



Customs Laboratories
European Network

ILIADe 578:2024 | CLEN Method

**Determination of cannabinoids in cannabis products
by gas chromatography**

Version 13 February 2024

**adopted as CLEN method
at the 26th CLEN Plenary Meeting on 13 February 2024**

Determination of cannabinoids in cannabis products by gas chromatography

1. Scope

This is a method in which the content of specific cannabinoids in a sample is quantified. In the case of carboxylated cannabinoids the combined concentration of the cannabinoid and the cannabinoid acid is measured. For instance, in the case of Δ -9-tetrahydrocannabinol (THC) and Δ -9-tetrahydrocannabinolic acid (THCA), the total concentration is measured with this method. This total THC content, and the concentration of other cannabinoids determines the legality of cannabis and cannabis products in many countries.

Cannabinoids that can be measured using this method include total Δ -9-THC total cannabidiol (CBD), total cannabigerol (CBG) and cannabigerolic acid (CBGA), and cannabinol (CBN).

This method can be applied to the analysis of dried plant material, hashish, oils and edibles made with the cannabis plant, next to possibly other THC containing products.

This method is based on the UNODC recommended method for the analysis of cannabis and cannabis products.^[1]

2. Principle

The cannabinoids are extracted out of the product with a suitable solvent and the sample is then injected on a GC (gas chromatograph). In the case of carboxylated cannabinoids, due to the temperature of the injector the carboxylated form will be decarboxylated. The cannabinoids and other molecules in the sample are separated on the GC column. Detection is performed with MS (mass spectroscopy) or FID (flame ionization detection). The cannabinoid peak is identified on the basis of the retention time. To quantify the level of the cannabinoid, an internal standard is used and a calibration curve (multiple or single point calibration), preferably using a solution of the cannabinoid of interest.

3. Reagents and materials

3.1 Carrier gas: helium or hydrogen

3.2 Extraction solvent, for example ethanol (absolute)

3.3 An internal standard, for example tribenzylamine (TBA) or dodecane

3.4 A certified reference sample of the cannabinoids of interest

All above reagents should be handled and stored according to their safety instructions.

4. Apparatus

4.1 A GC system, equipped with MS or FID as a detector. A column similar to the following one is recommended: 5% Diphenyl / 95% Dimethylpolysiloxane, 30 m, 0.3 mm, film thickness 0.25 μ m with precolumn. Depending on the matrix and the cannabinoids of interest, a medium polarity column can also be suitable.

4.2 Calibrated (automatic) pipettes

4.3 Calibrated glassware

4.4 Sonication bath

4.5 Vortex mixer

4.6 Analytical balance with a precision of 0.1 mg

4.7 Centrifuge which can obtain 1000 g or a filtration device

4.8 A blender or cutting device to reduce the material in size and to homogenise.

5. Procedure

5.1 Solutions and standards

5.1.1 Solution of the internal standard

A solution should be prepared of a concentration in the same order of magnitude as the concentration of interest of the cannabinoid of interest, which is usually near the legal limit.

For example, the following procedure can be used if TBA is selected as the internal standard: Make a solution of approximately 50 mg/l of TBA in ethanol. The exact concentration is noted for the final calculation. It is possible to work with a concentrated stock solution out of which this solution can be prepared, for example a solution of approximately 250 mg of TBA dissolved in ethanol to a total volume of 500 ml. In this last case, a working solution can be obtained by using 10 ml of this stock solution and diluting it to 100 ml. Both of these solutions should be stored in the refrigerator and should be used within 4 months after preparation of the initial solution.

5.1.2 Calibration solution

To the solution of the internal standard (5.1.1), a specific amount of one or more cannabinoids should be added to make the calibration solution. The concentration of the cannabinoids should be in the same order of magnitude as the concentration of interest when working with a single point calibration, which is usually the legal limit. It is also possible to specify the calibration and sample solutions in their mass fraction (mg/g). Small conversion errors are negligible.

