Technical Overview

Minimization of the Required Sample Volume for Agilent RapidFire High-Throughput Mass Spectrometry Systems

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Abstract

This application note describes analysis with the Agilent RapidFire high-throughput MS system using tubing with a smaller inner diameter (id). This way, the necessary sample volume could be minimized. Two small id tubing configurations were successfully applied within this methodology, reducing the sample requirements to just 10 and 5 µL per well. The optimized configurations displayed excellent reproducibility across whole 96- and 384-well plates, producing data with coefficients of variation (CV) between 2.3 and 4.0%.

Introduction

The Agilent RapidFire high-throughput mass spectrometry (MS) system is designed to perform online SPE of samples with maximum speed. Consistent with these goals, the id of the tubing used to collect each sample is intentionally large, allowing the liquid from each well to be rapidly sipped by aspiration; sample collection from each well is regularly achieved in less than 200 ms. A consequence of using sample collection tubing (composed of a sipper tube and a sample loop, Figure 1) with a relatively large id is that the volume of liquid sampled from each well is relatively large. Typically, 10 µL of sample is used for each measurement, but dead volume before (in the sipper tube) and after (for the sip sensor) the sample loop results in a total consumption of ~35 µL per sample. This study evaluated the potential for decreasing RapidFire sample consumption, primarily using sample collection tubing with the same outer diameter (od) but a smaller id. A robust method requiring only 5 µL per well, for 384- and 96-well formats, was achieved, representing a seven-fold reduction in sample needs.

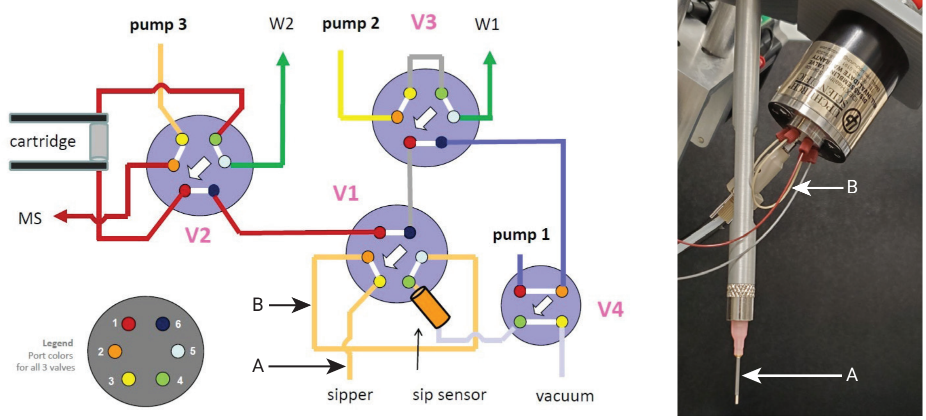

Figure 1. The Agilent RapidFire high-throughput MS comes standard with a beige sipper tube (A) and sample loop (B). The id of beige tubing is 0.015". This study replaced the sipper tube and sample loop with smaller id tubing (either gray with id 0.009" or red with id 0.005") to decrease the amount of sample required per well.

Experimental

Necessary parts and implementation

For 10 µL operation

Part Number	Quantity	Description	Notes
RF0052T	1	0.5 inch guide needle	Replaces 1.5 inch guide needle
RF0111T- 8	1	8 inch tubing, 0.009 inch id, gray	Cut to length (Table 1)
RF0094T	1 (10 pk)	Ferrule, 1/32 inch od, red	One per connection

For 5 µL operation

Part Number	Quantity	Description	Notes
RF0052T	1	0.5 inch guide needle	Replaces 1.5 inch guide needle
RF0112T-13	1	12 inch tubing, 0.005 inch id, red	Cut to length (Table 1)
RF0094T	1 (10 pk)	Ferrule, 1/32 inch od, red	One per connection

- Use the Agilent RapidFire sipper configuration wizard to teach the plate positions with the new needle configuration.
- Since vacuum levels can vary between labs, the optimal sip time for any volume must be empirically determined. Use the procedure described below to optimize the sip time for MS signal intensity and reproducibility.
- Best results were achieved using Greiner V-bottom 384-well plates (p/n 781280) and Greiner V-bottom 96-well plates (p/n 651201).

Modifications to RapidFire hardware

The RapidFire comes standard with a blunt ended 1.5" sipper guide needle, which allows the sipper to reach the bottom of deep-well plates, if necessary. For all the experiments, this needle was replaced with a 0.5" blunt ended needle (p/n RF0052T). The standard beige color (0.015" id) sipper tube and sample loop (Figure 1) were replaced with tubing that was either gray (0.009" id, p/n RF0111T-8) or red (0.005" id, p/n RF0112T-13). While mixing the id of the sipper tubing and the sample loop tubing could be useful, these remained matched in this study (both beige, gray, or red). Table 1 shows how the length and id of the sample collection tubing relates to the inner volume of the configuration.

