

## Rapid quantitative analysis of fermentation broth samples to assess efficiency of engineered yeast strain turnover

### Using Acoustic Ejection Mass Spectrometry on the Echo<sup>®</sup> MS System

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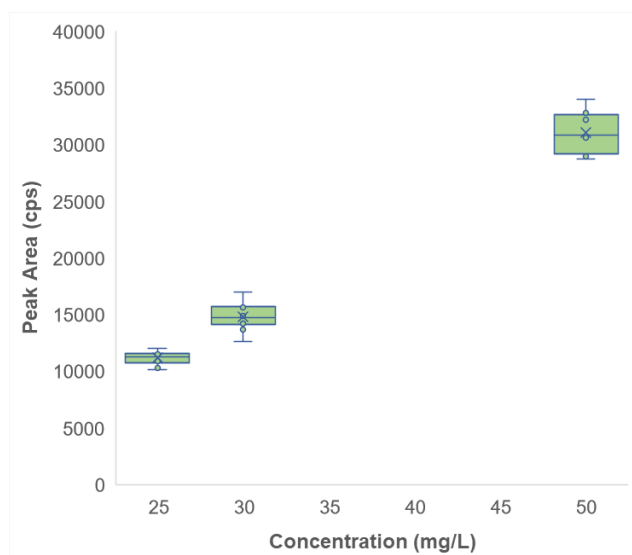
The field of synthetic biology has grown exponentially in recent years due to advances in genetic engineering, microbiology, biochemistry and molecular biology among various other disciplines. The goal is to engineer microbes to create a desired product; therefore the efficiency of production must be optimized through both selection of proper environmental conditions and selection of the highest producing strain of the organism. Many thousands of strains might need to be developed and assessed before selection of one for scale up and use in commercial production. This requires the quantitative analysis of the desired product in many thousands of samples; and this needs to be done as rapidly as possible to enable iterative strain refinement.

The SCIEX Echo MS System is a very high throughput, electrospray ionization mass spectrometry-based system that uses Acoustic Droplet Ejection (ADE) to transfer a precisely controlled sample droplet from a well plate into the liquid stream of the Open Port Interface (OPI), which is directly coupled to the SCIEX Triple Quad™ 6500+ Mass Spectrometer. This analytical technique has the sensitivity and compound coverage of



electrospray mass spectrometry with the ability to analyze a sample per second, in a non-contact, virtually carryover-free manner. The small droplet size (2.5nL) provides a significant dilution of matrix suppressing factors, which can be present in complex samples, that helps to reduce preparation needed prior to analysis.

Here, the ability to detect a target compound directly out of yeast fermentation broth, with minimal sample preparation, was investigated to assess the utility of the Echo MS System for fast screening of strains in a synthetic biology workflow.



**Figure 1. High reproducibility in quantification.** The ability to precisely quantify small differences in concentration of an analyte in broth is critical during strain screening. Here, 10 replicates at each concentration of angiotensin in broth were analyzed out of the 1x dilution sample. Statistically significant differences between the 25 and 30 mg/L samples were observed.

### Key features of Echo MS System for strain selection

- Samples were analyzed at 1 sec/sample, achieving high throughput for screening large numbers of samples
- Accurate quantification enabled differentiation between small differences in concentration of product (Figure 1)
- Minimal sample preparation was required to achieve the required throughput and quantitative accuracy
- Sensitivity of the Echo MS System provided high-quality data at all concentrations tested

## Methods

**Sample preparation:** Yeast fermentation broth samples, spiked with angiotensin (DRVYIHPFHL, MW=1295.6) were generously supplied by a collaborator. The samples were prepared by lysing the active broth with 4:1 acetonitrile/water and adding angiotensin standard to represent 25, 30 and 50 mg/L angiotensin in broth. The samples were then diluted to 40% acetonitrile/60% water by volume to 1x, 10x and 100x, centrifuged for 15 mins at 14000 rpm and transferred to a 96-well plate for shipping. Samples were stored at -40 °C until analysis.

Prior to analysis on the Echo MS System, 50 µL of each sample was transferred to an Echo 384-well plate (Beckman Life Sciences 384PP 2.0 Microplate).

