Rapid LC/MS/MS Detection of Opiates, Opioids, Benzodiazepines, Amphetamines, and Cannabinoids in Urine

Lauren E. Frick and Carrie J. Adler
Agilent Technologies, Inc
Lexington, MA

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Introduction

Liquid chromatography-mass spectrometry (LC/MS/MS) offers a sensitive method of determining the presence or absence of exogenous compounds in biological samples. It is generally more sensitive and more specific than other techniques used to achieve this goal. Wide adoption of LC/MS/MS for this purpose has been inhibited by the length of typical analytical methods looking at large panels of compounds. Here, an anlytical method has been developed to assess for the presence of 36 compounds representing several different classes and concentrations in a total method runtime (injection to injection) of 2.3 minutes.

A single calibrator, a positive sample (1.5 x calibrator concentration), and a negative sample (0.25 x calibrator concentration) were created by spiking drug standards into clean human urine. Blanks (solvent and matrix), the calibrator, samples, and controls were prepared for analysis through a simple dilution into water containing internal standards. Injection, separation of analytes, column cleaning, and column reequilibration were accomplished 2.3 minutes. One transition was monitored for each compound of interest, and 6 isotopically labeled internal standards were included to account for differential suppression across the chromatogram.

Calibration used a single concentration for each compound. An Average of Response Factors was used to generate a calibration equation. Samples were considered positive if they quantified above the calibrator and negative if they quantified below. Sample sets were prepared over three days (in triplicate once and in singleton twice) to ensure reproducibility.

Experimental

Sample Prep

Clean human urine was spiked with standards (Cerilliant) of the 36 compounds to achieve the calibration sample, the negative sample, and the positive sample. Diluent was created by spiking water with 6 deuterated internal standard compounds (Cerilliant) to concentrations of 2.5 ng/mL (oxycodone, methamphetamine, and fentanyl) or 10 ng/mL (morphine, oxazepam, and THC-COOH). 50 µL of sample, blank, or control (UTAK) were mixed with 950 µL of water (double blanks) or diluent (single blanks, samples, and controls). After being vortexed for 30 seconds, 5 µL were injected on to the LC/MS system.

LC/MS/MS Analytical Method

The LC/MS/MS system consisted of a 1290 binary pump, a thermostatted multisampler, a temperature controlled column compartment and a 6470 triple quadrupole mass spectrometer. Conditions used for the compound separation and column cleanup/reequilibration are given in Tables 1 and 2.

Table 1: LC Parameters

Analytical Column	Agilent Poroshell 120 EC-C18, 2.1 x 50 mm, 2.7 µm
Injection Volume	5 µL
Mobile Phase A	H_2O + 5 mM ammonium formate + 0.01% formic acid
Mobile Phase B	Methanol + 0.01% formic acid
Needle Wash	25:25:50 Isopropanol:Methanol:Water
Multisampler Temp	4 °C
Column Temp	55 °C
Flow Rate	0.9 mL/min
Stop Time	1.9 min
Post Time	Off
Overlapped Injection	On, at 1.8 min
Time Filtering	0.026
Divert to Waste	0 to 0.25 min

Table 2: Gradient

Time (min)	%B
0	20
0.9	98
1.4	98
1.41	20

The 6470 triple quadrupole mass spectrometer was used to detect the 36 compounds in dynamic MRM mode. Compromise MS source conditions were as in Table 3 and dMRM acquisition parameters as in Table 4. Positive/negative switching was utilized to monitor compounds of both polarities in a single injection. Unit resolution was used in both MS1 and MS2. The total cycle time was ~2.3 minutes injection to injection. Data were acquired with MassHunter Acquisition B.08.02 and analyzed with MassHunter Quantitative Analysis B.08.00 and Qualitative Analysis B.07.00.

Table 3: 6470 Agilent JetStream ESI Source Parameters

	Positive Mode	Negative Mode	Units
Gas Temp	325	325	°C
Gas Flow	9	9	L/min
Nebulizer Pressure	30	30	psi
Sheath Gas Temp	380	380	°C
Sheath Gas Flow	11	11	L/min
Capillary Voltage	3750	3500	V
Nozzle Voltage	0	1500	V
Delta EMV	0	600	V

Experimental

Table 4: MS transitions and dMRM acquisition details.

