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Separation of 1-(4-*tert.*-butylphenyl)-4-(4-hydroxypiperidinyl)-butan-1-one and its O-alkylated isomer by capillary zone electrophoresis

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Abstract

This paper describes the development and the validation of a capillary zone electrophoresis (CZE) method for the separation of position isomers: 1-(4-*tert.*-butylphenyl)-4-(4-hydroxypiperidinyl)-butan-1-one (hydroxybutyridine) and 1-(4-*tert.*-butylphenyl)-4-(piperidin-4-yloxy)-butan-1-one (O-alkylated compound). In order to fix the optimum buffer pH the theoretical estimation of pk_a is used. Rapid and efficient separation is shown with very low detection and quantification limits and excellent detection linearity. Good values of precision and accuracy were obtained as well as ruggedness and robustness.

Keywords: 1-(4-*tert.*-Butylphenyl)-4-(4-hydroxypiperidinyl)butan-1-one; Hydroxybutyridine; 1-(4-*tert.*-Butylphenyl-4-(piperidinyl-4-yloxy)butan-1-one

1. Introduction

Capillary electrophoresis (CE) has gained much popularity over the past decade mainly because of its high efficiency and resolution capacity [1–6,18]. CE comprises a family of related electrokinetic techniques, the principal ones in pharmaceutical analysis being capillary zone electrophoresis (CZE) [7–10] and micellar electrokinetic capillary chromatography (MECC) [11–13]. For the separation of water soluble and ionizable compounds the electrophoretic technique recommended is CZE.

In electrophoretic separations of ionizable compounds, buffer pH plays an important role as it determines the extent of ionization of each individual solute. The variation of electrosmotic flow by pH influences all components of the analysed sample in the same way, although it could individually affect the specific electrophoretic mobility of each compound [14].

This paper describes a possible strategy to find optimum separation conditions in the CZE method, estimating the pk_a values with a computerized expert system based on Perrin's calculation method [15].

In the physico-chemical development of a pharmaceutical product, one of the key points is the identification and quantification of related substances i.e. by-products of synthesis and degradation products. In the first group we can highlight the subgroup of position isomers because of their separation difficulties. In the synthesis of 1-(4-*tert.*-butylphen-

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yl)-4-(4-hydroxypiperidinyl)butan-1-one (hydroxybutyridine) it is possible to obtain as a by-product 1 - (4 - *tert.* - butylphenyl) - 4 - (piperidin - 4 - yloxy) - butan-1-one (O-alkylated isomer). With the aid of pk_a estimation a CZE method has been promptly developed and optimized to determine the O-alkylated isomer content as an impurity of hydroxybutyridine. By theoretical estimation it is possible to rapidly obtain the pk_a of different compounds with no product consumption. This fact is particularly important in the development of analytical methods for the determination of impurities due to the small quantity of product usually available.

In this case CZE method has a significant advantages over HPLC and GC. HPLC need long analysis time on the other hand, Hydroxybutyridine and O-alkylated compound can't be analyzed by GC due to his poor volatility.

The validation of the CZE optimized method described in this paper, demonstrates its suitability in the detection and quantification of the possible O-alkylated isomer present at low levels. Acceptable values of linearity over typical impurity range, detection limit, precision and accuracy were obtained, as well as robustness and ruggedness.

2. Experimental

2.1. Chemicals

Electrophoretic buffer reagents were obtained from Panreac (Barcelona, Spain); sodium tetraborate decahydrate (10 m*M*) and monobasic sodium phosphate dihydrate (10 m*M*); pH 9.5 is adjusted with 1 *M* sodium hydroxide also from Panreac. Water was obtained from a Milli-Q system (Millipore, Molsheim, France).

4-Phenyl-4-hydroxypiperidine (internal standard) was obtained from Aldrich (Steinheim, Germany). Hydroxybutyridine and O-alkylated compound standards and Hydroxybutyridine batches were supplied by Fine Chemical Plant of Lab. Almirall A.S. (S. Andreu de la Barca, Barcelona, Spain).

2.2. Apparatus

A P/ACE 2050 CE instrument (Beckman, Palo Alto, CA, USA) which was connected to an IBM PS/2 Model 40SX (Greenock, UK) with System Gold 711 V software (Beckman, San Ramón, USA) was used for CE analysis. PROLOG-D 1.0 software from CompuDrug Chemistry (Budapest, Hungary) installed in an IBM PS/2 Model 35SX was used to calculate pk_a.

