Supplementary information for:
Validated LC-MS/MS methodology for the quantification of CBD, trace level THCA and UK controlled cannabinoids (Δ9THC, Δ8THC, CBN and THCV) in food samples

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Supplementary Information 1 – Controlled Cannabinoids Chromatograms

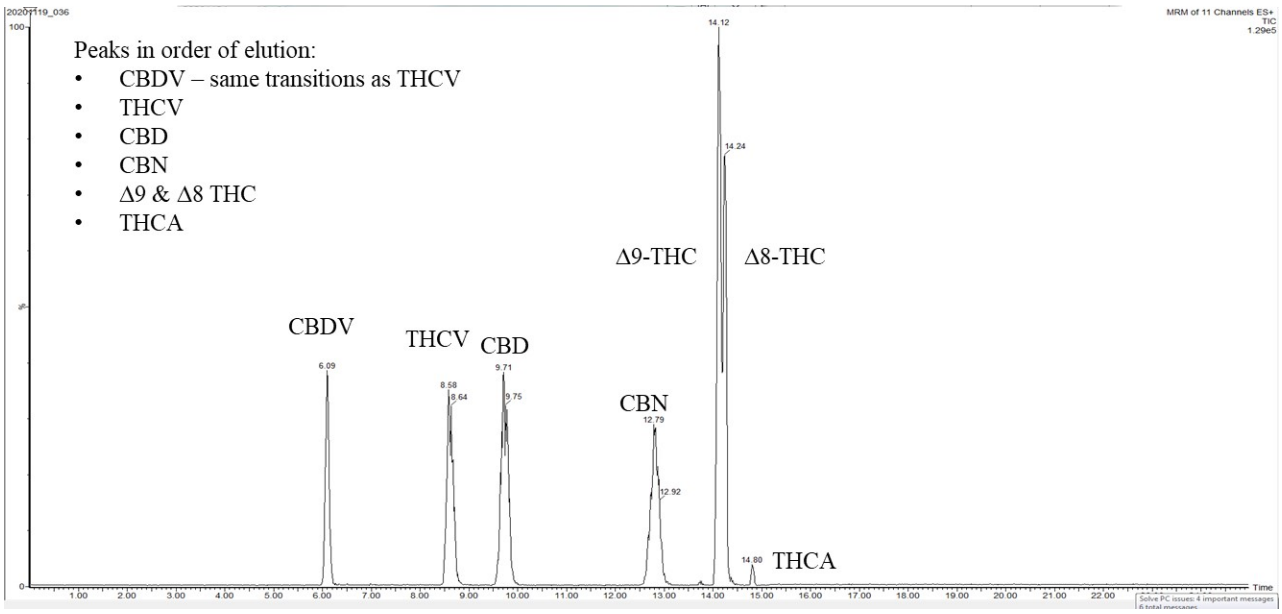

Figure S1 – Cannabinoids elution times on a Waters Quattro instrument coupled to HPLC. In the chromatogram the CBD peak shown to demonstrate where it was eluted to waste. Peak height of THCA was detected using positive mode so signal intensity was decreased.

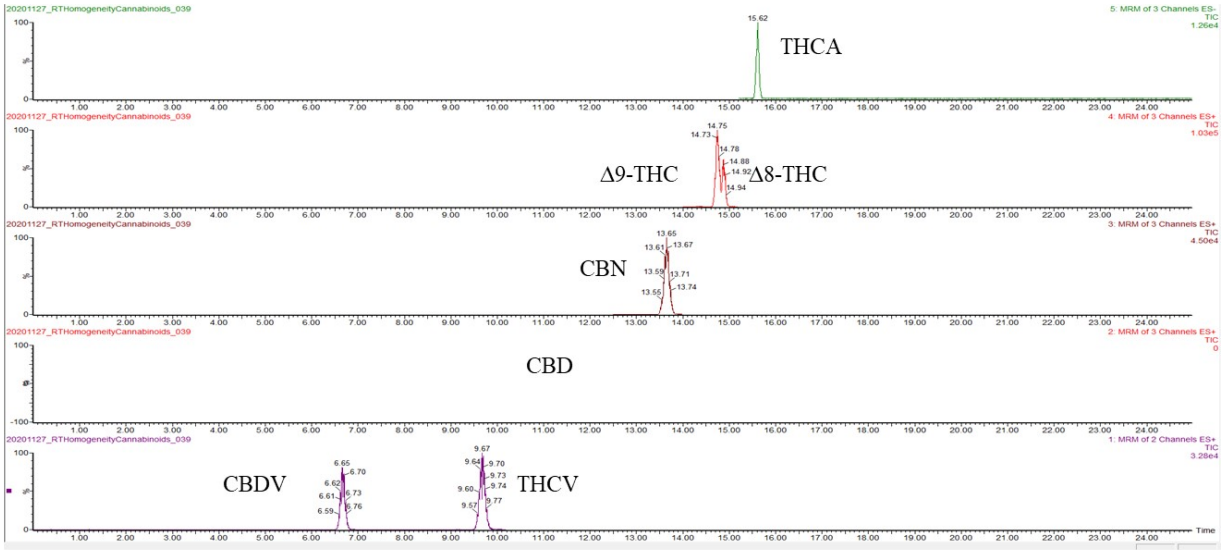

Figure S2 – Cannabinoid elution method on Sciex Qtrap with CBD cut to waste and THCA acquired in negative ESI mode. Time windows are in use to improve dwell time.

Supplementary Information 2 – LOQ CBD

The instrumental LOQ for CBD was 5 ng/g. The chromatograms (Figures S3 and S4) show that the S/N acceptance criteria were met for primary transitions and secondary transitions of CBD.

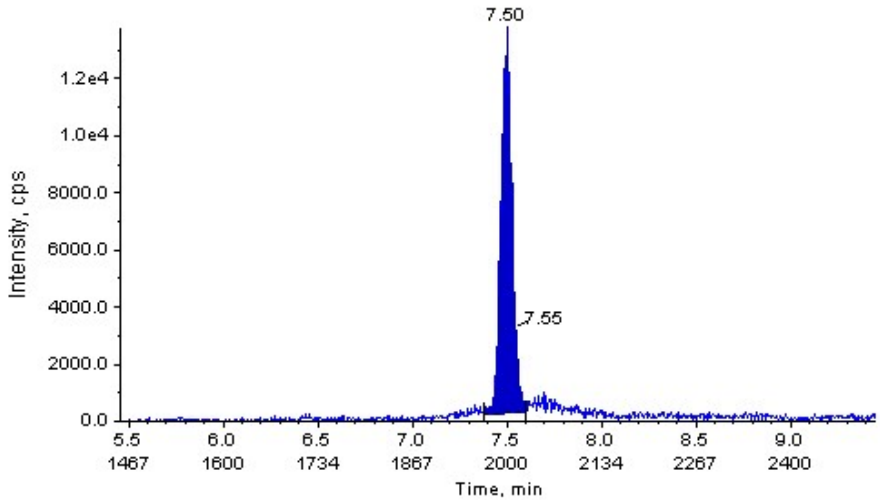

Figure S3 – Chromatogram for the primary transition of CBD (315.2/193.2 Da) for a 5 ng/g solvent standard.

