



Echo® MS System for High-Throughput Analysis

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LC-MS
data quality

Plate-reader
speed

Echo[®] MS

Direct sampling from high-density microplates

Low running cost



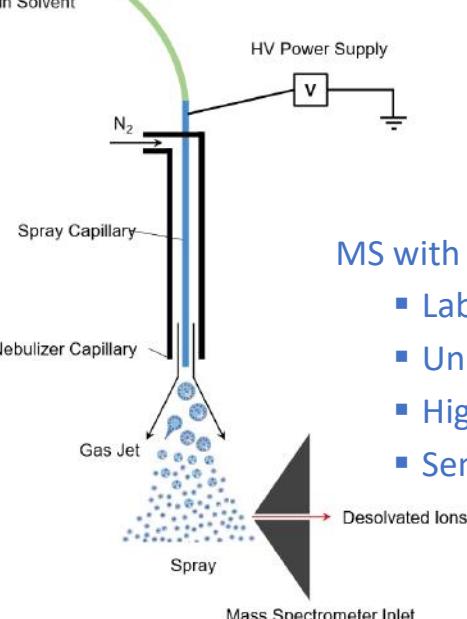
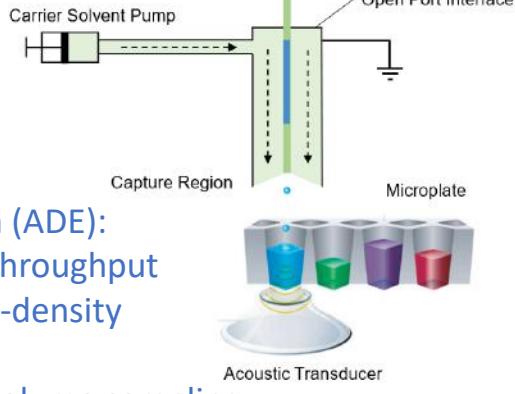
Minimum sample preparation or method development

High robustness without carryover

Wide assay coverage and
high versatility

Open-Port Interface (OPI):

- direct liquid transferring, no sample loss or carry over
- Significant matrix dilution, allowing the direct ejection of complex samples



MS with ESI ionization:

- Label free
- Universal detector
- High ionization efficiency
- Sensitivity and robustness

Acoustic Droplet Ejection (ADE):

- non-contact, high-throughput sampling from high-density microplates
- precision and low volume sampling

AEMS Workflow

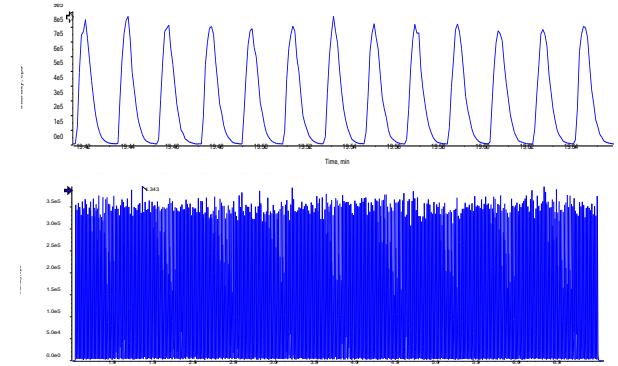
Echo® MS System



Echo® MS System Performance

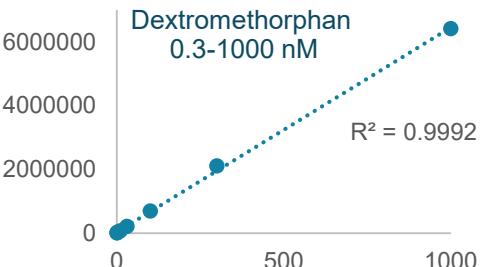
Echo® MS System

Throughput and Reproducibility



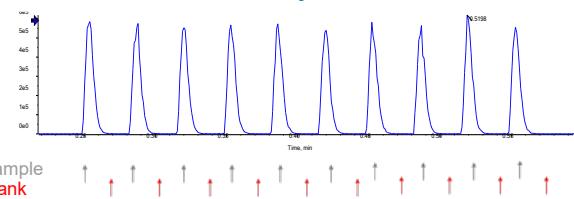
Baseline separation at 1 Hz (one sample per second)
Full-plate (384) CV = 1.98 %

