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Authentication of Antibiotics Using Portable Near-Infrared Spectroscopy and Multivariate Data Analysis

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Abstract

Counterfeit medicines represent a global public health threat warranting the development of accurate, rapid, and non-destructive methods for their identification. Portable near-infrared (NIR) spectroscopy offers this advantage. This work sheds light on the potential of combining NIR spectroscopy with principal component analysis (PCA) and soft independent modelling of class analogy (SIMCA) for authenticating branded and generic antibiotics. A total of 23 antibiotics were measured "nondestructively" using a portable NIR spectrometer. The antibiotics corresponded to six different active pharmaceutical ingredients being: amoxicillin trihydrate and clavulanic acid, azithromycin dihydrate, ciprofloxacin hydrochloride, doxycycline hydrochloride, and ofloxacin. NIR spectra were exported into Matlab R2018b where data analysis was applied. The results showed that the NIR spectra of the medicines showed characteristic features that corresponded to the main excipient(s). When combined with PCA, NIR spectroscopy could distinguish between branded and generic medicines and could classify medicines according to their manufacturing sources. The PCA scores showed the distinct clusters corresponding to each group of antibiotics, whereas the loadings indicated which spectral features were significant. SIMCA provided more accurate classification over PCA for all antibiotics except ciprofloxacin which products shared many overlapping excipients. In summary, the findings of the study demonstrated the feasibility of portable NIR as an initial method for screening antibiotics.

Keywords

Counterfeit medicines, antibiotics, near-infrared spectroscopy, principal component analysis, soft independent modelling of class analogy

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Introduction

Medicine counterfeiting represents a global expanding problem with increased morbidity and mortality worldwide. The impact of counterfeit medicines can result in lethal consequences in its worst. A counterfeit medicine is defined by the World Health Organization (WHO) as "deliberately/fraudulently misrepresent their identity, composition or source".[1] A substandard medicine is also known as poor quality medicine that fail to satisfy its manufacturing specifications.[1–3]

Medicine counterfeiting can occur to any class of medicines, of any formulation and of any source. Antibiotics represent one of the main classes of medicines sold in both developed and developing countries, and thus have high probability of being substandard or counterfeited.[4–8] Counterfeit and substandard antibiotics may not be limited to the lack of active pharmaceutical ingredients (APIs) but also may have defects in their excipients' constituents or in their physical characteristics. The consequences of using counterfeit antibiotics can range from decreased efficacy,[9,10] treatment failure,[11–14] antimicrobial resistance development,[5,15] and/or lethal consequences.[10,15,16]

The literature revealed various methods for antibiotics authentication. These methods range from simple color tests to mass spectrometric methods. Color tests and thin layer chromatography have been used for detecting

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macrolides,[17] amoxicillin and co-trimoxazole,[18] and fluoroquinolones.[19] Likewise, inexpensive test cards were used for the determination of beta-lactam antibiotics.[20] Color tests were also used alongside both the dissolution testing and the Global Pharma Health Fund (GPHF) Minilab for screening of specific classes of antibiotics such as amoxicillin and co-trimoxazole,[18] and/or multiple classes.[21,22] More sophisticated techniques used for analysis of counterfeit and substances antibiotics included high performance liquid chromatography,[18,23–25] ultra-high performance liquid chromatography,[26] liquid chromatography mass spectrometry,[27] and capillary electrophoresis.[28]

However, all the aforementioned techniques were destructive to the samples analyzed and/or required extensive method development. Portable near-infrared spectroscopy (NIRS) offers an advantage over the previous mentioned techniques in being rapid, mobile, and non-destructive. NIRS offers a further advantage over alternative chemical techniques in being able to characterize the physical properties alongside the chemical characteristics of the samples analyzed. Limited studies utilized NIRS for authenticating antibiotics such as ciprofloxacin,[29,30] fluoroquinolones,[31] and macrolides.[32] However, the three aforementioned studies focused on one class of antibiotics and utilized one multivariate data analysis algorithm at a time. Thus, there is still a need to look at a collective method that can authenticate diverse classes of antibiotics synchronously. This work aimed to evaluate NIRS and multivariate classification algorithms for authentication of antibiotics purchased worldwide.

Theory

Spectral Pre-Treatment

The multiplicative scatter correction-first derivative (MSC-D1) spectral pre-treatment approach was applied in order to correct for the offset and baseline in the spectra that changes depending on several factors including the sample age, thickness and optical properties, temperature, moisture content, and performance of the instrument.[33,34] MSC corrected the offset of the scattered light by construction of a new spectrum that is a linear combination of the original spectrum according to Eq. 1:[35,36]

$$y_{MSC,i} = \frac{(y_i - a)}{b} \quad (1)$$

where $y_{MSC,i}$ is the corrected spectrum value, y_i is the original spectrum value, a is the intercept of the line, and b is the slope of the line.