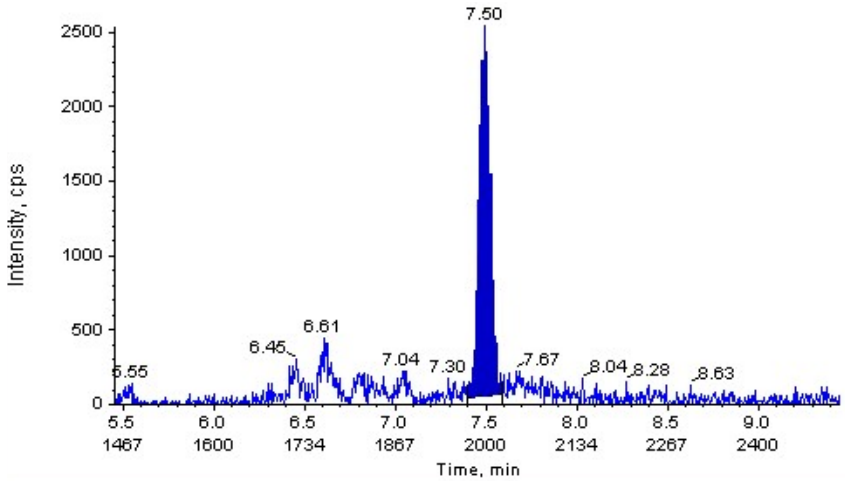

Figure S4 – Chromatogram for the secondary transition of CBD (315.2/235.2 Da) for a 5 ng/g solvent standard.

Supplementary Information 3 - LOQ Δ9THC

The instrumental LOQ for Δ^9-THC was 1 ng/g. The chromatograms (Figures S5 and S6) show that the S/N acceptance criteria was met for primary and secondary transitions of Δ^9-THC. All cannabinoids met this criteria (acceptance criteria stated in main paper).

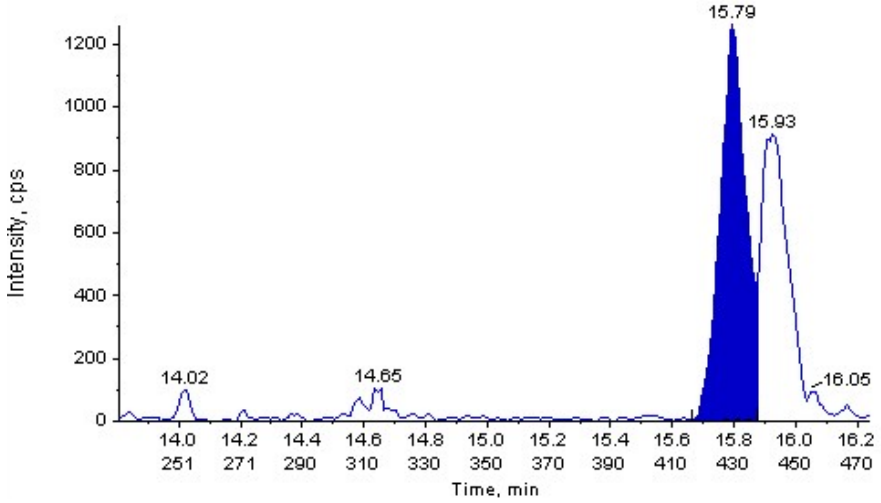

Figure S5 – Chromatogram showing the primary transition (315.2/193.2 Da) of Δ^9-THC at 1 ng/g in a mixed cannabinoid solvent standard. The Δ^9-THC peak is highlighted in blue. The double peak is because of two isomers, the other peak being Δ^8-THC.

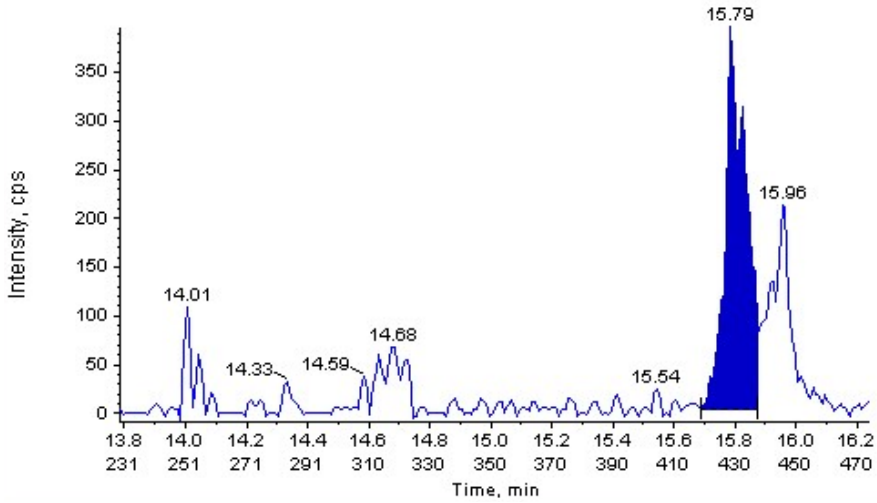

Figure S6 – Chromatogram displaying the secondary transition (315.2/235.2 Da) of Δ^9-THC at 1 ng/g in a mixed cannabinoid solvent standard. The Δ^9-THC peak is highlighted in blue.

Supplementary Information 4 – CBD Calibration lines

A calibration standard (as a quality control sample) and a blank were injected into the instrument after every six sample injections to confirm the accuracy of calculated concentrations. Calibration curves were plotted using Analyst processing software. All lines of best fit were calculated with linear regression and 1/x weighting.

The calibration curve for each batch had an R^2>0.995. An example is shown below in Figure S7.

