Dotmatics

Virscidian

Analytical Studio for Affinity Selection-Mass Spectrometry (AS-MS)

Purpose-built software for high-throughput AS-MS data processing, cross-hit filtering, and hit list generation.

AS-MS Screening Generates Complex Data. Your Vendor Software Wasn't Designed to Handle It

Affinity selection-mass spectrometry (AS-MS) is a powerful high-throughput screening technique for identifying small molecule binders to biological targets. Compound libraries containing hundreds of thousands of compounds, compressed into compound pools (commonly 96-or 384-well format), are incubated with a target protein, separated, and analyzed by LC-MS to detect which molecules bind.

Though the technique is proven, the data processing challenge is not trivial.

A single AS-MS screening campaign can generate chromatographic and mass spectral data for 250,000+ compounds distributed across microwell plates, with each well containing hundreds to thousands of different molecules. Identifying true binding hits within this data requires more than standard mass spectral analysis. It requires specialized cross-hit evaluation, isotopic pattern matching, retention time comparison, and systematic false positive removal across the entire plate.

Instrument vendor software (Waters™ MassLynx™ Mass Spectrometry Software, Waters Empower™ Chromatography Data Software (CDS), Thermo Scientific™ Xcalibur®, Thermo Scientific™ Chromeleon® Chromatography Data System (CDS), Agilent OpenLab CDS, etc.) was designed for general-purpose chromatographic and mass spectral data processing. None of these tools provide the compound library matching, plate-level cross-hit analysis, or automated false positive filtering that AS-MS workflows demand. The result: manual workarounds, spreadsheet-based tracking, inconsistent results across analysts, and false positives that consume expensive downstream follow-up resources.

Analytical Studio: Commercial Software Purpose-Built for AS-MS

Analytical Studio for AS-MS provides an integrated, vendor-neutral environment for processing, analyzing, and interpreting AS-MS screening data. From compound list import through cross-hit filtering to curated hit list export, Analytical Studio replaces fragmented manual workflows with a streamlined, reproducible pipeline.

Key Capabilities

Vendor-Neutral Data Processing

Import and process LC-MS data from most any instrument vendors, including Waters Corporation, Thermo Fisher Scientific Inc., and Agilent Technologies, Inc. One processing environment for your entire instrument fleet, regardless of manufacturer.

Cross-Hit Filtering

The only commercial software that performs cross-hit analysis across the entire experiment. Configurable parameters for retention time windows, peak height and area thresholds, isotopic pattern matching, and isomer tracking systematically identify and remove false positives.

Expression-Driven Decision Logic

NEW FEATURE

Define and enforce your laboratory's specific decision criteria using the same powerful expression framework available across all Analytical Studio workflows. Replaces the calculations panel from the previous version with a more flexible, consistent approach to how AS-MS results are calculated, displayed, and acted upon.

Compound Library Integration

Import compound libraries directly from CSV. Analytical Studio maps compounds to wells, associates metadata, and links results back to original compound identifiers throughout the workflow.

Interactive, Configurable Data Review

Customizable screen layouts, shown in Figure 1, display well-plate views, extracted ion chromatograms, mass spectra, and screening results simultaneously. Select any compound or well to instantly view the supporting analytical data.

Structured Export and Reporting

Export curated hit lists and underlying data to Excel or CSV for downstream analysis, project team review, or integration with ELN, something not possible with the previous version of Analytical Studio.

