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The Use of Portable Near Infrared Spectroscopy for Authenticating Cardiovascular Medicines

The use of portable near-infrared spectroscopy for authenticating cardiovascular medicines

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Abstract

The counterfeiting of medicines impacts the public health and contributes to morbidity and mortality worldwide. Counterfeiting can occur to any medicine class of any type whether it be lifestyle or lifesaving medicines. With lifesaving medicines the situation is more critical, especially when these medicines are used in patients with non-communicable diseases (NCDs) who are taking multiple medicines (polypharmacy) and have multiple comorbidities. According to the World Health Organization, NCDs are those that cannot be cured but can be controlled with appropriate intake of medicines and adjustment of lifestyle factors. NCDs include cardiovascular diseases that are associated with 31% of deaths worldwide. Consequently, any defects in cardiovascular medicines, whether in physical properties or chemical constituents, will impact the treatment effectiveness of cardiovascular medicines. Thus, a substandard cardiovascular medicine would not be of less harm than a counterfeit medicine especially when the medicine is of low active pharmaceutical ingredients. Near-infrared spectroscopy (NIRS) can not only identify chemical constituents in a medicinal formulation but also physical properties of medicines. Portable NIRS offer a further advantage in taking the laboratory to the sample as it measures the samples as they are with no treatment. Therefore, this work utilized portable NIRS for identifying counterfeit cardiovascular medicines focusing on both physical properties and chemical constituents.

Keywords: Counterfeit medicines, cardiovascular, non-communicable diseases, near infrared spectroscopy

Introduction

Medicine counterfeiting represents a global expanding problem that affects all countries worldwide. Counterfeit medicines are those which are deliberately or fraudulently mislabelled in relation to their identity or source (1,2). They may contain no active pharmaceutical ingredient (API), insufficient amount of API or the wrong API. On the other hand, substandard medicines are those who fail to match the manufacturer's specification and/or quality standards. The public health impact resulting from counterfeit or substandard medicines can range from treatment ineffectiveness to lethal effects (3).

Medicine counterfeiting is not exclusive to a class of medicines and can occur to all medicine classes whether lifestyle or lifesaving medicines (4). However, the effects with lifesaving medicines could be more pronounced especially among patients with non-communicable diseases (NCDs). NCDs are defined by the World Health Organization (WHO) as long-term diseases that have no permanent cure but could be controlled by modification of diet and lifestyle factors (5). NCDs include cardiovascular diseases (CVDs), diabetes, respiratory diseases and cancer. CVDs are diseases associated with the heart or blood vessels and contribute to morbidity and mortality worldwide (6). According to the WHO, CVDs contribute to 31% of global deaths. Patients with CVDs often have multiple morbidities and take multiple medicines and that could result in drug-drug interactions or drug-disease interactions (7).

Subsequently, having the wrong API or the wrong dose in a cardiovascular medicine can interfere not only with the treatment effectiveness but also with the adverse effects, drug-drug interactions and drug-disease interactions. CV medicines could be encountered anywhere across the supply chain including patients' homes, pharmacies and medicines. Therefore, a portable approach that carries the laboratory to the sample would be ideal (8,9), especially where a life-threatening situation is encountered. Portable near infrared spectroscopy (NIRS) offers the advantage of being mobile, rapid and non-destructive and this is ideal in medicine authentication (10, 11). NIRS has a further advantage over other techniques in not only detecting the chemical constituents of a sample but also the physical properties that are important for spotting a counterfeit or substandard medicine especially in cases of organized crime where a medicine of the sample source could be distributed in different countries worldwide (12).

Therefore, this work aimed to evaluate portable NIRS for authenticating cardiovascular medicines obtained from different countries worldwide. Moreover, the work further assessed the potential of portable NIRS for detecting differences in physical properties among authentic products of different manufacturers.

