Pilot Test Demonstrates Successful Development of Representative, Ready-to-Analyze, Method-Agnostic Proficiency Test Samples for Cannabis and Hemp Testing Laboratories

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The Need

The hemp and cannabis industry has experienced an economic boom in the last 10 years, with new products available every day. To keep pace, hemp and cannabis testing laboratories have had to swiftly evolve to ensure that analytical methodologies are fully addressing the needs of this rapidly growing industry and the changing regulatory landscape surrounding these materials.

Whether the focus is on ensuring products are free of dangerous pesticides, heavy metals, or microbials, or on characterizing potency and terpene content of hemp and cannabis related products, it is more imperative than ever for labs to prove to themselves, their customers, state and federal regulators, and the public that the results they deliver are defensible and accurate.

AOAC INTERNATIONAL (AOAC) and Signature Science, LLC (SigSci) have developed the AOAC Cannabis and Hemp Proficiency Test (PT) Program based on feedback from laboratories, regulatory and accreditation bodies, and stakeholders. The program offers uniquely representative ready-to-analyze PT samples in both hemp and cannabis matrices. Rigorous homogeneity and stability testing results support defensibility and interpretation of results. This is the only PT program currently capable of shipping > 0.3% delta-9 THC cannabis across state lines.

Creating Representative PT Samples

- 1. Hemp flower is dried/cured and inspected.
- 2. Flower is ground to an "oregano" grain size and homogenized thoroughly.
- 3. Aliquots of homogenized batches are analyzed to characterize the naturally occurring profile of cannabinoids and terpenes, moisture content, and possible contaminants.
- 4. Sample types that are representative of typical operational samples are created and packaged for distribution.
- 5. Homogeneity testing is performed and reviewed in detail between SigSci and AOAC prior to shipping.
- 6. Samples shipped to participants.
- Samples containing > 0.3% d9-THC are shipped in accordance with dangerous goods and DEA regulations.
- SigSci holds licensing required to ship cannabis samples to qualified domestic/international participants.
- 7. Stability testing is performed and reviewed in detail between SigSci and AOAC, and appropriately factored into participant grading.

All PT samples are prepared and verified in compliance with ISO 17043 requirements. Through internal R&D, SigSci developed and tested four hemp/cannabis analytical methods covering 119 compounds across multiple matrices. Sample preparation and verification SOPs were developed and validated and are the basis of the current AOAC Cannabis/Hemp PT Program.

The AOAC Cannabis/Hemp PT Pilot Study

- SigSci designed and created two sample types for analysis in accordance with validated in-house methods.
- PT samples were shipped to twenty participating domestic laboratories on May 2, 2022.
- Participants had three weeks to analyze and report results using any method of their choice.
- Homogeneity and stability testing was performed by SigSci (cannabinoids, terpenes, and pesticides) and a third-party ISO 17025 accredited laboratory (heavy metals and moisture).
- AOAC reviewed results and released grading and preliminary statistical reports in July 2022.
- Employed a statistical grading approach using reference laboratories for comparison
- Assigned values by sample type:
- Blue (naturally occurring profiles): consensus value based on median of reference laboratories
- Red (fortified with contaminants): known concentration
- based on spiking

Sample 1-Blue (10 g)

Naturally occurring profiles and contaminants

Analytical methods covered: cannabinoids, terpenes, heavy metals, and moisture content

