Virscidian

Virscidian's Analytical Studio software combined with Waters™ FractionLynx™ Increases Fraction Purity

Are you looking to increase the purity of your fractions while decreasing the person-hours required to obtain them? If so, look no further than Virscidian's Analytical Studio Professional software and Waters FractionLynx.

Introduction

High-throughput purification (HTP) aims to generate sufficient quantities of highly purified material — either for immediate characterization, downstream assays, or for long-term storage. When done correctly, HTP can deliver concentrated fractions of exceptional purity. When done poorly, however, it can lead to low yields, unwanted impurities, or even missing the compound of interest entirely. The stakes are high: if the target compound is lost or contaminated, labs can lose valuable time, resources, and opportunities for discovery.

How can laboratories ensure that their purification process remains both fast and automated, while consistently generating high-purity fractions? This becomes straightforward when using Virscidian's Analytical Studio Professional (AS-Pro) software in tandem with the Waters FractionLynx application manager. This powerful combination enables per-sample fraction triggers — meaning instead of relying on a one-size-fits-all set of criteria for the entire batch, each sample can have its own optimized settings. This flexibility minimizes contamination, reduces compound loss, and ensures fewer fractions are wasted.

One of the most common challenges scientists face when collecting fractions is identifying which peak corresponds to the compound of interest — and precisely determining when to begin and end the fraction collection. Traditionally, chemists start by injecting a few samples onto a preparative HPLC or LC-MS system, reviewing the resulting fractions, and then manually adjusting start and end times of fraction collection, as well as trigger intensity thresholds and even the detector used for triggering as needed to optimize fraction collection. Different plate-based chemistries can respond very differently to mass spectrometry (MS), UV, or analog detectors (CAD, ELSD), so a single set of trigger thresholds may not be optimized for every sample on the plate. Being able to specify unique fraction trigger criteria for each sample therefore improves both the number and the purity of the collected fractions, helping labs meet the central goal of collecting the fewest fractions with the highest possible purity.

> "The central goals in purification are: collect as few fractions and as high purity fractions as possible. AS-Pro combined with Waters FractionLynx helps lab obtain this goal automatically"

Most semi-automated or fully automated purification workflows use mass-directed purification, where the MS signal (often using extracted ion chromatograms) identifies peaks for collection. However, the instrument plumbing and the resulting extra-column band broadening inherent in mass-directed purification, can cause the MS signal to be delayed and broadened relative to the UV signal. Integrating MS and UV data offers a more precise approach: using MS to locate the compound of interest, then refining the fraction start and stop times with UV signals. This approach becomes even more critical when dealing with multiple components, isomers, or challenging chemistries.

Virscidian's AS-Pro is currently the only commercially available solution that supports per-sample fraction triggers. With its advanced logic, AS-Pro automatically determines which detector (MS, UV, or analog) and what intensity threshold should drive fraction collection. By giving each sample its own optimized trigger logic, AS-Pro boosts fraction purity, reduces missed compounds, and delivers tangible savings in both time and resources. The result is a high-throughput, automated workflow that consistently meets the demands of modern purification labs.

Detector Trigger Logic

Historically, fraction collection methods were limited to using either the MS signal or the UV signal for peak detection. While both are excellent chromatography detectors, neither alone covers every possible scenario:

- **MS trigger:** Provides compound specificity but may fail with poorly ionizing compounds. Splitting the flow for MS also causes band broadening, resulting in more dilute samples and larger fraction volumes. If your capacity is limited, critical material can be lost.
- **UV trigger:** Typically produces more concentrated fractions with smaller volumes. However, unrelated compounds with the same absorbance wavelength may be collected, and compounds without chromophores could be missed entirely.

