LZI SPICE II (UR-144/XLR-11) Enzyme Immunoassay – EU Only

REF 0510 (100/37.5 mL R_1/R_2 Kit)
0511 (1000/375 mL R_1/R_2 Kit)
2ºC – 8ºC

IVD For In Vitro Diagnostic Use Only

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) SPICE II (UR-144/XLR-11) Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of UR-144/XLR-11-type synthetic cannabinoids in human urine using UR-144 N-(5-hydroxypentyl) metabolite as calibrator at the cutoff value of 20 ng/mL. The assay is designed for use with a number of automated clinical chemistry analyzers.

The assay provides a rapid screening procedure for determining the presence of synthetic cannabinoids in urine. 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

In the past few decades, research has confirmed the presence of an endogenous endocannabinoid system, or ECS, in humans (3). Endocannabinoids are produced within the human body and activate two known cannabinoid receptors, CB_1 and CB_2 (4). The CB_1 receptor is localized primarily in the brain and is thought to be responsible for the euphoric and anticonvulsive effects of cannabis, whereas the CB_2 receptor is found primarily in the immune system and thought to be responsible for the anti-inflammatory effects of Δ^9-THC (5-7).

Due to the role the ECS may play in a number of physiological processes, much interest has been developed in the use of synthetic cannabinoids (synthetic ECS) ligands for therapeutic purposes, such as the CB_1 receptor mediated anti-emetic, appetite stimulating, and pain-relieving properties (7-10). Unfortunately, these compounds have become new drugs of choice world-wide, as many were not initially regulated and are not detected in common drug screening assays (11-13).

Early reports document undesirable symptoms not commonly associated with marijuana use, including serious acute kidney injuries associated with the synthetic cannabinoid XLR-11 (14-16).

Synthetic cannabinoids are predominantly excreted as metabolites in urine, often as hydroxyl and carboxy metabolites (17-24).

Assay Principle

The LZI SPICE II (UR-144/XLR-11) Enzyme Immunoassay is a homogeneous enzyme immunoassay 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 (25). 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, UR-144-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when drug is present in the sample, antibody would bind to free drug; the unbound UR-144-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.

Reagents Provided

Antibody/Substrate Reagent (R_1): Contains mouse monoclonal anti-UR-144 metabolite antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.
Enzyme-drug Conjugate Reagent (R_2): Contains UR-144-labeled glucose-6-phosphate dehydrogenase (G6PDH), stabilizers, and 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.*

SPICE II (UR-144 N-[-5-hydroxypentyl] metabolite) Calibrators	REF
THC Negative Calibrator	0002c
Low Calibrator: Contains 10 ng/mL, UR-144 N-(5-hydroxypentyl) metabolite	0512
Cutoff Calibrator: Contains 20 ng/mL, UR-144 N-(5-hydroxypentyl) metabolite	0513
Intermediate Calibrator: Contains 35 ng/mL, UR-144 N-(5-hydroxypentyl) metabolite	0514
High Calibrator: Contains 50 ng/mL, UR-144 N-(5-hydroxypentyl) metabolite	0515

SPICE II (UR-144 N-[-5-hydroxypentyl] metabolite) Controls	REF
Level 1 Control: Contains 15 ng/mL, UR-144 N-(5-hydroxypentyl) metabolite	0517
Level 2 Control: Contains 25 ng/mL, UR-144 N-(5-hydroxypentyl) metabolite	0518

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 (26).*
- *Do not use the reagents beyond their expiration dates.*

Reagent Preparation and Storage

The reagents are ready-to-use. No reagent preparation is required. All assay components should be stored 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 (27-29). Use fresh urine specimens for the test. If a sample cannot be analyzed immediately, it may be stored refrigerated at 2-8ºC for up to seven days. For longer storage, keep samples frozen at -20ºC and then thaw before use. Studies have shown JWH-type synthetic cannabinoid samples in urine are stable at -20ºC for up to 216 days (30). Samples should be equilibrated to room temperature (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 both samples should be forwarded 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 sample, mixing reagents, measuring enzyme rates at 340 nm 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 Beckman Coulter® AU480. If other instruments are used, performance will need to be validated by the laboratory (31, 32).

