June 4, 2020

Lin-Zhi International, Inc.
Bernice Lin
VP Operations
2945 Oakmead Village Court
Santa Clara, CA 95051

Re: k201223
Trade/Device Name: LZI Tramadol Enzyme Immunoassay
Regulation Number: 21 CFR 862.3650
Regulation Name: Opiate test system
Regulatory Class: Class II
Product Code: DJG
Dated: May 4, 2020
Received: May 6, 2020

Dear Bernice Lin:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's

requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Marianela Perez-Torres, Ph.D.
Acting Deputy Director
Division of Chemistry
and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K201223

Device Name

LZI Tramadol Enzyme Immunoassay

Indications for Use *(Describe)*

The LZI Tramadol Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of tramadol in human urine at the cutoff value of 100 ng/mL when calibrated against tramadol. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as gas or liquid chromatography/mass spectrometry (GC/MS or LC/MS) or (2) permitting laboratories to establish quality control procedures.

The assay provides only a preliminary analytical result. A more specific alternative chemical method (e.g., gas or liquid chromatography and mass spectrometry) must be used in order to obtain a confirmed analytical result. Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary of Safety and Effectiveness

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

510(k) Number

K201223

Prepared On

May 4, 2020

Introduction

According to the requirements of 21 CFR 807.92, the following information provides sufficient detail to understand the basis for a determination of substantial equivalence.

Submitter Name, Address, and Contact:

Lin-Zhi International, Inc.
2945 Oakmead Village Court
Santa Clara, CA 95051
Phone: (408) 970-8811
Fax: (408) 970-9030
e-mail: bclin@lin-zhi.com

Contact: Bernice Lin, Ph.D.
VP Operations

Device Name and Classification

Classification Name:	Enzyme Immunoassay, Opiates Class II, DJG (91 Toxicology), 21 CFR 862.3650
Common Name:	Homogeneous Enzyme Immunoassay
Proprietary Name:	LZI Tramadol Enzyme Immunoassay

Legally Marketed Predicate Device(s)

The LZI Tramadol Enzyme Immunoassay (EIA) is substantially equivalent to the ARK™ Tramadol Assay (k182280) manufactured by ARK Diagnostics, Inc. The LZI Tramadol Enzyme Immunoassay is identical or similar to its predicate in terms of intended use, method principle, device components, and clinical performance.

Device Description

The LZI Tramadol Enzyme Immunoassay is a homogeneous enzyme immunoassay with ready-to-use liquid reagents. 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. The drug-labeled G6PDH conjugate is traceable to a commercially available tramadol standard and referred to as tramadol-labeled G6PDH conjugate. 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, tramadol-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 would bind to free drug; the unbound tramadol-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 340 nm.

The LZI Tramadol Enzyme Immunoassay is a kit comprised of two reagents, an R_1 and R_2, which are bottled separately but sold together within the kit. The LZI Tramadol Enzyme Immunoassay is traceable to a commercially available tramadol standard.

The R_1 solution contains mouse monoclonal anti-tramadol antibody, glucose-6-phosphate (G6P) nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative. The R_2 solution contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with tramadol in buffer with sodium azide (0.09 %) as a preservative.

Intended Use

The LZI Tramadol Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of tramadol in human urine at the cutoff value of 100 ng/mL when calibrated against tramadol. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as GC/MS and LC/MS or (2) permitting laboratories to establish quality control procedures.

The assay provides only a preliminary analytical result. A more specific alternative chemical method (e.g., gas or liquid chromatograpy and mass spectrometry) must be used in order to obtain a confirmed analytical result. Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

Comparison to Predicate Device

The LZI Tramadol Enzyme Immunoassay is substantially equivalent to the ARK™ Tramadol Assay cleared by the FDA under the premarket notification k182280 for its stated intended use.

The following table compares LZI's Tramadol Enzyme Immunoassay with the predicate device.

