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Enantioseparation of (±)-*trans*-β-lactam Ureas by Supercritical Fluid Chromatography

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Abstract: In this study the enantioseparation of (±)-*trans*-β-lactam ureas **1a–g** by supercritical fluid chromatography (SFC) was examined using different polysaccharide based chiral stationary phases (CSPs), and CO_2/alcohol (70:30, *V*/*V*) as the mobile phase. The influence of CSP type (coated or immobilized), modifiers (alcohols), additive (isopropylamine), temperature and backpressure on enantioseparation were examined. From five tested columns, only the column filled with *tris*-(4-methylphenylcarbamoyl) cellulose selector proved superior in terms of broad range substrate acceptability and selectivity.

Keywords: β-lactam ureas, enantioseparation, SFC chromatography, chiral stationary phases (CSP's).

INTRODUCTION

SUPERCRITICAL fluid chromatography (SFC) is largely used in the pharmaceutical and food industry for drug analysis and purification,[1] and also in drug development monitoring where contaminants and degradation products are analyzed using this method. SFC is particularly widespread chromatographic technique for determining enantiomeric purity of chiral compounds,[2] and for separating enantiomers on a preparative scale.[3,4] Supercritical fluid chromatography is useful in the analysis of pesticides and other contaminants in soil, water, wastewater samples, and is used in the petrochemical industry for fuel, biodiesel and biomass analysis. Also, it is used for the analysis of natural compounds, such as lipids, vitamins, acylglycerols, sterols, alkaloids, coumarins, saponins, flavanoids, carotenoids, anthraquinones, etc. In the food industry, the SFC technique is useful in the analysis of pesticides and contaminants derived from packaging, in the cosmetics industry for the analysis of waxes containing esters with long hydrocarbon chains. There are a large number of compounds that can be used as fluids in SFC, but so far the most used is carbon dioxide because it is non-toxic, inexpensive,[5] inert, readily available, environmentally friendly,[6] non-flammable, non-corrosive[7] and missible with a large number of organic solvents.[6,7] Its critical temperature and pressure are relatively low (T_c = 31 °C, P_c = 73.8 bar).[5] Carbon dioxide has low viscosity, dielectric constant and surface tension[8] and poor UV absorption at low wavelengths (195, 205 and 210 nm when mixed with acetonitrile, methanol and ethanol).[7] The polarity of carbon dioxide is similar to hexane and heptane and this makes it suitable for use as mobile phases in the elution of non-polar compounds.[9] Problem with dissolving polar compounds and high molecular weight compounds can be overcome using additive, a polar organic solvent, called also a modifier.[10] The most commonly used modifiers are alcohols, such methanol, ethanol and propan-2-ol. In addition to alcohol modifiers, acetonitrile is also used.[10,11] However, the addition of an organic modifier to the mobile phase is sometimes not sufficient to elute highly polar and basic mixtures, so an extra additive is added to the mobile phase.[10] Those extra additives are added to concentration range from 0.1 vol. % to 1 vol. % for organic modifiers,[3] and from 1 vol. % to 5 vol. % for water. In SFC chromatography, an amine, usually isopropylamine, diethylamine or

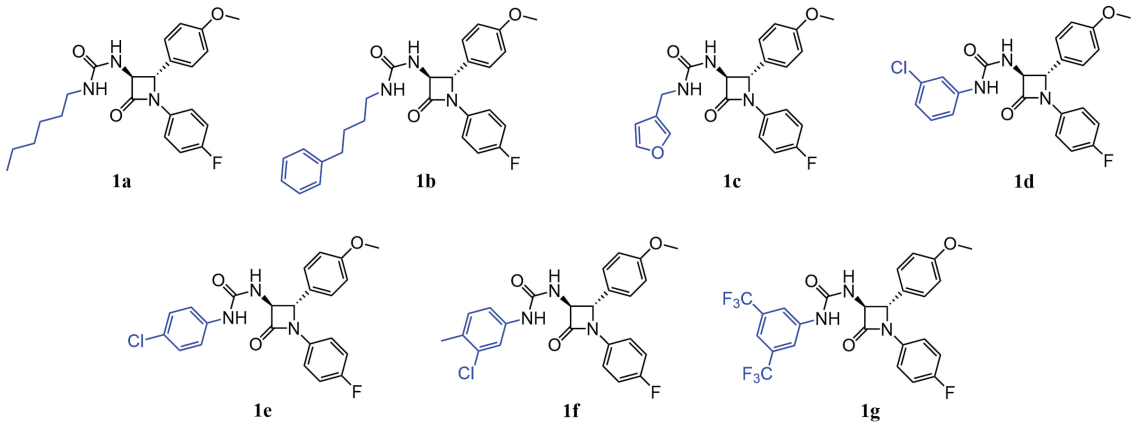


Figure 1. Structures of *trans*-β-lactam ureas **1a–g**.

trimethylamine, is added to the mobile phase for the analysis of basic compounds.[6] For the analysis of acidic compounds, trifluoroacetic acid, formic acid, acetic acid, ethanesulfonic or citric acid are usually used. SFC can be used with all stationary phases, polar and non-polar, and can be performed under normal or reverse phase mode.[10]

Recently, we reported on the synthesis, separation and absolute configuration determination of 3-amino-β-lactams and corresponding guanidines.[12,13] β-Lactams are an important group of heterocyclic compounds and can be found as a structural motif in biologically active natural products, and active pharmaceutical compounds.[14,15,16,17] β-Lactams are therefore extremely useful synthetic scaffolds and versatile precursors in medicinal chemistry due to their diverse biological activity.[15,18,19] In our current synthetic strategy towards chiral hydantoins, we examined the use of SFC chromatography for enantioseparation of different racemic *trans*-β-lactam ureas **1a–g**, Figure 1. The need of enantiopure intermediate *trans*-β-lactam ureas in our research comes from the fact that biological activity of the future hydantoins is closely related to its homochirality. The use of SFC technique in chiral separations of many pharmaceuticals and biomolecules is well established field,[20,21] but to the best of our knowledge, this is the first report describing the efficient application of SFC in enantioseparation of target β-lactam derivatives.

EXPERIMENTAL SECTION

Materials and Methods

The solvents used for SFC were of HPLC grade and were supplied by Honeywell or Merck. Carbon dioxide gas was supplied by Messer Austria and was of 4.5 grade. Racemic *trans*-β-lactam ureas **1a–g** were synthetized by standard procedure from 3-amino-β-lactams and corresponding isocyanates.[22] The final sample concentration used for SFC enantioseparation was 1 mg/mL in methanol.

