**Systemic Bias in In-Process Moisture Measurement Between Halogen Moisture Analyzer and Vacuum Drying Oven During Dry Cannabis Flower Production**

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***Abstract:***

*This study evaluates the performance of a Halogen Moisture Analyzer (HMA) in determining moisture content (%MC) in dry cannabis flowers, as In-Process Quality Control of the drying and curing production processes in comparison with Vacuum Drying Oven (DO) method, as per Ph.Eur. 2.2.32 and Ph.Eur. Cannabis Monograph No. 3028. Using 24 homogenized samples, moisture content (%MC) was measured in parallel with both methods. The HMA, operating at 105°C, yielded a mean %MC of 10.33% (SD = 0.61; RSD = 5.94%), while the DO method (40°C, 24-hour drying) reported 6.87% (SD = 0.57; RSD = 8.25%), revealing a systematic bias (Δ%MC = 3.46%) attributed to HMA-induced evaporation of volatile organic compounds (VOCs) such as terpenes. Statistical analysis confirmed non-overlapping 95% confidence intervals (HMA: 10.07–10.59%; DO: 6.63–7.11%) and a moderate Pearson correlation (r = 0.59), indicating that the methods measure distinct components (total volatiles vs. free water). Despite failing predefined accuracy criteria, the HMA demonstrated strong precision and operational efficiency, enabling real-time process monitoring. The regression model (ŷ = 0.55x + 1.21) highlighted limitations in direct method conversion, emphasizing the need for strain-specific correction factors. These findings underscore the HMA’s utility for in-process quality control but reaffirm the DO’s role as the regulatory benchmark. For the cannabis industry, we recommend adopting HMA for rapid drying/curing process adjustments while reserving DO for compliance testing. Future work should prioritize refining correction models and optimizing drying and curing protocols. Adopting this hybrid approach can enhance compliance, prevent over-drying, and optimize moisture control workflows in pharmaceutical cannabis production.*

***Keywords:*** *Moisture content, cannabis****,*** *Halogen Moisture Analyzer (HMA), Drying Oven (DO), Volatile Organic Compounds (VOCs), Quality Control*

1. **Introduction**

 Moisture content determination, *as In-Process* Quality Control of the drying and curing processes of a GMP production of dry Cannabis flowers (*Cannabis sativa L.*)*,* is a critical quality parameter, directly influencing product stability, microbial safety, and therapeutic efficacy, and of course yield [1]. Traditional methods, such as the vacuum Drying Oven (DO) outlined in the European Pharmacopoeia (*Ph.Eur. 2.2.32 and Cannabis Monograph No. 3028*), remain the gold standard due to their specificity for free water measurement [2]. However, the DO’s lengthy 24-hour analysis time and manual handling requirements limit its practicality as feasible In-Process Quality Control in high-throughput production environments [3]. In contrast, the Halogen Moisture Analyzer (HMA) offers rapid, automated results within minutes, and to achieve that, it operates at 105°C, raising concerns about its accuracy due to concurrent evaporation of volatile organic compounds (VOCs) such as terpenes and flavonoids [4], [6].

The cannabis industry faces a pressing challenge: balancing the need for rapid process monitoring for timely on-site process decision making, and to ensure regulatory compliance of the medicinal product. While the HMA’s efficiency is advantageous for real-time process adjustments during drying and/or curing, its tendency to overestimate moisture content risks non-compliance with pharmacopeial thresholds (e.g. Ph.Eur. <12%MC) and potential over-drying, which degrades product quality [7], [8]. Previous studies have highlighted similar discrepancies in botanical moisture analysis, where thermogravimetric methods conflate water loss with VOC evaporation [9], [10]. However, a systematic comparison of HMA and DO methods in cannabis—a matrix uniquely rich in heat-sensitive compounds—remains underexplored.

This study addresses this gap by evaluating the precision, accuracy, and practical applicability of HMA method as In-Process quality control compared to DO method across 24 cannabis samples of a single strain (Jack Herer). The key objectives were to quantify the systematic difference in moisture content measurement results, to assess the impact of sample heterogeneity and analyst variability.

1. **Material And Methods**

Bellow, we detail the specialized instrumentation, protocols, and acceptance criteria employed to compare the HMA and the vacuum DO reference method for moisture determination in dry cannabis flowers. The study encompassed 24 homogenized cannabis samples, each collected at the estimated endpoint of the curing process during dry cannabis flower production. They were analyzed in parallel with HMA and DO to evaluate accuracy, precision, and method bias. Table no.1 summarizes the equipment specifications, operating parameters, sample preparation steps, and acceptance criteria aligned with pharmacopeial guidelines, ensuring standardized conditions for reproducible data collection and robust statistical comparisons.

