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Comparison of the enantioseparation of racemic uridine analogs on Whelk-O 1 and ChiralPak-AD columns

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Abstract

The commercially available, brush-type (S,S)-Whelk-O 1 chiral stationary phase (CSP) has been used to separate 10 racemates of structurally related uridine analogs, potentially anti-viral agents, under various mobile phase compositions, using various temperatures. The enantioseparation was evaluated by comparing the Whelk-O 1 column performance with that of ChiralPak-AD column, reported previously. The comparison involved the role of some distinctive structural features of the racemates, type and composition of the solvent modifiers, as well as effect of temperature on the chiral discrimination. Despite the fact that both columns separate almost all the uridine analogs, significant differences were observed in their chiral recognition, as revealed from their retention, selectivity, resolution and elution order. The chiral recognition processes, responsible for enantioseparation on the Whelk-O 1 column, were relatively more systematic and easier to manipulate than on ChiralPak-AD column. Enantioseparation on the latter are of more complex nature and frequently gave results that were contradictory to the expectations. On the other hand, the performance in the ChiralPak-AD column was superior to that of the Whelk-O 1column. Limitations in column handling and maintenance (pressure and temperatures) as well as limited solvent choice lead to the preference of the Whelk-O 1 column, in spite of its lower (but adequate) performance.

Keywords: Enantiomer separation; Uridine

1. Introduction

In chiral liquid chromatography, the selection of an appropriate CSP column is probably the most substantial challenge in method development [1]. Problems associated with the use of certain columns include the instability of the CSP under some mobile phase conditions, restricted temperature ranges, pressure and flow-rate. Therefore, the availability of durable, stable and high-performance column is preferable in developing a rugged and reproducible method [2]. Among large numbers of commercially available CSP columns, the brush-type, π-donor π-acceptor Whelk-O 1 CSP exhibited relatively high level of enantioselectivity and compares favorably with the polysaccharide-based CSPs for its versatility

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[1]. In our earlier work, the carbamated amylose ChiralPak-AD CSP that is coated on silica support has been used for the separation of medicinally active cannabinoids [3,4]. The same CSP type was employed to separate anti-viral active agents of uridine analogs [5]. Successful separations were obtained in most cases, using this polysaccharide-based CSP [3–5]. We noticed, however, a significant difference on column-to-column reproducibility even when columns were purchased from the same manufacturer [4]. Upon replacement of the column, further adjustments of the mobile phase conditions were a necessity [4]. Moreover, proper care was carefully maintained, following the manufacturer's recommendations for modifier types and concentration, pressure limitations, flow-rate and temperature ranges to avoid the failure of such an expensive column.

Pirkle-type CSPs are more robust than the polysaccharide-based CSPs and generally have long lifetime durability [1]. Columns such as Whelk-O 1 have greater flexibility in operation, apparently as a result of the inherent stability of the covalent nature of this selector. This brings about a column that is compatible with all commonly used mobile phases, including the aqueous system, therefore minimizing the need for further adjustments of separation conditions when replacing a column.

The selector used in Whelk-O 1 CSP was initially designed for the separation of underivatized enantiomers of the non-steroidal anti-inflammatory drug (NSAID), naproxen [6]. The chiral recognition mechanisms responsible for the enantioseparation is relatively better understood than that of ChiralPak-AD, in which retention processes are complex and not yet adequately elucidated. This is probably due to the presence of multiple recognition sites on the macromolecular ChiralPak-AD CSP.

In the present study, we used the (S,S)-Whelk-O 1 CSP for the separation of the same family of uridine analogs that have been previously resolved on ChiralPak-AD CSP. The influence of varying the temperature on selectivity and resolution was investigated and compared for both chiral systems. The effect of various structural features of racemates, modifier type and composition on chiral recognition is discussed.

2. Experimental

2.1. Chemicals and reagents

All 10 racemates of uridine analogs were contributed by Professor Salo Gronowitz at the Chemical Center, Lund, Sweden.

All the solvents were HPLC grade; *n*-hexane and 2-propanol (Lab-Scan, Dublin, Ireland) and ethanol (Carlo-Erba, Milano, Italy).

2.2. Instrumentation

The HPLC analysis was performed by an HP1050 (Hewlett-Packard, Palo Alto, CA) instrument, equipped with a photodiode array UV-detector, and HPCHEM data station. A Rheodyne (Cotaty, CA) injection valve, equipped with a 20-μl loop was used. Circular dichroism (CD) spectra were recorded using a Jobin-Yvon (Longjuneau, France) model CD6.

2.3. Chiral columns

The commercially available chiral columns were ChiralPak-AD (10 μm), 250×4.6 mm I.D (Daicel Chemical Industries, Tokyo, Japan), the π-donor π-acceptor (S,S)-Whelk-O 1 column (5 μm), 250×4.6 mm I.D. (Merck, Darmstadt, Germany).

2.4. Procedure for chiral HPLC analysis

Flow rates of 1.00 and 0.85 ml/min were used with the Whelk-O 1 and the ChiralPak-AD columns, respectively. To get optimum separation reproducibility, column was thermostated at 10–35±1°C, using a circulating bath. Each run was monitored at two wavelengths simultaneously (250 and 320 nm). The racemates were injected, and the two separated peaks were collected. The two solutions were analyzed by CD to verify that the peaks represent enantiomers.

