

High-throughput bacterial profiling via MALDI spot analysis utilizing timsTOF fleX and MetaboScape 2026

Application of a spot-based MALDI-TIMS-MS workflow for precise characterization of antibiotic-induced metabolic responses in complex biological samples.

Abstract

Spot-based MALDI workflows are gaining traction in metabolomics for their speed and ease of automation. This Technical Note introduces a streamlined, fast, and selective MALDI spot workflow for metabolomics using the timsTOF fleX platform with Trapped Ion Mobility Spectrometry (TIMS). 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 improved data quality, while MetaboScape® 2026 provided a straightforward processing workflow combined with powerful statistical insights. Results demonstrate reproducible metabolite detection and clear clustering of treatment groups, confirming antibiotic-specific metabolic responses. The workflow combines speed, selectivity, and robust data analysis—ideal for biomarker discovery and antibiotic response profiling.

Keywords:
MALDI spot, Metabolomics,
TIMS, MetaboScape,
timsTOF fleX

Introduction

Matrix-Assisted Laser Desorption/Ionization (MALDI)-MS is a well-established technique in analytical laboratories due to its speed, sensitivity, and versatility in analyzing biomolecules and small compounds [1-3]. As the demand for high-throughput workflows continues to grow, rapid MALDI spot analysis has emerged as a complementary approach to spatially resolved MALDI Imaging [4]. Its efficient screening and profiling capabilities—combined with ease of automation and minimal sample preparation—make it ideally suited for high-throughput analysis [4-5].

In MALDI-based metabolomics, specificity is just as critical as speed and sensitivity. The timsTOF fleX platform, integrating MALDI with TIMS and Time-of-Flight (TOF) accurate-mass analysis, offers a powerful solution that combines rapid acquisition with TIMS-enhanced selectivity. The improved peak capacity of this platform enables efficient screening of complex biological samples with minimal preparation, supporting both untargeted and semi-targeted metabolomics workflows. Additionally, the dual ion source enables switching between MALDI spot analysis and LC-MS workflows, making the timsTOF fleX a versatile instrument for multimodal applications on a single device.

This Technical Note presents a streamlined MALDI spot analysis workflow that significantly reduces analysis time while maintaining high data quality and reproducibility. By leveraging acquisition speed, automation, and TIMS-enhanced selectivity, fast bacterial profiling was achieved. For straightforward data interpretation, the new MALDI spot processing tool in MetaboScape 2026 was utilized. This workflow is particularly suited for applications such as biomarker discovery and metabolic fingerprinting, where throughput, specificity, and reproducibility are essential.

Methods

Sample preparation

A 96-well plate containing bacterial extracts from *Escherichia coli* (*E. coli*), treated with various types and concentrations of antibiotics, was analyzed using MALDI-TIMS-MS. To enable accurate background subtraction, the cultivation medium (Mueller Hinton) was prepared as well and analyzed as a reference.

For sample preparation, *E. coli* was grown in Mueller–Hinton medium in four biological replicates. Each exponentially growing replicate was then transferred to one quarter of a plate containing one untreated control and three antibiotic-treated conditions. These conditions were randomly distributed within each quarter of the plate using an Echo liquid handler. Following cultivation, the samples were centrifuged to remove the supernatant, and metabolite extraction was performed using hot water. The optical density (OD) of each sample was normalized to ensure consistency across replicates. The plate was then subjected to a second centrifugation to discard the bacterial pellets. Finally, 15 μL aliquots were transferred to a new plate, where the samples were dried and reconstituted in 15 μL of H_2O .

