

High-Performance Liquid Chromatographic Enantioseparation of Flavanones on 2-Hydroxy-3-Methacryloyloxypropyl β -Cyclodextrin Copolymer Coated Silica Phase

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Abstract

Chromatographic resolution of four flavanones is achieved by reversed-phase high-performance liquid chromatography (HPLC) on a chiral stationary phase based on silica coated with a (2-hydroxy-3-methacryloyloxypropyl β -cyclodextrin-co-*N*-vinylpyrrolidone) copolymer. The influence of the mobile phase water content and the nature of the organic modifier on the retention and resolution is evaluated. Monosubstituted flavanones are better resolved than the unsubstituted one. Nevertheless, the 6- and 7-methoxy substituents enhance retention and chiral recognition to polymeric β -cyclodextrin stationary phase less than the 6-hydroxy group.

Introduction

Considering that pharmacological activity and metabolism of the two enantiomers of a certain drug are very often different, enantiomeric separation remains a significant challenge to separation science and an area of intense interest within the pharmaceutical industry. Cyclodextrin (CD)-containing chiral stationary phases (CSPs) have proved to be a powerful tool for the separation of a wide variety of enantiomers. There are several possibilities to immobilize native CDs or their derivatives on silica for the preparation of CSPs, such as the covalent linkage of CDs on silica (1–8) or the use of CD polymer coated CSPs (9–13).

Flavanoids have been recently the subject of significant interest as pharmaceutical components, showing possible anticancer properties and abilities to prevent cardiovascular problems, bacterial infections, and inflammations (14–17). Because flavanones are frequently extracted from plants either in a racemic or an optically active form, tremendous efforts have been expended to achieve their enantiomeric separation employing cellulose-coated silica gel (18,19) as well as columns packed with CD-bonded silica (20–23).

The primary objective of this work was to point out the enantioseparation ability of a new type of polymeric modified hydroxyl

propyl (HP) β -CD based chiral selectors, made of 2-hydroxy-3-methacryloyloxypropyl β -CD (modified HP β -CD) copolymers coated on silica phases, towards flavanone and three of its monosubstituted derivatives. The chromatographic behavior has been investigated as a function of the nature and composition of the mobile phase

Experimental

Chemicals

Racemic flavanone (98%) and 6-hydroxyflavanone (99%) were purchased from Avocado (La Tour du Pin, France). Racemic 6-methoxyflavanone (98%) and 7-methoxyflavanone (98%) were supplied by Sigma-Aldrich (L'Isle d'Abeau Chesnes, France). Acetonitrile, methanol, and water of high-performance liquid chromatographic grade, also supplied by Sigma-Aldrich, were filtered through a membrane filter with a 0.45- μ m pore size (Type HVLP, Millipore, Billerica, MA), and degassed by ultrasonic vibration prior to use.

CSPs preparation

Several CSPs based on (2-hydroxy-3-methacryloyloxypropyl- β -CD-co-*N*-vinylpyrrolidone) copolymers coated on silica were obtained and characterized as reported previously (13). The structure of the copolymeric chiral selector is shown in Figure 1. The present study is limited to the enantioselective properties of Si Copo VP 5 support, whose main characteristics are recalled in Table I.

Reproducibility and stability of the CSPs

Reproducibility of polymer adsorption

Using the Copo VP 5 sample, three different batches were prepared according to the adsorption method previously described (13). The amounts of deposited copolymer were 162.3, 170.4, and 174.7 mg/g, respectively, indicating satisfactory reproducibility.

The second batch, the characteristics of which are given in Table I, was used in the following.

The polymer coating was stable in the pH range from 7 (pH at which is performed the coating procedure) and 3.3 (water-methanol-acetic acid as mobile phase). After a 3-month use, the CSP was analyzed by thermogravimetric analysis to determine the amount of polymer coated. No significant change was detected, indicating that no desorption of the coated polymer occurred.

Reference separations were carried out periodically in order to control the long time reproducibility of the separations. This was done (i) with the position isomers of nitrophenol and (ii) with the enantiomers of flavanone. No significant shift of the separation factor (α) and resolution (R_s) values was detected over a 3-month period.

