Triple Quadrupole LC/MS Method Development Guide

Agilent
Trusted Answers

Background Reduction Protocol and Instrument Tuning

For detailed instructions please use the Simplified Background Reduction Protocol for Agilent Triple Quadrupole LC/MS Technical Overview.

Technical Overview

Materials Needed		
LC/MS grade water	Squirt bottles (2)	4000 grit paper
LC/MS grade methanol	Lint-free lab tissue	Sonicator
LC/MS grade isopropanol	Lint-free cloth	

Clean the source

- Disconnect the exhaust polyethylene tube
- Open the source and rinse with H2O:Methanol and Isopropanol; Wipe with lint free tissue
- Remove and clean the spray shield and capillary cap with isopropanol; Use 4000 grit paper if needed for discoloration; Sonicate in isopropanol and rinse with methanol
- Inspect and clean the nebulizer if needed
- Reinstall the nebulizer, cap, and spray shield and reconnect the exhaust tube.

Use MassHunter Data Acquisition for tuning

- Checktune — Daily/Weekly
- Autotune — Monthly/Quarterly/As Needed

Agilent 6400 Series LC/MS/MS Concepts Guide (pages 67–70)

Data Acquisition

Build a Background checkout method using MassHunter Data Acquisition

Configuration - LC	
Injection Volume	No injection
Analytical Column	Zero dead volume union
Column Temperature	Not controlled
Mobile Phase A	Water
Mobile Phase B	MeOH or AcN
Flow Rate	0.35-0.5 mL/min
Isocratic	50:50
Stop Time	1 minute

Configuration - MS	
Ionization Mode	Positive
Scan Type	MS2 Scan *m/z* 40 – 1000
Drying Gas Temperature	250 – 350 °C
Drying Gas Flow	11 L/min
Nebulizer Pressure	30 – 50 psi
Sheath Gas Temperature	300 – 400 °C
Sheath Gas Flow	11 L/min
Nozzle Voltage	0 V
Capillary Voltage	4000 V
Delta EMV	0 V

Review Results

After acquisition of the MS2 Scan, open the datafile using MassHunter Qualitative Analysis software. Review the total ion chromatogram (TIC) and observe the Y-axis for total number of counts compared to the table below.

If you want to improve your instrument background counts, please use the LC/MS cleaning protocol from the Technical Overview linked above, before beginning method development.

TIC background reference ranges for Agilent LC/TQ instruments.

LC/TQ	Extra Clean	Clean	Working Range	Dirty
Ultivo	< 1M	1M – 2M	2M – 6M	> 6M
6470	< 1M	1M – 2M	2M – 6M	> 6M
6495	< 20M	20 – 30M	30 – 100M	> 100M

www.agilent.com

DE22541104

This information is subject to change without notice.

Printed in the USA, May 13, 2022
5994-4797EN

For up-to-date information about Agilent's clinical research solutions and technologies, visit our webpage:

Compound Optimization

Step 1

- Prepare your samples
- Set up LC/MS method

Materials Needed		
Compound reference materials	LC/MS grade organic	Agilent LC Column
Internal Standard reference materials	LC/MS grade water	Zero dead volume union

Prepare standards

- Maximum number of compounds per mix – 10
- Compound concentrations between 1000 – 5000 ng/mL
- Ensure isobaric compounds are separated into different vials
- Prepare compound mixes in 50:50 Water:Organic

Build a short isocratic MS2 Scan "Optimizer Method"

- 50:50 Water:Organic
- Flow rate: 0.25 mL/min
- 1 – 10 µL injection

Step 2

- Create a project in Optimizer
- Follow the Tabs

Optimizer

Optimizer Setup | Precursor Ion Selection | Product Ion Selection | Compound Setup

Optimizer Setup

- Sample Introduction: Injection
- Fragmentor: Coarse Range 80-200 V; Fine Step 5 V
- Collision Energy: Range 5-40 V (Repeat any compound with 40 V CE at a higher range)
- Cell Accelerator Voltage(CAV): 4 V
- Direct acquisition method to the one built in Step 1