The following instrument was used in enantioselective analysis: Supercritical Fluid Chromatography Instrument 1260 Infinity II SFC/UHPLC Hybrid, manufactured by Agilent Technologies, Germany, consisting of the quaternary gradient pump G7111B, binary gradient pump G4782A, automatic sample feeder G4767A, column heater G7116A, scanning UV/VIS detector G7115A, RI detector G7162A, SFC module G4301A.

Typical SFC chromatographic conditions were following: the mobile phase consisting of CO_2/alcohol (70:30, *V*/*V*). The flow rate was 4.0 mL/min, the column operating temperature was 35 °C, and the backpressure was 11 MPa. The chromatogram recording was performed at a wavelength of 254 nm and an UV range of 190 to 400 nm.

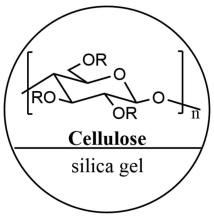
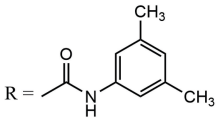
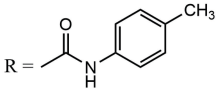
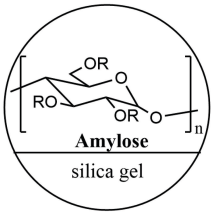
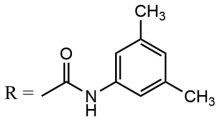
HPLC chiral columns Chiralcel OD-3, Chiralpak IB, Chiralpak IA, Chiralpak AD-3 were purchased from Daicel, Chiral Technologies Europe. HPLC chiral column Chirallica PST-10 was kindly provided by dr. Darko Kontrec.

Results and Discussion

The Effect of Chiral Stationary Phase on Enantioseparation

In this study we used polysaccharide chiral stationary phases (CSP's) based on *tris*-(3,5-dimethylphenylcarbamoyl) amylose (adsorbed and immobilized), *tris*-(3,5-dimethylphenylcarbamoyl) cellulose stationary phase (adsorbed and immobilized), and *tris*-(4-methylphenylcarbamoyl) cellulose stationary phase (adsorbed), Table 1. Initially we tested the influence of above-mentioned polysaccharide based CSP's on enantioseparation of (±)-*trans*-β-lactam ureas **1a–g**, Figure 1, Table 1. For each compound, the values of the retention factors k_1 and k_2, the separation factor α and the resolution R_s of the enantiomers achieved on each column are given. Selected examples **1a,g** of the highest enantioselectivities achieved are shown in the Figure 2. It is important to notice that β-lactam ureas **1a–g** have different substituents at the N1 position of the ureido group, which is attached *via* the

Table 1. Adsorbed and immobilized polysaccharide type CSP's based on cellulose and amylose derivatives

Chiral selector	Chemical structure of chiral selector		Commercial name	
			Coated	Immobilized
Cellulose *tris*-(3,5-dimethylphenylcarbamate)			Chiralcel OD-3	Chiralpak IB
Cellulose *tris*-(4-methylphenylcarbamate)			Chirallica PST-10	
Amylose *tris*-(3,5-dimethylphenylcarbamate)			Chiralpak AD-3	Chiralpak IA

N3-atom to the C3 position of the β-lactam ring. Different alkyl and aryl groups are attached to the N1 position, and in all cases the 4-methoxyphenyl group is attached to the C4 position of the β-lactam ring, and the 4-fluorophenyl group is attached to the N1 position. It should be taken into account that beside urea's structural differences also the structural differences between the amylose-based (helical) and cellulose-based (non helical) chiral stationary phases play an important role in enantiorecognition.[23] The differences between supramolecular structure of adsorbed and immobilized amylose-based and cellulose-based chiral stationary phases may also contribute to a different chiral

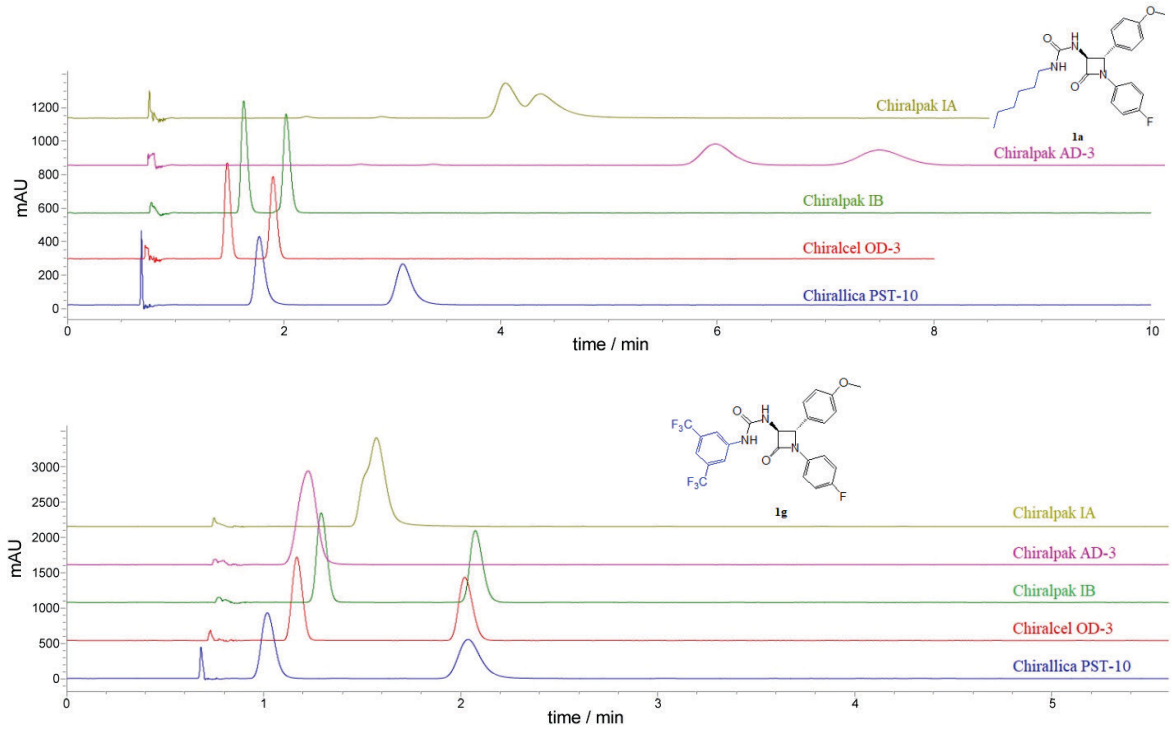


Figure 2. SFC chromatograms of enantiomers of (±)-*trans*-β-lactam ureas **1a** and **1g** on columns filled with different CSP's.

