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Standardized quantitative metabolomics with biocrates' MxP® Quant 500 XL kit on Waters Xevo TQ Absolute

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1 Introduction

Robust methods and reproducible results are the cornerstone of any scientific finding. Over the last decade, biocrates kit technology has emerged as the gold standard in quantitative metabolomics, equipping researchers with standardized and validated methods for the study of human health and disease. International ring trials have proven the quality and reproducibility of biocrates kit data[1], allowing researchers to conduct local sample measurements and contribute to the global scientific community with confidence.

MxP Quant 500 XL is a ready-to-use and quality-controlled kit for quantitative metabolic profiling. It targets 1,019 metabolites across 39 biochemical classes of lipids and small molecules (Figure 1) related to healthy aging, neurodegeneration, and cardiometabolic diseases. The WebIDQ workflow manager is a companion cloud software that guides users through the entire workflow and is easily accessible through a web browser. It combines machine learning-based peak picking, automated quantification, and visual validation features to standardize and increase confidence in acquired data. The integrated MetaboINDICATOR tool automatically calculates 474 predefined sums and ratios for advanced biological interpretation of enzyme activities and pathophysiological conditions resulting in a total of up to 1,493 metabolic features.

The kit performance was previously demonstrated across mass spectrometry platforms and laboratories from around the world through an international ring trial[2]. Most recently, methods were adapted from the Xevo TQ-XS to the new Xevo TQ Absolute and optimized with modifications and updates where required. The methods were tested using replicates of different sample types to determine the detectability and to verify reproducibility and comparability to Xevo TQ-XS. The performance results are presented in this application note.

2 Materials and method

The kit consists of two patented 96-well filter plates with internal standards already integrated, system suitability test samples, lyophilized calibration standards, and plasma-based quality controls (QCs), which were reconstituted according to protocol. Experimental samples, consisting of 11 human plasma samples (5 female, 6 male, age 17-65, absence of medical diagnosis), NIST SRM 1950, two NIST Candidate Reference Materials 8231 (pooled plasma from high triglyceride and diabetic samples), and three pooled fecal samples at different concentration levels from 30 human subjects, were registered in WebIDQ and arranged together with the calibration and plasma-based QC samples on a 96-well plate layout. All samples except the calibration standards were measured in replicates of three. Two additional, separate kit plates were prepared and measured containing the plasma-based QC samples and the feces pools, each in replicates of 6, in order to determine the inter-plate reproducibility for these matrix representatives. The worklist

was directly exported to the mass spectrometer software and the layout printed for kit preparation. Fecal samples were prepared according to the biocrates protocol for analyzing feces using a Precellys homogenizer and isopropanol as extraction solvent[3]. The kit was prepared according to the user manual with 10 µL of sample pipetted per well on each of the two kit plates followed by derivatization, extraction, and finally dilution into three separate measurement plates: one for LC-MS/MS and two for FIA-MS/MS (Quant 500 and XL part). The total run time was 54 hours (80 samples, 2 injections for each of the three measurement plates). Data files were directly processed in WebIDQ with automated quantification, validation, and normalization. Plasma-based QC samples at different concentration levels were used to automatically assess performance, checking both accuracy and reproducibility. The QCs were also used for data normalization as an essential part of the workflow to minimize any deviations caused by preparation or measurement. Quantified data was exported and evaluated in R.