The first derivative corrected both for the offset and baseline of the NIR spectra by using Savitzky–Golay method where a second-order polynomial was fitted to the data by least square using 13 data points.[35]

Correlation in Wavenumber Space

The correlation in wavenumber space (CWS) method matched the correlation coefficient (r) value of the test spectrum (A) and a reference spectrum (B). It was calculated as the momentum product (r_p) between both spectra according to Eq. 2[35,37]

$$r_p = \frac{\sum (A_i - \bar{A})(B_i - \bar{B})}{\sqrt{\sum (A_i - \bar{A})^2 (B_i - \bar{B})^2}} \quad (2)$$

where r_p is the correlation coefficient value, A_i is the test spectrum of A, $\bar{A}$ is the average spectrum of A, Bi is the reference spectrum of B, and $\bar{B}$ is the average spectrum of B.

An r value of −1 meant that the spectra were completely dissimilar whereas an r value of +1 meant that the spectra were identical. In this work, an r value of 0.95 was taken as a match among products because it was difficult to get +1 among identical samples due to noise in the spectra.[35,37] For the evaluation of CWS method, type I and type II errors were explored.[30] Type I errors (known as false positives) were encountered when an authentic antibiotic was misidentified by the algorithm (i.e., gave r values < 0.95). On the other hand, type II errors (known as false negatives) were encountered when a counterfeit sample was identified as authentic (i.e., gave r values > 0.95).

Principal Component Analysis

Principal component analysis (PCA) classified spectral data by reducing its dimensionality into two subspaces being scores and loadings. The scores showed the distribution of the antibiotics in multidimensional space and the loadings showed significant absorbance values corresponding to the significant constituents (influencers) within the models. PCA was applied to the MSC-D1 NIR spectra of the products in order to visualize patterns on classification among the products. As with CWS method, PCA was evaluated for type I and type II errors.[30] In this case, type I error was encountered when an authentic antibiotic was not clustered with authentic antibiotics. Moreover, a type II error was encountered when a counterfeit antibiotic was clustered with the authentic ones.

Soft Independent Modelling of Class Analogy

Soft independent modelling of class analogy, or SIMCA, is a chemometric approach, based on PCA, which models the variation within the collection of reference spectra for a given material, as well as the difference between spectra of different materials.[38] This allows SIMCA to be sensitive to small spectral differences, even batch-to-batch or sampling variations. New samples can then be classified to one (or none) of the established class models, based on their similarity to the respective model. This is achieved by investigating the size of its residual, as well as its location on the scores map.

Materials and Methods

Materials

A total of 23 antibiotic products containing six different APIs were used in this study (Table I; Figs. S1 to S25, Supplemental Material). The APIs of the antibiotics included: amoxicillin trihydrate and clavulanic acid, azithromycin dehydrate, ciprofloxacin hydrochloride, doxycycline hydrochloride, and ofloxacin. The antibiotic products were obtained from 11 different countries: Austria, France, Germany, Ghana, India, Italy, Jordan, Lebanon, Spain, United Arab Emirates (UAE), and the United Kingdom (UK). The products were either tablets or capsules and included both branded and generic medicines. Regarding the excipients, 19 products had between 7 and 10 excipients each (Table II). The excipients of the remaining four products were not reported. In total, 29 excipients were present in at least one or more products (Figs. S26 to S32). The recurrent excipients were: hypromellose, magnesium stearate, maize starch, and titanium dioxide.

Near-Infrared Spectroscopic Analysis

Near-infrared spectra of antibiotic products and their individual constituents were collected using the Spectrum Two N Fourier transform (FT)-NIR (PerkinElmer) instrument equipped with NIR reflectance module (NIRM). Tablet formulations were measured as received from both sides. The contents of each capsule formulation were emptied into glass vials and were measured through the vials. Likewise, excipients were powders and were measured using glass vials. Two spectra were collected per each tablet and three spectra per each vial over the wavenumber range of 10 000–4000 cm^{-1} with spectral resolution of 8 cm^{-1}. Each spectrum was the sum of 32 scans.

Data Analysis

Spectra were exported into Matlab R2018b where data pre-treatment was applied. Pre-treatment of NIR spectra was made using MSC-D1. Multivariate data analysis was conducted using CWS, PCA, and SIMCA methods. CWS was applied to the MSC-D1 NIR spectra in Matlab R2018b where the R values of products were compared and an R value of 0.95 was considered a threshold. PCA was applied in Matlab R2018b where clustering among antibiotics was evaluated. SIMCA analysis was carried out using PerkinElmer AssureID materials verification software to create five PCA models of the antibiotic products. A global PCA of all materials was also created to provide an overview of the complete model and understand relationships between material types. The threshold taken for intermaterial distances was 1.5 where a distance below 1.5 was considered a similarity.

