ACQUITY UPLC® Technology; On-Line solid phase extraction, Multi dimensional chromatography.
ACQUITY UPLC® Technology

- I Class
- UPC²
- H Class
- H Class Bio
ACQUITY UPLC® Technology

- HDx
- Clinical
- Nano UPLC
- PATrol
ACQUITY UPLC® Technology
Multi pump/Multi valve Solutions

UPLC® with On-Line SPE Technology

ACQUITY UPLC® System with 2D Technology

ACQUITY UPLC® Automated SPE System

2004 ACQUITY UPLC® technology 2012
ACQUITY UPLC® Technology
Multi pump/Multi valve Solutions

- Environmental
  - EPA 1694, EU
- Drinking Water Analysis
  - PAH’s
  - Personal and Pharmaceutical Care Product (PPCP)
  - Pesticides
  - Emerging contaminants
- 2 Modes of operation
  - On-Line SPE
  - UPLC®
- Tandem Quadrupole MS
ACQUITY UPLC® Technology
Multi pump/Multi valve Solutions

- Life Science
  - BioPharma,
  - Bioanalysis,
  - Lipidomics

- 3 modes of operation
  - Parallel column regeneration
  - On-Line sample preparation
  - Multi Dimension UPLC

- Tandem Quadrupole and TOF MS
- I Class, H Class, H Class Bio

ACQUITY UPLC® System with 2D Technology

©2012 Waters Corporation
ACQUITY UPLC® Technology
Multi pump/Multi valve Solutions

- MassTrack – IVD certification
  - Clinical
  - Forensic
  - Toxicology

- 4 modes of operation
  - On-Line SPE
  - Multidimensional SPE
  - Advanced method development
  - UPLC® mode

- Tandem Quadrupole MS

ACQUITY UPLC® Automated SPE System

©2012 Waters Corporation
Solid Phase Extraction,
On-Line SPE vs. Off-Line SPE
## Solid Phase Extraction, On-Line SPE vs. Off-Line SPE

<table>
<thead>
<tr>
<th>Common techniques</th>
<th>Sample Preparation Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLE</td>
<td>Less interference in the detector.</td>
</tr>
<tr>
<td>Protein Precipitation</td>
<td>Enhanced column life time.</td>
</tr>
<tr>
<td>Off-Line SPE</td>
<td>Enhance sensitivity.</td>
</tr>
<tr>
<td>On-Line SPE</td>
<td>Reduce matrix effects.</td>
</tr>
<tr>
<td></td>
<td>Reduce ion suppression.</td>
</tr>
<tr>
<td></td>
<td>Sample pre-concentration.</td>
</tr>
<tr>
<td></td>
<td>Robust assays.</td>
</tr>
</tbody>
</table>
Reduction in Steps, Closed System, Sample Utilization and Fixed Volume and Flow Rates

OFF-LINE

ON-LINE

"Switch valve"
Optimized elution conditions

Wash polar compounds

To LC column

Retain apolar compounds

Log Tr (Retention Time)

Organic Percentage

Retention Curve
Optimized elution conditions
Benefits of On-Line SPE

Enhanced Laboratory Productivity

• Better LC-MS and Human Resource Utilization
• Shorter Time to Result
• Minimizes Manual Intervention
• Accelerates Development of Robust Methods

Improved Assay Results

• Increase Assay Sensitivity with Minimal Sample
• Reduces Opportunity for Assay Variability
• Optimizes Sample Preparation Methods

Reduced Hazard Exposure

• Minimize Exposure to Harmful Solvents and Potential Biohazards

Appropriate for the Laboratory

• In Vitro Diagnostic Systems
• Forensics and toxicology applications
• Drinking Water Analysis
• Bioanalysis
ACQUITY UPLC® Technology
Purposeful innovation
Customer Enabled System
ACQUITY UPLC® Technology

UPLC with On-Line SPE Technology for Drinking Water Analysis
UPLC with On-Line SPE Technology for Drinking Water Analysis

- Analysis of compounds in drinking water including
  - PAHs
  - Pharmaceuticals
  - Pesticides

- Total analysis time can be reduced by 80%

- Better precision & accuracy (<15%)

- Laboratory flexibility
  - Switch between On-line SPE and UPLC
  - Detectors and chemistries choices for various analytes

- Simplify workflow in lab
  - Minimize or even eliminate manual sample preparation steps

- Increase throughput
  - Parallel processing of samples
  - Process more samples per day

- Reduce re-sampling and re-running
  - Minimize operator’s manual errors by using automation

- Increase lab efficiency
  - Free analyst’s time to do other tasks
Traditional Off-Line Analytical Approach For Analysis of Compounds of Concern in Water

Collection → Off-Line Sample Preparation → Analysis → Results

Most time consuming steps
New Analytical Approach For Analysis of Compounds of Concern in Water

Collection → On-Line Sample Preparation Analysis → Results

Sample concentration
Elution
Analysis
System diagram

- **Valve A:**
  - injection valve for online SPE UPLC

- **Valve B:**
  - switching valve for extraction columns

- **Valve C:**
  - injection valve for stand-alone UPLC
System control using MassLynx
sample preparation and elution sample manager

UPLC Binary Pump Interface

SPE Quaternary Pump Interface
Schematics of the process
Quanpedia

- Quanpedia is an application of MassLynx to facilitate method development and reporting

- A central data base for quantitative LC/MS methods

- A tool to aid MS method creation
  - Automatically creates data acquisition methods
  - Automatically creates data processing methods
Extracted ion chromatograms of pesticides at 10ppt. Peak to peak signal-to-noise (with no prior processing) are shown for each compound. illustrates the selectivity the system can offer.

