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Enantioselective potential of polysaccharide-based chiral stationary phases in supercritical fluid chromatography

Gabriela Kucerova | Kveta Kalikova | Eva Tesarova

Department of Physical and Macromolecular Chemistry, Charles University, Prague, Czech Republic

Correspondence
Kveta Kalikova, Department of Physical and Macromolecular Chemistry, Charles University, Albertov 6, Prague 2, 12843, Czech Republic.
Email: kveta.kalikova@natur.cuni.cz

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Abstract

The enantioselective potential of two polysaccharide-based chiral stationary phases for analysis of chiral structurally diverse biologically active compounds was evaluated in supercritical fluid chromatography using a set of 52 analytes. The chiral selectors immobilized on 2.5 μm silica particles were tris-(3,5-dimethylphenylcarmabate) derivatives of cellulose or amylose. The influence of the polysaccharide backbone, different organic modifiers, and different mobile phase additives on retention and enantioseparation was monitored. Conditions for fast baseline enantioseparation were found for the majority of the compounds. The success rate of baseline and partial enantioseparation with cellulose-based chiral stationary phase was 51.9% and 15.4%, respectively. Using amylose-based chiral stationary phase we obtained 76.9% of baseline enantioseparations and 9.6% of partial enantioseparations of the tested compounds. The best results on cellulose-based chiral stationary phase were achieved particularly with propane-2-ol and a mixture of isopropylamine and trifluoroacetic acid as organic modifier and additive to CO_2, respectively. Methanol and basic additive isopropylamine were preferred on amylose-based chiral stationary phase. The complementary enantioselectivity of the cellulose- and amylose-based chiral stationary phases allows separation of the majority of the tested structurally different compounds. Separation systems were found to be directly applicable for analyses of biologically active compounds of interest.

KEYWORDS

amylose, biologically active compounds, cellulose, chiral separation, chiral stationary phase, enantioselectivity, supercritical fluid chromatography

1 | INTRODUCTION

Supercritical fluid chromatography (SFC) has become a very successful technique for fast and efficient achiral or chiral separations of diverse compounds in the past few years. SFC is becoming a method of first choice in pharmaceutical applications concerning enantioseparations and/or purifications.[1-8] This is largely thanks to the commercialization of new-generation SFC systems, which offer enhanced sensitivity, robustness, and quantitative performance.[9] Supercritical CO_2 as the main part of the mobile phase (MP) is referred to as a "green solvent" and a further desirable MP component thanks to its properties such as density, solvating power, or viscosity.[1,10] From this point of view, SFC offers benefits such as higher throughput or lower analysis times than the conventional high-performance liquid chromatography (HPLC) technique.[1,11-13]

On the market, there are a number of chiral stationary phases (CSPs) including many of HPLC CSPs, that are suitable for enantioseparation in SFC. Regarding HPLC as well as SFC enantioseparations, polysaccharide-based CSPs are classified as the versatile ones and are extensively used.[5,14-24]

New-generation SFC systems offer better compatibility with modern stationary phases (SPs), such as those packed with sub-2 μm fully porous particles or sub-3 μm superficially porous particles.[9,25] In fact, SFC columns with 2.5 μm silica particles containing tris-(3,5-dimethylphenylcarbamate) of cellulose or amylose as chiral selectors (CSs) used in this study are recent products fully compatible with commercial UPC^2 (ultra-performance convergence chromatography) systems.

Organic modifiers, e.g., some alcohols, and basic or acidic additives added to the main MP component CO_2 are used to modulate the separation ability of the SFC system. A study has been performed on the effect of different alcohols in MP in separation systems with immobilized polysaccharide-based CSPs.[26] The authors demonstrated complementary separations of pharmaceuticals in MPs containing MEOH and propane-2-ol. However, in general the separation success rate for the studied pharmaceuticals was not very high in their work. Concerning the addition of basic isopropylamine (IPAM) and/or acidic trifluoroacetic acid (TFA) additives, the combination of both was reported to reduce nonspecific interactions and so to increase enantioselectivity. The dual addition also led to minimization of the memory effect of SP.[27] However, higher concentration of these additives could result in undesirable precipitation of the forming salt complexes.[28]

