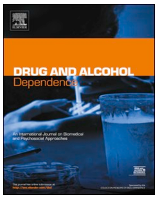
Drug and Alcohol Dependence 205 (2019) 107578

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep



Full length article

MicroNIR/Chemometrics: A new analytical platform for fast and accurate detection of Δ9-Tetrahydrocannabinol (THC) in oral fluids

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ARTICLE INFO

Keywords:
THC
MicroNIR
Chemometrics
Screening
Toxicology

ABSTRACT

Background: Δ^9-Tetrahydrocannabinol (THC) is already considered one of the most addictive substances since an increasing number of consumers/abusers of THC and THC based products are observed worldwide. In this work, the capabilities of a novel miniaturized and portable MicroNIR spectrometer were investigated in order to propose a practical and intelligible test allowing the rapid and easy screening of Δ^9-Tetrahydrocannabinol (THC) oral fluids without any pretreatment.

Methods: Specimens from volunteers were collected in order to consider any sources of variability in the spectral response and spiked with increasing amount of THC in order to realize predictive models to be used in real cases. Partial Least Square-Discriminant Analysis (PLS-DA) and Partial Least Square regression (PLSr) for the simultaneously detection and quantification of THC, were applied to baseline corrected spectra pre-treated by first derivative transform.

Results: Results demonstrated that MicroNIR/Chemometric platform is statistically able to identify THC abuse in simulated oral fluid samples containing THC from 10 to 100 ng/ml, with a precision and a sensitivity of about 1.51% and 0.1% respectively.

Conclusions: The coupling MicroNIR/Chemometrics permits to simplify THC abuse monitoring for roadside drug testing or workplace surveillance and provides the rapid interpretation of results, as once the model is assessed, it can be used to process real samples in a "click-on" device.

1. Introduction

Besides illicit drugs, the Δ^9-Tetrahydrocannabinol (THC) is one of the substances with the highest prevalence worldwide and its consumption is actually considered a social threat; in fact, it is frequently associated with the increased risk of becoming involved in traffic accident driving under the influence of psychoactive substances (Schulze et al., 2012; Romolo et al., 2015; Neavyn et al., 2014). According to the European Drug Report 2014, about 26.3% of the population (adults, 15–64 years old) has used cannabis in their lifetime and over 70% of the recovered seizures in Europe are associated to cannabis (EMCDDA, European Drug Report, 2018).

Therefore, new hemp-derivative food products containing trace levels of Δ^9-Tetrahydrocannabinol (THC) are continuously proposed for consumption and despite the European current legislation provides for hemp products claimed "THC-free" and sold as "Legal High", trace levels of the active THC may be recovered due to contamination during the maturation or harvesting or sale of illicit products (Abuhasira et al., 2018).

In addition, because of the high contents of ω-3 fatty acids and the therapeutic benefits of cannabis (Thomas and ElSohly, 2016) a number of galenic preparations are frequently sold or available on the pharmaceutical market for medical or illicit purposes (Downey et al., 2013; Liguori et al., 1998). As a consequence, there is a growing concern associated to the control and abuse of preparations derived from the Cannabis Sativa for medical purpose that may lead to an illicit use of galenic preparation containing THC (Cerda et al., 2012; Bramness et al., 2018). All these issues may result in increasing number of consumers/abusers of THC or THC based products and a consequent increasing level of recovered THC in biological specimens during roadside controls by law enforcement (Hayley et al., 2018; Newmeyer et al., 2017; Ramaekers et al., 2004).

Currently, oral fluid (OF) is widely accepted as alternative matrix to blood for illicit drugs detection, as it provides a valuable correlation of the THC levels and collection may be performed by non-medical personnel without embarrassment (Samyn et al., 1999; Dams et al., 2007; Gjerde et al., 2015). In particular, on-site testing for THC in oral fluids

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https://doi.org/10.1016/j.drugalcdep.2019.107578
Received 17 May 2019; Received in revised form 16 July 2019; Accepted 22 July 2019

includes screening using Drugwipe-5 + 1, RapidSTAT1, Cozart1, and Drug Test 50001 (Musshoff et al., 2014; Wille et al., 2010; Van der Linden et al., 2015; Cooper et al., 2005).

Nevertheless, despite the advances in the field, accurate methods permitting to achieve the required sensitivity for screening, in order to avoid true positive and false positive response, are still lacking. Therefore, providing personal screening systems able to detect illicit drug abuse in completely automated, miniaturized and one-touch device, is more and more required especially in cases involving workplace surveillance (Houwing et al., 2013; Risoluti et al., 2018).