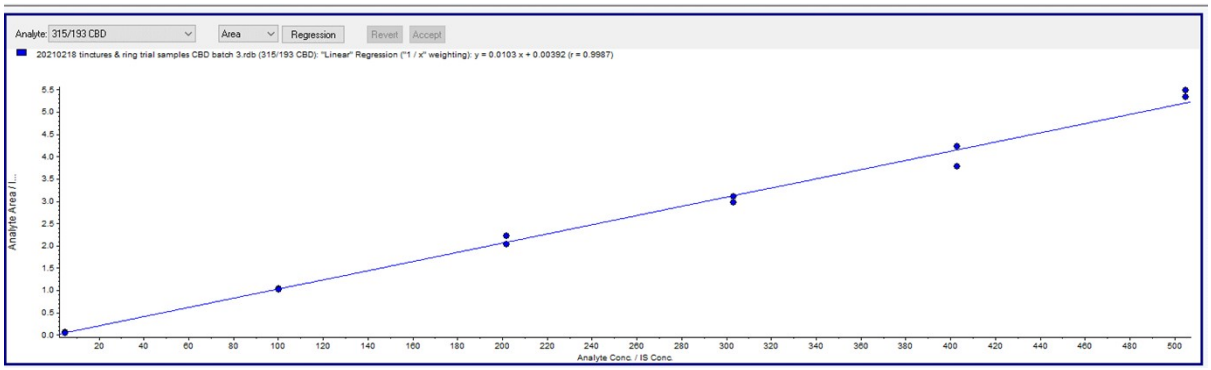

Figure S7 – CBD calibration curve 5-500 ng/g. R^2=0.9987

Supplementary Information 5 – Controlled cannabinoid calibration lines

Calibration curves of Δ^9-THC, Δ^8-THC, CBN, THCV and THCA had and R^2 greater than 0.995 for every batch. An example of a typical calibration curve for each analyte is shown in Figure S8 - S12.

