Application Note

Forensic Toxicology

Agilent

Trusted Answers

Automated Optimization and Intelligent Reflex Screening on the Agilent 6495D LC/TQ

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Abstract

Triple quadrupole LC/MS methods often require hours of manual parameter tuning and two-tier screening workflows that slow turnaround. Automated, intelligent features in Agilent MassHunter software—MassHunter Optimizer for analyte MRM transitions, MassHunter Source Optimizer, and the intelligent reflex fast screening workflow—streamline this process on the Agilent 6495D LC/TQ. Together, they automate compound and source optimization and perform a fast tier-1 screen. When thresholds are exceeded during the tier-1 screen, the software automatically reinjects the same sample/presumptive positive under a confirmation tier-2 method for quantitation and produces a combined report. This approach reduces hands-on time, increases throughput, and improves result consistency.

Introduction

Forensic toxicology panels evolve, and labs must update methods quickly without sacrificing confidence. Manual tuning of MRM transitions and source parameters is time-consuming and variable across analysts. Two-tier screening and confirmation often require separate runs and manual intervention. The onboard intelligence suite for Agilent LC/MS systems addresses these pain points. Automated compound and source optimization shorten method setup, and intelligent reflex applies real-time, data-driven decisions to trigger confirmatory runs and reporting without manual steps.

This application note establishes an orthogonal, automated workflow on the 6495D LC/TQ for forensic toxicology screening. The benefits of MassHunter Optimizer software to develop MRM transitions, MassHunter Source Optimizer software, and intelligent reflex fast screening reinjection logic are demonstrated.

Experimental

This workflow was performed on an Agilent 6495D LC/TQ equipped with an Agilent Jet Stream ESI source and paired with an Agilent 1290 Infinity II LC. MassHunter Acquisition and Quantitative Analysis software controlled the system and enabled automated optimization and intelligent reflex workflows.

Compound optimization for MRM transitions was completed using MassHunter Optimizer software, which automatically selected precursor/product ions and collision energies for each analyte. MassHunter Source Optimizer then refined LC/MS ionization conditions by scanning gas temperature, sheath gas temperature, and flow rates. These optimized parameters were applied to both screening and confirmation methods.

Tier-1 screening used a short Agilent InfinityLab Poroshell 120 EC-C18 column and a rapid gradient for fast presumptive detection, while tier-2 confirmation employed a longer InfinityLab Poroshell 120 Phenyl-Hexyl column for orthogonal selectivity. LC method parameters are shown in Table 1 for tier-1 and tier-2 methods. Both methods shared the same optimized LC/MS conditions.

Table 1. LC method parameters for tier-1 and tier-2 methods.

Parameter	Tier-1 Method	Tier-2 Method
LC System	Agilent 1290 Infinity II LC	Agilent 1290 Infinity II LC
LC Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 30, 2.7 µm (p/n 691775-902)	Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 2.1 × 100, 2.7 µm (p/n 695775-912)
Column Temperature	55 °C	55 °C
Mobile Phase	A) Water B) Methanol	A) Water B) Methanol
Flow Rate	0.5 mL/min	0.5 mL/min
Injection Volume	1 µL	1 µL
Gradient	Time (min) %B 0.0 10 4.0 98	Time (min) %B 0.0 10 6.0 98
Stop Time	4.0 min	6.0 min

Intelligent reflex was configured to monitor tier-1 results in real time and automatically reinject samples under tier-2 conditions when thresholds were exceeded. Combined reporting was enabled to merge tier-1 and tier-2 results into a single output.

Results and discussion

Automated MRM compound optimization reduces setup time and improves consistency

The workflow began with automated compound optimization using MassHunter Optimizer software. This tool systematically scanned precursor and product ion combinations for each forensic analyte, applying multiple collision energies to identify the most sensitive transitions (Figure 1). By automating this process, the method development time was reduced, and variability between analysts was lessened. Optimizer generated a ranked list of transitions and collision energies, which were directly imported into the acquisition method.

