Technical Overview

Agilent RapidFire Analyzer Software

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Introduction

Agilent RapidFire Analyzer (Analyzer) is a data analysis program that allows researchers to rapidly integrate, review, and report semiquantitative RF/MS/MS data acquired by an Agilent RapidFire high-throughput mass spectrometry system in multiple reaction monitoring (MRM) mode. Analyzer is designed to quickly evaluate large panels of results and automatically identify samples that require attention. Analyzer allows the researcher to view a large array of chromatographic peaks, review user-defined calculation and normalization values (with user-defined limits), and inspect flagged injections as an initial review of raw results. Composite reports are generated easily, allowing the integration, initial review, and exporting of thousands of data points in minutes (Figure 1).

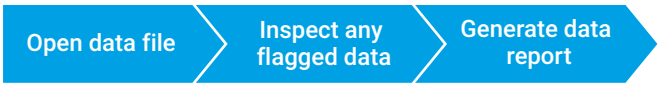

Figure 1. Standard operation of the Analyzer. Opening the data file automatically integrates every MRM for every injection using a preset and customizable method. The data can be reviewed by exception by simply looking for any injections that have been flagged for attention. A report for the entire batch is generated by a few clicks. These operations can be done in seconds, allowing thousands of injections to be analyzed quickly.

Integration

Analyzer software automatically parses all the injections in an opened data batch, and integrates each MRM chromatogram, for every injection, according to a preset method. The integration methods are customizable, giving the user control over standard peak baseline, tailing, and filter parameters (such as those parameters based on peak height, width, and area). This customization allows the user to fine tune the interpretation of experimental results for each assay (Figure 2). If desired, peaks can also be integrated manually, giving the user complete control of the data output.

Review

After opening a batch of data, there are four intuitive windows for selecting and reviewing the raw and integrated results (Figure 3).

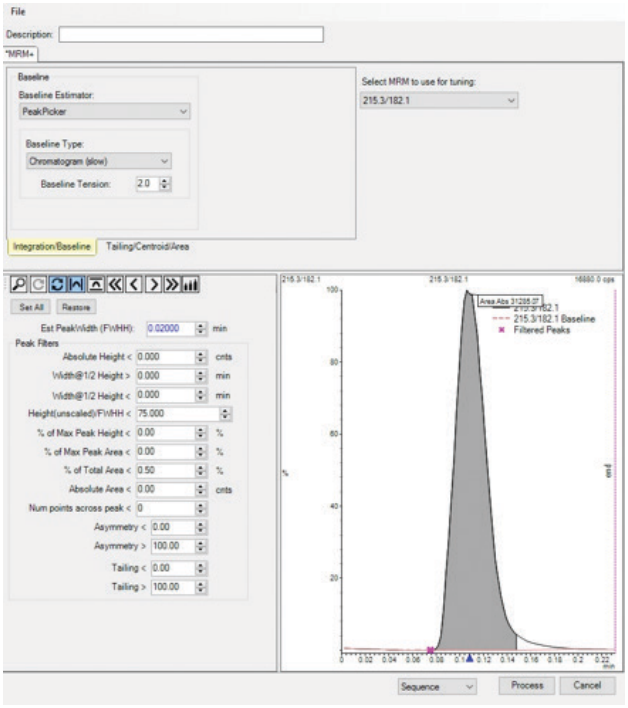

Figure 2. The Integration Method Editor provides control over standard peak baseline, peak tailing, and peak filtering parameters. Settings can be applied at the sequence level or to the entire batch of data, as desired.

Figure 3. Analyzer has four windows for selecting and reviewing data. The Plates window (A), the Wells window (B), and the Sequences window (C) allow the selection of injections for which to view MRM peaks in the Chromatogram window (D). Injections flagged in red may warrant manual inspection. This screenshot came from data that were intentionally burdened to highlight how flagging simplifies the identification of injections that warrant inspection.

The Plates window displays all the plates run within the batch, and allows view control for the Wells window by a selection status box.

The Wells window provides a simple plate-based option for selecting chromatogram data to view. Select injections by clicking individual wells, or by dragging a box around a group of wells. This action will result in the display of the corresponding MRM chromatograms.

The Sequences window complements the Wells window by organizing the injections by row, and controlling the chromatogram display by a selection status box. The Sequences window displays the integration results, acquisition parameters, and metadata (if sample information was imported before the batch run) for each injection. By displaying the data for each injection by row, the Sequences window allows rapid evaluation of the processed results for signal trends, controls, and outliers.

