DART-MS facilitated quantification of cannabinoids in complex edible matrices—Focus on chocolate and gelatin-based fruit candies

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Supplementary Information

This document contains the following: (1) THC and CBD calibration curves developed using semi-automated DART-HRMS capabilities and validated according to FDA guidelines; (2) tables featuring validation results for THC and CBD DART-HRMS quantification protocols; (3) DART-HR mass spectra of control and cannabinoid-infused edibles prepared in-house; (4) CBD calibration curves quantitative results developed to run alongside the extracts of CBD-infused chocolates and fruit chews; (5) example ion chromatograms demonstrating analyte signal responses in CBD-infused samples and the absence of an analyte signal for unspiked matrices; and (6) quality control (QC) results for the THC quantification experiments performed at IonSense Inc.

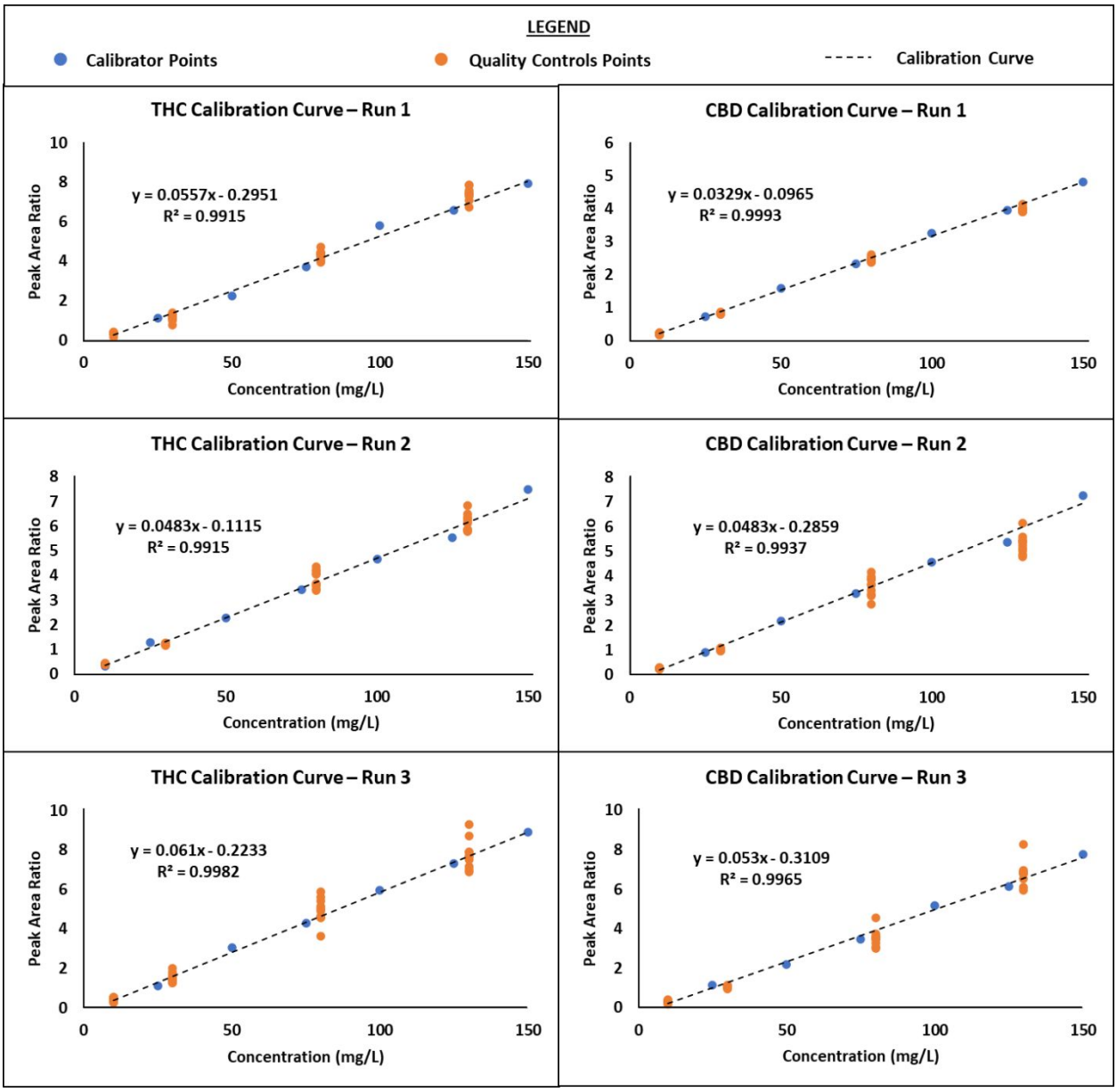

Figure S1. Calibration curves for THC (left) and CBD (right) quantification obtained from DART-HRMS data. All THC curves were developed with THC calibrators and THC-d_3 as the internal standard, and CBD curves were developed with CBD calibrators and CBD-d_9 as the internal standard. All quality control samples demonstrate precision and accuracy both within each run and between runs.

Table S1. Results for the THC calibration curve re-calculations with the relative error percentage for each concentration.

		Run 1		**Run 2**		**Run 3**	
	Conc. (mg/L)	Recalc. Conc. (mg/L)	Relative Error %	Recalc. Conc. (mg/L)	Relative Error %	Recalc. Conc. (mg/L)	Relative Error %
LLOQ	10	11.56	-15.57	9.43	5.73	10.01	-0.08
Point 1	25	25.09	-0.36	28.99	-15.98	21.93	12.28
