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Micro-flow Peptide Separations

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Introduction

Proteomics is the study of all proteins in an organism (proteome) or part of an organism. To study the proteome, Liquid Chromatography (LC) coupled to mass spectrometry (MS) has become the gold standard. Here, the role of LC is to provide the necessary resolution among peptides within a sample in order to be quantified or identified by MS.

An organism's proteome can be very complex. For example, a human cell contains about 10,000 protein producing coding genes, which in turn result in thousands of proteins with a myriad of different chemistries depending on their amino acid sequences and Post Translational Modifications (PTMs). In bottom-up proteomics, where an enzyme is used to digest the proteome prior to LC-MS analysis, understanding the chemistry of the sample being analyzed can be very beneficial. In this way, one can better choose the proper LC column phase chemistry to obtain the best resolution that provides the highest MS peptide identification and/or quantitation.

In this technical note, a mixture of 20 synthetic peptides with a wide mass and retention time range coverage (**Table 1**) was used to screen four micro-flow phase chemistries and gain insights into their peptide selectivity. A polar column that gives increased polar peptide retention, a biphenyl phase that provides aromatic selectivity, an F5 that gives aromatic plus electrostatic retention, and an XB-C18 with isobutyl side chains to improve peak shapes were used and behaved as expected.

Table 1. SCIEX PepCalMix Peptide Amino Acid Sequences Used and their Respective Monitored M+H Species.

Peptide Sequence	Analyte - m/z
AETSELHTSLK	408.5501+++
GAYVEVTAK	473.2602++
IGNEQGVSR	485.2530++
LDSTSIPVAK	519.7997++
AGLIVAEGVTK	533.3233++
LVGTPAEER	491.2656++
LGLDFDSFR	540.2734++
GFTAYYIPR	549.2863++
SGGLLWQLVR	569.8340++
AVGANPEQLTR	583.3136++
SAEGLDASASLR	593.8005++
VFTPLEVDVAK	613.3495++
VGNEIQYVALR	636.3527++
YIELAPGVDNSK	657.3450++
DGTFAVDGPGVIAK	677.8583++
YDSINNTEVSGIR	739.3615++
SPYVITGPGVVEYK	758.9105++
ALENDIGVPSDATVK	768.9034++
AVYFYAPQIPLYANK	589.3183+++
TVESLFPEEAETPGSAVR	643.6540+++

LC Conditions

Column: Kinetex™ 2.6 µm Biphenyl
Luna™ Omega 3 µm Polar C18
Kinetex 2.6 µm XB-C18
Kinetex 2.6 µm F5

Dimension: 150 x 0.3 mm

Part No.: 00F-4622-AC (Biphenyl)
00F-4760-AC (Polar C18)
00F-4496-AC (XB-C18)
00F-4723-AC (F5)

Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile

Gradient:

Time (min)	%B
0	2
60	40
60.1	80
65	80
65.1	2
70	2

Flow Rate: 10 µL/min

Injection: 1 µL

Temperature: 40 °C

Detector: Q Exactive™ Plus Orbitrap™

System: NanoLC™ 425 (SCIEX®)

Detection: MS

MS Conditions

Scan Type: Full MS SIM

Polarity: Positive

Resolution: 70,000

Scan Range: 400 to 1650 m/z

AGC Target: 3e6

Maximum IT: 100 ms

Results and Discussion

A 20 isotopically labeled peptide mix covering a wide range of retention times and masses was used to screen 4 different micro column chemistry phases (**Table 1**). First, a Kinetex™ 2.6 µm XB-C18 stationary phase, that employs core-shell technology to reduce peptide diffusion and silanol groups present on the stationary phase, gave narrower peaks and higher peak capacities than the rest of the screened columns (**Figure 1** and **Table 2**).

However, the first eluting peptide (IGNEQGVSR), which contains acidic groups like Glutamic Acid, was retained longer on a column with more polar character (**Figure 2**). In the case of peptides with aromatic character, the Kinetex Biphenyl column provided better resolution than the rest of the screened selectivities (**Figures 3-6**).

