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Short communication

Suppression of chiral recognition of 3-hydroxy-1,4-benzodiazepines during micellar electrokinetic capillary chromatography with bile salts

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Abstract

During the development of a micellar electrokinetic chromatographic screening method for 1,4-benzodiazepines, peak splitting and broadening were observed for some 3-hydroxy-1,4-benzodiazepines (oxazepam, lorazepam, temazepam and lormetazepam). This phenomenon occurred when the micellar phase consisted of bile salts and can be ascribed to the chiral nature of these surfactants. As the bile salts were applied in order to reduce the capacity factors to an appropriate level, enantiomer separation was not an objective and even disturbing. By increasing the analysis temperature, the chiral recognition of these compounds could be suppressed.

1. Introduction

Capillary electrophoretic techniques have been applied in pharmaceutical analysis with great success [1]. Separations of heterogeneous drug mixtures, containing a few benzodiazepines, have been reported [2,3] but only a limited number of studies were specifically dedicated to 1,4-benzodiazepines [4–7]. Except for the analysis of a few cationic species, which can be electrophores in an acidic buffer [4], neutral to mildly alkaline buffers containing an anionic surfactant have been applied [2,3,5–8]. However, with sodium dodecyl sulphate (SDS), the most popular surfactant in micellar electrokinetic capillary chromatography (MEKC), the capacity factors are high, generally above 20 [9]. Therefore, they elute close to the t_{mc} (retention time of the micelle) and resolution is limited. Some have organic modifiers added to the buffer to reduce capacity factors [3,5–8] but, through the reduction of the electroosmotic flow, this also leads to prolonged analysis times [6]. Another way to reduce k' values is to replace SDS with a less solubilizing surfactant, such as bile salts [10]. However, being chiral, bile salts also have the ability to interact differently with enantiomers [11]. This can lead to unwanted peak broadening or peak splitting. Pure 3-hydroxy-1,4-benzodiazepine enantiomers are difficult to isolate, are quickly racemized in aqueous medium [12,13] and are clinically used in racemic forms [14]. Therefore, enantiomeric recognition is not required for drug quality control or screening

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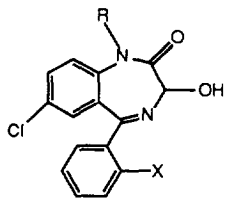

	R	X
Oxazepam	H	H
Temazepam	CH_3	H
Lorazepam	H	Cl
Lormetazepam	CH_3	Cl

Fig. 1. Benzodiazepines used in this study.

purposes, and is in fact a complicating factor. In this paper it will be shown that, for the 3-hydroxy-1,4-benzodiazepines (Fig. 1), chiral recognition can be simply suppressed by increasing the temperature during the MEKC analysis with bile salts.

2. Experimental

Electrokinetic chromatography was performed on a P/ACE 2100 system (Beckman, Palo Alto, CA, USA). Fused-silica capillaries (Beckman) were of 75 μm I.D., 375 μm O.D., 57 or 97 cm long, with the detection window 7 cm from the capillary outlet. The capillaries were liquid cooled at the temperatures indicated.

Temazepam, lorazepam, oxazepam and lormetazepam were obtained from Wyeth Labs. (Maidenhead, Berks., UK). Borate and borate–phosphate buffers were prepared with deionized water (Seralpur Pro 90 CN, Seral, Germany) and contained various amounts of the surfactants sodium cholate (SC) or sodium deoxycholate (SDC) (Sigma, St. Louis, MO, USA). All the buffer solutions were filtered through a 0.2-μm membrane filter.

Samples were introduced by pressure for 2 s, analysed with an applied voltage of 20 kV, unless stated otherwise, and detected at 214 nm. Between runs the capillary was flushed for 2 min with buffer and left to equilibrate for 5 min.

3. Results and discussion

During initial attempts at the MEKC analysis of a set of fourteen derivatives of 1,4-benzodiazepines with bile salts [9], some broadened peaks were observed. After having eliminated sample matrix effects, by preparing the sample in the separating buffer, and recognizing that the unusual peak shapes occurred only for the 3-hydroxy derivatives, it was obvious that chiral selectivity could be the reason for the peak broadening and splitting. The 3-hydroxybenzodiazepines were the only racemates in the mixture. From the literature, it is known that the enantiomers are easily racemized [12,13,15]. It is also known that, when the time scales of racemization and separation are comparable, typical peak interconversion profiles are obtained in which the signal between the elution of the two enantiomers forms a plateau [16], rather than returning to the baseline. This is caused by the fact that, during the analysis, a fraction of the enantiomer species is converted into its mirror image. They will therefore migrate during the first part of the analysis with the characteristics of one enantiomer, and during the remaining time with the characteristics of the other. This phenomenon has been observed in gas chromatography [16] and liquid chromatography [17] and in the MEKC analysis of diastereomeric rotamers [18,19].

By increasing the analysis time (by decreasing the applied voltage or, more efficiently, by increasing the column length, Fig. 2), the split and broadened peaks could be transformed into these typical interconversion patterns. The fact that the same relative intensities in those patterns were observed at four different wavelengths (200, 214, 254 and 280 nm) further contributes to the proof that the observed effects are related to enantiomeric recognition by the bile salt micellar phase. In those cases where peak splitting occurred, the second peak always had a higher intensity. It is known [13,14] that the racemization half-lives of 3-hydroxybenzodiazepines are increased in a less polar, aprotic medium. The hydrophobic micellar interior is enriched with the more retained enantiomer, which can be assumed to have, on average, an increased half-life. Consequently, the formation of a small enantiomeric excess during analysis can be expected.

