Recent Developments in UPLC Technology

UPLC Evolution
From 2004 to today

Sevilla, 3 de Noviembre 2011
UltraPerformance LC® Has Been Termed as a New Category of LC

- Years of focused research and development at Waters, in collaboration with LC scientists in academia and industry
- Introduced the first holistically designed UPLC system at PittCon™ 2004

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Chemistry</th>
<th>User Interface</th>
</tr>
</thead>
</table>
| • Binary Solvent Manager  
• Sample Manager  
• Column oven  
• TUV detector  
• PDA detector | • BEH C18  
• eCord | • Connections Insight  
• Empower  
• MassLynx  
• eCord |
Same Resolution and Selectivity with Increased Speed - Constant L/dp

5 µm – 150 mm
Injection = 5.0 µL
Flow rate = 0.2ml/min
Rs (2,3) = 2.28

3.5 µm – 100 mm
Injection = 3.3 µL
Flow rate = 0.3ml/min
Rs (2,3) = 2.32

2.5 µm – 75 mm
Injection = 2.5 µL
Flow rate = 0.5ml/min
Rs (2,3) = 2.34

1.7 µm – 50 mm
Injection = 1.7 µL
Flow rate = 0.6ml/min
Rs (2,3) = 2.29

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Traditional HPLC vs. UltraPerformance LC®

Traditional HPLC

UPLC®

UPLC® increases speed to get to right answers…. faster
What Were The Requests?

- From the introduction to January 2010, the ACQUITY UPLC was the only available core system
  - The definition was not changed
    - Binary solvent manager, fixed loop injector, ...
  - Many improvements were brought to the system
    - Needles, check valves, injection modes, ...
ACQUITY UPLC

Responding to customer feedback

There are thousands of ACQUITY UPLC Systems implemented successfully around the world, in every major industry, in hospitals, in universities, in public service laboratories, and research institutes.

The business benefits of ACQUITY show an outstanding return on investment (ROI), and will save money when compared to conventional HPLC.

UPLC has been adopted across business functions, from research and development to manufacturing and quality control.

One ACQUITY UPLC can replace multiple HPLC systems, bringing economies in capital investment, space, and overall running costs.

Introducing the newest member of the ACQUITY family, designed to meet those demands.
H- Class Introduction

- H-Class introduction in January 2010
  - New system with new definition:
    - Quaternary mixing: more solvent capability
    - Flow-Through-Needle (FTN) injection design: ease of use
    - New column management: better temperature control
  - But same performances as the ACQUITY UPLC!
### Continuous evolution driving adoption

Holistic system design has driven/accelerated innovation across product lines.

<table>
<thead>
<tr>
<th>2004</th>
<th>2005</th>
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<th>2007</th>
<th>2008</th>
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<tr>
<td>1 col. Oven</td>
<td>Sample Organizer</td>
<td>Column Mgr</td>
<td>FLR</td>
<td>Extended wavelength</td>
<td>Local Console</td>
<td>H-Class</td>
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<td>TUV &amp; PDA</td>
<td>New TUV</td>
<td>Column H/C</td>
<td>New CH</td>
<td>PDA</td>
<td>Controller</td>
<td>H-Class Bio</td>
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<td></td>
<td>New PDA ELSD</td>
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<td>Enhanced Pump Perf.</td>
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<td>Leak Sensors</td>
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<tr>
<td>BEH C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>BEH RP18</td>
<td>BEH300 C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>HSS C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>BEH300 C&lt;sub&gt;4&lt;/sub&gt;</td>
<td>BEH Amide</td>
<td>BEH200 SEC</td>
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<td>BEH Phenyl</td>
<td>BEH HILIC HSS T3</td>
<td>HSS C&lt;sub&gt;18&lt;/sub&gt; SB</td>
<td>BEH Glycan</td>
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<td>SYNAAPT</td>
<td>Xevo TQ</td>
<td>Xevo QTof</td>
<td>Xevo QT-S</td>
<td>Xevo G2</td>
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<td>SYNAPT G2</td>
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<td>MS Third Party</td>
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<td>AccQ•Tag Ultra for AAA</td>
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<td>Oligonucleotide</td>
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</tbody>
</table>
ACQUITY UPLC H-Class System

- Quaternary Solvent Manager (QSM)
- Sample Manager – Flow Through Needle (SM-FTN)
- Column Heater

- Supports all current ACQUITY UPLC detectors
  - PDA and PDAe
  - TUV
  - FLR
  - ELSD
  - MS systems
ACQUITY UPLC H-Class Quaternary Solvent Manager

- Operating characteristics
  - Maximum flow = 2mL/min
  - Maximum operating pressure = 15,000 psi