**Acoustic Ejection method:** Development of an optimal acoustic droplet ejection (ADE) method – the parameters involved in generating and transferring a sample droplet in the sample well to the ESI source, involves a few parameters. First is selection of an appropriate carrier solvent, analogous to the mobile phase in HPLC. 60:40 acetonitrile/water with 0.1% formic acid was selected for this work, and the flow rate was optimized to 450 µL/min.

**Mass spectrometry:** Echo MS System includes the SCIEX Triple Quad™ 6500+ LC-MS/MS System, and is controlled by SCIEX OS Software. Angiotensin (DRVYIHPFHL) was monitored using a single MRM transition (649.1 → 784.4 m/z, CE=40, Dwell time = 30 msec). Source parameters for the OptiFlow® Ion Source were GS1 of 90, GS2 of 70 and temperature of 600 °C.

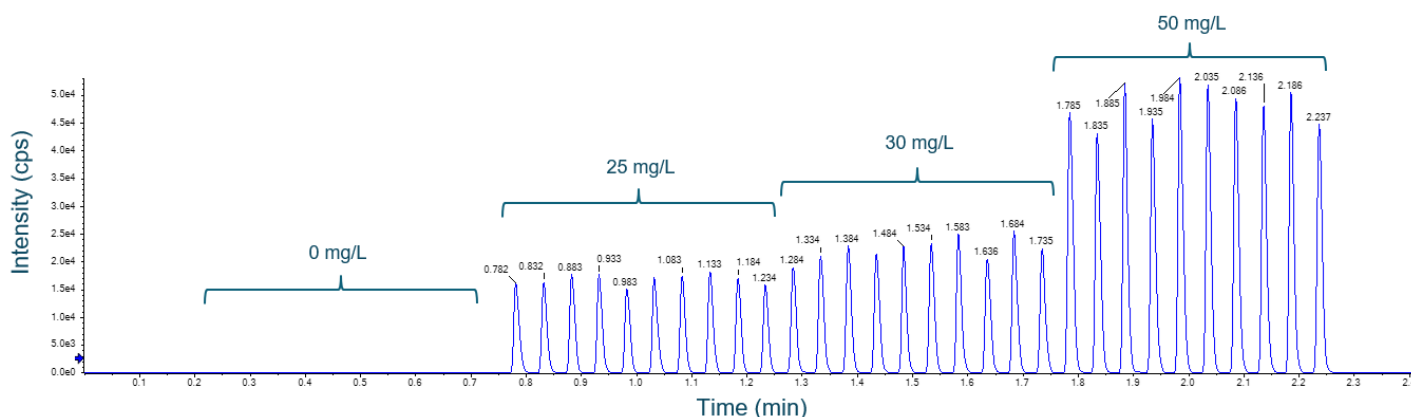
**Data processing:** Peak areas were integrated using SCIEX OS Software.

**Table 1. Peak area and reproducibility results.** 10 replicate ejections were performed at each concentration and each dilution. Very good reproducibility was observed across the dataset.

