

# Analysis of Marijuana by Liquid Chromatographic Techniques A Literature Survey, 1990 - 2015

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**ABSTRACT:** A survey of the liquid chromatographic analysis of marijuana, hemp, and related preparations (e.g., hashish, hash oil, “marijuana concentrates,” “cannabis smoke condensates,” etc.) for the major phytocannabinoids, as reported during the time frame 1990 through 2015,<sup>1</sup> is presented. 133 references are provided.

**KEYWORDS:** Marijuana, Cannabis, Hemp, Tetrahydrocannabinol, Phytocannabinoids, Liquid Chromatography, Forensic Chemistry.

## ***Introduction***

The qualitative and quantitative analysis of marijuana (*Cannabis sativa* L.) continues to be a significant task at most forensic laboratories. The most common techniques utilized for these analyses are GC/FID and GC/MS; however, while rapid and facile, these methodologies cannot handle thermally labile or non-volatile phytocannabinoids (such as  $\Delta^9$ -tetrahydrocannabinolic acid - A, THCA) without prior derivatization procedures - which tend to be time consuming, more expensive, and technique sensitive. An alternate approach for comprehensive analysis is to complement the GC-based methodology with a liquid chromatographic (LC-based) technique.

LC-based methodologies that have been utilized for this purpose range from basic (e.g., TLC with

UV or spray reagent detection) to highly sophisticated (e.g., UHPLC-MS/MS or UPC2). To the author’s knowledge, although this topic is lightly covered in several general reviews of the analysis of marijuana (e.g., 1,2,3), the last comprehensive survey of these methodologies appeared 30 years ago (4).

## ***Search Details***

Searches were conducted by the Chemical Abstracts Service’s Scientific & Technical Information Network (STN)<sup>®</sup>, Google<sup>®</sup>, PubMed, by reading select forensic journals, and/or by reviewing the reference citation lists of pertinent articles. The search terms for the analytical techniques included both their fully spelled-out names and their commonly utilized acronyms, as follows:

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<sup>1</sup> Through November, 2015. Due to (normal) publication and/or abstracting delays, a small number of pre-2015 and 2015 dated references will not appear in this survey.

Automated Multiple Development (AMD);  
 Capillary Electrophoresis (CE);  
 Capillary Electrochromatography (CEC);  
 Electrokinetic Chromatography (EKC);  
 Hydrophilic Interaction Liquid Chromatography (HILIC);  
 High Performance Liquid Chromatography (HPLC);  
 High Performance Thin Layer Chromatography (HPTLC);  
 Incremental Multiple Development (IMD);  
 Liquid Chromatography (LC);  
 Micellar Electrokinetic Capillary Chromatography (MECC);  
 Optimal Performance Laminar Chromatography (OPLC, also known as overpressured layer chromatography or forced-flow TLC);  
 Planar Chromatography (No Acronym);  
 Reversed Phase High Performance Liquid Chromatography (RP-HPLC);  
 Reversed Phase Liquid Chromatography (RP-LC);  
 Supercritical Fluid Chromatography (SFC);  
 Thin Layer Chromatography (TLC);  
 Ultra High Performance Liquid Chromatography (UHPLC / UPLC);  
 Ultra-Performance Convergence Chromatography (UPC2 / UPCC); and  
 Two Dimensional Liquid Chromatography (2D-LC).

The search terms for marijuana included: Cannabinoids, cannabis, hash oil, hashish, hemp, hempseed, marihuana, marijuana, phytocannabinoids, tetrahydrocannabinol, and THC; street terms were not utilized. Analyses of rare / trace-level phytocannabinoids, or of phytocannabinoids and their metabolites in post-ingestion biological matrices, are not included in this survey. With the exception of a few reports that were re-published in *LCGC* or *American Laboratory*, “application notes,” printed “infomercials,” and similar publications also are not included. While there are no reasons to doubt

the validity of the presented analyses in these latter studies, virtually all such studies are either from scientific instrumentation companies touting the capabilities of one of their instruments or are from commercial (i.e., non-government) laboratories offering for-fee testing services for “medical” or “recreational” marijuana, marijuana concentrates, or “marijuana edibles,” and are not appropriate for this survey. Finally, references concerning the heterogeneity (inhomogeneity) of cannabis and the sampling protocols to address it - pertinent especially for the accurate quantitation of “herbal” cannabis and similar preparations - are covered as a separate category.

