Smart note | 004171

Forensic toxicology

Behind the bench: Tips to simultaneously analyze THC, its metabolites, and other drugs of abuse using LC-MS

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THC, drugs of abuse, oral fluid, toxicology, forensics

Why include THC in your LC-MS toxicology assay?

After alcohol, cannabis is the second most commonly detected drug in impaired driving incidents.[1] In 2024, it was reported that nearly 22% of the American population 12 years or older (61.8 million people) alleged to have used marijuana in the last year.[2] This makes it imperative to have an assay that can accurately screen and simultaneously confirm THC and other drugs of abuse that can impair driving. Rather than developing a separate test for THC that would require a lab to perform multiple extractions and assays for different drug classes, it would save money, time, and resources to include THC in a larger drug panel.

What are the different forms of THC and what metabolites do I expect to find in each matrix?

After consumption, THC undergoes extensive hepatic metabolism primarily via cytochrome P450 enzymes, notably CYP2C9 and CYP3A4.[3] The major initial transformation is the oxidation of THC to 11-hydroxy-THC (11-OH-THC), an active and psychoactive metabolite, which is further oxidized by alcohol and aldehyde dehydrogenases to form 11-nor-9-carboxy-THC (THC-COOH), an inactive metabolite. THC-COOH can be conjugated with glucuronic acid by UDP-glucuronosyltransferases to form THC-COOH-glucuronide, a highly water-soluble compound that facilitates renal excretion.[4] THC will be detected in different forms based on the matrix being analyzed. While THC and 11-OH-THC are primarily found in blood and oral fluid and indicate recent use, THC-COOH and THC-COOH-glucuronide are the main targets in urine drug testing due to their relatively prolonged presence and accumulation in the body. The cutoff levels required for THC and its metabolites are matrix dependent and vary by organization guidelines (Table 1).

Table 1. Cutoff levels for THC and metabolites for standards based on matrix. Concentrations listed in ng/mL. Concentrations listed for confirmatory cutoffs.

	Matrix	SAMHSA[a]	National Safety Council[b]	ANSI/ASB Std 119 (Medicolegal Death Investigations)[c]	ANSI/ASB Std 120 (Impaired Driving Investigations)[d]	ANSI/ASB Std 121 (Drug-Facilitated Crime Investigations)[e]
THC	Oral fluid	2	1			
	Blood			2	1	
THC-COOH	Blood			10	5	
	Urine					10
11-OH-THC	Blood				1	

[a]Department of Health and Human Services, 42 CFR Chapter I, Mandatory Guidelines for Federal Workplace Drug Testing Programs
[b]National Safety Council Tier 1 Drugs
[c]ANSI/ASB Standard 119, Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Medicolegal Death Investigations
[d]ANSI/ASB Standard 120, Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations
[e]ANSI/ASB Standard 121, Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Urine in Drug-Facilitated Crime Investigations

Why is it challenging to measure THC and metabolites reliably by LC-MS?

Due to the strong lipophilic nature, THC and its metabolites tend to form non-specific binding to various components in biological matrices and are adsorbed to the non-polar surfaces of the consumables during LC-MS analysis. Thus, they can be difficult to extract due to their affinity for consumables. It is important to consider what extraction consumables and materials would prevent these hydrophobic interactions.

Extraction and consumables

Sample composition and consumable materials should be considered when developing an extraction protocol for THC and its metabolites. Extracting THC works best when MeOH is present at every stage of the extraction. This starts at the beginning following the collection step. Incorporating enough methanol (MeOH) in the sample and extraction process mitigates the affinity of THC and metabolites to consumables. This means that THC and metabolites will remain in the sample solution.

How much MeOH is necessary? This depends on the application and consumables used. In our experience, oral fluid requires adding MeOH-diluted internal standard stock, so the final volume of sample plus internal standard is 20% MeOH. Additionally, using 30% MeOH as a solid phase extraction (SPE) wash step improves the binding of THC to the SPE resin prior to its elution. Using 30% MeOH will help improve THC recovery without compromising the recovery of other drugs.

