Application note | 002827

Metabolomics

High-resolution quantitative metabolomics with the biocrates AbsoluteIDQ® p400 HR kit on Orbitrap Exploris mass spectrometers

Authors

Stephen Dearth[1], Agnes Scharrer[1], Tuan Hai Pham[1], Guohui Li[2], Susan Bird[2], Hoon Park[3], Maciej Bromirski[4]

[1]biocrates life sciences ag, Innsbruck, Austria

[2]Thermo Fisher Scientific, San Jose, CA, USA

[3]Thermo Fisher Scientific, Hunt Valley, MD, USA

[4]Thermo Fisher Scientific, Warsaw, Poland

Keywords

Orbitrap Exploris mass spectrometer, biocrates, AbsoluteIDQ p400 HR kit, metabolomics

Goal

To assess the biocrates AbsoluteIDQ® p400 kit for the Thermo Scientific™ Orbitrap Exploris™ series of mass spectrometers in terms of reproducibility and performance through quality controls, NIST reference material, and real plasma samples

Introduction

Metabolomics offers a valuable mechanism to explore the complex processes involved in cellular biology. However, the lack of standardized sample preparation and analytical protocols continue to present an ongoing challenge for researchers.

A solution to these challenges is the biocrates AbsoluteIDQ® p400 HR (p400) kit, a ready-to-use, standardized and quantitative metabolomics solution that targets 408 small molecules and lipids across 11 compound classes (Table 1). Specifically designed for Thermo Scientific™ Orbitrap™ mass spectrometers (MS), the kit comes with calibration and internal standards, quality controls (QCs), system test samples, and validated methods necessary for setting up and running targeted metabolomics experiments.

The kit and methods were originally developed for the Thermo Scientific™ Q Exactive™ Hybrid mass spectrometers and built on biocrates' proven kit technology. Through an international ring trial, standardized performance and data quality were demonstrated across labs from around the world.[1]

The biocrates WebIDQ companion workflow manager is designed to handle the demands of high-resolution data processing and assists the user through the entirety of the kit workflow, making the process straightforward and accessible from any computer. The software now includes 123 pre-programmed Metabolism Indicators (MI) specifically for the p400 kit. MIs are metabolites sums and ratios that have shown in the literature to either have biological significance or disease relevance and can be of great value to researchers in data analysis and biological interpretation.

The p400 instrument methods were optimized and tested on the Orbitrap Exploris mass spectrometers in collaboration with Thermo Fisher Scientific. The Orbitrap Exploris MS series improves on its successful Q Exactive MS predecessor to deliver the accuracy and selectivity required to obtain high quality results. The methods were optimized for the Orbitrap Exploris MS from the Q Exactive MS methods with modifications and updates as needed.

The p400 kit was prepared according to standard protocol and the results, which compared performance across the Thermo Scientific™ Orbitrap Exploris™ 120 MS, Thermo Scientific™ Orbitrap Exploris™ 240 MS, and Thermo Scientific™ Orbitrap Exploris™ 480 MS instruments, are summarized in this application note.

Experimental

The p400 kit consists of system suitability test samples and a patented 96-well filter plate with internal standards already integrated. The standard sample analysis consists of:

- Calibration standards:
  7 lyophilized concentration levels
- Quality controls:
  3 lyophilized plasma-based QC concentration levels for performance check and accurate data normalization
- Zero sample:
  PBS for limits of detection (LODs) calculation
- Instrument-specific methods:
  Optimized from validated Q Exactive MS methods and modified for each Orbitrap Exploris MS platform

The biocrates AbsoluteIDQ® p400 kit was applied to the measurement of small molecules and lipids through liquid chromatography and flow injection analysis using a Thermo Scientific™ Vanquish™ UHPLC system coupled to an Orbitrap Exploris MS. Calibration standards and plasma-based QCs at three concentration levels were used to optimize and validate the method for the Orbitrap Exploris MS family of instruments. Each QC level was run in replicates of five. Moreover, NIST standard reference material (SRM) 1950 and human plasma representing male, female, and lipemic samples were analyzed in replicates of three to determine coverage and reproducibility in real sample analogues. Plasma for male and female samples consisted of five unique individuals per group. Identical sample sets prepared and run on separate plates and measured on the Orbitrap Exploris 120, 240, and 480 MS platforms were used to assess intra- and inter-instrument variability (%CV). Evaluation was carried out at two different laboratories with the Orbitrap Exploris 120 MS and the Orbitrap Exploris 240 MS prepared together and the Orbitrap Exploris 480 MS at a separate site.

