

METHOD DEVELOPMENT
FOR SIMULTANEOUS ANALYSIS
OF AMYLOID β ($A\beta$) PROTEINS

Method development for simultaneous analysis of Amyloid β ($A\beta$) proteins

Amyloid β ($A\beta$) are peptides of 36–43 amino acids that are derived from the amyloid precursor protein (APP) by cleavage with β - and γ - secretase. $A\beta$ are the main components of amyloid plaques and therefore crucially involved in Alzheimer's disease. The biological function of $A\beta$ 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 $A\beta$ and for the research of Alzheimer's disease, it is important to develop a reliable analysis for $A\beta$.

However, this is not trivial as the $A\beta$ peptides such as $A\beta$ (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 $A\beta$ 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 $A\beta$ under ambient conditions using 0.1 % TFA as shown in Figure 1. $A\beta$ (1–42) and $A\beta$ (1–43) could not be separated.

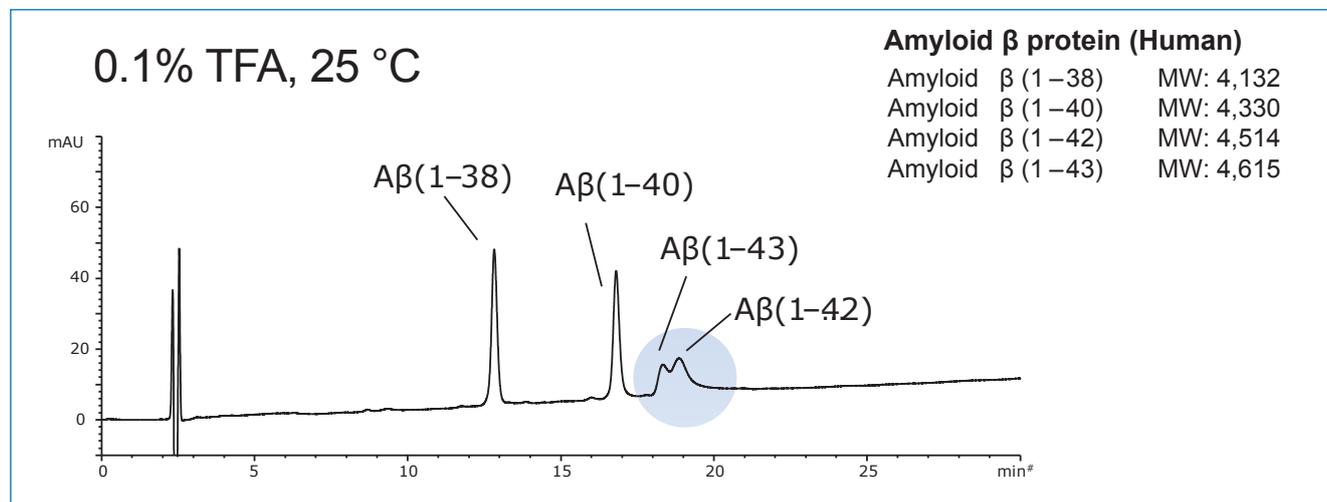


Figure 1: Analysis of 4 A with 0.1 % TFA at ambient temperature.

