Acquity UPLC Injection Techniques

Fixed Loop and Flow through Needle

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Acquity UPLC 2005
THE UPLC FAMILY
Sample limited to manufacturing

Capillary separations with 2D capability

In process monitoring

A full range of ACQUITY systems to meet specific business challenges
### I-Class FTN vs. I-Class FL: Comparison Summary

<table>
<thead>
<tr>
<th></th>
<th>ACQUITY</th>
<th>I-Class FTN</th>
<th>I-Class FL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dwell Volume</strong></td>
<td>120 µL</td>
<td>100 µL</td>
<td>95 µL</td>
</tr>
<tr>
<td><strong>Bandspread</strong></td>
<td>12 µL</td>
<td>&lt;9 µL</td>
<td>&lt;7 µL</td>
</tr>
<tr>
<td><strong>Carryover</strong></td>
<td>0.005 %</td>
<td>0.001 %</td>
<td>0.002 %</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td>&lt;0.3%</td>
<td>&lt;1% 0.2 to 1.9 µL</td>
<td>&lt;0.3% Full loop</td>
</tr>
<tr>
<td></td>
<td>&lt;1.0% PLUNO</td>
<td>&lt;0.5% 2 to 10 µL</td>
<td>&lt;1.0% PLUNO</td>
</tr>
<tr>
<td><strong>Cycle Time</strong></td>
<td>&lt; 15 sec (with load ahead)</td>
<td>&lt;30 sec</td>
<td>&lt; 15 sec (with load ahead)</td>
</tr>
<tr>
<td><strong>User Simplicity</strong></td>
<td>3 injection modes loop changes</td>
<td>Single injection mode</td>
<td>3 injection modes loop changes</td>
</tr>
</tbody>
</table>

*Measured with a complete system – Measurement on module only doesn’t make sense*
## 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
  - More robust sample transfer
- Low dispersion 1, 2, 5 and 10 μL loop design
  - Conventional 20, 50, 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 return line for the FTN
- Optional, conventional extension loops
- System volume <100 μL
Fixed-Loop Injector (FL)

- 2 Different Wash Solvents
  - Different purposes

- 3 Different Injection Modes
  - Full Loop
  - Partial Loop
  - Partial Loop with Needle Overfill

- Typical Carryover Performance with Injection Mode
  - Extra sample drawn in some modes

Partial Loop < Full Loop > or < Partial Loop Needle Overfill
Needle Wash Solvent Descriptions for the Fixed-Loop Injector

- There are two wash solvents
  - **Strong** Needle Wash
    - Tubing flushing
    - Elimination of components injected
    - Never injected
  - **Weak** Needle Wash
    - Strong solvent elimination
    - Used for transporting sample to sample loop
    - Injected with the sample in partial loop pressure assist mode
Selecting Needle Wash Solvents on a Fixed-Loop Injector

**Strong Needle Wash**
- Must be compatible with the weak wash solvent
- Strong enough to remove sample residue from the system
  - Must be able to dissolve the sample
- Should be at least equivalent strength to final gradient conditions
- May contain
  - Acidic or basic modifiers
  - Strong organic solvents like DMSO
    - Start with 10% first increase 10 by 10% until sample elimination

**Weak Needle Wash**
- Good starting point is initial gradient conditions
- Composition: Weak enough to prevent chromatographic perturbation
  - For injection modes that co-inject the solvent (partial loop)
- Volume big enough to ensure appropriate tubing flushing
- Compatible with sample
  - Avoid sample precipitation
    - Carry over effect
    - Poor recovery
- Use additives only if necessary
Carryover Example on ACQUITY UPLC I-Class (FL) Injector
SM-FL: New Chassis with No Window
SM-FL: New Chassis with No Pull-out Drawer
SM-FL: Common Rotary Carousel
SM-FL: Plate Sampling Mechanism

- Fewer moving parts than X, Y, Z transport mechanism
- RΘZ Sampling Mechanism
- Sample Tray continually rotates between injections for uniform sample temperature

New ‘R’ calibration tool for better accuracy on 384 well plates
SM-FL: Injection Port

- Injection port moved to front of SM-FL
- Needle volume reduced
- New routing of needle in sample manager
SM-FL: New Sample Needle

- New default needle volume
- Needle volume reduced
- New fittings

Available Sample Needles
- PEEK (Available Now)
- PEEKSil
- Steel
- FEP
SM-FL: Inject Valve

- Inject Valve
  - 18k psi compatibility
  - New rotor
    - Pod not interchangeable with ACQUITY UPLC
  - Minimized dispersion
  - Improved carryover

