Technical Overview

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Boost Efficiency and Productivity with Instrument Intelligence and Workflow Automation

For LC/TQ, LC/TOF, and LC/Q-TOF workflows

Introduction

Ever-increasing pressure for laboratories to minimize operating costs while maximizing output demands innovative approaches to their daily work. Recent developments in artificial intelligence (AI) and automation can help to alleviate this growing burden. The goal is to get technology to contribute more and more to day-to-day operations by reducing errors and ensuring consistent high-quality data generation. For workflows using Agilent LC/TQ and LC/(Q-)TOF instruments, this goal is becoming a reality.

The latest generation of Agilent LC/TQ and LC/(Q-)TOF instruments offers a suite of intelligent automation features to keep the instruments at peak performance. SWARM autotune allows instruments to be tuned for specific applications and desired performance. Auto- and check tunes can be scheduled to run during downtime so that the instruments are always ready to go. Instrument status is actively monitored using Early Maintenance Feedback (EMF) with constant feedback so that users can anticipate and plan for maintenance. Data are actively examined to ensure high quality. And, if data-related issues are suspected, Intelligent Reflex reinjection workflows automatically work to correct the situation. The culmination of these instrument intelligence features reduces errors, rework, and manual intervention—boosting laboratory efficiency and productivity.

Application-based SWARM optimized autotune

Application-based SWARM autotune ensures expert, application-specific results every time with Agilent LC/TQ and LC/(Q-)TOF instruments. Optimizing parameters one by one is time-intensive and might not find the true optimum settings. SWARM autotune obtains the absolute best system settings using multidimensional optimization. Adjustments in one element affect all other elements. Tuning the instrument this way captures the cross-interactions between each component, reducing instrument-to-instrument variability and providing the best overall instrument performance. SWARM autotune simplifies the optimization of multiple instrument parameters and is adjusted to allow for more ion transmission, more speed, and lower loss of fragile molecules. SWARM saves time and delivers the best accuracy and sensitivity for any application.

SWARM autotune was designed with flexibility in mind to meet any application needs. Users can choose from many application-based presets, ranging from small ions to large proteins, with the additional ability to adjust for fragile molecules and polarity as needed. Preconfigured instrument modes are shown in Figure 1. For most applications, the preconfigured instrument mode options will be sufficient. However, the ability to customize instrument performance for specific applications is available through the manual tune functionality.

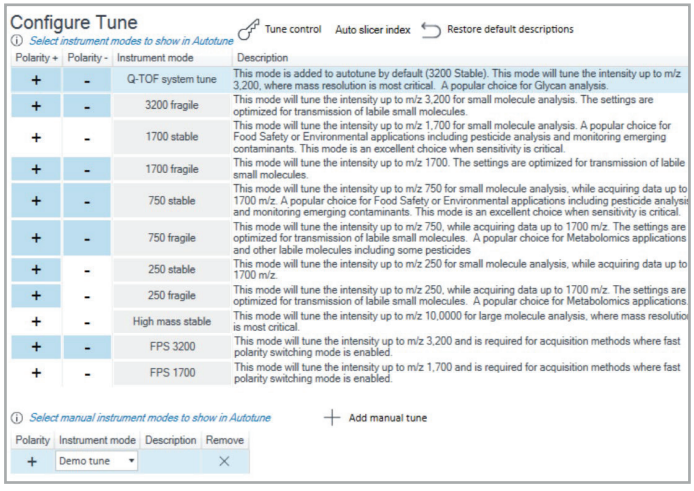

Figure 1. Preconfigured, application-based SWARM autotune options for an Agilent LC/Q-TOF system, with manual tune also available.

Instrument performance for high resolution mass spectrometry can favor either resolution or intensity—or a balance between the two. There is a big range between resolution and intensity, as shown in Figure 2, to tune the instrument to the exact analytical needs—allowing for application-based instrument mode optimization. For example, default system instrument modes typically favor resolution. However, for large proteins, high resolution is not always necessary, and maximizing intensity is often preferred. Figure 3 shows the advantages of an application-based SWARM autotune for large molecules. When the highest resolution is not required, this application-specific tuning greatly improves signal intensity for large molecules.

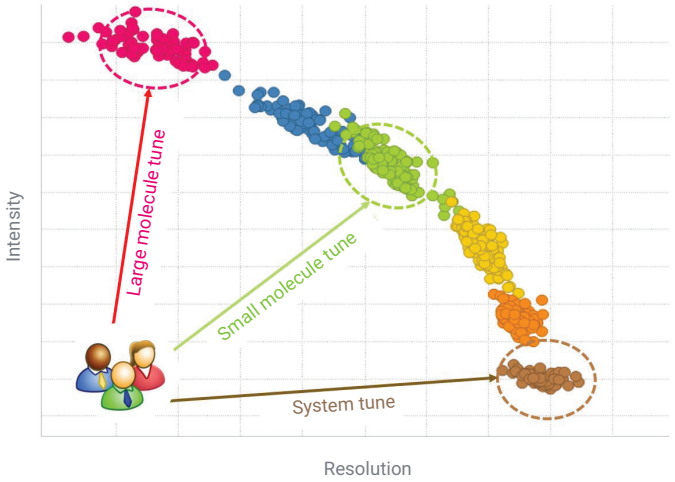

Figure 2. LC/MS systems can be tuned to favor intensity, resolution, or somewhere in between, depending on the desired application or workflow.

