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Active & iterative data-dependent reinjection logic for maintaining throughput, uptime, and consistency in triple quadrupole LC/MS analysis

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Introduction

Triple quadrupole LC/MS measurements are often associated with targeted, quantitative, large batch sample analysis with an emphasis on non-stop continuous operation. Such use cases are in 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 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 6475 triple quadrupole LC/MS system with MassHunter 12 includes an intelligent worklist reinjection logic feature called iReflex**

Herein, we present a technique utilizing an active and immediate data processing algorithm that evaluates and reinjects samples in a data-dependent manner based on the following intelligent reflex (iReflex) scenarios:

1. Detection of previous sample carryover
2. Detection of a sample outside of the calibration range
3. Fast analyte screening

Experimental

Measurements were carried out using a 6475 triple quadrupole LC/MS system (G6475A) and MassHunter 12 software system which is coupled to an Infinity II 1290 HPLC system.

MassHunter 12 features new iReflex workflows which enables a user to automatically add samples or blanks in a data-dependent manner. Ions were acquired in MRM mode to ensure that the signal was monitored as the analyte elutes. A worklist containing Blanks, Samples, and Calibration standards was created to stress test and demonstrate the data-dependent logic for all three iReflex workflows.

A specific Quantitative analysis method is created for each workflow and analyte concentration thresholds are set to trigger secondary injection. iReflex reinjection commands are defined as logical conditions in the Outliers section of the data analysis method and are based on the current abundance or concentration measurements for the sample or blank.

The unified Acquisition and DA analysis (iReflex parameters) method is created which is used to create worklists to demonstrate the workflow logic. If the logical commands are activated, a new injection in the worklist is appended or inserted to iterate on until a pass condition is met.

