EURLMP-method_009 (version 2)
Determination of cannabinoids in hemp seed and hemp seed products by LC-MS/MS

Analyte group:	**Plant toxins** – cannabinoids
Analyte(s):	Cannabidiol (CBD) Cannabidiolic acid (CBDA) Cannabigerol (CBG) Cannabigerolic acid (CBGA) Cannabinol (CBN) Δ^9-Tetrahydrocannabinol (Δ^9-THC) Δ^9-Tetrahydrocannabinolic acid (Δ^9-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:
In addition to Δ^9-THC and Δ^9-THCA, the method has been updated to include other cannabinoids such as CBD, CBDA, CBG, CBGA, and CBN, along with respective isotope labelled internal standards. The LOQs were lowered.

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Notices:
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Contents

1 Introduction

Delta-nine-tetrahydrocannabinol (Δ^9-tetrahydrocannabinol, Δ^9-THC) is derived from the hemp plant *Cannabis sativa*. In the growing and harvested plant and seeds, the precursors, delta-nine-tetrahydrocannabinolic acid (Δ^9-tetrahydrocannabinolic acid, Δ^9-THCA) is predominantly present, with Δ^9-THC itself occurring mostly at low concentrations. However, the plant contains other relevant cannabinoids like CBD (cannabidiol), CBG (cannabigerol), and CBN (cannabinol). CBD, for instance, has gained attention for its non-psychoactive nature and potential health benefits. Monitoring these cannabinoids is crucial for tailoring treatments, ensuring product safety, and optimizing cultivation practices.

Legal limits are proposed in the EU for THC in food and feed. The maximum levels (MLs) apply to the sum of Δ^9-THC and Δ^9-THCA expressed as Δ^9-THC equivalents (calculated as $\Sigma\Delta^9$-THC + 0.877 Δ^9-THCA, in case of a separate determination and quantification of Δ9-THC and Δ9-THCA). Maximum limits currently 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) currently under the discussion for the sum of Δ^9-THC and Δ^9-THCA 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 CBD, CBDA, CBG, CBGA, CBN, Δ^9-THC and Δ^9-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 0.1 to 10 mg/kg. Limit of quantification (LOQ) is determined at 0.1 mg/kg for each cannabinoid.

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 using solvent standards, and isotope-labeled internal standards are added *via* standard addition 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 Cannabidiol, CBD, e.g. standard solution in methanol, 1 mg/ml

4.1.2 Cannabidiolic acid, CBDA, e.g. standard solution in acetonitrile, 1 mg/ml

4.1.3 Cannabigerol, CBG, e.g. standard solution in methanol, 1 mg/ml

4.1.4 Cannabigerolic acid, CBGA, e.g. standard solution in acetonitrile, 1 mg/ml

4.1.5 Cannabinol, CBN, e.g. standard solution in methanol, 1 mg/ml

4.1.6 Δ^9-tetrahydrocannabinol, Δ^9-THC, e.g. standard solution in methanol, 1 mg/ml

4.1.7 Δ^9-tetrahydrocannabinolic acid, Δ^9-THCA, e.g. standard solution in acetonitrile, 1 mg/ml

4.1.8 Cannabidiol-D3 (CBD-D3), e.g. standard solution in methanol, 100 µg/ml

4.1.9 Cannabidiolic acid-D3 (CBDA-D3), e.g. standard solution in acetonitrile, 100 µg/ml

4.1.10 Cannabigerol-D3 (CBG-D3), e.g. standard solution in methanol, 100 µg/ml

4.1.11 Cannabigerolic acid-D3 (CBGA-D3), e.g. standard solution in acetonitrile, 100 µg/ml

4.1.12 Cannabinol-D3 (CBN-D3), e.g. standard solution in methanol, 100 µg/ml

4.1.13 Δ9-tetrahydrocannabinol-D3, Δ9-THC-D3, e.g. standard solution in methanol, 100 µg/ml

4.1.14 Δ9-tetrahydrocannabinolic acid-D3, Δ9-THCA-D3, e.g. standard solution in acetonitrile, 100 µg/ml

4.2 Chemicals

4.2.1 Formic acid (FA), 99%

4.2.2 Acetonitrile (ACN), LC-MS grade

4.2.3 Ammonium acetate (NH_4Ac), >99%

4.2.4 Ammonium formate (NH_4HCO_2), >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)
Pipette 200 µl of each of the stock solutions of 1 mg/ml (4.1.1-7) in a test tube (5.1.2). Add 600 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 Mixed isotope labelled internal standard solution MILIS (1 µg/ml)
Pipette 100 µl of each of the D3 labelled stock solutions (4.1.8-14) in a test tube (5.1.2). Add 9300 µl methanol (4.2.5) and mix.

