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Determination of sulfametoxazole, sulfadiazine and associated compounds in pharmaceutical preparations by capillary zone electrophoresis☆

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Abstract

A capillary zone electrophoresis method is presented to separate sulfadiazine, sulfamethoxazole, trimethoprim, bromhexine and guaiacol by using a fused-silica capillary (60.2 cm×75 μm I.D.). The separation was carried out at 30 kV and 25°C in a 15 m*M* phosphate buffer adjusted to pH 6.2 as electrolyte. Under these conditions, the run time was 6 min and the limits of quantification were about 1 mg/l for every component. The method was applied to pharmaceutical preparations and the results provided recoveries close to 100%.

Keywords: Pharmaceutical analysis; Sulfametoxazole; Sulfadiazine; Trimethoprim; Bromhexine; Guaiacol

1. Introduction

Sulfonamides have been widely used as anti-bacterial agents in medicine as well as in veterinary practice. Nowadays, pharmaceutical and veterinary commercials contain sulfonamides in conjunction with other compounds in order to increase their activities. Such compounds are called potentiators.

Illnesses related to the respiratory system, such as bronchitis, pneumonia or sinusitis are usually treated with the combinations sulfamethoxazole–trimethoprim (SMX–TMP) or sulfadiazine–trimethoprim (SDZ–TMP). In some commercials, the therapeutic action of these combinations can be completed with an expectorant and/or a pulmonary balsamic–antiseptic agent: bromhexine (BRO) and guaiacol (GUA), respectively.

The separation techniques provide analytical methods to resolve these combinations, with the purpose of identifying and quantifying these compounds in mixtures. Owing to the fact that the combination TMP–SMX is very common in the pharmaceutical and veterinary practices, several liquid chromatographic methods for the determination of both of them have been reported, not only in biological samples [1–3] but also in commercials [4,5]. TMP can be associated with SDZ, too. This mixture has been resolved in pharmaceutical commercials [6] by using UV absorption spectrophotometry and in mi-

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crobiological samples [7] by high-performance liquid chromatography (HPLC).

Some papers make reference to the separation of sulfonamides by capillary electrophoresis (CE). In these papers, the separation of a lot of sulfonamides is carried out in order to show the pH and pK_a its limitations in capillary zone electrophoresis (CZE) analysis and the high efficacy of this technique [8–11]. In addition, some works show methods for determining these compounds in milk [11] or pork meat extracts [12], even though no recoveries are shown. However, there are few papers reporting the determination of sulfonamides in pharmaceuticals by CZE. The introduction of modifiers such as β-cyclodextrins [13,14] and sodium dodecyl sulfate (SDS) as micellar phase [15] have contributed to widen the possibilities that CE has for the determination of these drugs and its application to commercial preparations.

In this way, our group has been doing research, for a long time, into the possibilities offered by CE for the determination of sulfonamides and their potentiators (or associated compounds) [16] in pharmaceutical commercials. As a result, regarding the routine analysis of these kinds of drugs, this paper presents a new, accurate and easy CZE method for the determination of the mentioned mixture.

2. Experimental

2.1. Apparatus

A Beckman MDQ CE system equipped with a diode array detector was used. The system was controlled by a personal computer equipped with P/ACE software.

The separations were carried out in a fused-silica capillary of 60.2 cm (50 cm effective length)×75 μm I.D.×375 μm O.D. housed in a cartridge with a 800×100 μm detector window. The capillary was conditioned by flushing first with 0.1 *M* NaOH for 30 min and then with water for 10 min before it was used for the first time.

A Crison micro-pH 2002 instrument was used for pH measurements.

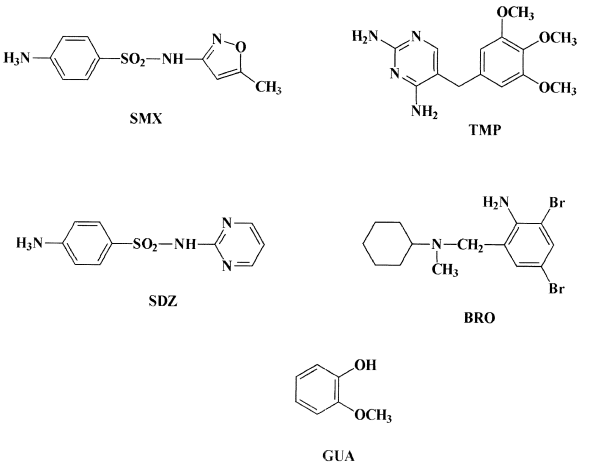

Fig. 1. Chemical structures of the mixture compounds.

