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Consistency of Label Claims of Internet-Purchased Hemp Oil and Cannabis Products as Determined using IMS and LC-MS: A Marketplace Survey

Ashley C. Ruth[a], Connie M. Gryniewicz-Ruzicka[a,*], Michael L. Trehy[a], Nicole Kornspan[b], Gary Coody[b]

[a] *US FDA/CDER/OPQ/OTR/DPA, St. Louis, MO*
[b] *US FDA/ORA/Office of Enforcement & Import Operations, Silver Spring, MD*

Abstract

In this paper we describe the use of ion mobility spectrometry (IMS) and liquid chromatography high resolution mass spectrometry (LC-HRMS) to screen for the presence of cannabinoids and other potential hazards in a set of products with hemp oil and/or cannabinoid label claims purchased via the internet. IMS was used as a preliminary screening tool to examine the products for the presence of cannabinoids, illicit drugs or undeclared pharmaceuticals. Detection of a cannabinoid by IMS was confirmed by subsequent LC-HRMS analysis, which qualitatively screened for the presence of nine common cannabinoids and quantified the following four cannabinoids: cannabidiol(CBD), (-)-Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^9-tetrahydocannabinolic acid (THCA), and cannabidiolic acid (CBDA). No other illicit drug or undeclared pharmaceutical was detected in any sample from IMS screening. Eighteen of 23 samples tested positive for the presence of at least one cannabinoid by LC-HRMS, with three products containing less than 0.01%(w/w) of a cannabinoid. Four products with explicit CBD label claims were found to not contain any CBD, while three products featured levels of cannabinoids below label claim.

Keywords: cannabinoids, LC-HRMS , IMS , hemp oil products

1. INTRODUCTION

Several cannabinoids have been designated by the U.S. Drug Enforcement Administration as Schedule I controlled substances, indicating a high abuse potential and no currently accepted medical use. However, several states have recently legalized the use of cannabinoid products for either medical and/or recreational use [1–3]. The main psychotropic cannabinoid responsible for hallucinogenic effects is THC (Δ^9-tetrahydrocannibinol) while cannabidiol (CBD) is a non-psychotropic cannabinoid purported to provide a therapeutic benefit[4, 5]. Recently, an increase in the number of hemp-based products with enriched levels of CBD has been observed. In the absence of appropriate regulations, concerns have been raised regarding the accuracy of cannabinoid content label claims[6] as well as the potential for other hazardous species to be present in the products.

Hemp refers to the *cannabis* sativa plant stalks which have significantly lower cannabinoid content than the leaves and buds; the THC content limit determined by Health Canada is not more than (NMT) 0.3% THC (w/w)[7] . However, several online retailers offer hemp oil products with label claims indicating elevated CBD levels. Ion mobility spectrometry (IMS) is a high throughput separation method that characterizes chemical substances based upon their gas phase ion mobilities. IMS instruments are easy to use and have been used as a detection device for the presence of trace amounts of illicit drugs including THC[8, 9] and for synthetic cannabinoids[10, 11]. IMS is an ideal screening tool for the detection of cannabinoids due to its high speed and low detection limits. Screening samples by IMS can help prioritize sample collection, detect undeclared ingredients in products and assist in reducing the number of samples undergoing testing using more time-intensive methods like LC-HRMS. The goal of this study is to perform a marketplace survey of hemp oil products with CBD label claims from internet-based retailers in order to inform the consumer of any imminent hazards that may be present as well as any label claim discrepancies. IMS was used to screen for the presence of cannabinoids as well as illicit drugs or any undeclared pharmaceuticals present, while LC-HRMS was used for specific cannabinoid screening and quantitation.

