

Comprehensive LC/MS Analysis of Opiates, Opioids, Benzodiazepines, Amphetamines, Illicits, and Metabolites in Urine

Application Note

Forensic Toxicology

Abstract

This application note demonstrates that the Agilent 6420 Triple Quadrupole LC/MS system can be used to simultaneously analyze a wide variety of drug classes in urine samples with minimal sample preparation while providing sufficient sensitivity, quantitative linearity, and accuracy. An analytical method for the quantitation of a comprehensive panel containing 65 drugs and metabolites was developed utilizing the Agilent dynamic MRM approach.

Performance measures including limit of quantitation and accuracy are presented for a wide variety of analytes. Extensive testing of the instrumentation substantiates method robustness. Linearity for all analytes described in this application note show an $R^2 \geq 0.980$. This method can be easily customized to include different analytes or smaller and more focused panels of analytes to enhance sample throughput.



Agilent Technologies

Introduction

This application note for forensic toxicology illustrates the use of LC/MS/MS in the simultaneous quantitation of more than 65 drug compounds referenced in Table 1. Compound classes analyzed in this method include amphetamines, analgesics, benzodiazepines, and opiates, in addition to other compounds and certain metabolites of interest.

Herein, we describe a forensic method for the rapid and targeted quantitation of a comprehensive suite of compounds in urine samples using LC/MS/MS. This method routinely surpasses forensic toxicology sensitivity requirements, often down to 10 % of the desired lower limit of quantification (LLOQ), while displaying excellent linearity and reproducibility. One of the key components of this method is the use of dynamic multiple reaction monitoring (dMRM)¹, which allows the LC/MS/MS instrument to analyze a higher number of analytes in a given time frame without compromising data integrity. In doing so, we are able to develop a highly comprehensive analyte method that is capable of faster throughput than that achieved using a traditional MRM method.

Experimental

Analytes

Table 1. Drugs and metabolites analyzed.

Opiates/Opioids	Benzodiazepines	Stimulants
6-Monoacetyl morphine	2-Hydroxyethylflurazepam	Benzoyllecgonine
Buprenorphine	7-Aminoclonazepam	Cocaethylene
Codeine	7-Aminoflunitrazepam	Cocaine
Dihydrocodeine	α -Hydroxymidazolam	Methylphenidate
EDDP	Alprazolam	<i>m</i> -Hydroxybenzoyllecgonine
Fentanyl	α -Hydroxyalprazolam	Ritalinic acid
Heroin	α -Hydroxytriazolam	Amphetamine
Hydrocodone	Chlordiazepoxide	Fenfluramine
Hydromorphone	Clonazepam	MDA
Meperidine	Desalkylflurazepam	MDEA
Methadone	Diazepam	MDMA
Morphine	Flunitrazepam	Meprobamate
Naloxone	Flurazepam	Methamphetamine
Naltrexone	Lorazepam	Phentermine
N-desmethyltramadol	Midazolam	Zopiclone
Norbuprenorphine	Nitrazepam	
Norfentanyl	Nordiazepam	
Normeperidine	Oxazepam	Others
Norpropoxyphene	Temazepam	Carisoprodol
<i>o</i> -Desmethyltramadol	Triazolam	PCP
Oxycodone		Trazodone
Oxymorphone		Verapamil
Propoxyphene		Zolpidem
Tapentadol		
Tramadol		

LC/MS/MS configurations and conditions

The LC/MS/MS system used in this study consisted of the following modules: an Agilent 1260 Infinity Binary Pump, an Agilent 1260 Infinity Thermostatted Column Compartment, an Agilent 1260 Infinity Thermostatted Autosampler, an Agilent 6420 Triple Quadrupole LC/MS equipped with a standard ESI source, and MassHunter Quantitative Analysis Software B.05.00. LC and MS/MS conditions are shown in Table 2.

Table 2. LC and MS/MS conditions.

LC conditions		
Column	Agilent Poroshell 120 EC C18, 2.1 × 100 mm, 2.7 μm	
Column temperature	55 °C	
Injection volume	5 μL	
Needle wash	flush port, 100 % methanol, 5 seconds	
Buffer A	5 mM ammonium formate + 0.01 % formic acid in water	
Buffer B	0.01 % formic acid in methanol	
Flow rate	0.5 mL/min	
Stop time	6.0 minutes	
Post time	1.5 minutes	
Gradient	Time (min)	%B
	0.00	10
	0.50	15
	3.00	50
	6.00	95
Total run time	7.5 minutes (This analysis time can be vastly reduced by using an Agilent 1290 Infinity LC system)	
MS/MS conditions		
Ion mode	electrospray, positive	
Gas temperature	350 °C	
Gas flow	12 L/min	
Nebulizer	50 psi	
Capillary voltage	2,000 V	
Δ EMV	0 V	
Dwell time	Variable (dynamic MRM)	

Method development with dMRM

When analyzing such a large panel of targets, developing traditional MRM methods can be challenging. As the number of simultaneously monitored MRM transitions increases, dwell time limitations emerge, adversely impacting data quality. dMRM methods provide an ideal solution to this challenge by assigning individual time windows to each transition and only monitoring transitions for compounds during the compound's elution window. Another important aspect of the dMRM approach is that MS cycle times are kept constant to ensure optimized sampling and consistently accurate quantitation.¹

A custom dMRM method was quickly created by importing MRM transitions from the Agilent Forensic Toxicology

Database, which contains optimized parameters for multiple MRM transitions for over 200 compounds. Table 3 shows an excerpt of the dMRM table generated.

Chemicals and reagents

All standards and labeled internal standards were purchased from Cerilliant, Round Rock, TX. Drug-free human urine was purchased from Golden West Biologicals, Temecula, CA. All other LC/MS grade solvents and reagents were purchased from Sigma-Aldrich, St. Louis, MO.

Sample preparation

Calibrators were prepared by spiking drug-free human urine with 1,000 ng/mL of all 65 compounds listed in Table 1. Serial two-fold dilutions were used to achieve the remaining standard calibrator concentrations.

A mixture of labeled internal standards was added to all samples. Samples then underwent an enzymatic hydrolysis followed by a 10x dilution using water. Samples were transferred to 96-well plates and injected onto the 6420 Triple Quadrupole LC/MS/MS system.

