Evaluating handheld spectroscopic techniques for identifying counterfeit branded and generic medicines worldwide

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Abstract

Counterfeit medicines represent a global public health problem which accounts for 10% of the world market including 50% in some countries. Medicine counterfeiting can occur to any class of medicines, any type of formulation and can be encountered anywhere in the world. Consequently, rapid methods are needed to identify counterfeit medicines at their site of origin. Handheld spectroscopic techniques offer this advantage.

This work features the use of near-infrared (NIR) and Raman spectroscopic methods for identification of counterfeit medicines obtained worldwide.

A total of 300 branded and generic medicines were measured using five spectroscopic instruments; being two NIR and three Raman (of different laser wavelength). Spectra obtained from these instruments were exported into Matlab v2014b where multivariate classification and regression algorithms were applied. The results showed that the selection of the technique depended on the type of medicine used. Thus, NIR was more successful in authenticating branded medicines where the physicochemical properties were of interest. On the other hand, Raman was ideal for authenticating generic medicines where the chemical signature of the API and/or excipient(s) were the subject of analyses. Furthermore, where adequate number of batches were available, the application of multivariate algorithms offered more accurate classification of the medicines.

In summary, both techniques alongside multivariate algorithms proposed rapid methods for identifying counterfeit branded and generic medicines worldwide.

Introduction

According to the World Health Organisation (WHO), counterfeit medicines are those which ‘are fraudulently and deliberately mislabelled according to identity or source’ [1]. Medicine counterfeiting represent a global health problem and account for around 10% of the world market; including 50% in some countries [2, 3]. The public health effects attributed to counterfeit medicines could range from treatment ineffectiveness to lethal effects. For example, counterfeit paracetamol containing diethylene glycol (a renal toxin) was attributed to the death of more than 500 children [4-6]. However, other long term effects can occur due to the use of counterfeit medicines such as drug resistance and epidemics [7].

Medicine counterfeiting can occur to any pharmacological class, any formulation type and to both branded and generic medicines. Hence, it can occur to both life-style and life-saving (antibiotics, anticancer) products. Life-style products are those intended to improve the image and/or performance such as medicines used for blood pressure, erectile dysfunction and hypercholesterolemia. Moreover, life-saving products are those used for serious conditions such as infection, cancer and AIDS. All the aforementioned products could be branded or generic medicines. It is noteworthy to mention that the branded medicine is the innovator product and patent owner; whereas the generic medicine is the interchangeable product marketed after the expiry of the patent [8]. The defects in counterfeit medicines could be attributed to wrong packaging, coating, active pharmaceutical ingredient (API) and/or excipients [9]. According to the WHO, 60% of counterfeit medicines contain no API, 17% contain too much or too little API and 16% contain wrong constituents [10].

Counterfeit medicines could be encountered anywhere across the wholesale supply chain in hospitals, industries, Internet, manufacturers, pharmacies, patient homes, retailers, street markets or with wholesalers. Furthermore, they can be found in any country or over the Internet. Thus, rapid, mobile and non-destructive methods are needed for identification of counterfeit medicines.

Handheld near-infrared and Raman spectroscopic techniques offer this advantage [11, 12] as they can give the required information at the site of analysis and require no sample preparation. Subsequently, both handheld NIR and Raman were used for identifying counterfeit life-style and life-saving medicines [11-23]. In this respect, two options were encountered with the use of handheld spectroscopic instruments; being

in-built identification algorithms or offline analysis. The inbuilt identification algorithms were quick and can give answers instantaneously yet less accurate option than offline analysis. Subsequently, the use of one or more multivariate algorithms offline offered more accurate identification [12].

Therefore, this study highlights the combination of NIR and Raman spectroscopic techniques with multiple spectral algorithms for the identification of counterfeit branded and generic medicines obtained from different countries worldwide.

Methods

A total of 300 branded and generic medicines obtained from 41 countries worldwide were used in this study. The corresponding APIs and excipients for these medicines were purchased from chemical suppliers.

NIR spectra of these medicines were collected using both a palm-sized and handheld NIR spectrometers over the wavelength ranges of 1600-2400 nm and 950-1650 nm respectively. Raman spectroscopy of medicines were collected using three handheld instruments with different laser wavelengths: 785 nm, 1064 nm and a dual laser. The wavenumber ranges used for the aforementioned three instruments were 250-2000 cm^{-1}, 250-2000cm^{-1} and 300-3200 cm^{-1}.