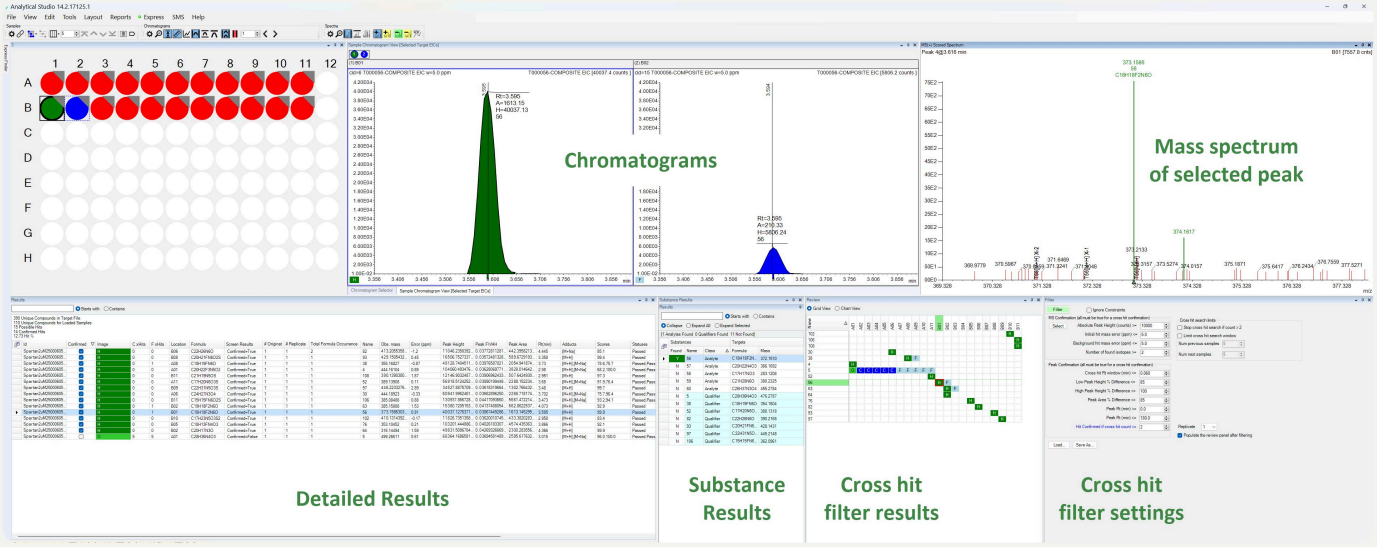

Figure 1. *Analytical Studio displays cross-hit filtering results alongside plate maps, chromatograms, and scored mass spectra, enabling rapid review and confident hit confirmation.*

From Compound List to Hit List: An Automated, Traceable Workflow

NEW FEATURE

Analytical Studio now has a fully integrated sample management and data processing pipeline for AS-MS workflows. Analytical Studio - Sample Management Services (AS-SMS) and Analytical Studio - Sample List Builder (AS-SLB), established components of the Analytical Studio ecosystem, are now available for AS-MS for the first time, automating the flow from compound list to processed results.

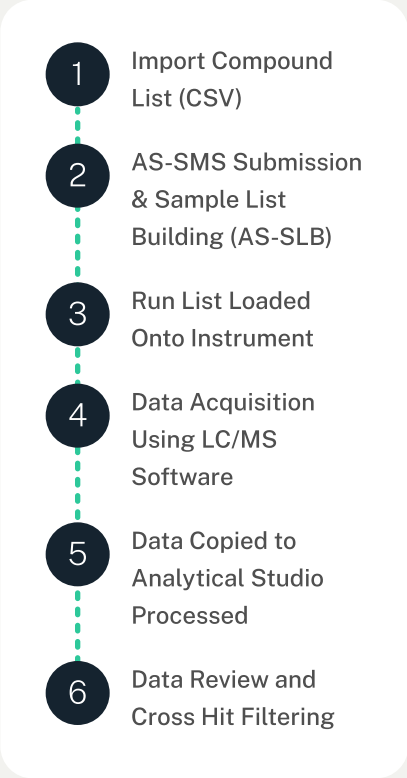

Figure 2. *AS-MS transforms a manual, file-based workflow into a fully automated, infrastructure-backed processing pipeline enabling automated sample submission, acquisition tracking, and cross-hit filtering.*

1 **Import Compound List**

Start from your standard compound CSV containing compound IDs, molecular formulas, well locations, and plate information. Create a submission directly within Analytical Studio, which organizes the metadata into AS-SMS for traceable storage and downstream reference.

2 **Generate Instrument-Ready Sample Lists**

AS-SLB creates injection sequences tailored to your specific instrument platform. We customize templates for your instruments, whether MassLynx, Empower CDS, Xcalibur, OpenLab CDS, Chromeleon CDS, or others, so that file names, sample positions, and method parameters automatically populate. The sample info string, previously constructed manually, is now generated automatically by AS-SMS.

