Application Note

Forensic Toxicology

Agilent

Trusted Answers

Intelligent Reflex Fast Screening for Drugs in Urine

Data-dependent reinjection logic for screening and confirmation of presumptive positives using the intelligent reflex fast screening function in MassHunter acquisition software

Authors

Lee Bertram and
Patrick Batoon
Agilent Technologies, Inc.

Abstract

The intelligent reflex protocol is a confidence and intelligence feature within Agilent MassHunter acquisition software that provides reflexive reinjection logic to ensure that results are immediately trustworthy and within operational limits. This application note demonstrates the use of the fast screening function within an innovative technology called intelligent reflex, available on Agilent triple quadrupole LC/MS instruments. This function can be used to improve analytical speed to rapidly screen for positive samples using an analytical method approximately 3 minutes in length. Prescreening a large batch of samples for presumptive positives using the fast screening workflow allows exclusion of negative samples from quantitative analysis, resulting in improved batch completion time.

Introduction

Intelligent reflex is a data-dependent, worklist-oriented, sample-reinjection logic protocol within MassHunter acquisition software that aims to provide intelligence to routine analysis workflows. Within MassHunter acquisition software version 12, intelligent reflex hosts three specific workflows enabled for triple quadrupole LC/MS (LC/TQ) instruments: the carryover detection and above calibration range functions are for sample assurance to protect measurement fidelity, and the fast screening function is for sample throughput to improve analytical speed.

A traditional method of analyzing urine for drugs of abuse includes a gradient of roughly 10 to 20 minutes to achieve good chromatographic separation for quantitative confirmation. However, for a plate of 96 samples, this would require roughly 16 to 32 hours (960 to 1,920 minutes) to complete a batch. It is not expected that 100% of samples contain the analytes of interest, so considerable time is wasted producing negative hit results.

The purpose of this experiment was to demonstrate the productivity gains and mechanics of the intelligent reflex fast screening workflow to rapidly screen for positive samples using a shortened analytical method (~3 minutes). Only samples marked as presumptive positives were analyzed using the longer confirmatory method for quantitation. This data-dependent workflow aims to shorten batch analysis time when positive hits are considered infrequent.

Fast screening allows the user to define two methods:

1. **Screening method**

   The screening method is used for general and broad detection for a threshold concentration value of various analytes. Excellent chromatography is not necessary; the method must simply be sensitive and selective enough to detect a desired target greater than the preset threshold.

2. **Confirmatory method**

   If a threshold concentration is met, the sample is marked for confirmatory analysis using a comprehensive, quantitative, highly selective liquid chromatography/mass spectrometry (LC/MS) method. This method must have calibration curves provided before setting up the worklist.

Data-dependent (intelligent reflex) triggers are configured through Agilent MassHunter Quantitative Analysis software version 12 as "outliers" to define trigger thresholds (concentration) for each analyte of interest. The quant method is then associated with the acquisition method in the DA tab. Method switching using the same chromatography system is made possible since column position can be configured via column compartment ("valve position") in the chromatography method. Each sample vial is analyzed via the screening method using the logic in Figure 1.