- Quaternary solvent delivery
  - Optional integrated Solvent Select Valve expands solvent choices to 9
  - Features Auto-Blend technology
    - Continuous blending of four solvents in any combination or proportion
    - Eliminates potential variability caused by manual mixing of solvents
Solvent Select Valve

- Adds 6 additional solvents to line D (3 + 6 solvents available)
Aldehydes and Ketones as DNPH Derivatives

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Water</th>
<th>% Acetonitrile</th>
<th>% THF</th>
<th>% Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>64</td>
<td>29</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>5.0</td>
<td>56</td>
<td>34</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>9.0</td>
<td>41</td>
<td>38</td>
<td>7</td>
<td>14</td>
</tr>
</tbody>
</table>
Developing method: Trying different TFA concentration

<table>
<thead>
<tr>
<th>TFA%</th>
<th>Water</th>
<th>Acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,025%</td>
<td>97,5%</td>
<td>0%</td>
</tr>
<tr>
<td>0,05</td>
<td>95%</td>
<td>0%</td>
</tr>
<tr>
<td>0,1%</td>
<td>90%</td>
<td>0%</td>
</tr>
</tbody>
</table>

**INITIAL CONDITIONS**

- **A**: 90% Water
- **B**: 0% Acetonitrile
- **C**: 0% Isopropanol
- **D**: 10% 1% TFA

**FINAL CONDITIONS**

- **A**: 40% Water
- **B**: 50% Acetonitrile
- **C**: 0% Isopropanol
- **D**: 10% 1% TFA

100% Water
0% Acetonitrile
0.1% TFA

50% Water
50% Acetonitrile
0.1% TFA
**Peptide Map Development**

**Varying TFA Concentration - %D**

- **0.025% TFA - 2.5% D**
- **0.05% TFA - 5% D**
- **0.1% TFA - 10% D**
Stability and Reproducibility
Peptide Mapping

Friday Night
Saturday Morning
Saturday Afternoon
Saturday Night
Sunday Morning
Sunday Afternoon
Sunday Night

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ACQUITY UPLC H-Class Sample Manager -FTN

- Programmable volume injection
  - Up to 10uL injection standard
  - Up to 250uL with extensions

- Needle in flow path design
  - Total volume sample injection

- Full range of plates and vials

- Supports load ahead and loop offline
  - For increased throughput
Injection Performance
2 Orders of Magnitude

No re-configuration necessary across the entire UPLC injection volume range

- 10.0µL @ 2µg/mL
- 1.0µL @ 20µg/mL
- 0.1µL @ 200µg/mL
Sample Manager – FTN Carryover Challenge

Stress Injection @ 2mg/mL
1st Blank Injection
Carryover = 0.0002%
Column Heater

- Temperature range: 20°C to 90°C
- Columns up to 4.6 mm x 150 mm with filter or guard column
- Active pre-heating standard
  - Ensures consistent control of temperature
  - Eliminates environmental influences on temperature
  - Reduced volume minimizes column bandspreading
  - Passive pre-heaters supported for legacy method transfer

- 4 - 90°C Multi-zone (Independent control of each compartment)
- Supports up to 2 columns, up to 4.6 mm x 150 mm with filter or guard column
- Active pre-heating standard
- Two 7-port, 6-position switching valves
- Stackable for support of up to 6 columns
Transferability Across Technology Platforms: UPLC-to-HPLC

1.8 µm 1.7 µm

3.5, 5 µm 2.5, 3.5, 5, 10 µm

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Method Transfer Tools

- Columns Calculator
  - HPLC to UPLC to HPLC
  - Ability to cut and paste conditions into Empower Methods Editor

- Pre-injection volume and Gradient Smart Start
  - Useful for accommodating systems with different dwell volumes

- New Method Transfer Chemistry Kits
Method Transfer Kits

- Method Transfer Kits are designed to take the guess work out of transferring a method from one LC to another.
  - Sustained selectivity between UPLC and HPLC particle sizes

- Each kit includes:
  - ACQUITY UPLC Columns calculator (multi-directional) that enables the customer to transfer from any technology platform to any other technology platform
    - HPLC-to-UPLC-to-HPLC
  - UPLC and HPLC column with equivalent selectivity and resolving power

Method Transfer Kit Options:
- 1.7/1.8 µm to 5 µm Transfer kit
  - 2.1 x 50 mm, 1.7/1.8 µm and 4.6 x 150 mm 5 µm
- 1.7/1.8 µm to 3.5 µm Transfer kit
  - 2.1 x 50 mm, 1.7/1.8 µm and 4.6 x 100 mm 3.5 µm
- 1.7/1.8 µm to 3.5 µm High Rs Transfer kit
  - 2.1 x 100 mm, 1.7/1.8 µm and 4.6 x 150 mm 3.5 µm
Transfer Methods With Ease

