An Integrated Gradient Capillary HPLC/MS System Incorporating Photodiode Array Detection

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Drug Discovery and Development Strategy

◆**Concept**

	How
Target ID	Genomics & proteomics
Lead ID	Combinatorial chemistry/parallel synthesis High throughput screening (HTS)
Lead Optimization	Synthesis scale-up (mg) Structure-activity relationships (SAR)
Lead Validation	PK/ADME screens

◆**Clinic**

Who Uses Capillary Chromatography?

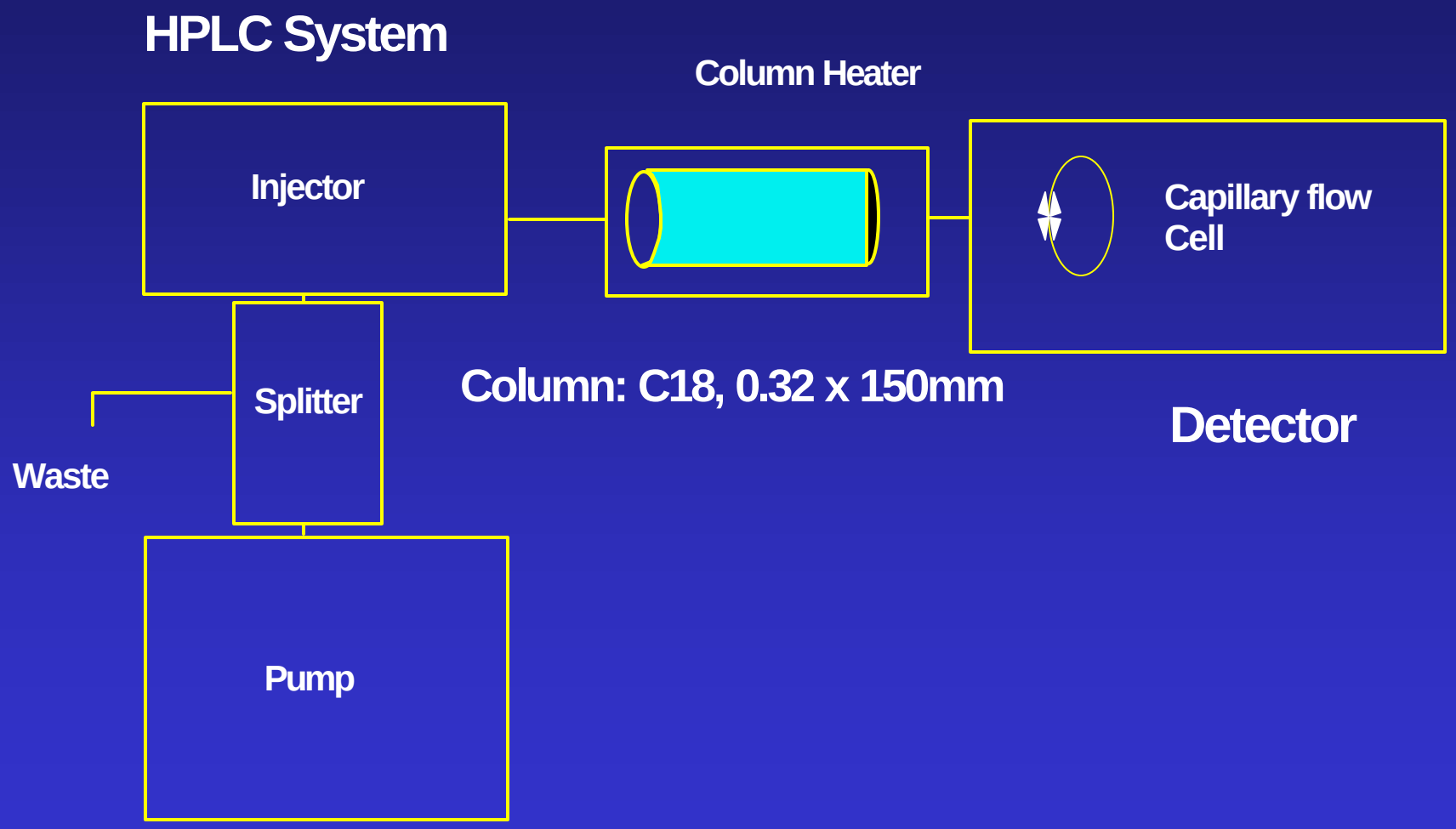
- **Proteomics/Biotechnology Laboratories**
  - Running 2-D gel electrophoresis
  - Performing library searching
  - Analyzing peptides by LC/MS

- **Pharmacokinetics Laboratories**
  - Using high potency drugs
  - Doing cassette dosing of drugs
  - Wishing to reduce the numbers of test animals

Why Switch to Capillary LC?

- Analysis Sensitivity
  - Microanalytical
  - Microprep
- Sample Limited
- Excellent MS Compatibility, Especially ESI
- Small Fraction Volume

Flow Diagram for a Typical Split Flow System



Current System Limitations

- Systems are not designed for microanalysis
  - Often use analytical LC with flow splitting
  - Assemble their own custom system
- Inconsistent instrument performance
- Sub-optimal flow cell design

What is Waters CapLC System?

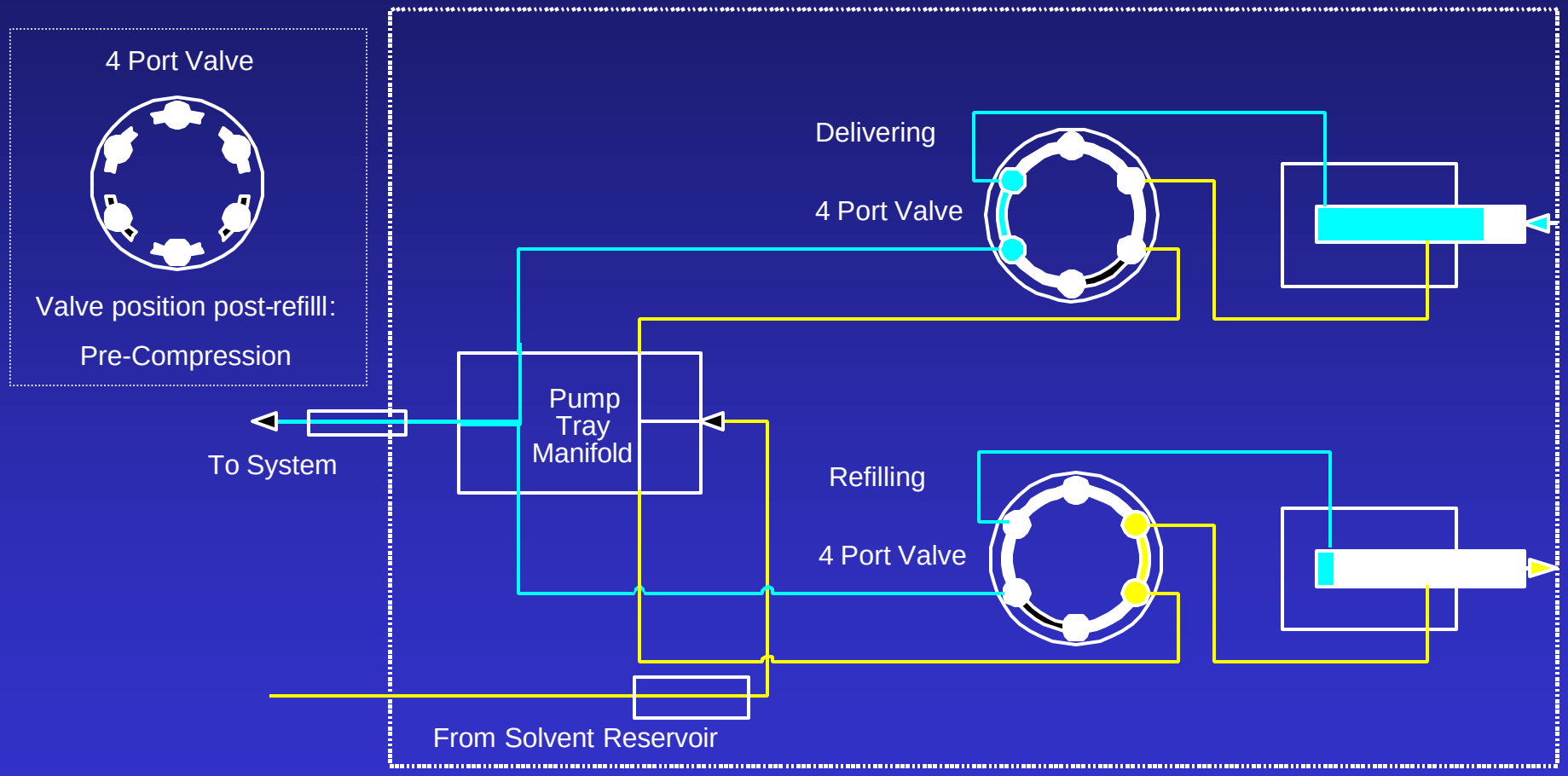
- Fully integrated, automated system with PDA detection that has been optimized for LC/MS(MS) applications
  - Innovative multisolvent delivery
  - Automated sample injection
  - Integrated capillary column heater
  - Photodiode array detection
  - MassLynx instrument control

Challenges of Low Flow System

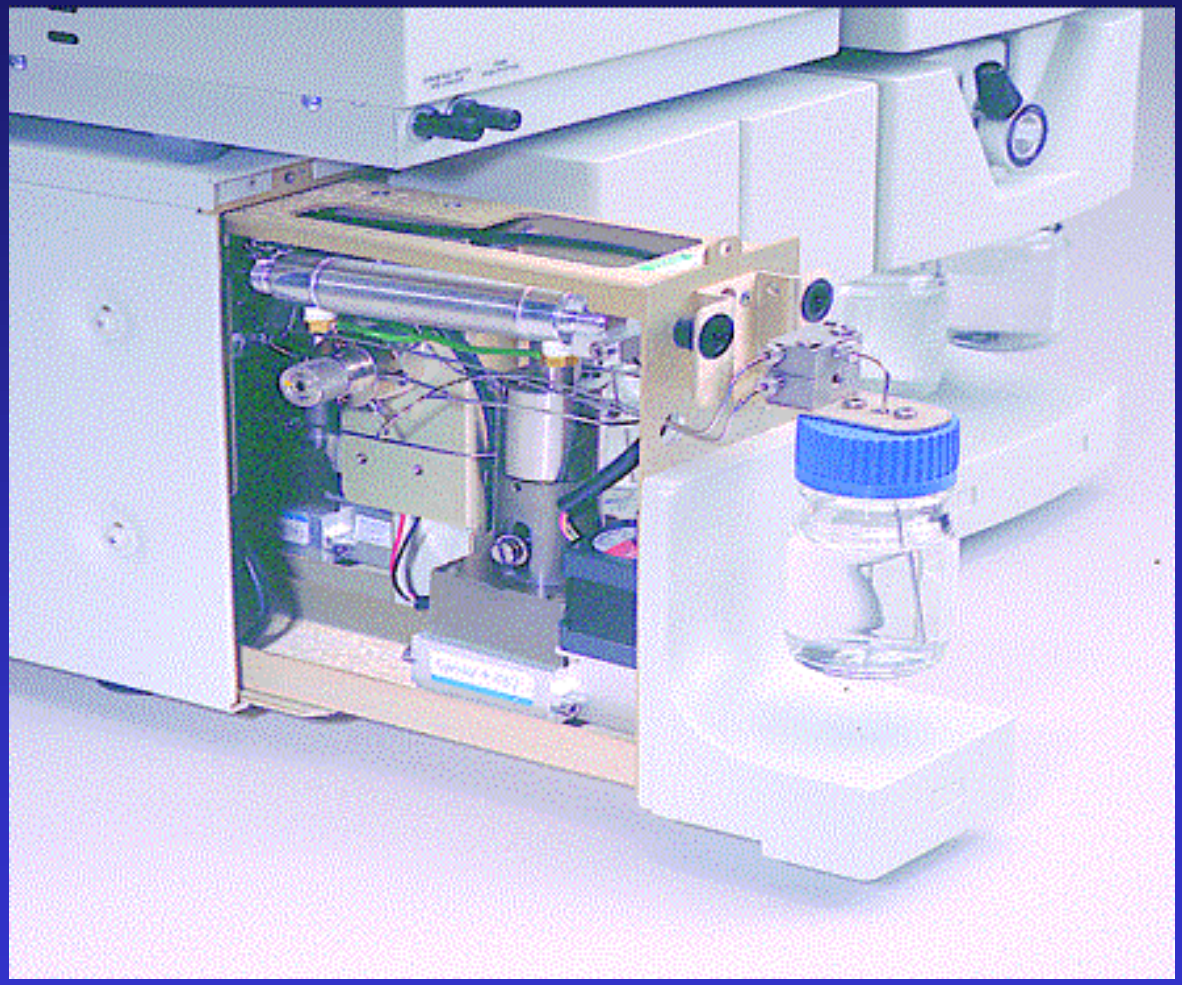
- Accurate, Precise Flow Delivery
- Low System Volume
- Gradient Difficulties
- Mixing Challenges