3. Results and discussions

Ten pairs of uridine analogs were separated and

studied on Whelk-O 1 CSP by using various proportions of ethanol or 2-propanol as modifiers with *n*-hexane, the bulk solvent. The effect of varying column temperature on chromatographic parameters, such as retention factor (k'), selectivity (α), resolution (R_s) and elution order, were also examined. The structures of the uridine analogs selected for this study are depicted in Fig. 1. These compounds are different from each other by specific structural features, particularly the type and position of the hetero-atom in the cyclopentadienyl ring (designated as X in Fig. 1, where X=O, S, Se) and the hydroxy versus the acetoxy group of the methyl cyclopentenyl moiety (designated as Y in Fig. 1, where Y=OH or $OCOCH_3$, respectively).

The enantioselective behavior was compared to similar observations, previously reported on the polysaccharide-base CSP, the ChiralPak-AD [5].

3.1. Performance of Whelk-O 1 CSP

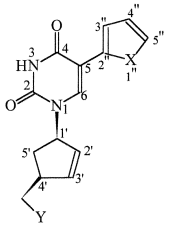
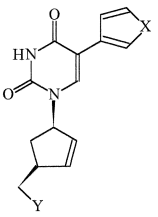
We investigated the effects of varying the composition of ethanol and 2-propanol with *n*-hexane on chiral discrimination. Generally, attempts to resolve uridine racemates directly by using the above-mentioned binary solvent mixtures was successful. As shown in Tables 1 and 2 and Figs. 2 and 3, a reasonable resolution was achieved for almost all of the uridine analogs on Whelk-O 1 CSP, using either ethanol (Fig. 2) or 2-propanol (Fig. 3) as the modifier. Base-line separations were obtained for all the acetylated uridine analogs, regardless of the modifier used.

3.1.1. Effect of type and composition of mobile phase modifiers

Organic modifiers are mainly used to control both retention capabilities and selectivity of the chiral columns. Pirkle-type CSPs are frequently utilized with classical normal-phase mobile phases, consisting of mixtures of protic modifiers like ethanol and/or 2-propanol and a non-polar solvent like *n*-hexane. Unlike the ChiralPak-AD column, the Whelk-O 1 column is stable and can readily tolerate the usual range of solvents, both organic and aqueous. Therefore, we examined various solvents for both reversed- and normal-phase chromatography. The normal-phase type of mobile phase that consists of *n*-hexane with ethanol or 2-propanol was found to be appropriate for our selected compounds.

3.1.1.1. Retention

The average retention of the 10 uridine racemates in an achiral native silica column was investigated

Uridine Structure	X=	Y=	Abbreviated Name	Uridine Structure	X=	Y=	Abbreviated Name
	O	OH	U_1		O	OH	U_7
	S	OH	U_2		S	OH	U_8
	Se	OH	U_3				
	O	OAc*	U_4		O	OAc	U_9
	S	OAc	U_5		S	OAc	U_{10}
	Se	OAc	U_6				

* OAC is an Acetoxy group = -$OCOCH_3$

Fig. 1. The structure of the 10 racemates of carbocyclic uridine analogs studied.

Table 1
Chromatographic parameters of the 10 pairs of nucleoside analogs showing the dependence of capacity factor (k'), selectivity factor (α) and resolution (R_s) of Whelk-O 1 and ChiralPak-AD CSPs on the 2-propanol composition in hexane[a]

Uridine analogs	Whelk-O 1 CSP								ChiralPak-AD CSP							
	15%				20%				15%				20%			
	$k'(-)$[b]	$k'(+)$[b]	α[c]	R_s[d]	$k'(-)$	$k'(+)$	α	R_s	$k'(-)$	$k'(+)$	α	R_s	$k'(-)$	$k'(+)$	α	R_s
U_1	7.19	8.25	1.15	1.09	4.47	5.20	1.16	1.12	7.82	9.36	1.20	2.39	4.32	5.24	1.21	2.27
U_2	10.00	11.30	1.13	0.98	6.26	7.11	1.14	0.98	10.28	10.62	1.03	0.41	5.55	5.85	1.05	0.55
U_3	8.62	9.96	1.15	1.16	5.37	6.21	1.15	1.13	9.78	10.00	1.02	0.28	5.47	5.49	1.00	0.03
U_4	10.20	13.10	1.28	2.13	7.43	9.50	1.28	2.06	6.82	6.51	1.05	0.43	4.5	4.36	1.03	0.22
U_5	16.80	22.80	1.35	2.62	11.60	16.00	1.35	2.71	9.21	8.76	1.05	0.68	6.05	5.79	1.05	0.52
U_6	16.00	21.80	1.36	2.74	11.00	15.01	1.36	2.70	10.26	8.77	1.17	1.18	6.86	5.82	1.18	1.74
U_7	7.27	8.03	1.10	0.82	4.22	4.72	1.11	0.81	8.01	8.55	1.07	0.74	4.37	4.69	1.07	0.65
U_8	10.20	11.10	1.09	0.74	6.13	6.73	1.10	0.77	9.92	11.75	1.18	2.39	5.41	6.50	1.20	2.33
U_9	12.50	16.40	1.31	2.47	8.20	11.00	1.31	2.35	8.24	9.33	1.13	1.94	5.36	6.12	1.14	1.88
U_{10}	19.00	26.40	1.39	2.92	12.60	18.01	1.39	2.98	10.11	11.74	1.16	2.24	6.57	7.73	1.18	2.24

[a] Flow rate, 1 ml/min with Whelk-O 1 CSP and 0.85 ml/min with ChiralPak-AD CSP; column temperature, 30°C; wavelength of detection, 250 nm.
[b] $k' = (t_R - t_0)/t_0$, where t_R is the retention time and t_0 is the void time.
[c] $\alpha = k'_{(l)}/k'_{(e)}$, where (l) is the later eluting enantiomer and (e) is the earlier eluting enantiomer.
[d] $R_s = 2(t_{R2} - t_{R1})/(W_1 + W_2)$, where t_{R1} and t_{R2} are the retention times of the first and the second adjacent enantiomer bands, W_1 and W_2 are their baseline widths.

