How to choose the most suitable phenyl column
Remember, not all phenyl columns are equal

Even though most phenyl phases are classified with USP L11, they can show huge differences in modification and as a result, big differences in selectivity. The USP classification L11 only requires "phenyl groups chemically bonded to porous silica particles – 1.5–10 µm in diameter", which encompasses a broad range of phase modifications. This technical note will discuss the selectivity of 3 different phenyl phases on the basis of their separation of peptides.

The following three phases will be compared:
- YMC-Triart Phenyl
  (hybrid silica base particle with a butyl linker)
- YMC-Pack Ph (silica based without linker)
- Luna Phenyl-Hexyl (silica based with a hexyl linker)

Table 1: Specifications of phenyl phases with differences in modification.

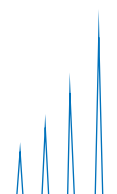|	YMC-Triart Phenyl	YMC-Pack Ph	Luna Phenyl-Hexyl
Base	organic/inorganic silica	silica	silica
Linker	butyl	none	hexyl
Particle size [µm]	1.9, 3, 5	3, 5	3, 5
Pore size [nm]	12	12	10
Specific surface area [m²/g]	360	330	400
Carbon content [% C]	17	9	17.5
pH range	1–10	2.0–7.5	1.5–9
Temperature limit [°C]	50	50	60*
End capping	multi-stage	standard	standard

*dependent on running parameters

From the specifications in the table above a different separation behaviour is to be expectable. The different base particles as well as linkers can lead to an alternate weighting between hydrophobic and π–π interactions. With a longer linker the hydrophobic interactions might outweigh the π–π interactions.

The analysis of five peptides shows that the three tested phenyl phases provide very different selectivities.

The following peptides sorted by elution order were tested:
- Oxytocin (1)
- Met-Enkephalin (2)
- Leu-Enkephalin (3)
- Neurotensin (4)
- γ-Endorphin (5)

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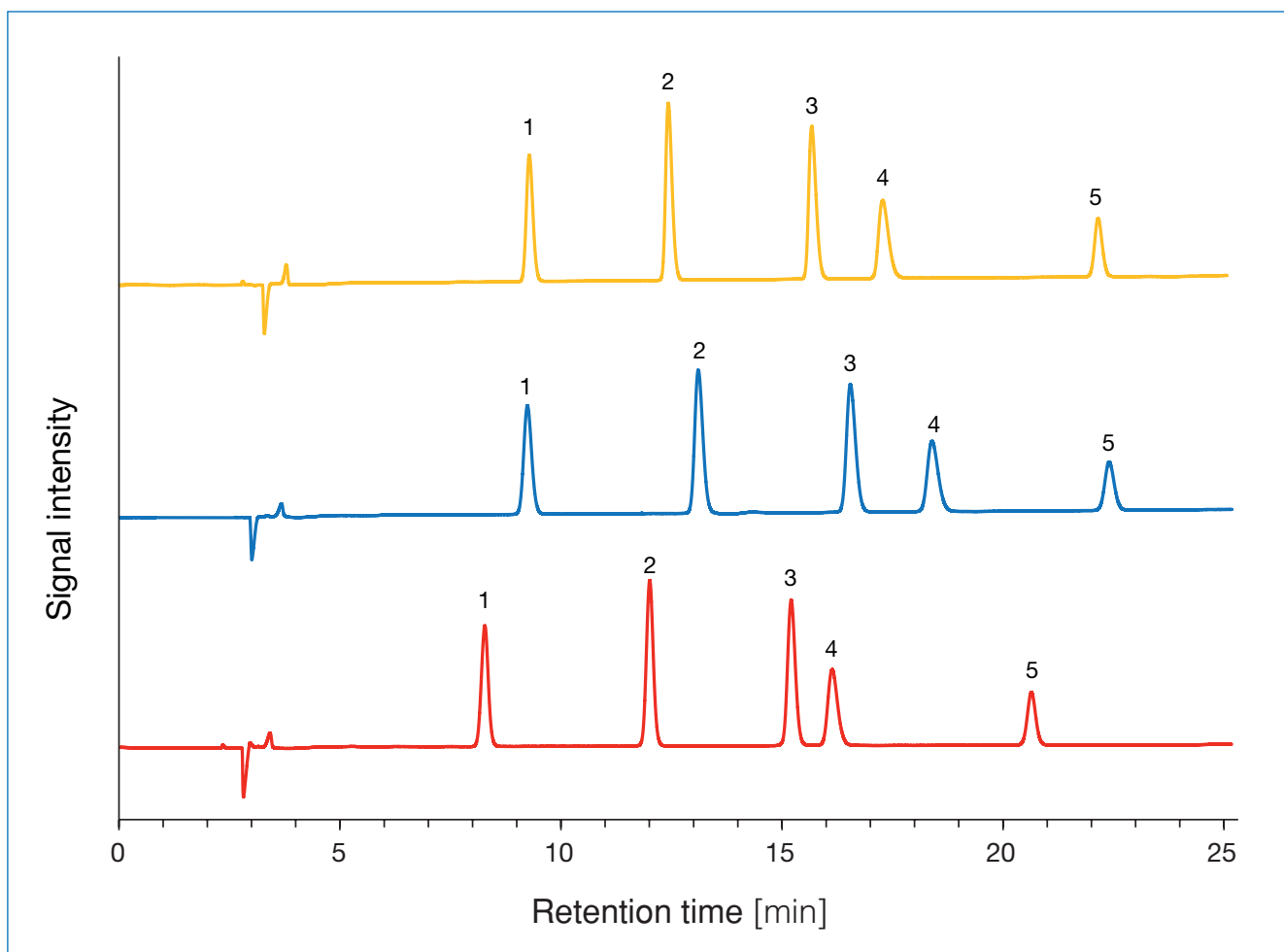
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Figure 1: Separation of 5 peptides using YMC-Pack Ph (yellow), YMC-Triart Phenyl (blue) and Luna Phenyl-Hexyl (red).