*Corresponding author:Connie M. Gryniewicz-Ruzicka, Phone:(314)539-3843. Fax: (314)539-3843. Email:connie.ruzicka@fda.hhs.gov, Address:US FDA/CDER/OPQ/OTR/Division of Pharmaceutical Analysis 645 S. Newstead Avenue, St. Louis, MO 63110

Product Name	Source	%CBD	%Δ9-THC	%THCA	%CBDA	Label Claim (%CBD)	Consistent w/Label Claim?	IMS Result Consistent?
RSHO Gold	hempmedspx.com	16.2%	1.0%			15.5%	Yes	Yes
RSHO Blue	hempmedspx.com	18.8%	0.8%			17.5%	Yes	Yes
RSHO Blue	hempmedspx.com	17.7%	0.8%			17.5%	Yes	Yes
21% CBD Hemp Oil	purecbd.net	*none detected*				21.0%	No	Yes
CBD Cannabis Extract Capsules	purecbd.net	*none detected*				Unknown	Unknown	Yes
Wiped Gold	hempoilcare.com	25.2%	1.2%		0.2%	23.42% (0.85% THC, 0.23%CBDA, 1.59 CBC, 0.64% CBG)	Yes	Yes
Ultra CBD	Ultracbd.com	*none detected above 0.1% (w/w)*				*Proprietary Blend	Unknown	Inconclusive
CBDY Hemp Oil Supplement Drops	calistores.com	0.1%				200 mg (5 mg/ dose)	Unknown	Yes
Hemp Honey CBD Oil	hempoilcare.com	*none detected*				21% (0.3% THC)	No	Yes
Arisi-Tol CBD Lozenges	arisitol.com	0.2%				3 mg	Yes	Yes
Hemp Honey CBD Vape Oil, Blueberries and Cream	hempoilcare.com	*none detected*				50 mg of 21%	No	Inconclusive
Hemp Pure Vape E-Drops, Peached	hempoilcare.com	*none detected*				50 mg	No	Inconclusive
Cibaderm Hemp Salve	hempoilcare.com				0.2%	Cannabis Sativa (Hemp) Seed Oil	Unknown	Inconclusive
Cibdex CBD Drops - Peppermint	hempoilcare.com	0.3%				1 mg/ 0.375mL	Yes	Yes
Cibdex CBD Drops - Vanilla	hempoilcare.com	0.3%				1 mg/ 0.375mL	Yes	Yes
Cibdex CBD Drops - Unflavored	hempoilcare.com	0.3%				1 mg/ 0.375mL	Yes	Yes
Extra Strength #4 Hemp Supplement for Cats and Dogs	www.cannaforpets.com	0.1%	0.1%	0.3%	0.9%	Phytocannabinoids	Unknown	Yes
Max CBD Capsules	www.canna-pet.com	2.6%	0.1%			Industrial Hemp	Unknown	Yes
Canna-Pet for Cats	www.canna-pet.com	0.5%				Industrial Hemp	Unknown	Yes
CBD Wedges for Dogs	www.canna-pet.com	*none detected above 0.1% (w/w)*				N/A	Unknown	Inconclusive
Ultra CBD (replicate order)	Ultracbd.com	0.02%	BQL		0.02%	200 mg/ 30 mL (proprietary blend)	No	
26% CBD Oil Supplement (replicate order)	purecbd.net	0.14%	0.45%	0.03%	0.05%	26%	No	
CBD Cannabis Extract Capsules (replicate order)	purecbd.net	0.5%	0.2%	0.03%	0.1%	50 mg	No	

2. MATERIALS AND METHODS

2.1. Standards

10 mg/mL standards of cannabidiolic acid (CBDA), Δ^9-tetrahydrocannabinolic acid (THCA), cannabichromene (CBC), cannabigerol (CBG) and tetrahydrocannabivarin (THCV) were obtained from Cayman Chemical. 1.0 mg/mL standards of (-)-Δ^8-tetrahydrocannabinol (Δ^8-THC), (-)-Δ^9-tetrahydrocannabinol (Δ^9-THC), Cannabidiol (CBD), and Cannabinol (CBN) were obtained from Cerilliant. Structures and MH+ *m/z* values are shown in Figure 1.

