Portable Transmission Raman Spectroscopy for At-Line Content Uniformity Testing of Pharmaceutical Tablets

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Introduction

Content uniformity (CU) testing is a crucial task in pharmaceutical manufacturing, as it ensures that each product that reaches a consumer contains a safe dosage of the active pharmaceutical ingredient (API). Traditionally, high performance liquid chromatography (HPLC) was performed off-line in a quality control lab to monitor the dosage of finished tablets due to its sensitivity and the ability to obtain a measurement from the entire volume of the tablet. However, HPLC analysis involves large amount of solvent and consumables, and often takes hours to complete. This slow and destructive technique requires highly trained personnel and can cause delays in the manufacturing process

Analytical methods to perform CU testing should ideally be fast, noninvasive and achieved with limited sample preparation. Recently, transmission near-infrared (NIR) spectroscopy and transmission Raman spectroscopy have both been explored as alternative methods for rapid and non-destructive on- and at-line CU testing with no sample preparation. Although quick and nondestructive, transmission NIR spectroscopy suffers from poor chemical selectivity and is sensitive to changes in the testing environment. Transmission Raman spectroscopy combined with chemometric modeling is quickly emerging as a valued technique for CU testing due to its high chemical specificity, which is particularly useful when dealing with complex pharmaceutical formulations that contain multiple components.

Conventional Raman spectroscopy collects back-scattered signal upon illumination of the sample with a laser, probing the surface (Figure 1a). While conventional Raman spectroscopy is quite suitable for identity tests of raw materials in the pharmaceutical industry, it does not provide an accurate quantitative measurement representative of the entire volume of a tablet- nor is it able to probe sufficiently beneath layers of coatings on finished tablets. Transmission Raman spectroscopy, however, is able to overcome the limitations of conventional Raman spectroscopy for content uniformity testing. Using transmission Raman spectroscopy, the sample is

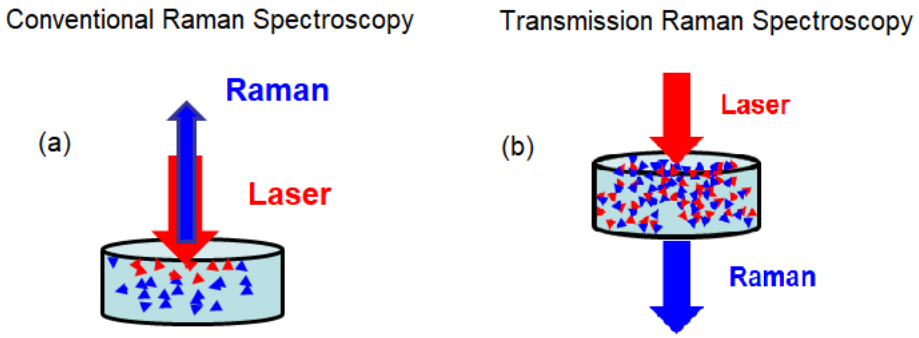

Fig. 1 (a) A conventional Raman spectroscopy setup versus (b) a transmission Raman spectroscopy setup

illuminated at one side, and signal is collected from the other side (Figure 1b). The signal collected contains photons with information that is representative of the full volume of the tablet. Through the use of chemometric models, an accurate quantitative prediction of the content within the tablet can be achieved.

Powered by B&W Tek's patent-pending STRaman™ technology, the QTRam™ allows for rapid and simple transmission Raman measurements to perform CU testing of pharmaceutical tablets. In addition to the transmission Raman capability, the QTRam is also equipped with convention Raman spectroscopy setup with a large laser spot size which makes it capable of assessing blend uniformity (BU) in any sample form, assisting in formulation development, and detecting pharmaceutical counterfeits.

In this case study, proof of concept is demonstrated for a CU analysis with a tablet formulation containing acetaminophen, mannitol, lactose, cellulose, and magnesium stearate. Data was collected on the QTRam, and chemometric models for quantitation of acetaminophen and lactose were created using BWIQ®, a chemometric model-building software. Models were then validated on the QTRam using BWAnalyst™-the 21CFR Part 11 compliant software that is on board the integrated touch screen tablet PC- to create quick and accurate predictive CU tests.

