Analysis of "Marijuana Edibles" – Food Products Containing Marijuana or Marijuana Extracts – An Overview, Review, and Literature Survey

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ABSTRACT: An overview and review of the analysis of food products containing marijuana or marijuana extracts, as reported in the scientific literature through 2016, is presented.

KEYWORDS: Marijuana Edibles, Marijuana Concentrates, Cannabis, Hemp, Tetrahydrocannabinol, THC, Analysis, Chromatography, Forensic Chemistry.

Introduction

Although still illegal under Federal and many state statutes, food products containing marijuana (*Cannabis sativa* L.) or marijuana extracts are currently common in states that either permit or decline to prosecute "medical" or "recreational" marijuana, and are increasingly being submitted to forensic laboratories for analysis – especially in neighboring states where marijuana statutes are still being enforced. Such products, generally referred to as "marijuana edibles," range from beverages to candies to baked goods, and can contain herbal cannabis ranging from entire leaves down to very finely ground material; semi-refined cannabis preparations such as hashish, sinsemilla, or cannabis resin; or moderately to highly refined cannabis extracts and concentrates such as hash oil, "butane honey oil" (BHO),[1] or similar preparations.[2] Due to the range of THC-containing adulterants, and the variability and complexity of their edible "support matrices," the qualitative and quantitative analyses of such products range from facile to significantly chal-lenging (1,2,3). An overview and review of this topic, with an emphasis on methods published from 2005 through 2016, is presented herein. To the author's knowledge, the analyses of marijuana edibles has not been previously reviewed or surveyed (4).

Search Details

Searches were conducted using the Chemical Abstracts Service's Scientific & Technical Information Network (STN)®, Google®, PubMed, by reading select forensic journals (notably the entire run of *Microgram*, *Microgram Bulletin*, and *Microgram Bulletin* LE 1967 to 2016), and/or by reviewing the reference citation lists of pertinent

[1] Utilized herein as a generic term for marijuana concentrates obtained via extraction using butane, supercritical CO_2, or an equivalent low polarity solvent or supercritical fluid.

[2] Including "budder", "errl", "marijuana rosin tech", "shatter", "wax", and other highly viscous or semi-solid, high THC concentrates. [Note that such slang / street names change constantly.]

articles or pertinent chapters of select reference texts. In general, on-line searches were conducted using four linked terms, one each from: A) Chromatography, electrochromatography, electrophoresis, spectrometry, or spectroscopy; B) marijuana or an equivalent term (cannabinoids, cannabis, hash oil, hashish, hemp, hempseed, marihuana, phytocannabinoids, tetrahydrocannabinol, tetrahydrocannabinolic acid, THC or THCA[3] – but no slang terms); C) food, foodstuffs, or a specific term (baked goods, beer, beverage(s), candy/ies, edible(s), liquor, milk, seed oil, tea, or wine); and D) analysis, analytical, or forensic. Followup searches were conducted as the results suggested. The STN and PubMed searches were limited from 1990 to 2016, while only the top 100 "hits" on Google were checked. No mass media sources (i.e., newspapers, magazines, radio, television, or their Internet equivalents) are cited.

An issue of note while conducting searches using Google was the significant number of pertinent, on-line "application notes," "infomercials," and similar reports. Nearly all of these have appeared in the past five years. With the exception of a few application notes that were re-published in *LC-GC* or *American Laboratory*, and two "cannabis industry" reports summarizing the salient issues with preparing marijuana edibles with accurate and consistent potency levels (*vide infra*), these are not included. While there are no reasons to doubt the validity of the presented information, virtually all of these reports are either from scientific instrumentation companies touting the capabilities of one of their instruments or from commercial analytical laboratories offering for-fee testing services, and (in the author's judgment) therefore are not appropriate for this review.

[3] THCA = Tetrahydrocannabinolic Acid (not 11-nor-9-Carboxy-THC). In this review, THCA is utilized to represent both THCA isomers (THCA-A and THCA-B).

The Development of Marijuana Edibles

Marijuana edibles can be arbitrarily divided into three generations. "First Generation Marijuana Edibles" are products that were illicitly produced for personal consumption or for small-scale sale on the black market, long before the advent of state-permitted/non-prosecuted medical or recreational marijuana (or even the term marijuana edibles).[4] These products enabled marijuana use without smoking, thereby reducing its detectability and/or providing an alternate consumption mechanism for users who were either adverse to smoking or who preferred the effects of orally consumed marijuana (5,6,7,8,9,10,11,12,13,14,15, 16,17; see also: 18). While already widespread – albeit low level – among marijuana users in the 1960s, the first such exhibit (cannabis resin smeared on bread) was not reported to *Microgram* until 1970 (19), suggesting only minimal interest among law enforcement personnel or forensic chemists. Until around 2000, most products of this type consisted of herbal cannabis, hashish, or cannabis resin in home-made baked goods such as brownies, cookies, fudge, and similar dessert-type items (e.g., 20,21,22,23,24).

"Second Generation Marijuana Edibles" started to appear soon after California legalized use of medical marijuana in 1996 (25); these products included various types of candies and other packaged foods. Many of these were provided in zip-lock plastic bags with homemade labels, while others were professionally packaged and labelled with names that mimicked well-known consumer products, e.g., "Stoners" (mimicking Snickers® candy bars) (26,27), "Buddafingas" (Butterfinger® candy bars) (28), "Splif" (Jif® peanut butter) (29), and "Budtella" (Nutella® hazelnut-chocolate spread) (30). Additional items included THC

[4] The first citations for marijuana edibles in PubMed appeared in 2013.

lollipops (31,32,33), THC candies (34,35,36,37), "pot butter" (or "ganja butter") (38,39,40,41), chewing gum (42,43), "pot shots" (hard liquor containing suspended herbal cannabis) (44; see also: 45), and others (46,47). The majority of these latter products contained a marijuana extract (i.e., hash oil or BHO) or concentrate, with the remainder containing plant material (i.e., herbal cannabis, sinsemilla, or hashish); many also included a small marijuana leaf logo on their labelling or packaging.

