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Capillary zone electrophoresis determination of loratadine in tablets

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A capillary electrophoretic method was developed for the determination of loratadine in pharmaceutical formulations. Capillary zone electrophoresis (CZE) separation and UV absorbance photometric detection were carried out in a 160 mm capillary tube with a 300 μm internal diameter, hydrodynamically (membrane) closed. The influences of pH, carrier cation and counter ion on the migration parameters of loratadine were studied and the following conditions were selected: 24 mmol/l glycine as a carrier cation, 1.6 mmol/l citric acid and 84 mmol/l acetic acid as counter ions at pH 3.2, 100 μA and 25 °C. The proposed electrophoretic method was successfully validated. It was convenient for the sensitive, simple, rapid and highly reproducible assay of loratadine. The determination of loratadine in tablet forms was demonstrated as an application of the method. CZE analysis was completed within 6 min. The detection limit of loratadine was 1.96 μmol/l at a 240 nm detection wavelength and the relative standard deviation for its determination was 0.6% for migration time and 1.1% for peak area. CZE in a hydrodynamically closed separation system, used for the first time for the analysis of loratadine, should also be convenient for complex biological sample applications, as it is easily combinable on-line with the purification CE modes (e.g. ITP).

1. Introduction

Loratadine (LOR), 4-(8-chloro-5,6-dihydro-11 H-benzo-[5,6] cyclohepta [1,2-*b*] pyridin-11-ylidene)-1-piperidine-carboxylic acid ethyl ester is a long-acting tricyclic antihistamine with selective peripheral histamine H_1-receptor antagonistic activity (Belaich et al. 1990; Mann et al. 1989). Several analytical methods have been described for LOR, such as HPLC (Gergov et al. 2001; Kunicki 2001; Rupérez et al. 2002; Sutherland et al. 2001; Zhong and Blume 1994), gas-liquid chromatography (Johnson et al. 1994), GC (Martens 1995), polarography (Ghoneim et al. 2001) and atomic absorption spectrometry (El-Kousy and Bebawy 1999). They have been used to quantify LOR in body fluids (column methods and polarography) and pharmaceutical preparations (polarography and spectrometry).

Recently, capillary electrophoresis (CE) has been used for the determination of LOR and its related impurities in raw materials and tablets (Fernández et al. 2003). The separations were carried out in a hydrodynamically open CE system using narrow-bore (50 μm I.D.) uncoated fused-silica capillary. The analysis time for LOR was ca. 14 min and the precision of the method was 11% (RSD). Validation parameters of the CE method were poorer than those described for HPLC (Rupérez et al. 2002) for the same compound. However, no attention has so far been paid to the use of CE for the analysis of LOR in a hydrodynamically closed separation system. Therefore, the aim of the present work was (i) to study the analytical capabilities of such a mode of CE, as an alternative to other separation techniques (HPLC, GC, open CE mode), for the determination of LOR and (ii) to apply such capillary electromigration method for the analysis of LOR in real matrices (tablet forms). The use of CZE in a hydrodynamically (membrane) closed separation system, employing a capillary of larger I.D., was preferred to the common hydrodynamically open CZE mode, as it enables some performance parameters to be enhanced (sensitivity, sample loadability, repeatability, reproducibility, see Kaniansky et al. (1997a) and references cited therein).

2. Investigations, results and discussion

The principal operating parameters optimized in the present CZE separation were electrolyte composition (type and concentration of carrier cation and counterions) and pH. Electrolyte systems (ES) used are given in Table 1. Other operating parameters optimized included driving current and wavelength.

