LD 48 Testimony
1.30.23

Good morning, Chair Hickman, Chair Supica and esteemed members of the VLA committee. My name is Brett Messer. I'm the owner and operator of Brigid Farm located in Saco. As I shared with you guys' last week, I am in the final semester of my Medical Cannabis Science Master's Degree at the University of Maryland, School of Pharmacy.

I tried to cram a bit too much for last week's testimony, so I'll keep it shorter and sweeter this week. And I will follow up with additional information via written testimony.

The lab testing issue before you today is rather simple. The scientific community has very different standards for analytical testing methodology in Cannabis than the Maine State Law. The understood variance is 20%, double our current state standard. I have attached two scientific journal articles which identify and confirm this statement.

Additionally, we have another major issue. Testing labs in Maine regularly report wildly varying results. This is largely due in part to not having standardized equipment and protocol for cannabis analytics. I did a study by submitting the same sample, to three labs, and I submitted the same sample to each lab twice. Not only were there greater than 10% variance between labs, but there were also instances of 10% variance at the same lab with the same sample. I have attached all the lab reports and a graph summarizing the study.

This shouldn't be decided based on the opinion of a business owner or lobbyist, the scientific community is already in agreement based on the currently available data.

On to packaging; let's start with some facts.

In 2019 the State of Maine had 145 alcohol related deaths, of those 42 were related to drunk driving, and 5 of those individuals were under the age of 21. 22% of Maine youth's aged 12 to 20 self-reported the use of alcohol which places Maine among the highest in the nation. This rate is currently rising year by year.

In contrast the self-reported rate of teen cannabis use is 9%. That number is in decline both here in Maine and nationwide as the conversation around cannabis changes. Additionally, there have been no reported cannabis related deaths in Maine since medical cannabis was first legalized in 1999, with nearly 3000 alcohol related deaths during that time.

So, this begs the question. Why do we seek regulate Cannabis to a higher degree than alcohol. Take a walk down the aisle of local Hannaford and take in the beer packaging. It's color, its artistic, its attractive among its competitors.

We seek to achieve the same within cannabis marketing. Our marketing efforts are already severely limited, and therefore we wish to make our products stand out on the shelf in stores, which already have age restricted entry requirements.

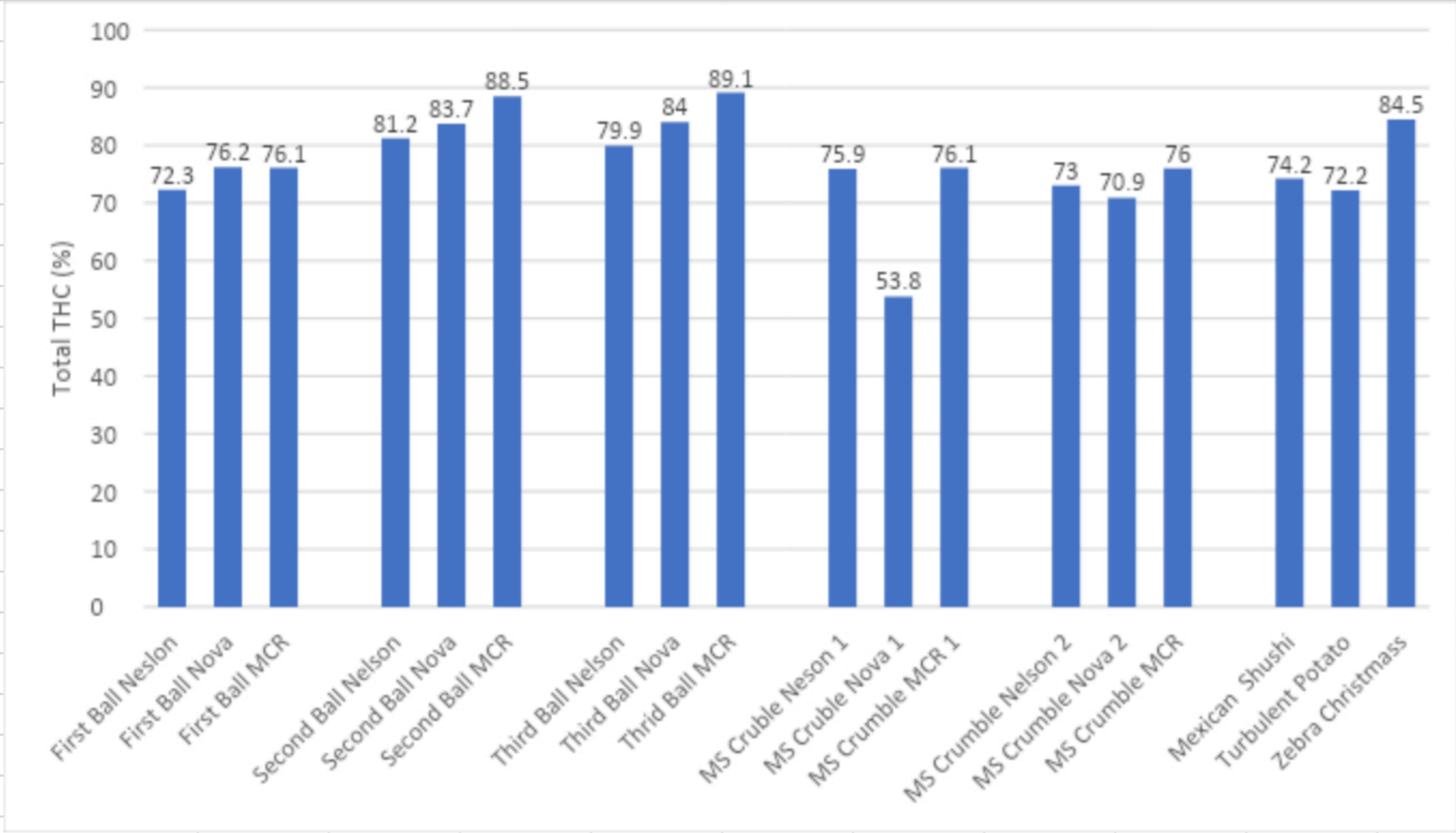
The Human, Animal and Fruit logo provision needs to be eliminated entirely, not modified. There is no data that supports this keeps cannabis out of the hands of children. The proposed changes make this part of the law even more vague and ambiguous than it is currently. The last thing we need in our cannabis laws is ambiguity, because as is, inspectors are notoriously known for interpreting things the way they see fit, which in my experience is usually not correct or a gross overstep of authority. Quite frankly, I think this is yet another play from the corporate, multi state cannabis operators to stimy their competition as they realize their large marketing teams still can't connect with consumers.

At my company we employ talented local artists to create packages that are attractive and stand out to consumers, much like the beer industry. Our average consumer at my business is 36 years old. We've never sought to create packaging attractive to children, though some of our packaging would be in violation of this proposed law. We make packaging that is attractive to adults. It's not possible to eliminate the possibility of someone under 18 being attracted to that package. Let's use Apple computers for example. They use a fruit. They use a cartoon style logo. Their core customer is not a child, its 25 to 34 years old, much like cannabis. It's absolutely silly that we are even wasting lawmakers time with any conversation other than eliminating this phrasing from the law entirely.

Thank you,
Brett Messer

Key	Nelson	Nova	MCR
First Ball	SPD	SPH	SD
Second Ball	SBTC	PTW	FYT
Third Ball	PT	CM	ZV
Midnight Snack Crumble	1C	1B	1A
	2C	2B	2A
Flower Sample	Mexican Shushi	Turbulent Potato	Zebra Christmass

Sample	Total THC
First Ball Neslon	72.3
First Ball Nova	76.2
First Ball MCR	76.1
Second Ball Nelson	81.2
Second Ball Nova	83.7
Second Ball MCR	88.5
Third Ball Nelson	79.9
Third Ball Nova	84
Thrid Ball MCR	89.1
MS Cruble Neson 1	75.9
MS Cruble Nova 1	53.8
MS Crumble MCR 1	76.1
MS Crumble Nelson 2	73
MS Crumble Nova 2	70.9
MS Crumble MCR	76
Mexican Shushi	74.2
Turbulent Potato	72.2
Zebra Christmass	84.5



RP221107016

120 York Street
Kennebunk, ME 04043
(207) 467-3478

NELSON ANALYTICAL LAB

ISO 17025:2017 Accreditation
ANAB Certificate Number: AT-2169
Maine CDC Accreditation MTF001
Office of Marijuana Policy MTF328

Report Date: 07 November 2022

Brigid Farm:
PO Box 1751 Saco ME , 04072:

Enclosed are the results of analytical testing performed on the following samples:

Laboratory ID	Sample Location	Date sampled	Date received
C22110129.01	**PT**	03-Nov-22 00:00	03-Nov-22 15:23
C22110129.02	**SBTC**	03-Nov-22 00:00	03-Nov-22 15:23
C22110129.03	**SPD**	03-Nov-22 00:00	03-Nov-22 15:23
C22110129.04	**1C**	03-Nov-22 00:00	03-Nov-22 15:23
C22110129.05	**2C**	03-Nov-22 00:00	03-Nov-22 15:23
C22110129.06	**Mexican Sushi**	03-Nov-22 00:00	03-Nov-22 15:23

If you have any questions concerning this report, please feel free to contact the laboratory at 207-467-3478.

Lorri Maling
Laboratory Director

120 York Street
Kennebunk, ME 04046
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Amount Received:

Collected by: Client

REPORT OF ANALYSIS

Brigid Farm

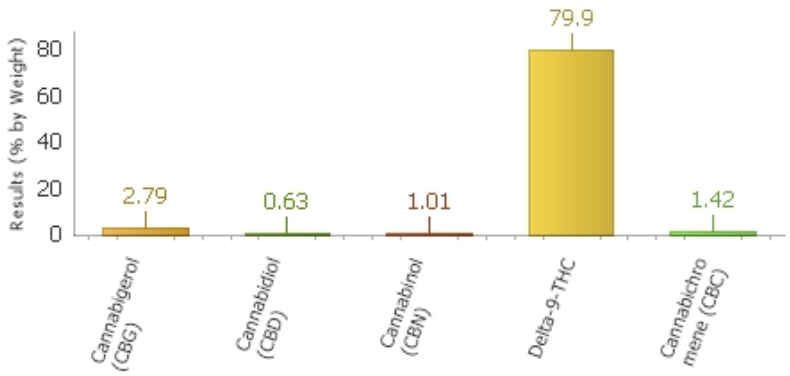
C22110129.01

PT(Concentrate)

Date sampled : 11/03/2022

Reported Date: 11/07/2022

Temp Received:



Cannabinoids by HPLC

Analyte	Result	Reporting Limit	Units	Q	Analyzed	Method	Analyst	Pass/Fail Limit	Test Remarks
Cannabidivarin (CBDV)	ND	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Cannabidiolic acid (CBDA)	ND	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Cannabigerolic acid (CBGA)	ND	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Cannabigerol (CBG)	**2.79**	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Cannabidiol (CBD)	**0.630**	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Tetrahydrocannabivarin (THCV)	ND	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Cannabinol (CBN)	**1.01**	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Delta-9-THC	**79.9**	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Delta-8-THC	ND	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Cannabichromene (CBC)	**1.42**	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
THCA-A	ND	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	

Total Cannabinoids by HPLC (Calculated)

Analyte	Result	Reporting Limit	Units	Q	Analyzed	Method	Analyst	Pass/Fail Limit	Test Remarks
CBD+CBDA- Calculated	**0.630**	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Total CBD-(Max CBD) Calculated	**0.630**	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
THC+THCA- Calculated	**79.9**	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Total THC-(Max THC) Calculated	**79.9**	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Total THC-(Max THC+D8) Calculated	**79.9**	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	
Total Cannabinoids- Calculated	**85.7**	0.1	% by Weight		11/04/2022 22:05	HPLC SOP-7	NRS	N/A	

Results as reported above relate only to samples as submitted, unless specifically noted otherwise.

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Amount Received:

Collected by: Client

REPORT OF ANALYSIS

Brigid Farm

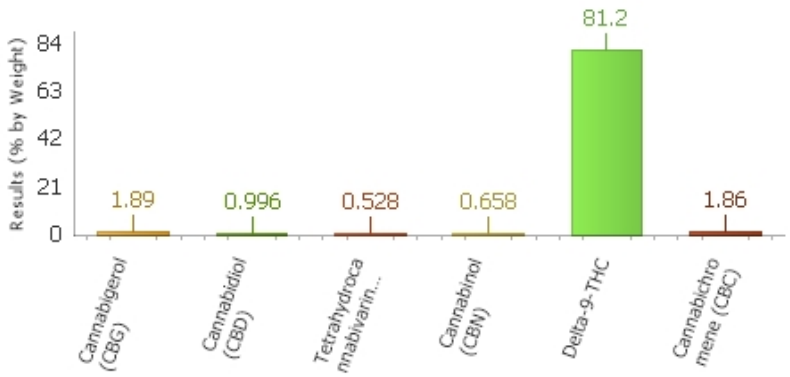
C22110129.02

SBTC(Concentrate)

Date sampled : 11/03/2022

Reported Date: 11/07/2022

Temp Received:



Cannabinoids by HPLC

Analyte	Result	Reporting Limit	Units	Q	Analyzed	Method	Analyst	Pass/Fail Limit	Test Remarks
Cannabidivarin (CBDV)	ND	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Cannabidiolic acid (CBDA)	ND	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Cannabigerolic acid (CBGA)	ND	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Cannabigerol (CBG)	**1.89**	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Cannabidiol (CBD)	**0.996**	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Tetrahydrocannabivarin (THCV)	**0.528**	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Cannabinol (CBN)	**0.658**	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Delta-9-THC	**81.2**	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Delta-8-THC	ND	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Cannabichromene (CBC)	**1.86**	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
THCA-A	ND	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	

Total Cannabinoids by HPLC (Calculated)

Analyte	Result	Reporting Limit	Units	Q	Analyzed	Method	Analyst	Pass/Fail Limit	Test Remarks
CBD+CBDA- Calculated	**0.996**	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Total CBD-(Max CBD) Calculated	**0.996**	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
THC+THCA- Calculated	**81.2**	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Total THC-(Max THC) Calculated	**81.2**	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Total THC-(Max THC+D8) Calculated	**81.2**	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	
Total Cannabinoids- Calculated	**87.1**	0.1	% by Weight		11/04/2022 22:57	HPLC SOP-7	NRS	N/A	

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REPORT OF ANALYSIS

Brigid Farm

C22110129.03

SPD(Concentrate)

Date sampled : 11/03/2022

Reported Date: 11/07/2022

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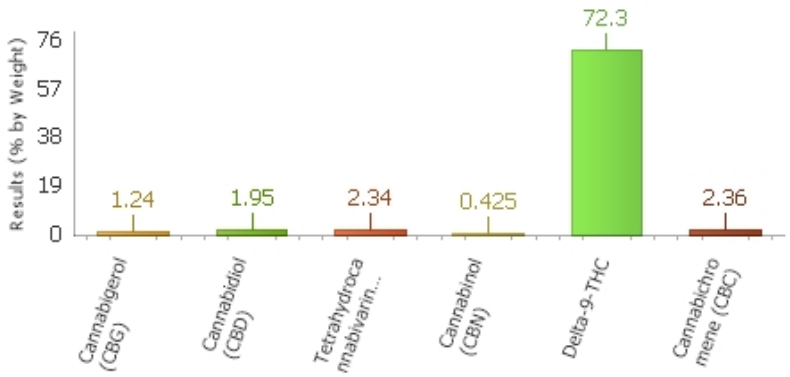

Cannabinoids by HPLC

Analyte	Result	Reporting Limit	Units	Q	Analyzed	Method	Analyst	Pass/Fail Limit	Test Remarks
Cannabidivarin (CBDV)	ND	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Cannabidiolic acid (CBDA)	ND	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Cannabigerolic acid (CBGA)	ND	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Cannabigerol (CBG)	**1.24**	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Cannabidiol (CBD)	**1.95**	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Tetrahydrocannabivarin (THCV)	**2.34**	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Cannabinol (CBN)	**0.425**	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Delta-9-THC	**72.3**	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Delta-8-THC	ND	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Cannabichromene (CBC)	**2.36**	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
THCA-A	ND	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	

Total Cannabinoids by HPLC (Calculated)

Analyte	Result	Reporting Limit	Units	Q	Analyzed	Method	Analyst	Pass/Fail Limit	Test Remarks
CBD+CBDA- Calculated	**1.95**	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Total CBD-(Max CBD) Calculated	**1.95**	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
THC+THCA- Calculated	**72.3**	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Total THC-(Max THC) Calculated	**72.3**	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Total THC-(Max THC+D8) Calculated	**72.3**	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	
Total Cannabinoids- Calculated	**80.6**	0.1	% by Weight		11/04/2022 23:08	HPLC SOP-7	NRS	N/A	

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REPORT OF ANALYSIS

Brigid Farm

C22110129.04

1C(Concentrate)

Date sampled : 11/03/2022

Reported Date: 11/07/2022

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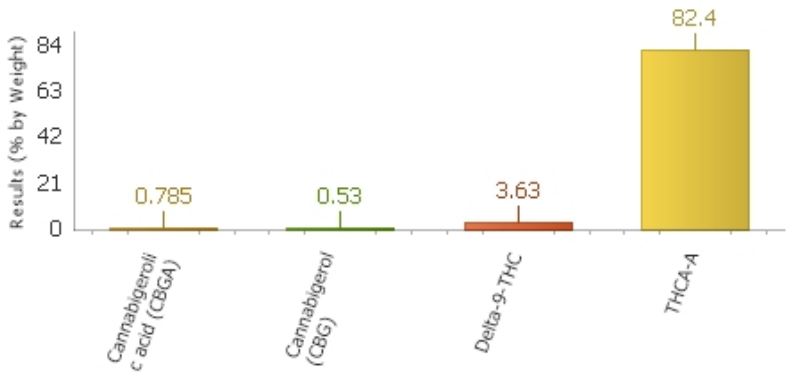

Cannabinoids by HPLC

Analyte	Result	Reporting Limit	Units	Q	Analyzed	Method	Analyst	Pass/Fail Limit	Test Remarks
Cannabidivarin (CBDV)	ND	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Cannabidiolic acid (CBDA)	ND	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Cannabigerolic acid (CBGA)	**0.785**	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Cannabigerol (CBG)	**0.530**	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Cannabidiol (CBD)	ND	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Tetrahydrocannabivarin (THCV)	ND	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Cannabinol (CBN)	ND	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Delta-9-THC	**3.63**	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Delta-8-THC	ND	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Cannabichromene (CBC)	ND	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
THCA-A	**82.4**	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	

Total Cannabinoids by HPLC (Calculated)

Analyte	Result	Reporting Limit	Units	Q	Analyzed	Method	Analyst	Pass/Fail Limit	Test Remarks
CBD+CBDA- Calculated	ND	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Total CBD-(Max CBD) Calculated	ND	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
THC+THCA- Calculated	**86.0**	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Total THC-(Max THC) Calculated	**75.9**	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Total THC-(Max THC+D8) Calculated	**75.9**	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	
Total Cannabinoids- Calculated	**87.3**	0.1	% by Weight		11/04/2022 23:18	HPLC SOP-7	NRS	N/A	

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Amount Received:

Collected by: Client

REPORT OF ANALYSIS

Brigid Farm

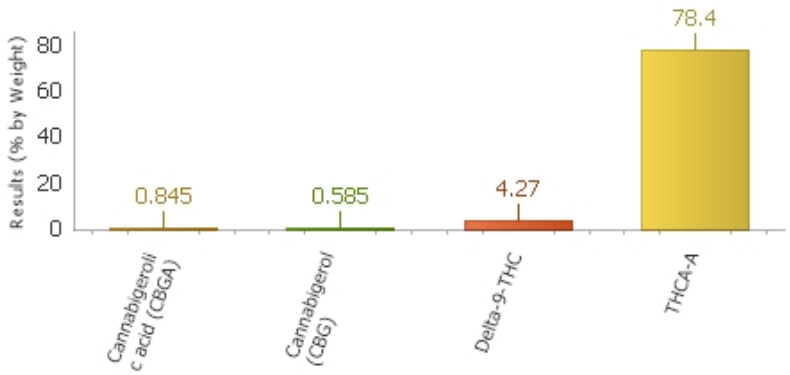
C22110129.05

2C(Concentrate)

Date sampled : 11/03/2022

Reported Date: 11/07/2022

Temp Received:



Cannabinoids by HPLC

Analyte	Result	Reporting Limit	Units	Q	Analyzed	Method	Analyst	Pass/Fail Limit	Test Remarks
Cannabidivarin (CBDV)	ND	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Cannabidiolic acid (CBDA)	ND	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Cannabigerolic acid (CBGA)	**0.845**	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Cannabigerol (CBG)	**0.585**	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Cannabidiol (CBD)	ND	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Tetrahydrocannabivarin (THCV)	ND	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Cannabinol (CBN)	ND	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Delta-9-THC	**4.27**	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Delta-8-THC	ND	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Cannabichromene (CBC)	ND	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
THCA-A	**78.4**	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	

Total Cannabinoids by HPLC (Calculated)

Analyte	Result	Reporting Limit	Units	Q	Analyzed	Method	Analyst	Pass/Fail Limit	Test Remarks
CBD+CBDA- Calculated	ND	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Total CBD-(Max CBD) Calculated	ND	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
THC+THCA- Calculated	**82.7**	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Total THC-(Max THC) Calculated	**73.0**	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Total THC-(Max THC+D8) Calculated	**73.0**	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	
Total Cannabinoids- Calculated	**84.1**	0.1	% by Weight		11/04/2022 23:28	HPLC SOP-7	NRS	N/A	

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Amount Received:

Collected by: Client

REPORT OF ANALYSIS

Brigid Farm

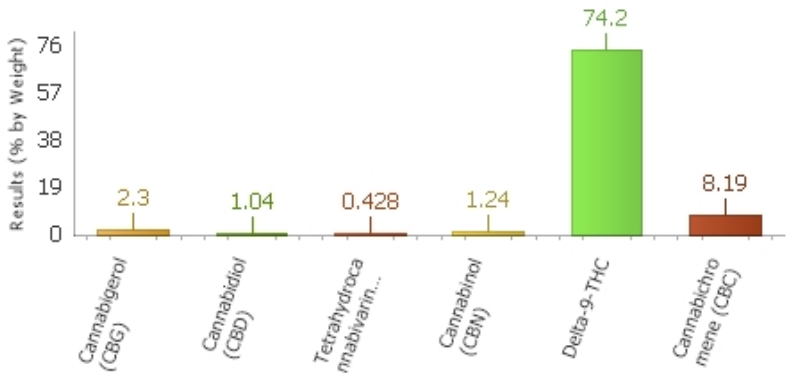
C22110129.06

Mexican Sushi(Concentrate)

Date sampled : 11/03/2022

Reported Date: 11/07/2022

Temp Received:



Cannabinoids by HPLC

Analyte	Result	Reporting Limit	Units	Q	Analyzed	Method	Analyst	Pass/Fail Limit	Test Remarks
Cannabidivarin (CBDV)	ND	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Cannabidiolic acid (CBDA)	ND	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Cannabigerolic acid (CBGA)	ND	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Cannabigerol (CBG)	**2.30**	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Cannabidiol (CBD)	**1.04**	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Tetrahydrocannabivarin (THCV)	**0.428**	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Cannabinol (CBN)	**1.24**	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Delta-9-THC	**74.2**	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Delta-8-THC	ND	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Cannabichromene (CBC)	**8.19**	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
THCA-A	ND	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	

Total Cannabinoids by HPLC (Calculated)

Analyte	Result	Reporting Limit	Units	Q	Analyzed	Method	Analyst	Pass/Fail Limit	Test Remarks
CBD+CBDA- Calculated	**1.04**	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Total CBD-(Max CBD) Calculated	**1.04**	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
THC+THCA- Calculated	**74.2**	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Total THC-(Max THC) Calculated	**74.2**	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Total THC-(Max THC+D8) Calculated	**74.2**	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	
Total Cannabinoids- Calculated	**87.4**	0.1	% by Weight		11/04/2022 23:39	HPLC SOP-7	NRS	N/A	

Results as reported above relate only to samples as submitted, unless specifically noted otherwise.

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Notes and Definitions

Note: All sample results are based on samples as they are received. Not all potential/existing hazards were evaluated. Unless otherwise noted below, analyses were performed without significant modifications and QC met the quality standards outlined in the methods reported. For purposes of reporting the terms marijuana and cannabis are used interchangeably. The Pass/Fail column on the report references Maine Adult Use acceptance limits. The State of Maine does not require Medical Marijuana or Hemp to meet these acceptance limits currently.

Results for the Maine Adult Use program are entered into the Metrc system. Due to reporting requirements some results are entered in Metrc as Zero. This is not scientifically accurate. Please refer to the final pdf report for the accurate reporting information and reporting limits.

Heat activation of cannabis products converts THCA to THC and CBDA to CBD in a time and temperature dependent manner. This conversion is known as decarboxylation and results from the loss of CO2 during heating.
Total THC (Max THC) = Delta 9 THC + (THCA x 0.877)- Calculation required for Maine Adult Use program
Total THC (Max THC+D8) = Delta 8 THC + Delta 9 THC + (THCA x 0.877)
Total CBD (Max CBD) = CBD + (CBDA x 0.877)

Nelson Analytical is accredited for testing by ISO/IEC 17025:2017 and certified by ME CDC for the following parameters only:
Cannabinoids: Cannabinol (CBN), Cannabidiol (CBD)*, Cannabidiolic Acid (CBDA)*, Cannabigerol (CBG), Cannabigerolic Acid (CBGA), Cannabichromene (CBC), delta-9-THC*, delta-8-THC, THCA-A*, Tetrahydrocannabivarin (THCV), Cannabidivarin (CBDV) by High Pressure Liquid Chromatography (HPLC). Internal SOP-1/SOP-7 Analysis of Cannabinoids *NOTE: ME CDC certification for CBD, CBDA, Delta 9 THC and THCA-A, Total THC and Total CBD. Edible samples for Maine Adult use may not exceed 10 mg/serving or 100 mg/package.

Homogeneity (Internal SOP-1/SOP-7 Analysis of Cannabinoids)- samples for edibles and concentrates must be within 15% for Maine Adult Use.

Visual Inspection - Foreign Material Testing (Internal SOP-24-Visual Inspection)

% Moisture (Loss on drying) (Internal SOP 59 - % Moisture)
Metals Preparation and Analysis: Arsenic, Cadmium, Lead and Mercury (SOP-17- ICP MS based on EPA 200.8)

Water Activity (SOP-53-Water Activity-based on ASTM D81918) For Maine Adult Use the water activity should be <0.65 for plant and <0.85 for edibles or other products.

Mycotoxins: Total Aflatoxin and Ochratoxin by ELISA - Internal SOP-4 Total Aflatoxin and Ochratoxin. For Maine Adult Use Total Mycotoxins are only evaluated after a yeast and mold failure. They must be 20 ppb or less for a passing result.

Yeast and Mold (based on AOAC Method 997.02/2014.05), Total Coliform and E. coli (based on AOAC Method 991.14) E. Coli P/A (based on AOAC 991.14 Modified with enrichment before plating), Aerobic Plate Count (based on AOAC Method 990.12), Enterobacteriaceae (based on OMA 2003.01), Salmonella (based on AOAC 2014.01) SOP-3-Microbiologial analysis by Petri Film.

Microbial limits for Maine Adult Use are as follows for all but concentrate samples:
Yeast and Mold 10,000cfu/g or less, Total Aerobic Bacteria 100,000 cfu/g or less, Total Coliform 1000 cfu/g or less, Enterobacteriaceae 1000 cfu/g or less, E. coli and Salmonella must be negative per gram.
For concentrates the microbial limits are as follows:
Yeast and Mold 1000 cfu/g or less, Total Aerobic Bacteria 10,000 cfu/g or less, Total Coliform 100 cfu/g or less, Enterobacteriaceae 100 cfu/g or less, E. coli and Salmonella must be negative per gram

Residual Solvents: (SOP-63 by GC/MS Headspace) The acceptance limits are in mg/kg in () next to the compound: Acetone(5000), Acetonitrile(410), Butanes(5000), Ethanol(5000), Ethyl Acetate(5000), Ethyl Ether(5000), Heptanes(5000), Hexane(290), Isopropyl alcohol(5000), Methanol(3000), Pentane(5000), Propane(5000), Toluene(890), Total Xylenes(2170), 1,2 Dichloroethane(1), Benzene(1), Chloroform(1), Ethylene Oxide(1), Methylene Chloride(1), Trichloroethylene(1).

< or ND - Analyte result not detected above the method reporting limit.
All sample results are reported on an “as received” basis.
Edibles are reported in mg/serving. The serving size is defined by the customer for Adult Use testing.
If the serving size is not defined by the customer (for R&D or Medical testing), the number reported is based on the weight of one unit of the product or as defined on the customer label.
The mg/serving reported are based on weights of the serving size taken at the laboratory or supplied by the customer. The mg/package results reported are based on information supplied by the customer.
Edible conversion calculation: mg/g in serving x weight of serving = mg per serving
Mg/package conversion: mg/serving x servings per package = mg/package
Laboratory uncertainty is calculated and updated on a regular basis and will be reported with lab results as needed or requested.
Samples are extracted and analyzed on the same day unless otherwise noted.
Cannabinoids, Residual Solvents and Terpene Analysis are based on laboratory developed methods. All other test methods are based on established EPA, USP or FDA methods.
Matrix matched quality control check samples for marijuana are available for microbiological analysis in a hemp-based QC. Other matrix matched quality control samples for most matrices may be available for hemp but do not currently exist in marijuana. Due to this unavailability, even ISO/IEC validated methods cannot be fully verified for the efficiency and accuracy of the marijuana extraction and analysis in any current Maine Testing facility.
To convert mg/ml to a % percentage move the decimal place one to the left.

QUALIFIER DEFINITION

NELSON ANALYTICAL LAB

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REPORT OF ANALYSIS

Laboratory ID: C22110129

NH ELAP Accreditation #NH2018
Maine State Certification # ME00015
Maine Radon Certification # ME17500

Qualifier Definition

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The analytical landscape of cannabis compliance testing

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ABSTRACT

Owing to the lack of federal oversight of recreational and medical cannabis in the United States, a patchwork of regulatory guidelines exists for compliance testing. Adding to this complexity is the fact that Canadian cannabis regulations differ from those in any of the state mandated regulatory jurisdictions and, at the time of writing, cannabis was only recently legalized in Mexico. Therefore, from a North American perspective, cannabis testing represents a significant regulatory landscape to navigate. This not only makes things confusing for those involved in cannabis production and processing, it also creates challenges for those in the analytical testing world when they have to understand and develop methods to be compliant with these various regulatory jurisdictions. In this review article, the current state of analytical chemistry knowledge for cannabis compliance testing is summarized, with an emphasis on suitable techniques and some common problems to avoid. This includes summaries of analytical methods for potency, terpenes, pesticides, mycotoxins, residual solvents, heavy metals and microbiology.

KEYWORDS

Cannabis; contaminants; microbiology; pesticides; potency

GRAPHICAL ABSTRACT

Introduction

Widely known and studied, *Cannabis sativa* L. (cannabis) produces a class of secondary metabolites called phytocannabinoids (heretofore referred to simply as cannabinoids); terpenophenolic compounds that are concentrated in a resinous secretion produced in the glandular trichomes of cannabis.[1] While only a handful of cannabinoids have been objectively characterized for their biological activity, more than 100 cannabinoid variants have been identified in cannabis.[2] Though used for its medicinal properties for hundreds of years, the characterization of the cannabinoids found in cannabis (and hemp) has been a focus of law

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enforcement for decades, as tetrahydrocannabinol (THC) has been classified as a Schedule 1 substance under the United States Controlled Substances Act since 1970. More recently, the US effectively legalized hemp, defined as having "the plant *Cannabis sativa Linnaeus* … with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis." in the Agriculture Improvement Act of 2018, commonly referred to as the Farm Bill.[3] Similar measures for hemp have been adopted by the European Union.[4] Meanwhile, the move to legalize cannabis consumption, for medical and/or recreational use in many North American regulatory jurisdictions has spurred research and development pertaining to cannabis. It has also lead to a burgeoning analytical testing landscape to support consumer safety and regulatory compliance.

Included in the various regulatory frameworks are requirements to test cannabis and cannabis-derived products for endogenous metabolites and exogenous contaminants. Paramount among the endogenous metabolites included in legislation are cannabinoids, and in some regulations, terpenes. For exogenous contaminants, all jurisdictions mandate testing for residual solvents (in derivative products), pesticides, mycotoxins, heavy metals and microbial contaminants. The particulars of these testing requirements change as a function of regulatory jurisdiction.

Due to its nascent status and heterogeneous regulatory requirements, the analytical testing infrastructure to support cannabis testing is evolving. This makes things confusing for those involved in cannabis production and processing and creates challenges for those in the analytical testing world when they have to understand and develop methods to be compliant with various (and evolving) regulatory requirements. In this review article, the current state of analytical chemistry in support of cannabis compliance testing is discussed. This includes thorough evaluations and commentary on published methods for potency, terpenes, pesticides, mycotoxins, residual solvents, heavy metals and microbiology.

Potency

There is a growing interest in cannabinoids beyond THC and (cannabidiol) CBD, led currently by δ8-THC. As a result, it has become increasingly important to implement methods for the quantitative measurement of cannabinoids in a variety of matrices. This includes so-called potency testing, defined here as the quantitation of THC, CBD, their respective acidic forms (tetrahydrocannabinolic acid [THCA] and cannabidiolic acid [CBDA]) and cannabinol (CBN), as well as the quantitation of more complex cannabinoid profiles (*vide infra*). The discussion herein on the quantitation of cannabinoids is a distinct analytical process from determining total THC on a dry weight basis. For viewpoints on dry weight determination, a critical aspect of hemp and cannabis legislation, we refer the reader to the work currently being done by the ASTM International Committee D37 and the Cannabis Analytical Science Program (CASP) coordinated by AOAC International.

Sample preparation for cannabinoid quantitation has recently gained focus as new methods and matrices reach the consumer marketplace. Traditionally, cannabis oil and flower samples used liquid-liquid and liquid-solid extraction procedures, respectively. The solvent systems have evolved from methanol/chloroform extractions,[1,5] most recently replaced by similar performing methanol/water or acetonitrile/methanol procedures to reduce the health risks for laboratory personnel when using chloroform.[5–7] Sampling and homogenization of plant materials is also a critical factor, especially for heterogenous materials like cannabis flower and concentrates. While sonication is the most common and supported technique,[8] a thorough understanding of sampling procedures and homogeneity should underpin all cannabis compliance testing programs.[9] As more matrices containing cannabinoids enter the marketplace (e.g., pet food, shampoo, bath bombs, cereal, etc.), there may be an evolving need for modified extraction procedures. For example, recent publications have focused on edible matrices like chocolates, where matrix effects have been shown to have an important impact on method accuracy.[10] Modified extraction procedures will have a measurable impact on method performance for such challenging matrices.[11]

The quantitative analysis of cannabinoids may by performed using high-pressure liquid chromatography (HPLC) and gas chromatography (GC) for analytical separation prior to detection, but their comparative suitability is a widely discussed topic.[1,4,9,12–14] Early focus of analytical analyses were related to the legality and criminalization of cannabis, led by forensic chemistry. Therefore, the focus was on the total THC content of a sample, defined as the sum of THC and THCA. Since GC relies on the thermal volatilization of samples during injection, it converts THC into THCA. However, determining total THC in this way requires the acidic cannabinoids to undergo *quantitative and reproducible* thermal decarboxylation in the GC.[12] The degree of decarboxylation during GC analysis depends on many factors including, but not limited to, inlet and oven temperatures, injection volume and diluents.[13] This typically makes it difficult to accurately quantitate THC and CBD using GC, let alone the quantitation of complex cannabinoid profiles.[1] A systemic analysis of cannabinoid decarboxylation during GC analysis has, to the authors knowledge, not been published. Derivatization, usually in the form of trimethylsilylated compounds,[13] can be used to stabilize acidic cannabinoids for GC analysis, but in a modern fee-for-service cannabis testing environment, the added material and labor costs associated with derivatization limit their adoption when throughput and testing costs are primary market drivers for cannabinoid quantitation.

Recently, HPLC and ultra-high pressure liquid chromatography (UHPLC)[15] have been implemented in laboratories in order to provide faster analysis times and additional information to the market. Complex profiling of the acidic and neutral cannabinoids can provide important information about the status of the plant material, including harvest maturity and storage conditions that could be valuable to manufacturers and cultivators.[13] While potency assays

typically have 5–11 target cannabinoids, more cannabinoids of interest and importance, biologically and economically, are being added to cannabinoid profiling analyses.[5,16] The addition of more cannabinoids is currently limited by the availability of analytical standards, which at the time of writing includes 24 analytes. The stability of mixed calibration standards should be monitored, as examples of significant changes in cannabinoid concentration at room temperature have been reported.[6] There are many published HPLC potency methods, with the vast majority of them using conventional C18 reversed phase column chemistry.[12] Both methanol and acetonitrile can be used as organic modifiers, yielding different retention factors due to elutropic strength and possibly divergent selectivity depending on the column phase.[17] It is clear from the literature that in order to achieve robust HPLC analyses with consistent chromatographic retention time, peak shape and resolution, precise pH and temperature control are required.[5,6,13] Buffer concentration and pH are especially important, as most methods prefer a pH $\sim$ 3.3 and many of the acidic cannabinoids have a $pK_a \sim 3.4$,[13] leading to the potential for peak shape and RT shifting since a given acidic cannabinoid will exist in solution in different charge states close to its pK_a.

Most published methods[1,4,6,7,13,14] use optical detection, typically photodiode arrays (PDAs), due to their ease of use and relatively low cost of operation. Additionally, PDAs can provide UV spectra for identification and peak purity information.[13] However, optical detection has some limitations in specificity, as many cannabinoids are structurally similar, and thus have similar UV spectra. While chromatography is able to address many interference challenges with optical detection, there are many examples of cannabinoids and/or other interferences co-eluting, causing problems in identification and quantification.[18] Such considerations become more important as the panel of cannabinoids is expanded and the number of matrices are increased, as the potential for chromatographic interferences increases. This is especially true when quantitating lower concentration cannabinoids like cannabigerol (CBG) or tetrahydrocannabivarin (THCV), where the relative impact of chromatographic interferences on quantitative accuracy would be higher. Recent publications are utilizing the advantages of mass spectrometry (MS) in series with liquid chromatography to overcome these challenges.[5] LC-MS methods come with selectivity advantages via the added dimension of mass-to-charge analysis and product ion ratios (i.e., multiple reaction monitoring [MRM]). Even in single-ion-monitoring (SIM) experiments, many selectivity challenges posed by interferences can be reduced or eliminated.[18]

LC-MS methodologies, however, have their own limitations. The largest of these being the cost increase and added laboratory expertise required for successful implementation of LC-MS methods.[6] Since cannabinoids like Δ^8-THC, Δ^9-THC, *exo*-THC, CBD, CBC, and CBL are structural isomers (i.e., isobaric), MS detection still requires chromatographic resolution, as these compounds all have the same mass-to-charge ratio and their product ion spectra are, for practical purposes, indistinguishable. Although, in general LC-MS methods are faster, despite the required isomer resolution since mass-resolved analytes typically can co-elute without negatively impacting method performance. Another MS-based method that could help overcome isomeric separation limitations is ion mobility. While not extensively explored, there is evidence that ion mobility can successfully differentiate THC and CBD.[19]

Terpenes

Second to cannabinoids as the dominant (by weight percent) secondary metabolite produced by cannabis, terpenes may constitute 0.5–5% w/w of the inflorescence.[9,20] Terpenes are classified according to the repeating number of five-carbon isoprene units used for their biosynthesis: mono- (2), sesqui- (3), di- (4) and triterpenoids (5).[21] While the term terpene includes compounds that are simple hydrocarbons (e.g., pinene) and terpenoids refers to compounds containing heteroatoms (e.g., linalool), it is common practice to use "terpenes" more generically to refer to this family of secondary metabolites. This nomenclature convention is used herein. To date more than 200 terpenes have been identified in cannabis, but the 58 monoterpenes and 38 sequiterpenes confirmed represent the compound classes found in the greatest frequency and abundance.[8] Parsing down these exhaustive lists further, cannabis chemotypes are generally dominated by a subset of 10–20 highly prevalent and highly abundant terpenes, with the most studied and frequently observed being β-myrcene, *d*-limonene, α-terpineol, γ-terpinolene, α/β-pinene, linalool, β-caryophyllene (the most abundant sesquiterpene), α-humulene and caryophyllene oxide (the chemical that narcotics control dogs smell when searching for cannabis[8]).[9,22]

Beyond their natural functions in plants (pest defense, etc.), terpenes represent the dominant organoleptic contributors to cannabis—it is these compounds that drive consumer preference for certain types of cannabis due to their distinct aroma and flavor profiles. Savvy cannabis brands have recognized this as an opportunity to curate a diverse product portfolio with consistent sensory attributes, that is highlighted not just by cannabinoid profiles and concentrations, but also terpene profiles and their relative concentrations. Notwithstanding their importance to consumer preference, it is their potential synergy with cannabinoids (and other secondary metabolites in cannabis) to elicit an "entourage" biological effect[21] that underscores their importance to the collective scientific understanding of cannabis. For example, while only a few terpenes have been adequately studied for their biological effects, pinene has been reported to aid memory, which may counteract some of the effects of THC, and β-caryophyllene interacts with the endocannabinoid system and may be responsible for some of the anti-inflammatory effects of cannabis.[22] Further, it has been proposed that terpene profiles, while highly influenced by environmental conditions during plant maturation,[23] could be leveraged to aid in the classification of cannabis chemotypes beyond the typical high THC and high CBD designations.[9] The need for accurate and precise quantitation of terpenes,

therefore, is driven by consumer preference, their potential biological impact and also the potential to aid in the classification of cannabis. As more is discovered about the biological impact of terpenes in cannabis products, it is also possible that policies similar to some states, where terpene quantitation is part of regulatory compliance testing, will be more widely adopted.

