New Quadrupole MS detection capabilities for Ultra Performance LC
Overview

- Refresher: What is UPLC?
- New Products overview:
  - SQ Detector
  - TQ Detector
The quality of the separation in HPLC today is both driven and limited by column chemistry. Optimized chemistry can bring speed, sensitivity and resolution benefits to the chromatographic process.

Particle size is a primary contributor to column performance.

The challenge in the laboratory is that today’s instrumentation cannot take advantage of tomorrow’s reality of smaller particles.
**Particle Size Evolution**

**Late 1960's**
- 40µm pellicular non-porous coated
- 100-500 psi (7-40 bar)
- **10,000 plates/meter**

**Early 1970's**
- 10µm Irregular micro-porous
- 1000-2500 psi (70-180 bar)
- **40,000 plates/meter**

**1980's to present day**
- 3.5 - 5µm spherical micro-porous
- 1500-4000 psi (110-280 bar)
- **80,000 - 115,000 plates/meter**
The promise of the van Deemter plot

**HPLC**

- 10 μm Particle 1970's
- 5 μm Particle 1980's
- 3.5 μm Particle 1990's
- 1.7 μm Particle 2004

**ACQUITY UPLC™ System**

<table>
<thead>
<tr>
<th>Flow Rate [mL/min]</th>
<th>Linear Velocity [u, mm/sec]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID = 1.0 mm</td>
<td>0.04 0.07 0.10 0.13 0.17 0.20 0.24</td>
</tr>
<tr>
<td>ID = 2.1 mm</td>
<td>0.15 0.30 0.45 0.60 0.75 0.90 1.05</td>
</tr>
<tr>
<td>ID = 4.6 mm</td>
<td>0.70 1.40 2.10 2.80 3.50 4.20 4.90</td>
</tr>
</tbody>
</table>
**UPLC™ increases speed by 9X**

**UPLC™ CONDITIONS**
- Column: 2.1 x 30 mm 1.7 µm, ACQUITY UPLC™ C18
- Flow: 0.6 mL/min
- Temperature: 32 oC
- Isocratic: 3% ACN with 0.1% Formic Acid
- Detector: UV at 280 nm, 20 points/sec, Tc = 0.1
- 2 uL injection
- Backpressure: 6460 psi
- Sample: Caffeine and metabolites

**HPLC CONDITIONS**
- Column: 2.1 x 100 mm 5.0 µm, Bridged Hybrid C18
- Flow: 0.2 mL/min
- Temperature: 32 oC
- Isocratic: 3% ACN with 0.1% Formic Acid
- Detector: UV at 280 nm, 20 points/sec, Tc = 0.1
- 2 uL injection
- Backpressure: 1130 psi
- Sample: Caffeine and metabolites
**Ultra Performance LC™ Sensitivity**

**UPLC™ provides increased sensitivity**

- **Column**: 2.1 X 50 mm 1.7 µm, ACQUITY UPLC™ C18 2.1 x 50 mm 5.0 µm, BH C18
- **Flow**: 0.5 mL/min
- **Temperature**: 32 °C
- **Isocratic**: 3% ACN with 0.1% Formic Acid
- **Detector**: UV at 280 nm, 20 points/sec, Tc = 0.1
- **2 uL injection**
- **Backpressure**: 2380 psi (5.0 µm), 7580 PSI (1.7 µm)
- **Sample**: Caffeine and metabolites

See more of your sample

Increased sensitivity using 1.7 µm particles

©2004 Waters Corporation
**UPLC™ offers 1.7X increase in resolution**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>2.1 x 50 mm 5.0 µm, Bridged Hybrid C18</td>
</tr>
<tr>
<td></td>
<td>2.1 x 50 mm 1.7 µm, ACQUITY UPLC™ C18</td>
</tr>
<tr>
<td>Flow</td>
<td>0.3 mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>38 °C</td>
</tr>
<tr>
<td>Gradient (30 min)</td>
<td>5-50% ACN with 0.018% TFA</td>
</tr>
<tr>
<td>Detector</td>
<td>UV at 214 nm, 20 points/sec, Tc = 0.1</td>
</tr>
<tr>
<td>Injection</td>
<td>10 µL injection</td>
</tr>
<tr>
<td>Backpressure</td>
<td>1400 psi (5.0 µm), 4500 psi (1.7 µm)</td>
</tr>
<tr>
<td>Sample</td>
<td>MassPREP™ Peptide Mixture</td>
</tr>
</tbody>
</table>