The influence of carrier cation on the migration characteristics of LOR (migration time and shape/area of the peak) was examined in several buffers, among them ES 1 and 2. An inorganic buffer like phosphate (Fernández et al. 2003) was unsuitable for use in the wide-bore capillary as its high conductivity generated a high Joule heat which dramatically decreased the separation efficiency (due to thermal dispersion). In our investigation, slowly migrating organic cations were found suitable in terms of low conductivity of the buffer (elimination of thermal disper-

Table 1: Electrolyte systems

Parameter	ES 1	ES 2	ES 3
Solvent	Water	Water	Water
Carrier cation	ε-ACA	Gly	Gly
Concentration (mmol/l)	20	20	24
Counter ion 1	AcH	HCl	AcH
Concentration (mmol/l)	–	–	84
Counter ion 2	–	–	CitH
Concentration (mmol/l)	–	–	1.6
pH	4.5	2.5	3.2
EOF suppressor	M-HEC	M-HEC	M-HEC
Concentration (%, w/v)	0.2	0.2	0.2
Voltage/100 μA (kV)	6.5	1.5	6.2

ε-ACA = ε-aminocaproic acid; Gly = glycine; AcH = acetic acid; CitH = citric acid; M-HEC = methylhydroxyethylcellulose

sion) and a good match of the effective mobilities of the migrants (elimination of electrodispersion).

The influence of pH on CZE analyses of LOR was studied in the pH range from 2.5 to 4.5 of the carrier cations listed in Table 1, glycine was suitable for lower pH values within this range and ε-ACA for higher ones. As a slowly migrating cation was being analyzed, a lower buffer pH was preferred in order to increase protonization of the analyte (compare the electropherograms in Fig. 1). However, at very low pH (ES 2) high ionic strength of the buffer decreased the charge transfer of LOR and high conductivity prevented the generation of an electric field in the capillary sufficient for a reasonable migration of LOR (see legend to Fig. 1). With regard to sufficient protonization of LOR and a reasonable ionic strength/conductivity of the buffer, pH 3.2 was chosen as an optimal value (ES 3). Here, a combination of glycine with weak acids (acetic, citric) as counterions provided better results in terms of the electric field generated during the separation in comparison with that when a strong acid (hydrochloric) was used.

The validation aspects assessed included performance parameters such as sensitivity, precision (repeatability and reproducibility) and linearity. Electrolyte system No. 3 (Table 1) was used to obtain validation data.

The limit of detection (estimated as 3σ) of LOR was 1.96 μmol/l when a 240 nm detection wavelength was used and the limit of quantification (estimated as 10 σ) was 6.53 μmol/l.

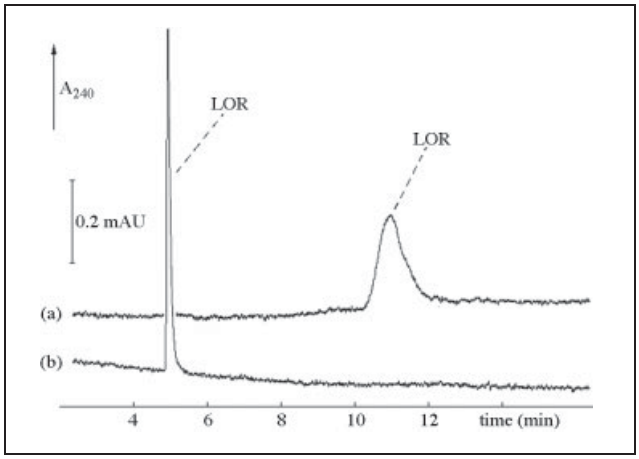

Fig. 1. Effect of CZE separating conditions on migration parameters of LOR. The separations were carried out in (a) ES 1 and (b) ES 3 (Table 1). In ES 2 no peak was obtained within the time interval presented here. Concentration of LOR in the standard sample analyzed was 100 μmol/l. The driving current was stabilized at 100 μA (for the corresponding driving voltage see Table 1). The detection wavelength was 240 nm

Table 2: Precision data for repeat injections of loratadine[a]

Factor	RSD (%)	
	Repeatability[b]	Reproducibility[c]
Migration time	0.46	0.61
Relative migration time[d]	0.38	0.47
Peak area	0.89	1.13
Peak-area ratio[d]	0.63	0.78

