ABSTRACT

Poor quality medicines represent an expanding global public health threat facilitated by the Internet. A recent survey showed that one in five students have used modafinil to enhance learning ability mainly purchased from Internet sources. The aim of this work was to develop on-the-spot and simple methods for the quantification of modafinil in generic medicines using Fourier transform-infrared (FTIR), near-infrared (NIR) and Raman spectroscopy along with partial least square regression (PLSR). Modafinil tablets were measured in intact form using NIR and Raman and in powdered form using FTIR, NIR and Raman. Additionally, powder mixtures of crushed modafinil tablets and excipient(s) were prepared either by diluting the crushed tablets with excipient(s), or sequentially adding excipient(s) to the crushed tablets. Three PLSR models were constructed in Matlab 2014a from powder mixtures and two from intact and powdered tablets. For FTIR and Raman spectroscopy, PLSR models based on tablets gave linear calibration curve with correlation coefficient (r^2) values above 0.94 and a root mean square error of calibration (RMSEC) below 0.96% m/m. Conversely, the PLSR model based on powder sequential addition gave the highest accuracy using the NIR spectra (r^2 = 0.99, RMSEC = 1.15% m/m). The latter model showed accuracy in predicting the concentration of the active pharmaceutical ingredient in modafinil generic medicines proving their authenticity. The overall results showed that the combination of the three spectroscopic methods with PLSR offered a rapid technique for authenticating generic modafinil medicines.

Keywords: Counterfeit medicines; infrared; near-infrared; Raman; spectroscopy; quantification; authentication; partial least square regression.

1. Introduction

Poor quality medicines represent a global threat to the public health that can result in treatment ineffectiveness, drug resistance, increased morbidity and mortality rate, economic loss and problems to the healthcare system [1,2]. Poor quality medicines can be degraded, substandard or counterfeit medicines [3]. Degraded medicines include those, which deteriorate from the poor-quality storage (humidity, temperature and light). Substandard medicines are those that encounter accidental defects in the manufacturing process and fail to fulfil the products' specifications. Counterfeit medicines are medicines which "are deliberately and fraudulently mislabelled with respect to identity and/or source" [4].

The Internet plays a major role in the spread of poor quality medicines, which could be over the counter products, prescription medicines, drugs of abuse and supplementary products [5-7]. This is partly due to the fact that the market of counterfeit online medicines is in continuous expansion [8]. According to the World Health Organisation (WHO), 50% of the medicines sold through illegal online pharmacies are counterfeits [9]. Thus, in 2013 the Interpol closed down over 9600 illegal online pharmacies and seized over 9.6 million prescription medicines that were worth over $41.1 million [10]. In this respect, the purchase of a counterfeit medicine could impose a public safety issue especially in case of drugs of abuse where medicines are frequently bought.

Smart drugs were one of the top classes that sales have increased (up to 50%) over the last decade and has been facilitated by the Internet (International Narcotics Control Board, 2016). Smart Drugs such include nootropics that have become particularly popular among students and healthcare professionals who have been under pressure of study/work [11,12]. Students and healthcare professionals have utilised nootropics for enhancement of the memory and learning abilities [13-15]. There are several different nootropics on the market e.g. adderall, dexedrine, ephedrine, methylphenidate, modafinil and piracetam. Modafinil has the same stimulant effects of nootropics however it has less abusive tendencies and side effects [13,14].

Modafinil is sold under the brand name Provigil [16], and comprises modafinil as the active pharmaceutical ingredient (API), and the following excipients: croscarmellose sodium, lactose monohydrate, magnesium stearate, maize starch and povidone. Nonetheless, the excipients in generic modafinil are not always known and this variation influences the process of authentication of branded and generic modafinil. Thus, when authenticating a branded medicine, the test medicine needs to match the physical and chemical properties of the reference medicine [17]. Generic medicines however only need to prove that they contain the exact API at the correct concentration in relation to the reference medicine [18]. Consequently, a quantitative approach in authenticating generic modafinil medicines is favoured.

