Featured Application: *Mycotoxins in Cannabis CBD Oil on Raptor Biphenyl*

High-Throughput Analysis of Mycotoxins in Cannabis CBD Oil Pairs Simplified Cleanup with LC-MS/MS Sensitivity

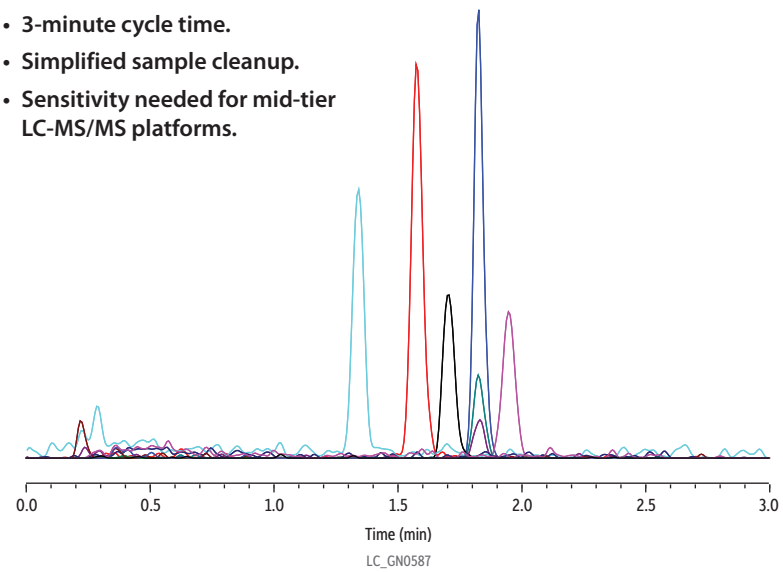
- Fast, 3-min total cycle time lets you analyze more samples per shift.
- Resprep SPE sample cleanup removes matrix interferences in one simple step.
- Excellent sensitivity down to 2 ng/g in matrix on legacy instrumentation.

Aflatoxins and ochratoxins are an emerging concern in the cannabis industry as these secondary fungal metabolites can cause disease and death if consumed. Crops are susceptible to fungal growth from seed through storage, so there are many opportunities for contamination to occur. As a result, a growing need is emerging for testing to detect the presence of mycotoxins in both raw plant materials and finished cannabis products in order to protect consumer health and safety. The analysis of mycotoxins in cannabis oils is particularly challenging because the lipids in the sample can contribute isobaric matrix interferences in addition to general ion suppression that can reduce accuracy, particularly at low levels.

Immunoaffinity columns (IACs) are commonly used for sample preparation when analyzing mycotoxins in cannabis; however, IACs also contribute significantly to the overall complexity, time, and cost associated with mycotoxin analysis. IAC methods are labor intensive, requiring numerous conditioning and washing steps. In addition, labs typically have to use multiple IACs in order to analyze all the mycotoxins of interest due to columns being specific for aflatoxins or ochratoxins.

In this study, a simple pass-through SPE sample cleanup was developed as an alternative to IACs and applied to lipid-rich CBD oils. Excellent chromatographic results were obtained for both aflatoxins and ochratoxins even at 2 ng/g, indicating that the simplified sample prep technique effectively removed lipid interferences. The SPE sample cleanup was paired with a rapid LC-MS/MS analysis utilizing a Raptor Biphenyl column. The polarizability and unique selectivity of the Raptor Biphenyl column effectively separated all target analytes in a fast, 3-minute analysis, making this method a better approach than IACs for high-throughput testing of mycotoxins in cannabis CBD oils.

