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Simultaneous determination of cloramphenicol, salicylic acid and resorcinol by capillary zone electrophoresis and its application to pharmaceutical dosage forms

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Abstract

This study demonstrates the separation of active ingredients in acne formulations (salicylic acid, cloramphenicol and resorcinol in presence of azulene) by capillary zone electrophoresis. Factors affecting their separations were the buffer pH and concentration, applied voltage, sample preparation, and presence of additives. Optimun results were obtained with a 50 mM sodium tetraborate–50 mM sodium phosphate, pH 9.0. The carrier electrolyte gave baseline separation with good resolution, short migration times (< 6 min), great reproducibility and accuracy. Calibration plots were linear over at least three orders of magnitude of analyte concentrations, the lower limits of detection being within the range 0.39–1.25 μg ml^{-1}. The procedure was fast and reliable and commercial pharmaceuticals could be analysed without prior sample clean-up procedure.

Keywords: Capillary zone electrophoresis; Salicylic acid; Cloramphenicol; Resorcinol monoacetate; Pharmaceutical quality control

1. Introduction

Acne is one of the most common of all skin problems. It affects most teenagers to some degree and even more adults. This one is a disease of the sebaceous glands that affects 80% of the population between 12 and 25 years old [1]. Astringent alcoholic lotions are generally used for its treatment. Common active compounds in acne formulations are antiseptics such as salicylic acid (SA), parabens and cresols useful against inflamation and seborrhea, and bacteriostatic agents such as cloramphenicol, tetracyclines and erythromicine [2–5].

The determination of ingredients in pharmaceuticals, is critical to keep the quality of the

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preparations high. To ensure the safety and efficacy of the final marketed product, it is important to characterize drug substance material and formulations.

Strict requirements by regulatory agencies [6–9] require fine characterization of drug substances, isomeric forms, degradations products, excipients, impurities, contaminants, and possible micromolecular interaction of components in finished pharmaceutical products. Furthermore, the requirements for stability tests [10–13], which are usually performed on hundreds of samples, require reliable, rugged, and fast analytical tests that are crucial in the control and research laboratories in order to meet these demands.

The measurement of chemical properties is routinely performed by HPLC and other chromatographic techniques [14,15]. Capillary electrophoresis is an effective tool for drug quality control [16–20]. It provides results with substantial advantages, not only in expediency, but also in ease of operation. Furthermore, relatively low volumes of electrolyte solution are required for the electrophoretic run.

The purpose of this study was to develop a CE methodology for the separation and simultaneous determination of SA (an antiseptic agent), resorcinol monoacetate (euresol (EU), an antiinflamatory) and chloramphenicol (CL, bacteriostatic), in presence of azulene. Separations of these compounds were obtained by capillary zone electrophoresis (CZE). The effects of pH, buffer concentration, sampling and separation modes were investigated. The best results were obtained with a BGE containing 50 mM borate–phosphate.

2. Experimental

2.1. Instrumentation

A Beckman P/ACE MDQ instrument (Beckman Instruments, Inc. Fullerton, CA) equipped with a diode array detector and a data handling system comprising and IBM personal computer and P/ACE System MDQ software. Detection was performed at 225 and 298 nm. The fused-silica capillaries were obtained from MicroSolv Technology Corporation and had the following dimensions: 67 cm total length, 50 cm effective length, 75 μm ID, 375 μm OD. The temperature of the capillary and the samples was maintained at 25 °C. The pH of the electrolyte was measured by an Orion 940 pHmeter equipped with a glass-combined electrode.

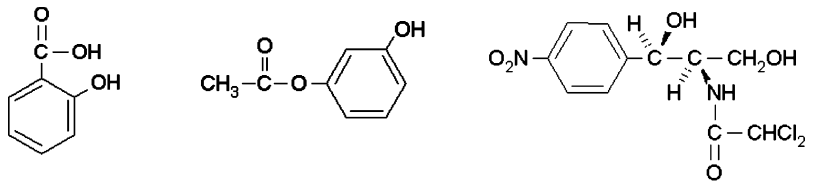
2.2. Chemicals

The structure and formulae of the compounds studied are shown in Fig. 1. SA, cloramphenicol, resorcinol monoacetate (EU) and azulene were purchased from Sigma Chemical Co. (St. Louis, MO). Acnoxin™ was manufactured and supplied by Androquímica Laboratories (San Luis, Argentina); sodium tetraborate ($Na_2B_4O_7 \cdot 10\ H_2O$) and sodium dihydrogenphosphate (NaH_2PO_4) by Mallinckrodt (St. Louis, USA). The water used in all studies was ultra-high-quality water obtained from a Barnstead Easy pure RF compact ultrapure water system. All other reagents and solvents were of analytical grade quality. All solutions were degassed by ultrasonication (Testlab, Argentina). Running electrolytes and samples were filtered through a 0.45-μm Titan syringe filters (Sri Inc., Eaton Town, NJ).

