Technical Overview

Forensic Toxicology

Robustness of the Agilent Ultivo Triple Quadrupole LC/MS with Standard ESI Ion Source for High-Throughput Testing of Drugs in Serum

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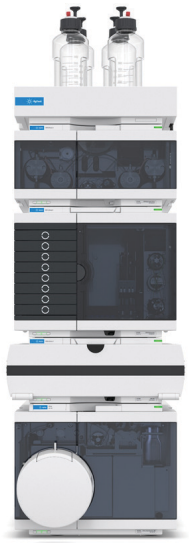

Figure 1. Agilent Ultivo LC/TQ with ESI source.

Abstract

This Technical Overview highlights the robustness of the Agilent Ultivo triple quadrupole LC/MS system (LC/TQ) with a standard ESI source coupled to an Agilent 1290 Infinity II LC for the detection of 26 drugs in human serum. Excellent reproducibility was observed for a six-day continuous run analyzing 1,625 individual injections. Observed RSD% for both raw peak area and calculated concentration of 1,400 QC samples averaged 4.3 and 4.4 %, respectively, for the 26 analytes. The exceptional robustness of Ultivo, along with its small size and reduced downtime for maintenance makes it an excellent tool for the high-throughput forensic toxicology laboratory.

Introduction

The field of forensic toxicology requires sensitive, robust, and reliable methods for routine testing of drugs in human serum. Drugs of interest must be accurately quantified in analytical worklists lasting up to several days. Ultivo has many features that benefit a high-throughput laboratory environment, such as early maintenance feedback (EMF) VacShield technology enabling the quick change of a capillary, and a small, stackable design. Ultivo has already proven to be robust and reliable for food testing[1], but this generation of Ultivo includes a standard ESI configuration, which can be valuable in a high-throughput forensic toxicology setting. The robustness of the ESI source coupled to the Ultivo LC/TQ was evaluated for 26 drugs in a human serum matrix.

Experimental

Reagents and chemicals

All reagents used were HPLC or LC/MS grade. Acetonitrile was purchased from Honeywell (Morristown, NJ, USA), and ultrapure water was sourced from a Milli-Q Integral system with an LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Drug standards were purchased from Cerilliant. Serum was obtained from Golden West Biologicals (Temecula, CA, USA).

Sample preparation

Serum for post spiking analytes was prepared by taking 250 µL of human serum and crashing it with 500 µL of cold acetonitrile, and vortexing for one minute[2]. The serum was then centrifuged for four minutes at 10,000 rpm, and 500 µL of serum supernatant was diluted with 500 µL of water[2]. Prepared serum was spiked with a stock solution of 26 drugs to a final concentration of 10 ng/mL QC standard. Calibration standards of 2, 5, 10, 20, and 50 ng/mL were prepared in series with prepared serum.

Instrumentation

Agilent 1290 Infinity II LC

- 1290 Infinity II high speed pump (G7120A)
- 1290 Infinity II multisampler with cooler (G7167B)
- 1290 Infinity II multicolumn thermostat (G7116B)

Agilent Ultivo triple quadrupole LC/MS system

- Electrospray ionization source (G1948B)

Method

Table 1 summarizes the 1290 Infinity II LC conditions, and Table 2 summarizes Ultivo ion source and instrument parameters. Dynamic multiple reaction monitoring (dMRM) was used for data collection. MS/MS transitions (Table 3) were either taken from the Agilent Forensic Toxicology database or optimized directly on Ultivo. Agilent MassHunter Quantitative Analysis software B.09 with the Quant-My-Way feature was used to accelerate and streamline the data analysis and review process.

Table 1. Agilent 1290 Infinity II LC parameters.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm
Column Temperature	50 °C
Injection Volume	1 µL
Mobile Phase	A) 0.1 % formic acid in water B) 0.1 % formic acid in acetonitrile
Flow Rate	0.4 mL/min
Gradient	Time (min) %B 0 5 0.5 35 1.0 45 2.0 60 2.5 100 3.5 100
Stop Time	3.5 minutes
Post Time	1.0 minute

Table 2. Ultivo ion source and mass analyzer parameters.

Parameter	Value
Gas Temperature	325 °C
Gas Flow	11 L/min
Nebulizer Pressure	60 psi
Capillary Voltage	4,000 V (+), 4,000 V (-)
Cycle Time	500 ms

Table 3. List of transitions and MS parameters used for the 26 drugs analyzed in this study.

