EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
LZI Carisoprodol Metabolite (Meprobamate) Enzyme Immunoassay

DECISION SUMMARY

A. DEN Number:

DEN170010

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the LZI Carisoprodol Metabolite (Meprobamate) Enzyme Immunoassay

C. Measurand:

Meprobamate

D. Type of Test:

Homogenous Enzyme Immunoassay, Qualitative and Semi-quantitative

E. Applicant:

Lin-Zhi International, Inc.

F. Proprietary and Established Names:

LZI Carisoprodol Metabolite (Meprobamate) Enzyme Immunoassay

G. Regulatory Information:

1. Regulation: 21 CFR 862.3590
2. Classification: Class II
3. Product code: QBK
4. Panel: Toxicology (91)

H. Intended Use:

1. Indications for use:

The LZI Carisoprodol Metabolite (Meprobamate) Enzyme Immunoassay is intended for

the qualitative and semi-quantitative determination of carisoprodol metabolite (meprobamate) in human urine at a cutoff value of 100 ng/mL when calibrated against meprobamate. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

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

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

2. Special conditions for use statement(s):

- For in vitro diagnostic use only.
- For prescription use only.

3. Special instrument requirements:

The assay was validated on the Beckman AU400e automated clinical chemistry analyzer. Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzyme rates at 340 nm and timing the reaction accurately can be used to perform this assay.

I. Device Description:

The LZI Carisoprodol Metabolite (Meprobamate) Enzyme Immunoassay is a homogeneous enzyme immunoassay with ready-to-use liquid reagents. The assay is a kit comprised of two reagents (antibody/substrate reagent R_1 and enzyme-drug conjugate reagent R_2), which are bottled separately but sold together within the kit. The R_1 solution contains mouse monoclonal anti-meprobamate antibody, glucose-6- phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative. The R_2 solution contains meprobamate-labeled glucose-6-phosphate dehydrogenase (G6PDH) in buffer with sodium azide (0.09 %) as a preservative.

J. Standard/Guidance Documents Referenced:

CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition.

K. Test Principle:

The LZI Carisoprodol Metabolite (Meprobamate) Enzyme Immunoassay is based on competition between meprobamate in the sample and the enzyme glucose-6-phosphate dehydrogenase (G6PDH) labeled with meprobamate for a fixed amount of antibody in the reagent. G6PDH enzyme activity decreases upon binding to the antibody, and thus meprobamate concentration in the sample is proportional to enzyme activity. In the absence of meprobamate in the sample, meprobamate-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. When meprobamate is present in the sample competes with drug-labeled G6PDH for binding to antibody; the unbound meprobamate-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.

L. Performance Characteristics (if/when applicable):

The following performance characteristics were obtained on the Beckman AU400e automated clinical chemistry analyzer.

1. Analytical performance:

a. Reproducibility/Precision

A precision study was performed over 22 days, with 2 runs per day in duplicate (n = 88) using pooled negative urine samples spiked with meprobamate to concentrations of 25, 50, 75, 100, 125, 150, 175, and 200 ng/mL. All concentrations for precision studies were confirmed by Gas Chromatography/Mass Spectrometry (GC/MS) testing. The results from samples assayed using the qualitative and semi-quantitative modes are summarized below.

Qualitative Analysis

Target meprobamate concentration (ng/mL)	**GC/MS meprobamate concentration (ng/mL)**	**% of Cutoff**	**# of Determinations**	**Result**
0	not determined	-100%	88	88 Neg / 0 Pos
25	24.7	-75%	88	88 Neg / 0 Pos
50	51.7	-50%	88	88 Neg / 0 Pos
75	76.8	-25%	88	88 Neg / 0 Pos
100	94.9	Cutoff	88	40 Neg / 48 Pos

Target meprobamate concentration (ng/mL)	GC/MS meprobamate concentration (ng/mL)	% of Cutoff	# of Determinations	Result
125	122.3	+25%	88	0 Neg / 88 Pos
150	149.4	+50%	88	0 Neg / 88 Pos
175	176.8	+75%	88	0 Neg / 88 Pos
200	211.0	+100%	88	0 Neg / 88 Pos

