Brazilian Journal of Forensic Sciences, Medical Law and Bioethics

Journal homepage: www.ipebj.com.br/forensicjournal

Application of Chemometric Tools on Cannabis Samples Analyzed by the FTIR-ATR Method

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Received 18 February 2020

Abstract. Marijuana is the most popular form of *Cannabis sativa* L. (*Cannabaceae*), popularly known, in Brazil, as the illicit drug. It is composed of the plant's aerial parts, such as the leaves and the inflorescences, which are dried, pressed and prepared as a mixture for smoking. Cannabis is the most consumed and illegally trafficked drug in the world, with an increasing number of users every year. The plant can be grown indoor and outdoor, and these differences may influence the drug's potency. In addition, marijuana can be mixed with diluents and/or adulterants such as aromatic plants, soil, commercial tobacco and feces that may contribute to cases of addiction and lead to serious health risks to its consumers. Studies involving the chemical profile of drug samples are important to provide evidence for trafficking, supporting the materiality of the crimes. The aim of this study is to analyze cannabis and marijuana seized samples by FTIR-ATR (range 1800-880 cm^{-1}), combined with unsupervised chemometric tools, to differentiate the plant's cultivation forms and to suggest the use of diluents. PCA and HCA showed relevant trends of separation between seized

http://dx.doi.org/10.17063/bjfs9(4)y2019477-498

samples from indoor and outdoor cultivation. Additionally, differences between samples containing pure cannabis and samples including diluents were observed, grouping the samples by their chemical similarity. The use of FTIR-ATR, combined with chemometric tools, can generate fast and sensitive data, providing relevant information for chemical profiles of drug abuse.

Keywords: *Cannabis sativa*; Marijuana; Chemical profiling; FTIR-ATR; Chemometric tools.

1. Introduction

Marijuana is composed of the aerial parts of the plant *Cannabis sativa* L., such as the leaves and the inflorescences, which are dried, pressed and prepared as a mixture for smoking cigarettes, pipes and/or hookahs[1]. In 2017, cannabis already accounted for 188 million of active users in the world, ranking first in drug use[1]. Just in 2014, the Brazilian Federal Police (BFP) seized more than 200 tons of marijuana in Brazilian territory[2]. Unlike other drugs, the number of seizures remained stable for cannabis over the years, but there is an increasing number of users and addicted consumers every year[1,3]. Brazilian Law[4,5] states that cannabis is prohibited in the country and it provides preventive measures for drug use, as well as highlighting attention and social reintegration of users; it also defines crimes and sets terms of repression for illegal drug trade. However, there is no effective ban on drug trafficking as well as drug use in the country. Recently, this law has changed, determining cannabidiol (CBD), a cannabis product, as a controlled substance for therapeutic purposes[6].

Furthermore, cannabis is a chemically complex plant with a diversity of compounds including flavonoids, mono and sesquiterpenoids, steroids, nitrogenous compounds and cannabinoids, a characteristic class of terpenophenols for the plant[7,8]. Over the years, cannabis has undergone enhancement of genetic and cultivation techniques, allowing the increase of its psychotropic cannabinoid, Δ9-Tetrahydrocannabinol (THC), along with its modulator CBD. The higher content of THC, and the addition of diluents and/or adulterants, frequently found in marijuana, may contribute to cannabis addiction[7].

Cole *et al*. in a review study of illicit drugs, the addition of diluents and / or adulterants was disregarded in clinical and forensic toxicology studies; the effects caused by these substances being ignored in the face of the effects of drugs[8]. Adulterants and diluents are deliberately added to increase bulk, enhance or mimic a pharmacological effect, or to facilitate drug delivery[8].

For marijuana, the main diluents added are: aromatic plants because they have a strong odor; soil from roots during cultivation and from an inadequate storage location, as accidental contamination; animal feces such as cows and horses, as the waste comes from a herbivorous diet and that dry visually resemble marijuana, being added to make the drug bulky; syrupy liquids like molasses, because cannabis has an oily extract to give it a dense and compact appearance for sale; aluminium for unknown reason, may have resulted from impure water supply and glass powder also for unknown reason, but potentially to improve apparent quality and increase weight[8,9,10]. Sometimes it is possible to use adulterants, like Tobacco, used to increase the volume and the addiction, due to nicotine presence[9-11]. Other adulterants come from the form of cannabis cultivation, such as pesticides or fungus that develop by a natural biological process and by the poor storage conditions, and can cause damage to health[9], but are not the direct objective of this study now.

The drug trafficking industry has become professionalized, requiring new analytical methodologies, capable of identifying and tracing its origin by the police force and the forensic scientists[12]. Therefore, the use of chemical profile studies are tools that could assist cannabis identification, providing sensitive data for tracking and grouping of seized samples, that can be used as evidence of trafficking, proving the materiality of the crime[12,13]. Infrared spectroscopy (IR) is a reliable methodology for detecting fingerprint regions of different compounds and can be widely used to analyze any sample that has organic functional groups (CH, NH, SH and OH)[14]. The FTIR-ATR methodology relies on the Fourier Transform, a mathematical operation that, through software, separates the frequencies of individual absorptions contained in the sample interferogram, also subtracting the background interferogram that is made from atmospheric gases active in IR (carbon dioxide and water vapor), producing a spectrum identical to that obtained by a dispersive spectrometer[15]. This is a desirable technique for this kind of research, since it requires only a small quantity of samples for the analysis and it is a non-destructive method, allowing sample re-processing if necessary. In addition, it has a faster sample preparation and a low cost, compared to other available methodologies[16]. These advantages fit the reality of different police forces throughout various regions of the world. Combined with exploratory data analysis, FTIR-ATR becomes a powerful tool in Forensic Science[10,14]. This method is already consolidated for cocaine analysis and its adulterants, for medicines falsification assessment and for adulteration of

documents[17], for example. Chemometrics is the application of statistical algorithms to chemical data. Chemometric algorithms have the advantage of tolerating overlapping peaks, so the models do not need to include the concentration of every chemical species present, and multiple analytes can be easily determined[18]. Using principal components analysis (PCA) and hierarchical cluster analysis (HCA) for data related to cannabis and marijuana samples it was possible to create, groups and/or isolates samples by criteria of their chemical and sectoral similarity can be identified[18]. Thus, it is possible to differentiate samples, and to infer the use of diluents and/or adulterants for drug yield increase.

FTIR-ATR presents a challenge for the infrared spectrum interpretation. Unique sample information is in the fingerprint region, which is in approximately 700 - 1800 cm^{-1}. In this region, it is possible to compare the spectrum of a standard sample to the questioned sample, which allows the sample's identity confirmation[19]. In this study, all cannabis and marijuana samples analyzed did not show a significant signal in the 2000-1800 cm^{-1} range and the range of 3600 - 2800 cm^{-1} was also not used, as the angular stretches of aromatic groups are observed in the fingerprint region, so this region of aromatic overtones was not included for the multivariate analysis. As a guide for identifying the main molecular clusters present in the samples in this study, we follow the Lopes and Fascio[19] scheme.

