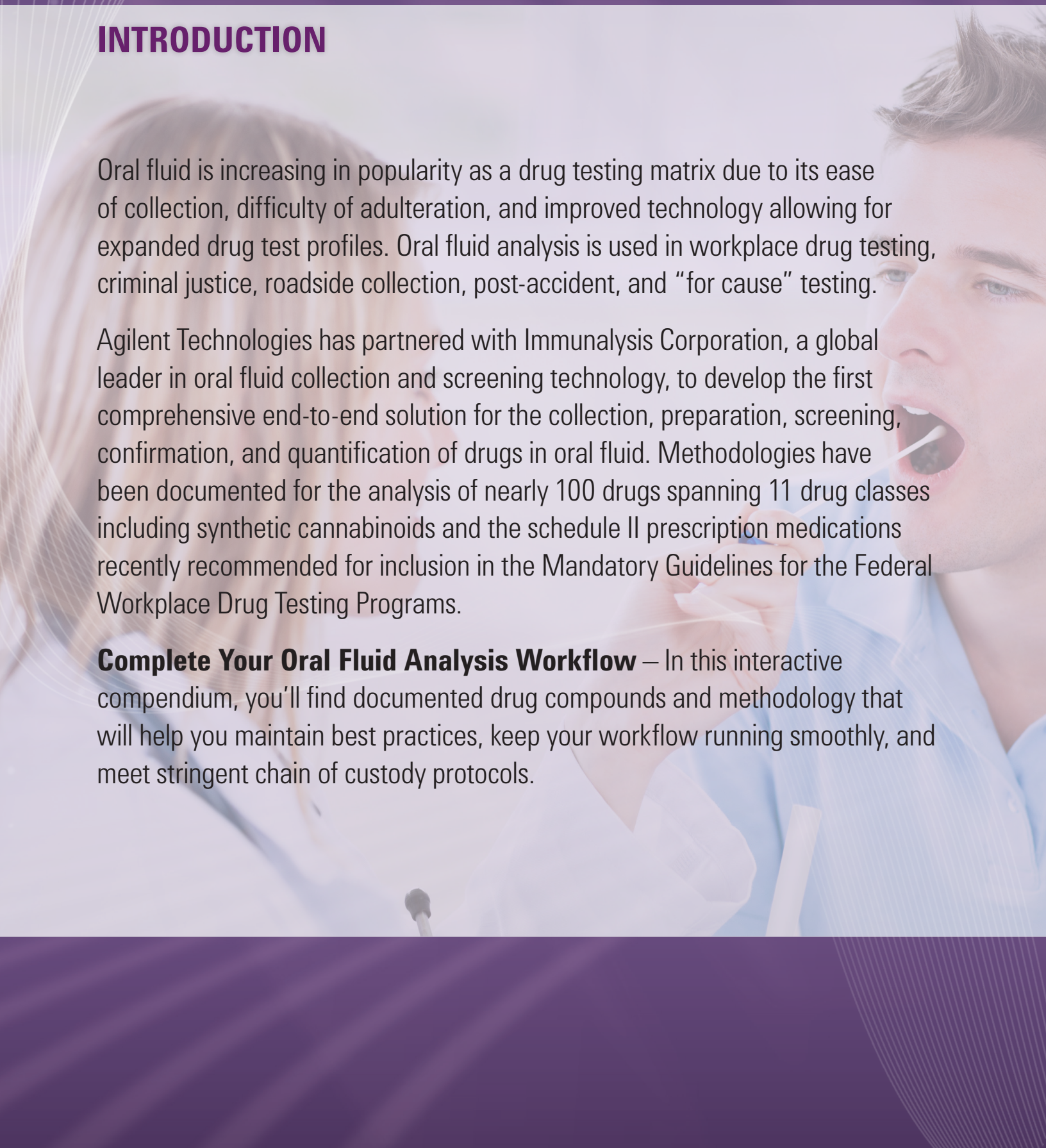
DRUGS AND METABOLITES IN ORAL FLUID: IMMUNOASSAY SCREENING AND LC/MS/MS CONFIRMATION AND QUANTIFICATION

Application Compendium

The Measure of Confidence

Agilent Technologies

INTRODUCTION



Oral fluid is increasing in popularity as a drug testing matrix due to its ease of collection, difficulty of adulteration, and improved technology allowing for expanded drug test profiles. Oral fluid analysis is used in workplace drug testing, criminal justice, roadside collection, post-accident, and "for cause" testing.

Agilent Technologies has partnered with Immunalysis Corporation, a global leader in oral fluid collection and screening technology, to develop the first comprehensive end-to-end solution for the collection, preparation, screening, confirmation, and quantification of drugs in oral fluid. Methodologies have been documented for the analysis of nearly 100 drugs spanning 11 drug classes including synthetic cannabinoids and the schedule II prescription medications recently recommended for inclusion in the Mandatory Guidelines for the Federal Workplace Drug Testing Programs.

Complete Your Oral Fluid Analysis Workflow – In this interactive compendium, you'll find documented drug compounds and methodology that will help you maintain best practices, keep your workflow running smoothly, and meet stringent chain of custody protocols.

COMPLETE YOUR ORAL FLUID ANALYSIS WORKFLOW

CLICK ON A PRODUCT BELOW TO LEARN MORE

SAMPLE COLLECTION

QUANTISAL™ (BY IMMUNALYSIS) »

SAMPLE PREPARATION

BOND ELUT PLEXA (SPE) »

BOND ELUT PLEXA PCX (SPE) »

BOND ELUT CERTIFY »

SAMPLE FILTRATION »

LC AND LC/MS COLUMNS AND SUPPLIES

POROSHELL 120 »
(HPLC AND UHPLC UP TO 600 BAR)

ZORBAX RRHD »
(FOR 1000+ BAR UHPLC)

FAST GUARDS FOR UHPLC »

CAPTIVA FILTER CARTRIDGES »

LC AND LC/MS SUPPLIES »

INSTRUMENTATION

6400 SERIES LC/QQQ »

1290 INFINITY II LC »

SOFTWARE

MASSHUNTER WORKSTATION SOFTWARE »

SERVICES

ADVANTAGE GOLD »

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Methods Compendium Content

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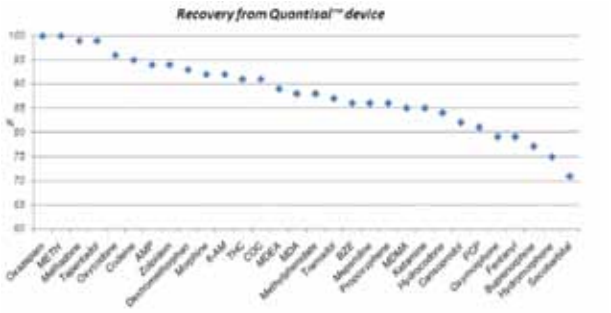
Introduction

Oral fluid is increasing in popularity as a drug testing matrix due to its ease of collection, difficulty of adulteration, and improved technology allowing for expanded drug test profiles. Oral fluid analysis is used in workplace drug testing, criminal justice, roadside collection, post-accident and "for cause" testing.

One of the main issues with the quantitation of drugs in oral fluid is the difficulty of collecting known adequate specimen volume, so that a wide range of compounds can be reliably quantified. Many devices do not give an indication of the amount of oral fluid collected, thereby rendering any quantitative results meaningless without further manipulation in the laboratory. Further, devices incorporating a pad or material for the saliva collection do not always indicate how much of each drug is recovered from the pad before analysis, again making the quantitative result problematic. The analytical methods described here use the Quantisal™ oral fluid collection device (Immunalysis Corporation, shown below), which collects a known amount of neat oral fluid. The efficiency of recovery of many drugs from the collection pad into the transportation buffer has been assessed and is shown below.



Laboratory analysis: It is recommended by the Society of Forensic Toxicologists (SOFT) and the American Academy of Forensic Sciences (AAFS) that the analysis of biological specimens for drugs be carried out using two separate techniques with two different chemical principles when possible. In general, laboratories employ immunoassay screening as the primary test; any presumptive positive results are confirmed using a mass spectrometric technique.

Sample Collection and Preparation

Oral fluid specimens were collected using the Quantisal™ oral fluid collection device (Immunalysis Corporation, Pomona, CA) following the manufacturer's instructions.

Sample Preparation for LC/MS/MS Confirmation and Quantification

The identification involves adding deuterated internal standards to an oral fluid sample, buffering, solid phase extraction (SPE), and analysis by liquid chromatography-tandem mass spectrometry (LC/MS/MS).

Basic drugs (used here for all drugs except the cannabinoids, carisoprodol and meprobamate)

SPE columns: Plexa PCX (p/n 12108601, 60 mg/1 mL or p/n 12108603, 60 mg/3 mL)

1. Remove an aliquot (1 mL) of oral fluid: Quantisal buffer mix, (equivalent to 0.25 mL of neat oral fluid)
2. Add appropriate internal standards and 0.1 M potassium phosphate buffer (pH 6.0; 1 mL) to sample
3. Condition: methanol (0.5 mL)
4. Add samples; do not allow columns to dry completely
5. Wash: 2% formic acid (1 mL); methanol (1 mL)
6. Dry columns under nitrogen pressure (5 min)
7. Elute: Ethyl acetate: ammonium hydroxide (98:2 v,v; 2 mL)
8. Evaporate to dryness; reconstitute in mobile phase (50 µL)

Acidic drugs (used here for the cannabinoids)

SPE columns: Plexa (p/n 12109301 30 mg/1 mL or p/n 12109303 30 mg/3 mL)

1. Remove an aliquot (1 mL) of oral fluid: Quantisal buffer mix, (equivalent to 0.25 mL of neat oral fluid)
2. Add appropriate internal standards and acetic acid (pH 4.0; 1 mL) to sample
3. Condition: methanol (0.5 mL); 0.1 M acetic acid (0.1 mL)
4. Add samples; do not allow columns to dry completely
5. Wash: DI water: acetic acid (80:20 v,v; 1 mL); DI water: methanol (40:60 v,v; 1 mL)
7. Dry columns under nitrogen pressure (5 min)
8. Elute: hexane: acetic acid (98:2 v,v; 1 mL)
9. Evaporate to dryness; reconstitute in mobile phase (50 µL)

Carisoprodol and meprobamate

SPE columns: Plexa (p/n 12109301 30 mg/1 mL or p/n 12109303 30 mg/3 mL)

1. Remove an aliquot (1 mL) of oral fluid: Quantisal buffer mix, (equivalent to 0.25 mL of neat oral fluid)
2. Add appropriate internal standards and 0.1 M potassium phosphate buffer (pH 6.0; 1 mL) to sample
3. Condition: methanol (2 mL), 0.1M potassium phosphate buffer (pH 6.0; 2 mL)
4. Add samples; do not allow columns to dry completely
5. Wash: DI water (2 mL); methanol:DI water 25:75 v/v (1 mL)
6. Dry columns under nitrogen pressure (30 psi, 5 min)
7. Wash: Hexane (1 mL)
8. Dry columns under vacuum (5 min)
9. Elute: Ethyl acetate: hexane 50:50 v/v (3 mL)
10. Evaporate to dryness; reconstitute in mobile phase (50 µL)

LC/MS/MS Instrumentation

The work described here was carried out on the following system:

Agilent 1200 Series UHPLC, consisting of:
G1379B micro vacuum degasser

G1312B SL (600 bar) binary pump with solvent selection valve; standard delay volume configuration

G1367D wellplate sampler, with 0.2µ inline filter (PN 5067-1553) between needle seat and valve

G1316B thermostatted column compartment, with 6-port column switching valve

Agilent 6430A triple quadrupole LC/MS/MS equipped with Agilent orthogonal ESI source. Ionization mode was positive ion unless otherwise noted for a particular compound.

The Agilent Zorbax RRHT (1.8µ particle size, 600 bar) UHPLC columns utilized in this work included:

Stationary Phase	i.d. x length, mm	Agilent part number
Eclipse Plus C18	4.6 x 50	959941-902
Eclipse Plus C18	2.1 x 50	959741-902
Extend-C18	2.1 x 50	727700-902

The column used for each compound is specified in the method descriptions in section VI

NOTES

1. In the Screening Methodology table, "No data" for cross-reactivity indicates that the compound has not been evaluated for that ELISA kit.

2. This compendium shows several LC methods with gradients which have %B actually *decreasing* in the last line of the gradient table after the gradient goes to high organic, e.g. 95% B. This is not a typographical error, but a technique which was used to sharpen late-eluting peaks for some difficult compounds.

3. In the LC-MS/MS transitions table for each drug, the transition used for quantification is shown in **bold** font, as in the example below:

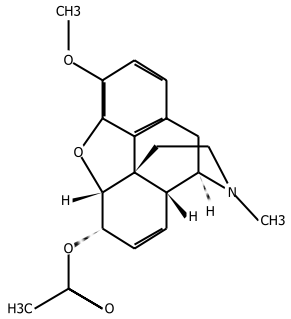
Compound	Precursor	Product	Fragmentor (V)	Collision Energy (V)
THC-d_3	318.3	196.3	125	20
THC	**315.4**	**193.3**	**150**	**20**
THC	315.4	123.3	150	30

Screening and Confirmation Methods by Compound

6-Acetylcodeine (6-AC)

Chemical name:	6-Monoacetylcodeine; 6-MAC; (IUPAC name) 3-methoxy-6-acetyl-(5α,6α)-7,8-didehydro-4,5-epoxy-17-methylmorphinan
Molecular formula:	$C_{20}H_{23}NO_4$
Molecular weight*:	341.4

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #207OF

Cut-off: 20 ng/mL	**Target Compound:** Morphine **Significant cross-reactivity:**	
	Morphine	100%
	6-AC	41%
	6-AM	83%
	Codeine	200%
	Hydrocodone	93%

Confirmatory methodology: LC-MS/MS
LOQ 4 ng/mL

LC Parameters

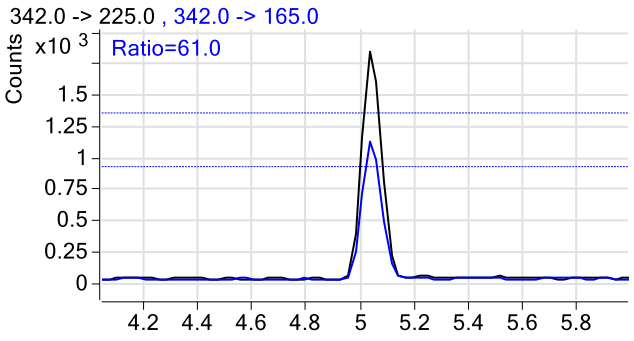
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	60 °C
Mobile phase	A = 20mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 6 min 78% B. Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
6-AM-d_3	331	165	160	35
6-AC	**342**	**225**	**160**	**30**
6-AC	342	165	160	40



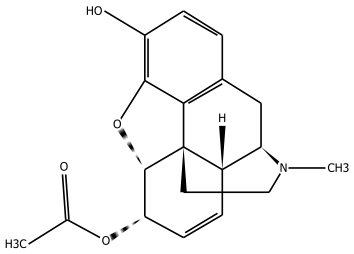
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

6-Acetylmorphine (6-AM)

Chemical name:	6-Monoacetylmorphine; 6-MAM; (IUPAC name) 6-*O*-Acetylmorphine
Molecular formula:	$C_{19}H_{21}NO_4$
Molecular weight*:	327.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #207OF

Cut-off: 20 ng/mL	**Target Compound:** Morphine **Significant cross-reactivity:**	
	Morphine	100%
	6-AM	83%
	Codeine	200%
	Hydrocodone	93%
	Dihydrocodeine	85%

Confirmatory methodology: LC-MS/MS
LOQ 4ng/mL

LC Parameters

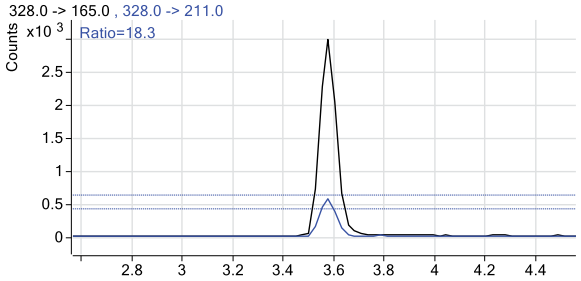
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	60 °C
Mobile phase	A = 20mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 6 min 78% B. Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
6-AM-d_3	331	165	160	35
6-AM	328	211	160	40
6-AM	**328**	**165**	**160**	**40**



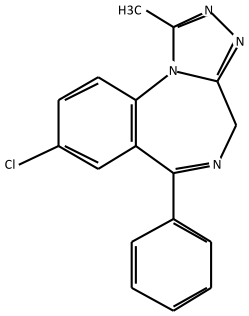
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Alprazolam

Chemical name:	(IUPAC Name) 8-Chloro-1-methyl-6-phenyl-4H-[1, 2, 4]-triazolo[4, 3-*a*][1,4]-benzodiazepine
Molecular formula:	$C_{17}H_{13}ClN_4$
Molecular weight*:	308.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Alprazolam	180%
	Clonazepam	70%
	Temazepam	200%
	Diazepam	70%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

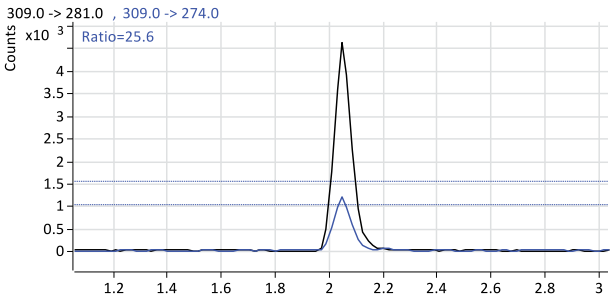
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Alprazolam-d_5	314	286	160	25
Alprazolam	**309**	**281**	**160**	**25**
Alprazolam	309	274	160	30



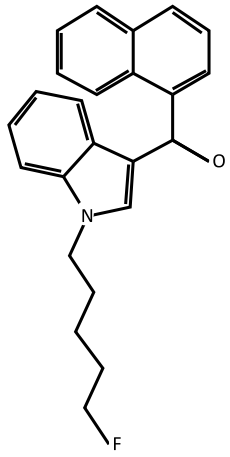
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

AM-2201

Chemical name:	(IUPAC Name) 1-[(5-fluoropentyl)-1H-indol-3-yl]-(naphthalen-1-yl)methanone
Molecular formula:	$C_{24}H_{22}FNO$
Molecular weight*:	359.435

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #244

Cut-off: 5 ng/mL	**Target Compound:** JWH-200 **Significant cross-reactivity:**	
	JWH-200	100%
	AM 2201	50%
	JWH-073	31%
	JWH-018	22%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

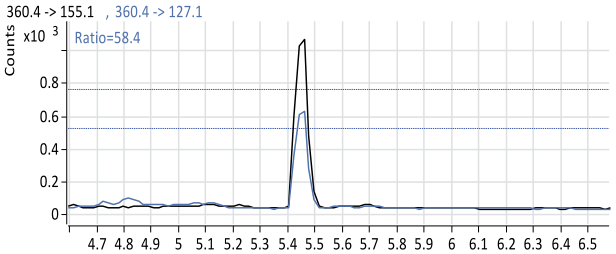
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 2.1 x 50 mm, 1.8 µm
Flow rate	0.4 mL/min
Column temperature	60 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Acetonitrile
Gradient	0 min 5% B 5 min 100% B 7 min 1% B 9 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	55 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	2500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
JWH-073-d_7	335.3	207.2	120	20
AM-2201	**360.4**	**155.1**	**160**	**25**
AM-2201	360.4	127.1	160	35



