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Method of Test for Δ^9-Tetrahydrocannabinol and Cannabidiol in Cosmetics

1. Scope

This method is applicable to the determination of Δ^9-tetrahydrocannabinol and cannabidiol in cosmetics.

2. Method

After extraction, cannabinoids are determined by liquid chromatograph/ tandem mass spectrometry (LC-MS/MS).

2.1. Equipments

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: electrospray ionization, ESI.

2.1.1.2. Column: ACQUITY BEH Shield RP18, 1.7 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.2. Ultrasonicator.

2.2. Chemicals

Methanol, HPLC grade;

Ammonium formate, AR grade;

Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);

Δ^9-Tetrahydrocannabinol (Δ^9-THC, 1 mg/mL in methanol) and cannabidiol (CBD, 1 mg/mL in methanol), reference standards;

Δ^9-Tetrahydrocannabinol-d_3 (Δ^9-THC-d_3, 100 μg/mL in methanol) and cannabidiol-d_3 (CBD-d_3, 100 μg/mL in methanol), internal standards.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL, 25 mL and 50 mL.

2.3.2. Membrane filter: 0.22 μm, PTFE.

2.4. Mobile phase

2.4.1. Solvent A:

Dissolve and dilute 0.63 g of ammonium formate with deionized water to 1000 mL, and filter with a membrane filter.

2.4.2. Solvent B: methanol.

2.5. Internal standard solution preparation

Mix 1 mL of Δ^9-THC-d_3 and CBD-d_3 isotope internal reference standards, and dilute with methanol to 20 mL as the internal standard solution.

2.6. Standard solution preparation

Accurately transfer 1 mL of Δ^9-THC and CBD reference standards to a 100-mL volumetric flask separately, dissolve and dilute to volume with methanol as stock solutions, and then store in a refrigerator. When to use, mix appropriate volume of the stock solutions and the internal standard solution, and dilute with methanol to 0.02~0.5 μg/mL (containing 0.01 μg/mL internal standard) as the standard solutions.

2.7. Sample solution preparation

Transfer about 1 g of the sample accurately weighed into a 50-mL volumetric flask. Add 40 mL of methanol, and ultrasonicate for 30 min. Dilute to volume with methanol and filter with a membrane filter. Take the filtrate as the sample solution.

2.8. Standard curve preparation

Accurately inject 3 μL of the standard solutions into LC-MS/MS, and operate LC-MS/MS according to the following conditions. Establish the standard curves of Δ^9-THC and CBD separately by the ratios of the peak area of Δ^9-THC and CBD to that of the internal standard vs. the added concentrations.

LC-MS/MS operating conditions(note):

Column: ACQUITY BEH Shield RP18, 1.7 μm, 2.1 mm i.d. × 10 cm.

Oven temperature: 30°C.

Mobile phase: a gradient program of solvent A and solvent B is as follows.

Time (min)	A (%)	B (%)
0 → 5	20 → 20	80 → 80
5 → 6	20 → 0	80 → 100
6 → 8	0 → 0	100 → 100
8 → 8.1	0 → 20	100 → 80
8.1 → 13	20 → 20	80 → 80

Flow rate: 0.3 mL/min.

Injection volume: 3 μL.

Capillary voltage: 2.8 KV.

Ionization mode: ESI^+.

Ion source temperature: 150°C.

Desolvation temperature: 350°C.

Cone gas flow rate: 50 L/hr.

