Chiral separation of oxazolidinone analogues by liquid chromatography on polysaccharide stationary phases using polar organic mode

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ABSTRACT

The enantioseparation of four oxazolidinone and one biosimilar thiazolidine derivatives was performed on seven different polysaccharide-type chiral stationary phases (Lux Amylose-1, Lux i-Amylose-1, Lux Amylose-2, Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4) differing in backbone (cellulose or amylose), substituent or the immobilization technologies (coated or immobilized). Polar organic mode was employed using neat methanol (MeOH), ethanol (EtOH), 2-propanol (IPA) and acetonitrile (ACN) either alone or in combinations as mobile phases. Amylose-based columns with ACN provided the highest enantioselectivities for the studied compounds. The replacement of an oxygen with a sulfur atom in the backbone of the studied analytes significantly alters the enantiomer recognition mechanism. Chiral selector-, mobile-phase-, and interestingly immobilization-dependent enantiomer elution order reversal was also observed. Reversal of elution order and hysteresis of retention and enantioselectivity was further investigated using different mixtures of IPA:MeOH and ACN:MeOH on amylose-type chiral stationary phases. Hysteresis of retention and enantioselectivity was observed on all investigated amylose-type columns and binary eluent mixtures, which can be further utilized for fine-tuning chiral separation performance of the studied columns.

1. Introduction

Commercialization of single enantiomeric drugs has attracted considerable attention in the last decades. Ever since it has been proven that enantiomers of a racemate may differ regarding their pharmacological, toxicological or pharmacokinetic aspects, there has been an increased pressure to obtain enantiopure compounds [1]. This tendency, however, also demands a continuous need to develop novel enantioseparation methods. Although there are numerous approaches to attain enantiodiscrimination, direct chromatographic methods are still considered the golden standard in this field. The direct approach uses chiral stationary phases (CSPs) and relies on the reversible transient diastereomer formation be-

tween the individual enantiomers and the chiral selector that is covalently attached or adsorbed to the surface of the solid support [2,3]. In spite of the increasing number of CSPs on the market, enantioseparation is still a challenging task, mostly based on a trial-and-error approach. Due to the increasing number of enantiopure drugs and also due to the increasingly strict regulatory requirements, there is an ever-increasing pressure on the shoulders of analytical scientists to develop newer and better enantioseparation methods. Under these circumstances, predictability of chiral separations could take some of the burden off the shoulder of analysts [4,5].

Among the numerous commercially available chiral columns, polysaccharide-type CSPs are probably the most commonly applied in LC enantioseparations, not just because of their high enantiorecognition capabilities, but also because of their multimodal applicability [6]. These columns can be operated in normal-phase, reversed-phase and polar organic mobile-phase (PO) modes. In PO mode only polar organic solvents, neat alcohols (methanol (MeOH), ethanol (EtOH) and 2-propanol (IPA)), neat acetonitrile (ACN) or their combinations are used as mobile phase. Polar organic mode

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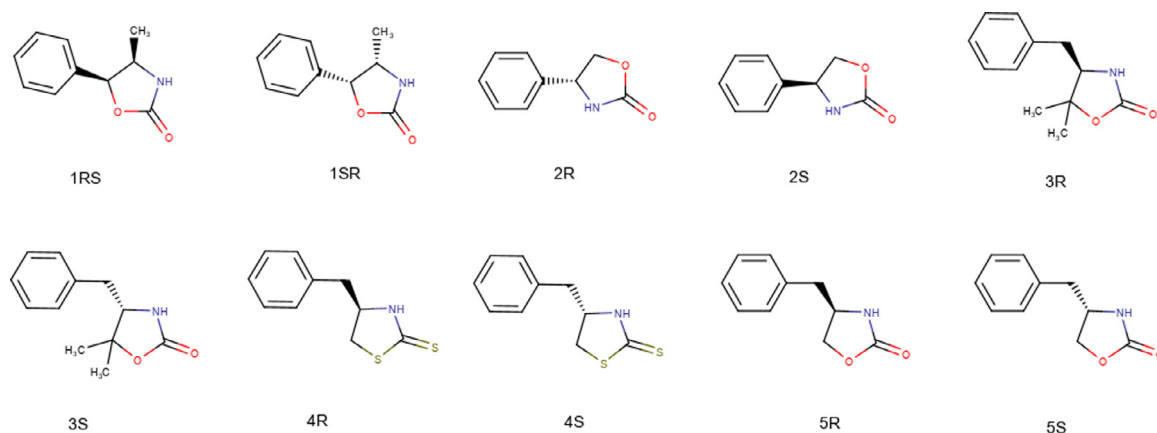
Fig. 1. The chemical structure of the analytes.

