Technical Note

Analysis of Fatty Acids in Marijuana (*Cannabis Sativa* Leaf)

Nadia Fucci, Ph.D.
Catholic University of the Sacred Heart
Institute of Legal Medicine
Largo Francesco Vito
1- 00168 Rome, Italy
[email: nadiafucci -at- rm.unicatt.it]

ABSTRACT: Various fatty acids (palmitic, myristic, oleic, and stearic acids) were identified in 20 marijuana (cannabis leaf) samples recently seized on the illicit market in Rome, Italy. Samples were analyzed by gas chromatography/mass spectrometry to determine delta-9-tetrahydrocannabinol, other minor cannabinoid congeners, and fatty acids. Although cannabis seeds and the oil derived from those seeds are known to be rich in fatty acids, this is believed to be the first study demonstrating the presence of fatty acids in marijuana. The potential value of the results in source determination and comparative analyses is discussed.

KEYWORDS: Marijuana, Fatty Acids, Myristic Acid, Palmitic Acid, Oleic Acid, Stearic Acid, Analysis, GC/MS, Forensic Chemistry

Introduction

Cannabis preparations, especially marijuana (cannabis leaf), are the most widely abused illicit drugs in the world. Because of the significant economic and social impact associated with abuse of marijuana and related products, extensive effort is expended to monitor their production, trafficking, and use. These efforts include source determination (i.e., geographic origin) [e.g., 1-4] and comparative analysis (i.e., sample - sample comparisons) [e.g., 5-8]. Approaches have included classic impurity profiling (including cannabinoid quants and ratios), various DNA-based analyses, and isotope ratio analyses (primarily based on $\delta^{13}C$ and $\delta^{15}N$) [e.g., 9].

Because hemp fiber, seeds, and seed oils all have potential economic value, analysis of *Cannabis sativa* has not been limited to leaf or leaf-derived products. One sub-topic of interest is the presence of fatty acids in the seeds and fruits of cannabis [10-13]. The seeds and seed oils of cannabis contain a wide variety of fatty acids in economically viable amounts. Samir *et al.* [12] reported the concentration of fatty acids and the relative percentage of unsaturated and saturated fatty acids in a number of different samples of cannabis seeds, and noted that climate and growing conditions seemed to influence the composition of these compounds in the different samples. Furthermore, Bagci *et al.* [13] showed that high amounts of individual fatty acids, along with various minor components (tocopherol and tocotrienols) was useful in assessing chemotaxonomic relationships among different varieties of cannabis. Collectively, these results suggest that fatty acids may be of value in source determination and/or comparative analysis of cannabis seeds or seed oils.

However, there do not appear to be any studies reporting the presence of fatty acids in marijuana itself (i.e., cannabis leaf). In this study, a simple gas chromatography/mass spectrometry (GC/MS) method was developed for the determination of fatty acids in marijuana, and was successfully demonstrated on 20 samples seized in Rome, Italy. The technique is sensitive and accurate. The potential value of the results in source determination and comparative analyses is discussed.

Experimental

Materials and Methods: All chemical and reagents employed were of analytical grade. Fatty acid standards were purchased from Sigma-Aldrich. A mixture of the standards, each at a concentration of 0.1 mg/mL, was used for method development. Twenty marijuana samples from the illicit Roman market were analyzed. The samples were stored in the dark in a dry-box prior to analysis. Individual samples (100 milligrams) were extracted with 0.5 mL of chloroform solution at room temperature, and an aliquot injected into the GC/MS.

Instrumentation: GC/MS analyses were performed on a Model Focus-HP Gas Chromatograph fitted with a split-splitless injector (270^{O}C) equipped with a HP-1 capillary column (12 m x 0.2 mm I.D.) coated with 0.3 μm thickness of methylsilicone. The temperature program ramped from 70^{O}C to 280^{O}C at 10^{O}C/min, with a 5 min final hold. Helium was employed as the carrier gas, at a column head pressure of 10 psi. The GC was connected to an HP 5971A Mass Analyzer operating at 70 eV EI over 40-500 a.m.u. in selected ion monitoring (SIM) mode.

Results and Discussion

Cannabis seeds and seed oils have been shown to contain up to 20 fatty acids [10-13]. Analysis of the 20 marijuana samples selected for this study confirmed the (varying) presence of myristic, palmitic, oleic, and/or stearic acids (see Tables 1-3 and Figures 1-4). Table 1 presents the IUPAC names and formulas for the respective acids. Table 2 presents the respective retention times and the ions selected for SIM analysis. Table 3 presents the results by sample (ratio'd against the combined cannabidiol (CBD) and cannabinol (CBN) content). Figures 1-4 display typical SIM chromatograms of the respective acids.

