

## 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



### What were the challenges!

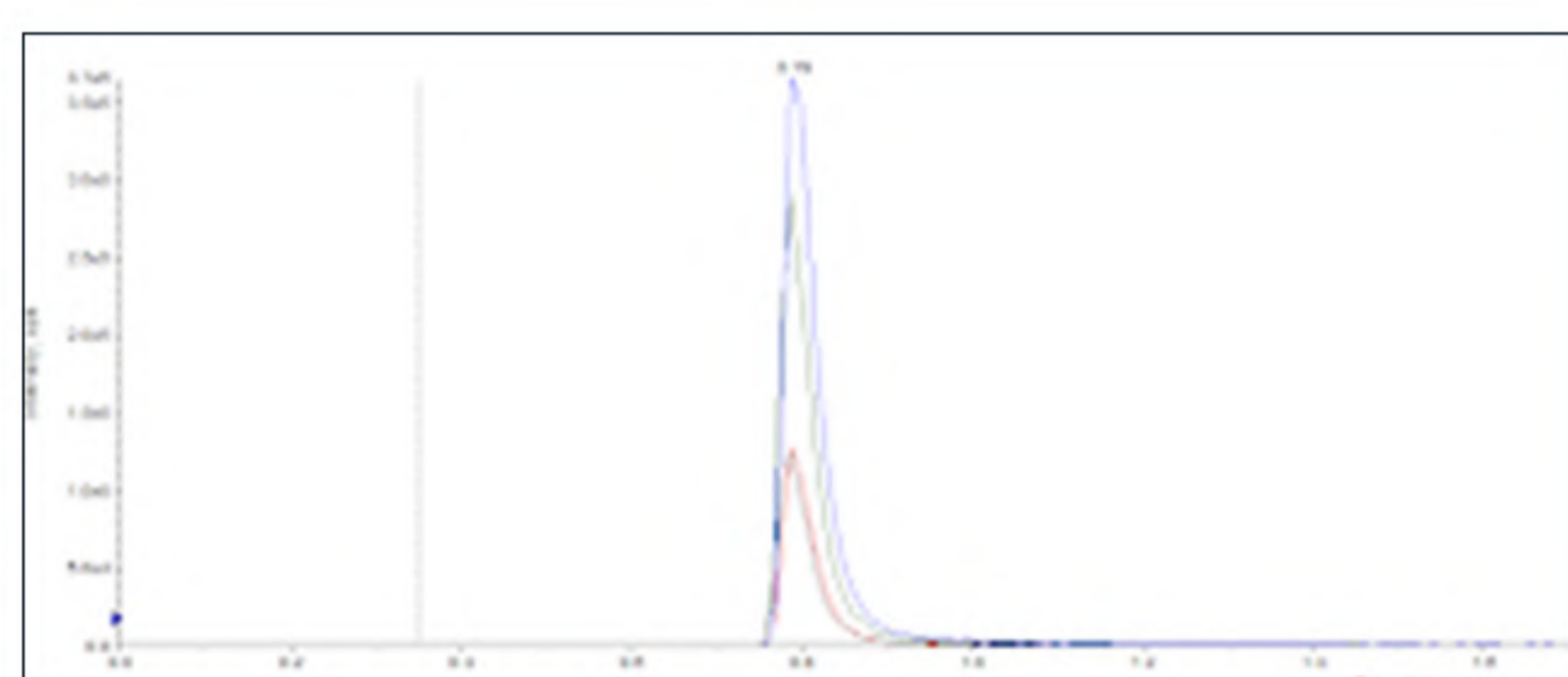
1. Low sensitivity - fragments generated were <200 amu
2. Matrix effect was occurring
3. There was low retention on column

## Aurigene's solutions:

1. Low sensitivity –
  - a. Different charge states were explored and the identified charged state with Q3 gave the maximum response
  - b. MS/MS parameters were hence optimized
2. Matrix effect –
  - a. Multiple SPE cartridges were tested
  - b. Preconditioning of HLB cartridges successfully helped clear the matrix effect
3. Low retention on column –
  - a. LC conditions were modified and different columns were used

