biocrates
The future of research and health

Standardized quantitative metabolomics with biocrates' MxP® Quant 500 XL kit on Thermo TSQ Altis™ Plus

Stephen Dearth, Markus Langsdorf, Gregor Ömer, Tuan Hai Pham;
biocrates life sciences ag, Innsbruck, Austria

Edmund Moy, Susan Bird;
Thermo Fisher Scientific, San Jose, CA

1 Introduction

Standardized protocols and methods capable of generating reproducible results are essential for producing high quality scientific findings. For the past decade, biocrates kit technology has been 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 kit is enhanced with the WebIDQ workflow manager, 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 biomarkers.

The kit performance was previously demonstrated across mass spectrometry platforms and laboratories from around the world through an international ring trial[2]. The instrument methods were adapted to the Thermo TSQ Altis™ and Altis Plus triple quadrupole mass spectrometers and optimized with modifications and updates where required. The methods were tested using replicates of different plasma and feces sample types to determine the detectability and to verify reproducibility and comparability to the other instruments. 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, three NIST Candidate Reference Materials 8231-1, 8231-2, and 8231-3 (plasma from high triglyceride, diabetic, and African American pools), 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. Three separate kit plates were prepared and measured containing the calibration standards and QC samples to monitor the inter-plate accuracy and reproducibility. 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. 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.

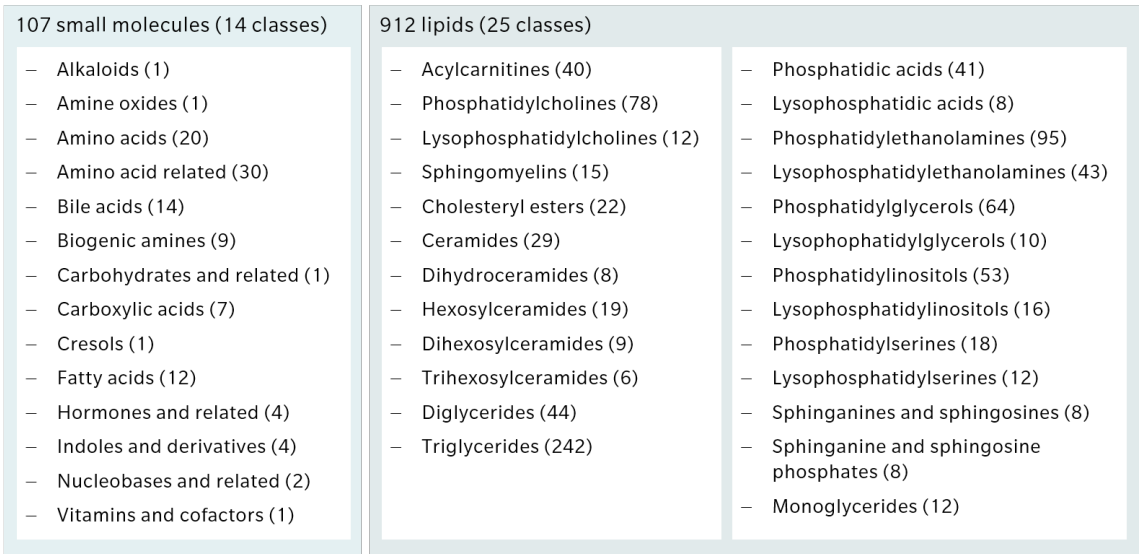

Figure 1: The MxP® Quant 500 XL kit metabolites by compound class.

3 Results and discussion

Up to 74% 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 reference samples. Lipid detectability showed coverage up to 82% in human feces and up to 75% in human plasma samples measured.

The precision across all measured sample types was within 20% for most analytes consistently detected above LOD.

Analyte coverage and concentrations were found to be highly comparable with other triple quadrupole mass spectrometer platforms previously evaluated with the same study samples.

The automated peak picking feature of WebIDQ simplified peak integration and accelerated metabolite quantification and data processing.

Detectability

Out of the total panel of 1,019 small molecules and lipids, the detectability in the 10 human plasma samples from different individuals showed a distribution of 68% (690 metabolites) to 74% (756 metabolites). Other sample types such as pooled feces samples measured from 63% (638 metabolites) to 83% (848 metabolites) and NIST reference and pooled samples measured from 71% (724 metabolites) to 74% (755 metabolites). (Figure 2). The detectability was defined as the number of metabolites above LOD with coefficient of variation (CV) below 30%.

