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Advances in Mass Spectrometry Instrument Intelligence

Overview

There is a global trend towards more self-aware, intelligent tools meant to make life easier. The field of liquid chromatography/mass spectrometry is no different with the move to automate difficult and challenging tasks, making mass spectrometry more accessible. These advances increase instrument uptime, streamline workflows, save time with immediate validated results and ultimately reduce cost-of-ownership.

In this poster collection, you will take a deep dive into the next evolution of our LC/MS instrument portfolio. The new 6475 triple quadrupole LC/MS includes easy-to-use, yet sophisticated onboard intelligence for routine analysis. We will demonstrate how the new intelligence features can improve analytical performance and lab productivity, providing you with peace of mind for day-to-day operation.

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Develop Methods Faster Using Our Intelligent Optimizer

An end-to-end software algorithm for LC/MS/MS method development, optimization, and QA/QC deployment

Authors: Anding Fan, Vicky He, James Pyke, Erik Lopez, Stephanie Aurand, Linfeng Wu, Patrick M. Batoon

Introduction

The act of developing targeted triple quadrupole LC/MS (LC/TQ) methods from start to finish is a complex and time-consuming, multi-step workflow. Method development becomes even more challenging if former mass spectrometry parameters for each analyte were not established – especially in the case of novel compounds. In such cases, "by-hand" optimization and characterization is needed to obtain the most effective MRM parameters.

Using the new 6475 triple quadrupole LC/MS system with MassHunter 12, intelligent workflows were added to ensure that users, particularly in routine analysis QA/QC lab environments, can maximize their operational efficiency. One aspect of LC/MS/MS analysis that may benefit from efficiency improvements is with the assisting and automation of method optimization. MassHunter 12 features an embedded 21CFR compliant, method-oriented, intelligent optimizer; allowing users to optimize MRMs and ion source parameters in a fully-automated or semi-automated fashion.

Using a modular end-to-end workflow approach, users may input chemical formula information which will result in (1) optimized MRM transitions for each compound and (2) optimal ion source parameters for the overall method. A unique feature of this algorithm is the ability to take advantage of the LC/TQ's speed to allow the simultaneous optimization of multiple compounds on a per-method basis, such that pre-production method development time is dramatically reduced compared to compound-by-compound approaches.

Experimental

Chemical formulas of the neutral analytes found in the LCMS 7-Analyte Sys Suitability Standard (amitriptyline hydrochloride, diethyl phthalate, diamyl phthalate, dihexyl phthalate, dioctyl phthalate, 8-bromoguanosine hydrate, and 4-chlorocinnamic acid) were entered into the method development interface to automatically calculate the potential [M+H]+ or [M-H]- precursor ions.

The optimization workflow was done unattended in two major phases. First, MRM optimization: precursor fragmentor voltage, RT determination (optional) Product Ion selection, and MRM collision energy voltage. Then, ion source optimization: drying gas heater, sheath gas heater, capillary voltage, nebulizer pressure, drying gas flow, sheath gas flow and nozzle voltage.

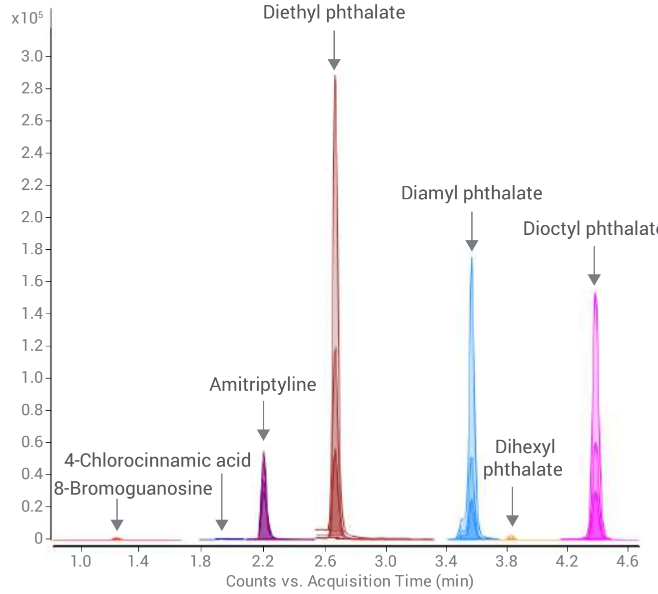

Figure 1: Fully optimized 7-analyte standard mixture chromatogram created from scratch.

MassHunter 12's Intelligent Optimizer enables comprehensive method development and parameter validation

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Results and Discussion

The End-to-End Optimization Workflow

The new intelligent optimization software algorithm, provides a complete, user-friendly, workflow approach to the tedious task of method development. Through this approach, a user may create a new method by inputting neutral analyte formulas, importing an existing MRM list from CSV, adding uncharacterized analytes to an existing method, or fine-tuning existing MRM or source parameters.