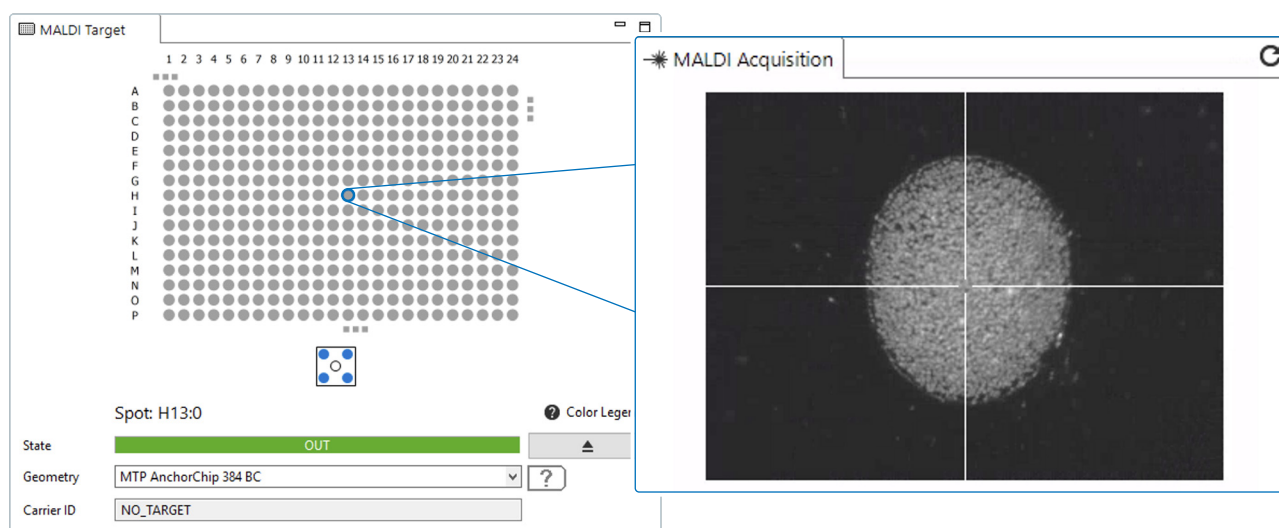


Figure 1. Scheme of the AnchorChip™ (800 μm) MALDI target plate used for bacterial profiling in timsControl acquisition software. One spot is visualized more closely using the camera of the timsTOF fleX, showing the homogeneous distribution of the MALDI matrix α -cyano-4-hydroxycinnamic acid (HCCA).

MALDI spot analysis

For MALDI spot analysis, a 1:10 dilution in H₂O (v/v) was prepared. The dried droplet technique was used to spot each sample onto an AnchorChip (800 μm) MALDI target plate (Bruker). Its unique anchor design traps the droplet in the exact center of each spot, ensuring consistent positioning and optimal laser targeting.

First, 0.5 μL of the diluted extract was applied to the target plate. After drying, 0.5 μL of MALDI matrix solution was added to each spot. The spots were left to dry thoroughly prior to the analysis. The matrix solution used for metabolomics profiling in positive ionization mode contained α-cyano-4-hydroxycinnamic acid (HCCA), supplemented with trifluoroacetic acid (TFA) and ammonium phosphate additives. In detail, 1.4 mg/mL HCCA was dissolved in a solvent mixture containing 85% ACN, 15% H₂O, 0.1% TFA, and 1 mM ammonium phosphate. Note that ammonium phosphate acts as an ion exchanger, which ultimately suppresses the formation of potassium and sodium adducts. However, usually still adducts are observed in MALDI spot analysis.

Table 1. MALDI spot acquisition parameters

MS		timsTOF fleX
Source	MALDI	
Ionization	Positive ion mode	
Matrix	HCCA with TFA and ammonium phosphate additives	
Acquisition mode	MALDI-TIMS-MS	
	Ramp time	500 ms
	Mobility range	0.45 – 1.45 1/K ₀
Laser settings	10 kHz smartbeam 3D laser, random walk	
	Application profile	MS Dried droplet
	MALDI-MS	4000 laser shots -> 0.4 s per sample spot
	MALDI-TIMS-MS	20 laser bursts, 400 shots each (1 burst per TIMS frame) -> 10 s per sample spot
Calibration	<i>m/z</i> : red phosphorus in MALDI mode TIMS: Agilent Tunemix in ESI mode	

MALDI spot analysis was performed using a timsTOF fleX mass spectrometer equipped with a smartbeam 3D laser. The frequency was set to 10 kHz during the automated run. The acquisition parameters, including ramp time, mobility range, and laser shot settings, are summarized in Table 1.

Data analysis

MetaboScape 2026 introduces a new software workflow for processing of MALDI(-TIMS) spot data. The T-ReX[®] workflow includes deisotoping, grouping of consistent features across spots, and ion deconvolution. The resulting feature tables were evaluated using multiple MetaboScape tools (Figure 2) such as blank subtraction.

