



Application Note: Automated DPX of THC and Metabolites in Blood (updated: 3/16/09)

Product: DPX-RP (1 mL, TA)

INTRODUCTION

The analysis of tetrahydrocannabinol (THC) and its metabolites, hydroxy-THC (OH-THC) and carboxy-THC (COOH-THC), in whole blood is one of the most tedious and time consuming methods in forensic toxicology. The reason for the analytical challenge is because limits of detection of 1 ng/mL for THC are required for this complex sample matrix. Most solid-phase extraction (SPE) methods require separate chromatographic analyses of THC and COOH-THC due to possible interfering sample matrix components, making the extractions and chromatographic analyses even longer than most methods.

Recently, Disposable Pipette Extraction (DPX) has been found to be a rapid and efficient SPE method for the analysis of THC and metabolites in whole blood.¹ The sorbent is loosely contained inside the pipette tip, and the sample solution is introduced through the bottom of the tip along with air to mix the analyte with the sorbent to provide efficient extractions without concerns of channeling or solution flow rates. Less solvent is required because conditioning steps are not required, which provides for much faster extractions. Using reverse phase DPX extraction mechanisms (DPX-RP), the GC/MS analysis has been shown to be efficient for the simultaneous extraction and analysis of THC and its metabolites.

This research² was performed to develop the fastest and least tedious method for the GC/MS analysis of THC and its metabolites from whole blood. The only manual requirement for this analysis involved spiking the samples with internal standard, protein precipitation with acetonitrile, and centrifugation. The rest of the method was completely automated, including extraction, concentration and chemical derivatization.

In this study, the DPX procedure was completely automated using a dual rail GERSTEL MPS-2 instrument. With this instrument, the automated extractions provide high recoveries and clean extracts because wash and elution solvents are introduced into the top of the DPX tips. This ensures a more thorough removal of sample matrix components and a more complete elution of analyte. To improve throughput, a GERSTEL cooled injection system (CIS) was utilized to simultaneously concentrate (solvent evaporate) and derivatize the analyte "in the inlet".

It was determined that BSTFA provided the best efficiency for "inlet derivatization" of THC and OH-THC; however, the efficiency and reproducibility for "inlet derivatization" of COOH-THC was much better using MTBSTFA. Hence, both derivatizing reagents were combined and used for "inlet derivatization" of THC and its metabolites. Limits of detection were found to be approximately 0.5 ng/mL for THC and OH-THC, and less than 2 ng/mL for COOH-THC. The coefficients of variation for the analysis of all 3 analytes were less than 5 % at 5 ng/mL concentrations for THC and OH-THC and 20 ng/mL for COOH-THC.



EXPERIMENTAL

Materials

BSTFA and MTBSTFA were purchased from Sigma Aldrich. Ampoules of COOH-THC (0.1 mg/mL), COOH-THC- d_3 (0.1 mg/mL), OH-THC (0.1 mg/mL), OH-THC- d_3 (0.1 mg/mL), THC (0.1 mg/mL), and THC- d_3 (0.1 mg/mL) were purchased from Cerilliant (Round Rock, TX). A working standard solution for each analyte was prepared by diluting the standard to 10 $\mu\text{g/mL}$ in acetonitrile.

Sample preparation: Dual rail GERSTEL MPS-2

Initial Sample Preparation

- Deuterated internal standards (d_3 -THC, d_3 -OH-THC and d_3 -COOH-THC) were added to 0.5 mL of blood samples resulting in concentrations of 5 ng/mL each.
- The samples were protein precipitated using 0.75 mL acetonitrile, centrifuged, and the resulting supernatants were transferred to clean, labeled sample tubes.
- 1 mL of deionized water and 50 μL of 0.1 M HCl were added to each tube.
- All tubes were then placed onto the GERSTEL MPS-2 sample tray.

Automated DPX

Automated DPX was performed using 1 mL DPX-RP-TA (reversed phase) tips with GERSTEL Transport Adaptor from DPX Labs, LLC.

