

# Development and application of ELISA for detection of Synthetic Cannabinoids: JWH-018, JWH-073, JWH-200,

## JWH-022, AM-2201, AM-2232, AM-1220, UR-144 and XLR-11 in oral fluid

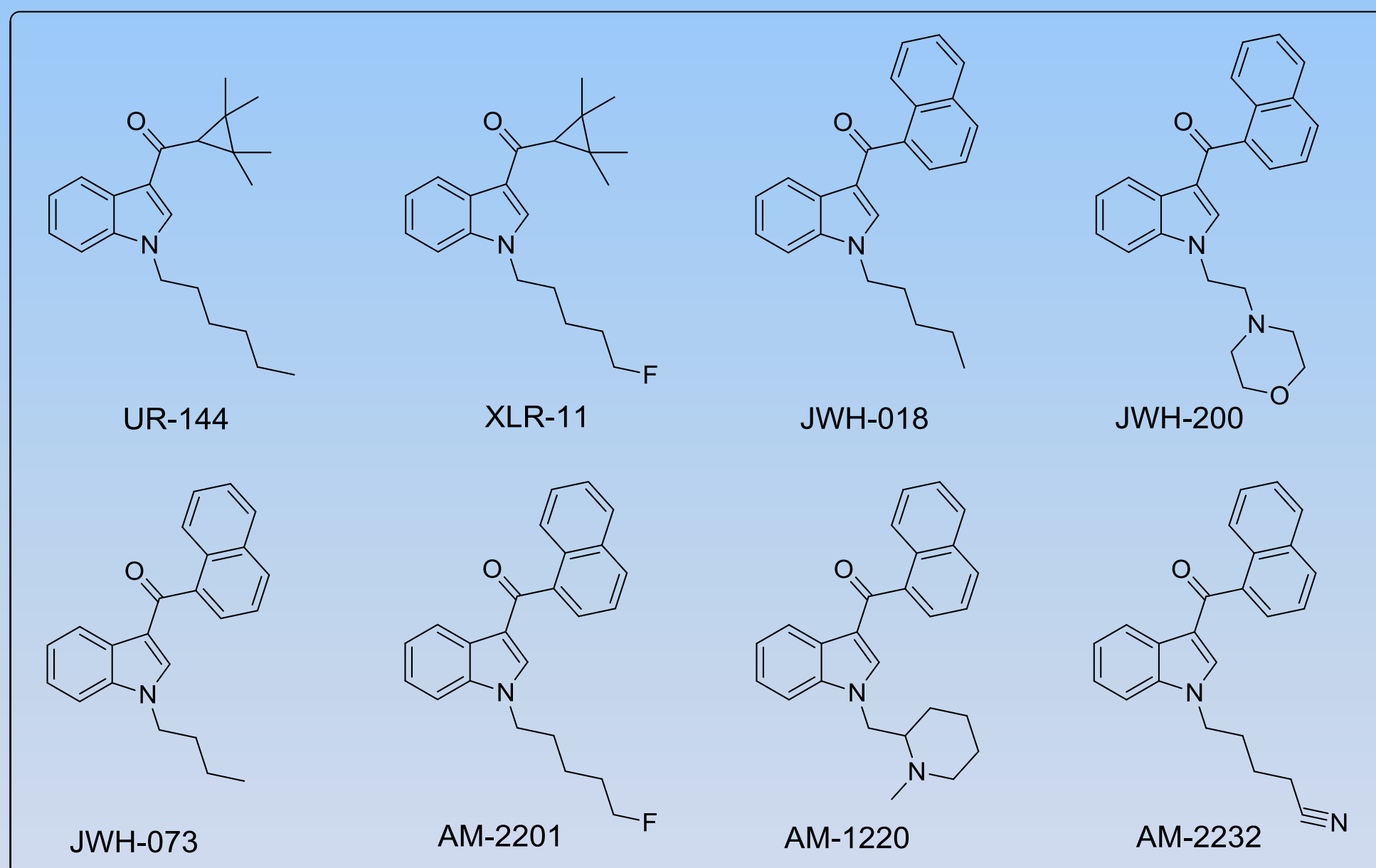
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### Background

Synthetic cannabinoids or “Spice” have emerged as potentially harmful drugs in recent years. Chemical modifications to the core indole ring structure have produced numerous compounds, capable of binding the CB<sub>1</sub> and CB<sub>2</sub> cannabinoid receptors in the brain. By June 2012, 15 “Spice” compounds had been placed on the DEA Sch I list, and by May 2013, 3 more compounds were placed in that category. EIA methods to detect these compounds in urine and blood have previously been developed, but so far detection in oral fluid has been limited to LC-MS methods.



