LZI Amphetamines 500 Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0100 (100/37.5 mL R_1/R_2 Kit)
0101 (1000/375 mL R_1/R_2 Kit)

2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Amphetamines 500 Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of methamphetamine and amphetamine in human urine at a cutoff value of 500 ng/mL when calibrated with *d*-methamphetamine. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

The assay provides only a preliminary analytical result. A more specific alternative analytical chemistry method must be used in order to obtain a confirmed analytical result. Gas or Liquid Chromatography/Mass Spectrometry (GC/MS or LC/MS) are the preferred confirmatory methods (1, 2). Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

Summary and Explanation of Test

Amphetamines are a class of phenethylamine drugs that have sympathomimetic activity, which imitate the stimulating actions of the endogenous neurotransmitter (3). The ability of amphetamines to alleviate fatigue, improve mental and physical performances, elevate mood, and produce euphoria has led to the abuse of these prescription drugs. Amphetamines are psychologically and physiologically addicting. Chronic, high dose abuse can lead to a psychotic condition indistinguishable to acute schizophrenia (4). The most common amphetamines include *d*-amphetamine, *d*- methamphetamine, and *d*,*l*-amphetamine (5). Due to its ease of manufacture and ready availability, methamphetamine is the most abused amphetamine. Analogs of methamphetamine and amphetamine such as methylenedioxy-methamphetamine (MDMA; Ecstasy) and 3, 4-methylendioxy-amphetamine (MDA) have recently become popular at rave parties in both the United States and Europe (3, 6).

Amphetamines can be taken orally, intravenously, or by smoking or snorting. They are rapidly absorbed from the gastrointestinal tract, and then either metabolized in liver or excreted in urine unchanged (3, 4). The presence of amphetamines may be detectable in urine for 3-4 days after administration (7).

Assay Principle

The LZI Amphetamines 500 assay is a homogeneous enzyme immunoassay with ready-to-use liquid reagent. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent (8). Enzyme activity decreases upon binding to the antibody, and the drug concentration in the sample is measured in terms of enzyme activity.

In the absence of drug in the sample, amphetamines-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when free drug is present in the sample, antibody binds to the free drug. The unbound amphetamines-labeled G6PDH then exhibits its maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at a 340 nm primary wavelength.

Reagents Provided

Antibody/Substrate Reagent (R_1): Contains mouse monoclonal anti-amphetamines antibodies, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R_2): Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with amphetamines in buffer with sodium azide (0.09 %) as a preservative.

Calibrators and Controls*

**Calibrators and controls are sold separately and contain negative human urine with sodium azide as a preservative.*

AMPHETAMINES 500 Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 250 ng/mL *d*-methamphetamine	0102
Cutoff Calibrator: Contains 500 ng/mL *d*-methamphetamine	0103
Intermediate Cal.: Contains 1000 ng/mL *d*-methamphetamine	0104
High Calibrator: Contains 2000 ng/mL *d*-methamphetamine	0105

AMPHETAMINES 500 Controls	REF
Level 1 Control: Contains 375 ng/mL *d*-methamphetamine	0107
Level 2 Control: Contains 625 ng/mL *d*-methamphetamine	0108

Precautions and Warning

- *This test is for in vitro diagnostic use only. Harmful if swallowed.*
- *Reagent contains sodium azide as a preservative, which may form explosive compounds in metal drain lines. When disposing such reagents or wastes always flush with a large volume of water to prevent azide build-up. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (9).*
- *Do not use the reagents beyond their expiration dates.*
- Rx ONLY *For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.*

Reagent Preparation and Storage

The reagents are ready-to-use. No reagent preparation is required. All assay components should be refrigerated at 2-8ºC when not in use.

Specimen Collection and Handling

Urine samples may be collected in plastic or glass containers. Some plastics may absorb drugs. Use of plastics such as polyethylene is recommended (10). Use fresh urine specimens for the test. If a sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to seven days (11, 12, 13). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown amphetamine analytes in urine are stable at -20ºC up to 24 months (13, 14). Samples should be equilibrated to room temperature of 18-25ºC for testing. Samples with high turbidity should be centrifuged before analysis.

Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and forward both samples to the laboratory for testing.

Handle all urine specimens as if they are potentially infectious.

