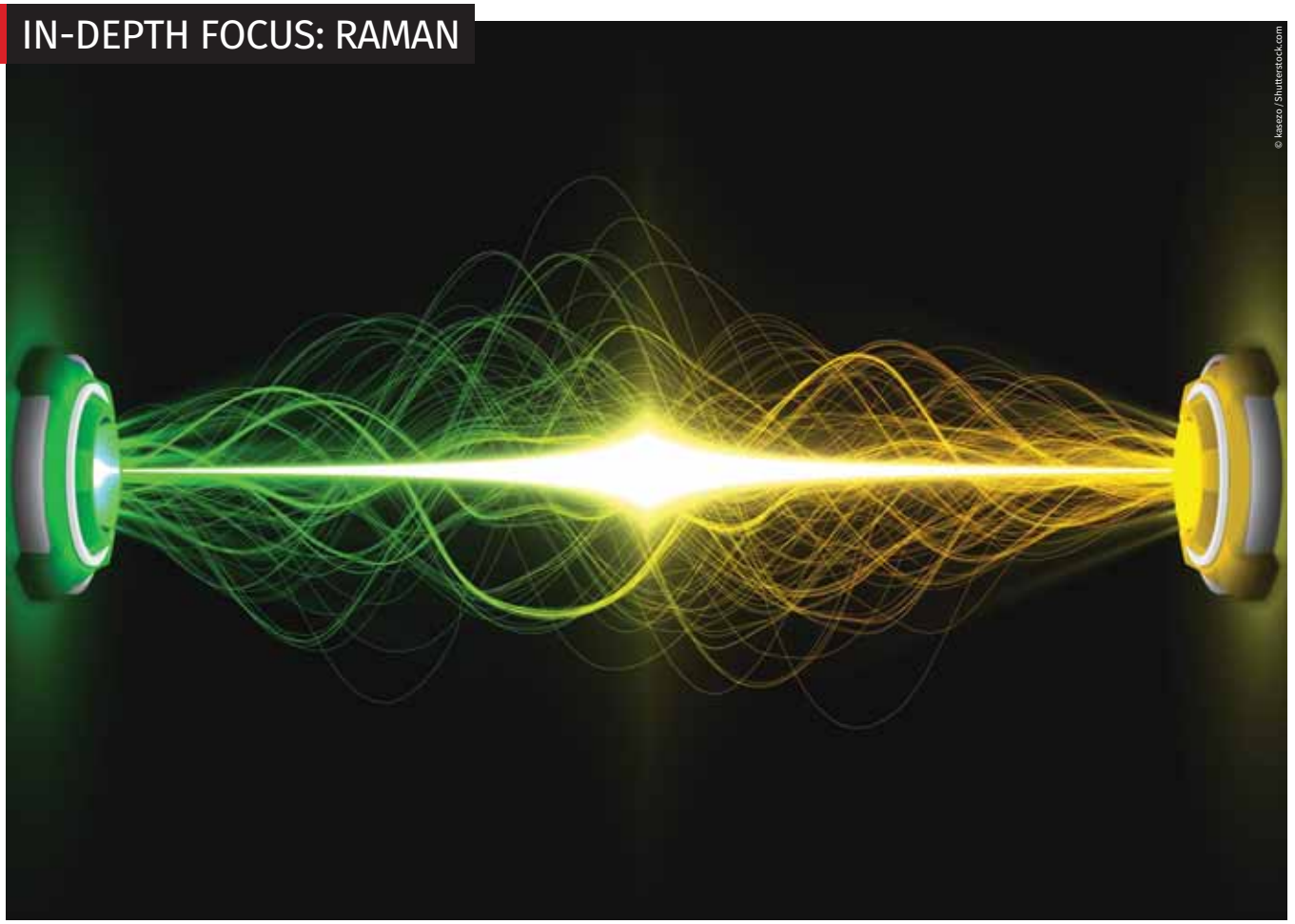
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Authenticating medicines with dual laser handheld Raman spectroscopy

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The World Health Organisation (WHO) defines counterfeit medicines as those that have been "deliberately and fraudulently mislabelled according to identity or source""[1]. Such illegitimate drugs can have defects in their active pharmaceutical ingredients (APIs), excipients or coating, or their packaging might have been tampered with. The harm resulting from them is unpredictable and can range from treatment ineffectiveness to drug resistance and even death. Additionally, drug counterfeiting is not limited to a class or formulation type and can be encountered anywhere in the wholesale supply chain. In this respect, rapid and mobile techniques that can authenticate medicines where they are encountered are favourable.