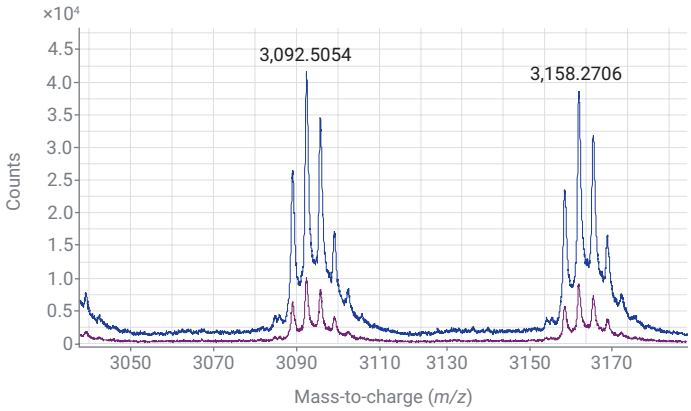

Figure 3. Spectra overlay showing the benefit of using an application-based SWARM autotune for large molecule analysis to increase signal intensity.

Streamline instrument tuning with active status monitoring, user roles and permissions, and scheduled auto- and check tunes

Once the appropriate instrument modes are selected and tuned, their status can be monitored through Agilent MassHunter Acquisition software for LC/MS systems. Traditionally, instrument tuning relied on user judgment, considering factors such as the date of the last tuning session and the current performance of the instrument. Now, the instrument provides feedback. Users can view the instrument mode status, when the instrument mode was last tuned, when it will expire, and the recommended next tune date (Figure 4). Lab managers can set tune time frames based on their laboratory needs or use preset/default time frames.

Importantly, lab managers can streamline the instrument mode tuning process based on user experience level. The acquisition software provides user roles and permissions that can simplify acquisition workflows for novice users, while allowing more advanced users to access advanced functionality. The permissions help to prevent new users from accidental instrument damage and wasting time and/or samples due to lack of experience. For tuning the LC/MS system, lab managers can preset the tune options so only the instrument modes that are relevant to particular analyses are displayed for selection by a technician—simplifying the tuning process for routine operation.

