Streamlined analysis: Combining mass spectral data analysis and endpoint calculations for drug discovery insights

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This technical note introduces an innovative software solution designed to combine mass spectral analysis and endpoint calculations, delivering quick and accurate quantitative results to support drug discovery (Figure 1). High-throughput analytical methods are essential in drug discovery as they accelerate compound screening and pharmacokinetic profiling. Mass spectrometry analytical techniques, including LC-MS and Echo® MS+ system, enable rapid data generation and reduce development timelines.

Automated data analysis in drug discovery faces significant challenges due to the sheer volume and complexity of high-throughput data. Integration across different platforms, such as high-throughput LC-MS and ultra-fast Echo® MS+ system with ZenoTOF 7600 system [Echo® MS+ system], further complicates the process. While standardized formats and interoperability are essential, variability in experimental conditions often hinders effective data analysis.

AI Quantitation software, a solution utilizing advanced algorithms, addresses these challenges by automatically selecting optimal MS and MS/MS signals based on compound structure and peak quality parameters. This approach helps ensure the best method to meet user-defined quantitative criteria and supports tailored endpoint calculations for specific research needs.

Key benefits of AI Quantitation software for drug discovery needs

- **Automatic selection of compound-related MS1 and MS/MS signal:** Automatic identification of optimal compound-related MS1 and MS/MS signals using the compound structure
- **Single data processing method:** Single data processing method for all compounds, generate quantitation results based on compound structures
- **Customizable endpoint calculation reports:** Customizable endpoint calculation reports to support various assays.
- **Streamlined data management**: Automated data processing in parallel to data acquisition using SCIEX mass spectrometers

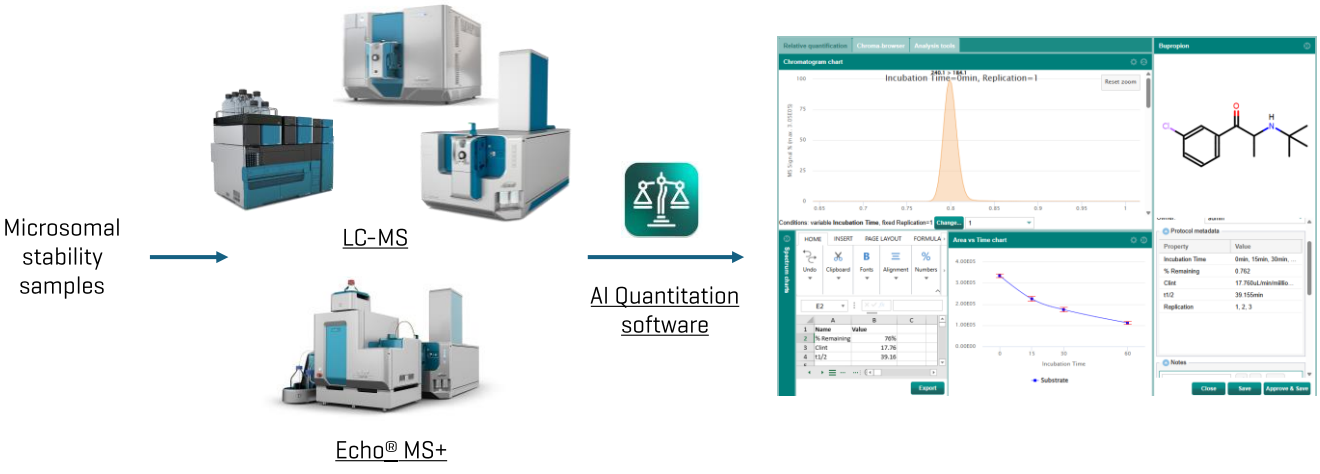

Figure 1. Workflow using AI Quantitation software on SCIEX MS platforms. The figure shows the workflow for the quantitation experiment to analyze microsomal stability samples using SCIEX mass spectrometers and data processing using AI Quantitation software with the final result layout.

Introduction

The drug discovery process involves experiments using various mass spectrometer platforms and methodologies, including MRM on nominal mass (NM) spectrometers and MRM^{HR}, Zeno MS1, SWATH DIA and TOF MS using high-resolution mass spectrometers (HRMSs).

High-throughput analytical techniques generate large volumes of data rapidly, providing quick insights. However, a significant hurdle in the overall process is managing this data to calculate biologically relevant endpoints. This process involves using multiple software tools to handle complex mass spectral data analysis and endpoint calculations, requiring manual data transfer between software to achieve the desired output. Streamlining this workflow through integrated software solutions could significantly enhance efficiency and accuracy, helping to reduce the time and effort needed for data management. Additionally, automation of data transfer processes can help minimize human error and improve the reproducibility of results.