has several advantages, such as shorter run times, high efficiency, and usually higher solubility of the analytes in the mobile phase. This mode also suits both analytical and preparative purposes as well [7,8]. The applicability of polar organic mode using neat alcohols or ACN has been already proven in several earlier studies [9–13]. In recent articles, the Németh group investigated the effect of eluent mixing on enantioseparation performance on amylose-type CSPs [14]. They found that eluent mixtures such as MeOH:IPA can result in better efficiency and different enantiomeric elution order compared to neat eluents. Hysteresis of the retention factor and the selectivity, another interesting phenomenon was also observed under the applied conditions, which can be further exploited in method development [14,15]. The aim of our work was to investigate the enantiorecognition capability of seven polysaccharide CSPs in polar organic mode using neat solvents and eluent mixtures towards four oxazolidinones and one thiazolidine derivatives. Our study focused on the separation capacity of the applied systems, on the elution order reversals, and on the possible appearance of the hysteresis phenomenon. Oxazolidinones were chosen as model molecules because of their widespread use as chiral building blocks in different antiepileptic (for example: trimethadione), antibiotic (for example: linezolid), and anticoagulants (for example: rivaroxaban) drugs [16,17]. To the best of our knowledge, enantiomeric separation of these compounds has not been studied.

2. Materials and methods

2.1. Materials

Enantiopure (4R,5S)-(+)-4-Methyl-5-phenyl-2-oxazolidinone (**1RS**), (4S,5R)-(-)-4-Methyl-5-phenyl-2-oxazolidinone (**1SR**), (R)-(-)-4-Phenyl-2-oxazolidinone (**2R**), (S)-(+)-4-Phenyl-2-oxazolidinone (**2S**), (R)-(+)-4-Benzyl-5,5-dimethyl-2-oxazolidinone (**3R**), (S)-(-)-4-Benzyl-5,5-dimethyl-2-oxazolidinone (**3S**), (R)-4-Benzylthiazolidine-2-thione (**4R**), (S)-4-Benzylthiazolidine-2-thione (**4S**), (R)-4-Benzyl-2-oxazolidinone (**5R**) and (S)-4-Benzyl-2-oxazolidinone (**5S**) were purchased from Sigma-Aldrich Hungary (Budapest, Hungary). The structure of the investigated molecules is depicted in Fig. 1 .

Gradient grade methanol (MeOH), ethanol (EtOH), 2-propanol (IPA) and acetonitrile (ACN) were purchased from Thomasker Finechemicals Ltd. (Budapest, Hungary). Lux Cellulose-1 (Cell1) (150 × 4.6 mm; particle size: 5 μm) [based on cellulose tris(3,5-dimethylphenylcarbamate)], Lux Cellulose-2 (Cell2) (150 × 4.6 mm; particle size: 5 μm) [based on cellulose tris(3-chloro-4-methylphenylcarbamate)], Lux Cellulose-3 (Cell3) (150 × 4.6 mm; particle size: 5 μm) [based on cellulose tris(4-methylbenzoate)], Lux Cellulose-4 (Cell4) (150 × 4.6 mm; particle size: 5 μm)

[based on cellulose tris(4-chloro-3-methylphenylcarbamate)] and Lux Amylose-1 (Am1) (150 × 4.6 mm; particle size: 5 μm) [based on amylose tris(3,5-dimethylphenylcarbamate)], Lux i-Amylose-1 (iAm1) (150 × 4.6 mm; particle size: 5 μm) [based on amylose tris(3,5-dimethylphenylcarbamate)], Lux Amylose-2 (Am2) (150 × 4.6 mm; particle size: 5 μm) [based on amylose tris(5-chloro-2-methylphenylcarbamate) were all the products of Phenomenex (Torrance, CA, USA). The chemical structures of the chiral selectors are in Fig. 2 .

2.2. LC-UV analysis

LC-UV analysis was carried out on a Jasco HPLC system consisting of PU-2089 plus quaternary pump, AS-4050 autosampler, MD-2010 diode array detector, Jetstream 2 Plus thermostat. JASCO ChromNAV software was used for instrument control and data analysis. All separations were performed at 25°C using 0.5 mL/min flow rate. UV detection was performed at 210 nm. All stock solutions were prepared at 1 mg/mL in MeOH and further dilutions were made with the same solvent. An injection volume of 1 μL was used and three parallel measurements were carried out in each case. For determination of elution order R-spiked samples were used, except compound **1**, where SR-isomer was used in higher concentration. In the screening phase, neat alcohols (MeOH, EtOH or IPA) and ACN were used. Whenever an experiment required pretreatment with either IPA, MeOH, EtOH or ACN it was brought about by pumping 10 column volumes (CV) of the corresponding solvent through the column. Hysteresis of retention time and enantioselectivity was investigated in binary eluent mixtures, starting with 100% MeOH, using 10% increments, until reaching 100% of the other eluent, and then 10% decrements until again, 100% MeOH was reached. In each case, 60 min conditioning was applied before injection [15].