Data analysis

MassHunter Quantitative Analysis software (B.05.00) was used for data analysis. A $1/x^2$ weighting factor was applied during linear regression of the calibration curves. Quantitation using MassHunter Quantitative Analysis software was performed by MRM peak area ratio to a known concentration of the internal standards.

Table 3. Excerpt of dMRM table.

Compound name	Prec ion	MS1 res	Prod ion	MS2 res	Frag (V)	CE (V)	RT	RT window
Oxymorphone	302.1	Unit	284.0	Unit	117	17	0.80	0.5
Oxymorphone	302.1	Unit	227.0	Unit	117	29	0.80	0.5
Morphine	286.2	Unit	165.1	Unit	158	41	0.85	0.7
Morphine	286.2	Unit	152.0	Unit	158	60	0.85	0.7
Hydromorphone	286.2	Unit	185.0	Unit	159	29	1.04	0.7
Hydromorphone	286.2	Unit	157.0	Unit	159	45	1.04	0.7
Codeine	300.2	Unit	165.1	Unit	158	45	1.77	0.5
Codeine	300.2	Unit	58.1	Unit	158	29	1.77	0.5
Naloxone	328.2	Unit	310.0	Unit	91	17	1.81	0.5
Naloxone	328.2	Unit	211.9	Unit	91	41	1.81	0.5
Oxycodone	316.2	Unit	298.1	Unit	143	17	1.91	0.5
Oxycodone	316.2	Unit	256.1	Unit	143	25	1.91	0.5
Naltrexone	342.2	Unit	324.0	Unit	117	21	1.99	0.5
Naltrexone	342.2	Unit	55.0	Unit	117	41	1.99	0.5
Hydrocodone	300.2	Unit	199.0	Unit	159	29	2.05	0.5
Hydrocodone	300.2	Unit	128.0	Unit	159	65	2.05	0.5
6-Monoacetyl morphine	328.2	Unit	165.1	Unit	158	41	2.07	0.5
6-Monoacetyl morphine	328.2	Unit	43.1	Unit	158	60	2.07	0.5
<i>d</i> -Amphetamine	136.1	Unit	119.1	Unit	66	5	2.18	0.5
<i>d</i> -Amphetamine	136.1	Unit	91.0	Unit	66	17	2.18	0.5
Methamphetamine	150.1	Unit	119.0	Unit	92	5	2.27	0.5
Methamphetamine	150.1	Unit	91.0	Unit	92	17	2.27	0.5
MDMA	194.1	Unit	163.0	Unit	97	9	2.28	0.5
MDMA	194.1	Unit	105.0	Unit	97	25	2.28	0.5

Results and Discussion

The chromatography conditions as outlined in the experimental section yield good chromatographic peak shape and separation for all analytes with a total analysis time of 6 minutes. An important chromatographic factor was to obtain good baseline separation for the isobaric analytes in this comprehensive suite. This was achieved for morphine/hydromorphone, codeine/hydrocodone and methamphetamine/phentermine using the chromatography conditions outlined in Table 2. Figure 1 shows an overlaid chromatogram that illustrates this using a matrix spiked sample of all analytes together with the corresponding isotopically labeled internal standards.

Calibrators were prepared by spiking drug-free human urine with a stock solution of the 65 compounds listed in Table 1, and their concentrations ranged from 1 to 10,000 ng/mL. An independently prepared stock solution was used to make quality control (QC) samples, in which three levels were prepared at 5, 100, and 750 ng/mL. Calibrators and QC were run in triplicate on three separate days to assess accuracy and reproducibility. All compounds showed accuracies between 90 and 110 % and coefficient of variation (CV) values less than 10 % (Table 4). Calibration curves showed excellent linearity over a wide range of concentrations (Figure 2). The LLOQ for each of the compounds analyzed can be found in Table 5.

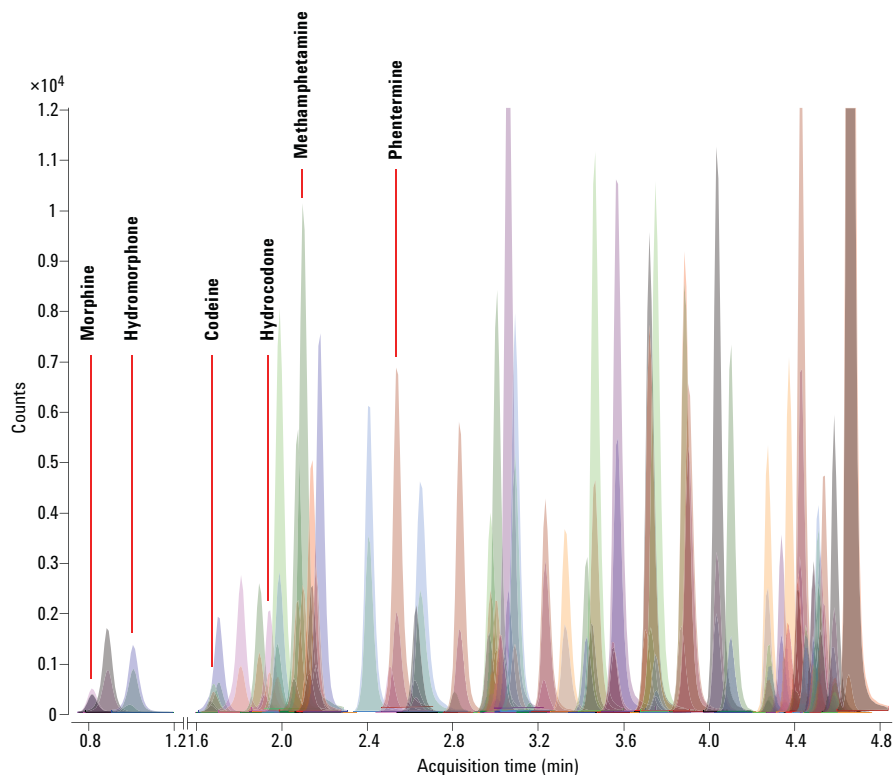


Figure 1. Chromatograph for 65 drugs and metabolites in a single run.

