Metabolomics - General

Fast polarity-switching untargeted metabolite workflow in a short run powered by Orbitrap Tribrid Apex Mass Spectrometer

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Abstract

Purpose: Metabolomics experiments, typically require positive and negative polarity runs increasing total analysis time. A short polarity switching experiment using the high speed of the Orbitrap™ Tribrid™ Apex is demonstrated through tea sample analysis

Methods: Six different tea extract samples were analyzed by LC-MS polarity switching experiments using 5- and 15-minute gradients on Thermo Scientific™ Orbitrap™ IQ-X™ Tribrid™ and Thermo Scientific™ Orbitrap™ Tribrid™ Apex mass spectrometers coupled with Thermo Scientific™ Vanquish™ Horizon LC and a Hypersil GOLD™ C18 column.

Results: Orbitrap™ Tribrid™ Apex demonstrated higher scan rate, acquired enough data points for quantification under polarity switching and annotated 73% of compounds despite 66% reduction in run time. PCA analysis grouped the compounds from tea flavors successfully.

Introduction

High-resolution mass spectrometry is widely used for untargeted metabolomics and lipidomics. However, separate positive and negative ionization mode analyses are typically required, increasing total run time. Although polarity switching can reduce analysis time, switching delays increase cycle time and reduce data points across chromatographic peaks. The Thermo Scientific™ Orbitrap™ Tribrid™ Apex mass spectrometer improves ion accumulation, synchronization and transfer efficiency, delivering a 40% increase in duty cycle. This enables a rapid untargeted workflow that acquires both positive and negative ionization mode data within a single short analytical run.

Materials and methods

Sample preparation

Six flavored tea bags; Black Tea, Earl Grey, Green Tea, Chamomile, Lemon Ginger (Twinings™), and Hibiscus (Care™) were steeped for 1 minute, cooled, centrifuged at 15,000xg for 15 min at 4˚ C, filtered through a 0.4 µm filter, and stored at -20 ˚ C prior to analysis.

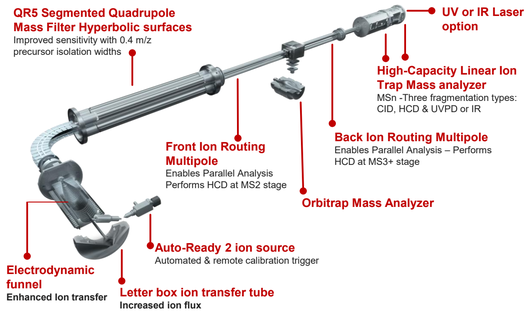
Test method(s)

Untargeted polarity-switching LC-MS experiments were performed on Orbitrap Tribrid Apex and Orbitrap IQ-X Tribrid mass spectrometers coupled with a Vanquish™ Horizon LC system. Tea samples were analyzed using Hypersil GOLD™ C18 150 mm and 50 mm columns with 5- and 15-minute gradients at 60K resolution in positive, negative, and polarity-switching modes, including pooled QC samples. Water and methanol containing 0.1% formic acid were used as mobile phases. Thermo Scientific AcquireX™ intelligent iterative MS² acquisition was applied to pooled tea samples for enhanced compound identification and characterization.

Data analysis

Polarity-switching data acquired on Orbitrap Tribrid mass spectrometers were evaluated in Thermo Scientific™ FreeStyle™ 1.8 software to assess scan rate performance. Thermo Scientific™ Compound Discoverer™ software was used for compound identification, annotation, and principal component analysis.

Figure 1. Ion Path of Orbitrap Tribrid Apex Mass Spectrometer



Orbitrap Tribrid Apex system is a next-generation Tribrid mass spectrometer designed for faster acquisition, higher sensitivity, and flexible MSⁿ workflows. The system integrates three mass analyzers
1. a quadrupole for precursor isolation, 2. high-resolution Orbitrap for high mass accuracy, and 3. a sensitive linear ion trap for trapping ions and alternate fragmentation, along with dual ion routing multipoles [front IRM (FIRM) and back IRM] for efficient ion storage, high collision fragmentation and ion transfer

Efficient parallelization of ion injection, accumulation, isolation, fragmentation, and detection enable continuous high-speed data acquisition with excellent mass accuracy and sensitivity. The combination of Orbitrap and ion trap analyzers with multiple activation options provides enhanced flexibility for advanced metabolomics and complex sample characterization.