Pump Tray Schematic

Single Pump Fluid Path



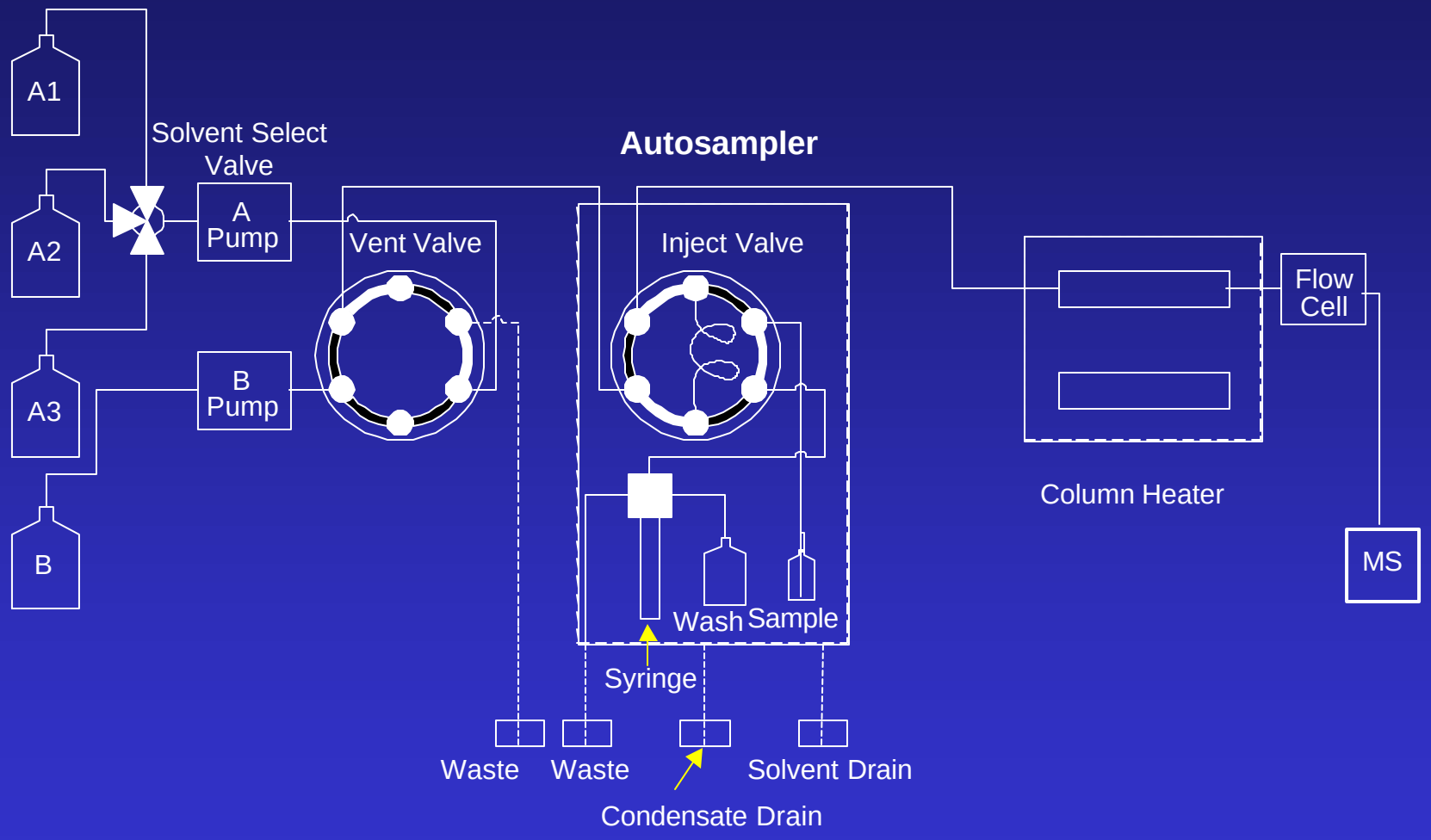
Solvent Delivery Module



Solvent Delivery Features

- Each solvent delivery module consists of two syringe pumps and active check valves controlled by embedded software
- Each syringe delivers 180 ml solvent
- Firmware control for solvent precompression following syringe refill and check valve actuation
- Inject synchronization enabled in software
- Firmware-embedded diagnostics
- Flow rate range for gradients @ 1 - 40 ml/min, isocratic to 200nl/min

