

Improved LC/MS Throughput with On-line Sample Cleanup, Peak Focusing and Composition and Flow Gradients

Jeanne B. Li, Eric H. Block & Michael P. Balogh
Waters Corporation, Milford MA 01757

On-line Cleanup Column Regeneration

Is it for me?
What is involved?

Introduction

More LC/MS samples need to be analyzed per day. Many samples contain complex matrices. Removing the matrix can 1) reduce ion suppression from salts, 2) keep the MS sample cone clean longer, 3) reduce background ions. This can lead to greater detection sensitivity.

For some basic analytes, a gradient separation at high pH is better for peak shape and resolution than acidic pH. The availability of solid phase extraction and analytical columns that are stable from pH 1 to 12 and can withstand rapid pressure changes (0 to 4000 psi) increases possibilities for methods development.

With column switching valves and flow programming, it is possible to perform on-line sample cleanup and fast gradient separations with cycle times of 3 to 5 minutes. Rapid equilibration and off-line column regeneration can decrease cycle time.

Analytical Methods

- Instrumentation

- Waters Alliance[®]HT LC/MS system consisting of a 2790 Separations Module, 996 photodiode array and ZSpray[™] mass detector (ZMD)
- Valves: 3-column selection valve or 10-Port, 2-position valve

- Columns

- Waters Oasis[®] HLB, 2.1x20 mm, 30 µm
- Waters XTerra[™] MS C₁₈, 2.1x10 or 2.1, 30 mm

- Mobile phases and solvents

- Acetonitrile and water gradients
- Additives: 0.1% formic acid or 5mM ammonium bicarbonate, pH 9.5

- Samples and Matrix

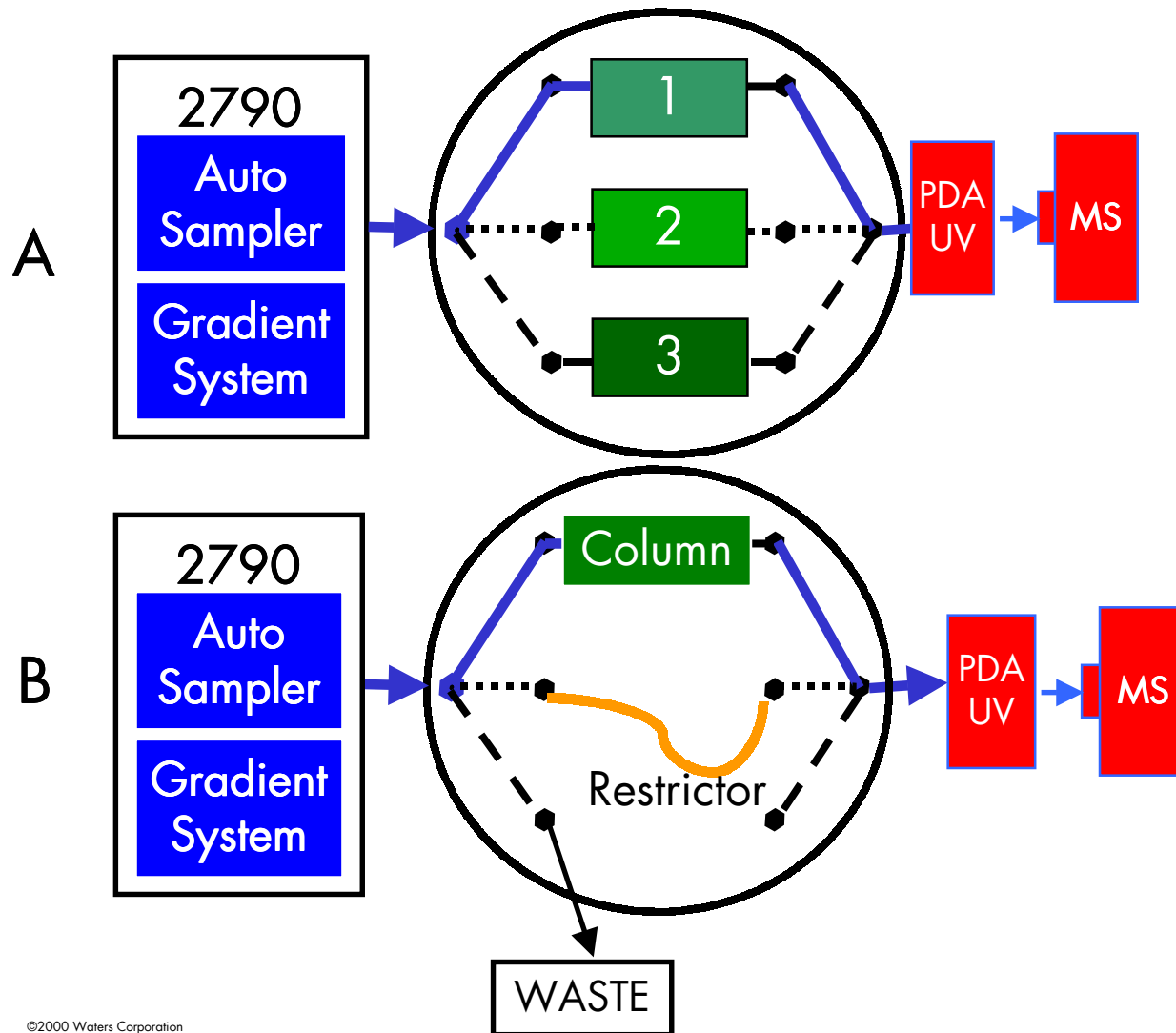
- Small molecules
- Hanks balanced salt solution (280 mosm/L) and calf serum

Uses for Valves

- Column Selection
 - To change columns
- Flow Diverting
 - To reduce reequilibration time
- Methods Switching
 - Methods development
 - Different analyses
 - Chromatography to flow injection analysis (FIA, loop injections)
- On-line Sample Preparation
 - Sample clean-up - remove matrix and ions
 - Sample preconcentration
- Column Regeneration
 - Simultaneous analysis and reequilibration of a second column

Column Selection & Method Switching

Built-in 8-Port, 3-Position Valve



ADVANTAGE

- Save time of changing columns or tubing

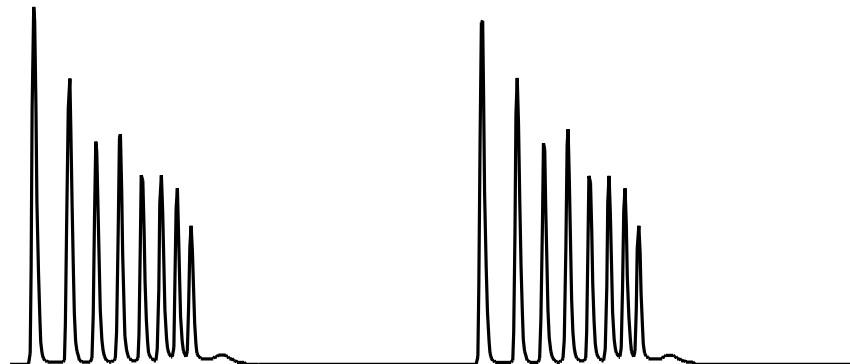
AUTOMATION

- Column selection (A)
- Column changing (A)
- Method switching (B)
 - HPLC
 - Flow Injection Analysis (FIA)