Table 4. Representative intraday and interday accuracy and precision for various drug compounds analyzed in urine; analytes were present at 100 ng/mL.

	Intraday (n = 3)		Interday (n = 9)	
	Ave accuracy (%)	CV (%)	Ave accuracy (%)	CV (%)
Buprenorphine	95.9	3.7	97.9	5.7
Carisoprodol	92.5	5.4	97.6	4.8
EDDP	97.3	2.9	96.2	3.0
Hydrocodone	96.8	3.5	98.4	7.5
Hydromorphone	96.8	1.9	96.6	4.9
Methadone	94.3	2.1	90.4	3.3
Morphine	101.2	3.8	99.9	3.1
Oxycodone	100.8	3.1	95.3	7.8
Oxymorphone	95.3	7.0	98.4	2.6
Propoxyphene	95.6	5.5	91.9	2.8

The robustness of the method was tested by making 3,000 sequential injections and monitoring the retention times of several analytes across the entire chromatographic run. All analytes showed a variation of less than 1 % (Table 6).

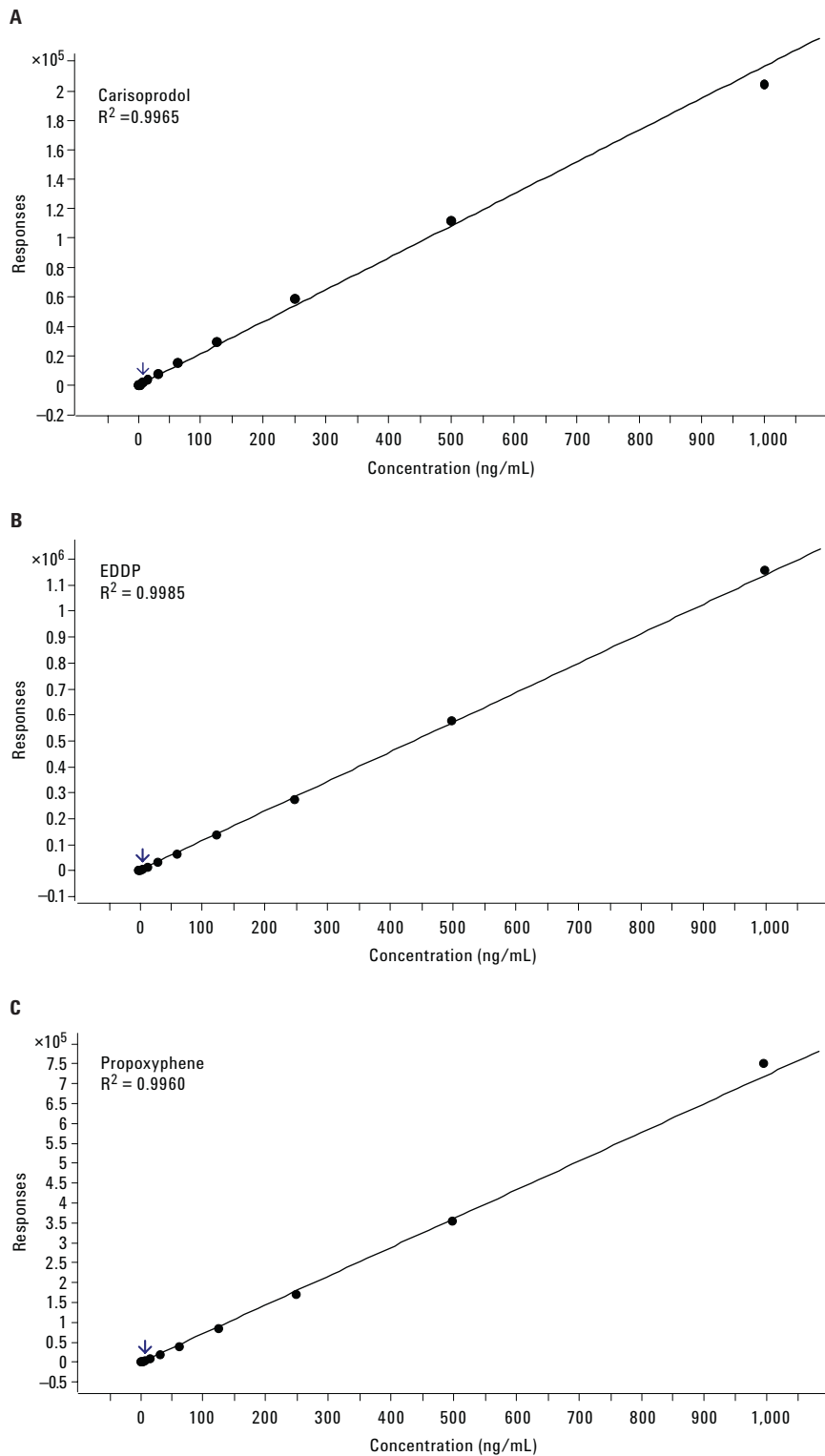


Figure 2. Representative calibration curves for carisoprodol (A) , EDDP (B), and propoxyphene (C).

Table 5. LLOQs for various drugs and metabolites.

Opiates/Opioids		Benzodiazepines		Stimulants	
Compound	LLOQ	Compound	LLOQ	Compound	LLOQ
6-Monoacetyl morphine	10	2-OH-Ethylflurazepam	200	Amphetamine	5
Buprenorphine	10	7-Aminoclonazepam	10	Benzoyllecgonine	5
Codeine	25	7-Aminoflunitrazepam	5	Cocaethylene	5
Dihydrocodeine	25	<i>alpha</i> -OH-Midazolam	10	Cocaine	5
EDDP	10	Alprazolam	10	Fenfluramine	1
Fentanyl	1	<i>a</i> -OH-alprazolam	20	MDA	5
Heroin	10	<i>a</i> -OH-triazolam	50	MDEA	1
Hydrocodone	10	Chlordiazepoxide	10	MDMA	5
Hydromorphone	5	Clonazepam	25	Meprobamate	10
Meperidine	5	Desalkylflurazepam	20	Methamphetamine	1
Methadone	10	Diazepam	10	Methylphenidate	5
Morphine	5	Flunitrazepam	10	<i>m</i> -Hydroxybenzoyllecgonine	10
Naloxone	5	Flurazepam	5	Phentermine	1
Naltrexone	10	Lorazepam	50	Ritalinic acid	5
N-Desmethyltramadol	10	Midazolam	10	Zopiclone	5
Norbuprenorphine	25	Nitrazepam	25	Others	
Norfentanyl	1	Nordiazepam	25	Compound	LLOQ
Normeperidine	5	Oxazepam	50	Carisoprodol	5
Norpropoxyphene	5	Temazepam	25	PCP	1
<i>o</i> -Desmethyltramadol	5	Triazolam	5	Trazodone	1
Oxycodone	10			Verapamil	2
Oxymorphone	5			Zolpidem	5
Propoxyphene	5				
Tapentadol	5				
Tramadol	1				

