

# Microfraction for Automated 2-D Capillary LC

## INTRODUCTION

Multidimensional chromatography allows for the separation of highly complex sample mixtures and is typically used when the separation efficiency of a single dimension is not sufficient. An attractive route towards comprehensive 2-D analysis is collecting fractions from the first dimension and subsequently analyze them on a second column packed with a different stationary phase. Alternatively or in compliment, a different mobile phase can be used, too.

## CONDITIONS

Capillary LC separation of tryptic digested fetuin

Injection: 5  $\mu$ L full loop injection  
 Detection: UV at 214 nm (U-Z View™ Flow Cell)  
 Column: 300  $\mu$ m I.D. x 15 cm, C18, 3  $\mu$ m (both dimensions)  
 Flow rate: 4.5  $\mu$ L/min (Acurate™ Microflow Processor)

### 1st Dimension

Mobile phase: A: 15 mM NH<sub>4</sub>Ac in 5:95 ACN:water  
 B: 15 mM NH<sub>4</sub>Ac in 20:80 water:ACN  
 Gradient: 0–40% B in 36 min

### 2nd Dimension:

Mobile phase: A: 0.1% TFA in 5:95 ACN:water  
 B: 0.08% TFA in 20:80 water:ACN  
 Gradient: 5–60% B in 36 min

## RESULTS AND DISCUSSION

Injection,  $\mu$ -fractionation and analysis were performed fully automatically on a FAMOS™ micro autosampler, using one of the additional column switching valves. The needle that aspirates the sample in the injection mode is switched to the detector outlet capillary when fractionation is required, as shown by the system setup in Figure 1. The FAMOS is programmed to move the needle with a programmable time interval into a series of consecutive collection vials.

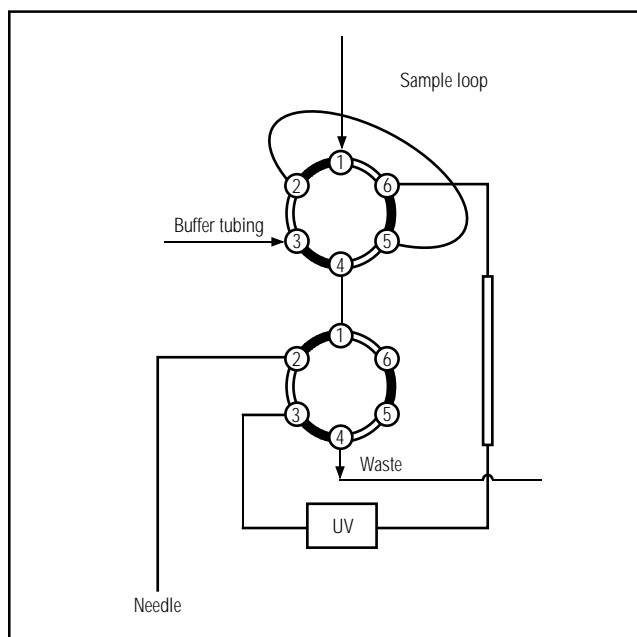


Figure 1. System setup for automated fractionation and 2-D analysis

The collected fractions from the first chromatographic dimension were automatically re-injected and chromatographed with a complementary mobile phase.

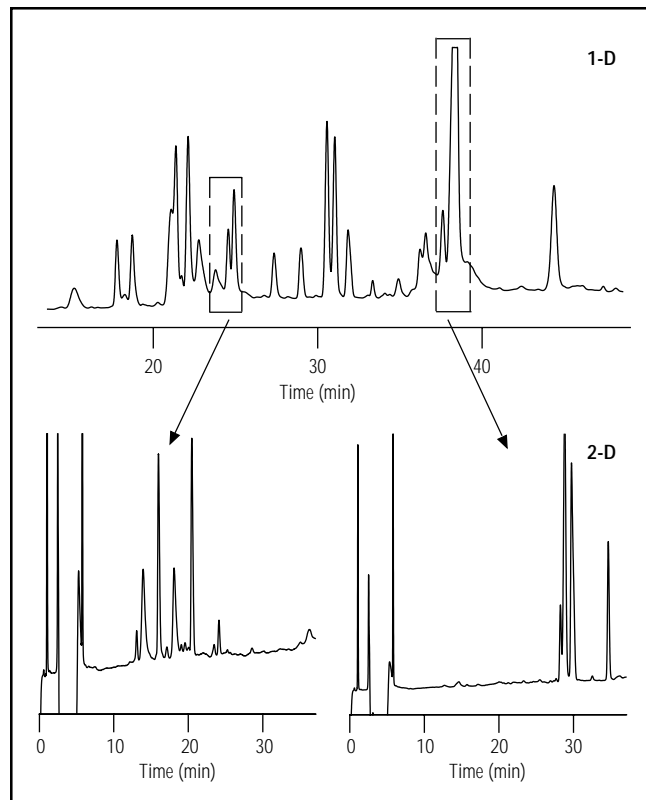


Figure 2.