Nowadays the SFC method is used for separation of neutral, acidic and also basic compounds.[29-31] Nevertheless, separation of basic compounds can be hampered by forming of ionic interactions with residual silanol-carrier groups.[1,32,33] Among basic, acidic, bifunctional, and neutral biologically active compounds (BACs) used in this study, we classify very well-known drugs like profens, thiazide diuretics, flavanone derivatives, and calcium channel blockers or phenothiazines and β-blockers.[22,34,35] Moreover, newly synthesized drugs called "legal highs" belong likewise to BACs.[36] The "legal highs" used in this work are derivatives of amphetamine or benzofuran. Mostly, they are "abused" similarly as prohibited addictive substances, with the difference that there is insufficient legislation that would punish this permitted activity. Recently, BACs based on amphetamine or benzofuran were successfully enantioseparated using amylose-based CSP in SFC.[1] Some enantiomers of β-blockers used in this study were previously separated on two different polysaccharide-based CSPs (Chiralpak IB-3 and Chiralpak AD columns) by SFC.[37,38] Almost 20 years ago, Berger and Wilson enantioseparated several phenothiazine substances using packed column SFC.[39]

The aim of this work was to find out and compare the enantioselective potential of new short polysaccharide-based columns (50 mm long), i.e., ACQUITY UPC^2 Trefoil CEL1 and ACQUITY UPC^2 Trefoil AMY1. The CSs are immobilized on 2.5 μm silica particles. The goal was to show differences in the chromatographic behavior between these two columns, as they differ in the nature of the polysaccharide backbone. For this purpose, a set of 52 structurally different BACs was tested under diverse SFC separation conditions, namely, different MP compositions, to examine the enantioseparation abilities and differences of these two columns. The other objective was to find the best/optimal mobile phases for enantioseparation of the tested chiral compounds.

2 | MATERIALS AND METHODS

2.1 | Chemicals and analytes

Methanol (MEOH, Chromasolv, gradient grade, ≥99.9%), propane-2-ol (PROH, Chromasolv for HPLC, ≥99.8%), isopropylamine (IPAM, ≥99.5%), trifluoroacetic acid (TFA, 99%), and tetrahydrofuran (THF, Chromasolv for HPLC) were supplied by Sigma-Aldrich (St. Louis, MO). Pressurized liquid CO_2 4.5 grade (99.995%) was purchased from Messer (Prague, Czech Republic). Chiral analytes: profen derivatives (PF1, ibuprofen; PF2, indoprofen; PF3, flurbiprofen; PF4, tiaprofenic acid; PF5, carprofen, PF6, suprofen; PF7, ketoprofen; PF8, fenoprofen), flavanone derivatives (F1, 6-hydroxyflavanone; F2, 7-hydroxyflavanone), thiazide diuretics (TD1, butizide; TD2, mefruside; TD3, chlorthalidone; TD4, trichlormethiazide; TD5, bendroflumethiazide), calcium channel blockers (CB1, amlodipine; CB2, nimodipine; CB3, nitrendipine; CB4, nicardipine; CB5, verapamil; CB6, nisoldipine), phenothiazines (PH1, thioridazine; PH2, promethazine), amphetamine derivatives (A1, 4-fluoromethcathinone; A2, 4-fluoroamphetamine; A3, 4-bromomethcathinone; A4, buphedrone; A5, ethylone; A6, 3-fluoroamphetamine; A7, 2-fluoromethcathinone; A8, methylendioxypyrovalerone), benzofury derivatives (B1, 5-(2-aminopropyl)benzofuran; B2, 6-(2-aminopropyl)benzofuran; B3, 5-(2-aminopropyl)-2,3-dihydrobenzofuran; B4, 6-(2-aminopropyl)-2,3-dihydrobenzofuran; B5, 1-(benzofuran-5-yl)-*N*-ethylpropan-2-amine; B6, 1-(benzofuran-6-yl)-*N*-ethylpropan-2-amine; B7, 1-(benzofuran-5-yl)-*N*-methylpropan-2-amine), β-blockers (BB1, propranolol; BB2, oxprenolol; BB3, metoprolol; BB4, metipranolol; BB5, acebutolol; BB6, pindolol; BB7, bopindolol; BB8, atenolol; BB9, alprenolol) and others (O1, BP34; O2, BP766; O3, thalidomide; O4, tramadol; O5, lorazepam) were purchased from Sigma-Aldrich or kindly donated from M.G. Schmid from Institute of Pharmaceutical Chemistry and Pharmaceutical Technology, Karl Franzens University, Graz, Austria. See Figures S1 in the Supporting Information for the structures of the compounds.