Table 1. Relationship between tubing length, inner diameter (id), and inner volume.

	Beige Tubing (1/32" od × 0.015" id)	Gray Tubing (1/32" od × 0.009" id)	Red Tubing (1/32" od × 0.005" id)
Sipper Tube (4.5 inches)	~13.2 µL	~4.7 µL	~1.5 µL
Sample Loop (3.5 inches)	~10.0 µL	~3.6 µL	~1.1 µL
Total (8 inches)	~23.2 µL	~8.3 µL	~2.6 µL

Table 2. Evaluation of sipping behaviors using beige, gray, and red tubing configurations.

	Beige Tubing	Gray Tubing	Red Tubing
Time to Sip 1 mL Water	~23 seconds	~1 minute	>10 minutes
Time to Trigger Sip Sensor	~190 milliseconds	~180 milliseconds	~1,500 milliseconds

Results and discussion

Sipping behavior

It is possible for the efficiency of RapidFire sipping to be affected by clogs, insufficient vacuum, or loose/overtightened ferrules. In these cases, it is common to evaluate the RapidFire sipping behavior by timing how long it takes to aspirate 1 mL of water. In the standard configuration with beige tubing, ~23 seconds is typical and indicative of an unobstructed sample collection path. For comparison, the time required to sip 1 mL of water using beige, gray, and red tubing configurations was tested. Table 2 shows 1 mL sip times averaged from six replicates were ~23 seconds, ~1 minute, and >10 minutes, respectively. As liquids travel easier through less constrictive capillaries, this general trend was expected.

The sipping behavior of all three configurations was also characterized by averaging the sip time, as recorded by the sip sensor, across 16 sample replicates. The standard beige configuration showed an average sample sip time of ~190 ms, while the gray and the red sample sip times were ~180 and ~1,500 ms, respectively (Table 2). These results were expected, as the wide differences in sipping efficiencies were offset by the total sipping volumes. For example, even though the time to sip 1 mL using gray tubing was nearly threefold greater than the time for beige tubing, the corresponding sample volume sipped using the gray tubing was nearly threefold less. The net effect was a roughly equal sample sip time for both the beige and gray tubing configurations.

Optimization of sample volume and sip time

To optimize the sample volume and sip time, the RapidFire was plumbed for direct injection (“blaze”) mode and samples containing S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), or both, were run. The plates used were either 384- (Greiner V-bottom, 781280) or 96-well (Greiner V-bottom, 651201) and centrifuged briefly prior to analysis to ensure that the liquid was at the well bottom. A sipper safe height of 1 mm was used. MS detection was conducted using the Agilent 6495C triple quadrupole MS.

Sample requirements can be reduced by a couple µL when the RapidFire sip sensor is disabled. In place of the sip sensor, the sip time for each configuration was optimized by measuring the MS signal as a function of RapidFire aspiration time and sample volume per well.

Using the optimization of the beige tubing configuration as an example, 30 µL of 500 nM SAM was added to each well, across multiple columns of a 384-well plate. Each column provided 16 replicates, and was analyzed using a different sip time (50, 75, 100, 125, 150, 175, and 200 ms). The average peak area for SAM was determined for each column and plotted. Once reproducible MS results for one or more sip times were confirmed, the experiment was repeated with a smaller volume per well (27.5, 25, or 22.5 µL). In this fashion, the optimization of sample volume and sip time was determined while monitoring the reproducibility of each condition.

The expectations in these experiments (Figure 2) were that sip times that were too short would result in less MS signal because the sample loop would not have time to fill completely. Likewise, sip times that were too long were also expected to result in less MS signal because some (or all) sample would have been aspirated through the sample loop to waste. The optimal sip time was therefore volume-dependent, where smaller sample volumes made the optimal sip time window narrower. Experimental results were consistent with these expectations.

Ultimately, each tubing configuration was optimized such that the required sample volume was just a couple µL more than the total tubing volume (Table 3). For the red tubing configuration, the optimized sample volume was just 5 µL per well, for both 384- and 96-well formats. These results represent a seven-fold reduction in sample requirements compared to when the RapidFire is used with the standard beige tubing and sip sensor enabled.

Reproducibility

Full 96- and 384-well plates were run to more thoroughly examine the robustness of each low sample volume method (Table 3). For each run, the plates were supplemented with a 2:1 mixture of SAM:SAH, and the area ratio was plotted for each injection. While the ratio of SAM to SAH was 2:1, the MS response factor for SAM was slightly greater than that for SAH, resulting in an average area ratio of ~2.3:1. Results showed excellent reproducibility for each configuration, with CVs between 2.3 and 4.0% (Figures 3, 4, and 5). No wells were missed during these analyses.