3 **Acquire Data** NEW FEATURE

Download the sample list to your instrument PC, load the autosampler, and run. The instrument agent now seamlessly detects completed acquisitions and automatically transfers raw data to the Analytical Studio database for processing.

4 **Review Processed Results** NEW FEATURE

Query the database from within Analytical Studio and download processed results. The review panel auto-populates, streamlining and simplifying the workflow by eliminating the need to manually load a targets file. Compound metadata, chromatographic data, and spectral data are linked and ready for review.

5 **Run Cross-Hit Filtering**

Apply cross-hit filtering with configurable parameters for mass confirmation, peak confirmation, and cross-hit search criteria. In this updated version of Analytical Studio, filtering speed has improved by 550-850% compared to the previous version, delivering the same results in a fraction of the time.

6 **Generate Instrument-Ready Sample Lists**

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Early hit lists often contain recurring features that appear across multiple wells due to background ions or shared signals. Without an automated cross-hit filter, researchers typically review wells manually or compare subsets of data to identify recurring peaks, an approach that becomes time-consuming and impractical if done over more than a few wells. Virscidian® implemented automated cross-hit filtering to expand the number of wells that could be searched to help identify more possible cross hits.

How It Works

When a compound's mass is detected in a given well, it could represent genuine target binding, or it could be background noise, isotopic interference, carryover from adjacent wells, or an isomeric compound producing a misleading signal. Cross-hit filtering systematically evaluates each potential hit against every other well on the plate. The filter parameters shown in Figure 3 enable you to tailor the filter to your specific preferences.

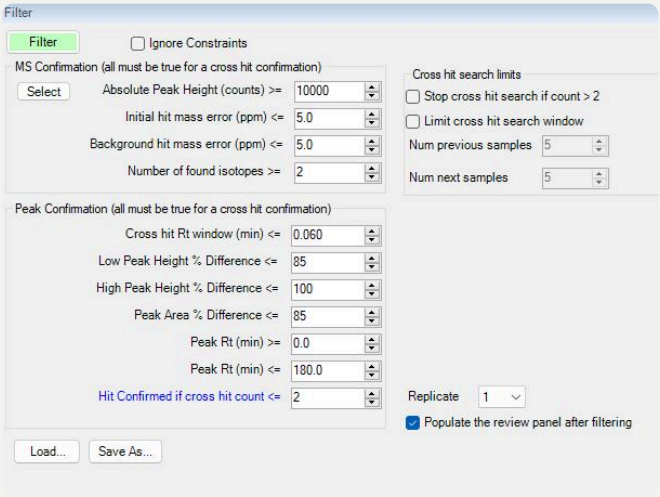


Figure 3. *Cross-hit filter settings can be adjusted based on your specific needs.*

Configurable, not black-box

Every filtering parameter is user-accessible and adjustable. Laboratories can define criteria that match their specific assay conditions, compound library characteristics, and confidence thresholds. Saved parameter sets ensure reproducibility across campaigns and analysts.

The cost of false positives

In an internal case study, results of which are shown in Figure 4, expanding cross-hit analysis from a limited window commonly used (±5 wells) to full-plate evaluation identified recurring/background signals that would otherwise appear as hits. This reclassified ~33% of the initial candidate hits (655 candidates in the representative dataset) as recurring/background rather than true binders, reducing avoidable follow-up assays and increasing hit confidence. Every false positive that advances to follow-up assays wastes time, reagents, and project team bandwidth.