[a] The separations were carried out in ES 3 (Table 1);
The RSD values of the migration parameters of the peaks were obtained from 7 parallel CZE runs repeated [b] on the same day, [c] on different days (two weeks between the series);
[d] relative migration data were obtained using ketotifen as an internal standard

Precision was assessed both with and without an internal standard. In our study, ketotifen (KET) served as the internal reference. Seven repeated injections of LOR with/without KET at 50 μmol/l concentrations of both compounds gave the data presented in Table 2. These results clearly indicated that CZE separations in a hydrodynamically closed separation system provided highly reproducible migration data [compare with those obtained in a corresponding open CE system (Fernández et al. 2003)] so that the use of the internal standard was not a prerequisite in order to obtain acceptable validation criteria.

The linearity of detector response (peak area) for LOR was assessed over the range 10–50 μmol/l. It represents an interval suitable for the evaluation of this drug in common preparations (after appropriate dilution). The correlation coefficient obtained for LOR was 0.9996 and the corresponding straight line equation was $y = 0.1429 + 7.6743x$. Use of the internal standard slightly improved the correlation coefficient, reducing the scatter of points due to random error.

A validated method was applied to the determination of LOR in Claritine® tablets (Fig. 2). Results from the examination are given in Table 3. The content of LOR obtained by the proposed method was in good agreement with that declared. Differences between determined and declared

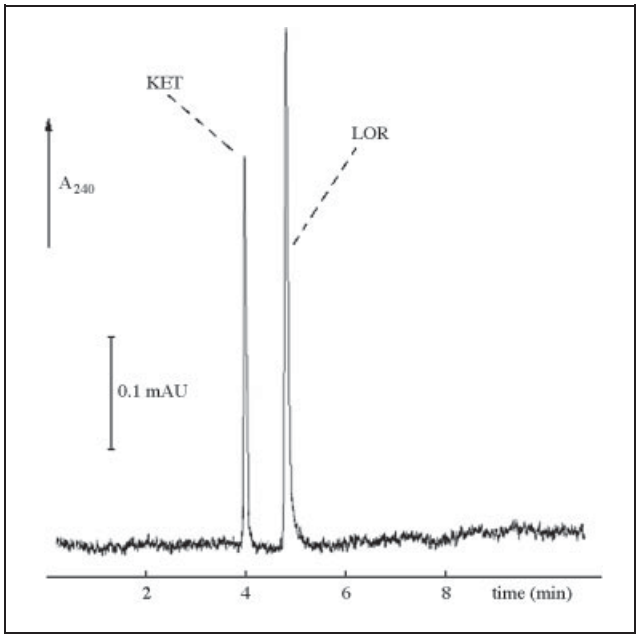

Fig. 2. Electropherogram from the determination of LOR in pharmaceutical tablets. The separation was carried out in ES 3 (Table 1). KET was used as an internal standard. LOR and KET were separated at 50 and 60 μmol/l concentrations, respectively. The driving current and detection wavelength were as those in Fig. 1. For the sample preparation see Experimental

Table 3: Determination of loratadine in tablets[a]

Parameter	1	2	3	4	5	6
Average content[b] (mg per tab.)	9.98	10.07	9.96	10.03	9.92	9.89
RSD[c] (%)	0.66	0.74	0.68	0.71	0.65	0.69

[a] The separations of LOR in 6 different batches of Claritine were carried out in the ES 3 (Table 1); LOR in each sample injected was at a 50 μmol/l concentration
[b] for calculations, relative migration data were used (ketotifen served as an internal standard)
[c] the RSD values of the peak areas were obtained from 7 parallel CZE runs (intra-assay)

contents of LOR in 6 different batches of Claritine ranged from 0.2 to 1.1%. No detection interference occurred when separating the preparation at a detection wavelength of 240 nm.

