ThP 650

High-resolution Quantitative Metabolomics with biocrates' AbsoluteIDQ® p400 HR kit on Thermo Scientific™ Orbitrap Exploris™ Hybrid Mass Spectrometry Platforms

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Abstract

Purpose: To optimize and validate the AbsoluteIDQ® p400 HR (p400 HR) kit, a ready-to-use, standardized, and quantitative protocol for targeted metabolomics study on high resolution, accurate mass Orbitrap Exploris™ (OE) line of mass spectrometers

Methods: The biocrates p400 HR kit was adapted and applied to the measurement of small molecules and lipids through liquid chromatography (LC) and flow injection analysis (FIA) using a Thermo Scientific™ Vanquish™ UHPLC system coupled to OE hybrid MS instruments

Results: The evaluation of the p400 HR kit for the OE series of mass spectrometers demonstrated overall good reproducibility, performance and comparability across OE120, OE240, and OE480 instruments

Introduction

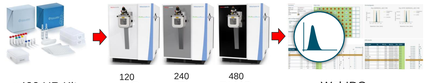
Metabolomics is a powerful tool for measuring the complex interactions of small, biologically relevant molecules, but a lack of standardized sample preparation and analytical protocols continue to be a challenge facing researchers. To address this, the p400 HR kit offers a ready-to-use, standardized and quantitative protocol that targets 43 small molecules and 365 lipids across 11 classes. The kit which incorporates calibration and internal standards, quality controls (QCs), and system test samples, was optimized and validated for the Thermo Fisher Scientific high resolution, accurate mass OE line of mass spectrometers. The NIST standard reference material (SRM) 1950 and human plasma representing male and female samples were evaluated to assess the accuracy, coverage, reproducibility, and performance across instruments.

Materials and methods

Sample Preparation: Calibration standards and plasma-based QCs at three concentrations levels, as well as the SRM 1950 were extracted and prepared according to the standard kit protocol. Identical sample sets were prepared and run on separate plates and measured on OE 120, 240, and 480 platforms to assess intra- and inter-instrument variability.

Test Methods: The p400 HR kit was adapted and applied to the measurement of small molecules and lipids through liquid chromatography (LC) and flow injection analysis (FIA) using a Thermo Vanquish UHPLC system coupled to OE hybrid MS instruments. FIA-MS data was collected in Full Scan mode and LC-MS data was collected in scheduled Full Scan and PRM modes

Data Analysis: Raw data was processed on the WebIDQ workflow management software with subsequent analysis in Excel and R.



p400 HR Kit | Orbitrap Exploris™ MS | WebIDQ

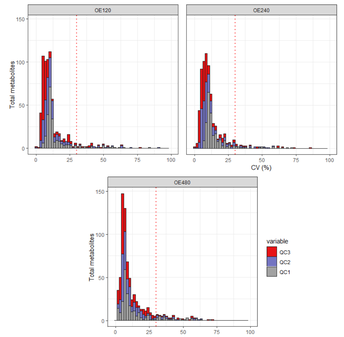
Results

Technical validation

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.

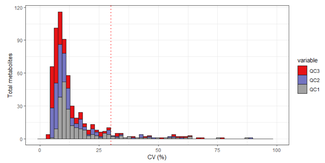
For each OE instrument across all QC levels, the median coefficient of variation (CV) was 9.3% for both OE120 and OE240 and 7.6% for OE480. Figure 1 shows the CV distributions for each QC level by instrument.

Figure 1. Intra-instrument coefficient of variation (CV, %) distribution across all QC levels, the median CV was 9.3% for both OE120 and OE240 and 7.6% for OE480.



The inter-instrument reproducibility was assessed across all three OE instruments. Median CV was 9.7% for all QC levels with average CV <15% for all metabolites. Analytes show good CV performance with most of the detected metabolites measuring well below 30%.

Figure 2. Inter-instrument CV (%) distribution across all QC levels, median CV was 9.7% with average CV < 15% for metabolites.



Biological application

Detectability: Human plasma samples representing average male and female populations as well as NIST SRM 1950 were analyzed to determine average metabolite detectability and coverage across the three instruments. These groups provide insight into approximate results in real samples.