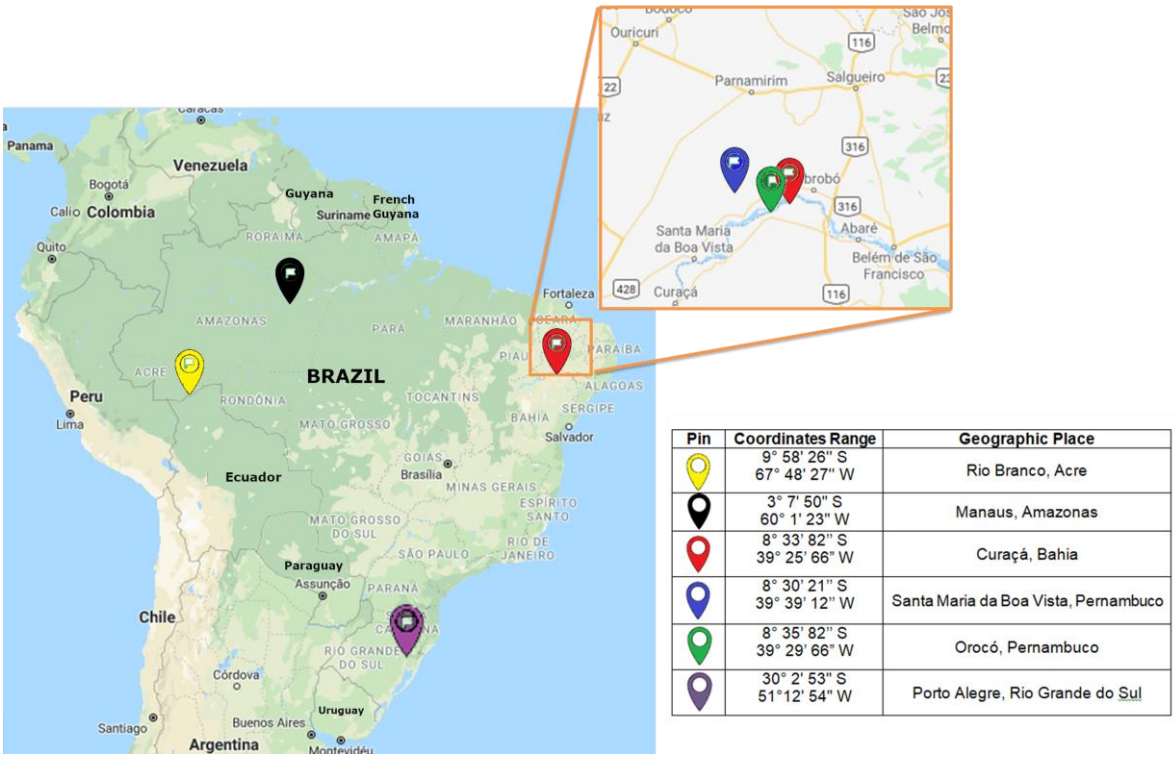
Thus, this study combines instrumental analysis methodologies from FTIR - ATR with exploratory tools to perform the analysis of marijuana and cannabis samples seized by the BFP with the addition of the following diluents: basil, cilantro, oregano, horse feces, soil and commercial tobacco. These diluents were chosen following the casuistry of the forensic institutes in the country and taking into account available and inexpensive materials that when added to cannabis had volume and aspect similar to marijuana without making the final product more expensive, aiming to mimic an adulterated real sample. This is a pioneering study for cannabis analysis that aims to pave the way for studies with adulterated real samples.

2. Methods

2.1. Seized samples and diluents

The cannabis and marijuana samples were provided by the BFP. All the research was observed by a federal criminal expert. Samples were separated into groups according to the region where they were seized and/or its geographic location.

Twenty-nine cannabis samples were provided from previous research on seed trafficking[16,20]. The seeds were grown in indoor way at the BFP station in Porto Alegre, Rio Grande do Sul, Brazil (30° 2' 53.30" S 51°12' 54.26" W), with authorization from the judiciary authorities[16,20]. Ten samples were seized in Manaus, Amazonas, Brazil (3° 7' 50"S 60° 1' 23" W) and they were sent to FPD in Rio Branco, Acre, Brazil (9° 58' 26" S 67° 48' 27" W). Nine samples of cannabis were obtained from seizures in the border region known as the São Francisco River Valley, on the border of the states of Bahia and Pernambuco (8º 35' 82'' S 39º 29' 66" W; 8º 33' 28'' S 39º 25' 53'' W; 8º 30' 21'' S 39º 39' 12'' W) as shown in Figure 1. Finally, three street samples of marijuana seized in different regions of Porto Alegre, lacking exact location, were used too. Table 1 shows all the information from the seized samples.



Pin	Coordinates Range	Geographic Place
	9° 58' 26" S 67° 48' 27" W	Rio Branco, Acre
	3° 7' 50" S 60° 1' 23" W	Manaus, Amazonas
	8° 33' 82" S 39° 25' 66" W	Curaçá, Bahia
	8° 30' 21" S 39° 39' 12" W	Santa Maria da Boa Vista, Pernambuco
	8° 35' 82" S 39° 29' 66" W	Orocó, Pernambuco
	30° 2' 53" S 51°12' 54" W	Porto Alegre, Rio Grande do Sul

Figure 1. Geographic location of seizure samples. Map source: Google Maps. Image organization adapted from González, M. (2018)[10].

The diluents for the samples were selected according to local reports by the BFP. The following were included: four samples of fresh horse feces, obtained from the Porto Alegre's Jockey Club Veterinary Hospital, four samples of commercial tobacco from popular cigarettes (Kent®, Minister®, L & M® and Marlboro®), three basil samples (*Ocimum basilicum* L.) – one fresh and two dried, – three samples of oregano (*Origanum vulgare* L.) - one fresh and two dried -, three samples of cilantro

(*Coriandrum sativum* L.) - one fresh and two dried -, and a sample of black soil enriched with dolomitic limestone (Table 2).

Table 1. Description of the seizure samples. *Exploratory analysis. AC = state of Acre; RS = state of Rio Grande Sul; BA = state of Bahia. SS = Street Samples. BFP = Brazilian Federal Police.

Seized Sample	Location	Number of Samples	Number for EA*	Add Information
Marijuana	AC	10	1	seized marijuana cigarette
Cannabis	RS	29	2	Indoor cultivation in BFP
Cannabis	BA	9	3	Outdoor cultivation
Marijuana	RS	3	4	SS

Table 2. Description of the diluents samples. * Exploratory Data Analysis.

Diluents Samples	Number of Samples	Name for EDA*	Add Information
Aromatic Plants	9	AP	Pool of basil, oregano and cilantro
Horse Feces	4	FZ	-
Soil	1	S	-
Commercial Tobacco	4	FM	Pool of Kent®, Minister®, L&M® and Marlboro®

2.2. Sample preparation

All the cannabis, marijuana and diluents samples were prepared using the same protocol. The samples were dried with heat at 60 °C for one hour in an oven (Biomatic®), and crushed using a hand crusher, followed by homogenization with a gral and a pistil. Then, they were sifted using a tamper and packed in a 1.5 ml plastic tube. Diluents samples were mixed according to the classes described in Table 2, with no distinction by origin.

2.3. Instrumentation

The infrared spectra of all seized samples and diluents were obtained in a Thermo Fisher Nicolet Avatar 370 DTGS Infrared Spectrometer (Thermo Fischer, San Diego, CA, USA) using a universal attenuated total reflectance (ATR) sampling accessory. Absorbance was measured in the spectral range of 4000 - 400 cm^{-1}, but the region of

analysis chosen was 1800 - 700 cm^{-1}, corresponding to the fingerprint region, in consensus, the authors observed that there is no relevant information in the regions of overtones and other expressive information such as aromatic groups can be seen in the fingerprint region[21,22.] The ground samples were directly analyzed. The spectra were acquired at random, in triplicate, with 32 scans and resolution of 4 cm^{-1}.

2.4. Chemometrics

ChemoStat® software was used for exploratory data analysis[23]. To test repeatability, all cannabis, marijuana and diluent samples analyses was performed in triplicate and its average spectra was used. The analysis range was set at 1800 – 880 cm^{-1} for it is a relevant region with less noise interference. The data was preprocessed using the Savitzky-Golay (SVG) algorithm (1st order polynomial, 13 points per window) and normalization, using the Chemostat® software. The standard normal variate (SNV) was applied to the spectra to remove vertical shifts, before exploratory analysis, and the spectra were mean-centered. PCA and HCA analysis were performed in the pre-processed spectra to investigate the similarities between the samples.

2.5. Analysis script

Exploratory data analysis was separated into stages, in order to know the differences between the seized samples: (a) comparison between cannabis and marijuana; (b) differentiation between indoor and outdoor cannabis; (c) diluents analysis; (d) comparison between samples of indoor cannabis mixed with diluents versus diluents and (e) comparison of all seizure samples, mixed samples and diluents. The results are exemplified in Figure 2.

3. Results and discussion

Plant materials like *Cannabis sativa* L. are rich in lignocellulosic biomass that is an abundant renewable resource that can provide biopolymers, fibers, chemicals and energy[24]. The tricky part of applying Beer's Law to cannabis analysis is that it contains many different molecules, and it is not always possible to find an infrared peak that is solely due to a specific analyte[25]. Factors that may alter the infrared spectrum in plant samples are: (i) different soil compositions; (ii) differences in harvest time; (iii) use of nitrogen fertilizers[22,24]. Figure 3 shows a cannabis FTIR-ATR spectrum after range selection of 1800 – 800 cm^{-1}.