4.4.6 Preparation of the calibration standards (CAL1-9)
The calibration standards are prepared by addition of mixed standard solutions MSS3 (4.4.3) ,MMS4 (4.4.4) and MILIS (4.4.5) to methanol (4.2.5) in autosampler vials as indicated in Table 1. Close the vials and mix the calibrants (5.1.7).

Table 1: Preparation of calibration standards, CAL1-9

Code	Concentration of cannabinoids in solvent (ng/ml)*	Concentration in sample (mg/kg)**	Mixed standard solution MSS4 100 ng/ml (4.4.4) (µl)	Mixed standard solution MSS3 1 µg/ml (4.4.3) (µl)	Mixed isotope labelled internal standard solution MILIS 1 µg/ml (4.4.5) (µl)	Methanol (4.2.5) (µl)
CAL1	1	0.055	10	-	100	890
CAL2	2.5	0.138	25	-	100	875
CAL3	5	0.275	50	-	100	850
CAL4	10	0.55	100	-	100	800
CAL5	25	1.38	-	25	100	875
CAL6	50	2.75	-	50	100	850
CAL7	100	5.5	-	100	100	800
CAL8	250	13.8	-	250	100	650
CAL9	500	27.5	-	500	100	400

*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 cannabinoids 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 cryogenically to obtain a homogenous 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 ± 0.05 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 two times a test portion of 1.0 ± 0.05 gram (5.1.5) in two 50 ml tubes (5.1.1). Use one of these two samples as QC blank sample (QC_{bl}). 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 (QC_{rec}). 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 ± 0.05 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 100 µl of the extract (organic phase) in a filter vial (5.1.3), add 50 µl mixed isotope labelled internal standard solution MILISS (1 µg/ml) (4.4.5) and 350 µl of methanol (4.2.5). An overview of the scheme is given in Table 2.

Table 2: Pipette scheme for extracts

	Sample ID	Extract (µl)	Methanol (4.2.5) (µl)	Mixed isotope labelled internal standard solution MILIS 1 µg/ml (4.4.5) (µl)
1	Sample	100	350	50

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 cannabinoids 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 higher 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, product ions 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 and tested by injecting calibration standard CAL4 (10 ng/ml) (4.4.6) at least 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 the cannabinoids can be measured at the validated level within given criteria.
- 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 (4.4.6) at least 2 times
- Extraction solvent acetonitrile (4.2.2)
- Calibration standards CAL1-9 (4.4.6)
- Extraction solvent acetonitrile (4.2.2)
- Quality control blank sample (QC_{bl} 6.3)
- Quality control recovery sample (QC_{rec} 6.3)
- Sample extracts (Sample) (6.4)
- Calibration standard CAL4 (4.4.6)

Inject calibration standard CAL4 (10 ng/ml) (4.4.6) 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 (4.4.6) 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 (4.4.6) 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 cannabinoids in the samples

Identify the cannabinoids in the samples by comparing retention time and ion ratio with that of the calibration standards CAL1-9 (4.4.6) according to Regulation (EU) 2023/2783 [1].

A cannabinoid is 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 (4.4.6)
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 (4.4.6) (=reference ion ratio) by using **Equation II** and **Equation III**.
c) in the blank QC sample (QC_{bl}) (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 (4.4.6) (min)
RT_{sample}	is the retention time of the analyte in the sample extract (6.4) (min)
RT_{avg}	is the average retention time of the analyte in the calibration standards CAL1-9 (4.4.6) (min)

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

$$IR = \left(\frac{A_{low}}{A_{high}}\right) x\ 100\%$$

where:

IR	is the ion ratio (%)
A_{low}	is the area of the product ion with the lowest intensity
A_{high}	is the area of the product ion with the highest intensity

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

$$D = \left(\frac{IR_{sample} - IR_{avg}}{IR_{avg}}\right) x\ 100\%$$

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 (4.4.6) (%)
IR_{sample}	is the ion ratio of the analyte in the sample (6.4) (%) (**Equation II**)
IR_{avg}	is the average ion ratio of the analyte in the calibration standards CAL1-9 (4.4.6) (%) (**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 cannabinoids in the samples

8.3.1 Quantification of cannabinoids in the samples

Quantification of cannabinoids is based on isotope labelled internal standard correction. Calculate the response factor (RF) of the analyte in the sample and in the CAL standards according to Equation IV. Calculate the concentration (C) of the analyte in the sample according to Equation V.

