Analytical Challenges in the Detection of Δ⁸-Tetrahydrocannabinol and Its Metabolites in Dried Urine Spots

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ABSTRACT

The emergence of products containing Δ⁸-tetrahydrocannabinol (Δ⁸-THC) has created the need for methods for its analysis and of its metabolites in toxicological specimens. Considering urine is routinely submitted for toxicological analysis, in this study, dried urine spots (DUS) are explored as a microsampling strategy to facilitate sample collection, preparation and analysis. The preliminary findings obtained with this pilot study revealed that Δ⁸-THC and its metabolites can be detected in DUS. However, this approach presents recovery and matrix effects limitations.

INTRODUCTION

There has been an increased dissemination of hemp-derived products containing cannabinoids such as Δ⁸-tetrahydrocannabinol (Δ⁸-THC). Δ⁸-THC is similar in structure to its isomer Δ⁹-THC. Given its occurrence in toxicological samples reported in the literature, there is a need for analytical methods. An alternative sampling technique gaining attention in forensic toxicology is dried urine spots (DUS). DUS provides the ability for easy storage and transportation as well as improved stability of analytes.

OBJECTIVE

The aim of this study was to investigate the extraction efficiency and matrix effects observed in the analysis of Δ⁸-THC and its metabolites in DUS using liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS).

MATERIALS & METHODS

Sample Preparation

An extraction workflow was developed based on previously published literature[1]:

1. Two DUS (25 µL/spot) were placed into a glass tube and 1 mL of methanol with 50 µL of 1 ng/µL Δ⁸-THC-d_3 was added.
2. Samples were vortexed and sonicated for 20 min.
3. The methanol extract was transferred to a clean tube and evaporated to dryness under nitrogen flow at 40°C.
4. Aliquots of 0.5 mL of 0.1 M acetate buffer solution (pH 4.5) were added to the glass tubes still containing the DUS.
5. The sample was sonicated for 20 min, and 1.5 mL of 90:10 (v/v) hexane/ethyl acetate were added.
6. Samples were extracted for 20 min at room temperature, followed by centrifugation for 10 min at 2500 rpm.
7. The organic layer was removed and transferred into the same glass tube where the methanol solution was evaporated in.
8. The supernatant was evaporated to dryness nitrogen flow at 40°C.
9. The extracts were reconstituted with 100 µL of mobile phase at initial conditions and injected (10 µL) into the LC-MS/MS.

RESULTS & DISCUSSION

Method Development and Optimization

- A chromatographic method was developed adapted from the literature[2].
- An acceptable separation was observed for the isomeric pairs (Figures 1 and 2), however, a recommended acceptance criteria is to compare the retention times of the analyte to the deuterated internal standard, for an additional level of confirmation.

Sensitivity Studies

- The greater the number of spots used per sample, the greater the peak area (Figure 3).
- Two spots per sample were selected considering the implementation in routine analysis.
- Three different concentrations were investigated as potential limits of detection (10, 25 and 50 ng/mL). Only DUS samples at 50 ng/mL met al the positive identification criteria.

Extraction and Matrix Effects Studies

- No differences in analytical response between overnight and 2h drying time (Figure 4).
- Recoveries ranged from 31.9 to 65.2% (at 50 ng/mL) and from 27.4 to 49.3% (at 200 ng/mL).
- Matrix effects (single source) were acceptable for Δ⁸-THC and 11-OH-Δ⁸-THC (within 20%) but unacceptable for Δ⁸-THC glucuronide and COOH-Δ⁸-THC at 50 and 200 ng/mL.

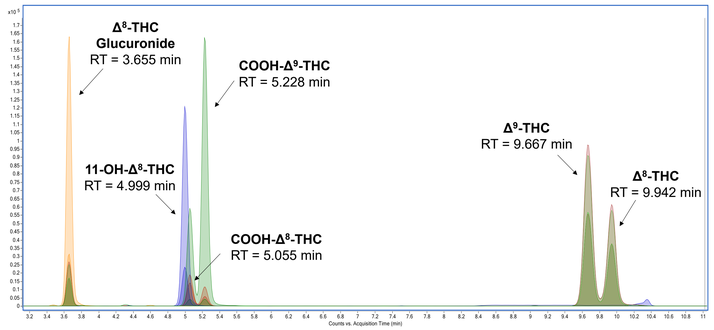


Figure 1. Optimized chromatographic separation of all analytes.