Point 2	50	45.30	9.40	49.55	0.91	54.04	-8.08
Point 3	75	72.17	3.78	73.27	2.31	74.33	0.89
Point 4	100	109.84	-9.84	99.04	0.96	101.26	-1.26
Point 5	125	123.44	1.25	117.18	6.26	123.64	1.09
ULOQ	150	147.60	1.60	157.55	-5.03	149.80	0.13

Table S2. QC calculations for the THC high point including mean, relative error percentage and coefficient of variation for between runs and within runs. QC1 (A1-A5) and QC2 (B1-B5).

			Calculated			**Between runs**		
		Conc. (mg/L)	Run 1	Run 2	Run 3	Mean	RE%	CV
High	A1	130.00	132.40	128.86	129.09	133.16	-2.43	6.88
	A2		136.48	131.22	116.95			
	A3		139.25	124.31	121.29			
	A4		141.40	136.68	118.65			
	A5		146.63	123.16	133.26			
	B1		126.25	134.01	132.97			
	B2		138.61	122.30	127.04			
	B3		139.06	143.90	146.27			
	B4		136.66	132.73	129.98			
	B5		146.48	123.07	156.00			
Within-run		mean	138.32	130.02	131.15			
		RE%	-6.40	-0.02	-0.88			
		CV	4.17	5.16	8.80			

Table S3. QC calculations for the THC medium point including mean, relative error percentage and coefficient of variation for between runs and within runs. QC1 (A1-A5) and QC2 (B1-B5).

			Calculated			**Between runs**		
		Conc. (mg/L)	Run 1	Run 2	Run 3	Mean	RE%	CV
Medium	A1	80.00	75.96	77.59	85.86	83.38	-4.22	8.88
	A2		84.72	86.43	100.25			
	A3		89.91	89.05	84.34			
	A4		83.28	86.83	95.85			
	A5		84.08	90.14	78.48			
	B1		78.25	92.68	79.38			
	B2		82.66	78.69	63.00			
	B3		85.35	72.77	87.96			
	B4		84.41	73.97	92.22			
	B5		80.98	76.24	79.95			
Within-run		mean	82.96	82.44	84.73			
		RE%	-3.70	-3.05	-5.91			
		CV	4.44	8.44	11.77			

Table S4. QC calculations for the THC low point including mean, relative error percentage and coefficient of variation for between runs and within runs. QC1 (A1-A5) and QC2 (B1-B5).