Table 6. Snapshot of retention time reproducibility over 3,000 injections on a single column.

Analyte	% CV	Analyte	% CV	Analyte	% CV
Morphine	0.7	Meperidine	0.4	Triazolam	0.0
Codeine	0.4	Zolpidem	0.3	Naltrexone	0.1
Hydrocodone	0.4	Fentanyl	0.1	Chlordiazepoxide	0.1
MDMA	0.3	EDDP	0.1	Desmethyl diazepam	0.1
Norfentanyl	0.2	Nitrazepam	0.1	Buprenorphine	0.3
Heroin	0.2	Propoxyphene	0.1	Cocaethylene	0.2
Methylphenidate	0.2	Buprenorphine	0.3	11-nor-9-carboxy- Δ 9-THC	0.0

Conclusions

An analytical method for the quantitation of 65 drugs and metabolites in human urine has been developed using the Agilent 6420 Triple Quadrupole LC/MS. The development of analytical methods, ranging from custom to comprehensive, is quick and easy using the Agilent Forensic Toxicology Database. The Agilent dynamic MRM approach ensures accurate and reproducible quantitation with this type of analysis.

This application note used a comprehensive panel of compounds to demonstrate the ultimate functionality of dynamic MRM and operational

quality of the Agilent system. A wide selection of analyte components and their isotopically labelled internal standards can be analyzed in 6 minutes. Smaller and more focused panels of analytes could reduce the analysis time and enhance sample throughput significantly.

All typical detection limits under these extreme acquisition conditions were met, with all analytes exhibiting better than 98 % accuracy for each respective linear range. Detection limits were measured conservatively by using signal-to-noise determinations along with %CV precision data for the lesser abundant qualifier ions associated with each analyte.

Future investigations will compare and contrast the sample preparation technique of dilute and shoot with other techniques such as solid phase extraction (SPE).

Reference

1. New Dynamic MRM Mode Improves Data Quality and Triple Quad Quantification in Complex Analyses. Agilent Publication 5990-3595EN.

Appendix A – MRM Transitions for All Analytes and ISTDs

Cpd name	ISTD?	Prec ion MS1 res	Prod ion MS2 res	Frag (V)	CE (V)	Cell acc (V)	Ret time (min)	Ret window	Polarity
11-nor-9-carboxy-Δ9-THC	No	345.2, Unit/Enh (6490)	327.2 Unit/Enh (6490)	140	18	7	5.01	0.5	Positive
11-nor-9-carboxy-Δ9-THC	No	345.2 Unit/Enh (6490)	299 Unit/Enh (6490)	140	18	7	5.01	0.5	Positive
11-nor-9-carboxy-Δ9-THC-d9	Yes	354.1 Unit/Enh (6490)	336.1 Unit/Enh (6490)	136	13	7	5	0.5	Positive
11-nor-9-carboxy-Δ9-THC-d9	Yes	354.1 Unit/Enh (6490)	308.2 Unit/Enh (6490)	136	17	7	5	0.5	Positive
2-OH-Ethylflurazepam	No	333.1 Unit/Enh (6490)	211 Unit/Enh (6490)	126	37	7	4.44	0.5	Positive
2-OH-Ethylflurazepam	No	333.1 Unit/Enh (6490)	109.1 Unit/Enh (6490)	126	29	7	4.44	0.5	Positive
6-Acetylmorphine-d6	Yes	334.2 Unit/Enh (6490)	211 Unit/Enh (6490)	168	25	7	1.9	0.5	Positive
6-Monoacetyl morphine	No	328.2 Unit/Enh (6490)	165.1 Unit/Enh (6490)	158	41	7	1.95	0.5	Positive
6-Monoacetyl morphine	No	328.2 Unit/Enh (6490)	43.1 Unit/Enh (6490)	158	60	7	1.95	0.5	Positive
7-Aminoclonazepam	No	286.1 Unit/Enh (6490)	222.1 Unit/Enh (6490)	141	25	7	3.01	0.5	Positive
7-Aminoclonazepam	No	286.1 Unit/Enh (6490)	121.1 Unit/Enh (6490)	141	29	7	3.01	0.5	Positive
7-Aminoclonazepam-d4	Yes	290.1 Unit/Enh (6490)	121.1 Unit/Enh (6490)	151	33	7	2.98	0.5	Positive
7-Aminoflunitrazepam	No	284.1 Unit/Enh (6490)	227 Unit/Enh (6490)	151	25	7	3.42	0.5	Positive
7-Aminoflunitrazepam	No	284.1 Unit/Enh (6490)	135.1 Unit/Enh (6490)	151	25	7	3.42	0.5	Positive
<i>alpha</i> -Hydroxyalprazolam	No	325.1 Unit/Enh (6490)	297 Unit/Enh (6490)	150	28	7	4.38	0.5	Positive
<i>alpha</i> -Hydroxyalprazolam	No	325.1 Unit/Enh (6490)	216 Unit/Enh (6490)	150	41	7	4.38	0.5	Positive
<i>alpha</i> -Hydroxyethylflurazepam	No	333.1 Unit/Enh (6490)	211 Unit/Enh (6490)	155	37	7	4.44	0.5	Positive
<i>alpha</i> -Hydroxyethylflurazepam	No	333.1 Unit/Enh (6490)	109 Unit/Enh (6490)	155	29	7	4.44	0.5	Positive
<i>alpha</i> -Hydroxymidazolam	No	342.1 Unit/Enh (6490)	324.1 Unit/Enh (6490)	148	21	7	4.51	0.5	Positive
<i>alpha</i> -Hydroxymidazolam	No	342.1 Unit/Enh (6490)	168.1 Unit/Enh (6490)	148	45	7	4.51	0.5	Positive
<i>alpha</i> -Hydroxytriazolam	No	359.1 Unit/Enh (6490)	331 Unit/Enh (6490)	169	29	7	4.34	0.5	Positive
<i>alpha</i> -Hydroxytriazolam	No	359.1 Unit/Enh (6490)	176 Unit/Enh (6490)	169	29	7	4.34	0.5	Positive
Alprazolam	No	309.1 Unit/Enh (6490)	281 Unit/Enh (6490)	179	25	7	4.5	0.5	Positive