The retention factor (k) was determined as $k = (t_R-t_0)/t_0$, where t_R is the retention time for the eluted enantiomer, t_0 is the dead time. The separation factor (α) was calculated as $\alpha = k_2/k_1$; k_1 and k_2 are the retention factor of the first- and second-eluted enantiomer, respectively. Resolution (R_s) was calculated with the following formula: $R_s = 2(t_2-t_1)/(w_1+w_2)$, where t_1 and t_2 are the retention times, w_1 and w_2 are the extrapolated peak widths at the baseline.

3. Results and discussion

3.1. General overview of the enantioseparations

140 different chromatographic conditions were investigated on the seven polysaccharide CSPs with neat eluents. All these mea-
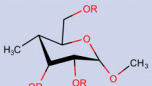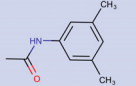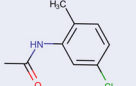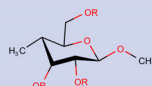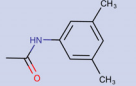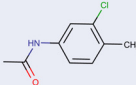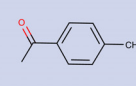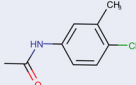
Column	Backbone	R	IUPAC name
Lux Amylose-1 (Am1) Lux iAmylose-1 (iAm1)	amylose		tris(3,5-dimethylphenylcarbamate)
Lux Amylose-2 (Am2)			tris(5-chloro-2-methylphenylcarbamate)
Lux Cellulose-1 (Cell1)			tris(3,5-dimethylphenylcarbamate)
Lux Cellulose-2 (Cell2)	cellulose		tris(3-chloro-4-methylphenylcarbamate)
Lux Cellulose-3 (Cell3)			tris(4-methylbenzoate)
Lux Cellulose-4 (Cell4)			tris(4-chloro-3-methylphenylcarbamate)

Fig. 2. The chemical structure of the chiral selectors.
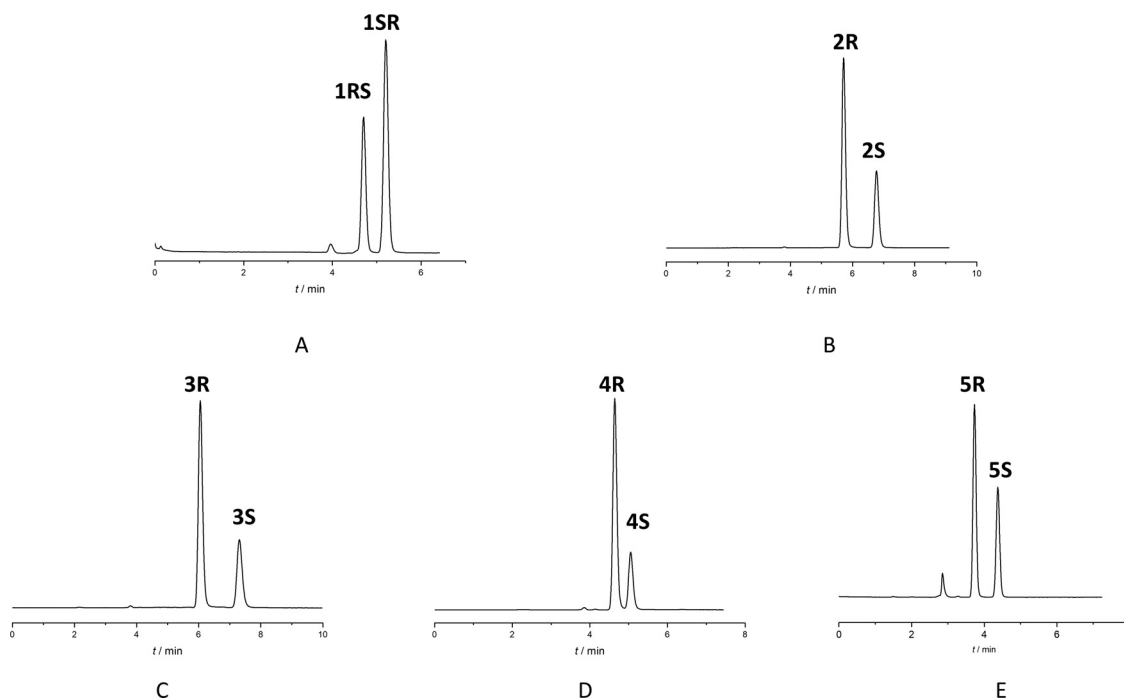
Fig. 3. The chromatograms with the highest resolution for each analyte. A: Compound 1, Am2 with ACN (R_s = 2.6); B: Compound 2, Am1 with ACN (R_s = 4.5); C: Compound 3, Am1 with ACN (R_s = 4.4); D: Compound 4, iAm1 with ACN (R_s = 2.0); E: Compound 5, Am2 with ACN (R_s = 4.3). (Column dimension: 150 × 4.6 mm; particle size: 5 μm, flow rate: 0.5 mL/min, temperature: 25°C).