			Calculated			**Between runs**		
		Conc. (mg/L)	Run 1	Run 2	Run 3	Mean	RE%	CV
Low	A1	30.00	19.11	28.49	24.71	27.41	8.63	10.84
	A2		23.26	28.65	27.02			
	A3		25.27	28.39	36.35			
	A4		24.42	28.25	30.79			
	A5		28.49	27.94	27.65			
	B1		27.78	27.22	33.31			
	B2		29.99	27.28	28.74			
	B3		28.77	27.31	26.70			
	B4		25.68	26.80	26.28			
	B5		26.12	27.07	24.53			
Within-run		mean	25.89	27.74	28.61			
		RE%	13.70	7.54	4.64			
		CV	11.65	2.32	12.66			

Table S5. QC calculations for the THC LLOQ (lower limit of quantification) point including mean, relative error percentage and coefficient of variation for between runs and within runs. QC1 (A1-A5) and QC2 (B1-B5).

			Calculated			**Between runs**		
		Conc. (mg/L)	Run 1	Run 2	Run 3	Mean	RE%	CV
LLOQ	A1	10.00	12.30	11.18	11.01	11.04	-10.43	10.99
	A2		11.71	11.54	12.48			
	A3		11.75	10.52	12.44			
	A4		11.44	10.81	9.10			
	A5		10.79	10.95	11.08			
	B1		12.16	11.13	8.69			
	B2		11.37	10.45	8.18			
	B3		12.69	11.28	12.40			
	B4		12.02	10.38	9.85			
	B5		8.07	11.35	12.18			
Within-run		mean	11.43	10.96	10.74			
		RE%	-14.29	-9.59	-7.41			
		CV	10.76	3.52	14.76			

Table S6. Results for the CBD calibration curve re-calculations with the relative error percentage for each concentration.

		Run 1		**Run 2**		**Run 3**	
	Conc. (mg/L)	Recalc. Conc. (mg/L)	Relative Error %	Recalc. Conc. (mg/L)	Relative Error %	Recalc. Conc. (mg/L)	Relative Error %
LLOQ	10	8.86	11.40	11.01	-10.14	11.05	-10.51
Point 1	25	25.24	-0.98	24.67	1.33	27.27	-9.07
Point 2	50	51.22	-2.45	51.28	-2.56	47.07	5.86
Point 3	75	73.96	1.38	74.19	1.09	71.68	4.43
Point 4	100	102.37	-2.37	100.56	-0.56	103.86	-3.86
Point 5	125	123.64	1.09	117.17	6.26	121.75	2.60
ULOQ	150	149.70	0.20	156.11	-4.08	152.33	-1.55

Table S7. QC calculations for the CBD high point including mean, relative error percentage and coefficient of variation for between runs and within runs. QC1 (A1-A5) and QC2 (B1-B5).

			Calculated			**Between runs**		
		Conc. (mg/L)	Run 1	Run 2	Run 3	Mean	RE%	CV
High	A1	130.00	125.32	117.58	129.34	124.75	4.04	8.77
	A2		124.61	118.89	117.98			
	A3		122.83	114.67	134.48			
	A4		125.91	112.13	134.29			
	A5		124.38	121.85	136.33			
	B1		122.07	117.22	134.48			
	B2		122.45	104.91	120.88			
	B3		129.50	133.06	135.08			
	B4		121.78	110.42	137.04			
	B5		124.36	106.64	161.86			
Within-run		mean	124.32	115.74	134.18			
		RE%	4.37	10.97	-3.21			
		CV	1.76	6.67	8.31			

Table S8. QC calculations for the CBD medium point including mean, relative error percentage and coefficient of variation for between runs and within runs. QC1 (A1-A5) and QC2 (B1-B5).

			Calculated			**Between runs**		
		Conc. (mg/L)	Run 1	Run 2	Run 3	Mean	RE%	CV
Medium	A1	80.00	77.63	81.30	62.49	76.95	3.82	9.40
	A2		75.32	72.22	72.85			
	A3		76.34	65.16	67.46			
	A4		75.52	88.14	91.55			
	A5		77.60	73.86	64.01			
	B1		82.46	86.50	71.44			
	B2		80.23	92.00	73.75			
	B3		79.13	85.74	71.40			
	B4		78.43	76.63	73.36			
	B5		77.24	81.87	76.77			
Within-run		mean	77.99	80.34	72.51			
		RE%	2.51	-0.43	9.37			
		CV	2.67	9.80	10.53			

Table S9. QC calculations for the CBD low point including mean, relative error percentage and coefficient of variation for between runs and within runs. QC1 (A1-A5) and QC2 (B1-B5).

