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Enantioseparation of alkyl tropate by high performance liquid chromatography using (2*R*, 3*R*)-di-*n*-propyl tartrate

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Abstract

Optically active tartaric acid and its diester derivatives are well known as effective chiral selectors. In the current studies, a self-prepared diester of tartaric acid (2*R*, 3*R*)-di-*n*-propyl tartrate (DPT) was used as a chiral mobile-phase additive (CMPA) for the enantioseparation of seven kinds of alkyl tropate on a silica gel column by high performance liquid chromatography (HPLC). Four kinds of alkyl tropate were successfully enantioseparated using this chiral system, indicating that DPT is a novel useful chiral selector. The influences of mobile-phase composition and solute structure on enantioseparation were extensively studied. Although different types of alcoholic additive or various concentrations of alcoholic additive, DPT and dichloromethane in the mobile phase had great influence on the retention factor (k') and resolution (R_s), the mobile-phase composition essentially had no effect on the separation factor (α). In contrast, the structure of alkyl tropate greatly influenced the separation factor (α). The results revealed that an increase in the bulkiness of the *O*-alkyl groups of tropic acid esters reduced the separation factors (α), indicating that inhibition of the formation of effective hydrogen-bond interaction between DPT and solutes by the bulkiness of the *O*-alkyl group decreases the retentivity difference between the transient diasterieomeric complex pairs. Our observations suggested that the hydrogen-bond interactions between chiral additives and solutes might be the major chiral recognition mechanism for DPT as CMPA.

Keywords: (2*R*, 3*R*)-Di-*n*-propyl tartrate; Chiral mobile-phase additive; Alkyl tropate; Enantioseparation; High performance liquid chromatography

1. Introduction

Diester and diamide derivatives of tartaric acid are well known as effective chiral selectors [1–6]. Because these derivatives are symmetric in C_2 axis and two kinds of functional groups, hydroxyl and carbonyl, attached to asymmetric carbons in these derivatives are stereochemically equivalent to those groups attached to other carbons; these structural features are favorable for them to be chiral selectors. (*R*, *R*)-diisopropyl tartrate or (*R*, *R*)-diisoamyl tartrate has been applied in solvent extraction as chiral selector to recognize enantiomers through hydrogen-bond associations [1]. Dobashi et al. [2,3] studied the enantioseparation of a wide range of enantiomers on a silica gel column using (*R*, *R*)-*N*,*N*′-diisopropyltartramide as a chiral mobile-phase additive (CMPA) and proposed that the enantioselections were based on dual diastereomeric hydrogen-bond associations between the chiral additive and the enantiomer to be resolved. The enantioseparation of acids, esters and alcohols of moderate hydrophobicity containing two hydrogen bonding functions was performed using (2*R*, 3*R*)-dicyclohexyl tartrate in phosphate buffer as mobile phase and porous graphitic carbon (Hypercarb) as stationary phase [5]. In the similar condition the chromatographic method for the determination of the enantiomeric purity of (*S*)-atropine was presented and had been applied to the analysis of tablets [6].

The anticholinergic activity of the two enantiomers of atropine was demonstrated in several papers [7–10], always with a higher potency for the (*S*)-enantiomer [11]. Tropic acid is the intermediate of atropine. The enantioseparation of tropic acid has been studied on cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) and (*S*, *S*)-Whelk-O 1 columns [12] and the chiral recognition of ethyl tropate

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was described using cellulose tribenzoate chiral stationary phase (CSP) in reversed-phase chromatography [13]. The ester derivatives of tropic acid are also useful in the study of chiral recognition mechanism since they may have different ester structure. We have recently compared the chiral recognition mechanism between cellulose derivative CSPs and (*S*, *S*)-Whelk-O 1 using ester derivatives of three kinds of 2-arylpropionic acid including tropic acid [14].

In the current studies, the enantioseparation of seven kinds of alkyl tropate were studied on a silica gel column using self-synthesized (2*R*, 3*R*)-di-*n*-propyl tartrate (DPT) as chiral mobile-phase additive (CMPA). Four kinds of alkyl tropate (methyl, ethyl, *n*-propyl and *iso*-propyl) were successfully enantioseparated, indicating that DPT is a novel useful CMPA. In order to investigate the chiral recognition mechanism, the influences of mobile-phase composition and solute structure on enantioseparation were extensively studied. The results suggested that the hydrogen-bond interactions between chiral additives and solutes might be the major chiral recognition mechanism for DPT as CMPA.