Virscidian's AS-Pro overcomes these drawbacks by analyzing the MS, UV, and analog signals from the crude (PreQC) data to generate optimized trigger collection settings. This logic is then applied by FractionLynx to collect the fractions. Below are examples of the trigger logic statements AS-Pro can use:

1. **UV only, MS only, MS OR UV**
    - When a compound either lacks a chromophore or ionizes poorly, the ability to switch on-the-fly between UV and MS triggers reduces the risk of missing the target. Although only one detector is ultimately used per sample, AS-Pro automatically selects the optimal one.
2. **MS AND UV**
    - This option uses the MS signal to confirm the compound of interest but relies on the UV signal to refine the start/stop timing for fraction collection. It offers compound specificity alongside more precise collection windows.
3. **Mass (A OR B)**
    - If the compound of interest presents multiple adduct masses (e.g., singly- and doubly-charged), either adduct can trigger fraction collection. This logic ignores the UV signal but is extensible to more than two masses if needed.
4. **Mass (A OR B) AND UVA**
    - Similar to "MS AND UV" but accounts for multiple adducts. Fraction collection start and stop times are triggered from the UV detector only if one of the target adduct masses is detected.
5. **Collect all isomer peaks or only the most intense isomer peak**
    - When compounds exist as isomers, you can collect only the major isomer peak (assuming this is the compound of interest) or collect every isomer present, depending on experimental goals.

Intensity Trigger Settings

AS-Pro also evaluates detector intensities (MS, UV, and analog) and categorizes them into intensity “bins” such as Low, Medium, and High. These bins map to named FractionLynx trigger-control files, which can then be customized to each instrument or user preference. In practice, many labs use a three-level scheme (Low/Med/High) for MS, UV, and analog detectors, generating files such as:

- MS-Low, MS-Med, MS-High
- MS-Low UV-Low, MS-Med UV-Low, MS-High UV-Low
- MS-Low UV-Med, MS-Med UV-Med, MS-High UV-Med
- MS-Low UV-High, MS-Med UV-High, MS-High UV-High

Even a reduced subset of these files can dramatically improve fraction-collection performance.

AS-Pro provides advanced logic and intensity triggers that are easy to use and adjustable by the users. Additionally, Virscidian’s expert scientists can collaborate directly with end users to ensure each workflow is set up correctly. With Virscidian’s unparalleled support, you can get assistance adjusting these settings any time your workflow changes.

Fraction Collection Triggers in Action

PreQC and Data Review

The purification workflow typically begins with a crude analysis or “PreQC” step, which helps identify chromatography conditions likely to purify the compound of interest. Upon completion, AS-Pro uses the resulting data to determine if the compound can be purified, which method will provide the highest level of purity, and what fraction triggers are optimal. Users can review the automated recommendations in several formats before proceeding. Figure 1 shows one such view, which displays five panes: a well plate view, an on-screen report, expressions output, mass spectra, and chromatograms.