			Calculated			**Between runs**		
		Conc. (mg/L)	Run 1	Run 2	Run 3	Mean	RE%	CV
Low	A1	30.00	29.30	27.20	26.18	27.03	9.89	4.94
	A2		27.46	26.29	24.79			
	A3		28.30	27.41	24.61			
	A4		28.08	25.79	26.75			
	A5		28.49	26.65	25.53			
	B1		27.63	26.01	27.79			
	B2		28.29	28.68	26.66			
	B3		28.74	27.22	25.13			
	B4		28.51	27.26	23.98			
	B5		28.38	27.51	26.38			
Within-run		mean	28.32	27.00	25.78			
		RE%	5.61	9.99	14.06			
		CV	1.76	2.98	4.31			

Table S10. QC calculations for the CBD LLOQ (lower limit of quantification) point including mean, relative error percentage and coefficient of variation for between runs and within runs. QC1 (A1-A5) and QC2 (B1-B5).

			Calculated			**Between runs**		
		Conc. (mg/L)	Run 1	Run 2	Run 3	Mean	RE%	CV
LLOQ	A1	10.00	8.70	10.52	13.15	10.76	-7.59	13.34
	A2		8.33	11.94	12.37			
	A3		8.03	11.50	11.71			
	A4		9.87	11.30	11.20			
	A5		9.08	12.09	13.26			
	B1		8.63	11.28	11.69			
	B2		9.39	11.58	11.00			
	B3		10.12	10.70	10.12			
	B4		9.14	11.75	13.09			
	B5		10.31	11.53	9.38			
Within-run		mean	9.16	11.42	11.70			
		RE%	8.38	-14.19	-16.97			
		CV	7.94	4.15	10.66			

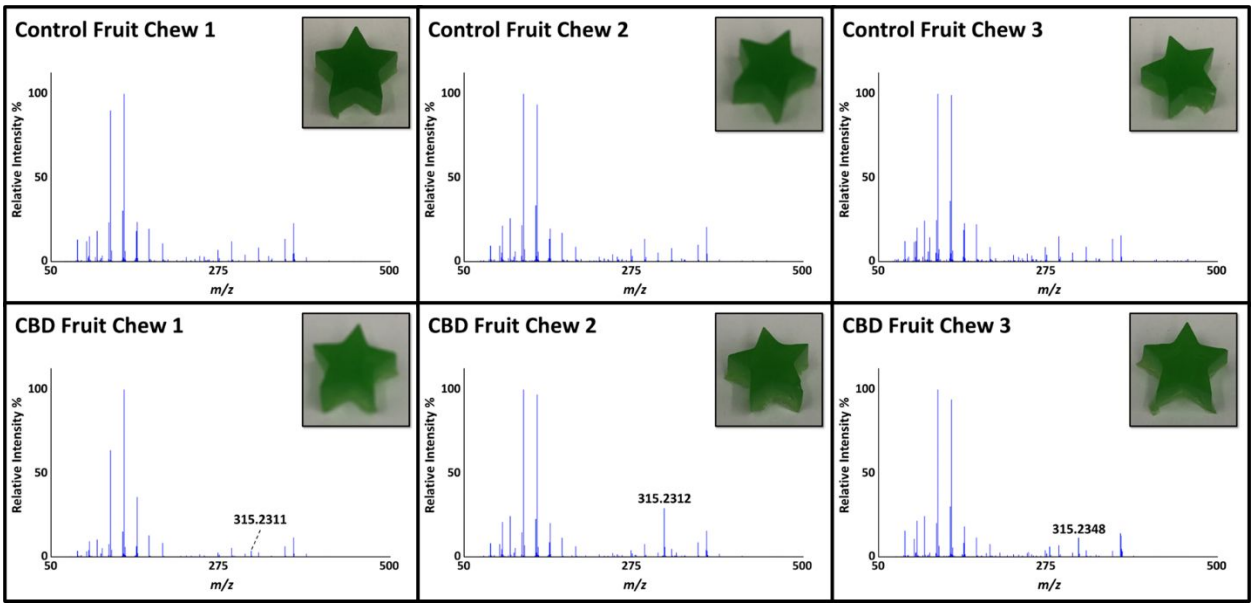

Figure S2. DART-HR mass spectra of control and CBD-infused fruit chews prepared in-house analyzed in positive-ion mode under soft ionization conditions (at an orifice 1 voltage of 20 V). The three control fruit chews (top) did not contain a peak at *m/z* 315, which confirms the absence of CBD in these samples. However, a peak at *m/z* 315 was detected in each of the CBD-infused fruit chews (bottom), which confirms the presence of CBD in these samples.