AI Quantitation software helps eliminate this barrier by employing innovative algorithms to simplify data processing, making it a preferred methodology for all drug discovery needs.[1]

Methods

Sample preparation: BioIVT provided ready-to-inject microsomal incubation samples (0 min, 15 min, 30 min, and 60 min) for this study.

Acoustic ejection: A total of 10 nL of sample was ejected in standard mode with 2-second intervals. 0.1% (v/v) formic acid in 70:30 (v/v) acetonitrile/water was used as a carrier solvent, and a flow rate of 400 µL/min was used for analysis.

Mass spectrometry: The MRM, Zeno MRM^{HR}, and Zeno MS1 methods were used for data acquisition on the SCIEX mass spectrometers with optimized conditions. Table 2 summarizes the list of compounds and methodology.

Chromatography: Analytical separation was performed on the ExionLC AE system using a Phenomenex Kinetex XB-C18 (2.1 × 50 mm, 2.6 µm) column at a 0.8 mL/min flow rate. Mobile phase A was 0.1% (v/v) formic acid in water and mobile phase B was 0.1% (v/v) formic acid in acetonitrile. The column temperature was set to 50°C. The gradient conditions used are summarized in Table 1.

Table 3. List of compounds and transitions (Q1/Q3) used for analysis.

Compound	LC-NM	LC-HRMS	Echo® MS+ system
Bupropion	240.1/184.0	240.1/184.1	240.1/184.1
Dextromethorphan	272.2/215.1	272.2/215.1	272.2/147.1
Midazolam	326.1/291.1	326.1/291.1	326.1/291.1
7-Ethoxycoumarin	191.1/163.0	191.1/163.0	191.1/163.0
Testosterone	289.2/109.1	289.2/109.1	289.2/289.2
Diclofenac	296.1/214.0	296.1/214.0	294.0/250.0

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

Compound	Methodology (NM, HRMS, Echo® MS+ system)
Bupropion	MRM, MRM^{HR}, MRM^{HR}
Dextromethorphan	MRM, MRM^{HR}, MRM^{HR}
Midazolam	MRM, MRM^{HR}, MRM^{HR}
7-Ethoxycoumarin	MRM, MRM^{HR}, MRM^{HR}
Testosterone	MRM, MRM^{HR}, ZenoMS1
Diclofenac	MRM, MRM^{HR}, MRM^{HR} (Negative)

Table 1. LC gradient conditions.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	90	10
0.2	90	10
1.1	5	95
1.5	5	95
1.6	90	10
2.0	90	10

Data processing: Data acquisition was performed using SCIEX OS software, version 3.4.5. Data processing was performed using AI Quantitation software. List of compounds and transitions used for quantitation is summarized in Table 3.

Mass spectral data analysis and endpoint calculations

MRM, MRM^{HR} and Zeno MS1 acquisition data files were processed using AI Quantitation software using the same processing methods and parameters for evaluation. The acquisition batch was composed of 3 replicates of each time point. AI Quantitation software enables an automated workflow for endpoint calculation using embedded formulas, analyzing $t_{1/2}$, clearance and %remaining.

All samples were processed using AI Quantitation software. The processing method includes a bond-breaking and MS algorithm that automatically assigns fragment ions (if present) per compound of interest and uses algorithm thresholds for peak selection (Figure 2). The workflow sheet uses the peak areas from the selected samples to generate endpoint numbers, such as $t_{1/2}$, clearance and %remaining.

The software automatically predicts the best fragments for analysis. Figure 4 shows examples of MRM^{HR} chromatograms from the LC-NM, LC-HRMS, and Echo® MS+ system using AI Quantitation software. The software uses the peak area from samples to calculate endpoints and displays an Area Vs Time chart in the final approved result experiment (Figure 3).