using ethanol or 2-propanol modifiers in the mobile phase, to examine the contribution of the non-selective portion of the stationary phase to the average retention of the compounds. Since the average retention factor of the acetylated analogs were compared to the corresponding OH compounds and exhibited unusual retention and selectivity behavior, it was important to verify their retention behavior in

Table 2
Chromatographic parameters of the 10 pairs of nucleoside analogs showing the dependence of capacity factor (k'), selectivity factor (α) and resolution (R_s) of Whelk-O 1 and ChiralPak-AD CSPs on the ethanol composition in hexane[a]

Uridine analogs	Whelk-O 1 CSP								ChiralPak-AD CSP							
	15%				20%				30%				40%			
	$k'(-)$	$k'(+)$	α	R_s	$k'(-)$	$k'(+)$	α	R_s	$k'(-)$	$k'(+)$	α	R_s	$k'(-)$	$k'(+)$	α	R_s
U_1	4.22	4.78	1.13	1.56	2.79	3.17	1.14	1.41	3.77	4.98	1.32	4.01	2.21	3.06	1.39	4.15
U_2	5.30	5.88	1.11	1.37	3.46	3.85	1.11	1.23	3.79	6.49	1.71	7.17	2.25	4.03	1.79	7.37
U_3	4.91	5.47	1.11	1.41	3.22	3.59	1.12	1.26	3.76	6.93	1.84	7.22	2.27	4.30	1.89	8.04
U_4	5.87	7.23	1.23	2.89	4.32	5.31	1.23	2.61	5.73	12.09	2.11	13.39	4.45	9.05	2.03	11.24
U_5	8.66	11.20	1.29	3.53	6.23	8.00	1.28	3.38	7.69	18.84	2.45	15.18	5.87	13.80	2.35	15.39
U_6	8.64	11.22	1.29	3.70	6.21	8.00	1.29	3.36	8.65	20.43	2.36	15.13	6.39	15.08	2.36	13.82
U_7	3.90	4.24	1.09	1.05	2.51	2.73	1.09	0.95	3.13	4.88	1.56	6.36	1.88	3.05	1.62	6.06
U_8	5.22	5.61	1.08	0.94	3.35	3.62	1.08	0.91	3.84	5.61	1.46	5.62	2.35	3.45	1.47	4.86
U_9	6.68	8.36	1.25	3.18	4.73	5.91	1.25	2.96	9.01	17.49	1.94	11.04	5.98	11.73	1.96	10.09
U_{10}	9.34	12.40	1.33	4.00	6.63	8.76	1.32	3.65	10.44	17.35	1.66	7.70	7.07	11.99	1.70	7.94

[a] Flow rate, 1 ml/min with Whelk-O 1 CSP and 0.85 ml/min with ChiralPak-AD CSP; column temperature, 30°C; wavelength of detection, 250 nm.

