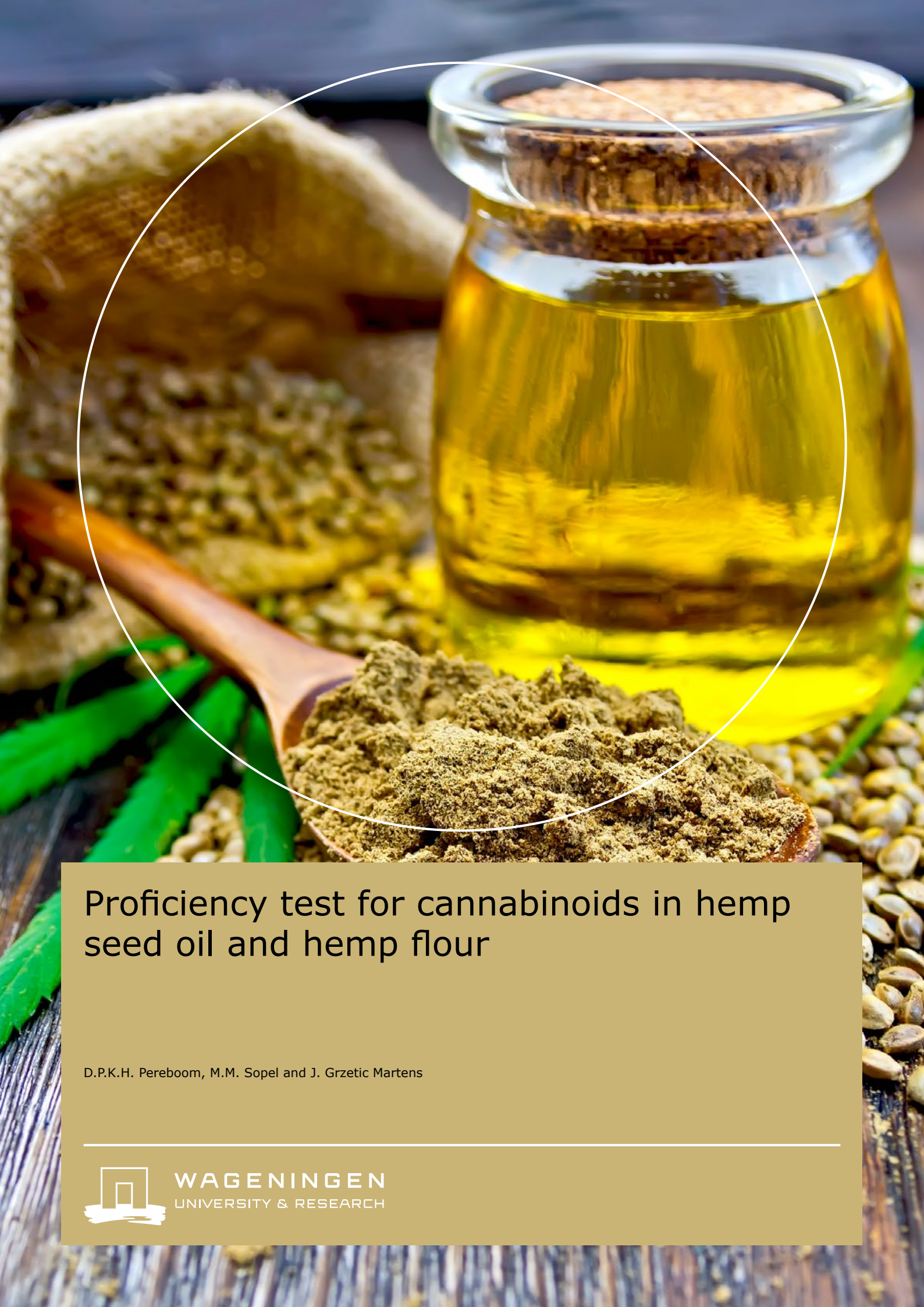

Proficiency test for cannabinoids in hemp seed oil and hemp flour

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This research has been carried out by Wageningen Food Safety Research, institute within the legal entity Wageningen Research Foundation subsidised by the Ministry of Agriculture, Fisheries, Food Security and Nature (project number 1237419201-2024 3.1. EURL MP PT13 Cannabinoids).

Wageningen, 03 February 2025

WFSR Report 2025.004

Pereboom, D.P.K.H., M.M. Sopel, J. Grzetic Martens, 2025. *Proficiency test for cannabinoids in hemp seed oil and hemp flour; EURLPT-MP12.* Wageningen, Wageningen Food Safety Research, WFSR Report 2025.004. 52 pp.; 12 fig.; 8 tab.; 11 ref.

Project number: 1237419201-2024 3.1. EURL MP PT13 THCs
BAS-code: WOT-02-001-064
Project title: EURL MP 2023/2024-101144001 (EURL proficiency test no. 13 on cannabinoids)
Coordinator proficiency tests: D.P.K.H. Pereboom (contact information: pt.wfsr@wur.nl)
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This report can be downloaded for free at https://doi.org/10.18174/684966 or at www.wur.eu/food-safety-research (under WFSR publications).

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WFSR report 2025.004 (final report)

Distribution list:
- Participating laboratories
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Contents

Summary

A proficiency test (PT) for the quantitative determination of cannabinoids Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinolic acid (THCA), cannabidiol (CBD) and cannabidiolic acid (CBDA) in hemp seed oil and hemp flour was organised from March 2024 to June 2024 by Wageningen Food Safety Research (WFSR) under accreditation (R013, Dutch Accreditation Council RvA, ISO/IEC 17043:2023) on behalf of the European Union Reference Laboratory for Mycotoxins & Plant toxins in food and feed (EURL MP).

Cannabinoids mandatory for quantification in this PT were THC and THCA. Additionally, the NRLs were encouraged, on a voluntary basis, to analyse the samples for the non-regulated cannabinoids CBD and CBDA.

The participants were provided with two samples: one hemp seed oil sample (material A), naturally contaminated with THC, THCA, CBD and CBDA, mixed with sunflower oil, and a second sample of hemp flour (material B) where THC, THCA, CBD and CBDA were spiked to the material. All analytes were sufficiently homogeneously divided and stable in the samples.

The primary goal of this PT was to assess the proficiency of the National Reference Laboratories (NRLs) and Official Laboratories (OLs) that participated.

Thirty-nine laboratories, of which 21 National Reference Laboratories (NRLs) for mycotoxins and/or plant toxins in food and feed (from 16 EU Member States, Serbia, Iceland, Norway and Switzerland) and 18 OLs (from five EU Member States and Switzerland) participated in this PT.

In this PT the robust mean was used as consensus value. The proficiency of the participants was assessed as z-scores in both materials, calculated using the consensus values and a relative target standard deviation for proficiency tests of 25%. Characteristics of the PT materials and the outcome of this PT are summarised in Tables 1a and 1b.
Results were calculated for THC, THCA, CBD, CBDA, the sum of THC and THCA expressed as THC + 0.877 THCA (THC eq.) and the sum of CBD and CBDA expressed as CBD + 0.877 CBDA (CBD eq). Maximum score was 4 out of 4 for the individual mandatory cannabinoids and for the sum of the mandatory cannabinoids 2 out of 2. False negative for the sum of THC and THCA in material A was considered as unsatisfactory z-score. Z-scores for the voluntary cannabinoids CBD, CBDA and the sum of these cannabinoids are for information only.

For material A, the consensus values of THC, THCA, CBD and CBDA were 12.4, 24.9, 45.4 and 166 mg/kg, respectively, and for material B 1.57, 3.28, 7.56 and 28.5 mg/kg, respectively. For material A, the interlaboratory reproducibilities (RSD_R) of the reported results were below the target standard deviation (25%), except for THCA (26.5%). For material B, higher RSD_R values were observed for the THCA (33%), THC eq. (33%), the CBDA (40%) and CBD eq. (43%). The RSD_R for THC (24%) and CBD (19%) were below the target standard deviation (25%).

A total of 38 participants out of 39 analysed material A. One participant analysed material A but made a reporting error. 86% of the results for THC, 89% of the results for THCA and 89% of the results for THC eq. were rated to have satisfactory z-scores ($|z| \leq 2$). All participants analysed material B and 79% of the results for both THC and THCA, and 76% for THC eq., were rated to have satisfactory z-scores ($|z| \leq 2$).
Of the 39 participants, 20 (51%) achieved optimal performance for both materials for the mandatory cannabinoids, the absence of false negative results and reporting within the deadline. For the sum of the mandatory cannabinoids, 26 (67%) participants demonstrated satisfactory performance for both materials.

Table 1a *Summary of proficiency materials parameters and participants' performance – number of laboratories reporting quantitative values, <LOQ and false negative (FN) results.*

Cannabinoids (mandatory)	Matrix	Consensus value (mg/kg)	Uncertainty (mg/kg)	Robust RSD_R[1)] (%)	# of labs out of 39 reporting		
					Quant. value	<LOQ	FN
THC	A	12.4	0.531	20.5	36		
	B	1.57	0.080	23.5	33	1	
THCA	A	24.9	1.40	**26.5**	35		
	B	3.28	0.229	**32.6**	34	1	
THC eq.	A	34.7	1.59	22.3	37		
	B	4.43	0.305	**33.1**	36	2	1
Cannabinoids (voluntary)							
CBD	A	45.4	1.92	17.6	27		
	B	7.56	0.336	18.8	28		
CBDA	A	166	5.95	13.5	22		
	B	28.5	2.93	**39.5**	23		
CBD eq.	A	184	7.53	16.3	25		
	B	30.2	3.19	**43.1**	26		

Matrix: A= hemp seed oil, B= hemp flour.

[1]) robust relative standard deviation (interlaboratory RSD based on participants' results).

Table 1b *Summary of proficiency materials parameters and participants' performance – evaluation of results, satisfactory, questionable and unsatisfactory z and z'-scores.*

Cannabinoids (mandatory)	Matrix	Consensus value (mg/kg)	z-scores[1] Satisfactory (%)	Questionable (%)	Unsatisfactory (%)
THC	A	12.4	86	2.8	11
	B	1.57	79	9.1	12
THCA	A	24.9	89	2.9	8.6
	B	3.28	79	5.9	15
THC eq.	A	34.7	89	0.0	11
	B	4.43	76	11	14
Cannabinoids (voluntary)					
CBD	A	45.4	89	0.0	11
	B	7.56	93	0.0	7.1
CBDA	A	166	91	0.0	9.1
	B	28.5	87	0.0	13
CBD eq.	A	184	84	4.0	12
	B	30.2	77	12	12

Matrix: A= hemp seed oil, B= hemp flour.

[1]) calculated using a fit-for-purpose target RSD for proficiency of 25%. False negatives were counted here as unsatisfactory z-score. The sum of % may deviating from 100% due to rounding.

Based on the results of this PT, it can be concluded that most of the participants have an analytical method capable of quantifying THC and THCA with sufficiently low LOQs in hemp seed oil, and that the interlaboratory variability is within the target RSD_R. However, for hemp flour, there was a considerable variation in the reported results. Further efforts are needed to improve the methods to ensure more reliable data.

1 Introduction

This proficiency test was organised under accreditation according to ISO 17043 (Conformity assessment – General requirements for the competence of proficiency testing providers - R013) [1].

The cannabinoids selected for mandatory quantification in this PT were THC and THCA. In addition, the NRLs were encouraged on a voluntary basis to also analyse the samples for the non-regulated cannabinoids CBD) and CBDA.

The EU has established maximum limits (MLs) for the cannabinoids THC and THCA in food - hemp seeds, ground hemp seeds and processed hemp seeds products and hemp seed oil, as described in Commission Regulation (EU) 2023/915 [2]. Currently, there are no EU limits set for other cannabinoids in food, such as CBD and CBDA. However, screening of other cannabinoids may become relevant due to their co-occurrence. For feed, no MLs or indicative values have been established yet, though the discussions are ongoing.

The ML for hemp seeds, ground hemp seeds and processed hemp seed products is set at 3 mg/kg THC eq. For hemp seed oil, the ML is 7.5 mg/kg THC eq. Following the regulation (EU) 2023/2783 [3] the required limit of quantification (LOQ) for individual cannabinoids is set at 0.75 mg/kg for ground hemp seeds, and 1.88 mg/kg for hemp seed oil.

Proficiency testing is conducted to provide participants with a tool to evaluate and demonstrate the reliability of the data that are produced by the laboratory. Proficiency testing is an important requirement and is demanded by ISO/IEC 17025:2017 [4]. Organisation of proficiency tests (PT) is one of the tasks of the European Union Reference Laboratories (EURLs) [5]. Here, the primary goal is to assess the proficiency of NRLs. To facilitate NRLs in their tasks, official laboratories (OLs) can also participate, in consultation with their NRL.

2 PT material

2.1 Scope of the PT

This proficiency test focused on cannabinoids in hemp seed oil and hemp flour as representative matrices for both food and feed. The mandatory cannabinoids for analysis were THC and THCA, while CBD and CBDA were voluntary. The hemp seed oil (material A) was naturally contaminated, while the hemp flour (material B) was spiked to reach target concentrations taking the regulatory limits into account (see Table 2).

Table 2 *Target concentrations mg/kg of the selected cannabinoids spiked to material B, hemp flour.*

	Target concentrations (mg/kg)
Cannabinoids	**Material B (hemp flour)**
THC	1.5
THCA	2.9
Sum THC + 0.877 x THCA	4.0
CBD	7.4
CBDA	33
Sum CBD + 0.877 x CBDA	36

2.2 Material preparation

The hemp seed oil (material A) was naturally contaminated with THC, THCA, CBD and CBDA, and was further diluted with sunflower oil. For the hemp flour (material B), a premix was prepared by spiking a portion of the blank material with THC, THCA, CBD and CBDA, and then mixing it with a larger portion of blank material.

For material A, 1 kg of hemp seed oil was diluted with 1 kg of sunflower oil and stored in a freezer until use. For material B, 200 g of hemp seed oil was dissolved in 500 mL of acetone, targeting the concentrations presented in Table 2. The premix for material B was prepared as follows: 1 kg of blank hemp flour was fortified by adding the diluted hemp seed oil in acetone. A slurry was made by mixing this material further with 300 mL of acetone and homogenised using an industrial mixer according to in-house standard operating procedures [6]. The fortified slurry was air-dried in a fume hood during the night, and subsequently homogenised in a Stephan cutter UMC12. For the final material B, 3 kg of blank hemp flour was mixed with 1 kg of the spiked premix in a rotating drum and stored in a freezer until use. The homogenisation of the final material B was carried out at Wageningen Evaluating Programs for Analytical Laboratories (WEPAL), which is accredited to ISO/IEC 17043 for the preparation of PT materials by the Dutch Accreditation Council (RvA, R002).

2.3 Sample identification

After homogenisation, materials A and B were divided into sub-portions of approximately 20 g and 50 g, respectively. Material A was stored in 50 mL airtight polypropylene tubes, while material B was stored in airtight polypropylene containers. Both materials were stored in the freezer until use.

