ARK™ PREGABALIN ASSAY ON BECKMAN COULTER AU480 ANALYZER

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Introduction

ABSTRACT

In Europe, pregabalin is approved for the treatment of epilepsy, neuropathic pain and generalized anxiety disorder. In the United States, pregabalin is approved for the treatment of epilepsy, diabetic neuropathy, postherpetic neuropathy, and fibromyalgia. In the United States pregabalin is classified as a schedule V drug by the Drug Enforcement Administration. In the European Union, pregabalin is not a controlled substance, but a warning related to its abuse potential was added to the Summary of Product Characteristics in June 2010. Reports suggest that pregabalin is a recreational drug and abuse potential may exist. Successful pain management demands consistent monitoring to ensure compliance as prescribed. Based upon illicit use of pregabalin and the need to verify treatment compliance, a urinary drug test applicable to general chemistry analyzers would be a useful for identifying pregabalin in urine. ARK™ Pregabalin Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, 5 calibrators (0, 100, 500, 1000, and 2000 ng/mL) and 2 controls (250, and 750 ng/mL). In addition to a semi-quantitative mode, the 500 ng/mL Calibrator can be used as a Cutoff reference in a qualitative mode for distinguishing "positive" from "negative" samples. Performance of this assay was evaluated using a Beckman AU480 analyzer. Precision, spike recovery, specificity and Histogram Overlap Analysis of Controls and Cutoff concentrations were evaluated. In the semi-quantitative mode, total precision ranged from 7.3% to 9.8%CV. Accuracy was determined by spiking pregabalin into pooled pregabalin-free urine. Using the semi-quantitative mode, spiked recovery of pregabalin ranged from 94.4% (1500 ng/mL) to 104.4% (200 ng/mL). Qualitative mode tests for gabapentin and 20 L-amino acids, which are structurally similar to pregabalin, did not result in false positive responses. Histogram overlap analysis showed no overlap between Cutoff and Control levels. ARK Pregabalin Assay measures pregabalin in human urine with acceptable performance in either the semi-quantitative or qualitative modes. The ability to measure urine levels of pregabalin with high accuracy and fast turn-around time makes this method ideal for identifying the presence of pregabalin in urine to detect misuse use or abuse and verify treatment compliance.

METHOD – BECKMAN COULTER AU480®

The ARK Pregabalin Urine Assay provides a simple and rapid analytical screening procedure for detecting pregabalin in urine.

Qualitative Mode: Quality controls, Cutoff Calibrator and urine samples are run with measurements expressed as enzymatic rate (mA/min). A specimen that gives a rate value equal to or greater than the ARK Pregabalin Urine Calibrator cutoff rate value is interpreted as positive, indicating that pregabalin is present. A specimen that gives a rate value less than the ARK Pregabalin Urine Calibrator cutoff rate value is interpreted as negative.

Semiquantitative Mode: To estimate the concentration of pregabalin, perform a 5-point calibration procedure; test calibrators in duplicate. Verify the calibration curve with ARK Low and High quality controls according to the established laboratory quality assurance plan.

Precision

Pooled human urine was spiked with pregabalin to achieve concentrations at 25% increments apart from the Cutoff Calibrator (500 ng/mL). Samples were tested in quadruplicate twice per day for 20 days (N=160). Qualitative and semiquantitative modes of analysis were evaluated. Precision data were calculated according to the Clinical Laboratory Standards Guideline Protocol EP05-A3.

QUALITATIVE ANALYSIS

Urine Spiked Samples		N=160	Within Run		Total	
(ng/mL)	% of cutoff	Mean (mA/min)	SD	CV (%)	SD	CV (%)
0	-100	426	2.1	0.5	2.6	0.6
250	-50	477	2.6	0.5	3.5	0.7
375	-25	488	3.2	0.6	3.7	0.8
500	cutoff	498	2.1	0.4	2.9	0.6
625	+25	508	2.2	0.4	3.2	0.6
750	+50	513	2.1	0.4	3.3	0.7
1000	+100	523	2.3	0.4	3.0	0.6

SEMIQUANTITATIVE ANALYSIS

Urine Spiked Samples		N=160	Within Run		Total	
(ng/mL)	% of cutoff	Mean (ng/mL)	SD	CV (%)	SD	CV (%)
0	-100	0.6	-	-	-	-
250	-50	248	20.2	8.2	26.1	10.5
375	-25	371	31.7	8.5	39.0	10.5
500	cutoff	478	27.5	5.8	35.7	7.5
625	+25	600	33.3	5.5	46.8	7.8
750	+50	705	33.7	4.8	52.2	7.4
1000	+100	917	50.9	5.5	69.7	7.6

Method Comparison

Sixty-nine (69) positive samples and sixty-seven (67) negative samples were analyzed qualitatively and semiquantitatively by ARK Pregabalin Urine Assay and by LC-MS/MS. Both methods used 500 ng/mL pregabalin as the cutoff concentration. Complete agreement between methods was observed.