recognition of *trans*-β-lactam urea's enantiomers. The Chirallica PST-10 column with *tris*-(4-methylphenylcarbamate) cellulose chiral selector showed to be the most effective for separating the enantiomers of target compounds, because the enantiomers of all (±)-*trans*-β-lactam ureas **1a–g** are separated on this column, Table 2. The columns filled with adsorbed and immobilized cellulose-based selector, Chiralcel OD-3 and Chiralpak IB columns, proved to be more effective than amylose analogs, Chiralpak AD-3 and Chiralpak IA columns. It is also interesting to note that chiral recognition of compounds **1d** and **1e** is completely absent on the column Chiralpak AD-3. The enantiomers of compound **1d** having Cl atom in the *meta*-position, and enantiomers of compound **1e** having Cl atom in the *para*-position achieved excellent chiral recognition on the cellulose-based stationary phase, i.e. on the Chiralcel OD-3 column, and also on its immobilized version, the Chiralpak IB column. Using Chiralpak AD-3 column, the best chiral recognition is achieved for the *meta*-analogue **1d**, while using its immobilized version, the Chiralpak IA column, the best chiral recognition is achieved for the *para*-analogue **1e**. From Table 2, a decrease in the enantiomer resolution values is observed in the sequence: R_s (*meta*, compound **1d**) > R_s (*para*, compound **1e**) on the Chirallica PST-10 column. These results suggest that the chiral selector of the Chirallica PST-10 column is more "suitable" or enantioselective when the chlorine atom is closer to the –NH and –C=O groups in particular *trans*-β-lactam urea.

Since in all cases of (±)-*trans*-β-lactam ureas enantioseparation, the Chirallica PST-10 column proved to be the most effective one, it was therefore chosen for the further study of chromatographic process.

Table 2. Effect of the CSP (column) on enantioselectivity of (±)-*trans*-β-lactam urea **1a–g**

(±)-*trans*-β-lactam urea	R	Column	t_{R1} (min)	t_{R2} (min)	k_1	k_2	α	R_s
1a	hexyl	Chirallica PST-10	1.77	3.09	1.60	3.54	2.21	6.30
		Chiralcel OD-3	1.47	1.9	0.92	1.48	1.61	3.75
		Chiralpak IB	1.63	2.02	1.11	1.62	1.45	3.40
		Chiralpak AD-3	5.98	7.49	7.02	9.04	1.29	2.36
		Chiralpak IA	4.05	4.37	4.39	4.82	1.10	0.75
1b	4-phenylbutyl	Chirallica PST-10	3.66	6.83	4.38	9.04	2.06	7.38
		Chiralcel OD-3	2.51	3.48	2.28	3.54	1.56	5.63
		Chiralpak IB	2.57	3.27	2.33	3.24	1.39	4.59
		Chiralpak AD-3	10.58	12.45	13.18	15.69	1.19	2.05
		Chiralpak IA	5.95	8.33	6.92	10.09	1.46	3.76
1c	furfuryl	Chirallica PST-10	3.01	3.86	3.43	4.68	1.36	3.04
		Chiralcel OD-3	1.71	2.06	1.23	1.69	1.37	2.83
		Chiralpak IB	1.9	2.19	1.46	1.84	1.26	2.38
		Chiralpak AD-3	5.05	9.75	5.77	12.07	2.09	8.69
		Chiralpak IA	3.33	5.61	3.43	6.47	1.88	6.75
1d	3-chlorophenyl	Chirallica PST-10	7.63	13.48	10.22	18.82	1.84	6.89
		Chiralcel OD-3	2.54	5.55	2.32	6.25	2.70	12.81
		Chiralpak IB	2.77	5.57	2.59	6.22	2.40	12.90
		Chiralpak AD-3	8.97	10.85	11.02	13.54	1.23	2.41
		Chiralpak IA	5.46	7.12	6.27	8.48	1.35	3.25
1e	4-chlorophenyl	Chirallica PST-10	7.55	11.87	10.10	16.46	1.63	5.49
		Chiralcel OD-3	2.53	5.36	2.30	6.00	2.60	12.36
		Chiralpak IB	2.68	5.39	2.47	5.98	2.42	13.23
		Chiralpak AD-3	12.53	12.53	15.80	15.80	1.00	0
		Chiralpak IA	6.01	8.54	7.00	10.37	1.48	4.26
1f	3-chloro-4-methylphenyl	Chirallica PST-10	3.03	8.60	3.46	11.65	3.37	11.04
		Chiralcel OD-3	2.73	5.92	2.56	6.73	2.62	12.77
		Chiralpak IB	2.54	5.03	2.29	5.52	2.41	12.35
		Chiralpak AD-3	4.93	6.11	5.61	7.19	1.28	2.56
		Chiralpak IA	5.46	8.32	6.27	10.08	1.61	5.88
1g	3,5-*bis*(trifluoromethyl) phenyl	Chirallica PST-10	1.01	2.04	0.49	2.00	4.12	6.08
		Chiralcel OD-3	1.17	2.02	0.53	1.64	3.10	7.25
		Chiralpak IB	1.29	2.07	0.67	1.68	2.51	7.09
		Chiralpak AD-3	1.22	1.22	0.64	0.64	1.00	0
		Chiralpak IA	1.57	1.57	1.09	1.09	1.00	0

Table 3. Effect of the alcohol modifiers on enantioselectivity of (±)-*trans*-β-lactam ureas **1a–g**

