Up to 10x increased throughput for metabolic stability assays

Featuring LeadScape® 3.0 Analyze software on the Echo® MS system

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In the search for new drug candidates, *in vitro* adsorption, distribution, metabolism, and excretion (ADME) profiling is used to determine the liabilities of candidates. Modern drug discovery environments depend on the accuracy and precision of results from HT-ADME studies to select those new chemical entities (NCEs) or new biological entities (NBEs) with a high potential for clinical success.

Medicinal chemistry labs can rapidly synthesize new compounds, putting higher throughput demands on ADME labs. Scientists are also challenged to develop sensitive and selective quantitative assays for drug candidates in complex matrices. Triple quadrupole instruments have demonstrated high selectivity and sensitivity and therefore are widely used for the quantitative analysis of ADME samples and running assays which serve as a critical decision gate for structural and functional lead optimization. Manual optimization of compounds and manual batch creation for analytical runs can occupy significant amounts of time reducing lab efficiency and increasing turnaround time.

Additionally, the emergence of complex therapeutics amplifies LC-MS/MS method development challenges and necessitates automated software tools to rapidly optimize MRM conditions and to enable acquisition of large sample sets quickly.

Traditionally, ADME profiling is performed on candidates later in the development cycle, after target and phenotypic-based high-

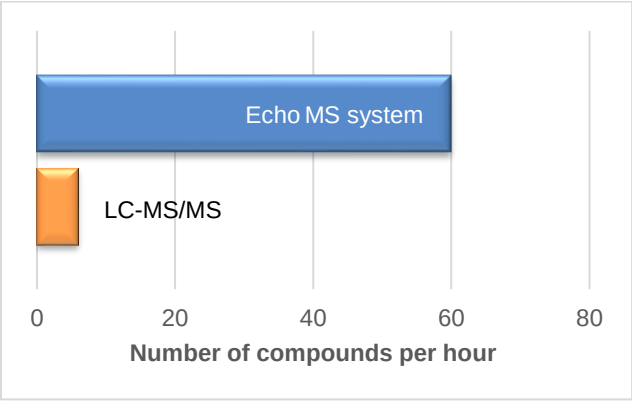

Figure 1. Throughput comparison. The optimized assay for metabolic stability assays using LC-MS/MS was transferred to the Echo® MS system. This led to an up to 10x increase in workflow throughput, based on the number of compounds that can be analyzed per hour.

throughput screening (HTS). Moving these screens to earlier in the cycle would provide significant benefits but increase the number of compounds to be tested by an order of magnitude. Supporting this effort would necessitate equivalent gains in throughput handling capability and capacity while maintaining data quality.

Acoustic Ejection Mass Spectrometry (AEMS) on the Echo® MS system has significantly higher throughput than traditional LC-MS/MS approaches because acoustic ejection permits fast sampling and flow injection analysis on a high sensitivity mass spectrometer.[1] Here, a suite of metabolic stability assays were developed and run using both LC-MS/MS and the Echo® MS system to compare the workflows.

Key features of the SCIEX Echo® MS system with LeadScape Analyze software

- Highly reproducible sample analysis using Acoustic Droplet Ejection and Open Port Interface[1]
  - Sample acquisition rates of up to 1 second per sample for a single analyte
  - Electrospray ionization for broad compound coverage
- High sensitivity and quantitative robustness using the SCIEX Triple Quad 6500+ mass spectrometer
- Metabolic stability assay screening on the Echo MS system yielded up to 10x greater throughput compared to LC-MS/MS (Figure 1)
- Automated MRM method building leveraging optimized compound parameters stored in DiscoveryQuant Optimize software databases
- Automated batch submission, acquisition, processing, and review using LeadScape Analyze software workflows

Methods

Sample preparation: The metabolic stability assay evaluates cytochrome P450 (CYP)-mediated metabolism of 23 commercially available compounds in human and rat microsomes. Solutions containing the test compounds at 3.5 mM in DMSO were diluted with acetonitrile to 50 µM. A 1 mg/mL microsomal suspension was prepared in 100 mM sodium phosphate pH 7.4 and 6.6 mM magnesium chloride. The suspension was pre-heated to 37°C and the diluted test samples were then added. The reaction was initiated by adding 17 µL of pre-heated 5 mM NADPH in 100 mM sodium phosphate at pH 7.4 into 153 µL of reaction mixture. The reaction mixture was mixed well and 75 µL was transferred into 150 µL of quench solution (100 µM 2,7 dimethyloxynaphthalene in acetonitrile) at the 0-minute time point (T0) and again after 10 minutes of incubation (T10). The quenched reaction mixtures were centrifuged for 15 minutes at 1500 RPM and 60 µL of the supernatant was transferred to a 384 well plate for LC-MS/MS. For the Echo® MS system analysis, 60 µL of the supernatant was transferred to a 384 well plate, evaporated to dryness and reconstituted in 60 µL of 95:5 water:methanol with 500 nM alprenolol as the internal standard. The plate was placed on the shaker for 5 minutes, then centrifuged for 2 minutes at 3500 RPM. 40 µL of the reconstituted samples was transferred to an Echo® MS Qualified 384 well plate, centrifuged for 5 minutes at 1500 RPM, then shaken for 5 minutes.