- Multiple extractions (3 total) of approximately 0.7 mL each was performed with the GERSTEL MPS 2.
- After extracting the analyte from the sample solutions, a wash step was performed by adding 0.5 mL of 10% acetonitrile in water to the top of the DPX tip and dispensed to a waste container.
- Elution of the analytes was accomplished using 0.7 mL of CH_2Cl_2 from the top of the DPX tip into a clean GC vial.
- The total extraction time was approximately 6 minutes per sample.

Instrumentation.

Analyses were performed using a 6890N GC equipped with a 5975 (inert XL) mass selective detector (Agilent Technologies), PTV inlet (CIS 4, Gerstel) and MPS 2 Prepstation with DPX option.



Analysis Conditions

Column: 30 m HP-5MS (Agilent) di = 0.25 mm, df = 0.25 μ m
 Pneumatics: He, Pi = 16.1
 Constant flow = 1.5 mL/min
 Oven: 100° C (1.5 min), 40° C/min, 200° C;
 8° C/min, 240° C (1 min); 10° C/min, 300° C (3 min)
 Total Runtime: 19 min
 PTV 1.0 min solvent vent (150 mL/min)
 80° C (1 min), 12° C/min, 280° C (3 min)
 Splitless
 MSD SIM mode

Analyte	Retention Time (min)	Target Ions (m/z)	Qualifier Ions (m/z)
THC-TMS	9.68	386	303, 371
THC-d ₃ -TMS	9.66	389	306, 374
OH-THC-2TMS	12.11	371	473, 488
OH-THC-d ₃ -2TMS	12.09	374	476, 491
COOH-THC-TMS/TBDMS	14.83	371	473, 515
COOH-THC-d ₃ -TMS/TBDMS	14.81	374	476, 518

Automated injection for concentration and derivatization

The derivatizing reagent contained 33% BSTFA and 33% MTBSTFA in acetonitrile. A “sandwich” injection of derivatizing reagent and eluent was performed by the left rail of the MPS 2 by first aspirating 20 μ L of derivatizing reagent, then aspirating 20 μ L of air, then aspirating 50 μ L of eluent. The injection speed was 1.32 μ L/s, allowing the solvent to evaporate and concentrate the analyte inside the injection port.

RESULTS AND DISCUSSION

For analysis of THC and metabolites in whole blood, the problem is exacerbated because high sensitivity is required (LOD of 1 ng/mL for THC and OH-THC, 2 ng/mL for COOH-THC),



whole blood extracts contain possible interferences, and the analytes require chemical derivatization. In order to maintain “in-line processing”, the GERSTEL cooled injection system (CIS-4) was used to simultaneously inject, concentrate and derivatize the analyte during the injection process.

The DPX extraction utilized 1 mL DPX-RP tips which were fitted with “transport adaptors” (Figure 1) for automation with the dual rail GERSTEL MPS-2 (Figure 2). The advantage of the use of the transport adaptors is that wash and elution solvents can be introduced into the “top” of the DPX tip, and this provides cleaner extracts and higher recoveries. It is important that the extracts are clean in order to prevent buildup of residue in the inlet liner which could have deleterious effects on subsequent chromatographic analyses.

Initially, the derivatizations utilized BSTFA. However, it was found that BSTFA seemed to provide varying degrees of derivatization efficiency for COOH-THC in particular, depending on the manufacturer and lot for the BSTFA reagents. This irreproducibility was observed for both on-column (“inlet”) and conventional “off-line” derivatization procedures. Use of MTBSTFA seemed to circumvent this problem, but the sensitivity for the analysis of THC and OH-THC was much worse than with BSTFA. We found through a series of studies that we could actually combine the two reagents and provide high derivatization efficiencies for all three analytes. By using both derivatizing reagents, we found that THC and OH-THC were derivatized with BSTFA, and COOH-THC was derivatized with both BSTFA and MTBSTFA.

The results of the study indicate good reproducibility and recovery (Table 1). It should be noted that the protein precipitation causes some loss of the analytes, so the actual extraction efficiency of DPX is better than what is indicated. There are three factors that limit the DPX extraction efficiency. The first is that the solution pH could be lowered further by addition of a strong acid to improve the recovery of COOH-THC, but this creates interference problems for the analysis of THC. A second factor is that CH₂Cl₂ was used for elution, and this is not the most suitable solvent for COOH-THC. However, this solvent is ideal for use with LVI and performed the best efficiency for “inlet derivatization”. The third factor is the volume of sample extractions could be reduced to provide higher recovery. For example, multiple extractions of just 0.5 mL instead of 0.7 mL provided higher recovery, but this also meant more extraction steps and hence more time for extraction.