"Third Generation Marijuana Edibles" refer to the current crop of state-permitted/non-prosecuted products. The passage of Amendment 64 in Colorado (48) and Initiative 502 in Washington (49), both in 2012, may be regarded as the break point between the second and third generations, as it marked the transition of marijuana edibles from a widespread cottage industry to large-scale, commercial production. While many of the products are highly similar to Second Generation Marijuana Edibles, their variety, quantities, THC potency levels, and marketing are unprecedented. In addition, based on an informal survey (by the author) of recipes and cannabis industry information, as of December, 2016 nearly all of the large-scale manufacturers of these items are utilizing liquid marijuana concentrates – not herbal cannabis – as the THC source in their products.

"Hemp Food Edibles"

A peripheral but pertinent subset of marijuana edibles are "hemp food edibles," i.e., foodstuffs containing the seeds, oil (from pressing the seeds), and/or the flour (from grinding the seeds) obtained from "industrial hemp" (henceforth hemp), a cultivar of *Cannabis sativa* L. that (usually) contain only trace to very low amounts of THC and THCA. Despite their deliberately innocuous names, however, hemp and hemp food edibles are legally suspect under Federal law; to wit, hemp and hemp food edibles that contain *any* detectable amounts of THC are still considered to be Schedule I materials under the U.S. Controlled Substances Act; i.e., they are in fact marijuana and marijuana edibles, albeit low potency (50).

Currently, hemp is a "niche" crop grown primarily in China, North Korea, Canada, a moderate number of European Union (EU) nations, and in lesser amounts elsewhere, including (with quite stringent restrictions, 51) in the U.S. (52,53,54,55,56).

The seeds, oil, and flour from hemp are touted (sometimes to excess) for their health benefits – especially the oil, a rich source of highly valued omega-3 fatty acids (57,58,59,60,61; see also: 62). Hemp food edibles (and numerous other non-edible, hemp-derived consumer products [5]) began to appear in greater numbers in the early to mid-1990s, as hemp cultivation was allowed, encouraged, and/or increased especially in Canada and the EU; they were initially popular, not for their potential health benefits or nutritional value, but rather for their novelty or shock impact (which has since faded, for obvious reasons).

Not surprisingly, the initial wave of hemp food edibles were often contaminated with phytocannabinoids. Although many of these products did in fact contain only trace to minor amounts of THC, some contained enough to result in positive drug tests (primarily urinalyses) for marijuana.[6]

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[5] Including soaps, shampoos, cosmetics, and biofuels made with hempseed oil, as well as paper, clothing, and other textiles made with hemp fiber (which is one of the strongest and most versatile plant-derived fibers known); these are not further addressed in this review (see References 52-56 for extensive information).

[6] A few others were inadvertently (or in some cases deliberately) produced with seeds, oil, or flour from marijuana instead of industrial hemp.

This resulted in numerous claims that positive tests for marijuana use were actually from consumption of hemp food edibles – even when those tests indicated THC metabolite levels several orders of magnitude higher than those that could possibly be caused by such products. Such claims in turn resulted in numerous articles either proving or disproving the likelihood of a positive test from consuming various products (not detailed in this review; see: 63). It was subsequently determined that inadequate cleansing of the seeds left residual cannabis resin on the seed exteriors, which would carry through to the hemp food edibles (64). These findings resulted in increasingly tighter regulations on acceptable THC levels on the seeds, forcing hemp cultivators to switch to cultivars with even lower native THC levels, and hemp processors to more thoroughly wash their seed stocks, significantly reducing the problem. The EU cutoff limit for THC in hemp is currently 0.2% (65), and the cultivars that meet this standard are published annually (66); *most* other hemp-growing nations have similar – though not as strict – regulations on domestically produced hemp and hemp-derived products.[7]

The analyses of hemp food edibles for THC was addressed in depth in multiple articles from 2000 to 2008 (67,68,69,70,71,72,73,74). Collectively, these studies provided useful insight into the subsequent analyses of marijuana edibles – in some cases, the only published workup procedures for certain products are those that were originally developed for hemp food edibles.

(Unadulterated) Food, Hemp Food Edible, or Marijuana Edible?

A disturbing consequence to the rapid increase in marijuana edibles is the concurrent increase in their accidental consumption (especially by children or pets) as unadulterated food products or less commonly as hemp food edibles. A number of overviews (75,76,77,78,79,80,81,82,83,84) and case reports (85,86,87,88,89,90,91) have been published in the scientific, medical, and veterinary literature,[8] a few of which included the analyses of the suspect items.

