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Research Article

Development and validation of a capillary electrophoresis method for determination of enantiomeric purity and related substances of esomeprazole in raw material and pellets

A capillary electrophoresis method using CDs for quality control of esomeprazole (ESO) in terms of enantiomeric purity and related substances in raw material and pellets was developed. ESO is the *S*-enantiomer of omeprazole (OMZ). Several parameters were evaluated, including type and concentration of buffer and CD, concentration of additives and electrolyte pH. Resolution between the enantiomers of OMZ obtained for each parameter tested was calculated and the presence of the main related substance such as OMZ sulfone was carefully monitored. The optimized system consisted of 100 mM Tris-phosphate buffer pH 2.5 with 20 mM 2-hydroxypropyl-β-CD, 1 mM sodium dithionite, temperature at 15°C, voltage at 28 kV, and UV detection at 301 nm. Once optimized, the electrophoretic system was validated according to ICH guidelines. The limits of detection and quantification for R-OMZ were 0.6 μg/mL (0.06% w/w of ESO) and 2.0 μg/mL (0.2% w/w of ESO), respectively. A mean concentration of R-OMZ <0.2% limit established by the United States Pharmacopeia (USP) was found in the raw material and six-pellet samples of ESO. No other impurities were found in the samples under these conditions. Therefore, the developed method was found to be appropriate not only for enantiomeric quality control of ESO but also for the analysis of ESO and the main related substance in raw material and pharmaceutical formulations as well as for stability indicating studies.

Keywords:
chiral capillary electrophoresis / enantiomeric purity / esomeprazole / omeprazole / related substances
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1 Introduction

Esomeprazole (5-methoxy-2-[(*R*)-[(4-methoxy-3,5-dimethylpyridin-2-yl)methane]sulfinyl]-1H-1,3-benzodiazole) (ESO) (Fig. 1) is a single optical isomer proton-pump inhibitor used in the treatment of acid-related diseases. It is a potent inhibitor of gastric acid secretion and it does not undergo chiral inversion in vivo [1]. ESO has demonstrated a better pharmacological effect than the racemic product, omeprazole (OMZ), due to its higher systemic absorption [2], since (*R*)-OMZ is stereoselectively hydroxylated by cytochrome P450 CYP2C19 enzyme [3]. ESO is a hydrophobic compound with weak basic properties and it can be degraded unless it is protected against acidic conditions [4]. Therefore, ESO is administered in gastroresistant formulations such as pellets or capsules.

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Abbreviations: ESO, esomeprazole; **OMZ**, omeprazole ; **R-OMZ**, (*R*)-omeprazole; **USP**, United States Pharmacopeia

Few analytical methods have been developed for the enantiomeric resolution of OMZ, using HPLC and CE as well. The United States Pharmacopeia (USP) official method for magnesium ESO involves the use of a HPLC with a CHIRALPAK® AGP column with UV detection for the determination of *R*-enantiomer impurity in raw material [5]. Other HPLC methods have already been reported for the enantioselective analysis of OMZ in biological samples or in bulk drug. These methods are based on chiral stationary phases, using mainly polysaccharide or protein-based columns [6–9]. The main disadvantage of the HPLC methods is that they usually involve the use of expensive chiral columns to cover a reasonably wide application range, which have a relatively short lifetime. Moreover, chiral HPLC systems require large volumes of organic solvents. Nevertheless, HPLC methods using chiral stationary phases were the preferred technique for this purpose until the advent of capillary electrophoresis in the last decade [10, 11]. CE has been successfully employed in chiral analyses for numerous enantiomeric compounds [12–15]. The main advantages of CE for this kind of analysis are the extremely high efficiency, instrumentation simplicity, low sample and reagent consumption, and speed in method development and analysis, particularly of charged, polar and chiral compounds, using chiral selectors [16]. Moreover, chiral CE has proven to be more versatile and much

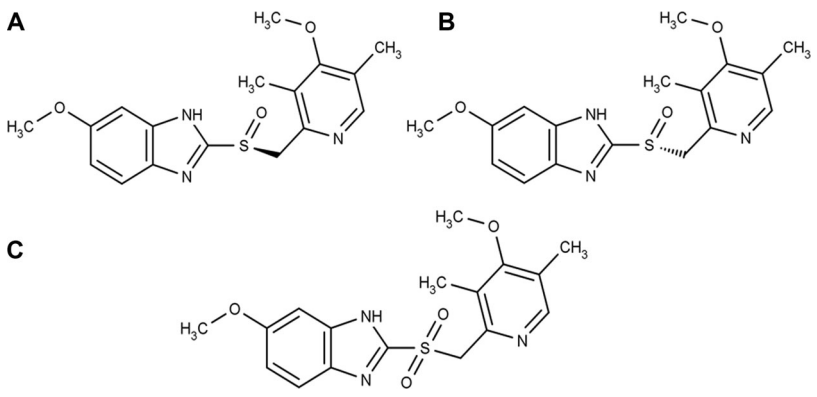

Figure 1. Chemical structures of ESO (A), R-OMZ (B), and OMZ sulfone (C). The sulfur atom is the stereogenic center.