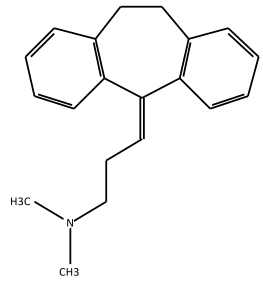
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Amitriptyline

Chemical name:	10, 11-Dihydro-N, N-dimethyl-5H-dibenzo[*a, d*] cycloheptene-Δ5, γ-propylamine
Molecular formula:	$C_{20}H_{23}N$
Molecular weight*:	277.4

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: 25 ng/mL	**Target Compound:** Nortriptyline **Significant cross-reactivity:**	
	Nortriptyline	100%
	Amitriptyline	200%
	Desipramine	200%
	Imipramine	200%
	Trimipramine	50%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

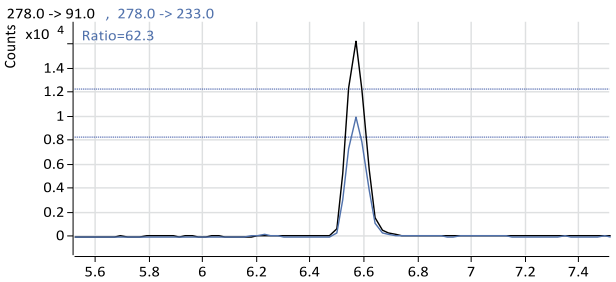
MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Nortriptyline-d_3	267	233	100	10
Amitriptyline	278	233	100	10
Amitriptyline	**278**	**91**	**100**	**20**

*Monoisotopic molecular weight

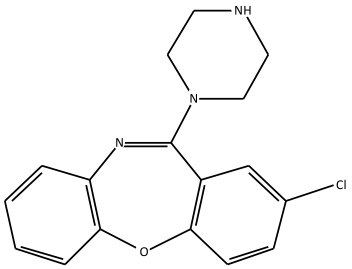


MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Amoxapine

Chemical name:	2-Chloro-11-(1-piperazinyl)dibenz[b, f]oxazepine
Molecular formula:	$C_{17}H_{16}ClN_3O$
Molecular weight*:	313.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog # 222

Cut-off: N/A	**Target Compound:** Nortriptyline **Significant cross-reactivity:**
	No data

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

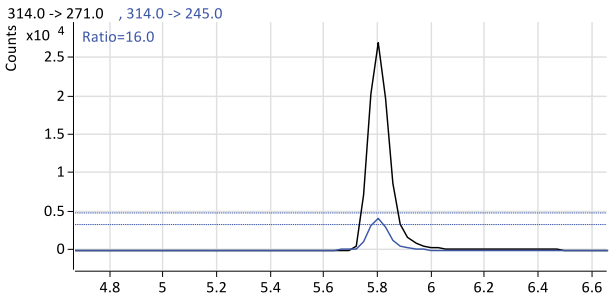
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Paroxetine-d_6	336	76	120	20
Amoxapine	**314**	**271**	**120**	**20**
Amoxapine	314	245	120	20



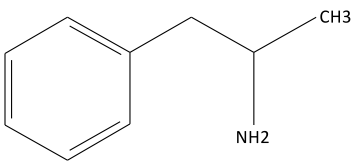
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Amphetamine

Chemical name:	Desoxynorephedrine; (IUPAC name) 1-Phenylpropan-2-amine
Molecular formula:	$C_9H_{13}N$
Molecular weight*:	135.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #209OF

Cut-off: 25 ng/mL	**Target Compound:** d-Amphetamine **Significant cross-reactivity:**	
	d-Amphetamine	100%
	l-Amphetamine	9.7%
	dl-MDA	178%
	Phentermine	89%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters (Method includes related compounds at longer retention times.)

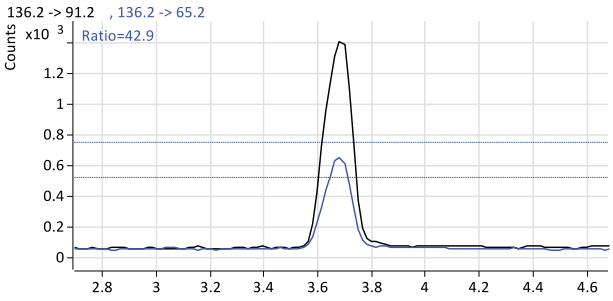
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 8 min 50% B 9 min 15% B 12 min Stop. Post-time 1 min
Injection volume	5 µL

MS Parameters

Nebulizer	40 psi
Drying gas flow	6 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Amphetamine-d_5	141.2	96.1	80	15
Amphetamine	**136.2**	**91.2**	**80**	**25**
Amphetamine	136.2	65.2	80	45



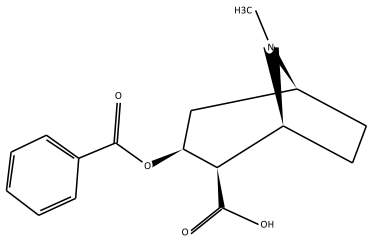
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Benzoylecgonine

Chemical name:	Ecgonine benzoate; (IUPAC name) 3-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid
Molecular formula:	$C_{16}H_{19}NO_4$
Molecular weight*:	289.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #212OF

Cut-off: 20 ng/mL	**Target Compound:** Benzoylecgonine **Significant cross-reactivity:**	
	Benzoylecgonine	100%
	Cocaethylene	90%
	Cocaine	70%
	Norcocaine	0.2%

Confirmatory methodology: LC-MS/MS
LOQ 2 ng/mL

LC Parameters

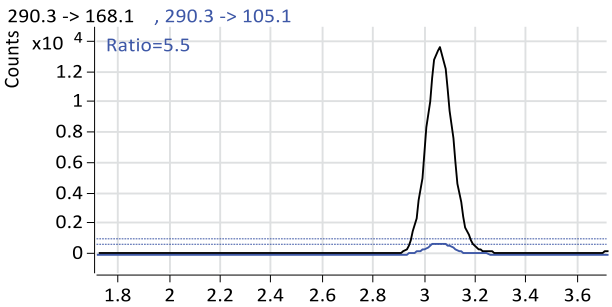
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.2 mL/min
Column temperature	60 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 80% B 4 min 30% 6 min Stop. Post-time 4 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Benzoylecgonine-d_3	293.3	171.2	120	20
Benzoylecgonine	**290.3**	**168.1**	**120**	**15**
Benzoylecgonine	290.3	105.1	100	15



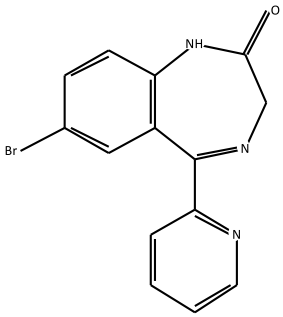
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Bromazepam

Chemical name:	7-Bromo-1, 3-dihydro-5-(2-pyridinyl)-2H-1, 4-benzodiazepin-2-one
Molecular formula:	$C_{14}H_{10}BrN_3O$
Molecular weight*:	315.0

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Bromazepam	70%
	Alprazolam	180%
	Temazepam	200%
	Diazepam	70%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

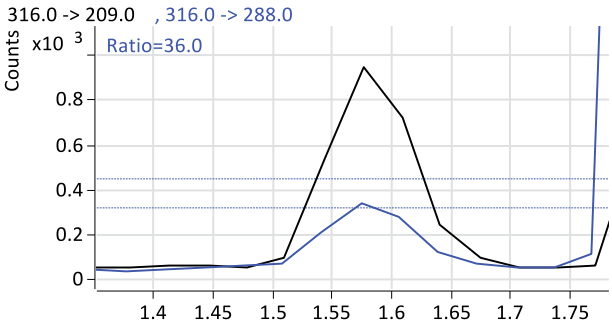
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Alprazolam-d_5	314	286	160	25
Bromazepam	316	288	160	20
Bromazepam	**316**	**209**	**160**	**30**



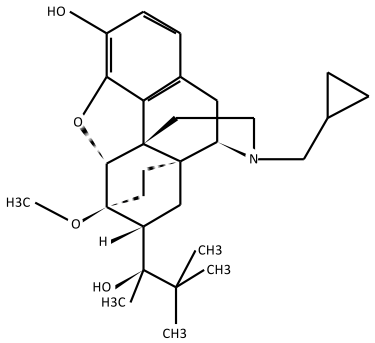
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Buprenorphine

Chemical name:	[5α, 7α(S)]-17-(cyclopropylmethyl)-α-(1, 1-dimethylethyl)-4, 5-epoxy-18, 19-dihydro-3-hydroxy-6-methoxy-α-methyl-6, 14-ethenomorphinan-7-methanol
Molecular formula:	$C_{29}H_{41}NO_4$
Molecular weight*:	467.3

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #236

Cut-off: 5 ng/mL	**Target Compound:** Buprenorphine **Significant cross-reactivity:**	
	Buprenorphine	100%
	Norbuprenorphine	120%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

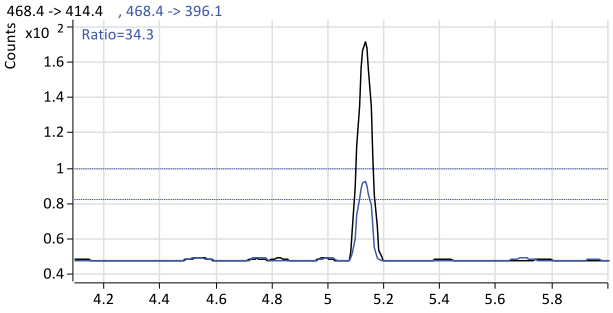
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.8 mL/min
Column temperature	50 °C
Mobile phase	A = 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 25% B 3 min 100% B 6 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300°C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Buprenorphine-d_4	472.5	400.4	240	45
Buprenorphine	**468.4**	**414.4**	**240**	**35**
Buprenorphine	468.4	396.1	240	55



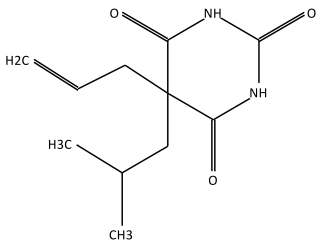
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Butalbital

Chemical name:	5-(2-Methylpropyl)-5-(2-propenyl)-2, 4, 6(1H, 3H, 5H)-pyrimidinetrione
Molecular formula:	$C_{11}H_{16}N_2O_3$
Molecular weight*:	224.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #210

Cut-off: 50 ng/mL	**Target Compound:** Secobarbital **Significant cross-reactivity:**	
	Secobarbital	100%
	Butalbital	83%
	Aprobarbital	89%
	Pentobarbital	83%
	Phenobarbital	50%
	Butabarbital	33%

Confirmatory methodology: LC-MS/MS
LOQ 25 ng/mL

LC Parameters

Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.8 mL/min
Column temperature	35 °C
Mobile phase	A = water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 5% B 0.2 min 5% B 5 min 95% B 5.2 min 95% B 6 min 5% 8.2 min Stop. Post-time Off
Injection volume	5 µL

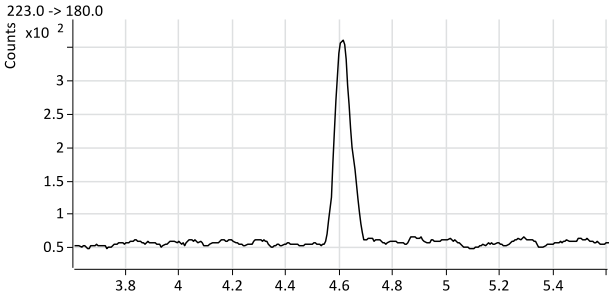
MS Parameters – negative ion

Nebulizer	35 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	4500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Pentobarbital-d_5	230	42	120	15
Butalbital	**223**	**180**	**100**	**4**

*Monoisotopic molecular weight

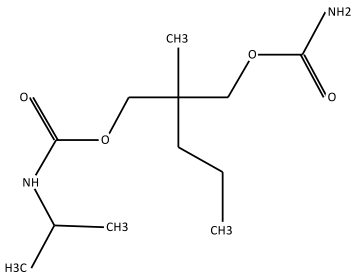


MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Carisoprodol

Chemical name:	N-isopropylmeprobamate; 2-[[(Aminocarbonyl)oxy]methyl]-2-methylpentyl(1-methylethyl)carbamate
Molecular formula:	$C_{12}H_{24}N_2O_4$
Molecular weight*:	260.17

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #231

Cut-off: 50 ng/mL	**Target Compound:** Carisoprodol **Significant cross-reactivity:**	
	Carisoprodol	100%
	Meprobamate	118%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

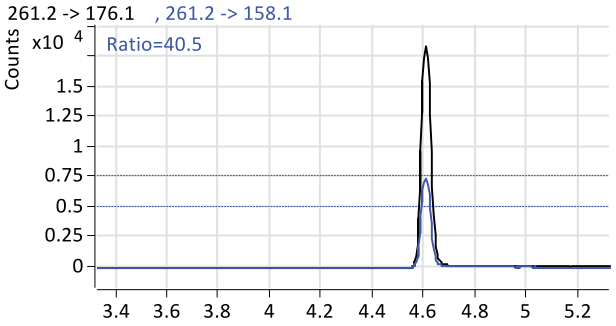
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 4 min 100% B 5 min 15% B 5.5 min Stop. Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	40 psi
Drying gas flow	8 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Carisoprodol-d_7	268.2	183.2	60	2
Carisoprodol	**261.2**	**176.1**	**60**	**2**
Carisoprodol	261.2	158.1	60	2



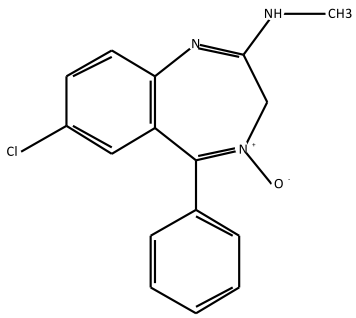
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Chlordiazepoxide

Chemical name:	7-chloro-N-methyl-5-phenyl-3H-1, 4-benzodiazepin-2-amine-4-oxide
Molecular formula:	$C_{16}H_{14}ClN_3O$
Molecular weight*:	300.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Chlordiazepoxide	30%
	Diazepam	70%
	Nordiazepam	50%
	Temazepam	200%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

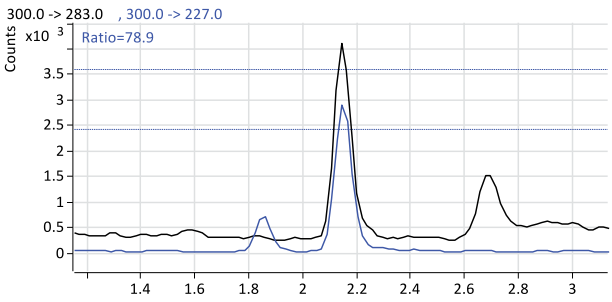
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Alprazolam-d_5	314	286	160	25
Chlordiazepoxide	**300**	**283**	**120**	**15**
Chlordiazepoxide	300	227	120	30



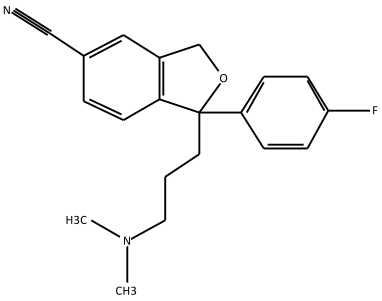
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Citalopram

Chemical name:	(IUPAC name) 1-[3-Dimethylamino)propyl]-1-(4-fluorophenyl)-1, 3-dihydro-5-isobenzofurancarbonitrile
Molecular formula:	$C_{20}H_{21}FN_2O$
Molecular weight*:	324.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: N/A	**Target Compound:** Nortriptyline **Significant cross-reactivity:**
	No Data

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

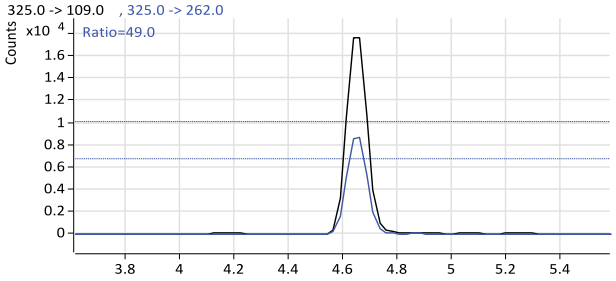
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Paroxetine-d_6	336	76	120	20
Citalopram	325	262	110	15
Citalopram	**325**	**109**	**110**	**20**



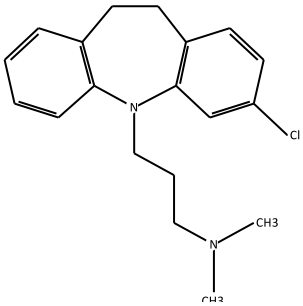
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Clomipramine

Chemical name:	Monochlorimipramine; 3-Chloro-10, 11-dihydro-N, N-dimethyl-5H-dibenz[b, f]azepine-5-propanamine
Molecular formula:	$C_{19}H_{23}ClN_2$
Molecular weight*:	314.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: 25 ng/mL	**Target Compound:** Nortriptyline **Significant cross-reactivity:**	
	Nortriptyline	100%
	Clomipramine	40%
	Desipramine	200%
	Imipramine	200%
	Trimipramine	50%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

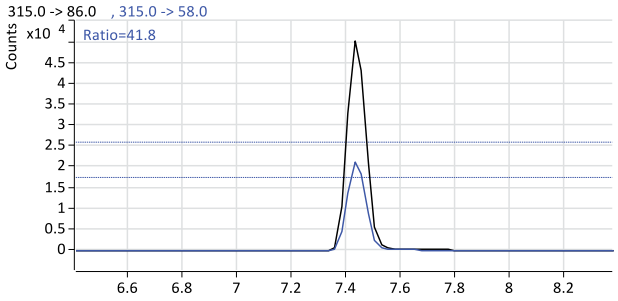
MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Paroxetine-d_6	336	76	120	20
Clomipramine	**315**	**86**	**110**	**15**
Clomipramine	315	58	110	35

*Monoisotopic molecular weight

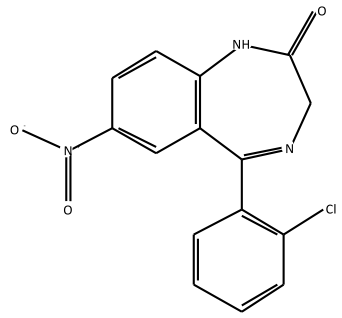


MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Clonazepam

Chemical name:	5-(2-Chlorophenyl)-1, 3-dihydro-7-nitro-2H-1, 4-benzodiazepin-2-one
Molecular formula:	$C_{15}H_{10}ClN_3O_3$
Molecular weight*:	315.0

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Clonazepam	70%
	Alprazolam	180%
	Temazepam	200%
	Diazepam	70%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

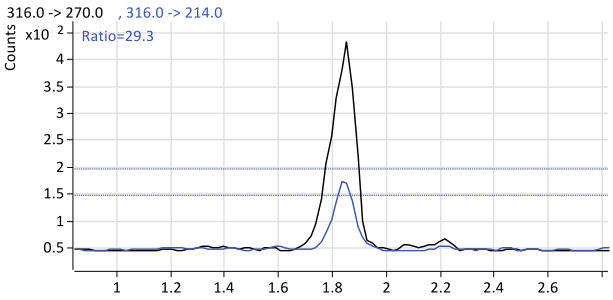
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Clonazepam-d_4	320	274	120	25
Clonazepam	**316**	**270**	**120**	**25**
Clonazepam	316	214	120	35



