



Selective and rapid water determination in Cannabis:

Reproducible results in less than 30 minutes



a xylem brand



SCALA
ELECTRA
For Laboratory Use
Medicinals
www.oxoid.com
OXOID
OXOID

Main menu
A: 0.3 µg/min
2.0
µg/min
With green pass
Method parameter
Select method / system
START
EDIT
MODE
ESC START
STOP

Keyboard

1. Abstract

The determination of the water content in cannabis is of great importance for the qualitative assessment of the active components (THC, CBD) as well as its storage stability. Karl Fischer titration offers a selective and reliable alternative to the drying method. Compared to the reference method of national and international pharmacopoeias, which require a determination of the loss on drying over 24 hours, the Karl Fischer method provides selective and reproducible results in less than 30 minutes with a significantly smaller sample quantity.

Depending on the permissible significance level, the oven method using a headspace oven and volumetric titration in boiling heat provide comparable results. Due to the better reproducibility, the significantly simpler sample handling and the lower equipment and measurement effort, the oven method is to be favoured.

2. Introduction

The interest on Cannabis and its products for medical purposes has increased over the past years. Hemp cultivation is an important component of the European Green Deal, in which the European Union has set itself the goal of creating “the transition to a modern, resource-efficient and competitive economy” [2]. As a result, the area available for hemp cultivation (industrial hemp) in the EU increased from around 20k ha in 2015 to around 35k ha in 2019. This corresponds to an increase of 75 % [1]. In the USA, cannabis has been legalised in several states, and medical access for a variety of different conditions has come into focus. In order to satisfy this growing importance, there is a need for general quality control standards. The aim is to avoid recalls due to impurities or incorrect cannabinoid compositions [6]. The analysis of cannabis is an important component in meeting these requirements.

In 2018 „The Agriculture Improvement Act of 2018“ gives a new definition for Cannabis and its products. The Act defines that “The term ‘hemp’ means the plant *Cannabis sativa* L. and any part of that plant [...] with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 % on a dry weight basis” [7]. This definition separates the terms hemp and marihuana and changes how these are treated under the Controlled Substances Act (CSA). As hemp is with this definition no longer a controlled substance the delta-9 THC concentration is of big importance. To standardize its concentration it is related, as written above, on a dry weight basis. This makes an exact determination of the water concentration so important [8]. As a too high concentration might lead to microbial growth it should also not exceed a level of 10 %.



Dr. Ing. Jens Hillerich
Head of Laboratory Application
Titration



Christian Funke
Application Titration

A monograph on cannabis was included in the European Pharmacopoeia (Ph. Eur.) with Supplement 11.5. Until then, there were no harmonised regulations for cannabis flowers and the regulations defined in the monograph “Herbal Drugs” applied. Supplement 11.5 comes into force on 01.04.2024. The German Pharmacopoeia (DAB) already had a monograph for cannabis flowers, which served as a basis for the European monograph [5].

In the European and German Pharmacopoeia (DAB), the water concentration is determined as the loss on drying (LOD) of 1 g of powdered sample over 24 h at 40 °C under vacuum using molecular sieve [4]. In order to minimize the evaporation of volatile components such as essential oils or terpenes, a medium vacuum is recommended in the European Pharmacopoeia. Nevertheless, the LOD does not only determine the water content. The essential oils and terpenes evaporate just like the water and lead to falsified results. The Karl Fischer titration offers a selective and reliable alternative to the LOD.

3. The Karl Fischer Titration

3.1. Theory

Compared to the drying method, Karl Fischer titration has the advantage of significantly faster determination and is highly selective.

The stoichiometry of the KF reaction was unclear for a long time. More recently, the mechanism has been investigated, resulting in the following reaction equation:



This means:

ROH An alcohol, e.g. Methanol, Ethanol,
Ethylene glycol monoethyl ether

R'N A base, e.g. Imidazol (formerly Pyridin)

The oxygen of the sulphate ester comes from the water molecule. Investigations of the mechanism show a change in the stoichiometry when working in other solvents. The addition of other solvents should therefore not exceed 50 % by volume. In addition to water, four components (an alcohol, sulphur dioxide, a base and iodine) are involved in the reaction: these must all be present for the reaction to take place.

Detection is carried out biamperometrically using a double platinum electrode to which a voltage of 20 - 200 mV is applied. Titration is carried out with an iodine solution. During the KF reaction, the iodine reacts directly to form iodide, so that no current flows at the double platinum electrode of the detection system. However, as soon as iodine is present in excess, a reversible redox system of iodine and iodide is present. A current flows, indicating the end of the titration.

3.2 The volumetric Karl Fischer titration

In volumetric KF titration, the iodine required for the reaction is dosed into the titration cell via a burette. Ready-to-use titrants are available for this purpose which, in addition to iodine, also contain all the components required for the Karl Fischer reaction (one-component reagent). The sample therefore only needs to be dissolved in a suitable solvent before titration. In addition, 2-component reagents are also available today, in which the solvent component contains an alcohol, a base and SO₂. The titration solution then consists of an iodine solution in an alcohol. This reagent has the advantage of better pH buffering and a higher concentration of all components on the left-hand side of the reaction equation.