Handheld Raman spectroscopy offers this advantage since these spectrometers are light in weight, have long battery lifetimes and can operate over a wide temperature range, including both hot and cold climates[2,3]. However, one issue that is often encountered with authenticating medicines using handheld Raman spectroscopy, and mainly attributed to the Raman activity of authenticated medicines, is that drugs with low API contents and high excipient concentrations often fluoresce and have weak Raman activity[3,4]. The latter phenomenon is often encountered when using a 785nm laser, which is used since it has the advantage of high sensitivity. Fluorescence can be mitigated by using a further wavelength laser (such as a 1064nm one); however, this risks lower sensitivity[5].

The use of dual laser handheld Raman spectroscopy was successful in the chemical characterisation and authentication of type 5

phosphodiesterase inhibitors, which contained concentrations of APIs in the range of 5-15% m/m[6]. This article describes the results that were obtained when handheld Raman was used to authenticate medicines with low API contents and of diverse classes.

Experimental design

The medicines investigated in this study included both lifesaving and lifestyle products; these five products were the antihistamine drug loratadine; antidepressant citalopram; the antihyperlipidemics atorvastatin and simvastatin; and vardenafil, a sexual stimulant (Table 1). All of these medicines had low doses of APIs, in the range of 5-15% m/m. Branded and generic batches of them were obtained from France, India, Lebanon, Turkey, the UK and via the Internet. Additionally, APIs and the main excipients commonly present in the drugs were purchased from Chemical Suppliers.

Raman spectra of medicines were acquired using the Bruker BRAVO handheld Raman instrument, equipped with dual laser and charged coupled device detector with thermo-electric cooling. Four spectra were collected from both sides of tablets such that tablets were rotated between measurements. Each spectrum was the sum of one scan over the wavenumber range of 300-3200cm^{-1}. Spectra of APIs and excipients were collected through transparent glass vials and each vial was shaken and repositioned after each measurement.

Next, the spectra of APIs, excipients and medicines were exported in Matlab 2014b where multivariate classification algorithms were applied. The two algorithms were: correlation in wavenumber space (CWS) and principal component analysis (PCA). For the CWS algorithm, the test medicine spectrum was compared against the spectra of each of the excipients and the authentic medicine. In this sense, a correlation coefficient value (r-value) of ≥ 0.95 indicated a match and vice versa.

For PCA, the first two scores plots of the authentic and counterfeit product were compared in relation to the 95% equal frequency ellipses. The 95% equal frequency ellipses were calculated using the authentic scores. In this respect, if an authentic score was encountered outside the ellipse, this was considered a type I error. However, if a counterfeit score was encountered inside the ellipse, this was considered a type II error.

