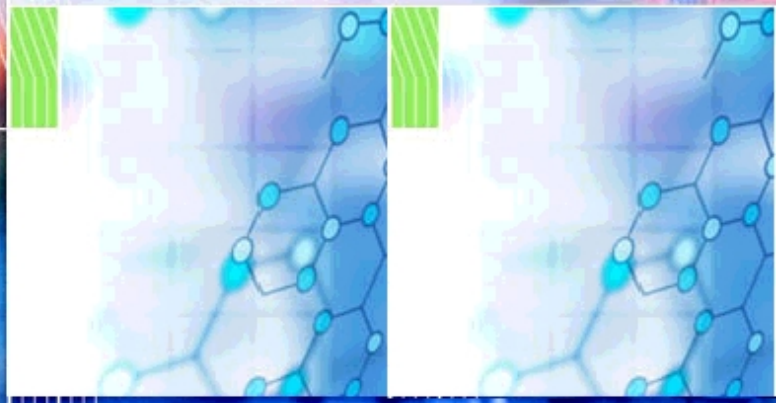
WA20267

Peptide Separations Employing Hybrid Packings



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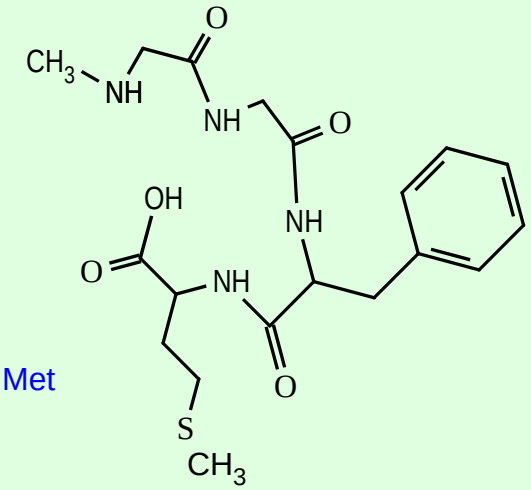
Abstract

We developed a separation for a model peptide mixture. We achieved successful separations employing hybrid particle materials at both low and high pH. These results indicate that the loadability and throughput can be highly improved at high pH. In addition, scale-up results are also shown.

Motivation

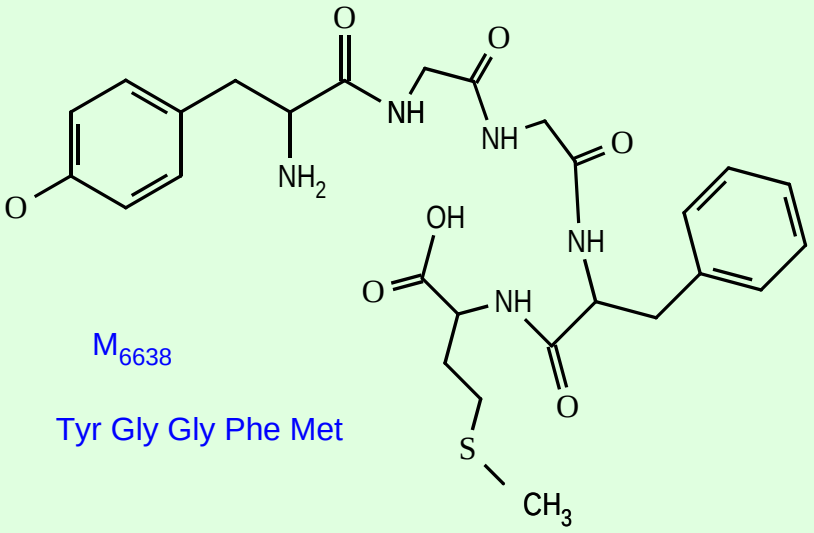
- Loadability of peptides onto a column has been historically challenging
- Peptides synthesized using combinatorial techniques are often difficult to purify due to the similar chemistries of the main compound and its impurities
- Evaluation of alternative chromatographic methods are required in order to improve yields and productivity

