Agilent

Trusted Answers

Agilent 6495C LC/TQ

Best practices guide

Get the highest performance from your Agilent 6495C triple quadrupole LC/MS

Your samples are precious, and turnaround time is critical to your organization's success. The rugged 6495C LC/TQ delivers reliable performance, day in and day out, for the highest levels of confidence.

To help you maximize instrument sensitivity and workflow efficiency, we've put together this eBook that contains dozens of valuable resources, all in one place. To access these resources, just use the convenient links provided.

Here are the topics that we cover

Prepping Your HPLC for High-Sensitivity Analysis 3

Optimizing Crucial Source Parameters 4

Optimizing MRM Parameters 6

Cleaning and Maintenance 8

Applications and Other Resources 9

Prepping Your HPLC for High-Sensitivity Analysis

HPLC best practices

By ensuring that your HPLC system is properly plumbed, conditioned, and primed, you can achieve the low detection limits you need for rigorous quantitative analysis. These guides will help you maximize resolution, sensitivity, and workflow efficiency.

- Best Practices for Efficient Liquid Chromatography (LC) Operations
  Discover straightforward techniques that you can use right away to ensure your most reliable results and maximize laboratory efficiency.
- Best Practices for Using an Agilent LC System
  This step-by-step guide walks you through critical tasks, such as solvent storage, sample preparation, choosing the right column, and more.

Minimizing sample carryover

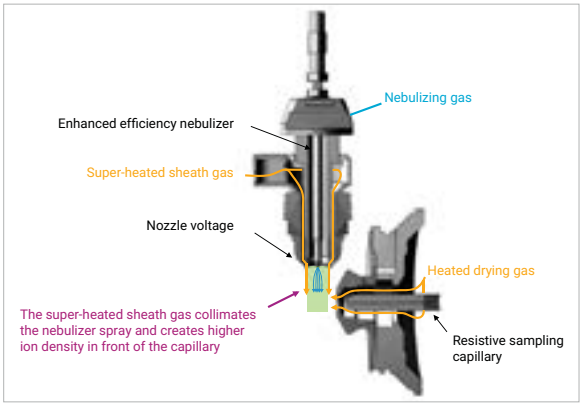
A clean baseline and clearly separated endogenous peaks are a must for quantitative accuracy. That's why it is critical to avoid sample carryover at every point in your LC flow path. You can reduce carryover to a negligible amount using the multiwash function of the 1290 Infinity II multisampler. These two guides will show you how.

- Tips and Tricks for Achieving Near-Zero Carryover Using the Multiwash Function of the Agilent 1290 Infinity II Multisampler
  Find out how you can reduce carryover originating from the autosampler to less than 10 parts per million (ppm)—or even eliminate it.
- Minimizing Sample Carryover Using the Multiwash Function
  See how we used washing procedures to reduce carryover to less than nine ppm, for caffeine and various pesticides.

Optimizing Crucial Source Parameters

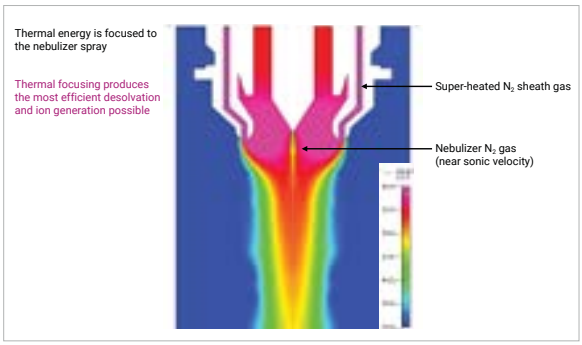
Agilent jet stream source

Agilent Jet Stream (AJS) thermal gradient focusing technology enhances ESI-MS sensitivity by improving ion desolvation and spatial focusing. Superheated nitrogen sheath gas confines the nebulizer spray to effectively concentrate and desolvate droplets in a thermal confinement zone. The benefits include less noise, reduced peak tailing, and the elimination of sample recirculation. What's more, improved ion production results in higher MS and MS/MS signal intensities and improved limits of detection.