Experimental

A total of 35 individual products of eight different cardiovascular medicines were evaluated in this study (Table 1). Reference products were purchased from the UK; whereas, test products were purchased from eight different countries: Australia, Egypt, France, India, Syria, Turkey and the UK. The medicines were of five pharmacological classes and were intended for three cardiovascular conditions being antiarrhythmic, anticoagulant and antihypertensive agents. The pharmacological classes of the medicines were angiotensin converting enzyme inhibitor (n = 2), angiotensin receptor blockers (n = 3), beta blockers (n = 2), P2Y12 platelet inhibitor (n = 1). Angiotensin converting enzyme inhibitors used were captopril and enalapril and angiotensin receptor blockers were losartan, olmesartan and valsartan. The two beta-blockers used were atenolol and propranolol. In addition the P2Y12 platelet inhibitor used was clopidogrel. The concentration of the API in the measured medicines varied in the range of 8.4 – 48.4% m/m.

The aforementioned products were tablets and were measured 'as received' from both sides by rotating and/or flipping the tablet after each measurement using the PerkinElmer Spectrum Two N™ FT-NIR spectrometer with the Near-Infrared Reflectance Module (NIRM). Each spectrum was the sum of 32 scans over the range of 10000 – 4000 cm^{-1}.

NIR spectra were exported into Matlab 2014b where spectral pre-treatment and treatment were applied. Spectral pre-treatment algorithms used were multiplicative scatter correction-second derivative (MSC-D1) and spectral treatment algorithms used were correlation in wavenumber space (CWS) and principle component analysis (PCA). For CWS method, the correlation coefficient (r) value of each test product was compared against the reference product. CWS method gave r values in the range of -1 to +1 where -1 indicated that the compared products were completely dissimilar and +1 indicated they were identical. The threshold taken for authenticity was r value above 0.95. On the other hand, PCA scores allowed visualisation of

clusters among reference and test products. Thus, the first two PCA scores of reference and test products were plotted and clusters of the products were compared.

Results and Discussion

The evaluated medicinal products were chosen on the basis of being used for critical cardiovascular conditions including arrhythmia, hypertension, ischemic heart disease and myocardial infarction. As patients with CVDs often have polypharmacy and suffer from multiple comorbidities, substandard/counterfeit medicines encountered will negatively impact the treatment outcome. It is also noteworthy to mention that the medicinal products had wide variations in colours, shapes and percentage of API. The percentage of API in the aforementioned medicinal products was in the range of 8.14 – 48.4% m/m. The number of excipients in the medicinal products ranged between three and 12 excipients. Yet, the most common excipients often encountered were lactose, maize starch and microcrystalline cellulose (MCC). Having the excipients alongside the API was key information for authenticating the medicinal products as part of the physicochemical fingerprinting of the products.

NIRS offered the advantage of inspecting the physicochemical properties of medicinal products non-destructively. Having a portable NIR device offered further benefits in mobility so the instruments could be carried to the sample without the need for distinctive laboratory settings. Additionally, obtained a spectral signature required few seconds to minutes. The software allowed spectra to be exported to external software where offline analysis could be applied.

Spectral evaluation of reference products

In this respect, Often spectra matched their main excipient especially among products with low % m/m of API. Hence, propranolol has lowest percentage of API (8.14% m/m) among the evaluated medicinal products and gave high r values for lactose, hypromellose and MCC that corresponded to 0.82, 0.76 and 0.72 respectively. However, none of the aforementioned values could be considered as they were below the threshold (i.e. 0.95). High r values were also observed for additional products with low % m/m of APIs (< 20%). In this respect, captopril (API of 13.9% m/m) and olmesartan (API of 13.2% m/m) gave r values of 0.71 and 0.7 for

lactose, and 0.79 and 0.88 for MCC respectively. As the % m/m of API in a product increased the r values against the excipients decreased but high r values were still observed for some of the excipients. Hence, losartan, clopidogrel and atenolol had % m/m of APIs of 22.1, 24.1 and 29.8% m/m respectively. Losartan gave rvalues of 0.79, 0.74 and 0.84 against hypromellose, lactose monohydrate and MCC. Clopidogrel that had higher % m/m of API showed lower r values for the aforementioned three excipients that were 0.71, 0.66 and 0.77 respectively. Atenolol had very low r values against all excipients that were below 0.51. Likewise, valsartan had 48.4% of API and gave r values below 0.65 for all excipients.

