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# Simultaneous determination of streptomycin and oxytetracycline in agricultural antimicrobials by CZE after an experimental design

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**Abstract**

A capillary zone electrophoresis (CZE) method was developed and validated for the simultaneous determination of both streptomycin (STP) and oxytetracycline (OTC) in bactericidal products to be used in agriculture. Using fused-silica capillaries, the influence of the electrolyte composition, pH and concentration, as well as temperature and applied voltage were investigated using a central composite design to optimize the method. The optimized electrophoretic conditions were as follows: 0.10 M sodium phosphate, pH 2.5, 7.0 kV and 20.0 °C. The method was validated for STP and OTC determination in agricultural formulations through the following performance criteria: linearity and linear range, sensitivity, selectivity, intra-day and inter-day precision, detectability, accuracy and ruggedness. This optimized CZE-method for the identification and quantification of STP and OTC is a potential alternative method to the HPLC methods described by the US Pharmacopeia, with the advantage that the same method could be used for the simultaneous determination of these different antibiotics.


*Keywords:* Bactericides; Streptomycin; Oxytetracycline; Capillary electrophoresis

## 1. Introduction

Antibiotics are chemical substances produced by microorganisms that kill or inhibit the growth of other organisms. Streptomycin (STP) belongs to the group of aminoglycosides that are an important class of antibiotics. The aminoglycosides are bactericidal drugs whose primary effect is to cause irreversible inhibition of bacterial protein synthesis. They are active against both Gram-positive and Gram-negative bacterial infections, have a low cost when compared with other antibiotics and consist of a disaccharide molecule with a streptidine aminocyclitol moiety. Streptomycin, the earliest of the aminoglycosides, is an antimicrobial organic base produced by *Streptomyces griseus* and has found widespread use in both human and veterinary medicine [1,2]. In agriculture it is used to control bacterial and fungal diseases of selected fruits, vegetables, seeds, specialized field crops, ornamental crops and in ornamental ponds and aquaria to control algae [3].

Oxytetracycline (OTC) is a member of the family of the tetracyclines, a group of clinically important natural products and semi-synthetic derivatives characterized by a broad spectrum of activity against pathogenic microorganisms, including Gram-positive and Gram-negative bacteria and protozoa. These compounds are bacteriostatic antibiotics that act by inhibiting the formation of proteins within the bacterial cell. They are used to control bacterial infections in humans and animals and have also found applications in preserving harvest fruits and vegetables, exterminating insect pests and supplementing animal feed. The chemical structures of this group of antibiotics are closely related and are derived from a common hydronaphthacene nucleus containing four fused rings. Oxytetracycline is produced industrially through fermentation by *Streptomyces rimosus* [4,5]. The representative molecular structures of the two antibiotics are given in Fig. 1.

Some countries, including Brazil, allow the use of these antimicrobials as fungicides and/or bactericides in agricultural commodities for protection against pests. Indeed, STP has regulatory status in the United States and in The Netherlands and OTC is registered for agricultural use in the United States [6]. In Brazil, the Ministry of Agriculture approved the use of STP and

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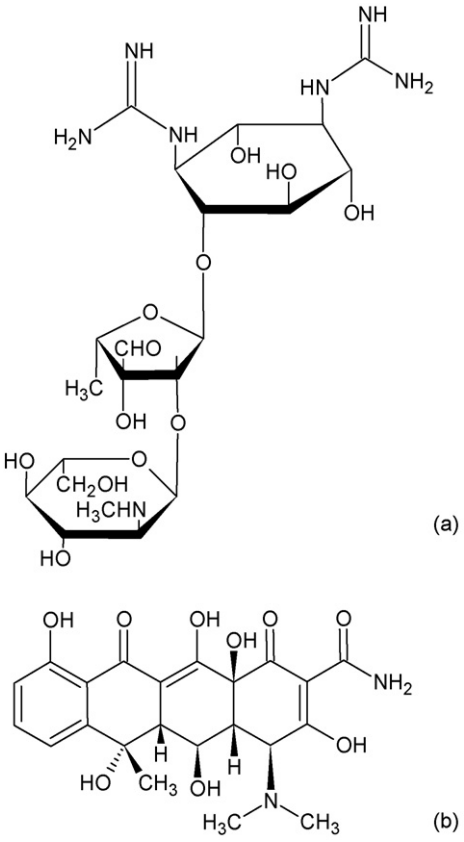


Fig. 1. Structures of (a) streptomycin and (b) oxytetracycline.