The fused-silica capillaries used in this study were purchased from Teknokroma (S. Cugat del Vallés, Barcelona, Spain).

Table 1
Capillary electrophoresis separation method

Step no.	Conditions
I	Rinse cycle I: water, 1 min
II	Rinse cycle II: run buffer, 5 min
III	Set detector
IV	3.0 s hydrodynamic injection
V	Operating voltage: + 10 kV
	Operating temperature: 30°C
	Capillary dimensions 57 cm×75 μm fused silica
	Run time: 15 min
	Wavelength: 214 nm
VI	Rinse cycle III: water, 2 min
VII	Rinse cycle IV: 0.1 *M* NaOH, 2 min

2.3. Procedure

Sample solutions of hydroxybutyridine were prepared at a concentration of 1 mg/ml in water with 10 μg/ml of internal standard. Sample solutions and buffer solutions were filtered through a 0.45-μm Nylon filter from Teknokroma (S. Cugat del Vallés, Barcelona, Spain).

The separation conditions are described in Table 1.

3. Results and discussion

3.1. Method development and optimization

In CZE the most important parameter to control separation is buffer pH. In order to discover quickly the optimum pH it is very useful to know the pk_a values of the compounds.

It is possible to estimate the pk_a values using the Prolog D expert system. This software package bases the estimation of pk_a on chemical structure, it recognizes acid and basic groups and takes into account the influence of substituents and calculates pk_a with Hammett and Taft equations [16,17]. Fig. 1

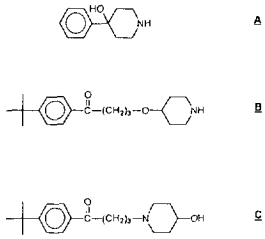

Fig. 1. Structures of hydroxybutyridine (C, pk_a=8.6), the O-alkylated isomer (B, pk_a=9.9) and internal standard (A, pk_a=9.8).

shows the chemical structure of position isomers and the internal standard. The pK_a value estimated by the computerized expert system for hydroxybutyridine and the O-alkylated compound are 8.6 and 9.9 respectively. Therefore a pH between the pk_as was selected, for instance 9.5. Fig. 2 shows an electropherogram obtained with an electrophoretic buffer at pH 9.5.

In order to check the optimum pH a standard solution has been analyzed under the same conditions with changing the buffer pH. The electropherograms obtained are presented in Fig. 3.

A mixture of sodium phosphate and sodium borate was used as run buffer to obtain good buffering capacity and a low current value. To reduce run time analysis voltage and temperature were increased accordingly [19]. To improve the system precision of the peak area and migration time reproducibility, and

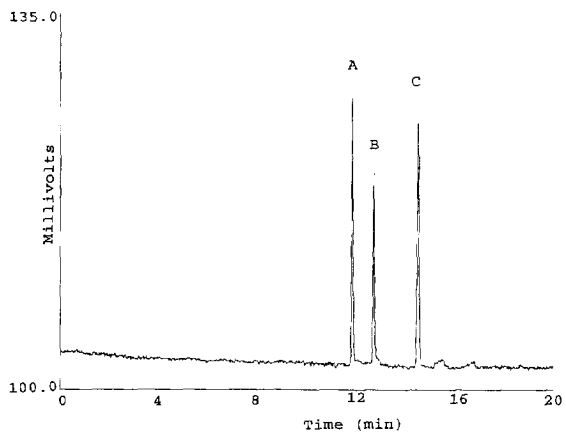

Fig. 2. Electropherogram of a standard solution of internal standard (A), O-alkylated isomer (B) and hydroxybutyridine (C). Separation conditions: 10 m*M* sodium tetraborate and 10 m*M* monobasic sodium phosphate pH 9.5; +5 kV; 30°C; 57 cm×75 μm fused silica (50 cm to detector); 214 nm; sample concentration 10 μg/ml; injection time 3 s.

