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DART-MS Facilitated Quantification of Cannabinoids in Complex Edible Matrices—Focus on Chocolates and Gelatin-Based Fruit Candies

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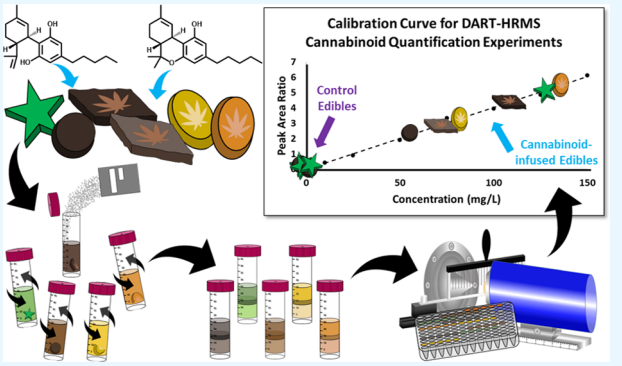
ABSTRACT: Traditional methods for detecting and quantifying cannabinoids in *Cannabis sativa* materials are most often chromatography-based, and they generally require extensive sample preparation protocols to render materials into a form that can be injected into the systems without the risk of contaminating or damaging the equipment. This challenge is amplified when interrogating the increasingly broad range of matrix types that cannabinoids are infused within, such as edibles that also contain sugars, fats, lipids, and carbohydrates. The requisite application of highly nuanced methods that must be developed for each matrix type is, in addition to being resource-intensive and time-consuming, highly impractical and unsustainable for crime laboratories endeavoring to perform such analyses in a routine manner, since they are often under-resourced while typically also confronting sample testing backlogs. A key to resolving this issue is to identify an analysis approach that avoids the requirement for nuanced method development by being applicable to a broader range of matrix types. Ambient ionization mass spectrometry (AIMS) methods have shown great promise in their ability to rapidly interrogate samples. Therefore, this study focused on developing validated protocols using AIMS (specifically, direct analysis in real time-high-resolution mass spectrometry, or DART-HRMS) to detect and quantify Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) in edible matrices. Calibration curves were developed using deuterated counterparts of THC and CBD as internal standards. Following the use of high cannabinoid recovery rate extraction protocols for chocolates and gelatin-based fruit candies or "gummies", the DART-HRMS approach was applied to quantify cannabinoid levels in commercially available cannabinoid-infused candies, yielding results similar to those reported on the product labels. Importantly, the developed method circumvented challenges encountered using traditional approaches. As the *Cannabis* field continues to evolve and new matrix types emerge on the market, the DART-HRMS detection and quantification protocols can be readily applied without the need for major procedural adaptations.



INTRODUCTION

Crime laboratories routinely analyze *Cannabis sativa* plant materials and products derived from it that contain Δ^9-tetrahydrocannabinol (THC), the plant's major psychoactive component. However, the explosive rise in *Cannabis* and THC-infused products, particularly edibles, has imposed immense challenges for analyses that are aimed at the detection and quantification of THC. Chromatography-based methods are often used to analyze *Cannabis* materials,[1,2] and with the diversity of product types available and the inherent complexity of their matrices, highly specialized methods must be developed for each material type.[3,4] This approach to *C. sativa*-related sample analysis is unsustainable for crime laboratories, since it involves lengthy run times, downtime resulting from contaminated or damaged instrumentation, and resource-intensive protocols which contribute to sample testing backlogs. Few major changes in sample analysis protocols have been introduced that significantly reduce the sample preparation steps, which can involve multiple extractions, filtrations, excess solvent usage, pH adjustments, grinding/homogenization, and chemical derivatization, before instrumental analysis.

The technical challenges associated with the measurement of the THC content in edibles are generally a consequence of the paucity of standardized or optimized methods for interrogation of all the types of edible matrices available on the market. This can lead to variable and inconsistent results; one study demonstrated that the THC content in various products deviated ±10% from the amount listed on their labels.[5]

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Another study, which utilized high-performance liquid chromatography/diode array detection/fluorescence detection (HPLC/DAD/FLD) to quantify cannabinoids and cannabinoid acids, found that of the 147 cannabidiol (CBD)-containing/hemp products analyzed, 55% had CBD concentrations that were ±20% of the amounts indicated on their labels.[6] These instances of inaccurate labeling further emphasize the need for the development of accurate and straightforward testing approaches for routine analysis.

Common conventional methods applied to the analysis of *Cannabis* matrix types include HPLC, GC−mass spectrometry (GC−MS), and GC−flame ionization detection (GC−FID) to quantify cannabinoids in *Cannabis* plant materials;[2,7−12] HPLC and GC−MS to quantify cannabinoids in *Cannabis*-infused products (e.g., oils, edibles, beverages, topicals, vapes, capsules);[6,13−20] and GC−MS and LC−MS to quantify cannabinoid metabolites in biological matrices (i.e., urine, oral fluid).[21−25] In general, for the aforementioned approaches, the sample preparation steps that are required before instrumental analysis are extensive, and the equipment (e.g., detectors, spectrometers) and consumables (e.g., columns, syringes, etc.) can become impaired or malfunction when exposed to residual amounts of matrix particulates. This is especially problematic when examining *Cannabis* edibles, which contain sugars, oils, and other ingredients that easily adhere to columns and syringes. LC and GC instruments are also incredibly sensitive to trace molecule contamination that can lead to carryover and false positives in subsequent runs. A key to addressing these challenges is to identify a method that can accommodate a broad range of sample types so that nuanced methods for different matrices are not required.