The samples provided to participants were randomly selected and coded using a web application designed for proficiency tests. The code used was "*2024/EURL PT MP/cannabinoids/xxx*", in which the three-digit number of the code was automatically generated by the WFSR Laboratory Quality Services web application. One sample set was prepared for each participant, consisting of one randomly selected sample of each material. The codes of the samples set are shown in Annex 2. Samples for homogeneity and stability testing were also randomly selected from materials A and B.

2.4 Homogeneity study

To verify the homogeneity of the PT materials, the contents of 10 tubes of material A and the content of 10 containers of material B were analysed in duplicate.

Method in brief: The cannabinoids were extracted from the homogenised material after addition of water by shaking with acetonitrile. Following a salt-induced phase partitioning step with magnesium sulphate, sodium chloride, and citrate salts, and subsequent centrifugation, an aliquot of the acetonitrile phase was diluted with methanol and filtered. The analysis was performed by high performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS).

The homogeneity of both materials was evaluated according to the International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [6] and ISO 13528 [7]. The between-sample standard deviation (s_s) and within-sample standard deviation (s_w) were compared with the standard deviation for proficiency assessment (σ_P). The method applied for homogeneity testing is considered suitable if $s_w < 0.5 * \sigma_P$, and a material is considered adequately homogeneous if $s_s < 0.3 * \sigma_P$. Both materials proved to be sufficiently homogeneous for this PT since the cannabinoids in the materials fulfilled the homogeneity requirements.

The results of the homogeneity study (grand means with the corresponding RSD_r) are presented in Table 3. The statistical evaluation is presented in Annex 3.

Table 3 *Concentrations of cannabinoids in materials A and B obtained during homogeneity testing.*

Compound	Material A: hemp seed oil		Material B: hemp flour	
	Conc. (mg/kg)	RSD_r (%)	Conc. (mg/kg)	RSD_r (%)
THC	11.4	1.91	1.43	3.49
THCA	17.1	2.42	2.80	2.42
CBD	47.9	3.87	7.87	3.36
CBDA	193	2.87	32.6	2.97

2.5 Stability of the materials

The stability of the cannabinoids in the PT materials was assessed [8,7]. On April 22nd, 2024, the day of distribution of the PT samples, six randomly selected tubes of material A and six randomly selected containers of material B were stored in an ultra-freezer (<-70°C). Under these conditions it is assumed that the cannabinoids are stable in the materials. Additionally, six samples of each material were stored in a freezer.

On July 3rd, 2024, 72 days after the samples were distributed, six samples of materials A and B stored in the ultra-freezer and freezer, were analysed in one batch. For each set of test samples, the average of the results and the standard deviation were calculated.

The potential instability of the analytes in the materials stored in the freezer was assessed [8,7]. Consequential instability is observed when the average concentration of an analyte in the freezer stored samples is more than $0.3\sigma_P$ below the average concentration of the analyte in the ultra-freezer stored samples.

The results of the stability testing for materials A and B are presented in Annex 4. For all analytes in both materials, none of the tested storage conditions resulted in consequential instability. Therefore, the cannabinoids in the materials were considered stable for the duration of the PT.

3 Organisational details

3.1 Participants

Invitations to the NRL network were sent on March 27th, 2024 (Annex 5). Forty laboratories registered for the PT and 39 participants (Annex 1) reported their results, although two reported after the deadline. One participant was unable to report results due to maintenance of the laboratory power systems.

Of the 39 participating laboratories, 21 were NRLs from 16 EU Member States, Serbia, Iceland, Norway and Switzerland. The remaining 18 were OLs from five EU Member States and Switzerland. Each participant was free to use their method of choice, reflecting their routine procedures. Participants were asked to report their results through a web application and complete a questionnaire, providing detailed information on the analytical methods used.

3.2 Material distribution and instructions

Each participant and sample received a randomly assigned code, generated by a web application designed for proficiency tests. The sample sets, consisting of two coded samples (Annex 2) were sent on dry ice to the participants on April 22nd, 2024. The participants were asked to store the samples in the freezer and to analyse the samples according to their routine method. As reported by participants, all samples were received in good order.

The samples were accompanied by a PT instruction letter detailing the required analysis (Annex 6) and an acknowledgement of receipt form. In addition, each participant received instructions by e-mail on how to use the web application to report the results. Details regarding the analytical methodology employed by participants for the quantification of cannabinoids were requested via a questionnaire. This included information on the sample processing, separation and detection technique, calibration strategies as well as the specified limits of quantification (LOQs) and recovery data.

The deadline for submitting the quantitative results was June 17th, 2024, allowing the participants 8 weeks for analysis of the test samples. With the exception of two participants, all results were submitted within the deadline.

4 Evaluation of results

The statistical evaluation was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [8], elaborated by ISO, IUPAC and AOAC and ISO 13528 [7] in combination with the insights published by the Analytical Methods Committee [9,10] regarding robust statistics.

The evaluation of results was based on consensus values and the standard deviation for proficiency assessment (σ_P). From this, z-scores were calculated to classify the participants' performance. Detailed information on the methods used for the statistical evaluation can be found in the background document 'EURL-MP-background doc_001 (v1.1). Performance assessment in proficiency tests organised by the EURL mycotoxins & plant toxins in food and feed' which is available from the EURL mycotoxins & plant toxins website [11].

4.1 Calculation of the consensus value (C)

The consensus value was determined using robust statistics [7 (Algorithm A),9,10] and was calculated based on the results provided by participating NRLs and OLs and was used as the consensus value. The values and their uncertainties are summarised in Table 1 in the Summary section. The sum, expressed as Δ^9-THC eq., refers to the sum of Δ^9-THC + 0.877 x Δ^9-THCA. The same applies to the sum of CBD and CBDA (CBD eq.).

The robust mean of the reported results of all participants, calculated from an iterative process that starts at the median of the reported results using a cut-off value depending on the number of results, was used as the consensus value [7,9].

4.2 Standard deviation for proficiency assessment (σ_P)

A fixed relative target standard deviation of 25% was used for proficiency assessment, irrespective of the cannabinoid, matrix or concentration. This generic fit-for-purpose value is considered to reflect current analytical capabilities and best practises for plant toxin determination in food and feed. The rationale behind this decision is provided in the background document 'EURL-MP PT performance assessment', available on the EURL-MP website [11].

4.3 Quantitative performance (z-scores)

For the evaluation of results, z-scores are calculated based on the consensus value, its uncertainty, and the standard deviation for proficiency assessment. When the uncertainty of the consensus value is negligible and no instability of the analytes in the material is observed, z-scores are calculated as follows:

$$z = \frac{x - C}{\sigma_p}$$ Equation 1

where:

z = z-score;
x = the result of the laboratory;
C = consensus value;
σ_P = standard deviation for proficiency assessment.

The z-score compares each participants' deviation from the consensus value, taking into account the target standard deviation accepted for the proficiency test. The performance of the laboratory is interpreted based on the z-score, as indicated in Table 4.

Table 4 *Classification of z-scores.*

$\|z\| \leq 2$	Satisfactory
$2 < \|z\| < 3$	Questionable
$\|z\| \geq 3$	Unsatisfactory

If the uncertainty of the consensus value, or any instability of the analyte in the PT material, is not negligible, this is taken into account in the determination of the z-score. In such cases, a z'-, z_i-, or z_i'-score is assigned. For further details, please refer to the background document 'EURL-MP PT performance assessment' on the EURL-MP website [11].

In this PT, the uncertainty of the consensus values for CBDA and the sum of CBD and CBDA in hemp flour were not negligible and this was taken into account in the assignment of the z-scores (z'). In all the other cases, the uncertainty of the consensus value was negligible. No instability of the analytes in the PT material was observed during the PT period.

4.4 Evaluation of non-quantified results

Reported non-quantified results, such as those labelled as 'detected', 'not detected' or '< LOQ', without a specified LOQ, were excluded from the evaluation. In these cases, the participant was considered to have no quantitative method available for the specific analyte or analyte group/matrix.

A proxy-z-score was calculated using equations IV and V from the background document 'EURL-MP-background doc_001' (for details, see the EURL-MP website), using the reported LOQ value as the result. Proxy-z-scores are for informational purposes only and are indicated as values between brackets. They are not included in the evaluation of results and do not count as satisfactory results. Proxy-z-score values [z<-2] were considered as false negatives (see 2.5).

4.5 False positive and false negative results

A false positive is a quantitative result reported by the participant while the analyte is not detected in the PT material by the organiser, and/or not detected by most of the other participants. A threshold may apply, below which results are not considered false positives, i.e. when the analyte concentration is below the LOQ of the organiser and/or the majority of the participants. False positives are to be interpreted as unsatisfactory performance.

When an analyte is present in the material, i.e. an consensus value has been established, and the participant reports the analyte as '<[value]', an assessment is made to determine whether such results should be classified as a false negative. This classification occurs when the 'proxy-z-score', calculated using the reported LOQ-value as the result, is <-2. False negatives are indicated as 'FN' and are also interpreted as unsatisfactory performance.

5 Performance assessment

5.1 Scope and LOQ

Annex 7 summarises the quantitative scope of each participant, with an indication of the LOQ for each cannabinoid.

Thirty-eight participants analysed both samples. The results for the two mandatory cannabinoids were reported by all participants, except PT7713, which didn't analyse THCA in either material. One participant did not quantify THC and THCA in material B and reported results as <LOQ for the sum.
Seven participants did not measure any cannabinoids from the voluntary scope. Five participants reported only CBD, of which two reported also the CBD eq. One participant reported only CBDA for material A.
Twenty-four participants included all cannabinoids in the scope of their analyses. PT7723 analysed both materials but made a reporting error for material A. Two participants reported only the cannabinoids equivalents. This artifact results from using a GC-based method, which does not allow for chromatographic separation of cannabinol and its respective acid.

The data of the LOQs provided by the participants were evaluated, reflecting on the new requirements outlined in EU Regulation 2023/2783 [3]. Based on the required LOQ levels and simplifying the evaluation process by disregarding the ratio within the sum of THC and THCA as the legal limit of THC equivalents, we established a value of 0.75 mg/kg for hemp flour and 1.88 mg/kg for hemp oil as benchmarks against which all reported LOQs were compared. Additionally, we derived preferable LOQ levels of 0.3 mg/kg for flour materials and 0.75 mg/kg for oil, calculated as ML/number of analytes * 5. The results of this exercise are summarized in Table 5.

Table 5 *Evaluation of participants reported LOQs for THC and THCA reflecting the requirements listed in EU 2023/2783 [3].*

OIL	LOQs Material A - Oil				FLOUR	LOQs Material B - Flour			
	THC		THCA			THC		THCA	
	participants numbers/%					participants numbers/%			
Not reported	3	9%	4	11%	Not reported	3	9%	4	11%
LOQ ≤ 1.88	**28**	**80%**	**27**	**77%**	**LOQ ≤ 0.75**	**24**	**69%**	**24**	**69%**
LOQ > 1.88	4	11%	4	11%	LOQ > 0.75	8	23%	7	20%
LOQ < 0.75	23	66%	23	66%	LOQ < 0.3	19	54%	19	54%

In conclusion, for mandatory cannabinoids, most participants reported LOQs that align with EU 2023/2783 [3]. However, some laboratories, when focusing on analysing food, need to lower the LOQ of their method for THC and THCA in hemp seed oil and hemp seeds to comply with this regulation.

5.2 Analytical methods

All participating laboratories were asked to complete a questionnaire regarding their accreditation, sample preparation, chromatographic separation, detection, quantification and calibration (Annex 8). Four participants did not provide information about their methods.

Of the 39 laboratories, 22 reported that their analytical methods were according to ISO 17025 accreditation, with most methods being developed in-house.

For hemp seed oil, the most often used sample size was 1 g (13), while 16 participants used 1 g for hemp flour. For the oil, 16 participants used 0.5 g or less, and 3 used 2 g or more. For hemp flour, 6 participants used 0.5 g or less, while 10 participants used 2 g or more.

The extraction solvents used for extraction of hemp oil were methanol or acetonitrile, each used by 8 participants. For hemp flour, methanol was used by 13 participants and acetonitrile by 7 participants. Other solvents included were acetone, ethanol, and iso-propanol, with acetonitrile and water being the most common combination (used by 6 participants). Extraction methods varied, including mechanical shaking, vortex mixing, and ultrasonic baths, with times ranging from 5 to 180 minutes. The most common extraction time was 30 minutes, reported by 15 participants.
For the identification and quantification of cannabinoids, most participants (28) used LC-MS/MS, GC-MS single (1), GC-MS/MS (1) and LC-HRMS (High Resolution Mass Spectrometry) (1). Sample extract purification methods included dilution, filtration or liquid-liquid partitioning (such as QuEChERS). Four participants applied a diode array detector (DAD) for identification and quantification.

Among the participants employing LC-MS/MS methods, 77% used isotope-labelled standards for quantification. These standards were added at different stages: 63% added them to the final extract, 21% before extraction, and 17% before clean-up. Quantification was most commonly performed using standards prepared in solvents (73%). Other LC-MS/MS methods used matrix-matched standards or standard addition after extraction for quantification. For LC-fluorescence based methods, quantification was done using solvent standards.

5.3 Performance

The quantitative performance was assessed through z-scores when 7 or more results were submitted by the participants and when uncertainty did not exceed $0.7\sigma_p$. The individual z-scores obtained by each participant, including their graphical representation, for the cannabinoids in materials A (hemp seed oil) and B (hemp flour) are summarised in Annex 9 and 10, respectively. A summary of the performance of the participants in this PT is provided in Annex 11.

The statistical evaluation of the PT results is presented in Tables 6 and 7, which include all relevant parameters: the consensus value (C), the uncertainty of the consensus value (u), the standard deviation for proficiency assessment (σ_p), and the robust (relative) standard deviation based on participants' results. In case the uncertainty of the consensus value exceeded the criterion $u \leq 0.3\sigma_p$, the uncertainty was taken into account in the evaluation by calculating the z'-score. Mostly, the uncertainty of the consensus value did comply with the criterion $u \leq 0.3\sigma_p$ and was therefore considered as negligible. Uncertainty of the consensus value (u) in the material B exceeded $0.3\sigma_p$ for CBDA and the CBD eq., and therefore, the uncertainty of the consensus value was taken into account by calculating the z'-scores.

Table 6 *Summary of statistical evaluation of the PT results on cannabinoids in material A (hemp seed oil).*

	THC	THCA	Sum THC + 0.877 THCA	CBD	CBDA	Sum CBD + 0.877 CBDA
C (mg/kg)	12.4	24.9	34.7	45.4	166	184
u (mg/kg)	0.531	1.40	1.59	1.92	5.95	7.53
σ_p (mg/kg) (25%)	3.10	6.23	8.67	11.3	41.4	46.1
$u>0.3\sigma_p$	No	No	No	No	No	No
robust σ (mg/kg)	2.55	6.61	7.73	8.00	22.3	30.1
robust σ (%)	20.5	26.5	22.3	17.6	13.5	16.3
# reported	36	35	37	28	24	26
"<", nd, detected, positive				1	2	1
# quantitative results	36	35	37	27	22	25
$\|z\|\leq 2$	31	31	33	24	20	21
$2<\|z\|<3$	1	1				1
$\|z\|\geq 3$	4	3	4	3	2	3
FN						
S z-scores (%)	86	89	89	89	91	84

S z-scores = satisfactory z-scores.