Compound and source parameters to be optimized are selected via the “Optimization parameters” page in the Method Editor with customizable user-input ranges and step sizes. An example of the optimization workflow is described in the diagram below.

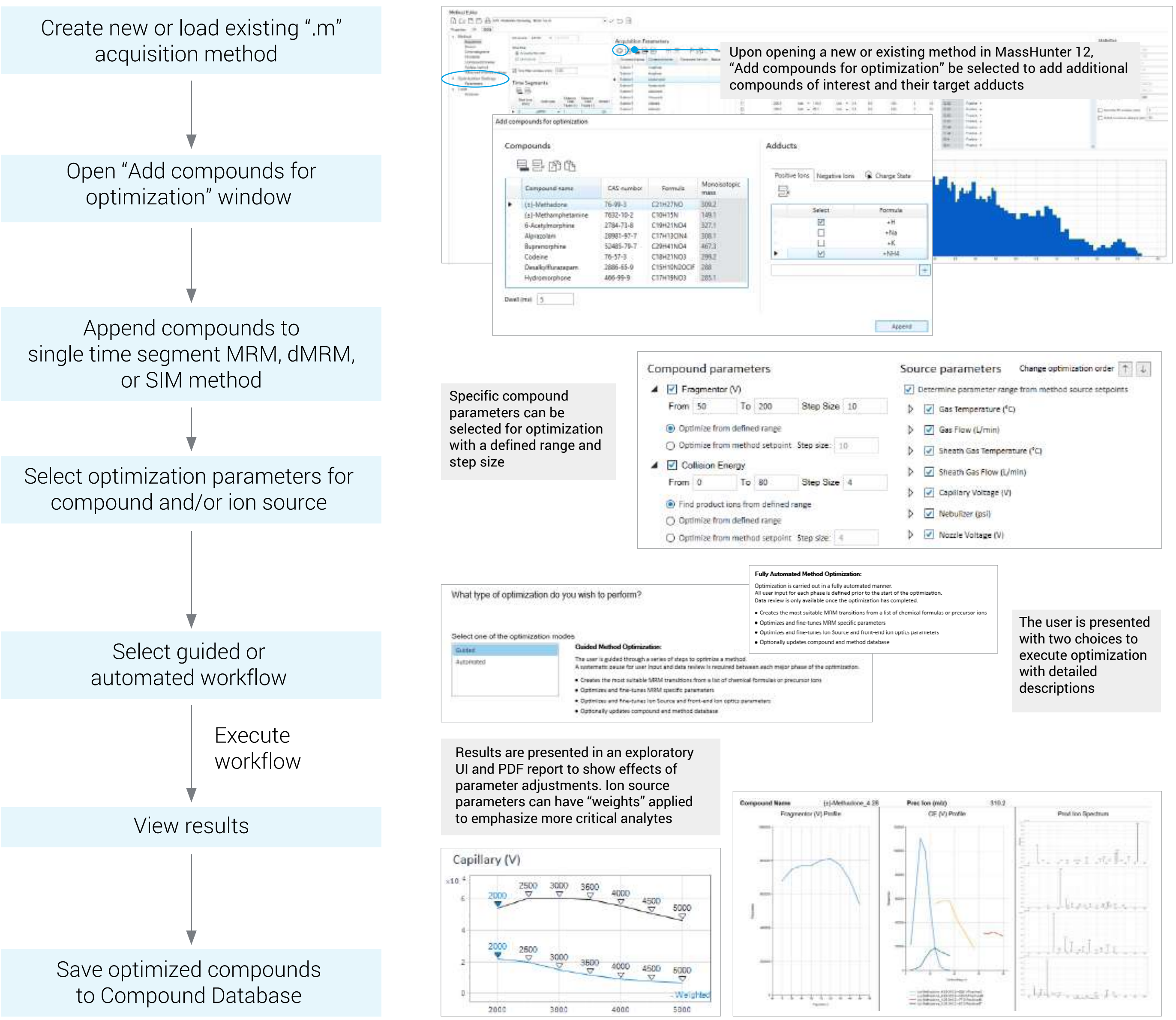


Figure 2: Screenshots of the method optimizer user interface from setup to results.

Results and Discussion

Guided Optimization Workflow

Through this workflow, optimization is carried out in two major steps.

1. MRM specific parameters are optimized to find the ideal product ions, fragmentor voltage, and collision energy voltage and retention time assignment (applied to injection with column) per analyte. The workflow will then pause to allow reviewing of data quality as it pertains to each compound.
2. Once ready, ion source parameters are optimized on a "global" basis to maximize the total ion current (unweighted - TIC based optimization) or with applied weights to priority analytes (weighted - EIC based optimization).