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

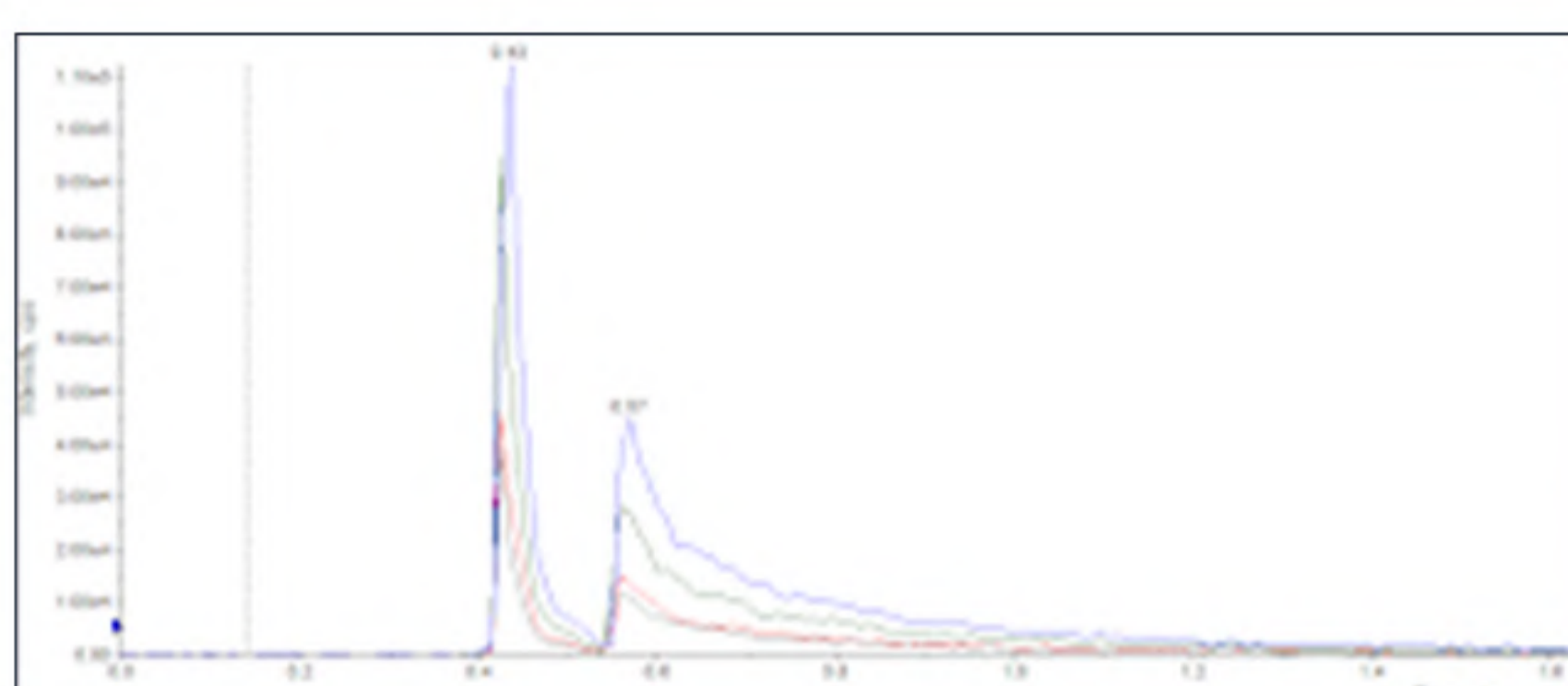
#### Trial 01



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