FN= False negative.

nd= not detected.

Table 7 *Summary of statistical evaluation of the PT results on cannabinoids in material B (hemp flour).*

	THC	THCA	Sum THC + 0.877 THCA	CBD	CBDA	Sum CBD + 0.877 CBDA
C (mg/kg)	1.57	3.28	4.43	7.56	28.5	30.2
u (mg/kg)	0.080	0.229	0.305	0.336	2.93	3.19
σ_p (mg/kg) (25%)	0.391	0.819	1.11	1.89	7.13	7.56
$u>0.3\sigma_p$	No	No	No	No	Yes	Yes
robust σ (mg/kg)	0.368	1.07	1.46	1.42	11.3	13.0
robust σ (%)	23.5	32.6	33.1	18.8	39.5	43.1
# reported	36	35	38	29	24	27
"<", nd, positive	3	1	2	1	1	1
# quantitative results	33	34	36	28	23	26
$\|z\|\leq 2$	26	27	28	26	20	20
$2<\|z\|<3$	3	2	4			3
$\|z\|\geq 3$	4	5	4	2	3	3
FN			1			
S z-scores (%)	79	79	76	93	87	77

S z-scores = satisfactory z-scores.

FN= False negative.

nd= not detected.

In material A, the percentages of satisfactory z-scores for THC and THCA were 86% and 89%, respectively. For the voluntary cannabinoids CBD and CBDA, the corresponding percentages were 89% and 91%. For material B, the percentages of satisfactory z-scores for THC and THCA were 79% in both cases. For CBD and CBDA, the percentages were 93% and 87%, respectively.

For the THC eq. in material A, 89% of the results were rated with satisfactory z-scores. In material B, this was 76%. For the CBD eq., 84% of the results in material A were satisfactory and for material B this was 77%.

An overview of the overall performance for each participant in this PT is provided in Annex 11. For both materials combined, a maximum of four satisfactory z-scores, based on the quantitative results of the individual mandatory cannabinoids, could be obtained. A '4 out of 4' score reflects optimal performance in terms of scope and capability for quantitative determination. Of the 39 participants, 20 achieved optimal

performance by correctly quantifying the individual cannabinoids, the absence of false negative (FN) results and reporting within the deadline. One participant achieved also satisfactory results for THC and THCA in both materials but reported after the deadline. For the remaining 18 participants, false negative results were reported, or one or more non-satisfactory z-score was obtained. With respect to the THC eq., 26 participants performed satisfactory, with one participant reporting satisfactory results but submitting after the deadline. In total, one FN result was reported for the sum THC eq. in material B.

5.4 Robust relative standard deviation

The robust relative standard deviation (RSD_R) was calculated according to ISO13528 [7] for informative purpose and used as an estimate of the interlaboratory variability in this study. The RSD_R values are presented in Table 1 (Summary section), Tables 6 and 7 (Section 5.3), in Annex 9 and 10, and Table 1 (Summary section).

For hemp seed oil, the RSD_R of the reported results was below or around the target standard deviation for proficiency (25%). For THC and THCA the RSD_R were 21 and 27%, respectively; for the THC eq. it was 22%. The RSD_R values of the voluntary cannabinoids CBD, CBDA and the sum of both were 18%, 14%, and 16%, respectively, and hence all below the target standard deviation (25%).

For the hemp flour, high RSD_Rs were observed for the THCA (33%), the THC eq. (33%), the CBDA (40%), and the CBD eq. (43%). However, the RSD_R for the THC (24%) and the CBD (19%) were below the target standard deviation of 25%. The increased variability observed for hemp flour samples, as indicated by higher RSD_R values, is likely attributable to the lower concentrations of cannabinoids present in the material compared to hemp seed oil. Despite this, the limit of quantification (LOQ) for cannabinoid analysis in most methods used by participants is 1 mg/kg or lower. This suggests that there is room for improvement in the analytical methods employed for hemp flour to enhance the consistency and accuracy of results.

6 Conclusions

Thirty-nine laboratories participated in the EURLPT-MP-12 on the quantitative determination selected cannabinoids in hemp seed oil and hemp flour. Of these laboratories, 21 were NRLs for mycotoxins and/or plant toxins in food and feed, representing 16 EU Member States and Serbia, Iceland, Norway and Switzerland. The remaining 18 were OLs from five EU Member States and Switzerland.

LC-MS/MS based methods were mostly used, though LC-MS single, LC-HRMS and diode array detector (DAD) methods were also employed for identification and quantification.

For both hemp materials, most of the participants reported LOQs that are in line with the EU 2023/2783 regulation. However, some laboratories, if focusing on analysing food, need to lower LOQs of their methods for THC and THCA in hemp seed oil and hemp seeds to comply with this regulation.

For the mandatory cannabinoids THC and THCA satisfactory results were reported by most of the participants (79 – 89%). A total of 20 (51%) participants achieved optimal performance by detecting these mandatory cannabinoids with correct quantification, the absence of false negative results and reporting within the deadline in both of the materials. With respect to the sum of the mandatory cannabinoids, for material A and B, respectively, 89% and 76% of the results were satisfactory. For the voluntary cannabinoids CBD and CBDA, 87 – 93% of participants reported satisfactory results, with 77 – 84% achieving satisfactory performance for the sum of these cannabinoids.

High variation in the reported results for THCA and the voluntary CBDA in hemp flour led to relatively high RSD_R values (33 - 40%). This variation may be related to the lower concentrations of these cannabinoids in hemp flour (3.28 mg/kg for THCA and 28.5 mg/kg for CBDA). This implies that improved performance of quantification methods is needed, especially for THCA (and CBDA) in hemp flour.

References

[1] ISO/IEC 17043:2023. Conformity assessment - General requirements for the competence of proficiency testing providers.

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[3] Commission Implementing 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 and repealing Regulation (EU) 2015/705 (europa.eu).

[4] ISO/IEC 17025:2017(E). 2017. General requirements for the competence of testing and calibration laboratories.

[5] Commission Regulation (EU) 2017/625 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, Art. 94.2. Official Journal of the European Union 7.4.2017, L95, 1-142.

[6] WFSR SOP-A-0989 – Preparation of PT materials and PT samples.

[7] ISO 13528:2022. Statistical methods for use in proficiency testing by inter-laboratory comparison, 1st edition.

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[9] Analytical Methods Committee. 1989. Robust statistics - How not to reject outliers Part 1. Basic concepts. Analyst 114:1693-1697.

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Annex 1 List of participants

Country	Organisation
AUSTRIA*	AGES GmbH
BELGIUM*	CER Groupe
CROATIA*	A. Stampar Teaching Institute of Public Health
CYPRUS*	State General Laboratory
CZECH REPUBLIC*	Czech Agriculture and Food Inspection Authority (CAFIA)
DENMARK*	Danish Veterinary and Food Administration
ESTONIA***	Estonian Forensic Science Institute
FINLAND*	Finnish Food Authority
FRANCE***	Inovalys
FRANCE*	Laboratoire SCL de Strasbourg
GERMANY***	Institut fur Hygiene und Umwelt
GERMANY***	Landesuntersuchungsanstalt Sachsen
GERMANY***	Bayerisches Landesamt fur Gesundheit und Lebensmittelsicherheit
GERMANY***	Chemisches und Veterinaruntersuchungsamt Munsterland-Emscher-Lippe (CVUA-MEL)
GERMANY**	Eurofins WEJ Contaminants
GERMANY***	Landeslabor Berlin-Brandenburg
GERMANY***	Landesamt fur Landwirtschaft, Lebensmittelsicherheit und Fischerei - MV
GERMANY***	CVUA Rhein Ruhr Wupper
GERMANY***	LUFA Speyer
GERMANY***	CVUA Karlsruhe
GERMANY*	Lower Saxony State Office for Consumer Protection and Food Safety (LAVES)
GERMANY*	Federal Institute fur Risk Assessment (BfR)
HUNGARY*	National Food Chain Safety Office
IRELAND*	The State Laboratory
IRELAND*	The Public Analyst's Laboratory
ITALY***	Agenzia Regionale per la Protezione dell'Ambiente Ligure
ITALY***	Istituto Zooprofilattico delle Venezie
ITALY*	IZSLER
LITHUANIA*	National Food and Veterinary Risk Assessment Institute
NORWAY**	Norwegian Institute of Bioeconomy Research (NIBIO)
POLAND*	National Veterinary Research Institute
SERBIA**	SP Laboratorija A.D.
SLOVENIA*	National Laboratory of Health, Environment and Food (NLZOH)
SPAIN***	Madrid Salud, Ayuntamiento de Madrid
SPAIN*	Spanish Agency for consumer affairs, Food Safety and Nutrition
SWITZERLAND***	Amt fur Lebensmittelsicherheit und Tiergesundheit Graubunden
SWITZERLAND***	Service de la consommation et des affaires veterinaires (SCAV)
SWITZERLAND***	Lebensmittelkontrolle Solothurn
SWITZERLAND**	Kantonales Laboratorium Bern

* National Reference Laboratory (NRL) of EU Member State.

** National Reference Laboratory (NRL) of the European Free Trade Association (Eurofins WEJ Contaminants = Iceland) and Serbia.

*** Official Laboratory (OL).

Annex 2 Coding of the samples

Participant's codes	Material A*	Material B*
PT7708	888	765
PT7709	309	203
PT7710	909	475
PT7711	476	218
PT7712	435	285
PT7713	811	728
PT7714	933	459
PT7715	336	452
PT7716	133	586
PT7717	643	240
PT7718	492	955
PT7719	977	208
PT7720	748	582
PT7721	778	333
PT7722	324	903
PT7723	640	297
PT7724	602	625
PT7725	836	278
PT7726	667	341
PT7727	470	379
PT7728	131	308
PT7729	207	830
PT7730	105	174
PT7731	256	373
PT7732	770	719
PT7733	412	901
PT7734	239	302
PT7735	433	876
PT7736	935	609
PT7737	437	848
PT7738	315	990
PT7739	185	562
PT7740	799	805
PT7741	831	823
PT7742	592	769
PT7743	276	528
PT7744	833	162
PT7745	358	289
PT7747	804	653

* All sample codes start with 2024/EURL PT MP/cannabinoids/.

Annex 3 Statistical evaluation of homogeneity data

	THC in material A (mg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	11.3	11.7
Hom/A002	11.8	11.2
Hom/A003	11.5	11.4
Hom/A004	11.4	11.4
Hom/A005	11.5	11.4
Hom/A006	11.4	11.8
Hom/A007	11.3	11.4
Hom/A008	11.5	11.2
Hom/A009	11.4	11.6
Hom/A010	11.2	10.9
Grand mean	11.4	
Cochran's test		
C	0.473	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	2.85	
s_x	0.151	
s_w	0.223	
s_s	0.000	
Critical= 0.3 σ_P	0.856	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

	THCA in material A (mg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	17.2	17.6
Hom/A002	17.5	16.7
Hom/A003	17.4	16.5
Hom/A004	16.9	17.1
Hom/A005	16.6	17.8
Hom/A006	17.3	17.7
Hom/A007	17.1	16.7
Hom/A008	17.0	17.2
Hom/A009	17.2	17.1
Hom/A010	16.4	16.5
Grand mean	17.1	
Cochran's test		
C	0.420	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.27	
s_x	0.280	
s_w	0.430	
s_s	0.000	
Critical= 0.3 σ_P	1.28	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	CBD in material A (mg/kg)	
	Replicate 1	Replicate 2
Hom/A001	47.3	51.8
Hom/A002	47.6	49.2
Hom/A003	51.8	48.0
Hom/A004	46.2	48.9
Hom/A005	47.9	48.0
Hom/A006	46.1	45.8
Hom/A007	50.3	48.8
Hom/A008	46.9	46.0
Hom/A009	47.1	48.1
Hom/A010	46.5	45.0
Grand mean	47.9	
Cochran's test		
C	0.401	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	12.0	
s_x	1.49	
s_w	1.59	
s_s	0.974	
Critical= 0.3 σ_P	3.59	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	CBDA in material A (mg/kg)	
	Replicate 1	Replicate 2
Hom/A001	183	198
Hom/A002	203	195
Hom/A003	198	198
Hom/A004	195	184
Hom/A005	192	199
Hom/A006	197	187
Hom/A007	197	188
Hom/A008	198	191
Hom/A009	192	196
Hom/A010	185	189
Grand mean	193	
Cochran's test		
C	0.328	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	48.3	
s_x	3.67	
s_w	5.87	
s_s	0.000	
Critical= 0.3 σ_P	14.5	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	THC in material B (mg/kg)	
	Replicate 1	Replicate 2
Hom/B001	1.36	1.46
Hom/B002	1.40	1.42
Hom/B003	1.38	1.48
Hom/B004	1.42	1.51
Hom/B005	1.43	1.49
Hom/B006	1.40	1.49
Hom/B007	1.36	1.42
Hom/B008	1.44	1.41
Hom/B009	1.47	1.39
Hom/B010	1.54	1.42
Grand mean	1.43	
Cochran's test		
C	0.191	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.358	
s_x	0.028	
s_w	0.058	
s_s	0.000	
Critical= 0.3 σ_P	0.108	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	THCA in material B (mg/kg)	
	Replicate 1	Replicate 2
Hom/B001	2.76	2.77
Hom/B002	2.65	2.91
Hom/B003	2.77	2.85
Hom/B004	2.77	2.83
Hom/B005	2.74	2.73
Hom/B006	2.84	2.82
Hom/B007	2.82	2.81
Hom/B008	2.79	2.87
Hom/B009	2.94	2.74
Hom/B010	2.88	2.79
Grand mean	2.80	
Cochran's test		
C	0.516	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.701	
s_x	0.034	
s_w	0.082	
s_s	0.000	
Critical= 0.3 σ_P	0.210	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	CBD in material B (mg/kg)	
	Replicate 1	Replicate 2
Hom/B001	7.55	7.81
Hom/B002	7.89	7.88
Hom/B003	7.70	7.99
Hom/B004	7.68	8.36
Hom/B005	7.84	7.60
Hom/B006	7.39	8.10
Hom/B007	8.22	7.74
Hom/B008	8.07	7.92
Hom/B009	8.21	7.54
Hom/B010	8.23	7.73
Grand mean	7.87	
Cochran's test		
C	0.240	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	1.97	
s_x	0.123	
s_w	0.325	
s_s	0.000	
Critical= 0.3 σ_P	0.590	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between sample standard deviation.

Sample No.	CBDA in material B (mg/kg)	
	Replicate 1	Replicate 2
Hom/B001	30.7	33.2
Hom/B002	31.5	32.9
Hom/B003	32.1	33.5
Hom/B004	32.3	33.6
Hom/B005	33.1	32.4
Hom/B006	31.6	34.2
Hom/B007	32.1	33.2
Hom/B008	32.3	30.9
Hom/B009	33.0	32.1
Hom/B010	34.1	33.0
Grand mean	32.6	
Cochran's test		
C	0.282	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.15	
s_x	0.546	
s_w	1.12	
s_s	0.000	
Critical= 0.3 σ_P	2.44	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Annex 4 Statistical evaluation of stability data

Stability evaluation for THC in material A.