Peptide Mixture



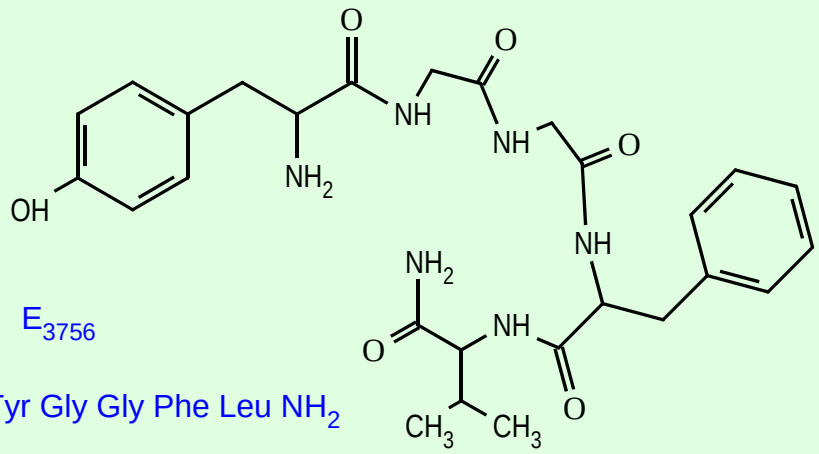
E_{2256}

Gly Gly Phe Met



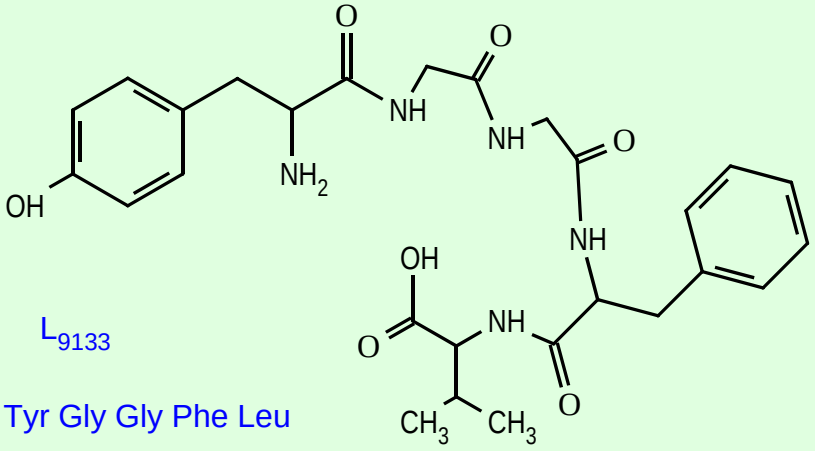
M_{6638}

Tyr Gly Gly Phe Met



E_{3756}

Tyr Gly Gly Phe Leu NH_2



L_{9133}

Tyr Gly Gly Phe Leu