surements were carried out uniformly using a 0.5 mL/min flow rate at 25°C. The results (retention times of the enantiomers, resolution values and enantiomeric elution order (EEO)) are summarized in Table 1. Based on our results all of the investigated molecules were separated both on cellulose- and amylose-based CSPs. The highest R_s values for all five drugs were measured on amylose-based CSPs using neat ACN as mobile phase. Chromatograms with the highest R_s for each substance are depicted in Fig. 3. To compare the enantioseparation capacity of the applied systems the sum of R_s values was calculated for each chromatographic system. Diagram is depicted in Supplementary Figure 1. It can be seen, that amylose-

type CSPs with ACN outperformed the other systems for the enantioseparation of the model analytes. iAm1 and Am1 columns with ACN provided the highest R_s values, while on the other end of the spectrum, Cell4 with MeOH and EtOH offered no observable chiral differentiation. It should be noted that using amylose tris(3,5-dimethylphenylcarbamate) CSP all of the studied compounds can be separated. These results further underline the earlier reported excellent applicability and high success rates of this chiral selector in polar organic mode [18–20]. It should be also observed that the retention times of the analytes are also very short, regardless of the CSP or eluent employed. The highest retention time is 7.33 min

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Table 1
Chromatographic data, enantiomeric elution order (EEO), retention times of the enantiomers and resolution of the mobile phase and CSP screening for the chiral separation of the model analytes in polar organic mode. Flow rate: 0.5 mL/min. Temperature: 25°C.