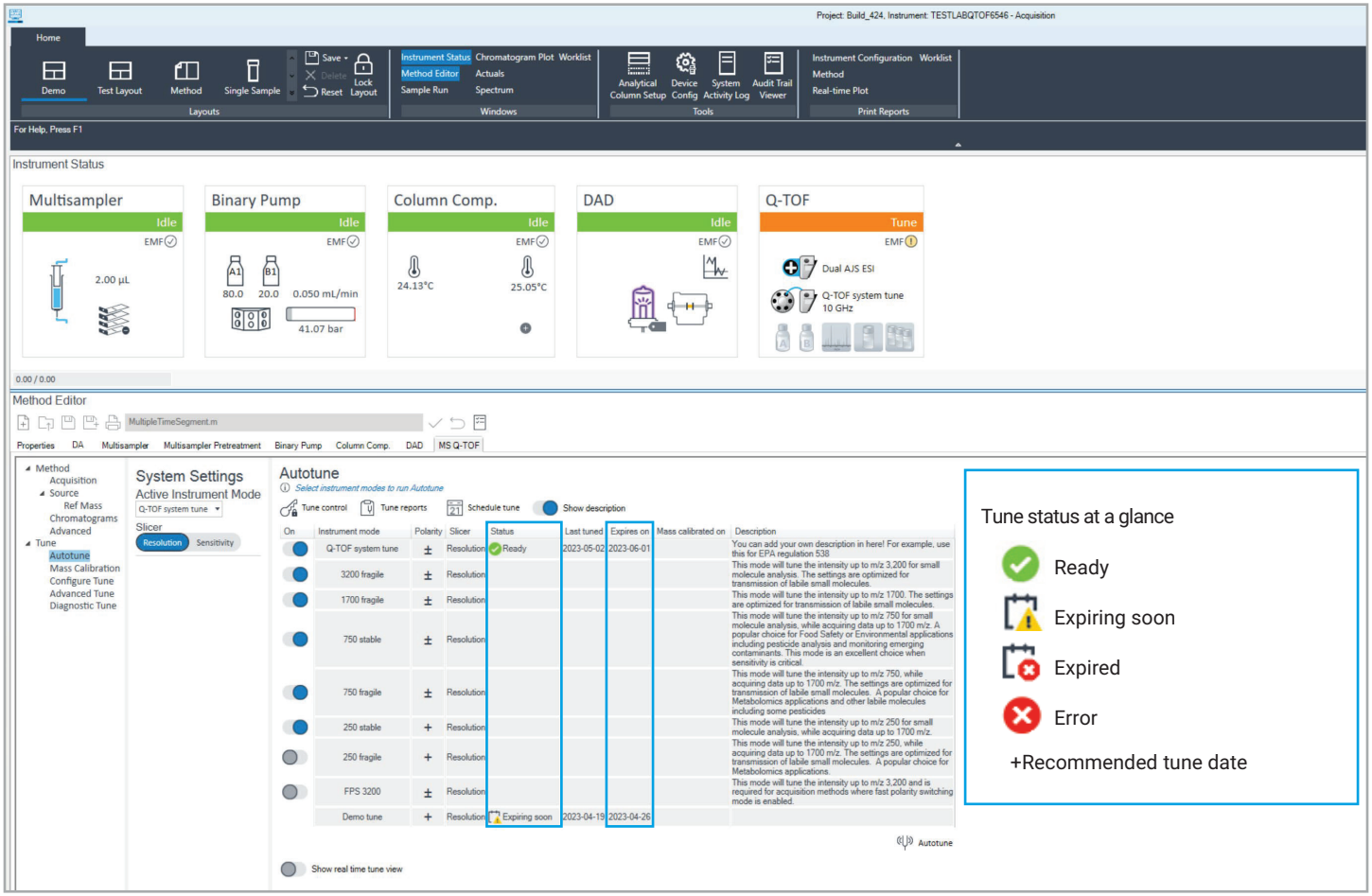

Figure 4. An LC/Q-TOF instrument mode tune status is displayed in Agilent MassHunter Acquisition software for LC/MS systems.

Scheduled Tune boosts laboratory productivity, helping to alleviate the burden of routine maintenance, so that the mass spectrometer is always at peak performance. Automated autotunes and check tunes save time and can be set to re-occur at a desired frequency when the instrument is not in use. For example, scheduled tuning can be programmed to run on Sunday nights, so that the instrument is immediately ready to run samples when the technicians arrive on Monday morning. Scheduled Tune can be set to meet any laboratory needs, whether weekly or monthly, as shown in Figure 5. System tunes may be performed less frequently—maybe monthly—and transmission tunes more frequently, as needed—such as weekly or biweekly.

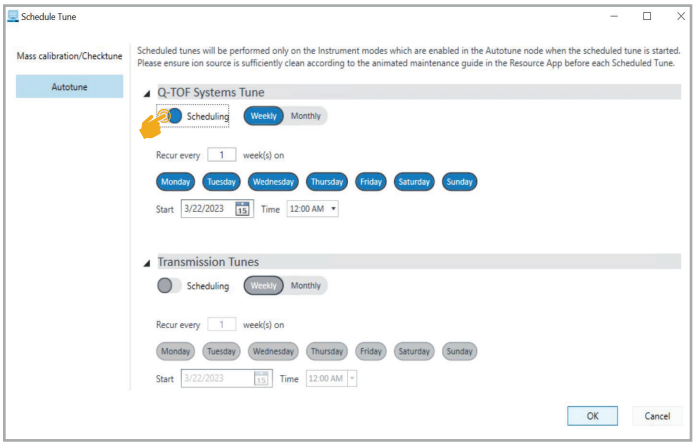

Figure 5. Agilent MassHunter Acquisition software for LC/MS systems allows users to schedule instrument tuning for their LC/MS systems to save time and ensure that the instrument is always operating at peak performance.

Early maintenance feedback

Early maintenance feedback (EMF) provides instrument health and status reports by reporting actively monitored metrics to anticipate downtime or address an immediate issue. EMF is displayed as a traffic light color-coded dashboard to provide insight at a glance and is present on all Agilent LC/MS instruments. EMF warnings do not stop the instrument from running, but rather alert the operator as to when maintenance should be performed based on the predicted lifetime of each monitored attribute.

Traditionally, laboratories use calendar-based maintenance schedules, which do not factor in the actual use of the instrument. EMF tracks simple metrics based on actual instrument use, such as liters pumped or valve switches, which is a better indicator of when maintenance is due. This feature allows users to get a reminder that maintenance is due on their instrument before it causes chromatography or hardware issues. With EMF, users can anticipate and plan for downtime, rather than reacting to unexpected interruptions. Insufficient instrument maintenance can lead to costly unplanned downtime or analysis failures that cause time-wasting sample remeasurement. Similarly, maintenance that is too frequent also wastes time and can increase the cost of consumable parts.

Instrument status and EMF notifications are displayed in Agilent MassHunter Acquisition software for LC/MS systems for each module in the Instrument Status window. EMF information is located directly under the instrument status bar for each module. When the EMF symbol is present with a check mark, it means that no maintenance is required, as displayed in Figure 6. However, when a yellow exclamation mark is displayed with the EMF symbol, it signals that maintenance is due (also Figure 6). Using a mouse, users can hover over the EMF symbol for details or right-click the symbol and select Maintenance for additional information, as shown in Figure 7.