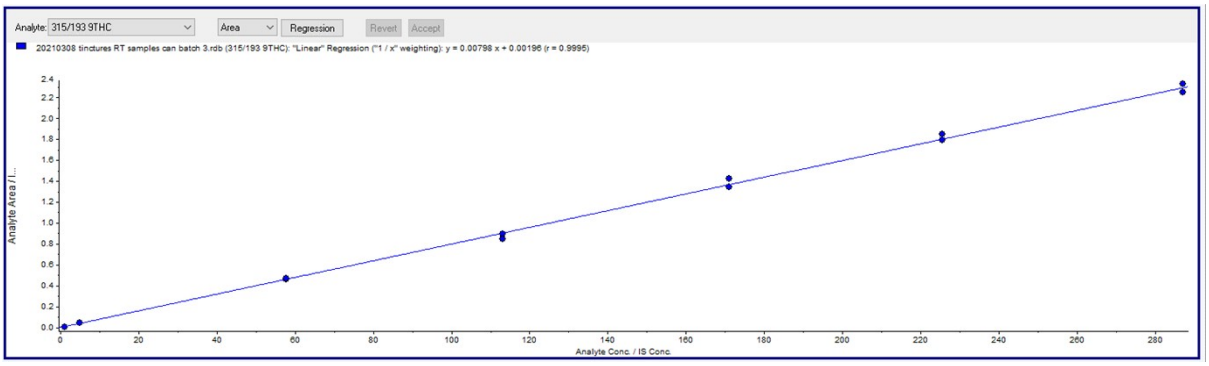

Figure S8 – Example calibration curve for Δ^9-THC 1-280 ng/g. R^2=0.9995

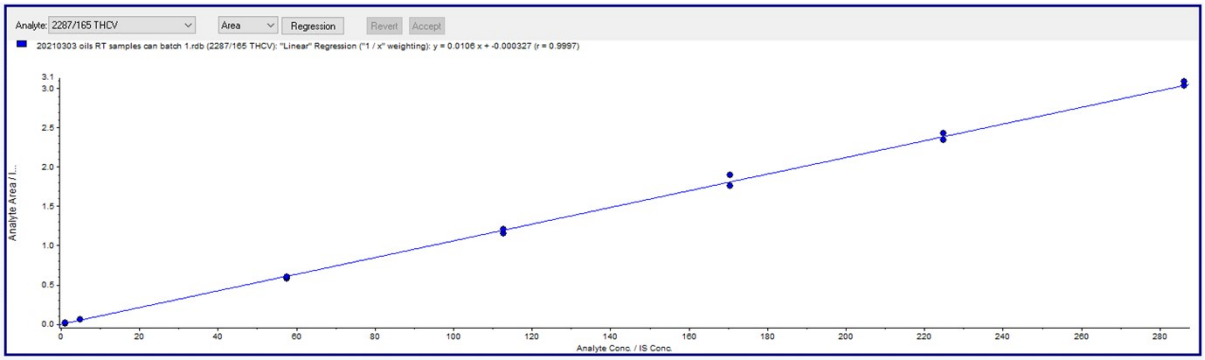

Figure S9 – Example calibration curve for THCV 1-280 ng/g. R^2=0.9997

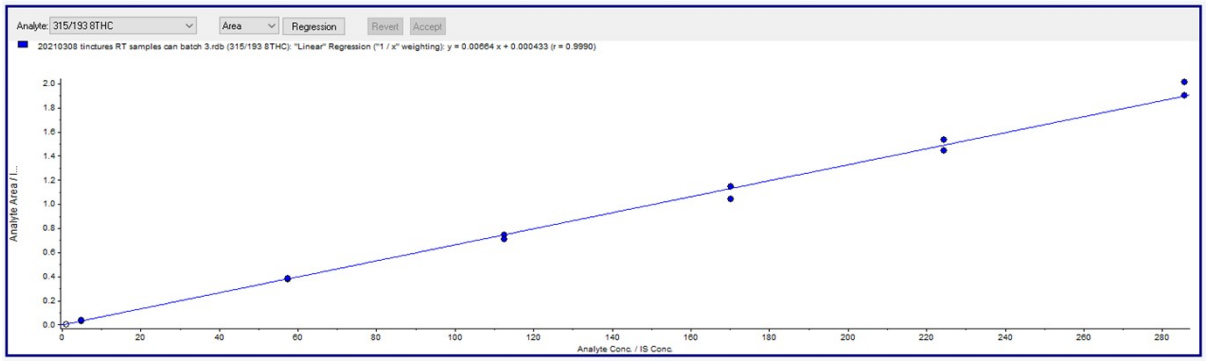

Figure S10 – Example calibration curve for Δ^8-THC 5-280 ng/g. R^2=0.9990

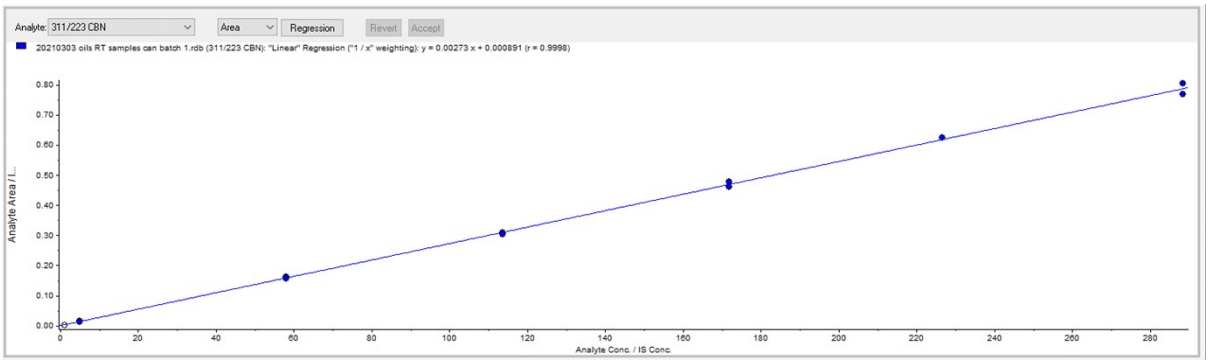

Figure S11 – Example calibration curve for CBN 5-280 ng/g. R^2=0.9998

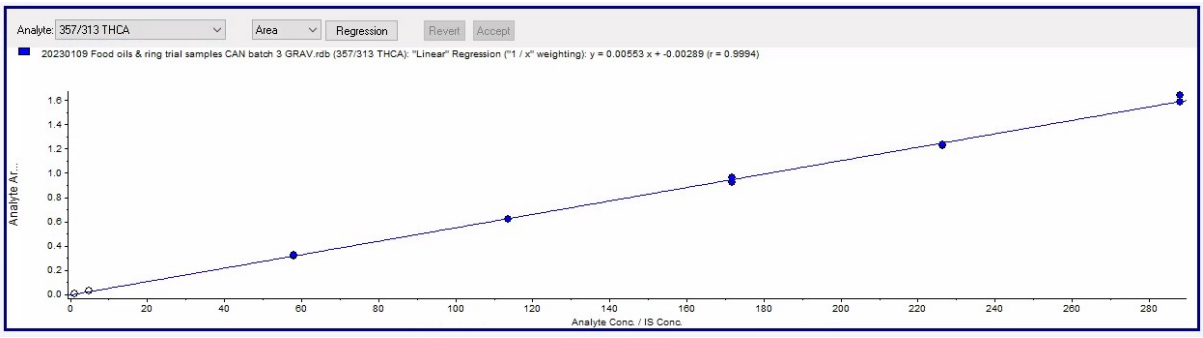

Figure S12 – Example calibration curve for THCA 50-280 ng/g. R^2=0.9994

Supplementary Information 6 – Robust testing analysts

Oil samples LGC-RT/20/A, LGC-RT/20/B were measured by two different analysts, each taking six analytical replicates. A one-way ANOVA (p = < 0.05) found that there was no statistically significant difference between the two sets of data.

LGC-RT/20/A: F(1,10) = 2.281, *p* = 0.16

LGC-RT/20/B: F(1,10) = 3.047, *p* = 0.11

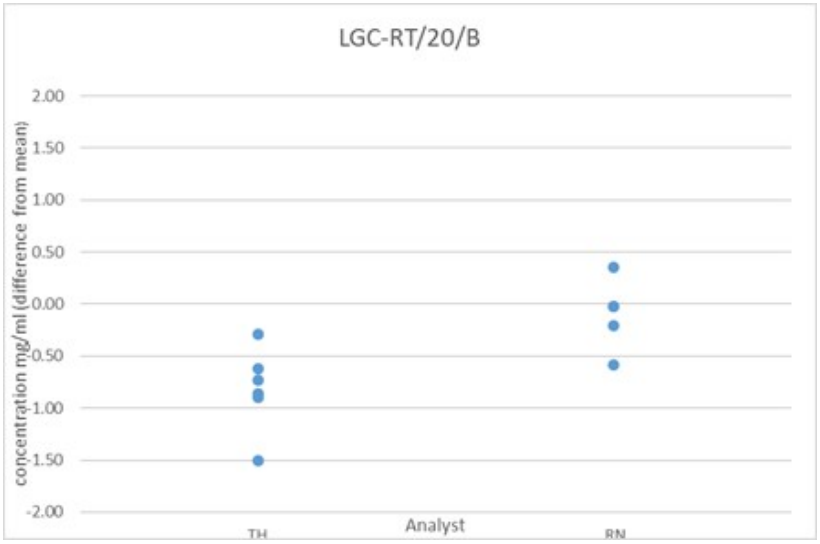

Figure S13 – CBD results for oil sample LGC-RT/20/A by two analysts on two separate days. The sample was analysed six times by each analyst.

Supplementary Information 7 – Alternate chromatography and columns

Mostly tested with H2O + 0.1% formic acid and MeCN + 0.1% formic acid mobile phases unless otherwise stated

- Acquity UPLC HSS C18 PFP 1.8µm 30 x 150mm – no separation of Δ^8-THC and Δ^9-THC
- Acquity UPLC BEH C18 1.7µm 2.1 x 100mm – separated Δ^8-THC and Δ^9-THC to baseline but then could not separate THCV and CBD (5mmol ammonium formate and H2O + 0.1% formic acid tested as well as normal mobile phases stated above)

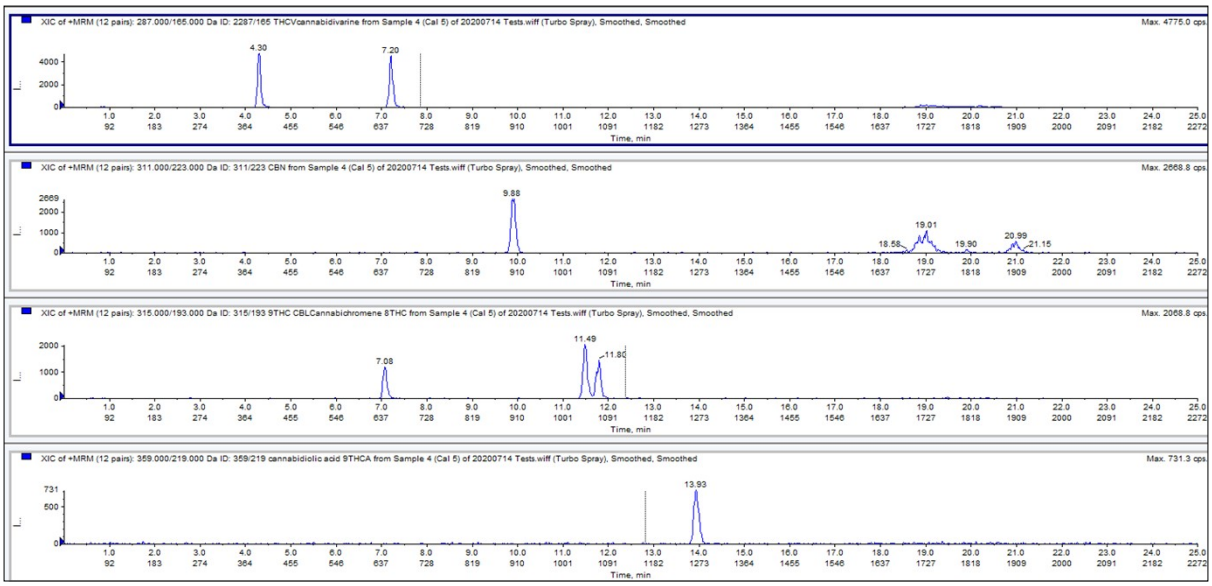

Figure S14. Acquity UPLC BEH C18 1.7µm 2.1 x 100mm with standard gradient. Top peaks: CBDV & THCV, Second: CBN, Third: CBD, Δ^9-THC, Δ^8-THC, Fourth: THCA.