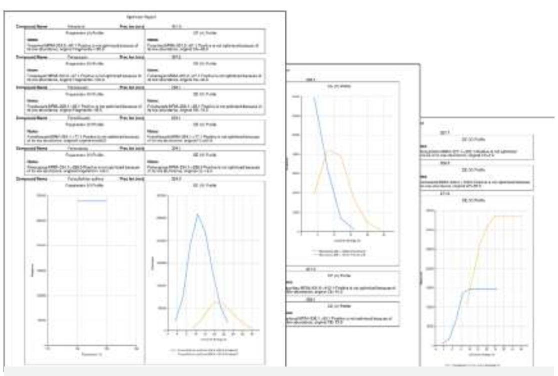
The method will be updated throughout the optimization process in stepwise fashion to create the final method. The user can save their transitions to the compound database to later apply to other methods.

Automated Optimization Workflow

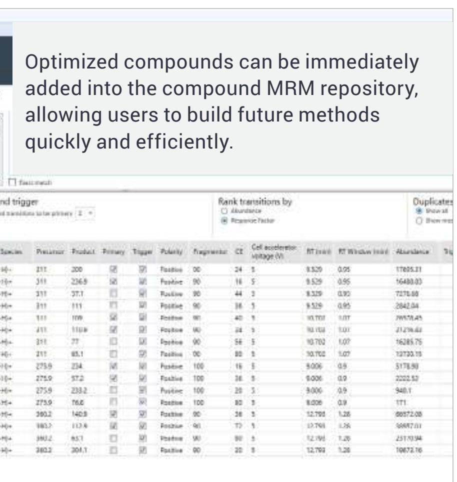
Through this workflow, optimization is carried out without pause, allowing the user optimize multiple methods in sequence.

Like Guided optimization, this workflow will also provide the user with a method that will include the best MRM transitions, collision energies, and voltages based on the parameters selected for optimization. It will also provide the user with fine-tuned source settings created specifically for that method. It can, however, run multiple-methods instead of a single one.

During this process, everything is completely automated and will transition from MRM to ion source optimization immediately. The user will not have the ability to view and update results or change the instrument settings.



Optimization reports are produced at the end of the workflow with the CE breakdown curves, Fragmentor response curve, and source parameter response curves.



Optimized compounds can be immediately added into the compound MRM repository, allowing users to build future methods quickly and efficiently.

Figure 3: **Finalized results are printed in an optimization report, stored as method revisions, and saved in a compound repositor.**

Conclusions

The new 6475 triple quadrupole LC/MS system with MassHunter 12 includes an intelligent MRM and source optimizer that is built into the method editor. The final result is a dMRM method with fully optimized parameters, MRM transitions, and retention time assignments.

The intelligent optimizer can be used to (1) Create a new method from scratch, (2) Add new compounds to an existing method, (3) Fine tune or verify parameters of an existing method.

Optimization results can be reviewed by the user after workflow completed. Any changes to the method are saved in an auditable fashion in accordance with to 21 CFR Part 11 compliance.

Ensure Confidence in Results While Processing Samples with Incredible Speed

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

Authors: Disha Shah, Emma E. Rennie, Lauren Seymour, Madhusudan Sharma, James S. Pyke, and Patrick Batoon

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 Intelligent Reflex.

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 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 Intelligent Reflex 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 Intelligent Reflex workflows.

A specific Quantitative analysis method is created for each workflow and analyte concentration thresholds are set to trigger secondary injection. Intelligent Reflex 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.

Intelligent Reflex is configured using the unified Acquisition and DA Analysis parameters. These parameters are used to create worklists that demonstrate and execute 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.