Utilize existing Assets
Transfer from UPLC-to-HPLC

Future-proof your lab
Run HPLC methods on ACQUITY UPLC H-Class

Retention Time Relative to Clozapine

<table>
<thead>
<tr>
<th>Separation Mode</th>
<th>Instrument</th>
<th>Impurity D</th>
<th>Impurity C</th>
<th>Impurity A</th>
<th>Impurity B</th>
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</thead>
<tbody>
<tr>
<td>UPLC</td>
<td>ACQUITY UPLC H-Class System</td>
<td>0.867</td>
<td>0.890</td>
<td>0.939</td>
<td>1.500</td>
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<tr>
<td>HPLC</td>
<td>Alliance 2695 System</td>
<td>0.865</td>
<td>0.895</td>
<td>0.950</td>
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<tr>
<td>HPLC</td>
<td>ACQUITY UPLC H-Class System</td>
<td>0.867</td>
<td>0.898</td>
<td>0.951</td>
<td>1.507</td>
</tr>
</tbody>
</table>
Delivers the proven business impact of UPLC into routine testing laboratories.

Replaces conventional HPLC as the chromatographic system of choice.

Will reproduce established HPLC methods while enabling seamless transfer to UPLC.

Is the system of choice for method development.

Future-proofs your LC laboratory with the widest range of chemistry, detection and services.

HPLC simplicity, UPLC performance.
What Were The Requests?

- Is it possible to achieve even better UPLC chromatographic performances?
  - Better efficiency, better peak capacity, better resolution?
    - We need to see more peaks!
  - Lower carryover specification?
    - To better take advantage of increased sensitivity
  - Shorter injection cycle time
    - To run more analysis
  - Manage 1 mm columns
    - Increased sensitivity and robustness with MS detectors

- Technology enablers:
  - Smaller extra column volume
  - Fast injection mechanism
  - Higher pressure limit
ACQUITY UPLC I-Class System

WHAT SEPARATES YOU FROM EVERYONE ELSE.
Why I-Class?

- Holistic configurations to ensure the best performance
  - Dwell volume
  - UV Cell
  - Ideal for ballistic gradients

- Providing the best performance in UPLC / MS
  - High End MS inlet of choice
  - Contributing to a better LOD
  - Chromatographic resolution

- Configuration flexibility
  - Choice of injector
  - Keeping the best performance
How do we take advantage of smaller particles?

- High pressure fluidic modules
  - Up to 15000 psi (1000 bars)
  - Low system volume → Reduce run time, equilibration time

- Reduced cycle time autosampler with minimum carryover → Reduce run time (injection to injection)

- Minimized system volumes and optimized flow paths → Reduce band broadening, Extra column effects

- High speed detectors; optical and mass
  - Low dispersion detector cell → Reduce band broadening, Extra column effects
  - High data rates
  - Low filter constant → Maintain resolution
The ACQUITY UPLC I-Class System represents the pinnacle of ultra performance separations technology, built upon seven years of engineering innovations fueled by customer input.

The ACQUITY UPLC I-Class System accomplishes new levels of analytical capabilities by maximizing peak capacity, advancing the impact of chromatographic separations and extending the performance of any MS detector.
ACQUITY UPLC I-Class Attributes - Meaningful Impact

Robust, proven performance built on 7 years of user based design innovation

Seamless transfer of existing UPLC methods

I-Class delivers the highest throughput without compromising performance

I-Class is designed to optimized the performance of any Mass Spectrometer

Pinnacle of Chromatographic performance
Why I-Class?

- Complex separation challenges require LC systems that are designed to maximize the benefits of sub 2µm particle columns.
  - Has minimized dispersion to enhance MS and UV performance
  - Lowest carryover complementing MS sensitivity and extending MS linear dynamic range
  - The system’s low dispersion, faster cycle-time allows complex separations can be accelerated without compromising chromatographic fidelity

Evolving analytical demands
Increasing regulatory demands
Quality and Consistency of results
-> Up time
<- Cost per sample
Why I-Class?

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Evolving analytical demands
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Impact of Low dispersion - Sensitivity

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Why I-Class?