Ambient ionization mass spectrometry (AIMS) approaches have shown great promise in their ability to rapidly interrogate complex matrix samples in a manner that circumvents some of the challenges associated with chromatography-based methods. Thus, the use of an AIMS technique, such as direct analysis in real time-high-resolution mass spectrometry (DART-HRMS), could be applied for *Cannabis* investigations in order to bypass some of the aforementioned challenges. This method, which demands little to no sample preparation, enables analysis of materials in their native form (for triage purposes) and requires minimal training for proper operation and data processing. Its speed enables high-throughput analysis that can be accomplished in a fraction of the time required for analysis by LC or GC methods. Furthermore, DART-HRMS has previously been shown to readily detect the presence of cannabinoids and other *Cannabis*-related molecules (e.g., terpenes) in complex matrices.[26]

In the present study, semiautomated DART-HRMS quantification protocols were developed with CBD and THC chemical standards. Deuterated counterparts were determined to be suitable as internal standards because they exhibited a response in the DART gas stream similar to that of their nondeuterated equivalents. In accordance with the Food and Drug Administration (FDA) Bioanalytical Method Validation: Guidelines for Industry,[27] protocols were then developed and validated for CBD and THC quantification by DART-HRMS. After successful validation, the method was applied to quantify CBD and THC in edibles that have proven challenging to routinely analyze by conventional approaches (i.e., chocolates, fruit chews/gelatin-based gummies) after the application of an extraction protocol using the QuEChERS DisQue CEN salts.[28] Edibles prepared in-house and recreational *Cannabis* products were prepared/acquired to determine their CBD/THC content by the developed DART-HRMS method. Images of representative control and CBD-infused edibles prepared in-house are featured in Figure 1. The results reveal a straightforward approach to circumvent the challenges often encountered when analyzing such complex matrices using conventional methods (i.e., lengthy run times, downtime resulting from contaminated/damaged instrumentation).

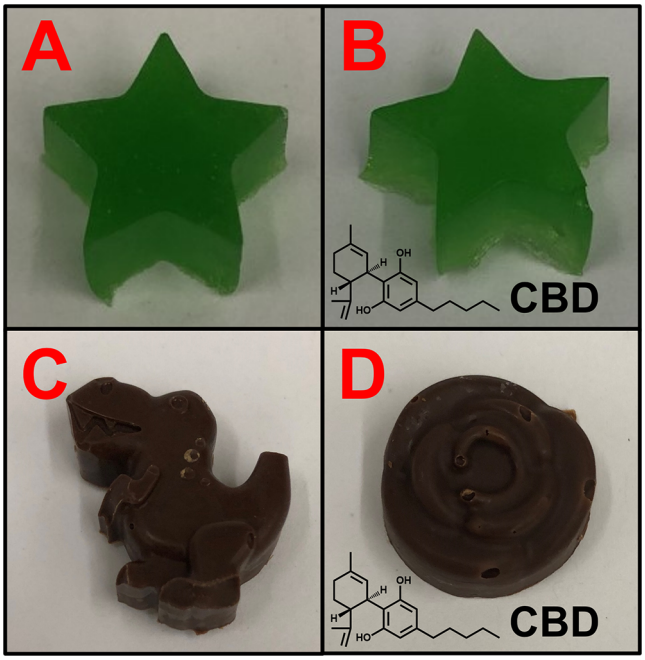

Figure 1. Representative photographs of the CBD-infused edibles prepared in-house for this study: a control lime fruit chew (A); a CBD-infused lime fruit chew (B); a control chocolate (C); and a CBD-infused chocolate (D).

RESULTS AND DISCUSSION

Validation of CBD and THC Calibration Curves. The CBD and THC calibration curves were developed and validated according to the FDA Bioanalytical Method Validation: Guidelines for Industry.[27] These guidelines were selected for the following reasons: (1) they are generally more stringent than other guidelines with regard to the development of novel methods; (2) they are more accommodating of heterogeneous and highly complex matrices in comparison to guidelines that are better suited for semi- or highly-purified samples; and (3) they have proven successful for the development of validated DART-HRMS quantification protocols for complex plant-based matrices. Future initiatives for the project will involve the validation of the developed protocols utilizing alternative guidelines such as those of the Scientific Working Group for the Analysis of Seized Drug (SWGDRUG) and the Association of Official Analytical Chemists (AOAC). Commercially available deuterated counterparts for both THC (THC-d_3) and CBD (CBD-d_9) were used as the internal standards for these experiments. When performing DART-HRMS quantification, peak area ratios (which were determined using the peak areas of the deuterated internal standard and the signals corresponding to the nondeuterated analytes) were plotted against their respective calibrator concentrations to create calibration curves. Following the recommended

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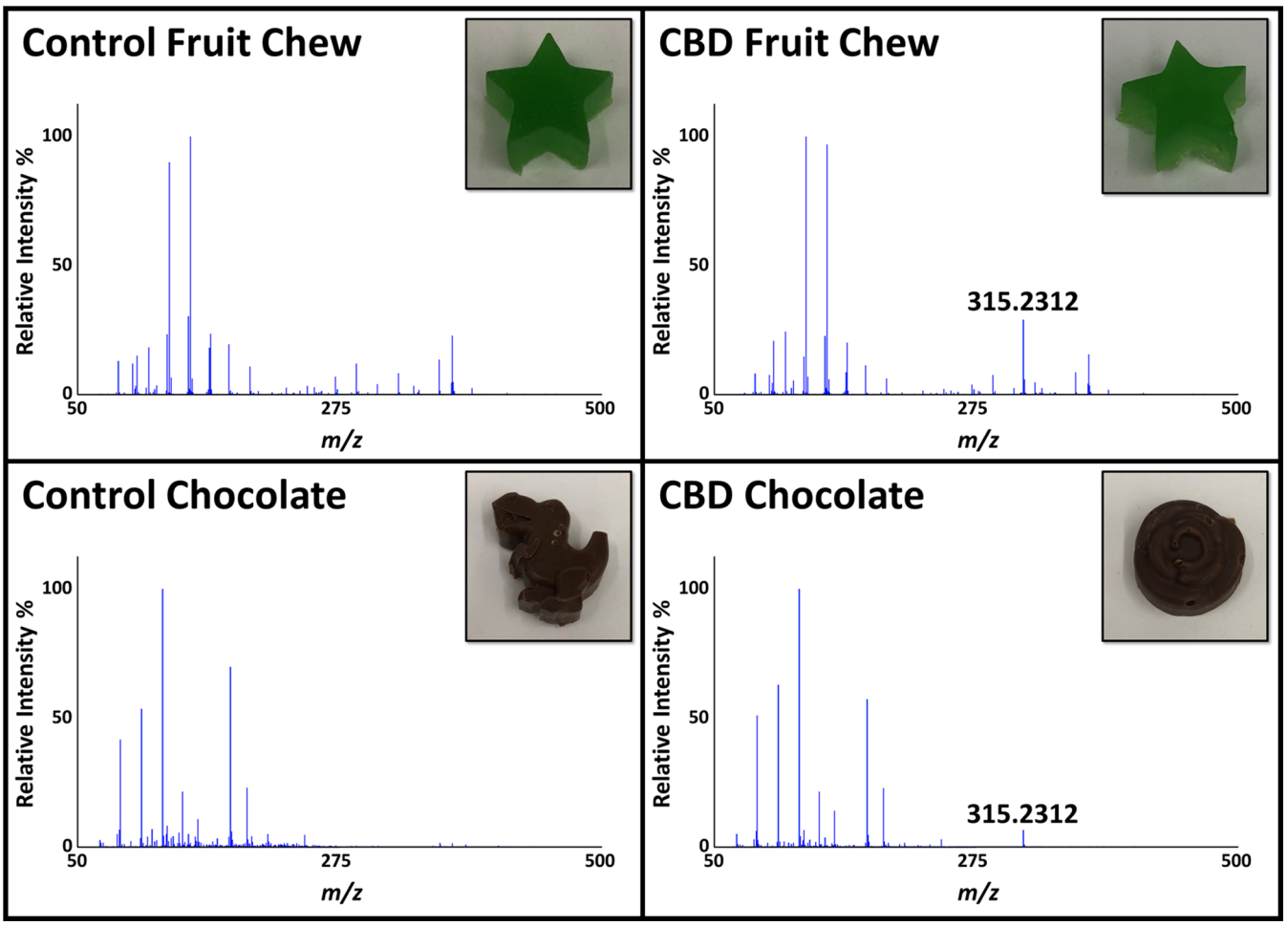