Table 1. The AbsoluteIDQ® p400 HR kit metabolite and lipid panel

43 small molecules (3 classes)	365 lipids (8 classes)		
• Amino acids (21)	• Acylcarnitines (55)	• Sphingomyelins (31)	• Diglycerides (18)
• Biogenic amines (21)	• Phosphatidylcholines (172)	• Ceramides (9)	• Triglycerides (42)
• Hexoses (1)	• Lysophosphatidylcholines (24)	• Cholesteryl esters (14)	

Results and discussion

Detectability

Human plasma samples representing male, female, and lipemic populations were analyzed to determine average metabolite detectability and coverage across the three instruments. The total coverage will depend on many factors including the nature and handling of the samples; however, these groups provide insight into approximate expected numbers in real samples.

Detectability was defined as the average number of metabolites in each group (male and female plasma n = 15, lipemic and NIST SRM 1950 n = 3, per instrument) measured above LOD. (Here, metabolites refers to the compounds annotated by the AbsoluteIDQ® p400 HR kit software that are referenced in Table 1.) The overall detectability across instrument types was similar for all plasma types (Figure 1). Male, female, and lipemic plasma averaged 230–250 metabolites across all instruments with the Orbitrap Exploris 480 MS measuring 5–10 more metabolites per group. The NIST sample averaged 209 metabolites for the Orbitrap Exploris 120 MS and the Orbitrap Exploris 240 MS, and 224 metabolites for the Orbitrap Exploris 480 MS.

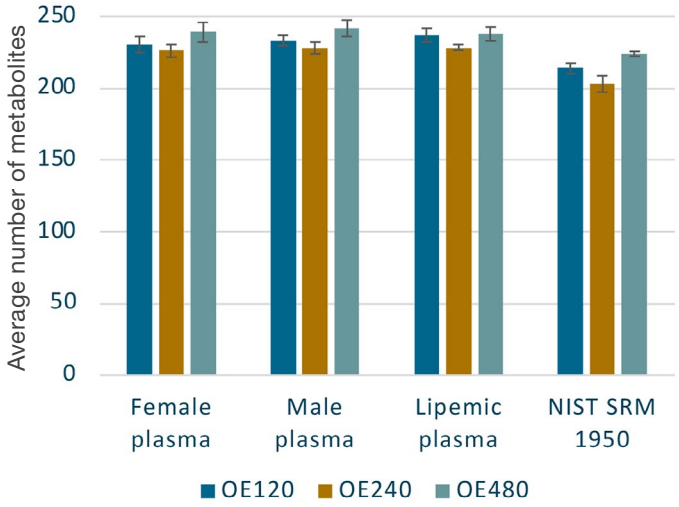

Figure 1. Detectability – Average number of metabolites above LOD out of 408 across different plasma samples. Error bars represent standard deviations.

Total coverage when comparing metabolites detected in at least two thirds of samples across all three instruments (Table 2) showed slightly higher but comparable results to those previously observed in the ring trial study on Q Exactive MS instruments.

Reproducibility

Precision and reproducibility were assessed for the metabolites and lipids in the QC samples measuring consistently above LOD being detected in at least two thirds of replicates. All data was normalized in the WebIDQ software to correct for any potential batch, preparation, or instrument variability.

Intra-instrument comparability

When considering instrument variability for each Orbitrap Exploris MS instrument individually, for all QC levels, the median coefficient of variation (CV) was 9.3% for both the Orbitrap Exploris 120 MS and the Orbitrap Exploris 240 MS and 7.6% for the Orbitrap Exploris 480 MS. Figure 2 shows the %CV distributions for each QC level by instrument. The vast majority of detected analytes showed good CV performance <30% for each instrument and QC level.

Inter-instrument comparability

Evaluating the reproducibility when considering all three Orbitrap Exploris MS instruments together, median CV was 9.7% for all QC levels with average CV <15% for metabolites. Figure 3 shows the distribution of %CVs for all QC levels and instruments combined. Again, analytes show good CV performance with most of the detected metabolites measuring well below 30%.

Table 2. Metabolite and lipid detectability by class for the four plasma sample types over the three instruments evaluated: Orbitrap Exploris 120 MS, Orbitrap Exploris 240 MS, and Orbitrap Exploris 480 MS (number of metabolites above LOD out of 408 detected in at least 60% of samples across the three instruments)

All instruments	Amino acids (21)	Biogenic amines (21)	Acylcarnitines (55)	Glycerides (60)	Glycerophospholipids (196)	Cholesterol esters (14)	Sphingolipids (40)	Sugars (1)	Total (408)
Female plasma (45)	21	11	14	29	102	10	33	1	221
Male plasma (45)	21	10	17	28	104	10	33	1	224
Lipemic plasma (9)	21	11	15	41	101	10	28	1	228
NIST SRM 1950 (9)	21	9	16	28	96	9	29	1	209