Compound Name	ISTD?	Precursor Ion	Product Ion	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	CAV	Polarity
6-MAM		328.2	165	0.53	0.5	132	44	2	Positive
7-Aminoclonazepam		286.1	121	0.77	0.5	107	32	4	Positive
Alprazolam		309.1	281	1.09	0.5	137	28	2	Positive
Amphetamine		136.1	91.1	0.63	0.5	82	16	2	Positive
Benzoylecgonine		290.1	168	0.7	0.5	82	16	2	Positive
Buprenorphine		468.3	55.2	1.1	0.4	167	64	2	Positive
Codeine		300.2	152	0.46	0.5	122	80	2	Positive
Diazepam		285.1	193	1.15	0.4	117	32	4	Positive
EDDP		278.2	234	0.95	0.46	132	32	4	Positive
Fentanyl		337.2	105.1	0.95	0.44	102	44	2	Positive
Fentanyl D5	✓	342.3	105.1	0.95	0.5	112	44	2	Positive
Flunitrazepm		314.1	268	1.04	0.5	157	24	2	Positive
Hydrocodone		300.2	199	0.53	0.5	152	28	2	Positive
Hydromorphone		286.2	185	0.35	0.5	132	28	2	Positive
JWH-018		342.2	214.1	1.35	0.4	100	24	4	Positive
JWH-073		328.2	200.1	1.3	0.4	100	24	4	Positive
Lorazepam		321.1	274.9	1.1	0.4	87	20	2	Positive
MDA		180.1	163	0.62	0.5	83	8	2	Positive
MDEA		208.1	163	0.68	0.5	102	8	2	Positive
MDMA		194.1	163	0.63	0.5	97	8	2	Positive
Methadone		310.2	265.1	1.05	0.4	62	12	2	Positive
Methamphetamine		150.1	91.1	0.64	0.5	82	16	2	Positive
Methamphetamine D5	✓	155.1	92.1	0.64	0.5	82	16	2	Positive
Midazolam		326.1	291	1.11	0.5	142	24	4	Positive
Morphine		286.2	152	0.3	0.5	122	68	2	Positive
Morphine D6	✓	292.2	152	0.3	0.5	122	72	2	Positive
Naloxone		328.2	310.1	0.45	0.5	97	16	2	Positive
Nitrazepam		282.1	236	1.03	0.5	102	24	2	Positive
Norbuprenorphine		414.3	55.2	0.89	0.5	157	80	2	Positive
Nordiazepam		271.1	140	1.15	0.4	112	28	2	Positive
Norfentanyl		233.2	84.2	0.75	0.5	67	16	2	Positive
Oxazepam		287.1	241	1.05	0.4	87	20	4	Positive
Oxazepam D5	✓	292.1	246	1.05	0.4	87	20	4	Positive
Oxycodone		316.2	298.1	0.5	0.5	97	16	2	Positive
Oxycodone D6	✓	322.2	304.1	0.5	0.5	100	18	2	Positive
Oxymorphone		302.1	284	0.33	0.5	97	16	2	Positive
PCP		244.2	86.1	0.9	0.43	86	9	7	Positive
Temazepam		301.1	255	1.1	0.4	82	20	2	Positive
THC-COOH		343.2	298.8	1.33	0.4	140	20	2	Negative
THC-COOH D9	✓	352.2	307.9	1.33	0.4	136	20	4	Negative
Zolpidem		308.2	235.1	0.88	0.5	156	37	4	Positive
Zopiclone		389.1	245	0.76	0.5	82	13	4	Positive