Homogeneity and Stability Results

Cannabinoid	CBD	CBG	CBDA	CBN	d9-THC	THCA	d8-THC	Terpene	β-Myrcene	Linalool	Terpineol	Caryophyllene	Guaiol
		Stability Sum	nmary Statistics						Stabilit	y Summary Statistic	s		
Mean (w/w%)	0.45	0.052	12.474		0.069	0.386		Mean (w/w%)	0.193	0.021	0.015	0.053	0.038
Mean (mg/g)	4.5	0.52	124.74	·	0.69	3.86		Mean (mg/g)	1.93	0.21	0.15	0.53	0.38
Std Dev using w/w%	0.01	0.01	0.36		0.00	0.01		Std Dev using w/w%	0.02	0.00	0.00	0.00	0.01
Total CV using w/w%	2%	14%	3%		4%	3%		Total CV using w/w%	8%	9%	8%	8%	13%
Min Measurement w/w% (mg/g)	0.44% (4.4)	0.04% (0.4)	11.89% (118.9)		0.07% (0.7)	0.37% (3.7)		Min Measurement w/w% (mg/g)	0.17% (1.7)	0.02% (0.2)	0.01% (0.1)	0.05% (0.5)	0.03% (0.3)
Max Measurement w/w% (mg/g)	0.46% (4.6)	0.06% (0.6)	12.76% (127.6)		0.07% (0.7)	0.39% (3.9)		Max Measurement w/w% (mg/g)	0.21% (2.1)	0.02% (0.2)	0.02% (0.2)	0.06% (0.6)	0.04% (0.4)
		Comparison to H	omogeneity Res	ults					Comparisor	n to Homogeneity R	esults		
Mean Homogeneity (w/w%)	0.445	0.037	12.306		0.082	0.407		Mean Homogeneity (w/w%)	0.237	0.021	0.014	0.059	0.041
Percent Difference (%)	0.981	41.067	1.358		-15.744	-5.206		Percent Difference (%)	-18.433	1.634	2.248	-9.972	-7.091
T-Test P Value	0.7263	0.0074	0.6918		0.0003	0.1658		T-Test P Value	0.0346	0.7772	0.6982	0.1095	0.2725
Conclusion	Pass	Pass	Pass		Pass	Pass		Conclusion	Pass	Pass	Pass	Pass	Pass

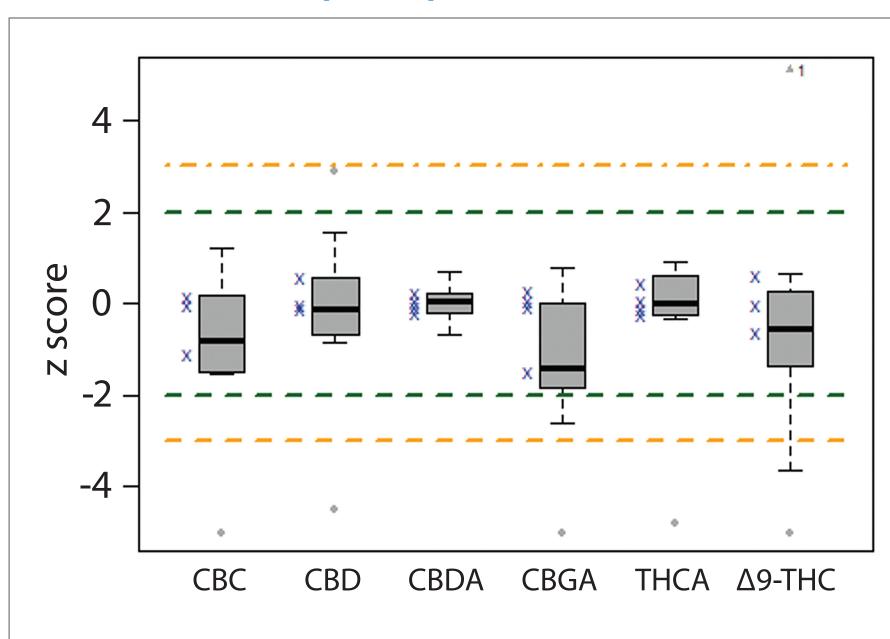


Sample 2-Red (2.5 g)