Storage temperature	Ultra-freezer	Freezer
Time (days)	0	72
Calculated amounts (mg/kg)	10.4	10.4
	10.4	10.6
	10.4	10.5
	10.9	10.8
	10.5	10.6
	10.5	10.5
Average amount (mg/kg)	10.5	10.6
n	6	6
st. dev (mg/kg)	0.194	0.137
Difference		-0.050
$0.3*\sigma_P$		0.789
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for THCA in material A.

Storage temperature	Ultra-freezer	Freezer
Time (days)	0	72
Calculated amounts (mg/kg)	16.3	15.5
	16.7	16.3
	16.4	16.5
	17.1	16.6
	17.0	16.7
	16.5	16.5
Average amount (mg/kg)	16.7	16.4
n	6	6
st. dev (mg/kg)	0.327	0.437
Difference		0.317
$0.3*\sigma_P$		1.25
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for CBD in material A.

Storage temperature	Ultra-freezer	Freezer
Time (days)	0	72
Calculated amounts (mg/kg)	41.3	41.0
	40.9	41.7
	42.1	40.2
	42.1	41.4
	42.0	42.1
	40.8	41.5
Average amount (mg/kg)	41.5	41.3
n	6	6
st. dev (mg/kg)	0.609	0.655
Difference		0.217
$0.3*\sigma_P$		3.12
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for CBDA in material A.

Storage temperature	Ultra-freezer	Freezer
Time (days)	0	72
Calculated amounts (mg/kg)	162	157
	159	161
	165	159
	164	163
	165	162
	164	165
Average amount (mg/kg)	163	161
n	6	6
st. dev (mg/kg)	2.32	2.86
Difference		2.00
$0.3*\sigma_P$		12.2
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for THC in material B.

Storage temperature	Ultra-freezer	Freezer
Time (days)	0	72
Calculated amounts (mg/kg)	1.44	1.47
	1.44	1.41
	1.44	1.41
	1.40	1.40
	1.45	1.46
	1.46	1.48
Average amount (mg/kg)	1.44	1.44
n	6	6
st. dev (mg/kg)	0.020	0.035
Difference		0.000
$0.3*\sigma_P$		0.108
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for THCA in material B.

Storage temperature	Ultra-freezer	Freezer
Time (days)	0	72
Calculated amounts (mg/kg)	2.79	2.83
	2.79	2.69
	2.73	2.80
	2.69	2.88
	2.78	2.72
	2.82	2.87
Average amount (mg/kg)	2.77	2.80
n	6	6
st. dev (mg/kg)	0.048	0.078
Difference		-0.032
$0.3*\sigma_P$		0.208
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for CBD in material B.

Storage temperature	Ultra-freezer	Freezer
Time (days)	0	72
Calculated amounts (mg/kg)	7.54	7.69
	7.93	7.61
	7.51	7.90
	7.61	7.60
	7.32	7.42
	7.61	7.78
Average amount (mg/kg)	7.59	7.67
n	6	6
st. dev (mg/kg)	0.199	0.165
Difference		-0.080
$0.3*\sigma_P$		0.569
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for CBDA in material B.

Storage temperature	Ultra-freezer	Freezer
Time (days)	0	72
Calculated amounts (mg/kg)	32.1	33.1
	33.0	33.5
	32.0	34.3
	31.1	34.0
	32.6	32.8
	32.5	33.6
Average amount (mg/kg)	32.2	33.6
n	6	6
st. dev (mg/kg)	0.655	0.554
Difference		-1.33
$0.3*\sigma_P$		2.42
Consequential difference? Diff < $0.3*\sigma_P$		No

Annex 5 Invitation letter

WAGENINGEN
UNIVERSITY & RESEARCH

European Union Reference Laboratory

P.O. Box 230 | 6700 AE Wageningen | The Netherlands

NRLs mycotoxins & plant toxins

Wageningen Food Safety Research

Natural toxins

DATE
March 27, 2024

SUBJECT
Invitation EURL mycotoxins & plant toxins proficiency test cannabinoids in hemp seed oil and hemp flour 2024 (EURLPT-MP12)

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Dear colleague,

I would like to invite you to participate in the proficiency test, organized by the EURL mycotoxins & plant toxins, at Wageningen Food Safety Research (WFSR), regarding cannabinoids in hemp seed oil and hemp flour (EURLPT-MP12).
For regulated cannabinoids (Δ9-tetrahydrocannabinol (THC) and Δ9-tetrahydrocannabinolic acid (THCA)) quantitative determination is mandatory. For other non-regulated cannabinoids reporting is optional (see Table 1). This proficiency test will be organised under accreditation according to ISO 17043 (Conformity assessment – General requirements for the competence of proficiency testing providers - R013).

The EU regulation for Δ9-tetrahydrocannabinol (THC) and Δ9-tetrahydrocannabinolic acid (THCA) in hemp seed and hemp seed oil is laid down in Commission Regulation (EU) 2023/915 and is applied from April 25, 2023. Currently there are no EU limits set for other cannabinoids e.g. cannabidiol (CBD) and cannabidiolic acid (CBDA). However, screening of this matrix/cannabinoids combination is of relevance, based on reports of co-occurrence and participants are encouraged to provide the results for these analytes as well.

The primary goal of this proficiency test is to give laboratories the opportunity to evaluate or demonstrate their performance regarding the analysis of cannabinoids in hemp seed oil and hemp flour. To quantify mandatory cannabinoids, laboratories are requested to use the methods routinely used for their enforcement, monitoring or control tasks.

According to Regulation (EU) 2017/625, all EU National Reference Laboratories (NRLs) responsible for plant toxins in food and/or feed are mandatory to participate.

The following matters are important for participation in this proficiency test:

1. Test materials
   Two test materials, hempseed oil and hemp flour, will be provided. The test amount sent for hempseed oil and hemp flour will be approximately 20 and 50 g, respectively.

2. Shipment of the test materials
   Test materials will be sent in April/May 2024. The distribution of the test materials will be announced by e-mail. The deadline for reporting is strict and will be six weeks

after the shipment of the samples. The participant should arrange the necessary import permits for the test materials.

3. Scope of the analysis
Both materials are to be analysed for the indicated regulated cannabinoids THC and THCA as defined in regulation (EU) 2023/915 and optionally for CBD and CBDA. The participants should provide their own analytical standards.

Table 1. Cannabinoids to be analysed in EURL-PT MP12.

Mandatory
Δ9-tetrahydrocannabinol (THC)
Δ9-tetrahydrocannabinolic acid (THCA)
Optional
cannabidiol (CBD)
cannabidiolic acid (CBDA)

The voluntary mycotoxins will not be benchmarked but will be evaluated for information purposes when reported by more than 7 particpants.

4. Questionnaire
A questionnaire will be sent electronically. In this questionnaire the particants will be asked to provide information on the laboratory method(s) used. This information is necessary to conduct a more in depth analysis of the results obtained in this proficiency test.

5. Report
- Preliminary results of this proficiency test will be reported to the participants in October 2024.
- The evaluation of the results will rely on the consensus value calculated exclusively from the data provided by NRLs and NRL-designated laboratories according to ISO 13528.
- Quantitative, qualitative, false positive, false negative results and z-scores will be taken into account for the evaluation of the performance.
- The report is expected to be published in December 2024 or latest in the beginning of 2025.
- Results of the proficiency test will be presented anonymously.
- Disclosure of the results of the NRLs to the representative of the European Commission is foreseen after the report is published.
- The report will feature a list of participating laboratories. This list will remain entirely separate and independent from any other data provided, ensuring there are no associations or correlations drawn between them.
- The follow-up protocol on proficiency tests from DG Santé will be applied.

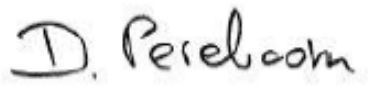
6. Additional information
- WFSR is allowed to use the anonymous results of the proficiency test in presentations, seminars and publications.
- WFSR will never inform third parties (e.g. accreditation bodies) on specific laboratory results without informing the laboratory first.
- WFSR can make use of the services of WEPAL, part of WUR, to homogenize test materials.

7. Costs
- Participation is free of charge for NRLs.

- Official laboratories (OLs) can participate as long as sufficient test material is available, at a first come first serve basis. The participation fee for OLs is €300.- (ex. VAT) as a compensation for the preparation and transportation of the samples.
- If an extra batch of samples is needed after the first shipping, the courier costs will be charged.

If you would like to participate, please fill out the accompanying participation form (preferably digitally) and send it back **before April 17th 2024**: pt.wfsr@wur.nl.

Looking forward to welcome you for this proficiency test,



Diana Pereboom
Proficiency tests

EURL mycotoxins & plant toxins in food and feed
Wageningen Food Safety Research

Annex 6 Instruction letter

WAGENINGEN
UNIVERSITY & RESEARCH

P.O. Box 230 | 6700 AE WAGENINGEN | The Netherlands

Dear Madam/Sir,

Thank you very much for your interest in the proficiency test for the analysis of cannabinoids in hemp seed oil and hemp flour.

The parcel shipped to you contains two PT materials:
- One material consisting of a hemp seed oil and another one consisting of hemp flour, weighing approximately 20 and 50 grams, respectively.

Instructions:
- After arrival the materials should be stored in the freezer.
- Please fill in the accompanied *'acknowledgement of receipt form'* and return it immediately upon receipt of the materials by e-mail (pt.wfsr@wur.nl).
- Before analysis, (re)homogenise the materials according to your laboratory's procedure.
- Treat the test material as a sample for routine analysis, according to your laboratory's procedure.

Mandatory:
Report for each material one or three results, depending of the type of the method used:

a. **One result**
1. the sum of delta-9-tetrahydrocannabinol (Δ^9-THC) and delta-9-tetrahydrocannabinolic acid (Δ^9-THCA), expressed as Δ^9-THC equivalents,
   if your method is e.g. GC based (and therefore does not chromatographically separates the individual analytes).

b. **Three results**
1. delta-9-tetrahydrocannabinol (Δ^9-THC),
2. delta-9-tetrahydrocannabinolic acid (Δ^9-THCA),
3. and the sum of Δ^9-THC and Δ^9-THCA expressed as Δ^9-THC equivalents. For the sum, a factor of 0.877 is applied to the level of Δ^9-THCA (Δ^9-THC + 0.877 × Δ^9-THCA),
   in the case of an LC based method and separate quantification of Δ^9-THC and Δ^9-THCA.

Wageningen Food Safety Research

DATE
April 22, 2024

SUBJECT
Instructions proficiency test regarding cannabinoids in hemp seed oil and hemp flour.

OUR REFERENCE
WFSR/2412272

POSTAL ADDRESS
P.O. Box 230
6700 AE WAGENINGEN
The Netherlands

VISITORS' ADDRESS
Wageningen Campus
Building 123
Akkermaalsbos 2
6708 WB WAGENINGEN

INTERNET
www.wur.nl/rikilt

CoC NUMBER
09098104

HANDLED BY
Diana Pereboom

TELEPHONE
+31 (0) 614323017

EMAIL
pt.wfsr@wur.nl

Optional :
Report for each material one or three results, depending of the type of the method used:

a. **One result**

1. the sum of cannabidiol (CBD) and cannabidiolic acid (CBDA), expressed as CBD (for GC technique),
   if your method is e.g. GC based (and therefore does not chromatographically separates the individual analytes).

b. **Three results**

1. cannabidiol (CBD),
2. cannabidiolic acid (CBDA),
3. and the sum of CBD and CBDA expressed as CBD equivalents. For the sum, a factor of 0.877 is applied to the level of CBDA (CBD + 0.877 × CBDA),
   in the case of an LC based method and separate quantification of CBD and CBDA.

Reporting:

- Report all analytical results in **mg/kg**.
- For cannabinoids below your LOQ, report as '**<[LOQ value mg/kg]**', e.g. < 5 mg/kg.
- In case a cannabinoid as listed above is not included in the scope of your method, so not measured, report as '**nt**' (not tested).

- Results reported in any other format (e.g. nd, detected, <LOQ, etc) will be regarded as not tested, 'nt'.
- Please use the web application to submit the results for the test materials (https://crlwebshop.wur.nl/ordsp/f?p=107:LOGIN). Instructions about the use of this web application was shared with you earlier by e-mail.
- Provide relevant information in the questionnaire on the analysis of the cannabinoids and the analytical method used (https://forms.office.com/e/nNqnHVPdM0).
- The deadline for submitting test-results for this proficiency test is **17th of June 2024.**

Information for Proficiency Test access:

- Your username is:
- Your password is:
- Your lab code to enter this proficiency test is:

Please contact me in case you have any questions or need any assistance.

With kind regards,

D. Pereboom

Diana Pereboom

Proficiency tests

EURL mycotoxins & plant toxins
Wageningen Food Safety Research (WFSR)
The Netherlands

Annex 7 Scope, LOQ and expanded measurement uncertainty (U)

	Mandatory cannabinoids				Voluntary cannabinoids			
	LOQ (mg/kg)		U (%)		LOQ (mg/kg)		U (%)	
Lab code	THC	THCA	THC	THCA	CBD	CBDA	CBD	CBDA
PT7708	5.0		10		5.0		15	
PT7709_A/B	6/2	6/2	20	20	6/2	6/2	20	20
PT7711	0.1	0.1	40	40				
PT7712_A/B	0.5/0.1	0.5/0.1	50	50	0.5/0.1	0.5/0.1	50	50
PT7714	1	0.001	18	17	0.001	0.001	20	21
PT7715	0.5	0.5	28	24	0.5	0.5	34	30
PT7716	0.1	0.1			0.1			
PT7717	1	1						
PT7719	0.06	0.06	30	30	0.12	0.06	30	30
PT7720	0.001	0.001	25	25	0.001	0.001	25	25
PT7721	0.01	0.01	20	20	0.01		18	
PT7722	0.65	0.58	16.4	15.5				
PT7723	0.1	0.1	30	30	0.1	0.1	30	30
PT7724	0.2	0.2			0.2	0.2		
PT7725	0.1	0.1	50	50	0.1	0.1	50	50
PT7726	0.01	0.025	30	30	0.01	0.01	30	30
PT7727	0.1	0.1	50	50	0.1	0.1	80	50
PT7728	1.2	1.2	34	34	1.2	1.2	41	22
PT7729	1	1	10	10	1	1	10	10
PT7732	0.1	0.1	15	15	0.1	0.1	15	15
PT7733	0.5	0.5	18.4	19.3	0.5	0.5		
PT7734	0.15	0.15	20	20	0.15	0.15	20	20
PT7735	0.02	20	25	25	20	20	25	25
PT7736	0.5	0.5	9.64	8.59	0.5	0.5	10.14	16.34
PT7737	2	4	25	25	4	4	25	25
PT7738	0.2	0.2	37	37	0.2	0.2	37	37
PT7739								
PT7740_A/B	2.5/0.4	2.5/0.4	25	20	2.5/0.4	2.5/0.4	30	30
PT7741								
PT7742								
PT7743	0.1	0.1			0.1			
PT7744	0.02	0.02	50	50	0.02		50	
PT7745_A/B	0.038/0.3	0.038/0.3	50	50	0.038/0.3		50	
PT7747	0.005	0.005			0.005	0.005		

* (A)= material A (hemp seed oil) and (B)= material B (hemp flour).