less expensive than the chiral HPLC methods [17]. The most used chiral selectors are the CDs, a family of torus-shaped malto-oligosaccharides have found many applications in recent years because of their ability to form inclusion complexes with a large number of molecules. Only a few reports on CE chiral separation of OMZ are presented, in this sense, separation of enantiomers in capsules of racemic OMZ (enantiomer relation *R*/*S* 1:1) was achieved using a BGE consisting of 40 mM phosphate buffer adjusted to pH 2.2, 30 mM methyl-β-CD, and 5 mM sodium disulfide to inhibit the oxidation of OMZ in acid medium. This method was mainly applied for the determination of OMZ racemate [18]. Another enantioselective analysis of racemic OMZ in tablets was carried out using 3% sulfated β-CD in 20 mM phosphate buffer pH 4.0, but without the use of any antioxidant. Also in this work, a comparison between chiral HPLC and chiral CE for the determination of OMZ enantiomers was made and the results presented show that both methods are suitable for the analysis of OMZ, but the HPLC method using a chiral column is more sensitive and achieves better resolution of OMZ enantiomers [17].

In the present work, we have developed a simple CE method for the separation and quantification of ESO, its *R*-impurity (R-OMZ), and its main related substances (OMZ sulfone, SFN) in esomeprazol raw material and pharmaceutical formulations such as gastroresistant pellets, using CDs as chiral selectors. The proposed method was optimized for different experimental parameters and validated according to ICH guidelines. To the best of our knowledge, there are no reports of any successful method described for the determination of enantiomeric purity and related substances of ESO in raw material and finished product.

2 Materials and methods

2.1 Chemicals and reagents

The racemic drug OMZ and ESO standards were supplied by INAME (National Institute of Drugs, Argentina). Enteric-coated ESO pellets were supplied by Laboratorios Richet Argentina. OMZ sulfone, a main related substance, was obtained from Sigma (St. Louis, MO, USA). For the purpose of our study, several CDs were tested as chiral selectors for the CE system. Sulfated β-CD, β-CD, (2,3,6-tri-O-methyl)-β-CD, and 2-hydroxypropyl-β-CD (1.0 molar substitution, average molecular weight = 1540, H107) were supplied by Sigma. Sodium dithionite, used to prevent the degradation of OMZ into its main degradation products under acidic conditions, as well as sodium hydroxide, potassium hydroxide, sodium borate, (2-amino-2-hidroxymethyl) propane-1,3-diol (Tris), phosphoric acid, and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). Water was purified in an EASY Pure™ RF equipment (Barnstead, Dubuque, IA, USA).

2.2 Standard and sample preparation

OMZ, R-OMZ, SFN, and ESO stock solutions were prepared in methanol to achieve a concentration of 1 mg/mL. Working standard solutions were prepared by diluting the corresponding stock solutions in 20 mM pH 9.2, sodium borate buffer according to the USP official method of OMZ [19, 20] to achieve a final concentration of 100 μg/mL for ESO, 5 μg/mL for R-OMZ and SFN, these solutions were used during the optimization of the system and sample analysis. OMZ working standard solution was prepared by diluting to a final concentration of 100 μg/mL. ESO samples were prepared by dissolving 100 mg of the enteric coated pellets (theoretical concentration: 21.8% w/w of ESO) with 2 mL of 0.1N NaOH, aided by 5 min of sonication, and then completed to a final volume of 25 mL with 20 mM sodium borate buffer pH 9.2. The resultant solutions were centrifuged and analyzed immediately. The final pH sample was carefully selected to avoid OMZ degradation [4].

2.3 Stress conditions

Oxidation: 25 mL of a 3% v/v hydrogen peroxide solution was added to 25 mg of ESO accurately weighted. Acidic: 20 mg of ESO with 100 mL of 0.5 M hydrochloric acid solution were refluxed for 1 h. Alkaline: similar condition to the method

described under "acidic" using 0.5 M sodium hydroxide solution. Light: 1 mg of ESO solution was exposed to white light for 1 wk. All samples were diluted with 20 mM sodium borate buffer pH 9.2 to obtain a final concentration of 100 μg/mL of ESO.

2.4 Equipment and analytical conditions

The electrophoretic separation was performed on a P/ACE ™ MDQ (Beckman Coulter) Karat V.8 software with diode array detection set at 301 nm. The separation was performed in an uncoated fused-silica capillary with 60 cm of length (effective length to the detector: 50 cm) and 75 μm of internal diameter. Standard and samples were introduced into the capillary by applying 0.5 psi of pressure for 5 s (sample volume injected approximately 19.6 nL calculated by Beckman Coulter CE Expert Lite software [https://www.beckmancoulter.com]). Capillary and sample temperature were set at 15°C, each run was performed at normal polarity (28 kV). New capillaries were conditioned by rinsing with 0.5 M potassium hydroxide for 10 min. Before each analysis, the capillary was washed with 0.1 M potassium hydroxide (2 min), water (2 min), and the running buffer (2 min). The electrophoretic separations were carried out in 100 mM Tris-phosphate buffer pH 2.5, containing 20 mM 2-HPβCD as chiral selector and 1 mM sodium dithionite to prevent degradation of the enantiomers of OMZ during the analysis.