Experimental

Samples

Formulations were blended with lab-grade acetaminophen, mannitol, lactose, microcrystalline cellulose (MCC), and magnesium stearate. Five different blends were created. The concentration of acetaminophen in these blends ranged from 10-18% (w/w) and the concentration of lactose ranged from 10.79-28.66% (w/w). Blends were compressed to create round tablets approximately 6 mm in diameter and 4.5 mm thick using a Gamlen tablet press. Tablets were pressed with an approximate force of 392 kg. Each pill had a typical mass of ~140 mg. Ten tablets were manufactured from each blend. Table 1 lists the concentration of ingredients for all blends.

Table 1. Concentrations of Ingredients in Sample Tablets (w/w%)

Blend #	APAP	Mannitol	Lactose	MCC	Magnesium stearate	Total
1	18	27.32	10.79	42.90	1.00	100
2	16	20.41	16.22	46.37	1.00	100
3	14	15.62	20.53	48.85	1.00	100
4	12	23.31	28.66	35.03	1.00	100
5	10	28.05	18.03	42.92	1.00	100

Measurements

The QTRam from B&W Tek (Figure 2) with BWAnalyst software was used for all analyses. The system uses a 785-nm laser with a maximum power of >340 mW. The spot size of the laser used was ~4 mm. For method development, method validation, and prediction analyses, all measurements used 100% of the maximum laser power and a 20 second integration time.

Measurements were performed on both sides of the tablets for an accurate representation of the full volume of the tablet. Spectra were exported into BWIQ®, B&W Tek's chemometric software for creating quantitative models.

Fig 2. QTRam portable Raman spectrometer with the QT-Sampler for transmission Raman measurements. BWAnalyst software (shown on the QT Ram) was used for all analyses.

Results

Method creation

Seven tablets from each blend were used to create a method in the BWAnalyst software. Three spectra were collected for each sample, totaling 105 method spectra. Spectra were imported into BWIQ software to create quantitative models for acetaminophen and lactose. Figure 3 shows 105 spectra as imported in BWIQ software. Due to the compositional differences between blends, the extent of the background signal varies between the spectra.

A random sampling algorithm was selected to designate each spectrum as a calibration or

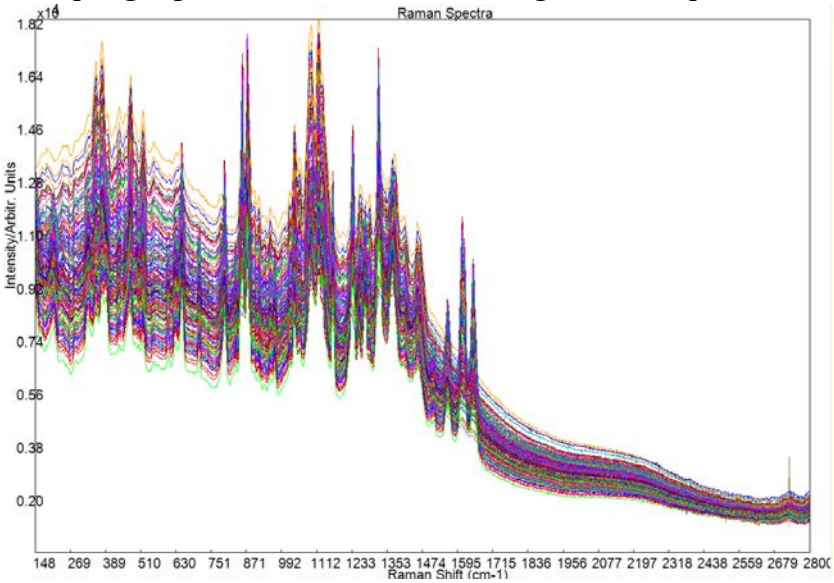

Fig. 3 Method spectra imported into BWIQ

validation sample. Seventy percent of the data was randomly designated as calibration spectra, and 30% were chosen as validation spectra. No statistical outliers were found after a leverage outlier test was performed.

Spectra regions corresponding to signal from acetaminophen and signal from lactose were isolated for chemometric modeling. Figure 4 shows the comparison of the transmission Raman spectrum from a tablet containing 18% acetaminophen and 10.79% lactose to the Raman spectra from the four main components in the formulation; the spectral regions used in the model are highlighted in blue and gray for lactose and acetaminophen, respectively. The spectral regions of 1190-1300 cm^{-1} and 1530-1700 cm^{-1} were isolated to model acetaminophen because their peaks correspond purely to acetaminophen. Spectral regions of 285-500 cm^{-1} and 840-890 cm^{-1} were isolated for lactose because their peaks correspond purely to lactose. All blends show signal for both acetaminophen and lactose in these regions.

