

SFC Separations with iHILIC-Fusion and iHILIC-Fusion(+) Columns

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Supercritical fluid chromatography (SFC), so-called “chromatography with carbon dioxide,” is one of the “green chromatographic” techniques that have been studied in the past decades. The high separation efficiency, combined with fast mobile phase gradients and a broad selectivity, make SFC an interesting complementary technique to liquid chromatography (LC) in many analytical fields. Recent studies also show that the range of separable compounds by SFC is significantly broader than that by reversed-phase LC (RPLC) or hydrophilic interaction liquid chromatography (HILIC) (1) alone. SFC can easily be used with UV and mass spectrometry (MS) detection to provide great benefits when aiming to separate compounds in complex samples. However, the retention mechanisms in SFC are more complex than in other LC modes (2). This often makes it necessary to screen different columns in method development and find the most suitable one for the given task. To compare the basic characteristics of various columns and evaluate the applicability for SFC, AFIN-TS GmbH has established a column screening model. Based on a generic separation method, columns can be investigated for their capability to retain and separate compounds with a broad range of polarity. The results from such a screening are provided in a specific certificate that is intended to give guidance when choosing columns for SFC method development.

In this study, iHILIC-Fusion and iHILIC-Fusion(+) from Hilicon were evaluated for SFC separations.

Experimental

A mixture of 114 standard compounds in a polarity range from logP -3.90 to +7.67 was used to evaluate the applicability of the columns for SFC. The final concentration for all compounds was 2 µmol/L in 50/50 (v/v) acetonitrile/H₂O solution. Please contact HILICON to get details on the used compounds.

SFC-MS/MS System: An Agilent 1260 Infinity I SFC system was used for the separations. The system consisted of a binary pump with solvent selector valve, an autosampler, a column oven with a column selection valve, a UV detector, and a back-pressure regulator. The SFC system was connected to an Orbitrap Exploris 120 MS (Thermo Fisher Scientific), which used a heated electrospray ionization (HESI) source with

Time (min)	B(%)
0	5
1	5
8	40
9	40
9.5	5
10	5

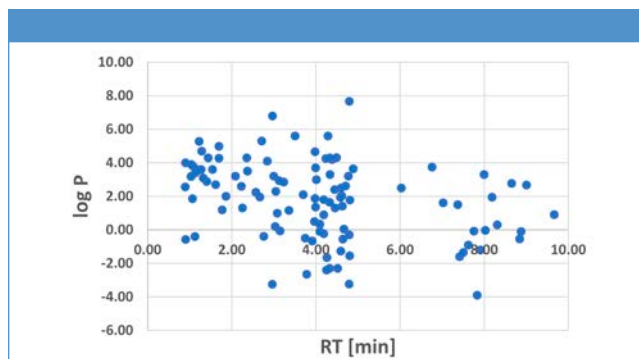


Figure 1: Retention time vs. logP plot of the analyzed standard compounds with the iHILIC®-Fusion column.

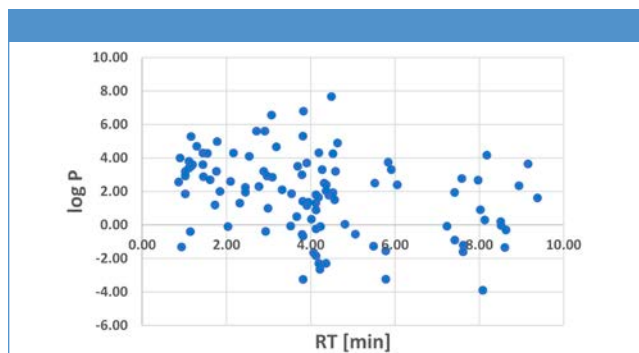


Figure 2: Retention time vs. logP plot of the analyzed standard compound with the iHILIC®-Fusion(+) column.

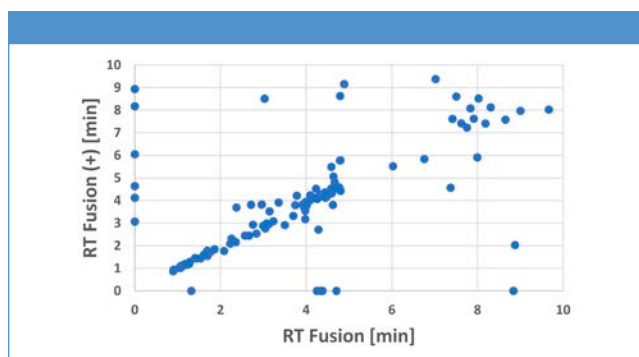


Figure 3: Orthogonality plot of the retention times of standard compounds with iHILIC®-Fusion and iHILIC®-Fusion(+) column.