For example, when comparing AGLIVAEGVTK and YIELAPGVDNSK (**Figures 3** and **4**), YIELAPGVDNSK (with it's aromatic Tyrosine) was fully resolved using a Kinetex Biphenyl column as opposed to the Kinetex XB-C18 or other selecitivities. The same can be said of SGGLLWQLVR and AVYFYAPQIPLYANK (**Figures 5** and **6**). AVYFYAPQIPLYANK contains two Tyrosine and one Phenylalanine, making it more aromatic than the SGGLLWQLVR peptide. The ability to resolve aromatic rich peptides may prove useful in proteomics applications that deal with W,Y, F, and H rich proteins. Examples include breast cancer biomarkers, DNA/RNA-protein binding, carbohydrate-protein binding, and cation-π protein interactions.

Amino acid composition can vary widely and choosing a column can be more complicated than shown in the above examples. Therefore, testing more than one phase can be beneficial. For instance, in the case of the separation of GFTAYYIPR and DGTFAVDGPGVIAK (**Figures 7** and **8**), the Kinetex Biphenyl phase did not provide the best resolving power. The Kinetex F5 column provided the resolution needed to separate the more polar, charged, and aromatic peptide DGTFAVDGPGVIAK from GFTAYYIPR.

Figure 1. Extracted Ion Chromatogram (XIC) of PepCalMix Peptides on a Kinetex 2.6 µm XB-C18 column.

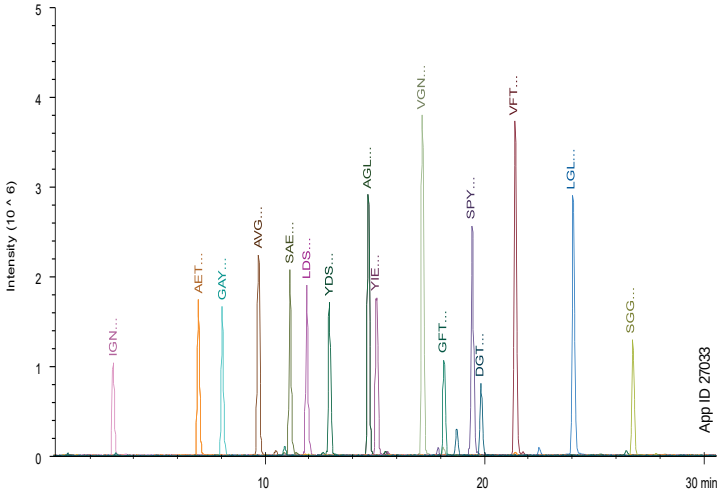


Table 2. Average Peak Widths and Peak Capacities for the 3 Kinetex Phases Used.

Column	Avg Peak Width	Peak Capacity
Kinetex 2.6 µm XB-C18	0.35	72
Kinetex 2.6 µm Biphenyl	0.42	68
Kinetex 2.6 µm F5	0.45	59

Figure 2. Retention Time Column Comparison Showing Retention Time Differences of Peptides when Using a Kinetex F5, a Kinetex Biphenyl, a Kinetex XB-C18 or a Luna™ Omega Polar Column.