OTC for cultivation of tomato, potato, beans, cucumber, coffee, peach, plum, passion fruit and pepper [7].

The determination of aminoglycosides typically requires the utilization of microbiological techniques that are more qualitative than quantitative [8–11]. Chemical methods of analysis have included high performance liquid chromatography (HPLC) with either pre-column [12] or post-column derivatization [13], HPLC with electrochemical detection [14], ion pair chromatography [15], agarose gel electrophoresis [16], immunoassay [17] and mass spectrometry [18,19]. These methods are expensive and time-consuming and interference from other materials is frequent. Thus, a simple, inexpensive method is desired for routine analysis.

The physicochemical properties of STP and OTC, their ionic nature, multiple ionization sites, and water solubility [20] make these compounds suitable for electrophoretic analysis. Capillary zone electrophoresis (CZE) is a powerful separation and quantitation technique that often provides higher resolving power, with shorter analysis times and at a lower cost than HPLC, because the consumption of organic solvents is lower, the quartz capillaries are inexpensive, in addition to the minimal environmental impact. CZE is also suitable for automation, high sample throughput and multiple detection modes [21].

Several studies using capillary electrophoresis for the determination of streptomycin [9,11,22,23,24] or oxytetracycline [4,25,26,27,28,29] in a variety of matrices have been reported, but not for both simultaneously. Hsiao et al. [30] proposed a CZE method for the simultaneous determination of STP and OTC, however these authors did not offer a detailed study about the influence of the composition of the electrolyte on the separation of the compounds or indicate the ruggedness of the method.

This paper describes the development of a simple CZE method for the simultaneous determination of both STP and OTC in bactericidal product to be used in agriculture, using experimental planning. The method was validated and its performance compared with the official HPLC method described in the US Pharmacopeia.

## 2. Experimental

### 2.1. Instrumental and operating conditions

The capillary electrophoresis was performed on a Hewlett-Packard 3D Capillary Electrophoresis system (Germany), equipped with a diode array detector (DAD). Streptomycin and oxytetracycline were detected at 195 nm. Data were collected using the HP 3D Chemstation software from Hewlett-Packard (Germany). The separations were carried out on a fused-silica capillary (75 μm i.d.) with effective (*l*) and total length (L) of 22.5 and 31.0 cm, respectively. Injection was done hydrodynamically for 30 s at 20 mbar. Measurements of pH were made with a DM-20 pH-meter from Digimed (Brazil), using a combined glass electrode. The pH of the electrolyte buffer and dilution buffer were adjusted using 0.10 M NaOH or 10% (v/v) $H_3PO_4$ before making up to volume.

### 2.2. Standards and reagents

Standards of streptomycin sulfate salt and oxytetracycline hydrochloride were purchased from Sigma (USA). Analytical grade disodium hydrogen phosphate, monobasic monohydrate sodium phosphate, sodium hydroxide, hydrochloride acid, phosphoric acid, hydrogen peroxide and methanol were purchased from Merck (Germany).

Throughout the study, water was obtained from a Milli-Q system from Millipore (USA). Before analysis, all the solutions were filtered through 0.45 μm nylon filters from Millipore (Brazil).

### 2.3. Samples

Ten samples of two commercial powder formulations from different batches were purchased at commercial agriculture stores in Alfenas, MG, Brazil. Five commercial samples contained STP and OTC and five commercial samples contained OTC only. All samples were provided from the same manufacturer (Pfizer).

