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Short communication

Detecting counterfeit antimalarial tablets by near-infrared spectroscopy

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ABSTRACT

Counterfeit antimalarial drugs are found in many developing countries, but it is challenging to differentiate between genuine and fakes due to their increasing sophistication. Near-infrared spectroscopy (NIRS) is a powerful tool in pharmaceutical forensics, and we tested this technique for discriminating between counterfeit and genuine artesunate antimalarial tablets. Using NIRS, we found that artesunate tablets could be identified as genuine or counterfeit with high accuracy. Multivariate classification models indicated that this discriminatory ability was based, at least partly, on the presence or absence of spectral signatures related to artesunate. This technique can be field-portable and requires little training after calibrations are developed, thus showing great promise for rapid and accurate fake detection.

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1. Introduction

Deliberate misrepresentation of medication has occurred throughout history, resulting in adverse effects, drug resistance, loss of confidence, as well as death [1]. Estimates of the proportion of counterfeit drug sales range from about 1% in developed countries to over 10% in developing countries, and possibly over 50% when medicines are purchased over the Internet [2]. Widespread infectious diseases such as malaria, as well as poor infrastructure and resources, provide fertile ground for counterfeiters in developing countries to market their fake products. Antimalarial drugs have been a particular target for counterfeiting, and of these, artesunate has been a focus. Counterfeit artesunate was first reported in 1998 in Cambodia when relatively cheap tablets were discovered and examined [3]. Subsequent studies revealed that about 50% of artesunate tablets sampled in S. E. Asia were counterfeit [1,4].

The increasing reports of counterfeit or substandard artemisinin derivative drugs, such as artesunate, in Africa, is of great public health concern as malaria control is increasingly relying on this class of medicines [5]. Suspicious packaging or inconsistent odor, taste, or shape of the drug can provide clues to its authenticity. These simple observations are rapid and economical, but are only effective if the patient is familiar with the authentic drug. Since good quality printing technology has become affordable, duplicating authentic packaging is relatively easy, but duplicating the proportions of pharmaceutical ingredients is much more problematic for the criminals. Therefore, chemical analysis of the pharmaceutical ingredients is important in determining if a product is counterfeit or genuine.

Counterfeit antimalarial drugs can be detected by technologies such as Raman spectroscopy [6], liquid chromatography–mass spectrometry (LC–MS) [7], Fourier-transform infrared spectroscopic imaging [8,9], and colorimetric assays [10]. Near-infrared spectroscopy (NIRS) was shown by Scafi and Pasquini [11] to be effective in discriminating between 27 different genuine and counterfeit drugs, but it has not yet been tested on counterfeit antimalarial tablets. Non-invasive spectroscopic techniques such as Raman and NIRS do not require the use of flammable or toxic reagents. Also, since sample preparation is not required, the product is not destroyed and sample throughput is high. The advent of portable battery-powered NIRS devices has enhanced this technique as a simple and low-cost method for quickly identifying counterfeit drugs in the field. Thus, the objective of this research was to determine if NIRS could be used to discriminate between genuine and counterfeit antimalarial tablets.

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2. Experimental

2.1. Genuine and counterfeit tablets

A set of genuine artesunate antimalarial tablets ($n = 62$) (Guilin Pharmaceutical Co. Ltd., Gulin, Peoples Republic of China, Dafra Pharma, Belgium, and Mediplantex, Hanoi, Vietnam) and counterfeit tablets ($n = 55$) were obtained from South East Asia as part of surveys of artesunate drug quality [1,4]. High-performance liquid chromatography (HPLC) and inspection of packaging were used to verify whether the tablets were genuine or counterfeit. The tablets were considered to be counterfeits if the packaging was inconsistent with known genuine samples and no measurable amount of the active ingredient, artesunate, was present [10]. For these confirmation tests, a half-tablet was analyzed and the other half was preserved for NIRS testing. The tablets were split approximately along the groove present on each one. Samples varied in chemical and physical composition, as extensively described in previous work [12]. NIR spectra of tablets were analyzed statistically with no opportunity for a priori knowledge to bias classifications.

2.2. NIRS method

Spectra were collected from individual half-tablets using a QualitySpec Pro spectrometer (ASD Inc., Boulder, CO). The spectrometer measures visible and NIR radiation from 350 to 2500 nm using silicon and indium–gallium–arsenide sensors. A Mikropack HL-2000 halogen light source (Mikropack Ostfildern, Germany) was used for illumination. All spectra were collected using a 6.3 mm diameter bifurcated probe, which has 78 fibers used for illumination and 78 fibers for collecting reflected radiation. Spectra were obtained by orienting the probe upward through a 6.3 mm diameter sleeve and towards a brass fixture that was positioned 4.8 mm above the probe tip. This brass fixture had a 4 mm diameter opening over which the smooth edge of each tablet was placed. The fixture ensured that each tablet was a uniform distance from the probe tip and that all external light was excluded. The instrument automatically optimized the sensor gain settings by analyzing the reflected energy from a 2.5 cm diameter spectralon (Labsphere, North Sutton, NH) plate positioned over the 4 mm diameter opening. A baseline was collected using the same fiber and spectralon configuration. The instrument was set to collect 20 spectra from each tablet which were then stored as an averaged spectrum. The procedure took less than 1 min per tablet, including sample positioning, data collection, and storage.

