



## **Application Note: Analysis of COOH-THC in Urine (updated: 12/30/08)**

### **Product: DPX-RP (5 mL)**

#### **INTRODUCTION**

The most pharmacologically active constituent of marijuana is  $\Delta^9$ -tetrahydrocannabinol (THC). The pathway for metabolism and excretion of THC leads primarily to the formation of 11-OH-THC which further metabolizes to 11-COOH-THC (or simply COOH-THC). Unchanged  $\Delta^9$ -THC exists in the urine in trace amounts, and 11-OH-THC accounts for only 2% of the excreted dose. COOH-THC and its conjugates are the major urinary metabolites of THC, and exposure to marijuana is therefore determined by identification of COOH-THC and its conjugates in urine.

In order to analyze the total amount of COOH-THC, the water soluble conjugates are first hydrolyzed to form COOH-THC. Subsequently, the sample solution is extracted by liquid-liquid or solid-phase extraction (SPE) methods.

The goal of this research was development of a rapid, reliable and sensitive extraction method to analyze COOH-THC in urine. This goal was accomplished by incorporating Disposable Pipette Extraction (DPX) for the SPE procedure. DPX is unique in that it incorporates mixing of the sorbent with the sample solution, and this reduces the time required for extraction by eliminating (or minimizing) a conditioning step and reducing the volume of solvent required for elution.

This procedure is used to analyze 1 mL of urine using 5 mL DPX-RP tips. Applications for 2 mL of urine can be accomplished by simply scaling the volumes accordingly and following the same procedures.

#### **EXPERIMENTAL**

##### *Materials.*

1. 12 M KOH
2. 10 M HCl
3. 0.1 M HCl
4. acetone (optional)
5. acetonitrile (ACN)
6. 30% ACN in DI water
7. 50/50 ACN and ethyl acetate
8. 50/50 hexanes and ethyl acetate
9.  $d_3$ -COOH-THC (internal standard)
10. BSTFA (derivatizing reagent)



*Sample Preparation.*

1. Spike 1 mL of urine with internal standard (i.s.), making 10 ng/mL concentration.
2. Carefully add 0.10 mL of 12 M KOH.
3. Incubate for 20 minutes at 70 °C. Let cool to room temperature before extraction.
4. Carefully add 100 µL of 10 M HCl to sample solution.
5. Add 200 µL of 0.1 M HCl to sample, and make sure solution is acidic with pH paper.
6. Add 300 µL of ACN to sample and vortex mix.
7. Optional (recommended): Centrifuge to remove possible particulate material.

*DPX Extraction for best results (“top” elutions):*

1. Optional: aspirate 1 mL acetone (draw in air to mix), and dispense to waste; this wets the sorbent and removes any possible contamination of the sorbent.
2. Aspirate sample solution (draw in and mix with air), let set about 30 s, then dispense (to waste).
3. Add 2 mL 30% ACN/H<sub>2</sub>O to top of DPX tip and push through sorbent to waste with attached syringe (or pipette).
4. Add 2 mL 50/50 ACN/Ethyl Acetate to top of DPX tip and push through sorbent into small test tube.
5. Dry elution solvent to ~1 mL under N<sub>2</sub> and heat then transfer to a GC vial.
6. Take sample to dryness with N<sub>2</sub> and heat.

*DPX Extraction for easiest processing (“bottom” elutions):*

1. Optional: aspirate 1 mL acetone (draw in air to mix), and dispense to waste; this wets the sorbent and removes any possible contamination of the sorbent.
2. Aspirate sample solution (draw in and mix with air), let set about 30 s, then dispense (to waste).
3. Aspirate 2 mL 30% ACN/Water and mix with air; dispense to waste.
4. Aspirate 2 mL 50/50 ACN/Ethyl Acetate and mix with air; dispense into small test tube.
5. Optional: repeat elution step 4.
6. Dry elution solvent to ~1 mL under N<sub>2</sub> and heat then transfer to a GC vial.
7. Take sample to dryness with N<sub>2</sub> and heat.



#### *Derivatization:*

1. Add 75  $\mu$ L of 50/50 Hexanes/Ethyl Acetate and 75  $\mu$ L of BSTFA to GC vial and cap under  $N_2$ .
2. Heat for 20 minutes at 80-85  $^{\circ}$ C.
3. Remove from heat, cool, and transfer to a GC vial insert.
4. Cap vial and place on autosampler.

#### GC/MS analysis:

Instrumentation: Agilent Technologies GC/MS: 6890 GC and 5975 Mass Selective Detector.

Column conditions: 30m DB-5MS, 0.25mm I.D., 0.25 $\mu$ m film thickness.