Results and Discussion

Diversity of the Sample Set Relating to the APIs and Excipients

In order to evaluate the identification potential of the method, 23 antibiotic products relating to 5 APIs were chosen. The products were of both branded and generic types, of tablet and/or capsule formulations and were obtained from different sources across the wholesale supply chain including community pharmacies, hospital pharmacies, humanitarian aid supply, online pharmacies, street market, and wholesalers (Table I). The APIs of the evaluated products were amoxicillin trihydrate and clavulanic acid (AMC), azithromycin dehydrate (AZ), ciprofloxacin hydrochloride (CIP), doxycycline hydrochloride (DOX), and ofloxacin (OFL). The numbers of products per antibiotic varied between 2 and 12 products for each API depending on availability and were: 2 for each of DOX and OFL, 3 for AMC, 4 for AZ, and 12 for CIP. In some cases, the aforementioned products had overlapping excipients (Table II). Excipients were always reported for branded but not generic products. Where reported, the minimum number of excipients per product was six and the maximum was 10. However, in most cases, the main excipients were consistent among products of the same API. For instance, AMC products (AMC1, AMC2, and AMC3) were from three different manufacturers in Lebanon, Spain and, the UK and had overlapping excipients being: Hypromellose, microcrystalline cellulose (MCC), magnesium stearate (MgS), and titanium dioxide. Likewise, OFL products (OFL1 and OFL2) were from two different manufacturers and had six common excipients being: croscarmellose sodium, hypromellose, lactose, maize starch, MCC, and titanium dioxide. CIP branded products (CIP1-CIP5) were all from the same manufacturer and had the same list of excipients. Three generic CIP products (CIP7, CIP8, and CIP9) had common excipients as branded CIP products being: crospovidone, colloidal anhydrous silica, hypromellose, macrogol 4000, maize starch, MgS, MCC, and titanium dioxide. On the other hand, AZ products (AZ1, AZ2, AZ3, and AZ4) were manufactured by two manufacturers and showed different excipients between both manufacturers. Moreover, CIP11 and CIP12 had different list of excipients to the other CIP products. The excipients were not reported for CIP6, CIP10, DOX1, and DOX2 that were manufactured by generic manufacturers.

Spectral Evaluation

The spectra of the antibiotic products showed characteristics for their main excipients that were keys in identifying the products using NIRS (Figs. S33 to S37). Hence, NIRS offered the advantage of giving more information on the samples' constituents including the API and excipients. Thus, it could serve as a fingerprinting in spectral identification.[39] This was

Table 1. Details of the antibiotics used in this study.

AN	API	Dose (mg)	B/G	Manufacturing place	Source	Formulation Type
AMC1	Amoxicillin trihydrate/clavulanic acid	500/125	B	UK	Lebanon/Community pharmacy	Tablet
AMC2	amoxicillin trihydrate/clavulanic acid	500/125	G	Lebanon	Lebanon/Humanitarian aid	Tablet
AMC3	Amoxicillin trihydrate/clavulanic acid	500/125	G	Spain	Lebanon/Humanitarian aid	Tablet
AZ1	Azithromycin dihydrate	250	G	UK	UK/wholesaler	Tablet
AZ2	Azithromycin dihydrate	250	B	Italy	Italy/wholesaler	Tablet
AZ3	Azithromycin dihydrate	250	B	Italy	Italy/wholesaler	Capsule
AZ4	Azithromycin dihydrate	250	B	Italy	Italy/wholesaler	Capsule
CIP1	Ciprofloxacin hydrochloride	500	B	Germany	UK/wholesaler	Tablet
CIP2	Ciprofloxacin hydrochloride	500	B	Germany	UK/wholesaler	Tablet
CIP3	Ciprofloxacin hydrochloride	750	B	Germany	UK/online pharmacy	Tablet
CIP4	Ciprofloxacin hydrochloride	500	B	Germany	UK/online pharmacy	Tablet
CIP5	Ciprofloxacin hydrochloride	250	B	Germany	UK/online pharmacy	Tablet
CIP6	Ciprofloxacin hydrochloride	500	G	Ghana	Ghana/street market	Tablet
CIP7	Ciprofloxacin hydrochloride	500	G	UAE	Saudi Arabia/hospital pharmacy	Tablet
CIP8	Ciprofloxacin hydrochloride	500	G	India	UK/wholesaler	Tablet
CIP9	Ciprofloxacin hydrochloride	500	G	UK	UK/community pharmacy	Tablet
CIP10	Ciprofloxacin hydrochloride	250	G	India	Lebanon/Humanitarian aid	Tablet
CIP11	Ciprofloxacin hydrochloride	500	G	UK	UK/community pharmacy	Tablet
CIP12	Ciprofloxacin hydrochloride	500	G	UK	UK/community pharmacy	Tablet
DOX1	Doxycycline hydrochloride	100	G	Jordan	Lebanon/Humanitarian aid	Capsule
DOX2	Doxycycline hydrochloride	100	G	Austria	Lebanon/community pharmacy	Capsule
OFL1	Ofloxacin	200	G	UK	UK/wholesaler	Tablet
OFL2	Ofloxacin	200	B	France	UK/wholesaler	Tablet