A spectrum of an artesunate standard was collected by orienting the fiber optic probe downward and positioning it 6.7 mm above the 2.5 cm dia. spectralon. The spectrometer was optimized and a baseline was collected in this configuration. The artesunate powder was then placed on the spectralon plate and a NIR spectrum collected.

ASD software RS^3 (Version 3.1) was used to collect all spectra. These were converted to GRAMS format (Thermo Galactic, Salem, NH, USA) using ASD ViewSpecPro. The Grams software PLSPlus/IQ was used to perform partial least squares (PLS) analysis on all data. For all analyses, the counterfeit tablets were assigned a value of "1" while the genuine tablets were assigned a value of "2". All spectra were mean-centered before analysis. Cross-validation was performed on the sample set, and the percent correct classification was determined for both counterfeit and genuine tablets. A tablet predicted as having a class value less than the midpoint (1.5) was considered as being counterfeit, and those with a predicted value greater than the midpoint were classed as genuine. In an alternate analysis, a classification model was developed with randomly selected tablets and used to predict an independent test set containing the remaining tablets.

The wavelengths useful in classifying the tablets as genuine or counterfeit were determined by examining the PLS regression coefficients and difference spectra. The accuracy of the classification models was determined using weighted correct classification, coefficient of determination (r^2) indicating the closeness of fit between the NIRS and reference data, and by the standard error of cross validation (SECV) using a leave-one-out procedure [13].

3. Results and discussion

A cross-validation with all tablets ($n = 117$) showed that distinction was possible between genuine or counterfeit with 100% accuracy when using five PLS latent variables (Fig. 1). This analysis only included the 700–2500 nm region and excluded visible wavelengths. The means of the predicted values were significantly different ($P < 0.05$) and 95% confidence intervals around the genuine and counterfeit predicted means did not overlap. If all wavelengths (350–2500 nm) were included in the model, the classification accuracy was slightly lower (98% correct, data not shown). Any discoloration, such as dust, on the tablet will introduce error into the classification model, and thus the visible region was excluded in subsequent analyses. To further test our ability to discriminate between genuine and counterfeit tablets, we randomly selected 40 genuine and 40 counterfeits and developed a new model with five PLS latent variables. The remaining 37 tablets were predicted as genuine or counterfeit with 100% accuracy using this calibration (data not shown).

Fig. 2 shows the PLS model regression coefficients and difference spectrum generated from all spectra and artesunate. The large positive and negative peaks in the regression coefficient plot indicate which wavelengths have larger weights in the classification model to distinguish tablets as genuine or counterfeit. The difference spectrum that was calculated by subtracting the average of the counterfeit spectra from the average of the genuine spectra shows many peaks that match features in the regression coefficient vector. This indicates that the model is classifying tablets based on unique spectral differences between the genuine and counterfeit tablets. The artesunate spectrum shows strong absorbance regions around 1200, 1360, 1700, and 2300 nm; these spectral features correspond to C–H 2nd, C–H combination, C–H stretch 1st, and C–H bend 2nd overtones, respectively [14]. These signals also agree with functional groups present in artesunate as reported by de Veij et al. [6].

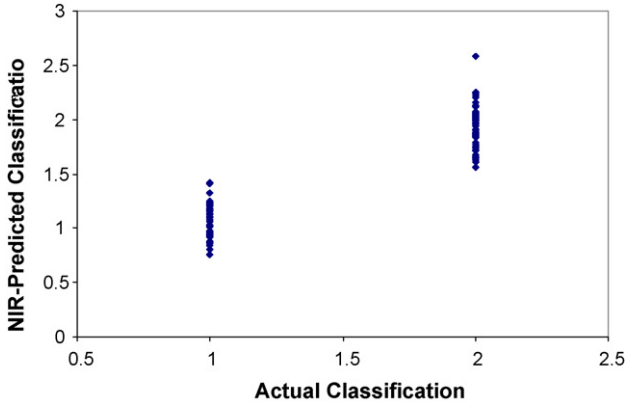

Fig. 1. Actual versus NIR-predicted classification of antimalarial tablets. Counterfeit tablets are assigned a value of "1" and genuine tables are assigned a value of "2". All tablets were correctly classed with this model that used five PLS latent variables.

