

Project Requirement:

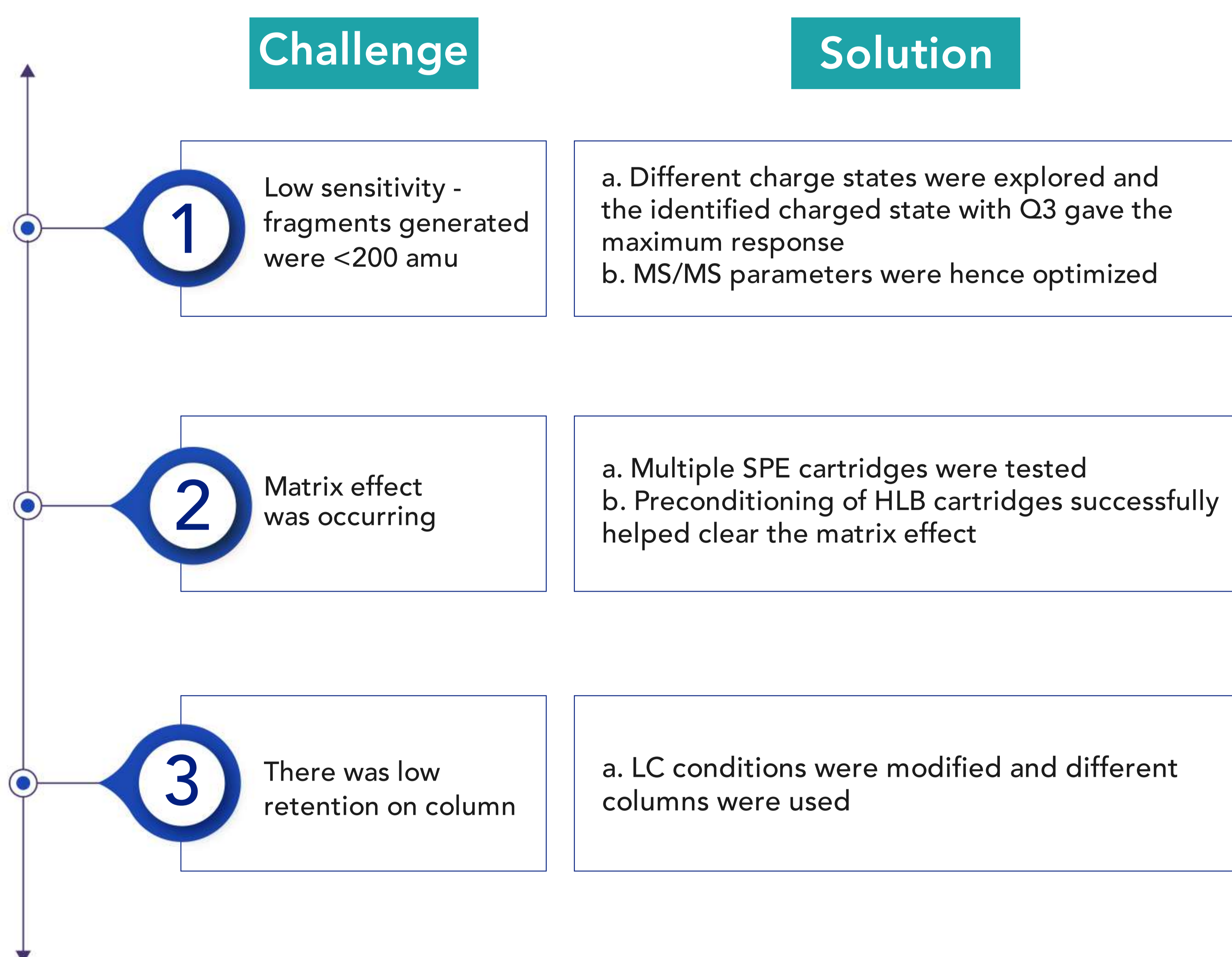
To develop an LC-MS/MS method for quantification of a highly polar, and rarely encountered peptide, for a biotech company's discovery process.