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

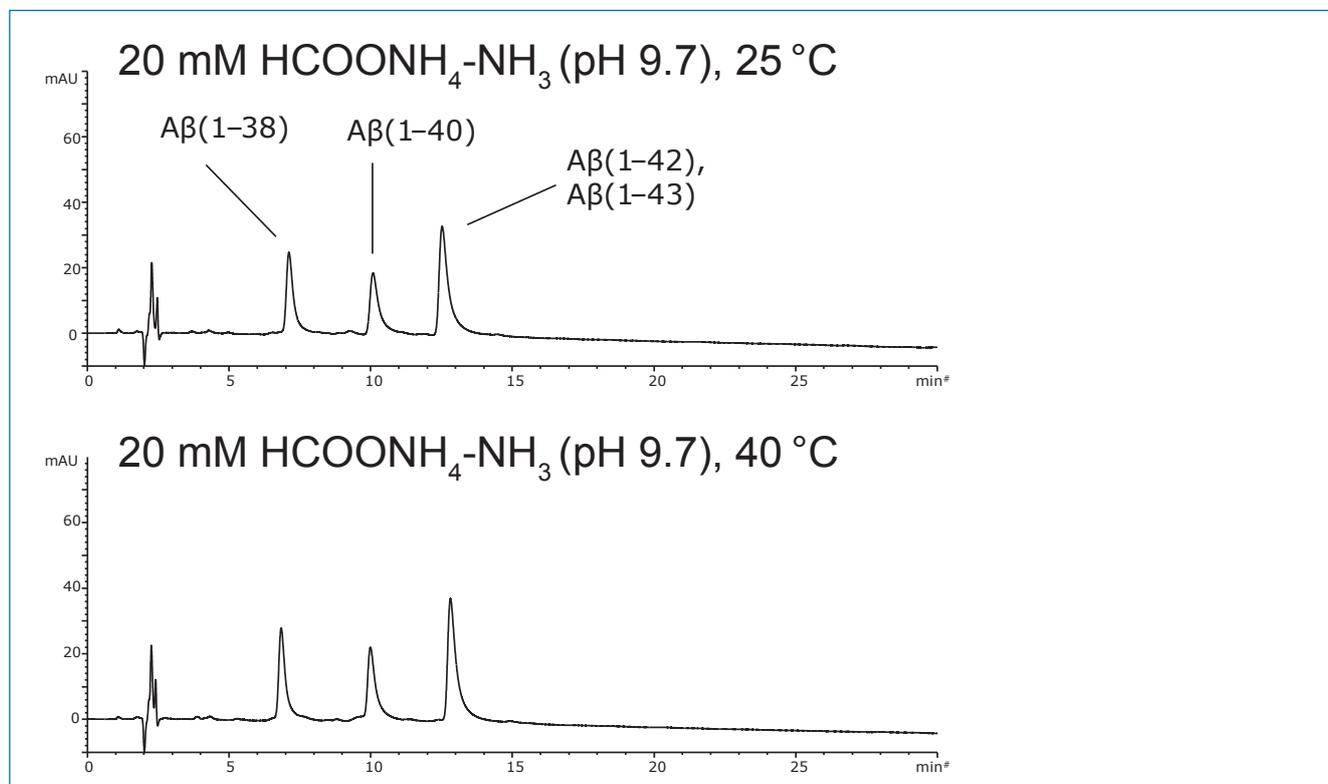


Figure 2: Chromatogram of 4 A β at higher pH value.

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

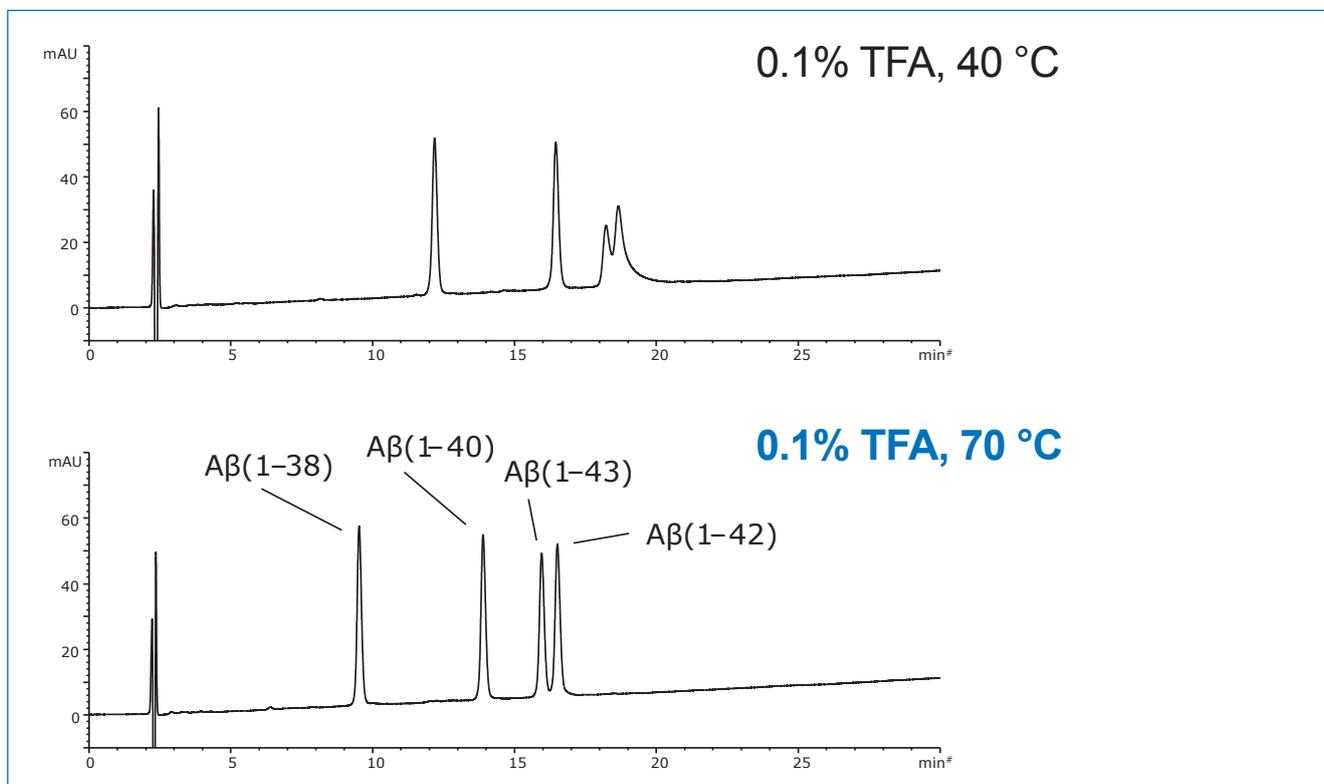


Figure 3: Separation of 4 A β with 0.1 % TFA at 70 °C.

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

Table 2: Final chromatographic conditions

Column:	YMC-Triart Bio C4 (5 μ m, 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 μ L (each 0.1 mg/mL)