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Original Article

Performance comparison of chlorinated chiral stationary phases in supercritical fluid chromatography for separation of selected pyrrolidone derivatives

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ABSTRACT

The effects of two chlorinated chiral stationary phases, namely, Lux Cellulose-2 and Lux i-Cellulose-5, flow-rate, percentage of co-solvent and chemical structures of the compounds on retention and resolution were studied within this article. In this work a backpressure of 150 bar, a temperature of 40 °C and 10% of methanol as co-solvent were chosen as operating conditions. The optimum flow-rate was 2 mL/min. The percentage of co-solvent was studied between 7.5% and 15%. We have observed that 15% of methanol gave the best results for most of the compounds. For all the derivatives, the Lux Cellulose-2 provided better resolutions going from 1.50 to 3.59 compared with Lux i-Cellulose-5.

1. Introduction

Supercritical fluid chromatography (SFC) is an important technique in drug discovery related analysis due to its many advantages compared to the commonly used high-performance liquid chromatography (HPLC) technique. The environmental-friendly CO_2 based mobile phase, the increased efficiency together with the short analysis time (due to high flow-rate) and the lower operational costs give SFC benefits over many analysis techniques related to drug development [1,2], particularly in chiral separation where preparative scale remains its main application run by industrial actors more than academic ones [3]. Halogenated polysaccharide based CSPs originally developed by Chankvetadze et al. for HPLC [4–6] have entered the market in 2005. The coated chlorinated carbamate phases were deeply characterized [7] and have found applications [8–12] as their immobilized versions [13–16]. In 2018, there were only two studies concerning the use of cellulose tris(3-chloro-4-methylphenylcarbamate) chiral column [17,18] and articles dealing with cellulose tris(3,5-dichlorophenylcarbamate) [19–21] in SFC. To compare the separation ability of these two columns, a pyrrolidin-2-one family, new class of antibacterial compounds under evaluation, was chosen (Fig. 1). In the context of green chemistry, many efforts have been made in the valorification of natural resources and industrial waste. The pyroglutamic acid is a raw material, derived from sugar beet molasses. The pyroglutamic acid (**1**) and its derivatives present a chiral center and like a great number of pharmaceutical molecules, each enantiomer has its own pharmacological properties, thus underlining the importance of their separation. The pyrrolidin-2-one scaffold is well known as a privileged synthon for a broad range of heterocyclic compounds as well as for high potential to afford small compounds with interesting biological activities. Therefore, simple pyrrolidones are often fundamental parts of the structure of antimicrobial [22], antiviral [23] and antitumor compounds [24], as well as products that target the central nervous system [25], or other biological systems. Considering the fact that the manufacturer presented baseline

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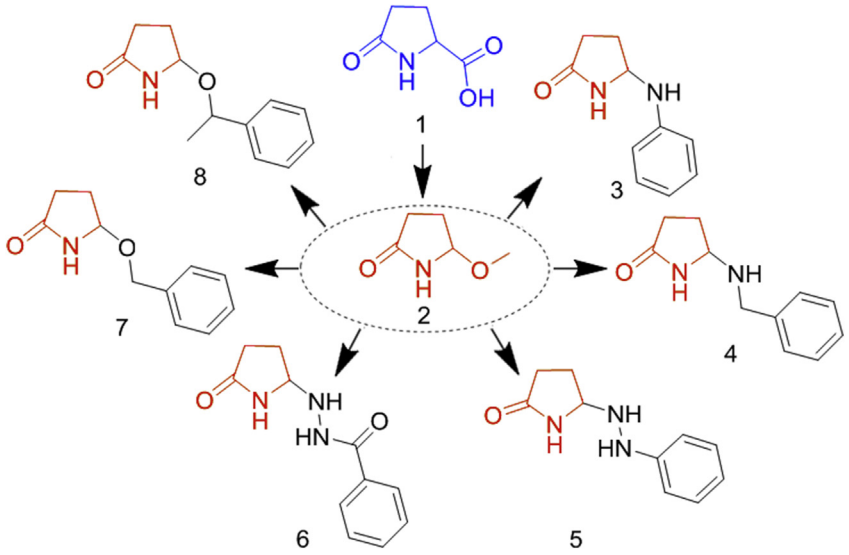

Fig. 1. Chemical structures of pyroglutamic acid (**1**), pyrrolidin-2-one (**2**) and antibacterial derivatives (**3** to **8**).

separation of thalidomide on the Lux Cellulose-2, and chlorthalidone on the Lux i-Cellulose-5, chemical compounds which show structural similarities with the pterolactame derivatives were used in this study, we expected a similar enantioseparation ability for our analytes. Therefore, the aim of this work was to compare the separation performances of each chiral selector, the influence of the flow-rate and percentage of methanol on retention and resolution.