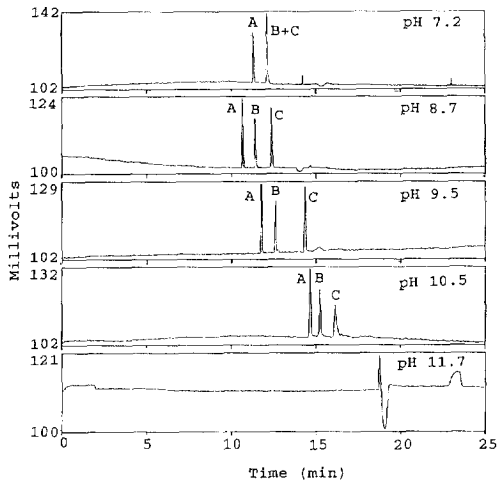

Fig. 3. Effect of increasing pH on effciency, resolution and run time during [CZE separation of standard solution internal standard (A), O-alkylated isomer (B) and hydroxybutyridine (C)]. Separation conditions see Fig. 2.

to increase capillary life it is useful to establish a rinse program before and after the analysis [20]. Prior to the analysis, the first step was a rinse cycle with water followed by a conditioning period with electrophoretic buffer. After the analysis, two rinse cycles are recommended: one with water and another with 0.1 *M* sodium hydroxide. By employing an internal standard (4-phenyl-4-hydroxypiperidine) injection reproducibility can be increased [21–23]. Fig. 4 shows an electropherogram of a standard solution obtained under final conditions.

3.2. Method validation

3.2.1. Selectivity

Test solutions containing known amounts of the position isomers and the internal standard were prepared and analysed under the conditions in Table 1. Good resolution between the two isomers and the internal standard was obtained in less than 10 min run-time. The product migration times are described in Table 2. It was proved that synthesis precursors don't interfere in the analysis.

3.2.2. Linearity

The linearity of the detector response between 2 μg/ml and 0.1 mg/ml (equivalent to 0.2% and 10%) was established. The linear calibration equations for

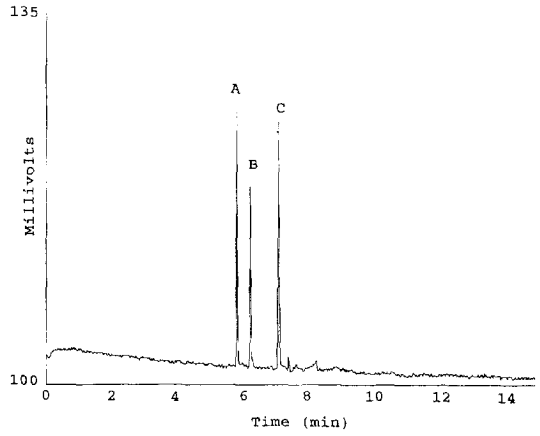

Fig. 4. Electropherogram of standard solution of internal standard (A), O-alkylated isomer (B) and hydroxybutyridine (C). Separation conditions: 10 m*M* sodium tetraborate and 10 m*M* monobasic sodium phosphate pH 9.5; +10 kV; 30°C; 57 cm×75 μm fused silica (50 cm to detector); 214 nm; concentration of each compound 10 μg/ml; injection time 3 s.

hydroxybutyridine and the O-alkylated isomer are respectively: $y=0.764x-0.0143$ and $y=0.996x-0.688$, where y is the relative concentration and x is the relative area.

3.2.3. Sensitivity

A detection limit of 0.1% (w/w) of hydroxybutyridine and the O-alkylated isomer (analysis concentration 1 mg/ml) was obtained with a signal-to-noise ratio greater than 3. This is equivalent to a detection limit of 1 μg/ml in the solution.

3.2.4. System precision

In order to measure the system precision six injections of the same standard solution (10 μg/ml, equivalent to 1%) were performed and results of relative areas to internal standard and migration times are presented on Table 2.

Table 2
System precision

		Internal standard	O-alkylated isomer	Hydroxybutyridine
Migration times		6.0	6.4	7.4
System precision: relative areas variation	Mean		0.6722	0.9705
	R.S.D. (%)		2.9	3.3
System precision: migration times variation	Mean	5.98	6.42	7.35
	R.S.D. (%)	0.4	0.5	0.6

Table 3
Method accuracy: recovery of O-alkylated compound added

Preparation	Recovery O-alkylated isomer added (μg/ml)	
	0.5%	1.0%
Mean	5.0	11.2
Theoretical value	5.0	10.6

Relative standard deviations of relative areas around 3% are acceptable values if we take into account that this is an impurity analysis. Migration time has good reproducibility.