Sensitivity and Linear Dynamic Range



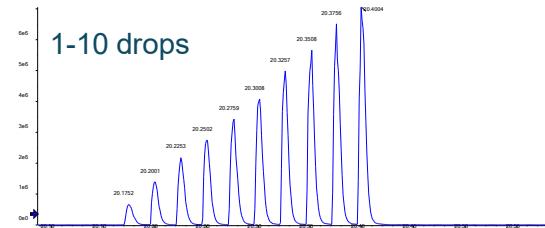
Single digit nM sensitivity for small molecules
>3 orders of the magnitude for LDR, even without IS

Zero Carryover



Sample Blank

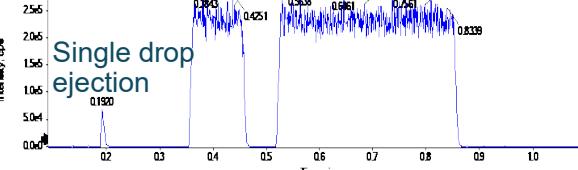
Flexible Ejection Volume



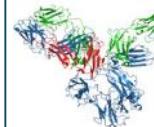
Flexible Ejection Duration

Infusion for different periods

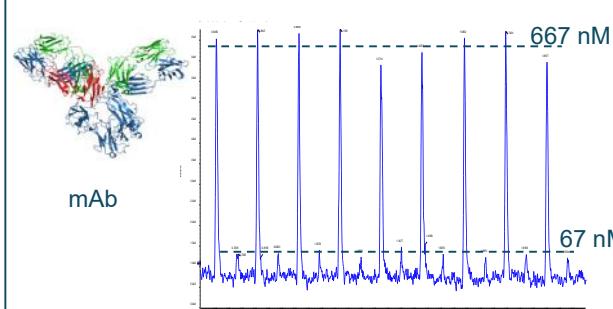
Single drop ejection



Wide Compound Coverage

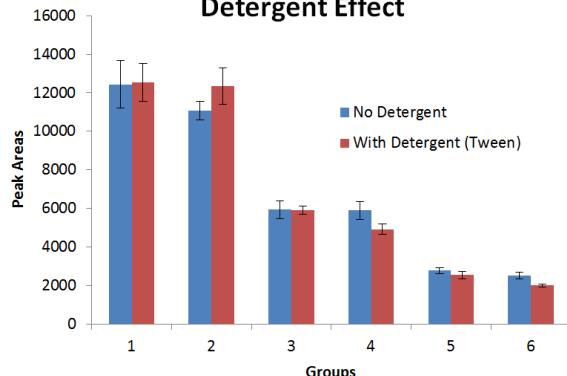


mAb



Matrix Tolerance

Detergent Effect



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The Power of Precision

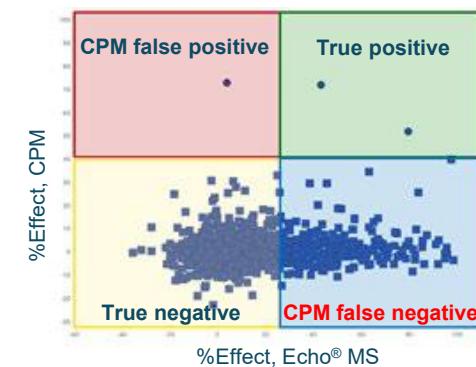
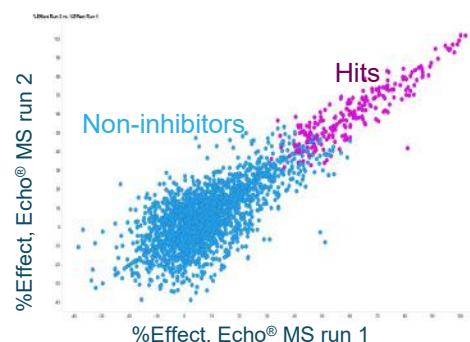
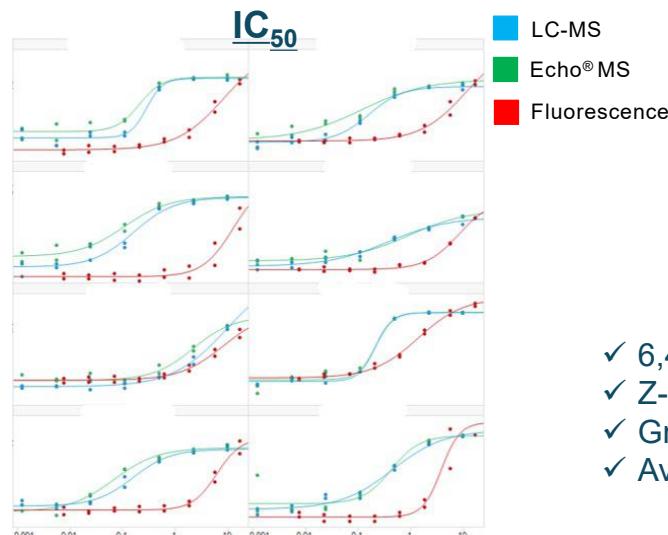
Echo® MS for High-Throughput Screening

Echo® MS System