### 2.4. Standard solutions

Standard stock solutions of STP and OTC were prepared by dilution of appropriate volumes of the standards in methanol to a final concentration of 1 mg $ml^{-1}$. These solutions were stored under refrigeration (4 °C) until use. Working solu-

Table 1
Nominal values corresponding to −1 and +1 in the first experimental design

| Variables | −1 | +1 |
|---|---|---|
| pH | 2.5 | 3.0 |
| Voltage (kV) | 5.0 | 6.0 |
| Temperature (°C) | 20.0 | 22.0 |

tions in the concentration range of 20–200 μg ml$^{-1}$ (STP) and 10–210 μg ml$^{-1}$ (OTC) were prepared daily by dilution of the standard stock solution in dilution buffer.

### 2.5. CZE procedure

Before daily use, the capillary was sequentially washed with water (10 min), 1 M NaOH (2 min), 0.10 M NaOH (3 min) and run electrolyte (5 min). During the analyses and after each determination the capillary was sequentially washed with water (1.5 min), 1 M NaOH (1 min), 0.10 M NaOH (1 min) and run electrolyte (2 min).

### 2.6. Experimental design for CZE optimization

A first simple factorial plan ($2^3$) was carried out to distinguish the significant parameters by analysis of the effects. The variables evaluated were pH (2.5–3.0), temperature (20.0–22.0 °C) and voltage (5.0–6.0 kV) (Table 1).

The results of this design were used to plan a subsequent higher order $2^2$ design with central composite, which was performed with the same procedure. The variables evaluated were temperature (19.6–22.4 °C) and voltage (5.8–7.2 kV) (Table 2). All statistical calculations were developed with the software Statistic, Statsoft Inc., v. 5.5 (USA).

The optimized separation conditions for the streptomycin and oxytetracycline determinations were 0.10 M monobasic monohydrate sodium phosphate at pH 2.5, voltage, 7.0 kV and temperature, 20.0 °C.

### 2.7. Method validation

The method was in-house validated using the following performance criteria: linearity and linear range, sensitivity, selectivity, intra-day and inter-day precision, detectability, accuracy and ruggedness. The linearity, linear range, sensitivity and detectability were established through the analytical curves obtained by triplicate analysis of STP and OTC at five concentration levels (20, 60, 100, 140 and 200 μg ml$^{-1}$ and 10, 60, 110, 160 and 210 μg ml$^{-1}$, respectively). The detectabilities for each antibiotic were obtained from three analytical curves and calculated using the following expression: $D = 3s_{y/x}/m$, where $s_{y/x}$ is the standard deviation of the residuals and $m$ is the slope of the analytical curve [31].

Table 2
Nominal values corresponding to −1.41, −1, 0, +1 and +1.41 in the second experimental design

| Variables | −1.41 | −1 | 0 | +1 | +1.41 |
|---|---|---|---|---|---|
| Voltage (kV) | 5.8 | 6.0 | 6.5 | 7.0 | 7.2 |
| Temperature (°C) | 19.6 | 20.0 | 21.0 | 22.0 | 22.4 |

The intra-day precision of the method, expressed as the relative standard deviation of peak area measurements ($n = 5$), was evaluated through the results obtained with the method operating over one day under the same conditions, using solutions of each analyte at a single concentration level: 100 μg ml$^{-1}$ for STP and 110 μg ml$^{-1}$ for OTC. The inter-day precision was determined at the same concentrations levels, and the analyses were performed for 5 days.

The selectivity of the method was evaluated by exposing STP and OTC, at concentration levels of 100 μg ml$^{-1}$ for STP and 110 μg ml$^{-1}$ for OTC, to the following stress conditions: 0.010 and 0.10 M HCl, 0.010 and 0.10 M NaOH, 3% (v/v) $H_2O_2$ and temperature (55 °C) for 1 h and 0.010 M HCl, 0.010 M NaOH, 3% (v/v) $H_2O_2$ for 24 h. The solutions were analyzed considering the resolution between analyte and other substances formed during the experiment and the analytical signal before and after the exposure of the analyte to the stress conditions, expressed as recovery [32].

The accuracy of the method was evaluated through analyses of samples of bactericidal products containing STP and OTC by the proposed CZE method using the optimized procedure and by HPLC according to the method described in the US Pharmacopeia [33] for OTC. Recovery tests were used for STP.

The susceptibility of the analytical method to changes was tested by evaluating the ruggedness of the method using a $2^2$ experimental design (Table 2).