The literature reported conventional methods for quantification of the API in modafinil, which range from simple spectrophotometric to chromatographic methods. A spectrophotometric method was reported for the quantification of modafinil in solid dosage forms and underlined measurement of the absorbance of modafinil at its maximum wavelength (236 nm) [19]. Chromatographic methods utilised mainly reverse phase high performance liquid chromatography (RP-HPLC) [19-22], and thin layer chromatography (TLC) [23]. The aforementioned techniques offered, sensitivity, selectivity and precision yet they were time-consuming, destructive and required extensive sample preparation. On the contrary, spectroscopic techniques including Fourier transform infrared (FTIR), near-infrared (NIR) and Raman spectroscopy have shown to be quicker, simpler and mobile [24-33]. When combined with multivariate regression analysis spectroscopic techniques offered rapid, on-spot and non-destructive quantification of APIs medicines [17,32]. To the best of our knowledge, no spectroscopic methods have yet been employed for quantification of modafinil in tablets.

Therefore, this work aimed at developing methods for the on-spot quantification of modafinil in generic medicines using FTIR, NIR and Raman spectroscopy along with PLSR.

2. Materials and Methods

2.1. Materials

Standard reference material including glucose, lactose, magnesium stearate, maize starch, microcrystalline cellulose, modafinil, povidone, sodium carboxymethylcellulose and sucrose were purchased from Sigma-Aldrich.

Eight modafinil generic batches of doses 100 and 200 milligrams (mgs) were bought from four Internet websites (Table 1). The percentage mass per mass (% m/m) of modafinil in the tablets was in the range of 57.6 – 72.7% m/m.

Reference analysis of modafinil API and tablets was performed using reverse phase-high performance liquid chromatography (RP-HPLC) by adopting the procedure given by Rao et al. 2007 [34].

2.2. Sample Preparation

Four types of samples were considered in this study. The first type included intact tablets, which were removed from the packaging and used ‘as received’ without any treatment. The second type comprised powdered tablets which had been crushed in a mortar, homogenised and stored in 4 mm glass vials. The third type of samples comprised powders of pure substances (API and excipients) and the fourth type included powdered mixtures that were prepared by mixing crushed modafinil tablets with excipient(s).

Three modafinil mixtures were prepared and included: M1 (modafinil lactose dilution), M2 (modafinil excipients dilution) and M3 (modafinil excipients sequential addition) (Table 2). M1 (modafinil lactose dilution) was prepared by adding aliquots of lactose (major excipient in modafinil tablets) to crushed modafinil tablets to get a % m/m of modafinil in the range of 9.59 – 62.5% m/m. Similarly, M2 was prepared by adding aliquots of different excipients (one at a time) to crushed modafinil tablets to get 15.4 – 52.9% m/m of modafinil. A third approach was adopted in mixtures (M3) which was made by adding excipients (one at a time) sequentially to aliquots of crushed modafinil tablets to get 15.9 – 62.5% m/m.

2.3. Instrumentation

FTIR spectra were recorded using the Bruker Alpha mobile-FTIR equipped with a single reflection pure diamond attenuated total reflectance (ATR) crystal sample interface. The spectral range of the instrument was 500 – 6000 cm^{-1}. NIR spectra were recorded employing the JDSU microNIR 1700 pro-spectrometer equipped with linear variable filter (LVF) dispersing element and 128-pixel cooled InGaAs photodiode array detector. Spectra were measured over the wavelength range of 900 – 1700 nm. Raman spectra were recorded using the Rigaku FirstGuard handheld Raman spectrometer equipped with 1064 nm laser power, thermoelectric cooling and charge coupled device detector. Spectra were collected over the wavenumber range of 250 – 2000 cm^{-1}.

2.4. Spectroscopic measurements

For FTIR measurements, a few milligrams from powdered samples or pure substances were measured by placing them in direct contact with the ATR crystal. Homogeneous preparations of samples were prepared using a Vortex mixer before each measurement. Four spectra were measured per sample such that a new aliquot was changed after each measurement. Each spectrum was the sum of 16 scans at a resolution of 4 cm^{-1}. For NIR and Raman measurements, intact tablets were measured ‘as received’ by placing them in direct contact with the spectrometers. Four spectra were taken from each tablet on both sides; such as two spectra were taken from each side rotating the tablet after each measurement. In addition, powders were measured through glass vials (after mixing with Vortex mixer) by placing them in direct contact with the instruments. Each spectrum was the sum of 32 scans for NIR and three scans for Raman respectively.

