Microspray and Microflow Liquid Chromatography: The Perfect Fit for Modern LC-MS Bioanalysis

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In 25 years, I believe the analytical measurements Pfizer performs will change drastically. The biggest change will be the miniaturization of analytical instruments, such as high-performance liquid chromatography and capillary electrophoresis. These and other instruments could be ‘pocket size’ to allow the immediate monitoring of clinical patients.

— Shane Needham, Drug Metabolism

In drug therapy, youth longevity, hair loss and memory; and drugs to aid in smoking cessation and alcohol abuse.

— David Tingley, Neurosciences
HPLC-MS/MS and MFLC-MS/MS chromatograms from the analysis of a 1.5 µL injection of a 5.00 ng/mL Propranolol solution on an AB SCIEX QTRAP® 5500

Notice band broadening on this first “prototype” MFLC ESI source.
MFLC-MS/MS IN REGULATED BIOANALYSIS

• Can MFLC-MS/MS methods be validated?

• Are the MFLC-MS/MS methods rugged?

• Do the MFLC-MS/MS method results compare well to HPLC-MS/MS methods?
METHOTREXATE (MTX)

- MW = 454

- Used in treatment of cancer, autoimmune diseases, ectopic pregnancy

  - Alturas previously validated a LC-MS/MS method in human plasma from 1-500 ng/mL

  - Acetonitrile precipitation
LC-MS/MS Overlayed Chromatograms (HPLC vs. MFLC) from Bioanalysis of Methotrexate (400 ng/mL) from Human Plasma Using Eksigent ekspert microLC 200 and a QTRAP® 5500

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9.6x Signal Increase!

HPLC - 700 μL/min, 2.0 mm ID x 50 mm
Injection volume = 2 μL

MFLC - 35 μL/min, 0.5 mm ID x 50 mm,
Injection volume = 2 μL

Similar linear velocities
Same stationary phase
Prontosil, C18, 3 μm

Methotrexate
## COMPARISON OF THE LC-MS/MS BIOANALYTICAL VALIDATION OF METHOTREXATE (MTX) FROM HUMAN PLASMA

<table>
<thead>
<tr>
<th>LC-MS</th>
<th>Inter A/P</th>
<th>Intra A/P</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conv.</td>
<td>105±10.2</td>
<td>*112±13.7</td>
<td>1.02</td>
</tr>
<tr>
<td>MFLC</td>
<td>93.3±12.9</td>
<td>*87.1±13.2</td>
<td>1.07</td>
</tr>
</tbody>
</table>

Dynamic range of 1-1000 ng/mL  *Includes LLOQ values
MFLC-MS/MS Chromatograms Overlayed from Beginning and End of Run of >350 injections of MTX Extracted from Human Plasma

Backpressure at beginning and end of run 1400 PSI

Peaks overlay perfectly and signal consistent
QTRAP® 5500 interface plate clean, before any injections
QTRAP® 5500 interface plate after approximately 400 extracted human plasma injections from the MFLC system – no switching valve
QTRAP® 5500 interface plate after approximately 150 extracted human plasma injections from the conventional HPLC system – no switching valve

Notice deposits and source contamination
FUTURE OF LC-MS/MS: COUPLING THE MS SOURCE AND MFLC

Why? Because it works! – simplifies LC-MS

- Natural progression of coupling analytical techniques
- Less connections-less band broadening
- Source is column heater
- No switching valve needed to divert waste
- SAMPLING ADVANTAGE

PicoFuze™ to be inserted into ESI-MS probe

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PicoFuze™ MS/MS on QTRAP® 6500

QTRAP® 6500 with the Microflow Eksigent ekspert microLC 200 System

“The source is the column, the column is the source”
SIGNAL COMPARISON: MTX

Notes:
• Maximum signal was achieved at 7 μL/min, but the RT and signal was inconsistent at this flow rate.
• Optimal signal, run time and robustness were achieved at 12 μL/min

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The LC/MS Experts™
MTX 400 ng/mL extracted from human plasma

“Plug and Play”

“First try at full bioanalytical validation on PicoFuze™”

After ~500 injections
MTX INTRA/INTERBATCH ACCURACY AND PRECISION

<table>
<thead>
<tr>
<th>Intrabatch A/P</th>
<th>LLOQ</th>
<th>LQC</th>
<th>MQC</th>
<th>HQC</th>
<th>ULOQ</th>
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<tbody>
<tr>
<td>Avg Accuracy (%)</td>
<td>97.9</td>
<td>108</td>
<td>111</td>
<td>101</td>
<td>94.0</td>
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<tr>
<td>%CV</td>
<td>10.4</td>
<td>9.3</td>
<td>4.4</td>
<td>7.7</td>
<td>4.3</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
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<tbody>
<tr>
<td>Avg Accuracy (%)</td>
<td>102</td>
<td>110</td>
<td>113</td>
<td>102</td>
<td>93.4</td>
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<tr>
<td>%CV</td>
<td>13.8</td>
<td>6.3</td>
<td>7.1</td>
<td>5.9</td>
<td>4.3</td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>18</td>
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Blank solvent injection showing ESI voltage “turned on” at 2.3 minutes and “turned off” at 3.4 minutes.
But wait, I can improve LLOQ by injecting more on my 2.1 mm ID and I can only inject 1-10 µL on my MFLC!!
≈1990-API III
- 4.6 mm ID, 1.0 ml/min, split, 100 µL injections

≈1996-API 3000
- 2.1 mm ID, 0.4 ml/min, 40 µL injections

≈2001-API 4000

≈2012-API 6500
- <1.0 mm ID, <100 µl/min, 1-10 µL injections

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The LC/MS Experts™
PicoFuze™ - Future of the LC-MS Source

- 4.6 mm ID, 1.0 ml/min, split, 100 µL injections
- <0.3 mm ID, <10 µl/min, 1-2 µL injections
- <1.0 mm ID, <100 µl/min, 1-10 µL injections
Consumer Driven “Convenience”!

Ease of use is “HIGH PERFORMANCE!”

Inject less volume with better signal for MFLC gives “peace of mind” – SAMPLING ADVANTAGE
OTHER BENEFITS OF MFLC-MS/MS

• Reduced source contamination due to less flow and lower injection volumes
  • More up time-increased PRODUCTIVITY
  • No need for a switching valve... -increased EFFICIENCY

• Reduced matrix effects?
  • Develop methods faster? More research needed....
  • Less potential failures?

• Saves solvent – “Goes Green”
  • 5 mL of organic mobile phase per day at 24/7 operation (normally 500 mL!) -increased EFFICIENCY
CONCLUSION: MFLC-MS/MS AND PICOFUZE™ BUZZ WORDS

It works! – “Ease of use is high performance”

“Peace of mind” – More injections per sample due to increased sensitivity – SAMPLING ADVANTAGE

“Plug and play” – minimal optimization for transferring methods from conventional LC or MFLC

Efficient, Productive and Convenient – “Source is the column, column is the source”

- Less connections-less band broadening, easier to use
- Source is column heater
- No switching valve needed to divert waste
CONCLUSION

MFLC-MS/MS and PicoFuze™-MS/MS coupled chromatography and MS will continue to develop

- More research needed
  - Expand user base, DATA, DATA, DATA
  - APCI???
  - Vendors develop HPLC pumps that work for micro and conventional flows in the same pump
  - Cost of “source column” should compete with cost of HPLC column
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