2.2. Sample Preparation

Hemp oil products were purchased from internet sources accessible within the United States (Table 1). Two aliquots of ~0.5 g of sample were prepared by diluting with 10 mL 99.5% ethanol, vortexing briefly and sonicating for 90 minutes. The samples were then filtered using 0.45 μm Nylon syringe filters (Millipore). From this solution, dilutions from 1:100 to 1:10000 in ethanol were made for IMS analysis. The 1:100 dilution was further diluted using 50:50 (v/v) Ethanol: Water for LC-HRMS using Optima LC-MS water (Fisher).

2.3. IMS Analysis

An IONSCAN-LS (Smiths Detection) benchtop ion mobility spectrometer with Instrument Manager (IM) software version 5.389 was used in this study. The instrument was equipped with isobutyramide as the internal calibrant. Solutions of 1μL were deposited onto a Teflon substrate using an autosampler and the volatile solvents were allowed to evaporate. The substrate was then introduced into the IMS system and placed on the desorber heater. Analyte molecules were vaporized and carried from the heated inlet to the ionization chamber in a flow of dry air carrier gas. The volatilized analyte molecules were selectively ionized by a ^{63}Ni β source in a controlled chemical ionization environment to produce molecular ions. The anal-

ysis time was 13.75 s with a scan period of 50 ms, a shutter grid width of 0.2 ms, 28 segments with 10 co-added scans per segment and a drift flow of 300 cc/min. The desorber, inlet and drift tube temperatures were set to 291, 289 and 232 °C, respectively.

Analyte ion drift times were measured relative to the drift time of the instruments internal isobutyramide calibrant. Analyte bands were identified visually and user-selected for analysis by the IM software. The software fit the selected bands to Gaussian band shapes and reported the band peak drift time (t_d), full width at half maximum, amplitude, and area, and computed the reduced ion mobility ($K_{O,A}$) from the band peak drift time. Identification of an analyte was based on its characteristic $K_{O,A}$ ($cm^2/(V \cdot s)$). $K_{O,A}$ was measured relative to the known reduced ion mobility of the internal isobutyramide calibrant, $K_{O,C}$, using Eq. (1). $K_{O,C}$, for isobutyramide was set to a value of 1.5022 $cm^2/(V \cdot s)$ for this study and was assumed to be exact.

$$K_{O,A} = K_{O,C} \times \frac{t_{d,C}}{t_{d,A}} \quad (1)$$

The $K_{O,A}$ for the following five standards: CBDA, Δ^8-THC, Δ^9-THC, CBD and CBN were determined during the IMS analysis of the certified reference standards. Alarms were programmed on the IMS instrument using the $K_{O,A}$ for each cannabinoid of interest. The $K_{O,A}$ of peaks observed in the sample were compared to the $K_{O,A}$ of cannabinoid standards for identification. The IMS instrument has a library that includes the $K_{O,A}$ values for 85 additional drug compounds consisting of the following classes: illegal narcotics, steroids, analgesics, antibiotics, male enhancement and weight loss. The additional alarms on the instrument could be turned on if additional peaks were observed in the mobility spectra.

2.4. LC-MS Analysis

LC-MS analysis was performed on a Waters Acquity UPLC-Synapt G2-Si QTOF system using a method adapted from Grabenauer and coworkers[12]. HPLC separation was performed using a Waters Acquity BEH C18 column, 2.1 × 50 mm, 1.7 micron, held at 50 - 55°C using a gradient consisting of 95% 10 mM NH_4OH:5% Methanol (MP A) and 95% Methanol:5% 10mM NH_4OH (MP B) as the mobile phases, flowing at 0.2−0.3 mL/min. An injection volume of 2 μL was used. The gradient was as follows: 50%B at 0 min, 70%B at 2 min, 95%B at 6 min and held until 9 min, returned to 50%B at 9.1 min, held at 50% until 12 minutes. The mass spectrometer was calibrated using NaI from m/z 50−2000 operating in Resolution mode using positive mode electrospray ionization with a spray voltage of 3.0 kV. Leucine encephalin was infused at 10 μL/min as a lock mass internal standard. Extracted ion chromatograms (EICs) were generated using a ±0.05 m/z extraction window. The MH+ - H_2O fragment, corresponding to the loss of water, was used for CBDA and THCA quantitation as MH+ was not stable under source conditions. Standard curves spanning the range from 50−1000 ng/mL were used for cannabinoid content determination. All samples were prepared in duplicate with duplicate analyses.