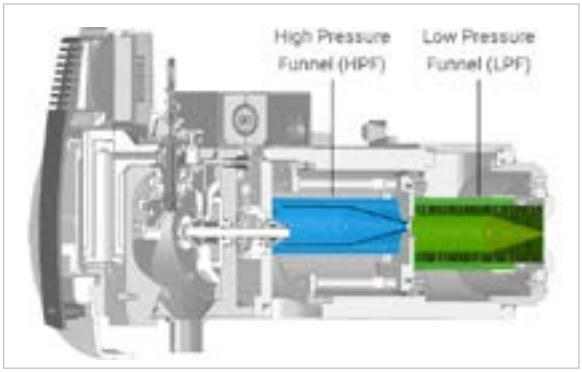
Agilent Jet Stream Thermal Gradient Focusing Technology
This technical note describes how Agilent Jet Stream thermal gradient focusing technology improves sensitivity up to 10-fold over ESI at conventional flow rates.

Jet Stream Proteomics for Sensitive and Robust Standard Flow LC/MS
Combining the Agilent Jet Stream source with ion funnel mass spectrometers makes it possible to use UHPLC for high-throughput, robust, and reproducible analysis of proteomics samples.



Dual stage iFunnel

Agilent iFunnel technology combines the efficient electrospray ion generation and focusing of Agilent Jet Stream technology with a hexabore capillary sampling array. Together, they enable a much larger fraction of the ESI spray plume to enter the mass spectrometer ion optics. A dual-stage ion funnel with optimized pressure allows increased ion capture and transmission for robust, precise quantitation at high MRM acquisition speeds requiring short dwell times. This innovative design greatly improves detection limits by collecting more ions while reducing system noise.



Optimizing Crucial Source Parameters

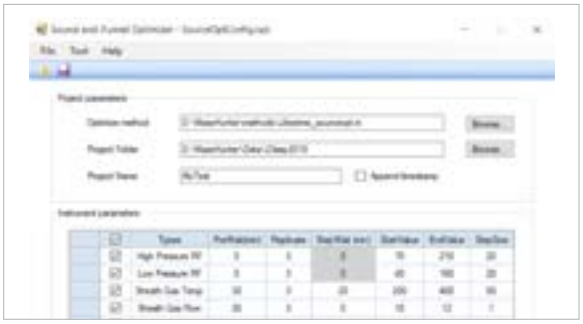
Automated and assisted source optimization

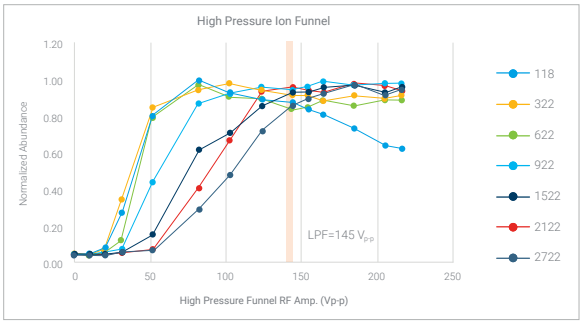
Parameter optimization is a lengthy process because you need time between temperature setpoints to achieve full thermal equilibration. Source and iFunnel Optimizer—included with Agilent MassHunter Acquisition software—helps you create and run source parameter ramps.

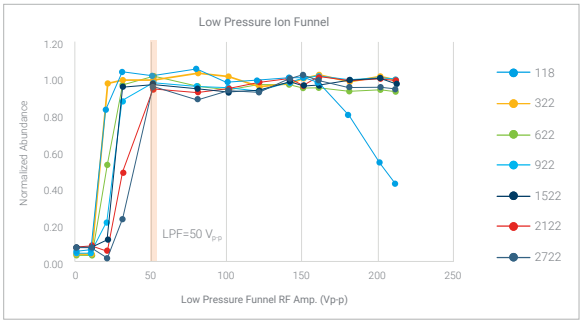
The software sets the number of replicates per experiment as well as parameter step size. It also allows you to set the desired time for thermal equilibration. Once all parameters are set, the program creates all methods and runs a worklist with these parameters. You can then analyze each data file to determine the best setpoints.

An appropriate RF amplitude ensures the highest possible transmission efficiency while minimizing precursor ion fragmentation. Source and iFunnel Optimizer can quickly create high- and low-pressure ramps for easier method development.

Although you can examine the TIC of each voltage setpoint, more information is gained by plotting "Abundance vs. RF Amplitude" for the precursor ions of interest. With this plot, you can find the most desirable funnel settings for your method. Keep in mind that an ideal voltage is not always possible. However, we recommend choosing the lowest required high- and low-pressure RF amplitudes that optimize transmission across the m/z range of ions being measured.



