COMMISSION REGULATION (EEC) No 421/86

of 25 February 1986

amending Regulation (EEC) No 771/74 and Regulation (EEC) No 2188/84 by prescribing a Community method for the quantitative determination of tetrahydrocannabinol in hemp

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Regulation (EEC) No 1308/70 of 29 June 1970 on the common organization of the market in flax and hemp [1], as last amended by Regulation (EEC) No 1430/82 [2], and in particular Article 4 (5) thereof,

Having regard to Council Regulation (EEC) No 2059/84 of 16 July 1984 laying down general rules relating to the import restrictions on hemp and hemp seed and amending Regulation (EEC) No 619/71 in respect of hemp [3], and in particular Article 4 thereof,

Whereas Article 3 (1) of Council Regulation (EEC) No 619/71 [4], as last amended by Regulation (EEC) No 2059/84, provides for the aid to be granted only for hemp grown from certified seed of varieties, the average tetrahydrocannabinol (THC) content of which has been found not to exceed certain acceptable limits; whereas Article 6a of Commission Regulation (EEC) No 771/74 of 29 March 1974 laying down detailed rules for granting aid for flax and hemp [5], as last amended by Regulation (EEC) No 2188/84 [6], provides in particular that the THC level shall be recorded, and samples taken for that purpose, temporarily and under certain conditions, in accordance with a method to be selected by Member States; whereas, in view of experience gained, Regulation (EEC) No 771/74 should be amended accordingly by prescribing a single method for the Community;

Whereas Article 2 of Regulation (EEC) No 2059/84 also lays down that raw hemp from third countries may be imported only if evidence is produced that its THC content does not exceed certain limits; whereas Article 2 (2) of Regulation (EEC) No 2188/84 provides that until such time as a Community method of analysis has been adopted for recording the weight of THC in relation to the weight of the representative sample taken for this purpose on import by the Member States, the latter shall apply a method of their choice; whereas, taking into account experience gained, the Community method should be prescribed and that Regulation amended accordingly;

Whereas the determination of Δ^9 THC has proved to be the most suitable method of measuring the THC content of hemp since that substance is composed very largely of that particular natural isomer and it should therefore be considered as representative of its total THC content; whereas this method should therefore be adopted;

Whereas the measures provided for in this Regulation are in accordance with the opinion of the Management Committee for flax and hemp,

HAS ADOPTED THIS REGULATION:

Article 1

Regulation (EEC) No 771/74 is hereby amended as follows:

1. Article 6a is replaced by the following:

   'Article 6a

   The tetrahydrocannabinol level shall be ascertained, and samples taken for that purpose, using the single method valid for the entire Community set out in Annex C'.

2. The following Annex C is inserted:

(1) OJ No L 146, 4. 7. 1970, p. 1.
(2) OJ No L 162, 12. 6. 1982, p. 27.
(3) OJ No L 191, 19. 7. 1984, p. 6.
(4) OJ No L 72, 26. 3. 1971, p. 2.
(5) OJ No L 92, 3. 4. 1974, p. 13.
(6) OJ No L 199, 28. 7. 1984, p. 23.

'ANNEX C

COMMUNITY METHOD FOR THE QUANTITATIVE DETERMINATION OF Δ^9 THC (TETRAHYDROCANNABINOL) IN CERTAIN VARIETIES OF HEMP

1. Purpose and scope

This method permits quantitative determination of Δ^9 tetrahydrocannabinol (Δ^9 THC) in certain varieties of hemp *(Cannabis sativa* L.) for the purpose of checking that the conditions laid down in Article 3 paragraph 1 of Regulation (EEC) No 619/71 are fulfilled.

2. Principle

Quantitative determination of Δ^9 THC by gas chromatography (GC) after extraction with a suitable solvent.

3 Apparatus.

— gas chromatography equipment with a flame ionization detector,

— glass column 2,50 m long and 3,2 mm in diameter (1,8") packed with a suitable support impregnated with a stationary phase phenyl-methyl-silicon (e.g. OV 17 at 3 %).

4. Sampling and reduction of sample

Sampling

In a standing crop of a given variety of hemp, take not less than 500 plants, preferably at different points buts not from the edges of the crop. Samples should be taken during the day after flowering has finished.

The pooled samples should be representative of the lot.

The plant material is then left to dry at ambient air temperature.

Reduction

Reduce the sample, obtained as described, to 500 stalks ; the reduced sample should be representative of the original sample. Divide the reduced sample into two portions.

Send one portion to the laboratory which is to determine the Δ^9 THC content. Keep the other portion for counter-analysis if necessary.

5. Reagents

— petroleum ether (40/65 °), or a solvent of comparable polarity,

— tetrahydrocannabinol (Δ^9 THC), pure for chromatographic purposes,

— solution of 0,1 % (w/v) androstene-3-17-dione in ethanol, pure for chromatographic purposes,.

6. Preparation of test sample

For the purposes of Δ^9 THC determination, retain the upper third of the plants in the portion of sample received. Stems and seeds must be removed from the plant material retained.

Dry the material in an oven, without exceeding 40 °C, to obtain a constant weight.

7. Extraction

Reduce the material obtained as described in point 6 to a semi-fine powder (sieve of 1 000 meshes per cm^2).

Take 2,0 g of well-mixed powder and extract with 30-40 ml petroleum ether (40-65 °C). Leave for 24 hours, then shake in a mechanical shaker for one hour, and then filter. The extraction process is carried out twice under the same conditions. Evaporate the petroleum ether solutions to dryness. Dissolve the residue in 10,0 ml of petroleum ether. The prepared extract is used for quantitative analysis by gas chromatography.