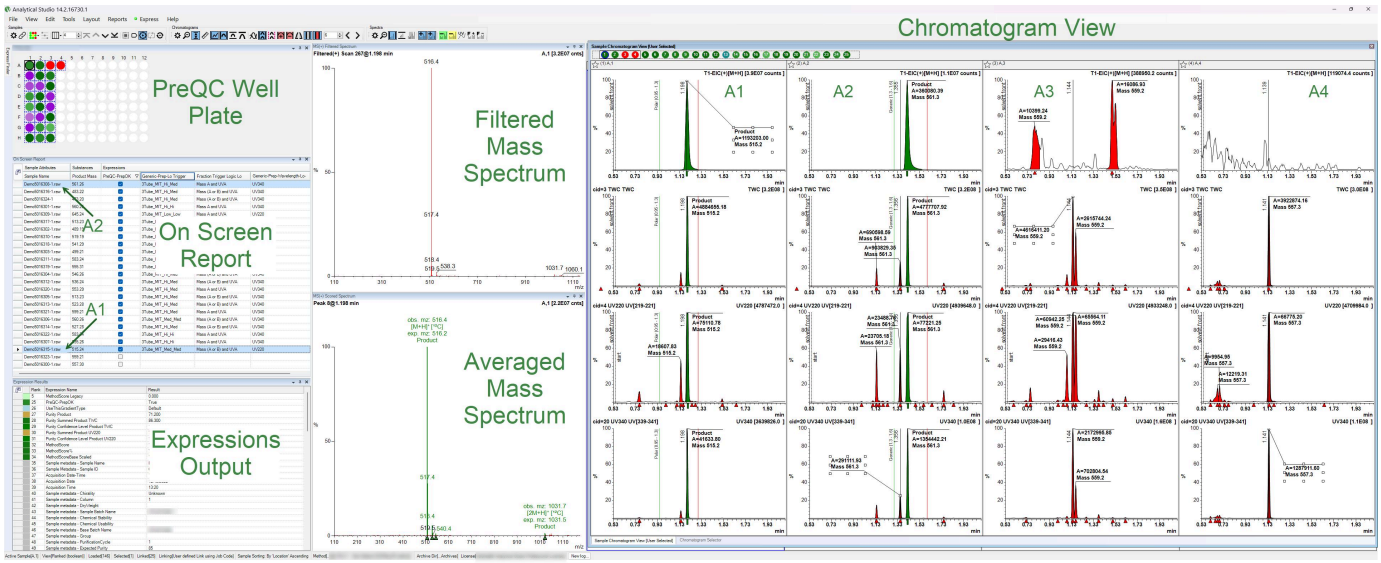

Figure 1. *AS-Pro Data Review Interface. A combined view displaying five key panes from a PreQC experiment — well plate view, on-screen report, expressions output, mass spectra, and chromatograms. This comprehensive view enables rapid assessment of purification strategies for multiple samples.*

Well Plate View

The well plate view, expanded in Figure 2, illustrates how AS-Pro leverages both color selection and color intensity to simplify data interpretation. Each well's color corresponds to the likelihood of obtaining a pure fraction:

- Green: Highest chance of purity
- Purple: Purification may proceed, but there is some risk of impurity
- Red: Target compound is not detected

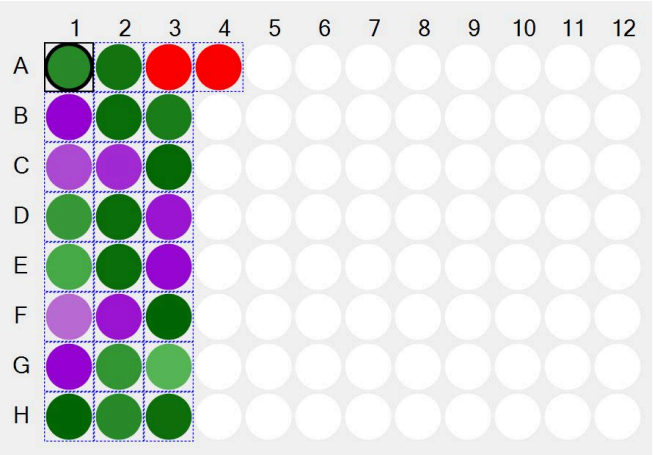

Figure 2. *Expanded Well Plate View. Color coding and intensity provide an "opportunity profile" indicating the likelihood of obtaining a pure fraction for each sample. Green wells suggest higher purity, purple implies moderate risk, and red indicates no detected target compound. Color intensity correlates with the relative abundance of the target compound, as shown by the contrast between wells A1 (darker green) and E1 (lighter green).*

Color intensity indicates the relative amount of the compound of interest. For example, wells A1 and E1 are green, indicating both may both be purifiable. However, E1, which is a lighter shade, will contain less of the compound of interest relative to the impurities. We can therefore think of the well plate view as an "opportunity profile" for the plate – how likely is it that a high concentration of a pure fraction can be collected for each sample. This upfront risk assessment saves time and money by alerting researchers early on if a compound is only present at low levels or is otherwise challenging to purify.

"Save time and money with the opportunity profile shown in the well plate view in AS-Pro"

Chromatogram Display

Figure 3 provides an enlarged view of the extracted ion chromatograms (EIC), total wavelength chromatogram (TWC), and UV traces (220 nm and 340 nm) for wells A1–A4:

- **A1 and A2** show green peaks (target compound) and red peaks (impurities) with good separation, confirming they are purifiable.
- **A3 and A4** lack any green peaks, indicating no detectable target compound.

These observations align with the "opportunity profile" shown in Figures 1 and 2.