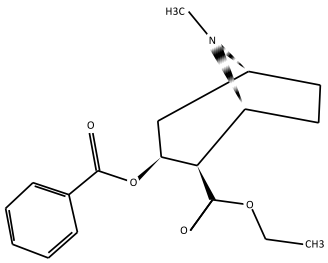
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Cocaethylene

Chemical name:	Ethylbenzoylecgonine; (IUPAC Name) Ethyl-(2R,3S)-3-benzoy-loxy-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate
Molecular formula:	$C_{18}H_{23}NO_4$
Molecular weight*:	317.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #212OF

Cut-off: 20 ng/mL **Target Compound:** Benzoylecgonine

Significant cross-reactivity:

Benzoylecgonine	100%
Cocaethylene	90%
Cocaine	70%
Norcocaine	0.2%

Confirmatory methodology: LC-MS/MS

LOQ 2 ng/mL

LC Parameters

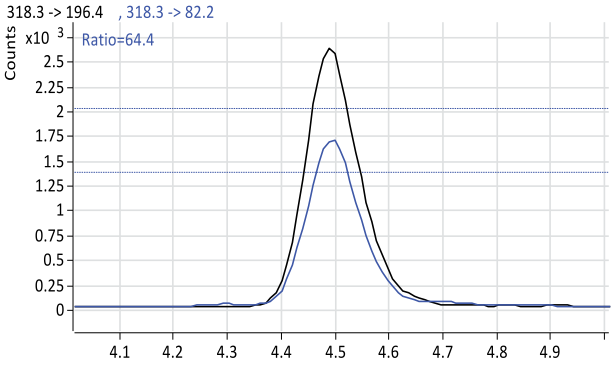
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.2 mL/min
Column temperature	60 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 80% B 4 min 30% 6 min Stop. Post-time 4 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	Precursor	Product	Fragmentor (V)	Collision Energy (V)
Cocaethylene-d_8	326.3	204.4	160	20
Cocaethylene	**318.3**	**196.4**	**120**	**25**
Cocaethylene	318.3	82.2	120	25



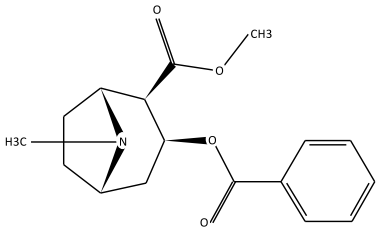
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Cocaine

Chemical name:	Methyl benzoylecgonine; (IUPAC name) Methyl-(1S, 3S, 4R, 5R)-3-benzoyloxy-8-methyl-8-azabicyclo[3.2.1]octane-4-carboxylate
Molecular formula:	$C_{17}H_{21}NO_4$
Molecular weight*:	303.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #212OF

Cut-off: 20 ng/mL	**Target Compound:** Benzoylecgonine **Significant cross-reactivity:**	
	Benzoylecgonine	100%
	Cocaethylene	90%
	Cocaine	70%
	Norcocaine	0.2%

Confirmatory methodology: LC-MS/MS
LOQ 2 ng/mL

LC Parameters

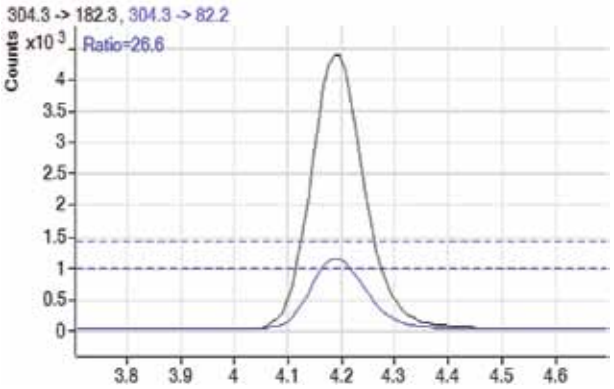
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.2 mL/min
Column temperature	60 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 80% B 4 min 30% 6 min Stop. Post-time 4 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Cocaine-d3	307.3	185.3	120	20
Cocaine	**304.3**	**182.3**	**120**	**20**
Cocaine	304.3	82.2	120	25



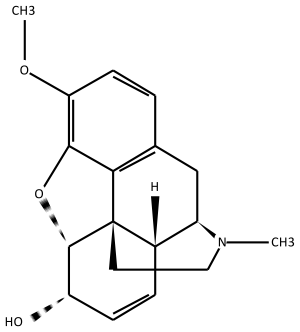
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Codeine

Chemical name:	(IUPAC Name) (5α, 6α)-3-Methoxy-17-methyl-7, 8-didehydro-4, 5-epoxymorphinan-6-ol
Molecular formula:	$C_{18}H_{21}NO_3$
Molecular weight*:	299.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #207OF

Cut-off: 20 ng/mL	**Target Compound:** Morphine **Significant cross-reactivity:**	
	Morphine	100%
	6-AM	83%
	Codeine	200%
	Hydrocodone	93%
	Dihydrocodeine	85%

Confirmatory methodology: LC-MS/MS
LOQ 4 ng/mL

LC Parameters

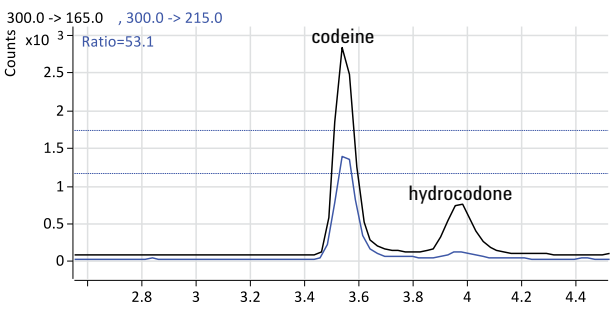
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	60 °C
Mobile phase	A = water 20 mM ammonium pH 6.4, B = Methanol
Gradient	0 min 15% B 6 min 78% B Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Codeine-d_3	303	165	140	40
Codeine	300	215	140	25
Codeine	**300**	**165**	**140**	**45**



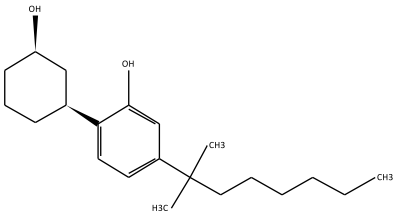
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

CP 47, 497

Chemical name:	(IUPAC Name) 1-[1R,3S)-3-Hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol
Molecular formula:	$C_{21}H_{34}O_2$
Molecular weight*:	318.5

Molecular Structure:



Screening methodology: Immunalysis ELISA in development

Cut-off: N/A	**Target Compound:** N/A **Significant cross-reactivity:**	
	None	N/A

Confirmatory methodology: LC-MS/MS
LOQ 2 ng/mL

LC Parameters

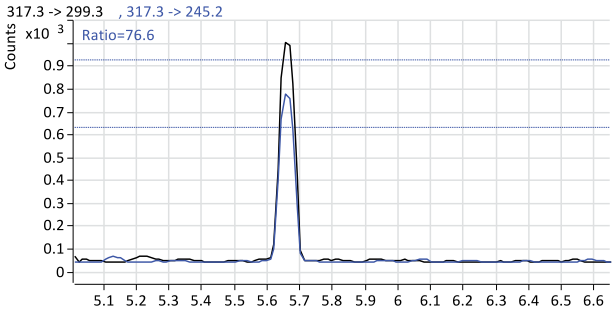
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 2.1 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	60 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Acetonitrile
Gradient	0 min 5% B 5 min 100% B 7 min 1% B 9.2 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters – negative ion

Nebulizer	55 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
JWH-073-d_7	335.3	207.2	120	20
CP 47497	**317.3**	**299.3**	**160**	**20**
CP 47497	317.3	245.2	160	30



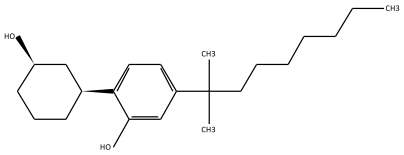
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

CP 47, 497-C8

Chemical name:	2-[(1R,3S)-3-hydroxycyclohexyl]-5-(2-methylnonan-2-yl) phenol
Molecular formula:	$C_{22}H_{36}O_2$
Molecular weight*:	332.3

Molecular Structure:



Screening methodology: Immunalysis ELISA in development

Cut-off: N/A	**Target Compound:** N/A **Significant cross-reactivity:**	
	None	N/A

Confirmatory methodology: LC-MS/MS
LOQ 2 ng/mL

LC Parameters

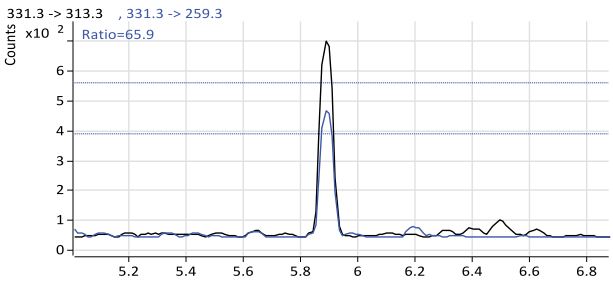
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 2.1 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	60 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Acetonitrile
Gradient	0 min 5% B 5 min 100% B 7 min 1% B 9.2 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters – negative ion

Nebulizer	55 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
JWH-073-d_7	335.3	207.2	120	20
CP 47497 C8	**331.3**	**313.3**	**160**	**25**
CP 47497 C8	331.3	259.3	160	35



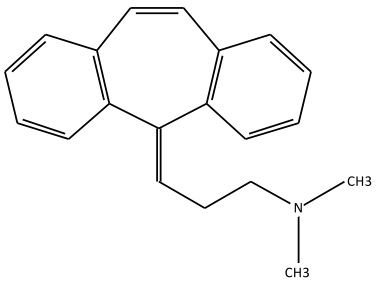
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Cyclobenzaprine

Chemical name:	Proheptatriene; (IUPAC name) 3-(5H-Dibenzo[*a, d*]cyclohepten-5-ylidene)-N, N-dimethyl-1-propanamine
Molecular formula:	$C_{20}H_{21}N$
Molecular weight*:	275.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #242

Cut-off: 25 ng/mL	**Target Compound:** Diphenhydramine **Significant cross-reactivity:**	
	Diphenhydramine	100%
	Cyclobenzaprine	200%
	Amitriptyline	100%
	Doxepin	50%
	Clomipramine	20%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

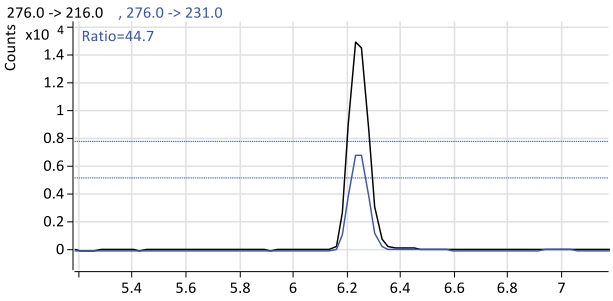
MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Paroxetine-d_6	336	76	120	20
Cyclobenzaprine	276	231	110	10
Cyclobenzaprine	**276**	**216**	**110**	**20**

*Monoisotopic molecular weight

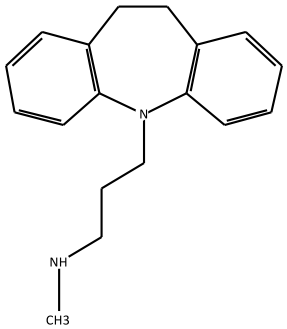


MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Desipramine

Chemical name:	Desmethylimipramine; (IUPAC name) 10, 11-Dihydro-N-methyl-5H-dibenz[*b, f*]azepine-5-propanamine
Molecular formula:	$C_{18}H_{22}N_2$
Molecular weight*:	266.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: 25 ng/mL **Target Compound:** Nortriptyline

Significant cross-reactivity:

Nortriptyline	100%
Amitriptyline	83%
Desipramine	10%
Imipramine	<1%
Trimipramine	<1%

Confirmatory methodology: LC-MS/MS

LOQ 10 ng/mL

LC Parameters

Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

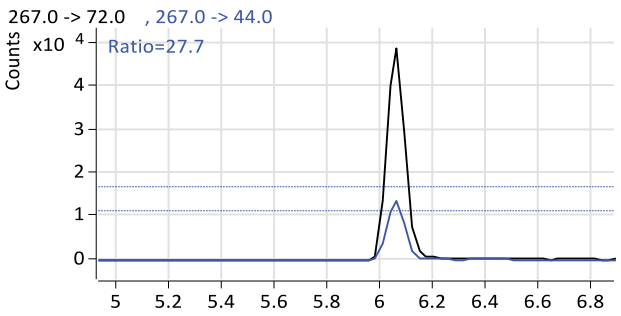
MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	Precursor	Product	Fragmentor (V)	Collision Energy (V)
Paroxetine-d_6	336	76	120	20
Desipramine	**267**	**72**	**110**	**15**
Desipramine	267	44	110	30

*Monoisotopic molecular weight

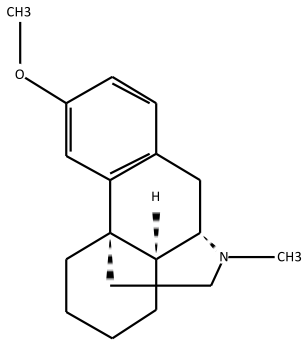


MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Dextromethorphan

Chemical name:	Deoxydihydrothebacodine
Molecular formula:	$C_{18}H_{25}NO$
Molecular weight*:	271.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #238

Cut-off: 50 ng/mL	**Target Compound:** Dextromethorphan **Significant cross-reactivity:**	
	Dextromethorphan	100%
	Dextrorphan	83%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

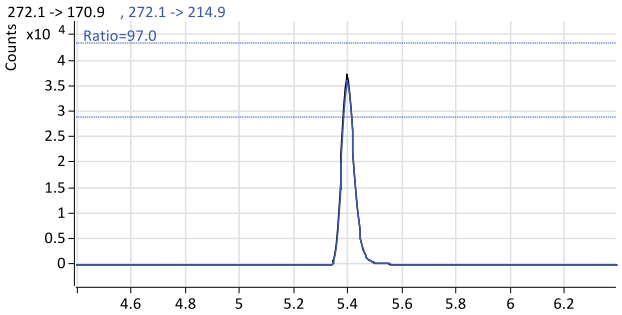
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	40 °C
Mobile phase	A = water 20mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 25% B 4 min 100% B 5 min 25% B 6.5 min Stop. Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350°C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Dextrorphan-d_3	261	156.9	120	40
Dextromethorphan	272.1	214.9	120	20
Dextromethorphan	**272.1**	**170.9**	**120**	**40**



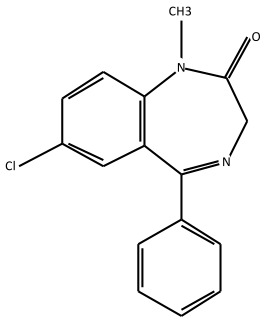
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Diazepam

Chemical name:	(IUPAC name) 7-Chloro-1, 3-dihydro-1-methyl-5-phenyl-3H-1, 4-benzodiazepin-2-one
Molecular formula:	$C_{15}H_{10}ClN_3O_3$
Molecular weight*:	315

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Diazepam	70%
	Nordiazepam	50%
	Temazepam	200%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

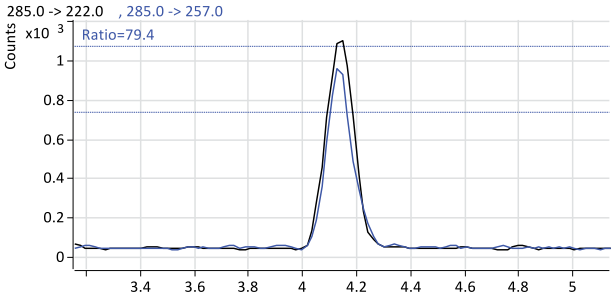
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Diazepam-d_5	290	262	160	25
Diazepam	285	257	160	25
Diazepam	**285**	**222**	**160**	**25**



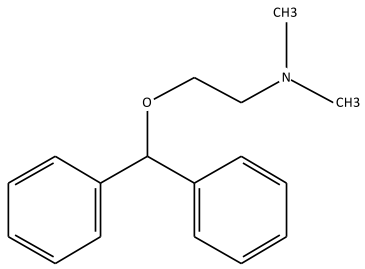
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Diphenhydramine

Chemical name:	Benzhydramine; (IUPAC name) 2-Diphenylmethoxy-N, N-dimethylethanamine
Molecular formula:	$C_{17}H_{21}NO$
Molecular weight*:	255.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #242

Cut-off: 25 ng/mL	**Target Compound:** Diphenhydramine **Significant cross-reactivity:**	
	Diphenhydramine	100%
	Cyclobenzaprine	200%
	Amitriptyline	100%
	Doxepin	50%
	Clomipramine	20%

Confirmatory methodology: LC-MS/MS
LOQ 5 ng/mL

LC Parameters

Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	60 °C
Mobile phase	A = water 0.2% Acetic Acid pH 4.0, B = Methanol
Gradient	0 min 0% B 2 min 0% B 6 min 100% B 7 min 0% B 10 min Stop. Post-time Off
Injection volume	5 µL

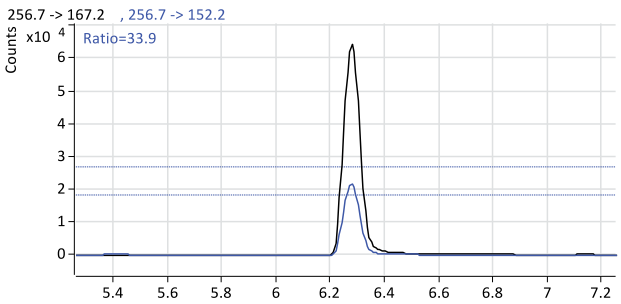
MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Diphenhydramine-d_3	259.7	165.2	80	35
Diphenhydramine	**256.7**	**167.2**	**80**	**15**
Diphenhydramine	256.7	152.2	80	35

*Monoisotopic molecular weight

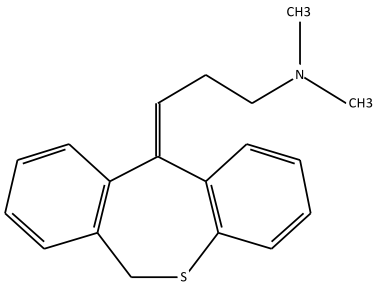


MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Dothiepin

Chemical name:	Dosulepin; 3-Dibenzo[*b, e*]thiepin-11(6H)-ylidine-N, N-dimethyl-1-propanamine
Molecular formula:	$C_{19}H_{21}NS$
Molecular weight*:	295.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: 25 ng/mL	**Target Compound:** Nortriptyline **Significant cross-reactivity:**	
	Nortriptyline	100%
	Dothiepin	25%
	Desipramine	200%
	Imipramine	200%
	Trimipramine	50%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

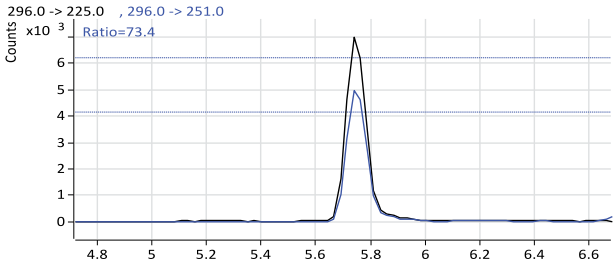
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Paroxetine-d_6	336	76	120	20
Dothiepin	296	251	120	15
Dothiepin	**296**	**225**	**120**	**15**



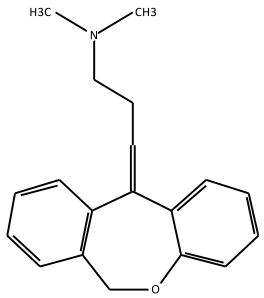
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Doxepin

Chemical name:	(IUPAC name) (3E)-3-(6H-Benzo[*c*][1]benzoxepin-11-ylidene)N, N-dimethylpropanamine
Molecular formula:	$C_{19}H_{21}NO$
Molecular weight*:	279.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: 25 ng/mL	**Target Compound:** Nortriptyline **Significant cross-reactivity:**	
	Nortriptyline	100%
	Doxepin	15%
	Desipramine	200%
	Imipramine	200%
	Trimipramine	50%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

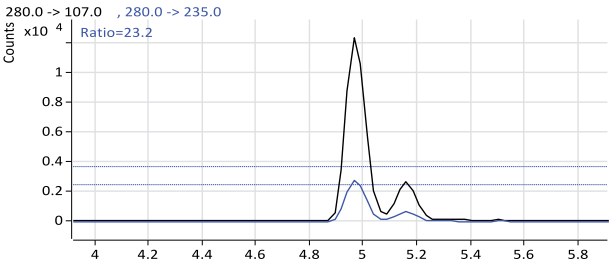
Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Paroxetine-d_6	336	76	120	20
Doxepin	280	235	120	10
Doxepin	**280**	**107**	**120**	**20**

*Monoisotopic molecular weight

Note: doxepin consists of cis and trans isomers which are separated by these LC conditions.

