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Direct classification of new psychoactive substances in seized blotter papers by ATR-FTIR and multivariate discriminant analysis☆

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ABSTRACT

Due to the general increase in drug trafficking crime rates, a high amount of drug samples is continuosly seized and requires forensic analysis. In order to cover the demand for this great amount of samples in forensic investigations, non-destructive, fast and direct analysis methods are desirable. A new supervised classification method using PLS-DA (*partial least squares discriminant analysis*) and ATR-FTIR (*attenuated total reflectance Fourier transform infrared spectroscopy*) was developed to identify NPS (new psychoactive substances) drugs in blotter papers. A multivariate model was built to classify NBOMe, 2C-H, LSD, MAL (methallylescaline) and discriminate them of blank papers. A submodel was also built to discriminate 25B-NBOMe, 25C-NBOMe and 25I-NBOMe inside NBOMe class. Both models were validated through the estimate of specific figures of merit. The average of reliability rate (RLR) was 88.9%, accordance (ACC) was 91.1% and concordance (CON) was 86.1%. For the NBOMe submodel RLR was 82.2%, ACC was 100% and CON was 94.4%. The model presented high correct classification rates for all the classes, with the exception of LSD, possibly due to its lower concentration on seized blotters. The proposed method has potential to be used on blotter screening routine. The analysis is cost-effective, rapid, 2 min per sample, and utilizes ATR-FTIR, a technique whose use is increasing on forensic laboratories around the world.

1. Introduction

The new psychoactive substances (NPS) are quickly spreading through the world, and the detection and identification of these drugs are fundamental to control and confront this market [1]. Most of these substances have undergone small chemical substitutions from known illicit drugs. These small changes in their chemical structures take some NPS out of the category of illegal substances, allowing them to be marketed legally instead of prohibited. For this reason, they are also called “legal highs”.

As NPS are emerging as a social problem, the prohibition or control of their use through scheduling has been a key step to tackle this drug problem. However, regulating NPS have proved to be difficult. These new drugs become less attractive to dealers as they are legally banned, and so, other “legal” drugs have emerged to replace the illegal ones [2,3]. The availability of different substituted drugs is broad, even if only the main classes of substituted NPS are considered, such as phenethylamines, tryptamines and cannabinoids [4,5]. The fast growth of forms originates additional problems at both the analytical and legislation levels [6]. At the analytical level, there is a need for developing rapid methods for chemical detection and characterization of new drugs. Specifically, forensic analysts must face the uncertainty about the identity of seized substances or products, and need to have access to pure standards and certified reference materials [7].

The phenethylamine class of drugs has been known for a long time [8]. Alexander and Ann Shulgin have given an important contribution to the knowledge about this class as they have described the synthesis and psychotropic effects of several substituted phenethylamines [9]. This class is named due to its basic structure, which comprises a phenyl group bounded to an amino group by a two carbons chain. Through this chemical backbone (Fig. S1A, Supplementary material) some favorable substitution sites are available. This class includes some drugs well known for their potential for abuse, such as methamphetamine [1,10], and others only recently introduced in the traffic.

NBOMe series and 2C series are substituted phenethylamines recently regulated in some countries, because they have been openly

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sold as "legal highs" and have become a public health problem [11,12]. In the NBOMe series, a 2-methoxybenzyl group is added to the nitrogen of the 2C series (Fig. S1B). The 2C series (Fig. S1C) is composed of primary derivatives of 2,5-dimethoxyphenethylamine (2C-H). Another drug that has attracted attention due to its potent hallucinogenic effect is methallylescaline (MAL) (Fig. S1D). This synthetic analog of mescaline was reported to be seized for the first time in Sweden in 2013 [13]. MAL has also been seized in Brazil [14], but it is not yet officially regulated.

These NPS are powerful agonists of serotonin receptors and potent hallucinogens with effects similar to LSD [15]. They are currently sold in blotter papers and drug dealers can mislead the users by selling them as LSD. Thus, users are consuming NPS without knowing the toxicological effects of the unknown drug. Several intoxication cases after ingestion of supposed LSD or "acid" blotters have been reported, including fatalities [11,15–17].

Forensic laboratories are dealing with increasingly large amounts of seized NPS blotter samples in recent years, but there are no currently reference color tests for these drugs [18]. The most common methods for detecting and characterizing NPS have been based on chromatographic techniques [17,19–22]. These methods have the disadvantages of requiring pretreatment steps, such as sample extraction, being destructive, expensive and time consuming. Other alternative methods have been based on mass spectrometry direct analysis [23–25]. Some papers have also characterized NPS by using spectroscopic techniques, such as Fourier transform infrared (FTIR), nuclear magnetic resonance (NMR) and Raman [17,22,26], but the relatively small number of samples analyzed did not allow to obtain broad and robust predictive models. For this aim, more sophisticated data analysis techniques based on multivariate statistics should be used.

