EURLMP-method_009 (version 1)

Determination of Δ⁹-tetrahydrocannabinol and Δ⁹-tetrahydrocannabinolic acid in hemp seed and hemp seed products by LC-MS/MS

Analyte group: Plant toxins - cannabinoids
Analyte(s):
- Δ⁹-Tetrahydrocannabinol (Δ⁹-THC)
- Δ⁹-Tetrahydrocannabinolic acid (Δ⁹-THCA)

Commodity group: Food and feed
Commodities validated: Hemp seed, hemp protein powder/hemp flour and hemp oil

Technique: Liquid Chromatography / Tandem Mass Spectrometry (LC-MS/MS)

Modifications compared to previous version:
Not applicable

Method drafted by:
EU Reference Laboratory for mycotoxins & plant toxins in food and feed (EURLMP)
Wageningen Food Safety Research (WFSR), part of Wageningen University & Research
Akkermaalsbos 2, 6708 WB, Wageningen, the Netherlands
eurl.mycotoxins-planttoxins@wur.nl

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Suggested Citation: EURLMP-method_009 v1, 2022, Determination of Δ⁹-tetrahydrocannabinol and Δ⁹-tetrahydrocannabinolic acid in hemp seed and hemp seed products by LC-MS/MS, EURL mycotoxins and plant toxins, WFSR Wageningen University & Research.
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1 **Introduction**
Delta-nine-tetrahydrocannabinol (Δ⁹-tetrahydrocannabinol, Δ⁹-THC) is derived from the hemp plant *Cannabis sativa*. In the growing and harvested plant and seeds, the precursors, delta-nine-tetrahydrocannabinolic acid (Δ⁹-tetrahydrocannabinolic acid, Δ⁹-THCA) is predominantly present, with Δ⁹-THC itself occurring mostly at low concentrations.

Legal limits are proposed in the EU for THC in food and feed. The maximum levels (MLs) apply the sum of Δ⁹-THC and Δ⁹-THCA (ΣΔ⁹-THC + 0.877 Δ⁹-THCA), expressed as Δ⁹-THC. Maximum limits currently under discussion in food are 3 mg/kg for hemp seed, ground hemp seed, (partially) defatted hemp seeds and other hemp seed derived products and 7.5 mg/kg for hemp seed oil. For feed products, the maximum levels (MLs) for the sum of Δ⁹-THC and Δ⁹-THCA currently considered are 3 mg/kg for hemp seed and hemp expeller, 7.5 mg/kg for hemp seed oil and 20 mg/kg for hemp flour and hemp fibre.

2 **Scope**
This method describes the quantitative determination of Δ⁹-THC and Δ⁹-THCA in hemp seed, hemp seeds oil and hemp seed derived products. The method was developed and validated for individual compounds in the range from 0.5 to 10 mg/kg. Limit of quantification (LOQ) is determined at 0.5 mg/kg for each of the two cannabinoids.

3 **Principle**
The cannabinoids are extracted from homogenised sample material using an acidified QuEChERS method. The samples are slurried with water and extracted with acetonitrile. A salt-induced phase partitioning step is performed by adding magnesium sulphate, sodium chloride and citrate salts, followed by vigorous shaking. An aliquot of the acetonitrile phase is diluted with methanol, filtered, and analysed by ultra-performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS). The compounds are quantified by standard addition of the analytical standards to the final extract.

4 **Reagents**
All reagents and solvents shall be of quality for LC analysis, unless otherwise specified.

4.1 **Analytical standards**
4.1.1 Δ⁹-tetrahydrocannabinol, Δ⁹-THC, e.g. standard solution in ethanol, 100 mg/ml
4.1.2 Δ⁹-tetrahydrocannabinolic acid, Δ⁹-THCA, e.g. standard solution in isopropanol, 1 mg/ml

4.2 **Chemicals**
4.2.1 Formic acid (FA), 99%
4.2.2 Acetonitrile (ACN), LC-MS grade
4.2.3 Ammonium acetate (NH₄Ac), >99%

4.2.4 Ammonium formate (NH₄HCO₂), >97%

4.2.5 Methanol (MeOH), LC-MS grade

4.2.6 QuEChERS-citrate-extraction-mix commercially available in portions of 6.5 g (1.0 g sodium chloride, 4.0 g magnesium sulphate, 1.0 g sodium citrate tribasic dihydrate, 0.5 g sodium citrate dibasic sesquihydrate)

4.2.7 Water Ultra LCMS, ULC grade

4.3 Solutions and reagents

4.3.1 Ammonium formate, 1.0 M
Dissolve 6.2 g of ammonium formate (4.2.4) in water (4.2.7) and make up to 100 ml with water (4.2.7) and mix. The shelf life is six months at room temperature.

4.3.2 Mobile phase A, 5 mM Ammonium formate + 0.1% formic acid in water
Pipette 5.0 ml of 1M ammonium formate solution (4.3.1) and 1.0 ml of formic acid (4.2.1) in a one-litre flask, fill up to the mark with water (4.2.7) and mix. The shelf life is one month at room temperature.

4.3.3 Mobile phase B, 0.1% formic acid in acetonitrile
Pipette 1.0 ml of formic acid (4.2.1) in a one-litre volumetric flask, fill up to mark with acetonitrile (4.2.2) and mix. The shelf life is six months at room temperature.

4.4 Standard solutions

4.4.1 Mixed standard solution MSS1 (100 µg/ml)
Dilute, if necessary, each of the stock solutions Δ⁹-THC (4.1.1) and Δ⁹-THCA (4.1.2) to a final concentration of 1 mg/ml. Subsequently, pipette 200 µl of each of the stock solutions of 1 mg/ml in a test tube (5.1.2). Add 1600 µl of methanol (4.2.5) and mix.

4.4.2 Mixed standard solution MSS2 (10 µg/ml)
Pipette 100 µl of the mixed standard solution MSS1 (4.4.1) in a test tube (5.1.2) and add 900 µl of methanol (4.2.5) and mix.

4.4.3 Mixed standard solution MSS3 (1 µg/ml)
Pipette 100 µl of the mixed standard solution MSS2 (4.4.2) in a test tube (5.1.2) and add 900 µl of methanol (4.2.5) and mix.

4.4.4 Mixed standard solution MSS4 (100 ng/ml)
Pipette 100 µl of the mixed standard solution MSS3 (4.4.3) in a test tube (5.1.2) and add 900 µl of methanol (4.2.5) and mix.
4.4.5 Preparation of the calibration standards (CAL1-9)

The calibration standards are prepared by addition of mixed standard solutions MSS3 (4.4.3) and MMS4 (4.4.4) to methanol (4.2.5) in autosampler vials (5.1.3) as indicated in Table 1. Close the vials (5.1.4) and mix the calibrants (5.1.7).

Table 1: Preparation of calibration standards, CAL1-9

<table>
<thead>
<tr>
<th>Code</th>
<th>Concentration of Δ⁹-THC and Δ⁸-THCA in solvent (ng/ml)*</th>
<th>Concentration in sample (mg/kg)**</th>
<th>Mixed standard solution MSS4 100 ng/ml (4.4.4) (µl)</th>
<th>Mixed standard solution MSS3 1 µg/ml (4.4.3) (µl)</th>
<th>Methanol (4.2.5) (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL1</td>
<td>1</td>
<td>0.11</td>
<td>10</td>
<td>-</td>
<td>990</td>
</tr>
<tr>
<td>CAL2</td>
<td>2.5</td>
<td>0.275</td>
<td>25</td>
<td>-</td>
<td>975</td>
</tr>
<tr>
<td>CAL3</td>
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<td>0.55</td>
<td>50</td>
<td>-</td>
<td>950</td>
</tr>
<tr>
<td>CAL4</td>
<td>10</td>
<td>1.1</td>
<td>100</td>
<td>-</td>
<td>900</td>
</tr>
<tr>
<td>CAL5</td>
<td>25</td>
<td>2.75</td>
<td>-</td>
<td>25</td>
<td>975</td>
</tr>
<tr>
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<td>50</td>
<td>5.5</td>
<td>-</td>
<td>50</td>
<td>950</td>
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<td>CAL7</td>
<td>100</td>
<td>11</td>
<td>-</td>
<td>100</td>
<td>900</td>
</tr>
<tr>
<td>CAL8</td>
<td>250</td>
<td>275</td>
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<td>250</td>
<td>750</td>
</tr>
<tr>
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<td>-</td>
<td>500</td>
<td>500</td>
</tr>
</tbody>
</table>

*concentration for each of the compounds
** Take into account 1 ml of the water phase dissolving in the organic phase after the partitioning step, adding up to a total volume of 11 ml.