Analysis of Marijuana Edibles – An Overview

The analyses of alkaloids (and other plant constituents, additives, and contaminants) in foodstuffs is a very heavily researched topic (see, e.g.: 92, 93,94,95,96,97).[9] As of December 2016, however, a universal, validated method for comprehensive, quantitative analysis for phytocannabinoids in marijuana edibles has not been published. This is not surprising, given the wide range and still increasing variety of such products; the broad array of ingredients in most prepared foods; the variety of THC sources being utilized in their preparation (as well as the heterogeneity of the plant material when that is used as the source [98, 99,100,101; see also: 102,103]); the thermal lability of THCA and the other acidic phytocannabinoids (104,105,106,107,108,109); the high affinity of the lipophilic phytocannabinoids for the fats and oils present in most foods; and the significant representative sampling challenges resulting from the inherent heterogeneity of most solid food products (compounded by the varied and sometimes amateurish marijuana edible preparation practices in current use [110, 111]).

In lieu of a universal method, a variety of

[7] The current USDA limit for THC in U.S. produced industrial hemp is 0.3% (51).

[8] A much larger number of examples have been reported in various mass media sources; these are not included in this review.

[9] In December, 2016 a PubMed search on "analysis of alkaloids in foods" returned over 6,500 citations.

procedures have been reported for specific subtypes of products (e.g., beverages); to date, however, in the majority of these studies the analytical methodology is presented for a single exhibit, a small set of virtually identical exhibits, or a small set of highly similar exhibits.

In the simplest case – i.e., a product that contains sizable/recoverable pieces of visible cannabis, but little or no other plant material(s) (112) – a physical separation and standard marijuana analysis may be conducted (i.e., microscopy, color testing, GC/FID, and/or GC/MS); however, this can be quite tedious and may give an ambiguous result or a false negative if the THC, THCA, and other major phytocannabinoids were *de facto* extracted from the plant material by the food matrix or by its preparation – which would be expected if the ingredients included significant amounts of ethanol or any lipophilic ingredient (butter, lard, oil, etc.), especially if typical baking temperatures were utilized. In such cases, additional workup of the "support matrix" would be required to confirm THC, THCA, CBD, etc.

For exhibits where cannabis is not visibly present – or is present but is not practically recoverable – sample prep is nearly always designed to obtain an extract for analysis. Liquids (including oils) are typically subjected to one or more liquid-liquid and/or solid phase extractions (LLEs or SPEs). Water-soluble solid samples (e.g., a sugar-based, hard or gummy candy) are either dissolved in water and extracted, or finely ground and triturated. More complex, solid samples are first homogenized and triturated, or mixed with a sorbent and homogenized, then triturated. The triturates are then isolated by filtering or centrifuging. Alternately, samples may be subjected to elution on a short column or a Soxhlet extractor. Problematic semi-solid or viscous samples may be extracted directly, or frozen at dry ice or liquid nitrogen temperatures prior to homogenization and workup. Vortexing or (with care) sonication can improve extraction or trituration efficiency. Derivatization, while advantageous for some analyses, at present is only occasionally employed.

Proper solvent selection is a critical aspect of the workup (113). Use of low polarity solvents usually result in reasonably clean triturates/extracts, but suffer from low recoveries, especially of the polar phytocannabinoids (most notably THCA, CBD, and CBDA). In contrast, use of high polarity solvents give good recoveries of the phytocannabinoids, but the triturates/extracts also contain a rich array of components from the support matrix. Back LLEs, SPEs, use of solvents or mixed solvents of intermediate polarity, and/or evaporation of extracts and reconstitution of the resulting residues in different solvents, are available options, but take additional time and resources. In general, if the intent of the analysis is merely to qualitatively prove the presence or absence of THC, the workup and analysis is usually facile; however, if a quantitative analysis of multiple phytocannabinoids is needed, then the *optimal* workup will likely vary for every different type of marijuana edible.[10,11]

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[10] Even (superficially) "identical" edible matrices may actually be quite different. Consider, e.g., two "nut brownies", one made using lard, cashews, and dark corn syrup, and the other made using butter, peanuts, and cane sugar – but otherwise prepared as similarly as possible with respect to the other ingredients, amounts, baking time, temperature, etc. Even if an identical amount of the same BHO concentrate was used in their preparation, and both exhibits were worked up by the same procedure, their dissimilar extraction characteristics (from the different sugars, fats, and oils present) and diverse array of matrix-derived contaminants would result in slightly to moderately differing quantitative results.

[11] A complete analysis would also determine pesticides, herbicides, fungicides, heavy metals, mycotoxins, residual solvents, etc.; however, these are not addressed in this review.

Analyses of the triturates/extracts or reconstituted residues are typically conducted by GC/FID, GC/MS, HPLC with UV, PDA, or LIF detection, or by a more sophisticated method, e.g., UHPLC-MS/MS. Of significant concern, if analyses are conducted on GC-based instrumentation, "dilute-and-shoot" injections of crude triturates/extracts (i.e., those obtained with high polarity solvents, especially those from substrates that contained high amounts of sugars) can result in fouling of injection ports, liners, and columns,[12] decomposition and loss of thermally labile phytocannabinoids, and poor chromatographic performance (114). In contrast, most LC-based methods are far more tolerant of such triturates/extracts, and are also much better able to handle sensitive components (115).

Finally, concentrated residues obtained from low polarity solvents (which therefore are reasonably clean) may be reconstituted in a deuterated solvent for NMR analysis, or even (for exhibits containing at least moderate amounts of THC) submitted to color testing and/or TLC analyses.

A Survey of Reported Analyses

In each case, the edible matrix, the focus of the analysis (i.e., THC, THC/THCA, THC/CBD, all major phytocannabinoids, etc.), the workup procedure, the analytical methodology/ies, and the reference citation, are specified. Where significantly different matrices with varying workup procedures are included in one article (e.g., a beverage and a baked good), where possible each matrix is detailed separately. Where multiple references for the same matrix (e.g., hempseeds) are cited, the presented order is chronological/most recent first. Peripherally pertinent references (i.e., that include some analytical details) are cited as "See also". Additional comments are provided in the reference citations as appropriate.

