Comparison of Metabolite Quantitation by a Triple Quadrupole vs. an Orbitrap-Class Mass Spectrometer

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Abstract

Shortly after the invention (1978)[1] and commercialization (1980's) of triple-quadrupole (QqQ) mass spectrometers, the instruments quickly became the reference platform for targeted quantitation of compounds in complex mixtures. Advantages of modern QqQ instruments include a multitude of ionization sources and scan modes (including timed-SRM), and relative ease of use and low cost. However, QqQ instruments are limited by the need to select specific precursor-to-product ion transitions for targeted quantitation. High-Resolution, Accurate-Mass (HRAM) instruments are able to be quantitative while scanning large mass ranges and provide an alternative strategy for quantitative metabolomics. Here, we compare HRAM-based quantitation of specific metabolites using an Orbitrap Fusion mass spectrometer and an Agilent 6460 QqQ mass spectrometer as the reference method.

Experimental Highlights

- Culture Media: 5% fetal bovine serum and 2 mM glutamine were added to Hyclone RPMI culture media to produce R5 media
- Metabolomic Cell Culture: OVCAR8/ADR ovarian cancer cells were incubated in R5 media at 37 °C at 90% relative humidity and 5% CO_2
- Culture Media Samples: Aliquots of R5 media were collected pre-seeding and at 24-hr, 48-hr, and 72-hr intervals post-seeding
- Metabolites Studied: 2-hydroxyglutarate, 2-oxoglutarate, aspartate, glutamate, glutamine, lactate, pyroglutamate, and pyruvate
- Internal Standards: [$^{13}C_5$]-2-oxoglutarate, [$^{13}C_4$, $^{15}N_1$]-aspartate, [$^{13}C_5$, $^{15}N_1$]-glutamate, [$^{13}C_5$]-glutamine, and [$^{13}C_3$]-lactate
- Neat Calibration standards: 0.0244, 0.0977, 0.390, 1.56, 6.25, 25.0, and 100 µM
- Instrumentation: Thermo Fisher Scientific Orbitrap Fusion and Agilent 6460 QqQ mass spectrometers
- Chromatography:
  Column: EMD Millipore pHILIC (150 x 2.1 mm, 3 micrometer)
  MPA: 90/10 acetonitrile/200 mM ammonium acetate, pH 5.8
  MPB: 50/40/10 acetonitrile/water/200 mM ammonium acetate, pH 5.8

Experimental Description

The instrument platform comparison was performed using sub-aliquots of media samples from ongoing metabolomic studies in our core facility. Media samples were extracted using a protein precipitation protocol followed by reconstitution at the initial mobile phase composition and contained all internal standard components. Neat calibration standards were also prepared at the initial mobile phase conditions with an equivalent amount of internal standard added. The final, reconstituted volume for each media sample was split equally into two sample vials, and was injected with calibration standards on each instrument platform using consistent chromatographic conditions.

The QqQ source and compound-specific acquisition parameters were tuned using post-column infusion of individual tuning solutions and the Agilent Optimizer software package. The Orbitrap Fusion was set with the following acquisition parameters: source voltage: -1,750 volts; full scan range: m/z 60 to m/z 220 in profile mode with Easy-IC turned on; resolution: 240,000. The analytical column and chromatographic gradient were held constant for both systems so that the metabolite retention factors were consistent. Analytical data acquired on the QqQ and Orbitrap Fusion mass spectrometers were processed using the MassHunter Quantitative Analysis software (Ver. B.06.00) and TraceFinder 3.3, respectively.

Results

6460 QqQ Analysis

Metabolite	ISTD	RT (mins)	Curve Range (uM)	Regression (WF)	LOD (S/N ~ 3)	LOQ (S/N ~ 10:1)
2-hydroxyglutarate	2-oxoglutarate-[$^{13}C_5$]	14.5	0.0244 - 100	Linear (1/x^2)	0.0034	0.011
2-oxoglutarate	2-oxoglutarate-[$^{13}C_5$]	14.2	0.0977 - 100	Linear (1/x)	0.074	0.25
aspartate	aspartate-[$^{13}C_4$, $^{15}N_1$]	13.9	0.0244 - 100	Linear (1/x)	0.013	0.045
glutamate	glutamate-[$^{13}C_5$, $^{15}N_1$]	13.5	0.0977 - 100	Linear (1/x)	0.031	0.10
glutamine	glutamine-[$^{13}C_5$]	11.2	0.391 - 100	Linear (1/x)	0.098	0.30
lactate	lactate-[$^{13}C_3$]	6.83	1.56 - 100	Linear (1/x)	0.36	1.2
pyroglutamate	glutamine-[$^{13}C_5$]	8.61	1.56 - 100	Linear (1/x)	0.39	2.7
pyruvate	lactate-[$^{13}C_3$]	4.30	1.56 - 100	Linear (none)	0.25	0.84