The Peptide:

- 40 amino acids, ~4915 da
- All D-amino acids
- 10 Arginines & 5 Lysines
- C-terminal acid and N-terminal amine
- Highly polar positively charged peptide

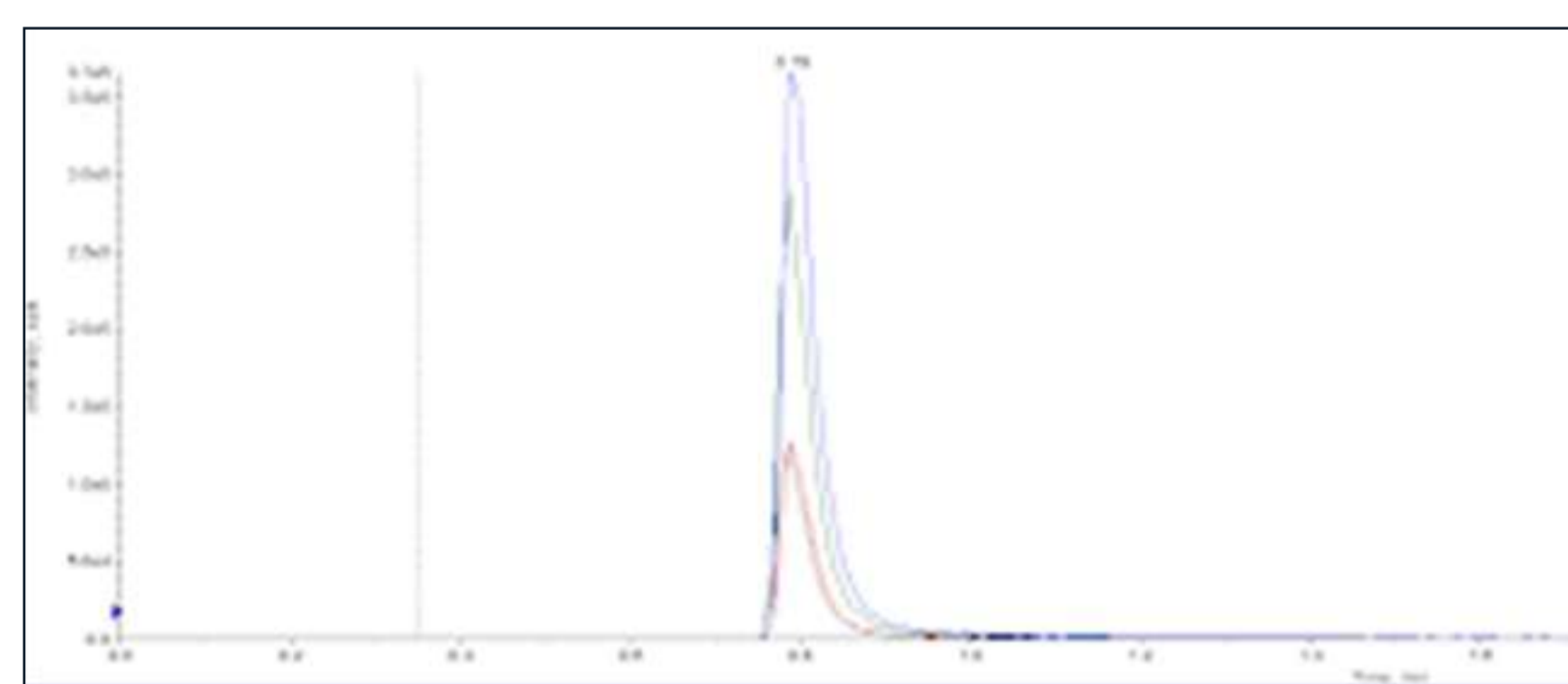


The challenges and Aurigene's solutions:



