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Simultaneous Determination of Loratadine, Desloratadine and Cetirizine by Capillary Zone Electrophoresis

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ABSTRACT

Purpose: The aim of the study was the development of a simple and rapid analytical procedure for the determination of the most frequently used antihistamine derivatives.
Methods: A capillary zone electrophoretic method was developed for the simultaneous separation of loratadine, desloratadine and cetirizine. Efforts were focused primarly on the optimisation of the experimental parameters: buffer composition and concentration, buffer pH, applied voltage, temperature, injection pressure and time.
Results: The optimised parameters for the separation were: 25 mM buffer electrolyte, buffer pH 2.5, voltage + 25 kV, temperature 25 °C, injection pressure 50 mbar, injection time 3 seconds, capillary 48 cm (effective length 40 cm) x 50 μm, detection at 240 nm. Under these conditions, the analysis time was below 5 minutes, the order of migration being: desloratadine, cetirizine and loratadine. The developed method was validated in terms of linearity, limits of detection and quantification, intra- and inter-day precision, selectivity and robustness.
Conclusion: Capillary zone electrophoresis proved to be a suitable method for the simulatneous determination of the three studied antihistamine derivatives.

Introduction

An H1 receptor antagonist is a histamine antagonist of the H1 receptor used in therapy to reduce or eliminate effects mediated by histamine, an endogenous chemical mediator released during allergic reactions.[1]

Second-generation H1 antihistamines are newer drugs that are much more selective for peripheral H1 receptors as opposed to the H1 receptors of the central nervous system (CNS) and cholinergic receptors. This selectivity significantly reduces the occurrence of adverse drug reactions, such as sedation, while still providing effective relief of allergic conditions. The reason for their peripheral selectivity is that most of these compounds are zwitterionic at physiological pH; consequently, they are polar compounds, meaning that they do not cross the blood–brain barrier and act mainly outside the CNS.[1]

Loratadine(LOR) (ethyl 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidinecarboxylate) is a second generation long acting H1 histamine antagonist drug, structurally related with tricyclic antidepressants. LOR undergoes extensive first pass metabolism in the liver, forming an active metabolite, **desloratadine (DSL)** (8-chloro-6,11-dihydro-11-(4-piperdinylidene)- 5*H*-benzo[5,6]cyclohepta[1,2-b]pyridine), which retains its antihistaminic activity.[1,2]

LOR and DSL are selective peripheral H1 receptor antagonists, which produce no substantial effect on the CNS. DSL exhibits similar pharmacodynamic activity with a relative potency of two to three-fold greater than LOR probably due to a higher affinity for histamine H1 receptors.[1,3]

Cetirizine (CET) ((±)-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid) is also a second generation long acting H1 antihistamine drug, the primary metabolite of the anxiolytic drug, hydroxizine. CET is zwitterionic and relatively polar and thus crosses only slightly the blood-brain barrier, exhibiting minimal effects on the CNS. The levorotatory enantiomer (R-enantiomer) of CET, levocetirizine is the more active form; and is marketed also as pure enantiomer.[1,3]

The chemical structures of the studied H1 antihistamines are presented in Figure 1.

In the literature several methods have been decribed for the simultaneous determination of different antihistamines from complex mixtures including thin layer chromatography,[4] high performance liquid chromatography,[5] and capillary electrophoresis.[6,7]

The simultaneous determination of LOR and DSL was also reported using liquid chromatography.[8,9] Capillary electrophoresis was used for the determination of LOR

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and its related impurities,[10] but there are no reports regarding the simultaneous electrophoretic determination of LOR and DSL.

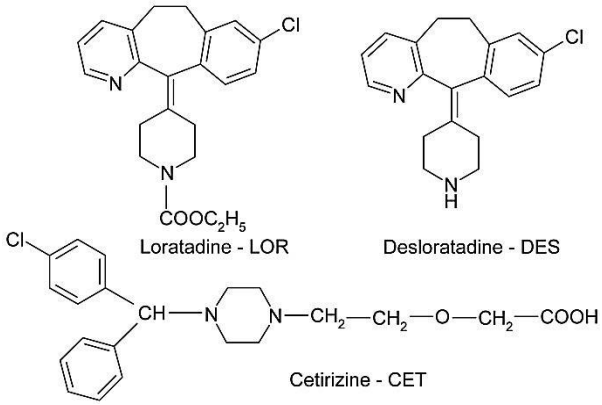

Figure 1. The chemical structures of the studied H1 antihistamines

In the last decade, capillary electrophoresis (CE) has proven to be an attractive alternative to high performance liquid chromatography because of its good selectivity and high separation efficiency in combination with short analysis times, low operational costs and fast method development.[11]

The aim of our work was the development of a simple and rapid procedure for the simultaneous separation of the three studied antihistamine derivatives and also the optimization of the analytical conditions in order to obtain a good separation resolution and a short analysis time.