LC-MS/MS analysis: A 4 µL sample of the supernatant was injected onto a C18 column (20 x 2 mm, 3 µm) and was eluted using a fast linear gradient (Table 1). The run time for the 8 sample injections per compound was 10 minutes (Figure 2).

Table 1. LC gradient.

Time (min)	%A	%B	Flow rate (mL/min)
0	*98*	*2*	*0.7*
0.20	*98*	*2*	*0.7*
0.40	*0*	*100*	*1.0*
0.70	*0*	*100*	*1.0*
0.73	*98*	*2*	*1.0*
1.00	*98*	*2*	*0.7*
1.10	*98*	*2*	*0.7*

Mobile phase A: 2 mM ammonium acetate / acetonitrile / formic acid (80:20:1 v/v/v)
Mobile phase B: 2 mM ammonium acetate / acetonitrile / formic acid (20:80:1 v/v/v)

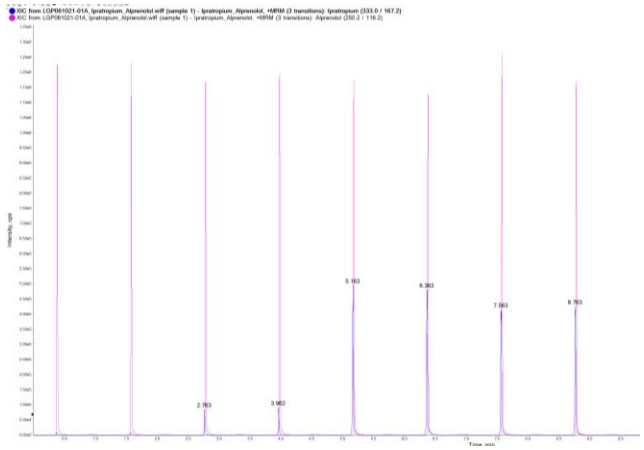

Figure 2. Example LC-MS/MS chromatogram. An example LC-MS/MS chromatogram shows 8 injections performed to analyze a single compound in duplicate, across 2 time points and 2 species. This analysis requires 10 minutes runtime to complete acquisition. Ipratropium (blue) and the IS alprenolol (pink) were monitored, peaks 1-4 for T0 and 5-8 for T10. The duplicate injections for rat are peaks 1-2 and 5-6, and for human are 3-4 and 7-8.

Mass spectrometry: Samples were analyzed using either the SCIEX Triple Quad 6500+ system coupled with an LC or the Echo® MS system. Optimal MRM parameters (Q1, Q3, DP, CE, CXP) for the test compounds were determined using DiscoveryQuant Optimize software and stored in its compound database. Acquisition methods for both LC-MS/MS and Echo® MS system workflows were created automatically by the LeadScape Analyze software version 3.0 using the optimized parameters for each compound previously stored in the compound database of DiscoveryQuant software. Batch creation and sample submission for acquisition was completed using the LeadScape Analyze software for both the LC-MS/MS and Echo MS system. Samples were analyzed in duplicate.

Echo® MS system analysis: Samples were ejected into the Open Port Interface using a carrier solvent of 100% methanol and operating at a flow rate of 400 µL/min (Figure 3). The ejection volume was 10 nL (4 drops of 2.5 nL sample each).

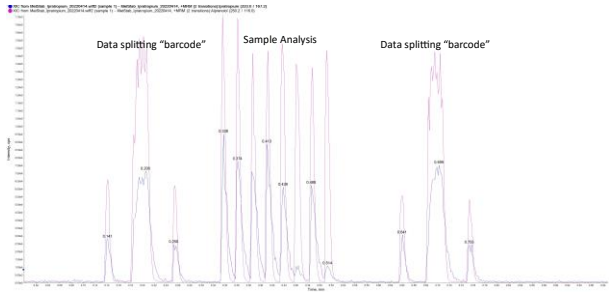

Figure 3. Example of an ejection series. An example traces generated by the Echo® MS system for the analysis of the same 8 samples analyzed by the LC-MS/MS analysis (Figure 2). The analysis requires only 1 minute of acquisition time on the Echo® MS system. Data splitting "barcodes" at the beginning and end of the run were used to calculate sample timing to allow ejections to be split into individual samples.