The acids varied dramatically by sample. All four acids were present in only 10 of the 20 samples (1,2,4,5,10,11,13,15,16, and 17). Three acids (myristic, palmitic, and stearic) were present in samples 3 and 18; two acids (myristic and palmitic) were present in samples 6,7,12, and 19; only one acid (oleic) was present in samples 9,14 and 20; and finally, no fatty acids were detected in sample 8. The geographic origin for samples 6 and 7 was alleged to be the Netherlands, and in fact those samples had similar delta-9-tetrahydrocannabinol contents (about 1%) and similar impurity profiles, and were also similar in their fatty acid profile (but with only two acids present and with actual origin unknown, the results are interesting but of only curiosity value). Nonetheless, these results suggest that the fatty acid profile of marijuana may be useful for comparative analyses (sample - sample comparisons). The analysis is easy, quick, and sufficiently sensitive for small sample amounts. Although unlikely [12], the method may also be useful for source determination; however, such an advance would require a much larger database of authentics (samples of known origin).

Additional research is planned for more sensitive determination of fatty acids in marijuana by derivatizing the acids prior to analysis. The results will be the subject of a future report.

References

1. Datwyler SL, Weiblen GD. Genetic variation in hemp and marijuana (Cannabis sativa L.) according to amplified fragment length polymorphism. Journal of Forensic Science 2006;51(2):371-75.

2. Stefanidou M, Athaneselis S, Alevisopoulos G, Koutselinis A. Δ^9-Tetrahydrocannabinol content in cannabis plants of Greek origin. Chemical Pharmaceutical Bulletin 2000;48:743-45.

3. United Nations. Special Issue on Cannabis. Bulletin on Narcotics 1985;37(4):1-94.

4. Toonen M, Ribot S, Thissen J. Yield of illicit indoor cannabis in the Netherlands. Journal of Forensic Science 2006;51(5):1050-4.

5. Kojoma M, Lida O, Makino Y, Sekita S, Satake M. DNA fingerprint of Cannabis sativa using Inter-Simple Sequence Repeat (ISSR) amplification. Planta Medica 2002;68:60-3.

6. Gilmore S, Peakall R, Robertson J. Short tandem repeat (STR) DNA markers are hypervariable and informative in Cannabis sativa: Implications for forensic investigations. Forensic Science International 2003;131(1):65-74.

7. Shibuya EK, Souza-Sarkis JE, Negrini-Neto O, Moreira MZ, Victoria RL. Sourcing Brazilian marijuana by applying IRMS analysis to seized samples. Forensic Science International 2006;160(1):35-43.

8. Kojoma M, Seki H, Yoshida S, Muranaka T. DNA polymorphisms in the tetrahydrocannabinol acid THCA synthase gene in "drug type" and "fiber type" Cannabis sativa. Forensic Science International 2006;159(2-3):132-140.

9. Denton TM, Schmidt S, Critchley C, Stewart GR. Natural abundance of stable carbon and nitrogen isotopes in Cannabis sativa reflects growth conditions. Australian Journal Plant Physiology 2001;28:1005-1012.

10. Molleken H, Theimer RR. Survey of minor fatty acids in Cannabis sativa L. fruits of various origins. Journal of the International Hemp Association 1997;4(1):13-17.

11. Deferne JL, Pate DW. Hemp seed oil: A source of valuable essential fatty acids. Journal of the International Hemp Association 1996;3(1):4-7.

12. Samir AR, ElSohly HN, ElKashoury EA, ElSohly MA. Fatty acids of Cannabis seeds. Phytochemical Analysis 1998:7(6):279-283.

13. Bagci E, Bruehl L, Aitzetmuller K, Altan Y. A chemotaxonomic approach to the fatty acid and tocochromanol content of Cannabis sativa L (cannabacee). Turkey Journal Botany 2003;27:141-47.

- - - - - - - - - -

Table 1. IUPAC Names and Formulas.

Common Name	Chemical Name	Formula
Myristic Acid	Tetradecanoic Acid	$C_{14}H_{30}O_2$
Palmitic Acid	Hexadecanoic Acid	$C_{16}H_{34}O_2$
Oleic Acid	9-Octadecenoic Acid[a]	$C_{18}H_{36}O_2$
Stearic Acid	Octadecanoic Acid	$C_{18}H_{38}O_2$

[a] Geometric Isomer (*cis* or *trans*) not determined.