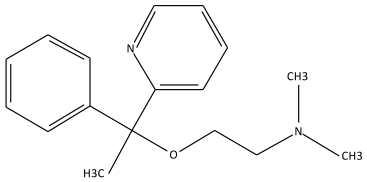


MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Doxylamine

Chemical name:	Histadoxylamine; N, N-Dimethyl-2-[1-phenyl-1-(2-pyridinyl) ethoxy]ethanamine
Molecular formula:	$C_{17}H_{22}N_2O$
Molecular weight*:	270.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #242

Cut-off: 25 ng/mL **Target Compound:** Diphenhydramine

Significant cross-reactivity:

Diphenhydramine	100%
Doxylamine	1%
Cyclobenzaprine	200%
Amitriptyline	100%
Doxepin	50%

Confirmatory methodology: LC-MS/MS
LOQ 5 ng/mL

LC Parameters

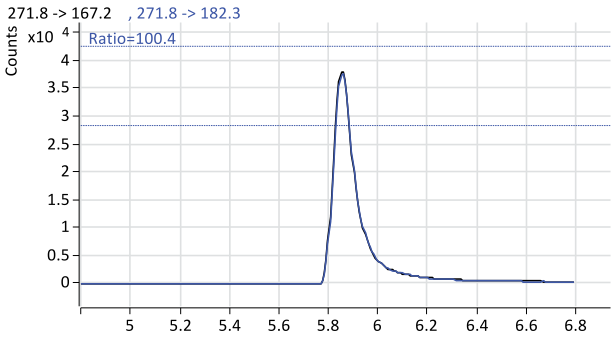
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	60 °C
Mobile phase	A = water 0.2% Acetic Acid pH 4.0, B = Methanol
Gradient	0 min 0% B 2 min 0% B 6 min 100% B 7 min 0% B 10 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	Precursor	Product	Fragmentor (V)	Collision Energy (V)
Chorpheniramine-d_6	281.8	118	80	35
Doxylamine	271.8	182.3	95	10
Doxylamine	**271.8**	**167.2**	**95**	**35**



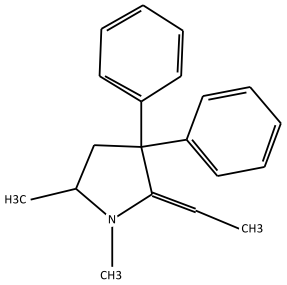
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

EDDP

Chemical name:	2-Ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine
Molecular formula:	$C_{20}H_{23}N$
Molecular weight*:	277.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #232

Cut-off: 50 ng/mL	**Target Compound:** Methadone **Significant cross-reactivity:**	
	Methadone	100%
	Methadol	50%
	EDDP	<5%
	LAAM	15%
	Nor-LAAM	<1%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

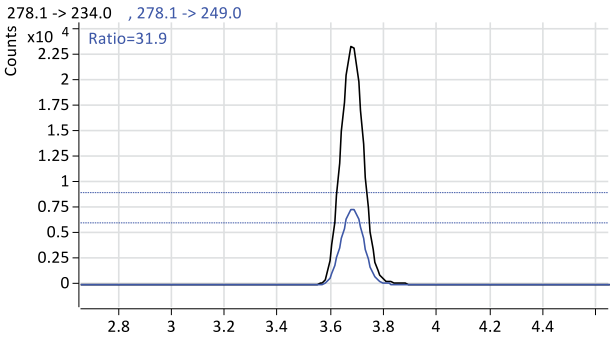
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	40 °C
Mobile phase	A = 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 50% B 4 min 100% B 5 min 25% B 6.5 min Stop. Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
EDDP-d_3	281.1	234	120	20
EDDP	278.1	249	120	20
EDDP	**278.1**	**234**	**120**	**30**



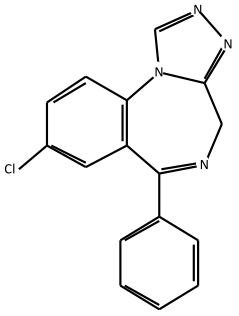
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Estazolam

Chemical name:	(IUPAC name) 8-Chloro-6-phenyl-4H-1, 2, 4-triazolo[4, 3α]-1, 4-benzodiazepine
Molecular formula:	$C_{16}H_{11}ClN_4$
Molecular weight*:	294.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Estazolam	70%
	Alprazolam	180%
	Temazepam	200%
	Diazepam	70%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

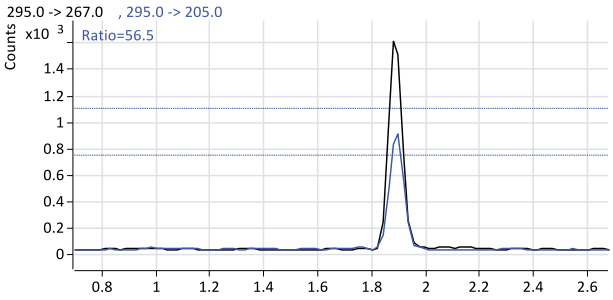
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Estazolam-d_5	300	272	160	25
Estazolam	**295**	**267**	**140**	**25**
Estazolam	295	205	140	35



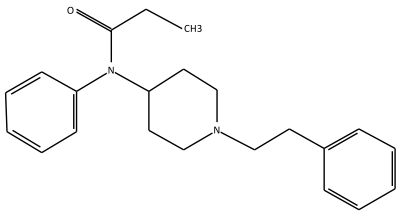
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Fentanyl

Chemical name:	N-Phenyl-N-[1-(2–phenylethyl)-4–piperidinyl]propanamide
Molecular formula:	$C_{22}H_{28}N_2O$
Molecular weight*:	336.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #218

Cut-off: 1 ng/mL	**Target Compound:** Fentanyl **Significant cross-reactivity:**	
	Fentanyl	100%
	Hydroxyfentanyl	83%
	Despropionylfentanyl	10%
	Norfentanyl	<1%
	Hydroxy norfentanyl	<1%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

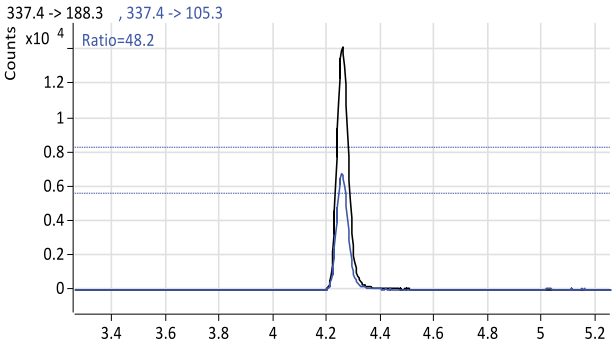
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 50% B 0.1 min 50% B 4 min 100% B 5 min 15% B 6 min Stop. Post-time 3 min
Injection volume	10 µL

MS Parameters – negative ion

Nebulizer	50 psi
Drying gas flow	12 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Fentanyl-d_5	342.4	221.5	160	20
Fentanyl	**337.4**	**188.3**	**160**	**25**
Fentanyl	337.4	105.3	160	25



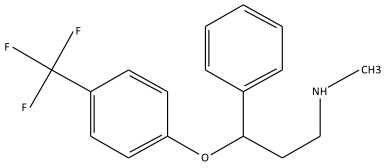
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Fluoxetine

Chemical name:	(IUPAC name) N-Methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy]propan-1-amine
Molecular formula:	$C_{17}H_{18}F_3NO$
Molecular weight*:	309.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #234

Cut-off: 50 ng/mL	**Target Compound:** Fluoxetine **Significant cross-reactivity:**	
	Fluoxetine	100%
	Norfluoxetine	25%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

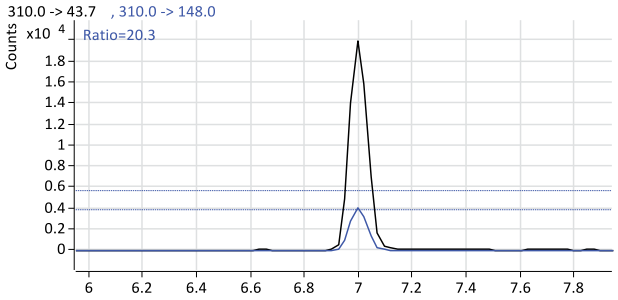
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Fluoxetine-d_6	316	154	100	2
Fluoxetine	310	148	100	1
Fluoxetine	**310**	**43.7**	**100**	**10**



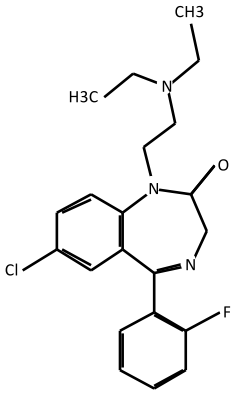
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Flurazepam

Chemical name:	7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1, 3-dihydro-2H-1, 4-benzodiazepin-2-one
Molecular formula:	$C_{21}H_{23}ClFN_3O$
Molecular weight*:	387.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Flurazepam	100%
	2-OH-ethylflurazepam	240%
	Flunitrazepam	30%
	N-desmethylflunitrazepam	90%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

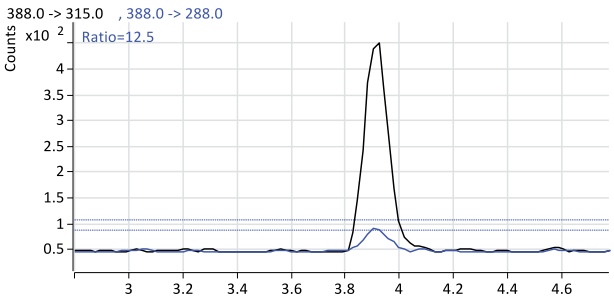
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Diazepam-d_5	290	262	160	25
Flurazepam	**388**	**315**	**160**	**25**
Flurazepam	388	288	160	25



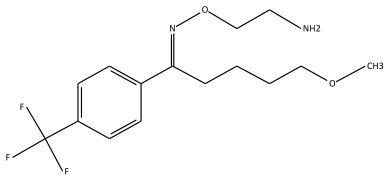
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Fluvoxamine

Chemical name:	(E)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone
Molecular formula:	$C_{15}H_{21}F_3N_2O_2$
Molecular weight*:	318.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: N/A	**Target Compound:** Nortriptyline **Significant cross-reactivity:**
	No Data

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

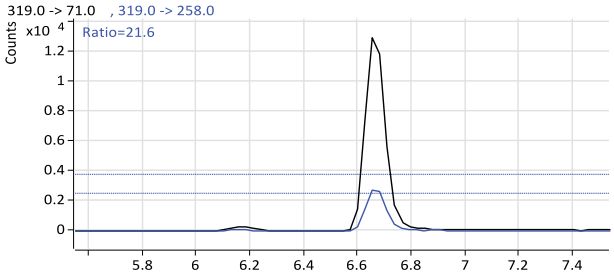
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Paroxetine-d_6	336	76	120	20
Fluvoxamine	319	258	100	2
Fluvoxamine	**319**	**71**	**100**	**6**



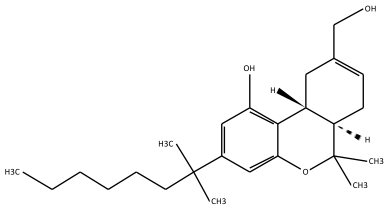
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

HU-210

Chemical name:	(6aR,10aR)-9-(Hydroxymethyl)-6, 6-dimethyl-3-(2-methyloctan-2-yl)-6a, 7, 10, 10a-tetrahydrobenzo [c] chromen-1-ol
Molecular formula:	$C_{25}H_{38}O_3$
Molecular weight*:	386.3

Molecular Structure:



Screening methodology: Immunalysis ELISA in development

Cut-off: N/A	**Target Compound:** N/A **Significant cross-reactivity:**	
	None	N/A

Confirmatory methodology: LC-MS/MS
LOQ 5 ng/mL

LC Parameters

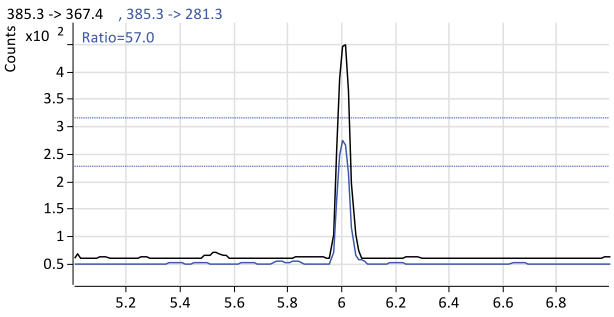
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 2.1 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	60 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Acetonitrile
Gradient	0 min 5% B 5 min 100% B 7 min 1% B 9.2 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters – negative ion

Nebulizer	55 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
JWH-073-d_7	335.3	207.2	120	20
HU-210	**385.3**	**367.4**	**120**	**30**
HU-210	385.3	281.3	120	45



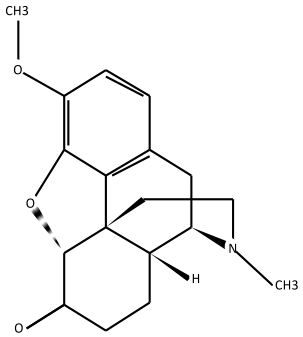
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Hydrocodone

Chemical name:	Dihydrocodeinone
Molecular formula:	$C_{18}H_{21}NO_3$
Molecular weight*:	299.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #207OF

Cut-off: 20 ng/mL	**Target Compound:** Morphine **Significant cross-reactivity:**	
	Morphine	100%
	6-AM	83%
	Codeine	200%
	Hydrocodone	93%
	Dihydrocodeine	85%

Confirmatory methodology: LC-MS/MS
LOQ 4 ng/mL

LC Parameters

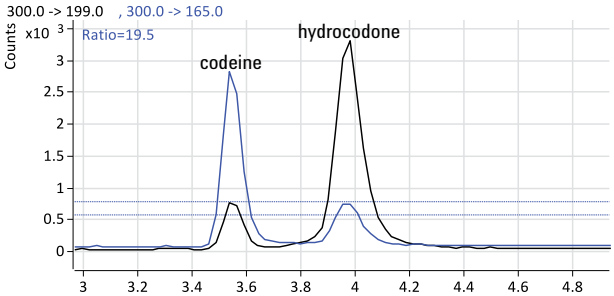
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	60 °C
Mobile phase	A = 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 6 min 78% B Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Codeine D_3	303	165	140	40
Hydrocodone	**300**	**199**	**160**	**35**
Hydrocodone	300	165	140	45



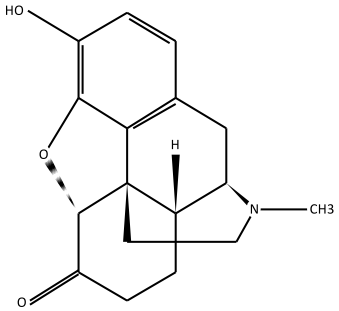
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Hydromorphone

Chemical name:	Dihydromorphinone; 4, 5-epoxy-3-hydroxy-17-methylmorphinan-6-one
Molecular formula:	$C_{18}H_{21}NO_3$
Molecular weight*:	299.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #207OF

Cut-off: 20 ng/mL	**Target Compound:** Morphine **Significant cross-reactivity:**	
	Morphine	100%
	6-AM	83%
	Codeine	200%
	Hydrocodone	93%
	Hydromorphone	81%

Confirmatory methodology: LC-MS/MS
LOQ 4 ng/mL

LC Parameters

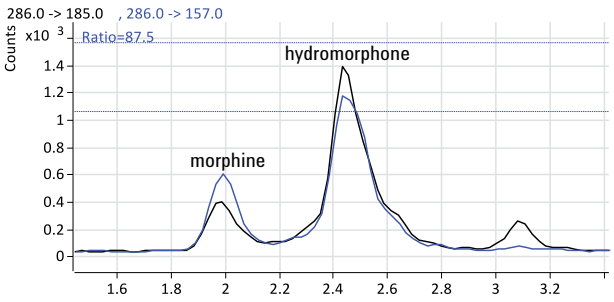
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	60 °C
Mobile phase	A = 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 6 min 78% B Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Morphine-d_3	289	165	130	40
Hydromorphone	**286**	**185**	**160**	**35**
Hydromorphone	286	157	160	50



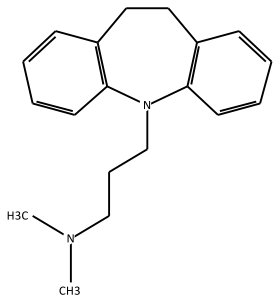
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Imipramine

Chemical name:	5-(3-Dimethylaminopropyl)-10, 11-dihydro-5H-dibenz[*b, f*]azepine
Molecular formula:	$C_{19}H_{24}N_2$
Molecular weight*:	280.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: 25 ng/mL **Target Compound:** Nortriptyline

Significant cross-reactivity:

Nortriptyline	100%
Amitriptyline	200%
Desipramine	200%
Imipramine	200%
Trimipramine	50%

Confirmatory methodology: LC-MS/MS

LOQ 10 ng/mL

LC Parameters

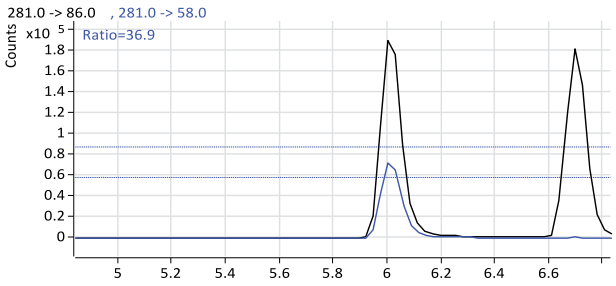
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	Precursor	Product	Fragmentor (V)	Collision Energy (V)
Paroxetine-d_6	336	76	120	20
Imipramine	**281**	**86**	**100**	**10**
Imipramine	281	58	100	30