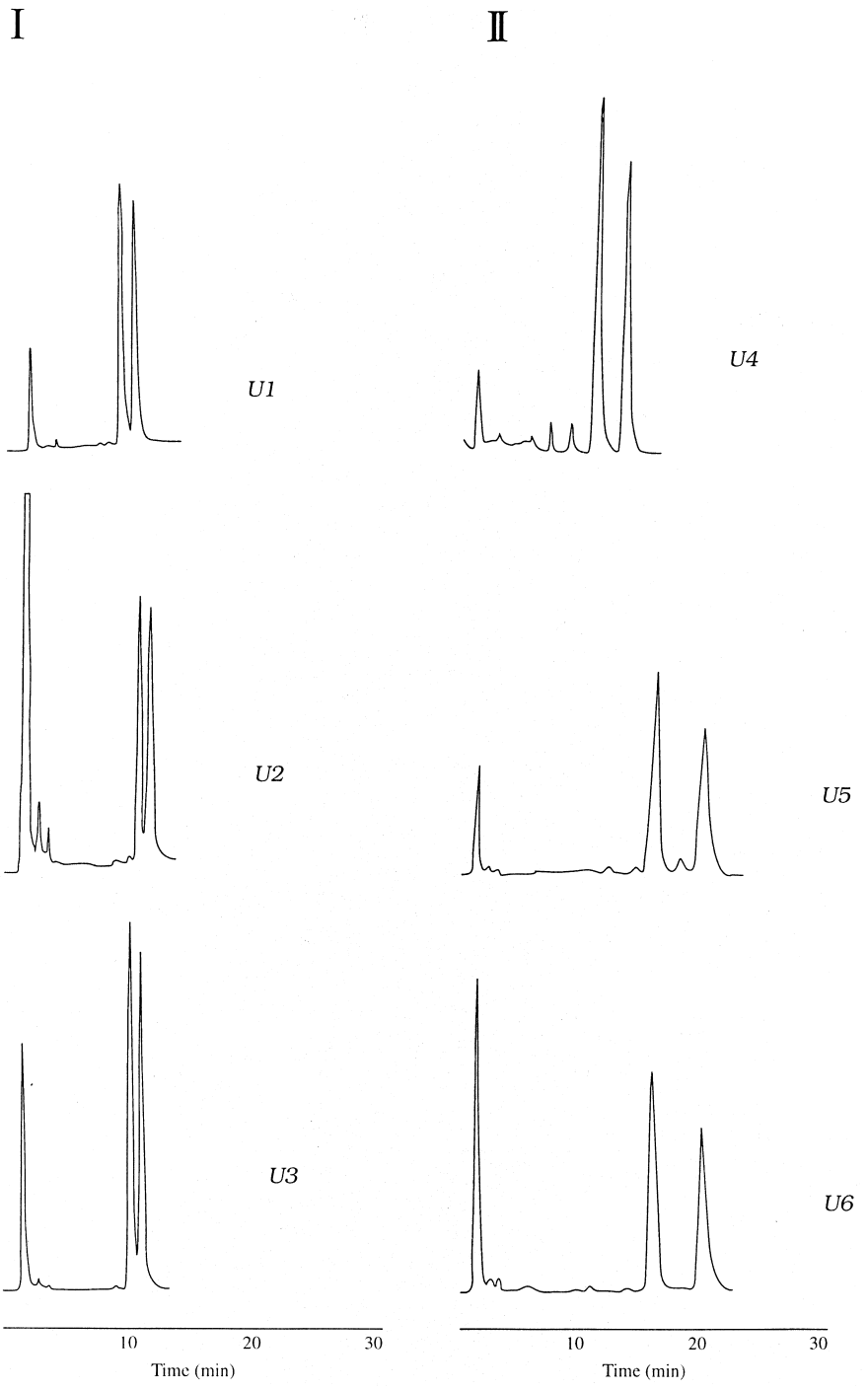


Fig. 2. Chromatograms of the uridine analogs on (S,S)-Whelk-O 1 CSP. (I) Pairs U_1, U_2, U_3; (II) pairs U_4, U_5, U_6; (III) pairs U_7, U_8; (IV) pairs U_9, U_{10}. Mobile phase: *n*-hexane–2-propanol (85:15, v:v); flow-rate, 1.00 ml/min; monitoring wavelength, 250 nm. (*Continued on next page*.)

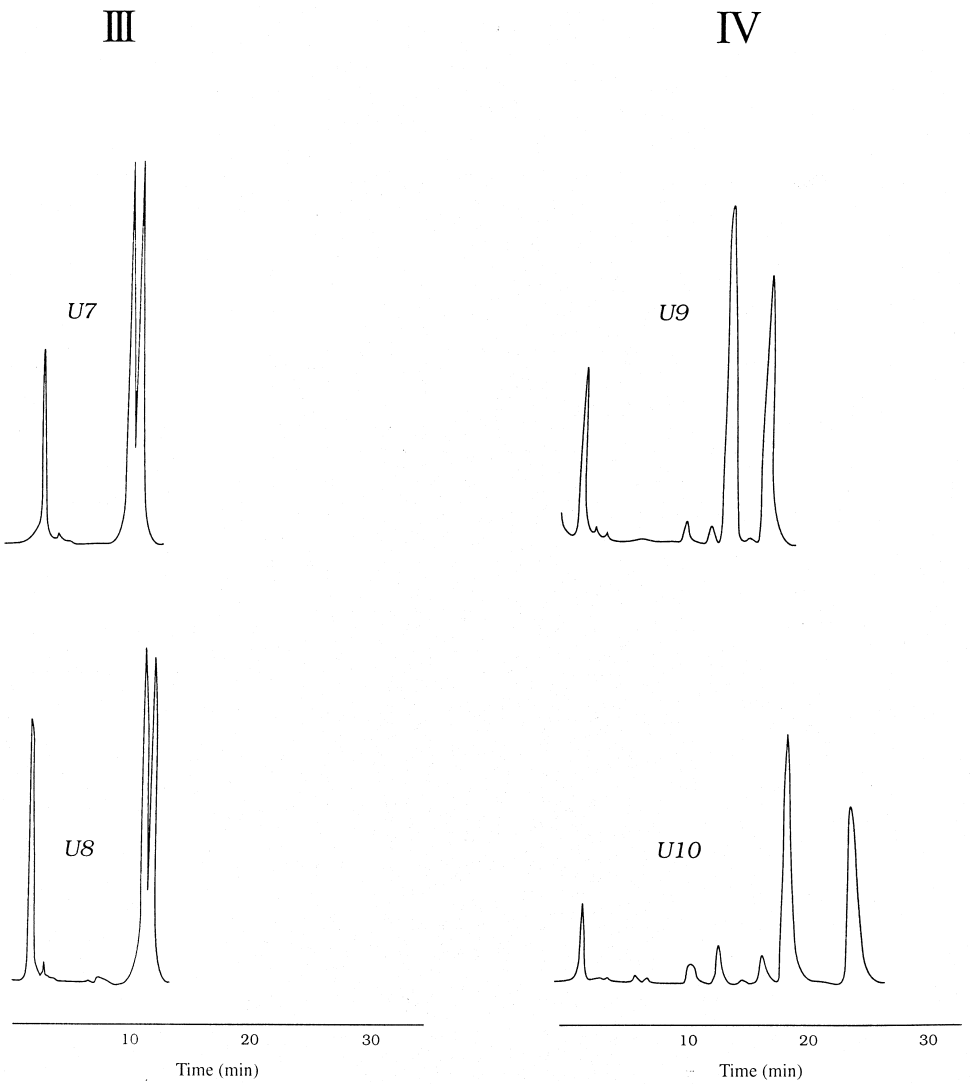


Fig. 2. (*continued*)

the achiral silica column. It was found that they behaved regularly as expected from normal-phase chromatography, in which acetoxy compounds elute before their corresponding OH compounds. Average retention factors were found to be relatively small in the non-selective system compared to those of the corresponding OH compounds in either the ChiralPak-AD CSP [5] or the Whelk-O 1 CSP column. The increase in retention indicates a potential binding of the racemates to the CSP selector, probably through a combination of interactions, such as hydrogen bonding, $\pi-\pi$ interaction and dipole stacking.