Clearly, a different selectivity towards the peptide separation from the fetuin digest was obtained, as shown by the results in Figure 2.

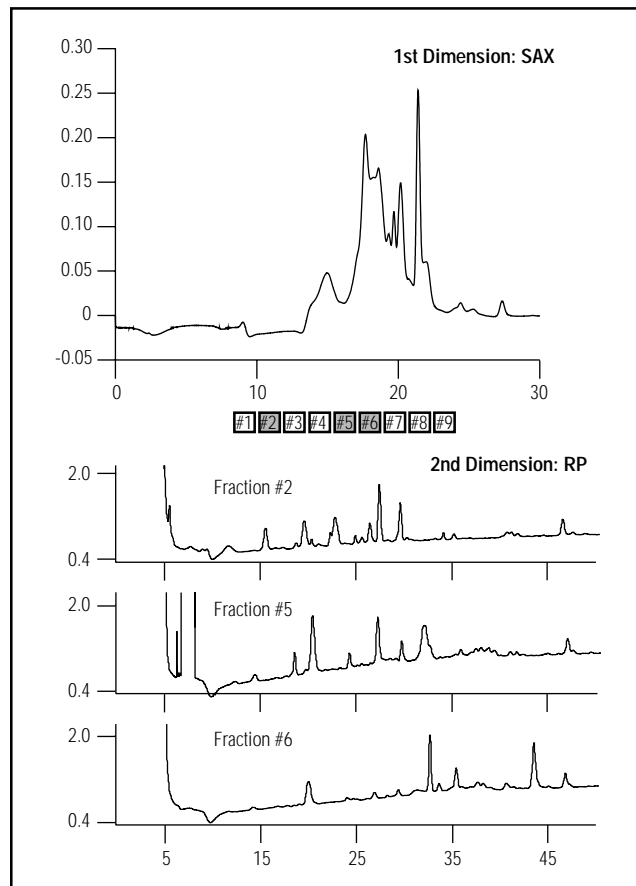


Figure 3.

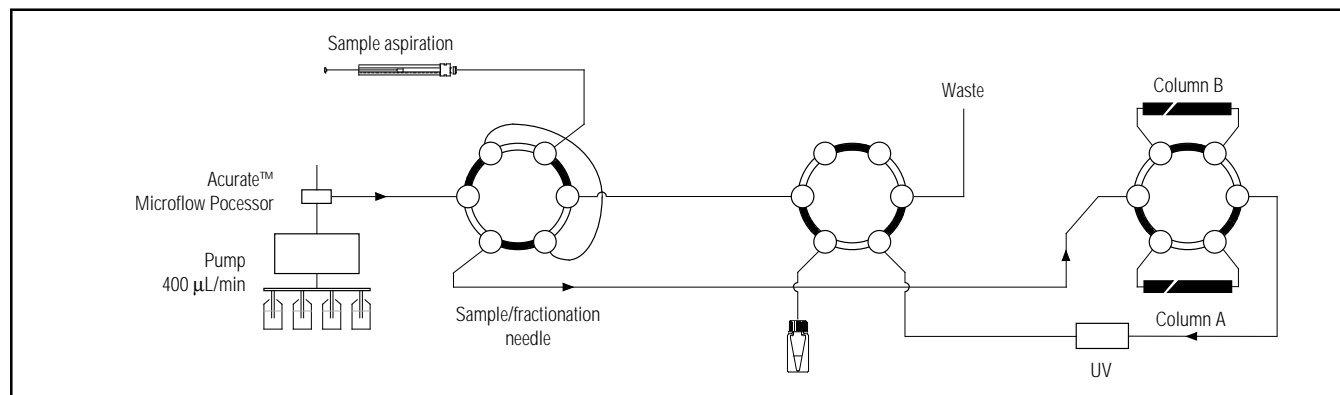


Figure 4. Fully automated 2-D capillary LC separations.



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\* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.



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