B144 The Use of Near-Infrared Spectroscopy (NIRS) and Chemometrics in Evaluating Growth Periods of Cannabis Seeds Seized and Cultivated in a Greenhouse

Bruna Tassi Borille, MS, Avenida Ipiranga 2752, Porto Alegre 90610000, BRAZIL; Taís Regina Fiorentin, BS, 210 Krewson Terrace, Willow Grove, Philadelphia, PA 19090; Rafael Scorsatto Ortiz, PhD, Avenida Ipiranga 1365, Porto Alegre 90160093, BRAZIL; Kristiane de Cássia Mariotti, PhD, Avenida Ipiranga 1365, Porto Alegre 90160093, BRAZIL; Marcelo Caetano Alexandre Marcelo, MS, Avenida Bento Gonçalves 9500, Porto Alegre 90040060, BRAZIL; Marco Flores Ferrão, PhD, Avenida Bento Gonçalves 9500, Porto Alegre 91501970, BRAZIL; and Renata P. Limberger, PhD, Avenida Ipiranga 2752, Porto Alegre 90610000, BRAZIL*

After attending this presentation, attendees will be able to assess the classification of cannabis seeds seized and cultivated in controlled conditions during their different growth stages.

This presentation will impact the forensic science community by providing a new and effective classification of cannabis plants by age and, consequently, by providing a fast and reliable solution to gathering information about indoor cultivation time, establishing a connection between the cultivation site, the trafficked seeds, and the route of cannabis trafficking.

In recent years, cannabis drug trafficking has been changing in Brazil. An exponential increase in the seizure of cannabis seeds sent by mail has been observed by the Brazilian Federal Police (BFP). This change in the cannabis trafficking scenario may be the result of BFP efforts to eradicate the cultivation of the plant in large-scale farms and the change of the outdoor setting for indoor cultivation. This same tendency is also observed in Europe.

Even though a simple chemical analysis of the seized drug is sufficient to identify cannabis as a drug for legal purposes, classification by age may be an important tool in forensic intelligence (e.g., to gather information regarding indoor cultivation time, establish a connection between the cultivation site and the trafficked seeds and route trafficking); however, the high intrinsic variability present in cannabis samples (brands, varieties, chemotypes and gender) makes it difficult to obtain a classification standard. Therefore, this study sought to develop a method for classifying cannabis germinated in a homemade greenhouse according to the growth period, using Near-Infrared Spectroscopy (NIRS) and chemometrics.

Twenty-nine seeds seized by BFP were cultivated in a homemade greenhouse, in three different growth periods and controlled conditions. The harvest was performed by removing the entire plant from the soil; the loss on drying was estimated by gravimetry until the sixth day post-harvest. The leaves, stems and inflorescence were ground in an agate mortar and directly analyzed by NIRS. The influence of the sample homogeneity on the classification was evaluated using triplicates, and at random.

The NIR spectra were obtained using a PerkinElmer® 400 IR spectrometer equipped with integrating sphere and Indium Gallium-Arsenic (InGaAs) detector. The spectra were measured with resolution of 4cm^{-1} in the range between 10,000 and 4,000 cm^{-1}. A total of 32 scans were performed for each sample.

Principal Component Analysis by intervals (*i*PCA) was performed to assess the spectral region. This provided the best separation between the groups. The PCA, Hierarchical Cluster Analysis (HCA), Partial Least Squares Discriminant Analysis (PLSDA) and Support Vector Machines Discriminant Analysis (SVMDA) were performed and the samples (n=87) were classified by age (5.5 weeks, 7.5 weeks, and 10 weeks). The software MATLAB with PLS_toolbox and iToolbox (http://www.models.kvl.dk) were used.

An absorption band above 8,000 cm^{-1} was observed, with only the absorption band associated with the third overtone of CH_3, CH_2 and CH being approximately 8,500 cm^{-1}. The absorption bands associated with the second and first overtone of CH_3, CH_2, and CH were observed in the area of 6,900 and 5,800 cm^{-1}. In the spectral region between 5,000 and 4,000 cm^{-1}, there was information associated with the combinations of CH_3, CH_2, CH, C-C, and CHO, among others.

The *i*PCA in the raw spectra demonstrated the best separation between the samples by age, identified in the spectral region 4,000-4,375 cm^{-1}, associated with CH_3, CH_2, CH, C-C, and CHO combinations. Both discrimination analysis algorithms were carried out in this interval. The PCA and HCA exhibited good separation between the three groups of cannabis with different growth periods. The PLSDA and SVMDA classified the samples with good results in terms of sensitivity and specificity. The sensitivity and specificity for SVMDA classification were equal to unity.

These results revealed that, in the early stages of the cultivation of indoor cannabis, the age of the plant can be predicted by NIRS and chemometric tools; however, a larger study may be needed to confirm this observation due to the low number of samples obtained.

Cannabis, Near-Infrared Spectroscopy, Chemometrics