High-throughput metabolite quantification for synthetic biology

Sensitive and robust results using the Echo® MS System

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Synthetic biology is a rapidly maturing interdisciplinary field that supports delivery of sustainably-produced products across many application areas, including human health (therapeutics, adjuvants), nutrition (vitamins, zero-calorie sweeteners, meatless meat), personal care (cosmetics, fragrances), agriculture (fertilizers), industrial chemicals (detergents, monomers), fuels and many others.[1,2] Products that are currently inaccessible due to cost, quality or supply constraints are now accessible at higher quality, at lower cost, and from an unconstrained supply.[3]

These developments are in large part due to technological improvements in the rapid genetic engineering of multiple types of host organisms, high-throughput culture, and analytical screening.[4,5,6] Currently, chromatography-based separation techniques (such as LC-MS/MS) are the most utilized approaches for strain analysis. However, these technologies are often not rapid enough to analyze large biological libraries, which can contain 10^5 entities or more, on a practical time scale.[7] With this advancing field comes the demand for an advanced technology to rapidly profile and quantify several analytes across a very large number of samples in a high-throughput approach.[8] The Echo MS System combines Acoustic Ejection Mass Spectrometry (AEMS) with the SCIEX Triple Quad™ 6500+ system to provide an integrated solution with the capability of quantifying approximately 1 sample per second.[9] The system delivers reproducible nanoliter-sized droplets from a complex matrix, in a 384- or 1536-well plate format, into a solvent stream for direct analysis by MS. This system provides the speed and accuracy to help circumvent the chromatographic bottleneck of high-throughput analysis.

Here, a quantitative approach for the rapid screening of 90 yeast strains using the Echo MS System was demonstrated, monitoring over 60 metabolites. The goal of publishing this workflow is to demonstrate the ability of the Echo MS System to rapidly screen a biological matrix for key metabolites of interest with significantly reduced analysis times (Figure 1).

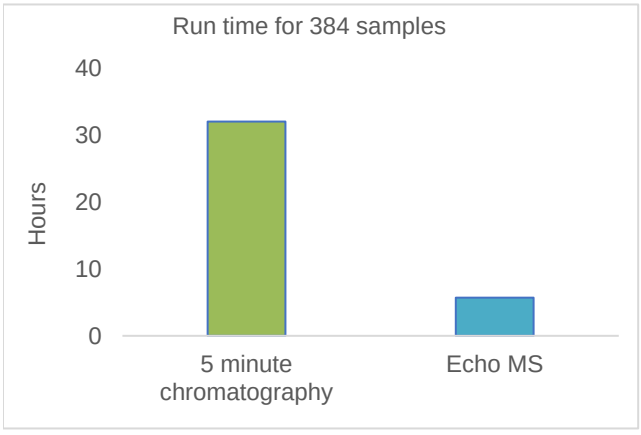

Figure 1. Reduction in analysis time. The speed of the Echo MS System allows for a reduction of analysis time by over 5x as compared to a similar experiment performed with LC-MS.

Key features of the Echo MS System for high throughput metabolomic quantification

- Rapid analysis of 67 endogenous metabolites in yeast extract in a 384-well plate in under 6 hours
- One sample analyzed every 3 seconds
- Low acoustic ejection sample volumes used - one 50 nL ejection per well for each method
- Good linearity demonstrated with excellent accuracy and reproducibility
- Minimal carryover due to AEMS and open port interface (OPI)

Methods

Sample preparation: *Saccharomyces cerevisiae* was grown in 4% sucrose media for 30 hours, quenched in cold methanol to halt metabolism, then spun down to separate extra- and intra-cellular matrices. Intracellular metabolites were extracted with 75:25 organic solvent/buffer solution. Both matrices were further diluted with water for a final organic solvent content of 50%, then filtered via a 3kD molecular cutoff membrane. Samples were stored at -70ºC until analysis.

Authentic standards were prepared separately in 50:50 methanol/water, then pooled per acquisition method and diluted in 50:50 methanol/water to 3 µg/mL, the top calibration concentration. Standard solutions were then serially diluted in the same diluent across a concentration range of 0.003 – 3 µg/mL. A total of 15 methods were used for the targeted panel.

Acoustic ejection method: Methanol was used as the carrier solvent at a flow rate of 425 µL/min. A volume of 50 nL (20 droplets) was acoustically ejected into the system.