### 2.8. Sample analysis

A proper amount of each formulation from the same batch was weighed into a 50 ml volumetric flask to result in a final concentration of 1 mg ml$^{-1}$ and diluted using the dilution buffer (0.010 M disodium hydrogen phosphate, pH 7.0). The mixture was sonicated for about 5 min, allowed to cool, diluted to volume and filtered through 0.45 μm membrane filters Millipore (USA). A proper aliquot was transferred to an autosampler vial. All samples were analyzed in quintuplicate.

The analyses by CZE were carried out as described above and quantitation was accomplished through an external calibration curve with five concentration levels in the range of 20–200 μg ml$^{-1}$ (STP) and 10–210 μg ml$^{-1}$ (OTC). All samples containing OTC were also analyzed by HPLC using the method described in the US Pharmacopeia [33].

## 3. Results and discussion

### 3.1. CZE method development

The running electrolytes recommended in the literature for the determination of OTC by CZE are sodium phosphate (pH 2.0) or sodium carbonate (pH 11.2) [25,26] and for STP are sodium tetraborate (pH 9.0 or 10.25) or sodium dihydrophosphate, boric acid and sodium tetraborate (pH 6.35) [9,11,22]. Thus, in order to optimize the simultaneous determination

of STP and OTC different compositions of background electrolyte were evaluated: sodium phosphate (pH 2.0 and 8.5), sodium carbonate (pH 8.5 and 10) and sodium tetraborate (pH 9.0).

Using 0.10 M phosphate at pH lower than 4.0 both compounds were separated. At higher pH values STP was not detected until 20 min. The other electrolytes did not separate the two components of the test mixture.

In acidic medium (pH < 4.0) the eletroosmotic flow is very low and the eletrophoretic flow is responsible for the movement of the ions through the capillary. Considering the p$K$ values of OTC ($pK_{a1} = 3.2$, $pK_{a2} = 7.5$ and $pK_{a3} = 8.9$) [34] and the basic character of STP, in pH lower than 4.0, OTC, as well as STP will be protonated, i.e. positively charged and the molecules move as a function of their eletrophoretic flows. Due to the differences in the charge/mass ratio, the separation is possible even in the absence of the eletroosmotic flow.

For the determination of the most relevant variables, a first experimental design using a $2^3$ design was conducted: pH (2.5–3.0), temperature (20.0–22.0 °C), and voltage (5.0–6.0 kV) (Table 1). The highest signals for STP and OTC were obtained at pH 2.5. At pH 2.0 and 3.0 the current produced in the capillary increased during the analysis. The same occurs at pH 4.0 where the signal of the peak of OTC decreased. This study showed that voltage has a significant negative influence. This means that a decrease in voltage improves the signal of the analytes but the migration times become too long. The temperature has a very low positive influence with no influence on migration times. Thus temperature = 21.0 °C and voltage = 6.5 kV as central points for the next plan were chosen.

The next step was the optimization of the concentration of the sodium phosphate electrolyte. Concentrations of 0.020, 0.050 and 0.10 M were evaluated. The best results were obtained using a solution prepared at 0.10 M. At 0.020 and 0.050 M the current obtained was very low, increasing the migration time and decreasing the peak areas.

The analytes were detected at 195 nm. Others wavelengths were also evaluated (200 and 205 nm). However, the sensitivity of STP decreased in comparison to detection at 195 nm.

After establishing the electrolyte composition, concentration and pH, a $2^2$ central composite design was performed for the two significant variables (temperature and voltage) in order to refine the optimal conditions for the separation of STP and OTC by CZE and to evaluate the ruggedness of the method. The experimental design was thus constructed by the use of a full $2^2$ factorial design with three central and four axial points ($\alpha = \pm 1.41$). This procedure offers an efficient route for determining the best resolution from a selected number of conditions [35]. The conditions are presented in Table 2.