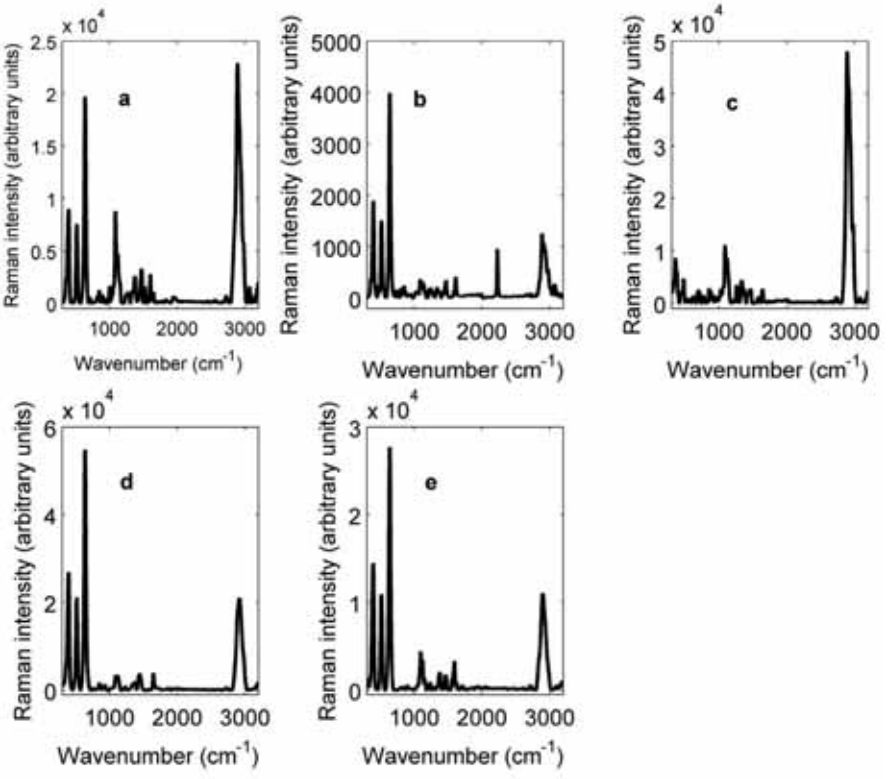

Figure 1: Raw Raman spectra of branded products of (a) atorvastatin, (b) citalopram, (c) loratadine, (d) simvastatin and (e) vardenafil, measured using the Bruker BRAVO handheld Raman spectrometer equipped with dual laser

Table 1: Details of the products used in the study

Product name	API	Dose (mg)	NTP	Purchase sources
Atorvastatin	atorvastatin calcium trihydrate	10	8	France, India, Lebanon, Turkey, UK
Citalopram	citalopram hydrobromide	10	10	Lebanon, UK
Loratadine	loratadine	10	11	Internet, UK
Simvastatin	simvastatin	20		Lebanon, UK
Vardenafil	vardenafil hydrochloride	20	30	India, Lebanon, Turkey, Internet, UK

Results and discussion

Raman activity of medicines with low APIs

Both branded and generic versions of each of the five medicines in the study were evaluated. All these medicines had API concentrations below 15% m/m and this introduced a challenge in the authentication process. Unlike branded medicines, the excipients in generic medicines were not always known[7]. Moreover, the Raman activity of the individual medicines depends on the activity of the API as well as the excipients.

> "Rapid and mobile techniques that can authenticate medicines where they are encountered are favourable"

In general, APIs are Raman-active provided they are present in a high concentration within the product[3]. Nonetheless, the Raman activity of excipients is often masked by fluorescence[3] that impacts the Raman activity of the overall medicine. One way of overcoming the fluorescence of excipients is to use sequential shifted excitation (SSE)[8]. In this work, SSE algorithm was used for authenticating medicines with multiple excipients and low doses of APIs. The algorithm proved successful in mitigating fluorescence of the medicines, yet had variable effects on the Raman activity of the medicines where the scattering intensity was a key factor. Though scattering intensity had arbitrary units, its value was directly proportional to the concentration(s) of Raman active substance(s)[9].

Hence, four parameters were taken into account when evaluating the Raman activity of the medicines. These were: fluorescence of the product, scattering intensity, range of scattering (over both lasers) and noise. Where known, the number of excipients present in the medicines