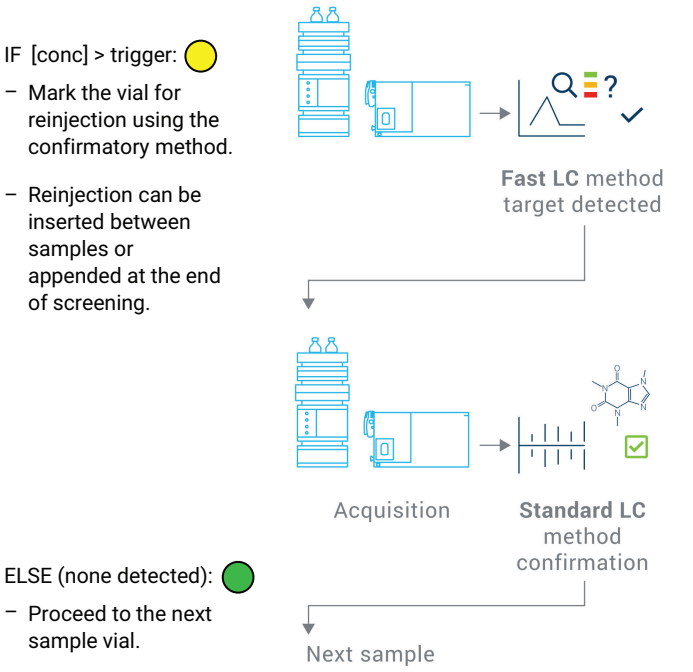

Figure 1. Visualization of intelligent reflex fast screening workflow.

Experimental

This experiment was crafted to simulate drug screening analysis using a 96-well plate template containing 10 positive-hit samples at randomized vial locations.

Chemicals and reagents

- Formic acid, LC/MS grade
- Acetonitrile (ACN), LC/MS grade
- Water, MS grade
- Agilent LC/MS forensic toxicology calibration mixture (part number 5190-0470)

Sample preparation

Negative samples were diluted synthetic urine. Presumptive positive samples were made by diluting the forensic toxicology calibration mixture spiked into synthetic urine to create a 10x diluted concentration.

Instrument configuration

This experiment was carried out using the following Agilent instrument configuration:

- 6475 Triple quadrupole LC/MS system (G6475A)
- MassHunter acquisition software for LC/TQ systems, version 12
- MassHunter Quantitative Analysis software, version 12
- 1290 Infinity II high-speed pump (G7120A)
- 1290 Infinity II multisampler (G7167B)
- 1290 Infinity II multicolumn thermostat (G7116B)

Mass spectrometer parameters

MS parameters are displayed in Table 1.

Table 1. Source parameters for the Agilent 6475 triple quadrupole LC/MS.

Parameter	Value
Ion Source	Agilent Jet Stream Electrospray ionization source
Polarity	Positive
Gas Temperature	350 °C
Drying Gas Flow	12 L/min
Nebulizer	50 psi
Atmospheric Pressure Chemical Ionization Source (APCI) Vaporizer Temperature	350 °C
Capillary Voltage	2,000 V
Scan Type	Multiple reaction monitoring (MRM)
Detector Gain Factor (+)	2

Targets of interest

Screening and confirmatory methods were established using the MRM transitions in Table 2. The dwell time for each MRM transition was 10 milliseconds.

Screening method (3 minutes)

The screening method was defined with relatively fast high performance liquid chromatography (HPLC) to provide rough separation of analytes above the defined concentration threshold. HPLC parameters for the shortened screening method are displayed in Table 3.

Table 2. MRM parameters for all target compounds.

Name	MS1	MS2	Frag (V)	CAV (V)	CE (V)	+/-
Alprazolam	309.1	281.1	156	4	40	+
Clonazepam	316.1	270.1	214	4	24	+
Cocaine	304.2	182.1	113	3	16	+
Codeine	300.2	128.1	166	4	60	+
Diazepam	285.1	193.1	166	4	32	+
Hydrocodone	300.2	199.1	161	4	26	+
Lorazepam	321	275	108	4	20	+
MDA	180.1	77.1	80	4	48	+
MDEA	208.1	163.1	98	4	8	+
MDMA	194.1	163.1	80	4	8	+
Meperidine (Pethidine)	248.2	220.1	131	4	20	+
Methadone	310.2	265.2	118	4	12	+
Methamphetamine	150.1	91.1	75	4	20	+
Nitrazepam	282.1	236.1	204	4	24	+
PCP	244.2	86.2	75	4	8	+
Phentermine	150.1	65.1	75	3	48	+
Proadifen	354.2	91.1	150	3	40	+
Strychnine	335.2	184.1	150	3	40	+
Temazepam	301.1	255.1	123	4	16	+
THC	315.2	193.1	150	3	20	+