Aqueous and Alcoholic Exhibits

Aqueous Extracts and Alcohol Tinctures – These are traditional forms of "medicinal" cannabis preparations, that are still occasionally submitted to forensic laboratories as unusual marijuana exhibits or as topical medications (116).

* Prepared Ethanolic Extracts; THC, THCA, CBN, CBD, CBDA, CBG, CBGA, cannflavin A/B, and total phenolics; herbal cannabis was extracted with 20%, 40%, or 80% ethanol/water, filtered, and analyzed by HPLC/DAD (117).

* Prepared Cold and Hot Water Extracts; THC and THCA; the aqueous solutions were filtered, extracted with hexane, and the extracts dried to residues and reconstituted in $CDCl_3$ for NMR analyses. Alternately, a hot water extract was freeze-dried, reconstituted in 80% aqueous methanol, and an aliquot was mixed with D_2O and analyzed by NMR. The NMR analyses included 1D and 2D (DOSY and NOESY) experiments with solvent peak suppression (118).

* Prepared Ethanolic Extracts; THC and THCA; herbal cannabis was extracted with 20%, 40%, or 80% ethanol, filtered, the respective filtrates evaporated to dryness, reconstituted with $CHCl_3$, methanol, or water, and an aliquot was mixed with D_2O and analyzed by NMR. The NMR analyses included 1D and 2D (DOSY and NOESY) experiments with solvent peak suppression (119).

Beverages – Of note, a growing number of commercially produced, marijuana-based alcoholic beverages (beers, wines, and hard liquors) are

[12] Anecdotal reporting to the author indicate that many forensic laboratories will not analyze marijuana edibles unless mandated to do so for prosecution, because of the fouling of their GC-based instruments often caused by such extracts.

being marketed as of December, 2016.

"Sodas" (carbonated); spiked THC, CBD, and CBN (and 35 spiked pesticides); an aliquot was degassed by sonication, added to 1:99 acetic acid/acetonitrile, the mixture added to a specialized mixture of "extraction salts" (the so-called QuEChERS technique (120)), vortexed, centrifuged, and the supernatant analyzed by LC-MS/MS (121).

* "Hemp Products" (beverages, including beer, tea, and vodka); trace THC; the solution was mixed with methanolic KOH, extracted with hexane, acidified with HCl, extracted with 1:9 ethyl acetate/hexane with vigorous mixing and centrifuging. The organic layer was evaporated to dryness under nitrogen, derivatized with BSTFA, and an aliquot analyzed by GC/MS (122).

* "Hempen Ale" – THC and 11-nor-9-carboxy-THC; the ale was subjected to SPE, derivatized with BSTFA, and analyzed both by standard GC/MS and GC/MS in SIM mode (123).

* See also: "Beverages" (124); "Hempen Ale" (125).

Milk – Milk is an unusually challenging matrix due to its high fat content. Although "marijuana milk" (usually prepared by boiling herbal cannabis in whole milk) has been reported (126), as of December, 2016 there are no reports of its analysis (however, see: 127). Trace-level analyses have been conducted on human breast milk obtained from lactating mothers who had been using marijuana (128,129,130), or on milk from lactating animals that had been foraging on wild cannabis/hemp or that had THC or marijuana extracts administered to them for study purposes.

* Human Breast Milk; ultra-trace THC, CBD, and CBN; the milk was saponified with methanolic NaOH, centrifuged, and the supernatant subjected to SPE. Qualitative analysis by Isotope Dilution UPLC-MS/MS (131).

* Human Breast Milk; trace THC, 11-hydroxy-THC, 11-nor-9-carboxy-THC; the milk was pasteurized, diluted 1:1 with methanol, centrifuged, and the supernatant subjected to SPE. Analysis by LC-MS/MS (132).

* Ewe's Milk; trace C-14-labelled THC; the milk was freeze-dried, extracted with ethanol, the extracts centrifuged, the supernatant was cooled (to precipitate some lipids), then isolated and evaporated to dryness under vacuum, reconstituted in water, then extracted with pet ether and then with diethyl ether. Qualitative analysis by radio-quantitation (scintillation counting) and separately by TLC (133).

* See also: Buffalo Milk (134); Human Breast Milk (135,136); Rat Milk (137); and Squirrel Monkey Milk (138).

Tea (i.e., Cannabis Tea) – Typically prepared by boiling herbal cannabis in water – is a simple but variable matrix due to the differing extraction efficiencies and solubilities of the phytocannabinoids in hot water (THC is poorly soluble even in boiling water), potentially complicated by the decarboxylation of THCA, CBDA, and several other acidic phytocannabinoids under extended heating conditions.

* Cannabis Tea; focus is on THC and THCA, but additional phytocannabinoids were observed in the chromatograms; the tea was freeze-dried, reconstituted in ethanol, and analyzed by HPLC/UV (139).

* Cannabis Tea; THC, THCA; an aliquot of the tea was diluted with methanol and analyzed by HPLC with UV and fluorescence detection (140).

Lipophilic (Oil) Exhibits

Oils are also an unusually challenging matrix due to the lipophilicity of the less polar phytocannabinoids (THC, CBN, etc.)

Hempseed Oil (cannabis oil, hemp oil) – Due to the very large number of studies on this product, only references from 2000 through 2016 are cited.

