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Multi-residue Method Development Using Agilent 6475 LC/TQ System Implemented with Intelligent Optimization Software: Application to Forensic Drug Screening

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Introduction

Developing Multiple Reaction Monitoring (MRM)-based screening methods for routine analysis is a common operation in analytical laboratories. To maximize analytical performance, researchers often need to optimize many LC and MS parameters. An intelligent software could assist method development from several aspects, such as cutting method development time, automating workflow, auto-selecting optimal parameters, tracking system to meet compliance requirement, etc.

Agilent developed a new triple quadrupole LC/MS system, 6475 LC/TQ, which contains software improvements to facilitate MRM-based method optimization workflow (Figure 1).

In this study, we demonstrated the new optimizer on developing multi-forensic drug screening methods and compared the final method to the one optimized using a previous system. Our results showed significant improvements on the software.

Experimental

Instrumentation

- 1290 Bio High-Speed Pump (G7132A)
- 1290 Bio Multisampler with Sample Cooler (G7137A)
- 1290 Multicolumn Thermostat (G7116B)
- 6475 Triple Quadrupole LC/MS (G6475A) with Jet Steam electrospray ionization source (G1958-65638)

Samples

All the forensic drug reference standards were purchased from Cerilliant.

Two types of standard solutions were prepared: single drug solution for all the used reference standards and a simple drug standard mixture contain 4 pairs of isobaric drugs and 4 drug standards which have unique mass (mass difference > 1 Da) (Table 1).

Compound name	Monoisotopic mass (Da)	Compound name	Monoisotopic mass (Da)
Methylphenidate	233.1	Codeine	299.2
Normeperidine	233.1	Hydrocodone	299.2
Hydromorphone	285.1	Alprazolam	308.1
Morphine	285.1	(±)-Methadone	309.2
Desalkylflurazepam	288	6-Acetylmorphine	327.1
Buprenorphine	467.3	Naloxone	327.1

Table 1. Compound names and masses in the mixture.

Experimental

Figure 1. 6475 Triple Quadrupole LC/MS with 1290 Infinity II Bio LC system

LC/MS analysis

All reference drug standards were optimized twice using two different injection methods: injection with column as Table 2 & 3 for complex mixture; automatic infusion using loop injection for single drug solution.

Agilent MassHunter Workstation for LC/TQ 12.0 was used for data acquisition and report.

1290 Infinity II Bio LC System		
Column	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm at 40 °C (p/n 695775-902)	
Sampler temp.	4 °C	
Mobile phase	A) 5 mM ammonium formate + 0.1% formic acid in water B) 5 mM ammonium formate + 0.1% formic acid in methanol	
Flow rate	0.500 mL/min	
Gradient program	Time	B (%)
	0.00	10
	0.50	15
	3.00	50
	4.00	95
	6.00	95
Post time	1 minutes	

Table 2. 1290 Infinity II Bio LC Method

6475 Triple Quadrupole Mass Spectrometer	
Ion source	Agilent Jet Stream (AJS) source
Polarity	Positive and Negative
Gas temperature	200 °C
Drying gas	11 L/min
Nebulizer gas	30 psi
Sheath gas	350 °C
Sheath gas flow	12 L/min
Capillary voltage	2000 ±V
Nozzle voltage	0 ±V
Q1/Q2 Resolution	Unit (0.7 amu)
Dwell time	5 ms

Table 2. 6475 Triple Quadrupole LC/MS Method

Intelligent optimizer software

The new optimizer software supports compound optimization by three types of algorithms, which match to three different injection approaches including injection with column, manual infusion and automatic infusion using loop injection. The workflow contains improvements on several aspects (Figure 2):

- Optimizing compounds and ion source in a seamless workflow
- Distinguishing chromatography-separated compounds that sharing the same precursor mass
- Supporting weighted compound signals for source optimization so that the final method favors less-responding compounds in a mixture
- Reducing sample consumption and instrument run time by 17%~80%
- Queue managing multiple optimization methods in automated workflow
- Exporting ready-to-run final method and PDF report
- Taking controls of data integrity and compliance