Fig. 2 shows the response surfaces and the influence of the parameters temperature and voltage on the signals for STP and OTC. Due to the great difference between the chemical structures of the STP and OTC, the response surfaces had distinct aspects. The equations of the model are: $Y = 268.47 - 18.15X_1 + 4.07X_2 - 31.42X_1^2 - 17.23X_2^2 - 19.50X_1X_2$ ($R^2 = 0.9203$) for STP and $Y = 1276.32 + 76.31X_1 + 139.89X_2 + 179.27X_1^2 + 96.10X_2^2 - 315.00X_1X_2$ ($R^2 = 0.8109$) for OTC.

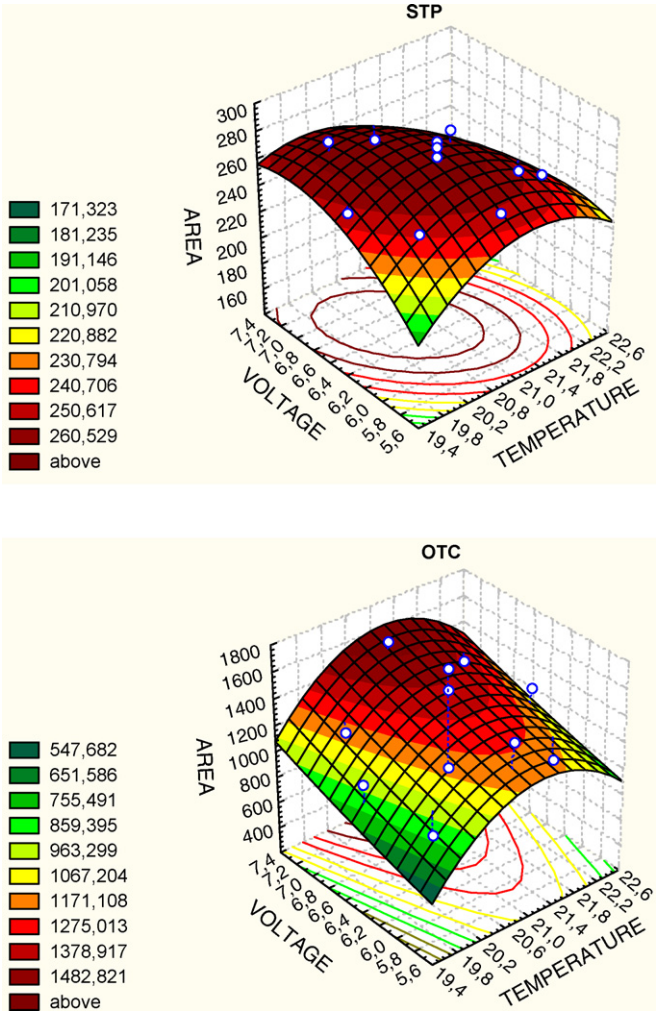


Fig. 2. Response surfaces for streptomycin (STP) and oxytetracycline (OTC) showing the area as a function of significant parameters voltage (kV) and temperature (°C), obtained using a $2^2$ experimental design with central composite design.

Considering the response surfaces, the best conditions for the simultaneous determination of OTC and STP were: running electrolyte: 0.10 M monobasic sodium phosphate, voltage: 7.0 kV, temperature: 20.0 °C and detection: 195 nm.

A typical electropherogram of STP and OTC using the optimized conditions established through the experimental design is presented in Fig. 3.

Before method validation, a system suitability test was performed. This test provides assurance that a system's performance is appropriate for the intended use. The following parameters were evaluated: plate count ($N$), resolution ($R_s$), and tailing factor ($T$). The results of resolution and tailing factor obtained were within the acceptable range ($R_s > 2$ and $T \leq 2$), according to Shabir [32]. The results are presented in Table 3.