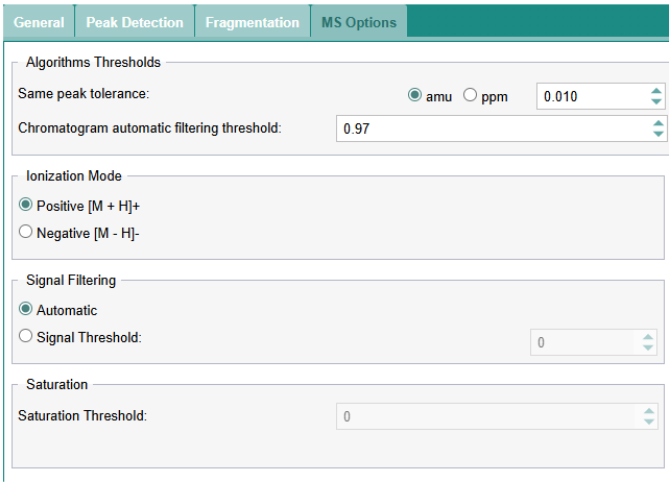

Figure 2. Relative quantitation MS option settings from AI Quantitation software for peak identification.

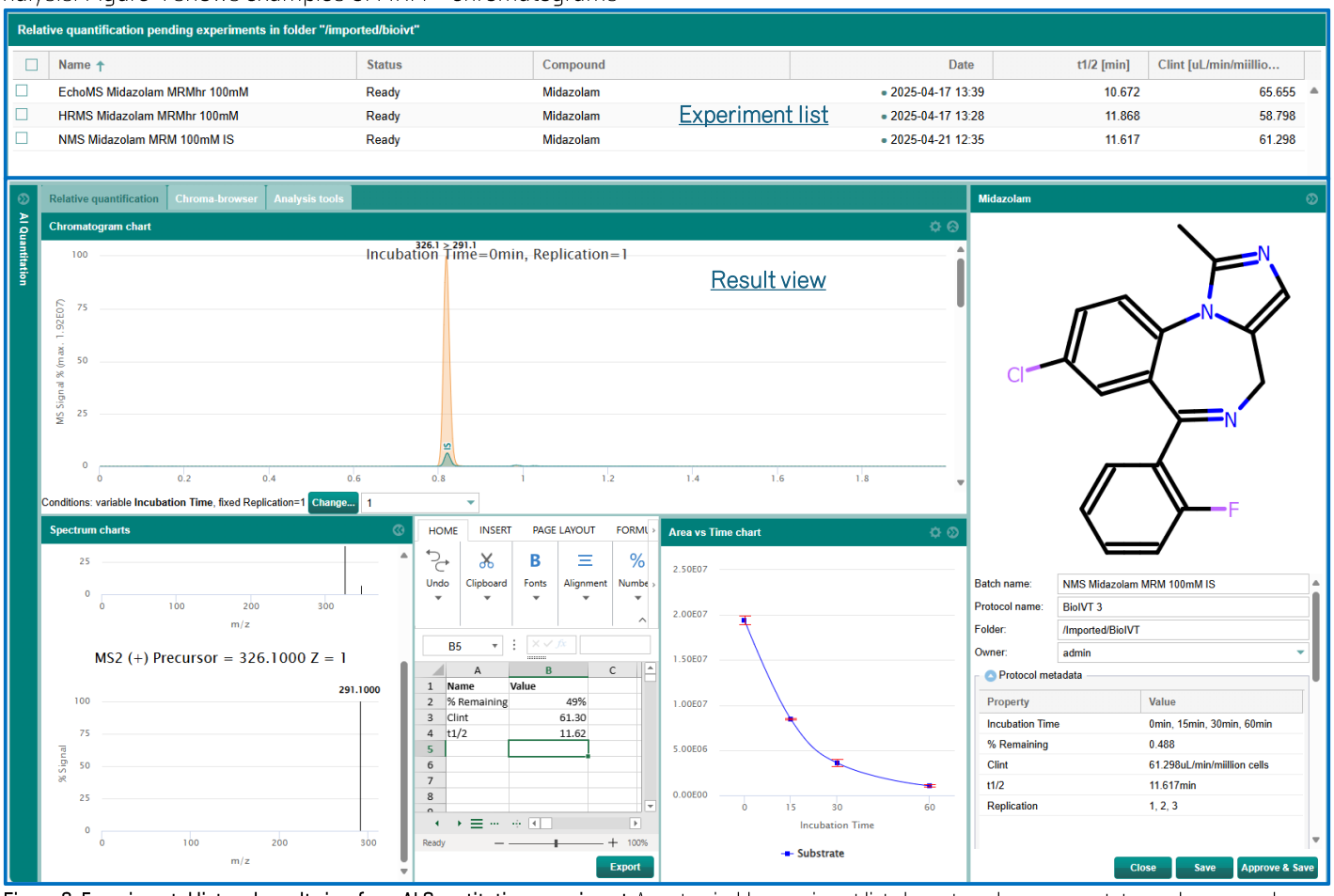

Figure 3. Experimental list and result view from AI Quantitation experiment. A customizable experiment list shows $t_{1/2}$ values, name, status and compound information (top). The results view shows the chromatogram, MS/MS information, structure, Area vs Time chart, and protocol metadata.