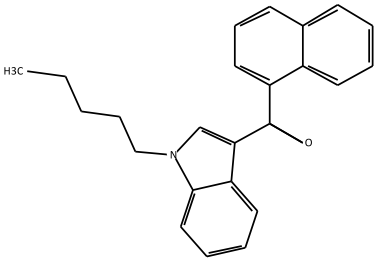
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

JWH-018

Chemical name:	1-Pentyl-3-(1-naphthoyl)indole; (IUPAC Name) Naphthalen-1-yl-(1-pentylindol-3-yl)methanone
Molecular formula:	$C_{24}H_{23}NO$
Molecular weight*:	341.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #244

Cut-off: 5 ng/mL	**Target Compound:** JWH-200 **Significant cross-reactivity:**	
	JWH-200	100%
	JWH-018	22%
	JWH-073	31%
	AM-2201	50%

Confirmatory methodology: LC-MS/MS
LOQ 2 ng/mL

LC Parameters

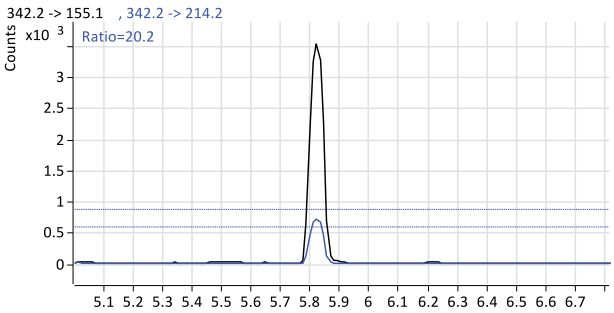
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 2.1 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	60 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Acetonitrile
Gradient	0 min 5% B 5 min 100% B 7 min 1% B 9.2 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	55 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
JWH-018-d_9	351.3	223.4	140	20
JWH-018	**342.2**	**214.2**	**120**	**20**
JWH-018	342.2	155.1	120	20



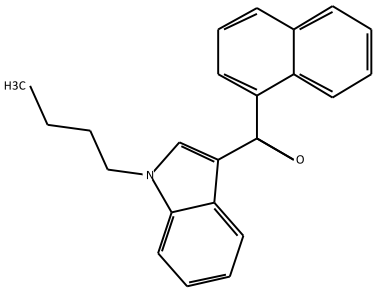
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

JWH-073

Chemical name:	(IUPAC Name) naphthalen-1-yl-(1-butylindol-3-yl)methanone
Molecular formula:	$C_{23}H_{21}NO$
Molecular weight*:	327.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #244

Cut-off: 5 ng/mL	**Target Compound:** JWH-200 **Significant cross-reactivity:**	
	JWH-200	100%
	JWH-073	31%
	JWH-018	22%
	AM 2201	50%

Confirmatory methodology: LC-MS/MS
LOQ 0.5 ng/mL

LC Parameters

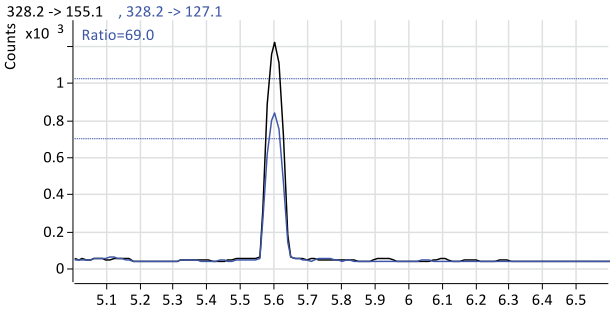
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 2.1 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	60 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Acetonitrile
Gradient	0 min 5% B 5 min 100% B 7 min 1% B 9.2 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	55 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
JWH-073-d_7	335.3	207.2	120	20
JWH-073	**328.2**	**155.1**	**120**	**20**
JWH-073	328.2	127.1	120	35



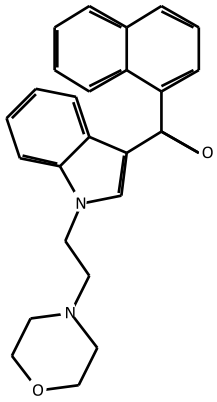
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

JWH-200

Chemical name:	(IUPAC Name) (1-(2-morpholin-4-ylethyl)indol-3-yl)-naphthalen-1-ylmethanone
Molecular formula:	$C_{25}H_{24}N_2O_2$
Molecular weight*:	384.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #244

Cut-off: 5 ng/mL	**Target Compound:** JWH-200 **Significant cross-reactivity:**	
	JWH-200	100%
	AM 2201	50%
	JWH-073	31%
	JWH-018	22%

Confirmatory methodology: LC-MS/MS
LOQ 0.5 ng/mL

LC Parameters

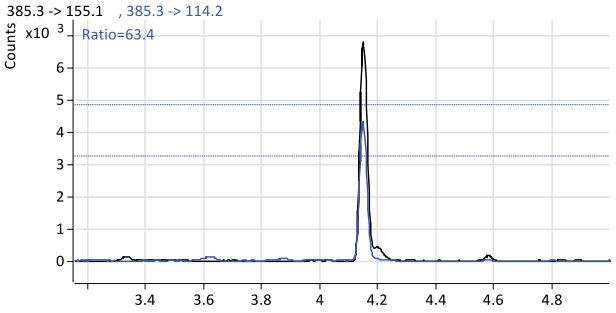
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 2.1 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	60 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Acetonitrile
Gradient	0 min 5% B 5 min 100% B 7 min 1% B 9.2 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	55 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
JWH-073-d_7	335.3	207.2	120	20
JWH-200	**385.3**	**155.1**	**140**	**20**
JWH-200	385.3	114.2	140	25



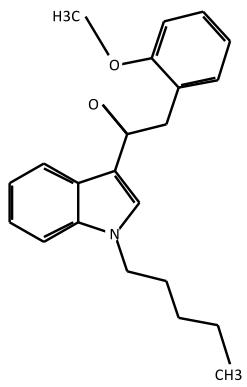
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

JWH-250

Chemical name:	(IUPAC Name) 2-(2-methoxyphenyl)-1-(1-pentylindol-3-yl) ethanone
Molecular formula:	$C_{22}H_{25}NO_2$
Molecular weight*:	335.2

Molecular Structure:



Screening methodology: Immunalysis ELISA in development

Cut-off: N/A	**Target Compound:** N/A **Significant cross-reactivity:**	
	None	N/A

Confirmatory methodology: LC-MS/MS
LOQ 5 ng/mL

LC Parameters

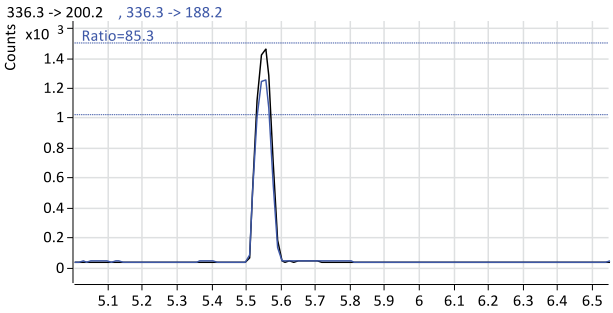
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 2.1 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	60 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Acetonitrile
Gradient	0 min 5% B 5 min 100% B 7 min 1% B 9.2 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	55 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
JWH-073-d_7	335.3	207.2	120	20
JWH-250	**336.3**	**200.2**	**120**	**20**
JWH-250	336.3	188.2	120	12



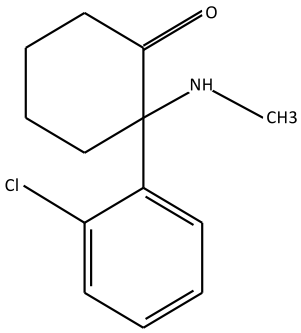
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Ketamine

Chemical name:	(IUPAC name) 2-(2-Chlorophenyl)-2-(methylamino) cyclohexanone
Molecular formula:	$C_{13}H_{16}ClNO$
Molecular weight*:	237.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #240

Cut-off: 10 ng/mL	**Target Compound:** Ketamine **Significant cross-reactivity:**	
	Ketamine	100%
	Norketamine	5%

Confirmatory methodology: LC-MS/MS
LOQ 3 ng/mL

LC Parameters

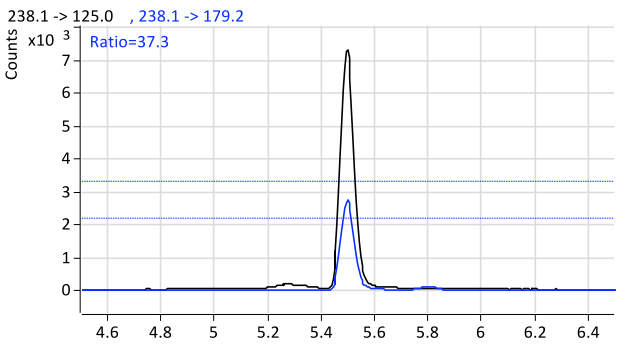
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	40 °C
Mobile phase	A = water 20mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 25% B 0.5 min 25% B 3.5 min 100% B 4.5 min 25% B 6.0 min Stop. Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	6 L/min
Drying gas temperature	350°C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Ketamine-d_4	242.2	129	80	30
Ketamine	238.1	179.2	120	15
Ketamine	**238.1**	**125**	**120**	**20**



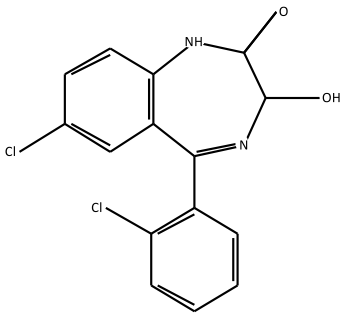
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Lorazepam

Chemical name:	7-Chloro-5-(2-chlorophenyl)-1, 3-dihydro-3-hydroxy-2H-1, 4-benzodiazepin-2-one
Molecular formula:	$C_{15}H_{10}Cl_2N_2O_2$
Molecular weight*:	320.01

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Lorazepam	90%
	Lormetazepam	120%
	Diazepam	70%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

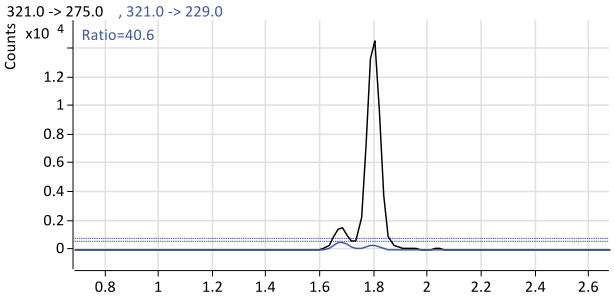
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Alprazolam-d_5	314	286	160	25
Lorazepam	**321**	**275**	**140**	**25**
Lorazepam	321	229	140	25



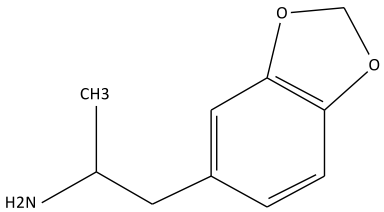
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

MDA

Chemical name:	Methylenedioxyamphetamine; α-Methyl-1, 3-benzodioxole-5-ethanamine
Molecular formula:	$C_{10}H_{13}NO_2$
Molecular weight*:	179.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #209OF

Cut-off: 50 ng/mL	**Target Compound:** d-Amphetamine **Significant cross-reactivity:**	
	d-Amphetamine	100%
	dl-MDA	178%
	l-Amphetamine	9.7%
	Phentermine	89%

Confirmatory methodology: LC-MS/MS
LOQ 25 ng/mL

LC Parameters (Method includes related compounds at longer retention times.)

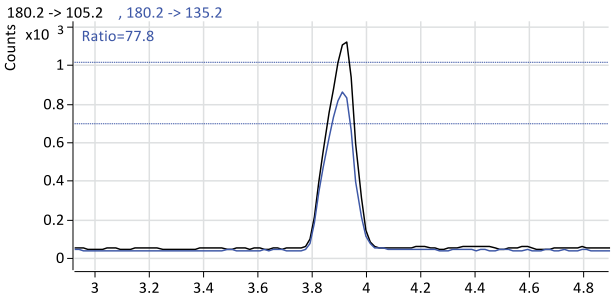
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 8 min 50% B 9 min 15% B 12 min Stop. Post-time 1 min
Injection volume	5 µL

MS Parameters

Nebulizer	40 psi
Drying gas flow	6 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
MDA-d_5	185.2	110.1	80	25
MDA	180.2	135.2	40	20
MDA	**180.2**	**105.2**	**40**	**20**



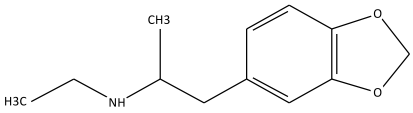
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

MDEA

Chemical name:	3, 4-Methylenedioxy-N-ethylamphetamine
Molecular formula:	$C_{12}H_{17}NO_2$
Molecular weight*:	207.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #2110F

Cut-off: 50 ng/mL	**Target Compound:** d-Methamphetamine **Significant cross-reactivity:**	
	d-Methamphetamine	100%
	l-Methamphetamine	3%
	d, l-MDMA	98%
	d, l-MDEA	6.4%

Confirmatory methodology: LC-MS/MS
LOQ 25 ng/mL

LC Parameters (Method includes related compounds at longer retention times.)

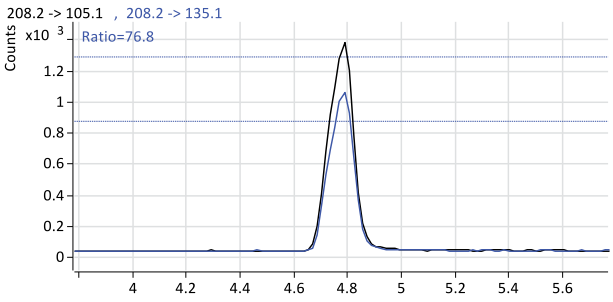
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 8 min 50% B 9 min 15% B 12 min Stop. Post-time 1 min
Injection volume	5 µL

MS Parameters

Nebulizer	40 psi
Drying gas flow	6 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
MDEA-d_5	213.2	105.1	120	30
MDEA	208	135.1	120	25
MDEA	**208**	**105.1**	**120**	**30**



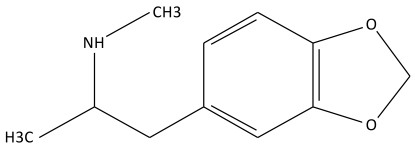
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

MDMA

Chemical name:	Methylenedioxymethamphetamine; (2-Benzol[1, 3]dioxol-5-yl-1-methyl-ethyl)-methyl-amine
Molecular formula:	$C_{11}H_{15}NO_2$
Molecular weight*:	193.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #211OF

Cut-off: 50 ng/mL	**Target Compound:** d-Methamphetamine **Significant cross-reactivity:**	
	d-Methamphetamine	100%
	l-Methamphetamine	3%
	d, l-MDMA	98%
	d, l-MDEA	6.4%

Confirmatory methodology: LC-MS/MS
LOQ 25 ng/mL

LC Parameters (Method includes related compounds at longer retention times.)

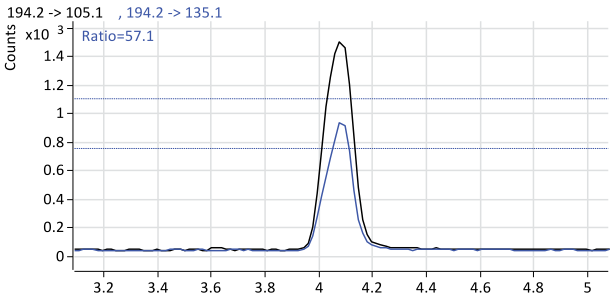
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 8 min 50% B 9 min 15% B 12 min Stop. Post-time 1 min
Injection volume	5 µL

MS Parameters

Nebulizer	40 psi
Drying gas flow	6 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
MDMA-d_5	199.2	107.1	80	25
MDMA	194	135.1	80	25
MDMA	**194**	**105.1**	**80**	**25**



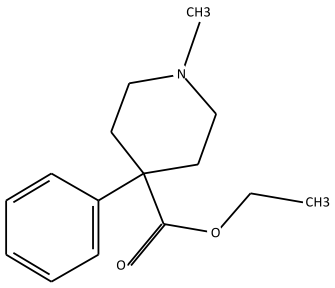
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Meperidine

Chemical name:	1-methyl-4-phenyl-4-piperidinecarboxylic acid ethyl ester
Molecular formula:	$C_{15}H_{21}NO_2$
Molecular weight*:	247.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #220

Cut-off: 25 ng/mL	**Target Compound:** Meperidine **Significant cross-reactivity:**	
	Meperidine	100%
	Normeperidine	7%
	Meperidinic Acid	3%
	6-Acetylcodeine	0.2%

Confirmatory methodology: LC-MS/MS
LOQ 25 ng/mL

LC Parameters

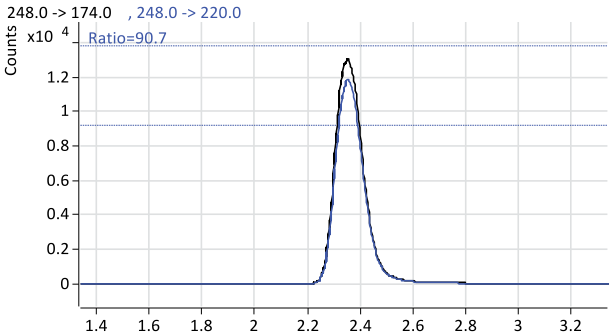
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 50% B 3 min 100% B 4 min 50% B 4.5 min Stop. Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Meperidine-d_4	252	178	160	20
Meperidine	248	220	160	20
Meperidine	**248**	**174**	**160**	**20**



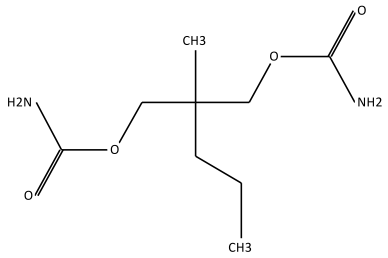
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Meprobamate

Chemical name:	Meprobamate; (IUPAC name) 2-Methyl-2-propyl-1, 3-propanediol dicarbamate
Molecular formula:	$C_9H_{18}N_2O_4$
Molecular weight*:	218.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #231

Cut-off: 50 ng/mL	**Target Compound:** Carisoprodol **Significant cross-reactivity:**	
	Carisoprodol	100%
	Meprobamate	118%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

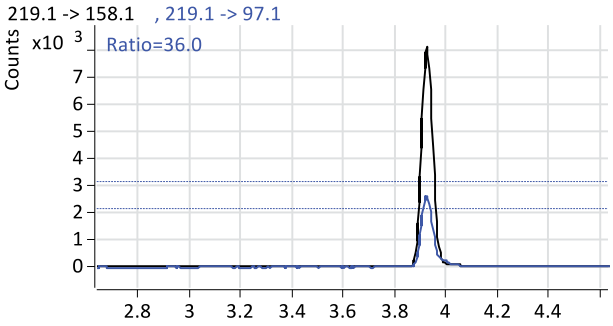
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 4 min 100% B 5 min 15% B 5.5 min Stop. Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	40 psi
Drying gas flow	8 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Carisoprodol-d_7	268.2	183.2	60	2
Meprobamate	**219.1**	**158.1**	**60**	**2**
Meprobamate	219.1	97.1	60	8



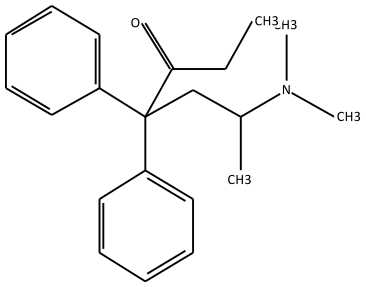
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Methadone

Chemical name:	6-Dimethylamino-4, 4-diphenyl-3-heptanone
Molecular formula:	$C_{21}H_{27}N_0$
Molecular weight*:	309.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #232

Cut-off: 50 ng/mL	**Target Compound:** Methadone **Significant cross-reactivity:**	
	Methadone	100%
	Methadol	50%
	EDDP	<5%
	LAAM	15%
	Nor-LAAM	<1%

Confirmatory methodology: LC-MS/MS
LOQ 2 ng/mL

LC Parameters

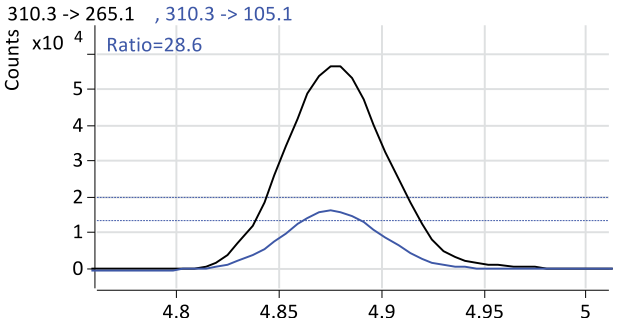
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 50% B 4 min 100% B 5 min 25% B 6.5 min Stop. Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Methadone-d_9	319.3	268.1	120	10
Methadone	**310.3**	**265.1**	**120**	**10**
Methadone	310.3	105.1	120	25



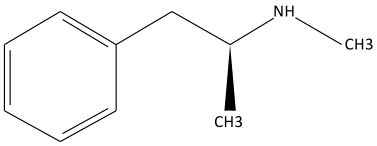
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Methamphetamine

Chemical name:	Phenylmethylaminopropane; (IUPAC name) (2S)-N-Methyl-1-phenylpropan-2-amine
Molecular formula:	$C_{10}H_{15}N$
Molecular weight*:	149.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #211OF

Cut-off: 50 ng/mL	**Target Compound:** d-Methamphetamine **Significant cross-reactivity:**	
	d-Methamphetamine	100%
	l-Methamphetamine	3%
	d, l-MDMA	98%
	d, l-MDEA	6.4%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters (Method includes related compounds at longer retention times.)