AM: amoxicillin; AN: antibiotic number; API: active pharmaceutical ingredient; AZ: azithromycin; B: branded; G: generic; CIP: ciprofloxacin; DOX: doxycycline; OFL: ofloxacin; UAE: United Arab Emirates

confirmed when the branded medicine of each antibiotic was compared against its main excipient (Fig. 1). However, the matching degree depended on the amount of API or excipients in the product. OFL1 showed spectral similarity for MCC and maize starch with correlation coefficient (r) values of 0.73 and 0.69, respectively, that confirmed that these excipients were present in adequate amounts. Likewise, DOX1 showed spectral similarity for MCC and

Table II. List of excipients studied in the investigated antibiotics.

Excipient/AN	AMC1	AMC2	AMC3	AZ1	AZ2	AZ3	AZ4	CIP1	CIP2	CIP3	CIP4	CIP5	CIP6	CIP7	CIP8	CIP9	CIP10	CIP11	CIP12	DOX1	DOX2	OFL1	OFL2
Butyl hydroxy toluene				■																			
Calcium hydrogen phosphate				■																			
Carmellose NS300																							■
Colloidal silicon dioxide	■	■	■	■				■	■	■	■	■		■	■	■						■	
Croscarmellose sodium		■	■													■						■	
Crospovidone		■	■					■	■	■	■	■		■		■		■	■				
Dimethicone	■																						
Ethanol 96%														■									
Ethyl cellulose		■																					
Gelatin					■	■	■																
Hyprolose																							■
Hypromellose	■	■	■					■	■	■	■	■		■	■	■		■	■			■	■
Lactose monohydrate					■	■	■											■	■			■	■
Macrogol 3000																						■	
Macrogol 4000	■							■	■	■	■	■		■	■	■		■	■				
Macrogol 6000	■																						
Macrogol 8000																							■
Maize starch					■	■	■	■	■	■	■	■			■							■	■
MCC	■	■	■					■	■	■	■	■		■	■	■							
MgS	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■						■	■
Propylene glycol			■																				
Sodium citrate																							
Sodium lauryl sulfate			■	■	■	■	■																
Sodium starch glycolate	■		■											■	■			■	■				
Sodium stearyl fumarate																		■	■				
Talc		■	■	■																			■
Titanium dioxide	■	■	■		■	■	■	■	■	■	■	■		■	■	■		■	■			■	■
Triacetin																						■	
Triethyl citrate		■																					
Total number of excipients	9	10	10	6	6	6	6	8	8	8	8	8	NR	9	8	8	NR	7	7	NR	NR	9	9

AMC: amoxicillin/clavulanic acid; AN: antibiotic number; AZ: azithromycin; CIP: ciprofloxacin; DOX: doxycycline; MgS: magnesium stearate; MCC: microcrystalline cellulose; NR: not reported; OFL: ofloxacin