2.2 | SFC instrumentation and columns

The Waters Acquity Ultra Performance Convergence Chromatography (UPC^2) system was equipped with a binary solvent delivery pump (MP flow rates up to 4 mL min^{-1}, pressures up to 6000 psi), an autosampler which included a partial loop volume injection system, a back-pressure (BP) regulator, a column oven, and a photodiode array detector (Waters, Milford, MA). The Empower 3 software was used for system control and data acquisition. Both columns: ACQUITY UPC^2 Trefoil CEL1 (CEL1) and ACQUITY UPC^2 Trefoil AMY1 (AMY1) were obtained from Waters. The CSs immobilized on 2.5 μm silica particles were tris-(3,5-dimethylphenylcarbamate) derivatives of cellulose (CEL1) or amylose (AMY1). The dimensions of both columns were 3.0 × 50 mm.

2.3 | General conditions

The chromatographic measurements were performed at a flow rate 2.5 mL min^{-1} based on our previous experience[1] and our preliminary measurements in this work (measurements were carried out in the range 1.5–3 mL min^{-1}). Both retention and resolution increased at lower flow rate. Thus, the best separation conditions were considered as a compromise between resolution and short analysis time. The column temperature was 35 °C, BP of 2000 psi and UV detection at 254, 260 and 280 nm. Void volume was determined using the solvent peak. Injection volume was in the range 0.6–1.0 μL depending on the detector response. Sample temperature was 10 °C. All measurements were performed in triplicate.

2.4 | Sample preparation

The stock solutions of profen derivatives, flavanones, thiazide diuretics, calcium channel blockers (except for CB6), phenothiazines, β-blockers, others (except for O3), and 3-fluoroamphetamine (A7) were prepared in MEOH at a concentration of 1.0 mg mL^{-1}. Amphetamine derivatives (except for A5, A6, and A7) were dissolved in MEOH at a concentration of 0.5 mg mL^{-1}. The stock solutions of benzofury derivatives were prepared at a concentration of 0.25 mg mL^{-1} in MeOH/THF 50/50 *(v/v)*. CB6 was dissolved in MEOH/THF 80/20 *(v/v)* at a concentration of 1.0 mg mL^{-1} and O3 in MEOH/THF 75/25 *(v/v)* at a concentration of 1.0 mg mL^{-1}.

2.5 | MP compositions

MPs composed of CO_2 and organic modifiers MEOH or PROH with the addition of basic additive IPAM and/or acidic additive TFA was prepared in various volume ratios. See Table 1 for exact MP compositions used in this work.

TABLE 1 MP compositions used for the enantioseparation of BACs on CEL1 and AMY1 CSPs

MP compositions:	Volume ratios *(v/v/v(/v))*:		
CO_2/MEOH/IPAM	90/10/0.1	95/5/0.1	98/2/0.1
CO_2/PROH/IPAM	90/10/0.1	95/5/0.1	98/2/0.1
CO_2/MEOH/IPAM/TFA	90/10/0.1/0.1	95/5/0.1/0.1	98/2/0.1/0.1
CO_2/PROH/IPAM/TFA	90/10/0.1/0.1	95/5/0.1/0.1	98/2/0.1/0.1
CO_2/MEOH/TFA	90/10/0.1	95/5/0.1	98/2/0.1
CO_2/PROH/TFA	90/10/0.1	95/5/0.1	98/2/0.1

3 | RESULTS AND DISCUSSION

The columns CEL1 and AMY1 were investigated under various MP compositions in order to evaluate their enantioselective ability for separation of acidic, basic, bifunctional, and neutral compounds. Temperature and BP were kept constant during measurements in order to monitor the impact of the CSP type and MP compositions. The best/optimized separation conditions were found for the majority of the studied compounds even without the additional optimization of temperature and BP. Some of the baseline separated compounds could be resolved under several MP compositions. However, other suitable MP compositions are not shown, particularly due to a higher duration of analysis that is claimed to be as short as possible. Chromatographic data collected from the measurements on the both CSPs at different MP compositions are summarized in Table S1 in the Supporting Information for better understanding and comparison. In general, a higher retention of analytes was obtained in MPs containing more hydrophobic PROH than those containing MEOH (comparing the same volume ratios). We did not observe the general effect of enhanced and decreased enantioselectivity on the cellulose-based and amylose-based CSPs, respectively, caused with the dual additives in the SFC separation systems that were reported by other authors.[28] It is obvious from Table 2 and Table S1 that different groups of tested analytes prefer different CSPs-polysaccharide backbone compared in this work.