Sample preparation for terpene analysis is divided into two categories: alkane-based solid-liquid extraction (SLE) and headspace-based (HS) techniques. To date, the most prevalent approach for the analysis of terpenes in cannabis (and indeed, in other plants and derivative products) has been SLE using a hydrocarbon solvent, typically pentane, hexane or ether.[22–28] The one exception to this was the recent work of Ibrahim et al., who found that ethyl acetate provided the highest extraction efficiency.[29] SLE is favored due to its scalability for throughput and the fact that it is simple and cost-effective. While it is generally assumed that quantitative extraction of terpenes is achieved with this approach, the dearth of certified reference materials (CRMs) for terpenes in cannabis and derivative products makes this assertion a challenge to validate. The principal drawback of SLE for terpene analysis is the fact that it is nonselective, so co-extracted matrix components that are not compatible with a given analytical technique can limit throughput capabilities due to an increased need for instrument maintenance (e.g., the GC inlet will become dirtier more readily than with more selective sample preparation techniques).

Several recent studies have eschewed SLE in favor of static HS[20,29] or HS solid-phase microextraction (HS-SPME).[26] These techniques have the advantage of only sampling volatile constituents in the sample matrix, so fouling of analytical instrumentation is minimized. HS-SPME takes this a step further and only samples volatile analytes that adsorb to a chosen stationary phase. Recent reports of bias in terms of the terpenes that adsorb to the SPME fiber[26,30] suggest that there are still method parameters to be evaluated with this approach before it could see wide adoption in a compliance testing environment. Moreover, SPME is frequently used as a preconcentration step. For terpenes, this is not necessary based on their concentrations in cannabis and could actually lead to instances of column overloading and/or detector saturation. These problems are potentially exacerbated in cannabis, as it can be a challenge to tell, *a priori*, what an appropriate sample size would be given the variability in terpene profiles and concentrations in cannabis. Static HS, on the other hand, will sample all analytes in an equilibrated gas phase, but may experience a sampling bias for high boiling terpenes, as such compounds may not have sufficient equilibrium partial pressures, especially in matrix. Nguyen et al. overcame this by using high-temperature HS sampling (180 °C) to force higher boiling terpenes (e.g., guaiol and α-bisabolol) into the gas phase.[26] This approach was utilized to successfully pass a terpene proficiency testing sample, indicating that acceptable bias for higher boiling terpenes was attained. It should be noted though, that not all HS autosamplers are capable of such high temperature incubations, nor do all laboratories have SPME hardware, so these techniques would not necessarily be accessible to a wide swath of compliance testing laboratories.

Another approach to reduce bias based on boiling point is to use a technique called full evaporative technique (FET) HS sampling. In this approach, a small quantity of sample is used to ensure complete evaporation into the gas phase. This low sample quantity facilities a full transfer to the gas phase due to an overall reduced partial pressure from other volatile analytes present in the sample (and diluent, etc.). At the same time, it eliminates disparity in gas phase equilibria for matrix samples in relation to solvent calibrators.[30] Shapira et al. successfully used this approach to quantitate 93 terpenes in cannabis.[31] However, the impact of small sample sizes relative to sample homogeneity and representative sampling need to be considered when using FET. For example, Shapira, *et al* only used 5 mg of solid sample or 10 µL of standard for their analyses. The potential for sampling bias and variability with such small sample quantities need to be systematically verified before FET methods are taken through method validation.

Chromatographic separation and detection of terpenes has historically been done using GC-FID,[23,24,27] a cost-effective detector with excellent dynamic range and a response that is directly proportional to carbon number (amenable to the analysis of hydrocarbon-based analytes like terpenes). However, with fee-for-service terpene panels of 30–40 analytes being the norm,[27–29] and the fact that 93 have been reported in other methods[2] and more than 200 identified overall,[8] the potential for chromatographic interferences with a nonspecific detector technology like FID is high.[20,28] For this reason, more current methods have focused on GC-MS[20,22,25,26]and GC-MS/MS,[28,29] both of which have improved selectivity in comparison to FID. But MS is not always the final solution to selectivity challenges for terpenes. This is due to the number of terpene isomers that can be present in a given sample and the high energy nature of electron impact (EI) ionization used for GC-MS analyses. These high energy reactions break the terpenes down to fragments that are generally shared within a given class and will also be shared for structural isomers. For example, pinene and myrcene are structural isomers (both have a formula of $C_{10}H_{16}$) and will have the same EI mass spectra, so they need to be chromatographically resolved. To provide an additional level of confirmation in terpene identification, especially in the absence of analytical standards, many methods use retention indexing (e.g., Kovats) in tandem with EI mass spectral matching.[20,22,23,26] An alternate approach, while seemingly more academic at present, is the use of cold EI,[25] which is analogous to chemical ionization for GC in that it provides a more stable molecular ion to aid compound identification.

A final point worth mentioning, is that all terpene analyses discussed thus far will report a sum of enantiomers present in a given sample, as conventional chromatographic columns do not resolve stereoisomers. This is an area of research that is likely to get more attention as the biological impact of new terpenes are investigated, especially since it is

known that enantiomers can elicit disparate biological effects (e.g., α/β-pinene have different perceived odors). Moreover, a proof-of-concept using terpene enantiomer profiles for product authenticity testing has been reported,[6] which is consistent with other terpene-rich products like tea tree oil where enantiomers have been used for geographic characterization.[32]

Pesticides

Pesticides are compounds manufactured with the intent of destroying insects or microorganisms that are harmful to plants. Pesticides are typically categorized based on their class of use or chemical class. The seven classes of use include insecticides, herbicides, fungicides, rodenticides, acaricides, molluscicides, and nematocides.[33] Pesticides can also be divided by their chemical structures, with organophosphorus compounds, carbamates, chlorinated hydrocarbons, pyrethroids, and heterocyclic compounds being the dominant groups. The Federal Insecticide Fungicide and Rodenticide Act (FIFRA) of 1947 requires pesticide registration for specific uses and establishes labeling requirements for these pesticides.[34] In the US, prior to its use, a pesticide must first be granted a residue tolerance or receive a tolerance exemption from the US Food and Drug Administration (FDA). Allowed uses for pesticides must be supported by data including toxicological, environmental fate, and lifecycle analyses. Given the federal status of cannabis as a Schedule 1 drug, none of these processes used for other plant commodities have been initiated and very little is known about best practices of pesticide application and use in cannabis cultivation.[35] While hemp has been effectively legalized in the US (*vide supra*), it too has the same lack of codified knowledge regarding best practices for pesticide application. As a result, most maximum residue limits (MRLs; often in the low part-per-billion range) are not due to toxicity studies or risk management, but often are based on the ability of laboratories to achieve them analytically.[36,37]

Pesticides are typically applied as a foliar spray during cannabis cultivation and their residues may remain with harvested flowers after cultivation. Interestingly, pesticide use may not even be beneficial for the cannabis plant, rendering their use a risk introduced during cultivation.[38] Even if pesticides are not applied directly to the plant though, they could still be introduced through the use of soil or irrigation sources contaminated with pesticides. If the cannabis inflorescence is further processed into cannabis oils, concentrates, or infused products, these pesticide residues could be concentrated, removed altogether, or chemically transformed depending on which manufacturing processes are utilized. Pesticide residues can also be altered after application due to photo oxidation, biological degradation (within the plant, or due to soil microbes, etc.), or volatilization[39] leading to variable concentrations throughout the cultivation and manufacturing processes.

Many of the pesticides found in cannabis and hemp samples are deemed moderately hazardous by the World Health Organization[33] and most of the documented toxic effects of pesticides are based on dermal contact. Cannabis, however, is frequently inhaled and there is scarce toxicological research on the fate of pesticides and pesticide metabolites during and after inhalational exposure. Some studies have measured the pesticide levels in mainstream smoke from 10% to 70% of the initial concentration on the flower.[40] While there is data on pesticide inhalation from cigarette smokers, cannabis users typically inhale up to two-thirds larger puffs by volume and hold the puff for much longer than cigarette smokers.[33] This indicates that pesticide exposure data from cigarettes may not correlate to cannabis users. As well, during pregnancy, the placenta and fetal brain are potential targets for both cannabinoids and organophosphate pesticides.[41] This is worrisome given that estimates from the Colorado Department of Public Health and Environment found 8.2% of new mothers had used cannabis shortly before their pregnancy. These data suggest that the risks of pesticides in cannabis and derivative products need to be better understood. One way to support this effort is the development of accurate quantitative methods for pesticides.

Cannabis products are also much more diverse than those offered by the tobacco or alcohol industries. Flowers are inhaled; oils and concentrates are dabbed at high temperatures or used in vaporizer cartridges and made into numerous infused products, beverages, topicals, and edibles. Most coil-based vaporizer cartridges can be operated at variable battery settings leading to vaporization temperatures ranging from 212 degrees F to 630 degrees F, depending on the degree of wick wetting.[42] This large temperature range covers pyrolysis temperatures and can lead to thermo-oxidative degradation of many pesticides and can yield toxic by-products. One of the most famous by-products is hydrogen cyanide, formed from the pyrolysis of the pesticide myclobutanil.[43]

The United States Pharmacopeia (USP) lists 561 compounds (including 108 pesticides) that consumable crops should be tested for, the National Organic Program (NOP) 2611-1 lists 188 residues, and the United States FDA lists over 750 pesticide residues in their compliance tests.[44] Given the high number of analytes, the lack of federal guidance on which residues to look for and the lack of any toxicological and environmental data, states are left with little guidance on how to figure out how to regulate pesticide use on cannabis on their own. Providing some guidance is a 2013 release by the American Herbal Pharmacopeia that included pesticides that were most likely to be used on cannabis and highlighted acaricides, insecticides, fungicides and plant growth regulators.[45] This document has helped some states begin to regulate pesticides, but variation between regulatory jurisdictions is the norm, rather than the exception. For example, California have mandated pesticide testing for 66 pesticides,[46] Oregon has 58 on their list,[47] Colorado has only 15 on their list, while Canada mandates testing for 96 pesticides.[37]

Beyond the challenges of inconsistent regulations, sample preparation is another area of concern for pesticide analysis.

The cannabis flower matrix is heterogeneous and includes over 550 compounds, many of which are capable of interacting[48]—flavonoids, fatty acids, phenols, phytosterols, alcohols, ketones, and lactones can all interfere with various extraction strategies and introduce ion suppression and ion enhancements when using MS for analyte detection. Common techniques include liquid-liquid extraction (LLE), solid-liquid extraction (SLE), solid-phase extraction (SPE), solid-phase microextraction (SPME), and quick, easy, cheap, effective, rugged, and safe (QuEChERS) extractions. LLE and/or SLE are quick and easy, but they are nonspecific, include high levels of solvent consumption and can require significant dilution schemes prior to injection to mitigate matrix effects. SPE uses less solvent, but is more laborious and can be costly depending on the SPE phase selected. SPME is a solvent free technique that is typically used only in GC analyses, limiting its usefulness in analyzing broad pesticide panels. Although, when its use is appropriate, SPME reduces background interferences that may have been present in LLE or SLE approaches. QuEChERS is a popular technique, appearing in a significant amount of the literature on pesticide quantitation in cannabis flower and derivative products. Given the many variables in using QuEChERS, this technique needs to be validated based on the type of salt, buffer, and organic/water phases used, as the chemistries of the pesticide panel under investigation will impact method recoveries (e.g., PSA will reduce the recovery of acidic pesticides and GCB will do the same for planar pesticides). A study investigating the effects of buffers on the QuEChERS preparation proved no clear trend in extraction efficiencies and recoveries across the three methods.[49] Another issue with QuEChERS is the fact that the organic solvents will also extract many hydrophobic compounds, like cannabinoids. Since the concentration difference between cannabinoids and pesticides in a given sample will likely be large, even with heavy dilution prior to analysis, the cannabinoids can present chromatographic interferences that are challenging to overcome. Indeed, while such interferences can frequently be addressed by the selectivity afforded by tandem mass-spectrometry analyses, this approach is not always successful. In some instances, it may require altered sample preparation and/or chromatography to remove such interferences. Therefore QuEChERS may not be the method of choice for some laboratories.

Given the variability in the physical and chemical properties of pesticides (polarity, solubility, thermal lability, pK_a, etc.), as well as the variability in maximum residue limits (MRLs) by regulatory jurisdiction, different technologies are utilized to quantitate pesticide residues. Since many pesticide MRLs are in the low part-per-billion range, most peer-reviewed articles have focused on LC-MS/MS and GC-MS/MS to quantitate these residues in cannabis and derivative products. GC is sensitive and selective for hydrophobic and volatile pesticides, but struggles with polar or thermally labile compounds. LC-MS/MS, excels at the analysis of polar and ionic compounds.[50] Such capital expenditures must be carefully considered, with financial capacity and testing needs influencing which instrumentation to purchase. One study from 2021 used LC-MS/MS to measure 82 of the 96 pesticide residues required by Health Canada.[36] This method shows how difficult pesticide testing can be when regulatory requirements need to be met. Fourteen pesticides were not able to meet Canadian requirements (seven were detectable but not quantifiable and the other seven were not detectable using ESI). A 2018 paper from Health Canada used three methods to validate and quantitate pesticides from various cannabis matrices and then inspect licensed cultivation sites to reveal unauthorized pesticide control products in use.[51] The first method detected 39 pesticides in cannabis leaves, the second 32 pesticides in cannabis flowers, and the third 40 pesticides in cannabis oils. All of these methods used a combination of LC/GC-MS/MS. Another interesting paper outlines possible pesticide remediation techniques for the cannabis industry.[52] The German research group used computer modeling to look at simultaneous CBD isolation and pesticide removal using liquid-liquid chromatography. The group outlines three solvent systems capable of achieving both goals and even produces a flowsheet on which solvent system to use based on the pesticide responsible for the contamination. Perhaps the most comprehensive study of pesticides in cannabis though, looked at 367 pesticides using QuEChERS cleanup and both LC-MS/MS (290 pesticides) and GC-MS/MS (78 pesticides) analyses. Most analytes had recoveries in the 70–120% range.[47]

Generally, the peer-reviewed literature sheds light on a couple analytical trends in cannabis testing laboratories regarding pesticides: (1) pesticide testing is difficult and requires advanced analytical instrumentation and highly skilled staff to meet regulatory demands with a robust, accurate and precise test method, (2) LC-MS/MS and GC-MS/MS are preferred by most groups, and (3) QuEChERS is the preferred sample clean up step.[53,54]

Mycotoxins

Cannabis and hemp, as with other commodity crops, is susceptible to contamination from fungi during growth, processing and storage.[55,56] Fresh cannabis has a water activity (A_w) of 0.95 A_w, but to prevent most fungal growth a water activity below 0.65 A_w is required.[57] For example, the *Botrytis* genus of fungi is most commonly found postharvest and has a water activity threshold of 0.85 A_w.[57] There are currently at least 88 known fungal species affecting cannabis during the growth stage.[58–60] Commonly found fungal genera include *Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium* and *Trichothecium.*[61] These pathogens can be found in the foliar and stem regions of the plant, but can also be soilborne.[61] It is for this reason microbial testing (*vide infra*) of cannabis is required in all regulated regions in Canada and the US. Certain genera of these fungi have the ability to produce secondary metabolites known as mycotoxins, which can elicit various forms of acute toxicity if consumed. For this reason, analysis of mycotoxins is also required in many regulatory jurisdictions.

The simplest and most common approach for mycotoxin sample preparation employs a broad spectrum solvent

extraction, typically with acetonitrile, using anywhere between 5 and 10 mL of solvent per gram of cannabis. Other solvents including toluene, ethyl acetate and methanol have shown reduced extraction efficiencies.[28] It has been found that wetting the sample prior to acetonitrile addition with a small volume (roughly 20% of the acetonitrile volume) of water or isopropyl alcohol can improve extraction yields and precision.[62–65]. The addition of this wetting step can increase the polar matrix co-extractables, which could result in matrix effects during analysis (i.e., by LC-MS/MS).[62] The addition of QuEChERS salts to separate the acetonitrile and water layers can mitigate this, while still showing high extraction efficiencies for mycotoxins.[62,66]

As mycotoxins are surface contaminants typically found at the foliar and stem junctions, it has been reported that measuring the whole flower bud without grinding was key for high extraction recoveries.[28] Of course, measuring exposure from a single flower presents questions about homogeneity across a given plant, row, crop, etc that would need to be weighed by regulatory agencies if/when they define sampling procedures for mycotoxin testing. Moreover, differences have been reported for measured extraction efficiencies between spiked samples (i.e., those used for method validation) and samples with incurred mycotoxins, especially without the inclusion of a wetting step.[62] For this reason, matrix-matched calibrations can be important for method accuracy and to compensate for matrix effects during analysis. This also highlights the importance for the development of certified reference materials to support cannabis testing programs (*vide infra*).

Because mycotoxins can be analyzed alongside pesticides using LC-MS/MS, employing selective clean-up steps increases the risk of analyte loss, especially in a multiresidue method. Although, depending on instrument sensitivity, it may be necessary to use SPE sorbents that are selective for mycotoxins to meet regulatory limits. A common dispersive solid-phase extraction (dSPE) cleanup step employs primary and secondary amine sorbents (PSA), graphitized carbon black (GCB) and $MgSO_4$. This has shown to result in complete loss of mycotoxins.[28] GCB is added to remove chlorophyll, but will adsorb planar analytes, such as the aflatoxins.[67] Flow-through SPE (where sample are loaded and eluted in an organic solvent in a single step) with reverse phase cartridges, typically with C_{18} sorbents, is becoming increasingly common for multi-residue methods.[51] However, this has also been shown to result in up to a 50% loss in mycotoxins during clean-up.[28] In instances where no loss of mycotoxins was observed, the flow-through SPE clean-up was not shown to improve matrix effects during LC-MS/MS analysis but may help with method robustness.[62]

Immunoaffinity columns (IACs) are designed for mycotoxin extraction and cleanup. These employ bound antibodies designed to specifically retain mycotoxins from complex matrices.[68] Since these columns are specifically designed for mycotoxins, they do not retain pesticides, and as such, are typically not used when LC-MS/MS is used for detection. They are, however, common when HPLC with fluorescence detection is used for mycotoxin quantification. Because IACs are so selective, it is often the case that multiple IACs must be used in tandem when multiple classes of mycotoxins are being analyzed.[57,69] Employing HPLC with fluorescence detection typically also necessitates multiple chromatographic methods. Multi-mycotoxin IACs have also been developed to mitigate the use of multiple columns. These have been used successfully in cannabis and show acceptable but variable recoveries, down to 60% for certain mycotoxin classes.[70–72]

As mycotoxins are commonly analyzed alongside pesticides, chromatography invariably employs cross-bonded or polar-embedded C_{18} stationary phases for separation, with mass spectrometry compatible mobile phases of methanol and water including ammonium formate and formic acid as the modifier.[28,62,65,66]. Mycotoxins have also been analyzed using an enzyme linked immune assay (ELISA). These methods show detection levels in the single-digit ng/g range (making them compliant with current regulations), but require an individual assay per analyte.[73] Further, these assays have the potential for up to 25% cross reactivity from other analytes, resulting in potential false positive results.[73] Notwithstanding these challenges, cannabis labs will sometimes employ ELISA-based methods as a rapid, high-throughput screen under the assumption that most samples will be negative. Assuming that appropriate quality control check points are used, such an approach represents an attractive method of increasing testing throughput.