5 Materials & equipment

Any reference to type and/or product is only to inform the user and to identify the equipment and does not imply exclusion of similar equipment.

Usual laboratory glassware and equipment, in particular, the following, can be used:

5.1 Materials

5.1.1 Centrifuge tubes, 50 ml, polypropylene, with screw cap

5.1.2 Centrifuge tubes, 10 ml, polypropylene, with screw cap

5.1.3 Mini-Uniprep™ PTFE filter, 0.45 μm, 500 µl

5.1.4 Compressor for filter vials

5.1.5 Laboratory balance, accuracy +/- 0.01 g

5.1.6 Mechanical shaker head-over-head, adjustable

5.1.7 Vortex mixer
5.1.8 Centrifuge

5.1.9 Micropipettes

5.2 LC-MS/MS system with the following components

5.2.1 LC pump, capable of delivering a binary gradient at flow rates appropriate for the analytical column in use with sufficient accuracy

5.2.2 Injection system, capable of injecting an appropriate volume of injection solution with sufficient accuracy, and cross-contamination below 0.1%

5.2.3 Analytical column, capable of retaining the target cannabinoids and capable of baseline separation of the analytes.

5.2.4 Column oven, capable of maintaining a constant temperature of 40 °C

5.2.5 Tandem mass spectrometer (MS/MS), capable of ionisation of the compounds in positive and negative mode, performing Multiple Reaction Monitoring (MRM), with a sufficiently wide dynamic range and capable of unit mass separation and equipped with a computer-based data processing system. Any ionisation source giving sufficient yield may be employed

6 Procedures
This document describes the quantification of Δ⁹-THC and Δ⁹-THCA in hemp seed and hemp seed products. The steps described in this section are presented in the format of a checklist in Annex A.

6.1 Preparation of the test sample
Samples are milled cryogenic to obtain a homogenic sample. The particle size needs to be equal to or less than 0.5 mm or smaller. Samples are stored in a freezer.

6.2 Test portion
The amount of homogenised test sample and oil examined is 1.0 ± 0.1 gram.

6.3 Quality control samples blank QCbl and recovery QCrec (3 mg/kg)
Use as blank sample in this method a matrix which is devoid of the cannabinoids, e.g. ground sesame seeds. Weigh twice a test portion of 1 ± 0.1 gram (5.1.5) in two 50 ml tubes (5.1.1). Use one of these two samples as QC blank sample (QCbl). To estimate recovery, add 30 µl of the mixed standard solution MSS1 (100 µg/ml) (4.4.1) to the sample in the tube to obtain a spike level of 3 mg/kg for each cannabinoid (QCrec). Wait 30 minutes to start the extraction procedure 6.4.
6.4 Extraction, clean-up and standard addition to prepare the test solutions

Weigh a test portion of 1.0 ± 0.1 gram (5.1.5) of each sample into a polypropylene tube of 50 ml (5.1.1). Add 5 ml of water (4.2.7) and mix using a vortex mixer (5.7). Add 10 ml of acetonitrile (4.2.2) and shake vigorously (5.1.7), make sure no dry sample remains on the walls of the tube. Place the tubes during 30 minutes in a rotary tumbling machine (5.1.6) at room temperature. Add the pre-weighed QuEChERS-citrate-extraction-mix (4.2.6) and mix using a vortex mixer (5.1.7). Centrifuge the tubes during 10 minutes at 3,600 rpm (5.1.8).

Pipet 50 µl of the extract (organic phase) in a filter vial (5.1.3) and add 450 µl of methanol (4.2.5) (Sample). To compensate for matrix effects on an individual sample basis, pipet a second portion of 50 µl of the extract to a second filter vial (5.1.3). Add a volume of 25 µl of the mixed standard solution MSS3 (1 µg/ml) (4.4.3) and add 425 µl of methanol (4.2.5) (Sample+add). Final concentration of the standard addition in the extract is 50 ng/ml \( (C_{\text{st.add}}) \), which equals to 5.5 mg/kg. An overview of the scheme is given in Table 2.