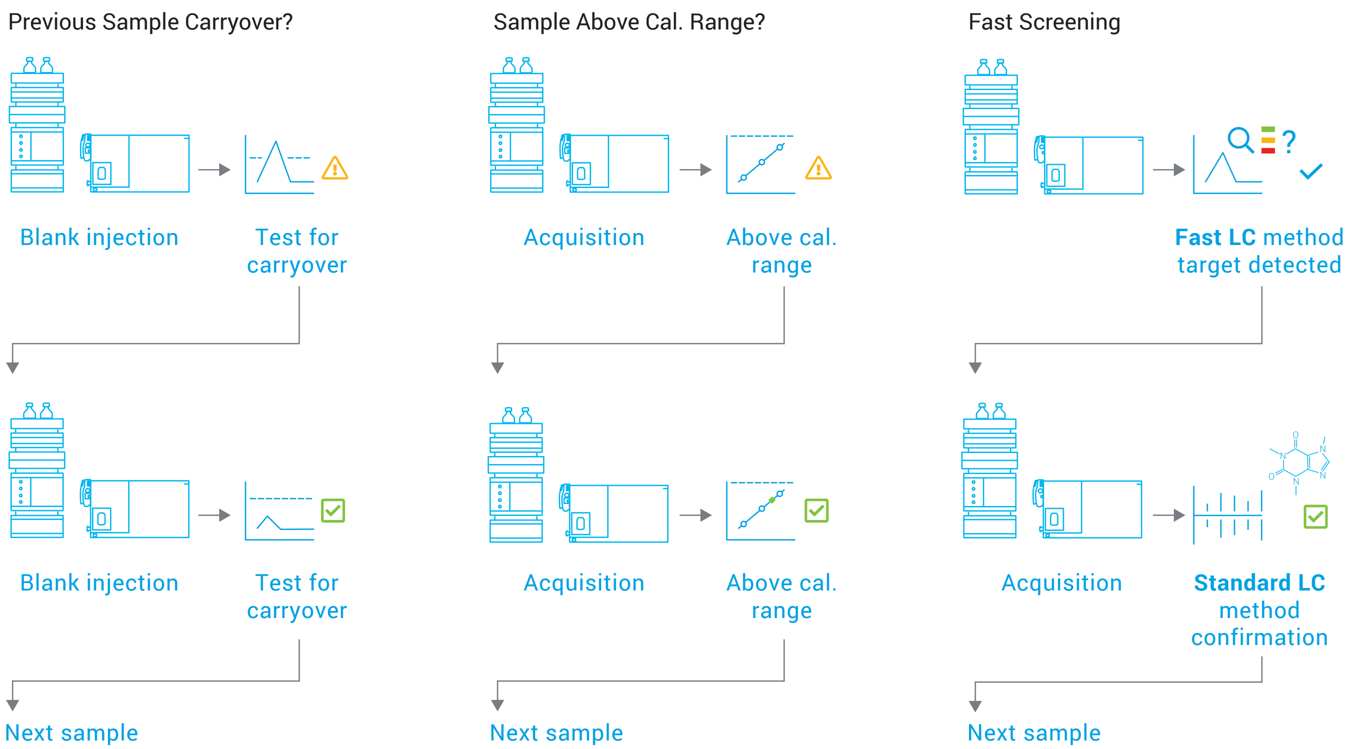


Figure 1: Intelligent Reflex Workflow logic.

Results and Discussion

Intelligent Reflex Workflows

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

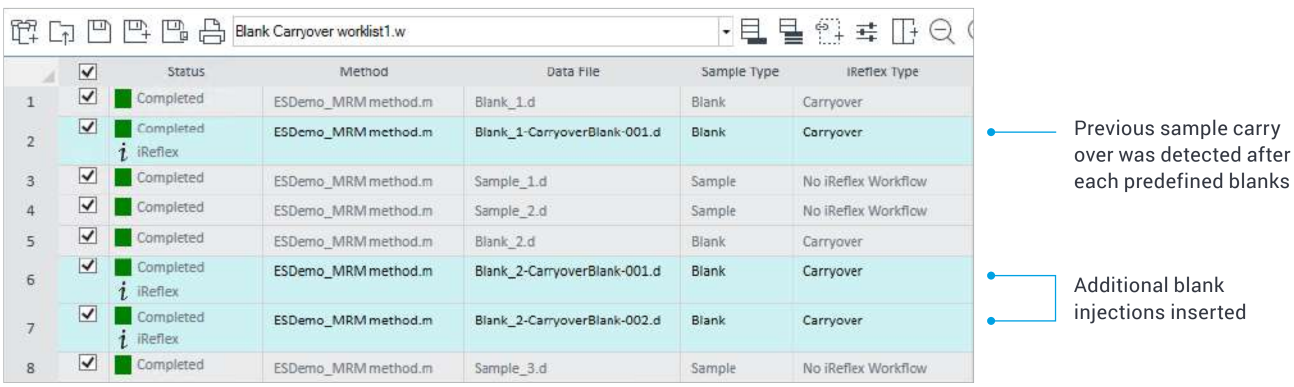
The Carryover Intelligent Reflex Workflows

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.