3 Results and discussion

3.1 Method optimization

The BGE was optimized to obtain the highest resolution with no degradation products under the conditions established. Type and concentration of buffer and CD, concentration of antioxidant and electrolyte pH were tested. Resolution between the enantiomers of OMZ obtained for each system tested was calculated and the presence of related substances was carefully monitored.

3.1.1 Choice of chiral selector and concentration

Based on literature, various CDs were tested (β-CD, sulfated β-CD, trimethyl-β-CD, and 2-HPβCD) in a concentration range of 10–40 mM. The selection of a suitable CD for chiral separation was made according to the shape and size of OMZ molecule and the respective CDs. From all CDs tested, only 2-HPβCD was able to separate OMZ enantiomers. The effect of different concentrations of 2-HPβCD (10–40 mM) on the resolution and migration times was investigated. Chiral separation was achieved for all cases, but 20 mM was the concentration chosen as a compromise between resolution and speed of analysis. From 10 to 30 mM, migration times obtained for OMZ were very similar and 20 mM being the concentration that gave the adequate resolution in a shorter time. Concentrations higher than 30 mM were difficult to achieve because of dissolution problems of the CD in the BGE. Therefore, the concentration of 30 mM was not chosen although it presented a minor improvement in resolution. At 40 mM, resolution between OMZ enantiomers increased significantly along with migration times (Table 1).

Table 1. Resolution values between ESO and its *R*-enantiomer obtained during method optimization by evaluating 2-HPβCD concentration (pH value fixed at 2.5) and evaluating the influence of the pH of the BGE (2-HPβCD concentration constant at 20 mM)

2-HPβCD concentration (mM)	R-OMZ migration time (min)	ESO migration time (min)	Resolution
10	14.4	14.6	1.72
20	16.4	16.8	2.32
30	18.5	18.9	2.44
40	24.3	25.0	6.45
BGE pH			
7	7.1	7.1	0
6	8.2	8.2	0
5	9.4	9.4	0
4	14.2	14.2	0
3	16.1	16.4	2.15
2.5	16.4	16.8	2.32
2	>60	>60	ND

Resolution was calculated according to USP [25]. Values "0" for resolution mean no separation at all. ND stands for not determined.

3.1.2 Effect of buffer pH

Knowing that OMZ suffers degradation at pH values <7.4 [4], different systems were initially tested in a pH range of 8–11. At alkaline pH values, the ESO, and R-OMZ are not charged (pKa1 = 4.0 pKa2 = 8.8, http://www.pulsus.com/cddw2001/PI.htm), so it was necessary to include in the electrolyte a charged pseudostationary phase such as SDS (plus a CD) to allow OMZ to run separately from the EOF. However, the high magnitude of EOF did not allow appreciable differentiation between the electrophoretic mobility of both enantiomers. The influence of the EOF in the resolution has been discussed a lot of times in previously published reports. It has been shown that for cations, the resolution decreases with increasing EOF. Despite the gain in efficiency, the lack of selectivity leads to a loss in resolution [21]. Analyzing the values of μ_{ap} (apparent mobility) obtained at pH 2.5, the difference between ESO and R-OMZ is of 2.5%. This difference becomes zero when the EOF increases 1.7 times at pH 4.0. Therefore, it was decided to work at an acidic pH value where the EOF is almost zero and both enantiomers are charged and migrate according to their own electrophoretic mobility. The pH was evaluated in a range of 2–7, 2.5 being the pH at which the highest resolution

