Raw material identification using dual laser handheld Raman spectroscopy

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Raw material identification is a significant and crucial stage in validating the authenticity of pharmaceutical products as pharmaceutical manufacturers have been recently moving into 100 per cent testing of incoming raw material[1]. The Falsified Medicines Directive (FMD) that came into light in July 2013 assured tough rules on the assessment of active pharmaceutical ingredients (APIs) and excipients[2].

Particularly in the UK, the Medicines and Health Regulatory Agency (MHRA) products strategy (2012 – 2015)[3] specifies that falsified finished medicines products seized in the UK have one or more of the following: 'No API, wrong API, too much API, too little API, always contain a range of unknown impurities, may use inappropriate excipients and often fail to dissolve at the correct rate when taken'. Consequently, manufacturer authorisation holders are required to verify the compliance of APIs with respect to good manufacturing practice and good distribution practices, and the suitability of the excipients for medicinal use. This will introduce a high demand of API / excipients (coming from outside the EU) authentication in a short time frame. This stimulates the need for rapid screening techniques for identification / authentication of raw materials.

Handheld Raman spectroscopy has been recognised as a powerful tool in pharmaceutical analysis particularly for raw material identity testing[1,4]. The advantages of handheld Raman spectroscopy include: simplicity, rapidity, portability and ease of use by non-skilled personnel[5-7]. In addition, handheld Raman spectroscopy offers a non-destructive measurement of materials regarding of their physical state (solid, semi-solid, liquid)[8]. Moreover, handheld Raman spectroscopy can measure the sample within its container whether glass or plastic[7].

Conventional Raman spectroscopy involves the use of 785 nm laser power. Although this laser power offers sensitivity in raw material identification, it has many disadvantages[9]. It introduces interferences due to fluorescence from Raman inactive materials such as cellulose and lactose. This affects the accuracy of identification of the material measured. In general, pharmaceutical excipients are Raman inactive, whereas pharmaceutical active ingredients (APIs) are Raman active[8,10]. However, the use of a longer wavelength laser (such as 1064 nm) can reduce and / or eliminate the fluorescence interference yet decreases the sensitivity[10].

The aim of this work is to compare the use of 785 nm to 1064 nm laser excitation wavelength for the accuracy of identification of raw materials.

Experimental

A total of 25 raw materials were measured through glass vials and included 13 APIs and 12 excipients. The APIs included Benzocaine (BEN); caffeine (CAF); indomethacin (IND); lidocaine hydrochloride (LID); nicotinamide (NIC); paracetamol (PAR); phenacetin (PHE); procaine hydrochloride (PRO); promethazine (PROM); quinine sulfate (QUI); sildenafil citrate (SIL); taurine (TAU) and theophylline (THE). In addition, the excipients included alginate sodium (ALG); dextrose monohydrate (DEX); hydroxyl ethyl cellulose (HEC); lactose monohydrate (LAC); maize starch (MAI); mannitol (MAN); microcrystalline cellulose

(MCC); polyvinyl pyrrolidone (PVP); saccharin sodium (SAC); sodium benzoate (SODB); sucrose (SUC) and talc (TAL).

Three spectra were collected from each vial using two handheld Raman spectrometers with laser excitation wavelengths of 785 nm and 1064 nm respectively. Both instruments were equipped with a thermoelectric cooled (TE) charged coupled device (CCD) detector.

The raw spectra were exported to Matlab R2007a where spectral quality and identification potential of the instruments were evaluated. The spectral quality was evaluated by comparing the number of peaks (n), maximum peak intensity and position and signal to noise ratio (S/N). The identification potential of the instruments were evaluated using correlation in wavelength space (CWS) and principal component analysis (PCA) were applied. The optimum value for the correlation coefficient (r) taken was 0.95.