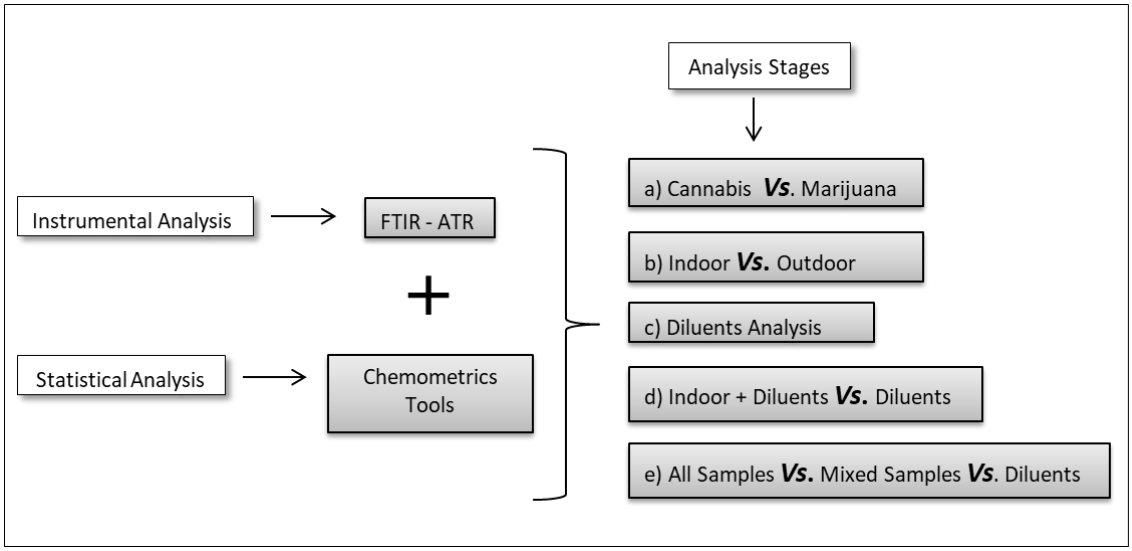


Figure 2. Sample classification for exploratory data analysis.

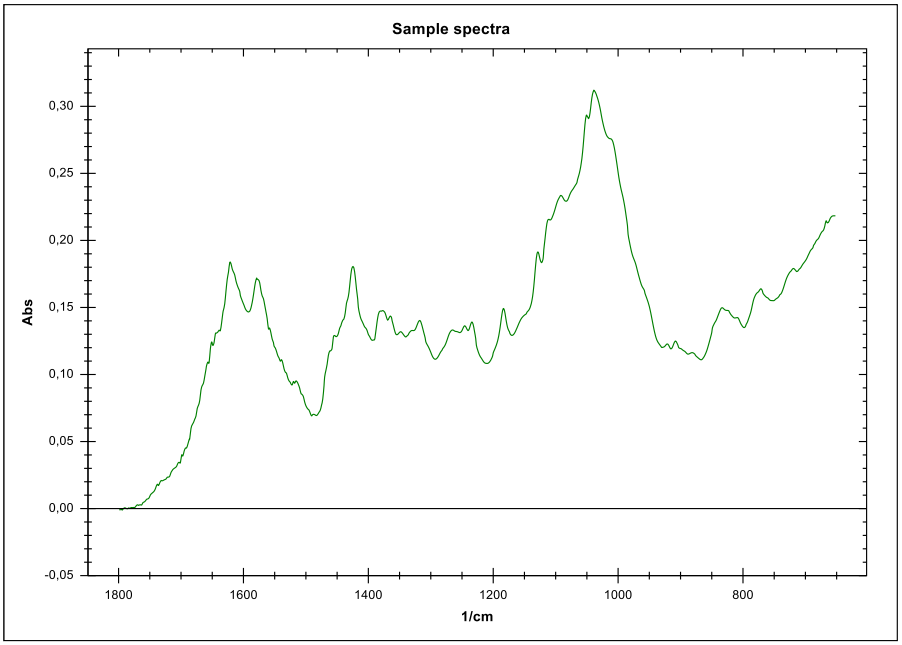


Figure 3. Region of interest in the spectra of cannabis samples.

Figure 4a shows the results for FTIR-ATR analysis of pure cannabis without any previous treatment of the data and without using the triplicate average for each sample, reveling four possible fields of interest and variation for the absorbance in the samples. To minimize the analysis noise, that can be caused by differences in deposition and pressure of the sample in the ATR crystal and environmental differences, we have used the preprocessing techniques Savitzky Golay (SVG) algorithm and normalization. The triplicates averages were used to reduce the sample set, which was relatively extensive, impairing the spectrum visualization.

However, in solid analyzes, the use of triplicates is recommended, since each sample may have a different behavior depending on the discussed conditions. These factors may change the response to analysis. When applying the pretreatment, it is possible to visualize the spectral signals uniformly (Figure 4b).

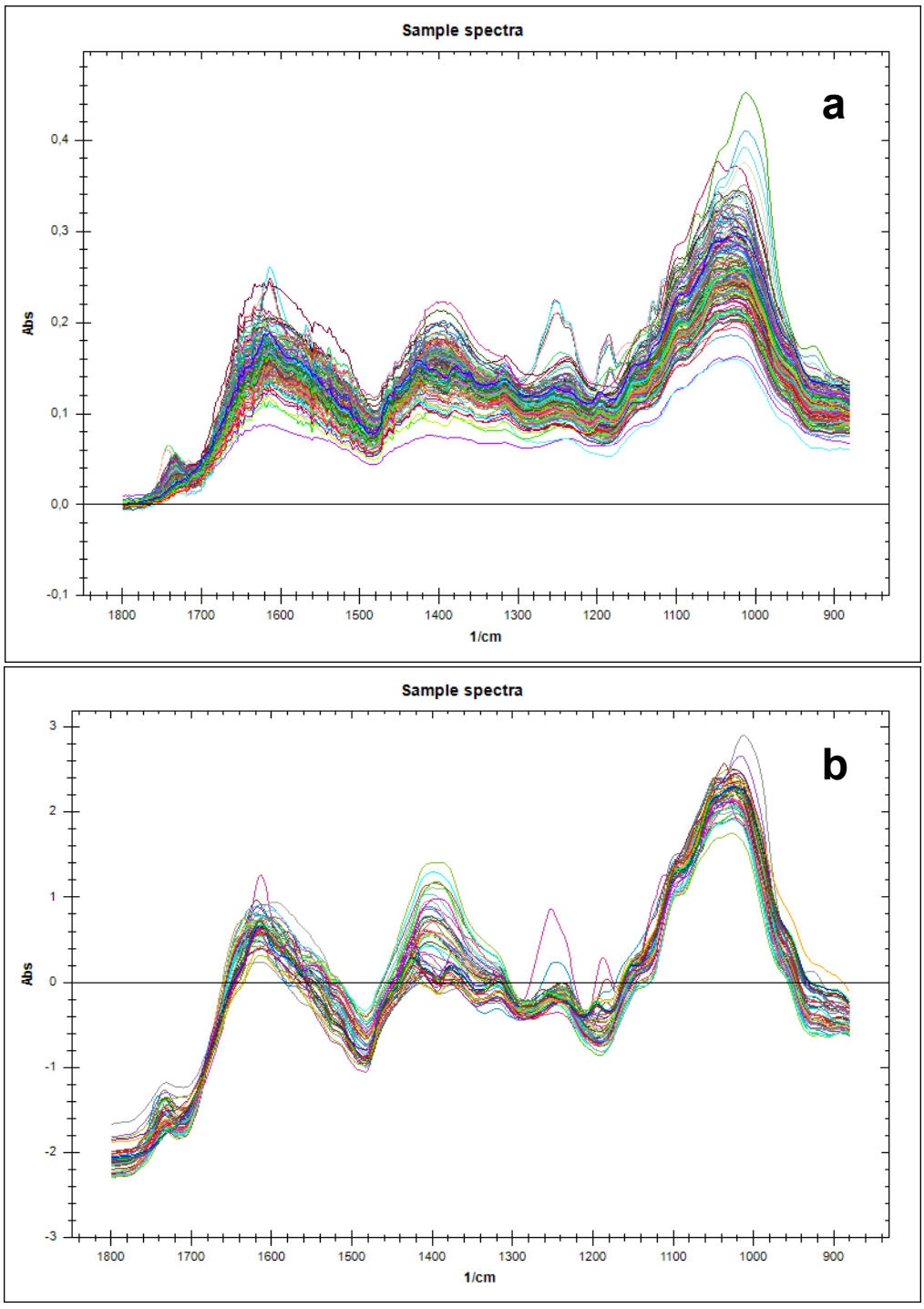


Figure 4. (a) FTIR-ATR spectra of *Cannabis* plants and marijuana samples in triplicate without preprocessing. (b) FTIR-ATR spectra of same samples with average and preprocessing.

