

## High-throughput bacterial profiling via MALDI spot analysis: Precise characterization of antibiotic-induced metabolic responses

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### Short Abstract for online program

Spot-based MALDI workflows are gaining traction in metabolomics for their speed and ease of automation. Our streamlined, fast, and selective MALDI spot workflow for metabolomics uses the timsTOF fleX platform with Trapped Ion Mobility Spectrometry (TIMS)-enabled separation to generate metabolic fingerprints. Designed for high-throughput screening, the method enables rapid profiling of bacterial samples treated with various antibiotics in a 96-well format. Background subtraction using the cultivation medium removed matrix-related features, while MetaboScape 2026 provided a straightforward, non-targeted processing workflow combined with powerful statistical insights. Results demonstrate reproducible fingerprints and clear clustering of treatment groups, suggesting antibiotic-specific metabolic responses. The workflow combines speed, selectivity, and robust data analysis — ideal for biomarker discovery and antibiotic response profiling.

### Introduction

MALDI-MS is widely used for rapid, sensitive screening of biomolecules and small compounds. In this context, MALDI spot analysis complements MALDI Imaging by enabling high-throughput profiling with minimal sample preparation and ease of automation. The timsTOF fleX platform integrates MALDI with Trapped Ion Mobility Spectrometry (TIMS) and accurate-mass analysis, enhancing selectivity and peak capacity for complex samples. This study presents a streamlined MALDI spot workflow for detecting antibiotic-specific metabolic responses in bacterial samples, using MetaboScape 2026 for efficient and intuitive data interpretation.

### Methods

*E. coli* was cultured in Mueller-Hinton medium (four biological replicates) and exposed to various antibiotics in a 96-well plate using an Echo liquid handler. After cultivation and extraction, supernatants were collected, dried, and reconstituted in H<sub>2</sub>O. Diluted samples (1:10) were spotted on an AnchorChip™ MALDI plate using the dried-droplet method with HCCA matrix solution (85% ACN, 0.1% TFA, 1 mM ammonium phosphate). MALDI-TIMS-MS analysis was performed in positive ionization mode on a timsTOF fleX with automated acquisition at 10 kHz. Data were processed in MetaboScape 2026 using the new “MALDI spot” workflow covering feature finding, normalization, and statistical analysis.

### Results

MALDI-TIMS-MS fingerprints of 96 bacterial samples treated with various antibiotics at various concentrations can be acquired at high speed (<10 s per spot) and reproducibility, which is essential for high-throughput metabolomics. Furthermore, software-guided MALDI background removal based on features present in the cultivation medium improved specificity, enabling confident detection of sample-related metabolites and metabolomic responses. Multivariate data analysis via Principal Component Analysis (PCA) revealed distinct clustering of samples representing Chloramphenicol and Penicillin G treatments, while Trimethoprim-treated samples clustered with controls, indicating less pronounced metabolic disturbance in the Trimethoprim group (consistent with *E. coli*'s known resistance to this treatment). Additionally, series plots confirmed concentration-dependent trends for antibiotic-specific features, demonstrating the high confidence and specificity of the developed MALDI spot analysis workflow.

### Conclusion

The timsTOF flex platform enables rapid, high-throughput MALDI-TIMS-MS acquisition with TIMS-enhanced selectivity for efficient and confident bacterial profiling. Combined with MetaboScape 2026, this workflow delivers robust data processing and intuitive interpretation of antibiotic-induced metabolic responses. This fingerprinting method provides the basis for further semi-quantitative analysis and detailed structural identification of statistically relevant features.