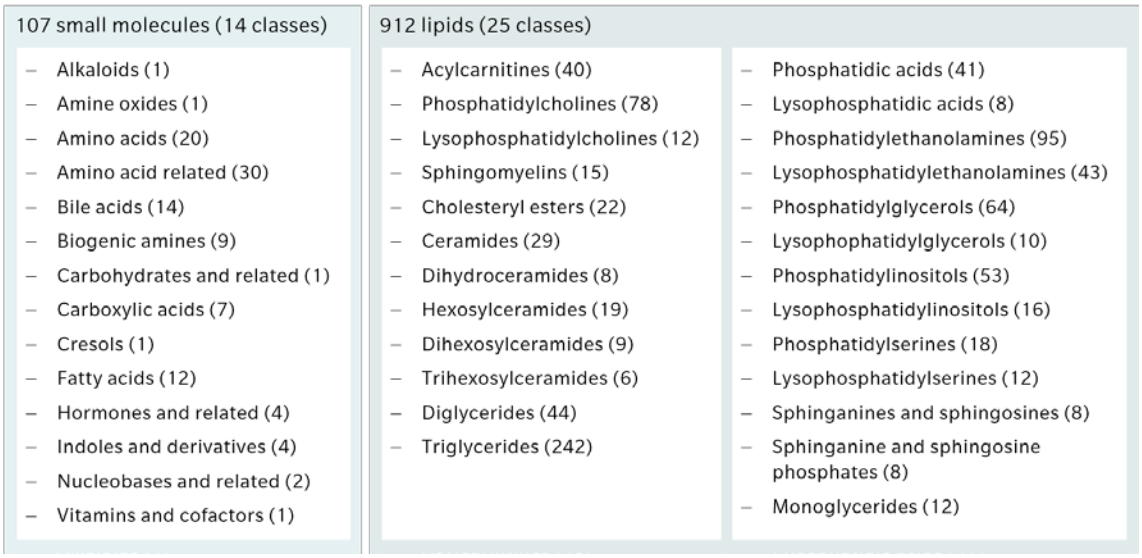

Figure 1: The MxP® Quant 500 XL kit metabolite panel.

3 Results and discussion

Up to 81% of the 1,019 metabolites were found to be above the limit of detection (LOD) in the analyzed human plasma samples, as well as NIST 1950. Lipid detectability showed coverage up to 89% in human feces and up to 82% in human plasma. The precision was within 20% for all measured matrices for most analytes above LOD. Analyte concentrations were comparable between Xevo TQ Absolute and TQ-XS. The automated peak picking feature of WebIDQ simplified peak integration and accelerated metabolite quantification.

Detectability

Out of the total panel of 1,019, the detectability from the 11 human plasma samples from different individuals showed a distribution of 53% (541 metabolites) to 81% (828 metabolites). All other sample types showed a detectability above 800 metabolites (Figure 2). The detectability was defined as the number of metabolites above LOD with coefficient of variation (CV) below 30%.