Mass spectrometry: The Echo MS System coupled with the SCIEX Triple Quad 6500+ system. SCIEX OS Software 1.6.10 was used to control the system.

Data processing: Peak areas and calculated concentrations were generated using SCIEX OS Software.

Accuracy and reproducibility of quantification

The MRM transitions for the selected metabolites of this study were optimized and then monitored in the yeast samples using the Echo MS System. Amino acids, nucleosides, organic acids, and other classes were included in the panel. A total of 15 methods were created in both positive and negative polarity to cover the 62 MRM transitions that monitored 67 metabolites. Isobars (leucine and isoleucine, for example) were monitored by a single MRM transition. There is no LC separation on the Echo MS System. Because the 62 MRM transitions account for a total of 67 metabolites, 5 transitions were reported as a sum for the isobar pair.

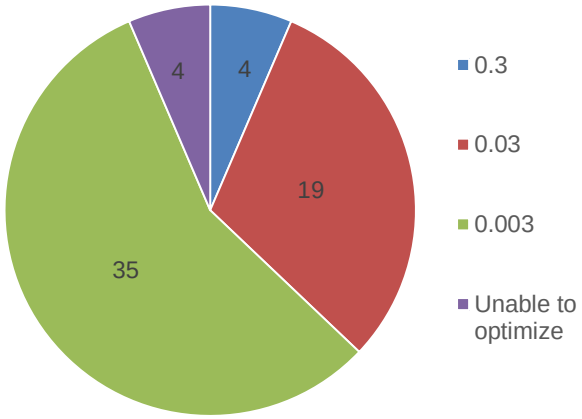

Figure 2. Distribution of limits of quantification. The number of analytes that hit the limit of quantification for each concentration is shown. The majority of analytes were detectable at the lowest concentration of 0.003 µg/mL.

The total run time required to analyze the 67 metabolites for each 384-well plate was just over 5.7 hours (including acquisition overhead) when using the Echo MS System. In contrast, the same analysis required 32 hours when using a 5 minute UHPLC-MS/MS screening method. Thus, the Echo MS approach significantly reduced the amount of time needed for this metabolite screen, ~5.6x faster than a UHPLC-MS approach (Figure 1).

Good sensitivity of detection of the standards was observed, with 35 out of the 67 metabolites detected at 0.003 µg/mL, the lowest concentration tested (Figure 2). An example of the quantitative accuracy is shown in Table 1 for ornithine across the 3 orders of linear dynamic range that was tested. All %CV values reported are less than ten percent for 3 replicate ejections from a well. Example extracted ion chromatograms (XICs) for ornithine are shown in Figure 3. Accuracy for the assay was measured at each concentration for each analyte, and the acceptance criteria was set to +/- 20 percent.

To illustrate reproducibility for other compounds, an example of sequential triplicate ejections at 0.003 µg/mL is shown in Figure 4. Valine, leucine/isoleucine, cysteine, and homoserine/threonine represent four MRM transitions that were monitored in a single method.

Table 1. Peak area and accuracy results for ornithine. 3 replicate ejections were performed at each standard concentration and each dilution in diluent. Very good reproducibility was observed across the dataset.

Concentration	Dilution	Avg peak area	%CV	Accuracy (%)
3 µg/mL	1x	974450	4.57	99.15
0.3 µg/mL	10x	10700	7.28	108.83
0.03 µg/mL	100x	9602	4.77	97.42
0.003 µg/mL	1000x	959	5.25	94.61