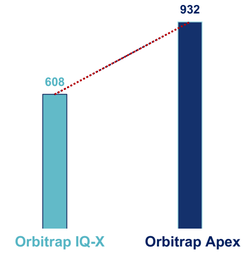
Results

Polarity switching

The Orbitrap Tribrid Apex mass spectrometer demonstrated substantially higher scan rates compared to the Orbitrap IQ-X. At 60K resolution, the Apex Tribrid system achieved significantly faster MS¹ acquisition rates in polarity-switching experiments.

During a 5-minute polarity-switching LC-MS run, the Orbitrap Tribrid Apex acquired more than 300 additional scans—approximately a 50% increase over the Orbitrap IQ-X system under identical conditions-highlighting its enhanced acquisition speed and improved duty cycle.

Figure 2. Number of MS1 scans- Polarity Switching - 60K resolution - 5-minute LCMS run



Short gradient vs. Long gradient

Untargeted metabolomics requires broad metabolome coverage with sufficient data points across chromatographic peaks for confident compound identification. This depends on both high scan speed and effective chromatographic separation.
The enhanced scan speed of the Orbitrap Tribrid Apex system was evaluated in a 5-minute polarity-switching LC-MS run at 60K resolution and compared with long-gradient data acquired on the Orbitrap IQ-X Tribrid under similar conditions. Raw data were assessed for peak sampling quality and consistent analyte detection.

Figure 3a-3b. Comparison of number of scan points collected – Orbitrap IQ-X - 15-minute LC-MS method; Orbitrap Apex – 5-minute LC-MS method - Positive ionization mode - 60K resolution

Fig 3a. Orbitrap IQ-X – 15-minute gradient

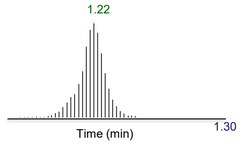
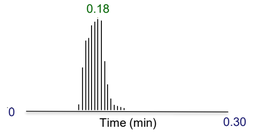


Fig 3b. Orbitrap Apex – 5-minute gradient



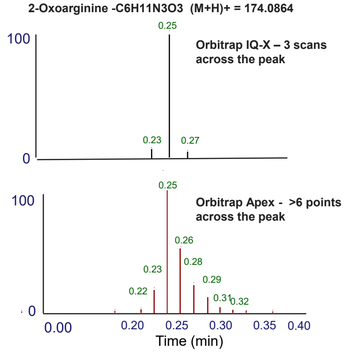
L-Threonine ($C_4H_9NO_3$; $[M+H]^+$ = 120.0655) demonstrates the improved scan rate performance of the Orbitrap Apex, with more than nine data points collected across the peak in a 5-minute LC-MS run with polarity switching—comparable to the number of scans obtained using a 15-minute gradient on the Orbitrap IQ-X.

Number of data points

The enhanced scan speed of the Orbitrap Tribrid Apex system is demonstrated further by the increased number of data points acquired across chromatographic peaks. Reliable quantitation of unknown compounds typically requires at least six data points per peak.

In a 5-minute polarity-switching run, the Orbitrap Tribrid Apex system has achieved >6 data points per peak, while the Orbitrap IQ-X Tribrid acquired only 3 under the same conditions. Similar trends were consistently observed across multiple compounds.

Figure 4. Comparison of number of scan points collected in 5-minute LC-MS method - Polarity Switching -60K resolution



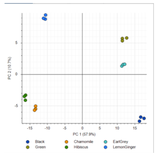
Sensitivity

The letterbox ion transfer tube enables a higher influx of ions, dramatically reducing ion injection time and enhancing sensitivity on the Orbitrap Apex system. Compound Discoverer analysis of the 5-minute gradient on the Orbitrap Tribrid Apex system annotated 60% of compounds detected with the long-gradient Orbitrap IQ-X Tribrid workflow, despite a 66% reduction in gradient time, highlighting enhanced sensitivity and scan speed. PCA of 5-minute gradient data successfully clustered compounds by similarity across six tea flavors.

Table 1. Number of Compounds annotated- 60K resolution at 5- & 15-minute gradients

	Orbitrap IQ-X	Orbitrap Apex
5-minute	223	431
15-minute	537	784

Figure 5. PCA analysis of Pooled Tea sample - 60K 5-minute gradient



Conclusions

Orbitrap Tribrid Apex system demonstrated higher scan rate, acquired enough data points for quantification under polarity switching and annotated 60% of compounds despite 66% reduction in run time. PCA analysis grouped the compounds from tea flavors successfully.

Acknowledgements

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Trademarks/licensing

Conflict of Interest: The authors BB, SY, RD, BA, SB, and RM are employees of Thermo Fisher Scientific whose instrumentation and software were used to acquire and process the data PO004841-2026-EN