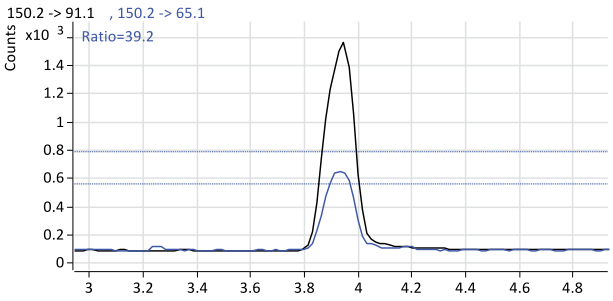
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 8 min 50% B 9 min 15% B 12 min Stop. Post-time 1 min
Injection volume	5 µL

MS Parameters

Nebulizer	40 psi
Drying gas flow	6 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Methamphetamine-d_5	155.2	92.1	80	25
Methamphetamine	**150.2**	**91.1**	**120**	**25**
Methamphetamine	150.2	65.1	120	50



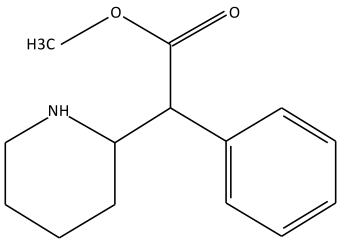
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Methylphenidate

Chemical name:	Methylphenidan; α-Phenyl-2-piperidineacetic acid methyl ester
Molecular formula:	$C_{14}H_{19}NO_2$
Molecular weight*:	233.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #219

Cut-off: 10 ng/mL	**Target Compound:** Methylphenidate **Significant cross-reactivity:**	
	Methylphenidate	100%
	Ritalinic acid	1%

Confirmatory methodology: LC-MS/MS
LOQ 5 ng/mL

LC Parameters (Method includes related compounds at longer retention times.)

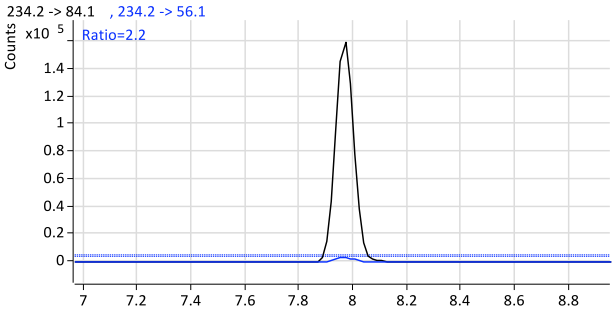
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 8 min 50% B 9 min 15% B 12 min Stop. Post-time 1 min
Injection volume	5 µL

MS Parameters

Nebulizer	40 psi
Drying gas flow	6 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Methamphetamine-d_5	155.2	92.1	80	25
Methylphenidate	**234.2**	**84.1**	**120**	**25**
Methylphenidate	234.2	56.1	120	25



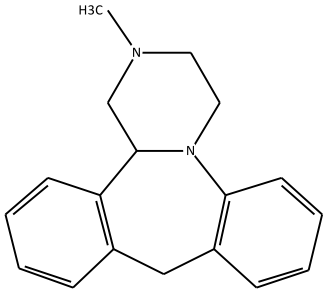
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Mianserine

Chemical name:	(IUPAC Name) 1, 2, 3, 4, 10, 14b-Hexahydro-2-methyldibenzo[*c, f*]-pyrazino[1, 2-*a*]azepine
Molecular formula:	$C_{18}H_{20}N_2$
Molecular weight*:	264.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: N/A	**Target Compound:** Nortriptyline **Significant cross-reactivity:**
	No Data

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

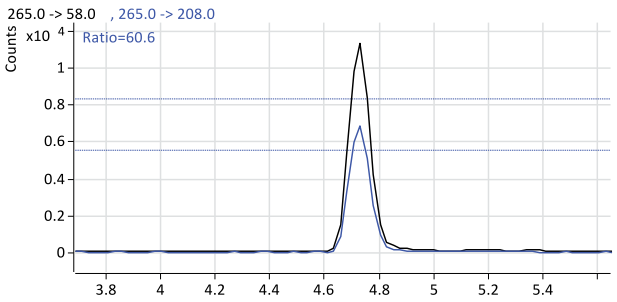
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Paroxetine-d_6	336	76	120	20
Mianserine	265	208	120	15
Mianserine	**265**	**58**	**120**	**25**



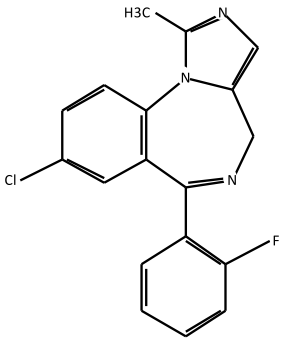
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Midazolam

Chemical name:	(IUPAC Name) 8-Chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo[1, 5-*a*][1, 4]benzodiazepine
Molecular formula:	$C_{18}H_{13}ClFN_3$
Molecular weight*:	325.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Midazolam	60%
	Medazepam	40%
	Diazepam	70%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

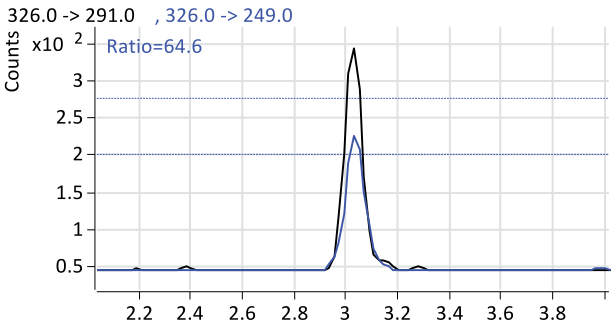
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Alprazolam-d_5	314	286	160	25
Midazolam	**326**	**291**	**200**	**30**
Midazolam	326	249	200	40



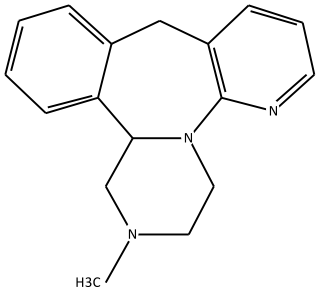
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Mirtazapine

Chemical name:	6-Azamianserin; (IUPAC Name) 1, 2, 3, 4, 10, 14*b*-Hexahydro-2-methylpyrazino[2, 1-*a*]pyrido[2, 3-*c*][2]benzazepine
Molecular formula:	$C_{18}H_{20}N_2$
Molecular weight*:	265.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: N/A	**Target Compound:** Nortriptyline **Significant cross-reactivity:**
	No Data

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

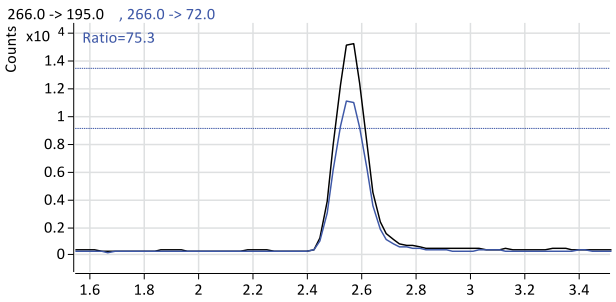
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Paroxetine-d_6	336	76	120	20
Mirtazapine	**266**	**195**	**100**	**25**
Mirtazapine	266	72	100	20



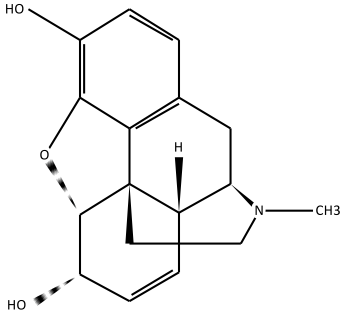
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Morphine

Chemical name:	(IUPAC Name) (5α, 6α)-17-Methyl-7, 8-didehydro-4, 5-epoxymorphinan-3, 6-diol
Molecular formula:	$C_{17}H_{19}NO_3$
Molecular weight*:	285.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #207OF

Cut-off: 20 ng/mL	**Target Compound:** Morphine **Significant cross-reactivity:**	
	Morphine	100%
	6-AM	83%
	Codeine	200%
	Hydrocodone	93%
	Dihydrocodeine	85%

Confirmatory methodology: LC-MS/MS
LOQ 4 ng/mL

LC Parameters

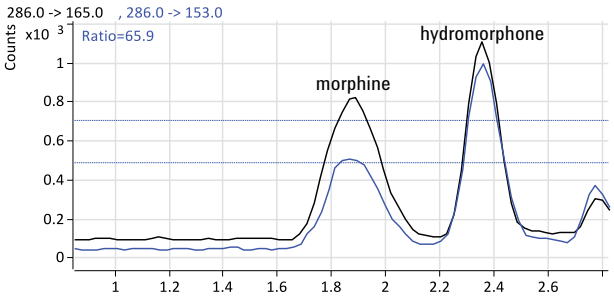
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	60 °C
Mobile phase	A = 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 6 min 78% B Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Morphine-d_3	289	165	130	40
Morphine	**286**	**165**	**170**	**35**
Morphine	286	153	150	45



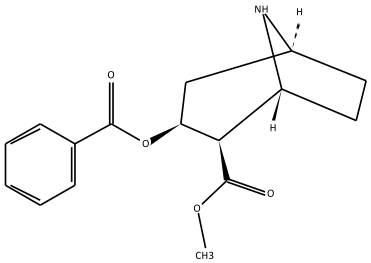
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Norcocaine

Chemical name:	(IUPAC Name) Methyl (1R, 2R, 3S, 5S)-3-(benzoyloxy)-8-azabicyclo[3.2.1]octane-2-carboxylate
Molecular formula:	$C_{16}H_{19}NO_4$
Molecular weight*:	289.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #212OF

Cut-off: 20 ng/mL	**Target Compound:** Benzoylecgonine **Significant cross-reactivity:**	
	Benzoylecgonine	100%
	Cocaethylene	90%
	Cocaine	70%
	Norcocaine	0.2%

Confirmatory methodology: LC-MS/MS
LOQ 2 ng/mL

LC Parameters

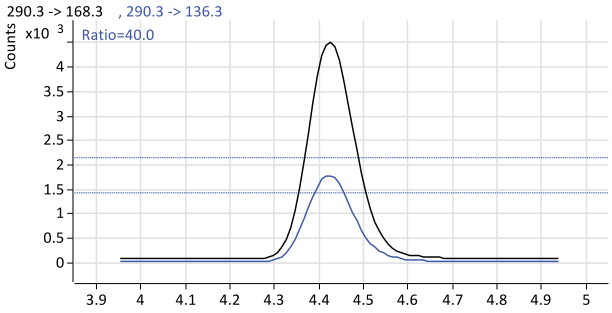
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.2 mL/min
Column temperature	60 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 80% B 4 min 30% 6 min Stop. Post-time 4 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Bezoylecgonine-d_3	293.3	171.2	120	20
Norcocaine	**290.3**	**168.3**	**120**	**15**
Norcocaine	290.3	136.3	120	25



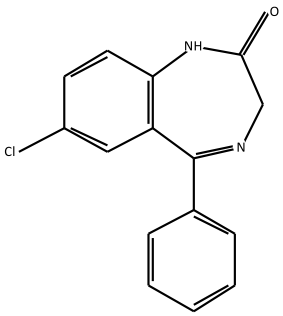
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Nordiazepam

Chemical name:	7-Chloro-1, 3-dihydro-5-phenyl-2H-1, 4-benzodiazepin-2-one
Molecular formula:	$C_{15}H_{11}ClN_2O$
Molecular weight*:	270.7

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Nordiazepam	50%
	Diazepam	70%
	Temazepam	200%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

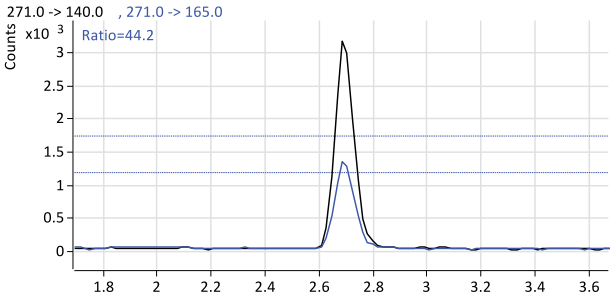
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Alprazolam-d_5	314	286	160	25
Nordiazepam	271	165	160	30
Nordiazepam	**271**	**140**	**160**	**30**



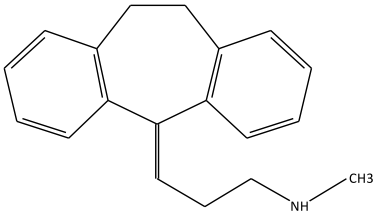
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Nortriptyline

Chemical name:	3-(10, 11-Dihydro-5H-dibenzo[*a, d*]cyclohepten-5-ylidine-N-methyl-1-propanamine
Molecular formula:	$C_{19}H_{21}N$
Molecular weight*:	263.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: 25 ng/mL	**Target Compound:** Nortriptyline **Significant cross-reactivity:**	
	Nortriptyline	100%
	Amitriptyline	200%
	Desipramine	200%
	Imipramine	200%
	Trimipramine	50%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

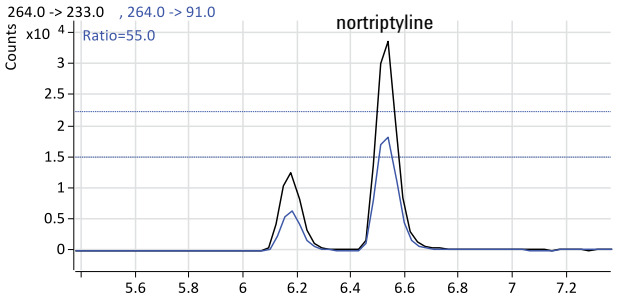
MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Nortriptyline-d_5	267	233	100	10
Nortriptyline	**264**	**233**	**100**	**10**
Nortriptyline	264	91	100	25

*Monoisotopic molecular weight

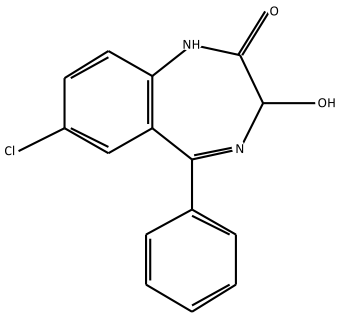


MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Oxazepam

Chemical name:	7-Chloro-1, 3-dihydro-3-hydroxy-5-phenyl-2H-1, 4-benzodiazepin-2-one
Molecular formula:	$C_{15}H_{11}ClN_2O_2$
Molecular weight*:	286.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Diazepam	70%
	Nordiazepam	50%
	Temazepam	200%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

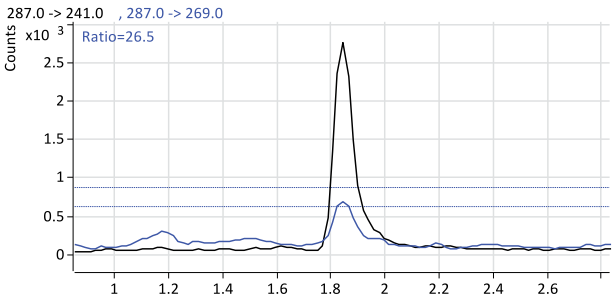
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Oxazepam-d_5	292	246	120	20
Oxazepam	287	269	120	20
Oxazepam	**287**	**241**	**120**	**20**



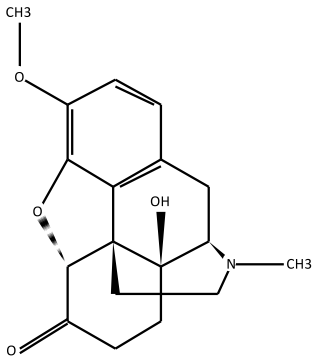
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Oxycodone

Chemical name:	7, 8-Dihydro-14-hydroxycodeinone
Molecular formula:	$C_{18}H_{21}NO_4$
Molecular weight*:	315.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #221B

Cut-off: 20 ng/mL	**Target Compound:** Oxycodone **Significant cross-reactivity:**	
	Oxycodone	100%
	Oxymorphone	88%
	Hydrocodone	10%
	Hydromorphone	3%

Confirmatory methodology: LC-MS/MS
LOQ 4 ng/mL

LC Parameters

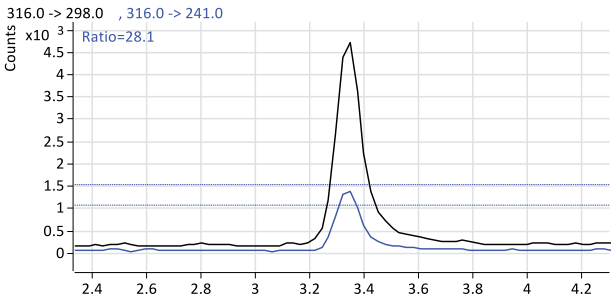
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	60 °C
Mobile phase	A = 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 6 min 78% B Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Oxycodone D_6	322	247	160	25
Oxycodone	**316**	**298**	**160**	**15**
Oxycodone	316	241	160	30