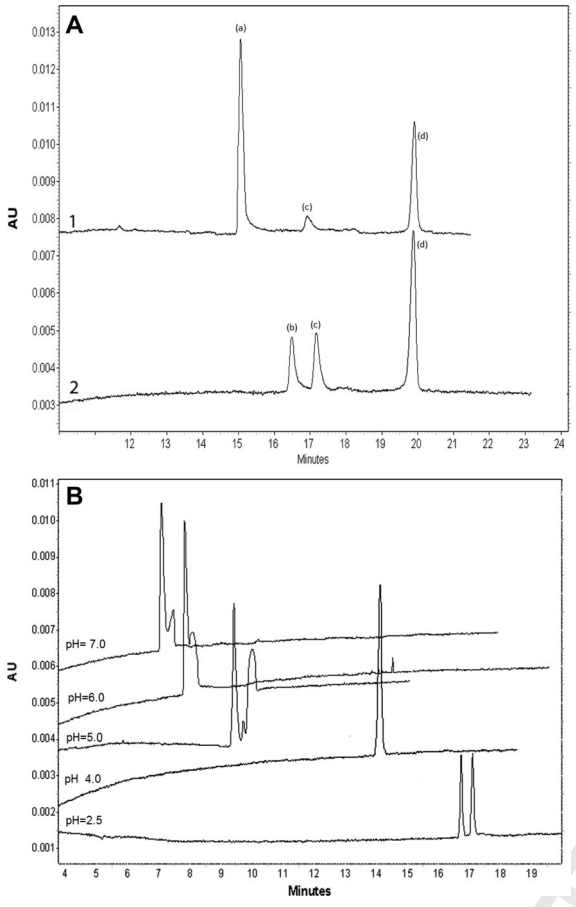

Figure 2. (A) Electropherogram 1 shows ESO subjected to acidic conditions. It can be seen SFN (d), ESO (c) and an unknown product (a). Electropherogram 2 shows the identification of the compounds, including R-OMZ (b) using the standards available under normal conditions. BGE conditions: 100 mM Tris-phosphate buffer pH 2.5 with 20 mM 2-HPβCD and 1 mM sodium dithionite. (B) Influence of pH on enantiomeric separation during method optimization. Separation of OMZ enantiomers is achieved from pH 3 to lower values. BGE conditions: 100 mM Tris-phosphate buffer pH 7 to 2.5 with 20 mM 2-HPβCD and 1 mM sodium dithionite.

was obtained with no significant increase in migration times (Table 1). In this range, pH values from 7 to 4 did not allow separation of the enantiomers, while pH values from 3 to 2.5 were sufficient to achieve separation (Fig. 2).

3.1.3 Choice and effect of buffer concentration

The effect of buffer type on the enantioseparation of OMZ was assessed by performing electrophoretic separation with the chosen chiral selector. Sodium phosphate buffer of pH 4.0 at a concentration of 20 mM was initially tested, but no separation was achieved under these conditions. Moreover, several products of degradation were observed due to the acidic conditions, for example the sulfone derivate. To prevent degradation, 20 mM sodium borate buffer pH 9.2 was tested. In this case, no separation was achieved, probably due to the high EOF. Finally, 100 mM Tris/phosphate buffer pH 2.5 was tested and separation was achieved but with the presence of degradation products, so a suitable antioxidant was selected to prevent it, since it seemed more likely to achieve separation only at low pH values due to the protonation of the pyridinium group in OMZ (pKa = 4.0) <pH 4. The dissociation equilibrium of the weakly acidic benzimidazole group (pKa = 8.8) is not affected in this pH range. Full chiral separation was achieved only at 100 mM of buffer concentration. Lower concentrations gave shorter migration times but with less resolution. The concentration established was chosen as a compromise between resolution and speed, being resolution the priority.

3.1.4 Effect of antioxidant addition

The optimized system consisted of 100 mM Tris-phosphate buffer pH 2.5 with 20 mM 2-HPβCD. However, degradation products were observed under these conditions, so to prevent degradation of ESO at this pH value, different antioxidants were tested by adding them to the BGE, sodium dithionite being the most effective (without affecting baseline stability) at a minimum concentration of 1 mM.

3.1.5 Effect of instrumental parameters on the resolution

Temperature and voltage parameters were investigated to evaluate the impact of these instrumental variations on enantiomer resolution. Temperature was tested in a range from 15 to 30°C whereas voltage was tested from 15 to 30 kV. An increase in temperature affected the resolution negatively since it may cause a decrease in the association constant for the enantiomer–CD complex and therefore, a decrease in enantioselectivity. A desirable migration time decrease was achieved with the temperature increment, but it is not enough to justify the lower resolution obtained. Voltage increase provided good resolution of OMZ enantiomers and shorter migration times with good peak shape. When 30 kV was tested, the current increased to an undesirable value, so a slight reduction was made to avoid high current values. A temperature of 15°C and a voltage of 28 kV (current: 110 μA) were selected for further analysis as a compromise between resolution and migration times.

3.2 Migration order

The migration order was established by analyzing a sample of OMZ standard (100 μg/mL) spiked with ESO (100 μg/mL). R-OMZ migrates first, which could be considered an advantage since peak tailing of ESO at sample concentration (1 mg/mL) might occur. In the analysis of enantiomeric purity of ESO samples, R-OMZ determination could be impaired if this enantiomer migrated in second place (Fig. 3).