(±)-*trans*-β-lactam urea	R	Modifier	t_{R1} (min)	t_{R2} (min)	k_1	k_2	α	R_s
1a	hexyl	MeOH	1.77	3.09	1.60	3.54	2.21	6.30
		EtOH	1.98	2.98	2.03	3.56	1.75	4.75
		2-PrOH	3.02	4.37	3.66	5.74	1.57	3.62
1b	4-phenylbutyl	MeOH	3.66	6.83	4.38	9.04	2.06	7.38
		EtOH	3.8	5.64	4.82	7.64	1.58	4.94
		2-PrOH	6.38	7.97	8.85	11.30	1.28	2.37
1c	furfuryl	MeOH	3.01	3.86	3.43	4.68	1.36	3.04
		EtOH	2.82	3.70	3.32	4.67	1.41	3.53
		2-PrOH	4.03	4.95	5.22	7.05	1.35	2.41
1d	3-chlorophenyl	MeOH	7.63	13.48	10.22	18.82	1.84	6.89
		EtOH	3.62	10.1	4.54	14.47	3.18	11.63
		2-PrOH	3.79	13.89	4.85	20.44	4.21	13.07
1e	4-chlorophenyl	MeOH	7.55	11.87	10.10	16.46	1.63	5.49
		EtOH	3.57	8.66	4.47	12.26	2.74	10.4
		2-PrOH	3.84	11.99	4.93	17.50	3.55	11.8
1f	3-chloro-4-methylphenyl	MeOH	3.03	8.60	3.46	11.65	3.37	11.04
		EtOH	3.18	8.16	3.87	11.50	2.97	10.92
		2-PrOH	4.19	12.55	5.47	18.37	3.36	11.05
1g	3,5-*bis*(trifluoromethyl) phenyl	MeOH	1.01	2.04	0.49	2.00	4.12	6.08
		EtOH	0.94	2.38	0.44	2.64	6.02	7.91
		2-PrOH	0.98	3.18	0.51	3.91	7.63	8.79

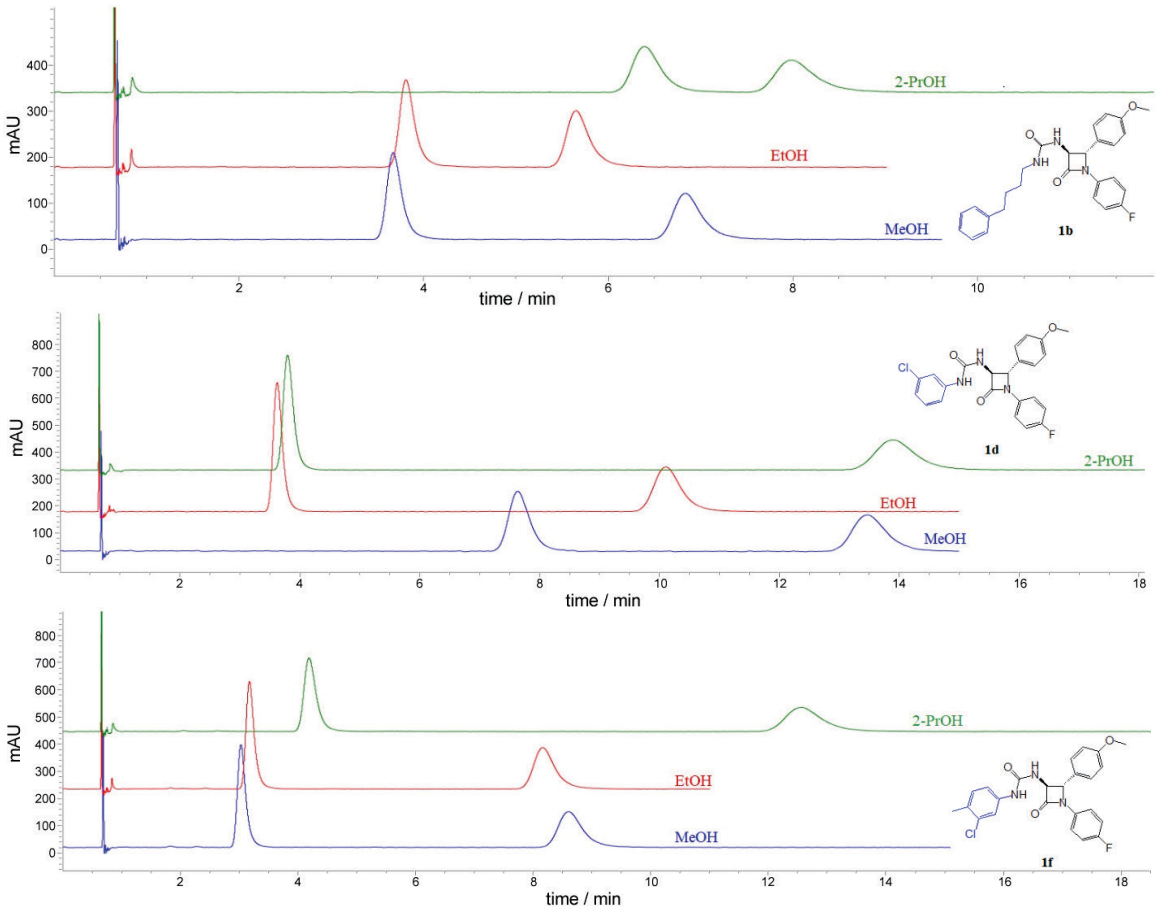


Figure 3. Influence of alcohol modifiers (methanol, ethanol and propan-2-ol) on the enantioseparation of (±)-*trans*-β-lactam ureas **1b**, **1d** and **1f** on Chirallica PST-10 column.

Table 4. The effect of isopropylamine volume fraction on enantioselectivity of (±)-*trans*-β-lactam ureas **1a–g**

(±)-*trans*-β-lactam urea	R	Isopropylamine %	t_{R1} (min)	t_{R2} (min)	k_1	k_2	α	R_s
1a	hexyl	0	1.77	3.09	1.60	3.54	2.21	6.3
		0.1	1.83	3.21	1.69	3.71	2.20	6.6
		0.2	1.82	3.18	1.66	3.65	2.20	6.49
1b	4-phenylbutyl	0	3.66	6.83	4.38	9.04	2.06	7.38
		0.1	3.84	7.15	4.64	9.50	2.05	7.74
		0.2	3.8	7.05	4.56	9.31	2.04	7.67
1c	furfuryl	0	3.01	3.86	3.43	4.68	1.36	3.04
		0.1	3.12	4.04	3.58	4.93	1.38	3.28
		0.2	3.11	4.00	3.55	4.85	1.37	3.21
1d	3-chlorophenyl	0	7.63	13.48	10.22	18.82	1.84	6.89
		0.1	6.57	12.62	8.65	17.53	2.03	6.97
		0.2	6.22	11.99	8.09	16.53	2.04	6.87
1e	4-chlorophenyl	0	7.17	11.41	9.54	15.78	1.65	5.47
		0.1	6.46	11.1	8.49	15.30	1.80	5.76
		0.2	6.14	10.58	7.98	14.47	1.81	5.79
1f	3-chloro-4-methylphenyl	0	3.03	8.6	3.46	11.65	3.37	11.04
		0.1	3.06	8.63	3.49	11.67	3.34	11.64
		0.2	3.09	8.54	3.52	11.49	3.27	11.47
1g	3,5-*bis*(trifluoromethyl) phenyl	0	1.01	2.04	0.49	2.00	4.12	6.08
		0.1	1.00	2.13	0.47	2.13	4.54	6.17
		0.2	0.99	2.06	0.45	2.01	4.50	6.23

