Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 125 (2014) 363–374

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Rapid identification of illegal synthetic adulterants in herbal anti-diabetic medicines using near infrared spectroscopy

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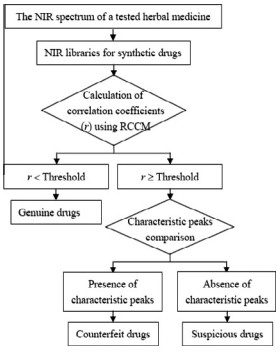
HIGHLIGHTS

- A NIR procedure to detect illicit adulterants in herbal medicines was constructed.
- This rapid, nondestructive procedure can reach an accuracy level over 80%.
- The strategy for RCCM threshold determination has been improved.

GRAPHICAL ABSTRACT

A NIR procedure for the rapid, nondestructive identification of herbal medicines containing illicit synthetic drugs was constructed during this study.



ARTICLE INFO

Article history:
Received 1 February 2013
Received in revised form 23 January 2014
Accepted 26 January 2014
Available online 8 February 2014

Keywords:
Near infrared spectroscopy
Reverse correlation coefficient method
Synthetic drugs
Adulterants
Herbal medicines

ABSTRACT

We created a rapid detection procedure for identifying herbal medicines illegally adulterated with synthetic drugs using near infrared spectroscopy. This procedure includes a reverse correlation coefficient method (RCCM) and comparison of characteristic peaks. Moreover, we made improvements to the RCCM based on new strategies for threshold settings. Any tested herbal medicine must meet two criteria to be identified with our procedure as adulterated. First, the correlation coefficient between the tested sample and the reference must be greater than the RCCM threshold. Next, the NIR spectrum of the tested sample must contain the same characteristic peaks as the reference. In this study, four pure synthetic anti-diabetic drugs (i.e., metformin, gliclazide, glibenclamide and glimepiride), 174 batches of laboratory samples and 127 batches of herbal anti-diabetic medicines were used to construct and validate the procedure. The accuracy of this procedure was greater than 80%. Our data suggest that this protocol is a rapid screening tool to identify synthetic drug adulterants in herbal medicines on the market.

Introduction

Herbal medicines are popular worldwide for their natural origins and healing properties. In contrast to conventional synthetic drugs, herbal medicines are generally perceived as safe, harmless and without deleterious side effects. However, nowadays,

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http://dx.doi.org/10.1016/j.saa.2014.01.117
1386-1425/

herbal medicines have not remained trustworthy as lots of evidences are coming into literature about adulteration of these products with synthetic drugs, in order to enhance the claims stated on the label [1]. Over the past several years, there have been various reports from Asia [2,3], Europe [4], Africa [5], USA [6] and Brazil [7], regarding this unethical trend. Such counterfeit drugs or adulterated products are commonly analyzed using high-performance liquid chromatography (HPLC) [8], mass spectrometry (MS) [9] or nuclear magnetic resonance (NMR) [10]. Recently voltammetry of microparticles [11] and high-performance thin layer chromatography (HPTLC) densitometry [12] have been used to detect these illicit drugs. Each of these techniques requires substantial time and complicated sample preprocessing, preventing opportunities for identifying and seizing such products.

Near infrared (NIR) is a powerful tool for counterfeit drug identification because it is a quick, non-destructive, and reagent-free technique [13]. Since 2002, the National Institutes for Food and Drug Control (NIFDC) has conducted a large scale study on NIR rapid drug screening and more than 300 mobile laboratory vehicles in China have equipped with NIR prescreening systems. These vehicles are used to quickly evaluate drug quality on site, in open market as well as in the distribution channel. The NIR prescreening system, utilizing a universal model, can successfully detect illegal common synthetic drugs [14,15]. Recently, we have used NIR to identify herbal medicines adulterated with synthetic drugs. Because herbal medicines are complex, NIR is more problematic for identifying adulterants. We did observe that in the NIR region of 6200–5500 cm^{-1}, the herbal medicine adulterated with sildenafil citrate had considerable similarity to the sildenafil citrate reference substance. Thus the NIR reverse correlation coefficient method (RCCM) was developed and it can detect sildenafil citrate illegally adulterated in herbal aphrodisiacs medicines accurately [16]. Moreover, Lu and co-workers [17] demonstrated infrared absorption differences between synthetic drugs which had sharp, abrupt peaks and herbal medicines that had broad, smooth peaks. Therefore, we hypothesized that NIR profiles of herbal medicines adulterated with a synthetic drug would have characteristic peaks of the corresponding synthetic adulterant. Upon validation of this hypothesis, construction of a robust NIR procedure, capable of detecting the most common synthetic adulterants in herbal medicines, is a worthwhile endeavor.