DGAT2 Assay – DGAT2 is a membrane protein catalyzing the rate limiting step in triglyceride synthesis



- Dilemma of choosing the analytical platform for the primary screening
 - CPM Fluorescence: high throughput but tends to be less potent compared to the label-free MS results. High risk for false negatives.
 - LC-MS: high confident readout but much lower throughput. Cannot screen a big sample set.
- Echo® MS provides both high throughput and high confident results.



- ✓ 6,400 compounds for the primary screening, selected based on chemical diversity
- ✓ Z-prime = 0.69, S/B = 30
- ✓ Great reproducibility on the assessment of %effect
- ✓ Avoided the false negative from the fluorescence assay

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SCIEX
The Power of Precision

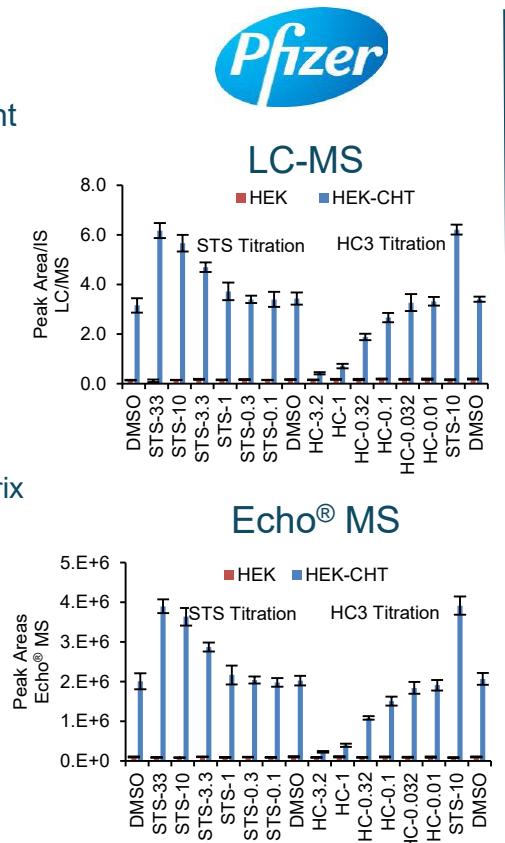
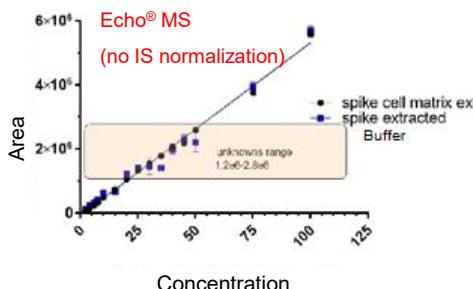
D. McLaren, SLAS 2020