As a result, the reaction is significantly faster and the reagents have a much longer shelf life. With the single-component reagent, it is possible to adjust the solvent to the solubility of the sample.

3.3. The coulometric Karl Fischer titration

The volumetric addition of reagent is preferably replaced by coulometrically generated iodine for low water contents. Coulometry is based on the same chemical reaction, but the iodine is not dosed using a burette. It is generated in situ at the anode of a generator electrode by oxidising iodide. Hydrogen is produced at the cathode by reduction. The amount of iodine produced is calculated by the titrator according to Coulomb's law.

Coulometry is an absolute method, so titer determination is neither necessary nor possible. KF volumetry and coulometry are the same except for the type of iodine addition respectively iodine generation.

3.4 The coulometric Karl Fischer titration with headspace oven

If water is to be determined in samples that are difficult to dissolve in the Karl Fischer solvent or lead to side reactions, an indirect method with a special oven can be used.

In the oven method, the sample is weighed in a glass vial and heated in a headspace oven. The water is transferred to the titration cell via a carrier gas stream. Either dried air or nitrogen can be used as the carrier gas. This prevents contamination of the cell with the sample.

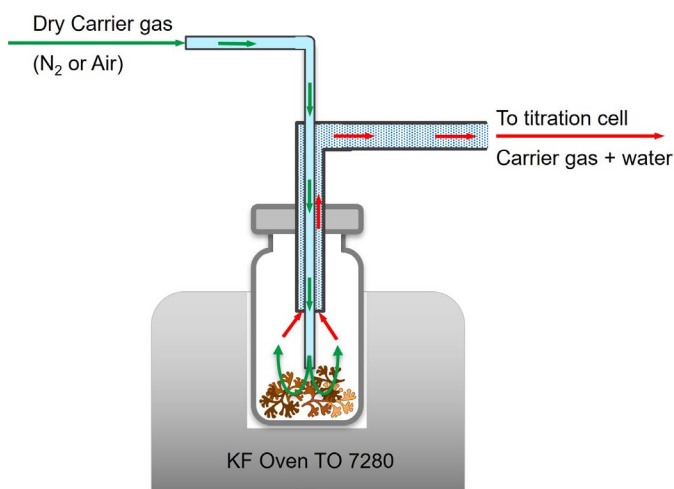


Figure 1: Schematic representation of the gas flow when using a KF headspace oven

4. Instrumentation and method description

YSI titrators from Xylem were used for the results published in this study. Two KF determination methods were to be compared with each other; the volumetric determination in the boiling heat with the coulometric determination by heating the water in a headspace oven.

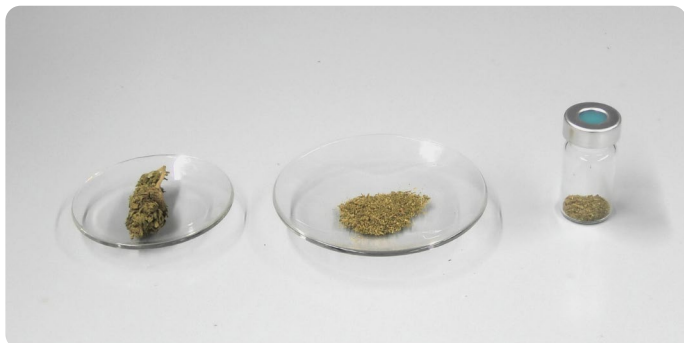


Figure 2: Hemp sample as a flower, ground and weighed in a glass vial for determination in the headspace oven (from left to right)

CBD-containing hemp with a CBD content of 2.9 % (Berry Silver Haze, THC < 0.2 %) was used for the repeat measurements. The sample was grinded using a commercially available coffee grinder and then homogenized by mixing. As losses due to heating can occur when using such grinders, it is recommended to use special cryogenic grinders for routine use. However, this study was not concerned with the absolute content of the sample, but with relative values for comparing two methods. Possible losses due to the grinding process were therefore accepted. The sample prepared in this way was used for both determination methods.

4.1. Volumetric titration at boiling heat

The volumetric system used was a TitroLine® 7500 KF with a 10 ml exchangeable head and a KF 1150 double platinum electrode as indicator system. The solvent was heated to boiling point using a heatable ECM 6 magnetic stirrer from CAT. During the titration, the sample was additionally grinded using a Polytron PT 1200 E homogenizer. Hydranal Methanol Rapid from Honeywell was used as the solvent and the titration reagent was Hydranal Composite 5.

For the volumetric titration, the solvent was heated to boiling point. The evaporated solvent was cooled using a reflux condenser and returned to the titration cell. The model TZ 1754 was selected as titration vessel (see Figure 3) in order to further minimize possible losses due to evaporation.