Table 2. Retention Times and Ions Chosen for Selected Ion Monitoring (SIM) Analysis.

SUBSTANCE	*R.T.*	*TARGET ION*
Myristic acid	8:06	228-185
Palmitic acid	10:06	256-213
Oleic acid	11:19	264-282
Stearic acid	11:34	284-241

Table 3. Results Obtained for the Marijuana Samples Analyzed in GC/MS.

SAMPLE	M/(CBD+CBN)	P/(CBD+CBN)	O/(CBD+CBN)	S/(CBD+CBN)
1	0.02	0.30	0.01	0.01
2	0.01	0.50	0.04	0.09
3	0.05	0.20	N	0.02
4	0.05	0.30	0.01	0.01
5	0.03	0.20	0.01	0.03
6	0.02	0.78	N	N
7	0.03	0.8	N	N
8	N	N	N	N
9	N	N	0.01	N
10	0.06	0.20	0.04	0.03
11	0.01	0.48	0.03	0.08
12	0.03	0.70	N	N
13	0.05	0.18	0.03	0.03
14	N	N	0.01	N
15	0.02	0.28	0.01	0.01
16	0.05	0.20	0.04	0.02
17	0.03	0.18	0.01	0.02
18	0.05	0.15	N	0.01
19	0.02	0.6	N	N
20	N	N	0.02	N

M = Myristic Acid
P = Palmitic Acid
O = Oleic Acid
S = Stearic Acid
CBD = Cannabidiol
CBN = Cannabinol
N = None Detected

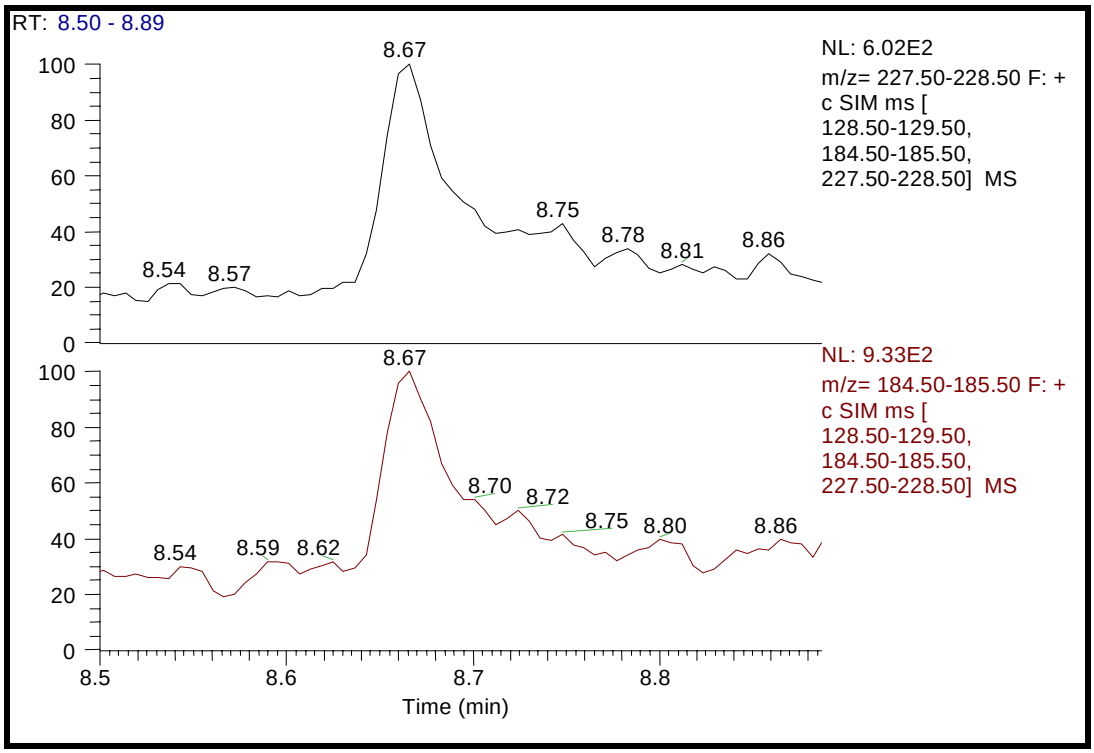

Figure 1. Myristic Acid (GC/MS Analysis in SIM Mode).