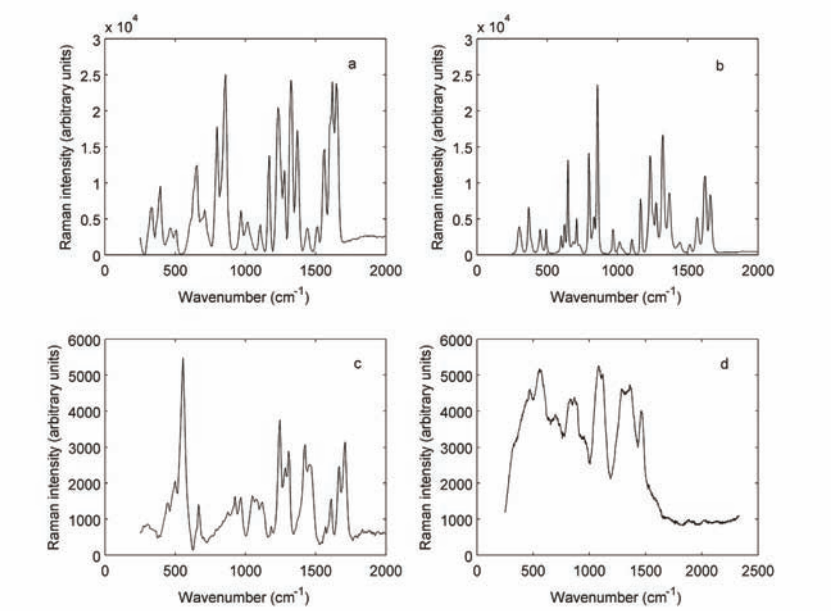

Figure 1 Raman spectra of (a) paracetamol measured using the 1064 nm instrument, (b) paracetamol measured using the 785 nm instrument, (c) saccharin measured using the 1064 nm instrument and (d) saccharin measured using the 785 nm instrument

Results and discussion

Both instruments offered a rapid and simple approach to identification of raw materials. Thus, a Raman spectrum could be obtained within a few seconds to one-minute intervals. This is advantageous when measuring large number of samples in the pharmaceutical industry such as raw materials identification. In addition, the simplicity in sample measurement makes these instruments easy to be used by non-skilled personnel.

Table 1 Spectral quality of raw materials measured using the Raman instrument equipped with 785 nm laser power

Raw material number	Raw material	n	Maximum Peak position (cm^{-1})	Maximum Peak intensity	S/N ratio	Raman activity
X1	Alginate sodium	8	566.8	6479	14.8	Medium
X2	Benzocaine	12	164	39670	118	Very strong
X3	Caffeine	14	558.1	32130	54.5	Strong
X4	Dextrose monohydrate	17	1331	4618	33.5	Strong
X5	Hydroxy ethyl cellulose	7	1079	5231	11.3	Medium
X6	Indomethacin	5	1592	19190	11.25	Medium
X7	Lactose	17	1087	6411	34	Strong
X8	Lidocaine hydrochloride	7	633.2	2861	26	Medium
X9	Maize starch	12	464.4	5261	30.5	Strong
X10	Mannitol	15	875	19860	146	Strong
X11	MCC	7	1092	9917	22	Medium
X12	NIC	13	1039	47700	140	Very strong
X13	PAR	20	8575	23630	142	Very strong
X14	PHE	24	1327	15850	72	Strong
X15	PRO	15	1261	23250	68	Strong
X16	PROM	17	1039	19810	73	Strong
X17	PVP	14	1426	4116	60	Strong
X18	QUI	12	1362	15870	62	Strong
X19	SAC	16	7011	53550	67	Strong
X20	SIL	15	1575	23580	70	Strong
X21	SODB	10	1002	45600	68	Strong
X22	SUC	7	556.9	8036	17.5	Medium
X23	TAL	6	673.1	13010	10	Weak
X24	TAU	17	1029	23220	69	Strong
X25	THE	17	547	30470	63	Strong

Spectral quality

The spectral quality varied between both instruments where the 785 nm instrument showed more fluorescence. This depended to a degree on the type of raw material measured. In general, APIs showed less fluorescence and higher Raman scattering than excipients when measured using the 785 nm instrument. In addition, the scattering due to glass vials was observed in some spectra. However, this did not affect the spectral quality of the raw material measured. **Figure 1** shows the Raman spectra of PAR (API) and SAC (excipient) measured using the two handheld Raman instruments.