It can be concluded that CZE in a hydrodynamically closed separation system is suitable for providing highly effective separations of tricyclic antihistamines. High precision and efficiency, short analysis time and low cost are advantages of the proposed method so that it can be used as an alternative to the commonly used separation methods (HPLC, GC, open CE mode). The larger I.D. (300 μm) capillary employed provided favorable conditions in terms of sensitivity of the photometric absorbance detection. The reported operating conditions are suitable for the routine assay of LOR present in pharmaceutical preparations like tablets. Moreover, the CE system used is advantageous as it is easily combinable on-line with related CE modes (e.g. ITP) that enable analyses of complex biological samples to be performed with minimal sample pretreatment (Kaniansky 2002 and refs. cited therein).

3. Experimental

3.1. Samples and reagents

Chemicals used for the preparation of the electrolyte solutions and standard samples were obtained from Merck (Darmstadt, Germany), Aldrich (Steinheim, Germany), CentralChem (Bratislava, Slovakia) and Fluka (Buchs, Switzerland) while water was demineralized by a Rowapure-Ultrapure water purification system (Premier, Phoenix, Arizona, U.S.A.). All chemicals used were of analytical grade or were additionally purified by the usual methods. Acetic acid was used for the preparation of the carrier electrolyte solution after isothermal distillation. Methylhydroxyethylcellulose 30000 (m-HEC), obtained from Serva (Heidelberg, Germany), served as an electroosmotic flow (EOF) suppressor in the carrier electrolyte solutions (Kaniansky et al. 1997b). The electrolyte solutions were filtered before use through disposable membrane filters (1.2 μm pore size) purchased from Sigma (St. Louis, MO, USA).

Loratadine (LOR) was obtained from Lek (Ljubljana, Slovenia) and ketotifen (KET) from Sandoz (Basel, Switzerland). The Claritine formulationanalysed, containing 10 mg of LOR per tablet, was produced by Schering-Plough (Heist-op-den-Berg, Belgium).

3.2. Instrumentation and CZE conditions

A CS Isotachophoretic Analyzer (Villa-Labeco, Spišská Nová Ves, Slovak Republic) was used with a single-column configuration of the separation unit. The separation unit consisted of the following modules: (i) a CZE injection valve with a 100 nl internal sample loop (Villa-Labeco); (ii) a column provided with a 300 μm I.D. (650 μm O.D.) capillary tube made of fluorinated ethylene-propylene copolymer (FEP) of 210 mm total length (160 mm to the photometric detector); (iii) a counter-electrode compartment with a hydrodynamically (membrane) closed connecting channel to the separation compartment (Villa-Labeco). The CZE column was provided with an LCD 2083 on-column photometric detector with variable wavelength, 190–600 nm (Ecom, Praha, Czech Republic). A high voltage power supply delivered the stabilized driving current. The signal from the detector was fed to a PC via a Unilab data acquisition unit (Villa-Labeco). ITP Pro32 Win software (version 1.0) obtained from KasComp (Bratislava, Slovak Republic) was used for data acquisition and processing.

Prior to use, the capillary was not given particular treatment to suppress electroosmotic flow (EOF). Dynamic coating of the capillary wall by means of 0.2% m-HEC in the background electrolyte solutions served for this purpose (Kaniansky et al. 1997b). CZE analyses were carried out in the cationic separation regime with direct injection of the samples. The experiments were performed in constant current mode (Kaniansky et al. 1997a). The driving current was 100 μA and the temperature 25 °C.

3.3. Sample and standard solution preparations

A total of 10 tablets containing LOR were weighed and finely powdered. A portion of the powder equivalent to 10 mg LOR was weighed accurately, transferred to a 25 ml volumetric flask and suspended in 1 ml of concentrated acetic acid and 10 ml of water. The flask was placed in an ultrasonic water bath for 15 min before making up to volume with water. The mixture was centrifuged (5000 rpm) for 10 min. The resulting solution was transferred to an appropiate flask and stored in a freezer at −18 °C. It was melted at a room temperature, suitably diluted with water prior to the analysis and filtered [1.2 μm pore size (Sigma)] before injection into the CE equipment. The aqueous stock solutions of LOR and KET working standards were prepared at 100 μmol/l concentrations in appropriately diluted acetic acid by the same procedure.

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