The Benefits of waters_connect™ MRM Processing Application, MS Quan

INTRODUCTION

A multitude of scientific analyses require a scientist to assess how much of a target compound or suite of compounds is present in a sample. This process is the same across different application areas such as Food and Environmental, Pharma, and Clinical Research, giving the scientist an answer to how much of their target substance is present.

Usually, the amounts analyzed are very small, sometimes in the parts per billion. Analysts are constantly asked to reach these low levels of quantitation due to local governmental guidelines or laws which, when met, will allow their tested commodity to be sold within a particular territory.

Quantitation itself is a very simple experiment. A clean matrix that does not contain the target compounds is used, then, known amounts of the targeted compounds are added to this matrix to make a series of standard solutions that are analyzed with the tandem quadrupole LC-MS system. These different concentration solutions give different sized peaks in the chromatograms produced. A small peak in the chromatogram relates to a small concentration and in a linear fashion, a large peak in a chromatogram relates to a large concentration of the targeted analyte being tested. The MS Quan software plots these peak areas on a graph against the sample concentration which forms what we call the calibration curve. Unknown samples are also injected and their peak areas from their chromatograms are compared to the calibration curve and thus the concentration of the compound present in the unknown samples is calculated.

The objective of this white paper is to discuss the data processing app called MS Quan that will deliver the ability to quantify and report data on the waters_connect platform for Waters™ tandem quadrupole mass spectrometer-based LC-MS systems.

THE MS QUAN APP IN WATERS_CONNECT

The waters_connect platform is the ecosystem for all the new Waters browser-based apps. The software uses a modular architecture with browser-based applications to minimize re-validation and deployment efforts so that any new updates, features and anomaly corrections are targeted to individual applications.

Several of these apps will work together to give users the ability to perform their MRM quantitation. Method development and routine quantitation workflows exist within the new product as electronic linkages and allow users to move their data from one app to another.
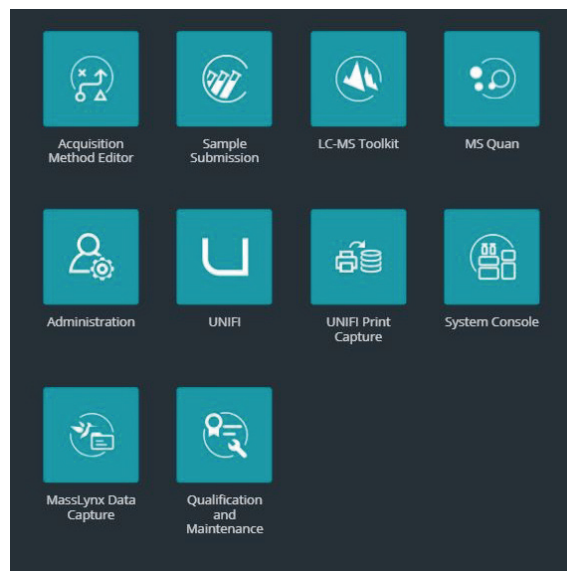

Figure 1. The apps that form waters_connect for quantitative analysis.

The waters_connect architecture is compliant ready. MS Quan also meets the compliance rules set out in 21 CFR Part 11, the U.S. Food and Drug Administration's (FDA) rule governing electronic records and electronic signatures, as discussed in the white paper *The Role of waters_connect in Assisting with Electronic Records Regulation Compliance* available on waters.com, document number 720006995en.

The MS Quan app focusses on MRM data produced by tandem quadrupole mass spectrometers (Xevo™ TQ-XS, Xevo TQ-S micro, and Xevo TQ-S cronos), the drivers for which are built into the waters_connect Software. Additionally, it is possible to import MassLynx™ v4.2 MRM data into the waters_connect Software via the MassLynx Data Capture app. This approach gives the user the flexibility to use the more modern MS Quan app and benefit from its updated functionality, while acquiring data on legacy MS systems.

Working within waters_connect, the app itself brings benefits such as the ability to add the latest updates without having to reinstall the entire waters_connect platform. Other apps within the waters_connect for quantitation platform also have this feature.

The MS Quan app, like all other MRM data processing software packages, requires a processing method to process the data. The integrated approach of the waters_connect platform means that the apps are linked, enabling the MS Quan processing method to be created in under 10 seconds, directly from the acquisition method editor. This electronic transfer of data between the two apps eliminates transcription errors, with all the masses and retention times electronically transcribed from one to the other.