Spectral quality was evaluated by taking four parameters into consideration (**Table 1** and **Table 2**, page 28). These included: the number of peaks, Raman scattering intensity, spectral range and S/N ratio. Based on these four criteria, the Raman activity of the material measured was classified as low, medium, strong or very strong.

The spectral range of scattering obtained using both instruments was between 250 – 2000 cm^{-1}. APIs showed stronger Raman activity than excipients in relation to the number of peaks, maximum scattering intensity and S/N ratio.

For the Raman spectra obtained using the 785 nm instrument, three APIs showed very strong Raman scattering. These were BEN, NIC and PAR and had S/N ratio of 118, 140 and 142

Table 2 Spectral quality of raw materials measured using the Raman instrument equipped with 1064 nm laser power

Raw material number	Raw material	n	Maximum Peak position (cm^{-1})	Maximum Peak intensity	S/N ratio	Raman activity
F1	Alginate sodium	9	1410	1701	10	Weak
F2	Benzocaine	13	1278	34670	15.5	Medium
F3	Caffeine	21	564	21790	61	Strong
F4	Dextrose monohydrate	14	518	3529	13.25	Medium
F5	Hydroxy ethyl cellulose	14	555	5487	12.6	Medium
F6	Indomethacin	17	1584	16790	31	Strong
F7	Lactose	16	356	9997	15.25	Medium
F8	Lidocaine hydrochloride	19	1449	8856	22.3	Medium
F9	Maize starch	10	480	3783	12.2	Medium
F10	Mannitol	13	875	9326	33.5	Strong
F11	MCC	8	1098	7672	22.3	Medium
F12	NIC	11	1033	20110	50	Strong
F13	PAR	21	858	25050	62	Strong
F14	PHE	18	1612	13600	71	Strong
F15	PRO	18	1255	30040	18	Medium
F16	PROM	13	1033	14720	70	Strong
F17	PVP	15	1425	3110	15.25	Medium
F18	QUI	12	1359	17060	12	Medium
F19	SAC	15	1702	2242	61	Strong
F20	SIL	17	1569	23020	32.5	Strong
F21	SODB	10	998	23220	69	Strong
F22	SUC	10	539	2410	10.3	Weak
F23	TAL	7	675	7064	21.3	Medium
F24	TAU	17	1033	26980	67	Strong
F25	THE	14	555	15230	56.5	Strong

respectively (**Table 1**, page 26). In addition, eight APIs showed strong Raman scattering and included CAF, PHE, PRO, PROM, QUI, SIL, TAU and THE. These had S/N ratios in the range of 54.5 to 73. Only two APIs showed medium Raman scattering and had S/N ratios of 34 (IND) and 30.5 (LID); whereas no APIs showed weak Raman scattering. The APIs which showed medium Raman scattering had the lowest number of peaks (**Table 1**, page 26). In this respect, IND and LID had five and seven peaks respectively. On the other hand, the highest number of peaks was observed for PHE and was 24 peaks. In addition, the maximum Raman scattering intensity of APIs ranged from 2861 to 47700 arbitrary units observed for LID and PHE respectively. This spectral range was wider than that obtained from the spectra using the 1064 nm instrument. Thus, the maximum Raman scattering intensity (for APIs) obtained through the 1064 nm instrument was observed for BEN and was 34670 arbitrary units (**Table 2**). Unlike the 785 nm instrument spectrum, BEN spectrum in this case showed medium Raman scattering and had S/N ratio of 15.5. For all APIs, the 1064 nm instrument showed lower S/N ratios, less number of peaks and lower scattering intensities. Whereas no API showed very strong Raman scattering, nine APIs showed strong Raman scattering and were: CAF, IND, NIC, PAR, PHE, PROM, SIL, TAU and THE. These had S/N ratios in the range of 31 (IND) to 71 (PHE), and maximum peak intensities in the range of 14720 (PROM) to 26980 (TAU). Also, the number of peaks of these eight APIs varied between 14 (THE) and 21 (CAF and PAR) peaks. The remaining four APIs showed medium Raman scattering and included BEN, LID, PRO and QUI. These had S/N ratios of 15.5, 22.3, 18 and 12 respectively. Moreover, the number of peaks and maximum scattering intensities for these four APIs ranged from 13 – 17 peaks and 8856 – 34670 arbitrary units respectively.