Using both NIR and Raman, tablets were measured as received from both sides. Capsule content, powders, creams and liquids were measured through transparent glass vials. For data analysis, NIR and Raman spectra were exported in Matlab v2014b where spectral pre-treatment and treatment were conducted. Spectral pre-treatment made for NIR spectra using standard normal variate and first derivatives (SNV-D1). Spectral treatment was made using multivariate classification and quantification algorithms, being: correlation in wavelength/wavenumber space (CWS), distance method, principal component analysis (PCA) and partial least square regression (PLSR).

Results and Discussion

Medicines included in this study were of diverse pharmacological classes, types, constituents and formulations. Pharmacological classes included antibiotics, anti-inflammatory as well as drugs working on cardiovascular, gastrointestinal, nervous and respiratory systems. Subsequently, the dose, constituents and therapeutic

margin were different for each medicine. Furthermore, the types of medicines included both branded and generic medicines. The medicines were obtained from difference sources across the wholesale supply chain. Sources of medicines included hospitals, pharmacies, street markets, the Internet and wholesalers. All the aforementioned factors implicated the authentication approach in relation to the technique of choice and the identification/quantification algorithms.

With the aforementioned scenario, an ideal technique would provide a rapid, portable and on-site approach for authenticating medicines [11, 22]. Handheld NIR and Raman spectroscopic techniques offered these advantages due to many factors including: Light weight (< 5 Kg), portability, operation under different environmental conditions (temperature, humidity), friendly interface, inbuilt algorithms and ability to export spectra for offline analysis [22]. Yet each spectroscopic technique had its advantages and disadvantages in relation to medicines' authentication. This depended to a degree on the type of medicine (branded versus generic), chemical make-up (API, excipients and doses) and physical properties (colour, shape and particle size). Subsequently, both techniques were complementary in authenticating medicines. In this respect, NIR confirmed the medicines' physicochemical properties; whereas, Raman inspected specific signatures for chemical constituents in medicines.

Near-infrared spectroscopy

NIR was ideal in authenticating branded medicines where the test product should match precisely the reference product with respect to its physicochemical properties. In this respect, the presence of the reference product was key in the authentication process. This was demonstrated by comparing the NIR spectra of the test and reference products. NIR was able to instantly detect specific differences in medicines due to physicochemical properties. These differences identified: Defects in coating, presence/absence of API/excipient(s), poor storage conditions (humidity), difference in grade of API/excipient(s) (particle size). Figure 1 shows the NIR spectra of authentic and counterfeit Plavix tablets with differences in coating, water content and API. The correlation coefficient (r) value between both spectra was 0.77 which identified the counterfeit batch.