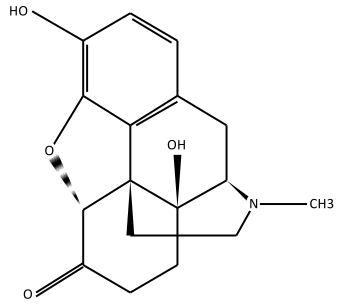
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Oxymorphone

Chemical name:	7, 8-Dihydro-14-hydroxymorphinone
Molecular formula:	$C_{17}H_{19}NO_4$
Molecular weight*:	301.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #221B

Cut-off: 20 ng/mL	**Target Compound:** Oxycodone **Significant cross-reactivity:**	
	Oxycodone	100%
	Oxymorphone	88%
	Hydrocodone	10%
	Hydromorphone	3%

Confirmatory methodology: LC-MS/MS
LOQ 4 ng/mL

LC Parameters

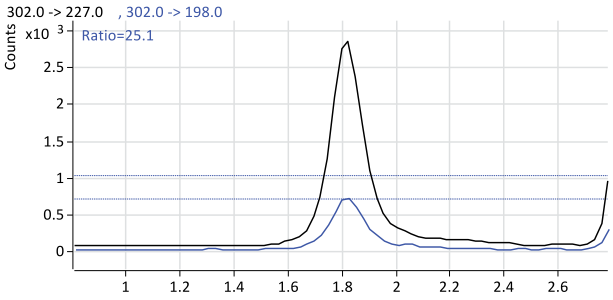
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	60 °C
Mobile phase	A = 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 6 min 78% B Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Oxymorphone-d_3	305	230	120	35
Oxymorphone	**302**	**227**	**140**	**30**
Oxymorphone	302	198	120	45



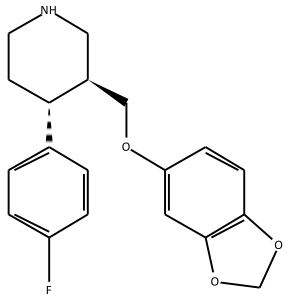
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Paroxetine

Chemical name:	(3S-trans)-3-[(1, 3-Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine
Molecular formula:	$C_{19}H_{20}FNO_3$
Molecular weight*:	329.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: N/A	**Target Compound:** N/A **Significant cross-reactivity:**
	N/A

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

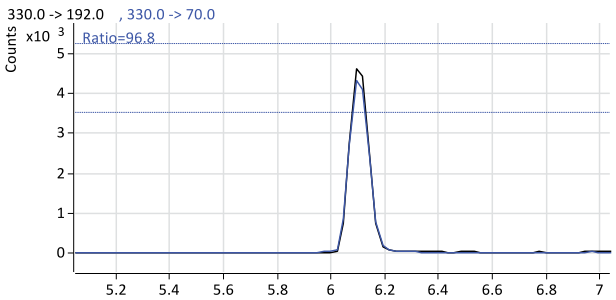
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Paroxetine-d_6	336	76	120	20
Paroxetine	**330**	**192**	**100**	**20**
Paroxetine	330	70	100	20



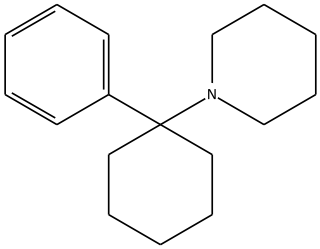
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Phencyclidine (PCP)

Chemical name:	1-(1-Phenylcyclohexyl)piperidine
Molecular formula:	$C_{17}H_{25}N$
Molecular weight*:	243.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #208

Cut-off: 10 ng/mL	**Target Compound:** Phencyclidine **Significant cross-reactivity:**	
	Phencyclidine	100%
	1[1-(2-thienyl)cyclohexyl]-piperidine	50%
	1[1-(2-thienyl)cyclohexyl]-morpholine	10%
	1-(1-phencyclohexyl)pyrollidine	10%
	1-(1-phencyclohexyl)morpholine	8%

Confirmatory methodology: LC-MS/MS
LOQ 5 ng/mL

LC Parameters

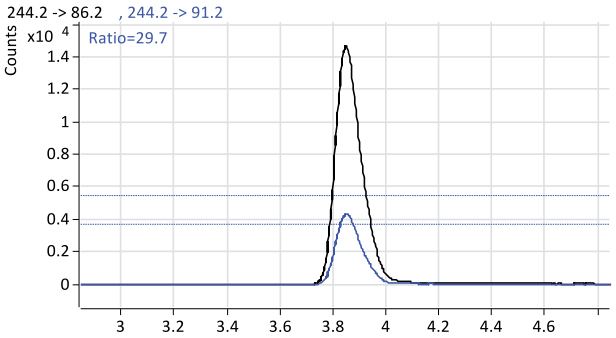
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 50% B 4 min 100% B 5 min 25% B 6 min Stop. Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	35 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Phencyclidine-d_5	249.2	96.1	5	30
Phencyclidine	244.2	91.2	5	15
Phencyclidine	**244.2**	**86.2**	**5**	**10**



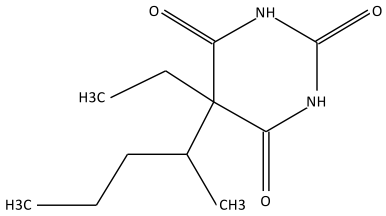
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Pentobarbital

Chemical name:	5-Ethyl-5-(1-methylbutyl)2, 4, 6(1H, 3H, 5H)-pyrimidinetrione
Molecular formula:	$C_{11}H_{18}N_2O_3$
Molecular weight*:	226.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Barbiturates Kit Catalog #210

Cut-off: 50 ng/mL	**Target Compound:** Secobarbital **Significant cross-reactivity:**	
	Secobarbital	100%
	Butalbital	83%
	Aprobarbital	89%
	Pentobarbital	83%
	Phenobarbital	50%
	Butabarbital	33%

Confirmatory methodology: LC-MS/MS
LOQ 25 ng/mL

LC Parameters

Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.8 mL/min
Column temperature	35 °C
Mobile phase	A = water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 5% B 0.2 min 5% B 5 min 95% B 5.2 min 95% B 6 min 5% 8.2 min Stop. Post-time Off
Injection volume	5 µL

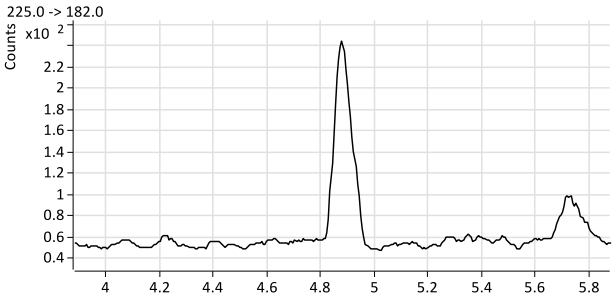
MS Parameters – negative ion

Nebulizer	35 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	4500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Pentobarbital-d_5	230	42	120	15
Pentobarbital	**225**	**182**	**80**	**4**

*Monoisotopic molecular weight

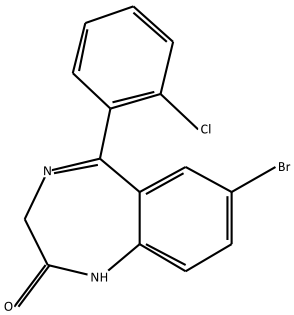


MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Phenazepam

Chemical name:	(IUPAC name) 7-bromo-5-(2-chorophenyl)-1, 3-dihydro-2H-1, 4-benzodiazepin-2-one
Molecular formula:	$C_{15}H_{10}N_2OBrCl$
Molecular weight*:	349.6

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Phenazepam	100%
	Flurazepam	100%
	Temazepam	200%
	Diazepam	70%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

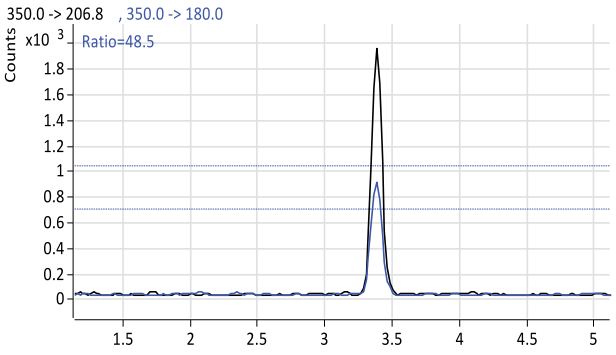
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Oxazepam-d_5	292	246	120	20
Phenazepam	**350**	**206.8**	**140**	**45**
Phenazepam	350	180	140	50



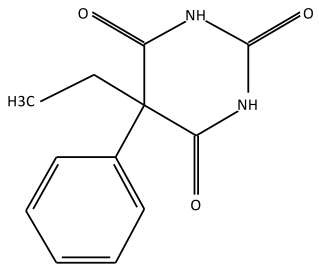
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Phenobarbital

Chemical name:	5-Ethyl-5-phenyl-2, 4, 6(1H, 3H, 5H)-pyrimidinetrione
Molecular formula:	$C_{12}H_{12}N_2O_3$
Molecular weight*:	232.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Barbiturates Kit Catalog #210

Cut-off: 50 ng/mL	**Target Compound:** Secobarbital **Significant cross-reactivity:**	
	Secobarbital	100%
	Butalbital	83%
	Aprobarbital	89%
	Pentobarbital	83%
	Phenobarbital	50%
	Butabarbital	33%

Confirmatory methodology: LC-MS/MS
LOQ 25 ng/mL

LC Parameters

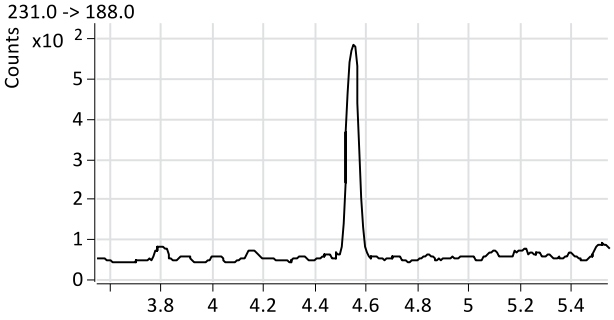
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.8 mL/min
Column temperature	35 °C
Mobile phase	A = water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 5% B 0.2 min 5% B 5 min 95% B 5.2 min 95% B 6 min 5% 8.2 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters – negative ion

Nebulizer	35 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	4500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Pentobarbital-d_5	230	42	120	15
Phenobarbital	**231**	**188**	**80**	**3**



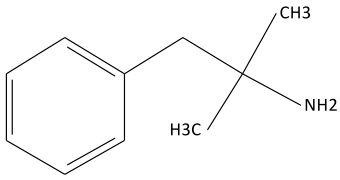
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Phentermine

Chemical name:	Phenyl-tertiary-butylamine; α, α-Dimethylbenzeneethanamine
Molecular formula:	$C_{10}H_{15}N$
Molecular weight*:	149.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #2090F

Cut-off: 50 ng/mL	**Target Compound:** d-Amphetamine **Significant cross-reactivity:**	
	d-Amphetamine	100%
	l-Amphetamine	9.7%
	dl-MDA	178%
	Phentermine	89%

Confirmatory methodology: LC-MS/MS
LOQ 25 ng/mL

LC Parameters (Method includes related compounds at longer retention times.)

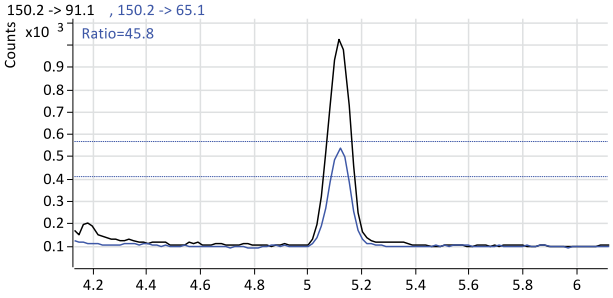
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 8 min 50% B 9 min 15% B 12 min Stop. Post-time 1 min
Injection volume	5 µL

MS Parameters

Nebulizer	40 psi
Drying gas flow	6 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Methamphetamine-d_5	155.2	92.1	80	25
Phentermine	**150.2**	**91.1**	**120**	**25**
Phentermine	150.2	65.1	120	50



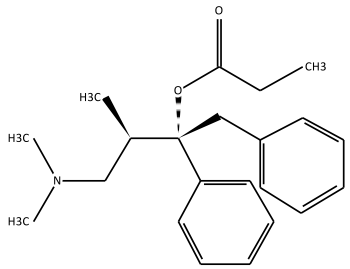
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Propoxyphene

Chemical name:	Dextropropoxyphene; (αS)-α-[(1R)-2-(Dimethylamino)-1-methylethyl]-α-phenylbenzene ethanol propanoate
Molecular formula:	$C_{22}H_{29}NO_2$
Molecular weight*:	339.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #237

Cut-off: 20 ng/mL	**Target Compound:** Propoxyphene **Significant cross-reactivity:**	
	Propoxyphene	100%
	Norpropoxyphene	10%

Confirmatory methodology: LC-MS/MS
LOQ 2 ng/mL

LC Parameters

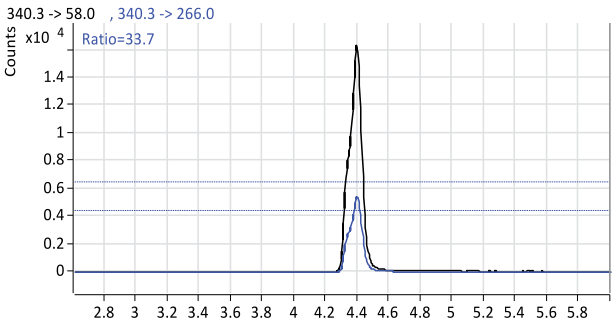
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = 0.2% Acetic Acid, B = Methanol
Gradient	0 min 15% B 4 min 100% B 5 min 15% B 6 min Stop. Post-time 3 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Propoxyphene-d_5	345.3	271	80	0
Propoxyphene	340.3	266	80	0
Propoxyphene	**340.3**	**58**	**80**	**20**



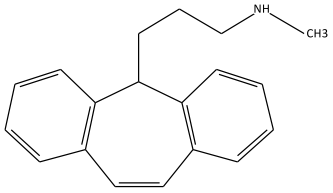
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Protriptyline

Chemical name:	N-Methyl-5H-dibenzo[*a, d*]cycloheptene-5-propanamine
Molecular formula:	$C_{19}H_{21}N$=263.4
Molecular weight*:	263.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: 25 ng/mL	**Target Compound:** Nortriptyline **Significant cross-reactivity:**	
	Nortriptyline	100%
	Protriptyline	25%
	Desipramine	200%
	Imipramine	200%
	Trimipramine	50%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

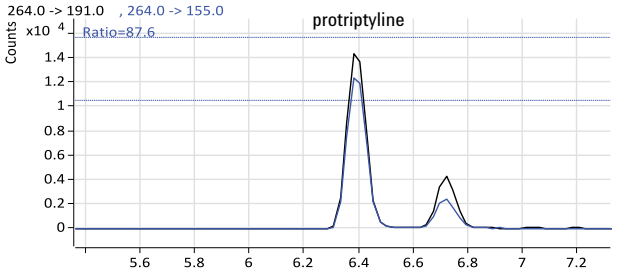
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Nortriptyline-d_5	267	233	100	10
Protriptyline	**264**	**191**	**110**	**30**
Protriptyline	264	155	110	20



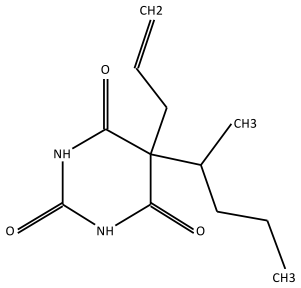
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Secobarbital

Chemical name:	5-(1-methylbutyl)-5-(2-propenyl)-2, 4, 6(1H, 3H, 5H)-pyrimidinetrione
Molecular formula:	$C_{12}H_{18}N_2O_3$
Molecular weight*:	238.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Barbiturates Kit Catalog #210

Cut-off: 50 ng/mL	**Target Compound:** Secobarbital **Significant cross-reactivity:**	
	Secobarbital	100%
	Butalbital	83%
	Aprobarbital	89%
	Pentobarbital	83%
	Phenobarbital	50%
	Butabarbital	33%

Confirmatory methodology: LC-MS/MS
LOQ 25 ng/mL

LC Parameters

Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.8 mL/min
Column temperature	35 °C
Mobile phase	A = water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 5% B 0.2 min 5% B 5 min 95% B 5.2 min 95% B 6 min 5% 8.2 min Stop. Post-time Off
Injection volume	5 µL

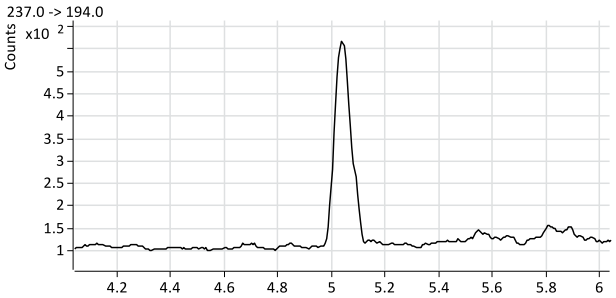
MS Parameters – negative ion

Nebulizer	35 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	4500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Pentobarbital-d_5	230	42	120	15
Secobarbital	**237**	**194**	**110**	**6**

*Monoisotopic molecular weight

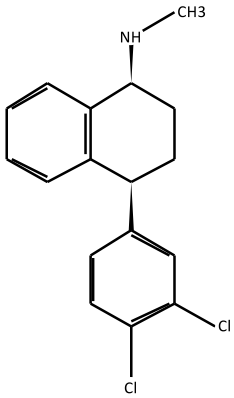


MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Sertraline

Chemical name:	(IUPAC name) (1S, 4S)-4-(3, 4-Dichlorophenyl)-1, 2, 3, 4-tetrahydro-N-methyl-1-naphthalenamine
Molecular formula:	$C_{17}H_{17}Cl_2N$
Molecular weight*:	305.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #235

Cut-off: 25 ng/mL	**Target Compound:** Sertraline **Significant cross-reactivity:**	
	Sertraline	100%
	Norsertraline	5%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

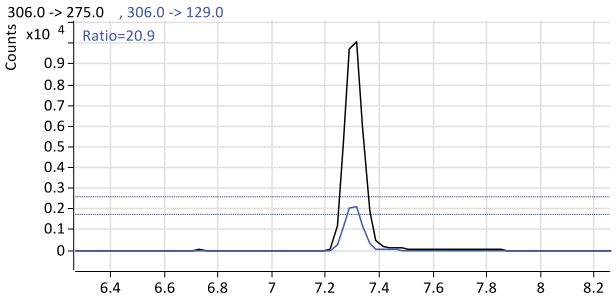
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Fluoxetine-d_6	316	154	100	2
Sertraline	**306**	**275**	**35**	**10**
Sertraline	306	129	35	20



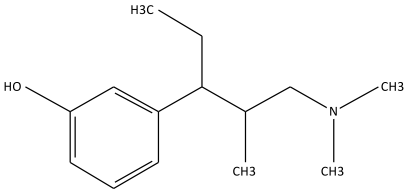
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Tapentadol

Chemical name:	3-[(1*R*, 2*R*)-3-(dimethylamino)-1-ethyl-2-methylpropyl]phenol hydrochloride
Molecular formula:	$C_{14}H_{23}N_{0}$
Molecular weight*:	221.3