Only a subset of mycotoxins including aflatoxins (B1, B2, G1, G2) and ochratoxin A are required to be monitored in Cannabis, based on current regulations in Canada and the US. These are primarily produced by *Aspergillus* and *Penicillium* genera.[61,74–77] The United States Pharmacopeia (USP) currently recommends an MRL of 20 ng/g for the summed concentration of aflatoxins, with an MRL of 5 ng/g for aflatoxin B1.[9] MRLs for ochratoxin A are commonly set at 20 ng/g, as with California state regulations.[78] Although as of July 2021, Colorado has an ochratoxin A limit in hemp of 5 ng/g. The European Pharmacopeia has implemented lower MRLs for aflatoxins in herbal drugs, set at 4 ng/g for total aflatoxins and 2 ng/g for aflatoxin B1. An MRL for ochratoxin A, however, is not established. *Fusarium* and *Trichothecium* fungi have been detected in cannabis plants, which have the ability to produce additional mycotoxins including fumonisons, deoxynivalenol, nivalenol and T2 toxin.[79–81] Methods looking for mycotoxins beyond regulated lists have also found direct evidence of T2 toxin, zearalenone, zearalenone, and various enniatins.[66] For this reason, it may be pertinent to expand mycotoxin testing beyond aflatoxins and ochratoxin A to support consumer health and safety as it pertains to cannabis consumption.

Residual solvents

As of the writing of this review, 34 states have legalized medical and/or recreational use of cannabis and cannabis products in the United States. Among these states, 22 of them developed laboratory or state mandatory residual

solvents testing for product safety.[82,83] Canada and some European countries also started to regulate residual solvent testing for cannabis oil, concentrate or inhalation products.[84,85] Because of its Schedule I status in the United States, the unified safety measures at the federal level are not applied to cannabis, leading to a lack of standardization in residual solvent testing. As a result, each state has selected its own residual solvents and associated MRLs. For example, in Colorado, eleven residual solvents are listed in the test regulations for cannabis concentrates with the action limits ranging from 2 ppm to 1000 ppm.[86] In California, twenty residual solvents are required to be tested in cannabis pre-rolls and products. The 20 residual solvents are divided into two categories based on their health concerns. Category 1 compounds have an MRL of 1 ppm and Category 2 compounds have MRLs ranging from 290 to 5000 ppm.[87] MRLs for residual solvents are generally established by referencing state standards (e.g., the Department of Cannabis Control in California) and federal organizations such as the Office of Environmental Health Hazard Assessment (OEHHA), the United States Environmental Protection Agency (USEPA), the Agency for Toxic Substances and Disease Registry (ATSDR), National Institute for Occupational Safety and Health (NIOSH) and the United States Pharmacopeial Convention (USP). Research from most common solvents and processing chemicals used in the cannabis industry and the results of exploratory laboratory testing are also used to establish MRLs. In Canada, twenty-eight residual solvents are required to be tested in cannabis oil with a blanket MRL for each compound of 5000 ppm.[85]

Certain chemicals and solvents, such as propane, butane, hydrocarbons, water, ethanol, isopropanol, acetone, and hexane are frequently employed during cannabis extraction processes.[40,88] Such solvents are used to reduce production costs and preserve incurred terpenes, in addition to their use by illicit extraction facilities.[89–91] Chemicals used during the processing of cannabis from machine operations and product packaging may also contaminate the end products,[92] with a recent study of 57 cannabis samples conducted by Raber et al. showing that over 80% of the concentrates evaluated contained residual solvents.[40] Producers of cannabis concentrates and derivative products are adding terpenes to tinctures, vaping oils, lotions, foods, and beverages to enhance the flavor, make claims of health benefits, or replicate the original terpene profile lost during the cannabis extraction process. Also, thinning/cutting agents such as propylene glycol, polyethylene glycol, or medium-chain triglycerides are added to vape oil to change product viscosity, and/or reduce the cost of production.[93] These added terpenes, be they cannabis, botanical, or synthetic in origin, create another potential source of residual solvents in cannabis products. Furthermore, added terpenes and thinning/cutting agents may interact with each other or undergo thermoxidative degradation during the vaping process to form, among other things, analytes included in residual solvent compliance testing.[94] Residual solvents formed in this manner are beyond the scope of current compliance regulations but nevertheless represent an important public health consideration.

Most of the published residual solvents test regulations reference USP <467>, which is a gold standard for pharmaceutical products.[95] The common solvents present in drug substances, excipients, and finished products were well defined and the testing methods described in USP <467> have been implemented for decades. However, manufacturing of cannabis products differs greatly from pharmaceuticals, especially in their use of compounds such as propane, butane and isobutane during the extraction process. Moreover, cannabis is a complex matrix compared to typical pharmaceuticals, and derivative products further complicate things with matrices including joints, concentrates/distillate, e-juice, topicals, oral supplements, tinctures, capsules, and infused edibles such as candies, chocolates, mint/chews, dried meat, crackers, dairy products and baked goods. Given these differences, USP < 467> is a good starting point, but does not have the necessary scope to deal with cannabis products.

There are limited methodologies for residual solvent testing in cannabis products. Gravimetric analysis (e.g., for class 3 volatile solvents specified in USP < 467> is simple, but needs larger amounts of samples and lacks specificity to identify the volatile analytes.[96] Spectroscopic and spectrometric tests have low sensitivity in general and are therefore not applicable for regulatory requirements that specify any Category I analytes. GC as a chief and preferred method of solvent analysis has been used by pharmaceutical manufacturers for decades and is the most appropriate, specific analysis for residual solvent testing.[97] Similarly, GC is the most dominant and successful tool in cannabis research and testing for residual solvents.[91,96] Many methods for residual solvent testing using headspace (HS) GC-MS or GC-FID to utilize sample introduction, GC separation, and analyte detection.[88,98–101]

Sample introduction has three main procedures and they are direct injection, HS analysis, and solid-phase microextraction (SPME). Direct injection is simple, reliable and a standard autosampler can be used. The samples are usually dissolved in an appropriate extraction solvent (i.e., not an analyte) prior to analysis.[97,102] Direct injection introduces liquid samples into the GC system and so care must be taken to mitigate matrix interferences and the potential for more frequent system maintenance due to contamination of the inlet and/or head of the analytical column. Because of these drawbacks, the direct injection method has become less popular and replaced by the HS sampling in residual solvent testing in recent years.

HS sampling is an indirect testing procedure. The analysis is conducted when a volume of gas above the sample matrix is collected and analyzed by a GC. The major types of headspace-sampling techniques are: dynamic HS sampling (also referred to as purge-and-trap) and static HS sampling. In dynamic HS sampling, a continuous gas flow is swept over the surface of a sample matrix. Volatiles from the sample matrix are conveyed into a trap where the volatile residual solvents are accumulated prior to analysis. Whereas

dynamic HS sampling is particularly suited for the determination of analytes at very low concentrations,[103] static HS sampling is the most widely used technique for residual solvent analysis in the cannabis industry. In static HS, a liquid or sometimes a solid sample is placed into a sealed vial where it is heated until a thermodynamic equilibrium between the sample and the gas phase is reached. A volume of the headspace gas is then sampled for analysis. The advantage of the HS sampling is simplified sample preparation, as homogenized or diluted samples can be directly aliquoted for analysis. Since only the gas phase of the samples are injected into GC system, matrix interferences are significantly reduced. The nonvolatile matrix components remain in the sample vial, which limits GC maintenance relative to other.[88,97,102] However, one disadvantage of the HS sampling is the possibility of over saturation of the detectors for analytes with high sensitives or concentrations depending on the liner dynamic range of the detector used. Another potential bias possibly introduced by HS techniques is that some residual solvents have higher boiling point, and this may contribute to partial pressure change in headspace gas phase due to incurred volatiles, especially in complex cannabis matrices. To overcome these potential matrix effects, full evaporation technique (FET) can be implemented into HS sampling. FET sample preparation involves the use of a very small sample amount (e.g., 5–20 mg), which effectively creates a single-phase gas system in the headspace vial at equilibrium, effectively mitigating the matrix issues discussed above.[98]

Finally, solid-phase microextraction (SPME) has made much progress in recent years for cannabis related research and testing.[96,104] In SPME, a silica fiber coated with a sorbent is used to collect and concentrate the volatile components of a sample prior to analysis. The selectivity of the fiber can be modified by changing the phase chemistry and/or thickness according to the characteristics of the analytes. In general, there are two types of SPME - headspace and immersion. Direct HS SPME has been demonstrated to be more sensitive than immersion SPME for polar residual solvents. In recent years, direct HS SPME has gained a sturdy reputation as a valid alternative to HS GC because of the simplicity of the procedure and the low cost of hardware.[105] However, its detection limits may be lower than headspace GC. In addition, the SPME fiber extraction efficiency changes with the wear (number of injections), which can cause poor inter-batch reproducibility. These facts may reduce the applicability of HS SPME in routine determinations of residual solvents in cannabis products.

MS and flame ionization detectors (FID) are the most common detectors for residual solvent testing.[97] GC-FID is adequately used for known specific residual solvents and chemicals in cannabis testing. However, the lack of specificity of the FID and its dependance on hydrocarbons in the analyte can create analytical challenges for certain regulatory jurisdictions. For example, DMSO has low sensitivity using FID and can challenge to chromatographically separate some residual solvents sufficiently (e.g., without the added specificity of an MS detector). In some instances, it may not be possible to overcome these limitations through a single GC column phase. For complicated separations like this, two capillary columns with different stationary phases can be combined in series (tandem) to obtain tunable selectivity. This two-dimensional GC chromatography improves chromatographic separation, which may be necessary in complex cannabis matrices, especially when dealing with large lists of residual solvents.[98,106] The most utilized capillary columns for GC separation are composed of 6% cyanopropylphenyl, 94% dimethylpolysiloxane stationary phases (624-type columns). This type of MS column is designed specifically for volatiles analysis and is widely used for the analysis of residual solvents.[98] As specified in USP < 467>, USP phase G43 (polyethylene glycol stationary phase; WAX-type columns) are the usual choice, as they represent an orthogonal column chemistry to the 624-type columns when trying to resolve co-eluting residual solvents.

GC-MS can be used for the determination of known residual solvents or processing chemicals in cannabis products and it also has the capability to identify unknowns using mass spectral libraries. Based on the unique mass spectrum of each analyte, GC-MS data processing software can deconvolute coeluting compounds and permit accurate integration of each component. MRLs for residual solvents generally range from 1 ppm (detection limit) to 5000 ppm. To cover such a wide concentration range, both full scan and single ion monitoring (SIM) modes may be used simultaneously in one MS method,[107] or a SIM only method can be used.[100] For example, compounds such as propane and ethylene oxide, as well as chloroform, benzene, and 1,2-dichloroethane are notoriously difficult to quantitate accurately due to their high volatility and poor response - SIM can help reduce chemical background to achieve sufficient sensitivity for such compounds. However, GC-MS may suffer from detector saturation for analytes with high sensitivities and/or high MRLs, especially when trying to implement multi-residue methods with Class 1 solvents. In addition, SIM may not have suitable specificity for low molecular weight residual solvents, those that share hydrocarbon backbones (e.g., pentane and hexane) and those with common functional groups. In such cases, orthogonal column chemistries (vide supra) or alternate MS techniques (e.g., GC-MS/MS) may be necessary. The need for these alternate techniques may be exacerbated in the presence of high concentrations of terpenes.[108,109]

Standard preparation and storage represents a unique challenge for residual solvent quantitation, especially for highly volatile compounds such as propane, butane, ethylene oxide, etc. Gas tight vials and gas tight syringes need to be used during their preparation, and it is advisable to store and open standards cold (e.g., −20 °C) to limit losses due to volatility. Even with these precautions, standards (supplied in sealed ampules) are only good for a single use. Therefore, efficiency from sample preparation to data reporting in residual solvent testing is key. The authors have developed and validated a screening method to detect and semi-quantitate the residual solvents listed in the California test regulations (data not yet published). In this method, a single-point

calibration and average response factor are used to identify and estimate residual solvent concentrations. Owing to this semiquantitative screening approach, a full sequence with multiple calibration points and quality control samples are only carried out for samples above compliance MRLs.

Heavy metals

Toxic elements with densities greater than 5 g/cm^3 and atomic numbers higher than 11 have traditionally been called heavy metals.[110] Of the 90 naturally occurring elements, 53 can be classified as metals (and one metalloid, As).[111] These elements can include lead, cadmium, arsenic, mercury, cobalt, vanadium, nickel, lithium, antimony, barium, molybdenum, copper, tin, and chromium among others. Low concentrations of these metals (lower even than 0.01 mg/L have been shown to be toxic to humans.[110] Some of these elements are essential plant nutrients (Cr, Mn, Mo, Zn, Fe, Co, Cu, Al, Ni) and some are non-essential elements that can lead to toxicity even at trace levels (Cd, Pb, As, Hg).[112,113] Many species exist in different oxidation states and will have different biological impacts depending on their route of exposure; inhalation *versus* ingestion of cannabis products is an excellent example of this, as is the relative toxicity of Cr^{6+} *versus* Cr^{3+}.

Metals are found in the Earth's crust in varying concentrations. They are introduced through uncontrolled anthropic activities such as mining, transportation, smelting, and agricultural applications.[113] Typically, they appear in food and cannabis products through plant bioaccumulation, cross contamination during processing, or post-process adulteration.[114] There are ways to use cannabis and cannabis products to remediate soils and water contaminated with toxic elements[115,116] but those have not been adopted at industrial scales yet. Metals can contaminate soils through runoff from industrial manufacturing plants, direct applications of fertilizers and pesticides, the application of animals wastes or sludges, and the atmospheric deposition of metal-containing particulate matter. It is also known that wildfires and volcanic eruptions in one location can deposit heavy metal containing ash on crops hundreds of miles away.[117]

In soil, where healthy pH is typically 5.5–7, metal cations are bound to negatively charged particles such as clay, humus, and other organic matter. Only when unbound, are metals bioavailable. Several studies have discussed the mobility and availability of metals under specific soil conditions including the effects of rhizosphere specific bacteria on plant uptake.[113] At low pH, metals like lead, copper, mercury, and cadmium are more available for plant uptake. Some specific transport systems can also transport metals, such as phosphate systems for arsenic and cadmium and lead through divalent metal transporters (used for iron, calcium, and zinc typically).[111,113]

Cadmium has been shown to be enriched in some phosphate-based fertilizers[114] and thus available for plant uptake. High concentrations of cadmium in soil are associated with iron deficiency in some plants[113] leading to higher application of fertilizers and a feedback loop of increasing contaminant concentrations. Similarly, metal uptake can cause signs of disease in plants, such as spots on the leaf surface,[110] which can again cause cultivators to apply more fertilizers, thinking the cause of the disease was a nutrient deficiency. Mercury can enter plant stomatal openings through gas exchange (mercury is volatile at around 30 Celsius) and can also enter plant roots in certain oxidation states.[113] Metals can hyperaccumulate in plants with levels of cadmium and selenium seen over 100 ppm in dry leaf tissue, chromium, and copper over 300 ppm, while lead and arsenic and nickel can reach 1000 ppm.[113] It is not uncommon for metal concentrations in Cannabis to bioaccumulate by tissue location, with higher levels in roots and leaves than in the stems themselves.[118]

Most metals do not degrade quickly or easily leading to their ability to bioaccumulate up the food chain. Cadmium is a bluish white metal usually from zinc ore processing. Chronic cadmium exposure can cause Itai-Itai disease, characterized by intense bone pain and numbness in extremities.[119] Lead is a silver to gray soft metal. It is widely used and can exist over 30 years in bones. Lead is a calcium analog that interferes with ion channels. In Leipzig Germany, illicit lead was added to Cannabis to increase its mass, and therefore its value. Hundreds of patients exhibited nausea, acute colic, loss of appetite and weight, and chronic fatigue.[119] Mercury is a silver metal that is liquid at standard temperature (0 °C). Mercury vapors can cause forgetfulness, irritability, tremors, and paranoia. Arsenic is colorless or gray and can lead to skin cancer and lesions, diabetes, cardiovascular disease and infant mortality.[119,120]

The International Agency for Research on Cancer (IARC) has classified metals into three categories based on carcinogenicity in humans. As, Cd, Cr, and Ni are all group 1 (proven carcinogens). Inorganic lead and methyl mercury are in group 2 (probable carcinogens), while Organic lead and metallic mercury are in group 3 (possible carcinogens).[117] Similarly, the international Agency for Toxic Substances and Disease Registry (ATSDR) has ranked As, Pb, Hg, and Cd as the 1st, 2nd, 3rd, and 7th most hazardous substances for toxicological profiles.[111]

Given the high toxicity of metals at low concentrations, it is important to quantitate them at low levels (part per billion or lower). Common methods to prepare samples to run historically included the addition of concentrated acid followed by heating such as EPA methods 200.2, 200.7 or 200.8.[121–123] More common in high throughput laboratories is to digest material in microwaves (microwave digestion[124]). Many traditional methods to detect metals have included atomic absorption spectrometry, x-ray fluorescence and proton induced x-ray emission but struggle to meet these detection limits.[125] More common in today's laboratories are inductively coupled plasma (ICP) with either optical emission spectrometry (ICP-OES) or mass spectrometry (ICP-MS)[117] Due to many stringent testing requirements for heavy metals, ICP-MS has become the preferred technique, as it enables higher throughput, can reach lower detection limits, can detect multiple elements concurrently and has a larger linear range than ICP-OES. Some groups

have turned to electrochemical microfluidics techniques[126] and observed part-per-billion detection levels. These techniques have not seen mass adoption, however, due to high costs and increasing complexity.

Multiple studies have used ICP-MS to detect metal concentrations in cannabis plants and products.[112,120,124,125,127] Studies in Greece have found low part-per-million concentrations of arsenic, chromium, lead, and cadmium in cannabis flower samples.[120] A screen of CBD products in the UK found lead and arsenic in the low part-per-million range[124] while studies in Poland have detected lead, cadmium, arsenic, aluminum, and nickel in a screen of dietary supplements and dried medicinal plants.m[112] Further screens in the United States have also confirmed arsenic, cadmium, and lead in CBD pet products[127] and the authors own network of laboratories regularly finds metal contamination in cannabis and hemp products in multiple states (data not published).

Given the propensity for cannabis plants to accumulate metals from contaminated soils[115,128] and the fact that the cheapest method of cultivation is to grow outdoors, it is likely metal contamination is a problem that is not going away soon. Without federal oversight, states are left to themselves to regulate which metals to test for and at what levels. Most states are testing for As, Cd, Hg, and Pb, which are categorized as Class 1 elements by the US FDA guidance.[129] Maryland has added chromium, selenium, barium and silver to their list while New York has added nickel, chromium, copper, antimony, and zinc. NIST has recently created hemp reference material which includes lead, cadmium, arsenic, mercury, beryllium, cobalt, vanadium, chromium, manganese, molybdenum, nickel, selenium, and uranium.[129] In 2022, Colorado will mandate the emissions of vaping products be tested for arsenic, lead, cadmium, and mercury. This is increasingly interesting, as the e-juice is in contact with various metal parts in the vaping device, for example, the heating coil. The fate of metals in different oxidation states that may be produced under pyrolysis and vaping conditions (high temperatures but not pyrolysis) is unknown. Due to vaping metal component interactions Cr, and Ni are under study by various groups in cannabis-containing e-juice and may be under consideration for cannabis regulation in states beyond Colorado.[130,131]

Microbiological testing

Identifying and monitoring the diversity of microbiota in cannabis inflorescence and cannabis products can shed light on not only the quality attributes of cannabis, but also the prevalence of pathogens that have the potential to pose public health and safety concerns, especially in immunocompromised populations. Studies on the microbiome of cannabis inflorescence reveal a natural abundance of a wide variety of fungal and bacterial microorganisms, some of which can be pathogenic to humans.[132] Additionally, cultivation practices, product manufacturing processes, and ingredients involved in manufacturing cannabis infused products introduce potential external sources of microbial contamination for consideration. Noting the natural and external sources of microbes to cannabis, it is imperative that adequate methodologies are employed to test cannabis and cannabis products for the presence, and when applicable, concentration of microbes to ensure quality and health standards are met.[9,114]

In the current regulatory landscape within markets that have legal cannabis industries, *Salmonella spp.* and *Escherichia coli,* particularly Shiga toxin-producing *Escherichia coli* (STEC), are considered to be important enteric pathogens due to their potential to cause human disease and outbreak and their prevalence in a variety of agricultural, food, and general environmental settings. *Salmonella spp*. can serve as the causative agents of salmonellosis, typhoid fever, and paratyphoid fever, among other potentially fatal diseases. STEC are also facultative anaerobes that express virulence factors Shiga toxin type 1 and type 2 and when hosted in humans can cause hemolytic uremic syndrome (HUS), often indicated by bloody or non-bloody diarrhea, that can progress to a life threatening state. Additionally, *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and bile tolerant gram negative (BTGN) bacteria have been implicated as pathogens of interest when considering testing requirements. Because all of these pathogens have prevalence in the environment and animals and can be transmitted through the production, processing, manufacturing, and dispensing of cannabis and cannabis products, it is critical that these types of products and processes are adequately and efficiently monitored to protect human and environmental health.