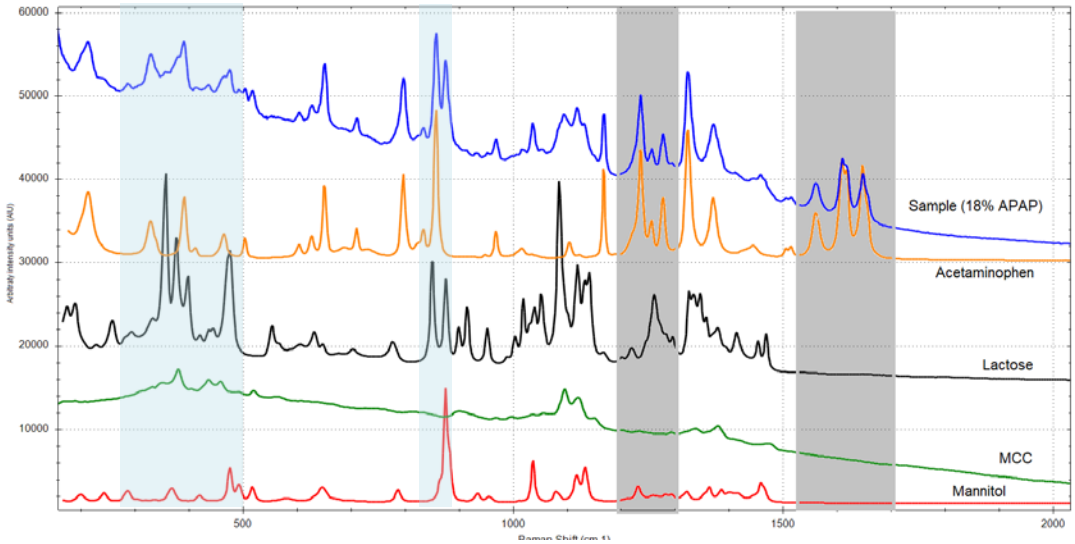

Fig. 4 Transmission Raman spectrum from a tablet sample compared to the four main components of the tablets. The blue regions correspond to the areas for lactose modeling. The gray regions correspond to the areas for acetaminophen modeling. Spectra are manually offset.

Chemometric Model Building

Several preprocessing steps were applied to the data in order to minimize background effects and baseline offsets. An adaptive iteratively reweighted Penalized Least Squares (air-PLS) background removal was used in order to mitigate the background effects from the excipients, and a multiplicative scatter correction (MSC) was used to offset effects from baseline differences. A first derivative (Savitzky-Golay, window 11, order 3) was also applied to the data. The data was modeled using a partial least squares regression (PLS). Figure 5 shows the method spectra after preprocessing steps were applied.

Figure 6 shows the plots of the predicted vs. measured values for both the calibration and validation samples for the acetaminophen and lactose PLS models. Four factors were used in the

acetaminophen model, while five factors were used in the lactose model. The R^2 coefficient is a measure of how close the data are to the regression line. The R^2 value of the calibration spectra for acetaminophen and lactose models are 0.9929 and 0.99149, respectively. The root mean square error (RMSE) of the acetaminophen calibration curve is 0.23282 and the RMSE of the lactose calibration curve is 0.53141.

Method Validation

The BWIQ models were imported into the BWAnalyst software. A method containing the models for acetaminophen and lactose was constructed. Each model in the method is given upper and lower limits, for a simple "Pass" or "Fail" result. For the acetaminophen component, the lower limit was 10% and the upper limit was 14%. For the lactose component, the lower limit was 18% and the upper limit was 30%. Separate validation sets (samples not included in the calibration model) were collected directly on QTRam following the method generation work flow. Two tablets per blend were chosen as validation samples, with three spectra collected for each sample. The result for the prediction of the validation samples are listed in Table 2 for acetaminophen model and Table 3 for lactose model.