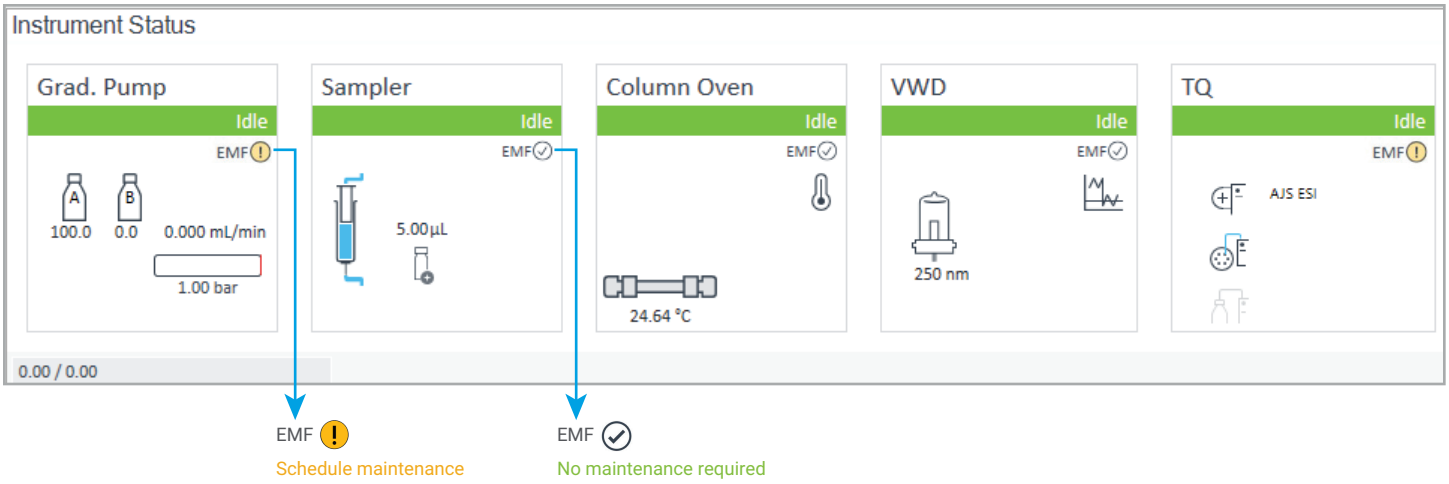

Figure 6. LC/TQ instrument status and EMF as displayed in Agilent MassHunter Acquisition software for LC/MS systems.

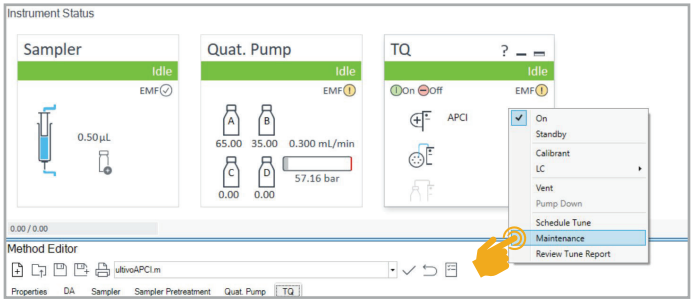

Figure 7. Right-clicking the EMF warning allows users to find additional information regarding instrument health.

For LC/TQ and LC/(Q-)TOF instruments, EMF provides the status for various attributes, including autotune, check tune, injection count, diverter valve switches, rough pump, gas filter, detector health, nebulizer status, capillary status, and spray stability status. Figure 8 shows EMF counters for each attribute on an LC/TQ. In this example, the check tune is expired, which is noted by the red status color. Also, both the rough pump and gas filter show that they will be due for maintenance soon, as illustrated by the yellow status color. All attributes with green status are still operating within their expected lifetime ranges and do not currently need attention.

The EMF counters monitor many instrument metrics and can be adjusted to suit the type of method or samples that are typically run. So, users can ensure that their maintenance schedule is appropriate to maintain their specific instrument and its desired performance. For example, if samples with high dissolved solids are typically run, the instrument will need more frequent maintenance than one running drinking water samples. Similarly, if users run harsh solvents or operate at very high pressure, the instrument may also need more frequent maintenance.