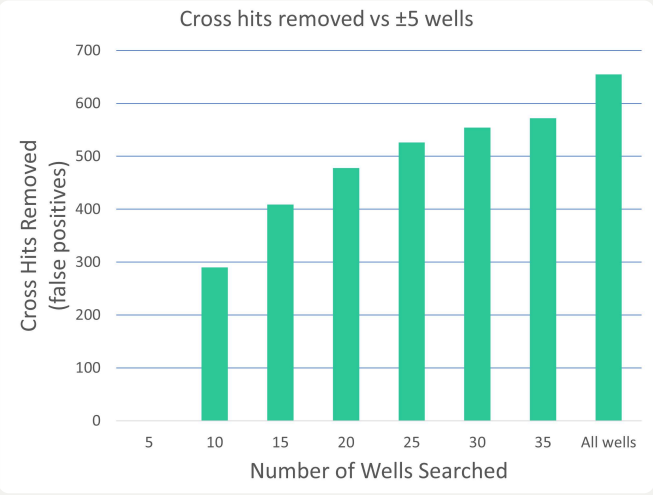


Figure 4. *Expanded cross-well analysis removes recurring background compounds and increases hit confidence.*

Why the speed improvement matters

Even with automated cross-hit filtering, screening across a full 96-or 384-well plate with thousands of compounds is computationally intensive and time consuming. For even larger sample sets, automated cross-hit filtering, though very useful, can become almost impractical. In the previous version of AS-MS, this step represented a significant time investment, particularly for large campaigns. The 550-850% speed improvement in this updated version means that a plate previously requiring 30 minutes for complete cross-hit filtering can be completed in 5 minutes, with no sacrifice in performance. This dramatic improvement in speed enables analysts to process more campaigns per day and makes full-plate and/or full-experiment cross-hit analysis practical for even the largest studies.

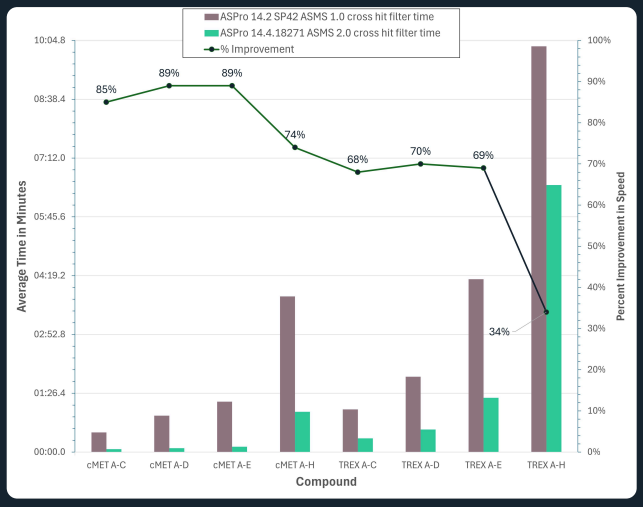


Figure 5. *The cross-hit filter capacities have been significantly upgraded in this updated release. For this test set of samples, speed improvements of up to 89% were seen (846% faster than previously possible).*

Due to the high compressions rates found in AS-MS experiments, isomeric species frequently appear. Mass spectrometers have a difficult time differentiating between isomeric species because they have the same exact mass. Analytical Studio's Isomer Tracking capability accurately flags and manages possible isomers through the analysis process, eliminating false positives due to isomer interference, ensuring high-quality results. Figure 6 illustrates a well with isomeric compounds, peaks 38 and 73. Instead of assuming Peak 38 is the target due to its larger peak area, Analytical Studio assesses each peak separately and searches across all samples for matches in mass and retention time to identify potential cross-hits.

The result is the filtered hit list shown in Figure 7 where each compound is classified as a confirmed hit (H), cross-hit/filtered (C), or other status, with full traceability back to the underlying chromatographic and spectral evidence.