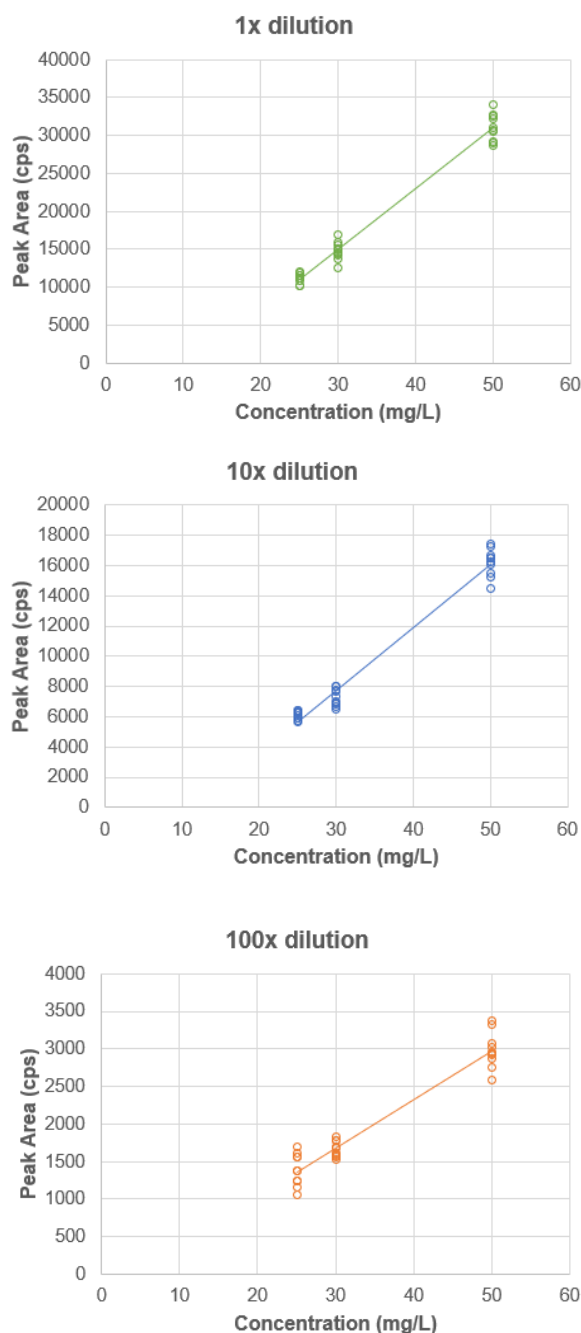
Concentration in broth	Dilution	# of droplets	Avg peak area	Peak area %CV
25 mg/L	1x	1	11174	5.2
30 mg/L	1x	1	14787	7.9
50 mg/L	1x	1	31078	5.5
25 mg/L	10x	4	6106	4.2
30 mg/L	10x	4	7269	7.5
50 mg/L	10x	4	16202	5.2
25 mg/L	100x	10	1386	14.6
30 mg/L	100x	10	1664	5.7
50 mg/L	100x	10	2982	7.5

## Accuracy of quantification

Samples were provided as a series of three 10x (1x, 10x and 100x) dilutions of three concentrations of angiotensin in broth (25, 30 and 50 mg/L). Samples were analyzed in replicates of 10 to show reproducibility of the AEMS technique in this matrix (Figure 2). Very high reproducibility was observed across all dilutions, even the least dilute sample (1x), indicating that minimal sample preparation could be used in this type of experiment to achieve desired results (Table 1). In addition, the reproducibility was high enough to easily distinguish between the same concentration differences used here (Figure 1), which is key when making critical decisions during strain selection.



**Figure 2. Raw MS data for angiotensin peptide in undiluted 1x sample.** Ten replicates were analyzed for each concentration, acquired with a single droplet ejection method. Very high reproducibility was achieved for all 3 concentrations with minimal preparation of the sample (Table 1).



**Figure 3. Concentration curve for angiotensin in all three broth dilutions.** Good linearity and reproducibility were observed for the sample concentrations out of the minimally prepared samples.

Linearity and precision is shown for the three dilution series in Figure 3. As a sample is more diluted, analyte signal can be increased by increasing the droplet count for sample ejection. For the 10x diluted samples, a 4 droplet method was used to achieve good results. For the 100x diluted samples, a 10 droplet method was used, also with good results, although the lowest point for that series is showing higher variability than might be desired. But in all 3 dilutions, good linearity was observed.

## Conclusions

The results of this study show that the Echo MS System is capable of rapidly producing high-quality results for the analysis of a target peptide in a minimally prepared, common fermentation broth matrix. The rapid analysis time of this platform will enable faster generation of quantitative data to assess microbe strain efficiency, which should allow for more rapid strain selection removing a costly bottleneck in the important and growing field of synthetic biology.

## References

1. Rapid MS/MS analysis with Acoustic Ejection Mass Spectrometry (AEMS) - Using the SCIEX Echo<sup>®</sup> MS System to break bottlenecks in quantitative mass spectrometry throughput. SCIEX technical note RUO-MKT-02-11385-A.

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