The chromatography was then changed to be isocratic for the first 5 minutes to separate CBD and THCV, however that increased the LOD due to wider peaks of CBD and THCV.

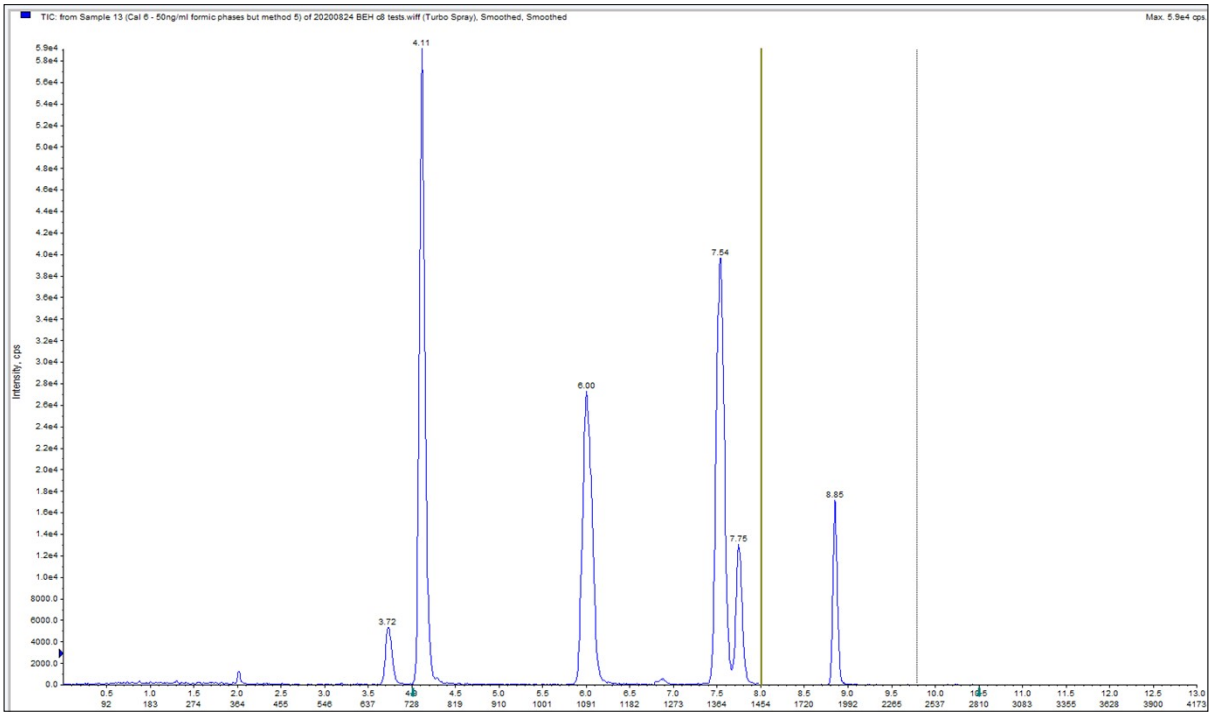

Figure S15. Acquity UPLC BEH C18 1.7µm 2.1 x 100mm with 70% isocratic hold for the first 5 minutes to separate CBD and THCV. Elution order: CBD, THCV, CBN, Δ^9-THC, Δ^8-THC, THCA

- Xbridge C8 BEH Column 2.5µm 100 x 2.1 – separated CBD and THCV – doesn't baseline resolve Δ^8-THC and Δ^9-THC but there is a defined split between both peaks
- Ace C18 PFP 3µm 150 x 2.1mm – no separation of Δ^8-THC and Δ^9-THC
- Ace C18-Amide 2µm 100 x 2.1mm – baseline separation of Δ8THC and Δ9THC and good separation of all peaks but they are much wider so a higher limit of detection

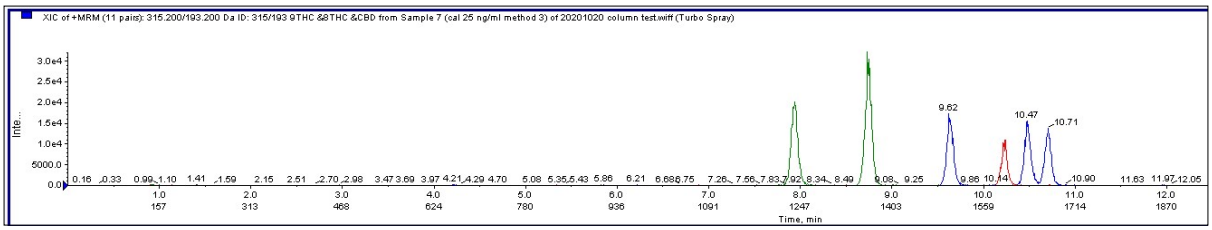

Figure S16. C18 Amide column: elution order - CBDV, THCV, CBD, CBN, 9THC, 8THC, No THCA peak present

- Ace 2µm Super C18 100 x 2.1mm - Only 30 second separation between THCV and CBD but good separation of Δ^8-THC and Δ^9-THC

The following were also trialled and did not achieve the desired separation of THCV & CBD and Δ^8-THC and Δ^9-THC:

- Poroshell HPH-C18 2.7µm 4.6 x 50mm
- Poroshell 120 EC-C18 2.1 x 30 mm 2.7µm
- Fortis C18 1.7µm 100 x 2.1mm
- Luna 3µm C18 150 x 2mm
- Luna 5µm phenyl hexyl 150 x 2 mm
- Allure PFP propyl 5µm 2.1 x 100mm