The most important results from this study are the reproducibility and limits of detection (LOD), which are shown in Table 2. The results show very good reproducibility based on the calibration plots (Fig. 3-5), with % C.V. of less than 5 % for all three analytes. The LOD was found to be 0.5 ng/mL for both THC and OH-THC, based on a signal-to-noise (S/N) of 5 for the lowest intensity qualifier ion. In Fig. 6, the qualifier ion of 303 has a S/N of approximately 10 at 0.5 ng/mL, clearly showing that this analyte is readily detected at this low concentration.

For OH-THC (Fig. 7), the qualifier ion of 474 has a S/N of about 8 at 0.5 ng/mL. The target ion, however, has a much greater S/N and could be detected at concentrations less than 0.1 ng/mL. It is noted that there is some intensity for the 372 ion being contributed for *d*₃-OH-THC, and it is readily apparent at the low concentration of 0.5 ng/mL. If it is deemed necessary to have a third



qualifier ion for OH-THC, then a lower concentration of 2 ng/mL for *d*₃-OH-THC (instead of 5 ng/mL) should make it possible to detect this ion without interference at the LOD of 0.5 ng/mL.

The detection of COOH-THC was problematic when trying to derivatize with BSTFA, which seemed to be due to the quality of the commercial derivatizing reagents. This was not the case with MTBSTFA, but this reagent did not provide results as good as with THC and OH-THC. By combining MTBSTFA and BSTFA, we could reproducibly detect COOH-THC-TMS/TBDMS without any apparent difficulties. In Fig. 8, the extracted ion chromatogram of COOH-THC-TMS/TBDMS is shown at 2 ng/mL.



Table 1. Recovery data for THC and its metabolites.

compound	concentration	Recov.
THC	5 ng/mL	51%
OH-THC	5 ng/mL	59%
COOH-THC	20 ng/mL	41%

Table 2. Reproducibility and LOD data.

compound	%C.V.	LOD ng/mL	LOQ ng/mL
THC	2.0	0.5	1.0
OH-THC	3.2	0.5	1.0
COOH-THC	3.1	2	2

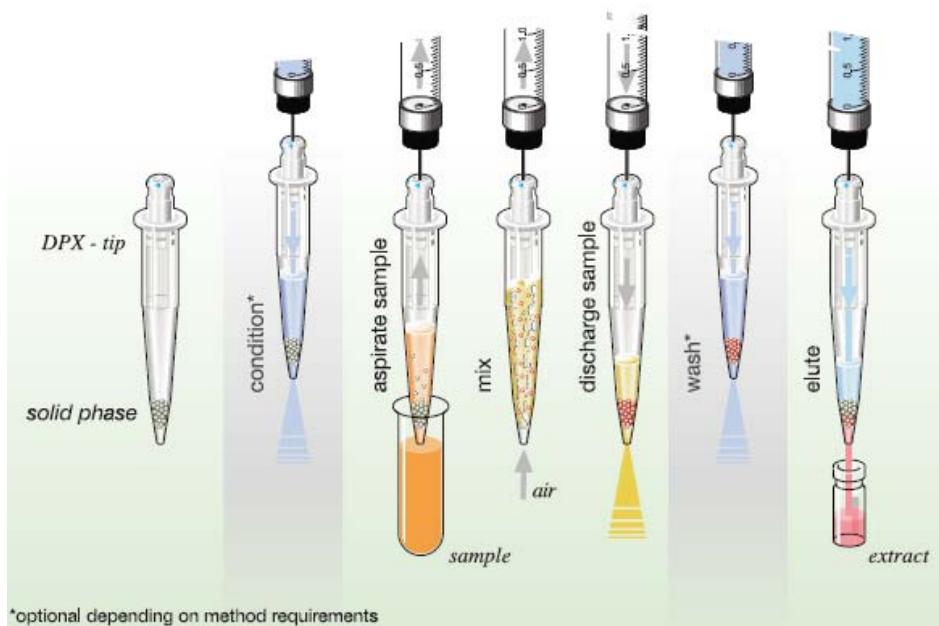


Figure 1. Schematic of the steps used in the automated DPX extraction method. The DPX tip is fitted with a transport adaptor (left), which permits a syringe and holder to process the extraction steps.