Thus, it is possible to confirm that there are three regions of important vibrations for cannabis. The regions comprising at 1800 – 1500 cm^{-1}; at 1400 – 1200 cm^{-1} and at 1100 – 950 cm^{-1} are responsible for the sample characterization (Figure 5), which are related to C=C type stretch vibrations, stretching and deformation =C-H, -C-H, stretch vibrations =C-O-C, C-O-H and strain vibrations =C-H. The fingerprint region comprising from 1800 cm^{-1} to 700 cm^{-1} is associated with angular deformation of OH, CH, CH_2 and CH_3 of aromatic and aliphatic chains. At the fingerprinting region, it is possible to identify plant's macronutrients, such as lipids, proteins and carbohydrates[15,21,22]. A primary amide from a protein is observed in stretching vibrations C=O (1637 cm^{-1}). Carbohydrates possess strong and characteristic IR absorptions between 1200 and 750 cm^{-1}, relevant to coupling and combining of stretching/deformation or vibrational modes of individual bonds in the molecular structure[22]. The intense band at 1160 cm^{-1} is probably due to the vibration of the cellulose ring-breathing, with its intensity altered systematically[22], Table 3 shows a summary of the main assignments of the signals.

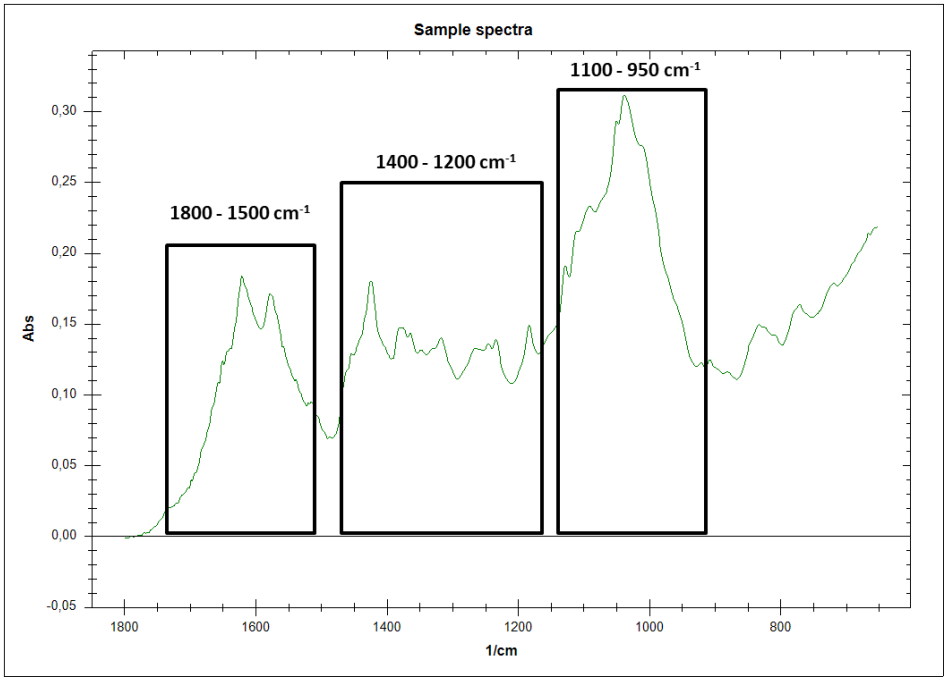


Figure 5. Regions of the main signs of cannabis and marijuana samples.

A dendrogram is a diagram that shows clusters formed by grouping samples according to their levels of similarity, based on Euclidean distance. In Figure 6, it is possible to observe the dendrogram resulting from all the seizure samples used in the study. The first observation that we can notice is where the samples grown by

BFP are divided in two clusters and the samples from the state of Acre are disposed in another cluster. These results answer the questions (a) and (b) proposed in the analysis script (Figure 2), making it possible to differentiate cannabis from marijuana samples and to establish differences between indoor and outdoor cannabis samples.

Table 3. Correlation between the bands identified in the spectra and their respective functional groups. Adapted from Siano, 2018 and Colthup *et al*. 1990[15,21].

IR Absorption Frequencies (cm^{-1})	Assignments
1680 - 1640	Alkenes C=C stretching
1460, 1375	Alkane C–H bending
1000 – 675	Alkene C–H bending
1600, 1500	Aromatic C=C stretching (two bands)
870 – 675	Aromatic C–H bending
1300 – 1080	Alcohols C–O stretching
1690 – 1600	Amide C=O Primary – two bands

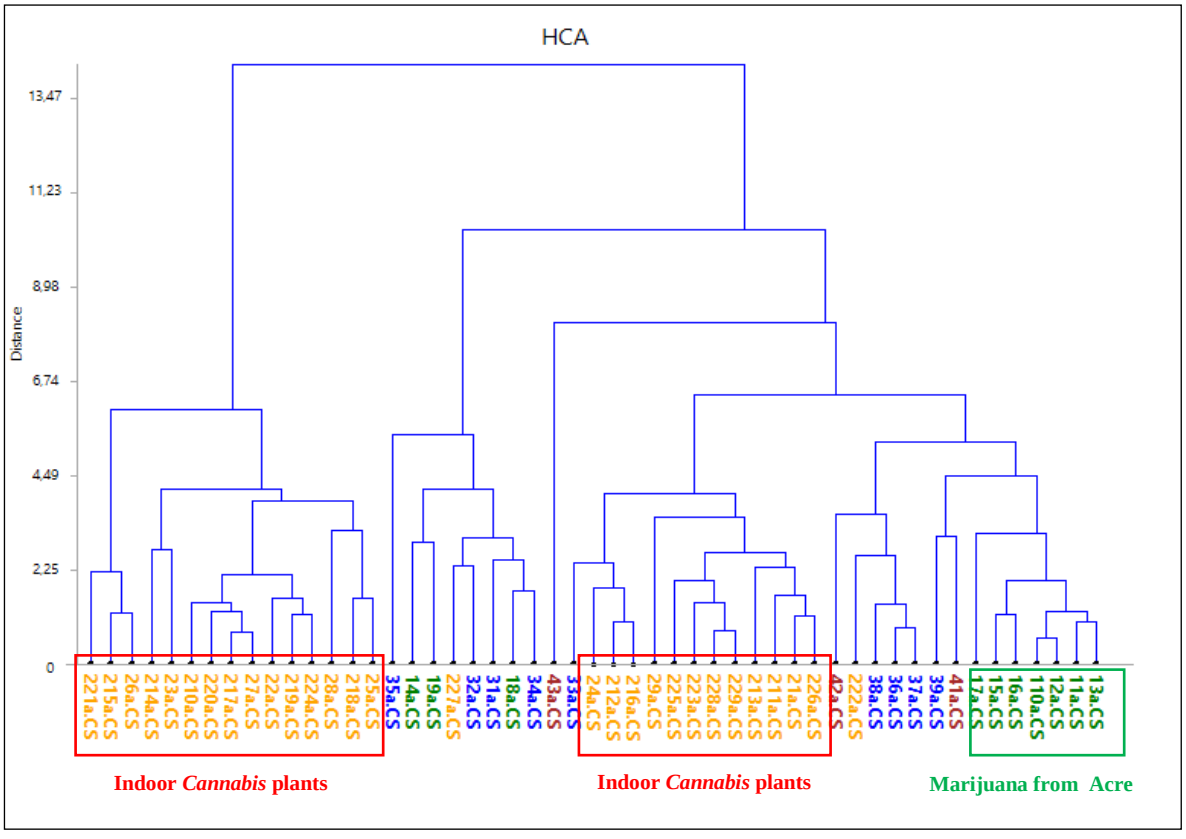


Figure 6. Dendrogram of cannabis and marijuana samples. Highlighted in red are the clusters of the indoor cannabis plants samples and highlighted in green the cluster of the marijuana samples from Acre state. All Samples starting with number 1 are marijuana from Acre; 2: Indoor cannabis plants; 3: Outdoor cannabis plants from Bahia and 4: street samples from Rio Grande do Sul.

Figure 7 shows PCA analysis results for cannabis and marijuana of four groups of seized samples. In this figure, it is possible to observe that the first principal component (PC1), which explains 70.51% of variance, is responsible for the separation of indoor culture samples from BFP grown samples. When analyzing PC2, it shows the tendency of a differentiation between outdoor cultivation samples from Bahia and marijuana samples from Acre. In addition, there is a separation, in the graph's second quadrant, of street samples, and two of the three seized samples are closer to each other, suggesting a higher similarity. It is also possible to observe, in the first quadrant, the separation of samples from drug seizures in Rio Grande do Sul. Although there are only three samples (41a, 42a, 43a) included in the study when the analyzes was already in progress and this number is not expressive for solid conclusions in discriminatory analysis, it is possible to suggest that the method is also useful for separating street samples, in addition to showing results for indoor cultivation samples and discrimination between marijuana and cannabis.

