Analysis of contaminants in hemp using LC and GC coupled to MS/MS

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Introduction

- Hemp is a class of Cannabis sativa that contains significantly lower levels of tetrahydrocannabinol (THC), and may have higher levels of cannabidiol and cannabigerol (CBD and CBG).
- Like cannabis and other crops, dried hemp plant material may contain various contaminants that are harmful to humans.
- The high complexity of hemp and cannabis samples, and the broad range of contaminants being regulated at minimum required performance levels (MRPL) in the order of parts per billion (ppb), demands for robust, fast, and effective analytical methods.
- This work describes a complete workflow for the analysis of diverse contaminants in hemp using hydrophilic lipophilic balanced (HLB) cartridges to clean-up organic hemp extracts, and using LC and GC coupled to MS/MS for reliable instrumental analysis.

Goal

To provide an effective workflow for the analysis of pesticides and mycotoxins in hemp and cannabis plant material by using a single extract and LC-MS/MS and GC-MS/MS.

Method development: sample preparation

Weigh 1 g of pulverized plant material in a 15 mL plastic tube.

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Spike isotopically labeled internal standards (for recovery assessment, target analytes should be spiked at this stage).

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Add 5 mL of acetonitrile acidified with 1% acetic acid and vortex for 5 min at 2500 rpm.

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Add 200 µL of water to a 6 mL hydrophilic lipophilic balanced (HLB) cartridge (200 mg) (Restek cat.# 28451). Then transfer 3 mL of the supernatant.

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Apply vacuum or positive pressure to collect the cleaned extract.

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Stop vacuum/positive pressure. Add 300 µL of methanol and re-apply vacuum/positive pressure to rinse the cartridge. Collect the solvent with the rest of the extract.

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For LC-MS/MS analysis: dilute the extract in a ratio 1:1 with a 2:2:1 methanol:acetonitrile (1% acetic acid):water solution. Inject 1.5 µL.

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For GC-MS/MS analysis: transfer 1 mL of cleaned supernatant to a dSPE tube containing magnesium sulfate and C18 (cat.# 26242). Vortex briefly and centrifuge for 5 min. Dilute the extract in a ratio 1:1 with a 1:1 hexane:acetone (1% acetic acid) solution. Inject 1µL.

Figure 1. Sample preparation workflow for hemp samples

Method development: LC/GC-MS/MS

Table 1. LC-MS/MS conditions (ionization: ESI)

Column	Raptor ARC-18 2.7 µm, 150 mm x 2.1 mm (cat.# 9314A62)			
Guard Column	Raptor ARC-18 EXP Guard Column Cartridge 2.7 µm, 5 x 2.1 mm (cat.# 9314A0252)			
Mobile Phase A	Water, 2 mM ammonium formate, 0.1% formic acid			
Mobile Phase B	Methanol, 2 mM ammonium formate, 0.1% formic acid			
Time Program	Time (min.)	%B	Time (min.)	%B
	0	5	11	75
	1.0	50	11.5	80
	2.5	50	13.5	80
	4.0	65	15.5	95
	7.0	65	16.5	100
	7.5	70	19.5	100
	9.0	70	19.6	5
	9.5	75		
Other parameters	Column T: 40°C; autosampler T: 10°C; flow: 0.4 mL/min; injection volume: 1.5 µL			
Instrument	Shimadzu LCMS-8045			

Table 2. GC-MS/MS conditions (ionization: EI)

GC Column	Rxi-5ms 30 m x 0.25 mm x 0.25 µm (cat.# 13423)
Injection	Splitless, 1 µL (0.5 min splitless time, 7 mL/min split flow)
Liner	Topaz 4.0 mm ID Single Taper Inlet Liner w/ Wool (cat.# 23447)
Inj. T	250°C
Purge Flow	5 mL/min
Oven	70°C (hold 1 min) to 220°C by 30°C/min; to 240°C by 5°C/min; to 315°C (hold 10 min) by 10°C/min
Carrier Gas	He, at a constant flow of 1.4 mL/min
Transfer line T	290°C
Source T	330°C
Instrument	Thermo Trace 1310-TSQ 8000