## ***Results***

References are organized first by general category (e.g., Planar Techniques), then by year (in reverse chronological order), and within year by author (in alphabetical order). Except for methodologies that are deemed (by this author) to be “advanced,” techniques that are interfaced with specialized detection methods are reported under the parent technique (e.g., HPLC-MS is reported under HPLC, but HPLC-MS/MS is reported under Advanced). References that report the use of more than one LC technique are reported under both/all categories. It should be noted that in many cases the LC technique is not the focus of the referenced article, but rather was used to complement or confirm the results acquired via a different methodology. Similarly, in many other cases the analysis of cannabis is only a minor aspect of a broader technique study.

### Planar Techniques

**2015** - by TLC/MS, for determination of cannabinoids and pesticides in cannabis (5); **2014** - by TLC, as a complement to a detailed study of the Duquenois-Levine color test (6); **2013** - by TLC (7); **2010** - by TLC, to analyze the components of ayurvedic (a folk remedy from the

Indian subcontinent that includes cannabis) (8); **2009** - by HPTLC, for the quantitation of THC and the qualitative analysis of other main neutral cannabinoids in cannabis (9); by TLC, as a comparison with and complement to a detailed study of *Salvia divinorum* and other salvia species (10); by TLC (from the UNODC Monograph on cannabis) (11); by TLC, for determination of cannabinoids in cannabis and hemp (12); **2007** - by TLC, for determination of cannabinoids in cannabis (13); by TLC (an effort to determine optimal mobile phases for select drugs, including cannabis) (14); **2006** - by TLC, as part of a more comprehensive analysis of cannabis (15); by TLC, for determination of cannabinoids in cannabis and hemp (16); **2005** - by TLC, as part of a more comprehensive analysis of cannabis (17); by TLC (from the SWGDRUG Monograph on cannabis) (18); by HPTLC, for determination of cannabinoids in both commercially available and cannabis oils stored long term (19); **2004** - by AMD, OPLC, and TLC (a comparative study of the analysis of cannabis by different planar chromatography techniques) (20); by TLC, for determination of cannabinoids in cannabis (21); **2003** - by OPLC (a technique study of a specialized OPLC instrument capable of simultaneously running 4 to 8 samples, including cannabis) (22); **2002** - by AMD, OPLC and TLC (a preliminary version of the work detailed in Reference #20, as conducted on different plants, including cannabis) (23); by TLC, for determination of cannabinoids in “monoecious” hemp (24); by OPLC, for determination of neutral cannabinoids in hemp (25); **1998** - by TLC, for analyses of cannabis resin and cannabis in unsmoked, handrolled cigarettes (26); by TLC, for the qualitative and quantitative analyses of cannabinoids in cannabis seeds (27); **1997** - by IMD (a technique study, including cannabis) (28); **1995** - by IMD (a feasibility study, including cannabis) (29); **1994** - by TLC, as part of a comprehensive chromatographic analysis of

cannabis (30); **1993** - by HPTLC (a technique study, including cannabis) (31); **1992** - by TLC, to determine the cannabinoid content of UK-grown plants (up to the 6th generation) from Moroccan, Sri Lankan, and Zambian seedstock (32); by HPTLC (a feasibility study, including cannabis) (33); by HPTLC, as part of the determination of cannabinoids in cannabis oil (34); **1990** - by TLC, for the analysis of derivatized cannabinoids (35); and by TLC, for the qualitative and quantitative analyses of cannabinoids in cannabis seeds (36).

#### Normal Phase LC / HPLC Techniques

**2015** - by LC/MS, (37); by HPLC/DAD, for the analysis of cannabinoids and terpenes in cannabis (38); by HPLC, for the determination of 11 cannabinoids in biomass and in extracts of different varieties of cannabis (39); by HPLC and LC/MS (an overview of the marijuana testing rules in Colorado, the methods used for testing, and test results to date for “recreational marijuana”) (40); by HPLC (an overview of the marijuana testing rules in Colorado and the methods used for testing) (41); by HPLC/DAD (presenting two new, validated HPLC/DAD methods for identification and extract profiling based on the main patterns of cannabinoids and other phenolics in cannabis) (42); by HPLC, for the determination of the relative percentage of THCA and THC in cannabis, and the impact of different storage temperatures on stability (43); by LC/MS, as a complement to a DNA genotyping study (44); by HPLC, for determination of cannabinoids in “marijuana edibles” (45); by HPLC, for determination of cannabinoids in “marijuana edibles” (a feasibility study with spiked samples) (46); **2014** - by HPLC, for identification and quantitation of cannabinoids in cannabis (47); by HPLC/DAD, for quantitation of THC, THCA, CBN, and CBD in seized cannabis (48); by HPLC, to determine THC and THCA (49); by HPLC/DAD, to determine THC in hempseed oil (50); **2013** - by HPLC, for

