From compound optimization to endpoint decisions: high-throughput metabolic stability workflows for faster decision-making

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This technical note describes an integrated workflow for metabolic stability assays on Echo® MS+ system with SCIEX Triple Quad 6500+ system. This workflow features a seamless progression from method development to endpoint calculations, highlighting efficient compound optimization with LeadScape® software and automated data processing with AI Quantitation software (Figure 1).

Metabolic stability assays are a critical component of early drug discovery, providing rapid insight into compound clearance, half-life, and overall suitability for progression. However, conventional workflows can be limited by sequential compound optimization, fixed acquisition methods, and manual data processing steps that slow turnaround times. These challenges are amplified in high-throughput (HT) ADME environments, where large numbers of structurally diverse compounds must be evaluated efficiently and reproducibly. The integration of LeadScape® software and AI Quantitation software with acoustic ejection mass spectrometry (AEMS) offers a HT solution with minimal method development and automated data processing, accelerating drug discovery pipelines.

Key benefits of metabolic stability analysis using LeadScape® software and AI Quantitation software on Echo® MS+ system with SCIEX Triple Quad 6500+ system:

- **Streamlined compound tuning:** Streamline MRM transition optimization with LeadScape® software, reducing development effort.
- **Streamlined data processing:** Perform integrations and automate endpoint readouts without manual intervention with AI Quantitation software.
- **Ultra-high throughput:** HT analysis (up to 1 second per sample) with no residual carryover effects enabled by acoustic ejection technology.
- **Confident metabolic stability profiling:** Generate intrinsic clearance (CL_{int}), half-life ($t_{1/2}$), and percent remaining values consistent with published clearance ranges across diverse compounds.

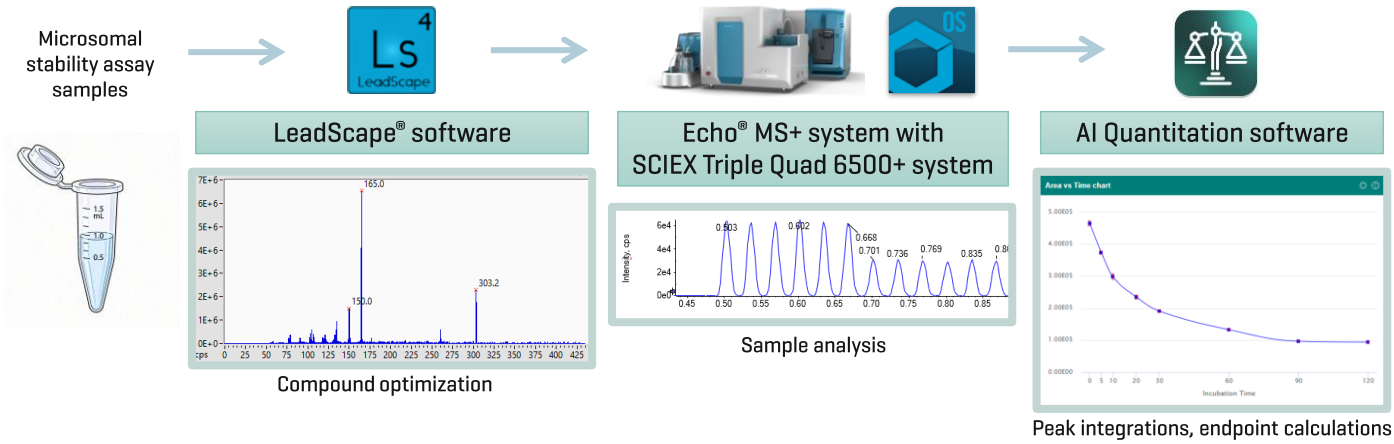

Figure 1. Metabolic stability workflow using Leadscape® software and AI Quantitation software on the Echo® MS+ system with SCIEX Triple Quad 6500+ system. High sample throughput for metabolic stability is achieved with accelerated method optimization, rapid sample analysis, and automated data processing.

Introduction

Metabolic stability assessment is critical in early drug discovery but is often constrained by sequential method development, static acquisition, and fragmented data processing. This study demonstrates an integrated workflow that connects streamlined compound optimization, sample-driven MRM acquisition, and automated endpoint generation on the Echo® MS+ system with SCIEX Triple Quad 6500+ system. This approach is designed to improve throughput, reproducibility, and confidence while accelerating data-driven compound progression decisions.