3.1 | Profen derivatives

From the set of eight profen derivatives, seven were baseline separated under optimized conditions on the CSP with amylose backbone AMY1, while just three exhibited a resolution higher than 1.5 on CEL1 CSP. Enantiomers of ibuprofen (PF1) could not be resolved on any of these CSPs. Despite the general observation that better enantioseparation of profens can be achieved on the amylose-based column, certain separations show opposite results, as demonstrated in Figure 1B. Comparison of the separation of flurbiprofen

TABLE 2 Chromatographic data and the best MP compositions for enantioseparation of studied compounds on CEL1 and AMY1 CSPs

		CEL1 CSP					AMY1 CSP				
Compounds		$t_{r,1}$ (min)	k_1	α	R_s	MP composition *(v/v/v(/v))*	$t_{r,1}$ (min)	k_1	α	R_s	MP composition *(v/v/v(/v))*
Profen derivatives	**PF1**	X	X	X	X	X	X	X	X	X	X
	PF2	1.41	9.71	1.19	**2.25**	**C/M/T 90/10/0.1**	15.08	134	1.22	**2.73**	C/P/T 90/10/0.1
	PF3	3.95	32.50	1.13	**1.52**	C/P/T 98/2/0.1	0.39	2.53	1.35	**2.89**	**C/M/T 90/10/0.1**
	PF4	14.29	120	1.10	1.36	C/P/T 98/2/0.1	3.24	28.50	1.86	**6.71**	**C/P/I/T 95/5/0.1/0.1**
	PF5	3.38	24.60	1.23	**2.07**	**C/P/I/T 90/10/0.1/0.1**	5.42	46.97	1.20	**2.34**	C/M/I/T 90/10/0.1/0.1
	PF6	2.53	21.02	1.11	1.27	C/P/T 95/5/0.1	1.17	9.42	1.26	**2.55**	**C/M/T 90/10/0.1**
	PF7	X	X	X	X	X	1.36	11.37	1.17	1.47	**C/P/I/T 95/5/0.1/0.1**
	PF8	X	X	X	X	X	5.61	41.80	1.17	**2.13**	**C/P/T 98/2/0.1**
Flavanones	**F1**	1.61	11.18	1.11	**1.51**	C/M/I/T 95/5/0.1/0.1	1.73	14.99	1.48	**3.86**	**C/P/I/T 90/10/0.1/0.1**
	F2	X	X	X	X	X	1.55	13.38	1.18	**1.80**	**C/P/I/T 90/10/0.1/0.1**
Thiazide diuretics	**TD1**	2.69	19.36	1.52	**4.73**	**C/M/I/T 90/10/0.1/0.1**	15.17	133	1.24	**1.81**	C/P/I/T 90/10/0.1/0.1
	TD2	X	X	X	X	X	2.63	22.86	1.70	**3.85**	**C/M/I 90/10/0.1**
	TD3	3.40	24.83	1.23	**2.53**	**C/M/I/T 90/10/0.1/0.1**	12.18	87.91	1.36	**2.64**	C/M/I 90/10/0.1
	TD4	5.75	49.41	1.11	**1.51**	**C/M/T 90/10/0.1**	18.74	165	1.13	**8.68**	C/P/I/T 90/10/0.1/0.1
	TD5	17.39	130	1.09	1.05	C/P/I 90/10/0.1	3.36	28.69	1.26	**2.03**	**C/M/I/T 90/10/0.1/0.1**
Calcium channel blockers	**CB1**	7.16	52.75	1.24	**1.70**	**C/P/I/T 95/5/0.1/0.1**	X	X	X	X	X
	CB2	10.72	89.83	1.09	1.05	C/P/T 98/2/0.1	0.72	5.43	3.37	**4.23**	**C/P/T 90/10/0.1**
	CB3	X	X	X	X	X	X	X	X	X	X
	CB4	X	X	X	X	X	X	X	X	X	X
	CB5	0.73	4.50	1.28	**1.87**	**C/M/I/T 90/10/0.1/0.1**	0.85	6.11	1.14	0.84	C/P/I 90/10/0.1
