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High-throughput quantitative analysis of isoprenol (IP) by using an Agilent RapidFire-QqQ system

Forked from High-throughput quantitative analysis of isoprenyl acetate (IPA) by using an Agilent RapidFire-QqQ system

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We use this protocol and it's working

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Disclaimer

This protocol is for research purposes only.

Abstract

This protocol details steps in high-throughput measurement of isoprenol (IP) with the Agilent RapidFire 400 coupled with Agilent 6460 QqQ system, and subsequent data processing using the Agilent Quantitative analysis software. This analytical assay provides accurate and high-throughput quantitation of IP from a broad variety of sample types, such as microbial fermentation cultures, chemical overlays, intracellular cell lysate, etc. The protocol provides flexible options for measurements of small number of samples or ultra high-throughput screening of thousands samples per day.

Materials

- Acetonitrile LCMS quality **JT Baker Catalog #**9829-02
- Water LC-MS grade B&J Brand **VWR International (Avantor) Catalog #**BJLC365-2.5
- Cis-3-Hexenyl Acetate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**W317101
- 3-METHYL-3-BUTEN-1-YL ACETATE **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S881171

- Bio-Rad hard shell 96-well PCR plate (Bio-Rad part number HSP9601) or other equivalent RapidFire compatible 96-Well plate.
- LC compatible non adhesive alumni seal (Agilent part number: 06644-001)
- PlateLoc Thermal Microplate Sealer or equivalent type
- BioMek FX or any equivalent liquid transfer automation platform

Equipment

RapidFire 400	NAME
High-throughput liquid chromatography	TYPE
Agilent	BRAND
G9532AA	SKU
https://www.agilent.com/en/product/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-instruments/high-throughput-lc-ms/rapidfire-400	LINK

Troubleshooting

Safety warnings

- Wear proper PPE (gloves, safety goggles, and lab coat), and prepare solvents in a chemical fume hood.
- Store organic solvents in a flammable solvent storage cabinet when not in use.
- Always prepare necessary control samples to validate sample preparation procedures.
- Dispose of used solvents and samples in accordance with your institute's Environmental, Health, and Safety requirements.

Before start

Refill RapidFire pumps with sufficient volumes of the following solvents to complete the analysis:

- LCMS grade water for pump 1.
- LCMS grade water for pump 2.
- LCMS grade methanol for pump 3.

Verify MS status:

- Perform Check-tune of QqQ system to ensure it passes calibration criteria. Service the QqQ and perform autotune if check-tune fails.
- Perform a quality control (QC) run to ensure QC compound peak full width at half maximum (FWHW), shape, and intensity are within quality standard range.

Isoprenol (IP) standard preparation

1 Prepare internal standard (IS) stock of Cis-3-Hexenyl Acetate (CAS: 3681-71-8) in methanol at [M] 10 millimolar (mM) .

2 Prepare general diluent solution of [M] 50 micromolar (µM) IS in [M] 5 % volume Methanol (MeOH). This general diluent is used for diluting samples and preparing calibration standard diluent.

3 Prepare sample matrix that contains everything that is present in the typical sample except IP.

Note

A typical sample suitable for this protocol could be but not limited to microbial fermentation broth, IP trapping overlay such as dodecane, or extracted intracellular lysate of a microbial culture.

4 Prepare the calibration standard diluent (diluent used for generating calibration standards at a serial of concentrations) by spiking the same volume ratio of sample matrix into general diluent solution used to prepare test samples.

Note

Proper determination of sample dilution factor is important to make sure IP measurement is in calibration linear range.

5 Prepare IP standard stock at [M] 100 millimolar (mM) in MeOH. Prepare the highest concentration IP calibration standard at [M] 5 millimolar (mM) (430 mg/L) by diluting IP standard stock in calibration standard diluent.

Note

IP standard calibration range can be adjusted to fit estimated sample IP amount and chosen sample dilution factor.