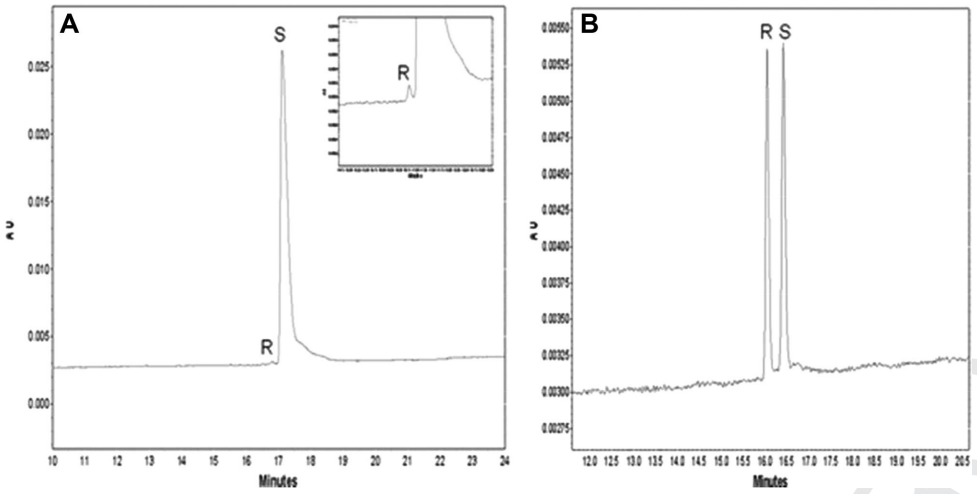

Figure 3. (A) Electropherogram of ESO pellets sample. *R*-enantiomer standard (5 μg/mL) can be seen on the electropherogram at the upper right corner along with ESO standard (1000 μg/mL). The *R*-enantiomer concentration in the sample is lower than the 0.2% limit. (B) OMZ standard at a concentration of 100 μg/mL. Migration order was established, R-OMZ migrating first, then ESO. BGE conditions (A) and (B) 100 mM Tris-phosphate buffer pH 2.5 with 20 mM 2-HPβCD and 1 mM sodium dithionite.

3.3 Method validation for ESO

Once optimized, the electrophoretic optimal system consisting of 100 mM Tris-phosphate buffer pH 2.5 with 20 mM 2-HPβCD and 1 mM sodium dithionite was validated evaluating the specificity, linearity, LOD, LOQ, precision, accuracy, and robustness of the method according to ICH guidelines [22]. All validation results are shown in Table 2.

The ability of the method to indicate chemical stability was studied by accelerated stress conditions (acid, alkaline, oxidation, and light) and specificity was also examined by comparing electropherograms of excipient blanks of the pharmaceutical formulation tested (pellets). Degradation was observed when ESO was exposed to acidic conditions. The presence of OMZ sulfone was detected in the electropherograms (Fig. 2). Oxidation conditions also lead to significant degradation. Exposure to light stress and alkaline conditions resulted in minimal degradation of ESO.

Also, to detect impurities on the electrophoretic peaks of the samples corresponding to the enantiomers, peak homogeneity was measured and evaluated, using different combined techniques available in a DAD system [23]. Absorbance at two different wavelengths was measured and spectra from several peak sections were compared. Both techniques demonstrated that the peak corresponding to ESO in the samples present a high level of purity, with no interferences of the excipients involved in the pellets samples. Pharmaceutical formulation excipients of pellets samples did not interfere with the assay.

Linearity for ESO was evaluated in a concentration range from 20 to 200 μg/mL ($n = 5$). In the case of R-OMZ, SFN were prepared in a concentration range of 2.0–6.0 μg/mL ($n = 5$) in the presence of 1 mg/mL of ESO. Each standard solution was analyzed in triplicate and plots of peak area versus concentrations were constructed for each compound.

Table 2. Parameters of validation of the method for R-OMZ, SFN, and ESO

Parameter	R-OMZ			SFN			ESO		
Linear range (μg/mL)	2.00–6.00			2.00–6.00			20.0–200.0		
R^2	0.9843			0.9955			0.9912		
LOD (μg/mL)	0.6			0.2			1.4		
LOD (% w/w of ESO)	0.06			0.02					
LOQ (μg/mL)	2.0			0.6			4.6		
LOQ (% w/w of ESO)	0.20			0.06					
Precision (%RSD)									
Intraday ($n = 6$)									
Migration time	0.3			0.7			0.5		
Peak area	1.9			1.5			0.9		
Interday ($n = 18$)									
Migration time	0.8			1.0			0.8		
Peak area	1.5			1.3			1.7		
Accuracy									
Spiked levels (%)	80	100	120	0.2	0.3	0.5	0.2	0.3	0.5
Recovery in pellets (%)	95.5	97.5	98.8	97.5	98.9	98.9	97.9	00.4	99.8

Table 3. Percentage of recovery of R-OMZ enantiomer for samples prepared at different ratios of enantiomer concentration

R/*S* enantiomer proportion	R-OMZ recovery (%)	RSD (%) $n = 3$
0.01/1	99.2	1.7
0.05/1	99.7	1.3
0.10/1	100.4	0.7
0.20/1	98.9	1.2
0.50/1	100.8	0.9
1.00/1	98.3	1.2

The LOD and LOQ were determined, based on S/N . A relation of 3:1 was used for estimating the LOD, whereas a 10:1 relation was used for the LOQ. Limit concentrations of each impurity were expressed in microgram per microliter as well as percentage weight in weight of ESO.