The Above Calibration Range Intelligent Reflex Workflow

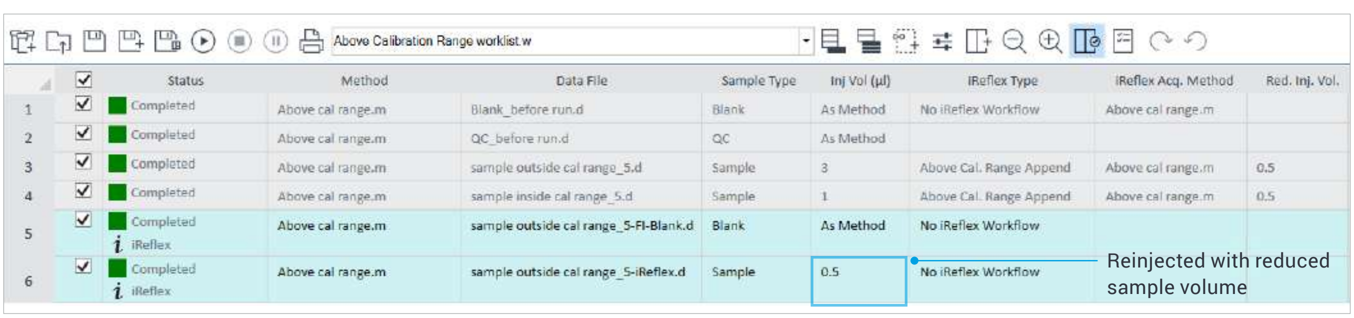
Ensuring a target analyte concentration is within the calibration curve range is critical when quantifying analytical analytes. If an analyte is above the upper limit of quantitation (ULOQ), it is necessary to either dilute the sample or reduce the injection volume to bring the concentration within quantitation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

Detection of an analyte in a sample above the calibration range 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.


Blank Carryover worklist1.w

		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.


Above Calibration Range worklist.w

		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 Intelligent Reflex 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.

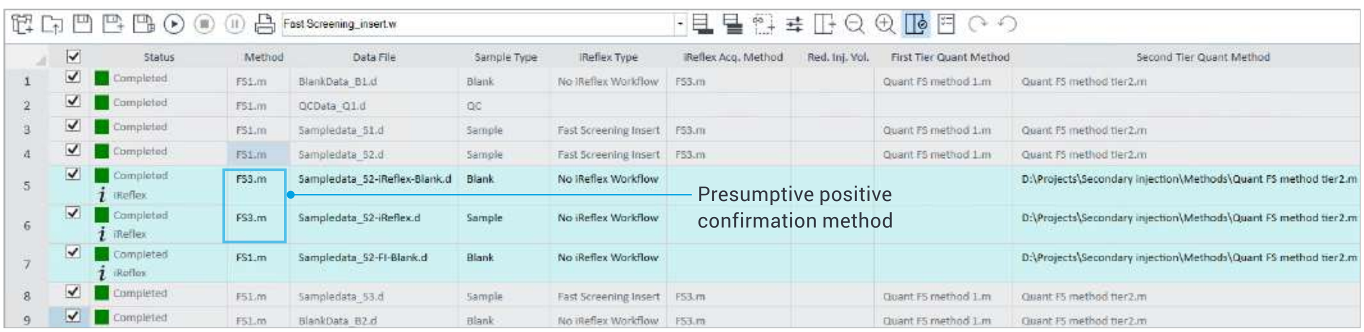
The fast screening Intelligent Reflex workflow produces two different data batches; the 1st tier consists of the original worklist with the fast screening method. The 2nd tier batch consists of reinjected samples which are acquired and analyzed with a different, usually longer and comprehensive, confirmation 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 analyses.

– 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.


Fast Screening_insert.w

	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

Conclusions

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

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

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

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Maximize Uptime While Anticipating Downtime

Accelerated lifetime testing with real-time Early Maintenance Feedback (EMF) diagnostic monitoring on the 6475 triple quadrupole LC/MS system

Authors: Michael B. Pastor, Ryan Rademacher, Patrick M. Batoon

Introduction

Triple quadrupole LC/MS systems have become widely accepted as a platform for targeted, large-batch, sample analysis on a day-to-day basis. A primary concern for routine/targeted analysis is instrument stability; which can vary over time due to the soiling of crucial ion optics components. While incorporating an internal standard and measuring abundance ratio may help alleviate signal drift from a data-analysis standpoint, it does not give key indications to the quality of the instrument's health.

To help alleviate concerns on instrument health and longevity, the new Agilent 6475 triple quadrupole LC/MS system was designed with onboard intelligence that actively reports on the instrument health and status through Early Maintenance Feedback (EMF).

EMF reports various aspects pertaining to instrument maintenance such as "last tuned", number of samples injected, number of diverter valve switches, last rough pump oil change, last gas filter change, and real time reports on detector health, nebulizer blockage, ion injector blockage, and spray stability status.

Here we present a use case to trigger Early Maintenance Feedback (EMF) events to simulate heavy instrument use through 10,000 sample injections of spiked bovine urine. The sample matrix was specifically chosen due the challenging endogenous components that may cause measurement issues (salts, metabolites, fats, proteins, etc.).

As this is not a true analytical method, the intention of this poster is primarily to stress the Early Maintenance Feedback mechanisms, test the instrument's response to heavy matrix accumulation, stability of tune parameters, and recovery of instrument tuning if out of spec.

