

Government Chemist CBD Food and Cosmetic Ring Trial Final Report

Cannabidiol and controlled cannabinoids

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1 Contents

1	Contents	4
2	Summary	5
3	Samples.....	6
3.1	LGC-RT/20/A.....	6
3.2	LGC-RT/20/B.....	6
3.3	LGC-RT/20/C.....	6
4	Homogeneity.....	7
5	Shipping.....	8
6	Ring Trial Results.....	8
6.1	Technologies	8
6.2	CBD.....	10
6.2.1	Method Information	10
6.2.2	CBD Consensus Values	11
6.2.3	CBD z-Score Calculations and Interpretations	12
6.3	Controlled Cannabinoids.....	19
6.3.1	Method Information	19
6.3.2	Cannabinoid Consensus Values	20
6.3.3	Cannabinoid Z-Score Calculations and Interpretations	21
6.3.5	THCV Results.....	22
6.3.6	CBN Results	25
6.3.7	Δ 9-THC.....	31
6.3.8	Δ 8-THC.....	36
6.3.9	Δ 9THCA-A.....	37
6.4	Result Trends	37
6.5	Controlled Status in the UK.....	38
7	Conclusions	38
8	Participating Laboratories	39
9	Appendix 1 - Protocol.....	40
10	Appendix 2 – Results Template.....	60

2 Summary

Cannabidiol (CBD) products have rapidly entered the UK market in a variety of forms, including food and cosmetics. Laboratories across the UK need to be able to accurately measure the CBD content as well as the controlled cannabinoid content in commercially available products. CBD and cannabinoids have been highlighted as difficult compounds to analyse, and so a ring trial began under the Government Chemist programme to assess laboratories' performance in measuring these analytes.

The aim of the ring trial was to share and compare methods for quantifying CBD and controlled cannabinoids in food and cosmetics among testing laboratories. LGC shared details of their CBD and controlled cannabinoid methods. Participating laboratories could either use LGC's method or their own to analyse provided samples for CBD and, optionally, controlled cannabinoids (Table 1).

Three commercial samples were sourced from a high street retailer and analysed for the presence of any CBD or controlled cannabinoid content. Each sample was mixed in bulk and then distributed into > 40 bottles. The samples were then tested for homogeneity.

The bottles were dispatched to 35 laboratories (16 UK, 19 International). Participants were asked to analyse the samples in singlicate on three separate days. A reporting template was provided for participants to fill out and return to LGC. 32 result templates were returned to LGC (14 UK, 18 international), however, not all of them were completed sets.

Overall, the ring trial was a successful international study. There was good agreement of results between most laboratories. An interesting set of data was shared including instrument types and limits of detection which helped assess the capability of testing laboratories.

Table 1. Cannabinoids of interest.

Cannabinoid	Abbreviation	CAS Number	Controlled Status
Cannabidiol	CBD	13956-29-1	No
Delta-9-tetrahydrocannabinol*	Δ^9 -THC	1972-08-3	Yes
Delta-9-tetrahydrocannabivarin*	THCV	31262-37-0	Yes
Cannabinol*	CBN	521-35-7	Yes
Delta-8-tetrahydrocannabinol*	Δ^8 -THC	5957-75-5	Yes
Delta 9-Tetrahydrocannabinolic acid*	Δ^9 THCA-A	23978-85-0	No - but readily converts to THC

*Optional

3 Samples

The samples were sourced from a high street retailer in November, 2020. The samples were stored at ambient temperature throughout analysis and shipping. The samples were prepared as follows:

3.1 LGC-RT/20/A

- 5% CBD Hemp Oil, 30 mL (Food)

After initial testing, the sample was found to contain high levels of controlled cannabinoids. It was therefore diluted five times in olive oil. ~ 60 mL of sample (two bottles) were diluted in 240 mL olive oil in a DURAN® bottle. The sample was mixed overnight with a magnetic stirrer bar. While the sample was still being mixed, 5.2 mL aliquots were transferred into 8 mL plastic bottles labelled LGC-RT/20/A CBD Hemp Oil. The sample was aliquoted in bottle order 1 – 41. The sample was then reanalysed and determined suitable for shipping. Sample A had an expiry date of September, 2022 stated on the commercial bottle.

3.2 LGC-RT/20/B

- 400 mg CBD in MCT Oil Spray, 30 mL (Food)

The sample was initially analysed and found to have suitable levels of controlled cannabinoids for shipping. ~240 mL of sample (eight bottles) was decanted into a DURAN® bottle and mixed overnight with a magnetic stirrer. While the sample was still being mixed, 5.1 mL aliquots were transferred into 8 mL plastic bottles labelled LGC-RT/20/B CBD Oil. The sample was aliquoted in bottle order 1 – 41. Sample B had an expiry date of August, 2021 stated on each commercial bottle.

3.3 LGC-RT/20/C

- CBD Body Wash, 100 mL (Cosmetic)

The sample was initially analysed and found to have suitable levels of controlled cannabinoids for shipping. ~300 mL of sample (three bottles) was decanted into a DURAN® bottle and mixed overnight with a magnetic stirrer. The bottle was sonicated and then put back on the magnetic stirrer while 5.2 mL aliquots were transferred into 8 mL plastic bottles labelled LGC-RT/20/C CBD Cosmetic. The sample was aliquoted in bottle order 1 – 41. Sample C did not have an expiry date stated on the commercial bottle.

One 8 mL bottle of each sample was placed into a plastic bag and stored at ambient temperature until being shipped to participating laboratories.

4 Homogeneity

All samples were tested for homogeneity using LGC's analysis method for CBD. Three bottles were taken from the beginning, middle and end of the bottling numbers (1, 20 and 41) and analysed in triplicate (Figure 1). Using a one way ANOVA ($p < 0.05$), it was found there was no statistically significant difference between the bottles, and therefore they were all considered homogeneous.

Sample A: $F(2,6) = 3.017, p = 0.12$

Sample B: $F(2,6) = 1.406, p = 0.32$

Sample C: $F(2,6) = 3.041, p = 0.12$

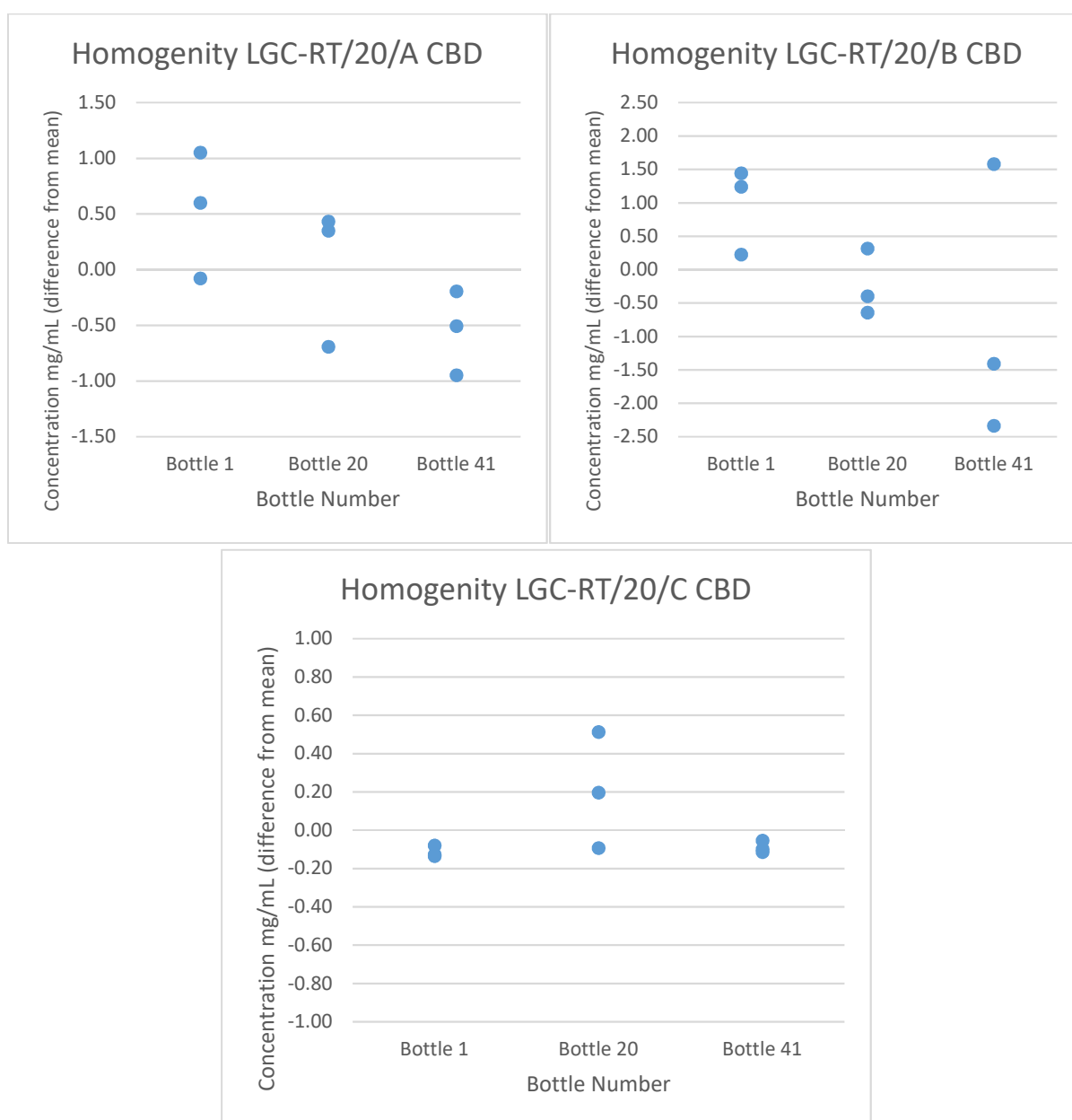


Figure 1. CBD homogeneity results for three ring trial samples – each bottle was analysed in triplicate for CBD.

5 Shipping

The bottled samples were distributed to 35 laboratories (16 UK, 19 International) at room temperature during January and February, 2021. Participants were asked to quantify the samples for CBD and, optionally, controlled cannabinoids in singlicate on three separate days. A reporting template was provided to participants to fill out and return to LGC. The reporting template required: method details (Extraction Type, Dilution Factor, Instrument LOQ, and Analysis Techniques), analysis details (Bottle Number, Extraction and Analysis Dates, and Internal Standard) and results (CBD Concentration [mg/mL], Controlled Cannabinoid Concentrations [$\mu\text{g/mL}$], Concentration [per pack] and Controlled Status of Samples [UK only]).

6 Ring Trial Results

32 result templates were returned to LGC (14 UK, 18 international), however, not all of them were completed sets.

6.1 Technologies

A range of technologies were used by testing laboratories to measure CBD and controlled cannabinoids (Table 2, 3 and Figure 2). There was good split between using either Mass Spectrometry or Spectroscopy techniques.

Table 2. Acronyms for measurement technologies.

Acronym	Measurement Technology	Technique
LC-DAD/PDA	Liquid Chromatography with Diode Array Detector or Photodiode Array Detector	Spectroscopy
LC-UV	Liquid Chromatography with Ultraviolet Detection	Spectroscopy
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry	Mass Spectrometry
LC-QTOF-MS	Liquid Chromatography with Quadrupole Time-of-Flight Mass Spectrometry	Mass Spectrometry
GC-MS	Gas Chromatography with Mass Spectrometry	Mass Spectrometry
GC-FID	Gas Chromatography with Flame Ionisation Detection	Flame Ionisation

Table 3. Extraction method and analysis technique for each participating laboratory.

Institute/ Laboratory Number	CBD Extraction Method	CBD Analysis Technique	Cannabinoid Extraction Method	Cannabinoid Analysis Technique
1	Dilution	LC-MS/MS	Dilution	LC-MS/MS
2	Liquid-Liquid Extraction	LC-DAD/PDA	Liquid-Liquid Extraction	LC-DAD/PDA
3	Dilution	LC-UV	Dilution	LC-MS/MS
4	Dilution	LC-MS/MS	Dilution	LC-MS/MS
5	Not Reported	LC-DAD/PDA	Not Reported	LC-DAD/PDA
6	Solid-Phase Extraction	LC-DAD/PDA	Solid-Phase Extraction	LC-DAD/PDA
7	Dilution	LC-DAD/PDA	Dilution	LC-DAD/PDA
8	Liquid-Liquid Extraction	LC-MS/MS	Liquid-Liquid Extraction	LC-MS/MS
9	Dilution	LC-MS/MS	Dilution	LC-MS/MS
10	Dilution	LC-DAD/PDA	Dilution	LC-DAD/PDA
11	Dilution	LC-MS/MS	-	-
12	Dilution	LC-QTOF-MS	Dilution	LC-QTOF-MS
13	Liquid-Liquid Extraction	LC-UV	Liquid-Liquid Extraction	LC-UV
14	Dilution	LC-MS/MS	Dilution	LC-MS/MS
15	Dilution	LC-UV	Dilution	LC-UV / GC-MS*
16	Dilution	LC-MS/MS	Dilution	LC-MS/MS
17	Dilution	GC-MS	Dilution	GC-MS
18	Dilution	LC-UV	Dilution	LC-MS/MS
19	Dilution	GC-MS	Dilution	GC-MS
20	Dilution	LC-MS/MS	Dilution	LC-MS/MS
21	Dilution	GC-FID / LC- DAD/PDA	-	-
22	Dilution	LC-MS/MS	Dilution	LC-MS/MS
23	Dilution	LC-MS/MS	Dilution	LC-MS/MS
24	Dilution	LC-MS/MS	Dilution	LC-MS/MS
25	Solid-Phase Extraction	LC-UV	Solid-Phase Extraction	LC-UV
26	Dilution	LC-DAD/PDA	Liquid-Liquid Extraction	LC-MS/MS
27	Liquid-Liquid Extraction	LC-DAD/PDA	Liquid-Liquid Extraction	LC-DAD/PDA
28	Dilution	LC-DAD/PDA	Dilution	LC-MS/MS
29	Dilution	Not Reported	Dilution	Not Reported
30	Dilution	LC-MS/MS	Dilution	LC-MS/MS
31	QuEChERS	LC-MS/MS	-	-
32	Dilution	LC-MS/MS	Dilution	LC-MS/MS
				*GC-MS used for Δ9-THC only

