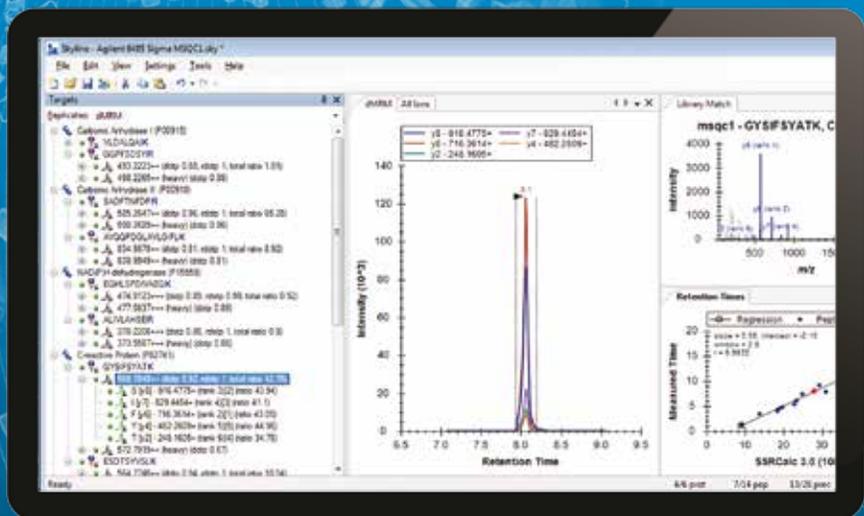


From the MacCoss Group at the University of Washington

SKYLINE TARGETED PROTEOMICS SOFTWARE

DISCOVER NEW PATHWAYS WITH AGILENT'S SOLUTIONS

The Measure of Confidence



Agilent Technologies

CHOOSING THE RIGHT TOOLS FOR YOUR RESEARCH

For targeted protein analysis and quantitation, the right tools can make all the difference. The right instruments with the right software can accelerate your research and make your life easier. Consider, for example, Agilent LC/MS instruments and Skyline software from the MacCoss group at the University of Washington in Seattle.

Widely used and highly regarded (thanks to input from top labs around the world), Skyline is a freely available Windows client application. It is also exceptionally well integrated with Agilent instruments and software.

Customized capabilities

Skyline software is designed specifically for proteomics research. The interface takes you from protein to peptides to MRM transitions—a very intuitive progression for anyone working in proteomics.

We know you appreciate that.
So does Agilent!

As long-time supporters of Skyline, Agilent has developed a close relationship with the scientists in the MacCoss lab, which has resulted in customized capabilities for Agilent instrumentation. Together we created utilities in Skyline to link Agilent automation software with this open-source software.

Seamless integration

Agilent has developed unique software called Agilent Automation that simplifies MRM method development—increasing your productivity. Agilent Automation connects an Agilent triple quadrupole system and Skyline to create a fully optimized retention-time scheduled MRM method—all without user intervention.

A tale of two programs

Quantitation-focused meets protein-centric:

MassHunter

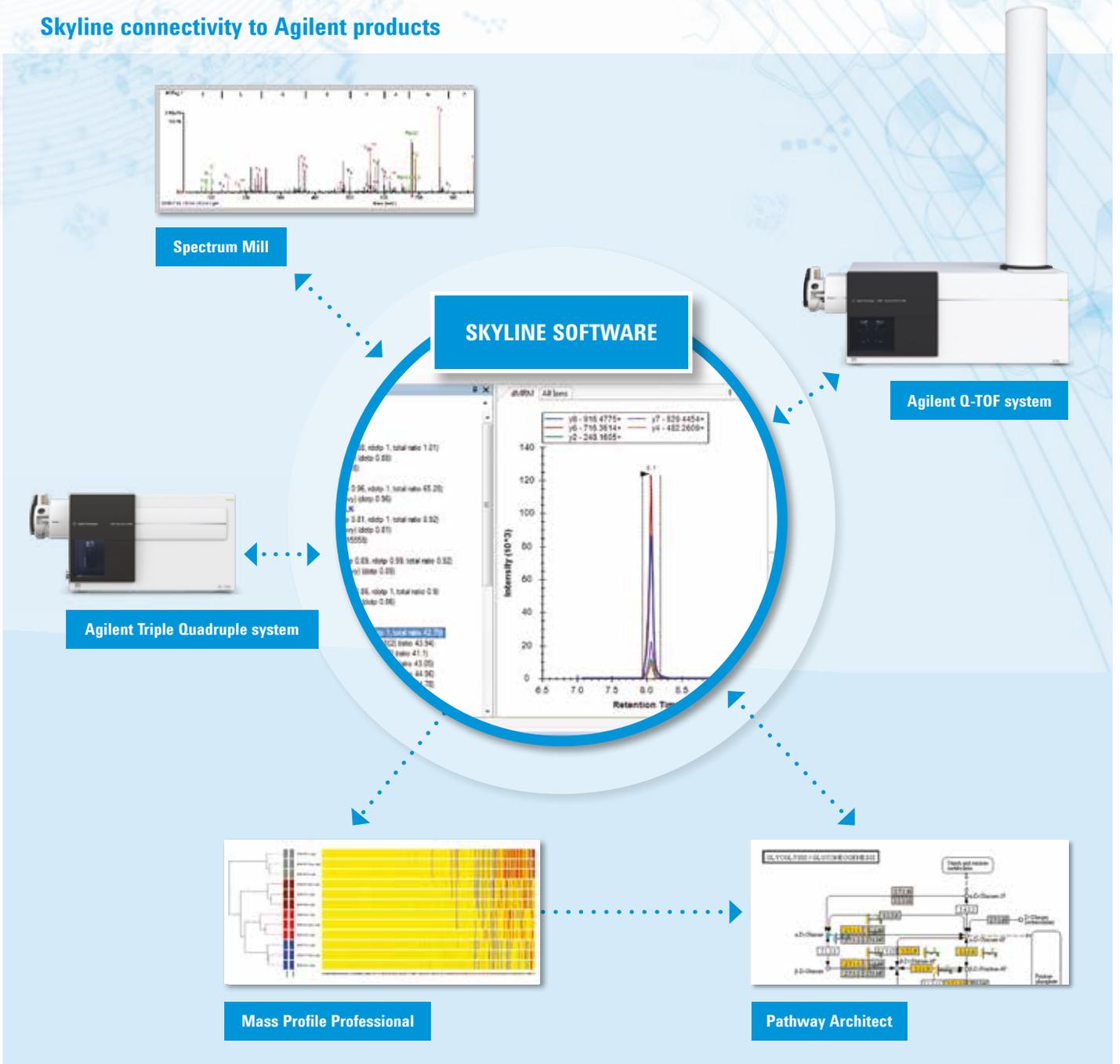
- Routine quantitation
- Useful flagging and visualization tools for detecting outliers
- Confirm peptides by matching tMRM spectra to library

Skyline

- Protein-centric organization
- Excellent visualization tools for data review
- Valuable peptide MRM prediction
- Powerful tools for predicting retention time and for spectral matching
- Continuously improving based on user feedback

Skyline offers an excellent environment for developing peptide methods and protein-centric data review. MassHunter Quant software is ideal for routine absolute quantitation of peptides.

Skyline connectivity to Agilent products



EXCEEDING THE LIMITS OF CONVENTIONAL MRM

For the quantitative analysis of known proteins, scientists have regularly used triple quadrupole mass spectrometry. But, as sample complexity increases, the ability to acquire the desired quantifier and qualifier signals has reached the practical limits of multiple reaction monitoring (MRM).

As a result, proteomics researchers have been attacking the problem in a number of different ways, often with the help of Agilent instruments and Skyline software.



Two modes

Skyline supports the two modes Agilent has developed to exceed the limits of conventional MRM on our triple quadrupole systems:

The first is dynamic MRM acquisition, which acquires MRM transitions only when the peptide is eluting. This allows you to tackle large multi-peptide assays and to accurately quantify narrow peaks. Dynamic MRM yields better results as the number

of transitions which are monitored at any point is dramatically reduced relative to time segment methods, allowing much faster MS scan cycle times and longer dwell times.

The second is triggered MRM acquisition, which adds data-dependent logic to measure additional transitions for peptide confirmation. Triggered MRM is faster and more sensitive than conventional data-dependent MS/MS scanning, and produces a pseudo MS/MS spectrum that MassHunter Quant can use for library matching.

