

High-Throughput (Sub-2.5 Second) Direct Injection Analysis by Mass Spectrometry

Using a modified Agilent RapidFire 365
high-throughput MS system

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Abstract

Due to large numbers of samples and the associated analysis time for each, using mass spectrometry (MS) as a primary screening technique can be a long, tedious process. This Application Note presents a modified Agilent RapidFire 365 high-throughput MS system (RapidFire) that bypasses SPE desalting and enables a sub-2.5 second sample throughput rate, making the analysis of 35,000 samples a day possible.

Introduction

The role of MS in early drug discovery, and especially in functional biochemical and binding assays, is well established. However, even fast techniques such as UHPLC or SPE/MS face challenges when primary screens of several hundreds of thousands of compounds need to be performed. With an eight seconds per sample throughput, a RapidFire can sample, desalt, and analyze 10,000 samples in 24 hours. However, a large screen of several hundreds of thousands of compounds still requires many weeks of effort.

We have modified a RapidFire to bypass the SPE desalting step, and inject samples directly into the MS at a throughput of less than 2.5 seconds per sample. This modified system enables the analysis of 35,000 samples, without cleanup, in 24 hours.

Experimental

Methods

The RapidFire was modified to achieve direct injection (Figure 1). The line between valves 1 and 2 was replaced with one that connected valve 1 directly to the MS. This resulted in pump 1

going to the MS, either around the sampling loop or through it depending on the position (load versus inject) of valve 1. Pumps 2 and 3 were plumbed to recycle water. The configuration files were altered to minimize unnecessary valve toggling. The modified system was connected to an Agilent 6470 triple quadrupole LC/MS. Samples, made to be representative of a high-throughput screen, were diluted and transferred to microplates. Plates were run using modified and conventional modes. AUC values were generated by Agilent RapidFire Integrator software, and analyzed using Microsoft Excel.