Silanized glass test tubes (Cat. No. CTS-13100) and Thermo Scientific™ SureSTART™ Conical Silanized Glass Vial Inserts (Cat. No. 6PME03C1SSP) may be the best option to minimize adsorption of THC and metabolites to the surface of the consumables.

Finally, if drying down and reconstituting during the extraction, be sure to add the organic portion of the reconstitution solvent first and vortex, followed by the remaining aqueous portion of the reconstitution solvent. This will ensure that the extracted THC remains in solution.

LC columns

When choosing a column for a THC assay, there are specific stationary phases that will perform better. C18 columns often require a higher percentage of organic mobile phases to elute THC and metabolites, which ostensibly is when many matrix interferences are washed off the column. This may impact the sensitivity achieved. For this reason, it may be necessary to consider other stationary phases. Biphenyl columns (Thermo Scientific™ Accucore™ Biphenyl Reversed Phase HPLC Columns) will elute THC at 80%–85% organic mobile phase, which will precede many possible matrix interferences. Pentafluorophenyl (PFP) columns can help separate THC isomers (more on this to come). These two columns may be better options for THC and metabolite assays.

THC oral fluid application

In a recent technical note TN003851, THC and 30 other drugs were extracted from oral fluid using DPX® XTR™ tips with mixed mode SCX/WAX chemistry from DPX Technologies in order to achieve high recovery of each drug.[5] Figure 1 shows the extraction protocol used in this technical note followed by detection using the Thermo Scientific™ Vanquish™ Horizon Ultra-High Performance Liquid Chromatography (UHPLC) System and Thermo Scientific™ Stellar™ Mass Spectrometer.