2.5. Data treatment

Spectra from the three instruments were exported to Matlab 2014a for analysis. Spectral pre-treatment was made using multiplicative scatter correction second derivative (MSC-D1). The similarity between spectra was assessed using correlation in wavelength space (CWS) method. In this respect, a correlation coefficient (r) value greater than or equal to 0.95 showed similarity. In addition, quantitative models were developed using partial least square regression (PLSR). PLSR has been considered as ideal in this case where univariate regression had not been possible. This was because the absorbance and scattering intensities in FTIR/NIR and Raman differed according to the physicochemical properties of the measured samples and not proportional to the concentration of the analyte of interest (Burns and Ciurczak, 2007) [35]. In this respect, PLSR offered a multivariate approach for quantifying the APIs in the aforementioned products. PLSR models predicted the concentrations of the different mixtures and/or products from multiple variables (absorbance intensities of scattering intensities measured at the full wavelength range). PLSR models find components (latent variables) in the absorbance and/or scattering intensities that relate to the concentrations. A PLSR model eventually assigns loadings (small and large) to the aforementioned latent variables. Latent variables with small loadings are rejected and vice versa. This is done by finding factors that capture variation among the data such that each factor is added as one at a time. In this sense, the first factor capture the highest variance, the second factor the second highest variance and so on. The following equations illustrate a PLSR model [36-37]:

$$X = T.P + E$$

$$c = T.\, q + f$$

Such as
X absorbance or scattering intensities at different wavelengths
c concentrations
q loading vector
T spectral score vector
p spectral loading vector

3. Results and Discussion

The present study explored a swift quantification of medicines purchased from several Internet sources using handheld instruments. This was first study that had utilised quantitative PLSR models (non-destructive) with portable handheld FTIR, NIR and Raman spectroscopy, as well as a powder form of formulations with FTIR for the quantification of modafinil in branded and generic tablets. The aforementioned PLSR models were not limited to conventional dilution models; but also included more complex mixtures based on standard and sequential additions of constituents to crushed tablets.

Eight modafinil products (from different batches) were purchased from four websites. The eight products were selected based on assessing the differences of authenticity of products between websites as well as within each website. Four of these products had a label claim of modafinil 100 mg; while the remaining four had a label claim of modafinil 200 mg. The concentration range of modafinil in the four products was 57.6 – 72.7% m/m (Table 1). The tablets were compared in relation to the major constituents (API and excipients) expected to be present in branded and generic modafinil tablets. Excipients present in branded modafinil tablets (Provigil) include lactose monohydrate (main excipient), pregelatinised maize starch, croscarmellose sodium, povidone K29/32 and magnesium stearate [15]. The excipient content of generic tablets may be variable and not always known [23], therefore additional excipients were measured including glucose, maize starch, microcrystalline cellulose, sodium carboxymethylcellulose and sucrose. The spectra of modafinil tablets were compared to the spectra of the API, excipient(s) and caffeine using the three techniques.

3.1. FTIR, NIR and Raman activity of modafinil tablets

Prior to spectral evaluation the FTIR, NIR and Raman activity of modafinil tablets and their main constituents had been investigated. When comparing the three spectroscopic techniques in relation to medicines' identification, it is well known that APIs are more Raman active whereas excipients are more IR/NIR active where the Raman activity of excipients is often masked by fluorescence [24]. If the medicine contains high concentrations of an excipient then the Raman activity of the medicine could be masked by the fluorescence exhibited by the excipient. One way of overcoming fluorescence of excipients was by using a longer wavelength laser such as 1064 nm, and that had been adopted in the current work.