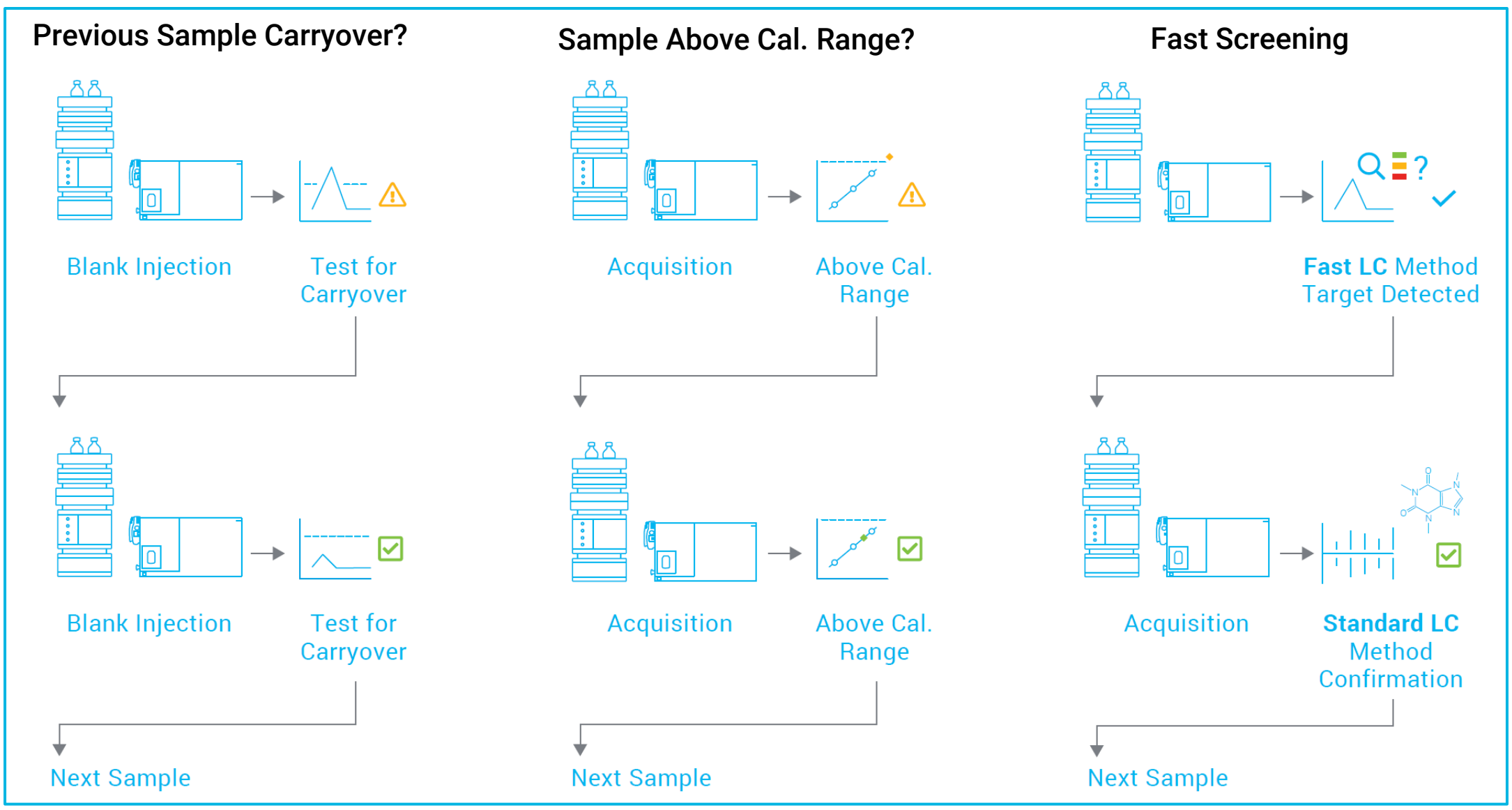


Figure 1: Intelligent Reflex (iReflex) Workflow logic.

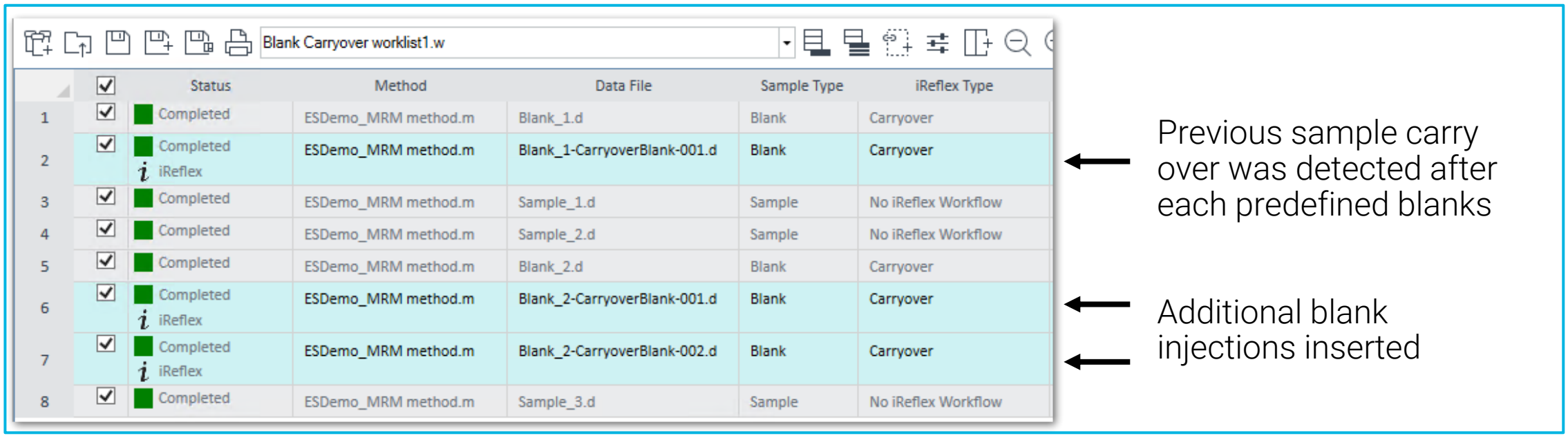
iReflex Workflows

MassHunter 12 Intelligent Reflex workflows evaluate and reinject blanks and samples in a data-dependent manner within a running worklist. The iReflex Workflows:

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

The Carryover iReflex Workflows

Sample Carryover or contamination is a very common problem which could be due to insufficient washout, contaminated wash vial, or overloading sample on column. Detection of carryover in a blank above the outlier threshold, will trigger the workflow to insert up to *n* blanks. An additional option to pause the worklist if the maximum user defined *n* limit is met prevents contamination of samples.



