ASMS 2024 MP537

# A system suitability scheme for assessment of longitudinal LC-TIMS-MS performance to promote reproducibility in *OMICS* sciences

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## Introduction

Data reproducibility is a cornerstone of all scientific areas, yet it is challenging to implement in untargeted "omics" mass spectrometry. Typically, QC workflows are applied to check performance of analytical instrumentation prior to and during the experiment to ensure precise and accurate measurements. Standardization between instruments and labs is necessary to validate results used to assess biological outcomes.[1]

As mass spectrometry-based metabolomics and lipidomics are used in large sample cohorts from biobanks and epidemiological studies, there is a need to develop quality control metrics of system performance to ensure consistency of data over long periods of analysis time.

In this study, we present the development of a complete solution for automated system suitability testing on LC-TIMS-MS. The **QSee Performance Test** software (Figure 1.) is tailored to small molecules for large cohort 4D-Metabolomics and 4D-Lipidomics studies. Based on a mixture of synthetic reference standards, defined LC columns, and fixed standard methods, the software automatically processes and reports system suitability data for validation of longitudinal and inter-lab studies.

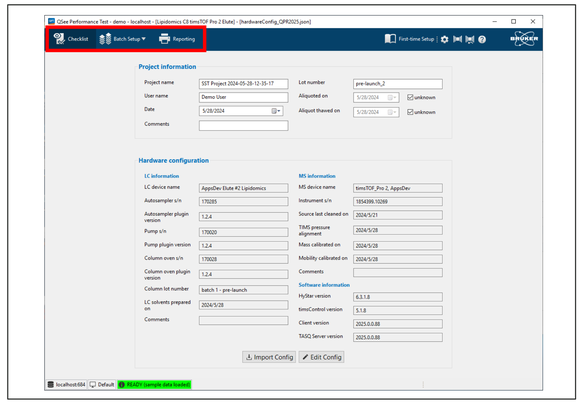


Fig 1. QSee Performance Test software with perspectives for Checklist (shown) to control the collection of metadata, Batch Setup for triggering data acquisition, and Reporting for review of QC results.

## Methods

A reference mixture was developed jointly with Polymer Factory (Sweden) consisting of 8 synthetic dendrimers in the mass range of 290 to 1200 m/z. Compounds were diluted to 5 µg/mL with LC-MS grade $H_2O$ for timsTOF HT (or 10 µg/mL for timsTOF Pro 2) and frozen in aliquots.

| Peak | Chemical Formula | Neutral Mass | Retention Time (min) | CCS (Å²) |
|---|---|---|---|---|
| 1 | C12H22O8 | 294.1315 | 1.10 | 162 |
| 2 | C25H44O16 | 600.2629 | 1.17 | 219.2 |
| 3 | C30H52O20 | 732.3052 | 1.17 | 244.4 |
| 4 | C40H70O25 | 950.4206 | 1.24 | 282.5 |
| 5 | C20H36O12 | 468.2207 | 1.26 | 198.1 |
| 6 | C50H84O30 | 1164.505 | 1.48 | 309.6 |
| 7 | C17H30O10 | 394.1839 | 1.53 | 182.7 |
| 8 | C37H62O22 | 858.3733 | 1.63 | 266 |

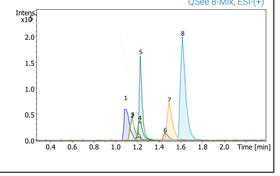


Fig 2. QSee mixture components (left) and extracted ion chromatograms in ESI positive mode (right) highlighting rapid separation for system suitability testing.

Gradients were developed for rapid qualification of LC-TIMS-MS systems in both polarities for Bruker Bio-LP (C8) and Bio-AQ (C18) 100 mm columns: 5–99% B in 2.6 minutes, total runtime 5 minutes, solvent A: $H_2O$+0.1% FA, solvent B: ACN+0.1% FA. Positive mode shown in Fig 2.

Scheduled precursor lists with optimized collision energies for prm-PASEF acquisition were developed for use with different MS and LC combinations using a VIP-HESI source used as interface (timsTOF Pro 2 or timsTOF HT, Agilent 1290 Infinity II or Bruker Elute UHPLC).

System suitability assessment was performed in a cross-lab study between Bremen (Apps Dev lab, Demo lab) and Tubingen using data from different hardware combinations and labs. The overall data reproducibility was investigated using TASQ 2024b processing methods. Parameters of interest were e.g. mass accuracy, retention time, CCS values, isotopic pattern, and LC peak shapes.

