

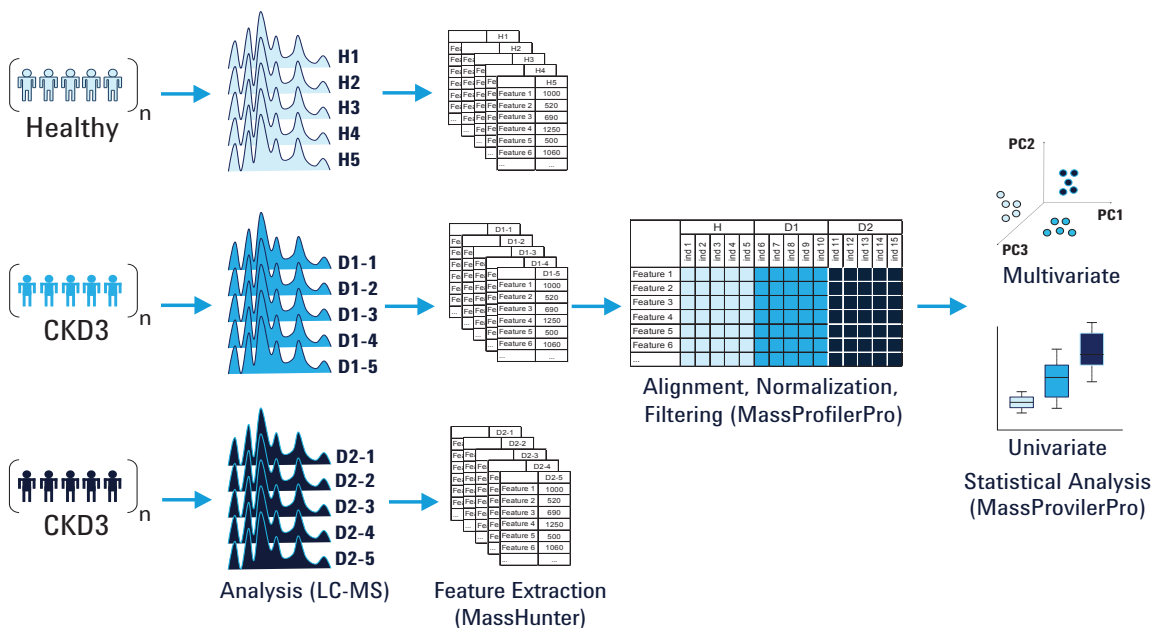
METABOLOMIC RESEARCH USING THE AGILENT 6545 Q-TOF LC/MS SYSTEM

Metabolomics allows researchers to interrogate thousands of potential biomarkers simultaneously, without a priori knowledge of the underlying biology or pathophysiology. Due to the complexity of the samples, people need outstanding mass resolution, mass accuracy, and sensitivity as well as sophisticated data analysis software. Agilent 6545 Q-TOF LC/MS system and Agilent MassHunter software are the perfect combination to fulfill the requirements.

In this application, serum extracts from healthy subjects and patients with chronic kidney disease (CKD) at stage 3 and stage 5 are analyzed with 6545 LC/Q-TOF and 1290 Infinity LC system. The mass spectrometer is tuned using the novel Swarm Autotune for optimal performance in the m/z range of the metabolites. Data was acquired in both positive and negative ionization modes with reference mass correction. The typical metabolomics workflow is illustrated here.

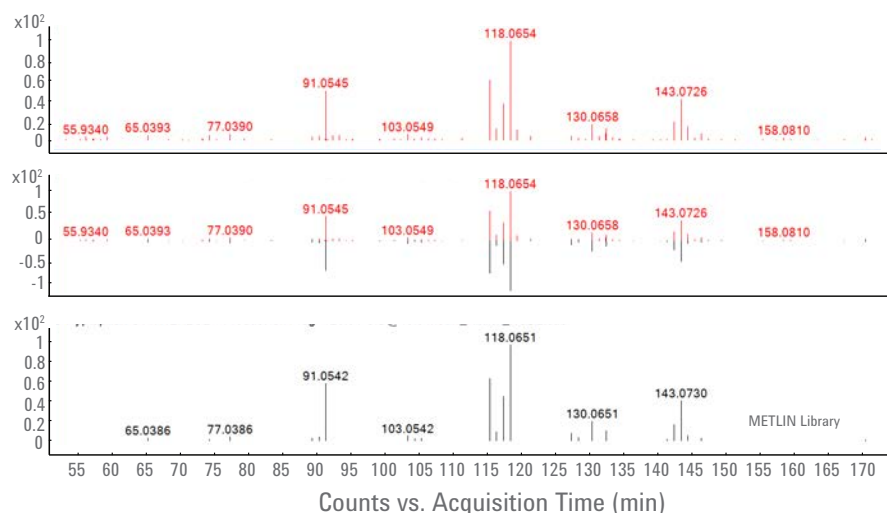
Big sensitivity gains improve results

The improved sensitivity of the 6545 Q-TOF LC/MS enables us to extract 5,817 features as opposed to 2,102 features on the previous generation of Q-TOF under the same column load in positive ionization mode. Even with a five-fold reduction of column load, we are still able to extract 2,489 features.