Precision was evaluated for intraday ($n = 6$) and interday ($n = 18$) assay of OMZ *R*-enantiomer and SFN (2 μg/mL of R-OMZ and SFN with 1 mg/mL of ESO) and ESO (50 μg/mL) after applying the sample preparation procedure, and it was expressed as RSD for retention times and areas.

Accuracy was calculated from recovery studies. Placebo samples prepared with all excipients contained in the pellets formulation were spiked with ESO at 80, 100, and 120% concentration levels. Preparations of each level were assayed by triplicate. Percentages of recovery values were obtained in the range of 97.9–100.4% (Table 2). For R-OMZ and SFN, placebo samples were spiked with a concentration equivalent to 0.2, 0.3, and 0.5% w/w respect to ESO (1 mg/mL). Preparations of each level were assayed by triplicate. Percentages of recovery values were obtained in the range of 95.5–98.8% and 97.5–98.9%, respectively.

To identify possible sources of error when the specified conditions are slightly changed, robustness studies were conducted by making small but deliberate changes in electrophoretic conditions. The method's capacity to remain unaffected was studied for ESO samples and standard solutions, and parameters selected for two level variations (upper and lower) were buffer pH (2.5 ± 0.2), 2-HPβCD concentration (20 ± 2 mM), temperature (15 ± 2°C), and voltage (28 ± 2 kV). The effects of variations in the electrophoretic parameters were evaluated statistically applying Student's *t*-test according to international guidelines [24, 25] using migration time, mean theoretical plates, resolution between enantiomers and ESO, R-OMZ, and SFN concentration in samples. The data obtained in these experiments did not show a significant difference of means (*p*-value >0.05). Only buffer pH was statistically significant at the levels evaluated for migration time. pH values <2.5 considerably increased the migration time, whereas higher pH values decreased it, but compromising the resolution. Therefore, buffer pH should be carefully controlled during analysis. All other parameters did not affect the separation and determination of enantiomers concentration significantly.

Table 4. Comparison between the methods already available

Method	Detection	System				Validation data					Resolution	Product
		Chiral selector	Capillary effective length	Buffer or mobile phase	Antioxidant	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Precision intraday (%RSD)			
									Migration time	Area		
HPLC [17]	UV 302 nm	Chiralpack AD column		Hexane:ethanol (40:60 v/v)	No	25.0–150.0	0.0061	0.020	ND	1.7	3.3	Tablets
HPLC [9]	UV 299 nm	Chiralpack IA column		MtBE-EA-EtOH-DEA (60:40:5:0.1 v/v/v/v)	0.1% DEA	0.5–25	0.17	0.51	0.17	0.27	Unknown	Tablets
CZE [18]	UV 301 nm	30 mM methyl-β-CD	21 cm	40 mM phosphate pH 2.2	5 mM sodium disulfide	2.0–40.0	0.31	1.0	<2	<2.5	1.54	Capsules
CZE [17]	UV 202 nm	3% sulfated-β-CD	53 cm	20 mM Phosphate pH 4.0	No	25.0–150.0	1.3	4.3	ND	1.8	1.5	Tablets
CZE[a)]	UV 301 nm	20 mM 2-HP-β-CD	50 cm	100 mM Tris-phosphate pH 2.5	1 mM sodium dithionite	2.00–6.00	0.6	2.0	0.26	2.02	2.32	Pellets

"ND" stands for not determined.
a) Method proposed in this research.

The evaluation of stability of both OMZ enantiomers standard solutions (ESO and R-OMZ standard) at different time intervals (up to 6 h) in the autosampler presented a RSD value of 1.9% for the content of R-OMZ and a RSD value of 3.7% for ESO. These results show that there is no significant variation in the content of each enantiomer standard from time of preparation to conclusion of the analyses, so these standard preparations were considered stable for 6 h in the autosampler.

3.4 Application: Analysis of ESO pharmaceutical formulations

The electrophoretic system performance on pharmaceutical samples was evaluated for the determination of R-OMZ impurity in ESO raw material as well as enteric-coated pellets (Fig. 3). Preparation of standards and samples is shown in the Section 2.2 described previously. Triplicate determinations of the content of the R-OMZ in the samples were performed. A mean concentration below the 0.2% limit established by the USP for R-OMZ was found in the raw material and six-pellet samples [5]. No other impurities were found in the samples under these conditions. Moreover, the capability of the method in the analysis of pharmaceutical samples containing different ratios of R-OMZ was evaluated. *R*/*S* enantiomer proportions were prepared at 0.01/1, 0.05/1, 0.1/1, 0.2/1, 0.5/1, and 1/1. These samples were prepared by triplicate and analyzed. Percentages of recovery for these samples are presented in Table 3.