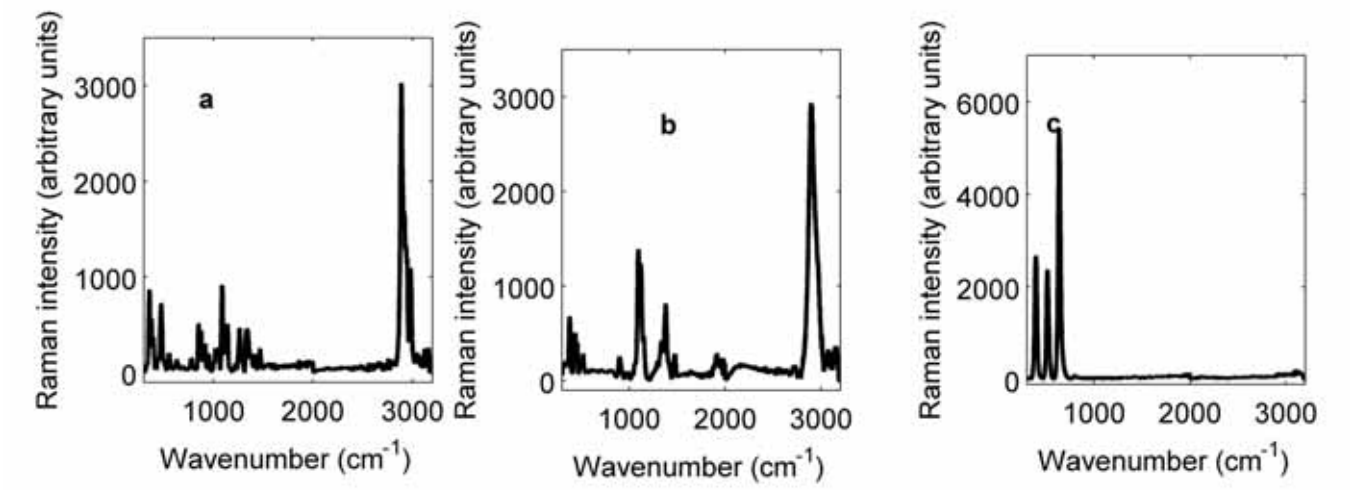

Figure 2: Raw Raman spectra of branded products of (a) lactose, (b) MCC and (c) TiO2, measured using the Bruker BRAVO handheld Raman spectrometer equipped with dual laser

ranged between four and 20 (**Table 2**). Hence, the multiple constituents present in the medicines affected the Raman activity of them. It is noteworthy to mention that the number of excipients did not affect the Raman activity of the medicines (**Figure 1**; page 13). Hence, all the evaluated medicines, except for citalopram, had scattering intensities in the range of 25,000 (observed for atorvastatin) and 60,000 arbitrary units (observed for simvastatin).

> "handheld dual laser Raman equipped with SSE was successful in characteristing medicines with low amounts of APIs"

Citalopram had a scattering intensity of 5,000 arbitrary units; however, it showed numerous peaks over the two laser wavenumbers (the first laser up to 2,000 cm^{-1} and the second between 2,000 and 3,000cm^{-1}). The remaining four medicines showed scattering mainly in the first laser range (7-12 peaks), and showed only one peak in the second laser range corresponding to the CH group. None of the medicines showed fluorescence within their spectra. Moreover, all medicines' spectra showed minimum noise (S/N ratio in the range of 10-40).

Table 2: Constituents of the products used in the study

Product	API	Main excipient	List of excipients
Atorvastatin	atorvastatin calcium trihydrate	lactose monohydrate	calcium carbonate, microcrystalline cellulose, lactose monohydrate, croscarmellose sodium, polysorbate 80, hydroxypropyl cellulose, magnesium stearate, hypromellose, macrogol 8000, titanium dioxide, talc, simethicone emulsion
Citalopram	citalopram hydrobromide	microcrystalline cellulose	mannitol, microcrystalline cellulose, colloidal anhydrous silica, magnesium stearate, hypromellose, titanium dioxide, macrogol 6000
Loratadine	loratadine	lactose monohydrate	lactose monohydrate, microcrystalline cellulose, maize starch, magnesium stearate
Simvastatin	simvastatin	lactose monohydrate	butylated hydroxyanisole, ascorbic acid, citric acid monohydrate, microcrystalline cellulose, pregelatinised maize starch, lactose monohydrate, magnesium stearate, hypromellose, hydroxy propyl cellulose, titanium dioxide, talc, iron oxide yellow, iron oxide red
Vardenafil	vardenafil hydrochloride	microcrystalline cellulose	crospovidone, magnesium stearate, microcrystalline cellulose, colloidal anhydrous silica, macrogol 400, hypromellose, titanium dioxide, ferric oxide yellow, ferric oxide red