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- **Lowest carryover complementing MS sensitivity and extending MS linear dynamic range**

- The system’s low dispersion, faster cycle-time allows complex separations can be accelerated without compromising chromatographic fidelity

Evolving analytical demands

Increasing regulatory demands

Quality and Consistency of results

->Up time

<-Cost per sample
Carryover Performance: I-Class with Xevo TQ-S

- >4 orders of magnitude
- Assurance that the quantitation represents only the presence of analyte, not carryover
- Exploit the full linear dynamic range
- Ability to analyze disparate levels using the full sensitivity range of the MS
Why I-Class

- Has minimized dispersion to enhance MS and UV performance
- Lowest carryover complementing MS sensitivity and extending MS linear dynamic range
- The system’s low dispersion, faster cycle-time allows complex separations can be accelerated without compromising chromatographic fidelity
Accelerate Ballistic Separations
I-Class vs. Competitor – No compromise

Competitive UHPLC System: 21μL extra column band spread

Average Peak Capacity at 5σ: 42

ACQUITY UPLC I-Class: 5μL extra column band spread

Average Peak Capacity at 5σ: 70

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Complex separation challenges require LC systems designed to maximize the benefits of sub 2μm particle columns. The ACQUITY UPLC I-Class system:

- Maximized MS, UV detection sensitivity
- Generating robust, highest performance binary based methods
- Increased throughput without compromising chromatographic fidelity maximizing return on investment

Evolving analytical demands
Increasing regulatory demands
Quality and Consistency of results
-> Up time <- Cost per sample

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ACQUITY UPLC I-Class System

- What is it?

- Binary Solvent Manager
- Sample Manager (2 options)
  - Fixed-Loop (FL) Sample Manager
  - Flow-Through-Needle (FTN) Sample Manager
- Column Management (2 options)
  - Single Column Heater
  - Dual Column Manager
    - Max 2 columns with optional 2D Technology feature
- Detection
  - MS
  - TUV or PDA only – new lower dispersion flow cells
- New ACQUITY UPLC Systems Driver Pack (DP 3)
- Existing ACQUITY UPLC Chemistries
The Widest UPLC Column Offering
- Now supporting 15 – 18 K PSI

- **Five particle substrates**
  - 130Å, 200Å and 300Å BEH [Ethylene Bridged Hybrid], HSS [High Strength Silica] and CSH [Charged Surface Hybrid]
  - All are available in HPLC and UPLC particle sizes

- **Wide and growing selection of column chemistries**
  - 14 scalable stationary phases
  - BEH 130Å C₁₈, C₈, Shield RP₁₈, Phenyl, HILIC and Amide
    - BEH 300Å C₁₈ and C₄
    - HSS C₁₈, T₃, C₁₈ SB
  - CSH C₁₈, Fluoro-Phenyl and Phenyl-Hexyl

- **Proven application-based solutions**
  - SEC, AAA, OST, PST, PrST and Glycan

- **Transferability between HPLC and UPLC**
  - XBridge HPLC and ACQUITY UPLC BEH columns
  - HSS HPLC and ACQUITY UPLC HSS columns
  - XSelect HPLC and ACQUITY UPLC CSH columns

- **VanGuard Pre-columns**

- **eCord Technology**
NEW Binary Solvent Manager

ACQUITY UPLC I-Class BSM

- High pressure Binary mixing
  - 18,000 PSI maximum
    - 2mL/min at 12,000 PSI
  - 4 solvents (A1 or A2 and B1 or B2)
- New seals, check valves and intelligent Intake Valves (i²V)
- Extended automatic, compressibility compensation
- New vent valve for convenience even at higher pressures
- New higher pressure mixers
  - 50 µL default, 100 µL and 380 µL
### Fixed Loop
- New EverFlow inject valve to enable higher pressures
- H-Class chassis and robust rotary sample tray/plate mechanism
- Compatible with newest Sample Organizer (18 shelves)
- New low dispersion fittings, shorter sample path for the FL (10 µL)
  - More robust sample transfer
- Low dispersion 1, 2, 5 and 10 µL loop design
  - Conventional 20, 100 and 250 µL available
- System volume <95 µL

### Flow Through Needle
- New EverFlow inject valve design to enable higher pressures
- H-Class chassis and robust rotary sample tray/plate mechanism
- Compatible with newest Sample Organizer (18 shelves)
- New low dispersion fittings, lower dispersion needle seal for the FTN
- Optional, conventional extension loops
- System volume <100 µL
I-Class FTN vs. I-Class FL: Comparison Summary

- When ultimate UPLC performance or throughput is the user’s paramount concern, position the Fixed Loop Sample Manager
- Best for 1.0 mm columns
- Great carryover performance

- When usability, flexibility and lowest possible carryover matter most position the FTN Sample Manager
- Excellent dispersion
- Runs ballistic gradients well
- Longer cycle time
NEW Column Heater

- New 0.003” ID tubing
- Robust 18K psi fitting
- Excellent method transfer between I-Class systems
- CH-30A is compatible where HPLC columns must be supported
  - H-Class performance only

Increasing regulatory demands
Quality and Consistency of results

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UPLC H-Class to UPLC I-Class Method Transfer Example