* Hempseed Oil (commercial-grade foodstuff); THC, CBD, CBN; the oil was homogenized, added to acetonitrile, sonicated, cooled to -15°C, and an aliquot of the acetonitrile layer analyzed by GC/MS (141).

* "Edible Vegetable Oil"; trace THC; the oil was extracted with methanol, submitted to SPE, and the eluant analyzed by UPLC-negative ESI-MS/MS (142).

* "Edible Oil" (commercial-grade hempseed oil); THC, CBD, CBN; the oil was extracted with methanol, submitted to SPE, and the eluant analyzed by UPLC-MS/MS (143).

* "Hemp Products" (44 different oils); trace THC; the oil was mixed with methanolic KOH, extracted with hexane, acidified with HCl, extracted with 1:9 ethyl acetate/hexane with vigorous mixing and centrifuging. The organic layer was evaporated to dryness under nitrogen, derivatized with BSTFA, and an aliquot analyzed by GC/MS (144).

* "Cannabis Oil" (commercial-grade hempseed oil); THC, CBD, CBN, CBC; the oil was added to n-hexane and extracted several times with acetonitrile, the combined extracts washed with 2% aqueous NaCl, then with hexane. The acetonitrile was dried under nitrogen, reconstituted in an unspecified solvent (presumably acetonitrile), and analyzed by HPTLC and GC/MS (145).

* "Hemp Oils" (several different products); THC, CBD, CBN; the sample was extracted 3 times with methanol with sonication, the extracts isolated and evaporated to dryness under nitrogen, derivatized with MSTFA, and analyzed by GC/MS (146).

* Hempseed Oil (health supplements); THC; the oil was added to acetonitrile, mixed thoroughly, cooled to -70°C, centrifuged, the acetonitrile layer isolated, dried under nitrogen, derivatized with MSTFA, centrifuged again, and the supernatant analyzed by GC/MS. Alternately, the oil was added to acetonitrile, mixed thoroughly, an aliquot of the acetonitrile layer removed and dried under nitrogen, the residue reconstituted in hexane and submitted to SPE. The eluant was dried under nitrogen, reconstituted in 20% ethyl acetate/hexane, and analyzed by GC/MS (147).

* Hempseed Oil; THC, THCA; an aliquot of the oil was diluted with methanol and analyzed by HPLC with UV and fluorescence detection (148).

* See also: Hempseed Oil (149).

Hemp seeds (cannabis seeds) – As previously noted (*vide supra*), virtually all of the THC and other phytocannabinoids "in" hemp seeds is actually due to cannabis resin adhering to the exteriors of the seeds; however, trace levels of phytocannabinoids have been identified within the seeds (*vide infra*). Due to the very large number of studies on this product, only references from 2000 through 2016 are cited.

* "Hemp Nuts" (containing cannabis seeds); trace THC, CBD, CBN; the nuts were extracted with 60% isopropanol, and the extracts were analyzed by HPLC-MS/MS (150; see also: 151).

* Drug and Fiber Type Cannabis Seeds; trace THC; the seeds were added to 99:1 chloroform/

methanol, homogenized, centrifuged, and the supernatant was separated and evaporated to dryness. The residue was reconstituted in methanol, centrifuged, and the supernatant mixed with 1N KOH in methanol and 9:1 hexane/ethyl acetate and vortex mixed. The upper layer was isolated, evaporated to dryness, reconstituted in hexane and submitted to a short silica gel column. The appropriate fraction of the eluant was analyzed by GC/MS (152).

* Hempseeds; THC, THCA; the seeds were homogenized, extracted with 9:1 methanol/methylene chloride with sonication, an aliquot of the supernatant diluted with methanol and analyzed by HPLC with UV and fluorescence detection (153).

Pharmaceuticals – Includes Federally approved pharmaceuticals only. Although these are not marijuana edibles, they are included due to their close similarity to hemp oil samples and other oil-based supplements containing significant amounts of phytocannabinoids.

* Dronabinol Capsules (synthetic THC in sesame oil); THC; the oil was removed from the capsule, diluted 9:1 chloroform/methanol and further with 9:1 trichloroethane/methanol, and an aliquot analyzed by HPLC/UV (154).

* Dronabinol Capsules (synthetic THC in sesame oil; includes solutions in vials); THC; the oil was removed from the capsule (or vial), diluted with absolute ethanol, and aliquots analyzed: (a) by TLC with confirmation with Fast Blue BB after development; or (b) by HPLC/UV (155).

* Dronabinol Capsules (synthetic THC in sesame oil); THC, CBN; the oil was removed from the capsule, diluted with absolute ethanol, and an aliquot analyzed: (a) by HPLC with variable wavelength UV or PDA; or (b) by GC/FID (156).

* In different pharmaceutical "vehicles" (support agents); THC; the sample was diluted with an "appropriate solvent" containing an internal standard, and analyzed by HPLC (157).

Solid, Complex Exhibits

* Brownies (prepared using many different consumer mixes); stability study on spiked THC and CBD; after preparation (baking and cooling), a small portion of the brownie was added to methanol, thoroughly mixed, centrifuged, and an aliquot of the supernatant was analyzed by UPLC-MS/MS (158).

* Marijuana Edibles (hard candies, chocolates, "gummies", "cookie and cream bar", brownies, oils; spiked THC, CBD, and CBN (and 35 spiked pesticides); the sample was mixed with water, then mixed with 1:99 acetic acid/acetonitrile, the mixture added to a specialized mixture of "extraction salts" (QuEChERS), vortexed (shaken with the assistance of metal balls if necessary), centrifuged, and the supernatant analyzed by LC-MS/MS (159).

