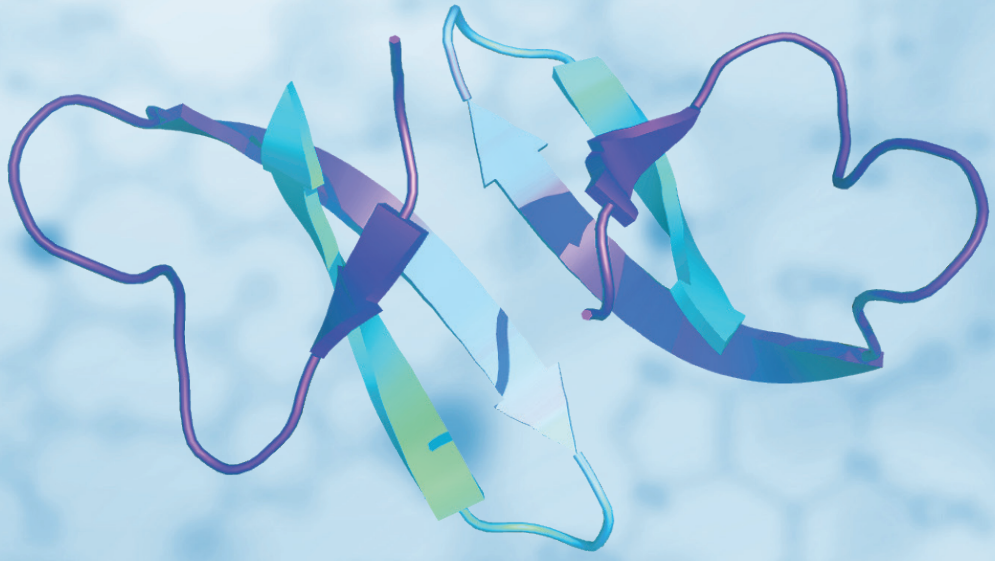
Tips for Peptide Separation



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Tips for Peptide Separation

The separation of very similar peptides often represents a challenge to RP-LC method development. In this case study the effects of two major method parameters are introduced:

- **Temperature**
- **Mobile Phase**

The following example shows defensin peptides, which play an important role in defending micro biotic organisms in animals and plants. The three selected defensins are very similar as they differ only in one amino acid.

α-Defensin-1: MW 3,442	**A**	**CYCRIPACIAGERRYGTCIYQGRLWAFCC**
α-Defensin-2: MW 3,371		**CYCRIPACIAGERRYGTCIYQGRLWAFCC**
α-Defensin-3: MW 3,486	**D**	**CYCRIPACIAGERRYGTCIYQGRLWAFCC**

Difference in amino acid residue on N-terminal

Even a suitable UHPLC C18 column is not able to separate these three forms from each other at standard conditions. A method optimisation is required.

Column	YMC-Triart C18, (1.9 µm, 12 nm) 50 x 2.0 mm ID
Part No.	TA12SP9-0502PT
Eluent	A) Water / TFA (100/0.1) B) Acetonitrile / TFA (100/0.1) 25-45%B (0-5 min)
Flow rate	0,4 mL/min
Detection	220 nm
Temperature	40 °C