2. Material and methods

2.1. Chemicals

The molecules used are 5-methoxy-2-pyrrolidone **2** derivatives (racemic compound **3**: 5-anilino-pyrrolidin-2-one, racemic compound **4**: 5-(benzylamino)pyrrolidin-2-one, racemic compound **5**: 5-(2-phenylhydrazino)pyrrolidin-2-one, racemic compound **6**: N'-(5-oxopyrrolidin-2-yl)benzohydrazide, racemic compound **7**: 5-(benzyloxy)pyrrolidin-2-one, racemic compound **8**: 5-(1-phenylethoxy)pyrrolidin-2-one), synthesized in the frame of this project. Unlike compounds **1** to **7** bearing a single asymmetric center, compound **8** has two asymmetrical carbons and the complete separation of the four expected stereoisomers was also attempted on CSPs.

The used methanol, ethanol, isopropanol and acetonitrile were of HPLC grade and were purchased from VWR (Strasbourg, France). Carbon dioxide (CO_2) with purity of 99.995% was purchased from Linde (Saint-Priest, France).

2.2. Sample solutions

For analytical screening, solutions of the samples were prepared in methanol at 1 mg/mL. The solutions were always degassed by an ultrasonic bath and filtered on a 0.45 m PTFE syringe-filter (15 mm diameter) prior to use.

2.3. Chiral supercritical fluid chromatography apparatus

2.3.1. Stationary phases

Two chiral analytical columns, Lux Cellulose-2 and Lux i-Cellulose-5, were used for this study and purchased from Phenomenex® (Le Pecq, France) having dimensions 250 mm × 4.6 mm i.d. with 5 µm or 3 µm particle size (Fig. 2). The silica gel of the Lux Cellulose-2 column is coated with cellulose tris(3-chloro-4-methylphenylcarbamate) chiral selector, and the silica gel of the Lux i-Cellulose-5 column is immobilized with cellulose tris(3,5-dichlorophenylcarbamate) chiral selector.

2.3.2. Chromatographic system and conditions

The chromatographic system used was an SFC-PICLAB hybrid 10–20 apparatus (PIC Solution, Avignon, France) equipped with an autosampler comprising a 48-vials plate (model Alias, Emmen, Netherlands), three model 40 P pumps: two for CO_2 and a third for the modifier (Knauer, Berlin, Germany), a column oven with a Valco ten-position column selection valve, and a Valco six-position solvent switching valve. The pump head used for pumping the CO_2 was cooled to −8 °C by a cryostat (model Minichiller, Huber, Offenburg, Germany). The system was also composed of a Smartline 2600 diode array detector (DAD) (Knauer, Berlin, Germany). After the detector, the outlet pressure was controlled by a back-pressure regulator (BPR). The outlet regulator tube was heated to 55 °C to avoid ice formation during the CO_2 depressurization. The system was controlled and the data were acquired with the SFC PicLab Analytic Online v.3.1.2 software and the data were processed with

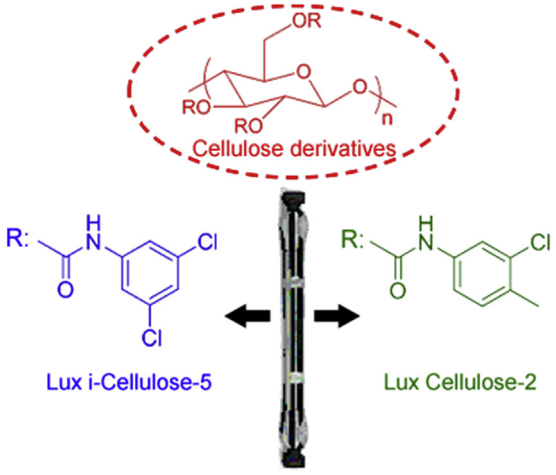

Fig. 2. Chemical structures of studied chiral stationary phases.

the Analytic Offline v.3.2.0 software (PIC Solution, Avignon, France). During the separation screening, the mobile phase was always CO_2-modifier mixtures with 20% of either methanol, ethanol, *iso*-propanol or acetonitrile and during the separation optimization, the mobile phase was always CO_2-modifier mixtures with the proportion of methanol ranging from 7.5% to 15%, the flow rate ranged between 2 and 4 mL/min. All analyses were run in isocratic mode. The column oven temperature was 40 °C and the outlet pressure was 150 bar. The wavelength was 210 nm and the injected volume was 20 μL.