	CB6	8.00	58.84	1.09	1.23	**C/P/I 98/2/0.1**	X	X	X	X	X
Phenothiazines	**PH1**	X	X	X	X	X	2.85	22.73	1.24	**2.04**	**C/P/I 90/10/0.1**
	PH2	1.41	9.73	1.10	0.93	C/P/I 98/2/0.1	1.95	12.73	1.26	**1.56**	**C/P/I 98/2/0.1**
Amphetamine derivatives	**A1**	X	X	X	X	X	1.65	12.85	1.30	1.20	**C/P/T 95/5/0.1**
	A2	X	X	X	X	X	10.53	88.23	1.15	**1.83**	**C/P/I 98/2/0.1**
	A3	X	X	X	X	X	0.43	2.80	1.51	**1.78**	**C/P/I/T 90/10/0.1/0.1**
	A4	2.25	18.72	1.16	1.43	C/M/T 98/2/0.1	3.05	25.07	1.35	**2.68**	**C/P/I 95/5/0.1**
	A5	X	X	X	X	X	1.75	14.92	1.66	**2.54**	**C/P/I/T 95/5/0.1/0.1**
	A6	X	X	X	X	X	3.06	25.14	1.27	**2.15**	**C/P/I 95/5/0.1**
	A7	X	X	X	X	X	3.06	25.50	1.26	**2.66**	**C/P/I 95/5/0.1**
	A8	1.34	9.12	1.27	**2.25**	C/P/I/T 95/5/0.1/0.1	0.56	3.80	1.24	**1.84**	**C/P/I 95/5/0.1**
Benzofury derivatives	**B1**	1.45	9.99	1.24	**1.80**	**C/P/I/T 95/5/0.1/0.1**	7.25	60.97	1.31	**3.72**	C/P/I 95/5/0.1
	B2	1.45	9.93	1.23	**1.68**	**C/P/I/T 95/5/0.1/0.1**	7.28	61.18	1.32	**3.48**	C/P/I 95/5/0.1
	B3	2.71	19.70	1.92	**7.33**	**C/M/I 98/2/0.1**	7.58	63.79	1.11	1.37	C/P/I 95/5/0.1
	B4	0.54	3.05	1.50	**2.42**	**C/P/I/T 90/10/0.1/0.1**	0.80	5.94	1.35	**1.97**	C/M/I/T 95/5/0.1/0.1
	B5	X	X	X	X	X	1.99	16.04	1.24	**2.38**	**C/P/I 95/5/0.1**
	B6	X	X	X	X	X	2.08	16.81	1.13	1.42	**C/P/I 95/5/0.1**
	B7	3.27	23.78	1.12	1.01	C/P/I 98/2/0.1	2.92	23.96	1.19	**2.07**	**C/P/I 95/5/0.1**
β-blockers	**BB1**	0.80	5.09	2.00	**6.36**	**C/M/I/T 90/10/0.1/0.1**	2.48	21.37	1.50	**2.92**	C/P/I 90/10/0.1
	BB2	0.31	1.35	2.44	**4.14**	**C/M/I/T 90/10/0.1/0.1**	1.25	10.25	1.28	**1.63**	C/P/I 90/10/0.1
	BB3	0.28	1.13	4.02	**5.00**	**C/M/I/T 90/10/0.1/0.1**	1.83	15.46	1.34	**1.94**	C/P/I 90/10/0.1
	BB4	0.60	3.54	1.30	**2.26**	**C/M/I/T 95/5/0.1/0.1**	13.58	115	1.20	**2.92**	C/P/I 95/5/0.1
	BB5	5.43	40.10	1.15	**1.71**	**C/M/I/T 95/5/0.1/0.1**	15.69	140	1.26	**1.62**	C/P/I 90/10/0.1
	BB6	2.01	14.25	4.46	**14.2**	**C/M/I/T 90/10/0.1/0.1**	X	X	X	X	X
	BB7	1.76	12.25	1.72	**5.66**	**C/M/I/T 90/10/0.1/0.1**	5.56	47.4	1.86	**5.52**	C/M/I/ 95/5/0.1
	BB8	1.55	10.61	2.31	**8.62**	**C/M/I/T 90/10/0.1/0.1**	X	X	X	X	X
	BB9	0.23	0.76	1.79	**2.05**	**C/M/I/T 90/10/0.1/0.1**	0.93	7.35	1.41	**2.07**	C/P/I 90/10/0.1
Others	**O1**	2.70	19.41	1.41	**1.92**	**C/P/I/T 95/5/0.1/0.1**	3.46	27.83	1.40	**1.68**	C/P/I 90/10/0.1
	O2	X	X	X	X	X	2.02	15.97	1.31	**1.58**	**C/P/T 95/5/0.1**