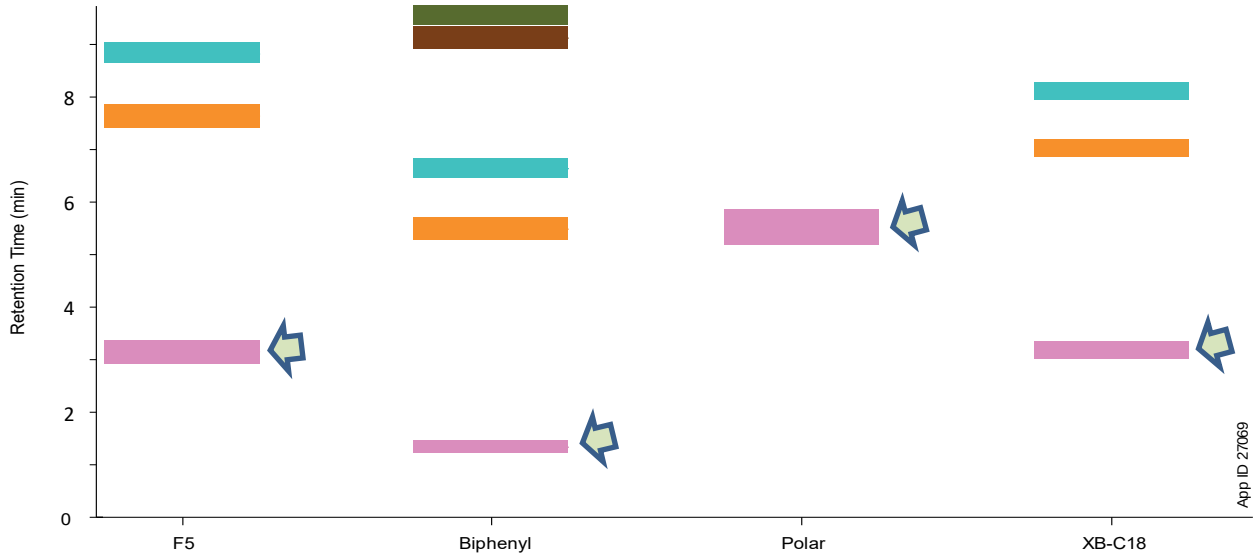


Figure 3. XIC of Peptides 1)AGLIVAEGVTK and 2)YIELAPGVDNSK on Kinetex™ 2.6 µm XB-C18 Column.

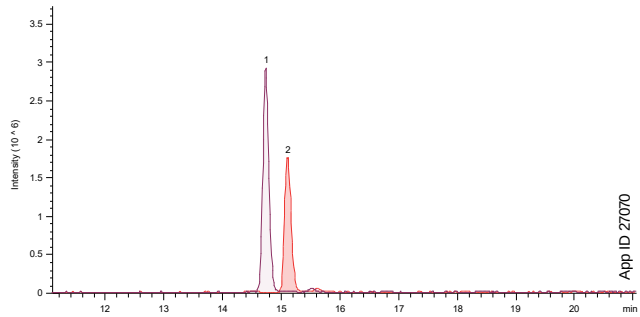


Figure 4. XIC of Peptides 1)AGLIVAEGVTK and 2)YIELAPGVDNSK on a Kinetex 2.6 µm Biphenyl Column.

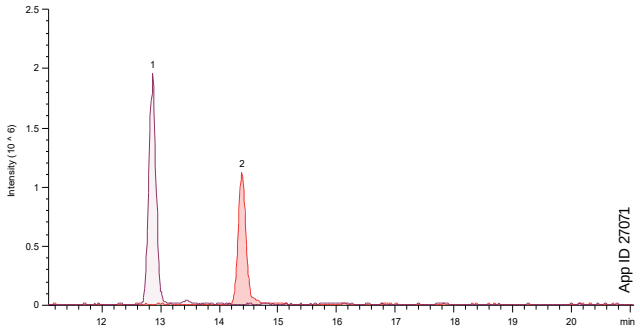


Figure 5. XIC of Peptides 1)SGGLLWQLVR and 2)AVYFYAPQIPLYANK on Kinetex 2.6 µm XB-C18 Column.

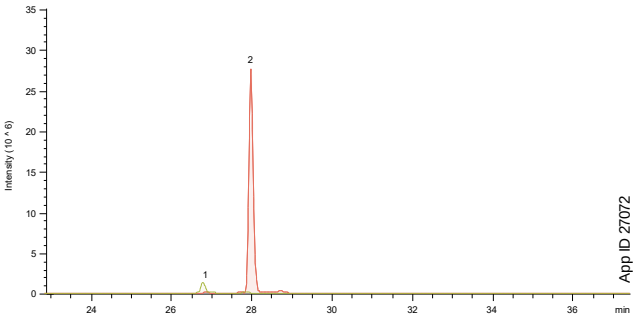


Figure 6. XIC of Peptides 1)SGGLLWQLVR and 2)AVYFYAPQIPLYANK on Kinetex 2.6 µm Biphenyl Column.

