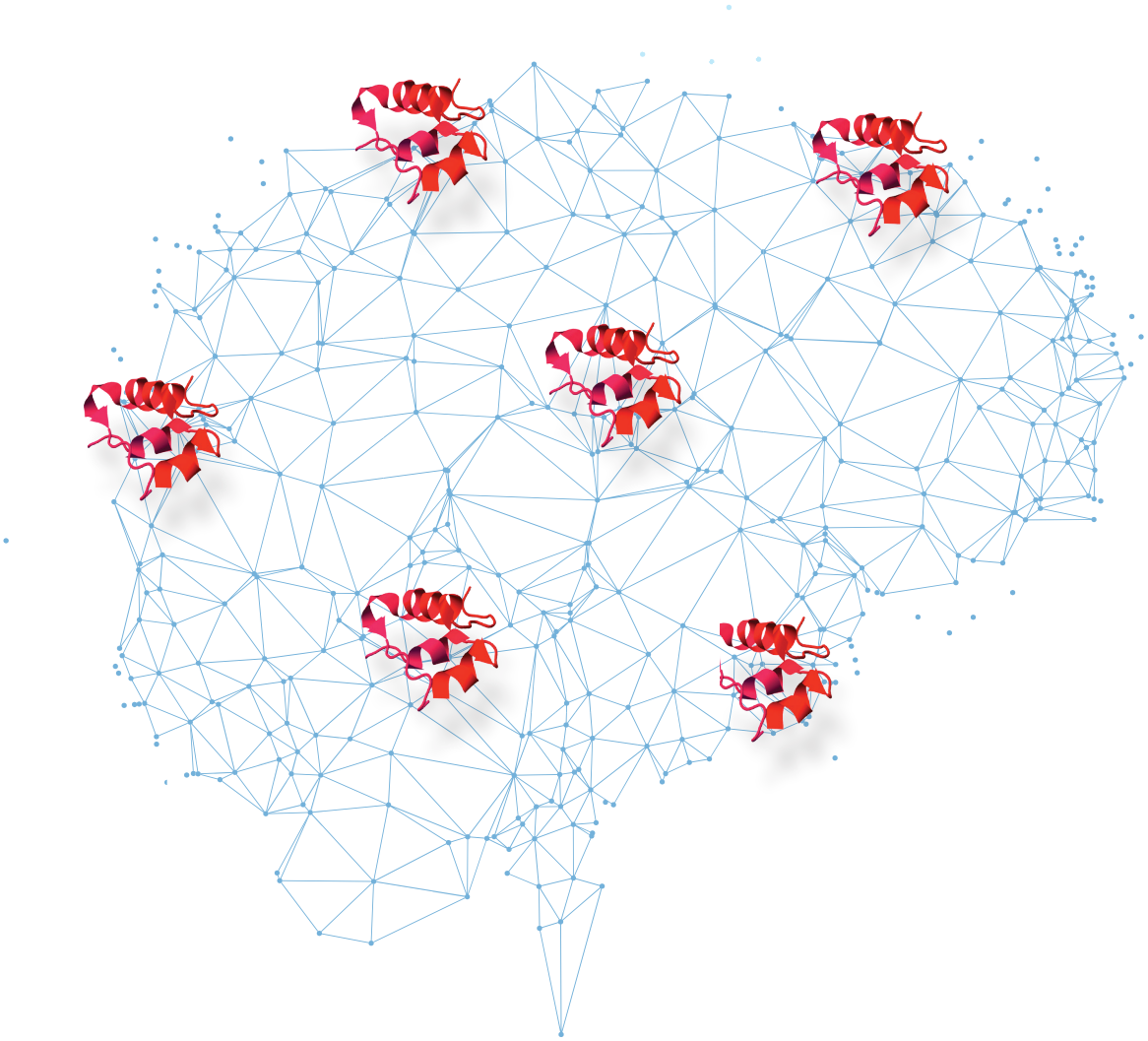
APPLICATION NOTE



Method development for simultaneous analysis of amyloid β (Aβ) peptides

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Method development for simultaneous analysis of amyloid β (Aβ) peptides

Amyloid β (Aβ) are peptides of 36–43 amino acids that are derived from the amyloid precursor protein (APP) by cleavage with β- and γ- secretase. Aβ are the main components of amyloid plaques and therefore crucially involved in Alzheimer's disease. The biological function of Aβ is still unexplained, but they are claimed to be neurotoxic as they form extracellular deposits in the brain and so can harm the cellular membrane.

To understand the function of the Aβ and for the research of Alzheimer's disease, it is important to develop a reliable analysis for Aβ.

However, this is not trivial as the Aβ peptides such as Aβ (1–42) are highly hydrophobic which leads to the formation of aggregates.