ACQUITY UPLCH-Class

System Volume Not Adjusted

ACQUITY UPLC I-Class

Increasing regulatory demands

ACQUITY UPLC H-Class

-> Up time

Adjusted System Volume

< - Cost per sample

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NEW Column Manager

- Two (2) Columns plumbed right or left only
- New 0.003” ID tubing
- Robust 18 K PSI fitting
- Excellent Method transfer between I-Class systems
- 2D Technology supported

Increasing regulatory demands
Quality and Consistency of results
->Up time <-Cost per sample
ACQUITY UPLC I-Class

- ACQUITY UPLC I-Class is a system designed to meet evolving analytical demands, by
  - Maximized detection sensitivity, especially MS
  - Generating robust, highest performance binary based methods
  - Increasing productivity without compromising chromatographic fidelity to maximize return on investment sooner

- Clear where I-Class fits, for the user, wherever sensitivity, particularly MS, UPLC performance and productivity are critical to the user
Accelerate Ballistic Separations
ACQUITY and ACQUITY I-Class

Instrument Method Name: 30sec_10_95p600mL_90C ACQUITY

Average peak capacity at 5σ = 57

Instrument Method Name: 20sec_NO hold 10_95_p904mL_90C

Average peak capacity at 5σ = 70

-> Up time
<- Cost per sample

Evolving analytical demands
Maintaining minimum USP resolution with fastest run time of an anti-depressant medication

ACQUITY UPLC with PDAD Detector
ACQUITY UPLC BEH C_{18}^r
2.1 x 30 mm; 1.7 μm

0.86 injection-to-injection cycle time
Average peak width ~ 0.44 sec
ACQUITY UPLC I-Class
Faster Gradients for LC/MS

Faster with better resolution
Systems designed to meet your needs.

<table>
<thead>
<tr>
<th>ACQUITY UPLC I-Class</th>
<th>ACQUITY UPLC H-Class</th>
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<tbody>
<tr>
<td>Ultimate performance</td>
<td>Method development</td>
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<td>MS inlet</td>
<td>Methods transfer</td>
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<td>High throughput</td>
<td>Routine analysis</td>
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<tr>
<td>Binary solvent管理</td>
<td>Multiple solvent management</td>
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</table>
Acquity UPLC H-Class for Food Analysis
Beverage – Additives Analysis
Experimental Overview

**Aims:**
- To rapidly analyze 6 additives in soft drinks with minimum sample preparation
  - Acesulfame potassium (ASK) - Sodium saccharin - Caffeine - Sodium benzoate – Aspartame - Potassium sorbate
- To rapidly analyze 14 compounds (vitamins and dyes) in soft drinks (with minimal / no sample preparation)
- To develop a rapid method for a new sweetener
  - Detect compound and ID impurities

**Results:**
- High-quality results
- Increased laboratory productivity
- Reduced cost / sample
Beverage Analysis

Common Additives – H-Class HPLC mode

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<tr>
<th>Time</th>
<th>%age A</th>
<th>%age B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>1.50</td>
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<td>6.00</td>
<td>82</td>
<td>18</td>
</tr>
<tr>
<td>6.10</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

No | Compound                  
---|---------------------------|
1  | Acesulfame-K (ASK)        |
2  | Sodium Saccharin          |
3  | Caffeine                  |
4  | Sodium Benzoate           |
5  | Aspartame                 |
6  | Potassium Sorbate         |
## Beverage Analysis

### Common Additives – H-Class UPLC mode

<table>
<thead>
<tr>
<th>Time</th>
<th>%age A</th>
<th>%age B</th>
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<tbody>
<tr>
<td>0.00</td>
<td>95</td>
<td>5</td>
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<tr>
<td>1.50</td>
<td>66</td>
<td>34</td>
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<tr>
<td>1.60</td>
<td>95</td>
<td>5</td>
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<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acesulfame-K (ASK)</td>
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<td>2</td>
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<td>3</td>
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</tr>
<tr>
<td>4</td>
<td>Sodium Benzoate</td>
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<tr>
<td>5</td>
<td>Aspartame</td>
</tr>
<tr>
<td>6</td>
<td>Potassium Sorbate</td>
</tr>
</tbody>
</table>

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Beverage Analysis

Additives - Alcoholic & soft drink examples

Diet Cola
1- ASK- 88.9 mg/L
2- Caffeine- 108.0 mg/L
3- Aspartame- 255.2 mg/L
4- Potassium Sorbate- 98.6 mg/L (73.0 )**
*- Unknown compound

Root Beer
3- Sodium Benzoate- 532.4 mg/L ( 447.2 )**
*- Unknown compound

Diet Fruit Flavored Soft Drink
1- ASK- 100.5 mg/L
2- Caffeine- 157.2 mg/L
3- Sodium Benzoate- 392.3mg/L (329.5 )**
4- Aspartame- 306.9 mg/L
*- Unknown Compound
Beverage Analysis
Column Selectivity – pH 4.7