Annex 8 Analytical methods details

Lab code	Column	Column length (mm)	Total run time (min)	Mobile phase	Detection technique	Retention time (min)			
						THC	THCA	CBD	CBDA
PT7708	DB-5; (5% Phenyl) - methylpolysiloxane 60 m x 0.25 mm, 0.25 µm	60000	33		MS (single)	18.507		16.551	
PT7709	Macherey & Nagel Nucleoshell, C18, 150 x 3 mm, 2.7 µm	150	15	A: water + 0.1% formic acid (v/v) B: acetonitrile + 0.1% formic acid (v/v)	UV/DAD	8	10.7	4	3.2
PT7711	Agilent ZORBAX Eclipse Plus, C18, 2.1 × 100 mm, 1.8 µm	100	10	A: 0.01% formic acid and 5mM ammonium formate in water B: 0.01% formic acid in acetonitrile	MS/MS	8.38	8.33		
PT7712	Restek Raptor ARC-C18 150 x 2.1 mm, 2.7 µm	150	11	A: water + 0.1% formic acid + 12 mM ammonium formate B: acetonitrile/methanol (1:1, v/v) + 0,1% formic acid	MS/MS	4.54	8.14	2.26	2.41
PT7714	YMC TriArt C18, 2.0 x 100 mm	100	26	A: water B: methanol	MS/MS	10.9	22.2	5.2	6.46
PT7715	Waters, Acquity UPLC BEH Shield, RP18, 2.1 x 100 mm, 1.7 µm	100	13	A: water with 0.1% formic acid B: acetonitrile	MS/MS	6.19	7.06	5.24	5.51
PT7716	Waters, Acquity HSS T3, 100 x 2.1 mm, 1.8 µm	100	12	A: 5 mM ammonium formate + 0.1% formic acid in water B: 0.1% formic acid in acetonitrile	MS/MS	6.5	6.8	5.7	
PT7717	Acquity HSS T3, C18, 100 x 2.1 mm, 1.8 µm	100	12	A: 5 mM ammonium formate + 0.1% formic acid in water B: 0.1% formic acid in acetonitrile	MS/MS	8.597	9.021		
PT7719	Phenomenex, Kinetex, C18, 100 x 2.1 mm, 2.6 µm	100	19.1	A: water with 0.1% formic acid B: Methanol with 0.1% formic acid	MS/MS	9.4	14.0	6.7	7.2
PT7720	ZorBAX SB, C18, 2.1 x 10 mm, 1.8 µm	10	27	A: water + 0.1% formic acid; B: acetonitrile + 0.1% formic acid	MS/MS	22.4	24.8	17.2	15.9
PT7721	Phenomenex, Kinetex, C18, 100 x 2.1 mm, 2.6 um	100	25	A: 0.3% formic acid in water B: 0.3% formic acid in methanol	MS/MS	9.7	11.4	7.2	
PT7722	Waters, Acquity HSS T3, 100 x 2.1 mm, 1.8 µm	100	12	A: water + 5mM ammonium formate + 0.1% formic acid B: acetonitrile + 0.1% formic acid	MS/MS	5.8	6.1		
PT7723	Restek, Raptor ARC, C18		16	A: water + 0.1% formic acid B: acetonitrile + 0.1% formic acid	MS/MS	10.38	12.66	6.6	5.71
PT7724	Phenomenex, Kinetex, C18, 2.1 x 100 mm	100	35	A: water + 0.1% formic acid B: methanol + 0.1% formic acid	MS/MS	10.3	11.2	9.4	9.8
PT7725	Thermo Scientific, Hypercil Gold, C18, 2.1 x 50 mm, 1.9um	50	12	A: 5mM ammonium formate + 0.1% formic acid in water B: 5mM ammonium formate + 0.1% formic acid in methanol	MS/MS	8.47	9.01	7.9	8.02

Lab code	Column	Column length (mm)	Total run time (min)	Mobile phase	Detection technique	Retention time (min)			
						THC	THCA	CBD	CBDA
PT7726	Restek, Raptor ARC, C18, 100 x 2.1mm, 1.8 µm	100	24	A: 0.1% formic acid in wate B: 0.1% formic acid in acetonitrile	HRMS	5.3	7.1	2.9	2.5
PT7727	Phenomenex, Kinetex Polar C18, 150 x 2.1 mm, 2.6 µm	150	35	A: water + 0.1% formic acid B: acetonitrile + 0.1% formic acid	MS/MS	16.3	17.9	13.4	12.8
PT7728	Phenomenex ZB5, 30 m, 0.25 mm, 0.25 µm	30000	18		MS/MS	15.2	16.6	14.2	15.4
PT7729	Phenomenex Kinetex, C18, 250 x 4.6 mm, 5 µm	250	60	A: water + 0.5 g H_3PO_4 B: acetonitrile + 0.5 g H_3PO_4	UV/DAD	15	18	9	8
PT7732	Restek, Raptor ARC, C18, 150 x 2.1 mm, 1.8 µm	150	12	A: water + 0.1% Formic acid +5 mM Ammonium formate (38%) B: acetonitrile + 0.1% formic acid (62%)	MS/MS	4.93	6.41	2.82	2.47
PT7733	Waters, Acquity UPLC BEH, C18, 2.1 x 100 mm, 1.7 µm	100	11	A: water + with 0.1% formic acid (v/v) B: acetonitrile + 0.1% formic acid (v/v)	MS/MS	7.10	7.99	5.35	4.90
PT7734	Restek, Raptor ARC, C18, 150 x 4.6 mm, 2.7 µm	150	25	A: 75 mmol H_3PO_4: acetonitrile (3:7) B: acetonitrile	UV/DAD	7.08	9.42	3.91	3.29
PT7735	Agilent, Zorbax SB-C18 Rapid res. HT, 2.1 x 50 mm, 1.8 µm	50	23	A: water + 5 mM ammonium formate B: MeOH + 5 mM ammonium formate	MS/MS	9.5	8.7	7.5	6.9
PT7736	YMC tri-art, C18, 2.0 x 100 mm	100	22	A: 0.1% acetic acid in water B: 0.1% acetic Acid in methanol	MS/MS	6.73	14.78	3.18	4.01
PT7737	Restek, Raptor ARC, C18, 150 x 3.0 mm, 2.7 µm	150	21	A: 2.5 g H_3PO_4 + 300 ml water + 700 ml acetonitrile B: acetonitrile	FLD; UV/DAD	8.6	11.6	4.7	4.0
PT7738	Phenomenex, Kinetex C18, 100 x 2.1 mm, 1.7 µm	100	12	A: 0.1% formic acid in water B: 0.1% formic acid in methanol	MS/MS	7.34	9.46	5.24	5.82
PT7739	Phenomenex, Kinetex, C18, 150 x 2.1 mm, 5 µm	150	29	A: water + 1% formic acid B: acetonitrile + 1% formic acid	MS/MS	15.95	19.9	10.0	9.1
PT7740	Phenomenex, Kinetex XB, C18, 2.1 x 50 mm, 2.6 µm	50	15	A: water + 0.1% formic acid B: methanol + 0.1% formic acid	MS/MS;	5.6	7.2	4.1	4.5
PT7741	Restek, Raptor, ARC, C18, 150 x 2.1 mm, 2.7 µm	150	30	A: 0.1% formic acid in water B: 0.1% formic acid in methanol	MS/MS				
PT7742	Waters, XBridge BEH, C18, 2.1 x 150 mm, 2.5 µm	150	21.5	A: 0.1% formic acid + 5 mM ammonium formate in water B: 0.1% formic acid + 5 mM ammonium formate in methanol	MS/MS	13.48	14.5	12.4	12.5
PT7743	Waters, Acquity HSS T3, 100 x 2.1 mm, 1.8 µm	100	20	A: 5 mM ammonium formate / 0.1% formic acid in water B: 0.1% formic acid in acetonitrile	MS/MS	8.00	8.50	7.5	
PT7744	Phenomenex, EVO Kinetex, 150 x 2.1 mm, 1.7 µm	150	30	A: water + 0.1% formic acid B: acetonitrile + 0.1% formic acid	MS/MS;	12.04	13.84	9.82	
PT7745	Water, HSS T3, 2.1 x 100 mm, 1.8 µm	100	8.5	A: water + 5 mM ammonium format + 0.1% formic acid B: acetonitrile + 0.1% formic acid	MS/MS	4.73	5.38	3.23	
PT7747	Waters, Acquity UPLC® BEH, C18, 150 × 2.1 mm, 1.7 µm	150	18	A: water + 0.1% formic acid (v/v) B: acetonitrile + 0.1% formic acid (v/v)	MS/MS	9.17	10.61	6.54	6.04

Lab code	Sample weight (g)	Extraction solvent	Extraction solvent volume (ml)	Extraction conditions	Extraction time (min)	Sample clean-up	SPE cartridge	Volume extract loaded on SPE (ml)	Matrix equivalent final extract (g/ml)	Internal standards
PT7708	2	acetonitrile	10	vortex; ultrasonic	15	filters				Tributylamine
PT7709	A: 0.1 B: 1	methanol	20	ultrasonic	30	SPE	Macherey & Nagel CHROMABOND HR-X, 85 µm, 6 mL/200 mg	20	A: 0.2 B: 2	
PT7711	0.050	ethanol	5	vortex; ultrasonic	30	dilution			0.001	THC-D3, CBD-D3
PT7712	A: 0.5 B: 5	methanol	A: 5 B: 50	ultrasonic	15	dilution				Δ9-THC-D3, Δ9-THCA-D3, 11-or-9-carboxy-Δ9-THC-D9, CBD-D3
PT7714	0.1	2-propanol, acetonitrile, methanol.		vortex; ultrasonic	50	dilution				Δ9-THC-D3, CBD-D3, CBN-D3, THCA-A-D3,
PT7715	1	acetonitrile	10	vortex	10	QuEChERS				none
PT7716	1	5 mL water and 10 mL acetonitrile	15	vortex	30	QuEChERS			0.067	
PT7717	1	5 mL water and 10 mL acetonitrile	15	mechanical shaking	30	QuEChERS			0.067	THC-D3
PT7719	0.1 - 2.5	QuEChERS extraction with water and acetonitrile	10	mechanical shaking	10	QuEChERS;			0.01 - 0.25	Δ9-THC-D3, Δ9-THCA-D3, CBD-D3, CBDA-D3
PT7720	2	acetone	300	other	180	other			0.04	Δ9-THC-D3, Δ9-THCA-D3, 11-OH-Δ9-THC-D3, 11 nor-9-carboxy-Δ9-THC-D3, CBD-D3, CBN-D3
PT7721	1	methanol	10	mechanical shaking; ultrasonic	10	dilution; filters			0.1	THC-D3
PT7722	1	water, acetonitrile	15	mechanical shaking; vortex	30	QuEChERS				
PT7723	1	15.0 mL methanol + 1 mL citric acid buffer pH5 (0.4 mol/L water free citric acid and NaOH in water)	16	mechanical shaking; ultrasonic	20	none				Δ9-THC-D3, Δ9-THCA-A-D3, CBD-D3, CBDA- Δ9
PT7724	0.2	methanol, water, formic acid	20	mechanical shaking; vortex; ultrasonic;	45	filters; other			0.01	THC-D3, THCA-D3, CBD-D3,
PT7725	1.0	acetonitrile	10	mechanical shaking	30	QuEChERS			0.02	CBD-D3
PT7726	1	iso-propanol	10	vortex; ultrasonic; shaking by hand	30	dilution; filters				

Lab code	Sample weight (g)	Extraction solvent	Extraction solvent volume (ml)	Extraction conditions	Extraction time (min)	Sample clean-up	SPE cartridge	Volume extract loaded on SPE (ml)	Matrix equivalent final extract (g/ml)	Internal standards
PT7727	1	methanol	50	mechanical shaking	120	dilution			1/125	Δ9-THC-D3, THCA-A-D3, CBD-D3, Cannabinol D3
PT7728	0.5 - 1.0	methanol	50	mechanical shaking	30	none			1,25*10^-5g/ml	Δ9-THC-D3, CBD-D3
PT7729	A: 0.1 B: 1	A: acetonitrile; B: methanol	A: 1 B: 10	ultrasonic	30	SPE	Agilent BondElut Mega BE-C18 1g 6ml	1	0.1	
PT7732	0.1	methanol	10	ultrasonic	10	dilution			0.1/10	Δ9-THC-D3, 11-nr-9-carboxy-Δ9-THC-D3, CBD-D3
PT7733	A: 0.5 B: 2.0	10 mL water and 20 mL acetonitrile	30	mechanical shaking	30	QuEChERS				"
PT7734	A: 0.5 B: 2	methanol:dichloromethane = 9:1	25 or 30	ultrasonic; mechanical shaking	60	none				
PT7735	1	acetonitrile	15	mechanical shaking	30	QuEChERS				THC-D3, CBD-D3
PT7736	1	acetone	10	mechanical shaking	60	none			0.1	NA
PT7737	0.5	A: methanol + dichloromethane (9+1) B: methanol	10	vortex; ultrasonic;	5	other			0.05	
PT7738	1	acetonitrile	10	mechanical shaking; ultrasonic	30	dilution				Δ9-THC-D3, Δ9-THCA-D3, CBD-D3
PT7739	0.5-1	methanol	2 x 10	mechanical shaking	2 x 30	dilution; filters			0.001-0.000005	
PT7740	A: 0.2 B: 5	A: acetone; B: methanol	A: 10 B: 40	A: shaking by hand; B: mechanical shaking;	A:short shaking; B: 60	none			A: 0.0004; B: 0.0025	Δ9-THC-D3, CBD-D3
PT7741	A: 0.2 B: 5	A: methanol and isooctane B: methanol	50	mechanical shaking; ultrasonic	30	dilution				Δ9-THC-D3, Δ9-THCA-A-D3
PT7742	A: 0.5 B: 5	acetonitrile/water/formic acid (79/20/1)	2 x 25	mechanical shaking	2 x 30	dilution; filters				Δ9-THC-D3, THCA-D3, CBDA-D3, CBD-D3
PT7743	1	5 mL water and 10 mL acetonitrile	15	mechanical shaking	30	QuEChERS				THC-D3, THCA-A-D3, CBD-D3

Lab code	Sample weight (g)	Extraction solvent	Extraction solvent volume (ml)	Extraction conditions	Extraction time (min)	Sample clean-up	SPE cartridge	Volume extract loaded on SPE (ml)	Matrix equivalent final extract (g/ml)	Internal standards
PT7744	5	acetonitrile	50	mechanical shaking	2 x 30	other			0.1	Δ9-THC-D3, Δ9-THCA-A-D3, CBD D3, Cannabinol-D3
PT7745	A: 0.2 B: 1	acetonitrile	A: 2 B: 10	mechanical shaking	15	none; dilution			A: 0.1 B: 0.01	Δ9-THC D3, CBD D9, Cannabinol-D3
PT7747	A: 0.2 B: 3	A: acetone B: methanol	A: 10 B: 2 x 10	vortex; ultrasonic	B: 2 x 15	dilution; filters			A: 0.02 B: 0.15	Δ9-THC-D3, THCA-D3, CBD-D3, Cannabinol-D3

A= material A (hemp seed oil); B= material B (hemp flour); ACN = acetonitrile; MeOH = methanol; NaOH = sodium hydroxide.