Detectability was defined as the average number of metabolites in each group (male and female plasma n = 15 and NIST SRM 1950 n = 3, per instrument) measured above LOD. Male and female plasma averaged between 230-250 metabolites across all instruments. NIST SRM 1950 averaged 209 metabolites for the OE120 and OE240, and 224 for the OE480.

Figure 3. Detectability in human plasma. Average number of metabolites above LOD in each group (male and female plasma n = 15 and NIST SRM 1950 n = 3, per instrument) . Error bars represent standard deviations.

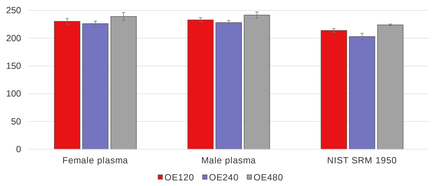


Figure 4. Average metabolite and lipid detectability by class for the three plasma sample types over the three instruments evaluated: OE120, OE240, OE480 (number of metabolites above LOD out of 408 detected in at least 60% of samples across the 3 instruments).

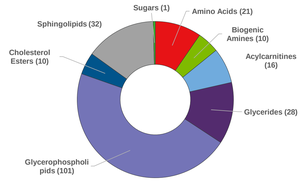
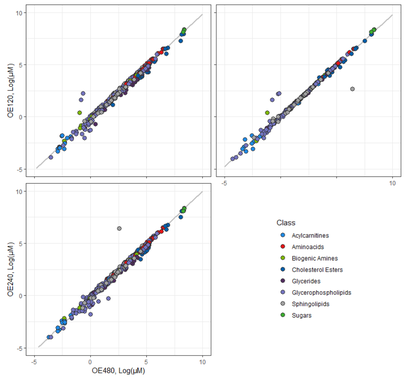


Table 1. Metabolite and lipid panel in the p400 HR kit with total numbers.

Small molecules (43, 3 classes)	Lipids (365, 8 classes)	
– Amino acids (21)	– Acylcarnitines (55)	– Sphingolipids:
– Biogenic amines (21)	– Glycerophospholipids:	– Sphingomyelins (31)
– Sugars (1)	– Phosphatidyl-cholines (172)	– Ceramides (9)
	– Lysophosphatidyl-cholines (24)	– Glycerides:
	– Cholesteryl esters (14)	– Diglycerides (18)
		– Triglycerides (42)

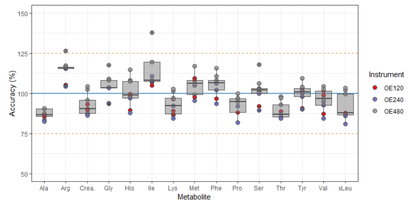
Correlation: Biological samples showed good correlation across instruments for male, female, and NIST SRM 1950 plasma samples. The data shows good trends across all instrument platforms for metabolites measuring above LOD and CV <30% within respective plates. The correlations of OE120 and OE240 to OE480 maintained especially good correlation considering these samples were prepared and run in different laboratories.

Figure 5. Correlation of plasma sample concentrations (averaged technical replicates) of each instrument compared to one another. Colors represent individual compound class.



Accuracy: The measured amino acid and biogenic amine concentrations across the three instruments were evaluated against NIST SRM 1950 values for metabolites where reference information was available[1]. The accuracies showed generally good alignment compared to reported concentrations with measured values differing on average by less than 6% from the expected values.

Figure 6. Amino acid and biogenic amine concentrations compared to NIST SRM 1950 reference values for each instrument.



Conclusions

The evaluation of the AbsoluteIDQ® p400 HR kit on the Thermo Scientific™ Orbitrap Exploris™ series of mass spectrometers demonstrated overall good reproducibility and performance through quality controls, NIST reference material, and human plasma samples demonstrating a successful method adaptation..

The methods produced a robust, quantitative analysis with comparability across the Exploris family of instruments which was consistent with results reported on QE instruments from the international ring trial[2].

References

1. Phinney KW, et al. Development of a Standard Reference Material for metabolomics research. Anal Chem. 2013 Dec 17; 85(24):11732-8.

2. Thompson JW, et al. International Ring Trial of a High Resolution Targeted Metabolomics and Lipidomics Platform for Serum and Plasma Analysis. Anal Chem. 2019 Nov 19; 91(22):14407-14416.

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