When working with a multiple point calibration, the concentration of interest should be within the range of calibration solutions.

For example, the following procedure can be used for the detection of total THC at low concentrations using a single point calibration: To 5 ml of a TBA solution in ethanol (50mg/l) add 150 µl of a certified reference solution of 1 mg/ml of THC. This solution should be stored in the refrigerator and should be used within one month, or within the expiration period of the TBA solution that was used.

It is possible, although not recommended, to calibrate using a different cannabinoid than the one of interest. It is for example possible to use CBD or CBN for calibration when measuring total THC. When using another cannabinoid to calibrate it is recommended to use FID as a detector rather than MS. For example, a calibration curve of CBN in the concentration range 0.1 to 16% can be used to quantify total THC by GC-FID.^[1]

5.2 Sample preparation

Depending on the nature of the sample, it should be homogenized using a blender or other such device. In a sealable vessel, an appropriate amount of the homogenized sample should be weighed to a precision of 0.1 mg and the weight noted for the final calculation. A precise amount of the solution of the internal standard should be added and homogenized thoroughly.

In the case of samples that are not completely soluble or inhomogeneous samples, these should be placed in a sonication bath for at least 10 minutes. The sample should then be thoroughly mixed before sonicating further. To clean up the solution from the suspended material, a separation step can be introduced. This can be done via centrifuge or filtering of the solution.

It should be noted that in the case of fresh flowers and other fresh plant material, the samples should first be dried until constant weight.

For example, the following procedure can be used: Weigh between 140 and 170 mg of the homogenized sample in a centrifuge tube and note the weight. Add to this 10 ml of the TBA solution (50 mg/l) and place it in a sonication bath for 10 minutes. Mix the sample with a vortex mixer and sonicate for another 10 minutes. Then, place the centrifuge tube in a centrifuge and separate at 1000 g for at least 5 minutes. Transfer the clean solution to a GC vial and seal it. The remaining solution can be kept in the refrigerator until the results are validated. When a reanalysis is needed afterwards, a new GC vial can be filled with this solution.

In the case of a watery solution (such as in a cannabinoid infused soft drink), the solution can be extracted with heptane or a similar solvent. This fraction can then be reduced in volume and treated further as described above.

5.3 GC analysis

5.3.1 Calibration, tuning and conditioning

If the GC has not been used continuously for this analysis, it is suggested to perform a conditioning. When a MS detector is used, it is recommended to tune before launching a sequence. The conditioning can be performed by the injection of two blank solutions, followed by some random samples with cannabinoid concentrations in the range of interest, followed by another blank solution.

A calibration (single point or multiple points) should then be performed using the calibration solutions (5.1.2).

5.3.2 GC settings

A GC temperature program suitable to separate the cannabinoids and any other components should be employed.

For example, a starting temperature of 100°C, increasing to 320°C at 15°/minute, followed by a hold time of 15 minutes can be used.

Another example of a temperature program is to start at 180°C, hold for 0.5 minutes, then increase at 5°C/minute to 270°C, followed by a hold of 10 minutes.

The injector temperature should be sufficient to transform any cannabinoid acids into their decarboxylated form and to volatilize the sample. For example, a temperature of 260-280°C is usually sufficient.

The injection volume is recommended to be 0.5-1 µl.

A split of 1:15 or 1:25 can be used.

Both helium and hydrogen can be used as carrier gas.

A pressure in the range of 50 to 90 kPa is recommended.

5.3.3 Checks of blanks and calibration solution

At regular intervals during the measurement series, a measurement check of the blank and calibration solutions can be performed (see 5.4 Quality control).

It is recommended to add additional blank measurements after measuring samples with a high content of the cannabinoids of interest to ensure that this does not contaminate other samples in the series. Also after measuring matrices which can influence the following measurements, for example hashish or oils, an extra blank can be added.