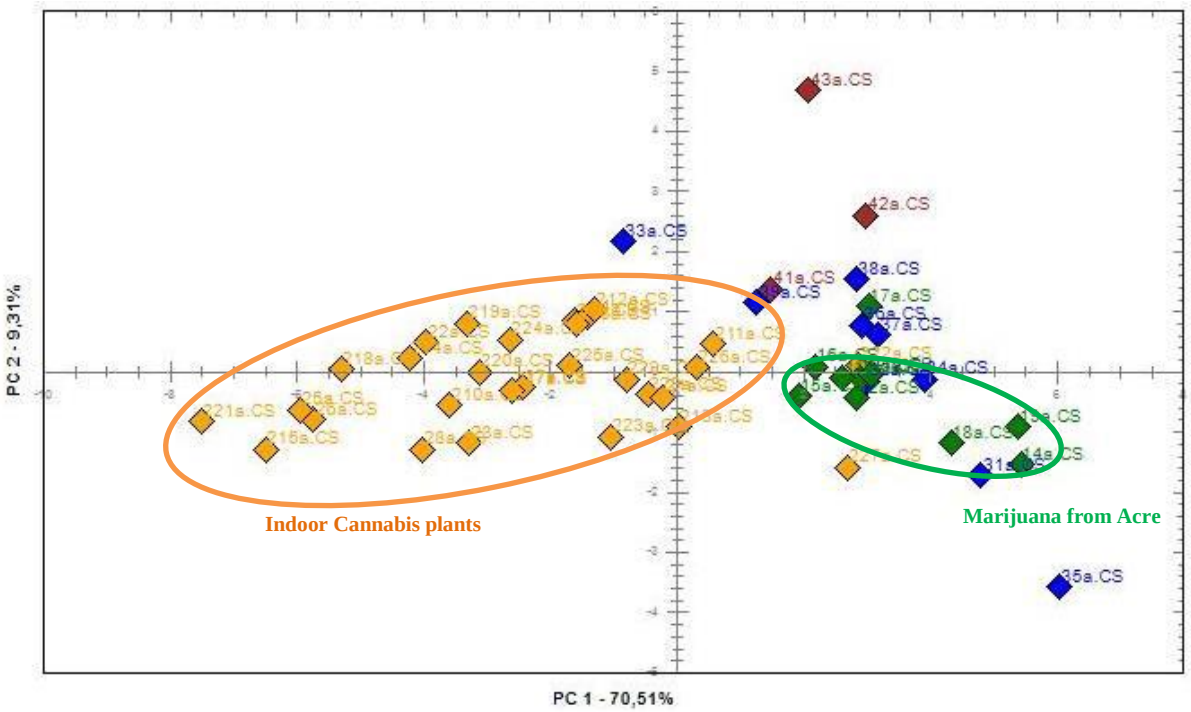


Figure 7. Scores of PCA (PC1xPC2) for cannabis and marijuana FTIR-ATR spectra of four groups of seized samples. Samples named number 1: marijuana from Acre; samples named number 2: indoor cannabis plants; samples named number 3: outdoor cannabis plants from Bahia; samples named number 4: street samples from Rio Grande do Sul.

Figure 8 shows PC1 (70.51%) and PC3 (8.14%) graph, where the difference between cannabis and marijuana samples is more evident. The indoor samples are concentrated in the quadrants three and four on the graph, while 90% of Acre's samples are in the first quadrant and Bahia's samples are in the fourth quadrant.

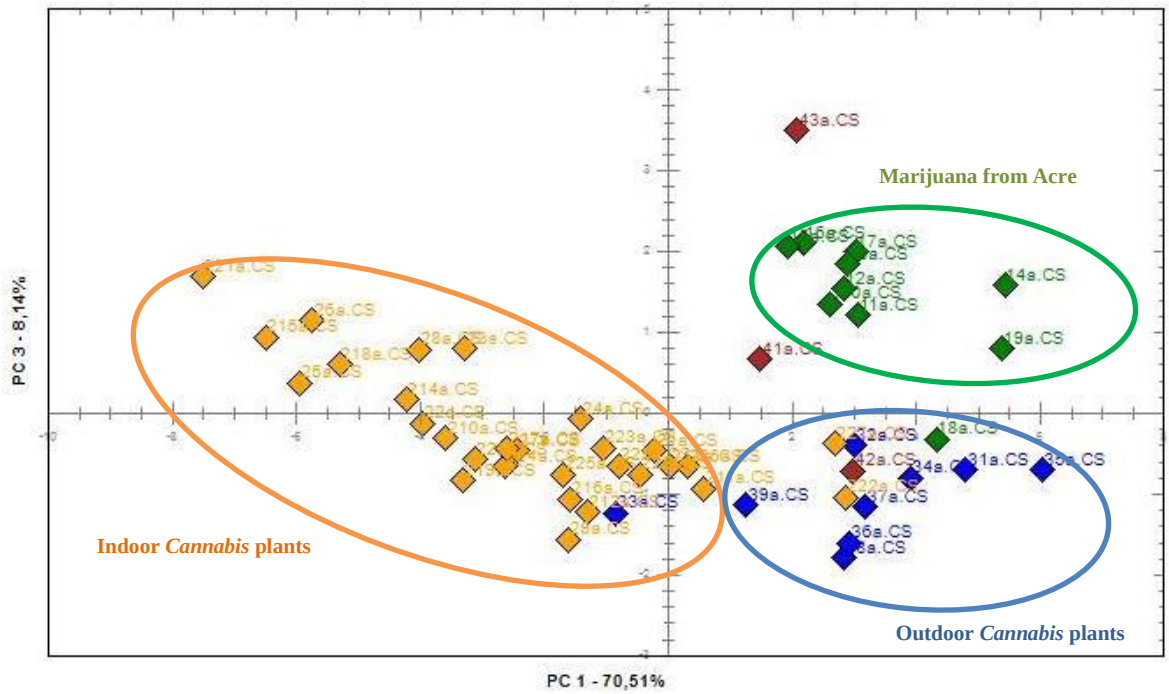
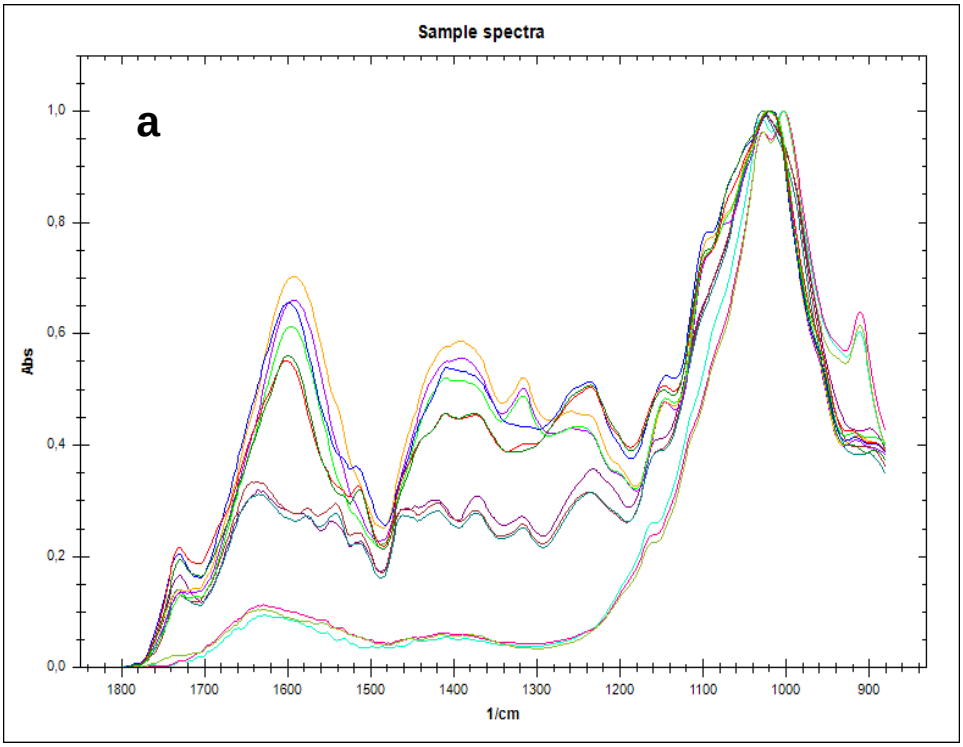
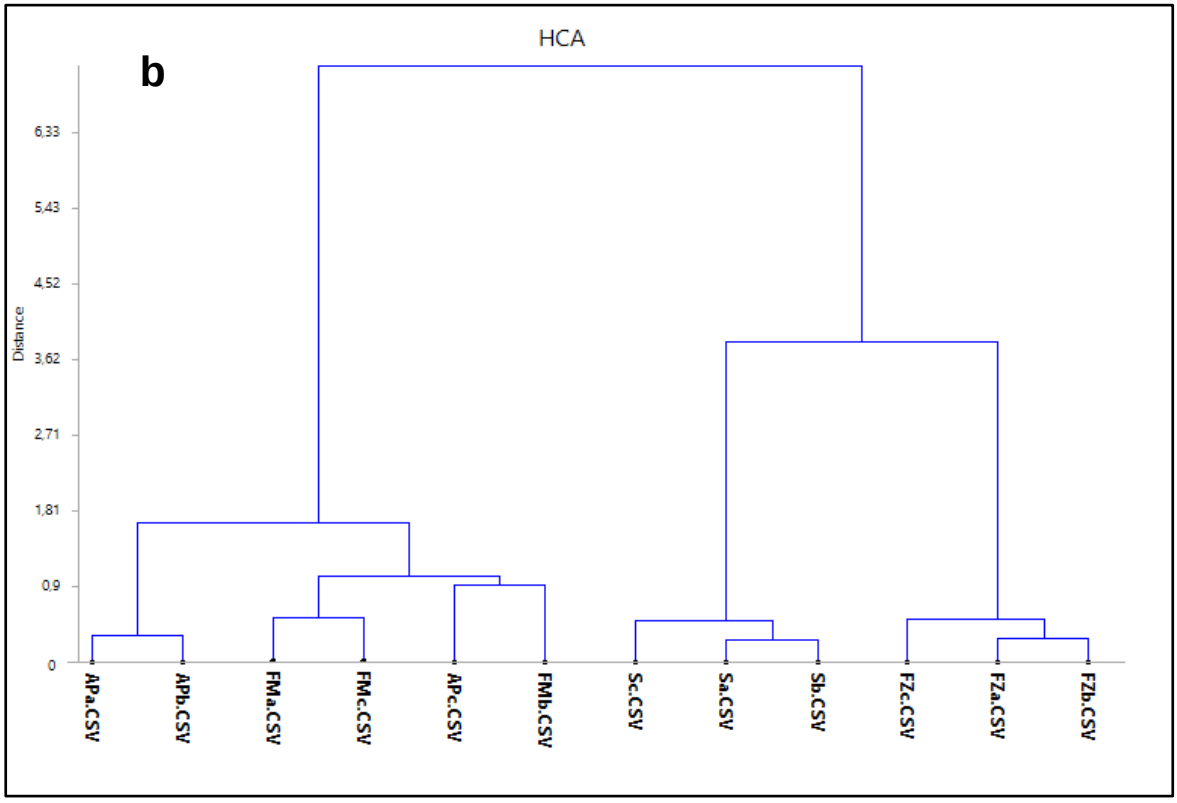