- New VDD (Volume Detection Device) design

- New sample loops
SM-FL: New Loops and VDD

- New sample loops
  - Pre-swaged to prevent poor installation
  - Smaller o.d. tubing with more consistent i.d. for better loop to loop consistency
  - Better carryover performance

- New VDD design
  - More robust
  - Lower volume
  - New materials
  - More chemically compatible
SM-FL: Injection Mode Recommendations

- **Full loop**
  - Best precision
  - Requires lots of sample - 3x or more

- **Partial loop with pressure assist**
  - Weak wash injected along with the sample
  - Inject up to 50% of loop volume
  - Recommended only for 20 and 50µL loops
  - Recommended for fast injection-to-injection cycles
  - No extra sample consumed

- **Partial loop needle overfill**
  - Widest injection volume range: 10-75% of loop volume
  - More reproducible than partial loop with pressure assist
  - Requires minimum 3 µL more than the injection volume
    - Default will be less than 15µL, TBD
SM-FL: Full Loop - 1
Sample Drawn

- Drawn Up Needle
- Sample 3X
- Weak wash
- Air gap
- Injector
- Sample Syringe
- Column
- BSM
SM-FL: Full Loop - 2
Sample Loaded

Pressure assist, 150 psi, delivery of weak wash

Air gap

Sample Syringe

BSM

Column

Injector

1 2 3 4 5 6

Needle

Weak wash  Sample 3X  Weak wash

Air gap
SM-FL: Full Loop – 3 Sample Injected

Valve turns, injection made
Only sample is in the loop

Needle

Sample Syringe

Column

BSM

Weak wash
Sample 3X
Weak wash

Air gap
SM-FL:
Partial Loop (Pressure Assist) - 1
Sample Drawn

- Sample Drawn
- Weak wash
- Air gap 4 µL
- Inject

- Drawn Up Needle
- Injector
- Sample Syringe
- Column
- BSM

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SM-FL: Partial Loop - 2
Sample Positioned

Sample
Weak wash
Inject

Weak wash
Pressure Assist

Air gap compressed by Pressure Assist

150 psi

BSM

Column

Sample Syringe

Injector
SM-FL: Partial Loop - 3
Sample Injected

NOTE: Air gap injected
SM-FL: Partial Loop Needle Overfill (PLUNO) – 1 Sample Drawn

**Note:** Since ICOP version 1.30 the default setting is 15µL extra sample per injection. This can be modified.
Sample Positioned

- Sample positioned after the Injector Valve in the Volume Detector tube

**Diagram Descriptions:**
- **Sample:**
  - Pre 14 µL
  - Post 1 µL
- **Weak wash**:
- **Air gap**
- **Inject**
- **Volume Detector**
- **Sample Syringe**
- **Column**
- **BSM**
SM-FL: Partial Loop Needle Overfill – 3
Sample Loaded

- Sample pushed into the Injector loop
- Mobile phase fills remainder of loop

Sample

Weak wash

Inject

Mobile phase

Post 1µL

Pre 14µL

Air Gap

Volume Detector

Syringe

Detector

BSM

Column

Air

Pre 1µL

Load Sample

Sample

Mobile phase

1 µL

14 µL

Inject

2 µL

Air Gap
SM-FL: Partial Loop Needle Overfill – 4
Sample Injected

- Sample injected with some mobile phase
- No weak wash solvent injected
- No air injected
After injection and cleaning of the valve and sampling mechanism there is a part of the valve fact that is not cleaned (Noted in RED)

By actuating the valve during the run the material is pushed into the sample loop and then ‘injected’ during the end of the gradient
SM-FL: ‘Double-Click’ Method
SM-FL: MS Carryover Improvement with ‘Double Click’

<table>
<thead>
<tr>
<th>Carryover</th>
<th>PLNO</th>
<th>PLNO with Double Click</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1: Diphenhydramine</td>
<td>0.0005%</td>
<td>0.0001%</td>
</tr>
<tr>
<td>#2: Oxybutynin</td>
<td>0.0011%</td>
<td>0.0001%</td>
</tr>
<tr>
<td>#3: Terfenadine</td>
<td>0.0053%</td>
<td>0.0004%</td>
</tr>
</tbody>
</table>

1st Blank Injection after High Concentration Standard PLNO
1st Blank Injection after High Concentration Standard PLNO with ‘Double Click’
SM-FL: UV Carryover Improvement with ‘Double Click’ - Caffeine

Partial Loop Needle Overfill (PLNO)