Table 3. HPLC parameters for shortened screening method.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm
Valve Position	Position 2 (Port 2 → 2')
Sampler Temperature	4 °C
Mobile Phase A	dH_2O + 0.1% formic acid
Mobile Phase B	ACN + 0.1% formic acid
Flow Rate	0.6 mL/min
Injection Volume	1 µL
Column Temperature	55 °C
Post-Time	0.50 min
Gradient Program	Time (min) %B 0.0 5 0.15 5 0.35 30 1.15 60 1.50 95 2.00 95 2.10 5

Confirmatory method

The confirmatory method used a traditional chromatography run time and gradient to provide high resolution, selectivity, and sensitivity for each analyte. HPLC parameters for the longer confirmatory method are displayed in Table 4.

Table 4. HPLC parameters for longer confirmatory method.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 1.9 µm
Valve Position	Position 3 (Port 3 → 3')
Sampler Temperature	4 °C
Mobile Phase A	dH_2O + 0.1% formic acid
Mobile Phase B	ACN + 0.1% formic acid
Flow Rate	0.6 mL/min
Injection Volume	1 µL
Column Temperature	40 °C
Post-Time	2.00 min
Gradient Program	Time (min) %B 0.0 5 0.50 5 1.50 30 6.50 60 10.00 95 12.00 95 12.10 5

Intelligent reflex enablement

The Intelligent Reflex Enabled check box must be selected under the DA tab while in the Method Editor of MassHunter acquisition software (Figure 2). Depending on the circumstances of the application, Fast Screening Append or Fast Screening Insert can be selected:

- **Fast Screening Insert:** Inserts reflexed injections between two samples vials (i.e., confirmatory analysis will immediately run after the sample is deemed a presumptive positive).
- **Fast Screening Append:** Appends reflexed injections to the end of the existing worklist. This causes all confirmatory analysis to be run after all samples have been screened.

For this experiment, the Fast Screening Append workflow was selected from the drop-down menu.

Results and discussion

Quality control injections for screening and confirmatory method

The chromatograms in Figure 3 are quality control (QC) injections for the screening and confirmatory methods for all analytes of interest. Each analyte can be detected acceptably, but with greater sensitivity, selectivity, and specificity in the confirmatory method.

