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Robust analysis of the hydrophobic basic analytes loratadine and desloratadine in pharmaceutical preparations and biological fluids by sweeping—cyclodextrin-modified micellar electrokinetic chromatography

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ABSTRACT

The analysis of hydrophobic basic analytes by micellar electrokinetic chromatography (MEKC) is usually challenging because of the tendency of these analytes to be adsorbed onto the inner capillary wall in addition to the difficulty to separate these compounds as they exhibit extremely high retention factors. A robust and reliable method for the simultaneous determination of loratadine (LOR) and its major metabolite desloratadine (DSL) is developed based on cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) with acidic sample matrix and basic background electrolyte (BGE). The influence of the sample matrix on the reachable focusing efficiency is studied. It is shown that the application of a low pH sample solution mitigates problems associated with the low solubility of the hydrophobic basic analytes in aqueous solution while having advantages with regard to on-line focusing. Moreover, the use of a basic BGE reduces the adsorption of these analytes in the separation compartment. The separation of the studied analytes is achieved in less than 7 min using a BGE consisting of 10 mmol L^{-1} disodium tetraborate buffer, pH 9.30 containing 40 mmol L^{-1} SDS and 20 mmol L^{-1} hydroxypropyl-β-CD while the sample solution is composed of 10 mmol L^{-1} phosphoric acid, pH 2.15. A full validation study of the developed method based on the pharmacopeial guidelines is performed. The method is successfully applied to the analysis of the studied drugs in tablets without interference of tablet additives as well as the analysis of spiked human urine without any sample pretreatment. Furthermore, DSL can be detected as an impurity in LOR bulk powder at the stated pharmacopeial limit (0.1%, w/w). The selectivity of the developed method allows the analysis of LOR and DSL in combination with the co-formulated drug pseudoephedrine. It is shown that in CD-MEKC with basic BGE, solute–wall interactions are effectively suppressed allowing the development of efficient and precise methods for the determination of hydrophobic basic analytes, whereas the use of a low pH sample solution has a positive impact on the attainable sweeping efficiency without compromising peak shape and resolution.

1. Introduction

Capillary electromigration separation techniques are characterized by their high versatility, short run times, selectivity, extremely high efficiency and minimum solvent consumption. The use of capillary electrophoretic methods for the analysis of hydrophobic basic analytes suffers from some difficulties. One of these difficulties is the adsorption of these analytes onto the inner capillary wall, which can lead to a number of disturbances such as instability of the electroosmotic flow (EOF) velocity, poor figures-of-merit, peak deformation, sample loss, deterioration of separation efficiency and irreproducible migration times. The main driving forces for the adsorption of analytes onto the capillary wall are hydrophobic and/or electrostatic interactions [1]. Analysis of hydrophobic basic analytes by capillary zone electrophoresis is usually performed at acidic pH to get the analytes charged with positive electrophoretic mobility as well as to bring them dissolved in solution. The electrostatic interaction of these positively charged solutes with the inner capillary wall makes the adsorption problem more severe. Several approaches have been utilized to overcome this problem such as the use of extreme pH rinsing, manipulation of the ionic strength of the BGE, dynamic coating of the inner capillary surface with organic molecules or use of a permanently coated fused-silica capillary [2–5].

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Another problem, which is usually encountered with the analysis of highly hydrophobic analytes by MEKC, is their high retention factors that render their separation very difficult [6]. To overcome this problem, different approaches have been investigated e.g. the use of CD-MEKC [7–11]. The addition of a CD to the BGE alters the apparent retention factor of the analytes by introducing an additional equilibrium (the complex-formation) to the system. To understand the processes involved upon addition of CD to the BGE in CD-MEKC, different equilibria must be taken into account: the acid-base equilibrium of the weak base, the micelle-formation equilibrium, the distribution equilibria and the complex-formation equilibria. The distribution equilibria involve the distribution of both the ionized and the non-ionized forms of the analyte between the aqueous phase and the micellar phase. The complex-formation equilibria involve the formation of inclusion complexes between CD and both the ionized and non-ionized forms of the analyte as well as the surfactant monomer [12]. Therefore, by addition of a CD to the BGE, the apparent distribution coefficient $K_{D,app}$ of the analyte between the micellar pseudophase and the aqueous phase is reduced by increasing the fraction of analyte in the non-micellar phase resulting in a significant decrease in the apparent retention factor $k_{BGE,app}$ [6,9,12]. Moreover, the CD can form an inclusion complex with the SDS monomer and hence the micellization of SDS molecules is affected resulting in an increase of the apparent critical micelle concentration (CMC_{app}) of SDS, which is another reason for the significant decrease of $k_{BGE,app}$ upon addition of CD to the BGE [13–16].

Reducing the retention factor and avoiding the adsorption of hydrophobic analytes onto the capillary wall can be achieved by the addition of an organic solvent to the BGE [17]. CDs being added to the BGE similarly do not only reduce the apparent retention factor but also effectively suppress the adsorption of analytes onto the inner capillary wall and hence are reported to improve the efficiency and reproducibility of the separation method [18]. Consequently, the addition of a CD to the BGE is a viable alternative to the addition of an organic solvent to the BGE offering potential advantages over solvent-modified MEKC regarding the tunability of the retention factor, precision and/or efficiency. Applications of CD-MEKC in the analysis of hydrophobic analytes have been reviewed in several articles [19,20].