Supplementary Information 8 – Sample data sets

Table S1. Sample data sets from LGC and KSS

Matrix type	CBD stated pack concentration (mg/g)	Measured concentration of CBD (mg/g)	Measured concentration of THCA (ng/g)	Measured concentration of CBN (ng/g)	Measured concentration of THCV (ng/g)	Measured concentration of THC (ng/g)	Sum of controlled CBN, THCV, THC (ng/g)	Pack states THC Free
Capsule / Supplement	23	23.1	< LOQ	< LOQ	< LOQ	2780	2780	
Capsule / Supplement	50	45.5	< LOQ	< LOQ	< LOQ	< LOQ		Yes
Capsule / Supplement	10	13.8	< LOQ	178000	1410	89700	269110	Yes
Capsule / Supplement	61	56.2	< LOQ	26900	1490	102000	130390	
Capsule / Supplement	12	34.5	13500	5960	777	40800	47537	
Capsule / Supplement	45	52.4	< LOQ	15600	108	23800	39508	
Capsule / Supplement	106	0.1	965	913	< LOQ	4260	5173	
Capsule / Supplement	3	6.0	< LOQ	2460	477	34800	37737	
Capsule / Supplement	10	17	< LOQ	2480	3390	< LOQ	5870	
Capsule / Supplement*	10	16	< LOQ	410000	10100	< LOQ	420100	
Chewing Gum	Not declared	0.0003	< LOQ	< LOQ	< LOQ	< LOQ		
Chocolate	Not declared	0.01	< LOQ	< LOQ	< LOQ	< LOQ		
Chocolate	Not declared	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ		
Chocolate	1.2	0.8	< LOQ	< LOQ	< LOQ	< LOQ		No
Chocolate	0.15	0.1	< LOQ	< LOQ	< LOQ	< LOQ		
Chocolate	10	0.1	5440	562	101	2660	3323	
Chocolate	0.7	0.4	4680	721	160	12300	13181	
Chocolate	Not declared	0.001	< LOQ	< LOQ	< LOQ	< LOQ		
Cookie	Not declared	0.01	< LOQ	< LOQ	< LOQ	< LOQ		No
Cookie	Not declared	0.004	< LOQ	< LOQ	< LOQ	< LOQ		No
Cookie	Not declared	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ		
Cookie	Not declared	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ		No
Cookie	Not declared	0.001	< LOQ	< LOQ	< LOQ	< LOQ		No
Cookie	Not declared	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ		
Cookie	Not declared	0.0003	< LOQ	< LOQ	< LOQ	< LOQ		

Cookie	Not declared	0.01	< LOQ	< LOQ	< LOQ	< LOQ		
Cookie	Not declared	0.0005	< LOQ	< LOQ	< LOQ	< LOQ		Yes
Cookie	2	0.0002	< LOQ	< LOQ	< LOQ	< LOQ		
Cookie	0.14	<LOQ	< LOQ	< LOQ	< LOQ	< LOQ		
Cookie	Not declared	<LOQ	< LOQ	< LOQ	< LOQ	< LOQ		
Cookie	Not declared	<LOQ	< LOQ	< LOQ	< LOQ	< LOQ		
Cookie	Not declared	<LOQ	< LOQ	< LOQ	< LOQ	< LOQ		
Drink	0.06	0.04	< LOQ	< LOQ	< LOQ	< LOQ		No
Drink	0.4	0.3	< LOQ	< LOQ	< LOQ	< LOQ		
Drink	0.04	0.03	< LOQ	< LOQ	< LOQ	< LOQ		No
Drink	0.1	0.1	< LOQ	< LOQ	< LOQ	1210	1210	No
Drink	0.01	0.04	< LOQ	< LOQ	< LOQ	1290	1290	
Drink	0.04	0.01	< LOQ	< LOQ	544	221	765	
Drink	Not declared	<LOQ	< LOQ	< LOQ	< LOQ	< LOQ		Yes
Drink	0	0.04	< LOQ	< LOQ	< LOQ	< LOQ		
Drink	0	0.1	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	4	1.0	< LOQ	< LOQ	< LOQ	< LOQ		Yes
Gummy Sweet	4	0.1	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	3.5	1.6	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	2.5	1.7	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	3.5	2.7	< LOQ	< LOQ	< LOQ	< LOQ		No
Gummy Sweet	7	2.6	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	5.6	4.8	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	9.3	1.1	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	0.16	0.1	< LOQ	< LOQ	< LOQ	171	171	No
Gummy Sweet	3	1.3	< LOQ	< LOQ	< LOQ	< LOQ		Yes (<0.2%)
Gummy Sweet	3.1	0.2	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	3.1	0.9	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	8.4	1.9	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	3.5	0.8	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	3.2	1.5	< LOQ	< LOQ	< LOQ	< LOQ		

Gummy Sweet	4.8	2.1	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	9.4	3.1	< LOQ	< LOQ	< LOQ	< LOQ		
Gummy Sweet	1	0.8	<LOQ	<LOQ	<LOQ	217	217	
Gummy Sweet*	7.3	1.6	< LOQ	458	< LOQ	1320	1778	
Gummy Sweet*	5	1.0	< LOQ	148	< LOQ	309	457	
Gummy Sweet*	2.3	1.6	< LOQ	< LOQ	< LOQ	409	409	
Gummy Sweet*	2.6	0.6	< LOQ	< LOQ	< LOQ	1280	1280	
Gummy Sweet*	4	2.0	< LOQ	< LOQ	< LOQ	1090	1090	
Gummy Sweet*	3.3	3.0	< LOQ	< LOQ	< LOQ	484	484	
Gummy Sweet*	3	2.4	< LOQ	399	< LOQ	2840	3239	
Gummy Sweet*	5	3.0	< LOQ	< LOQ	< LOQ	983	983	
Hard Sweet	Not declared	0.01	< LOQ	< LOQ	< LOQ	< LOQ		
Hard Sweet	2.5	2.1	< LOQ	< LOQ	< LOQ	< LOQ		No
Hard Sweet	Not declared	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ		
Hard Sweet	Not declared	0.0003	< LOQ	< LOQ	< LOQ	< LOQ		
Hard Sweet	Not declared	0.0008	< LOQ	< LOQ	< LOQ	< LOQ		
Honey / Jam / Spread	10	6.4	< LOQ	< LOQ	< LOQ	1810	1810	Yes
Honey / Jam / Spread	6.6	0.2	< LOQ	< LOQ	3430	< LOQ	3430	No
Honey / Jam / Spread	0.66	0.1	< LOQ	516	< LOQ	444	960	Yes
Honey / Jam / Spread	7.1	0.8	< LOQ	< LOQ	< LOQ	< LOQ		Yes
Honey / Jam / Spread	1	0.0	< LOQ	< LOQ	< LOQ	< LOQ		
Oil	28	13.0	202000	25000	< LOQ	591000	616000	No
Oil	28	29.3	< LOQ	< LOQ	< LOQ	< LOQ		Yes
Oil	Not declared	< LOQ	< LOQ	7.7	15.3	< LOQ	23	
Oil	50	46.6	< LOQ	169000	6000	561000	736000	Yes (<0.2%)
Oil	100	100.0	< LOQ	< LOQ	< LOQ	13800	13800	
Oil	40	12.3	< LOQ	1050000	< LOQ	10300	1060300	Yes
Oil	33	31.7	4870	9370	1250	94000	104620	
Oil	500	0.0004	2360	375	1110	1660	3145	
Oil	40	36.2	100	1130	66.6	7950	9146.6	Yes (<0.2%)
Oil	30	26.6	< LOQ	3750	243	19800	23793	

