ELSEVIER

Journal of Chromatography B, 751 (2001) 29–36

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Analytical control of a pharmaceutical formulation of sodium picosulfate by capillary zone electrophoresis

M. Blanco*, J. Coello, H. Iturriaga, S. Maspoch, M.A. Romero

Departament de Química, Unitat de Química Analítica, Facultat de Ciències, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Spain

Received 13 June 2000; received in revised form 2 August 2000; accepted 2 August 2000

Abstract

A new procedure for the analytical control of a pharmaceutical formulation by capillary zone electrophoresis (CZE) is proposed. It allows the simultaneous determination of the major compounds in the formulation: active compound (sodium picosulfate) and preservative (methylparaben), and the degradation products of the preservative, which slowly degrades by hydrolysis or by transesterification with sorbitol (sweetener in excess in the formulation) yielding *p*-hydroxybenzoic acid and sorbitolparaben, respectively. UV–Vis detection in the absorption maxima of the analytes and 20 m*M* borate solution at pH 10 as background electrolyte are used. Results are compared with those provided by the HPLC procedure. The method has also been validated using the HPLC procedure as the reference method, evaluating selectivity, accuracy, linearity and precision. The CZE procedure developed is sufficiently accurate and the precision achieved is about 1% for major and 3% for minor compounds.

Keywords: Sodium picosulfate

1. Introduction

Since it was developed, capillary electrophoresis (CE) has been applied to the separation and determination of a wide range of substances. In the beginning, most of these applications were of biochemical interest, but great technical advances have allowed CE to become an automated high-performance technique, which is becoming an interesting alternative to chromatographic analytical tools such as HPLC. CE is rapidly gaining popularity in the pharmaceutical industry [1–5], because of the wide range of compounds that can be analysed and the high efficiency and resolution achieved. CE also presents other advantages such as: analytical expeditiousness, simplicity, flexibility and versatility, reduced operation costs and minimal waste generation. Applications of CE in the pharmaceutical industry include active compound determinations [2], separation of preservatives and sweeteners [6,7], and chiral determinations [8]. CE has also been accepted by regulatory bodies for quality control of pharmaceuticals [9].

In this work we have studied the separation and determination of the active compound (sodium picosulfate, SPS) and the preservative (sodium methylparaben, MPB) in the formulation Evacuol®. Sodium picosulfate (disodium 4,4′-disulfoxy-

*Corresponding author. Tel.: +34-93-581-1017; fax: +34-93-581-2477.

E-mail address: iqan8@blues.uab.es (M. Blanco).

0378-4347/01/$ – see front matter
PII: S0378-4347(00)00443-6

diphenyl-(2-pyridyl)methane) is a synthetic laxative which acts by contact on the colon mucous membrane. MPB (methyl 4-hydroxybenzoate) is an antimicrobial preservative very common in pharmaceuticals, cosmetics and food products. In spite of its wide use, it is well known that, in aqueous solution, MPB slowly hydrolyses yielding *p*-hydroxybenzoic acid (PHBA). In the presence of a high concentration of a polyol, MPB may also transesterificate [10–12]. Sorbitol is present in the studied formulation as a sweetener, so it is to be expected that it will slowly react with methylparaben to give different isomers of sorbitolparabens (SPB), which can also hydrolyse to give PHBA. Since this could affect the antimicrobial activity (PHBA is known to have no antimicrobial activity), these compounds also have to be quantified.

The separation and determination of all compounds of the formulation, the structure of which can be seen in Fig. 1, has been achieved not only by capillary zone electrophoresis (CZE) but also by HPLC [13]. The results obtained by these two different techniques are compared in this work.