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Acesulfame-K (ASK)</td>
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<tr>
<td>2</td>
<td>Sodium Saccharin</td>
</tr>
<tr>
<td>3</td>
<td>Caffeine</td>
</tr>
<tr>
<td>4</td>
<td>Sodium Benzoate</td>
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<td>Aspartame</td>
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<tr>
<td>6</td>
<td>Potassium Sorbate</td>
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- HSS T3
- CSH-C18
- CSH Phenyl-Hexyl
- CSH Fluoro-Phenyl

©2011 Waters Corporation
Beverage Analysis
Column Selectivity – pH 3.75

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<th>Compound</th>
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<td>Caffeine</td>
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<td>Potassium Sorbate</td>
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Beverage Analysis

Column Selectivity – pH 3.75

CSH Fluoro-Phenyl

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<tr>
<td>4</td>
<td>Sodium Benzoate</td>
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<td>5</td>
<td>Aspartame</td>
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Beverage Analysis

Column Selectivity: Samples

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<tr>
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<tr>
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<td>Sodium Saccharin</td>
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<td>3</td>
<td>Caffeine</td>
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<tr>
<td>4</td>
<td>Sodium Benzoate</td>
</tr>
<tr>
<td>5</td>
<td>Aspartame</td>
</tr>
<tr>
<td>6</td>
<td>Potassium Sorbate</td>
</tr>
</tbody>
</table>

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Dairy Products: Sugar Analysis

Standards using ELS detection

Acquity H-Class with ELS Detector

<table>
<thead>
<tr>
<th>Column</th>
<th>Acquity BEH Amide 1.7 um, 2.1 x 100mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column temp</td>
<td>35C</td>
</tr>
<tr>
<td>Run-time</td>
<td>18 min</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>5 uL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>%age A</th>
<th>Curve</th>
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<tbody>
<tr>
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<td>15</td>
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<td>2.00</td>
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<td>11.00</td>
<td>50</td>
<td>4</td>
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<td>18.00</td>
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<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Fructose</td>
<td>5.75</td>
</tr>
<tr>
<td>Sorbose</td>
<td>5.99</td>
</tr>
<tr>
<td>Glucose</td>
<td>6.69</td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.99</td>
</tr>
<tr>
<td>Maltose</td>
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</tr>
<tr>
<td>Lactose</td>
<td>8.63</td>
</tr>
<tr>
<td>Maltotriose</td>
<td>9.64</td>
</tr>
<tr>
<td>Maltodextrase</td>
<td>10.42</td>
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![Graph showing sugar analysis results]
Dairy Products: Sugar Analysis

Samples using ELS detection

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<thead>
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<th>Concentration (mg/g)</th>
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<td>Sucrose</td>
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<tr>
<td>Lactose</td>
<td>140.1</td>
</tr>
<tr>
<td>Maltotriose</td>
<td>1.9</td>
</tr>
<tr>
<td>Maltodextrate</td>
<td>1.3</td>
</tr>
<tr>
<td>Lactose</td>
<td>3.1</td>
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</table>
## Dairy Analysis: Vitamins Using UV detection

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<th>%age B</th>
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<tbody>
<tr>
<td>0.00</td>
<td>99</td>
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<td>1.50</td>
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<tr>
<td>1.60</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>3.00</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>5.50</td>
<td>45</td>
<td>55</td>
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<tr>
<td>5.60</td>
<td>45</td>
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<td>98</td>
</tr>
<tr>
<td>7.60</td>
<td>99</td>
<td>1</td>
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<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
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<tbody>
<tr>
<td>1</td>
<td>Thiamine-HCl (B1)</td>
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<tr>
<td>2</td>
<td>Ascorbic acid (C)</td>
</tr>
<tr>
<td>3</td>
<td>Nicotinic Acid (B3-OH)</td>
</tr>
<tr>
<td>4</td>
<td>Niacinamide (B3-NH2)</td>
</tr>
<tr>
<td>5</td>
<td>Pyridoxine-HCl (B6)</td>
</tr>
<tr>
<td>6</td>
<td>Pantothenate (B5)</td>
</tr>
<tr>
<td>7</td>
<td>Cyanocobalamin (B12)</td>
</tr>
<tr>
<td>8</td>
<td>Folic Acid (B9)</td>
</tr>
<tr>
<td>9</td>
<td>Biotin (B7)</td>
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<tr>
<td>10</td>
<td>Riboflavin (B2)</td>
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Dairy Analysis: Vitamins