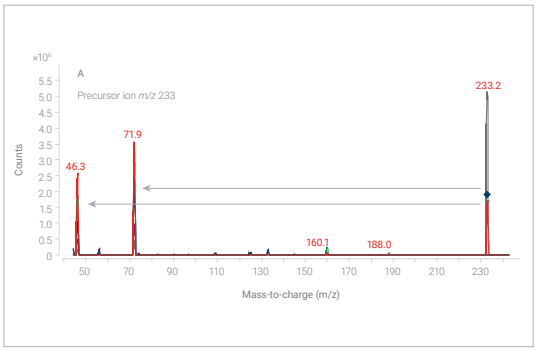


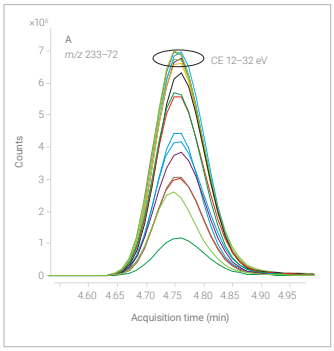


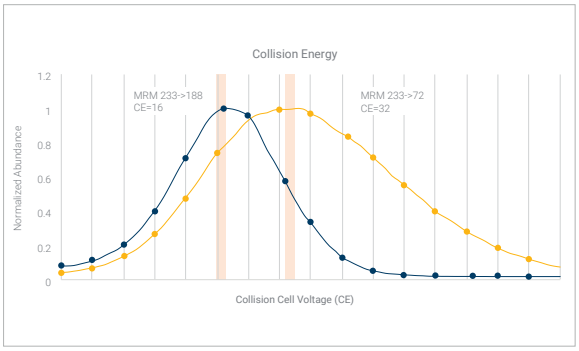
Optimizing MRM Parameters

MRM transitions and collision energies

When developing targeted methods, or when following methods such as EPA 537.1, it is crucial to choose at least two of the most abundant product ions.



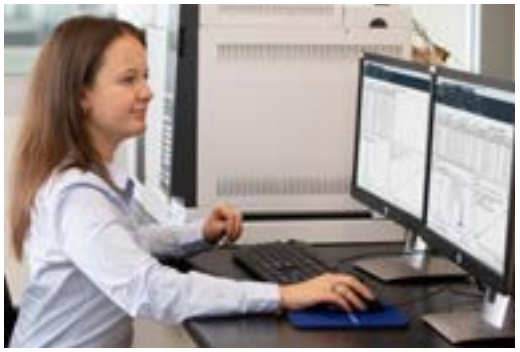




Once you have chosen the unique MRM transitions, collision energies (CE) should be ramped systematically to determine the maximum signal produced with good peak shape. Similarly, by plotting abundance versus collision cell voltage, a clearer picture of the effects of MRM collision energy emerges. In this example, the ideal CE values are 16 and 32 V.

Automated and assisted MRM optimization

To simplify method development, MassHunter software includes an optimizer tool that helps you develop MRM parameters and manage libraries. To learn more, use these links:



MassHunter Optimizer Quick Start Guide
This handy resource walks you through basic toolbars and menus, plus more complex operations like setting optimization parameters.

Triggered MRM LC/MS/MS Method Development—Practical Considerations for MRM Optimization Using Agilent MassHunter Optimizer Software
This technical overview gives you practical advice about routine compound optimization using MassHunter optimizer. It draws from our experience developing a large pesticide multiresidue tMRM LC/MS/MS method.

Manually Optimizing LC/MS/MS Acquisition Methods with MassHunter
In this Agilent University course, we'll review development strategies for LC/MS/MS MRM methods. We'll also explore optimization parameters, including fragmentor voltage, collision energy, and iFunnel.

Optimizing MRM Parameters

Peptide-specific MRM optimization

The analysis of peptides poses unique challenges that are better addressed using third-party software. MassHunter easily integrates with open-source Skyline software, helping you develop MRM methods for peptide sequences. To gain insights for peptide-centric workflows, check out these resources.

LC/TQ Integration with Skyline and MassHunter's Study Manager
We'll show you how to automate LC/MS method development for targeted proteomics and peptide quantitation.