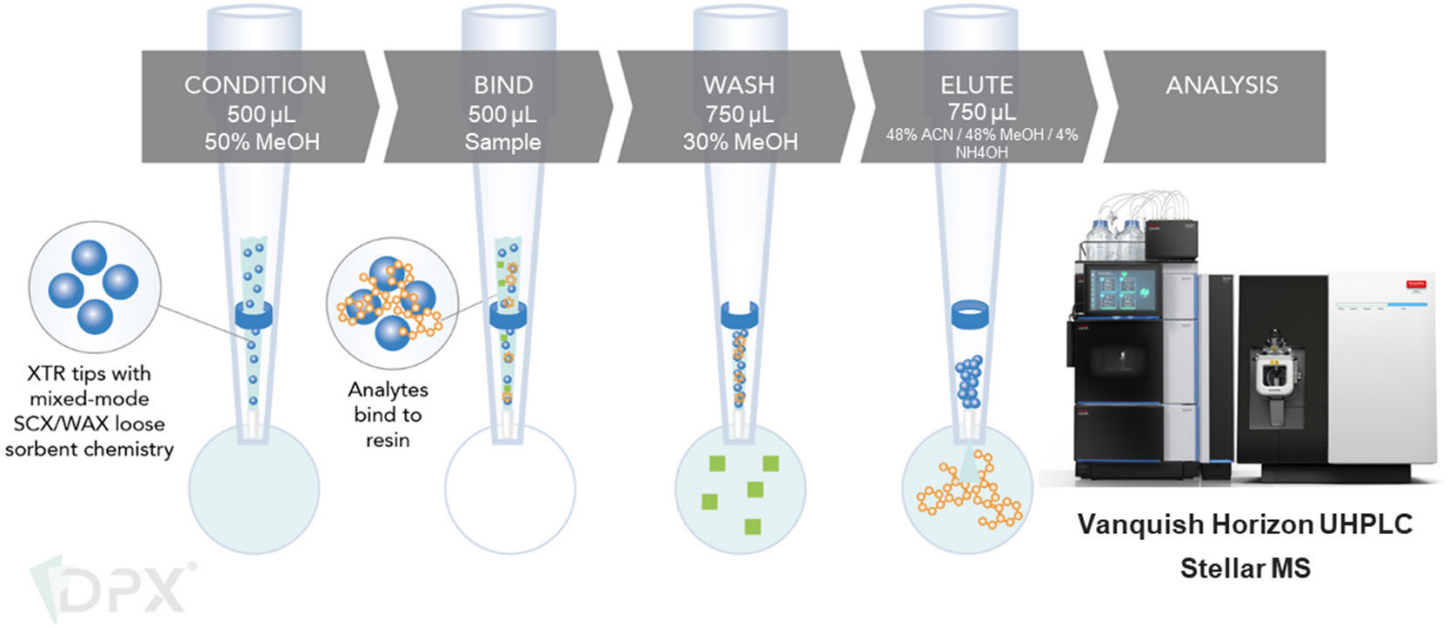


Figure 1. Schematic of XTR tip extraction protocol with mixed mode SCX/WAX chemistry for INTip™ dispersive solid phase extraction followed by analysis using the Vanquish Horizon UHPLC system and Stellar mass spectrometer. Artwork provided by DPX Technologies.

By following each of these tips and tricks to improve THC recovery and signal, we were able to improve the signal by 7x as seen in Figure 2. The changes made between the before and after peaks include the following:

1. Increasing volume of MeOH-diluted internal standard stock from 10% to 20% of collected and aliquoted oral fluid sample volume
2. Changing from 10% MeOH to 30% MeOH for the SPE wash step
3. Switching from a C18 to a Biphenyl column
4. Adding the organic portion of the reconstitution solvent first before the aqueous portion

Before **After**

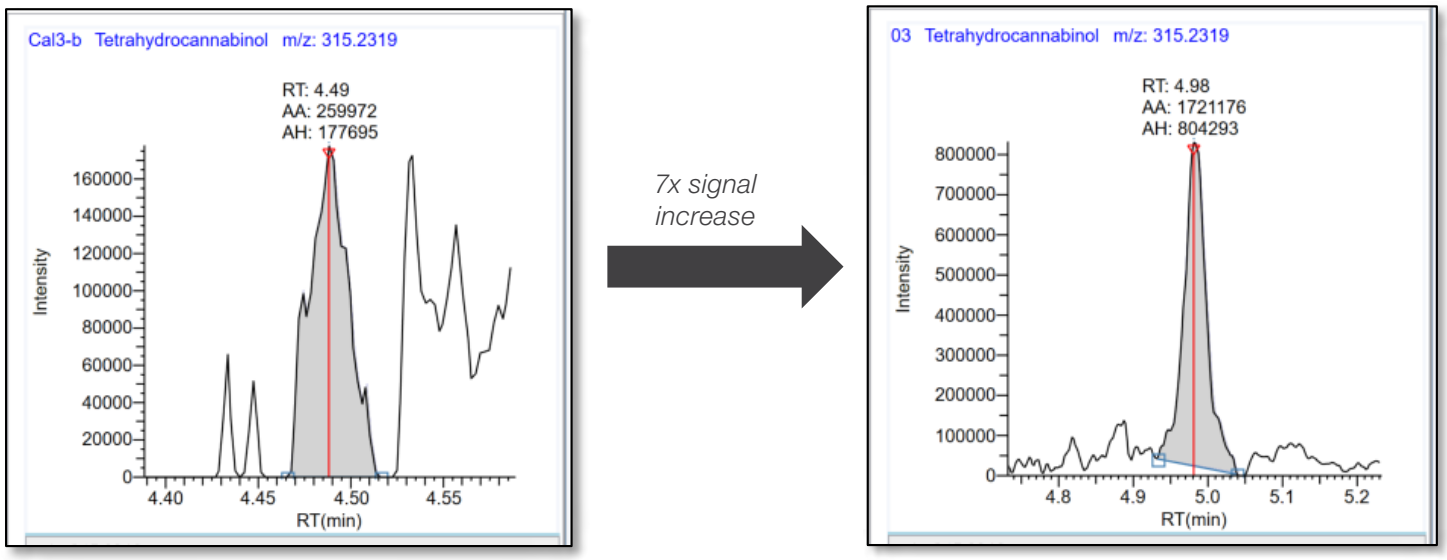


Figure 2. THC signal in extracted oral fluid sample before and after changes were made to increase signal by 7x.