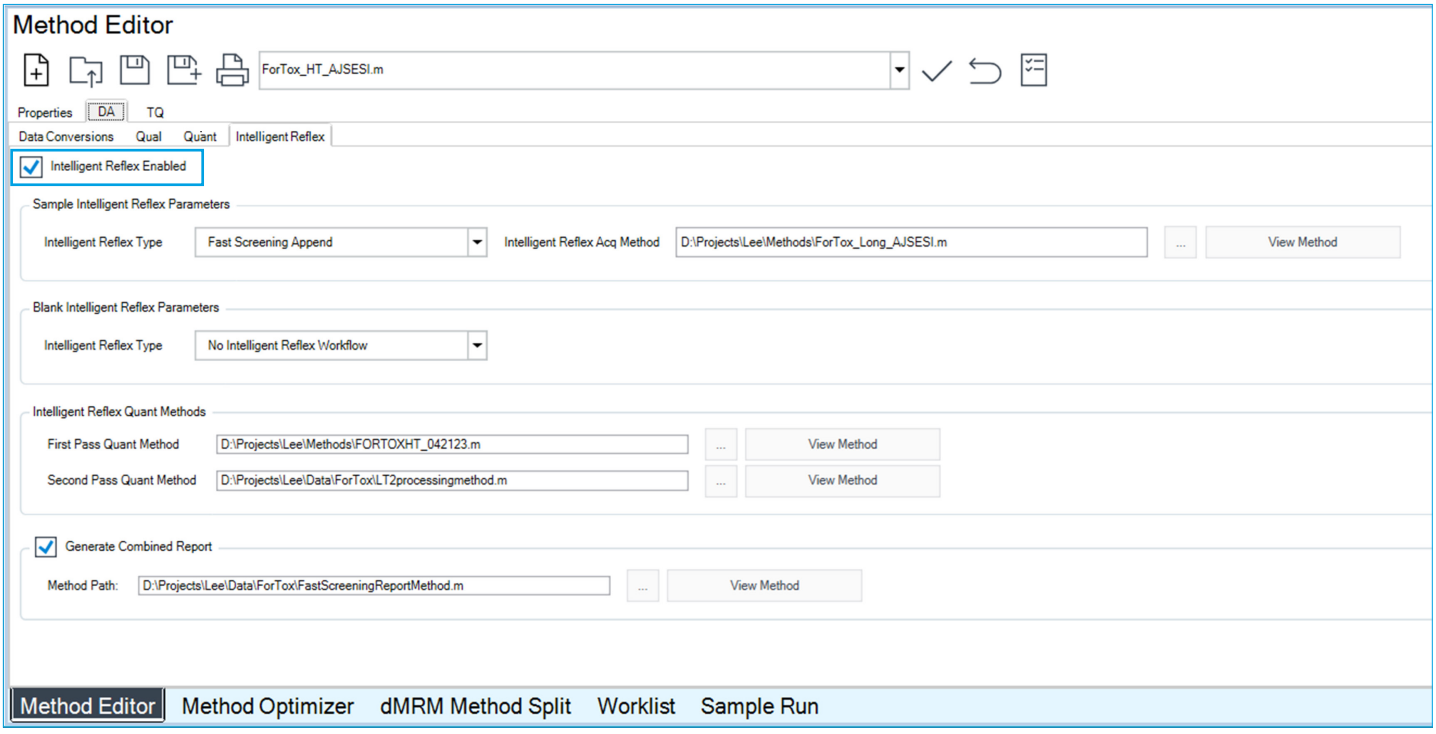

Figure 2. The Method Editor page of Agilent MassHunter acquisition software showing the selection of Intelligent Reflex Enabled.

Screening for targets of interest in a 96-sample batch

Of the 96 sample vials analyzed, 86 samples did not contain any targets of interest. In a traditional analysis, all sample vials would be analyzed using the comprehensive method, resulting in 1,440 minutes (15 minutes × 96 samples) of continuous run time. Instead, the analysis of this batch took only 468 minutes (3 minutes × 96 samples + 15 minutes × 12 samples) of continuous run time because not all samples produced the threshold necessary to mark for comprehensive analysis. In this example, data-driven automation enabled a time savings of 68%. Figure 4 demonstrates a negative sample and a presumptive positive sample. Figure 5 shows the samples that were automatically appended for reinjection labeled and highlighted in teal.

