

# Enabling High-Throughput MRM Based Biomarker Validation Studies Through A Vendor Neutral Application



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## Abstract

A modern current trend in biomarker validation is to profile well known systems using specific higher sensitivity assays based on quantitative mass spectrometry MRM experiments. Chemical biomarkers evaluated using these tools range from multiple peptide targets in plasma to panels of small molecules (e.g. biogenic amines) in cerebral spinal fluid. In all cases the potential biomarkers were quantified based on reference standards with a goal to better understand the response of the patient to therapeutic treatments. The software tools developed and applied to this study improved the success and throughput of these targeted assay studies.

## Background

As the number of patient samples and the size of the panels increase, data processing becomes a bottleneck. Each instrument vendor provides a mechanism for extracting the raw integration areas from the chromatograms. Some vendors are better than others. But the lack of consistency across platforms, combined with the tedious manual steps often required, introduced delay, error and inconsistency in the final results.

A new workflow application was developed in a few months based on an existing flexible analytical data handling and processing framework, Analytical Studio. The application allows the user to acquire data from various instrument vendor formats. The scientist can then quickly setup and run MRM studies with hundreds of samples significantly reducing the time to process a study. For larger studies, the time to process the study was reduced from 3 days to 15 minutes.

To improve quantification accuracy, a flexible MRM project builder and processing engine was implemented to allow each transition to reference a unique corresponding internal standard for retention time determination and analyte area normalization. This approach also resulted in higher quantitative reproducibility by automatically adjusting integration parameters to overcome drift in chromatography.

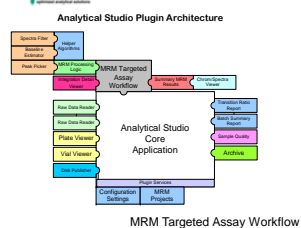
A new improved baseline estimation peak picking algorithm was used to improve quantitative accuracy. An intuitive interface allowed the scientist to interact with the algorithms to tune the parameters to the data set prior to running the automated data processing against hundreds of samples.

Once the project settings were acceptable, they could be saved as an XML document and reused or modified for other studies. Reports were generated from the results to assess the expected versus calculated ratios of the panel components.

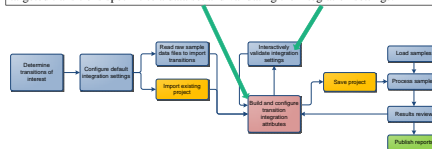
## Analytical Studio Architecture

Analytical Studio MRM Targeted Assay workflow deployed as a full featured client application with multiple plug-ins to support multiple raw vendor readers and multiple custom reports. Plug-ins can be easily "wired up" and coordinated by a hosting application to automate most workflows. As workflow requirements grow, the application can be extended to include more workflow support tools. Using this approach, 100% of the workflow requirements can be met with optimal performance

### Analytical Studio Plugin Architecture



The primary bottlenecks of an MRM experiment are building a large number of targeted transitions specific to a data set and validating the integration settings.



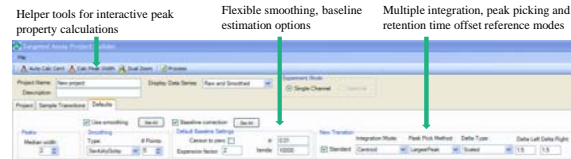
## Building an MRM Project

The project builder is the work horse for setting up the experiment. The scientist starts by importing all transitions found in one or many sample files or importing an existing project.



All experiment transitions from multiple samples are automatically available to make configuration easy

Default settings save time by eliminating repetitive actions required to configure large numbers of transitions. Users can also apply default settings to any combination of preconfigured transition records.

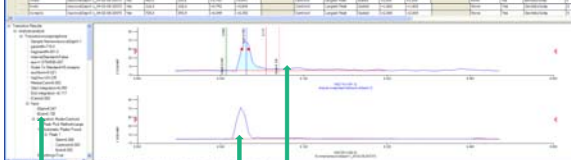
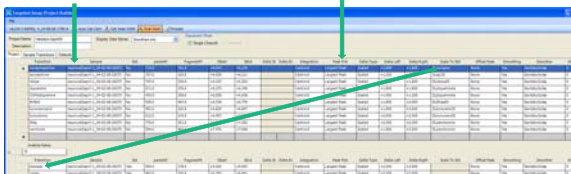


Helper tools for interactive peak selection, flexible smoothing, baseline estimation options, multiple integration, peak picking and retention time offset reference modes

Challenging integrations from dirty mixtures are solved with the flexible integration parameters, including user specified relative retention time settings which are employed during processing.

Simple easy to use interactive real-time validation of integration parameters with side-by-side reference standard viewing

Transitions configured with unique corresponding reference standard



Detailed results and integration settings

Side-by-side internal standard with transition

Interactive real-time integration configuration and calculation. Click on the chromatogram to specify start/end integration times and receive instantaneous processing based on the new settings

For extreme efficiency in high throughput MRM analysis, the devil is in the details for how easy it is to build and configure the experiment.