MALDI spectra acquired from blank cultivation medium served as the background reference, and features with intensities ≤10 were excluded from statistics. Furthermore, feature tables were normalized using probabilistic quotient normalization [6] followed by principal component analysis (PCA) to identify treatment-specific clustering and metabolic responses.

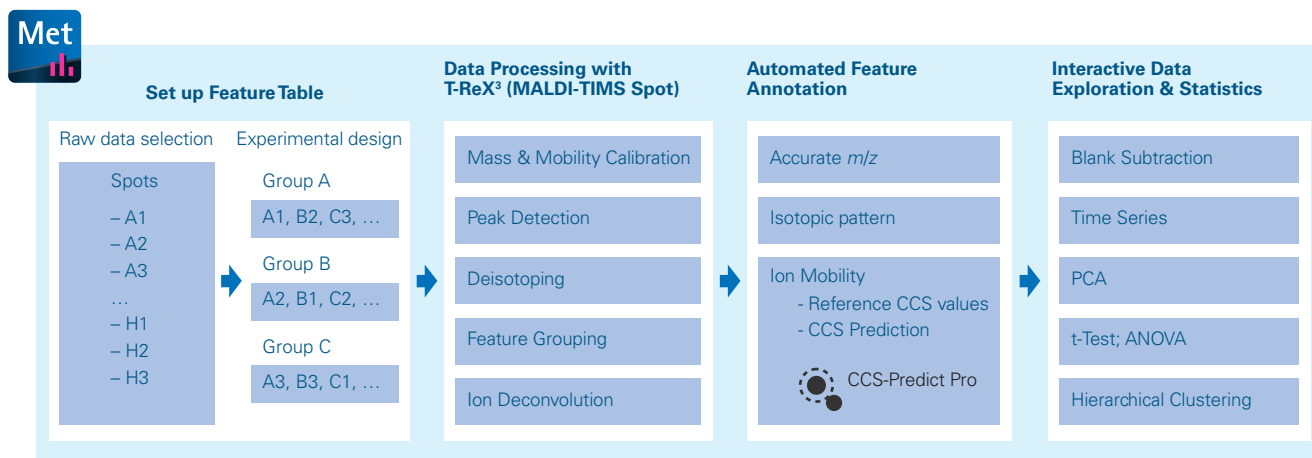


Figure 2. MALDI-TIMS Spot analysis data processing in MetaboScape 2026.

In MetaboScape 2026, a new comprehensive T-ReX workflow for MALDI Spot and MALDI-TIMS Spot analysis data was introduced providing a fully integrated pipeline from setting up feature tables to interactive data exploration and statistics.