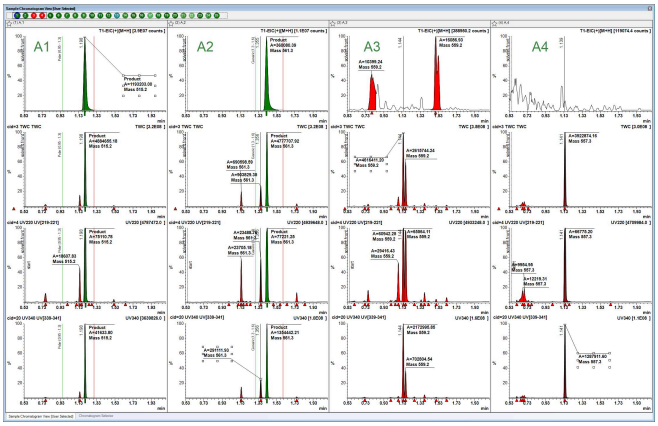

Figure 3. *Chromatogram Display for Wells A1–A4. Shown are extracted ion chromatograms (EIC), total wavelength chromatogram (TWC), and UV traces (220 and 340 nm). Green peaks represent target compounds, while red peaks indicate impurities. Wells A1 and A2 display well-separated target compounds suitable for purification, while A3 and A4 show no detectable target compounds.*

On-Screen Report

The left side of Figure 1 shows the On-Screen Report (expanded in Figure 4), which summarizes the fraction collection triggers across all samples and aids manual curation. Figure 5 highlights a small portion of the information shown in Figure 4 for wells A1 and A2:

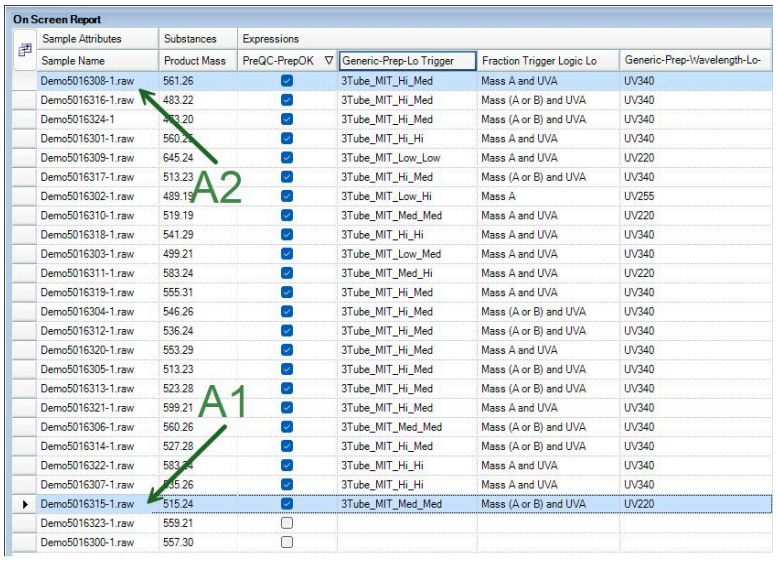

Figure 4. *On-Screen Report (Expanded View). Comprehensive on-screen report summarizing fraction collection parameters for all samples. The report details trigger logic, wavelength selections, and threshold settings that will be used by FractionLynx during purification.*

- **Generic-Prep Trigger Column:** Lists a generic FractionLynx file (e.g., 3Tube_MIT_...) used for fraction collection. FractionLynx converts the Low, Medium, and High trigger nomenclature addended after the FractionLynx file name to count-based thresholds unique to each prep instrument. Here, they are listed in UV_MS order such that A2 corresponds to a Hi UV trigger threshold and a Med MS trigger threshold while A1 uses a Med trigger threshold for both detectors.
- **Fraction Trigger Logic:** A1 uses “Mass (A or B) AND UV,” meaning if the compound of interest is found at one of two masses (A or B), and the UV signal (at 220 nm as displayed in the **Generic-Prep-Wavelength** column) exceed the medium threshold, fractions are collected. A2 uses “Mass A AND UVA” (at 340 nm) so that if the mass if found above the medium threshold and there is a UV signal at 340 nm above the Hi threshold, fraction collection begins.