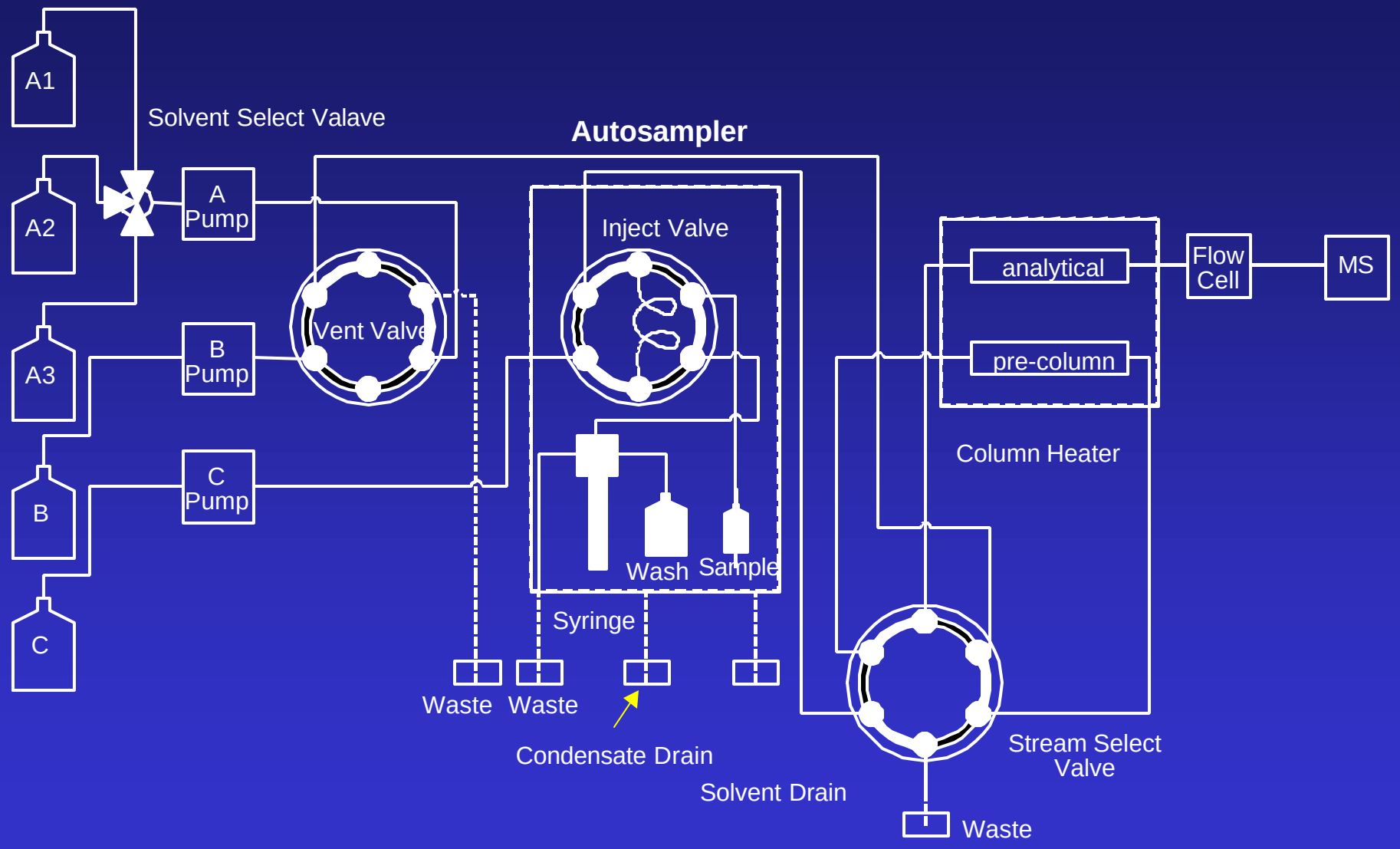
Binary Gradient System



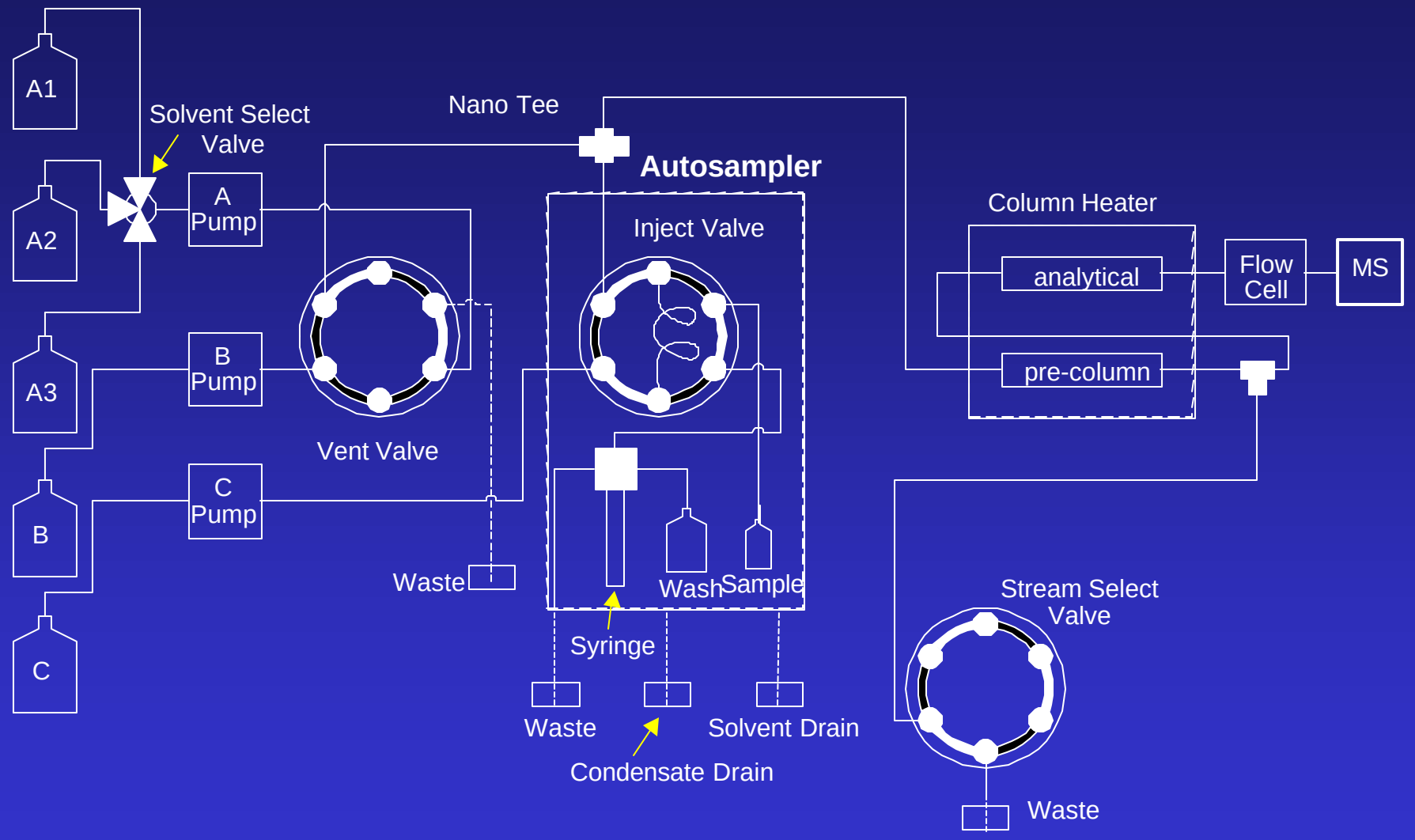
System Details

- Third solvent delivery module option for ternary gradient or solvent addition
  - Modular design enables plug-in installation
- Automated 3-solvent select valve upstream of A pump
- Automated vent valve
- System volume <12 µl
- Microvolume mixer option
  - <5 µl additional volume