Compound	Precursor (*m/z*)	Product (*m/z*)	RT (min)	RT Window (min)	Fragmentor (V)	CE (V)	Average Dwell (ms)	Polarity
Morphine	286.2	165	0.41	0.68	141	35	108.0	Positive
Morphine	286.2	153	0.41	0.68	141	45	108.0	Positive
6-Acetylmorphine	328.2	211	1.11	0.91	194	24	18.7	Positive
6-Acetylmorphine	328.2	165	1.11	0.91	194	44	18.7	Positive
Methamphetamine	150.1	119.1	1.15	1.14	75	8	20.5	Positive
Methamphetamine	150.1	91.1	1.15	1.14	75	20	20.5	Positive
MDMA	194.1	163.1	1.16	1.14	80	8	19.9	Positive
MDMA	194.1	105.1	1.16	1.14	80	24	19.9	Positive
Atropine	290.2	124.1	1.21	1.14	171	24	17.7	Positive
Atropine	290.2	93.1	1.21	1.14	171	32	17.7	Positive
Benzoylecgonine	290.1	168	1.23	1.01	123	16	16.6	Positive
Benzoylecgonine	290.1	77	1.23	1.01	123	60	16.6	Positive
Ketamine	238.1	179.1	1.23	1.12	103	12	16.9	Positive
Ketamine	238.1	125	1.23	1.12	103	28	16.9	Positive
Norfentanyl	233.2	84.1	1.23	1.09	123	16	16.9	Positive
Norfentanyl	233.2	55.2	1.23	1.09	123	40	16.9	Positive
Cocaine	304.2	182.1	1.36	1.12	113	16	14.9	Positive
Cocaine	304.2	82	1.36	1.12	113	48	14.9	Positive
Clozapine	327.1	270.1	1.45	1.14	141	20	14.1	Positive
Clozapine	327.1	192.1	1.45	1.14	141	44	14.1	Positive
Alfentanil	417.3	268.1	1.53	0.99	128	16	12.4	Positive
Alfentanil	417.3	197	1.53	0.99	128	24	12.4	Positive
Fentanyl	337.2	188	1.55	1.09	146	24	13.3	Positive
Fentanyl	337.2	105	1.55	1.09	146	40	13.3	Positive
Buprenorphine	468.3	414.2	1.62	1.03	199	40	12.8	Positive
Buprenorphine	468.3	55.2	1.62	1.03	199	60	12.8	Positive
Promethazine	285.1	86.1	1.67	1.14	118	16	13.3	Positive
Promethazine	285.1	71.1	1.67	1.14	118	48	13.3	Positive
Protriptyline	264.2	191	1.73	1.29	130	32	14.6	Positive
Protriptyline	264.2	155	1.73	1.29	130	20	14.6	Positive
Nortriptyline	264.2	105.1	1.76	1.32	110	24	15.1	Positive
Nortriptyline	264.2	91.1	1.76	1.32	110	28	15.1	Positive
Maprotiline	278.2	250.1	1.78	1.14	140	20	14.1	Positive
Maprotiline	278.2	191	1.78	1.14	140	40	14.1	Positive
Amitriptyline	278.2	105.1	1.80	1.19	120	28	14.8	Positive
Amitriptyline	278.2	91.1	1.80	1.19	120	28	14.8	Positive
Oxazepam	287.1	269.1	1.85	1.07	131	12	14.7	Positive
Oxazepam	287.1	241.1	1.85	1.07	131	20	14.7	Positive
Chlorpromazine	319.1	86.1	1.89	1.07	150	20	15.3	Positive
Chlorpromazine	319.1	58.1	1.89	1.07	150	40	15.3	Positive
Alprazolam	309.1	281.1	1.92	0.81	156	40	14.8	Positive
Alprazolam	309.1	240	1.92	0.81	156	40	14.8	Positive
Clonazepam	316.1	270.1	1.92	1.14	214	24	18.3	Positive
Clonazepam	316.1	241	1.92	1.14	214	32	18.3	Positive
Triazolam	343.1	308.1	1.96	1.12	176	24	20.3	Positive
Triazolam	343.1	239	1.96	1.12	176	44	20.3	Positive
Temazepam	301.1	283.1	2.10	0.96	123	8	29.1	Positive
Temazepam	301.1	255.1	2.10	0.96	123	16	29.1	Positive
Diazepam	285.1	193.1	2.30	1.13	166	32	63.5	Positive
Diazepam	285.1	154.1	2.30	1.13	166	24	63.5	Positive
Δ^9-THC	343.2	299.2	3.08	0.8	190	20	218.2	Negative
Δ^9-THC	343.2	245.2	3.08	0.8	190	32	218.2	Negative

Results and discussion

Peak area and quantitative robustness

The stability of the Ultivo system with ESI source was evaluated for 1,400 injections of a QC standard of 27 drugs at 10 ng/mL spiked into human serum. The raw area signal responses of seven selected MRM transitions at 10 ng/mL are plotted over the 1,400 QC injections in Figure 3, demonstrating the excellent signal stability of Ultivo with the ESI source over six days of continuous, uninterrupted run time.