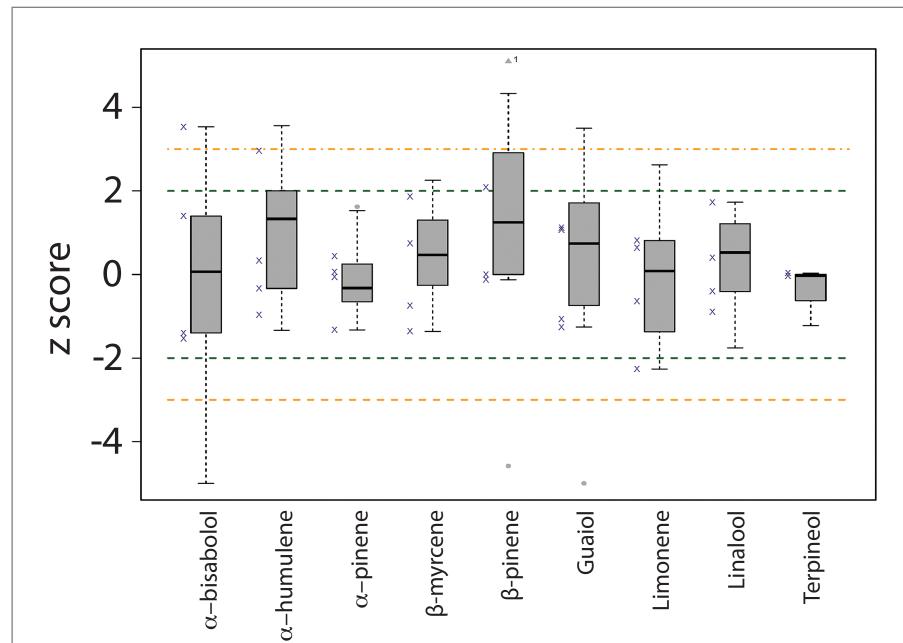
Fortified with contaminants Analytical methods covered: pesticide residues

Participant Results

Cannabinoids (Blue)



Terpenes (Blue)

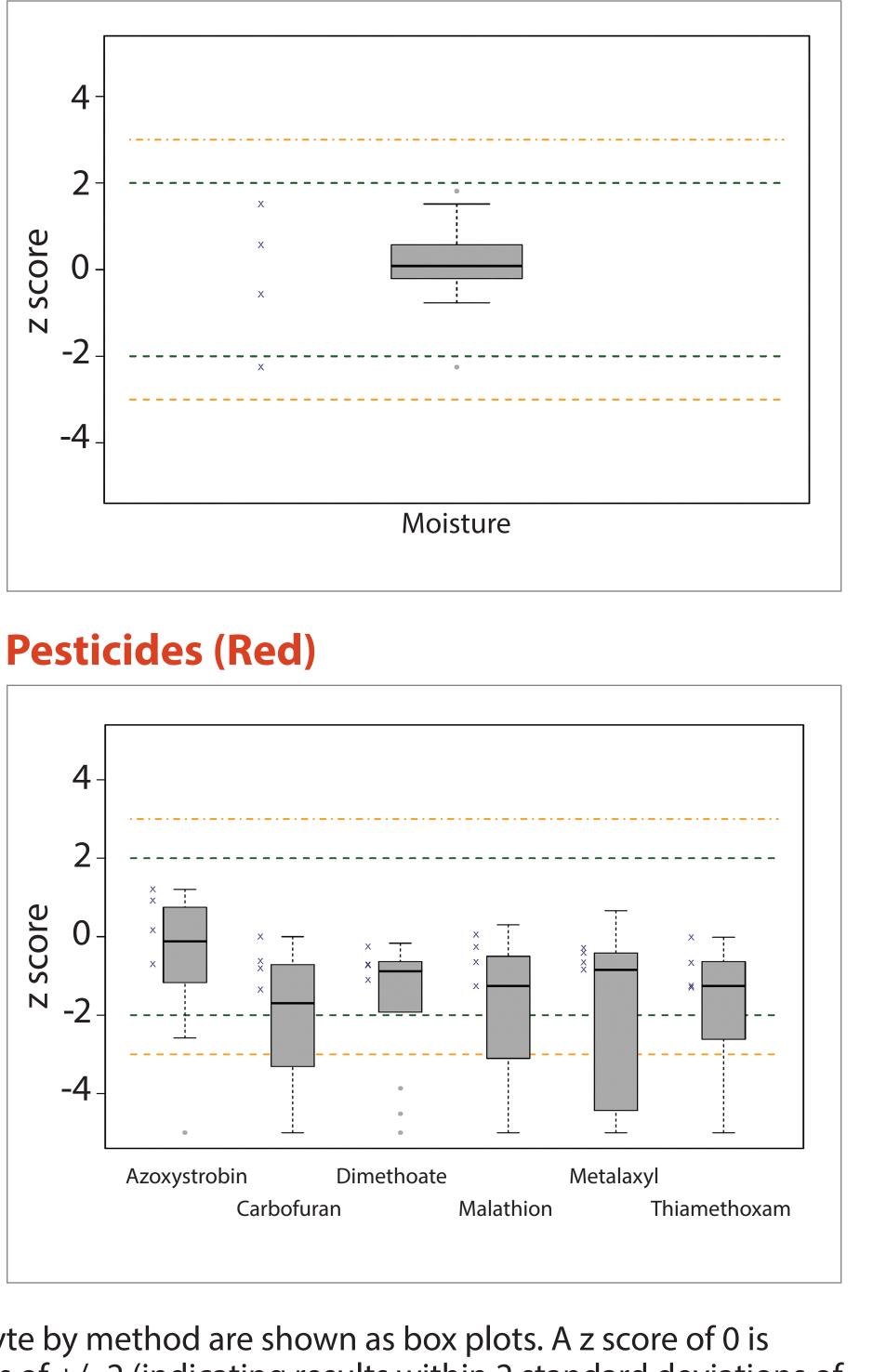


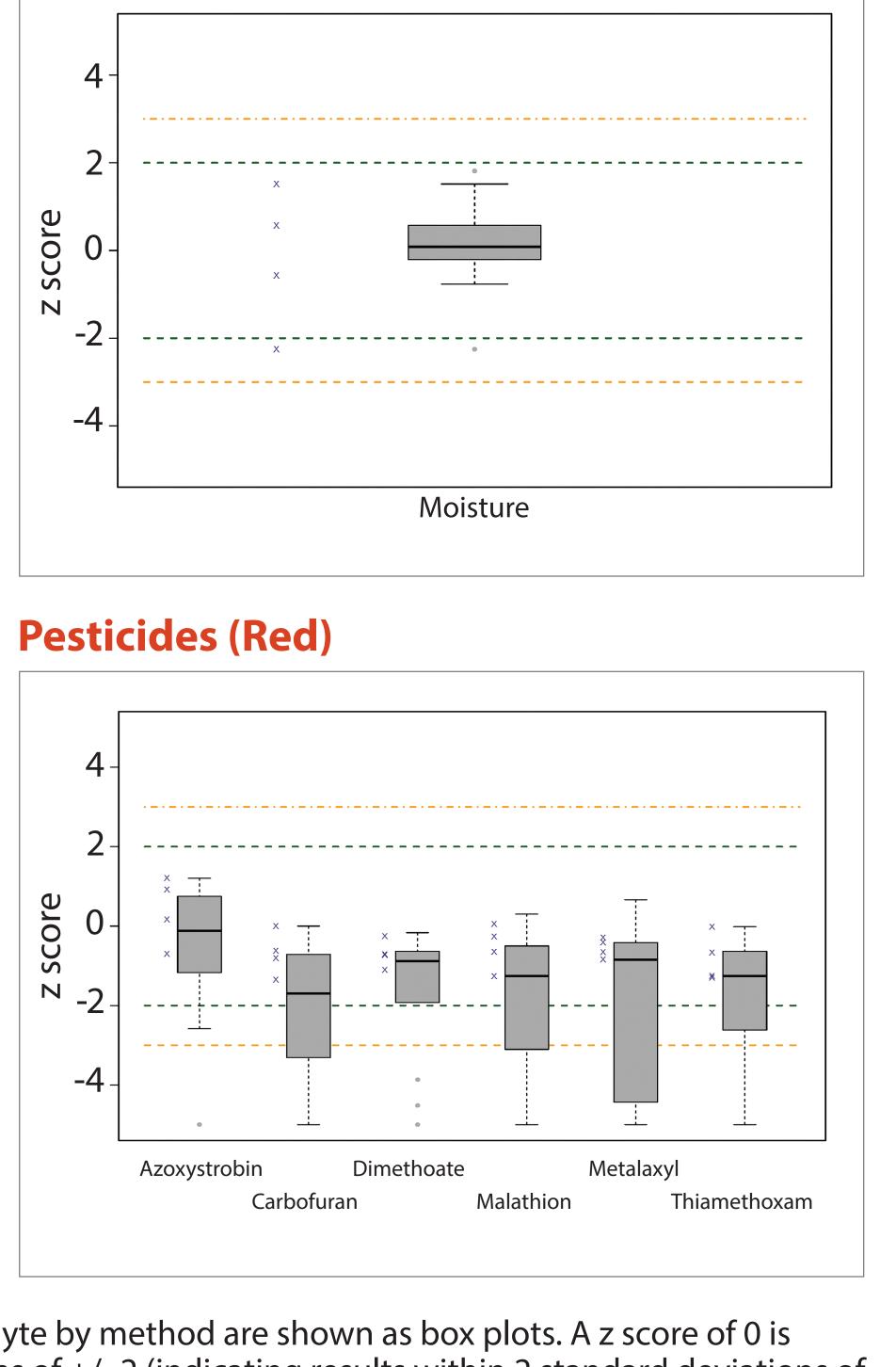
Results (z scores) across all participants for each scored analyte by method are shown as box plots. A z score of 0 is equal to the assigned value. Green lines are drawn at z scores of +/-2 (indicating results within 2 standard deviations of the assigned value). Orange lines are drawn at z scores of +/- 3 (indicating results between 2 and standard deviations of the assigned value). Blue x's adjacent to boxplots show z scores for reference laboratories.

Homogeneity and stability criteria for all methods (cannabinoids, terpenes, moisture, and pesticides) were met during the pilot study.

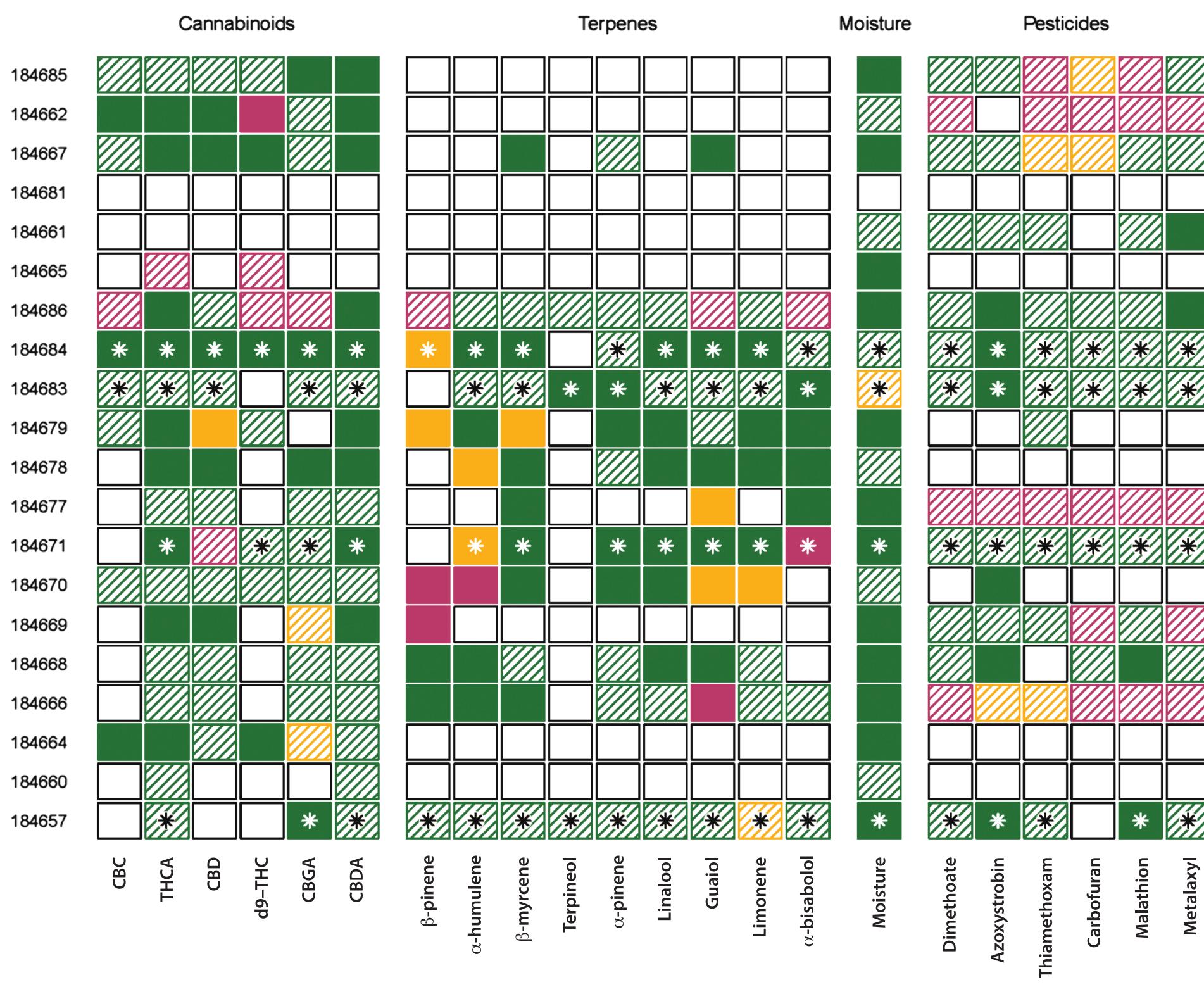
Results by Method

Moisture (Blue)





AOAC INTERNATIONAL, Rockville, MD: Shane Flynn • Chad Stephan



Moisture Content					
Stability Summary Statistics					
Mean (w/w%)	15.02				
Std Dev using w/w%	0.13				
Total CV using w/w%	1%				
Min Measurement (w/w%)	14.9				
Max Measurement (w/w%)	15.2				
Comparison to Homogeneity Results					
Mean Homogeneity (w/w%)	12.93				
Percent Difference (%)	16.13				
T-Test P Value	< 0.0001				
Conclusion	Pass				

Pesticide Target Concentration (ng/g, ppb) 1200 Stability Stability 128.0 896.6 Mean (ng/g, ppb 106.7% 74.7% Mean % Recovery 6.04 50.94 Std Dev using ppb Total CV using ppb 5% 819.4 (68 Min Measurement ppb (% Recovery) Max Measurement ppb (% Recovery) Comparisor 103.23 Mean Homogeneity (ng/g, ppb) 24.04 Percent Difference (% 12.07 **T-Test P Value** 0.0008 0.0132

Results by Lab and Method





Box Color and Fill Represent z Score +/-2 +/- 2 to 3 +/-3 or more ND or no value reported * * * * * Solid: z Score > 0 (Result > Assigned Value) Striped: z Score < 0 (Result < Assigned Value) Reference Labs

Conclusions and Path Forward

Signature science

The pilot study demonstrated that that SigSci was able to create representative, ready-to-analyze, homogeneous, and stable hemp PT samples.

Feedback from participants included:

"Participating in the pilot round was a refreshing experience."

"Real PT samples are a game changer for the cannabis industry."

> "Thank you for making this process so simple."

AOAC Cannabis/Hemp PT Program Current Offerings:

Hemp and Cannabis (> 0.3% d9-THC) Flower Cannabinoids, Terpenes, Heavy Metals, Moisture, Water Activity, Pesticides, Mycotoxins

Planned Expansions:

New Matrices: Oils, Gummies, Chocolate, Beverages

Additional Offerings: Microbiology PT Program in development, including Aspergillus, STEC, Yeast and Mold, and Salmonella (Qualitative and Quantitative)



More about the AOAC Cannabis Hemp Program https://www.aoac.org/scientific-solutions/ _ cannabis-hemp-pt-program/

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uran	Dimethoate	Malathion	Metalaxyl	Thiamethoxam	
)	1200	300	1200	300	
ummai	ry Statistics				
6	983.0	240.7	1137.9	173.5	
%	81.9%	80.2%	94.8%	57.8%	
4	64.63	25.72	54.58	15.12	
	7%	11%	5%	9%	
58%)	877.2 (73%)	209.0 (70%)	1058.8 (88%)	156.3 (52%)	
/8%)	1036.5 (86%)	266.4 (89%)	1209.1 (101%)	195.5 (65%)	
Homo	geneity Results				
6	928.42	229.82	968.56	175.47	
7	5.88	4.74	17.48	-1.13	
2	0.1371	0.4078	0.0021	0.7857	
	Pass	Pass	Pass	Pass	