Design and Integration of a High-Performance Micro-flow LC-MS/MS system

Brendon Kapinos1, John Janiszewski1, Bernhard Nemec2, Werner Dobelin2, Wayne Lootsma3, Steve Ainline3
1Pfizer, Groton, CT; 2Prolab Instruments, Reinach, Switzerland; 3Sound Analytics, Niantic, CT

Worldwide Research & Development

Abstract

Biopharmaceutical R&D is challenged to reduce operating costs in spite of increasingly demanding portfolio needs. Sample volume, solvent consumption, waste generation, and cycle time are key considerations. Micro-flow (µF) LC-MS/MS reduces solvent consumption and waste for lower cost per analysis while delivering enhanced sensitivity. However, few µF-LC-MS/MS systems exist with optimal configurability, flexibility, and sample throughput. Parameters for high-performance micro-flow were identified, and drove design of a fully-integrated LC-MS/MS platform with very low system volume to support various LC-MSMS analyses, including micro-flow. Instrument software automated batch building using conditions from a centralized DiscoveryQuant 3.0 database, and integrated micro-flow pump delivered precise gradient separations at 3-5µL/min.

Materials and Methods

- SCIEX 6500 TripleQuad mass spectrometer with Analyst 1.7 software
- TurboV VonDrive and OptiFlow MS/MS sources equipped with 25µ electrodes
- Prolab Zirconium Ultra micro-flow pump with flow control
- LeadSampler (LS-1) and LeadScape software
- 25 and 50µ ID, 1/32” OD tubing and fittings from Analytical Sales and Services
- 25 and 50µ ID, 1/16” OD NanoViper tubing from Thermon
- Luna Omega PS C18 50x0.3mm 3µ columns from Supelco
- Ascent Express C18 50x0.2mm 2.7µ columns from Supelco
- Method development cocktail in 10% methanol containing propranolol, zaleplon, diazepam, verapamil, diltiazem, tobramycin, terfenadine and diclofenac

Micro-flow LC-MS/MS System Design and Integration

Fig 1. Micro-flow system configuration and components.

- SCIEX 6500 TripleQuad
- Prolab Zirconium Ultra micro-flow pump
- LS-1 autosampler
  - High-speed, UHPLC-ready
  - DiscoveryQuant database integration

Table 1. Comparison of flow path delay volumes across system configurations

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Pump to Autosampler</th>
<th>Injector Port to Injection</th>
<th>Total Delay Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20 µL x 150mm</td>
<td>20 µL x 550mm</td>
<td>0.22</td>
</tr>
<tr>
<td>B</td>
<td>50 µL x 150mm</td>
<td>50 µL x 550mm</td>
<td>1.37</td>
</tr>
</tbody>
</table>

Table 2. LC gradient program

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow (µL/min)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>170</td>
<td>4</td>
<td>85</td>
</tr>
<tr>
<td>190</td>
<td>4</td>
<td>85</td>
</tr>
<tr>
<td>195</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 3. MS/MS Source Assessment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
</table>
| Mobile phase | A: 0.1% formic acid in water  
| B: 0.1% formic acid in acetonitrile |
| Column | Supelco Ascent Express C18 50x2.7µ 3µm, 50µL column |
| Injection Volume | 5 µL/injection |
| Cycle time | 5 minutes/injection |

Fig 2a. Components of the micro-flow LC-MS/MS system: 1) LS-1 injection port 2) Prolab Zirconium LC 3) MS/MS source

Fig 2b. Flow path configurations for LC-MS/MS analyses: A) SCIEX 6500 TripleQuad MS/MS equipped with either TurboV VonDrive (A) or OptiFlow source (B) for analysis of a standard cocktail. Both sources were outfitted with 25µ electrodes, however, 1/32” OD, 25µ ID x 50mm tubing with 1/16” OD NanoViper tubing and valves were installed on NanoDrive source to interface the column with 1/32” electrode coupling.

Fig 3a. Injections of standard cocktail with instrument Configuration A (top) and B (bottom).

Fig 3b. Impact of delay volume on MS/MS flow performance was examined. The system was configured with 25 or 50µ ID NanoViper tubing for these assessments.

Fig 4a. Table 3a. SCIEX 6500 TripleQuad MS/MS was equipped with either TurboV VonDrive (A) or OptiFlow source (B) for analysis of a standard cocktail. Both sources were outfitted with 25µ electrodes, however, 1/32” OD, 25µ ID x 50mm tubing with 1/16” OD NanoViper tubing and valves were installed on NanoDrive source to interface the column with 1/32” electrode coupling.

Fig 4b. SCIEX 6500 TripleQuad MS/MS was equipped for low flow performance (1-5µL/min). Flow path and LC methods were identical (Table 2 and 3). SCIEX 6500 TripleQuad MS/MS was equipped with either TurboV VonDrive (A) or OptiFlow source (B) for analysis of a standard cocktail. Both sources were outfitted with 25µ electrodes, however, 1/32” OD, 25µ ID x 50mm tubing with 1/16” OD NanoViper tubing and valves were installed on NanoDrive source to interface the column with 1/32” electrode coupling.

Fig 5. An internal standard cocktail containing 9 small molecule analytes was injected on the system in each delay volume configuration. Analytes eluted ~100% earlier, on average, in Configuration B compared to Configuration A. Methods were identical for all injections, 4L/min flow rate, linear gradient from 4% B over 2 minutes was programmed through Prolab Zirconium software and cycle time reduced to 3 minute/injection.

Fig 6. Multiple-injected, micro-flow LC-MS/MS acquisitions of standards (n=4 injections) revealed good throughput and reproducibility using the OptiFlow micro-flow MS/MS source. Flow rate was 5µL/min and 5 minutes/injection cycle time (A). An expanded view of injection 3 shows sharp peak widths (3-5A) and near baseline separation of all casestandard analytes (B). Method parameters were identical to those listed in Table 3. A linear LC gradient from 5-85% B over 2 minutes was programmed through Prolab Zirconium software and cycle time reduced to 3 minute/injection.

Conclusions

- A micro-flow LC-MS/MS system was purposefully designed with integrated, high-performance components to minimize dead volume while maximizing throughput and flexibility.
- Delay volume was reduced by close placement of system components (valves, pump, and MSMS sources) and reduction in tubing ID.
- An innovative MSMS source design (SCIEX OptiFlow) reduced post-column volume to ~60L, and consistently supported high-performance micro-flow analyses at 1-5µL/minute.
- Integration of LS-1 LeadScape, Prolab Zirconium pump, and SCIEX OptiFlow source allowed multi-injected acquisitions at very low flows while achieving good throughput (6µL/minute flow rate, 3 minutes/injection).