(*Continues*)

TABLE 2 (Continued)

Compounds		CEL1 CSP $t_{r,1}$ (min)	k_1	α	R_s	MP composition *(v/v/v(/v))*	AMY1 CSP $t_{r,1}$ (min)	k_1	α	R_s	MP composition *(v/v/v(/v))*
	O3	2.43	20.29	1.13	**1.70**	**C/M/T 95/5/0.1**	3.40	29.11	1.30	**2.34**	C/P/I/T 90/10/0.1/0.1
	O4	0.53	2.99	1.18	**1.56**	C/M/I/ 95/5/0.1	0.38	2.13	1.29	**1.58**	**C/P/I 90/10/0.1**
	O5	1.46	10.07	1.27	**2.72**	**C/M/I/T 90/10/0.1/0.1**	1.96	13.77	1.30	**2.36**	C/M/I/T 90/10/0.1/0.1

In the case of baseline separation, the optimized chromatographic data are reported with respect to the shortest analysis time; resolution value in bold indicates baseline separation; MP composition in bold indicates the best chromatographic conditions (including CSP) for enantioseparation, C: CO_2, M: MEOH, P: PROH, I: IPAM, T: TFA; $t_{R,1:}$ retention time of the first eluted enantiomer, k_1: retention factor of the first eluted enantiomer, $\alpha_{:}$ selectivity, R_S: resolution of the two enantiomers, X: no indication of enantioseparation.

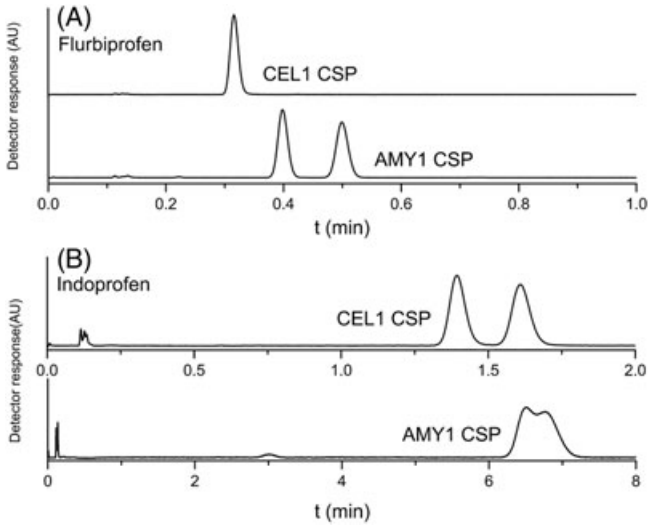

FIGURE 1 Analyses of flurbiprofen **A**, and indoprofen **B**, on the polysaccharide-based CSPs. MP composition: CO_2/MEOH/TFA 90/10/0.1 *(v/v/v)*

and indoprofen in the same MP composed of CO_2/MEOH/TFA 90/10/0.1 *(v/v/v)* supports this statement, i.e., better result of indoprofen enantioseparation obtained on CEL1 CSP. Moreover, indoprofen (PF2) was baseline enantioseparated in all the MPs tested using CEL1 CSP (Table S1 in the Supporting Information). MPs with basic IPAM additive were not suitable for enantioseparation of profen derivatives. The basic additive increases "dissociation" of acidic profens, while compounds to be separated on the polysaccharide-based CSPs are nondissociated. Thus, addition of TFA or a combination of IPAM and TFA resulted in improved enantioseparation. Certain complementarity of the enantioseparation ability of the compared CSPs is clearly seen from the obtained results (Figure 1).

