System suitability testing of LC-IMS-HRMS for metabolomics applications

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Introduction

Liquid chromatography-mass spectrometry (LC-MS) produces a vast amount of data from complex samples, particularly in the diverse chemical space of metabolomics. Extracted information required for analysis includes LC retention times, accurate masses, isotope patterns, MS/MS fragmentation patterns and, for systems with ion mobility spectrometry (IMS), collisional cross section (CCS) values. Ultimately, these parameters are used to confidently annotate and quantify metabolites of interest in a given study.

It is challenging, and often overlooked, to monitor system performance for accuracy and precision during the long experimental sequences and multiple batches without substantial manual data curation. To ensure downstream metabolomic data analysis will be meaningful and comparable across a sample cohorts, instrument performance should be appropriately qualified prior to and during data acquisition using standards relevant to the sample cohort – in this case small molecule metabolites.

Here we propose a System Suitability Test (SST) using an aqueous metabolite reference mixture and quality control method that validates system performance, in this case a timsTOF Pro 2, specifically for metabolomics experiments. For this purpose, 18 reference standards were selected from literature with the prerequisites of being relevant to endogenous metabolism, water soluble, and non-hazardous.

MS	timsTOF Pro 2
Source	VIP HESI source
Ionization	ESI (+) and (–) modes
Scan range	20 – 1300 m/z
Mobility range	0.1 – 1.5 1/K_0
Acquisition mode	prm-PASEF
Calibration	Mass calibration through automation, sodium formate Mobility calibration through automation, Agilent TuneMix

HPLC	Agilent 1290 Infinity II
Column	Avantor® ACE® Excel® AQ, 2.1x 100 mm, 2 µm
Column Temp	50 °C
Flow Rate	600 µL/min
Mobile Phases	A: 0.1% FA in H_2O B: 0.1% FA in ACN
Injection Volume	2 µL
Gradient	0 min 99% B 0.1 min 99% B 2.5 min 40% B 2.6 min 1% B 3.3 min 1% B 3.4 min 99% B 5 min 99% B
Data processing	TASQ 2023b

Ion polarity	Acquired data
ESI-(+)	3x blank H_2O
	5x Reference std. mix
ESI (–)	3x blank H_2O
	5x Reference std. mix
	3x blank H_2O

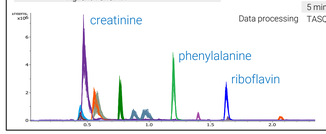

Figure 1. LC-MS setup and methods (tables); overlayed EIC traces of 42 injections of reference compounds (chromatogram).

Methods

Reference standards were selected with the aim to cover the small molecule mass range of ~ 100 – 550 *m/z*, be easy to dissolve for simple preparation and safe to ship or transfer between labs. Standards were procured from Cambridge Isotope Laboratories and mixed at defined concentrations. The dried mixture was dissolved in 1 mL of deionized water and aliquoted. An Agilent 1290 Infinity II LC system and a Bruker timsTOF Pro 2 trapped ion mobility – quadrupole time of flight mass spectrometer equipped with a vacuum insulated probe heated electrospray source (VIP-HESI) were used to run a fast 5-minute LC-MS gradient on a 100 mm, 100% aqueous solvent compatible reversed-phase column (Figure 1).

The chromatographic method was developed to deliver reliable separation of leucine and isoleucine (Figure 2A) at a total runtime of less than 7 minutes. Analyte selection and MS method optimization took into consideration that detection be possible in both ion polarities. The trisaccharides melezitose and maltotriose were specifically selected to test for mobility separation of co-eluting isomers (Figure 2B). To obtain the best signal intensity for MS1 precursor ions and best MS/MS fragment spectra quality for target compounds (Figure 2C), a targeted prm-PASEF acquisition method was developed. For data acquisition, multiple injections of ESI-(+) and ESI-(–) modes were combined in a sequence of about 90 minutes. To test the standard mixture for system qualification, and to evaluate the consistency of precision and accuracy metrics during repeated injections, TASQ RealTimeQC 2023b (*Bruker*) was used (Figure 3). A batch containing 10 consecutive sequences were acquired for a total of 50 runs in each positive and negative mode. TASQ 2023b was used for targeted evaluation of the compound findings and for reporting of the quality factors.