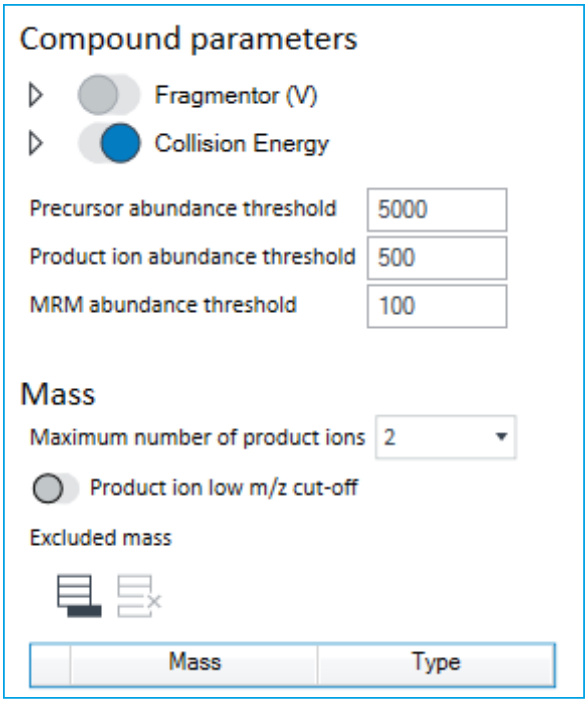


Figure 1. Compound optimizer in Agilent MassHunter software.

Automated source optimization delivers robust spray and signal across the panel

Following compound optimization, MassHunter Source Optimizer was used to refine ionization conditions (Figure 2). The software executed a controlled ramp of key parameters, including gas temperature, sheath gas temperature, and flow rates, across a defined range (Table 2).

Table 2. Source optimization parameters.

Name	Pre-Wait (min)	No. of Replicates	Step Wait (min)	From	To	Step Size
Gas Temperature	0.0	1	15.0	180 °C	290 °C	10 °C
Sheath Gas Temperature	0.0	1	15.0	230 °C	350 °C	10 °C
Gas Flow	0.0	1	0.0	12 L/min	15 L/min	0.5 L/min
Sheath Gas Flow	0.0	1	0.0	10 L/min	12 L/min	0.5 L/min

Each setting was evaluated for signal intensity and stability. The result was a single set of source conditions that supported all compounds in the panel, ensuring robust spray and reproducible response. These optimized parameters are shown in Table 3 and were applied to both tier-1 and tier-2 methods, creating consistency across the workflow.

Table 3. Optimized LC/MS source conditions.

Parameter	Value
Gas Temperature	190 °C
Gas Flow	12 L/min
Nebulizer	33 psig
Sheath Gas Temperature	300 °C
Sheath Gas Flow	10 L/min
Capillary Voltage	2,000 V
Nozzle Voltage	100 V
Detector Gain Factor	+2

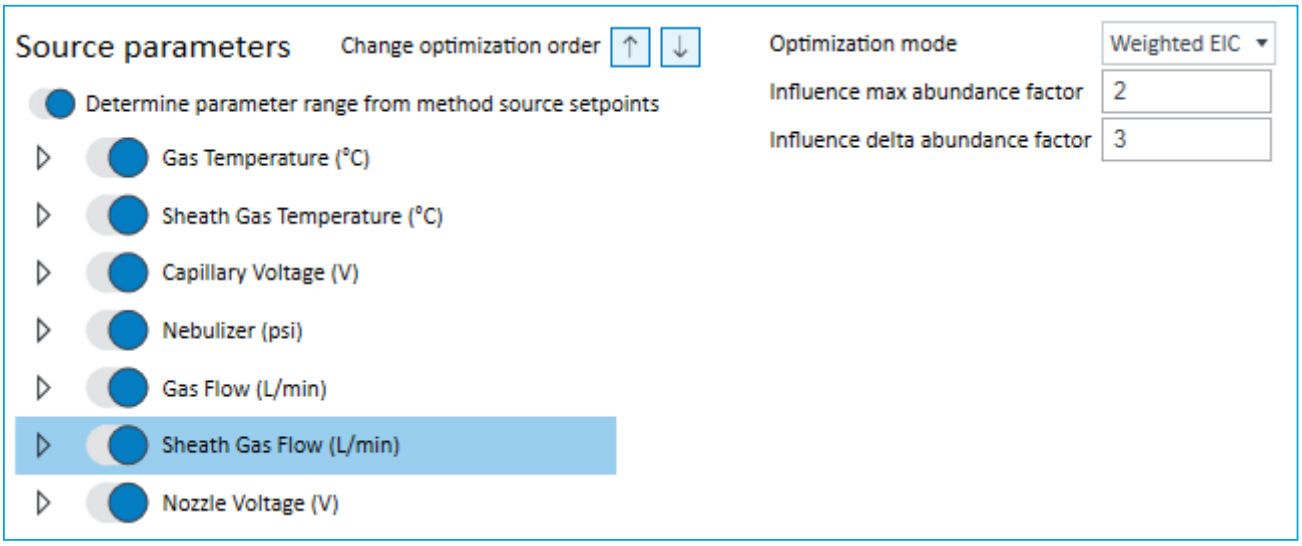


Figure 2. Source optimizer in Agilent MassHunter software.

