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Simultaneous multiresponse optimization applied to epinastine determination in human serum by using capillary electrophoresis

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Abstract

Experimental design and optimization techniques were implemented for the development of a rapid and simple capillary zone electrophoresis method (CZE) for the determination of epinastine hydrochloride in human serum. The effects of five factors were studied on the resolution between the peaks for the target analyte (epinastine hydrochloride) and lidocaine hydrochloride, used as internal standard, as well as on the analysis time. The factors were the concentration and pH of the buffer, the injection time, the injection voltage and the separation voltage. The separation was carried out by using an uncoated silica capillary with 50 μm i.d. and total length 64.5 cm (150 μm of path length) and UV detection (200 nm).

Multiple response simultaneous optimization by using the desirability function was used to find experimental conditions where the system generates desirable results. The optimum conditions were: sodium phosphate buffer solution, 16.0 mmol L^{-1}; pH 8.50; injection voltage, 20.0 kV; injection time, 30 s; separation voltage, 26.7 kV.

The method was confirmed to be linear in the range of 2.0–12 ng mL^{-1}. The injection repeatability of the method was evaluated by six injections at three concentration levels, while intra-assay precision was assessed by analysing a single concentration level, yielding a CV's of ca. 1% for standard and 2% for serum samples. Accuracy was evaluated by recovery assays and by comparing with an HPLC method, the results being acceptable according to regulatory agencies. The rudgeness was evaluated by means of an experimental Plackett–Burman design, in which the accuracy was assessed when small changes were set in the studied parameters. Clean-up of human serum samples was carried out by means of a liquid–liquid extraction procedure, which gave a high extraction yield for epinastine hydrochloride (93.00%).

Keywords: Multiresponse optimization; Capillary electrophoresis; Epinastine

1. Introduction

Whenever a new capillary electrophoretic method is being developed, optimization is usually applied to reduce the analysis time and efforts, without losing the resolution between the peaks originated by the analyte migration. Moreover, the need of simultaneously taking into account different aspects of the analysis calls for the use of multi-criteria optimization. In order to carry out this type of study, experimental design is a valuable tool, specifically response surface analysis [1]. In addition, when different objective functions have to be optimized, the so-called Derringer's desirability function is a valuable tool to be considered [2]. The latter function requires to define which results are acceptable for each individual response, and which results are not acceptable at all.

Epinastine hydrochloride (EPN) (9,13b-dihydro-1*H*-dibenz[*c*,*f*]imidazo[1,5-*a*]azepin-3-amine hydrochloride, CAS 80012-43-7) is a novel anti-allergic, non-sedative drug, that acts as histamine H_1 receptor antagonist [3]. The use of EPN is gaining importance owing to the fact that it does not penetrate the blood/brain barrier (based on its physicochemical properties, such as hydrophilicity and cationic charge at the physiological pH range). Therefore, it is not expected to induce side effects of the central nervous system [4]. On the other hand, EPN ophthalmic solutions are applied to prevent itching of the eyes caused by allergic conjunctivitis (a condition in

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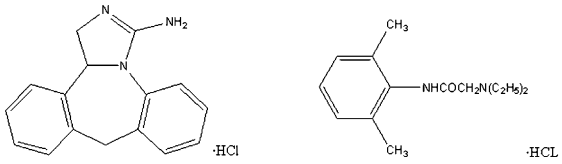

Epinastine hydrochloride Lidocaine hydrochloride

Fig. 1. Chemical structures of epinastine hydrochloride and lidocaine hydrochloride.

which the eyes become itchy, swollen, red, and teary when they are exposed to certain substances in the air) [5].

A recent pharmacokinetic study in pediatric patients, in which dosage was determined based on the body weight, showed that the average plasmatic concentration (C) is 25.6 ± 6.9 ng mL^{-1}, similar to those for adults after administration of 20 mg ($C = 26.9 \pm 9.1$ ng mL^{-1}) [4]. The pharmacokinetic properties of EPN make it a potential replacement for conventional non-sedating antihistamines, and provide it with great clinical relevance. In conclusion, precise pharmacokinetic properties should be investigated under several clinical states.