THC and metabolite stability

Degradation is a common cause for concern when analyzing THC and metabolites, whose stability is heavily dependent on matrix, temperature, and the storage container. For instance, THC and metabolites in urine within polyethylene plastic vials at room temperature are not stable, and these same compounds in blood within Venoject™ tubes with rubber stoppers at room temperature decrease in concentration significantly after 2–8 weeks.[6] By storing at -20°C in green-top (sodium herapin) tubes, THC and metabolites can be stable for 6-12 months.

If the matrix being extracted is oral fluid, it is necessary to pick a collection device and buffer that will work best for THC. A buffer is necessary to ensure stability of drugs for days to weeks. Quantisal™ collection buffer stabilizes THC without introducing interferences that may impact the detection of the drug and is used by labs across the country that perform oral fluid assays. Compared to another common oral fluid extraction buffer, Quantisal produced 150x greater peak areas for THC (Figure 3).

What are the steps we used to optimize the THC Oral Fluid extraction protocol?

When developing a new extraction protocol to include THC, it is best to work backwards during the extraction to see what steps may impact recovery. Before introducing matrix, start with neat samples. Here is an example taken from the development of our THC Oral Fluid assay with XTR tips.

Dry down test

To ensure no drug loss is occurring during the dry down step, use the steps highlighted in Figure 4. This test compares neat samples diluted in starting mobile phase conditions with neat samples diluted in MeOH that will undergo a dry down. For our method, the starting mobile phase condition was 15% MeOH. Sample A can be injected immediately. Sample B undergoes a dry down and reconstitution with the starting mobile phase conditions. Be sure to use the same concentration for both samples A and B. If recovery is low for a compound of interest, a dry down step may not be the best option.

Sequential elution testing

To determine if the correct volume of eluant is being used to ensure all analytes elute from the SPE, make iterative elutions of the same volume into tubes/wells (Figure 5). Dry down and inject to determine which sample still has analytes and that is the volume required for a complete elution.

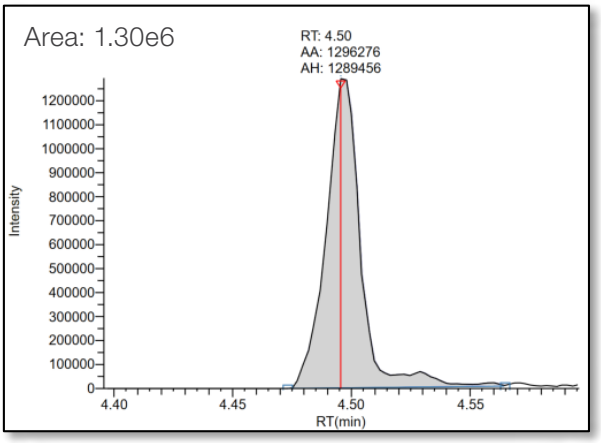
Wash test

To decide the wash step organic components to use, test in triplicate variations of wash solvents. For example: H_2O, 10% MeOH, 20% MeOH, 30% MeOH. Start by conditioning the SPE, load the neat sample, wash with different wash solvents, elute, and dry down. Evaluate the highest percentage organic that can be used without significant analyte loss. THC tends to prefer a wash step with a higher percentage of MeOH for better binding, whereas opioids tend to get washed off the SPE more easily at higher percentage organic.