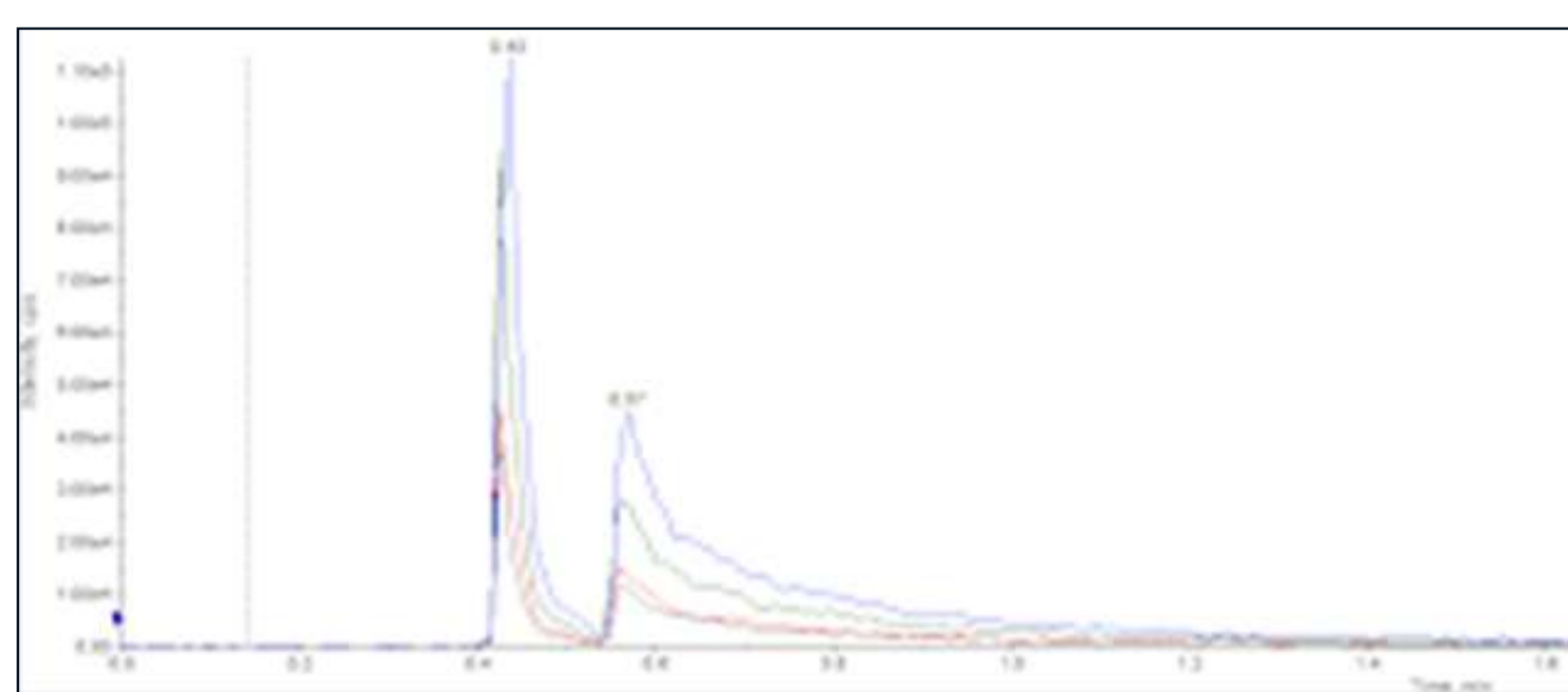
Here are the trial results towards the optimization of LC parameters -

Trial 01



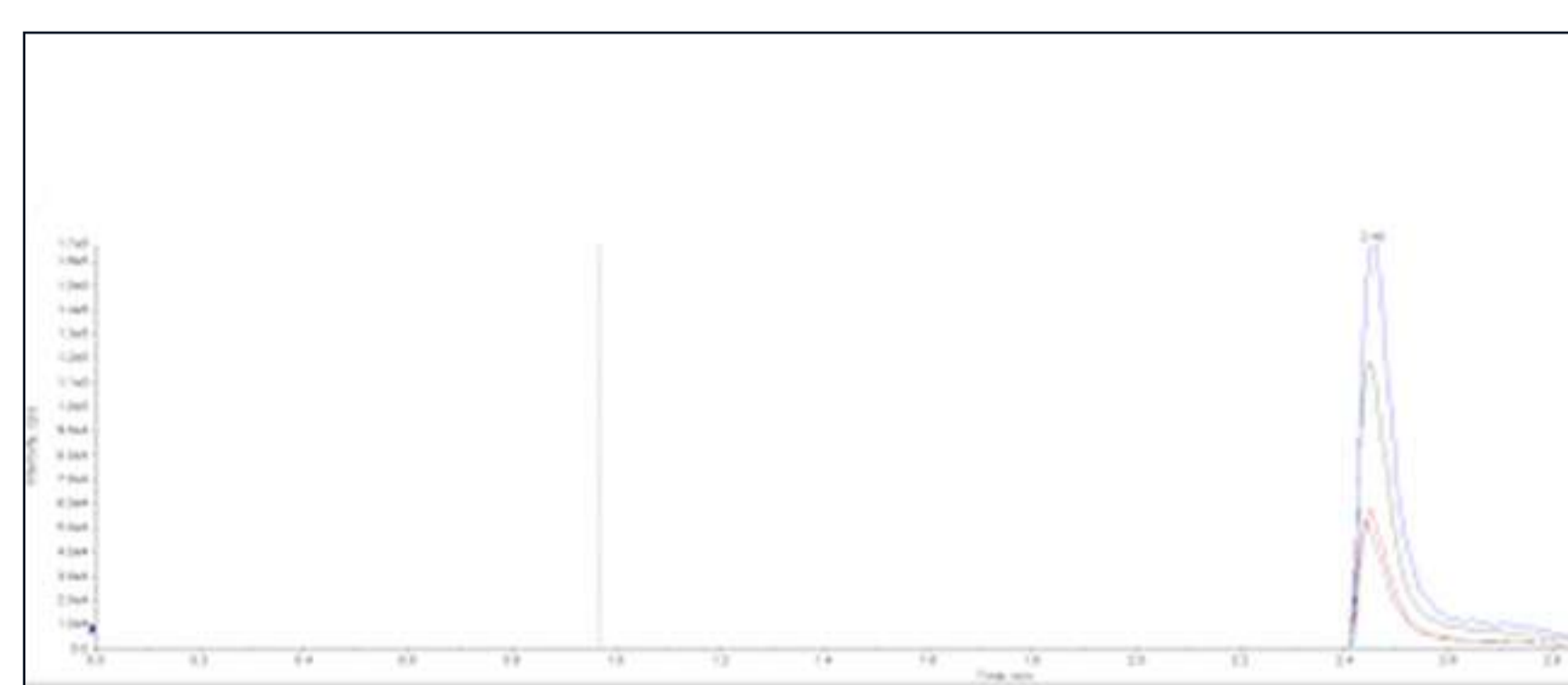
SPE-HLB; SB Phenyl 4.6 X 150 mm 3.5 μm;
Isocratic; 70:30 Formic acid in H2O + Formic acid in MeOH
Good peak shape and response.
Analyte eluted in void volume in 50 & 150 mm columns