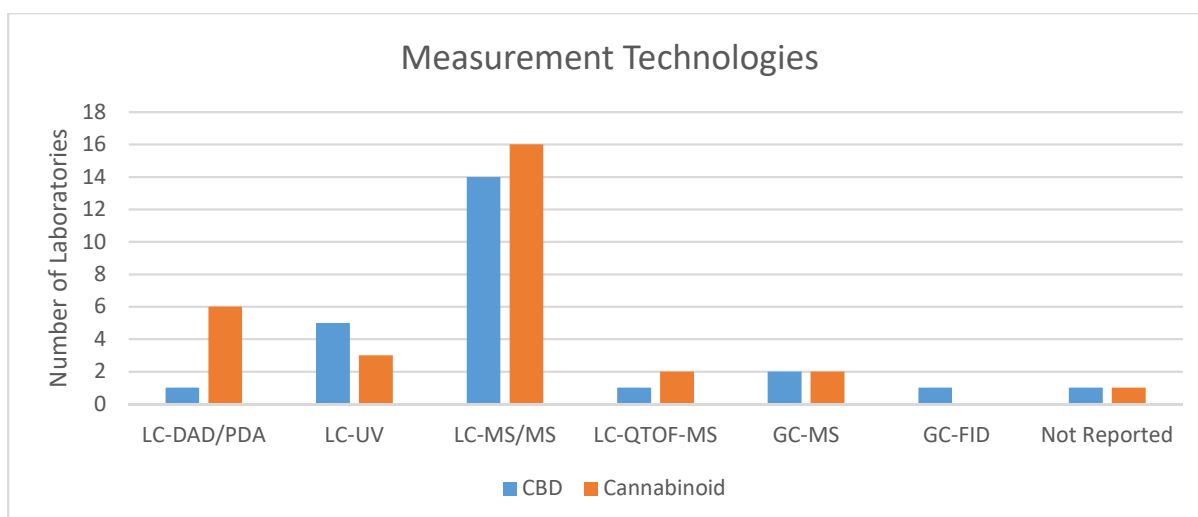


Figure 2. Instruments used by participating laboratories in the analysis of CBD and cannabinoids.

6.2 CBD

6.2.1 Method Information

30 laboratories reported results for the quantification of CBD in all three samples. One laboratory reported results for LGC-RT/20/A and LGC-RT/20/B only. A further one laboratory reported results for LGC-RT/20/C only. Therefore, 31 results were received for each sample. The majority of laboratories used their own extraction and analysis methods, with a few using LGC's method or modifying the LGC method (Table 4). The most popular extraction technique was dilution with liquid-liquid, solid-phase and QuEChERS also being used by a few laboratories (Table 5). A range of instrument limit of detections (LODs) were reported (Figure 3). Total lower limit of quantifications (LLOQs) were not reported because laboratories used different dilution factors for each sample. A range of internal standards were used, however, the majority of laboratories did not use an internal standard (Table 6).

Table 4. Methods used when analysing ring trial samples for CBD.

	LGC	Modified LGC	Own method
CBD Extraction Method	6	2	23
CBD Analysis Method	3	1	27

Table 5. Type of extraction used by laboratories for extracting CBD from ring trial samples.

Extraction	Number of Laboratories
Dilution	24
Liquid-Liquid	4
Solid Phase	2
QuEChERS	1
N/R	1

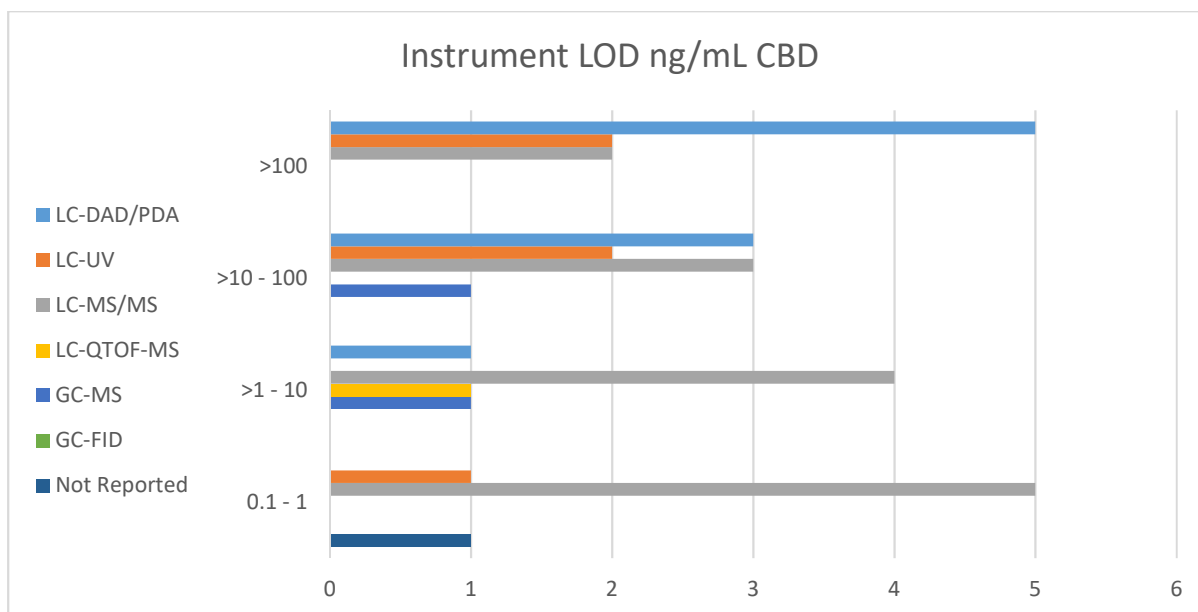


Figure 3. Instrument limit of detection of participating laboratories when measuring CBD.

Table 6. Internal Standards used by participating laboratories when measuring CBD.

Internal Standard	Number of Laboratories
Δ 9-THC-D3	1
Benzonitrile	1
CBD-D3	9
N/R	2
None	16
Squalene	1
Tribenzylamine	1

6.2.2 CBD Consensus Values

Overall, there was good agreement with the majority of laboratories.

The data was examined using plots and tables. Figures 4 to 6 (in section 6.2.3) show the CBD concentration measurements obtained by the different laboratories for sample A, B and C. In each case there is a clear consensus level, but the results cover more than one order of magnitude.

From the shape of the plots, the distribution is approximately symmetrical, but departs from normal distribution. This is expected behaviour for inter-laboratory studies, which typically contain outliers and long distribution tails.

Given the lack of normal distribution and the presence of extreme values in the data, a calculation of the mean and standard error is inappropriate. Of the two possible approaches (outlier removal or robust statistics), the latter is preferred because it avoids decisions regarding the removal of extreme data while reducing their influence. Robust methods are

also standard practice in inter-laboratory studies, and the same approach has been followed here.

Since the dispersion between institutes is relatively large compared to their standard uncertainties, only a single estimate for each institute is needed to compute a consensus value for each analyte, typically the mean or median.

The most commonly used robust estimator for the population mean is the median, which has a standard uncertainty given by equation 1.

$$u = \sqrt{\frac{\pi}{2n}} \text{MAD}_E$$

Equation 1.

Where MAD_E is the median absolute deviation of the individual estimates, scaled to be an equivalent estimator for the population standard deviation, and n is the number of replicate values. Here, n is the number of institutes.

Table 7 shows the CBD results. In all three cases, every institute submitted either a minimum of two positive results or three non-detect results; There were no mixtures of non-detects and detected results. The institute means were therefore used to compute the consensus values. Degrees of freedom were sufficient for a coverage factor $k = 2$ to be used for the expanded uncertainty.

Table 7. Calculated CBD concentration consensus value and uncertainty, expressed as mg/mL. The median is the median of institute means and n is the number of institutes which reported a result for the indicated sample.

Sample	Median (mg/mL)	n	MAD_E (mg/mL)	u	k	U	$U(\%)$
Sample A	9.583	31	2.4758	0.5573	2	1.115	11.63
Sample B	12.167	31	1.6406	0.3693	2	0.739	6.07
Sample C	0.468	30	0.0542	0.0124	2	0.025	5.29

6.2.3 CBD z-Score Calculations and Interpretations

The CBD z-scores for each laboratory were calculated by equation 2.

$$z = \frac{(x - x_a)}{\sigma_p}$$

Equation 2.

Where x is the mean result reported by the participant, x_a is the consensus value and σ_p is the robust estimate of the standard deviation. This is the median absolute deviation (MAD) which is calculated as follows: for each result, the absolute difference between its value and the overall median is calculated. The MAD is the median of the absolute differences. To obtain a scaled equivalent to the standard deviation, the MAD is then multiplied by 1.483.

In normal circumstances, ~ 95 % of z-scores will be in the range $|z| \leq 2$. These scores are designated “satisfactory”.

A rate of 1 in 20 would be expected for z-scores in the range $2 < |z| \leq 3$. These scores may require further investigation.

Scores where $|z| > 3$ are to be expected at a rate of about 1 in 300. These z-scores indicate that the result is not fit-for-purpose and certainly requires investigation.

6.2.3.1 LGC-RT/20/A

The stated CBD concentration on the package was 50 mg/mL, however the sample was diluted five times before shipping and therefore the concentration should be ~ 10 mg/mL. Overall, there was good agreement with the majority of laboratories. Participants were asked to run each sample in singlicate on three separate days. Generally, there was good agreement between replicates – this is shown by the error bars in Figure 4.

Notably, Institutes 3, 17 and 19 have submitted very low values.

After sharing the preliminary results, laboratories 3, 17, 19 and 32 expressed that they had found a dilution error in their calculations. As the corrected concentrations were received after the results were no longer blinded, the amended results were not accepted. However, this explains why these results are outliers. The results did not need to be removed from the calculation of the consensus value due to the use of robust statistics, meaning the outlier results do not affect the consensus value. These z-scores have been highlighted in red as known errors.

27 laboratories had satisfactory z-scores, out of 31 laboratories that submitted a results this equates to 87%. If the results are calculated excluding the laboratories that have found errors, the z-score increases to 96%.

Table 8. Individually calculated z-scores for CBD in LGC-RT/20/A. Where green are satisfactory scores and orange are scores that need further investigation. Laboratories marked in red had already found a calculation error after preliminary results were shared but before the final report.

Sample LGC-RT/20/A CBD		
Laboratory	Mean concentration (mg/mL)	z-score
1	10.7	0.43
2	9.7	0.07
3	0.0	-3.87
4	7.6	-0.82
5	12.1	1.02
6	9.4	-0.08
7	10.2	0.23
8	12.2	1.06
9	9.7	0.03
10	9.6	0.00
11	9.4	-0.06
12	10.1	0.21
13	9.0	-0.22
14	11.3	0.67
15	8.5	-0.44
16	9.3	-0.11
17	0.2	-3.81
18	9.3	-0.11
19	0.1	-3.82
20	1.0	-3.46
21	9.7	0.04
22	7.2	-0.96
23	13.9	1.74
24	10.3	0.28
25	7.8	-0.71
26	9.6	0.00
27	11.5	0.79
28	11.9	0.92
29	12.7	1.24
30	7.2	-0.96
32	4.8	-1.95

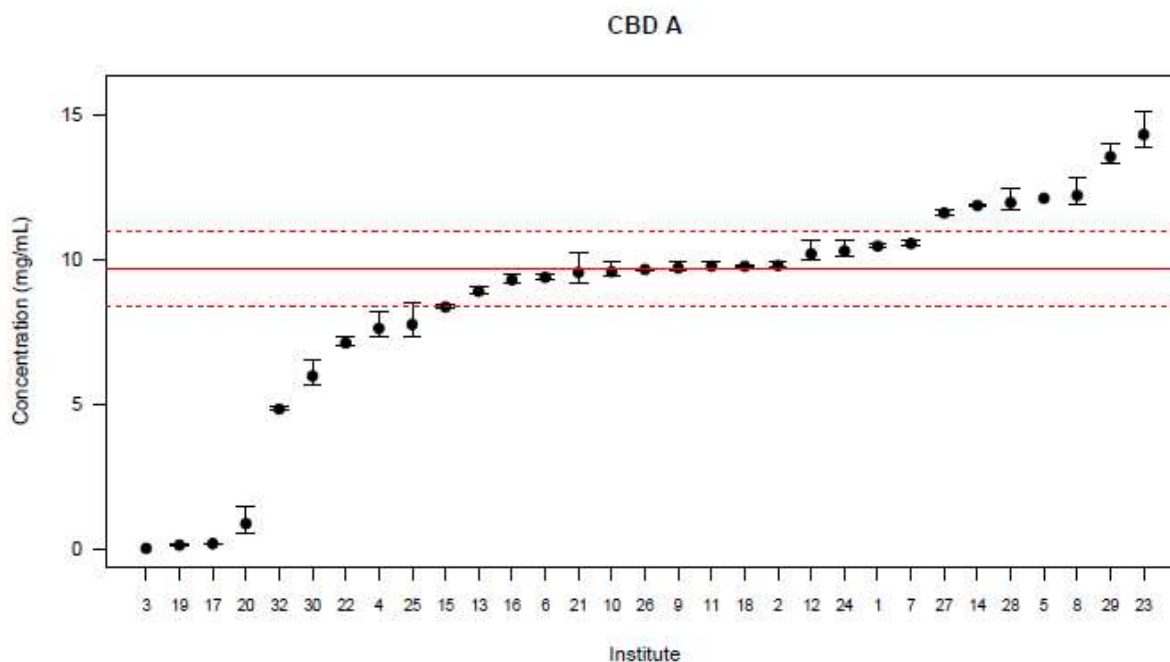


Figure 4. Calculated concentrations of CBD in LGC-RT/20/A by all participating laboratories. Error bars represent the standard uncertainty of the laboratories' replicate results.

6.2.3.2 LGC-RT/20/B

The stated CBD concentration on the package was 13.3 mg/mL. Overall, there was good agreement with the majority of laboratories. Participants were asked to run each sample in singlicate on three separate days. Generally there was good agreement between replicates – this is shown by the error bars in Figure 5.