**Table no.1:** Structured overview of Equipment used, Method Parameters, Sample Preparation,

Experimental Procedure and Acceptance Criteria for both HMA & DO methods.

|  |  |  |
| --- | --- | --- |
| **Category** | **HMA Method** | **DO Method** |
| **Equipment** | **Halogen Moisture Analyzer:** Mettler Toledo HE73/01 (Serial No: C244177463, QC#003); Calibration Cert: V240148 (15.03.2024) | **Drying Oven:** Binder VD56 (Serial No: 20230000006861, QC#007); **Analytical Scale:** Mettler Toledo MS205DU (Serial No: C311839656, QC#001; Calibration Cert: V240146, 15.03.2024); |
| **Method Parameters** | Sample Size: 2.0g ±10% Drying Program: Standard Temperature: 105°C; Switch-Off Criterion: 0.001g/20s | Sample Size: ~1.0000g Temperature: 40°C Pressure: 20 mbar (15-25 mbar) Drying Duration: 24 hours (Ph.Eur 2.2.32, Cannabis Monograph No. 3028) |
| **Sample Preparation** | Uniformly Milled (but not powdered) cannabis flower (~10g). Homogenized and stored in airtight containers samples were sampled for the HMA and DO at the same time and analyzed promptly to maintain integrity |
| **Experimental Procedure** | 2.0g ±10% sample placed in an aluminum pan Drying at 105°C until weight loss stabilized Conducted by two analysts at different times (morning/evening) | ~1.00000g sample dried at 40°C, 20 mbar for 24 hours; Samples cooled in a desiccator and weighed on an analytical scale (as described in Ph.Eur 2.2.32 LoD). Conducted by a single analyst in one session |
| **Parallel Analysis & Data Collection** | Tested in parallel with DO method and LoD values recorded for direct comparison | Tested in parallel with HMA method and LoD values recorded for direct comparison |
| **Acceptance Criteria****for HMA Method** | **Accuracy Based on Absolute %MC Difference:***Excellent:* ∆%MC ≤ 0.1% *Good:* 0.1% < ∆%MC ≤ 0.2% *Acceptable:* 0.2% < ∆%MC ≤ 0.4% *Failed:* ∆%MC > 0.4% | **Precision Criterion:** Standard Deviation Ratio (Q): Q ≤ 1.5 |

1. **Result**

Bellow, we present the outcomes from analyzing 24 cannabis samples using both the HMA and DO methods. Table no.2 reports individual sample measurements alongside absolute differences, indicating potential method bias. Table no.3 details the calculated statistical parameters—such as mean, standard deviation, confidence intervals, and correlation coefficients—which quantify variability and help assess whether these two methods meet predetermined accuracy and precision criteria. Finally, Table no.4 consolidates the accuracy and precision evaluations, illustrating how these parameters guide conclusions on method suitability for routine In-Process quality control during dry cannabis flower production.

**Table no2:** HMA & DO %MC Measurement Results of samples n1-n24 and Δ%MC|DO – HMA| with Acc. Levels

| **Sample** | **%MCHMA (** | **%MCDO (** | **Δ%MC****|DO – HMA|** | **Acc. Levels** | **Sample** | **%MCHMA (** | **%MCDO (** | **Δ%MC****|DO – HMA|** | **Acc. Levels** | **Sample** | **%MCHMA (** | **%MCDO (** | **Δ%MC****|DO – HMA|** | **Acc. Levels** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| n1 | 9.68 | 6.38 | 3.30 | Fail | n9 | 10.58 | 6.10 | 4.48 | Fail | n17 | 11.14 | 7.53 | 3.61 | Fail |
| n2 | 9.74 | 6.67 | 3.07 | Fail | n10 | 9.84 | 6.73 | 3.11 | Fail | n18 | 10.14 | 6.69 | 3.45 | Fail |
| n3 | 10.46 | 6.60 | 3.86 | Fail | n11 | 9.50 | 6.63 | 2.87 | Fail | n19 | 10.69 | 7.32 | 3.37 | Fail |
| n4 | 10.93 | 6.70 | 4.23 | Fail | n12 | 11.02 | 7.21 | 3.81 | Fail | n20 | 10.25 | 7.46 | 2.79 | Fail |
| n5 | 9.01 | 5.94 | 3.07 | Fail | n13 | 11.11 | 6.85 | 4.26 | Fail | n21 | 11.08 | 7.72 | 3.36 | Fail |
| n6 | 10.04 | 5.88 | 4.16 | Fail | n14 | 11.23 | 7.08 | 4.15 | Fail | n22 | 10.28 | 6.95 | 3.33 | Fail |
| n7 | 9.79 | 6.29 | 3.50 | Fail | n15 | 9.87 | 7.14 | 2.73 | Fail | n23 | 10.82 | 7.40 | 3.42 | Fail |
| n8 | 9.58 | 6.33 | 3.25 | Fail | n16 | 10.63 | 7.19 | 3.44 | Fail | n24 | 10.48 | 8.10 | 2.38 | Fail |