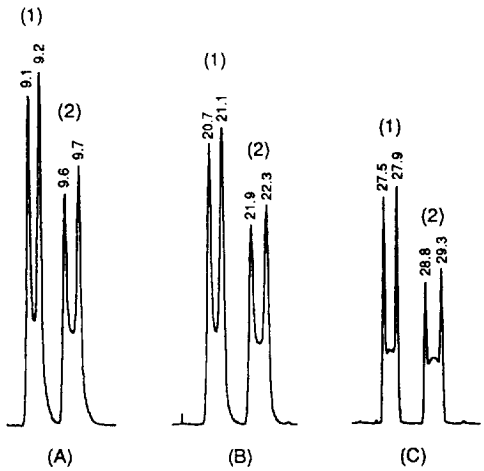

Fig. 2. MEKC separation of (1) temazepam and (2) oxazepam in a bile salt buffer as a function of analysis time [20 m*M* borate–phosphate (pH 8.0), 60 m*M* SC, 20°C]. (A) 20 kV, column length 57 cm; (B) 10 kV, 57 cm; (C) 20 kV, 97 cm.

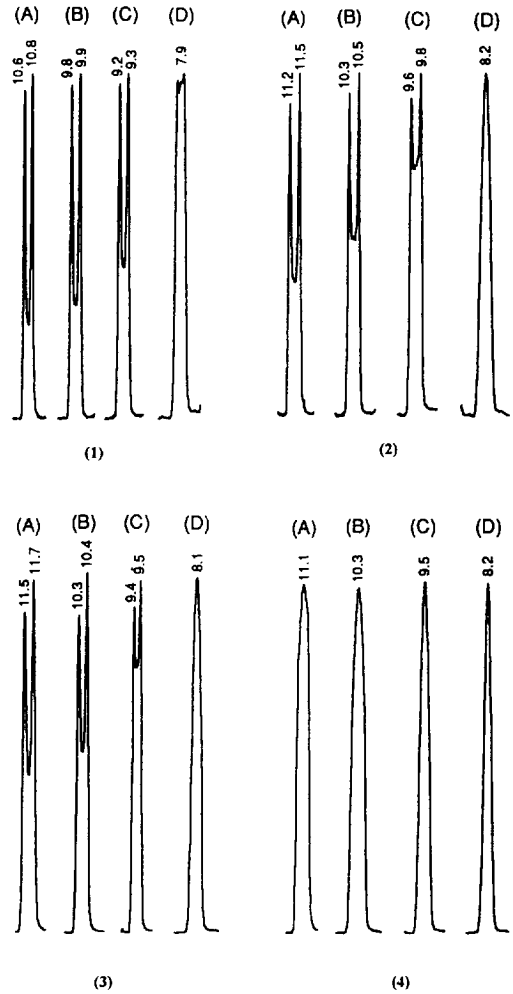

Fig. 3. MEKC separation of (1) temazepam, (2) oxazepam, (3) lorazepam and (4) lormetazepam in a bile salt buffer as a function of temperature [20 m*M* borate (pH 8.0), 50 m*M* SC, 20 kV, column length 57 cm]. Temperature: (A) 20; (B) 22; (C) 25; (D) 30°C.

In contrast, increasing the temperature leads to an increase in the racemization speed. Once fast enough, in relation to the analysis time, chiral recognition is no longer observed and single, sharp peaks are obtained (Fig. 3). The results shown were obtained with SC as surfactant, but comparable results have also been obtained with SDC. Among the four species, lormetazepam enantiomers are known to have the shortest half-life (0.7 min at 37°C and pH 7.5 [15]), and this benzodiazepine is also the easiest to obtain as a sharp single peak. The reported half-lives for the other species at 37°C and pH 7.5 (oxazepam 3.0 min, lorazepam 1.3 min, temazepam 3.4 min [15]) do not correlate well with the degree of peak splitting that is observed. Of course, apart from the different temperature and pH conditions in our experiments, differences in enantioselectivity and capacity factors will also play a role. For lorazepam the half-life at 23°C (also at pH 7.5) was reported to be 5.0 min [15]. This is comparable to the time scale of the analyses shown in Fig. 3.

In the series shown in Fig. 3, some peak splitting can still be observed for temazepam. This could be avoided by further increasing the temperature to 33°C, at which a single, sharp peak was obtained at any pH investigated from 7 to 11. Fig. 4 shows the separation of four 3-hydroxybenzodiazepines after optimization in 20 m*M* borate buffer (pH 10.2) containing 50 m*M* SC and 10% acetonitrile with a fused-silica capillary of length 57 cm at 33°C.

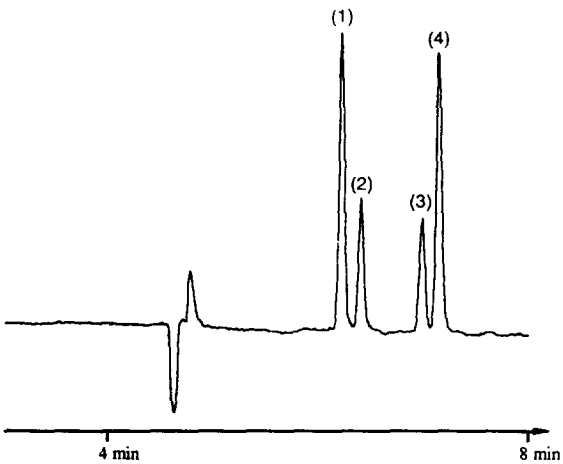

Fig. 4. MEKC separation of 3-hydroxybenzodiazepines [buffer, 20 m*M* borate (pH 10.2), 50 m*M* SC, 10% acetonitrile; 20 kV, column length 57 cm, 33°C]. Peaks: 1 = temazepam; 2 = lormetazepam; 3 = oxazepam; 4 = lorazepam.

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