## WORK INSTRUCTIONS

#### 6.1.2 Confirmation of Cannabinoids in Blood and Urine

#### **REFERENCE MATERIAL**

#### **Calibrators and Controls**

- <u>Stock Solutions</u>
  1 mg/mL or 100 μg/mL single component cannabinoid-class reference solutions.
- <u>Calibrator/Control Working Solutions</u> The calibrator solutions must contain  $\Delta^9$ -THC, 11-nor- $\Delta^9$ -THC-9-COOH, 19-hydroxy- $\Delta^9$ -THC, Cannabinol, Cannabidiol
  - $\circ$  <u>1.0 µg/mL Target Mix in methanol</u>

Add  $10\mu$ L each (1mg/mL) or  $100\mu$ L (100 $\mu$ g/mL) Stock Solution to  $\cong$  6mL MeOH in 10mL ball flask *EXCEPT carboxy-THC*. QS with MeOH. Solution is stable for one-year when stored in the freezer.

 0.1/0.5 μg/mL Target Mix in methanol Add 1mL 1.0 μg/mL Target Mix + 50 μL (100 μg/mL) carboxy-THC stock solution to ≅ 8mL MeOH in 10mL ball flask. QS with MeOH. Solution is stable for one-year when stored in the freezer.

(NOTE: Alternative calibrator/control working solution preparation options are listed in Appendix 1 of the Analytical Method.)

#### • <u>Calibrator Preparation</u>

Add the volume of working calibrator working solution to appropriate tube as indicated below.

Sample Type	0.1/05 µg/mL	1.0 μg/mL	100 µg/mL	or
	Target Mix	Target Mix	c-THC stock	1.0/5.0 μg/mL
				Target Mix
Blank		-	-	-
1/5 ng/mL Carl	10 µL	-	-	-
2/10 ng/mL Cal 2	20 µL	-	-	-
5/25 ng/mL Cal 3	50 µL	-	-	-
10/50 mg/mL Cal 4	<b>C</b> 100 μL	-	-	-
25/125 ng/mL Cal 5	<b>2</b> 50 μL	-	-	-
50/250 ng/mL Cal	-	50 µL	2.5 μL	50 μL
00/500 ng/mL Cal 7	-	100 µL	5 µL	100 µL

#### • <u>Positive Control</u>

Negative urine or blood can be spiked with working solutions, but the compounds in that solution **cannot** be the same lot as was used for the calibrators. At minimum, the control must contain two compounds included in the scope of the method.

- For urine, as single positive control between the approximate concentrations of 10-75 ng/mL is acceptable.
- For blood, two positive controls are required. The low concentration control shall fall between the LOD and the next highest calibrator; the mid- or high-concentration control shall have an approximate concentration between the 4<sup>th</sup> and 7<sup>th</sup> calibrators.

#### **Conjugated Controls (Urine samples only)**

- Spiked Negative urine (must be the same lot for calibrators and controls) •
- Stock Solution • 100µg/mL Carboxy-THC Glucuronide
- *Working Glucuronide Solution (10ng/µL)* Add 10µL 100µg/mL Stock Solution to 990µL MeOH or Acetonitrile. Solution is stable for one week when stored under refrigeration.

#### **Internal Standard**

- Stock Solution 100  $\mu$ g/mL  $\Delta^9$ -THC-D3, 11-nor- $\Delta^9$ -THC-9-COOH-D3, 11-hydroxy- $\Delta^9$ -THC -D3, Cannabinol-D3. Cannabidiol-D3
- Working Internal Standard Solution (1.0 µg/mL ISTD mix in methanol Add 100µL of 100µg/mL stock solution to  $\cong$  9mL MeOH, in 10mL ball flask. QS with MeOH. procedures of the second secon Solution is stable for one year when stored under refrigeration.

#### **COMMENTS**

This method has instructions for the preparation of both urine and blood casework samples.

## WORK INSTRUCTIONS

# 6.1.2 Confirmation of Cannabinoids in Blood and Urine SAMPLE PREPARATION:



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#### 6.1.2 Confirmation of Cannabinoids in Blood and Urine

# **Revision History**

Revision No.	Issue Date	Revision/Comments
0	08/31/2015	Original Issue.
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