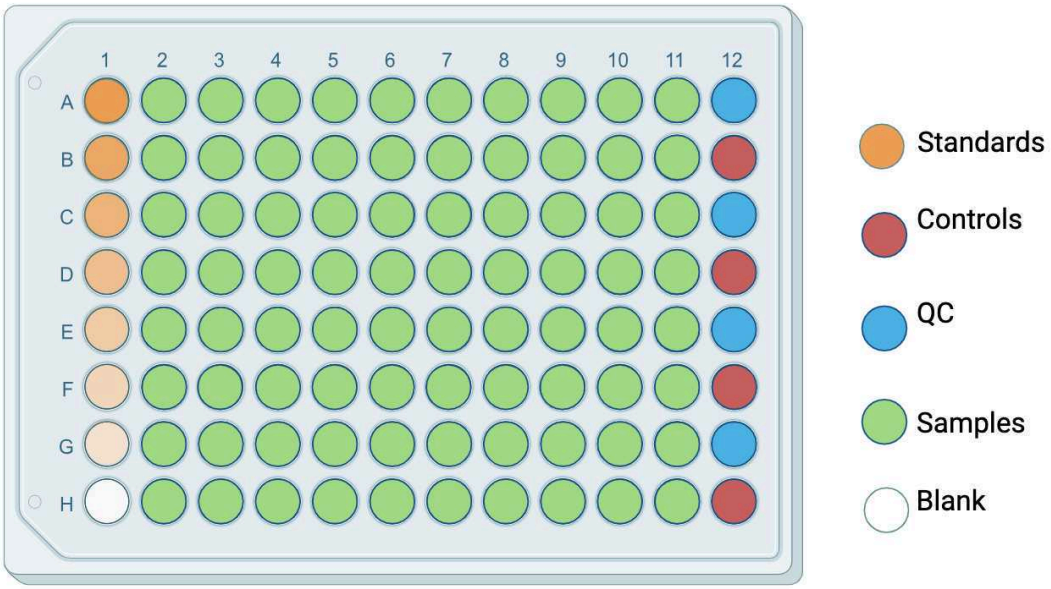
6 Perform serial dilution of the highest concentration standard in calibration standard diluent by a dilution factor of 2 .

7 Leave one well containing Calibration standard diluent as Blank. At least 50 µL of blank and each standard are needed.

Sample preparation

8 Calculate the volumes of general diluent solution and test samples that are needed to prepare 100 µL Per sample with predetermined sample dilution factors. The following steps use dilution factor of 2 as an example.

9 Transfer 50 uL of general diluent solution (5% Methanol with the internal standard) to wells of Bio-Rad 96-well PCR plate that were designated for samples. Leave wells designated to Blank, QC, Calibration standards, and controls empty.



Example analytical plate layout. Calibration standards could be arranged together with samples in one plate, or be prepared in a separate plate. For every plate that contains test samples, controls and QC are required, but their well locations could be random.

Note

Using automated liquid handler reduces human error and increases accuracy and throughput, especially when multiple dilution factors are used and sample locations are randomized.

10 Transfer the test samples (at least 300 uL per well) to the sample well locations of 96 deep-well plates, then centrifuge at 3,200 rcf at 4 °C for 30 minutes.

> Note
>
> The centrifugation time could be reduced when spinning at a higher centrifugation force. Alternatively, samples matrix could be removed through filtration process using commercially available filter plates, such as Agilent Captiva EMR-Lipid plate (Part Number: 5190-1001).

11 Transfer 50 uL of test samples from 96 deep-well sample plate, into the Bio-Rad 96-well PCR plate containing the general diluent solution (see Step 9).

> Note
>
> Using automated liquid handler reduces human error and increases accuracy and throughput, especially when multiple dilution factors are used and sample locations are randomized.

12 Finish preparing analysis plates by manually filling Blank, QC, Calibration standards and controls, then sealing the sample plate with LC compatible non adhesive alumni seal. Store the plates in -20 °C if they are not processed immediately.

Prepare RapidFire-MS system

13 Condition RapidFire pumps by purging pumps 1, 2, and 3 at 5 mL/min flow rate, and pump 4 at 10 RPM for 5 minutes.

14 Change the flow rates of pump 1, 2, and 3 to that of the running method, and change pump 4 speed to 3 RPM. Close the purge valves of pump 1, 2, and 3 afterwards.

	A	B
	RapidFire pump	Flow rate (mL/min)

	A	B
	Pump 1	1.25
	Pump 2	0.5
	Pump 3	0.5

RapidFire pump flow rates for IP and internal standard quantitation

15 Check the back pressure of RapidFire high-throughput mass spectrometry system. Monitor all pump's pressure until they are stabilized at expected range.

	A	B	C	D
	RapidFire state	Pump 1 pressure (Mpa)	Pump 2 pressure (Mpa)	Pump 3 pressure (Mpa)
	Aspirate	4.06 ~ 4.15	0.67 ~ 0.72	1.55 ~ 1.70
	Load	4.08 ~ 4.15	0.67 ~ 0.72	1.55 ~ 1.70
	Extra Wash	0.72 ~ 0.74	1.46 ~ 1.47	1.55 ~ 1.70
	Elute	0.72 ~ 0.74	0.72 ~ 0.79	2.20 ~ 2.23

RapidFire-QqQ system pumps back pressures at the method flow rates in each cycle state

Note

Pay close attention to the pressure of all the pumps. Make sure they are stable at the expected range before starting your run. Diagnose and service RapidFire if pump pressure exceeds normal range.