<table>
<thead>
<tr>
<th>Table 2: Pipette scheme for extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>1 Sample</td>
</tr>
<tr>
<td>2 Sample+add</td>
</tr>
</tbody>
</table>

7 LC-MS/MS analysis

Chromatographic and Mass Spectrometric conditions may be chosen freely. The optimal measurement conditions strongly depend on the instrumentation used. However, important criteria and parameters with respect to the chromatographic separation and detection of the analytes are:

The chosen column dimensions and chromatographic conditions should be appropriate to obtain base line separation of \( \Delta^9 \)-THC and \( \Delta^9 \)-THCA from compounds with the same mass-to-charge ratio. The injection volume should be optimised for the column dimension and the sensitivity of the mass spectrometric system. The use of large volume injections may result in distorted peak shapes. The chosen mass spectrometric conditions should be appropriate to measure the analytes with sufficient sensitivity and specificity. Preferably, the protonated molecular parent ion should be selected as precursor ion and the product ions should be specific for the compound. Preferably, productions that are formed by the loss of water from the protonated molecular parent ion should not be selected. Select at least two precursor-to-product ion combinations to be included in the multiple reaction monitoring (MRM) method. Each chromatographic peak should be composed of at least 10 data points.

The LC-MS system is conditioned by injecting calibration standard CAL4 (10 ng/ml) (4.4.5) twice, followed by the injection of extraction solvent acetonitrile (4.2.2). It should be verified that the system produces stable analyte retention times and that the sensitivity of the detector is sufficient and stable.

These injections should meet the following criteria:
- Retention times should be stable.
- Sensitivity should be sufficient and fit-for-purpose. Sensitivity is sufficient if \( \Delta^9 \)-THC and \( \Delta^9 \)-THCA can be measured at the reporting limit level.
- Carry-over: The presence of the target compounds in the solvent injection is assessed. If the target compounds are present in the solvent injection, the system should be cleaned before starting the analysis series, since this might lead to a false-positive results.

Example LC-MS/MS conditions and example LC-MS/MS chromatograms are given in Annex B.

7.1 Injection sequence
Analyse the sample extracts in the order as given below.

- Calibration standard CAL4 (4.4.5) at least 2 times
- Extraction solvent acetonitrile (4.2.2)
- Calibration standards CAL1-9 (4.4.5)
- Extraction solvent acetonitrile (4.2.2)
- Quality control blank sample (QCbl 6.3)
- Quality control recovery sample (QCrec 6.3)
- Sample extracts (Sample) (6.4)
- [when required: Sample extracts spiked (Sample+add) (6.4)]
- Calibration standard CAL4 (4.4.5)

Inject calibration standard CAL4 (10 ng/ml) (4.4.5) at least after every 10 sample extracts.

8 Evaluation and calculations
Peak areas are used for all subsequent calculations. For each injection, check peak assignment and integration for all measured transitions and adjust if needed.

8.1 Verification of linearity of LC-MS/MS measurement
The calibration standards CAL1-9 (4.4.5) are used to determine the linearity of the LC-MS/MS system. Plot the response of the quantifier of all individual calibration standards CAL1-9 (4.4.5) against the corresponding concentrations in ng/ml. Construct a calibration curve using (weighted) least-square regression with all individual data points obtained. Linearity has been demonstrated and the calibration curve is fit-for-purpose when the deviation of the back-calculated concentrations of the calibration standards from the true concentrations, using the calibration equation, do not exceed 20%.

8.2 Identification of Δ^9-THC and Δ^9-THCA in the samples
Identify Δ^9-THC and Δ^9-THCA in the samples by comparing retention time and ion ratio with that of the calibration standards CAL1-9 (4.4.5) according to SANTE/11312/2021 [1].