Echo® MS for High-Throughput Screening



Cell uptake assay

- Echo® MS vs LC-MS on the assessment of choline transporter uptake function
 - Superimposable results
 - >10x times faster
 - >1000x less sample consumption
 - Tighter CV even without internal standard
 - No ionization suppression for cell matrix
 - No sample preparation

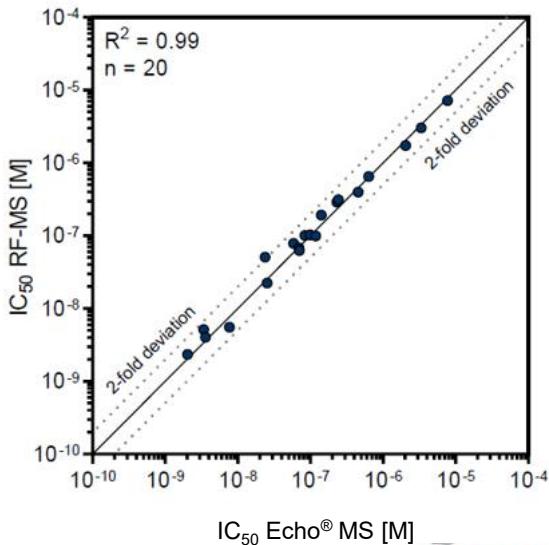


Enzyme inhibitor assay (CNS)



Boehringer
Ingelheim

- ✓ Echo® MS provided the same IC_{50} information as RapidFire, while consumed much less sample, and was much faster.

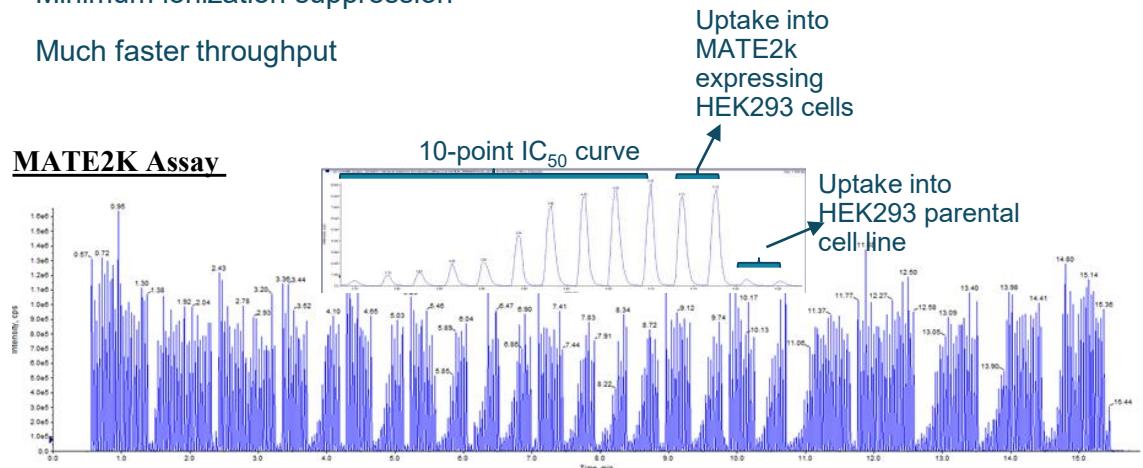


Echo® MS for High-Throughput ADME

Echo® MS System

Transporter Assay

- Very polar compounds such as metformin is often used as the substrate of multiple transporter assays.
- It is a significant challenge to develop the LC-MS workflow due to the poor chromatographic retention, resulting in the severe ionization suppression from co-eluting matrix.
- Echo® MS solved the problem
 - Minimum ionization suppression
 - Much faster throughput