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzyme rates at a 340 nm primary wavelength and timing the reaction accurately can be used to perform this homogeneous immunoassay. Performance characteristics presented in this package insert have been validated on the Hitachi 717 and the Synermed IR500 clinical analyzers.

Assay Procedure

Refer to the specific parameters used for each analyzer before performing the assay. For qualitative analysis, use the 500 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. Recalibration should be performed after reagent bottle change or if there is a change in calibrators or reagent lot. Two levels of controls are also available for monitoring the cutoff level: 375 ng/mL and 625 ng/mL.

Calibration and Quality Control

Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) daily to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument system manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters, or contact LZI technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

Results

Note: A preliminary positive test result does not necessarily mean a person took illegal drugs and a negative test result does not necessarily mean a person did not take illegal drugs. There are a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator, which contains 500 ng/mL of *d*-methamphetamine, is used as a reference for distinguishing a preliminary positive from negative samples. A sample with a change in absorbance (ΔmA/min) equal to or greater than that obtained with the cutoff calibrator is considered a preliminary positive. A sample with a change in absorbance (ΔmA/min) lower than that obtained with the cutoff calibrator is considered negative.

Semi-Quantitative: The semi-quantitative mode is for the purpose of (1) enabling laboratories to determine an appropriate dilution of the specimen for verification by confirmatory methods such as GC/MS or LC/MS or (2) permitting laboratories to establish quality control procedures.

When an approximation of concentration is required, a calibration curve can be established with five calibrators. The concentration of amphetamines in the sample may then be estimated from the calibration curve.

Limitations

1. *A preliminary positive result from the assay indicates only the presence of amphetamines. The test is not intended for quantifying these single analytes in samples.*
2. *A preliminary positive result does not necessarily indicate drug abuse.*
3. *A negative result does not necessarily mean a person did not take amphetamines.*
4. *There is a possibility that other substances and/or factors not listed above may interfere with the test and cause incorrect results (e.g., technical or procedural error, fluid intake, endogenous or exogenous interferents).*
5. *Preliminary positive results should be confirmed by other affirmative, analytical chemistry methods (e.g., chromatography), preferably GC/MS or LC/MS.*
6. *The test is designed for use with human urine only*.
7. *The test is not for therapeutic drug monitoring.*
8. *There is a possibility that metabolites of other phenethylamine drugs may interfere with the test.*

Typical Performance Characteristics

The assay's range from 0 ng/mL to 2000 ng/mL was tested in qualitative (mA/min) and semi-quantitative (ng/mL) modes using a modified NCCLS protocol. Results shown below were obtained by testing all samples in replicates of two, two runs per day, for 22 days on the Hitachi 717 automated clinical chemistry analyzer.

Precision: *d*-methamphetamine

Semi-quantitative analysis: Typical results (ng/mL) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	5.6	5.3	116.6 %	5.6	7.5	132.1 %
125 ng/mL	128.3	6.4	5.0 %	128.3	8.4	6.6 %
250 ng/mL	252.9	6.1	2.4 %	252.9	9.1	3.6 %
375 ng/mL	369.6	11.4	3.1 %	369.6	14.6	4.0 %
500 ng/mL	489.9	11.7	2.4 %	489.9	15.9	3.2 %
625 ng/mL	605.3	17.6	2.9 %	605.3	19.3	3.2 %
750 ng/mL	746.5	16.3	2.2 %	746.5	18.9	2.5 %
875 ng/mL	867.7	22.9	2.6 %	867.7	25.6	3.0 %
1000 ng/mL	1024.2	34.0	3.3 %	1024.2	41.8	4.1 %

Concentration	Within Run (N=22)		Total Precision (N=88)	
	Mean	Qualitative Response	Mean	Qualitative Response
0 ng/mL	5.6	-	5.6	-
125 ng/mL	128.3	-	128.3	-
250 ng/mL	252.9	-	252.9	-
375 ng/mL	369.6	-	369.6	-
500 ng/mL	489.9	-	489.9	-
625 ng/mL	605.3	+	605.3	+
750 ng/mL	746.5	+	746.5	+
875 ng/mL	867.7	+	867.7	+
1000 ng/mL	1024.2	+	1024.2	+