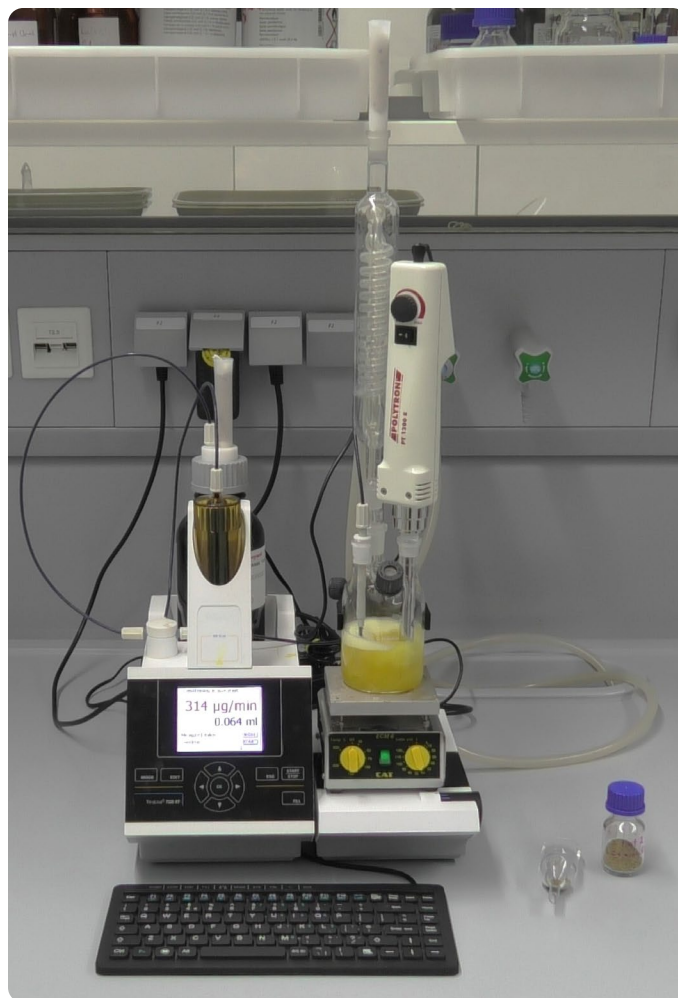


Figure 3: Volumetric Karl Fischer TitroLine® 7500 KF system with TZ 1754 titration vessel, reflux condenser, PT 1200 E homogenizer and ECM 6 heating element

After conditioning the solvent sample (titrating the water contained in the solvent), the heating stirrer was switched off so that the solvent could cool down. As soon as it no longer boiled, the sample was transferred to the titration cell via a weighing funnel. After switching on the homogenizer and the heating plate, the sample was extracted for 300 s and then titrated.

4.2. The coulometric determination with oven method

The coulometric titration was carried out using the TitroLine® 7500 KF Trace titrator. The TZ 1752 model without diaphragm was used as the generator electrode and the indication was carried out using the KF 1150 double platinum electrode. The oven system was the TO 7280 headspace oven with TW 7650 sample changer. Hydranal Coulomat AG Oven from Honeywell was used as reagent. The system was controlled via a PC and the TitrSoft titration software, Version 3.5.3. The sample was weighed into a 5 ml crimp rim bottle ND20 and sealed with an aluminium crimp cap (see also Figure 2). The aluminium cap has a 10 mm centre hole which is sealed with a silicone membrane. This is pierced with the double needle system of the oven and the heated water is transferred to the titration cell using nitrogen as carrier gas.



Figure 4: Coulometric system TitroLine® 7500 KF Trace with oven TO 7280 and sample changer TW 7650

5. Results

5.1. Determination of the heating temperature and linearity test

5.1.1. Temperature ramp

In order to determine the correct oven temperature for determining the water content of cannabis inflorescence, a temperature ramp in the range from 40 °C to 200 °C was measured. The heating rate was 1 °C/min. A value was recorded every 10 s. For comparison, two varieties, Skunk Haze and Berry Silver Haze, were measured (see figure 5 and 6). Approx. 0.08 g was weighed in for both varieties. The Berry Silver Haze variety was used for the following repeat trials (see chapter 5.2 Reproducibility and statistical comparison of methods).