		LC-MS/MS 500 ng/mL Cutoff	
		(+)	(-)
ARK Pregabalin 500 ng/mL Cutoff	(+)	69	0
	(-)	0	67*

*Three (3) negative samples contained pregabalin between 250 to 500 ng/mL

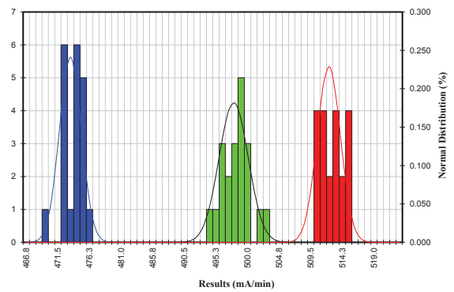
High Sample Dilution

Samples containing pregabalin in excess of the calibration range were analyzed. Spiked urine samples (5, 50 and 100 µg/mL) were diluted 1:10, 1:100, and 1:200 respectively with negative human urine. Each sample was assayed 10 replicates using the semi-quantitative mode on AU480. Recovery after dilution ranged 95.5 to 99.5%.

Tested Samples Conc. (µg/mL)	% of cutoff	Mean (µg/mL)	SD	CV (%)	Recovery (%)	N
5	10	493.4	45.88	9.3	98.7	10
50	100	497.4	46.15	9.3	99.5	10
100	200	477.5	33.71	7.1	95.5	10

Histogram Overlap Analysis

Frequency of distribution of pregabalin values for each sample is shown by histogram analysis. Twenty (20) replicates each of Negative Control (250 ng/mL), Cutoff Calibrator (500 ng/mL), and Positive Control (750 ng/mL) were assayed together in a single run. One run each was performed by the qualitative mode or semiquantitative mode. The distributions of measurements did not overlap.



Limit of Quantitation and Linearity

Limit of Quantitation (LoQ) and linearity were estimated by measuring pregabalin in semiquantitative mode. Pooled human urine was supplemented with pregabalin to give concentrations from 100 to 2000 ng/mL. The semiquantitative measurement range of the ARK Pregabalin Urine Assay is 200 to 2000 ng/mL based on linearity and recovery.

Estimated Value (ng/mL)	Results (ng/mL)	Results (ng/mL)	1st Order Predicted Results	2nd Order Predicted Results	Difference (%)
100	107.5	107.5	88.5	113.2	27.9
200	200.8	100.4	186.9	201.9	8.0
300	299.7	99.9	285.4	291.8	2.3
400	375.1	93.8	383.8	382.9	-0.2
600	574.2	95.7	580.6	568.7	-2.1
800	743.1	92.9	777.5	759.2	-2.3
1000	977.8	97.8	974.3	954.6	-2.0
1200	1162.7	96.9	1171.1	1154.8	-1.4
1600	1520.0	95.0	1564.8	1569.6	0.3
2000	2010.7	100.5	1958.5	2003.5	2.3

Specificity - Interfering Substances

Pooled human urine was supplemented with pregabalin to contain 250 ng/mL or 750 ng/mL (equivalent to the quality control concentrations; ± 50% of the cutoff concentration respectively) and then potentially interfering substances were spiked at the concentrations tested.

QUALITATIVE ANALYSIS

The ARK Pregabalin Urine Assay correctly identified the mean rate of the control at -50% of the cutoff as negative 100% of the time and the mean rate of the control at +50% of the cutoff as positive 100 % of the time.

SEMIQUANTITATIVE ANALYSIS

In the semiquantitative analysis, the two levels of controls at ± 50% of the cutoff concentration did not yield a false response relative to the cutoff.

Interfering Substance	Level Tested (mg/dL)	Tested Substance Low (-50%) Control (ng/mL)	Tested Substance High (+50%) Control (ng/mL)
Acetaminophen	10	231.1	648.4
Acetone	1000	261.6	698.6
Acetylsalicylic Acid	10	260.0	642.0
Ascorbate	200	245.7	688.1
Caffeine	10	250.4	704.0
Creatinine	400	272.4	683.5
Ethanol	10	232.9	687.1
Galactose	10	226.7	681.6
Glucose	3000	231.0	663.3
Hemoglobin	300	188.6	536.6
Human Serum Albumin	500	259.8	626.6
Ibuprofen	10	235.5	655.4
Oxalic Acid	30	228.1	674.3
Riboflavin	3.75	236.0	648.9
Sodium Chloride	900	244.9	680.0
Urea	1000	237.9	738.4

Specific Gravity and pH

Urine samples with specific gravity values from 1.003 to 1.035 g/mL and pH values ranging from 4.0 to 10.0 were tested in the presence of 250 and 750 ng/mL of pregabalin. No interference was observed.