Compound	LC-MS/MS IC ₅₀ (μ M)	Echo® MS IC ₅₀ (μ M)
1	23.61	25.55
2	>50.00	>50.00
3	>50.00	>50.00
4	>50.00	>50.00
5	>50.00	>50.00
6	>50.00	>50.00
7	>50.00	56.80
8	>50.00	>50.00
9	>50.00	>50.00
10	38.61	>50.00
11	36.17	36.67
12	>50.00	>50.00
13	>50.00	>50.00
14	>50.00	>50.00
15	>50.00	>50.00
16	>50.00	>50.00
17	0.54	0.47
18	0.28	0.29
19	0.32	0.36
20	>50.00	>50.00
21	0.10	0.10
22	1.53	1.51
23	>50.00	>50.00
24	>50.00	>50.00

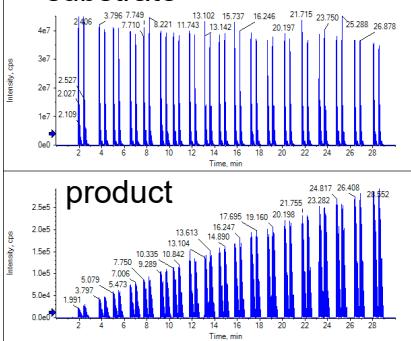
Echo® MS for High-Throughput ADME



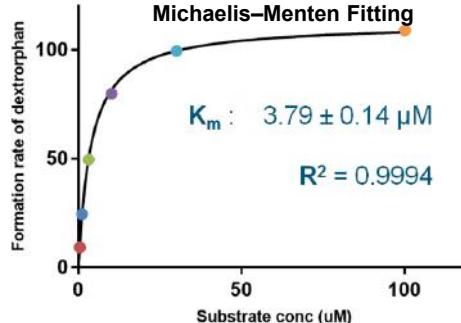
Metabolic stability assay, in-situ kinetics



substrate



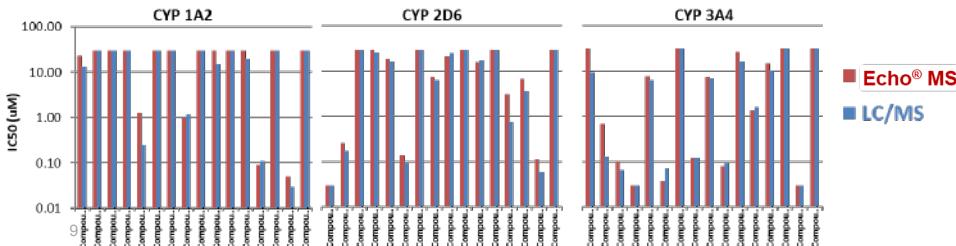
product



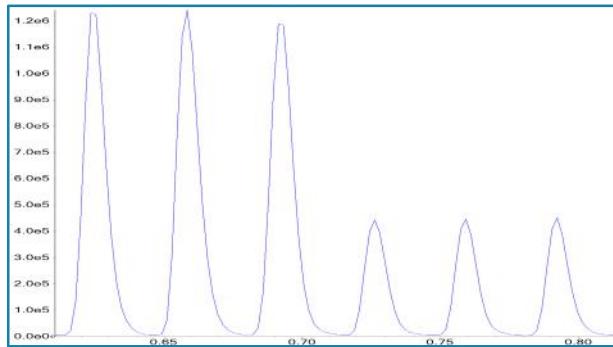
Live monitoring, no sample prep or clean up needed

CYP inhibition assay

IC₅₀ Comparison



Protein binding assay



- Echo® MS vs LC-MS
 - Superimposable results
 - >10x faster
 - >1000x less sample consumption
 - No sample cleanup
 - In-situ kinetics

