# Development and validation of a new homogeneous immunoassay for the detection of UR-144 metabolites in urine

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## Introduction and Objective

Synthetic cannabinoids are consistently being synthesized worldwide with the intention of evading detection in biological matrices. The objective of this project was to develop and validate a new high throughput homogeneous enzyme immunoassay (HEIA) for the rapid detection of the urinary metabolites of the latest synthetic cannabinoids such as UR-144 and XLR-11. Despite the fact that UR-144, XLR-11, JWH-018, and AM2201 belong to a drug class that share a core indole-ring moiety, it is challenging to develop an immunoassay to cross react with all of them due to the significant difference in structures of the substituted groups. The current commercially available homogeneous immunoassay targeted at JWH- metabolites has very low cross reactivity for UR-144 and XLR-11, thus it was necessary to develop a new screening method to detect UR-144, XLR-11 and their metabolites in urine.

## Advantages

1. Ready to use reagents suitable for high throughput instruments
2. Assay working range : 0 to 40ng/mL, at a cutoff of 10ng/mL
3. Specific assay with accuracy >98% based on 65 urine specimens

## Results and Discussion

***Precision: Daily Calibration Required***

The qualitative precision was determined by assaying calibrators and controls in synthetic urine for 20 days, 2 runs per day in duplicates (N=80). The results are summarized below.

| Conc. ng/mL | Mean Conc. (ng/ml) | C.V.% |
|---|---|---|
| 5 ng/mL (Control LOW) | 537.2 | 4.6 |
| 10 ng/mL Calibrator | 564.2 | 5.0 |
| 15ng/mL (Control HIGH) | 585.8 | 4.7 |

***Cross Reactivity:***

Structurally related compounds that are potentially found in urine were tested using the 10 ng/mL cutoff calibrator.

| Structurally Related Compounds | | | |
|---|---|---|---|
| Compound | Analyte Conc. (ng/mL) | UR-144 N-pentanoic acid (ng/mL) | Cross-Reactivity (%) |
| UR-144 N-pentanoic acid | 10 | 10 | 100 |
| UR-144 | 20 | 10 | 50 |
| UR-144 N-(5hydroxypentyl)β-d-glucuronide | 30 | 10 | 33 |
| UR-144 N-(5-bromopentyl) | 25 | 10 | 40 |
| UR-144 N-(5-chloropentyl) | 20 | 10 | 50 |
| UR-144 N-heptyl | 40 | 10 | 25 |
| UR-144 N-(5hydroxypentyl) metabolite | 20 | 10 | 50 |
| XLR-11 | 20 | 10 | 50 |
| XLR-11 N-(4-pentenyl) | 20 | 10 | 50 |
| XLR-11 N-(4-hydroxypentyl) metabolite | 70 | 10 | 14 |

| Structurally Related Compounds | | | |
|---|---|---|---|
| Compound | Analyte Conc. (ng/mL) | UR-144 N-pentanoic acid (ng/mL) | Cross-Reactivity (%) |
| AB-005 | 30 | 10 | 33 |
| A-834735 | 20 | 10 | 50 |
| JWH-250 5 hydroxyindole metabolite | 100,000 | 10 | ND |
| JWH-250-N(hydroxypentyl) metabolite | 20,000 | 10 | 0.05 |
| JWH-250-N(4-hydroxypentyl) metabolite | 50,000 | 10 | 0.02 |
| JWH-250-N(5-carboxypentyl) metabolite | 50,000 | 10 | 0.02 |
| RCS-4-2 methoxy isomer | 10,000 | 10 | 0.10 |
| JWH-250 | 100,000 | 10 | ND |
| JWH-210 | 100,000 | 10 | ND |
| JWH-018 N(5-hydroxypentyl)metabolite | 3,000 | 10 | 0.3 |
| AM2201-6-hydroxyindole metabolite | 100,000 | 10 | ND |
| JWH-007 | 100,000 | 10 | ND |
| JWH-019 | 100,000 | 10 | ND |
| AM-2232 | 100,000 | 10 | ND |
| JWH-081 | 100,000 | 10 | ND |
| AM-2201-N-4-hydroxypentyl metabolite | 100,000 | 10 | ND |
| AM-2233 | 10,000 | 10 | 0.1% |
| 1-Naphthoyl indole | 100,000 | 10 | ND |
| JWH-073 6-hydroxyindole metabolite | 100,000 | 10 | ND |
| JWH-073 | 100,000 | 10 | ND |
| JWH-122 | 100,000 | 10 | ND |
| JWH-018 N(5-hydroxypentyl β-d-glucuronide | 100,000 | 10 | ND |
| JWH-201 | 100,000 | 10 | ND |
| AM-2201 | 100,000 | 10 | ND |
| Cannabipiperidiethanone | 50,000 | 10 | 0.02% |
| JWH-018 4-hydroxyindole | 100,000 | 10 | ND |
| JWH-022 | 100,000 | 10 | ND |
| JWH-073 N-butanoic acid | 100,000 | 10 | ND |
| JWH-018 5-hydroxyindole | 100,000 | 10 | ND |
| 3-1 napthoyl-1H-indole | 100,000 | 10 | ND |
| JWH-018 pentanoic acid | 100,000 | 10 | ND |
| JWH-018 | 100,000 | 10 | ND |

***Authentic specimens:***

- 65 urine specimens previously confirmed by an outside laboratory by LC-MS/MS were analyzed with this Immunalysis UR-144 PA EIA assay
- Cutoff concentration: 10 ng/mL for both EIA and confirmation method
- 40 specimens were negative by both methods
- 24 specimens were positive by both methods
- The sensitivity, specificity and accuracy were 96%, 100%, and 98%, respectively

| | | LC-MS/MS + | LC-MS/MS - |
|---|---|---|---|
| HEIA | + | 24 | 0 |
| | - | 1 | 40 |

•Negative result: absorbance rate reading just below cutoff

## Summary

A high throughput HEIA has been developed for the detection of UR-144 metabolites in human urine which correlates well with LC-MS/MS.

## References

•Grigoryev, A., Kavanagh, P., Melnik, P., Savchuk, S., Simonov, A.; Gas and Liquid Chromatography-Mass Spectrometry Detection of the Urinary Metabolites of UR-144 and Its Major Pyrolysis Product; *Journal of Analytical Toxicology*, April 16, 2013: doi:10.1093/jat/bkt028.

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