Similarly, the excipients spectra showed stronger Raman activity with the 785 nm instrument. Thus, seven excipients had strong Raman activity and were: DEX, LAC, MAI, MAN, PVP, SAC and SODB. These excipients had S/N ratios in the ratio of 30.5 – 146 (**Table 1**, page 26). In addition, four excipients showed medium Raman activity (ALG, HEC, MCC and SUC) and only one excipient showed weak Raman activity (TAL). The minimum number of peaks observed for excipients was six (TAL) and the maximum was 17 (LAC). Moreover, the maximum peak intensity ranged from minimum of 4116 arbitrary units (PVP) to a maximum of 53550 arbitrary units (SAC). Both SAC and PVP had strong Raman activity which was explained by the high noise in SAC spectrum, along with the high signal. On the other hand, the 1064 nm instrument showed lower number of peaks, peak intensities and S/N ratios. These ranged between 7 (TAL) – 16 (LAC), 1701 (ALG) – 23020 (SODB) arbitrary units, and 10 (ALG) – 69 (SODB) respectively. In this respect, only three excipients had strong Raman activity when measured by the 1064 nm instruments and were MAN, SAC and SODB. These three excipients had S/N ratios of 33.5, 61, 69 and maxima peak intensities of 9326, 2242 and 23220 arbitrary units respectively. In addition, two excipients had weak Raman activity and were ALG (S/N = 10)

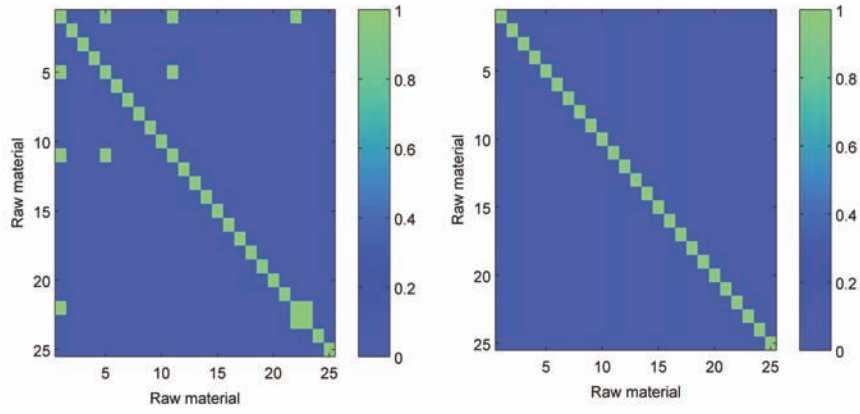

Figure 2 Correlation map of the raw Raman spectra measured using (a) the 785 nm instrument and (b) the 1064 nm instrument of: (1) ALG, (2) BEN, (3) CAF, (4) DEX, (5) HEC, (6) IND, (7) LAC, (8) LID, (9) MAI, (10) MAN, (11) MCC, (12) NIC, (13) PAR, (14) PHE, (15) PRO, (16) PROM, (17) PVP, (18) QUI, (19) SAC, (20) SIL, (21) SODB, (22) SUC, (23) TAL, (24) TAU and (25) THE