Studies regarding the mycoflora present in cannabis inflorescence demonstrate the prevalence of *Aspergillus spp., Penicillium spp.*, *Botrytis cinerea,* and *Fusarium spp.,* among others.[114,132–135] Of these fungal organisms, *Aspergillus spp.,* in particular the human pathogenic species *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus,* and *Aspergillus terreus*, have been a focal point of public health concern and subsequent testing regulations for cannabis. This is due to their known ubiquity in the environment, soil, and natural plant products, and incidence in clinical cases as the causative agent of aspergillosis in immunocompromised patients who had reported cannabis use.[9,119,136] Aspergillosis is a disease that ranges in medical effects from invasive lung infection to the formation of aspergillomas (fungal balls) to allergic reactions, and cannabis use via inhaled modes of consumption indicates a higher risk for infection, especially in users with already compromised immune function. For this reason, *Aspergillus spp.* is more commonly required for testing in cannabis and cannabis products, although other fungal pathogens may also be of concern.

As testing for microbial contaminants in cannabis is a relatively nascent development, cannabis testing facilities and method developers have both looked to analogous industries for testing standards and guidance, as well as emerging technologies to meet the evolving needs of the cannabis space. Most commonly, either qualitative presence/absence tests or quantitative general microbial count tests

are applied to cannabis products, although the need for additional confirmatory tests and metagenomics analyses have proven important in both confirming presumptive results and surveying cannabis materials for the prevalence of certain microorganisms.

In general, contaminants are known to be pathogenic to humans, such as *Salmonella spp.,* STEC, and *Aspergillus spp.* require a qualitative presence/absence analysis, whereas tests evaluating the overall quality attributes of cannabis or cannabis products, such as viable yeast and mold count (TYMC) and total aerobic count (TAC) involve a quantitative overview of microbial populations within a given sample. Method references such as the FDA Bacteriological Analytical Manual Chapters 4 and 5 have provided the foundation of *Salmonella spp.* and STEC analysis for many emerging methods in cannabis.[137,138] Furthermore, entities like AOAC International have developed guidance documents to aid in the establishment of method performance requirements to assist in the development, validation, and approval of microbial contaminant analysis methods specifically in cannabis.[139–141] In addition, USP general chapters <61>, <62>, and <1111> provide direction for the establishment of appropriate limits for general count tests and also methodological guidance.[9,142–144] This has influenced testing methodologies for microbial contaminants in the cannabis space to revolve around culture-based isolation and identification and quantitative polymerase-chain reaction (qPCR) analysis, although other culture-based, mass spectral, and molecular technologies are being developed and implemented.

As cannabis and the process of its cultivation, harvesting, product manufacturing, and distribution are further studied, an increasing understanding of prevalent microbial contaminants and their respective implications on public and environmental health will be better understood. Currently, there is some research into the microbiome populations that occur naturally on the cannabis plant, and as well as other microbial pathogens that have susceptibility to contamination through manufacturing and handling practices in the cannabis and analogous industries that have guided the establishment of testing regulations and corresponding microbial analysis methodologies. As the industry matures, the development of inter-laboratory, reproducible reference methods in conjunction with the evolution of new testing technologies will continue to drive innovation in rapid, accurate, and consistent cannabis microbial analysis to support overall confidence in the quality attributes of cannabis and cannabis products, for both the general consumer and medical patients.

Certified reference materials and method standardization

The lack of method standardization in potency testing has led to a variety of test methods being used for cannabinoid quantitation, adding to the potential for variability in results between testing.[5,8,145] Not only is this an issue of analytical accuracy and consumer safety (e.g., does the consumer really know the potency of a product given interlaboratory variability?), it also undermines the confidence that cannabis growers and processors have in the entire testing process. While organizations like AOAC International have codified methods for the quantitation of cannabinoids in flower,[6] concentrates and oils[146] and more recently in edible chocolates (methods not yet published), regulatory agencies have, as yet, not mandated the use of specific methods. For example, water quality test methods in the US are based on standard Environmental Protection Agency, with all laboratories contracted to do this testing running the same method and adhering to the same method validation and data quality criteria. Moreover, these labs are required to demonstrate ongoing proof of performance for a given test method through participation in interlaboratory studies and proficiency testing programs. This type of standardization would provide a clear quality management framework within which cannabis testing laboratories should operate. Not only would this improve data quality and consistency across the cannabis industry, it would also increase consumer confidence in the analytical results provided by different laboratories. Hindering this type of effort is the secrecy with which laboratories guard their test methods due to the perception that they are private intellectual property. This lack of open scientific communication hinders method standardization (e.g., developing consensus methods). While used as an example, this problem is not unique to potency testing—all of the test methods discussion herein are facing the same challenges when it comes to a lack of method standardization.

Closely tied to the lack of method standardization, is the fact that no consistent guidance on method validation is provided for cannabis compliance testing. To be fair, method validation is inherently ambiguous, with a plethora of ways to approach the evaluation of the core parameters of linearity, accuracy, precision, repeatability and robustness. Not to mention to myriad ways to calculate the limit of detection and/or limit of quantitation for a given test method.[147] This lack of standardization across the industry also adds to the potential for disparate results when comparing data between laboratories (as many cannabis growers and processors rightly do).

Finally, connecting method standardization and method validation is the challenge of developing certified reference materials (CRMs) to support compliance testing labs with materials to document the validity of their method validation results and test methods in general. The importance of CRMs was highlighted in a recent mycotoxin study, where differences were observed for the measured extraction efficiencies between spiked samples and samples with incurred mycotoxins, especially without the inclusion of a wetting step.[62] This study highlights a common limitation of spike-recovery studies that are used for method validation and emphasis the need for CRMs that can be used to assess method performance internally. The same challenge has been raised for pesticides, where the question of sequestration in the plant during maturation and the effect that may have on extraction efficiency is an important consideration. And for volatile analytes (e.g., residual solvent and terpenes),

the development of CRMs may present logistical challenges related to analyte retention during storage, transport and prior to analysis on-site at a cannabis testing laboratory.

It is worth noting that there are several cannabis/hemp proficiency testing programs, and participation in such programs is actually required as part of ISO/IEC 17025 accreditation. Such programs are not a substitute for CRMs though. Rather, they are another arm of a robust quality management system meant to facilitate method validity (*versus* consensus values) and data transparency. While invaluable, the reliance on a consensus value is not a measure of trueness, *per se*, as it could be influenced by systematic issues. For example, in 2020 a major supplier of analytical standards provided notification to cannabis testing laboratories that their certificate of authenticity label claim for a particular lot of THC standard was not accurate. This created the potential for a whole segment of testing laboratories (that used that particular lot of THC from that vendor) to have a systematic bias in their THC values reported to clients, and possibly as part of a given proficiency testing study. A recent initiative by the National Institute for Standards and Technology (NIST) is working to address reference materials through their CannaQAP program,[148] which covers hemp potency and, for those with a DEA exemption, cannabis potency. In the second round of this program, NIST also included toxic elements (i.e., heavy metals) and moisture. Not only is this program free to participate in, but NIST openly publish the aggregate interlaboratory results. This type of transparency will help move the cannabis industry beyond some the issues observed today, including the much-discussed challenge of "lab shopping."[149]

Conclusions

Analytical technologies have generally found their respective places as they pertain to potency, terpenes and chemical/biological contaminants in cannabis and derivative products. However, as more testing data are acquired, more proficiency testing programs are evaluated, regulations change, *etc*., there will be a continuing need to evolve the collective approach to cannabis testing. Doing so will provide continued support to consumer safety and will also guide the evolving regulations and testing requirements for hemp and derivative products. As an example, consider potency, which continues to be a primary driver for broad consumer preference in the cannabis industry. As the cannabis derivatives market matures, increased regulatory control as well as increases in the number of such diverse matrices will continue to challenge testing laboratories and their support services (e.g., proficiency testing providers, certified reference material manufacturers, etc.). Moreover, as more cannabinoids, like δ8-THC, come under regulatory control, new tools in the laboratory may be necessary to meet method specificity requirements. Similar sets of challenges exist for the other test methods discussed herein.

It is also worth noting that secondary metabolites of interest in cannabis are not limited to cannabinoids and terpenes. There are variety of other chemicals of interest, flavonoids[150] for example, that may enter the cannabis testing space. When these demands arise, the analytical testing community will need to leverage what has been learned about the challenges of cannabis as a matrix to develop suitable testing methods. Luckily, our understanding of the boundaries of analytical testing in cannabis and derivative products has made significant progress in recent years. This is a direct result of changes in the regulatory landscape bringing cannabis science into the commercial marketplace—when there is money to be made, the push toward robust, efficient testing regimes will always be hastened, albeit not without some growing pains due to a lag in the availability of the necessary testing infrastructure.

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References

[1] De Backer, B.; Debrus, B.; Lebrun, P.; Theunis, L.; Dubois, N.; Decock, L.; Verstraete, A.; Hubert, P.; Charlier, C. Innovative Development and Validation of an HPLC/DAD Method for the Qualitative and Quantitative Determination of Major Cannabinoids in Cannabis Plant Material. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2009**, *877*, 4115–4124. DOI: 10.1016/j.jchromb.2009.11.004.

[2] ElSohly, M. A.; Gul, W. Constituents of *Cannabis sativa*. In *Handbook of Cannabis*; Oxford University Press: Oxford, 2014; pp 3–22. DOI: 10.1093/acprof:oso/9780199662685.003.0001.

[3] US Public Law 115-334. Agriculture Improvement Act of 2018. https://www.govinfo.gov/content/pkg/PLAW-115publ334/pdf/PLAW-115publ334.pdf. 2018.

[4] Sgrò, S.; Lavezzi, B.; Caprari, C.; Polito, M.; D'Elia, M.; Lago, G.; Furlan, G.; Girotti, S.; Ferri, E. N. Delta9-THC Determination by the EU Official Method: Evaluation of Measurement Uncertainty and Compliance Assessment of Hemp Samples. *Anal. Bioanal. Chem.* **2021**, *413*, 3399–3410. DOI: 10.1007/s00216-021-03283-x/Published.

[5] McRae, G.; Melanson, J. E. Quantitative Determination and Validation of 17 Cannabinoids in Cannabis and Hemp Using Liquid Chromatography-Tandem Mass Spectrometry. *Anal. Bioanal. Chem.* **2020**, *412*, 7381–7393. DOI: 10.1007/s00216-020-02862-8.

[6] Mudge, E. M.; Murch, S. J.; Brown, P. N. Leaner and Greener Analysis of Cannabinoids. *Anal. Bioanal. Chem.* **2017**, *409*, 3153–3163. DOI: 10.1007/s00216-017-0256-3.

[7] Gul, W.; Gul, S. W.; Radwan, M. M.; Wanas, A. S.; Mehmedic, Z.; Khan, I. I.; Sharaf, M. H. M.; ElSohly, M. A. Determination of 11 Cannabinoids in Biomass and Extracts of Different Varieties of Cannabis Using High-Performance Liquid Chromatography. *J. AOAC Int.* **2015**, *98*, 1523–1528. DOI: 10.5740/jaoacint.15-095.

[8] Giese, M. W.; Lewis, M. A.; Giese, L.; Smith, K. M. Development and Validation of a Reliable and Robust Method for the Analysis of Cannabinoids and Terpenes in Cannabis. *J. AOAC Int.* **2015**, *98*, 1503–1522. DOI: 10.5740/jaoacint.15-116.

[9] Sarma, N. D.; Waye, A.; ElSohly, M. A.; Brown, P. N.; Elzinga, S.; Johnson, H. E.; Marles, R. J.; Melanson, J. E.; Russo, E.; Deyton, L.; et al. Cannabis Inflorescence for Medical Purposes: USP Considerations for Quality Attributes. *J. Nat. Prod.* **2020**, *83*, 1334–1351. DOI: 10.1021/acs.jnatprod.9b01200.

[10] Dawson, D. D.; Martin, R. W. Investigation of Chocolate Matrix Interference on Cannabinoid Analytes. *J. Agric. Food Chem.* **2020**, *68*, 5699–5706. DOI: 10.1021/acs.jafc.0c01161.

[11] Favell, J. W.; Hayward, R.; O'Brien, E.; Riordan-Short, S.; Sagar, N.; O'Brien, R.; Noestheden, M. Quantitating Cannabinoids in Edible Chocolates Using Heated Ultrasonic-Assisted Extraction. In *Comprehensive Analytical Chemistry*; Elsevier B.V.: Amsterdam, 2020; Vol. 90, pp 397–414. DOI: 10.1016/bs.coac.2020.04.009.

[12] Pourseyed Lazarjani, M.; Torres, S.; Hooker, T.; Fowlie, C.; Young, O.; Seyfoddin, A. Methods for Quantification of Cannabinoids: A Narrative Review. *J. Cannabis Res.* **2020**, *2*, 35. DOI: 10.1186/s42238-020-00040-2.

[13] Križman, M. A Simplified Approach for Isocratic HPLC Analysis of Cannabinoids by Fine Tuning Chromatographic Selectivity. *Eur. Food Res. Technol.* **2020**, *246*, 315–322. DOI: 10.1007/s00217-019-03344-7.

[14] Nahar, L.; Onder, A.; Sarker, S. D. A Review on the Recent Advances in HPLC, UHPLC and UPLC Analyses of Naturally Occurring Cannabinoids (2010–2019). *Phytochem. Anal.* **2020**, *31*, 413–457. DOI: 10.1002/pca.2906.

[15] Noestheden, M.; Friedlander, G.; Anspach, J.; Krepich, S.; Hyland, K. C.; Zandberg, W. F. Chromatographic Characterisation of 11 Phytocannabinoids: Quantitative and Fit-to-Purpose Performance as a Function of Extra-Column Variance. *Phytochem. Anal.* **2018**, *29*, 507–515. DOI: 10.1002/pca.2761.

[16] Berman, P.; Futoran, K.; Lewitus, G. M.; Mukha, D.; Benami, M.; Shlomi, T.; Meiri, D. A New ESI-LC/MS Approach for Comprehensive Metabolic Profiling of Phytocannabinoids in Cannabis. *Sci. Rep.* **2018**, *8*, 14280. DOI: 10.1038/s41598-018-32651-4.

[17] Croes, K.; Steffens, A.; Marchand, D.; Snyder, L. R. Relevance of Pi-Pi and Dipole-Dipole Interactions for Retention on Cyano and Phenyl Columns in Reversed-Phase Liquid Chromatography. *J. Chromatogr. A* **2005**, *1098*, 123–130. DOI: 10.1016/j.chroma.2005.08.090.

[18] Nie, B.; Henion, J.; Ryona, I. The Role of Mass Spectrometry in the Cannabis Industry. *J. Am. Soc. Mass. Spectrom.* **2019**, *30*, 719–730. DOI: 10.1007/s13361-019-02164-z.

[19] Hädener, M.; Kamrath, M. Z.; Weinmann, W.; Groessl, M. High-Resolution Ion Mobility Spectrometry for Rapid Cannabis Potency Testing. *Anal. Chem.* **2018**, *90*, 8764–8768. DOI: 10.1021/acs.analchem.8b02180.

[20] Micalizzi, G.; Vento, F.; Alibrando, F.; Donnarumma, D.; Dugo, P.; Mondello, L. C. *Cannabis sativa* L.: A Comprehensive Review on the Analytical Methodologies for Cannabinoids and Terpenes Characterization. *J. Chromatogr. A* **2021**, *1637*, 461864. DOI: 10.1016/j.chroma.2020.461864.

[21] Russo, E. B. Taming THC: Potential Cannabis Synergy and Phytocannabinoid-Terpenoid Entourage Effects. *Br. J. Pharmacol.* **2011**, *163*, 1344–1364. DOI: 10.1111/j.1476-5381.2011.01238.x.

[22] Sommano, S. R.; Chittasupho, C.; Ruksiriwanich, W.; Jantrawut, P. The Cannabis Terpenes. *Molecules (Basel, Switzerland). NLM (Medline)* **2020**, *25*, 5792. DOI: 10.3390/molecules25245792.

[23] Amirav, A.; Neumark, B.; Margolin Eren, K. J.; Fialkov, A. B.; Tal, N. Cannabis and Its Cannabinoids Analysis by Gas Chromatography–Mass Spectrometry with Cold EI. *J. Mass Spectrom.* **2021**, *56:e4726*, DOI: 10.1002/jms.4726.

[24] Krill, C.; Rochfort, S.; Spangenberg, G. A High-Throughput Method for the Comprehensive Analysis of Terpenes and Terpenoids in Medicinal Cannabis Biomass. *Metabolites* **2020**, *10*, 276–214. DOI: 10.3390/metabo10070276.

[25] Casano, S.; Grassi, G.; Martini, V.; Michelozzi, M. Variations in Terpene Profiles of Different Strains of *Cannabis sativa* L. *Acta Hortic.* **2011**, *925*, 115–121. DOI: 10.17660/ActaHortic.2011.925.15.

[26] Nguyen, T. D.; Riordan-Short, S.; Dang, T. T. T.; O'Brien, R.; Noestheden, M. Quantitation of Select Terpenes/Terpenoids and Nicotine Using Gas Chromatography-Mass Spectrometry with High-Temperature Headspace Sampling. *ACS Omega* **2020**, *5*, 5565–5573. DOI: 10.1021/acsomega.0c00384.

[27] Booth, J. K.; Bohlmann, J. Terpenes in *Cannabis sativa* – From Plant Genome to Humans. *Plant Sci.* **2019**, *284*, 67–72. DOI: 10.1016/j.plantsci.2019.03.022.

[28] Brown, A. K.; Xia, Z.; Bulloch, P.; Idowu, I.; Francisco, O.; Stetefeld, J.; Stout, J.; Zimmer, J.; Marvin, C.; Letcher, R. J.; et al. Validated Quantitative Cannabis Profiling for Canadian Regulatory Compliance – Cannabinoids, Aflatoxins, and Terpenes. *Anal. Chim. Acta* **2019**, *1088*, 79–88. DOI: 10.1016/j.aca.2019.08.042.

[29] Ibrahim, E. A.; Wang, M.; Radwan, M. M.; Wanas, A. S.; Majumdar, C. G.; Avula, B.; Wang, Y. H.; Khan, I. A.; Chandra, S.; Lata, H.; et al. Analysis of Terpenes in *Cannabis sativa* L. Using GC/MS: Method Development, Validation, and Application. *Planta Med.* **2019**, *85*, 431–438. DOI: 10.1055/a-0828-8387.

[30] Micalizzi, G.; Alibrando, F.; Vento, F.; Trovato, E.; Zoccali, M.; Guarnaccia, P.; Dugo, P.; Mondello, L. Development of a Novel Microwave Distillation Technique for the Isolation of *Cannabis sativa* L. Essential Oil and Gas Chromatography Analyses for the Comprehensive Characterization of Terpenes and Terpenoids, Including Their Enantio-Distribution. *Molecules* **2021**, *26*, 1588. DOI: 10.3390/molecules26061588.

[31] Shapira, A.; Berman, P.; Futoran, K.; Guberman, O.; Meiri, D. Tandem Mass Spectrometric Quantification of 93 Terpenoids in Cannabis Using Static Headspace Injections. *Anal. Chem.* **2019**, *91*, 11425–11432. DOI: 10.1021/acs.analchem.9b02844.

[32] Davies, N. W.; Larkman, T.; Marriott, P. J.; Khan, I. A. Determination of Enantiomeric Distribution of Terpenes for Quality Assessment of Australian Tea Tree Oil. *J. Agric. Food Chem.* **2016**, *64*, 4817–4819. DOI: 10.1021/acs.jafc.6b01803.

[33] Taylor, A.; Birkett, J. W. Pesticides in Cannabis: A Review of Analytical and Toxicological Considerations. *Drug Test. Anal.* **2020**, *12*, 180–190. DOI: 10.1002/dta.2747.