Automated optimization

Agilent's Skyline automation tool makes creating new methods much simpler by automating the important method development and optimization process. Skyline and MassHunter work together to provide an easy solution: just put your sample in a vial, set up the parameters, and let the software do the rest. Come back a few hours later and your method is ready to run samples.

When changing columns or methods, adjusting retention times in complex methods can be time-consuming. The retention time calibration tool in MassHunter is designed to easily adjust methods to address the changes in retention time. Skyline also includes iRT capabilities to store and predict peptide retention-times.

Agilent MassHunter and Skyline software work together to make your peptide quantitation analyses faster, easier, and more productive.

Automated MRM in three easy steps using the Agilent Automation tool

1 Determine RT

- Create Skyline document
- Add proteins/peptides
- Export MRM method
- Run MRM analyses

2 Optimize CE

- Import MRM results
- Export CE optimization method
- Run CE optimization

3 Create final method

- Import CE optimization results
- Export final dynamic MRM method
- Run final method



The Agilent Automation tool allows the user to select the steps and parameters for MRM method development.

TURNING DATA INTO ANSWERS

A triple quadrupole mass spectrometer is the preferred instrument for targeted, quantitative proteomics for a small number of proteins. On the other hand, if you wish to monitor a large number of proteins, a Q-TOF mass spectrometer is the instrument of choice for targeted MS/MS proteomics. Targeted MS/MS proteomics gathers peptide MS/MS spectra in a data-independent mode. It identifies peptides from specific proteins of interest by checking the spectra it has acquired against a peptide MS/MS library.

Flexible data acquisition

Both Skyline and MassHunter support data-independent analysis, including wideband isolation, which can help you reduce complexity by isolating and fragmenting a series of narrow mass ranges. In addition, Agilent's data-independent analysis also includes All Ions MS/MS. All Ions MS/MS does not use quadrupole isolation, and fragments ions in the collision cell with two or more collision energies. Both of these approaches work well because of the very precise mass measurements produced by the Agilent Q-TOF, which facilitates accurate mining and matching of spectral data. What's more, Skyline is able to use publicly available and privately created peptide MS/MS libraries, such as those generated by Agilent's Spectrum Mill software.