Δ^9-THC and Δ^9-THCA are considered present and identified when:

a) the retention time (RT) of the peak observed for the cannabinoid in the sample extract differs not more than 0.1 min from the average retention time as calculated (Equation I) from the calibration standards CAL1-9 (4.4.5)

b) the relative deviation of the ion ratio (D) is less than 30%. Compare the ion ratio (IR) of the cannabinoids is in the sample extract (IR_{sample}) with the average ion ratio (IR_{avg}) calculated from
the calibration standards CAL1-9 (4.4.5) (=reference ion ratio) by using **Equation II** and **Equation III**.

c) in the blank QC sample (QC<sub>bl</sub>) (6.3), the peak for the quantifier ion at the retention time of the cannabinoid is below 30% of the limit of quantification;

**Equation I**: Deviation of the retention time (ΔRT)

\[
\Delta RT = RT_{sample} - RT_{avg}
\]

where:
- ΔRT is the deviation of the retention time of the analyte in the sample extract (6.4), compared to the average retention time in the calibration standard CAL1-9 (4.4.5) (min)
- RT<sub>sample</sub> is the retention time of the analyte in the sample extract (6.4) (min)
- RT<sub>avg</sub> is the average retention time of the analyte in the calibration standards CAL1-9 (4.4.5) (min)

**Equation II**: Calculation of ion ratio for each cannabinoid in the sample

\[
IR = \left( \frac{A_{low}}{A_{high}} \right) \times 100\%
\]

where:
- IR is the ion ratio (%)  
- A<sub>low</sub> is the area of the product ion with the lowest intensity  
- A<sub>high</sub> is the area of the product ion with the highest intensity

**Equation III**: Relative deviation of the ion ratio (D)

\[
D = \left( \frac{IR_{sample} - IR_{avg}}{IR_{avg}} \right) \times 100\%
\]

where:
- D is the relative deviation of the ion ratio of the analyte in the sample (6.4), compared to the average ion ratio of the analyte in the calibration standards CAL1-9 (4.4.5) (%)
- IR<sub>sample</sub> is the ion ratio of the analyte in the sample (6.4) (%) (**Equation II**)  
- IR<sub>avg</sub> is the average ion ratio of the analyte in the calibration standards CAL1-9 (4.4.5) (%) (**Equation II**)  

*Note: for calculation of the reference ion ratio use only responses with an S/N > 10. For the higher concentrations, exclude peak areas exceeding the linear range from calculation of the reference ion ratio.*
8.3 Quantification of $\Delta^9$-THC and $\Delta^9$-THCA in the samples

8.3.1 Recovery
Calculate the recovery ($R$) for $\Delta^9$-THC and $\Delta^9$-THCA with Equation IV. The recovery should be between 70-120%.

**Equation IV** Recovery for $\Delta^9$-THC or $\Delta^9$-THCA:

$$R = \left( \frac{C_{QCrec-calculated}}{C_{QCrec-spiked}} \right) \times 100\%$$

Where:
- $R$ is the recovery (%)
- $C_{QCrec-calculated}$ is the calculated concentration in the QC$_{rec}$ sample (6.3) using CAL1-9 (4.4.5) in mg/kg
- $C_{QCrec-spiked}$ is the concentration spiked to the QC$_{rec}$ sample in mg/kg (6.3)

8.3.2 Quantification
The concentration of $\Delta^9$-THC and $\Delta^9$-THCA in the sample extracts is calculated from the 1-point standard addition (6.4) using Equation V.

**Equation V**: Calculation of the concentration of each cannabinoid

$$C_{sample} = \frac{A_{sample}}{A_{sample+add} - A_{sample}} \times C_{st.add} \times F \times \frac{V_{es}}{m_s} \times \frac{1}{1000}$$

Where:
- $C_{sample}$ is the concentration of the cannabinoid in the sample in mg/kg
- $A_{sample}$ is the area of the quantifier in the sample extract (Sample) (6.4)
- $A_{sample+add}$ is the area of the quantifier in the sample extract to which standard is added (Sample+add) (6.4)
- $C_{st.add}$ is the concentration of the cannabinoid added to the sample extract in ng/ml (6.4)
- $F$ is the dilution factor
- $V_{es}$ is the volume of the extraction solvent, in ml (*here: 11.0 ml*)
- $m_s$ is the weight of the test portion in g

**Take into account 1 ml of the water phase dissolving in the organic phase after the partitioning step, adding up to a total volume of 11 ml.**

If the calculated concentration exceeds the linear range, the extract should be diluted and reanalyzed.