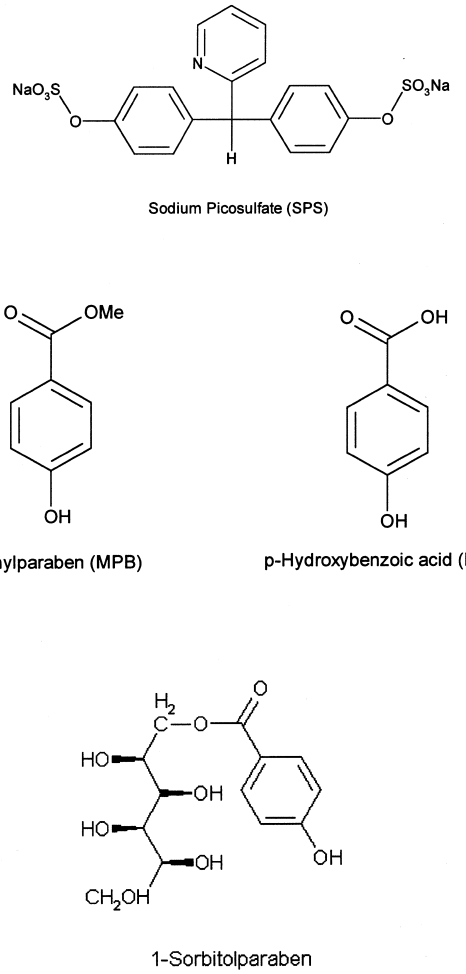

Fig. 1. Chemical structures of compounds in the formulation Evacuol®. Only one of the six possible isomers of sorbitolparaben is shown. Five more isomers can be formed with the other hydroxyls.

2. Experimental

2.1. Apparatus

CE measurements were made on a Model HP ^{3D}CE instrument (Hewlett-Packard, Waldbronn, Germany) equipped with a diode-array detector, automatic injector and sampler. Hydrodynamic injection at the anode end (accomplished by applying pressure to the injection vial) was used. A Sugelabor S.A. (Madrid, Spain) fused-silica capillary, 50 μm I.D. with a length of 64.5 cm (effective length 56 cm), was used in all experiments. The instrument was controlled, and data acquired and processed, using a HP ^{3D}CE Chemstation.

The HPLC system used consisted of a pumping system model LC-10AD (Shimadzu, Kyoto, Japan), a six-way injection valve Model 7725i with a 20 μl injection loop (Rheodyne, Cotati, CA, USA), a C_{18} Tracer Spherisorb column 5 μm, 15×0.4 cm (Teknokroma, St. Cugat del Vallés, Spain) and a UV–Vis diode-array detector Model 1040A (Hewlett-Packard). Data were acquired and processed using a Data Station Model 9153C (Hewlett-Packard).

2.2. Reagents

The reagents used included sodium picosulfate from Laboratorios Almirall (Sant Andreu de la Barca, Spain), methylparaben and 4-hydroxybenzoic acid, both from Fluka (Buchs, Switzerland), D(−)-sorbitol from Panreac (Montcada i Reixac, Spain), disodium tetraborate decahydrate from Merck (Darmstadt, Germany), HPLC-grade methanol from Promochem (Wesel, Germany), sodium sulfate from

Probus (Badalona, Spain) and sodium hydroxide from Carlo Erba (Milan, Italy). Milli-Q water from a water purification system (Millipore, Molsheim, France) was used throughout.

2.3. Sample

The pharmaceutical preparation studied was Evacuol (Laboratorios Almirall, St. Andreu de la Barca, Spain), available as an aqueous solution of sodium picosulfate (7.5 mg/ml) as the active compound, sodium methylparaben (2.2 g/l) as an antimicrobial preservative and an excess of sorbitol as a sweetener. Two different batches were studied in this work, which were analysed 1 and 2 years since manufacture, respectively.

The same sample treatment was used for both CZE and HPLC: 2 ml of sample was diluted in 50 ml of water. The solution was filtered and degassed for 5 min.

2.4. Working conditions

The experimental conditions used in the analysis by CZE were as follows: 20 m*M* borate buffer at pH 10 as background electrolyte (BGE), temperature 25°C and voltage 22 kV. Samples were injected by applying 50 mbar pressure to the injection vial for 5 s. Detection was performed at the wavelength of the absorption maxima: 265, 275 and 300 nm for SPS, PHBA and parabens, respectively.

HPLC was used as reference analytical procedure. The method consisted of an isocratic HPLC separation with 0.05 *M* sodium sulfate–methanol (70:30) as mobile phase in a constant flow of 1 ml/min. The addition of the inorganic electrolyte increased the retention time of SPS yielding a symmetric peak, perfectly resolved [13]. The injection volume was 20 μl and the detection wavelength was 254 nm for all substances.