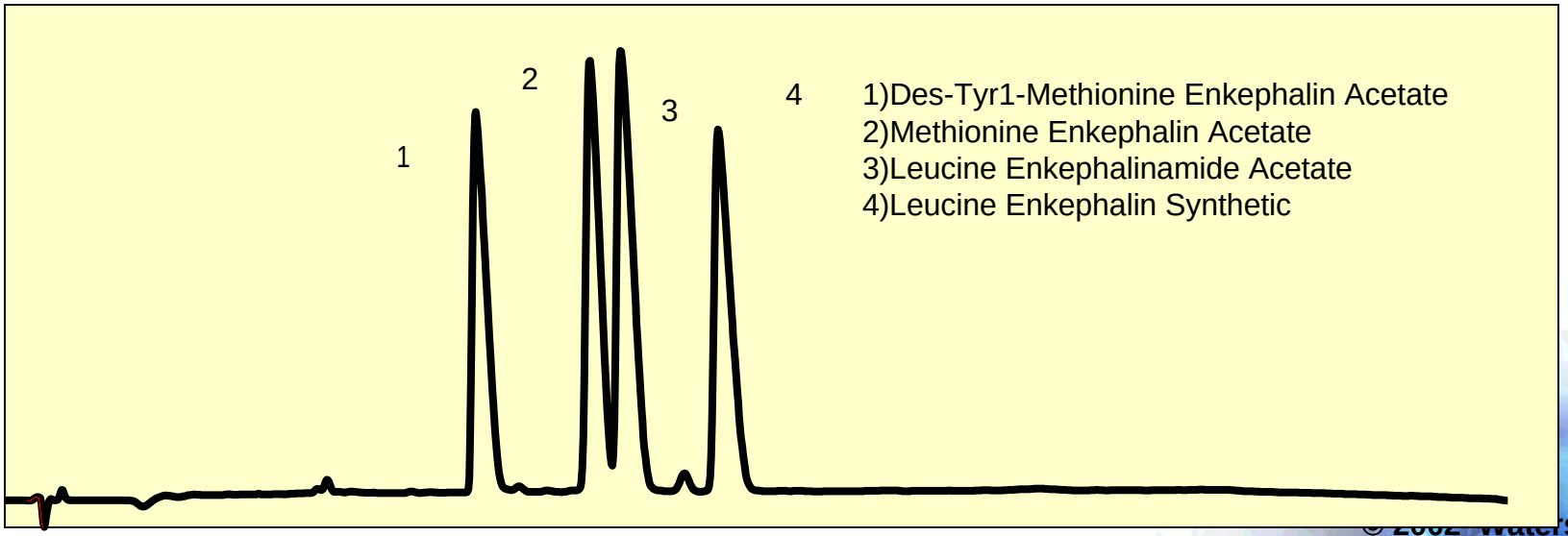
Method Development at Low pH

XTerra® MS C_{18} 4.6 X 50 mm, 5 µm
Linear Gradient
Gradient Table

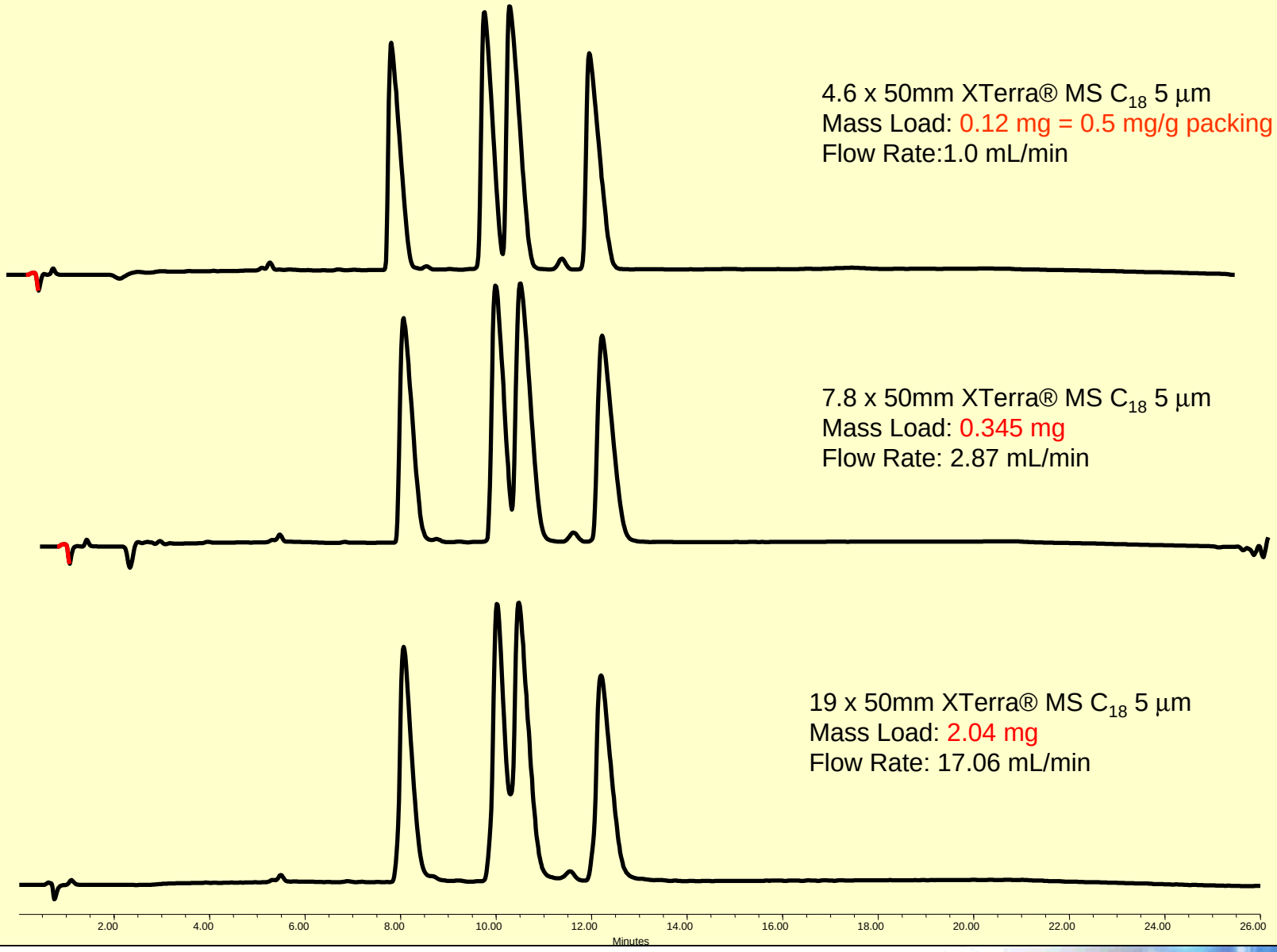
Time	%A	%B	%C
0	90	0	10
5	76	14	10
20	60	30	10
25	90	0	10
26	90	0	10