Figure 2. DART high-resolution mass spectra of control and CBD-infused fruit chews and chocolates prepared in-house and analyzed in positive-ion mode under soft ionization conditions (at an orifice 1 voltage of 20 V). The two control edibles (left) did not contain a peak at nominal m/z 315, which confirms the absence of CBD in these samples. However, a peak at m/z 315 was detected in each of the CBD-infused edibles (right), which confirms the presence of CBD in these edibles. Images in the insets feature pictures of the fruit chews and chocolates.

guidelines, both calibration curves were created with a blank calibrator (no analyte or internal standard) and a zero calibrator (blank calibrator spiked with an internal standard). Thus, the requirement that the blank and zero calibrators not interfere with the analyte peak(s) of interest was met. Seven non-zero calibrators were created that covered the range of quantification (10−150 mg/L). The lower limit of quantification (LLOQ) was 10 mg/L, which was determined to be approximately 10 times the previously identified instrument detection limit (IDL) for both THC and CBD.[26] This calibrator produced an analyte response greater than five times that of the zero calibrator. The concentrations of the remaining six curve calibrators were 25, 50, 75, 100, 125, and 150 mg/L. For a calibration curve to be considered acceptable, a minimum of six non-zero calibrators must be within ±15% of their nominal (theoretical) concentrations, except at the LLOQ where the calibrator should be within ±20% of the nominal concentrations. Two sets of quality control (QC) standards from separate stocks were made fresh each day that the curves were analyzed. Four QC levels were prepared: (1) LLOQ; (2) low, which is defined as three times the LLOQ; (3) medium, which is defined as mid-range; and (4) high, classified as high-range. The chosen concentrations were 10, 30, 80, and 130 mg/L, respectively, for both the THC and CBD calibration curves. For each curve acquisition, calibrators were analyzed in triplicate, while QC standards were analyzed in replicates of five. For an acquisition to be considered validated, ≥50% of the QC standards at each level (i.e., LLOQ, low, medium, and high) should be within ±15% of their nominal concentrations, and ≥67% of the QC standards overall should be within ±15% of the nominal values. These requirements were met for each of the three acquisitions of the THC and CBD calibration curves. The curves created for these validations are shown in Figure S1, with the curve and QC quantitation results presented in Tables S1−S10. The successful validation of these curves acquired by DART-HRMS analysis indicated that subsequent quantification experiments for use in determining the CBD and THC content in infused samples could be accomplished.

DART-HRMS Screening of CBD Edibles Prepared In-House. To examine the accuracy of the validated quantification protocols, control (containing no CBD) and CBD-infused chocolates and fruit chews were prepared in-house. Before initiating the extraction protocol, the three control and three CBD-infused fruit chews were screened by DART-HRMS in positive-ion mode under soft ionization conditions (at an orifice 1 voltage of 20 V). The detection of a peak at m/z 315.2324 (within 5 millimass units (mmu)) would indicate the presence of protonated $[M + H]^+$ CBD ($[C_{21}H_{30}O_2 + H]^+$). Figure 2 (top spectra) shows representative DART-HR mass

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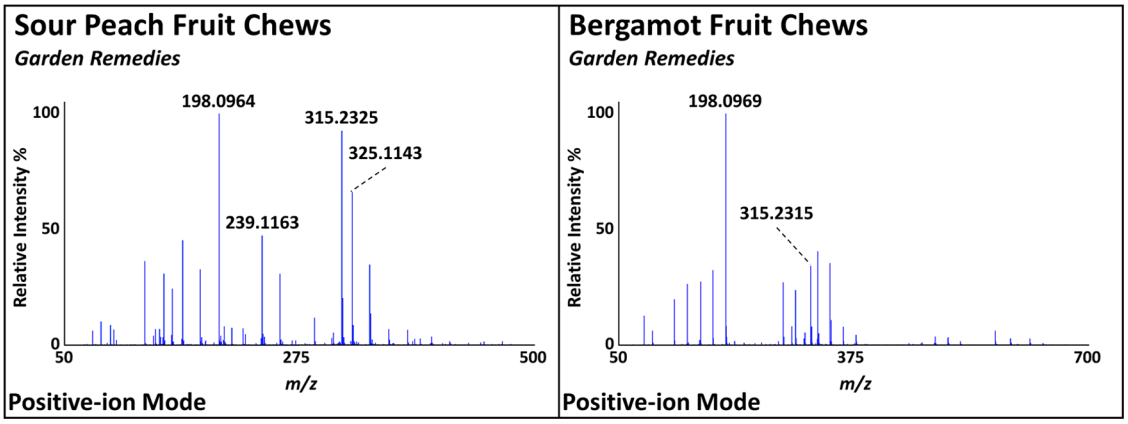

Figure 3. DART high-resolution mass spectra of recreational *Cannabis* fruit chews analyzed in positive-ion mode under soft ionization conditions (at an orifice 1 voltage of 20 V). Each spectrum contains a peak at nominal m/z 315, which is consistent with the protonated mass of THC.