2.3.3. Chromatographic parameters

The resolution factor from our study was calculated using $R_s = 2(t_{R2}\text{-}t_{R1})/(\omega_1+\omega_2)$, where t_{R1} and t_{R2} are the retention times of the peaks of enantiomers and ω_1 and ω_2 are the peak widths measured at the baseline between tangents drawn to the peak sides. The retention (or capacity) factor (k) is a mean of measuring the retention of an analyte on the chromatographic column for each enantiomer, k_1 and k_2 are calculated by $k = (t_R\text{-}t_0)/t_0$, where t_0 was measured using 1, 3, 5-tri-*tert*-butylbenzene (TTBB).

3. Results and discussion

3.1. Selection of stationary and mobile phases

Two columns packed with two types of chiral selectors Lux Cellulose-2 and Lux i-Cellulose-5 were examined for their separative performances towards six antibacterial compounds. In SFC and in HPLC as well, polysaccharide stationary phases play a crucial role in chiral separation. Thus the two columns were first tested at 4 mL/min with 10% of either acetonitrile, *iso*propanol, ethanol and methanol. As previously reported, aprotic solvent as acetonitrile leads to the highest retention time, followed by *iso*propanol and then ethanol for which almost all compounds were not separated. In terms of resolution and analysis time, best results were observed with methanol and are summarized for the six derivatives in Tables 1 and 2.

From these results it can be seen that compounds **7** and **8** are not separated on Lux i-Cellulose-5, CSP. In addition, on this column, three derivatives, *i.e* **3**, **4** and **5**, were only partially separated ($R < 1.5$). This behavior was also observed on Lux Cellulose-2 for compounds **3**, **5**, **7** and **8**. The effect of a decrease of the flow-rate on the resolution was further explored.

3.2. Flow-rate selection

Six different flow-rates were tested between 2 and 4 mL/min with step of 0.5 mL/min on the two columns. All the results are summarized in Tables 1 and 2. On Lux Cellulose-2 CSP, all the compounds benefited from this decrease of flow-rate leading to higher resolution values. For example, for pyrrolidone derivative **3**, the resolution value goes from 1.15 to 1.47 at 4 and 2 mL/min, respectively. Following the Purnell's equation resolution mainly depends on the efficiency value and for all the compounds, on the two CSPs, this parameter increased with a decreased flow-rate (Tables 1 and 2). However, this improvement was not sufficient for compounds **7** and **8**, for which resolution values were equal to 1.19 and 1.00 respectively at this lower flow-rate. It must be noticed that except for pyrrolidone derivative **6**, the resolution values obtained on Lux Cellulose-2 were higher than on Lux i-Cellulose-5. In addition, one could notice the unusual high values of retention factors for derivative **6** in comparison to other compounds, whatever the CSP was. This behavior can be explained by the presence of a supplementary carbonyl moiety on this structure inducing supplementary dipole-dipole interactions and hydrogen bonding with the CSP.

It is noteworthy to say that lower flow-rates (*i.e* 1.0 and 1.5 mL/min) were also tested, but led to large peaks and long retention time (data not shown). In order to improve further the resolution, particularly for derivatives **7** and **8**, the influence of the percentage

Table 1
Chromatographic parameters obtained for all compounds under different flow-rates with 10% of MeOH on Lux Cellulose-2, 5 μm.