In addition to the processing method, MS Quan also uses a rule set that can be customized according to individual laboratory SOPs and protocols. Rules guiding the user on the variance of the LLOQ (lower limit of quantitation) (eg ±20%) and then all other calibration standards (eg ±15%) are easily set. Additional rules, such as the peak area in a blank sample compared to the LLOQ, can also be set. Metrics around quality control samples, internal standards and ion ratios are also possible.
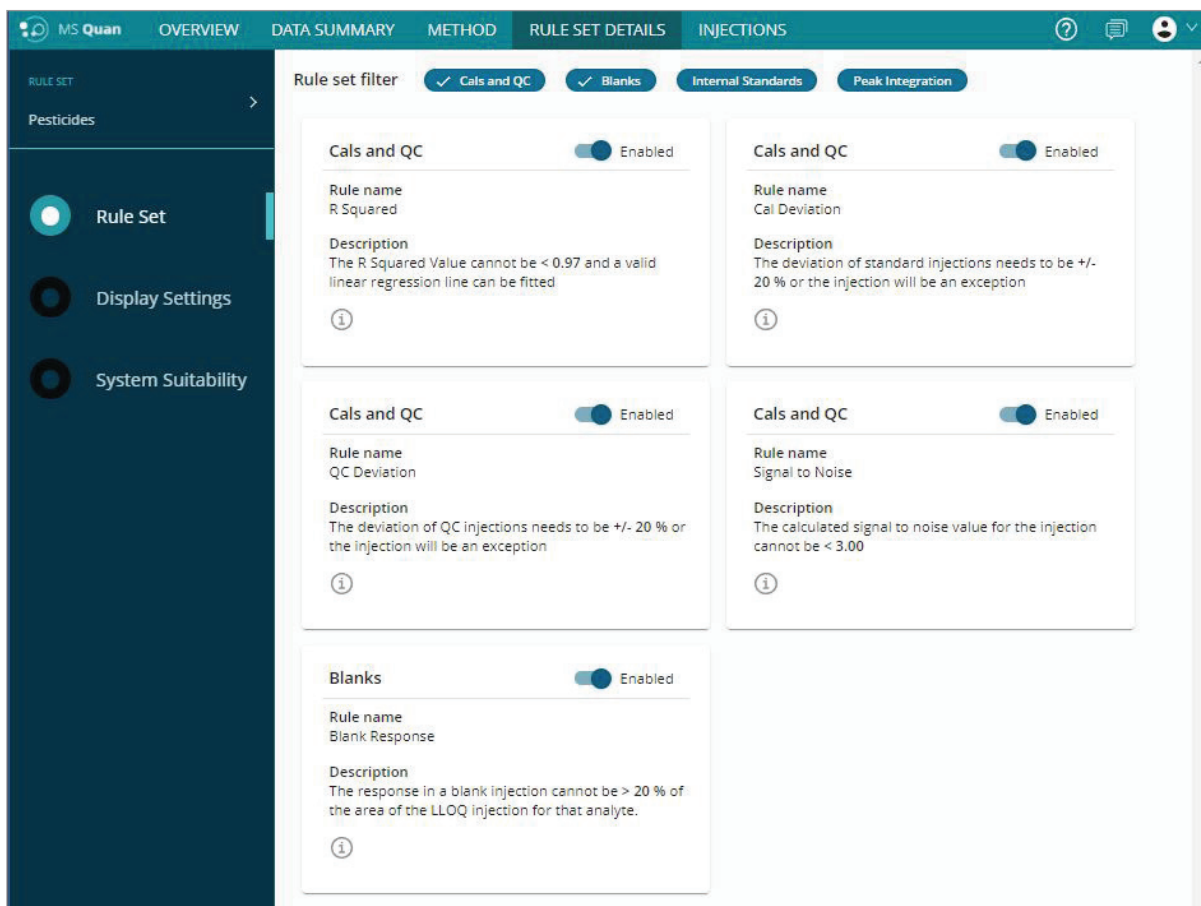

Figure 2. The MS Quan app features user defined rules that reflect the lab SOP.

The Exception Focused Review (XFR) functionality increases the quality and efficiency of the data review process and will lead to fewer errors being found in QA data checks that happen after the analyst has fully processed the dataset. The rule sets can be shared between analysts and can be locked by system administrators who may choose to make the rule sets reflect their organizational review criteria. Once the rule set has been made and applied to the data set at the time of processing, the user receives the processed data. This is where the similarity between MS Quan and other data processing software ends.

Initially the user can see a high level view of the batch that forms the start of a workflow for processing the data (Figure 3). There are several different aspects that are visible on this dashboard showing the user if there are any issues with the construction of their calibration curves or possible contamination/carryover in their blank samples.

The dashboard highlights how much work is needed to process the data set and achieve reportable results. The dashboard is also continually updated as the batch is processed to give the current status at all times. Users can click through from this dashboard to specific sections of interest. For example, the calibration standards section will allow them to navigate to the start of the Exception Focused Review.

The XFR clearly shows areas of the data set which require immediate attention. These exceptions are areas that fall outside the rule set specified by the user when processing the original data.