Skyline Targeted Proteomics Software
Skyline software is designed specifically for proteomics research. Discover how this intuitive software can take you from protein to peptides to MRM transitions.

Agilent Triple Quadrupole LC/MS Peptide Quantitation with Skyline
This overview shows you how to use Skyline software to optimize collision energies, review and report your results, and save time with automation.

Application notes

Quantification of Host Cell Protein Impurities Using the Agilent 1290 Infinity II LC Coupled with the 6495B Triple Quadrupole LC/MS System

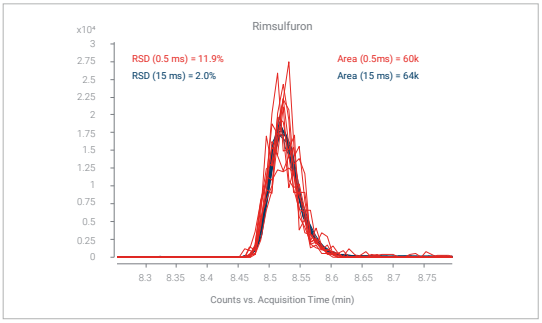
Quantification of Host Cell Protein Impurities Using the Agilent 6495C Triple Quadrupole LC/MS

Human Breast Cancer Cell Line Phosphoproteome Revealed by an Automated and Highly Selective Enrichment Workflow

Automated MRM Method Optimizer for Peptides: Optimizing Mass Spectrometry Parameters for High-Throughput Protein Quantitation

Optimizing MRM dwell times

To maximize instrument sensitivity, it is important to consider MRM dwell time, which influences the stochastic sampling of the ion beam. Generally, limits of detection are characterized by considerable time spent sampling the ion beam, which allows a stable, consistent flux of ions to hit the detector.



Due to the increased need for throughput, lab productivity, and regulatory compliance, labs are monitoring analytes using shorter chromatographic runs. As a result, more MRM transitions are being monitored simultaneously to reduce dwell times. Therefore, it is important to understand the impact of dwell time and ion flux on your analytical assay precision.

At lower instrument dwell times (typically <5 ms), ion beam sampling becomes less precise due to the insufficient number of ions involved in the measurement. In the context of chromatographic peak area, lower dwell times can increase the uncertainty (% RSD) of measurements within a set of replicates. The figure compares the results for the same analyte at the same concentration, but with different dwell times—emphasizing its effect on % RSD.

These references explore dwell time in greater detail.

Impact of Dwell Time and Ion Flux on Multiple Reaction Ion Monitoring (MRM) Measurement Precision
In this poster, we explore how MRM dwell time and ion flux impact precision and detection limits. We also give you recommendations for selecting dwell times when developing LC/MS/MS assays.

Instrument Detection Limit at Ultrashort Dwell Times Demonstrated on the Agilent 6495C Triple Quadrupole LC/MS
This technical overview compares instrument detection limits at two MRM dwell time conditions: with sufficient ion sampling time and with minimum allowed ion sampling time.

Cleaning and Maintenance

Getting acquainted with our Resources App

Need help with familiarization and troubleshooting? Use our convenient triple quadrupole LC/MS Resources App, which you'll find in the "Agilent" folder within the Start Menu. The app includes a collection of resources that cover:

- Software installation
- MassHunter Data Acquisition software and workflow
- Mass spectrometer maintenance, including consumable part numbers