Using UV detection

<table>
<thead>
<tr>
<th>Time</th>
<th>%age A</th>
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<tbody>
<tr>
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<td>90</td>
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<td>90</td>
</tr>
<tr>
<td>3.00</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>7.00</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>7.50</td>
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<td>90</td>
</tr>
<tr>
<td>10.00</td>
<td>10</td>
<td>90</td>
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<table>
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<tr>
<th>t&lt;sub&gt;R&lt;/sub&gt;</th>
<th>Compound</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Vitamin A1</td>
</tr>
<tr>
<td>2</td>
<td>Vitamin K2</td>
</tr>
<tr>
<td>3</td>
<td>Vitamin D2</td>
</tr>
<tr>
<td>4</td>
<td>Vitamin D3</td>
</tr>
<tr>
<td>5</td>
<td>Vitamin E</td>
</tr>
<tr>
<td>6</td>
<td>Vitamin E acetate</td>
</tr>
<tr>
<td>7</td>
<td>Vitamin K1</td>
</tr>
</tbody>
</table>
Dairy Products: Preservatives Analysis

Sorbic acid: 0.06\%RSD
Benzoic acid: 0.09\%RSD
Food Safety Applications

Analysis of PAHs in edible oils and fish samples
Background

- Commonly found in the environment
  - Carcinogenic properties
  - Analysis is mandated WW
- Official method use HPLC procedures
  - Detection: UV and fluorescence.
  - MRL – can be challenging (2.0 ppb)

Solution:

- ACQUITY H-Class with eλPDA and FLR-LVFC
  - Rapid screening of PAHs:
    - Edible oils & Fish
### PAHS PDA and FLR conditions

<table>
<thead>
<tr>
<th></th>
<th>UV</th>
<th>Ex</th>
<th>Em</th>
<th></th>
<th>UV</th>
<th>Ex</th>
<th>Em</th>
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</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>220</td>
<td>276</td>
<td>331</td>
<td>Benzo(j)fluoranthene *</td>
<td>223</td>
<td>239</td>
<td>511</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>229</td>
<td>-</td>
<td>-</td>
<td>Benzo(b)fluoranthene</td>
<td>256</td>
<td>298</td>
<td>437</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>226</td>
<td>298</td>
<td>329</td>
<td>Benzo(k)fluoranthene</td>
<td>307</td>
<td>301</td>
<td>407</td>
</tr>
<tr>
<td>Fluorene</td>
<td>261</td>
<td>293</td>
<td>306</td>
<td>Benzo(a)pyrene</td>
<td>296</td>
<td>364</td>
<td>408</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>250</td>
<td>248</td>
<td>363</td>
<td>Dibenzo(a,l)pyrene *</td>
<td>314</td>
<td>314</td>
<td>427</td>
</tr>
<tr>
<td>Anthracene</td>
<td>251</td>
<td>248</td>
<td>400</td>
<td>Benzo(g,h,i)perylene</td>
<td>296</td>
<td>297</td>
<td>395</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>235</td>
<td>246</td>
<td>488</td>
<td>Dibenzo(a,h)anthracene</td>
<td>299</td>
<td>298</td>
<td>422</td>
</tr>
<tr>
<td>Pyrene</td>
<td>240</td>
<td>312</td>
<td>390</td>
<td>Indeno(1,2,3-cd)pyrene</td>
<td>249</td>
<td>305</td>
<td>500</td>
</tr>
<tr>
<td>Cyclopenta(c,d)pyrene *</td>
<td>223</td>
<td>-</td>
<td>-</td>
<td>Dibenzo(a,e)pyrene *</td>
<td>302</td>
<td>271</td>
<td>398</td>
</tr>
<tr>
<td>Benzo(a)anthracene</td>
<td>287</td>
<td>282</td>
<td>392</td>
<td>Dibenzo(a,i)pyrene *</td>
<td>240</td>
<td>370</td>
<td>436</td>
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<tr>
<td>Chrysene</td>
<td>266</td>
<td>267</td>
<td>367</td>
<td>Dibenzo(a,h)pyrene *</td>
<td>309</td>
<td>309</td>
<td>453</td>
</tr>
<tr>
<td>5-Methylchrysene *</td>
<td>268</td>
<td>268</td>
<td>377</td>
<td></td>
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</table>
## Analysis of PAHs
*(EU and US regulated)*

<table>
<thead>
<tr>
<th>Acquity H-Class with eλ PDA &amp; FLR-LVFC</th>
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<tbody>
<tr>
<td><strong>Column</strong></td>
<td>Waters PAH 3 um, 4.6 x 50mm</td>
<td></td>
</tr>
<tr>
<td><strong>Column temp</strong></td>
<td>35°C</td>
<td></td>
</tr>
<tr>
<td><strong>Run-time</strong></td>
<td>7.0 min</td>
<td></td>
</tr>
<tr>
<td><strong>Injection Volume</strong></td>
<td>10 uL</td>
<td></td>
</tr>
<tr>
<td><strong>Solvent A</strong></td>
<td>Water</td>
<td></td>
</tr>
<tr>
<td><strong>Solvent B</strong></td>
<td>Methanol</td>
<td></td>
</tr>
<tr>
<td><strong>Solvent C</strong></td>
<td>Acetonitrile</td>
<td></td>
</tr>
<tr>
<td><strong>Solvent D</strong></td>
<td>Isopropanol</td>
<td>21</td>
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### Analyte Table