Trial 02



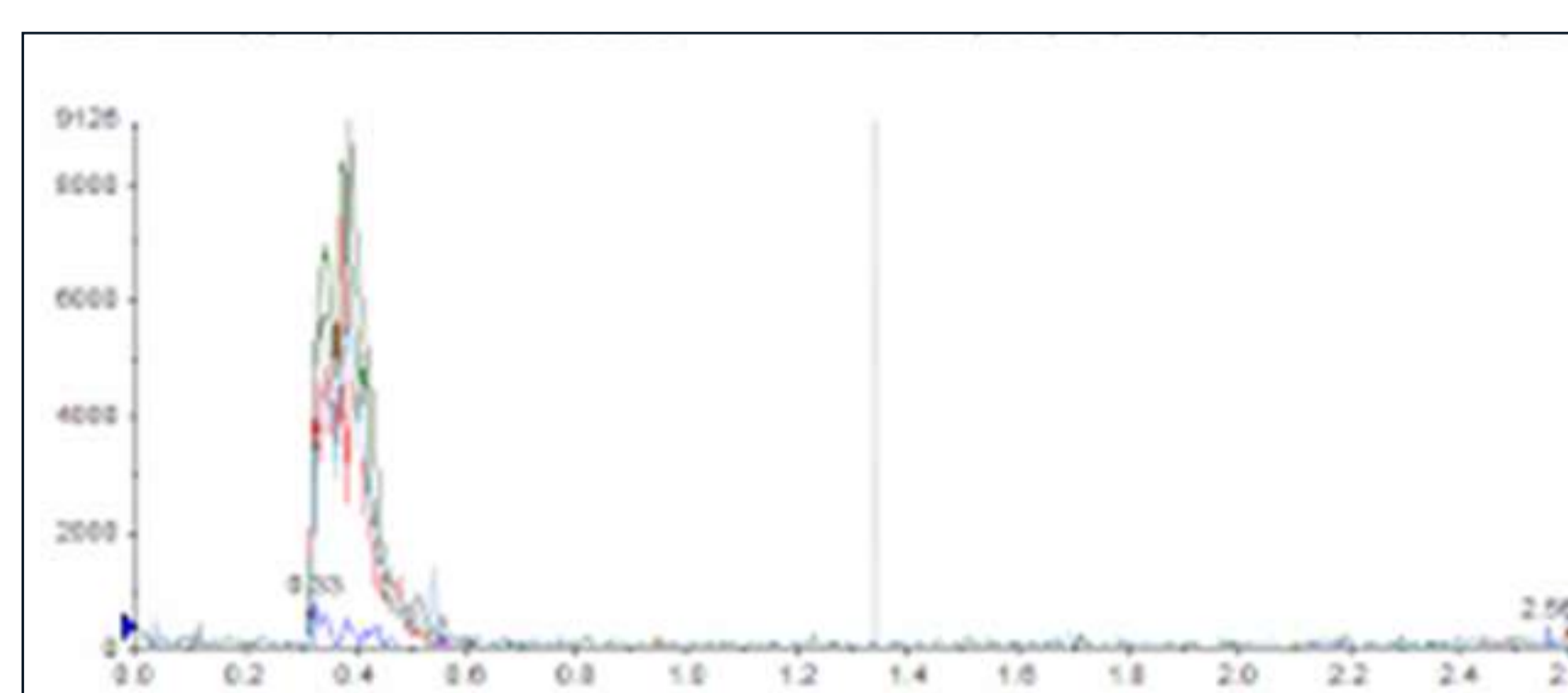
SPE-HLB; SB Phenyl 4.6 X 150 mm 3.5 μm;
Isocratic; 50:50 Formic acid in H2O + Formic acid in MeOH
Split peak observed