Assay Procedure

Typical assay parameters used for the Beckman Coulter AU480 analyzer include a 12 µL sample, 120 µL of antibody reagent (R_1), 45 µL of enzyme conjugate reagent (R_2), 10 µL dilution with de-ionized water (DI H_2O) following addition of R_2 in 37 ºC incubation temperature, 16-20 reading points, Fixed Mode, 340 nm primary wavelength, and 410 nm secondary wavelength.

For qualitative analysis, use the 20 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. Recalibration should be performed after reagent bottle change or a change in calibrators or reagent lot. Two levels of controls are also available for monitoring the cutoff level: 15 ng/mL and 25 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) 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 20 ng/mL of UR-144 N-(5-hydroxypentyl) metabolite, is used as a reference for distinguishing preliminary positive from negative samples. A sample with a change in absorbance (ΔmAU) equal to or greater than that obtained with the cutoff calibrator is considered positive. A sample with a change in absorbance (ΔmAU) lower than that obtained with the cutoff calibrator is considered negative.

Semi-Quantitative: The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for verification by a confirmatory method 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 UR-144 N-(5-hydroxypentyl) metabolite in the sample may then be estimated from the calibration curve.

Limitations

1. *A positive result from the assay indicates only the presence of UR-144, AB-005, A-796260, A-834735, FAB-144, FUB-144, XLR-11, or XLR-12 or similar analogs and their metabolites.*
2. *The test is not intended for quantifying these single analytes in samples.*
3. *A preliminary positive result does not necessarily indicate drug abuse.*
4. *A negative result does not necessarily mean a person did not take illegal drugs.*
5. *Care should be taken when reporting results, as numerous factors (e.g., fluid intake, endogenous or exogenous interferents) may influence the urine test results.*
6. *Preliminary positive results should be confirmed by other affirmative, analytical chemistry methods (e.g., chromatography), preferably GC/MS or LC/MS.*
7. *The test is designed for use with human urine only.*
8. *The test is not designed for therapeutic drug monitoring.*

Typical Performance Characteristics

The results shown below were performed with a single Beckman Coulter AU480 chemistry analyzer.

Precision:

Semi-quantitative analysis: The following concentrations were determined with reference curves from five calibrators. Typical results (ng/mL) are as follows:

Concentration	Within Run (N=20)			Run-to-Run (N=80)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	0.0	0.3	N/A	0.0	0.3	N/A
5 ng/mL	4.2	0.3	6.0 %	4.2	0.3	7.6 %
10 ng/mL	9.9	0.3	3.1 %	9.9	0.4	3.9 %
15 ng/mL	14.3	0.3	2.0 %	14.3	0.4	2.5 %
20 ng/mL	19.7	0.2	1.2 %	19.7	0.4	1.9 %
25 ng/mL	24.9	0.3	1.0 %	24.9	0.3	1.4 %
30 ng/mL	30.0	0.3	0.9 %	30.0	0.4	1.3 %
35 ng/mL	35.3	0.4	1.0 %	35.3	0.5	1.5 %
40 ng/mL	41.8	0.4	1.0 %	41.8	0.6	1.4 %

20 ng/mL Cutoff Result:		Within Run (N=20)		Total Precision (N=80)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	20	20 Neg	80	80 Neg
5 ng/mL	25 %	20	20 Neg	80	80 Neg
10 ng/mL	50 %	20	20 Neg	80	80 Neg
15 ng/mL	75 %	20	20 Neg	80	80 Neg
20 ng/mL	100 %	20	13 Neg/ 7 Pos	80	61 Neg/ 19 Pos
25 ng/mL	125 %	20	20 Pos	80	80 Pos
30 ng/mL	150 %	20	20 Pos	80	80 Pos
35 ng/mL	175 %	20	20 Pos	80	80 Pos
40 ng/mL	200 %	20	20 Pos	80	80 Pos

Qualitative analysis: The following concentrations were evaluated. Typical qualitative results (measured by ΔOD, mAU) are as follows:

Concentration	Within Run (N=20)			Run-to-Run (N=80)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	-5.6	1.7	-30.4 %	-5.6	1.9	-34.5 %
5 ng/mL	19.6	1.6	8.1 %	19.6	1.8	9.3 %
10 ng/mL	54.7	1.7	3.1 %	54.7	2.1	3.8 %
15 ng/mL	94.9	2.4	2.5 %	94.9	2.8	3.0 %
20 ng/mL	143.5	2.8	2.0 %	143.5	3.3	2.3 %
25 ng/mL	197.3	3.0	1.5 %	197.3	3.8	1.9 %
30 ng/mL	249.8	2.5	1.0 %	249.8	3.1	1.3 %
35 ng/mL	302.8	3.0	1.0 %	302.8	3.8	1.3 %
40 ng/mL	342.7	2.6	0.8 %	342.7	3.0	0.9 %