Oil	25	10.9	125000	56400	3470	334000	393870	
Oil	50	43.1	72800	37800	3270	325000	366070	
Oil	100	77.5	< LOQ	27900	12500	44300	84700	
Oil	7.3	7.4	< LOQ	152000	< LOQ	2650	154650	
Oil	5	4.7	< LOQ	4440	1990	131000	137430	No
Oil	160	151.0	< LOQ	1480	< LOQ	7270	8750	Yes
Oil	28	19.3	75300	77000	10100	998000	1085100	
Oil	7.5	8.2	< LOQ	2930	< LOQ	3400	6330	
Oil	30	31.5	1760	< LOQ	< LOQ	4410	4410	
Oil	10	10.0	< LOQ	< LOQ	< LOQ	823	823	
Oil	40	32.2	< LOQ	575	2210	10100	12885	
Oil	100	95.5	< LOQ	3650	< LOQ	13100	16750	
Oil	66	12.6	4580	828	1460	2200	4488	
Oil	Not declared	0.02	4500	322	229	3140	3691	
Oil	100	103.0	< LOQ	5330	< LOQ	21900	27230	
Oil	16.7	20.1	< LOQ	418	< LOQ	13000	13418	
Oil	100	96.1	< LOQ	7630	102	43300	51032	
Oil	45.7	49.5	< LOQ	< LOQ	< LOQ	< LOQ		
Oil	26	28	< LOQ	414	< LOQ	8360	8774	
Oil	200	197	< LOQ	10600	< LOQ	13800	24400	
Oil	50	48	86500	22100	2090	109000	133190	
Oil	0	0.0	283	< LOQ	< LOQ	< LOQ		
Oil	0	23	26900	4670	349	24600	29619	
Oil	25	25	< LOQ	4220	851	4410	9481	
Oil	17	17	< LOQ	793	105	10000	10898	
Oil	67	77	< LOQ	4510	123	14800	19433	
Oil	50	53	<LOQ	100395	15776	464882	581053	
Oil	20	19	<LOQ	12229	4197	16941	33366	
Oil	20	15	<LOQ	2594	290378	21488	314460	
Oil	33	35	<LOQ	<LOQ	448	4105	4552	
Oil	2	3.5	<LOQ	<LOQ	<LOQ	503	503	

Oil	90	120	<LOQ	17936	<LOQ	21723	39659	
Oil	10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ		
Oil	100	116	<LOQ	<LOQ	1026	38563	39589	
Oil	66.7	71	<LOQ	28502	2251928	121170	2401600	
Oil	20	20	<LOQ	1675	<LOQ	6510	8185	
Oil	400	411	<LOQ	<LOQ	<LOQ	100832	100832	
Oil	20	26	<LOQ	49326	4147	239751	293223	
Oil	100	118	<LOQ	<LOQ	<LOQ	<LOQ		
Oil	100	136	<LOQ	<LOQ	<LOQ	8191	8191	
Oil	300	155	<LOQ	93953	11550644	395154	12039750	
Oil	100	125	<LOQ	<LOQ	<LOQ	10382	10382	
Oil	33.3	42	<LOQ	<LOQ	<LOQ	7971	7971	
Oil	50	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ		
Oil	100	103	<LOQ	<LOQ	<LOQ	16141	16141	
Oil	100	161	<LOQ	15465	910486	145948	1071900	
Oil	100	75	<LOQ	59362	6437	439570	505368	
Oil	250	252	<LOQ	109890	16522	678207	804618	
Oil	26	32	<LOQ	3187	<LOQ	20970	24157	
Oil	10	12	<LOQ	69492	2711	98461	170664	
Oil	13.3	15	<LOQ	1301	<LOQ	5064	6365	
Tea / Coffee	Not declared	<LOQ	4550000	310000	39000	2920000	3269000	
Tea / Coffee	3	1.2	< LOQ	616	< LOQ	2660	3276	
Tea / Coffee	1	0.7	3480	2570	494	30200	33264	
Tea / Coffee	26.7	2.9	165000	24300	2250	132000	158550	
Tea / Coffee	26.7	1.5	36300	27000	132	23200	50332	
Tea / Coffee	0.076	0.1	1690	< LOQ	< LOQ	4670	4670	
Tea / Coffee	1	0.9	< LOQ	< LOQ	< LOQ	< LOQ		
Tea / Coffee	6.5	6.5	2800000	13800	1950	809000	824750	
Tea / Coffee	26.7	1.6	254000	55000	9070	167000	231070	
Tea / Coffee	2.5	2.1	< LOQ	985	< LOQ	2560	3545	