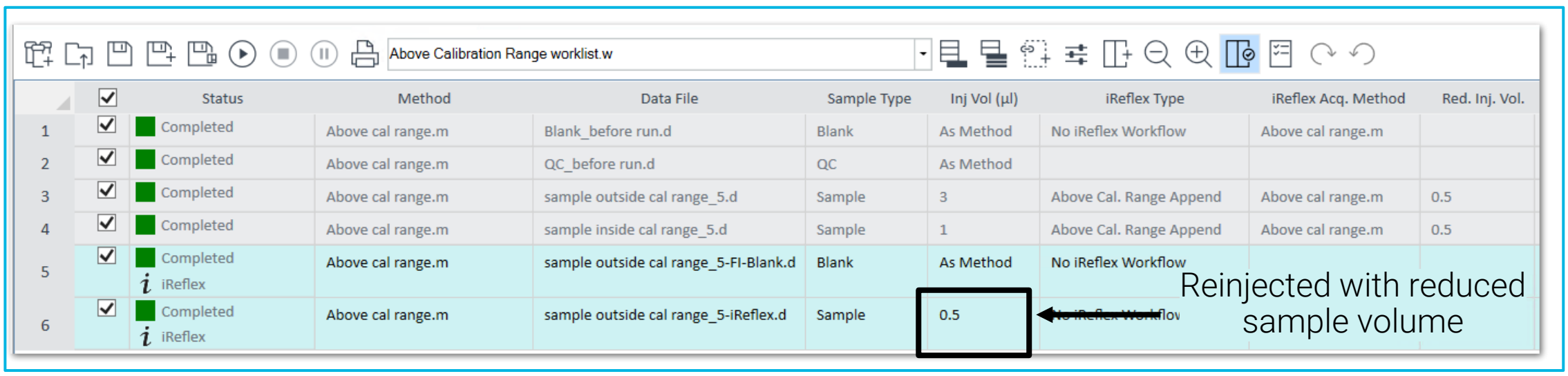
	Status	Method	Data File	Sample Type	iReflex Type
1	Completed	ESDemo_MRM method.m	Blank_1.d	Blank	Carryover
2	Completed, iReflex	ESDemo_MRM method.m	Blank_1-CarryoverBlank-001.d	Blank	Carryover
3	Completed	ESDemo_MRM method.m	Sample_1.d	Sample	No iReflex Workflow
4	Completed	ESDemo_MRM method.m	Sample_2.d	Sample	No iReflex Workflow
5	Completed	ESDemo_MRM method.m	Blank_2.d	Blank	Carryover
6	Completed, iReflex	ESDemo_MRM method.m	Blank_2-CarryoverBlank-001.d	Blank	Carryover
7	Completed, iReflex	ESDemo_MRM method.m	Blank_2-CarryoverBlank-002.d	Blank	Carryover
8	Completed	ESDemo_MRM method.m	Sample_3.d	Sample	No iReflex Workflow

Figure 2 : Insertion of blanks when carryover is detected during ongoing analysis.

The Above Calibration Range iReflex 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 will trigger an insert/append re-injection with reduced volume to provide an estimated concentration. Figure 3 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 appended before sample reinjection to ensure there is no carryover. The reduced injection volume is displayed in the worklist for each reinjected sample.



	Status	Method	Data File	Sample Type	Inj Vol (µl)	iReflex Type	iReflex Acq. Method	Red. Inj. Vol.
1	Completed	Above cal range.m	Blank_before run.d	Blank	As Method	No iReflex Workflow	Above cal range.m	
2	Completed	Above cal range.m	QC_before run.d	QC	As Method			
3	Completed	Above cal range.m	sample outside cal range_5.d	Sample	3	Above Cal. Range Append	Above cal range.m	0.5
4	Completed	Above cal range.m	sample inside cal range_5.d	Sample	1	Above Cal. Range Append	Above cal range.m	0.5
5	Completed, iReflex	Above cal range.m	sample outside cal range_5-FI-Blank.d	Blank	As Method	No iReflex Workflow		
6	Completed, iReflex	Above cal range.m	sample outside cal range_5-iReflex.d	Sample	0.5	~~No iReflex Workflow~~		

Figure 3: Appending a reinjection with lower injection volume due to original measurement reporting above ULOQ.