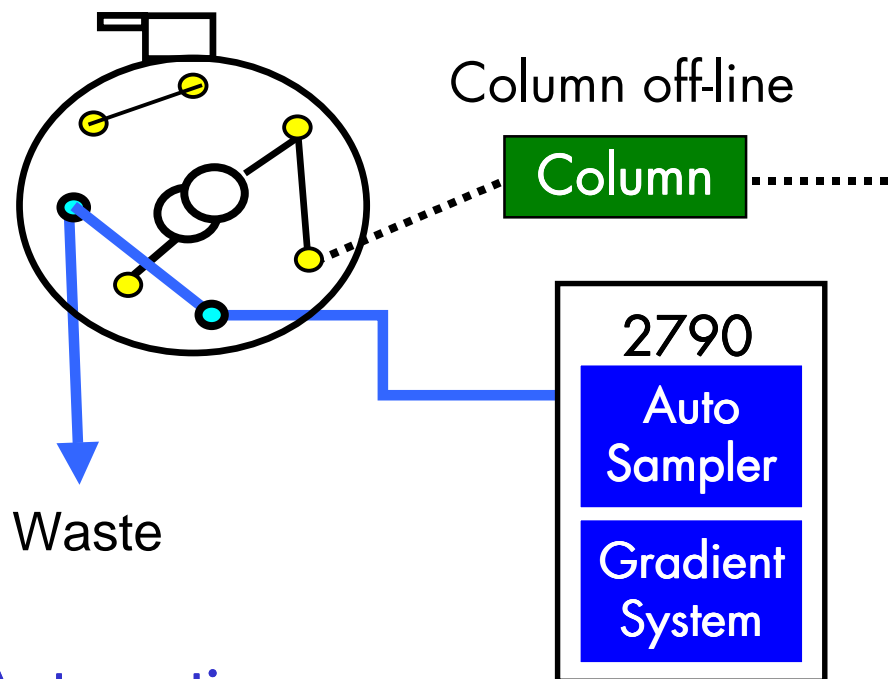
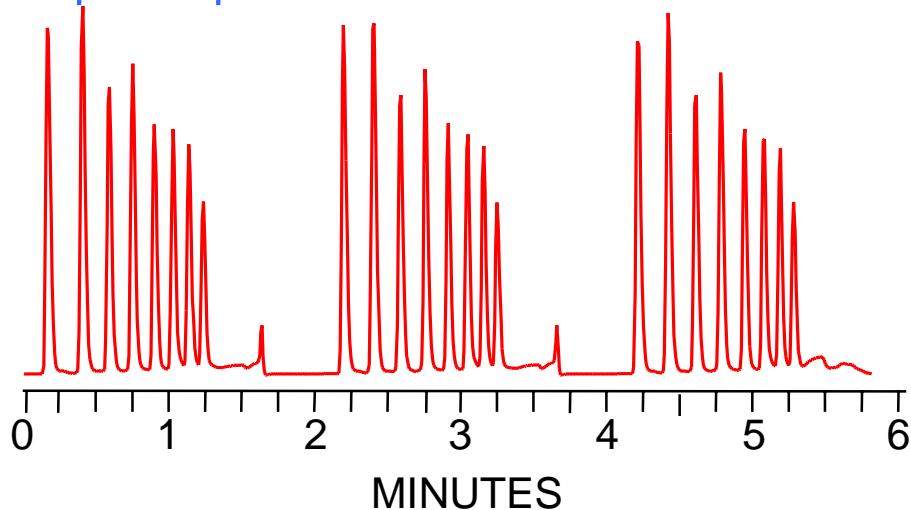
Rapid Equilibration

2790 Injector Valve

Traditional



Rapid Equilibration

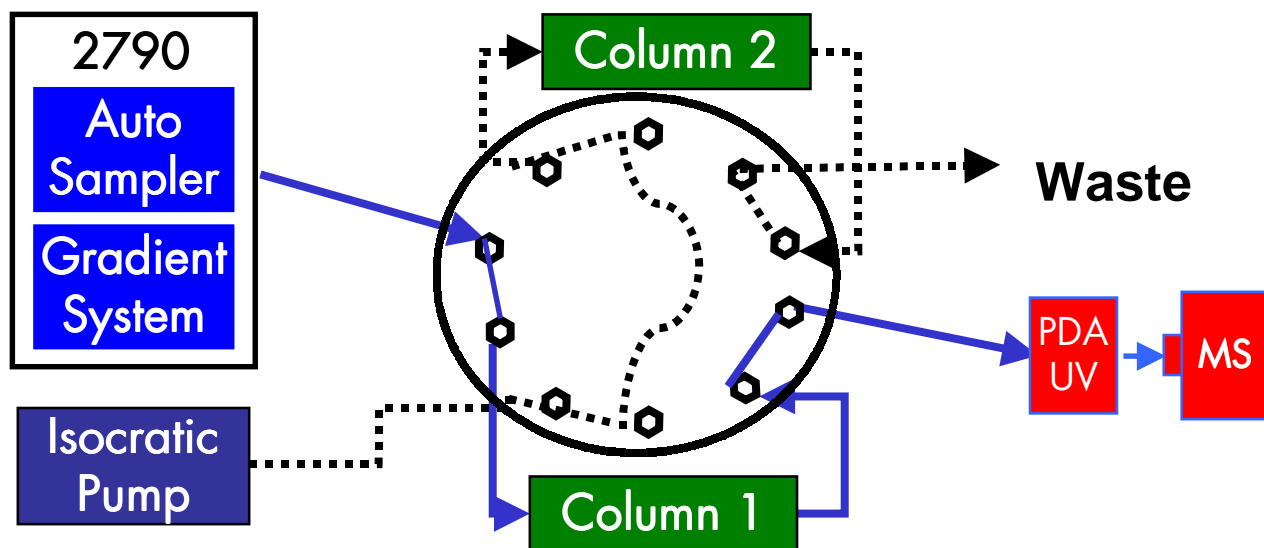


Automation

- Automatic valve switching
- Initial gradient composition
- Flow ramping from to 5 mL/min, 0.2 min
- Return to chromatographic conditions (1 mL/min)