Cpd name	ISTD?	Prec ion MS1 res	Prod ion MS2 res	Frag (V)	CE (V)	Cell acc (V)	Ret time (min)	Ret window	Polarity
Alprazolam	No	309.1 Unit/Enh (6490)	205 Unit/Enh (6490)	179	49	7	4.5	0.5	Positive
Alprazolam-d5	Yes	314.1 Unit/Enh (6490)	286.1 Unit/Enh (6490)	166	25	7	4.48	0.5	Positive
Benzoylcegonine	No	290.1 Unit/Enh (6490)	168.1 Unit/Enh (6490)	118	17	7	2.64	0.5	Positive
Benzoylcegonine	No	290.1 Unit/Enh (6490)	77 Unit/Enh (6490)	118	60	7	2.64	0.5	Positive
Benzoylcegonine-d8	Yes	298.2 Unit/Enh (6490)	171.1 Unit/Enh (6490)	124	17	7	2.59	0.5	Positive
Buprenorphine	No	468.3 Unit/Enh (6490)	396.2 Unit/Enh (6490)	200	41	7	4.51	0.5	Positive
Buprenorphine	No	468.3 Unit/Enh (6490)	55.1 Unit/Enh (6490)	200	60	7	4.51	0.5	Positive
Buprenorphine-d4	Yes	472.3 Unit/Enh (6490)	59.2 Unit/Enh (6490)	198	57	7	4.39	0.5	Positive
Carisoprodol	No	261.2 Unit/Enh (6490)	176.1 Unit/Enh (6490)	67	5	7	4.4	0.5	Positive
Carisoprodol	No	261.2 Unit/Enh (6490)	55.1 Unit/Enh (6490)	67	29	7	4.4	0.5	Positive
Carisoprodol-d7	Yes	268.2 Unit/Enh (6490)	183.1 Unit/Enh (6490)	72	5	7	4.39	0.5	Positive
Chlordiazepoxide	No	300.1 Unit/Enh (6490)	282.1 Unit/Enh (6490)	117	21	7	4.53	0.5	Positive
Chlordiazepoxide	No	300.1 Unit/Enh (6490)	227 Unit/Enh (6490)	117	21	7	4.53	0.5	Positive
Chlordiazepoxide-d5	Yes	305.1 Unit/Enh (6490)	286.1 Unit/Enh (6490)	124	25	7	4.51	0.5	Positive
Clonazepam	No	316.1 Unit/Enh (6490)	270.1 Unit/Enh (6490)	158	25	7	4.27	0.5	Positive
Clonazepam	No	316.1 Unit/Enh (6490)	214 Unit/Enh (6490)	158	41	7	4.27	0.5	Positive
Clonazepam-d4	Yes	320.1 Unit/Enh (6490)	274 Unit/Enh (6490)	136	25	7	4.26	0.5	Positive
Cocaethylene	No	318.2 Unit/Enh (6490)	196.1 Unit/Enh (6490)	123	17	7	3.45	0.5	Positive
Cocaethylene	No	318.2 Unit/Enh (6490)	82.1 Unit/Enh (6490)	123	33	7	3.45	0.5	Positive
Cocaethylene-d8	Yes	326.2 Unit/Enh (6490)	204.1 Unit/Enh (6490)	126	17	7	3.38	0.5	Positive
Cocaine	No	304.2 Unit/Enh (6490)	182.1 Unit/Enh (6490)	138	17	7	2.98	0.5	Positive
Cocaine	No	304.2 Unit/Enh (6490)	77 Unit/Enh (6490)	138	61	7	2.99	0.5	Positive
Cocaine-d3	Yes	307.2 Unit/Enh (6490)	185.1 Unit/Enh (6490)	129	17	7	2.93	0.5	Positive
Codeine	No	300.2 Unit/Enh (6490)	165.1 Unit/Enh (6490)	158	45	7	1.67	0.5	Positive
Codeine	No	300.2 Unit/Enh (6490)	128 Unit/Enh (6490)	158	60	7	1.67	0.5	Positive
Codeine-d6	Yes	306.2 Unit/Enh (6490)	218.1 Unit/Enh (6490)	151	25	7	1.58	0.5	Positive
<i>d</i> -Amphetamine	No	136.1 Unit/Enh (6490)	119.1 Unit/Enh (6490)	66	5	7	1.96	0.5	Positive
<i>d</i> -Amphetamine	No	136.1 Unit/Enh (6490)	91 Unit/Enh (6490)	66	17	7	1.96	0.5	Positive
Desalkylflurazepam	No	289.1 Unit/Enh (6490)	226 Unit/Enh (6490)	131	29	7	4.48	0.5	Positive
Desalkylflurazepam	No	289.1 Unit/Enh (6490)	140 Unit/Enh (6490)	131	29	7	4.48	0.5	Positive
Desmethyl diazepam	No	271.1 Unit/Enh (6490)	165 Unit/Enh (6490)	189	29	7	4.58	0.5	Positive