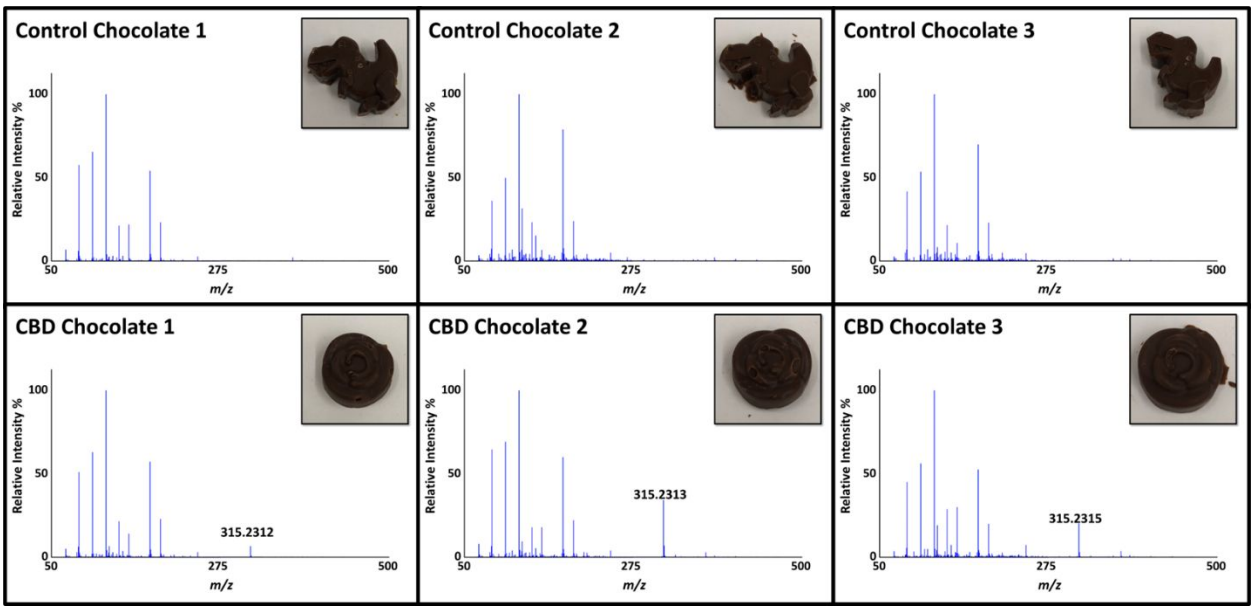

Figure S3. DART-HR mass spectra of control and CBD-infused chocolate prepared in-house analyzed in positive-ion mode under soft ionization conditions (at an orifice 1 voltage of 20 V). The three control chocolates (top) did not contain a peak at *m/z* 315, which confirms the absence of CBD in these samples. However, a peak at *m/z* 315 was detected in each of the CBD-infused chocolates (bottom), which confirms the presence of CBD in these samples.