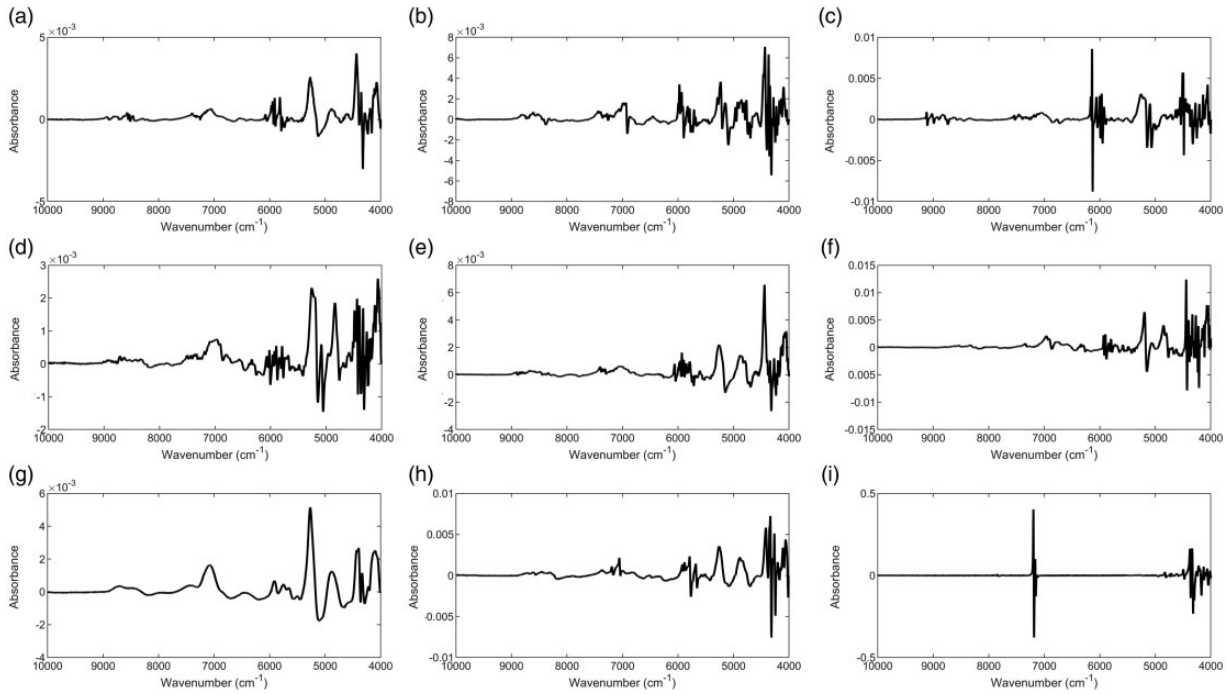

Figure 1. MSC-D1 NIR spectra of (a) amoxicillin/clavulanic acid, (b) azithromycin, (c) ciprofloxacin, (d) doxycycline, (e) ofloxacin branded antibiotic products and their main excipients including (f) lactose, (g) maize starch, (h) MCC, and (i) talc measured using the PerkinElmer Spectrum Two N FT-NIR instrument equipped with NIRM.

maize starch with r values of 0.71 and 0.75, respectively. However, excipients that were present in low amounts within a tablet did not show peaks in the NIR spectra of the tablets. For instance, talc was present in OFL1 but no characteristic peak for it was seen within its spectra.

Authentication of Branded Antibiotic Products

Principal component analysis was successful in showing the chemical variation between different antibiotics. The PCA model showed good classification following MSC-D1 treatment of the NIR spectra of the products. The first three PCs contributed to 89.2% of the variance with 76.4% of the variance explained by PC1 and PC2. Figure 2 shows the 2D and 3D scores plots of AMC1, AZ1, CIP1, DOX1, and OFL1. A distinct cluster was observed for each antibiotic product and that showed the effectiveness of PCA in differentiating between the five authentic products (Fig. 2). The highest variance on PC1 was observed for the CIP1 cluster. This was followed by the clusters corresponding to AMC1, DOX1, and OFL1 that were neighboring each other. AMC1 and OFL1 contained around 50% of API and 50% of excipients. Two excipients were common among both products and were MgS and hypromellose. This also could indicate that DOX had similar excipients to AMC and OFL. To interpret the influences of individual constituents on antibiotic products, PC loading plots were visualized. Figure 3 shows the PC1 loading plot of the different antibiotic PCA model that corresponded to 51.2% of the variance. The aforementioned PC1 loading showed contribution over the wavenumber ranges of 9172–8124 cm^{-1}, 7572–6502 cm^{-1}, 6260–5632 cm^{-1}, 5340–4880 cm^{-1}, and 4752–4016 cm^{-1}. The aforementioned five regions showed spectral features corresponding to MgS, ciprofloxacin and MCC, ciprofloxacin and lactose, amoxicillin, and ciprofloxacin (Figs. S33 to S37). This suggested that the five antibiotic products could be principally separated on the basis of differences in their APIs and excipients.

Taking the aforementioned model forward, the next step was to classify the branded and generic medicines for each antibiotic and look into tracking their manufacturing sources (Figs. 4 and 5). The discriminative capability of PCA depended on sample size and sample type.[37] For both AMC and AZ products, two distinct clusters were seen between the branded and generic products (Figs. 4a and 4b). AMC1, AMC2, and AMC3 showed three distinct clusters that confirmed their three distinct manufacturing sources being the UK, Lebanon, and Spain. The PC1 loading (95.2% of the variance) showed characteristic features for amoxicillin, MCC, and talc. Amoxicillin spectral features were seen in the regions of 8910–8378 cm^{-1}, 6178–5636 cm^{-1}, and 5334–5082 cm^{-1}. Talc spectral features were featured at 7318–6992 cm^{-1}, whereas MCC spectral features were seen at 4550–4000 cm^{-1}. Moreover, the PCA

