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FULL PAPER

Identification of Δ^9-Tetrahydrocannabinol (Δ^9-THC) in *Cannabis* Seeds by Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI(-)FT-ICR MS)

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Abstract:

The identification of cannabinoids directly in seeds is very controversial. According to the Recommended Methods for the Identification and Analysis of *Cannabis* and *Cannabis* products, "*the seeds themselves do not contain Δ^9-THC*". It is defended the idea that the detection of Δ^9-THC when it possibly occurs by contact of the seed with its bracts and/or flowers of the plant, which has a high content of this cannabinoid. In this paper, we reported a simple method of extraction and fast detection of Δ^9-THC in the internal part of *Cannabis* seeds by negative-ion mode Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI(-)FT-ICR MS). Results showed that the method proposed allowed the chemotaxonomic forensic examination to prove the identification of Δ^9-THC in *Cannabis* seeds.

Keywords: *cannabis* seeds; ESI(-)FT-ICR; Δ^9-tetrahydrocannabinol

1. Introduction

Cannabis sativa L. (*Cannabis*, Cannabaceae), popularly called marijuana, is one of the oldest plants known to man. It has more than 700 natural constituents identified [1], which more than 100 are classified as cannabinoids [2]. *Cannabis* is the illicit drug most commonly used worldwide. In Brazil, *Cannabis* is an outcast, and drug trafficking is punished with 5 to 15 years in prison. However, the drug user has alternative penalties, such as the provision of community services [3].

Many drug users see indoor *Cannabis* cultivation as a way of not getting involved with traffickers and crime. The indoor cultivation is one of the reasons why seed traffic by logistics postal has significantly increased in recent years. The seizure of *Cannabis* seeds by Brazilian Federal Police (BFP) showed an exponential increase: 34 in 2010, 137 in 2011, 194 in 2012, 1157 in 2013 and 2192 in 2014, resulting in an increase of 6,088.9% during five years [3,4].

To characterize the nature of these seeds, if they are in fact *Cannabis* seeds, the seized

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material is sent to a forensics section, where the botanical identification (forensic biology) is performed, or the seeds are sent to the forensic chemistry laboratory where it is germinated, cultivated, and, subsequently, the compounds present in seeds are identified from *Cannabis sativa* L. species (i.e., the cannabinoids) [2]. This second option is laborious. Our research group established a protocol that allows the germination, cultivation, and identification of the Δ^9-tetrahydrocannabinol (Δ^9-THC – the main psychoactive cannabinoid) by gas chromatography coupled with mass spectrometry detection (GC-MS) in 15 days [4].

But why not chemically identify Δ^9-THC directly in seeds suspected of being the species *Cannabis*? The identification of cannabinoids directly in seeds is very controversial [5-7]. According to the Recommended Methods for the Identification and Analysis of *Cannabis* and *Cannabis* products [8] "*the seeds themselves do not contain Δ^9-THC*". It is defended the idea that the detection of Δ^9-THC when it possibly occurs by contact of the seed with its bracts and/or flowers of the plant, which has a high content of this cannabinoid. However, ElSohly et al. [9], a well-known research group in this area, suggested the existence of Δ^9-THC in the *Cannabis* seeds, but in low concentrations (< 0.5 μg g^{-1}).

In this work, we report a simple method of extraction and fast detection of Δ^9-THC in the internal part of *Cannabis* seeds by negative-ion mode Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI(-) FT-ICR MS). This technique has already been used in previous studies by our research group to identify cannabinoids in street samples [5] and indoor grown plants from seized *Cannabis* seeds [10].

Ultra-high resolution and accuracy mass spectrometry, such as FT-MS techniques, are a powerful tool that allows complex identification without long and complicated preparation steps, with applications in different areas of "omics" sciences (i.e., metabolomics, proteomics and petroleomics), enabling analysis at the molecular level. Accurate mass measurements define a unique elemental composition ($C_cH_hN_nO_oS_s$) from singly charged ions, such as $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$, $[M-H]^-$, and $[M+Cl]^-$, where M corresponds the neutral molecule [5, 10-13].

2. Results and Discussion

The chemical composition of *Cannabis* has been studied extensively, and approximately 100 phytocannabinoids have been identified, of which Δ^9-THC is one of the most abundant, being found in carboxylic acid precursor form (Δ^9-THCA) in fresh plant material [4, 5, 9, 10].

ESI(-)FT-ICR mass spectra, in the region of *m/z* 200-400 of the three parts of the seed (whole seed, shell and internal part of the seed), generate a lot of signals (**Figure 1**), where a total of 58 compounds were identified. They correspond to sugars, fatty acids, and twenty-nine cannabinoids, where their molecular formula, theoretical *m/z*, double bound equivalent (DBE) and mass errors values are reported in **Table S1** (supplementary material). The species were primarily identified as deprotonated anions followed by chlorine adducts [5, 10]. The cannabinoids species more abundant detected are cannabinol (CBN), cannabitriol (CBT) and Δ^9-THCA. The identification of Δ^9-THCA and CBN in seeds are an indication of the presence of Δ^9-THC since they correspond to precursor and degradation product of the Δ^9-THC, respectively [4, 5, 9, 10]. The last not exist naturally in the plant [14]. In this communication, we will only address the identification of the Δ^9-THC cannabinoid in the seeds.

