Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 189 (2018) 1–7

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Dynamic surface-enhanced Raman spectroscopy and Chemometric methods for fast detection and intelligent identification of methamphetamine and 3, 4-Methylenedioxy methamphetamine in human urine

Shizhuang Weng [a,*], Ronglu Dong [b], Zede Zhu [b], Dongyan Zhang [a], Jinling Zhao [a], Linsheng Huang [a], Dong Liang [a,*]

[a] Anhui Engineering Laboratory of Agro-Ecological Big Data, Anhui University, Hefei 230601, China
[b] Hefei Institute of Physical Science, Chinese Academy of Sciences, Hefei, 230031,China

ARTICLE INFO

Article history:
Received 9 December 2016
Received in revised form 23 May 2017
Accepted 1 August 2017
Available online 02 August 2017

Keywords:
Dynamic surface-enhanced Raman spectroscopy
Chemometric methods
Methamphetamine
3
4-methylenedioxy methamphetamine
Fast identification

ABSTRACT

Conventional Surface-Enhanced Raman Spectroscopy (SERS) for fast detection of drugs in urine on the portable Raman spectrometer remains challenges because of low sensitivity and unreliable Raman signal, and spectra process with manual intervention. Here, we develop a novel detection method of drugs in urine using chemometric methods and dynamic SERS (D-SERS) with mPEG-SH coated gold nanorods (GNRs). D-SERS combined with the uniform GNRs can obtain giant enhancement, and the signal is also of high reproducibility. On the basis of the above advantages, we obtained the spectra of urine, urine with methamphetamine (MAMP), urine with 3, 4-Methylenedioxy Methamphetamine (MDMA) using D-SERS. Simultaneously, some chemometric methods were introduced for the intelligent and automatic analysis of spectra. Firstly, the spectra at the critical state were selected through using K-means. Then, the spectra were proposed by random forest (RF) with feature selection and principal component analysis (PCA) to develop the recognition model. And the identification accuracy of model were 100%, 98.7% and 96.7%, respectively. To validate the effect in practical issue further, the drug abusers'urine samples with 0.4, 3, 30 ppm MAMP were detected using D-SERS and identified by the classification model. The high recognition accuracy of >92.0% can meet the demand of practical application. Additionally, the parameter optimization of RF classification model was simple. Compared with the general laboratory method, the detection process of urine's spectra using D-SERS only need 2 mins and 2 μL samples volume, and the identification of spectra based on chemometric methods can be finish in seconds. It is verified that the proposed approach can provide the accurate, convenient and rapid detection of drugs in urine.

1. Introduction

Drug abuse is one of the worldwide and most concerned problems in the field of public safety. Particularly, MAMP and MDMA are the most widely circulated drugs for low cost, ease of synthesis and strong addictive properties [1]. Identification of drug addict is the main mean of drug testing generally through detecting the human body fluid, such as blood, urine, saliva and so on [2,3], which the urine is the most common [4,5]. The conventional and accurate detection methods include gas chromatography with mass spectrometry [6,7], high-performance liquid chromatography [5,8] and enzyme-linked immunosorbent assay [9]. However, expensive and huge instruments, complicated pretreatment, skilled operating personnel and stable laboratory environment are needed. Additionally, it usually takes a few hours or a few days to obtain the detection result. The rapid detection method, which is authenticated by the inspection and quarantine departments of China, is the immue collideal gold technique [10]. It is a subjective color perception method and has a high probability of misjudgement [11,12]. Meanwhile, as the synthesis of corresponding material is needed for the detection objects in this method, the large reagent consumption and limited detection range are unavoidable. Consequently, to detect the drugs more conveniently and accurately, exploring a fast, simple, practical and high sensitive method is of great significance.

SERS is a vibrational spectroscopy, and it has been one of the most powerful analytical methods for the high sensitivity and unique spectroscopic fingerprint [13,14,15]. SERS is mainly applied to the detection of chemicals and biological molecule, especially for explosive particulates [16], pesticide or herbicide residues [17], molecule in livings [18] and drugs in human body fluids [2,3,4]. However, there are still some difficulties for SERS detection of drugs in human urine. Most

* Coressponding authors.
E-mail addresses: weng1989@mail.ustc.edu.cn (S. Weng), dliang@ahu.edu.cn (D. Liang).

http://dx.doi.org/10.1016/j.saa.2017.08.004
1386-1425/

importantly, the poor reproducibility of SERS, which is due to the Raman hot spots of random distribution [19,20], can cause poor uniformity of target analyte spectra. Meanwhile, as for the multiple components and low concentration of target analyte in urine, SERS signal of analyte is probably be covered up or overlapped [21]. Due to these problems, the fast analysis of drugs is not feasible in human urine using SERS directly.

Recently, we developed a novel method called dynamic SERS (D-SERS) [22,23,24], which originated from the two conventional approaches as the dry film-based and solution-based methods. Dry film-based SERS detection is such a process like this: place the general colloidal nanoparticles on a solid substrate (silicon, glass wafer), dry the sample on the substrate, and obtain the SERS spectra. Generally, it can gain the enormous SERS response accompanied with weak reproducibility and stability for fabrication of complicated substrates and damage of laser. Solution-based detection involves that the Raman probe is mixed with colloidal particles and hot spots are generated through the addition of an external agent that induces particle aggregation prior to the SERS measurement. It can hardly ensure high sensitivity for the hot spots of random distribution and low density. However, the D-SERS measurement depends on the translation-based Raman substrate state from wet to dry [22]. During the transition from the wet state to dry state, an optimal surface plasmon peak of a nanostructure that resonates sharply with excitation wavelength will certainly emerge. And the nanostructures can self-close to form hot spots driven by the solvent capillary forces. D-SERS not only produces giant Raman enhancement of at least two orders of magnitude larger than that of dried substrates but also provides more excellent repeatability. The earlier experiments have proved that D-SERS can provides reproducible, stable and sensitive SERS signals for at least 100 s [24].