Annex 9 Results material A (hemp seed oil)

Material A						
	THC **C: 12.4 mg/kg** **u: 0.531 mg/kg** **σ_p: 3.10 mg/kg (25%)** **robust σ: 2.55 mg/kg (20.5%)**		**THCA** **C: 24.9 mg/kg** **u: 1.40 mg/kg** **σ_p: 6.23 µg/kg (25%)** **robust σ: 6.61 mg/kg (26.5%)**		**Sum THC and THCA** **C: 34.7 mg/kg** **u: 1.59 mg/kg** **σ_p: 8.67 mg/kg (25%)** **robust σ: 7.73 mg/kg (22.3%)**	
Lab code	**Result mg/kg**	**z-score**	**Result mg/kg**	**z-score**	**Result mg/kg**	**z-score**
PT7708	nt	-	nt	-	41.75	0.82
PT7709	11	-0.46	20	-0.79	29	-0.65
PT7710	-	-	-	-	29	-0.65
PT7711	13.783	0.44	27.995	0.50	38.335	0.42
PT7712	9.96	-0.79	16.65	-1.33	24.56	-1.17
PT7713	40	**8.88**	nt	-	nt	-
PT7714	10.39	-0.65	21.79	-0.50	29.49	-0.60
PT7715	10.21	-0.71	25.35	0.07	32.43	-0.26
PT7716	15	0.83	38	**2.10**	48	1.54
PT7717	10.73	-0.54	30.05	0.83	37.08	0.28
PT7718	13	0.19	23.5	-0.23	36.7	0.24
PT7719	13.8	0.44	25.7	0.13	36.3	0.19
PT7720	1.56	**-3.50**	2.93	**-3.53**	4.13	**-3.52**
PT7721	52.2	**12.81**	30.9	0.96	79.3	**5.15**
PT7722	13.1	0.22	34.9	1.60	42.7	0.93
PT7723						
PT7724	11.69	-0.24	21.96	-0.47	30.94	-0.43
PT7725	15.25	0.91	30	0.82	41.56	0.80
PT7726	12	-0.14	26	0.18	34	-0.08
PT7727	10.8	-0.52	24.8	-0.02	32.6	-0.24
PT7728	12.4	-0.01	23.4	-0.24	32.8	-0.21
PT7729	12.8	0.12	19.8	-0.82	30.2	-0.51
PT7730	20.4	**2.57**	35.1	1.64	51.2	1.91
PT7731	10.3	-0.68	25.4	0.08	32.6	-0.24
PT7732	12.32	-0.03	26.86	0.31	35.88	0.14
PT7733	16.5	1.31	27	0.34	40.22	0.64
PT7734	13.9	0.48	24.9	0.00	35.7	0.12
PT7735	6.2	-2.00	147	**19.61**	135	**11.58**
PT7736	13	0.19	26.1	0.19	35.8	0.13
PT7737	12.4	-0.01	19.7	-0.84	29.7	-0.57
PT7738	8.53	-1.25	15.42	-1.52	22.05	-1.46
PT7739	12.1	-0.10	12.6	-1.98	23.2	-1.32
PT7740	13.2	0.25	25.3	0.06	35.4	0.09
PT7741	14.5	0.67	26.3	0.22	37.6	0.34
PT7742	15.5	0.99	30.2	0.85	42	0.85
PT7743	13	0.19	25	0.01	35	0.04
PT7744	9.706	-0.87	16.736	-1.31	24.382	-1.19
PT7745	12.07	-0.11	29.88	0.80	38.28	0.42
PT7747	1.3	**-3.58**	2.3	**-3.63**	3.4	**-3.61**

* Results presented in this table are as reported by the participants. Results reported in other formats (e.g. nd, detected, <LOQ, positive, etc) will be regarded as not tested, 'nt'.

C = consensus value (robust mean), u = uncertainty of consensus value, σ_p = target standard deviation for proficiency test, robust σ = robust (relative) standard deviation based on participants' results, nt = not tested, Participant PT7723 did analyse material A but made a reporting error.

Material A						
	CBD C: 45.4mg/kg u: 1.92 mg/kg σ_p: 11.3 mg/kg (25%) robust σ: 8.00 mg/kg (17.6%)		CBDA C: 166 mg/kg u: 5.95 mg/kg σ_p: 41.4 µg/kg (25%) robust σ: 22.3 mg/kg (13.5%)		Sum CBD and CBDA C: 184 mg/kg u: 7.53 mg/kg σ_p: 46.1 mg/kg (25%) robust σ: 30.1 mg/kg (16.3%)	
Lab code	**Result mg/kg**	**z-score**	**Result mg/kg**	**z-score**	**Result mg/kg**	**z-score**
PT7708	nt	-	nt	-	208.5	0.52
PT7709	46	0.06	157	-0.21	184	-0.01
PT7710	-	-	-	-	125	-1.29
PT7711	positive	-	positive	-	positive	-
PT7712	42.31	-0.27	151.09	-0.35	174.82	-0.21
PT7713	nt	**-**	nt	-	nt	-
PT7714	-	-	145	-0.50	-	-
PT7715	50.24	0.43	detected	-	-	**-**
PT7716	36	-0.83	nt	**-**	36	**-3.22**
PT7717	nt	-	nt	-	nt	-
PT7718	nt	-	nt	-	nt	-
PT7719	44.2	-0.10	172	0.15	195	0.23
PT7720	7.92	**-3.30**	24.9	**-3.40**	29.8	**-3.35**
PT7721	65	1.73	nt	-	nt	**-**
PT7722	-	-	-	-	-	-
PT7723						
PT7724	48.73	0.30	173.66	0.19	201.03	0.36
PT7725	9.22	**-3.19**	200	0.83	184.6	0.00
PT7726	42	-0.30	180	0.35	200	0.34
PT7727	61.2	1.40	178	0.30	217	0.71
PT7728	60.1	1.30	196	0.73	232	1.03
PT7729	44.2	-0.10	148	-0.43	174	-0.23
PT7730	nt	**-**	nt	-	nt	-
PT7731	nt	-	nt	-	nt	-
PT7732	42.35	-0.27	166.29	0.02	188.17	0.08
PT7733	50.3	0.44	179	0.32	207.28	0.50
PT7734	45.4	0.00	192	0.64	214	0.64
PT7735	32	-1.18	153	-0.31	166	-0.40
PT7736	47.2	0.16	164.1	-0.04	191.1	0.14
PT7737	44.9	-0.04	152	-0.33	178.1	-0.14
PT7738	40.61	-0.42	111.41	-1.31	138.32	-1.00
PT7739	41.7	-0.32	178	0.30	198	0.29
PT7740	50.2	0.43	171.5	0.14	200.6	0.35
PT7741	nt	-	nt	-	nt	-
PT7742	59.3	1.23	184	0.44	221	0.79
PT7743	51	0.50	nt	-	nt	**-**
PT7744	45.155	-0.02	nt	-	nt	**-**
PT7745	50.9	0.49	nt	-	50.9	**-2.90**
PT7747	6.8	**-3.40**	25.9	**-3.37**	29.5	**-3.36**

* Results presented in this table are as reported by the participants. Results reported in other formats (e.g. nd, detected, <LOQ, positive, etc) will be regarded as not tested, 'nt'.

C = consensus value (robust mean), u = uncertainty of consensus value, σ_p = target standard deviation for proficiency test, robust σ = robust (relative) standard deviation based on participants' results, nt = not tested, Participant PT7723 did analyse material A but made a reporting error.

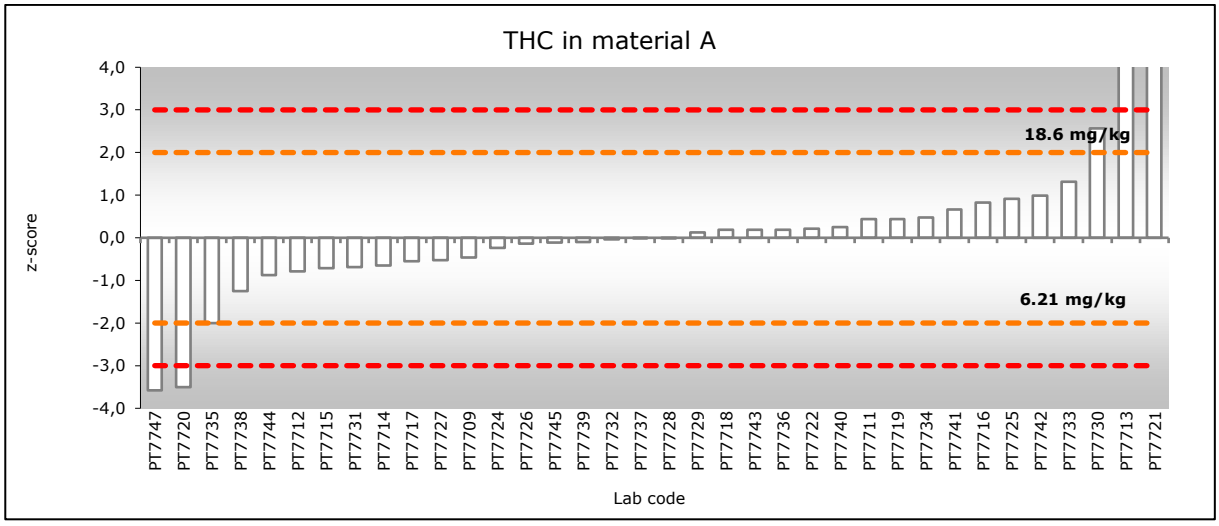


Figure 1 *Graphical representation of the z-scores for THC in the material A. Dotted lines show PT performance boundaries ± 2 (also in mg/kg) and ± 3.*

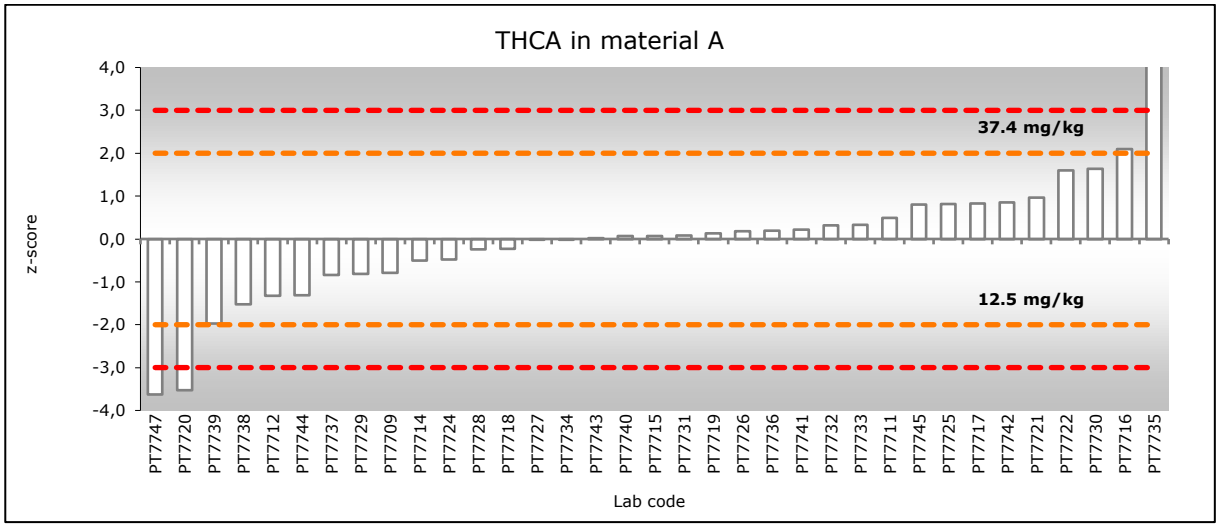


Figure 2 *Graphical representation of the z-scores for THCA in the material A. Dotted lines show PT performance boundaries ± 2 (also in mg/kg) and ± 3.*

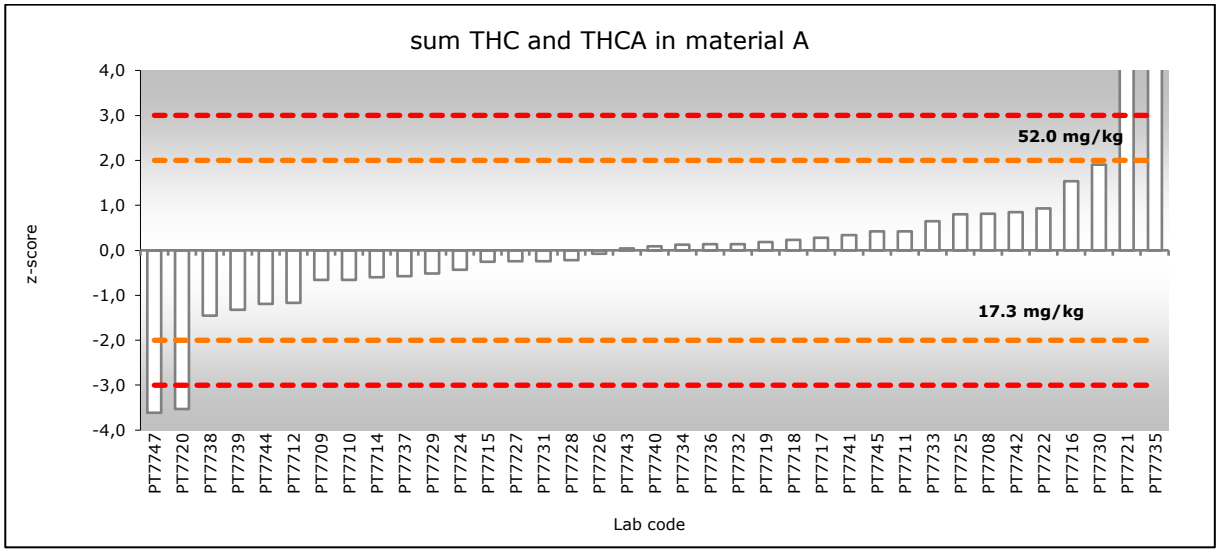


Figure 3 *Graphical representation of the z-scores for the sum of THC and THCA in the material A. Dotted lines show PT performance boundaries ± 2 (also in mg/kg) and ± 3.*