A particularly promising technique for qualitative and quantitative analysis of NPS is FTIR. Modern FTIR spectrophotometers provide rapid determinations with adequate signal-to-noise ratios, allowing direct and non-destructive analysis of solids or liquids when an attenuated total reflectance (ATR) accessory is available. Although FTIR is a very useful technique for extracting structural information from pure substances [27], IR spectra of complex real matrices cannot be satisfactorily analyzed only by simple spectral matching and univariate methods. Hence, the use of multivariate statistics is necessary. A previous paper [14] has analyzed some NPS by FTIR using spectral matching and discriminant analysis. However, this work has not developed a robust model by adopting systematic criteria for selecting training and test samples, by conducting a robust validation and by performing the spectral characterization of the model. Similarly, other recent paper [28] has applied near infrared spectroscopy (NIRS) and PCA for discriminating synthetic cannabinoids from phenethylamines, but no supervised classification model was developed. Supervised classification chemometric methods, mainly partial least squares discriminant analysis (PLS-DA), are the most appropriate alternative to build robust predictive models applied to discriminate forensic samples. They should be used within an entire multivariate strategy, including proper data preprocessing,

Table 1
Number of samples in each class for the training and test sets.

Class	Training set	Test set
Main model		
NBOMe	17	8
2C-H	6	3
LSD1	7	3
LSD2	6	3
MAL	13	7
Paper	14	7
NBOMe sub-model		
25B-NBOMe	3	2
25C-NBOMe	5	3
25I-NBOMe	8	4

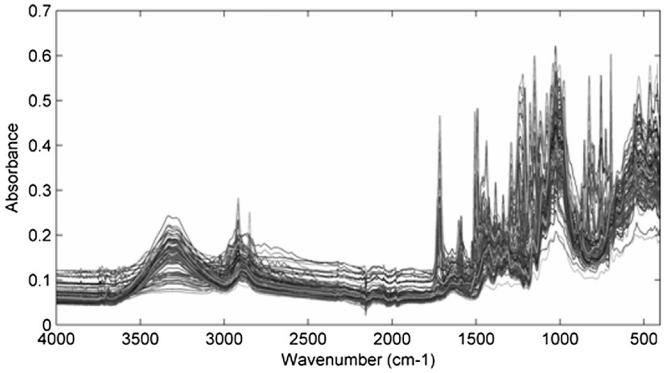

Fig. 1. ATR-FTIR spectra of 73 seized blotter samples and 21 paper samples.

representative criteria for splitting samples in training and test sets, estimate of specific figures of merit for method validation and identification of the most discriminant variables by inspecting model informative vectors. Recently, several papers have developed PLS-DA models for the discriminant analysis of forensic samples, such as drugs [29–31], explosives [32], fuels [33], soils [34], documents [35], adulterated food [36] and counterfeit beverages [37].

As quantitative methods, supervised classification methods can be submitted to a full analytical validation in order to obtain official recognition. However, multivariate qualitative validation has received only very recently attention in the literature [38–40]. Since the responses provided by qualitative methods are discrete, statistical tests and procedures used differ from the ones used in quantitative validation. The most common qualitative figures of merit (FOM) are false-positive rate (FPR), false-negative rate (FNR), sensitivity rate (SNR) and selectivity rate (SLR). The former two FOM are related to the trueness of the methods, while the latter two are related to their qualitative selectivity. All of these are easily calculated from the confusion matrix. Other more general FOM are used to evaluate trueness, such as the rate of correctly classified samples (%CC) [41] and the reliability rate (RLR) [39]. The former is calculated as the ratio between the sum of true-positives plus true-negatives and all the results, while the latter is more robust and is calculated with result rates of misclassified samples. The qualitative precision is estimated as accordance (ACC) at the repeatability level, and concordance (CON) at the level of intermediary precision or reproducibility [42]. Both of these FOM are calculated by combinatory possibilities of two concordant results.

The aim of this paper was to develop rapid and non-destructive supervised classification methods for discriminating seized blotter samples from four different classes of synthetic drugs, NBOMe, 2C-H, LSD and MAL, and blank papers, based on PLS-DA and ATR-FTIR spectra. In a hierarchical strategy, a PLS-DA submodel was built in the sequence to differentiate three specific NBOMe molecules. All the developed methods were validated through the estimate of proper FOM. In

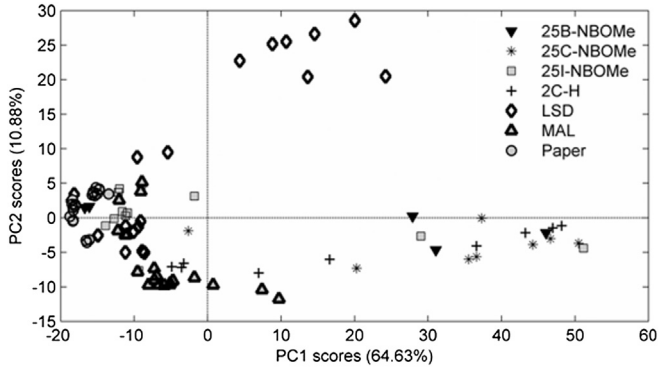

Fig. 2. PCA model including all the samples. PC1 vs. PC2 scores.