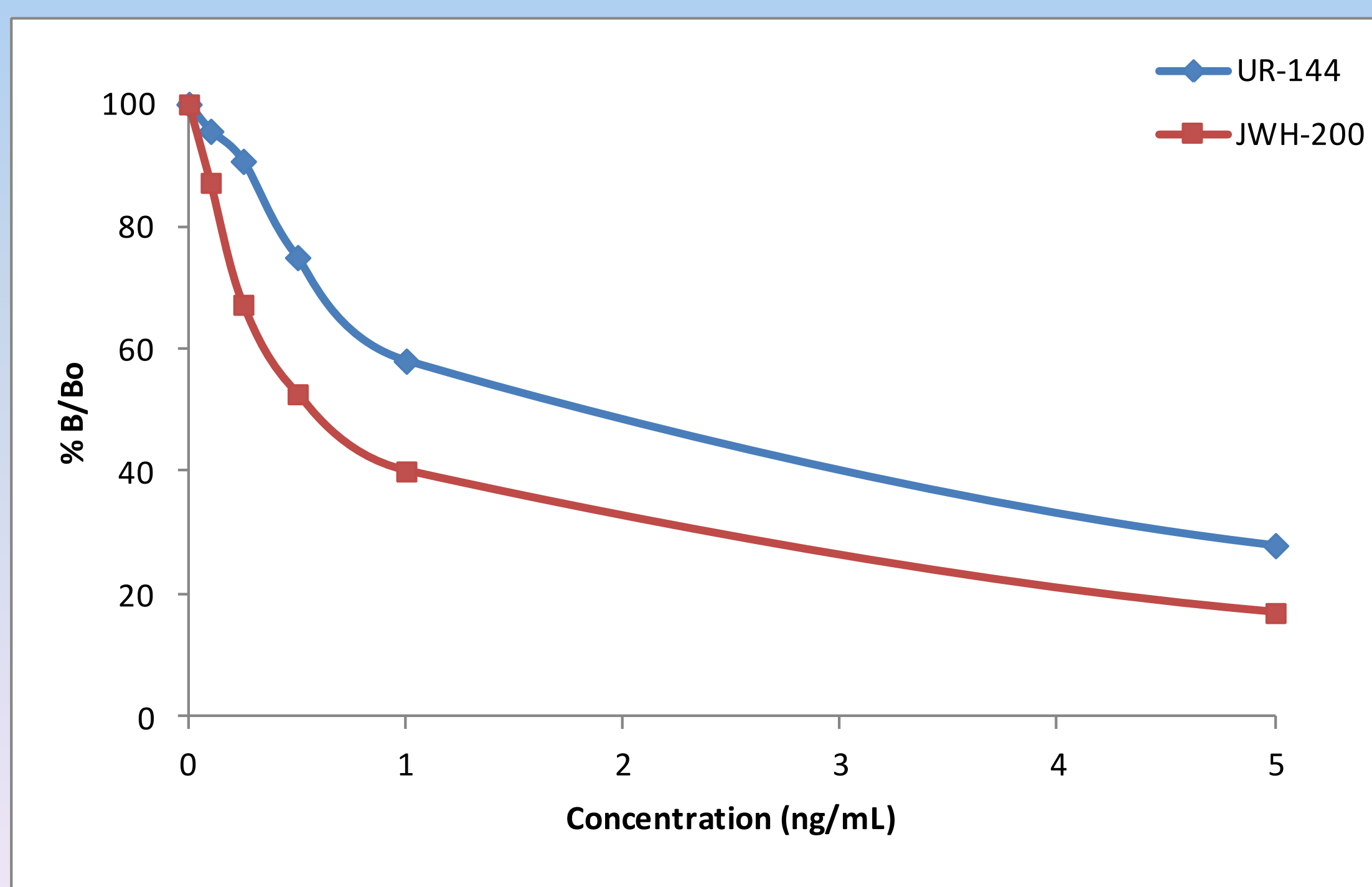
### Objective

- To develop methods to screen for JWH-018, JWH-073, JWH-200, JWH-022, AM-2201, AM-2232, AM-1220, UR-144 and XLR-11 in oral fluid.
- Two assays necessary, since it is virtually impossible to elicit a single antibody response to all such compounds.

### Methods

- Standard competitive binding ELISA protocol used for both assays.
- Assay sensitivity was achieved using 75 µL sample, with a critical **30 minute pre-incubation step**.
- 1 mL oral fluid sample was collected with Quantisal™ device and diluted with 3 mL stabilization buffer.
- Assay 1: JWH-200 calibrator **0.25 ng/mL** cutoff
- Assay 2: UR-144 calibrator **1 ng/mL** cutoff

### Results



### Cross-reactivity

#### Assay 1: Naphthoyl indole related compounds

Compound	Fortified conc (ng/mL)	JWH-200 (ng/mL)	% cross-reactivity
JWH-200	0.25	0.25	100
JWH-018	1.75	0.25	14.2
JWH-073	0.40	0.25	63.5
JWH-022	0.85	0.25	29.1
AM-1220	0.35	0.25	69
AM-2201	0.60	0.25	43
AM-2232	2.50	0.25	8.6
AM-2233	11.50	0.25	2.2
JWH-015	2.1	0.25	11.9
JWH-019	2.95	0.25	8.5
JWH-122	11	0.25	2.3
JWH-210	57	0.25	0.44
JWH-250	835	0.25	0.03
JWH-398	24	0.25	1.04
3-(1-naphthoyl)-1H-indole	1.9	0.25	13.5

#### Assay 2: Cyclopropanoyl indole related compounds

Compound	Fortified conc (ng/mL)	UR-144 (ng/mL)	% cross-reactivity
UR-144	1	1	100