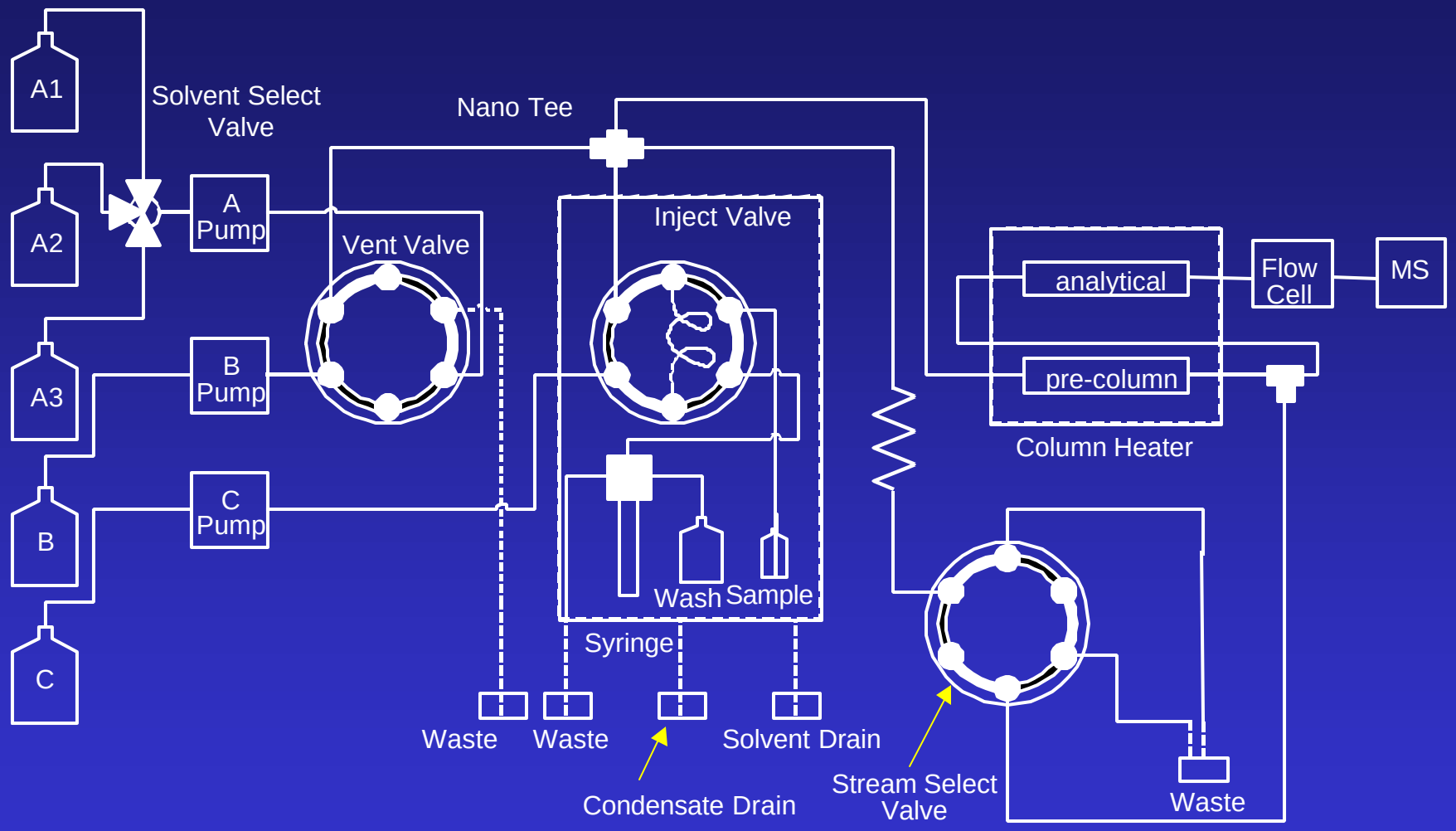
Sample Focussing with Backflush



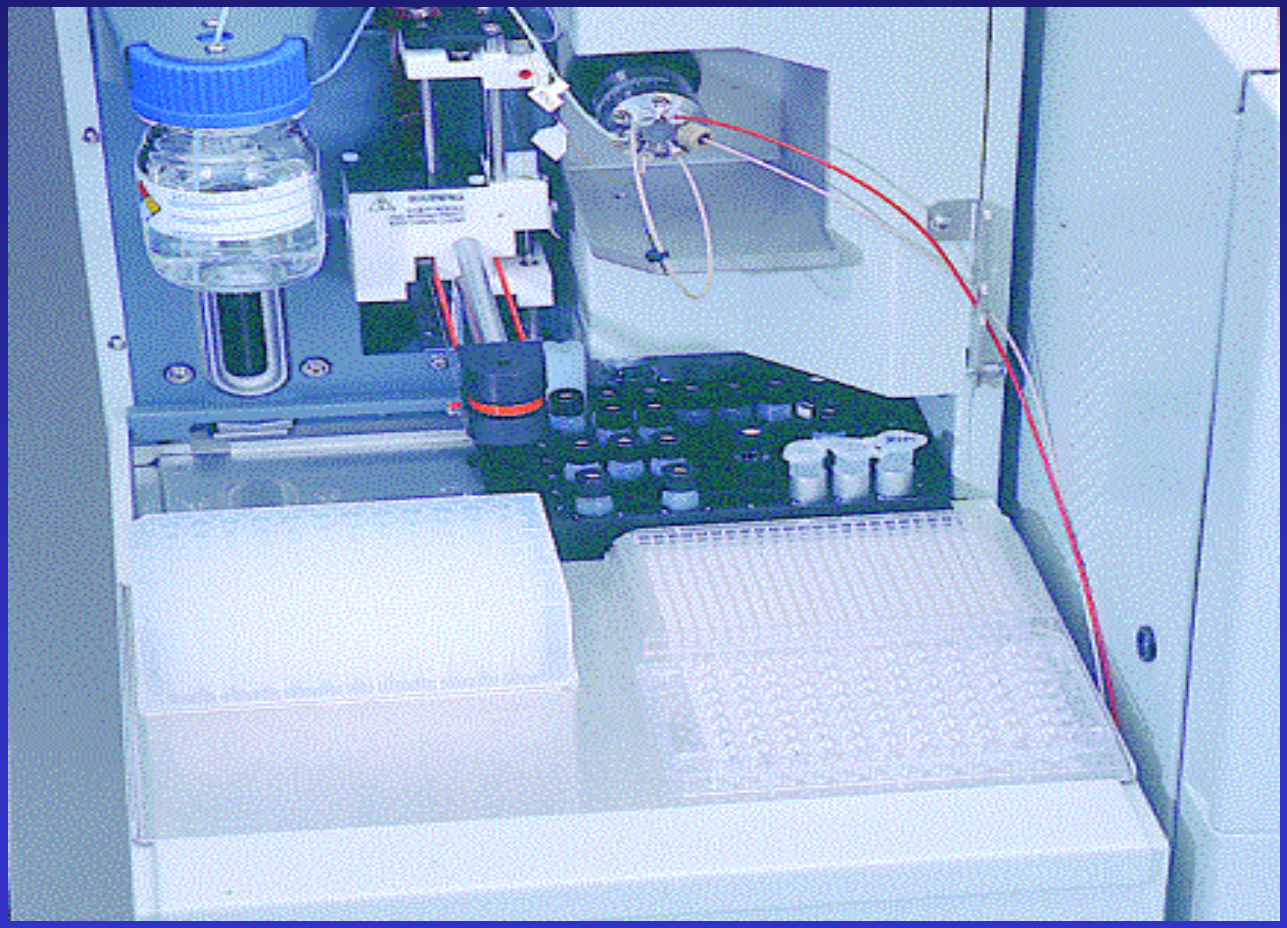
Sample Focussing/Cleanup: Microflow



Sample Focussing/Cleanup: Nanoflow



Sample Injection

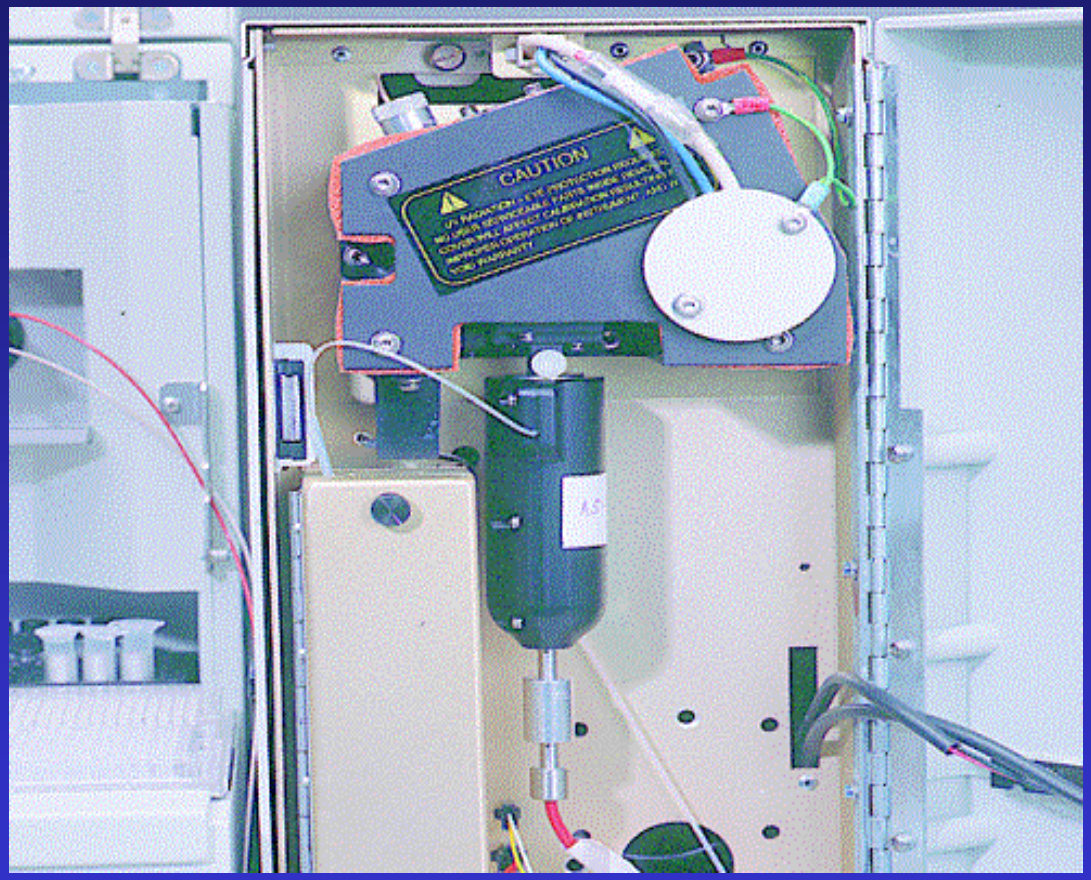


Sample Injection

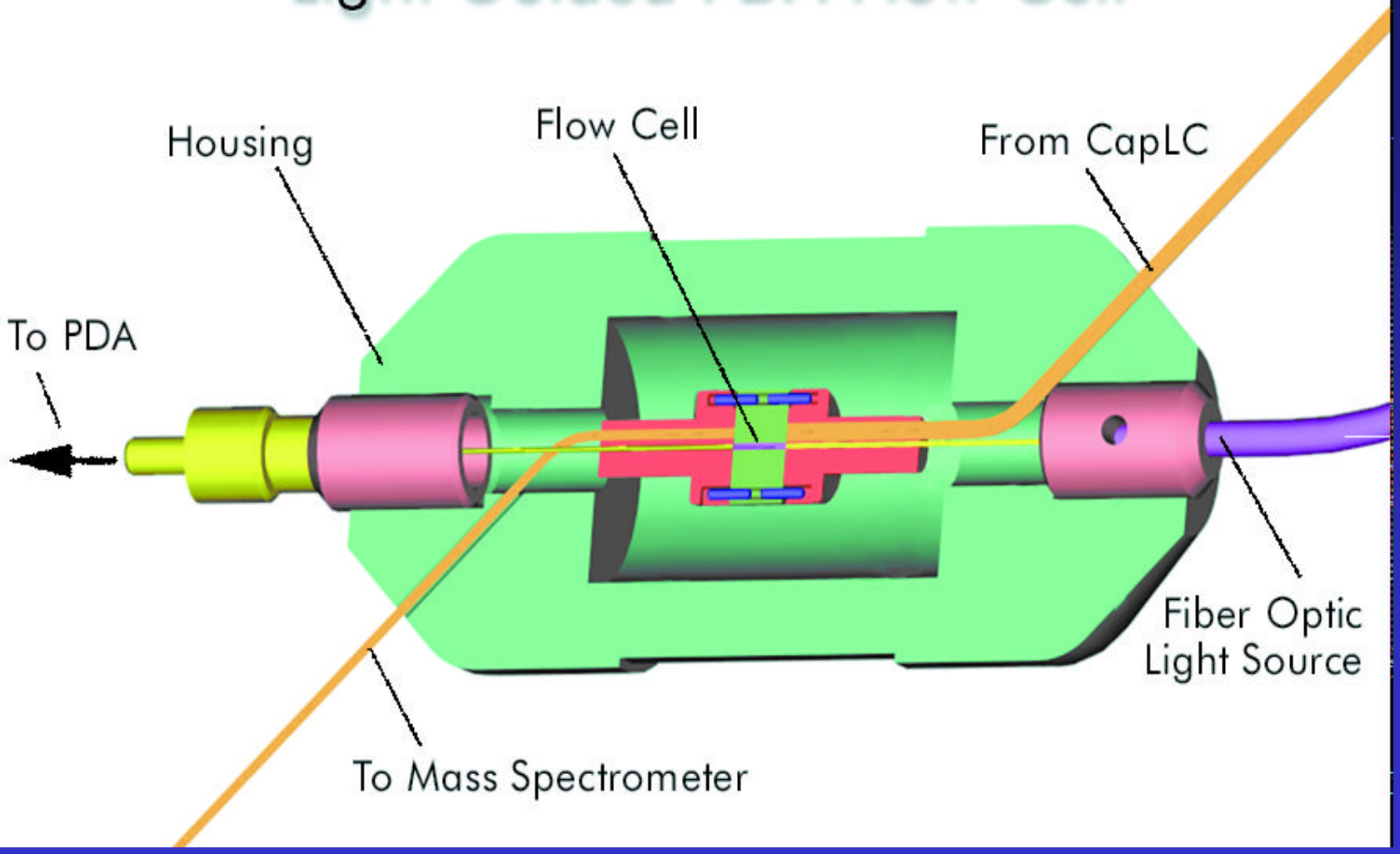
- **Flexible format vial positioning system**
  - **(1) 96- or 384-well microtiter plate**
  - **Tray for 48 x 1.5mL vials**
  - **Eppendorf tubes**

- **Low volume injection capability**