3.2 | Flavanones

The two flavanone derivatives with very similar structures (the difference lies only in the position of the hydroxy group in their molecule) were successfully enantioseparated using AMY1 CSP and MP composed of CO_2/PROH/IPAM/TFA 90/10/0.1/0.1 *(v/v/v/v)* (Table 2). The combination of both MP additives (IPAM and TFA) was very supportive for fast enantioseparation of both analytes on AMY1 CSP. On the other hand, they were not baseline enantioresolved on CEL1 CSP under the above-mentioned MP. However, enantioresolution $R_S = 1.5$ for F1 enantiomers was achieved on CEL1 CSP in MP with higher CO_2 content. The representative chromatograms of analysis of 7-hydroxyflavanone on both columns in the same MP composition are depicted in Figure 2.

3.3 | Thiazide diuretics

All the enantiomers of analytes from the group of thiazide diuretics could be baseline separated under optimized separation conditions on AMY1 CSP. However, their retention and thus analysis time was too long for practical purposes. On the other hand, butizide (TD1), chlorthalidone (TD3), and trichlormethiazide (TD4) enantiomers could be baseline separated on CEL1 CSP in a significantly shorter analysis time. Concerning the MPs, the most suitable composition varies by CSP used and analyte of interest (Table 2).

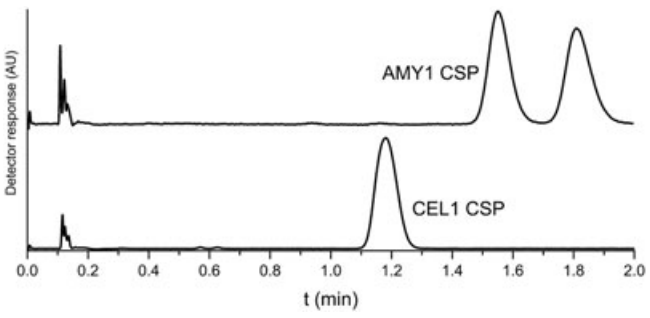

FIGURE 2 Analyses of 7-hydroxyflavanone on the polysaccharide-based CSPs. MP composition: CO_2/PROH/IPAM/TFA 90/10/0.1/0.1 *(v/v/v/v)*

3.4 | Calcium channel blockers

Neither AMY1 nor CEL1 CSPs were suitable for enantioseparation of this group of analytes. Despite the fact that calcium channel blockers possess in their molecules structurally similar motifs, we observed rather different results for diverse derivatives. Whereas amlodipine, verapamil, and nisoldipine enantiomers were baseline separated on CEL1 CSP, nimodipine could be enantioseparated on AMY1 CSP. No CSP could be preferred; moreover, the best MP composition for analysis of the calcium channel blockers differed in the type of organic modifier as well as in MP additive.

3.5 | Phenothiazines

AMY1 CSP better suited for separation of phenothiazine enantiomers than CEL1 CSP. Thioridazine (PH1) and promethazine (PH2) were both baseline resolved in MP containing CO_2, PROH, and IPAM (Table 2). A better resolution value was achieved for PH1 than for PH2 with amylose-based column, while the opposite result was observed on cellulose-based CSP. On the latter, partial enantioseparation was obtained for PH2, while no enantioseparation was achieved for PH1.

3.6 | Amphetamine derivatives

All amphetamine derivatives were baseline enantioseparated, except of 4-F-methcathinone (A1) enantiomers, which were partially separated using AMY1 CSP. PROH was a better organic modifier for separation of all the analytes from this group and also the best MP composition was mostly the same, i.e., CO_2/PROH/TFA 95/5/0.1 *(v/v/v)* (Table 2). Using CEL1 CSP, all these analytes eluted in very short retention times and no enantioseparation was observed (except for A4 and A8) (Table 2 and Table S1 in the Supporting Information). Only one amphetamine derivative, A8, was baseline enantioresolved using CEL1 CSP. Nevertheless, faster baseline separation of A8 was observed using AMY1 CSP. Comparing the observed results AMY1 CSP is definitely the better choice for the enantioseparation of amphetamine derivatives in SFC.

3.7 | Benzofury derivatives

The measurements of the group of benzofury derivatives brought interesting results. No general trends could be related to the structure of these analytes, to the structure of the polysaccharide backbone of the CSP, or to MP composition. On the one hand, enantiomers of B1, B2, B3, and B4 were baseline enantioseparated using CEL1 CSP. On the other hand, no enantioseparation of B5 and B6 was observed in any of the MPs tested on CEL1 CSP, and just partial enantioseparation was achieved for B7 with this CSP. The use of MP composed of PROH and both basic IPAM and acidic TFA additives seemed to be advantageous. On the other hand, MP consisted of CO_2, PROH and just the basic additive, mostly in the volume ratio CO_2/PROH/IPAM 95/5/0.1 *(v/v/v)*, was a better choice if AMY1 CSP was used. A special result was observed with compound B3 (Table 2). The highest resolution value (R_S = 7.33) of all the compounds of this group was achieved with cellulose-based CSP, while on an amylose-based column the lowest resolution value (R_S = 1.37) of B3 was obtained under the "best" MP compositions for a given system.