16 Load all sample plates in RapidFire stacker.

RapidFire plate stacker with loaded sample plates

RapidFire and QQQ method parameters

17 Create and save a RapidFire method using the following RapidFire Cycle parameters and pump flow rates:

	A	B	C
	State	Time (ms)	Flow rate (mL/min)
	Aspirate	600	
	Load	3000	1.25
	Extra Wash	0	0.5

	A	B	C
	Elute	5000	0.5
	Reequilibrate	500	1.25

RapidFire Cycle duration and flow rates for IP and internal standard quantitation

18 Install and condition RapidFire C18 4 µL Type C cartridge (Agilent Part Number: G9205A).

Note

Tracking sample counts on the working C18 cartridge and Install multiple ones if the number of total injection samples is greater than the maximum allowed injections per cartridge. Use the same cartridge type name if multiple C18 cartridges are installed.

19 Create and save a Agilent 6460 QqQ acquisition method that uses the following source parameters:

	A	B
	Gas Flow (L/min)	10
	Gas Temperature	300
	Vaporizer Temperature	300
	Nebulizer pressure	30
	Capillary Voltage	3500
	Corona Current (uA)	4

Source parameters setting for IP and internal standard quantitation

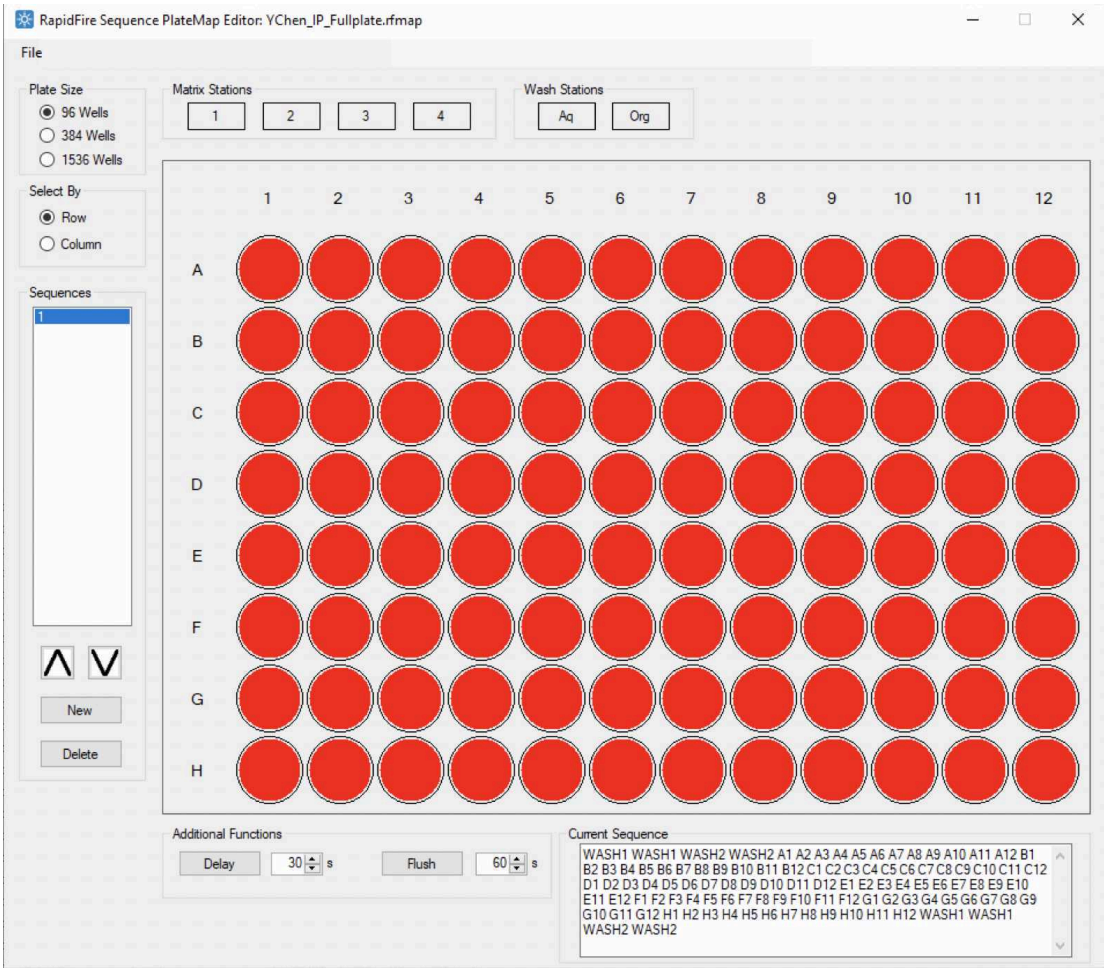
20 Update the Agilent 6460 QqQ acquisition method with the following MRM acquisition parameters:

	A	B	C	D	E	F
	Compound name	Precursor (m/z)	Product (m/z)	Dwell time (ms)	CE	Polarity
	Cis-3-Hexenyl Acetate	143.1	83.2	50	5	Positive
	Isoprenol	69.2	41.2	50	12	Positive