2.2. Reagents

All solvents and reagents were of analytical grade unless indicated otherwise. Solutions were prepared with deionized water (Milli-Q quality). Potassium salt of guaiacol (GUA), bromhexine hydrochloride (BRO), sulfamethoxazole (SMX) and trimethoprim (TMP) (see Fig. 1) were from Sigma (Germany and USA). Sulfadiazine (SDZ) (Fig. 1) was from Vorquímica (Vigo, Spain).

Stock solutions (100 mg/l) of TMP and SMX were prepared in ethanol–water (50:50), BRO in ethanol–water (10:90) whereas SDZ and GUA stock solutions were prepared in water.

Buffer solutions were prepared by dissolving the adequate quantity of NaH_2PO_4 in deionized water and then adjusting with HCl or NaOH to the required pH. All these reagents were from Panreac (Barcelona, Spain).

3. Results and discussion

3.1. Preliminary experiments

A solution containing 20 mg/l of each component, which will be called ''Q'', was prepared by diluting the stock solutions of the five compounds.

In order to select a pH for the separation, it was necessary to take into account that BRO is not

soluble at pH>7. In addition, using pH 5.5 (acetate buffer), the peaks of BRO and TMP overlapped partially. This behavior made us work in a very narrow pH range, from 5.5 to 7.0. At a pH close to 6.0 the BRO and TMP peaks start to separate from each other, but at pH 6.5, the BRO peak begins to decrease, which is the first signal of precipitation.

As a conclusion, it was decided to use phosphate buffer to adjust the pH about 6.0. Every compound in the mixture has either a positive or negative charge at pH 6.0. That is why they all can be separated by CZE. In order to study the influence of different analytical and instrumental parameters on the separation, some initial conditions were selected: 15 m*M* phosphate buffer (pH 6.0), 25 kV voltage, 20°C and 5 s injection time (0.5 p.s.i.; 1 p.s.i.= 6894.76 Pa).

3.2. Effect of pH

The effect of the pH of the buffer on the migration times was studied. For this purpose, five 15 m*M* phosphate buffer solutions, adjusted to pH 5, 5.5, 6.0, 6.2 and 6.5 were prepared. Electropherograms of the ''Q'' solution were performed in accordance with the conditions above. The response at different pH values versus migration time is plotted in Fig. 2. From the migration time of the electroosmotic flow (EOF), we can state that BRO and TMP are in their cationic forms and that SDZ, SMX and GUA are in their anionic forms. Moreover, it can be seen that the BRO and TMP peaks overlap in the pH range from 5 to 5.5 and also that GUA shows high migration time. At pH 6.5, good resolutions were obtained for all peaks, nevertheless that of BRO begins to decrease. Consequently, pH 6.2 was selected as suitable and was used for further separations.

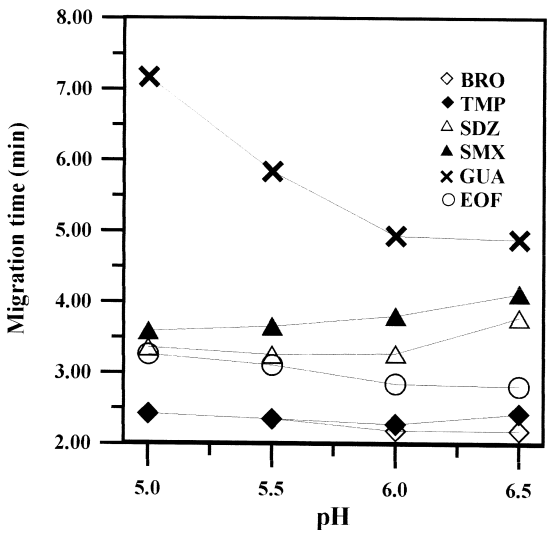

Fig. 2. Influence of the electrolyte pH on the migration time.