The FTIR, NIR and Raman spectra of the medicinal products were compared to those of the API (modafinil) and the major excipient (lactose monohydrate) in modafinil tablets. Modafinil API was present in high amounts in all of the measured products (57 – 72% m/m) which minimised the effect of the excipients [24]. This was confirmed in the spectra of modafinil products, its API and lactose using the three techniques (Fig. 1). In this respect, the modafinil tablets' spectra showed representation of the modafinil API rather than lactose. The FTIR spectrum of modafinil tablet (Fig. 1a) showed higher similarity for the API spectrum ($r = 0.95$) than the lactose spectrum ($r = 0.82$). Likewise the modafinil tablet NIR spectrum showed higher representation for the API spectrum ($r = 0.99$) than lactose spectrum ($r = 0.77$). The modafinil Raman spectrum showed higher similarity for the API spectrum ($r = 0.95$) but dissimilarity to the lactose spectrum ($r = 0.01$). This could be attributed to the strong Raman activity of modafinil API that had not been affected by the fluorescence of lactose. Subsequently, the high representation of the API in the tablets' spectra contributed to the accuracy of quantification of tablets. Crushing the tablets into powders was needed to facilitate data collection and while this process may affect the physical properties of the powder (such as the particle size), our observations showed that the spectroscopic data were not affected. Some properties such as polymorphic nature of API are likely to be affected if strong physical processing was applied however, in our experiments we used gentle processing to ensure minimal energy is applied on tablets. Such delicate processing avoids any polymorphic changes (such as recrystallization or amorphous form formation). The particles should be representative of the tablets content regardless of the size of generated particles; hence reproducibility was not affected by sample preparation.

3.2. PLSR model construction

PLSR was applied to the MSC-D1 FTIR, NIR and Raman spectra over the full wavenumber/ wavelength in each technique. Four models were created using the FTIR spectra and five models were created using the NIR and Raman spectra (Table 3).

FTIR models included: FTIRM1 (modafinil lactose dilution), FTIRM2 (modafinil excipients dilution), FTIRM3 (modafinil excipients sequential addition) and FTIRM4 (modafinil powdered tablets model). FTIRM1, FTIRM2 and FTIRM3 were constructed using a calibration validation (C: V) ratio of 2:1. Moreover, the calibration ranges used were 9.49 – 62.5, 15.4 – 52.9 and 15.9 – 62.5% m/m respectively. The modafinil powdered tablet model (FTIRM4) was constructed with a C: V ratio of 3:1, four factors and a range of 54.9 – 62.4% m/m.

NIR models included NIRM1 (modafinil lactose dilution), NIRM2 (modafinil excipients dilution), NIRM3 (modafinil excipients sequential addition), NIRM4 (modafinil powdered tablets model) and NIRM5 (modafinil intact tablet model). NIRM1, NIRM2 and NIRM3 were constructed with a C: V ratio of 2:1, and had calibration ranges of 9.49 – 62.5, 15.4 – 52.9 and 15.9 – 62.5% m/m respectively. In addition, NIRM4 and NIRM5 were created with a C: V ratio of 3:1 and calibration range of 54.9 – 62.5% m/m respectively.

The Raman models used were: RamanM1 (modafinil lactose dilution), RamanM2 (modafinil excipients dilution), RamanM3 (modafinil excipients sequential addition), RamanM4 (modafinil powdered tablets model) and RamanM5 (modafinil intact tablet model). RamanM1, RamanM2 and RamanM3 were made with a C: V ratio of 2: 1, and had calibration range of 9.49 – 62.5, 15.4 – 52.9 and 15.9 – 62.5% m/m respectively. Furthermore, RamanM4 and RamanM5 were constructed with a C: V ratio of 3:1 and calibration range of 54.9 – 62.5% m/m respectively.

3.3. PLSR model validation

The linearity of the models was evaluated by internal validation criteria calculated using the calibration and internal validation sets. For internal validation, the criteria considered were the regression correlation coefficient (r^2), root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) of the internal validation set. The r^2 and RMSEC were calculated by interpreting the relationship between the predicted concentration and the nominal concentration of the calibration set. Likewise, the RMSEP was calculated by interpreting the relationship between the predicted concentration and the nominal concentration of the validation set. If the model was a good fit, the relationship would be linear and an r^2 value close to 1 would be obtained. There was no optimal value for the RMSEC and RMSEP however, the lower they were the more accurate was the model. A more accurate judgement was made by evaluating the relative standard error of prediction (RSEP); which was calculated as the percentage of the ratio of RMSEP to the mean value of the prediction set. A threshold value of ±5% was taken for RSEP.