Compound	Flow-rate (mL/min)	$t_{R1\ (min)}$	$t_{R2\ (min)}$	k_1	k_2	α	R_s	N
3	2	28.42	31.5	17.90	19.94	1.11	1.47	3643
	2.5	24.25	28.41	20.29	23.94	1.18	1.97	2899
	3	19.93	23.44	19.76	23.42	1.19	1.88	2517
	3.5	16.85	19.79	19.06	22.57	1.18	1.67	2012
	4	15.32	17.22	19.27	21.79	1.13	1.15	1731
4	2	24.88	29.79	15.54	18.81	1.21	2.83	4703
	2.5	19.36	23.26	16.00	19.42	1.21	2.72	4220
	3	16.05	19.33	15.72	19.13	1.22	2.44	3320
	3.5	13.58	16.35	15.17	18.47	1.22	2.16	2599
	4	11.80	14.19	14.62	17.78	1.22	1.87	1976
5	2	11.04	12.53	6.34	7.33	1.16	1.71	3312
	2.5	8.69	9.84	6.63	7.64	1.15	1.51	2670
	3	7.21	8.19	6.51	7.53	1.16	1.42	2256
	3.5	6.03	6.89	6.19	7.20	1.16	1.34	1894
	4	5.39	6.09	6.14	7.07	1.15	1.15	1593
6	2	50.31	68.64	32.46	44.64	1.38	3.59	2895
	2.5	38.42	52.24	32.73	44.86	1.37	3.47	2754
	3	31.50	43.22	31.82	44.02	1.38	3.32	2402
	3.5	26.53	36.31	30.58	42.23	1.38	3.05	2049
	4	22.73	31.34	29.08	40.46	1.39	2.88	1761
7	2	8.15	8.92	4.42	4.93	1.12	1.19	3063
	2.5	6.39	7.01	4.61	5.15	1.12	1.16	2793
	3	5.33	5.84	4.55	5.08	1.12	1.04	2277
	3.5	4.54	4.97	4.40	4.91	1.12	0.89	1702
	4	3.93	4.29	4.20	4.68	1.11	0.79	1398
8	2	11.36	12.5	6.55	7.31	1.12	1.00	1913
	2.5	8.92	9.92	6.84	7.71	1.13	0.95	1447
	3	7.44	8.26	6.75	7.60	1.13	1.01	1671
	3.5	6.31	6.99	6.52	7.33	1.12	1.08	1974
	4	5.49	6.05	6.26	7.01	1.12	0.97	1717

Table 2
Chromatographic parameters obtained for all compounds under different flow-rates with 10% of MeOH on Lux i-Cellulose-5, 5 μm.

Compound	Flow-rate (mL/min)	$t_{R1\ (min)}$	$t_{R2\ (min)}$	k_1	k_2	α	R_s	N
3	2	29.06	32.19	18.76	20.90	1.11	1.50	3770
	2.5	22.62	25.23	17.85	20.03	1.12	1.30	2516
	3	18.49	20.56	17.07	19.09	1.12	1.14	2057
	3.5	15.57	17.15	16.49	18.27	1.11	0.97	1764
	4	13.34	14.80	20.52	22.87	1.11	0.96	1521
4	2	24.34	27.89	15.61	18.04	1.16	1.80	3183
	2.5	19.15	21.92	14.96	17.27	1.15	1.46	2133
	3	15.61	17.98	14.26	16.58	1.16	1.31	1577
	3.5	13.04	14.86	13.66	15.70	1.15	1.10	1297
	4	11.21	12.83	17.08	19.69	1.15	1.05	1109
5	2	11.93	13.81	7.14	8.42	1.18	1.84	2934
	2.5	9.38	10.82	6.81	8.02	1.18	1.68	2509
	3	7.65	8.85	6.48	7.65	1.18	1.59	2211
	3.5	6.56	7.56	6.37	7.49	1.18	1.41	1828
	4	5.64	6.49	8.09	9.46	1.17	1.24	1434
6	2	59.53	84.85	39.63	56.92	1.44	4.79	4120
	2.5	45.82	67.78	37.18	55.48	1.49	4.68	3338
	3	37.06	54.68	35.22	52.45	1.49	4.12	2614
	3.5	30.53	45.21	33.29	49.80	1.50	3.76	2142
	4	26.28	39.15	41.38	62.14	1.50	3.43	1741
7	2	7.42	–	4.07	–	–	–	–
	2.5	5.93	–	3.94	–	–	–	–
	3	4.88	–	3.76	–	–	–	–
	3.5	4.09	–	3.59	–	–	–	–
	4	3.56	–	4.74	–	–	–	–
8	2	11.15	–	6.61	–	–	–	–
	2.5	8.79	–	6.32	–	–	–	–
	3	7.21	–	6.04	–	–	–	–
	3.5	6.05	–	5.79	–	–	–	–
	4	5.28	–	7.51	–	–	–	–

of methanol with a flow-rate conserved at 2 mL/min was explored on the Lux Cellulose-2.