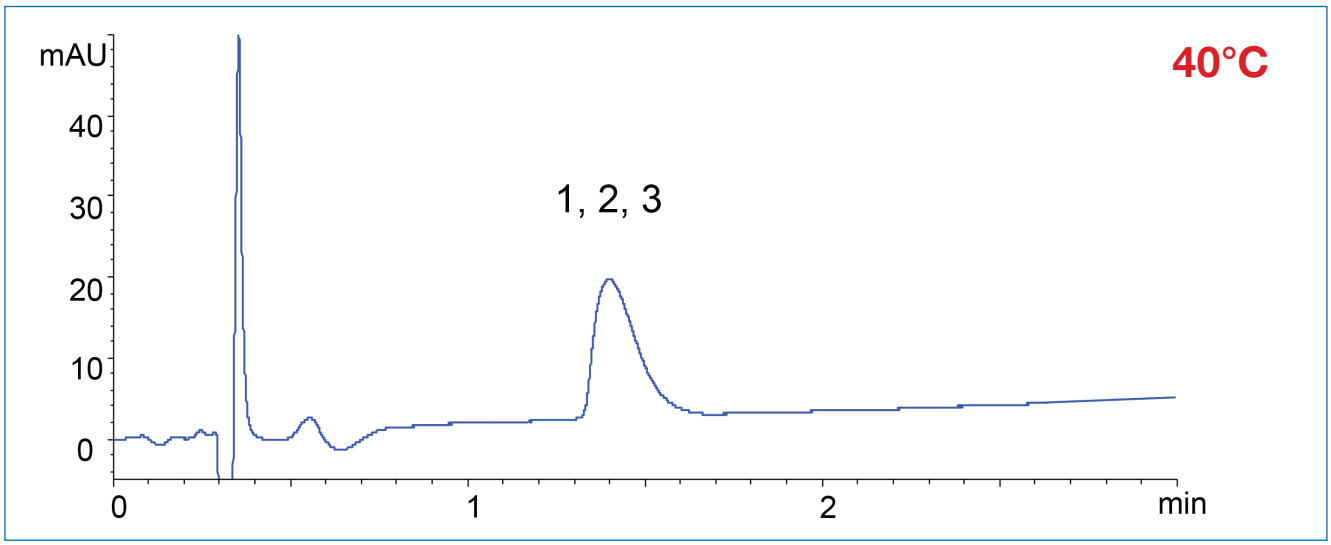

Figure 1: Initial UHPLC chromatogram of the 3 defensins

1. α-Defensin-1
2. α-Defensin-2
3. α-Defensin-3

• Temperature

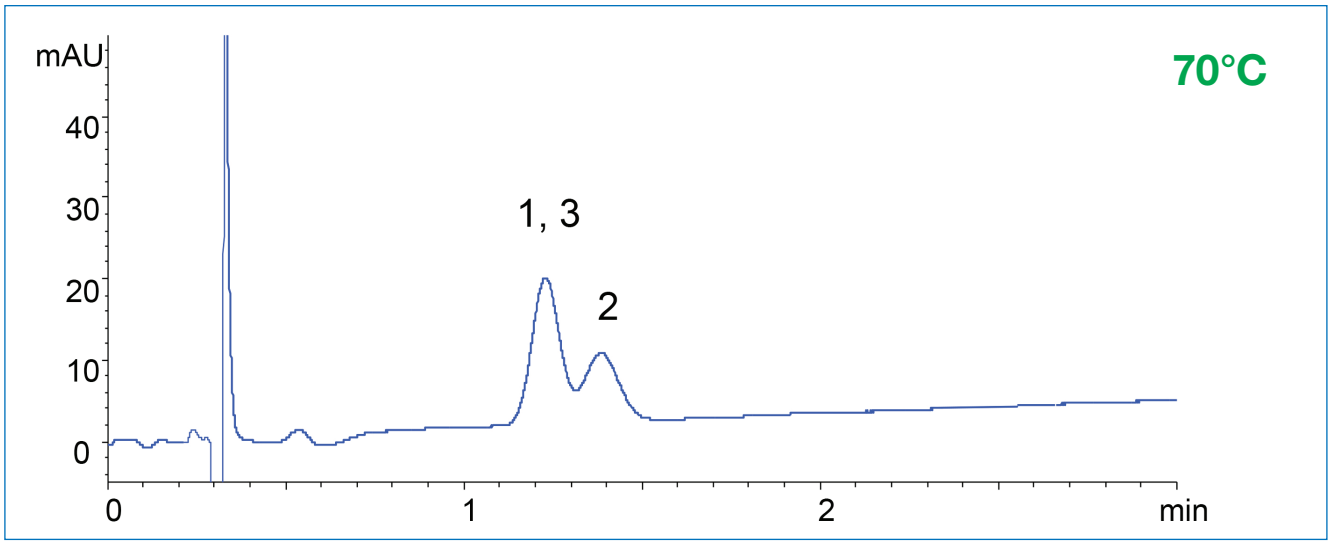

Figure 2: Temperature optimisation

• Mobile Phase

Due to the ionic characteristic of peptides, type and concentration of the acid additive influences the separation and can be used for method development.