Calibration curves of 2, 5, 10, 20, and 50 ng/mL were run in triplicate after every 100 QC injections to imitate the type of analysis and stability needed in a high-throughput forensic toxicology laboratory setting, where laboratory technicians will run multiple calibration curves over the course of a multiday run to ensure data integrity. This resulted in a total of 1,625 injections for this experiment. The RSD% of the 1,400 QC samples were evaluated both with the raw area MRM and the calculated concentration based on the periodic calibration curves during the six-day run. The average RSD% for all compounds evaluated in this study are nearly identical between raw area and calculated concentration (Table 4).

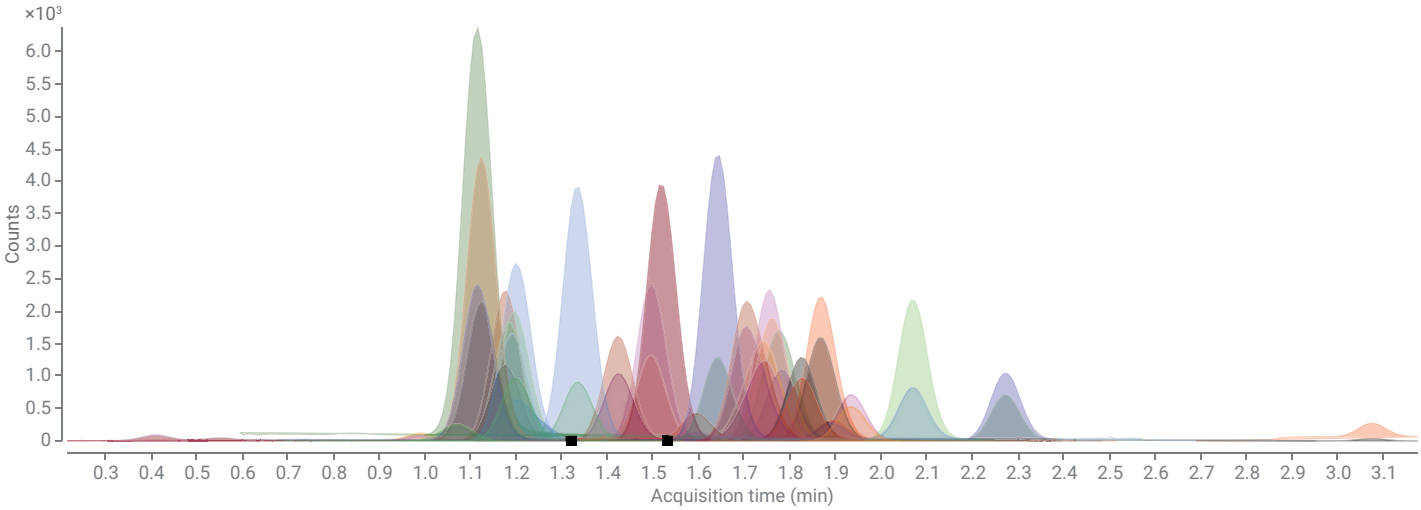

Figure 2. Chromatogram of 26 drugs in serum at a 10 ng/mL.