2.3. Procedure

The electrolyte solution was prepared daily and filtered through a 0.45-μm titan syringe filters (Sri Inc.). At the beginning of the day, the capillary was conditioned with 0.1 mol l^{-1} NaOH for 5 min, followed by water for 5 min, and then with running electrolyte for 10 min before sample injection. To achieve high reproducibility of migration times and to avoid solute adsorption, the capillary was washed between analyses with sodium hydroxide for 2 min, followed by water for 2 min, then equilibrated with the running buffer for 4 min. Samples were pressure-injected at the anodic side at 0.5 psi for lengths of time 3–7 s. A constant voltage was used for all the experiments.

Stock standard solutions containing SA, EU and CL were prepared in buffer at concentrations of 10–100 mg l^{-1}. For the case of EU, it was



1 2 3

Fig. 1. Chemical structures of (1) SA; (2) EU and (3) CL.

previously dissolved in ethanol, and then suitable diluted in buffer to obtain a final ethanol concentration of 30%. All solutions were filtered through a 0.45 μm membrane prior injection.

A combined standard solution containing SA, EU and CL was prepared by accurately weighing 10 mg of each powder and made up to 100 ml.

A diluted solution of the commercial formulation was prepared as follows: 2 ml of the lotion (Acnoxin) were carefully measured into a volumetric flask and diluted to 1000 ml with buffer solution. The solution was mixed and filtered through a 0.45-μm membrane.

3. Results and discussion

3.1. Method development

In order to propose a specific and accurate way of analysing pharmaceuticals products containing SA, EU and CL, by using CZE, it is essential to find the best experimental conditions in which the analytes can be separated from each other. The optimization was performed using a synthetic mixture of SA, EU and CL. The following parameters were consecutively optimized: samples conditioning, pH, BGE composition and concentration, samples and capillary temperatures, and other electrophoretic parameters.

3.2. Effect of pH

The buffer pH plays an important role for improving selectivity in CE especially for closely related compounds, because it affects both the overall charges of the solute and the electroosmotic flow (EOF).

The pK_a-values are 5.5 for CL; 3.0 (25 °C) for SA and 10.1 (20 °C) for resorcinol monoacetate. Thus, the effect of the buffer pH was investigated within the range of 6.0–10.0 at a fixed buffer concentration, adjusted by 0.1 mol l^{-1} NaOH and 0.1 mol l^{-1} HCl. It was found that when the pH was lower than 8.5, the resolution was poor (Table 1). At pH 9.0 baseline separation was achieved.

3.3. Effect of buffer composition and concentration

Buffer concentration has also a significant effect on the separation performance through its influence on the EOF and the current produced in the capillary. Different BGEs have been tested, but one producing the best results considering selectivity, reproducibility, baseline and current performance, was sodium dihydrogenphosphate–sodium tetraborate.

Keeping other parameters constant (pH: 9.0, 25 kV, 25 °C) the buffer concentration was varied from 10 to 75 mM. Increases in migration times as well as current were observed when the concentration of buffer increased. Resolution also increased

Table 1
Effects of pH on the migration times and electrophoretic mobilities (μ_e)

	pH 6.0		pH 9.0	
	tm (min)	μ_e (cm^2 V^{-1} s^{-1})	tm (min)	μ_e (cm^2 V^{-1} s^{-1})
EU	4.19	1.17×10^{-5}	3.26	-5.89×10^{-5}
CL	5.22	-1.05×10^{-4}	4.28	-2.22×10^{-4}
SA	5.26	-1.08×10^{-4}	5.10	-3.06×10^{-4}

EU, euresol; CL, chloramphenicol; SA, salicylic acid. Conditions: capillary, 67 cm full length, 50 cm effective length, 75 μm ID, 375 μm OD; hydrodynamic injection at 0.5 psi, 5 s; 25 kV constant voltage; 25 °C, detection by UV absorbance at 225 nm for salicylic acid and 298 nm for the other compounds.

for higher buffer concentrations, but no appreciable improvements were observed for buffer concentrations above 50 mM. So, baseline separation was obtained with 50 mM sodium dihydrogenphosphate–sodium tetraborate buffer, pH 9.0 (Fig. 2).