Herbal anti-diabetic medicines containing undeclared pharmaceuticals are a significant problem because patients taking these illicit products may experience potentially fatal adverse effects. Such illicit herbal medicines usually contain undeclared, registered and/or banned, oral anti-diabetic agents, including metformin, gliclazide, glibenclamide and glimepiride etc. [18]. Here herbal anti-diabetic medicines dosed with synthetic adulterants were tested to investigate the usefulness of RCCM in this application. Based on these findings, a general detection procedure for common synthetic adulterants found in herbal medicines was developed using the RCCM in tandem with NIR characteristic peaks comparisons.

Materials and methods

Sample preparation

Both genuine herbal medicines and counterfeit ones adulterated with synthetic drugs were obtained from the Chinese market by the NIFDC, the Beijing Institute for Drug Control, and the Yulin Institute for Drug Control in Guangxi Province. Most of the herbal medicines used here were capsules and the others were tablets. All samples were identified according to official laboratory methods. Here genuine herbal medicines were the samples passed the examination of its official laboratory method and counterfeit herbal medicines referred to herbal medicines adulterated with synthetic drugs. Reference substances of metformin, gliclazide, glibenclamide and glimepiride were obtained from the NIFDC.

To determine if synthetic anti-diabetic drugs had characteristic regions when adulterated into herbal medicines, five kinds of anti-diabetic herbal medicines, named Qi Lu Wen Shen Jiao Nang, Gan Lu Xiao Ke Jiao Nang, Pu Nuo Ning Si Jiao Nang, Zhong Yan Wan Tong Jiao Nang and Sang Ge Jiao Nang, were selected as blanks. These products were removed from capsules and milled into small granules before using as blanks. Laboratory samples were made by mixing given amounts of pure synthetic drugs into blank samples. See Table 1 for the composition of the laboratory samples.

Instrumentation and data acquisition

FT-NIR spectrometers, MATRIX-F, from Bruker Optik GmbH (Ettlingen, Germany) were used for all experiments. All spectrometers are equipped with a 1.5 m fiber-optic diffuse reflectance probe and an extended TE-cooled indium gallium arsenide (InGaAs) detector. Data were collected and processed using OPUS software (version 6.5).

Diffuse reflectance spectra were recorded using a 1.5 m fiber optic probe at 8 cm^{-1} resolution with 32 co-added scans over the spectral range of 4000–12,000 cm^{-1}. For capsule samples, contents were transferred into glass bottles and a fiber-optic probe was inserted to record spectra. For tablet samples, spectra were recorded via a randomly selected tablet surface scan.

Spectral pretreatment

Data pretreatment was performed to reduce the influence of physical parameters. First, the average spectrum was calculated using six original spectra from each sample. Then a second derivation was performed on the averaged spectra with 17-point smoothing followed by vector normalization, to enhance spectral information and reduce baseline drift. Only the preprocessed, averaged spectra were used in the construction and validation of these methods.

Reverse correlation coefficient method

RCCM is a new detection method for illicit synthetic adulterants in herbal medicines using NIR spectroscopy [16]. RCCM and the traditional correlation coefficient method use the same formula to calculate the correlation coefficient (r) (Eq. 1). However, significant differences exist between the two methods: RCCM uses a pure adulterant spectrum as the reference spectrum, and can detect herbal medicines from different manufacturers simultaneously, whereas the traditional correlation coefficient method requires a spectrum of a genuine sample from one manufacturer as the reference spectrum (Fig. 1). Because different manufacturers produce various products, one traditional correlation coefficient method can be only used for detecting the corresponding manufacturer's product.

$$r = \frac{Cov(y_1(k), y_2(k))}{\sigma_{y_1} \cdot \sigma_{y_2}} \quad (1)$$

$y(k)$: all Y data points, when a spectrum is presented as a data point table (X, Y), $Cov(y_1(k), y_2(k))$: the covariance of $y_1(k)$ and $y_2(k)$ and σ_y: the standard deviation of $y(k)$.

The key parameter of RCCM is the threshold. By comparing the r and a preset corresponding threshold, we could identify whether the tested herbal medicine is adulterated with synthetic drugs. The minimum effective concentration (MEC) of an adulterant was used to set the RCCM threshold (Eq. 2) as we previously reported