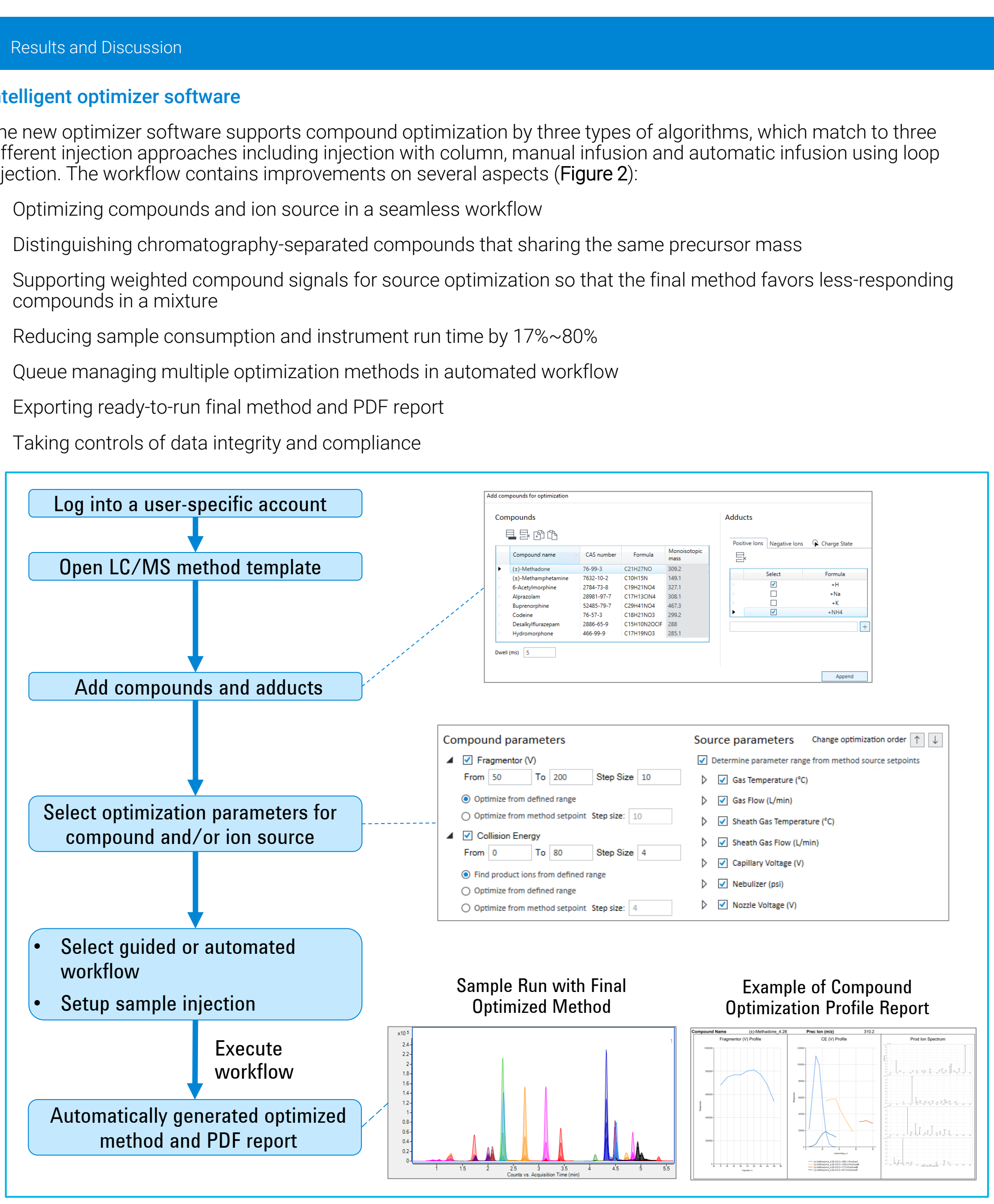

Figure 2. Overview of the new optimizer workflow.

