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Quantitative Analysis of β-Blockers in Pharmaceutical Preparations by Capillary Electrophoresis

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ABSTRACT A simple capillary electrophoresis method was developed for the analysis of four β-blockers (atenolol, metoprolol, pindolol, and propranolol) in pharmaceutical preparations. The method was validated regarding accuracy, precision, linearity, and detection/quantification limits, and the obtained values were in accordance to those reported in the literature. The method was applied to the determination of the drugs in commercial tablet preparations and proved to be fast and reliable for the quantitative analysis of the β-blockers.

KEYWORDS β-blockers, Pharmaceutical preparations, Capillary electrophoresis

INTRODUCTION

Capillary electrophoresis (CE) is a recently developed analytical technique based on the use of a strong electric field applied in a capillary to separate charged compounds as well as a noncharged one. Compared to high-performance liquid chromatography (HPLC), CE offers a completely different mechanism of separation; yet it also offers similar levels of accuracy, precision, and sensitivity. Because of this, HPLC and CE are often referred to as complementary techniques.

The simplicity and versatility of CE, together with its minimal costs (due to the low solvent consumption and use of cheap capillaries) and separation power, make it suitable for the separation of a wide range of substances including inorganic ions, drugs, pollutants, peptides, proteins, and DNA (Issaq, 2002). The analysis of pharmaceutical preparations is one of its most common applications (Altria & Elder, 2004; Altria et al., 1998a, 1998b). Now CE is a routine technique in many industries and CE methods and monographs have begun to appear in some pharmacopoeias (Altria & Elder, 2004). In Brazil, the technique has not been used frequently for this purpose, but interest in it has increased markedly (Bonato & Paias, 2004; Prado et al., 2002, 2004; Steppe et al., 2003).

β-Blockers are among the most frequently prescribed drugs for the treatment of various cardiovascular disorders, such as cardiac arrhythmia, angina pectoris, and hypertension (Hardman et al., 1996): The United States

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Pharmacopeia (1999) and British Pharmacopeia (1999) describe titrimetric, spectrophotometric, and chromatographic methods of assay for the analysis of β-blockers. Other methods based on flow injection analysis–chemiluminescence (Park et al., 2002), indirect atomic absorption spectrometry (Khalil & Borham, 2000; Khalil & El-Rabiehi, 2000), and potentiometry (Hassan et al., 2003) have also been reported in the pertinent literature. Although β-blockers are frequently used as drug models for CE separations (Nguyen & Siegler, 1996), only one validated method for atenolol analysis in bulk and tabled forms is reported (Shafaati & Clark, 1996).

Thus, the aim of this work was the development and validation of a capillary zone electrophoresis (CZE) method for the quantitative analysis of atenolol, metoprolol, pindolol, and propranolol in tablets.

EXPERIMENTAL

Chemicals and Standard Solutions

The methanol used for sample preparation was of HPLC grade and supplied by EM Science (Gibbstown, NJ, USA). All other reagents were of p.a. grade, obtained from Merck (Rio de Janeiro, Brazil) or Mallinckrodt (Baker IC., Phillipsburg, NJ, USA).

β-Blocker powders were kindly supplied by the laboratories responsible for their distribution in Brazil, i.e., Laboratórios Biossintética Ltda, (atenolol), Astrazeneca (propranolol and metoprolol), and Sandoz (pindolol). Stock solutions of the four drugs were prepared in methanol at a concentration of 1.0 mg mL^{-1}. Working solutions were prepared at a concentration of 50–300 μg mL^{-1} by appropriate dilution with methanol.

CE Conditions

Crystal CE equipment from Thermo Separations Products Inc. (Eberline Instruments, Santa Fe, NM, USA), equipped with a UV detector set at 225 nm, was employed for the analyses of atenolol, pindolol, and metoprolol. Injection was performed by applying pressure to the anodic side of the capillary. The separation was performed in an ultraviolet transparent fused-silica capillary CElect UVT75 (Supelco, Bellefonte, PA, USA). Due to equipment limitation, temperature was not controlled.

For the analysis of propranolol, the experiments were carried out using an Agilent capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany) provided with an autosampler device, temperature control set at 25°C, and a diode array detector also set at 225 nm. The silica-fused capillary employed was obtained from Agilent technologies.