The Chromatogram window displays the MRM peaks for selected injections, facilitating the inspection of the raw peak shape. Each column in the Chromatogram window is from a single injection, while each row in the Chromatogram window is from a single MRM (as defined by the MS acquisition method). This display layout enables the peaks from any MRM to be inspected between injections by scrolling from left to right. The blue fill indicates the integration of each peak, enabling the suitability of integration parameters to be evaluated and optimized easily.

Calculations

Custom calculation templates can be applied to the experimental results to simplify the review process (Figure 4). Two common user-defined calculations are:

- The normalization of one analyte signal to its corresponding internal standard
- The expression of product signal as percent conversion [= product signal × 100/(product signal + substrate signal)]

The user can set the low and high limits for each calculation. If the calculation value for any injection falls outside of the defined limits, the injection is flagged for user review (Figure 4). The calculations editor also allows low and high limits to be defined for the integration values of the individual MRMs. The editor facilitates the identification of injections where signal-to-noise or signal saturation could affect data interpretation.

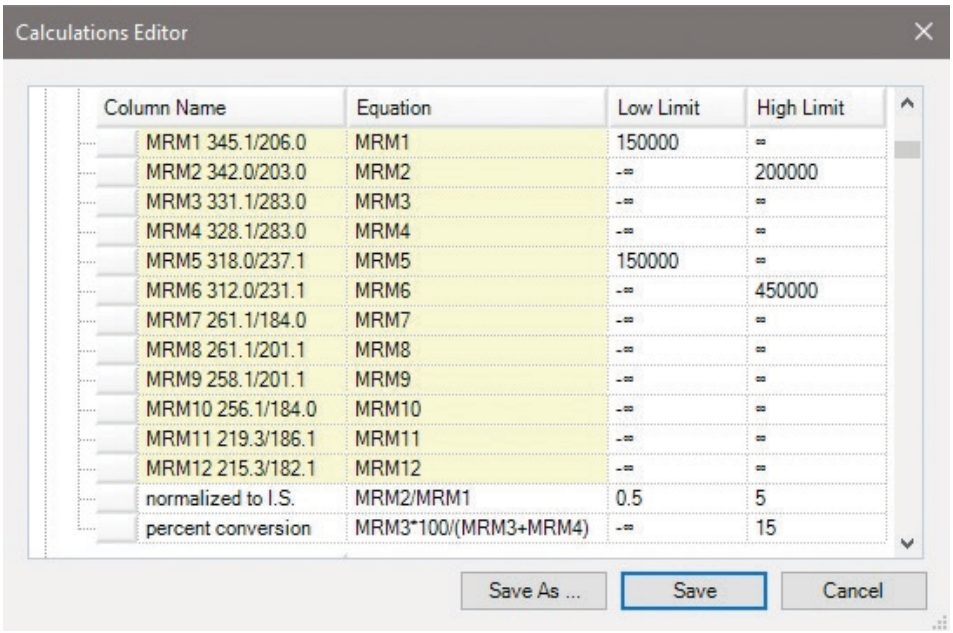

Figure 4. The Calculations Editor allows user-defined calculations to be applied to the experimental results. Low or high limits can be set for individual MRMs or for calculations values. If the calculation value for any injection falls outside of the defined limits, the injection is flagged for user review.

Flagging for review by exception

Analyzer automatically flags compromised samples, allowing review by exception, which greatly facilitates the processing of large batches of data (Figure 3). Injections are flagged by color in the Wells, Sequences, and Chromatograms viewing windows if:

- The calculation value falls outside of the user-defined limits (red flag)
- The sip sensor did not confirm sample collection from the plate during RapidFire analysis (red flag)
- The integration of any MRM chromatogram from that injection has been manually manipulated by the user (yellow flag)

Hovering over any flag in the chromatogram window identifies the reason for that flag. The user can quickly identify the injections that deserve manual attention, and gain a sense of the data quality before complete analysis.

Reporting

All injection data can be easily reported with just a few clicks. Reports are saved as Excel workbook files (.xls) and contain (optionally) all information about each injection including:

- Experimental results for each MRM for each well address
- User-defined calculation values for each sequence
- RapidFire method settings used during analysis
- Mass spectrometry method used during analysis

If sample information was imported before the run, metadata can be displayed as well.

Conclusions

Agilent Analyzer allows large numbers of samples, acquired by RapidFire triple quadrupole mass spectrometry, to be integrated, reviewed, and exported easily and quickly. Automatic and customizable integration provides high-quality data. Injection flagging minimizes the time it takes for preliminary results review by highlighting the wells that deserve manual inspection. Customized reports are generated quickly, allowing thousands of samples to be processed in minutes.

Definitions

Batch: One or more sequences that were run on one or more plates.

Sequence: One or more injections that were analyzed (from a single plate) using a user-specified injection order (plate map), RapidFire method, cartridge type, and mass spectrometry method.

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