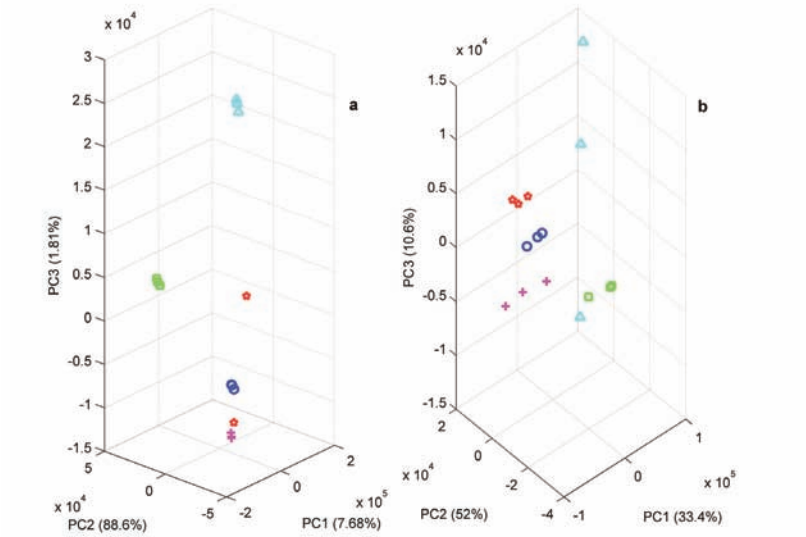

Figure 3 PCA scores plot the Raman spectra of ALG (blue), HEC (red), MCC (green), SUC (magenta) and TAL (cyan) measured using (a) the 785 nm instrument and (b) the 1064 nm handheld Raman spectrometers

and SUC (S/N = 10.3). These two excipients had maxima peak intensities of 1701 and 2410 arbitrary units respectively. In addition, the remaining nine excipients had medium Raman activity and included: DEX, HEC, LAC, MAI, MCC, PVP and TAL. The S/N ratios of these excipients ranged from 12.2 – 33.5, and the maxima Raman intensities ranged from 3110 – 9997 arbitrary units.

Identification of raw material

The identification of raw materials using both instruments was evaluated using CWS and PCA algorithms.

For the CWS algorithm, the r values of the mean spectra of the 25 raw materials (APIs and excipients) were compared. In this respect, an r value above 0.95 indicated strong similarity; whereas a lower r value indicated dissimilarity. The results of CWS were visualised as a correlation map, where a green colour indicated an r > 0.95. Otherwise, a blue colour was observed. **Figure 2** (page 28) shows the correlation map obtained from the spectra measured using (a) 785 nm instrument and (b) 1064 nm instrument of: (1) ALG, (2) BEN, (3) CAF, (4) DEX, (5) HEC, (6) IND, (7) LAC, (8) LID, (9) MAI, (10) MAN, (11) MCC, (12) NIC, (13) PAR, (14) PHE, (15) PRO, (16) PROM, (17) PVP, (18) QUI, (19) SAC, (20) SIL, (21) SODB, (22) SUC, (23) TAL, (24) TAU and (25) THE respectively. In this respect, green colour was expected among the spectra of the same raw material and blue colour between spectra of different material. This was observed among spectra measured with the 1064 nm instrument (**Figure 2b**, page 28). On the other hand, the spectra measured using the 785 nm instrument showed green colour among spectra of five different excipients: ALG, HEC, MCC, SUC and TAL. The mismatches were as follows: ALG against MCC (r = 0.97), ALG against HEC (r = 0.96), ALG against SUC (r = 0.96), HEC against MCC (r = 0.96) and SUC against TAL (r = 0.97).

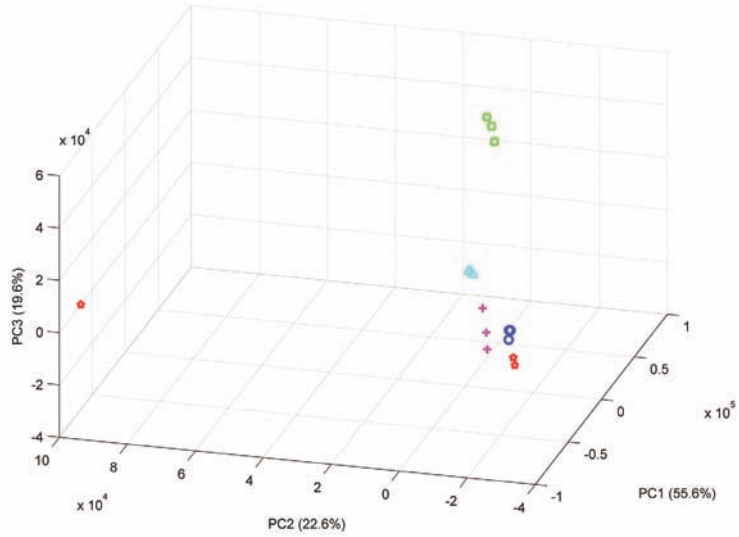

Figure 4 PCA scores plot of the combined data of ALG (blue), HEC (red), MCC (green), SUC (magenta) and TAL (cyan) obtained from both the 785 nm and the 1064 nm handheld Raman spectrometers