Equation IV: Response factor (RF)

$$RF = \frac{A_x}{A_{IS}}$$

where:

RF	is the response factor
A_x	is the peak area of the product ion of the analyte in the sample
A_{IS}	is the peak area of the product ion of the isotope labelled analogue in the sample

Equation V: Calculation of the concentration of each cannabinoid

$$C_{sample} = \left(\frac{RF_{sample} - b}{a}\right) \times F \times \frac{V_{es}}{m_s} \times \frac{1}{1000}$$

Where:

C_{sample}	is the concentration of the cannabinoid in the sample in mg/kg
RF_{sample}	is the response factor for the analyte in the sample extract (6.4)
a	is the slope of the calibration curve
b	is the intercept of the calibration curve
F	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 re-analyzed.

8.3.2 Recovery

Calculate the recovery (R) for a cannabinoid with **Equation VI**. The recovery should be between 70-120%.

Equation VI: Recovery for cannabinoids

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

Where:

R	is the recovery (%)
$C_{QCrec-calculated}$	is the calculated concentration in the QC_{rec} sample (6.3) using CAL1-9 (4.4.6) in mg/kg
$C_{QCrec-spiked}$	is the concentration spiked to the QC_{rec} sample (6.3) in mg/kg

8.4 Final result

The concentration of cannabinoids in the samples is advised to be expressed in mg/kg.
One should note that according to the EU 915/2023 the maximum level refers to the Δ^9-THC equivalents calculates as a sum of Δ9-THC + 0,877 × Δ9-THCA (in case of a separate determination and quantification of Δ9-THC and Δ9-THCA)

9 References

[1] Regulation (EU) 2023/2783 of 14 December 2023 laying down the methods of sampling and analysis for the control of the levels of plant toxins in food
[2] Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food

Annex A. Checklist for sample preparation of cannabinoids in hemp seed and hemp seed food products

Technician:	
Date:	
Lab. journal / page:	

A.1 Preparation of calibration standards in sovent (4.4.6)

- ☐ Mix in an autosampler vial (5.1.3) the calibrant standards (4.4.6) 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

Code	Concentration of cannabinoids in solvent (ng/ml)*	Concentration in sample (mg/kg)**	Mixed standard solution MSS4 100 ng/ml (4.4.4) (µl)	Mixed standard solution MSS3 1 µg/ml (4.4.3) (µl)	Mixed isotope labelled internal standard solution MILIS 1 µg/ml (4.4.5) (µl)	Methanol (4.2.5) (µl)
CAL1	1	0.055	10	-	100	890
CAL2	2.5	0.138	25	-	100	875
CAL3	5	0.275	50	-	100	850
CAL4	10	0.55	100	-	100	800
CAL5	25	1.38	-	25	100	875
CAL6	50	2.75	-	50	100	850
CAL7	100	5.5	-	100	100	800
CAL8	250	13.8	-	250	100	650
CAL9	500	27.5	-	500	100	400

*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, QC_{bl} and QC_{REC} (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, QC_{bl} (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

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 the sample 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

	Sample ID	Extract (µl)	Methanol (4.2.5) (µl)	Mixed isotope labelled internal standard solution MILIS 1 µg/ml (4.4.5) (µl)
1	Sample	100	350	50

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:	Sciex
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 B.1 Gradient for the LC system

Time (min)	Mobile phase A (4.3.2) %	Mobile phase B (4.3.3) %
0.0	50	50
1.0	50	50
6.0	0.0	100
9.0	0.0	100
9.5	50	50
12	50	50

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.