### 3.2. Method validation

The CZE method was in-house validated for the analyses of the STP and OTC by evaluation of the following parameters: lin-

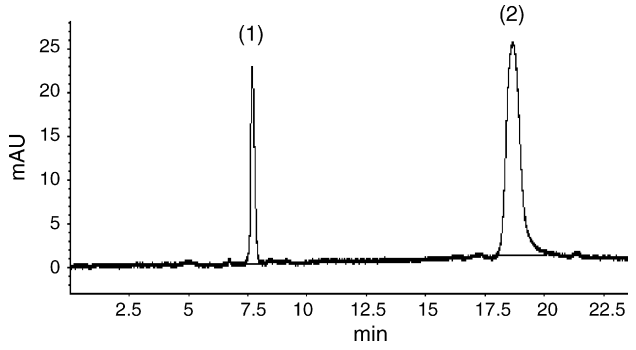


Fig. 3. A typical electropherogram of 25 μg ml$^{-1}$ of (1) streptomycin and (2) oxytetracycline ($t_{m1}$ 7.7 and $t_{m2}$ 18.7 min, respectively). Capillary: uncoated fused silica; background electrolyte, a solution of 0.10 M sodium phosphate, pH 2.5; temperature, 20.0 °C; applied voltage, 7.0 kV; detection wavelength, 195 nm.

Table 3
System suitability parameters for CZE

| Parameters | STP | OTC |
|---|---|---|
| Plate number/*m* | 102276 | 155087 |
| Resolution ($R_s$) | 2.6 | – |
| Tailing factor (*T*) | 0.92 | 1.13 |
| Migration time (min) | 7.7 | 18.7 |

ear range, linearity, sensitivity, detectability, intra- and inter-day precision. The results are summarized in Table 4. The accuracy was evaluated by comparing the results obtained from the analysis of bactericidal products by the proposed CZE method with those obtained using the recommended HPLC method described in the US Pharmacopeia [33] for OTC. Recovery tests were used to evaluate the accuracy of STP [31,32]. The results are shown in Tables 5 and 6.

The linearity, linear range and sensitivity were obtained from analytical curves at five concentration levels for each analyte under study, with triplicate analyses. The linearity was tested using a pure error lack of fit test with simple regression, which was not significant at the 5% level.

The precision of the method for STP and OTC was evaluated using the results obtained over 1 day of operation under the same conditions (intra-day) and for 5 days (inter-day). The results are expressed as relative standard deviations (R.S.D.) in Table 4 and were lower than 2.0% for both intra- and inter-day evaluations. Considering that regulatory agencies [31,32,33] recommend that

Table 4
Quantitative features for STP and OTC

| Parameter | STP | OTC |
|---|---|---|
| Linear range (μg ml$^{-1}$) | 20–200 | 10–210 |
| Sensitivity (aU μg ml$^{-1}$) ($P < 0.05$) | 10.77 | 34.76 |
| Linearity (*r*) | 0.9997 | 0.9998 |
| Intercept ($P < 0.05$) | −50.7 | 30.7 |
| Intra-day precision, $n = 5$ (R.S.D. %)[a] | 0.22 | 0.71 |
| Inter-day precision, $n = 5$ (R.S.D. %)[a] | 1.13 | 1.75 |
| Detectability (μg ml$^{-1}$) | 6 | 2 |

R.S.D.: relative standard deviation.
[a] Standard concentration: 100 μg ml$^{-1}$ (STP) and 110 μg ml$^{-1}$ (OTC).

Table 5
OTC content determined in commercial samples of bactericidal formulations by CZE and HPLC

| Sample | CZE | | HPLC | |
|---|---|---|---|---|
| | Average content[a] (g kg$^{-1}$) | R.S.D. (%) | Average content[a] (g kg$^{-1}$) | R.S.D. (%) |
| 1 | 16.0 | 0.4 | 16.5 | 0.4 |
| 2 | 16.1 | 0.2 | 16.4 | 0.1 |
| 3 | 15.1 | 0.3 | 15.3 | 0.1 |
| 4 | 16.2 | 0.2 | 16.6 | 0.1 |
| 5 | 15.4 | 0.6 | 15,5 | 0.2 |
| 6 | 227.8 | 4.9 | 231.9 | 6.0 |
| 7 | 231.9 | 5.1 | 232.2 | 1.3 |
| 8 | 230.7 | 4.3 | 237.8 | 1.0 |
| 9 | 230.2 | 9.4 | 228.8 | 1.8 |
| 10 | 231.7 | 7.0 | 229.0 | 2.1 |

Nominal value: samples 1–5 = 15 g kg$^{-1}$; samples 6–10 = 200 g kg$^{-1}$.
[a] $n = 5$; R.S.D., relative standard deviation.

the precision should be lower than 2%, the values obtained by the CZE method are acceptable.