- **3-minute cycle time.**
- **Simplified sample cleanup.**
- **Sensitivity needed for mid-tier LC-MS/MS platforms.**



Peaks	tR (min)	Precursor Ion	Product Ion 1	Product Ion 2
1. Aflatoxin G2-13C17	1.340	348.3	330.3	—
2. Aflatoxin G1-13C17	1.576	346.3	257.3	—
3. Aflatoxin B2-13C17	1.703	332.3	303.3	—
4. Ochratoxin A	1.824	404.3	239.1	358.3
5. Ochratoxin A-13C20	1.825	424.3	250.2	—
6. Aflatoxin B1-13C17	1.947	330.3	301.4	—

Column: Raptor Biphenyl (cat.# 9309A52); Dimensions: 50 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor Biphenyl EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252); Temp.: 35 °C; **Sample**: Diluent: 45:55 Water:Methanol; Inj. Vol.: 5 µL; **Mobile Phase**: A: Water, 2 mM ammonium formate, 0.1% formic acid; B: Methanol, 2 mM ammonium formate, 0.1% formic acid; **Gradient (%B):** 0.00 min (65%), 2.00 min (90%); 2.01 min (65%); 3.00 min (65%); **Flow:** 0.7 mL/min; **Detector**: MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument:** UHPLC; **Notes:** 0.25 g of commercially available hemp-derived CBD oil was weighed into a 2 mL vial. A working internal standard was prepared using 13C labeled analogs at a concentration of 250 ng/mL in methanol. 10 µL of the working internal standard was aliquoted into the sample followed by vortexing for 10 seconds at 3,000 rpm. 1 mL of 45:55 H_2O:MeOH was added to the sample. The sample was vortexed for 30 seconds at 3,000 rpm. The sample was then centrifuged at 3,000 xg for 5 min at 10 °C. 750 µL of the supernatant was transferred to a conditioned (1 mL 45:55 water:methanol) Resprep bonded reversed phase SPE cartridge (Restek cat.# 26030). The sample was pulled through under vacuum into an autosampler vial for LC-MS/MS analysis. **Notes:** Want even better performance when analyzing metal-sensitive compounds? Check out Inert LC columns at www.restek.com/inert

Resprep SPE Cartridges (Bonded Reversed Phases)

Hydrophobic (nonpolar) silica-based adsorbents; used to extract hydrophobic analytes from polar matrices, such as water (e.g., pesticides from water).

All cartridges are manufactured using high-density polypropylene and have polyethylene frits unless otherwise noted.
Cartridges may be processed by any one or all of these techniques: positive pressure, sidearm flask, centrifuge, or vacuum manifold.

	1 mL/100 mg (100-pk.)	3 mL/200 mg (50-pk.)	3 mL/500 mg (50-pk.)	6 mL/500 mg (30-pk.)	6 mL/1,000 mg (30-pk.)	60 mL/10 g (16-pk.)
C18 (high load, end-capped)	26030	26031	24050	24052	24051	26035

Resprep 12- or 24-Port SPE Manifolds

- Use with any standard male luer end SPE cartridges.
- Inert, PTFE sample guides reduce cross-contamination and carryover.
- Flexible sample collection rack will accommodate a variety of receiving vessels.
- Quick vacuum-release valve for better system control.
- Individual valves allow vacuum control for each cartridge, improving reproducibility.

Description	qty.	cat.#
Complete Resprep SPE Manifold, 12-Port (Includes: glass basin with built-in vacuum regulator (1); polypropylene lid with 12 individual control valves (1); 12-position collection rack (1); PTFE sample guides (12); and waste container (1).)	kit	26077

Note: Replacement waste container not included in 24-port manifold kit (cat.# 26080).
*Waste container (shown in tank) and top shelf for round-bottom flasks are not included in 24-port manifold kit (cat.# 26080).

2.0 mL, 8 mm Autosampler Vial Convenience Kits

- Vials packaged in a clear-lid tray.
- Preassembled caps with septa packaged separately in a plastic bag.
- Black polypropylene open-hole caps and 8 mm red PTFE/silicone septa, 0.065".

Description	100-pk. cat.#	1,000-pk. cat.#
Amber 2.0 mL Vial, Untreated, Black Cap, Red PTFE/Silicone Septa, 0.065"	21194	21195

Bluestem Glass Solvent Filter

- Restek Bluestem glass solvent filter provides clean mobile phase to extend the life of columns and pump seals.
- 15 µm borosilicate glass frit sits lower than conventional glass filters to draw more mobile phase from each bottle.
- Blue filter stem allows instant visual confirmation of upright filter orientation.
- Connects to standard 1/8" OD (3.2 mm) PTFE tubing using your existing frit adaptor (also sold separately as cat.# 26392).

