Gas Chromatographic Determination of Tetrahydrocannabinol in Cannabis

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PRINCIPLE OF THE METHOD

The THC and THCA in cannabis plant material and resin are extracted from the plant matrix by sonicating or rotating in toluene. The extraction is repeated three times to afford as complete an extraction as practicable. The combined toluene extracts are injected into a gas chromatograph with flame ionization detection. Δ^9-tetrahydrocannabinolic acid (THCA), if present in the extracts, is converted, by decarboxylation, to THC in the injector of the gas chromatograph. Δ^9-tetrahydrocannabinol (THC) is quantified.

INSTRUMENTATION AND EQUIPMENT

1. Gas chromatograph 5890 Hewlett-Packard equipped with:

   a split/splitless injector,

   a flame ionization detector,

   a DB-5 capillary column (15m x 0.25 mm i.d., 0.25µm film thickness) available from J & W Scientific,

   a 7673A Hewlett-Packard autosampler,

   a HP 5895A GC Chemstation.

2. Ultrasonic bath: 250 ULTRAsonik^R from NEY or a multi-purpose rotator model 150 V (Scientific Industry Inc.)

3. Centrifuge: IEC Model HN-SII Centrifuge at about 1500 rpm (Damon/IEC Division)

4. Laboratory refrigerator set at 4^{o}C with a separate freezer compartment

5. 15 mL screw-cap disposable centrifuge tube (borosilicate glass with O.D.(mm): 17, height(mm): 126)

6. Screw caps: phenolic - PTFE-faced rubber liner (Canlab)

7. Volumetric flasks: 10 mL
   25 mL
   100 mL
   500 mL

8. Volumetric pipettes: 1.0 mL (graduated)
   2.0 mL
   5.0 mL

9. Pasteur pipettes

10. 12 x 32mm autosampler vials (clear borosilicate glass with crimp top teflon/rubber septum (11 mm))

11. Electronic balance: Mettler 100

12. Pipette rubber bulb

REAGENTS

1. Toluene, distilled in glass, Caledon Laboratories Ltd.

2. Helium gas supply, high purity

3. Nitrogen gas supply, prepurified

4. Air gas supply, medical grade

5. Hydrogen gas supply, prepurified

MATERIALS REQUIRED

1. THC standard stock solution - a 10.0 mg/mL solution of Δ^9-tetrahydrocannabinol (Δ^9-THC) in ethanol sufficient for the preparation of solutions for the determination of linearity of the method and the analysis of samples prepared from a single solution of THC to ensure the same concentration in all laboratories.

2. THC resolution solution - a solution of about 0.1 mg/mL Δ^8-THC, about 0.1 mg/mL Δ^9-THC and about 0.8 mg/mL lidocaine sufficient for the determination of the resolution of the Δ^8 and Δ^9 isomers of THC.

3. Δ^9-THC acid solution (NOTE 1) - a 9.78 mg/mL solution of Δ^9-tetrahydrocannabinolic acid (THCA) in ethanol for the determination of the degree of conversion of the acid to THC in the injector of the gas chromatograph.

4. Internal standard - lidocaine base (Lot # 105F-0009), Sigma Chemical Company - sufficient for the preparation of all solutions

SOLUTIONS

1. Internal standard stock solution.
   Weigh an appropriate amount of lidocaine base into a volumetric flask to give a final concentration of about 8 mg/mL. Dilute the flask to volume with toluene and mix well.

2. Internal standard extraction solution

Dilute the internal standard stock solution by a factor of ten using a suitable volumetric flask and pipette to give a concentration of about 0.8 mg/mL.

3. Standard solutions Store all solutions below 0°C.

A. Solutions for standard curve

i. Prepare a 2.0 mg/mL solution of THC in toluene by adding to a 10 mL volumetric flask 2.0 mL of the THC standard stock solution. Add 1.0 mL of the internal standard stock solution and make the flask to volume. Mix well.

ii. Prepare a 1.0 mg/mL solution of THC in toluene by adding to a 10 mL volumetric flask 1.0 mL of the THC standard stock solution. Add 1.0 mL of the internal standard stock solution and make the flask to volume. Mix well.

iii. Prepare a 0.75 mg/mL solution of THC in toluene by adding to a 10 mL volumetric flask 0.75 mL of the THC standard stock solution. Add 1.0 mL of the internal standard stock solution and make the flask to volume. Mix well.

iv. Prepare a 0.50 mg/mL solution of THC in toluene by adding to a 10 mL volumetric flask 0.50 mL of the THC standard stock solution. Add 1.0 mL of the internal standard stock solution and make the flask to volume. Mix well.

v. Prepare a 0.2 mg/mL solution of THC in toluene by adding to a 10 mL volumetric flask 0.20 mL of the THC standard stock solution. Add 1.0 mL of the internal standard stock solution and make the flask to volume. Mix well.