spectra of a control and a CBD fruit chew, while mass spectra for all control and CBD-infused fruit chews analyzed in this study are featured in Figure S2. No peak at nominal m/z 315 was detected in any of the control fruit chews. This not only confirmed that CBD was not present in the samples but also indicated that there was no analyte in the fruit chew matrix with an m/z value that would interfere with the quantification experiments. The three control and three CBD-infused chocolates were also screened by DART-HRMS using instrument parameters identical to those employed in the analysis of the fruit chews. Figure 2 (bottom spectra) shows representative mass spectra for a control and a CBD-infused chocolate, with the DART-HR mass spectra for the other chocolate replicates presented in Figure S3. As was observed with the fruit chews, no peak at m/z 315 was detected in the control chocolates, which confirmed the absence of CBD in the controls. Not only did these results demonstrate the rapid detection of CBD in complex edibles that were analyzed in their native forms, but it also showed that, similar to the fruit chew matrix, there were no analytes in the chocolate matrix that would interfere with the quantification of the CBD content in the CBD-infused chocolates.

DART-HRMS Screening of Recreational THC Edibles. Four recreational products were selected for this study: (1) Sour Peach Fruit Chews; (2) Bergamot Fruit Chews; (3) Dark Chocolate; and (4) Milk Chocolate. These products were selected because the only cannabinoid (reported on the product label) that would contribute to the peak at m/z 315 was THC ($[C_{21}H_{30}O_2 + H]^+$), and as such, they would serve as suitable samples to test the developed DART-HRMS quantification protocols.

Two recreational THC chocolates (i.e., Dark Chocolate and Milk Chocolate) were screened for THC. The results revealed a peak at m/z 315 consistent with the mass of protonated THC. The two recreational THC fruit chews (i.e., Sour Peach Fruit Chews and Bergamot Fruit Chews) were analyzed by DART-HRMS to confirm the presence of THC, before initiating quantification experiments. Because THC and CBD are cannabinoid isomers (i.e., they share the same molecular formula), and because these products did not contain any CBD, the detection of a peak at nominal m/z 315 would indicate the presence of THC. Shown in Figure 3 are the DART-HR mass spectra obtained when the two fruit chews were analyzed in positive-ion mode under soft ionization conditions (20 V). The peak at m/z 315 in each confirmed the appropriateness of subjecting them to subsequent extraction and quantification experiments. As was demonstrated by the DART-HRMS analysis of the CBD-infused edibles prepared in-house, the detection of THC in these recreational samples was accomplished without the need for sample pretreatment steps (which would have been required to perform this assessment by chromatography-based methods).

DART-HRMS Quantification of CBD in Edibles Prepared In-House. The validated DART-HRMS quantification protocol was used to quantify the CBD content in the edibles prepared in-house, (i.e., chocolates and fruit chews) following the extraction protocol described in the Materials and Methods section. In short, the extraction process used DisQue CEN salts and the manufacturer-recommended solvents (i.e., water and acetonitrile). After all chocolates and fruit chews prepared in-house were extracted, CBD calibration curves were developed to run alongside the extracts. Since the chocolate and fruit chew experiments were performed on different days, separate CBD calibration curves and QC sets were created and analyzed. Figures S4 and S5 show the calibration curves created for the quantification of CBD in chocolates and fruit chews. The quantitative results associated with the calibration curves developed for the CBD-infused chocolates and fruit chews are shown in Tables S11 and S12, respectively. The curves and QC standards passed validation, which indicates that the quantification results obtained for the edibles extracts could be considered accurate. The quantification experiments also confirmed the absence of CBD in the control extracts (i.e., chocolates and fruit chews that were not infused with CBD). The quantification results for the CBD-infused edibles prepared in-house are reported in Table 1. The determined quantitative values for the CBD-infused candies ranged from 76.2 to 84.3% (chocolates) and 96.7 to 99.0% (fruit chews) of the actual amount spiked into the samples, and were measured with relative standard deviations (RSDs) of less than 10%. The RSDs of the CBD calibrators used to develop the calibration curves were less than 10%, and no analyte signal was observed in the unspiked chocolate and gummy matrices (example shown in Figure S6). Several studies (presented in Table 2) have reported the successful quantification of cannabinoids in *Cannabis*-infused matrices such as oils/liquids,[6,14,15,17−19,25,29−35] food products,[6,13,15,16,19,20,36−38] concentrates,[18] capsules,[6,17,19] and vape products.[17,19] Ciolino et al.[19] reported the average percent (%) recovery of five cannabinoids in various spiked matrices, including edible oils,

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Table 1. Quantification Results for Determination of the CBD Content in Chocolates and Fruit Chews Prepared In-House[a]

Edible	Actual CBD (mg)	Avg. experimentally determined CBD (mg)	Recovery (%)	SD	RSD (%)
CBD chocolate 1	4.81	4.06	84.3	0.360	8.87
CBD chocolate 2	5.65	4.30	76.2	0.194	4.50
CBD chocolate 3	5.78	4.70	81.4	0.175	3.72
CBD fruit chew 1	5.07	5.02	99.0	0.129	2.56
CBD fruit chew 2	4.31	4.19	97.3	0.277	6.62
CBD fruit chew 3	5.47	5.29	96.7	0.333	6.30

[a]The actual amount of CBD that was present in the candies, the percent of CBD that was recovered from the candies (in comparison to the amount that was used to prepare the samples), and the standard deviation (SD) and corresponding relative standard deviation (RSD) are also reported.

foods, topicals, oral OTC (over-the-counter) pharmaceuticals, beverages, and dairy foods.[19] Included within the food product category were milk and dark chocolate bars (extracted with acetonitrile) and a hard candy (extracted with 83% aqueous acetonitrile).[19]