Device Characteristics	**Subject Device** LZI Tramadol Enzyme Immunoassay	**Predicate Device (k182280)** ARK™ Tramadol Assay
Intended Use	Same	The ARK Tramadol Assay is an immunoassay intended for the qualitative and/or semiquantitative determination of tramadol in human urine at a cutoff concentration of 100 ng/mL. The assay is intended for use in laboratories with automated clinical chemistry analyzers. This in vitro diagnostic device is for prescription use only. The semiquantitative mode is for the purpose of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method, such as Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS), or (2) permitting laboratories to establish quality control procedures. The ARK Tramadol Assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed positive analytical result. Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be exercised with any drug test result, particularly when the preliminary test result is positive.
Analyte	tramadol	tramadol
Cutoff	100 ng/ml	100 ng/mL
Matrix	Urine	Urine
Calibrators Level	0, 50, 100, 225, and 400 ng/mL	0, 100, 200, 500, and 1000 ng/mL
Controls Level	75 ng/mL and 125 ng/mL	75 ng/mL and 125 ng/mL
Storage	2-8 ºC until expiration date	2-8 ºC until expiration date

Performance Characteristics Summary:

All validation studies below were conducted on the Beckman Coulter® AU480 Analyzer

Precision: 100 ng/mL Cutoff

The assay was tested in qualitative (ΔOD, mAU) and semi-quantitative (ng/mL) mode using a modified NCCLS-EP5 protocol. Tramadol sample concentrations were prepared by spiking a tramadol standard into a pool of negative human urine at concentrations ±25 %, ±50 %, ±75 %, and ±100 % of the cutoff concentration.

Results shown below were obtained by testing all samples in replicate of two, two runs a day (one in the morning and one in the afternoon) for 22 days on one Beckman Coulter© AU480 automatic clinical analyzer for a total of 88 replicates. Samples were evaluated against the OD of the cutoff calibrator in the qualitative mode and evaluated against the assay's calibration curve in the semi-quantitative mode. One single lot of reagents, calibrators, and controls were used and stored at 2-8ºC when not in use.

Semi-Quantitative Precision Analysis Summary: Qualitative Results

Tramadol Concentration	Within Run (N=22)		Total Precision (N=88)	
	Mean	Qualitative Response	Mean	Qualitative Response
0 ng/mL	-1.0	-	-1.0	-
25 ng/mL	20.2	-	20.2	-
50 ng/mL	49.8	-	49.8	-
75 ng/mL	74.6	-	74.6	-
100 ng/mL	101.5	+	101.5	+
125 ng/mL	130.5	+	130.5	+
150 ng/mL	162.7	+	162.7	+
175 ng/mL	186.2	+	186.2	+
200 ng/mL	210.2	+	210.2	+

Semi-Quantitative Positive/Negative Results:

100 ng/mL Cutoff Result:		Within Run (N=22)		Total Precision (N=88)	
Tramadol Concentration	% of Cutoff	Number of Determination	Immunoassay Result	Number of Determination	Immunoassay Result
0 ng/mL	0 %	22	22 Negative	88	88 Negative
25 ng/mL	25 %	22	22 Negative	88	88 Negative
50 ng/mL	50 %	22	22 Negative	88	88 Negative
75 ng/mL	75 %	22	22 Negative	88	88 Negative
100 ng/mL	100 %	22	16 Pos/6 Neg	88	53 Pos/35 Neg
125 ng/mL	125 %	22	22 Positive	88	88 Positive
150 ng/mL	150 %	22	22 Positive	88	88 Positive
175 ng/mL	175 %	22	22 Positive	88	88 Positive
200 ng/mL	200 %	22	22 Positive	88	88 Positive

Performance Characteristics Summary (continued):

Beckman Coulter® AU480 Analyzer

Qualitative Positive/Negative Results:

100 ng/mL Cutoff Result:		Within Run (N=22)		Total Precision (N=88)	
Tramadol Concentration	**% of Cutoff**	**Number of Determination**	**Immunoassay Result**	**Number of Determination**	**Immunoassay Result**
0 ng/mL	0 %	22	22 Negative	88	88 Negative
25 ng/mL	25 %	22	22 Negative	88	88 Negative
50 ng/mL	50 %	22	22 Negative	88	88 Negative
75 ng/mL	75 %	22	22 Negative	88	88 Negative
100 ng/mL	100 %	22	15 Pos/7 Neg	88	48 Pos/40 Neg
125 ng/mL	125 %	22	22 Positive	88	88 Positive
150 ng/mL	150 %	22	22 Positive	88	88 Positive
175 ng/mL	175 %	22	22 Positive	88	88 Positive
200 ng/mL	200 %	22	22 Positive	88	88 Positive

Analytical Recovery:

To demonstrate recovery of the entire assay range, a drug free–urine pool spiked with tramadol at 400 ng/mL was serially diluted. Each sample was run in 10 replicates and the average was used to determine percent recovery compared to the expected target value.