Results and Discussion

Compound optimization

In real world analysis, there is a need to separate isobaric compounds or background interference when performing optimization with compound mixtures. The new algorithm in compound optimizer for injection with column is suitable for such scenarios. The 12-drugs mixture was used to demonstrate the new algorithm. The workflow covering fragmentor optimization, finding product ion and collision energy optimization was carried out for both positive (+H) and negative (-H) adducts. All the forensic drugs were also optimized using their corresponding single analyte solution as a gold standard to exam result accuracy.[1,2] The sample run with the final exported method was shown in Figure 3:

- A total of 15 analyte peaks were found correctly matching to all the chromatography-separated 12 drugs and 3 unknown background interferences, which were labeled with their corresponding retention times.
- The MRM transitions for each detected peak were optimized independently

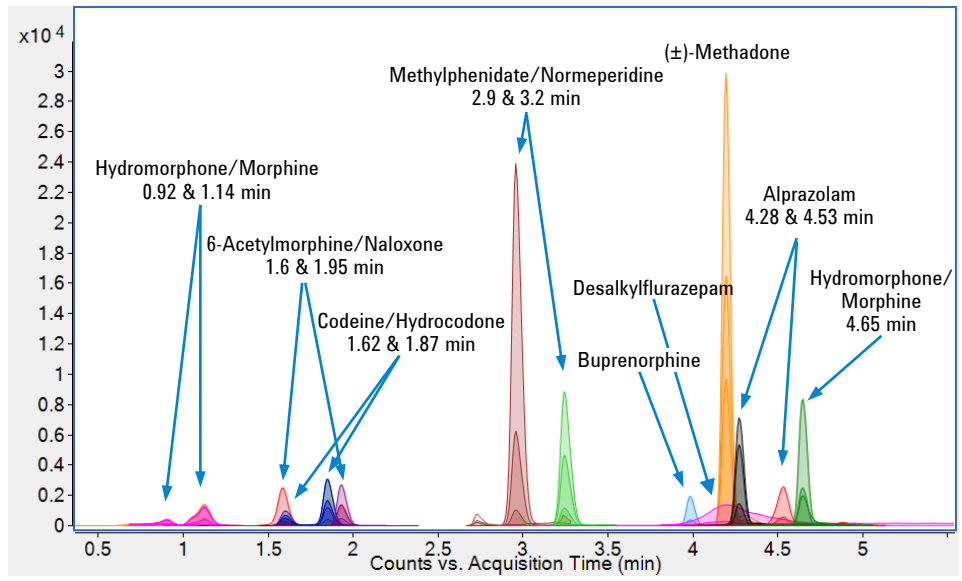

Figure 3. Chromatograms of the 12-drug mix using the automatically optimized method.

Ion source optimization

The new optimizer supports source optimization using weighted compound signals so that the final optimized parameters are in favor of less-responding compounds in a complex mixture. Figure 4 shows an example of optimization curves for one source parameter using a 68-drugs mixture. The apex of optimization curve using weighted signal is not always the same as the one using average signal.