Selective sample switching for easy viewing and validation

Transition ion pairs selection from imported raw data or csv file

Add a new standard and it is automatically available as a reference standard

Modify the reference standard integration settings automatically updates the linked transition settings

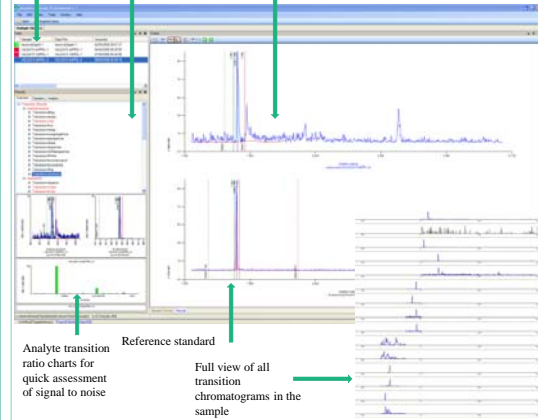
Determine retention time from a separate transition peak AND scale to an internal standard



## Powerful Data Review and Validation Tools

The next critical bottle neck in high-throughput biomarker validation is to ensure the integrations are performed as expected for a well known data set. Color coding of individual transitions gives a quick visual feedback where integration exceptions exist (e.g. transition was not found in the sample or no peak was picked in the specified region).

Apply the project to any number of samples  
Detailed integration settings and results for each transition  
Single exploded or compact view of integration results, baseline, smoothing and raw data with side-by-side integrated reference standard



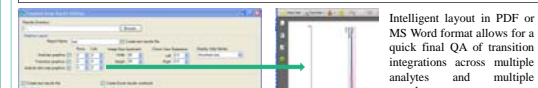
Analyze transition ratio charts for quick assessment of signal to noise

Reference standard

Full view of all transition chromatograms in the sample

## Flexible Reporting

Unlimited reporting through custom plug-ins enables customers to receive their results in the format that meets their needs. By developing a custom report plug-in, a multi-sample formatted is generated to the specification of the client. Using well defined layout of analyte ratios charts and transition integrations the scientist can quickly perform a final QA on the results.



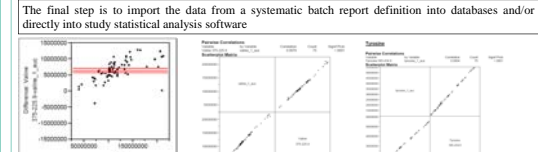
Intelligent layout in PDF or MS Word format allows for a quick final QA of transition integrations across multiple analytes and multiple samples



Easy to read transition ratio charts



The final step is to import the data from a systematic batch report definition into databases and/or directly into study statistical analysis software



## Benefits

The use of Analytical Studio to solve workflow and processing challenges related to biomarker validation has resulted in significant benefits including:

- Considerable time savings for configuring and processing large numbers of sample using large-sized panels
- Vendor neutral solution allows us to choose the equipment that is best for the job
- Flexibility in selection and scheduling the use of the lab instruments
- Higher quality results in a high-throughput operation with efficiency and higher reproducibility
- Easy integration to meet client's workflow needs (i.e. sample lists, data processing requirements, report formats)
- Real-time interactive UI for tuning integration parameters against test data
- Features to easily determine and configure transitions with unique reference standards viewed alongside the transition of interest.
- Detailed description on precisely how the results were generated, neighboring peaks and individual transition integration parameters
- Flexible reporting formats for a single sample or batches of samples
- Automated processing and report generation including detailed data review to validate integration settings and easily integrate with study analysis software
- Extensible design to allow for additional pre-processing workflow steps (MRM hunting and spectral signal to noise enhancement).
- Full suite of interrogation tools for viewing raw data, smoothed data, baseline response along side of processed data for improved decision making, trouble shooting and tuning and validating quantification results
- Robust peak picking and baseline estimation with validation tools to allow optimized settings specifically for the data set

## Conclusions

The purchase and operation of analytical instrumentation is often assumed to be the only cost of operating a high throughput analytical lab. However, there is significant cost associated with ensuring quality results are produced from the acquired data. This cost is directly related to quality of results and complexity of workflow.

Investing in building a general purpose analytical data handling framework helped Virscidian deliver a more efficient and flexible application without compromising the quality of results returned to customers.

The targeted assay workflow is just one example of how Analytical Studio has been applied to a complex set of steps across a variety of instrumentation to save costs associated with processing analytical data.

## Acknowledgments

We want to thank Rick Higgs, Jim Eickstein, and Brad Ackerman of Eli Lilly and Company for their contributions to the usability and design of the User Interface, the validation of the integration algorithms and insight into identifying the painful bottlenecks of high-throughput MRM experiments.

## For Further Information

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