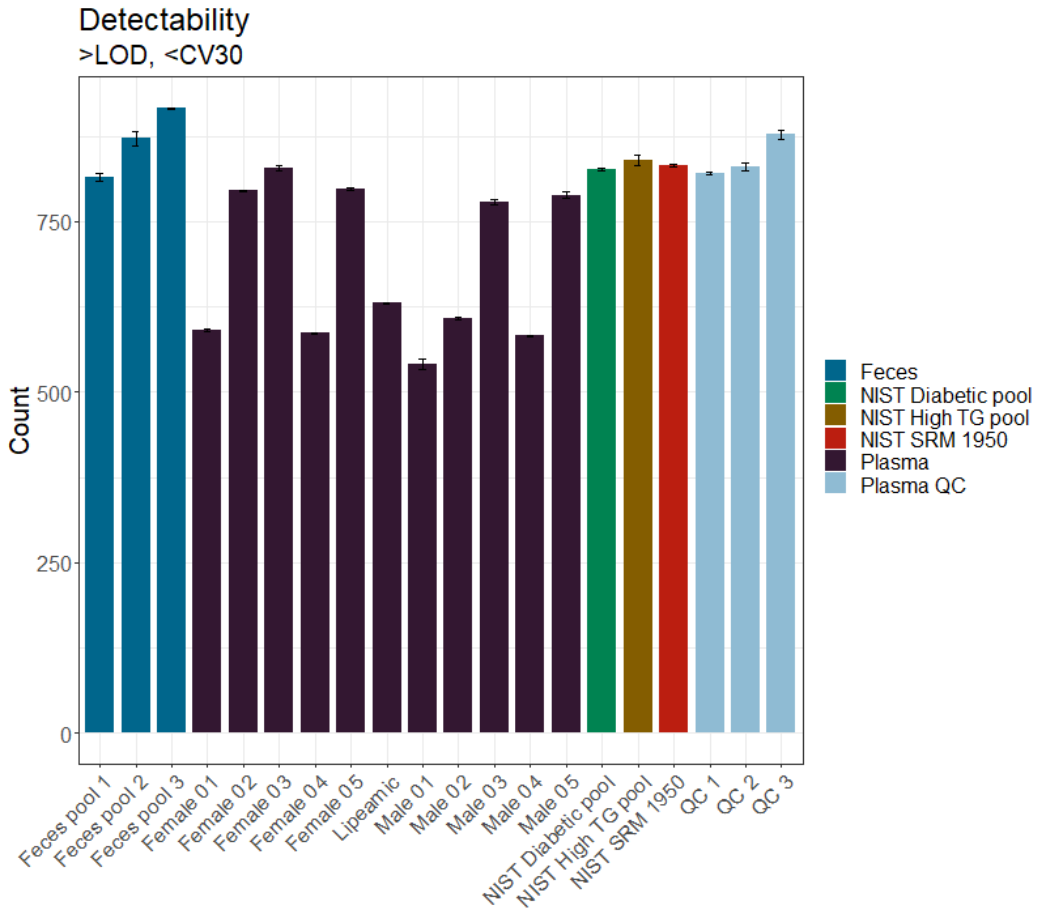

Figure 2: Detectability – Number of metabolites above LOD out of 1,019 and CV<30%.

Intra-plate reproducibility

All samples measured in triplicates showed a median CV below 10% for metabolites measured above LOD (Figure 3). Depending on the sample matrix, 76% to 85% of analytes were below 30% CV and 74% to 83% were below 20% CV. Figure 4 shows the NIST SRM 1950, the plasma-based QCs, and the feces pools across the different measurement plates, demonstrating excellent CVs across all methods and sample types.

Inter-plate reproducibility

Inter-plate %CVs across three individual kit plates prepared and measured at different time points were comparable to the intra-plate %CVs, demonstrating the robustness of the assay and of the Waters Xevo TQ Absolute (Figure 5).

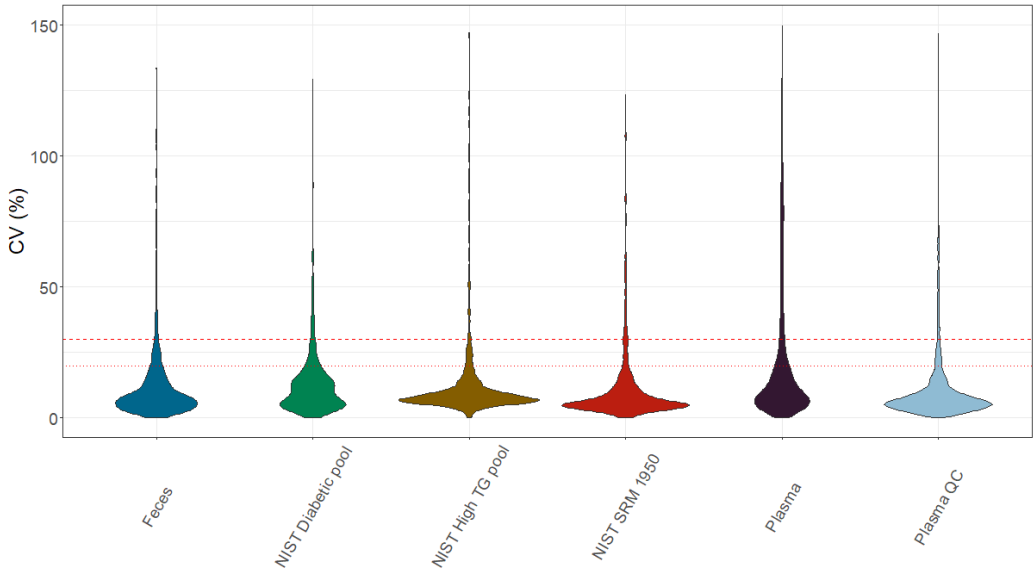

Figure 3: Intra-plate CVs of all samples. Upper red dotted line = 30% CV, lower red dotted line = 20% CV