MRM acquisition parameters setting for IP and internal standard quantitation

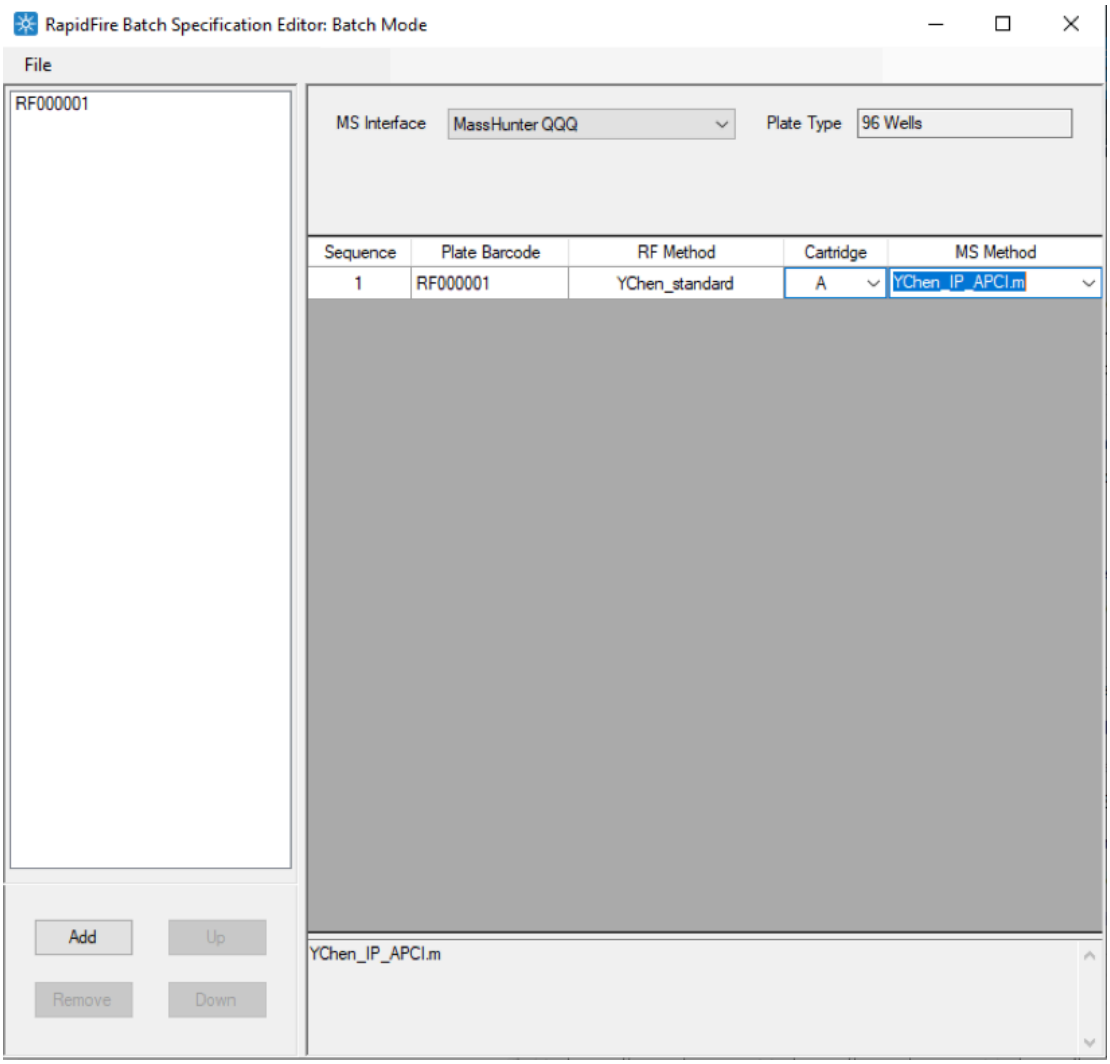
Set up RapidFire-MS analysis

21 Create plate maps that contain the desired RapidFire injection sequences.



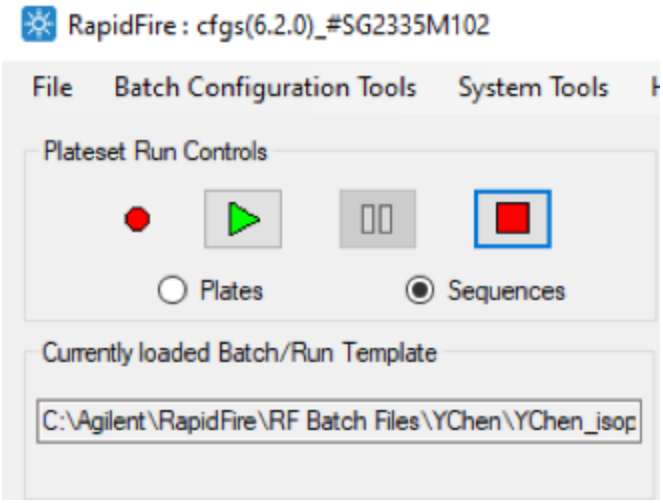
Example platemap that contains RapidFire injection sequence of a full 96-well sample plate

22 Create and save a batch file when the application runs single plate analysis or plates with multiple different injection sequences.



