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Application of Immobilized Amylose and Cellulose Chiral Stationary Phases for the Enantioseparation of Methoxyflavanones Enantiomers by Liquid Chromatography

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The HPLC enantiomeric separation of four methoxyflavanones substituted in positions; 4', 5, 6 and 7 respectively was accomplished in the normal-phase mode using tow polysaccharide-derived chiral stationary phases immobilized on silica Chiralpak IA and Chiralpak IB and various *n*- hexane/alcohol mobile phases. The chiral recognition mechanism of each stationary phase is suggested based on the chemical nature and conformation of the chiral selector.

Keyword: Methoxyflavanones, HPLC, Enantioseparation, chiral stationary phase.

1. Introduction

Flavanones have been a potential source in the search for lead compounds and biologically active components and have been the focus of much researches and development in the last 30 years. Flavanones present a unique structural feature known as chirality, which distinguishes them from all other classes of flavonoids[1-8]. A numbers of publications in recent years have reviewed the multiple scientific achievements in the field of the polysaccharide-derived CSPs and their applications in the separation of enantiomers[24,28]. The most applicable chiral stationary phases are based on the linear derivatized polysaccharide family of chiral selectors such as cellulose and amylose coated or immobilized on silica support. These chiral selectors have been commercialized as the Chiralpak and Chiralcel CSPs[24,27]. Krause and Galensa 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 column using hexane-2-propanol (90:10, vol./vol.) as the mobile phase. For the same compounds, B. H. Shao et al are studied the influence of different alcohol modifiers in mobile phase on the chiral separation on cellulose tris (3, 5-dimethylphenylcarbamate) column, using hexane-*tert*-butanol (1.31 mol L-1) as the mobile phase, those three methoxylflavanones were excellently separated[13]. Recently, K. Si-Ahmed *et al* were used the phenyl-carbamate-propyl-β-CD stationary phase in the nano-LC and achieved the separation of enantiomers and diastereoisomers of flavanones with good results. In the present study, four methoxyflavanones were separated by HPLC into their enantiomers on tow kind of chiral stationary phases. flavanones was then evaluated by using normal mode. Different chromatographic parameters including composition of the mobile phase, nature of organic solvent and flow rate were

optimized to obtain the complete enantioseparation of all studied compounds.

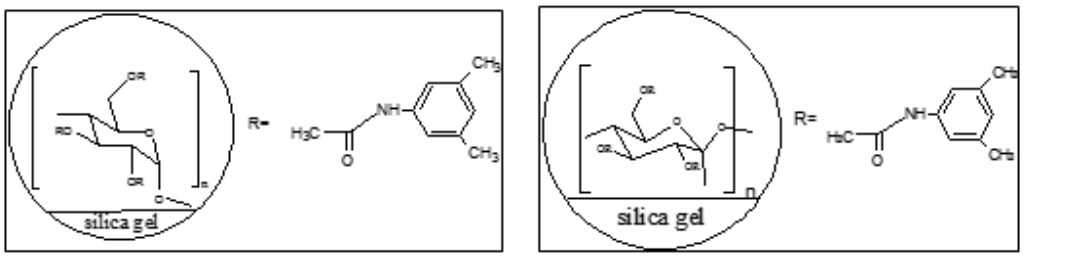
2. Experimental

2.1 Instrumentation and Chromatographic Conditions

The analytical chromatographic instrument used in this study is an SHIMADZU series apparatus equipped with a pump LC-20A, a degasser DGU-20 A_5, a multiple wavelength UV detector SPD-20A and LCsolution software. The mobile phase for LC was filtered through a Millipore membrane filter (0.5 µm) and degassed before use. Four different mobile phase systems were investigated in this study. All of them were composed of commonly used organic HPLC solvents: (1) ethanol–hexane mixtures; (2) isopropanol–hexane mixtures; (3) pure ethanol; (4) pure isopropanol; The proportion of each mobile phase component was always measured by volume. The chromatographic runs were performed at a room temperature ~ 25 ◦C. Sample injection was done by using injection valve (20µl).

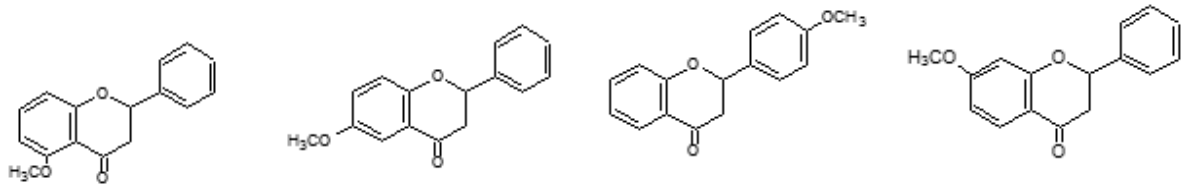
2.2 Chemicals

The solvents used for chromatography were of HPLC grade, isopropanol from MERCK KGaA (darrnstadt germany), n-hexane and ethanol were purchased from Sigma-Aldrich (Seelze, Germany). The selected flavanones (4'-methoxyflavanone, 5-methoxyflavanone, 6-methoxyflavanone and 7-methoxyflavanone) were from Sigma-Aldrich (St. Louis, MO, USA). Standard solutions of each flavanone (1 mg/mL) were prepared in MeOH. Detection was carried out at 254nm.



Amylose tris (3, 5-dimethylphenylcarbamate) Cellulose tris (3, 5-dimethylphenylcarbamate)

Fig 1: The chiral selectors based on tris (3, 5-dimethylphenylcarbamate) of amylose and cellulose[29-31].