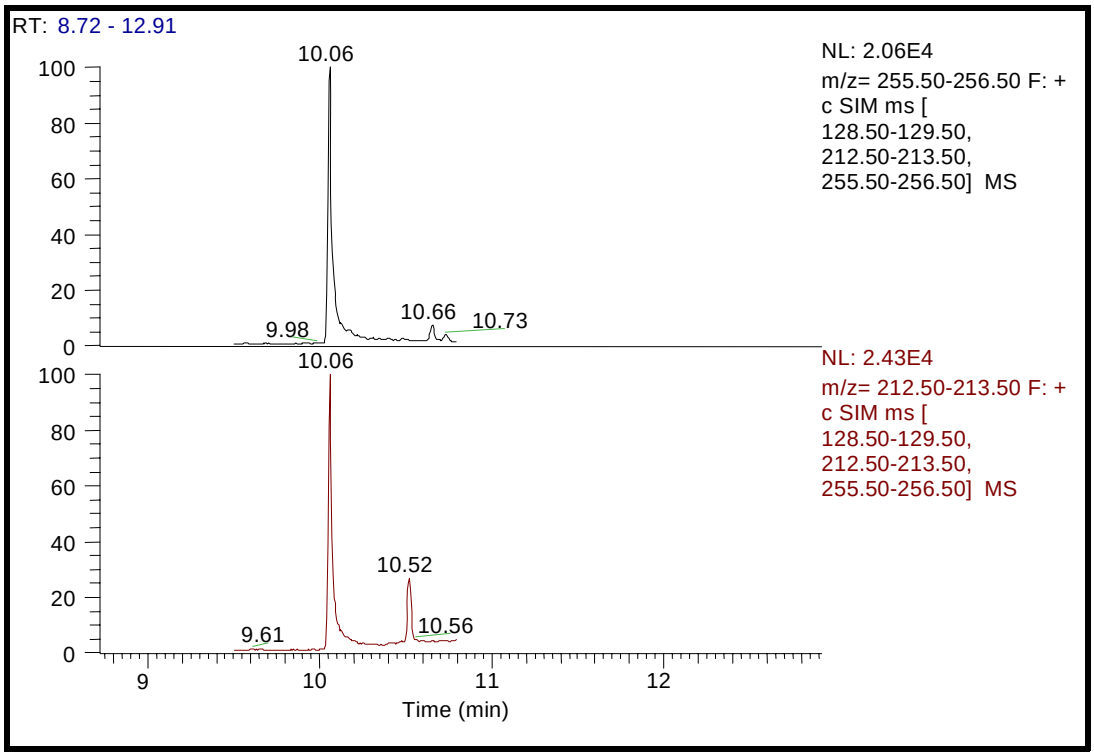

Figure 2. Palmitic Acid (GC/MS Analysis in SIM Mode).