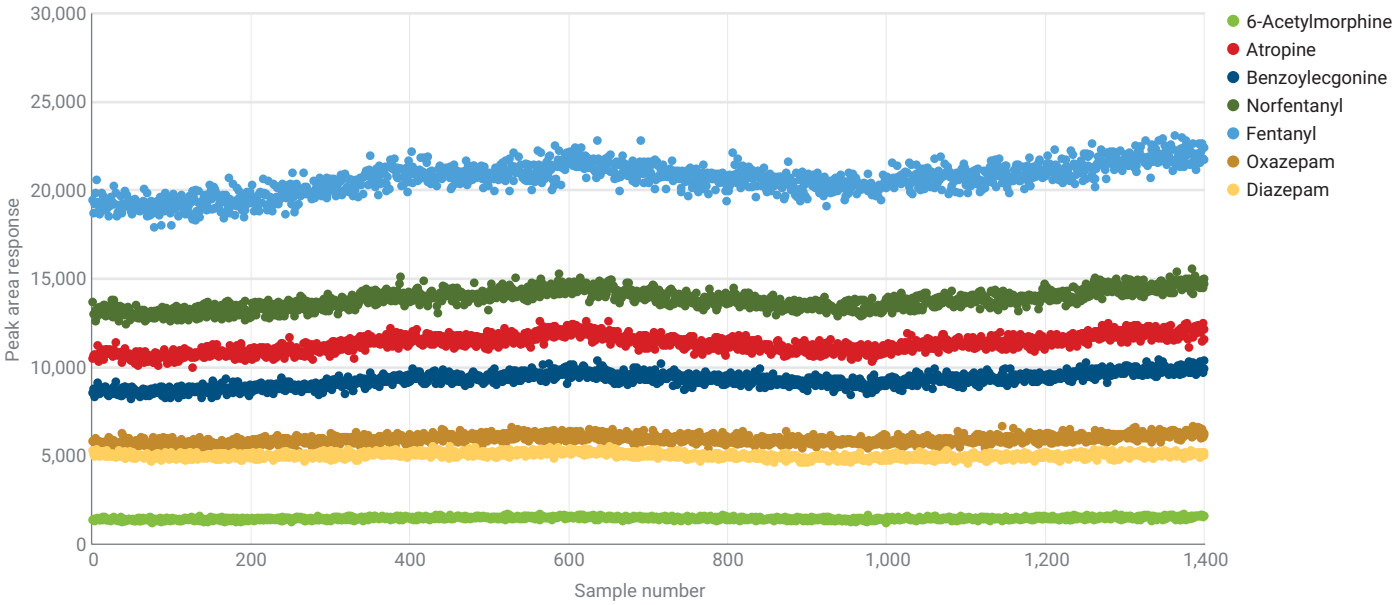

Figure 3. Peak area stability of the primary MRM transition for seven selected compounds over 1,400 injections.

Table 4. Reproducibility of peak area and calculated concentration of 1,400 injections over six days.

	Raw Area RSD (%) n = 1400	Calculated Concentration RSD (%) n = 1400
Morphine	6.9	5.3
6-Acetylmorphine	5.6	6.0
Methamphetamine	3.4	3.5
MDMA	3.6	3.7
Atropine	4.2	3.7
Benzoylecgonine	4.4	3.9
Ketamine	3.8	3.7
Norfentanyl	3.9	3.9
Cocaine	3.6	3.7
Clozapine	4.3	4.5
Alfentanil	3.6	4.0
Fentanyl	4.3	3.7
Buprenorphine	5.7	6.4
Promethazine	3.4	4.1

	Raw Area RSD (%) n = 1400	Calculated Concentration RSD (%) n = 1400
Protriptyline	3.6	3.6
Nortriptyline	3.9	4.1
Maprotiline	3.5	3.9
Amitriptyline	3.9	4.1
Oxazepam	4.0	4.1
Chlorpromazine	3.6	4.1
Clonazepam	7.1	7.3
Alprazolam	5.8	5.8
Triazolam	4.0	4.3
Temazepam	3.0	3.2
Diazepam	3.1	3.8
Δ^9-THC*	5.0	5.0
Average	**4.3**	**4.4**

* Reproducibility for Δ^9-THC calculated over 600 samples – significant analyte degradation was observed after three days of analysis.

Linearity and calibration curve stability

Figure 4 shows the exceptional stability of the calibration curves over the 1,625 injections during this experiment, where calibrations from the beginning, middle, and end of the six-day run are presented overlaid for three representative compounds. All calibration curves had R^2 values of 0.99 or greater for all 15 calibration curves for each of the 26 compounds studied.

Conclusions

This Technical Overview demonstrates that the Ultivo triple quadrupole LC/MS, with standard ESI source and a 1290 Infinity II LC, is a robust and reliable analysis tool for the high-throughput forensic laboratory. Over six days of acquisition and 1,625 samples, the Ultivo exhibited low peak area variation and exceptional accuracy in quantitation for 26 drugs in human serum.

References

1. Sartain, M.; Sosienski, T.; Yang, D. Robustness of the Agilent Ultivo Triple Quadrupole LC/MS for Routine Analysis in Food Safety, *Agilent Technologies Technical Overview*, publication number 5991-8741EN, **2017**.
2. Grossman, J. N.; Yang, Y. Analytical Determination of Drugs in Serum Using the Ultivo Triple Quadrupole LC/MS, *Agilent Technologies Application Note*, publication number 5994-0056EN, **2019**.