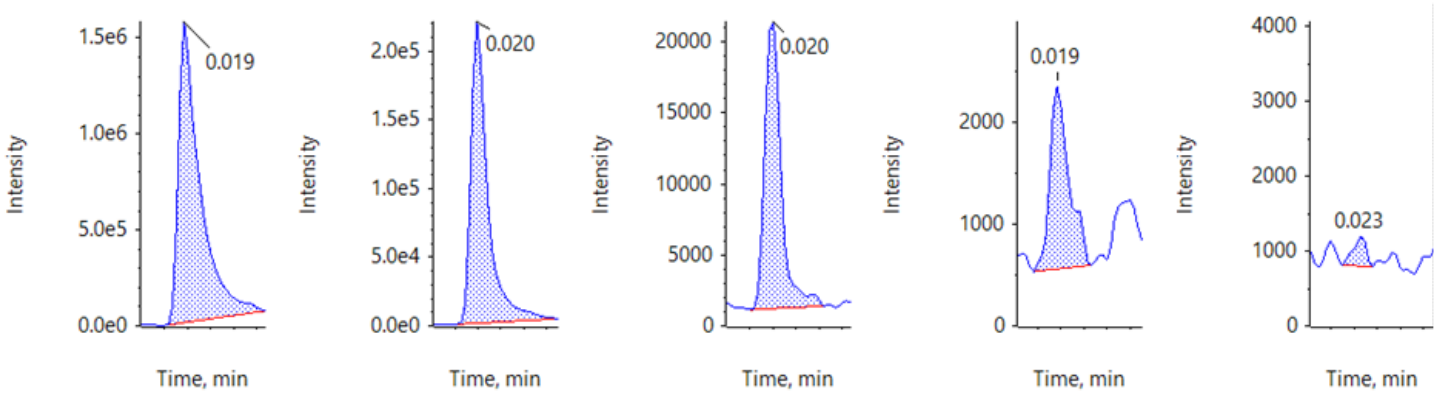

Figure 3. Raw MS data for dilution series of ornithine. Three replicates were analyzed for each concentration; one replicate is shown here. Concentration range was from 3.0 µg/mL down to 0.003 µg/mL (left to right) with the last ejection shown being the 50% methanol blank ejection. All data was acquired with a 50 nL droplet ejection method. >90% reproducibility was achieved for all four concentrations.

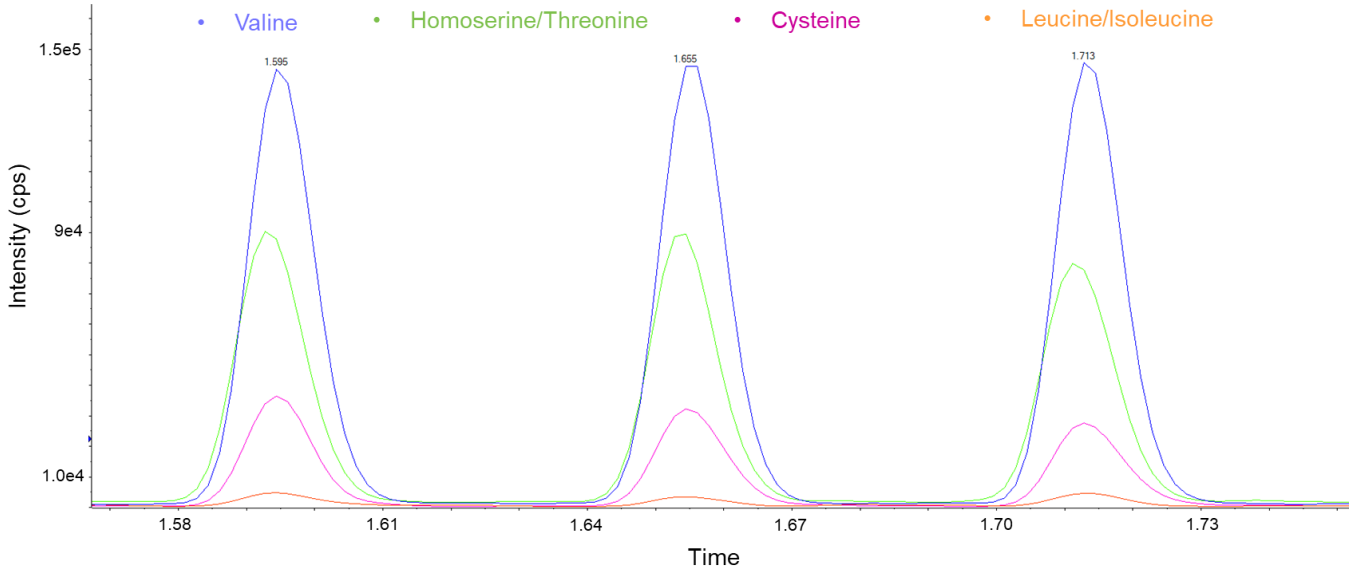

Figure 4. Excellent reproducibility. The overlaid ejection traces for 4 compounds (valine, homoserine/threonine, cysteine and leucine/isoleucine) are shown at a concentration of 0.003 µg/mL. Percent CV of each compound is less than 10 percent for triplicate ejections (data not shown).