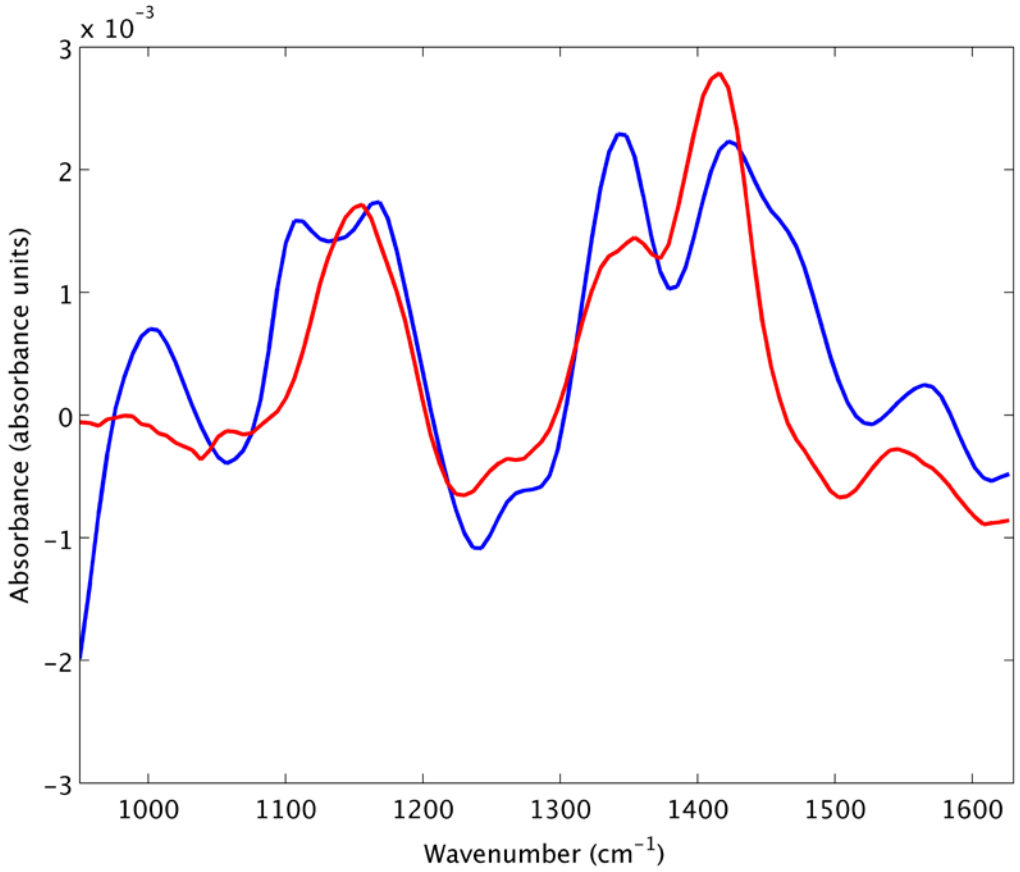

Figure 1 SNV-D1 treated NIR spectra of authentic (blue) and counterfeit (red) Plavix tablets measured using the palm-sized NIR spectrometer.

Handheld NIR was also able to accurately classify manufacturing source of medicines despite being purchased from different countries. This was possible when adequate number of batches (> 20) of a medicine were available [23]. For instance, the combination of NIR and PCA was able to classify authentic and counterfeit Viagra medicines obtained from different sources worldwide (Figure 2). Thus, the authentic scores incorporated inside the 95% equal frequency ellipses which indicated the same manufacturing source.

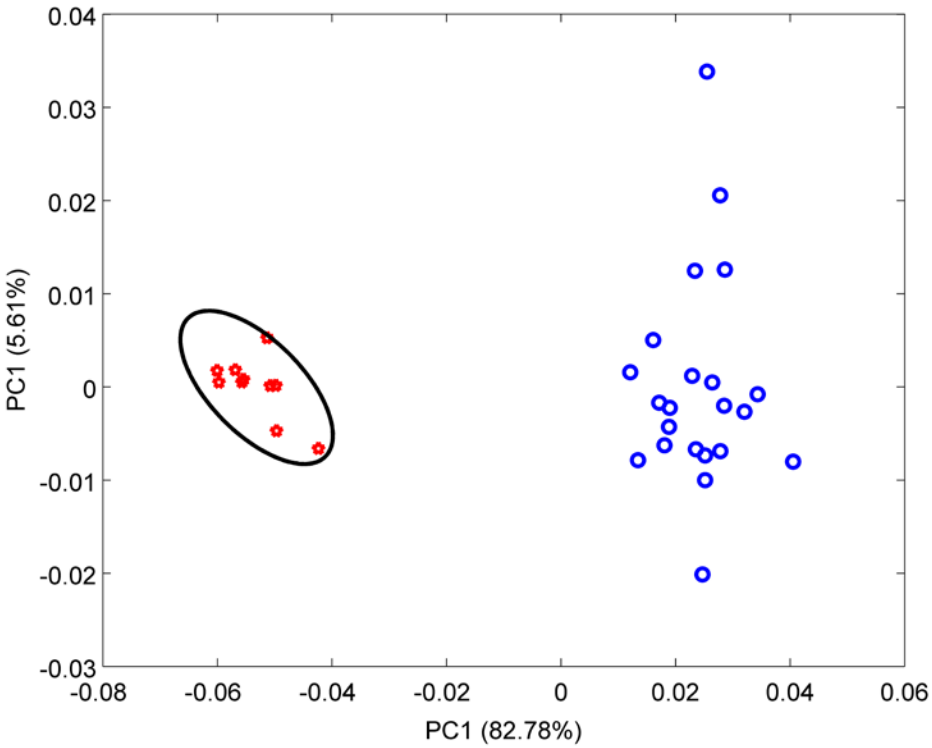

Figure 2 PCA scores plot of the SNV-D1 treated NIR spectra of authentic (red) and counterfeit (blue) Viagra tablets measured using the handheld NIR spectrometer with 95% equal frequency ellipses drawn around the authentic scores.