A comparison between the methods already available in literature and the method we propose is shown in Table 4. The reported HPLC methods present better resolution, precision and lower LOD and LOQ than the ~~CZE~~ methods. However, they involve the use of expensive columns and higher solvent consumption, making these methods much less versatile than CZE methods. Moreover, determination of related substances is not described. USP official method involves the use of three different HPLC systems for assay, chromatographic purity, and enantiomeric purity, respectively, while our CZE system allows performing all three determinations at the same time, which is a great advantage [5]. The other CZE methods reported [17, 18] present lower resolution values for enantiomer separation and do not show separation of related substances (sulfone and other degradation products).

4 Concluding remarks

The method developed is simple and robust for quality control of enantiomeric purity of ESO raw material and finished product allowing the resolution of the enantiomers and the main related substances such as the OMZ sulfone encoded in USP. This method has been validated for the analysis of raw material and pellet samples without any excipient or degradation products interference. To the best of our knowledge, this is the first method using CE applied to the determination of R-OMZ impurity and its main degradation products in ESO pellets samples. This method is applicable for quality control of ESO products in pharmaceutical industry and a stability indicating method, being a valuable alternative to chiral analyses that employ HPLC, which are much more expensive and less versatile compared to chiral capillary electrophoresis.

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The authors have declared no conflict of interest.

5 References

[1] Mckeage, K., Blick, S. K. A., Croxtall, J. D., Lyseng-Williamson, K. A., Keating, G. M., *Drugs* 2008, *68*, 1571–1607.

[2] Saccar C. L., *Expert Opin. Drug Metab. Toxicol.* 2009, *9*, 1113–1124.

[3] Tybring, G., Bottiger, Y., Widen, J., Bertilsson, L., *Clin. Pharmacol. Ther.* 1997, *62*, 129–137.

[4] Mathew, M., Das Gupta, V., Bailey, R., *Drug Develop. Ind. Pharm.* 1995, *21*, 965–971.

[5] United States Pharmacopoeia 32 Revision. *United States Pharmacopeial Convention*, Rockville, MD, USA 2009, p. 2298.

[6] Balmer, K., Persson, B. A., Lagerstrom, P. O., *J. Chromatogr. A* 1994, *660*, 269–273.

[7] Tanaka, M., Yamazaki, H., Hakusui, H., *Chirality* 1995, *7*, 612–615.

[8] Cass, Q.B., Degani, A. L. G., Cassiano, N., *J. Liq. Chromatogr. Rel. Technol.* 2000, *23*, 1029–1038.

[9] Zanitti, L., Ferreti, R., Gallinella, B., La Torre, F., Sanna, M. L., Mosca, A., Cirilli, R., *J. Pharm. Biomed. Anal.* 2010, *52*, 665–671.

[10] Nishi, H., *Electrophoresis* 1999, *20*, 3237–3258.

[11] Maier, N. M., Franco, P., Lindner, W., *J. Chromatogr. A* 2001, *906*, 3–33.

[12] Tonon, M. A., Bonato P. S., *Electrophoresis* 2012, *33*, 1606–1612

[13] Aguiar, F. A., de Gaitani C. M., Borges K. B., *Electrophoresis* 2011, *32*, 2673–2682.

[14] Fakhari, A. R., Nojavan, S., Haghgoo, S., Mohammadi, A., *Electrophoresis* 2008, *29*, 4583–4592.

[15] Guan, J., Yan, F., Shi, S., Wang, S., *Electrophoresis* 2012, *33*, 1631–1636.

[16] Blaschke, G., Chankvetadze, B., *J. Chromatogr. A* 2000, *875*, 3–25.

[17] Bonato, P. S., Paias, F. O., *J. Braz. Chem. Soc.*, 2004, *15*, 318–323.

[18] Berzas Nevado, J. J., Castañeda Peñalvo, G., Rodriguez Dorado, R. M., *Anal. Chim. Acta*. 2005, *533*, 127–133.

[19] United States Pharmacopoeia 31 Revision, *United States Pharmacopeial Convention*, Rockville, MD, USA 2009, p. 2851.

[20] Flor, S., Tripodi, V., Scioscia, S., Revello, L., Lucangioli, S., *J. Liq. Chromatogr. Relat. Technol.* 2010, *33*, 1666–1678.

[21] Kenndler, E., *J. Microcolumn Sep.* 1998, *10*, 273–279.

[22] Guidance Q2 (R1), *Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonization.* Final version 200

[23] Farbe, H., Lebris, A., Blanchin, M., *J. Chromatogr. A* 1995, *697*, 81–88.

[24] Dejaegher B., Vander Heyden Y., *J. Chromatogr. A* 2007, *1158*, 138–157.

[25] United States Pharmacopoeia 32 Revision, *United States Pharmacopeial Convention*, Rockville, MD, USA 2009, p. 227.

Q9

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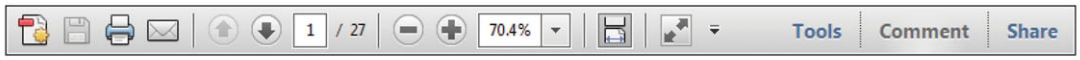

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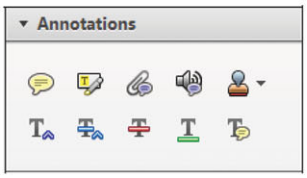

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Strikes a line through text and opens up a text box where replacement text can be entered.