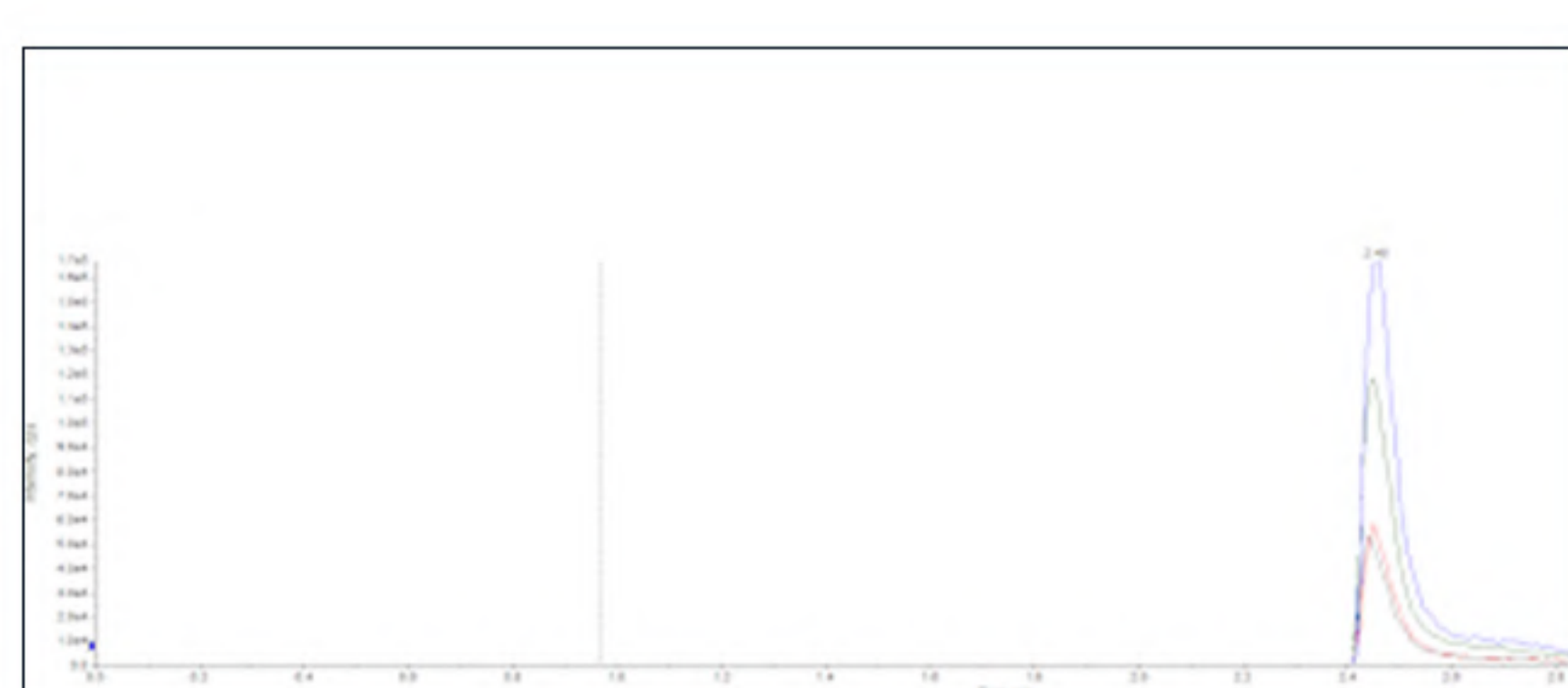
#### Trial 02



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

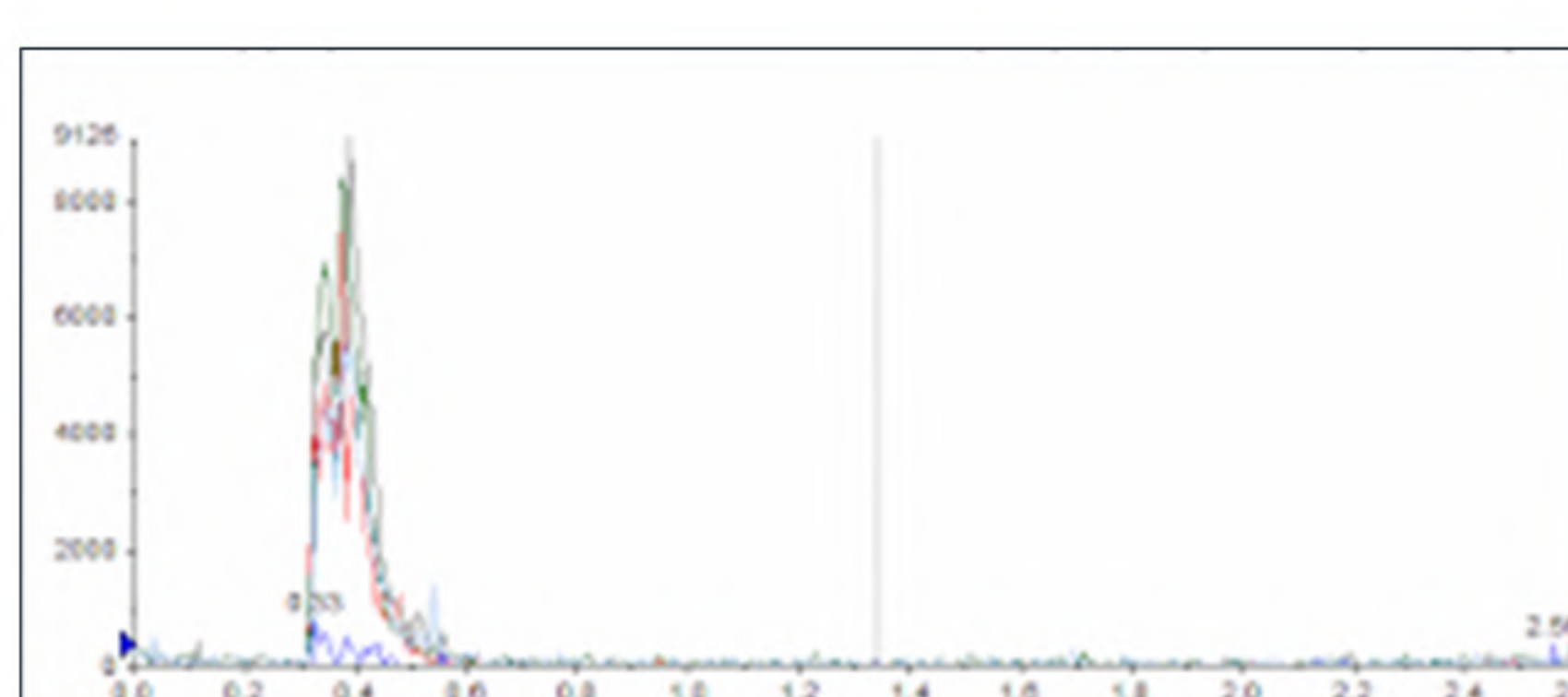