Analyzing the expansion of the ESI(-)FT-ICR mass spectra, in the region of *m/z* 312-314 (**Figure 2**), it is noted the presence of three isobars (of equal nominal mass) with *m/z* of 313, where the Δ^9-THC ($[C_{21}H_{30}O_2-H]^-$ ion) presents *m/z* 313.2175, DBE = 7 and mass accuracy lower than -0.69 ppm for the three samples.

Cannabis seeds are rich in fatty acids, which other two isobars of Δ^9-THC were identified, being distinguished from Δ^9-THC, as α-linolenic and octadecanedioic acids, **Figure 3**, ($[C_{18}H_{30}O_2+Cl]^-$ and $[C_{18}H_{34}O_4-H]^-$ ions, respectively). They have mass defects of -23 ppb and 22 ppb in relation to the ion of *m/z* 313.21726 ($[\Delta^9$-THC–H$]^-$). FT-ICR MS can identify chemical constituents in complex organic mixtures in forensic chemistry, being a valuable tool to assign molecular formula of cannabinoids.

However, there is some limitation regarding the unambiguous identification of isomers of Δ^9-THC, which can be overcome by performing ESI-MS/MS, NMR, ion mobility MS, and LC-MS/MS analyses [5, 10, 15].

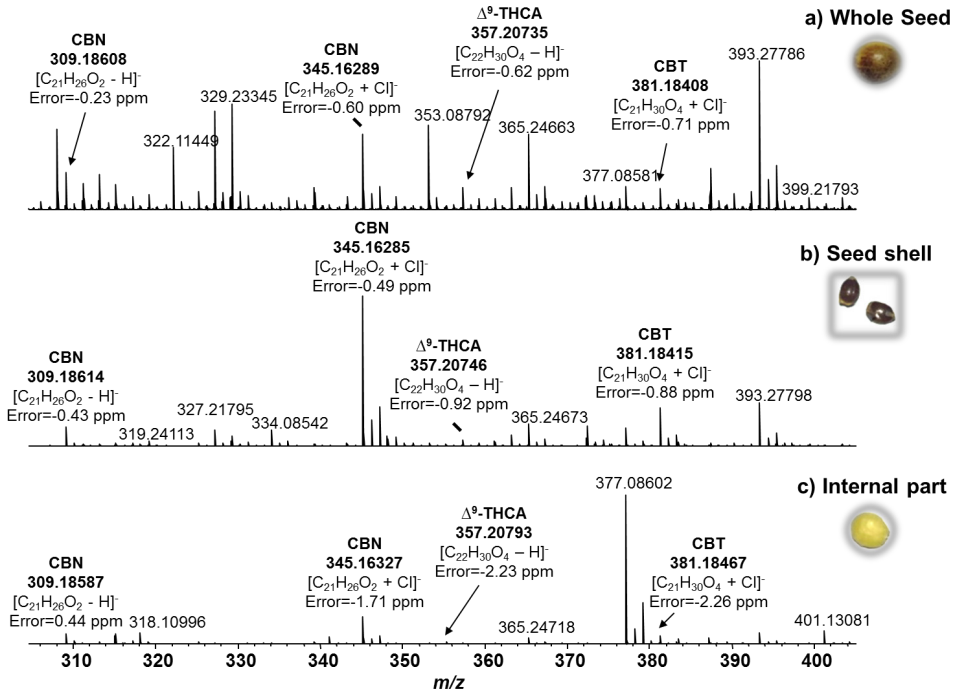


Figure 1. ESI(-)FT-ICR mass spectra, in the region *m/z* 300-420, of the three parts of the seed: a) whole seed, b) seed shell, and c) internal part.