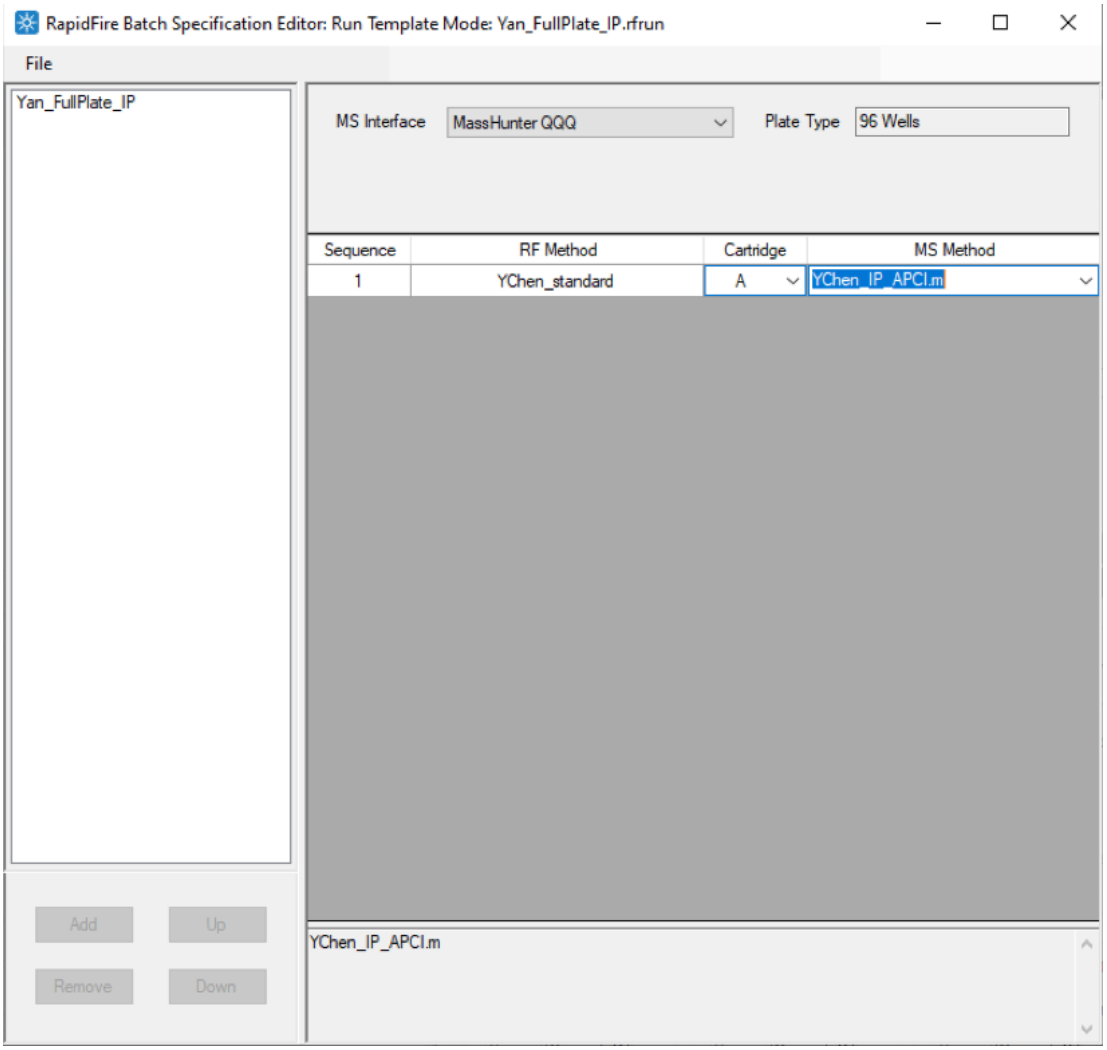
Example Batch file that contains plate info(Barcode etc) and their respective injection sequence, RapidFire method, Cartridge choice, and MS method.

23 Load the saved batch file under RapidFire UI File drop down menu. Click the green arrow button and follow the prompt to start the plateset.



RapidFire UI plateset run control panel

24 Create and save a Run Template file when the application runs high-throughput screen assay that analyze numerous full sample plates or plates with same injection sequence.