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<td>16</td>
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<td>19</td>
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<tr>
<td>Pyrene</td>
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<td>20</td>
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<tr>
<td>Cyclopenta(c,d)pyrene</td>
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<tr>
<td>Benzo(a)anthracene</td>
<td>10</td>
<td>22</td>
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<tr>
<td>Chrysene</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>5-Methylchrysene</td>
<td>12</td>
<td>* Additional analytes for EU</td>
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</tbody>
</table>

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PAHs in edible oil
*US regulated only*

**H-Class, eλ PDA, FLR-LVFC**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
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<tbody>
<tr>
<td>Column</td>
<td>Waters PAH - 3μm 4.6 X 50mm</td>
</tr>
<tr>
<td>Col. temp</td>
<td>35°C</td>
</tr>
<tr>
<td>Run-time</td>
<td><strong>3.5 min</strong></td>
</tr>
<tr>
<td>Inj. Volume</td>
<td>10 μL</td>
</tr>
<tr>
<td>Solvent A</td>
<td>Water</td>
</tr>
<tr>
<td>Solvent B</td>
<td>Methanol</td>
</tr>
<tr>
<td>Solvent C</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>Solvent D</td>
<td>Isopropanol</td>
</tr>
</tbody>
</table>

**Unspiked hazelnut oil**

**Spiked hazelnut oil**

**Unspiked olive oil**

**Spiked olive oil**

**Olive Oil**

**Olive Oil spiked at 2.0 ppb BaP**

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Alternative samples?
E.g. fish?

**DisQuE Workflow**

**AOAC QuECHERS Method Sample Preparation Protocol**

**Benefits of DisQuE**

1. Pre-filled tubes eliminate sample pre-preparation bottlenecks.
2. Clean sorbents and DisQuE devices ensure clean extracts.
3. Premium tubes and quality caps ensure a leak-free fit under ALL extraction conditions.
4. High temperature process eliminates organic contaminants and residual water.

- Sample is homogenized by blender or homogenizer.
- Transfer 15 g sample to 50 mL tube.
- Add 15 mL 1% acetic acid in acetonitrile + salt mixture.
- Add internal standards.
- Shake vigorously for 1 min. Centrifuge > 1500 rpm for 1 min.
- Transfer 1-8 mL to tube with 150 mg MgSO4 + 50 mg PSA per mL extract and shake for 30 seconds.
- Centrifuge > 1500 rpm for 1 min.

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PAHs in fish samples
US regulated only

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Food Safety Applications

Aflatoxins Analysis in Foodstuffs
Aflatoxins
Brief Overview

- Routinely analyzed using HPLC in different food products
- HPLC methods
  - Long
  - Require derivatization step to achieve low levels of detection
- Limits number of samples analyzed

**Solution:**

- **ACQUITY H-class with Fluorescence detection & Aflatoxin Analysis Application Kit**
Aflatoxins
Experimental Overview

- **Aim:**
  - **Business:** Increase lab throughput / productivity compared to HPLC
  - **Analytical:** Meet regulations & eliminate derivatization step
    - Levels of detection
    - Improve chromatographic resolution between compounds

- **Results:**
  - **Analytical:**
    - High sensitivity – able to meet regulatory requirements
    - Fast analysis time increases throughput
    - No derivatization necessary
    - Ternary mixing allows chromatographic separation to be optimized
  - **Business:** Reduced cost / sample
Aflatoxins

<table>
<thead>
<tr>
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<th>Aflatoxins</th>
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<tbody>
<tr>
<td>1</td>
<td>Aflatoxin M1</td>
</tr>
<tr>
<td>2</td>
<td>Aflatoxin G2</td>
</tr>
<tr>
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<td>Aflatoxin G1</td>
</tr>
<tr>
<td>4</td>
<td>Aflatoxin B2</td>
</tr>
<tr>
<td>5</td>
<td>Aflatoxin B1</td>
</tr>
<tr>
<td>Analyte</td>
<td>RSDs (Corn)</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td></td>
<td>RT</td>
</tr>
<tr>
<td>M1</td>
<td>-</td>
</tr>
<tr>
<td>G2</td>
<td>0.06</td>
</tr>
<tr>
<td>G1</td>
<td>0.04</td>
</tr>
<tr>
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</tr>
<tr>
<td>B1</td>
<td>0.03</td>
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</table>

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