Off-Line Column Regeneration

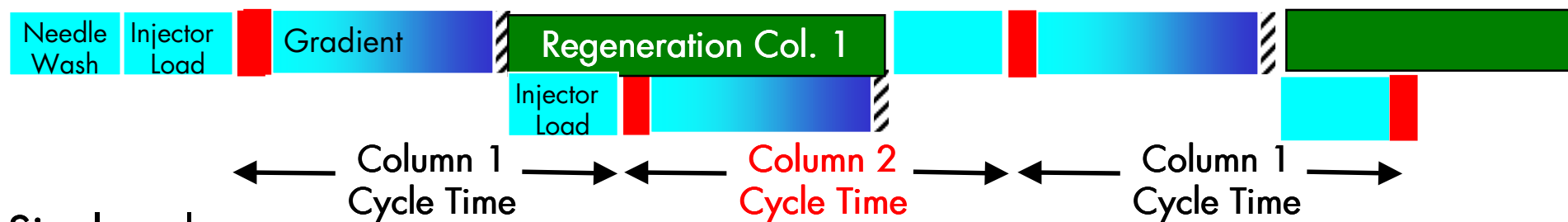
2 Analytical Columns



ADVANTAGES

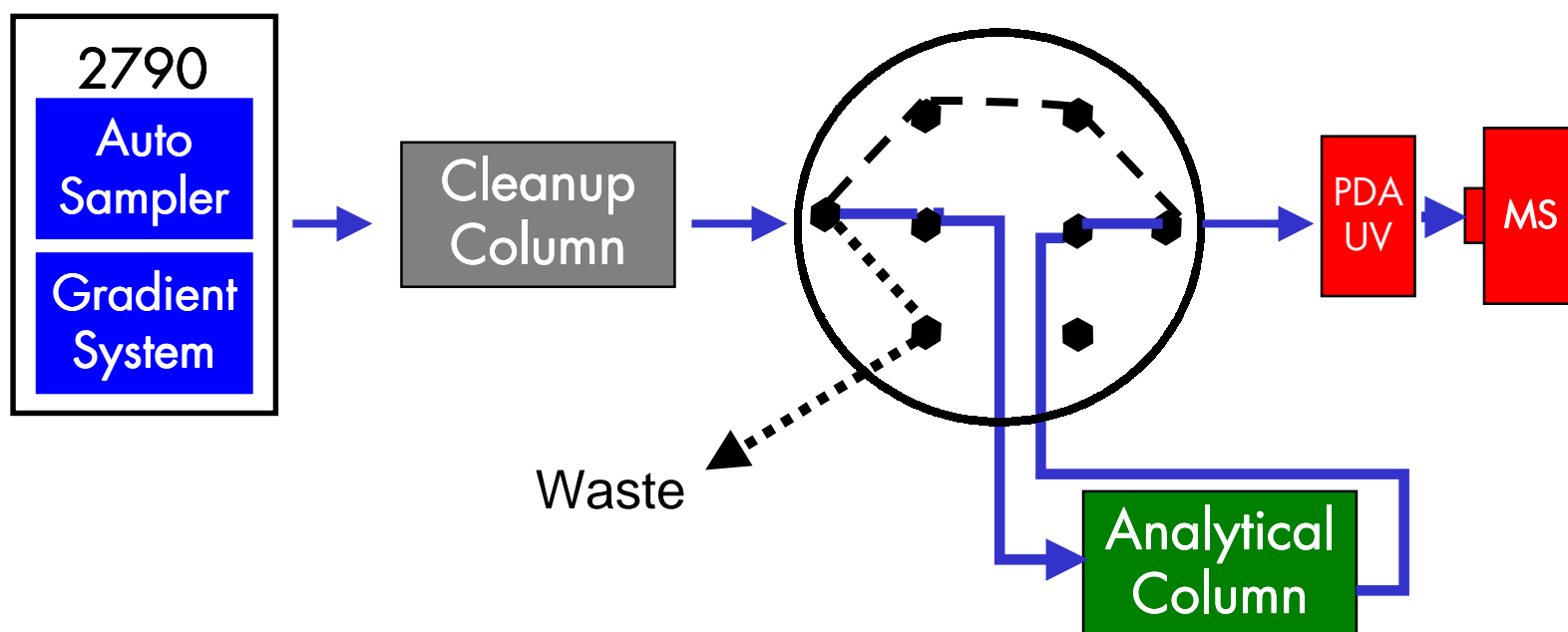
- No additional reequilibration time
- Continuous data from the mass spectrometer

2 columns with Regeneration Valve



On-Line Sample Cleanup and Gradient Analysis

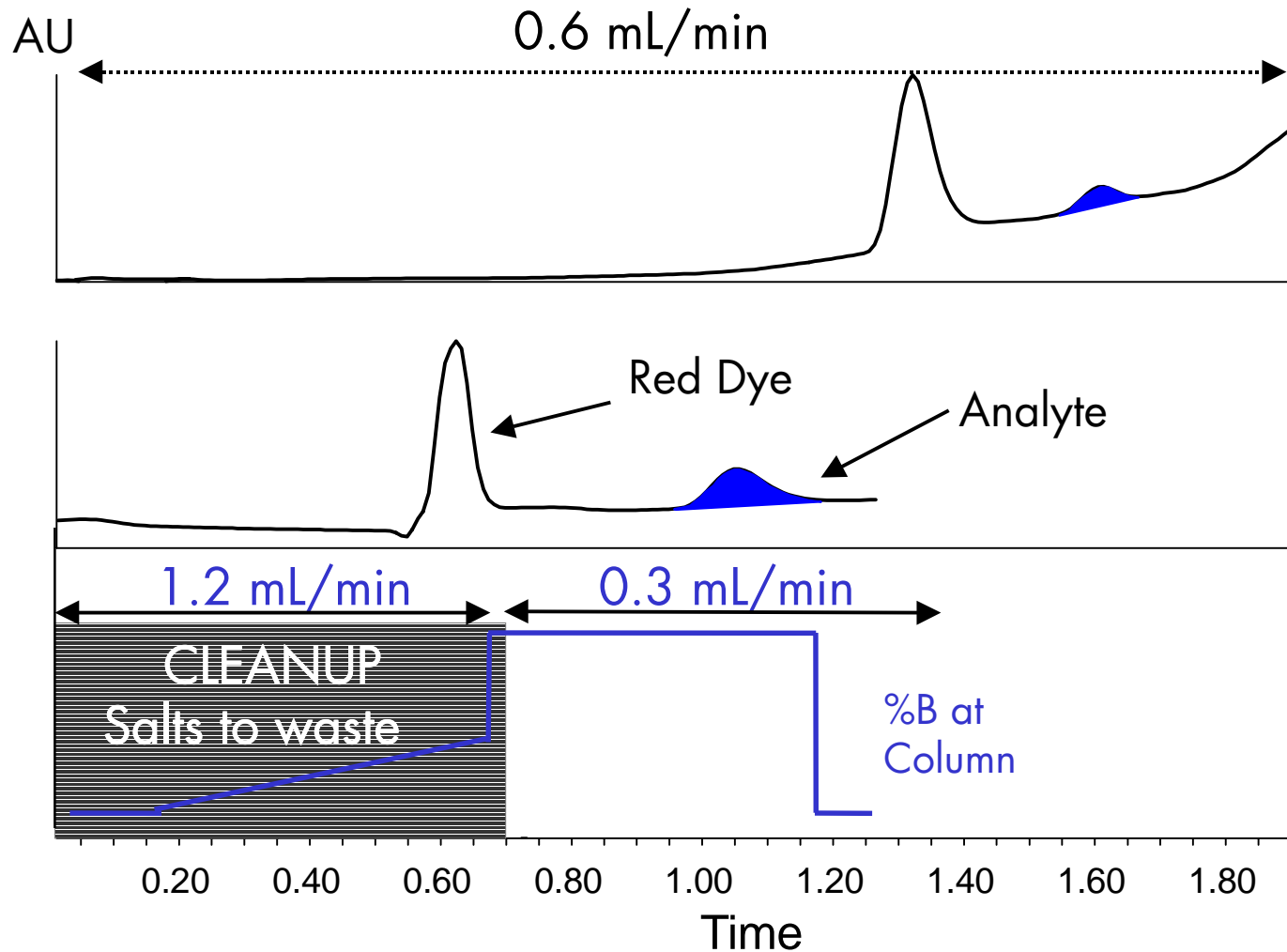
2790 Built-in 8-Port, 3-Position Valve



- Cleanup - matrix to waste (dotted line)
- Analytes to separation column ——— (solid line)
- or Directly to detectors - - - (dashed line)
- Alternative: solid phase extraction

