Poster Reprint

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# Intelligent Reflex Fast Screening for Drugs in Urine: data-dependent reinjection logic for screening and confirmation of presumptive positives

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## Introduction

Intelligent reflex is a data-dependent, worklist-oriented sample reinjection logic protocol that provides “intelligence” to routine analysis workflows. Within MassHunter 12, intelligent reflex hosts 3 specific workflows enabled for LC/TQ instruments.

**Sample Assurance – Protects measurement fidelity**

1. Carryover Detection
2. Above Cal. Range

**Sample Throughput – Improves Analytical Speed**

3. Fast Screening

A traditional method of analyzing urine for drugs of abuse includes chromatographic separation, roughly 10-20 minutes, to achieve good chromatographic separation for quantitative confirmation. However, for a plate of 96 samples this would require roughly 16-32 hours (960-1920 min) to complete a batch. It is not expected that 100% of samples contain the analytes of interest, so considerable time is wasted to produce a “negative hit” result.

**The purpose of this experiment is 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 are analyzed using the longer confirmatory method for quantitation.

This data-dependent workflow aims to shorten batch analysis when positive hits are considered infrequent.

### MassHunter 12’s Intelligent Reflex feature present on the Ultivo LC/TQ, 6475 LC/TQ, 6495 LC/TQ, and select LC/Q-TOF systems

6495 triple quadrupole LC/MS system (G6495D)

6475 triple quadrupole LC/MS system (G6475A)

Ultivo triple quadrupole LC/MS system (G6465B)

### Intelligent Reflex: Fast Screening

Fast screening allows the user to define two methods:

- (1) Screening Method

  The screening method is used for general & broad detection for a threshold [conc] of various analytes. Since excellent chromatography is not necessary, the method must simply be sensitive and selective enough to detect a desired target.

- (2) Confirmatory Method

  If a threshold [conc] is met, the sample is marked for confirmatory analysis using a comprehensive, quantitative, highly selective chromatography/MS method. This method must have calibration curves provided

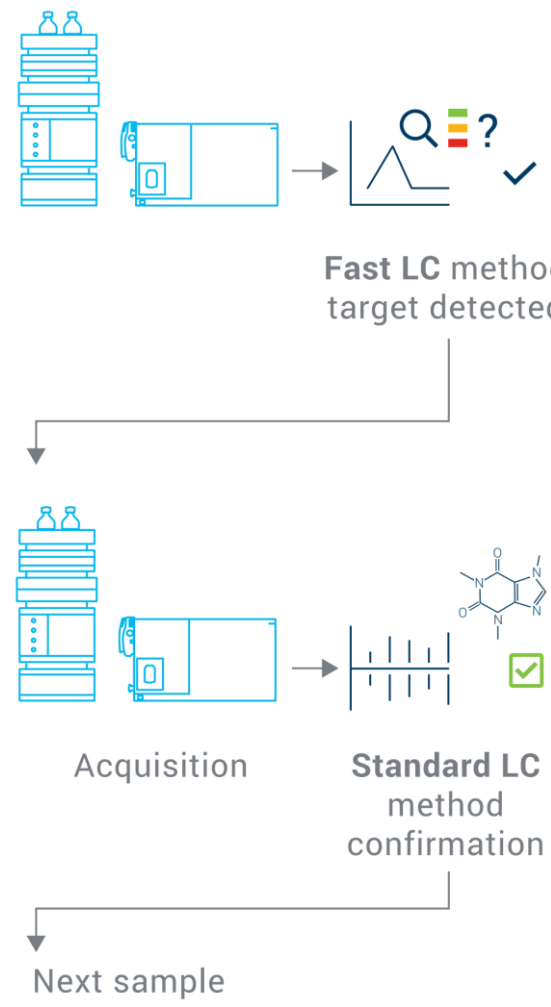
Trigger concentrations are configured through MassHunter 12 Quantitative Analysis as “outliers” to define trigger thresholds (concentrations) for each analyte of interest. The quant method is then associated with the acquisition method in the data analysis (DA) tab.