2. Experimental

2.1. Apparatus and column

Enantioseparations were performed using Waters 2690 Separations Module equipped with a Waters 996 Photodiode Array Detector and Waters Millennium32 System (Waters Co., Milford, MA, USA). The silica gel column (Lichrosorb Si60, 10 μm, 250 × 4.0 mm i.d.) was purchased from Merck Co. (Darmstadt, Germany). All chromatographic analyses were carried out at ambient temperature and the UV absorbance signal was monitored at 259 nm.

2.2. Materials and reagents

All the reagents were of analytical grade except that *n*-propanol and *sec*-butyl alcohol were of chemical grade. *n*-Hexane was purchased from Hangzhou Refinery Factory (Hangzhou, PR China). Dichloromethane and ethanol were obtained from Huipu Chemical Industry Apparatus Co. (Hangzhou, PR China). Methanol and *iso*-propanol were purchased from Shanghai Ludu Reagent Factory (Shanghai, PR China). *n*-Propanol and *sec*-butanol were obtained from Shanghai Chemical Reagent Factory (Shanghai, PR China). *n*-Butanol and *tert*-butanol were purchased from the Shanghai First Reagent Factory (Shanghai, PR China). Tropic acid (99%) was provided by Hangzhou Minsheng Pharmaceutical Group Co. (Hangzhou, PR China). (*R*, *R*)-tartaric acid (99%) was obtained from Wenzhou Longwan Plant (Wenzhou, PR China). Alkyl tropate and tartaric acid ester were self-synthesized as previously described methods [13,15]. All other chemicals were analytical grade reagents produced in PR China.

2.3. Preparation of sample solutions and mobile phases

Sample solutions (1 mg/ml) were prepared by dissolving 1 mg of solute in 1 ml of dichloromethane. All sample solutions were filtered through a 0.2 μm membrane filter (Millipore, Waters Co., USA) before the injection. The mobile-phases were composed of indicated volume ratio of *n*-hexane and dichloromethane and containing indicated concentrations of alcoholic additives and DPT and were filtered through a 0.45 μm membrane filter (Millipore, Waters Co., USA) and degassed before use.

3. Results and discussion

3.1. Influence of alcoholic additives

The results from the preliminary experiments revealed that none alkyl tropate can be enantioseparated on silica gel column using the mobile phase composed of *n*-hexane and dichloromethane containing DPT without alcoholic additive. However, after adding alcoholic additives in the mobile phase, four kinds of alkyl tropate (methyl, ethyl, *n*-propyl and *iso*-propyl) obtained enantioseparation. Then the influences of different type and various concentration of alcoholic additive in mobile phase were extensively studied.

Table 1 lists the influence of different type of alcoholic additive in mobile phase on the enantioseparation of alkyl tropate. The mobile phase was composed of *n*-hexane and dichloromethane (20:80, v/v) containing 2 mM DPT and 3% alcoholic additive. As shown by the data in Table 1, as the

Table 1
Influence of different type of alcoholic additive on the enantioseparation of alkyl tropate

Alcoholic additive	Methyl tropate			Ethyl tropate			*n*-Propyl tropate			*iso*-Propyl tropate		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
Ethanol	2.47	1.16	1.78	1.94	1.26	3.94	2.28	1.10	1.87	1.95	1.12	1.53
n-Propanol	2.56	1.18	2.88	2.53	1.33	4.57	2.66	1.14	2.33	2.31	1.12	2.03
iso-Propanol	3.25	1.19	2.93	2.59	1.28	4.60	2.80	1.18	3.01	3.14	1.17	2.73
n-Butanol	4.09	1.23	3.73	3.44	1.28	5.94	3.88	1.22	4.02	3.82	1.16	3.02

The k_1' is the retention factor of eluting first enantiomer. Chromatographic conditions: mobile phase, dichloromethane/*n*-hexane (80:20, v/v) containing 2 mM DPT and 3% alcoholic additives, flow rate, 0.5 mL/min.