More information from complex samples
• Small, pressure-tolerant particles
• High pressure fluidic modules (up to 15,000 psi)
• Minimized system volumes and optimized flow paths
• Reduced cycle times
• Negligible carryover sample management
• High speed detectors (optical and mass)
• Software designed for system integration
  – Novel communication protocols
  – Advanced diagnostics
Detectors:
- Optical and/or Mass Spec
- Tunable UV or Photodiode Array
- Optimized flow cell for UPLC™
- High speed detection
- Low dispersion design
- UPLC pressure capabilities

Sample Manager:
- Low dispersion XYZZ’ Format
- Fast cycle times
- Low carryover
- Plates and/or vials
- Optional Sample Organizer
- UPLC pressure capabilities

Column Manager:
- Innovative pivot design for MS Interface
- Column temperature control
eCord technology

Solvent Manager:
- High pressure blending
- Binary gradients
- Four solvent choices
- On-line degassing
- Low dispersion design
- UPLC pressure capabilities
• UPLC provides the following benefits for MS:

  – Improved resolution for complex mixture analysis

  – Improved resolution can reduce MS ion suppression by separating species that co-elute in conventional HPLC – improved MRM detection limits

  – Shorter analytical run times without compromising chromatographic resolution – increasing sample throughput

  – Narrower chromatographic peaks effectively increase concentration of analytes entering the MS source – increasing signal intensity and improving MRM detection limits.
UPLC-MS/MS vs HPLC-MS/MS

ESI+, Alprazolam

Peak height increase 3.7 times.
Signal to noise ratio 4.7 times increase
UPLC Compatibility: Narrow peaks = less data points?

100 ms Dwell Time, 10 ms Delay

Convert the x axis to scan number

Peak Width = 1.8 s
Points Across Peak = 7
UPLC Compatibility:
Narrow peaks = less data points?

100 ms Dwell Time, 10 ms Delay
Peak Width = 1.8 s
Points Across Peak = 7

5 ms Dwell Time, 5 ms Delay
Peak Width = 1.8 s
Points Across Peak = 60
New MS Detection for ACQUITY UPLC

ACQUITY SQD
featuring the SQ Detector
Single Quadrupole MS
Introduced Pittcon 2006

ACQUITY TQD
featuring the TQ Detector
Tandem Quadrupole MS
Introduced ASMS 2006
New MS Detection for UPLC

- SQ and TQ Detectors designed to be:
  - UPLC Compatible
  - Smaller in size
  - Multi-mode
  - Easier to operate
Peak at 1.41 min 1.5 sec at baseline
10000da/sec 23 scans across peak

150-650m/z
50ms scan time

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.400</td>
<td>2227573</td>
</tr>
<tr>
<td>1.401</td>
<td>2625689</td>
</tr>
<tr>
<td>1.403</td>
<td>3262307</td>
</tr>
<tr>
<td>1.404</td>
<td>4705320</td>
</tr>
<tr>
<td>1.405</td>
<td>6698501</td>
</tr>
<tr>
<td>1.406</td>
<td>8489564</td>
</tr>
<tr>
<td>1.407</td>
<td>9407464</td>
</tr>
<tr>
<td>1.408</td>
<td>10572410</td>
</tr>
<tr>
<td>1.410</td>
<td>12789670</td>
</tr>
<tr>
<td>1.411</td>
<td>15031861</td>
</tr>
<tr>
<td>1.412</td>
<td>16597485</td>
</tr>
<tr>
<td>1.413</td>
<td>16926088</td>
</tr>
<tr>
<td>1.414</td>
<td>16159314</td>
</tr>
<tr>
<td>1.415</td>
<td>12984572</td>
</tr>
<tr>
<td>1.417</td>
<td>10160362</td>
</tr>
<tr>
<td>1.418</td>
<td>8654048</td>
</tr>
<tr>
<td>1.419</td>
<td>8560126</td>
</tr>
<tr>
<td>1.420</td>
<td>6905351</td>
</tr>
<tr>
<td>1.421</td>
<td>5542828</td>
</tr>
<tr>
<td>1.422</td>
<td>4519957</td>
</tr>
<tr>
<td>1.424</td>
<td>4161767</td>
</tr>
<tr>
<td>1.425</td>
<td>3235327</td>
</tr>
<tr>
<td>1.426</td>
<td>2770925</td>
</tr>
</tbody>
</table>
Waters TQ Detector
Smallest tandem quadrupole on the bench