#### Trial 03



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

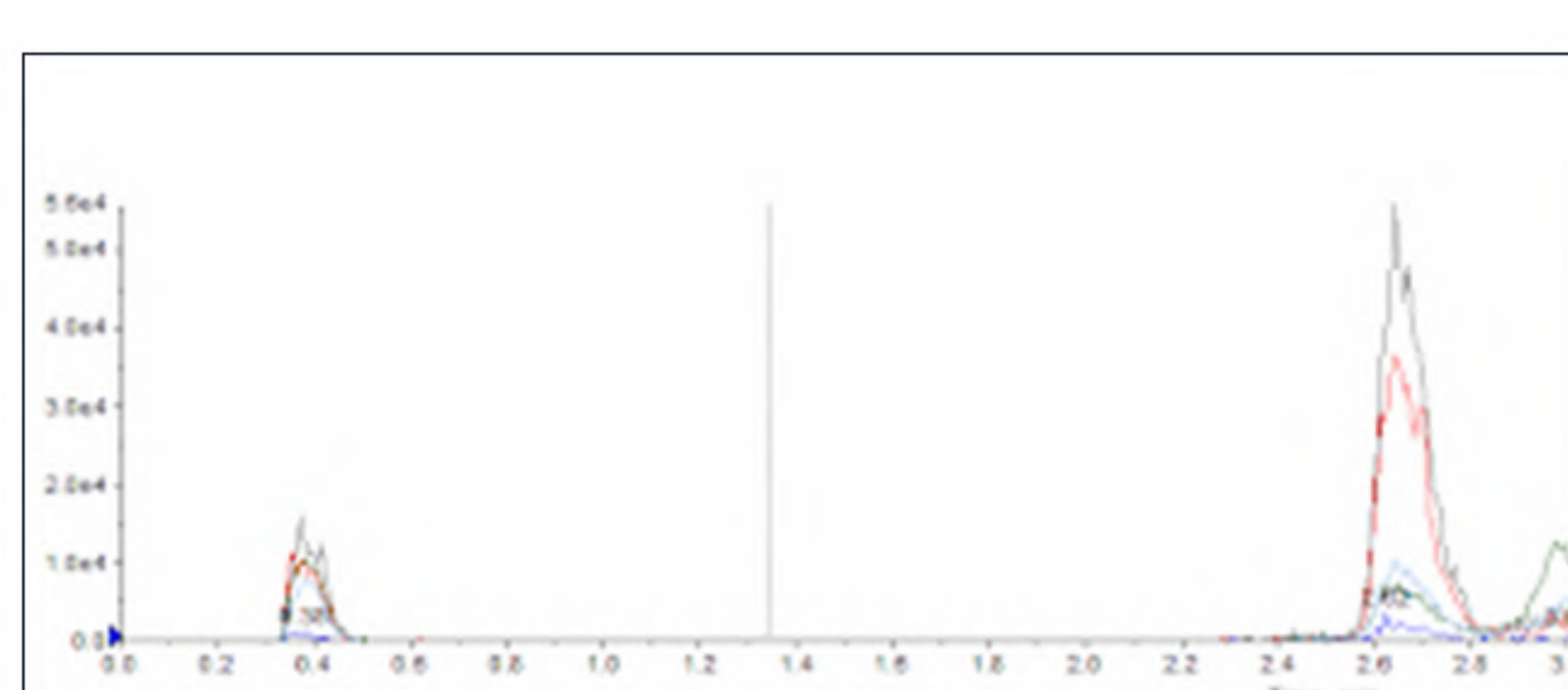
#### Trial 04