Table 2: Chromatographic conditions.

Columns:	YMC-Triart Phenyl (5 µm, 12 nm) 250 x 4.6 mm ID
	YMC-Pack Ph (5 µm, 12 nm) 250 x 4.6 mm ID
	Luna Phenyl-Hexyl (5 µm, 10 nm) 250 x 4.6 mm ID
Part Nos. (YMC):	TPH12S05-2546PTH
	PH12S05-2546WT
Eluents:	A) Water + 0.1 % TFA
	B) Acetonitrile + 0.1 % TFA
Gradient:	20–40 % B (0–5 min)
Flow rate:	1 mL/min
Temperature:	25 °C
Injection:	10 µL
Sample:	0.167 mg/mL per peptide dissolved in water
Detection:	UV at 220 nm

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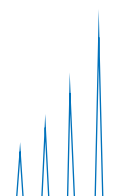
Table 3: Separation factor α

Peak pair	YMC-Triart Phenyl	YMC-Pack Ph	Luna Phenyl-Hexyl
1,2	1.56	1.48	1.63
2,3	1.32	1.33	1.33
3,4	1.13	1.12	1.07
4,5	1.25	1.34	1.33

Table 4: Resolution Rs

Peak pair	YMC-Triart Phenyl	YMC-Pack Ph	Luna Phenyl-Hexyl
1,2	11.3	11.4	12.8
2,3	9.4	11.2	10.4
3,4	4.4	4.4	2.5
4,5	9.4	13.1	11.9

The figure above shows that all three phases are suitable for the separation of these peptides, but clear differences can be observed. YMC-Pack Ph shows the best overall selectivity and resolution with sharp peaks, whereas YMC-Triart Phenyl shows slightly higher retention for peaks 2 to 4. Regarding the critical peaks 3 and 4 the lowest resolution is obtained using Luna Phenyl-Hexyl. Furthermore the column shows lower retention for all peptides even though the higher specific surface area in combination with the high carbon load could lead to the assumption of higher retention. But for these peptides hydrophobicity seems to have only a limited impact on retention.

For this application the use of a phase where π–π interactions predominate seems to be the best solution as the peptides with aromatic residues give longer retention on YMC-Pack Ph and YMC-Triart Phenyl which leads to the assumption that phenyl columns without or with a shorter linker are more suitable for the separation of peptides with more aromatic residues.

In general, it is a good idea to choose a phase where hydrophobic and π–π interactions are balanced, such as YMC-Triart Phenyl, which also generated excellent results for this separation.

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