Chromatographic methods, including High Performance Liquid Chromatography followed by Mass Spectrometry (HPLC-MS) (Catauro et al., 2018; Mortier et al., 2002; Zancanaro et al., 2012) and Gas Chromatography coupled to Mass Spectrometry (GC–MS) (Langel et al., 2011; Cognard et al., 2006) are the most common approaches, since provide the required sensitivity and specificity to serve as official methods, especially if result has to be used in medico-legal situations (Pichini and Pacifici, 2013). On the other hands, spectroscopic techniques demonstrated to be promising in characterizing complex matrices, offering the advantages to be easy-to-use and non destructive without requiring any pretreatment (Véstia et al., 2019; Materazzi et al., 2017a,b; NunezSánchez-Carnerero et al., 2018). Recently, Raman spectroscopy has been involved as not destructive method for the study of biological matrices such as oral fluids (D'Elia et al., 2018a, b).

Nevertheless, Near Infrared spectroscopy has the advantage over Raman in detecting analytes in oral fluid composition such as amino acids. In addition, the coupling with chemometric tools provides models of prediction for qualitative as well as quantitative response with the required accuracy since the interference of the matrix in the spectral signal may be evaluated (Meesa et al., 2018; Risoluti et al., 2016; Materazzi et al., 2014a, b; Materazzi et al., 2014c, 2015; Kordi et al., 2017).

The latest miniaturized NIR instrument is the MicroNIR, an ultra compact (45 mm in diameter and 42 mm in height) and portable device developed and distributed by Viavi Solutions (JDSU Corporation, Milpitas, CA), that weighs about 60 g and it is entirely powered (5 V) and controlled by a USB port of a portable computer (Risoluti et al., 2018; Risoluti and Materazzi, 2018).

In this work, a novel analytical platform based on MicroNIR spectroscopy associated to chemometric analysis is proposed for a fast and accurate detection of THC in non-pretreated oral fluids. The main advantage of this compact system is related to the miniaturized portable spectrometer operating in the Near Infrared region that permits to obtain comparable outcomes as the laboratory one because of its geometry and optical resolution (Papadopoulos et al., 2016; Modrono et al., 2017; Risoluti et al., 2017; Risoluti and Materazzi, 2018; Basri et al., 2017; da Silva et al., 2017).

In addition, the association with chemometrics provided an automated and not destructive tool able to simultaneously detect and quantify traces of THC in oral fluids and permitted to further investigate the same oral fluid sample from consumer with the reference official procedure (GC–MS) for confirmatory analyses.

2. Materials and methods

2.1. Materials and samples collection

Δ9-Tetrahydrocannabinol (THC) reference standard was purchased from Sigma-Aldrich (St. Louis, Missouri United States) as methanolic solution at the concentration of 1 mg-ml. All the standards were pure at 99%. Oral fluid (OF) specimens were collected from 50 anonymous volunteers by passive drool (spitting) and no salivary stimulation was simulated, taking into account heterogeneity of the gender (32 males and 18 females) and age (from 18 to 53 years old). In order to consider potential interferences in the platform response, different habits of volunteers were considered, and subjects were asked about eating or drinking as usual (caffeine, sugars, chewing-gum, alcohol). A pool of oral fluids was considered for model calibration and divided into 50 specimens analyzed as such and spiked with increasing amount of THC in the range 10–100 ng-ml. In addition, the same number of specimens was considered for model validation. A number of ten spectra were recorded for each sample in order to evaluate reproducibility and the average was considered for calculations. MicroNIR measuremets did not require any sample pre-treatment and OFs (1 ml) were directly processed by the platform after collection in a MicroNIR cuvettes, while a proper clean-up was put in place for GC–MS analysis in order to accomplish the confirmatory analyses.

