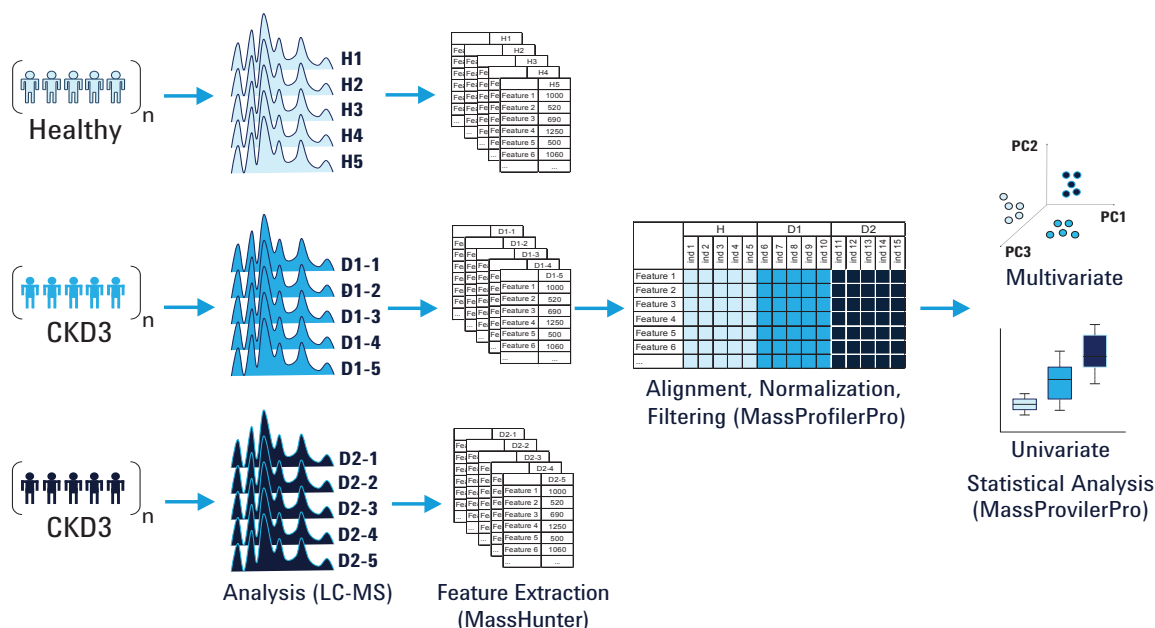# METABOLOMIC RESEARCH
USING THE AGILENT 6545 Q-TOF LC/MS SYSTEM

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 samples 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.
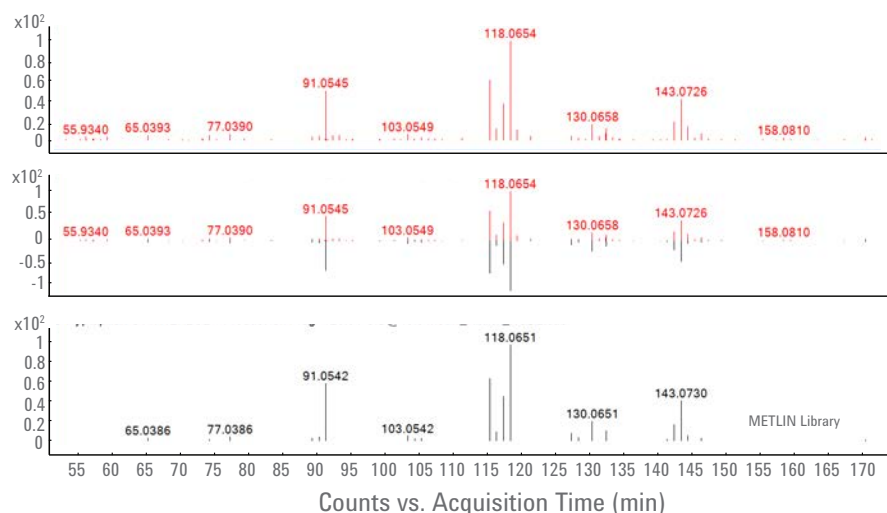
Big sensitivity gains improve results

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.

Metabolomics workflow: Healthy, CKD3, CKD3 — Analysis (LC-MS) — Feature Extraction (MassHunter) — Alignment, Normalization, Filtering (MassProfilerPro) — Multivariate, Univariate Statistical Analysis (MassProvilerPro)
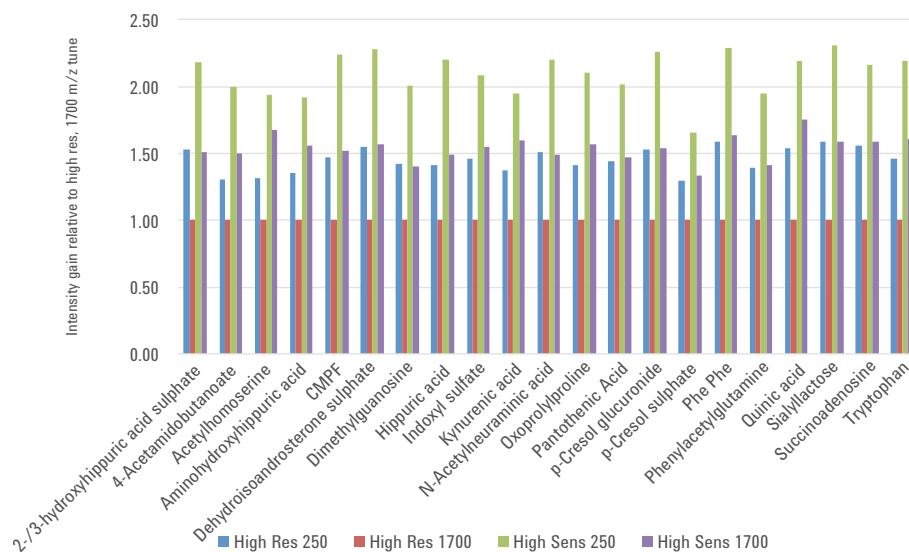
Agilent Technologies

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
Oxoprolylproline ↑	-	-	-	106715	16747	6.37	3721863	483834	7.69
N-Threonylcarbamoyl Adenosine ↑	72366	16027	4.52	176765	37413	4.72	1636856	274936	5.95
Nicotinuric 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

Counts vs. Acquisition Time (min)

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.

The Measure of Confidence

Agilent Technologies