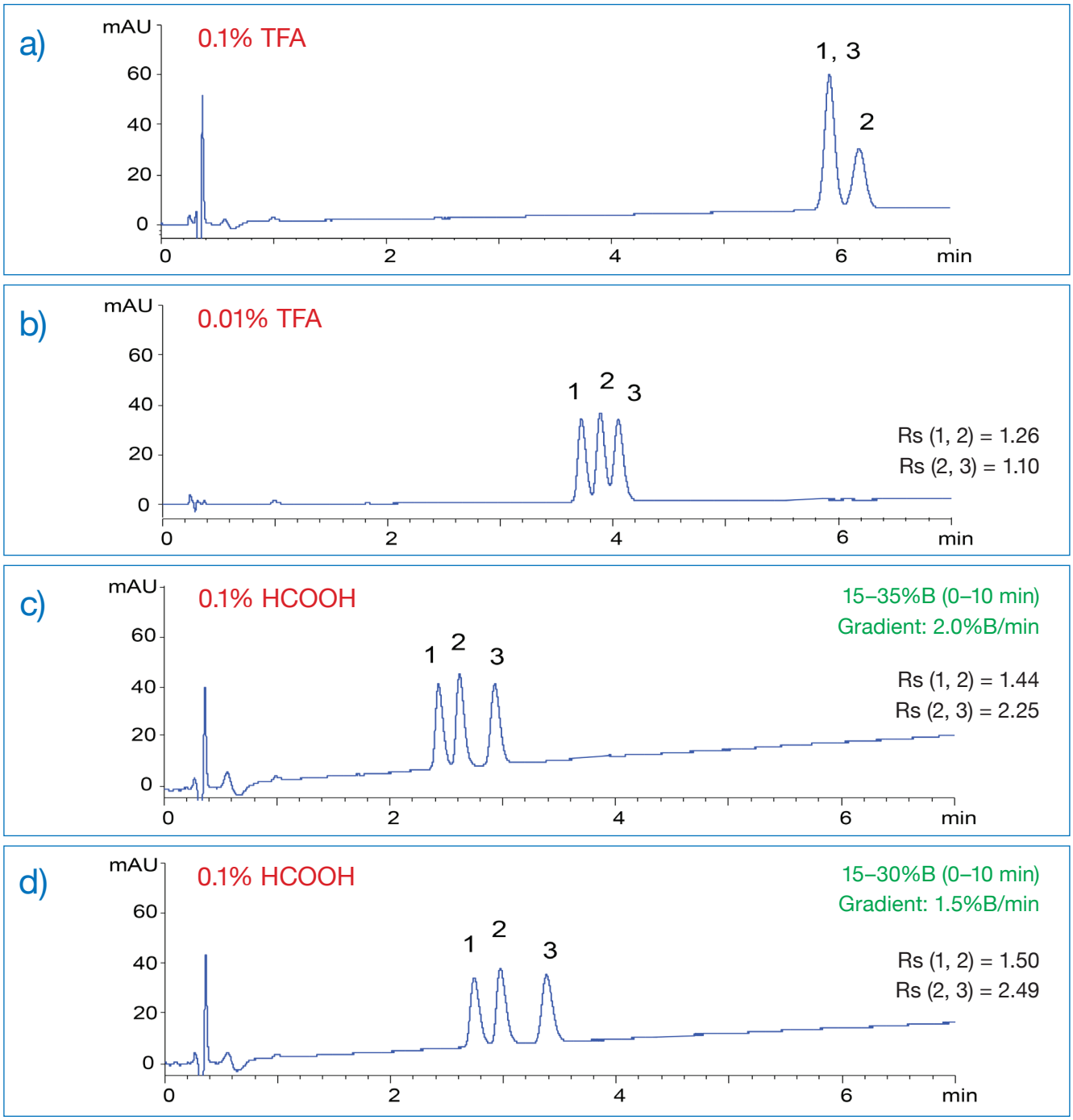

Figure 3: Mobile phase optimisation (a-d)

1. α-Defensin-1
2. α-Defensin-2
3. α-Defensin-3

In addition to modifying the gradient, changing the organic solvent can be a further tool.