Desolvation flow rate: 780 L/hr.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, cone voltage and collision energy are shown as follows:

Analyte	Precursor ion (*m/z*) > Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
Δ^9-THC	315 > 193*	40	20
	315 > 259	40	15
CBD	315 > 193*	40	20
	315 > 259	40	15
Δ^9-THC-d_3 (I.S.)	318 > 196	40	15
CBD-d_3 (I.S.)	318 > 196	40	15

Note: all the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.9. Identification and quantification

Accurately inject 3 μL the sample solution and the standard solutions into LC-MS/MS separately, and operate according to the conditions described in section 2.8. Identify Δ^9-THC and CBD based on the retention time and the relative ion intensities(note). Calculate the amount of Δ^9-THC or CBD in the sample by the following formula:

$$\text{The amount of } \Delta^9\text{-THC or CBD in the sample (ppm)} = \frac{C \times V}{M}$$

Where,

C: the concentration of Δ^9-THC or CBD in the sample solution calculated by the standard curve (μg/mL)

V: the final make-up volume of the sample (mL)

M: the weight of the sample (g)

Note: Relative ion intensities are calculated by peak areas of quantitative ions divided by peak areas of qualitative ions (≤100%). Maximum permitted tolerances of relative ion intensities by LC-MS/MS are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20

>	20 ~ 50	± 25
>	10 ~ 20	± 30
≤	10	± 50

Remark

1. The limits of quantification (LOQs) for Δ^9-THC and CBD are 1 ppm.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. Meng, Q., Buchanan, B., Zuccolo, J., Poulin, M. M., Gabriele, J. and Baranowski, D. C. 2018. A reliable and validated LC-MS/MS method for the simultaneous quantification of 4 cannabinoids in 40 consumer products. PLoS ONE 13: e0196396.
2. Molnar, A., Lewis, J., Doble, P., Hansen, G., Prolov, T. and Fu, S. 2012. A rapid and sensitive method for the identification of delta-9-tetrahydrocannabinol in oral fluid by liquid chromatography-tandem mass spectrometry. Forensic Sci. Int. 215: 92-96.

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Reference chromatograms

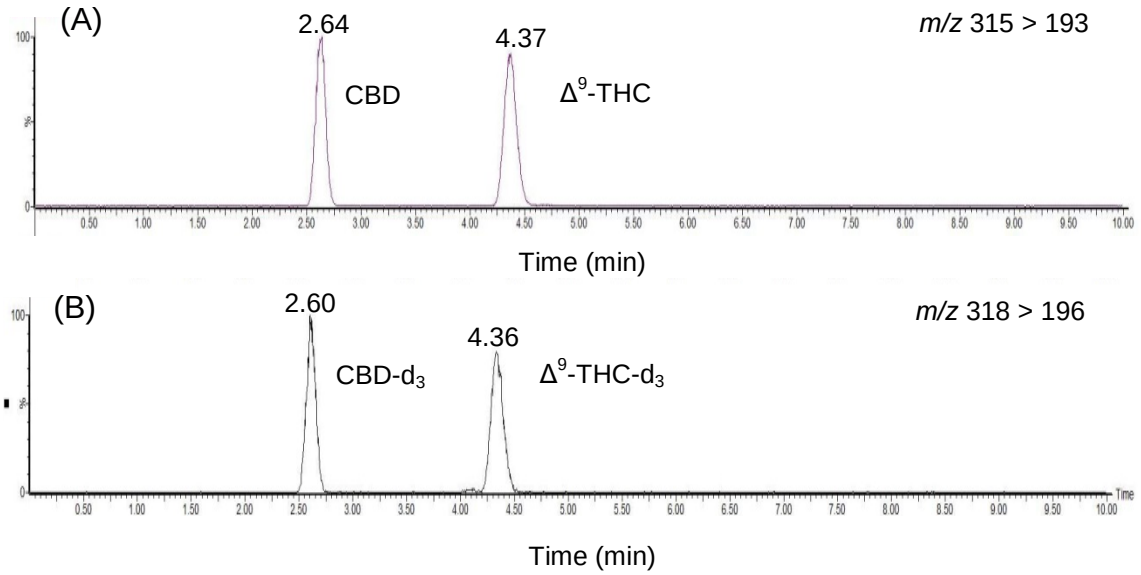

Figure. MRM chromatograms of Δ^9-THC and CBD standards (A) and the isotopic internal standards (B) analyzed by LC-MS/MS.