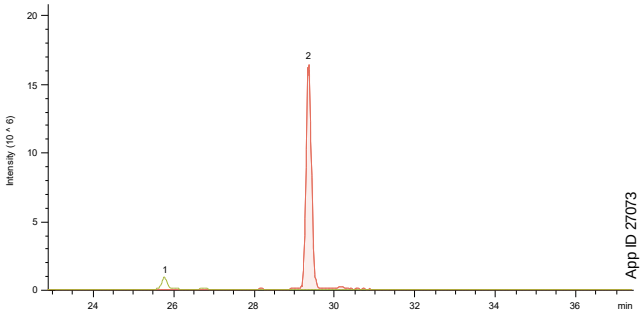


Figure 7. XIC of Peptides 1)GFTAYYIPR and 2)DGTFAVDGPGVIAK on Kinetex 2.6 µm Biphenyl Column.

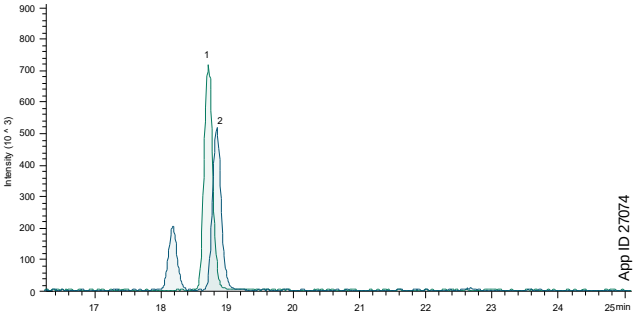
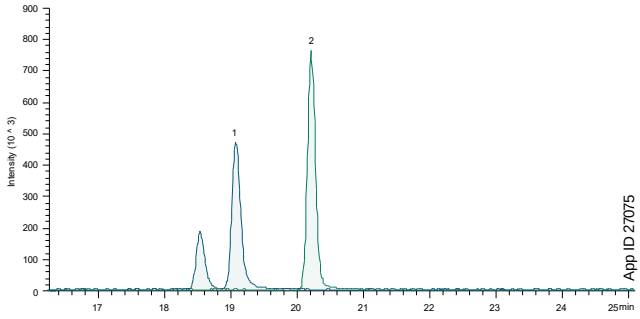


Figure 8. XIC of Peptides 1)DGTFAVDGPGVIAK and 2)GFTAYYIPR on Kinetex 2.6 µm F5 Column.



Conclusions

Proteomics samples are composed of thousands of proteins, and in the case of bottom-up proteomics, thousands of peptides. These peptides are made of many different amino acid sequences in addition to PTMs that give those peptides unique chemistries. In order to get the most out of an LC-MS workflow, the column phase chemistry should be carefully explored. As shown here, when dealing with early eluters, a polar column may be the best choice. In the case of aromatic peptides, a Biphenyl or an F5 column could provide the optimal resolution. When in doubt, an XB-C18 column can be a good place to start to obtain optimal peak shape and determine specific needs depending on the sample chemistry.

Kinetex™ Micro LC Columns Ordering Information

2.6 µm Micro LC Columns (mm)						
Part	Phases	30 x 0.3	50 x 0.3	100 x 0.3	150 x 0.3	50 x 0.5
XB-C18	00A-4496-AC	00B-4496-AC	00D-4496-AC	00F-4496-AC	00B-4496-AF	00F-4496-AF
Biphenyl	—	00B-4622-AC	—	00F-4622-AC	00B-4622-AF	—
C18	00A-4462-AC	00B-4462-AC	—	00F-4462-AC	00B-4462-AF	—
EVO C18	—	00B-4725-AC	—	00F-4725-AC	00B-4725-AF	—
F5	—	00B-4723-AC	00D-4723-AC	00F-4723-AC	00B-4723-AF	—

Luna ™ Omega Micro LC Columns Ordering Information

3 µm Micro LC Columns (mm)							Trap Column
Phases	50 x 0.30	100 x 0.30	150 x 0.30	50 x 0.50	100 x 0.50	150 x 0.50	20 x 0.3
Polar C18	00B-4760-AC	00D-4760-AC	00F-4760-AC	00B-4760-AF	00D-4760-AF	00F-4760-AF	—
PS C18	00B-4758-AC	00D-4758-AC	00F-4758-AC	00B-4758-AF	00D-4758-AF	00F-4758-AF	05M-4758-AC

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