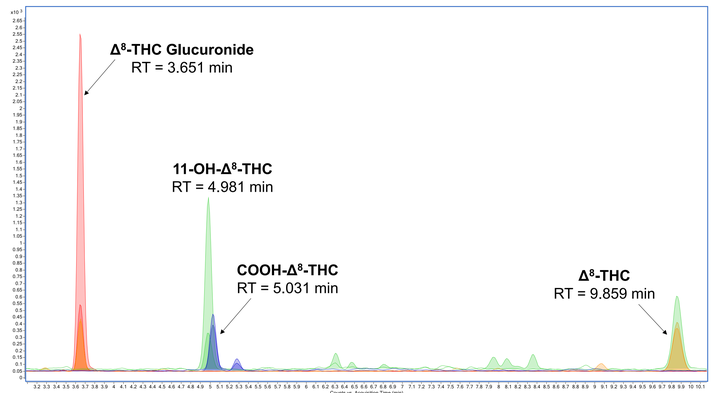


Figure 2. Chromatogram of a DUS sample containing target analytes at 50 ng/mL.

Stability Studies

- The stability of the target cannabinoids appears to be inversely proportional to storage time. As shown in Figure 5 for Δ⁸-THC, the longer cannabinoids remain on the paper surface, the more adsorbed they become resulting in a time-dependent recovery.

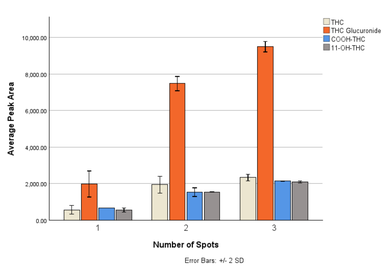


Figure 2. Average peak area (n=2) for DUS using 1, 2, and 3 spots per sample. SD: standard deviation.

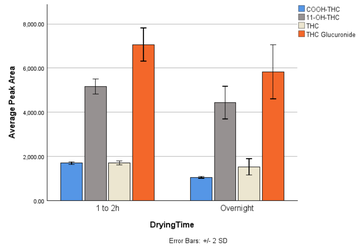


Figure 3. Average peak area (n=3) for DUS at 50 ng/mL dried overnight or for 1 to 2h. Each condition was performed on a different run (day, not sequentially), in triplicate.

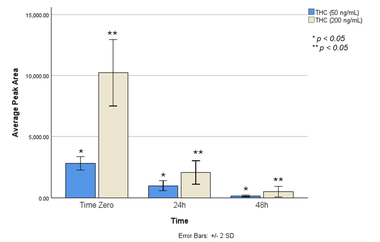
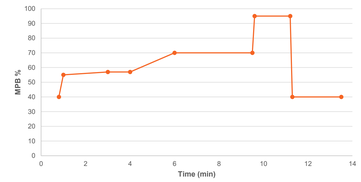


Figure 4. Average peak area (n=5) for Δ⁸-THC in DUS at 50 ng/mL (LQC and 200 ng/mL (HQC), analyzed at time zero and after 24h and 48h of storage at room temperature.

MATERIALS & METHODS

Instrumental Analysis

Instrumentation	Agilent 1290 Infinity II Liquid Chromatograph coupled to Agilent 6470 triple quadrupole MS
Column	Agilent Poroshell 120 EC-C18 (2.1 x 100 mm, 2.7 µm) with matching guard column, at 35 °C.
Mobile Phase	Water (MPA) and acetonitrile (MPB) both containing 0.1% formic acid
Gradient Elution Flow Rate: 0.5 mL/min	
MS Parameters Positive ESI	Gas Temperature: 350 °C Gas Flow: 11 L/min Nebulizer: 35 psi Sheath Gas Heater: 400 °C Sheath Gas Flow: 12 L/min Capillary: 4500 V Nozzle Voltage: 2000 V

CONCLUSIONS

- Our preliminary findings indicate low recovery and matrix effects in DUS as main limitations.
- The stability of the target analytes in DUS demonstrated a time-dependent decrease after 24h and 48h at room temperature, suggesting that the extraction efficiency is diminished over time.
- This study demonstrated that Δ⁸-THC and its metabolites can be detected in DUS (> 50 ng/mL).
- Future investigations into the matrix effects, long-term stability and recovery beyond 48h and the possibility to incorporate conjugate hydrolysis are recommended.

DISCLOSURE

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

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2. Reber, J.D., Karschner, E.L., Seither, J.Z., Knittel, J.L., Dozier, K. V and Walterscheid, J.P. (2022) An Enhanced LC–MS-MS Technique for Distinguishing Δ8- and Δ9-Tetrahydrocannabinol Isomers in Blood and Urine Specimens. Journal of Analytical Toxicology, 46, 343–349.

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