B. THCA to THC conversion efficiency

Diluted Δ^9-THC acid solution

Dilute the Δ^9-THC acid solution in toluene by adding to a 10 mL volumetric flask 1.0 mL of this solution and 1.0 mL of the internal standard stock solution. Make the flask to volume with toluene. Mix well.

ANALYTICAL METHOD

Extraction of THC from cannabis preparations.

A. Cannabis marihuana and cannabis resin (hashish)

Weigh accurately an approximate amount of cannabis preparation as indicated for each sample in Table 1 into a 15 mL disposable centrifuge tube. Add a 5 mL quantity of the internal standard extraction solution and sonicate or rotate for 30 minutes. Centrifuge the tube and its contents for about 1 minute. Transfer as much as possible of the toluene, without disturbing the insoluble material, into a 25 mL volumetric flask. To the tube add another 5 mL of internal standard extraction solution and repeat the sonication or rotation of the tube for 30 minutes. Centrifuge the tube and its contents again and transfer as much as possible of the toluene to the same volumetric flask containing the first portion of toluene. Repeat the extraction and centrifugation a third time with a fresh portion of the internal standard extraction solution and combine the toluene in the same volumetric with the two previous extracts. Fill the volumetric flask containing the toluene extracts to the volumetric mark with internal standard extraction solution. Transfer sufficient solution to an autosampler vial for injection into the gas chromatograph or inject 1 µL of the solution manually into the chromatograph.

B. Cannabis resin (liquid)

Weigh accurately an approximate amount of liquid resin as indicated for each sample in Table 1 into a 10 mL volumetric flask. Add a 5-6 mL quantity of the internal standard extraction solution washing as much as possible of the liquid resin from contact with the glass. Swirl the flask to mix. Fill the volumetric flask to the volumetric mark with internal standard extraction solution and mix well. Transfer sufficient solution to an autosampler vial for injection into the gas chromatograph or inject 1 µL of the solution manually into the chromatograph.

CHROMATOGRAPHY

Analyze all samples in duplicate, i.e., two weighings and two complete extractions for each sample. (NOTE 2)

A. Chromatographic conditions

Install and condition the column according to the manufacturers' instructions.

Adjust the flow rates of the gases to following:

Column (helium) 1.5 mL/min.

Flame Ionization Detector (hydrogen) 30 mL/min.

Flame Ionization Detector Auxiliary Gas (nitrogen) 30-40 mL/min.

Flame Ionization Detector (air) 400 mL/min.

Adjust the temperature of the injector to 240°C.

Adjust the temperature of the detector to 275°C.

Adjust the split ratio of the injector to give about 30:1.

Make all injections 1 µL.

Program the temperature of the column from 200°C, after a 1 minute hold, to 295°C at 20°C/min; maintain final column temperature for 1 min.

Acquire and process the peak integrations using the instrument data system or other suitable integrator.

B. Chromatography suitability tests

i. Resolution

Inject the THC resolution solution . Calculate the resolution of the peaks due to the two isomers using the following equation:

$$\text{Resolution (R)} = \frac{2(t_2 - t_1)}{W_2 + W_1}$$

where t_2 and t_1 are the retention times of the Δ^9 and Δ^8 peaks respectively and

W_2 and W_1 are the corresponding widths of the bases of the peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline

The resolution obtained should be not less than 2.

ii. Linearity

Inject each of the linearity solutions and calculate the relative response of the THC peak to that of the internal standard for each solution. Calculate the square of the correlation coefficient (R^2) from the relative response of each solution and the mass of THC injected for each solution.

The R^2 value obtained should not be less than 0.98.

iii. Conversion of THCA to THC

Inject, three times each, the 1.0 mg/mL solution of the THC standard prepared for the standard curve (ii, above) and the diluted Δ^9-THC acid solution. Calculate the % conversion of the THC acid to THC by the following formula:

$$\% \text{ Conversion} = \frac{R_{THCA}}{R_{THC}} \times \frac{100}{0.978} \times \frac{358}{314} =$$

where R_{THCA} = Ratio of the area counts of the THC peak obtained from the injection of the diluted Δ^9-THC acid solution to the area counts of the internal standard obtained from the same solution,

R_{THC} = Ratio of the area counts of the THC peak obtained from the injection of the THC standard solution to the area counts of the internal standard obtained from the same solution,

0.978 is the concentration of the diluted Δ^9-THC acid solution and

358 and 314 are the molecular weights of THCA and THC.

The % conversion should be not less than 90%.

NOTES

1. This solution contains 7% THC as determined by HPLC.

2. The quantification of the samples may be performed by the injection of the 1 mg/mL solution of THC as the standard. Assurance of the daily correct performance of the instrument is at the discretion of the analyst. However, it is recommended that three injections of this solution are made to ensure the reproducible detection of the internal standard and the THC in this solution.