Results and Discussion

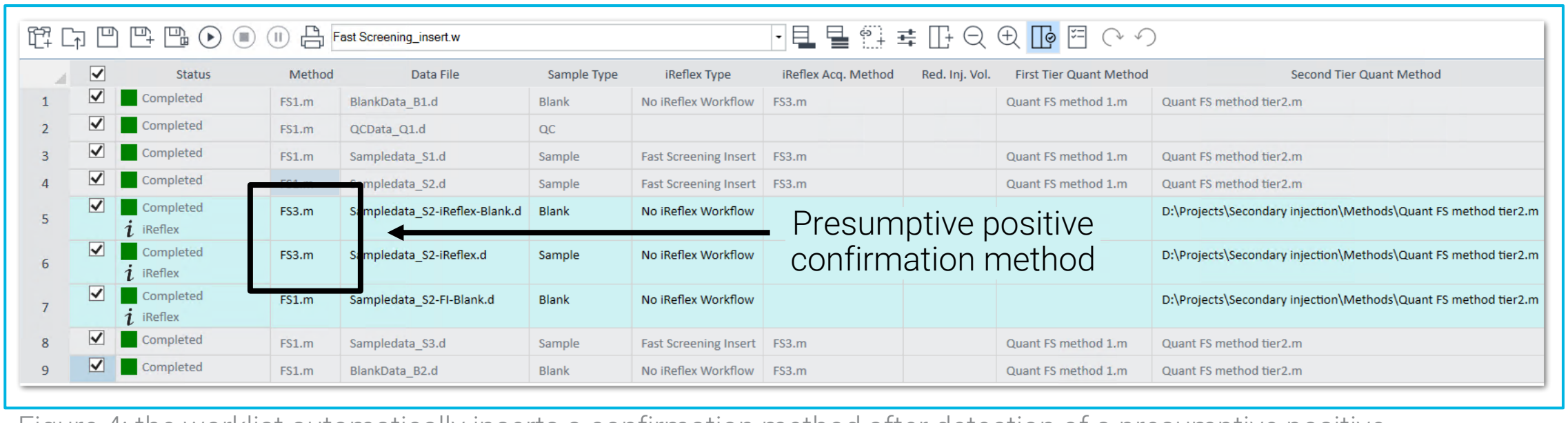
The Fast Screening iReflex Workflow

Fast screening methods are commonly used to increase sample throughput. These methods are short, on the order of seconds to minutes, and identify presumptive positive samples which are then manually scheduled for reinjection and analyzed using a longer confirmation method. Automating the reinjection and analysis of a presumptive positive is critical to increasing throughput allowing labs to analyze more samples for more targets.

Upon detection of a presumptive positive in a fast screening method, this workflow will either insert or append a reinjection with a different analysis method for target confirmation. The insert action is used for confirmation methods with the same LC method, while the append action is used when a different LC method and/or column will be used for confirmation. If the insert action has been chosen, then a blank with be automatically inserted before and after the sample.

The fast screening iReflex workflow produces two different data batches; the 1st tier consists of the original worklist with the fast screening method. The 2nd tier batch consists of reinjected samples which are acquired and analyzed with a different, usually longer and comprehensive, confirmation method. Additional options are available to tailor these workflows to each unique analysis and lab SOP:

- Automatically produce a combined report created from the 1st and 2nd tier batch analyzes.
- Append a blank before every appended 2nd tier sample or only before the first 2nd tier sample.
- Append a QC after *n* number of reinjections are appended to the worklist.
- Pause the worklist after the 1st tier analysis has completed for manual verification.



	Status	Method	Data File	Sample Type	iReflex Type	iReflex Acq. Method	Red. Inj. Vol.	First Tier Quant Method	Second Tier Quant Method
1	Completed	FS1.m	BlankData_B1.d	Blank	No iReflex Workflow	FS3.m		Quant FS method 1.m	Quant FS method tier2.m
2	Completed	FS1.m	QCData_Q1.d	QC					
3	Completed	FS1.m	Sampledata_S1.d	Sample	Fast Screening Insert	FS3.m		Quant FS method 1.m	Quant FS method tier2.m
4	Completed	FS1.m	Sampledata_S2.d	Sample	Fast Screening Insert	FS3.m		Quant FS method 1.m	Quant FS method tier2.m
5	Completed iReflex	FS3.m	Sampledata_S2-iReflex-Blank.d	Blank	No iReflex Workflow				D:\Projects\Secondary injection\Methods\Quant FS method tier2.m
6	Completed iReflex	FS3.m	Sampledata_S2-iReflex.d	Sample	No iReflex Workflow				D:\Projects\Secondary injection\Methods\Quant FS method tier2.m
7	Completed iReflex	FS1.m	Sampledata_S2-FI-Blank.d	Blank	No iReflex Workflow				D:\Projects\Secondary injection\Methods\Quant FS method tier2.m
8	Completed	FS1.m	Sampledata_S3.d	Sample	Fast Screening Insert	FS3.m		Quant FS method 1.m	Quant FS method tier2.m
9	Completed	FS1.m	BlankData_B2.d	Blank	No iReflex Workflow	FS3.m		Quant FS method 1.m	Quant FS method tier2.m

Figure 4: the worklist automatically inserts a confirmation method after detection of a presumptive positive.

The new Agilent 6475 triple quadrupole LC/MS system and MassHunter 12 Data Acquisition software

https://explore.agilent.com/asms

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Conclusions

The 6475 triple quadrupole LC/MS system with MassHunter 12 features an intelligent workflow called iReflex.

iReflex is an intelligent automated worklist reinjection logic tool to maximize analytical throughput or ensuring samples are within tolerance.

The three iReflex workflows shown can operate concurrently in one worklist to ensure samples are measured within SOP guidelines.

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