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.

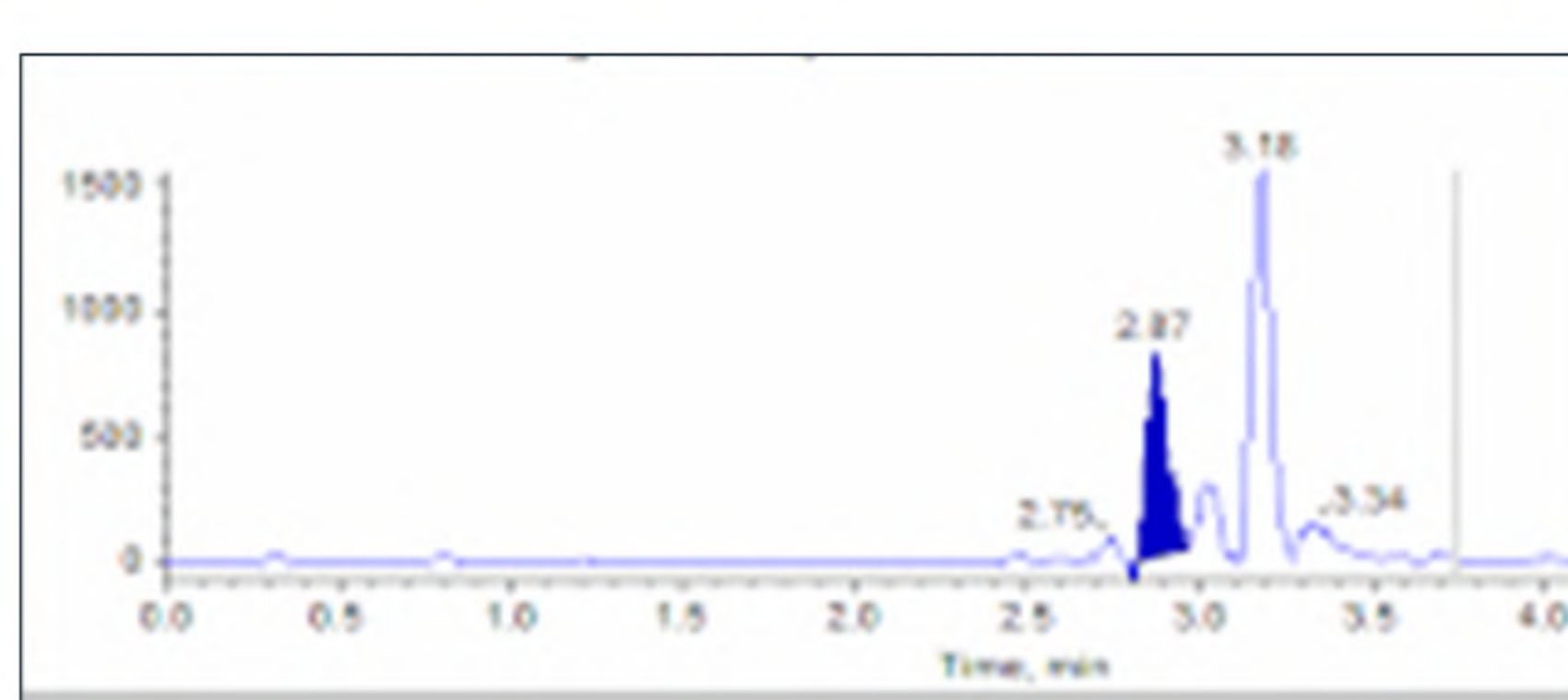
#### Trial 05



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