Recovery with matrix

To determine the total percent recovery this method produces, spike matrix pre- and post-extraction to the same final concentration (Figure 6). This will help determine if there is significant loss from the extraction process. Using Equation 1, percent recovery can be calculated for each of the drugs in the method. For the oral fluid drugs of abuse method, our recovery of THC was calculated to be greater than 70% while ensuring that each of the 31 drugs were able to achieve their required cutoff levels.

Other oral fluid buffer



150x increase

Quantisal

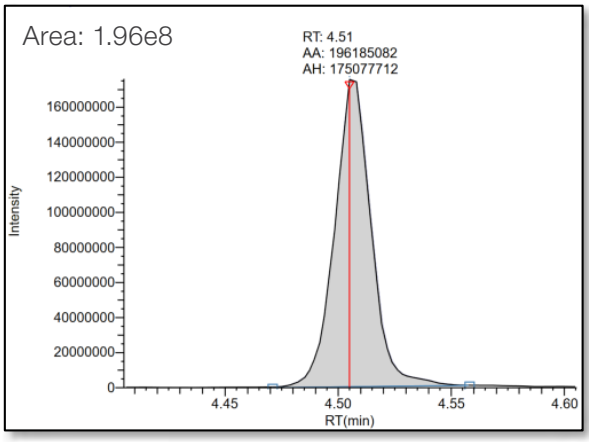


Figure 3. Extracted ion chromatogram of THC using common oral fluid buffer compared to Quantisal extraction buffer.

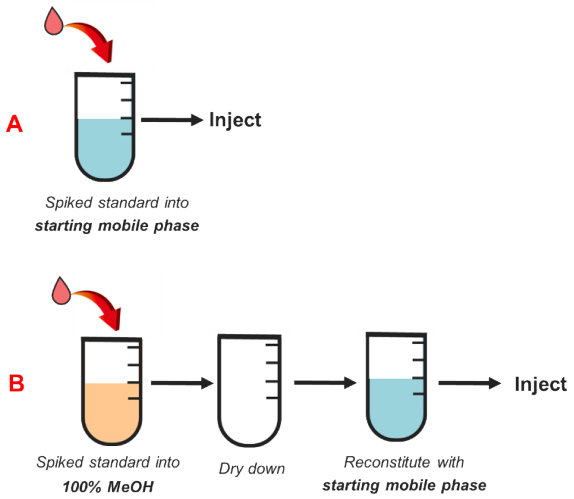


Figure 4. Schematic of a dry down test to check if a dry down and reconstitution causes significant recovery loss.

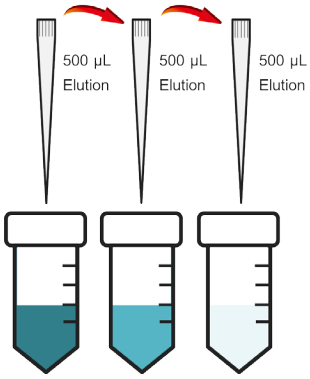


Figure 5. Schematic of sequential elution testing using the same SPE tip.

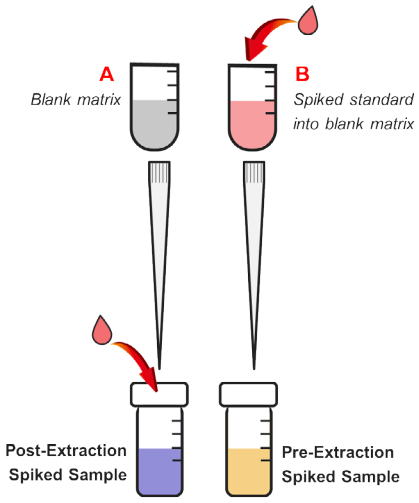


Figure 6. Schematic of recovery study.

Equation 1

$$\textbf{Percent recovery} = \left(\frac{\textbf{Response B}}{\textit{Extracted sample}} \Big/ \frac{\textbf{Response A}}{\textit{Post-extraction spiked sample}} \right) * 100\%$$

How to separate Delta 8 and Delta 9 THC isomers?