As the reference medicinal products had common excipients, CWS was applied to observe any type I or type II errors that could be encountered in identification of the aforementioned medicines. Type I error was encountered when a medicinal product was misidentified as itself (i.e. gave r value < 0.95 against its reference spectra) and type II error was encountered when a medicinal product was misidentified as another product (i.e. gave r value > 0.95 against another product's spectra). In this respect, no type I error was observed for individual tablets of each reference product. However, type II error was observed for propranolol spectra that were identified as olmesertan (r = 0.95). This could be due to the common excipients among both products.

Tracking authenticity of products

Subsequently, CWS was adopted for evaluating the authenticity of test products. In this respect, the spectra of the test medicinal products were compared against the reference products and actual/potential constituents within each product (Table II). Only one test product was evaluated for atenolol, captopril and enalapril but the three products passed against the reference and gave r values of 0.9999. On the other contrary, match failures were seen among losartan, propranolol and clopidogrel test products and that could indicate the presence of counterfeit or substandard products. Two out of three losartan test products failed against the authentic reference and gave r values of 0.9189 and 0.8349. Only one propranolol product (P3) passed with r value of 0.9503. The remaining propranolol products gave r values in 0.8917 – 0.9111. Only four clopidogrel products passed and gave r values in the range of 0.9859 – 0.9949. The remaining clopidogrel products that failed gave r values in the range of 0.7016 – 0.9365. Two of the failed clopidogrel products (P2 and P3) had close r values against the

reference product (0.7016 and 0.7226) and similar r values against each other (r = 0.9746). When the spectrum of P3 was compared to the authentic product spectra, they were quiet dissimilar (Figure 2). It is noteworthy to mention that P3 spectrum showed a peak at 7196 cm^{-1} corresponding to talc that was not an excipient in reference clopidogrel product (Figure 2).

The r value matches among clopidogrel products was also reflected in the correlation map that compared the r values of the 13 different clopidogrel products. Figure 3 shows the details of the correlation map with colours ranging from dark blue (r values 0.64 – 0.73), light blue (0.74-0.8), green (0.8 – 0.85), yellow (0.85 – 0.89), orange (0.89 – 0.95) and dark red (0.95 – 1). Therefore, the darkest colour indicated highly similar products and showed that the products had the same matrix of APIs and excipients. Not only high matches were observed among the two potential counterfeit clopidogrel products (i.e. P2 and P3), but also among authentic products P4 – P6 had r values above 0.99 against each other and against P1 (reference product). Also, P8 had r values above 0.98 against the aforementioned authentic products and that indicated authenticity of the products relating to their physicochemical properties.

Tracing physicochemical properties of products

Physicochemical properties among the products were further confirmed when PCA was applied to the products with authentic and potential counterfeit/substandard samples. Figure 4 shows the PCA scores plots of propranolol, clopidogrel, losartan and valsartan. For clopidogrel, the PC scores reflected the results obtained by the CWS method where authentic products' scores were grouped together and separated from counterfeit products' scores. It is noteworthy to mention that the PCA results did not always match the CWS method and this could be due to the different manufacturing sources of the individual products and/or suitability of the PCA model. Two clusters were seen for propranolol products where individual scores of two products were seen in two clusters and this could be due to lack of accuracy of the model. For instance, all valsartan products were confirmed authentic using CWS method (r values between 0.9965 – 0.9988) but they showed three distinct clusters when measured using PCA method and this could indicate different manufacturing sources. Three distinct clusters were also seen among the three losartan products which were obtained from three sources i.e. India, Lebanon and the UK. Hence, PCA method showed classified spectra according to variances rather than comparing the whole spectra taking into account both physical and chemical differences

reflected within spectra. Therefore, PCA on its own may not indicate authenticity where different manufacturing sources of products were involved.

Conclusion

Portable NIRS offered a rapid, mobile and cost-effective method for authenticating cardiovascular medicines and that could serve as alternative to laboratory-based instruments where different laboratory settings are involved. CWS method was successful in authenticating a cardiovascular product and raising alertness for potential counterfeit and/or substandard products. However, PCA showed to be complementary to CWS in authenticating products where it uncovered detailed variability relating to physicochemical among individual products whether authentic or counterfeit products.