500 ng/mL Cutoff Result:		Within Run (N=22)		Total Precision (N=88)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
125 ng/mL	25 %	22	22 Neg	88	88 Neg
250 ng/mL	50 %	22	22 Neg	88	88 Neg
375 ng/mL	75 %	22	22 Neg	88	88 Neg
500 ng/mL	100 %	22	19 Neg/ 3 Pos	88	70 Neg/ 18 Pos
625 ng/mL	125 %	22	22 Pos	88	88 Pos
750 ng/mL	150 %	22	22 Pos	88	88 Pos
875 ng/mL	175 %	22	22 Pos	88	88 Pos
1000 ng/mL	200 %	22	22 Pos	88	88 Pos

Qualitative analysis: Typical results (ΔmA/min) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	273.5	2.8	1.0 %	273.5	3.4	1.2 %
125 ng/mL	314.5	2.2	0.7 %	314.5	3.0	0.9 %
250 ng/mL	359.0	2.2	0.6 %	359.0	3.4	0.9 %
375 ng/mL	398.2	3.0	0.8 %	398.2	4.1	1.0 %
500 ng/mL	431.3	3.0	0.7 %	431.3	4.3	1.0 %
625 ng/mL	459.6	3.8	0.8 %	459.6	5.2	1.1 %
750 ng/mL	489.2	3.9	0.8 %	489.2	5.4	1.1 %
875 ng/mL	509.7	4.1	0.8 %	509.7	5.1	1.0 %
1000 ng/mL	533.1	3.7	0.7 %	533.1	4.8	0.9 %

Additional Qualitative analysis: The following table summarizes the absorbance (mA/min) as being positive or negative results:

500 ng/mL Cutoff Result:		Within Run (N=22)		Total Precision (N=88)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
125 ng/mL	25 %	22	22 Neg	88	88 Neg
250 ng/mL	50 %	22	22 Neg	88	88 Neg
375 ng/mL	75 %	22	22 Neg	88	88 Neg
500 ng/mL	100 %	22	9 Neg/ 13 Pos	88	34 Neg/ 54 Pos
625 ng/mL	125 %	22	22 Pos	88	88 Pos
750 ng/mL	150 %	22	22 Pos	88	88 Pos
875 ng/mL	175 %	22	22 Pos	88	88 Pos
1000 ng/mL	200 %	22	22 Pos	88	88 Pos

Precision: *d*-amphetamine

Semi-quantitative analysis: Typical results (ng/mL) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	18.2	15.1	77.6 %	18.2	17.8	98.1 %
125 ng/mL	165.5	10.1	6.1 %	165.5	14.3	8.6 %
250 ng/mL	297.4	8.1	2.7 %	297.4	14.0	4.7 %
375 ng/mL	409.0	8.2	2.0 %	409.0	15.7	3.8 %
500 ng/mL	529.9	13.7	2.6 %	529.9	17.3	3.3 %
625 ng/mL	631.3	10.6	1.7 %	631.3	18.5	2.9 %
750 ng/mL	714.0	19.0	2.7 %	714.0	23.0	3.2 %
875 ng/mL	809.1	15.4	1.9 %	809.1	20.7	2.6 %
1000 ng/mL	910.6	20.2	2.2 %	910.6	26.6	2.9 %

Concentration	Within Run (N=22)		Total Precision (N=88)	
	Mean	Qualitative Response	Mean	Qualitative Response
0 ng/mL	18.2	-	18.2	-
125 ng/mL	165.5	-	165.5	-
250 ng/mL	297.4	-	297.4	-
375 ng/mL	409.0	-	409.0	-
500 ng/mL	529.9	+	529.9	+
625 ng/mL	631.3	+	631.3	+
750 ng/mL	714.0	+	714.0	+
875 ng/mL	809.1	+	809.1	+
1000 ng/mL	910.6	+	910.6	+

500 ng/mL Cutoff Result:		Within Run (N=22)		Total Precision (N=88)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
125 ng/mL	25 %	22	22 Neg	88	88 Neg
250 ng/mL	50 %	22	22 Neg	88	88 Neg
375 ng/mL	75 %	22	22 Neg	88	88 Neg
500 ng/mL	100 %	22	22 Pos	88	5 Neg/ 83 Pos
625 ng/mL	125 %	22	22 Pos	88	88 Pos
750 ng/mL	150 %	22	22 Pos	88	88 Pos
875 ng/mL	175 %	22	22 Pos	88	88 Pos
1000 ng/mL	200 %	22	22 Pos	88	88 Pos