**Table no3:** Summary Statistics Results.

| **Parameter** | **HMA** | **DO** | **Comparison, Interpretation & Significance** |
| --- | --- | --- | --- |
| **Moisture Content (%MC) Mean** | 10.33% | 6.87% | HMA consistently reports higher %MC due to additional volatile loss. |
| **Standard Deviation (SD)** | 0.61 | 0.57 | Both methods demonstrate similar internal precision. |
| **Relative Standard Deviation (RSD)** | 5.94% | 8.25% | HMA provides slightly better repeatability compared to DO. |
| **95% Confidence Intervals****(t-distribution)** | 10.07 – 10.59 | 6.63 – 7.11 | Non-overlapping intervals confirm a statistically significant difference. |
| **Absolute ∆%MC (HMA - DO)** | 3.46% | Reference Standard | Indicates systematic differences in moisture determination. |
| **Pearson Correlation Coefficient (r)** | 0.59 | Moderate correlation between methods, Systematic offset observed. |
| **Correlation (Regression Equation)** |  = **%MCHMA** |  | Can be used for conversion between methods. |
|  **- Slope of the best-fit line.** | 0.55 | Small changes in **%MCHMA** produce relatively large changes in  |
|  **- y-intercept** | 1.21 | Serves as a baseline for the linear relationship |

**Table no4:** Results of Accuracy & Precision Assessment

|  |
| --- |
| **Accuracy Acceptance Criterion** |
| **Accuracy Based on Absolute ∆%MC** | **HMA Results** | **DO Reference** | **Comparison, Interpretation & Significance** |
| **Excellent (∆%MC ≤ 0.1%)** | 0 samples | Reference Standard | No sample met the excellent threshold. |
| **Good (0.1% < ∆%MC ≤ 0.2%)** | 0 samples | Reference Standard | No sample classified as good. |
| **Acceptable (0.2% < ∆%MC ≤ 0.4%)** | 0 samples | Reference Standard | No sample classified as acceptable. |
| **Failed (∆%MC > 0.4%)** | 24 samples | Reference Standard | All samples failed based on accuracy criteria. |
| **Precision Acceptance Criterion** |
| **SD Ratio (Q)** | 1.08 | Variability between methods is comparable. |
| **Analyst I & II Variability** | **t-test****(t-stat. & p-value)** | **Levene’s Test****(F-stat. & p-value)** | Analyst I1's mean measurements and Analyst II2's variability are not significantly different from their respective comparisons, suggesting consistency and stability in their data. |
| **Analyst I (n1-n16)** | *t* = -1.47 | *p* = 0.674 |
| **Analyst II (n17-n24)** | *p* = 0.157 | *F* = 0.18 |

1. **Discussion**

The systematic comparison of HMA and vacuum DO methods for moisture content determination in dry cannabis flowers reveals critical insights into the interplay between analytical methodology, material composition, and operational practicality. The HMA method, operating at 105°C, reported a mean moisture content (%MC) of 10.33% ± 0.61%, while the DO method, adhering to Ph.Eur. 2.2.32 guidelines, yielded a significantly lower mean of 6.87% ± 0.57% [11]. This 3.46% absolute discrepancy Δ%MC|DO – HMA| is not an analytical artifact but a consequence of fundamental thermodynamic and chemical interactions inherent to cannabis’s complex matrix. At 105°C, the HMA volatilizes not only free and bound water but also low-boiling-point volatile organic compounds (VOCs), including monoterpenes and sesquiterpenes, which constitute up to 3% of dry cannabis weight [12], [14]. These findings align with prior studies demonstrating that thermogravimetric methods like HMA conflate moisture loss with VOC evaporation, particularly in botanicals rich in heat-sensitive volatiles [15], [16].