## Acknowledgments

We thank Christoph Trautwein and his research group at the University of Tübingen for testing the workflow and for valuable discussions and feedback.

We thank Elin Mignérus and Jens Sommertune from the Polymer Factory for support and joint developments.

## References

[1] M. Lewis *et al*., 2022, 10.26434/chemrxiv-2022-nq9k0

## Results

QSee Performance Test software was developed for use with the 8-component QSee mixture to monitor system performance over time. Testing the mixture between large cohort experiments ensures measurements are accurate and consistent. Figure 3 highlights reproducibility of dendrimer $C_{17}H_{30}O_{10}$ for LC performance by monitoring peak area and retention time deviations, while MS performance is assessed using the known accurate mass and CCS value. For all 8 components across >75 test runs, retention times matched within 0.1 min deviation, with mass accuracy below 2 ppm, and CCS accuracy below 1.5% Å². Figure 4 demonstrates the inter-lab reproducibility between different hardware configurations using PCA plot.

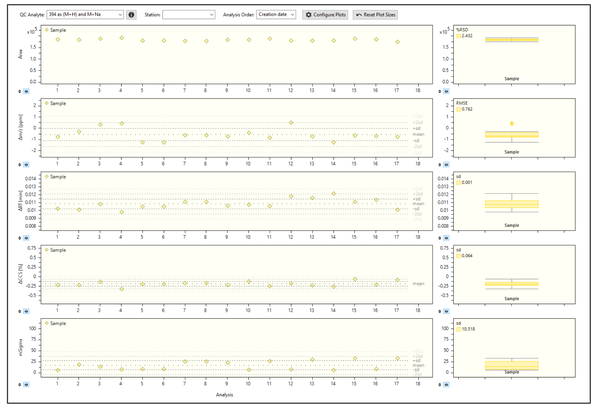


Fig 3. Sample of reproducibility for QSee peak 7 at 394 m/z as [M+H]+ and [M+Na]+ over 17 test injections from Bremen Lab A. Individual metrics are tracked on the left and Deviations in component performance are displayed as % relative standard deviation (%RSD), root mean standard deviation (RMSD), or standard deviation SD as box plots on the right.

The inter-lab reproducibility in Figure 4 shows good accuracy within a given hardware configuration but indicates the need for system tuning to produce consistent results between labs. When used in combination with the cloud-based database TwinScape, the QSee Performance Test workflow will provide guidance on system performance so researchers can undertake interventions to improve reproducibility, such as tuning parameter optimization (e.g. voltages, gas flows) and preventative maintenance schedules (e.g. source cleaning, changing columns).

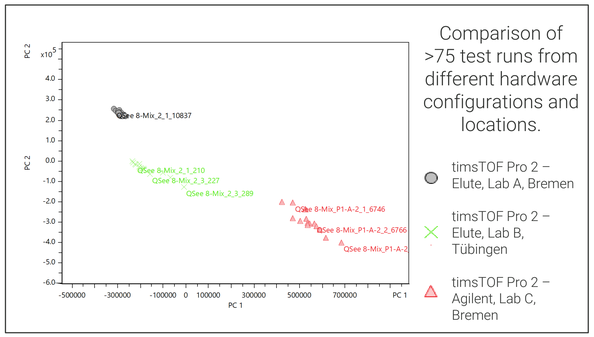


Fig 4. Principal Component Analysis shows clustering of test runs from different timsTOF Pro 2 hardware combinations and labs.

## Summary

The 8-component QSee mixture and QSee Performance Test surveys real-time status of data quality metrics as well as the long-term performance of LC-TIMS-MS devices in a streamlined workflow. System qualification as a standard procedure prior to data acquisition of large sample cohorts is needed to ensure high quality, reproducible data to generate meaningful results. Our results demonstrate the usability of the workflow in testing system performance and data quality across different labs.

## Conflict of Interest

All contributing authors for this work are currently employed by Bruker Daltonics GmbH & Co. KG, which is a supplier of commercial MS instruments

## Conclusion

- The QSee reference mixture enables performance testing for different LC and MS hardware configurations
- Regular system performance testing enables trend analysis in real-time and longitudinally
- The QSee performance Test Software features a streamlined workflow to simplify system monitoring in intra- and inter-lab studies

LC-TIMS-MS Performance Testing