Results and discussion

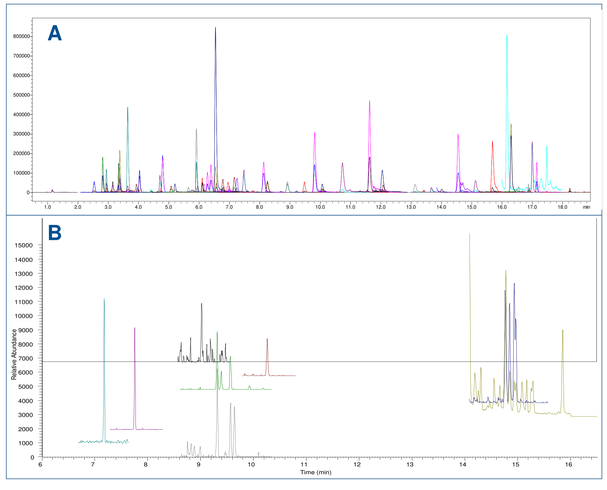

Figure 2. Chromatograms corresponding to LC (A) and GC (B) amenable contaminants extracted from a hemp sample spiked at 100 ng/g

Table 3. Figures of merit corresponding to pesticides and mycotoxins analyzed in hemp (CBG variety)

Contaminant	California Action level, µg/g	Canada Action level, µg/g	Method LOQ, µg/g	R^2	0.1 µg/g (n=4) Accuracy (RSD)	Contaminant	California Action level, µg/g	Canada Action level, µg/g	Method LOQ, µg/g	R^2	0.1 µg/g (n=4) Accuracy (RSD)
Daminozide	0.1	0.1	0.02	0.9974	80 (6)	Spinosad- spinosyn A	0.1*	0.1*	0.00355	0.9963	74 (14)
Acephate	0.1	0.02	0.005	0.9996	100 (4)	Diazinon	0.1	0.02	0.005	0.9990	96 (4)
Thiamethoxam	5	0.02	0.005	0.9994	102 (3)	Coumaphos	0.1	0.02	0.01	0.9989	84 (9)
Methomyl	1	0.05	0.005	0.9989	105 (5)	Clofentezine	0.1	0.02	0.02	0.9844	38 (12)
Oxamyl	0.5	3	0.005	0.9980	96 (2)	Spinosad - spinosyn D	0.1*	0.1*	0.0029	0.9961	74 (20)
Imidacloprid	5	0.02	0.01	0.9983	93 (11)	Spinetoram - spinosyn J	0.1^	0.02^	0.0042	0.9960	73 (16)
Dimethoate	0.1	0.02	0.005	0.9979	101 (2)	Spinetoram - spinosyn L	0.1^	0.02^	0.001	0.9959	75 (13)
Acetamiprid	0.1	0.1	0.005	0.9993	100 (4)	Trifloxystrobin	0.1	0.02	0.005	0.9987	106 (6)
Thiacloprid	0.1	0.02	0.005	0.9993	100 (8)	Prallethrin	0.1	0.05	0.05	0.9993	98 (9)
Aldicarb	0.1	1	0.005	0.9977	101 (5)	Hexythiazox	0.1	0.01	0.005	0.9915	75 (26)
Naled	0.1	0.1	0.02	0.9988	83 (12)	Cyfluthrin	2	0.2	0.15	0.9943	-
Mevinphos (I, II)	0.1	0.05	0.02	0.9976	99 (5)	Etoxazole	0.1	0.02	0.005	0.9965	90 (6)
Carbofuran	0.1	0.02	0.005	0.9983	102 (5)	Chlorpyrifos	0.1	0.04	0.02	0.9961	97 (7)
Carbaryl	0.5	0.05	0.02	0.9979	96 (6)	Permethrins	0.5	0.5	0.01	0.9979	77 (6)
Dichlorvos	0.1	0.1	0.1	0.9983	99 (12)	Fenpyroximate	0.1	0.02	0.005	0.9977	97 (7)
Propoxur	0.1	0.02	0.005	0.9982	100 (3)	Bifenthrin	3	1	0.005	0.9974	93 (7)
Chlorantraniliprole	10	0.02	0.005	0.9953	94 (3)	AbamectinB1a	0.1	0.1	0.01	0.9973	94 (11)
Imazalil	0.1	0.05	0.01	0.9987	80 (11)	Cypermethrin	1	0.3	0.075	0.9966	90 (10)
Metalaxyl	2	0.02	0.005	0.9992	105 (5)	Etofenprox	0.1	0.05	0.005	0.9977	78 (6)
Azoxystrobin	0.1	0.02	0.005	0.9967	105 (4)	Pyridaben	0.1	0.05	0.005	0.9991	92 (7)
Myclobutanil	0.1	0.02	0.005	0.9959	100 (4)	Acequinocyl	0.1	0.03	0.02	0.9961	80 (16)
Phosmet	0.1	0.02	0.005	0.9991	79 (16)	Flonicamid	0.1	0.05	0.05	0.9968	87 (17)
Spiroxamine	0.1	0.1	0.005	0.9920	72 (15)	Fipronil	0.1	0.06	0.01	0.9985	106 (10)
Fenoxycarb	0.1	0.02	0.005	0.9988	89 (5)	Fludioxonil	0.1	0.02	0.02	0.9958	83 (6)
Methiocarb	0.1	0.02	0.005	0.9958	97 (3)	Aflatoxin G2	0.02#	0.002	0.02	0.9905	87 (6)
Spiromesifen	0.1	3	0.02	0.9981	108 (12)	Aflatoxin G1	0.02#	0.002	0.005	0.9969	88 (12)
Boscalid	0.1	0.02	0.02	0.9949	86 (8)	Aflatoxin B2	0.02#	0.002	0.01	0.9958	87 (8)
Paclobutrazol	0.1	0.02	0.01	0.9957	102 (7)	Aflatoxin B1	0.02#	0.002	0.005	0.9959	85 (7)
Malathion	0.5	0.02	0.01	0.9954	97 (4)	Ochratoxin A	0.02	0.02	0.02	0.9966	83 (8)
Dimethomorph (I,II)	2	0.05	0.01	0.9955	93 (8)	Captan (GC)	0.7	-	0.075	0.9829	78 (25)
Tebuconazole	0.1	0.05	0.005	0.9988	101 (4)	Chlordane (GC)	0.1	-	0.02	0.9941	81 (1)
Bifenazate	0.1	0.02	0.005	0.9970	100 (5)	Chlorfenapyr (GC)	0.1	0.05	0.02	0.9939	98 (9)
Fenhexamid	0.1	-	0.02	0.9942	79 (12)	Methyl parathion (GC)	0.1	0.05	0.005	0.9969	97 (2)
Propiconazole	0.1	0.1	0.02	0.9985	98 (18)	PCNB (GC)	0.1	0.02	0.01	0.9965	81 (8)
Spirotetramat	0.1	0.02	0.005	0.9993	98 (4)	Cyfluthrin (GC)	2	0.2	0.02	0.9927	92 (6)
Ethoprophos	0.1	0.02	0.005	0.9995	98 (5)	Cypermethrin (GC)	1	0.3	0.05	0.9897	97 (7)
Kresoxym-methyl	0.1	0.02	0.02	0.9980	91 (8)						