Materials and Methods

The analyzed antihistamines were purchased from different distributors: loratadine (Tonira Pharma Limited, India), desloratadine (Morepen Laboratories, India) and cetirizine (RA Chem Pharma Limited, India). All substances were of pharmaceutical grade.

For the determinations from pharmaceutical products we used the following commercial preparation: Symphoral tablets (Gedeon Richter, Romania) containing 10 mg LOR, Aerius tablets (Schering Plough, USA) containing 5 mg DESL, Zyrtec tablets (UCB Pharma, Germany) containing 10 mg CET.

The following reagents of analytical grade were used: phosphoric acid 85%, sodium tetraborate, disodium hydrogenophosphate, sodium didydrogenophosphate (Merck, Germany), methanol, sodium hydroxide (Lach Ner, Czech Republic). Purified water was provided by a Milli-Q Plus water purification system (Millipore, USA).

The separation was performed on an Agilent 6100 CE system (Agilent, Germany) equipped with a diode array UV detector. The separation was carried out on an uncoated fused-capillaries of 48 cm (effective length 40 cm) x 50 μm I.D. The electropherograms were recorded and processed by Chemstation 7.01 (Agilent, Germany). The pH of the buffer solutions was determined with the Terminal 740 pH–meter (Inolab, Germany).

Stock solutions containing 100 μg/ml of each compound were prepared in methanol and later were diluted conveniently for the analysis. The samples were introduced in the system at the anodic end of the capillary by hydrodynamic injection. All samples and buffers were filtered through a 0.45 μm syringe filter and degassed by ultrasound for 5 minutes before use.

To determine the studied antihistamines from tablets, twenty tablets from the same batch product were weight and pulverized in a mortar, and an amount of powder equivalent to the average weight of a tablet was accurately weighed and used. The powder was dissolved in methanol, and then the solution was diluted to the appropriate concentration sonicated for 10 minutes and filtered through 0.45 μm syringe filter. The samples was centrifuged at 3500 rpm for 10 minutes, the supernatant was diluted; further the same procedures were followed as for the preparation of standard solutions for the CE separation.

The capillaries were conditioned before use with 0.1 M sodium hydroxide for 30 minutes and with the background electrolyte used in the analysis for 30 minutes. The capillary was rinsed for 1 minute with 0.1M sodium hydroxide and buffer solutions before each electrophoretic separation.

Results and Discussion

Preliminary analysis

Electrophoretic mobilities and ionization behavior of analytes are the key factors driving separations in capillary zone electrophoresis (CZE). In CZE, the selectivity of the method is fundamentally based on charge-to-volume ratios, as separation occurs due to the differences between the own electrophoretic mobilities of the analytes. Knowledge of these basic physicochemical properties of analytes gives valuable information about their nature and makes it easier to choose appropriate experimental conditions for their separation.

In order to find the suitable conditions for the separation a series of preliminary experiments were conducted at different pH and buffer compositions. In the preliminary analysis we used 25 mM phosphoric acid (pH –2.1), 25 mM disodium hydrogenophosphate – 25 mM sodium didydrogenophosphate (pH – 7) and 25 mM sodium tetraborate (pH – 9.3) background electrolytes (BGEs) respectively and we adjusted the pH of the buffer by adding a 0.1M sodium hydroxide solution.

We applied some “standard” electrophoretic conditions for a CZE analysis: temperature 20 °C, applied voltage + 20 kV, injection pressure/time 50 mbar/3 sec, sample concetration 10 μg/ml.We recorded previously the UV spectra of three antihistamines in methanol and found absorption maximum at 232 nm for LOR, at 244 nm for DSL and 250 nm for CET; consequently an intermediate value of 240 nm was elected as detection wavelength in the CE separations.