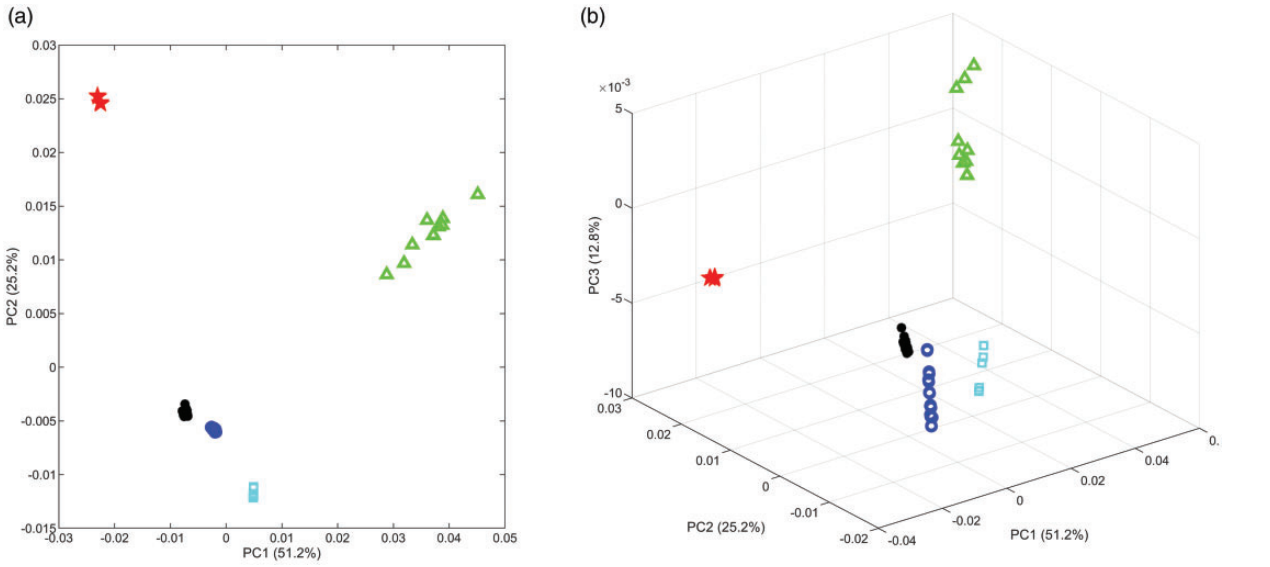

Figure 2. (a) Two-dimensional and (b) three-dimensional PCA scores plots of the MSC-D1 NIR spectra of branded antibiotic products of amoxicillin/clavulanic acid (blue), azithromycin (red), ciprofloxacin (green), doxycycline (cyan), and ofloxacin (black) measured using the PerkinElmer Spectrum Two N FT-NIR instrument equipped with NIRM.

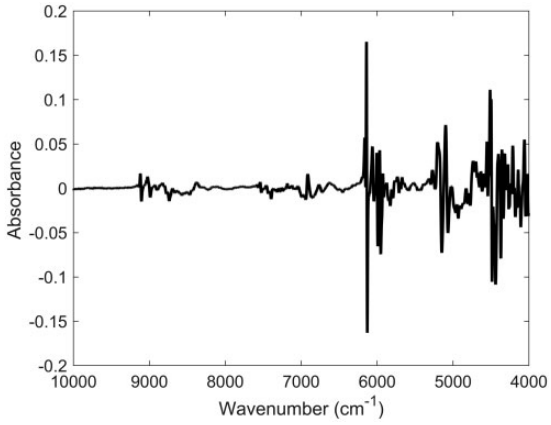

Figure 3. PC1 loading plot of the different brands that contributed to 51.2% of the variance among the data.

scores plot of AZ showed three distinct clusters that corresponded to both their manufacturing sources and formulation type. In this respect, AZ3 and AZ4 products were clustered together where both products were capsules and manufactured by the same manufacturer. Two distinct clusters were seen for AZ1 and AZ2 which were both of tablet formulation but manufactured by two different manufacturers. It is noteworthy to mention here that AZ2 had the same manufacturer as AZ3 and AZ4 but was of tablet instead of capsule formulation. This confirmed the ability of NIR to distinguish physical differences between samples of the same chemical makeup.[40] The PC1 loading plot of AZ products (75.2% of the variance) showed characteristic peak between 7270–7138 cm^{-1}, which corresponding to talc that was an excipient in AZ1 (of tablet formulation). Additional spectral features in the PC1 loading plot were seen in the regions of 8804–8350 cm^{-1}, 7074–6800 cm^{-1}, 6584–6290 cm^{-1}, 6064–5646 cm^{-1}, 5334–5004 cm^{-1}, 4984–4668 cm^{-1}, and 4550–4668 cm^{-1}. The aforementioned seven regions corresponded to lactose. DOX products scores plot showed type I error in the cluster of one product (Fig. 4c). Hence, DOX1 and DOX2 products were separated in three clusters (instead of two) where DOX1 was separated in two distinct clusters. The PC1 loading of DOX products (90.7% of the variance) showed characteristic features for talc in the region of 7242–7088 cm^{-1}. Other features for this PC1 loading were seen in the region of 6156–5670 cm^{-1}, 5348–4750 cm^{-1}, and 4650–4000 cm^{-1}. The aforementioned three regions corresponded to lactose and MCC. Nonetheless, OFL1 and OFL2 products were clustered into two distinct clusters that corresponded to their manufacturing sources being the UK and France, respectively (Fig. 4d). However, type I error was encountered in this latter PCA score plot where both products had outlier(s) within their score plot. The PC1 loading (82.9% of the variance) of OFL products showed characteristic spectral features for talc in the region of 7246–7136 cm^{-1}. Additional peaks were seen in the regions of 6170–5598 cm^{-1}, 5312–5124 cm^{-1}, and 4752–4000 cm^{-1}. The aforementioned three regions corresponded to lactose.