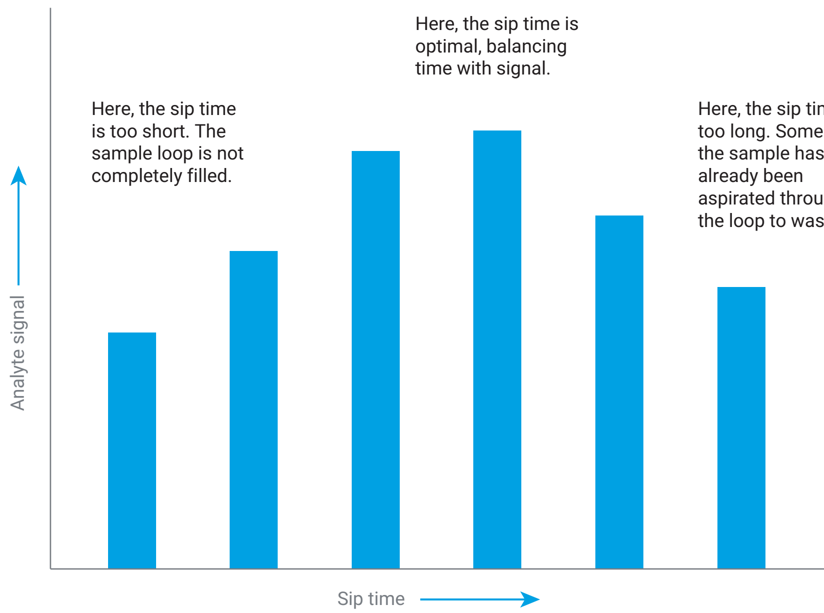

Figure 2. Analyte MS signal as a function of sip time. When the RapidFire sip sensor is off, which was done in this study to decrease material needs, the sip time must be optimized. If the sip time is too short, the sample loop will not be filled completely. If the sip time is too long, the liquid in the well will get aspirated all the way through the sample loop and leave it partially/completely empty. Determination of the sip time range that renders the sample loop full is critical for MS signal intensity and reproducibility.

Table 3. Optimized sample volume and sip time for the beige, gray, and red tubing configurations.

	Beige Tubing	Gray Tubing	Red Tubing
Optimized Sample Volume per Well	25 µL	10 µL	5 µL
Optimized Sip Time (Sip Sensor Off)	125 milliseconds	125 milliseconds	1,250 milliseconds

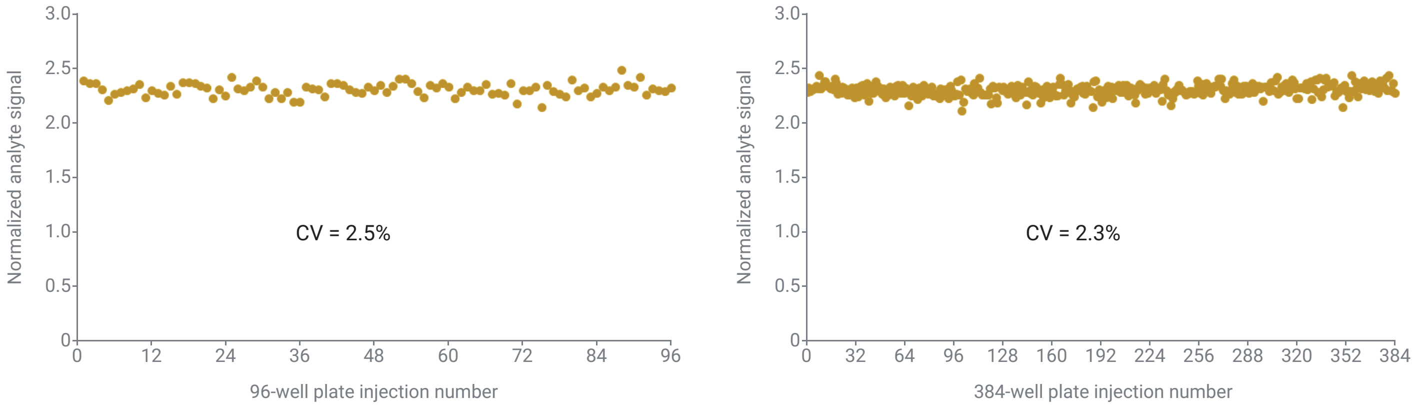

Figure 3. Reproducibility data from 96- (left) and 384-well (right) plates, for the optimized beige tubing configuration (25 µL per well).