5-methoxyflavanone 6-methoxyflavanone 4'-methoxyflavanone 7-methoxyflavanone

Fig 2: Chemical structures of studied flavanones

2.3 Chiral Stationary Phases

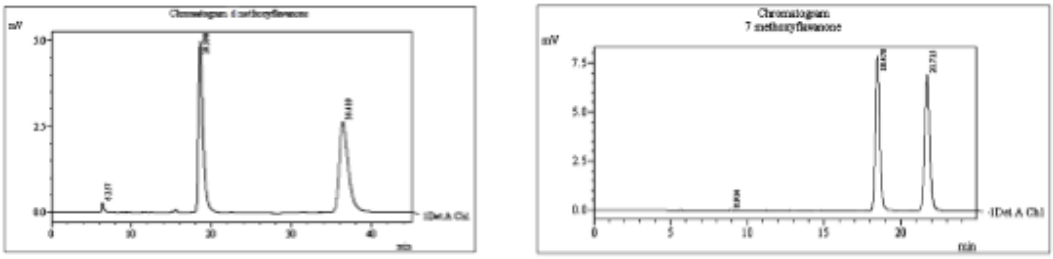
The columns Chiralpak IA, Chiralpak IB, were obtained from Chiral Technologies Europe (Illkirch Cedex, France). Chiralpak® IA and Chiralpak® IB are the first in a series of polysaccharide derived chiral chromatographic columns from Daicel compatible with all ranges of organic miscible solvents. These new immobilized chiral stationary phases show a unique solvent flexibility and excellent chiral recognition ability.

Compound	CSP	Eluent (alcohol%)	Flow rate	t_{r1}	t_{r2}	Rs	α
5-Methoxyflavanone	Chiralpak® IA	100[a]	0.2	11.357	12.514	2.327	1.240
5-Methoxyflavanone	Chiralpak® IB	100[b]	0.2	30.117	34.984	2.999	1.236
6-Methoxyflavanone	Chiralpak® IB	100[b]	0.2	27.950	29.818	1.687	1.161
6-Methoxyflavanone	Chiralpak® IA	100[a]	0.5	18.590	36.419	11.950	2.457
4'-Methoxyflavanone	Chiralpak® IA	100[a]	0.5	17.656	38.231	17.929	2.403
4'-Methoxyflavanone	Chiralpak® IB	100[b]	0.2	29.141	30.069	0.752	1.074
4'-Methoxyflavanone	Chiralpak® IB	05[b]	0.6	18.183	18.967	1.521	1.472
7-Methoxyflavanone	Chiralpak® IA	50[a]	0.6	12.855	28.602	22.600	5.215
7-Methoxyflavanone	Chiralpak® IB	05[b]	0.6	18.478	21.715	5.721	1.339

Note: CSP, Chiral Stationary Phase, T= 25 °C; [a] Hexane/ethanol, [b] Hexane/isopropanol

3. Results and Discussion

To optimize the conditions for obtaining the separation of enantiomers or diastereomers of all flavanones studied we used commercialised compounds in the chiral separation screening on the 2 CSPs and various (n-hexane/ethanol or isopropanol) mobile phases. Table 1 illustrates the best chromatographic results obtained for the separation of (2R/2S) - flavanones using the two CSPs. Chiralpak IA shows the best selectivity and resolution values with short retention time for all studied flavanones. The elution orders of those flavanones on the two phases are similar; this may be related to their common structural feature. Slight differences in elution order may, however, be attributed to differences in the chemical nature and physical properties of chiral stationary pahses[35]. The difference in the chiral recognition ability between the amylose and the cellulose may be due to the different volumes of the helical groove of the cellulose derivative and the amylose derivative, because it is well known that amylose-derived phases possess a wider and more compact helix[36].



(a) (b)

Fig 3: Chromatograms of chiral separations of (a)6-methoxyflavanone on Chiralpak IA. Mobile Phase: ethanol, flow rate: 0.5ml/min, injection volume: 20 µL, détection: 254nm, α=2.457, *Rs*= 11.950, (b) 7- methoxyflavanone on Chiralpak IB. Mobile Phase: hexane/isopropanol 95:05, flow rate: 0.6ml/min, injection volume: 20 µL, détection: 254nm, α=1.339, *Rs*= 5.721.

3.1 Molecular Structure and Chiral Recognition

The phenyl ring of the CSP should be a π-base because of the two methyl groups. For the methoxyflavanones, 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 the CSP and the phenyl ring with methoxyl group of flavanones is weaker than that between the phenyl ring of the CSP and the phenyl ring without methoxyl group of the flavanones. For 5-,6- and 7-methoxyflavanone the phenyl ring without methoxyl group is connected with the chiral carbon but for 4′-methoxyflavanone, the corresponding phenyl ring is not connected with the chiral carbon. This may be the reason of the worst enantioseparation of 4′-methoxyflavanone on Chiralpak IB. So the π-π

interaction might play an important role in the enantioseparation of the flavanones on cellulose tris (3, 5-dimethylphenylcarbamate) chiral stationary phase[17]. Therefore, in correlation with the molecular structure, the lower chiral discrimination of 5-methoxyflavanone than the other three methoxyflavanones is probably resulted from the spatial configuration of the 5-methoxyl substituent which may form an intramolecular hydrogen bonding with the carbonyl group.

4. Conclusion

Our results from this study clearly demonstrate that the chromatographic system based on polysaccharide derivatives CSPs immobilized on silica provides a powerful analytical tool for identification and quantification of isomeric mixtures of title compounds. The amylose derivative shows a best resolution of all studied compounds. The recognition of these molecules implies the inclusion phenomena, π-π interactions; meanwhile the hydrogen-bond interaction is also important for the enantioseparation of all the four methoxyl flavanones on both cellulose and amylose derivatives CSPs.

5. Acknowledgments

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