8.4 Final result
The concentration of $\Delta^9$-THC and $\Delta^9$-THCA in the samples is expressed in mg/kg.

The summed concentration of $\Delta^9$-THC and $\Delta^9$-THCA, according to the proposed EU regulation, should be expressed in mg/kg as the sum of the concentration of $\Delta^9$-THC and $0.877 \times \Delta^9$-THCA.
9 References

Annex A  Checklist for sample preparation of Δ⁹-THC and Δ⁹-THCA in hemp seed and hemp seed food products

Technician: 
Date: 
Lab. journal / page: 

Annex A. Checklist for sample preparation of Δ⁹-THC and Δ⁹-THCA in hemp seed and hemp seed products

A.1 Preparation of calibration standards in solvent (4.4.5)

☐ Mix in an autosampler vial (5.1.3) the calibrant standards (4.4.5) as indicated in table A.1
☐ Close the vials (5.1.4) and mix the content of the vials (5.1.7)

Table A.1: Preparation of calibration standards, CAL1-9

<table>
<thead>
<tr>
<th>Code</th>
<th>Concentration of Δ⁹-THC and Δ⁹-THCA in solvent (ng/ml)*</th>
<th>Concentration in sample (mg/kg)**</th>
<th>Mixed standard solution MSS4 100 ng/ml (4.4.4) (µl)</th>
<th>Mixed standard solution MSS3 1 µg/ml (4.4.3) (µl)</th>
<th>Methanol (4.2.5) (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL1</td>
<td>1</td>
<td>0.11</td>
<td>10</td>
<td>-</td>
<td>990</td>
</tr>
<tr>
<td>CAL2</td>
<td>2.5</td>
<td>0.275</td>
<td>25</td>
<td>-</td>
<td>975</td>
</tr>
<tr>
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<td>5</td>
<td>0.55</td>
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<td>550</td>
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<td>500</td>
<td>500</td>
</tr>
</tbody>
</table>

*concentration for each of the two standards
** Take into account 1 ml of the water phase dissolving in the organic phase after the partitioning step, adding up to a total volume of 11 ml.

A.2 Quality samples for and recovery, QCbl and QCREC (3 mg/kg) (6.3)

☐ Use a matrix which is devoid of the cannabinoids, e.g. ground sesame seeds
☐ Weight 2 portions of 1.0 ± 0.1 grams (5.1.5) into 50 ml PP tubes (5.1.1)
☐ Use the first portion as blank, QCbl (6.3)
☐ Add to the second portion 30 µl of the mixed standard solution MMS1 (100 µg/ml) (4.4.1) (6.3)
☐ Continue with A.3
Annex A. Checklist for sample preparation of Δ⁹-THC and Δ⁹-THCA in hemp seed and hemp seed products

A.3 Extraction, clean-up and standard addition to prepare the test solutions (6.4)

- Weigh a test portion of 1.0 ± 0.1 gram (5.1.5) into a polypropylene tube of 50 ml (5.1.1)
- Add 5 ml of water (4.2.7) and mix using a vortex mixer (5.1.7)
- Add 10 ml of acetonitrile (4.2.2) and shake vigorously (5.1.7), make sure no dry sample remains on the walls of the tube
- Place the tubes in a rotary tumbling machine (5.1.6) and extract for 30 minutes
- Add the pre-weighed QuEChERS-citrate-extraction-mix (4.2.6) and mix for ca. 1 minute using a vortex mixer (5.1.7)
- Centrifuge the tubes at 3,600 rpm for 10 minutes (5.1.8)
- Dilute two portions of the extract in filter vials (5.1.3) according to the scheme in Table A.2
- Close the vial (5.4) and mix the contents of the vial

Table A.2: Pipette scheme for extracts

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Extract (µl)</th>
<th>Methanol (4.2.5) (µl)</th>
<th>Mixed standard solution MSS3 1 µg/ml (4.4.3) (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Sample</td>
<td>50</td>
<td>450</td>
<td>-</td>
</tr>
<tr>
<td>2 Sample+add</td>
<td>50</td>
<td>425</td>
<td>25</td>
</tr>
</tbody>
</table>
Annex B  Example of LC-MS/MS conditions

B.1  LC conditions
The equipment and measuring conditions shown here are provided as an example. Other analytical equipment, columns, mobile phases and gradient conditions may work equally well.