  - **Piercing needle for opening sealed well or vial**
  - **Protected insertion of injection needle**
  - **Fused silica capillary**
  - **Needle travels to bottom of vial for microsampling**
  - **Spring-loaded retainer holds well or vial in place**
  - **µl-Pickup routine enables injection from 1 µl**

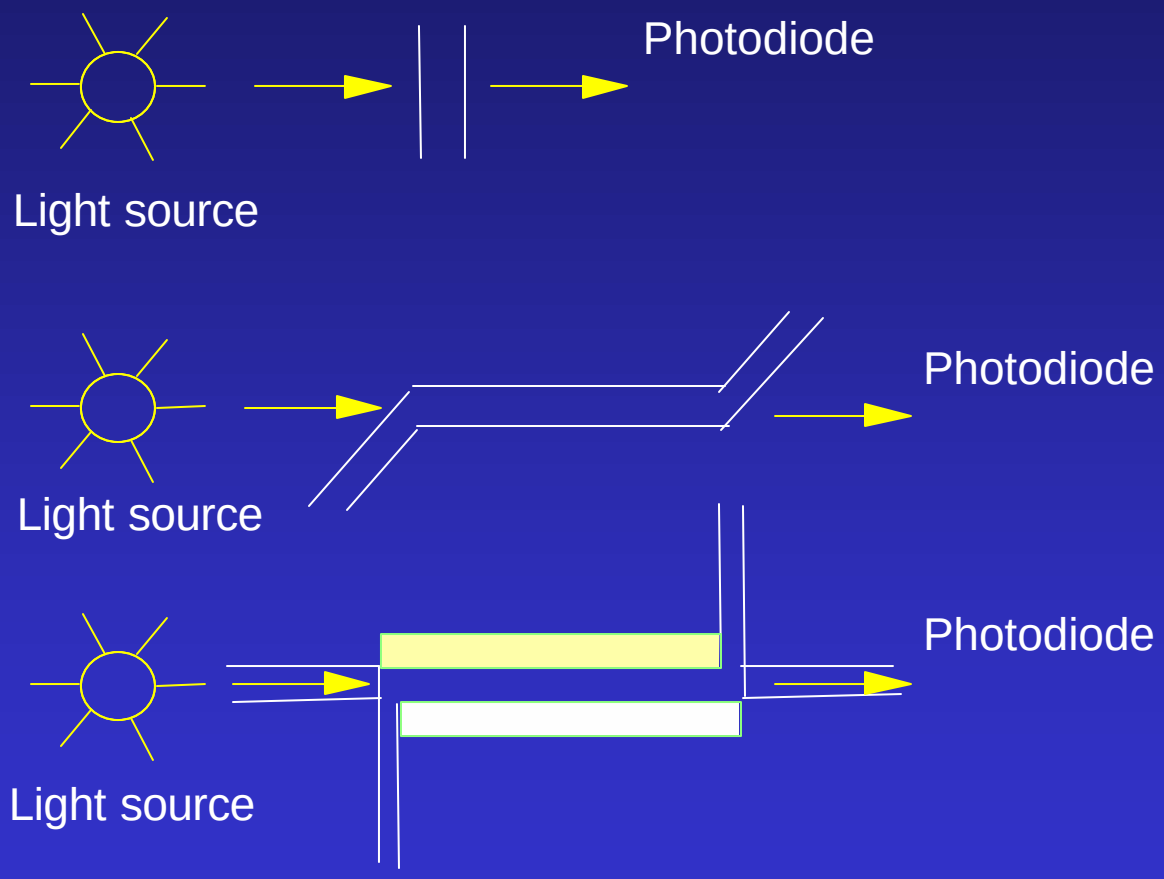
Photodiode Array Detection



Light Guided PDA Flow Cell



Evolution of Capillary Detector Design



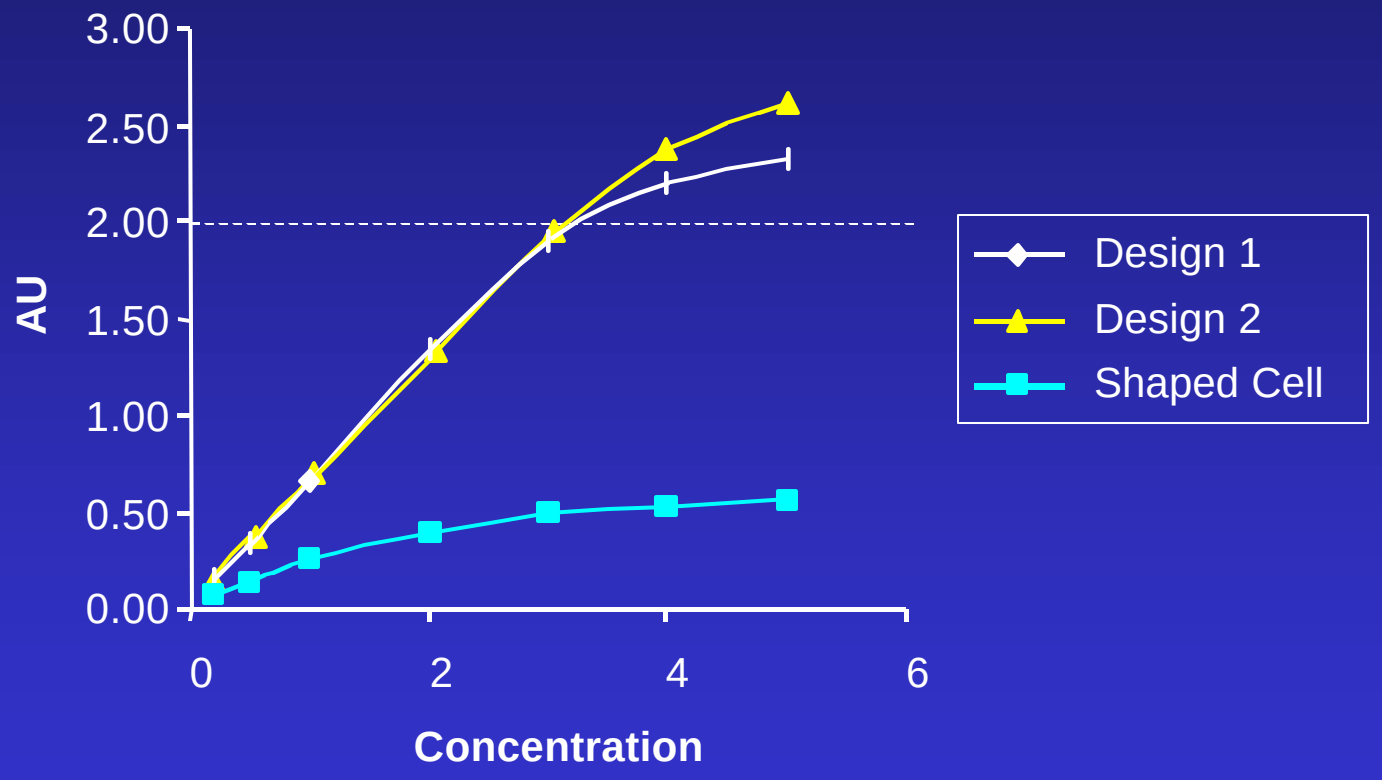
Transverse Illumination
- short pathlength
- low sensitivity