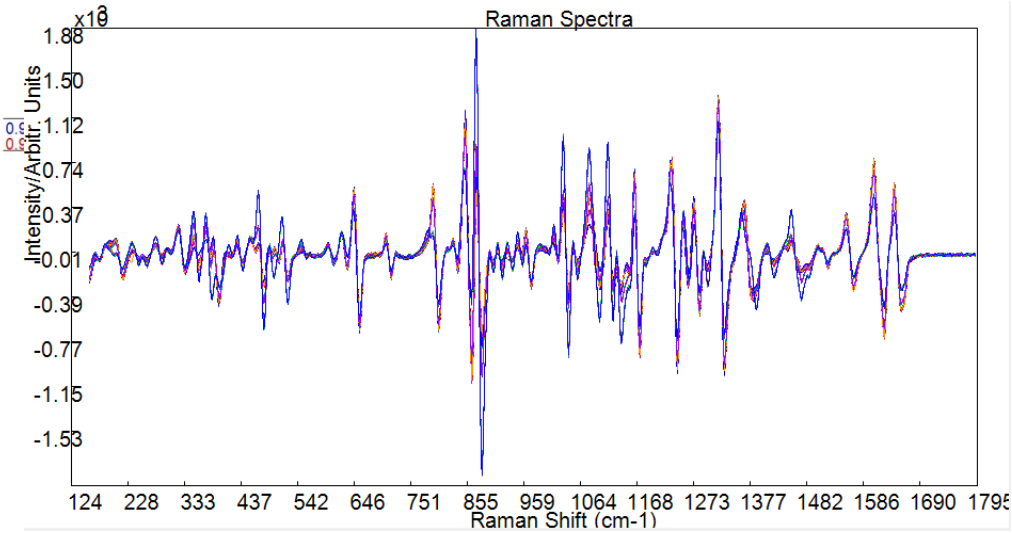

Fig. 5 Method spectra after air-PLS background correction, MSC, and Savitsky-Golay first derivative.

(a)

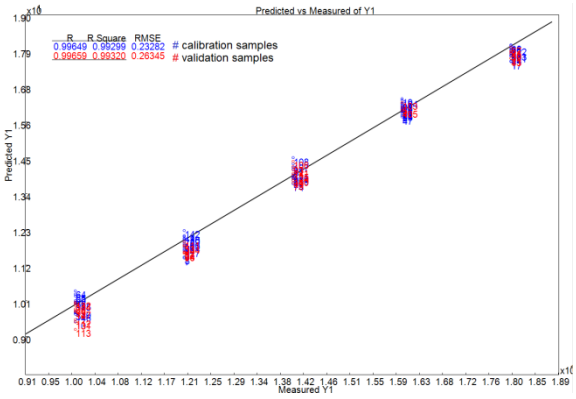

Fig. 6a Predicted vs measured plot of the calibration and validation samples for the acetaminophen PLS model. The wavenumber regions used in the model are from 1190-1300 cm^{-1} and 1530-1700 cm^{-1}. Four principal components are used in the model.

(b)

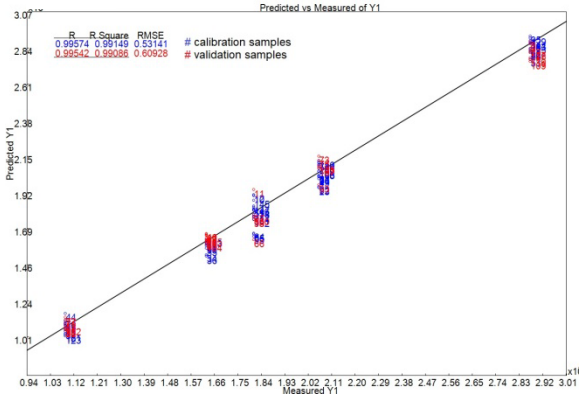

Fig. 6b Predicted vs measured plot of the calibration and validation samples for the lactose PLS model. The wavenumber regions used in the model are from 285-500 cm^{-1} and 840-890 cm^{-1}. Five principal components are used in the model.

Table 2 Validation results for acetaminophen PLS model

Blend	True acetaminophen concentration (%)	Average (n=3) acetaminophen concentration prediction of validation samples (%)	Standard deviation (n=3) (%w/w)
1	18	17.61	0.13
		17.66	0.06
2	16	15.67	0.14
		15.74	0.11
3	14	14.31	0.08
		14.37	0.17
4	12	11.90	0.06
		11.89	0.02
5	10	9.95	0.16
		9.88	0.08

By executing the Method Validation function on the BWAnalyst, the method performance parameters are generated from the method validation process and included in the method validation report. Table 4 summarizes the method parameters.