* "Hemp Foods" (unspecified products); trace "characteristic cannabinol"; the sample was extracted with methanol, the extract concentrated and submitted to SPE, the eluant evaporated to near dryness under nitrogen, reconstituted in 77:23 methanol/water, and analyzed by UHPLC-MS/MS (160).

* "Baked Goods" (a brownie and a cookie); THC, CBD, CBN; a small portion of the brownie or cookie was added to methanol, thoroughly mixed, filtered, the eluant centrifuged, the supernatant isolated and filtered again, and an aliquot of the filtrate analyzed by UHPLC/MS (161; includes multiple references).

* "Hemp Products" (solid products, many

different types); trace THC; the solid was mixed with methanolic KOH, homogenized, extracted with hexane, acidified with HCl, extracted with 1:9 ethyl acetate/hexane with vigorous mixing and centrifuging. The organic layer was evaporated to dryness under nitrogen, derivatized with BSTFA, and an aliquot analyzed by GC/MS (162).

* "Biscuits" (the British term for cookies – several types); THC, THCA; a portion of the biscuit was homogenized, extracted with 9:1 methanol/methylene chloride with vigorous mixing, filtered, an aliquot of the supernatant diluted with methanol and analyzed by HPLC with UV and fluorescence detection (163).

See also: "Edibles" (Gummies, Chocolate, Brownies, Oil, Caramels) and "Topical Lotions" (164); "Edibles" (165); and "Edible Medical Cannabis Products" (Baked Goods, Candies, and Chocolates) (166).

Multiple Matrices (studies that provide general procedures for workup and analysis)

* "Cannabis-Based Products" (20 different products, including oral supplements, vapes, topicals, and veterinary items, with 3 duplicates for repeat analyses); THC, CBD, THCA, CBDA; the product was extracted with 99.5% ethanol, vortexed, sonicated, filtered, and an aliquot evaporated and screened by IMS; those products that tested positive had aliquots analyzed by UPLC-QTOF-HRMS (167).

* "Hemp Food Products" (included multiple different solutions and solid products, numbers not specified in the article); trace to low-level THC, CBD, CBN; the sample was homogenized, extracted with 9:1 hexane/isopropanol, vortexed, centrifuged, the organic layer isolated and evaporated to dryness under nitrogen, derivatized with MSTFA, and analyzed by GC/MS (168).

* "Hemp Products" (included 9 solid foods and 16 beverages); trace to low-level THC; solid products were homogenized, extracted with methanol, the extracts were filtered, concentrated, reconstituted in methanol and screened by immunoassay (EMIT-II). Samples that tested positive were analyzed by GC/MS in SIM mode. Liquids were screened (undiluted) by immunoassay (EMIT-II). Samples that tested positive were subjected to SPE, with analysis by GC/MS in SIM mode (169).

* "Hemp Food Products" (included 30 different liquid and solid products); THC, CBD, CBN; Method 1 (HS-SPME) – the sample was homogenized, hydrolyzed with a mixture of aqueous sodium hydroxide and sodium carbonate, heated with vigorous agitation, and the resulting mixture was subjected to HS-SPME, derivatized with MSTFA, and analyzed by GC/MS. Method 2 (LLE, done for comparison against Method 1) – the sample was added to an equal amount of 9:1 hexane/ethyl acetate, homogenized with sonication, centrifuged, and the organic layer isolated, evaporated to dryness under nitrogen, derivatized with MSTFA, and analyzed by GC/MS. Method 1 was determined to be superior (170).

A Note Concerning Ongoing Developments

The intent of this review was to provide a "snapshot" of the analyses of marijuana edibles as of December, 2016 – not to make any specific recommendations for such analyses. As is typical with reviews of dynamic topics, it will be rapidly superceded by ongoing research – as well as by ongoing developments in the cannabis industry (especially the recent surge in cannabis-based oral supplements). Of note, the American Chemical Society (ACS) initiated a Cannabis Chemistry Subdivision in 2015 (171), and approximately three dozen cannabis-related presentations were made at the 2015 and 2016 ACS Annual Meetings (172); few of these, however, presented analyses

of any marijuana edibles. The AOAC International solicited for standard methods for analyses of marijuana and marijuana edibles in 2016, at the 130th AOAC Annual Meeting and Exposition (173). The U.S. Food and Drug Administration (FDA) has analyzed cannabis-based products for THC and/or CBD (174), and several publications providing broadly applicable methods are in preparation (175). In short, the next five years should see significant advances in this field.

* * * * *

Acknowledgments

The assistance of DEA Librarians Kristin Carr and Rose Russo in acquiring numerous references, and Laura Ciolino, U.S. FDA, for valuable discussions, are gratefully acknowledged.

References and Additional Notes

[Note: In order to minimize the odd spacings created by the use of fully justified columns for references, they and the author's associated notes are provided in full page, left-justified format.]

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134. Ahmad GR, Ahmad N. Passive consumption of marijuana through milk: A low level chronic exposure to delta-9-tetrahydrocannabinol (THC). Journal of Toxicology – Clinical Toxicology 1990;28(2):255-260. [Notes: This is an ambiguous study. The analytical focus is on detection of trace 11-nor-delta-9-THC-9-carboxylic acid (i.e., the primary metabolite from THC) in buffalo milk and urine; however, the writeup implies several times that the THC in the milk was also determined – though not reported. The THC and deuterium-labelled THC (IS) were extracted by an (unspecified) organic solvent after alkaline hydrolysis, derivatized by bis-trimethyltrifluoro-acetamide, and analyzed by GC/MS. Due to the ambiguity and lack of experimental details, this reference is included as "pertinent background" only.]

