none of these variables affected significantly the assay of lansoprazol enantiomers. Therefore, the proposed method can be deemed robust and effective for the analysis of lansoprazole enantiomers in commercial pharmaceutical formulations.

3.3 Application of the proposed method to commercial pharmaceutical formulations

The proposed method was applied to the determination of lansoprazole in three different pharmaceutical preparations, namely Davur, Alter and Cinfa. Samples were prepared as

Table 2. Accuracy of the proposed method

Pharmaceutical formulation	Lansoprazole added (ppm)	Recovery (%) (mean ± SD)	
		Enantiomer *R*	Enantiomer *S*
Cinfa	2	97.8 ± 1.6	92.2 ± 2.0
	4	94.3 ± 1.0	96.1 ± 1.4
	6	97.0 ± 0.7	94.4 ± 1.0
	10	93.9 ± 1.4	94.5 ± 2.1
	16	92.1 ± 0.9	93.4 ± 1.5
Davur	2	93.8 ± 1.3	95.8 ± 0.9
	4	95.0 ± 0.7	94.9 ± 1.1
	6	93.3 ± 1.4	93.0 ± 1.7
	10	92.2 ± 1.3	96.1 ± 2.0
	16	103.2 ± 2.1	102.6 ± 2.5
Alter	2	99.3 ± 1.8	101.5 ± 2.2
	4	100.9 ± 0.9	101.4 ± 2.3
	6	102.2 ± 0.6	96.1 ± 1.0
	10	94.5 ± 1.9	93.9 ± 1.7
	16	91.4 ± 1.4	97.7 ± 0.9

Table 3. Application of the proposed method to pharmaceutical formulations

Pharmaceutical formulation			Found (ppm) ± SD	
Drug	Enantiomer *R* (mg)	Enantiomer *S* (mg)	Enantiomer *R*	Enantiomer *S*
Cinfa Lansoprazol (20 mg)	10	10	9.6 ± 0.2	10.3 ± 0.5
Davur Lansoprazol (20 mg)	10	10	10.1 ± 0.6	9.8 ± 1.1
Alter Lansoprazol (20 mg)	10	10	9.3 ± 1.0	10.1 ± 1.4

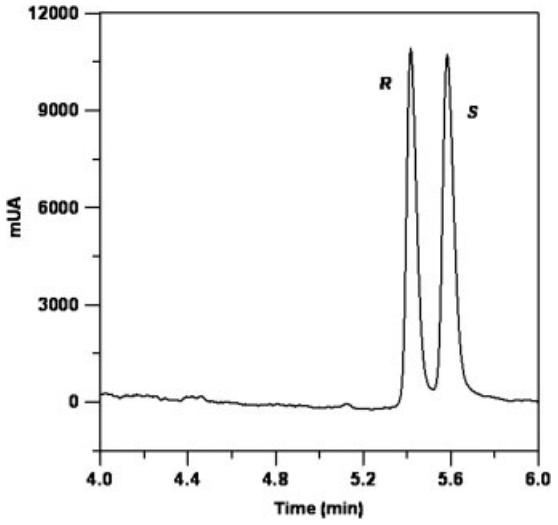

Figure 6. Electropherogram for the lansoprazole enantiomers in the formulation Alter.

described in Section 2 and analysed in triplicate. As can be seen from Table 3, the results were reproducible; also, the capsule contents were within the officially established limits [45]. Figure 6 shows a selected electropherogram for one of the pharmaceutical formulations.

4 Concluding remarks

A straightforward, expeditious, reliable CZE method for the determination of lansoprazole enantiomers in pharmaceutical formulations was developed and validated. The method performs quite well as regards specificity, linearity, accuracy, precision and robustness in the determination of lansoprazole enantiomers. Also, it can be directly applied to pharmaceutical formulations with no interference from the excipients. Chromatographic methods typically use large amounts of organic solvents, which are expensive and environmentally toxic; also, they require highly specific chiral selectors and expensive columns. By contrast, CZE uses only a few millilitres of electrolyte buffer and inexpensive capillaries, and features short analysis times. The proposed method can be used for the routine quality control of lansoprazole enantiomers in pharmaceutical formulations.

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The authors have declared no conflict of interest.

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