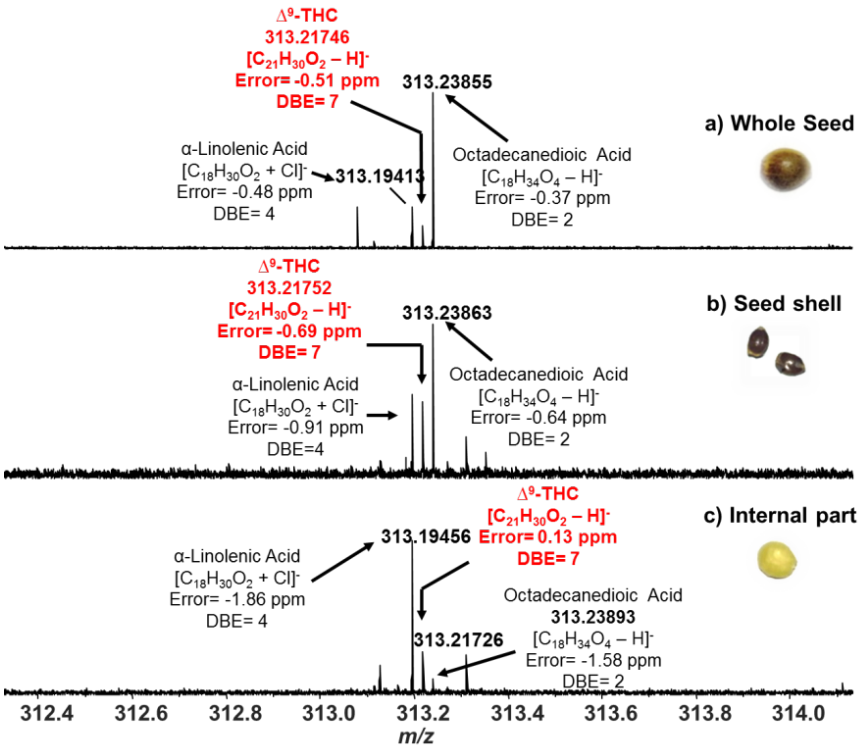
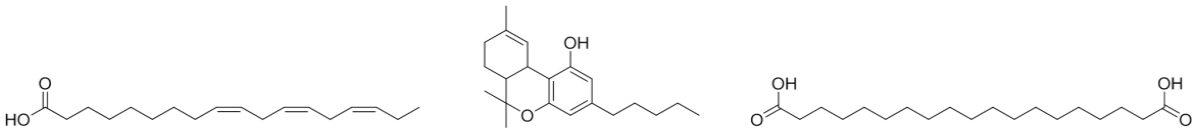


Figure 2. Expansion of ESI(-)FT-ICR mass spectra in the region of *m/z* 313 of the three parts of the seed: a) whole seed, b) seed shell and c) internal part.



α-Linolenic Acid
$[C_{18}H_{30}O_2 + Cl]^-$
M = 313.19398
DBE 4

Δ^9-THC
$[C_{21}H_{30}O_2 - H]^-$
M = 313.21730
DBE 7

Octadecanedioic Acid
$[C_{18}H_{34}O_4 - H]^-$
M = 313.23843
DBE 2

Figure 3. Chemical structures of the three isobaric molecules of *m/z* 313 detected in Figure 2: α-linolenic acid, Δ^9-THC, and octadecanedioic acid, respectively.

3. Material and Methods

Two seeds of the same seizure, seized and provided by BFP, were randomly selected and analyzed to verify the possible existence of Δ^9-THC. The seeds had no lesions. One intact seed was designated as "whole seed." With the use of tweezers, scalpel, and surgical gloves, the other seed was divided into two parts: the seed shell named “seed shell” and the inner part or core of the seed, labeled as “internal part.” This step was taken to prevent the inner part of the seed, and its shell comes into contact. The whole seed, seed shell, and internal part were extracted using 1 mL of methanol for each (Vetec® Química Fina Ltda, Brazil, analytical purity superior to 99.5%).

FT-ICR MS analysis was performed using a mass spectrometer (model 9.4 T Solarix, Bruker Daltonics, Bremen, Germany) set over a mass range of *m/z* 150–1500 in the ESI(-) mode. The negative mode was chosen due to the acidity of terpenophenolic compounds (such as cannabinoids) that are easily deprotonated; forming negative ions when analyzed by ESI (-) [5, 10]. For each analysis, the injection flow rate was 5 μL min^{-1}. The remaining parameters of the ESI(-) source were: (i) capillary voltage (cone): 3500–4100 V; (ii) end plate offset = 500 V; (iii) drying gas temperature and flow rate: 250 °C and 2 μL min^{-1}; (iv) nebulizer gas pressure: 1 bar; (v) skimmer = 15 V; and (vi) collision voltage = (±) 1 V. In the ion transmission, ion accumulation times in the hexapole and time-of-flight were 0.02 s and 0.9 ms, respectively. Each spectrum was acquired by the accumulation of 16 scans of time-domain signals in 4 M (mega-point). All mass spectra were externally calibrated using an arginine solution (*m/z* from 150 to 1500). The resolving power was of approximately 500,000 at *m/z* 400, providing the unambiguous molecular formula assignments for singly charged molecular ions. The FT-ICR mass spectra were acquired and processed using the software Data Analysis (Bruker Daltonics, Bremen, Germany).

4. Conclusions

We present a simple and fast method to detect Δ^9-THC in *Cannabis* seed by applying for an ultra-high resolution and mass accuracy mass spectrometry (ESI(-)FT-ICR MS). Our results show that the method proposed allows the chemotaxonomic forensic examination to prove the nature of the suspected *Cannabis sativa* L. seed, analyzing the seized seeds directly without any botanical identification (forensic biology). Future work will be done with the aim to quantify the Δ^9-THC molecule in the parts of the seed.

Supporting Information

Table S1. Molecular formula, theoretical m/z, DBE, proposed compound and mass error identified in the *Cannabis* seeds by ESI(-)FT-ICR MS.

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