Orthogonal two-tier chromatography balances speed and selectivity

Tier-1 screening employed a short EC-C18 column and a rapid four-minute gradient to deliver presumptive results quickly. This fast screen minimized instrument time per sample while maintaining adequate separation for initial detection. Tier-2 confirmation used a longer Phenyl-Hexyl column and a six-minute gradient to provide orthogonal selectivity. Both methods shared the same optimized source conditions, reducing complexity and avoiding additional tuning. Figures 3 and 4 present representative chromatograms from each tier, highlighting the orthogonal nature of the methods.

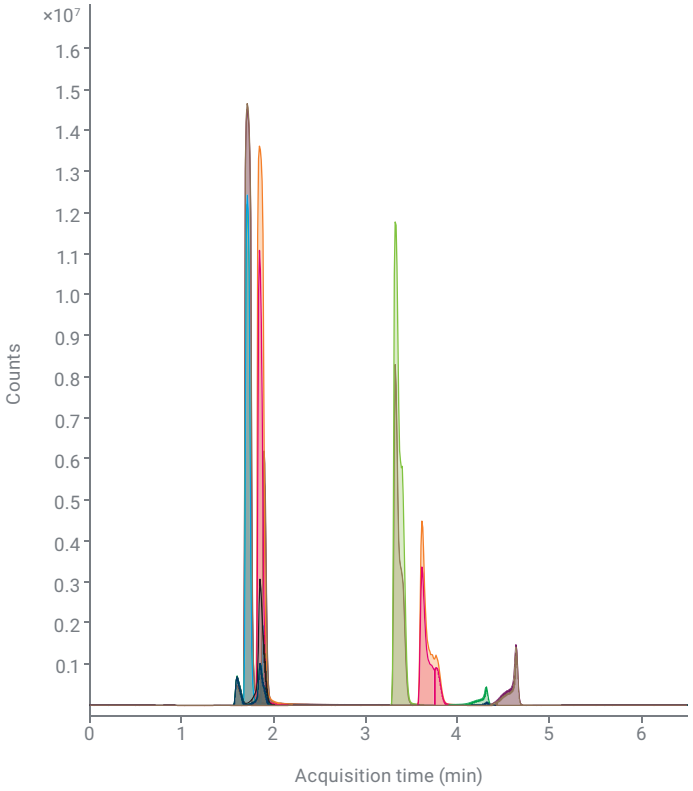


Figure 4. Tier-2 method on an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column.