[34] Office of Pesticide Programs. Insecticide Fungicide and Rodenticide Act (FIFRA). https://www.epa.gov/enforcement/federal-insecticide-fungicide-and-rodenticide-act-fifra-and-federal-facilities.

[35] Stone, D. Cannabis, Pesticides and Conflicting Laws: The Dilemma for Legalized States and Implications for Public Health. *Regul. Toxicol. Pharmacol.* **2014**, *69*, 284–288. DOI: 10.1016/j.yrtph.2014.05.015.

[36] Craven, C. B.; Birjandi, A. P.; Simons, B.; Jiang, P.; Li, X. F. Determination of Eighty-Two Pesticides and Application to Screening Pesticides in Cannabis Growing Facilities. *J. Environ. Sci.* **2021**, *104*, 11–16. DOI: 10.1016/j.jes.2020.11.004.

[37] Health Canada. Mandatory Cannabis Testing for Pesticide Active Ingredients – Requirements. https://www.canada.ca/en/public-health/services/publications/drugs-health-products/cannabis-testing-pesticide-requirements.html.

[38] Sandler, L. N.; Beckerman, J. L.; Whitford, F.; Gibson, K. A. Cannabis as Conundrum. *Crop Prot.* **2019**, *117*, 37–44. DOI: 10.1016/j.cropro.2018.11.003.

[39] Sall, E. D.; Huang, K.; Pai, N.; Schapaugh, A. W.; Honegger, J. L.; Orr, T. B.; Riter, L. S. Quantifying Dicamba Volatility under Field Conditions: Part II, Comparative Analysis of 23 Dicamba Volatility Field Trials. *J. Agric. Food Chem.* **2020**, *68*, 2286–2296. DOI: 10.1021/acs.jafc.9b06452.

[40] Sullivan, N.; Elzinga, S.; Raber, J. C. Determination of Pesticide Residues in Cannabis Smoke. *J. Toxicol.* **2013**, *2013*, 378168–378166. DOI: 10.1155/2013/378168.

[41] Leung, M. C. K.; Silva, M. H.; Palumbo, A. J.; Lohstroh, P. N.; Koshlukova, S. E.; DuTeaux, S. B. Adverse Outcome Pathway of Developmental Neurotoxicity Resulting from Prenatal Exposures to Cannabis Contaminated with Organophosphate

Pesticide Residues. *Reprod. Toxicol.* **2019**, *85*, 12–18. DOI: 10.1016/j.reprotox.2019.01.004.

[42] Chen, W.; Wang, P.; Ito, K.; Fowles, J.; Shusterman, D.; Jaques, P. A.; Kumagai, K. Measurement of Heating Coil Temperature for E-Cigarettes with a "Top-Coil" Clearomizer. *PLoS One* **2018**, *13*, e0195925. DOI: 10.1371/journal.pone.0195925.

[43] Voelker, R.; Holmes, M. *Pesticide Use on Cannabis Pesticide Use on Cannabis*; Cannabis Safety Institute, 2015.

[44] Administration, U. S. F. and D. *Pesticide Residue Monitoring Program Fiscal Year 2017 Pesticide Report*; 2017.

[45] American Herbal Pharmacopiea. Cannabis Inflorescence. https://herbal-ahp.org/online-ordering-cannabis-inflorescence-qc-monograph/.

[46] Seltenrich, N. Into the Weeds: Regulating Pesticides in Cannabis. *Environ. Health Perspect.* **2019**, *127*, 42001. DOI: 10.1289/EHP5265.

[47] Maguire, W. J.; Call, C. W.; Cerbu, C.; Jambor, K. L.; Benavides-Montes, V. E. Comprehensive Determination of Unregulated Pesticide Residues in Oregon Cannabis Flower by Liquid Chromatography Paired with Triple Quadrupole Mass Spectrometry and Gas Chromatography Paired with Triple Quadrupole Mass Spectrometry. *J. Agric. Food Chem.* **2019**, *67*, 12670–12674. DOI: 10.1021/acs.jafc.9b01559.

[48] Atapattu, S. N.; Johnson, K. R. D. Pesticide Analysis in Cannabis Products. *J. Chromatogr. A* **2020**, *1612*, 460656. DOI: 10.1016/j.chroma.2019.460656.

[49] Pérez-Parada, A.; Alonso, B.; Rodríguez, C.; Besil, N.; Cesio, V.; Diana, L.; Burgueño, A.; Bazzurro, P.; Bojorge, A.; Gerez, N.; et al. Evaluation of Three Multiresidue Methods for the Determination of Pesticides in Marijuana (*Cannabis sativa* L.) with Liquid Chromatography-Tandem Mass Spectrometry. *Chromatographia* **2016**, *79*, 1069–1083. DOI: 10.1007/s10337-016-3029-9.

[50] Craven, C. B.; Wawryk, N.; Jiang, P.; Liu, Z.; Li, X. F. Pesticides and Trace Elements in Cannabis: Analytical and Environmental Challenges and Opportunities. *J. Environ. Sci.* **2019**, *85*, 82–93. DOI: 10.1016/j.jes.2019.04.028.

[51] Moulins, J. R.; Blais, M.; Montsion, K.; Tully, J.; Mohan, W.; Gagnon, M.; McRitchie, T.; Kwong, K.; Snider, N.; Blais, D. R. Multiresidue Method of Analysis of Pesticides in Medical Cannabis. *J. AOAC Int.* **2018**, *101*, 1948–1960. DOI: 10.5740/jaoacint.17-0495.

[52] Luca, S. V.; Roehrer, S.; Kleigrewe, K.; Minceva, M. Approach for Simultaneous Cannabidiol Isolation and Pesticide Removal from Hemp Extracts with Liquid-Liquid Chromatography. *Ind. Crops Prod.* **2020**, *155*, 112726. DOI: 10.1016/j.indcrop.2020.112726.

[53] Kasperkiewicz, A.; Pawliszyn, J. Multi-Class Pesticide Analysis in Cannabis Oil Using Coated Blade Spray and Solid-Phase Microextraction with Liquid Chromatography Coupled to Mass Spectrometry. *Talanta* **2021**, *225*, 122036. DOI: 10.1016/j.talanta.2020.122036.

[54] Michlig, N.; Lehotay, S. J.; Lightfield, A. R.; Beldoménico, H.; Repetti, M. R. Validation of a High-Throughput Method for Analysis of Pesticide Residues in Hemp and Hemp Products. *J. Chromatogr. A* **2021**, *1645*, 462097. DOI: 10.1016/j.chroma.2021.462097.

[55] Cuypers, E.; Vanhove, W.; Gotink, J.; Bonneure, A.; Van Damme, P.; Tytgat, J. The Use of Pesticides in Belgian Illicit Indoor Cannabis Plantations. *Forensic Sci. Int.* **2017**, *277*, 59–65. DOI: 10.1016/j.forsciint.2017.05.016.

[56] Roggen, M. Healthy and Concentrated Cannabis Plants: How to Use Acronyms to Optimize Production. *J AOAC Int.* **2019**, *102*, 421–426. DOI: 10.5740/jaoacint.18-0205.

[57] Wilcox, J.; Pazdanska, M.; Milligan, C.; Chan, D.; Macdonald, S. J.; Donnelly, C. Analysis of Aflatoxins and Ochratoxin a in Cannabis and Cannabis Products by LC-Fluorescence Detection Using Cleanup with Either Multiantibody Immunoaffinity Columns or an Automated System with In-Line Reusable Immunoaffinity Cartridges. *J. AOAC Int.* **2020**, *103*, 494–503. DOI: 10.5740/jaoacint.19-0176.

[58] McPartland, J. M.; Clarke, R. C.; Watson, D. P. *Hemp Diseases and Pests: Management and Biological Control – An Advanced Treatise.*; CABI: Wallingford, 2000. DOI: 10.1079/9780851994543.0000.

[59] Punja, Z. K. Flower and Foliage-Infecting Pathogens of Marijuana (*Cannabis sativa* L.) Plants. *Can. J. Plant Pathol.* **2018**, *40*, 514–527. DOI: 10.1080/07060661.2018.1535467.

[60] Punja, Z. K.; Scott, C.; Chen, S. Root and Crown Rot Pathogens Causing Wilt Symptoms on Field-Grown Marijuana (*Cannabis sativa* L.) Plants. *Can. J. Plant Pathol.* **2018**, *40*, 528–541. DOI: 10.1080/07060661.2018.1535470.

[61] Jerushalmi, S.; Maymon, M.; Dombrovsky, A.; Freeman, S. Fungal Pathogens Affecting the Production and Quality of Medical Cannabis in Israel. *Plants* **2020**, *9*, 882. DOI: 10.3390/plants9070882.

[62] Dzuman, Z.; Zachariasova, M.; Lacina, O.; Veprikova, Z.; Slavikova, P.; Hajslova, J. A Rugged High-Throughput Analytical Approach for the Determination and Quantification of Multiple Mycotoxins in Complex Feed Matrices. *Talanta* **2014**, *121*, 263–272. DOI: 10.1016/j.talanta.2013.12.064.

[63] Rudrabhatla, M.; George, J. E.; Hill, N. R.; Siantar, D. P. Detection of Relevant Mycotoxins in Wheat Beer and Sake by LC-MS/MS Using Prototype Immunoaffinity Column Clean-Up: A Preliminary Study. ACS Symposium Series; 2008; Vol. 1001, pp 241–251. DOI: 10.1021/bk-2008-1001.ch012.

[64] Lacina, O.; Zachariasova, M.; Urbanova, J.; Vaclavikova, M.; Cajka, T.; Hajslova, J. Critical Assessment of Extraction Methods for the Simultaneous Determination of Pesticide Residues and Mycotoxins in Fruits, Cereals, Spices and Oil Seeds Employing Ultra-High Performance Liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2012**, *1262*, 8–18. DOI: 10.1016/j.chroma.2012.08.097.

[65] Reyes-Garcés, N.; Myers, C. Analysis of the California List of Pesticides, Mycotoxins, and Cannabinoids in Chocolate Using Liquid Chromatography and Low-Pressure Gas Chromatography-Based Platforms. *J. Sep. Sci.* **2021**, *44*, 2564–2576. DOI: 10.1002/jssc.202001265.

[66] Narváez, A.; Rodríguez-Carrasco, Y.; Castaldo, L.; Izzo, L.; Ritieni, A. Ultra-High-Performance Liquid Chromatography Coupled with Quadrupole Orbitrap High-Resolution Mass Spectrometry for Multi-Residue Analysis of Mycotoxins and Pesticides in Botanical Nutraceuticals. *Toxins* **2020**, *12*, 114. DOI: 10.3390/toxins12020114.

[67] Zhang, L.; Dou, X.-W.; Zhang, C.; Logrieco, A.; Yang, M.-H. A Review of Current Methods for Analysis of Mycotoxins in Herbal Medicines. *Toxins* **2018**, *10*, 65. DOI: 10.3390/toxins10020065.

[68] Senyuva, H. Z.; Gilbert, J. Immunoaffinity Column Clean-up Techniques in Food Analysis: A Review. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2010**, *878*, 115–132. DOI: 10.1016/j.jchromb.2009.05.042.

[69] Trucksess, M.; Weaver, C.; Oles, C.; D'Ovidio, K.; Rader, J. Determination of Aflatoxins and Ochratoxin a in Ginseng and Other Botanical Roots by Immunoaffinity Column Cleanup and Liquid Chromatography with Fluorescence Detection. *J. AOAC Int.* **2006**, *89*, 624–630. DOI: 10.1093/jaoac/89.3.624.

[70] Lattanzio, V. M. T.; Ciasca, B.; Powers, S.; Visconti, A. Improved Method for the Simultaneous Determination of Aflatoxins, Ochratoxin A and Fusarium Toxins in Cereals and Derived Products by Liquid Chromatography-Tandem Mass Spectrometry after Multi-Toxin Immunoaffinity Clean Up. *J. Chromatogr. A* **2014**, *1354*, 139–143. DOI: 10.1016/j.chroma.2014.05.069.

[71] Trucksess, M. W.; Weaver, C. M.; Oles, C. J.; Fry, F. S.; Noonan, G. O.; Betz, J. M.; Rader, J. I. Determination of Aflatoxins B1, B2, G1, and G2 and Ochratoxin a in Ginseng and Ginger by Multitoxin Immunoaffinity Column Cleanup and Liquid Chromatographic Quantitation: Collaborative

Study. *J. AOAC Int.* **2008**, *91*, 511–523. DOI: 10.1093/jaoac/91.3.511.

[72] Greaves, A.; Maddison, K.; Doran, M.; Lin, S.; Geiling, B. Single-Laboratory Validation of an Immunoaffinity Column Cleanup LC Method for the Analysis of Aflatoxins and Ochratoxin a in Cannabis Plant Material, Resins, Vapes, Isolates, and Edible Products. *J. AOAC Int.* **2021**, 104(5), 1264–1271. DOI: 10.1093/jaoacint/qsab057.

[73] Di Nardo, F.; Cavalera, S.; Baggiani, C.; Chiarello, M.; Pazzi, M.; Anfossi, L. Enzyme Immunoassay for Measuring Aflatoxin B1 in Legal Cannabis. *Toxins* **2020**, *12*, 265. DOI: 10.3390/toxins12040265.

[74] Llewellyn, G. C.; O'Rear, C. E. Examination of Fungal Growth and Aflatoxin Production on Marihuana. *Mycopathologia* **1977**, *62*, 109–112. DOI: 10.1007/BF01259400.

[75] Brera, C.; De Santis, B.; Debegnach, F.; Miraglia, M. Mycotoxins. In *Comprehensive Analytical Chemistry*; Elsevier: Amsterdam, The Netherlands, 2008; pp 363–427.

[76] Domijan, A.; M.; Peraica, M. Carcinogenic Mycotoxins. In *Comprehensive Toxicology*; Elsevier: Oxford, UK, 2018; pp 154–167.

[77] Ashiq, S.; Hussain, M.; Ahmad, B. Natural Occurrence of Mycotoxins in Medicinal Plants: A Review. *Fungal Genet. Biol.* **2014**, *66*, 1–10. DOI: 10.1016/j.fgb.2014.02.005.

[78] California Bureau of Cannabis Control-Testing Laboratories. https://bcc.ca.gov/licensees/testing_labs.html.

[79] D'Mello, J. P. F.; Placinta, C. M.; Macdonald, A. M. C. Fusarium Mycotoxins: A Review of Global Implications for Animal Health, Welfare and Productivity. *Anim. Feed Sci. Technol.* **1999**, *80*, 183–205. DOI: 10.1016/S0377-8401(99)00059-0.

[80] Edwards, S. G. Influence of Agricultural Practices on Fusarium Infection of Cereals and Subsequent Contamination of Grain by Trichothecene Mycotoxins. *Toxicol. Lett.* **2004**, *153*, 29–35. DOI: 10.1016/j.toxlet.2004.04.022.

[81] Placinta, C. M.; D'Mello, J. P. F.; Macdonald, A. M. C. A Review of Worldwide Contamination of Cereal Grains and Animal Feed with Fusarium Mycotoxins. *Anim. Feed Sci. Technol.* **1999**, *78*, 21–37. DOI: 10.1016/S0377-8401(98)00278-8.

[82] Perry Johnson Consulting. States That Require Cannabis Testing. https://www.pjcinc.com/?s=Cannabis+testing.

[83] Leafly Staff. Cannabis Testing Regulations: A State-by-State Guide. https://www.leafly.com/news/health/leaflys-state-by-state-guide-to-cannabis-testing-regulations.

[84] Europe Cannabis Testing Market to 2025 – Regional Analysis and Forecasts by Type; Services; End User and Country. https://www.reportlinker.com/p05764200/Europe-Cannabis-Testing-Market-to-Regional-Analysis-and-Forecasts-by-Type-Services-End-User-and-Country.html#backAction=1.

[85] Government of Canada Regulations under the Cannabis Act. https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/laws-regulations/regulations-support-cannabis-act.html.

[86] State of Colorado. Medical Merijuana Rules – Code of Colorado Regulations https://www.sos.state.co.us/CCR/GenerateRulePdf.do?ruleVersionId=7502&fileName=1CCR212-1.

[87] State of California Bureau of Cannabis Control, Text of Regulations, California Code of Regulations Title 16. https://www.bcc.ca.gov/law_regs/.

[88] Marotta, L.; Kwoka, T.; Scott, D.; Snow, M.; Astill, T. Fast, Quantitative Analysis of Residual Solvents in Cannabis Concentrates. https://www.perkinelmer.com/libraries/APP_Analysis_of_Residual_Solvents_in_Cannabis_Concentrates_Application_Note_014321A_01. 2018.

[89] Seltenrich, N. Cannabis Contaminants: Regulating Solvents, Microbes, and Metals in Legal Weed. *Environ. Health Perspect.* **2019**, *127*, 82001. DOI: 10.1289/EHP5785.

[90] Romano, L. L.; Hazekamp, A. Cannabis Oil: Chemical Evaluation of an Upcoming Cannabis-Based Medicine. *Cannabinoids* **2013**, *1*, 1–11.

[91] Tatera, K. *Residual Solvent Analysis: Ensuring the Safety of Cannabis Extracts* https://www.analyticalcannabis.com/articles/residual-solvent-analysis-ensuring-the-safety-of-cannabis-extracts-289021.

[92] Association of Public Health Laboratories (APHL). Guidance for State Medical Cannabis Testing Programs. https://www.aphl.org/aboutAPHL/publications/Documents/EH-Guide-State-Med-Cannabis-052016.pdf. 2017.

[93] Erickson, B. E. Cannabis Industry Gets Crafty with Terpenes. *Chem. Eng. News* **2019**, *97*, *18-20*.

[94] Troutt, W. D.; DiDonato, M. D. Carbonyl Compounds Produced by Vaporizing Cannabis Oil Thinning Agents. *J. Altern. Complement. Med.* **2017**, *23*, 879–884. DOI: 10.1089/acm.2016.0337.

[95] USP. Chemical Tests/<467> Residual Solvents. https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/generalChapter467Current.pdf.

[96] Vas, G. Chapter Four – State of the Art Solventless Sample Preparation Alternatives for Analytical Evaluation of the Volatile Constituents of Different Cannabis Based Products. In *Analysis of Cannabis*; Ferrer, I., Thurman, E. M., Eds.; Comprehensive Analytical Chemistry; Elsevier: Amsterdam, 2020; Vol. 90, pp 105–137. DOI: 10.1016/bs.coac.2020.04.012.

[97] Grodowska, K.; Parczewski, A. Analytical Methods for Residual Solvents Determination in Pharmaceutical Products. *Acta Pol. Pharm.* **2010**, *67*, 13–26.

[98] Hilliard, C.; Rigdon, A.; Schroeder, W.; Schroeder, C.; Flood, T. App Note (Restek) – Residual Solvents in Cannabis Concentrates. https://www.restek.com/en/chromablography/chromablography/analyzing-residual-solvents-in-cannabis-concentrates-a-sticky-situation/. 2014.

[99] Clifford, B.; Lock, N.; Karbowski, R.; Johnson, V.; Sandy, A.; Lugo-Morales, L.; Owens, A.; Sheehan, C. Utilizing GC-MS and GC Instrumentation for Residual Solvents in Cannabis and Hemp. In *Cannabis Laboratory Fundamentals*; Springer International Publishing: Cham, 2021; pp 191–222DOI: 10.1007/978-3-030-62716-4_10.

[100] Harper, T.; Hollis, J. S.; Fausett, E.; Macherone, A. Novel Residual Solvents Analysis of Cannabinoid Products with the Agilent Headspace-GC/MS System. https://www.agilent.com/cs/library/applications/application-cannabis-testing-agilent-headspace-gcms-5994-1926en-agilent.pdf. 2020.

[101] Shimadzu. Oregon Residual Solvent Analysis Method for Cannabis/Hemp Using a Single Quad GCMS with Headspace. https://www.ssi.shimadzu.com/sites/ssi.shimadzu.com/files/Industry/Literature/2004-Oregon-Residual-Solvent-Analysis-Method-Cannabis-Hemp.pdf. 2020.

[102] B'Hymer, C. Residual Solvent Testing: A Review of Gas-Chromatographic and Alternative Techniques. *Pharm. Res.* **2003**, *20*, 337–344. DOI: 10.1023/a:1022693516409.

[103] Camarasu, C. C.; Mezei-Szüts, M.; Varga, G. B. Residual Solvents Determination in Pharmaceutical Products by GC-HS and GC-MS–SPME. *J. Pharm. Biomed. Anal.* **1998**, *18*, 623–638. DOI: 10.1016/S0731-7085(98)00276-3.