XLR-11	0.15	0.91	607
AB-005	0.10	0.79	790
A-834735	0.075	0.90	1200
A-796260	0.07	0.88	1257
UR-144 N-pentanoic acid	0.04	0.76	1900
UR-144 N-(5-hydroxypentyl)	0.04	0.67	1675
UR-144 N-(5-hydroxypentyl)-β-D-glucuronide	0.1	0.92	920
XLR-11 N-(4-hydroxypentyl)	1	0.75	75

### Method Validation

96 specimens were collected using Quantisal™, of which 32 were screened with assay #1 and 64 with assay #2.

#### Assay #1: JWH-200

ELISA	LC-MS/MS	
	+	-
+	21	0
-	5	6

#### Assay #2: UR-144

ELISA	LC-MS/MS	
	+	-
+	23	0
-	1	40

4 specimens contained < 0.5 ng/mL AM-2201; 1 contained JWH-210

1 specimen contained < 0.3 ng/mL XLR-11

### Summary

These ELISA assays can be used for screening oral fluid specimens for the newer compounds: UR-144 and XLR-11, as well as JWH-018, JWH-073, JWH-200, JWH-022, AM-2201, AM-2232, AM-2233 and AM-1220, to indicate synthetic cannabinoid usage.

### References

- Detection of synthetic cannabinoids in oral fluid using ELISA and LC-MS/MS, Rodrigues, W.C.; Catbagan, C.; Rana, S.; Wang, G.; Moore, C. *J. Anal. Toxicol.* **2013**, *37*, 526-533.
- Synthetic cannabinoids in oral fluid, Coulter, C.; Garnier, M.; Moore, C. *J. Anal. Toxicol.* **2011**, *35*, 424-430.

**Disclosure:** Immunoanalysis Corporation manufactures and distributes the immunoassay products described in this presentation