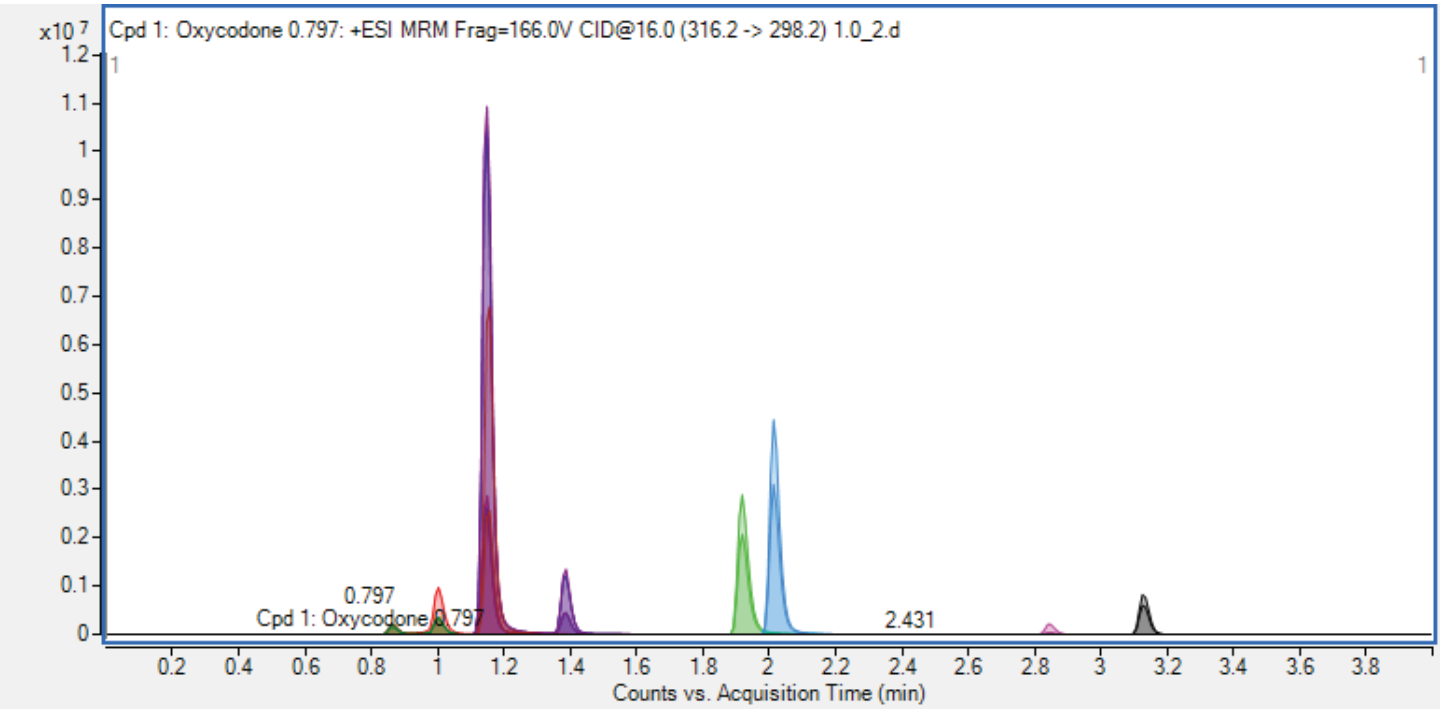


Figure 3. Tier-1 method on an Agilent InfinityLab Poroshell 120 EC-C18 column.

Intelligent reflex automates trigger-to-confirmation decisions

Intelligent reflex was then configured in MassHunter Acquisition (Figure 5). The fast screening workflow monitored tier-1 results in real time. When a compound exceeded its predefined high threshold, the system automatically equilibrated the second column and reinjected the same sample under tier-2 conditions. This reinjection logic, shown in Figure 6, occurred without analyst intervention, and could be scheduled as an insert or append to the worklist.

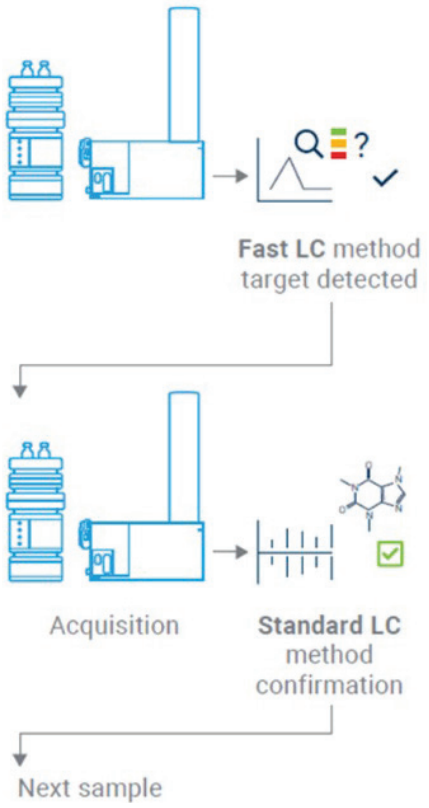


Figure 6. Intelligent reflex fast screening workflow reinjection logic.