When looking at the exceptions, the user can filter on calibration point deviation. In the batch shown in Figure 4, there are 19 compounds, with 9 showing an out of tolerance deviation. If the user clicks on the "cal deviation" exception filter, the software filters the data to leave the 9 compounds that require attention. Ultimately, this reduces the time needed for the analyst to process the data when compared to other quantitation packages.
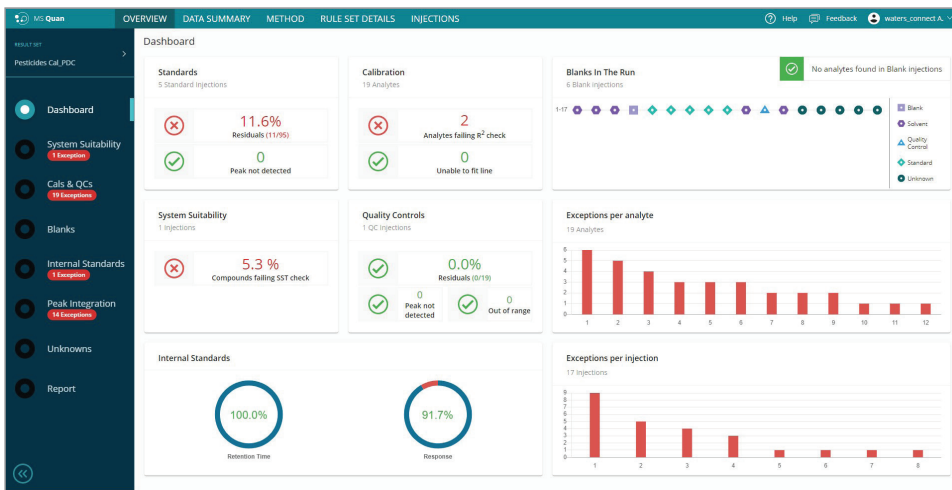

Figure 3. The dashboard instantly gives the user a view of how likely they are to get a passed batch from their dataset.
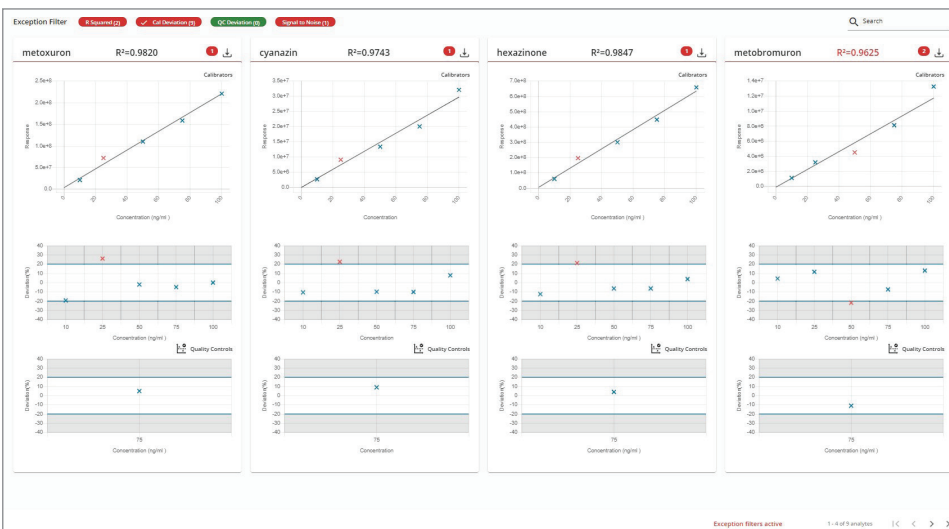

Figure 4. The Exception Focused Review filters out the calibration curves that meet the rule set.

Another new feature within the MS Quan app is the integration between two retention times. Analysts often struggle with integrating low intensity peaks, or peaks where two isomers co-elute. Instead of time-consuming manual integration, the user can set their start and end integration times and apply them to their peak of interest as shown in Figure 5. When the analyst is satisfied with the integration interval, they can apply this setting to the method.

This applies all the settings within the MS Quan software to all peaks in the data set for this compound, meaning nothing has been individually (manually) integrated as all peaks have been processed with the same processing parameters for the compound of interest.