Sample Cleanup - Salts Solution (1)

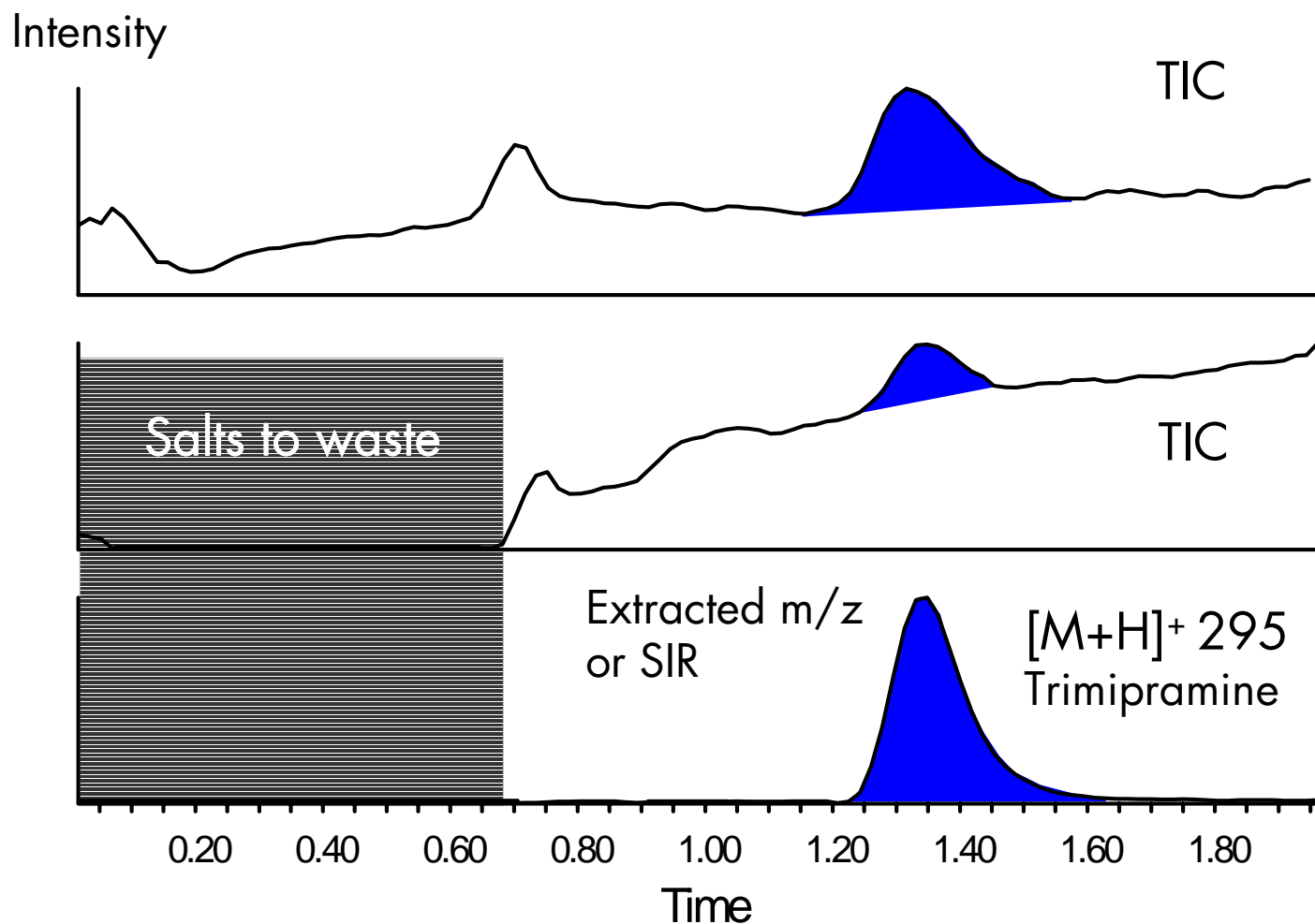
Valving and Flow Programming



- XTerra™ MS C₁₈ column 2.1x10 mm
- Hanks Balanced Salts Solution
- Drug
- Gradient
MeCN-H₂O + 0.1% formic acid
- Flow programming
- UV detection

Sample Cleanup - Salts Solution (2)