3.3. Influence of phosphate buffer concentration

Some experiments were carried out in order to study the influence of buffer concentration on the separation. Thus, the concentration of the pH 6.2 phosphate buffer was varied in successive separations.

As ionic strength increased, the total run time did too, nevertheless, no changes in resolution were reported.

Accordingly, a 15 m*M* phosphate buffer concentration was considered as suitable because it showed enough buffer capacity. Moreover, the run time remained under 6 min and the current was moderate (64 μA).

3.4. Influence of voltage

The effect of varying the voltage from 15 to 30 kV was investigated under the conditions selected above. The influence of the voltage on the migration time of the drugs is plotted in Fig. 3. An applied voltage of 30 kV yielded the best compromise in terms of run time, current generated and linearity between voltage and current. This voltage was used in subsequent stages of the method development.

3.5. Effect of temperature

The effect of the temperature on the separation was tested between 20 and 35°C. Temperatures lower than 20°C were not considered because the temperature regulation with our instrument is not efficient beyond 4°C under room temperature.

A decrease in temperature resulted in a decrease in EOF and an increase of migration times due to the lower electrolyte viscosity. According to resolution,

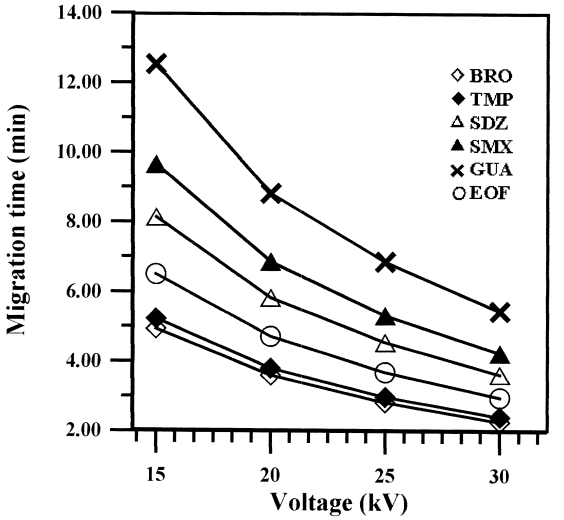

Fig. 3. Influence of the voltage on the migration time.

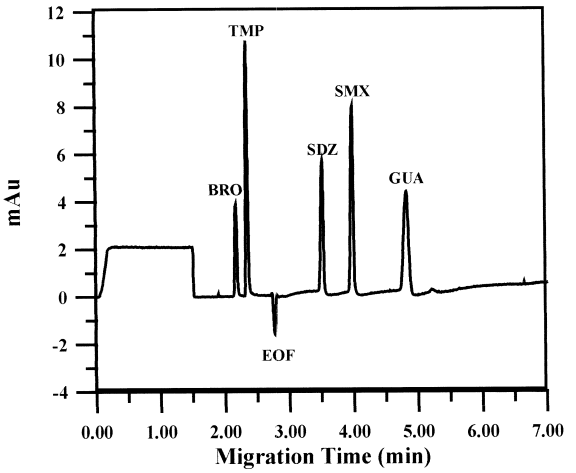

Fig. 4. Electropherogram of a "Q" sample obtained under the optimized conditions at 250 nm.

run time and current generated (62 μA), 25°C was selected as suitable.

3.6. Selected conditions

From the studies carried out before, we suggest that the procedure summarized below is convenient to separate the mixture properly: a fused-silica capillary of 60.2 cm×75 μm I.D.; 15 m*M* phosphate buffer at pH 6.2 as electrolyte; 25°C; 30 kV; 150 kV/min in 0.2 min as voltage ramp; auto-zero at 1.5 min and a detection window of 800×100 μm.

The electropherogram obtained in the separation under the selected conditions is presented in Fig. 4. It is remarkable that all peaks have good resolution in a run time as short as 5 min.

3.7. Quantitative aspects

3.7.1. Limits of detection and quantification

Limits of detection (LODs) and quantification (LOQs) were estimated in accordance with the baseline noise. The baseline noise was evaluated by recording the detector response over a period about 10 times the peak width. The LOD was obtained as the sample concentration that caused a peak with a height three times the baseline noise level and the LOQ was calculated as 10 times the baseline noise level.