The pKa value of LOR is 5 while the pKa values for DSL are 4.2 corresponding to the pyridine functional

group and 9.7 corresponding to the piperidine functional group.[12]

CET has three ionizable moieties resulting in pKa values of 2.2, 2.9 and 8.0, and depending on the pH it predominantly exists as a zwitterion. CET will be negatively or positively charged depending on the pH of the environment, as it posses two basic and one acidic functions, offering the possibility of using either an acidic or an alkaline running buffer for its determination.[13] This was one of the main reasons why CET was elected in this separation, as a substance whose electrophoretic behavior is in contrast with the ones of LOR and DSL.

Optimization of the electrophoretic separation

Selectivity in CZE can be controlled by background electrolyte (BGE) concentration, pH, organic modifiers, applied voltage, temperature, injection parameters and capillary length. All these parameters were varied and results are summarized below.

The first optimization step involved selecting the optimal buffer solution and buffer pH for the separation. Using the previously selected BGEs we conducted a systematic study over a pH range between 2-11, in which the charge of the analytes changes, as indicated by the pKa values presented above, and the electroosmotic flow (EOF), thus influencing the ionic mobilities of the analytes. Figure 2 shows the migration times of the studied antihistamines over the studied pH range. We may observe that LOR can be determined over a pH range 2-5, while DSL and CET can be detected on the whole studied pH range. Over the pH range 7-9 CET migrates very close or even with the EOF. The migration times of the analyzed substances increased over the pH range 2-5, were almost similar over the pH range 5-9, and decreased over the pH range 9-11. Buffer concentration ranging from 25 mM to 100 mM were tested, higher buffer concentrations increased migration times of the analytes, without significant influence on the separation. A buffer containing 25 mM phosphoric acid at a pH of 2.5 was elected as optimum for the separation.

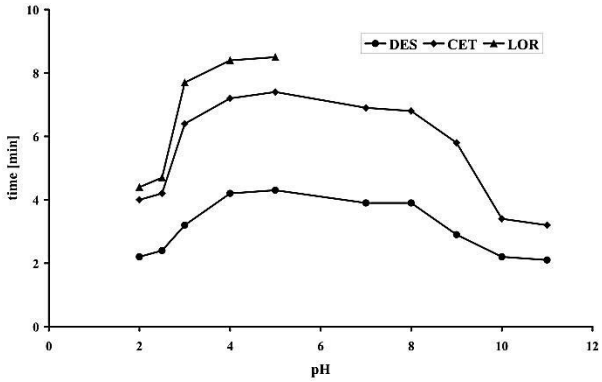

Figure 2. Effect of buffer pH on the separation of the studied antihistamines

At such a low pH, the ionisation of the acidic silanol groups on the capillary surface is slight and, therefore, the EOF flow rate and its influence on the separation is not significant. Consequently the separation is based only on the differences between the own electrophoretic mobilities of the analytes.

We used methanol and acetonitrile as organic modifiers but the results indicated that the addition of these modifiers had a detrimental effect on the separation.

Migration times and the resolution of the separation are influenced by the applied voltage and the system temperature. A higher voltage leads to more efficient separation and a shorter analysis time, but high currents can be a limiting factor for the separation, the limit depending on the system's ability to dissipate heat generated during electrophoresis. An increase in temperature causes a slight decrease in migration times because of the decrease in the viscosity of the buffer. Basically it is essential to establish a balance between the applied voltage and the temperature of the system. The optimum voltage was set at + 25 kV while the optimum temperature was set at 20 °C, in order to obtain a good resolution and a short analysis time.

A high injection pressure and a fast injection time may improve selectivity of the separation and also the shape and amplitude of the peaks. The optimum injection parameteres were set at a 50 mbar injection pressure and a 2 seconds injection time.

Using buffer solution containing 25 mM phosphoric acid, at a pH of 2.5, applying a voltage of + 25 kV at a temperature of 20 °C, we achieved the simultaneous separation of the studied antihistamines in approximately 5 minutes, the order of separation being: DES, CET, LOR (Figure 3).