Determined concentration averages were obtained and all averages were ±15 % of the target concentrations. The determined average percent recovery (Determined Concentration Average divided by the Target Concentration) were considered acceptable between 85 – 115 %.

The recovery of tramadol spiked to various concentrations was evaluated and was found to range between 89 % - 116 %.

Target Concentration (ng/mL)	Determined Concentration Range (ng/mL)	Determined Concentration Average (ng/mL)	Average % Recovery
400	395.2 – 433.3	412.7	103.2 %
360	350.3 – 404.5	377.5	104.9 %
320	316.5 – 371.6	346.9	108.4 %
280	277.6 – 313.0	297.8	106.4 %
240	221.7 – 279.7	245.1	102.1 %
200	202.0 – 215.5	210.0	105.0 %
160	168.2 – 180.2	174.5	109.1 %
120	122.8 – 134.2	130.1	108.4 %
80	78.0 – 84.6	80.9	101.1 %
40	35.6 – 43.0	39.3	98.2 %
0	-4.6 – 1.3	-2.2	N/A

Performance Characteristics Summary (continued):

Beckman Coulter® AU480 Analyzer

Method Comparison - Clinical Samples:

A total of eighty-six (86) unaltered clinical samples were tested with the LZI Tramadol Enzyme Immunoassay on the Beckman Coulter® AU480 automated clinical analyzer. Samples were evaluated against the OD of the cutoff calibrator in the qualitative mode and evaluated against the assay's calibration curve in the semi-quantitative mode. All samples were tested in singlet.

All samples were confirmed with LC/MS for tramadol concentrations. Samples were collected by Lin-Zhi International, Inc. (LZI) and and the University of California at San Francisco (UCSF, San Francisco).

Semi-Quantitative Results:

Tramadol Results **100 ng/mL Cutoff**	**Negative**	**< 50 % of the cutoff** concentration by LC/MS analysis	**Near Cutoff Negative** (Between 50 % below the cutoff and the cutoff concentration)	**Near Cutoff Positive** (Between the cutoff and 50 % above the cutoff concentration)	**High Positive** (Greater than 50 % above the cutoff concentration)	**% Agreement**
Positive	0	0	1*	9	33	97.7 %
Negative	20	7	15	1**	0	97.7 %

Discrepant samples determined when comparing LC/MS tramadol results with tramadol EIA results on the Beckman Coulter® AU480 automated clinical analyzer.

Sample #	**LC/MS Tramadol (ng/mL)**	**Pos/ Neg Result**	**LC/MS O-desmethyl tramadol (ng/mL)**		**Pos/ Neg Result**
38*	71	-	118		+
46**	114	+	57		-

* Discrepant between 50% below cutoff and cutoff concentration (50 – 99.9 ng/mL)

** Discrepant between cutoff and 50% above cutoff concentration (100 – 149.9 ng/mL)

Performance Characteristics Summary (continued):

Beckman Coulter® AU480 Analyzer

Method Comparison - Clinical Samples (continued):

Qualitative Accuracy Study:

Tramadol Results 100 ng/mL Cutoff	**Negative**	**< 50 % of the cutoff** concentration by LC/MS analysis	**Near Cutoff Negative** (Between 50 % below the cutoff and the cutoff concentration)	**Near Cutoff Positive** (Between the cutoff and 50 % above the cutoff concentration)	**High Positive** (Greater than 50 % above the cutoff concentration)	**% Agreement**
Positive	0	0	2*	9	33	97.7 %
Negative	20	7	14	1**	0	95.3 %

Discrepant samples determined when comparing LC/MS tramadol results with tramadol EIA results on the Beckman Coulter® AU480 automated clinical analyzer.

Sample #	**LC/MS Tramadol (ng/mL)**	**Pos/ Neg Result**	**LC/MS O-desmethyl tramadol (ng/mL)**		**Pos/ Neg Result**
38*	71	-	118		+
42*	85	-	56		+
46**	114	+	57		-

* Discrepant between 50% below cutoff and cutoff concentration (50 – 99.9 ng/mL)

** Discrepant between cutoff and 50% above cutoff concentration (100 – 149.9 ng/mL)

Performance Characteristics Summary (continued):

Beckman Coulter® AU480 Analyzer

Cross-reactivity

The cross-reactivity of various potentially interfering drugs were tested by spiking various concentrations of each substance into a pool of negative human urine and then evaluated against the assay's calibration curve in both qualitative and semi-quantitative modes. All samples were tested in duplicates.