For FTIR models, the highest accuracy was observed for FTIRM1 (modafinil powdered tablet model), which showed r^2 values of 0.98 and 0.97 for the calibration and validation sets respectively (Table 2). FTIRM1 also showed the high precision among the models with close RMSEC and RMSEP values, which were 0.52 and 0.78% m/m respectively. Moreover, the RSEP value of FTIRM4 was 1.33%. The worst model in relation to accuracy and precision among the FTIR models was FTIRM2. Thus, this model showed very low r^2 values which were 0.51 and 0.49 for both the calibration and validation sets respectively. Moreover, the model showed high RMSEC, RMSEP and RSEP values of 11.2% m/m, 11.6% m/m and 29.8% respectively. This indicated that although the model was repeatable, it had low precision as the error values were not satisfactory. Similarly, FTIRM3 (modafinil excipients dilution) showed close RMSEC and RMSEP values of 6.57 and 4.63% m/m respectively; yet, high RSEP value of 13.55%. FTIRM3 showed low accuracy of calibration with r^2 value of 0.75. The same pattern was observed with FTIRM4 that had close values of RMSEC (6.29% m/m) and RMSEP (7.03% m/m) and high RSEP value (19.8%). The lower accuracy in models based on mixtures rather than tablets could be attributed to the small amount of measurements (few milligrams) taken per sample. In this respect, the higher the complexity of the sample (as the case of powdered tablets), the more representation of the sample was in the FTIR spectrum.

NIR models showed the highest accuracy for NIRM3 (modafinil excipients sequential addition) which gave r^2 values for the calibration and validation sets of 0.99 and 0.99 respectively (Table 3). NIRM3 showed high precision with RMSEC and RMSEP values of 1.15 and 1.21 correspondingly. Moreover, it showed an RSEP value of 3.45%. Additionally, the two tablet based models showed high precision but slightly lower accuracy than NIRM3. These included NIRM4 (modafinil powdered tablet model) and NIRM5 (modafinil intact tablet model) which had r^2 values of calibration of 0.77 and 0.69 individually. Both models were highly precise and showed RMSEC values below 2% m/m and RSEP values below 4%. The remaining two powder models (NIRM1 and NIRM2) showed slightly lower accuracy but very poor precision. Thus, the r^2 values of calibration for NIRM1 (modafinil lactose dilution) and NIRM2 (modafinil excipients dilution) were 0.72 and 0.84. Both of these models showed good repeatability with very close RMSEC and RMSEP values. Thus, NIRM1 showed RMSEC and RMSEP values of 8.45 and 8.82% m/m respectively. Likewise, NIRM2 showed RMSEC and RMSEP values of 5.26 and 5.25% m/m but had very poor external precision in the range of 15 – 23%.

Raman models showed the highest accuracy/precision for RamanM4 (modafinil powdered tablet model) and Raman M5 (modafinil intact tablet model) (Table 3). The aforementioned two models showed r^2 value of calibration of 0.98 and 0.94. In addition, the RMSEC and RMSEP values for RamanM4 were 0.54 and 0.82% m/m, whereas for RamanM5 these values were 0.96 and 0.91% m/m respectively. RamanM4 provided a more precise model as it showed ten times lower RSEP value (1.4%) than RamanM5 (12.1%). The models based on powdered mixtures gave lower accuracy and precision than tablet based models. In this sense, RamanM1 (modafinil lactose dilution), RamanM2 (modafinil excipients dilution) and RamanM3 (modafinil excipients sequential addition) had low r^2 values of calibration which were 0.70, 0.84 and 0.76 respectively. The three aforementioned models had high RSEP values which were in the range of 17 – 24%. All three models showed close agreement between their RMSEC and RMSEP values (Table 3).

3.4. Prediction of modafinil in generic tablets

Test sets of powdered and intact tablets were used to examine the external predictive ability of the models. The predicted value was converted into label claim and the percentage label claim of each product was assessed. The pharmacopoeia acceptable deviation of the API for tablets is usually ±5% of the label claim in order to allow variation in production, degradation during shelf life of the product and accuracy of the analytical method. In this work the range was extended to ±30% of the label claim to compensate for difficulty in setting up a calibration in the spectra and account for the noise generated by the instrument/spectral algorithms [16].

For powdered tablets, all the eight products were predicted through the powdered dilution models (Table 4). In this respect, the best predictive ability was observed for NIRM3 which showed a mean prediction of 98.2% label claim (RSD = 2.35%) for all batches. This was followed by FTIRM3 and NIRM1, which showed mean prediction values of 97.9 and 97.2% label claim respectively. Additionally, FTIRM2 and NIRM2 showed good predictive ability with values of 91.1 and 90.2% label claim respectively. The remaining models (FTIRM1, RamanM1, RamanM2 and RamanM3) exhibited poor predictive ability below 70%.