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 following logic in the diagram below

**IF [conc] > Trigger:**

- Mark vial for reinjection using Confirmatory method
- Reinjection can be inserted between samples or appended at the end of screening




**ELSE (No Detect):**

- Proceed to next sample vial

## Experimental

This experiment was crafted to simulate drug screening analysis using a 96-well plate template containing 12 positive-hit samples at randomized vial locations.

### Sample Composition

Negative samples were diluted synthetic urine

Presumptive positive samples were made by diluting the Agilent ForTox Standard (5190-0470) spiked into synthetic urine to create a 10x diluted concentration .

### Instrument Configuration

This experiment was carried out using the following instrument configuration:

- 6475 triple quadrupole LC/MS system (G6475A)
- MassHunter 12 Acquisition for LC/TQ
- MassHunter 12 Quantitative Analysis
- Infinity II 1290 Binary Pump (G7120A)
- Infinity II 1290 Multisampler (G7167B)
- Infinity II 1290 Column Compartment (G7116B)

### Mass Spectrometer Parameters

| Parameter | Value |
|---|---|
| Ion Source | Agilent ESI source |
| Polarity | Positive |
| Gas Temperature | 350 °C |
| Drying Gas Flow | 12 L/min |
| Nebulizer | 50psi |
| APCI Vaporizer Temperature | 350 °C |
| Capillary Voltage | 2000 V |
| Scan Type | MRM |
| Detector gain factor (+) | 2 |

### Targets of Interest

Screening and confirmatory methods were established using the following MRM transitions below. Dwell time for each MRM transition = 10 ms

| 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 | + |

### Screening Method (3 min)

The screening method was defined with relatively fast chromatography to provide rough separation of analytes for detection above the defined [conc] threshold.

| Parameter | Value | |
|---|---|---|
| Column | Agilent Poroshell 120 EC-C18, 2.1 X 50mm, 1.9 µm | |
| Valve Position | Position 2 (Port 2 -> 2') | |
| Sampler Temperature | 4 °C | |
| Mobile Phase A | $ddH_2O$+0.1% Formic acid | |
| Mobile Phase B | ACN+0.1% Formic acid | |
| Flow Rate | 0.6 mL/min | |
| Injection Volume | 1µL | |
| Column Temperature | 55°C | |
| Post time | 0.50 minutes | |
| 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 |

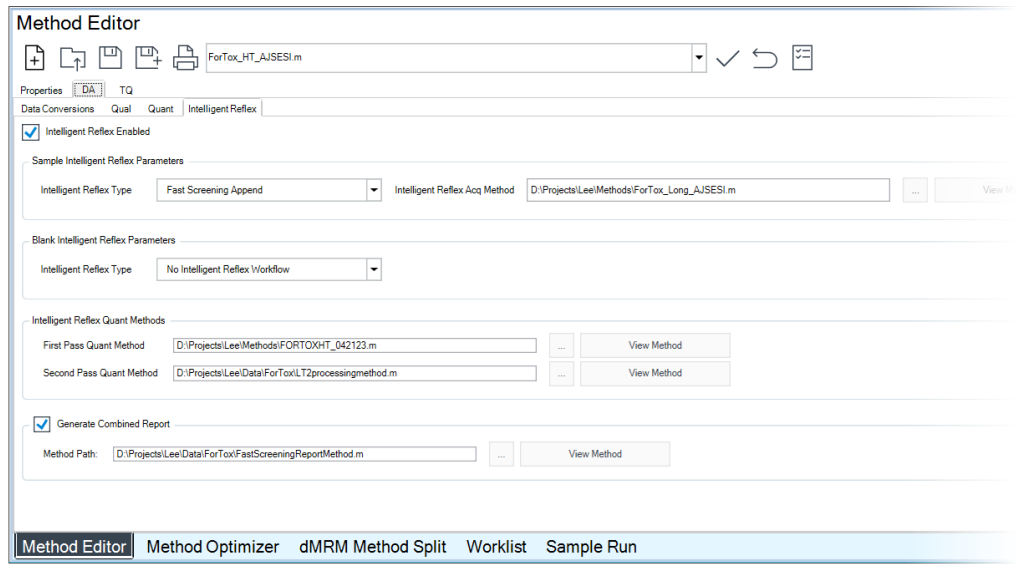
### Confirmatory Method (15 min)