Qualitative analysis: Typical results (ΔmA/min) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	330.4	2.5	0.8 %	330.4	3.2	1.0 %
125 ng/mL	362.6	1.7	0.5 %	362.6	2.7	0.8 %
250 ng/mL	400.5	2.6	0.6 %	400.5	3.5	0.9 %
375 ng/mL	429.9	2.3	0.5 %	429.9	3.4	0.8 %
500 ng/mL	458.9	4.3	0.9 %	458.9	4.9	1.1 %
625 ng/mL	484.6	2.3	0.5 %	484.6	3.6	0.7 %
750 ng/mL	498.9	3.2	0.6 %	498.9	4.4	0.9 %
875 ng/mL	515.5	2.8	0.5 %	515.5	4.1	0.8 %
1000 ng/mL	533.2	2.6	0.5 %	533.2	4.4	0.8 %

Additional Qualitative analysis: The following table summarizes the absorbance (mA/min) as being positive or negative results:

500 ng/mL Cutoff Result:		Within Run (N=22)		Total Precision (N=88)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
125 ng/mL	25 %	22	22 Neg	88	88 Neg
250 ng/mL	50 %	22	22 Neg	88	88 Neg
375 ng/mL	75 %	22	22 Neg	88	88 Neg
500 ng/mL	100 %	22	7 Neg/ 15 Pos	88	40 Neg/ 48 Pos
625 ng/mL	125 %	22	22 Pos	88	88 Pos
750 ng/mL	150 %	22	22 Pos	88	88 Pos
875 ng/mL	175 %	22	22 Pos	88	88 Pos
1000 ng/mL	200 %	22	22 Pos	88	88 Pos

Limit of Detection: The lowest concentration that can be differentiated from the negative urine with 95 % confidence is determined as 50 ng/mL for both *d*-methamphetamine and *d*-amphetamine, which is well below the cutoff concentration of 500 ng/mL.

Accuracy: *d*-methamphetamine

Eighty-six (86) unaltered clinical urine specimens were tested with the LZI Amphetamines 500 Enzyme Immunoassay and confirmed with LC/MS. Specimens having a *d*-methamphetamine concentration greater than 500 ng/mL by LC/MS are defined as positive, and specimens with lower concentrations by LC/MS are defined as negative in the table below. The correlation results are summarized as follows (near cutoff samples are defined as ± 50 % of the cutoff value):

Semi-Quantitative Accuracy Study:

500 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agree-ment
Positive	0	4*	10**	9	34	100 %
Negative	4	20	5	0	0	67.4 %

Discrepant Sample #	GCMS or LCMS AMP Result (ng/mL)	GCMS or LCMS MAMP Result (ng/mL)	Total GCMS or LCMS Result AMP + MAMP (ng/mL)	EIA Result (ng/mL)
5*	584.0	**16.1**	600.1	521.8
10*	621.0	**113.6**	734.6	692.7
12*	1029.0	**133.0**	1162.0	1358.9
22*	956.6	**183.9**	1140.5	945.6
30**	397.2	**268.7**	665.9	690.5
33**	243.5	**306.4**	549.9	709.7
36**	235.3	**385.3**	620.6	643.5
37**	177.8	**387.8**	565.6	583.7
38**	225.5	**401.7**	627.2	513.2
39**	146.8	**409.1**	555.9	631.7
40**	708.0	**413.0**	1121.0	2070.2
41**	129.6	**413.4**	543.0	562.7
42**	180.0	**457.8**	637.8	638.7
43**	108.0	**471.0**	579.0	574.0

Qualitative Accuracy Study:

The Qualitative Cutoff value is 435.0 mA/min at 500 ng/mL cutoff concentration.