Notably, Institutes 3, 17 and 19 have submitted very low values.

After sharing the preliminary results, laboratories 3, 17, 19 and 32 expressed that they had found a dilution error in their calculations. As the corrected concentrations were received after the results were no longer blinded, the amended results were not accepted. However, this explains why these results are outliers. The results did not need to be removed from the calculation of the consensus value due to the use of robust statistics, meaning the outlier results do not affect the consensus value. These z-scores have been highlighted in red as known errors.

21 laboratories had satisfactory z-scores, out of 31 laboratories that submitted a results, this equates to 68%. If the results are calculated excluding the laboratories that have found errors, the z-score increases to 78%.

Table 9. Individually calculated z-scores for CBD in LGC-RT/20/B. Where green are satisfactory scores and orange are scores that need further investigation. Laboratories marked in red had already found a calculation error after preliminary results were released but before the final report.

Sample LGC-RT/20/B CBD		
Laboratory	Mean concentration (mg/mL)	z-score
1	14.0	1.14
2	12.8	0.39
3	0.0	-7.41
4	7.0	-3.18
5	15.7	2.17
6	11.4	-0.48
7	13.8	1.01
8	15.8	2.21
9	13.0	0.49
10	12.4	0.12
11	12.3	0.07
12	12.5	0.23
13	11.8	-0.21
14	13.3	0.67
15	11.1	-0.67
16	12.0	-0.13
17	0.2	-7.28
18	13.1	0.55
19	0.2	-7.32
20	2.4	-5.96
21	12.2	0.00
22	9.7	-1.48
23	17.2	3.06
24	11.2	-0.57
25	11.0	-0.70
26	12.5	0.23
27	12.7	0.30
28	16.0	2.36
29	11.5	-0.41
30	9.4	-1.66
32	5.4	-4.15

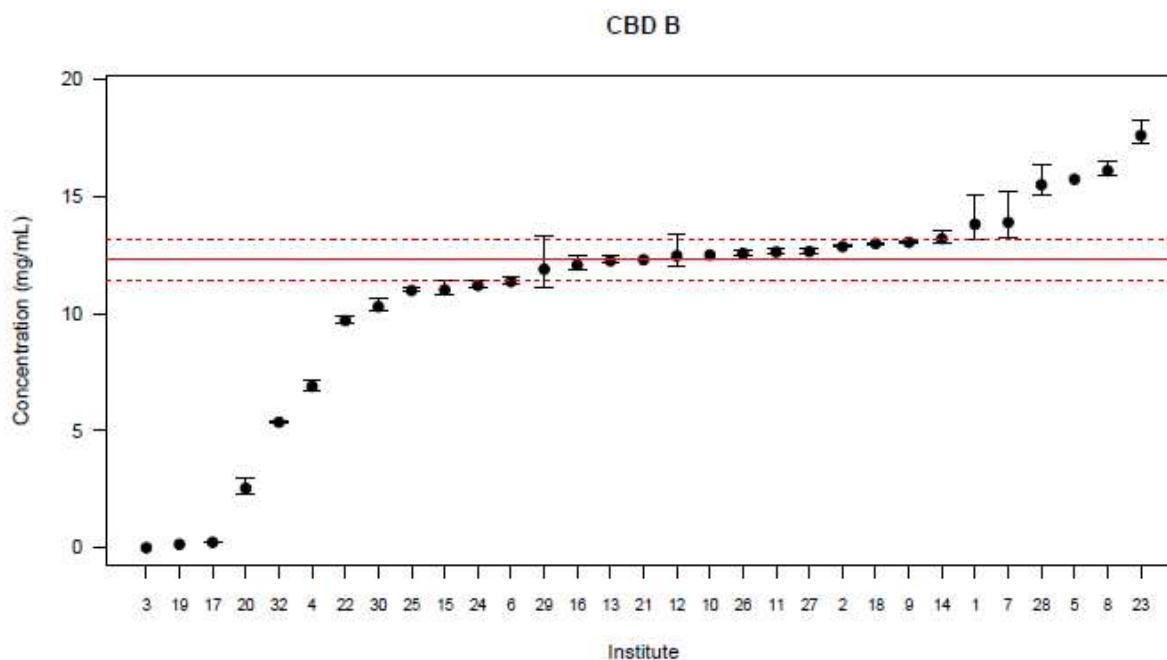


Figure 5. Calculated concentrations of CBD in LGC-RT/20/B by all participating laboratories. Error bars represent the standard uncertainty of the laboratories' replicate results.

6.2.3.3 LGC-RT/20/C

The stated CBD concentration on the package 0.5 mg/mL. Overall, there was good agreement with the majority of laboratories. Participants were asked to run each sample in singlicate on three separate days, generally there was good agreement between replicates – this is shown by the error bars in Figure 6.

Notably, Institutes 3 and 19 have submitted very low values and institute 17 stated CBD was either 'not detected' or below LOQ (N/D). Conversely, Institute 24 reported a high outlier compared to the others. Institute 26 did not report a result (N/R).

After sharing the preliminary results, Institutes 3, 19 and 32 expressed that they had found a dilution error in their calculations. As the corrected concentrations were received after the results were no longer blinded, the amended results were not accepted. However, this explains why these results are outliers. The results did not need to be removed from the calculation of the consensus value due to the use of robust statistics, meaning the outlier results do not affect the consensus value. These z-scores have been highlighted in red as known errors.

20 laboratories had satisfactory z-scores, out of 30 laboratories that submitted a results, this equates to 67%. If the results are calculated excluding the laboratories that have found errors, the z-score increases to 74%. Results that were reported as not detected, zero or below LOQ (N/D) are included in this calculation. However, results that were not reported (N/R) were not included.

Table 10. Individually calculated z-scores for CBD in LGC-RT/20/C. Where green are satisfactory scores and orange are scores that need further investigation. Laboratories marked in red had already found a calculation error after preliminary results were released but before the final report.

Sample LGC-RT/20/C CBD		
Laboratory	Mean concentration (mg/mL)	z-score
1	0.58	2.06
2	0.47	0.05
3	0.00	-8.64
4	0.47	0.12
5	0.82	6.51
6	0.44	-0.56
7	0.48	0.26
9	0.46	-0.21
10	0.48	0.22
11	0.49	0.40
12	0.48	0.22
13	0.74	5.08
14	0.45	-0.37
15	0.44	-0.48
16	0.47	-0.05
17	N/D	
18	0.57	1.88
19	0.01	-8.46
20	0.46	-0.15
21	0.44	-0.58
22	0.30	-3.11
23	0.54	1.29
24	1.60	20.96
25	0.44	-0.50
26	N/R	
27	0.49	0.34
28	0.53	1.14
29	0.43	-0.76
30	0.36	-1.94
31	0.63	3.07
32	0.24	-4.21

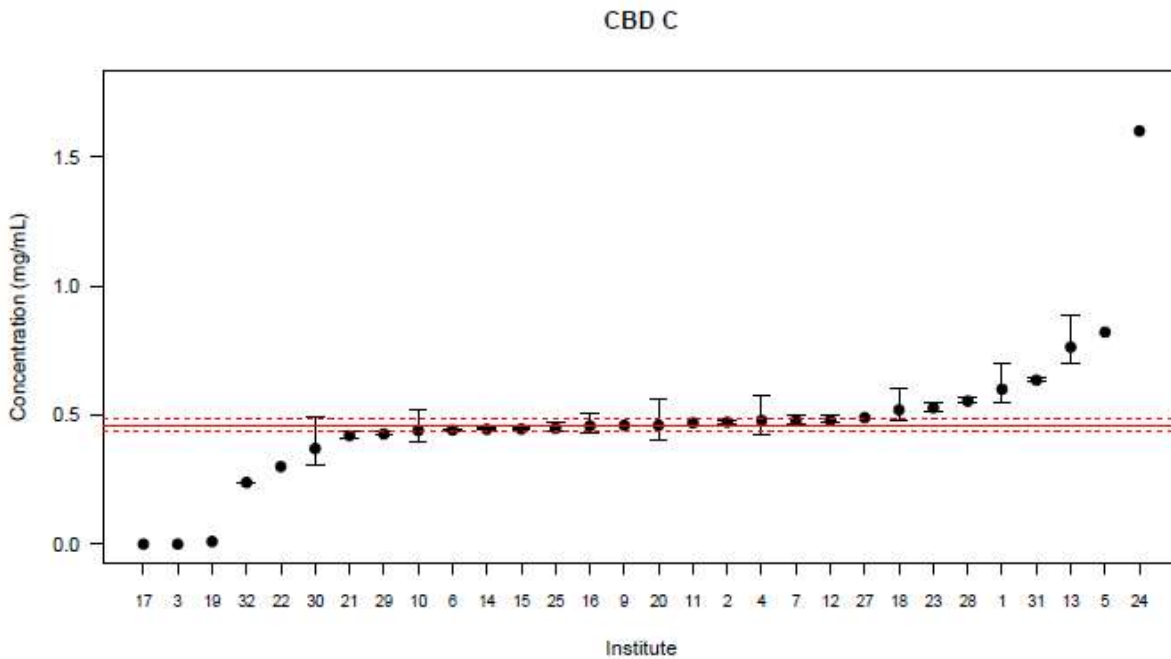


Figure 6. Calculated concentrations of CBD in LGC-RT/20/C by all participating laboratories. Error bars represent the standard uncertainty of the laboratories' replicate results.

6.3 Controlled Cannabinoids

6.3.1 Method Information

29 laboratories reported cannabinoid results for the quantification of all three samples, although not every laboratory looked for all five cannabinoids. The majority of laboratories used their own extraction and analysis methods, with a few using LGC's method or modifying the LGC method (Table 11). The most popular extraction technique was dilution with liquid-liquid and solid-phase extractions also being used by a few laboratories (Table 12). A range of instrument LODs were reported (Figures 7, 10, 14, 18 and 19). Total LLOQs were not reported because laboratories used different dilution factors for each sample. A range of internal standards were used, however, the majority of laboratories did not use an internal standard (Table 13).

Table 11. Methods used when analysing ring trial samples for controlled cannabinoids.

	LGC	Modified LGC	Own Method
Cannabinoid Extraction Method	4	2	22
Cannabinoid Analysis Method	2	1	25

Table 12. Type of extractions used when extracting cannabinoids from the ring trial samples.

Extraction	Number of Laboratories
Dilution	21
Liquid-Liquid	5
Solid Phase	2
N/R	1

Table 13. Internal standards used when analysing the samples for controlled cannabinoids.

	Number of Laboratories				
	CBN	THCV	Δ^8 -THC	Δ^9 -THC	Δ^9 THCA-A
None	10	9	9	10	11
CBN-D3	6	3	0	0	0
Δ^9 -THC-D3	4	3	8	12	1
CBD-D3	1	1	0	1	1
Δ^9 -THCA-A-D3	0	0	0	0	6
Tribenzylamine	0	0	0	1	0
Benzonitrile	0	0	0	1	0
N/R	8	13	12	4	10

6.3.2 Cannabinoid Consensus Values

The data was examined using plots and tables. Figures 8, 9, 11, 12, 13, 15, 16 and 17 (in sections 6.3.5 and 6.3.6) show the CBN, Δ^9 -THC and THCV concentration measurements obtained by the different laboratories for sample A, B and C. Laboratories were asked to run each sample in singlicate on three separate days. Generally there was good agreement between replicates – this is shown by the error bars on these figures. Although all three samples were analysed for 5 analytes (20 datasets), only three datasets had sufficient usable data for a meaningful consensus and uncertainty to be computed. These were from Sample A. Not enough usable data for statistical analysis was obtained from sample B or C due to the amount of ‘not detected’ and ‘not reported’ results. Therefore Table 14 has only three rows instead of 20.

From the shape of the useable plots, the distribution is approximately symmetrical, but departs from normal distribution. This is expected behaviour for inter-laboratory studies, which typically contain outliers and long distribution tails.

Given the lack of normal distribution and the presence of extreme values in the data, a calculation of the mean and standard error is inappropriate. Of the two possible approaches (outlier removal or robust statistics), the latter is preferred because it avoids decisions regarding the removal of extreme data while reducing their influence. Robust methods are also standard practice in inter-laboratory studies, and the same approach has been followed here.

Since the dispersion between institutes is relatively large compared to their standard uncertainties, only a single estimate for each institute is needed to compute a consensus value for each analyte, typically the mean or median.

The most commonly used robust estimator for the population mean is the median, which has a standard uncertainty given by equation 1.

$$u = \sqrt{\frac{\pi}{2n}} \text{MAD}_{\bar{x}}$$

Equation 1.

Where MAD_E is the median absolute deviation of the individual estimates, scaled to be an equivalent estimator for the population standard deviation, and n is the number of replicate values. Here, n is the number of institutes.

Table 14 Calculated cannabinoid consensus concentrations, expressed as $\mu\text{g/mL}$ and shown by sample and analyte. Only results for which both a quantifiable estimate and a non-zero uncertainty were obtained are included. Degrees of freedom were sufficient for a coverage factor $k = 2$ to be used for the expanded uncertainty.

Sample	Analyte	Median ($\mu\text{g/mL}$)	MAD_E ($\mu\text{g/mL}$)	n	u	k	U	$U(\%)$
Sample A	CBN	65.2375	10.0635	22	2.6892	2.0796	5.5925	8.57
Sample A	THCV	1.9160	2.8405	16	0.8901	2.1314	1.8972	99.01
Sample A	Δ^9 -THC	80.3380	22.8432	28	5.4108	2.0518	11.1021	13.82

6.3.3 Cannabinoid Z-Score Calculations and Interpretations

The cannabinoid z-scores for each laboratory were calculated by

$$z = \frac{(x - x_a)}{\sigma_p}$$

Equation 2.

Where x is the mean result reported by the participant, x_a is the consensus value and σ_p is the robust estimate of the standard deviation. This is the median absolute deviation (MAD) which is calculated as follows: for each result, the absolute difference between its value and the overall median is calculated. The MAD is the median of the absolute differences. To obtain a scaled equivalent to the standard deviation, the MAD is then multiplied by 1.483.

In normal circumstances, ~ 95 % of z-scores will be in the range $|z| \leq 2$. These scores are designated “satisfactory”.

A rate of 1 in 20 would be expected for z-scores in the range $2 < |z| \leq 3$. These scores may require further investigation.

Scores where $|z| > 3$ are to be expected at a rate of about 1 in 300. These z-scores indicate that the result is not fit-for-purpose and certainly requires investigation.

6.3.5 THCV Results

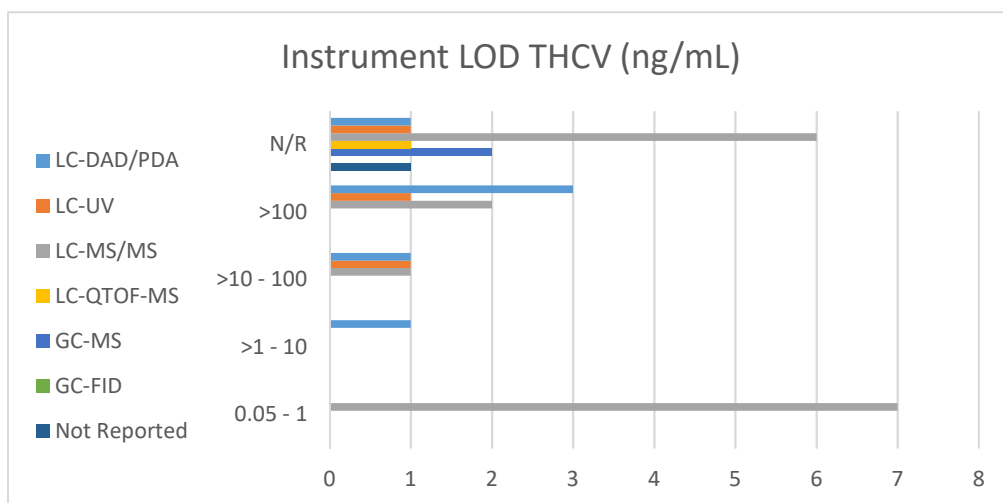


Figure 7. Instrument limit of detection of participating laboratories when measuring THCV.

6.3.5.1 LGC-RT/20/A

9 laboratories detected THCV in the sample. 7 laboratories reported they either could not detect THCV, it was zero, or <LOQ (N/D). 13 laboratories did not report THCV (N/R).

Although a consensus value for THCV in LGC-RT/20/A was calculated, the observed relative standard deviation was over 100% suggesting a skewed distribution. Therefore, individual z-scores were not calculated because it is assumed that z is a normally distributed variable. Therefore, only the reported concentrations have been listed in Table 15.

Notably, Institute 24 has reported a high result. However, the result did not need to be removed from the calculation of the consensus value due to the use of robust statistics, meaning the outlier result does not affect the consensus value. The data is shown both with and without institute 24 in Figure 8 and 9.

Of the nine laboratories that reported a concentration for THCV, eight of them used LC-MS/MS whereas one used LC-DAD/PDA, suggesting LC-MS/MS is the more sensitive technique for THCV analysis.

Table 15. Median reported concentrations for THCv in LGC-RT/20/A.

Sample LGC-RT/20/A THCv	
Laboratory	Median concentration (µg/mL)
1	2.4
2	13.1
3	2.9
4	N/R
5	N/D
6	N/R
7	N/D
8	N/R
9	N/R
10	N/D
12	N/R
13	N/D
14	6.4
15	N/D
16	2.1
17	N/R
18	N/D
19	N/R
20	N/R
22	3.2
23	N/R
24	212.3
25	N/R
26	2.2
27	N/R
28	N/R
29	N/R
30	N/D
32	1.8

Cannabinoid A (THCV)

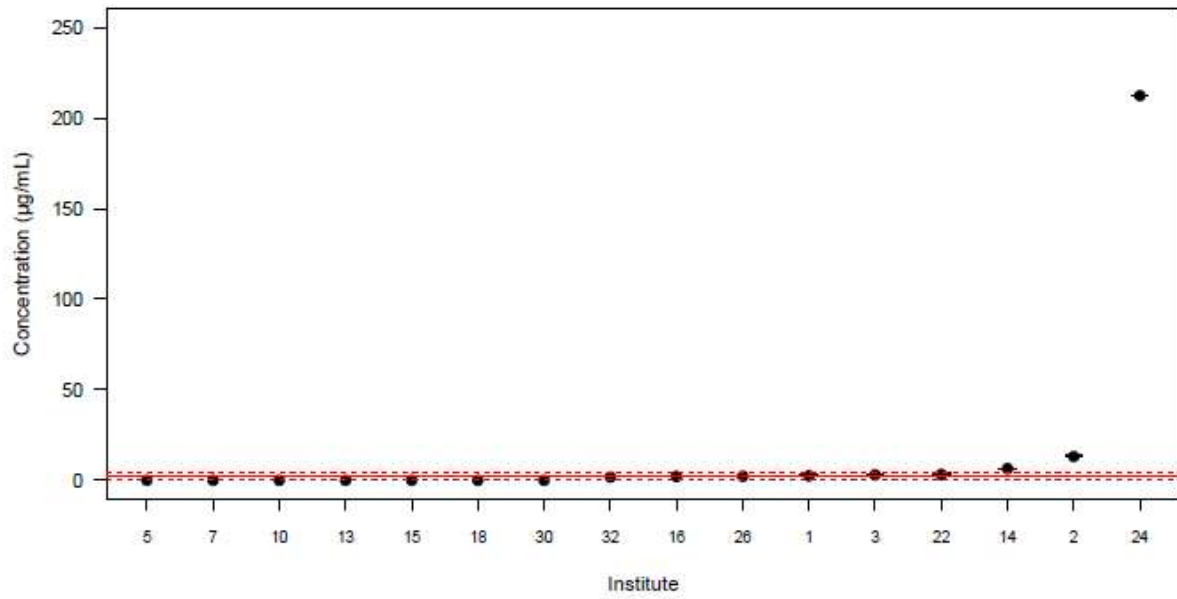


Figure 8. Reported concentrations of THCV in LGC-RT/20/A by all participating laboratories. Error bars represent the standard uncertainty of the laboratories' replicate results (K=1).

Cannabinoid A (THCV)

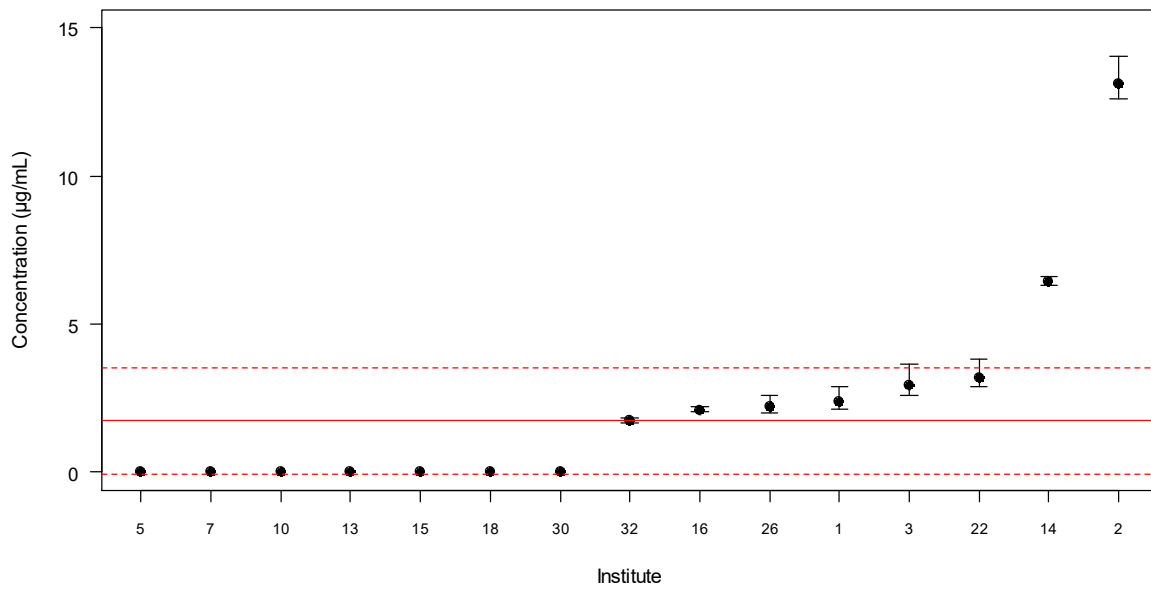


Figure 9. Reported concentrations of THCV in LGC-RT/20/A where **laboratory 24 has been removed**. Error bars represent the standard uncertainty of the laboratories' replicate results (K=1).

6.3.5.2 LGC-RT/20/B

2 laboratories detected THCv in the sample. Notably, Institute 24 reported a high result of 252.1 µg/ml, whereas Institute 14 reported a low result of 0.1 µg/ml. 14 laboratories reported they either could not detect THCv, it was zero, or <LOQ. 13 laboratories did not report THCv.

6.3.5.3 LGC-RT/20/C

Zero laboratories detected THCv.

6.3.6 CBN Results

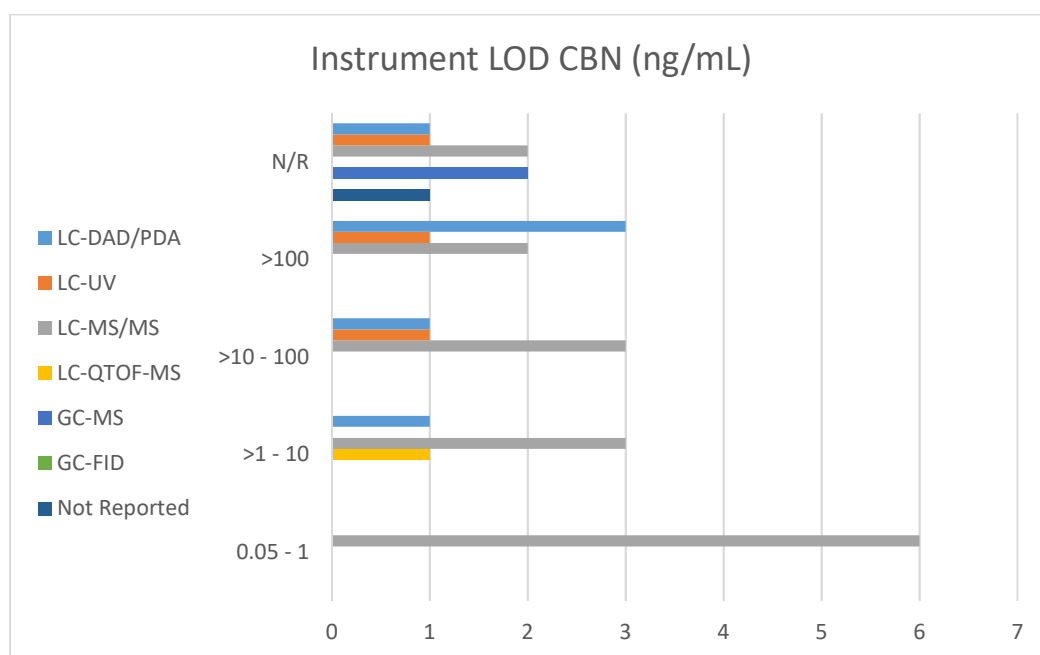


Figure 10. Instrument limits of detection when analysing for CBN.

6.3.6.1 LGC-RT/20/A

21 laboratories detected CBN in the sample. 1 laboratory reported they either could not detect CBN, it was zero, or <LOQ (N/D). 7 laboratories did not report CBN (N/R).

After sharing the preliminary results, Institute 27 found a dilution factor error in their calculations. As the corrected concentration was received after the results were no longer blinded, the amended result was not accepted. However, this explains why this result is an outlier. The result did not need to be removed from the calculation of the consensus value due to the use of robust statistics, meaning the outlier result does not affect the consensus value. This z-score has been highlighted in red as a known error.

16 laboratories had satisfactory z-scores, out of the 22 laboratories that submitted a results, this equates to 73%. Results that were reported as not detected, zero or below LOQ (N/D) are included in this calculation. However, results that were not reported (N/R) were not included. If you calculate the results without the laboratory that found an error, the z-score increases to 76%.

Table 16. Individually calculated z-scores for CBN in LGC-RT/20/A. Where green are satisfactory scores and orange are scores that need further investigation. Laboratories marked in red had already found a calculation error after preliminary results were released but before the final report.

Sample LGC-RT/20/A CBN		
Laboratory	Median concentration (µg/mL)	z-score
1	63.0	-0.22
2	67.8	0.26
3	77.8	1.25
4	N/R	
5	77.0	1.17
6	N/R	
7	69.1	0.39
8	68.7	0.34
9	70.3	0.51
10	65.5	0.03
12	56.8	-0.84
13	41.3	-2.38
14	110.6	4.50
15	25.3	-3.97
16	65.5	0.02
17	N/R	
18	65.0	-0.02
19	N/R	
20	N/R	
22	60.3	-0.49
23	85.8	2.04
24	N/D	
25	N/R	
26	66.4	0.12
27	33.0	-3.20
28	48.1	-1.70
29	N/R	
30	48.5	-1.66
32	61.5	-0.37

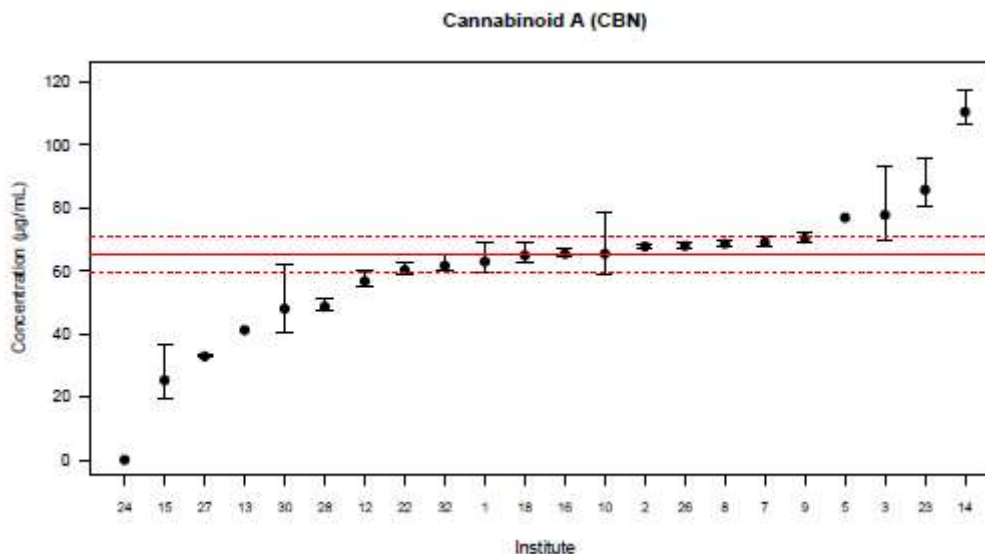


Figure 11. Reported concentrations of CBN in LGC-RT/20/A by all participating laboratories. Error bars represent the standard uncertainty of the laboratories' replicate results (K=1).

6.3.6.2 LGC-RT/20/B

11 laboratories detected CBN in the sample. 10 laboratories reported they either could not detect CBN, it was zero or <LOQ. 8 laboratories did not report CBN. Institute 15 has reported a notably high result that has therefore been removed from Figure 13.

Due to the lack of reported concentrations, there was not enough statistically significant data to calculate a consensus value or z-scores. Therefore, only the concentrations are listed in Table 17.

Table 17. Median concentrations for CBN in LGC-RT/20/B.

Sample LGC-RT/20/B CBN	
Laboratory	Median concentration (µg/mL)
1	1.0
2	N/D
3	3.5
4	N/R
5	N/D
6	N/R
7	N/D
8	1.8
9	1.7
10	N/D
12	N/D
13	N/D
14	1.8
15	52.6
16	1.7
17	N/R
18	N/D
19	N/R
20	N/R
22	1.6
23	N/D
24	N/D
25	N/R
26	1.5
27	N/R
28	N/D
29	N/R
30	3.6
32	1.6

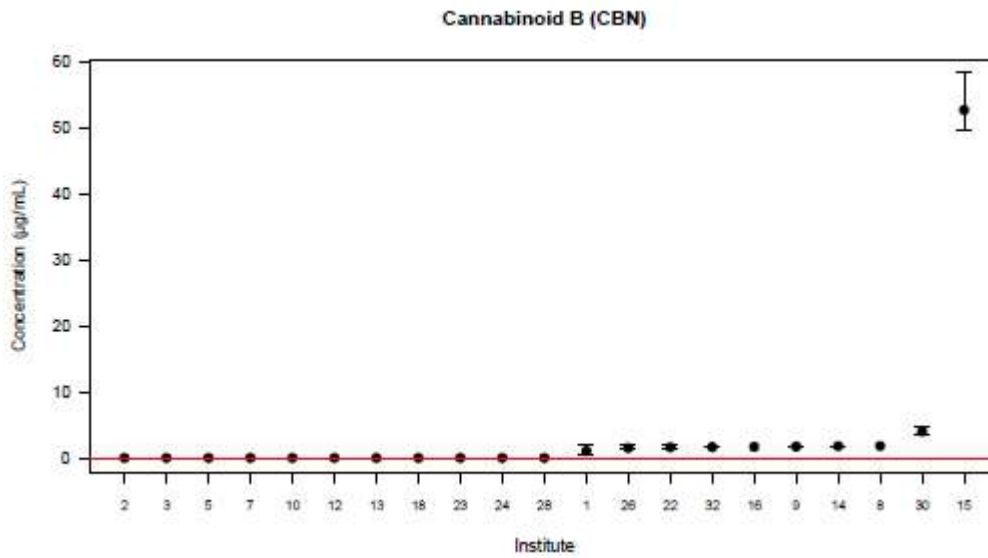


Figure 12. Reported concentrations of CBN in LGC-RT/20/B by all participating laboratories. Error bars represent the standard uncertainty of the laboratories' replicate results (K=1).

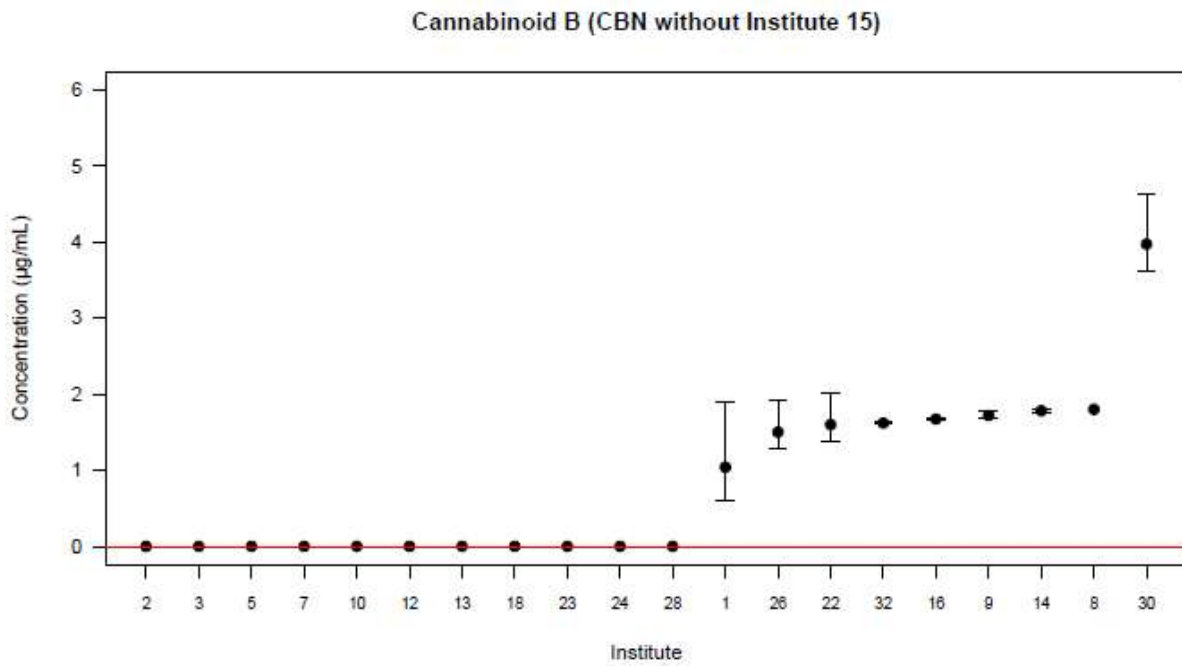


Figure 13. Reported concentrations of CBN in LGC-RT/20/B **without laboratory 15**. Error bars represent the standard uncertainty of the laboratories' replicate results (K=1).

6.3.6.3 LGC-RT/20/C

Zero laboratories detected CBN in LGC-RT/20/C.

6.3.7 Δ^9 -THC

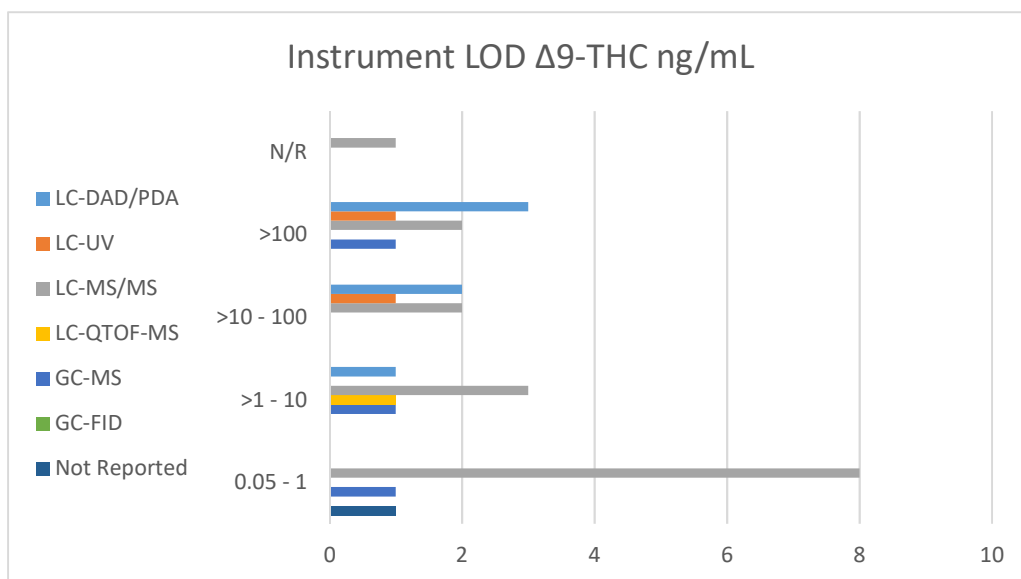


Figure 14. Instrument limits of detection when analysing for Δ^9 -THC.

6.3.7.1 LGC-RT/20/A

25 laboratories reported a concentration for Δ^9 -THC. 1 laboratory combined the Δ^8 -THC and Δ^9 -THC concentration but is included in the Figure 15 and Table 18. One laboratory could not confirm the presence of Δ^9 -THC, one laboratory could not detect Δ^9 -THC (N/D), and one laboratory did not report Δ^9 -THC (N/R).

24 laboratories had satisfactory z-scores out of the 28 laboratories that submitted a result, this equates to 86%. Results that were reported as not detected, zero or below LOQ (N/D) are included in this calculation. However, results that were not reported (N/R) were not included.

Table 18. Individually calculated z-scores for Δ^9 -THC in LGC-RT/20/A. Where green are satisfactory scores and orange are scores that need further investigation.

Sample LGC-RT/20/A Δ^9 -THC		
Laboratory	Median concentration ($\mu\text{g}/\text{mL}$)	z-score
1	Not confirmed	
2	73.6	-0.30
3	96.3	0.70
4	64.5	-0.69
5	85.0	0.20
6	81.0	0.03
7	96.2	0.70
8	84.3	0.17
9	82.1	0.08
10	71.3	-0.40
12	60.9	-0.85
13	82.9	0.11
14	89.6	0.41
15	102.2	0.96
16	80.7	0.01
17	17.7	-2.74
18	76.0	-0.19
19	N/D	
20	56.1	-1.06
22	99.6	0.84
23	N/R	
24	150.6	3.08
25	58.1	-0.97
26	80.0	-0.01
27	68.8	-0.51
28	65.3	-0.66
29	93.4	0.57
30	38.1	-1.85
32	101.0	0.91

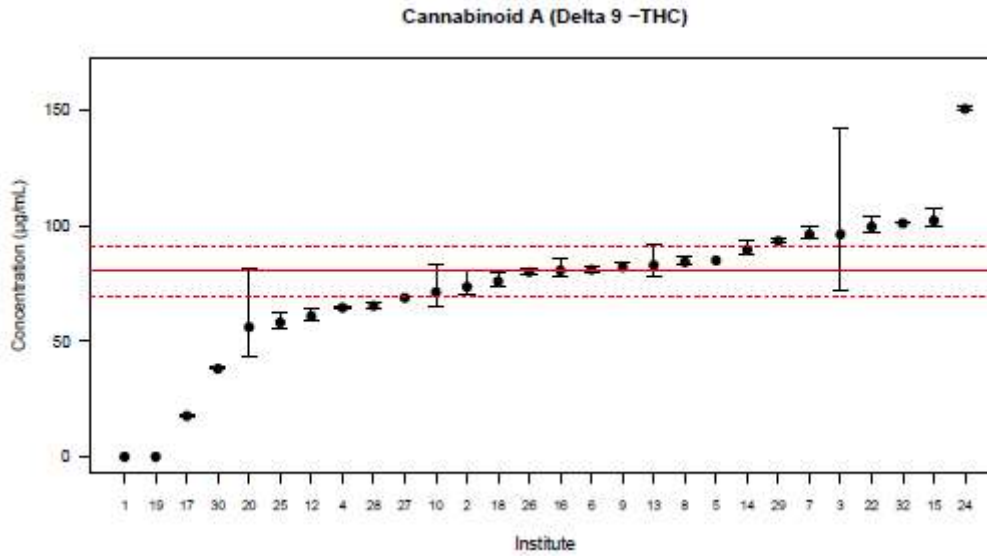


Figure 15. Reported concentrations of Δ^9 -THC in LGC-RT/20/A by all participating laboratories. Error bars represent the standard uncertainty of the laboratories' replicate results ($K=1$).

6.3.7.2 LGC-RT/20/B

11 laboratories reported a concentration for Δ^9 -THC. 1 laboratory combined the Δ^8 -THC and Δ^9 -THC concentration but is included in Figure 16 and Table 19 as the combined value. 14 laboratories either could not detect Δ^9 -THC, reported it was zero or reported as <LOQ. One laboratory could not confirm the presence of Δ^9 -THC and two laboratories did not report Δ^9 -THC.

Due to the lack of reported concentrations, there was not enough statistically significant data to calculate a consensus value or z-scores. Therefore, only the concentrations are listed in Table 19.

Table 19. Median concentrations for Δ^9 -THC in LGC-RT/20/B.