3.3. Percentage of methanol in the mobile phase

In a general manner, in SFC the modifier plays a key role and its effects are numerous: i) the organic solvent changes the polarity of the mobile phase, ii) it changes the density of the mobile phase, particularly when pressure and temperature conditions are such that fluid compressibility is high, that is to say, close to the critical point and when the fluid is more gas-like, iii) the solvent changes the polarity and possibly the three dimensional structure of the CSP through its extensive adsorption on the CSP surface [26]. In SFC, the percentage of co-solvent affects very strongly the retention time and then the resolution. The percentage of methanol varied between 7.5% and 15% by step of 2.5%. All results are summarized in Table 3. An increase in co-solvent proportion generally leads to a decrease of the retention factors. This behavior is observed in Table 3. In addition, one can see that the decrease in retention factor is more and more diminished when the co-solvent percentage is increased: large variation in retention is observed at small percentages of modifier. For instance, k_1 goes from 34.38 to 15.54 when the percentage ranges from 7.5% to 10%, whereas further increase in modifier proportions causes less modification (k_1 varying from 8.42 to 5.49 when the percentage ranges from 12.5% to 15%) for compound **4**, this being a classical behavior in SFC. Best Rs/t_{R2} ratio depends on each compound. For derivatives **3**, **4** and **6**, the best resolutions in shorter analysis time were achieved with 15% of methanol and for **5** and **8** under 7.5% of co-solvent. All the five optimized chromatograms are presented in Fig. 3. Considering the separation of compound **7** on the Lux Cellulose-2 packed with the 5 μm particles, this latter is not fully baseline resolved.

Table 3
Chromatographic parameters obtained for all compounds at 2 mL/min under different modifier percentages on Lux Cellulose-2, 5 μm.

Compound	MeOH (%)	$t_{R1\ (min)}$	$t_{R2\ (min)}$	k_1	k_2	α	R_s
3	7.5	50.44	59.51	34.77	41.21	1.18	1.89
	10	28.42	31.5	19.30	21.50	1.11	1.47
	12.5	21.67	25.42	14.70	17.42	1.18	1.91
	15	16.26	18.81	10.87	12.73	1.17	2.83
4	7.5	41.75	50.77	28.61	35.01	1.22	1.87
	10	24.88	29.79	16.77	20.28	1.21	2.83
	12.5	17.53	20.96	11.70	14.19	1.21	1.90
	15	13.30	15.68	8.71	10.45	1.20	1.69
5	7.5	16.03	18.7	10.37	12.26	1.18	1.72
	10	11.04	12.53	6.89	7.95	1.15	1.61
	12.5	8.26	9.36	4.99	5.78	1.16	1.10
	15	6.67	7.54	3.87	4.50	1.16	1.09
6	7.5	–	–	–	–	–	–
	10	50.32	68.64	34.94	48.03	1.37	3.59
	12.5	31.44	42.71	21.78	29.95	1.37	2.97
	15	21.42	28.81	14.64	20.03	1.37	2.50
7	7.5	17.01	19.12	11.06	12.56	1.14	1.02
	10	11.36	12.5	7.11	7.93	1.11	1.00
	12.5	8.66	9.48	5.28	5.87	1.11	0.98
	15	6.96	7.55	4.08	4.51	1.11	1.06
8	7.5	11.74	13.2	7.33	8.36	1.14	1.79
	10	8.20	8.96	4.86	5.40	1.11	1.17
	12.5	6.38	7.03	3.62	4.09	1.13	1.10
	15	5.20	5.64	2.80	1.75	0.63	0.90

3.4. Reduction of the particle size effect on resolution

Since 2010, there were some studies concerning the use of chiral columns packed with smaller particles [27–33]. The mean diameter or particle size (d_p), of the spherical supports used for the stationary phase of a column is a physical dimension that has a significant impact on the performance of the column. Cellulose