2.5. Procedure

The CZE procedure was as follows: borate buffer was prepared by dissolving di-sodium tetraborate decahydrate in Milli-Q water, pH 10, adjusted with 0.1 *M* NaOH. The resulting solution was passed through a filter of 0.2 μm pore size and degassed. Prior to each experiment batch, the capillary was successively flushed with 1 *M* NaOH, 0.1 *M* NaOH, Milli-Q water and BGE for 5 min each, and then equilibrated at an applied voltage of 22 kV for 10 min. Before each injection the capillary was flushed with 0.1 *M* NaOH, Milli-Q water and BGE for 3, 5 and 5 min, respectively. The system was then equilibrated at a voltage of 22 kV for 10 min.

For HPLC, the mobile phase was filtered and degassed. Prior to each experiment batch, the column was flushed with MeOH and H_2O for 30 min each solvent. Then the mobile phase was flushed until stabilization of the column pressure (approx. 15 min). Before injection, the column was flushed with H_2O for 15 min and again with the mobile phase until pressure stabilization. It is important to wash the column with water before each new injection, because the formulation contains 35% sorbitol which is retained in the column, causing a decrease in the efficiency and changes of elution times.

2.6. Calibration samples

SPS, MPB and PHBA stock solutions were prepared by weighing and dissolution of pure products. Appropriate amounts of these solutions were used to prepare the calibration samples. The calibration sample set consisted of 10 solutions containing SPS and MPB in the range ±25% and ±50% of the nominal value. Different quantities of a PHBA stock solution in the range 0 to 40% of the equivalent to the nominal value of MPB were added to the calibration set solutions.

Sorbitolparabens are not available as commercial products. To obtain solutions with known total sorbitolparaben concentrations, solutions with known concentrations of MPB and an excess of sorbitol were prepared at pH ≈8 in order to accelerate the transesterification reaction [11]. These solutions were analysed by HPLC and CZE on different days and the total SPBs was calculated from the difference between the initial value of the MPB concentration, the experimental MPB concentration and the PHBA concentration:

$$[\mathrm{SPB}]_t = [\mathrm{MPB}]_0 - [\mathrm{MPB}]_t - [\mathrm{PHBA}]_t \quad (1)$$

