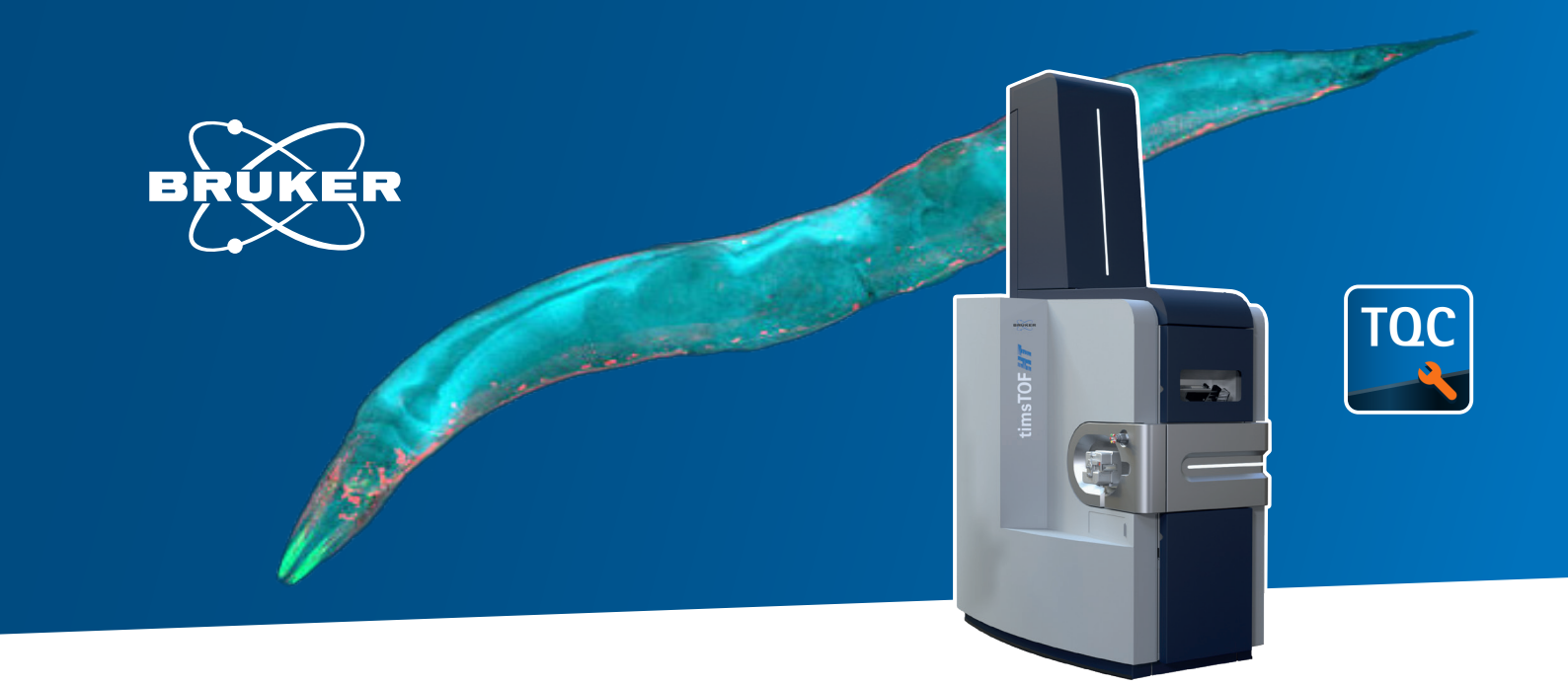

Metabolomics data quality tracking assures precision measurement and reveals trends across replicated experiments

Benefit: No time to waste! Easily determine if data is suited for further untargeted metabolomics analysis.

Keywords:
TASQ RealTimeQC,
precision measurement,
TIMS

Challenge

Assess the quality of LC-MS measurements for untargeted metabolomics experiments before detailed statistical evaluation and data annotation.

Solution

TASQ RealTimeQC module enables to readily track e.g., retention time drift, mass drift, CCS drift and further quality factors for selected QC analytes.