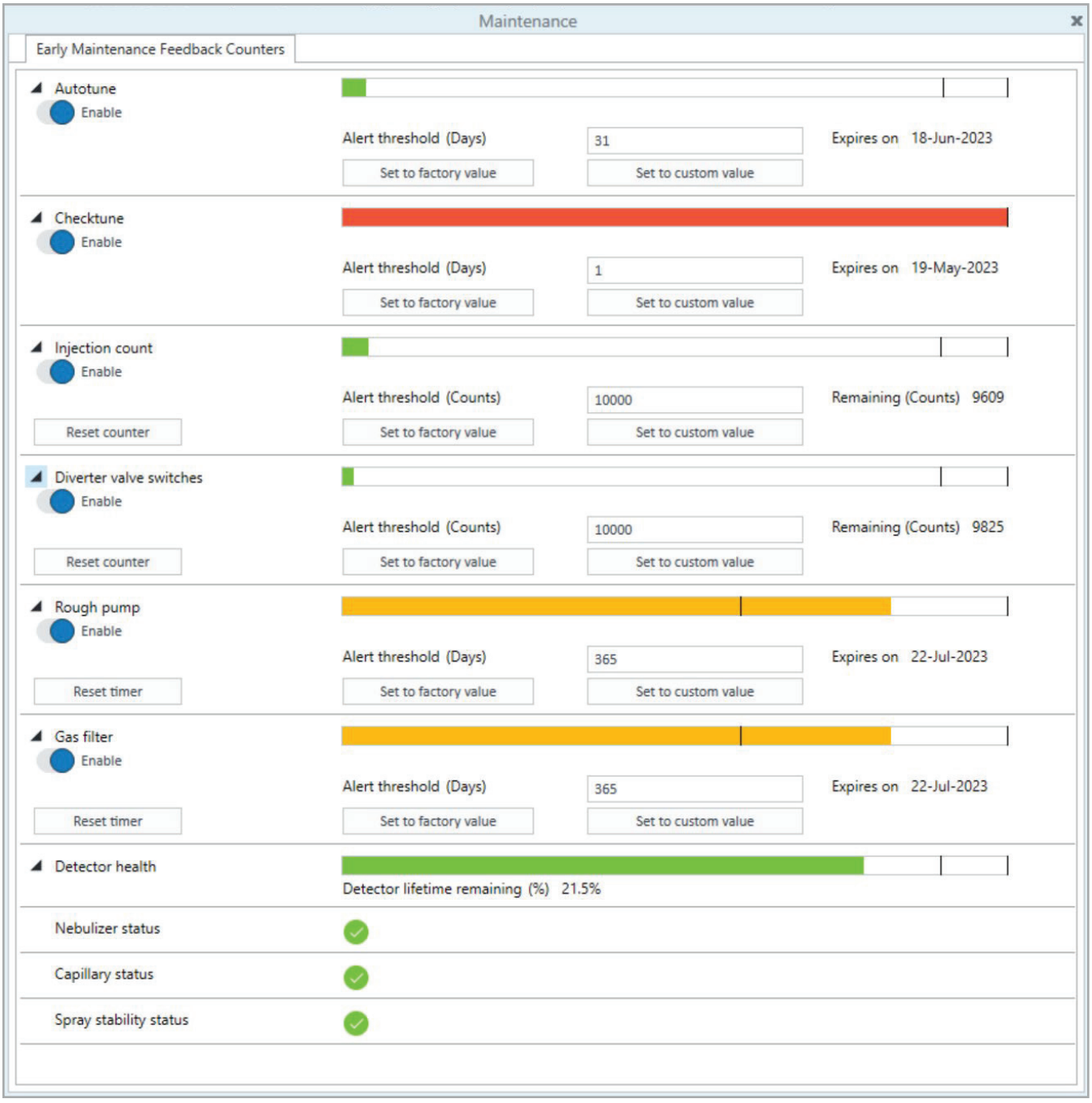

Figure 8. EMF counters and statuses for LC/TQ attributes.

Intelligent reflex workflows

Intelligent reflex is a data-dependent worklist reinjection algorithm. This algorithm enhances data quality in real time and dramatically speeds up sample analysis throughput through several logic-based workflows. Five intelligent reflex workflows are available; details for each workflow are discussed later in this technical overview.

These sample reinjection protocols boost efficiency in high-throughput and routine laboratory scenarios for LC/TQ and LC/(Q-)TOF users. Users can also improve identification confidence with two LC/Q-TOF-only intelligent reflex workflows. All workflows assume that there is a normal, routine worklist structure with QCs, blanks, and samples.

The workflows use MassHunter Quantitative Analysis methods, pre-established calibration curves, and outlier thresholds. Hardcoded rules prevent carryover and allow users to follow their laboratory standard operating procedures. Intelligent reflex can be set up on a per-method basis or a per-worklist basis, as suits the laboratory (Figure 9).

Reinjections added as a result of intelligent reflex workflows are visible at a glance on a walk through the lab. Intelligent reflex reinjection rows in the MassHunter worklist are visible from approximately six feet or two meters. These worklist rows are colored teal, as shown in Figure 10, for easy identification. All reinjection rows are also labeled with an icon and text in the Status column.