Semi-Quantitative Analysis

Sample concentration (ng/mL)	GC/MS meprobamate concentration (ng/mL)	% of Cutoff	# of Determinations	Result
0	not determined	-100%	88	88 Neg / 0 Pos
25	24.7	-75%	88	88 Neg / 0 Pos
50	51.7	-50%	88	88 Neg / 0 Pos
75	76.8	-25%	88	88 Neg / 0 Pos
100	94.9	Cutoff	88	60 Neg / 28 Pos
125	122.3	+25%	88	0 Neg / 88 Pos
150	149.4	+50%	88	0 Neg / 88 Pos
175	176.8	+75%	88	0 Neg / 88 Pos
200	211.0	+100%	88	0 Neg / 88 Pos

b. Linearity/assay reportable range:

A recovery study was performed by spiking a pool of negative urine with a high dose of meprobamate and generating serial dilutions to achieve the following concentrations: 10, 40, 80, 120, 160, 200, 240, 280, 320, 360, and 400 ng/mL meprobamate. Each sample was run in replicates of 10 in semi-quantitative mode with a calibration curve established with five meprobamate calibrators (0, 50, 100, 200, and 400 ng/mL). Percent recovery was calculated using the mean concentration of the 10 replicates relative to the expected concentration as shown below.

Expected Concentration (ng/mL)	Mean Observed Concentration (ng/mL)	Mean Recovery (%)	Range of Recovery (%)
0	2.2	N/A	N/A
10	6.7	66.6	38.0 – 87.0
40	39.9	99.7	93.8 – 104.8
80	81.8	102.2	98.0 – 108.4
120	123.3	102.8	99.9 – 107.3
160	163.7	102.3	98.3 – 107.5
200	195.3	97.7	95.4 – 99.7
240	251.6	104.8	95.3 – 110.5
280	305.7	109.2	102.0 – 114.3
320	348.9	109.0	105.5 – 111.9
360	386.6	107.4	102.3 – 111.8
400	412.5	103.1	100.3 – 105.4

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The LZI Carisoprodol Metabolite (Meprobamate) Enzyme Immunoassay is traceable to a commercially available meprobamate source material having 99% analytical purity as determined by LC/MS.

Specimen stability information was provided to support the following specimen storage conditions: storage at 2-8ºC for up to 1 week and at -20ºC for up to 17 months.

d. Detection limit

Not applicable.

e. Analytical specificity:

Endogenous Compounds: Potential interference from endogenous compounds was evaluated in the qualitative and semi-quantitative modes by spiking these compounds (at the test concentrations shown in the table below) into urine containing either 75 ng/mL or 125 ng/mL meprobamate (±25% of the assay cutoff). Samples were tested in duplicate. The results, which were the same for the qualitative and semi-quantitative modes, are shown below.

Endogenous Compound	Concentration Tested (mg/dL)	-25% Cutoff (75 ng/mL)	+25% Cutoff (125 ng/mL)
Acetone	1000	Negative	Positive
Ascorbic acid	1500	Negative	Positive
Beta-hydroxybutyric acid sodium salt	100	Negative	Positive
Bilirubin	2	Negative	Positive
Calcium chloride dihydrate	300 (saturated solution)	Negative	Positive
Citric acid	800	Negative	Positive
Creatinine	500	Negative	Positive
Ethanol	1000	Negative	Positive
Galactose	10	Negative	Positive
γ-Globulin	500	Negative	Positive
Glucose	3000	Negative	Positive
Hemoglobin	300	Negative	Positive
Human Serum Albumin	500	Negative	Positive
Oxalic Acid	100	Negative	Positive
Potassium chloride	6000	Negative	Positive
Riboflavin	0.3	Negative	Positive
Urea	6000	Negative	Positive
Uric acid monosodium salt	10	Negative	Positive
Sodium Chloride	6000	Negative	Positive
Sodium phosphate dibasic salt	300	Negative	Positive

Urine sample preservatives: To evaluate potential interference from common urine sample preservatives, device performance in qualitative and semi-quantitative modes was tested by spiking sodium azide (1% w/v), sodium fluoride (1% w/v), or boric acid (1% w/v) into negative urine to ± 25% of the 100 ng/mL meprobamate cutoff (75 ng/mL and 125 ng/mL). Samples were tested in duplicate. Boric acid was found to cause false negative results at +25% of the 100 ng/mL cutoff (125 ng/mL) and up to +125% of the 100 ng/mL cutoff (225 ng/mL) in both the qualitative and semi-quantitative modes. The following statement is provided in the limitations section of the labeling: Boric Acid at 1% w/v may cause false negative results. Boric Acid is not recommended as a preservative for urine.