3.4. Method validation

The calibration plots were measured under the optimal experimental conditions over the concentration range 10–100 μg ml^{-1} for EU, SA and CL. The migration times for EU, CL and SA were 3.26, 4.28 and 5.1 min, respectively. They were obtained representing the ratio of the corrected areas vs concentration. Six point of calibration curve were

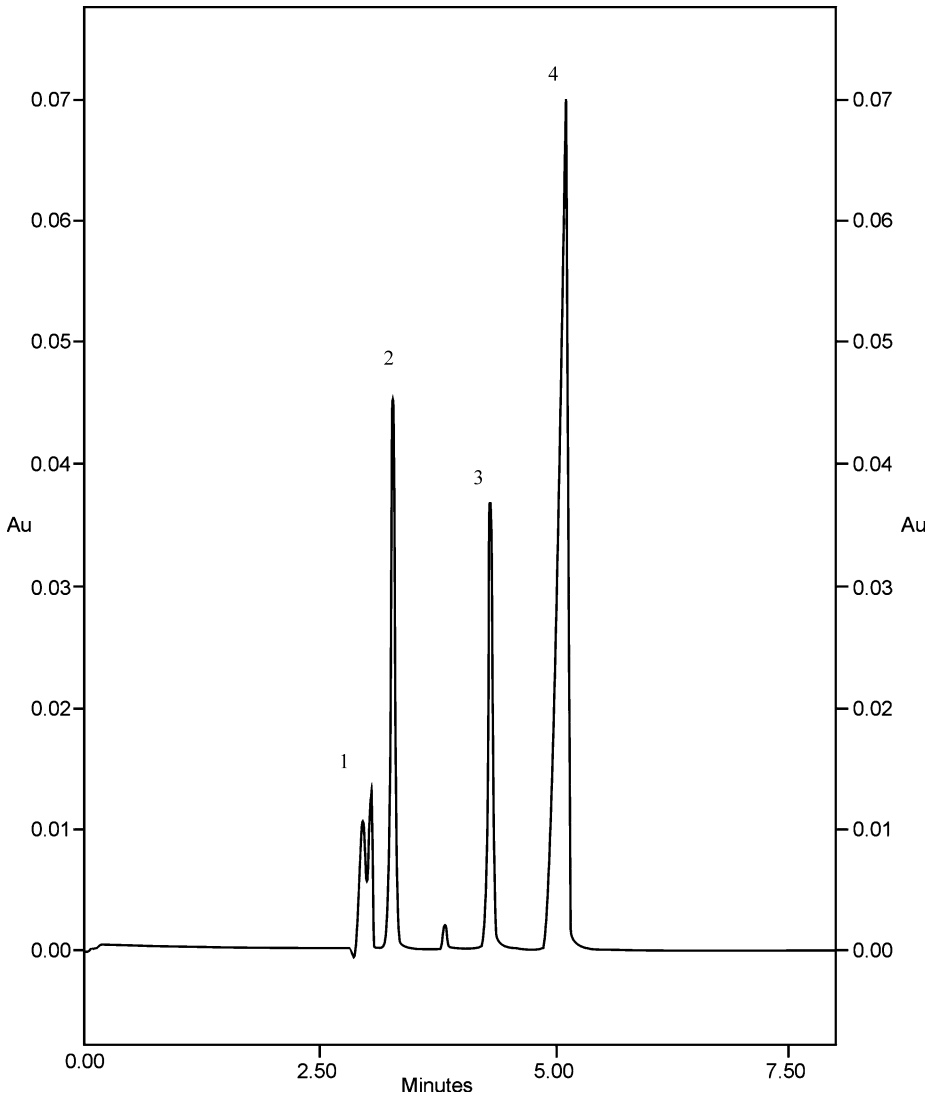


Fig. 2. Electropherogram of a diluted commercial formulation (Acnoxin™). Conditions: 50 mM sodium tetraborate–sodium dihydrogenphosphate buffer, pH 9.0; capillary, 67 cm full length, 50 cm effective length, 75 μm ID, 375 μm OD; hydrodynamic injection at 0.5 psi, 5 s; 25 kV constant voltage; 25 °C, detection by UV absorbance at 298 nm. Peak identification: (1) azulene; (2) EU; (3) CL; (4) SA.