20 ng/mL Cutoff Result:		Within Run (N=20)		Total Precision (N=80)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	20	20 Neg	80	80 Neg
5 ng/mL	25 %	20	20 Neg	80	80 Neg
10 ng/mL	50 %	20	20 Neg	80	80 Neg
15 ng/mL	75 %	20	20 Neg	80	80 Neg
20 ng/mL	100 %	20	14 Neg 6 Pos	80	49 Neg/ 31 Pos
25 ng/mL	125 %	20	20 Pos	80	80 Pos
30 ng/mL	150 %	20	20 Pos	80	80 Pos
35 ng/mL	175 %	20	20 Pos	80	80 Pos
40 ng/mL	200 %	20	20 Pos	80	80 Pos

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from the negative urine with 95 % confidence, was determined to be 3 ng/mL in both qualitative and semi-quantitative analyses.

Analytical Recovery: To demonstrate linearity for the purposes of sample dilution and quality control (see semi-quantitative results section), a drug-free pool of processed urine was spiked with UR-144 N-(5-hydroxypentyl) metabolite to 50 ng/mL and subsequently diluted, as shown in the table below. Each sample was run in 10 replicates and the average was used to determine percent recovery compared to the expected target value. When comparing the determined (y) and target (x) value, using the least squares regression technique, the regression equation and correlation are as follows:

$y = 1.0366x - 0.4316$, $r^2 = 0.9978$

% Dilution	Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
100 %	0	0.2	N/A
94 %	3	2.6	87.0 %
90 %	5	4.5	90.6 %
80 %	10	10.3	103.2 %
70 %	15	14.7	98.2 %
60 %	20	20.2	101.1 %
50 %	25	25.1	100.6 %
40 %	30	30.3	100.9 %
30 %	35	34.6	98.7 %
20 %	40	42.2	105.5 %
10 %	45	47.8	106.3 %
0 %	50	50.4	100.9 %

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine calibrator matrix individually to various concentrations and evaluated against the cutoff calibrator.

The table below lists either the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator, or the maximal concentration of the compound tested that gave a response below the response of the cutoff calibrator.

Structurally Related Synthetic Cannabinoid Compounds:

Compound	Spiked [ ] (ng/mL)	EIA [ ] (ng/mL)	% Cross-reactivity
UR-144 N-(5-hydroxypentyl) metabolite	20	20.0	100.00 %
UR-144	50	23.9	47.70 %
XLR-11	25	19.7	78.80 %
UR-144 N-(pentanoic acid) metabolite	25	23.5	93.80 %
XLR-11 N-(4-hydroxypentyl) metabolite	25	22.0	88.00 %
UR-144 N-(5-hydroxypentyl) β-D-glucuronide	50	24.0	48.00 %
AB-005	50	21.9	43.70 %
A-796260	20	25.0	125.00 %
A-834735	35	24.5	70.00 %
FAB-144	70	22.4	32.00 %
FUB-144	50	22.9	45.70 %
M-144 (XLR-11 2-methylindole analog)	150	20.0	13.30 %
UR-144 N-(5-chloropentyl) analog	200	23.0	11.50 %
UR-144 N-(5-bromopentyl) analog	350	22.4	6.40 %

Structurally Related Synthetic Cannabinoid Compounds, continued:

Compound	Spiked [ ] (ng/mL)	EIA [ ] (ng/mL)	% Cross-reactivity
XLR-11 N-(4-pentenyl) analog	200	28.3	14.13 %
XLR-12	50	23.5	47.00 %
(1H-Indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone	50	32.7	65.40 %
AB-PINACA	10,000	12.8	0.13 %
AM-2201 6-hydroxyindole metabolite	10,000	0.3	0.00 %
AM-2201 N-(4-hydroxypentyl) metabolite	10,000	14.5	0.14 %
AM-694 N-(5-hydroxypentyl) metabolite	500	20.9	4.17 %
JWH-007	10,000	0.8	0.01 %
JWH-015	10,000	6.6	0.07 %
JWH-022	10,000	1.1	0.01 %
(±) JWH-018 N-(4-hydroxypentyl) metabolite	10,000	13.2	0.13 %
JWH-018 5-hydroxyindole metabolite	10,000	0.0	0.00 %
JWH-018 N-(5-hydroxypentyl) metabolite	10,000	13.9	0.14 %
JWH-018 N-(5-hydroxypentyl)-b-D-glucuronide	10,000	8.0	0.08 %
JWH-018 N-(pentanoic acid) metabolite	10,000	10.8	0.11 %
JWH-019 N-(5-hydroxyhexyl) metabolite	10,000	14.5	0.14 %
(±) JWH-073 N-(3-hydroxybutyl) metabolite	10,000	-0.1	0.00 %
JWH-073 6-hydroxyindole metabolite	10,000	10.2	0.10 %
JWH-073 N-(4-butanoic acid) metabolite	10,000	12.9	0.13 %
JWH-073 N-(4-hydroxybutyl) metabolite	10,000	14.1	0.14 %
JWH-073 N-(4-hydroxybutyl)-b-D-glucuronide	10,000	8.8	0.09 %
JWH-081 N-(5-hydroxypentyl) metabolite	10,000	7.9	0.08 %
JWH-122 N-(5-hydroxypentyl) metabolite	10,000	10.6	0.11 %
JWH-203 N-(5-hydroxypentyl) metabolite	10,000	15.6	0.16 %
JWH-210 N-(5-hydroxypentyl) metabolite	10,000	6.9	0.07 %
JWH-250 N-(5-hydroxypentyl) metabolite	10,000	12.9	0.13 %
JWH-398 N-(5-hydroxypentyl) metabolite	10,000	5.3	0.05 %
MAM-2201 N-(4-hydroxypentyl) metabolite	10,000	8.7	0.09 %
1'-Naphthoyl Indole	10,000	4.1	0.04 %
RCS-4 N-(5-hydroxypentyl) metabolite	10,000	4.3	0.04 %
THJ	10,000	0.1	0.00 %
THJ-018	10,000	0.3	0.00 %
THJ-2201	10,000	-0.2	0.00 %

Structurally Unrelated Pharmacological Compounds: Various structurally unrelated compounds that are potential interferents were tested for cross-reactivity with the assay. Test compounds were spiked into a pool of processed drug free urine to the desired concentrations and then UR-144 N-(5-hydroxypentyl) metabolite was spiked to a final concentration of 0 ng/mL, the negative control concentration of 15 ng/mL, or the positive control concentration of 25 ng/mL. The table below lists the concentration measured of each test compound.

Interfering Substances	Spiked [ ] (ng/mL)	UR-144 N-(5-hydroxypentyl) metabolite (ng/mL)		
		0 ng/mL (ng/mL)	15 ng/mL Control (ng/mL)	25 ng/mL Control (ng/mL)
Acetaminophen	100,000	-0.2	13.5	23.2
6-Acetylmorphine	10,000	-2.2	13.2	25.2
Acetylsalicylic Acid	100,000	-0.2	14.7	26.0
Amitryptyline	100,000	-0.6	13.9	25.2
Amobarbital	100,000	-0.4	14.5	25.7
Amphetamine	100,000	-0.4	14.1	25.2
Benzoylecgonine	100,000	-0.6	14.6	26.0
Buprenorphine	20,000	0.1	13.4	24.2
Burpropion	100,000	-0.1	14.1	25.5
Caffeine	100,000	-0.4	13.6	27.1
Chlorpheniramine	100,000	-0.4	14.2	25.8
Chlorpromazine	100,000	-0.7	14.4	25.9
Cocaine	100,000	-0.2	14.9	26.6
Codeine	100,000	-0.4	14.6	25.9
Dextromethorphan	100,000	-0.5	14.5	26.0
Diazepam	10,000	1.6	15.5	25.9
Ecgonine Methyl Ester	100,000	-0.4	14.5	25.7
d,l-Ephedrine	100,000	-0.5	14.6	25.8
Hydrocodone	100,000	-0.4	13.8	24.2
Hydromorphone	100,000	-0.4	14.8	25.3
α-Hydroxy-alprazolam	10,000	-0.4	14.1	25.0
Imipramine	100,000	-0.6	14.5	25.4
Lidocaine	100,000	-0.4	14.8	25.5
Lorazepam	100,000	-0.5	14.5	25.6
MDMA	100,000	-1.1	14.0	24.3
Meperidine	100,000	-0.5	14.3	25.3
Methadone	100,000	-0.7	14.2	25.3
Methamphetamine	100,000	-0.3	14.6	25.7