pH: To evaluate potential interference from the pH of urine, device performance in the qualitative and semi-quantitative modes was tested using a range of urine pH values (3, 4, 5, 6, 7, 8, 9, 10, and 11). Test samples were prepared in negative urine, which was spiked to ±25% of the 100 ng/mL meprobamate cutoff (75 ng/mL and 125 ng/mL), and tested in duplicate. No positive or negative interference was observed at

urine pH values ranging from 3 to 11 for the qualitative and semi-quantitative modes.

Specific Gravity: To evaluate potential interference from the specific gravity of urine, device performance in the qualitative and semi-quantitative modes was tested using a range of urine specific gravities (1.002, 1.003, 1.005, 1.006, 1.007, 1.008, 1.012, 1.014, 1.015, 1.019, 1.025, and 1.029). These 12 negative urine samples were spiked with meprobamate to ±25% of the 100 ng/mL meprobamate cutoff (75 ng/mL and 125 ng/mL), and tested in duplicate. No positive or negative interference was observed at urine specific gravities ranging from 1.002 to 1.029 for the qualitative and semi-quantitative modes.

Cross-reactivity of structurally related compounds: The cross-reactivity of various structurally related drugs was evaluated in qualitative and semi-quantitative modes by spiking each substance into negative urine. The following table shows the quantity of each potential cross-reacting compound that produced assay reactivity equivalent to the 100 ng/mL meprobamate cutoff, or the maximum concentration tested (concentration tested), and calculated cross-reactivity for each compound (% cross-reactivity). The results were the same for the qualitative and semi-quantitative modes.

Compound	**Concentration Tested (ng/mL)**	**% Cross-Reactivity**
Carisoprodol	110	90.9
Darunavir	200,000	< 0.1
Efavirenz	200,000	< 0.1
Felbamate	400	25.0
Hydroxymeprobamate	65,000	0.2
Meprobamate	100	100.0
Meprobamate-N-Glucuronide	20,000	0.5
Methocarbamol	200,000	< 0.1
Mitocycin C	200,000	< 0.1
Neostigmine bromide	200,000	< 0.1
Retigabine	200,000	< 0.1
Ritonavir	100,000	< 0.1
Rivastigmine tartrate	200,000	< 0.1
Zafirlukast	200,000	< 0.1

Interference by structurally unrelated compounds: The potential for positive or negative interference by various structurally unrelated compounds was evaluated by spiking these compounds (at the test concentrations shown in the table below) into urine containing either 75 ng/mL or 125 ng/mL meprobamate (±25% of the assay cutoff). Samples were tested in duplicate. The results, which are the same for the qualitative and semi-quantitative modes, are shown below.

Compound	Concentration Tested (ng/mL)	-25% Cutoff (75 ng/mL)	+25% Cutoff (125 ng/mL)
Acetaminophen	100,000	Negative	Positive
6-Acetylmorphine	10,000	Negative	Positive
Acetylsalicylic Acid	100,000	Negative	Positive
Albuterol(Salbutamol)	100,000	Negative	Positive
Amitriptyline	100,000	Negative	Positive
d-Amphetamine	100,000	Negative	Positive
Benzoylecgonine	100,000	Negative	Positive
Buprenorphine	15,000	Negative	Positive
Bupropion	100,000	Negative	Positive
Caffeine	100,000	Negative	Positive
Carbamazepine	100,000	Negative	Positive
Cetirizine	20,000	Negative	Positive
Chlorpheniramine	100,000	Negative	Positive
Chlorpromazine	100,000	Negative	Positive
Clomipramine	100,000	Negative	Positive
Codeine	100,000	Negative	Positive
Cyclobenzaprine	100,000	Negative	Positive
Desipramine	100,000	Negative	Positive
Diphenhydramine	100,000	Negative	Positive
Ephedrine	100,000	Negative	Positive
Fentanyl	10,000	Negative	Positive
Fluoxetine	100,000	Negative	Positive
Fluphenazine	100,000	Negative	Positive
Hydrocodone	100,000	Negative	Positive
Hydromorphone	100,000	Negative	Positive
Ibuprofen	100,000	Negative	Positive
Imipramine	100,000	Negative	Positive
Lidocaine	100,000	Negative	Positive
Loratadine	100,000	Negative	Positive
Maprotiline	30,000	Negative	Positive
MDA (3,4-methylenedioxyamphetamine)	100,000	Negative	Positive
MDEA	100,000	Negative	Positive
MDMA (3,4-methylenedioxymethamphetamine)	100,000	Negative	Positive
Meperidine	100,000	Negative	Positive
Methadone	100,000	Negative	Positive
d-Methamphetamine	100,000	Negative	Positive
Methapyrilene	100,000	Negative	Positive
Methaqualone	100,000	Negative	Positive