Sample LGC-RT/20/B Δ^9 -THC	
Laboratory	Median concentration ($\mu\text{g/mL}$)
1	Not confirmed
2	N/D
3	5.6
4	N/D
5	N/D
6	8.1
7	N/D
8	3.4
9	3.4
10	N/D
12	N/D
13	11.6
14	4.2
15	N/D
16	5.5
17	N/D
18	N/D
19	N/D
20	2.6
22	9.4
23	N/R
24	N/D
25	N/D
26	6.9
27	N/R
28	N/D
29	3.1
30	N/D
32	6.8

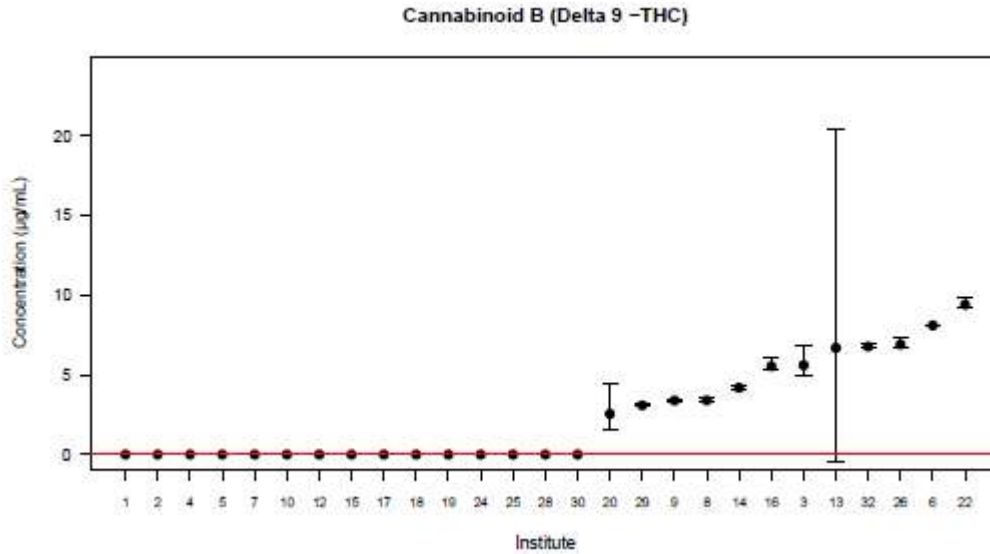


Figure 16. Reported concentrations of Δ^9 -THC in LGC-RT/20/B by all participating laboratories. Error bars represent the standard uncertainty of the laboratories' replicate results (K=1).

6.3.7.3 LGC-RT/20/C

5 laboratories reported a concentration for Δ^9 -THC, two of which were high results (0.2 µg/ml, 0.2 µg/ml, 0.4 µg/ml, 75 µg/ml, and 143 µg/ml). 22 laboratories either could not detect Δ^9 -THC, reported it was zero or reported as <LOQ. Two laboratories did not report Δ^9 -THC.

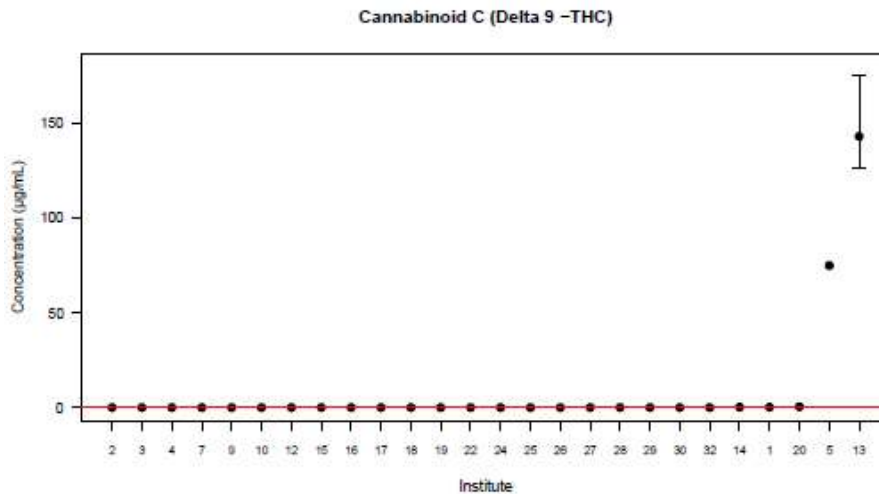


Figure 17. Reported concentrations of Δ^9 -THC in LGC-RT/20/C by all participating laboratories. Error bars represent the standard uncertainty of the laboratories' replicate results (K=1).

6.3.8 Δ^8 -THC

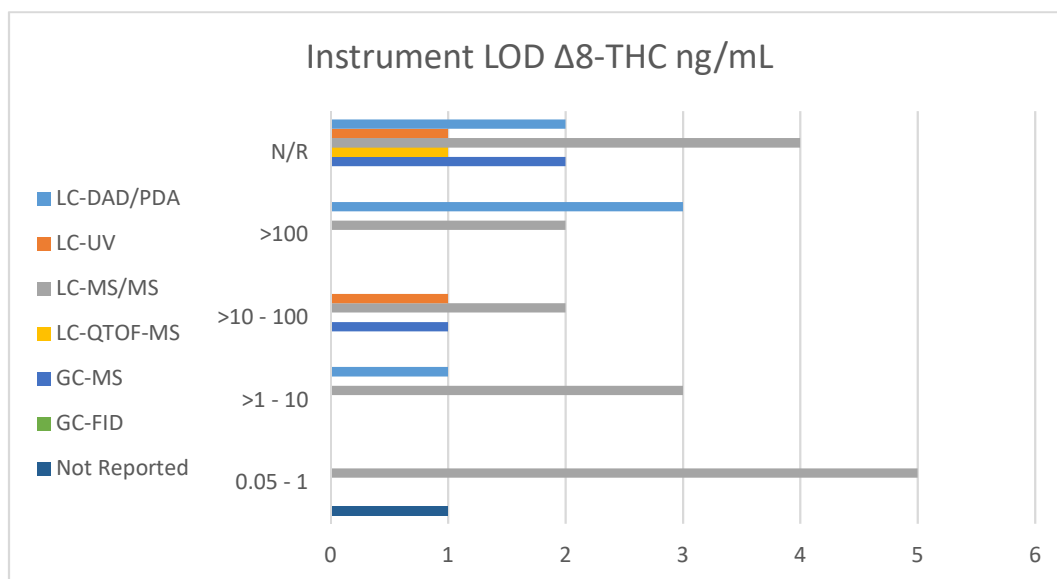


Figure 18. Instrument limits of detection when analysing for Δ^8 -THC.

6.3.8.1 LGC-RT/20/A

6 laboratories reported a concentration for Δ^8 -THC over a wide range (3 $\mu\text{g/ml}$, 5 $\mu\text{g/ml}$, 13 $\mu\text{g/ml}$, 24 $\mu\text{g/ml}$, 43 $\mu\text{g/ml}$ and 62 $\mu\text{g/ml}$). 1 laboratory combined the Δ^8 -THC and Δ^9 -THC. One laboratory could not confirm the presence Δ^8 -THC. 10 laboratories either could not detect Δ^8 -THC, reported it was zero or reported as <LOQ. 11 laboratories did not report Δ^8 -THC.

6.3.8.2 LGC-RT/20/B

3 laboratories reported a concentration for Δ^8 -THC (2 $\mu\text{g/ml}$, 4 $\mu\text{g/ml}$ and 7 $\mu\text{g/ml}$). 1 laboratory combined the Δ^8 -THC and Δ^9 -THC concentration. 14 laboratories either could not detect Δ^8 -THC, reported it was zero or reported as <LOQ. 11 laboratories did not report Δ^8 -THC.

6.3.8.3 LGC-RT/20/C

Zero laboratories detected Δ^8 -THC.

6.3.9 Δ^9 THCA-A

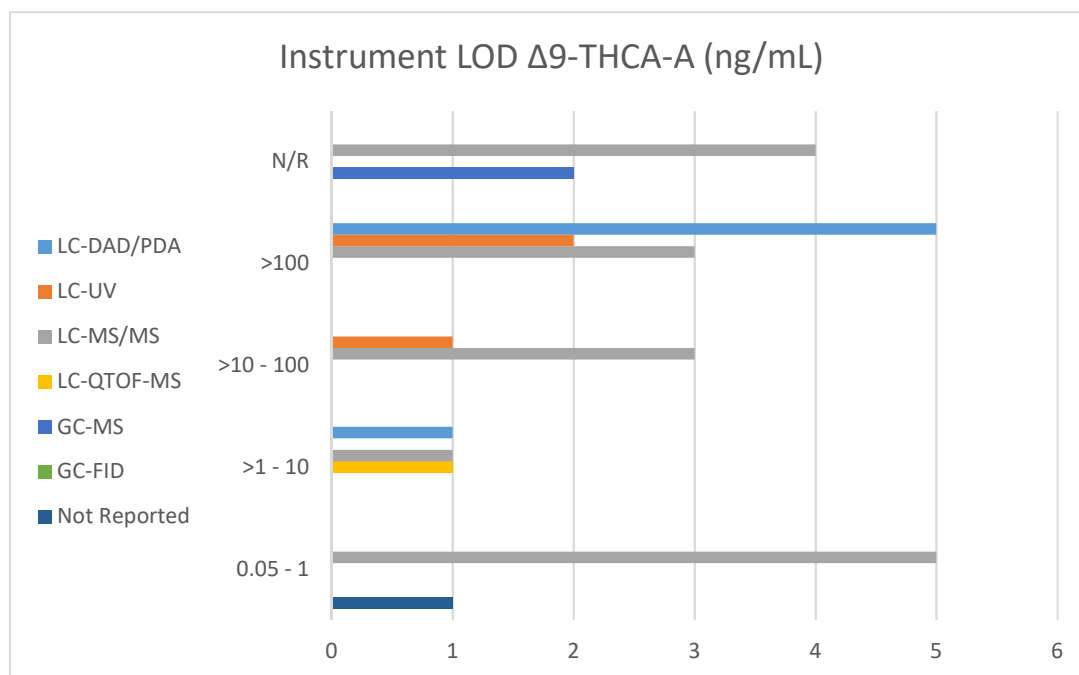


Figure 19. Instrument limits of detection when analysing for Δ^9 -THCA-A.

6.3.9.1 LGC-RT/20/A

3 laboratories reported a concentration for Δ^9 -THCA-A over a large range (0.1 $\mu\text{g/ml}$, 131 $\mu\text{g/ml}$ and 339 $\mu\text{g/ml}$). 19 laboratories could not detect or confirm Δ^9 -THCA-A, reported it was zero or reported as <LOQ. 7 laboratories did not report Δ^9 -THCA-A.

6.3.9.2 LGC-RT/20/B

Zero laboratories detected Δ^9 -THCA-A.

6.3.9.3 LGC-RT/20/C

Zero laboratories detected Δ^9 -THCA-A.

6.4 Result Trends

Trends in results when compared with instrumentation type and use of internal standard were initially plotted but the results were scattered and no deeper analysis was completed. However, further statistical analysis may be able to pull out trends in the reported results.

It is, however, noted that LC-MS/MS is the more sensitive instrumentation to be used according to instrument LODs. This could be crucial in controlled cannabinoid analysis as these analytes can be present at lower levels. This is shown in this study in sample LGC-RT/20/A for analyte THCV, where 8 of the 9 laboratories that reported a concentration were using LC-MS/MS. Although there were no trends in internal standard use, it is best practice to use an internal standard, preferably labelled, to account for matrix effects. Since the CBD food and cosmetics come in a variety of forms, there is always a possibility of matrix effects.

6.5 Controlled Status in the UK

Of the 14 UK laboratories, 13 reported results for controlled cannabinoids. 10 laboratories reported that sample LGC-RT/20/A should be controlled. 1 reported that LGC-RT/20/B should be controlled. Zero reported that LGC-RT/20/C should be controlled.

7 Conclusions

The ring trial was very successful. 35 international laboratories signed up and samples were sent to all laboratories. 32 data sets were returned. There was an interesting range of methods and instrument types shared. The data shows good agreement between most laboratories.

CBD measurement was considered a success, with most institutes agreeing. The consensus concentrations were similar to the stated pack concentrations. On average, 82% of laboratories reported satisfactory results for CBD.

Controlled cannabinoid measurement was more varied with a wider spread of results. Most laboratories quantified CBN and Δ 9-THC in LGC-RT/20/A, with 76% and 86% of laboratories respectively reporting satisfactory results. The THCv consensus value in LGC-RT/20/A was calculated as a low concentration, which lead to more variable results and a large portion of laboratories reporting they could not detect THCv. All cannabinoids in LGC-RT/20/B and LGC-RT/20/C had variable results; many of laboratories reported they could not detect the cannabinoids and therefore there was not enough statistically significant data to calculate consensus values.

From the limit of detection data shared, LC-MS/MS is the more sensitive instrumentation to be used. This could be crucial in controlled cannabinoid analysis as these analytes can be present at lower levels, as shown in this study in sample LGC-RT/20/A for analyte THCv. There did not appear to be significant trends in technology or internal standard used, however, it is best practice to use an internal standard to account for matrix effects.

8 Participating Laboratories

The data is anonymous and therefore randomised. The participating laboratories listed below are not named in order of laboratory number.