In addition to identifying manufacturing source and discriminating branded from generic medicines, the potential for NIR and PCA for spotting a potential counterfeit product

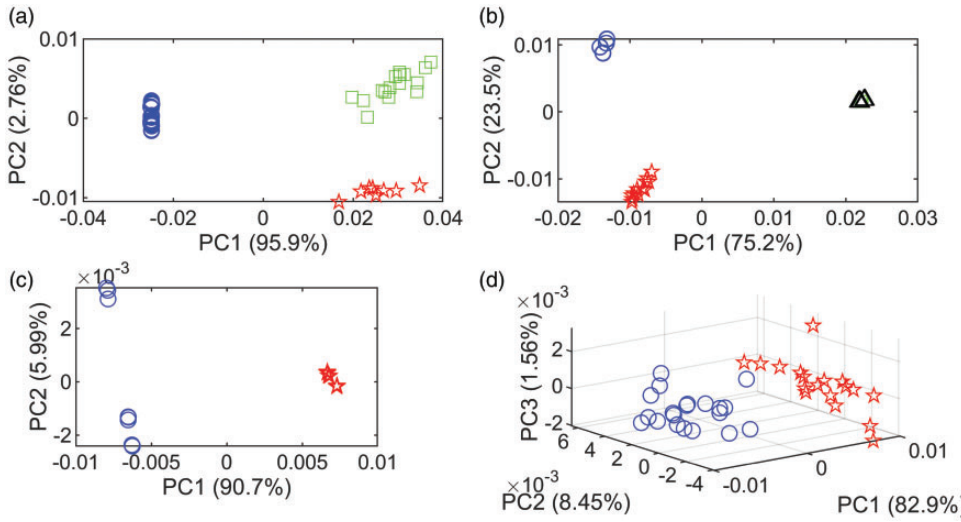

Figure 4. PCA scores plots of the MSC-D1 spectra of antibiotics products including (a) amoxicillin/clavulanic acid, (b) azithromycin, (c) doxycycline, and (d) ofloxacin measured using the PerkinElmer Spectrum Two N FT-NIR instrument equipped with NIRM. The first three PCA scores plots were two-dimensional, whereas the latter score plot was three-dimensional.

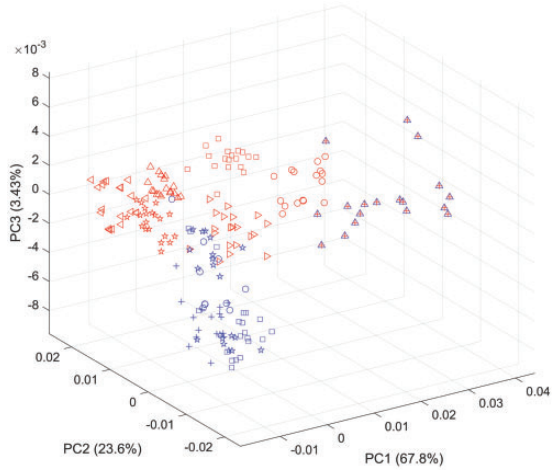

Figure 5. PCA scores plot of the MSC-D1 NIR spectra of branded (blue) and generic (red) ciprofloxacin batches measured using the PerkinElmer Spectrum Two N FT-NIR instrument equipped with NIRM.

was demonstrated through the PCA scores plot of CIP products (Fig. 5). In this sense, the PCA score of a CIP branded product (CIP5) overlapped with one of the generic products. In order to address this overlap, the PC1 loading (67.8% of the variance) of the CIP products had been examined and had shown a major influence of 7260–7150 cm^{-1} that is characteristic for talc.[41] It is noteworthy to mention in this case that talc was not listed in the label claim of any of the branded products. Talc had been found in counterfeit antibiotics as it is cheap and increases the bulk of the medicine.[20,42] Therefore, CIP5 did not match the manufacturers' specification relating to the identity and could be counterfeit[30]