Yeast strain analysis

Separate 384-well plates of intracellular and extracellular yeast matrices were screened for this study. Plates were provided in eight of the 96-well format and transferred into two of the 384-well format. 90 different yeast strains were each analyzed for all 67 metabolites. Figure 5 illustrates a heat map of metabolite peak areas from both matrices for valine, homoserine/threonine, cysteine, and leucine/isoleucine shown in a 96-well plate format for reporting. At 3 seconds per sample, the dataset for these chosen analytes for one 384-well plate took just 19.2 minutes. Concentrations for these metabolites are reported in µg/mL. Blank samples are shown in wells H12.

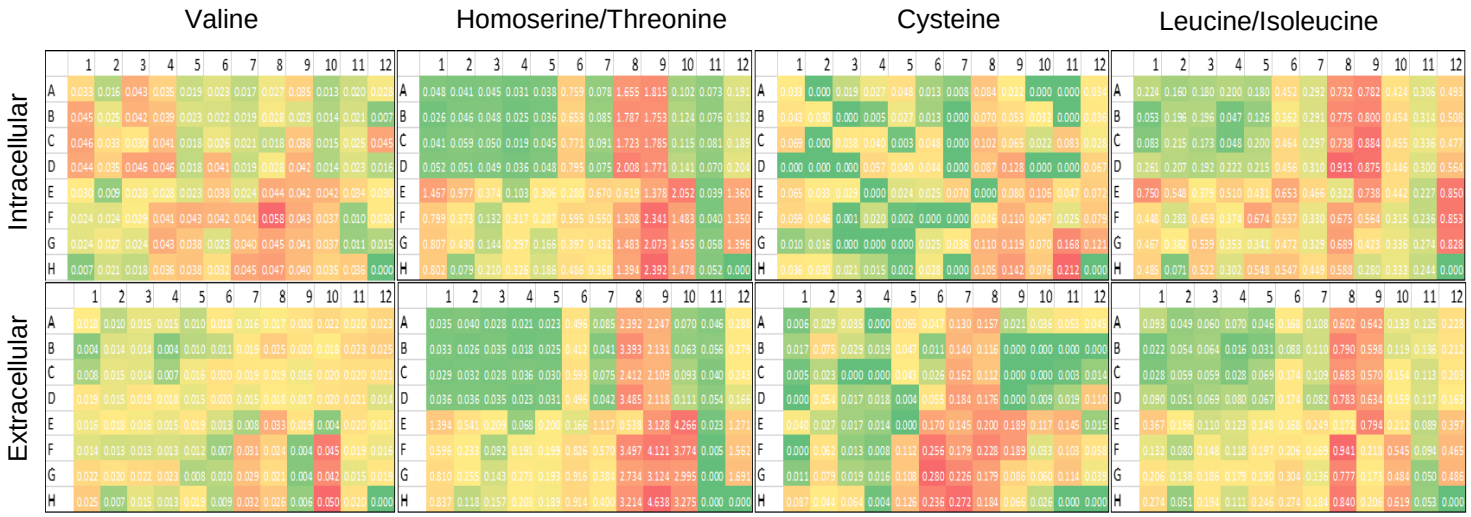

Figure 5. Variation in calculated concentrations for selected metabolites across the 90 yeast strains. Heat maps for calculated concentrations of valine, homoserine/threonine, cysteine, and leucine/isoleucine in intracellular (top row) and extracellular (bottom row) yeast matrix shown in 96-well plate format. The entire dataset for these four metabolites across two sets of 384 sample wells took 38.4 minutes of acquisition time (not including the standard plate acquisition overhead).

Conclusions

The utility of the Echo MS System to rapidly profile and quantify synthetic biology samples has been demonstrated here. The intracellular and extracellular matrices for 90 different yeast strains were analyzed and a total of 67 metabolites were monitored in about 5.7 hours. This high-throughput, robust workflow consumed a total of 750 nL of sample volume per well. The amount of analysis time saved was considerable. The Echo MS System analysis was completed 5.6x faster than the traditional LC-MS analysis. The reproducibility and sensitivity demonstrated are sufficient for strain profiling, making the Echo MS System workflow a powerful solution for synthetic biology.

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