Structurally Unrelated Pharmacological Compounds, continued:

Interfering Substances	Spiked [ ] (ng/mL)	UR-144 N-(5-hydroxypentyl) metabolite (ng/mL)		
		0 ng/mL (ng/mL)	15 ng/mL Control (ng/mL)	25 ng/mL Control (ng/mL)
Methaqualone	20,000	2.2	16.1	26.6
Morphine	100,000	-0.1	14.2	25.7
Nordiazepam	10,000	0.0	14.3	25.3
l-11-Nor-Δ9-THC-9-Carboxylic Acid	10,000	-0.3	14.3	25.8
Nortriptyline	100,000	-0.6	13.9	25.2
Oxazepam	100,000	0.1	14.3	25.2
Oxycodone	10,000	-0.4	14.4	25.5
Oxymorphone	10,000	-0.3	14.5	25.6
Phencyclidine	100,000	-0.3	14.4	24.8
Phenobarbital	100,000	-0.2	14.5	25.7
Promethazine	100,000	-0.5	13.8	25.0
Propoxyphene	100,000	-0.2	14.3	26.0
Ranitidine	100,000	-0.3	14.5	26.0
Secobarbital	100,000	-0.4	14.3	25.7
Valproic Acid	100,000	-0.3	14.4	25.5
Zolpidem	100,000	-0.8	13.7	24.7
Zopiclone	5000	-0.2	12.5	23.5

It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results.

Interference: Endogenous Substances
The following potentially interfering compounds were spiked into a pool of processed drug-free urine to the desired concentrations, and UR-144 N-(5-hydroxypentyl) metabolite was then spiked to a final concentration of 0 ng/mL, the negative control concentration of 15 ng/mL, or the positive control concentration of 25 ng/mL. The spiked solution is evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiologically relevant concentrations, as all spiked samples gave correct responding positive/negative results against the cutoff value of 20 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked [ ] (mg/dL)	UR-144 N-(5-hydroxypentyl) metabolite (ng/mL)		
		0 ng/mL (ng/mL)	15 ng/mL Control (ng/mL)	25 ng/mL Control (ng/mL)
Acetone	1000	-0.6	13.1	23.7
Ascorbic Acid	400	-0.3	13.7	24.6
Creatinine	500	-0.4	14.0	25.0
Ethanol	1000	-0.8	13.8	24.9
Galactose	10	-0.4	14.4	25.2
γ-Globulin	500	-0.3	14.0	24.6
Glucose	3000	-0.5	14.1	25.4
Hemoglobin	200	-0.1	14.4	24.7
Human Serum Albumin	500	-0.3	15.1	26.0
Oxalic Acid	100	-0.4	14.2	25.0
Riboflavin	0.3	-0.3	14.5	25.4
Urea	2000	-0.8	14.0	24.4
Sodium Chloride	2000	-2.0	13.5	23.8
pH 3	N/A	-0.3	13.9	24.3
pH 4	N/A	-0.3	14.8	25.5
pH 5	N/A	-0.5	14.3	25.2
pH 6	N/A	-0.2	14.1	25.0
pH 7	N/A	-0.3	14.8	25.6
pH 8	N/A	-0.1	14.7	25.4
pH 9	N/A	-0.1	15.0	25.7
pH 10	N/A	0.0	14.4	24.8
pH 11	N/A	0.1	14.0	24.5

Specific gravity: Samples ranging in specific gravity from 1.001 to 1.028 were spiked with UR-144 N-(5-hydroxypentyl) metabolite to a final concentration of 0 ng/mL, the negative control concentration of 15 ng/mL, or the positive control concentration of 25 ng/mL. No interference was observed.