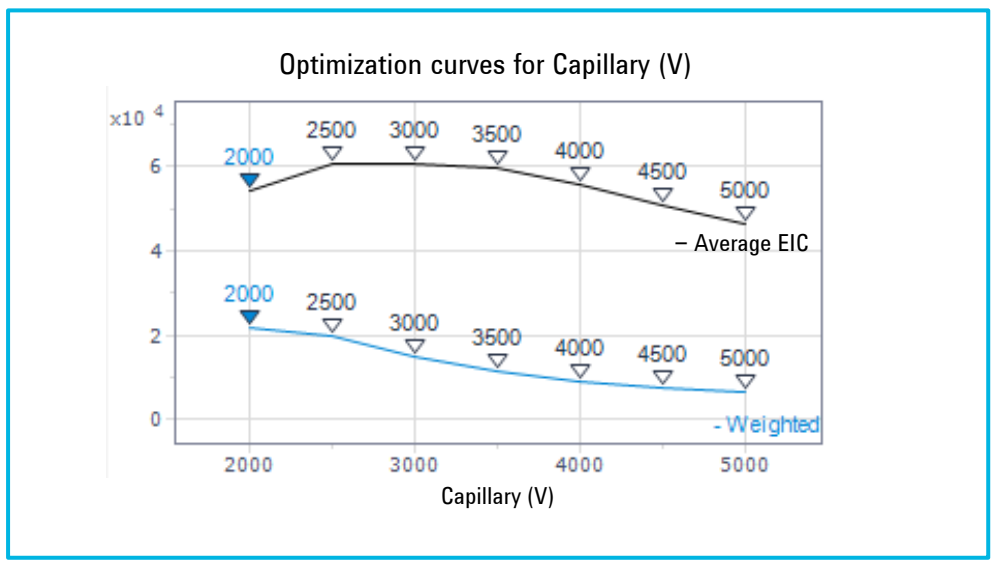

Figure 4. Optimization curves for capillary voltage using average EIC and weighted average EIC.

Data Integrity and Compliance

The new system allows users to take control of data integrity and compliance (Figure 5):

- Assign roles to provide unique levels of access for each user
- Protect records
- Ensure end-to-end work attribution
- Document audit trail reviews

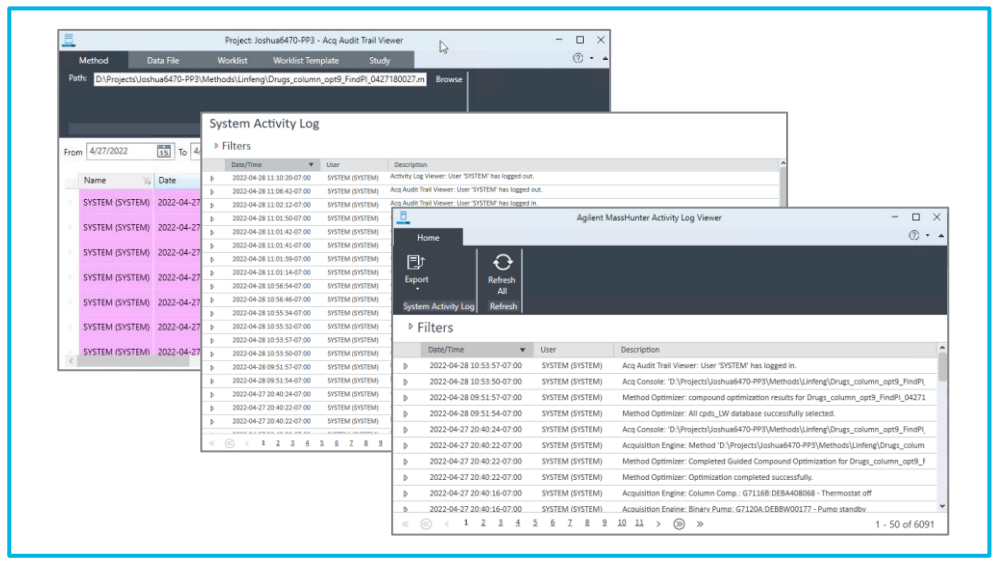

Figure 5. Screenshots of acquisition audit trail viewer and activity log viewers.

Conclusions

MRM-based multiple-residue LC/MS method development for forensic drug quantitation using the new optimizer software on the Agilent 6475 LC/TQ demonstrated several improvements on the new acquisition software.

https://explore.agilent.com/asms

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References

[1]Comprehensive LC/MS Analysis of Opiates, Opioids, Benzodiazepines, Amphetamines, Illicits, and Metabolites in Urine. Agilent Publication Part Number: 5991-1667EN

[2]Agilent Ultivo ESI for High-Throughput Detection of Drugs in Urine and Serum. Agilent Publication Part Number: 5994-1545EN

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