API: Active pharmaceutical ingredient

Spectral evaluation of medicines

In both laser ranges, the spectra of the medicines showed corresponding peaks to the API, main excipient and titanium dioxide where present (**Figures 1 and 2**). Corresponding APIs' peaks showed scattering in the range of 1,000-2,000cm^{-1} and around 3,000cm^{-1}. The main excipients (being lactose and microcrystalline cellulose) showed scattering over the full wavelength range when titanium dioxide was not present. Thus, the presence of titanium dioxide masked the signal between 300 and 700cm^{-1}. In this case, the presence of titanium dioxide was detected in all products except for loratadine, which was not film coated (**Figure 1**). This is useful since it enables the thickness of coating to be detected by observing the scattering of titanium dioxide[6].

Regarding the main excipients, lactose was present in three products: atorvastatin, loratadine and simvastatin (**Table 2**) with featured peaks around 1,000cm^{-1} Furthermore, microcrystalline cellulose was featured in the spectra of citalopram and vardenafil (between 1,000 and 1,500cm^{-1}) as it was their main excipient. The featuring of the main excipient in the spectra of medicines was useful in allowing one to compare when the generic medicine contained the same main excipient as the branded medicine. This was the case in this work, where lactose and MCC spectral features were seen in the spectra of the generic test medicines.

Spectral authentication of medicines

Subsequently, the spectra of the test medicines were compared against the authentic medicines, titanium dioxide and the main excipients' spectra. For atorvastatin medicines tested, six products matched the spectra of the authentic medicine (r value = > 0.98), and showed r values in the range of 0.85-0.92 and 0.17-0.32 for lactose

and titanium dioxide, respectively. One atorvastatin test medicine failed the identification as it mismatched the authentic product and showed an r value of 0.78 against the authentic reference (**Figure 3**). The aforementioned test medicine showed r values for lactose and titanium dioxide of 0.69 and 0.29, respectively. This indicated that this batch had the main ingredients listed but in different amounts than the authentic reference.

Figure 4 (page 16) shows the correlation map of test atorvastatin tablets against the reference medicine, lactose and titanium dioxide. A high match (ir values of 0.95-1) is displayed in a dark red colour, whereas a high dissimilarity (r values of around 0) is shown in dark blue. In this respect, authentic batches (columns and rows 13-50) showed r values of above 0.98. On the other hand, the counterfeit batch (columns and rows 51-60) showed r values in the range of 0.77-0.79 against the authentic reference.