Experimental

Bovine urine diluted 1:1 in acetonitrile/water and were delivered to the system using an Infinity II 1290 HPLC with dual injector setup in overlapped injection mode with isocratic flow of 90:10 acetonitrile/water + 0.1% Formic acid.

To generate sufficient backpressure for stable HPLC operation and to simulate the use of an analytical HPLC column, a ZORBAX Extend-C18, 80Å, 2.1 mm, 1.8 µm, 1200 bar pressure limit, UHPLC guard column was used (821725-107), as shown in Figure 1.

Figure 1: A ZORBAX Extend-C18, 80Å, 2.1 mm, 1.8 µm, 1200 bar pressure limit, UHPLC guard column was appended directly to the nebulizer to simulate the passing of a sample through a chromatographic column.

MRM signals of various analytes were recorded to ensure that ions were reaching the detector. This current served to "age" the Electron Multiplier horn as if it were in standard/normal operation.

Early Maintenance Feedback (EMF) provided real time monitoring of the instrument health. EMF intelligence is incorporated in the systems firmware to monitor for crucial points along the ion path such as ion injector blockage, precipitation on the nebulizer, and detector's estimated lifetime. Additionally, the instrument automatically monitored for commonly disruptive potential maintenance events such as poor spray stability and ion beam blockage events originating at the nebulizer or ion injector.

No cleaning or removal of the nebulizer, ion injector, or ion source chamber and spray shield was carried out over the course of the injection series.

Post-Experiment Investigations

Upon completion of the injection series, an examination of the ion source and desolvation assembly was carried out to identify regions of ion burn, salt accumulation, broad matrix deposition, or potential modes of failure.

Tune ion abundances that were recorded during the Checktune procedure were plotted to evaluate the effects of matrix over time.

The new Agilent 6475 triple quadrupole LC/MS system coupled with the 1290 Infinity II LC

Results and Discussion

Pre-experiment to Post-experiment Physical Attributes

A. Sample vial with diluted matrix (Bovine urine 1:1 acetonitrile/water).

B. Sample overlaid MRM TIC every 1,000 injections.

C. MS inlet after 10,000 injections. Spray shield and capillary cap with heavy contamination while maintaining ion injector performance.

D. Skimmer with desolvation assembly removed. Cotton swabs with IPA to highlight matrix contamination in vacuum region after ion injector (front of skimmer, back of skimmer, octopole).

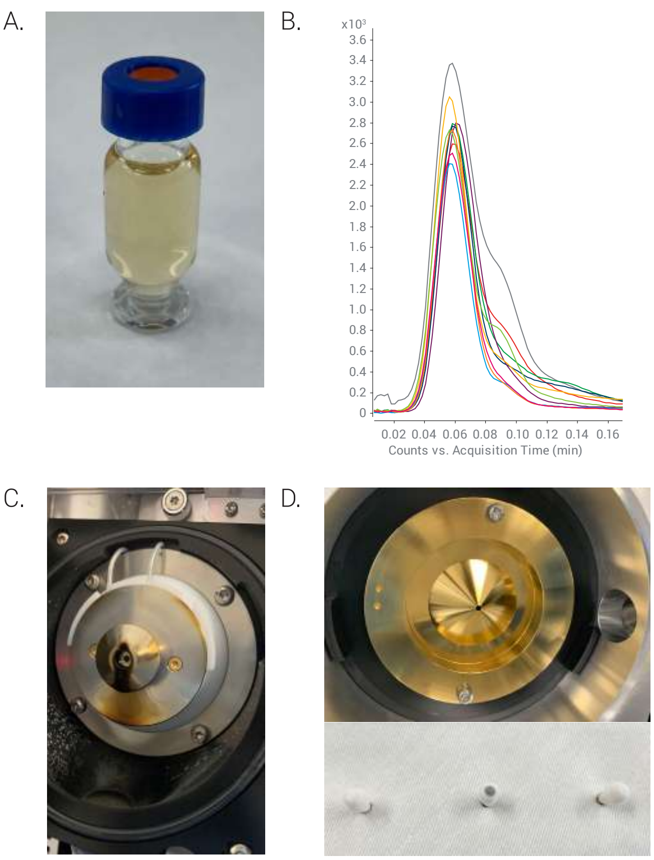

Figure 2: Experiment physical attributes.

Tune Ion Tolerances as Matrix Components Accumulated Onto the Spray Chamber

Checktunes were performed every 1,000 injections with no cleaning of the nebulizer or ion injector (Figure 3). Despite heavy front-end contamination, mass calibration (m/z drift) and mass spectral peak width (FWHM) remained within tolerance and stable over the 10,000 injections. Tolerances for m/z<1000, mass calibration must remain within ±0.1 Da, while peak width must remain ±0.14 Da. Over the course of injection series, the instrument reported an "Out of Tolerance" event at injection 6,000; this was remedied by running the Autotune procedure before proceeding to the next series of injections.

