High-throughput (sub-2.5 second) direct injection using a modified RapidFire 365 HTMS system

Introduction

The role of mass spectrometry in early drug discovery and especially in functional biochemical and binding assays is well established. Even fast techniques such as UHPLC or SPE-MS face challenges when primary screens of several hundred thousand compounds need to be performed. At a throughput of 8 seconds per sample, an Agilent RapidFire 365 system can analyze 10,000 samples in 24 hours. However, a large screen of several hundred thousand compounds still requires many weeks of effort. We have modified a RapidFire system to facilitate the direct injection of samples at a throughput of less than 2.5 seconds per sample enabling the analysis of 35,000 samples in 24 hours.

Experimental

Modifications to RapidFire plumbing

- 10 mL sample loop (between V1 port 2 and 5) was replaced with a 2 mL sample loop
- Tubing attached to V1 port 1 was disconnected
- Tubing attached to V2 port 2 was connected to V1 port 1
- To recycle mobile phase for pumps 2 and 3:
  - Plumb a tube from V2 port 2 to a recycling bottle
  - Submerge the intake lines for pumps 2 and 3 into the recycling bottle
  - Direct the waste line from pump 2 into the recycling bottle
- To disengage flow for the peristaltic pump (pump 4), the conduits were detached from the rollers

Modifications to RapidFire configuration files

- A copy of the configuration files folder was made
- The configuration files “Pump2” and “Pump3” were individually modified such that the “MIN_ALLOWABLE_PRESSURE=0.00;”
- The configuration file “SampleInterface” was modified such that the valve positions were set as
  - STATE1=[0,0,0,...]
  - STATE2=[1,1,0,...]
  - STATE3=[0,0,0,...]
  - STATE4=[0,0,0,...]
  - STATE5=[0,0,0,...]
- The configuration file “Column Changer” was modified such that “auto column switch = 0”, not 1
- The configuration file “Sipper” was modified such that “sip sensor present = 0”, not 1

Flowpath diagrams for the standard state 1 (aspirate, top left) and 2 (load/wash, top right) and the modified state 1 (aspirate, bottom left) and 2 (elute, bottom right). In standard mode the RapidFire aspirates sample from the plate into the sampling loop during state 1 and loads/washes the sample onto the cartridge during state 2. In direct injection mode the RapidFire aspirates sample from the plate into the sampling loop during state 1 and elutes that sample to the mass spectrometer (MS) during state 2.
Experimental

**RapidFire Method: Direct Injection Mode**
- Pump 1: 1.25 ml/min
- Pump 2: 0.1 ml/min
- Pump 3: 0.1 ml/min
- State 1 (aspirate): 150 msec
- State 2 (elute): 500 msec
- Elute buffer: 90% acetonitrile + 0.1% formic acid

**RapidFire Method: Standard Mode**
- Pump 1: 1.5 ml/min
- Pump 2: 1.25 ml/min
- Pump 3: 1.25 ml/min
- State 1 (aspirate): 150 msec
- State 2 (load/wash): 3000 msec
- State 3 (extra wash): 0 msec
- State 4 (elute): 3000 msec
- State 5 (reequilibrate): 500 msec

**Load/wash buffer:** water + 0.1% trifluoroacetic acid (TFA)
**Elute buffer:** 80% acetonitrile + 0.1% TFA
**Cartridge type:** D

**MS Method: 6470 Triple Quadrupole**
Standard small molecule conditions were used. No optimization was done.

---

**Results and Discussion**

![Representative MRM chromatogram data for S-adenosylhomocysteine (SAH), demonstrating the sampling and measurement of 60 injections in ~2 minutes.](image)

**Concentration response curve for SAH.** Thirteen 2-fold serial dilutions of SAH were made in water + 0.1% formic acid starting from a concentration of 8000 nM. Each of these 14 stock solutions (8000, 4000, 2000, 1000, 500, 250, 125, 62.50, 31.25, 15.62, 7.81, 3.91, 1.95, and 0.98 nM) was aliquoted into a multi-well plate for analysis. One hundred replicate measurements were conducted on each concentration requiring a 52 minute run time. Data were integrated and exported in 1 minute using RapidFire Integrator. Error bars are shown and indicate the standard error of the mean. The plot of the entire concentration range is shown on the left. A zoom-in of the lower concentration data is shown on the right.
Results and Discussion

Replicate data for three concentrations of SAH. Bulk solutions of 100, 500, and 1000 nM SAH were made and each was supplemented with 500 nM internal standard (S-adenosylmethionine). Each solution was aliquoted into a multi-well plate for analysis. For each of the three plates, 1,920 replicate measurements were made requiring a 72 minute run time. Data for each run were integrated and exported in 1 minute using RapidFire Integrator. The AUC for the SAH MS signal was divided by the AUC of the internal standard MS signal.

<table>
<thead>
<tr>
<th>Concentration (nM)</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 nM SAH</td>
<td>1.63</td>
<td>1.17</td>
<td>1.3</td>
</tr>
<tr>
<td>500 nM SAH</td>
<td>0.86</td>
<td>0.01</td>
<td>1.2</td>
</tr>
<tr>
<td>100 nM SAH</td>
<td>0.22</td>
<td>0.00</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Conclusions

- The RapidFire system was modified to perform direct injection of samples to improve the throughput of the system further.
- The throughput of the modified system was ~2.2 seconds per sample, representing a 3- to 5-fold improvement over standard configuration analyses.
- The modified system demonstrated:
  - A broad and linear concentration response over nearly 4 orders of magnitude with an $R^2=0.9997$.
  - C.V.s were between 1 and 2% for 1,920 replicate measurements, of three different concentrations, showing excellent reproducibility.
  - Near perfect correlation with data acquired in standard mode. $R^2=0.9983$ over 4 test concentrations.
- The RapidFire system could be converted between modes in less than 5 minutes.
- The ability to interconvert the RapidFire system between modes allows the user to balance the throughput and sensitivity of their specific screens.

Correlation between standard mode and direct injection mode MS data. Four solutions of SAH were made (125, 250, 500, and 1000 nM) and each was supplemented with 500 nM internal standard (SAM). Ninety-six replicates of each solution were measured with the RapidFire in direct injection mode first and standard mode second. The normalized data for each mode were plotted against each other.

For Research Use Only. Not for use in diagnostic procedures.