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Customizable Intelligent Reflex Workflows: Embedding User-Defined Reaction Logic in Automatic High Throughput LC/MS Analysis

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Introduction

LC/MS measurements are often associated with targeted, quantitative, large batch sample analysis with an emphasis on non-stop, continuous operation. Examples include the continuous processing of QA/QC samples for contaminants in pharmaceuticals, pesticides and veterinary drug detection in foods, or measurements of biological analytes from a sizeable population. Regardless of the application, consistent results, high sample throughput, and avoidance of sample reprocessing is highly desired.

To aid in the acquisition of high-quality data and high throughput measurement, the LC/MS systems with MassHunter 13 acquisition have improved the automated reinjection logic feature called **intelligent reflex** by:

1. Adding **new sample dilution** options for the Above Cal. Range workflow.
2. Allowing the Carryover workflow to be performed on all blanks in the worklist, including reinjected blanks from another intelligent reflex workflow.
3. Adding user flexibility by removing hardcoded rules requiring the use of blanks or QCs.
4. Adding a new flexible **Custom workflow** for ultimate flexibility allowing the user to tailor their workflow to their unique needs.

Experimental

Measurements were carried out using Agilent LC/MS triple quadrupole (TQ) and quadrupole time of flight (Q-TOF) systems with MassHunter 13 acquisition software coupled to an Infinity III 1290 HPLC system.

[Agilent MassHunter Acquisition for LC/MS](#) version 13 features a new intelligent reflex workflow which enables a user to automatically reinject blanks, quality controls and samples or stop or pause the worklist in a data-dependent manner.

A worklist containing blanks, samples, QCs and calibration standards was created to stress test and demonstrate the data-dependent logic for the new intelligent reflex workflow functionality.

Specific Quantitative analysis methods were created for each workflow with pre-established calibration curves, and outlier thresholds which are based on the chromatographic or mass spectrometric characteristics for the blank, QC or sample analyte such as concentration or retention time window.

Intelligent reflex commands are defined as logical conditions in the acquisition and Outliers section of the data analysis method and if the logical commands are activated a reflex action is performed, such as a new line in the sample queue is appended or inserted, the worklist is paused, or the worklist is stopped.

