Agilent 6430 Triple Quadrupole LC/MS System

Ideal Quantitative LC/MS for UHPLC with Dynamic MRM and Fast Polarity Switching, plus Unsurpassed Sensitivity with Chip LC

Summary

The Agilent 6430 Triple Quadrupole LC/MS System has been specifically designed and engineered to be fully compatible with ultra high pressure liquid chromatography (UHPLC) separations and as an ideal interface for Agilent's proprietary HPLC Chip technology. The 6430 Triple Quad is a feature-packed new addition to the Agilent triple quadrupole product family, with:

- Reliable MassHunter software
- MassHunter Optimizer for automated compound optimization
- 1 ms dwell times with no collision cell cross talk
- Dynamic MRM methods for up to 4,000 transitions
- 30 ms polarity switching for fast analyses
- Low attomole sensitivity in peptide quantitation with Agilent's advanced HPLC-Chip Nano LC system

Controlled by proven MassHunter software, the new 6430 Triple Quad joins the Agilent 6410 Triple Quadrupole LC/MS System, an entry-level system for routine quantitative analysis, and the flagship Agilent 6460 Triple Quadrupole LC/MS System with unrivalled sensitivity powered by Agilent Jet Stream technology, to create a powerful suite of products to meet the quantitation demands of any application or budget. The 6430 Triple Quad offers the same combination of superior performance, exceptional reliability, and excellent overall cost-of-ownership for which Agilent is famous.

Our measure is your success.

Fast MRM Analysis and Polarity Switching (Plus Sensitivity) — Perfect for UHPLC

The 1290 Infinity LC System for UHPLC offers tremendous productivity gains versus conventional HPLC. UHPLC with sub-2-micron columns can dramatically reduce separation times and maximize chromatographic resolution. This frequently results in very narrow peaks which may be less than a second wide. The new 6430 Triple Quad has been engineered to achieve optimal quantitation of narrow UHPLC peaks.

Reliable peak integration requires at least ten data points across a peak, mandating MS cycle times as low as 100 ms for a second-wide peak. Complex, multi-analyte assays require very short dwell times. The 6430 Triple Quad can keep up! It features extremely fast ion monitoring and polarity switching. The enhanced electronics in the 6430 Triple Quad enable 1 ms dwell times with no collision cell cross talk. Further, the introduction of dynamic MRM methods allows dynamic selection of MRM transitions during a method, based on retention time windows, to optimize instrument cycle time and maximize collection of high quality quantitative data.[1] This allows hundreds of compounds to be analyzed in a single LC/MS run. Samples containing compounds with both positive and negative ion transitions require fast polarity switching – the 6430 Triple Quad can change polarity and begin acquiring data in just 30 ms. The improvement in polarity switching times is derived from a new, resistively coated capillary that reacts very quickly to voltage changes.

In addition to fast analysis capabilities, the 6430 Triple Quad is more sensitive than its predecessor – it has a standard second turbo pump in the Q3 region to enhance ion transmission by reducing chemical noise.

Designed as an IDEAL SYSTEM for UHPLC/MS Analyses

- 1200 bar pressure (up to 2 mL/min) for the fastest separations
- 30 ms polarity switching for fast peaks
- 1 ms dwell times for analyzing hundreds of compounds
- Dynamic MRM methods for analyzing thousands of ion transitions
- Improved sensitivity

Figure 1: The Agilent 1290 Infinity LC System and the Agilent 6430 Triple Quadrupole LC/MS System. The 6430 Triple Quad is an ideal MS system for UHPLC separations.

As part of a metabolic stability assay[2] (**Figure 2**), several pharmaceutical compounds and their metabolites were separated using a 1.5 min method with the Agilent 1290 Infinity LC System, using a sub-2-micron column and pressure of 1060 bar. The diclofenac ion transition was monitored along with four other positive ion transitions and one negative ion transition. The cycle time for six ion transitions and positive/negative polarity switching was **less than 115 ms**. This narrow peak was sampled 9 times, creating an excellent basis for peak area determination - the area RSD value for diclofenac was less than 5%.