Background

Quality control (QC) of LC-MS measurements is crucial for both targeted and untargeted metabolomics experiments, as reliable measurements are the basis for further analysis. Generation of a reliable data matrix with mainly unknown features is only possible when drifts in e.g. retention time and mass accuracy can be tracked and stay within a certain confidence range. Only with a set of reliable measurements it is possible to start evaluating unknown changes in untargeted metabolomics.

Introduction

C. elegans is an excellent model organism to study the interaction of diet and aging because of its short life cycle and ease of handling for multiomic analysis [1, 2]. Several genetic mutations can extend lifespan some of which are linked to dietary restriction or fasting [3, 4]. However, the underlying mechanisms are not fully understood. One aim of this study is to unravel new metabolic pathways linking dietary regimes to increased lifespan using untargeted metabolomics.

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Methods

C. elegans stratified across fifteen genetic, age, and feeding condition combinations were analyzed to investigate the dynamic change of metabolism during fasting and refeeding:

- Wild type and mutant strains
- Ages range from 2 days to 35 days old
- Dietary regimes include ad libitum fed; fasted; re-fed

Samples from this fifteen-condition study design were analysed sequentially in five biological replicates, with QC measurements before and after each biological replicate. A 10 min reversed-phase gradient using water and acetonitrile with formic acid as buffer system was applied to separate metabolite extracts. LC-PASEF MS/MS data was acquired on a timsTOF Pro 2 in positive ionization mode. Several "QC analyte" metabolites chosen from an in-house database were selected to assess quality of LC-MS measurements and track experiment status. The acquired data was processed using TASQ 2023b and evaluated using TASQ RealTimeQC 2023b.