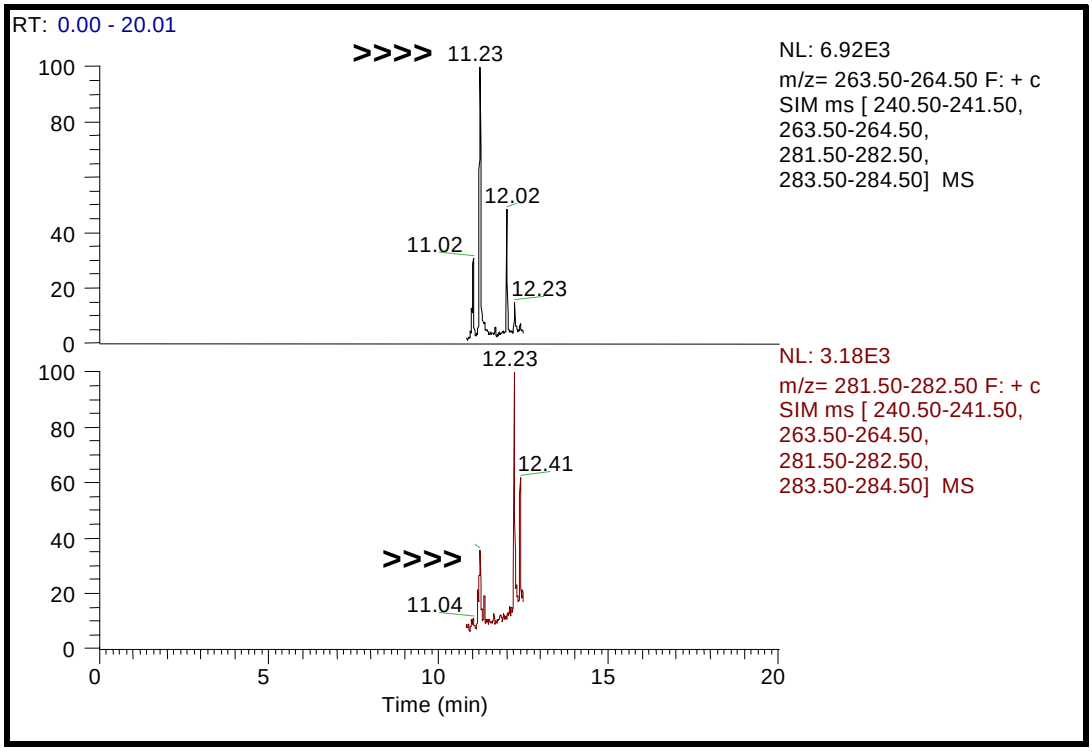

Figure 3. Oleic Acid (GC/MS Analysis in SIM Mode).

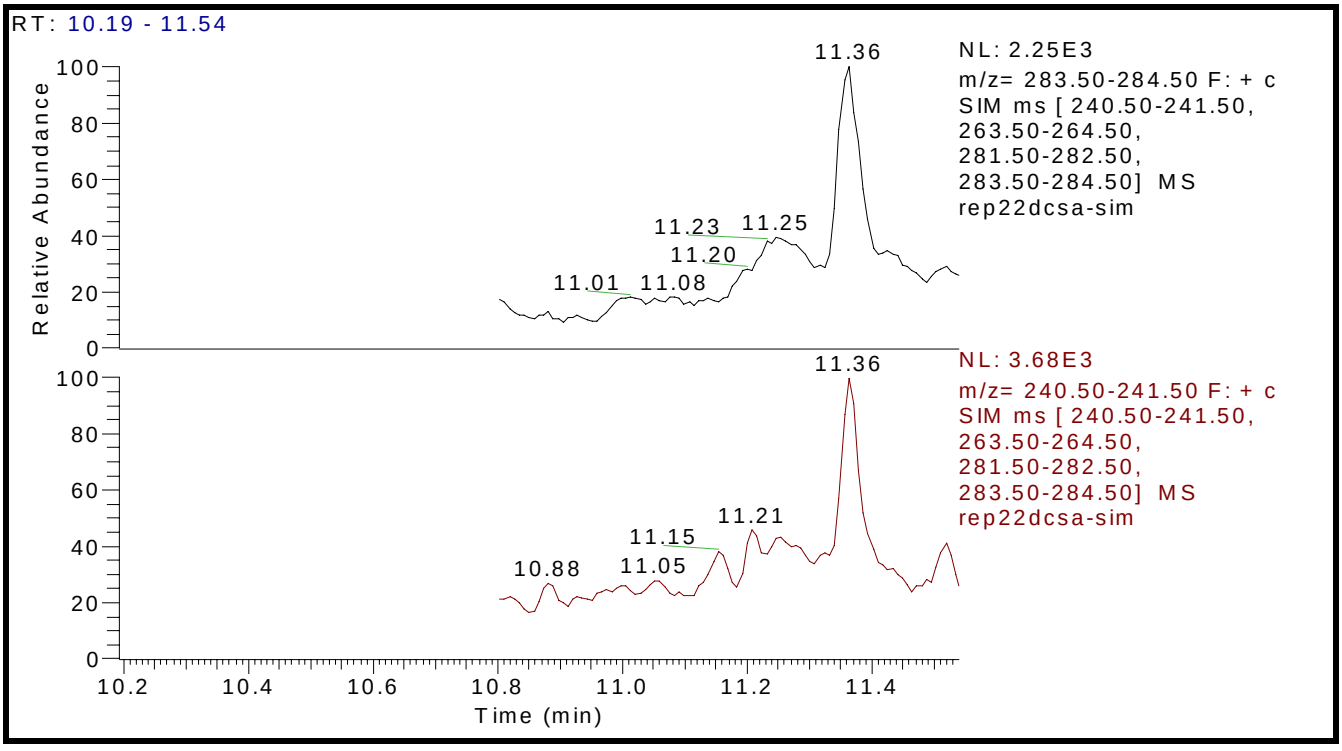

Figure 4. Stearic Acid (GC/MS Analysis in SIM Mode).