Specific Gravity Value	UR-144 N-(5-hydroxypentyl) metabolite (ng/mL)		
	0 ng/mL	15 ng/mL Control	25 ng/mL Control
1.001	0.7	12.7	20.7
1.005	0.4	12.9	21.5
1.008	0.8	12.7	21.3
1.010	-0.8	11.6	20.4
1.012	0.7	12.9	22.0
1.015	-0.2	13.3	21.5
1.018	-0.8	11.7	20.8
1.020	-0.5	12.7	21.8
1.025	-1.3	11.6	21.2
1.028	-1.9	10.7	21.1

Bibliography:

1. Urine Testing for Drug of Abuse, National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Mandatory Guidelines for Federal Workplace Drug Testing Program, National Institute on Drug Abuse, Federal Register, **53**(69): 11970 (1988).
3. Onaivi, E.S., Suguira, T., and Marzo, V. Di, (Eds): Endocannabinoids: The Brain and Body's Marijuana and Beyond. CRC Press, Taylor and Francis, London, UK; FL, USA (2006).
4. Galligan, J.J. Cannabinoid signalling in the enteric nervous system. Neurogastroenterol Motil. **21**(9): 899-902 (2009).
5. Massi, L., Elezgarai, I., Puente, N., Reguero, L., Grandes, P., et al. Cannabinoid receptors in the bed nucleus of the stria terminalis control cortical excitation of midbrain dopamine cells in vivo. *J Neurosci.* **28**:10496–10508 (2008).
6. Matsuda, L.A. Molecular aspects of cannabinoid receptors. *Crit Rev Neurobiol* **11**(2-3):143-66 (1997).
7. Nagarkatti, P., Pandey, R., Rieder, S.A., Hegde, V.L., and Nagarkatti, M. Cannabinoids as novel anti-inflammatory drugs. *Future Med Chem.* **1**(7):1333-1349 (2009).
8. Salazar, M., et. al. Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. *J Clin Invest.***119**(5):1359-72 (2009).
9. Malfitano, A.M., Ciaglia, E., Gangemi, G., Gazzerro, P., Laezza, C., and Bifulco, M.. Targets Update on the endocannabinoid system as an anticancer target. *Expert Opin Ther* **15**(3):297-308 (2011).
10. Iversen, LL. The Science of Marijuana. 2nd ed. New York: Oxford University Press, Inc. (2008).
11. Fattore, L., Fratta, W., Beyond THC: The new generation of cannabinoid designer drugs, *Front Behav. Neurosci*, **5**:60 (2011).
12. Sedefov, R., Gallegos, A., Kind, L., Lopez, D., Auwarter, V., and Hughes, B., European Monitoring Centre for Drugs and Drug Addiction EMCDDA 2009 thematic paper, Understanding the "Spice' Phenomenon, Office for Official Publications of the European Communities, 2009. www.emcdda.europa.eu/attachements.cfm/att_80086_EN_SpiceThematicpaperfinalversion.pdf (accessed August 2014)
13. Kikura-Hanajiri, R., Uchiyama, N., Kawamura, M., Ogata, J., and Goday, Y., Prevalance of new designer drugs and their legal status in Japan, *Yakugaku Zasshi*, **133**: 31-40 (2013).
14. Center for Disease Control and Prevention (CDC), Acute kidney injury associated with synthetic cannabinoid use. *Morb Mortal Wkly Rep*, **62**:93-98 (2013).
15. Thornton, S.L., Wood, C., Friesen, M.W., Gerona, R.R., Synthetic cannabinoid use associated with acute kidney injury, *Clin Tox*, **51**:189-190 (2013).
16. Meyer, M.R., Peters, F.T., Analytical toxicology of emerging drugs of abuse – an update, *Ther. Drug Monit*., **34**:615-621 (2012).
17. Chimalakonda, K.C., Seely, K.A., Bratton S.M., Brents, L.K., Moran, C.L., Endres, G.W. and et. al., Cytochrome P450-mediated oxidative metabolism of abused synthetic cannabinoids found in K2/Spice: identification of novel cannabinoid receptor ligands. *Drug Metab Dispos* **40**:2174–2184 (2012).
18. Grigoryev. A., Kavanagh. P., and Melnik, A. The detection of the urinary metabolites of 1-[(5-fluoropentyl)-1h-indol-3-yl]-(2-iodophenyl)methanone (AM-694), a high affinity cannabimimetic, by gas chromatography-mass spectrometry. *Drug Test Anal* **5**:110–115 (2012).
19. Grigoryev, A., Kavanagh, P., and Melnik, A., The detection of the urinary metabolites of 3-[(adamantan-1-yl)carbonyl]-1-pentylindole (AB-001), a novel cannabimimetic, by gas chromatography-mass spectrometry. *Drug Test Anal* **4**:519–524 (2012).
20. Grigoryev, A., Melnik, A., Savchuk, S., Simonov, A., and Rozhanets, V., Gas and liquid chromatography-mass spectrometry studies on the metabolism of the synthetic phenylacetylindole cannabimimetic JWH-250, the psychoactive component of smoking mixtures. *J Chromatogr B Analyt Technol Biomed Life Sci* **879**:2519–2526 (2011).
21. Hutter, M., Broecker, S., Kneisel, S., and Auwarter, V. Identification of the major urinary metabolites in man of seven synthetic cannabinoids of the aminoalkylindole type present as adulterants in "herbal mixtures" using LC-MS/MS techniques. *J Mass Spectrom* **47**:54–65 (2012).
22. Kavanagh, P., Grigoryev, A., Melnik, A., and Simonov, A., The identification of the urinary metabolites of 3-(4-methoxybenzoyl)-1-pentylindole (RCS-4), a novel cannabimimetic, by gas chromatography-mass spectrometry. *J Anal Toxicol* **36**:303–311 (2012).
23. Sobolevsky, T., Prasolov, I., and Rodchenkov, G., Detection of urinary metabolites of AM-2201 and UR-144, two novel synthetic cannabinoids. *Drug Test Anal* **4**:745–53 (2012).
24. Wohlfarth, A., Pang, S., Zhu, M., Gandhi, A.S., Scheidweiler, K.B., Liu, H., and Huestis, M.A., First Metabolic Profile of XLR-11, a Novel Synthetic Cannabinoid, Obtained by using Human Hepatocytes and High-Resolution Mass Spectrometry. *Drug Monit and Tox*. **59**(11):1638-1648 (2013).