The presented metabolic stability workflow demonstrates reduced need for manual intervention from method setup through data review. LeadScape® software drives compound-specific MRM optimization and automated peak integration with endpoint calculations using AI Quantitation software provides a direct path from raw acquisition to decision-ready results. Together, these capabilities support the rapid analysis of multiple compounds within a single sample batch while maintaining the assay performance required for metabolic stability measurements.[1]

Methods

Sample preparation: 6 small molecule compounds spanning a range of intrinsic clearance values (prazosin, chlorpromazine, diltiazem, erythromycin, buspirone, verapamil) were evaluated. Samples containing compound (1 µM), human liver microsomes (0.5 mg/mL), and an NADPH-regenerating system in 100 mM potassium phosphate buffer were incubated at 37°C for 0, 5, 10, 20, 30, and 60 minutes. Reactions were stopped with 3 volumes of ice-cold acetonitrile, followed by vortexing, centrifugation, and supernatant recovery for analysis.

AEMS analysis: Samples were transferred to an Echo® MS qualified 384-well plate for AEMS analysis using Echo® MS+ system (SCIEX). Carrier solvent was 0.1% formic acid in methanol with a flow of 360 µL/min. 25 nL of each sample was ejected using wide peak mode at 20 Hz. AEMS parameters are shown in Table 1.

MRM optimization: LeadScape® software was used to optimize MRM transitions for each target compound using neat standards (Table 2). Assay sample data were acquired with SCIEX OS software using the Echo® MS+ system with SCIEX Triple Quad 6500+ system.

Data processing: Data were processed using AI Quantitation software, which performed all integrations and endpoint calculations, including Cl_{int}, $t_{1/2}$, and parent compound depletion over time (% remaining).

Table 1. AEMS parameters.

Parameter	Value
Carrier solvent	0.1% formic acid in methanol
Carrier solvent flow rate	360 µL/min
Acoustic ejection	25 nL at 20 Hz
Polarity/workflow	Positive/small molecule
Ion spray voltage	5000 V
Ion source gas 1	90 psi
Ion source gas 2	40 psi
Curtain gas	40 psi
Source temperature	300°C

Table 2. List of compounds and transitions used for analysis.

Compound	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)
Prazosin	384.2	212.8	15
Erythromycin	734.4685	576.3	28
Diltiazem	415.1686	178.0	40
Chlorpromazine	319.1027	86.0	25
Buspirone	386.2548	122.0	45
Verapamil	455.2904	165.0	40

Optimization with LeadScape® software

High analytical sensitivity is crucial for stability assays as parent compound concentrations decrease over incubation time, particularly for high-clearance molecules. Optimization of MRM transitions through compound tuning is essential to ensure low-abundance compounds are accurately measured. To streamline this process for ADME workflows, LeadScape® (Optimize) was used to tune target compounds, determining optimal product ions and collision energies (CEs) in positive mode. Neat compounds were tuned directly from the Echo® MS+ system, bypassing the need for time-consuming manual infusion. Tuning results were evaluated through a graphical review panel that displayed the corresponding spectra along with the optimized CEs and declustering potentials (DPs) (Figure 2). LeadScape® software imports text files to create a batch for tuning and generate tuning results,