3.8 | β-blockers

It is obvious from Table 2 as well as from Table S1 that for separation of enantiomers of β-blockers cellulose-based CSP should be considered the column of first choice. All β–blockers were baseline enantioseparated with very high resolution values in very short retention times (except for BB5) using CEL1 CSP (Table 2). The best MP compositions for the enantioseparation of β-blockers on the CEL1 CSP were: CO_2/MEOH/IPAM/TFA 90/10/0.1/0.1 or 95/5/0.1/0.1 *(v/v/v/v)*. MPs suited for enantioseparation of β–blockers on AMY1 CSP contained PROH as organic modifier (except for BB7). The presence of the less polar alcohol PROH instead of MEOH in MP caused longer analyses on AMY1 than on CEL1 CSP (Table S1). Illustrative chromatograms of enantioseparation of metoprolol (BB3) are shown in Figure 3.

3.9 | Others

The last tested analytes are structurally less similar compounds than those in the other groups. Thus, any general discussion cannot be performed. However, we show these results that can be helpful to those who have to carry out separations of these enantiomers because these are compounds of interest (thalidomide, tramadol, lorazepam) for analyses

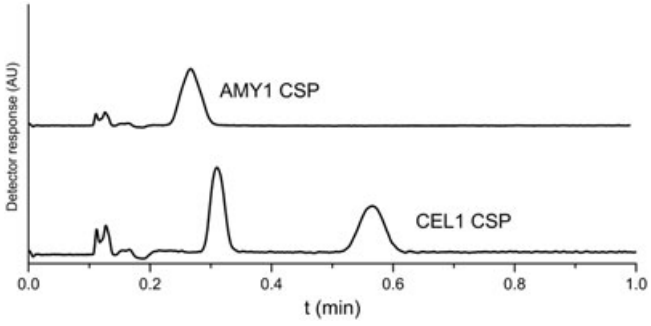

FIGURE 3 Analyses of metoprolol on the polysaccharide-based CSPs. MP composition: CO_2/MEOH/IPAM/TFA 90/10/0.1/0.1 *(v/v/v/v)*

in clinical or pharmaceutical laboratories. As can be seen from Table 2, both columns are applicable for fast enantioseparation of thalidomide, tramadol, as well as lorazepam enantiomers.

4 | CONCLUSION

A set of 52 structurally different chiral BACs were used to reveal the enantioselective potential of two polysaccharide-based CSs immobilized on 2.5 μm silica particles, i.e., CEL1 and AMY1 CSPs in SFC. We monitored the influence of the type of CS backbone, the type and amount of organic modifier, as well as MP additives on enantioresolution of the studied compounds. MPs were composed of CO_2, organic modifier, i.e., MEOH or PROH and MP additive, i.e., IPAM and/or TFA. The results showed that the tris-(3,5-dimethylphenylcarbamate) derivatives of amylose and cellulose show very broad and complementary enantiorecognition abilities. In general, tris-(3,5-dimethylphenylcarbamate) of amylose was more suitable for enantioseparation of the studied compounds than tris-(3,5-dimethylphenylcarbamate) of cellulose. However, certain enantiomers could be better resolved using the CSP with cellulose backbone. We obtained baseline and partial enantioseparations of 45 and 4 tested compounds, respectively, even without further BP and temperature optimization. Three compounds were not enantioseparated under any conditions used.

In summary, 27 analytes were baseline enatioresolved on CEL1 CSP, whereas 40 on AMY1 CSP. Furthermore, eight and five analytes were partially enantioseparated on CEL1 and AMY1 CSPs, respectively. We were not able to achieve enantioseparation of 17 analytes on CEL1 CSP, while seven on AMY1 CSP. Some complementary behavior of the CSPs was observed. Thus, the combination of these two CSPs offers a powerful tool for enantioseparation of different types of BACs in SFC.

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