LC/MS Drug in Hanks Solution

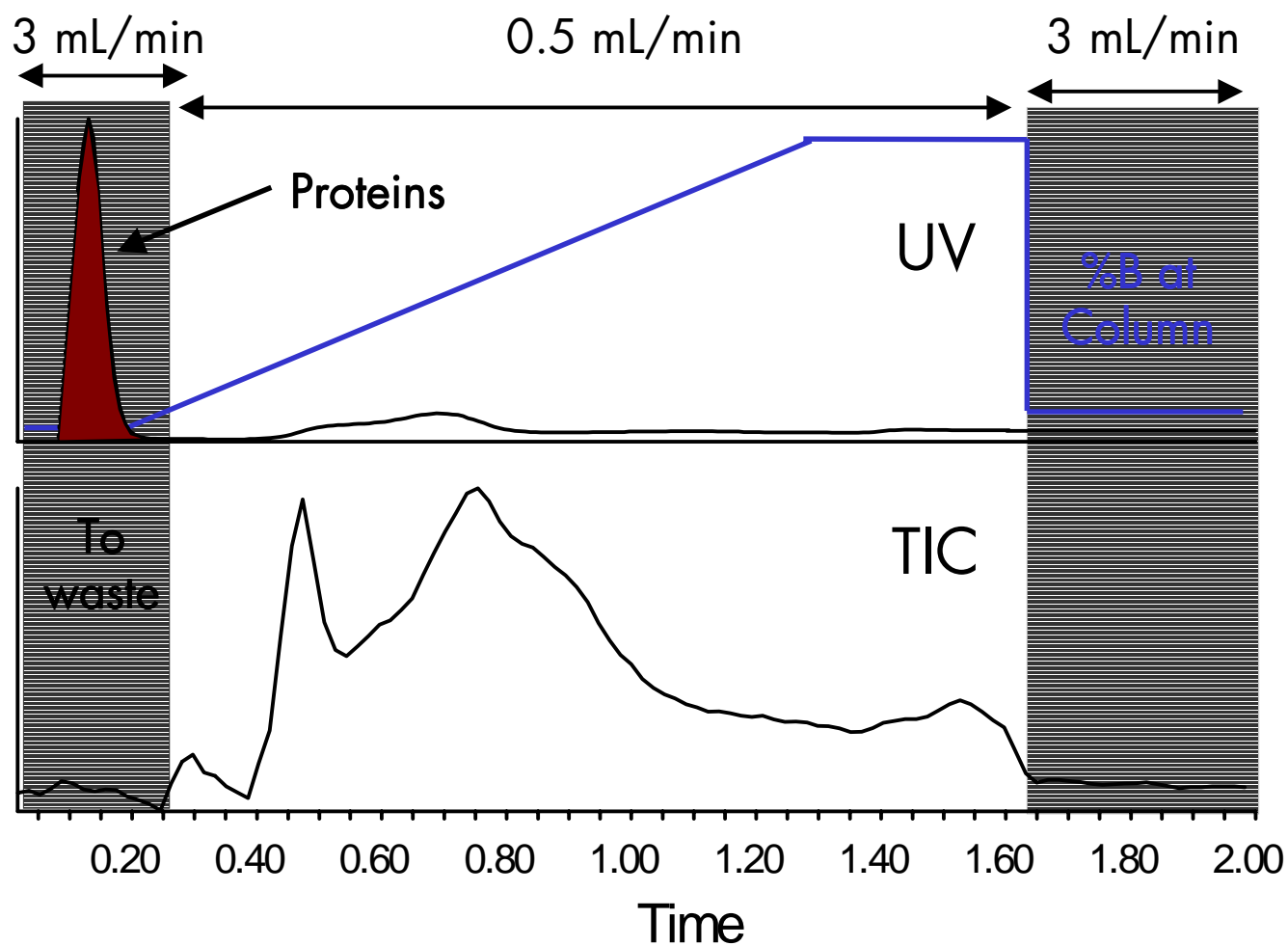


ADVANTAGES

- Rapid separation
- Removal of matrix
- Reduction of ion suppression
- Increased method ruggedness
- Keeps MS source clean

Sample Cleanup - Serum (1)

Removal of Protein

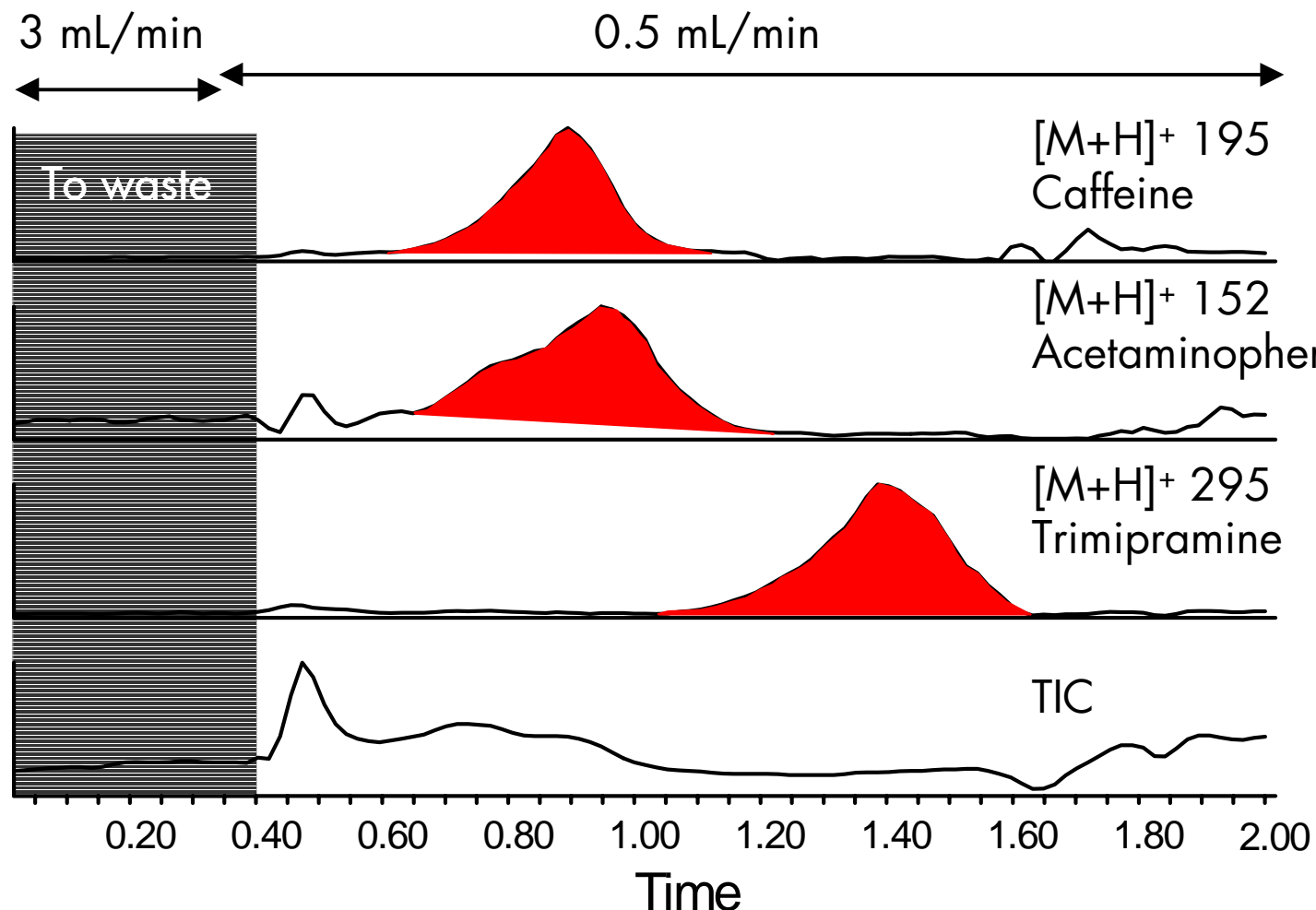


ADVANTAGES

- On-line clean-up
- Flow programming - no flow splitting
- Oasis[®]HLB column
2.1x20 mm
- Calf serum, 10 μ L
- Gradient
MeCN-H₂O + 0.1% formic acid

Sample Cleanup - Serum (2)