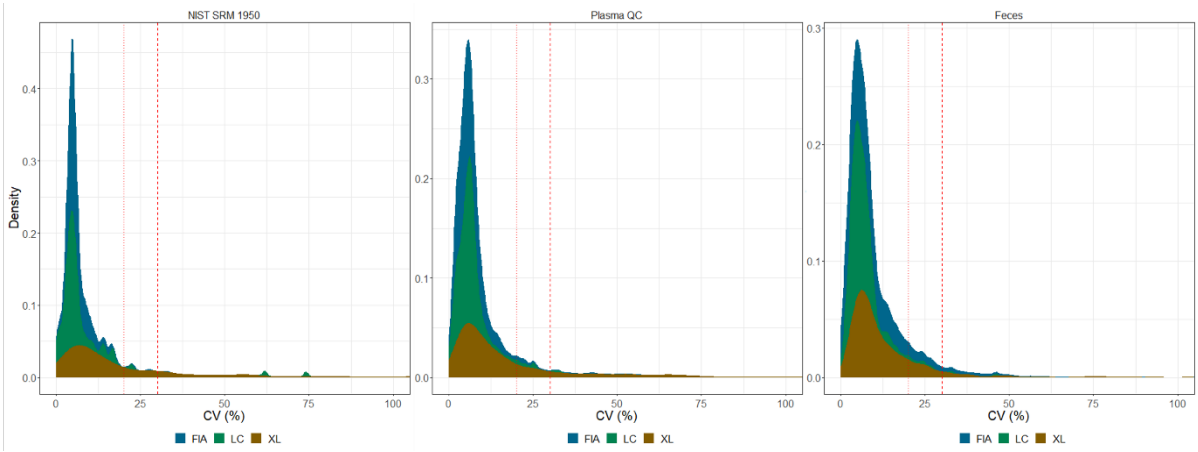

Figure 4: Intra-plate CVs of the NIST SRM 1950, plasma-based QC levels and feces pools. Right red dotted line = 30% CV, left red dotted line = 20% CV

Accuracy

The accuracies were determined in relation to the reference values defined for each QC level. Most accuracies were within 85-115% across all QC levels of different concentrations. Figure 6 displays the accuracies of amino acids, amino acid-related metabolites and biogenic amines.

Correlation with Xevo TQ-XS

Figure 7 shows an excellent correlation of concentration values of the Waters Xevo TQ Absolute with the predecessor Xevo TQ-XS (metabolites above LOD and CV below 30%).

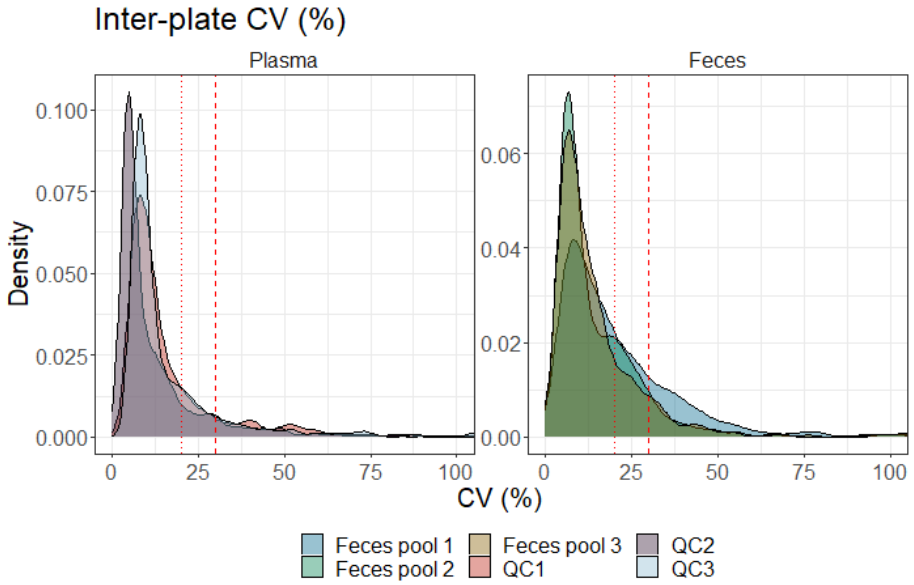

Figure 5: Intra-plate CVs of plasma based QCs and feces pools. Right red dotted line = 30% CV, left red dotted line = 20% CV