500 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agree-ment
Positive	0	4*	9**	9	34	100 %
Negative	4	20	6	0	0	69.8 %

Discrepant Sample #	GCMS or LCMS AMP Result (ng/mL)	GCMS or LCMS MAMP Result (ng/mL)	Total GCMS or LCMS Result AMP + MAMP (ng/mL)	EIA Result (mA/min)
5*	584.0	**16.1**	600.1	435.5
10*	621.0	**113.6**	734.6	477.6
12*	1029.0	**133.0**	1162.0	575.0
22*	956.6	**183.9**	1140.6	531.3
30**	397.2	**268.7**	666.0	476.0
33**	243.5	**306.4**	549.9	485.1
36**	235.3	**385.3**	620.6	465.3
37**	177.8	**387.8**	565.5	459.5
38**	225.5	**409.1**	555.9	462.9
40**	708.0	**413.0**	1121.0	621.7
41**	129.6	**413.4**	542.9	455.0
42**	180.0	**457.8**	637.8	466.7
43**	108.0	**471.0**	579.0	449.4

Accuracy: *d*-amphetamine

One-hundred and eleven (111) unaltered clinical urine specimens were tested with the LZI Amphetamines 500 Enzyme Immunoassay and confirmed with LC/MS. Specimens having a *d*-amphetamine concentration greater than 500 ng/mL by LC/MS are defined as positive, and specimens with lower concentrations by LC/MS are defined as negative in the table below. The correlation results are summarized as follows (near cutoff samples are defined as ± 50 % of the cutoff value):

Semi-Quantitative Accuracy Study:

500 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agree-ment
Positive	0	0	0	10	45	98.2 %
Negative	12	22	21	1*	0	100 %

Discrepant Sample #	GCMS or LCMS AMP Result (ng/mL)	GCMS or LCMS MAMP Result (ng/mL)	Total GCMS or LCMS Result AMP + MAMP (ng/mL)	EIA Result (ng/mL)
58*	531.0	**0.0**	531.0	442.6

Qualitative Accuracy Study:

The Qualitative Cutoff value is 435.0 mA/min at 500 ng/mL cutoff concentration.

500 ng/mL Cutoff	Neg	<50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agree-ment
Positive	0	0	0	9	45	96.4 %
Negative	12	22	21	2*	0	100 %

Discrepant Sample #	GCMS or LCMS AMP Result (ng/mL)	GCMS or LCMS MAMP Result (ng/mL)	Total GCMS or LCMS Result AMP + MAMP (ng/mL)	EIA Result (mA/min)
56*	510.0	**0.0**	510.0	428.6
58*	531.0	**0.0**	531.0	424.5

Analytical Recovery: To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section), a drug-free urine pool spiked with either *d*-methamphetamine or *d*-amphetamine was serially diluted. Each sample was run in 10 replicates and the average is used to determine the functional linearity range of the assay. When comparing the result (y) and target (x) value, using the least squares regression technique, the regression equation and correlation are as follows:

***d*-methamphetamine**

$y = 0.9717x + 5.7341$, $r^2 = 0.9971$

% Dilution	Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
100%	0	5.7	N/A
98.75%	25	36.0	144.1 %
97.50%	50	56.2	112.4 %
90.00%	200	205.8	102.9 %
85.00%	300	293.6	97.9 %
80.00%	400	380.7	95.2 %
75.00%	500	489.4	97.9 %
70.00%	600	564.8	94.1 %
62.50%	750	732.3	97.6 %
50.00%	1000	974.9	97.5 %
25.00%	1500	1552.3	103.5 %
0.00%	2000	1894.9	94.7 %

***d*-amphetamine**

$y = 0.9447x + 24.34$, $r^2 = 0.9978$

% Dilution	Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
100.0 %	0	4.1	N/A
98.75 %	25	30.1	120.4 %
92.50 %	150	193.7	129.2 %
85.00 %	300	346.6	115.5 %
80.00 %	400	438.9	109.7 %
75.00 %	500	485.5	97.1 %
70.00 %	600	562.2	93.7 %
62.50 %	750	734.9	98.0 %
50.00 %	1000	926.9	92.7 %
30.00 %	1400	1340.8	95.8 %
0.00 %	2000	1934.6	96.7 %

Specificity: Cross-reactivity of various potential interfering drugs were tested by spiking a final concentration of 100,000 ng/mL of each substance into drug-free urine, and then evaluated with the assay's calibrated dose-response curve. The assay detects equally well for *d*-amphetamine and *d*-methamphetamine at the 500 ng/mL cutoff. The following table summarizes the approximate quantity of each compound that is equivalent in assay reactivity to the 500 ng/mL amphetamines cutoff or the maximal concentration of the compound tested that gave a response with cross-reactivity below the response of the cutoff calibrator.