Cpd name	ISTD?	Prec ion MS1 res	Prod ion MS2 res	Frag (V)	CE (V)	Cell acc (V)	Ret time (min)	Ret window	Polarity
Desmethyl diazepam	No	271.1 Unit/Enh (6490)	140 Unit/Enh (6490)	189	29	7	4.58	0.5	Positive
Diazepam	No	285.1 Unit/Enh (6490)	193.1 Unit/Enh (6490)	131	33	7	4.65	0.5	Positive
Diazepam	No	285.1 Unit/Enh (6490)	154 Unit/Enh (6490)	131	25	7	4.65	0.5	Positive
Diazepam-d5	Yes	290.1 Unit/Enh (6490)	198.1 Unit/Enh (6490)	131	33	7	4.64	0.5	Positive
Dihydrocodeine	No	303.2 Unit/Enh (6490)	200.1 Unit/Enh (6490)	136	33	7	1.64	0.5	Positive
Dihydrocodeine	No	303.2 Unit/Enh (6490)	129.1 Unit/Enh (6490)	136	65	7	1.64	0.5	Positive
EDDP	No	278.2 Unit/Enh (6490)	234.1 Unit/Enh (6490)	158	33	7	4.02	0.5	Positive
EDDP	No	278.2 Unit/Enh (6490)	219.1 Unit/Enh (6490)	158	45	7	4.02	0.5	Positive
EDDP-d3	Yes	281.2 Unit/Enh (6490)	234.1 Unit/Enh (6490)	168	29	7	3.96	0.5	Positive
Fenfluramine	No	232.1 Unit/Enh (6490)	159 Unit/Enh (6490)	104	21	7	3.55	0.5	Positive
Fenfluramine	No	232.1 Unit/Enh (6490)	109 Unit/Enh (6490)	104	45	7	3.55	0.5	Positive
Fenfluramine-d10	Yes	242.2 Unit/Enh (6490)	161 Unit/Enh (6490)	114	25	7	3.48	0.5	Positive
Fentanyl	No	337.2 Unit/Enh (6490)	188.1 Unit/Enh (6490)	143	21	7	3.86	0.5	Positive
Fentanyl	No	337.2 Unit/Enh (6490)	105.1 Unit/Enh (6490)	143	41	7	3.86	0.5	Positive
Fentanyl-d5	Yes	342.3 Unit/Enh (6490)	188.1 Unit/Enh (6490)	146	21	7	3.78	0.5	Positive
Flunitrazepam	No	314.1 Unit/Enh (6490)	268.1 Unit/Enh (6490)	153	25	7	4.33	0.5	Positive
Flunitrazepam	No	314.1 Unit/Enh (6490)	239.1 Unit/Enh (6490)	153	37	7	4.33	0.5	Positive
Flurazepam	No	388.2 Unit/Enh (6490)	317.1 Unit/Enh (6490)	158	17	7	4.08	0.5	Positive
Flurazepam	No	388.2 Unit/Enh (6490)	315 Unit/Enh (6490)	158	21	7	4.08	0.5	Positive
Heroin	No	370.2 Unit/Enh (6490)	268.1 Unit/Enh (6490)	149	37	7	2.97	0.5	Positive
Heroin	No	370.2 Unit/Enh (6490)	165 Unit/Enh (6490)	149	61	7	2.97	0.5	Positive
Heroin-d9	Yes	379.2 Unit/Enh (6490)	61.2 Unit/Enh (6490)	168	29	7	2.9	0.5	Positive
Hydrocodone	No	300.2 Unit/Enh (6490)	199 Unit/Enh (6490)	159	29	7	1.92	0.5	Positive
Hydrocodone	No	300.2 Unit/Enh (6490)	128 Unit/Enh (6490)	159	65	7	1.92	0.5	Positive
Hydrocodone-d6	Yes	306.2 Unit/Enh (6490)	202.1 Unit/Enh (6490)	161	29	7	1.85	0.5	Positive
Hydromorphone	No	286.2 Unit/Enh (6490)	185 Unit/Enh (6490)	159	29	7	0.97	0.5	Positive
Hydromorphone	No	286.2 Unit/Enh (6490)	157 Unit/Enh (6490)	159	45	7	0.98	0.5	Positive
hydromorphone-d6	Yes	292.2 Unit/Enh (6490)	185 Unit/Enh (6490)	166	33	7	0.95	0.5	Positive
Lorazepam	No	321 Unit/Enh (6490)	303 Unit/Enh (6490)	124	9	7	4.43	0.5	Positive
Lorazepam	No	321 Unit/Enh (6490)	275 Unit/Enh (6490)	124	17	7	4.43	0.5	Positive
MDA	No	180.1 Unit/Enh (6490)	163 Unit/Enh (6490)	61	5	7	2.04	0.5	Positive