Blank1
Blank1 with DC

Partial Loop Pressure Assist (PL)

Blank1
Blank1 with DC

Injection Mode | Double-Click | Carryover
---|---|---
PLNO | No | 0.00100%
PLNO | Yes | 0.00062%
PL | No | 0.00064%
PL | Yes | 0.00024%
FTN | -- | 0.00034%
SM-FL: Injection Linearity
10μL Loop

Partial Loop Needle Overfill (PLNO)
Benzocaine – 0.99987
Procaine – 0.99971
Tetracaine – 0.99972

Partial Loop Pressure Assist (PL)
Benzocaine – 0.99984
Procaine – 0.99983
Tetracaine – 0.99977
Flow-Through Needle Injector (FTN)

- This style of injector is not typically prone to carryover
  - Only the sample drawn is injected
  - Sample is not transferred to a loop and therefore additional lines are not contaminated
  - Inside of needle is washed with the gradient

- Uses two solvents in the injector
  - Needlewash
    - Responsible for carryover management of exterior of needle
  - Purge Solvent
    - Acts only as a solvent for the metering syringe
      - Never contacts sample
Selecting Needle Wash Solvents on a Flow-Through Needle Injector

**Needle Wash Solvent**

- This does not contact sample so composition is less important
- Use a mixture that has some organic solvent to prevent bacterial growth
- Can use needlewash
- Try to avoid additives

**Purge Solvent**

- Sample should be entirely soluble in the wash solvent
- Should be compatible with the sample diluent
- Can contain modifiers
- Very poor selection of wash solvent can lead to contamination
- 50/50 Water/Acetonitrile is typically a good starting point
  - But check solubility
SM-FTN Front View
SM-FTN Injection Port Location
SM-FTN Mechanics: Sealing Procedure

- Hardware in injection port stays the same
- Sealing force increased
- Return line reduced from 0.004” to 0.003” to reduce dispersion
SM-FTN Sample Loading

Syringe / Sample Metering

Inject Valve Load Position

To Waste

To Column

From Pump

Sample

Injection Port

From Rinse Pump

To Waste
SM-FTN Sample Injection
<table>
<thead>
<tr>
<th>Cycle Steps</th>
<th>Inject-to-Inject Cycle Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Cycle Time (with Empower Overhead)</td>
<td>~17s</td>
</tr>
<tr>
<td>Time/µL</td>
<td>+2s/µL</td>
</tr>
<tr>
<td>Post Wash</td>
<td>0s</td>
</tr>
<tr>
<td>Pre Wash</td>
<td>time configured</td>
</tr>
<tr>
<td>PDA</td>
<td>+3s (average)</td>
</tr>
<tr>
<td>Pre-Injection Volume</td>
<td>0s (if time to deliver is subtracted from re-equil time)</td>
</tr>
<tr>
<td>Load Ahead (with Empower Overhead)</td>
<td>~17s</td>
</tr>
</tbody>
</table>
Using the Sample Manager - FTN

- Priming Recommendations

To Refresh Solvents

For New Solvents

- Selecting Wash Solvents
  - Sample should be very soluble in the wash solvent
  - Purge solvent does not contact sample (use wash solvent)
  - Purge solvent should not contain additives
SM-FTN: Low Volume Injection Linearity

- Benzocaine $R^2 = 0.99996$
- Procaine $R^2 = 0.99995$
- Tetracaine $R^2 = 0.99992$
SM-FTN: MS Carryover of Omeprazole

Omeprazole 10ng 0403

Blank1 10ng 0403

MRM of 1 Channel ES+
346.083 > 198.068 (Omeprazole)
7.06e7

MRM of 1 Channel ES+
346.083 > 198.068 (Omeprazole)
4.42e3

1µL Injection of High Concentration Standard @ 10ng/mL
Compound name: Omeprazole
Correlation coefficient: $r = 0.999985$, $r^2 = 0.999970$
Calibration curve: $134101 \times x -12.3789$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

SM-FTN: Low Carryover Extends Quantification Ranges in MS
When do we recommend what?

Sample limited?

FTN - Flow through Needle design. Only injects what you program

If you know you have very sticky compounds, prone to carryover and cycle time is critical?

FL – Fixed loop. More flexibility in washing solution

Both sample limited and prone to carryover?

FTN – Flow through Needle design. Ads cycle time as gradient washing step (high % ) needs to increase.

Methods with various injection volumes?
FTN-Flow Through Needle design. Injectes 0.1-10ul without configuration change. 0.1-50ul with extension loop....