2.2. MicroNIR platform

The MicroNIR (Fig. 1) operates in the spectral region 900–1700 nm and consists of a linear variable filter (LVF) directly connected to a 128

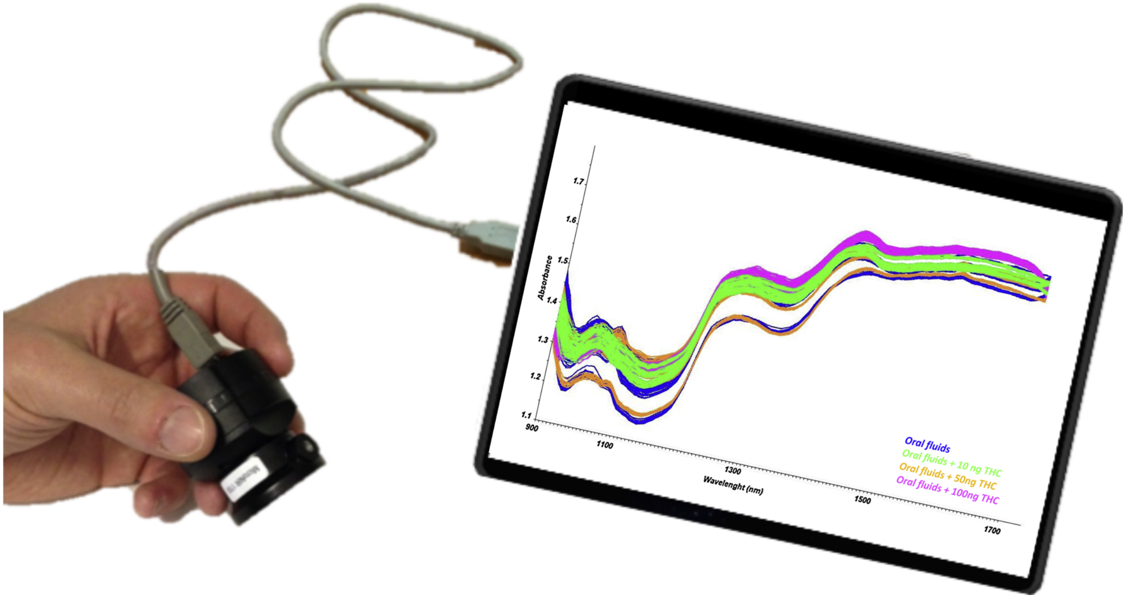

Fig. 1. MicroNIR device used to collect spectra and to develop the model of prediction for THC in oral fluids.

pixel linear InGaAs array detector and two tungsten light bulbs used as the radiation source. All the collected spectra were recorded at a nominal spectral resolution of 6.25 nm, as the most performing conditions. Spectralon was used as the NIR-reflectance standard (the blank) with a 99% diffuse reflectance, while the dark reference was obtained from a fixed point in the room. Collection of spectra was performed with an integration time of 10 ms, resulting in a total measurement time of 2.5 s per sample. The control was achieved by MicroNIR Pro software (JDSU Corporation, Milpitas, CA) and the chemometric analysis was performed by VJDSU Unscrambler Lite (Camo software AS, Oslo, Norway). A model of prediction for THC in oral fluids was developed by Partial Least Square- Discriminant Analysis (PLS-DA) and its ability to correctly detect THC was checked against true-positive and false positive response. In addition, a quantification model based on Partial Least Square regression (PLS) was also pointed out in order to simultaneously quantify the psychoactive molecule. Different chemometric spectral pretreatments were investigated in order to provide the best separation of samples: among these, Standard Normal Variate (SNV) transform, multiplicative scatter correction (MSC), and normalization (Barnes et al., 1989; Geladi et al., 1985; Wold and Sjöström, 1977) were evaluated, whereas the Savitzky–Golay (SG) polynomial derivative filter (Savitzky and Golay, 1964) was considered as spectral derivation technique.

2.3. GC–MS confirmatory analyses

Confirmatory analyses were performed using a Perkin Elmer (Waltham, MA) GC system interfaced with a mass selective detector. Separation was achieved by a HP-5MS column (30 m × 0.25 mm × 0.25 mm). A constant flow of helium at 1 mL/min was used as the carrier gas. The injection was made in split mode with a split ratio of 50:1. Regardless to the method, the oven temperature was set at 120 °C for 1 min, ramped to 240 °C at 30 °C/min and then increased to 290 °C at 10 °C/min for 10 min. A post run was made at 300 °C for 5 min. The mass spectrometer parameters were as follows: transfer line at 250 °C, ion source at 230 °C and quadrupole at 150 °C. Mass spectral data were collected in the scan mode from 44 to 450 m/z. Identification of analytes was performed in Selected Ion Monitoring (SIM) mode considering 303, 371 and 386 m/z as characteristic mass fragments for THC (Pichini and Pacifici, 2013).