With these samples, the average cannabinoid recoveries were 83% (milk chocolate), 95% (dark chocolate), and 74% (hard candy). The recoveries of the two chocolates analyzed by Ciolino et al.[19] are very similar to the recoveries of CBD from chocolates analyzed in the present study (76.2−84.3%). Furthermore, the RSDs for the experimental CBD amounts were less than 10%, which indicates similarities between the five replicates for each chocolate extract, as well as the reproducibility of the results. The aforementioned report did not analyze fruit chew (i.e., gummy) samples and there are no other reported fruit chew/gummy samples to which to compare the present results. The hard candy cannabinoid recovery (74%)[19] is less than the recoveries observed here for the CBD-infused fruit chew candies (96.7−99.0%). In addition, RSDs for the experimental CBD amounts in the CBD-infused fruit chews were less than 10%, indicating similarity and reproducibility between the five replicates for each fruit chew extract. Although CBD can be quantified as a total of the CBD and cannabidiolic acid (CBDA) content, the purpose of this experiment was to examine the ability of DART-HRMS to determine the CBD content of edibles using a percent recovery approach. Therefore, the preparation of the chocolates and fruit chews in-house with CBD as the only cannabinoid present enabled the investigation of this question. One study that did investigate the recovery of cannabinoids from gelatin-based gummies obtained percent recoveries of 92.3% (CBD) and 93.7% (THC).[38] These results, which were obtained using the Agilent EU QuEChERS extraction kit and Agilent polytetrafluoroethylene (PTFE) filters,[38] are similar to the results presented here for CBD recovery from the fruit chews prepared in-house. The success observed for extracting and quantifying CBD from edibles prepared in-house illustrates the utility of DART-HRMS as a facile approach to the accomplishment of the quantification of CBD in edibles. Importantly, the results were obtained while circumventing the lengthy run times often encountered when utilizing LC- or GC-based methods to quantify cannabinoids. Furthermore, they are promising for the development of quantification experiments focused on determining the CBD content in other products, such as commercial hemp materials and recreational *Cannabis* samples.

DART-HRMS Quantification of THC Content in Recreational Edibles. THC calibrators and QC standards were prepared (as outlined in the Materials and Methods section below) for the quantification experiments that were focused on determining the THC content in the recreational candies. From DART-HRMS experiments using a semi-automated approach, the calibration curve displayed in Figure 4 was developed. All calibrators, which ranged from 10 to 150 mg/L, were within 15% of their nominal (theoretical) concentration, indicating that the curve passed validation. Furthermore, the R^2 value was >0.99, which confirmed that the calibration curve was suitable for quantification experiments. Shown in Figure S7 are the QC standard results for the quantification experiments performed. Greater than 50% of the QC standards at each level (high, medium, low, and LLOQ) were within an acceptable percentage of their theoretical concentration, and greater than 67% of the QC standards overall passed validation. In Figure S7, the replicates highlighted in red were outside the acceptable percentage range for that respective QC level. In the aggregate, the results indicate the following: (1) the calibration curve passed validation; and (2) the curve could be used to determine the unknown THC content in the edibles extracts that were analyzed alongside the curve, which were prepared as described below.

To determine the THC concentration in the extracts, the peak area ratios for the THC and THC-d_3 $[M + H]^+$ peaks at m/z 315.2324 and 318.2512 respectively, from the analysis of the fruit chew and chocolate extracts, were obtained and plotted against the calibration curve. When coupled with the dilution factors and the extraction solvent volume information, the %THC content in the edibles could be determined. This value was calculated by comparing the THC content to the total mass of the edible/sample. The results are featured in Table 3. It is important to note that the values in the "reported THC (%)" column are those printed on the product label (i.e., no further significant figures are shown after the decimal points listed in the table). The amount of THC quantified in each edible was similar to the values reported on the product labels, indicating the utility of DART-HRMS as an approach to quantification of THC in *Cannabis* products.

When considering the product label information provided for the fruit chews, it is possible that with additional decimal information these values might have been closer to the actual %THC in the fruit chews (i.e., 0.1% THC in the Bergamot Fruit Chew may actually have been 0.14% THC (for example), but this could not be determined since the amounts were only reported to one decimal place).

With regard to the quantification of THC in the chocolates, the results for the %THC determined from the analysis of the recreational milk (0.155% THC) and dark chocolate (0.143% THC) are slightly below the %THC values reported on the product labels (0.17 and 0.15% THC). For the gummy/fruit chews, the %THC quantified in the Bergamot Fruit Chew (0.102% THC) and the Sour Peach Fruit Chew (0.116% THC) are slightly above the %THC reported on the product labels (0.1 and 0.11% THC; −0.002 and −0.006% THC

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Table 2. Reported Methods for the Identification, Analysis, and Quantification of Cannabinoids in *Cannabis*-Infused/Derived Products by LC- and GC-Based Methods

Methods	Analytes	Products
HPLC	THC; cannabigerolic acid (CBGA); cannabichromenic acid (CBCA); tetrahydrocannabinolic acid (THCA); cannabigerol (CBG); cannabichromene (CBC)	*Cannabis* oils[34]
HPLC	CBD	CBD oils and hemp seed oils[32]
HPLC	cannabidivarin (CBDV); CBD; CBG; THC; tetrahydrocannabivarin (THCV); cannabidiolic acid (CBDA); CBGA; cannabinol (CBN); Δ^8-THC; CBC; THCA	hemp oil, CBD oil, concentrates, tinctures, and resins[18]
HPLC/DAD/FID	CBD; Δ^8-THC; CBC; CBG; CBGA; CBDV; THCV; THCA; CBDA; CBN; THC	CBD-containing/hemp products (capsules (oil and powder containing), liquids (oils, tinctures, beverages, honey), gummies, candies)[6]
HPLC−MS/MS	CBD; THC; CBDA; THCA	hemp-containing products (oils, cosmetics, food products, supplements, tinctures)[15]
HPLC−MS/MS	CBD; CBN; THC	marijuana edibles (beverages, candies, baked goods, oils)[16]
HPLC−photodiode array (PDA)	CBDA; CBGA; CBG; CBD; THCV; CBN; THC; CBC; CBDV; THCA	medical marijuana products (capsules, tablets, tinctures, vape cartridges)[17]
HPLC−UV/DAD	CBGA; CBG; CBD; CBDA	hemp oil, balms, and extracts[14]
hydrophilic interaction liquid chromatography (HILIC)−HRMS	CBD	hemp seed oil[35]
LC−HRMS	CBD; THC; THCA; CBDA	hemp oil products[30]
LC−quadrupole time-of-flight (Q-TOF)-MS	CBD; CBDV; CBDA; cannabidivarinic acid (CBDVA); THC; THCV; THCA; CBN; CBGA; CBC; CBCA	hemp oil samples[31]
LC−electrospray ionization (ESI)−tandem MS	THC; CBD; THCA; CBDA; CBN	galenic oil preparations[33]
LC/UV	THC; CBD	infused beverages[36]
LC/UV	THC; CBD	gummies and candies[38]
LC−UV−MS/MS	CBD; CBDA; THC; Δ^8-THC; THCA; CBN; CBG; THCV; CBDV; CBC; CBGA	*Cannabis*/hemp concentrates (oil, distillate, wax)[39]
reverse-phase (RP)-HPLC−UV	THC; THCA	hemp food products[13]
ultra-HPLC/MS (UHPLC/MS)	THC; CBD; CBN	baked goods[20]
UPLC−MS/MS	THC; CBD	brownies spiked with cannabinoids[37]
UPLC−UV; UPLC−MS	THC	hard candies, sugar-based drinks, and brownies spiked with cannabinoids[40]
GC−FID	THCA; CBGA; CBCA; THCA; CBG; CBC	*Cannabis* oils[34]
GC−MS	THC	hemp seed oil products[25]
GC−MS; GC−FID	THC; THCA	*Cannabis* olive oil preparations[29]
GC−MS	CBD; CBDA; THC; THCA; CBN; Δ^8-THC; CBG; CBGA; CBDV; THCV; CBC	commercial products (oral supplements, foods, candies, beverages, topicals, vapes/e-liquids); recreational marijuana plants, hemp seed oils, and dronabinol capsules[19]