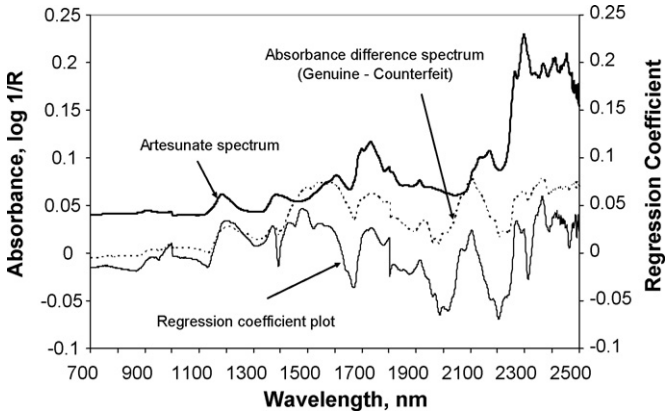

Fig. 2. Plots showing the regression coefficients used to classify antimalarial tablets as counterfeit or genuine, the difference spectrum when subtracting the average counterfeit spectrum from the average genuine spectrum, and the absorbance spectrum of pure artesunate which is the active ingredient in genuine tablets.

A comparison of the artesunate spectrum with the regression coefficient plot provides evidence that the model classifies the tablets at least partly because of the absorbance of NIR radiation by the artesunate present in the genuine tablets. The artesunate peaks around 1200, 1400, 1730, and 2300 nm correspond to large positive or negative regression coefficients at those wavelengths, indicating that the model is recognizing the absence of artesunate in the counterfeit drugs. Other wavelengths, such as those around 1940 nm, do not correspond to artesunate peaks and may be due to chemicals that are substituted for the artesunate in the counterfeit drugs [15]. Of particular interest are the large negative and positive peaks at 2314 and 2370 nm, respectively. These correspond to a strong absorbance region for calcium carbonate [13], which Hall et al. [7] showed was present in many counterfeit tablets, but is not used in genuine Guilin Pharmaceutical brand tablets.

We conducted additional statistical analyses using only the data acquired over the 700–1100 nm region, which simulates a low-cost silicon detector that could be more easily made field-portable. In a cross-validation analysis with all 117 tablets, the model was able to correctly classify all tablets when using six PLS latent variables (data not shown). The regression coefficients (Fig. 3) show intense features around the 900 and 1000 nm regions. The 900 nm region corresponds to the 3rd C–H overtone region, which agrees with the 1st and 2nd overtone regions identified above when using all wavelengths. These 3rd overtone peaks are not used by models using longer wavelengths since these peaks are much weaker than those for 1st and 2nd overtones, and are thus dominated by those stronger overtones. However, this analysis does show that this 700–1100 nm region can still be used to classify tablets with good accuracy even in the absence of absorbance of the 1st and 2nd overtones.

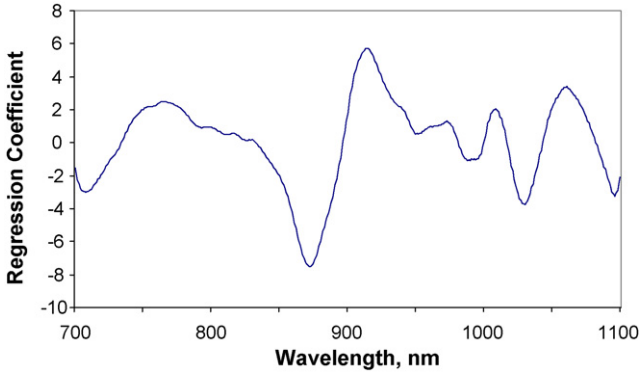

Fig. 3. Plot of the regression coefficients when using the 700–1100 nm region. All genuine and counterfeit tablets were correctly classed when using this region and six PLS latent variables.

Further work is required to examine how NIRS performs with counterfeits containing small quantities of the active ingredient and substandard medicines which, by definition, contain too little or too much active ingredient due to quality assurance faults. Further work is also needed to determine if this technique could detect counterfeits through blister packs or bottles. While this research utilized an instrument that costs about $45,000 US, additional work should include a lower cost silicon-based instrument that can be less than $5000 US. The training required to use this instrument is minimal after initial calibrations are developed, and would require only basic computer skills.

4. Conclusions

NIRS was used to identify antimalarial tablets as genuine or counterfeit with 100% accuracy when using a broad near-infrared wavelength range (700–2500 nm) or even when confining the analysis to the spectral region where the silicon detector is responsive (700–1100 nm). The classification models indicated that the discrimination was based at least partly on the presence or absence of artesunate. These results indicate that it may be possible to detect counterfeit antimalarial tablets containing no artesunate using technology that is user-friendly and field-portable.

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Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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