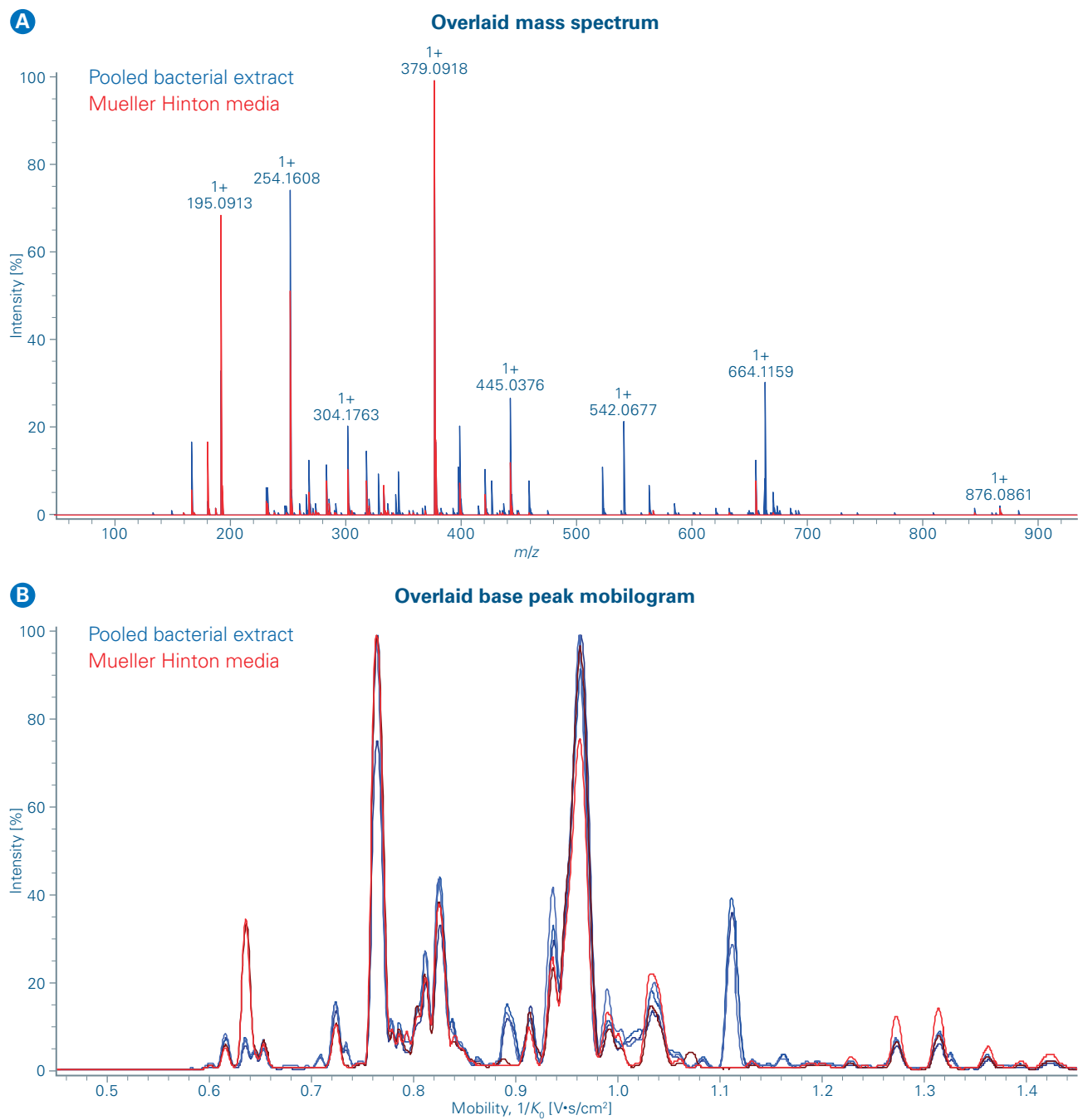


Figure 3. MALDI-(TIMS-)MS profile obtained from a pooled bacterial extract sample.

Overlaid mass spectra **(A)** and base peak mobilograms **(B)** of pooled bacterial extract (blue) and Mueller Hinton medium (red) using MALDI-TIMS-MS. Using the Mueller Hinton medium as MALDI background spectrum, multiple mass signals and mobility peaks specific to the bacterial samples were revealed.

Results

MALDI-(TIMS)-IMS Spot analysis for fast and efficient bacterial profiling

Figure 3 provides a comparison of MALDI-TIMS-MS profiles acquired from a pooled bacterial extract sample and a cultivation medium sample, respectively. The latter is being used as a blank for background removal. Software-guided background removal performed in Mataboscape 2026 enhanced the discriminative power and level of confidence of MALDI data significantly resulting in robust and sensitive detection of treatment-specific response marker metabolites.

High-throughput MALDI spot analysis results are shown in Figure 4 in the shape of overlaid base peak mobilograms. A total of 96 samples, including *E. coli* extracts treated with various antibiotics at various concentrations, were analyzed using MALDI-TIMS-MS. Data acquisition was performed as a fully automated sequence at an acquisition speed of 10 s per sample spot.