Bibliography, continued:

25. Rubenstein, K.E., Schneider, R.S., and Ullman, E.F., Homogeneous Enzyme Immunoassay: A New Immunochemical Technique, *Biochem Biophys Res Commun*, **47**: 846 (1972).
26. Sodium Azide. National Institute for Occupational Safety (NIOSH). Pocket Guide to Chemical Hazards. Third Printing, September 2007. Available online at: https://www.cdc.gov/niosh/npg/default.html
27. Blanc, J.A., Manneh, V.A., Ernst, R., Berger, D.E., de Keczer, S.A., Chase, C., Centofanti, J.M., and DeLizzza, A.J., Adsorption losses from urine-based cannabinoid calibrators during routine use. *Clinical Chemistry*. **39**(8): 1705-12 (1993).
28. Stout, P.R., Horn, C.K., and Lesser, D.R., Loss of THCCOOH from urine specimens stored in polypropylene and polyethylene containers at different temperatures. *J Anal Toxicol*. **24**(7): 567-71 (2000).
29. Giardino, N. J., Stability of 11-nor-delta-9-tetrahydrocannabinol in negative human urine in high-density polyethylene (Nalgene). *J Anal Toxicol*. **20**(4):275-6 (1996).
30. Davies, B. B., Bayard, C., Larson, S. J., Zarwell, L. W., and Mitchell, R. A., Retrospective Analysis of Synthetic Cannabinoid Metabolites in Urine of Individuals Suspected of Driving Impaired. Journal of Analytical Toxicology, **40**(2), 89–96 (2015).
31. CDRH Guidance for Industry and FDA Staff: Replacement Reagent and Instrument Family Policy (2003).
32. Nichols, J., Instrument Validation: The Road to Success. CLN's Lab 2004: From Basic to Advanced Series. 14-16 (2004).

Additions, deletions, or changes are indicated by a change bar in the margin.

For technical assistance please call: (408) 970-8811

Manufacturer:
Lin-Zhi International, Inc.
2945 Oakmead Village Court
Santa Clara, CA 95051
USA
Tel: (408) 970-8811
Fax: (408) 970-9030
www.lin-zhi.com

EC REP **Authorized European Rep. within the EU:**
CEpartner4U
Esdoornlaan 13
3951 DB Maarn
The Netherlands
www.cepartner4u.eu
CE