Trial 03



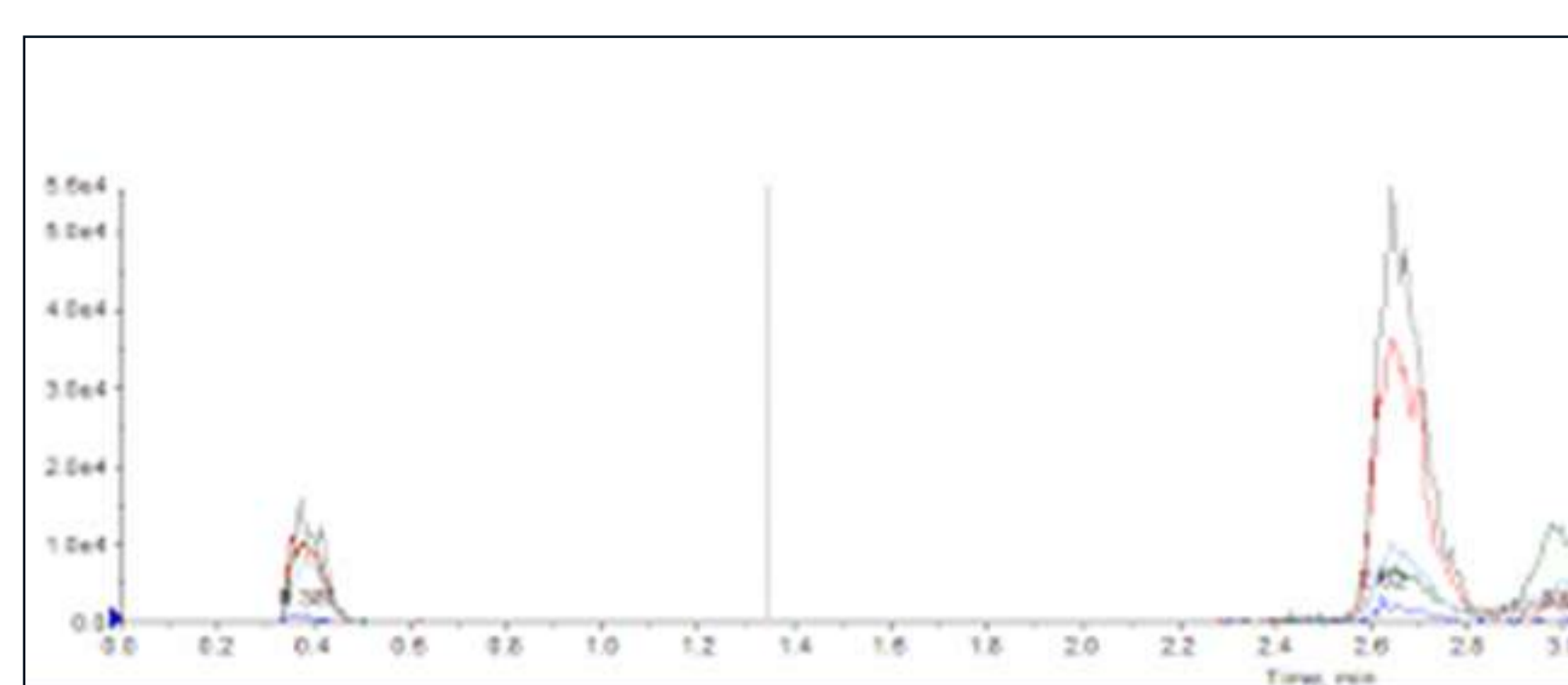
SPE-HLB; SB Phenyl 4.6 X 150 mm 3.5 μm;
Gradient; Formic acid in H2O + Formic acid in MeOH
Poor peak shape and low response. Tailing observed