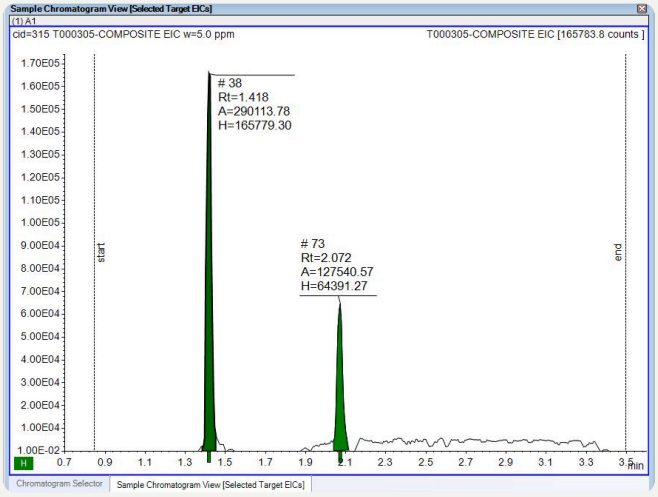
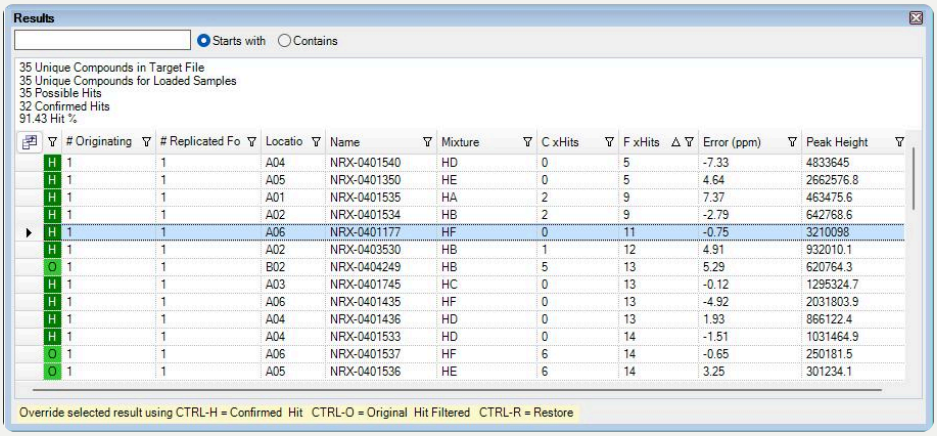
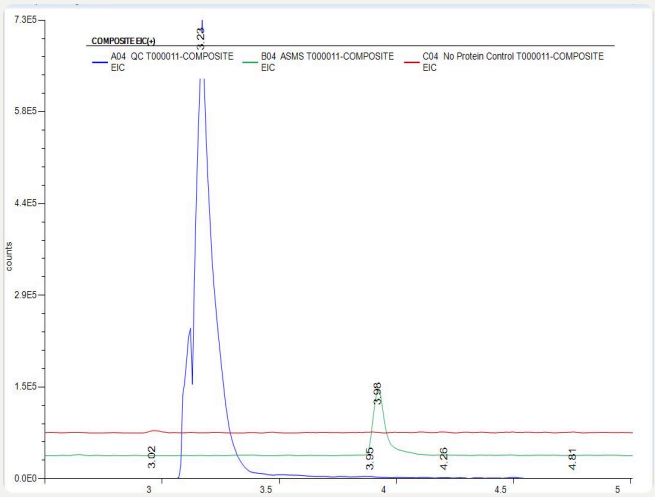


Figure 6. *During cross-hit analysis, isomeric peaks are evaluated independently, ensuring that the correct isomer peak isn't overlooked even if it's the smaller peak.*



	# Originating	# Replicated Fo	Locatio	Name	Mixture	C xHits	F xHits	Error (ppm)	Peak Height
H	1	1	A04	NRX-0401540	HD	0	5	-7.33	4833645
H	1	1	A05	NRX-0401350	HE	0	5	4.64	2662576.8
H	1	1	A01	NRX-0401535	HA	2	9	7.37	463475.6
H	1	1	A02	NRX-0401534	HB	2	9	-2.79	642768.6
H	1	1	A06	NRX-0401177	HF	0	11	-0.75	3210098
H	1	1	A02	NRX-0403530	HB	1	12	4.91	932010.1
O	1	1	B02	NRX-0404249	HB	5	13	5.29	620764.3
H	1	1	A03	NRX-0401745	HC	0	13	-0.12	1295324.7
H	1	1	A06	NRX-0401435	HF	0	13	-4.92	2031803.9
H	1	1	A04	NRX-0401436	HD	0	13	1.93	866122.4
H	1	1	A04	NRX-0401533	HD	0	14	-1.51	1031464.9
O	1	1	A06	NRX-0401537	HF	6	14	-0.65	250181.5
O	1	1	A05	NRX-0401536	HE	6	14	3.25	301234.1

Figure 7. *Cross-hit filtering transforms initial potential hits into a curated, high-confidence hit list by systematically evaluating each signal against the full plate.*



Comprehensive Data Review Tools

Analytical Studio contains several tools in addition to cross-hit filters that help analysts interpret AS-MS results faster and more consistently. Analysts can compare multiple injections side-by-side or overlaid (Figure 8) to assess binding signals versus background, click into any well or compound to immediately view chromatograms, spectra, and metadata, and save/share customized screen layouts tailored for screening review, hit confirmation, or reporting.