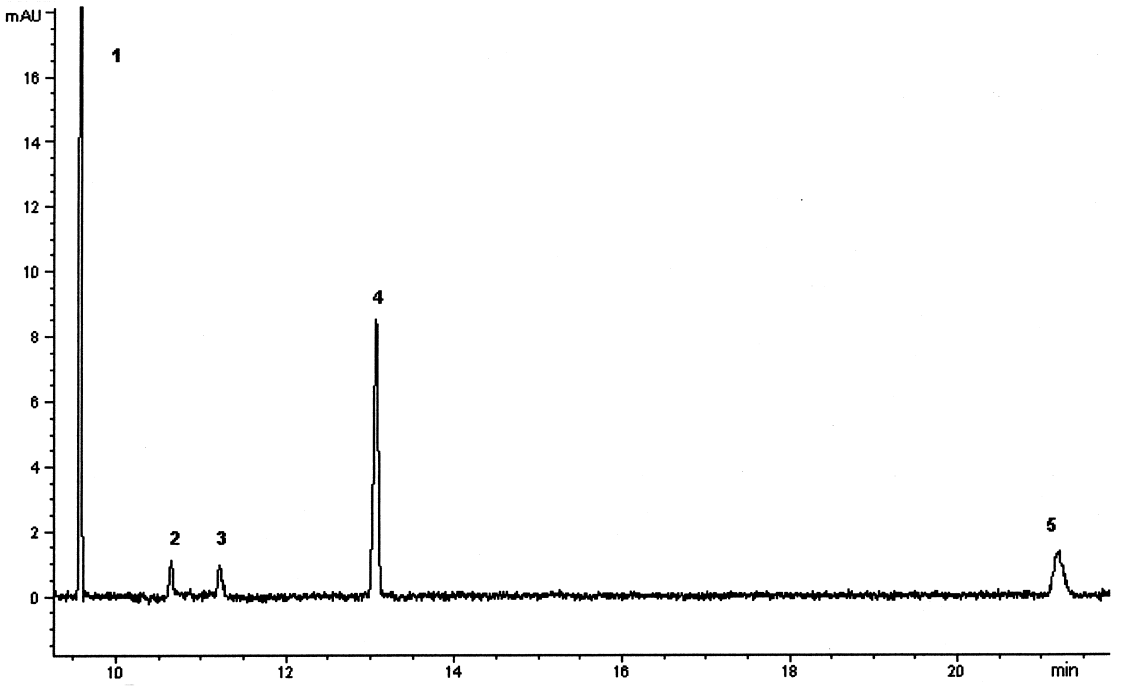

Fig. 2. Separation of a sample of Evacuol by CZE. Conditions: 20 m*M* borate buffer at pH 10, 22 kV (43 μA), 25°C. Detection wavelength, 300 nm. Peaks: 1=methylparaben, 2,3=sorbitolparaben isomers, 4=sodium picosulfate, 5=*p*-hydroxybenzoic acid.

3. Results

Fig. 2 shows an electropherogram of a sample recorded under the above conditions. It can be seen that a complete separation of all components in the formulation was obtained by CZE, not only for the major compounds but also for the degradation products.

At pH 10, the phenolic hydroxyl group of paraben is ionized ($pK_a \simeq 8.5$), so MPB and SPBs acquire a negative charge, while SPS and PHBA acquire two negative charges. The order of elution for MPB, SPS and PHBA substantially agrees with the electrophoretic separation pattern of anions in a system with electroosmotic flow (EOF) directed towards the cathode. PHBA is eluted last due to its two negative charges and its smaller size compared with SPS. According to this criterion, SPB should elute before MPB but, as shown in Fig. 1, it elutes later in two peaks with practically the same area. This can be attributed to a complexing reaction between the different isomers of SPB and borate ions. Sorbitol is a C_6 polyol, so the transesters formed have five hydroxyl groups which can be complexed by borate ions. The complexing interaction between polyols and borate has been described and in some cases is used to separate different carbohydrates by CZE [14,15]. However, the chemistry involved in the polyol–borate interaction is very complex and many different situations may occur as a function of temperature and pH. Also, the steric hindering effect of the MPB group may affect the borate–SPB complexation considerably.

A screening of applied voltages was performed in order to optimize the total time of analysis. Applying the maximum voltage (30 kV), the migration time of the last analyte (PHBA) was slightly shorter than at lower voltages (only about 1 min shorter), but the noise increased considerably. A voltage of 22 kV was selected because a good signal-to-noise ratio was obtained.

