Certificate

The stationary phase

YMC-Triart Diol

Dimension: 100 x 3 mm, 1.9 µm, 12 nm

P/N: TDN 12SP9-1003PT

has successfully been tested for supercritical fluid chromatography (SFC) separations

in the range of 0.5 mL to 4.0 mL/min flow rate
and 100-200 bar back pressure

Methodology

The stationary phase was tested for the applicability in SFC separations. An isocratic separation method was used to evaluate the retention of a set of 17 compounds. This generic separation method was not optimized for a most efficient separation of all compounds. An exemplary chromatogram is shown in Figure 1.

The results can be used to assess the retention characteristics and the selectivity of the stationary phase.

A subset of the tested compounds was used to gain information about the influence of mobile phase flow rate and back pressure on retention and selectivity. The results are summarized in Figure 2 and 3 and indicate optimal flow rate ranges at different back pressures.

Retention

Tested conditions:

Isocratic carbon dioxide vs. isopropanol (85/15)

Temperature: 40°C

Back pressure: 130 bar

Flow rate: 2 mL/min

Number of tested compounds: 17

t_0 = 0.31 min

Retention factor: $k' = \frac{t_R - t_0}{t_0}$

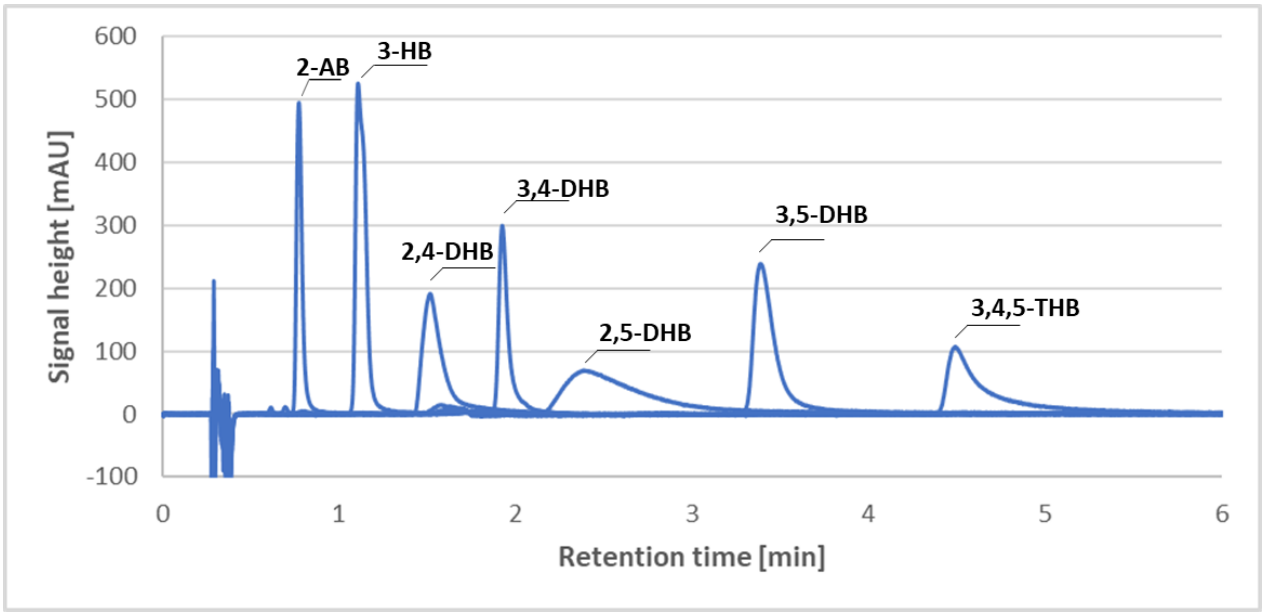

Figure 1: Chromatogram of 7 of the 17 compounds, which were tested under isocratic conditions. The chromatographic conditions were not optimized.