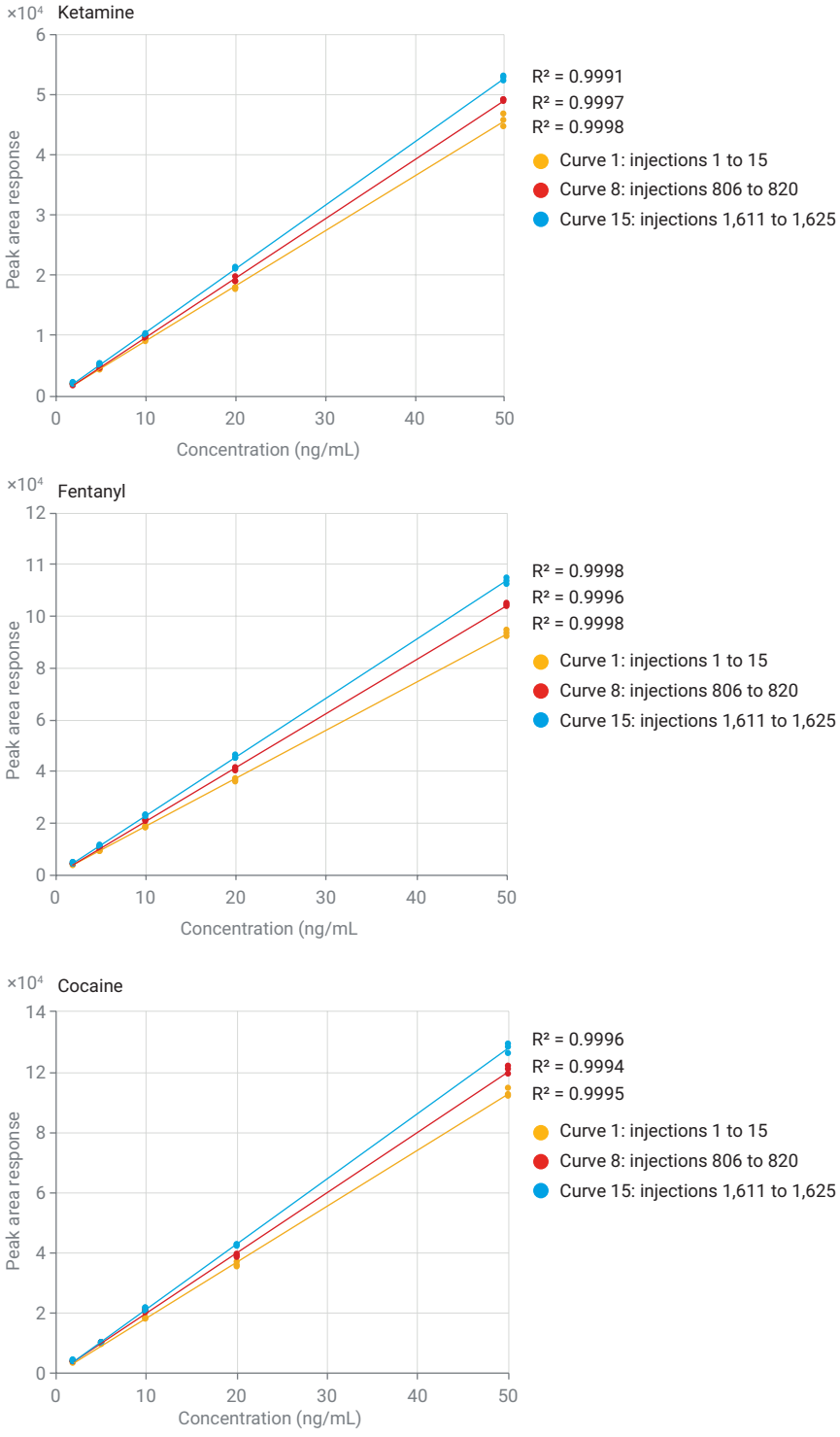

Figure 4. An overlay of calibration curve 1 (injections 1 to 15), calibration curve 8 (injections 806 to 820), and calibration curve 15 (injections 1,611 to 1,625) for three representative compounds analyzed in this study demonstrating the calibration curve integrity over the course of this experiment. Each calibration point was run in triplicate.

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