Echo® MS for Bioanalysis

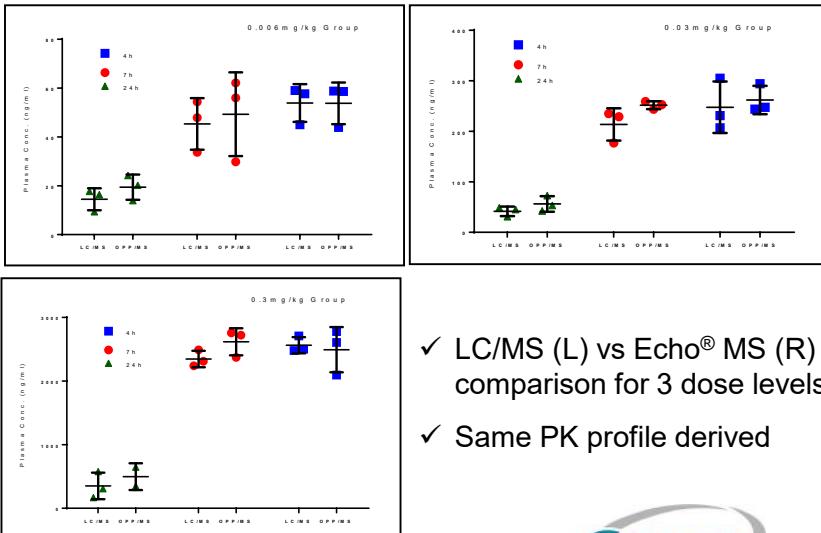
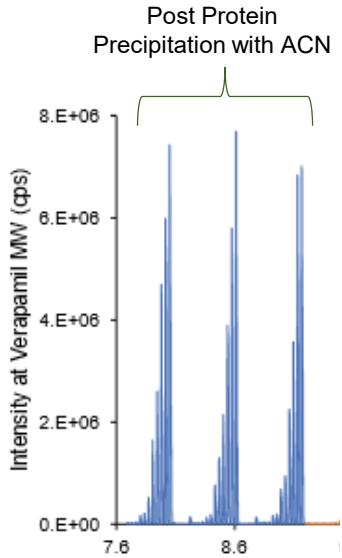
Echo® MS System

Plasma pharmacokinetic (PK) study



- The matrix tolerance of Echo® MS allows the direct analysis of plasma samples (even with PEG) without tedious sample preparation (protein precipitation, centrifuge, etc.)

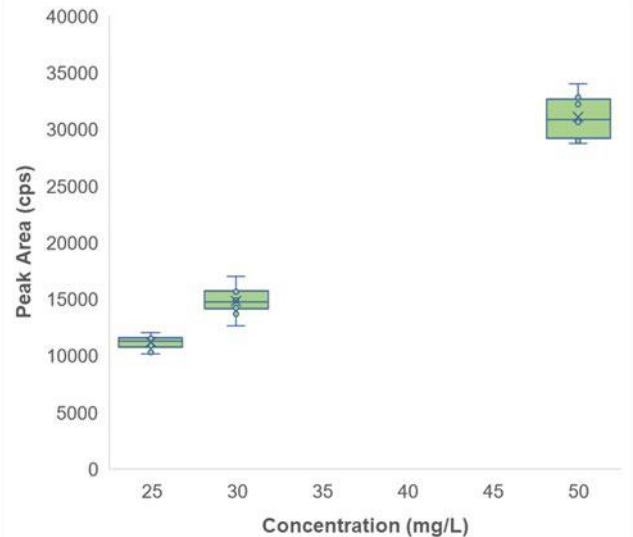
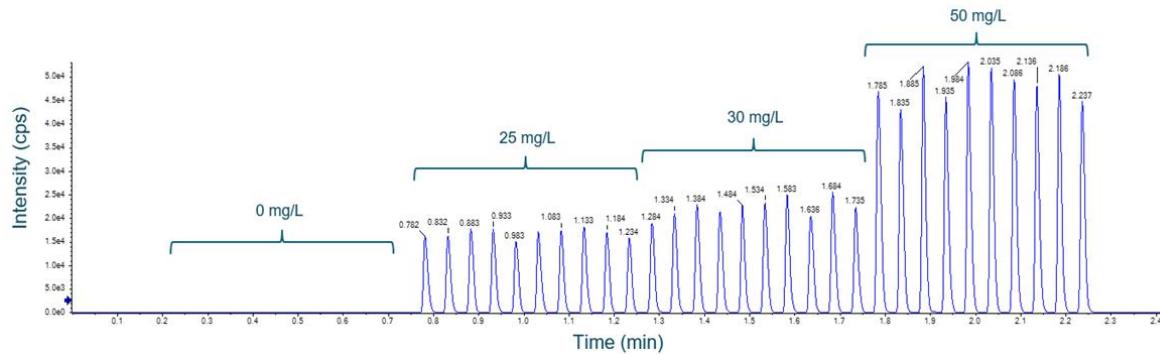
Verapamil in Plasma: 4nM – 20μM