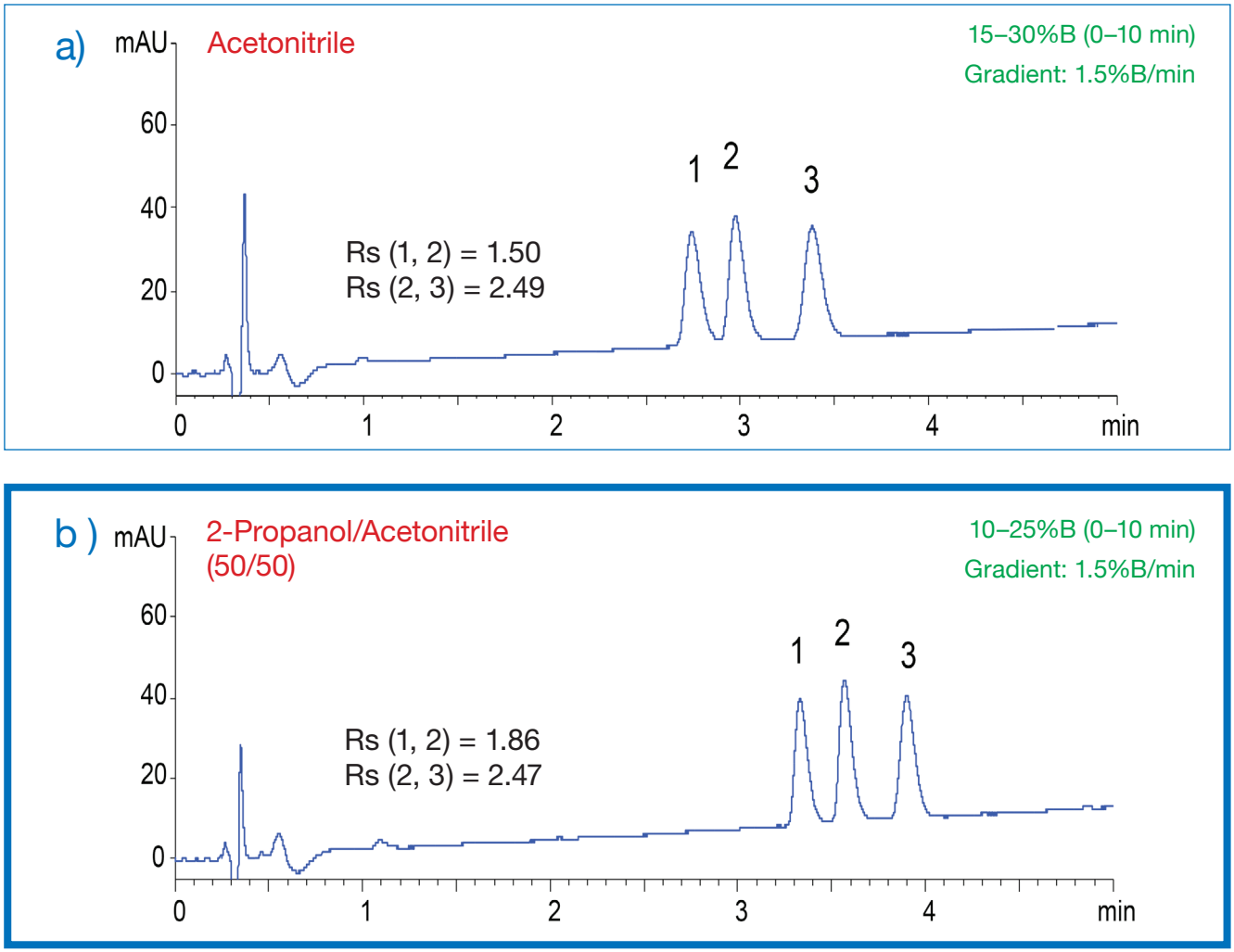

Figure 4: Mobile phase optimisation (a-b)

1. α-Defensin-1
2. α-Defensin-2
3. α-Defensin-3

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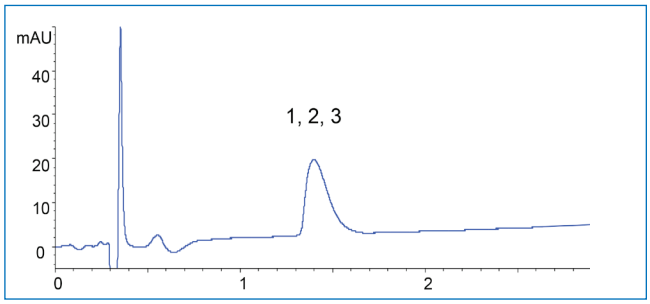
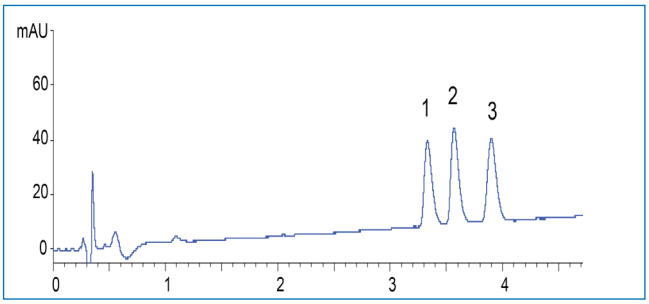
Initial method



Eluent: A) Water / TFA (100/0.1)
B) Acetonitrile / TFA (100/0.1)
Flow rate: 0.4 mL/min
Temperature: 40 °C
Detection: 220 nm

Optimised method



Eluent: A) Water / Formic Acid (100/0.1)
B) 2-Propanol / Acetonitrile / Formic Acid (50/50/0.08)
Flow rate: 0.4 mL/min
Temperature: 70 °C
Detection: 220 nm

The three defensins can be baseline separated, although they differ by only one amino acid. The main parameters to consider for optimisation of peptide separation:

- **Temperature**
- **Mobile Phase**

Therefore it is important to choose a column that provides flexibility for method development as well as robustness in use. For this application example a pH- and temperature- stable column was used.
YMC-Triart C18 is the ideal choice for peptide separations and should be included in your column screening set, not only for peptide analysis but for all analytes.

Ask for your YMC-Triart free trial column now!

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