8. **Quantitative analysis by gas chromatography**

(a) *Preparation of assay solutions*

The extraction residue dissolved in 10,0 ml of petroleum ether is subjected to quantitative analysis to determine the Δ^9 THC content. This is performed with the aid of an internal standard and calculation of the peak areas.

Evaporate to dryness 1,0 ml of the petroleum ether solution. Dissolve the residue in 2,0 ml of a solution of 0,1 % androstene-3-17-dione in ethanol (internal standard with a retention time distinctly higher than that of other cannabinoids, and in particular twice that of Δ^9 THC).

calibration ranges

0,10, 0,25, 0,50, 1,0 and 1,5 mg of Δ^9 THC in 1 ml of a solution of 0,1 % androstene-3-17-dione in ethanol.

(b) *Experimental conditions*

Oven temperature	240 °C.
Injector temperature	280 °C.
Detector temperature	270 °C.
Nitrogen flowrate :	25 ml/min,
Hydrogen flowrate :	25 ml/min,
Air flowrate :	300 ml/min,
Volume injected :	1 µl of the final ethanol solution.

The relative retention time of Δ^9 THC is calculated in relation to the andostene.

9. **Expression of the results**

The result is expressed in g of Δ^9 THC per 100 g of the laboratory sample dried to constant weight.

The result is subject to a tolerance of 0,03 g per 100 g.'

Article 2

Regulation (EEC) No 2188/84 is hereby amended as follows :

1. The last subparagraph of Article 2 (2) is replaced by the following :

'The weight of THC in relation to the weight of the sample shall be recorded using the single method valid for the entire Community set out in the Annex to this Regulation'.

2. The following Annex is inserted :

'ANNEX

COMMUNITY METHOD FOR THE QUANTITATIVE DETERMINATION OF Δ^9 THC (TETRAHYDROCANNABINOL) IN HEMP

1. **Purpose and scope**

This method permits quantitative determination of Δ^9 tetrahydrocannabinol (Δ^9 THC) in raw hemp (Cannabis sativa L.) for the purpose of checking that the conditions laid down in Article 2 (1) of Regulation (EEC) No 2059/84 are fulfilled.

2. **Principle**

Quantitative determination of Δ^9 THC by gas chromatography (GC) after extraction with a suitable solvent.

3. **Apparatus**

— gas chromatography equipment with a flame ionization detector

— glass column 2,50 m long and 3,2 mm in diameter (1,8") packed with a suitable support impregnated with 3 % stationary phase phenyl-methyl-silicon (e.g. OV 17 at 3 %).

4. **Sampling and reduction of sample**

Sampling

In a standing crop of a given variety of hemp, take not less than five spot samples each weighing not less than 1 000 g from different parts of the lot for inspection. The pooled samples should be representative of the lot.

Reduction

Reduce the sample, obtained as described, to 1 000 g; the reduced sample should be representative of the original sample. Moist material is dried in air. Divide the reduced sample into two portions.

Send one portion to the laboratory which is to determine the Δ^9 THC content. Keep the other portion for counter-analysis if necessary.

5. **Reagents**

— petroleum ether (40/65 °), or a solvent of comparable polarity,

— tetrahydrocannabinol (Δ^9 THC), pure for chromatographic purposes,

— solution of 0,1 % (w/v) androstene-3-17-dione in ethanol, pure for chromatographic purposes.

6. **Preparation of test sample**

For the purposes of Δ^9 THC determination, retain the upper third of the plants in the portion of sample received. If the third cannot be isolated, retain the entire plants. Stems and seeds must be removed from the plant material retained.

Dry the material in an oven, without exceeding 40 °C, to obtain a constant weight.

7. **Extraction**

Reduce the material obtained as described in point 6 to a semi-fine powder (sieve of 1 000 meshes per cm^2).

Take 2,0 g of well-mixed powder and extract with 30-40 ml of petroleum ether (40-65 °C). Leave for 24 hours, then shake in a mechanical shaker for one hour, and then filter. The extraction process is carried out twice under the same conditions. Evaporate the petroleum ether solution to dryness. Dissolve the residue in 10,0 ml of petroleum ether. The prepared extract is used for quantitative analysis by gas chromatography.

8. **Quantitative analysis by gas chromatography**

(a) *Preparation of assay solutions*

The extraction residue dissolved in 10,0 ml of petroleum ether is subjected to quantitative analysis to determine the Δ^9 THC content. This is performed with the aid of an internal standard and calculation of the peak areas.

Evaporate to dryness 1,0 ml of the petroleum ether solution. Dissolve the residue in 2,0 ml of a solution of 0,1 % androstene-3-17-dione in ethanol (internal standard with a retention time distinctly higher than that of other cannabinoids, and in particular twice that of Δ^9 THC).

Calibration ranges

0,10, 0,25, 0,50, 1,0 and 1,5 mg of Δ^9 THC in 1 ml of a solution of 0,1 % androstene-3, 17-dione in ethanol.

(b) *Experimental conditions*

Oven temperature	240 °C
Injector temperature	280 °C
Detector temperature	270 °C.
Nitrogen flowrate :	25 ml/min
Hydrogen flowrate :	25 ml/min
Air flowrate :	300 ml/min
Volume injected :	1 µl of the final ethanol solution.

The relative retention time of Δ^9 THC is calculated in relation to the andostene.

9. **Expression of the results**

The result is expressed in g of Δ^9 THC per 100 g of the laboratory sample dried to constant weight.

The result is subject to a tolerance of 0,03 g per 100 g.'

Article 3

This Regulation shall enter into force on the third day following its publication in the *Official Journal of the European Communities.*

It shall apply from 1 July 1986.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 25 February 1986.

For the Commission

Frans ANDRIESSEN

Vice-President