A method for the simultaneous determination of four Amyloid β using YMC-Triart Bio C4 has been developed by YMC. As the hydrophobic Aβ have the tendency to form aggregates a stationary phase with lower hydrophobicity and larger pore is needed, therefore YMC-Triart Bio C4 was chosen. Due to the high chemical stability of YMC-Triart Bio C4 it is possible to do rapid method optimisation for complex mixtures of peptides and proteins utilising the wide pH and temperature range available. YMC-Triart Bio C4 is available with 3 and 5 µm for HPLC, as well as with fully scalable 1.9 µm for UHPLC.

Table 1: Chromatographic conditions

Column:	YMC-Triart Bio C4 (5 µm, 30 nm), 150 x 3.0 mm ID
Part.no.:	TB30S05-1503PTH
Eluent:	A) water/buffer
	B) acetonitrile/buffer
Gradient:	25–40%B (0–30 min), 90%B (30–40 min)
Detection:	UV at 220 nm
Flow rate:	0.4 mL/min
Injection:	4 µL (each 0.1 mg/mL)

It is difficult to separate the Aβ under ambient conditions using 0.1 % TFA as shown in Figure 1. Aβ (1–42) and Aβ (1–43) could not be separated.

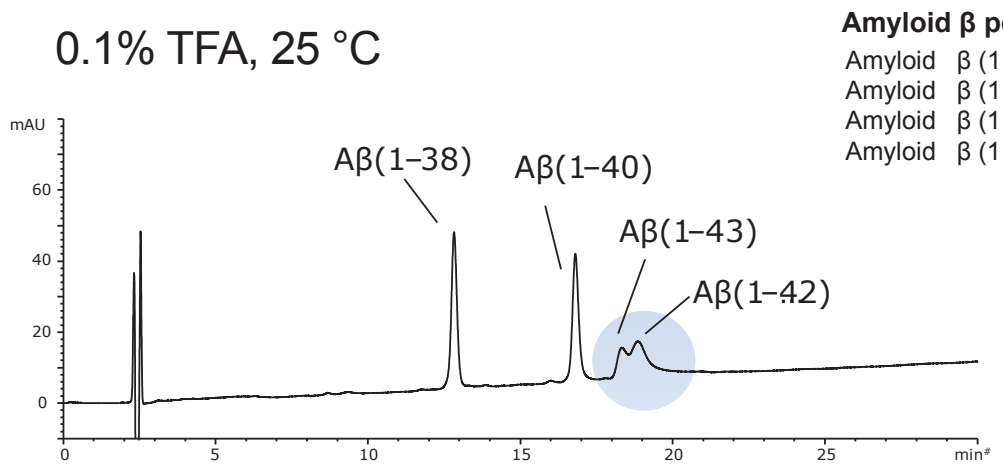

Figure 1: Analysis of 4 Aβ with 0.1 % TFA at ambient temperature.

With a change to higher pH values it is not possible to separate Aβ (1-42) and Aβ (1-43), even at higher temperatures. This is shown in Figure 2.

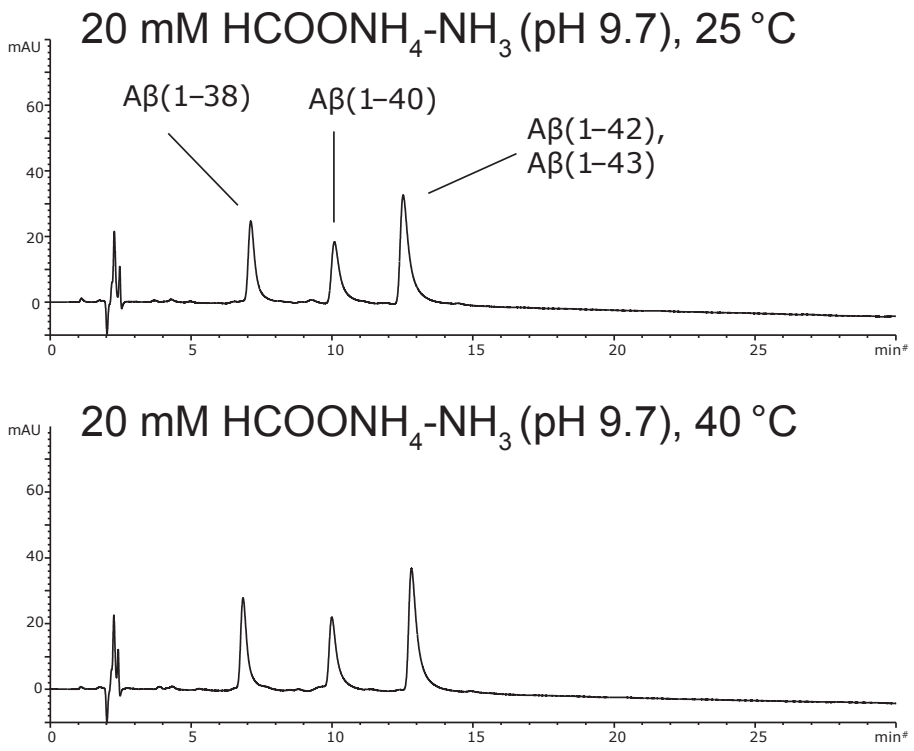

Figure 2: Chromatogram of 4 Aβ at higher pH value.