Figure 2. A picture of the dual rail MPS-2 system, which stands on top of the GC/MS system. The left syringe performs large volume injections, and the right syringe performs automated DPX extractions.

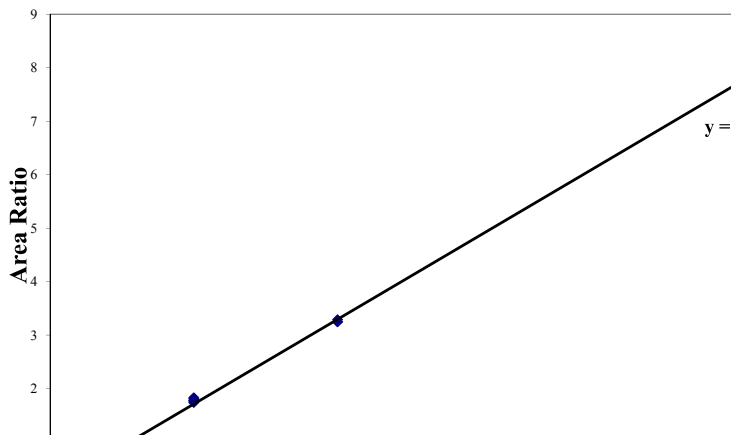


Figure 3. Calibration plot of THC extracted from whole blood.

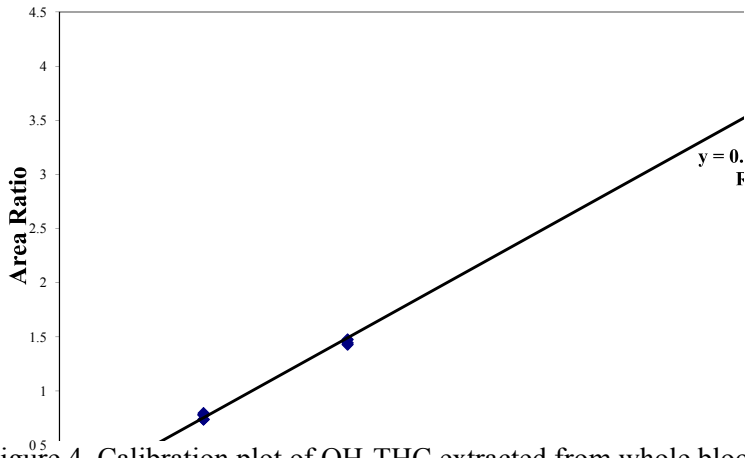


Figure 4. Calibration plot of OH-THC extracted from whole blood.

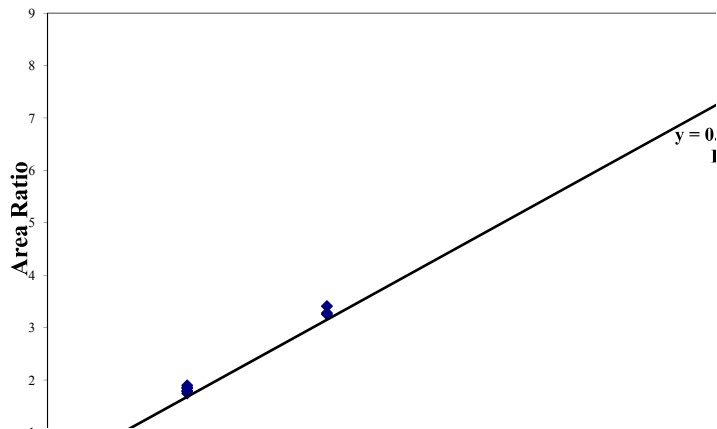


Figure 5. Calibration plot of COOH-THC extracted from whole blood.