- ✓ LC/MS (L) vs Echo® MS (R) comparison for 3 dose levels:
 - ✓ Same PK profile derived

H. Zhang, et al. bioRxiv doi: <https://doi.org/10.1101/2020.01.28.923938>; R. Kern, et al. Sciex Tech Note

Synthetic biology – direct analysis of fermentation broth to assess efficiency of yeast strain turnover



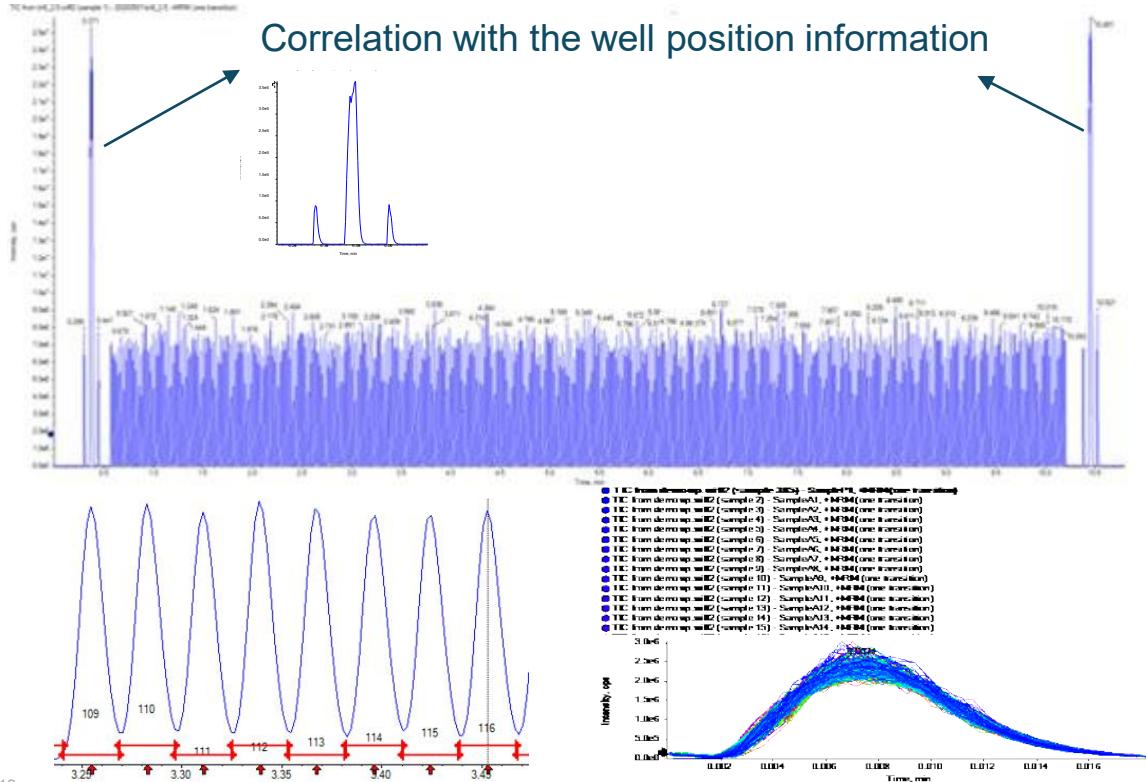
Echo® MS enables

- Direct analysis from yeast fermentation broth without sample cleanup
- Enough sensitivity covering the required concentration range
- Great reproducibility allowing the statistically distinguish between small differences in concentration of angiotensin in broth

Echo® MS Data Processing and Output



Automated data processing



Auto-generation of the result table

SampleNa	Well	Posit	Compound	Peak Area	Peak Heig	Peak Width
test	A1		Dextrometophan	287547.3	551142	0.016333
test	A1		Olanzapine	625918	1267019	0.016333
test	A1		Propranolol	145421.2	242126	0.016333
test	A1		Terfenadine	67407.34	150055	0.016333
test	A2		Dextrometophan	244768	445079	0.01635
test	A2		Olanzapine	602600.7	1131460	0.01635
test	A2		Propranolol	129844	280733	0.01635
test	A2		Terfenadine	58963.11	104825	0.01635
test	A3		Dextrometophan	264182.2	576012	0.016333
test	A3		Olanzapine	671599.8	1441817	0.016333
test	A3		Propranolol	133808.2	279352	0.016333
test	A3		Terfenadine	57012.06	109476	0.016333
test	A4		Dextrometophan	224330.3	466889	0.01635
test	A4		Olanzapine	580772.4	1220027	0.01635
test	A4		Propranolol	131373.1	280611	0.01635
test	A4		Terfenadine	53681.46	132671	0.01635
test	A5		Dextrometophan	230785.5	491891	0.016333
test	A5		Olanzapine	598921.5	1178736	0.016333