3.2.5. Accuracy

To guarantee the method recuperability, two series of 5 sample solutions were added with known amounts of the O-alkylated compound (5 and 10 μg/ml, corresponding to 0.5% and 1% in relation to the hydroxybutyridine concentration). Table 3 shows the results obtained. Good recuperability in a typical impurity range is shown in Table 3.

3.2.6. Robustness

To study methods robustness 11 experiments were carried out with modified voltage, temperature, pH and ionic strength. Table 4 shows the migration times obtained and demonstrates method robustness.

3.2.7. Ruggedness

To determine method ruggedness, the system precision experience was carried out in a second instrument, a Beckman 5500 equipped with a diode array detector and a new capillary. This experiment was performed on two different days and the results are shown in Table 5.

Table 4
Robustness. Migration times

Condition	Internal standard	O-alkylated compound	Hydroxy-butyridine
10Ph-10Tb pH 9.5, 10 kV, 30°C[a]	6.48	7.01	8.02
10Ph-10Tb pH 9.5, 10 kV, 27°C[a]	6.95	7.51	8.60
10Ph-10Tb pH 9.5, 10 kV, 33°C[a]	6.14	6.62	7.55
10Ph-10Tb pH 9.5, 9 kV, 30°C[a]	7.31	7.90	9.03
10Ph-10Tb pH 9.5, 11 kV, 30°C[a]	5.88	6.35	7.26
10Ph-10Tb pH 9.0, 10 kV, 30°C[a]	6.06	6.55	7.39
10Ph-10Tb pH 10, 10 kV, 30°C[a]	7.44	7.88	8.62
9Ph-10Tb pH 9.5, 10 kV, 30°C[a]	6.70	7.20	8.22
11Ph-10Tb pH 9.5, 10 kV, 30°C[a]	6.90	7.43	8.44
10Ph-9Tb pH 9.5, 10 kV, 30°C[a]	6.70	7.19	8.15
10Ph-11Tb pH 9.5, 10 kV, 30°C[a]	6.92	7.42	8.46

[a] Ph: m*M* phosphate, Tb: m*M* tetraborate.

Table 5
Ruggedness

		Internal standard		O-alkylated isomer		Hydroxybutyridine	
		Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Migration times	Mean	6.493	6.454	6.996	6.974	8.016	7.954
	R.S.D. (%)	0.26%	0.30%	0.23%	0.46%	0.27%	0.36%
Corrected areas	Mean	0.0243	0.0226	0.0157	0.0144	0.0176	0.0184
	R.S.D. (%)	1.7%	1.8%	1.7%	2.0%	0.9%	2.1%

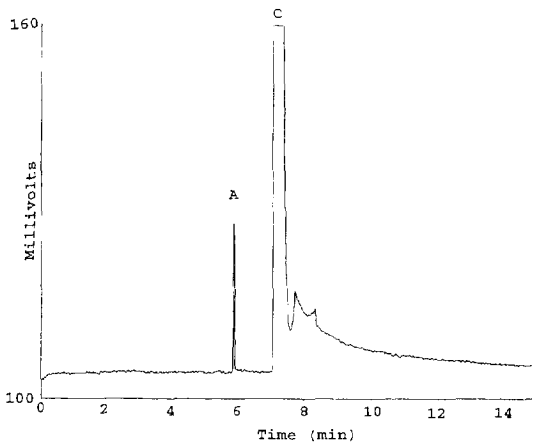

Fig. 5. Typical electropherogram of a sample of hydroxybutyridine. Separation conditions: 10 m*M* sodium tetraborate and 10 m*M* monobasic sodium phosphate pH 9.5; +10 kV; +30°C; 57 cm×75 μm fused silica (50 cm to detector); 214 nm; concentration 1 mg/ml; injection time 3 s.

3.3. Batch analysis

Different batches of hydroxybutyridine were analyzed under the conditions described in Table 1. The presence of the O-alkylated isomer (by-product of synthesis) has not been detected in any analyzed batches (i.e., it was below the detection limit). Fig. 5 shows an electropherogram of a hydroxybutyridine batch analysis.

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