EXPLANATIONS AND METHODS

Explanations covering several characteristics

Characteristics containing the following key in the second column of the Table of Characteristics should be examined as indicated below:

(a) Observations should be done in the period between the beginning of flowering (growth stage 2101, 2201 or 2301, whichever is earliest) and the beginning of seed maturity.

(b) Observations should be done on the last opposite, fully expanded leaves

(c) Observations should be done on the internode below the last opposite leaves of female and/or hermaphrodite plants only.

Explanations for individual characteristics

Ad. 5: Leaf: number of leaflets

Few is less than 7 leaflets.
Medium number of leaflets is 7 (predominant number of leaflets).
Many is more than 7 leaflets.

Ad. 8: Time of male flowering

Monoecious varieties: 50 % of all plants with first male flower open.
Other varieties: 50 % of all male plants with first male flower open.

First male flowers mostly appear from the axils of the leaves on the main stem. Male flowers usually appear about 2 weeks before the styles of female flowers are visible.

Ad. 10: Inflorescence: THC content

The method to determine the THC content is based on a quantitative determination of Δ^9-tetrahydrocannabinol by gas chromatography after extraction with a suitable solvent.

Sampling
The sample (mixture of 20 plants) should be taken from the upper 30 cm of the main stem, containing the female inflorescence. Sampling should be carried out in the period from 20 days after the beginning of female flowering up to the end of flowering. The sample should be dried as soon as possible (within 48 hours) at a temperature below 70º C. Samples should be dried to a constant weight and to a moisture content of 8 – 13 %. After drying samples can be stored (without crushing) at below 25º C in a dark place.

Determination of THC content (see also Cole, 2003).

1. Preparation of the test sample
Remove stems and seeds over 2 mm in size from the dried samples.
Grind the dried samples to obtain a semi-fine powder (passing through a 1 mm mesh sieve).
The powder may be stored for 10 weeks at below 25º C in a dark dry place.

2. Reagents and extraction solution
Reagents
- Δ^9-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. Extraction of Δ^9-tetrahydrocannabinol

Weigh 100 mg of the powdered test sample, place in a centrifuge tube and add 5 ml of extraction solution containing the internal standard.

Place in an ultrasound bath and leave for 20 minutes. Centrifuge for 5 minutes at 3,000 r.p.m. and then remove the supernatant THC solution. Inject the solution into the chromatograph and carry out a quantitative analysis.

4. Gas chromatography

(a) Apparatus

- gas chromatograph with a flame ionization 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 including points 0.04 and 0.50 mg/ml Δ^9-THC in extraction solution.

(c) Experimental conditions

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

oven temperature	260º C
injector temperature	300º C
detector temperature	300º C

(d) Injection volume: 1 µl

Results

THC should be determined to two decimals in grams of Δ^9-THC per 100 grams of analytical sample dried to constant weight. A tolerance of 0.03 g per 100 grams applies. The results are expressed in % dry weight.

Although varietal differences for THC content are consistent, absolute levels of THC content are sensitive to environmental variation. States of expression need to be calibrated by Example varieties.

Ad. 11, 12 and 13: Plant: proportion of hermaphrodite plants, female plants and male plants resp.

Cannabis sativa L. is dioecious by nature, containing approximately equal proportions of male and female plants. Hermaphrodite plants (male and female flowers on one plant) occasionally occur naturally but are specially created by breeding activity (Bócsa, 1998). Several intersexual forms exist and sex expression can be modified by environmental factors.

Hermaphrodite plants:	plants with both male and female flowers
Female plants:	plants with female flowers only
Male plants:	plants with male flowers only

Proportion	Note	Ranges (percentage)
low	1	<= 5 %
low to medium	2	6-35 %
medium	3	36-65 %
medium to high	4	66-95 %
high	5	>= 96 %

Proportion should be based on at least 200 plants for seed propagated varieties and at least 40 plants for vegetatively propagated varieties (numbers are rounded to whole numbers).

Ad. 14: Plant: natural height

Natural height should be observed on female and/or hermaphrodite plants including inflorescence.

Ad. 19: Main stem: pith in cross-section

1
absent or thin

2
medium

3
thick

Ad. 22: Seed: marbling

Marbling of testa: black mosaic patterns

1
weak

2
medium

3
strong