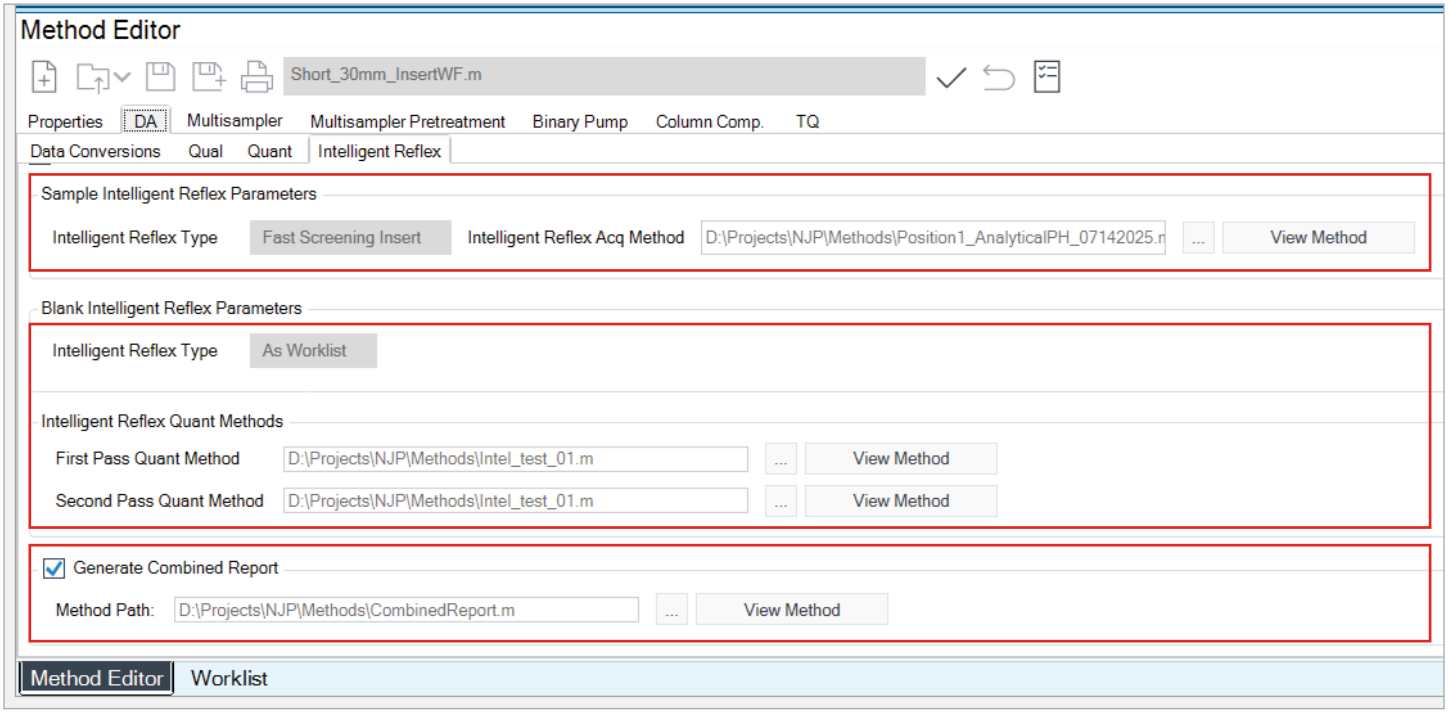


Figure 5. Intelligent reflex setup in Agilent MassHunter Acquisition software.

Figure 7 shows the real-time execution of this process. By removing manual decision points, intelligent reflex reduced turnaround time and eliminated the need for duplicate sample preparation.