No Critical Early Maintenance Feedback Events Were Triggered Over the Course of This Investigation

Early Maintenance Feedback continuously monitors for the most common sources of addressable issues pertaining to heavy routine analysis use. Over the course of this investigation, none of these events were triggered with the exception of "Injection Count" set to a threshold of 10,000 injections.

Despite constant bombardment of ions, detector health was observed to be stable and did not change to a considerable degree, the nebulizer and ion injector remained unclogged, and the spray stability remained consistent.

Detector EMV	Positive Mode	Negative Mode
Start	1212 V	1232 V
End	1198 V	1232 V

Checktune Results Per 1,000 Injections

Checktune report after 10,000 injections shown in Figure 4 with passing result. Results for both positive and negative, MS1 and MS2, as well as various scan speeds and peak widths shown in a single page report (detailed also available).

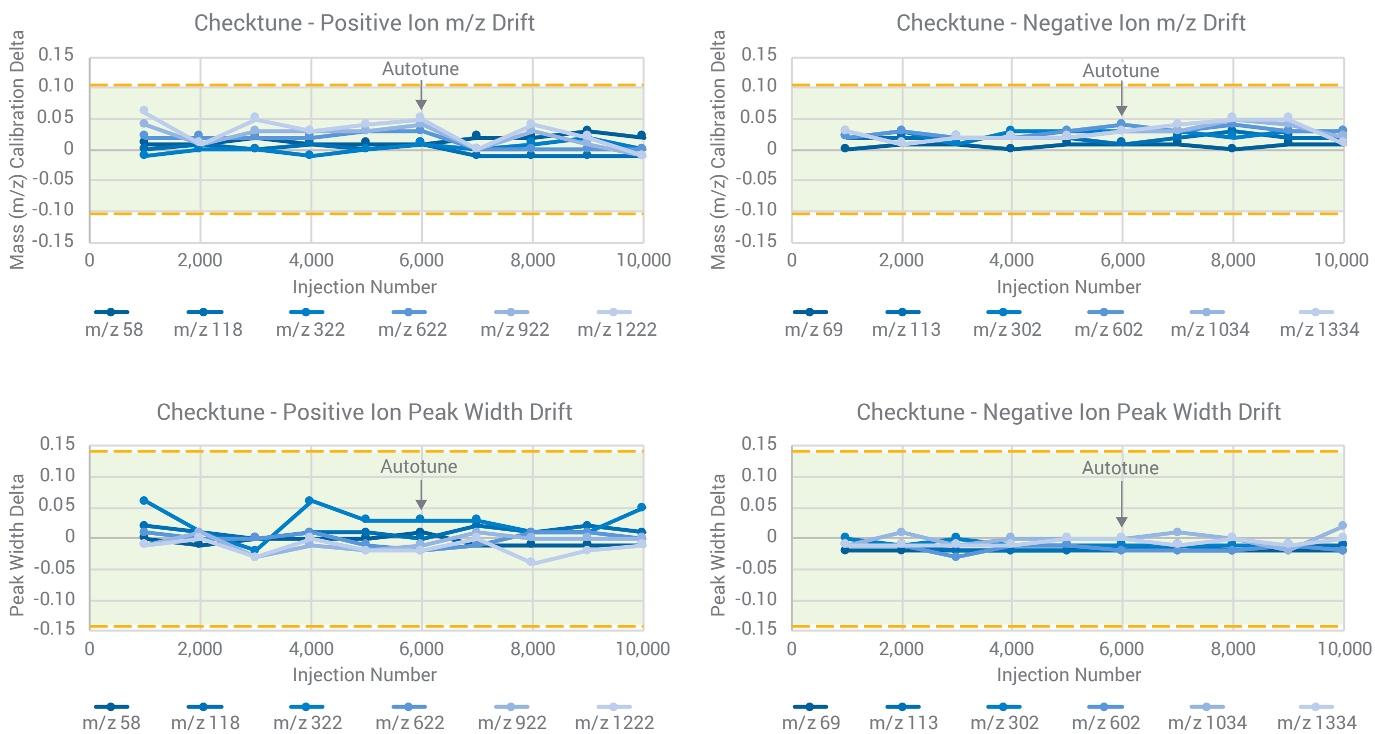

Figure 3: Checktune tolerance results performed every 1,000 injections.