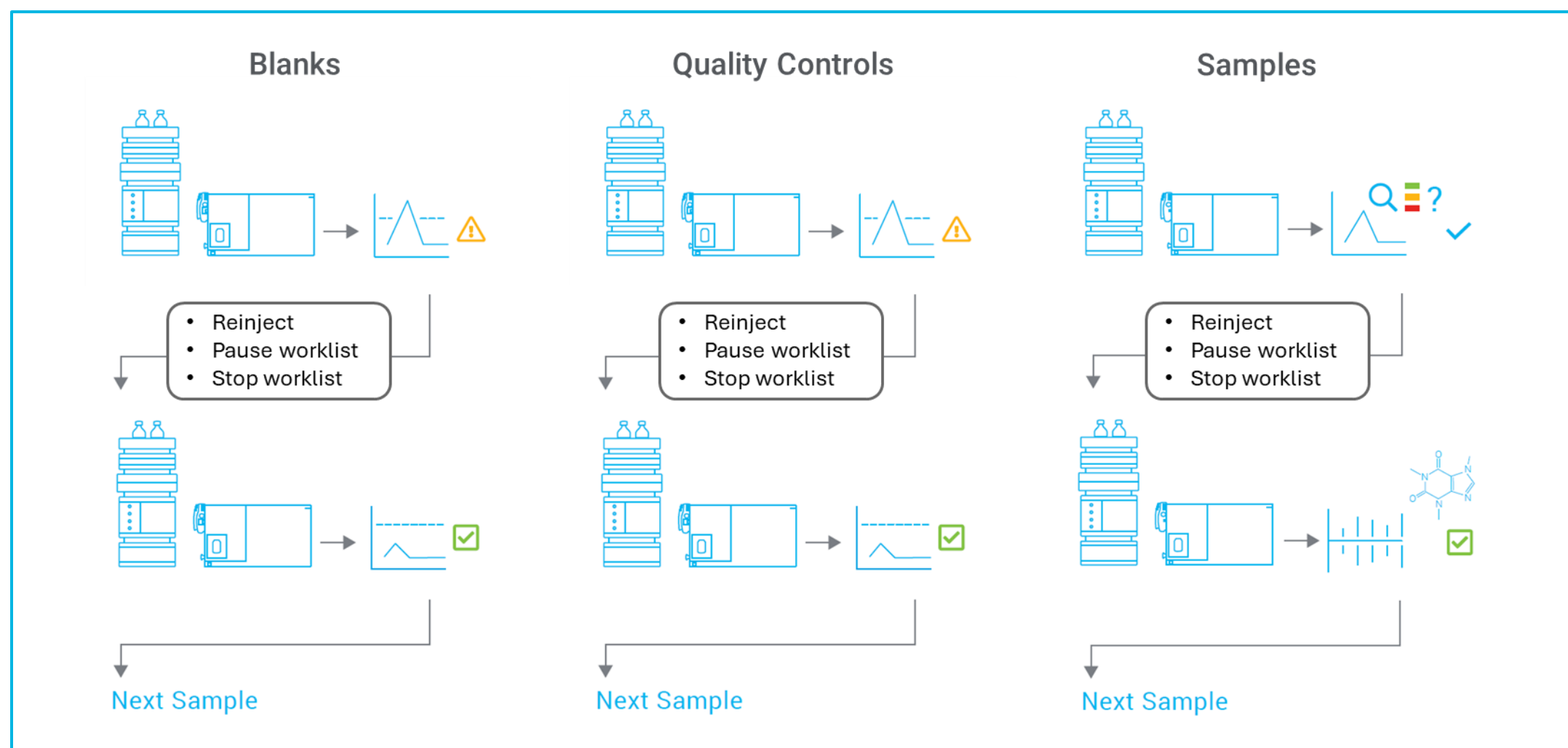


Figure 1: New Custom Intelligent Reflex Workflow Logic

Intelligent Reflex Workflows

MassHunter Acquisition for LC/MS version 13 intelligent reflex workflows evaluate and reinject Blanks, QC's and Samples in a data-dependent manner within a running worklist in real time. The intelligent reflex workflows:

- Enhance throughput for large batch sample analysis through automation.
- Boost lab productivity by reducing manual intervention, user error and sample reprocessing.
- Save valuable sample material by automatically preventing carryover from contaminating a batch analysis.

LC/MS TQ, TOF and Q-TOF Workflows:

- Carryover
- Above Cal. Range
- Fast Screening
- **New Custom Intelligent Reflex Workflow**

Additional LC/MS Q-TOF Workflows:

- Targeted MS/MS Confirmation
- Iterative MS/MS

The New Custom Intelligent Reflex Workflow

The new custom intelligent workflow allows the user to design their own workflow for greater flexibility in analysis:

- Choose the quantitation analysis method QC/blank/sample outliers.
- Choose from available reflex actions, such as pause, stop, or reinsert an injection or append an injection.
- Choose to add a blank before and/or after each inserted or appended reinjection.
- Chose to add a QC after *n* number of appended QCs.