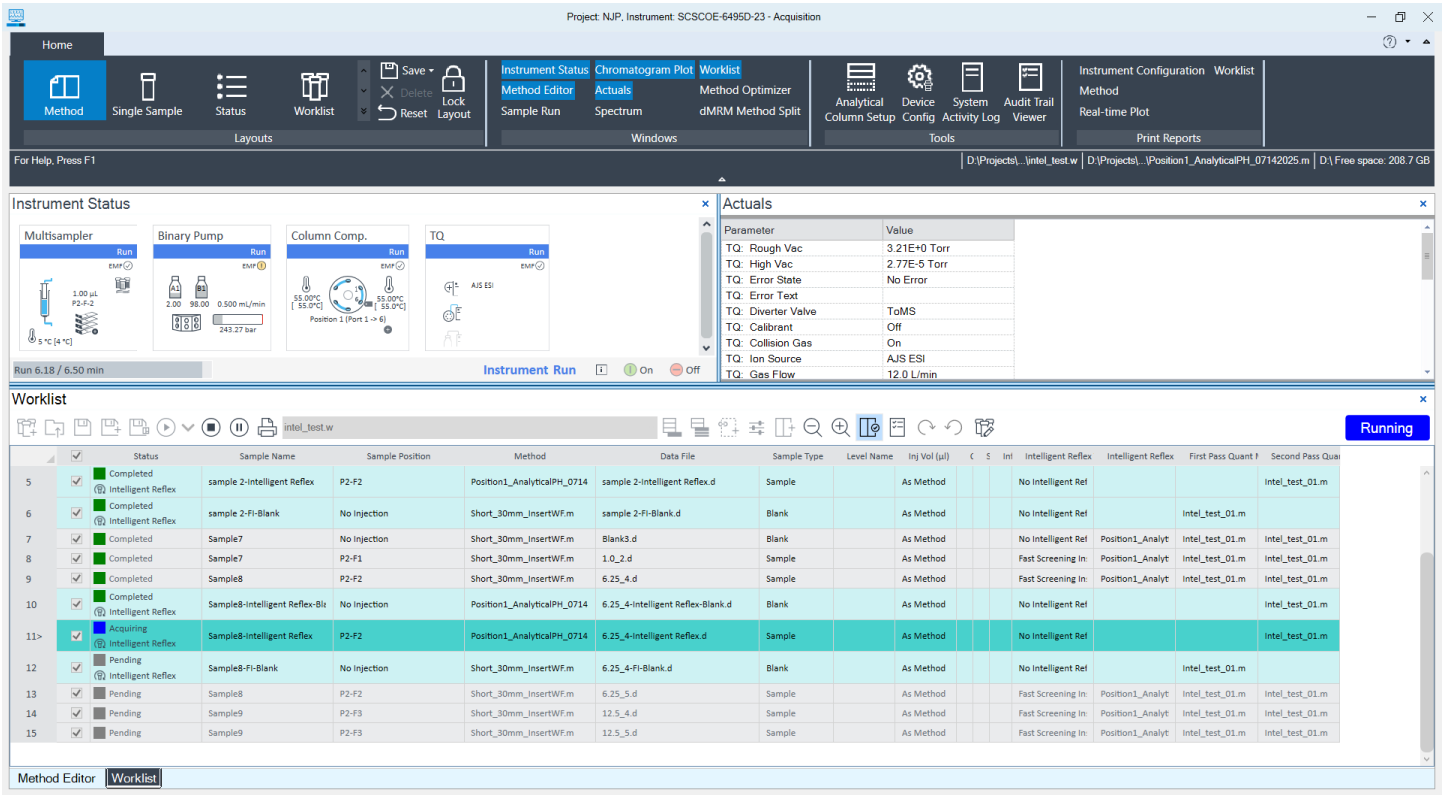


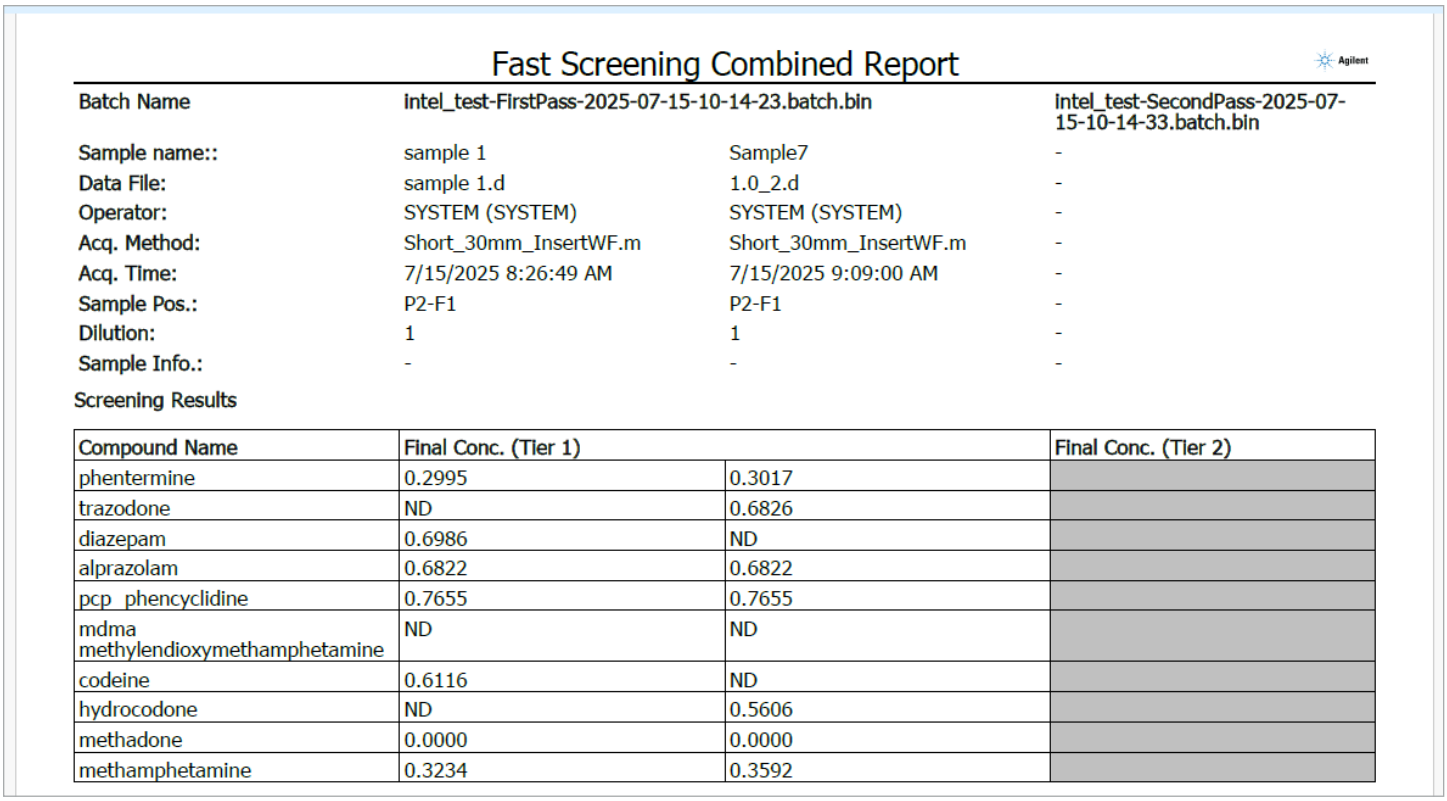
Figure 7. Intelligent reflex running in Agilent MassHunter software.

Data review and combined reporting simplifies workflow

Finally, MassHunter Quantitative Analysis compiled tier-1 and tier-2 results into a combined report. This consolidated output simplified review and supported faster release of results. Figure 8 shows an example layout of the combined report, which aligns screening and confirmation data for each compound in a single view.

Conclusion

Automated, intelligent features in Agilent MassHunter software on the Agilent 6495D LC/TQ shorten method development and make two-tier screening routine. MassHunter Optimizer and Source Optimizer deliver consistent transitions and robust source settings. Intelligent reflex converts tier-1 screen results into automatic tier-2 confirmations and a single combined report—with no manual intervention. The result is faster turnaround, less rework, and higher confidence from orthogonal chromatographic confirmation—using one instrument and one workflow.