MS Checktune Report - G6475A

Instrument Information

Model	G6475A	Checktune Date	2022-09-25T16:08:08-07:00
Serial Number	SG2222S001	SW/FW Version	3.0.1424/8.1.34
Ion Source	AJS ESI	Ionization Mode	ESI
Last Autotune Date	2022-08-29T11:36:41-07:00	Last Tuned By	SYSTEM (SYSTEM)
Vacuum Pressure	2.25E+0 [R] (Torr); 2.90E-5 [H] (Torr)	Overall Result	Passed

Positive Ion Mode

MS1 Peak Width Unit, Scan Speed Normal	Result	Passed
MS2 Peak Width Unit, Scan Speed Normal	Result	Passed
MS1 Peak Width Narrow, Scan Speed Normal	Result	Passed
MS2 Peak Width Narrow, Scan Speed Normal	Result	Passed
MS1 Peak Width Wide, Scan Speed Normal	Result	Passed
MS2 Peak Width Wide, Scan Speed Normal	Result	Passed
MS1 Peak Width Widest, Scan Speed Normal	Result	Passed
MS2 Peak Width Widest, Scan Speed Normal	Result	Passed
MS2 Scan Speed Fast	Result	Passed
MS2 Scan Speed Ultra	Result	Passed
MS1 Lag Factor	Result	Passed
MS2 Lag Factor	Result	Passed
Gain	Result	Passed

Negative Ion Mode

MS1 Peak Width Unit, Scan Speed Normal	Result	Passed
MS2 Peak Width Unit, Scan Speed Normal	Result	Passed
MS1 Peak Width Narrow, Scan Speed Normal	Result	Passed
MS2 Peak Width Narrow, Scan Speed Normal	Result	Passed
MS1 Peak Width Wide, Scan Speed Normal	Result	Passed
MS2 Peak Width Wide, Scan Speed Normal	Result	Passed
MS1 Peak Width Widest, Scan Speed Normal	Result	Passed
MS2 Peak Width Widest, Scan Speed Normal	Result	Passed
MS2 Scan Speed Fast	Result	Passed
MS2 Scan Speed Ultra	Result	Passed
MS1 Lag Factor	Result	Passed
MS2 Lag Factor	Result	Passed
Gain	Result	Passed

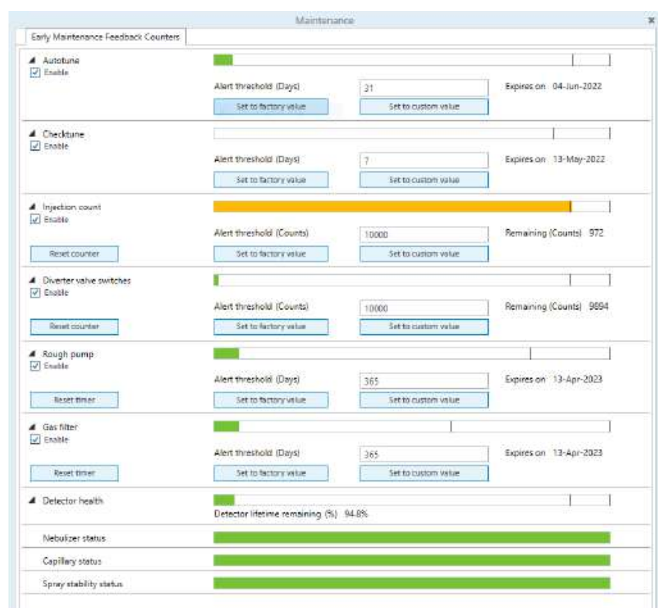

Figure 4: Checktune report results demonstrating that all parameters pass (left) and Early Maintenance Feedback letting the user know that the instrument has exceeded 10,000 injections (right).

Conclusions

- Instrument robustness over 10,000 injections was demonstrated using a heavy matrix (bovine urine) sample.
- Checktunes were recorded to verify instrument stability. Tune ion Mass Calibration and Peak Widths were recorded every 1,000 injections and were within tolerance criteria for good performance.
- Nebulizer spray, ion injector capillary, and spray stability triggered no adverse events.
- Constant ion bombardment through MRM acquisition did not age the detector in a significant manner.

References

1. Robustness of the Agilent Ultivo Triple Quadrupole LC/MS with the ESI Source, Agilent Technologies, Inc. 2019, 5994-0671EN.
2. Metzger, Benedikt; Willmann, Lucas; Simplify your Method Development using the Agilent 1260 Infinity Prime LC System and InfinityLab LC/MSD iQ, Agilent Technologies, Inc. 2020, 5994-2124EN.
3. Sartain, Mark; Sosienski, Theresa; Yang, Dan-Hui Dorothy; Robustness of the Agilent Ultivo Triple Quadrupole LC/MS for Routine Analysis in Food Safety, Agilent Technologies, Inc. 2017, 5991-8741EN.

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