Figure 8. *Comparison of the extracted ion chromatograms from three experiments: blue trace shows the analysis of the sample containing only the potential lead compound, the green trace shows when the compound was subjected to the full affinity selection process before MS analysis, and the red trace shows results for a compound that underwent the affinity selection process without protein prior to MS analysis.*

Optional Add-On: Kd and Dose-Response Curve Analysis

For laboratories that need binding affinity data earlier in the screening process, Analytical Studio offers a K_d and dose-response curve (DRC) analysis add-on that operates directly within the AS-MS environment. For more information on dissociation constant and DRC analysis, please see the brochure Automated Kd Analysis in Affinity Selection-Mass Spectreomtry.

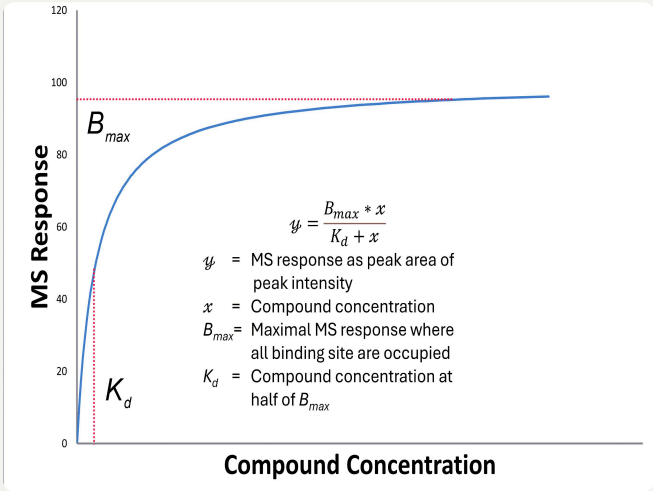


Figure 9. *Dose-response curve example showing response vs. concentration with the binding model equation.*

Key capabilities of the Kd add-on:

Automated DRC Processing

Dose-response curves are processed automatically when data is opened. K_d, B_{max}, and R-squared values are calculated for both peak area and peak intensity.

Quality-of-Fit Metrics

R-squared values assess how well the experimental data matches the binding model. Linear fit metrics and K_d ratios (peak intensity vs. peak area) provide additional quality indicators. These metrics help analysts quickly identify reliable measurements and flag data that warrants further review.

Interactive Visualization

Customizable layouts link well-plate views, extracted ion chromatograms, mass spectra, and DRC plots. Selecting a data point on the dose-response curve highlights the corresponding well, chromatogram, and spectrum for rapid verification.

Seamless Export

All results and underlying data can be exported to Excel with DRC plots automatically generated, keeping data portable for downstream pharmacology, informatics, or reporting workflows.

