Analysis of the California list of pesticides, mycotoxins, and cannabinoids in edibles using LC and GC-based platforms

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Introduction

- Cannabis legalization has led to the development of a variety of cannabis-infused products with edibles being one of the most popular.
- The state of California has implemented comprehensive cannabis testing regulations requiring the analysis of cannabinoids (potency) and contaminants, such as pesticides and mycotoxins, in any type of cannabis good.
- In this work, we propose analytical workflows for the quantification of the California list of pesticides and mycotoxins, as well as six cannabinoids, in infused chocolate and gummies.

Goal

Provide effective workflows for the analysis of the California list of pesticides, mycotoxins, and cannabinoids, in infused edibles by using a single extract and LC-MS/MS, low pressure- LPGC-MS/MS and HPLC-UV instrumental platforms.

Method development: sample preparation

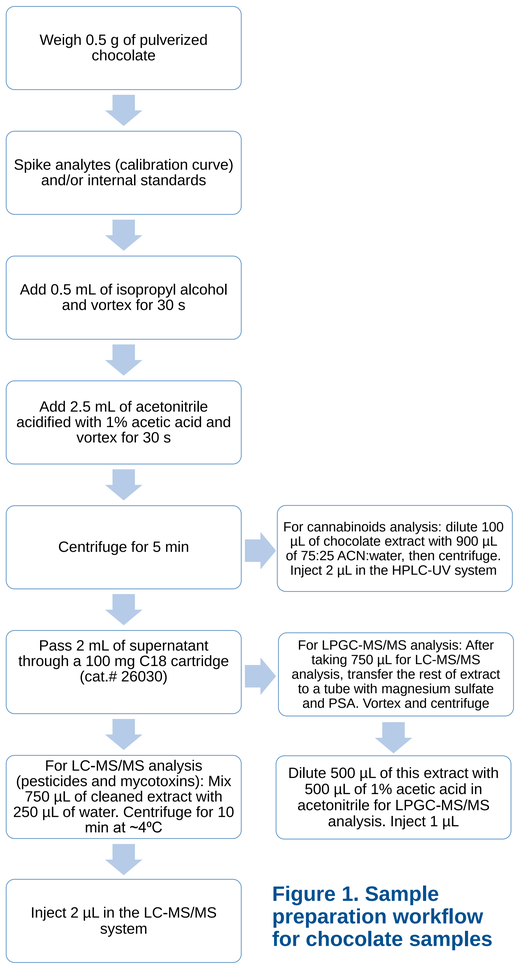

Figure 1. Sample preparation workflow for chocolate samples

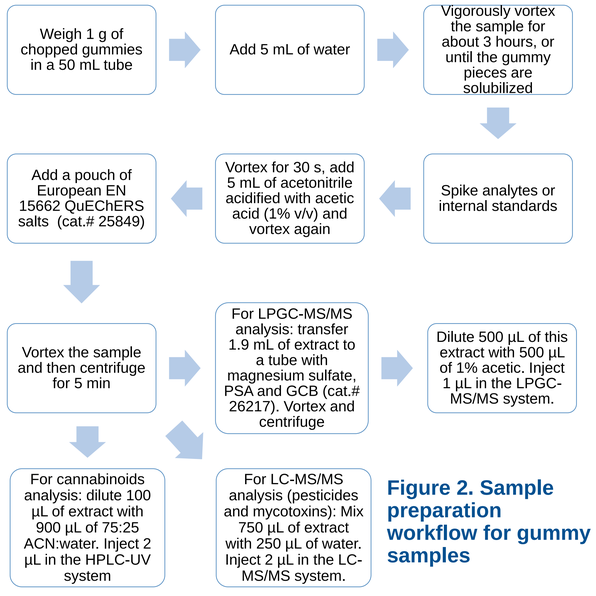

Figure 2. Sample preparation workflow for gummy samples

Method development: LC/LPGC-MS/MS

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

Column	Raptor ARC-18 2.7 µm, 100 mm x 2.1 mm (cat.# 9314A12)			
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	10.5	100
	1.5	65	10.6	5
	8.5	95	12.0	5
	9.5	100		
Other parameters	Column T: 40°C; autosampler T: 10°C; injection volume: 2 µL			
Instrument	Shimadzu LCMS-8060			

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

LPGC Column	Rtx-5ms 15m x 0.53mm x 1.0µm w/ Hydroguard 5m x 0.18mm (cat.# 11800)
Injection	Splitless, 1 µL (0.5 min splitless time, 20 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	80°C (hold 1 min) to 330°C (hold 5.50 min) by 45 °C/min
Carrier Gas	He, at a constant flow of 2.0 mL/min
Transfer line T	290°C
Source T	325°C
Instrument	Thermo Trace 1310-TSQ 8000

Results and Discussion

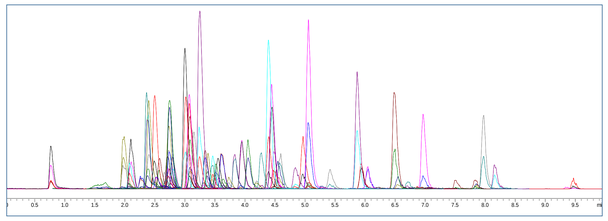

Figure 3. LC-MS/MS amenable contaminants extracted from a chocolate sample spiked at 100 ng/g.