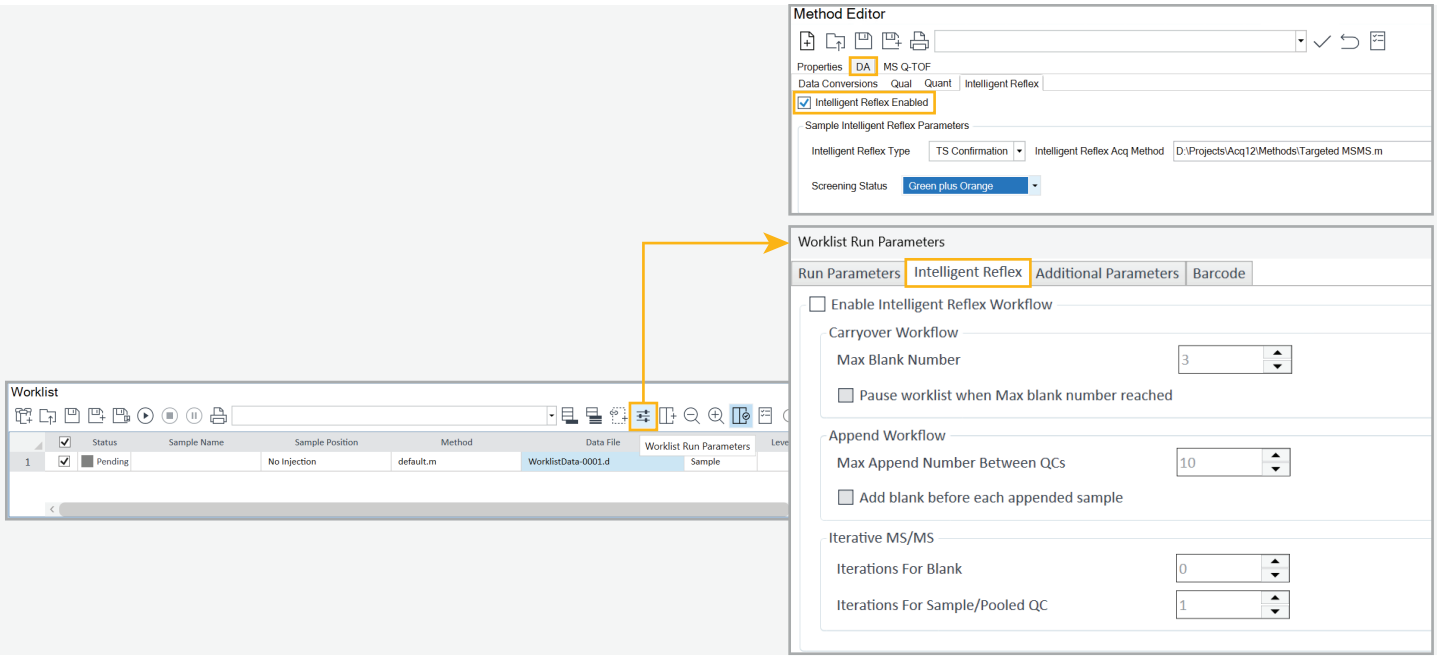

Figure 9. Intelligent reflex workflows can be set up on either a per-method basis or a per-worklist basis.

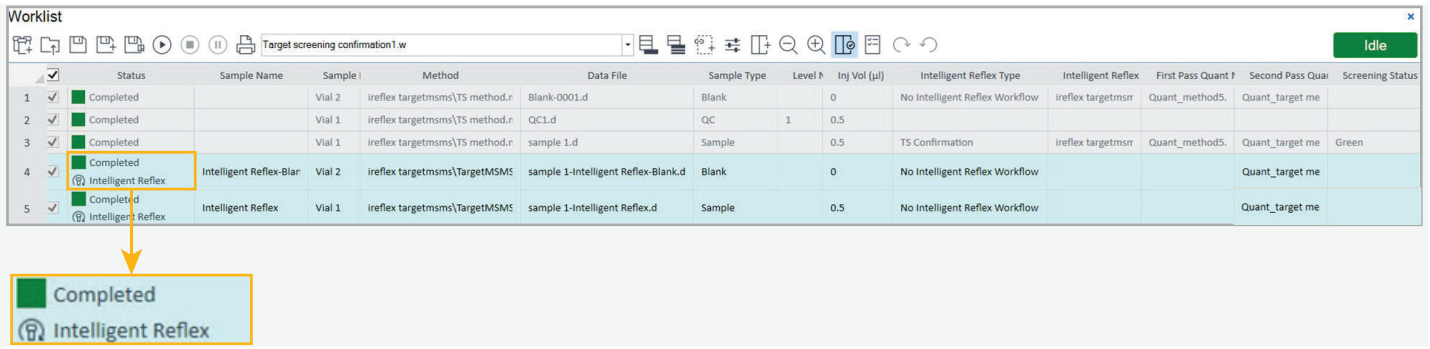

Figure 10. As intelligent reflex workflows run, their worklist rows in Agilent MassHunter Acquisition software for LC/MS systems are displayed in teal for easy identification, and their status is listed.

Carryover detection workflows

Intelligent reflex carryover detection workflows prevent carryover from contaminating multiple samples in a worklist. A blank sample is inserted into the worklist if carryover is present. If carryover is not present, the worklist continues as scheduled. If carryover is detected, the system will inject up to a user-defined maximum number of blanks to attempt to overcome the contamination. When carryover is no longer detected, the worklist continues as scheduled (Figure 11). If the maximum number of user-defined blanks is run, and carryover is still present, the worklist and LC pumps may optionally be programmed to pause to prevent further loss of mobile phase and samples. The same acquisition and quantitative methods are used for all analyses (original injection and reinjections) in intelligent reflex carryover detection workflows. The Blank Concentration Outlier in MassHunter Quantitative Analysis software is used to determine whether carryover is present in the blank samples.

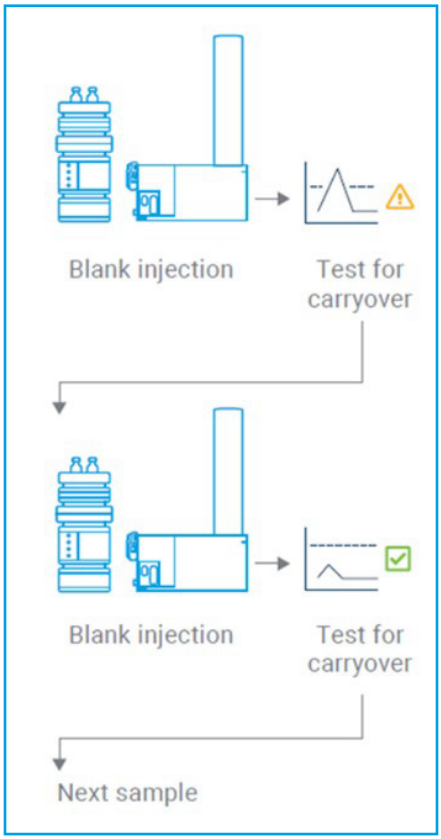

Figure 11. Intelligent reflex carryover detection workflow logic for Agilent LC/TQ and LC/(Q-)TOF instruments.