Orbitrap Fusion Analysis

Metabolite	ISTD	RT (mins)	Curve Range (uM)	Regression (WF)	LOD (S/N ~ 3)	LOQ (S/N ~ 10:1)
2-hydroxyglutarate	2-oxoglutarate-[$^{13}C_5$]	14.5	0.0977 - 100	Quadratic (1/x^2)	0.059	0.020
2-oxoglutarate	2-oxoglutarate-[$^{13}C_5$]	14.2	0.0977 - 100	Quadratic (1/x^2)	0.030	0.098
aspartate	aspartate-[$^{13}C_4$, $^{15}N_1$]	13.9	0.0977 - 100	Linear (1/x^2)	0.059	0.20
glutamate	glutamate-[$^{13}C_5$, $^{15}N_1$]	13.5	0.0977 - 100	Quadratic (1/x^2)	0.059	0.20
glutamine	glutamine-[$^{13}C_5$]	11.2	0.0977 - 100	Linear (1/x^2)	0.059	0.20
lactate	lactate-[$^{13}C_3$]	6.83	0.0977 - 100	Linear (1/x)	0.044	0.15
pyroglutamate	glutamine-[$^{13}C_5$]	8.61	0.390 - 100	Linear (1/x)	0.086	0.29
pyruvate	lactate-[$^{13}C_3$]	4.22	0.0977 - 100	Quadratic (1/x)	0.059	0.20

Table 1. Metabolite Chromatographic Retention Time (RT), curve range, regression model and weighting factor, and estimated analytical detection limits for the two instrument platforms, which were estimated from calibration standards near the lowest level neat calibraiton standard (S/N > 5:1).

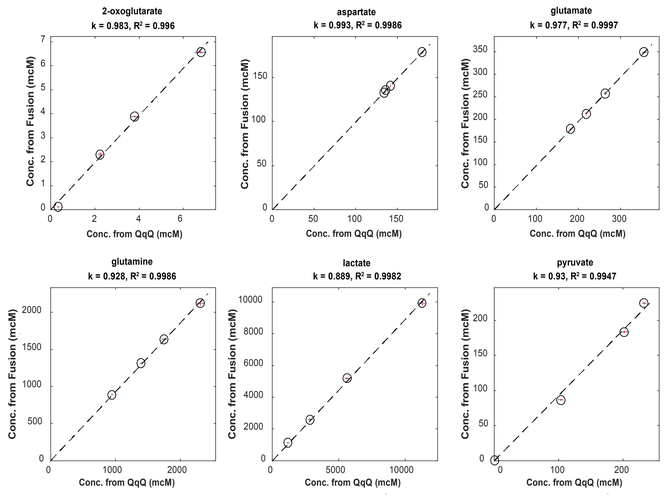

Figure 1. Correlative analysis of six metabolites. T-tests were performed on the slopes and R-squares with a null hypothesis that they are equal to 1.0. For all six metabolites shown, R-squared is equal to 1 with a p-value of 0.063 > 0.05 and a confidence interval of [0.9962 1.0001]. The average slope of all plots is equal to 1 with a p-value of 0.066 > 0.05 and a confidence interval of [0.9152, 1.0043] if lactate is excluded.

Discussion

- Table 1: The calibration range includes only those neat standards that had a S/N>5:1 for each metabolite.
- Table 1: The Limit of Detection (LOD) and Limit of Quantition (LOQ) were estimated from neat calibration standards for S/N ratios of approximately 3 and 10, respectively.
- Table 1: The LOD and LOQ are generally better for the Orbitrap Fusion than for the Agilent 6460 QqQ mass spectrometer across the spectrum of metabolites and calibration ranges studied.
- Figure 1: 2-oxoglutarate, aspartate, glutamate, glutamine, and pyuvate levels exhibited strong correlation coefficients across the two instrument platforms, and absolute quantitation was consistent for all media samples across time points.
- Figure 1: Lactate and glutamine levels were above the limit of quantitation (ALQ) for all samples at the 10x dilution factor used for this study. Better correlation between the measurements probably could have been achieved by diluting the samples further for these analytes.
- Comparative analysis for 2-hydroxyglutarate and pyroglutamate were omitted because of the presence of severe ion suppression by co-eluting matrix compenents.

Conclusions

We compared the Agilent 6460 triple quadrupole and Orbitrap Fusion mass spectrometers in terms of dynamic range, regression model and weighting factor, and detection limits for eight individual metabolites present in neat calibration standards and in used cell culture media samples harvested in an ongoing study in our lab. In general, the two platforms yielded comparable results for six of the eight metabolites studied. All lactate and glutamine levels were measured Above the Limit of Quantitation (ALQ) for the 10x dilution factor used; further dilution of these samples may have provided better correlation for these metabolites. Comparative analysis for 2-hydroxyglutarate and pyroglutamate were excluded from this study because of the presence of co-eluting matrix compounds present in the used culture media that produced severe ion suppression. Based on these insights, alterations will be made to the bioanalytical method to make conditions more favorable for the quantitation of these two metabolites.

Overall, these preliminary results suggest that high-resolution, accurate mass instruments appear to provide a quantitative capability that is comparable to that of triple quadrupole instruments for metabolomics based studies. Our labatory will continue to investigate these comparisons with an extended set of metabolites in a variety of different matricies (e.g., blood, plasma, urine, and tissue).

Future Directions

- Investigate the influence of source parameters on the Orbitrap Fusion and their effects on reliable quantitation.
- Evaluate additional metabolites using the existing workflow.
- Test performance across a variety of matrices including whole blood, plasma, urine, tissue and cell extracts.
- Multiplex HRAM-based targeted method with non-targeted workflows.

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References

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