135. Escuder-Vieco D, Garcia-Algar O, Joya X, Marchei E, Pichini S, Pacifici R, Pallas-Alonso CR. Breast milk and hair testing to detect illegal drugs, nicotine, and caffeine in donors to a human milk bank. Journal of Human Lactation 2016;32(3):542-545. [Note: Analyses were conducted similarly to the procedures by Marchei, Escuder, et al., Reference #132.]

136. Perez-Reyes M, Wall ME. Presence of Δ9-tetrahydrocannabinol in human milk. New England Journal of Medicine 1982;307(13):819-820. [Notes: This was a "Letter to the Editor", not a full article. Analysis by GC/MS (reported erroneously by Friguls, Joya, et al., Reference #129, as by LC/MS). No workup details were provided; therefore, this reference is included as "pertinent background" only.]

137. Jakubovic A, Hattori T, McGeer PL. Radioactivity in suckled rats after giving 14C-tetrahydro-cannabinol to the mother. European Journal of Pharmacology 1973;22(2):221-223.

138. Chao FC, Green DE, Forrest IS, Kaplan JN, Winship-Ball A, Braude M. The passage of 14C-delta-9-tetrahydrocannabinol into the milk of lactating squirrel monkeys. Research Communications in Chemical Pathology and Pharmacology 1976;15(2):303-317. [Note: Analyses were conducted similarly to the procedures by Jakubovic, Tait , et al., Reference #133.]

139. Hazekamp A, Bastola K, Rashidi H, Bender J, Verpoorte R. Cannabis tea revisited: A systematic evaluation of the cannabinoid composition of cannabis tea. Journal of Ethnopharmacology 2007;113(1):85-90. [Note: This article was included in Hazekamp's thesis, posted at: https://openaccess.leidenuniv.nl/bitstream/handle/1887/12297/Thesis.pdf?#page=113 Date of Most Recent Access: December, 2016].

140. See: Zoller, Rhyn, et al., Reference #67.

141. See: Petrovic, Debeljak, et al., Reference #58.

142. Zhang A, Wang Q. Determination of THC in edible vegetable oil by ultra-high performance liquid chromatography – electrospray tandem mass spectrometry. Shipin Kexue 2011;32(10):194-198. [Note: Written in Chinese.]

143. Zhang A, Wang Q, Mo S. Simultaneous determination of Δ-9-tetrahydrocannabinol, cannabidiol and cannabinol in edible oil using ultra performance liquid chromatography – tandem mass spectrometry. Sepu 2010;28(11):1015-1019. [Note: Written in Chinese.]

144. See: Holler, Bosy, et al., Reference #74.

145. Yotoriyama M, Ishiharajima E, Kato Y, Nagato A, Sekita S, Watanabe K, Yamamoto I. Identification and determination of cannabinoids in both commercially available and cannabis oils stored long term. Journal of Health Science 2005;51(4):483-487.

146. See: Lachenmeier, Kroener, et al., Reference #69.

147. Bosy TZ, Cole KA. Consumption and quantitation of Δ9-tetrahydrocannabinol in commercially available hemp seed oil products. Journal of Analytical Toxicology 2000;24(7):562-566.

148. See: Zoller, Rhyn, et al., Reference #67.

149. Zhang G, Guo J, Bi K. Study on the extraction process for cannabinoids in hemp seed oil by orthogonal design. Zhong Yao Cai 2005;28(5):417-418. [Notes: Determined that the "best" procedure for extracting cannabinoids from hempseed oil was two extractions with methanol, 15 minutes each. Not clear (from the abstract) how the extracts were analyzed. Written in Chinese.]

150. Chang CW, Tung CW, Tsai CC, Wu YT, Hsu MC. Determination of cannabinoids in hemp nut products in Taiwan by HPLC-MS/MS coupled with chemometric analysis: Quality evaluation and a pilot human study. Drug Testing and Analysis 2017;9(6):888-897. [Note: Although dated 2017, this article was actually posted on-line in late 2016.]

151. Zhou W-J, Song J-Z, Fu W-W, Tan H-S, Bian Z-X, Xu H-X. Chemical comparison of two dosage forms of hemp seed pills by UHPLC-Q-ToF-MS/MS and multivariate statistical techniques. Journal of Pharmaceutical and Biomedical Analysis 2013;84:59-68. [Notes: Although this article presents the analysis of (apparently) the same type "hemp nut" products as referenced by Chang, Tung, et al. (Reference #150), no cannabinoids were identified among the constituents – possibly because the products were only one component in a six herb mixture, and as a result the target phytocannabinoids were below the lower detection limit. Therefore, although the presented analytical procedures may be useful, this reference is included as "pertinent background" only.]

152. See: Ross, Mehmedic , et al., Reference #64. [Note: This article includes numerous pre-2000 citations regarding the analysis of hempseeds.]

153. See: Zoller, Rhyn, et al., Reference #67.

154. Wempe MF, Oldland A, Stolpman N, Kiser TH. Stability of Dronabinol capsules when stored frozen, refrigerated, or at room temperature. American Journal of Health-System Pharmacy 2016;73(14):1088-1092.

155. USP Monographs for Bulk Drug Substances and Other Ingredients; 2016 edition (USP 40-NF 35); Dronabinol (#3907) and Dronabinol Capsules (#3908).