Dynamic MRM Methods Enable Complex Multi-Residue Analyses

Dynamic MRM methods dramatically reduce MS cycle times for complex multi-analyte assays. MRM transition lists are built dynamically during the LC separation based on a retention time window for each analyte. Using the dynamic MRM method capabilities of the 6430 Triple Quad, 224 pesticides were analyzed in only 7 min as shown in **Figure 3**. The average peak widths were about 1 sec wide, requiring an MS cycle time of about 100 ms for precise and accurate quantitation of all compounds. The minimum dwell time was a very fast 2.5 ms. The excellent retention time precision of the 1290 Infinity LC allowed very narrow retention time windows and reduced the number of required ion transitions at any point during the chromatogram to fewer than 20. The precision of this very fast pesticide assay was excellent, with an average peak area RSD of less than 5%.

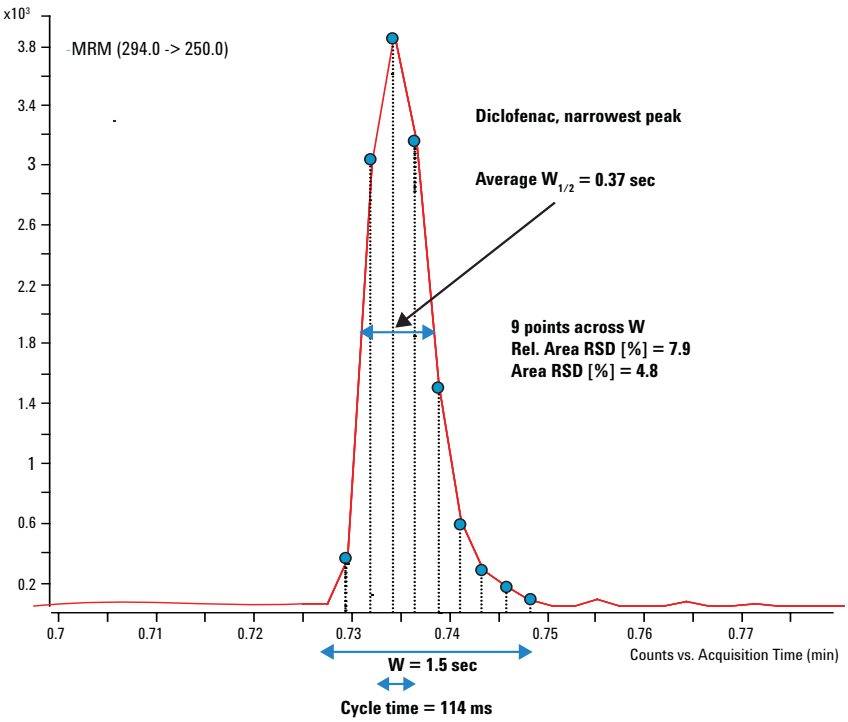

Fig. 2: MRM chromatogram of diclofenac obtained using a flow rate of 1.5 mL/min. The graphic shows the peak width at half height (0.37 sec) and the number of data points collected across the peak (9 data points).

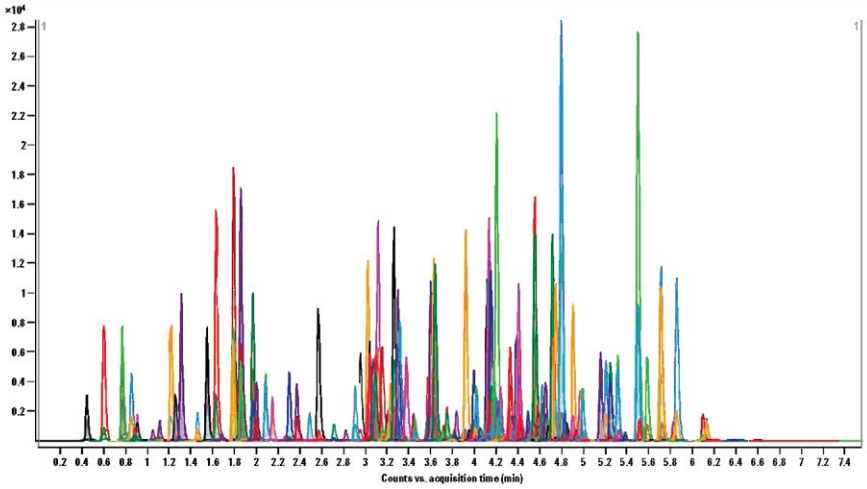

Figure 3. Fast analysis of 224-pesticides at the 500 ppt level in less than 7 min using the new Agilent 1290 Infinity LC and 6430 Triple Quad with dynamic MRM. Average peak area RSD was less than 5%.