Column	Mobile phase	Compound 1				Compound 2				Compound 3				Compound 4				Compound 5			
		EEO	t_1	t_2	R_s	EEO	t_1	t_2	R_s	EEO	t_1	t_2	R_s	EEO	t_1	t_2	R_s	EEO	t_1	t_2	R_s
Cell1	ACN	SR>RS	4.61	4.95	1.4	-	4.73	-	-	-	4.57	-	-	-	3.85	-	-	-	4.53	-	
	MeOH	-	4.47	-	-	R>S	4.91	5.13	0.4	S>R	5.17	5.39	0.3	-	5.84	-	-	-	5.20	-	
	EtOH	-	4.58	-	-	-	5.12	-	-	S>R	5.22	5.65	0.8	-	3.89	-	-	R>S	5.28	5.47	0.3
	IPA	-	4.84	-	-	-	5.36	-	-	S>R	5.44	6.00	1.2	R>S	6.22	6.69	0.92	R>S	6.21	6.61	0.4
Cell2	ACN	SR>RS	5.56	6.04	2.1	R>S	5.20	5.49	1.3	R>S	5.56	5.99	1.9	-	4.55	-	-	R>S	4.99	5.20	1.3
	MeOH	SR>RS	4.52	4.77	1.1	-	4.60	-	-	-	4.88	-	-	-	5.13	-	-	-	4.76	-	-
	EtOH	SR>RS	4.85	5.29	1.3	-	5.04	-	-	-	3.75	-	-	-	5.26	-	-	-	5.24	-	-
	IPA	SR>RS	5.41	6.18	2.1	-	5.80	-	-	S>R	6.88	7.30	0.9	-	6.51	-	-	-	7.10	-	-
Cell3	ACN	SR>RS	4.21	4.35	0.4	-	4.21	-	-	-	4.15	-	-	-	4.36	-	-	-	4.05	-	-
	MeOH	SR>RS	4.40	4.61	0.5	-	4.45	-	-	-	4.52	-	-	R>S	5.65	5.96	0.6	-	4.40	-	-
	EtOH	-	4.48	-	-	-	4.59	-	-	-	4.71	-	-	R>S	5.81	6.47	1.4	R>S	4.60	4.82	0.4
	IPA	-	4.24	-	-	S>R	4.33	4.53	0.5	S>R	4.29	4.53	0.3	R>S	5.65	6.06	0.9	R>S	4.72	4.92	0.3
Cell4	ACN	-	5.24	-	-	-	5.12	-	-	R>S	5.13	5.35	1.0	-	4.49	-	-	-	4.71	-	
	MeOH	-	4.53	-	-	-	4.64	-	-	-	4.77	-	-	-	4.95	-	-	-	4.61	-	
	EtOH	-	4.76	-	-	-	5.06	-	-	-	5.20	-	-	-	5.05	-	-	-	5.00	-	
	IPA	SR>RS	5.09	5.32	0.7	S>R	5.35	5.52	0.2	-	5.70	-	-	-	5.50	-	-	-	6.42	-	
Am1	ACN	SR>RS	6.35	6.60	1.1	R>S	5.71	6.76	4.5	R>S	6.04	7.33	4.4	-	5.16	-	-	R>S	6.02	6.48	2.0
	MeOH	RS>SR	4.69	4.89	1.3	S>R	4.93	5.05	0.5	R>S	4.99	5.45	1.5	-	5.51	-	-	R>S	5.15	5.48	1.6
	EtOH	-	4.93	-	-	S>R	5.47	5.84	1.3	R>S	4.89	5.83	3.3	-	5.37	-	-	R>S	4.99	5.39	1.8
	IPA	-	4.19	-	-	-	4.21	-	-	R>S	4.41	5.08	1.6	R>S	4.71	4.99	1.1	R>S	4.28	4.52	0.9
iAm1	ACN	RS>SR	5.71	5.87	0.8	R>S	4.91	5.63	2.8	R>S	5.07	5.89	3.4	R>S	4.64	5.04	2.0	R>S	5.15	5.24	0.2
	MeOH	-	4.32	-	-	-	4.23	-	-	R>S	4.35	4.64	1.5	-	5.62	-	-	R>S	4.39	4.48	0.3
	EtOH	-	4.50	-	-	-	4.58	-	-	R>S	4.58	5.15	2.1	-	5.08	-	-	R>S	4.61	4.81	0.7
	IPA	-	4.51	-	-	R>S	4.55	4.75	0.5	R>S	4.60	5.13	2.0	R>S	5.21	5.61	1.33	-	4.66	-	-
Am2	ACN	RS>SR	4.73	5.20	2.6	R>S	4.77	5.03	1.4	R>S	4.37	4.56	1.1	-	4.59	-	-	R>S	4.95	5.77	4.3
	MeOH	SR>RS	3.80	4.41	2.3	-	4.35	-	-	-	4.28	-	-	-	4.53	-	-	-	4.29	-	-
	EtOH	-	4.81	-	-	R>S	4.78	5.04	1.0	R>S	4.91	6.22	4.5	-	4.74	-	-	R>S	4.83	5.16	1.5
	IPA	SR>RS	4.45	4.96	1.8	R>S	4.53	5.97	4.4	R>S	4.85	4.95	1.34	-	4.72	-	-	R>S	4.69	5.13	1.7

in the case of **3** on the Am1 column with ACN (R_s=4.4) (Fig. 3C). Our study further underlines one of the main advantages of polar organic mode, that high resolution can be achieved within short analysis times.

As the analytes in this study present both hydrogen-donor and hydrogen-acceptor groups, hydrogen-bonding seems as a possible interaction between the chiral selector and the analytes. This can be clearly observed upon comparing the effect of the applied mobile phases on retention and resolution values. Higher retention time and resolution was observed in the cases where the aprotic ACN was applied, which implies hydrogen-bonding types of interactions taking place between the chiral selector and the analytes [2]. As alcohols compete for hydrogen bonding sites, application of these solvents resulted in general in decreased retention and in our case, decreased resolution also. Comparison of alcohol-type eluent shows that IPA and EtOH present the highest R_s values, while MeOH seems to be the least beneficial for enantioseparation of these compounds. MeOH and EtOH may seem similar as eluents, however, several examples of alternative enantioseparations were observed using these mobile phases. For example, **1** was baseline resolved on the Am2 column using MeOH with R_s=2.3 but with EtOH, no enantiorecognition was observed. Opposite result was observed for example in the case of **3** on Am2 CSP.