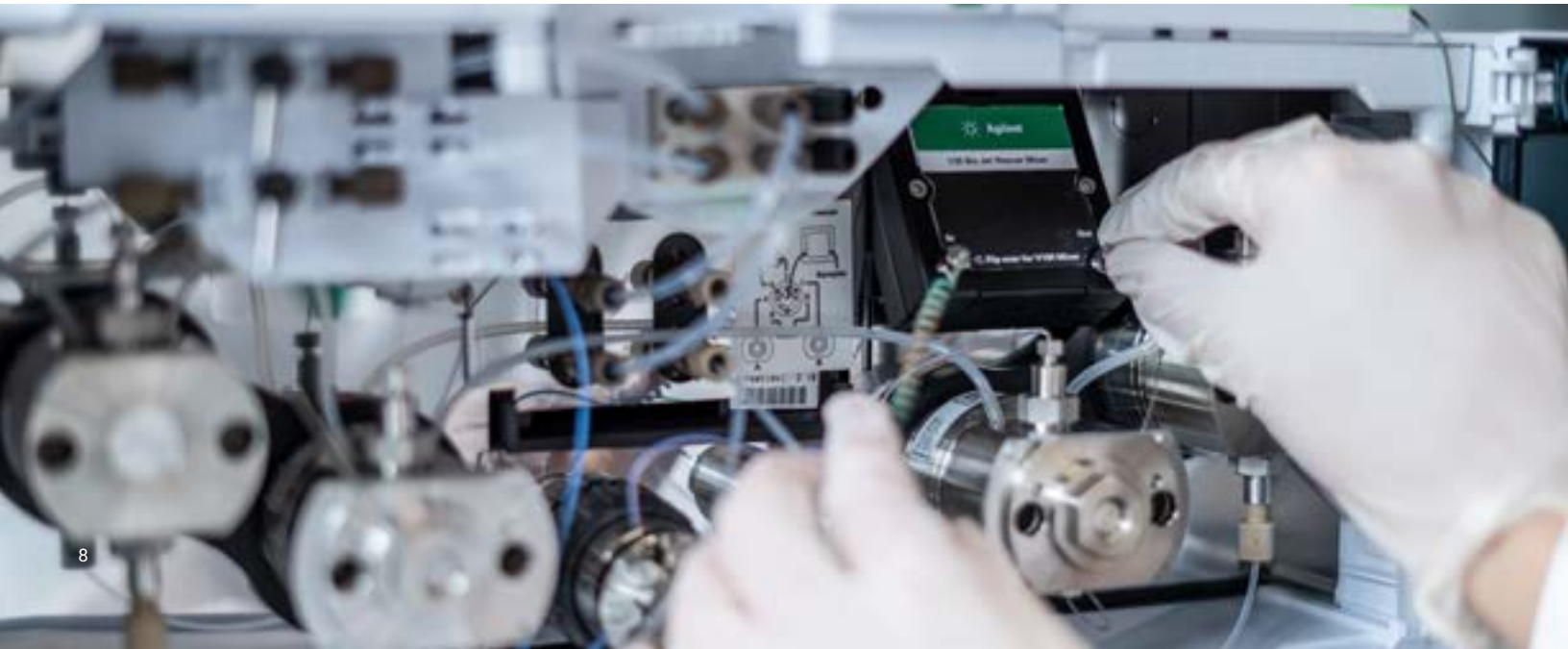
Other HPLC maintenance guides

Agilent 1290 Infinity II Easy Maintenance Pump Head

Find easy-to-follow instructions for tasks such as pump head removal and assembly, seal exchange, and piston cleaning.

Best Practices for Addressing Problems Associated with Unstable Solvents in an (U)HPLC Environment

This overview explains how to safely use unstable solvents in an (U)HPLC environment and how to avoid damage to the instrument or column.



Applications and Other Resources

Agilent application notes: Pharma and biopharma

These application notes will help you maximize instrument sensitivity and guide your sample analysis.

- Quantification of Host Cell Protein Impurities Using the Agilent 6495C Triple Quadrupole LC/MS
- Fast and Sensitive Pharmacokinetic Assessment Using an Agilent LC/MS Triple Quadrupole System
- Benefits of 2D-LC/MS/MS in Pharmaceutical Bioanalytics

Agilent application notes: Food and environmental

These application notes will help you maximize instrument sensitivity and guide your sample analysis.

Food

- Multiclass Residue Screening of 217 Veterinary Drugs in Milk and Milk Powder
- Analysis of Formamidine Pesticides and Metabolites in Pork and Porcine Liver Using Agilent Captiva EMR-Lipid and LC/MS/MS
- Direct Analysis of Glyphosate, AMPA, and Other Polar Pesticides in Food
- Determination of Eight Estrogens in Milk by UHPLC and the Agilent 6495 Triple Quadrupole Mass Spectrometer
- Multi-Residue Pesticide Screening and Quantitation in Difficult Food Matrixes Using the Agilent 6495 Triple Quadrupole Mass Spectrometer
- Fast Data Acquisition Speed and High Quantitative Performance in the Simultaneous Determination of Mycotoxins, Illegal Dyes, and Pesticides in Spices
- Simultaneous Determination of 16 Mycotoxins in Cereals Using an Agilent Triple Quadrupole LC/MS System and E-Method
- Analysis of Veterinary Drugs in Meat with the Agilent 6495 Triple Quadrupole LC/MS
- Multiclass Determination of Organic Contaminants in Red Chili and Turmeric Powders
- Determination of Paralytic Shellfish Toxins and Tetrodotoxin in Shellfish Using HILIC/MS/MS