Routinely, to obtain the information of analyte from the spectra, the professionals are necessary. But the manual intervention limits the application of spectroscopy on the fast detection to some extent. Meanwhile, there is tiny difference between the complex media with and without the target analyte, the useful information is hard to acquire just relying on the artificial efforts. Therefore, some powerful chemometric methods are desperately needed. Successful applications have been achieved in the analysis of food additives [25], herbicides/pesticides [26], living cell [27] and pharmaceutical manufacturing [28]. Firstly, D-SERS is measured during the process of state change, and the SERS spectra, which are used for the subsequent analysis, are obtained at the critical state. Then, the K-means clustering algorithm was adopt to select the spectra obtained at the critical state. To identify the analyte accurately and intelligently, some discriminant algorithms, such as linear discriminant analysis, artificial neural networks, random forests (RF), support vector machines (SVM) and so on, are chose to develop the classification model combined with the selected spectra. Among them, RF and SVM are superior to the others methods for the powerful learning ability (high prediction accuracy) and good generalization [29,30]. However, for SVM the normalization of training data and complex parameter optimization are still necessary, but RF do not need these treatments. Moreover, RF also exhibits some advantages: [1] the estimate of the variables importance in the classification; [2] the maintenance of accuracy even when a large proportion of the data are missing; [3] the unbiased estimate of generalization error [31]. Herein, RF was used to build the classification model for realizing the intelligent identification. Additionally, PCA is adopted to extract the main information and reduce the dimension of variables for the better identification result.

Taking advantage of D-SERS and chemometric methods, we expect to achieve fast detection of drugs in human urine with a portable spectrometer. Firstly, the mPEG-SH coated GNRS were used as the substrate, and the spectra of urine and urine with MAMP or MDMA were obtained using D-SERS method. Then the spectra were filtered by K-means, and the processed spectra were used to develop the classification model through RF. Meanwhile, combining with the importance of features, the classification models which are built with different features are compared to get the best performance one. Moreover, the PCA is adopted to improve the identification performance of the model further.

2. Experiments and Methods

2.1. Synthesis of Gold Nanorods (GNRS) and Sampling Produce

The synthesis of GNRs was prepared by the seed-mediated growth method [32]. In this paper, the mPEG-SH was used to displace CTAB from the GNRs surface because that it can induce self-assembly and prevent aggregation of GNRs and during D-SERS measurement.

Simulative samples: 50 urine samples were collected from 50 volunteers who were non-drug abusers. MAMP or MDMA of different concentration were added to the original urine for simulating the drug abusers' urine. The concentration of MAMP in urine was 50, 25, 10, 1, 0.1 ppm, and concentration of MDMA was as same as the former. Real samples: 3 drug abusers' urine was obtained from the Institute of Pharamacology and Toxicology, Academy of Military Medical Sciences, China. The urine samples contained 30, 3, 0.4 ppm MAMP, respectively.

2.2. Spectral Measurement

Spectra were measured by the conventional Raman spectrometer (Horiba Jobin-Yvon LabRAM HR800, Japan) and the portable Raman spectrometer (B&WTEK, i-Raman® Plus, USA), respectively. A diode laser of 785 nm were illuminated on the sample surface during the measurement. Urine sample and GNRs colloid were mixed adequately, and the mixture of 2 μL were dropped on the silicon chip. During the state from wet to dry, 20 spectra were obtained with the integration time of 5 s.

2.3. Random Forest and Data Analysis

Random Forest is an integrated learning algorithm based on the decision tree and bagging learning technology, which is originally developed by Leo Breiman [33]. Generally, RF contains multiple decision trees which are trained through bagging learning technology, and the final analysis results are determined by the voting output of the trees. Thus, RF was used to develop classification model for intelligent identification of drugs in human urine. Additionally, it can also evaluate the importance of features when deciding the category of samples. The high importance represents the features play a more positive and important role for the classification, and the features of low importance are insignificant or negative. Through retaining the features of high importance, the number of features is reduced, and the analysis accuracy can get a certain improvement.

Before the intelligent recognition of spectroscopy, some spectral pre-processing and feature extraction methods are also necessary. Due to the measurement of D-SERS from wet state to dry state, the obtained spectra are of obvious difference during the whole procedures. And the spectra in critical state shows the high repeatability [22,23]. K-means, which is a classical clustering algorithm based on variation, can easily divide the objects of high similarity into a group, so it is introduced to select the spectra which are measured in the critical state and avoid the negative effect of spectral nonuniformity. As the original spectra include the invalid information which can affect the subsequent analysis, PCA is adopted to eliminate invalid information and extract the main information with accumulating contribution rate up 99.9%.

Firstly, the selected spectra of 50 group urine with and without drugs (MAMP and MDMA) were directly used to develop the recognition model through RF, and the model was evaluated by cross-validation method. Then, combining the importance of features given by RF with PCA, the training data can be further processed for the model of higher identification accuracy. During the above process, the double cross validation was adopted to estimate the results. The whole data set was selectively divided into two subsets (4:6), the one was for the