** = QuECheERS extraction method used*

Table S2. Sample data sub-sets displaying dilution factors

Matrix type	Pack concentration	Dilution factor						Measured concentration						Sum of controlled
	CBD	CBD	THCA	CBN	THCV	Δ^9 THC	Δ^8 THC	CBD	THCA	CBN	THCV	Δ^9 THC	Δ^8 THC	CBN, THCV, Δ^9& Δ^8 THC
	mg/g							mg/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Oil	50	195070	495	2473	495	2473	495	53	<LOQ	100395	15776	464882	No peak	581053
Oil	20	93000	191	191	191	191	191	19	<LOQ	12229	4197.0	16941	No peak	33366
Oil	20	94703	1936	1936	1936	1936	1936	15	<LOQ	2594.0	290378	21488	No peak	314460
Oil	33	85432	285	285	285	285	285	35	<LOQ	<LOQ	447.5	4104.8	No peak	4552.3
Oil	2	9747	35	35	35	35	35	3.5	<LOQ	<LOQ	<LOQ	502.7	No peak	502.7
Oil	90	491927	996	996	996	996	996	120	<LOQ	17936	<LOQ	21723	No peak	39659
Oil	10	40162	84	84	84	84	84	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	No peak	<LOQ
Oil	100	491927	996	996	996	996	996	116	<LOQ	<LOQ	1026.3	38563	No peak	39589
Oil	67	186114	762	762	11431	762	762	71	<LOQ	28502	2251928	121170	No peak	2401600
Oil	20	89795	185	185	185	185	185	20	<LOQ	1675	<LOQ	6510.4	No peak	8185.3
Oil	400	1785814	3791	3791	3791	3791	3791	411	<LOQ	<LOQ	<LOQ	100832	No peak	100832
Oil	20	96479	197	197	197	1965	197	26	<LOQ	49326	4146.5	239751	No peak	293223
Oil	100	434399	902	902	902	902	902	118	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Oil	100	491927	996	996	996	996	996	136	<LOQ	<LOQ	<LOQ	8190.9	No peak	8190.9
Oil	300	861147	2763	2763	55266	2763	2763	155	<LOQ	93953	11550644	395154	No peak	12039750
Oil	100	465260	953	953	953	953	953	125	<LOQ	<LOQ	<LOQ	10382	No peak	10382
Oil	33	96479	393	393	393	393	393	42	<LOQ	<LOQ	<LOQ	7971.5	No peak	7971
Oil	50	185993	471	471	471	471	471	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	No peak	<LOQ
Oil	100	372828	967	967	967	967	967	103	<LOQ	<LOQ	<LOQ	16141.3	No peak	16141
Oil	100	473778	967	967	9665	967	967	161	<LOQ	15465	910486	145948	No peak	1071900
Oil	100	482660	981	981	981	1962	981	75	<LOQ	59362	6436.6	439570	No peak	505368
Oil	250	991598	2550	2550	2550	2550	2550	252	<LOQ	109890	16522	678207	No peak	804618
Oil	26	91366	376	376	376	376	376	32	<LOQ	3186.9	<LOQ	20970	No peak	24157
Oil	10	49008	502	502	502	502	502	12	<LOQ	69492	2711.0	98461	No peak	170664
Oil	13	59344	96	96	96	96	96	15	<LOQ	1301.2	<LOQ	5064.2	No peak	6365.3
Gummy Sweet	1	4547	12	12	12	12	12	0.8	<LOQ	<LOQ	<LOQ	217.2	<LOQ	217.2

Supplementary Information 9 – Commercially available CBD standard impurities

A commercially available CBD standard with the stated purity of 99.52% was analysed using the controlled cannabinoids method on a Waters Xevo TQA system. The retention times were compared to a mixed cannabinoid calibration standard to make an identification. The chromatogram shows the presence of THCV, CBN and THC and the extracted ion chromatograms contain other peaks that could be other cannabinoids. The baseline of THC is also raised due to the highloading of the CBD.

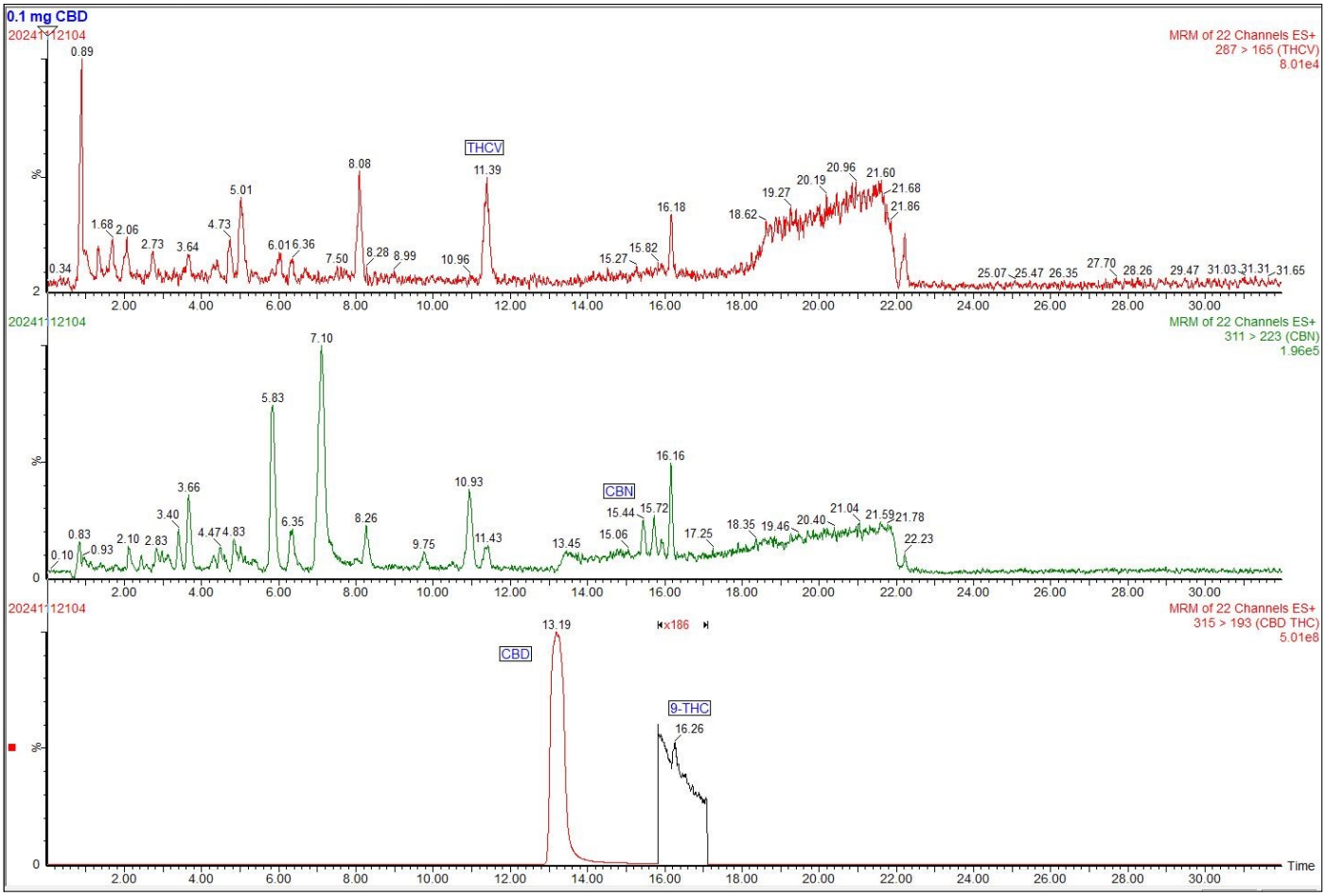

Figure S17. Chromatograph of a CBD standard solution analysed at 0.1 mg/g CBD showing trace levels of controlled cannabinoids THCV, CBN and THC. The THC trace is enhanced x186 to show the peak, which has increased noise due to the overloading of the CBD peak.