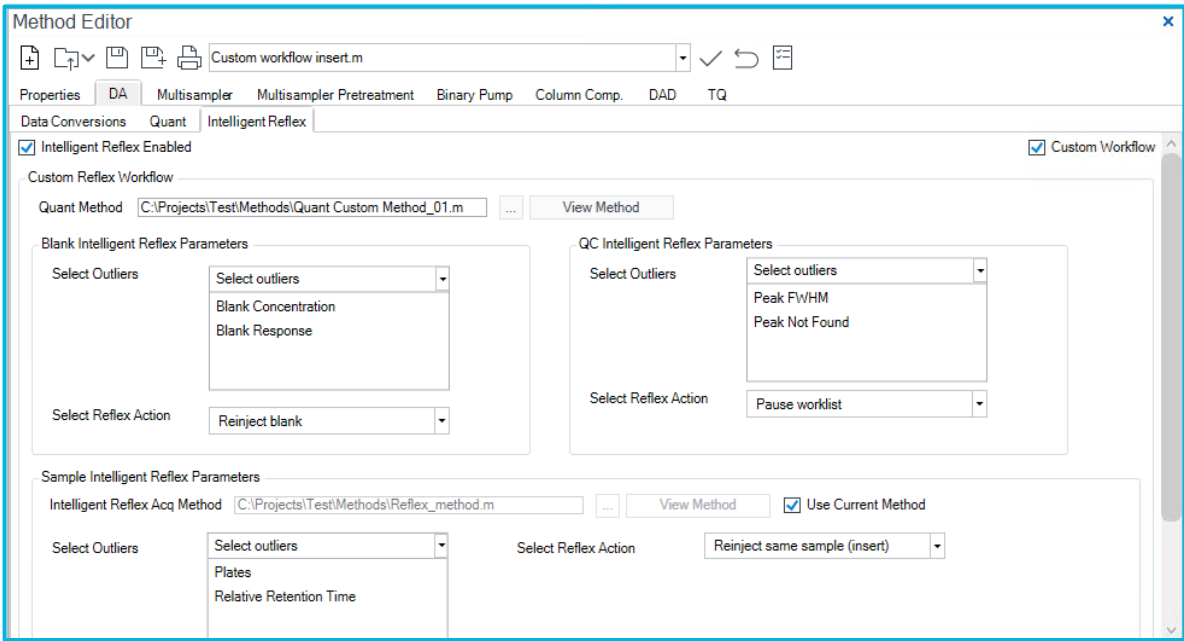


Figure 2: Custom Intelligent Reflex Workflow Method Setup

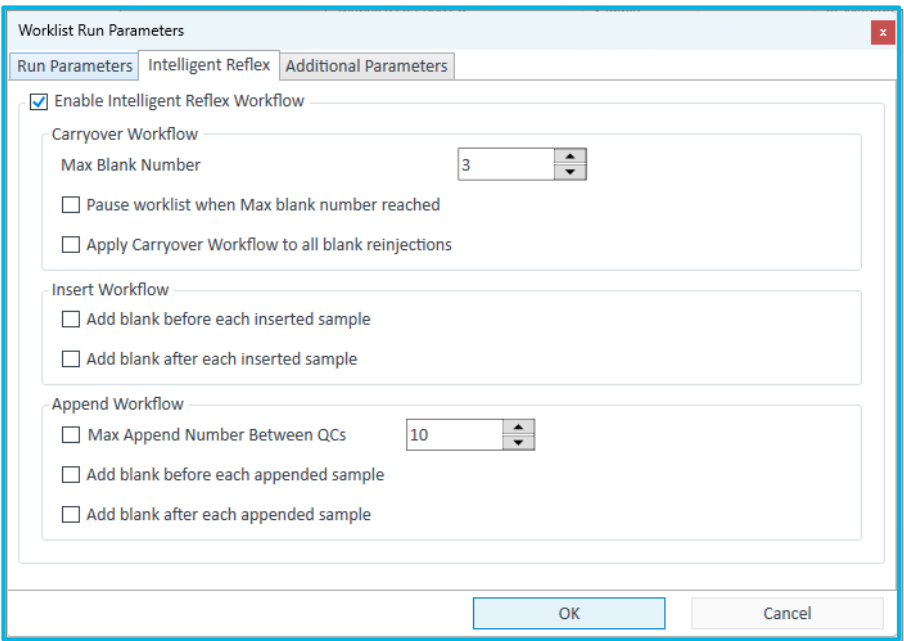


Figure 3: Blank and QC options

Worklist									
Custom WF.w									
	Status	Sample Name	Sample Position	Method	Data File	Sample Type	Intelligent Reflex Type	Intelligent Reflex Acq. Method	First Pass Quant Method
1	Completed	Blank_001	P1-A1	Custom workflow insert.m	Blank_001.d	Blank	Custom Workflow	Custom workflow insert.m	Quant Custom Method_01.m
2	Completed	CaI_001	P1-A2	Custom workflow insert.m	CaI_001.d	Cal			
3	Completed	CaI_002	P1-A3	Custom workflow insert.m	CaI_002.d	Cal			
4	Completed	CaI_003	P1-A4	Custom workflow insert.m	CaI_003.d	Cal			
5	Completed	CaI_004	P1-A5	Custom workflow insert.m	CaI_004.d	Cal			
6	Completed	CaI_005	P1-A6	Custom workflow insert.m	CaI_005.d	Cal			
7	Completed	Blank_002	P1-A1	Custom workflow insert.m	Blank_002.d	Blank	Custom Workflow	Custom workflow insert.m	Quant Custom Method_01.m
8	Completed Intelligent Reflex	Blank_002-Intelligent Reflex	P1-A1	Custom workflow insert.m	Blank_002-Intelligent Reflex.d	Blank	No Intelligent Reflex Workflow		Quant Custom Method_01.m
9	Completed	QC_001	P1-A7	Custom workflow insert.m	QC_001.d	QC	Custom Workflow	Custom workflow insert.m	Quant Custom Method_01.m
10	Completed	QC_002	P1-A8	Custom workflow insert.m	QC_002.d	QC	Custom Workflow	Custom workflow insert.m	Quant Custom Method_01.m
11	Completed	Sample-001	P1-A9	Custom workflow insert.m	Sample-001.d	Sample	Custom Workflow	Custom workflow insert.m	Quant Custom Method_01.m
12	Completed	Sample-002	P1-A10	Custom workflow insert.m	Sample-002.d	Sample	Custom Workflow	Custom workflow insert.m	Quant Custom Method_01.m
13	Completed Intelligent Reflex	Sample-002-Intelligent Reflex	P1-A10	Custom workflow insert.m	Sample-002-Intelligent Reflex	Sample	No Intelligent Reflex Workflow		
14	Completed	Sample-003	P1-A11	Custom workflow insert.m	Sample-003.d	Sample	Custom Workflow	Custom workflow insert.m	Quant Custom Method_01.m
15	Completed	Sample-004	P1-A12	Custom workflow insert.m	Sample-004.d	Sample	Custom Workflow	Custom workflow insert.m	Quant Custom Method_01.m

Carry over was detected in blank, new blank was inserted

Outlier for retention time window failed, sample was reinjected

Figure 4: Completed Worklist with Blank and Sample Reflex Reinjection

Results and Discussion

The Above Calibration Range Intelligent Reflex Workflow

Ensuring a target analyte concentration is within the calibration curve range is critical when quantifying analytical analytes. If an analyte is above the upper limit of quantitation (ULOQ), it is necessary to either dilute the sample or reduce the injection volume to bring the concentration within quantitation limits.