Sweeping is one of the most important sample preconcentration techniques in MEKC. It is based on the concentration enrichment of analyte by the pseudostationary phase (PSP) that penetrates the sample zone being void of PSP [21]. The final enrichment factor due to sweeping depends on both the retention factor in the sample zone and the retention factor in the BGE compartment [21,22]. In a previous publication [22], we introduced the term "retention factor gradient effect (RFGE)" to express the additional focusing or defocusing effect that arises if the distribution coefficient and hence the retention factor of the analyte is different in the sample and BGE compartments [22]. Sweeping can also be combined with dynamic pH junction to improve the focusing efficiency for certain analytes with regard to the focusing efficiency reached for these analytes with either exclusively dynamic pH junction or exclusively sweeping [23,24].

Loratadine (Fig. 1) or ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)piperidine-1-carboxylate is a long-acting non-sedating antihistaminic drug used for the symptomatic relief of allergic conditions. LOR is also co-formulated with the decongestant drug pseudoephedrine [25]. Chemically, LOR is a weak base with a pK_a of 5.25 at 25 °C [26] and an octanol/water partition coefficient log*P* of 5 [27]. LOR is insoluble in water and soluble in acids and alcohol [28]. Desloratadine (Fig. 1) or 8-chloro-6,11-dihydro-11-(4-piperidylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine is also a long-acting non-sedating antihistaminic drug. Its oral dose is half

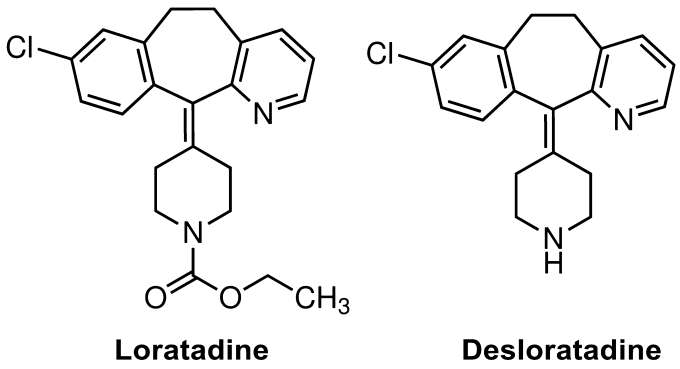

Fig. 1. Chemical structures of the studied analytes.

of the LOR dose. DSL has the same medicinal uses as LOR and is also co-formulated with pseudoephedrine [25]. Besides being an antihistaminic drug, DSL is the active metabolite of LOR and is one of its potential impurities. DSL is reported by the European Pharmacopeia [29] and the United States Pharmacopeia [30] as one of the related substances of LOR that must pass a liquid chromatographic limit test. The maximum allowed limit of DSL as an impurity in LOR powder is 0.1% (w/w) [29,30]. Chemically, DSL is a weak base having two pK_a values, 4.41 and 9.97 at 25 °C [26] and an octanol/water partition coefficient log*P* of 3.2 [31]. DSL is slightly soluble in water and well soluble in acids, ethanol and propylene glycol [28]. DSL is synthesized by decarboxylation of LOR [32–36]. Therefore, LOR may be contained as an impurity in DSL powder due to incomplete reaction or purification.

The high structural and physicochemical similarities between LOR and DSL render the simultaneous analysis of both drugs difficult. Different analytical methods for the simultaneous determination of LOR and DSL have been published in the literature. These include UPLC [37], HPLC [38–54], HPTLC [55], TLC [56], GC [57] and spectrophotometric [58] methods. Most of the chromatographic methods reported for the simultaneous determination of LOR and DSL depend on mass spectrometric detectors which are expensive and not readily accessible in many laboratories.

Regarding capillary electromigration separation techniques, Fernandez et al. [59] developed a method for the determination of loratadine and its related impurities including desloratadine based on capillary zone electrophoresis (CZE) using an uncoated fused-silica capillary and a BGE consisting of 100 mmol L^{-1} phosphoric acid pH 2.5 containing 10% (v/v) acetonitrile. Fernandez et al. [59] reported that their developed method suffered from poor figures-of merit especially regarding the precision. They also reported that the poor precision of their method provided some validation parameters, which did not meet the official requirements. They attributed the reason of this problem to analyte-wall interactions (adsorption of the analyte onto the inner capillary wall). Different strategies to solve this problem were developed by the authors, however, with insignificant improvement. The final conclusion drawn by Fernandez et al. [59] was that the developed CZE method is only suitable as a complementary tool for the impurity profiling of LOR during stability tests. Moreover, they stated that the validation parameters of this method are poorer than those described for an HPLC method for the same compounds and therefore HPLC would be preferable to CZE for quantitation purposes.

The aim of the present work is to develop a robust, precise and reliable capillary electromigration separation method for the simultaneous determination of LOR and DSL based on CD-MEKC with acidic sample matrix and basic BGE (pH 9.30). In order to reduce the reported problems due to solute-wall interactions, a basic pH of the BGE is utilized as the studied analytes are non-ionized at this pH and hence the ionic interaction with