3. Results and discussion

A pre-analytical phase was put in place, in order to provide a tool able to detect THC in oral fluids. All the experiments were carried out with the aim of developing a model of prediction as robust as possible. As a consequence, any sources of variability in the spectral response were taken into consideration. The spectroscopic signal was calibrated to provide the most performing outcome and the interferences due to the complexity of the matrix were considered by evaluating a pool of oral fluids collected from volunteers with different habits (caffeine, sugars, chewing-gum, alcohol) and characteristics (gender and age). The same specimens were fortified with increasing amount of THC in order to simulate THC intake over time. This procedure would avoid the results being dependent of the different concentration, thus ensuring to detect THC even few hours since abuse. The typical behavior of the samples (raw data) is reported in Fig. 2, where overlapped spectra of oral fluids (blue lines) and fortified oral fluids with THC (orange lines) are reported.

A significant effect of the heterogeneity and complexity of oral fluids may be observed in Fig. 2, as the recorded spectra resulted in overlapped signals apparently not distinguishable. Chemometric analysis was applied to select the diagnostic variables reducing the contribution of the matrix as not all the wavelengths contribute equally to samples separation. Stepwise decorrelation of variables was applied to the spectral dataset in order to identify variables with the largest Fisher weight (Substance Abuse Mental Health Services Administration (SAMHSA, 2015).

Among the investigated chemometric tools, spectra were baseline corrected and pretreated by first derivative transform in the range of wavelengths 960–1180 nm, as a result of the loadings investigation by PCA. Overlapped signals of the average pre-treated spectrum of oral

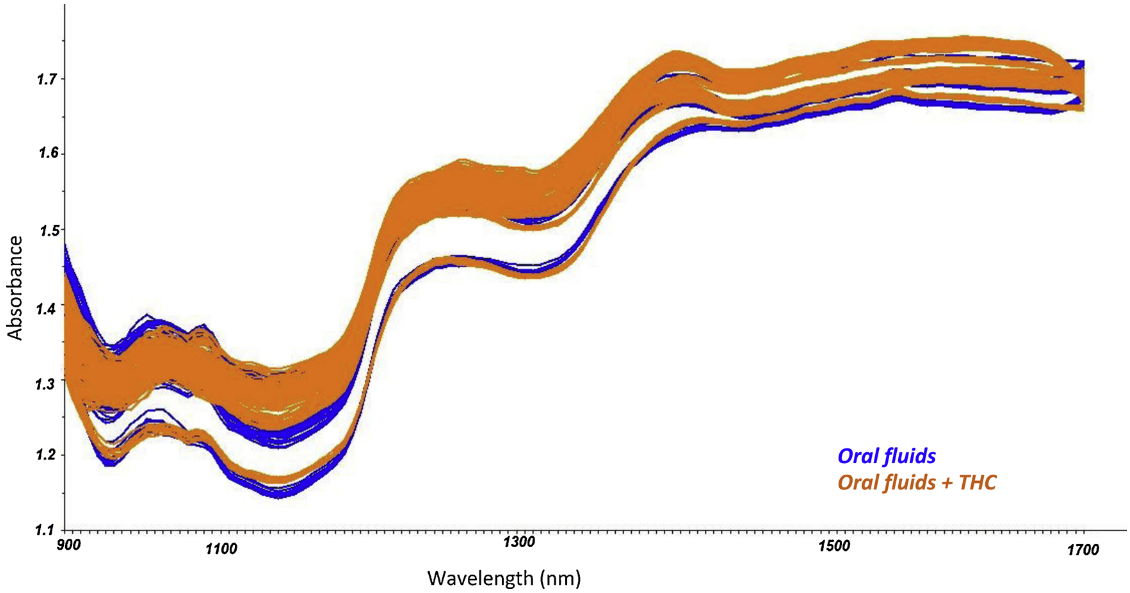

Fig. 2. MicroNIR spectra of collected oral fluids (blue) and spiked oral fluids with THC (orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