Compound	Concentration Tested (ng/mL)	-25% Cutoff (75 ng/mL)	+25% Cutoff (125 ng/mL)
Metronidazole	100,000	Negative	Positive
Morphine	100,000	Negative	Positive
Nicotine	100,000	Negative	Positive
Nortriptyline	100,000	Negative	Positive
Oxazepam	100,000	Negative	Positive
Oxycodone	100,000	Negative	Positive
Oxymorphone	100,000	Negative	Positive
PCP (phencyclidine)	10,000	Negative	Positive
Pentazocine	20,000	Negative	Positive
Phenobarbital	100,000	Negative	Positive
d-Propoxyphene	100,000	Negative	Positive
Propranaolol	100,000	Negative	Positive
Ranitidine	100,000	Negative	Positive
Sertraline	100,000	Negative	Positive
THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid)	1,000	Negative	Positive
Thioridazine	100,000	Negative	Positive
Tramadol	100,000	Negative	Positive
Valproic Acid	100,000	Negative	Positive

f. Assay cut-off:

Characterization of how the device performs analytically around the claimed cutoff concentrations of 100 ng/mL is described in the precision section, M.1.a. above.

2. Comparison studies:

a. Method comparison:

A method comparison study was performed using 127 unaltered clinical urine samples from subjects that were (b) (4) taking prescribed or non-prescribed Carisoprodol. Each sample was run in singlicate using the LZI Carisoprodol Metabolite (Meprobamate) Enzyme Immunoassay on the AU400e automated clinical analyzer and the result was compared to that obtained by LC/MS or GC/MS. Samples with meprobamate concentrations < 100 ng/mL by LC/MS or GC/MS were defined as "negative." Samples with meprobamate concentrations ≥ 100 ng/mL by LC/MS or GC/MS were defined as "positive." The results are summarized below.

Qualitative analysis

Candidate device results	**Negative** (Drug-free or less than 50% of the cutoff concentration)	**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	2*	11	56	98.5
Negative	43	14	1**	0	96.6

Sample #	**LC/MS or GC/MS Meprobamate (ng/mL)**	**LC/MS or GC/MS result**	**Candidate device result**
58*	92	Negative	Positive
59*	98	Negative	Positive
60**	103	Positive	Negative

Semi-Quantitative analysis

Candidate device results	**Negative** (Drug-free or less than 50% of the cutoff concentration)	**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	1*	11	56	98.5
Negative	43	15	1**	0	98.3

Sample #	**LC/MS Meprobamate (ng/mL)**	**LC/MS result**	**Candidate device result**
59*	98	Negative	Positive
60**	103	Positive	Negative

b. Matrix comparison:

Not applicable. Urine is the only claimed matrix for the candidate device.

3. Clinical studies:

a. Clinical Sensitivity:
Not applicable.

b. Clinical Specificity:
Not applicable.

c. *Other clinical supportive data (when a. and b. are not applicable):*
Not applicable.

4. Clinical cut-off:

The assay has a clinical cut-off of 100 ng/mL meprobamate in human urine. To support the clinical validity of this cut-off, the sponsor submitted information from pharmacokinetic studies of carisoprodol and meprobamate in plasma following single dose administration and detailed information about meprobamate elimination in urine as a first-order elimination. This information indicates that meprobamate levels above the cutoff of this assay (100 ng/mL) are present in the urine for up to a few days following a single dose of drug. The information provided supported the clinical validity of the claimed cutoff of this device.