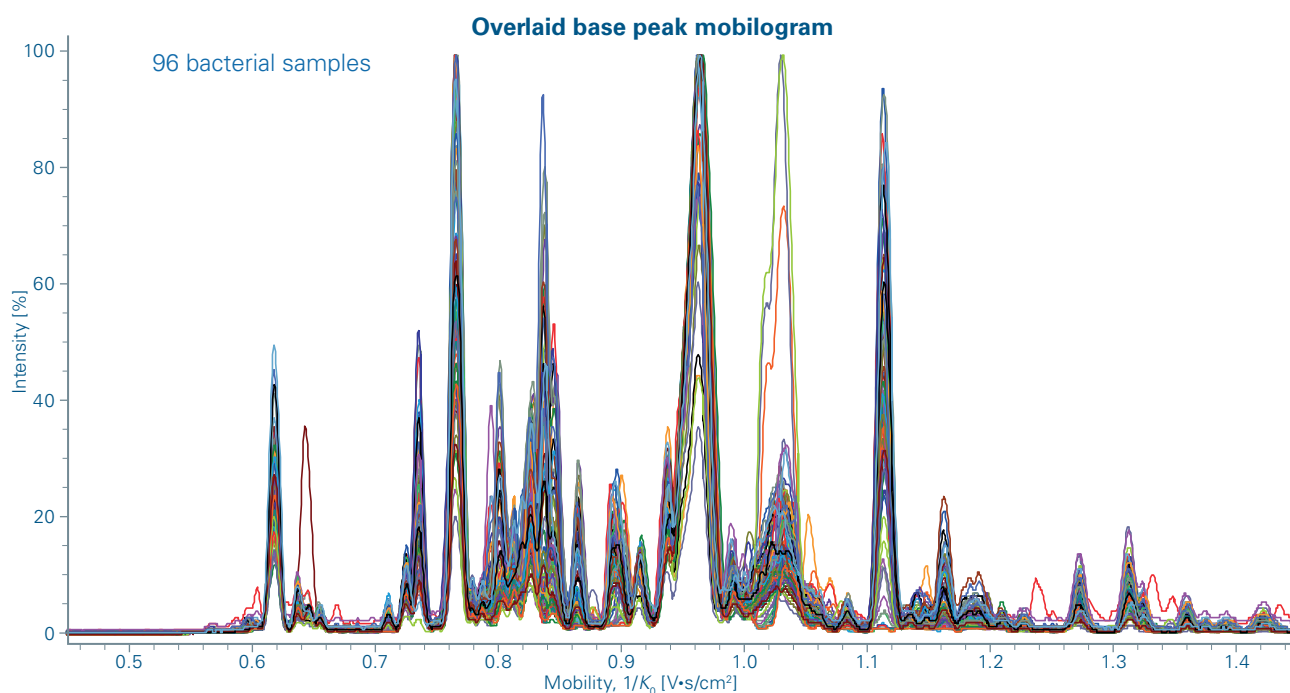


Figure 4. Fast generation of metabolic fingerprints.

Overlaid base peak mobilograms of 96 bacterial samples (color-coded) analyzed by MALDI-TIMS-MS show reproducible mobility values, enabling straightforward metabolic fingerprint characterization.

The MALDI-TIMS-MS results (Figure 5) demonstrate high reproducibility, a critically important requirement for reliably detecting metabolic changes following antibiotic treatment.

Advanced MetaboScape workflow for MALDI spot data analysis

MetaboScape 2026 introduces a new software workflow tailored for MALDI spot analysis. The workflow covers multiple steps such as feature finding, normalization and statistical analysis using Principal Component Analysis (PCA).

Figure 7 presents the MetaboScape results obtained from analysis of MALDI-TIMS-MS profiles acquired from the set of 96 bacterial extract samples. PCA analysis revealed distinct clustering of samples representing Chloramphenicol (CLO, light green) and Penicillin G (PENG, violet) treatments. In contrast, Trimethoprim (TMP, blue) samples clustered near the untreated control (red), showing limited TMP-induced metabolic response after treatment.