Above calibration range workflows

If a target analyte is detected above the predetermined calibration curve range, the intelligent reflex above calibration range workflow can be used to estimate a calculated concentration. When an analyte exceeds the calibration range, first a blank is injected to ensure that there is no carryover. Then, the sample is reinjected with a reduced injection volume to bring it back within the range of the calibration curve (Figure 12).

Users can decide whether to insert the above calibration range workflow reinjections into the worklist or append them to the end of the worklist. If appending the reinjections to the worklist, users can choose to append a blank before each appended sample and to append a QC after every n-number of samples. The same acquisition and quantitative methods are used for all injections (original injection and reinjections) in this workflow. Whether a sample falls outside of the predetermined calibration range is assessed by the Calibration Range Outlier in MassHunter Quantitative Analysis software.

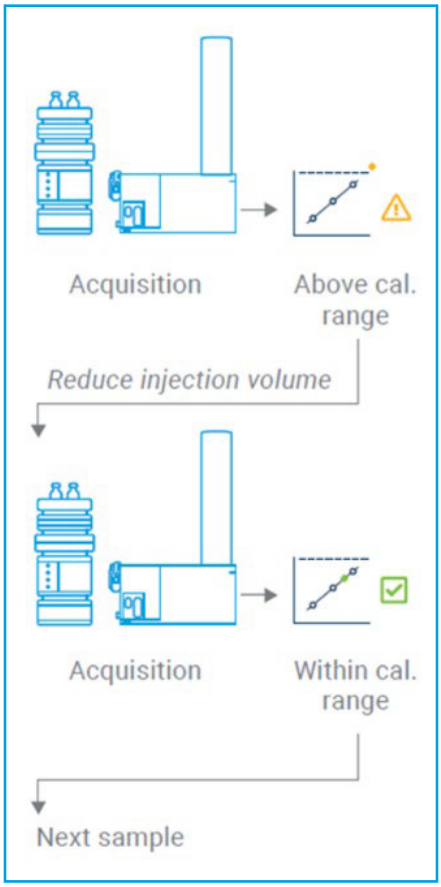

Figure 12. Intelligent reflex above calibration range workflow logic for Agilent LC/TQ and LC/(Q-)TOF instruments.

Fast screening workflows

Intelligent reflex fast screening workflows are used to confirm a presumptive positive. These workflows boost productivity and save time for worklists where there is a low expected number of positive samples. For example, in forensic toxicology samples, there is likely an expectation that most samples will be clean, with a low percentage of samples returning positive results. Fast screening workflows typically use two different acquisition and quantitative methods. In this scenario, the first-tier method is fast, often a ballistic gradient. This fast method is used to rapidly screen all samples. If a run positively detects a target analyte, that sample is re-analyzed using a longer method for true confirmation (Figure 13).

Re-analyses can be either inserted into the worklist or appended to the end of the worklist. When inserting the re-analysis workflow, the same column and LC gradient should be used for the first- and second-tier analyses. To change the column and/or LC method between the first- and second-tier analysis, then append the re-analysis workflow. If the reinjection is appended to the worklist, a blank is always run before the reinjected sample to ensure that the column for the second-tier method is flushed and fully equilibrated. If appending the reinjections to the worklist, users can optionally choose to append a blank before each appended sample and to append a QC after every n-number of samples. The Sample Amount Outlier is used in MassHunter Quantitative Analysis software to determine whether the confirmation analysis should be run. Using the, often longer, confirmation method only as needed saves time and costs for each worklist, especially when few samples are expected to positively detect target analytes.

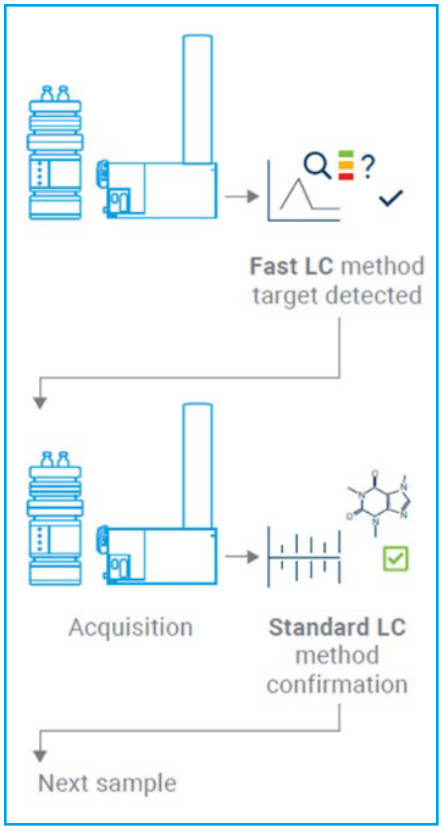

Figure 13. Intelligent reflex fast screening workflow logic for Agilent LC/TQ and LC/(Q-)TOF instruments.