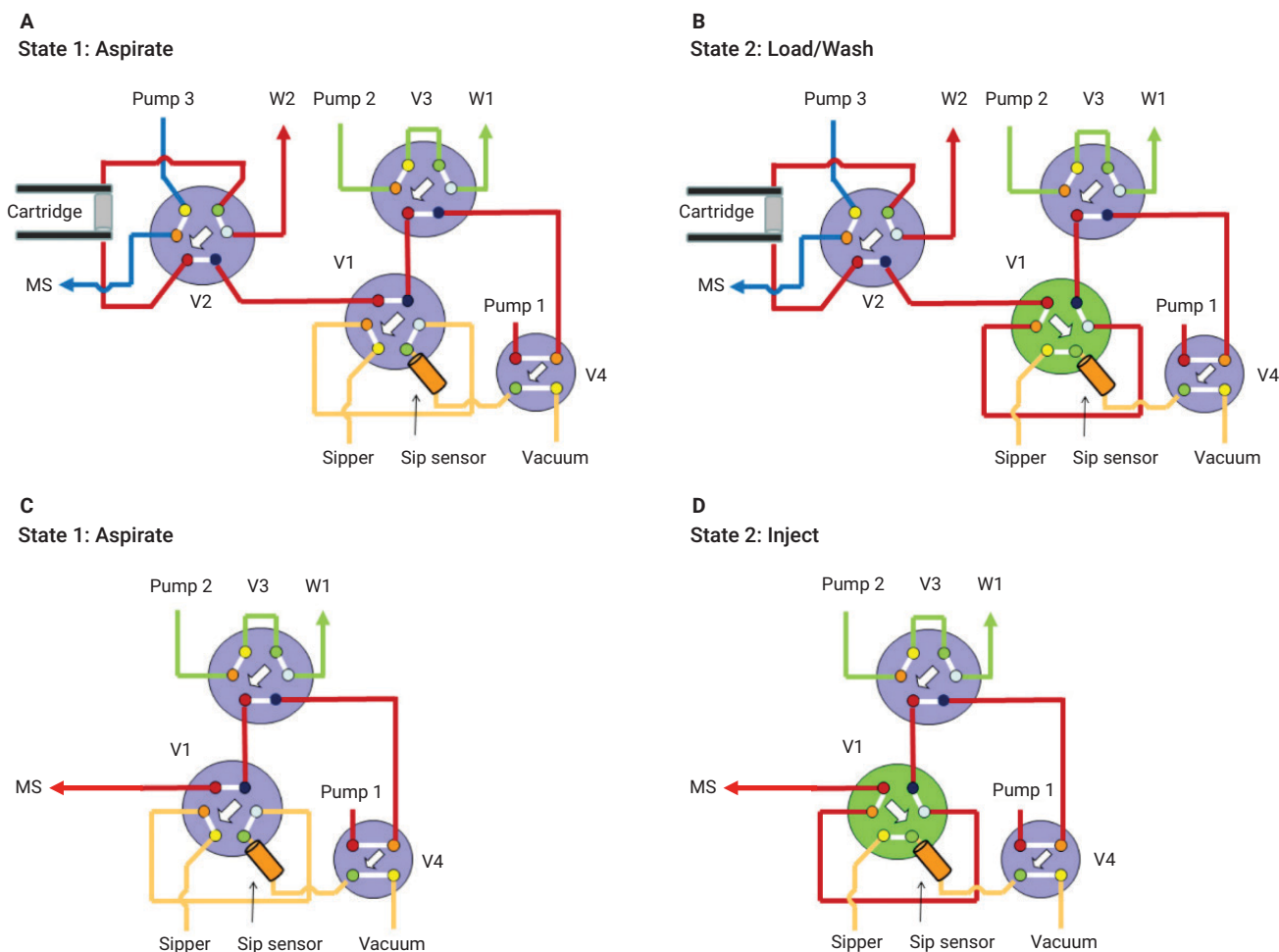


Figure 1. Flowpath diagrams for the RapidFire in standard and direct injection modes. In standard mode, the RapidFire aspirates sample from the plate into the sampling loop during state 1 (A) and loads/washes the sample onto the cartridge during state 2 (B). In subsequent states, not shown, the analytes are eluted into the MS. In direct injection mode, the RapidFire aspirates sample from the plate into the sampling loop during state 1 (C) and elutes that sample into the MS during state 2 (D).

Results and discussion

RapidFire in direct injection mode can sustain a throughput of just over two seconds per sample

To examine the speed of RapidFire in direct injection mode (Figure 2), replicate injections were made from a microplate containing a single dilution of S-adenosylhomocysteine (SAH). The flow rate of the elution pump and the elution time were optimized to balance throughput, peak-to-peak separation, and MS signal. Ultimately, a pump flow rate of 1.25 mL/min and an elution time of 500 msec was configured. The optimized method demonstrated that 60 injections could be measured in just over two minutes, sustaining a throughput of just over two seconds per sample.

Faster flow rates did result in greater peak-to-peak separation, which in turn allowed the method to be sped up further. However, faster flow rates also decreased the MS signal slightly. These results (data not shown) illustrate that the direct injection method can be tuned according to how different assay parameters are prioritized by the user.

RapidFire in direct injection mode provides a good concentration response

To demonstrate the concentration response of RapidFire in direct injection mode (Figure 3), 13 two-fold serial dilutions of SAH were made in water with 0.1 % formic acid starting from a concentration of 8,000 nM. Each of these 14 stock solutions (8,000, 4,000,

2,000, 1,000, 500, 250, 125, 62.50, 31.25, 15.62, 7.81, 3.91, 1.95, and 0.98 nM) was aliquoted into a microplate for analysis. One hundred replicate measurements were conducted of each concentration. These 1,400 injections were analyzed in 52 minutes. Data were integrated and exported in one minute using RapidFire Integrator. Results show a broad (spanning nearly four orders of magnitude) and linear ($R^2 = 0.9997$) concentration response.