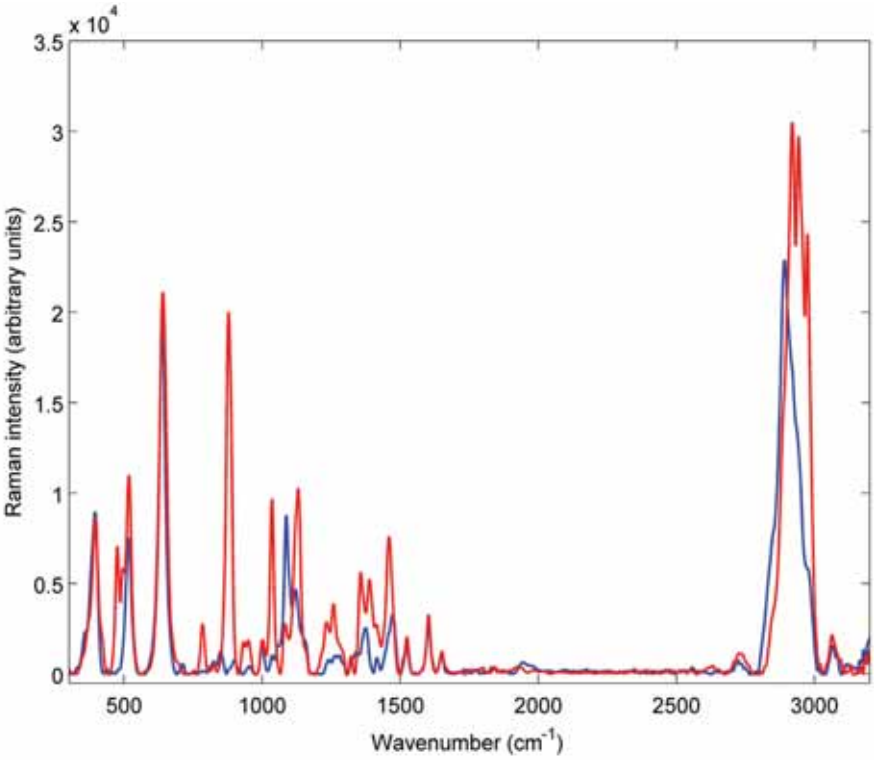

Figure 3: Raw Raman spectra of authentic (blue) counterfeit (red) atorvastatin products measured using the Bruker BRAVO handheld Raman spectrometer equipped with dual laser

Similarly, mismatches were also encountered when authenticating vardenafil products. In this respect, authentic vardenafil batches gave r values in the range of 0.96-0.98, 0.46-0.58 and 0.70-0.92 against the reference medicine, lactose and titanium dioxide. Conversely, counterfeit vardenafil batches gave r values in the range of 0.78-0.92, 0.13-0.83 and 0.38-0.95 against the reference medicine, lactose and titanium dioxide. This indicated the diversity and inconsistency of ingredients present in the counterfeit batches evaluated.

On the other hand, no mismatches were observed for citalopram,

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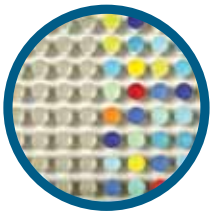
HTS Screening

785 nm – 1 Wavelength, 1 Instrument
Transmission to Millimeters to Microns

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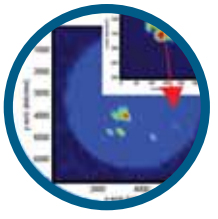
- Transmission Raman (Variable Spot Size)
- *P*^{h}*AT* Technology (mm)
- Video Capture & Image Overlay
- Robust, Reliable, Reproducible Raman
- Qualitative & Quantitative Analysis


Polymorph Screening


Transmission Backscatter


P^{h}*AST* Map

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loratadine and simvastatin medicines evaluated. Accordingly, the ten citalopram medicines evaluated gave r values of above 0.99 for the citalopram reference medicine. Additionally, they gave r values in the range of 0.72-0.92 and 0.3-0.41 for titanium dioxide and microcrystalline cellulose, respectively. The identical match between the aforementioned ten batches and the authentic reference could indicate they were from the same manufacturing source as the authentic product.

Likewise, loratadine and simvastatin medicines gave r values of above 0.96 against their authentic references. Additionally, loratadine gave r values in the ranges of 0.93-0.95 and 0.01-0.012 for lactose and titanium dioxide. This was because loratadine medicines had high concentrations of lactose in their formulations and no titanium dioxide. On the other hand, simvastatin medicines showed r values in the ranges of 0.48-0.63 and 0.70-0.84 for lactose and titanium dioxide, which corresponded to the excipient content and coating.