The confirmatory method uses a “traditional” chromatography runtime to provide good separation, selectivity, and sensitivity for each analyte.

| Parameter | Value | |
|---|---|---|
| Column | Agilent Poroshell 120 EC-C18, 2.1 X 100mm, 1.9 µm | |
| Valve Position | Position 3 (Port 3 -> 3') | |
| Sampler Temperature | 4 °C | |
| Mobile Phase A | $ddH_2O$+0.1% Formic acid | |
| Mobile Phase B | ACN+0.1% Formic acid | |
| Flow Rate | 0.6 mL/min | |
| Injection Volume | 1µL | |
| Column Temperature | 40°C | |
| Post time | 2.00 minutes | |
| 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

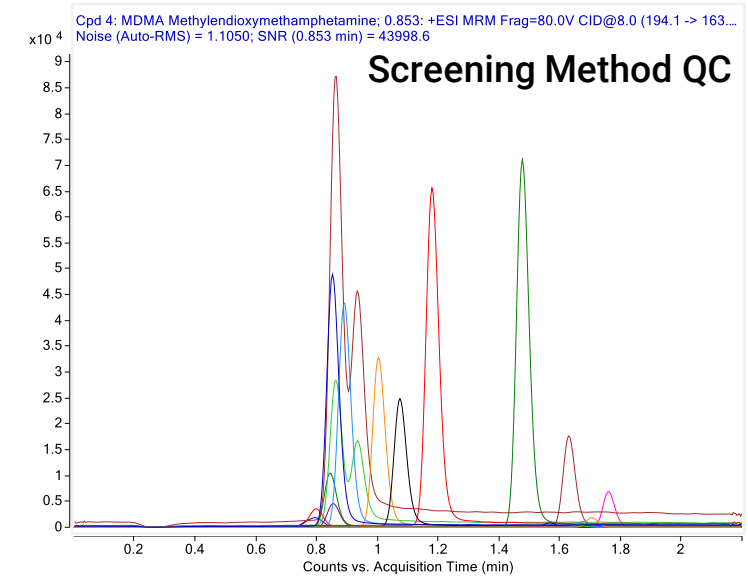
Intelligent reflex must be enabled in the DA tab of the acquisition system. The “Fast Screening Append” workflow was selected