Thus, NIR was ideal for identifying counterfeit branded medicines when the label claim was indicated and a reference medicine was available. However, this approach was not successful in authenticating generic medicines with difference physicochemical properties but similar APIs.

Raman spectroscopy

Raman offered an advantage in authenticating generic medicines where the signature of API and/or excipient(s) was of interest. Raman spectroscopy showed spectral features specific to Raman active constituents in a medicine product [13, 24].

Yet the Raman activity of the constituents was dependent on the laser wavelength used. Using the 785 nm wavelength, the Raman activity of the medicine was mainly

dependent on the API [13]. Thus, APIs showed Raman active signatures provided they were present in high concentrations. Figure 3 shows the Raman spectra of paracetamol (generic) and Panadol (branded) which showed signature specific to their corresponding API (paracetamol).

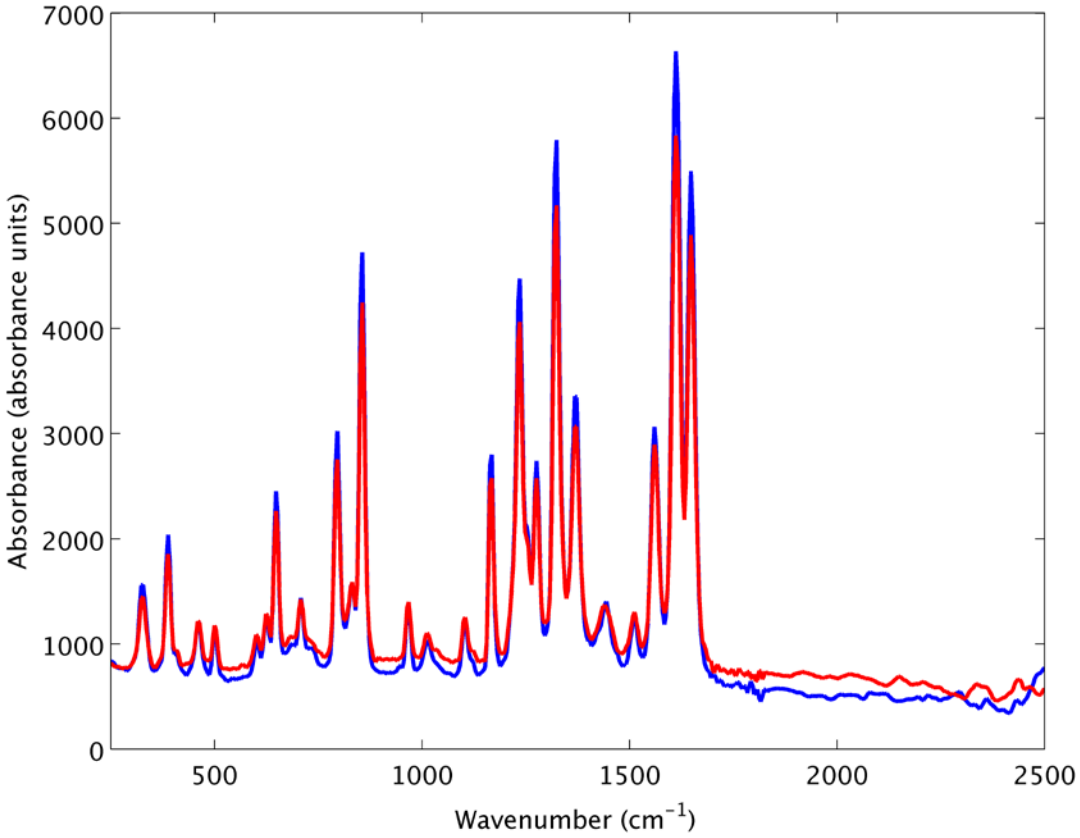

Figure 3 Raw Raman spectra of Panadol (blue) and test paracetamol (red) which proved authenticity (r = 0.99) measured using a handheld Raman spectrometer equipped with 785 nm laser wavelength.