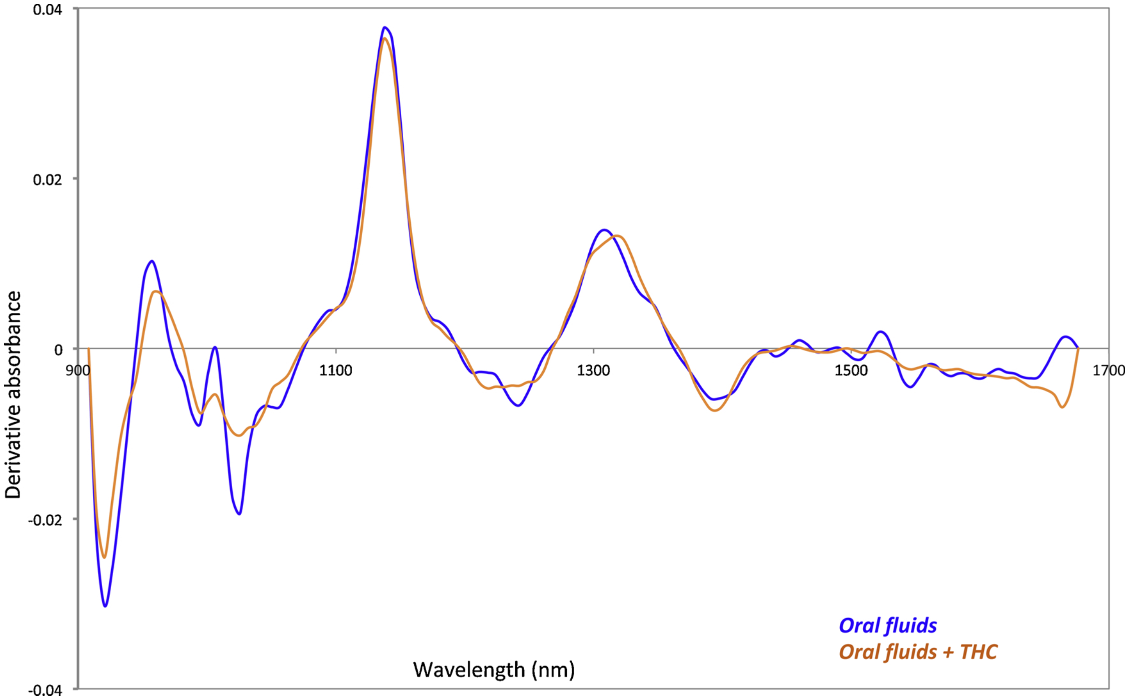

Fig. 3. Overlapped spectra of oral fluids (blue) and fortified oral fluids with THC, after chemometric processing. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fluids (blue lines) and fortified oral fluids with THC (orange lines) are reported in Fig. 3.

This procedure provided the suitable separation of all the processed samples, as shown in the scores plot reported in Fig. 4, obtained from the Principal Component Analysis (PCA).This preliminary unsupervised analysis suggested to further investigate the possibility to develop a model of prediction for THC identification in oral fluids, as all the investigated samples were located in two different side of the plot according to PC 2 (17% of explained variance), thus according to the presence (spiked oral fluids, orange lines) or to the absence (unspiked oral fluids, blue lines) of THC. To this aim, Partial Least Square-Discriminant Analysis (PLS- DA) was considered in order to validate a