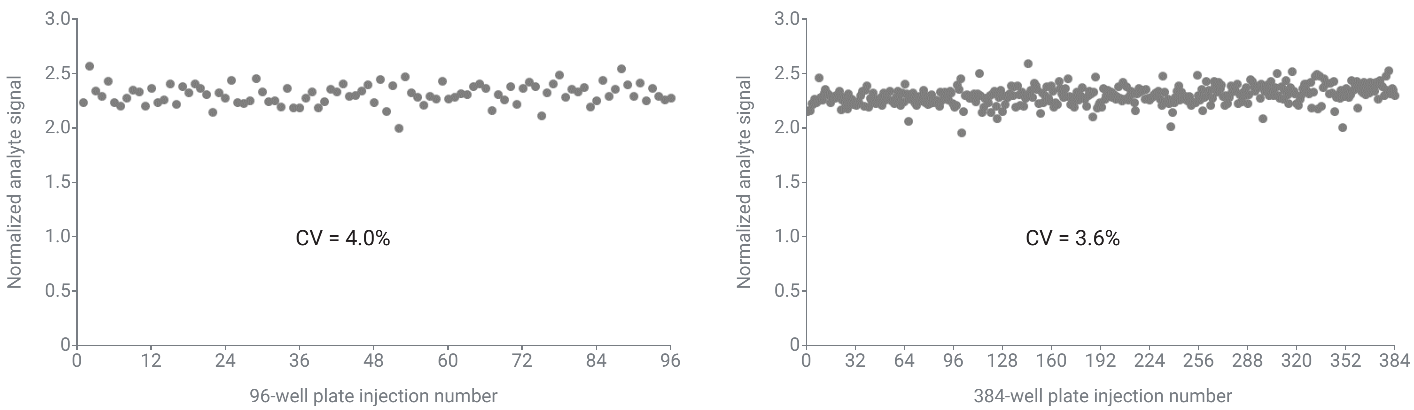

Figure 4. Reproducibility data from 96- (left) and 384-well (right) plates, for the optimized gray tubing configuration (10 µL per well).

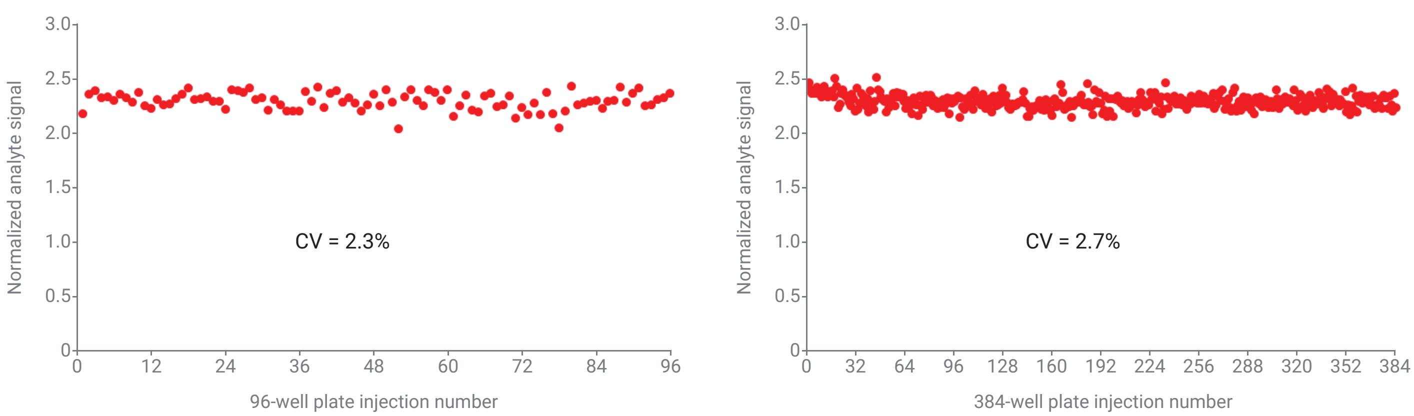

Figure 5. Reproducibility data from 96- (left) and 384-well (right) plates, for the optimized red tubing configuration (5 µL per well).

Conclusion

The goal of these studies was to use nonstandard sample collection tubing with a smaller id to decrease the material consumption of Agilent RapidFire MS analyses. Two smaller id tubing configurations were tested, and each was successfully optimized to decrease sample needs. In comparison to the standard beige tubing configuration, which requires 35 µL sample per well with the sip sensor is enabled, the gray and red tubing configurations decreased sample needs to 10 and 5 µL per well, respectively. These optimized methods performed reproducibly across entire plates and provided data with low CV.

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DE44243.1229398148

Printed in the USA, February 23, 2021
5994-1679EN