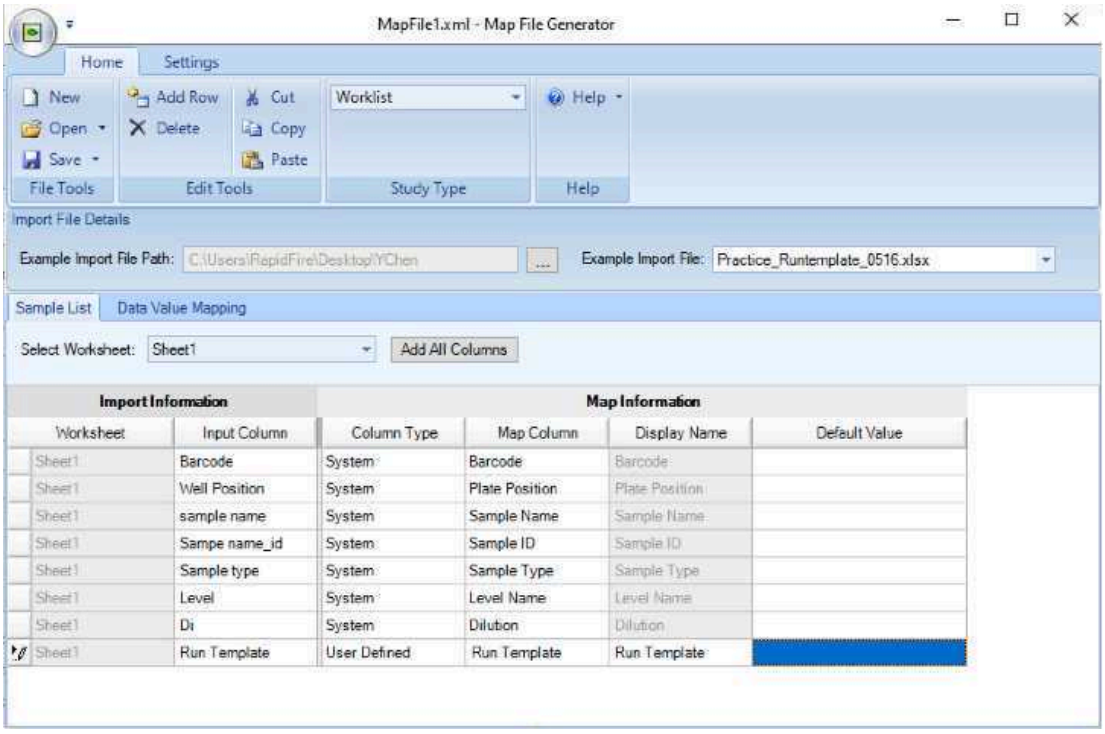
Example Run Template file that contains RapidFire injection sequence, RapidFire method, Cartridge choice, and MS method.

25 Create sample path file according to analytical sample plate layout. Fill in applied barcode information, well position, Sample name (File name), Sample type, assigned Calibration standard Levels and sample dilution factor(s).