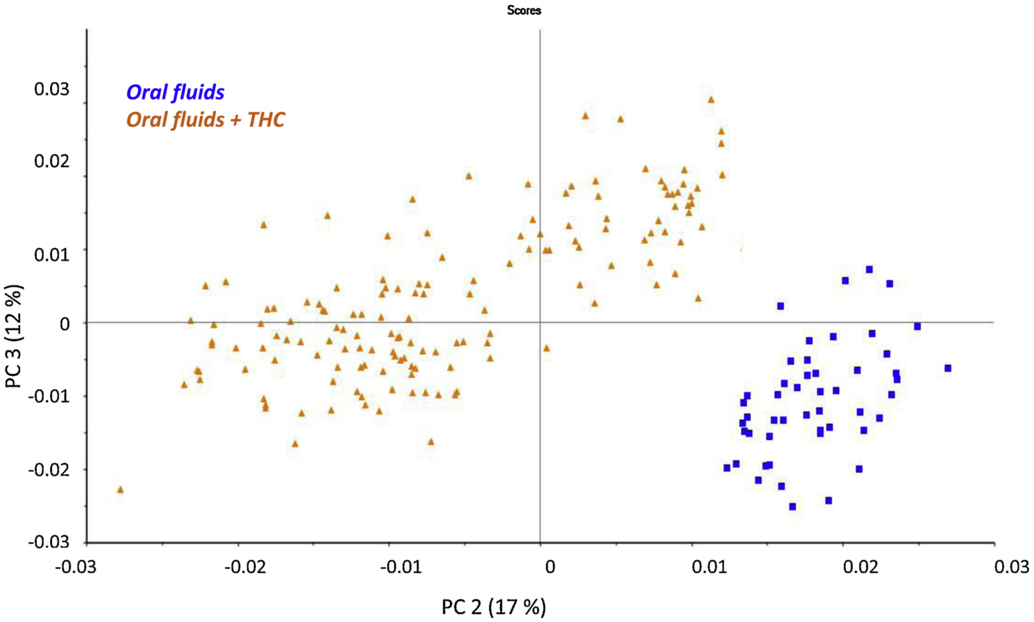

Fig. 4. Resulting scores plot by PCA of oral fluids (blue) and spiked oral fluids (orange), for the identification of positive samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

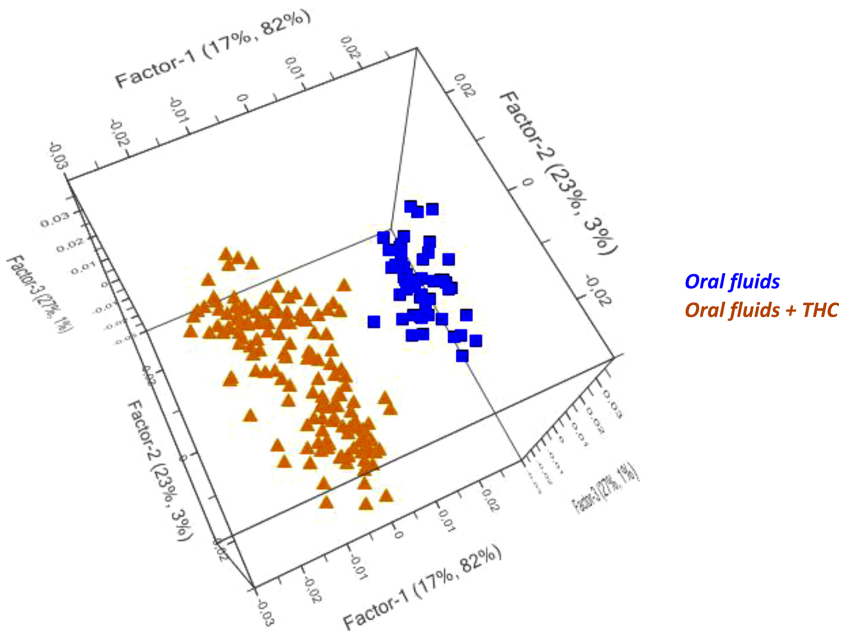

Fig. 5. 3D-scores plot from PLS-DA model for oral fluids (blue) and spiked oral fluids with THC (orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Figures of merit obtained by PLD-DA model for THC.

	Calibration		Validation		Prediction	
	Blank	*Blank + THC*	*Blank*	*Blank + THC*	*Blank*	*Blank + THC*
NER (%)	98.0	100	98.0	100	100	100
Sp (%)	100	99.3	100	99.3	100	100
RMSE	0.16	0.11	0.17	0.12	0.16	0.20

model of prediction for THC to be used as first level test for roadside controls by law enforcement. The resulting 3-D scores plot is reported in Fig. 5.

Satisfactory calibration results were obtained for the THC in oral fluids, as shown in Table 1. The performances of the model were evaluated considering the figures of merit, calculated as Non Error Rate (NER %), Specificity (Sp%) and Root Mean Square Error of Calibration (RMSEC), Validation (RMSECV), and Prediction (RMSEP). Interestingly, the model provided for accuracy and specificity not lower than 98.0% and 99.3% respectively and an error of prediction not exceeding 0.17% both in calibration and validation. Prediction of unknow samples resulted in 100% of accuracy and specificity of the model, since all the samples were correctly predicted as belonging to the right class and provided for an error of about 0.2%.

Results demonstrated the feasibility of the MicroNIR/Chemometric platform to recognize THC abuse in oral fluids for at the first level tests, with accuracy of the reference official method as all the results were in accordance with those obtained by the parallel GC–MS analyses on the same specimens (even for samples close to the cut-off level).

