

Reducing False Positives from Isotopic Misidentification in AS-MS

In affinity selection-mass spectrometry (AS-MS), one of the more challenging-to-discern causes of false positives is compound misidentification due to isotopic pattern overlap. These errors occur when the X+1 of one compound is perceived as the C12 of another compound. This can also happen at the X+2 level. This error is particularly perilous because it can affect both the original hit as well as the background hit.

Experimental

Scientists used Analytical Studio's automated X-1 and X-2 cross-hit filter check during isotopic pattern matching to determine its impact on reducing false positives.

Key Finding

Enabling the automated X-1 and X-2 cross-hit filter check during isotopic pattern matching resulted in a 5% reduction in confirmed hits. This implies that 5% of the initial reported hits were likely false positives caused by isotopic confusion.

	No X-1 Check	With X-1 Check
Total Original Hits	3936	3154
Possible Hits	3165	2555
Confirmed Hits	1385	1315

Figure 2

Impact of enabling X-1 and X-2 isotopic misidentification checks. Without the check, 1385 hits were reported. With the check, hits dropped to 1315, a 5% reduction due to filtering false positives.

Recommended Practice

Always enable X-1 and X-2 misidentification checks during compound assignment. If your AS-MS data processing software doesn't have this capability, perhaps it's time to look for alternative tools. This simple processing setting can eliminate a significant portion of false positives at the source, improving the quality of your final hit list.



Figure 1

Example of peak mis-identification. The peak at m/z 338 falsely identified as the C12 of the (M+Na)+ peak of the possible hit when it is actually the M+1 peak from the compound at m/z 337.