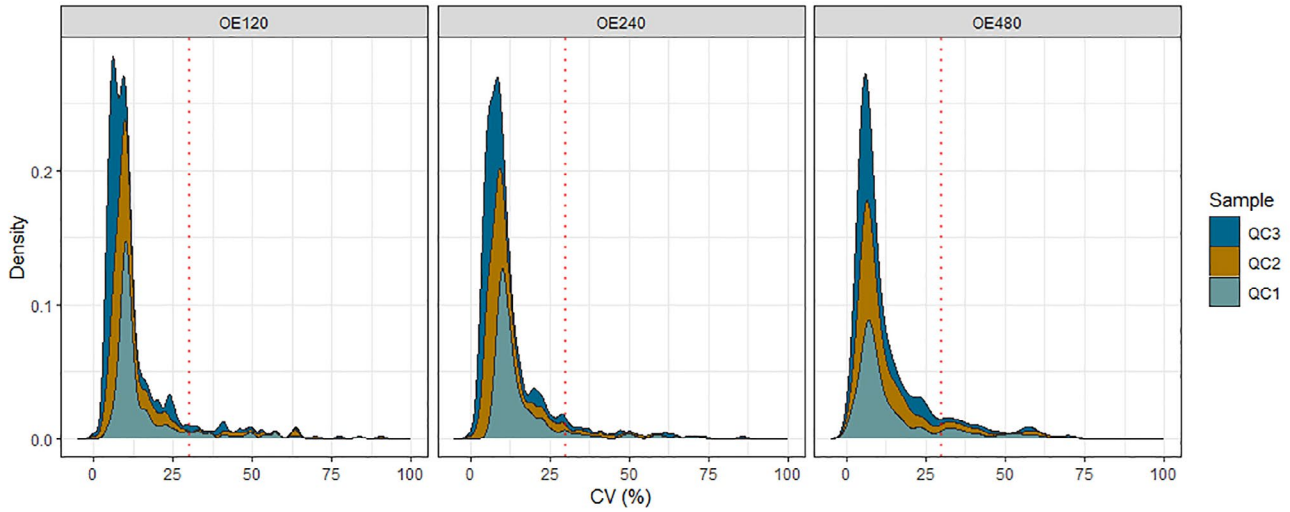

Figure 2. Intra-instrument CV (%) distribution across all QC samples

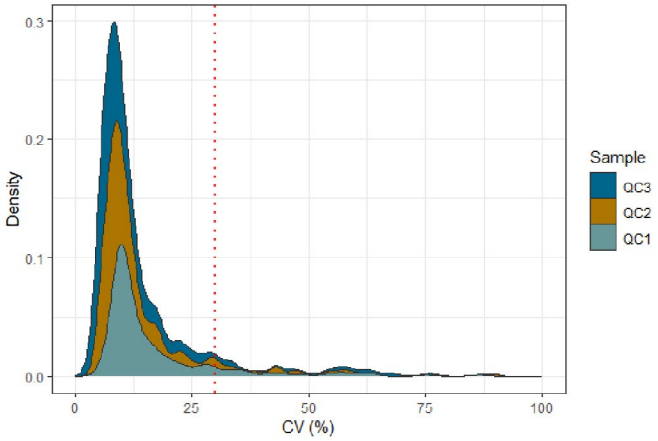

Figure 3. Inter-instrument CV (%) distribution across all QC samples

Inter-instrument correlation

Figure 4 shows the correlation of concentration values including male, female, and NIST plasma samples of each instrument compared to one another. Overall, the data shows good correlation trends across all instrument platforms for metabolites measuring above LOD and CV <30%. The correlations of the Orbitrap Exploris 120 MS and the Orbitrap Exploris 240 MS to the Orbitrap Exploris 480 MS maintained especially good correlation considering these samples were prepared and run by users in different laboratories.