Direct LC/MS Analysis - 1 Column

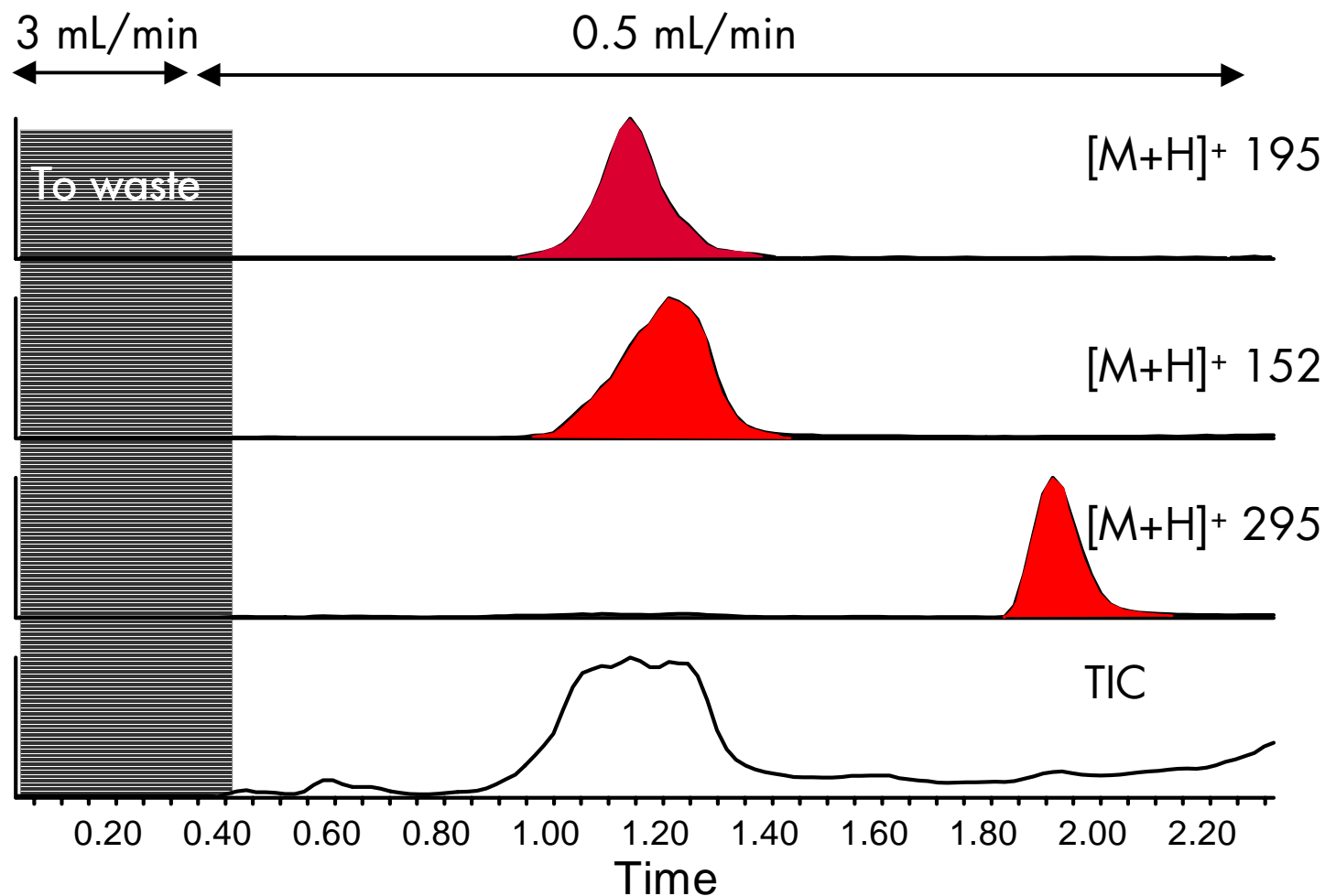


ADVANTAGES

- Some separation
- Removal of proteins
- Oasis[®] HLB column
2.1x20 mm
- Calf serum, 10 µL
- Gradient
MeCN-H₂O + 0.1% formic acid
- ESI⁺

Sample Cleanup and Analytical Separation

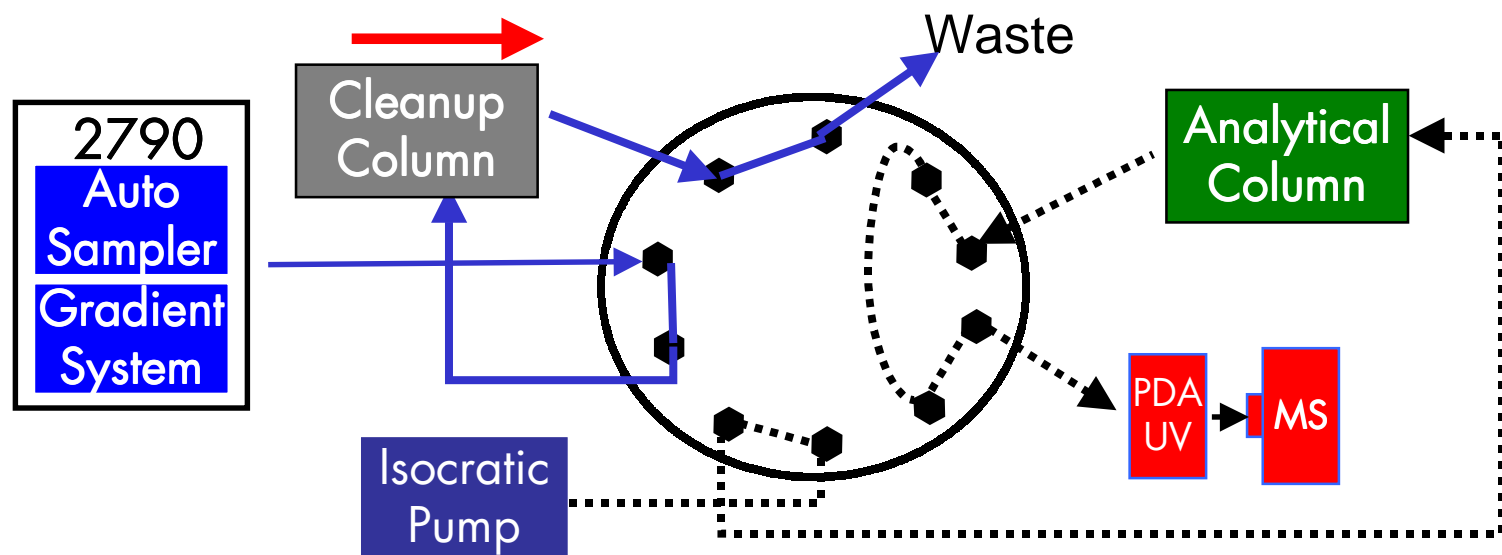
LC/MS Analysis - 2 Columns



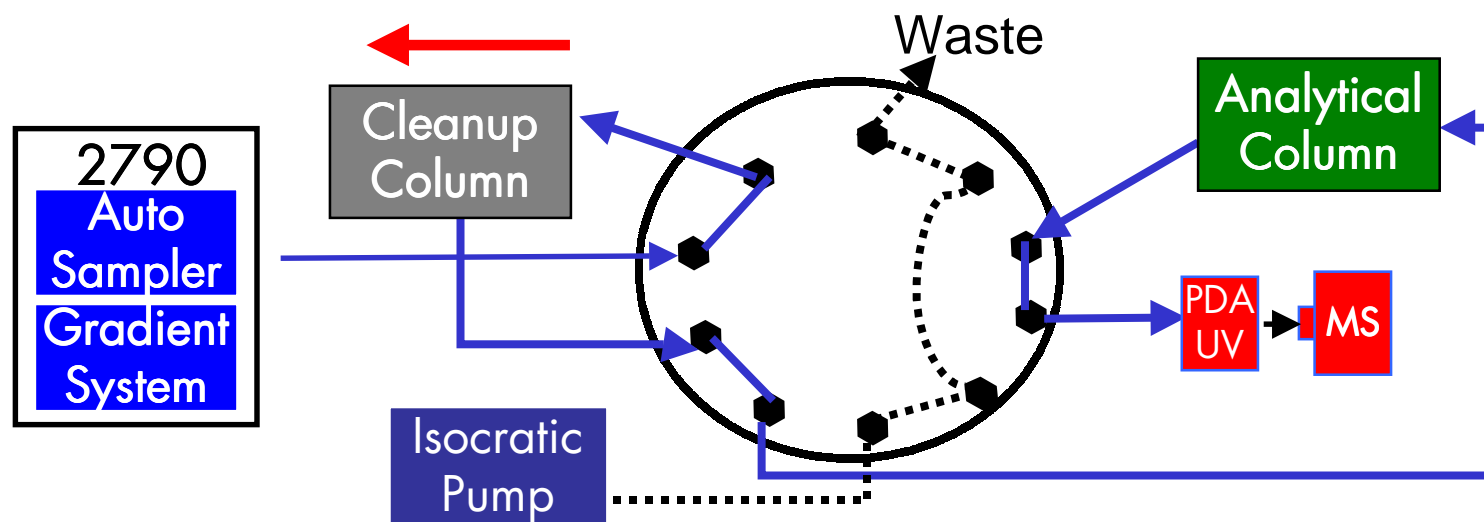
ADVANTAGE

- Better separation
- Improved peak shape
- Oasis[®] HLB column 2.1x20 mm
- XTerra[™] MS C₁₈ column 2.1x30 mm
- Calf serum+drugs
- Gradient
- ESI⁺

