

Influence of Mobile Phase Composition on the Enantioseparation of Methoxyl Flavanones with Self-prepared CDMPC Column and Chiral Recognition Mechanism

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Abstract: The influence of different alcohol modifiers in mobile phase on the chiral separation of 4'-methoxyl flavanone, 5-methoxyl flavanone and 6-methoxyl flavanone on cellulose tris (3, 5-dimethylphenylcarbamate) (CDMPC) column was studied and the chiral recognition mechanism was discussed. Using hexane-*tert*-butanol (1.31 mol L⁻¹) as the mobile phase, those three methoxyl flavanones were excellently separated on CDMPC chiral column.

Keywords: Cellulose tris (3,5-dimethylphenylcarbamate), methoxyl flavanone, chiral stationary phase, enantioseparation.

Chiral stationary phases (CSPs) based on the cellulose derivatives have proven to be one type of the most useful CSPs in high-performance liquid chromatography because of their versatility, durability and loadability^{1, 2}. Flavanone was often used as a standard chiral compound for evaluating cellulose derivative for chiral columns^{1, 3}. Krause and Galensa⁴ reported the enantioseparation of flavanone and its seven derivatives on six kinds of commercial chiral column. 4'-Methoxyl flavanone, 5-methoxyl flavanone and 6-methoxyl flavanone were enantioseparated best on Chiralcel OD (CDMPC) column using hexane-2-propanol (90:10, vol./vol.) as the mobile phase. But the chiral recognition mechanism of methoxyl flavanones on the cellulose derivative CSPs has not been reported. In this paper we studied the influence of different alcohol modifiers in mobile phase on the enantioseparation of the three methoxyl flavanones with self-prepared CDMPC column and the chiral recognition mechanism was discussed.

The CDMPC chiral column was prepared as previously reported^{5, 6}. The three methoxyl flavanones and silica gel were kindly donated by Prof. Dr. J.N. Kinkel. The structures of CDMPC and the three flavanone derivatives are illustrated in **Figure 1**. Enantioseparations were performed using Waters 2690 Separations Module equipped with a Waters 996 PDA Detector and Waters Millennium³² System.

The influence of different alcohol modifier in mobile phase on the chiral separation of methoxyl flavanones with CDMPC column is listed in **Table 1**. As seen in **Table 1**, (1) the enantioseparation of 4'-methoxyl flavanone on CDMPC is the worst compared with that of other two methoxyl flavanones; (2) for 4'-methoxyl flavanone, the

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enantioseparation with *iso*-propanol, *sec*-butanol or *tert*-butanol as the alcohol modifier is better than that with ethanol, *n*-propanol or *n*-butanol and for all the three methoxyl flavanones, the longest enantioseparation factor was obtained when *tert*-butanol was used as the alcohol modifier.

Figure 1 Structures of CDMPC and three kinds of flavanone derivatives

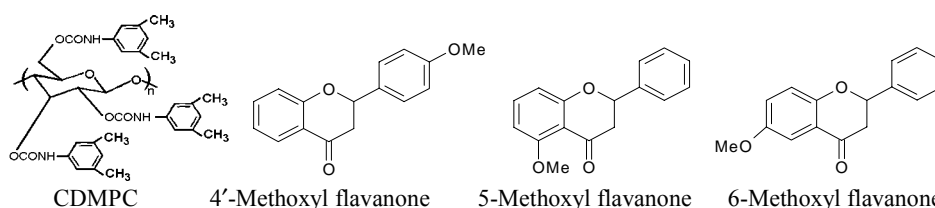


Table 1 Influence of different alcohol modifiers in mobile phase on the chiral separation of methoxyl flavanones with CDMPC column

Alcohol modifier	4'-methoxyl flavanone			5-methoxyl flavanone			6-methoxyl flavanone		
	k_1' *	α	R_S	k_1' *	α	R_S	k_1' *	α	R_S
Ethanol	1.55	1.08	1.35	2.23	1.29	4.60	1.23	1.19	2.80
<i>n</i> -Propanol	1.54	1.08	1.31	2.10	1.31	4.32	1.17	1.22	2.98
<i>n</i> -Butanol	1.50	1.07	1.07	1.88	1.32	4.01	1.08	1.30	3.64
<i>iso</i> -Propanol	1.79	1.16	2.45	2.71	1.31	4.75	1.39	1.22	3.21
<i>sec</i> -Butanol	1.70	1.13	2.04	2.54	1.31	4.46	1.24	1.22	2.91
<i>tert</i> -Butanol	2.24	1.30	4.62	4.14	1.38	4.24	1.73	1.36	5.03

* The retention factor of first eluted enantiomer; the dead time (t_0) was determined using 1,3,5-tri-*tert*-butyl benzene.