Figure 8. Scores of PCA (PC1xPC3) for cannabis and marijuana FTIR-ATR spectra, highlighting three main groups of samples. Samples named number 1: marijuana from Acre; samples named number 2: indoor cannabis plants; samples named number 3: outdoor cannabis plants from Bahia; samples named number 4: street samples from Rio Grande do Sul.

Adding diluent to the analysis was an attempt to differentiate marijuana samples that did not clearly separate in the initial analysis (Figure 9). First, the diluents were analyzed without any marijuana sample, and a pool of each diluent was analyzed in triplicates using data preprocessing as it was used for cannabis samples. Figure 9 shows that the diluent samples had different components and it also possible to differentiate each one, thus answering question (c) of script analysis (Figure 2). It was possible to differentiate diluents made up of a vegetal material – due to the separation of aromatic plants samples and commercial tobacco – from samples of soil and feces, whose components are different.

PCA scores chart (Figure 9c) confirms the findings of HCA (Figure 9b), placing each group of diluents in a different graph's quadrant. PC1 is responsible for 95.77% of the variance.





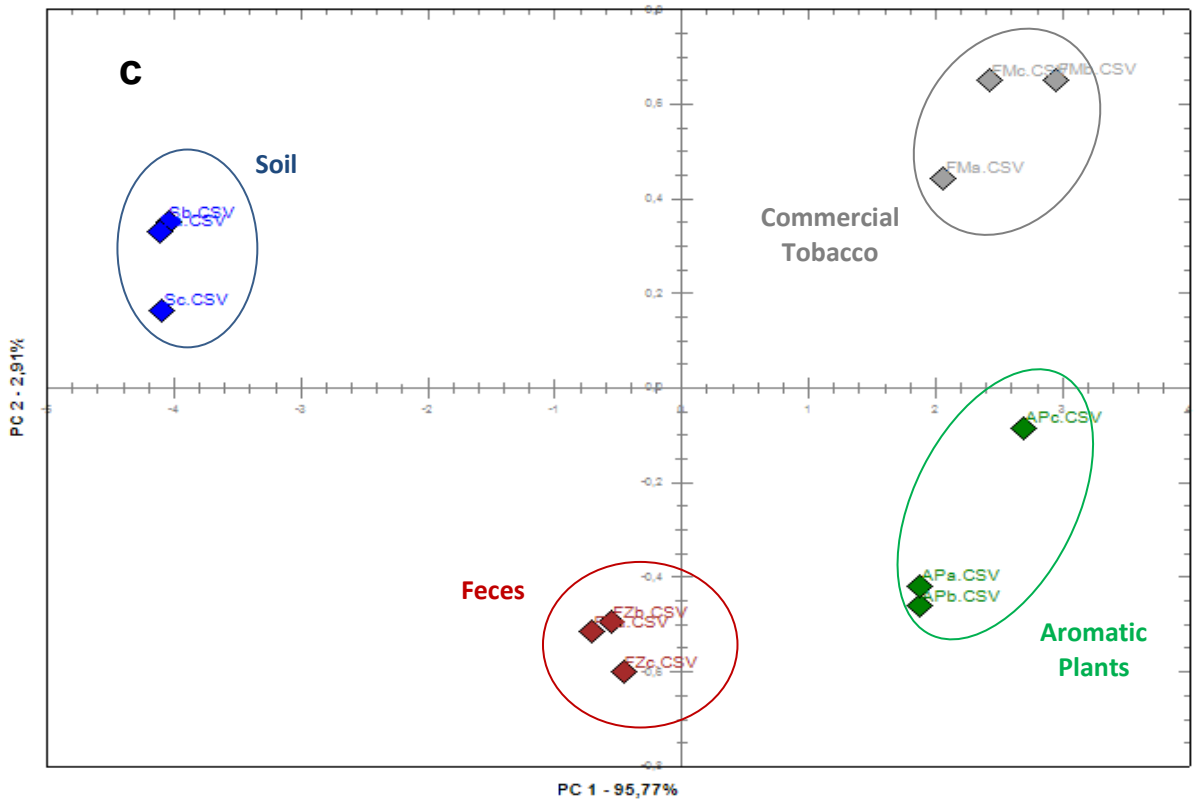
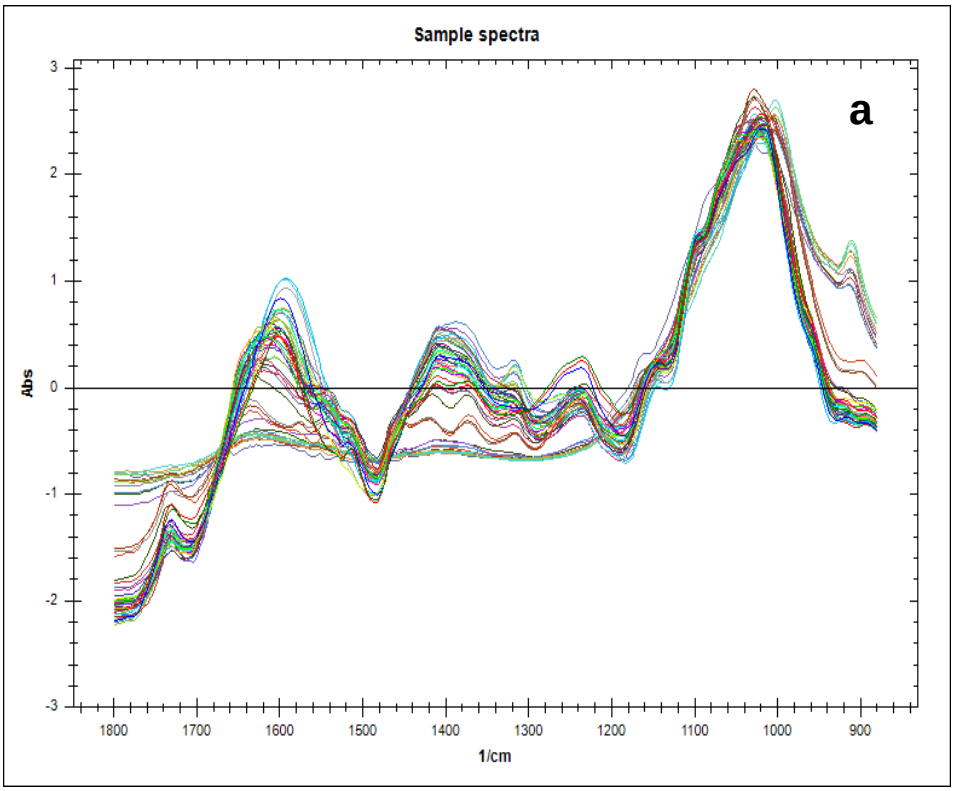