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List of Tables

Medicine	API	Dose (mg)	Concentration of API (% m/m)	Excipients	N
Antiarrythmic (beta-blocker)					
Atenolol	atenolol	100	24.1	Gelatin Heavy Magnesium Carbonate Magnesium Stearate Microcrystalline Cellulose Maize Starch Sodium Laurylsulfate Talc	2
Propranolol	propranolol hydrochloride	10	8.14	Maize starch Lactose monohydrate Microcrystaline cellulose Magnesium stearate Hypromellose Titanium dioxide	6
Anticoagulant					
Clopidogrel	clopidogrel hydrogen sulfate	75	29.8	Mannitol Macrogol 6000 Microcrystalline cellulose Hydrogenated castor oil Low substituted hydroxypropylcellulose Hypromellose Lactose monohydrate Triacetin Titanium dioxide Red iron oxide Carnauba wax	13
Antihypertensive					
Angiotensin converting enzyme inhibitor					
Captopril	captopril	50	13.9	Lactose monohydrate Pregelatinised Starch Microcrystalline Cellulose Stearic Acid	2
Enalapril	enalapril maleate	20		Lactose monohydrate Maize starch Glycerol distearate	2
Angiotensin receptor blocker					
losartan	losartan potassium	50	22.1	Microcrystalline cellulose Lactose monohydrate Maize starch Magnesium stearate Hyprolose Hypromellose	3

olmesartan	olmesartan	10	13.2	Microcrystalline cellulose Lactose monohydrate Low substituted hyprolose Hyprolose Magnesium stearate Tablet coat Talc Hypromellose Titanium dioxide Iron oxide (yellow, red)	2
valsartan	valsartan	80	48.4	Microcrystalline cellulose Colloidal silicon dioxide Crospovidone Hydroxypropyl methylcellulose Iron oxide (yellow, black and/or red) Magnesium stearate Polyethylene glycol 8000 Titanium dioxide	5

N: number of products that include one reference product for each medicine.

Table II. Correlation coefficient values of test products against their corresponding reference products

Product	Product	Source	r value	Result
Atenolol	P1	Lebanon	0.9999	P
Propranolol	P1	UK	0.9111	F
	P2	UK	0.8922	F
	P3	Lebanon	0.9503	P
	P4	Saudi Arabia	0.8917	F
	P5	Saudi Arabia	0.8924	F
Clopidogrel	P1	Syria	0.9129	F
	P2	Egypt	0.7016	F
	P3	France	0.7226	F
	P4	France	0.9931	P
	P5	Turkey	0.9907	P
	P6	UK	0.9949	P
	P7	Lebanon	0.7858	F
	P8	Australia	0.9859	P
	P9	Syria	0.9052	F
	P10	India	0.9365	F
	P11	India	0.7838	F
	P12	India	0.9149	F

Captopril	P1	Lebanon	0.9999	P
Enalapril	P1	Lebanon	0.9999	P
Losartan	P1	UK	0.9189	F
	P2	Lebanon	0.8349	F
Olmesartan	P1	India	0.9999	P
Valsartan	P1	Lebanon	0.9988	P
	P2	Turkey	0.9965	P
	P3	Turkey	0.9973	P
	P4	Turkey	0.9977	P