determination of THC following cloud point extraction of cannabis resin (51); by LC/MS, for analysis of cannabinoids in laser-microdissected trichomes of “medicinal” cannabis (52); by HPLC, for determination of potency and cannabinoid profiles (53); **2012** - by HPLC, as a complement to the voltametric determination of THC (54); by HPLC/DAD, for the determination of THC and other major cannabinoids in cannabis cuttings and seedlings during plant growth (55); by HPLC with chemiluminescence detection, for determination of CBD in industrial-grade hemp (stated to be applicable for determination of THC in cannabis) (56); by Nano-LC, for determination of synthetic cannabinoids and THC in herbal blends (focus is on synthetic cannabinoids) (57); by LC/MS (a review of the use of LC/MS for the detection and quantitation of cannabinoids) (58); by HPLC, for the determination of cannabinoids in cannabis oil during long-term storage (59); by HPLC, for the determination of cannabinoids in cannabis resin during long-term storage (60); by HPLC, for the identification and characterization of “special types of herbal cannabis” (61); by HPLC (a study to determine the optimal solvent and conditions for extraction of THC, CBD, and CBN from cannabis resin) (62); by HPLC, for determination of THC in cannabis (63); **2011** - by HPLC, for the determination of cannabinoids in cannabis during different storage conditions (64); by HPLC/DAD, for determination of THC and (separately) THCA from cannabis after isolation via two different flash chromatography systems (65); **2010** - by HPLC/DAD, for monitoring the long term stability of cannabis resin and cannabis extracts (66); **2009** - by HPLC/DAD, for the qualitative and quantitative determination of the major cannabinoids in cannabis (67); by HPLC, as a complement to an HPTLC study (68); by LC/MS, as a complement to a DART-TOF-MS-based screening for THC in cannabis (69); by High-Temperature LC (a technique study, including analysis of THC in cannabis) (70); by

HPLC, to determine cannabinoids in vaporized cannabis (71); by HPLC (from the UNODC Monograph on cannabis) (72); by HPLC, to determine the effects of tobacco on the levels of cannabinoids in vaporized cannabis (73); **2008** - by HPLC, to determine the effects of different preparation methods on the levels of cannabinoids in vaporized cannabis (74); 2007 - by HPLC, for the determination of the major cannabinoids in “medicinal grade” cannabis (75); by HPLC, for the determination of the major cannabinoids in cannabis tea (76); by HPLC, for the determination of cannabinoids in hemp (77); **2006** - by HPLC, for the determination of the major cannabinoids in “medicinal grade” cannabis (78); by HPLC, as a complement to a DNA study of drug-type versus fiber-type cannabis (79); **2005** - by HPLC/DAD and HPLC with fluorescence detection (as part of a detailed chromatographic and spectroscopic analysis of the cannabinoids in cannabis) (80); by HPLC at elevated pressure (a technique study, including analysis of THC in cannabis) (81); by HPLC/DAD, for analysis of the cannabinoids in cannabis (82); **2004** - by HPLC, for analysis of the cannabinoids in cannabis (83); by HPLC/ESI-MS (a technique study, including analysis of the cannabinoids in cannabis) (84); **2003** - by HPLC, for determination of CBD in hempseed oil (85); **2002** - HPLC, for the determination of cannabinoids in cannabis (86); by HPLC, as a complement to a DNA (ISSR) study (87); by HPLC (an optimization study of the HPLC separation conditions for cannabinoids in cannabis, including  $\Delta^9$ - versus  $\Delta^8$ -THC) (88); **2001** - by HPLC, as a complement to a study of the supercritical fluid extraction of cannabis (89); **2000** - by Capillary LC with electrochemical detection, for determination of the cannabinoids in cannabis (90); by HPLC, for determination of the cannabinoids in hashish (91); by HPLC, for determination of the cannabinoids in cannabis (92); by HPLC with UV or fluorescence detection, for determination of THC and THCA in hemp-

containing foods (93); **1998** - by HPLC, for analysis of cannabinoids in hemp (94); **1997** - by HPLC, for the qualitative and quantitative analyses of cannabinoids in cannabis (95); **1996** - by LC/MS, for determination of the cannabinoids in hashish (96); **1995** - by HPLC, as a complement to a DNA (RAPD) study of different samples of cannabis (97); by HPLC/DAD, for the qualitative and quantitative determination of neutral and acidic cannabinoids in cannabis (for profiling purposes) (98); **1994** - by HPLC, as part of a comprehensive chromatographic analysis of cannabis (99); by HPLC, as a complement to a study of the supercritical fluid extraction of cannabis and hashish (100); **1992** - by HPLC, to determine the cannabinoid content of UK-grown plants (up to the 6th generation) from Moroccan, Sri Lankan, and Zambian seedstock (101); **1991** - by HPLC, to determine the uniformity of hashish samples (102); by HPLC (as a complement to an MECC technique study, including analysis of cannabis) (103); and **1990** - by HPLC with UV or fluorescence detection, following derivatization (a technique study, including analysis of cannabis) (104).