test	A5		Propranolol	134864.7	227162	0.016333
test	A5		Terfenadine	54950.7	126976	0.016333
test	A6		Dextrometophan	280594	589247	0.01635
test	A6		Olanzapine	562932.0	1285588	0.01635
test	A6		Propranolol	138059.6	252664	0.01635
test	A6		Terfenadine	67867.14	165310	0.01635
test	A7		Dextrometophan	250399.2	474476	0.016333
test	A7		Olanzapine	503185.9	1133176	0.016333
test	A7		Propranolol	121654.8	232397	0.016333
test	A7		Terfenadine	53150.86	118756	0.016333
test	A8		Dextrometophan	241965.2	540094	0.01635
test	A8		Olanzapine	599506.9	1324475	0.01635
test	A8		Propranolol	130018.5	270023	0.01635
test	A8		Terfenadine	60794.71	135237	0.01635
test	A9		Dextrometophan	258771.7	587895	0.016333
test	A9		Olanzapine	568511.4	1131052	0.016333
test	A9		Propranolol	117596.6	228702	0.016333

Summary



Echo® MS for high-throughput analysis

- Plate reader speed
 - 10-50x faster than LC-MS
 - 384- and 1536- formats
- Universal detection
 - Label free
 - Standard ESI
 - Small molecules; peptides; proteins and mAb
- Robust data quality
 - High sensitivity, selectivity, specificity and reproducibility
- Internal standard optional and minimum sample preparation
 - Fast overall speed and minimum method development
- Matrix tolerance
 - Solvent independent
 - Tolerant to high salt, protein, detergent, etc.
- Wide applications
 - Drug discovery and development
 - Synthetic biology and others
- In-situ kinetics
 - Significant reagent saving
 - Full kinetics analysis within a single run
- Automation integration
 - Automated data processing
 - Integrated with liquid handling, incubation, etc

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