2.5. Additional Analyses

Additional screening for heavy metals was performed using XRF and ICP-MS using published methods[13, 14] . Residual solvent testing was performed using a headspace GC-FID or GC-MS method adapted from USP[15]. As no sample tested positive for the presence of a heavy metal above the International Council for Harmonization (of Technical Requirements for Pharmaceuticals for Human Use)(ICH) Q3D Guideline for Elemental Impurities permitted daily exposure limit (PDE), no ICH Class I residual solvents were detected, and no ICH Class IIA/B residual solvents were present above the ICH PDE, no additional discussion of the results is provided.

3. RESULTS AND DISCUSSION

Twenty products suspected of containing CBD or any cannabinoid were analyzed using IMS and LC-HRMS. Of these 20 samples, three products in which no cannabinoids were detected above 0.1%(w/w) were reordered for a second round of analysis. These three samples were analyzed only by LC-HRMS, as noted in Table 1.

3.1. IMS Screening

While lacking specificity, IMS can be used as a rapid preliminary screening tool in complement with confirmatory LC-HRMS analysis. The five cannabinoid standards were analyzed by IMS across a series of concentrations to determine the reduced mobility alarm parameters for each compound, as shown in Figure 2. With the exception of CBN, the cannabinoid standards could not be independently resolved from one another, a reflection of the similarity of their structures. Thus two cannabinoid alarms were programmed on the instrument: one corresponding to CBN and one corresponding to the other four cannabinoids.

In all samples, no additional peaks were detected by IMS so additional alarms to screen for other classes of compounds were not needed. In total, thirteen of twenty samples tested positive for cannabinoids by IMS. One product tested positive by IMS but was determined to be a false positive by LC-HRMS. Results for four products were inconclusive, which may be attributable to differences in the product matrices and/or the presence of plant-based material in these products. In those instances, broader signals and variations in the peak shape were observed in the cannabinoid region thus preventing conclusive determinations. IMS results are summarized in Table 1, where "failed" indicates the sample tested positive for a cannabinoid.

3.2. LC-HRMS Analysis

Samples were screened for the presence of nine cannabinoids using LC-HRMS. Samples were received and analyses were performed over nine months with standard curves generated and system suitability re-established for each sample set. For the first 20 samples, a cannabinoid content of less than 0.1%(w/w) for a 0.2 g sample could be quantitated. For the three re-ordered samples, a quantitation limit of ~0.01%(w/w) for 0.2 g sample was obtained. Chromatographic conditions