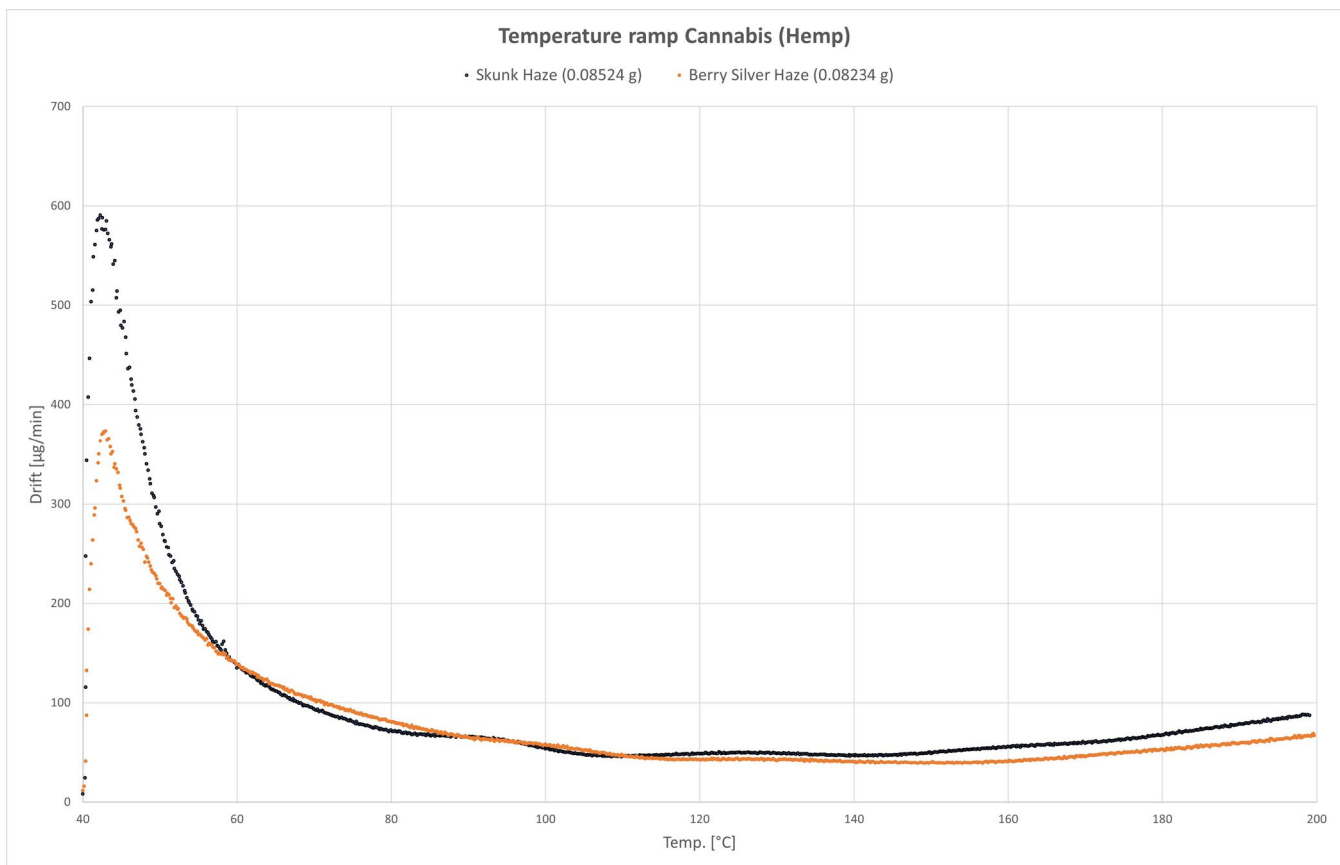


Figure 5: Temperature ramps of two cannabis varieties (hemp), recorded from 40 to 200 °C with a heating rate of 1 °C/min.