Sample Cleanup, Backflush and Analysis (1)

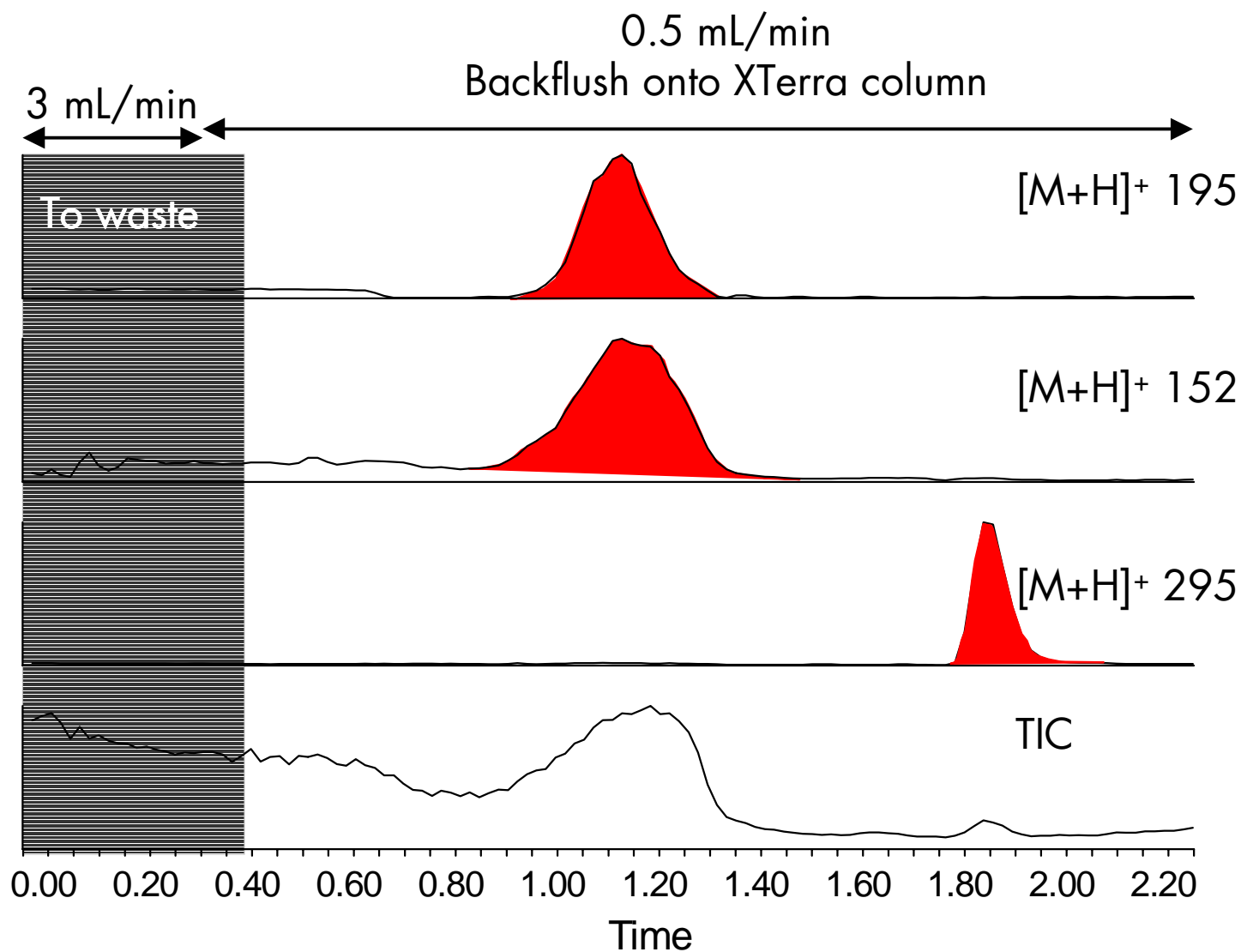


- On-line sample cleanup
- Simultaneous equilibration of analytical column



- Backflush analytes on to analytical column
- Increased complexity

Sample Cleanup, Backflush and Analysis (2)



ADVANTAGES

- Improved peak shape
- Calf serum
- Oasis[®] HLB column 2.1x20 mm
- XTerra[™] MS C₁₈ column 2.1x30 mm
- Gradient MeCN-H₂O + 0.1% formic acid
- ESI⁺

Considerations - Tradeoffs

- Time savings
 - Equilibration time
 - Sample preparation time
 - Changes in plumbing
- On-line sample cleanup
 - Versus batch off-line cleanup (e.g. 96-well plate SFE)
- Peak focusing
 - Chemistry versus valves
- Automation
- Mass spectrometer
 - Flow rate requirements
 - Sample concentration
- Complexity of valves
 - More valves, pumps, columns
 - More tubing and system volume (more peak bandspreading)
- Column requirements
 - Ruggedness - flow and pressure
 - Column life
 - Size
 - Sample capacity
 - Number and types of columns needed
- Methods development
 - Additional time required
- Equipment maintenance