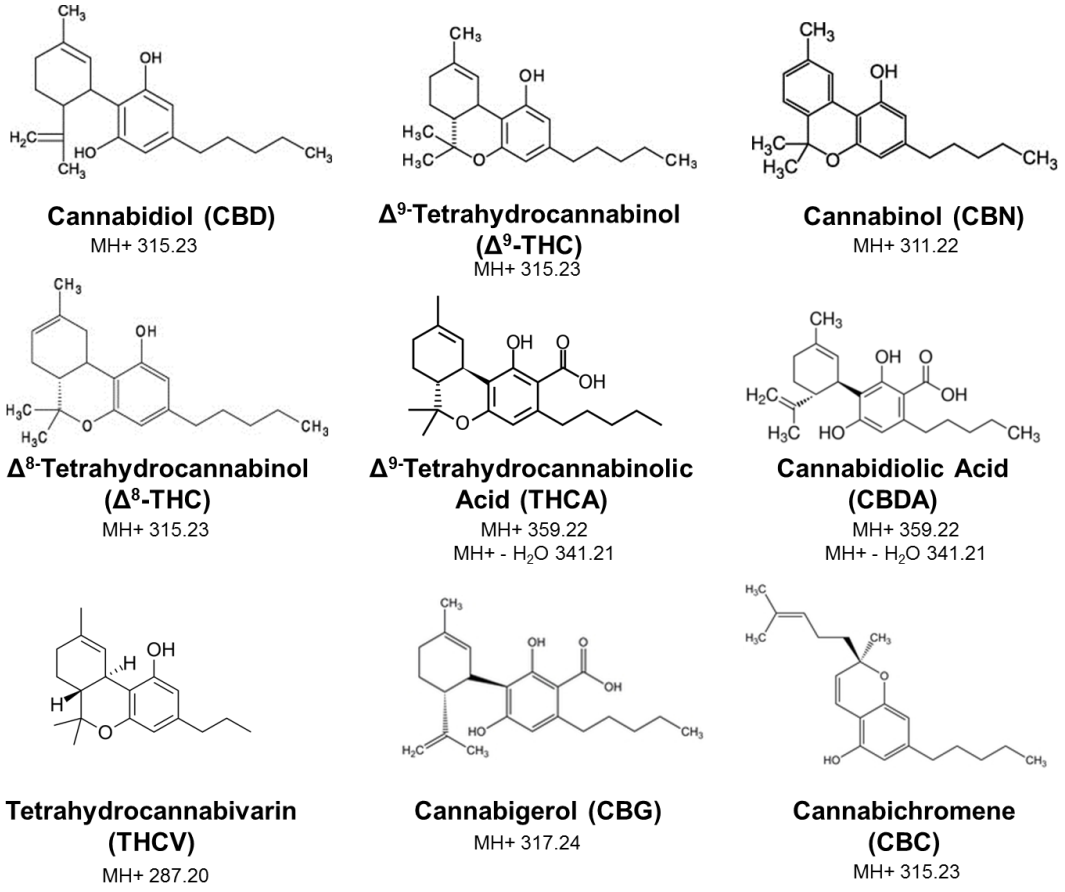

Figure 1: Structures and *m/z* values for nine cannabinoid standards used

allowed for isobaric cannabinoid standards to be resolved. Retention times and accurate masses were used to make identifications.

The EICs of the nine cannabinoid standard mixture are shown in the bottom six panels in Figure 2. While the retention times between the CBD and CBG peaks differ by only ~0.07 minutes, the two species are nearly completely baseline resolved from one another in the standard when present in equivalent concentrations. As CBD is more prevalent and concentrated in hemp oil products, the ability to detect and quantitate the CBD present should not be affected by the presence of CBG. While the presence or absence of CBG in the presence of CBD can be determined by interpretation of isotopic distributions, alternative methods may be necessary for accurate quantitation of CBG. While no other cannabinoid standards used are isobaric to CBG, three low intensity additional peaks are detected in the CBG EIC corresponding to the $^{13}C_2$ isotopes of the *m/z* 315 species.

Quantitative results for four of the most commonly detected (and most abundant) cannabinoid species (CBD, Δ^9-THC, CBDA and THCA) are summarized in Table 1 along with comparisons to product label claims. LC-HRMS results were consistent with those from HPLC-UV, which are to be published by a collaborating laboratory in a separate report. The %RSD of replicate injections was less than 0.5% for most samples and less than 3.0% RSD for all samples. For products where cannabinoid or CBD content were not explicitly stated, a determination as to whether or not a product is consistent with label claim is denoted as unknown. In total, 18 of 23 samples tested positive for the presence of at least one cannabinoid based on LC-HRMS, three of which contained less than 0.1%(w/w) of any cannabinoid. Despite the large number of samples testing positive for cannabinoids, only eight products were consistent with label claim. These results highlight the need for consumers to be aware of the variability in CBD content and product quality across similarly labeled products.