All of the investigated compounds are structurally similar, as they present an oxazolidinone core structure, except **4**, which is a 2-thiazolidine-2-thiol, being the thio-analogue of **5** (see Fig. 1). It is very conspicuous that the lowest number of successful enantioseparation was observed in the case of the thiazolidine compound **4**. For example, all oxazolidinone compounds are separated on Am2 or Am1 column using ACN, but **4** not. The difference in enantiodiscrimination may be explained by the larger size and lower electronegativity of sulfur, that could influence the spatial structure of the thio-analogue and consequently the binding to the chiral selector. In addition, it should be noted that sulfur shows a marked preference for a more "perpendicular" direction of approach to the donor atom [21]. These differences may result in decreased enantiorecognition. **3** and **5** differ from each other only by a dimethyl group at position 3. It can be seen that the dimethyl substitution reduces the enantioselectivity on Am1 and iAm1 column using ACN as mobile phase, however an opposite effect can be seen on Am2 column using the same eluent. It is also interesting that this small difference in the structure can lead to opposite EEO for example on Cell1 column with IPA.

3.2. Enantiomer elution order reversals

Changes in EEO suggest significant changes in the enantiorecognition mechanisms. Therefore, mapping of EEO reversals offers valuable information upon the interaction between the analyte and CSPs. In our work three types of EEO reversals were observed: chiral selector-dependent reversal, immobilization dependent reversal as well as mobile phase-dependent reversal. All of the EEO reversals are summarized in Supplementary Table 1. It is not surprising that the change in chiral selector can often lead to different enantiorecognition mechanism, which then translates to EEO reversal. Either changing the backbone or the substituent of the chiral selector, EEO reversal could be observed [22–24]. A good example of the latter case is the different EEO of **3** on Cell1 (containing cellulose tris(3,5-dimethylphenylcarbamate)) and on Am1 (containing amylose tris(3,5-dimethylphenylcarbamate)) column using IPA as mobile phase. The chiral selector-dependent reversal of elution order observed between amylose tris(3,5-dimethylphenylcarbamate and cellulose tris(3,5-dimethylphenylcarbamate containing CSP is frequently explained by the conformational difference between these CSPs. The different linkage type ($\beta(1\rightarrow4)$ linked D-glucose units for cellulose and $\alpha(1\rightarrow4)$ glycosidic bonds for amylose) results larger

chiral cavities and weaker intrapolymer H-bond in the cellulose derivative, when compared with the amylose-based polymer, that could lead different affinity pattern of the CSPs towards the enantiomers [25]. Substituent dependent reversal of EEO can be found in the case of **2** on Am1 and Am2 columns using EtOH as well as for **1** on the same two columns using ACN.

A unique type of EEO reversal, based on immobilization of the polysaccharide-type chiral selector. EEO of **1** differs on Lux Amylose-1 vs. Lux i-Amylose-1 column using ACN as the mobile phase in both cases. These two columns contain the same amylose tris(3,5-dimethylphenylcarbamate) chiral selector, however, the immobilization process differs. In the first case, the chiral selector is coated on the surface of porous silica, while in the latter, it is covalently attached to it. A literature survey reveals only a few cases regarding EEO reversal based on immobilization type [26,27]. However, it is unequivocal that the covalent attachment of the chiral selector to silica influences its spatial structure. Thus, immobilization processes can impact the chiral recognition [26,28,29].

The supramolecular structure may also vary in different solvents, which could be the base of the mobile phase dependent EEO reversal [13,30,31]. The mobile phase dependent EEO reversal was observed in six cases mainly using amylose-type CSPs (Supplementary Table 1). Changing ACN to alcohol-type eluent could result in the opposite EEO. This type of EEO reversal was observed twice on the Am1 column, twice on the Am2 column and interestingly once on the Cell2 column. The reason for the mobile phase dependent EEO could be the different spatial structure of the chiral selector or for example the different types of secondary interactions based on the applied mobile phase.

3.3. Measurement in polar organic eluent mixtures - hysteresis

Often in polar organic mode neat eluents are applied instead of mixtures [32–34]. This approach poses several advantages, mainly related to their ease of use and simplicity. However, it is known that the composition of solvent mixtures used as eluents can provide several possible conformations of the chiral selector, which result in different selectivity of the separation systems. This means that an appropriate eluent mixture can provide better enantioselectivity than each of the neat eluents individually [15,35]. In their recent publications Horváth et al. investigated the effect of the eluent mixture on amylose tris(3,5-dimethylphenylcarbamate)-based chiral columns [15]. The authors observed that selectivity and retention times strongly depend on column history, that is the eluents in which it was previously used. The hysteretic behaviour was rationalized by the spatial alteration of the CSP upon changes in polar organic mixtures and upon the direction from which a certain composition of eluent is approached. The observation - that different separations using the same CSP-eluent combination depend on the preceding eluent compositions - have been interpreted as hindered transitions between different higher order structures of the CSP. It has been speculated that the explanation of the hindrance may reside in different helical structures of the polysaccharide backbone with different H-bond systems in MeOH as opposed to IPA. The various stable states of the CSP can be utilized in method development using amylose tris(3,5-dimethylphenylcarbamate)-based columns. In our study MeOH:IPA and MeOH:ACN mixtures were examined with 10% increments and decrements with all the compounds on Am1, iAm1 and Am2 columns. Some representative retention factor vs eluent composition and separation factor vs. eluent composition curves depicted in Fig. 4 and Supplementary Figure 2, while some chromatograms are presented in Fig. 5 .