Trial 04



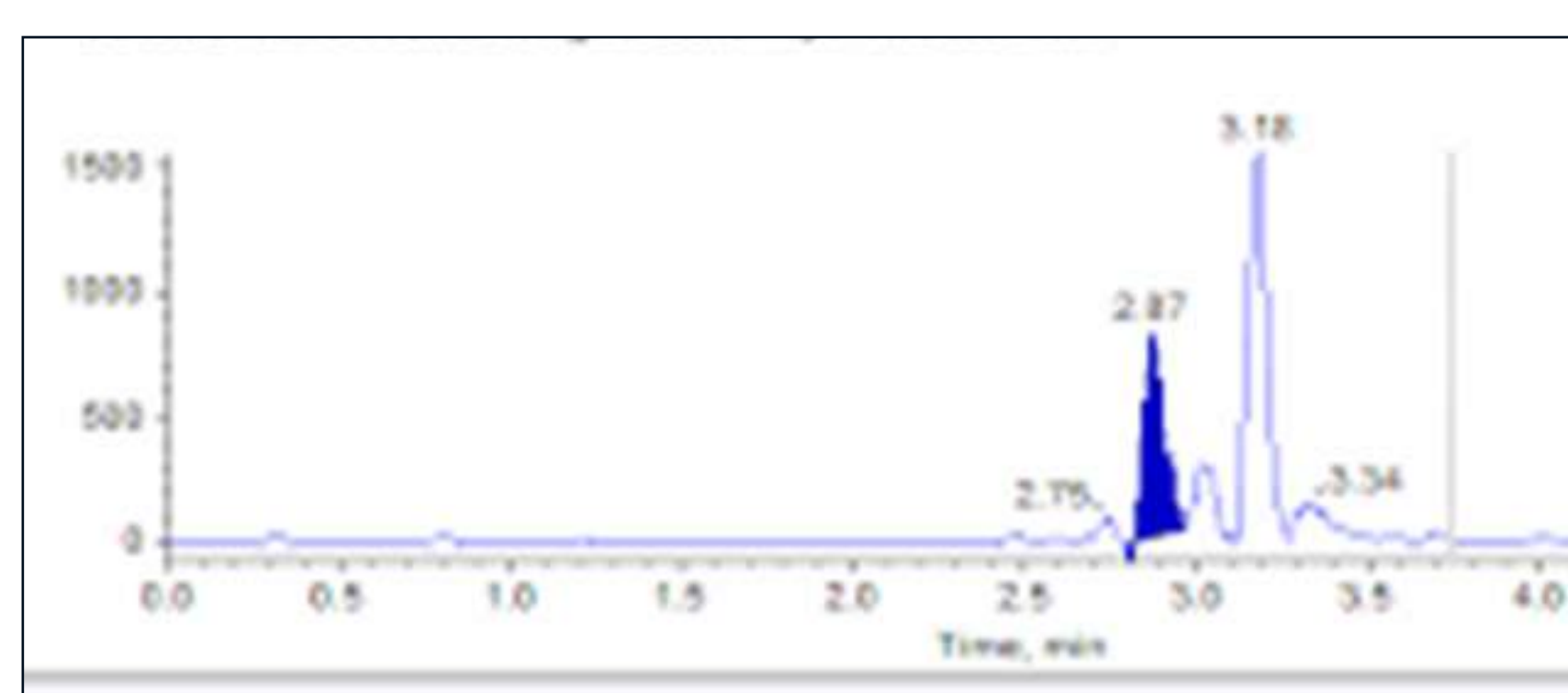
SPE-HLB; Waters Xbridge BEH; 4.6*50mm; 3.5μm; Isocratic;
Formic acid in H2O + Formic acid in ACN
Good peak shape and response.
Analyte eluted in void volume.

Trial 05



SPE-HLB; Waters Xbridge BEH; 4.6*50mm; 3.5μm; Gradient;
Formic acid in H2O + Formic acid in ACN
Split peak observed

Trial 06



SPE-HLB; Waters Xbridge BEH; 4.6*50mm; 3.5μm; Gradient;
Formic acid in H2O + Formic acid in MeOH
Good peak shape and response
Analyte retained; LLOQ 20 ng/mL