Moreover, retention times in ethanol were lower than in 2-propanol and acetoxy compounds eluted at lower retention factors than their corresponding OH compounds, as expected from achiral normal-phase systems.

Examining the effect of solvent modifiers on the separation using the Whelk-O 1 column, it was found that the solvents behaved just like regular normal-

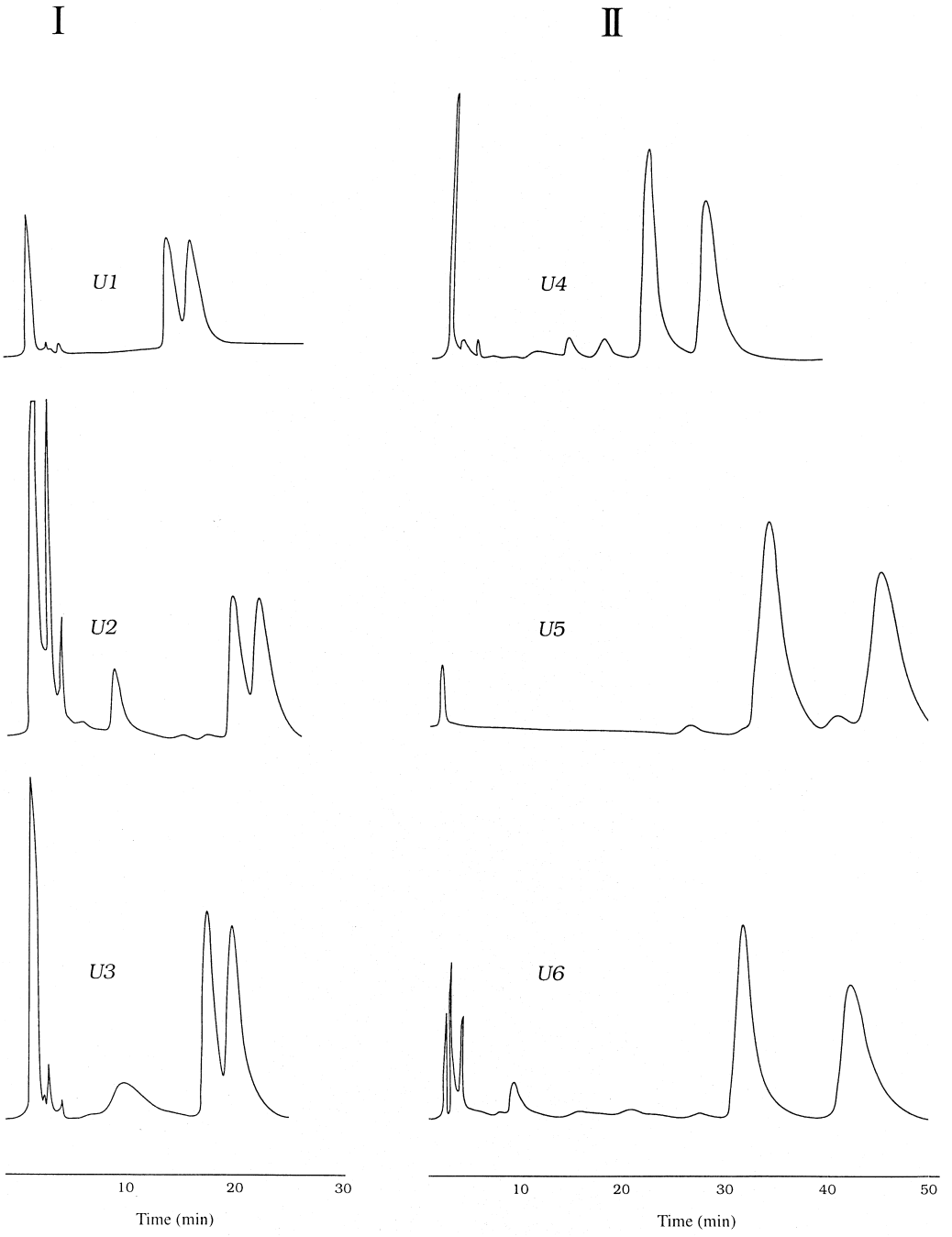


Fig. 3. Chromatograms of the uridine analogs on (S,S)-Whelk-O 1 CSP. (I) Pairs U_1, U_2, U_3; (II) pairs U_4, U_5, U_6; (III) pairs U_7, U_8; (IV) pairs U_9, U_{10}. Mobile phase: *n*-hexane–ethanol (85:15, v:v); flow-rate, 1.00 ml/min; monitoring wavelength, 250 nm. (*Continued on next page*.)