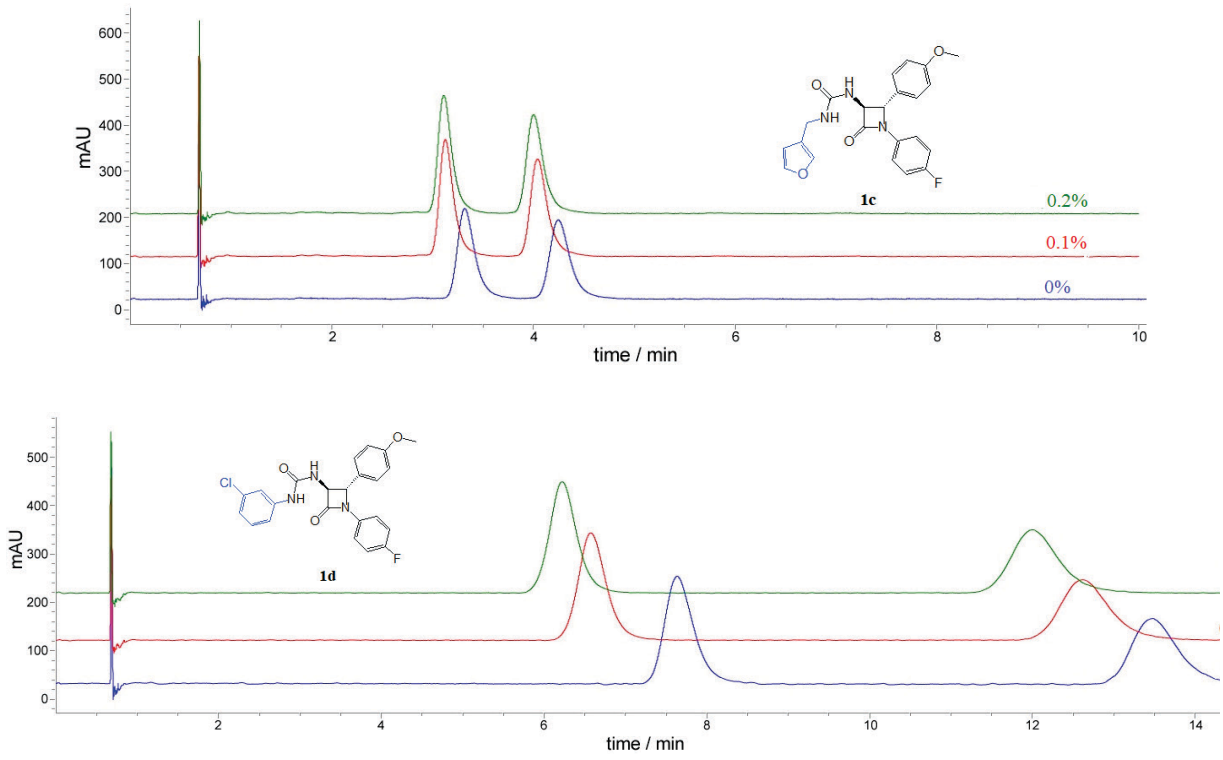


Figure 4. Influence of isopropylamine volume fraction in the mobile phase on enantioseparation of (±)-*trans*-β-lactam ureas **1c** and **1d** on Chirallica PST-10 column.

The Effect of Alcohol Modifier on Enantioseparation

In this work, the influence of the type of alcohol modifier (methanol, ethanol and propan-2-ol) on the separation of enantiomers of (±)-*trans*-β-lactam urea **1a–g** was also investigated, Table 3. It is known that the higher order structure of the polysaccharide stationary phases varies depending on the type of alcohol modifier used in the mobile phase.[24] Ethanol and propan-2-ol affect the tertiary structure of the polysaccharide stationary phase differently by changing the size and shape of chiral cavities where 'accommodate' the enantiomers of an analyte. As a result of these changes, the chiral recognition of enantiomers with a chiral stationary phase is different.[25] The influence of the volume fraction (30 %) of methanol, ethanol or propan-2-ol in the mobile phase was examined. Changing the alcohol modifier affects the polarity of the mobile phase.[26] The Figure 3. shows the effect of alcohol modifiers on the separation of enantiomers of (±)-*trans*-β-lactam ureas **1b**, **1d** and **1f** on a Chirallica PST-10 column. It can be observed that by changing the alcohol modifier from methanol to ethanol, the R_s value increases for the enantiomers of compounds **1d** and **1f**, and decreases for compound **1b**. By replacing the mobile alcohol phase modifier from methanol to propan-2-ol, the R_s value increases for the enantiomers of compounds **1d** and **1f**, while decreases for the **1b**. Such a result indicates that the mechanism of chiral recognition depends on the polarity of the alcohol modifier and also on the type of substituent attached to the nitrogen atom of the ureido group of *trans*-β-lactam urea.

The Effect of Isopropylamine on Enantioseparation

Further examination of the influence of the volume fraction of additive, isopropylamine, in the mobile phase on the enantioseparation of (±)-*trans*-β-lactam ureas **1a–g** on a Chirallica PST-10 column, showed that chiral recognition

Table 5. Effect of the column temperature on enantioselectivity of (±)-*trans*-β-lactam ureas **1a-g**

