Introduction

Agilent RapidFire Analyzer software (Analyzer) and Agilent RapidFire Integrator software (Integrator) are distinct data analysis programs that allow researchers to integrate and report semiquantitative RF/MS/MS data acquired by an Agilent RapidFire high-throughput mass spectrometry system in multiple reaction monitoring (MRM) mode. Integrator provides the basic elements for data integration and reporting, while Analyzer accelerates and expands these features by enabling data processing using faster, more reliable, and more information-rich workflows. This Technical Overview describes how Analyzer is superior for processing data acquired by a RapidFire system with a triple quadrupole (TQ) mass spectrometer. This Technical Overview presumes the reader has a fundamental understanding of Integrator, and has already read the Technical Overview Agilent RapidFire Analyzer Software, publication number 5991-9005EN.
Analyzer software integration is fast and automatic

Upon opening a batch of data, Analyzer automatically parses all the injections in the batch and integrates each MRM chromatogram, for each injection, according to a preset method. In contrast, the Integrator requires a peak to be manually selected and defined for each sequence. Therefore, the time savings of Analyzer, over Integrator, become more pronounced as the number of sequences in the batch increases.

To compare speed, the average times associated with opening and integrating an eight-sequence batch (representing 8 × 384-well plates from one work day of operation) using each program three times were calculated and graphed (Figure 1). Analyzer opened and integrated this batch of data 49 times faster than an experienced user on Integrator.

To illustrate the power of Analyzer, a batch of data containing 24,224 injections over 66 sequences was processed multiple times on a personal laptop computer. On average, the data integration process was completed in 102 ±2 seconds. This processing rate corresponds to over 470 AUC values (number of injections × 2 acquired MRMs per injection) per second.

Analyzer provides experimental results that are fool-proof

Analyzer integrates each MRM chromatogram according to a preset method that can be fine-tuned for each assay (described further below). As a result, unlike Integrator, no user intervention is required during the integration process. This hands-off processing eliminates peak definition variability from user to user and from time to time. Also, Analyzer parses the injection times from each other, precluding incorrect assignment of peaks.

To demonstrate the precision of Analyzer, eight sequences containing a total of 682 injections were analyzed twice. The experimental results from the first processing run were plotted against those from the second processing run (Figure 2). The runs produced identical values for each injection, as shown by a unity line with an $R^2 = 1$. An additional eight analyses were conducted, each time yielding identical results (data not shown).

Figure 1. Demonstration of the time associated with opening and integrating data using Integrator and Analyzer software. An eight-sequence batch (representing 8 × 384 wells plates run during a typical eight-hour shift) was opened/integrated using each program three times, and the averages were graphed. RapidFire Analyzer afforded a time savings of 49-fold.

Figure 2. Correlation plot of experimental data from a single batch of data processed twice with Analyzer. Results show that the two processing runs produced identical results.
Analyzer is customizable

While the default integration parameters in Analyzer work well, the software does allow the integration methods to be customized (Figure 3). Analyzer provides control over situations where peak baseline, tailing, or splitting abnormalities are observed. Unique integration methods can be saved and applied to the peaks from a single sequence, a single plate, or the entire batch. Integrator allows peak start and stop times to be defined, but these preferences are manually set by the user, and are subject to variability (user to user and time to time).

Using Analyzer, custom calculation templates can be applied to the experimental results to simplify data review (Figure 4). Two common user-defined calculations are the normalization of one analyte signal to its corresponding internal standard and the expression of product signal as a percent conversion. 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 5). The calculations editor allows low and high limits to be defined for the integration values of the individual MRMs. This definition facilitates the identification of injections where signal-to-noise or signal saturation could affect data interpretation. Integrator does not support calculations, limits, or flagging.

Figure 3. The Integration Method Editor provides control over standard peak baseline, peak tailing, and peak filter parameters. Settings can be applied to a single sequence, a single plate, or to the entire batch of data, as desired.