Barcode	Well Position	sample name	Sampe name_id	Sample type	Level	Di	Run Template
RF_000043	A1	Culture_Tube_24C_0hr_001	Culture_Tube_24C_0hr_001	Sample		2	Yan_FullPlate_IP
RF_000043	A2	Culture_Tube_24C_0hr_002	Culture_Tube_24C_0hr_002	Sample		2	Yan_FullPlate_IP
RF_000043	A3	Culture_Tube_24C_0hr_003	Culture_Tube_24C_0hr_003	Sample		2	Yan_FullPlate_IP
RF_000043	A4	Culture_Tube_24C_0hr_004	Culture_Tube_24C_0hr_004	Sample		2	Yan_FullPlate_IP
RF_000043	A5	Culture_Tube_24C_0hr_005	Culture_Tube_24C_0hr_005	Sample		2	Yan_FullPlate_IP
RF_000043	A6	Culture_Tube_24C_0hr_006	Culture_Tube_24C_0hr_006	Sample		2	Yan_FullPlate_IP
RF_000043	A7	Culture_Tube_24C_0hr_007	Culture_Tube_24C_0hr_007	Sample		2	Yan_FullPlate_IP
RF_000043	A8	Culture_Tube_24C_0hr_008	Culture_Tube_24C_0hr_008	Sample		2	Yan_FullPlate_IP
RF_000043	A9	Culture_Tube_24C_0hr_009	Culture_Tube_24C_0hr_009	Sample		2	Yan_FullPlate_IP
RF_000043	A10	Culture_Tube_24C_0hr_010	Culture_Tube_24C_0hr_010	Sample		2	Yan_FullPlate_IP
RF_000043	A11	Culture_Tube_24C_0hr_011	Culture_Tube_24C_0hr_011	Sample		2	Yan_FullPlate_IP
RF_000043	A12	Culture_Tube_24C_0hr_012	Culture_Tube_24C_0hr_012	Sample		2	Yan_FullPlate_IP
RF_000043	B1	Culture_Tube_24C_0hr_013	Culture_Tube_24C_0hr_013	Sample		2	Yan_FullPlate_IP
RF_000043	B2	Culture_Tube_24C_0hr_014	Culture_Tube_24C_0hr_014	Sample		2	Yan_FullPlate_IP
RF_000043	B3	Culture_Tube_24C_0hr_015	Culture_Tube_24C_0hr_015	Sample		2	Yan_FullPlate_IP
RF_000043	B4	Culture_Tube_24C_0hr_016	Culture_Tube_24C_0hr_016	Sample		2	Yan_FullPlate_IP
RF_000043	B5	Culture_Tube_24C_0hr_017	Culture_Tube_24C_0hr_017	Sample		2	Yan_FullPlate_IP
RF_000043	B6	Culture_Tube_24C_0hr_018	Culture_Tube_24C_0hr_018	Sample		2	Yan_FullPlate_IP
RF_000043	B7	Culture_Tube_24C_0hr_019	Culture_Tube_24C_0hr_019	Sample		2	Yan_FullPlate_IP
RF_000043	B8	Culture_Tube_24C_0hr_020	Culture_Tube_24C_0hr_020	Sample		2	Yan_FullPlate_IP
RF_000043	B9	Culture_Tube_24C_0hr_021	Culture_Tube_24C_0hr_021	Sample		2	Yan_FullPlate_IP
RF_000043	B10	Culture_Tube_24C_0hr_022	Culture_Tube_24C_0hr_022	Sample		2	Yan_FullPlate_IP
RF_000043	B11	Culture_Tube_24C_0hr_023	Culture_Tube_24C_0hr_023	Sample		2	Yan_FullPlate_IP
RF_000043	B12	Culture_Tube_24C_0hr_024	Culture_Tube_24C_0hr_024	Sample		2	Yan_FullPlate_IP
RF_000043	C1	Culture_Tube_24C_0hr_025	Culture_Tube_24C_0hr_025	Sample		2	Yan_FullPlate_IP
RF_000043	C2	Culture_Tube_24C_0hr_026	Culture_Tube_24C_0hr_026	Sample		2	Yan_FullPlate_IP
RF_000043	C3	Culture_Tube_24C_0hr_027	Culture_Tube_24C_0hr_027	Sample		2	Yan_FullPlate_IP
RF_000043	C4	Culture_Tube_24C_0hr_028	Culture_Tube_24C_0hr_028	Sample		2	Yan_FullPlate_IP
RF_000043	C5	Culture_Tube_24C_0hr_029	Culture_Tube_24C_0hr_029	Sample		2	Yan_FullPlate_IP
RF_000043	C6	Culture_Tube_24C_0hr_030	Culture_Tube_24C_0hr_030	Sample		2	Yan_FullPlate_IP
RF_000043	C7	Culture_Tube_24C_0hr_031	Culture_Tube_24C_0hr_031	Sample		2	Yan_FullPlate_IP
RF_000043	C8	Culture_Tube_24C_0hr_032	Culture_Tube_24C_0hr_032	Sample		2	Yan_FullPlate_IP

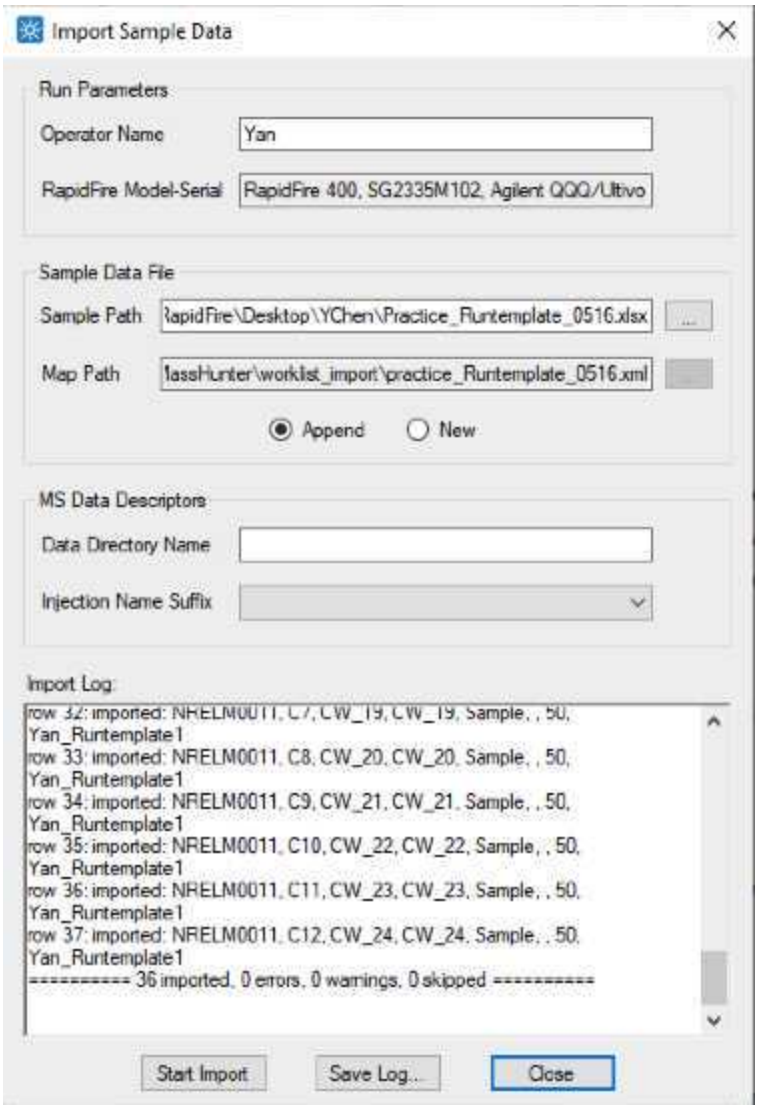
Example sample path file. Barcode column information needs to match applied barcode to corresponding plate. Well position, sample name, sample name_id and sample type are parsed from sample plate layout information. Calibration standard Levels are required for setting up quantitation method in Agilent QQQ quantitative analysis software. Dilution factor is a variable that could be modified accordingly. Run template file is generated and updated according to step 24.