Name	Compound	CAS	log D (pH 5.5)	Retention time [min]	k'
Propylparaben	PP	94-13-3	2.87	0.517	0.67
Methylparaben	MP	99-76-3	2.14	0.639	1.06
2-hydroxybenzoic acid	2-HB	69-72-7	-0.56	0.734	1.37
2-aminobenzoic acid	2-AB	118-92-3	0.39	0.772	1.49
Vanillic acid	VAN	121-34-6	0.16	0.941	2.04
3-hydroxybenzoic acid	3-HB	99-06-9	-0.03	1.108	2.57
Syringic acid	SYR	530-57-4	-0.06	1.129	2.64
4-hydroxybenzoic acid	4-HB	99-96-7	0.51	1.333	3.30
2,4-dihydroxybenzoic acid	2,4-DHB	89-86-1	-1.10	1.516	3.89
2,3-dihydroxybenzoic acid	2,3-DHB	303-38-8	-1.47	1.613	4.20
4-aminobenzoic acid	4-AB	150-13-0	0.15	1.784	4.75
3,4-dihydroxybenzoic acid	3,4-DHB	99-50-3	-0.29	1.924	5.21
2,5-dihydroxybenzoic acid	2,5-DHB	490-79-9	-1.42	2.386	6.70
3,5-dihydroxybenzoic acid	3,5-DHB	99-10-5	-0.74	3.388	9.93
3-amino-,4-hydroxybenzoic acid	3-A,4-HB	1571-72-8	-0.12	3.591	10.58
3,4,5-trihydroxybenzoic acid	3,4,5-THB	149-91-7	-0.85	4.488	13.48
3,4-diaminobenzoic acid	3,4-DAB	619-05-6	-0.27	6.814	20.98

Separation efficiency and selectivity

Tested conditions: Isocratic carbon dioxide vs. isopropanol (85/15)

Temperature: 40°C

Back pressure: 100, 150 and 200 bar

Flow rate: 0.5 to 4 mL/min

Tested compounds:

Compound	Name	CAS	log D (pH 5.5)
3-hydroxybenzoic acid	3-HB	99-06-9	-0.03
3,4-dihydroxybenzoic acid	3,4-DHB	99-50-3	-0.29
3,5-dihydroxybenzoic acid	3,5-DHB	99-10-5	-0.74
3,4,5-trihydroxybenzoic acid	3,4,5-THB	149-91-7	-0.85

Plate number: $N = 5.54\,(\frac{t_R}{w_{1/2}})^2$

Selectivity: $\alpha = \frac{k'_2}{k'_1}$

Highest observed plate numbers:

Compound	Chromatographic conditions	Plate number
3-hydroxybenzoic acid	3.50 mL/min, 100 bar back pressure	2045
3,4-dihydroxybenzoic acid	1.25 mL/min, 200 bar back pressure	10233
3,5-dihydroxybenzoic acid	1.75 mL/min, 150 bar back pressure	10470
3,4,5-trihydroxybenzoic acid	0.75 mL/min, 150 bar back pressure	4235

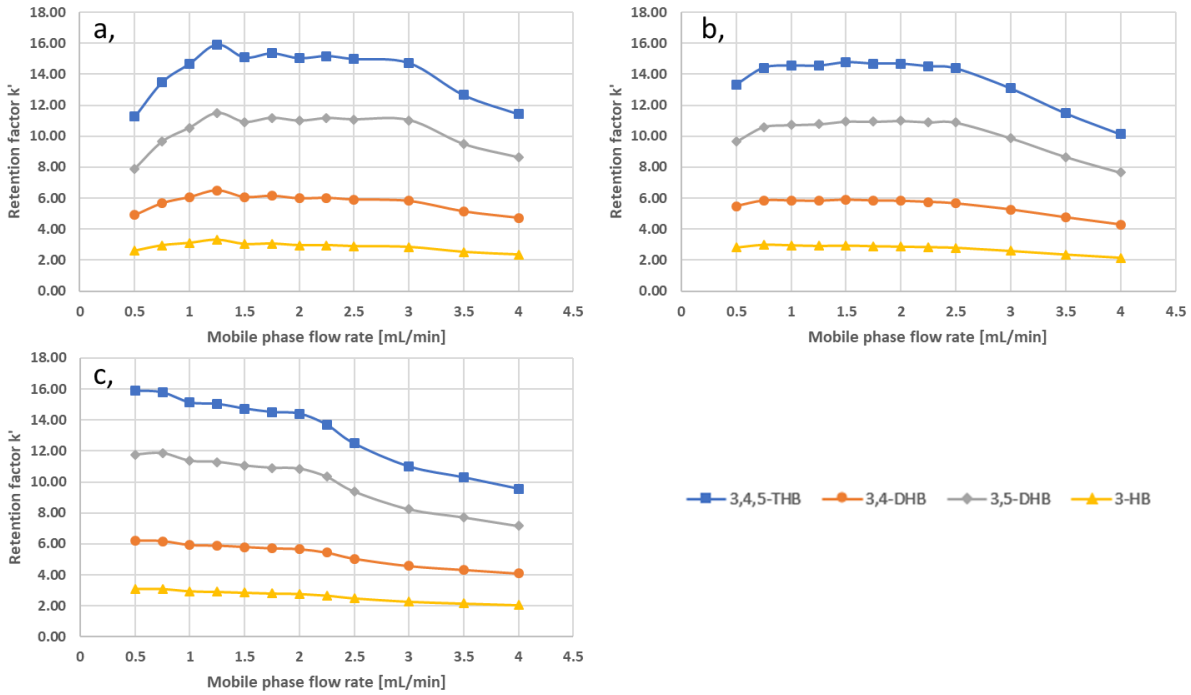

Figure 2: Retention dependency of mobile phase flow rate at different back pressure levels (a, 100 bar; b, 150 bar; c, 200 bar back pressure)