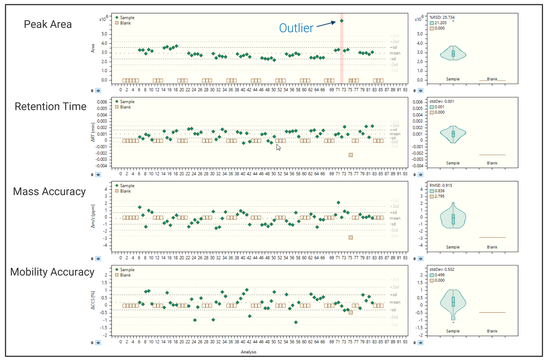

Figure 3. Key parameters plotted for the example of riboflavin in TASQ RealTimeQC 2023b

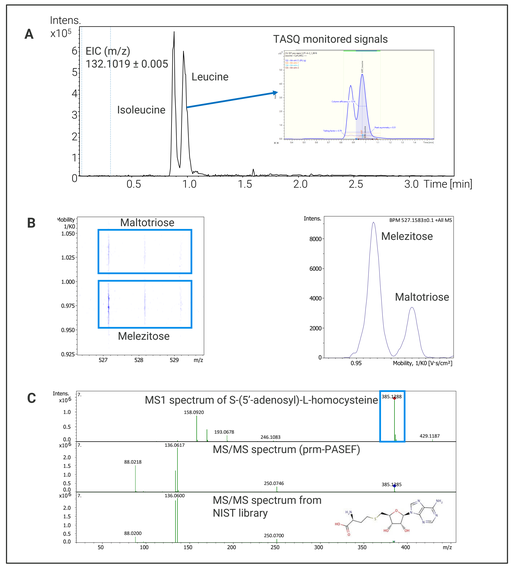

Figure 2. (A) Chromatographic separation of leucine and isoleucine reporting LC performance parameters; mobility separation of co-eluting trisaccharides melezitose and maltotriose (B); Comparison of MS/MS spectra with NIST based library (C)

Results

A metabolomics-specific system suitability test solution was developed and thoroughly tested. The project was conducted on Bruker MS instrumentation, but the concept can be transferred to other system configurations. A sequence consisting of 10 subsequently run replicates of the test procedure (50 runs in positive and negative mode, respectively, see Figure 1) was conducted showing excellent data quality for all 18 analytes of interest. Figure 3 shows the example of riboflavin with peak area, retention time, mass accuracy, MS/MS fragmentation pattern match, and CCS ensuring high quality data over a long sequence. Figure 4 lists information on analyte species within the system suitability test and some of the evaluated qualifiers.

Metabolite	Molecular formula	Retention time [min]	CCS [Å²]	Concentration [mM]
Adenosine 5'-monophosphate	C10H14N5O7P	0.58	171.3	0.30
Adenosyl homocysteine	C14H20N6O5S	0.77	182.93	0.25
Argininosuccinic acid	C10H18N4O6	0.46	165.84	0.25
Benzoic acid	C7H6O2	1.64	132.02	0.40
Caffeine	C8H10N4O2	1.59	143.05	0.15
Creatinine	C4H7N3O	0.49	121.58	0.20
Cytidine	C9H13N3O5	0.58	150.54	0.40
Glutamic acid	C5H9NO4	0.45	131.24	0.20
Glutamine	C5H10N2O3	0.45	130.0	0.25
Hippuric acid	C9H9NO3	1.64	140.35	0.25
Isoleucine	C6H13NO2	0.87	134.04	0.15
Leucine	C6H13NO2	0.97	132.5	0.15
Maltotriose	C18H32O16	0.50	201.9	0.50
Melezitose	C18H32O16	0.50	209.8	0.50
Phenylalanine	C9H11NO2	1.20	139.63	0.30
Tryptophan	C11H12N2O2	3.64	147.5	0.15
Vitamin B2 (riboflavin)	C17H20N4O6	1.63	188.67	0.20
Vitamin B9 (folic acid)	C19H19N7O6	1.40	197.2	0.20

Figure 4. List of Reference standards and parameters used for rating

Summary and Outlook

A 5-min chromatographic gradient was developed for a system suitability test mixture containing 18 aqueous endogenous metabolites. This SST is designed for instrument qualification after system installation and to be used regularly to check for data quality prior to and during long sequences. Chromatographic separation of LEU / ILE and mobility separation of melezitose and maltotriose were selected for testing both chromatographic separation of difficult to separate analytes and co-eluting isomeric species, respectively.

The timsTOF Pro 2 system showed reproducible accuracy and precision parameters at the beginning and throughout multiple sample sequences including peak area, mass accuracy, isotope pattern, retention time and CCS accuracy. When used as a QC mixture, the SST mixtures will be able to discover degrading performance trends or acute hardware issues in advance. We plan to add more parameters such as LC peak shapes and backpressure and the automatic visualizations for long-term tracking of system performance.

Conclusion

- Development of a standardized test solution enabling the evaluation and long-term surveillance of the LC-TIMS-MS system performance
- Within about 90 minutes, the performance of the complete setup is tested in both ion polarities, checking the main qualifiers
- The batch setup, processing and reporting is done in one tool to simplify the QC housekeeping

QC of LC-TIMS-MS/MS