The limit of detection represents the lowest concentration of an analyte in sample solutions to be introduced in the CE equipment that can be detected and has been included only to provide information about the detectability of the method. The quantitation limit of the method is not presented, due to the fact that the active compound is the major constituent of the formulations and this parameter is not required for method validation for the quality control of bactericidal products. Furthermore, this limit would depend on sample dilution before analysis.

The selectivity of the method indicates the ability of the method to accurately measure the analyte response in the presence of all potentially interfering sample components or degradation products [32]. In this study the selectivity was evaluated by exposing the analyte to stress conditions, such as temperature, acid, base and an oxidizing medium. The solutions were analyzed considering the resolution between the analyte and other substances formed during the experiment and the analytical signal before and after exposure of the analyte to the stress conditions. The results are presented in Fig. 4. The stability of the analytes depends on their chemical structure and many differ-

Table 6
STP content determined in commercial samples of bactericidal formulations by CZE and recovery tests

| Sample | CZE | | Recovery[b] | |
|---|---|---|---|---|
| | Average content[a] (g kg$^{-1}$) | R.S.D. (%) | Average content[a] (g kg$^{-1}$) | R.S.D. (%) |
| 1 | 178.8 | 5.3 | 176.6 | 1.7 |
| 2 | 175.4 | 6.3 | 175.7 | 2.1 |
| 3 | 173.3 | 1.3 | 172.2 | 2.0 |
| 4 | 171.4 | 2.9 | 170.7 | 3.8 |
| 5 | 177.0 | 4.5 | 175.5 | 3.8 |

Nominal value: samples 1–5 = 150 g kg$^{-1}$.
[a] $n = 5$; R.S.D., relative standard deviation.
[b] Amount added: 20, 40 and 60 g kg$^{-1}$.

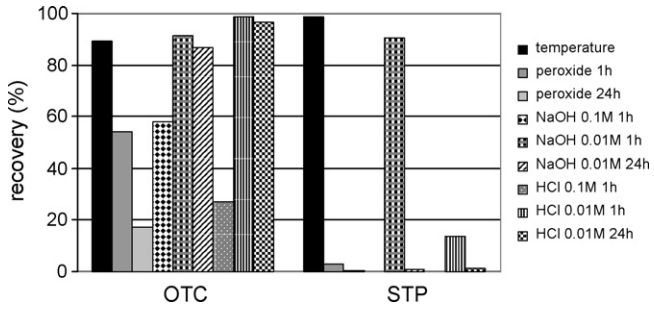


Fig. 4. Recoveries (%) of streptomycin (STP) and oxytetracycline (OTC) after exposure of standard solutions (100 μg ml$^{-1}$) to temperature (55 °C), 0.010 and 0.10 M HCl, 0.010 and 0.10 M NaOH and 3% (v/v) $H_2O_2$ for 1 h and 0.010 M HCl, 0.010 M NaOH and 3% (v/v) $H_2O_2$ for 24 h.

ences between STP and OTC recoveries were observed. Under the four stressing conditions, OTC was more stable than STP under the same conditions, except for temperature. In some conditions (0.10 M NaOH and 0.10 M HCl for 1 h) of this study, STP degrades completely. The degradation products formed under all the stress conditions had migration times significantly different from their corresponding parent analyte, thus confirming the selectivity of the method.

The accuracy of the method was assessed for STP and OTC by analyzing ten samples of two commercial formulations: five commercial samples containing STP and OTC (numbers 1–5) and five commercial samples containing only OTC (numbers 6–10). All samples were analyzed by the CZE method developed and by the US Pharmacopeia method recommended to detect and quantify OTC. The samples, which contained STP in their formulation were also analyzed using recovery tests. The mean values obtained using the proposed and the reference method did not differ significantly ($P < 0.05$) for all samples (Tables 5 and 6). Some of the results obtained for the OTC content in commercial samples, by both CZE and HPLC, were not in compliance with the Pharmacopeia recommendation (not less than 90.0 percent and not more than 110.0 percent of the labeled amount of oxytetracycline hydrochloride) [33]. The nominal values for OTC were 15 and 200 g kg$^{-1}$ for samples 1–5 and 6–10, respectively. The amount determined in relation to the labeled amount was in the range of 100.7–115.9% (using CZE) and 102.0–118.9% (using HPLC). In relation to STP, the Pharmacopeia has not yet included the specific monograph for soluble powder. Nevertheless, the amount determined in relation to the labeled amount (nominal value is 150 g kg$^{-1}$) was 114.3–119.2% (using CZE) and 113.8–117.7% (after recovery tests).