positive/negative switching. The source and gas parameters were set as follows: ion spray voltage at +3.5 kV/ -3.0 kV, sheath gas flow rate at 50 arb units, aux gas flow at 8 arb units, ion transfer tube temperature at 320 °C, vaporizer temperature at 350 °C. Data

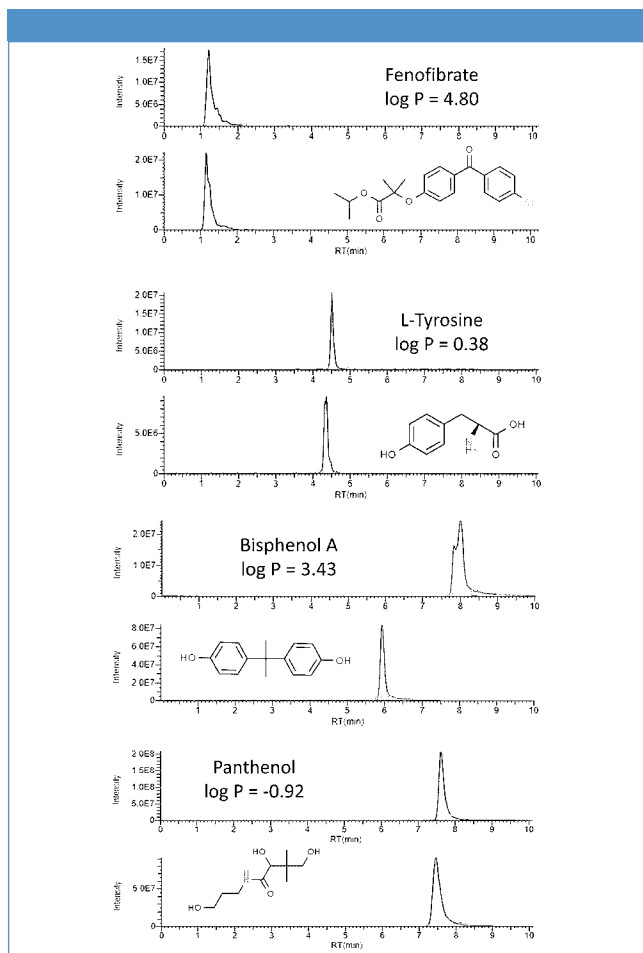


Figure 4: Extracted ion chromatograms of four standard compounds, separated with iHILIC-Fusion (top, trace with name) and iHILIC-Fusion(+) (bottom, trace with structure).

was acquired in full scan mode with data dependent MS2 acquisition.

SFC Separations: Columns: 1) 100×3 mm, 3.5µm, iHILIC®-Fusion (P/N 114.103.0310, HILICON) 2) 100×3 mm, 3.5 µm, iHILIC®-Fusion(+) (P/N104.103.0310, HILICON) Eluents: (A) Carbon dioxide (3.5 grade);(B) Isopropanol (gradient grade); Flow Rate: 1.50 mL/min; Column temperature: 40 °C; Back pressure: 100 bar; Injection volume: 10 µL.

Results and Discussion

Selectivity in SFC is driven by several factors, including not only mobile phase composition and stationary phase chemistry but also

the system pressure and temperature (2). To compare the columns, a generic separation method was used for screening and assessing their applicability in SFC separations. Figure 1 and Figure 2 show the retention time vs. logP plots of iHILIC-Fusion and iHILIC-Fusion(+) column, respectively. In contrast to RPLC, the hydrophobicity of compounds seems not to be the key parameter to dominate the retention in SFC. As a result, there is no strict correlation between retention time and logP.

When comparing the retention times of compounds on both iHILIC columns, which is shown in Figure 3, there is a linear correlation for many compounds. However, the iHILIC-Fusion(+) tends to show stronger retention for some later eluting compounds than the iHILIC-Fusion column. Furthermore, both columns could separate compounds exclusively (points on y- or x-axis, respectively), which indicates a certain degree of complementarity between these two types of columns. This could be helpful in method development where slight differences in selectivity can be used to optimize separations of critical peak pairs. The range of separable compounds with the investigated columns includes the whole spectra of tested compounds from polar to non-polar compounds. Figure 4 shows the extracted ion chromatograms of four exemplary compounds with the iHILIC columns.

Both iHILIC-Fusion and iHILIC-Fusion(+) are very well suitable for SFC separations. A broad range of separable compounds make both columns qualified for screening applications. In addition, the differences in selectivity between the iHILIC columns are valuable for specific SFC method developments.

References

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- (2) Lesellier, E.; West, C. The Many Faces of Packed Column Supercritical Fluid Chromatography – A Critical Review. *J. Chromatogr. A.* 2015, 1382, 2–46.



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