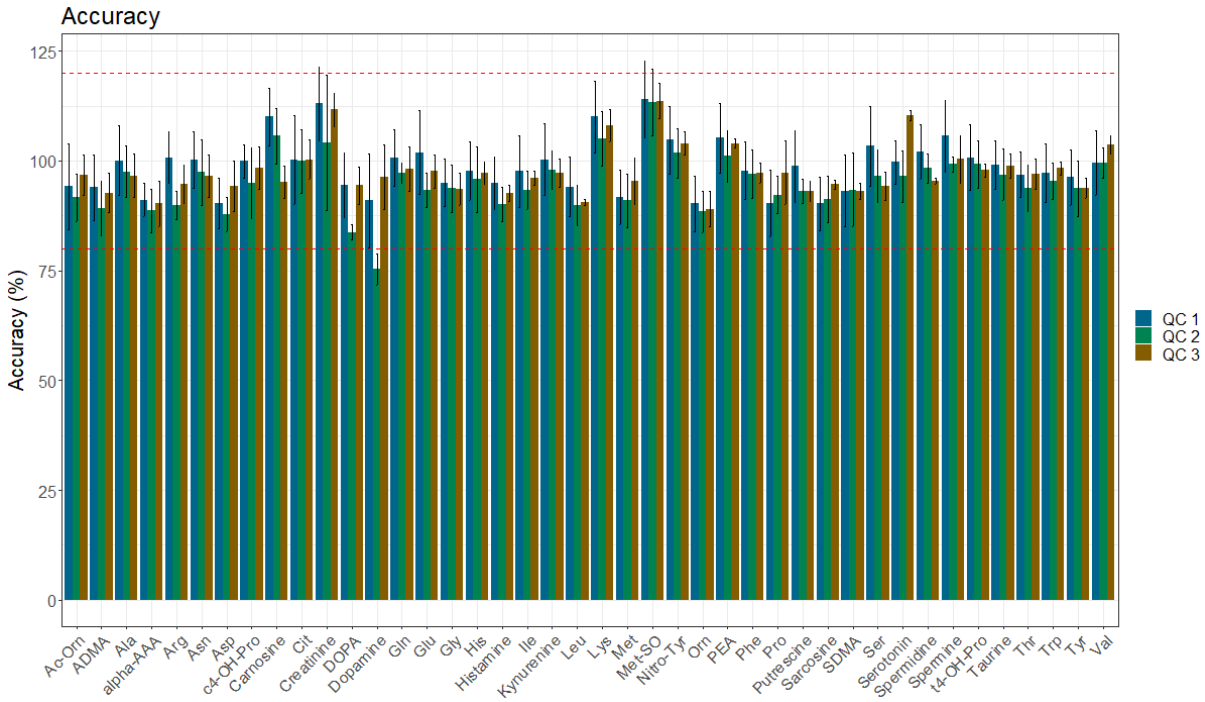

Figure 6: Accuracies of amino acids, amino acid-related metabolites and biogenic amines in QC levels of different concentrations. Upper red dotted line = 120%, lower red dotted line = 80%

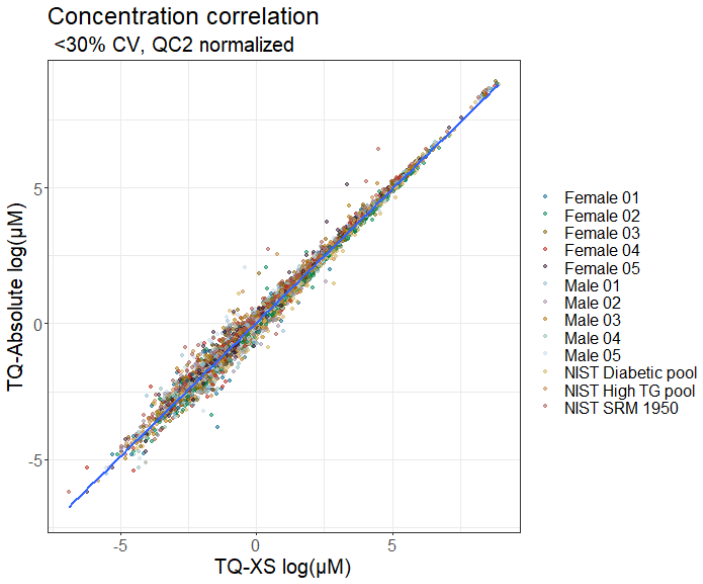

Figure 7: Correlation of sample concentrations obtained on Waters Xevo TQ Absolute compared to concentrations from predecessor Xevo TQ-XS.

4 Conclusions

The results showed high intra- and inter-plate reproducibility over time and across different sample types, demonstrating the robustness of the assay and of the Waters Xevo TQ Absolute platform. Good detectably was observed for all sample types tested. It was demonstrated that the MxP® Quant 500 XL kit in combination with the Waters Xevo TQ Absolute shows an outstanding analytical performance with excellent precision and comparability of concentration values to the predecessor Xevo TQ-XS.

5 References

1. Siskos et al. Interlaboratory Reproducibility of a Targeted Metabolomics Platform for Analysis of Human Serum and Plasma. Anal. Chem. 2017; 89(1):656–65.

2. biocrates application note: Standardized quantitative metabolomics using the biocrates MxP® Quant 500 XL kit across mass spectrometer platforms

3. biocrates sample preparation protocol (SOP): Analyzing human feces with biocrates kits