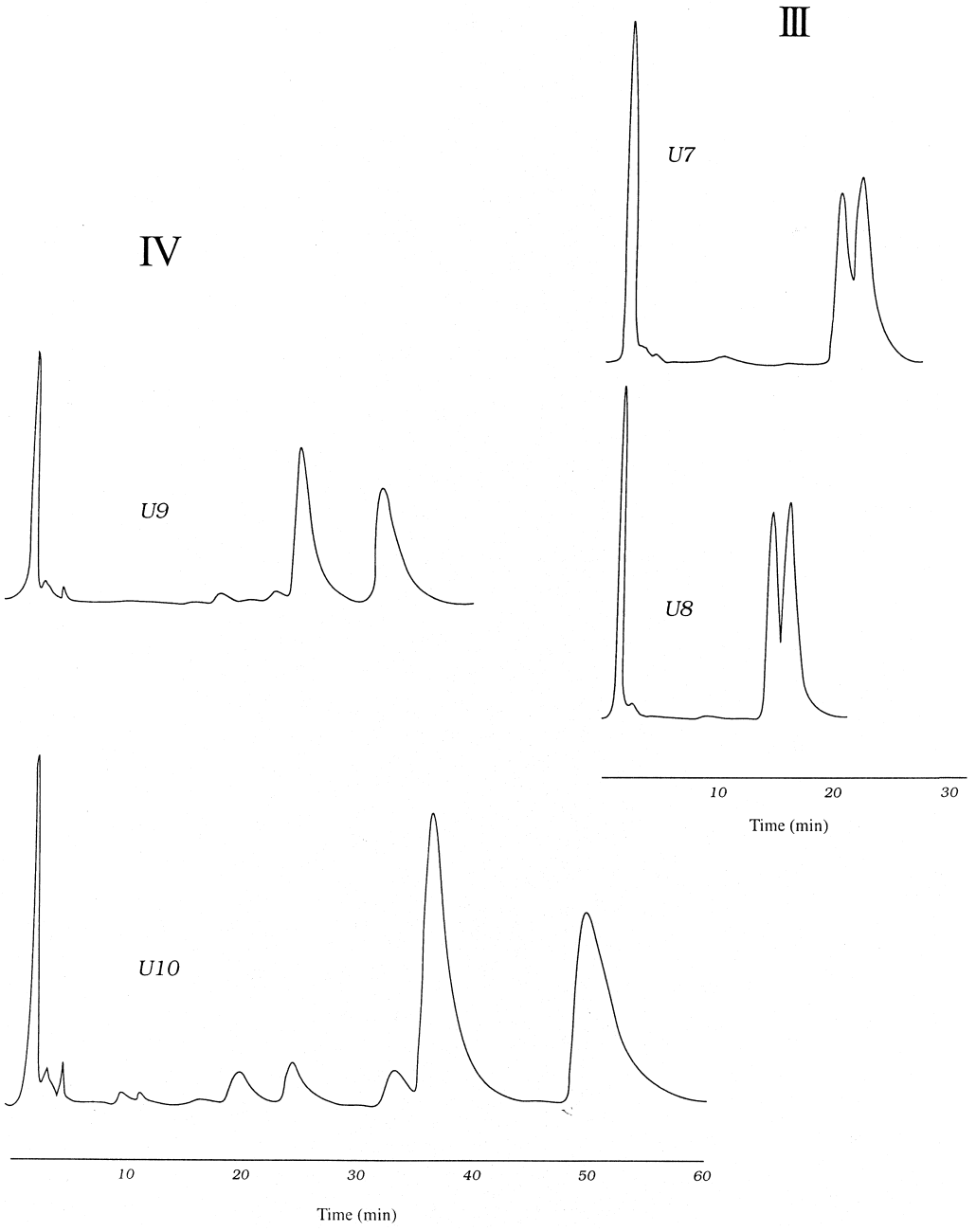


Fig. 3. (*continued*)

phase chromatography. Ethanol was the stronger solvent, as can be seen in Tables 1 and 2, comparing ethanol to 2-propanol, using them at 15 and 20% in the mobile phase. Also typical was the decrease in retention with the increase of modifier percentages in the mobile phase. In contrast to this regularity, a

puzzling retention behavior was observed on ChiralPak-AD CSP, in which all racemates were retained longer in ethanol than in 2-propanol [5].

Another puzzling observation can also be seen in Tables 1 and 2. Longer retention times were obtained for all the acetylated analogs (U_4, U_5, U_6 U_9, U_{10}) in both modifiers, in comparison to the more polar hydroxy analogs (U_1, U_2, U_3 U_7, U_8). A similar result was obtained on the ChiralPak-AD column, but only when ethanol was used as the modifier. This observation cannot be easily explained, in light of the fact that these compounds exhibited a normal retention order in the achiral silica column. The higher retention of the acetylated analogs probably indicates the presence of non-polar interactions between the acetoxy group and the CSP, akin to reversed-phase behavior.

Another interesting observation is the order of retention of racemates bearing different hetero-atoms. We initially anticipated longer retention for the more polar uridines (electronegativities of the hetero atoms are in the order O>S>Se). Retentions obtained, however, contradicted our expectations. Uridines bearing S and Se atoms were retarded more compared to their O analogs. This unusual pattern of elution may be attributed to the fact that the thiophene ring is much more aromatic in character than the furan ring, therefore, enhancing the π–π interactions with both types of CSPs.

3.1.1.2. Selectivity and resolution

The uridine racemates were resolved much better with ethanol modifier compared to 2-propanol modifier on the ChiralPak-AD column (Tables 1 and 2). The higher selectivity and resolution may indicate the participation of ethanol in altering the CSP conformation leading to a better 'lock and key' fit of enantiomers into the chiral selectors causing better enantioselectivity.

In comparison, when Whelk-O 1 CSP was used, the role of ethanol modifier was much less pronounced (Tables 1 and 2, and Figs. 2 and 3). The selectivity values were almost the same for ethanol and 2-propanol, whereas column efficiency, hence the resolution factor, were better in ethanol. These observations indicate that the modifiers are not so significantly involved in the chiral recognition process on the Pirkle type CSP as was the case in the polysaccharide column.

3.1.1.3. Elution order

The Whelk-O 1 column has maintained the same elution order under all conditions. The (−) enantiomer preceded the (+) enantiomer (see Tables 1 and 2). In contrast, reversal of elution order was observed in many cases when the ChiralPak-AD CSP was used. For example, in the pairs U_4, U_5 and U_6, the (−) enantiomer precedes the (+) enantiomer in ethanol, whereas the (+) enantiomer precedes the (−) enantiomer in 2-propanol. The reversal of elution order on ChiralPak-AD CSP is another indication that the type of modifier may sometimes cause a profound alteration of the chiral recognition mechanism.