Environmental

- Analysis of >50 Legacy and Emerging PFAS in Water
- Determination of Endocrine-Disrupting Chemicals in Drinking Water at Sub ng/L Levels Using the Agilent 6495 Triple Quadrupole Mass Spectrometer
- Highly Sensitive Detection of Pharmaceuticals and Personal Care Products (PPCPs) in Water
- Determination of Iodinated Contrast Media by Direct-Injection LC/MS/MS
- Increased Throughput in the Determination of PPCPs in Water Using Optimized MS Cycle Times in a High Sensitivity UHPLC-Triple Quadrupole System

Miscellaneous applications

- Extended Mass Range Triple Quadrupole for Routine Analysis of High Mass-to-Charge Peptide Ions
- Instrument Detection Limit at Ultrashort Dwell Times Demonstrated on the Agilent 6495C Triple Quadrupole LC/MS

Applications and Other Resources

Online resources and support

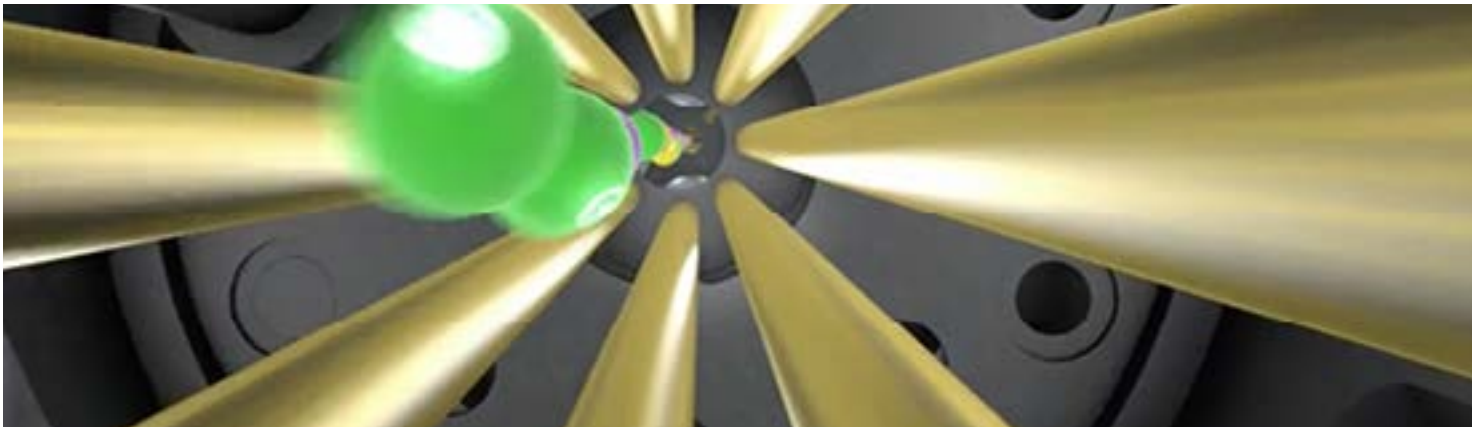
Agilent Resource Center: Expert advice, just a click away

Pressed for time? Use our easy-access collection of guides, instructional videos, and online selection tools to support your analysis.

Agilent Community: Connect, collaborate, and share insights

The Agilent Community is the best place to seek help with applications, instruments, and more—and to find in-depth content about topics relevant to your analysis.

Video tour



How It Works: Agilent 6495C Triple Quadrupole LC/MS

Elevate productivity to a new level. Watch how the Agilent 6495C triple quadrupole LC/MS brings unprecedented sensitivity, accuracy, and reproducibility to your lab.

Agilent CrossLab: Supporting Your Success

CrossLab is an Agilent capability that integrates services and consumables to support workflow success, improve productivity, and enhance operational efficiency. In every interaction, we strive to provide insight that help you achieve your goals. We offer a wide range of products and services—from method optimization and training to full-lab relocations and operations analytics—to help you manage your instruments and your lab for best performance.

Learn more about CrossLab at www.agilent.com/crosslab

Agilent CrossLab
From Insight to Outcome

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