The peak integration page uses a multiple chromatogram layout that is uniform and carefully designed to let users detect any issues with their peak's shape and integration. Figure 6 shows this view filtered by sample type, illustrating that users can view only the unknown samples if desired. Analysts with multiple compounds in their data set can also use the search function to go directly to their compound of choice. Analysts can also focus on exceptions to the rule set, a red marker will appear if an exception exists, and the user can click on the chromatogram to display a detailed view showing the issue.
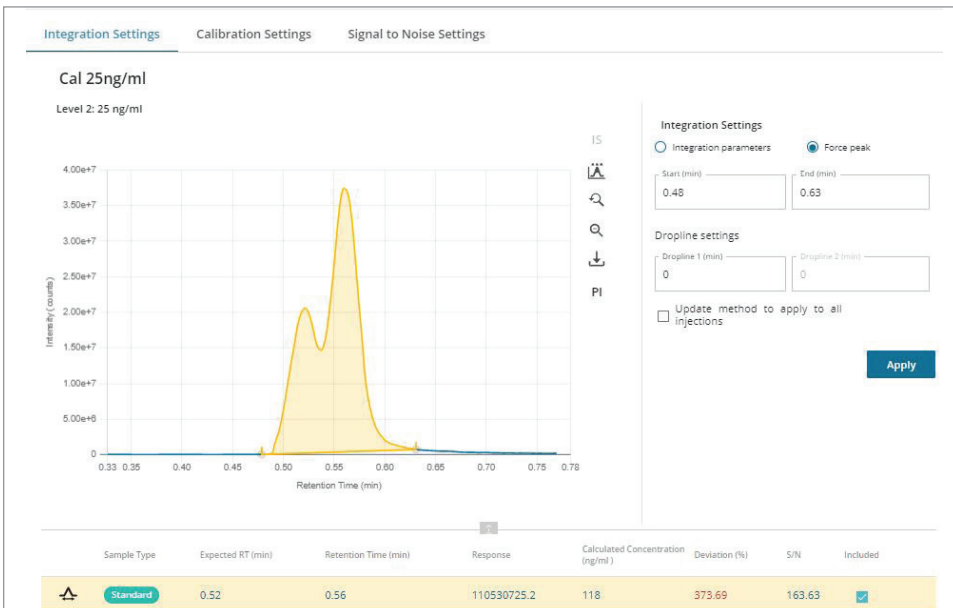

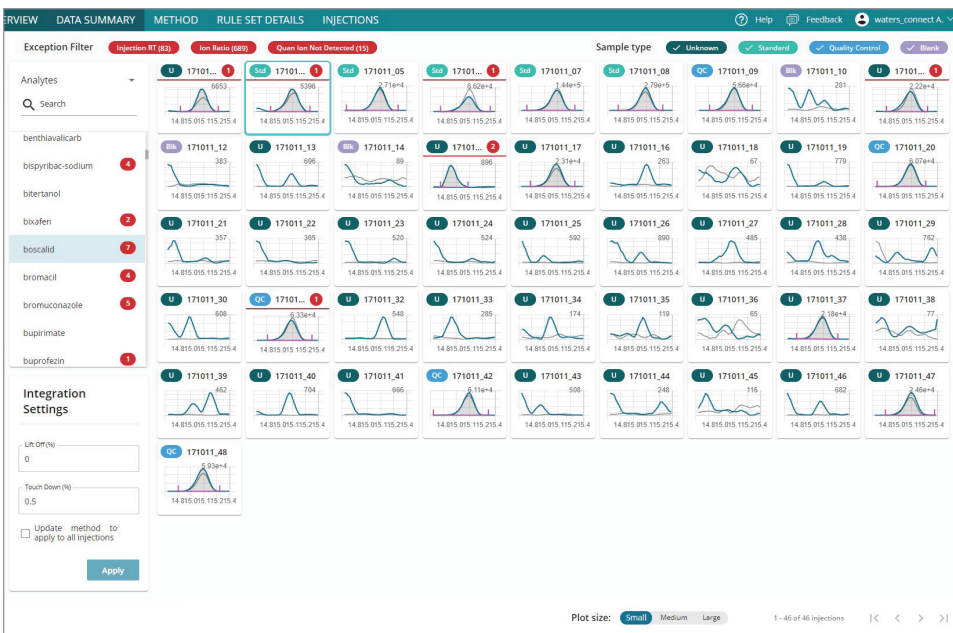Figure 5. Integrating between two timepoints is easy in MS Quan.

Figure 6. reviewing multiple peaks is easier when they are all viewed together.

In addition the software automatically gives visibility of the blank MRM trace for an analyte overlaid with any calibrator, QC or unknown sample peak allowing the user to more accurately view the level of the analyte in the sample relative to the blank response.

CONCLUSION

The overriding benefit of MS Quan is that analysts can reduce review time which is one of the largest bottlenecks in their laboratory to improve throughput, and overall efficiency.

Exception Focused Review, working in conjunction with the multiple peak integration page, enables the analyst to quickly process their batch. The peak integration between the two retention timepoints also reduces the data review time as there are fewer complex peaks to integrate at the lower end of the MS Quan calibration curve where time-consuming manual integrations are often required.

The MS Quan software flags exceptions to the rule set defined by the laboratories SOP's and protocols resulting in improved data quality. Improving the workflow for quantitative LC-MS/MS data review is critical to many analytical laboratories and this new software helps reduce data review time to a minimum.

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