Using HPLC, the major compounds of the formu-

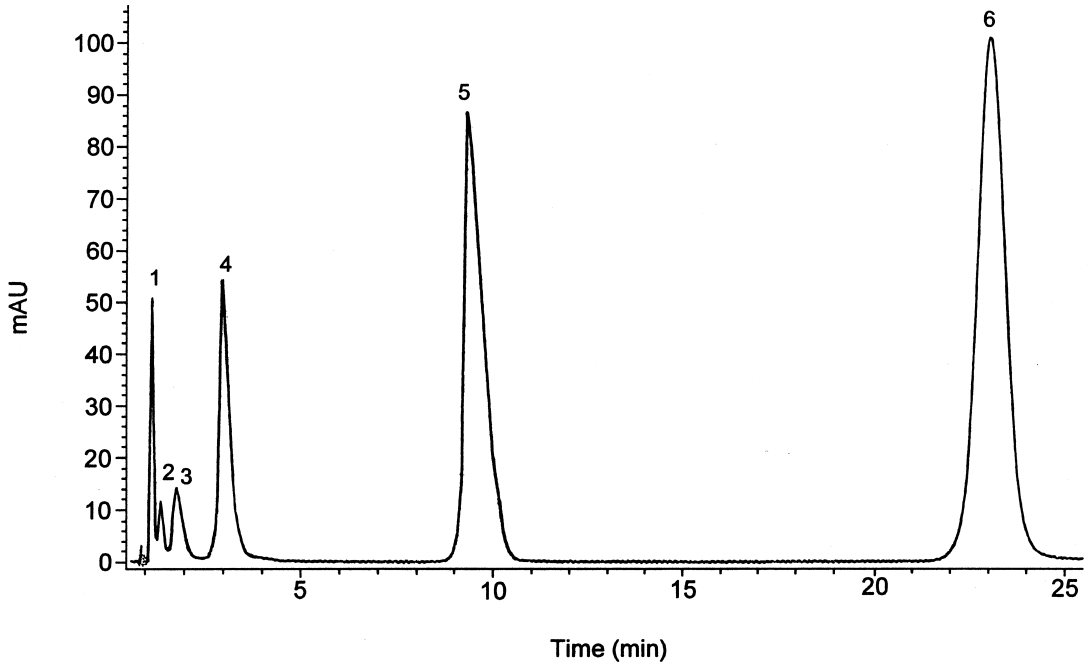

Fig. 3. Separation of a sample of Evacuol obtained by HPLC. Conditions: Na_2SO_4 0.05 *M*–MeOH (70:30) 1 ml/min, detection wavelength 254 nm. Peaks: 1=*p*-hydroxybenzoic acid, 2,3,4=sorbitolparaben isomers, 5=sodium picosulfate, 6=methylparaben.

lation (MPB and SPS) were well resolved, but minor compounds (PHBA and SPBs) eluted partially overlapped (Fig. 3). SPB appeared in three peaks, in the ratio of 5, 20 and 75%. It has been reported [10] that the major peak corresponds to a mixture of the primary isomers of SPB.

The elution time of the last peak by HPLC is comparable to that of the last peak in CZE. Incomplete washing of the HPLC system considerably reduces the efficiency of the HPLC column, increasing the overlapping of minor compounds, in contrast to CZE, which is not affected by the possible absorption of sorbitol in the capillary. The higher efficiency of the CZE system can be computed in terms of the number of theoretical plates (*N*). The results obtained, using in both cases the MPB peak, were $N_{HPLC} = 800$ and $N_{CZE} = 120\,000$.

3.1. Calibration curves

As usual, the analytical signal for quantitative analysis was the corrected peak area (area/migration time) for CZE and the peak area for HPLC. Each calibration sample was injected in triplicate. Both parameters, normalized peak area and peak area, were found to be linear within the concentration ranges studied. Tables 1 and 2 show the figures of merit for all CZE and HPLC calibration curves. For SPB, the sum of the corrected area of the two peaks was used in CZE and the sum of the three peaks in HPLC.

Limits of detection (LOD) were calculated for all compounds using the curve parameters [16]. LODs by CZE and HPLC are shown in Tables 1 and 2, respectively. Although lack of sensitivity is a typical problem in CZE, in this case the LODs are of the same order as those obtained by HPLC.

From the intercepts of the different curves, it can be seen that CZE calibration curves do not present any systematic error.

The standard errors were estimated to be about 1.5–2% from the mean normalized peak area measured by CZE and about 2–2.5% from the mean peak area measured by HPLC.

Table 1
Figures of merit of the calibration curves obtained by CZE[a]

Substance	Concentration range (mg/ml)	Data points	Slope	Intercept	*r*	Standard error	LOD (mg/ml)
SPS	0.150–0.450	10	18.1±0.6	−0.04±0.18	0.9992	0.08	$1.5\cdot10^{-2}$
MPB	0.044–0.132	10	205.2±6.3	0.05±0.57	0.9993	0.27	$3.8\cdot10^{-3}$
PHBA	0–0.013	7	178.6±3.9	0.00±0.02	0.9998	0.15	$1.3\cdot10^{-4}$
SPB	0–0.067	9	105.9±3.9	−0.18±0.18	0.9991	0.10	$3.8\cdot10^{-3}$

[a] Confidence intervals are given ($\alpha = 0.05$). Conditions: BGE, 20 m*M* borate buffer, pH 10; temperature, 25°C; applied voltage, 22 kV. Wavelength detection at 300 nm for MPB and SPBs, 265 nm for SPS and 275 nm for PHBA.