- **Carbendazim**
  - S/N 10415
  - 192>160

- **Atrazine**
  - S/N 744
  - 216.1>174

- **Metolachlor**
  - S/N 3916
  - 284.3>252.1
Reproducibility of pesticide results (n=6) at 100 ppt level
- % RSD is less than 15%.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Calculated Concentration</th>
<th>% RSD on area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simazine</td>
<td>103.3</td>
<td>4.7</td>
</tr>
<tr>
<td>Alidcarb</td>
<td>100.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Propoxure</td>
<td>98.5</td>
<td>3.9</td>
</tr>
<tr>
<td>Propachlor</td>
<td>102.6</td>
<td>7.2</td>
</tr>
<tr>
<td>Simetryn</td>
<td>104.2</td>
<td>8.5</td>
</tr>
<tr>
<td>Atrazine</td>
<td>101.5</td>
<td>4.7</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>98.7</td>
<td>6.9</td>
</tr>
<tr>
<td>Methiocarb</td>
<td>99.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Propazine</td>
<td>95.1</td>
<td>8.1</td>
</tr>
<tr>
<td>Terbuthylazine</td>
<td>96.2</td>
<td>11.5</td>
</tr>
<tr>
<td>Cyanazine</td>
<td>95.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Prometryn</td>
<td>104.0</td>
<td>5.4</td>
</tr>
<tr>
<td>Metolachlor</td>
<td>99.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>105.8</td>
<td>6.9</td>
</tr>
<tr>
<td>Propiconazole</td>
<td>96.6</td>
<td>12.5</td>
</tr>
</tbody>
</table>
Reproducibility of pharmaceutical results (n=6) at 100 ppt level
- % RSD is less than 15%.

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>Calculated Concentration</th>
<th>% RSD on area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamezapine</td>
<td>106.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>98.8</td>
<td>7.4</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>95.2</td>
<td>11.2</td>
</tr>
<tr>
<td>Atenolol</td>
<td>97.8</td>
<td>10.2</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>101.4</td>
<td>7.1</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>92.8</td>
<td>10.6</td>
</tr>
<tr>
<td>Tripolidine</td>
<td>99.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Trimethoprimine</td>
<td>97.3</td>
<td>9.1</td>
</tr>
<tr>
<td>Terbinafine</td>
<td>97.1</td>
<td>11.3</td>
</tr>
<tr>
<td>Codeine</td>
<td>100.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Cocaine</td>
<td>95.1</td>
<td>11.5</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>100.6</td>
<td>9.0</td>
</tr>
<tr>
<td>Miconazole</td>
<td>99.8</td>
<td>10.7</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>93.8</td>
<td>8.7</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>99.3</td>
<td>7.4</td>
</tr>
</tbody>
</table>
ACQUITY UPLC® Technology

MassTrak™ On-Line SPE Analyzer

MassTrak™ Clinical Solutions
Use of the On-Line SPE Systems offers several key benefits

- Increased Laboratory Productivity
  - Better Use of Human and Instrument Resources
  - Faster Time to Result
  - Quick and Efficient Method Development
  - Minimizes Opportunity for Sample Processing Errors and Variability
  - Reduced Exposure to Chemical and Biological Hazards

- Improved LC/MS Assays
  - Improved Sample Clean Up
  - Increased Assay Sensitivity
  - More Efficient and Robust Methods

- Appropriate for the Clinical and Forensics Laboratories
What is the System?

- ACQUITY UPLC Sample Manager
- ACQUITY UPLC Binary Solvent Manager
- ACQUITY UPLC Column Manager – A
- **ACQUITY UPLC Online SPE Manager**
- ACQUITY TQ Detector -IVD
- Xevo TQD, Xevo TQ MS, Xevo TQ-S
- Online SPE Cartridges

The System uniquely integrates SPE sample preparation with LC/MS in a single, easy to use platform that significantly increases overall laboratory productivity and improves results for Clinical and Forensics/Toxicology LC/MS based assays
On-Line SPE Cartridges

- 10 x 1 mm Cartridges
- RFID Equipped Racks of 96 Cartridges
- Exclusively for use with OSM
- Single Use Cartridges
- Available in Three Sorbents:
  - C18 (10 µm)
  - C8 (10 µm)
  - WCX (30 µm)

RFID tag embedded in the cartridge rack to log cartridge type, batch number, and number of cartridge uses.
Advanced Software, Ease of Use Operation
Improved sample clean-up, Immunosuppressant

Reduction of Matrix effects

Whole Blood without SPE

Whole Blood with Online SPE

Blood extracted with OSM

50% aqueous/methanol

100 fold reduction in the level of phospholipids

Net improvement both assay sensitivity and robustness
Manual Method Development Vs DoE: Sensitivity and solvent reduction

25% increase in sensitivity using Design Of Experiment

Mycophenolic Acid 100ng/mL

Faster Elution of Analyte

Manually Optimized SPE Method

DoE Optimized Method with Online SPE
ACQUITY UPLC® Technology

ACQUITY Systems with 2D Technology
ACQUITY Systems with 2D Technology

- Need more sensitivity?
- Need for higher throughput?
- Should you perform LLE?
- Need to improve the quality of your separation?
- Need to reduce the down time (cleaning) of your MS detector?