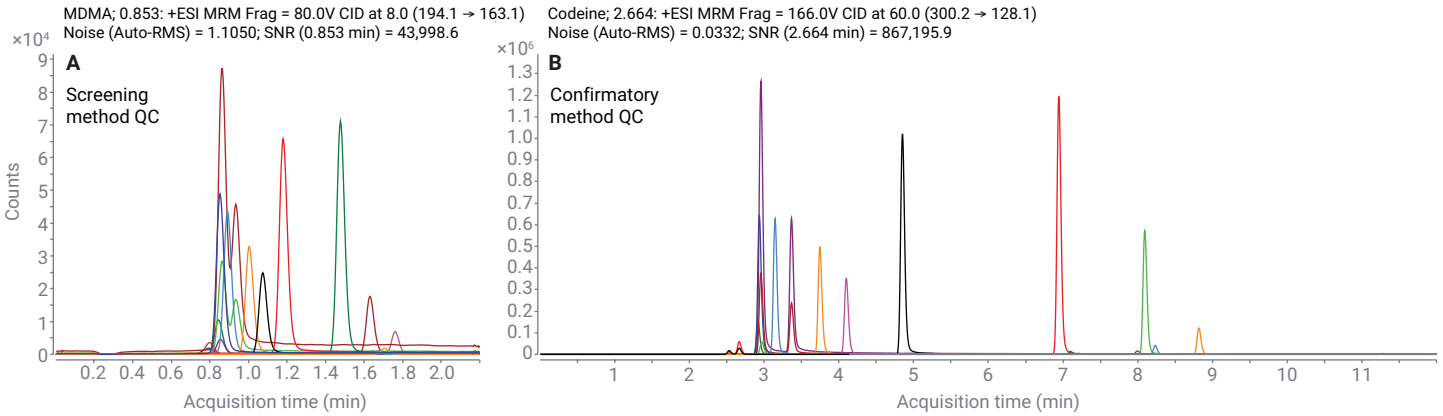

Figure 3. Representative chromatograms of QC injections containing all compounds of interest.

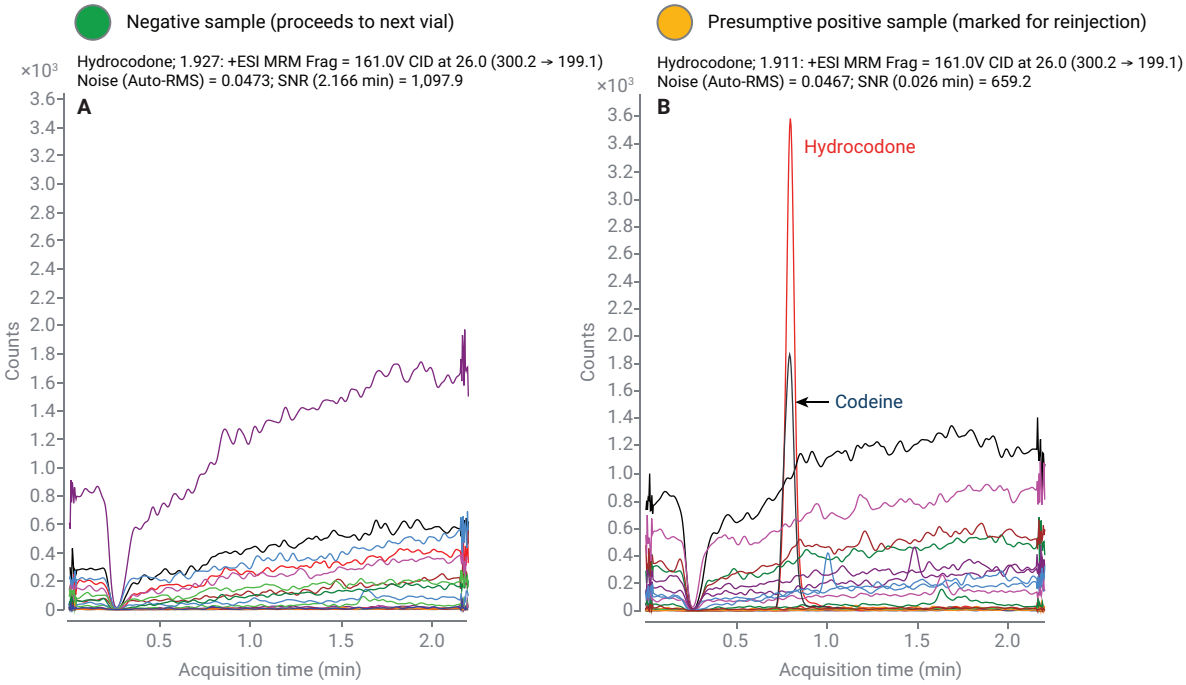

Figure 4. Demonstration of a negative sample and a presumptive positive sample.

