

**Title:** Advancing metabolomics with ion mobility: distinguishing isovalerylcarnitine and 2-methylbutyrylcarnitine with a novel timsTOF mass spectrometer

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### **Short abstract:**

Accurate identification of small molecules is crucial for studying enzymatic function in clinical research. We evaluated a novel quadrupole time-of-flight (Q-TOF) mass spectrometer with trapped ion mobility spectrometry (TIMS), optimized for metabolomics. Using butylated acyl carnitine standards and plasma samples, TIMS enabled separation of isomeric isovalerylcarnitine and 2-methylbutyrylcarnitine without prior chromatography. The new timsTOF device matched triple quadrupole MS sensitivity, offered high mass resolution, and enabled isomer differentiation. Integrating TIMS with Q-TOF MS enhances analytical precision in metabolite characterization and enables high-throughput in clinical research.

### **Abstract:**

### **Introduction:**

Accurate identification of small molecules is essential for studying enzymatic function in clinical research, especially when isomeric metabolites such as C5-acylcarnitines are concerned. We evaluated the utility of a novel quadrupole time-of-flight (Q-TOF) mass spectrometer integrated with trapped ion mobility spectrometry (TIMS) for small molecule analysis in metabolomics studies. The system's hardware enhancements facilitate increased sensitivity and improved structural resolution of isomeric acyl carnitines.

### **Methods:**

Butyl-derivatized plasma extracts and standard mixtures of butylated acylcarnitines were analyzed using the timsMetabo platform (Bruker Daltonics) coupled to an Agilent UHPLC inlet via a VIP-HESI source. Direct infusion and loop injection workflows were employed and timsTOF parameters, including source settings, mass range, TIMS ramp time, and collision energy, were optimized for acylcarnitine detection and their mobility separation. Data were acquired in "manual reaction monitoring" -mode, and processed using DataAnalysis™ software for CCS-based annotation.

### **Results:**

We assessed the instrument's performance using butylated standards and plasma samples representing disturbances in acyl carnitine pathways. Even in the complex samples, and without prior chromatography,

TIMS facilitated the separation of isomeric isovalerylcarnitine and 2-methylbutyrylcarnitine based on their respective, distinct molecular shapes and resulting collisional cross sections (CCS). The new timsMetabo system demonstrated sensitivity comparable to unit resolution MS platforms, while providing accurate mass detection and added isomer selectivity via ion mobility separation.

Compared to standard MS workflows, TIMS-based ion sorting resulted in cleaner spectra, enhanced differentiation of isomeric species, and more accurate identification in both standard mixtures and complex biological samples.

**Conclusion:**

Our findings show that integrating ion mobility spectrometry with Q-TOF MS significantly improves the characterization of polar metabolites, including isomeric and isobaric compounds. Chromatography-free workflows enable high-throughput clinical research studies and fast clinical translation into routine patient care.