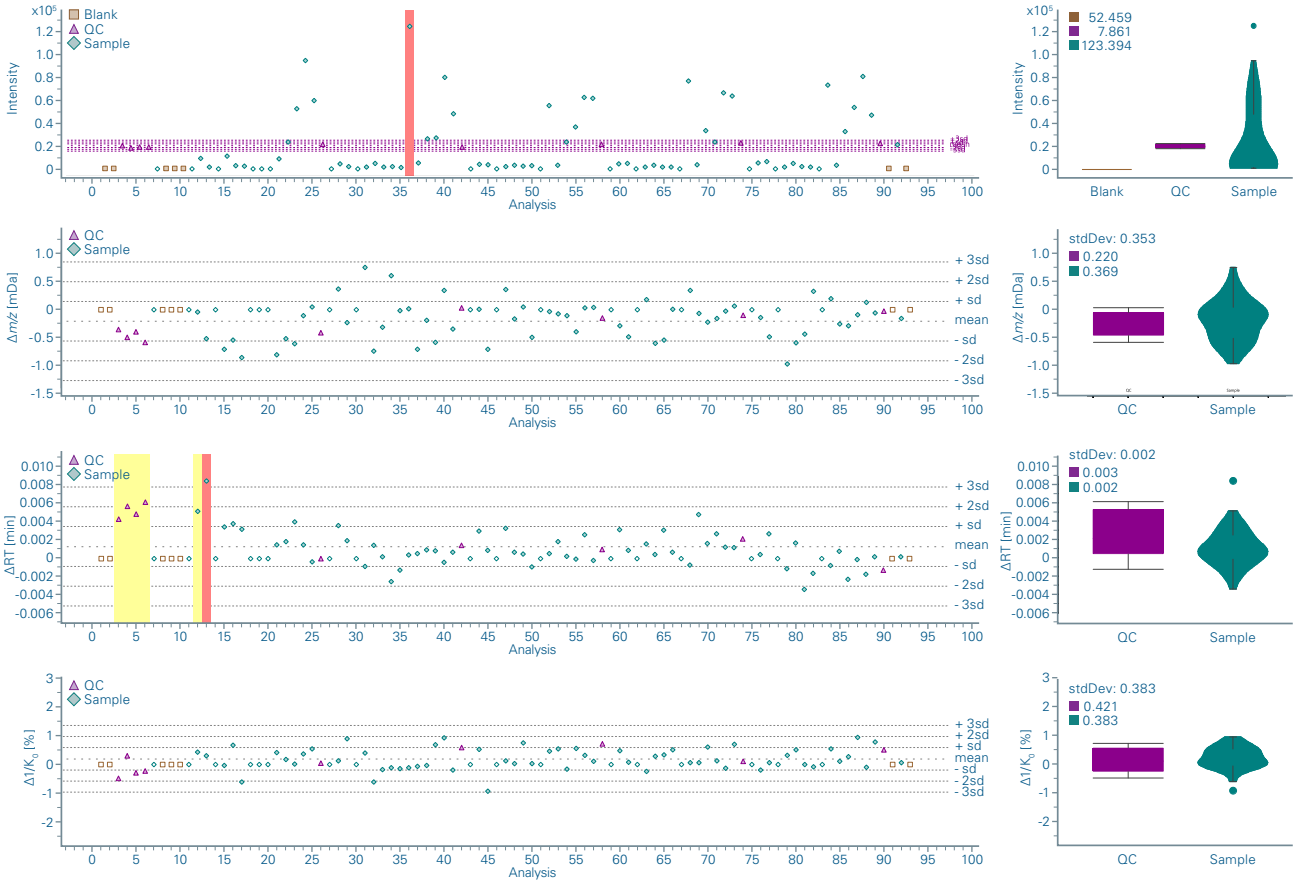

Figure 1

Experiment-wide TASQ RealTimeQC plots of LC and MS quality markers for the QC analyte myristoylcarnitine. Outliers are automatically highlighted by red or yellow bars.

Results

Figure 1 highlights the QC analyte myristoylcarnitine investigated along all samples of the experiment. Myristoylcarnitine was chosen due to its expected presence in most samples, making it an effective marker for assessing stability of system performance. The investigation encompassed changes in peak intensity, mass drift, retention time drift, and mobility drift (shown top to bottom). The scatter plots (left) display individual data points, while the violin plots (right) summarize the measurement distributions and provide a comprehensive overview of the entire experiment that facilitates easy evaluation of data quality. Outliers are automatically highlighted by red or yellow bars. An outlying measurement in the intensity plot was further scrutinized by analyzing several additional QC analytes, as detailed in Figure 2.

The low variability across measurements of a pooled quality control (QC) sample, represented by purple triangles in the scatter plots, indicate the analytical precision achieved (intensity RSD = 7.8%).

Figure 2 presents Peak Intensity plots of two amino acid and two carnitine QC analytes. Interestingly, the observed intensity patterns of these were found to correlate with the experimental design, coded in the sample analysis run order, exhibiting a recurring pattern across the five consecutive biological replicates. Within each biological replicate, the first nine samples are "young" worms, aged two days, followed by "older" worms aged 10, 21, and 35 days. This observation already indicates a clear age-related effect and points towards potential interest in further downstream data analysis.