- The first ever ACQUITY UPLC System with 2D Technology that provides the proven UPLC® benefits of Resolution, Sensitivity and Productivity, now for multidimensional applications.
Purposeful Innovations to enable ACQUITY UPLC® with 2D Technology
What Is This?

3 types of applications

- Real 2D LC
- On-Line Sample Preparation
- Parallel Column Regeneration

Column 2 is regenerating while column 1 is used for the analysis

- Increase sample throughput
- More flexibility, Faster ROI, Faster Decision Making, Less Waiting Time...
- Hardware Requirements (2 SM, 2x6PHPV)
Business Benefits – Parallel Column Regeneration

Contract Research Lab performing Metabolite Profiling looking to improve throughput

<table>
<thead>
<tr>
<th></th>
<th>Typical UPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection to Injection Cycle Time</td>
<td>12 Minutes</td>
</tr>
<tr>
<td>Samples Analyzed in 24 Hours</td>
<td>120</td>
</tr>
</tbody>
</table>

8 Minute Separation and 4 Minute Re-Equilibration

50% Increase in Throughput

“We have an ROI in 3 days, it’s a no brainer!”
Annie E. – Customer has purchased 6 UPLC 2D Systems and is looking to purchase an additional 3 more!
2D Chromatography With UPLC®
What Is This?

3 types of applications

Real 2D LC
On-Line Sample Preparation
Parallel Column Regeneration

Trap & Elute

Sample of interest is trapped and concentrated on the cartridge
Impurities can be trapped on the cartridge

Increased sensitivity
Increased robustness

Hardware Requirements
(2 SM, 1x6PHPV)

Hardware Requirements
(2 SM, 2x6PHPV)

Column 2 is regenerating while column 1 is used for the analysis

Increase sample throughput
More flexibility
Fast ROI...
Increase Sample Loading and Sensitivity with Trap and Back-Transfer Configuration

250 uL Clozapine 1 ppb
1D – 3 min gradient
Peak distortion
Volume Overload

20 uL Clozapine 1 ppb
1D – 3 min gradient

MRM of 6 Channels ES+
327 > 269.9
1.46e6
### 3 types of applications

<table>
<thead>
<tr>
<th>Application</th>
<th>Description</th>
<th>Benefits</th>
<th>Hardware Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Real 2D LC</strong></td>
<td>1/ Sample passes through the first column&lt;br&gt;2/ Portions of the effluent are sent to the second column</td>
<td>Increased selectivity&lt;br&gt;Increased sensitivity&lt;br&gt;Increased robustness&lt;br&gt;Hardware Requirements (2 SM, 1x6PHPV)</td>
<td></td>
</tr>
<tr>
<td><strong>On-Line Sample Preparation</strong></td>
<td>Sample of interest is trapped and concentrated on the cartridge&lt;br&gt;Impurities can be trapped on the cartridge</td>
<td>Increased sensitivity&lt;br&gt;Increased robustness&lt;br&gt;Hardware Requirements (2 SM, 1x6PHPV)</td>
<td></td>
</tr>
<tr>
<td><strong>Parallel Column Regeneration</strong></td>
<td>Column 2 is regenerating while column 1 is used for the analysis</td>
<td>Increase sample throughput&lt;br&gt;More flexibility&lt;br&gt;Hardware Requirements (2 SM, 2x6PHPV)</td>
<td></td>
</tr>
</tbody>
</table>
Typical LC Separation versus Separation Using 2D Heart Cutting

Typical LC Separation

desired peptides (co-eluting)  phospholipids interference

ACQUITY UPLC® with 2D Technology

desired peptides
### UPLC® On-Line SPE Systems

#### MassTrak™ On-Line SPE Analyzer
- One time use SPE cartridge
- Simplified Operation/Method Development for On-Line SPE
- Parallel Operation – Multi dimensional SPE
- Tandem Quad Compatible Only

#### ACQUITY UPLC® with 2D Technology
- Reusable SPE Column
- Flexibility to perform various 2D Operations – including at-column dilution
- Serial Operation
- MS/Detector Options

©2012 Waters Corporation
Conclusion

- ACQUITY UPLC® Technology
  - On-Line solid phase extraction
  - Multi dimensional chromatography

- New capability that is available on our award winning, best in class ACQUITY UPLC® Systems.
  - Increased Laboratory Productivity
  - Increased Laboratory flexibility
  - Improved LC/MS Assays
    - Selectivity
    - Sensitivity
    - Robustness