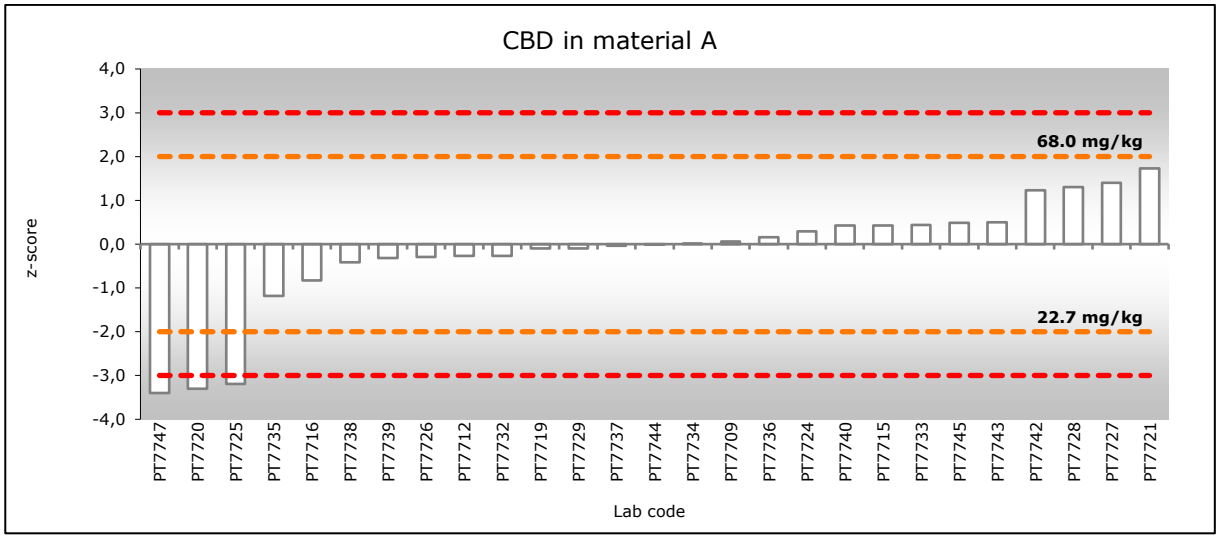


Figure 4 *Graphical representation of the z-scores for CBD in the material A. Dotted lines show PT performance boundaries ± 2 (also in mg/kg) and ± 3.*

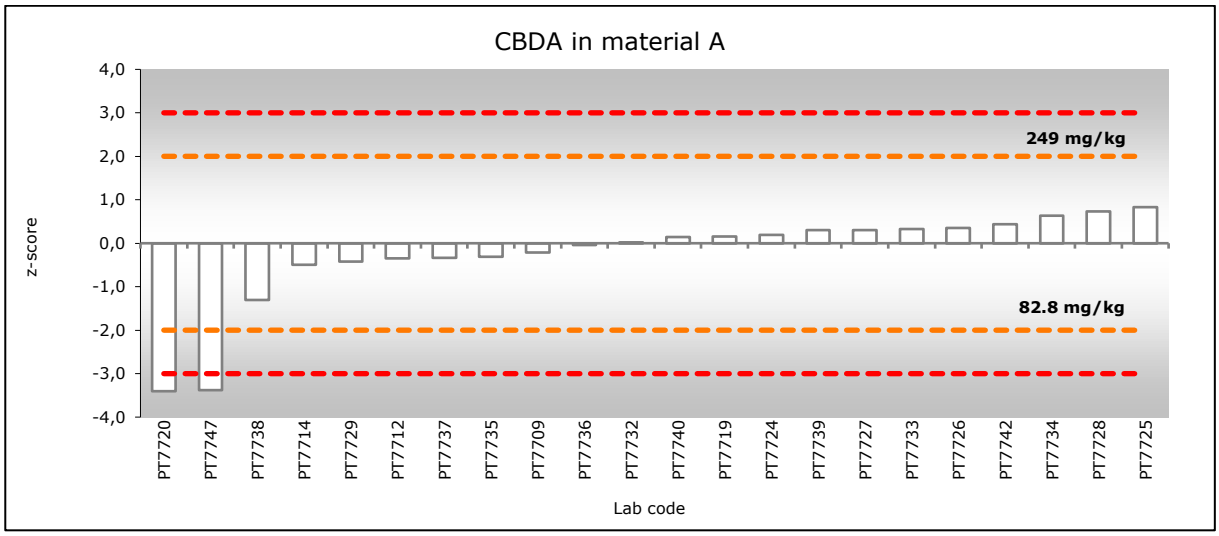


Figure 5 *Graphical representation of the z-scores for CBDA in the material A. Dotted lines show PT performance boundaries ± 2 (also in mg/kg) and ± 3.*

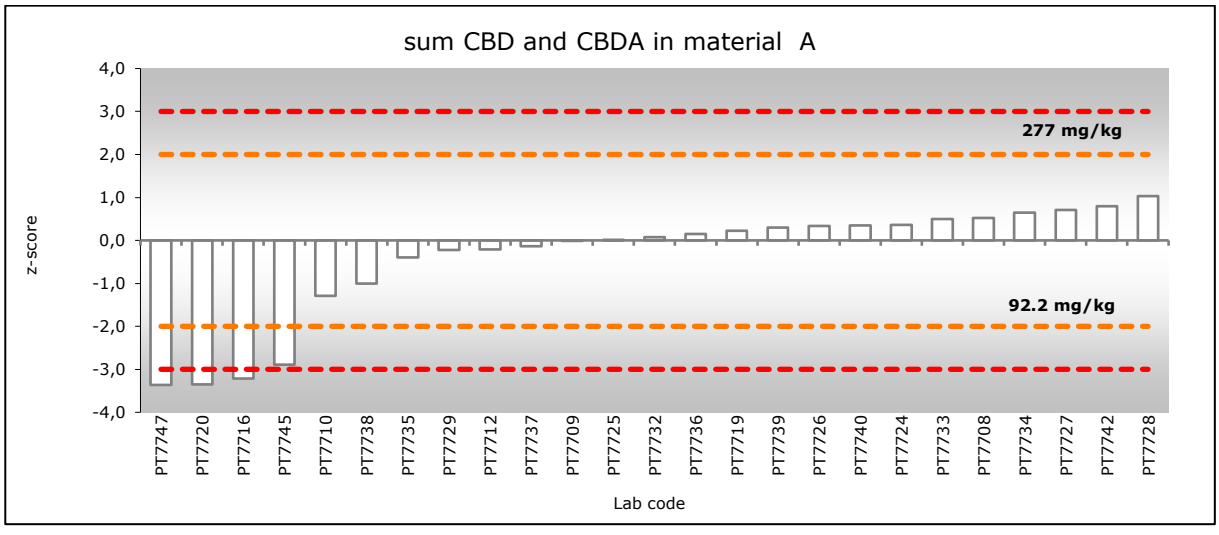


Figure 6 *Graphical representation of the z-scores for the sum of CBD and CBDA in the material A. Dotted lines show PT performance boundaries ± 2 (also in mg/kg) and ± 3.*

Annex 10 Results material B (hemp flour)

Material B						
	THC		**THCA**		**Sum THC and THCA**	
	C: 1.57 mg/kg		**A: 3.28 mg/kg**		**C: 4.43 mg/kg**	
	u: 0.080 mg/kg		**u: 0.229 mg/kg**		**u: 0.305 mg/kg**	
	σ_p: 0.391 mg/kg (25%)		**σ_p: 0.819 mg/kg (25%)**		**σ_p: 1.11 mg/kg (25%)**	
	robust σ: 0.368 mg/kg (23.5%)		**robust σ: 1.07 mg/kg (32.6%)**		**robust σ: 1.46 mg/kg (33.1%)**	
Lab code	**Result mg/kg**	**z-score**	**Result mg/kg**	**z-score**	**Result mg/kg**	**z-score**
PT7708	nt	-	nt	-	6.2	1.60
PT7709	<LOQ	-	4	0.88	3.5	-0.84
PT7710	-	-	-	-	2	**-2.19**
PT7711	1.434	-0.34	4.295	1.24	5.202	0.70
PT7712	1.2	-0.93	2.52	-0.92	3.41	-0.92
PT7713	30	**72.66**	nt	-	nt	**-**
PT7714	-	-	-	-	<1.0	**[-3.10]FN**
PT7715	1.32	-0.63	3.53	0.31	4.41	-0.01
PT7716	1.5	-0.17	2.5	-0.95	3.7	-0.66
PT7717	1.78	0.55	4.87	1.94	6.05	1.47
PT7718	1.4	-0.42	2.8	-0.58	4	-0.39
PT7719	1.46	-0.27	3.1	-0.22	4.18	-0.22
PT7720	13	**29.22**	26.5	**28.34**	36.2	**28.71**
PT7721	4.5	**7.50**	2.8	-0.58	7	**2.33**
PT7722	1.7	0.34	4	0.88	5.1	0.61
PT7723	1.41	-0.40	3.06	-0.27	4.09	-0.30
PT7724	1.1	-1.19	2.65	-0.77	3.42	-0.91
PT7725	2	1.11	4.1	1.00	5.6	1.06
PT7726	1.6	0.09	3	-0.34	4.2	-0.20
PT7727	1.2	-0.93	3.14	-0.17	3.96	-0.42
PT7728	1.47	-0.24	3.83	0.67	4.82	0.36
PT7729	nd	-	2.05	-1.50	1.8	**-2.37**
PT7730	2.4	**2.13**	3.7	0.52	5.6	1.06
PT7731	2.7	**2.90**	7.6	**5.28**	9.3	**4.40**
PT7732	1.33	-0.60	2.44	-1.02	3.47	-0.86
PT7733	1.8	0.60	3.5	0.27	4.86	0.39
PT7734	1.75	0.47	3.09	-0.23	4.46	0.03
PT7735	0.7	**-2.21**	22	**22.85**	20	**14.07**
PT7736	1.7	0.34	4.2	1.13	5.4	0.88
PT7737	<2	[1.11]	<4	[0.88]	< 5.5	[0.97]
PT7738	1.11	-1.16	1.96	-1.61	2.83	-1.44
PT7739	1.55	-0.04	1.42	**-2.27**	2.79	-1.48
PT7740	1.2	-0.93	2.6	-0.83	3.4	-0.93
PT7741	1.7	0.34	2.7	-0.70	4.1	-0.29
PT7742	1.69	0.32	4.27	1.21	5.43	0.91
PT7743	1.4	-0.42	3.3	0.03	4.4	-0.02
PT7744	1.223	-0.87	0.689	**-3.16**	1.827	**-2.35**
PT7745	1.32	-0.63	1.24	**-2.49**	2.41	-1.82
PT7747	11.6	**25.64**	19.2	**19.43**	28.5	**21.76**

* Results presented in this table are as reported by the participants. Results reported in other formats (e.g. nd, detected, <LOQ, positive, etc) will be regarded as not tested, 'nt'.

C = consensus value (robust mean), u = uncertainty of consensus value, σ_p = target standard deviation for proficiency test, robust σ = robust (relative) standard deviation based on participants' results, nt = not tested.

Material B						
	CBD C: 7.56 mg/kg u: 0.336 mg/kg σ_p: 1.89 mg/kg (25%) robust σ: 1.42 mg/kg (18.8%)		CBDA C: 28.5 mg/kg u: 2.93 mg/kg σ_p: 7.13 mg/kg (25%) robust σ: 11.3 mg/kg (39.5%)		Sum CBD and CBDA C: 30.2 mg/kg u: 3.19 mg/kg σ_p: 7.56 mg/kg (25%) robust σ: 13.0 mg/kg (43.1%)	
Lab code	**Result mg/kg**	**z-score**	**Result mg/kg**	**z'-score**	**Result mg/kg**	**z'-score**
PT7708	nt	-	nt	-	29.18	-0.13
PT7709	7	-0.30	30	0.19	33	0.34
PT7710	-	-	-	-	13	**-2.10**
PT7711	positive	-	positive	-	positive	-
PT7712	6.63	-0.49	18.82	-1.26	23.14	-0.86
PT7713	nt	**-**	nt	-	nt	-
PT7714	-	-	-	-	-	-
PT7715	7.12	-0.23	27.49	-0.13	-	-
PT7716	7.9	0.18	nt	**-**	7.9	**-2.72**
PT7717	nt	-	nt	-	nt	-
PT7718	nt	-	nt	-	nt	-
PT7719	7.58	0.01	30.6	0.27	34.4	0.51
PT7720	48.8	**21.82**	177	**19.26**	204	**21.18**
PT7721	10	1.29	nt	-	nt	**-**
PT7722	-	-	-	-	-	-
PT7723	7.31	-0.13	29.8	0.17	33.5	0.40
PT7724	6.28	-0.68	23.06	-0.71	26.5	-0.45
PT7725	9.37	0.96	35	0.84	40.06	1.20
PT7726	7.9	0.18	29	0.06	33	0.34
PT7727	8.74	0.62	39.4	1.41	43.3	1.59
PT7728	7.72	0.08	30.2	0.22	34.2	0.48
PT7729	7.1	-0.24	20.1	-1.09	24.8	-0.66
PT7730	nt	**-**	nt	-	nt	-
PT7731	nt	-	nt	-	nt	-
PT7732	6.07	-0.79	18.35	-1.32	22.16	-0.98
PT7733	8.2	0.34	31.4	0.37	35.78	0.68
PT7734	8.17	0.32	32.9	0.57	37.02	0.83
PT7735	5	-1.35	21	-0.98	24	-0.76
PT7736	9.9	1.24	96.4	**8.81**	94.5	**7.83**
PT7737	4.9	-1.41	13.1	-2.00	16.4	-1.69
PT7738	6.46	-0.58	15.04	-1.75	19.65	-1.29
PT7739	6.47	-0.58	15.3	-1.71	19.9	-1.26
PT7740	6.3	-0.67	21.5	-0.91	25.1	-0.63
PT7741	nt	-	nt	-	nt	-
PT7742	10.8	1.71	38.2	1.26	44.3	1.72
PT7743	7.8	0.13	nt	-	nt	**-**
PT7744	6.912	-0.34	nt	-	nt	**-**
PT7745	6.7	-0.46	nt	-	6.7	**-2.87**
PT7747	39.6	**16.95**	155	**16.41**	175.5	**17.71**

* Results presented in this table are as reported by the participants. Results reported in other formats (e.g. nd, detected, <LOQ, positive, etc) will be regarded as not tested, 'nt'.

C = consensus value (robust mean), u = uncertainty of consensus value, σ_p = target standard deviation for proficiency test, robust σ = robust (relative) standard deviation based on participants' results, nt = not tested.