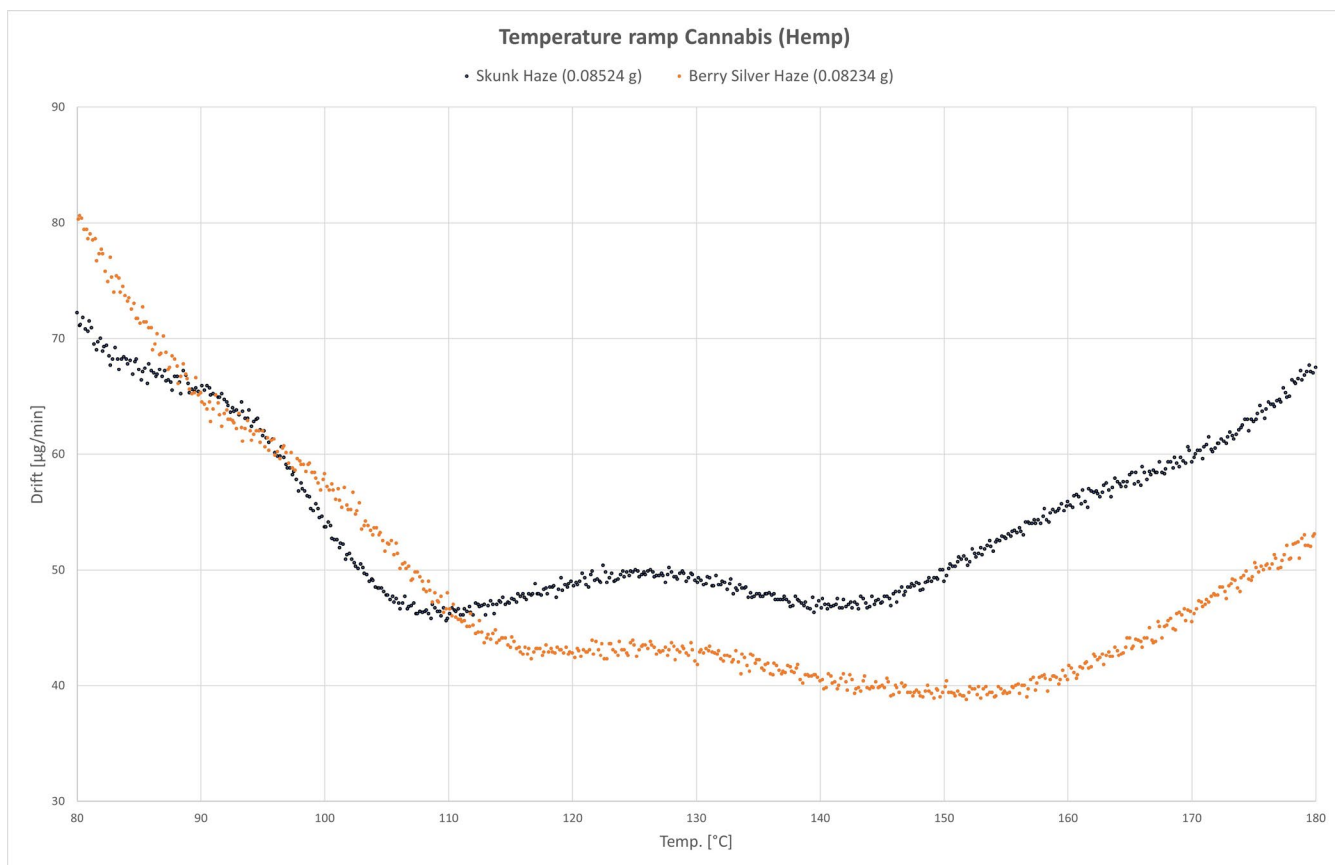


Figure 6: Temperature ramps of two cannabis varieties (hemp), recorded from 40 to 200 °C with a heating rate of 1 °C/min., detail between 80 and 180 °C.

The evaluation of the ramp suggests a temperature of 110 °C. The subsequently observed increase in drift presumably results from decomposition processes or side reactions such as polycondensation. Two linearity tests at 110 °C and at an alternative temperature of 150 °C were carried out to verify the correct temperature. The linearity test was recorded with the Berry Silver Haze variety, as were the subsequent repeat tests.

5.1.2. Linearity test oven method

The results of the linearity test are shown in table 1 below.

Temp. 110 °C				Temp. 150 °C			
Weight	µg	µg - Blank	H ₂ O [%]	Weight	µg	µg - Blank	H ₂ O [%]
0.08369	7756.7	7676.1	9.17	0.08330	8560.2	8482.2	10.18
0.06950	6481.0	6400.4	9.21	0.06977	7139.4	7061.4	10.12
0.06193	5767.5	5686.9	9.18	0.06136	6200.9	6122.9	9.98
0.05334	4962.7	4882.1	9.15	0.05331	5415.0	5337.0	10.01
0.04024	3756.6	3676.0	9.14	0.04059	4064.5	3986.5	9.82
0.02332	2200.2	2119.6	9.09	0.02374	2400.2	2322.2	9.78