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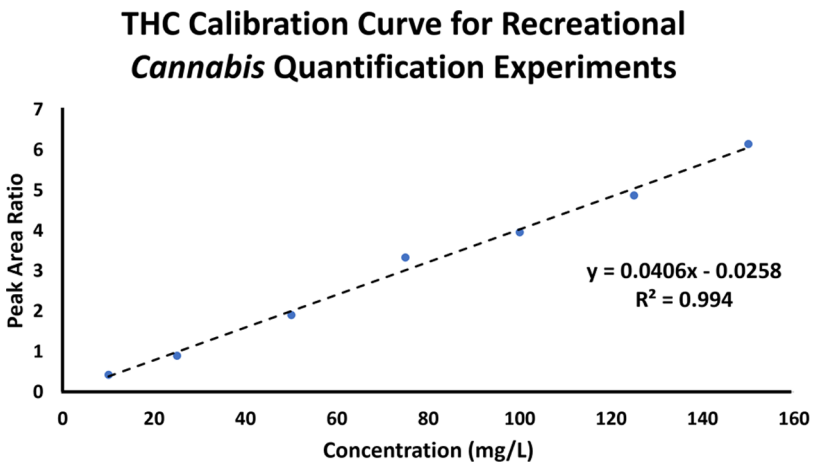

Figure 4. THC calibration curve developed using DART-HRMS data that were generated using a semiautomated approach. All seven calibrators passed the validation requirements, and the R^2 value was >0.99, which makes the curve suitable for determining the THC content in the extracts of recreational edibles.

Table 3. Quantification Results for Determination of the THC Content in Recreational *Cannabis* Chocolates and Fruit Chews. Information Featured in this Table also Includes the Experimentally Determined %THC, Experimental Standard Deviations and Relative Standard Deviations, %THC Reported on the Product Labels, and the Difference between the Experimentally Determined and Label-Reported Results

Edible product	Sample weight (mg)	Product label-reported THC per piece (mg)	Exp. determined THC (mg)	Exp. determined THC (%)	Product label-reported THC (%)	Relative standard deviation (RSD) (%)	Diff. between exp. and rep. %THC
milk chocolate	1752	5.16	2.72 ± 0.06	0.155 ± 0.003	0.17	1.99	0.015
dark chocolate	2162	4.44	3.09 ± 0.18	0.143 ± 0.008	0.15	5.79	0.007
bergamot fruit chew	1904	4.44	1.94 ± 0.07	0.102 ± 0.004	0.1	3.75	−0.002
sour peach fruit chew	2126	4.12	2.47 ± 0.16	0.116 ± 0.007	0.11	6.30	−0.006

difference observed, respectively). The reported %THC for the dark chocolate and both fruit chew types fell within the standard deviation of the experimentally determined %THC, with only the reported %THC for the milk chocolate falling outside the experimentally determined standard deviation. In addition, not only were the RSDs for each of the THC products less than 10%, but they were also very similar to the RSDs obtained in the previous experiments in which CBD was quantified in chocolates and fruit chews prepared in-house. There are several possible reasons for the observation of experimentally determined %THC values that are greater than that reported on the fruit chew product labels. One is the limited information provided on the product labels, which listed the concentrations with limited decimal information. Thus, it is possible that with additional significant figures, the reported %THC may be closer to the experimentally determined %THC observed here. Another, which could be applied to all of the recreational products analyzed in this study, is sample heterogeneity. Although it is presumed that the THC content in the edibles is uniform, it is possible that some portions of the candies contained higher concentrations of THC than others. For example, the portions of the fruit chews that were extracted may have contained a higher concentration of THC than that indicated on the product label, with other areas containing less. Nonetheless, the goal of these experiments was to test the DART-HRMS quantification methods developed in-house with samples that only contained THC, which was the reason these fruit chews and chocolates were selected. The results of this study will serve as the foundation for optimizing the method to enable total THC content (i.e., for samples which contain THC and tetrahydrocannabinolic acid (THCA)). In summary, the results observed in this study demonstrate the successful application of DART-HRMS for the rapid quantification of major cannabinoids in extracts derived from complex edible matrices.

