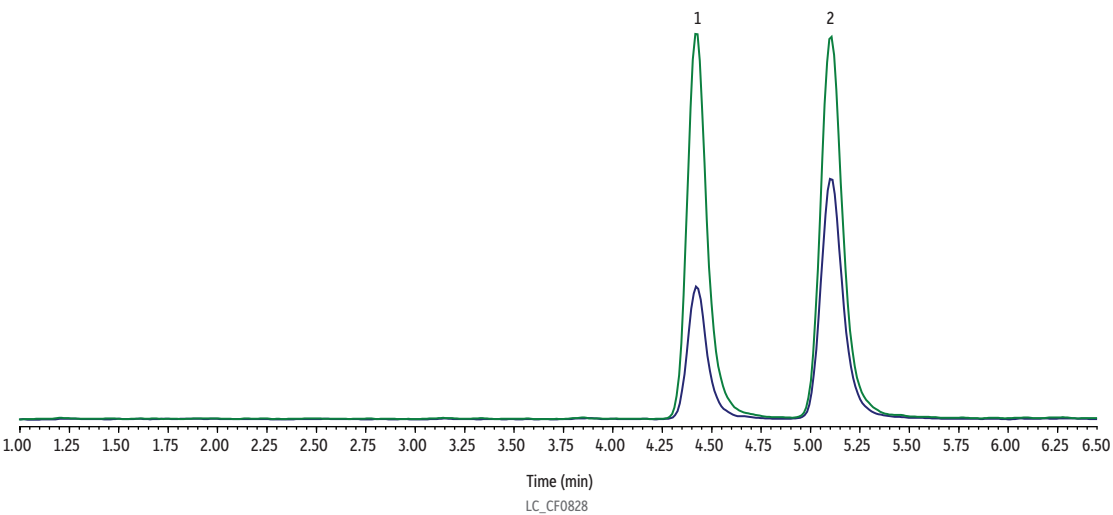


Δ8-THC-COOH and Δ9-THC-COOH Isomers in Urine on Raptor FluoroPhenyl



| Peaks          | tr (min) | Precursor | Product 1 | Product 2 |
|----------------|----------|-----------|-----------|-----------|
| 1. Δ8-THC-COOH | 4.44     | 345.1     | 327.0     | 299.2     |
| 2. Δ9-THC-COOH | 5.12     | 345.1     | 327.0     | 299.2     |

|                 |                                                                                                 |
|-----------------|-------------------------------------------------------------------------------------------------|
| Column          | Raptor FluoroPhenyl (cat.# 9319A1E)                                                             |
| Dimensions:     | 100 mm x 3 mm ID                                                                                |
| Particle Size:  | 2.7 μm                                                                                          |
| Pore Size:      | 90 Å                                                                                            |
| Guard Column:   | Raptor FluoroPhenyl EXP guard column cartridge 5 mm, 3 mm ID, 2.7 μm (cat.# 9319A0253)          |
| Temp.:          | 40 °C                                                                                           |
| Standard/Sample | (±)11-nor-9-carboxy-Δ-9-THC (Δ9-THC-COOH) (cat.# 34068)<br>Other compounds obtained separately. |
| Diluent:        | 40:60 Water:Methanol, both with 0.1% formic acid (v/v)                                          |
| Conc.:          | 50 ng/mL                                                                                        |
| Inj. Vol.:      | 1 μL                                                                                            |
| Mobile Phase    |                                                                                                 |
| A:              | Water, 0.1% formic acid                                                                         |
| B:              | Methanol, 0.1% formic acid                                                                      |

| Time (min) | Flow (mL/min) | %A | %B  |
|------------|---------------|----|-----|
| 0.00       | 0.8           | 35 | 65  |
| 6.50       | 0.8           | 35 | 65  |
| 6.60       | 0.8           | 0  | 100 |
| 7.50       | 0.8           | 0  | 100 |
| 7.60       | 0.8           | 35 | 65  |
| 8.50       | 0.8           | 35 | 65  |

|                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Max Pressure:      | 385 bar                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Detector           | Shimadzu 8045 MS/MS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Ion Mode:          | ESI+                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Instrument         | Shimadzu Nexera X2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Sample Preparation | Blank urine was spiked across the calibration range. Five hundred microliters of sample was added to a glass test tube. Fifty microliters of internal standard was added to each sample and vortexed. Prior to extraction, alkaline hydrolysis was performed on the samples by adding 40 μL of 10 N NaOH to each sample. Samples were capped, vortexed, and heated at 60 °C for 20 minutes. After cooling, 25 μL of glacial acetic acid was added to neutralize the pH of the samples. After hydrolysis, the samples were extracted by LLE. Five hundred microliters of HPLC grade water was added to each tube and vortexed. One hundred microliters of 10% acetic acid was added to each tube and vortexed. Two-and-a-half milliliters of 80:20 hexanes:ethyl acetate was added to each tube, capped, and vortexed until visibly combined. Samples were centrifuged at 2800 rpm for 15 minutes or until the two layers had completely separated. The supernatant was pulled off the top of each sample and transferred to a clean test tube. Samples were dried down under nitrogen. Samples were reconstituted in 100 μL of 40:60 water:methanol, both with 0.1% formic acid, and vortexed. Samples were transferred to 2 mL short-cap, screw-thread vials (cat.# 21143) with glass vial inserts (cat.# 21776) and capped with short-cap, screw-thread closures (cat.# 24498). |
| Notes              | The column was stored in 100% acetonitrile when not in use.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |