Validation of Moisture Content and Water Activity Methods in Cannabis Flower

Technical White Paper

J. Brian Leapard
Laboratory Director

Prepared by:
Modern Canna Laboratories

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Modern Canna Laboratories
www.moderncanna.com

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Summary

This white paper validates moisture content and water activity methods for dried cannabis flower using Modern Canna Laboratories' data and AQUALAB provided instrumentation (ROS1, TDL2). We compared Karl Fischer (KF) (100 °C), Vacuum Oven (VO) (40 °C), Loss on Drying Low Temperature (ROS1, LoD LT) (80 °C), Loss on Drying High Temperature (Convection Oven, LoD HT) (103 °C), and Vacuum Desiccation (VD) (22 °C), established water activity–moisture relationships across six equilibration levels and evaluated decarboxylation and terpene loss. Outcomes support the use of water activity as a predictive proxy for moisture and recommend KF, VO, or LoD LT for reference calibration, while discouraging LoD HT due to analyte loss as well as vacuum desiccation due to consistently lower values than those obtained by the three recommended methods for determining water content. A model was created for the AQUALAB TDL2 to represent the relationship between water activity and moisture content based on the experimental data.

Introduction

Moisture content (MC) and water activity (a_w) govern stability, microbial risk, and reported potency in cannabis. Moisture content is the total amount of water contained within a sample. Water activity is defined as the ratio of the vapor pressure in a material compared to the vapor pressure of pure water at the same temperature. Therefore, water activity is the measure of “free” water within a sample and is an important indicator of stability and shelf life of a product.

Cured cannabis flower typically has a moisture content of 5 - 9% and a water activity of 0.45 - 0.60. In most states the regulatory limit is 0.65 a_w for cannabis flower to ensure that microbial growth does not occur, ensuring a suitable product shelf life. We examined various methods for determining moisture content and equilibrated cannabis flower to known water activity levels to establish the relationship between water activity and total moisture content.

Methods and Discussion

Moisture Content Determination Comparison

The study covered >100 unique homogenized flower samples across five moisture techniques to compare the results and determine if any methods introduce bias to the data. Method conditions and procedural steps are summarized in Table 1. The full data set plot is shown in Figure 1. The average moisture is shown in Figure 2. To determine the relative standard deviation (RSD) of the techniques, a single homogenized aliquot was analyzed 9 times by each technique on the same day. Those results are presented in Figure 3.

Table 1. Moisture Technique Method Conditions

Technique	Key Conditions
Loss on Drying (LoD HT)	Aluminum dishes, 0.3 - 0.5 g, 103 °C for 3.5 h, convection oven
Vacuum Desiccation (VD)	Aluminum dishes, 0.3 - 0.5 g, room temp under vacuum, 3.5 h
Vacuum Oven (VO)	Aluminum dishes, 0.3 - 0.5 g, 40 °C under vacuum for 24 h
ROS1 Loss on Drying (ROS1 LoD LT)	ROS1 moisture analyzer at 80 °C to constant weight (~1.0 g)
Karl Fischer (KF)	Headspace vials, 0.2 - 0.3 g, heated to 100 °C and titrated (blank and drift corrected)

The ROS1 (LT), Vacuum Oven and Karl Fischer yielded very similar results across the data set. The LoD (HT) produced results that were significantly higher due to the decarboxylation of the sample and loss of volatile terpenes (Figures 4 and 5). With Karl Fischer titration, the terpene loss and decarboxylation do not present a source of error since the titration is specific for water and the weight loss does not factor into the result. Analysis using the AQUALAB TDL2 is conducted at 25°C and therefore should not result in decarboxylation of the sample.

Moisture Content vs Water Activity

To investigate the moisture content of cannabis samples at known water activities, samples of ground cannabis were equilibrated in sealed chambers containing saturated salt solutions which hold the chamber at a specific humidity and then analyzed with the Aqualab TDL2 after equilibration. Equilibration studies used six salt solutions spanning ~0.22 to 0.75 a_w, triplicate analysis per chamber and 72 - 144 h total equilibration. The salts and their theoretical a_w used in the experiment are shown in Table 2.

Table 2. Salt Calibration Levels and Corresponding Equilibration Water Activity

CALIBRATION LEVEL	SALT UTILIZED	WATER ACTIVITY
1	Potassium Acetate	0.225
2	Magnesium Chloride	0.328
3	Potassium Carbonate	0.432
4	Sodium Bromide	0.576
5	Potassium Iodide	0.689
6	Sodium Chloride	0.753

When equilibrating a material to a specific water activity, the hysteresis region must be taken into consideration, therefore samples should be brought to a water activity above what they will be equilibrated to and given the opportunity to drop. The steps of the equilibration procedure are detailed below:

- Weigh samples in triplicate for each of the chambers being used for calibration.
- Place samples in appropriate pre-equilibration chamber for 24 hours.
- Move samples to corresponding equilibration chambers and begin checking WA levels after 72 hours.
    1. Levels 1-5: Total Equilibration Time - 120-144 hours
    2. Level 6: Total Equilibration Time - 72 hours
- Once fully equilibrated, the samples can be analyzed for Water Activity and Moisture Content.

Special care must be taken with very low moisture content samples as these will quickly pick up atmospheric moisture as soon as they are removed from the equilibration chamber. To combat this effect, the study was conducted in a humidity-controlled room with the relative humidity held at 30%. The samples were immediately analyzed upon removal from the chamber.

Results

From the samples ran against all five moisture methods simultaneously, three techniques gave very comparable results while the other two gave drastically different values. The Karl Fischer titration, 40°C vacuum oven and the ROS1 at 80°C all agreed very well against each other across the sample set. The LoD at 103°C overestimated the total moisture and the room temperature vacuum desiccation produced consistently lower results compared to the other techniques. Given that Karl Fischer titration is considered to be the gold standard for water content determination, it is assumed that volumetric KF utilizing an oven-based autosampler should be the most accurate and free from interferences due to the titration's specificity for water. However, given the more complex nature of the KF analysis, the 80°C loss on drying or vacuum oven may be much more applicable approaches for commercial high throughput facilities.

Since most laboratories and producers are measuring water activity as well, we set out to try to establish a relationship between the free water (a_w) and the moisture content in cannabis flower. The isotherms were created by equilibrating samples as described previously and concurrently running each level for a_w with the TDL2 and for MC by the three most promising techniques. The entire experiment was repeated three times, and the results of each replicate are shown in Tables 5-7. Based on these results, an isotherm derived moisture model was created to estimate moisture content in relation to the measured water activity using the AQUALAB TDL2 (Figure 10).

Plots of the moisture content versus the water activity are shown in Figures 6-9. When performing a coefficient of determination on the plots using all three replicate determinations, the R^2 was >0.98 for both the ROS1 at 80°C and the vacuum oven at 40°C. The fit for the KF determinations was not as good, likely due to the limitation on sample weight that could be used. A maximum of 300 mg of plant

material can be used without encountering excessively long titration times. With smaller sample weights, the homogeneity of the plant material becomes crucial to getting consistent results. Unfortunately, there is a limit to how fine the plant material can be ground before the properties of the material itself start to be impacted.

In conclusion, using the TDL2 for water activity measurements and moisture content is a viable and efficient way of obtaining both data points. This is especially useful for cannabis and hemp producers to evaluate cured samples quickly and accurately. This approach eliminates the need for a second test to measure moisture content by an additional technique. The TDL2 can save time and eliminate the cost associated with determining moisture content as the relationship has been established using the actual matrix of interest.

Figure 1. Moisture Levels Across the Dataset of 119 samples by Multiple Techniques

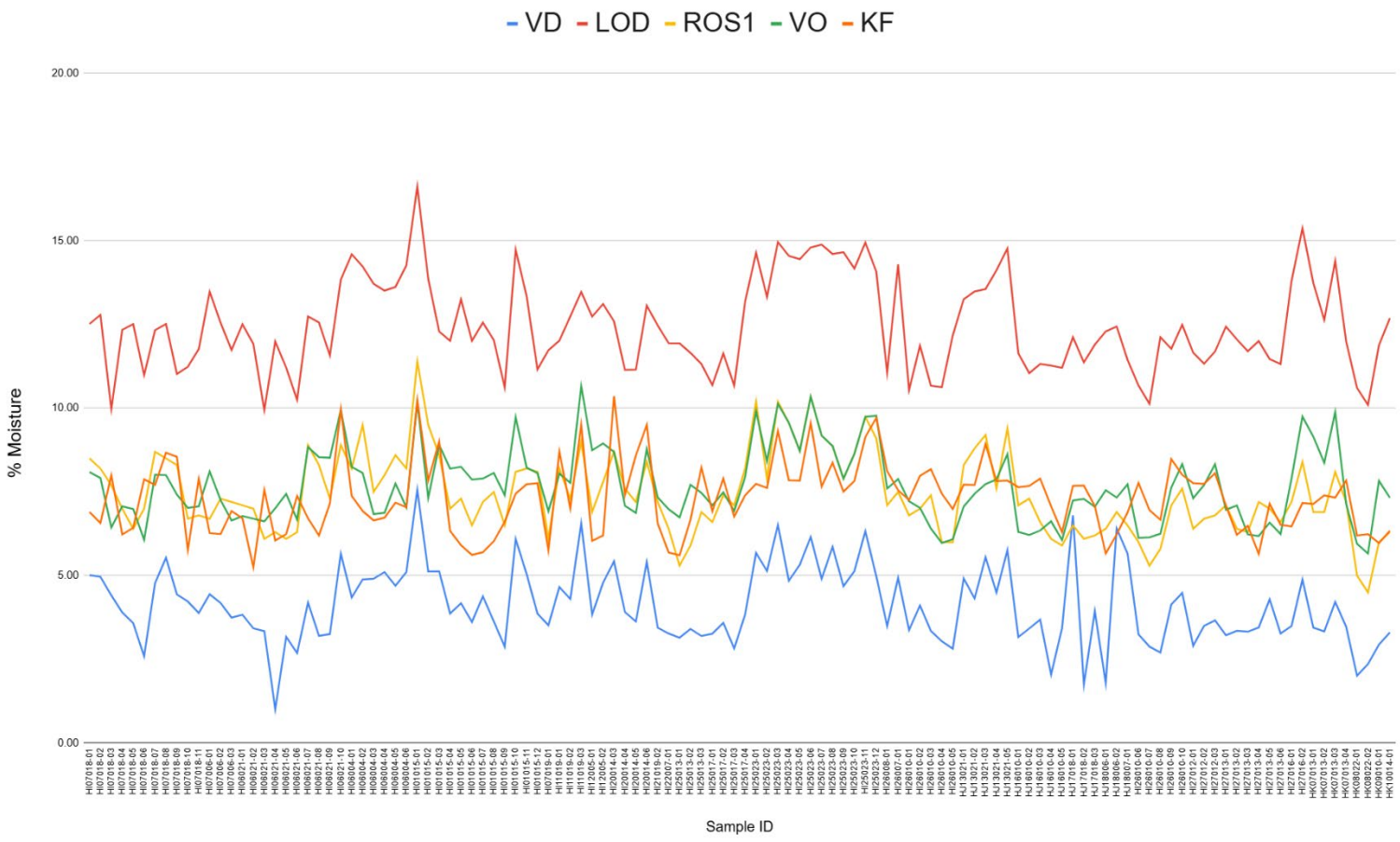


Figure 2. Average Moisture by Technique: Average percent moisture across the dataset (119 individual samples).

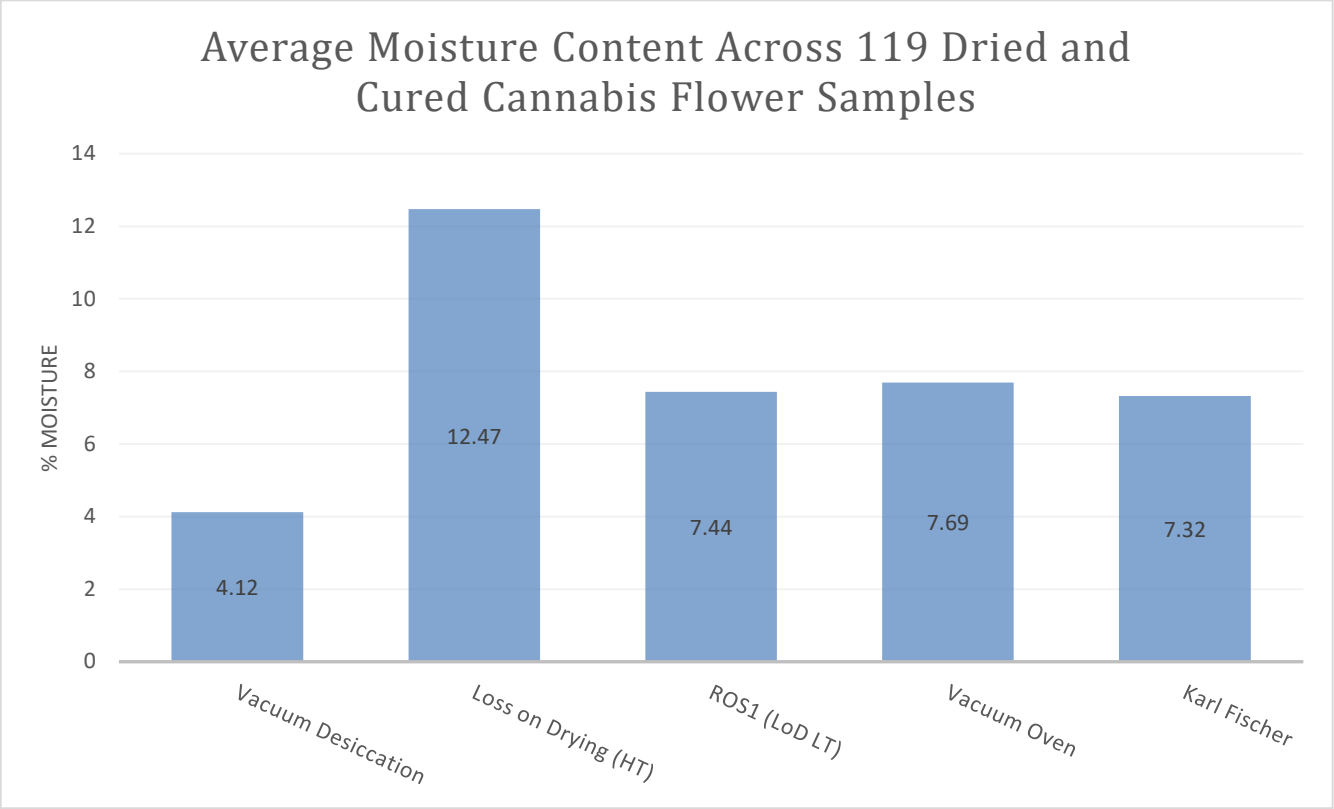


Table 3. Average Percent Moisture Across the Dataset

Method	Average	Min	Max
Vacuum Desiccation (VD)	4.12	1.01	7.57
Loss on Drying (HT)	12.47	9.94	16.62
ROS1 (LoD LT)	7.44	4.5	11.4
Vacuum Oven (VO)	7.69	5.66	10.66
Karl Fischer (KF)	7.32	5.25	10.36

Figure 3. Reproducibility: Standard Deviation (9 Replicates per Technique).

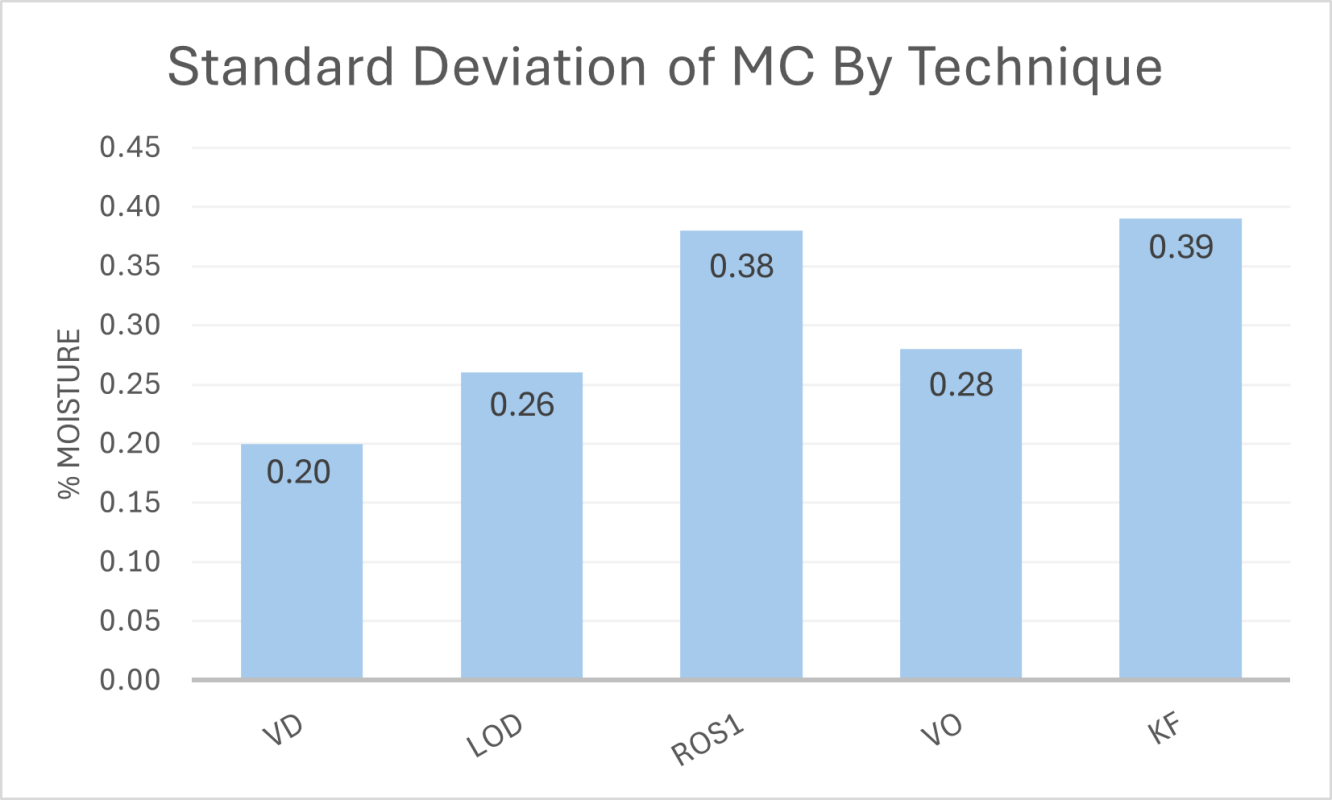
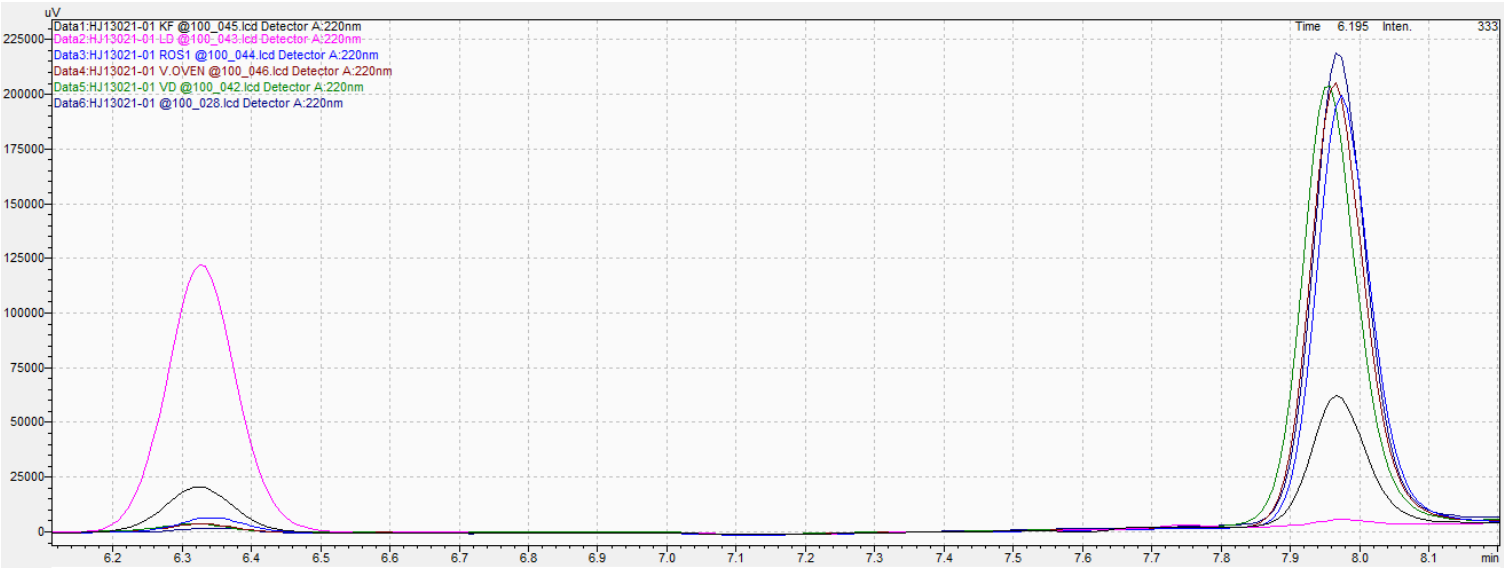


Table 4. Reproducibility Summary

Method	Average Moisture (%)	Std Dev (%)
Vacuum Desiccation (VD)	3.75	0.2
Loss on Drying (HT)	13.14	0.26
ROS1 (LT)	6.5	0.38
Vacuum Oven (VO)	8.36	0.28
Karl Fischer (KF)	6.43	0.39

Figure 4. Total % Decarboxylation across all MC Methods



	Original	VD	LoD (HT)	ROS1 (LT)	VO	KF
THCa	20.5%	20.3%	0.277%	19.0%	19.8%	13.4%
D9-THC	0.383%	0.531%	17.4%	1.00%	0.590%	7.15%

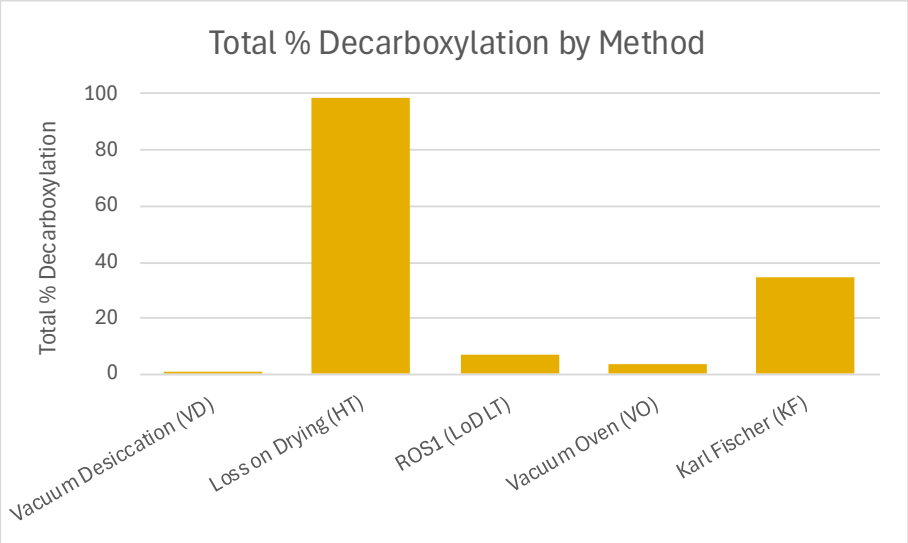
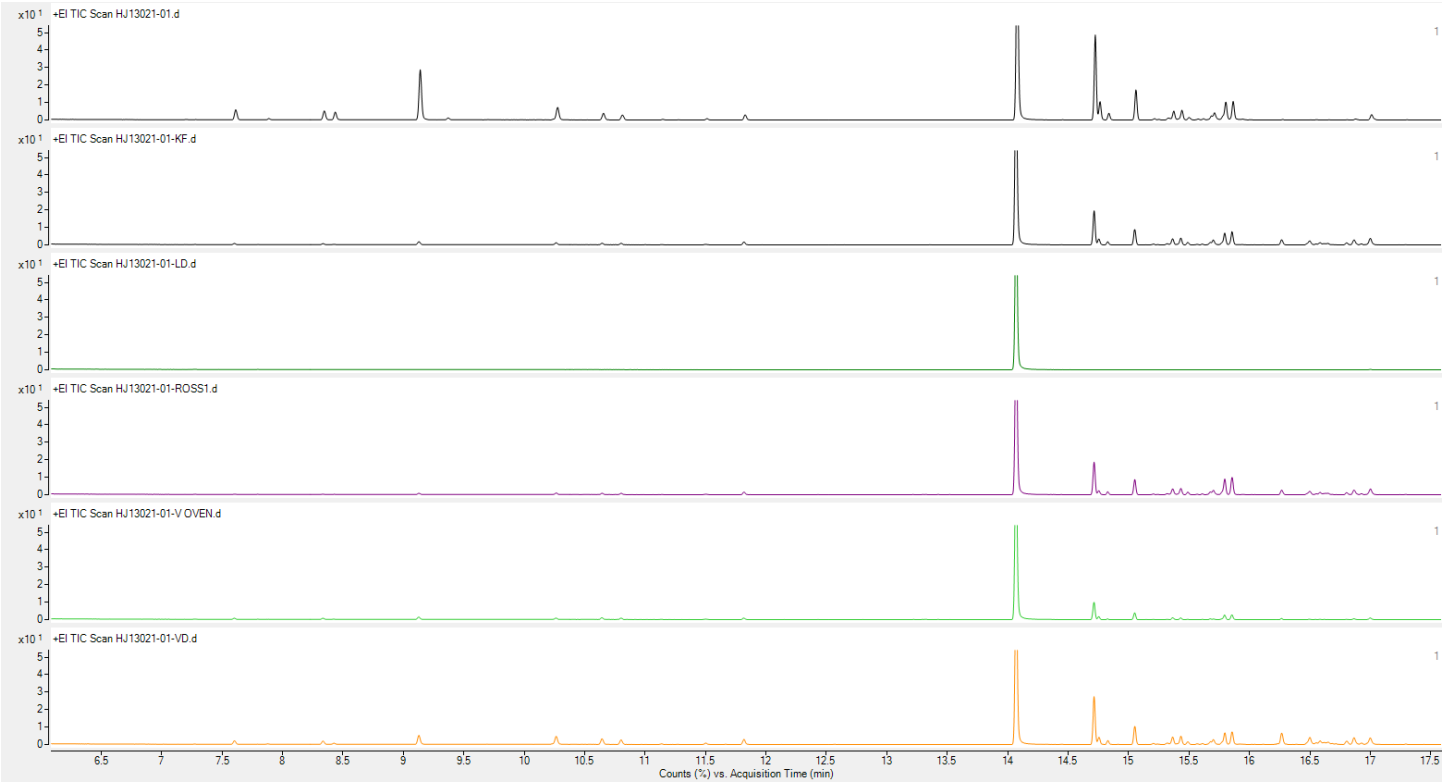
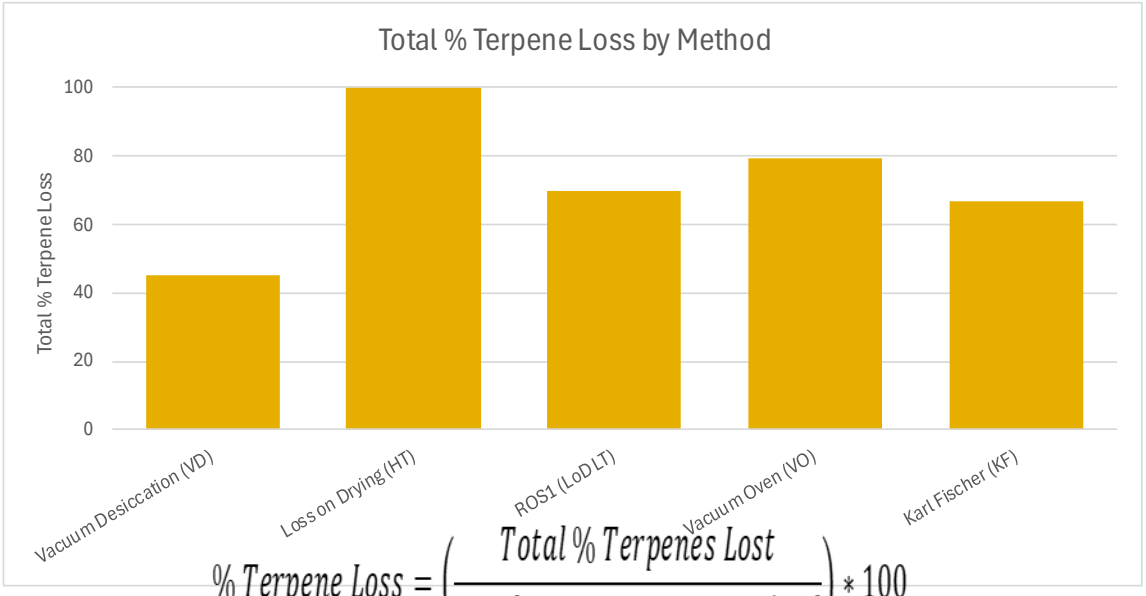


Figure 5. Total % terpene loss across methods



Total Terpenes (%)	Original	VD	LoD (HT)	ROS1 (LoD LT)	VO	KF
	1.59	0.87	0	0.48	0.33	0.53



$$\%\,Terpene\ Loss = \left(\frac{Total\,\%\,Terpenes\ Lost}{Total\,\%\,Terpenes\ as\ Received}\right) * 100$$

Table 5. Water Activity–Moisture Correlation

Calibration Results: Replicate 1

Calibration Level	Karl Fischer		Vacuum Oven		ROS1 (LT)	
	Water Activity (a_w)	Moisture (%)	Water Activity (a_w)	Moisture (%)	Water Activity (a_w)	Moisture (%)
1	0.2414	5.37	0.2390	4.90	0.2351	4.10
2	0.3278	6.36	0.3279	5.41	0.3301	4.60
3	0.4257	7.17	0.4307	6.32	0.4339	5.20
4	0.5813	9.22	0.5811	8.18	0.5937	7.40
5	0.6858	11.5	0.6799	10.22	0.6887	9.80
6	0.7570	13.3	0.7468	11.1	0.7561	12.1

Table 6. Water Activity–Moisture Correlation

Calibration Results: Replicate 2

Calibration Level	Karl Fischer		Vacuum Oven		ROS1 (LT)	
	Water Activity (a_w)	Moisture (%)	Water Activity (a_w)	Moisture (%)	Water Activity (a_w)	Moisture (%)
1	0.2427	4.14	0.2416	4.07	0.2358	3.50
2	0.3293	4.94	0.3291	4.96	0.3308	4.30
3	0.4264	5.79	0.4244	5.67	0.4242	4.70
4	0.5744	7.93	0.5774	7.35	0.5723	7.40
5	0.6871	10.1	0.6878	9.37	0.6897	9.00
6	0.7481	12.3	0.7555	12.2	0.7522	11.30

Table 7. Water Activity–Moisture Correlation

Calibration Results: Replicate 3

Calibration Level	Karl Fischer		Vacuum Oven		ROS1 (LT)	
	Water Activity (a_w)	Moisture (%)	Water Activity (a_w)	Moisture (%)	Water Activity (a_w)	Moisture (%)
1	0.2513	4.06	0.2505	4.78	0.2602	3.60
2	0.3416	4.61	0.3386	5.23	0.3353	4.20
3	0.4318	5.49	0.4342	5.94	0.4346	4.90
4	0.5806	7.41	0.5818	7.70	0.5788	6.60
5	0.6901	9.50	0.6853	9.77	0.6873	8.60
6	0.7547	11.0	0.7543	11.8	0.7524	11.0

Figure 6. MC–a_w relationship by Low Temperature LoD (ROS1)

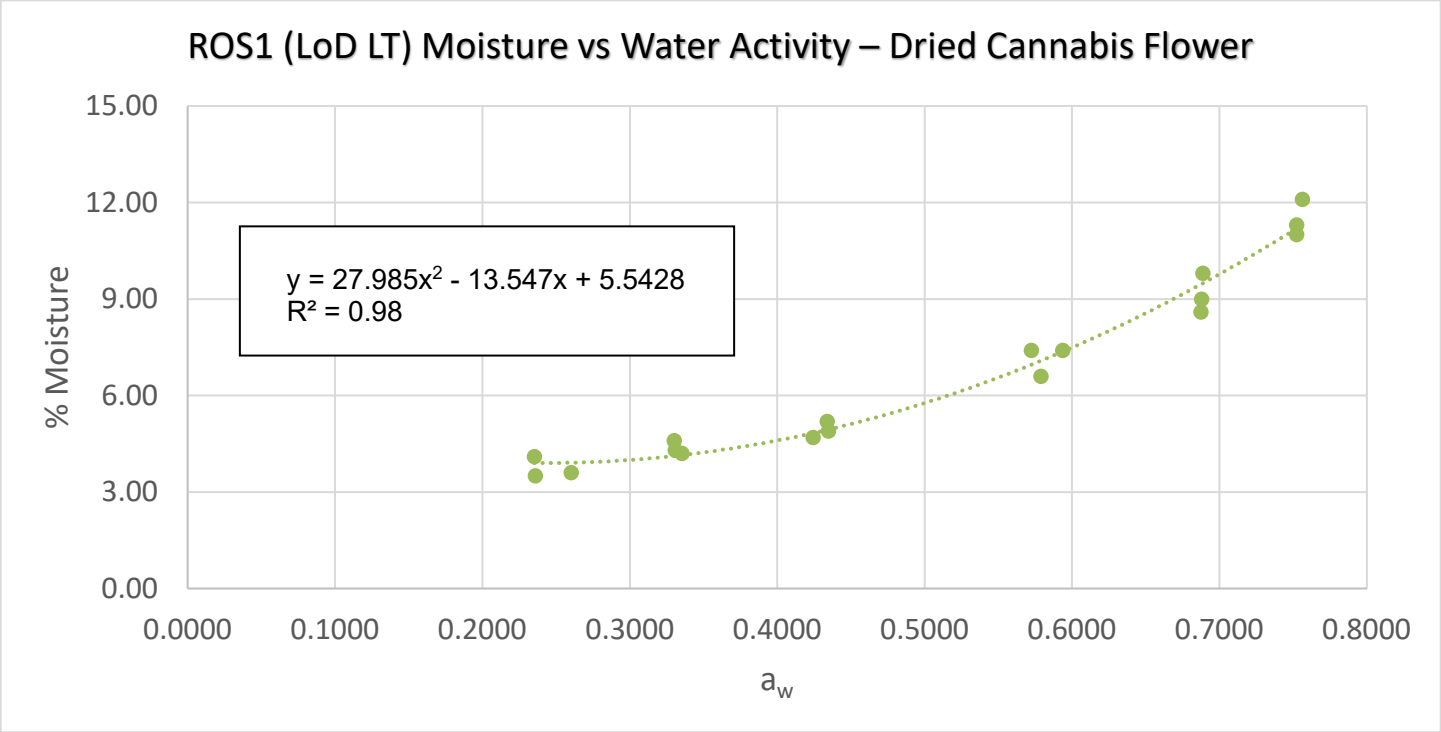


Figure 7. MC-a_w relationship by Vacuum Oven

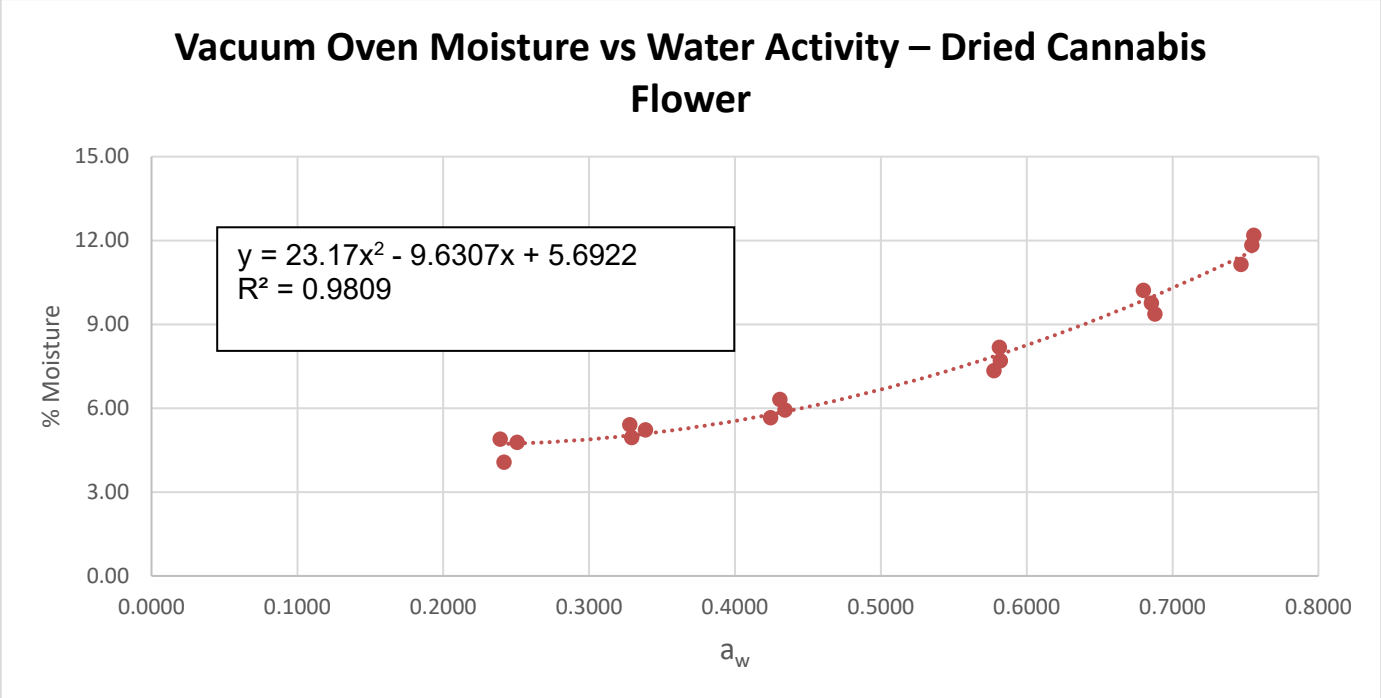
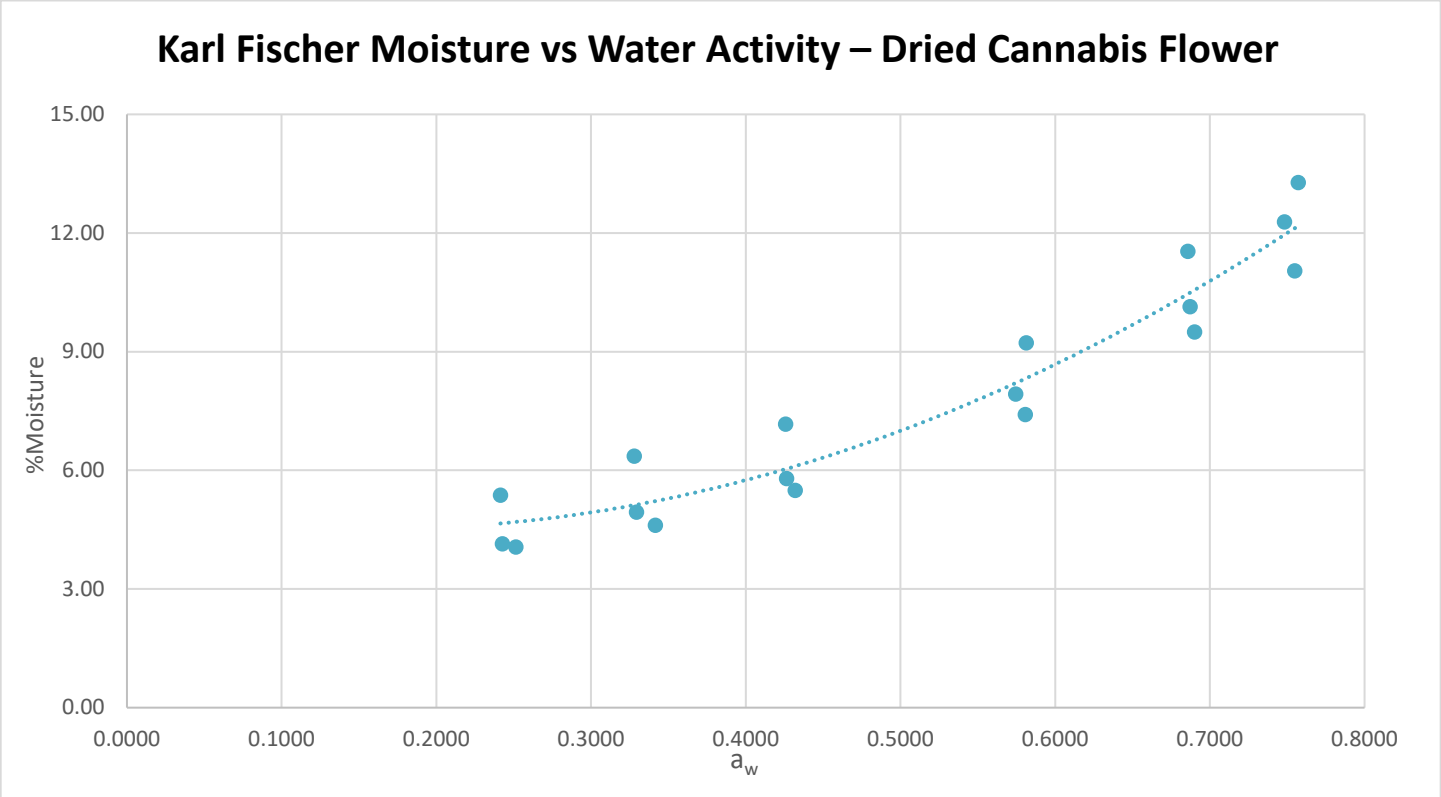


Figure 8. MC-a_w relationship by Karl Fischer Titration



$y = 21.528x^2 - 6.9024x + 5.0684$
$R^2 = 0.923$

Figure 9. MC vs a_w Overlay of all Methods.

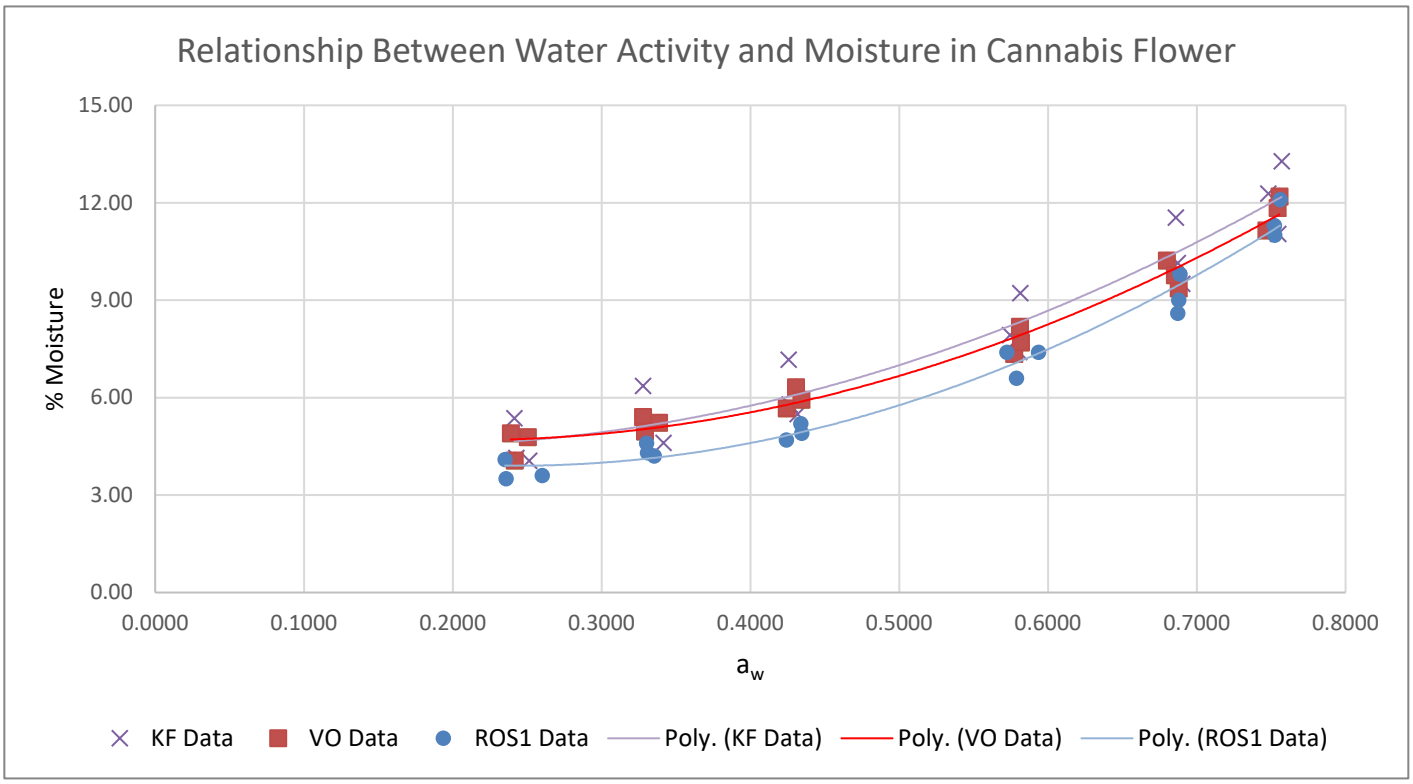
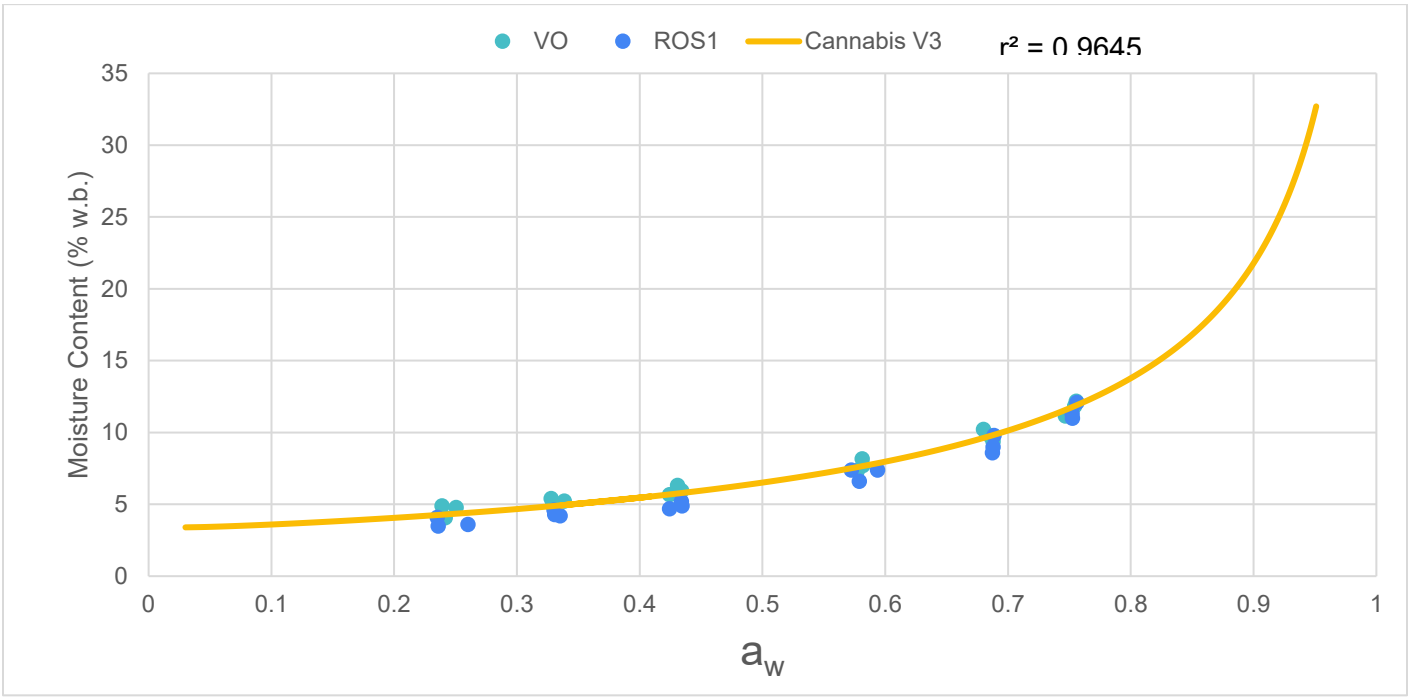


Figure 10. Moisture Model Cannabis V3 for AQUALAB TDL2



About Modern Canna Laboratories

Modern Canna Laboratories is an analytical testing laboratory specializing in cannabis and hemp products. Founded with a commitment to scientific rigor and data integrity, Modern Canna provides accurate and defensible laboratory testing services for cultivators, manufacturers, and regulatory stakeholders.

Modern Canna's scientific leadership and laboratory staff bring extensive experience in analytical chemistry, method validation, and quality systems. The laboratory operates under strict quality control protocols designed to produce reproducible and defensible data. Modern Canna also played a historic role in Florida's medical cannabis program, serving as the first laboratory involved in rule development and the first lab in the state to test medical marijuana.

Through research, collaboration, and continuous method development, Modern Canna Laboratories works to advance analytical standards within the cannabis industry and promote greater transparency, consistency, and consumer safety. Today, the laboratory is widely recognized as one of the most trusted cannabis testing laboratories in the United States.

Modern Canna Laboratories

Lakeland, Florida
United States

www.moderncanna.com