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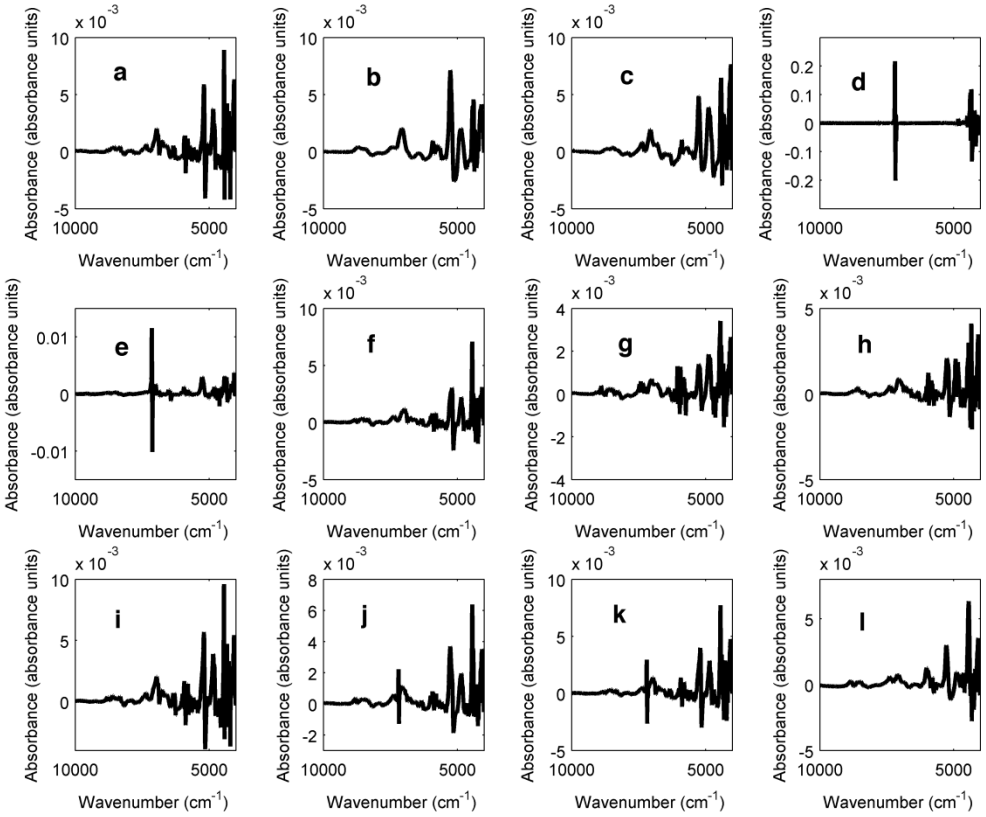

Figure 1 MSC-D1 treated NIR spectra of (a) lactose, (b) maize starch, (c) MCC, (d) talc, (e) atenolol, (f) propranolol, (g) clopidogrel, (h) captopril, (i) enalapril, (j) losartan, (k) olmesartan and (l) valsartan measured using the PerkinElmer Spectrum Two N™ FT-NIR spectrometer with the Near-Infrared Reflectance Module (NIRM).