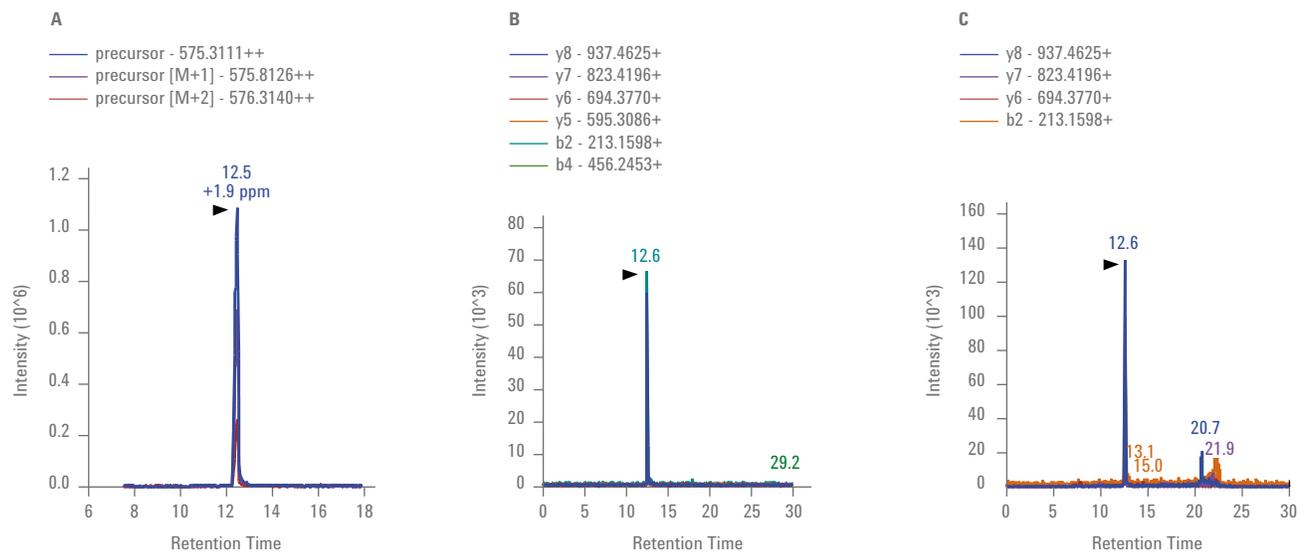
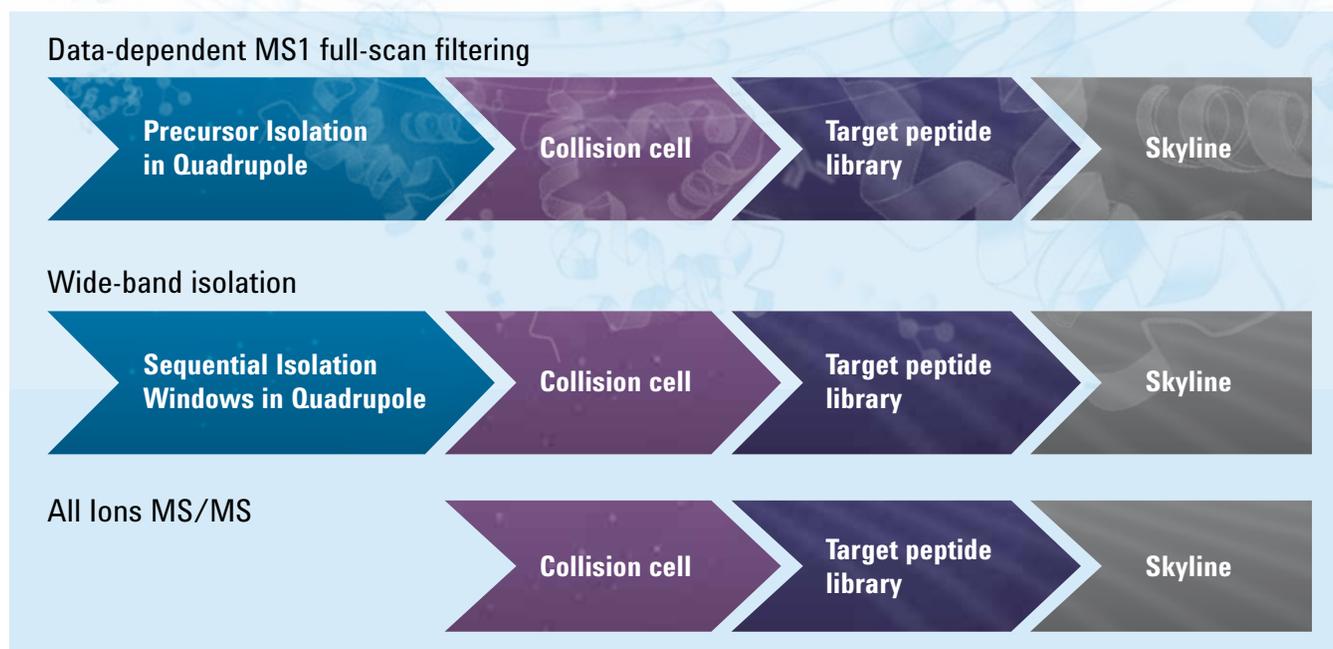
Skyline also supports MS1 full scan filtering of data-dependent acquisition on Agilent TOF and Q-TOF instruments. In this scenario, the MS1 data is used to quantitate specific peptides of interest while a spectral library from the MS/MS spectra is used to identify the peptide.

The Agilent Q-TOF is a very versatile instrument that can be used for discovery proteomics and for targeted MS/MS proteomics. While the triple quadrupole in MRM mode provides the ultimate sensitivity, the Q-TOF's ability to both identify and quantify may be more important to your lab. Skyline software is designed to seamlessly process Q-TOF data when operated in targeted quantitation mode.

Gaining insights

Regardless of your approach, data is just the beginning. When you need to turn data into answers, Agilent Mass Profiler Professional (MPP) software enables multivariate analysis of complex protein experiments to pinpoint the key proteins that are correlated to the biological difference. Skyline software easily exports Agilent results to MPP. Because Skyline is protein-centric, the exported results contain protein accession numbers that allow visualization of proteins onto biological pathways within the Pathway Architect module of MPP.

The different Agilent Q-TOF acquisition modes are all supported in Skyline



Skyline processing of a peptide from A) data-dependent results with MS1 Full-scan filtering, B) data-independent analysis using wide-band isolation; and C) data-independent analysis using Agilent's All Ions MS/MS.

Learn more

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