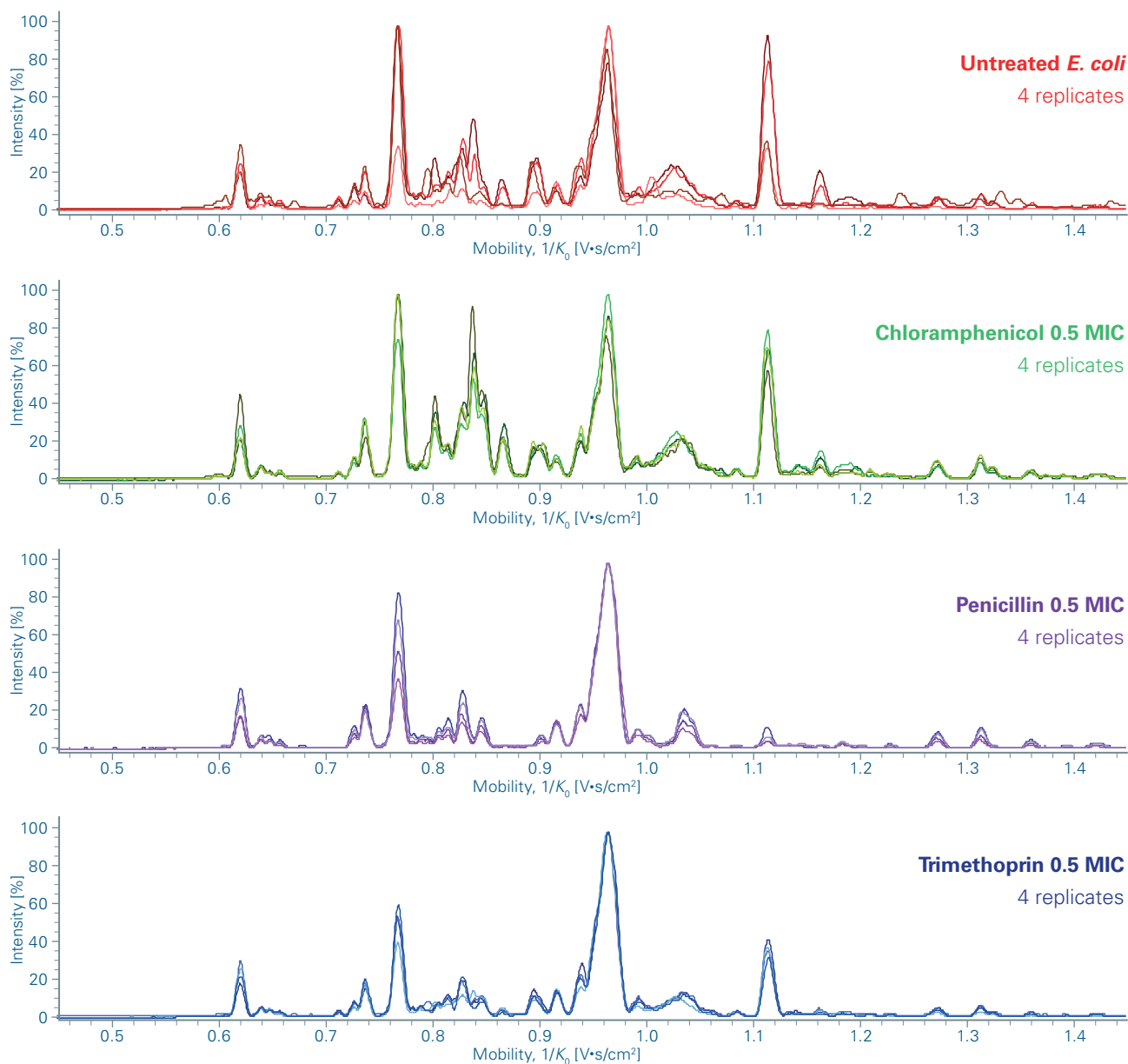


Figure 5. Demonstration of reproducibility achieved in MALDI-TIMS-MS analysis of bacterial extract samples.

Overlaid base peak mobilogram of biological replicates ($n=4$) for untreated (red), Chloramphenicol (green), Penicillin G (violet) and Trimethoprim-treated (blue) *E. coli* samples analyzed by MALDI-TIMS-MS demonstrate high reproducibility, a cornerstone of high-quality metabolomics.

To further prove the sample related specificity of features resulting from statistical analysis, the series plot tool was used. *E. coli* samples were treated with increasing antibiotic concentrations based on minimum inhibitory concentration (MIC) values. The series plot tracks feature intensity trends across concentrations. Sample-specific features show consistent upward or downward trends, reflecting metabolic changes. For example, one feature (Figure 8) shows a downward trend with increasing PENG concentration, sustaining its specificity and indicating the high level of confidence provided by the MALDI spot analysis results.