The statistical robustness of this divergence is underscored by non-overlapping 95% confidence intervals (HMA: 10.07–10.59%; DO: 6.63–7.11%), confirming that the methods measure distinct material properties. While the Pearson correlation coefficient (r = 0.59) indicates a moderate linear relationship, the regression equation highlights a systematic bias, suggesting HMA values cannot be directly equated to DO results without correction and thus cannot be considered as method equivalent to the DO [17], [18]. This aligns with agricultural studies where rapid moisture analyzers require crop-specific calibration to account for volatile solids [19], [20]. The HMA’s precision (RSD = 5.94% vs. DO’s 8.25%) and operational efficiency (<20 minutes vs. 24 hours) make it advantageous for real-time In-Process quality control monitoring, yet its overestimation poses risks in regulatory contexts where compliance with pharmacopeial thresholds is critical (e.g. <12% MC; Ph.Eur. 2.2.32) [21], [22]. For instance, a batch measuring ≥12%MC with HMA likely exceeds the true pharmacopeial limit when adjusted for VOC loss, risking over-drying and terpene degradation, which compromises product quality [23], [24].

Analyst variability, assessed through independent measurements by two analysts, revealed no statistically significant difference in mean %MC for the first 16 samples (t = -1.47, p = 0.157) and consistent variances in the final 8 samples (Levene’s F = 0.18, p = 0.674). These findings indicate that any difference in HMA vs. DO readings is likely not driven by inconsistent application of the methods between analysts, but rather by other systematic factors. [25], [26]. These observations mirror pharmaceutical quality control paradigms, where operator training and protocol harmonization are essential even when statistical significance is absent [27], [28].

The HMA’s ability to rapidly detect moisture trends is invaluable in high-throughput environments, particularly during curing, where real-time data can prevent microbial growth or over-drying [29], [30]. However, its limitation as a standalone regulatory tool is evident in the universal failure of all 24 samples (∆%MC > 0.4%) to meet accuracy criteria. This necessitates a hybrid workflow: HMA for in-process quality control checks and DO for final release testing—a strategy successfully employed in herbal drug manufacturing [31], [32]. Such an approach balances efficiency with compliance, leveraging HMA’s speed while reserving DO’s specificity for critical quality milestones.

The broader implications of this study extend to botanical analysis, where method selection must account for matrix complexity. Cannabis’s heterogeneity—varied trichome density, moisture distribution, and VOC profiles—exacerbates measurement variability, particularly in larger samples (2.0g for HMA vs. 1.0g for DO) [33], [34]. Smaller samples, as used in the DO method, mitigate this by ensuring uniform heat penetration, whereas larger samples risk incomplete drying in resin-rich regions [35], [36]. This aligns with research on plant material drying, where sample size inversely correlates with measurement consistency [37], [38].

Furthermore, the role of water binding states cannot be overlooked. The DO method’s vacuum environment and low temperature selectively remove free water, while HMA’s higher temperature desorbs bound water integrated into cellulose and proteins [39], [40]. This differential extraction explains the HMA’s elevated readings and underscores the need for method transparency in reporting “total volatiles” versus “free moisture” [41], [42]. Regulatory bodies must recognize these distinctions to avoid conflating safety thresholds (e.g., microbial growth linked to free water) with product quality metrics (e.g., terpene retention) [43], [44].

1. **Conclusion**

This study underscores the critical trade-offs between analytical precision, operational efficiency, and regulatory compliance in moisture determination for Cannabis sativa L. The Halogen Moisture Analyzer (HMA) method, while offering rapid results and superior precision (RSD = 5.94%), systematically overestimates moisture content by 3.46% compared to the pharmacopeial Drying Oven (DO) method due to the volatilization of terpenes and other low-boiling-point compounds at 105°C. This discrepancy highlights the importance of distinguishing between total volatiles (HMA) and free moisture (DO) in cannabis quality control, particularly in regulatory contexts where compliance with pharmacopeial thresholds (e.g. Ph.Eur. <12% MC) is paramount.

The hybrid workflow proposed in this study—leveraging HMA for real-time process monitoring and reserving DO for final compliance testing—offers a pragmatic solution to balance speed and accuracy. This approach aligns with ISO 17025 guidelines for herbal drug manufacturing, where method suitability is context dependent. Furthermore, the findings emphasize the need for method-specific calibration to account for matrix complexity, particularly in heterogeneous botanicals like cannabis, where sample size and water binding states significantly influence measurement outcomes.

Future research should focus on developing VOC-specific correction models for HMA measurements, integrating advanced analytical techniques such as gas chromatography-mass spectrometry (GC-MS) to quantify terpene loss and refine moisture determination protocols. Additionally, the adoption of non-destructive methods like near-infrared (NIR) spectroscopy could bridge the gap between rapid analysis and regulatory precision, preserving both efficiency and product quality.

In conclusion, while the HMA method is not a pharmacopeial substitute, and cannot be said that the method is an equivalent to the DO method, its integration into cannabis quality systems offers significant operational advantages. By adopting a hybrid workflow and advancing method-specific calibration, cannabis producers can achieve a balance between regulatory compliance and production efficiency, ensuring both product safety and therapeutic efficacy.

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