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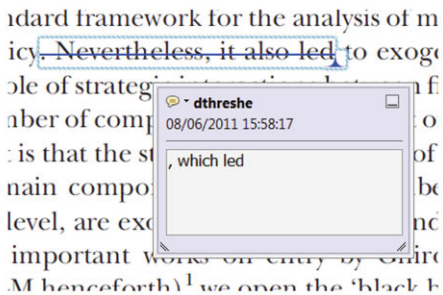

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How to use it

- Highlight a word or sentence.
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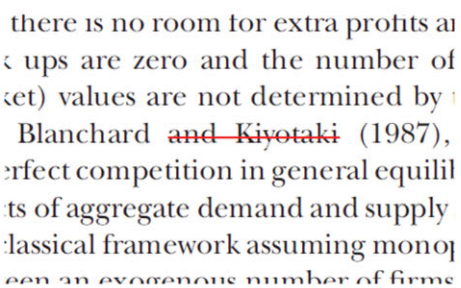

3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

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- Click on the Add note to text icon in the Annotations section.
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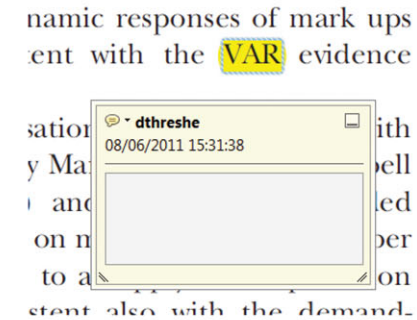

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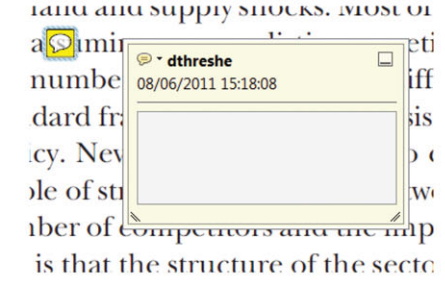
Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.



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5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
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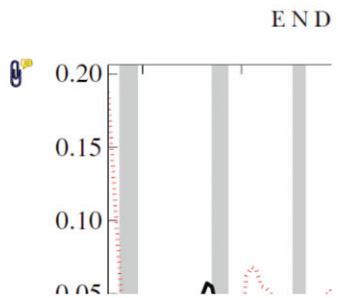
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



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Inserts a selected stamp onto an appropriate place in the proof.

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- Click on the Add stamp icon in the Annotations section.
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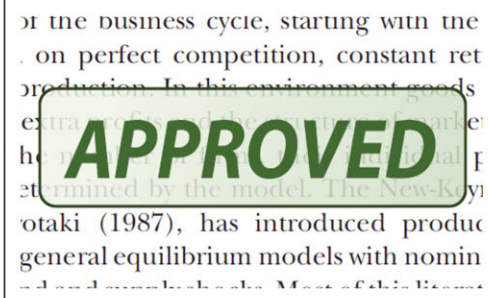

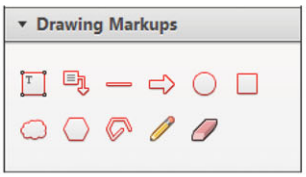

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How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
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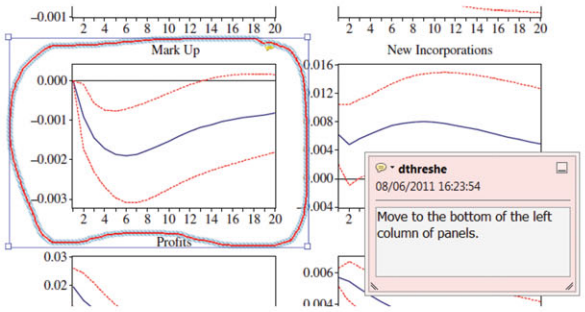
- Double click on the shape and type any text in the red box that appears.



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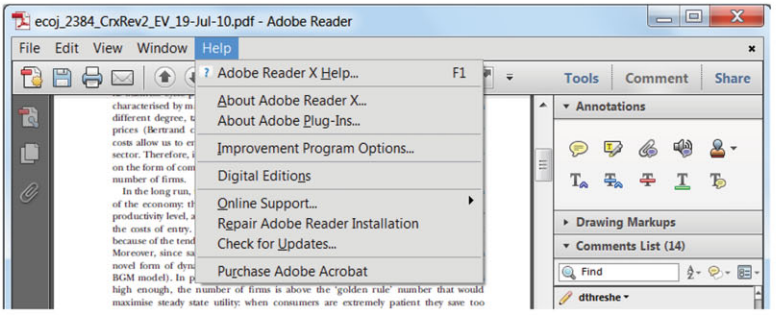

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