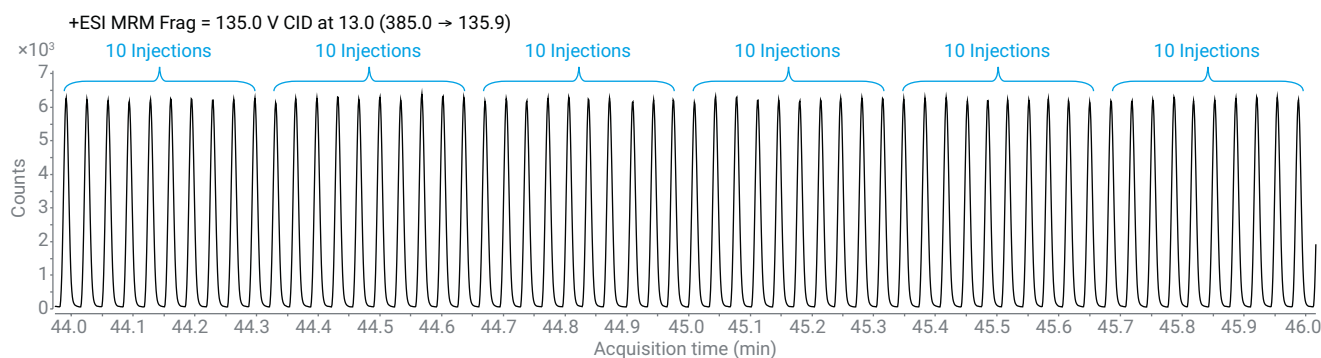


Figure 2. MRM chromatogram data for SAH analyzed by RapidFire-MS in direct injection mode, demonstrating the sampling and measurement of 60 injections in ~two minutes, or two seconds per sample

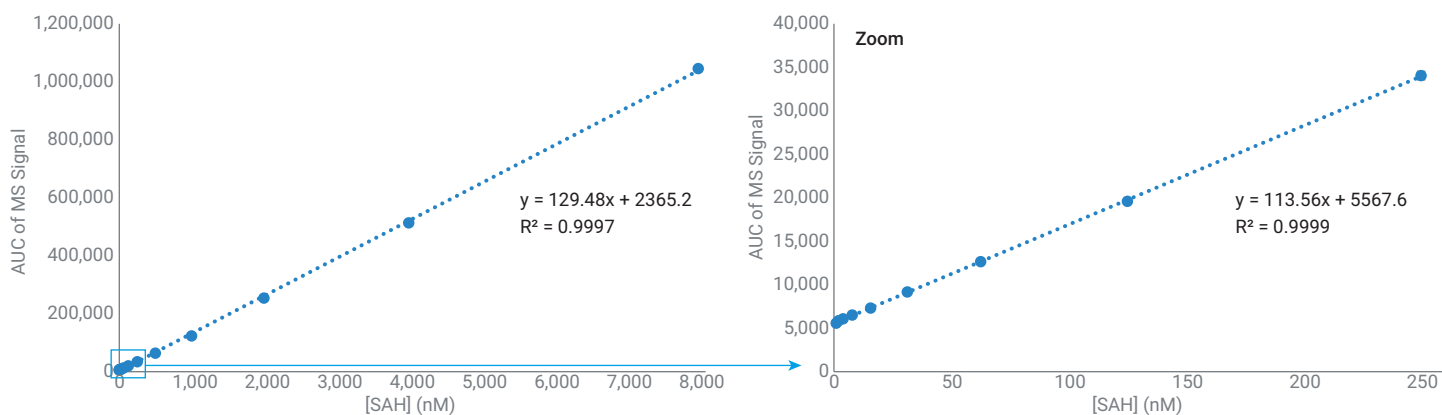


Figure 3. RapidFire-MS in direct injection mode provides a good concentration response. Error bars are shown to indicate the standard error of the mean. The plot of the entire concentration range is shown on the left. A zoom-in of the lower concentration data is shown on the right.

RapidFire in direct injection mode is reproducible

To test the reproducibility of the RapidFire in direct injection mode (Figure 4), bulk solutions of 100, 500, and 1,000 nM SAH were made, and each was supplemented with 500 nM S-adenosylmethionine (SAM) as an internal standard. Each solution was aliquoted into an individual microplate for analysis. For each of the three plates, 1,920 replicate injections were made in 72 minutes. In total, 5,760 injections were analyzed in ~3.5 hours. Data for each run were integrated and exported in one minute using RapidFire Integrator. Results showed excellent reproducibility. Coefficients of variation (CVs) were between 1 and 2 % for all concentrations tested.

Data collected by RapidFire in direct injection mode correlate well with data from standard mode

To investigate the extent to which data collected by direct injection mode correlate with data from standard mode (Figure 5), four solutions of SAH were made (125, 250, 500, and 1,000 nM). Each was supplemented with 500 nM internal standard SAM, and 96 replicates of each solution were measured in both modes. The normalized data for the four samples, from each mode, were plotted against each other. In total, 768 measurements were used to generate the four-point unity plot, which illustrated good correlation between the data from the two RapidFire modes. The best fit line of the plot had an $R^2 = 0.9983$.