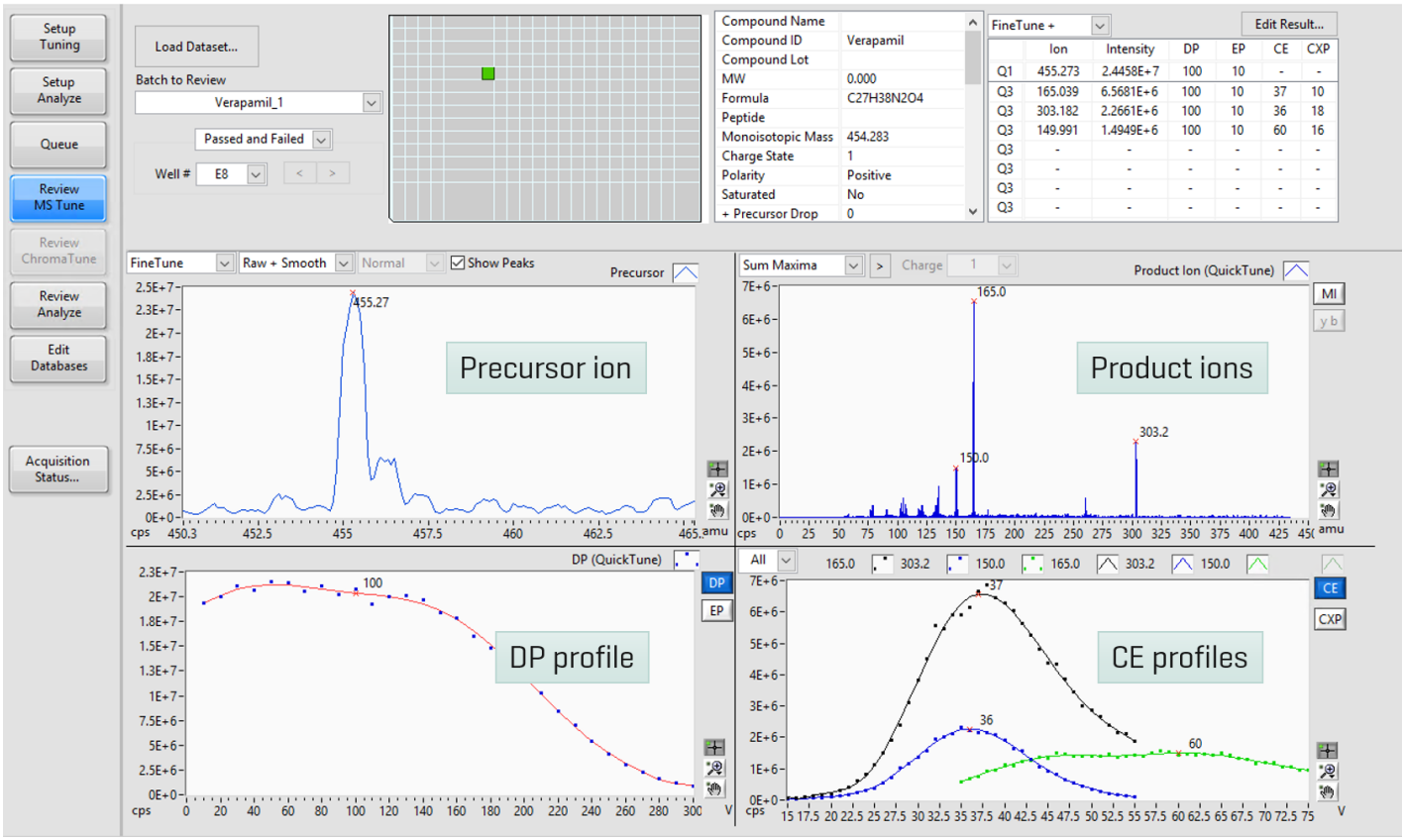

Figure 2. The tuning results review panel on LeadScape® software helps visualize results for easy review. Precursor ion spectrum and DP profile are displayed in the left panels, while the panels on the right show product ions and their respective CE profiles.

allowing integration with both upstream and downstream workflow processes.

Data processing with AI Quantitation software

AI Quantitation software automates data processing, quickly converting HT metabolic stability measurements into reliable data with minimal human intervention. Determination of Cl_{int}, $t_{1/2}$, and % remaining are among the multitude of output possibilities enabled by AI Quantitation software (Figure 3).

Stability profiles obtained in this study are consistent with published values[2], demonstrating the suitability of this workflow for metabolic stability profiling. Table 3 lists Cl_{int} values obtained in this study, along with published values and their clearance classification.