The optimised conditions using 0.1 % TFA at higher temperatures, as shown in Figure 3, allow Aβ (1–43) and Aβ (1–42) to be base line separated at 70 °C.

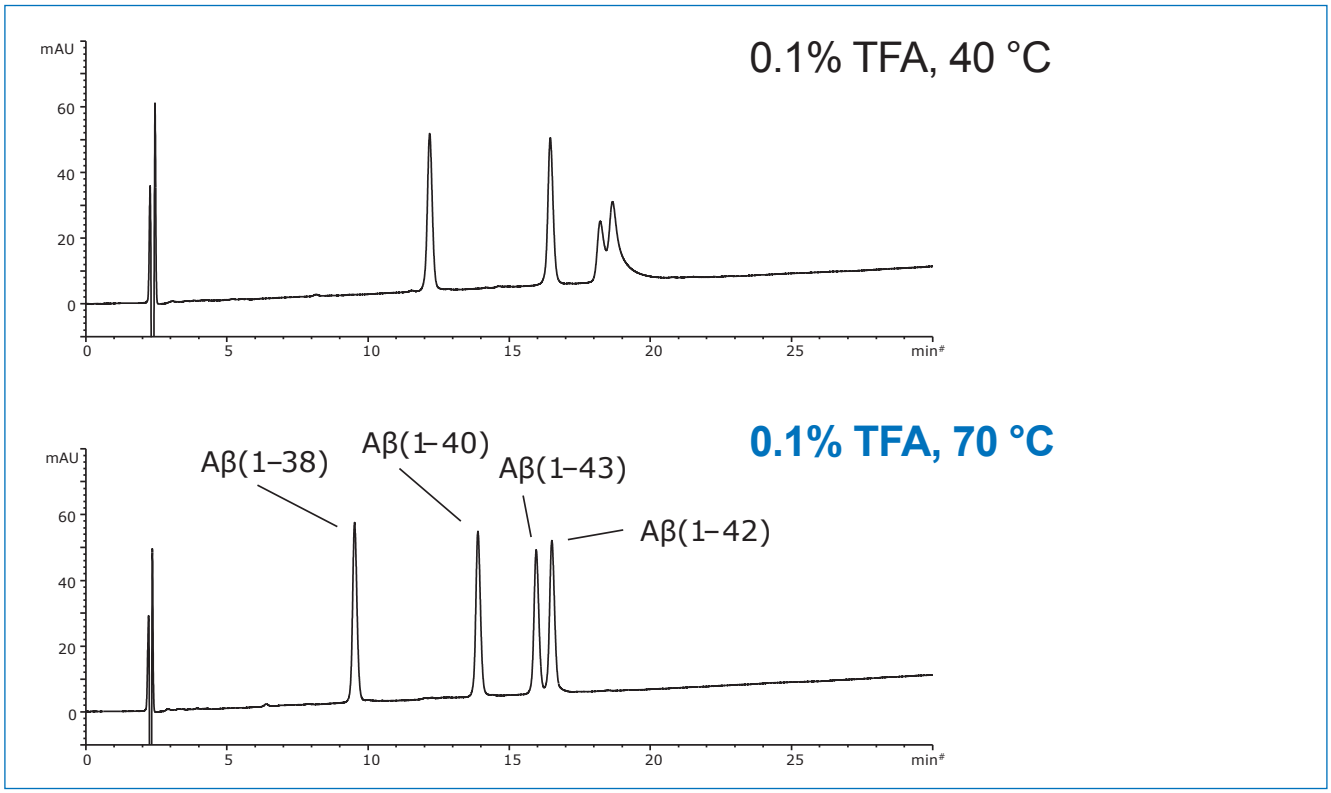

Figure 3: Separation of 4 Aβ with 0.1 % TFA at 70 °C.

The final conditions (shown in Table 2) contain 0.1 % TFA and the analysis was performed at 70 °C, which allow all four Aβ to be separated.

Table 2: Final chromatographic conditions

Column:	YMC-Triart Bio C4 (5 µm, 30 nm) 150 x 3.0 mm ID
Part.no.:	TB30S05-1503PTH
Eluent:	A) water + 0.1 % TFA
	B) acetonitrile + 0.1 % TFA
Gradient:	25–40%B (0-30 min), 90%B (30–40 min)
Temperature:	70 °C
Detection:	UV at 220 nm
Flow rate:	0.4 mL/min
Injection:	4 µL (each 0.1 mg/mL)