(±)-*trans*-β-lactam urea	R	Column temperature (°C)	t_{R1} (min)	t_{R2} (min)	k_1	k_2	α	R_s
1a	hexyl	29	1.88	3.48	1.76	4.12	2.33	6.38
		32	1.82	3.28	1.70	3.87	2.27	6.28
		35	1.77	3.09	1.60	3.54	2.21	6.30
		38	1.70	2.91	1.52	3.32	2.18	6.11
		41	1.67	2.78	1.59	3.31	2.08	5.96
1b	4-phenylbutyl	29	3.98	7.80	4.85	10.47	2.16	7.23
		32	3.82	7.32	4.68	9.88	2.11	7.21
		35	3.66	6.83	4.38	9.04	2.06	7.38
		38	3.48	6.34	4.16	8.41	2.02	7.28
		41	3.37	6.00	4.22	8.30	1.97	7.27
1c	furfuryl	29	3.31	4.24	3.87	5.24	1.35	2.74
		32	3.18	4.05	3.73	5.02	1.35	2.79
		35	3.01	3.86	3.43	4.68	1.36	3.04
		38	2.86	3.66	3.24	4.43	1.37	3.07
		41	2.76	3.52	3.28	4.46	1.36	3.12
1d	3-chlorophenyl	29	7.31	14.58	9.75	20.44	2.10	7.37
		32	6.81	13.41	9.12	18.93	2.08	7.32
		35	7.63	13.48	10.22	18.82	1.84	6.89
		38	6.47	12.66	8.60	17.78	2.07	7.86
		41	5.77	11.36	7.95	16.61	2.09	8.09
1e	4-chlorophenyl	29	7.08	12.47	9.41	17.34	1.84	5.79
		32	6.76	11.83	9.04	16.58	1.83	5.99
		35	7.55	11.87	10.10	16.46	1.63	5.49
		38	6.43	11.28	8.54	15.74	1.84	6.31
		41	5.64	10.05	7.74	14.58	1.88	6.93
1f	3-chloro-4-methylphenyl	29	3.23	9.89	3.75	13.54	3.61	10.60
		32	3.10	9.21	3.61	12.68	3.52	10.69
		35	3.03	8.60	3.46	11.65	3.37	11.04
		38	2.90	8.00	3.30	10.87	3.29	11.23
		41	2.81	7.51	3.36	10.64	3.17	11.29
1g	3,5-*bis*(trifluoromethyl) phenyl	29	1.05	2.39	0.54	2.51	4.62	6.02
		32	1.04	2.29	0.55	2.40	4.41	6.18
		35	1.01	2.04	0.49	2.00	4.12	6.08
		38	1.01	2.19	0.50	2.25	4.51	6.18
		41	0.99	2.02	0.53	2.13	3.99	6.29

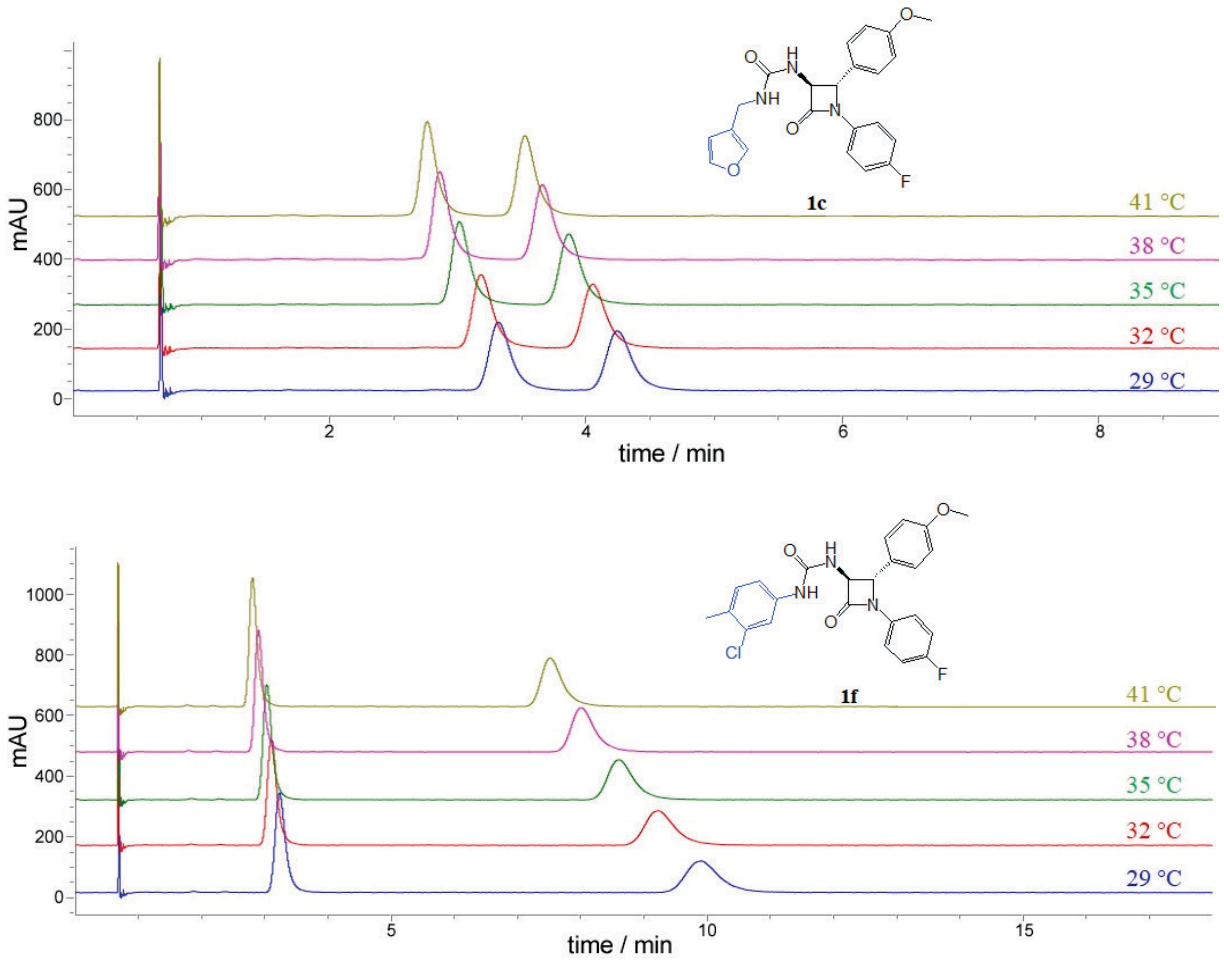


Figure 5. Influence of column temperature on the enantioseparation of (±)-*trans*-β-lactam ureas **1c** and **1f** on Chirallica PST-10 column.

depends on the type of substituent attached to the N atom of the ureido group and the volume fraction of isopropylamine in mobile phase, Table 4, Figure 4. In all examined cases the addition of isopropylamine revealed quite weak effect on enantioseparation without disturbing overall chromatographic parameters.

The Effect of Temperature on Enantioseparation

The influence of temperature on chiral separation was examined in the temperature range from 29 °C to 41 °C, Table 5, Figure 5. Based on the results obtained, it was determined that the influence of column temperature on the retention time of enantiomers R_t, separation factor α, and resolution R_s of enantiomers on used CSP is unpredictable and in dependance of supstituents attached to the N atom of the ureido group. The structures of *trans*-β-lactam ureas apparently have a strong influence on the thermodynamics of enantioselective adsorption of analytes on the chiral selector of the Chirallica PST-10 column.