The table below lists the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator (as positive) or the maximal concentration of the compound tested that gave a response below the response of the cutoff calibrator (as negative). Compounds tested at high concentration (100,000 ng/mL) with results below the cutoff value were listed as Not Detected (ND). Compounds tested below the high concentration (100,000 ng/mL) that gave a result below the cutoff value were given a "< %" value.

Compound	Test Concentration (ng/mL)	% Cross-reactivity
Tramadol	100	100.00 %
O-desmethyl cis-tramadol HCl	166	60.24 %
N-desmethyl-cis-tramadol	10,000	1.00 %
Rac N, O-didesmethyl tramadol	15,000	0.67 %
O-desmethyl tramadol beta-D-glucoronide	90	111.11 %
Ketamine	100,000	ND
Dehydronorketamine	10,000	< 1.00 %
Norketamine	20,000	< 0.50 %
Phencyclidine (PCP)	100,000	ND
Venlafaxine	100,000	ND
O-desmethylvenlafaxine	100,000	ND

Cross-reactivity (continued)

Structurally unrelated compounds were additionally spiked into pooled negative human urine to desired concentrations (as described above). These solutions were then split into three portions; one without tramadol, and the remaining two that were further spiked with tramadol standards to a final tramadol concentration of 75 ng/mL or 125 ng/mL (as negative or positive controls, ±25 % of the cutoff concentration, respectively). Samples were then evaluated against the assay's calibration curve in both qualitative and semi-quantitative modes. All samples were tested in duplicates. Compounds tested at high concentration (100,000 ng/mL) with results below the cutoff value were listed as Not Detected (ND). Compounds tested below the high concentration (100,000 ng/mL) that gave a result below the cutoff value were given a "< %" value.

Structurally Unrelated Pharmacological Compounds:

Compound	Test Concentration (ng/mL)	0 ng/mL Tramadol	-25 % Tramadol Cutoff (75 ng/mL)	+25 % Tramadol Cutoff (125 ng/mL)
		% Cross	Result	Result
6-Acetylmorphine	100,000	ND	Neg	Pos
Acetaminophen	100,000	ND	Neg	Pos
Acetylsalicylic Acid	100,000	ND	Neg	Pos
Aliemazine Tartrate	100,000	ND	Neg	Pos
Amitriptyline	100,000	ND	Neg	Pos
Amlodipine Besylate	100,000	ND	Neg	Pos
d-Amphetamine	100,000	ND	Neg	Pos
Amoxicillin	100,000	ND	Neg	Pos
Atorvastatin	100,000	ND	Neg	Pos
Benzoylecgonine	100,000	ND	Neg	Pos
Buprenorphine	100,000	ND	Neg	Pos
Bupropion	100,000	ND	Neg	Pos
Caffeine	100,000	ND	Neg	Pos
Carbamazepine	100,000	ND	Neg	Pos
Cetirizine	100,000	ND	Neg	Pos
Chlorpheniramine	100,000	ND	Neg	Pos
Chlorpromazine	100,000	ND	Neg	Pos
Clomipramine	100,000	ND	Neg	Pos
Codeine	100,000	ND	Neg	Pos
Desipramine	100,000	ND	Neg	Pos
Diphenhydramine	100,000	ND	Neg	Pos
Doxylamine Succinate	100,000	ND	Neg	Pos

Structurally Unrelated Pharmacological Compounds, continued:

Compound	Test Concentration (ng/mL)	0 ng/mL Tramadol	-25 % Tramadol Cutoff (75 ng/mL)	+25 % Tramadol Cutoff (125 ng/mL)
		% Cross	Result	Result
Duloxetine	100,000	ND	Neg	Pos
Etavirenz	100,000	ND	Neg	Pos
Fentanyl (citrate)	100,000	ND	Neg	Pos
Fluoxetine	100,000	ND	Neg	Pos
Fluphenazine	100,000	ND	Neg	Pos
Gabapentin	100,000	ND	Neg	Pos
Hydrocodone	100,000	ND	Neg	Pos
Hydromorphone	100,000	ND	Neg	Pos
Hydroxyzine Pamoate	100,000	ND	Neg	Pos
Ibuprofen	100,000	ND	Neg	Pos
Imipramine	100,000	ND	Neg	Pos
JWH-073 (SPICE I)	100,000	ND	Neg	Pos
Lisinopril	100,000	ND	Neg	Pos
Loratidine	100,000	ND	Neg	Pos
Lorazepam	100,000	ND	Neg	Pos
Losartan	100,000	ND	Neg	Pos
MDA (3,4-methylenedioxyamphetamine)	100,000	ND	Neg	Pos
MDEA	100,000	ND	Neg	Pos
MDMA (3,4-methylenedioxymethamphetamine)	100,000	ND	Neg	Pos
Meperidine	100,000	ND	Neg	Pos
Metformin	100,000	ND	Neg	Pos
Methadone	100,000	ND	Neg	Pos
d-Methamphetamine	100,000	ND	Neg	Pos
Methapyrilene HCl	100,000	ND	Neg	Pos
Metoprolol	100,000	ND	Neg	Pos
Morphine	100,000	ND	Neg	Pos
Nicotine	100,000	ND	Neg	Pos
Niflumic Acid	100,000	ND	Neg	Pos

Structurally Unrelated Pharmacological Compounds, continued:

Compound	Test Concentration (ng/mL)	0 ng/mL Tramadol	-25 % Tramadol Cutoff (75 ng/mL)	+25 % Tramadol Cutoff (125 ng/mL)
		% Cross	Result	Result
Nortriptyline	100,000	ND	Neg	Pos
Omeprazole	100,000	ND	Neg	Pos
Oxazepam	100,000	ND	Neg	Pos
Oxycodone	100,000	ND	Neg	Pos
Oxymorphone	100,000	ND	Neg	Pos
Phenobarbital	100,000	ND	Neg	Pos
Prochloreazine	100,000	ND	Neg	Pos
d-Propoxyphene	100,000	ND	Neg	Pos
(1S,2S)-(+)Pseudoephedrine	100,000	ND	Neg	Pos
Quetiapine	100,000	ND	Neg	Pos
Ranitidine	100,000	ND	Neg	Pos
Salbutamol (Albuterol)	100,000	ND	Neg	Pos
Sertraline	100,000	ND	Neg	Pos
THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid)	100,000	ND	Neg	Pos
l-Thyroxine	100,000	ND	Neg	Pos
Trioridazine	100,000	ND	Neg	Pos
(+)Verapamil HCl	100,000	ND	Neg	Pos
Zolpidem	1,250	< 0.10 %	Neg	Pos
Zolpidem phenyl-4-carboxylic acid	10,000	< 0.10 %	Neg	Pos
Zolpidem-6-carboxylic acid	10,000	< 0.10 %	Neg	Pos

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 procedural errors

Performance Characteristics Summary, continued:

Beckman Coulter® AU480 Analyzer

Endogenous and Preservative Compound Interference:

Endogenous and Preservative compounds were spiked into pooled negative human urine to desired concentrations. These solutions were then split into three portions; one without tramadol, and the remaining two that were further spiked with tramadol standards to a final tramadol concentration of 75 ng/mL or 125 ng/mL (as negative or positive controls, ±25 % of the cutoff concentration, respectively). Samples were then evaluated against the assay's calibration curve in both qualitative and semi-quantitative modes. All samples were tested in duplicates.

Interfering Substance	Concentration of Compound (mg/dL)	0 ng/mL Tramadol	-25 % Tramadol Cutoff (75 ng/mL)	+25 % Tramadol Cutoff (125 ng/mL)
Acetone	1000	Neg	Neg	Pos
Ascorbic Acid	1500	Neg	Neg	Pos
Bilirubin	2	Neg	Neg	Pos
Boric Acid	1000	Neg	Neg	Neg
Calcium Chloride (CaCl2)	300	Neg	Neg	Pos
Ciprofloxacin	1	Neg	Neg	Pos
Citric Acid (pH 3)	800	Neg	Neg	Pos
Creatinine	500	Neg	Neg	Pos
Ethanol	1000	Neg	Neg	Pos
Galactose	10	Neg	Neg	Pos
γ-Globulin	500	Neg	Neg	Pos
Glucose	3000	Neg	Neg	Pos
Hemoglobin	300	Neg	Neg	Pos
β-hydroxybutyric Acid	100	Neg	Neg	Pos
HSA	500	Neg	Neg	Pos
Oxalic Acid	100	Neg	Neg	Pos
Potassium Chloride	6000	Neg	Neg	Pos
Riboflavin	0.3	Neg	Neg	Pos
Urea	6000	Neg	Neg	Pos
Uric Acid	10	Neg	Neg	Pos
Sodium Azide	1000	Neg	Neg	Pos