Table 2
Figures of merit of the calibration curves obtained by HPLC[a]

Substance	Concentration range (mg/ml)	Data points	Slope	Intercept	*r*	Standard error	LOD (mg/ml)
SPS	0.150–0.450	10	$(7.5\pm0.1)10^3$	−95.6±36.5	0.9991	48.7	$1.9\cdot10^{-2}$
MPB	0.044–0.132	10	$(6.9\pm0.1)10^4$	329.4±97	0.9991	151.4	$6.6\cdot10^{-3}$
PHBA	0–0.013	7	$(5.8\pm0.1)10^4$	−2.56±5.7	0.9997	5.4	$1.6\cdot10^{-4}$
SPB	0–0.067	9	$(3.5\pm0.3)10^4$	−39.2±11.4	0.9998	30.9	$1.5\cdot10^{-3}$

[a] Confidence intervals are given ($\alpha = 0.05$). Conditions: Na_2SO_4 0.05 *M*–MeOH (70:30), 1 ml/min; wavelength detection, 254 nm.

3.2. Quantitative analysis of Evacuol

Each batch of Evacuol was injected in triplicate by both CZE and HPLC. The three components were analysed in the same run, storing the three signals and analysing them after interpolation of the calibration curves. The values obtained, expressed as mg/ml in the formulation, are shown in Table 3. The standard deviation of the three injections is shown in parentheses. The concentrations of PHBA and SPB, degradation products of methylparaben, expressed in molar percentage of the nominal value of methylparaben, are given in Table 4. Results show that the older batch (batch A) has higher values for degradation products than the other batch, as expected. This suggests that the preservative continues reacting, and shows the necessity to control the degradation.

Table 3
Quantitation of Evacuol. Values are given in mg/ml in the formulation[a]

Substance	Batch	CZE	HPLC
Sodium picosulfate	A	8.03 (0.06)	7.96 (0.07)
	B	8.12 (0.04)	8.23 (0.06)
Sodium methylparaben	A	1.891 (0.003)	1.881 (0.017)
	B	2.046 (0.013)	2.066 (0.019)
p-Hydroxybenzoic acid	A	0.076 (0.003)	0.078 (0.002)
	B	0.070 (0.004)	0.070 (0.001)
Sorbitolparaben	A	0.421 (0.007)	0.398 (0.018)
	B	0.223 (0.018)	0.198 (0.019)

[a] Each batch was injected in triplicate. Standard deviations are given in brackets.

3.3. Validation of the CZE method

The CZE method was validated following the ICH guidelines for validation of analytical procedures [17]. The parameters evaluated were linearity, selectivity, repeatability, intermediate precision and accuracy.

Table 4
Molar percentages of SPB and PHBA, degradation products of the preservative

Batch	% SPB	% PHBA
A	11	5
B	6	4

3.3.1. Selectivity

For chromatographic procedures, representative chromatograms should be used to demonstrate selectivity. Similar considerations should be given to other separation techniques. In the CZE method presented in this paper, the electropherogram in Fig. 2 shows a complete resolution of all compounds in the formulation. All the pure available compounds were positively identified by comparison of migration times and UV–Vis spectra.

3.3.2. Linearity

All compounds were found to have linear relationships between the corrected peak areas and the concentrations, as shown in Table 1, with high correlation coefficients (>0.999).

3.3.3. Precision

Two parameters were studied for the assessment of precision: repeatability and intermediate precision, both calculated for the major compounds MPB and SPS. The RSDs of migration times and concentrations were used to evaluate these parameters.

3.3.3.1. Repeatability. The same sample was injected six times consecutively. RSDs of migration times were 0.5% for MPB and 0.9% for SPS. Concentration RSDs were found to be 0.3% for MPB and 1% for SPS.

3.3.3.2. Intermediate precision (day-to-day). In this case, we only studied the effect of analysing on different days. For this purpose, the same sample was injected in triplicate on three different days. Results obtained show migration time RSDs of 1.9% for MPB and 2.7% for SPS, and concentration RSDs of 0.5 and 1.9% for MPB and SPS, respectively.