The Effect of Backpressure on Enantioseparation

Furthermore, the effect of backpressure on the enantioseparation of (±)-*trans*-β-lactam urea **1a–g** was investigated in this study, Table 6, Figure 6. The pressure ranged from 11 MPa to 15 MPa. It can be observed that the separation factor remains almost unchanged with increasing backpressure in the system. The results show that the values of retention factors k_1 and k_2 for most *trans*-β-lactam ureas **1a–g** decrease with increasing back pressure in the system. This indicates that when the backpressure in the system increases, the enantiomers of compounds **1a–g** remain shorter time on the Chirallica PST-10 column. The increase of the backpressure in the system caused an increase of the density of the mobile phase, as well as an increase in the solvation ability, which led to a faster elution of the enantiomers from the column. At the tested backpressures, the value of the separation factor α and the resolution of the enantiomer R_s changed slightly. The enantiomers of compound **1g** bearing a 3,5-*bis* (trifluoromethylphenyl) group showed the best enantioselective recognition.

Table 6. Effect of the backpressure on enantioselectivity of (±)-*trans*-β-lactam ureas **1a–g**

(±)-*trans*-β-lactam urea	R	Backpressure (MPa)	t_{R1} (min)	t_{R2} (min)	k_1	k_2	α	R_s
1a	hexyl	11	1.77	3.09	1.60	3.54	2.21	6.30
		12	1.75	3.05	1.58	3.51	2.21	6.10
		13	1.74	3.03	1.55	3.44	2.22	6.13
		14	1.75	3.04	1.54	3.42	2.21	6.07
		15	1.74	2.99	1.51	3.31	2.19	6.10
1b	4-phenylbutyl	11	3.66	6.83	4.38	9.04	2.06	7.38
		12	3.61	6.73	4.33	8.94	2.06	7.29
		13	3.55	6.59	4.21	8.66	2.06	7.44
		14	3.60	6.69	4.23	8.72	2.06	7.22
		15	3.47	6.4	4.01	8.24	2.06	7.19
1c	furfuryl	11	3.01	3.86	3.43	4.68	1.36	3.04
		12	3.00	3.8	3.43	4.61	1.34	2.84
		13	2.97	3.76	3.35	4.51	1.35	2.83
		14	2.99	3.79	3.35	4.51	1.35	2.89
		15	2.92	3.69	3.21	4.32	1.35	2.81
1d	3-chlorophenyl	11	7.63	13.48	10.22	18.82	1.84	6.89
		12	6.40	12.48	8.45	17.43	2.06	7.39
		13	6.41	12.32	8.40	17.06	2.03	7.19
		14	6.39	12.19	8.29	16.72	2.02	7.09
		15	6.33	12.03	8.13	16.36	2.01	6.99
1e	4-chlorophenyl	11	7.55	11.87	10.10	16.46	1.63	5.49
		12	6.30	10.96	8.31	15.19	1.83	6.14
		13	6.31	10.83	8.25	14.88	1.80	5.96
		14	6.27	10.68	8.11	14.52	1.79	5.82
		15	6.21	10.53	7.96	14.19	1.78	5.75
1f	3-chloro-4-methylphenyl	11	3.03	8.6	3.46	11.65	3.37	11.04
		12	2.95	8.4	3.36	11.41	3.40	10.97
		13	2.92	8.3	3.28	11.17	3.40	10.95
		14	2.89	8.18	3.20	10.89	3.40	10.91
		15	2.93	8.15	3.23	10.76	3.33	10.97
1g	3,5-*bis*(trifluoromethyl) phenyl	11	1.01	2.04	0.49	2.00	4.12	6.08
		12	1.01	2.16	0.49	2.19	4.45	6.10
		13	1.00	2.12	0.47	2.11	4.52	5.92
		14	1.00	2.09	0.45	2.04	4.49	5.84
		15	1.00	2.08	0.44	2.00	4.52	5.79

CONCLUSION

Successful enantioseparation of (±)-*trans*-β-lactam ureas **1a–g** is achieved using Chirallica PST-10 column filled with *tris*-(4-methylphenylcarbamate) cellulose chiral selector under SFC conditions. This column is proved to be the most effective for separating the enantiomers of examined racemates, because the enantiomers of all (±)-*trans*-β-lactam ureas **1a–g** are efficiently separated on this column. Cellulose-based columns with adsorbed and immobilized selector, Chiralcel OD-3 and Chiralpak IB columns, proved to be more effective than corresponding amylose analogs, Chiralpak AD-3 and Chiralpak IA columns. The mechanism of chiral recognition, in addition to hydrogen bonds, is dominated by π–π interactions, dipole-dipole interactions and inclusion effects within chiral cavity. Chiral recognition largely depends on the type of substituent attached to the N1 atom of the ureido group, the type of chiral selector, and the polarity of the mobile phase. The influence of alcohol modifier on enantioseparation is mainly based on alcohol polarity, setting methanol as the best choice. The impact of three other examined parameters; temperature, addition of isopropylamine and backpressure, showed little or no influence on overall chromatographic process.