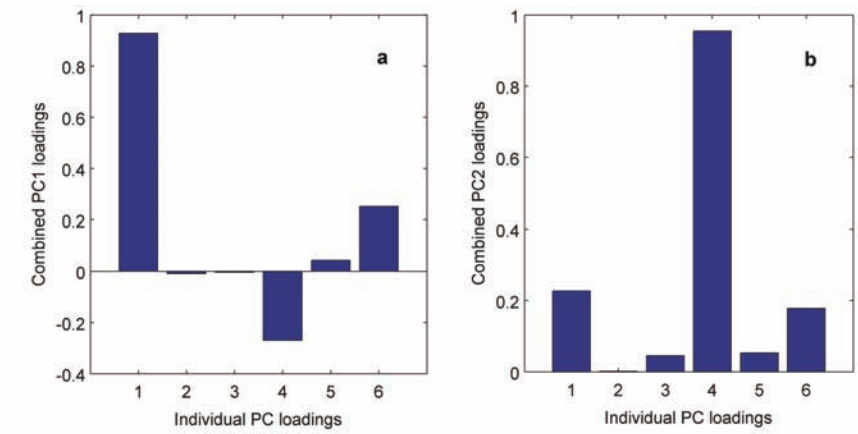

Figure 5 PCA loading plot of the combined data obtained from both the 785 nm instrument and the 1064 nm handheld Raman spectrometers

This may be due to interferences from fluorescence encountered in the Raman spectra of these excipients.

Consequently, PCA was applied to these five excipients' Raman spectra measured using both the 785 and 1064 nm instruments. **Figure 3** shows the PCA scores plot of the Raman spectra obtained using both instruments. In both cases, discrimination was observed among the five excipients. However, the 785 nm instrument showed more accurate identification between individual excipients scores (**Figure 3a**). In this respect, HEC and talc score showed type I error with one score distinct from the other two scores in both cases. Likewise, type I error was observed between PCA scores of ALG, MCC, SUC and TAL when measured using the 1064 nm instrument (**Figure 3b**).

However, this was overcome when the PCA scores from the individual PCAs of the Raman spectra obtained using both instruments were combined. Consequently, the scores of the first three PCs obtained from each instrument were used as variables to construct the new PCA. **Figure 4** (page 30) shows the combined PCA scores of the five excipients. Four of these excipients showed accurate identification and were: ALG, MCC, SUC and TAL. On the other hand, type I error was encountered only among one HEC score. The loadings of the combined PCA (785 and 1064 nm) showed contribution from both individual PCAs (**Figure 5**, page 30). This showed that the use of both instruments gave more accurate identification.

Conclusion

Handheld Raman spectroscopy offered a simple, rapid and non-destructive approach for identification of raw materials. The use of a 785 nm laser showed higher sensitivity and better spectral quality despite the fluorescence and glass interference in some cases. However, the fluorescence encountered from these spectra affected the accuracy of identification of raw material. The use of a 1064 nm laser showed better identification due to less fluorescence encountered in the spectra. However, combining the data obtained from both instruments gave more accurate identification of raw materials. Consequently, the use of both 785 nm and 1064 nm lasers in unison will be a better option for raw material identification.

Biography

Dr Sulaf Assi is an Associate Lecturer in Forensic Sciences at Bournemouth University, Bournemouth, UK. She obtained her Bachelor and MSc in Pharmacy from Beirut Arab University, Beirut- Lebanon. After this, she pursued her PhD in pharmaceutical analysis from the School of Pharmacy University of London, London, UK. Then she undertook a postdoctoral fellowship position in drug misuse and abuse at the department of Pharmacy, University of Hertfordshire, Hatfield, UK.

Her recent research work is dedicated to developing rapid and non-destructive techniques for the identification of counterfeit medicines. Her research interests include pharmaceutical analysis, counterfeit medicines, novel psychoactive substances, spectroscopy and chemometrics.

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