Example conditions

| LC system: | Waters Acquity LCMS (UPLC grade) |
| Analytical column: | Acquity HSS T3 1.8 µm 100 x 2.1 mm |
| Column temperature: | 40 °C |
| Mobile phase solvent A: | 5 mM NH4Fm / 0.1% FA in water (4.3.2) |
| Mobile phase solvent B: | 0.1% FA in acetonitrile (4.3.3) |
| Flow rate: | 0.4 ml/min |
| Injection volume: | 5 µl |
| Injection temperature | 10 °C |
| Gradient program: | Table B.1 |

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (4.3.2) %</th>
<th>Mobile phase B (4.3.3) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>1.0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>6.0</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>9.0</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>9.5</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Table B.1 Gradient for the LC system

See Annex B.3 for an example LC-MS/MS chromatogram.

B.2  MS conditions
The conditions given in Table B.2.1 are guidelines; in practice adjusted settings may be required to obtain an optimal performance of the LC-MS/MS system.

Example for MS conditions

<table>
<thead>
<tr>
<th>Ionisation mode</th>
<th>ESI +/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary voltage</td>
<td>2.50 kV</td>
</tr>
<tr>
<td>Cone voltage</td>
<td>20.0 V</td>
</tr>
<tr>
<td>Source Offset (V)</td>
<td>30 V</td>
</tr>
<tr>
<td>Source temperature</td>
<td>150°C</td>
</tr>
<tr>
<td>Desolvation temperature</td>
<td>600°C</td>
</tr>
<tr>
<td>Cone gas flow</td>
<td>150 L/hr</td>
</tr>
<tr>
<td>Desolvation gas flow</td>
<td>800 L/hr</td>
</tr>
<tr>
<td>Collision Gas Flow (ml/Min)</td>
<td>0.17 (ml/min)</td>
</tr>
<tr>
<td>Nebuliser Gas Flow (Bar)</td>
<td>800 (Bar)</td>
</tr>
</tbody>
</table>

The precursor ions fragment to structurally related products ions. In Table B.2 the theoretical masses of the precursor ion and corresponding product ions are shown. Depending on the instrument, a deviation of ± 0.3 Da is allowed. All transitions shown in Table B.2 are included in the MS method.
installed on the LC-MS/MS. The retention times can differ from column to column and between LC systems. The retention times shown in Table B.2 are therefore indicative.

Table B.2 Example for MS conditions

<table>
<thead>
<tr>
<th>Analyte/ionisation mode</th>
<th>Indicative RT (min)</th>
<th>Precursor ion (m/z)</th>
<th>Cone voltage (V)</th>
<th>Product ion (m/z) (eV)</th>
<th>Collision energy (eV)</th>
<th>Dwell time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>∆⁹-THC (qn) pos</td>
<td>5.80</td>
<td>315.3</td>
<td>20</td>
<td>193.10</td>
<td>20</td>
<td>0.011</td>
</tr>
<tr>
<td>∆⁹-THC (ql) pos</td>
<td>5.80</td>
<td>315.3</td>
<td>20</td>
<td>123.00</td>
<td>30</td>
<td>0.011</td>
</tr>
<tr>
<td>∆⁹-THCA (qn) neg</td>
<td>5.98</td>
<td>356.9</td>
<td>20</td>
<td>313.73</td>
<td>27</td>
<td>0.011</td>
</tr>
<tr>
<td>∆⁹-THCA (ql) neg</td>
<td>5.98</td>
<td>356.9</td>
<td>20</td>
<td>245.17</td>
<td>28</td>
<td>0.011</td>
</tr>
</tbody>
</table>
B.3 LC-MS/MS example chromatogram of Δ⁹-THC and Δ⁹-THCA in hemp seed at 0.5 mg/kg

TOXS_EURL_211109_Cannabinoiden_080

F1: MRM of 11 channels, ES+
315.3 > 193.1
7.300e+005

THC
5.73
30456.38

TOXS_EURL_211109_Cannabinoiden_080

F1: MRM of 11 channels, ES+
315.3 > 123
5.599e+005

THC
5.73
22227.31

TOXS_EURL_211109_Cannabinoiden_080

F2: MRM of 10 channels, ES-
356.9 > 313.73
3.055e+005

THCA
6.02
14534.98*

TOXS_EURL_211109_Cannabinoiden_080

F2: MRM of 10 channels, ES-
356.9 > 245.17
3.447e+005

THCA
6.02
16074.55*