Batch creation, sample submission for acquisition, and automated data processing were completed using the LeadScape Analyze software version 3.0.

Data processing: To facilitate automated data processing following acquisition, metadata is included as part of batch submission when using LeadScape Analyze software version 3.0. Peak integration and area calculation were automatically completed on the Echo® MS system by SCIEX OS software. Peak integration and area calculations for the LC-MS/MS workflow were completed by Analyst software. Peak review for both workflows was completed in the LeadScape Analyze software version 3.0.

Equivalent results with up to 10x throughput using Echo MS system

Samples were prepared to investigate the metabolic stability of 23 standard compounds. Samples were measured in duplicate using both standard LC-MS/MS and Echo MS system assays. The standard LC-MS/MS assay required 10 minutes per compound, totaling ~230 minutes of acquisition time to analyze 23 compounds (see Figure 2 for analysis of a single compound). In contrast, the assay using the Echo MS system required 1 minute per compound and therefore took only ~23 minutes of acquisition time to analyze the same 23 compounds (see Figure 3 for analysis of a single compound). The peak areas for each compound were measured in duplicate for each species at T0 and T10. Peak areas for the duplicate runs were averaged and metabolic stability (% remaining) was calculated for each species (Figure 4).

From the peak areas measured, the metabolic stability was calculated for each of the test compounds for both human and rat microsomes (Figure 4). Comparable peak areas were observed between the LC-MS/MS results and the very fast Echo® MS system results. The correlation of results between the LC-MS/MS and Echo® MS system data is plotted in Figure 5 for both the human and the rat studies. The results show that the Echo® MS system provided equivalent results to LC-MS/MS with good correlation, but with ~10x faster throughput in this case.

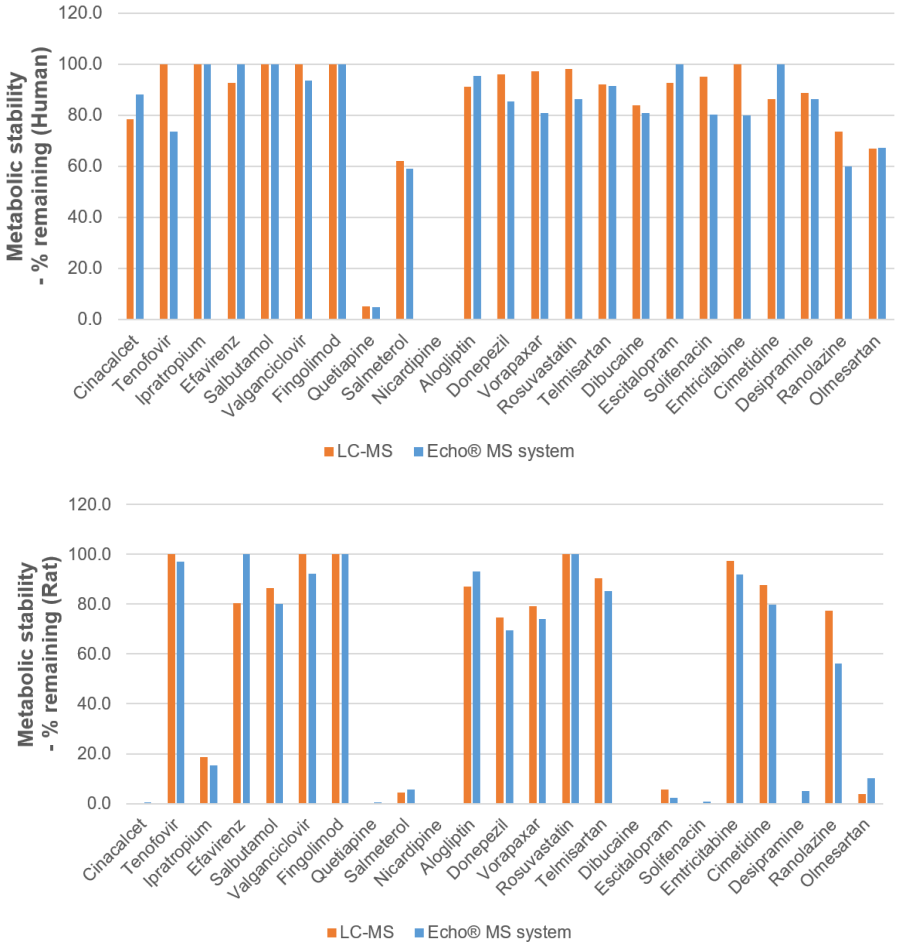

Figure 4. Comparison of metabolic stability (% remaining) in human microsomes. Results for % drug remaining are shown for both human (upper chart) and rat (lower chart) microsomes. Good comparability of metabolic stability results was observed between LC-MS/MS approach and the assay performed on the Echo® MS system.

These results show that well-established HT-ADME workflows run on the Echo MS system with the combination of DiscoveryQuant Optimize software and LeadScape Analyze software to automate compound parameter optimization, method building, batch submission and data review brings HT-ADME assay time-to-result to a new level.[2]

Conclusions

AEMS is a very high throughput quantitative technique that is ideal for labs performing *in vitro* ADME. LeadScape Analyze software helps to overcome the manual method creation, batch building and submission bottleneck for sample sets with large number of compounds, hence empowering customers to deploy the full potential of AEMS. LC-MS/MS has traditionally been used for performing metabolic stability studies and thus was used here as the benchmark to compare high throughput acquisition on the Echo® MS system. Very similar results were obtained with the 2 approaches but the Echo® MS system provided ~10x higher throughput in this example. This demonstrates that the Echo® MS system with LeadScape Analyze software can deliver the needed sample throughput and data quality to perform metabolic stability and similar ADME assays earlier in the drug development process to significantly accelerate and de-risk drug discovery programs.

Key to the success of the full workflow is the software to streamline the acquisition and processing across many compounds and many samples. Here, DiscoveryQuant Optimize software was used to determine the optimal parameters for sensitive and selective MRM detection of the target compounds. These MRM conditions were stored in the globally accessible compound database of DiscoveryQuant Optimize software which LeadScape Analyze software then used to create the batches for both LC-MS/MS and Echo® MS system analysis and automatically build the required acquisition methods. This work describes a fully automated workflow that requires minimal user intervention. Using a metabolic stability assay as an example we were able to demonstrate an up to 10x increase in sample throughput using the Echo® MS system coupled with LeadScape Analyze software while retaining data quality for rapid decision making.

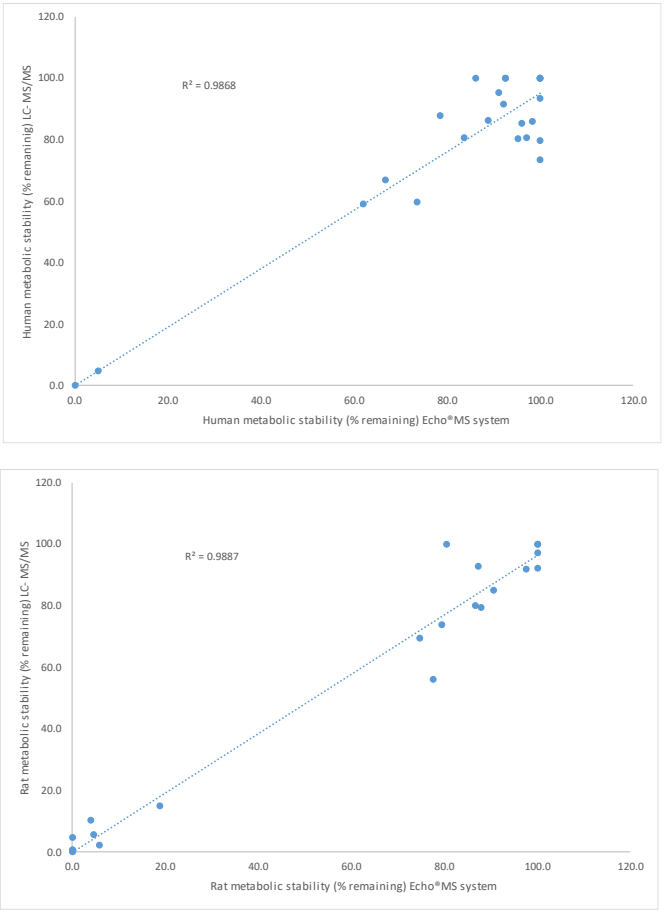

Figure 5. Correlation plots of metabolic stability. A high correlation was observed for metabolic stability in both the human (top) and rat (bottom) microsomes between the LC-MS/MS and Echo MS system analyses.

References

1. Rapid MS/MS analysis with Acoustic Ejection Mass Spectrometry (AEMS). SCIEX technical note RUO-MKT-02-11385-A.
2. Accelerating turnaround time in high-throughput ADME pipelines. SCIEX technical note RUO-MKT-02-14235-A.

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