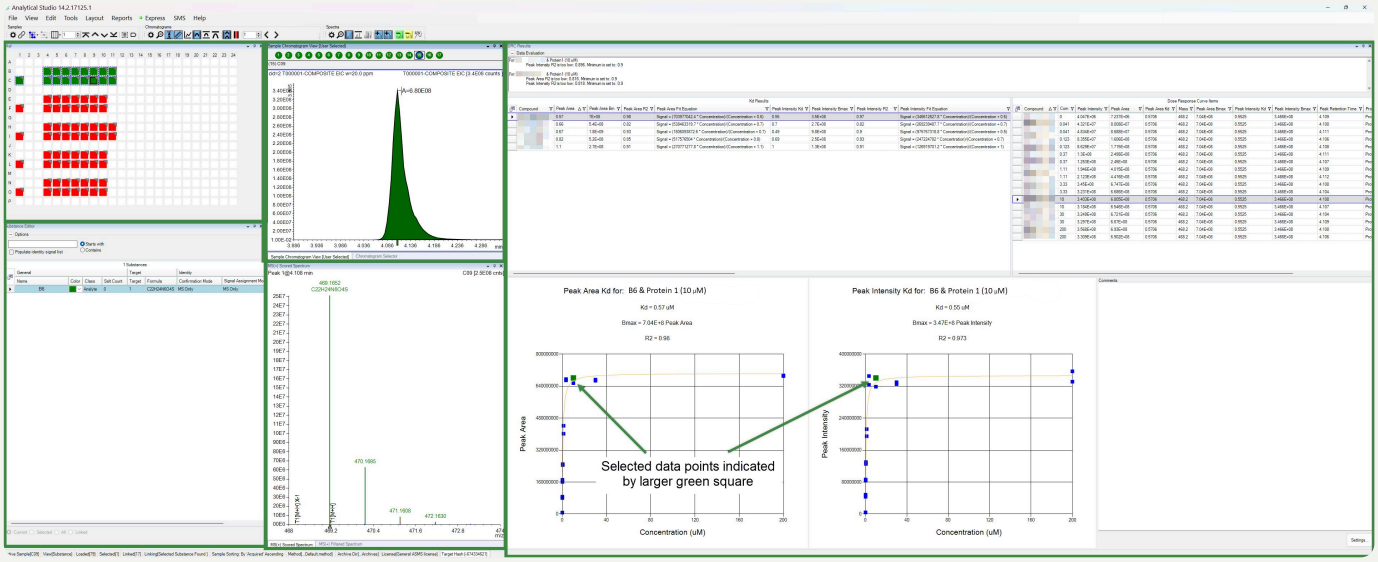


Figure 10. *Analytical Studio screen layout showing K_d analysis with well-plate view, EIC, substance editor, mass spectrum, and DRC results panel.*

Early affinity ranking, not surface plasmon resonance (SPR) replacement

The K_d add-on is designed to provide actionable affinity ranking data during hit screening, enabling smarter prioritization before investing in full biophysical characterization. It complements rather than replaces downstream SPR or other K_d measurements.

Built for Your Lab's Environment

Vendor-Neutral Architecture

Analytical Studio processes data from most chromatography or mass spectrometry vendor. Whether your AS-MS platform runs on Waters, Thermo, Agilent, or a multi-vendor configuration, Analytical Studio provides a single, consistent processing environment. Instrument-specific templates in AS-SLB handle the translation between your compound data and each vendor's required format.

Integration with Laboratory Systems NEW FEATURE

Analytical Studio connects with ELNs, LIMS, and enterprise informatics platforms. API connectivity and structured data exports (Excel, CSV) support downstream workflows including compound registration, project reporting, and data archiving. As part of the Dotmatics® portfolio, Analytical Studio aligns with broader scientific informatics strategies.

Infrastructure for AS-MS Workflows

The AS-MS workflow operates within the Analytical Studio ecosystem using AS-SMS for metadata management and AS-SLB for creating instrument sample lists. Organizations already using AS-SMS and AS-SLB for other Analytical Studio workflows (such as HTP or QC) can extend their existing infrastructure to support AS-MS. For organizations new to the Analytical Studio ecosystem, Virscidian provides implementation support to configure AS-SMS, AS-SLB, and instrument connectivity for your specific environment.

Configurable and Scalable

From single-instrument setups to multi-site, multi-vendor screening operations, Analytical Studio scales with your AS-MS program. Expression-driven decision logic, saved parameter sets, and reusable templates ensure that as your screening volume grows, your data processing remains consistent, fast, and reproducible.

Analytical Studio for AS-MS: Confidently Transform Screening Data into Decisions

Analytical Studio is the industry leading software purpose-built for AS-MS data analysis with cross-hit filtering. It delivers automated, vendor-neutral data processing, and configurable decision logic in a single, integrated environment, enabling your team to produce reliable hit lists faster and with fewer false positives.

Ready to see it in action?

Contact Virscidian® to schedule a demonstration with your data.