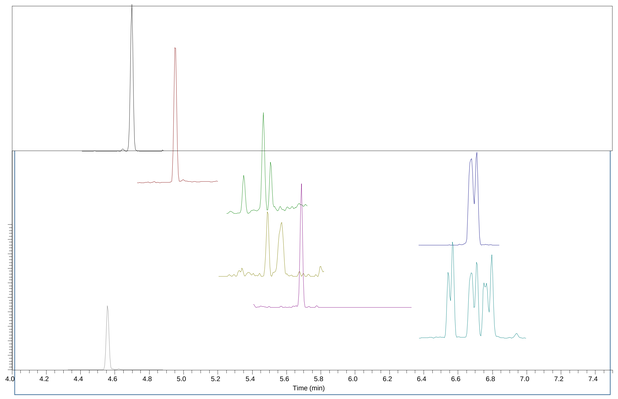

Figure 4. LPGC-MS/MS amenable pesticides extracted from a chocolate sample spiked at 100 ng/g.

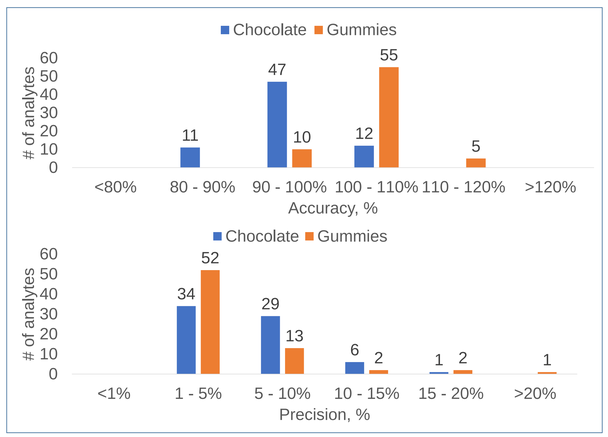

Figure 5. Accuracy and precision estimated for all the LC amenable contaminants spiked at 100 ng/g in matrix (n=4).

- The proposed workflows showed satisfactory results in the quantification of all the pesticides and mycotoxins regulated by the state of California. The LOQ values obtained were significantly below the action levels established by the state of CA in cannabis goods (not inhalable).
- In the case of chocolate samples, satisfactory results were obtained for all target analytes with good linearity (>0.99), accuracy (81-114%), and precision (RSD values <23%).

Table 3. Figures of merit corresponding to GC amenable pesticides analyzed in chocolate and gummies via LPGC-MS/MS.

Contaminants	Action level, ng/g	Choc. LOQ, ng/g	Gummy LOQ, ng/g	Low conc. (10 ng/g, n=4)		Medium conc. (100 ng/g, n=4)	
				Choc. Accuracy (%RSD)	Gummy, Accuracy (%RSD)	Choc. Accuracy (%RSD)	Gummy, Accuracy (%RSD)
PCNB	200	10	20	104 (8)	-	95 (9)	110 (11)
Methyl parathion	100	5	5	110 (5)	108 (6)	97 (4)	106 (5)
Chlordane	100	50	50	-	-	101 (17)	119 (13)
Chlorfenapyr	100	10	20	110 (11)	-	107 (18)	110 (23)
Cyfluthrin	1000	5	5	110 (10)	111 (6)	102 (10)	110 (16)
Cypermethrin	1000	10	5	115 (8)	117 (13)	100 (13)	108 (18)

Table 4. Results corresponding to cannabinoids analysis in chocolate and gummy samples via HPLC-UV (n=3).

Cannabinoid	Spiking conc. 0.2 mg/g		Spiking conc. 0.5 mg/g	
	Chocolate	Gummy	Chocolate	Gummy
Cannabidiolic acid (CBDA)	0.2 ± 0.01	0.2 ± 0.01	0.5 ± 0.01	0.5 ± 0.01
Cannabigerol (CBG)	0.2 ± 0.01	0.2 ± 0.004	0.5 ± 0.02	0.5 ± 0.03
Cannabidiol (CBD)	0.2± 0.01	0.2± 0.003	0.5 ± 0.02	0.5 ± 0.03
Cannabinol (CBN)	0.2± 0.01	0.2± 0.01	0.5 ± 0.01	0.5 ± 0.02
Delta 9 tetrahydrocannabinol (Delta 9 THC)	0.2± 0.01	0.2± 0.01	0.5 ± 0.01	0.5 ± 0.02
Tretrahydrocannabinolic acid (THCA)	0.2± 0.01	0.2± 0.01	0.5 ± 0.02	0.5 ± 0.01

- For gummies, RSDs values below 24% were obtained for all the analytes at all the concentration levels tested. Accuracy values were within 75–118%, and coefficients of determination (R2) were all above 0.99.
- Gas chromatography amenable pesticides were analyzed using LPGC-MS/MS which allowed for a total method run of 12 min. Both LC and LPGC-MS/MS methods had the same analysis time, ensuring satisfactory sample throughput.
- The results for cannabinoids analysis demonstrated that extracts collected for contaminants determination is also suitable for potency testing. As can be seen in Table 4, all the cannabinoids were successfully quantified at 0.2 and 0.5 mg/g by employing a calibration curve prepared in solvent.

Conclusions

Easy and effective workflows for the analysis of pesticides, mycotoxins, and cannabinoids in cannabis edibles were developed. Satisfactory results in terms of figures of merit were obtained for all the target contaminants. Fast GC analysis was possible by employing an LPGC setup that enabled faster elution of analytes of interest and co-extracted interferences while maintaining resolution. Overall, the presented workflow streamlines work for cannabis testing labs by enabling the satisfactory quantitation of multiple analyte classes in chocolate and gummy samples using a single extract.

References

- Reyes-Garcés N, Myers C. J Sep Sci. 2021; 44: 2564–2576. https://doi.org/10.1002/jssc.202001265
- Reyes-Garcés N. Analysis of Pesticides, Mycotoxins, and Cannabinoids in Cannabis Gummies. Technical article: https://www.restek.com/en/technical-literature-library/articles/analysis-of-pesticides-mycotoxins-and-cannabinoids-in-cannabis-gummies/?term=gummies