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Standardized quantitative metabolomics using biocrates' MxP® Quant 1000 kit across mass spectrometer platforms

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1 Introduction

Standardized protocols and methods capable of generating reproducible results are essential for producing high quality scientific findings. The MxP® Quant 1000, a new ready-to-use, quality-controlled kit, has been developed and validated for quantitative metabolomics profiling. The kit targets 1,233 analytes across 49 biochemical classes including 327 small molecules and 906 lipids related to microbiome, metabolic health, and disease metabolism. The kit was designed with a modular concept, with the small molecule panel and lipid panel available as separate kits: MxQuant and LxQuant, respectively. Methods were developed and optimized on key triple quadrupole mass spectrometer platforms. Project management and data processing was performed in the WebIDQ workflow manager, the companion cloud-based software guiding from sample registration through metabolite quantification to statistical analysis and biological interpretation.

2 Materials and method

The kit consists of three patented 96-well filter plates, system suitability test mixtures, calibration standards, internal standards and quality controls (QCs), which were reconstituted according to protocol. Small molecules are analyzed across two plates, one designed for 3-NPH (3-nitrophenyl-hydrazin) and one for PITC (phenyl isothiocyanate) derivatization. Lipids are analyzed by an individual plate without derivatization. Experimental samples, consisting of 11 human plasma samples (5 female, 6 male, age 17-65, absence of medical diagnosis), and NIST SRM 1950 were registered in WebIDQ and arranged together with the calibration and QC samples on a 96-well plate layout. All samples except the calibration standards were measured in replicates of three. The worklist was directly exported to the mass spectrometer software. The kit was prepared according to the user manual with a total of 40 µL of each sample pipetted per well across the three plates (20 µL for the 3-NPH plate, 10 µL for the PITC and lipid plates), Following sample preparation, extraction, and dilution, two measurement plates were prepared for small molecule analysis (3-NPH and PITC) and two for lipid analysis. Small molecules were analyzed using optimized LC-MS/MS methods, with one injection per plate: the 3-NPH plate in negative mode and the PITC plate in positive mode. Lipids were analyzed using optimized FIA-MS/MS methods, with two injections per plate: three in positive mode and one in negative mode. Plates were measured on the following triple quadrupole instruments, three located at biocrates, one located in another laboratory: Agilent 6495C, SCIEX 5500+ and 6500+, and Waters Xevo TQ-XS. Data files were directly processed in WebIDQ with automated AI-driven peak picking, quantification, validation, and normalization. Plasma-based quality control samples at different concentration levels were used to automatically assess performance, checking both accuracy and reproducibility. The quantified data was exported to R for plotting and evaluating analytical performance.

3 Results and discussion

Up to 82% of the 327 small molecules and up to 83% of the 906 lipids were found to be above the limit of detection (LOD) in the human plasma samples analyzed. Analyte concentrations were comparable across laboratories and LC-MS platforms. The automated AI-driven peak picking tool of WebIDQ simplified peak integration and accelerated metabolite quantification.

Detectability

The detectability from the 11 human plasma samples (blue bars in Figure 1) showed a distribution of 76% (937 metabolites) to 83% (1,023 metabolites) across all instruments. The detectability was defined as the number of metabolites above LOD in all triplicates and with a CV below 30%.

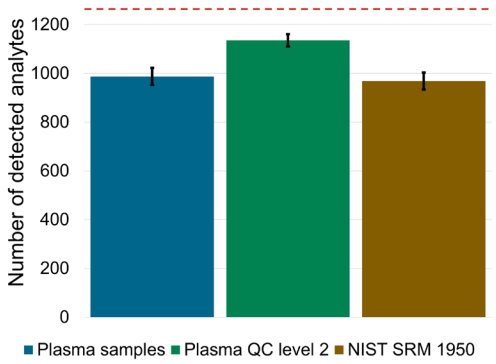

Figure 1: Detectability – Number of metabolites above LOD out of the total panel of 1,233 (red dotted line) and CV<30%.

Reproducibility

All samples measured in triplicates showed comparable CV distribution across all sample types and instruments (Figure 2). The precision was below 10% for most small molecules above LOD and below 20% for most lipids independent of the MS platform and sample.

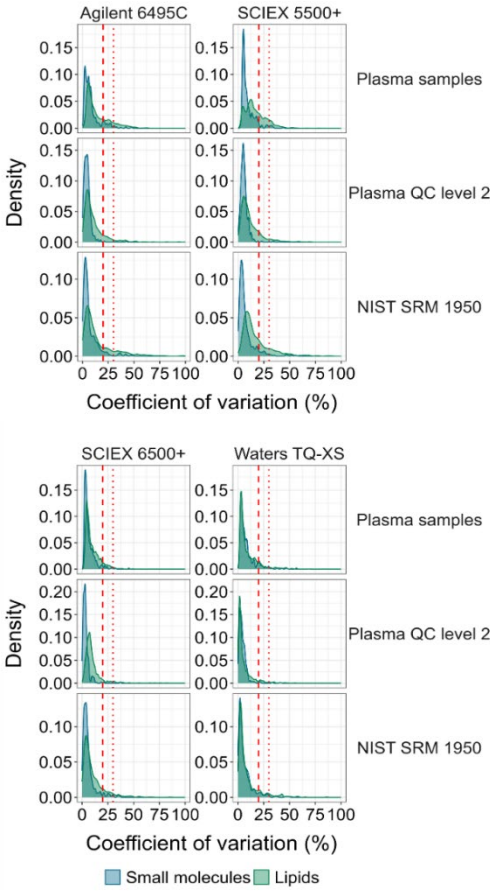

Figure 2: Intra-laboratory CVs of all samples across all laboratories and instrument platforms.

