Automated capillary HPLC systems for mass spectrometric analyses of proteomic and metabolomic samples

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Overview

- High-throughput automated LC-MS/MS platforms targeted at the analysis of either proteomic or metabolomic samples have been designed and developed at PNNL for proteomics applications[1,2]
- Automated systems include: 4-column, dual mixer; 4-column, single mixer; 2-column, single mixer for metabolomics samples; and another 2-column single mixer for phosphoproteomic samples

Introduction

Proteomics relies heavily on capillary LC-MS/MS for protein identification and quantitation. Automation of these processes benefits proteomics in three main ways:

Throughput - As many samples as possible should be processed as rapidly as possible so that:

- Results can be obtained in a timely fashion
- 'Confounding factors', i.e., the effects of electronic drift in instrument electronics, can be minimized
- Maximum benefit of the funds invested in instrumentation can be obtained

Reproducibility – All samples can (ideally) be processed in an identical manner.

QA/QC - Quality assurance and quality control standards can be easily incorporated into the processing of large samples sets.

Fully automated capillary LC systems designed to tackle specific challenges have been developed in the laboratory:

- A multi-column, multi-mixer LC system with a ~100% duty cycle for the analysis of proteomic or metabolomic samples
- A multi-column high-throughput system for the analysis of 'simple' proteomic samples (e.g., the proteins obtained from a pulldown experiment, i.e., ~10 proteins)
- A dual-column system for the analysis of metabolomic samples
- A system used for the analysis of phosphoprotemic samples with non-metallic fluidic components

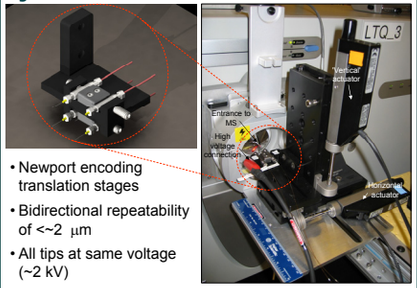
The throughput of all of these systems is increased by overlapping the processing of different samples. A system that utilizes multiple mixers is capable of achieving a duty cycle of nearly 100%.

Methods

System components

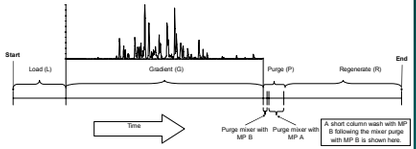
- Constant pressure systems that utilize a mixing bomb and a splitter which leads to an exponential gradient
- ISCO pumps capable of providing 10,000 psi (20,000 psi being evaluated)
- CTC HTS PAL autosampler
- VICI Valco valves rated to 5,000 psi or 10,000 psi
- Novel positioning system for the ESI emitters (described further below)
- Versatile LC-control software developed in-house
  - Controls any valve configuration as well as several peripheral devices
  - Allows integration of the LC system with a variety of mass spectrometers including: ThermoFinnigan LTQ, LTQ FT and LCQ, Bruker 9.4T FT-ICR, Micromass QTOF

Automated ESI-emitter positioning system



- Newport encoding translation stages
- Bidirectional repeatability of <~2 μm
- All tips at same voltage (~2 kV)

Steps of LC analysis



Steps in each LC separation process:

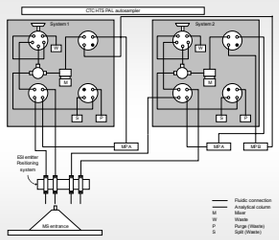
- Sample load onto the head of the analytical column (either directly or via an on-line SPE column)
- Separation using an aqueous-to-organic gradient
- Mixer purge following the gradient
- Column regeneration following the gradient

Results

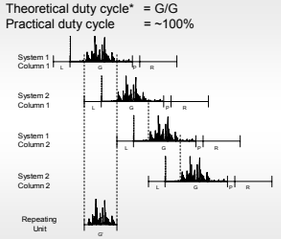
4-column, dual mixer[3]

Developed to provide a duty cycle of ~100%. **Proteomics or metabolomics**.

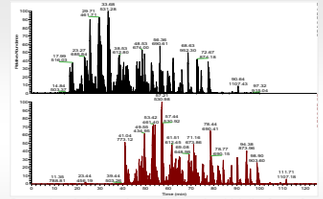
- Dead time at the beginning of the gradient is bypassed (i.e., "elution hopping").
- Mobile phases: A = H_2O with 0.2% acetic acid and 0.05% TFA, B = 90:10 Acetonitrile:H_2O (v:v) with 0.1% TFA
- 4 analytical columns
  - 65 cm x 360 μm x 50 μm
  - 3 μm diameter C_{18}-bonded particles
- ESI emitters chemically etched from fused silica capillary ~3-4 cm x 150 μm x 20 μm



Fluidic design of the dual-mixer, 4-column system



Timing diagram of the dual-mixer, 4-column system

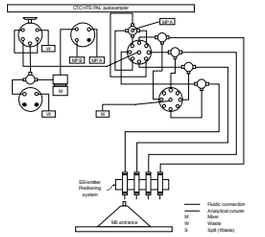


Comparison between chromatograms in which the front of the gradient has been bypassed ('elution hopping') and one in which the entire elution has been analyzed. Data was acquired on an LTQ ion trap. Relative LC elution times are globally aligned during data processing.

