



Letter to the Editor

Large batch size effect on signal stability and retention time consistency in LC-MS/MS analyses: A technical observation

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Dear Editor,

Large batch analysis is commonly used in high throughput LC-MS/MS workflows in routine clinical laboratories; however, the potential impact of expanded batch analysis size on the analytical process has not been sufficiently reported. In addition to known factors contributing to signal variability, such as sample preparation variability, matrix effect, carryover, and ion source contamination, we have occasionally observed that long batches (≥ 120 samples) can also independently affect ionization stability and chromatographic behavior.

In our laboratory, stable internal standard (ISTD) peak areas, consistent retention times, and no deviation were observed in small batches (20-30 samples). In contrast, in larger batches, stepwise ion suppression, decreased ISTD signal intensity, and slight retention time (RT) shifts towards the end of the sequence were observed in some analyses. Such gradual suppression trends observed over long analysis times have been attributed to the accumulation of endogenous residues in the ion source or in the early sections of the column (1,2). Additionally, prolonged automated sampling times have been associated with subtle temperature variations that can alter the distribution of analytes and matrix components (3).

These cumulative effects led to falsely low measurement results in the later portions of large batches in some analytes. Previous studies have demonstrated that even small decreases in ionization efficiency can significantly affect LC-MS/MS quantification, particularly in analyses involving drugs with narrow therapeutic ranges, and have important implications for clinical decision-making (4). Furthermore, even minimal progressive RT drift can impair peak integration consistency and quantifying/qualifying ion ratios in long analytical runs (5).

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To minimize analytical bias related to batch size, inclusion of mid-batch and especially end-batch quality control samples, observation of ISTD peak area trends throughout the study, inclusion of batch size effect as a parameter during method validation in accordance with current LC-MS/MS guidelines (6), more aggressive or periodic ion source cleaning during high-throughput periods and monitoring RT drift tolerance and adjusting peak integration rules accordingly can be suggested.

In conclusion, introducing an extended batch size as a preliminary analytical variable can help improve accuracy, robustness, and reproducibility in high-throughput LC-MS/MS analyses.

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