3.1. Quantification model

On the basis of the previously achieved results, further experiments were performed in order to evaluate the possibility to quantify THC. Oral fluids specimens were spiked with increasing amount of THC (10, 50 and 100 ng-ml) in order to mimic THC intake over time and spectra were recorded by MicroNIR. The previously chemometric treatment was tested and Principal Component Analysis was applied, confirming that the baseline correction followed by the first derivative transform in the range 960–1180 nm, provided a clear separation of samples according to the first two Principal Components (overall explained variance of 56%), as reported in Fig. 6. A suitable separation of the samples was achieved since all the measurements resulted located in the same side of the plot as a function of the different amount of THC in the specimen.

As a consequence, the same OF specimens were prepared in triplicate in order to ensure that results are not batch-dependent and Partial Least Square regression (PLSr) was used for model assessment. Samples were divided into training set (75% of samples) and evaluation set (25% of samples) in order to develop a model of prediction of the amount of THC. The resulting 3D-scores plot is reported in Fig. 7, where colors are used to represent samples belonging to different classes.

The performances of the model were evaluated considering as figures of merit, the correlation (R^2), the Root Mean Squared Error of Calibration (RMSEC), the Root Mean Squared Error of Cross-Validation (RMSECV) and the Root Mean Squared Error of Prediction (RMSEP). Precision and sensitivity were also determined as well as the Minimum Detectable Concentration (MDC) in order to compare results with those provided by the actual screening tests for roadside control of THC in oral fluids. Results of the prediction ability of the PLS model are summarized in Table 2. Satisfactory validation outcomes were achieved for RMSEs by the mean of 7 latent variables. The most important result consists of the detection limit of the model that permits to correctly identify THC at the cut-off level (10 ng) (Substance Abuse Mental Health Services Administration (SAMHSA, 2015). In addition, the PLS model proved to be suitable for fast and accurate screening of THC since the model provided for a precision and a sensitivity of about 1.51% and 0.1%, respectively.

3.2. Prediction of real samples

A number of 4 real samples collected from volunteers were processed by the validated platform in order to assess its performances and results were compared to those obtained from GC–MS analyses. Among the investigated samples, one of these was found to be negative to THC while the remaining three were found to be about 11.5 ng-ml, 19.3 ng-ml and 2.9 ng-ml.

A good correlation among the data from the two methods was observed since all the samples were correctly predicted by the model and

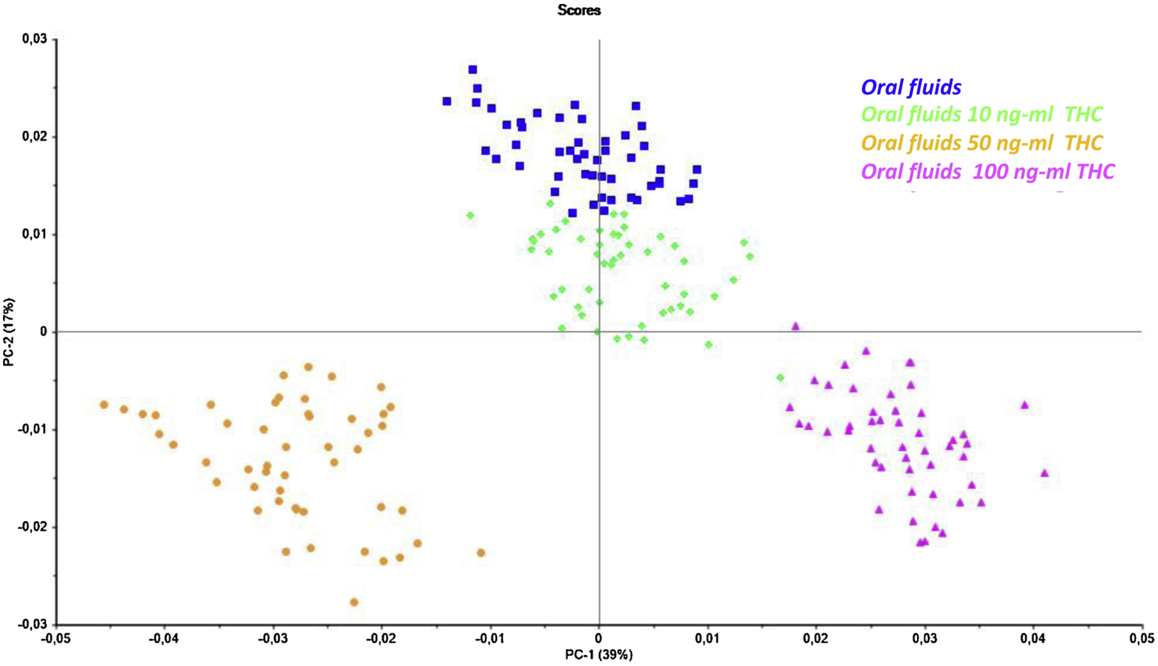

Fig. 6. Resulting scores plot by PCA of oral fluids (blue) and spiked oral fluids with 10 ng (green), 50 ng (orange) and 100 ng (pink) of THC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the correlation coefficient resulting from the analysis of the predicted (GC–MS outcomes) versus measured (MicroNIR measurements) samples was about 0.997.