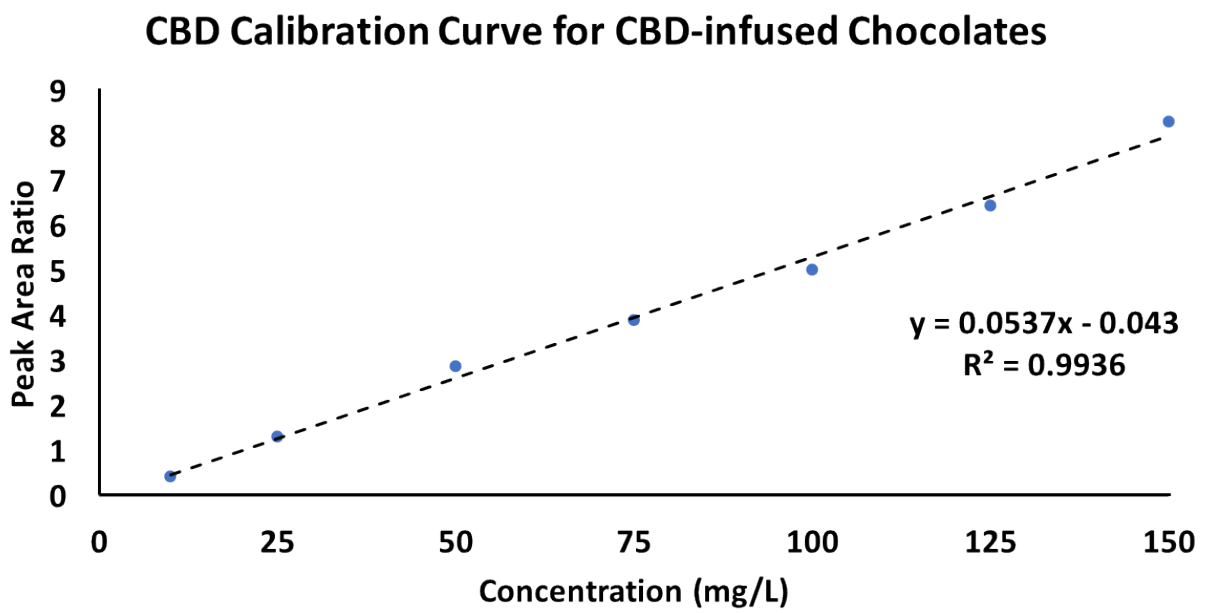

Figure S4. CBD calibration curve developed using DART-HRMS data that were generated using a semi-automated approach. Because all seven calibrators passed the validation requirements, and the R^2 value was >0.99, the curve was suitable for determining the CBD content in the extracts of CBD-infused chocolates, which were analyzed in the same acquisition as the curve shown here.

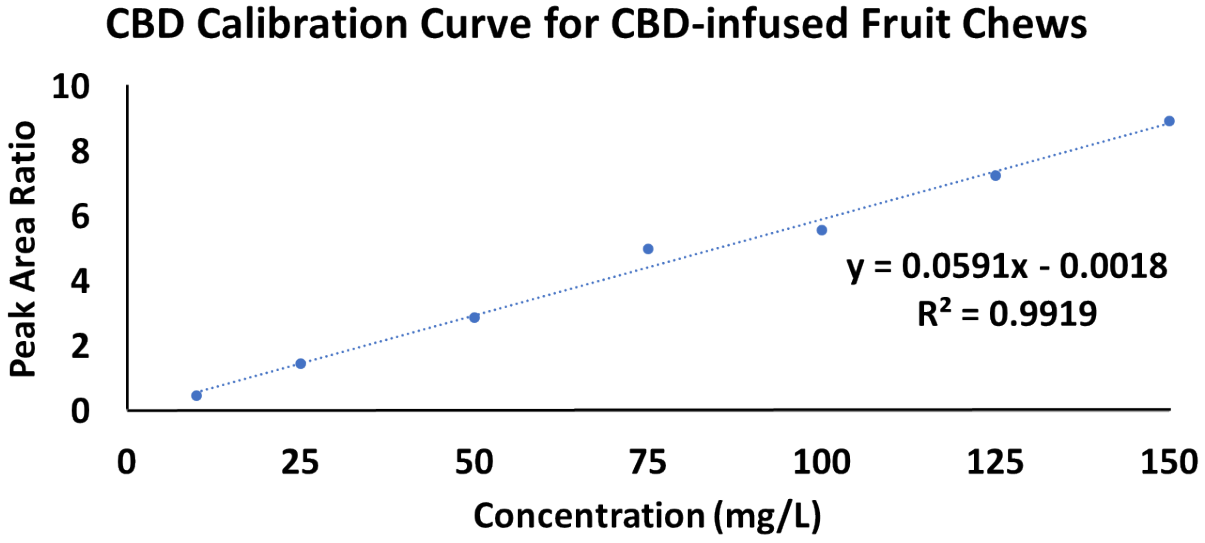

Figure S5. CBD calibration curve developed using DART-HRMS data that were generated using a semi-automated approach. Because all seven calibrators passed the validation requirements, and the R^2 value was >0.99, the curve was suitable for determining the CBD content in the extracts of CBD-infused fruit chews, which were analyzed in the same acquisition as the curve shown here.