3.1.2. Effect of structural features

As shown in Fig. 1, the common features to all the 10 racemates investigated are the pyrimidine (uracil) moiety. However, the racemates vary in the type and position of the hetero-atom (designated as X, where X=O, S, Se) attached to the 5-cyclopentadienyl hetero-ring. Another different feature is the hydroxy versus acetoxy groups (designated as Y, where Y= OH or $OCOCH_3$, respectively) attached to the methyl-2′-cyclopentenyl moiety. We examined the effect of these structural features on chiral recognition, using either ethanol or 2-propanol as a mobile phase modifier.

3.1.2.1. Type and position of the hetero-atom

The hydroxy analogs were divided to two groups (U_1, U_2, U_3 together and U_7, U_8 together) and the corresponding acetoxy analogs were also divided to two groups (U_4, U_5, U_6 together and U_9, U_{10} together) to compare the effect of type of the hetero-atom on the separation. Likewise, to explore the effect of position of the hetero-atom, the following hydroxy pairs were divided to two groups (U_1, U_7 together, U_2, U_8 together) and acetoxy pairs were divided to two groups (U_4, U_9 together, and U_5, U_{10} together) for comparison.

When ethanol was used for the separation of the hydroxy analogs (U_1, U_2, U_3 U_7, U_8), we noticed that the effect of type and position of hetero-atom on separation were almost insignificant (Table 2 and

Fig. 3). Similar results of retention, selectivity and resolution were obtained. Moreover, the efficiency of the column (number of theoretical plates) was not changed significantly. When the same modifier was used to separate the acetoxy analogs (U_4, U_5, U_6 U_9, U_{10}), the effect of structural features (type of hetero-atom) were more prominent. Higher retention and better selectivity and resolution were obtained when the hetero-atom was S or Se. The effect of the positions of these hetero-atoms was insignificant.

When 2-propanol was the modifier, the same observations were noticed, but the effects of hetero atoms on the separation were more pronounced in the hydroxy analogs group rather than in the acetoxy analogs (Table 1 and Fig. 2).

The Whelk-O 1 system, as reflected from the chromatographic behavior findings, gave less confusing results compared to the ChiralPak-AD, where the effect of hetero-atom type and its position were very significant but difficult to analyze because of the inconsistency of patterns obtained [5].

3.1.2.2. Hydroxyl versus acetoxy group

The hydroxy and acetoxy analogs were compared in terms of their retention, selectivity, resolution and column efficiency as revealed from Tables 1 and 2 and Figs. 2 and 3. When the hydroxyl groups were blocked by acetylation, they showed a baseline separation for all of the enantiomeric pairs regardless of the type of modifier used.

The effect of acetoxy group versus the corresponding hydroxy group on the separation of the racemates was solvent dependent as depicted in Tables 1 and 2 and Figs. 2 and 3. Using the Whelk-O 1 CSP, the acetoxy analogs were retained significantly longer in either ethanol or 2-propanol, and the values of selectivity and resolution were also increased. We have noticed, however, a decrease in column efficiency (number of theoretical plates) using 2-propanol as a mobile phase modifier. Similar but less consistent results were noticed on the previous chiral system, the ChiralPak-AD.

Based on the results accumulated so far, the mechanism by which uridine analogs interact selectively with the Whelk-O 1 CSP is rather complicated. It seems likely that lipophilic interactions occur between the enantiomers and the CSP, as well as basic hydrogen-bonding with the uracil moiety (through the amide and extra carbonyl). Apparently, the uracil residues contribute insignificantly to enantioselectivity. The modifiers in this column play a less significant role in the chiral recognition process than in the amylose-based stationary phase.

3.1.3. Effect of temperature

Commonly, ambient temperature is used as a starting point for chiral method development. However, when the enantiomers are partially resolved, one possible recourse is to lower column temperature [7]. In many cases retention and selectivity are significantly increased at temperatures below ambient. Nevertheless, improved resolution is not always granted upon lowering temperature due to the decrease in column efficiency [8]. The influence of the temperature on the selectivity and resolution of the 10 racemates of uridine analogs using ethanol and 2-propanol on both Whelk-O 1 and ChiralPak-AD CSPs is shown in Tables 3 and 4, respectively.

3.1.3.1. Selectivity

As the temperature of the Whelk-O 1 column is reduced, the selectivity is increased for all the racemates, using either modifiers without any exceptions. The effect was more significant, however, in the acetoxy analogs compared to their corresponding hydroxy analogs. As shown in Table 3, we observed similar temperature influence on selectivity using the ChiralPak-AD column with a few exceptions. For example, U_5 (using 30% 2-propanol) and U_{10} (using 30% ethanol) racemates showed a decrease in selectivity upon reducing temperature. The U_4 racemate shows no effect when the temperature was reduced using 2-propanol modifier.

3.1.3.2. Resolution

As shown in Table 4, in most cases, lowering the temperature of the Whelk-O 1 column from 30 to 10°C decreased the resolution of the uridine racemates using either modifier. The decrease in resolution is probably due to the slower adsorption–desorption kinetics which caused peak broadening, i.e., reduced the efficiency of the column. Such mass transfer characteristics seem to be typical to polymeric stationary phases or those who are based on

Table 3
Selectivity factor (α) of the 10 enantiomeric pairs of uridine analogs using different temperatures[a]