Targeted MS/MS confirmation workflows

Like fast screening workflows, the intelligent reflex targeted MS/MS confirmation workflow offers automatic confirmation. The targeted MS/MS confirmation workflow is only available for LC/Q-TOF instruments. The targeted MS/MS confirmation workflow confirms questionable or identified compounds in data-independent acquisition (DIA) All Ions data with targeted MS/MS spectra (Figure 14). Two different acquisition and quantitative methods are used for this intelligent reflex workflow. The first-tier method uses All Ions (DIA) data, while the second-tier method uses MS/MS data for confirmation. For the first time, this intelligent reflex workflow automatically connects DIA and data-dependent (or targeted MS/MS) data into one workflow.

The targeted MS/MS confirmation workflow can only be appended to the end of a worklist. A blank is always run before the confirmation (second-tier) analysis. Users can optionally choose to append a blank before each appended sample and to append a QC after every n-number of samples. The targeted MS/MS confirmation workflow uses the Screening Status in the LC Screener tool in MassHunter Quantitative Analysis software to flag suspected analytes for confirmation.

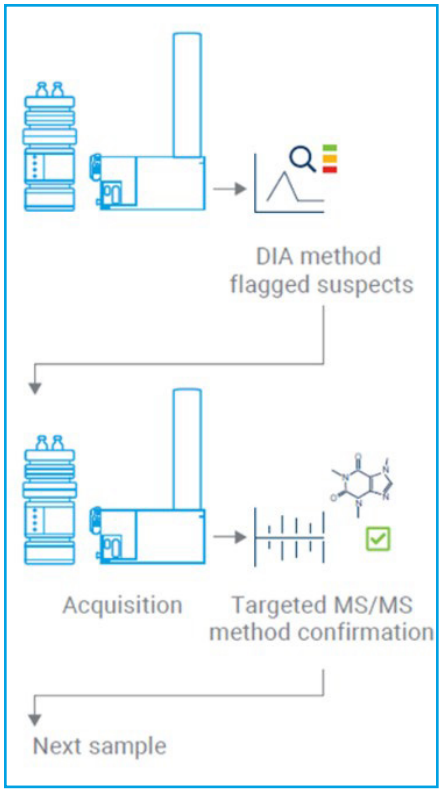

Figure 14. Intelligent reflex targeted MS/MS confirmation workflow logic for Agilent LC/Q-TOF instruments.

Iterative MS/MS workflows

Intelligent reflex iterative MS/MS workflows offer thorough characterization of a sample or pooled QC by LC/Q-TOF. Users can boost identification confidence by iteratively excluding higher abundant precursors (Figure 15). For example, the first analysis detects precursors A, B, and C. The second analysis uses the same method but will exclude A, B, and C. If the second analysis detects precursors D and E, then the third analysis will exclude A, B, C, D, and E—and so on, until the user-defined number of iterations is run. The iterative MS/MS workflow inserts reinjections into the worklist. Optionally, the user can start with matrix blank iterations to ensure that high-abundant matrix precursors are not selected during sample runs.

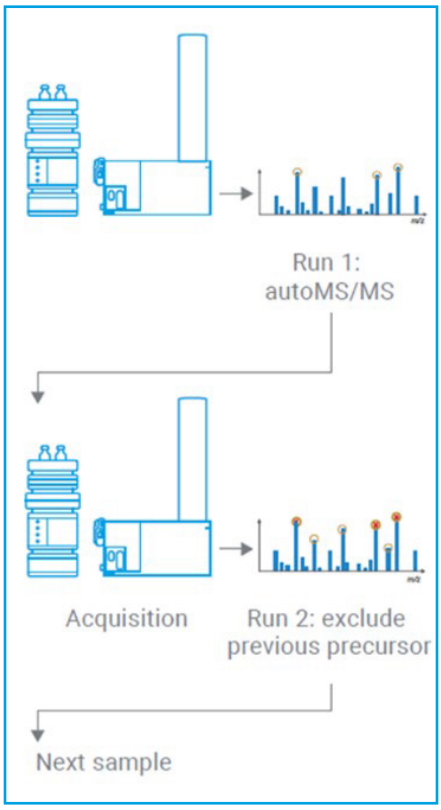

Figure 15. Intelligent reflex iterative MS/MS workflow logic for Agilent LC/Q-TOF instruments.

Conclusion

Agilent LC/TQ and LC/(Q-)TOF instruments include numerous intelligence and workflow automation features to boost productivity and efficiency of demanding routine analysis applications. These intelligence features:

- Maximize instrument performance and analytical sensitivity (application-based SWARM autotune)
- Minimize downtime (early maintenance feedback and scheduled tuning)
- Maximize sample throughput (intelligent reflex and fast screening workflows)
- Ensure that injected samples do not require repeat work (intelligent reflex carryover detection and above calibration range workflows)
- Improve sample characterization (intelligent reflex targeted MS/MS confirmation and iterative MS/MS workflows)

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DE02631466

Printed in the USA, July 30, 2024
5994-7430EN

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