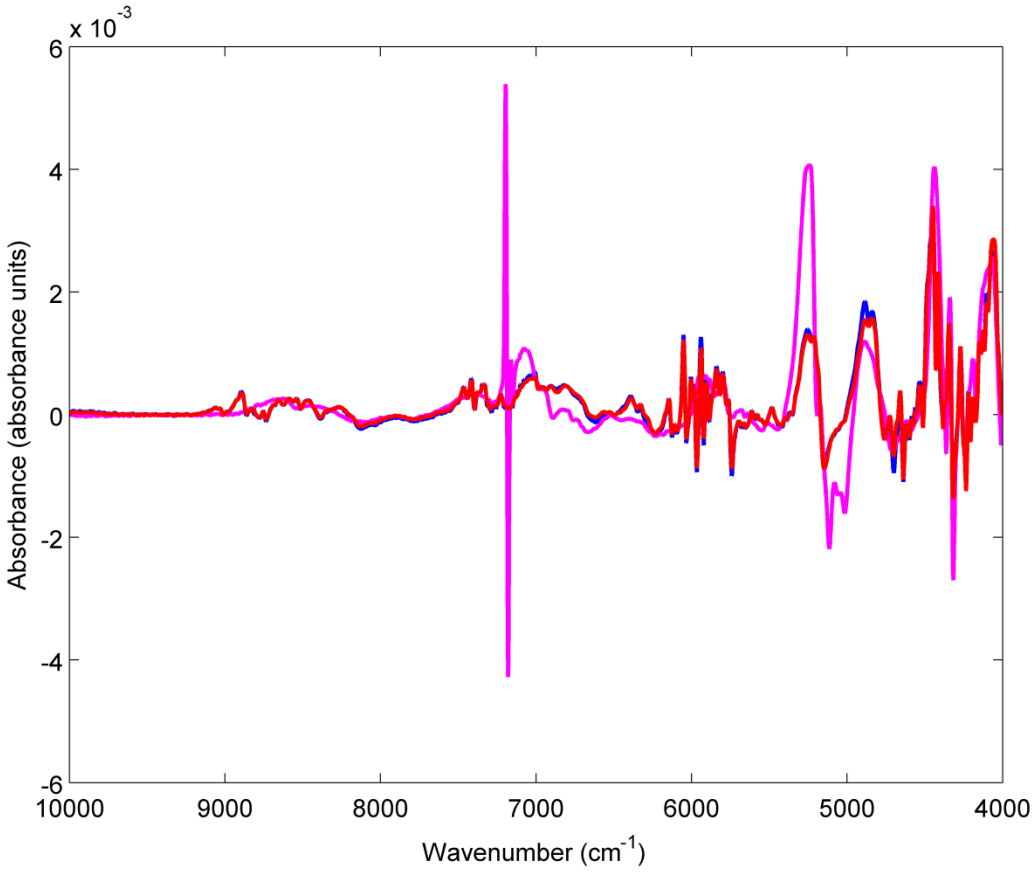

Figure 2 MSC-D1 treated NIR spectra of clopidogrel reference product (blue), authentic test product (red, r = 0.7227) and counterfeit test product (magenta, r = 0.9931) measured using the PerkinElmer Spectrum Two N™ FT-NIR spectrometer with the Near-Infrared Reflectance Module (NIRM).