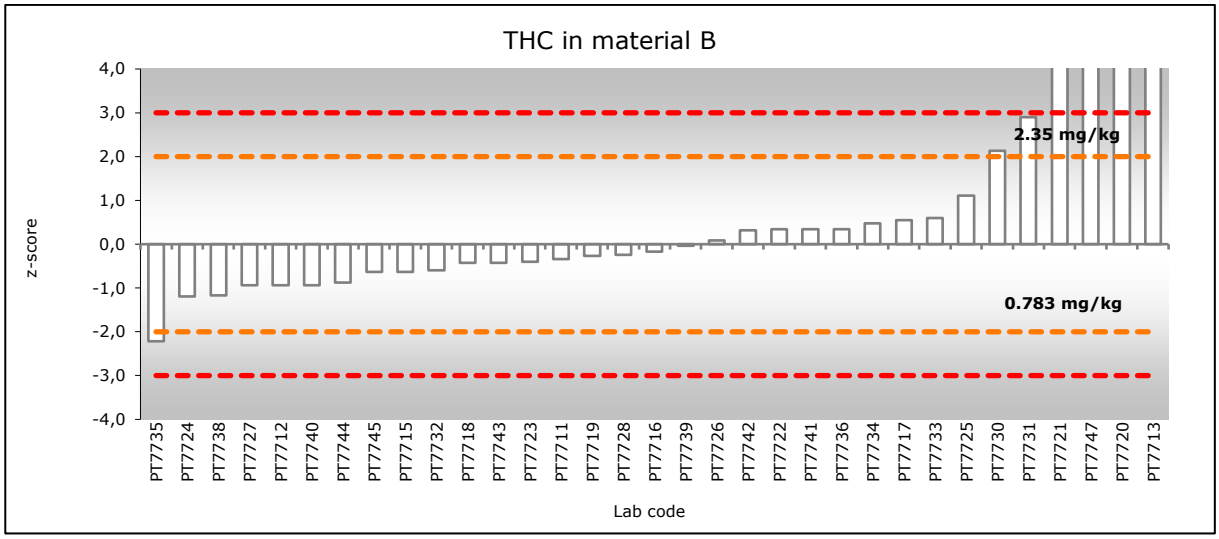


Figure 7 *Graphical representation of the z-scores for THC in the material B. Dotted lines show PT performance boundaries ± 2 (also in mg/kg) and ± 3.*

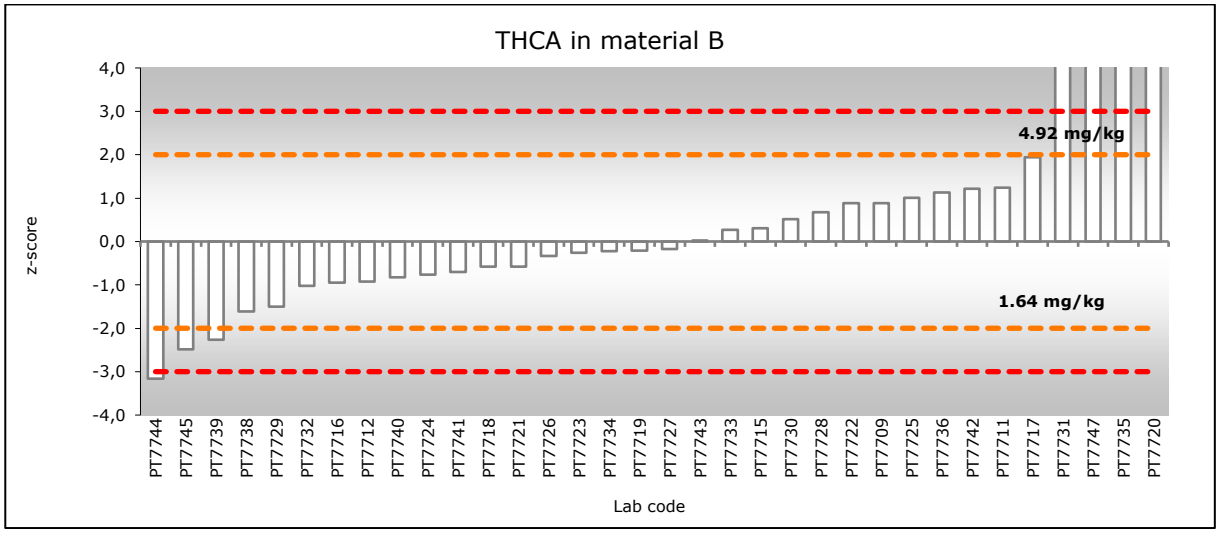


Figure 8 *Graphical representation of the z-scores for THCA in the material B. Dotted lines show PT performance boundaries ± 2 (also in mg/kg) and ± 3.*

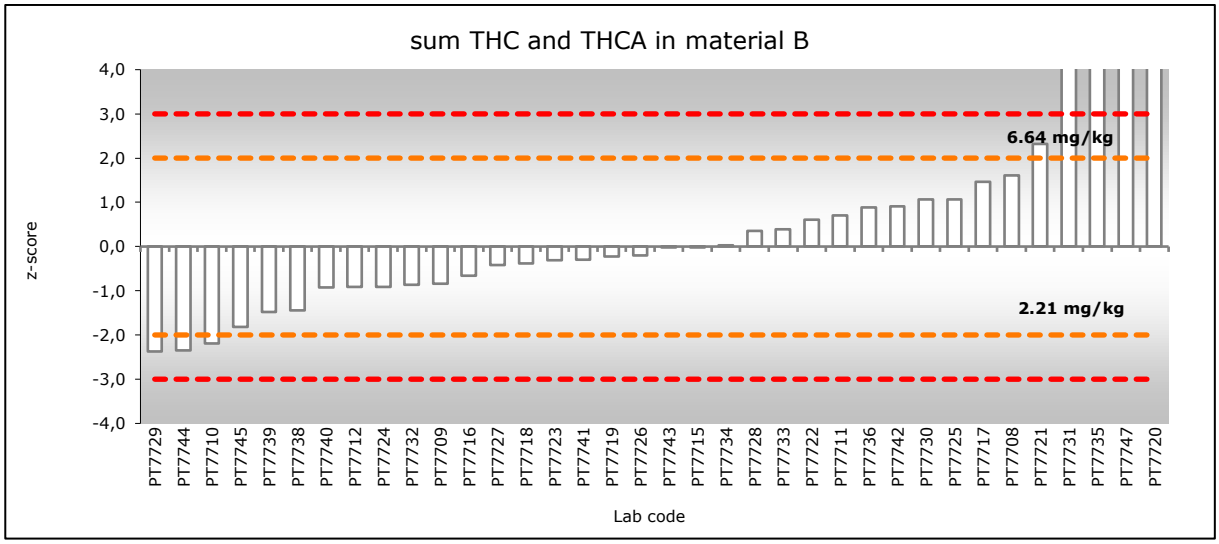


Figure 9 *Graphical representation of the z-scores for the sum THC and THCA in material B. Dotted lines show PT performance boundaries ± 2 (also in mg/kg) and ± 3.*

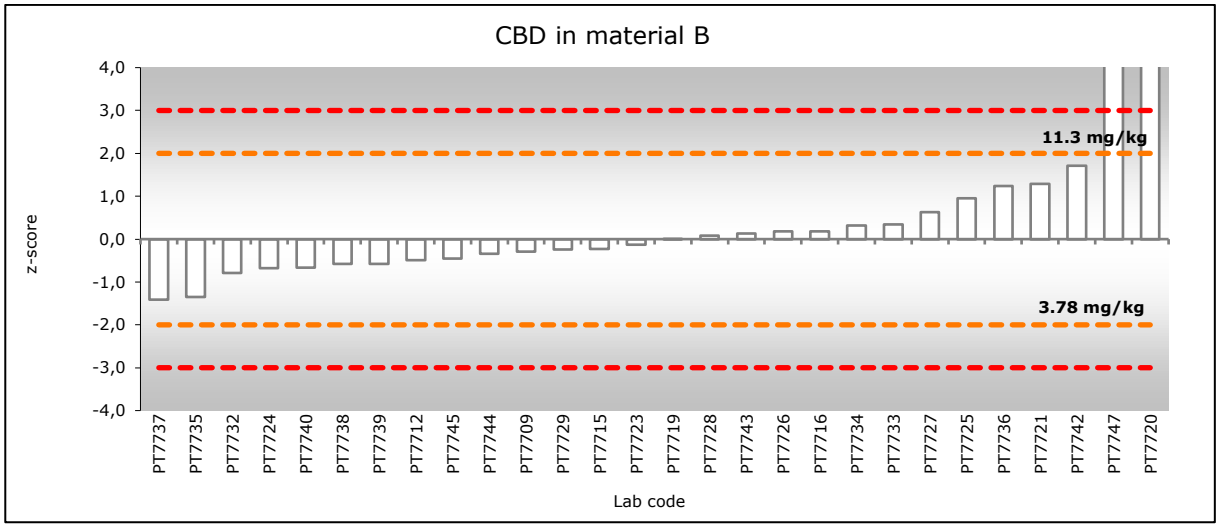


Figure 10 *Graphical representation of the z-scores for CBD in the material B. Dotted lines show PT performance boundaries ± 2 (also in mg/kg) and ± 3.*

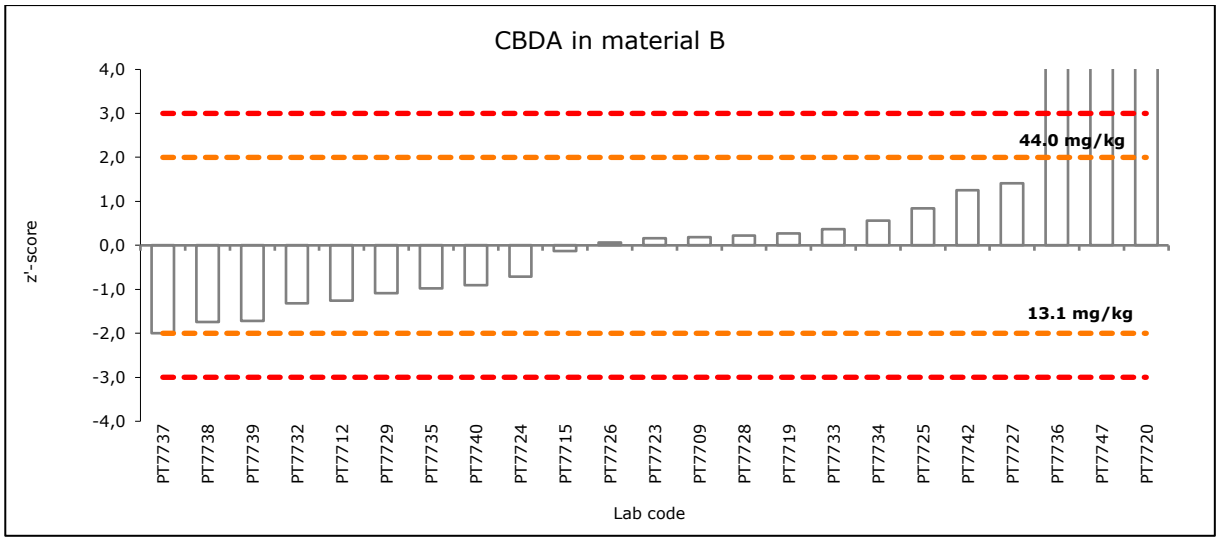


Figure 11 *Graphical representation of the z'-scores for CBDA in the material B. Dotted lines show PT performance boundaries ± 2 (also in mg/kg) and ± 3.*

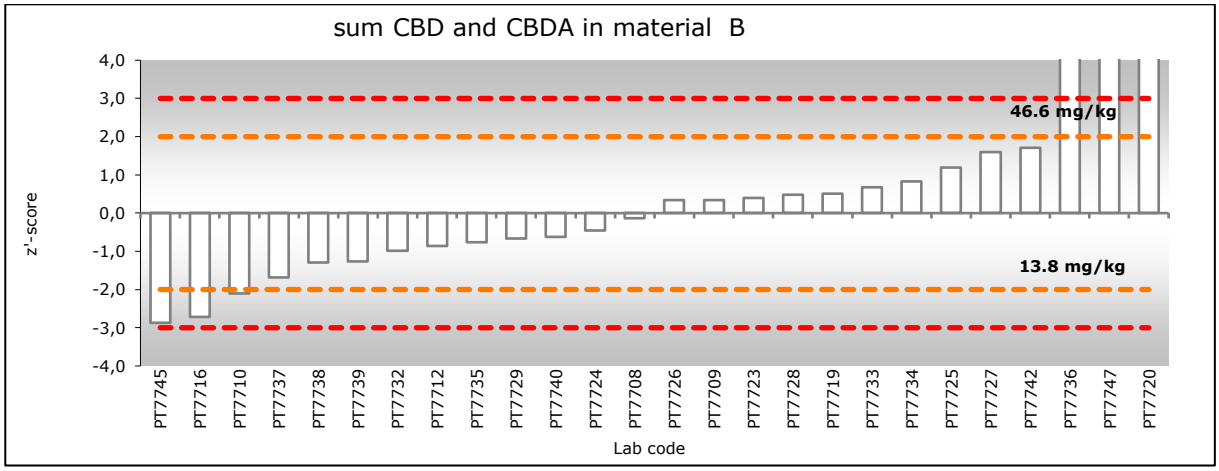


Figure 12 *Graphical representation of the z'-scores for the sum of CBD and CBDA in material B. Dotted lines show PT performance boundaries ± 2 (also in mg/kg) and ± 3.*

Annex 11 Overview performance per laboratory

	satisfactory performance mandatory individual cannabinoids	satisfactory performance mandatory sum cannabinoids	FN
PT7708		**2 out of 2**	
PT7709	3 out of 4	**2 out of 2**	
PT7710		1 out of 2	
PT7711	**4 out of** 4	**2 out of 2**	
PT7712	**4 out of 4**	**2 out of 2**	
PT7713	0 out of 4**	0 out of 2**	
PT7714	2 out of 4	1 out of 2	FN
PT7715	**4 out of 4****	**2 out of 2****	
PT7716	3 out of 4	**2 out of 2**	
PT7717	**4 out of 4**	**2 out of 2**	
PT7718	**4 out of 4**	**2 out of 2**	
PT7719	**4 out of 4**	**2 out of 2**	
PT7720	0 out of 4	0 out of 2	
PT7721	2 out of 4	0 out of 2	
PT7722	**4 out of 4**	**2 out of 2**	
PT7723	2 out of 4	1 out of 2	
PT7724	**4 out of 4**	**2 out of 2**	
PT7725	**4 out of 4**	**2 out of 2**	
PT7726	**4 out of 4**	**2 out of 2**	
PT7727	**4 out of 4**	**2 out of 2**	
PT7728	**4 out of 4**	**2 out of 2**	
PT7729	3 out of 4	1 out of 2	
PT7730	2 out of 4	**2 out of 2**	
PT7731	2 out of 4	1 out of 2	
PT7732	**4 out of 4**	**2 out of 2**	
PT7733	**4 out of 4**	**2 out of 2**	
PT7734	**4 out of 4**	**2 out of 2**	
PT7735	1 out of 4	0 out of 2	
PT7736	**4 out of 4**	**2 out of 2**	
PT7737	2 out of 4	1 out of 2	
PT7738	**4 out of 4**	**2 out of 2**	
PT7739	3 out of 4	**2 out of 2**	
PT7740	**4 out of 4**	**2 out of 2**	
PT7741	**4 out of 4**	**2 out of 2**	
PT7742	**4 out of 4**	**2 out of 2**	
PT7743	**4 out of 4**	**2 out of 2**	
PT7744	3 out of 4	1 out of 2	
PT7745	3 out of 4	**2 out of 2**	
PT7747	0 out of 4	0 out of 2	

* Satisfactory performance means a quantitative result with a satisfactory z-score was obtained for the individual mycotoxins or the total sum of cannabinoids present in material A and B. Results reported as <LOQ, nd, positive, detected are not considered a satisfactory z-score.

** reported results after the deadline.

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WFSR Report 2025.004

The mission of Wageningen University & Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 7,700 employees (7,000 fte), 2,500 PhD and EngD candidates, 13,100 students and over 150,000 participants to WUR's Life Long Learning, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.

To explore the potential of nature to improve the quality of life

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