# Determination of Δ9-Tetrahydrocannabinol and 11-Nor- Δ9-Tetrahydrocannabinol-9-carboxylic acid in Human Urine Using Automated ITSP Solid Phase Extraction and Liquid Chromatography Mass Spectrometric Detection

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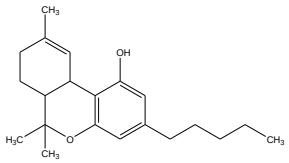
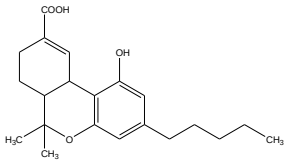
## Abstract

A quantitative analytical method has been developed for the determination of 9-Tetrahydrocannabinol (THC) and 11-Nor- D9-Tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in human urine. THC-COOH is the primary metabolite of THC found in urine and is the species used to confirm THC use (15 ng/mL confirmatory cutoff for workplace drug testing)[1]. Standards of THC and THC-COOH were prepared in both solvent and urine and the analytes were quantified using reversed phase ($C_{18}$) ITSP solid phase extraction (SPE) and liquid chromatography mass spectrometry (LC-MS). Single ion monitoring (SIM) was used for the analysis and deuterated analogs of the two analytes were used for quantitation.




THC
Chemical Formula: $C_{21}H_{30}O_2$
Exact Mass: 314.2 Da




THC-COOH
Chemical Formula: $C_{21}H_{28}O_4$
Exact Mass: 344.2 Da

## Experimental

### Sample Preparation

THC and THC-COOH standards and deuterated analogs were obtained from Cambridge Isotope Laboratories, Inc. Two sets of standards were prepared, one set in 70:30 Water:Acetonitrile and one set in urine. Calibration standards were prepared at nominal concentrations of 1, 5, 9, 23, 45, 91, 455 and 909 ng/mL with a constant concentration of the deuterated analogs at 45 ng/mL.

### ITSP SPE Method

ITSP Cartridges: SSPE µLplate w/C18 10mg (Product No.: 07-C1810-20A)

A CTC Analytics PAL HTS sample handler was used to prepare the samples. The PAL was configured with a 100 µL L-Mark syringe and two tray holders. Each tray holder held 2 microplates, one of which was designed to hold the ITSP hardware kit (Product No.: 07-ITSP-HW). The extraction protocol was as follows:

| Step | Solvent | Volume (µL) | Flowrate (µL/sec) |
|---|---|---|---|
| Wash | B | 80 | 10 |
| Condition | A | 80 | 10 |
| Load | Sample | 100 | 5 |
| Wash | C | 100 | 5 |
| Aspirate | Air | 80 | 20 |
| Elute | D | 100 | 5 |
| Aspirate | Air | 80 | 20 |

Solvent A: Water
Solvent B: Acetonitrile
Solvent C: 40% Acetonitrile in Water
Solvent D: 100% Isopropanol

Samples were prepared off-line and analyzed separately with the following method:

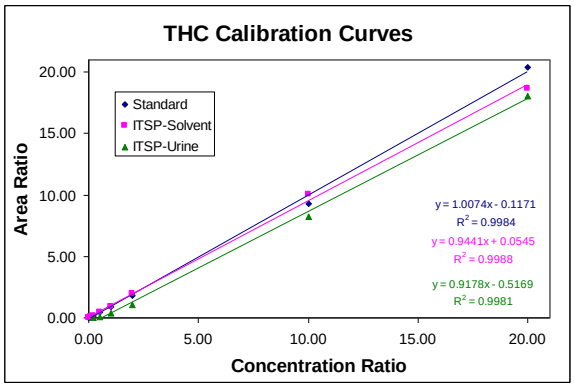
### Analysis Method

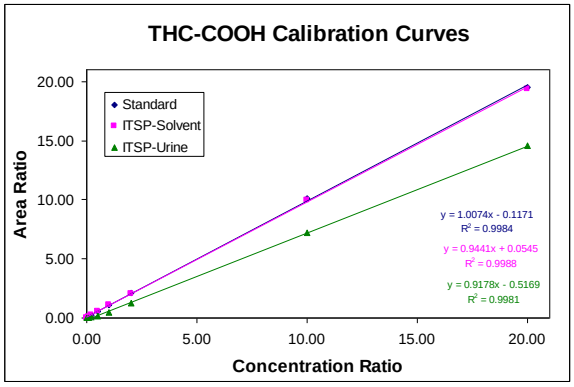
| | |
|---|---|
| Instrument: | Sciex API3000 Triple Quadrupole with Shimadzu Prominence HPLC System |
| Solvent A: | Water with 0.05% TFA |
| Solvent B: | Methanol with 0.05% TFA |
| Column: | Shimadzu Shim-pack XR-ODS, 3.0x30mm, 2.2 µm particles |
| Column Temp.: | 50 °C |
| Flowrate: | 1.5 mL/min |
| Gradient: | 0.00 min (5% B), 3.0 min (100% B), 3.30 min (100% B), 3.35 min (5% B) |
| Ionization Mode: | Positive and Negative Ion Electrospray |

MRM Channels: m/z +315.2/193.2 (THC)
m/z +318.2/196.2 (THC-d3)
m/z -343.2/299.1 (THC-COOH)
m/z -346.2/302.1 (THC-COOH-d3)

## Results

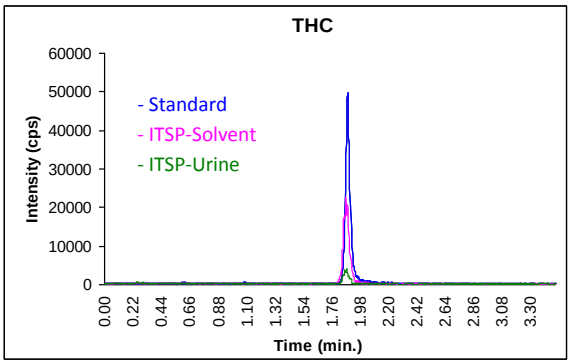
Standards of THC and THC-COOH were prepared in solvent and in urine over the range of 1 ng/mL – 909 ng/mL and processed with the ITSP cleanup procedure. The calibration curves that were obtained for each are shown below plotting the analyte to internal standard area ratio versus the concentration ratio.

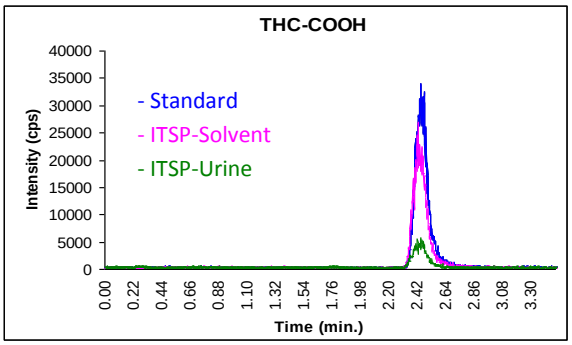



The detection limit for THC was 1 ng/mL for the unprocessed Standards as well as for the ITSP processed Solvent standards. However, the lowest detectable level in the ITSP processed urine was 9 ng/mL. For the THC-COOH metabolite, a similar detection limit was observed for the unprocessed and ITSP processed Standards in solvent and was 5 ng/mL in ITSP processed urine. Although the response was linear over the range of detectable standards, there appears to be a negative bias in the urine matrix. The recovery of THC and THC-COOH in ITSP processed solvent ranged from 36-76% and 65-83%, respectively. Lower recoveries were observed from the ITSP processed urine samples ranging from 0-23% and 0-38% for THC and THC-COOH, respectively.

Example TIC chromatograms of the MRM channels for THC (m/z 315.2/193.2) and THC-COOH (m/z 343.2/299.1) for the 45 ng/mL (Conc. Ratio 1.00) standards in both solvent and urine with ITSP cleanup are shown below.




## Conclusions

A method for the determination of THC and THC-COOH in human urine has been developed using reversed phase ($C_{18}$) ITSP solid phase extraction (SPE) for sample cleanup and LC-MS/MS for detection. An alternate ITSP packing or elution solvent system may be required to improve recovery and reduce the urine matrix affect.

[1]Mandatory Guidelines for Federal Workplace Drug Testing Programs, Federal Register notice published April 13, 2004 (69 FR 19644) effective Nov. 1, 2004.