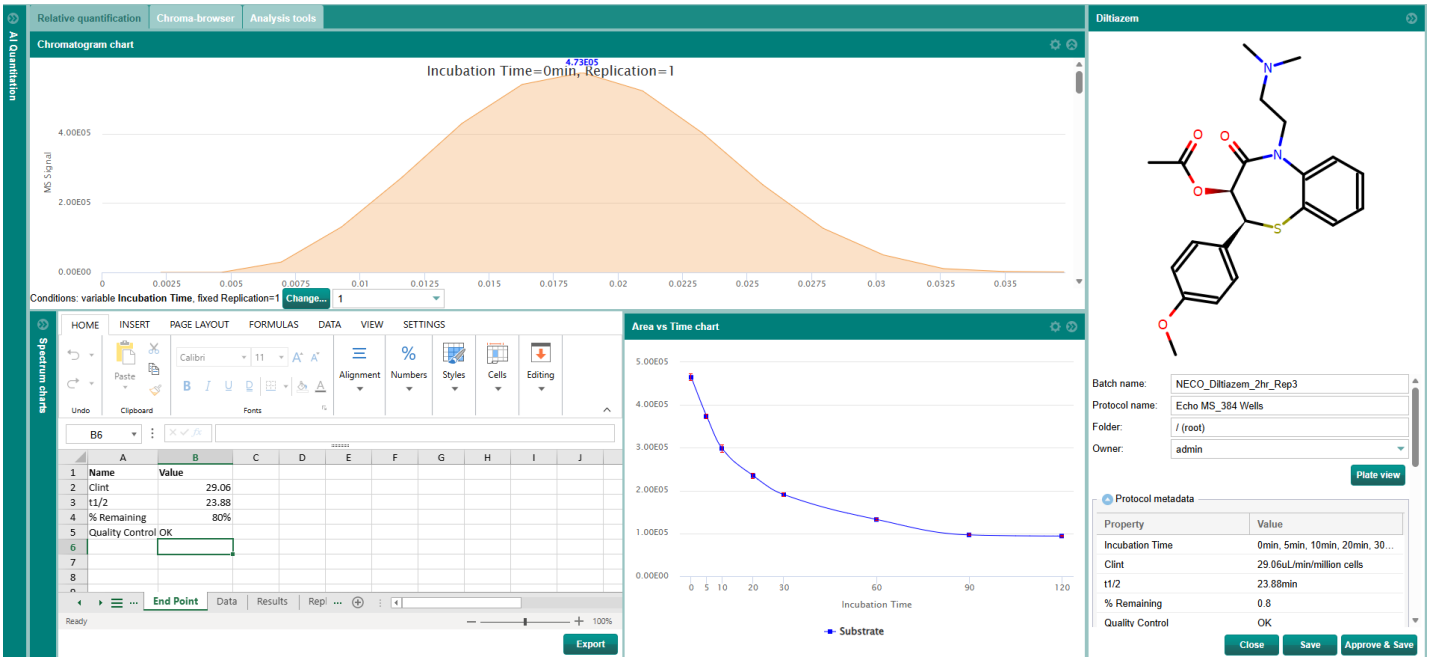

Figure 3. Overview of the results from AI Quantitation software for diltiazem. Automatically integrate and calculate critical metabolic stability readouts such as CL_{int}, $t_{1/2}$, % remaining, and trace peak area against incubation time using AI Quantitation software.

Table 3. Calculated Cl_{int}, published Cl_{int}, and clearance classification of the tested compounds.

Compound	Calculated CL_{int} (µL/min/mg)	Published CL_{int} range (µL/min/mg)
Prazosin	11.55	5-20 (low)
Erythromycin	19.52	10-40 (medium)
Diltiazem	29.06	30-80 (medium)
Chlorpromazine	11.15	20-50 (medium)
Buspirone	148.75	80-200 (high)
Verapamil	121.57	120-250 (high)

Conclusions

- Metabolic stability assessments of 6 small molecules demonstrated intrinsic clearance values consistent with expected ranges when evaluated on Echo® MS+ system with SCIEX Triple Quad™ 6500+ system.
- Enable faster compound optimization with automated tuning in LeadScape® software, reducing development effort while maintaining data quality.
- Accelerate automated data processing and endpoint calculation with AI Quantitation software, reducing time from analysis to results for metabolic stability assays.
- Increase sample throughput for high-volume ADME workflows using acoustic ejection technology for rapid, contactless sample introduction.

References

1. Accelerating ADME workflows: AEMS for high-throughput metabolic stability studies, SCIEX technical note, MKT-35763-A
2. Cyprotex. Microsomal Stability Assay. https://www.evotec.com/solutions/drug-discovery-preclinical-development/cyprotex-adme-tox-solutions/adme-pk/drug-metabolism/microsomal-stability

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