Precursor Ion Selection

- Select positive and/or negative ions (+H, -H, etc.)
- Can exclude masses or set a minimum abundance (Optional)

Product Ion Selection

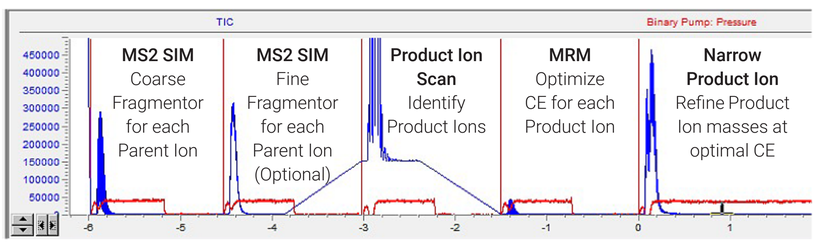
- Set max number product Ions, Recommended number: 6
- Set low mass cutoff: *m/z* 40
- Can exclude masses or losses (Optional)

Compound Setup

- Name your compound and group if desired (If re-running a compound input a unique name)
- Enter the formula to calculate the nominal mass
- Specify sample position

Step 3

- Start Optimizer – An automatic 4 Step Process
- MS2 SIM > Product Ion > MRM > Narrow Product Ion



Optimizer Report is generated; Results can be imported to MassHunter Data Acquisition

Source Optimization

Step 1

- Open Source Optimizer Program
- Set up Project and Instrument Parameters

Source Optimizer

Project Parameters

- Optimize Method: Load the acquisition method to be optimized
- Project Folder: Choose where to save the results
- Project Name: Name your project

Worklist Parameters

- Name your samples and specify sample position

Instrument Parameters

- Input all the information from the table below. Ranges may vary based on instrument model.

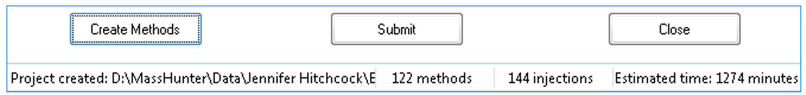
Project	Type	Pre-Wait (min)	Replicate	Step-Wait (min)	Start Value	End Value	Step Size
1	Capillary	0	1	0	1000	6000	500
1	Nozzle Voltage	0	1	0	0	2000	500
1	Drying Gas Temp	10	2	10	100	290	25
1	Drying Gas Flow	5	1	5	10	20	1
2	Sheath Gas Temp	10	2	10	100	400	25
2	Sheath Gas Flow	5	1	5	5	12	1
3	Nebulizer	0	1	0	15	60	5
4	High Pressure RF	0	1	0	70	210	20
4	Low Pressure RF	0	1	0	40	160	20

Step 2

- Group Parameters as shown above into 4 Projects
- Review results and update the method as you go

Start the First Project (Capillary/Nozzle Voltage/Drying Gas Temp & Flow)

- Check the boxes of the parameters to optimize, grouped by "Project" in the table above
- Calculate the "injections" multiplied by the injection volume to ensure enough sample volume to complete the project (e.g. 20µL x 144 = 2.88 mL)
- Click "Create Methods" and "Submit".



- The Study Manager application is now open.
- A script may be added to the end of the run if needed, for example to shutdown if running over night. Once everything is set, click , "Start"

Review Study Results in Quantitative Analysis

- Open Batch – The instrument parameters are listed as .s files – Select and open the study folder and subsequent pre-created batch file based on your project name
- Locate the Final Conc. Column in the Batch table – Find the highest value in that column and enter corresponding source condition into the acquisition method you are optimizing. Update and save the method before moving onto the next project.
- With multiple compounds, settings compromise may be needed. It is recommended to skew the settings toward less responsive compounds.

Next Project

- Redirect Optimizer to the updated acquisition method so it uses the updated parameters
- Check the boxes for the next project group and repeat the steps above until you have completed all 4 projects and an optimized method is achieved.
- iFunnel Optimizer will be performed for 6490 and 6495 users only.