Helium carrier gas at 1 mL/min @ constant flow. Injection temperature was set at 280  $^{\circ}$ C and detector was set at 300  $^{\circ}$ C. The oven temperature program was set at an initial temp. of 200  $^{\circ}$ C for 1 min., then ramped at 20  $^{\circ}$ C/min to 300  $^{\circ}$  and held for 6 min. for a total run time of 12.0 min.

*Selected ion Monitoring (SIM) Parameters:* 371, \*473, 488 for derivatized COOH-THC and \*476 for i.s. (\* denotes target ions for quantitation). The dwell time was 20 ms for each ion.

## **RESULTS AND DISCUSSION**

Two methods of performing the DPX extraction are delineated. The first method, listed as “best results”, provides cleaner extracts and higher recoveries because the wash and elution solvents are being passed through the sorbent in one direction to thoroughly elute matrix components and analyte, respectively. To perform the extraction with “top” washes and elutions, the syringe (or pipette) must be attached and detached multiple times to perform the extractions. This makes the method more tedious to perform. However, it should be noted that this “tedious” aspect is not an issue with semi- or automated DPX extractions, which are highly recommended for use.

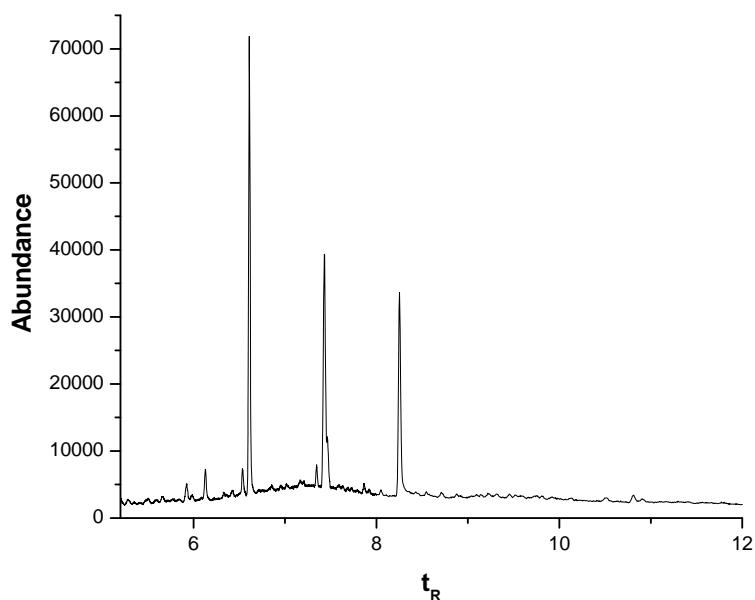
The second method involves aspirating and dispensing all solutions from test tubes from the narrow bottom end of the DPX tip, analogous to using standard pipette tips. This method is much faster, taking less than 3 minutes to perform. The results obtained from this method are very similar, except the recoveries are slightly less. With elutions from the “bottom”, two separate elution steps are recommended to obtain high recoveries.

All results reported below were obtained using the first method (“top” elutions). With semi-automation using the DPX extractor or automation using the GERSTEL MPS-2, the “top” washes and elutions are readily performed, providing non-tedious and rapid

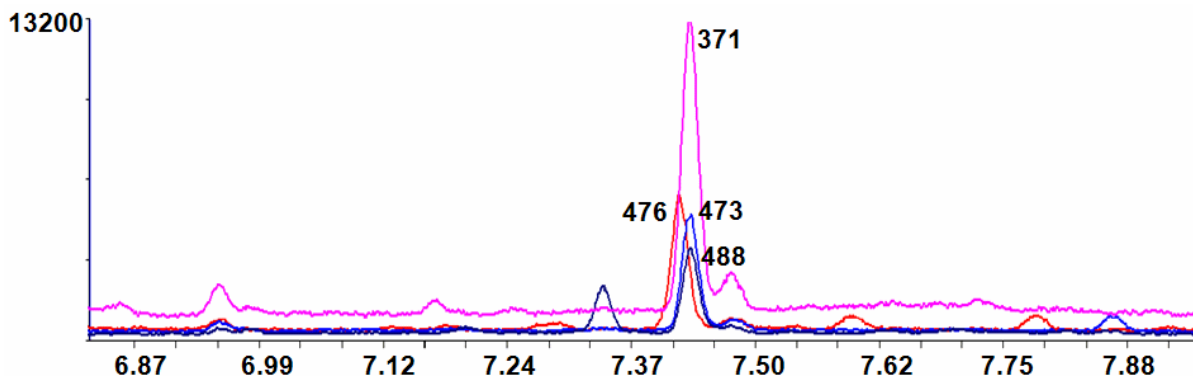


extractions with optimal results. With semi-automation or automation, the analysis is unsurpassed in terms of speed and ease of use.