Specificity - Structurally Unrelated Compounds

Pooled human urine was supplemented with pregabalin to contain 250 ng/mL or 750 ng/mL (equivalent to the quality control concentrations; ± 50% of the cutoff concentration respectively) and then compounds that are not structurally related to pregabalin were spiked at the concentrations tested. None of the compounds tested were crossreactive.

QUALITATIVE ANALYSIS

The ARK Pregabalin Urine Assay correctly identified the mean rate of the control at -50% of the cutoff as negative 100% of the time and the mean rate of the control at +50% of the cutoff as positive 100 % of the time.

SEMIQUANTITATIVE ANALYSIS

In the semiquantitative analysis, the two levels of controls at ± 50% of the cutoff concentration did not yield a false response relative to the cutoff.

Compound	Level Tested (µg/mL)	Tested Substance Low (-50%) Control (%) Crossreactivity	Tested Substance High (+50%) Control (%) Crossreactivity
6-Acetyl morphine	10	-0.11	-0.27
Amitriptyline	100	0.00	0.09
Amoxicillin	100	0.04	0.03
Amphetamine	100	0.21	0.46
Benzoylecgonine	100	0.00	-0.14
Carbamazepine	100	0.13	0.19
Chlorpromazine	100	0.04	0.03
Clomipramine	100	0.01	-0.03
Cimetidine	500	0.00	0.00
Codeine	100	-0.02	-0.01
Desipramine	100	-0.01	-0.05
Dextromethorphan	200/150	-0.01	-0.05
Dihydrocodeine	100	-0.03	-0.11
Doxepin	200	-0.01	-0.07
Ephedrine	200/150	-0.03	-0.07
Fentanyl	100	-0.04	-0.10
Fluoxetine	100	-0.02	-0.10
Fluphenazine	100	0.03	-0.12

Compound	Level Tested (µg/mL)	Tested Substance Low (-50%) Control (%) Crossreactivity	Tested Substance High (+50%) Control (%) Crossreactivity
Heroin	100	-0.06	-0.20
Hydrocodone	200	-0.03	-0.09
Hydromorphone	200	-0.02	-0.08
Imipramine	100	-0.03	-0.03
Levorphanol	50	-0.01	-0.07
Meperidine	100	-0.03	-0.08
Maprotiline	100	-0.01	-0.09
Methadone	100	-0.03	-0.08
Metronidazole	300	-0.01	0.01
Morphine	100	-0.03	-0.13
Morphine-3-glucuronide	50	-0.03	-0.20
Nalbuphine	100	-0.02	-0.02
Naltrexone	50	-0.05	-0.15
Norcodeine	50	0.01	-0.11
Normorphine	50	-0.03	-0.15
Nortriptyline	50	0.11	-0.07
Oxazepam	100	-0.02	-0.11
Oxycodone	100	-0.04	-0.13
Pentazocine	50	-0.03	-0.17
Phencyclidine	50	0.13	-0.17
Phenobarbital	100	-0.03	-0.14
Ranitidine	100	0.02	-0.06
Secobarbital	100	-0.04	-0.05
Thioridazine	100	-0.03	-0.03
Tramadol	100	-0.02	-0.06

Specificity - Structurally Related Compounds

The structures of twenty L-amino acids and gabapentin are similar to that of pregabalin. None of these compounds were crossreactive (≤0.04%) at the concentrations tested in human urine and did not give a false positive result relative to the 500 ng/mL Cutoff Calibrator in the semiquantitative mode. Extremely high concentrations of gabapentin (>4.5 mg/mL) may cause a false positive result.

Compound	Level Tested (µg/mL)	Compound	Level Tested (µg/mL)
Gabapentin	4,000	L-Phenylalanine	200
L-Arginine	200	L-Serine	200
L-Asparagine	200	L-Threonine	200
L-Aspartic Acid	200	L-Tyrosine	200
L-Cysteine	200	L-Alanine	200
L-Glutamic Acid	200	L-Lysine	200
L-Glycine	200	L-Proline	200
L-Histidine	200	L-Valine	200
L-Isoleucine	200	L-Tryptophan	200
L-Leucine	200	L-Glutamine	200
L-Methionine	200		

Conclusions

ARK Pregabalin Urine Assay measures pregabalin in human urine with acceptable performance in either the qualitative or semiquantitative mode. Ability to determine pregabalin accurately in human urine with fast turn-around time makes this method ideal for detecting possible misuse/abuse or to verify treatment compliance.

INTENDED USE

The ARK Pregabalin Urine Assay is intended for the qualitative and/or semiquantitative determination of pregabalin in human urine at a cutoff concentration of 500 ng/mL. The assay provides a simple and rapid analytical screening procedure for detecting pregabalin in urine and is designated for professional use on automated clinical chemistry analyzers.

The ARK Pregabalin Urine Assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) are the preferred confirmatory methods. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

REGULATORY STATUS

Europe: The ARK Pregabalin Urine Assay is available for export only with CE/IVD status.

USA: The ARK Pregabalin Urine Assay is available For Criminal Justice and Forensic Use Only.