Cpd name	ISTD?	Prec ion MS1 res	Prod ion MS2 res	Frag (V)	CE (V)	Cell acc (V)	Ret time (min)	Ret window	Polarity
MDA	No	180.1 Unit/Enh (6490)	105 Unit/Enh (6490)	61	21	7	2.04	0.5	Positive
MDA-d5	Yes	185.1 Unit/Enh (6490)	168.1 Unit/Enh (6490)	87	5	7	1.98	0.5	Positive
MDEA	No	208.1 Unit/Enh (6490)	163 Unit/Enh (6490)	107	9	7	2.39	0.5	Positive
MDEA	No	208.1 Unit/Enh (6490)	105 Unit/Enh (6490)	107	25	7	2.39	0.5	Positive
MDMA	No	194.1 Unit/Enh (6490)	163 Unit/Enh (6490)	97	9	7	2.12	0.5	Positive
MDMA	No	194.1 Unit/Enh (6490)	105 Unit/Enh (6490)	97	25	7	2.12	0.5	Positive
MDMA-d5	Yes	199.1 Unit/Enh (6490)	165 Unit/Enh (6490)	82	9	7	2.06	0.5	Positive
Meperidine	No	248.2 Unit/Enh (6490)	220.1 Unit/Enh (6490)	128	21	7	3.21	0.5	Positive
Meperidine	No	248.2 Unit/Enh (6490)	174.1 Unit/Enh (6490)	128	17	7	3.21	0.5	Positive
Meperidine-d4	Yes	252.2 Unit/Enh (6490)	224.1 Unit/Enh (6490)	141	17	7	3.15	0.5	Positive
Meprobamate	No	219.1 Unit/Enh (6490)	158.1 Unit/Enh (6490)	65	5	7	3.54	0.5	Positive
Meprobamate	No	219.1 Unit/Enh (6490)	55.1 Unit/Enh (6490)	65	21	7	3.54	0.5	Positive
Methadone	No	310.2 Unit/Enh (6490)	265.1 Unit/Enh (6490)	112	9	7	4.42	0.5	Positive
Methadone	No	310.2 Unit/Enh (6490)	105 Unit/Enh (6490)	112	29	7	4.42	0.5	Positive
Methadone-d9	Yes	319.3 Unit/Enh (6490)	268.1 Unit/Enh (6490)	89	13	7	4.39	0.5	Positive
Methamphetamine	No	150.1 Unit/Enh (6490)	91 Unit/Enh (6490)	92	17	7	2.07	0.5	Positive
Methamphetamine	No	150.1 Unit/Enh (6490)	65.1 Unit/Enh (6490)	92	45	7	2.07	0.5	Positive
Methylphenidate	No	234.1 Unit/Enh (6490)	84.1 Unit/Enh (6490)	112	21	7	3.04	0.5	Positive
Methylphenidate	No	234.1 Unit/Enh (6490)	56.1 Unit/Enh (6490)	112	53	7	3.04	0.5	Positive
Methylphenidate-d9	Yes	243.2 Unit/Enh (6490)	93.2 Unit/Enh (6490)	109	21	7	2.97	0.5	Positive
<i>m</i> -Hydroxybenzoylcegonine	No	306.1 Unit/Enh (6490)	168 Unit/Enh (6490)	124	17	7	2.14	0.5	Positive
<i>m</i> -Hydroxybenzoylcegonine	No	306.1 Unit/Enh (6490)	121 Unit/Enh (6490)	124	33	7	2.14	0.5	Positive
Midazolam	No	326.1 Unit/Enh (6490)	291.1 Unit/Enh (6490)	194	29	7	4.57	0.5	Positive
Midazolam	No	326.1 Unit/Enh (6490)	249.1 Unit/Enh (6490)	194	41	7	4.57	0.5	Positive
Midazolam-d4	Yes	330.1 Unit/Enh (6490)	295.1 Unit/Enh (6490)	156	29	7	4.54	0.5	Positive
Morphine	No	286.2 Unit/Enh (6490)	165.1 Unit/Enh (6490)	158	41	7	0.79	0.5	Positive
Morphine	No	286.2 Unit/Enh (6490)	152 Unit/Enh (6490)	158	60	7	0.79	0.5	Positive
Morphine-d6	Yes	292.2 Unit/Enh (6490)	152 Unit/Enh (6490)	173	65	7	0.72	0.5	Positive
Naloxone	No	328.2 Unit/Enh (6490)	310.1 Unit/Enh (6490)	129	17	7	1.68	0.5	Positive
Naloxone	No	328.2 Unit/Enh (6490)	212 Unit/Enh (6490)	129	41	7	1.68	0.5	Positive
Naltrexone	No	342.2 Unit/Enh (6490)	324.1 Unit/Enh (6490)	126	17	7	1.87	0.5	Positive
Naltrexone	No	342.2 Unit/Enh (6490)	55.1 Unit/Enh (6490)	126	41	7	1.87	0.5	Positive
N-Desmethyltramadol	No	250.2 Unit/Enh (6490)	232.1 Unit/Enh (6490)	92	5	7	3.14	0.5	Positive
N-Desmethyltramadol	No	250.2 Unit/Enh (6490)	121 Unit/Enh (6490)	92	33	7	3.14	0.5	Positive