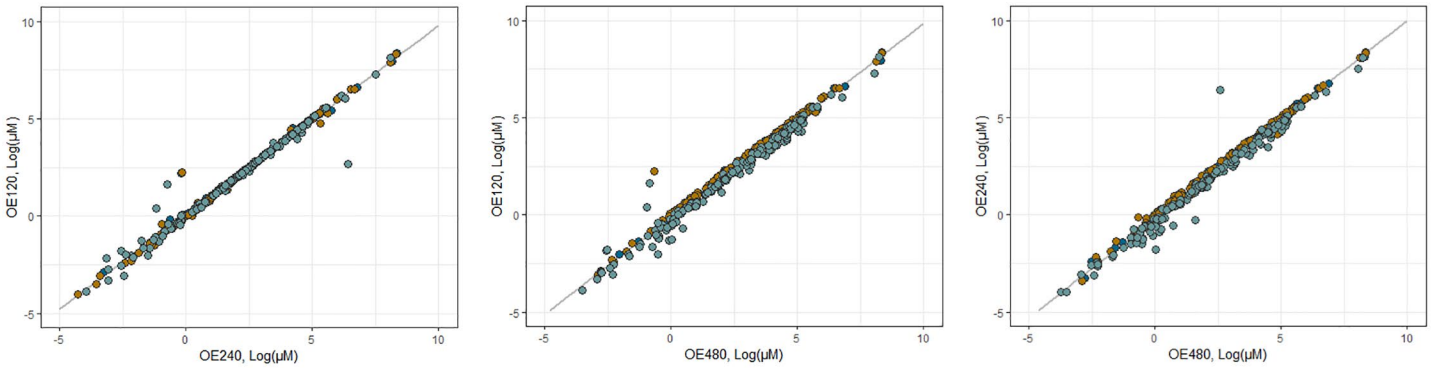

Figure 4. Correlation of plasma sample concentrations (averaged technical replicates) for all instruments compared to concentrations from all other instruments. Colors represent individual plasma groups, blue: female, green: male, yellow: NIST SRM 1950.

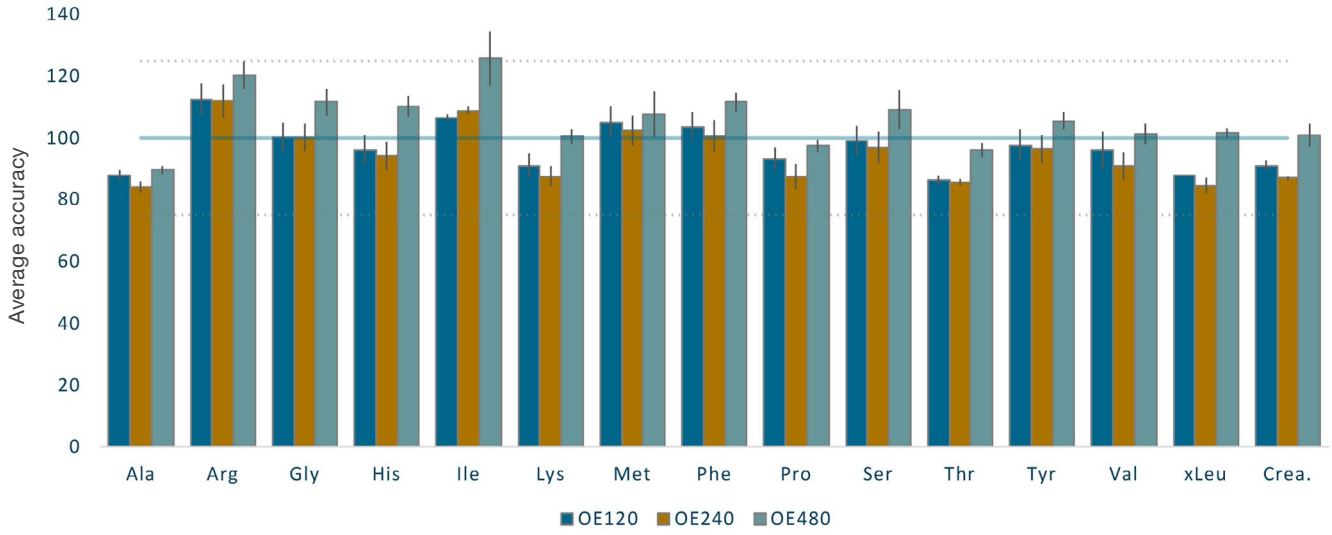

Figure 5. Amino acid and biogenic amine concentrations compared to NIST SRM 1950 reference values

Accuracy of measurements

The measured amino acid and biogenic amine concentrations across the three instruments were evaluated against NIST SRM 1950 values[2] for metabolites where reference information was available (Figure 5). The accuracies showed generally good alignment compared to reported concentrations with measured values differing on average by less than 6% from the expected values.

There were some minor, higher differences in the concentration values for the Orbitrap Exploris 480 MS compared to the Orbitrap Exploris 120 MS and the Orbitrap Exploris 240 MS. This may be more due to pre-analytical factors related to preparation as these were prepared in different labs, as otherwise other samples and data were highly correlated.

Conclusion

The evaluation of the AbsoluteIDQ® p400 kit for the Orbitrap Exploris series of mass spectrometers demonstrated overall good reproducibility and performance through quality controls, NIST reference material, and real plasma samples. The methods produced a robust, quantitative analysis with comparability across the Orbitrap Exploris family of instruments, which was consistent with results reported on Q Exactive MS instruments from the international ring trial.[1] The p400 kit was successfully adapted to the Orbitrap Exploris 120 MS, Orbitrap Exploris 240 MS, and Orbitrap Exploris 480 MS instruments.

References

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2. Phinney, K.W. et al. Development of a Standard Reference Material for Metabolomics Research. *Anal. Chem.* **2013** Dec 17, *85*(24), 11732–11738.

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