

Retention Time Windows: Why 0.05 Minutes Is the Sweet Spot for Background Hit Filtering

When identifying true binding events in AS-MS workflows, background noise is the enemy, and the retention time (Rt) windows play a critical role in deciding what counts as “similar” between an original hit and a potential cross-hit.

Experiment

Using data from a high-throughput screen of 250,000 compounds across 96 samples, Virscidian took a deep dive into the data to determine the typical retention time window between cross-hits.

Key Finding

Virscidian found that over 99% of background peaks that were true cross-hits occur within 0.05 minutes of the retention time of the original peak. When the retention time window was reduced below 0.05 min, real cross-hits were missed, potentially leading to inflated hit counts. On the other hand, using retention time windows greater than 0.05 min risked pulling in isomeric peaks without offering any meaningful gain in finding cross hits.

Recommended Practice

Use an automated cross-hit filter such as the one in Analytical Studio and set its retention time filter to ± 0.05 minutes to maximize cross-hit detection without increasing isomer-related misclassification.

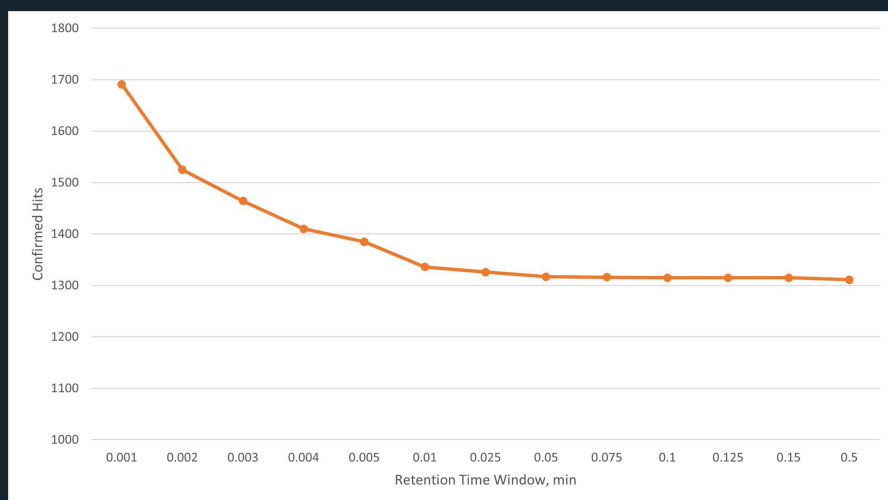


Figure 1

Plot of retention time search window vs number of confirmed hits. Further analysis found that 99% of background ions identified as cross-hits fall within ± 0.05 min of the RT of the original hit.