Fast HILIC Analysis of Hydrophilic Dipeptides Using a YMC-Triart SIL Column

Hydrophilic compounds usually show poor retention when using reversed phase (RP) columns for their separation. Fortunately, as a chromatographer one can resort to other separation techniques that allow for better retention of hydrophilic compounds. For example, when using HILIC (hydrophilic interaction chromatography) as a separation mode the elution order is completely reversed compared to RP resulting in high retention times for hydrophilic compounds which would have been eluted (too) early using RP columns.

Therefore, HILIC is the perfect choice for separating hydrophilic dipeptides. The analysis of four dipeptides using YMC-Triart SIL column is shown in Figure 2.

L-Carnosine and L-anserine for example can be found in muscle and brain tissues in mammals. Both peptides have several physiological effects in the human body and further properties are discussed such as in the context of Alzheimer's disease. Gly-Gly is used in peptide synthesis whereas Gly-Asp is commonly known as a metabolite.

As the logP values of the analysed peptides are far below 0 they show excellent retention using HILIC conditions without sacrificing valuable analysis time. A fast separation in less than four minutes could be obtained using a 50 x 2.1 mm ID column. All peaks were well-resolved even for the challenging peptides L-carnosine and L-anserine which are structurally very similar as they only differ in a methyl group.

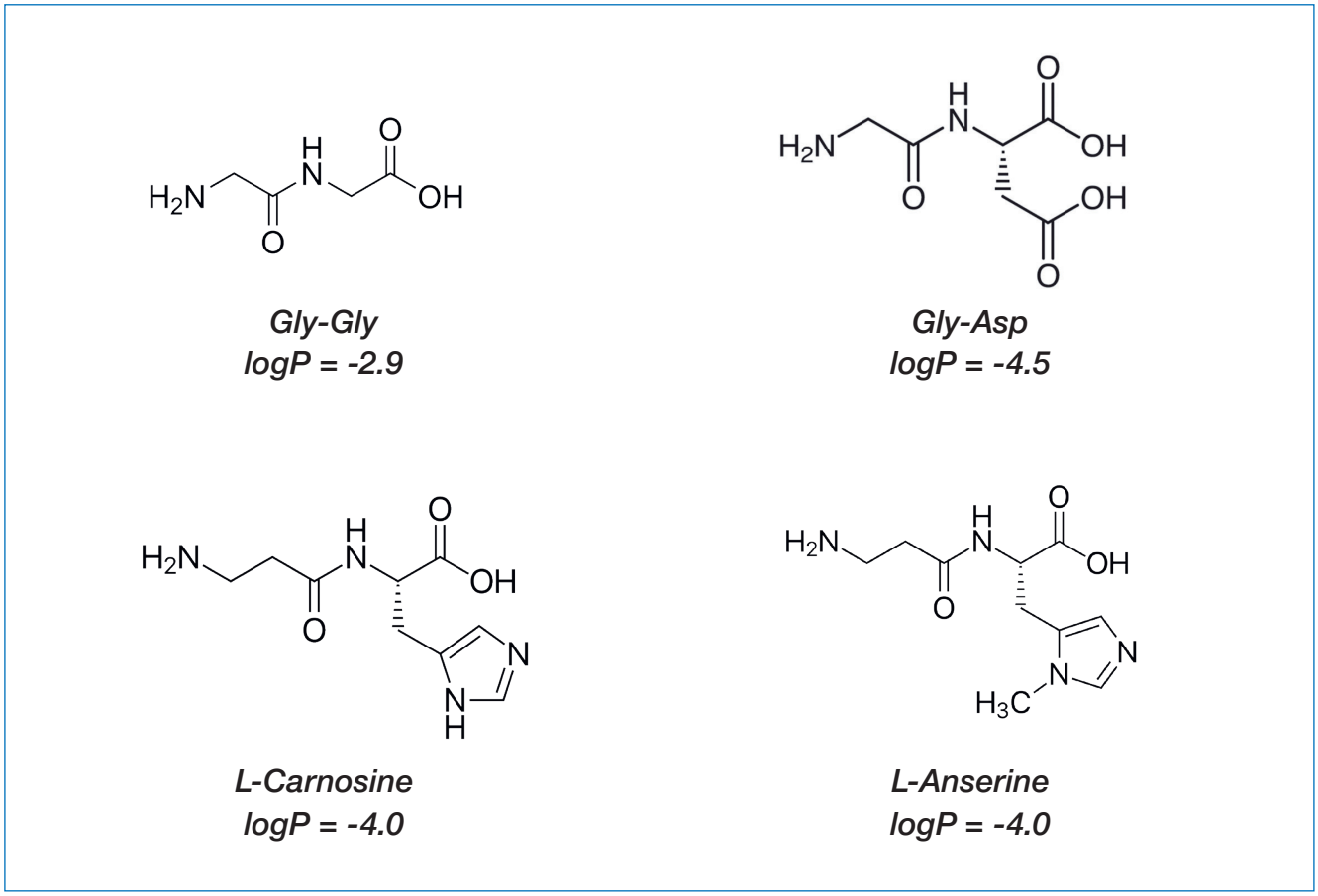

Figure 1: Molecular structure of four hydrophilic dipeptides.