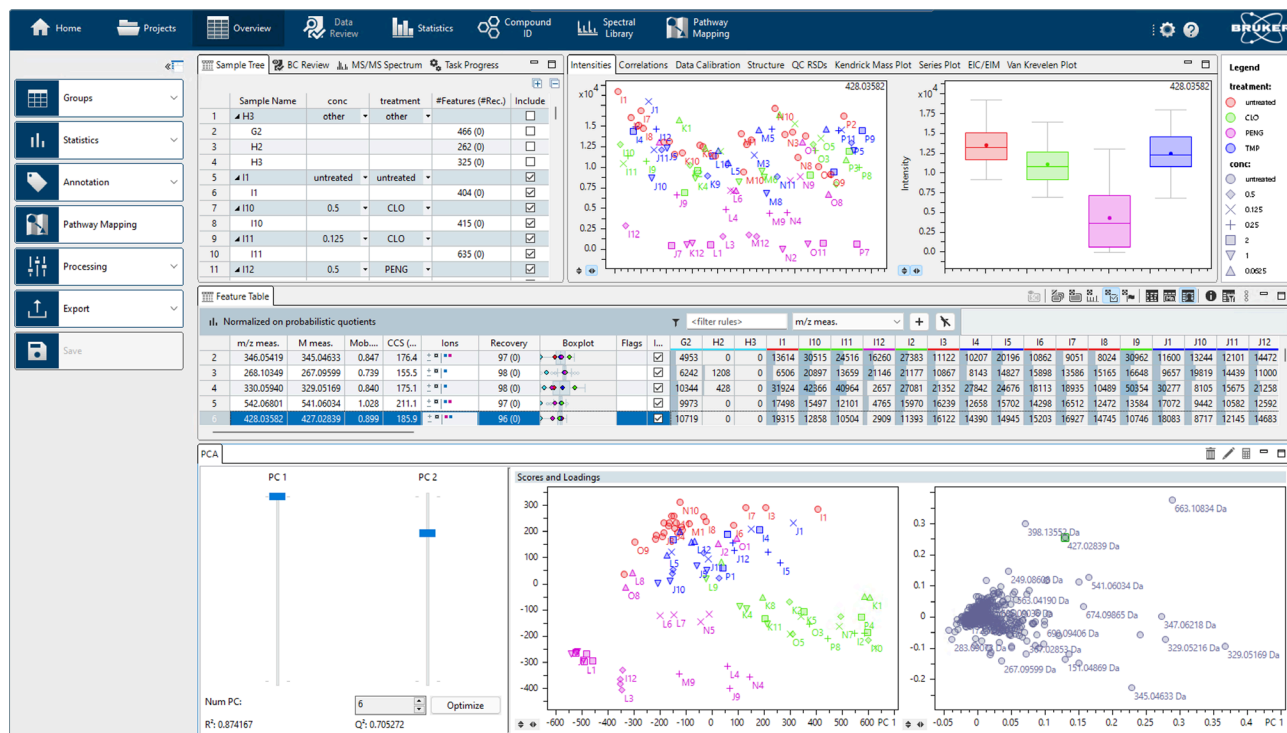


Figure 6. Instant insights in antibiotic-specific responses provided by fast MALDI-TIMS-MS profiling of bacterial extracts. Screenshot from MetaboScape 2026 shows clear clustering of PENG (violet) and CLO (light green) treatment groups and no clustering after TMP treatment (blue), confirming antibiotic-specific metabolic responses.