5.4 Quality control

The quality control can be performed by one (or a combination) of the following actions:

- Verification if decarboxylation is complete during GC analyses;
- Evaluation of measured peaks (symmetry, tailing, baseline separation of cannabinoids of interest);
- Evaluation of blank sample (e.g. solvents used without internal standard or cannabinoids);
- Evaluation of the calibration;
- Evaluation of duplicate measurements;
- Measurement of a control sample: use of a CRM is advised to assess the quality of all the procedure.

It should be noted that it is known that over time the total THC content in a cannabis product will degrade. It is therefore strongly recommended that if a control sample is used, it is used for six months or less, before another sample is used or the concentration in the control sample is recalculated.^[1,2]

6. Calculation

The corresponding peaks for the cannabinoid and internal standard (IS) are detected and their peak area is integrated. The peak area of the cannabinoid can be recalculated to a cannabinoid concentration, using the calibration line and results of the internal standard. The procedure below could be applied in order to calculate the result manually:

- A calibration line is plotted with the area ratio (measured cannabinoid peak area / measured IS peak area) in function of the used quantity ratio (used cannabinoid concentration (mg/mL) / used IS concentration (mg/mL)). Calculate the slope of the linear regression;
- The area ratio of the sample is calculated (measured cannabinoid peak area / measured IS peak area);
- The quantity ratio of the cannabinoid over IS in the sample is calculated from the area ratio using the calibration line;
- The mass fraction of the cannabinoid can be calculated by multiplying this quantity ratio by the quantity of IS (g) used for dissolving the sample, divided by the sample amount. The mass percentage (g cannabinoid / 100 g sample) is obtained by multiplying the mass fraction by 100%.

7. Expression of the results

The results are expressed with 3 significant digits (i.e. 2 decimal places if the content is below 10 % of total mass). Alternatively, the result can be expressed as higher or lower than the legal limit.

8. Precision

Will be completed later (preferably after a proficiency test performance, with results obtained by this ILIADe 578 method).

9. Remarks

- During the validation of every new method, it should be confirmed that the decarboxylation step of the cannabinoid of interest is performed completely during the GC program. This can be done for example by incubation of the sample in an oven prior to analyses.^[3]
- During validation, it should be checked that all cannabinoids of interest are well separated in the chromatogram (e.g. different isomers of THC, different natural cannabinoids and semi-synthetic cannabinoids) so that no interference is possible. This can be verified by analysing reference materials or by MS identification.
- Quantification of the acid form of the cannabinoid by GC can be performed but is outside the scope of this method. In this case, the cannabinoid needs to be derivatized prior to analyses (e.g. by MSTFA: N-methyl-N-trimethylsilyltrifluoroacetamide or BSTFA/TMCS: N,O-bis(trimethylsilyl)trifluoroacetamide/trimethylchlorosilane (1%)). Otherwise, a HPLC method could also be used (suitable for measurement of both acid and decarboxylated form without derivatization).^[1]
- Acidic conditions must be avoided as acidity can cause isomerization of THC (especially crucial when sample would be derivatized).^[4]

References

- [1] UNODC, "Recommended methods for the identification and analysis of cannabis and cannabis products", 2022, (https://www.unodc.org/documents/scientific/Recommended_methods_for_the_Identification_and_Analysis_of_Cannabis_and_Cannabis_products.pdf)
- [2] UNODC, Ross S. A., Elsohly M. A., "CBN and D9-THC concentration ratio as an indicator of the age of stored marijuana samples", 1999, (https://www.unodc.org/unodc/en/data-and-analysis/bulletin/bulletin_1997-01-01_1_page008.html#5)
- [3] Decarboxylation of Tetrahydrocannabinolic acid (THCA) to active THC (Kerstin Iffland, Michael Carus and Dr. med. Franjo Grotenhermen, nova-Institut GmbH)
- [4] Conversion of Cannabidiol (CBD) into Psychotropic Cannabinoids Including Tetrahydrocannabinol (THC): A Controversy in the Scientific Literature (Patricia Golombek y, Marco Müller y, Ines Barthlott, Constanze Sproll and Dirk W. Lachenmeier)