Table B.2.1 Example for MS conditions

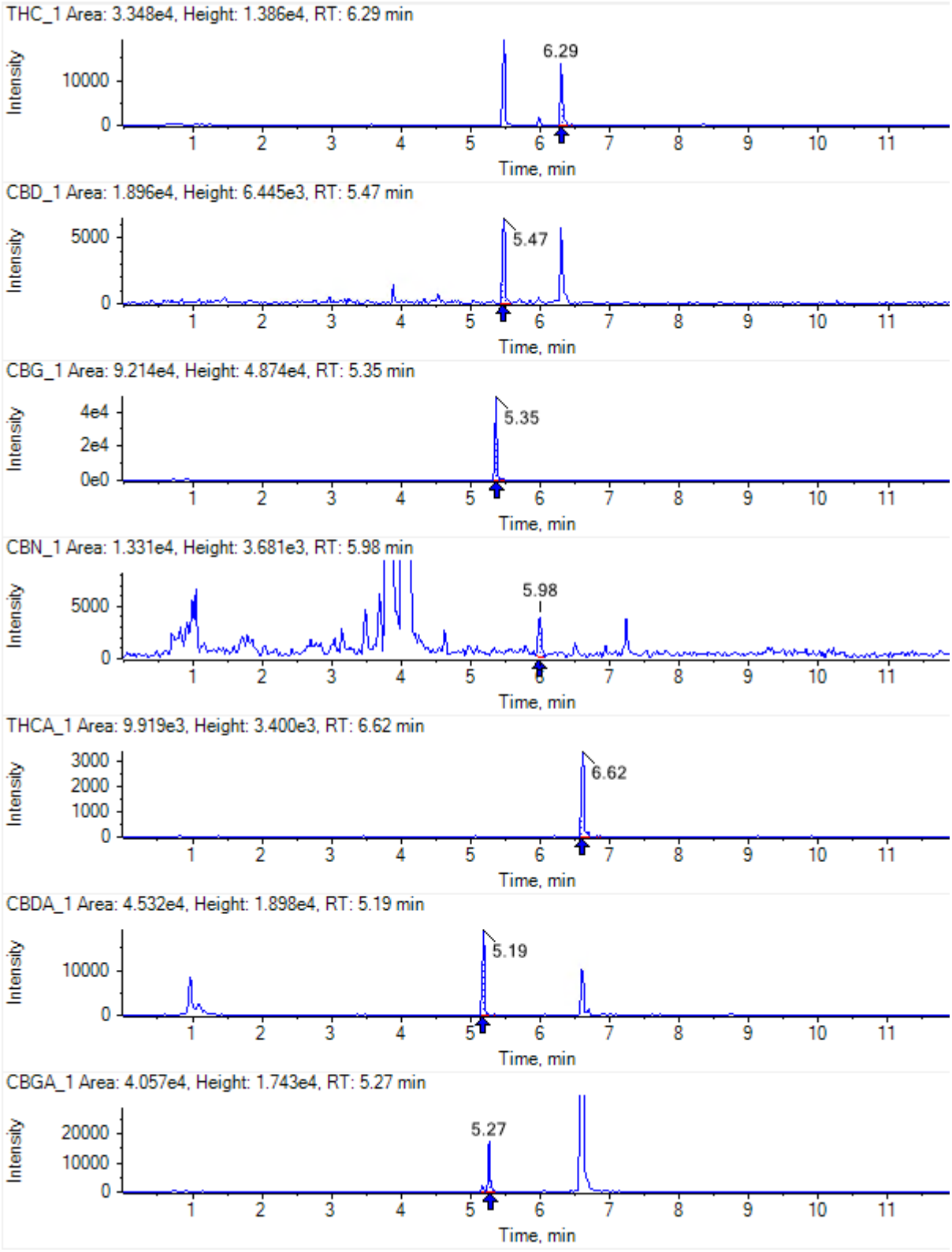
Mass spectrometer	e.g. Sciex QTRAP6500
Ionisation mode	ESI +/-
Ion spray voltage	4000V
Source temperature	400°C
Collision Gas Flow (ml/Min)	0.17 (ml/min)

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

Analyte / ionisation mode		Indicative RT (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	DP (V)	CE (eV)	CXP (V)
CBG (qn)	pos	5.35	317.3	193.2	41	17	22
CBG (ql)	pos	5.35	317.3	123.1	41	43	14
CBG-D3-1	pos	5.35	320.2	196.1	41	17	22
CBD (qn)	pos	5.45	315.3	193.1	26	31	26
CBD (ql)	pos	5.45	315.3	123.0	26	41	12
CBD-D3	pos	5.45	318.3	196.1	26	31	26
CBN (qn)	pos	6.0	311.3	223.3	30	27	6
CBN (ql)	pos	6.0	311.3	208.0	30	43	22
CBN-D3	pos	6.0	314.3	223.1	30	27	6
THC (qn)	pos	6.3	315.3	193.2	60	29	34
THC (ql)	pos	6.3	315.3	123.1	60	39	8
THC-D3	pos	6.3	318.3	196.2	60	29	34
CBDA (qn)	neg	5.15	357.2	339.3	-35	-28	-49
CBDA (ql1)	neg	5.15	357.2	245.2	-35	-40	-27
CBDA-D3	neg	5.15	360.2	248.2	-35	-40	-27
CBGA (qn)	neg	5.25	359.1	341.3	-60	-30	-23
CBGA (ql)	neg	5.25	359.1	315.2	-60	-28	-23
CBGA-D3	neg	5.25	362.1	344.3	-60	-30	-23
THCA (qn)	neg	6.6	357.1	313.2	-5	-32	-23
THCA (ql)	neg	6.6	357.1	245.2	-5	-42	-13
THCA-D3	neg	6.6	360.1	316.2	-5	-27	-35

B.3 LC-MS/MS example chromatogram of cannabinoids spiked at 0.1 mg/kg in sesame seed



B.4 LC-MS/MS example chromatogram of cannabinoids in a standard solution 10 ng/ml corresponding to 0.5 mg/kg