Subsequently, Raman spectroscopy (with 785 nm wavelength) was ideal for authenticating generic medicines with high concentration of API. However, in medicines with low concentration of APIs, the Raman spectra of the medicine were often masked by the fluorescence of the excipients [11]. In this respect, the choice of a longer laser wavelength (such as 1064 nm) or a dual laser would be an option. The use of 1064 nm laser wavelength removed fluorescence but resulted in less spectral resolution and lower sensitivity [13]. Nonetheless, the use of dual laser overcome all the issues in relation to fluorescence, spectral resolution and sensitivity. In this

respect, the signature of the medicine showed spectral features corresponding to API and excipient(s). Figure 4 shows the Raman spectra of Viagra tablets which contained 16 % m/m of sildenafil citrate and showed spectral features for titanium dioxide, lactose (main excipient) and sildenafil citrate (Assi et al 2015). This was key in differentiating authentic and counterfeit Viagra tablets. Thus, the counterfeit tablets contained excess amount of sildenafil citrate, no lactose (main excipient) and had thinner film coating. Subsequently, the Raman spectra of authentic counterfeit Viagra against sildenafil citrate, lactose and titanium dioxide showed r values of 0.57, 0.47 and 0.86 respectively. On the other hand, the counterfeit Viagra tablets showed r values of 0.83, 0.15 and 0.56 against sildenafil citrate, lactose and titanium dioxide respectively. Thus dual laser Raman was ideal in identifying chemical differences between authentic and counterfeit medicines when a reference product (from the same manufacturer of the test product) was available.

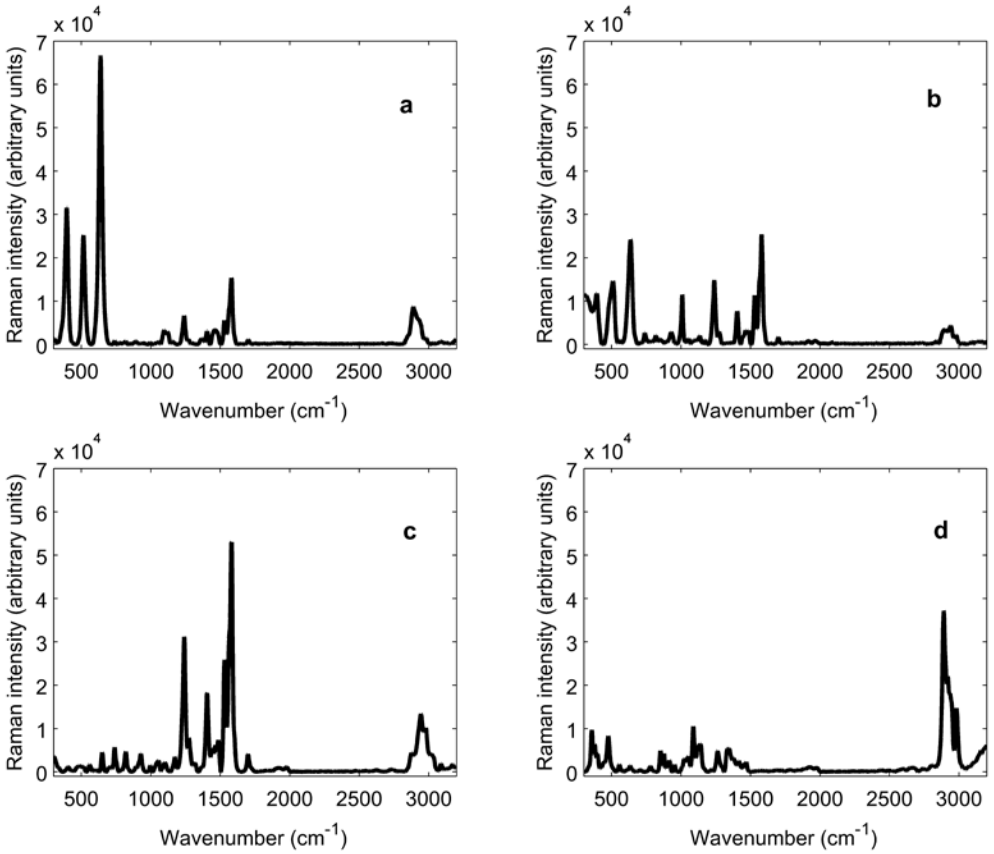

Figure 4 Raw Raman spectra of (a) authentic Viagra tablet, (b) counterfeit Viagra tablet, (c) sildenafil citrate and (d) lactose measured using a handheld Raman instrument equipped with a dual laser wavelength [16].