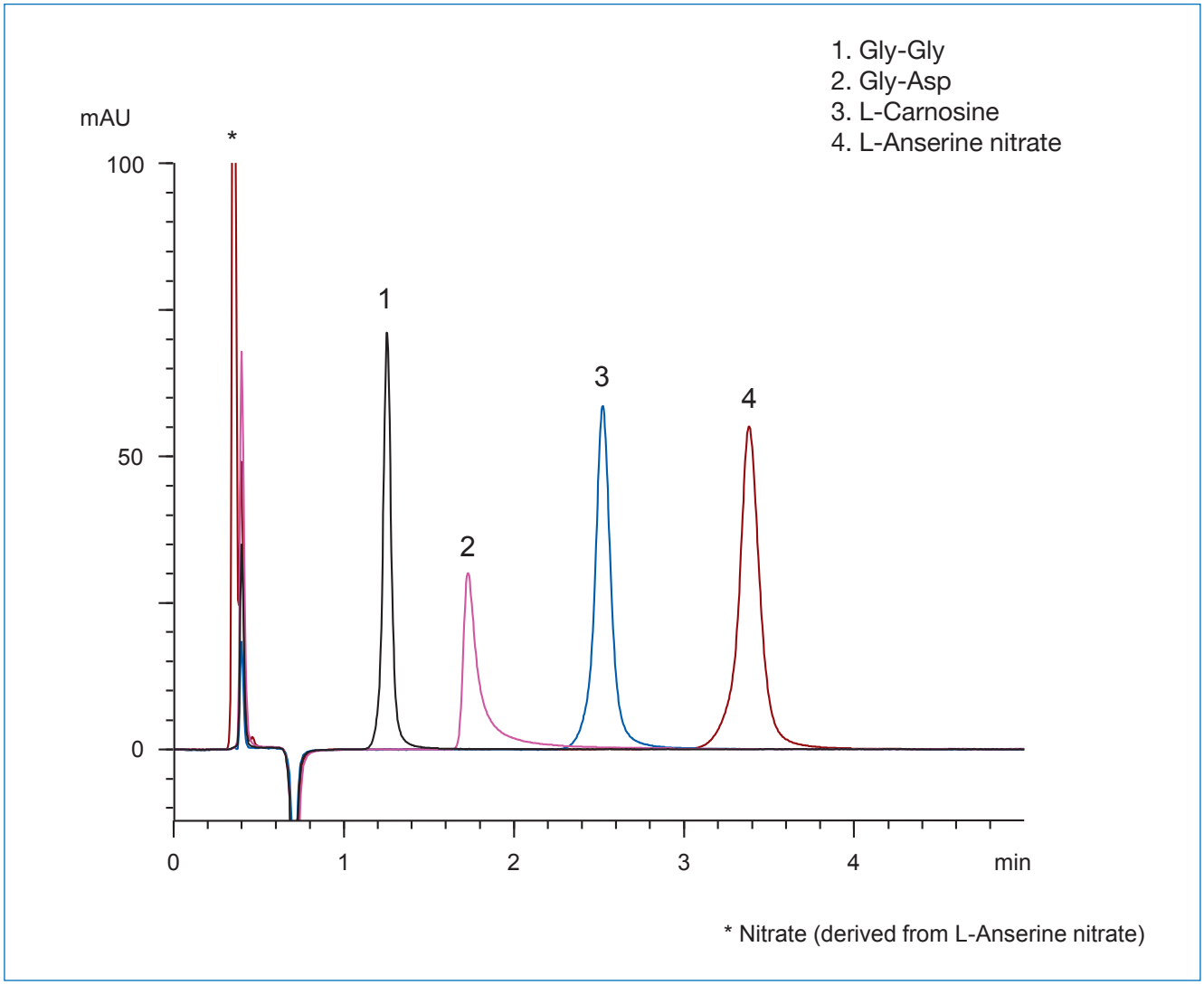

Figure 2: Separation of four hydrophilic dipeptides.

Table 1: Chromatographic conditions.

Column:	YMC-Triart SIL (3 µm, 12 nm) 50 x 2.1 mm ID
Part No.:	TS12S03-05Q1PTH
Eluent:	40 mM $HCOONH_4$ (pH 6.5) / acetonitrile (25/75)
Flow rate:	0.4 mL/min
Temperature:	40° C
Detection:	UV at 210 nm
Injection	0.5 µL (0.1 mg/mL)