- Advanced Development and Safety Laboratory Ltd, UK
- AGES Institute for Food Safety Vienna, Austria
- Amt für Verbraucherschutz und Veterinärwesen (AVSV), Switzerland
- British American Tobacco Group R&D, UK
- Bridge Farm Group, UK
- CBD Health Foods Ltd, UK
- Croatian Institute of Public Health, Croatia
- CVUA Karlsruhe Kosmetic, Germany
- CVUA Karlsruhe ZSL-HPLC, Germany
- Eurofins Food Testing UK Ltd, UK
- Fera Science Ltd, UK
- Glasgow Scientific Services, UK
- Health Sciences Authority, Singapore
- Institute of Public Health of Belgrade, Serbia
- Kent County Council, UK
- King's Forensics, UK
- Landeslabor-bbb, Germany
- LGC, UK
- Masdiag/HempEkspert, Poland
- MHRA, UK
- Minton Treharne & Davies Limited (MTD), UK
- National Analytical Forensic Services (NAFS), South Africa
- National Laboratory of Health, Environment and Food, Slovenia
- Phytovista Laboratories, UK
- Sample Control d.o.o., Croatia
- SOCOTEC, UK
- State General Laboratory of Cyprus
- The Public Analyst Laboratory Dublin, Ireland
- The State Laboratory, Ireland

9 Appendix 1 - Protocol

Ring Trial Protocol - Analysis of Cannabidiol and Controlled Cannabinoids in Food and Cosmetic Samples

T. Hambidge





Ring Trial Protocol - Analysis of Cannabidiol and Controlled Cannabinoids in Food and Cosmetic Samples

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10 Contents

1	Contents	4
2	Summary	5
3	Samples.....	6
3.1	LGC-RT/20/A.....	6
3.2	LGC-RT/20/B.....	6
3.3	LGC-RT/20/C.....	6
4	Homogeneity.....	7
5	Shipping.....	8
6	Ring Trial Results.....	8
6.1	Technologies	8
6.2	CBD.....	10
6.2.1	Method Information	10
6.2.2	CBD Consensus Values	11
6.2.3	CBD z-Score Calculations and Interpretations	12
6.3	Controlled Cannabinoids.....	19
6.3.1	Method Information	19
6.3.2	Cannabinoid Consensus Values	20
6.3.3	Cannabinoid Z-Score Calculations and Interpretations	21
6.3.5	THCV Results.....	22
6.3.6	CBN Results	25
6.3.7	Δ 9-THC.....	31
6.3.8	Δ 8-THC.....	36
6.3.9	Δ 9THCA-A.....	37
6.4	Result Trends	37
6.5	Controlled Status in the UK.....	38
7	Conclusions	38
8	Participating Laboratories	39
1	Ring Trial Introduction.....	6
2	Timeline.....	6
3	LGC Supplied Samples.....	7
3.1	LGC-RT/20/A Food.....	7
3.2	LGC-RT/20/B Food.....	7
3.3	LGC-RT/20/C Cosmetic	7
4	Ring Trial Requirements	7

4.1	CBD	7
4.2	Controlled Cannabinoids (Optional)	7
5	Reporting Results	8
6	Appendix 1 – CBD Method	9
6.1	Scope	9
6.2	Background and Method Principle	9
6.3	Apparatus	9
6.4	Reagents	10
6.5	Calibration Preparation	10
6.5.1	Stock Standard	10
6.5.2	Intermediate Standard	10
6.5.3	Calibration Line	10
6.5.4	Autosampler Vial	10
6.6	Internal Standard Preparation	10
6.6.1	Stock Standard	10
6.6.2	Intermediate Standard	10
6.6.3	Working Standard	10
6.7	Sample Preparation	11
6.7.1	Food Oil Samples	11
6.7.2	Cosmetic Body Wash Samples (no centrifuging)	11
6.8	HPLC Conditions	12
6.9	MS Condition	13
7	Appendix 2 - Controlled Cannabinoid Method	14
7.1	Scope	14
7.2	Background and Method Principle	14
7.3	Apparatus	14
7.4	Reagents	15
7.5	Calibration Preparation	15
7.5.1	Stock Standard	15
7.5.2	Individual Intermediate Standard	15
7.5.3	Mixed Intermediate Standard	15
7.5.4	Calibration Line	15
7.5.5	Autosampler Vial	15
7.6	Internal Standard Preparation	16
7.6.1	Stock Standard	16
7.6.2	Individual Intermediate Standards	16

7.6.3	Working Standard	16
7.7	Sample Preparation	16
7.7.1	Food Oil Samples	16
7.7.2	Cosmetic Body Wash Sample (No Centrifuging).....	17
7.8	HPLC Conditions	18
7.9	MS Conditions	18

1 Ring Trial Introduction

Cannabidiol (CBD) products have rapidly entered the UK market in a variety of forms, including food and cosmetics. As regulations are introduced to help govern the influx of CBD products; laboratories across the UK need to be able to accurately measure the CBD content as well as the controlled cannabinoid content in commercially available products. This ring trial aims to share and compare methods for quantifying CBD and controlled cannabinoids in food and cosmetics among UK testing laboratories. While CBD analysis is required, controlled cannabinoid analysis is optional.

LGC will provide the protocol and details of their CBD and controlled cannabinoid methods. Laboratories will receive three different samples to analyse consisting of two foods and one cosmetic. Participating laboratories may use LGC's method or their own method to analyse the samples for CBD and controlled cannabinoids (optional) listed in Table 1. A report template will be provided to fill out and return to LGC once analysis is completed. Participating laboratories will be anonymised when results are reported.

Table 20. Cannabinoids of Interest

Cannabinoid	Abbreviation	CAS Number	Controlled Status
Cannabidiol	CBD	13956-29-1	No
Delta-9-tetrahydrocannabinol*	Δ^9 -THC	1972-08-3	Yes
Delta-9-tetrahydrocannabivarin*	THCV	31262-37-0	Yes
Cannabinol*	CBN	521-35-7	Yes
Delta-8-tetrahydrocannabinol*	Δ^8 -THC	5957-75-5	Yes
Delta 9-Tetrahydrocannabinolic acid*	Δ^9 THCA-A	23978-85-0	No - but readily converts to THC

*Optional

2 Timeline

Table 21. Ring Trial Timeline

Date	Action
29 th January, 2021	Deadline for Registration
18 th January – 5 th February, 2021	Sample Distribution
12 th March, 2021	Deadline for Submission of Results
April, 2021	Reporting of Results

3 LGC Supplied Samples

3.1 LGC-RT/20/A Food

Sample Type: CBD Hemp Food Oil
CBD range: 0.01 – 5% CBD
Volume of commercial package: 30 mL
Volume supplied to participating laboratories: 5 mL

3.2 LGC-RT/20/B Food

Sample Type: CBD Food Oil
CBD range: 0.01 – 5% CBD
Volume of commercial package: 30 mL
Volume supplied to participating laboratories: 5 mL

3.3 LGC-RT/20/C Cosmetic

Sample Type: CBD Cosmetic
CBD range: 0.01 – 5% CBD
Volume of commercial package: 100 mL
Volume supplied to participating laboratories: 5 mL

4 Ring Trial Requirements

4.1 CBD

- You may use your own method or LGC's
- Extract each sample in singlicate on three separate days
- Quantify all three samples for CBD content
- Please report your results in the report template provided in a separate email and return to LGC (Section 6).

4.2 Controlled Cannabinoids (Optional)

- You may use your own method or LGC's
- Extract each sample in singlicate on three separate days
- Quantify all three samples for the five cannabinoids listed in Table 1 (Δ^9 -THC, THCV, CBN, Δ^8 -THC and Δ^9 THCA-A)
- Please report your results in the report template provided in a separate email and return to LGC (Section 6).

5 Reporting Results

Please report results via the excel report template that is provided.

Results required – method details (limits of detection, dilution factors, instrumentation, internal standards, etc) and calculated concentrations of CBD and optional cannabinoids of each sample per mL and per pack.

Please return your completed results template to tabatha.hambidge@lqcgroup.com and christopher.hopley@lqcgroup.com by 12th March, 2021.

6 Appendix 1 – CBD Method

6.1 Scope

This method is suitable for the quantification of Cannabidiol (CBD) in CBD food oil and cosmetic body wash.

6.2 Background and Method Principle

Cannabidiol (CBD) products have recently entered UK food markets. Laboratories need to be able to accurately measure the CBD content to check authenticity of samples and consumer safety.

The food and cosmetic matrices are diluted into propan-2-ol, acetonitrile and then acetonitrile/water 50/50 v/v. The extracts are then analysed via LC-MS/MS.

6.3 Apparatus

- General laboratory glassware, including beakers, bottles and cylinders
- Falcon Tubes and Eppendorfs of various sizes
- 2 mL autosampler vials and vials caps, PTFE lined
- Positive displacement pipettes, delivering volumes in the range of 10 μ L – 1000 μ L
- Larger volume pipettes, multi-pipettes or volumetric glassware capable of dispensing larger volumes (1-50 mL)
- Vortex Mixer
- Sonicator
- Centrifuge capable of 3220 G and 15700 G
- HPLC column: BEH C8 2.5 μ m, 2.1x100 mm or equivalent.
- HPLC System
- Mass Spectrometer Detector

6.4 Reagents

All reagents must be of analytical reagent quality or better.

1. Methanol (Honeywell, Fisher UK)
2. Stock Cannabidiol (Cerilliant, part numbers listed in 6.5.1, 6.6.1)
3. Acetonitrile (Honeywell, Fisher UK)
4. Ultra high purity water ($\geq 18.2\text{m}\Omega$) e.g. Elga
5. Acetonitrile/Water 50/50 v/v
Mix 200 mL of acetonitrile with 200 mL of water. This solution is stable for one month when stored at room temperature.
6. Propan-2-ol (Fisher, UK)
7. Formic Acid (Sigma-Aldrich, UK)
8. Mobile Phase A - 0.1 % Formic Acid in Water
Mix 1 L of water with 1 mL of formic acid. This solution is stable for one week when stored at room temperature.
9. Mobile Phase B - 0.1 % Formic Acid in Acetonitrile
Mix 1 L of acetonitrile with 1 mL of formic acid. This solution is stable for two weeks when stored at room temperature.

6.5 Calibration Preparation

6.5.1 Stock Standard

The stock is acquired from Merck - Cerilliant 1 mg/mL CBD solution P/N: C-045

6.5.2 Intermediate Standard

An intermediate solution in 100% methanol is prepared to form a 100 $\mu\text{g}/\text{mL}$ solution.

6.5.3 Calibration Line

The intermediate solution is then used to prepare a calibration line in acetonitrile/water 50/50 v/v at 500, 400, 300, 200, 100 and 5 ng/mL.

6.5.4 Autosampler Vial

In an autosampler vial place 500 μL of calibration standard (6.5.3) and 100 μL Internal Standard working solution (6.6.3).

6.6 Internal Standard Preparation

6.6.1 Stock Standard

The stock is acquired from Merck - Cerilliant 100 $\mu\text{g}/\text{mL}$ CBD-D3 solution P/N: C-084.

6.6.2 Intermediate Standard

An intermediate solution in 100% methanol is prepared to form a 10 $\mu\text{g}/\text{mL}$ solution.

6.6.3 Working Standard

The intermediate solution is then used to prepare a working solution in acetonitrile/water 50/50 v/v at 500 ng/mL.

6.7 Sample Preparation

For each sample, dilute to fit within the calibration curve.

6.7.1 Food Oil Samples

6.7.1.1 Dilution 1

1. Vigorously vortex mix the sample, if bubbles form, wait for bubbles to dissipate before taking an aliquot for analysis.
2. Take a minimum of 500 μL of sample.
3. Dilute firstly with propan-2-ol.
4. Vortex mix.
5. Sonicate for 30 minutes.
6. Vortex mix.
7. Centrifuge for 10 minutes at 4000 RPM (3220 G).

6.7.1.2 Dilution 2

8. If layers are formed, aliquot from the top layer of previous dilution.
9. Dilute with acetonitrile.
10. Vortex mix.
11. Sonicate for 10 minutes.
12. Vortex mix.
13. Centrifuge for 10 minutes at 4000 RPM (3220 G).

6.7.1.3 Dilution 3

14. If layers are formed, aliquot from the top layer of previous dilution.
15. Dilute with acetonitrile/water 50/50 v/v.
16. Vortex mix.
17. Sonicate for 10 minutes.
18. Vortex mix.
19. If the solution is clear then centrifuge at 4000 RPM (3220 G) for 10 minutes. If the solution is cloudy, centrifuge at 12000 RPM (15700 G) for 10 minutes.
20. Add 500 μL of sample to 100 μL of internal standard working solution (6.6.3) in an autosampler vial.

6.7.2 Cosmetic Body Wash Samples (no centrifuging)

For each sample, dilute to fit within the calibration curve.

6.7.2.1 Dilution 1

1. Vigorously vortex mix the sample, if bubbles form, wait for bubbles to dissipate before taking an aliquot for analysis.
2. Take a minimum of 500 μL of sample.
3. Dilute firstly with propan-2-ol.
4. Vortex mix.
5. Sonicate for 30 minutes.
6. Vortex mix.

6.7.2.2 Dilution 2

7. Dilute with acetonitrile.
8. Vortex mix.
9. Sonicate for 10 minutes.
10. Vortex mix.

6.7.2.3 Dilution 3

11. Dilute with acetonitrile/water 50/50 v/v.
12. Vortex mix.
13. Sonicate for 10 minutes.
14. Vortex mix.
15. Add 500 µL of sample to 100 µL of internal standard working solution (6.6.3) in an autosampler vial.

6.8 HPLC Conditions

LC: Accela UPLC

Column: BEH C8 2.5 µm, 2.1x100 mm

Mobile phases:

A – Deionised water with 0.1% formic acid (v/v),

B – Acetonitrile with 0.1% formic acid (v/v).

Column temperature: 30 °C

Sample temperature: 10 °C

Injection volume: 10 µL

Flow Rate: 0.2 mL/min

Gradient Elution:

Table 22. CBD Gradient Elution

Time (min)	%A	%B
0.00	50	50
1.00	50	50
8.00	0	100
11.00	0	100
11.10	50	50
15.00	50	50

6.9 MS Condition

Mass Spectrometer: Thermo Scientific TSQ Vantage

Ionisation mode: ESI+

S-Lens: 35

Table 4. CBD MRMs

Analyte	Transition 1 Pre-cursor (m/z)	Transition 1 Product (m/z)	Transition 2 Pre-cursor (m/z)	Transition 2 Product (m/z)	Collision energy (V)
CBD	315.2	193.2	315.2	235.2	20
CBD-D3	318.2	196.2	318.2	238.2	20

7 Appendix 2 - Controlled Cannabinoid Method

7.1 Scope

This method is suitable for the quantification of controlled cannabidiol (CBN, THCV, Δ^9 -THC, Δ^8 -THC and Δ^9 THCA-A in CBD food oil and cosmetic body wash.

7.2 Background and Method Principle

Cannabidiol (CBD) products have recently entered UK food markets. Laboratories need to be able to accurately measure the controlled cannabinoid content of the products to check the compliance of samples to the Home Office regulations as well as consumer safety.

The food and cosmetic matrices are diluted into propan-2-ol, acetonitrile and then acetonitrile/water 50/50 v/v. The extracts are then analysed via LC-MS/MS.

7.3 Apparatus

- General laboratory glassware, including beakers, bottles and cylinders
- Falcon Tubes and Eppendorfs of various sizes
- 2 mL autosampler vials and vials caps, PTFE lined
- Positive displacement pipettes, delivering volumes in the range of 10 μ L - 1000 μ L
- Larger volume pipettes, multi-pipettes or volumetric glassware capable of dispensing larger volumes (1-50 mL)
- Vortex Mixer
- Sonicator
- Centrifuge capable of 3220 G and 15700 G
- HPLC column: BEH C8 2.5 μ m, 2.1x100 mm or equivalent
- HPLC System
- Mass Spectrometer Detector

7.4 Reagents

All reagents must be of analytical reagent quality or better.