Apparatus and chromatographic conditions

The stainless steel column (10-cm \times 4.6-mm i.d.) was home-packed by using a Touzard & Matignon model slurry packer as follows: 1.25 g of silica support was supported in 10 mL of carbon tetrachloride (Acros-Organics, Noisy Le Grand, France), sonicated for 1 min and packed into the column at 200 bars with dichloromethane (Sigma-Aldrich). The column was then washed with methanol before use. The high-performance liquid chromatographic (HPLC) system was purchased from Merck-Eurolab

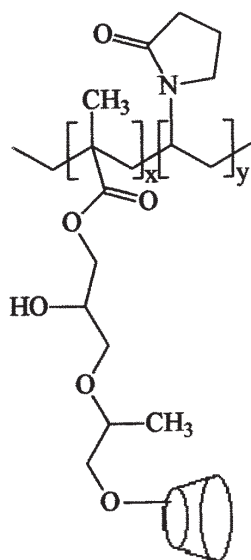


Figure 1. Structure of (2-hydroxy-3-methacryloyloxypropyl β -CD-co-N-vinylpyrrolidone) copolymer.

Table I. Composition of (2-Hydroxy-3-Methacryloyloxypropyl β -CD-co-N-Vinylpyrrolidone) Copolymer Copo VP 5 and Characteristics of Coated Silica Phase Si Copo VP 5

Copolymer	x	y	CSP	Deposited copolymer (mg/g)	Modified HP β -CD (μ mol/g)	N-Vinyl pyrrolidone (μ mol/g)	Specific area (m^2/g)	Porous volume (cm_3/g)
Copo VP 5	0.12	0.88	Si Copo VP 5	170.4	78	581	229	0.71

(Nogent sur Marne, France) and consisted of a L6200A Intelligent Pump, a Rheodyne model 7125 injector with a 20- μ L loop, a L4250 UV-vis variable-wavelength spectrophotometric detector and a D2500 Chromato-Integrator (Cotati, CA). The operating conditions were: flow rate of 1 mL/min, room temperature, and UV detector wavelength 280 nm. The void volume was determined by injecting saturated potassium nitrite solution.

Preparation of sample solutions

Sample solutions were obtained by dissolving solutes in methanol-water (v/v) mixtures at 100 μ g/mL. They were stored at room temperature and filtered through a filter membrane (0.45 μ m, Type HVLP, Millipore) before injection.

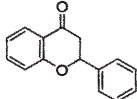
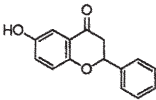
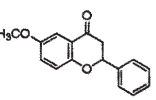
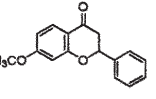
Results and Discussion

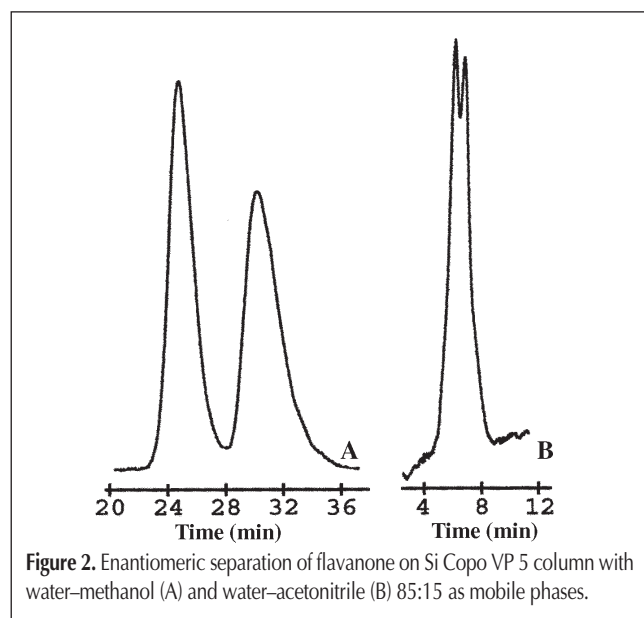
The effect of environmental changes on the separation process was examined by modifying the percentage and type of the organic cosolvent (methanol or acetonitrile) added to water. Such eluent mixtures have been successfully applied to provide chiral separation of neutral flavanone compounds using CD-based CSPs under reversed-phase HPLC (22,23). However, it should be mentioned that our modified HP β -CD support was stable when using various buffer-methanol or buffer-acetonitrile mixtures as mobile phases. The chromatographic data for flavanone and three monosubstituted flavanones resolved on the Si Copo VP 5 phase are summarized in Table II. In all instances, an increase of the retention factors (k) was observed, as the methanol percentage decreased, in agreement with the behavior predicted for reversed-phase chromatography using CD-based CSPs (24,25). Moreover, there was a substantial improvement in the separation factors (α) and resolutions (R_s) obtained on the Si Copo VP 5 column as the methanol content of the eluent phase was reduced. It was found that under comparable conditions, monosubstituted flavanones had larger k and α values and were better resolved than nonsubstituted flavanone. For instance, the latter one was not resolved using a water-methanol (45:55) mixture. Therefore, it is assumed that the presence of a substituent in the 6 or 7 position is responsible for additional interactions between the solutes and the modified HP β -CD copolymer selector that selectively influence the chiral recognition mechanism, leading to greater resolution. However, the Si Copo VP 5 CSP used in the present work led to better chiral separations, especially in the case of flavanone as compared with the ureido-bonded methylated β -CD CSPs developed by Ng et al. (23). Surprisingly, under comparable elution