\bar{x}	9.16
s	0.04
RSD [%]	0.42

\bar{x}	9.98
s	0.15
RSD [%]	1.45

Table 1: Result of the linearity test at 110 °C and 150 °C

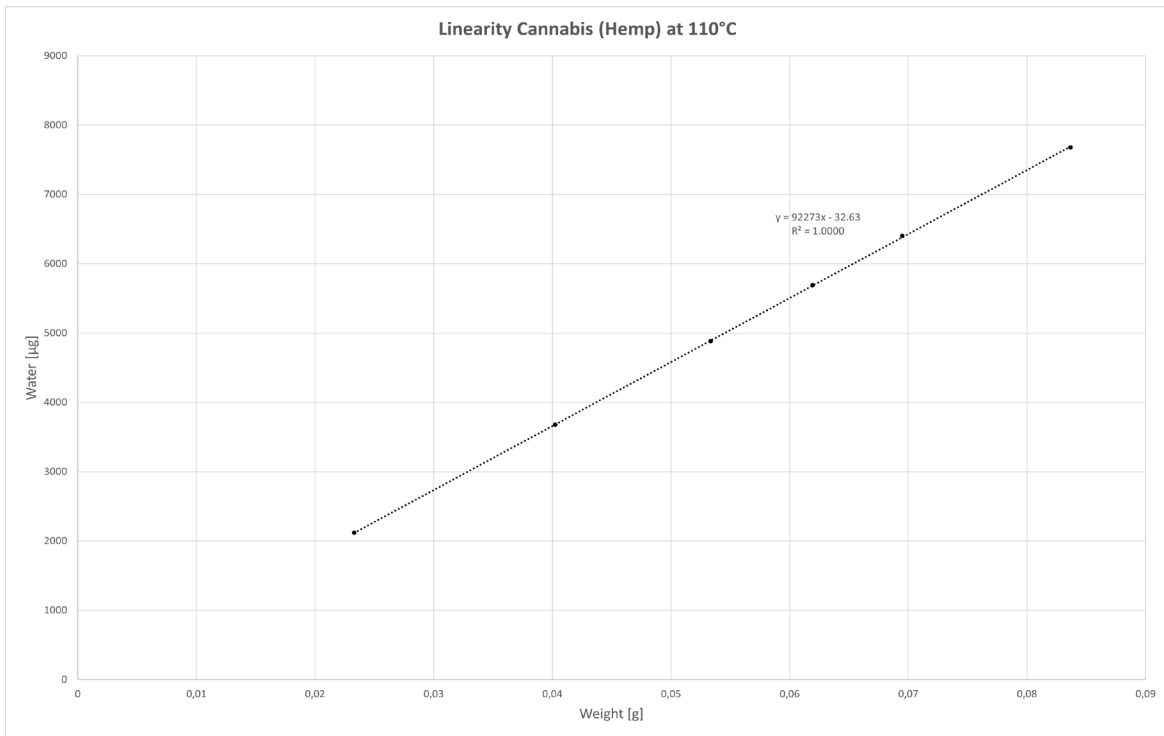


Figure 7: Graphical plot and calculation of the correlation coefficient and ordinate intercept of the linearity test at 110 °C

It can be seen that the mean value of the measured water content at 150 °C is higher than at 110 °C, as expected. The standard deviation as a measure of the fluctuation range is wider at the higher temperature of 150 °C than at 110 °C. If the values are sorted in ascending order of weight, an increasing trend is recognizable at 150 °C.

For further evaluation, the values were plotted graphically and a linear straight fit was calculated. The correlation coefficient and the ordinate intercept (amount of water at a theoretical initial weight of 0) served as the basis for evaluating the linearity test.

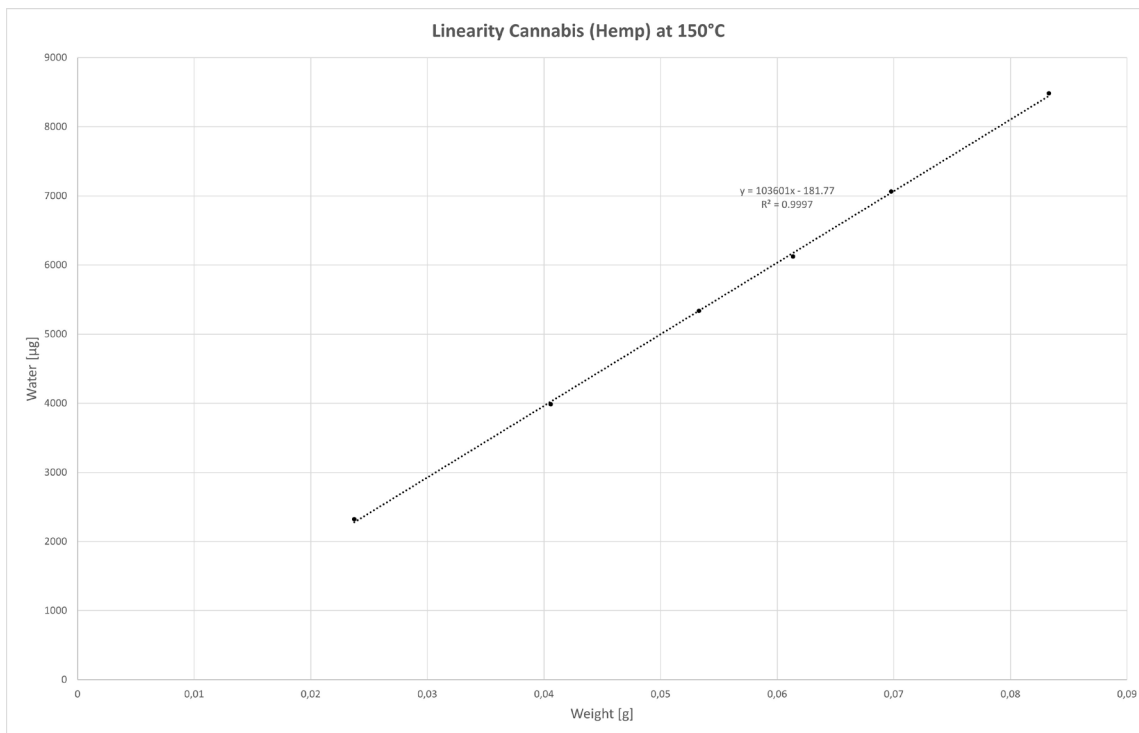


Figure 8: Graphical plot and calculation of the correlation coefficient and ordinate intercept of the linearity test at 150 °C

It can be seen that a very good correlation is observed at both temperatures, but the ordinate intercept at 110 °C is significantly closer to 0 than in the case of the higher temperature of 150 °C. Experience has shown that an ordinate intercept of up to $\pm 50 \mu\text{g}$ is acceptable, especially in the case of natural samples. The ordinate intercept together with the trend observed in the results of the linearity test confirms the assumption that temperatures higher than 110 °C lead to decomposition reactions.