26 Generate a Map File under RapidFire UI File drop down menu.



Example map file that parses information from sample path file to data file.

27 Import sample data file based on the map file generated above. Click import sample data under RapidFire UI file drop down menu. Select sample path file generated in step 25 and Map file generated in step 26 to fill in Sample Data File section.



Example result after import sample data.

Note

Check the end of the import log to make sure that all samples are imported without errors, warnings and skipped samples.

28 Click the green arrow button and follow the prompt to start the plateset.

Data analysis

29 Convert RapidFire sequence data file(s) to individual injection data files.

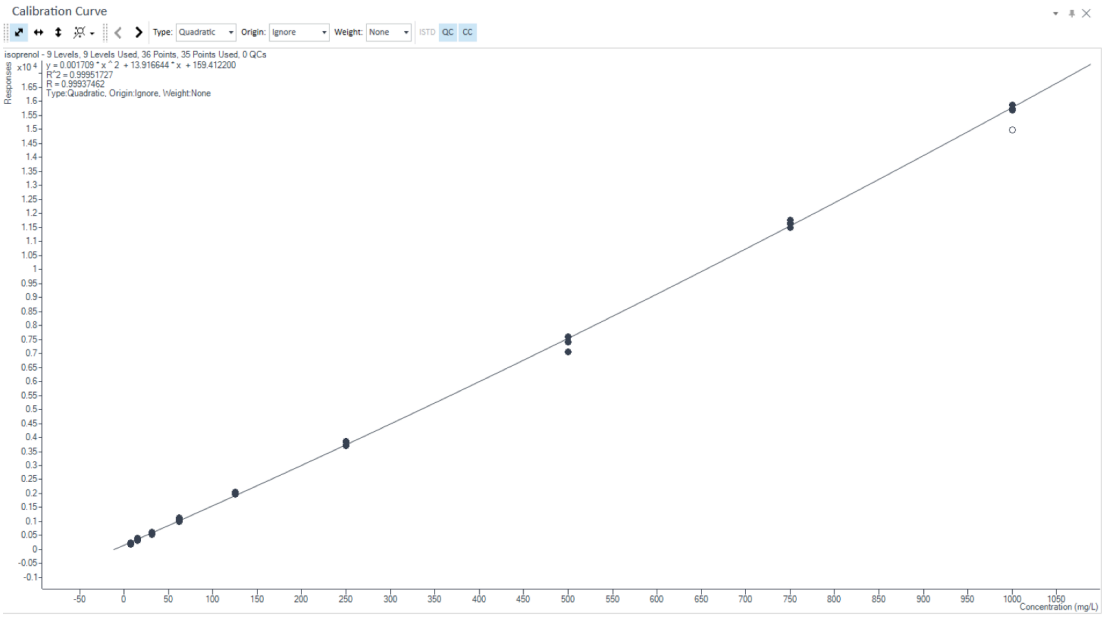
> Note
>
> Sample data files could miss if 'Do not covert missed sips' option in the converting process is checked.

30 Create a new batch in Agilent MassHunter QQQ quantitative analysis software, and load all individual injection data files. If this is the first analysis of IP, create a new quantitative method from acquired IP standard data file.

> Note
>
> Other software such as the Agilent *RapidFire* Integrator could be also used for analyzing RapidFire MS data. Refer to each software user guide for details.

31 Validate and save the quantitative method after completing all tasks in the method setup sections. Apply the quantitative method and analyze the sample batch.

> Note
>
> The calibration standard levels and expected concentrations must match the levels defined in the sample batch table. Refer to MassHunter QQQ quantitative analysis software user manual for more details on setting up the quantitative method.



Example IP calibration curve that shows linear fitting ranging from 7.8 mg/L to 1000 mg/L.

32 Save sample batch and Export quantitative reports from batch table for further data processing and visualization.

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Competing Interests:
BAS has a financial interest in Illium Technologies, Caribou Biofuels, and Erg Bio. None of the other authors have an outside financial interest to disclose.