In addition, in a parallel study focusing on the effectiveness of MicroNIR in detecting illicit drugs (Risoluti et al., 2019), no interferences in the spectral response were observed, demonstrating the ability of the platform to correctlydetect THC, avoiding false positive nor false negative outcomes.

4. Conclusions

A MicroNIR/Chemometric platform was proposed and validated for the on-site detection of THC in oral fluids. Two models of prediction based on PLS-DA and PLS regression were developed to identify and quantify THC in simulated samples and the prediction ability of the models were checked by comparing results with those obtained from the parallel GC–MS analysis. Results demonstrated that the spectroscopic investigation of oral fluids by MicroNIR device provided a not destructive, solvent-less and fast tool to perform an accurate and timely screening of THC abusers.

The coupling with chemometric tools provided the optimization of models of prediction for THC in a completely automated device, with comparable performances as the reference procedures since the limit of detection and accuracy were found to be those required for confirmatory tests. In fact, the platform was entirely designed with the aim of simplify law enforcement procedures and to facilitate the rapid interpretation of results.

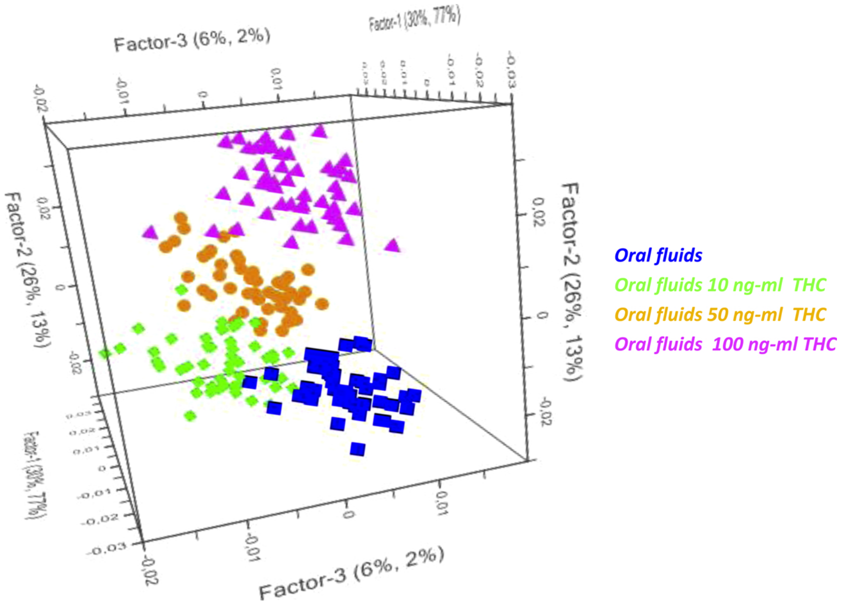

Fig. 7. 3D-scores plot for THC detection in oral fluids by PLS regression of oral fluids (blue), spiked oral fluids with 10 ng (green), 50 ng (orange) and 100 ng (pink) of THC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Figures of merit obtained by PLS regression model for THC quantification in oral fluids.

Figures of merit	
RMSEC	2.9
RMSECV	1.1
RMSEP	1.3
LV[a]	7
R^2 Validation	0.989
MDC[b] (ng)	10
Precision (%)	1.51
Sensitivity (%)	0.10

[a] Latent variables.
[b] Minimum detection concentration.

Despite that the results are preliminary, all these outcomes suggest the opportunity of considering the proposed approach into procedures (to be used alongside another technique), permitting to simplify THC abuse monitoring for roadside drug testing or workplace surveillance.

Role of funding source

No funding sources are associated to this research.

Contributors

R.R. and S.M. conceived the study and wrote the manuscript.

Data were obtained through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of Competing Interest

No conflict declared.

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