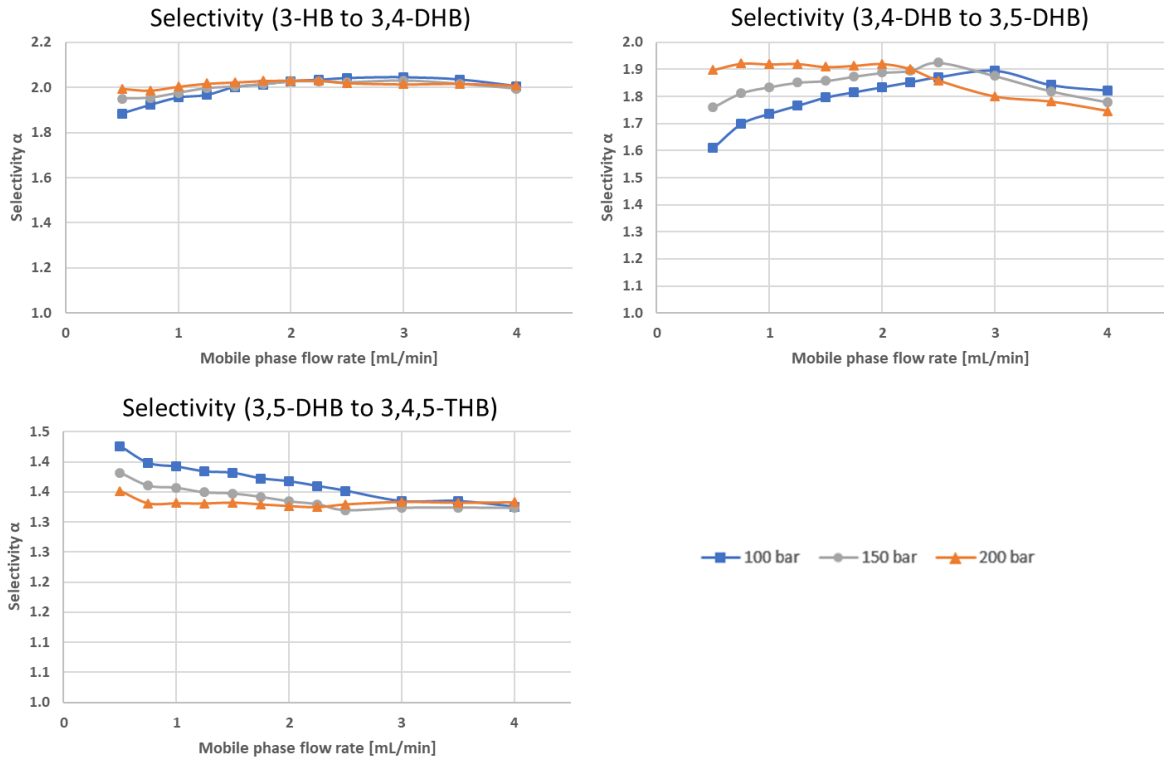

Figure 3: Selectivity dependency of mobile phase flow rate and back pressure level

Conclusions

The tested stationary phase is fully applicable in investigated flow rate range of 0.5 to 4.0 mL/min. All 17 investigated compounds could be retained under (unoptimized) isocratic conditions, using 15% isopropanol in the mobile phase.

The separation efficiency studies showed up to 10470 theoretical plates (unoptimized separation conditions).

The influence of the back pressure on the retention of compounds was investigated and optimal flow rate ranges were identified. At a back pressure of 100 bar, flow rates between 1 and 3 mL/min provide highest retention. The optimal flow rate range decreases with increasing back pressure to 1 to 2.5 mL/min for 150 bar and 0.5 to approximately 2 mL/min at 200 bar back pressure.

The substance specific influence of back pressure and flow rate on the selectivity was evaluated. It can be concluded that both, flow rate and back pressure impact the selectivity of the investigated stationary phase. As a consequence, both can be used in SFC method development for separation optimization.

The testing of this stationary phase was conducted in February 2019 by AFIN-TS GmbH

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