3.3.4. Accuracy

Accuracy was evaluated by means of the Student's *t*-test of comparison of means, using the values obtained by HPLC as the reference method. Using the data in Table 3, the experimental *t* values were calculated and all experimental *t* were lower than the tabulated *t* (Table 5), which means that CZE and HPLC do not provide significantly different results.

Table 5
Student's *t*-test for comparison of means obtained in the quantitation of the different compounds in the formulation by CZE and by HPLC[a]

Substance	Batch	Calculated *t*
Sodium picosulfate (SPS)	A	1.31
	B	2.60
Methylparaben (MPB)	A	1.00
	B	1.53
p-Hydroxybenzoic acid (PHBA)	A	0.11
	B	0.00
Sorbitolparaben (SPB)	A	2.06
	B	1.65

[a] Degrees of freedom in all cases is 4. The tabulated *t* (four degrees of freedom, $P = 0.05$) = 2.78.

4. Conclusions

CE, in its separation mode known as CZE, allowed the simultaneous separation and determination of the active compound, preservative and degradation products of a commercial formulation. Results obtained by CZE were accurate and precise, and very similar to those obtained by HPLC. CZE presents substantial advantages such as high resolution, high efficiency, good reproducibility in elution times and also short times of analysis. Capillaries were not affected by the large amount of sorbitol present in the formulation, so their efficiency was not reduced. The validation of the CZE method confirms that CZE could be used for the quality control of Evacuol instead of HPLC. After extensive use, results confirm that, although methylparaben reacts by hydrolysis and by transesterification to give PHBA and SPBs, respectively, this reaction occurs very slowly, and even in a batch 2 years old, the amount of MPB remaining was sufficient to assure Evacuol conservation.

Acknowledgements

The authors are grateful to Spain's DGICyT (project PB96-1180) for funding this work. M.A. Romero also acknowledges additional funding from Spain's Ministry of Education and Culture in the form of an FPI grant.

References

[1] K.D. Altria, M.A. Kelly, B.J. Clark, Trends Anal. Chem. 17 (1998) 204.
[2] K.D. Altria, M.A. Kelly, B.J. Clark, Trends Anal. Chem. 17 (1998) 214.
[3] K.D. Altria, J. Chromatogr. A 735 (1996) 43.
[4] A.S. Cohen, S. Terabe, Z. Deyl (Eds.), Capillary Electrophoretic Separations of Drugs, Elsevier, Amsterdam, 1996.
[5] S.M. Lunte, D.M. Radzik (Eds.), Pharmaceutical and Biomedical Applications of Capillary Electrophoresis, Pergamon, Oxford, 1996.
[6] R.J. Geise, N.I. Machnicki, J. Cap. Elec. 2 (1995) 69.
[7] CZE analysis of artificial sweeteners and preservatives in drinks, Hewlett-Packard Application Note, Publication Number 12-5963-1122E.
[8] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, C. Pérez Maseda, J. Chromatogr. A 799 (1998) 301.
[9] A. Pluym, W. Van Ael, M. De Smet, Trends Anal. Chem. 11 (1992) 27.
[10] B. Runesson, K. Gustavii, Acta Pharm. Suec. 23 (1986) 151.
[11] M.J. Thompson, A.F. Fell, B.J. Clark, M.L. Robinson, J. Pharm. Biomed. Anal. 11 (1993) 233.
[12] A. Hensel, S. Leisenheimer, A. Muller, E. Busker, E. Wolf-Heuss, J.J. Engel, J. Pharm. Sci. 84 (1995) 115.
[13] M. Massaccesi, Ann. Chim. 77 (1987) 515.
[14] S. Hoffstetter-Kuhn, A. Paulus, E. Gassmann, H.M. Widmer, Anal. Chem. 63 (1991) 1541.
[15] T. Soga, Capillary zone electrophoresis of carbohydrates by borate complexation utilizing EOF reversal, Hewlett-Packard Application Note, Publication Number 12-5964-1817E, 1995.
[16] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, Ellis Horwood, Chichester, 1988.
[17] Guidance for Industry, Q2B Validation of Analytical Procedures: Methodology, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Washington, DC, 1996.