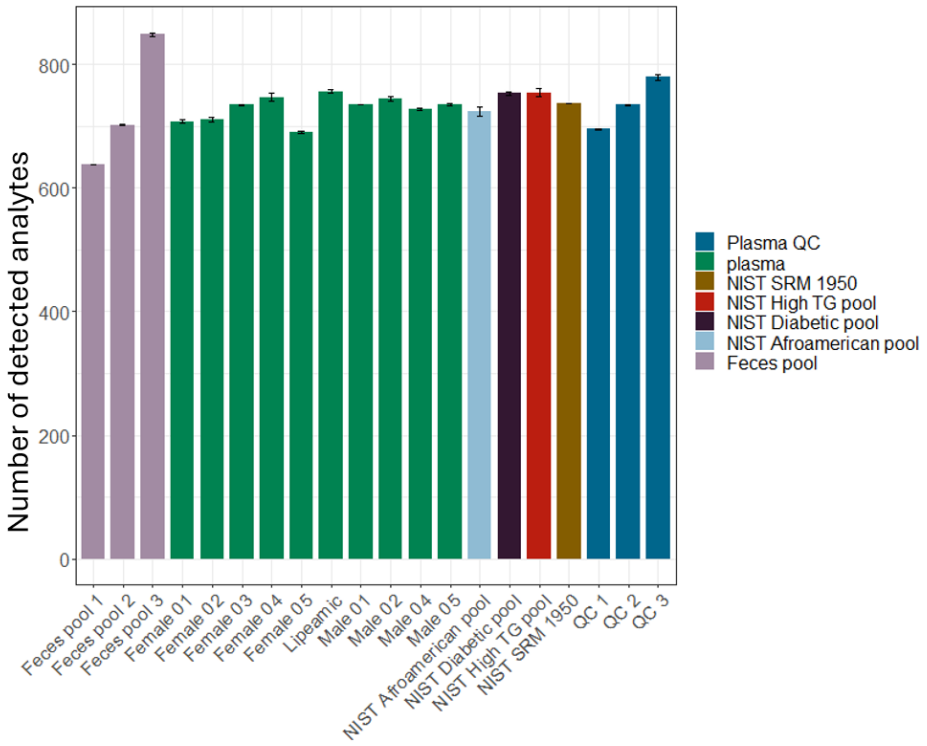

Figure 2: Detectability – Number of metabolites above LOD out of 1,019 with CV < 30% for the sample groups analyzed. Each bar represents a different individual sample (n = 3)

Reproducibility

All samples measured in triplicates showed a median CV below 10% for metabolites measured above LOD (Figure 3). Across all sample matrices measured (plasma and feces), approximately 88% of analytes were below 30% CV and 77% were below 20% CV.

The plasma-based samples, NIST reference and pooled samples, and feces pools demonstrated exceptional CVs across all types and levels for each analysis type (Figure 4).

Taken together, all sample matrices had comparable reproducibility for all measured analytes (Figure 5).

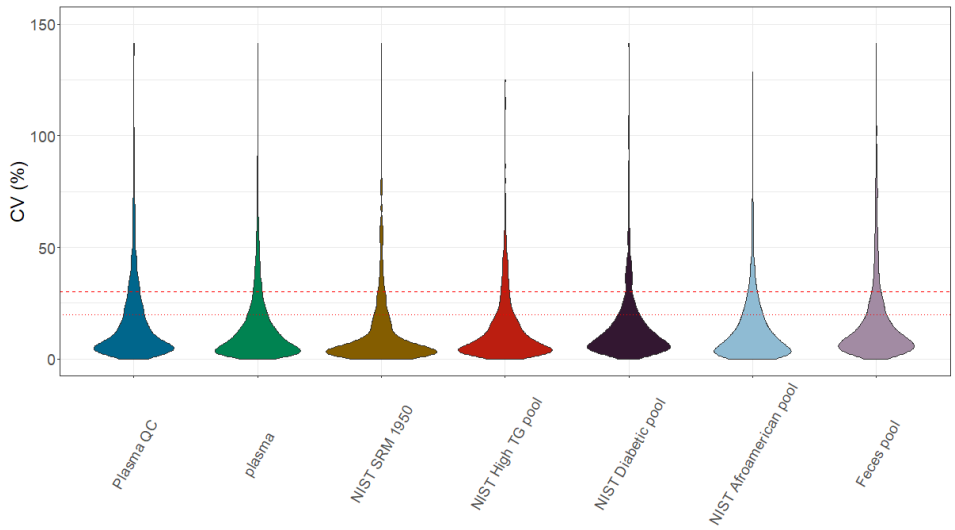

Figure 3: Combined CVs of all sample types. Upper red dotted line = 30% CV, lower red dotted line = 20% CV