The conditions used for the analyses of these drugs are shown in Table 1. The same buffer solution was used for the analysis of all drugs. The solution was prepared by dissolving Tris (hydroxymethyl)aminomethane (Mallinckrodt, Baker IC) in water purified with a Milli-Q Plus system (Millipore Co., Bedford, MA, USA) at the concentration of 100 mmol L^{-1} and the pH was adjusted to 2.9 using phosphoric acid (Merck, Rio de Janeiro, Brazil).

TABLE 1 **Analysis Parameters**

Drug	Capillary (effective length)	Voltage (KV)	Hydrodynamic injection[a]	Pre-run wash
Atenolol	80 cm × 75 μm (30 cm)	28	20 mbar × 6 s	NaOH 1 mol L^{-1}—5 min Water—2 min Running buffer—2 min
Metoprolol	80 cm × 75 μm (30 cm)	28	20 mbar × 6 s	NaOH 1 mol L^{-1}—5 min Water—2 min Running buffer—2 min
Pindolol	70 cm × 75 μm (30 cm)	27	20 mbar × 6 s	NaOH 1 mol L^{-1}—5 min Water—2 min Running buffer—2 min
Propranolol	30 cm × 50 μm (25 cm)	20	20 mbar × 10 s	NaOH 0.1 mol L^{-1}—1 min Water—1 min Running buffer—1 min

[a] Hydrodynamic injection at the anodic end of the capillary.

TABLE 2 **Sample Preparation**

Drug	Amount weighed	Sample concentration	Amount analyzed	Internal standard
Atenolol	5 mg	50 μg mL^{-1}	200 μL	Pindolol 100 μg mL^{-1}
Metoprolol	10 mg	100 μg mL^{-1}	100 μL	Propranolol 100 μg mL^{-1}
Pindolol	1 mg	50 μg mL^{-1}	200 μL	Atenolol 200 μg mL^{-1}
Propranolol	5 mg	100 μg mL^{-1}	100 μL	Metoprolol 300 μg mL^{-1}

Before first use, the capillaries were washed for 30 min with a 1-mol L^{-1} NaOH (Merck, Rio de Janeiro, Brazil) solution, and then washed with water for 20 min. Before each analysis, the capillaries were washed with NaOH solution, water, and the running buffer (Table 1). All solutions were filtered through 0.45-um membranes (Millipore Co., Bedford, MA, USA) and sonicated for 5 minutes prior to use. The analysis parameters are specified in Table 1.