13" (330mm) of linear bench space
Tandem Quadrupole MS/MS Advantages

- **High Selectivity**
  - reduce or eliminate matrix interferences

- **High Sensitivity**
  - trace levels - low reporting limits

- **Quantitative Accuracy**
  - reproducibility, stability and dynamic range
  - accurate quantitation of targets at low levels in matrix

- **Robustness**
  - complex sample matrices, reduced sample clean-up
SIR vs MRM for complex matrices

MRM

SIR

©2004 Waters Corporation
Waters TQ Detector
Key Features

- Tandem quadrupole mass spectrometer
- Small footprint:
  - Only 13” wide
  - 25% smaller box than Quattro micro
- Z-Spray ion sources
  - ESI and ESCi are standard
  - IonSabre APCI, APPI options
  - Integrated infusion fluidics
  - Tool free routine maintenance
- 2 to 2000 Da mass range
- T-Wave collision cell for Hi-Speed UPLC-MS/MS
- Scan rate up to 10,000 Da per second
- Polarity switching in 20 ms

©2004 Waters Corporation
The cycle time per MRM transition is composed of two parameters:
- a dwell period where ions are monitored
- an inter-channel delay period between successive MRM transitions to allow ions to be cleared from the collision cell.

Following the inter-channel delay, the collision cell must be rapidly filled with ions of the next transition.

If this does not occur rapidly enough (i.e., when the dwell time is very short) the signal will fall in intensity.
T-Wave™ Technology

- Fast MRM acquisitions
  - 100 data points/second
  - Maintains sensitivity
  - Minimises cross-talk

- Improved scanning data
  - Precursor ion mode
  - Neutral loss mode

The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).
Do you need a high MRM acquisition rate?

Travelling Wave Ion Transport
The effect of MRM acquisition rate on signal intensity

100 data points per second
T-Wave for Fast MS/MS scanning
Improved Precursor and CNL scans

Neutral loss scans can be acquired at very high scan speeds with almost no loss in signal intensity.
UPLC Compatibility Summary

- New digital electronics delivers fast scan capability
- T-Wave delivers fast MS/MS capability
- New design delivers mode switching speed
  - ESCi
  - Pos/Neg
• IntelliStart is designed to automatically monitor Instrument ‘health’ to ensure it is ready for use.

• Where possible, the system may also take corrective action to rectify any failed system checks

• IntelliStart comprises:
  – Automated system checking software
  – Diagnostic electronics
  – Integrated fluidics device
  – 2 built-in vial locations for set-up/user solutions
IntelliStart software continuously monitors selected instrument parameters:

- Operate status
- Gases
- Voltages
- Temperatures
- Vacuum
- Inlet communications
- Network availability/disk space
- Tune check
- Calibration check
- Service date elapsed
- Fuses
- Interlocks
IntelliStart: MassLynx Toolbar

- System status displays Readiness state to the user
- System check failure results in red light
- System help describes the failed system check
- Mouse hover-over reveals reason and corrective action

**Click **IntelliStart** to launch System Console**
Designed to simplify user interaction with the system, enabling many actions to be automated
- Displays detailed information on system readiness with possible corrective action. Progress bar shows time required to rectify.
- Enables automated tuning, calibration and system performance checks to be performed
- Fluidics can be operated manually from the Console or automated as part of an IntelliStart sequence
IntelliStart also features a system performance check

6 replicate injections of a known compound are made from the LC system with known chromatographic retention time

Data quality measurements are made by OpenLynx processing to produce a pass/fail report

Users may define tolerances for pass criteria

Results are logged in the System Console and reports are produced in OpenLynx (electronic) and printed form.