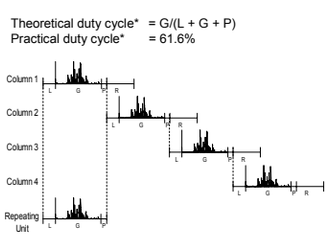
4-column, single mixer

Developed for fast analysis of **'simple' proteomic** samples (e.g., proteins obtained from a pulldown experiment)

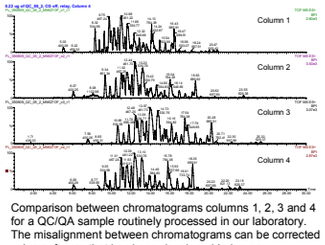
- Mobile phases: A = H_2O with 0.2% acetic acid and 0.05% TFA, B = 90:10 Acetonitrile:H_2O (v:v) with 0.1% TFA
- 4 analytical columns
  - 10 cm x 360 μm x 100 μm
  - 3 μm diameter C_{18}-bonded particles
- ESI emitters chemically etched from fused silica capillary ~3-4 cm x 150 μm x 20 μm



Fluidic design of the single mixer, 4-column system



Timing diagram of the single-mixer, 4-column system

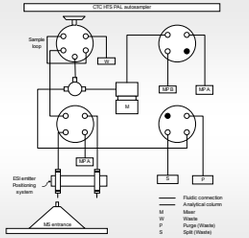


Comparison between chromatograms columns 1, 2, 3 and 4 for a QC/QA sample routinely processed in our laboratory. The misalignment between chromatograms can be corrected using software that has been developed in-house.

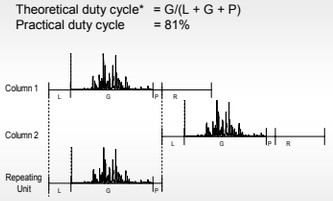
2-column, single mixer[3]

This system is dedicated to the analysis of **metabolomics** samples.

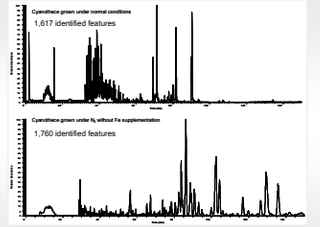
- Mobile phases: A = 10mM ammonium acetate in H_2O, B = 10 mM ammonium acetate in 90:10 Acetonitrile:H_2O v:v
- 2 analytical columns
  - 65 cm x 360 μm x 150 μm
  - 4 μm diameter C_{18} with imbedded polar group
- ESI emitters chemically etched from fused silica capillary ~3-4 cm x 150 μm x 20 μm



Fluidic design of the single mixer, 2-column system



Timing diagram of the single-mixer, 2-column system

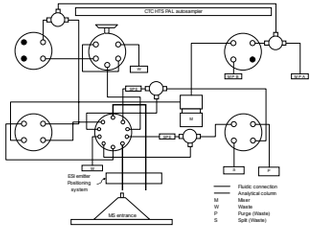


Comparison between chromatograms of Cyanothece grown under two different conditions.

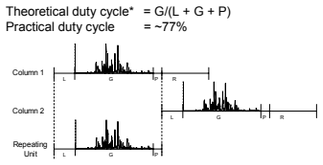
2-column, single mixer[4]

Developed for **phosphoproteomic** samples. Sample flow path is non-metallic except for stainless steel syringe of the autosampler.

- Mobile phases: A = 0.1 mM acetic acid in H_2O, B = 0.1 mM acetic acid in 30:70 H_2O:Acetonitrile (v:v)
- 2 analytical columns
  - 40 cm x 360 μm x 50 μm
  - 5 μm diameter C_{18}-bonded particles
- ESI emitter integrated into the end of the column. The final tip diameter is ~10 μm

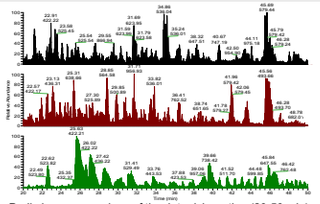


Fluidic design of the single-mixer, 2-column system that utilizes on-line SPE



Timing diagram of the single-mixer, 2-column system that utilizes on-line SPE

*Calculations of theoretical duty cycle do not include the time needed to alternate between ESI emitters or the time required to perform pump refills. Calculations of practical duty cycle are based on times typically used in our laboratory.



Preliminary comparison of the data rich portion (20-50 min) between LC-MS/MS analyses of Geobacter sulfurreducens grown with three different materials as the end electron receptor; fumarate (top), soluble Fe citrate (middle) and insoluble Fe oxide (bottom).

Conclusions

- A variety of automated systems that are appropriate for analysis of proteomic and metabolomic samples have been designed and developed:
  - 4-column, dual-mixer system with a duty cycle of ~100% that routinely operates at 10,000 psi with 50 μm i.d. analytical columns
  - 4-column, single-mixer system that is utilized for the analysis of 'simple' proteomic samples (e.g., that resulting from pulldown experiments)
  - 2-column, single-mixer system dedicated to the analysis of metabolomic samples
  - 2-column, single-mixer system that has a metal-free sample flow-path (other than the stainless steel syringe used by the autosampler) utilized for the analysis of phosphoproteomic samples
- Future directions will take advantage of recent technological developments that enable high pressure LC and the increased sensitivity provided by improved nano ESI emitters (see Poster #060 by Kelly, et al., ThP04)

Acknowledgements

Portions of this research were supported by the U. S. Department of Energy (DOE) Office of Biological and Environmental Research (Project 40601 and GTL:Genomes to Life Contract De-AC05-00OR22725) and by the NIH National Center for Research Resources (RR18522). Experimental portions of this research were performed in the Environmental Molecular Sciences Laboratory, a DOE national scientific user facility located at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. PNNL is a multiprogram national laboratory operated by Battelle for the DOE under Contract No. DE-AC05-76RLO 1830.

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