Development of SIMCA Classification Models

To further address the type I error encountered with PCA, PCA was taken forward and SIMCA models were constructed. The first SIMCA model showed agreement with PCA Model 1. Hence, distinct classification of the five branded products was observed with no overlapping materials. SIMCA provided a further advantage over PCA in detecting type I and type II errors in the classification of different products.[43] In this respect, the distances between the five products were calculated and were found above zero and this showed no type I or type II errors (Table III). Hence, Table III shows all distances above the threshold that was 1.5. Successively, individual SIMCA models were applied to each antibiotic (Fig. 6). For AMC products, the global PCA showed three distinct PCs for AMC1, AMC2, and AMC3 that confirmed their different manufacturing sources. The four AZ products showed three distinct clusters: one corresponding to AZ1, second to AZ2, and the third to AZ3 and AZ4. AZ3 and AZ4 were of the same formulation (both capsules) and had the same manufacturer but purchased in different countries; therefore, SIMCA was further successful in detecting differences in manufacturing sources and formulation. On the other hand, misclassification was observed among CIP branded and generic products where no clear clustering was observed between both groups of products. Two products were misclassified and seen as two distinct clusters (CIP 6 and CIP 10) and that denoted type I error. Moreover, the aforementioned model

Table III. Intermaterial distances explained by the SIMCA models.

Material	Doxycycline	Ofloxacin	Ciprofloxacin	Amoxycillin	Azithromycin
Doxycycline	–	24	30.5	28.2	28.7
Ofloxacin	24	–	28.1	19.3	16.3
Ciprofloxacin	30.5	28.1	–	25.4	34.5
Amoxicillin	28.2	19.3	25.4	–	23.5
Azithromycin	28.7	16.3	34.5	23.5	–

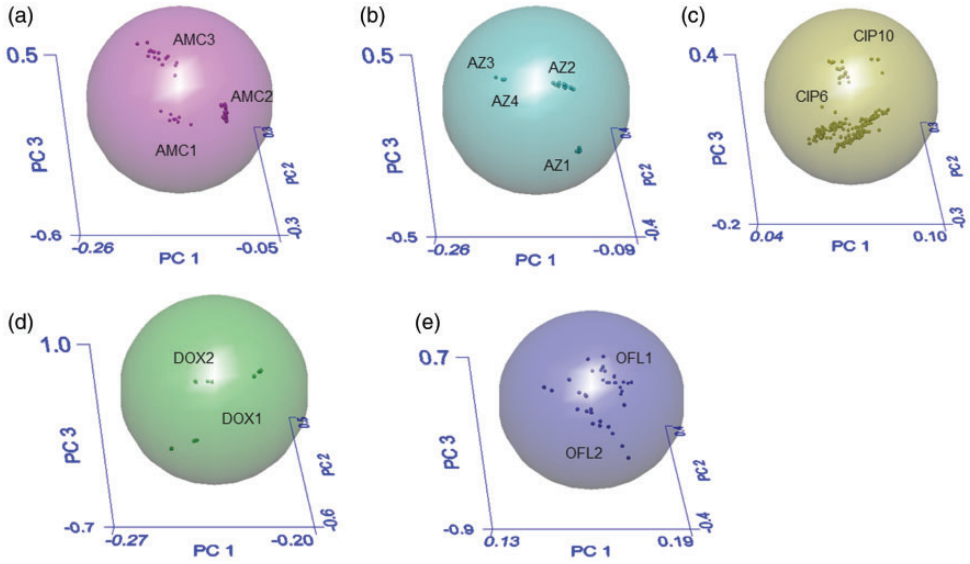

Figure 6. SIMCA models of the MSC-D1 spectra of antibiotics products including (a) amoxicillin/clavulanic acid, (b) azithromycin, (c) ciprofloxacin, (d) doxycycline, and (e) ofloxacin measured using the PerkinElmer Spectrum Two N FT-NIR instrument equipped with NIRM.

could not distinguish the counterfeit CIP batch (CIP 5) that indicated type II error. Likewise, type I error was observed for DOX global PCA where DOX1 was scattered in two distinct clusters. On the other hand, OFL1 and OFL2 products were separated between two individual clusters that corresponded to their different manufacturing sources.

Conclusion

The findings of the study demonstrated the effectiveness of portable NIRS and chemometrics as a tool in authenticating antibiotics. The combination of NIRS with PCA and SIMCA proved to be efficient in discriminating branded from generic medicines and in tracking the manufacturing sources of medicines. Moreover, the algorithms could give initial indication for the presence of a potential counterfeit. However, some limitations were encountered in this study. The first limitation related to sample size and sourcing of the samples that had been a challenge especially that the medicines had been sought from different countries. The second limitation related to the precision of classifying authentic products particularly with large datasets with overlapping excipients such as CIP. Other limitations were associated with the sensitivity of NIRS for characterizing constituents where constituents with low amounts in a medicine will not show spectral features. In summary, portable NIRS could serve as an initial screening method for authentication of antibiotics saving time and money associated with importing the samples to the laboratory. However, for identity confirmation of the API in antibiotics, more quantitative techniques are needed.

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Supplemental Material

All supplemental material mentioned in the text including spectra of APIs, excipients, antibiotics and PCA loading are available in the online version of the journal.

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