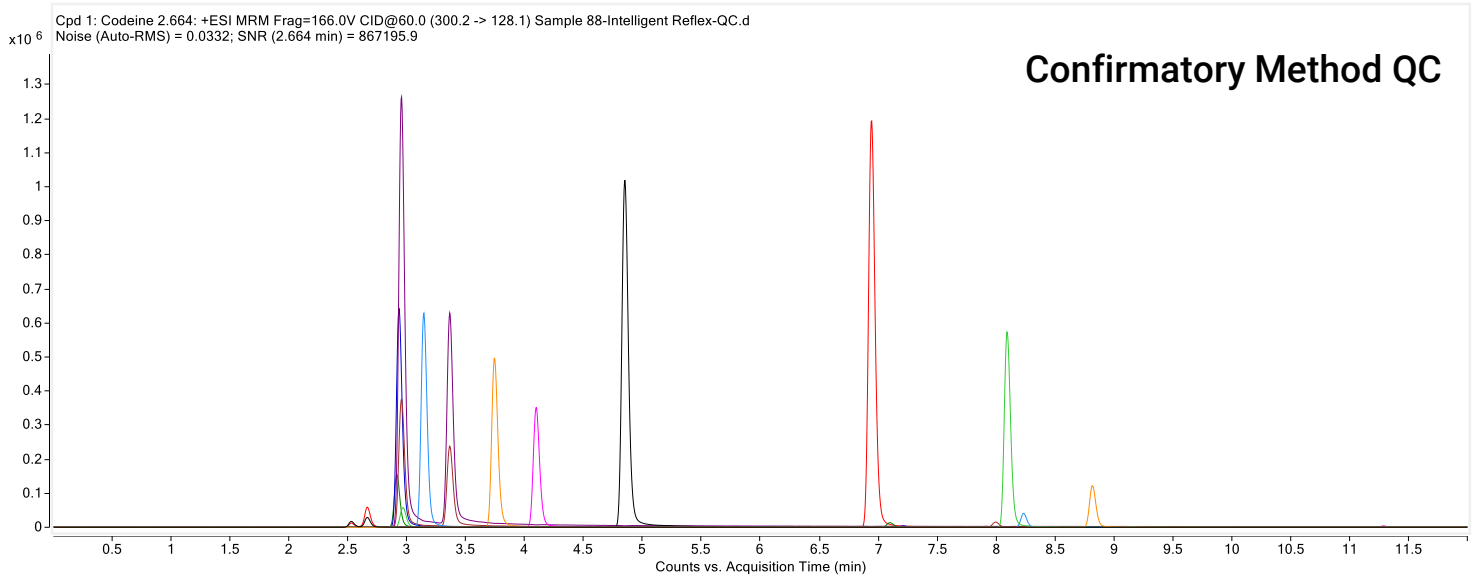


## Results and Discussion

### QC Injections for Screening and Confirmatory Method

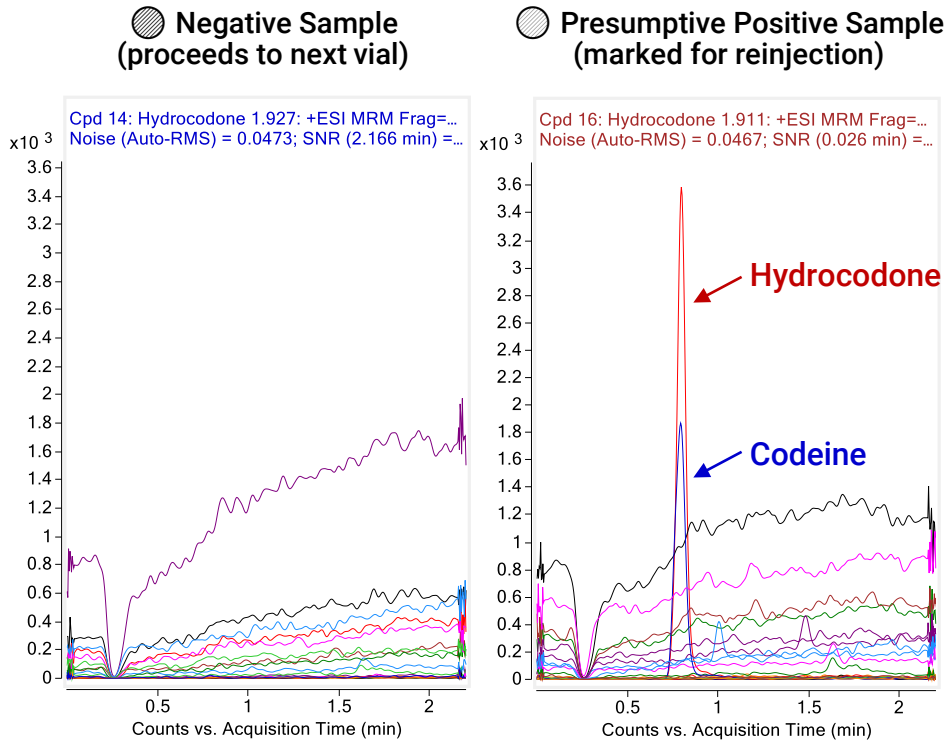
The chromatograms below are QC injections for the Screening and Confirmatory method for all analytes of interest. Each analyte can be acceptably detected; but with greater sensitivity, selectivity, and specificity in the confirmatory method.