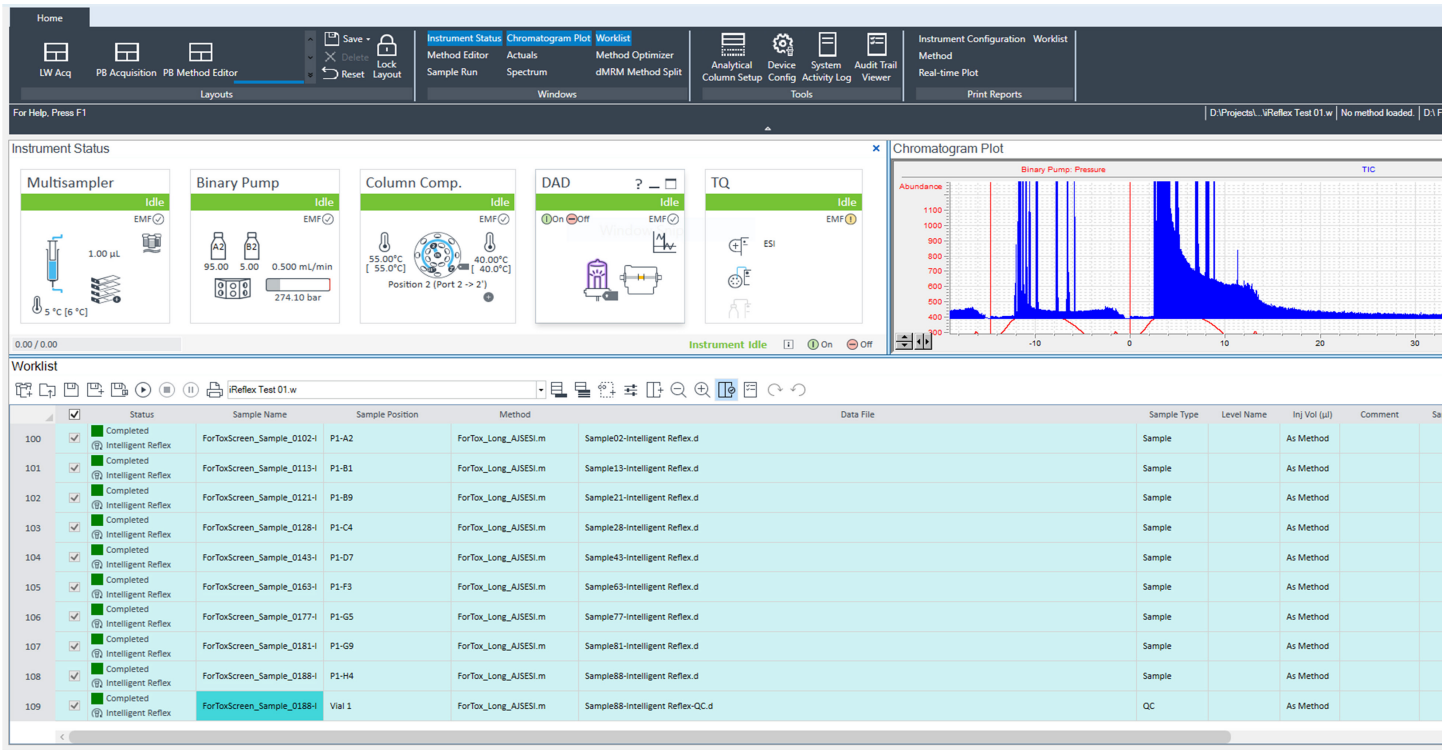

Figure 5. Agilent MassHunter acquisition software showing the automatically appended samples for reinjection.

Conclusion

This experiment demonstrates the effectiveness of the intelligent reflex fast screening append workflow to accurately identify and reinject 10 randomly positioned sample vials (presumptive positive hits) in a full 96-well plate sample set. Using a workflow based on this innovative technology, a laboratory will be able to save time by using the data-driven automation that is built into the instrument intelligence of all Agilent triple quadrupole LC/MS systems that operate using Agilent MassHunter acquisition software version 12.

www.agilent.com

RA45203.4451851852

Printed in the USA, October 13, 2023
5994-6724EN

Agilent
Trusted Answers