The enlarged view of the chromatograms, Figure 6, illustrates the power of changing the trigger wavelength and threshold for each sample individually. The sample in A1 showed no absorbance at 340 nm, while the signal for A2 at 340 nm is roughly 100 times greater than the signal at 220 nm.

Sample Location	Generic-Prep Trigger	Fraction Trigger Logic	Generic-Prep-Wavelength	Product Mass
A2	3Tube_MIT-Hi_Med	Mass A and UVA	340 nm	561
A1	3Tube_MIT_Med_Med	Mass (A or B) and UV	220 nm	515

Figure 5. *Detailed Triggers for Wells A1 and A2. Detailed view of fraction collection parameters for wells A1 and A2, highlighting the sample-specific trigger logic and threshold settings. The report shows how different wavelengths (220 nm for A1, 340 nm for A2) and trigger combinations are optimized for each sample.*

The ability to automatically select the best detector, wavelength, and trigger threshold setting makes a critical impact in maximizing both the purity and the amount of compounds collected in high-throughput purification.

Optimize your Resources by Running Multiple Prep Instruments

A single LC/MS instrument running PreQC can feed data to multiple preparative systems — ranging from micro-scale to large-scale — without manual recalculations. That's because AS-Pro's trigger threshold output (e.g., Low, Med, High) is mapped to numeric intensity settings on each prep instrument independently. This feature is especially valuable in high-throughput labs, where preparative chromatography times can create bottlenecks.

Conclusion

By combining Virscidian's Analytical Studio with Waters FractionLynx, you gain exceptional flexibility in designing fraction collection triggers. Each sample's triggers can be configured independently, improving both recovery and purity. The result is a nimble workflow that adapts seamlessly to varying chemistries, scales, and throughput demands — ensuring you maximize the value of every purification run.

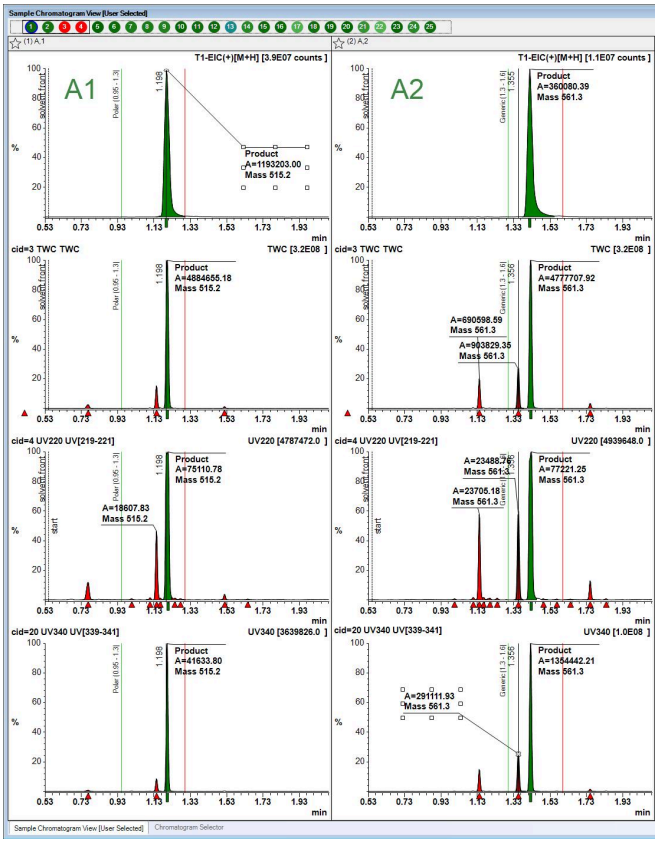

Figure 6. *Customized Chromatogram Analysis. Expanded chromatographic comparison of wells A1 and A2, demonstrating the importance of sample-specific wavelength selection. A1 shows minimal absorption at 340 nm, while A2's signal at 340 nm is approximately 100-fold stronger than at 220 nm, justifying the different wavelength selections for optimal fraction collection.*

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