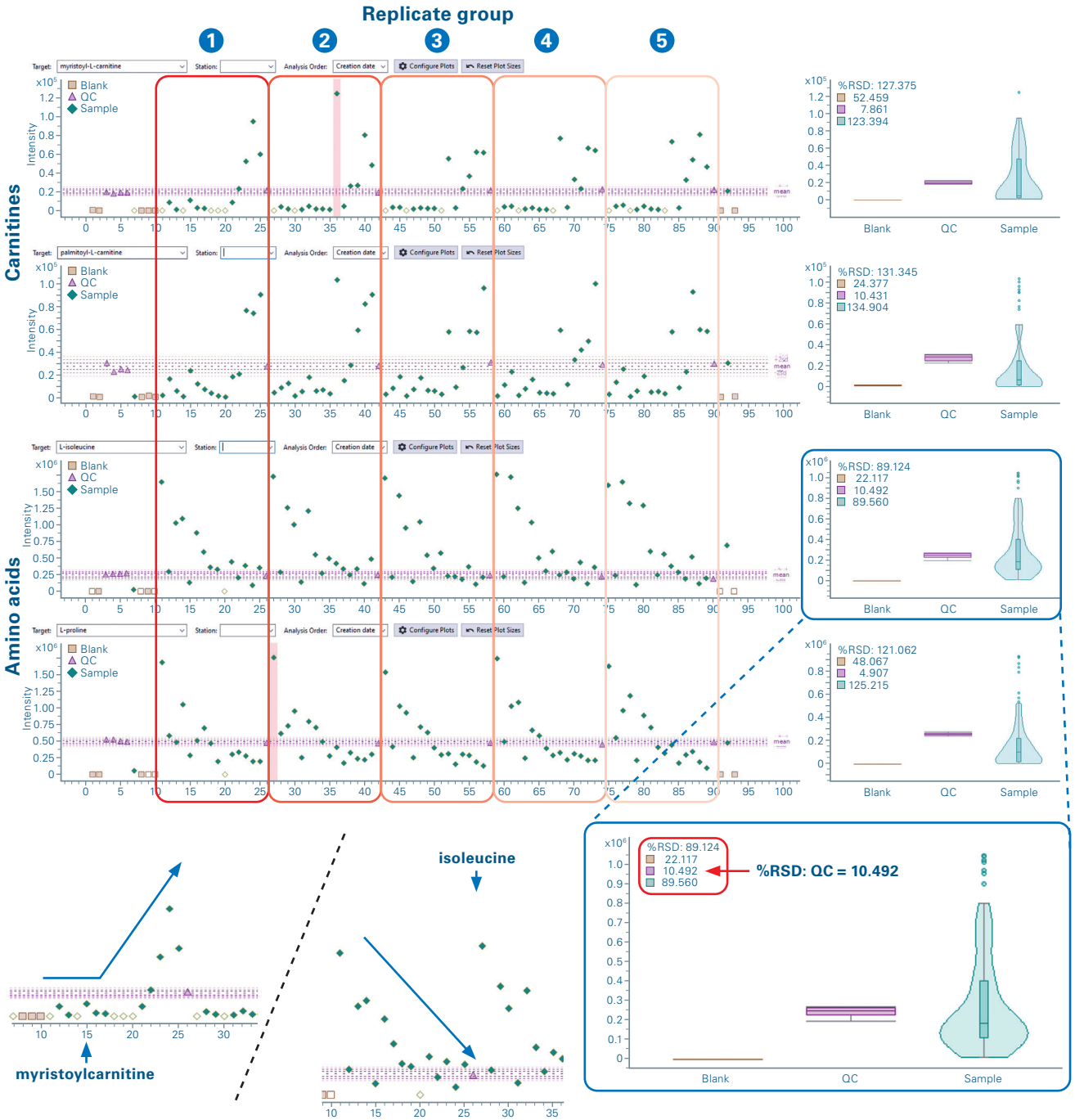

Figure 2

Intensity plot of selected amino acids and carnitines in TASQ RealTimeQC.
Red bars in the plots highlight automatically flagged outliers.

Conclusion

In summary, evaluation of the QC Analytes (Figures 1 and 2), coupled with the observation that peak intensities for several characteristic metabolites align with the experimental design (Figure 2), give confidence that the data emerging is of high technical quality and contains meaningful study-design related trends in metabolite measurements. This is substantiated by a low relative standard deviations measured across technical QC sample replicates (4.9% - 10.5%), notably lower than the %RSD of the study samples. These findings provide a robust basis for subsequent non-targeted data exploration in MetaboScape.

Dr. Frederik Dethloff

Scientist Metabolomics Core Facility of the Max Planck Institute for Biology of Ageing, Cologne, Germany, as he summarizes:

"With the help of TASQ RealTime QC we are now able to access the quality of our measurement within a very short time and determine if the data is suited for further untargeted Metabolomics analyses."

TASQ RealTimeQC: Your guide to high quality data acquisition (youtube.com)

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