Detection of an analyte in a sample above the calibration range can trigger an insert/append re-injection with the following options.

- 1. **Reduced Injection Volume**
 - Specify a reduced injection volume to provide an estimated concentration.
- 2. **New Reinjection Dilution Factor**
 - Specify the desired dilution factor and the dilutant vial position and your sample will be diluted and automatically reinjected.
- 3. **New Sample Preparation Method**
 - Add your own customized sample preparation method.

Figure 5 shows a worklist where an analyte has been detected as being above the calibration range set in the data analysis method. An additional blank is automatically inserted before and after sample dilution and reinjection to ensure there is no carryover. The dilution factor is displayed in the worklist for each reinjected sample.

Worklist

Sample dilution.w

	✓	Status	Sample Position	Method	Data File	Sample Type	Intelligent Reflex Type	First Pass Quant Method	Above Cal Range Mode	Reinjection Dilution Factor	Diluent Vial Position
2	✓	Completed	P1-A2	Sample Dilution Factor.m	Cal_001.d	Cal					
3	✓	Completed	P1-A3	Sample Dilution Factor.m	Cal_002.d	Cal					
4	✓	Completed	P1-A4	Sample Dilution Factor.m	Cal_003.d	Cal					
5	✓	Completed	P1-A5	Sample Dilution Factor.m	Cal_004.d	Cal					
6	✓	Completed	P1-A6	Sample Dilution Factor.m	Cal_005.d	Cal					
7	✓	Completed	P1-A1	Sample Dilution Factor.m	Blank_002.d	Blank	No Intelligent Reflex Workflow	QuantMethod_02.m			
8	✓	Completed	P1-A7	Sample Dilution Factor.m	QC_001.d	QC					
9	✓	Completed	P1-A8	Sample Dilution Factor.m	QC_002.d	QC					
10	✓	Completed	P1-A9	Sample Dilution Factor.m	Sample-001.d	Sample	Above Cal. Range Insert	QuantMethod_02.m	Reinjection Dilution Factor	2	P1-D1
11	✓	Completed	P1-A10	Sample Dilution Factor.m	Sample-002.d	Sample	Above Cal. Range Insert	QuantMethod_02.m	Reinjection Dilution Factor	2	P1-D1
12	✓	Completed	P1-A1	Sample Dilution Factor.m	Sample-002-In	Blank	Carryover	QuantMethod_02.m			
13	✓	Completed	P1-A1	Sample Dilution Factor.m	Sample-002-In	Blank	Carryover	QuantMethod_02.m			
14	✓	Completed	P1-A10	Sample Dilution Factor.m	Sample-002-In	Sample	No Intelligent Reflex Workflow	QuantMethod_02.m			
15	✓	Completed	P1-A1	Sample Dilution Factor.m	Sample-002-Fi	Blank	Carryover	QuantMethod_02.m			
16	✓	Completed	P1-A11	Sample Dilution Factor.m	Sample-003.d	Sample	Above Cal. Range Insert	QuantMethod_02.m	Reinjection Dilution Factor	2	P1-D1
17	✓	Completed	P1-A12	Sample Dilution Factor.m	Sample-004.d	Sample	Above Cal. Range Insert	QuantMethod_02.m	Reinjection Dilution Factor	2	P1-D1
18	✓	Completed	P1-B1	Sample Dilution Factor.m	Sample-005.d	Sample	Above Cal. Range Insert	QuantMethod_02.m	Reinjection Dilution Factor	2	P1-D1

Sample conc. above cal. curve. Sample diluted and reinjected with blanks before and after to prevent carryover

Carry over was detected in blank, new blank was inserted

Figure 5: Completed Worklist with Above Cal. Range Reflex Reinjection

Conclusions

- The new Custom Intelligent Workflow empowers users to define their own outlier criteria and reflex actions, enabling tailored workflows for blanks, QCs, and samples.
- Intelligent reflex workflows provide real-time automation and live decision-making during acquisition, helping keep samples within SOP limits while maximizing throughput.
- Multiple reflex workflows can operate concurrently within a single worklist, reducing the need for constant monitoring, protecting valuable samples, and minimizing costly re-runs.

<https://www.agilent.com/en/promotions/asms>

This information is subject to change without notice.

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