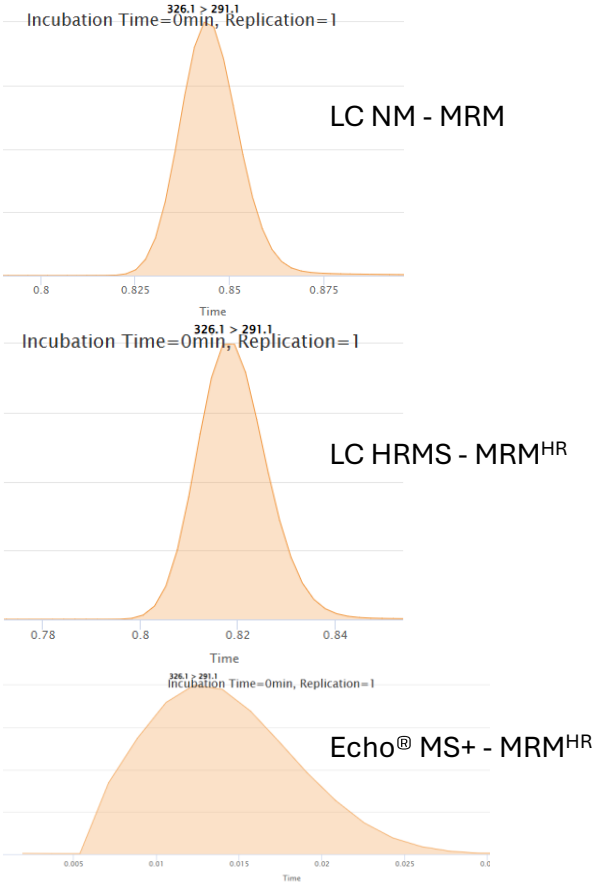

Figure 4. Example midazolam MRM^{HR} chromatograms from the LC-NM, LC-HRMS, and Echo® MS+ system using AI Quantitation software.

Results from analyzed microsomal incubation samples using LC-NM, LC-HRMS and Echo® MS+ system were compared for their $t_{1/2}$ values and all were found very close to each other with a % variation of <10 (Figure 5).

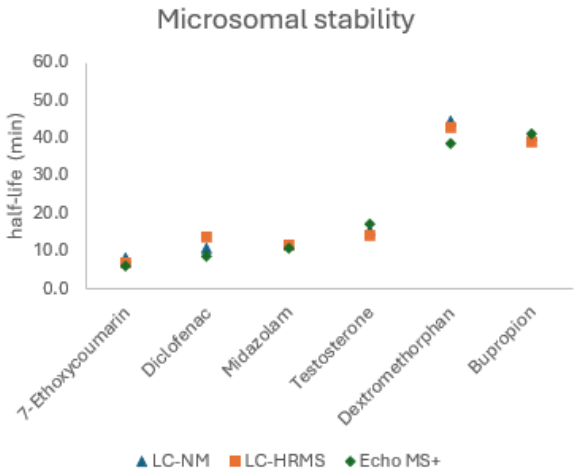

Figure 5. Microsomal stability data showing half-life (min) of 6 tested compounds using various instruments.

Conclusions

- Streamlined data processing and endpoint calculation were demonstrated using AI Quantitation software on SCIEX mass spectrometers
- Comprehensive quantitative solutions offered compatibility with various experiment types, including MRM, MRM^{HR} and Zeno MS1 for quantitation experiments
- Automated identification of fragment ions helped ensure the enhanced quantitative results were achieved within the user-defined criteria
- Data equivalency between the 3 most popular quantitation methodologies, MRM, MRM^{HR} and Zeno MS1, with automated data processing, enabled flexibility to choose data acquisition types based on specific needs while maintaining excellent precision and reliability

References

1. Fabien F, Luca M, Ken A, Bernard C, Ismael Z, Kevin B. Molecular Structure and Mass Spectral Data Quality–Driven Processing of High-Resolution Mass Spectrometry for Quantitative Analysis. *Rapid Commun Mass Spectrom*. 2025 Feb 10:e10000. doi: 10.1002/rcm.10000

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