CONCLUSIONS

The DART-HRMS-validated calibration curves for THC and CBD were developed in alignment with FDA guidelines using a semiautomated protocol. These procedures were used to quantify the following: (1) CBD in chocolates and fruit chews prepared in-house; and (2) THC in recreational *Cannabis* chocolate and fruit chew products. The CBD quantified in chocolates (76.2−84.3% recovery) and fruit chews prepared in-house (96.7−99.0% recovery), and the THC quantified in recreational chocolates (0.155 and 0.143% THC) and fruit chews (0.102 and 0.116% THC) demonstrate the successful application of the validated DART-HRMS quantification protocols to complex edible matrices. These results also compare well with previously reported quantification studies for THC and CBD from similar edible sample types. The results demonstrate proof-of-concept for the application of AIMS techniques, such as DART-HRMS-based methods, for the quantification of cannabinoids in complex *Cannabis* matrices. Furthermore, the developed approach highlights the ability of AIMS techniques to circumvent laborious, time-

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ACS Omega 2023, 8, 14459−14469

consuming, and resource-intensive analyses (i.e., various chromatography-based methods). One limitation of analyses conducted by DART-HRMS under soft ionization conditions is the inability to distinguish between isomeric compounds (i.e., molecules with the same chemical formula). Therefore, to address the applicability of this method to edibles/complex matrices infused with more than one cannabinoid, a potential approach would be to incorporate the use of a chemical derivatization step into the extraction protocol. This step would be useful for differentiating between THC and CBD cannabinoid isomers before quantification by DART-HRMS. Furthermore, the use of tandem mass spectrometry coupled to DART may offer additional assistance toward differentiating other cannabinoid isomers (i.e., Δ^9-THC and Δ^8-THC). However, because DART-HRMS is a nonchromatographic method, this deficit is offset by eliminating the risk of contaminating and/or clogging columns and syringes, which could introduce carryover into subsequent runs and cause instrument downtime for cleaning and repairs. The developed quantification protocol is also compatible with various extraction methods and solvent systems and therefore offers analysts multiple options for combining already validated extraction protocols with DART-HRMS quantification. This approach also has the versatility to be applicable to other complex *Cannabis* matrices, such as plant materials (i.e., leaf, flower, stem), edibles, topicals, and oils, among others, that will continue to emerge as the *Cannabis* industry evolves, demonstrating the universality and robustness of DART-HRMS-based methods.

MATERIALS AND METHODS

Chemical Standards. Cannabidiol (CBD), Δ^9-tetrahydrocannabinol (THC), CBD-d_9, and THC-d_3 chemical standards (1 mg/mL) and solid CBD were purchased from Cayman Chemical (Ann Arbor, MI). Methanol was obtained from Pharmco (Brookfield, CT). Polyethylene glycol (PEG 600) was purchased from Sigma-Aldrich (St. Louis, MO). Nitrogen and ultra-high-purity helium gases were acquired from Airgas (Albany, NY).

Development of CBD and THC Calibrator Solutions. Calibration curves were developed using cannabinoid (CBD, THC) calibrators and deuterated internal standards (CBD-d_9 and THC-d_3) using methanol as the diluting solvent. CBD and THC certified reference materials (CRMs) (1000 mg/L in methanol) were used as the stock solutions. Serial dilutions from the stocks were made to prepare the calibrator solutions ranging from 20 to 300 mg/L, all with a final volume of 100 μL. To create a 50 mg/L working stock solution of CBD-d_9 and THC-d_3, 250 μL of the 1000 mg/L CBD-d_9 and THC-d_3 stock solutions was diluted to 5000 μL (5 mL). Each CBD and THC calibrator solution was spiked with 100 μL of the 50 mg/L CBD-d_9 and THC-d_3 internal standard working stock solutions, respectively, to bring the final volume of each calibrator to 200 μL. Therefore, the final concentration of each calibrator was diluted to half the concentration made during the serial dilution process. The final concentration of the CBD-d_9 and THC-d_3 internal standards was 25 mg/L. The final concentrations of the calibration curve calibrators ranged from 10 to 150 mg/L, and these solutions were used to develop the respective calibration curves. A blank standard (containing only methanol) and a zero calibrator (containing only 100 μL of methanol and 100 μL of internal standard solution) were run with the calibrators of each calibration curve.

Development of CBD and THC Quality Control Standards. In alignment with the stipulations of the Food and Drug Administration (FDA) Bioanalytical Method Validation: Guidelines for Industry,[27] fresh stock solutions (CRMs at 1000 mg/L) were used to prepare the quality control (QC) standard stock solutions. Two CBD CRMs and two THC CRMs were used as the stock solutions to prepare fresh QC standards each day the curve was analyzed. Serial dilutions were made to generate concentrations of 20, 60, 160, and 260 mg/L with 50 μL from each solution being transferred to a new 0.6 mL Eppendorf tube. Each of these solutions was spiked with 50 μL of either the CBD-d_9 or the THC-d_3 internal standard stock solutions to create the final QC standards. This brought the QC standards to their final volume (100 μL) and concentrations of 10, 30, 80, and 130 mg/L, with the standard of lowest concentration representing the lower limit of quantification (LLOQ). QC standards were prepared fresh each of the three days that the curves were analyzed during the validation process, and each day that the curve was analyzed to quantify THC/CBD in edibles extracts. Although the stock solutions were not prepared fresh each day, no signal decrease in the DART high-resolution mass spectra or degradation was observed over the one-week time frame during which the validation processes occurred.

Edibles Prepared In-House. Baking ingredients (chocolate chips, flavored and unflavored gelatin) were purchased from a local grocery store. A Wilton Candy Melts Candy Melting Pot and candy molds were purchased from Walmart (Albany, NY). Gummy/fruit chew matrix was prepared with water, flavored (lime) gelatin, and unflavored gelatin following manufacturer guidelines. Three CBD fruit chews were prepared with 5 mg CBD (non-CRM formulation) each. In addition, three control fruit chews were prepared with no CBD. To prepare the experimental chocolates, chocolate chips were melted in a melting pot to a smooth consistency. Three CBD chocolates were prepared with 5 mg CBD (non-CRM formulation). In addition, three control chocolates were prepared with no CBD. All edible candies were allowed to solidify under refrigeration before sample analysis.

Recreational Cannabis Products. Sour Peach Fruit Chews, Bergamot Fruit Chews, Dark Chocolate, and Milk Chocolate products were purchased from Garden Remedies Marijuana Dispensary (Melrose, MA). These products were selected for quantification experiments because THC was the only cannabinoid reported that would exhibit a peak at m/z 315 in the mass spectral analysis (i.e., no other cannabinoid molecules that would interfere with quantifying the THC content were listed on the label).