#### Reversed Phase LC and HPLC Techniques

**2015** - by RP-HPLC (on three different columns), for determination of the cannabinoids in cannabis and a marijuana concentrate (105); **1996** - by RP-HPLC, for analysis of “drugs of abuse,” including cannabis (106); **1994** - by RP-HPLC (a technique study, including analysis of THC in cannabis) (107); **1993** - by RP-HPLC, for determination of THC in cannabis (108); and **1990** - by RP-HPLC, to characterize the lipophilicity of natural and synthetic analogs of THC (109).

#### Electrokinetic Techniques

**2004** - by CE/ESI-MS (a technique study, also covering HPLC/ESI-MS, including analysis of the

cannabinoids in cannabis) (110); **1998** - by CEC (a technique study, including analysis of THC in cannabis) (111); by CEC/DAD, for the determination of the cannabinoids in cannabis (112); and **1991** - by MECC, for the determination of the cannabinoids in cannabis (113).

#### Advanced Techniques

**2015** - by HILIC, as part of an RP-HPLC study for the determination of the cannabinoids in cannabis and a marijuana concentrate (114); by 2D-LC with chemiluminescence detection, for screening of cannabinoids in industrial-grade hemp (115); by EI-LC/MS with supersonic molecular beams (an introductory technique study, including cannabis) (116); by UPC2, for the determination of the cannabinoids in cannabis (117); **2014** - by HPLC/MS and HPLC-MS/MS, for identification and quantitation of cannabinoids in cannabis (118); **2013** - by UHPLC/MS (a technique review, including analysis of a mixture of drug standards including THC, CBD, and CBN, plus analyses of two different baked goods that contained THC) (119); by LC-MS/MS, for screening of “botanicals” (including cannabis) in food supplements (120); by UHPLC-MS/MS, for determination of THC in hemp food (121); by UHPLC-Q-ToF-MS/MS, for determination of cannabinoids in hemp seed pills (a traditional Chinese medication) (122); **2012** - by LC/ESI-MS/MS, to investigate the isomerization of CBD and THC during positive ESI analyses (123); **2011** - by HILIC (a technique study, including analysis of THC in cannabis) (124); by LC-MS/MS, for determination of cannabinoids in industrial hemp (125); by UPLC-MS/MS, for determination of THC in edible vegetable oil (126); **2010** - by UHPLC/MS (a technique study, including analysis of THC in cannabis) (127); by UPLC-MS/MS, for determination of cannabinoids in edible oil (128); **2009** - by LC/MS and LC-MS/MS, to evaluate microwave-assisted derivatization of THC (129); by UHPLC/MS, for

determination of cannabinoids in baked goods (130); **2004** - by LC-Ion Trap-MS/MS, for analysis of the cannabinoids in cannabis (131); **2000** - by SFC, for the determination of the cannabinoids in cannabis (132); by SFC (a technique study, including analysis of THC in cannabis) (133); **1998** - by LC with thermospray-MS detection and by LC-MS/MS, for determination of the cannabinoids in cannabis (134); **1997** - by SFC with APCI-MS detection, for analysis of THC in cannabis (135); and **1993** - by HPLC with post-elution photoirradiation followed by DAD or thermospray MS detection (a technique study, including analysis of THC in cannabis) (136).

#### Heterogeneity (Inhomogeneity) of Cannabis and Sampling Protocols to Reduce its Impact

Although the “within sample” heterogeneity of “herbal” cannabis and the resulting, inherent variability on their quantitative analyses are widely recognized (e.g., 137,138), to date there

have been few studies that specifically addressed these issues (139,140). Reflecting the importance of this topic, however, a comprehensive, three-part study was recently published (141,142,143). The heterogeneity of all plant materials utilized for medicinal, nutritional, or similar purposes has also been addressed in more general terms by the U.S. Food and Drug Administration (144) and the World Health Organization (145,146).

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[Note: In order to avoid the occasional overly wide spacings created by the use of fully justified columns, the references are provided in full page, left-justified format.]

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or in the associated text; however, it apparently is from the following presentation: Hudalla C. Analytical testing for the cannabis industry: Application of Ultra Performance Convergence Chromatography. Presented at: The 2014 Conference on Small Molecule Science (CoSMoS), Williamsburg, VA:August 11-13, 2014.

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