Table S11. Quantitation results associated with the CBD calibration curve developed for the quantification of CBD in CBD-infused chocolates.							
CBD Concentrations (mg/L)	10	25	50	75	100	125	150
Peak Area Ratios	0.45	1.33	2.91	3.92	5.05	6.46	8.33
Standard Deviation	0.01	0.01	0.02	0.05	0.21	0.15	0.21
Relative Standard Deviation	1.79	0.97	0.54	1.16	4.18	2.32	2.51

Table S12. Quantitation results associated with the CBD calibration curve developed for the quantification of CBD in CBD-infused fruit chews.							
CBD Concentrations (mg/L)	10	25	50	75	100	125	150
Peak Area Ratios	0.48	1.46	2.90	4.99	5.57	7.26	8.94
Standard Deviation	0.03	0.05	0.03	0.27	0.16	0.30	0.73
Relative Standard Deviation	6.17	3.39	1.12	5.37	2.96	4.12	8.16

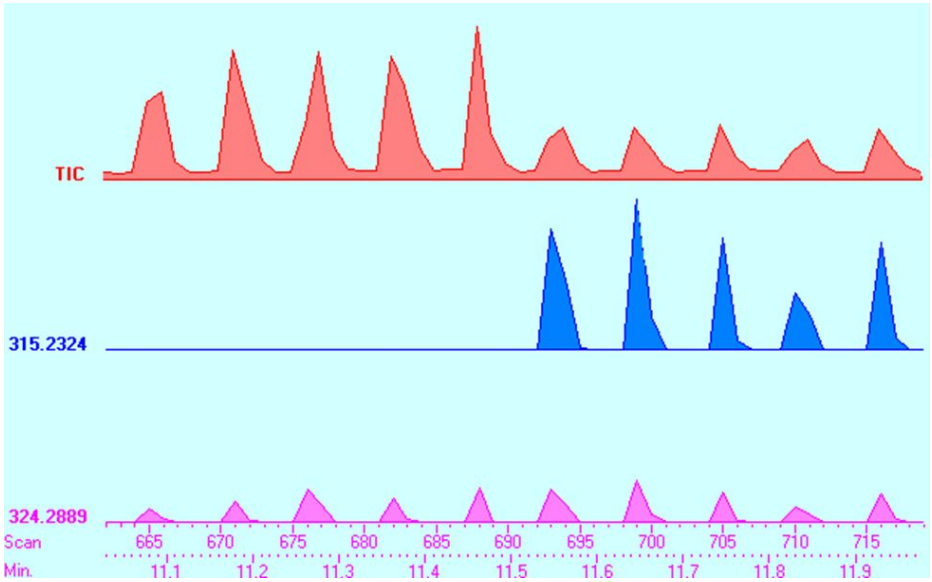

Figure S6. Example total ion chromatogram (TIC) (top); extracted ion chromatogram for the analyte of interest (CBD at *m/z* 315.2324) (middle); and extracted ion chromatogram for the internal standard (CBD-d_9) at *m/z* 324.2889 (bottom); which were used to determine the peak area ratios. The first five replicates represent un-spiked matrix (i.e., experimental blank extracts) for which no analyte signal (at *m/z* 315) was observed. However, peaks were detected at the internal standard signal (at *m/z* 324) for each replicate. The second five replicates represent CBD calibrators which contain both the analyte of interest (at *m/z* 315) and the internal standard (at *m/z* 324).