Results and Discussion

Chromatography

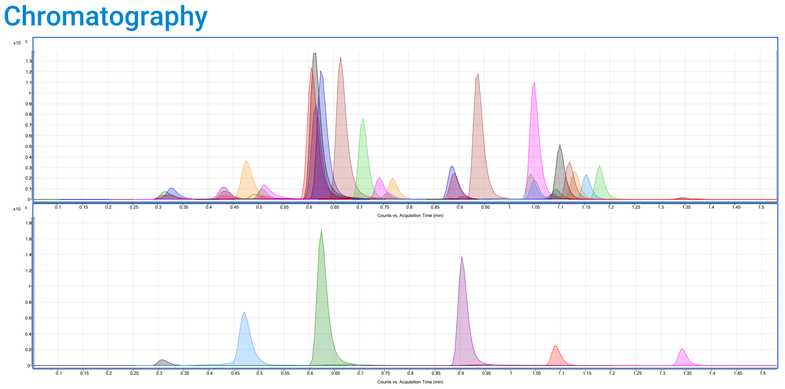

Figure 1: Example dMRM chromatograms showing elution of the 36 compounds (top) and 6 internal standards (bottom).

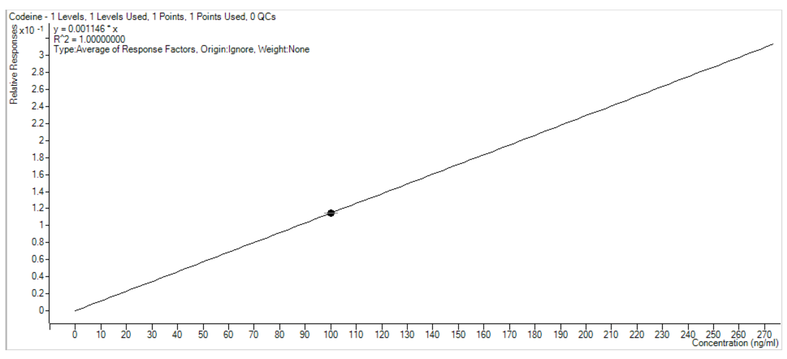

Figure 2: Example single-point calibration curve

Results and Discussion

In-House Matrix Matched Sample Example Results

	Calibrator	Neg Sample (1/4 x calibrator)			Pos Sample (1.5x calibrator)		
	ng/mL	Expected concentration	Calculated concentration	Result	Expected concentration	Calculated concentration	Result
6-MAM	100	25	26.7	negative	150	159.7	positive
7-Aminoclonazepam	100	25	25.5	negative	150	158.4	positive
Alprazolam	100	25	25.6	negative	150	162.2	positive
Amphetamine	100	25	27.9	negative	150	151.9	positive
Benzoylecgonine	100	25	26.0	negative	150	159.8	positive
Buprenorphine	5	1.25	1.3	negative	7.5	8.2	positive
Codeine	100	25	26.2	negative	150	158.2	positive
Diazepam	100	25	26.0	negative	150	164.9	positive
EDDP	100	25	26.8	negative	150	155.7	positive
Fentanyl	2	0.5	0.5	negative	3	3.0	positive
Flunitrazepm	100	25	25.2	negative	150	155.9	positive
Hydrocodone	100	25	25.6	negative	150	162.0	positive
Hydromorphone	100	25	26.8	negative	150	156.3	positive
JWH-018	20	5	6.0	negative	30	36.3	positive
JWH-073	20	5	7.1	negative	30	35.8	positive
Lorazepam	100	25	28.2	negative	150	164.1	positive
MDA	200	50	56.6	negative	300	308.3	positive
MDEA	200	50	52.3	negative	300	322.3	positive
MDMA	200	50	52.7	negative	300	320.9	positive
Methadone	100	25	25.6	negative	150	168.8	positive
Methamphetamine	100	25	25.9	negative	150	158.7	positive
Midazolam	100	25	26.5	negative	150	164.9	positive
Morphine	100	25	24.5	negative	150	157.9	positive
Naloxone	50	12.5	13.9	negative	75	79.6	positive
Nitrazepam	100	25	26.9	negative	150	156.1	positive
Norbuprenorphine	20	5	5.8	negative	30	35.3	positive
Nordiazepam	100	25	26.1	negative	150	155.4	positive
Norfentanyl	20	5	5.0	negative	30	32.1	positive
Oxazepam	100	25	28.4	negative	150	155.7	positive
Oxycodone	100	25	26.1	negative	150	161.8	positive
Oxymorphone	100	25	27.2	negative	150	154.0	positive
PCP	25	6.25	6.7	negative	37.5	38.3	positive
Temazepam	100	25	25.9	negative	150	157.0	positive
THC-COOH	20	5	5.1	negative	30	31.9	positive
Zolpidem	20	5	5.4	negative	30	31.1	positive
Zopiclone	20	5	5.2	negative	30	32.2	positive

Commercial Control Results

Commercially available control samples were prepared and analyzed over three days, in singleton on two days and in triplicate on one day, for a total of 5 datasets.