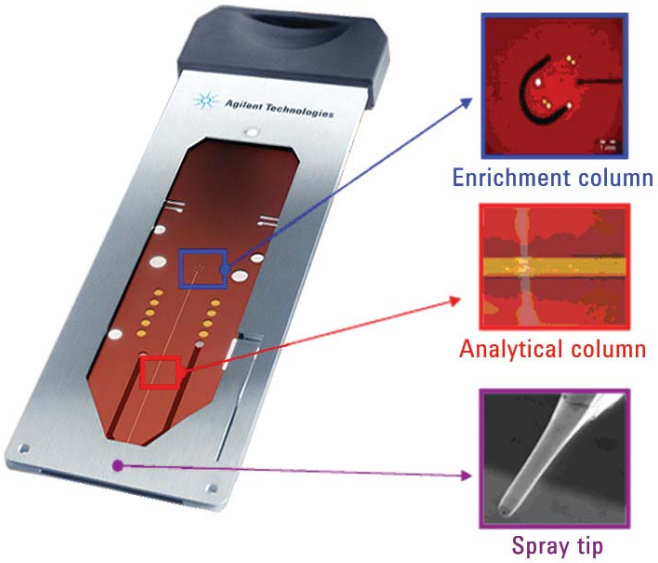

Figure 4. ChipCube with integrated valves, columns and spray tip.

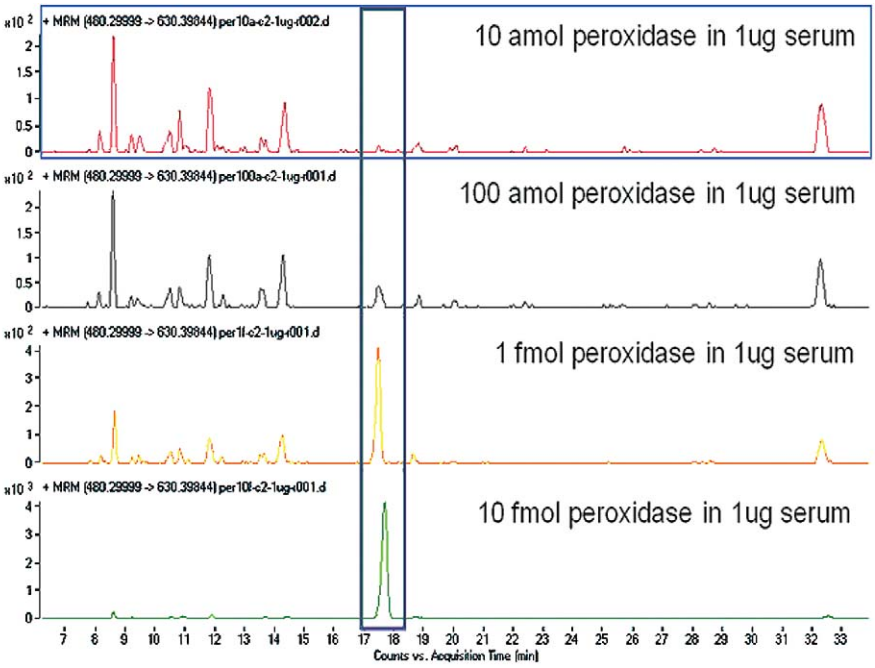

Figure 5. Attomolar detections limits for exogenous peroxidase protein spiked into immunodepleted human plasma, analyzed with the HPLC-Chip coupled with a 6430 Triple Quad.