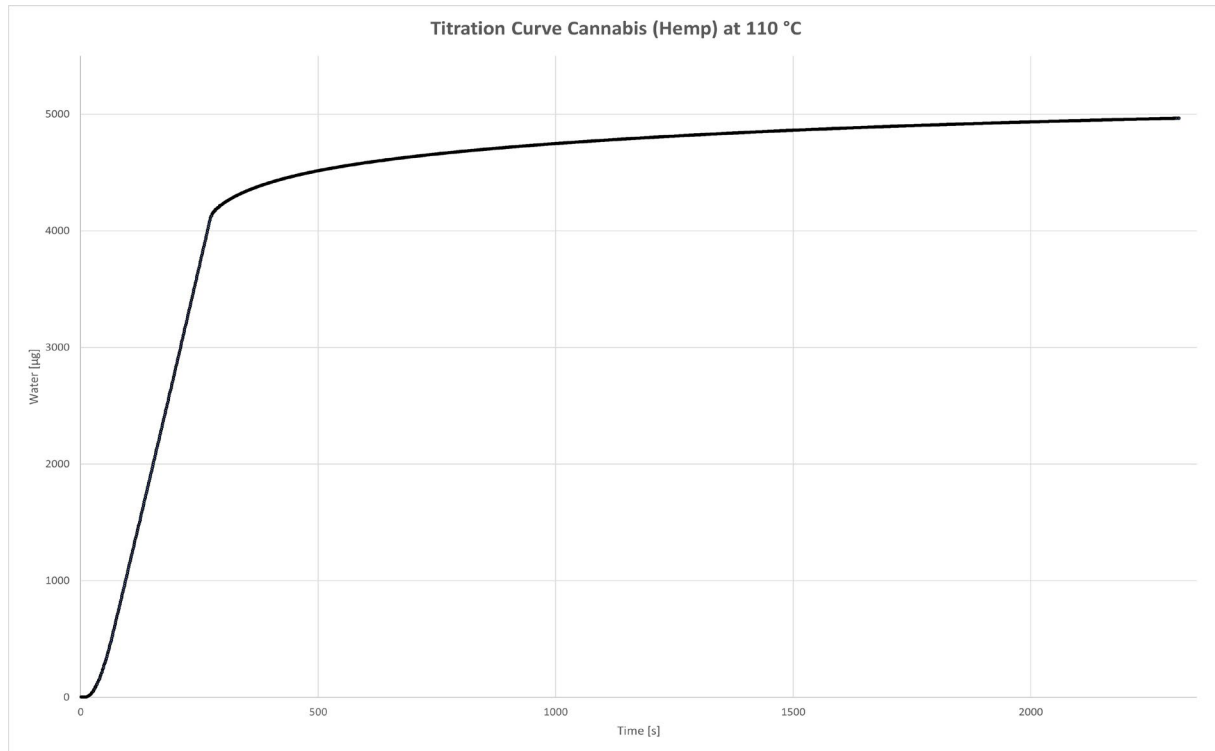


Figure 9: Example titration curve of Cannabis (Hemp) at 110 °C

5.2. Reproducibility and statistical method comparison

To determine the repeatability and as a data basis for the statistical comparison of the measurement methods, a 10-fold repeat measurement of the cannabis sample was carried out. The mean value and standard deviation were calculated from this.



5.2.1. Oven method at 110 °C

Weight [g]	H ₂ O [µg]	H ₂ O [%]
0.04726	4359.2	9.22
0.04954	4579.7	9.12
0.04750	4421.5	9.17
0.04974	4596.6	9.11
0.04765	4390.2	9.08
0.04910	4565.4	9.17
0.04882	4512.7	9.11
0.05582	5225.2	9.25
0.04744	4401.4	9.14
0.04804	4459.1	9.15

Table 2: Measurement results of the repeat measurement using the headspace oven method at 110 °C

\bar{x}	9.15
s	0.05
RSD [%]	0.57

Table 3: Calculated mean value, standard deviation and relative standard deviation of the oven method

Weight [g]	EP [ml]	H ₂ O [%]
0.15097	2.685	9.09
0.14883	2.609	8.96
0.12244	2.174	9.07
0.20545	3.681	9.15
0.16874	2.685	8.13
0.12077	2.100	8.88
0.14224	2.508	9.01
0.13033	2.252	8.83
0.11648	2.005	8.79
0.10998	1.812	8.42

Table 4: Measurement results of the repeat measurement using volumetric water determination in the boiling heat

\bar{x}	8.83
s	0.32
RSD [%]	4.66

Table 5: Calculated mean value, standard deviation and relative standard deviation of the volumetric water determination in the boiling heat

5.2.2. Volumetric method in the boiling heat

5.2.3. Statistical analysis

A two-sample t-test was used to statistically assess the two determination methods. The data was first checked for the requirements of the t-test. A significance level of 95 % was used for all tests.

