Triple Quadrupole LC/MS Method Development Guide

Background Reduction Protocol and Instrument Tuning

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



Clean the source

- Disconnect the exhaust polyethylene tube

- Inspect and clean the nebulizer if needed

- Checktune - Daily/Weekly

Sonicate in isopropanol and rinse with methanol

Use MassHunter Data Acquisition for tuning

– Autotune – Monthly/Quarterly/As Needed

Materials Needed LC/MS grade water LC/MS grade methanol

LC/MS grade isopropanol

- 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;

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Squirt bottles (2) Lint-free lab tissue Lint-free cloth

4000 grit paper Sonicator











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Agilent 6400 Series LC/MS/MS Concepts Guide (pages 67–70)	Data Acquisition	
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– Reinstall the nebulizer, cap, and spray shield and reconnect the exhaust tube.

onfiguration - LC					Optimizer Setup	Precursor Ion Selection	n Product lo	on Selection	Compound Setup	
			Configuration - MS							
jection Volume	No injection		Ionization Mode	Positive	Optimizer Setup		Product Ion S	Selection		
nalytical Column	Zero dead volu	ume union	Scan Type	MS2 Scan m/z 40 – 1000	– Sample Introduction: Injec	otion	– Set max nun	nber product Ions, R	Recommended number: 6	
olumn Temperature	Not controlled		Drying Gas Temperature	250 – 350 °C	– Fragmentor: Coarse Rang	e 80-200 V; Fine Step 5 V	– Set low mas	s cutoff: <i>m/z</i> 40		
lobile Phase A	Water		Drying Gas Flow	11 L/min	- Collision Energy: Range 5	40 V (Repeat any compound with	– Can exclude	masses or losses (Optional)	
lobile Phase B	MeOH or AcN		Nebulizer Pressure	30 – 50 psi	– Cell Accelerator Voltage((AV): 4 V	Compound Se	etup		
low Rate	0.35-0.5 mL/m	nin	Sheath Gas Temperature	300 – 400 °C	 Direct acquisition method 	 Direct acquisition method to the one built in Step 1 Precursor Ion Selection 		 Name your compound and group if desired (If re-running compound input a unique name) Enter the formula to calculate the nominal mass 		
socratic	50:50		Sheath Gas Flow	11 L/min	Precursor Ion Selection					
top Time	1 minute		Nozzle Voltage	0 V	– Select positive and/or neg	 Select positive and/or negative ions (+H, -H, etc.) 		 Specify sample position 		
			Capillary Voltage	4000 V	– Can exclude masses or se	et a minimum abundance (Optiona))			
			Delta FMV	0 V						
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C/TQ	Extra Clean	Clean	Working Range	Dirty
lltivo	< 1M	1M – 2M	2M – 6M	> 6M
470	< 1M	1M – 2M	2M – 6M	> 6M
495	< 20M	20 – 30M	30 – 100M	> 100M









- Prepare your samples - Set up LC/MS method

aterials Needed

Compound reference materials	LC/MS grade organic	Agilent LC Column
nternal 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
- $-1 10 \,\mu\text{L}$ injection

- Create a project in Optimizer - Follow the Tabs Optimizer

Step 1

Project Parameters

2 2

Step 2

Project c

- 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

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.





Source Optimization

_	Open Source Optimizer Program
_	Set up Project and Instrument Parameter



- 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.

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

- 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".

Create Methods	Submit	(Close	
reated: D:\MassHunter\Data\Jennifer Hitch	ncock\E 122 methods	144 injections	Estimated time: 1274 minu	tes

- 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