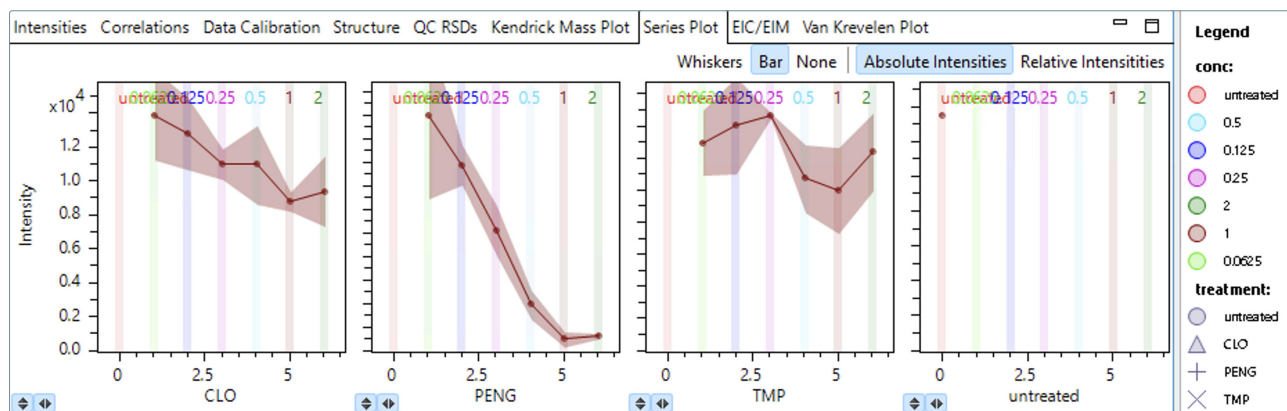


Figure 7. Series plot for trend confirmation. Screenshot from MetaboScape 2026's series plot tool highlights metabolic response trends across antibiotic concentration gradients, confirming specificity of MALDI-TIMS-MS spot profiling data.

Conclusions

- **Fast and efficient profiling:** MALDI-TIMS-MS workflow using timsTOF fleX enabled acquisition times <10 s per sample spot.
- **TIMS-enhanced selectivity:** The TIMS dimension improved peak capacity and selectivity, allowing confident identification of sample-specific metabolites in complex samples.
- **Smart background subtraction:** Software-guided background removal function in MetaboScape 2026 eliminates non-specific, matrix-related features, thus enhancing the discrimination power of MALDI-TIMS-MS data.
- **Tailored T-ReX workflow in MetaboScape 2026:** New MALDI spot analysis workflow allowed for fast and intuitive high-throughput data analysis to reveal biological effects such as antibiotic-specific metabolic responses.

References

- [1] Djambazova KV, Klein DR, Migas LG, Neumann EK, Rivera ES, Rempel D, Caprioli RM, Spraggins JM (2020). *Resolving the Complexity of Spatial Lipidomics Using MALDI TIMS Imaging*. Anal. Chem. **92** (20), 13290–13297, DOI: 10.1021/acs.analchem.0c02288
- [2] Heuckeroth S, Behrens A, Wolf C, Fütterer A, Nordhorn ID, Kronenberg K, Brungs C, Korf A, Richter H, Jeibmann A, Karst U, Schmid R (2023). *On-Tissue Dataset-Dependent MALDI-TIMS-MS2 Bioimaging*. Nat. Commun. **14**, 1–9, DOI: 10.1038/s41467-023-43298-9
- [3] Gruber L, Schmidt S, Enzlein T, Vo HG, Bausbacher T, Cairns JL, Ucal Y, Keller F, Kerndl M, Abu Sammour D, Sharif O, Schabbauer G, Rudolf R, Eckhardt M, Iakab SA, Bindila L, Hopf C (2025). *Deep MALDI-MS Spatial Omics Guided by Quantum Cascade Laser Mid-Infrared Imaging Microscopy*. Nat. Commun. **16**, 59839, DOI: 10.1038/s41467-025-59839-3
- [4] Liang T, Leung LM, Opene B, Fondrie WE, Lee YI, Chandler CE, Yoon SH, Doi Y, Ernst RK, Goodlett DR (2019). *Rapid Microbial Identification and Antibiotic Resistance Detection by Mass Spectrometric Analysis of Membrane Lipids*. Anal. Chem. **91** (2), 1286–1294, DOI: 10.1021/acs.analchem.8b02611
- [5] Rudt E, Froning M, Heuckeroth S, Ortmann L, Diemand J, Hornschemeyer L, Pleger A, Vinzelberg M, Schmid R, Pluskal T (2025). *Rapid MALDI-MS/MS-Based Profiling of Lipid A Species from Gram-Negative Bacteria Utilizing Trapped Ion Mobility Spectrometry and m/zmine*. Anal. Chem. **97**, 7781–7788, DOI: 10.1021/acs.analchem.4c05989
- [6] Dieterle F, Ross A, Schlotterbeck G, Senn H (2006). *Probabilistic Quotient Normalization as Robust Method to Account for Dilution of Complex Biological Mixtures. Application in ¹H NMR Metabonomics*. Anal. Chem. **78** (13), 4281–4290. DOI: 10.1021/ac051632c

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