[104] Guo, W.; Vrdoljak, G.; Liao, V.-C.; Moezzi, B. Major Constituents of Cannabis Vape Oil Liquid, Vapor and Aerosol in California Vape Oil Cartridge Samples. *Front. Chem.* **2021**, *9*, 694905. DOI: 10.3389/fchem.2021.694905.

[105] Coran, S. A.; Giannellini, V.; Furlanetto, S.; Bambagiotti-Alberti, M.; Pinzauti, S. Improving Gas Chromatographic Determination of Residual Solvents in Pharmaceuticals by the Combined Use of Headspace Solid-Phase Microextraction and Isotopic Dilution. *J. Chromatogr. A* **2001**, *915*, 209–216. DOI: 10.1016/S0021-9673(01)00658-6.

[106] Górecki, T.; Harynuk, J.; Panić, O. The Evolution of Comprehensive Two-Dimensional Gas Chromatography (GC x GC). *J. Sep. Sci.* **2004**, *27*, 359–379. DOI: 10.1002/jssc.200301650.

[107] Guo, W. Development of a Residual Solvent Test Method for California Cannabis Test Regulations Using Headspace-GC-MS. Cannabis Science Conference; 2018.
[108] Stevens, D. M. Analysis of Residual Solvents in Hemp Oil Using Headspace Sampling and Atmospheric Pressure GC-MS/MS. Waters Corporation, Application Note. https://www.waters.com/nextgen/in/en/library/application-notes/2021/analysis-of-residual-solvents-in-hemp-oil-using-headspace-sampling-and-atmospheric-pressure-gc-ms-ms.html. 2021.
[109] SepSolve Analytical Ltd. White Paper 033 - Quantitation of Residual Solvents and Terpenes in Cannabis Products – A Combined Approach. https://theanalyticalscientist.com/fileadmin/tas/app-notes/2020/SepSolve_White_Paper_033__Cannabis_terpenes_and_residual_solvents_-_GC-TOF_MS_and_GCxGC-TOF_MS_FID___1_.pdf. 2020.
[110] Sall, M. L.; Diaw, A. K. D.; Gningue-Sall, D.; Efremova Aaron, S.; Aaron, J. J. Toxic Heavy Metals: Impact on the Environment and Human Health, and Treatment with Conducting Organic Polymers, A Review. *Environ. Sci. Pollut. Res. Int.* **2020**, *27*, 29927–29942. DOI: 10.1007/s11356-020-09354-3.
[111] Rahman, Z.; Singh, V. P. The Relative Impact of Toxic Heavy Metals (THMs) (Arsenic (As), Cadmium (Cd), Chromium (Cr)(VI), Mercury (Hg), and Lead (Pb)) on the Total Environment: An Overview. *Environ. Monit. Assess.* **2019**, *191*, 419. DOI: 10.1007/s10661-019-7528-7.
[112] Filipiak-Szok, A.; Kurzawa, M.; Szłyk, E. Determination of Toxic Metals by ICP-MS in Asiatic and European Medicinal Plants and Dietary Supplements. *J. Trace Elem. Med. Biol.* **2015**, *30*, 54–58. DOI: 10.1016/j.jtemb.2014.10.008.
[113] Gall, J. E.; Boyd, R. S.; Rajakaruna, N. Transfer of Heavy Metals through Terrestrial Food Webs: A Review. *Environ. Monit. Assess.* **2015**, *187*, 201. DOI: 10.1007/s10661-015-4436-3.
[114] Dryburgh, L. M.; Bolan, N. S.; Grof, C. P. L.; Galettis, P.; Schneider, J.; Lucas, C. J.; Martin, J. H. Cannabis Contaminants: Sources, Distribution, Human Toxicity and Pharmacologic Effects. *Br. J. Clin. Pharmacol.* **2018**, *84*, 2468–2476. DOI: 10.1111/bcp.13695.
[115] Khan, A. G. Promises and Potential of in Situ Nano-Phytoremediation Strategy to Mycorrhizo-Remediate Heavy Metal Contaminated Soils Using Non-Food Bioenergy Crops (*Vetiver zizinoides* & *Cannabis sativa*). *Int. J. Phytoremediation* **2020**, *22*, 900–915. DOI: 10.1080/15226514.2020.1774504.
[116] Omidi, A. H.; Cheraghi, M.; Lorestani, B.; Sobhanardakani, S.; Jafari, A. Biochar Obtained from Cinnamon and Cannabis as Effective Adsorbents for Removal of Lead Ions from Water. *Environ. Sci. Pollut. Res. Int.* **2019**, *26*, 27905–27914. DOI: 10.1007/s11356-019-05997-z.
[117] Suvarapu, L. N.; Baek, S.-O. Determination of Heavy Metals in the Ambient Atmosphere. *Toxicol. Ind. Health.* **2017**, *33*, 79–96. DOI: 10.1177/0748233716654827.
[118] Radosavljevic-Stevanovic, N.; Markovic, J.; Agatonovic-Kustrin, S.; Razic, S. Metals and Organic Compounds in the Biosynthesis of Cannabinoids: A Chemometric Approach to the Analysis of *Cannabis sativa* Samples. *Nat. Prod. Res.* **2014**, *28*, 511–516. DOI: 10.1080/14786419.2014.880912.
[119] Montoya, Z.; Conroy, M.; Vanden Heuvel, B. D.; Pauli, C. S.; Park, S.-H. Cannabis Contaminants Limit Pharmacological Use of Cannabidiol. *Front. Pharmacol.* **2020**, *11*, 571832. DOI: 10.3389/fphar.2020.571832.
[120] Zafeiraki, E.; Kasiotis, K. M.; Nisianakis, P.; Machera, K. Macro and Trace Elements in Hemp (*Cannabis sativa* L.) Cultivated in Greece: Risk Assessment of Toxic Elements. *Front. Chem.* **2021**, *9*, 654308. DOI: 10.3389/fchem.2021.654308.
[121] US EPA. Method 206.5, Arsenic (Sample Digestion Prior to Total Arsenic Analysis by Silver Diethyldithiocarbamate or Hydride Procedures). https://www.epa.gov/sites/default/files/2015-08/documents/method_206-5_1978.pdf. 1978.
[122] US EPA. Method 200.8, Revision 5.4: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma – Mass Spectrometry. https://www.epa.gov/esam/epa-method-2008-determination-trace-elements-waters-and-wastes-inductively-coupled-plasma-mass. 1994.
[123] US EPA. Method 200.2, Revision 2.8: Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements. https://www.epa.gov/sites/default/files/2015-08/documents/method_200-2_rev_2-8_1994.pdf. 1994.
[124] Liebling, J. P.; Clarkson, N. J.; Gibbs, B. W.; Yates, A. S.; O'Sullivan, S. E. An Analysis of Over-the-Counter Cannabidiol Products in the United Kingdom. *Cannabis Cannabinoid Res.* **2020**. DOI: 10.1089/can.2019.0078. Online ahead of print.
[125] Ali, N.; Hadi, F.; Ali, M. Growth Stage and Molybdenum Treatment Affect Cadmium Accumulation, Antioxidant Defence and Chlorophyll Contents in *Cannabis sativa* Plant. *Chemosphere* **2019**, *236*, 124360. DOI: 10.1016/j.chemosphere.2019.124360.
[126] Li, S.; Zhang, C.; Wang, S.; Liu, Q.; Feng, H.; Ma, X.; Guo, J. Electrochemical Microfluidics Techniques for Heavy Metal Ion Detection. *Analyst* **2018**, *143*, 4230–4246. DOI: 10.1039/c8an01067f.
[127] Wakshlag, J. J.; Cital, S.; Eaton, S. J.; Prussin, R.; Hudalla, C.; Cannabinoid. Terpene, and Heavy Metal Analysis of 29 over--the-Counter Commercial Veterinary Hemp Supplements. *Vet. Med. Res. Rep.* **2020**, *11*, 45–55. DOI: 10.2147/vmrr.s248712.
[128] Husain, R.; Weeden, H.; Bogush, D.; Deguchi, M.; Soliman, M.; Potlakayala, S.; Katam, R.; Goldman, S.; Rudrabhatla, S. Enhanced Tolerance of Industrial Hemp (*Cannabis sativa* L.) Plants on Abandoned Mine Land Soil Leads to Overexpression of Cannabinoids. *PLoS One* **2019**, *14*, e0221570. DOI: 10.1371/journal.pone.0221570.
[129] International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. Guideline for Elemental Impurities - Q3D(R1). https://database.ich.org/sites/default/files/Q3D-R1EWG_Document_Step4_Guideline_2019_0322.pdf. 2019.
[130] Wagner, J.; Chen, W.; Vrdoljak, G. Vaping Cartridge Heating Element Compositions and Evidence of High Temperatures. *PLoS One* **2020**, *15*, e0240613. DOI: 10.1371/journal.pone.0240613.
[131] Hess, C. A.; Olmedo, P.; Navas-Acien, A.; Goessler, W.; Cohen, J. E.; Rule, A. M. E-Cigarettes as a Source of Toxic and Potentially Carcinogenic Metals. *Environ. Res.* **2017**, *152*, 221–225. DOI: 10.1016/j.envres.2016.09.026.
[132] Thompson, G. R.; Tuscano, J. M.; Dennis, M.; Singapuri, A.; Libertini, S.; Gaudino, R.; Torres, A.; Delisle, J. M. P.; Gillece, J. D.; Schupp, J. M.; Engelthaler, D. M. A Microbiome Assessment of Medical Marijuana. *Clin. Microbiol. Infect.* **2017**, *23*, 269–270. DOI: 10.1016/j.cmi.2016.12.001.
[133] Punja, Z. K. The Diverse Mycoflora Present on Dried Cannabis (*Cannabis sativa* L., Marijuana) Inflorescences in Commercial Production. *Can. J. Plant Pathol.* **2021**, *43*, 88–100. DOI: 10.1080/07060661.2020.1758959.
[134] Vujanovic, V.; Korber, D. R.; Vujanovic, S.; Vujanovic, J.; Jabaji, S. Scientific Prospects for Cannabis-Microbiome Research to Ensure Quality and Safety of Products. *Microorganisms* **2020**, *8*, 290. DOI: 10.3390/microorganisms8020290.
[135] Punja, Z. K.; Collyer, D.; Scott, C.; Lung, S.; Holmes, J.; Sutton, D. Pathogens and Molds Affecting Production and Quality of *Cannabis sativa* L. *Front. Plant Sci.* **2019**, *10*, 1120. DOI: 10.3389/fpls.2019.01120.
[136] Remington, T. L.; Fuller, J.; Chiu, I. Chronic Necrotizing Pulmonary Aspergillosis in a Patient with Diabetes and Marijuana Use. *CMAJ* **2015**, *187*, 1305–1308. DOI: 10.1503/cmaj.141412.
[137] Andrews, W. H.; Wang, H.; Jacobson, A.; Ge, B.; Zhang, G.; Hammack, T. S. Salmonella. In *Bacteriological Analytical*

Manual; Food & Drug Administration: Washington, DC, 2018.

[138] Feng, P.; Weagant, S.,D.; Grant, M.,A.; Burkhardt, W. Enumeration of *Escherichia coli* and the Coliform Bacteria. In *Bacteriological Analytical Manual*; Food & Drug Administration: Washington, DC, 2017.

[139] AOAC International. Standard Method Performance Requirements (SMPR®) for Detection of Aspergillus in Cannabis and Cannabis Products – SMPR 2019.001. https://www.aoac.org/resources/smpr-2019001/.

[140] AOAC International. Standard Method Performance Requirements (SMPRs®) for Detection of Shiga Toxin-Producing Escherichia coli in Cannabis and Cannabis Products – SMPR 2020.012. https://www.aoac.org/wp-content/uploads/2021/02/SMPR-2020_012.pdf.

[141] AOAC International. Standard Method Performance Requirements (SMPRs®) for Detection of Salmonella species in Cannabis and Cannabis Products – SMPR 2020.002. https://www.aoac.org/wp-content/uploads/2020/07/SMPR-2020_002.pdf.

[142] USP. Microbiological Examination of Nonsterile Products/ <61> Microbial Enumeration Tests. https://www.usp.org/sites/default/files/usp/document/harmonization/gen-method/q05b_pf_ira_34_6_2008.pdf.

[143] USP. Microbiological Examination of Nonsterile Products/ <62> Tests for Specified Microorganisms. https://www.usp.org/sites/default/files/usp/document/harmonization/gen-method/q05a_pf_ira_34_6_2008.pdf.

[144] USP. Microbiological Examination of Nonsterile Products/ <1111> Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use. https://www.usp.org/sites/default/files/usp/document/harmonization/gen-method/q05c_pf_ira_33_2_2007.pdf.

[145] McLaren, J.; Swift, W.; Dillon, P.; Allsop, S. Cannabis Potency and Contamination: A Review of the Literature. *Addiction* **2008**, *103*, 1100–1109. DOI: 10.1111/j.1360-0443.2008.02230.x.

[146] AOAC International. Quantitation of Cannabinoids in Cannabis Dried Plant Materials, Concentrates, and Oils - 2018.11. http://www.eoma.aoac.org/methods/info.asp?ID=51760. 2018.

[147] Kruve, A.; Rebane, R.; Kipper, K.; Oldekop, M.-L.; Evard, H.; Herodes, K.; Ravio, P.; Leito, I. Tutorial Review on Validation of Liquid Chromatography-Mass Spectrometry Methods: Part II. *Anal. Chim. Acta.* **2015**, *870*, 8–28. DOI: 10.1016/j.aca.2015.02.016.

[148] NIST. NIST Tools for Cannabis Laboratory Quality Assurance. https://www.nist.gov/programs-projects/nist-tools-cannabis-laboratory-quality-assurance.

[149] Lochhead, C. Marijuana Testing Data Points to Lab Shopping in Nevada. https://www.reviewjournal.com/news/pot-news/marijuana-testing-data-points-to-lab-shopping-in-nevada-1910269/.

[150] Bautista, J. L.; Yu, S.; Tian, L. Flavonoids in *Cannabis sativa*: Biosynthesis, Bioactivities, and Biotechnology. *ACS Omega* **2021**, *6*, 5119–5123. DOI: 10.1021/acsomega.1c00318.

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Variation among hemp (*Cannabis sativus* L.) analytical testing laboratories evinces regulatory and quality control issues for the industry

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THC

ABSTRACT

Validated analytical testing of cannabinoid content for regulatory purposes is critical to farming high-cannabidiol (CBD) hemp (*Cannabis sativus* L.), as these methods are key to determine whether a crop is federally compliant by containing < 0.3% THC or must be destroyed at the time of harvest. This report identifies the sources and extent of variation in reported cannabinoid content after flower selection and conducted a survey of ten accredited hemp regulatory testing laboratories, including one state-owned laboratory. The results indicate that total tetrahydrocannabinol (THC) content is reported inconsistently due to an insufficient standardization of sample preparation and testing methods, thus likely leading to erroneous data. This work represents the early phase of ongoing research to enhance the consistency of cannabinoid analyses of hemp flower samples as an essential tool in Cannabis crop development.

1. Introduction

Although hemp and marijuana plants belong to the same group of three species, *Cannabis sativa, C. indica,* and *C. ruderalis*, recent agricultural legislation in the U.S. has reclassified hemp, removing it from the Schedule 1 controlled substances list and distinguishing it from marijuana by using the total THC level as a threshold. These levels are limited to "a Δ9-tetrahydrocannabinol concentration of not more than 0.3% on a dry weight basis" in any part of the hemp plant and any products derived from its cultivation (USDA Agricultural Marketing Service, 2019). This definition of hemp, tied to plant chemotype rather than plant genotype, stems from a 1976 taxonomical study distinguishing industrial hemp (used commonly at the time for fiber and food) from psychoactive, high-THC cultivars of (mostly) *C. sativa* marijuana (Small and Cronquist, 1976). However, hemp is known to be genetically distinguishable from marijuana because it lacks the allele coding for THCA-synthase (De Meijer et al., 2003; Sawler et al., 2015).

While industrial fibrous hemp remains an important crop, an increasing number of farmers are cultivating medicinal cultivars of hemp to meet consumer demand for CBD and other cannabinoid products such as cannabigerol (CBG) and cannabichromene (CBC). These products have been shown to have pharmacological activity providing purported therapeutic potential for antiepileptic, anxiolytic, antipsychotic, anti-inflammatory, and neuroprotective effects (Bridgeman and Abazia, 2017). Hemp flowers grown for medicinal use today contain up to 15% of CBD plus additional non-THC/non-CBD cannabinoids by dry weight. Depending on the growing conditions that favor cannabinoid production, these variants often approach or slightly exceed the 0.3% THC limit for classification as hemp at the time of harvest—despite having no psychotropic effects compared with marijuana, which is cultivated for the production of THC at dry-weight percentages commonly in excess of 20%. In general, a level of about 1% THC is considered the threshold for cannabis to have a psychotropic effect (Small and Marcus, 2002).

The industry faces several challenges as it attempts to scale cultivation to meet consumer demand for cannabinoid products. For example, categorizing cultivars becomes difficult due to variation in testing protocols and discrepancies in results between testing laboratories. However, these results are critical when quantifying the amount of THC in hemp flower and hemp-derived extracts, and certificates of analysis (COA) are compulsory for legal hemp harvest. In most states, hemp farmers are required to submit a request to the state 30 days before the anticipated harvesting time, so an official sampler can collect flowers from multiple plants of the same strain on the farm for compliance testing (CA Code of Regulations, 2021).

The selection methods to control for the uniformity of flower

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sampling from each registered strain of hemp prepared for harvesting can vary slightly based on a local regulator's protocol. The samples are then sent to an analytical lab, either operated by the state or individually contracted, to determine the concentration of cannabinoids in each varietal (strain) of hemp sampled. Industry or other standard operating procedures for these labs are missing. In addition, none of the commercial labs we evaluated provided experimental details about the applied methods or validation data. This may be related to the lack of regulations and reflect a means of maintaining a competitive advantage in a competitive environment. The lack of validated convention methods means that generally accepted standards for instruments, calibration, or sampling replicates are missing. However, in any state, if the test results reveal a total THC concentration below 0.3%, the farmer is approved to harvest on their listed date. However, if the THC test result is above 0.3% it is considered "hot", and the farmer may have to destroy the entire crop.

The federal mandate distinguishes between hemp and marijuana based on plant chemotype, using a single marker compound (THC), but without establishing a convention method that is "fit for purpose" for this definition. This is despite the existence of fit-for-purpose chemotype identification convention methods in the literature (Sarma et al., 2020). The lack of a validated convention method representing the official standard is particularly problematic because measurement of the THC concentration involves quantitation at a relatively low level, which poses analytical challenges, especially when performed outside highly controlled laboratory settings. With limited federal regulation over hemp analytical laboratory accreditation policies or standardized analysis protocols, the quantitation of cannabinoid composition reported by commercial hemp testing laboratories has the potential to vary greatly, including for identical samples. The sources of potential variation extend beyond analytic techniques and include sample storage upon receipt, homogenization and extraction methods, instrumentation, and calibration procedures. Collectively, these factors all influence the test results and, thereby, the ultimate decision about crop validation versus destruction. The development of different analytical methods to quantify cannabinoids in plant products allows individual laboratories to compete in a growing market for hemp analysis. However, this practice also interferes with transparency, exacerbates inter-laboratory variation in analysis results, diminishes the validity of crop regulation, and calls the overall value of COAs into question.

The attention focus on a single marker compound, THC, also raises the question about options and rationales for a more holistic analysis of the cannabinoid metabolome, which in fact is practically feasible. Potential approaches including methods for the quantitation of 13 individual cannabinoids have been compiled by the USP Cannabis Expert Panel (Sarma et al., 2020). However, with the legal definition of the Controlled Substance Act (CSA) in place, this does still not address the question of standardization of the analytical methodology used to determine cannabinoid content, including threshold CBD values. In this context, it should be pointed out that pharmacopeial approaches worldwide involve the establishment of convention methods, which are developed via a consensus building process and considered fit-for-purpose. While this approach provides a more definitive analytical framework, it does not necessarily address the levels of uncertainty involved in the analyses and are typically not embedded into metrological frameworks by inclusion of primary reference standards (such as NIST benzoic acid PS1) and traceable certified reference materials (Nelson et al., 2018).

2. Experimental

2.1. Laboratory survey

A total of 45 laboratories were identified via a Google search of the term "analytical cannabis testing labs" and contacted about their participation in a research survey of their analytical methods, prioritizing labs that provided services to regulatory agencies above those used only by the industry. Of the 45 labs contacted, 11 agreed to participate. One of the participating laboratories was eliminated because it did not offer hemp testing. The 10 participating laboratories were asked to extract and analyze three hemp flower samples and to return their cannabinoid potency test results. Surveyed laboratories were also asked to provide information about their instrumentation, standards, extraction protocols, and methods of analysis. While all of the participating laboratories chose to withhold their extraction and analysis protocols, they disclosed the manufacturer and make of their analytical instrumentation and source of reference standards employed for calibration.