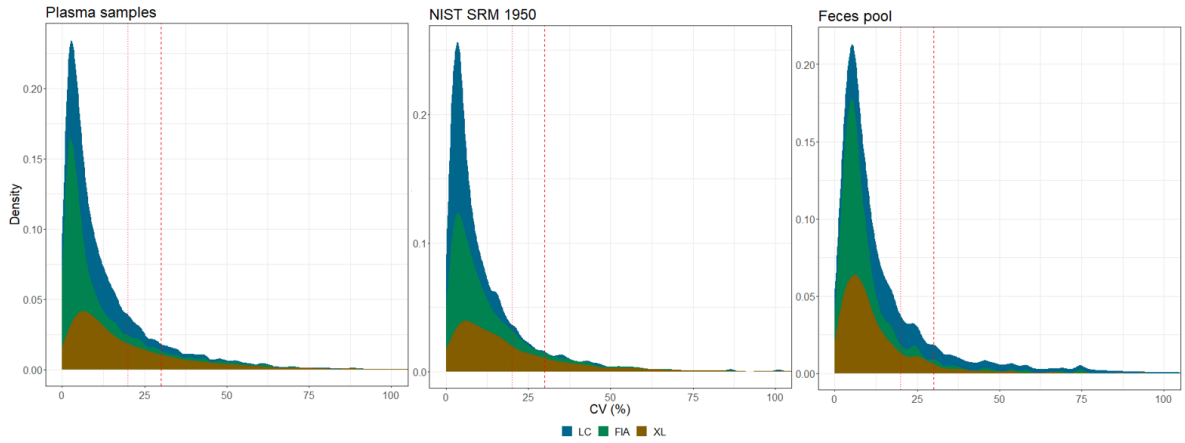

Figure 4: CVs of the, the different plasma samples (left), NIST pools (center), and feces pools (right) by run type. Right red dotted line = 30% CV, left red dotted line = 20% CV

Correlation with other platforms

The dataset evaluated was compared with previously measured data on other mass spectrometers and demonstrated excellent correlation.

Figure 7 reveals the correlations among Agilent 6495C, Waters Xevo TQ-XS, and SCIEX 5500+ instruments all running identical sample sets (metabolites above LOD and CV below 30%).

The results not only highlight the strong reproducibility across mass spectrometer instruments, but also minimal effects from preparation, and operator variability.

.

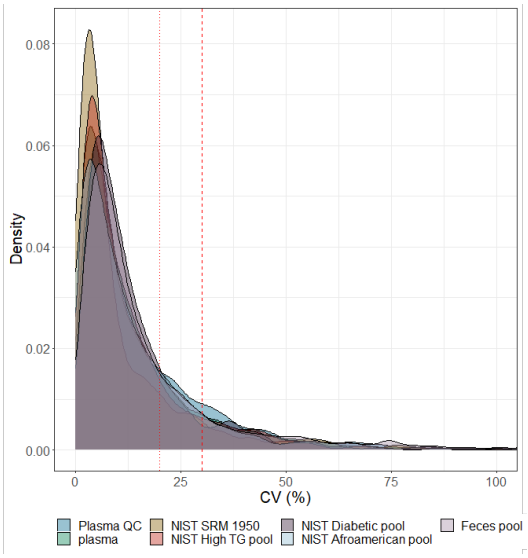

Figure 5: CV density of plasma-based QCs, human plasma samples, NIST materials, and feces pools. Right red dotted line = 30% CV, left red dotted line = 20% CV

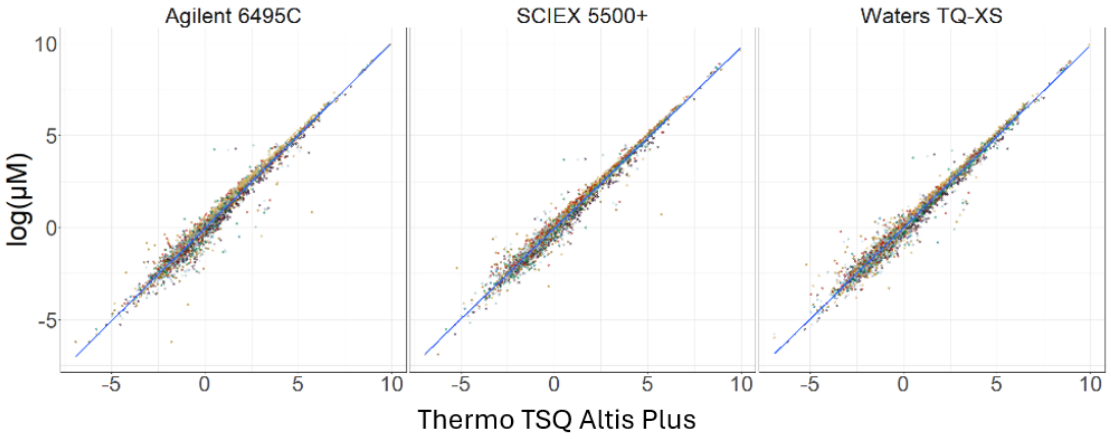

Figure 6: Correlation of sample concentrations obtained on Thermo Altis™ Plus compared to concentrations from other Agilent 6495C (left), SCIEX 5500+ (center), and Waters TQ-XS (right) instruments. Colors represent individual plasma samples.

4 Conclusions

The MxP® Quant 500 XL kit methods were successfully adapted and results showed high reproducibility and good detectability across plasma and feces samples. Exceptional correlation was observed when compared with other LC-MS platforms, highlighting the robustness of the assay in combination with the Thermo TSQ Altis™ Plus platform.

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, 2023: Standardized quantitative metabolomics using the biocrates MxP® Quant 500 XL kit across mass spectrometer platforms