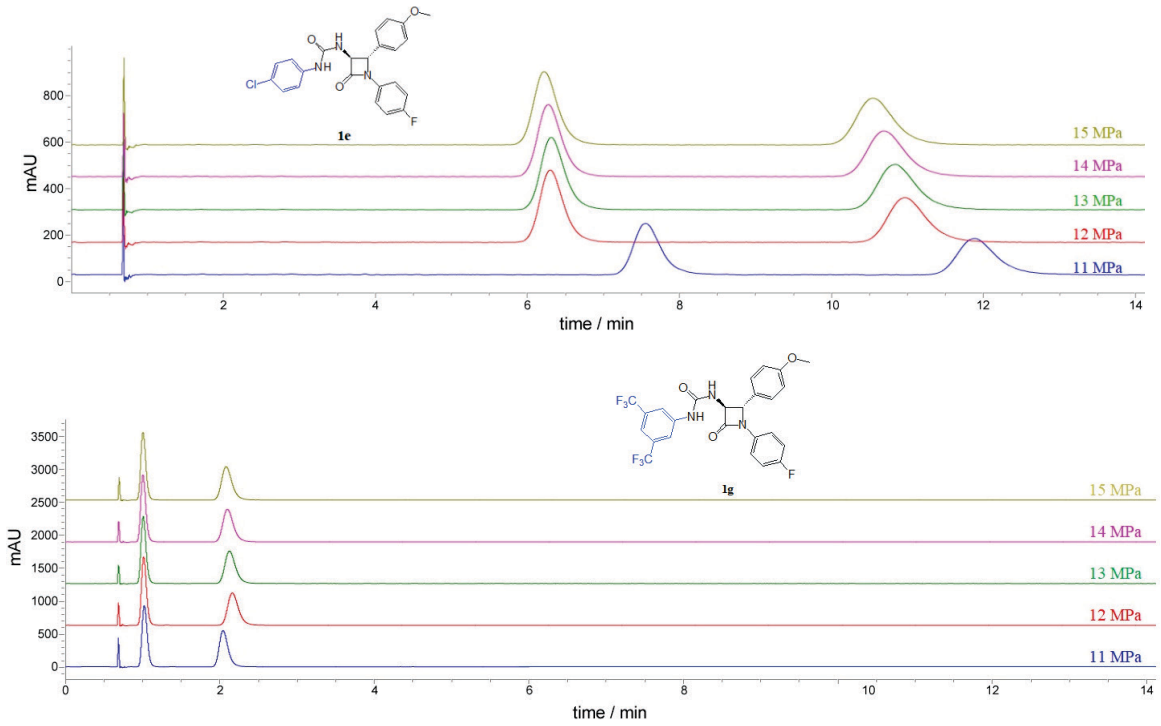


Figure 6. Influence of backpressure on the enantioseparation of (±)-*trans*-β-lactam ureas **1e** and **1g** on Chirallica PST-10 column.

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REFERENCES

[1] B. S. Sekhon, *Int. J. Pharmtech Res.* **2010**, *2*, 1595–1602.

[2] P. Gopaliya, P. R. Kamble, R. Kamble, C. S. Chauhan, *Int. J. Pharm. Sci. Rev. Res.* **2014**, *3*, 59–66.

[3] G. Kucerova, K. Kalikova, E. Tesarova, *Chirality* **2017**, *29*, 239–246. https://doi.org/10.1002/chir.22701

[4] M. M. Wong, W. B. Holzheuer, G. K. Webster, *Curr. Pharm. Anal.* **2008**, *4*, 101–105. https://doi.org/10.2174/157341208784246288

[5] N. Sethi, A. Anand, G. Jain, K. S. Srinivas, K. K. Chandrul, *Chron. Young Sci.* **2010**, *1*, 12–22.

[6] E. Lemasson, S. Bertin, C. West, *J. Sep. Sci.* **2016**, *39*, 212–233. https://doi.org/10.1002/jssc.201501062

[7] L. Laboureur, M. Ollero, D. Touboul, *Int. J. Mol. Sci.* **2015**, *16*, 13868–13884. https://doi.org/10.3390/ijms160613868

[8] J. Peach, J. Eastoe, *Beilstein J. Org. Chem.* **2014**, *10*, 1878–1895. https://doi.org/10.3762/bjoc.10.196

[9] G. M. Fassauer, R. Hofstetter, M. Hasan, S. Oswald, C. Mode, W. Siegmund, A. Link, *J. Pharm. Biomed. Anal.* **2017**, *146*, 410–419. https://doi.org/10.1016/j.jpba.2017.09.007

[10] E. Lessllier, *J. Chromatogr. A* **2009**, *1216*, 1881–1890. https://doi.org/10.1016/j.chroma.2008.10.081

[11] L. T. Taylor, *J. Supercrit. Fluids* **2009**, *47*, 566–573. https://doi.org/10.1016/j.supflu.2008.09.012

[12] T. Dražić, M. Roje, M. Jurin, G. Pescitelli, *Eur. J. Org. Chem.* **2016**, *24*, 4189–4199. https://doi.org/10.1002/ejoc.201600641

[13] T. Dražić, K. Molčanov, M. Jurin, M. Roje, *Synth. Commun*. **2017**, *47*, 764–770. https://doi.org/10.1080/00397911.2017.1283525

[14] P. D. Mehta, N. P. S. Sengar, A. K. Pathak, *Eur. J. Med Chem* **2010**, *45*, 5541–5560. https://doi.org/10.1016/j.ejmech.2010.09.035

[15] T. Dražić, M. Roje, *Chem. Heterocycl. Compd.* **2017**, *53*, 953–962. https://doi.org/10.1007/s10593-017-2156-z

[16] A. Kamath, I. Ojima, *Tetrahedron* **2012**, *68*, 10640–10664. https://doi.org/10.1016/j.tet.2012.07.090

[17] A. Jarrahpour, P. Shirvani, V. Sinou, C. Latour, J. M. Brunel, *Med. Chem. Res.* **2016**, *25*, 149–162. https://doi.org/10.1007/s00044-015-1474-x

[18] D-J. Fu, Y-F. Zhang, A-Q. Chang, J. Li, *Eur. J. Med. Chem.* **2020**, *201*, 112510. https://doi.org/10.1016/j.ejmech.2020.112510

[19] A. Bhalla, G. Modi, S. S. Bari, A. Kumari, D. Narula, S. Berry, *Tetrahedron Asymmetry* **2017**, *28*, 307–316. https://doi.org/10.1016/j.tetasy.2016.12.007

[20] K. De Klerck, D. Mangelings, Y. Vander Heyden, *J. Pharm. Biomed. Anal.* **2012**, *69*, 77–92. https://doi.org/10.1016/j.jpba.2012.01.021

[21] L. C. Harps, J. F: Joseph, M. K. Parr, *J. Pharm. Biomed. Anal.* **2019**, *162*, 47–59. https://doi.org/10.1016/j.jpba.2018.08.061

[22] V. Mehra, V. Kumar, *Tetrahedron Lett.* **2013**, *54*, 6041–6044. https://doi.org/10.1016/j.tetlet.2013.08.101

[23] M. N. Rebizia, K. Sekkouma, N. Belboukharia, A. Cheritib, H. Y. Aboul-Eneinc, *Egypt. Pharm. J.* **2016**, *15*, 88–97. https://doi.org/10.4103/1687-4315.190399

[24] S. Khater, C. West, *J. Chromatogr. A* **2014**, *1373*, 197–210. https://doi.org/10.1016/j.chroma.2014.11.033

[25] R. N. Rao, K. N. Kumar, B. S. Kumar, *J. Sep. Sci.* **2012**, *35*, 2671–2677. https://doi.org/10.1002/jssc.201200410

[26] J. Ding, M. Zhang, H. Dai, C. Lin, *Chirality* **2018**, *30*, 1245–1256. https://doi.org/10.1002/chir.23018