Chromatographic conditions: stationary phase, CDMPC (column dimension, 30cm \times 0.4cm i.d.; particle diameter of silica gel, 5 μ m; pore diameter of silica gel, 20 nm); mobile phase, hexane-alcohol (the concentration of alcohol modifier in hexane, 1.31 mol L⁻¹); flow rate, 0.5 ml min⁻¹; temperature, 30°C; detection, Waters 996 PDA detector (254 nm).

There exist π - π , hydrogen-bond and dipole-dipole interactions between CDMPC and the three methoxyl flavanones. The phenyl ring of CDMPC should be a π -base because of the two methyl groups. For the three methoxyl flavanones, the phenyl ring with methoxyl group is a strong π -base because of the methoxyl group. So the π - π interaction between the phenyl ring of CDMPC and the phenyl ring with methoxyl group of flavanones are weaker than that between the phenyl ring of CDMPC and the phenyl ring without methoxyl group of the flavanones. On the other hand, we can see from **Figure 1** that the phenyl ring without methoxyl group is connected with the chiral carbon for 5-methoxyl flavanone and 6-methoxyl flavanone, but for 4'-methoxyl flavanone, the corresponding phenyl ring is not connected with the chiral carbon. This may be the reason of the worst enantioseparation of 4'-methoxyl flavanone on CDMPC. So the π - π interaction might play an important role in the enantioseparation of the three methoxyl flavanones on CDMPC chiral stationary phase.

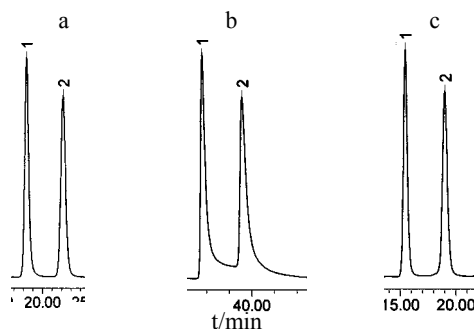
However, when *tert*-butanol was used as the alcohol modifier in the mobile phase, the enantioseparation of 4'-methoxyl flavanone was also excellent and so were the other two solutes. This result shows that the mobile phase composition greatly influences the enantioseparation of the three methoxyl flavanones on CDMPC chiral column, especially

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for 4'-methoxyl flavanone. The alcohol modifiers in the mobile phase compete for the hydrogen-bond dot on CDMPC chiral stationary phase with solutes. The competition ability of *iso*-propanol, *sec*-butanol or *tert*-butanol is weaker than that of ethanol, *n*-propanol or *n*-butanol and the competition ability of *tert*-butanol was the weakest among all the alcohol modifiers. This may be the reason that for 4'-methoxyl flavanone the enantioseparation with *iso*-propanol, *sec*-butanol or *tert*-butanol as the alcohol modifier is better than that with ethanol, *n*-propanol or *n*-butanol as the alcohol modifier and, for all the three methoxyl flavanones, the longest enantioseparation factor was obtained when *tert*-butanol is used as the alcohol modifier. The alcohol modifier influence results show that, besides the π - π interaction, the hydrogen-bond interaction is also important for the enantioseparation of all the three methoxyl flavanones on CDMPC chiral stationary phase.

Figure 2 shows the best enantioseparation chromatograms of the three flavanones on CDMPC column.

Figure 2 The enantioseparation of three kinds of flavanone derivative on coated CDMPC column



(a) 4'-methoxyl flavanone; (b) 5-methoxyl flavanone; (c) 6-methoxyl flavanone
Chromatographic condition: mobile phase, hexane-*tert*-butanol (1.31 mol L⁻¹); for other conditions, see **Table 1**.

In conclusion, our results show that the mobile phase composition greatly influences the enantioseparation of the three methoxyl flavanones on CDMPC chiral stationary phase. The three methoxyl flavanones were excellently separated on CDMPC chiral column when hexane-*tert*-butanol (1.31 mol L⁻¹) was used as the mobile phase. The π - π interaction might play an important role in the enantioseparation, meanwhile the hydrogen-bond interaction is also important for the enantioseparation of all the three methoxyl flavanones on CDMPC chiral stationary phase especially for the enantioseparation of 4'-methoxyl flavanone.

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