Uridine analogs	Whelk-O 1 CPS						ChiralPak-AD CPS					
	20% Ethanol			20% 2-Propanol			30% Ethanol		30% 2-Propanol			
	10°C	20°C	30°C	10°C	20°C	30°C	10°C	30°C	10°C	20°C	30°C	35°C
U_1	1.17	1.15	1.14	1.20	1.18	1.16	1.40	1.30	1.32	1.29	1.22	1.23
U_2	1.13	1.12	1.11	1.17	1.15	1.14	1.90	1.70	1.14	1.12	1.07	1.06
U_3	1.13	1.12	1.12	1.18	1.16	1.15	2.00	1.80	1.10	1.07	1.03	1.00
U_4	1.28	1.25	1.23	1.35	1.32	1.28	2.30	2.10	1.00	1.00	1.00	1.00
U_5	1.35	1.31	1.28	1.44	1.39	1.35	2.70	2.50	1.00	1.00	1.02	1.11
U_6	1.35	1.32	1.29	1.44	1.40	1.36	2.60	2.40	1.18	1.15	1.13	1.11
U_7	1.10	1.09	1.09	1.13	1.12	1.11	1.70	1.60	1.10	1.10	1.08	1.08
U_8	1.10	1.08	1.08	1.12	1.11	1.10	1.60	1.50	1.33	1.28	1.23	1.21
U_9	1.30	1.27	1.25	1.38	1.35	1.31	2.20	1.90	1.23	1.20	1.16	1.16
U_{10}	1.40	1.35	1.32	1.49	1.44	1.39	1.60	1.70	1.26	1.23	1.20	1.19

[a] Flow rate, 1 ml/min with Whelk-O 1 CSP and 0.85 ml/min with ChiralPak-AD CSP; wavelength of detection, 250 nm.

'chiral cavities' [9]. The ChiralPak-AD column which is based on carbamated amylose fits into both mentioned categories. Therefore, variation of temperature may alter the conformation of such a CSP, thus affecting resolution. No systematic pattern of resolution behavior was observed using the ChiralPak-AD column upon reducing the temperature (Table 4).

3.2. Summary of the comparison of the enantioseparation on Whelk-O 1 and ChiralPak-AD CSPs

It was shown above that normal-phase mode behavior of average retention is not always to be expected in the two chiral systems under investigation, although normal-phase solvents were used

Table 4
Resolution (R_s) between the enantiomeric pairs of uridine analogs using different temperatures[a]

Uridine analogs	Whelk-O 1 CPS						ChiralPak-AD CPS					
	20% Ethanol			20% 2-Propanol			30% Ethanol		30% 2-Propanol			
	10°C	20°C	30°C	10°C	20°C	30°C	10°C	30°C	10°C	20°C	30°C	35°C
U_1	1.50	1.42	1.41	1.03	1.14	1.12	3.70	4.01	2.46	2.48	1.91	2.09
U_2	1.21	1.21	1.23	0.94	1.01	0.98	7.98	7.17	1.10	1.07	0.42	0.69
U_3	1.23	1.22	1.26	0.97	1.07	1.13	7.88	7.22	0.89	0.69	0.21	0.00
U_4	2.54	2.93	2.61	1.96	2.07	2.06	11.24	13.39	0.00	0.00	0.24	0.00
U_5	2.90	3.21	3.38	2.62	2.54	2.71	16.09	15.18	0.00	0.00	0.21	1.35
U_6	2.99	3.30	3.36	2.47	2.68	2.70	14.04	15.13	1.96	1.73	1.37	1.27
U_7	0.87	0.88	0.95	0.73	0.78	0.81	6.16	6.36	0.85	0.90	0.57	0.79
U_8	0.90	0.88	0.91	0.72	0.75	0.77	5.22	5.62	2.49	2.50	2.05	2.08
U_9	2.77	3.00	2.96	2.29	2.40	2.35	11.11	11.04	2.51	2.25	1.78	1.80
U_{10}	5.33	3.54	3.65	2.22	2.84	2.98	7.31	7.70	2.70	2.63	2.16	2.27

[a] Flow rate, 1 ml/min with Whelk-O 1 CSP and 0.85 ml/min with ChiralPak-AD CSP; wavelength of detection, 250 nm.

in conjunction with relatively polar stationary phases. For example, the acetoxy analogs were retained longer compared to their corresponding hydroxy analogs in both ChiralPak-AD and Whelk-O 1 columns. Also, uridine analogs were retained considerably longer in the ChiralPak-AD column when ethanol was used as mobile phase modifier rather than 2-propanol.

As to the chiral separation, unexpected solvent effects were observed. For example, enantiomers were much better resolved using ethanol as the mobile phase modifier rather with 2-propanol, although less pronounced in the Whelk-O 1 column. Reversal of elution order was often noticed on the ChiralPak-AD column, either as a solvent effect or as a result of a structural change in the enantiomers. No reversal of elution order was observed on the Whelk-O 1 column. These observations support the assumption that solvents may participate significantly in the chiral recognition process on the ChiralPak-AD column.

It seems that temperature plays also an important role in the chiral recognition process and can, therefore, influence selectivity and resolution on both columns. Effects were more extreme and less systematic in the ChiralPak-AD column.

4. Conclusion

We have resolved racemates of carbocyclic analogs of 5-substituted uracil into enantiomers, using an (S,S)-Whelk-O 1 column. It is difficult to understand or predict the effect of a structural features of the enantiomers on their chromatographic behavior in the system under study, as it is generally the case in chiral liquid chromatography. These experiments show that the ChiralPak-AD column was superior in performance than the Whelk-O 1 column.

In spite of its lower chromatographic performance in terms of resolution factor, the column of choice for the separation of this group of racemates (excluding U_7 and U_8) was the Whelk-O 1 column, using ethanol as the modifier. It was more predictable and systematic in its results, analysis times were much shorter in general, it was much easier to handle and maintain (far less limitations in solvent choice, pressure and temperature ranges), and it was far less costly.

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