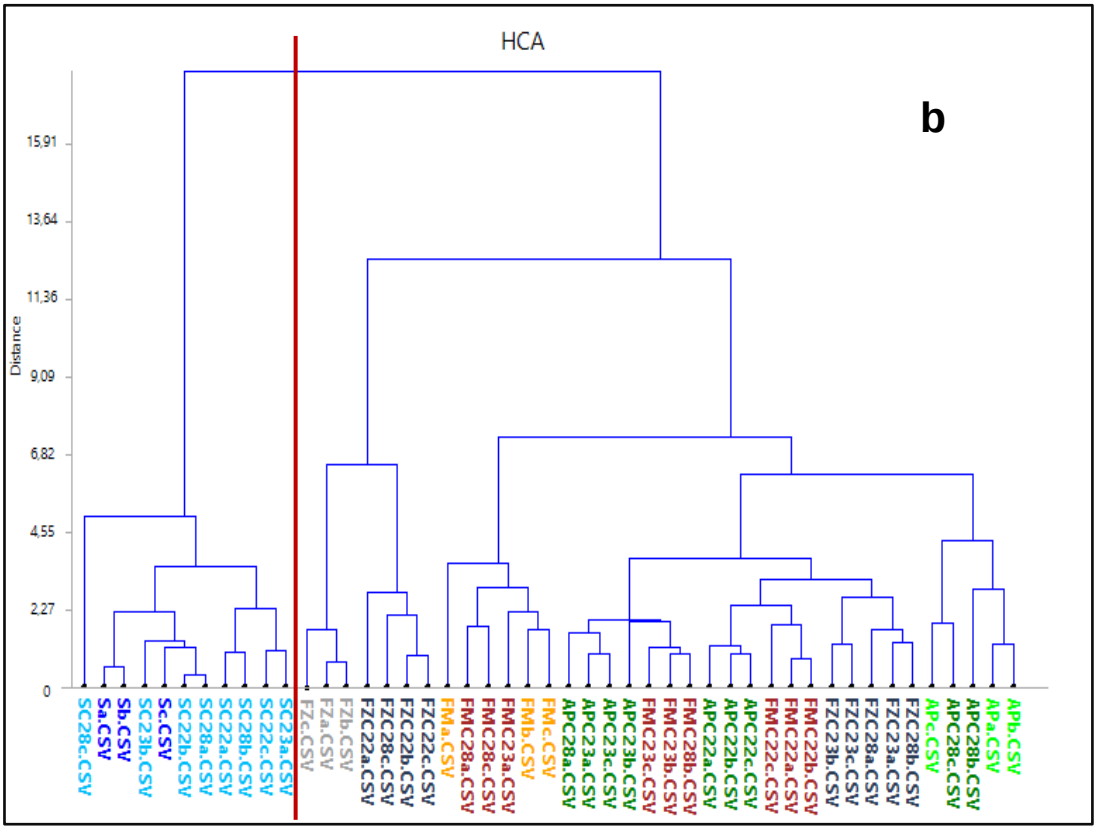
Figure 9. (a) FTIR-ATR spectra of diluents samples in triplicate with preprocessing. (b) Dendrogram of diluents samples, highlighting the differences between plant material from soil and feces. (c) PCA scores show diluents samples in opposite quadrants. Legend: AP – Aromatic Plants; FM – Commercial Tobacco; FZ – Feces; S – Soil. The letters "ab, "b" and "c" identify the triplicates.

Afterwards, three indoor cannabis samples were randomly chosen and mixed with each group of diluents in a 1:1 ratio, separately, and analyzed in triplicate. The results were treated and compared with diluent samples results (Figure 10). Different classes of diluents separated cannabis samples according to the similarities of the diluents chemical similarities (Figure 10c). The mixture of samples with commercial tobacco and aromatic plants shows that it is possible to have deliberate or accidental contamination with diluents in the seized samples, responding in a relevant way question (d) of the script analysis (Figure 2).

When adding diluents and adulterants, it is more difficult to verify the discrimination between the samples, but there is a clear separation between the organic matter of the plants and the feces in relation to the samples named with soil. The aromatic plants were not analyzed separately; they were mixed in a 1:1 ratio and then added to cannabis in the same proportion. The cannabis and marijuana samples in this part of the survey were chosen at random, with no direct specification

of sample information to try to simulate a real sample. Separately, aromatic plants and cannabis had differences in the spectrum in the fingerprint region, but many signals are common because they are plant material.





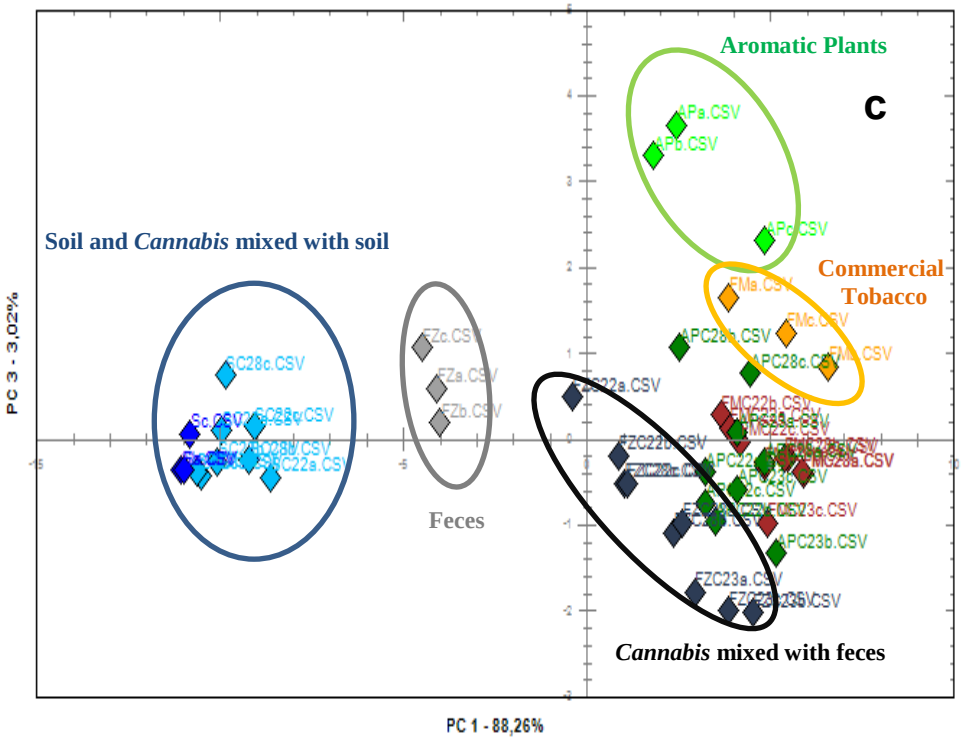


Figure 10. (a) FTIR-ATR spectra of diluents samples and cannabis samples mixed with diluents in triplicate with preprocessing. (b) Dendrogram showing the separation between soil and cannabis mixed with soil (samples named with "S", on the left) to other diluents and mixed samples. (c) PCA scores showing 88.26% of variance in PC1 to justify the separation between soil and feces samples that are in the negative part of PC1 from the different class of organic material (plants, other diluents, mixed samples and feces).

In order to analyze all samples (seized drugs, mixtures and diluents) it was necessary to average the results triplicate for each sample, before the pretreatment. Due to the number of samples and their similarity, the results overlapped, and a clear identification of the groups was not observed (Figure 11a).