156. Ray G, Crook M, West N, Kwoka M, Rehagen G, Cox J, Murrill E, Flora K. Comparison of the analysis of delta 9-tetrahydrocannabinol capsules by high-performance liquid chromatography and capillary gas chromatography. Journal of Chromatography 1984;317:455-462.

157. Flora KP, Cradock JC, Davignon JP. Determination of delta-9-tetrahydrocannabinol in pharmaceutical vehicles by high-performance liquid chromatography. Journal of Chromatography 1981;206(1):117-123. [Note: The "vehicles" (support matrices) included sesame oil USP, polyvinylpyrrolidone, Emulphor EL620, and Cremophor EL.]

158. Wolf CE, Poklis JL, Poklis A. Stability of tetrahydrocannabinol and cannabidiol in prepared quality control medible brownies. Journal of Analytical Toxicology 2017;41(2):153-157. [Notes: The authors used a modification of the procedures by Jiang, Stenzel, et al. (Reference #161; see below). The study indicated that THC and CBD were not affected by the matrix or the baking temperatures (300^{O}C); however, while a valuable contribution, in this author's opinion the study would have been more insightful if THCA and CBDA standards (which are thermally labile) had been included. Although dated 2017, this article was actually posted on-line in late 2016.]

159. See: Wang and Fanning, Reference #121.

160. Wang Q-l, Zhang A-z. UHPLC-MS/MS determination of characteristic cannabinol in hemp food. Lihua Jianyan, Huaxue Fence 2013;49(6):720-724. [Notes: Not clear from the abstract what products were analyzed, or whether "characteristic cannabinol" actually was CBN, or if THC was intended – the authors' other articles indicated THC and other phytocannabinoids (see Zhang and Wang, References #s 142 and 143). Written in Chinese.]

161. (a) Jiang G, Stenzel JR, Chen R, Elmashni D. UHPLC/MS analysis of illicit drugs. Chapter 9 in: Ultra-High Performance Liquid Chromatography and its Applications, Q.A. Xu, Editor, John Wiley & Sons, Inc., Hoboken, NJ: 2013, pps. 253-269. This procedure was also published in the two following, short communications: (b) Jiang G, Stenzel JR. Identification of cannabinoids in baked goods by UHPLC-MS. LC-GC North America, Sep 1, 2009. (c) Stenzel JR, Jiang G. Identification of cannabinoids in baked goods by UHPLC/MS. LC-GC North America, Dec 2, 2008.

162. See: Holler, Bosy, et al., Reference #74.

163. See: Zoller, Rhyn, et al., Reference #67.

164. (a) Marcu J, Kababick JP, Wilcox MJ, Jacyno M. Use of flash chromatography to "clean up" samples prior to analysis: Improving quality control methods for cannabis using flash chromatography. Abstracts of Papers, 251st ACS National Meeting & Exposition, San Diego, CA, March 13-17, 2016: AGFD-51. (b) Wilcox M, Marcu J, Kababick J, Jacyno M. Rapid front end cleanup of cannabis-infused edibles using automated flash column chromatography. Abstracts of Papers, 251st ACS National Meeting & Exposition, San Diego, CA,, March 13-17, 2016: AGFD-142. See also: (c) Wilcox MJ, Marcu J, Kababick JP, Jacyno M, Pryor EM. Improving quality control methods for cannabis using flash chromatography. Abstracts, Joint 41st Great Lakes and 46th Central Regional Meeting of the American Chemical Society, Grand

Rapids, MI, May 27-30, 2015: JGLCRM-71.

165. Riggle J, Nilsson Z, Spikerman D. Extraction and quantitation of cannabinoids in locally grown medicinal cannabis flowers and other extraction products. Abstracts of Papers, 251st ACS National Meeting & Exposition, San Diego, CA, March 13-17, 2016: CHED-520.

166. See: Vandrey, Raber, et al., Reference #111a. [Notes: Included multiple, unspecified products; THC and CBD were the primary focus, but other cannabinoids were also determined; two samples of each respective product were homogenized and analyzed by HPLC (the extracting solvent and workup procedure were not identified).]

167. See: Ruth, Gryniewicz-Ruzicka, et al., Reference #111b.

168. See: Pellegrini, Marchei, et al., Reference #73. [Note: The presented method was subjected to a limited validation study.]

169. See: Below, Rosenstock, et al., Reference #70.

170. See: Lachenmeier, Kroener, et al., Reference #69. [Note: Method 1 was subjected to a limited validation study.]

171. See: The Cannabis Chemistry Subdivision of the American Chemical Society. https://dchas.org/cann/ [Date of Most Recent Access: December, 2016.]

172. For a selection of the more pertinent presentations (citations only) see: Klein RFX. The 2016 "Research on Drug Evidence" Report [From the 18th ICPO / INTERPOL Forensic Science Symposium]. Microgram Journal 2016;13(1-4):609-817. [Note: There is an extensive section in this triennial review that covers marijuana.]

173. Anonymous. AOAC and industry partners to set voluntary consensus standards for cannabis potency. Inside Laboratory Management 2016;November/December:44-45.

174. See: (a) Ruth, Gryniewicz-Ruzicka, et al., Reference #111b. (b) U.S. Food and Drug Administration. Warning Letters and Test Results for Cannabidiol-Related Products. Posted at: https://www.fda.gov/newsevents/publichealthfocus/ucm484109.htm [Date of Most Recent Access: December, 2016.]

175. Laura Ciolino, U.S. Food and Drug Administration, personal communication.

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DEA PRB 01-18-18-05