Thus, LODs and LOQs are shown in Table 1 for each compound.

3.7.2. Linearity range and calibration curves

The linearity of the assay was checked by injecting standard solutions of each drug in the range from 1 to 25 mg/l. In all cases, the separation was carried out by using the optimized electrophoretic procedure. The calibration curves were obtained for each component by plotting the corrected area, measured at the maximum absorption wavelengths, 241, 262, 205, 213 and 204 nm for SDZ, SMX, TMP, BRO and GUA, respectively, versus their concentrations.

A good linear relationship was obtained between concentration and corrected area for each component. In Table 1, equations, determination coefficients and linearity ranges for the calibration curves are presented. In all cases the intercepts were estimated as negligible by using the Student's *t*-test ($\alpha = 0.05$).

3.7.3. Repeatability and reproducibility

Repeatability was assessed under the previously selected conditions by means of 12 replicates of a solution containing 12 mg/l of each component. Reproducibility was evaluated over 2 days by performing 12 replicates each day.

Table 1
LODs and LOQs

	BRO	TMP	SDZ	SMX	GUA
LOD (mg/l)	0.3	0.1	0.3	0.2	0.2
LOQ (mg/l)	1.1	0.4	1.0	0.6	0.7
Intercepts (CAU[a])	−109±43	−2±18	20±69	−14±60	4±15
Slope (CAU×l/mg)	202.0±3.3	549±13	188.3±5.1	181.7±4.3	387±12
r^2	0.9987	0.9970	0.9964	0.9972	0.9954
Linear range (mg/l)	1.2–24	1.2–24	1.2–25	1.3–26	1.1–23

Linear regression calibration curves.
[a] CAU, Corrected area unit.

The results showed that the repeatability for every component in each day is satisfactory. In terms of reproducibility, the comparison of averages with the Snedecor test did not provide any significant difference between both days series, for $\alpha=0.05$ ($n=12$).

3.8. Application

The presented method was tested to determine the mentioned compounds in pharmaceutical preparations.

Ten capsules of each commercial were emptied, weighed accurately and the contents were mixed thoroughly. A quantity of the powder equivalent to one tablet was dissolved in 400 ml of water, then 500 ml of ethanol were added and later on, diluted to 1 l (''X'' solution).

For the determination of SMX–TMP and SDZ–TMP, three aliquots of 1 ml were taken from ''X'' and placed in three 25-ml volumetric flasks, where the pH was set to 6.2 by adding 2.5 ml of phosphate buffer, and then diluted with water.

For the determination of BRO, no dilution of ''X'' was necessary and it was then directly analyzed.

In the analysis of the commercials, the found amounts and recoveries were achieved by comparing with standard solutions containing the same concentrations than expected for commercials, according to their claimed levels. The standard solutions were prepared from the stock solutions after convenient dilution.

The results, presented in Table 2, show agreement between the claimed and found values.

Table 2
Application results

Commercial	Claimed (mg/l)	Found (mg/l)	Recovery (%)
Brongenit	SMX 400	404.0±9.3	101.0
	TMP 80	81.6±1.9	102.1
Triglobe	SDZ 820	811±17	99.3
	TMP 180	178.8±8.6	98.9
Balsoprim	SMX 400	386.9±8.2	97.7
	TMP 80	78.3±2.6	97.9
	BRO 5	4.91±0.19	98.2
Pulmosterín	SMX 400	394.9±3.5	98.7
	TMP 80	79.9±1.4	99.9
	BRO 5	5.148±0.043	102.9
Eduprim	SMX 400	390±10	97.6
	TMP 80	78.1±2.5	97.6
Septrín	SMX 400	410±14	102.5
	TMP 80	81.5±2.5	101.9

4. Conclusion

The results show that CZE is a valuable technique for the determination of sulfonamides and their associated compounds. CZE complies with the requirements of drug quality control, in terms of reproducibility and accuracy, and it is also useful for routine analysis. In addition, it offers advantages such as simplicity of operation, flexibility and low cost.

The presented CZE method to determine SMX, SDZ, TMP, BRO and GUA was easy to apply to commercials because there are no previous sample treatments, only the dissolution of the commercials in ethanol–water (50:50).

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