Extraction of Cannabinoids from Edibles Matrices. First, 50 mL centrifuge tubes (VWR, part of Avantor, Radnor, PA) were weighed before use. Each control and CBD-infused edible was deposited into a separate tube, and these were then reweighed to obtain the total weight of the edible. Whole edibles were extracted in order to determine an accurate percent recovery and avoid the potential influence of heterogeneity in the results. The edibles were broken into smaller fragments using a metal spatula to expose a greater surface area to the extraction solvents. Water (10 mL) was added to each centrifuge tube, and the suspension was vortexed for 1 min and sonicated for an additional 20 min. Acetonitrile (10 mL) was then added to each tube and the contents were vortexed for another 1 min. One packet of DisQue CEN salts (1 g trisodium citrate dihydrate, 0.5 g

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ACS Omega 2023, 8, 14459−14469

disodium hydrogen citrate sesquihydrate, 1 g NaCl, and 4 g $MgSO_4$) (Waters, Milford, MA) was added to each of the tubes, and the resulting suspension was vortexed for 1 min. After allowing the tubes to stand for several minutes, three distinct layers began to appear in the centrifuge tube. A 500 μL aliquot from the top acetonitrile layer was centrifuged for 5 min at 3000*g*. A 50 μL aliquot of each (i.e., the gummy and chocolate extracts) was diluted with 50 μL of acetonitrile before the addition of internal standard (100 μL of 50 mg/L CBD-d_9).

The sample protocol described was used to extract the THC content from the four recreational *Cannabis* products (i.e., Sour Peach Fruit Chews, Bergamot Fruit Chews, Dark Chocolate, and Milk Chocolate). However, whole pieces of the candies were not extracted. The fruit chews were sectioned into halves, and one half of each chew was extracted (approximately 2000 mg) according to the aforementioned protocol. The chocolate squares (i.e., two servings) were sectioned into quarters, and one half of a piece (i.e., one serving size) of chocolate (approximately 1500 mg) was extracted for both chocolates. Following the extraction, 100 μL of the fruit chew extracts were diluted with 100 μL of acetonitrile and vortexed. A 100 μL aliquot of the resulting solution was spiked with the internal standard (100 μL of 50 mg/L THC-d_3). For the chocolates, 100 μL of the extract was spiked with the internal standard (100 μL of 50 mg/L THC-d_3). The four candy extracts were analyzed in replicates of five alongside the curve calibrators and QC standards.

DART-HRMS Mass Spectral Data Acquisition and Data Analysis. Samples were analyzed by DART-HRMS at one of two locations: (1) the University at Albany − State University of New York (SUNY) (Albany, NY); or (2) IonSense Inc. (Saugus, MA). An IonSense DART ion source SVP coupled to a JEOL AccuTOF high-resolution mass spectrometer (Peabody, MA) was used for all mass spectral analyses, which were conducted in positive-ion mode (20 V). Ultra-high-purity helium at a DART gas temperature of 350 °C and a flow rate of 2 L/min was used for all DART acquisitions. The following parameters were used at both locations: ring lens, 5 V; orifice 1 voltage, 20 V; orifice 2 voltage, 5 V; peak voltage, 600 V; and detector voltage, 2000 V. The DART ion source at the University at Albany was operated at a grid voltage of 250 V, while the DART ion source at IonSense was operated at a grid voltage of 350 V.

Materials for screening purposes (i.e., determining the presence of cannabinoids for identification purposes) were sampled using a capillary tube technique. The closed end of a glass melting point capillary tube was inserted into the sample, and the coated surface was presented to the DART gas stream for approximately 5 s. This process was repeated three times, after which the three replicates were averaged to produce a mass spectrum representative of all replicates. Sampling protocols for quantification experiments are outlined in the detail below.

Data were collected over a mass range of m/z 60−1000, and PEG 600 was used as the mass calibrant for all mass spectral acquisitions. TSSPro 3.0 software (Shrader Software Solutions, Grosse Pointe, MI) was used for the calibration, spectral averaging, background subtraction, and peak centroiding of mass spectral data. Data collected at the University at Albany by DART-HRMS were translated and calibrated before data processing. Data collected at IonSense were calibrated (using a reference mass in the PEG 600 acquisition), processed, and evaluated at the University at Albany. A millimass unit (mmu) tolerance of within ±5 mmu was used when determining the presence of a peak consistent with the mass of THC/CBD during screening experiments. The Mass Mountaineer software suite (RBC Software, Portsmouth, NH) was used for the generation of all mass spectra.

DART-HRMS Semiautomated Quantification. To perform semiautomated DART-HRMS quantification experiments, a 24-Pin Liquid Sampler (IonSense, Saugus, MA) was used for all mass spectrometric measurements (i.e., calibration curves, QC standards, edibles extracts). This apparatus consists of 24 small metal pins affixed to a handle that is then suspended on a moving automated linear rail system. This enables samples to enter the DART gas stream at a user-defined speed, which in these experiments was optimal at 0.8 mm/s. Samples for analysis were dispensed into 384-well plates (Eppendorf, Enfield, CT) in 10 μL aliquots. The pins of the 24-Pin Liquid Sampler were then immersed in the 24-sample wells within a 384-well plate for 5 s. This resulted in the pins being coated with the sample when the 24-Pin Sampler was removed from the 24-sample-containing wells. With the pins now coated with the sample, the 24-Pin Sampler was suspended on the linear rail system for semiautomated analysis. All calibrators (including the blank and zero calibrators) were analyzed in triplicate, while QC standards and edibles extracts were analyzed in replicates of five.

TSSPro 3.0 software enables the integration of individual peaks in extracted ion chromatograms (EICs) based on selected high-resolution m/z values. To develop CBD calibration curves, the peak area ratios of CBD to CBD-d_9 $[M + H]^+$ at m/z 315.2324 and 324.2889, respectively, were plotted against the CBD calibrator concentrations. To develop the THC calibration curves, the peak area ratios of THC to THC-d_3 $[M + H]^+$ at m/z 315.2324 and 318.2512, respectively, were plotted against the THC calibrator concentrations. These ratios were used to confirm the CBD and THC concentrations in QC standards and accurately determine the cannabinoid content in edibles prepared in-house and commercial recreational products.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c08172.

THC and CBD calibration curves developed using semiautomated DART-HRMS capabilities and validated according to FDA guidelines; tables featuring validation results for THC and CBD DART-HRMS quantification protocols; DART high-resolution mass spectra of control and cannabinoid-infused edibles prepared in-house; CBD calibration curve quantitation results developed to run alongside the extracts of CBD-infused chocolates and fruit chews; example ion chromatograms demonstrating analyte signal responses in CBD-infused samples and the absence of an analyte signal for unspiked matrices; and quality control (QC) results for the THC quantification experiments performed at IonSense Inc (PDF)

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Notes

The authors declare no competing financial interest.

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