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 calculation values. If the calculation value for any injection falls outside of the defined limits, the injection is flagged for user review.
Analyzer facilitates preliminary data review

After opening a batch of data, there are several software features that can communicate a general view of the data quality (Figure 5).

- **First**, the Wells window flags injections (red coloring) that have calculation values outside of the user-defined limits. The Sequences window expands this information by displaying the calculation value, the calculation limit, and a message if the limit was not met or exceeded.

- **Second**, the Wells window flags injections (red coloring) where the sip sensor did not confirm sample collection during analysis. Correspondingly, the sip field in the Sequences window displays *Error* for these injections.

- **Third**, by displaying the data for each injection by row, the Sequences window allows rapid manual evaluation of the processed results for signal trends, controls, and outliers.

- **Fourth**, the Chromatogram window displays the integrated MRM peaks for selected injections: (a) facilitating the inspection of raw peak shape, (b) enabling the comparison of integration results between MRMs or injections, and (c) illustrating the accuracy and precision of the integration method.

As a result of these indicators, any injections that deserve manual attention can quickly be identified, and a general sense of the data quality can quickly be gained. In comparison, Integrator does not flag injections, provide display control for specific injections, or show integration results. This comparison shows that the preliminary inspection of results is more user-friendly with Analyzer.

Figure 5. Analyzer has four windows for selecting and reviewing data. The Plates window (upper left, A), the Wells window (upper left, B), and the Sequences window (lower left, C) allow the selection of injections for which to view MRM peaks in the Chromatogram window (right side of screen, D). This screenshot came from data that were intentionally burdened, to highlight how flagging simplifies the identification of injections that warrant inspection.
Analyzer provides superior reporting options

Data reports generated by both Analyzer and Integrator include the experimental results from wells, wash, and matrix stations. However, reports from Analyzer can also include calculation data, acquisition information, and metadata, which is optionally imported by the user before analysis. Examples of acquisition information include the acquisition time, instrument name, cartridge type, cartridge injection number, method name, and MS method name. Examples of metadata include injection-specific information such as sample name, group, or concentration. These options enrich the overall quality and usefulness of data reports from Analyzer.

Analyzer provides data that correlate well with Integrator

Data correlation between software platforms is critical, especially for laboratories who are considering the adoption of Analyzer midproject. The correlation between data outputs from Integrator and Analyzer was examined, and the results were exceptional. Samples from 22 data point inhibition curves for 26 compounds, plus controls, were analyzed by RapidFire-TQ, and the resulting data were processed with Analyzer and Integrator individually. The AUC values were graphed using Prism software to generate the IC\textsubscript{50} values (Figure 6). The IC\textsubscript{50} values from the Analyzer output (X-axis) were plotted against those from Integrator (Y-axis), and the graph shows a near perfect correlation (R\textsuperscript{2} = 0.9993) between the software outputs. The small deviation observed is likely from human error (specifically, the definition of peak start and stop times) within the Integrator workflow, as iterations of this process yielded variance in precision. Conversely, as demonstrated above, multiple iterations of Analyzer processing yielded identical IC\textsubscript{50} results.

In summary, Analyzer provides multiple advantages over Integrator for processing RapidFire-TQ data including time savings, precision, preliminary data review, and reporting options (Table 1).

![Graph showing correlation between Analyzer and Integrator IC\textsubscript{50} values]

Table 1. Comparison of features for Integrator and Analyzer software.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Integrator</th>
<th>Analyzer</th>
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<tbody>
<tr>
<td>Easy to use</td>
<td>yes</td>
<td>yes</td>
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<tr>
<td>Accommodates large batches of data</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Automatic peak integration</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Plates-based visualizations</td>
<td>no</td>
<td>yes</td>
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<tr>
<td>Calculations</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Review by exception (Flagging of injections that were missed and/or resulted in calculation values out of limits)</td>
<td>no</td>
<td>yes</td>
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