conditions, the separation factors and the resolutions obtained with the three monosubstituted flavanones were in the same range, although 6-hydroxyflavanone was better resolved. Only the retention factors were strongly affected by substitution. However, the influence of substitution on the retention appeared to be dependent on the nature (proton donor or acceptor) of the substituent and its position (6 or 7) in the aromatic ring fused with the hetero ring. The hydroxylated flavanone was more retained than the methoxy-

lated ones and the retention factors for 7-methoxyflavanone were greater than those observed for 6-methoxyflavanone. This behavior is different from the situation on Cyclobond I and acetylated Cyclobond I, where the introduction of a hydroxy group in 6-position decreased the retention factors (22). The influence of the nature of the organic cosolvent was evaluated by using acetonitrile in place of methanol. In all cases, it was found that both retention and enantioselectivity were greater with water–methanol mixtures than with water–acetonitrile ones (Figure 2 as an example). This can be explained by the fact that

Table II. Chromatographic Data for the Enantioseparation of Flavanone and Its Derivatives on Si Copo VP 5 Column with Water–Methanol Mixtures as Mobile Phases

Compound	H ₂ O–MeOH	k	α	R _s
Flavanone 	45:55	0.54	1	–
	55:45	1.27	1.10	0.57
	65:35	3.05	1.14	0.92
	75:25	6.64	1.20	1.28
	85:15	14.27	1.24	1.42
6-Hydroxy flavanone 	45:55	0.56	1.06	–
	55:45	1.40	1.13	0.71
	65:35	3.80	1.19	1.11
	75:25	9.71	1.28	1.48
	85:15	21.73	1.33	1.71
6-Methoxy flavanone 	45:55	0.56	1.07	–
	55:45	1.11	1.13	0.72
	65:35	3.23	1.20	1.03
	75:25	8.38	1.27	1.43
	85:15	18.96	1.33	1.61
7-Methoxy flavanone 	45:55	0.50	1.07	–
	55:45	1.18	1.13	0.73
	65:35	3.47	1.20	1.09
	75:25	9.05	1.26	1.38
	85:15	20.32	1.31	1.57



methanol is more hydrophilic and thus interacts less strongly with the interior of the β -CD cavity and is consequently regarded as the weaker displacer of analytes from CD cavity (23). The ratio of acetonitrile in the water–acetonitrile mixture was reduced down to 96:4 in order to obtain retention times around 24 mn for the first peak, which was comparable to the separation with 85:15 water–methanol. Even in this case the resolution of flavanone was poor.

Conclusion

The use of a new chiral stationary phase based on a (2-hydroxy-3-methacryloyloxypropyl β -CD-co-*N*-vinylpyrrolidone) copolymer coated on silica was extended to the enantiomer separation of non- and monosubstituted flavanones. The present study, as well as other works, showed that substituents that increase hydrogen bonding with CD chiral selector tend to enhance retention and resolution of flavanone derivatives. This effect was more pronounced when the substituent can act as a proton donor. A more comprehensive investigation of the separation mechanism of the flavanones studied here, as well as polysubstituted flavanones and coumarine derivatives, is now in progress and the results will be presented elsewhere.

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