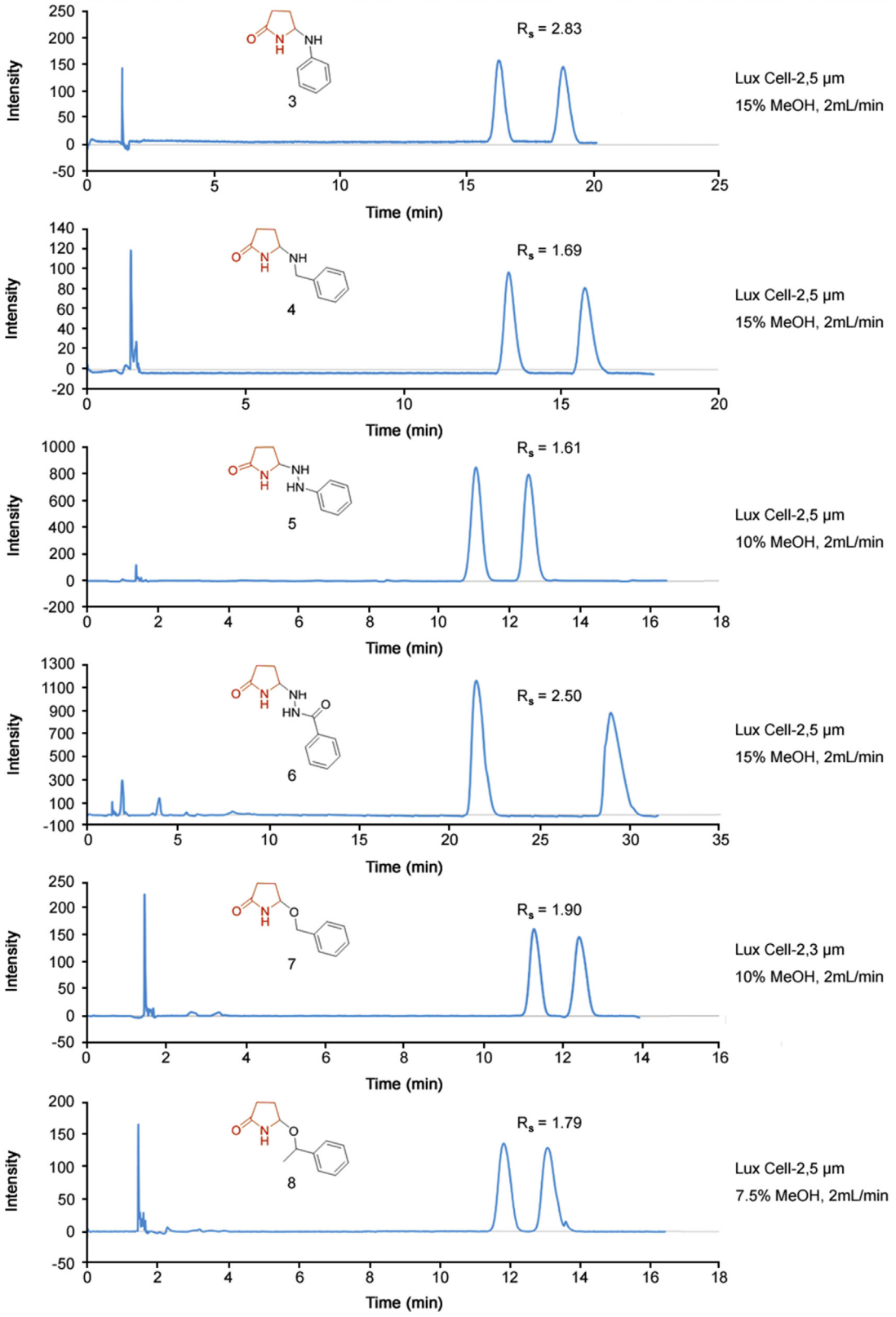

Fig. 3. Detailed chromatograms showing the best chromatographic performance for the six derivatives on Lux Cellulose-2 column with 5 or 3 µm particle size stationary at 2 mL/min; various percentages of methanol, temperature 40 °C, UV detection: 210 nm, $P_{out} = 150$ bar.

tris(3-chloro-4-methylphenylcarbamate) columns with 3 μm particle are now commercially available from different suppliers. Thus this particle size was tested to improve the separation of derivative **7**. It can be seen from Fig. 3 that the 3 μm stationary phase provides slightly faster analysis times and improved resolution equal to 1.90.

4. Conclusive remarks

The performance of Lux i-Cellulose-5 (5 μm) and Lux Cellulose-2 (3 and 5 μm), 250 mm × 4.6 mm columns with respectively an immobilized and a coated chlorinated polysaccharide stationary phases were evaluated towards six pyrrolidone derivatives, with respect to flow-rate and methanol concentration using supercritical fluid chromatography. The optimum linear velocity corresponded to a low flow-rate equal to 2 mL/min. The Lux Cellulose-2 (5 μm) CSP was found to separate the enantiomers of five derivatives out of six thanks to various percentages of methanol as a co-solvent. Concerning the last derivative **7**, the benefit of small particle (3 μm) leading to improved efficiency resulting in better chiral separation was highlighted in this experimental study. This study result is of practical significance for future separation of similar analytes.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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