Amphetamines Compounds:

Compound	Target [ ] (ng/mL)	Observed Value (ng/mL)	% Cross-Reactivity
d-amphetamine	500	480.7	96.1 %
d-methamphetamine	500	489.4	97.9 %

Structurally Related Amphetamines Class Compounds*:

Compound	Target [ ] (ng/mL)	Observed Value (ng/mL)	% Cross-Reactivity
l-Amphetamine	12,000	271.6	2.3 %
Atomoxetine	500,000	75.15	0.0 %
Benzphetamine	500,000	121.4	0.0 %
d-Ephedrine	150,000	400.7	0.3 %
l-Ephedrine	200,000	409.1	0.2 %

Structurally Related Amphetamines Class Compounds, continued*:

Compound	Target [ ] (ng/mL)	Observed Value (ng/mL)	% Cross-Reactivity
Fenfluramine	4,000	433.3	10.8 %
3-Hydroxy-Tyramine	500,000	300.5	0.1 %
Isoxsuprine	500,000	83.9	0.0 %
Mephentermine	25,000	57.4	0.2 %
l-Methamphetamine	5,000	386.1	7.7 %
para-Methoxyamphetamine (PMA)	400	433.8	108.4 %
Methylenedioxy-amphetamine (MDA)	1,400	306.0	21.9 %
Methylenedioxyethyl-amphetamine (MDEA)	10,000	441.9	4.4 %
Methylenedioxymeth-amphetamine (MDMA)	1,250	427.1	34.2 %
Phendimetrazine	150,000	213.5	0.1 %
Phenethylamine	25,000	411.4	1.7 %
Phenmetrazine	40,000	313.2	0.8 %
Phentermine	20,000	416.3	2.1 %
Phenylephrine	400,000	453.4	0.1 %
d,l-Phenylpropanolamine	150,000	403.9	0.3 %
d-Pseudoephedrine	150,000	422.3	0.3 %
l-Pseudoephedrine	200,000	106.9	0.1 %
Tranylcypromine	50,000	399.1	0.8 %
Tyramine	400,000	423.5	0.1 %

Structurally Unrelated Pharmacological Compounds*:

Compound	Target [ ] (ng/mL)	Observed Value (ng/mL)	% Cross-Reactivity
Acetaminophen	500,000	108.6	0.02 %
Acetylsalicylic Acid	500,000	97.8	0.02 %
Amobarbital	500,000	100.1	0.02 %
Benzoylecgonine	500,000	103.1	0.02 %
Bromopheniramine	500,000	125.0	0.02 %
Bupropion	500,000	194.0	0.04 %
Buspirone	500,000	170.0	0.03 %
Caffeine	500,000	105.1	0.02 %
Chlorpheniramine	500,000	129.7	0.03 %
Chlorpromazine	500,000	159.7	0.03 %
Codeine	500,000	109.4	0.02 %
Dextromethorphan	500,000	107.2	0.02 %
Doxepine	500,000	240.4	0.05 %
Meperidine	500,000	30.8	0.01 %
Methadone	500,000	121.2	0.02 %
Methapyrilene	500,000	191.5	0.04 %
Methaqualone	500,000	101.6	0.02 %
Morphine	500,000	62.9	0.01 %
Oxazepam	500,000	101.2	0.02 %
Phencyclidine	500,000	29.3	0.01 %
Phenobarbital	500,000	99.7	0.02 %
Phenothiazine	500,000	65.0	0.01 %
Procainamide	500,000	412.4	0.08 %
Promethazine	500,000	122.0	0.02 %
Propoxyphene	500,000	92.2	0.02 %
Propranolol	500,000	102.3	0.02 %
Ranitidine	80,000	799.8	1.00 %
Scopolamine	500,000	105.6	0.02 %
Secobarbital	500,000	92.3	0.02 %
Sertraline	500,000	194.1	0.04 %
Thioridazine	500,000	103.6	0.02 %
Trazodone	500,000	251.6	0.05 %
Trifluoperazine	500,000	123.0	0.02 %
Triflupromazine	500,000	127.8	0.03 %
Valproic Acid	500,000	88.3	0.02 %

** It is possible that other substances and/or factors not listed above may interfere with the test and cause false results, e.g., technical or procedure errors.*

Interference: Endogenous Substances *d*-methamphetamine

The following endogenous compounds were spiked into a pool of negative urine or a pool of negative urine spiked with standards to the following two levels of *d*-methamphetamine concentrations (375 ng/mL and 625 ng/mL) for the assay. The spiked solution is evaluated against the *d*-methamphetamine calibration curve. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding positive/negative results against the cutoff value of 500 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked [ ] (mg/dL)	0 ng/mL (ng/mL)	375 ng/mL Control (ng/mL)	625 ng/mL Control (ng/mL)
None	N/A	0.0	358.8	634.9
Acetone	1000	7.5	364.0	632.9
Ascorbic Acid	1500	0.0	330.3	548.9
Creatinine	500	11.9	368.0	622.5
Ethanol	1000	3.7	347.7	618.8
Galactose	10	5.4	383.7	624.9
γ-Globulin	500	0.0	370.9	619.3
Glucose	1500	2.6	376.4	639.7
Hemoglobin	300	27.2	396.4	653.3
Human Serum Albumin	500	5.1	392.5	662.3
Oxalic Acid	100	7.8	380.3	647.8
Riboflavin	2.5	0.0	399.1	647.0
Sodium Chloride	6000	0.0	356.1	583.0
Urea	2000	2.6	364.2	613.9
pH 3	N/A	4.8	376.7	647.1
pH 4.5	N/A	13.8	376.2	631.1
pH 5	N/A	5.1	375.6	633.9
pH 6	N/A	7.0	373.8	640.9
pH 7	N/A	12.7	380.0	642.6
pH 8	N/A	3.7	379.0	648.8
pH 11	N/A	7.3	379.4	644.0

Interference: Endogenous Substances *d*-amphetamine

The following endogenous compounds were spiked into a pool of negative urine or a pool of negative urine spiked with standards to the following two levels of *d*-amphetamine concentrations (375 ng/mL and 625 ng/mL) for the assay. The spiked solution was evaluated against the *d*-methamphetamine calibration curve. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding positive/negative results against the cutoff value of 500 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked [ ] (mg/dL)	0 ng/mL (ng/mL)	375 ng/mL Control (ng/mL)	625 ng/mL Control (ng/mL)
None	N/A	30.7	401.2	622.4
Acetone	1000	54.0	417.2	654.4
Ascorbic Acid	1500	0.0	365.7	536.8
Creatinine	500	54.1	428.0	648.6
Ethanol	1000	24.6	419.9	632.1
Galactose	10	33.7	427.9	621.7
γ-Globulin	500	30.4	425.6	614.4
Glucose	1500	12.7	424.1	627.7
Hemoglobin	300	58.8	452.6	663.1
Human Serum Albumin	500	33.3	421.0	645.1
Oxalic Acid	100	12.8	415.4	626.5
Riboflavin	7.5	12.6	430.8	636.9
Sodium Chloride	6000	0.0	344.4	506.6
Urea	2000	21.5	428.4	562.6
pH 3	N/A	8.4	408.2	621.5
pH 4.5	N/A	19.9	432.1	647.1
pH 5	N/A	25.7	427.2	584.1
pH 6	N/A	28.0	424.1	588.3
pH 7	N/A	14.1	387.5	574.1
pH 8	N/A	34.5	394.9	636.2
pH 11	N/A	0.0	385.8	662.6

Specific Gravity: Samples ranging in specific gravity from 1.002 to 1.030 were tested with the assay in the presence of 0 ng/mL, 375 ng/mL, and 625 ng/mL of *d*-methamphetamine or *d*-amphetamine, and no interference was observed.

Note: All endogenous substances listed above, including specific gravity, were also tested in qualitative mode. No interference was observed. The results are identical to those determined by semi-quantitative mode, as all samples gave correct positive/negative results corresponding to the cutoff value of 500 ng/mL.

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