ANNEX

## Community method for the quantitative determination of Δ9-tetrahydrocannabinol content in hemp varieties

### 1. Scope and area of application

This method seeks to determine the Δ9-tetrahydrocannabinol (hereinafter referred to as THC) content of varieties of hemp (*Cannabis sativa L.*) As appropriate, the method involves applying procedure A or B herein described.

The method is based on the quantitative determination of Δ9-THC by gas chromatography (GC) after extraction with a suitable solvent.

1.1. *Procedure A*

Procedure A shall be used for checks on production as provided for in Article 32(6) of Regulation (EU) No 1307/2013 and Article 30(g) of this Regulation.

1.2. *Procedure B*

Procedure B shall be used in cases as referred to in Article 32(6) of Regulation (EU) No 1307/2013 and Article 36(6) of this Regulation.

### 2. Sampling

2.1. *Samples*

(a) Procedure A: in a standing crop of a given variety of hemp, a 30 cm part containing at least one female inflorescence of each plant selected shall be taken. Sampling shall be carried out during the period running from 20 days after the start of flowering to 10 days after the end of flowering, during the day, following a systematic pattern to ensure that the sample is representative of the field but excluding the edges of the crop.

Member States may authorise sampling to be carried out during the period from the start of flowering to 20 days after the start of flowering provided that, for each variety grown, other representative samples are taken in accordance with the first subparagraph during the period from 20 days after the start of flowering to 10 days after the end of flowering.

(b) Procedure B: in a standing crop of a given variety of hemp, the upper third of each plant selected shall be taken. Sampling shall be carried out during the 10 days following the end of flowering, during the day, following a systematic pattern to ensure that the sample is representative of the field but excluding the edges of the crop. In the case of dioecious varieties, only female plants shall be taken.

2.2. *Sample size*

Procedure A: the sample shall comprise parts of 50 plants per field.

Procedure B: the sample shall comprise parts of 200 plants per field.

Each sample shall be placed in a fabric or paper bag, without crushing it, and be sent to the laboratory for analysis.

The Member State may provide for a second sample to be collected for counteranalysis, if required, to be kept either by the producer or by the body responsible for the analysis.

2.3. *Drying and storage of the sample*

Drying of the samples shall begin as soon as possible and, in any case, within 48 hours using any method below 70 °C.

Samples shall be dried to a constant weight and to a moisture content of between 8 % and 13 %.

After drying, the samples shall be stored without crushing them at below 25 °C in a dark place.

## 3. Determination of THC content

### 3.1. *Preparation of the test sample*

Stems and seeds over 2 mm in size shall be removed from the dried samples.

The dried samples shall be grinded to obtain a semi-fine powder (passing through a 1 mm mesh sieve).

The powder may be stored for 10 weeks at below 25 °C in a dark, dry place.

### 3.2. *Reagents and extraction solution*

Reagents

— Δ9-tetrahydrocannabinol, pure for chromatographic purposes,

— Squalane, pure for chromatographic purposes, as an internal standard.

Extraction solution

— 35 mg of squalane per 100 ml hexane.

### 3.3. *Extraction of Δ9-THC*

100 mg of the powdered test sample shall be weighed, be placed in a centrifuge tube and 5 ml of extraction solution shall be added containing the internal standard.

The sample shall be placed in an ultrasound bath and be left for 20 minutes. It shall be centrifuged for five minutes at 3 000 r.p.m. and then the supernatant THC solution shall be removed. The solution shall be injected into the chromatograph and a quantitative analysis shall be carried out.

### 3.4. *Gas chromatography*

(a) Apparatus

— gas chromatograph with a flame ionisation detector and a split/splitless injector,

— column allowing good separation of cannabinoids, for example a glass capillary column 25 m long and 0,22 mm in diameter impregnated with a 5 % non-polar phenyl-methyl-siloxane phase.

(b) Calibration ranges

At least three points for procedure A and five points for procedure B, including points 0.04 and 0.50 mg/ml Δ9-THC in extraction solution.

(c) Experimental conditions

The following conditions are given as an example for the column referred to in (a):

— oven temperature 260 °C

— injector temperature 300 °C

— detector temperature 300 °C

(d) Volume injected: 1 µl

## 4. Results

The findings shall be expressed to two decimal places in grams of Δ9-THC per 100 grams of analytical sample dried to constant weight. A tolerance of 0,03 g per 100 g shall apply.

— Procedure A: one determination per test sample.

However, where the result obtained is above the limit laid down in Article 32(6) of Regulation (EU) No 1307/2013, a second determination shall be carried out per analysis sample and the mean value of the two determinations shall be taken as the result.

— Procedure B: the result shall correspond to the mean value of two determinations per test sample.

**Status:**
Point in time view as at 17/07/2014.

**Changes to legislation:**
There are outstanding changes not yet made to Commission Implementing Regulation (EU) No 809/2014. Any changes that have already been made to the legislation appear in the content and are referenced with annotations.