Reviewing the measurement results, it can be concluded at first that the hysteresis phenomenon on the investigated amylose-type columns is general. It can be observed not only on the pre-
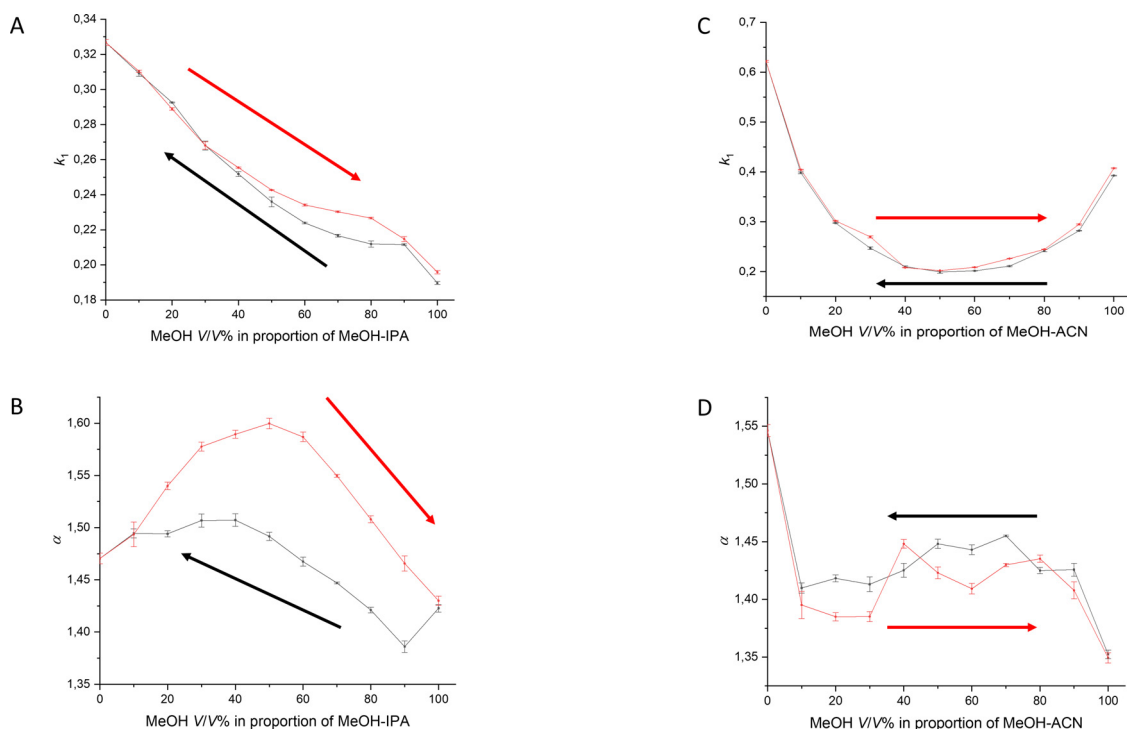
Fig. 4. Some representative graphs of retention factor/separation factor vs. eluent composition. A: Retention factor of 3R enantiomer in different MeOH:IPA compositions on iAm1 column. B: Separation factor of compound 3 enantiomers in different MeOH:IPA compositions on iAm1 column. C: Retention factor of 3R enantiomer in different MeOH:ACN compositions on Am1 column. D: Separation factor of compound 3 enantiomers in different MeOH:ACN compositions on Am1 column. (Flow rate: 0.5 mL/min, temperature: 25°C).
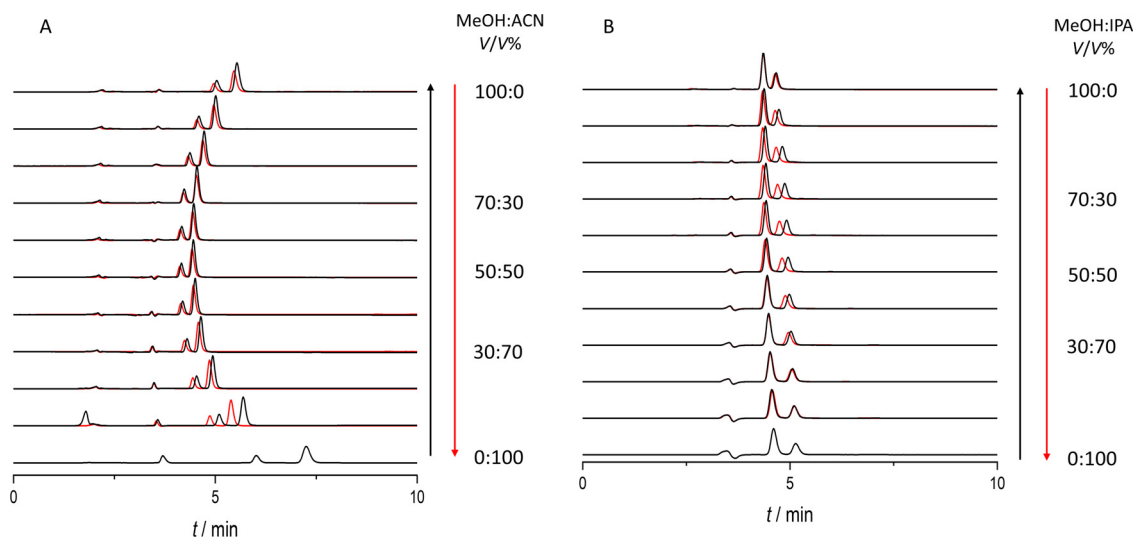
Fig. 5. Chromatograms observed in different eluent compositions during the hysteresis study. A: Enantioseparation of compound 5 in different MeOH:ACN eluent mixtures using Am1 CSP. B: Enantioseparation of compound 3 in different MeOH:IPA eluent mixtures using Am2 CSP. (Flow rate: 0.5 mL/min, temperature: 25°C).