THC has a few common isomers, which have the same chemical formula but a different arrangement of atoms or double bonds. Two of the most common THC isomers are Δ8 and Δ9, which differ by a double bond location. For some assays, it is necessary to separate these two isomers. For example, in urine samples, Δ8-THC-COOH and Δ9-THC-COOH can both be detected (Figure 7). Using the correct column and mobile phases, separation can be achieved. PFP columns efficiently separate the isomers using H_2O + 0.1% formic acid (Mobile phase A) and MeOH + 0.1% formic acid (Mobile phase B). In a study to separate these isomers in human urine samples, the gradient listed in Table 2 was used to achieve the separation seen in Figure 8. A Vanquish Horizon UHPLC system coupled with the Thermo Scientific™ Orbitrap Exploris™ 120 Mass Spectrometer was used to analyze these isomers.

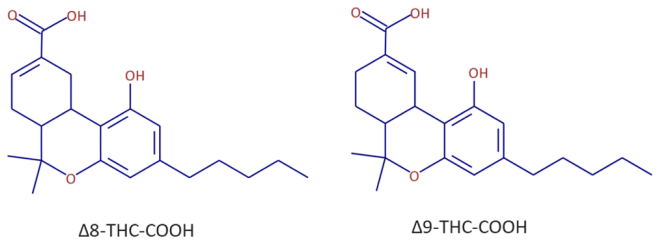


Figure 7. Structures of isomers Δ8-THC-COOH and Δ9-THC-COOH.

Table 2. LC gradient for separation of Δ8-THC-COOH and Δ9-THC-COOH with a Raptor™ FluoroPhenyl 2.7 µm 100 x 2.1 mm column (Restek Corp.).

Time (min)	Flow rate (mL/min)	% A	% B	Curve
0.000	0.4	36	64	5
5.000	0.4	36	64	5
5.000	0.4	1	99	5
5.500	0.4	1	99	5
5.500	0.4	36	64	5
6.000	36	36	64	5

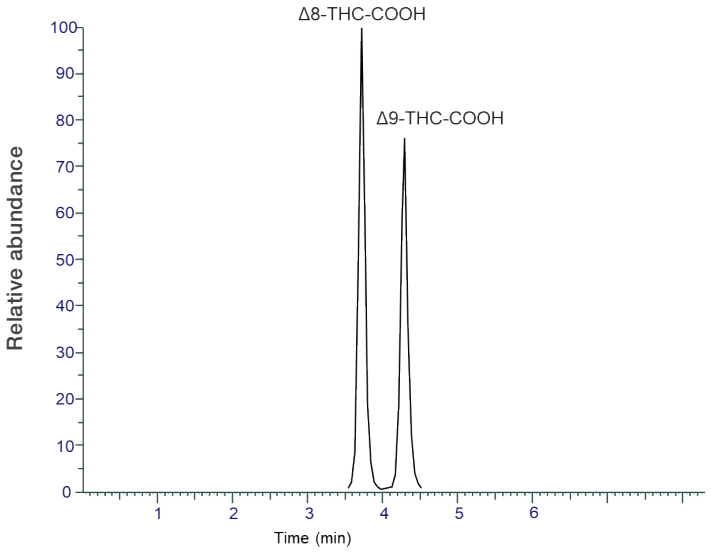


Figure 8. Extracted ion chromatogram of Δ8-THC-COOH and Δ9-THC-COOH separation.

Conclusion

By including THC and metabolites in a broader drug panel, laboratories can save resources and streamline processes. Careful consideration of extraction methods, column selection, and matrix effects is crucial to ensure accurate detection and quantification of THC and its metabolites. Utilizing appropriate consumables and optimizing extraction protocols will enhance recovery and sensitivity, making your assay robust and reliable for forensic toxicology applications.

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