5. Expected values/Reference range:

Not applicable.

M. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809 and the special controls for this device type.

O. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device. In general, patients benefit from reliable tests to screen for the presence of drugs of abuse.

P. Identified Risks to Health and Required Mitigations

Identified Risks to Health	Required Mitigations
Clinical action based on incorrect test results (false positive results, false negative results) may lead to inappropriate clinical decision making.	Special controls (1), (2), and (3)
Incorrect understanding of the device, including the results, may lead to inappropriate clinical decision making.	Special controls (2) and (3)

Q. Benefit/Risk Analysis

Summary	
Summary of the Benefit(s)	There are benefits related to the detection of the presence of the carisoprodol metabolite or meprobamate drug in human urine. While the test is not indicated for acute clinical management of patients it may have value in settings of drug treatment programs, including decreasing the risk of potentially dangerous medication interactions. For example, identifying the presence of meprobamate may help prevent drug interactions with other sedating drugs that could potentiate the sedating effect. The test also has benefit for use in substance abuse programs to monitor the illicit use of carisoprodol or meprobamate which have abuse potential and are categorized as Schedule IV controlled substances by the Drug Enforcement Administration (DEA).
Summary of the Risk(s)	The risk of the test is limited to false negative or false positive test results and the incorrect understanding of how to interpret the results. A false positive result could result in the misidentification of a patient as having taken carisoprodol or meprobamate; however, device labelling states that positive results must be confirmed with a more specific analytical testing method; and when performed this confirmatory testing mitigates the risk of false positive results. A false negative result could increase the risk of drug interactions if other drugs which contribute to or potentiate the side effects are prescribed. However, the analytical performance of the test appears mitigates the risk of false negative results. Negative agreement among the 127 samples tested was 96.6% and the one false negative result in the study showed a level of meprobamate (established by a confirmatory analytical method) that was very near the cut-off. Misinterpretation of a positive test as indicative of toxicity, when the test is used in an acute care setting, could delay appropriate treatment if the result leads to confusion in the diagnosis of another acute condition that is present. This risk is mitigated by labelling of the device which specifically limits against the use of the test in being used for diagnosing drug intoxication or for the purposes of determining appropriate therapy.
Summary of Other Factors	The studies conducted included assessment of the accuracy, specificity, and precision around the cutoff of the device.
Conclusions Do the probable benefits outweigh the probable risks?	
Given the device's indications for use, required general controls and special controls established for this device, the probable benefits outweigh the probable risks.	

R. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 862.3590. FDA believes that the stated special controls, and applicable general controls, including design controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code:	QBK
Device Type:	Meprobamate test system
Class:	II (special controls)
Regulation:	21 CFR 862.3590

(a) *Identification*. A meprobamate test system is a device intended to measure meprobamate in human specimens. Measurements obtained by this device are used to detect the presence of meprobamate to diagnose the use or overdose of meprobamate or structurally-related drug compounds (e.g., prodrugs).

(b) *Classification*. Class II (special controls). The special controls for this device are:

1) Design verification and validation must include:

(i) Robust data demonstrating the accuracy of the device when used in the intended specimen matrix. The accuracy data must include a comparison between the meprobamate test system results and meprobamate results that are measured on an FDA-accepted measurement method that is specific and accurate (e.g., gas or liquid chromatography combined with tandem mass spectrometry).

(ii) Robust analytical data demonstrating the performance characteristics of the device, including, but not limited to, specificity, cross-reactivity to relevant endogenous and exogenous substances, and the reproducibility of analyte detection around the cutoff(s).

2) The intended use of the device must not include an indication for use in monitoring therapeutic drug concentrations or informing dosing adjustment decisions.

3) Your 21 CFR 809.10 labeling must include the following:

(i) If indicated for use as a screening test to identify preliminary results for further confirmation, the intended use must state "This assay provides only a preliminary analytical result. A more specific alternative chemical confirmatory method (e.g., gas or liquid chromatography and mass spectrometry) must be used to obtain a confirmed analytical result. Clinical consideration and professional judgment must be exercised with any drug of abuse test, particularly when the preliminary test result is positive."

(ii) A limiting statement that reads as follows: "This test should not be used to monitor therapeutic drug concentrations or to inform dosing adjustment decisions."