Prevent the particulates and microbial growth in your LC solvents from entering your instrument with the new Restek Bluestem glass solvent filter.

Description	Similar to Agilent Part #	qty.	cat.#
Glass Solvent Filter, 15 µm frit	5041-2168	ea.	26431
Frit Adaptor, PTFE	5062-8517	4-pk.	26392

Raptor Biphenyl LC Columns (USP L11)

Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
1.8 µm Columns			
30 mm	9309232	—	—
50 mm	9309252	930925E	—
100 mm	9309212	930921E	—
150 mm	9309262	—	—
2.7 µm Columns			
30 mm	9309A32	9309A3E	9309A35
50 mm	9309A52	9309A5E	9309A55
100 mm	9309A12	9309A1E	9309A15
150 mm	9309A62	9309A6E	9309A65
5 µm Columns			
30 mm	—	930953E	—
50 mm	9309552	930955E	9309555
100 mm	9309512	930951E	9309515
150 mm	9309562	930956E	9309565
250 mm	—	—	9309575

Want even better performance when analyzing mycotoxins? Check out Inert LC columns at **www.restek.com/inert**

Raptor Inert Biphenyl HPLC Columns

- Inert LC column technology reduces nonspecific binding of chelating analytes, enabling sensitive analysis and smooth integration of peaks.
- Ideal for the analysis of metal-sensitive compounds, such as mycotoxins.
- Increased response and analyte recovery, allowing lower detection limits.
- Improved peak shape without additional passivation or mobile phase additives.
- Part of Restek's Raptor Biphenyl column line featuring 2.7 µm SPP core-shell silica.

ID	Length	Particle Size	Units	Cat.#
2.1 mm	100	2.7 µm	ea.	9309A12-T
3.0 mm	100	2.7 µm	ea.	9309A1E-T
2.1 mm	50	2.7 µm	ea.	9309A52-T
3.0 mm	50	2.7 µm	ea.	9309A5E-T

Raptor EXP Guard Column Cartridges

- Free-Turn architecture lets you change cartridges by hand without breaking inlet/outlet fluid connections—no tools needed.
- Patented titanium hybrid ferrules can be installed repeatedly without compromising high-pressure seal.
- Auto-adjusting design provides ZDV (zero dead volume) connection to any 10-32 female port.

To help protect your investment and further extend the life of our already-rugged LC columns, Restek offers the patent-pending guard column hardware developed by Optimize Technologies. A Restek LC guard cartridge in an EXP direct connect holder is the ultimate in column protection, especially when using dilute-and-shoot or other limited-sample preparation techniques.

Description	Particle Size	qty.	5 x 2.1 mm cat.#	5 x 3.0 mm cat.#	5 x 4.6 mm cat.#
Raptor Biphenyl EXP Guard Column Cartridge	UHPLC	3-pk.	9309U0252	9309U0253	
Raptor Biphenyl EXP Guard Column Cartridge	2.7 µm	3-pk.	9309A0252	9309A0253	9309A0250
Raptor Biphenyl EXP Guard Column Cartridge	5 µm	3-pk.	930950252	930950253	930950250

Maximum cartridge pressure: 1,034 bar/15,000 psi* (UHPLC), 600 bar/8,700 psi (2.7 µm); 400 bar/5,800 psi (5 µm)

* For maximum lifetime, recommended maximum pressure for UHPLC particles is 830 bar/12,000 psi.

EXP Direct Connect Holder

Description	qty.	cat.#
EXP Direct Connect Holder for EXP Guard Cartridges (includes hex-head fitting & 2 ferrules)	ea.	25808

Maximum holder pressure: 20,000 psi (1,400 bar)

Hybrid Ferrule U.S. Patent No. 8201854, EXP Holders U.S. Patent No. 8696902, EXP2 Wrench U.S. Patent No. D766055. Other U.S. and Foreign Patents Pending. The EXP, Free-Turn, and the Opti- prefix are registered trademarks of Optimize Technologies, Inc.

RESTEK
Pure Chromatography

Questions? Contact us or your local Restek representative (www.restek.com/contact-us).

www.restek.com

Lit. Cat.# FFSS2946A-UNV