	Calibrator	UTAK DAU 1		UTAK DAU 2		UTAK PM 100	
	ng/mL	Expected conc	Result	Expected conc	Result	Expected conc	Result
6-MAM	100		negative (5/5)		negative (5/5)		negative (5/5)
7-Aminoclonazepam	100		negative (5/5)		negative (5/5)		negative (5/5)
Alprazolam	100		negative (5/5)		negative (5/5)		negative (5/5)
Amphetamine	100		negative (5/5)		negative (5/5)		negative (5/5)
Benzoylecgonine	100	50	negative (5/5)	112.5	positive (5/5)		negative (5/5)
Buprenorphine	5		negative (5/5)		negative (5/5)	100	positive (5/5)
Codeine	100		negative (5/5)		negative (5/5)	100	negative (5/5)
Diazepam	100		negative (5/5)		negative (5/5)		negative (5/5)
EDDP	100		negative (5/5)		negative (5/5)	100	positive (5/5)
Fentanyl	2		negative (5/5)		negative (5/5)	10	positive (5/5)
Flunitrazepm	100		negative (5/5)		negative (5/5)		negative (5/5)
Hydrocodone	100		negative (5/5)		negative (5/5)	100	negative (5/5)
Hydromorphone	100		negative (5/5)		negative (5/5)	100	positive (5/5)
JWH-018	20		negative (5/5)		negative (5/5)		negative (5/5)
JWH-073	20		negative (5/5)		negative (5/5)		negative (5/5)
Lorazepam	100		negative (5/5)		negative (5/5)		negative (5/5)
MDA	200		negative (5/5)		negative (5/5)		negative (5/5)
MDEA	200		negative (5/5)		negative (5/5)		negative (5/5)
MDMA	200		negative (5/5)		negative (5/5)		negative (5/5)
Methadone	100	50	negative (5/5)	112.5	positive (5/5)	100	positive (5/5)
Methamphetamine	100	225	positive (5/5)	375	positive (5/5)		negative (5/5)
Midazolam	100		negative (5/5)		negative (5/5)		negative (5/5)
Morphine	100	225	positive (5/5)	375	positive (5/5)	100	positive (5/5)
Naloxone	50		negative (5/5)		negative (5/5)		negative (5/5)
Nitrazepam	100		negative (5/5)		negative (5/5)		negative (5/5)
Norbuprenorphine	20		negative (5/5)		negative (5/5)	100	positive (5/5)
Nordiazepam	100		negative (5/5)		negative (5/5)		negative (5/5)
Norfentanyl	20		negative (5/5)		negative (5/5)	10	negative (5/5)
Oxazepam	100	50	negative (5/5)	75	negative (5/5)		negative (5/5)
Oxycodone	100		negative (5/5)		negative (5/5)	100	negative (5/5)
Oxymorphone	100		negative (5/5)		negative (5/5)	100	positive (5/5)
PCP	25	5	negative (5/5)	9.375	negative (5/5)		negative (5/5)
Temazepam	100		negative (5/5)		negative (5/5)		negative (5/5)
THC-COOH	20	10	negative (5/5)	15	positive (5/5)		negative (5/5)
Zolpidem	20		negative (5/5)		negative (5/5)		negative (5/5)
Zopiclone	20		negative (5/5)		negative (5/5)		negative (5/5)

Conclusions

- A 2.3 minute LC/MS/MS method has been developed for analytical detection of the presence or absence of exogenous compounds in urine.
- Single point calibration was sufficient to differentiate positive from negative samples.
- Preparation and analysis of commercial controls gave consistent results over multiple days and multiple runs within a single day.
- Alternative sources of human urine will be evaluated for interferences.

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