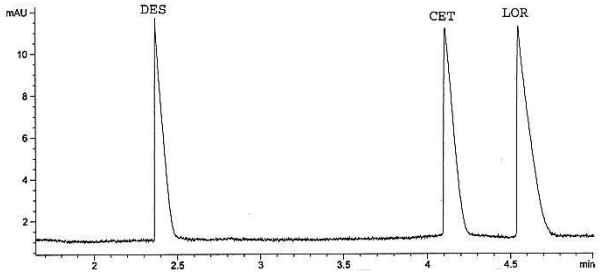

Figure 3. Electropherogram of the separation of the studied antihistamines using the optimized analytical conditions

Analytical performance

The optimized separation method was evaluated on the basis of precision (migration times and peak areas), linearity, robustness, limit of detection (LOD) and limit of quantification (LOQ).

Very similar migration times and peak areas and heights were obtained for six repeated measurements of the three analytes, the RSD values being smaller than 1% (Table 1).

The individual linear regression equations for each antihistamine was calculated according to six concentrations in a specific range (2.5 - 50 µg/mL) and

three replicates per concentration (Table 2). The linear regression coefficients were always above 0.99.

LOD and LOQ were estimated as: standard deviation of regression equation/slope of the regression equation multiplied by 3.3 and 10, respectively (Table 2).

Table 1. Analytical parameters of the studied antihistamines separation (n = 6, sample concentration = 10 µg/mL)

Substance	Migration time (min)	RSD (%)		
		RSD (%) migration time	RSD (%) peak area	DSR (%) peak height
DES	2.275	0.027	0.804	0.675
CET	3.925	0.061	0.906	0.659
LOR	4.308	0.058	0.874	0.798

Table 2. Linearity regression data for the separation of the studied antihistamines (n=3, concentration range=2.5-50 µg/mL)

Substance	Regression equation	Correlation coefficient	LOD (µg/ml)	LOQ (µg/ml)
DES	y = 1.9195x + 2.622	0.993	1.25	3.50
CET	y = 1.8798 + 1.9175	0.998	1.35	3.90
LOR	y = 2.3931x + 4.3726	0.993	1.30	3.70

The intra-day (average of 6 measurements taken on the same day) and inter-day precision (average of 6 measurements taken over 5 days) at three different concentrations (2.5, 5, 10 µg/mL) was also determined. Precision, expressed as relative standard deviations (RSD%) and accuracy expressed a relative error, were lower than 2.5% for all analytes (Table 3). This indicates the ability of the developed method to be used for the analysis of the studied substances in pharmaceutical preparations.

Table 3. Intra and inter-day precision of the studied antihistamines separation

Substance	precision		
	Day 1 (n=6)	Day 3 (n=6) RSD (%)	Day 5 (n=6)
DES	0.25	1.14	1.66
CET	0.60	1.57	2.05
LOR	0.55	1.44	2.12

The robustness of the method was examined by analyzing a mixture of the analytes (n = 3) by making slight changes to the following parameters: buffer pH (2.5-3.0), buffer concentration (25-30 mM), applied voltage (22-25 kV) and injection pressure (40-50 mbar), taking in consideration the variation of migration times. The slight variation of these parameter does not significantly modify the migration times (RSD<2.5%).

The optimized procedure was applied to the analysis of the studied individual antihistamines found in pharmaceutical preparations. Ten samples of each pharmaceutical formulation were analysed, and three injections were done to obtain the average values of the drug concentration. All the label claims were in the range of 95.5–101.5%, and the results were in agreement with the contents declared by the manufactures. The peaks obtained from the samples prepared from tablets were very similar with those obtained from standard and there were no noticeable interference from the matrix. It is important that tablets excipients do not interfere in the determination of the studied antihistamines, since they allow direct injections, thus involving minimum handling.

Conclusion

A CZE procedure with UV detection has been developed for the determination of the three most frequently used H1 antihistamine derivatives (DES, LOR, CET). Discussions have been focused on the optimization of the separation conditions by considering the following experimental parameters: buffer composition, buffer pH, voltage, temperature, pressure injection and time; under the criteria of maximum resolution and minimum analysis time. The proposed method was validated according to ICH guidelines. All the values were good enough for the method to be used in routine analysis.

The method proved to be robust, precise, simple and specific and is suitable for the practical determination of the studied antihistamines from pharmaceuticals and maybe also from biological samples.

Conflict of Interest

The authors declare that they have no conflict of interest.

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