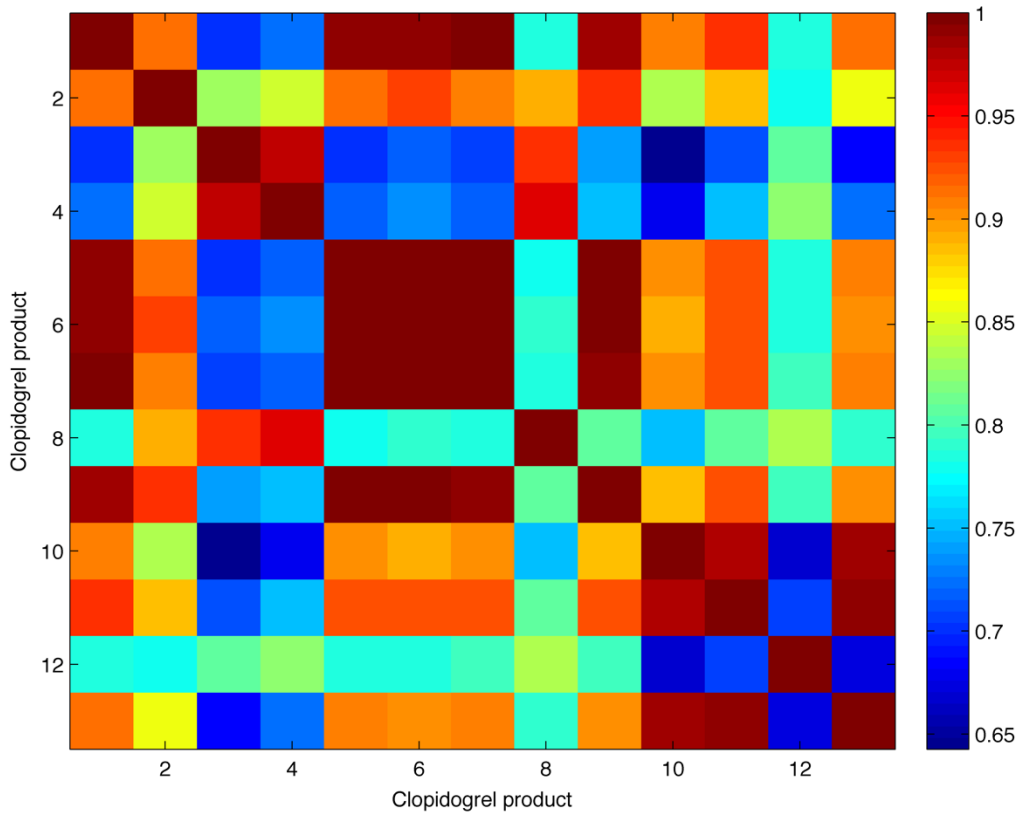

Figure 3 Correlation map of clopidogrel products including reference product and test products being P1 (Syria), P2 (Egypt), P3 (France), P4 (France), P5 (Turkey), P6 (UK), P7 (Lebanon), P8 (Australia), P9 (Syria), P10 (India), P11 (India) and P12 (India) respectively. The correlation map shows r value range between 0.6 (dark blue) and up to 1 (dark red).

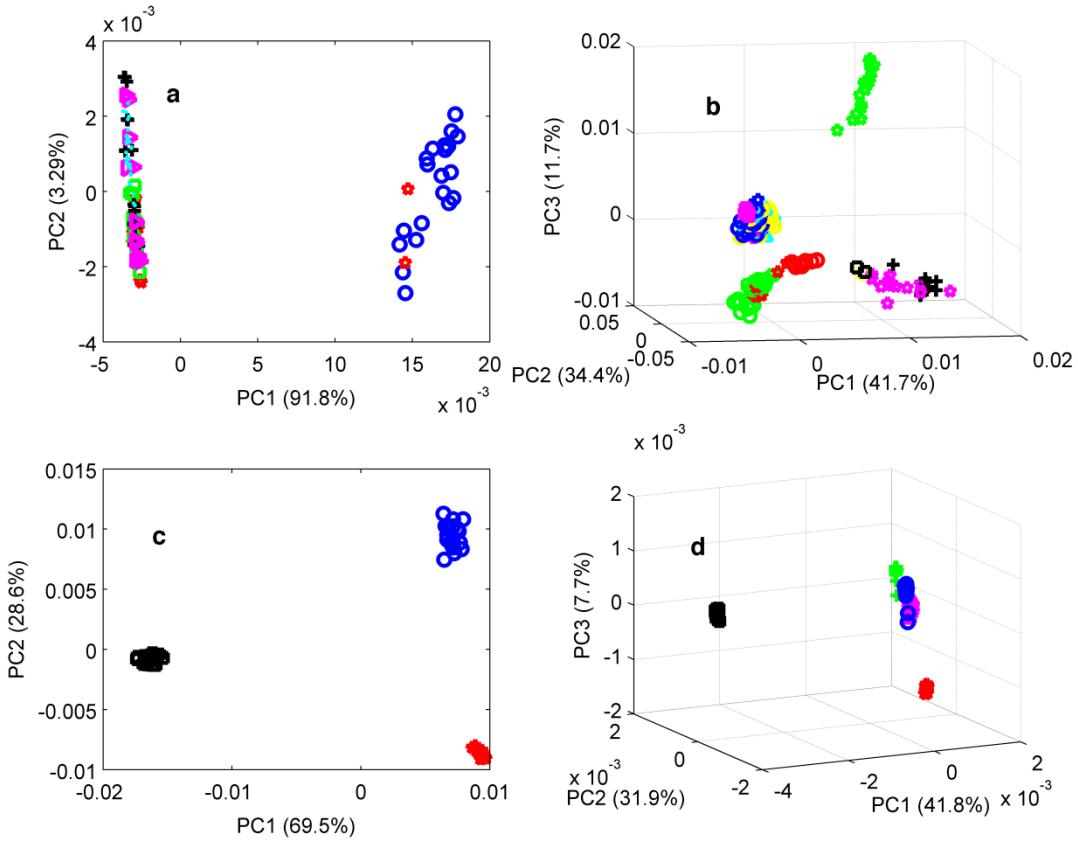

Figure 4 PCA scores plot of the MSC-D1 NIR spectra of authentic (blue) and test products of (a) propranolol, (b) clopidogrel, (c) losartan and (d) valsartan medicines measured using the PerkinElmer Spectrum Two N™ FT-NIR spectrometer with the Near-Infrared Reflectance Module (NIRM).