Table 2
Quantitative parameters of the analysis of SA, EU and CL by CZE in pharmaceutical samples

	EU	CL	SA
Calibration range (μg ml^{-1})	10–100	10–100	10–100
Slope of the calibration curve ± ts[a] (95%; $n=6$)	$0.8\times10^{6}\pm1.0\times10^{4}$	$1.6\times10^{6}\pm1.4\times10^{4}$	$4.8\times10^{6}\pm1.9\times10^{4}$
Intercept ± ts[a] (95%; $n=6$)	$2120\pm0.9\times10^{2}$	$1383\pm1.1\times10^{2}$	$12\,277\pm7.2\times10^{2}$
Regression coefficient of the calibration (r^2)	0.997	0.999	0.999
Detection limit (μg ml^{-1})	1.25	0.39	0.42
Quantitation limit (μg ml^{-1})	4.16	1.30	1.42

[a] Standard deviation

determined, and three replicate injections of standards at each concentration level were performed. The calibration equations were calculated by the least-squares linear regression method, and unknown concentrations were calculated by interpolation. The detection and quantitation limits were calculated as the analyte concentrations that give rise to peak heights with a signal-to-noise ratio of 3 and 10, respectively. The LOD was determined by injecting standard combined solution at three different level concentrations for each analyte (15, 30 and 50 μg ml^{-1}) Table 2 shows the concentration ranges for calibration curves of each analyte, regression parameters and limits of detection and quantitation.

In order to determine the repeatability (within-day precision) of the method, replicate injections ($n=6$) of 50 μg ml^{-1} combined solution containing SA, EU and CL were carried out. In all cases, the precision was better than 0.7% for the migration time and 3.4% for the peak area. Good peak area precision was achieved without adding any internal standard.

Intermediate precision (between-day precision) was also evaluated over 3 days by performing six injections each day. Intermediate precision

Table 3
Commercial formulation[a] recovery test

	Base value (μg ml^{-1})	Quantity added (μg ml^{-1})	Quantity found[b] (μg ml^{-1})	Recovery (%)[c]
Aliquot I				
SA	–	0.0	19.86	–
EU	–	0.0	39.79	–
CL	–	0.0	10.10	–
Aliquot II				
SA	19.86	50.0	69.83	99.94
EU	39.79	0.0	–	–
CL	10.10	0.0	–	–
Aliquot III				
SA	19.86	0.0	–	–
EU	39.79	50.0	89.62	99.66
CL	10.10	0.0	–	–
Aliquot IV				
SA	19.86	0.0	–	–
EU	39.79	0.0	–	–
CL	10.10	50.0	59.87	99.54

EU, euresol; CL, chloramphenicol; SA, salicylic acid.
[a] Diluted solution of the commercial formulation, Acnoxin™.
[b] Mean value ($n=6$).
[c] 100 × ((Found-base)/added).

(R.S.D.) on the basis of migration time and peak area were better than 0.52 and 2.71%, respectively.

Repeatability of the method was performed by two analysts (six determinations) using the proposed method and the same instrumentation. The results showed no significant differences: 0.8 (R.S.D.%).

3.5. Assay of a commercial product. Recovery test

Once the conditions for separation and quantitation were established, the CE method was applied to the determination of cloramphenicol, SA and EU in a commercial formulation (Fig. 2). The electropherogram in Fig. 2 shows no interference between active compounds and excipients of the commercial samples.

In order to determine the accuracy of this method, a 50 m1 of the diluted solution of the commercial formulation was collected and divided into 10 portions of 5 ml each. The proposed method was applied to six portions and the average concentrations determined for each compound (CL, SA and EU) were taken as a base value. Then, known quantities of the analytes were added to the other aliquots, and the active compounds determined following the recommended procedure (Table 3).

4. Conclusion

CZE was investigated for the separation of three pharmaceutical ingredients. The active compounds were determined with high efficiency in a short period of time (<6 min). The sample excipients did not interfere with the proposed method. This method may be considered for the routine analysis of a large number of samples. It has been validated and shows a good performance with respect to selectivity, intermediate precision, linearity and accuracy, and it offers a simple, fast, inexpensive and precise way for the determination of SA, EU and CL in a pharmaceutical formulation.

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