HIGH	Nominal Concentration	Run 1 Analyte PA	Standard PA	PAR	Calculated C	RE%
A1	130.00	700	149	4.69798658	116.389387	10.4697027
A2	130.00	626	120	5.21666667	129.169023	0.639213
A3	130.00	987	241	4.09543568	101.543277	21.889787
A4	130.00	1192	253	4.71146245	116.721415	10.2142959
A5	130.00	996	204	4.88235294	120.931946	6.9754262
B1	130.00	801	177	4.52542373	112.137651	13.7402684
B2	130.00	1923	337	5.70623145	141.231295	-8.63945739
B3	130.00	794	157	5.05732484	125.243037	3.65920203
B4	130.00	1110	220	5.04545455	124.950568	3.8841785
B5	130.00	894	165	5.41818182	134.134107	-3.18008266
	mean			122.2451706		
Within-run	mean RE%			5.965253356		
	CV			8.823043367		
Meets 50% crit?				TRUE		
All criteria met for level				TRUE		

MEDIUM	Nominal Concentration	Run 1 Analyte PA	Standard PA	PAR	Calculated C	RE%
A6	80.00	207	64	3.2343750	80.3278055	-0.40975687
A7	80.00	790	293	2.6962457	67.0689652	16.1637935
A8	80.00	206	64	3.2187500	79.9428248	0.071469
A9	80.00	409	116	3.5258621	87.5096868	-9.38710844
A10	80.00	847	279	3.0358423	75.4362049	5.70474387
B6	80.00	327	125	2.6160000	65.0918095	18.6352381
B7	80.00	296	80	3.7000000	91.8002302	-14.7502878
B8	80.00	334	96	3.4791667	86.3591697	-7.94896216
B9	80.00	842	246	3.4227642	84.9694833	-6.21185415
B10	80.00	948	271	3.4981550	86.8270184	-8.53377294
	mean			80.53331983		
Within-run	mean RE%			-0.666649786		
	CV			10.49930355		
Meets 50% crit?				TRUE		
All criteria met for level				TRUE		

LOW	Nominal Concentration	Run 1 Analyte PA	Standard PA	PAR	Calculated C	RE%
A11	30.00	201	174	1.15517241	29.0988226	3.00392475
A12	30.00	187	127	1.47244094	36.9159272	-23.0530907
A13	30.00	203	196	1.03571429	26.1555219	12.814927
A14	30.00	225	203	1.10837438	27.9457769	6.84741019
A15	30.00	188	172	1.09302326	27.5675441	8.10818631
B11	30.00	406	318	1.27672956	32.0938405	-6.97946823
B12	30.00	243	195	1.24615385	31.3404927	-4.46830888
B13	30.00	388	292	1.32876712	33.3759817	-11.2532725
B14	30.00	159	153	1.03921569	26.2417921	12.5273598
B15	30.00	204	154	1.32467532	33.2751649	-10.9172163
	mean			30.40108645		
Within-run	mean RE%			-1.336954848		
	CV			11.12460658		
Meets 50% crit?				TRUE		
All criteria met for level				TRUE		

LLOQ	Nominal Concentration	Run 1 Analyte PA	Standard PA	PAR	Calculated C	RE%
A16	10.00	276	565	0.48849558	12.6727289	-26.7272893
A17	10.00	196	464	0.42241379	11.0445555	-10.4455547
A18	10.00	75	174	0.43103448	11.2569586	-12.5695861
A19	10.00	63	164	0.38414634	10.1016927	-1.01692735
A20	10.00	59	149	0.39597315	10.3930908	-3.93090793
B16	10.00	197	570	0.34561404	9.15230431	8.47695688
B17	10.00	253	721	0.35090153	9.28258155	7.17418453
B18	10.00	267	656	0.4070122	10.6650791	-6.65079119
B19	10.00	268	691	0.3878437	10.1927912	-1.92791199
B20	10.00	339	676	0.50147929	12.9926316	-29.9263162
	mean			10.77544143		
Within-run	mean RE%			-7.754414335		
	CV			11.22690211		
Meets 50% crit?				TRUE		
All criteria met for level				TRUE		

	Run meets 67% crit?
	TRUE
All QC's for run meet criteria	TRUE

Figure S7. Quality control (QC) standard results for the quantification experiments performed. These QC standards were run alongside the calibration curve featured in Figure 4. Greater than 50% of the QC standards at each level were within an acceptable percentage of their theoretical concentrations. Greater than 67% of the QC standards overall passed validation. The replicates highlighted in red were outside the acceptable percentage range for that respective QC level. These results indicate that: (1) the calibration curve overall passed validation; and (2) the curve can be used to determine the unknown THC content in the edibles extracts that were analyzed alongside the curve.