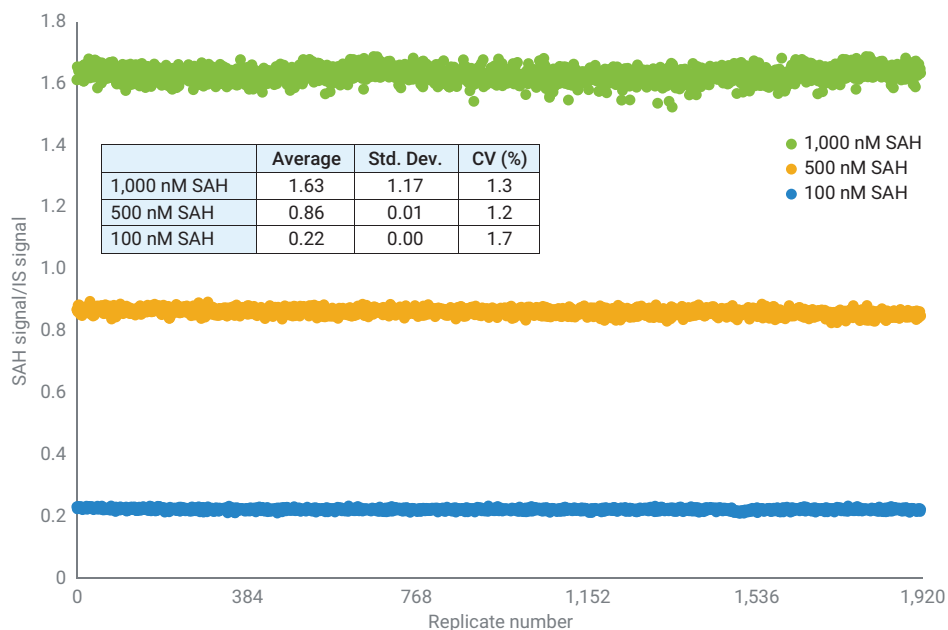


Figure 4. RapidFire-MS in direct injection mode is reproducible. Replicate data for three concentrations of SAH were collected. The AUC for the SAH MS signal was divided by the AUC of the internal standard MS signal and plotted.

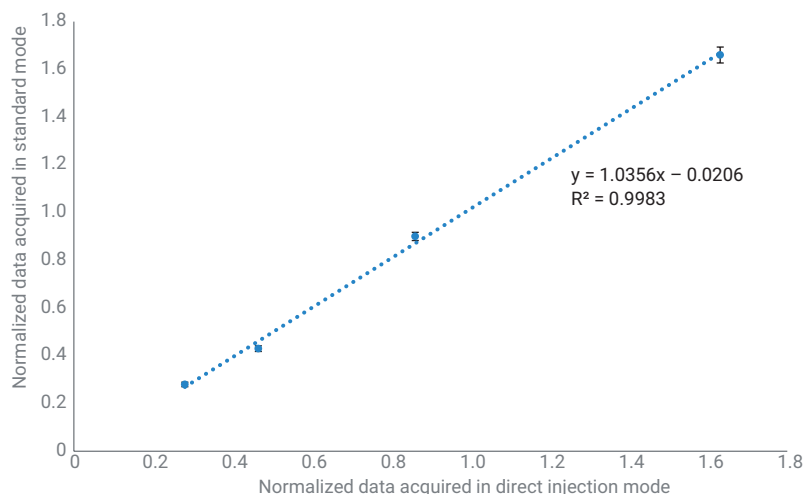


Figure 5. Data collected by RapidFire-MS in direct injection mode correlate well with data from standard mode.

Conclusions

The RapidFire was modified to bypass the SPE cartridge and perform direct injection of samples. This modification resulted in increased throughput, and could easily sustain a cycle time of less than 2.5 seconds per sample, representing a three to five-fold improvement over standard configuration analyses. The modified system also demonstrated a broad and linear concentration response, excellent reproducibility, and near perfect correlation with data acquired in standard mode. The RapidFire could be interconverted in less than 10 minutes, allowing users to balance the throughput and sensitivity requirements of their specific screens.

Though not described in detail here, the modified system can be beneficial in ways beyond increased throughput. As one example, because the sample composition is not subjected to SPE enrichment (which can affect the relative concentrations of analytes delivered to the MS), the modified system enables the analysis of diverse analyte panels in each sample. As another example, because the bind/elute process has been circumvented, the modified system allows the analysis of samples that are not amenable to SPE. That is, if a suitable SPE packing material cannot be identified, or if the sample matrix (high organic content, nonspecific binders, and so on) spoils the SPE binding process.

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Printed in the USA, April 11, 2019
5994-0781EN