Test	Result	Conclusion
Normal distribution (Shapiro-Wilk)	The distribution is normal	Requirement fulfilled
Outlier test (Grubbs)	No outlier	Requirement fulfilled
Trend test (Neumann)	No trend	Requirement fulfilled
Variance homogeneity (F-Test)	The variances are not identical	A two-sample t-test according to Welch is used

Table 6: Result of the test for the requirements for the two-sample t-test

As can be seen from table 6, the variances of the two methods cannot be regarded as identical. Therefore, a modified Welch two-sample t-test was used to compare the methods. The methods can be considered identical if the test value Q is smaller than or equals the critical value KW of the corresponding significance level.

	Oven Method	Volumetric boiling heat
\bar{x}	9.152	8.832
n	10	10
s	0.052	0.32316
s_d	0.1035	
Q	3.0955	
KW (α=5 %)	2.2622	
KW (α=1 %)	3.2498	

Table 7: Calculation of the test variables and result of the two-sample t-test according to Welch

Table 7 shows that the results obtained using oven method and volumetric titration in boiling heat can be regarded as identical, taking into account an error probability of 1 %. If a 5 % probability of error is used as a criterion, the results are not statistically identical.

6. Advantages of the Karl Fischer oven method

- Selective results, only water is determined
- Fast results in less than 30 minutes
- Small sample quantities for fewer sample losses
- Reproducible results
- Simple sample handling due to direct weighing into a glass vial

7. Literature

- [1] Hanfanbau in der EU, https://agriculture.ec.europa.eu/farming/crop-productions-and-plant-based-products/hemp_de, retrieved 12.2023
- [2] Ein europäischer grüner Deal, https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal_de, retrieved 12.2023
- [3] Qualität auf allen Ebenen, Qualitätsanforderungen an medizinischen Cannabis – Eine Momentaufnahme, <https://www.deutsche-apotheker-zeitung.de/daz-az/2021/daz-8-2021/qualitaet-auf-allen-ebenen>, abgerufen 12.2023
- [4] Europäisches Arzneibuch, Deutscher Apotheker Verlag, Govi-Verlag – Pharmazeutischer Verlag, Online-Version der 9. Ausgabe, Grundwerk 2017 inkl. 1. Und 2. Nachtrag (Ph.Eur. 9.2).
- [5] Deutsches Arzneibuch 2022, Deutscher Apotheker Verlag, Avoxa – Mediengruppe Deutscher Apotheker, Gesamtwerk 2022
- [6] Cannabis Inflorescence for Medical Purposes: USP Considerations for Quality Attributes, Journal of Natural Products, Cannabis Inflorescence for Medical Purposes: USP Considerations for Quality Attributes | Journal of Natural Products (acs.org)
- [7] Agriculture Improvement Act of 2018, [BILLS-115hr2enr.pdf](https://www.congress.gov/bills/115/hr/2/enr/pdf) (congress.gov)
- [8] Cannabis and Cannabis-Derived Compounds: Quality Considerations for Clinical Research, January 2023, Cannabis and Cannabis-Derived Compounds: Quality Considerations for Clinical Research (fda.gov)
- [9] Sachs, L & Hedderich, J, 2006, Angewandte Statistik: Methodensammlung mit R, 12. vollständig neu bearbeitete Auflage, Springer-Verlag, Berlin, Heidelberg.
- [10] Kromidas, S, 1999, Validierung in der Analytik: Die Praxis der instrumentellen Analytik, Wiley-VCH Verlag GmbH, Weinheim.

Xylem |'zīləm|

- 1) The tissue in plants that brings water upward from the roots;
- 2) a leading global water technology company.

We're a global team unified in a common purpose: creating advanced technology solutions to the world's water challenges. Developing new technologies that will improve the way water is used, conserved, and re-used in the future is central to our work. Our products and services move, treat, analyze, monitor and return water to the environment, in public utility, industrial, residential and commercial building services settings. Xylem also provides a leading portfolio of smart metering, network technologies and advanced analytics solutions for water, electric and gas utilities. In more than 150 countries, we have strong, long-standing relationships with customers who know us for our powerful combination of leading product brands and applications expertise with a strong focus on developing comprehensive, sustainable solutions.

For more information on how Xylem can help you, go to www.xylem.com.



YSI, a Xylem brand
1725 Brannum Lane
Yellow Springs, OH 45387

 +1.979.690.1711

 info@ysi.com

 ysi.com

Microsoft® and Windows are registered trademarks of Microsoft Corporation.

Silcosteel is a registered trademark of Restek

Teflon is a registered trademark of E.I. DuPont de Nemours, Inc.

Peek is a registered trademark of Victrex PLC

OI Analytical® is a registered trademark of Xylem, Inc.

© 2024 Xylem, Inc. XA00334 0824



ysi.com