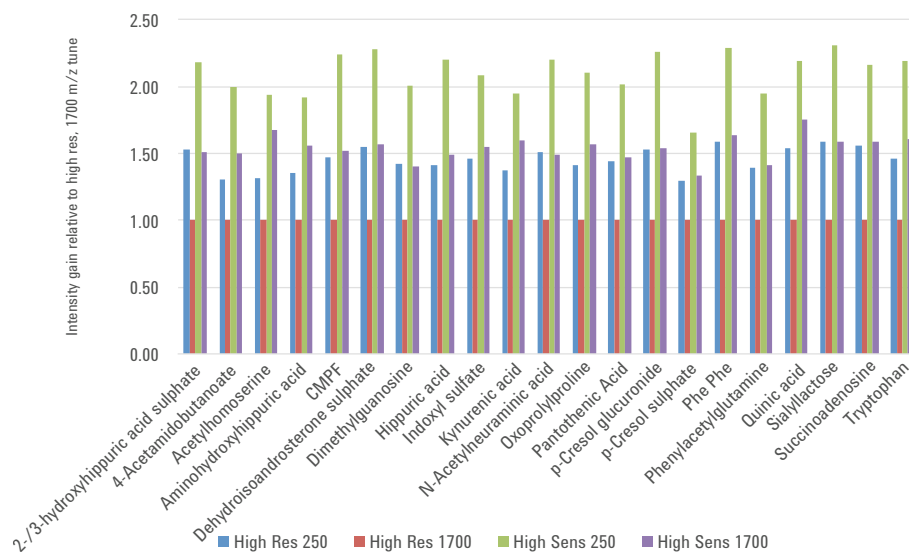


Here we achieve two-to-seven-fold sensitivity improvement for identified biomarkers in positive ionization mode. Some compounds were only detected with the 6545 Q-TOF. Mass accuracies on these measurements are typically below 1 ppm.

| Marker Evolution | Healthy | | | CKD3 | | | CKD5 | | |
|--|-----------|--------------------------------|-------------------------------------|-----------|--------------------------------|-------------------------------------|-----------|--------------------------------|-------------------------------------|
| | Area 6545 | Area Previous Generation Q-TOF | Area 6545/Previous Generation Q-TOF | Area 6545 | Area Previous Generation Q-TOF | Area 6545/Previous Generation Q-TOF | Area 6545 | Area Previous Generation Q-TOF | Area 6545/Previous Generation Q-TOF |
| Tryptophan ↓ | 31960076 | 12840877 | 2.49 | 27059409 | 11084681 | 2.44 | 10591592 | 5028854 | 2.11 |
| Succinoadenosine ↑ | 102176 | 17157 | 5.96 | 221093 | 44063 | 5.02 | 1521394 | 282713 | 5.38 |
| Sialyllactose ↑ | 66575 | 0 | ∞ | 160999 | 31472 | 5.12 | 1083488 | 180847 | 5.99 |
| Quinic acid ↑ | - | - | - | 255901 | 120752 | 2.12 | 899575 | 385270 | 2.33 |
| Oxopropylproline ↑ | - | - | - | 106715 | 16747 | 6.37 | 3721863 | 483834 | 7.69 |
| N-Threonylcarbamoyl Adenosine ↑ | 72366 | 16027 | 4.52 | 176765 | 37413 | 4.72 | 1636856 | 274936 | 5.95 |
| Nicotinic acid or Isonicotinylglycine ↑ | - | - | - | 95482 | 0 | ∞ | 1570370 | 469079 | 3.35 |
| Kynurenic acid ↑ | 84257 | 19330 | 4.35 | 208262 | 48814 | 4.27 | 4252626 | 833638 | 5.10 |
| Glutamylphenylalanine ↑ | 314998 | 87567 | 3.60 | 347270 | 76350 | 4.55 | 762331 | 170651 | 4.47 |
| Cinnamoylglycine ↑ | 296651 | 81692 | 3.63 | 448622 | 124378 | 3.61 | 983804 | 269980 | 3.64 |



The 6545 Q-TOF enables the sensitive detection of metabolite markers and structural confirmation by MSMS fragmentation using spectra library search or Molecular Structure Correlator (MSC) with ChemSpider search. See left, the identification of tryptophan, a down-regulated marker of CKD progression.



The increase in ion transmission by Swarm autotune and slicer position can increase analyte detection.

The 6545 Q-TOF enables detection of metabolites using less precious biological samples.

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