The ruggedness of the method is assessed when introducing small changes to the procedure and examining the effect on the results (analytical signal). In this study, ruggedness was observed by the counter plot obtained by the $2^2$ central composite design described in 2.7. The counter plots for STP and OTC are presented in Fig. 5. Whereas the analytical signal of STP was less influenced by small variations in temperature and applied voltage, OTC was more affected by small variations in these parameters. In order to guarantee consistent results, it is important that the range of each variable that produces acceptable results be incorporated in the analytical procedure. The results obtained in this study allowed establishing the following parameters: voltage 7.0 ± 0.2 and temperature 20.0 ± 0.5 °C. This small zone of ruggedness is common with electrophoretic methods.

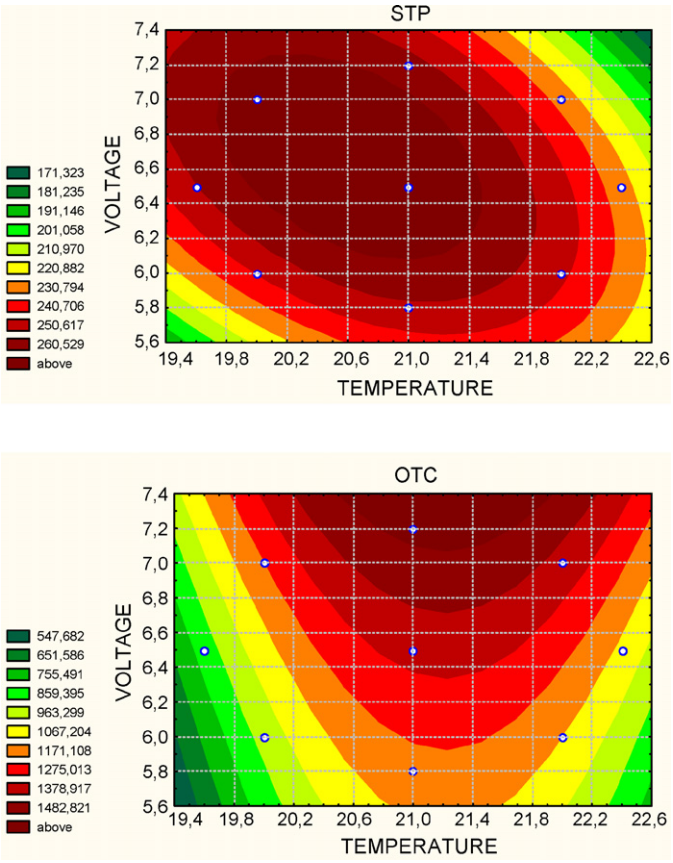


Fig. 5. Counter plots for streptomycin (STP) and oxytetracycline (OTC) used to evaluate the ruggedness of the method selected by the $2^2$ experimental design as a function of significant parameters voltage (kV) and temperature (°C).

## 4. Conclusions

This paper describes a relatively simple, rapid and accurate CZE method for the determination of streptomycin and oxytetracycline in bactericidal products to be used in agriculture. This method may offer an alternative to the procedures currently required in the monographs described in the US Pharmacopeia, which recommends analysis by microbial assays that are quite time consuming or HPLC that requires the use of large volumes of HPLC-grade solvents.

The results obtained in this work confirm that the CZE method, when properly optimized and validated, fulfills all the pre-established requirements based on international regulations and is adequate to be used in the quality control of bactericidal products with the advantage that the same method could be used for the simultaneous determination of these structurally different antibiotics streptomycin and oxytetracycline.

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