Accuracy

Four different aliquots of NIST SRM 1950 sample were measured. Results were compared with certified values. The accuracies were within 80-120% for almost all analytes and platforms (Figure 3).

Inter-instrument correlation

Figure 4 shows the correlation of concentration values across the four different instrument platforms (metabolites above LOD and CV below 30%). The results show an excellent inter-instrument correlation.

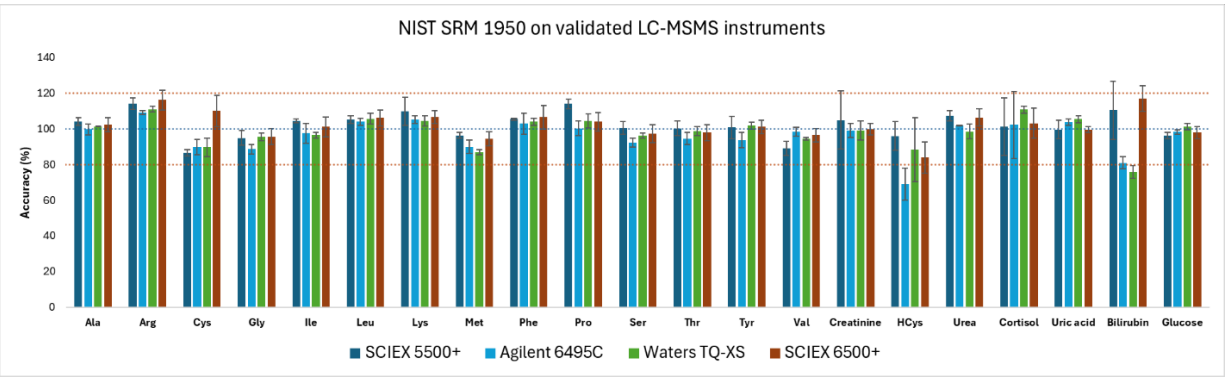

Figure 3: Accuracies of NIST SRM 1950 measured with MxP Quant 1000 kit.

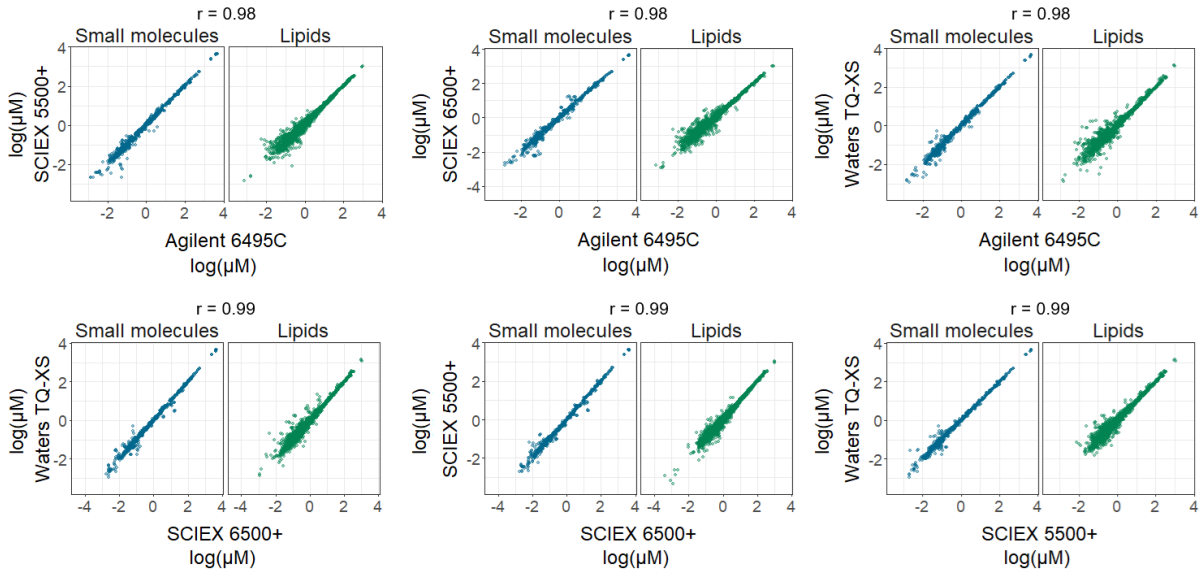

Figure 4: Correlation of plasma sample concentrations across all laboratories and instrument platforms.

4 Conclusions

The results showed high reproducibility and correlation across all laboratories and mass spectrometers used. All instruments displayed good detectably and comparable coverage. It was demonstrated that the MxP® Quant 1000 kit enabled robust and reproducible quantification across multiple MS vendor platforms.

The cloud-based WebIDQ workflow manager – with integrated AI-driven peak picking, automated quantification, validation, and normalization – streamlined the workflow and ensured highly reproducible results, independent of the MS system used.

Together, the total solution of kit and software empowers researchers to generate high-quality data and accelerates quantitative metabolomics studies.

5 Acknowledgement

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