In the absence of a reference medicine, dual laser Raman was still able to identify counterfeit medicines if multiple authentic batches were available. In this respect, a clustering method (such as PCA or distance method) would be able to differentiate between authentic and counterfeit medicines. Figure 5 shows the clustering of authentic and counterfeit Cialis tablets. The counterfeit tablets showed higher distances than the authentic medicines; which indicated higher variability due to different manufacturers.

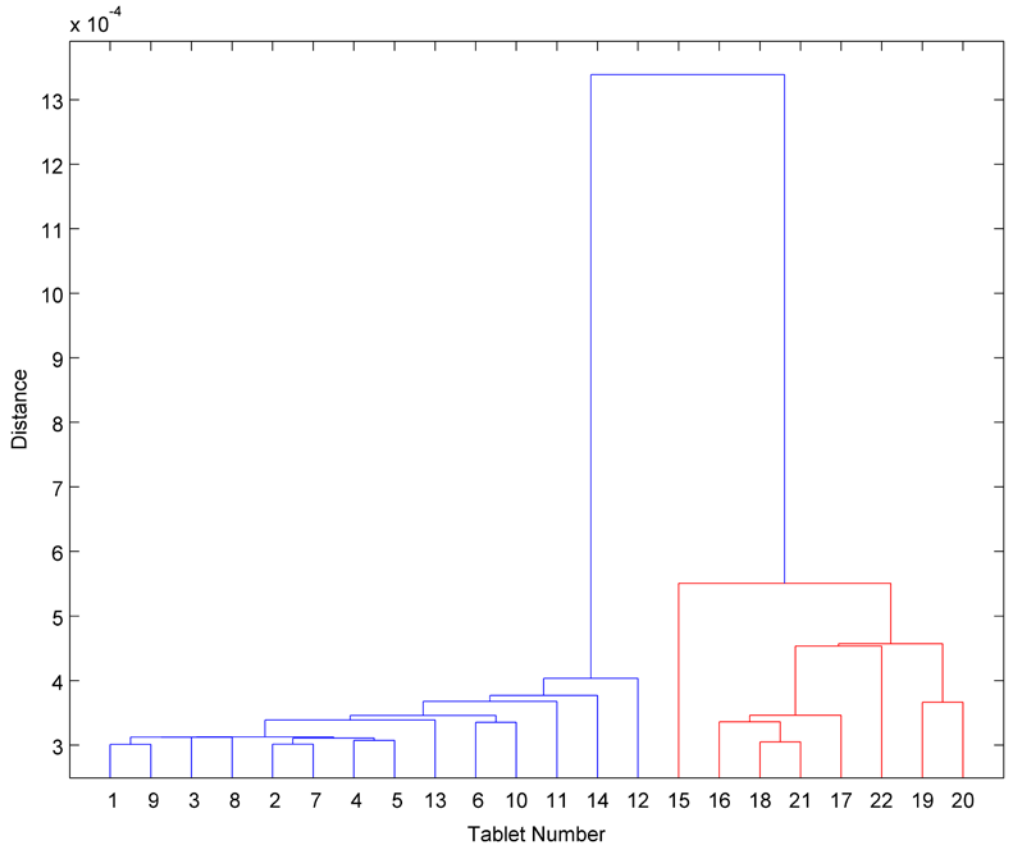

Figure 5 Distance plot showing the clustering of the authentic (blue) and counterfeit (red) Cialis tablets measured using a handheld Raman instrument equipped with a dual laser wavelength.

However, clustering was not effective in all cases especially with the diversity of genetic batches available on the market. Subsequently, a more quantitative approach was needed where the presence of API as well as its concentration were of interest. For instance, PLSR applied to Raman spectra of branded Ciproxin tablets

was able to quantify the API in both branded and generic tablet obtained worldwide [25]. The accuracy of the prediction was variable between different batches and this was attributed partly to the diversity of the samples as well as the noise generated by the Raman instrument.

Conclusion

In summary, handheld spectroscopic techniques offered rapid, mobile and non-destructive approach for identifying counterfeit medicines worldwide. Both NIR and Raman were complementary in authenticating branded and generic medicines. NIR gave an overview on the medicines' physicochemical properties and hence was more suitable for authenticating branded medicines. On the other hand, Raman spectroscopy showed specific chemical signatures to constituents in the medicines and thus was more suitable for authenticating generic medicines.

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