1. Methanol (Honeywell, Fisher UK)
2. Stock Cannabinoids (Cerilliant, part numbers listed in 7.5.1, 7.6.1)
3. Acetonitrile (Honeywell, Fisher UK)
4. Ultra high purity water ($\geq 18.2\text{m}\Omega$) e.g. Elga
5. Acetonitrile/Water 50/50 v/v
Mix 200 mL of acetonitrile with 200 mL of water. This solution is stable for one month when stored at room temperature.
6. Propan-2-ol (Fisher, UK)
7. Formic Acid (Sigma-Aldrich, UK)
8. Mobile Phase A - 0.1 % Formic Acid in Water
Mix 1 L of water with 1 mL of formic acid. This solution is stable for one week when stored at room temperature.
9. Mobile Phase B - 0.1 % Formic Acid in Acetonitrile
Mix 1 L of acetonitrile with 1 mL of formic acid. This solution is stable for two weeks when stored at room temperature.

7.5 Calibration Preparation

7.5.1 Stock Standard

The stock is acquired from Merck - Cerilliant 1 mg/mL CBN (P/N: C-046), THCV (P/N: T-094), Δ^9 -THC (P/N: T-4764), Δ^8 -THC (P/N: T-032) and Δ^9 THCA-A (P/N: T-093) solution.

7.5.2 Individual Intermediate Standard

Individual intermediate solutions in 100% methanol are prepared to form 5 individual 100 $\mu\text{g/mL}$ solutions.

7.5.3 Mixed Intermediate Standard

The intermediates are then mixed to make a working solution containing all 5 cannabinoids in acetonitrile/water 50/50 v/v at 10 $\mu\text{g/mL}$.

7.5.4 Calibration Line

The mixed working solution is used to prepare a calibration line in acetonitrile/water 50/50 v/v to form the following points: 250, 200, 150, 100, 50, 5, and 1 ng/mL.

7.5.5 Autosampler Vial

In an autosampler vial place 200 μL of calibration standard (7.6.34) and 50 μL internal standard working solution (7.6.3).

7.6 Internal Standard Preparation

7.6.1 Stock Standard

The stock is acquired from Merck - Cerilliant 100 µg/mL CBN-D3 (P/N: C-115), Δ^9 THCA-A-D3 (P/N: T-145), Δ^9 -THC-D3 (P/N: T-003) solutions.

7.6.2 Individual Intermediate Standards

Individual intermediate solutions in 100% methanol are prepared to form 3 individual 10 µg/mL solutions.

7.6.3 Working Standard

Dilute further and combine in acetonitrile/water 50/50 v/v to a mixed working solution of 500 ng/mL.

7.7 Sample Preparation

As cannabinoid concentrations are completely unknown – initially aim to dilute all samples to maximum of 0.1 mg/mL CBD concentration. The majority of the CBD peak should then be redirected to waste at its retention time. This is because overloading CBD causes contamination issues in the mass spectrometer. The dilution can then be altered if the samples are over calibration range.

7.7.1 Food Oil Samples

7.7.1.1 Dilution 1

1. Vigorously vortex mix the sample, if bubbles form, wait for bubbles to dissipate before taking an aliquot for analysis.
2. Take a minimum of 500 µL of sample.
3. Dilute firstly with propan-2-ol.
4. Vortex mix.
5. Sonicate for 30 minutes.
6. Vortex mix.
7. Centrifuge for 10 minutes at 4000 RPM (3220 G).

7.7.1.2 Dilution 2

8. If layers are formed, aliquot from the top layer of previous dilution.
9. Dilute with acetonitrile.
10. Vortex mix.
11. Sonicate for 10 minutes.
12. Vortex mix.
13. Centrifuge for 10 minutes at 4000 RPM (3220 G).

7.7.1.3 *Dilution 3*

14. If layers are formed, aliquot from the top layer of previous dilution.
15. Dilute with acetonitrile/water 50/50 v/v.
16. Vortex mix.
17. Sonicate for 10 minutes.
18. Vortex mix.
19. Centrifuge for 10 minutes at 12000 RPM (15700 G).
20. Add 200 μ L of sample to 50 μ L of internal standard working solution (7.6.3) in an autosampler vial.

7.7.2 Cosmetic Body Wash Sample (No Centrifuging)

7.7.2.1 *Dilution 1*

1. Vigorously vortex mix the sample, if bubbles form, wait for bubbles to dissipate before taking an aliquot for analysis.
2. Take a minimum of 500 μ L of sample.
3. Dilute firstly with propan-2-ol.
4. Vortex mix.
5. Sonicate for 30 minutes.
6. Vortex mix.

7.7.2.2 *Dilution 2*

7. Dilute with acetonitrile.
8. Sonicate for 10 minutes.
9. Vortex mix.

7.7.2.3 *Dilution 3*

10. Dilute with acetonitrile/water 50/50 v/v.
11. Vortex mix.
12. Sonicate for 10 minutes.
13. Vortex mix.
14. Add 200 μ L of sample to 50 μ L of internal standard working solution (7.6.3) in an autosampler vial.

7.8 HPLC Conditions

LC: Acquity H-Class UPLC

Column: BEH C8 2.5 μm , 2.1x100 mm

Mobile phases:

A – Deionised water with 0.1% formic acid (v/v),

B – Acetonitrile with 0.1% formic acid (v/v).

Column temperature: 30 °C

Sample temperature: 10 °C

Injection volume: 20 μL

Flow Rate: 0.3 mL/min

Table 5. Cannabinoid Gradient

Time (min)	%A	%B
0.0	50	50
12.0	43	57
15.5	20	80
17.0	0	100
20.0	0	100
20.5	50	50
28	50	50

A divert valve cuts the flow to waste from 12 - 13.7 minutes to cut out the CBD peak.

7.9 MS Conditions

Mass Spectrometer: ABI Sciex 4000 Qtrap

Ionisation mode: ESI+ and ESI-

Temperature: 650°C

Ion spray voltage: 5500 V

Ion source gas 1&2: 50

Curtain gas: 10

Entrance potential: 10 V

Collision cell exit potential: 15 V

MRM windows are used to limit the number of analytes scanned for. Dwell times are adjusted to give a reasonable amount of points across the peaks.

Table 6. Cannabinoid MRMs

Analyte	Transition 1 Pre-cursor (m/z)	Transition 1 Product (m/z)	Transition 2 Pre-cursor (m/z)	Transition 2 Product (m/z)	Declustering potential (V)	Collision energy (V)
THCV	287.2	165.1	287.2	231.2	60	30
CBN	311.0	223.5	311.0	241.5	75	30
CBD*	315.2	193.2	315.2	235.0	60	30
Δ^9 -THC	315.2	193.2	315.2	235.2	60	30
Δ^8 -THC	315.2	193.2	315.2	235.2	60	30
Δ^9 THCA-A **	359.5 Pos	219.5	359.5	261.5	80	40
	357.2 Neg	313.2	357.2	245.0	-50	-35
CBN-D3	314.0	223.0	314.0	241.0	75	30
Δ^9 -THC-D3	318.0	196.0	318.0	238.0	60	30
Δ^9 THCA-A-D3 **	362.5 Pos	264.5	362.5	222.5	80	40
	360.0 Neg	316.0	360.0	248.0	-50	-35

*CBD should not be observed in chromatogram – the peak should elute when the flow is cut to waste

** Δ^9 THCA-A can be analysed in either positive ESI or negative ESI depending on instrument capability and sensitivity.

10 Appendix 2 – Results Template

Instructions -

- Type of extraction: Indicate which type of extraction was used (e.g. Dilution, Solid-Phase, Liquid-Liquid).
- Dilution Factor: State the dilution factor used for each sample.
- Instrument Limit of Quantification: Please specify your instrument's limit of quantification.
- Number of samples between calibration lines: How many samples were analysed between calibration lines.

CBD Method Details -

Please specify type of extraction:		
Dilution Factor -		
LGC-RT.20.A:		
LGC-RT.20.B:		
LGC-RT.20.C:		
Instrument Limit of Quantification - CBD:		ng/mL
Number of samples between calibration lines:		

Controlled Cannabinoid Method Details -

Please specify type of extraction:		
Dilution Factor -		
LGC-RT.20.A:		
LGC-RT.20.B:		
LGC-RT.20.C:		
Instrument Limit of Quantification -	CBN:	ng/mL
	THCV:	ng/mL
	Δ^8 -THC:	ng/mL
	Δ^9 -THC:	ng/mL
	Δ^9 THCA-A:	ng/mL
Number of samples between calibration lines:		

Meta Data Instructions -

Institute: State the name of your institute.
 Replicate: Extract your sample in **singlicate** on **three separate days**.
 Bottle Number: Indicate the bottle number of the sample you received.
 Extraction Date: Date sample was extracted.
 CBD Extraction Method: Indicate which institutes extraction method was used (e.g. LGC, Own Method).
 CBD Analysis Method: Indicate which institutes analysis method was used (e.g. LGC, Own Method).
 Instrument Analysis Date: Date sample was analysed on the instrument.
 Technique: Indicate the analysis technique used (e.g. LC-MS/MS, GC-MS, GC-FID).
 Internal Standard: Indicate which internal standard was used (e.g. CBD-d3) or, if no internal standard was used for the analyte, type none.
 CBD Calculated Concentration mg/mL: Calculate the concentration of CBD in the sample in mg/mL.
 CBD Calculated Concentration mg per pack: Calculate the concentration of CBD in the sample in mg per pack.

LGC-RT/20/A CBD Hemp Food Oil - CBD Results

Pack Size: 30 mL

Institute	Replicate	Bottle Number	Extraction Date	CBD Extraction Method	CBD Analysis Method	Instrument Analysis Date	Technique	Internal Standard	CBD Calculated Concentration mg/mL	CBD Calculated Concentration mg per pack
	1									
	2									
	3									

LGC-RT/20/B CBD Food Oil - CBD Results

Pack Size: 30 mL

Institute	Replicate	Bottle Number	Extraction Date	CBD Extraction Method	CBD Analysis Method	Instrument Analysis Date	Technique	Internal Standard	CBD Calculated Concentration mg/mL	CBD Calculated Concentration mg per pack
	1									
	2									
	3									

LGC-RT/20/C CBD Cosmetic - CBD Results

Pack Size: 100 mL

Institute	Replicate	Bottle Number	Extraction Date	CBD Extraction Method	CBD Analysis Method	Instrument Analysis Date	Technique	Internal Standard	CBD Calculated Concentration mg/mL	CBD Calculated Concentration mg per pack
	1									
	2									
	3									

Meta Data Instructions -

Institute:	State the name of your institute.
Replicate:	Extract your sample in triplicate on three separate days.
Bottle Number:	Indicate the bottle number of the sample you received.
Extraction Date:	Date sample was extracted.
Cannabinoid Extraction Method:	Indicate which institutes extraction method was used (e.g. LGC, Own Method).
Cannabinoid Analysis Method:	Indicate which institutes analysis method was used (e.g. LGC, Own Method).
Instrument Analysis Date:	Date sample was analysed on the instrument.
Technique:	Indicate the analysis technique used (e.g. LC-MS/MS, GC-MS, GC-FID).
Internal Standard:	Indicate which internal standard was used (E.g. CBN-d3, Δ^9 -THC-d3, Δ^9 -THCA-A-d3) or, if no internal standard was used for the analyte, type none.
Calculated Concentration $\mu\text{g}/\text{mL}$:	Calculate the concentration of each analyte in the sample in $\mu\text{g}/\text{mL}$.
Calculated Concentration μg per pack:	Calculate the concentration of each analyte in the sample in μg per pack.
Controlled Status:	State whether this sample - in its commercial pack size - should be controlled.

LGC-RT/20/A CBD Hemp Food Oil - Cannabinoids Results

Pack Size: 30 mL

Institute	Replicate	Bottle Number	Extraction Date	Cannabinoid Extraction Method	Cannabinoid Analysis Method	Instrument Analysis Date	Technique	Internal Standard				
								CBN	THCV	Δ^8 -THC	Δ^9 -THC	Δ^8 THCA-A
	1											
	2											
	3											

LGC-RT/20/B CBD Food Oil - Cannabinoids Results

Pack Size: 30 mL

Institute	Replicate	Bottle Number	Extraction Date	Cannabinoid Extraction Method	Cannabinoid Analysis Method	Instrument Analysis Date	Technique	Internal Standard				
								CBN	THCV	Δ^8 -THC	Δ^9 -THC	Δ^8 THCA-A
	1											
	2											
	3											

LGC-RT/20/C CBD Cosmetic - Cannabinoids Results

Pack Size: 100 mL

Institute	Replicate	Bottle Number	Extraction Date	Cannabinoid Extraction Method	Cannabinoid Analysis Method	Instrument Analysis Date	Technique	Internal Standard				
								CBN	THCV	Δ^8 -THC	Δ^9 -THC	Δ^8 THCA-A
	1											
	2											
	3											

Calculated Concentration $\mu\text{g/mL}$					Calculated Concentration $\mu\text{g per pack}$					Should this sample be controlled?	Drop Down List
CBN	THCV	$\Delta^8\text{-THC}$	$\Delta^9\text{-THC}$	$\Delta^8\text{THCA-A}$	CBN	THCV	$\Delta^8\text{-THC}$	$\Delta^9\text{-THC}$	$\Delta^8\text{THCA-A}$		

Calculated Concentration $\mu\text{g/mL}$					Calculated Concentration $\mu\text{g per pack}$					Should this sample be controlled?	Drop Down List
CBN	THCV	$\Delta^8\text{-THC}$	$\Delta^9\text{-THC}$	$\Delta^8\text{THCA-A}$	CBN	THCV	$\Delta^8\text{-THC}$	$\Delta^9\text{-THC}$	$\Delta^8\text{THCA-A}$		

Calculated Concentration $\mu\text{g/mL}$					Calculated Concentration $\mu\text{g per pack}$					Should this sample be controlled?	Drop Down List
CBN	THCV	$\Delta^8\text{-THC}$	$\Delta^9\text{-THC}$	$\Delta^8\text{THCA-A}$	CBN	THCV	$\Delta^8\text{-THC}$	$\Delta^9\text{-THC}$	$\Delta^8\text{THCA-A}$		