viously reported amylose tris(3,5-dimethylphenylcarbamate)-based column, but also on the Am2 column containing amylose tris(5-chloro-2-methylphenylcarbamate) chiral selector as well. In addition, it should also be noted that no hysteresis phenomenom was observed on cellulose CSPs (Supplementary Figure 3). Using amylose-based CSPs, the effect was not only observed in the case of MeOH:ACN mixtures, but also in MeOH:IPA eluents. However, in the latter case, the hysteresis effect is much more pronounced. It can be seen that the retention profiles using MeOH:ACN mixtures are different than in MeOH:IPA mixtures. In general U-shape curve can be observed in MeOH:ACN mixture, while in MeOH:IPA mixture the inverted S-shape is also common. In MeOH:ACN mix-

ture the best resolution can be measured at one of the extreme values (100% MeOH or 100% ACN). In MeOH:IPA there are more examples where the best separation is at an intermediate value. Based on this, it can be assumed that the spatial structure of the chiral selector in MeOH:IPA changes and may exist in several conformational states. The enantiomeric recognition of each stable conformer differs, which allows us to increase the selectivity or even change the EEO by using only one column. Although it should be noted that there was no EEO change in our case. In an ACN:MeOH mixture, it is conceivable that the structure of the chiral selector does not change at the intermediate states. The U-shaped retention profiles obtained may be ex-

plained by the different H-bridge-forming ability of the eluents used.

4. Conclusion

Enantioseparation of oxazolidinone analogues were carried out on amylose- and cellulose-based CSPs in polar organic mode. Best separation was observed on amylose-type columns with ACN. Our work focused on the investigation of EEO and studying the phenomenon of selectivity- and retention-hysteresis. During our study chiral selector-, mobile-phase- and immobilization-dependent EEO reversals were observed. The latest example clearly shows that the immobilization conditions produce chemical and/or physical alteration of the selector, and the Am1 and iAm1 columns are not interchangeable. The investigation of hysteresis shows that it is a general phenomenon on amylose-based columns. In polar organic mode using the mixture of polar organic solvents allows us to expand the boundaries of each amylose-based column. In eluent mixture the amylosed-based chiral selector could exist more conformational states each with different enantiorecognition mechanisms. This finding can pave the way to a novel, easier and cheaper chiral method development approach.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

CRediT authorship contribution statement

Máté Dobó: Investigation, Methodology. **Mohammadhassan Foroughbakhshfasaei:** Investigation, Formal analysis. **Zoltán-István Szabó:** Conceptualization, Methodology, Writing – original draft. **Gergő Tóth:** Conceptualization, Methodology, Investigation, Supervision, Funding acquisition.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2021.462741.

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