A: Water B: Acetonitrile C: 1%TFA

Flow Rate: 1.0mL/min
Mass Load: 0.12 mg = 0.5 mg/g packing
Detection: 214 nm



Scale-up of Peptide Separation at Low pH



Method Development at High pH

XTerra® MS C_{18} 4.6 X 50 mm, 5 µm

Linear Gradient

Gradient Table

Time	%A	%B	%C
0	88	2	10
2	88	2	10
4.5	76	14	10
14	60	30	10

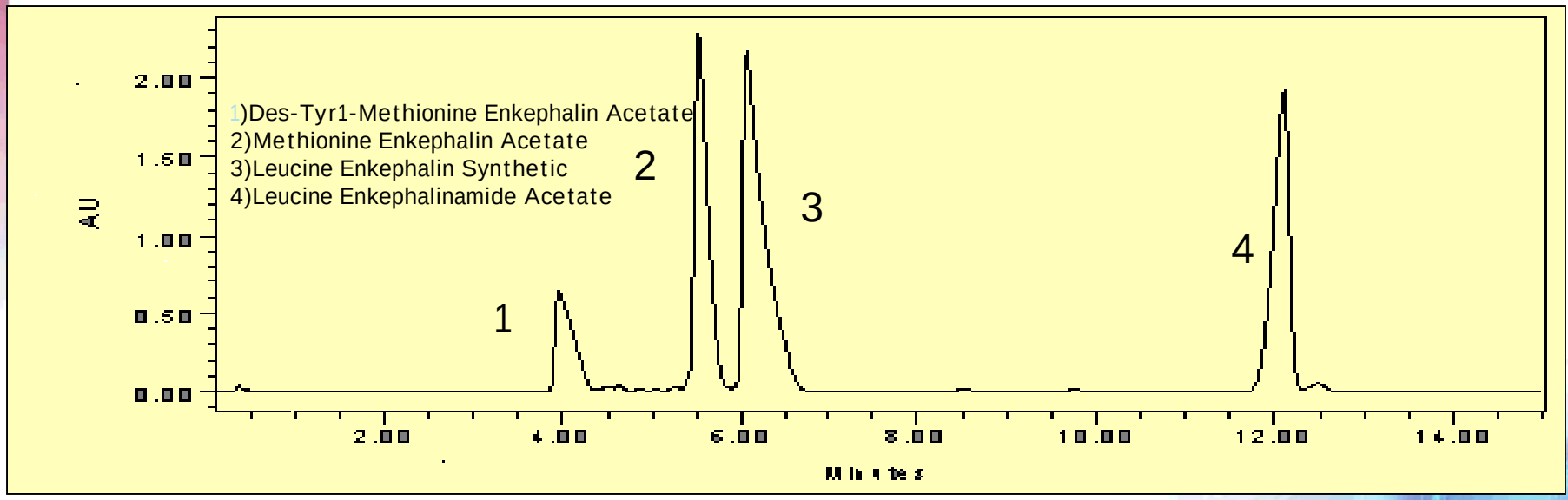
A: Water B: Acetonitrile C: 100mM NH4OH

Flow Rate: 1.8 mL/min