Table 3 Validation results for lactose PLS model

Blend	True lactose concentration (%)	Average (n=3) lactose concentration prediction of validation samples (%)	Standard deviation (n=3) (%w/w)
1	10.79	10.64	0.20
		10.83	0.20
2	16.22	15.49	0.22
		16.06	0.13
3	20.53	20.44	0.19
		20.36	0.26
4	28.66	28.34	0.17
		27.88	0.36
5	18.03	17.63	0.33
		18.66	0.35

Table 4 Method characteristics from method validation result

Method	Accepted Range (% w/w)	Linearity	Accuracy (%w/w)
Acetaminophen	10 – 14	0.9889	0.2883
Lactose	18 – 30	0.9923	0.5040

Prediction

The method was subsequently released in the BWAnalyst software, which allows Operator-level users to load the method for prediction of new samples. If the value calculated for either of the components is outside of the set lower and upper limits, the software will present a “Fail” message (Figure 7a), signaling the user that a sample is not within the guidelines that have been set. If both components are found to be within the set lower and upper limits, the sample will “Pass” (Figure 7b).

One tablet per blend was chosen as an independent sample to test the prediction of the acetaminophen and lactose content using the models created. Three spectra were collected for each sample. The prediction results for each tablet are listed in Table 5. The tablet from blend 4 is the only sample that meets the set Pass/Fail criteria set in BWAnalyst.

Table 5 Prediction results for acetaminophen and lactose

Blend	True acetaminophen value (%w/w)	Average (n=3) predicted acetaminophen content (%w/w)	Standard deviation (%w/w)	True lactose value (%w/w)	Average (n=3) predicted lactose content (%w/w)	Standard deviation n=3 (%w/w/)	BWAnalyst Result
1	18	17.6	0.17	10.79	11.41	1.67	**Fail**
2	16	16.11	0.17	16.22	15.43	0.07	**Fail**
3	14	14.39	0.03	20.53	20.71	0.40	**Fail**
4	12	11.95	0.24	28.66	28.09	0.16	**Pass**
5	10	9.94	0.03	18.03	18.41	0.25	**Fail**

(a) (b)

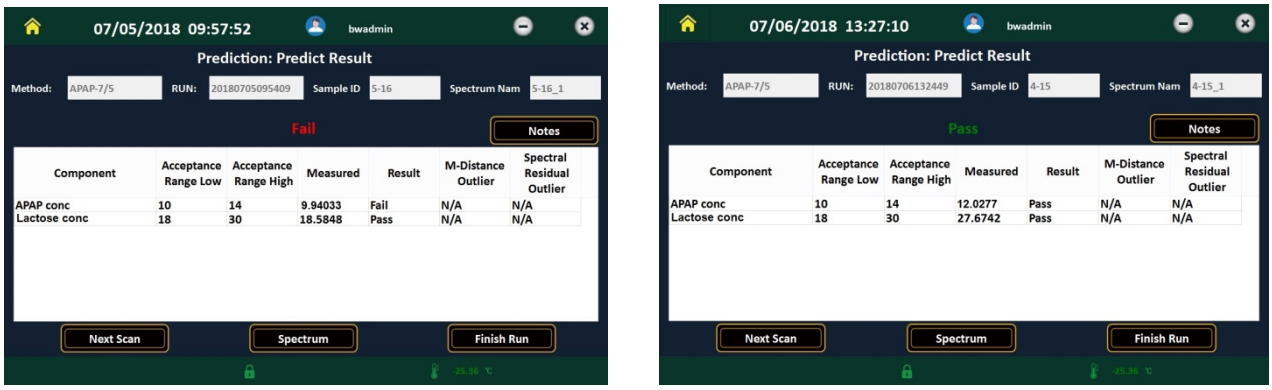

Fig. 7 BWAnalyst software prediction interface. In 7a, the acetaminophen content is outside of the set lower and upper limits, yielding a "Fail" result. In 7b, both lactose and acetaminophen concentrations are within the set lower and upper limits, yielding a "Pass" result.

Conclusion

Portable transmission Raman spectroscopy is an effective tool for rapid, at-line content uniformity testing. It is demonstrated through a matrix of tablets of varying compositions that the QTRam from B&W Tek, along with the softwares BWAnalyst and BWIQ, is able to build methods that can accurately quantitate the acetaminophen and lactose components. As an alternative method of content uniformity testing, transmission Raman spectroscopy provides a simple and non-destructive analysis method with no solvent consumption and no consumables.