Remarkably, a limited number of publications deal with methods for the determination of EPN in human serum. They are exclusively based in high performance liquid chromatography (HPLC) with UV detection, most of them presenting poor sensitivity [6–8]. HPLC is an established technique with concentration sensitivity in the nanomolar range. However, capillary electrophoresis (CE), due to its high efficiency, offers a real and attractive alternative to HPLC, and appears as an appropriate technique for the analysis of biological samples, as demonstrated in several published papers in this area, in which CE has been shown to be a valuable alternative technique for their separation [9–12].

In this work, a CE method was developed, optimized and validated for the determination of EPN in human serum, reaching a sufficiently high sensitivity to follow the drug kinetics. The multiple response criteria were successfully used to optimize the separation of two analytes: EPN and lidocaine hydrochloride (LID), used as internal standard (Fig. 1). To the best of our knowledge, there seems to be no reports concerning methods for the determination of EPN in human serum by CE.

2. Experimental

2.1. Apparatus

All experiments were carried out on a capillary electrophoresis system (Agilent Technologies), equipped with a diode array detector. The instrument was operated under positive polarity (injection end of capillary). A PC Athlon 2.2 microcomputer was used for data handling. Electrophoretic separation was carried out with uncoated fused-silica capillary provided by Agilent Technologies with an inner diameter of 50 μm (150 μm of path length) and a total length of 64.5 cm (56 cm to detector). The pH of the buffers were adjusted by means of an Orion 9165 BN model 710a with Ag/ClAg, KCl electrode.

2.2. Software

A CE chemstation (Hewlett–Packard) was used for instrument control and data acquisition. Experimental design, data analysis and desirability function calculations were performed by using the software Stat-Ease Design-Expert trial Version 7.0.3.

2.3. Reagents

All the reagents were of analytical-reagent grade. They were preserved at 4 °C in the darkness during the experiments. Milli-Q quality water was used in all the CE experiments. Sodium phosphate, sodium hydroxide, sodium carbonate and lidocaine hydrochloride were obtained from Merck. All the buffers were filtered through a 0.45 μm nylon membrane (Sartorius-Germany) and degasified before use. The EPN standard was obtained from the commercial tablet Flurinol (Boehringer Ingelheim) by extraction and subsequent purification.

2.4. Electrophoretic conditions

The capillary, when new, was washed for 10 min with filtered 1 mol L^{-1} sodium hydroxide solution, for 10 min with 0.1 mol L^{-1} sodium hydroxide solution, for 10 min with Milli-Q water and for 10 min with electrolyte buffer solution.

At the beginning of the working day, the capillary was washed with sodium hydroxide 0.1 mol L^{-1} solution, Milli-Q water and finally with running buffer solution during 10 min.

Between runs, the capillary was washed successively with 0.1 mol L^{-1} sodium hydroxide solution, followed by Milli-Q water and then with running buffer solution for 2 min. At the end of the day, a last washing with 0.1 mol L^{-1} of sodium hydroxide solution and with Milli-Q water was performed.

All the solutions were degassed in an ultrasonic bath and filtered though 0.45 μm membrane filter before use. The electrolyte buffer solution was prepared at the beginning of the day. Samples were introduced into the capillary via electrokinetic injection by applying 20.0 kV during 30 s. A constant voltage of 26.7 kV was used for all experiments. The wavelength used for recording the electropherograms was 200 nm. The capillary was thermostated at 25.0 °C.

2.5. Method validation

EPN and LID were dissolved in water reaching final concentrations of 0.20 and 0.57 mg L^{-1}, respectively and stored as stock solutions, in the darkness at 4 °C. The standard solutions were prepared every day by dilution in Milli-Q water.

2.5.1. Calibration curves

The calibration curves were built by dilution of known amounts of analyte standard solutions in Milli-Q water. The concentration levels were: 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 ng mL^{-1}.The ratios between the peak areas for EPN and LID were plotted against the corresponding concentrations