Cpd name	ISTD?	Prec ion MS1 res	Prod ion MS2 res	Frag (V)	CE (V)	Cell acc (V)	Ret time (min)	Ret window	Polarity
Nitrazepam	No	282.1 Unit/Enh (6490)	236.1 Unit/Enh (6490)	148	25	7	4.27	0.5	Positive
Nitrazepam	No	282.1 Unit/Enh (6490)	180 Unit/Enh (6490)	148	41	7	4.27	0.5	Positive
Nitrazepam-d5	Yes	287.1 Unit/Enh (6490)	241.1 Unit/Enh (6490)	151	25	7	4.24	0.5	Positive
Norbuprenorphine	No	414.3 Unit/Enh (6490)	101.1 Unit/Enh (6490)	188	41	7	3.74	0.5	Positive
Norbuprenorphine	No	414.3 Unit/Enh (6490)	83.1 Unit/Enh (6490)	188	53	7	3.74	0.5	Positive
Norcodeine	No	286.2 Unit/Enh (6490)	268.1 Unit/Enh (6490)	143	17	7	0.79	0.5	Positive
Norcodeine	No	286.2 Unit/Enh (6490)	165.1 Unit/Enh (6490)	143	45	7	0.79	0.5	Positive
Nordiazepam	No	271.1 Unit/Enh (6490)	165 Unit/Enh (6490)	138	25	7	4.58	0.5	Positive
Nordiazepam	No	271.1 Unit/Enh (6490)	140 Unit/Enh (6490)	138	29	7	4.58	0.5	Positive
Norfentanyl	No	233.2 Unit/Enh (6490)	84.1 Unit/Enh (6490)	97	13	7	2.82	0.5	Positive
Norfentanyl	No	233.2 Unit/Enh (6490)	55.1 Unit/Enh (6490)	97	37	7	2.81	0.5	Positive
Norfentanyl-d5	Yes	238.2 Unit/Enh (6490)	84.1 Unit/Enh (6490)	114	17	7	2.74	0.5	Positive
Norfludiazepam	No	289.1 Unit/Enh (6490)	226.1 Unit/Enh (6490)	117	25	7	4.48	0.5	Positive
Norfludiazepam	No	289.1 Unit/Enh (6490)	140.1 Unit/Enh (6490)	117	29	7	4.48	0.5	Positive
Normeperidine	No	234 Unit/Enh (6490)	160 Unit/Enh (6490)	81	13	7	3.29	0.5	Positive
Normeperidine	No	234 Unit/Enh (6490)	56 Unit/Enh (6490)	81	21	7	3.29	0.5	Positive
Norpropoxyphene	No	326.2 Unit/Enh (6490)	252.2 Unit/Enh (6490)	87	5	7	4.4	0.5	Positive
Norpropoxyphene	No	326.2 Unit/Enh (6490)	91 Unit/Enh (6490)	87	49	7	4.4	0.5	Positive
<i>o</i> -Desmethyltramadol	No	250.2 Unit/Enh (6490)	232.1 Unit/Enh (6490)	97	9	7	2.16	0.5	Positive
<i>o</i> -Desmethyltramadol	No	250.2 Unit/Enh (6490)	58.1 Unit/Enh (6490)	97	17	7	2.16	0.5	Positive
Oxazepam	No	287.1 Unit/Enh (6490)	269 Unit/Enh (6490)	114	21	7	4.43	0.5	Positive
Oxazepam	No	287.1 Unit/Enh (6490)	241 Unit/Enh (6490)	114	9	7	4.43	0.5	Positive
Oxycodone	No	316.2 Unit/Enh (6490)	298.1 Unit/Enh (6490)	143	17	7	1.8	0.5	Positive
Oxycodone	No	316.2 Unit/Enh (6490)	241.1 Unit/Enh (6490)	143	29	7	1.8	0.5	Positive
Oxymorphone	No	302.1 Unit/Enh (6490)	284 Unit/Enh (6490)	117	17	7	0.87	0.5	Positive
Oxymorphone	No	302.1 Unit/Enh (6490)	227 Unit/Enh (6490)	117	29	7	0.86	0.5	Positive
PCP	No	244.2 Unit/Enh (6490)	91 Unit/Enh (6490)	86	41	7	3.71	0.5	Positive
PCP	No	244.2 Unit/Enh (6490)	86.1 Unit/Enh (6490)	86	9	7	3.71	0.5	Positive
PCP-d5	Yes	249.2 Unit/Enh (6490)	86.1 Unit/Enh (6490)	87	9	7	3.63	0.5	Positive
Phentermine	No	150.1 Unit/Enh (6490)	91.1 Unit/Enh (6490)	70	21	7	2.53	0.5	Positive
Phentermine	No	150.1 Unit/Enh (6490)	65.1 Unit/Enh (6490)	70	45	7	2.53	0.5	Positive
Phentermine-d5	Yes	155.2 Unit/Enh (6490)	96.1 Unit/Enh (6490)	72	21	7	2.43	0.5	Positive
Propoxyphene	No	340.2 Unit/Enh (6490)	266.2 Unit/Enh (6490)	92	5	7	4.37	0.5	Positive

Cpd name	ISTD?	Prec ion MS1 res	Prod ion MS2 res	Frag (V)	CE (V)	Cell acc (V)	Ret time (min)	Ret window	Polarity
Propoxyphene	No	340.2 Unit/Enh (6490)	58.1 Unit/Enh (6490)	92	25	7	4.37	0.5	Positive
propoxyphene-d5	Yes	345.3 Unit/Enh (6490)	58.1 Unit/Enh (6490)	87	17	7	4.32	0.5	Positive
Ritalinic acid	No	220.1 Unit/Enh (6490)	84.1 Unit/Enh (6490)	95	20	7	2.63	0.5	Positive
Ritalinic acid	No	220.1 Unit/Enh (6490)	56.1 Unit/Enh (6490)	95	41	7	2.63	0.5	Positive
Tapentadol	No	222.2 Unit/Enh (6490)	107.1 Unit/Enh (6490)	119	25	7	3.08	0.5	Positive
Tapentadol	No	222.2 Unit/Enh (6490)	77.1 Unit/Enh (6490)	119	57	7	3.08	0.5	Positive
Tapentadol-d3	Yes	225.2 Unit/Enh (6490)	107.1 Unit/Enh (6490)	129	25	7	3.02	0.5	Positive
Temazepam	No	301.1 Unit/Enh (6490)	255.1 Unit/Enh (6490)	117	29	7	4.51	0.5	Positive
Temazepam	No	301.1 Unit/Enh (6490)	177 Unit/Enh (6490)	117	45	7	4.51	0.5	Positive
tramadol- ¹³ C-d3	Yes	268.2 Unit/Enh (6490)	58.2 Unit/Enh (6490)	104	17	7	2.9	0.5	Positive
Trazodone	No	372.2 Unit/Enh (6490)	176 Unit/Enh (6490)	159	25	7	3.89	0.5	Positive
Trazodone	No	372.2 Unit/Enh (6490)	148 Unit/Enh (6490)	159	37	7	3.89	0.5	Positive
Triazolam	No	343.1 Unit/Enh (6490)	308.1 Unit/Enh (6490)	140	25	7	4.48	0.5	Positive
Triazolam	No	343.1 Unit/Enh (6490)	239 Unit/Enh (6490)	140	45	7	4.48	0.5	Positive
Verapamil	No	455.3 Unit/Enh (6490)	165 Unit/Enh (6490)	158	37	7	4.26	0.5	Positive
Verapamil	No	455.3 Unit/Enh (6490)	150 Unit/Enh (6490)	158	45	7	4.27	0.5	Positive
Zolpidem	No	308.2 Unit/Enh (6490)	235.6 Unit/Enh (6490)	156	25	7	3.74	0.5	Positive
Zolpidem	No	308.2 Unit/Enh (6490)	235.1 Unit/Enh (6490)	156	37	7	3.74	0.5	Positive
Zolpidem-d6	Yes	314.2 Unit/Enh (6490)	235.1 Unit/Enh (6490)	168	37	7	3.61	0.5	Positive
Zopiclone	No	389.1 Unit/Enh (6490)	245 Unit/Enh (6490)	82	13	7	2.96	0.5	Positive
Zopiclone	No	389.1 Unit/Enh (6490)	217 Unit/Enh (6490)	82	33	7	2.96	0.5	Positive
Zopiclone-d4	Yes	393.1 Unit/Enh (6490)	217 Unit/Enh (6490)	97	33	7	2.88	0.5	Positive

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© Agilent Technologies, Inc., 2014, 2017
Published in the USA, January 25, 2017
5991-1667EN



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