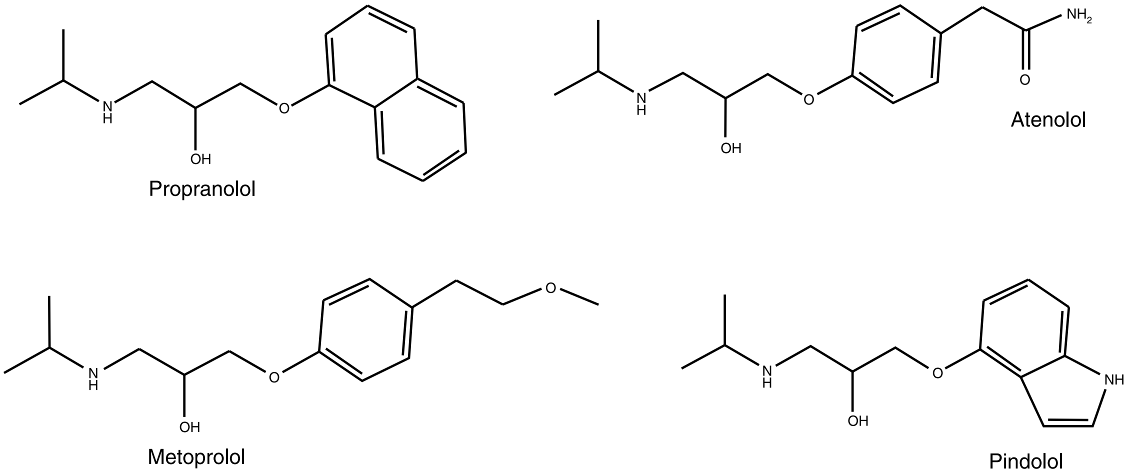
Sample Preparation

Ten tablets were weighed and pulverized by gentle grinding. An accurate weight of the powder was transferred to a volumetric flask of 100, 50, or 20 mL according to the desired concentration of the sample. The flask was filled up to 70% of its volume with methanol, sonicated for 45 minutes, and then completed to its total volume. Since the tablets contained insoluble materials, a portion of the solution was filtered through a 0.45-um membrane and a part of the filtered solution was transferred to clean tubes, containing 100 uL of the internal standard solution. The solvent was evaporated under airflow and the residues were dissolved in 200 uL of a 10-mmol mL^{-1} TRIS-phosphate buffer solution and analyzed. Details of the sample preparation are shown in Table 2.

Drug Determination and Method Validation

Calibration curves were prepared by analyzing standard solutions of the drugs spiked with the suitable internal standard. Volumes of 100 μL of the standard solutions of the drugs in concentrations of 50, 75, 150, and 300 μg mL^{-1} and 100 μL or 200 μL of the internal standard solution (Table 2) were transferred to clean tubes and the solvent was evaporated to dryness under air flow at room temperature. The residues were dissolved in 200 uL of a 10-mmol mL^{-1} TRIS-phosphate buffer solution and analyzed. Plots of drug concentrations vs. peak height ratio were constructed and the linear regression lines were used for the determination of the drugs in the samples. The calibration curves were based on peak height instead of peak area due to higher reproducibility obtained.

The precision and accuracy of the method were evaluated by determining the drugs in commercial tablet samples containing 10 mg of pindolol, 40 mg of propranolol, 100 mg of metoprolol, and 50 mg of atenolol. Ten tablets were weighed, powdered, and



Propranolol

Atenolol

Metoprolol

Pindolol

FIGURE 1 **β-blocker Structures.**

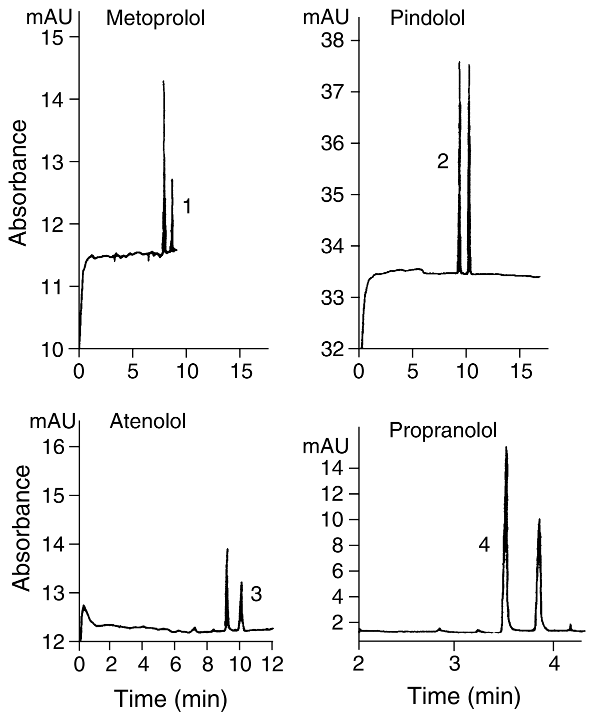


FIGURE 2 Analyses of Commercially Aavailable Samples. See Table 1 for Conditions. 1) Metoprolol, 2) Pindolol, 3) Atenolol, 4) Propranolol. Unnumbered Peaks: Internal Standard (Table 1).

TABLE 4 Detection Limits and Quantification Limits of the Methods

Drug	Detection limit (μg mL^{-1})	Quantification limit (μg mL^{-1})
Metoprolol	1.9	6.3
Atenolol	7.5	25.1
Pindolol	3.9	13.2
Propranolol	2.8	9.4

homogenized. For the evaluation of within-day precision and accuracy, 10 aliquots were analyzed on the same day according to the procedure previously described. Between-day experiments were carried out by analyzing aliquots of these samples for 5 days.

Detection and quantifications limits were determined by analyzing diluted solutions of the drugs and determining the signal-to-noise ratio.