*MRPL for total spinosad; ^MRPL for total spinoteram; # AG2+AG1+AB1+AB2<0.002µg/g

- Hemp and cannabis extracts are characterized for having a high concentration of hydrophobic constituents. By mixing 3 mL of extract with 200 µL of water prior to SPE clean-up with HLB it was possible to remove major hydrophobic interferences. The addition of 300 µL of methanol helped in the elution of all target pesticides.
- Calibration curves to cover a range of 0.005 and 1.5 µg/g in matrix (10 points) were prepared by post-spiking blank hemp extract with target analytes at various concentrations, and internal standards (9 compounds). All analytes, except clofentezine and captan, showed R^2 >0.99. It is recommended to use deuterated analogues for these two compounds.
- Accuracy and precision were assessed by spiking hemp samples at 0.01, 0.05, 0.1, and 0.5 µg/g (n=4), and estimating their concentration using the calibration curve prepared in hemp extract. Accuracy and precision values for the great majority of pesticides were within 70 – 130% and below 30%, respectively.
- Sample prep, extract dilution, injection volume, chromatographic separation were all critical in resolving analytes from interferences as to minimize possible matrix effects and reach the required MRPLs.
- In total 9 deuterated analytes were used to account for sample prep and instrumental variation.
- The use of dSPE containing magnesium sulfate was essential to remove any water left in extracts after the first clean-up step.
- Pyrethrins I and II, and piperonyl butoxide were present in the hemp samples used for this work, so they were excluded from the table.
- Overall, the proposed workflow showed satisfactory results in the quantification of all target pesticides and mycotoxins. For the great majority of the compounds the LOQ values were significantly below the action levels established by the state of CA in inhalable cannabis, and comply with Canada regulations.

Conclusions

An easy and effective workflow for the analysis of pesticides and mycotoxins in hemp was developed. Satisfactory results in terms of figures of merit (LOQs, R^2 , accuracy, and precision) were obtained for the great majority of target contaminants.

References

- Reyes-Garcés N, Myers C. J Sep Sci. 2021; 44: 2564–2576. https://doi.org/10.1002/jssc.202001265