Shaped Cell
- long pathlength
- better sensitivity
- stray light
- poor linearity

Light Guided
- long pathlength
- good linearity
- near theoretical signal/noise

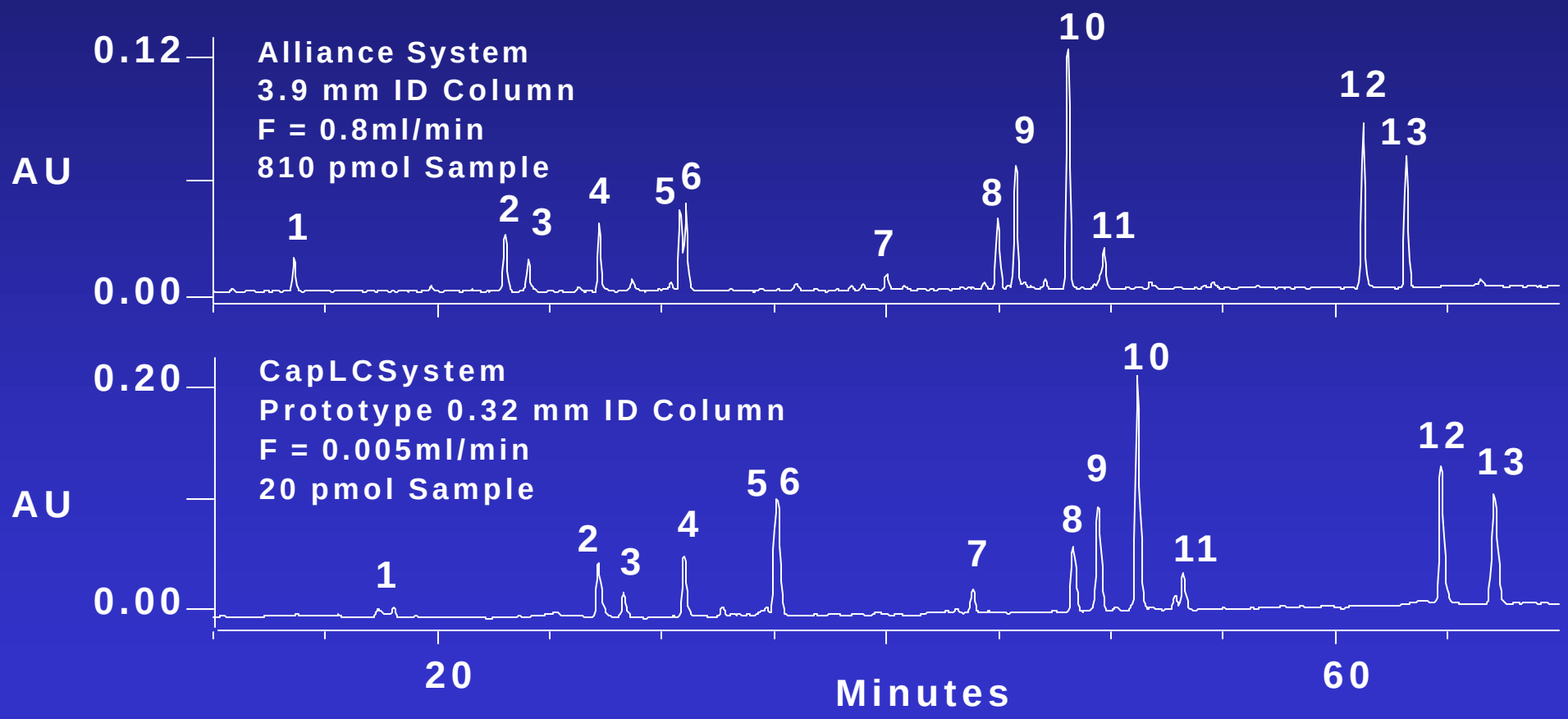
Comparison of Flow Cell Performance

Linearity at 205 nm

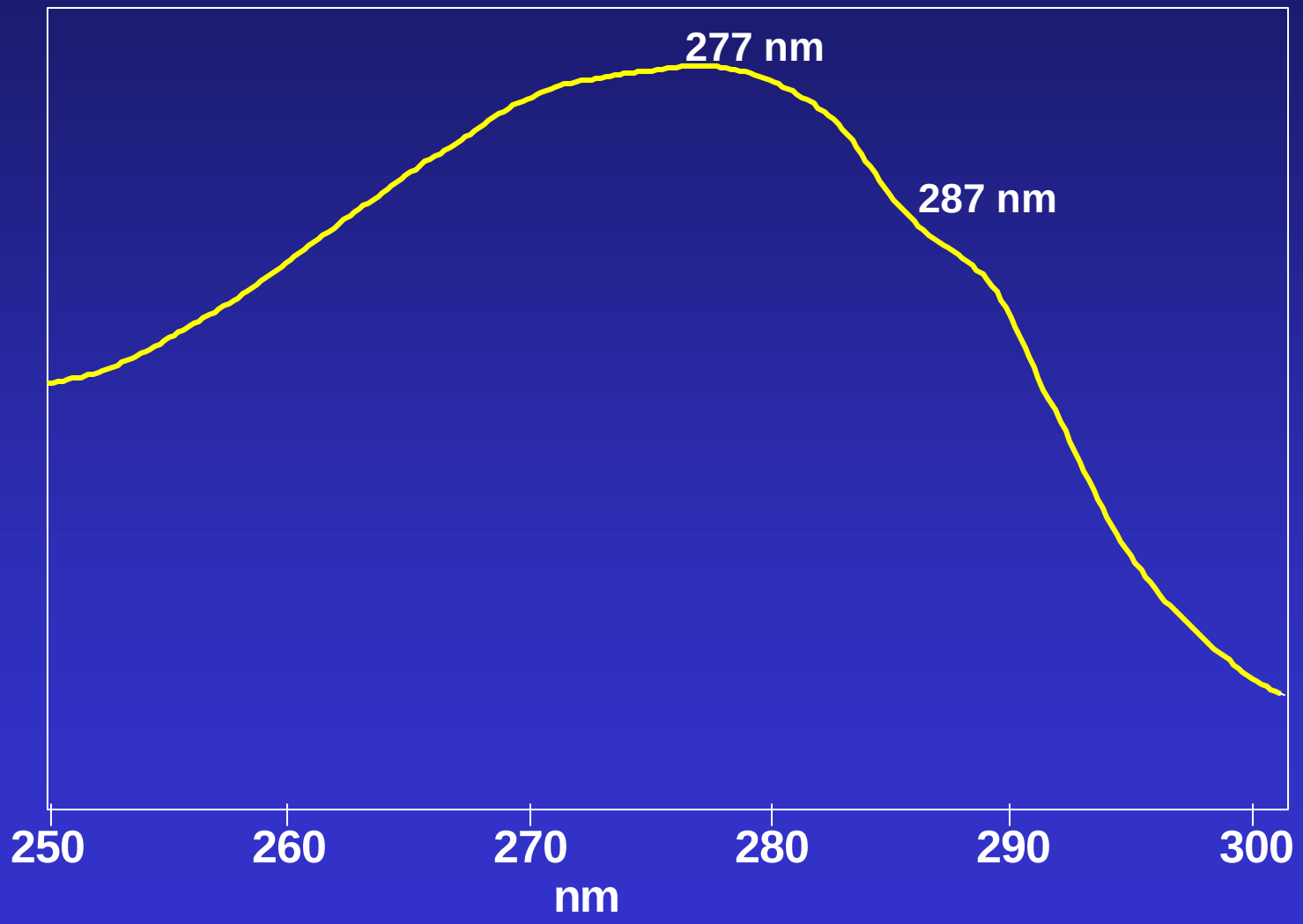


Comparison of Capillary to Standard Chromatography

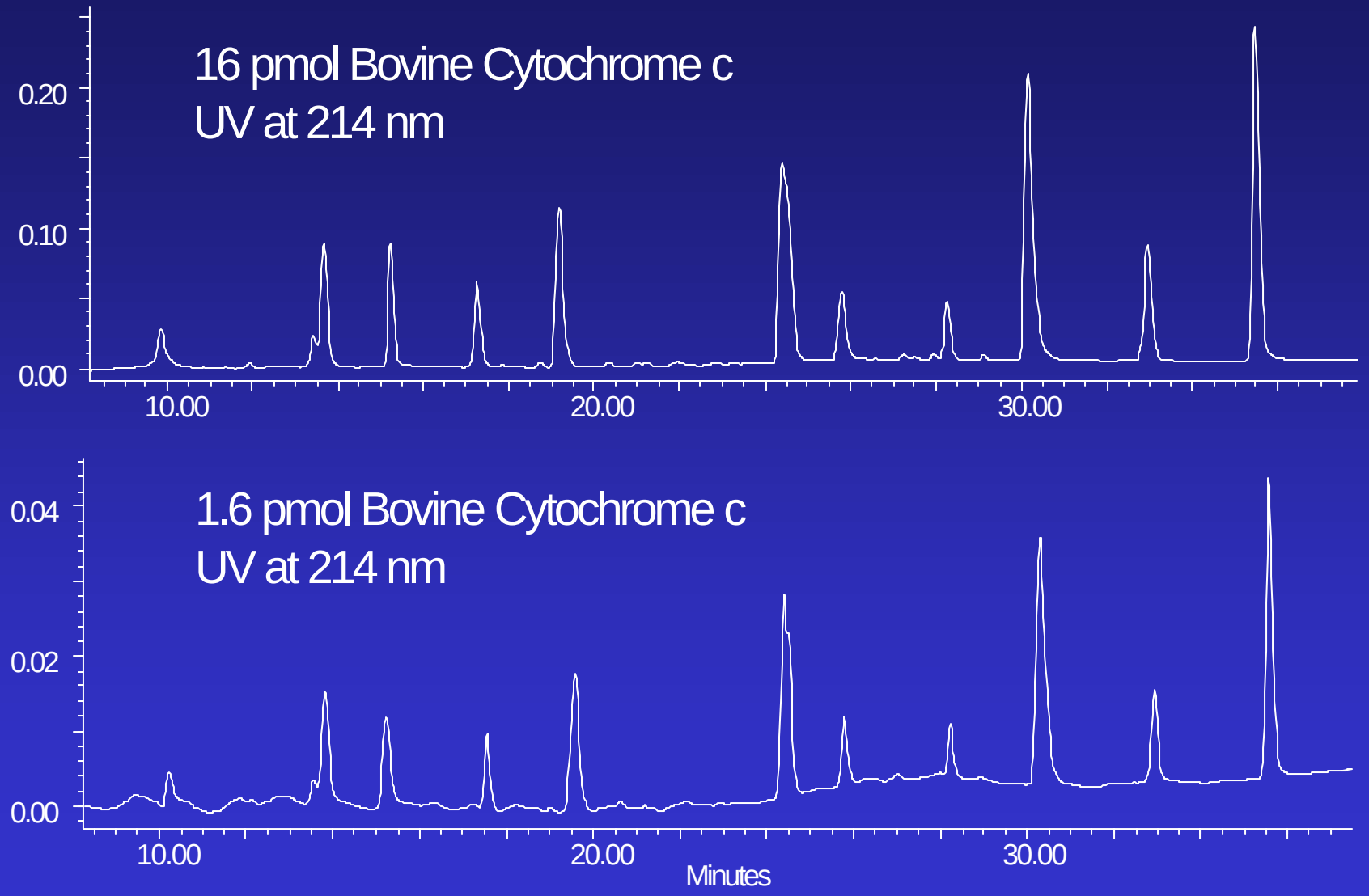
Columns: Waters Symmetry300™ Wide Pore



T13 - Trp Peptide Spectrum

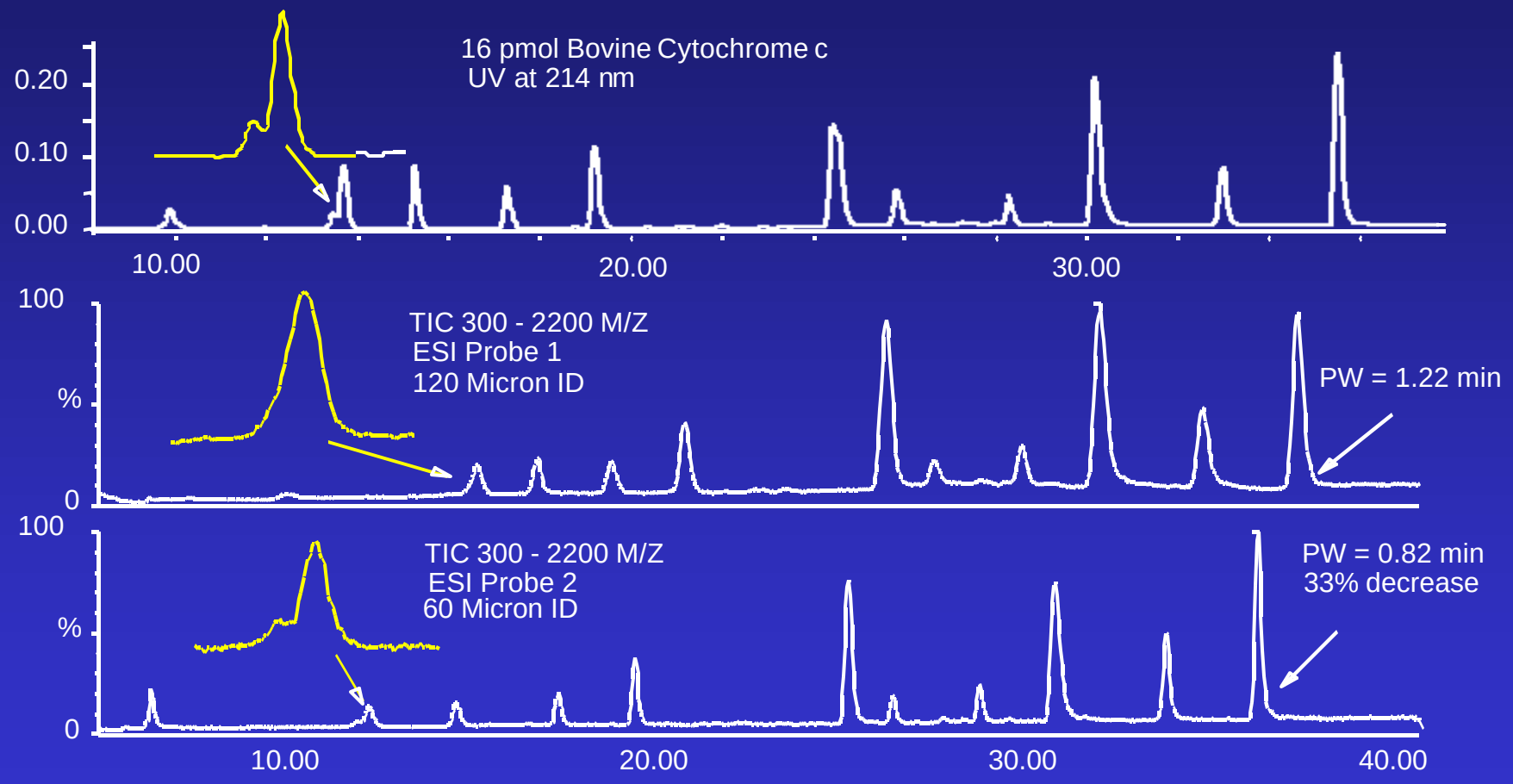


High Sensitivity UV Analysis

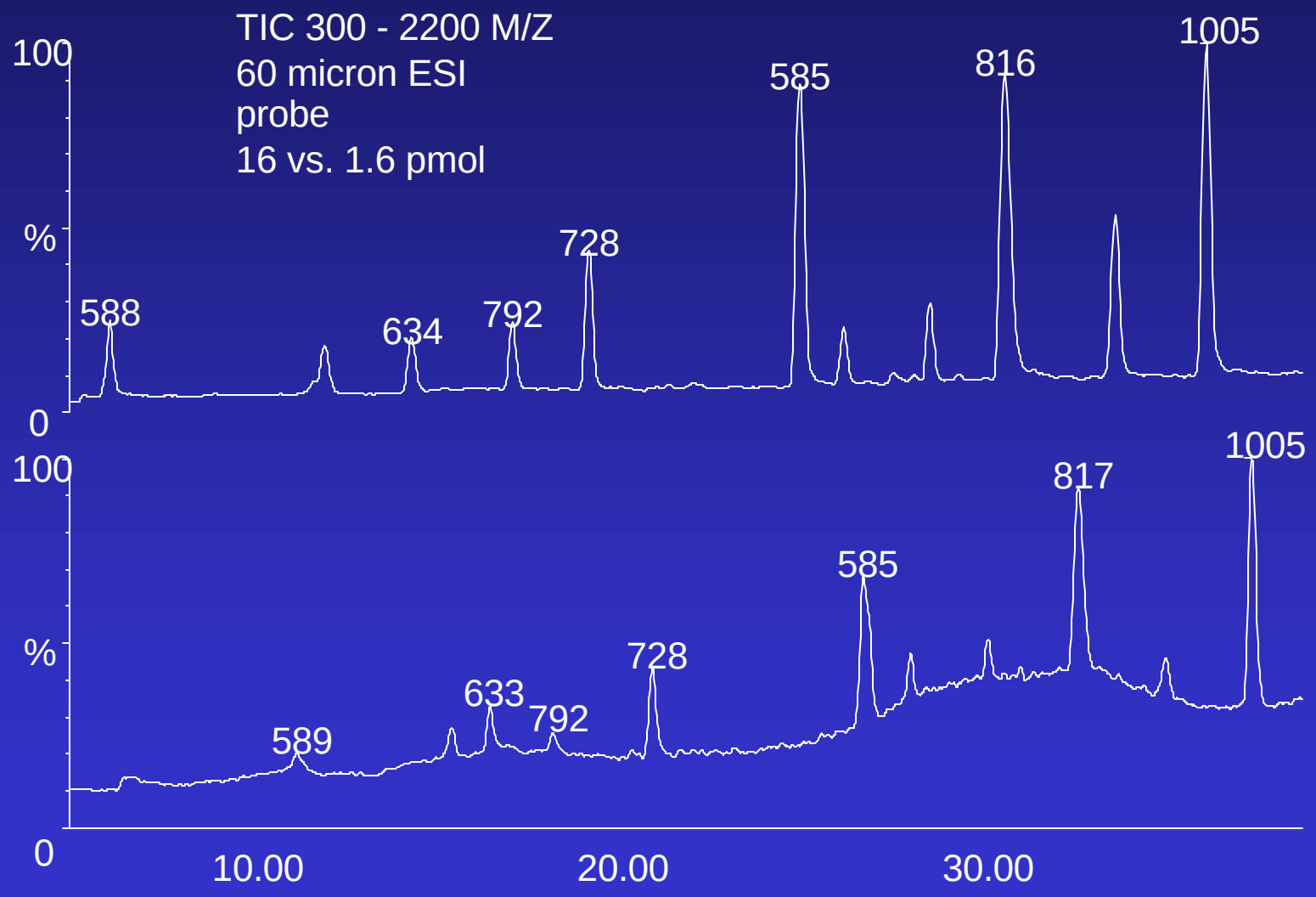


Capillary LC/MS:

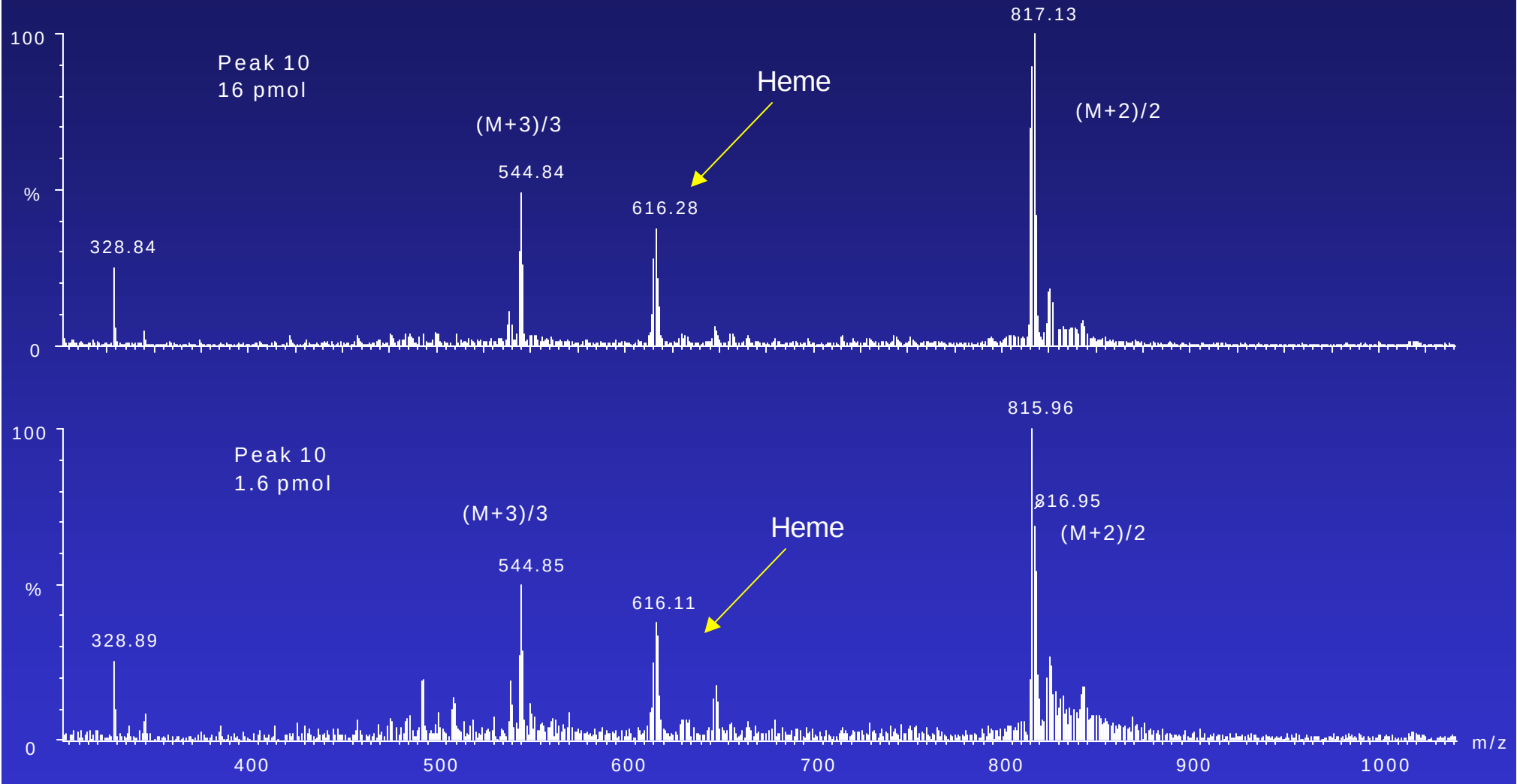
Analytical vs. Capillary ESI Probe



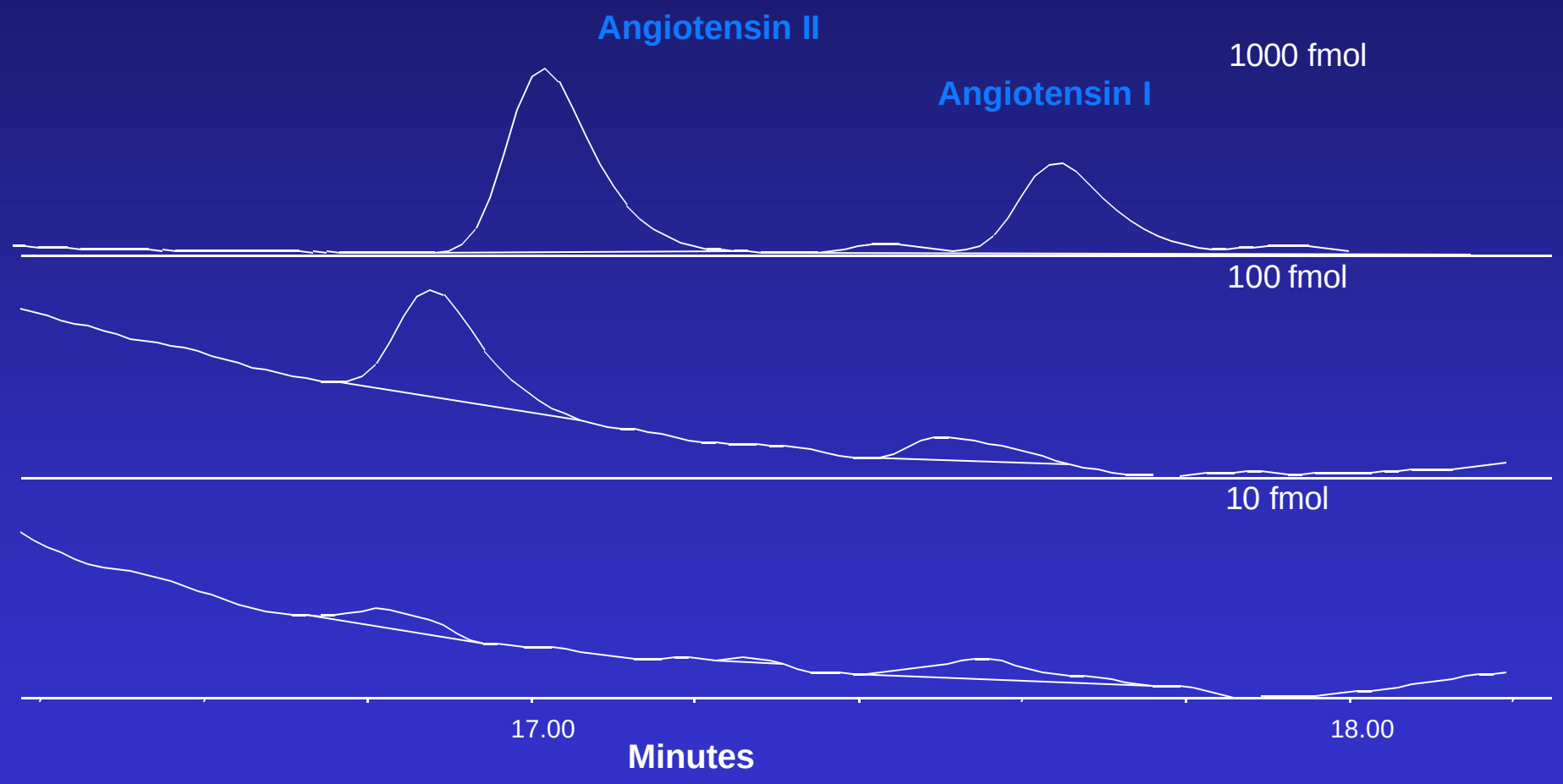
High Sensitivity MS Detection



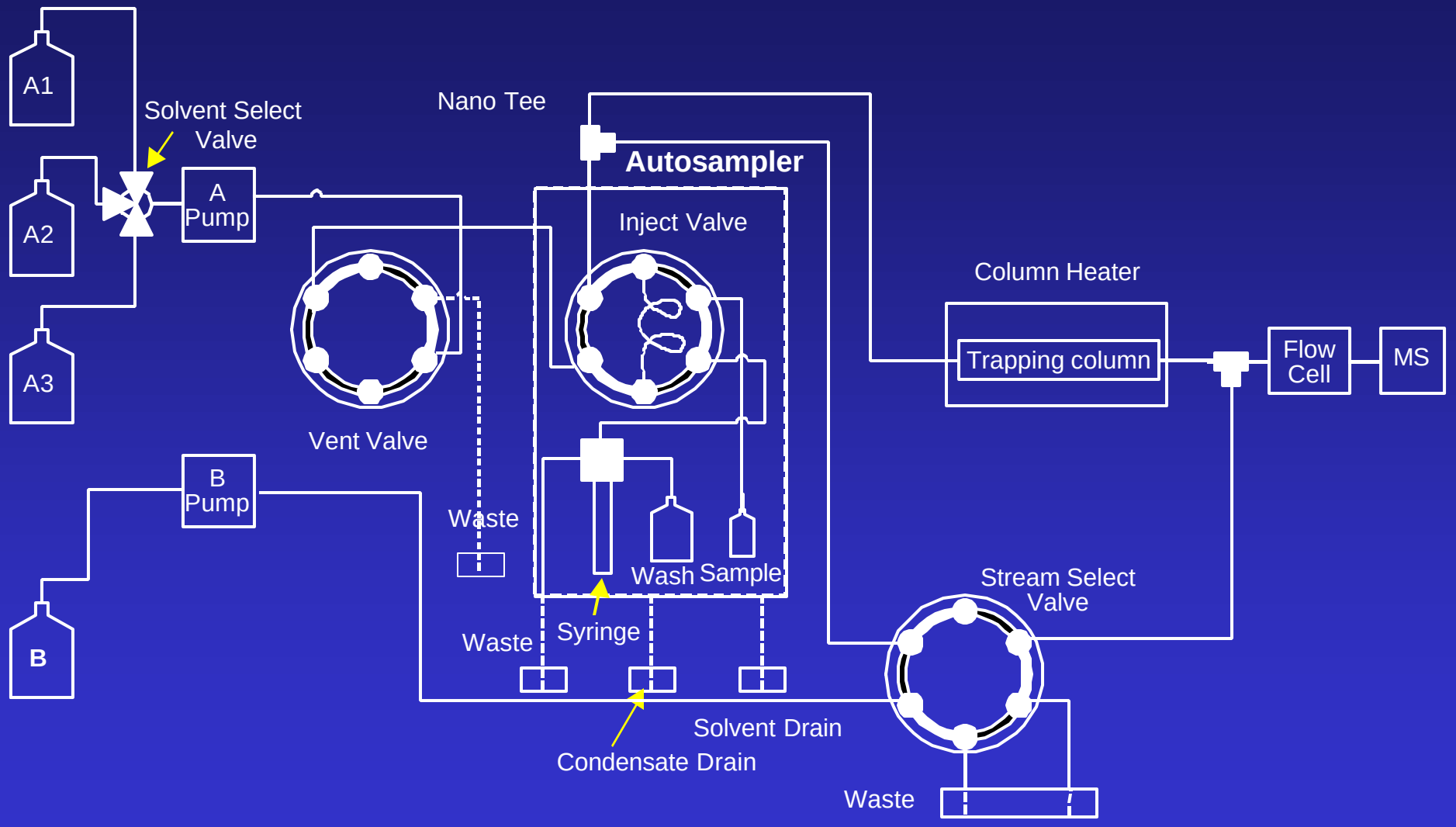
Mass Spectra for Peak 10



Ultra-High Sensitivity Detection of Angiotensin I and II



High Throughput Two Pump System



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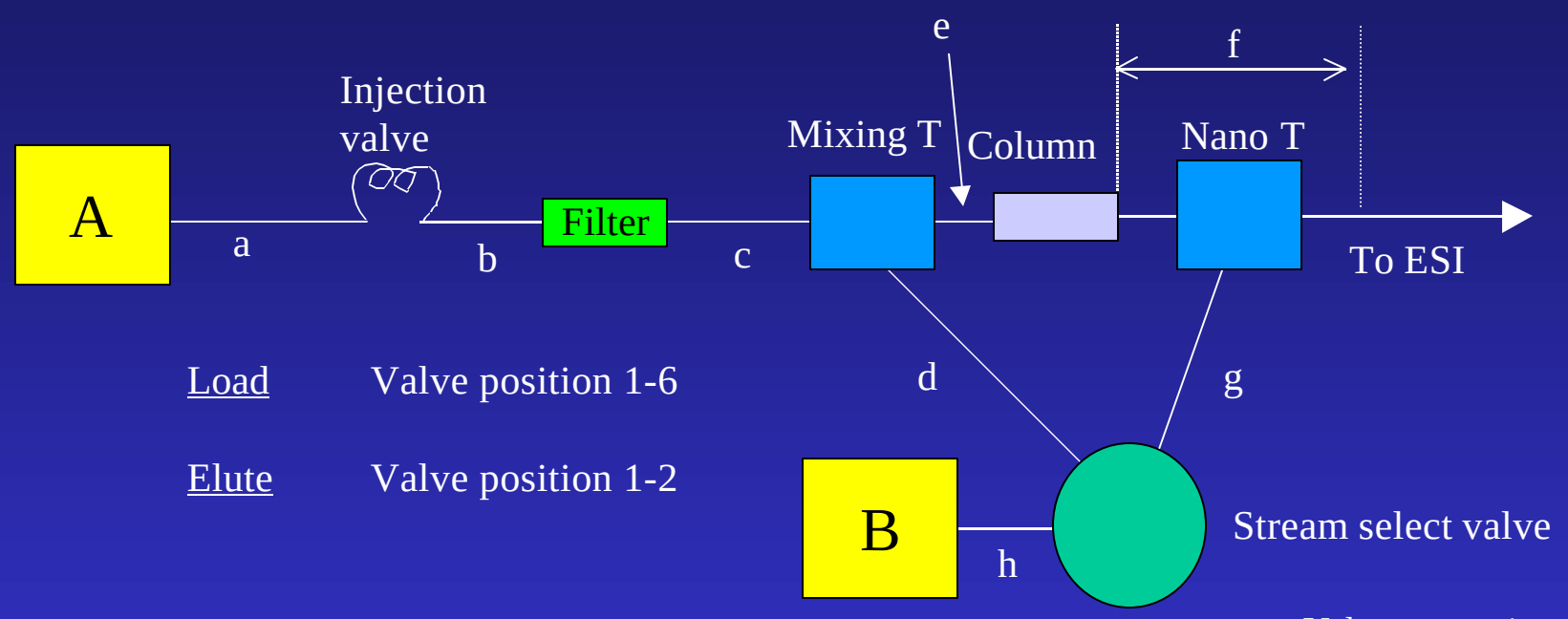