The chromatograms obtained by DPX extractions are characterized with little to no interferences. The total ion chromatogram of the SIM analysis is shown in Fig. 1. The peak at approximately 7.4 min. is the analyte, with no interferences noted.



**Figure 1:** TIC of COOH-THC extracted from hydrolyzed urine using RP-5. The COOH-THC peak is at app. 7.4 min, with little to no interferences noted.



**Figure 2.** EIC of COOH-THC taken from 10 ppb reproducibility study

The extracted ion chromatogram of 10 ng/mL COOH-THC is shown in Fig. 2. The signal-to-noise at this level is over 100 for the 488 ion, the lowest intensity qualifier ion. The LOD is estimated to be approximately 0.5 ng/mL based on this chromatographic data using a S/N ratio of 5.



The calibration plot of COOH-THC extracted from urine is shown in Fig. 3. Data was recorded at concentrations ranging from 1 ng/mL (using six replicates) up to 100 ng/mL. The plot is linear with a squared linear regression value of 0.9982. From this data, the LOD is calculated to be 0.4 ng/mL, which is shown in Table 1. The %RSD was found to be just 1.8%, indicating very good reproducibility.

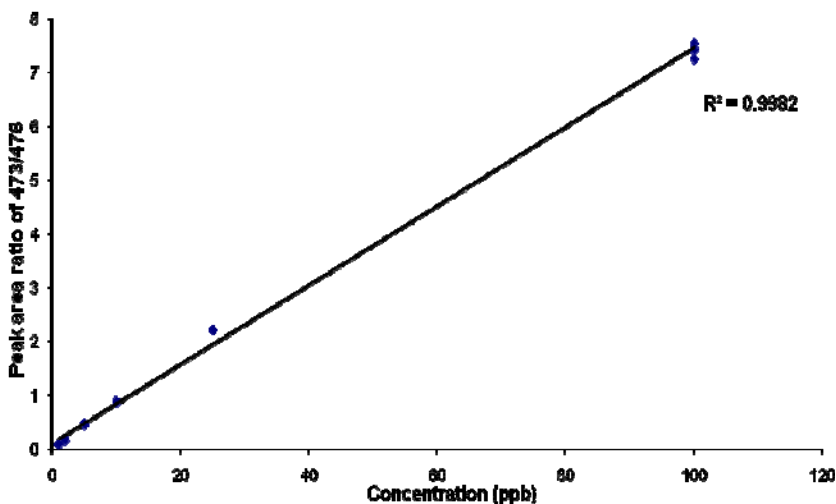


Figure 3. Calibration plot of COOH-THC extracted from hydrolyzed urine using 5 mL DPX-RP.

Table 1: Validation results for COOH-THC. Concentration used for recovery and reproducibility studies is bolded. LOD based on ion 473 at a concentration of 1 ppb.

Comp.	Int. std.	Target (m/z)	Qual.	Conc. (ng/mL)	Recov.	%RSD	R <sup>2</sup>	LOD (ng/mL)
COOH-THC-2TMS	d <sub>3</sub> -COOH-THC-2TMS	473 (476)	371, 488 (374)	1, 2, 5, 10, 25 & 100	85.8%	1.8	0.9982	0.4

The recovery was determined in a separate study by adding the deuterated analogue of COOH-THC to the eluent after the DPX extraction of COOH-THC (ie, external standard). The recovery was determined to be 85.8%. It may be possible to improve the recovery by incorporating an anion exchange mechanism as opposed to reversed phase, but the RP sorbent provides high recoveries and clean extracts, as shown in this study. The DPX-RP tips are much easier to utilize and provide ample recoveries that it should not be necessary to use an alternative extraction method.

## CONCLUSIONS

This DPX method has been validated for use in forensic case samples for the analysis of COOH-THC in urine. This method is very reliable, fast, and easy to perform.



## REFERENCES

J. L. Schroeder, L. J. Marinetti, R. K. Smith, W. E. Brewer, B. L. Clelland and S. L. Morgan, "The Analysis of ( $\delta$ 9)Tetrahydrocannabinol and Metabolite in Whole Blood and 11-Nor-( $\delta$ 9)-Tetrahydrocannabinol-9-Carboxylic Acid in Urine Using Disposable Pipette Extraction with Confirmation and Quantification by Gas Chromatography-Mass Spectrometry", *J. Anal. Toxicol.*, **32**, 659 (2008).

J. L. Schroeder, L. J. Marinetti, W. E. Brewer, B. L. Clelland and S. L. Morgan, "Rapid Analysis of THC and Metabolites Using Disposable Pipette Extraction", Abstract at the American Association of Forensic Sciences, 2007, San Antonio, TX.