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Effect of low concentration sodium dodecyl sulfate on the electromigration of palonosetron hydrochloride stereoisomers in micellar electrokinetic chromatography

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ABSTRACT

The effect of low concentrations of sodium dodecyl sulfate (SDS) on the separation of palonosetron hydrochloride (PALO) stereoisomers by micellar electrokinetic chromatography (MEKC) has been investigated. It was found that the addition of SDS prolongs the migration time and the migration order of four stereoisomers changes regularly with the SDS concentration. Good separations for all the four stereoisomers were achieved at appropriate SDS concentration. The effect of SDS on the electromigration (mobilities) of PALO stereoisomers has been studied, in order to explain its effect on the separation by MEKC. It was found that low concentrations of SDS added into the separation media forms negatively charged complexes with PALO stereoisomers and hence reverses their electromigration direction. Furthermore, the migration order between two enantiomeric pairs is also reversed because the enantiomeric pair with a bigger positive mobility than that of another pair turns to have a bigger negative mobility when bound with SDS. Based on these results, the effect of SDS on the MEKC separation of PALO stereoisomers was elucidated reasonably. The performance of the developed chiral MEKC method was validated by the analysis of a real sample.

1. Introduction

Palonosetron hydrochloride (PALO (3aS, 2S)), (3aS)-2-[(*S*)-1-azabi-cyclo[2.2.2]oct-3-yl]-2,3,3*a*, 4,5,6-hexahydro-1*H*-benz[*de*]-isoquinolin-1-one hydrochloride, is a highly selective second generation 5-HT3 receptor antagonist used for the prevention of nausea and vomiting associated with chemotherapy [1–4]. It contains two chiral centers in molecular structure (see also Appendix A, Fig. S1) and thus has four stereoisomers belonging respectively to two pairs of enantiomers, i.e. PALO (3aS, 2S), PALO (3aR, 2R), PALO (3aS, 2R) and PALO (3aR, 2S). Among them only PALO (3aS, 2S) possesses pharmacological activity [5,6]. Therefore, development of enantiomeric separation methods for PALO stereoisomers is of great importance for the control of its enantiomeric impurities and avoiding unwanted pharmaceutical and toxicological side effects [5–8].

Micellar electrokinetic chromatography (MEKC), as a combination of chromatographic and electrophoretic mechanisms, is an important separation mode of capillary electrophoresis (CE) [9–12]. The separation based on the former mechanism depends on the selectivity, relative strength of affinity of solutes for the micelles. The separation based on the latter mechanism depends on the mobility difference between two solutes. Separation of the four stereoisomers of PALO by MEKC using sodium cholate (SC) as surfactant and chiral selector has been reported [13,14], which includes the separation in each enantiomeric pair (chiral separation) and the separation between enantiomeric pairs (achiral separation). Our previous research [14] found that the migration orders in each enantiomeric pair are 3aS, 2S < 3aR, 2R and 3aS, 2R < 3aR, 2S (the sequence of migration time), determined by selectivity (chromatographic mechanism). The enantiomeric pair (3aS, 2S), (3aR, 2R) (as a whole) is eluted before enantiomeric pair (3aS, 2R), (3aR, 2S) due to the mobility difference (electrophoretic mechanism). Because of the offset of the two mechanisms which give opposite migration orders, the peaks of PALO (3aR, 2R) and (3aS, 2R), the second enantiomer of the first pair and the first enantiomer of the second pair, coalesce in pure MEKC at the original

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pH of borate buffer (pH 9.20). Therefore, the separation for all of the four stereoisomers is not achieved, although the resolution in each pair of enantiomers is very good. In order to achieve the separation between PALO (3aR, 2R) and (3aS, 2R), Tian et al. [13] added methanol of high concentration (solvent modified MEKC) and we added butanol of low concentration (cosurfactant modified MEKC) into the micellar solution [14]. The mechanism of separation is tipping the balance between two effects by weakening the chromatographic effect while strengthening the electrophoretic effect, according to the measured selectivity and mobility data. In this work, a commonly used anionic surfactant sodium dodecyl sulfate (SDS) was employed as an alternative to organic solvents in selectivity tuning of MEKC. The separation between PALO (3aR, 2R) and (3aS, 2R) was obtained by adding low concentration of SDS into the separation media. The mechanism of separation is altering the electromigration (mobilities) of PALO stereoisomers through the formation of negatively charged complexes with SDS.

2. Material and methods

2.1. Chemicals and reagents

The four enantiomerically pure PALO stereoisomers were purchased from J&K Scientific Ltd. (Beijing, China). Sodium cholate (SC) was purchased from Serva Feinbiochemica (Heidelberg, Germany). PALO injection (PALO (3aS, 2S) content, 50 μg mL^{-1}), a real sample for the validation of the method, was the product of Chia Tai Tianqing Pharmaceutical Co. Ltd. (Jiangsu, China). Other chemicals used were of analytical regent grade and were used without further purification.

2.2. Preparation of separation media (BGE) and sample solutions

Micelle solutions for MEKC were prepared by dissolving appropriate quantities of SC surfactant, SDS if necessary, and sodium tetraborate buffer in distilled water to the desired volume in a flask. The solution was sonicated while covered for 15 min to form a transparent micelle solution.

The sample solutions of enantiomerically pure standards, used in the development of the method, were prepared by dissolving appropriate quantities of each enantiomerically pure PALO steroisomer mixedly in appropriate background electrolyte (BGE) to a concentration of 0.1 mg mL^{-1}. A small amount of methanol was added as an electroosmotic flow (EOF) marker. The spiked sample solutions, for the validation of the developed method, were prepared by adding appropriate quantities of enatiomeric impurity standards mixedly into the PALO injection to concentrations ranging from 0.5 to 5.0 μg mL^{-1}.

All solutions were filtered through a 0.45 μm filter prior to use.

2.3. Electrophoresis experiments

A TH-3100 capillary electrophoresis system equipped with a UV detector (Tianhui Institute of Separation Science, Hebei, China) was employed for all electrophoresis (CE) experiments. The detection wavelength was 214 nm. An uncoated fused silica capillary of id 50 μm × od 365 μm (Yongnian Photoconductive Fiber Factory, Hebei, China) was used, with a total length (L_{tot}) of 60.0 cm and an effective length (L_{eff}) of 50.0 cm. New capillaries were pretreated by flushing in sequence with distilled water for 5 min, 1.0 M NaOH for 10 min and distilled water for 5 min again at 140 kPa (approx. 20 psi). Between injections, the capillary was rinsed in sequence with distilled water, 1.0 M NaOH, distilled water again and finally BGE for 2 min each at 140 kPa. The capillary cartridge temperature was maintained at 20 °C. Injections were performed hydrodynamically at 10 kPa (approx. 1.5 psi) for 1 s and 5 s, for the solutions of enantiomerically pure standards and the spiked sample solutions, respectively. An applied voltage of 25 kV was used for all experiments.

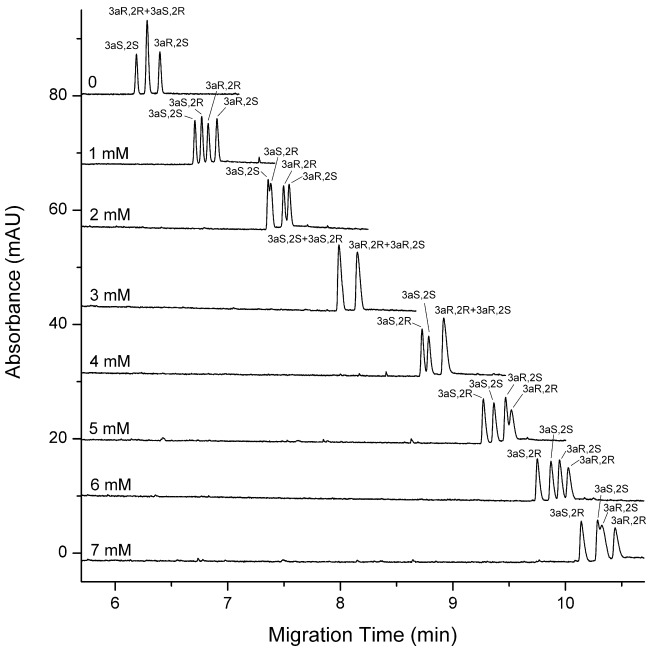

Fig. 1. Effect of SDS concentration on separation of PALO stereoisomers by MEKC. Composition of micellar solution is 30 mM SC in 30 mM sodium tetraborate of pH 9.20. Detection wavelength: 214 nm. Capillary: id 50 μm, L_{tot} 60.0 cm, L_{eff} 50.0 cm. Capillary temperature: 20 °C. Hydrodynamic injection at 10 kPa for 1 s. Applied voltage: 25 kV.

2.4. UV spectrometry tests

The UV spectra of PALO stereoisomers in the presence of varying concentrations of SC or SDS surfactant in 30 mM sodium tetraborate buffer were recorded in the range from 200 to 300 nm with a UV-3200 spectrophotometer (Shanghai Mapada Instruments Co. Ltd., Shanghai, China), using quartz cells with 1 cm path length. The reference cell contained the same concentrations of surfactant and buffer as the sample.

3. Results and discussion

3.1. Effect of SDS on separation of PALO stereoisomers by MEKC

At the top of Fig. 1 is the electropherogram of PALO stereoisomers obtained by pure MEKC (no SDS addition) using a 30 mM SC micelle solution. As mentioned earlier, the peaks of PALO (3aR, 2R) and (3aS, 2R) coalesce due to the offset of chromatographic mechanism and electrophoretic mechanism, so the migration order of four stereoisomers is 3aS, 2S < 3aR, 2R = 3aS, 2R < 3aR, 2S (the sequence of migration time). When low concentration of SDS is added into the separation media, the migration time of stereoisomers is prolonged and it increases with the concentration of SDS. Furthermore, the migration order between two enantiomeric pairs is reversed, i.e., the peaks of enantiomeric pair (3aS, 2S), (3aR, 2R) (as a whole) move gradually to the rear of those of another pair (3aS, 2R), (3aR, 2S) (Fig. 1).

As can be seen in Fig. 1, when 1 mM of SDS is added into the micelle solution, the coalesced peak of PALO (3aR, 2R) and (3aS, 2R) is resolved and the peaks belonging to two enantiomeric pairs begin to interlace, i.e., the first enantiomer of the second pair, PALO (3aS, 2R), is eluted before the second enantiomer of the first