RamanM2 and RamanM3 showed better prediction for intact tablets (Table 5). Thus, the mean prediction of intact tablets using the two models were 108 and 84% label claim respectively. Moreover, NIRM2 showed good predictive ability for intact tablets with a mean prediction of 103% label claim. The remaining models included NIRM1, NIRM3 and RamanM1 had poor prediction above 130% label claim.

4. Conclusions

The findings demonstrated that the combination of handheld FTIR, NIR and Raman spectroscopy with PLSR offered a rapid method for quantifying modafinil in branded generic medicines with minimal sample preparation. NIR and Raman techniques were non-destructive, however FTIR required powdering the tablets prior to measurement. In comparison to NIR, FTIR and Raman showed that models based on tablets were more accurate than those based on powder mixtures. Among the powder mixture models, modafinil excipients sequential addition offered the highest accuracy and precision for the quantification of powdered tablets using FTIR and NIR spectroscopy. Modafinil excipient dilution models offered the highest accuracy and precision for the quantification of intact tablets using NIR and Raman spectroscopy. Consequently, the choice of the powder model depended to a degree, on the technique used as well as the sample quantified. Subsequently, this may represent a challenge in the generalisability of the method to other nootropics that could be of different concentration and have different formulation. Hence, future work should consider the accuracy of quantification for different formulation types (tablets, capsules, caplets) and/or closely related analogues of drugs.

List of abbreviations:

Active pharmaceutical ingredient (API)

Correlation in Wavelength Space (CWS)

Fourier transform-infrared (FTIR)

High performance liquid chromatography (HPLC)

Near-infrared (NIR)

Partial least square regression (PLSR)

Relative standard error of calibration (RSEC)

Relative standard error of prediction (RSEP)

Root mean square error of calibration (RMSEC)

Thin layer chromatography (TLC)

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List of tables

Table 1. Modafinil tablets purchased from the Internet

Website number	Batch number	Dose (mg)	Modafinil concentration (% m/m)
1	1a	100	57.6
1	1b	100	62.9
2	2a	200	65.9
2	2b	200	72.7
3	3a	200	63.8
3	3b	200	66.3
4	4a	100	57.7
4	4b	100	62.8

Table 2. Details of the powdered mixtures prepared

DN	Modafinil tablet amount (mg)	Diluent	Diluent amount (mg)	Total weight (mg)	API (% m/m)
M1V1	201.3	LAC	0	201.3	62.5
M1V2	181.6	LAC	19.6	201.2	56.4
M1V3	168.5	LAC	32.6	201.1	52.4
M1V4	159.5	LAC	43.3	202.8	49.1
M1V5	151.2	LAC	53.1	204.3	46.2
M1V6	129	LAC	71.7	200.7	40.2
M1V7	110.9	LAC	90.6	201.5	34.4
M1V8	99.3	LAC	98.9	198.2	31.3
M1V9	81	LAC	119.9	201	25.2
M1V10	71.8	LAC	129.7	201.5	22.3
M1V11	50.8	LAC	150	200.8	15.8
M1V12	30.6	LAC	170.7	201.3	9.5
M1V13	0	LAC	199.6	199.6	0
M2V1	169.6	LAC/ POV	30.6	200.2	52.9
M2V2	163	LAC/POV/ MgS	52.4	215.4	47.3
M2V3	121.9	LAC/ POV/ MgS/ MAI	79.4	201.3	37.8
M2V4	104.1	LAC/POV/ MgS/MAI/ MCC	97.2	201.3	32.3
M2V5	76.8	LAC/MCC/ NaCMC	134.9	211.7	22.7
M2V6	51.8	LAC/POV/ MgS/MAI/ MCC/NaCMC	158.9	210.7	15.4
M3V1	201.3	0	0	201.3	62.5
M3V2	180.3	LAC	43.2	223.5	50.1

M3V3	134.4	POV	78.8	213.1	39.4
M3V4	100.7	MgS	97.2	197.8	31.8
M3V5	87.2	MAI	128	215.2	25.3
M3V6	71	MCC	149.1	220.1	20.2
M3V7	52.7	NaCMC	154.5	207.2	15.9

DN: dilution number, M1: modafinil lactose dilution, M2: modafinil excipients dilution, M3: modafinil excipients sequential addition, LAC: lactose, POV: povidone, MgS: magnesium stearate, MAI: maize starch, MCC: microcrystalline cellulose, NaCMC: sodium carboxymethylcellulose.