4. CONCLUSIONS

A marketplace survey of 23 internet-purchased hemp oil products was presented in this study. While IMS can be used for rapid screening of cannabinoid-containing compounds, the

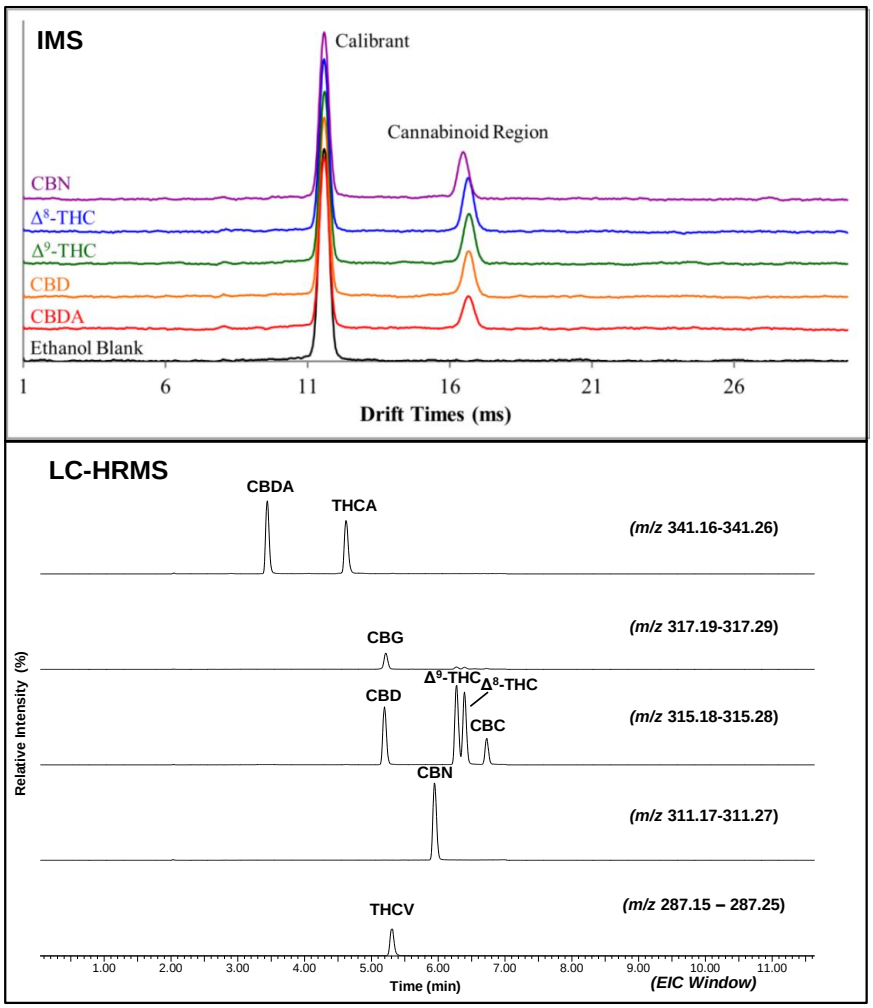

Figure 2: IMS Ion Chromatograms and LC-HRMS EICs for Cannabinoid Standards

use of an additional orthogonal technique (LC-HRMS) for specific cannabinoid identification and quantitation was necessary. Eighteen of twenty-three products tested positive for the presence of at least one cannabinoid present at 0.1%(w/w) or higher using LC-HRMS. Quantitative results for the four most abundant cannabinoids observed in this study (CBD, CBDA, THCA and Δ^9-THC) were consistent between LC-HRMS and HPLC-UV methods. However, only eight products were explicitly consistent with the product CBD label claim. While the lack of toxic metals, residual solvents and elemental impurities across all products were considered positive results, consumers should be aware in the variability of cannabinoid or CBD content that may exist across these unregulated products. This work highlights potential quality issues with hemp oil or cannabis derived products available in the US marketplace in the absence of regulation.

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6. Article Information

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