Robust, Reliable, Sensitive Nanospray with HPLC-Chip

The highest possible sensitivity in LC/MS is achieved with the smallest column diameters. Nano LC columns with an i.d. of 75 microns offer an obvious route to high sensitivity but have historically suffered from a lack of robustness and precision. Agilent's revolutionary HPLC-Chip/MS technology provides nanoscale LC on a plug-and-play microfluidic chip, minimizing leaks and dead volumes. The chip contains a fully integrated electrospray emitter for unprecedented spray stability.

The 6430 Triple Quad with an HPLC-Chip/MS interface is ideal for targeted protein quantitation using dynamic MRM methods. This system can achieve low attomolar sensitivity (see **Figure 5**) in plasma protein digests.

Dynamic MRM methods on the HPLC-Chip/6430 Triple Quad system can be used to quantitate hundreds or thousands of peptides, as shown in the analysis of the complex peptide digest shown in **Figure 6**. More than 2,000 ion transitions were monitored during this LC/MS analysis.

Conclusions

The new Agilent 6430 Triple Quad LC/MS features seamless integration with the Agilent 1290 Infinity LC System and the proprietary Agilent HPLC-Chip/MS nanospray interface. This new system features:

- Extensive sampling capabilities for narrow UHPLC peaks
- Fast 1 ms MRM dwell times
- Dynamic MRM methods for complex, multi-analyte assays
- Fast 30 ms polarity switching times
- Attomole sensitivity for biomarker validation studies using the HPLC-Chip and 6430 Triple Quad
- Rugged, reliable MassHunter software control
- Automated method optimization using MassHunter Optimizer and Peptide Optimizer software
- 21 CFR Part 11 support for validated methods

With legendary Agilent robustness and reliability, and a commitment to highest quality service and support, the 6430 Triple Quad is a productive new addition to the Agilent 6400 Series Triple Quadrupole LC/MS product family.

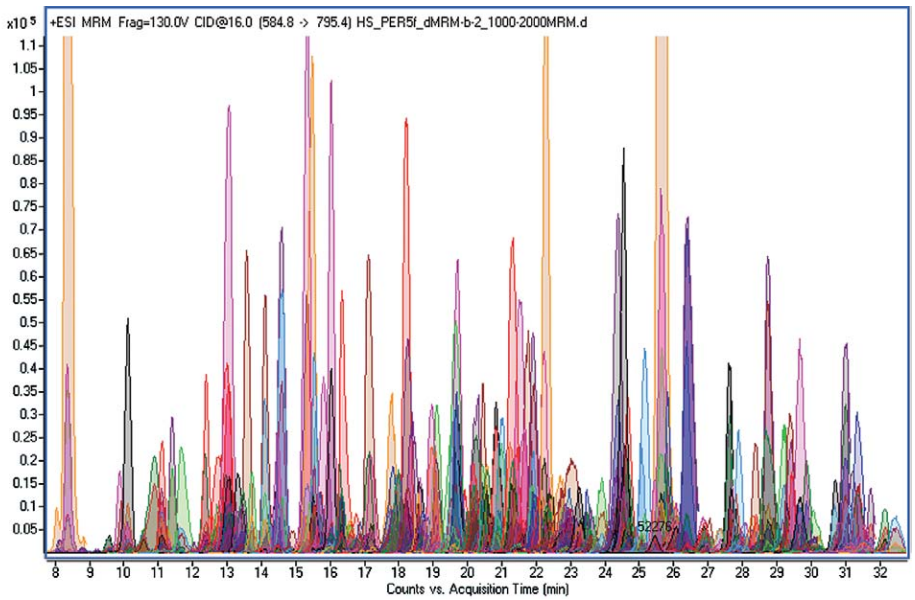

Figure 6. 2,000 Dynamic MRM transitions from the tryptic digest of immunodepleted human plasma using the HPLC-Chip/6430 Triple Quad system.

References

1. Peter Stone, Thomas Glauner, Frank Kuhlmann, Tim Schlabach and Ken Miller, "New Dynamic MRM Mode Improves Data Quality and Triple Quad Quantification in Complex Analyses," Agilent publication number 5990-3595EN, **2009**.

2. Anabel S. Fandiño, Edgar Naegele, "Metabolic Stability Study Using Cassette Analysis and Polarity Switching in a UHPLC Triple Quad LC/MS System," Agilent publication number 5990-4469EN, **2009**.

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