Fast Screening Combined Report

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Batch Name	Intel_test-FirstPass-2025-07-15-10-14-23.batch.bin		Intel_test-SecondPass-2025-07-15-10-14-33.batch.bin
Sample name::	sample 1	Sample7	-
Data File:	sample 1.d	1.0_2.d	-
Operator:	SYSTEM (SYSTEM)	SYSTEM (SYSTEM)	-
Acq. Method:	Short_30mm_InsertWF.m	Short_30mm_InsertWF.m	-
Acq. Time:	7/15/2025 8:26:49 AM	7/15/2025 9:09:00 AM	-
Sample Pos.:	P2-F1	P2-F1	-
Dilution:	1	1	-
Sample Info.:	-	-	-

Screening Results

Compound Name	Final Conc. (Tier 1)		Final Conc. (Tier 2)
phentermine	0.2995	0.3017	
trazodone	ND	0.6826	
diazepam	0.6986	ND	
alprazolam	0.6822	0.6822	
pcp phencyclidine	0.7655	0.7655	
mdma methylendioxymethamphetamine	ND	ND	
codeine	0.6116	ND	
hydrocodone	ND	0.5606	
methadone	0.0000	0.0000	
methamphetamine	0.3234	0.3592	

Figure 8. Combined report showing both tier-1 and tier-2 results.

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