Table 3. Results of the PLSR models constructed using the three techniques

Model number	F	C:V ratio	r^2calib	RMSEC (% m/m)	r^2valid	RMSEP (% m/m)	RSEP (%)
FTIRM1	3	25:11	0.98	0.52	0.97	0.78	1.33
FTIRM2	1	12:60	0.51	11.24	0.49	11.61	29.9
FTIRM3	1	14:70	0.75	6.57	0.93	4.63	16.5
FTIRM4	4	60:20	0.84	6.29	0.80	7.03	19.8
NIRM1	1	25:11	0.72	8.45	0.70	8.82	23.3
NIRM2	1	12:60	0.84	5.26	0.84	5.25	15.2
NIRM3	3	14:70	0.99	1.15	0.99	1.21	3.45
NIRM4	1	60:20	0.77	1.77	0.69	2.05	3.51
NIRM5	1	48:16	0.69	1.91	0.76	1.71	2.85
RamanM1	1	25:11	0.70	8.49	0.80	6.74	19.0
RamanM2	1	12:60	0.84	5.52	0.83	6.57	17.8
RamanM3	1	14:70	0.76	7.62	0.83	9.16	23.9

RamanM4	4	60:20	0.98	0.54	0.95	0.82	1.40
RamanM5	4	48:16	0.94	0.96	0.93	0.91	12.1

FTIRM1, NIRM1and RamanM1: modafinil lactose dilution, FTIRM2, NIRM2 and RamanM2: modafinil excipients dilution, FTIRM3, NIRM3and RamanM3: modafinil excipients sequential addition, FTIRM4, NIRM4 and RamanM4: modafinil powdered tablets model, NIRM5 and RamanM5: modafinil intact tablet model. C:V: calibration:validation ratio, F: number of factors, r^2: correlation coefficient, RMSE:root mean square error.

Table 4. Results of the predicted powdered tablets

	Predicted label claim (%)							
	1a	1b	2a	2b	3a	3b	4a	4b
BN								
Dose (mg)	100	100	200	200	200	200	100	100
FTIRM1	24.9	57.7	49.4	43.8	70.2	49.4	28.1	42.6
FTIRM2	98.5	96.4	86.8	87.8	88.0	87.5	91.2	92.9
FTIRM3	105	104	93.1	94.1	94.6	93.9	98.4	100
NIRM1	100	98.6	95.3	95.2	95.9	93.4	101	98.3
NIRM2	92.0	89.5	88.8	88.4	89.6	87.1	94.4	91.4
NIRM3	97.6	94.7	99.2	99.2	101	96.9	101	96.0
RamanM1	91.1	60.7	55.4	55.8	56.0	55.2	59.1	60.6
RamanM2	74.0	46.2	44.9	44.9	44.9	44.5	46.7	48.7
RamanM3	80.6	65.9	64.0	63.2	65.8	64.0	67.5	68.4

BN: Batch number

Table 5. Results of the predicted intact tablets

BN	Predicted label claim (%)					
	2a	2b	3a	3b	4a	4b
Dose (mg)	200	200	200	200	100	100
NIRM1	134	135	137	136	133	133
NIRM2	107	107	108	110	95.4	89.1
NIRM3	144	145	148	150	125	118
RamanM1	186	188	170	182	205	210
RamanM2	101	106	97.6	104	119	122
RamanM3	77.9	81.0	72.5	77.7	94.5	100.9

BN: Batch number

Figure legend

Fig. 1. MSCD1 treated (a) FTIR spectrum modafinil tablets, (b) FTIR spectrum of pure modafinil, (c) FTIR spectrum lactose monohydrate, (d) NIR spectrum modafinil tablets, (e) NIR spectrum of pure modafinil, (f) NIR spectrum lactose monohydrate, (g) Raman spectrum modafinil tablets, (h) Raman spectrum of pure modafinil and (i) Raman spectrum lactose monohydrate measured using the Bruker Alpha FTIR, JDSU microNIR and Rigaku handheld Raman instruments respectively.