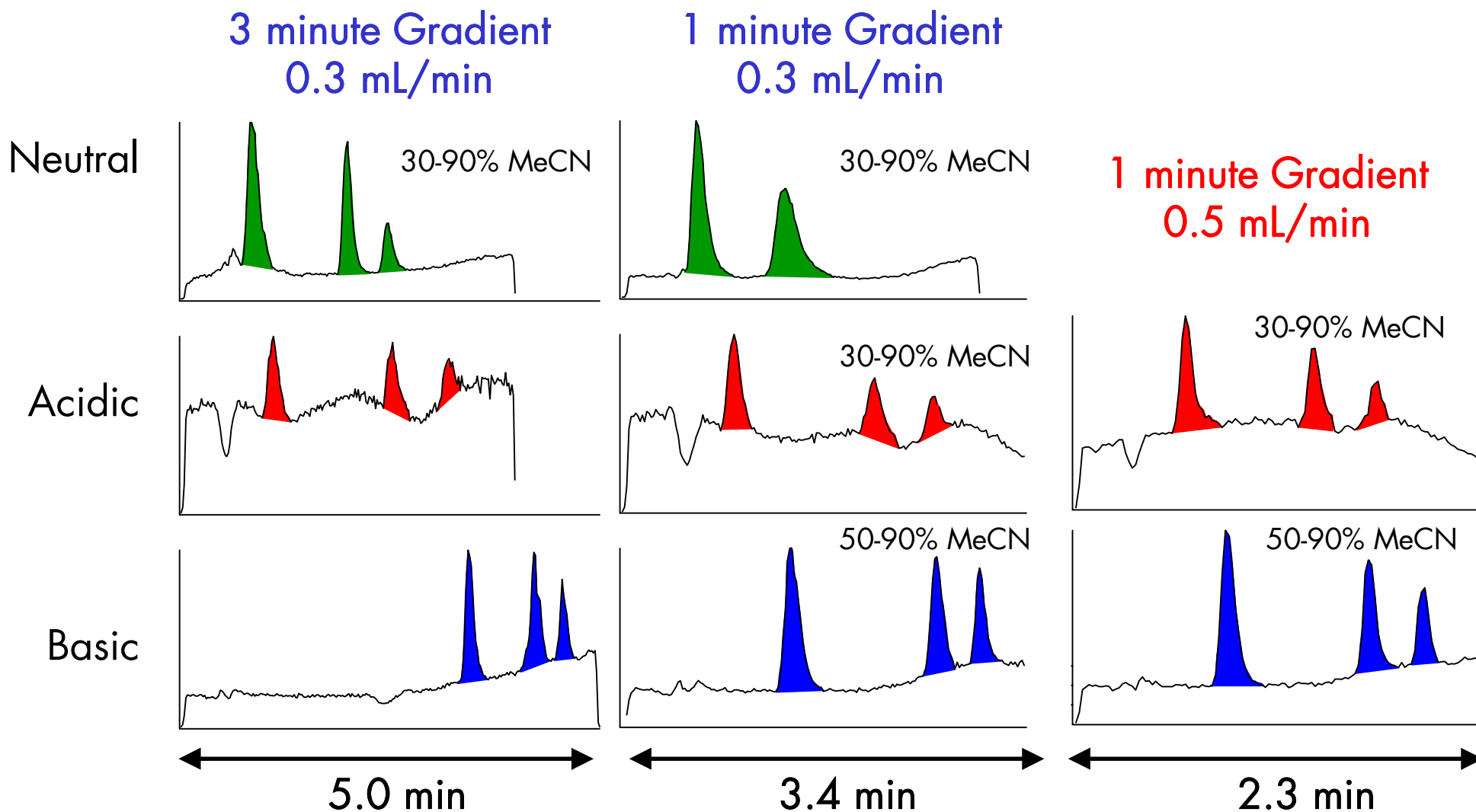
Effect of pH on Amine Separation (1)

Experimental Conditions

- **Purpose:** To determine the fastest separation with the sharpest peaks for maximum sensitivity
- **Compounds:** Diphenhydramine, Oxybutynin, Terfenadine
 - $[M+H]^+$ 256, 358, 472, respectively
- **Column**
 - XTerra™ MS C₁₈, 2.1x30mm, 3.5μm
 - Stable at pH 1-12
- **Gradients**
 - 30-90% or 50-90% Acetonitrile in water or 0.1% formic acid or 5 mM ammonium bicarbonate/carbonate, pH 9.5.
 - 1 or 3 minute linear gradients at 0.3 or 0.5 mL/min
- **Detection:** ESI⁺, scan 100-600 amu, extracted masses

Effect of pH on Amine Separation (2)

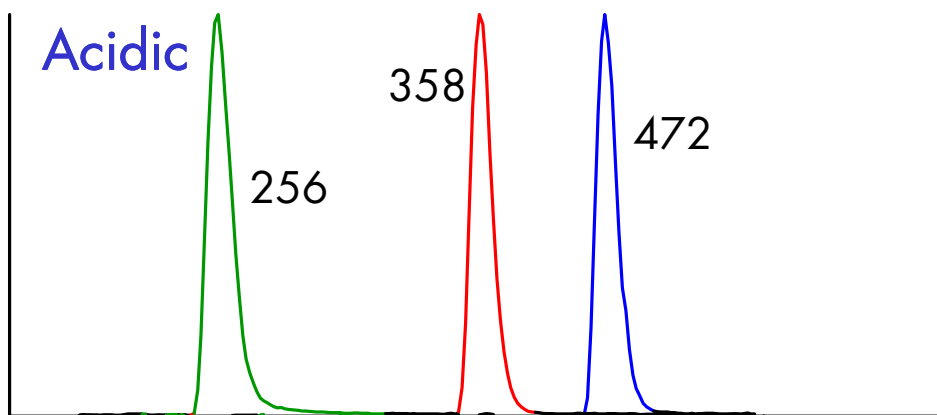
Separation in neutral, acidic and basic solutions (TIC)



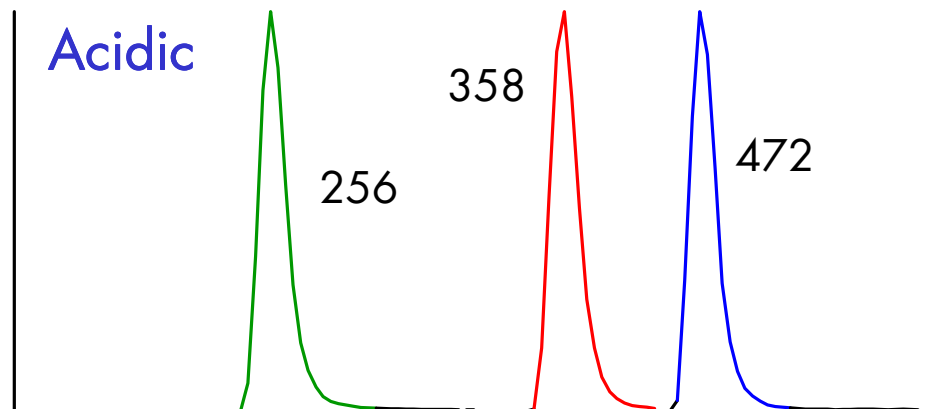
Effect of pH on Amine Separation (3)

Peak Shape of Extracted Masses, $[M+H]^+$

3 minute Gradient, 0.3 mL/min
5 minute Run

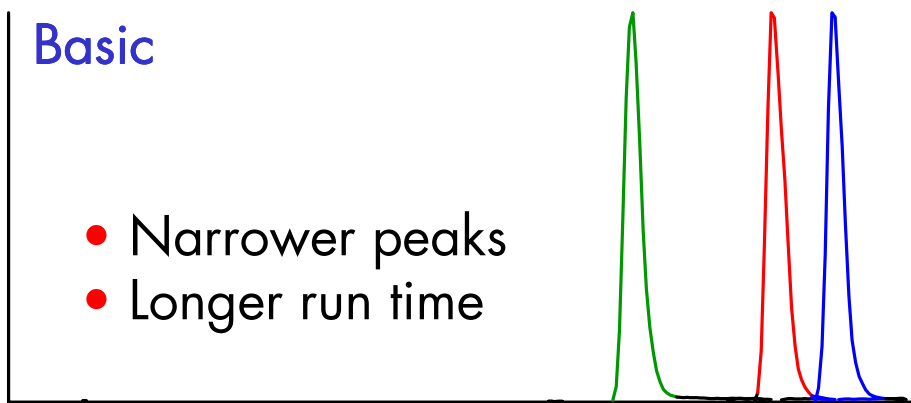


1 minute Gradient, 0.5 mL/min
2.3 minute Run



Basic

- Narrower peaks
- Longer run time



Basic

- No difference