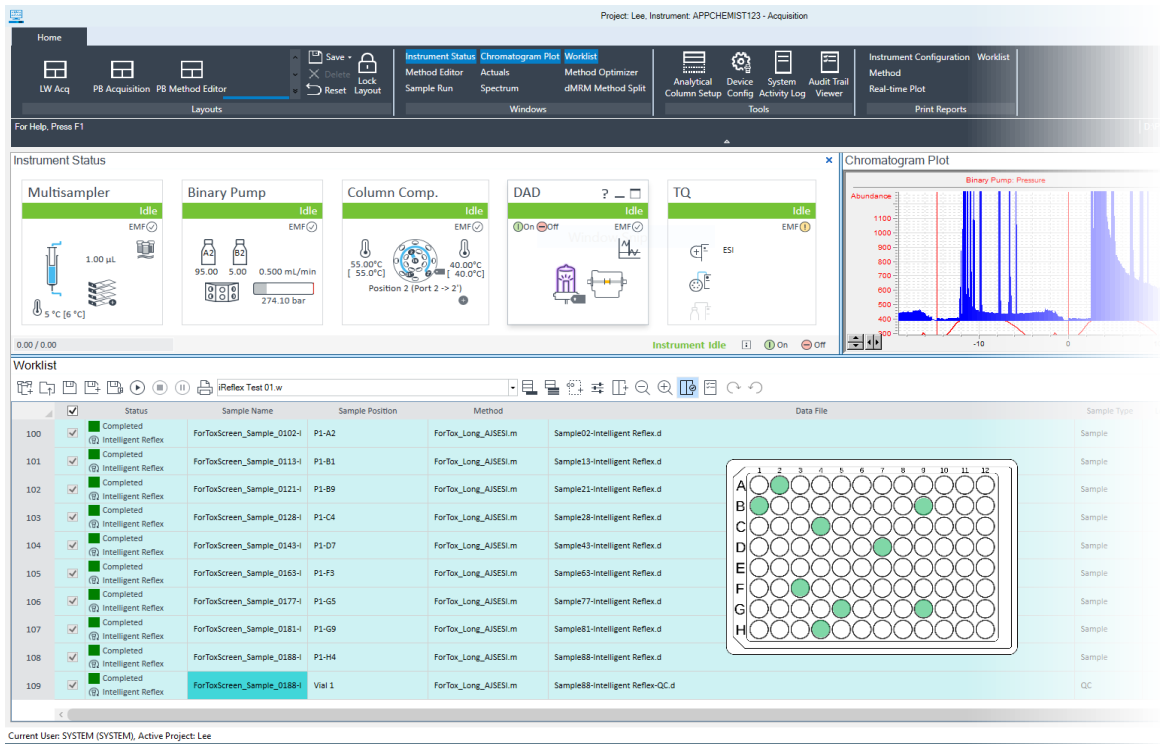

### Screening for targets of interest in a 96-sample batch

Of the 96 sample vials analyzed, 84 samples did not contain any targets of interest. In a traditional analysis, all sample vials would be analyzed using the comprehensive method, resulting in 1440 min (15 min × 96 samples) of continuous runtime. Instead, the analysis of this batch resulted in 468 min (3 min × 96 samples + 15 min × 12 samples) of continuous runtime since all samples did not reach the threshold for comprehensive analysis. The example below demonstrates a Negative sample, Presumptive Positive, and the automatically appended samples for reinjection










## Conclusions

- Intelligent Reflex is a data-dependent worklist reinjection logic system built into MassHunter 12 for LC/TQ and LC/QTOF. The Fast screening workflow was demonstrated to showcase potential productivity gains for large sample batches.
- The 96-sample batch contained 12 randomly-positioned positive samples within the vial tray. Intelligent Reflex was able to properly identify their positions while completing the analysis of all vials.
- Using Intelligent Reflex Fast Screening the batch was completed in 468 min, which is 67% faster than a traditional method of analyzing all samples using a comprehensive method.

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