RESULTS AND DISCUSSION

As shown in Fig. 1, the selected drugs are basic compounds, so we selected an acidic buffer for the analysis, in order to have them in the protonated form. Phosphate and tris-phosphate buffers were evaluated in the pH range of 2–5 (concentrations of 20–100 mmol L^{-1}). During method development, we also studied the effect of voltage and temperature.

Figure 2 shows the electropherograms for the analysis of the four β-blockers under the best electrophoretic conditions. Although the conditions used for the analysis were different for each drug, including the use of different equipment (Table 1), the same buffer solution was used for the analysis of the four drugs, representing an important advantage for methods used in drug assay.

Although there is no official guideline for the validation of CE methods, generally the results reported are similar to those applied to HPLC assays (Fabre & Altria, 2001; ICH, 2004; Shabir, 2003). The methods were validated in terms of linearity, accuracy, precision, and detection/quantification limits.

The linearity of an analytical procedure is its ability, within a given range, to achieve results that are directly proportional to the concentration of analyte in the sample. To evaluate the linearity of the method, calibration curves were obtained by duplicate injections of standard solutions in the concentration range of 50–300 μg mL^{-1}. The graphs of the ratio analyte/internal standard peak height vs. concentration were plotted

TABLE 3 Precicion and Accuracy of the Methods

	Between-day (n=5)			Within-day (n=10)		
Drug	mg/tablet	Precision (CV %)	Accuracy (%)	mg/tablet	Precision (CV %)	Accuracy (%)
Metoprolol	99.77	1.5	0.2	98.4	1.3	1.6
Atenolol	49.60	1.9	0.8	50.11	2.1	0.2
Pindolol	9.82	2.1	1.8	10.14	1.9	1.4
Propranolol	39.96	1.0	0.1	39.59	1.7	1.0

Note: n, number of determinations; CV, coefficient of variation.

and linear regression results showed adequate values of correlation coefficient (r = 0.999) for all four drugs.

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple analyses of the same homogeneous sample, and it was expressed in this work as the coefficient of variation (CV). The accuracy of an analytical procedure expresses the closeness of agreement between a value that is accepted either as a conventional true value or an accepted reference value and the value found, and it was evaluated through the percentage of relative errors. Between-day precision and accuracy was evaluated through the duplicate analysis of samples prepared as described previously for 5 days and the within-day experiment was carried out by analyzing 10 samples on the same day. The results are shown in Table 3. Despite performing the analysis of atenolol, metoprolol, and pindolol with equipment without temperature control, the results obtained in the precision and accuracy studies were as good as those obtained for propranolol, analyzed with temperature control.

Table 4 shows the detection limit and quantification limit obtained for the analysis of the four drugs. The detection limit is defined as the lowest concentration of an analyte in a sample that can be detected, not quantified. It was expressed as a concentration that results in signal-to-noise-ratio of 3. Similarly, for the quantification limit, the signal-to-noise ratio was established at 10 (Shabir, 2003).

The proposed method was applied to the analysis of different pharmaceutical preparations containing β-blockers (Table 5). One of the metoprolol samples was an association with the diuretic hydrochlorothiazide. The analysis of a tablet of this compound prepared with the same procedure of the samples showed no interference.

TABLE 5 **Results of Analyses of Commercially Available Samples**

Drug	Concentration expected (mg/tablet)	Concentration determined (mg/tablet)	Accuracy (%)
Atenolol			
A	50	49.78	0.4
B	50	49.99	0.2
C	50	50.79	1.3
D	50	48.85	2.0
Metoprolol			
A	100	99.68	0.3
B	100	100.80	0.8
C	100	97.66	2.0
Pindolol			
A	5	4.91	1.8
B	10	10.10	1.0
C	10	10.07	0.7
Propranolol			
A	40	39.25	1.8
B	40	40.48	1.2

CONCLUSION

The proposed method was simple, fast, and suitable for the quantification of the selected β-blockers in commercially available tablets with adequate values of accuracy and precision. The preparation of the samples was easy and efficient. Except for the use of methanol during sample preparation, the method does not require the use of organic solvents with their inherent environmental and cost problems. The use of two different pieces of equipment represents an additional advantage of the method, making it suitable for application in any laboratory with minor changes in operational parameters.

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