Molecular Structure:



Screening methodology: Immunalysis ELISA in development

Cut-off: N/A	**Target Compound:** N/A **Significant cross-reactivity:**
	N/A N/A

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

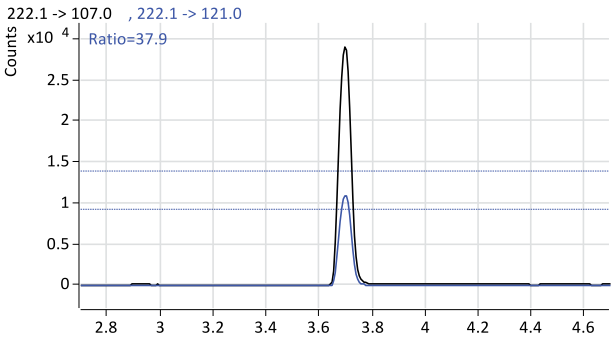
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = 20 mM Ammonium Formate pH 6.4, B = Methanol
Gradient	0 min 15% B 4 min 100% B 5 min 15% B 8 min Stop. Post-time Off
Injection volume	2 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Methamphetamine-d_5	155.2	92	80	25
Tapentadol	222.1	121	120	15
Tapentadol	**222.1**	**107**	**120**	**20**



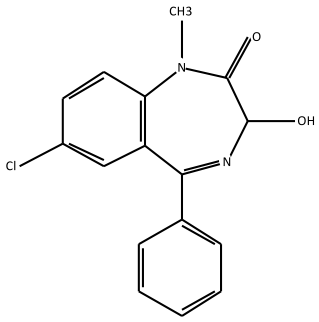
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Temazepam

Chemical name:	7-Chloro-3-hihydro-3-hydroxy-1-methyl-5-phenyl-2H-1, 4-benzodiazepin-2-one
Molecular formula:	$C_{16}H_{13}ClN_2O_2$
Molecular weight*:	300.1

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Temazepam	200%
	Diazepam	70%
	Nordiazepam	50%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

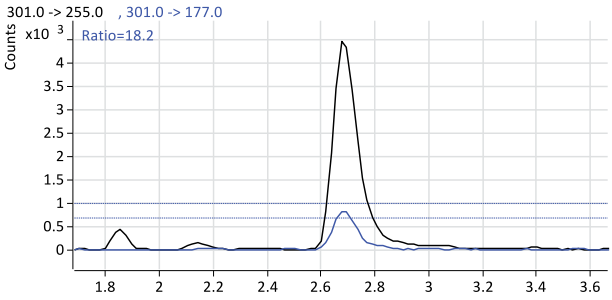
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Temazepam-d_5	306	260	120	25
Temazepam	**301**	**255**	**120**	**35**
Temazepam	301	177	120	40



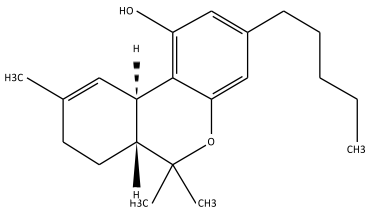
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

THC

Chemical name:	Δ^9-THC; (-)-trans-Δ^9-Tetrahydrocannabinol
Molecular formula:	$C_{21}H_{30}O_2$
Molecular weight*:	314.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #224

Cut-off: 4 ng/mL	**Target Compound:** Δ^9-THC **Significant cross-reactivity:**	
	Δ^9-THC	100%
	Δ^8-THC	66.7%
	Cannabinol	4%
	Cannabidiol	50%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

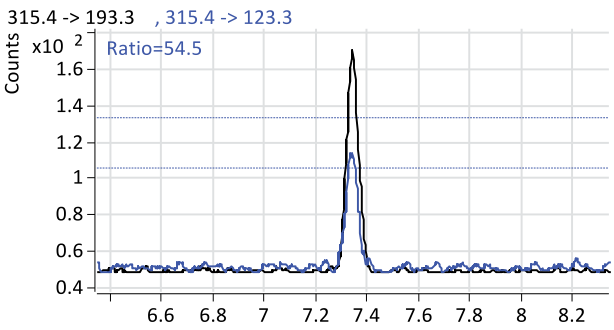
Column	Zorbax Extend-C18 Rapid Resolution HT 2.1 x 50 mm, 1.8µm
Flow rate	0.3 mL/min
Column temperature	40 °C
Mobile phase	A = 20mM Ammonium Formate pH 8.6, B = Methanol
Gradient	0 min 70% B 5 min 90% B 8 min 100% B 8.5 min Stop. Post-time 6 min
Injection volume	5 µL

MS Parameters

Nebulizer	35 psi
Drying gas flow	10 L/min
Drying gas temperature	350°C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
THC-d_3	318.3	196.3	125	20
THC	**315.4**	**193.3**	**150**	**20**
THC	315.4	123.3	150	30



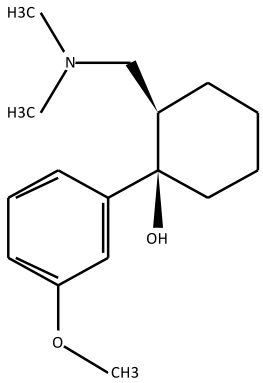
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Tramadol

Chemical name:	(IUPAC name) (1*R*, 2*R*)-2-(Dimethylaminomethyl)-1-(3-methoxyphenyl)cyclohexanol-1-ol
Molecular formula:	$C_{16}H_{25}NO_2$
Molecular weight*:	263.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #225

Cut-off: 50 ng/mL	**Target Compound:** Tramadol **Significant cross-reactivity:**	
	Tramadol	100%
	O-Desmethyl-tramadol	<1%
	N-Desmethyl-tramadol	30%
	Venlafaxine	<0.05%
	O-Desmethyl-venlafaxine	Not detectable
	N-Desmethyl-venlafaxine	Not detectable

Confirmatory methodology: LC-MS/MS
LOQ 20 ng/mL

LC Parameters

Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 15% B 4 min 100% B 5 min 15% B 4 min Stop. Post-time 2.5 min
Injection volume	5 µL

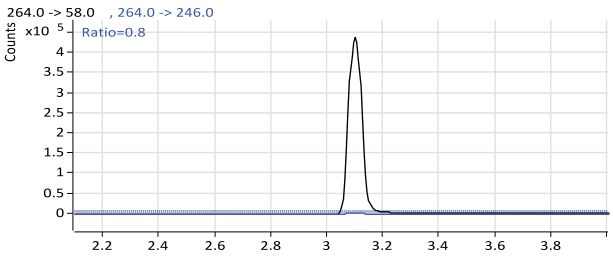
MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Tramadol C13-d_3	268	58	100	15
Tramadol	264	246	100	5
Tramadol	**264**	**58**	**100**	**15**

*Monoisotopic molecular weight

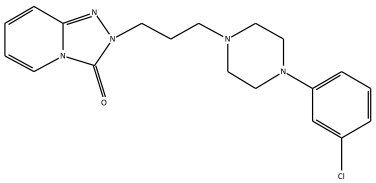


MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Trazodone

Chemical name:	2-[3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl]-[1, 2, 4] triazolo[4, 3-*a*]pyridine-3-one
Molecular formula:	$C_{19}H_{22}ClN_5O$
Molecular weight*:	371.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: N/A	**Target Compound:** Nortriptyline **Significant cross-reactivity:**
	No data

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

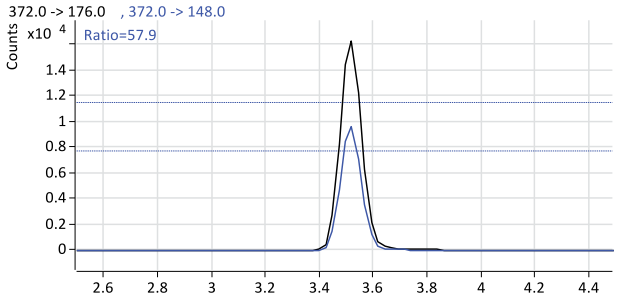
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Paroxetine-d_6	336	76	120	20
Trazodone	**372**	**176**	**100**	**25**
Trazodone	372	148	100	30



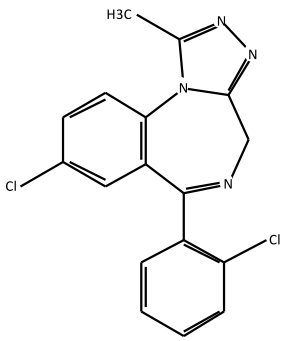
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Triazolam

Chemical name:	Clorazolam; (IUPAC Name) 8-Chloro-6-(2-chlorophenyl)-1-methyl-4H-1, 2, 4-triazolo[4,3-*a*]-1, 4-benzodiazepine
Molecular formula:	$C_{17}H_{12}Cl_2N_4$
Molecular weight*:	342.0

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #214

Cut-off: 5 ng/mL	**Target Compound:** Oxazepam **Significant cross-reactivity:**	
	Oxazepam	100%
	Triazolam	75%
	Alprazolam	180%
	Temazepam	200%
	Diazepam	70%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

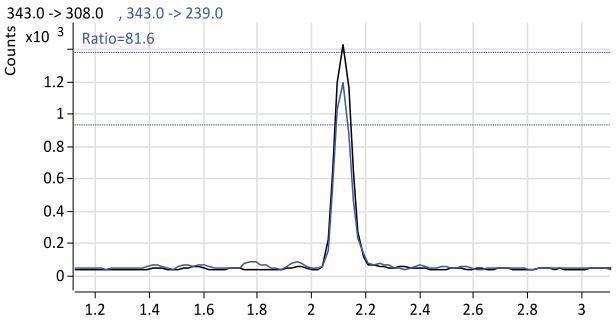
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.5 mL/min
Column temperature	35 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 8.6, B = Acetonitrile
Gradient	0 min 50% B Isocratic 5.5 min Stop. Post-time Off
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	10 L/min
Drying gas temperature	300 °C
Capillary Voltage	3500 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Alprazolam-d_5	314	286	160	25
Triazolam	**343**	**308**	**120**	**35**
Triazolam	343	239	120	35



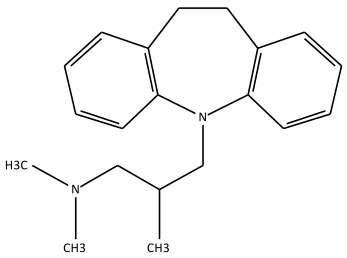
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Trimipramine

Chemical name:	Trimeproprimine; (IUPAC name) 10, 11-Dihydro-N, N, β-trimethyl-5H-dibenz[b,f]azepine-5-propanamine
Molecular formula:	$C_{20}H_{26}N_2$
Molecular weight*:	294.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #222

Cut-off: 25 ng/mL	**Target Compound:** Nortriptyline **Significant cross-reactivity:**	
	Nortriptyline	100%
	Amitriptyline	200%
	Desipramine	200%
	Imipramine	200%
	Trimipramine	50%

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

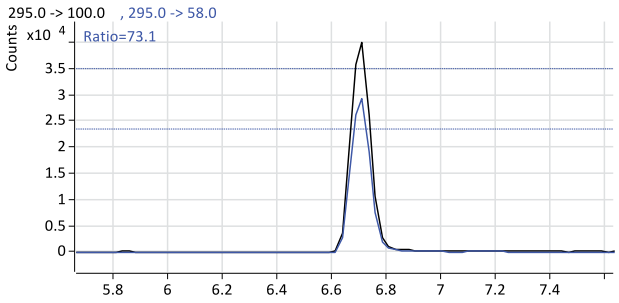
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Paroxetine-d_6	336	76	120	20
Trimipramine	**295**	**100**	**110**	**10**
Trimipramine	295	58	110	30



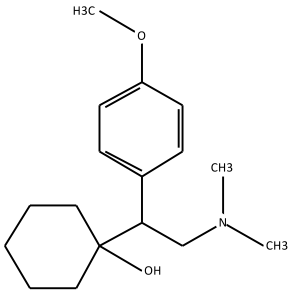
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Venlafaxine

Chemical name:	(IUPAC name) 1-[2-(Dimethylamino)-1-(4-methoxyphenyl)ethyl] cyclohexane-1-ol
Molecular formula:	$C_{17}H_{27}NO_2$
Molecular weight*:	277.2

Molecular Structure:



Screening methodology: None

Confirmatory methodology: LC-MS/MS

LOQ 10 ng/mL

LC Parameters

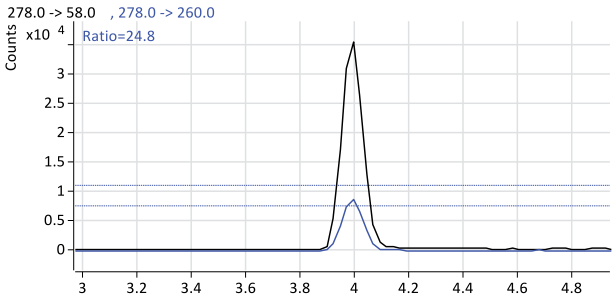
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	1 mL/min
Column temperature	45 °C
Mobile phase	A = Water 0.2% Acetic Acid, B = Methanol
Gradient	0 min 30% B 0.1 min 30% B 8 min 70% B 9 min 30% B 11 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	11 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	Precursor	Product	Fragmentor (V)	Collision Energy (V)
Paroxetine-d_6	336	76	120	20
Venlafaxine	278	260	100	5
Venlafaxine	**278**	**58**	**100**	**15**



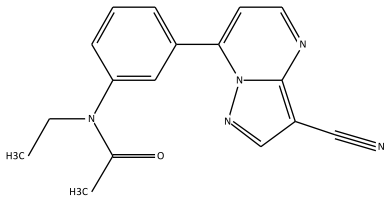
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Zaleplon

Chemical name:	N-[3-(3-Cyanopyrazolo[1, 5-a]pyrimidin-7-ylphenyl]-N-ethylacetamide
Molecular formula:	$C_{17}H_{15}N_5O$
Molecular weight*:	305.1

Molecular Structure:



Screening methodology: Immunalysis ELISA in development

Cut-off: N/A	**Target Compound:** N/A **Significant cross-reactivity:**	
	None	N/A

Confirmatory methodology: LC-MS/MS
LOQ 10 ng/mL

LC Parameters

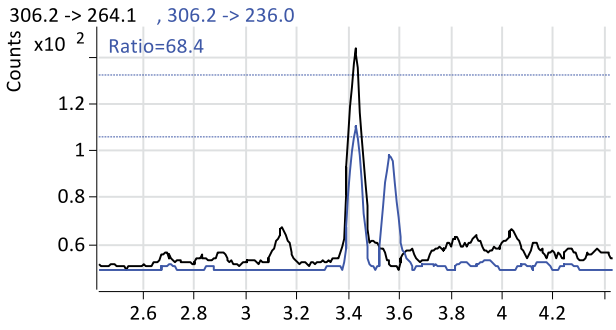
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Acetonitrile
Gradient	0 min 15% B 4 min 100% B 5 min 15% B 5.5 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	6 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	Precursor	Product	Fragmentor (V)	Collision Energy (V)
Zolpidem-d_6	313.9	235.1	100	30
Zaleplon	**306.2**	**264.1**	**80**	**20**
Zaleplon	306.2	236	80	30



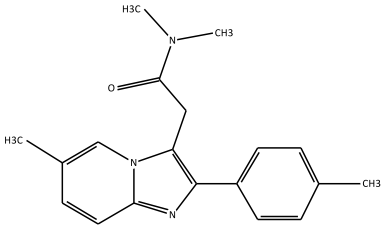
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Zolpidem

Chemical name:	N, N-6-trimethyl-2-(4-methylphenyl)imidazo[1, 2-*a*]pyridine-3-acetamide
Molecular formula:	$C_{19}H_{21}N_3O$
Molecular weight*:	307.2

Molecular Structure:



Screening methodology: Immunalysis ELISA Catalog #233

Cut-off: 5 ng/mL	**Target Compound:** Zolpidem **Significant cross-reactivity:**	
	Zolpidem	100%

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

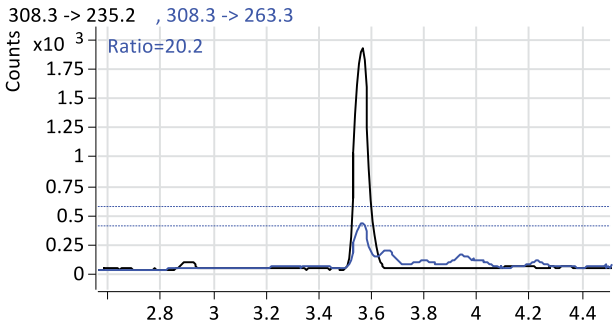
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Acetonitrile
Gradient	0 min 15% B 4 min 100% B 5 min 15% B 5.5 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	6 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Zolpidem-d_6	313.9	235.1	100	30
Zolpidem	308.3	263.3	100	20
Zolpidem	**308.3**	**235.2**	**100**	**20**



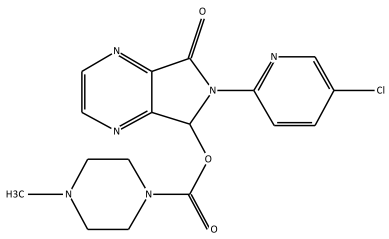
*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

Zopiclone

Chemical name:	4-Methyl-1-piperazine-carboxylic acid-6-(5-chloro-2-pyridinyl)-6, 7-dihydro-7-oxo-5H-pyrrolo[3, 4-b]pyrazin-5-yl ester
Molecular formula:	$C_{17}H_{17}ClN_6O_3$
Molecular weight*:	388.1

Molecular Structure:



Screening methodology: Immunalysis ELISA in development

Cut-off: N/A	**Target Compound:** N/A **Significant cross-reactivity:**	
	None	N/A

Confirmatory methodology: LC-MS/MS
LOQ 1 ng/mL

LC Parameters

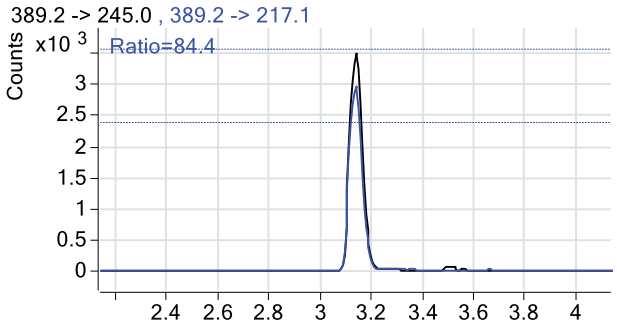
Column	Zorbax Eclipse Plus C18 Rapid Resolution HT 4.6 x 50 mm, 1.8 µm
Flow rate	0.7 mL/min
Column temperature	40 °C
Mobile phase	A = water 20 mM Ammonium Formate pH 6.4, B = Acetonitrile
Gradient	0 min 15% B 4 min 100% B 5 min 15% B 5.5 min Stop. Post-time 2 min
Injection volume	5 µL

MS Parameters

Nebulizer	50 psi
Drying gas flow	6 L/min
Drying gas temperature	350 °C
Capillary Voltage	4000 V

LC-MS/MS transitions:

Compound	**Precursor**	**Product**	**Fragmentor (V)**	**Collision Energy (V)**
Zolpidem-d_6	313.9	235.1	100	30
Zopiclone	**389.2**	**245.1**	**100**	**10**
Zopiclone	389.2	217.1	100	30



*Monoisotopic molecular weight

MRM chromatograms shown at LOQ with ± 20% ion ratio limits

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Printed in the USA
January 15, 2016
5991-6633EN

Agilent Technologies