Raw data and experimental details are also stored.
System check:

- Retention time
- Peak area
- Peak height
- Peak width
- Signal-to-noise
Sample 1

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Peak Area</th>
<th>Peak Height</th>
<th>Peak Width</th>
<th>Signal to Noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.52</td>
<td>111254</td>
<td>2156784</td>
<td>0.31</td>
<td>1143:1</td>
</tr>
</tbody>
</table>

Summary

<table>
<thead>
<tr>
<th>.</th>
<th>Pass/Fail</th>
<th>Setpoint</th>
<th>Value</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Time</td>
<td>Pass</td>
<td>10.5</td>
<td>10.52</td>
<td>+/- 0.2 mins</td>
</tr>
<tr>
<td>Peak Area</td>
<td>Pass</td>
<td>110000</td>
<td>1.2% RSD</td>
<td>10%</td>
</tr>
<tr>
<td>Peak Height</td>
<td>Not Tested</td>
<td>N/A</td>
<td>5.2% RSD</td>
<td>N/A</td>
</tr>
<tr>
<td>Peak Width</td>
<td>Pass</td>
<td>0.3</td>
<td>0.31</td>
<td>+/- 0.05 mins</td>
</tr>
<tr>
<td>Signal to Noise</td>
<td>Pass</td>
<td>.</td>
<td>1235:1</td>
<td>1000:1</td>
</tr>
</tbody>
</table>
• Connections® INSIGHT™ uses proven Intelligent Device Management (IDM) technology that has been successfully implemented in highly regulated, maximum security environments, ranging from high tech to healthcare industries.

• Connections® INSIGHT™ leverages the Internet to proactively and securely connect the ACQUITY UPLC™ Systems to Waters service experts.

• Connections® INSIGHT™ builds an instrument usage profile over time so we can deliver customized service solutions.

• Connections® INSIGHT™ creates the “virtual presence” of a service Engineer in the lab that monitors customers’ ACQUITY UPLC™ systems to ensure maximum uptime and system performance.

• Now extended to include SQD and TQD systems.
For the first time, a Tandem Quadrupole MS will be available on both MassLynx and Empower platforms.

- Scalable, networked CDS Solution
- Embedded relational database
- Support for regulated laboratory environments
- Full system suitability reporting
- Method Validation Manager

- Dedicated MS Software platform
- Customized Application-managers
- Automated System check
- QuanOptimize
- Open Access quantitation
Summary

• UPLC has demanded improved acquisition speed from mass spectrometry

• New single quadrupole and tandem quadrupole MS systems designed for UPLC compatibility

  – Faster…multi-mode complex acquisitions in single UPLC run
  – Easier…IntelliStart automated system monitoring & set-up
  – Smaller…>25% smaller than previous generation
Many thanks to all on the ACQUITY SQD and TQD project team
Thank you

Any questions?
## Quattro micro vs TQ Detector

<table>
<thead>
<tr>
<th></th>
<th>Quattro micro</th>
<th>TQ Detector</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mass range</strong></td>
<td>4-2000amu</td>
<td>4-2000amu</td>
<td>-</td>
</tr>
<tr>
<td><strong>Scan Speed</strong></td>
<td>5,000amu/s</td>
<td>10,000amu/s</td>
<td>2x</td>
</tr>
<tr>
<td><strong>Polarity switching speed</strong></td>
<td>100ms</td>
<td>20ms</td>
<td>5x</td>
</tr>
<tr>
<td><strong>Minimum Dwell time</strong></td>
<td>10ms</td>
<td>5ms</td>
<td>2x</td>
</tr>
<tr>
<td><strong>Minimum dwell time without signal loss</strong></td>
<td>100ms</td>
<td>5ms</td>
<td>20x</td>
</tr>
<tr>
<td><strong>Minimum inter-channel delay</strong></td>
<td>10ms</td>
<td>5ms</td>
<td>2x</td>
</tr>
<tr>
<td><strong>ESCi switching speed</strong></td>
<td>100ms</td>
<td>20ms</td>
<td>5x</td>
</tr>
<tr>
<td><strong>IonSabre &amp; APPI options</strong></td>
<td>Yes – requires external control unit</td>
<td>Yes – plug&amp;play</td>
<td>Integrated</td>
</tr>
<tr>
<td><strong>Autotune</strong></td>
<td>Autotune wizard</td>
<td>IntelliStart</td>
<td>System checks</td>
</tr>
</tbody>
</table>