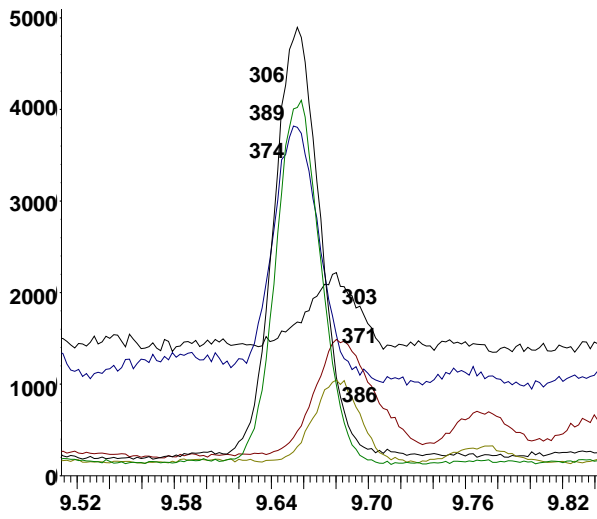


Figure 6. Extracted ion chromatogram of THC-TMS at 0.5 ng/mL, showing target and qualifier ions.

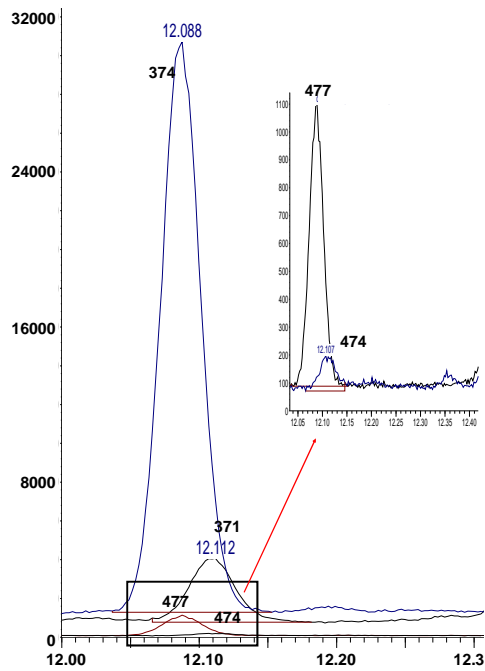


Figure 7. Extracted ion chromatogram of OH-THC-2TMS, showing target and qualifier ions.

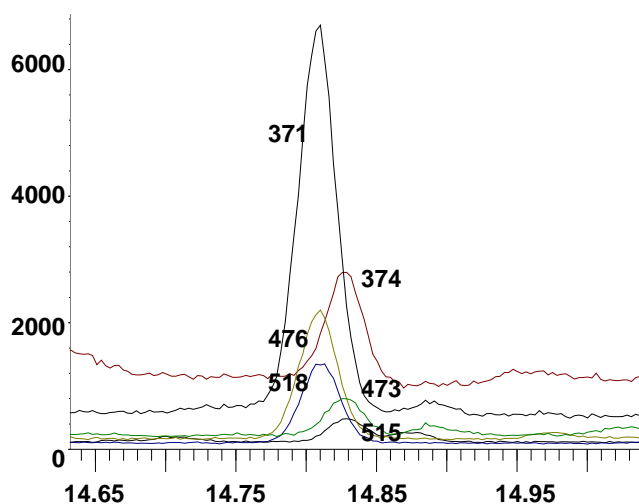


Figure 8. Extracted ion chromatogram of COOH-THC-TMS/MTBDMS, showing target and qualifier ions.

Conclusions

The DPX method is rapid and readily automated using the GERSTEL MPS2, and the Maestro software is easy to use to set up the DPX methods. The DPX extractions take approximately six minutes to perform, so the extraction is performed while the previous sample is being chromatographically analyzed. The automated injections with the CIS-4 (inlet system) combine concentration and derivatization steps with the injection process, thereby eliminating time consuming concentration, evaporation and derivatization steps. The limits of detection are less than 1 ng/mL for THC and OH-THC, and less than 2 ng/mL for COOH-THC with less than 5 % C.V. using automated DPX with inlet derivatization with BSTFA and MTBSTFA. Automated DPX with the dual rail GERSTEL MPS-2 provides the highest throughput available for this application using GC/MS analysis.

REFERENCES

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