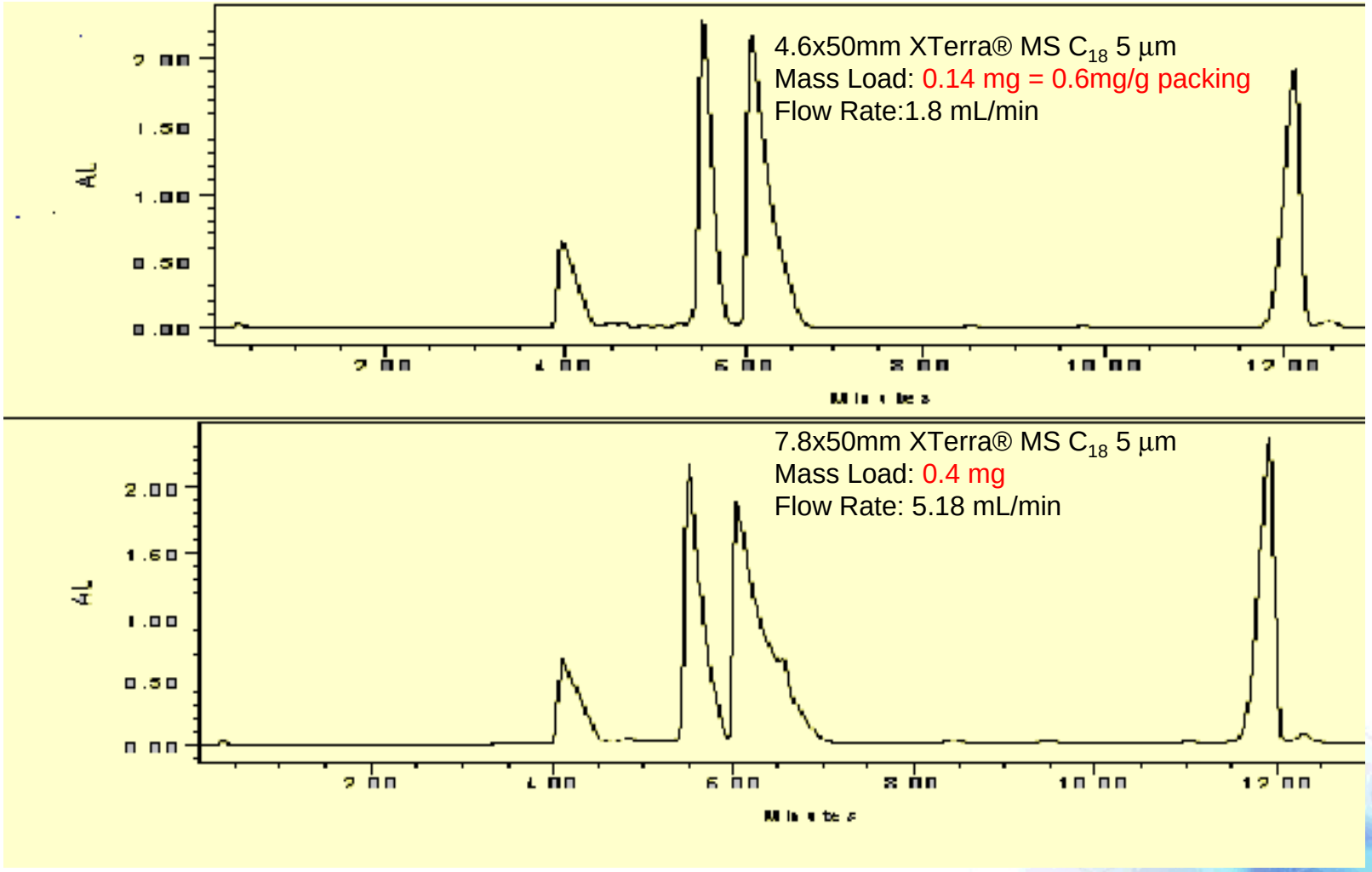
Mass Load: 0.14 mg = 0.6 mg/g packing

Detection: 214 nm

Selectivity reversal of peaks 3 & 4 from low to high pH

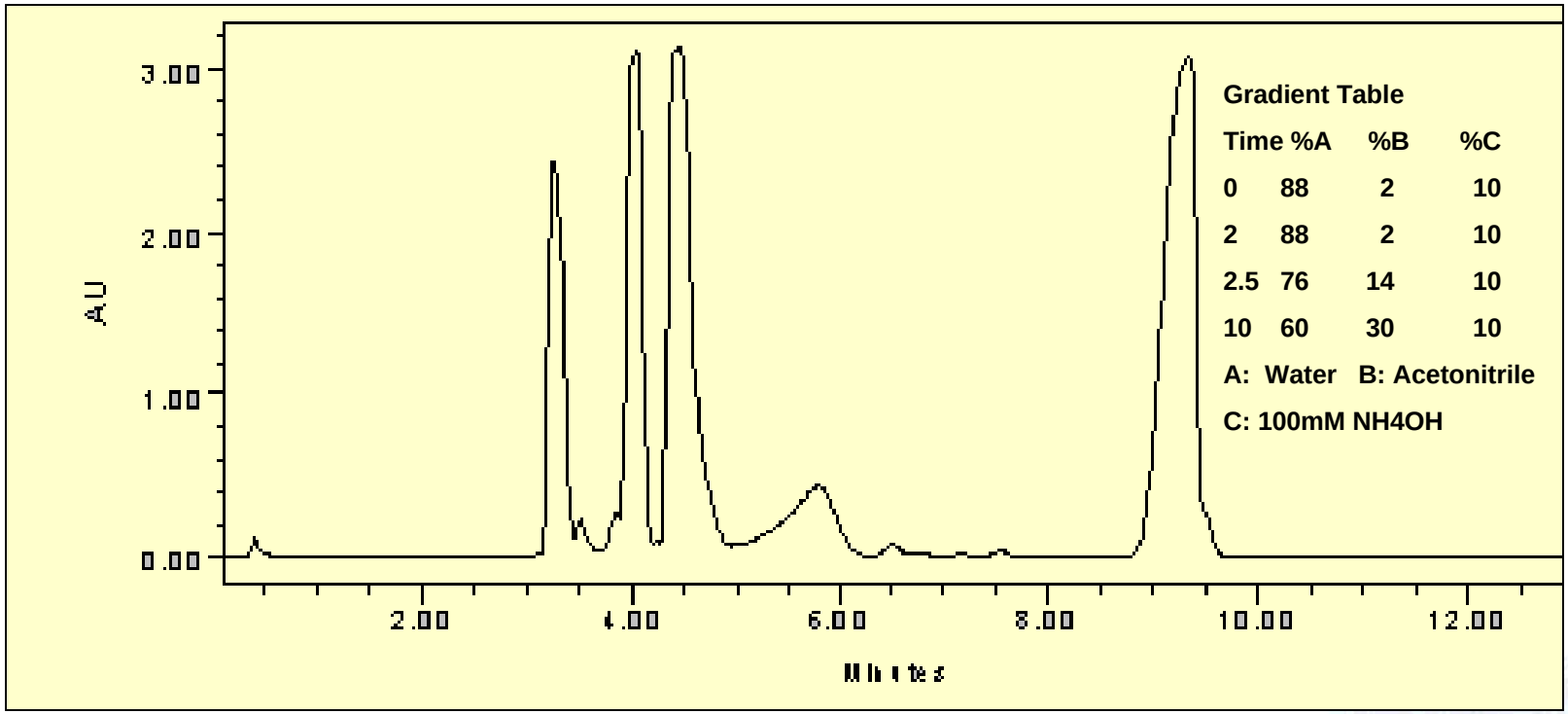


Scale-up of Peptide Separation at High pH



Increase in Loadability at High pH

4.6x50mm XTerra® MS C_{18}, 5µm
Mass Load: 0.4 mg = 1.6 mg/g packing
Flow Rate:1.8 mL/min



At high pH the peaks are better resolved allowing the loading to increase by a 2.8 factor.

Conclusions

- Peptides can be effectively separated at both low and high pH using hybrid particle packing.
- Loading is highly improved at high pH.
- Selectivity reversals have been observed for the model peptide mixture when running at low and high pH, indicating that mobile phase conditions can be exploited for changes in selectivity.
- Scale-up to 7.8 x 50 mm columns has been demonstrated.