Performance Characteristics Summary, continued:

Beckman Coulter® AU480 Analyzer

Endogenous and Preservative Compound Interference (continued):

Interfering Substance	Concentration of Compound (mg/dL)	0 ng/mL Tramadol	-25 % Tramadol Cutoff (75 ng/mL)	+25 % Tramadol Cutoff (125 ng/mL)
Sodium Chloride	6000	Neg	Neg	Pos
Sodium Fluoride	1000	Neg	Neg	Pos
Sodium Phosphate	300	Neg	Neg	Pos

The following endogenous and preservative compounds which showed interference at ±25 % of cutoff concentrations were then spiked into negative urine and at ±50 % of the cutoff concentration (50 ng/mL and 150 ng/mL) for the assay.

Interference was observed with Boric Acid at 1 % w/v. No other significant undesired cross-reactants or endogenous/preservative substance interference was observed.

Interfering Substance	Concentration of Compound (mg/dL)	0 ng/mL Tramadol	-50 % Tramadol Cutoff (50 ng/mL)	+50 % Tramadol Cutoff (150 ng/mL)
Boric Acid	1000	Neg	Neg	Neg

Performance Characteristics Summary, continued:

Beckman Coulter® AU480 Analyzer

Specific Gravity Interference:

Samples ranging in specific gravity from 1.000 to 1.030 were split into three portions each and either left un-spiked or further spiked to a final tramadol concentration of either 75 ng/mL or 125 ng/mL (as negative or positive controls, ±25 % of the cutoff concentration, respectively). These samples were then evaluated in both semi-quantitative and qualitative modes. No interference was observed.

Specific Gravity Value	0 ng/mL Tramadol	-25 % Tramadol Cutoff (75 ng/mL)	+25 % Tramadol Cutoff (125 ng/mL)
1.000	Neg	Neg	Pos
1.003	Neg	Neg	Pos
1.005	Neg	Neg	Pos
1.006	Neg	Neg	Pos
1.007	Neg	Neg	Pos
1.008	Neg	Neg	Pos
1.009	Neg	Neg	Pos
1.010	Neg	Neg	Pos
1.011	Neg	Neg	Pos
1.012	Neg	Neg	Pos
1.013	Neg	Neg	Pos
1.015	Neg	Neg	Pos
1.018	Neg	Neg	Pos
1.020	Neg	Neg	Pos
1.025	Neg	Neg	Pos
1.028	Neg	Neg	Pos
1.030	Neg	Neg	Pos

Performance Characteristics Summary, continued:

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pH Interference:

Negative urine and urine spiked with tramadol to the final tramadol concentration of either 75 ng/mL or 125 ng/mL (as negative or positive controls, ±25 % of the cutoff concentration, respectively) were adjusted to the following pH levels and tested by the assay. The pH adjusted solutions were evaluated in both qualitative and semi-quantitative modes.

No major interference was observed between pH 3 to pH 11. Results are summarized in the following table:

Interfering Substance	0 ng/mL Tramadol	-25 % Tramadol Cutoff (75 ng/mL)	+25 % Tramadol Cutoff (125 ng/mL)
pH 3	Neg	Neg	Pos
pH 4	Neg	Neg	Pos
pH 5	Neg	Neg	Pos
pH 6	Neg	Neg	Pos
pH 7	Neg	Neg	Pos
pH 8	Neg	Neg	Pos
pH 9	Neg	Neg	Pos
pH 10	Neg	Neg	Pos
pH 11	Neg	Neg	Pos

Summary:

The information provided in this pre-market notification demonstrates that the LZI Tramadol Enzyme Immunoassay is substantially equivalent to the legally marketed predicate device for its general intended use. Substantial equivalence was demonstrated through comparison of intended use and physical properties to the commercially available predicate device as confirmed by chromatography/mass spectrometry (GC/MS or LC/MS), an independent analytical method. The information supplied in this pre-market notification provides reasonable assurance that the LZI Tramadol Enzyme Immunoassay is safe and effective for its stated intended use.