Considering all sample set, some trends are disclosed, such as: 1) PC1 showed 89.68% of variance and clearly separates soil and cannabis mixed with soil samples from the others, as shown in the loadings graph (Figure 11b and 11c). Samples in the negative portion of PC1 correspond to the wave number around 900 cm^{-1} in the spectra; 2) indoor cannabis samples form a well-defined group on the positive portion of PC1 and in the negative portion of PC2; 3) marijuana samples from Acre also have a characteristic profile, forming a group on the positive portion of PC1. *Cannabis* samples from Bahia are different from those grown by BPF, confirming the difference between indoor and outdoor cultivation forms; 4) due to the high distances in the graph, the unknown samples, are from different types of

marijuana; 5) the feces samples did not form a separate group but are almost equidistant in the negative portion of PC2, relatively separate from the other samples.

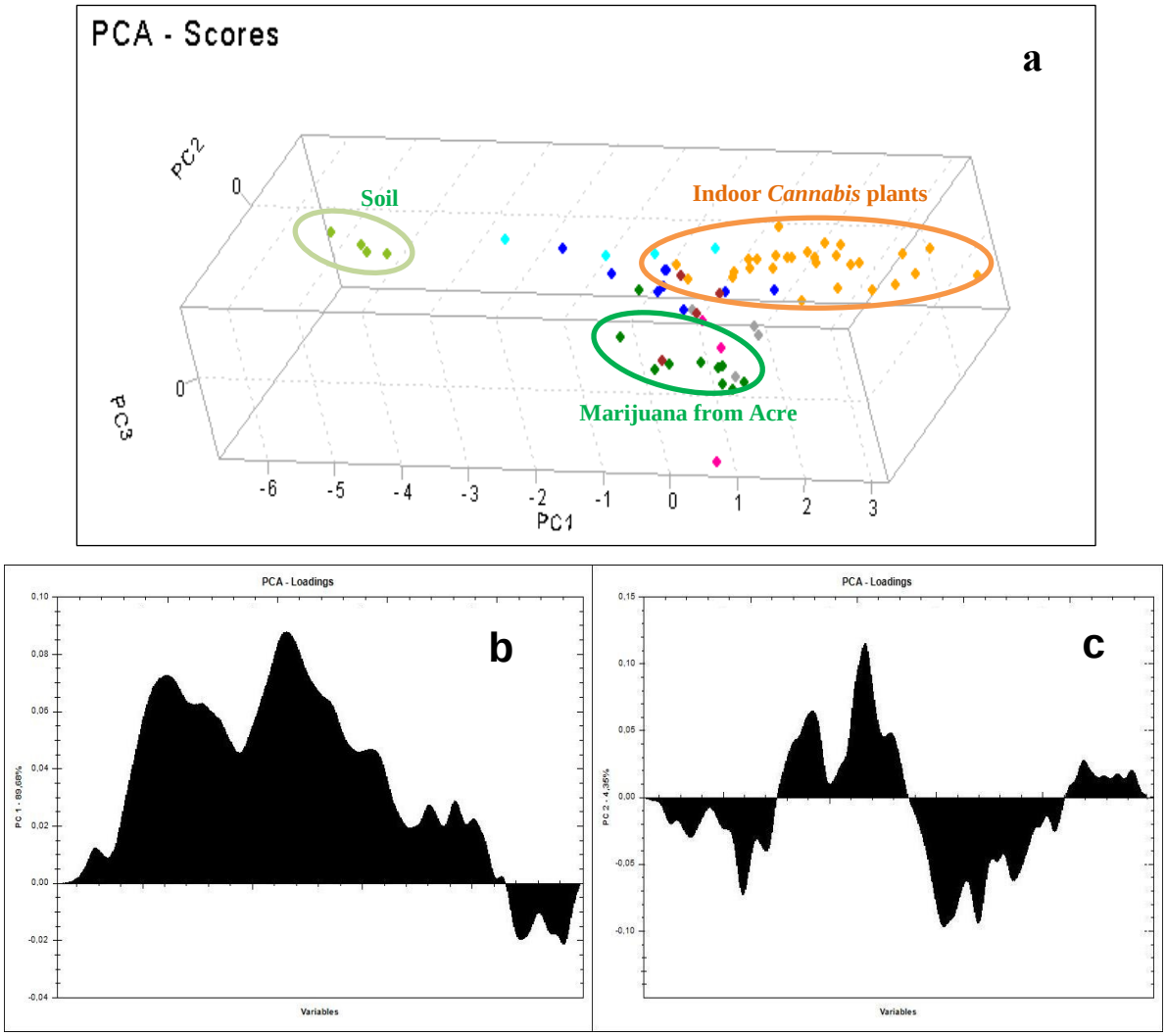


Figure 11. (a) FTIR-ATR average of spectra of cannabis, marijuana, diluents and cannabis mixed with diluents samples. (b) PCA score plot 3D of cannabis, marijuana, diluents and cannabis mixed with diluents samples. (c) Loadings of contributions of variance of the PC1, PC2, PC3. (light green: soil and soil mixed with cannabis; green: marijuana from Acre light blue: feces and feces mixed with cannabis; orange: cannabis indoor from BFP; grey: commercial tobacco and commercial tobacco with cannabis; pink: unknown samples; burgundy: aromatic plants and aromatic plants with cannabis.

Thus, the chemometric findings were able to compare the sample sets, answering question (e) of the analysis script (Figure 2); 6) as a pioneering study and a moderate universe of samples, the study can also be applied to differentiate street samples, as seen in Figure 8, in which samples 41a, 42a and 43a are separated.

Siano *et al*., analyzed the biochemical and chemical parameters of a type of cannabis aimed at the food industry and performed FTIR-ATR analyzes showing the differences in functional groups identified in each presentation[22]. Garside and Whyeth used the FTIR-ATR technique to differentiate cellulosic fibers from cannabis and linen for the textile industry[26] Using the same method, it is also possible to monitor lignocellulosic substrates with the modifications of carbon and nitrogen with the use of microbial transformation in cannabis samples for the paper industry[24]. These studies show that in the literature, research on direct analysis of cannabis and its adulterants for forensic purposes is scarce and lacking in results. This research arose from the chemical profile study carried out by BFP for the research of adulterants in cocaine[17]. As research on cannabis has been expanded in recent years for therapeutic purposes, new studies have analyzed the properties of plant presentations such as seeds, oil and leaves[28]. However, there is also a need for extensive research on the methods of cultivation, harvesting, storage and illegally trafficking and how these factors influence the content of cannabinoids and how they can be harmful to health. In France, a case report study of cannabis adulteration with sand and glass powder resulted in patients with upper and lower airway ulcerations and acute inhalation pneumonitis[27]. Borille *et al*. discriminated cannabis at different times of cultivation using the Near Infrared Spectroscopy (NIR) combined with chemometrics method, showing the versatility and establishment of the use of a spectroscopic method combined with multivariate analyzes[16]. Cole *et al*. conducted a review study focused on adulterants of illicit drugs that provides important data on which adulterants are most common, mentioning cannabis, the use of aluminum and glass[8]. The use of aromatic herbs is more common and is reported in a specific chapter of the book Cannabis and Cannabinoids: Pharmacology, Toxicology, and Therapeutic Potential, the aromatic herbs used are different in various different parts of the world[9]. Focusing on the value of the drug in Brazil, the diluents and adulterants chosen in the study were selected due to their common availability and because they do not significantly increase the end value of the drug[10,22].

4. Conclusions

A methodology of separation for cannabis plants and marijuana, with additional analysis of possible diluents present in the seized samples, was proposed by performing FTIR-ATR analysis and exploratory data analysis. Unsupervised methods

were used because of the amount of samples available; the supervised methods were not used because they depend on a set of samples for the construction of the analytical models and another set of samples for external validation, which was not the case. The unsupervisioned methods of PCA and HCA showed that it is possible to separate cannabis and marijuana samples and to differentiate indoor culture from outdoor samples. Diluent analysis disclosed that contamination of seized marijuana samples is possible. FTIR-ATR methodology is quick and easy to apply, it requires a small sample volume and, mainly, it preserves the sample from destruction, an important detail in Forensic Sciences. Chemometrics, obtained through infrared analysis, were reliable and satisfactory, gathering several relevant pieces of information for the chemical profile of cannabis, in a short period of time.

Acknowledgments

We would like to thank to CNPq and CAPES for the financial support and to Brazilian Federal Police for the scientific partnership.

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