<u>Load</u> Valve position 1-6

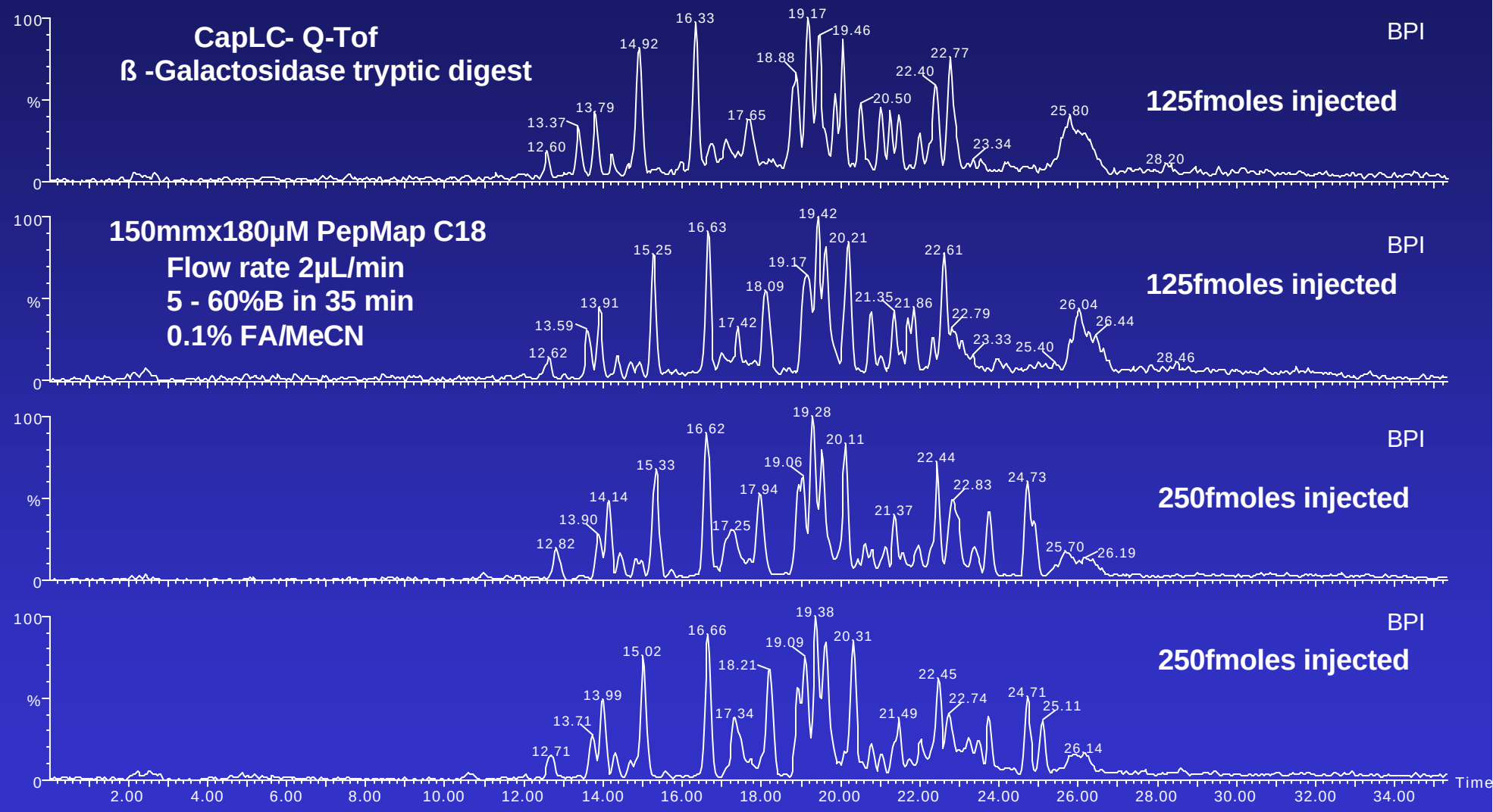
<u>Elute</u> Valve position 1-2

a: 75 micron x 500mm
b: 75 micron x 350mm
c: 75 micron x 80mm
d: 25 micron x 150mm
e: 50 micron x 100mm
f: 50 micron x 170mm (nanoT)

g: 75 micron x 100mm
h: 75 micron x 400mm

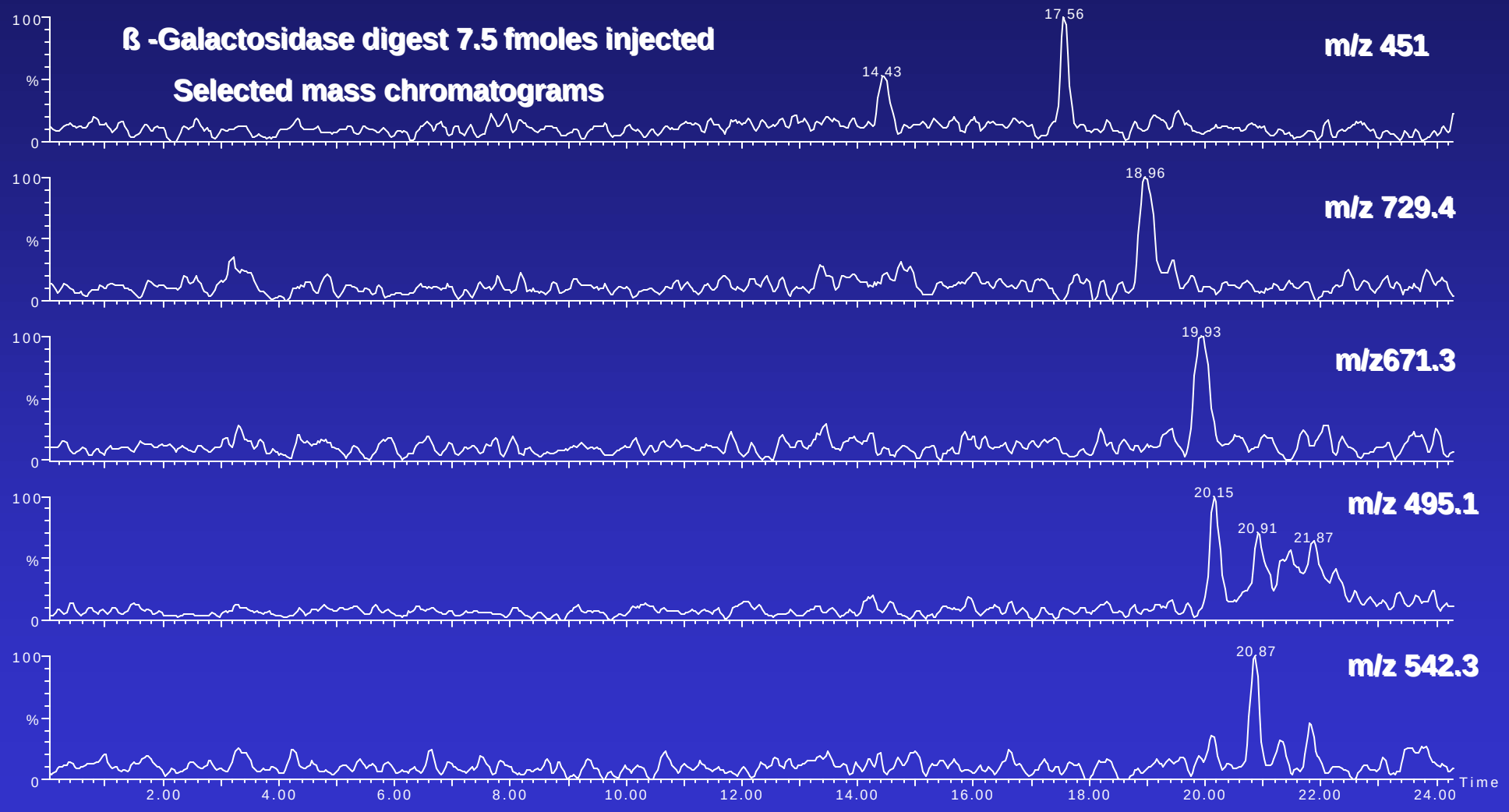
<u>Valve connections</u>
1 From nano T
2 Plugged
3 To mixing T
4 From pump B
5 B waste
6 A waste

Nanospray LC/MS Peptide Map

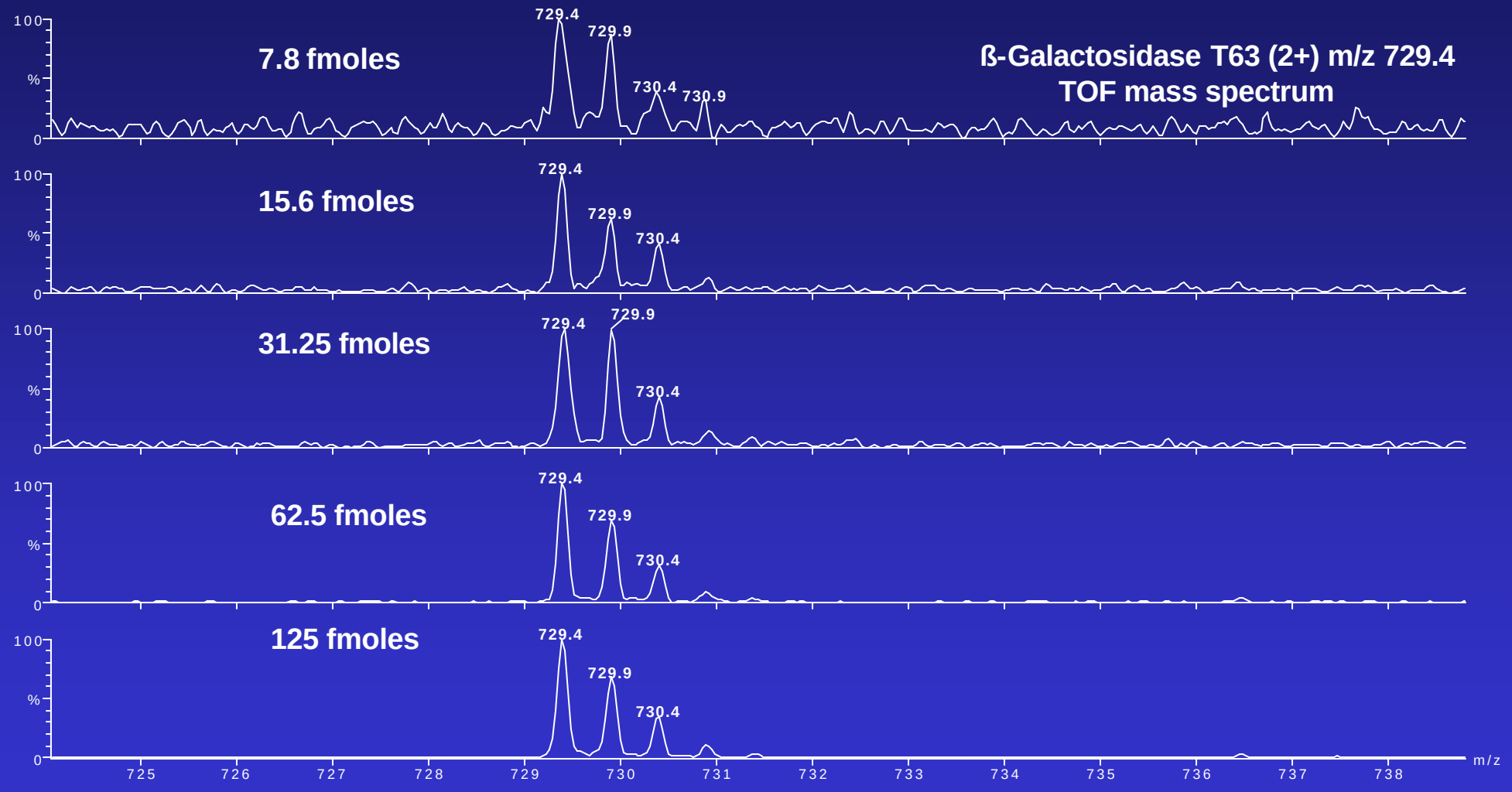


Extracted Ion Chromatograms

QTof with Nanospray



Nanospray Dilution Series



Waters CapLC System

- Fully integrated, automated system with PDA detection
- Sensitive, high performance detector design
- Innovative solvent delivery
- Separation performance comparable to analytical HPLC
- Easy integration with mass spectrometers

Acknowledgements

- Engineering
  - Joe Luongo
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