2.2. Preparation

Two strains of hemp, Sample A, a high-CBG chemotype, and Sample B, a high-CBD chemotype, were cultivated for this survey. Samples from each strain were harvested on the same day. The samples were prepared from buds removed from the stalks and trimmed of surrounding leaves. The samples from each strain were collected into a bag and shaken to randomly distribute flowers collected from individual plants for distribution to the testing facilities. Homogenized samples were ground using a Spex 2010 Geno/Grinder 115 V. Flowers from each sample were added to 50 mL centrifuge tubes along with three steel balls. Each sample was run at 1000 strokes per minute for one minute, then manually stirred and visually inspected to ensure all flowers were ground. The grinding process was repeated at 1000 strokes per minute for one minute. All ground flower from each sample was combined in a designated container and mixed again to ensure a completely homogenized distribution, minimizing variability due to flower selection from one individual plant or a portion thereof to another given the same strain.

2.3. Extraction control experiment

To determine variation in cannabinoid results due to extraction solvents, an internal analysis was performed using various extraction solvents. Homogenized flowers from Sample B were extracted nine times using each of the solvents reportedly used by the labs in the survey. About 200 mg of homogenized flowers was added to each of nine 50 mL polypropylene centrifuge tubes. The exact mass of Sample B was recorded for each tube, and each sample was extracted with 20 mL ethanol (3), 20 mL isopropanol (3), or 20 mL 1:1 isopropanol and acetonitrile (3). Each tube was mixed with a FisherBrand VWR Multi-tube vortexer at speed 9 for 5 min. Each of the tubes was centrifuged in a Q-Sep 3000 Centrifuge 110 V for 5 min to separate residual solids from the supernatant. 1.5 mL of the suspended supernatant was removed and syringe-filtered through a Millex GP 0.22 μm filter to remove solid particulates. 50 μl of the supernatant was added to a 1.5 mL amber HPLC vial and diluted with 950 μl of the respective extraction solvent. The samples were analyzed using a Shimadzu LC2030–3D plus with a PDA detector equipped with a NexLeaf CBX C18 column and mobile phase 0.085% phosphoric acid in acetonitrile. The sample was compared to calibration standards "Certified Standard-11 Components (CRM) in Acetonitrile" sourced from Cayman Chemicals. The 250 μg/mL standard mixture was diluted to 0.5 ppm, 1 ppm, 5 ppm, 10 ppm, 50 ppm, and 500 ppm ($R2 > 0.999$ for each of the 11 cannabinoids).

2.4. Sample B extract preparation

200.4 mg of homogenized Sample B was added to a 50 mL polypropylene centrifuge tube and extracted with 20 mL ethanol. The mixture was mixed with a FisherBrand VWR Multi-tube vortex at speed 9 for 5 min. The sample was centrifuged in a Q-Sep 3000 Centrifuge 110 V for 5 min. The suspended supernatant was drawn into a syringe and filtered using a 0.22 μm Millex GP filter. We sent each surveyed lab 1 mL of the filtered extract in a vial sealed using parafilm and shipped in

insulated, light-proof packaging. Participating laboratories were instructed to dilute the sample further using ethanol as necessary prior to the cannabinoid potency determination.

2.5. Data analysis

Data provided by participating laboratories (n = 10) were compiled into spreadsheets using the following variables to organize reported results for the three strains: Extraction Solvent, Instrument used, Total Cannabinoids, Total CBD, CBDa, CBD, CBDv, CBDva, Total THC, d9THC, d8THC, THCa, THCv, THCva, Total CBG, CBG, CBGa, Total CBC, CBCa, CBC, CBL, CBLa, CBN, CBNa. Total THC/CBD was calculated using the following formulas to take into account the loss of a carboxyl group during decarboxylation step:

Total THC = Δ9THC +(THCa (0·877))

Total CBD = CBD + (CBDa (0·877))

Descriptive statistics were calculated, including mean, standard deviation (SD), relative SD, and range; missing data (for variables not tested or non-reported results) were ignored. Median values and interquartile range were plotted using boxplots. Statistical analyses were conducted with Microsoft Excel and OriginPro 9.0 SR2 (v2013), which was also used to generate Figs. 1 and 2.

3. Results and discussion

For the survey used in this report, two strains of hemp, Sample A, a high-CBG chemotype, and Sample B, a high-CBD chemotype, were collected as whole flowers and distributed to laboratories that agreed to participate in a blind study of their analytical results and a survey of their testing protocols. The samples were also analyzed in our laboratory to further study the effects of homogenization and extraction protocols, and compare the results from the labs against a carefully controlled and transparent sample preparation, calibration, and analytic procedure. The reported total THC content of Sample A varied among ten surveyed laboratories (Fig. 1) with a mean of 0.11% (dry weight basis), a SD of 0.10%, and three labs returning a non-detect (ND) result, indicating that total THC content was below the limits of their detection methods (for the purposes of statistical analysis, ND results were treated as a result of 0%). Total THC/CBD is calculated using the following formulas to take into account the loss of a carboxyl group during decarboxylation step:

Total THC = Δ9THC +(THCa (0·877)) and Total CBD = CBD + (CBDa (0·877))

Of the ten surveyed laboratories, one reported a result of 0.29% of

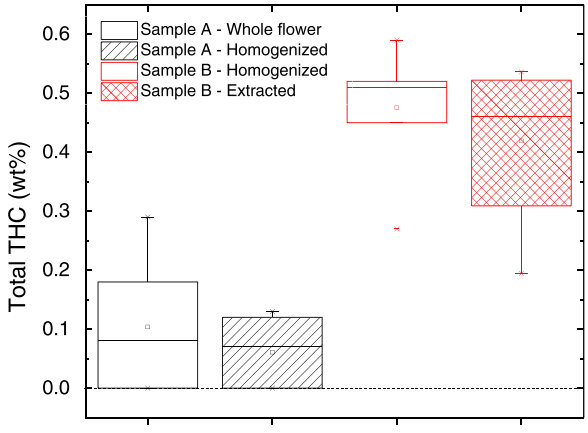

Fig. 1. Total THC content distributions as reported by ten participating commercial hemp analytical laboratories in the U.S.

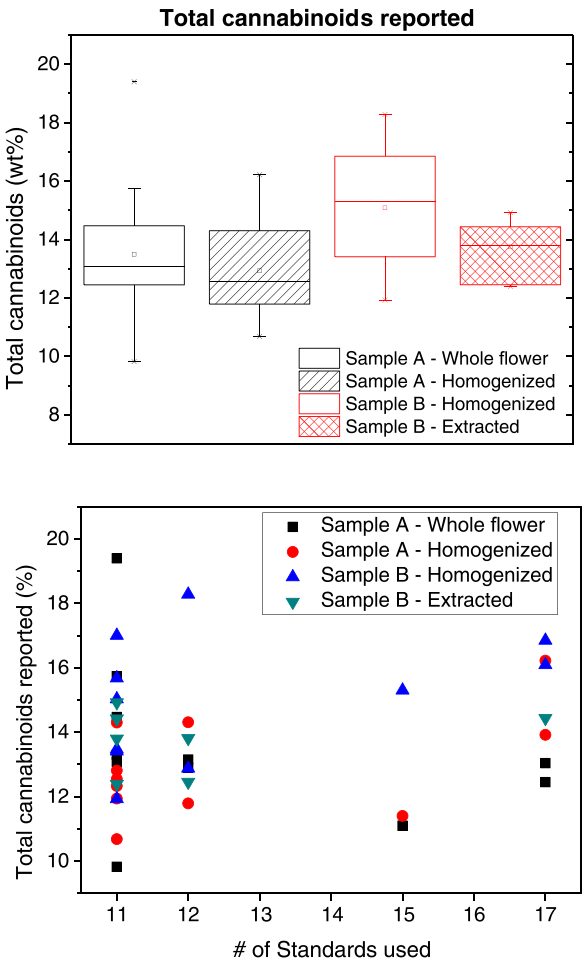

Fig. 2. Total cannabinoid concentration reported (top) and a comparison between total cannabinoids reported and number of standards used in quantitation (bottom).

THC, a value just below the legal threshold for crop destruction. The range of reported total THC (including THCA) content in this sample therefore spans 96.7% of the legally permissible total THC detection range for hemp, with a relative SD of 95% of the mean. This suggests that the applied analytical methods for total THC quantitation across surveyed laboratories are not sufficiently precise at the levels required for the analysis of THC content in hemp. Our own analysis yielded a total THC content of 0.09%, close to the mean reported by the sample of surveyed laboratories.

From harvest of flower samples to sample preparation, homogenization, extraction, analysis, and quantitation, multiple factors can contribute to the variation in the reported total THC content, independently of the analytical method used. We probed these potential sources of variation by sending participating laboratories a homogenized (ground) Sample A, which was identified to participating laboratories as a third sample, Sample C. Although the plant material was identical to Sample A, the variation in reported results significantly decreased (SD = 0.10% in whole flower and SD = 0.05% in homogenized flower), indicating that, in addition to potential flower-to-flower variation, different homogenization methods between laboratories also contributed to significant variation in the quantified total THC content.

Sample B was shipped to participating laboratories homogenized using a GenoGrinder. In this sample, the total THC content measured in our laboratory (0.51%) was higher than that of Sample A. Again, only the mean of the total THC content reported by the ten participating laboratories yielded a result similar to control (0.48%), whereas the reported values ranged from 0.27% (compliant) to 0.59% (non-

compliant), with a relative SD of 21% of the mean. Although the relative SD was lower in this sample relative to Sample A, the SD remained as high as 0.10% dry weight in terms of total THC content).

While all external laboratories reported using HPLC with UV–vis photodiode array detectors, the solvent used to extract flower samples varied: nine laboratories used methanol, one laboratory a 1:1 mixture of isopropanol and acetonitrile. In our internal reference analysis, ethanol was employed as the extraction solvent. In an extraction control experiment, nine samples of homogenized sample B were extracted with either ethanol (3), methanol (3), or isopropanol/acetonitrile (3). It was found that ethanol yielded the most exhaustive extraction, regardless of whether only total THC (Table 1) or total cannabinoid content were compared (15.7% ± 0.5, 14.3% ± 0.2, 15.1% ± 0.8 for ethanol, methanol, and isopropanol/acetonitrile, respectively).

Given the success in reducing analytical variation by pre-homogenizing flower samples, we anticipated that extracting the homogenized flower and shipping the extract to participating laboratories would significantly reduce variation. However, the variation in reported total THC content among participating laboratories increased when pre-extracted samples versus whole flower samples were shipped to and analyzed by participating laboratories (Sample B homogenized flower SD = 0.10% and extracted SD = 0.12%).

A pre-extracted sample should show the highest degree of homogeneity (lowest variation) between aliquots sampled by participating laboratories, as it controls for all aspects of sample harvesting and preparation up to analytical method and quantitation. One reason for the observed variations of results may be that participating laboratories employ sample preparation and analysis protocols for whole flower samples rather than liquid samples sent from customers. Here, variation may be due to lack of consistency of liquid sample handling, dilution procedures, and time to complete the analysis.

The outcomes of this survey highlight the potential issues resulting from variation among laboratories testing THC content. Farmers cultivating hemp strains for medicinal applications, however, will be interested not only in the THC content reported in COAs, but in the content of many other cannabinoids quantified in hemp samples. The variation in total cannabinoid content is shown in Fig. 2, where it can be seen that for the whole flower Sample A, reported cannabinoid content spans a range from 9.8% to 19.4%—a huge variation. This once more highlights the importance of considering a wider spectrum of cannabinoids when evaluating cannabis materials (Sarma et al., 2020).

In addition to unavoidable inter-laboratory variation, the observed inconsistencies of the analytical outcomes can be inherent to the method by which cannabinoids are measured: HPLC with photodiode array detection is a comparative technique that relies on efficient separation, which in turn depends on many factors such as the specific column and chromatographic conditions (mobile phase solvents, solvent gradient, flow rate, temperature). To achieve quantitation, the HPLC chromatogram of a sample is compared to a chemically identical reference material ("standard") that is analyzed under identical conditions to establish internal or external calibration. Provided that multiple calibrants are available, complex mixtures can be separated and individual components quantified. However, peaks overlap is one major source of error, especially when analyzing chemically complex mixtures such as Cannabis extracts. For example, similar or fully overlapping retention times under the applied conditions—as is often the case, for instance with Δ8- vs. Δ9-THC or the *cis* and *trans* isomers — affect the specificity of the quantitation. Moreover, the reference materials used for calibration can undergo chemical change (degradation), which may or may not be captured in both externally and internally calibrated methods. In fact, as terpenoids, cannabinoids are known to be relatively unstable compounds and commonly change after exposure to heat, light, or air (not counting the common decarboxylation reaction of the acid forms).

It should be noted that analytical methods are available that are independent of chemically identical reference materials (calibrants). For example, quantitative nuclear magnetic resonance (qNMR) has this capability as it represents a (relative) primary analytical technique. Its suitability for natural product and pharmaceutical analysis is widely acknowledged and the fitness of the application of quantum mechanics-based qNMR has recently been demonstrated for CBD (Pauli et al., 2014; Nelson et al., 2020).

The laboratories surveyed in this study used reference standards from Restek, Cerilliant, LGC, and Cayman. While most laboratories employed 11 standards, up to 18 standards were used to quantify various cannabinoids in hemp samples. Facilities that quantified a larger number of individual cannabinoids tended to report greater means in total cannabinoid content. However, no correlation was found between the number of calibration standards and the reported total percent content quantified across all facilities and all four samples. This kind of inconsistency highlights the analytical challenges hemp farmers face when evaluating the reliability of COA results and assessing the value of their crops.

Table 1
Total THC content measured in identical samples extracted with various solvents.

	Ethanol	Methanol	Isopropanol/acetonitrile (1:1)
Sample 1	0.520	0.445	0.45
Sample 2	0.507	0.463	0.51
Sample 3	0.502	0.459	0.47
Average	0.510 ± 0.007	0.456 ± 0.008	0.48 ± 0.03

4. Conclusion

This study represents a preliminary survey of the variation in reported total THC content among ten commercial hemp testing facilities. While early, the presented evidence strongly suggests that inconsistencies exist in reported total THC content, which leads to a regulatory liability. While the total THC content is currently the key legal metric defining whether a crop is harvestable or must be destroyed, it is not tied to, for example, a convention method that ensures validity and reproducibility of analytical outcomes, such as the methods found in pharmacopeias. The present data suggest that total THC content is frequently reported imprecisely, most likely due to analytical inconsistency of sample preparation and testing methods. Even under the carefully controlled conditions of the in-house laboratory, the relative SD of total THC content as measured by HPLC using commercially available calibrants varied between 2% and 7% of the mean, depending on the extraction protocol employed. This variation is within the range of what can be expected for the quantitation of a minor (<1%) constituent embedded in a complex analytical matrix and indicates the intrinsic limitations in peak purity as the likely key factor in HPLC-UV-based quantitation.

These results suggest a significant likelihood of a hemp crop being marked for destruction due to inaccurate COA reporting – as well as a crop being labeled as compliant despite actually containing elevated THC levels. To support this burgeoning industry those exposed to the risk of growing this relatively new crop in the U.S., it would be a step forward to revise enforcement of COA results such that they account for the variation in reported total THC content, introduce methodology that establishes a reproducible linkage with metrological reference materials, and eliminate the inherent variability of results from different analytical methods. To do this, however, several factors would need to be carefully considered. The current regulatory specifications are simple numbers that do not include measurement uncertainties. Determining the threshold values may have involved accounting for measurement uncertainty, but the values do not express that. In pharmacopeial monographs and standards, general rules exist about precision and accuracy, for example for the difference between 0.3% and 0.30%. Threshold values such as NMT 0.3%, however, are agnostic to statistics as they are expressed with only one significant number. It is always good

to define the specifications of an analytical method to avoid this confusion, for example by clarifying that threshold values or ranges include three significant numbers, two true and one uncertain, as is common in analytical chemistry (Ellison and Williams, 2012). This will also allow testing labs to demonstrate their fitness for analytical purposes. Considering our results, from the viewpoint of statistics alone, it may be adequate to introduce ranges of two SD of the legal limit when defining the thresholds that determine the legal designation of a crop as hemp vs. marijuana.

Until these points are addressed, the definition of hemp based on an arbitrary number with one significant figure as upper boundary ("0.3%") of total THC content remains flawed, due to the demonstrated variability in the reported measurements of that value and the difficulty and cost of making the measurement for each crop. It should also be borne in mind that genotyping, rather than chemotyping, offers a valuable approach to the distinction of hemp from marijuana. Legal definitions that consider the existing analytical evidence will help protect hemp farming from the undue liability of misrepresented or inaccurate strain identification and flawed chemical analysis, and will foster the rational development of hemp crops with expanded utility by means of specific chemotype.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Bridgeman, M.B., Abazia, D., 2017. Medical cannabis: history, pharmacology, and implications for acute care settings. Pharm. Ther. 42 (3), 180–188. ⟨https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5312634/⟩.

CA Code of Regulations, 2021. California Industrial Hemp Law and Regulations. Title 3, Div. 4, Ch. 8, Art. 3. January 19, 2021. ⟨https://www.cdfa.ca.gov/plant/industrialhemp/docs/CaliforniaIndustrialHempLawandRegulations.pdf⟩.

De Meijer, E.P.M., Bagatta, M., Carboni, A., Crucciti, P., Moliterni, V.M.C., Ranalli, P., Mandolino, G., 2003. The inheritance of chemical phenotype in Cannabis sativa L. Genetics 163, 335–346. https://doi.org/10.1093/genetics/163.1.335.

Ellison, S.L., Williams, A., 2012. Quantifying uncertainty in analytical measurement. Eurachem. CITAC.

Nelson, K.M., Bisson, J., Singh, G., Graham, J.G., Chen, S.N., Friesen, J.B., Dahlin, J.L., Niemitz, M., Walters, M.A., Pauli, G.F., 2020. The essential medicinal chemistry of cannabidiol (CBD. J. Med. Chem. 63 (21), 12137–12155. https://doi.org/10.1021/acs.jmedchem.0c00724.

Nelson, M.A., Waters, J.F., Toman, B., Lang, B.E., Rück, A., Breitruck, K., Obkircher, M., Windust, A., Lippa, K.A., 2018. A new realization of SI for organic chemical measurement: NIST PS1 primary standard for quantitative NMR (Benzoic Acid). Anal. Chem. 90, 10510–10517. https://doi.org/10.1021/acs.analchem.8b02575.

Pauli, G.F., Chen, S.N., Simmler, C., Lankin, D.C., Gödecke, T., Jaki, B.U., Friesen, J.B., McAlpine, J.B., Napolitano, J.G., 2014. Importance of purity evaluation and the potential of quantitative 1H NMR as a purity assay: miniperspective. J. Med. Chem. 57 (22), 9220–9231. https://doi.org/10.1021/jm500734a.

Sarma, N.D., Waye, A., ElSohly, M.A., Brown, P.N., Elzinga, S., Johnson, H.E., H.E, Marles, R.J., Melanson, J.E., Russo, E., Deyton, L., Hudalla, C., Vrdoljak, G.A., Wurzer, J.H., Khan, I.A., Kim, Nam-Cheol, Giancaspro, G.I., 2020. Cannabis inflorescence for medical purposes: USP considerations for quality attributes (https:/doi.org/). J. Nat. Prod. 83 (4), 1334–1351. https://doi.org/10.1021/acs.jnatprod.9b01200.

Sawler, J., Stout, J.M., Gardner, K.M., Hudson, D., Vidmar, J., Butler, L., Page, J.E., 2015. PLoS One 10, 1–9. https://doi.org/10.1371/journal.pone.0133292.

Small, E., Cronquist, A., 1976. A practical and natural taxonomy for Cannabis. Taxon 25, 405–435. https://doi.org/10.2307/1220524.

Small, E., Marcus, D. 2002. Hemp: A New Crop with New Uses for North America. Trends in New Crops and New Uses, ed. J. Janick and A. Whipkey, Alexandria, VA. American Society for Horticultural Science Press: 284–326. ⟨https://www.hort.purdue.edu/newcrop/ncnu02/v5–284.html⟩.

USDA Agricultural Marketing Service, 2019. Establishment of a domestic hemp production program. Fed. Reg. 84 (211), 58522–58564.