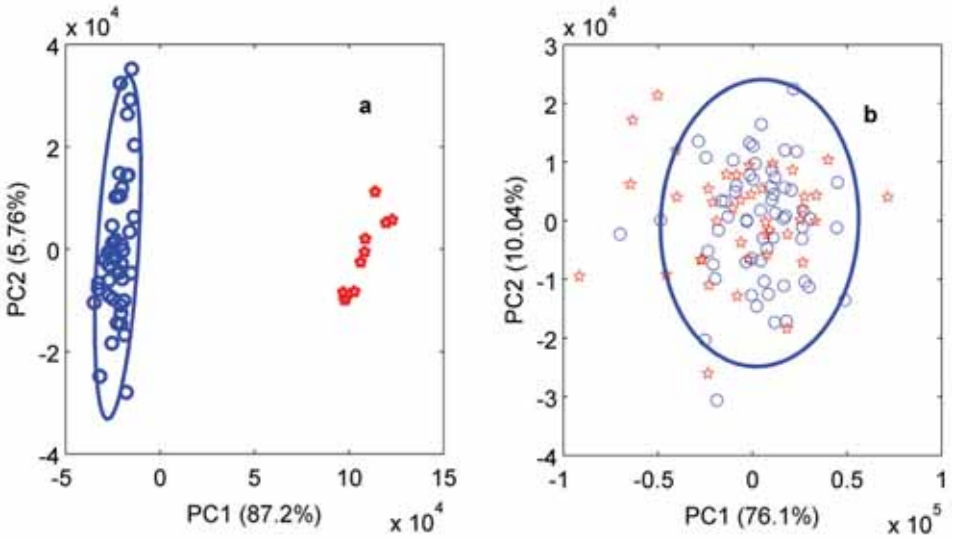

Figure 5: PCA scores plot of the Raman spectra authentic (blue) and counterfeit (red) of (a) atorvastatin and (b) loratadine medicines with the 95% equal frequency ellipses plotted around the authentic scores

To confirm the reliability of the correlation method in authenticating the aforementioned medicines, PCA was applied to the spectra of authentic and counterfeit batches. For most medicines, PCA was able to confirm the correlation results with no type I or type II errors. Type I errors were only encountered when authenticating atorvastatin and loratadine medicines (Figure 5). Two authentic atorvastatin batches and seven authentic loratadine batches were misclassified using the 95% equal frequency ellipse. This could be attributed to the diversity of matrices among the medicines. Subsequently, future work should look into a more quantitative approach for APIs and main excipients in medicines with diverse matrices.

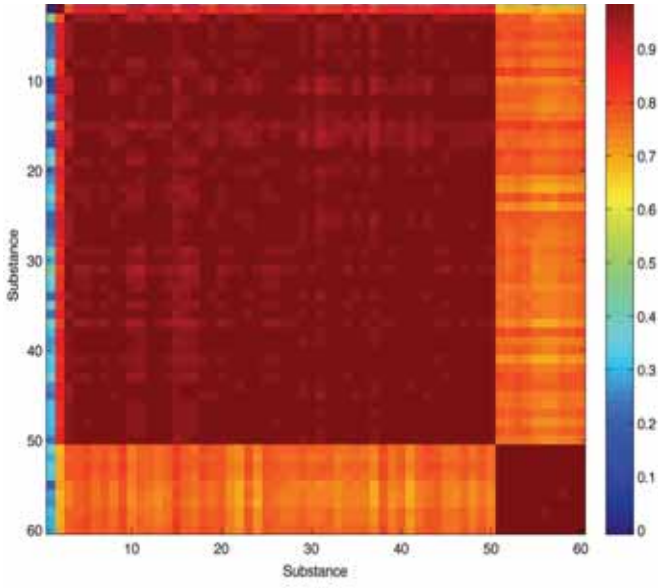

Figure 4: Correlation map of the Raman spectra of (1) titanium dioxide, (2) lactose, (3-50) authentic atorvastatin and (51-60) counterfeit atorvastatin. The colour map on the right shows the corresponding range to the r values. A perfect match (r = 1) is displayed in dark red, whereas a perfect mismatch (r = negative) is displayed in dark blue

Conclusion

In summary, handheld dual laser Raman equipped with SSE was successful in characteristing medicines with low amounts of APIs. The SSE algorithm showed to be successful in mitigating the fluorescence encountered when multiple excipients were present in the formulation. Additionally, correlation and the PCA method were successful in authenticating the majority of the products. Few products showed type I errors when classified using PCA. Thus, future work will involve the development of quantitative models for authenticating branded and generic medicines.

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