Application Note

Pharmaceuticals

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Evaluating Repeatability and Sensitivity of Transmission Raman Spectroscopy

Comparing measurements of API standards by Agilent TRS100 quantitative pharmaceutical analysis system with HPLC

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Abstract

Transmission Raman Spectroscopy (TRS) is a scattering technique that provides structural information in terms of quantity and identity. Unlike conventional Raman, TRS is a bulk analysis technique as laser light diffuses across solid materials, making it suitable for Uniformity Content Analysis (UCA) of pharmaceuticals. This work demonstrates how to evaluate the reproducibility and sensitivity of the Agilent TRS100 instrument based on a real case scenario of a drug containing 17% active pharmaceutical ingredient (API).

Introduction

Raman spectroscopy is a light-scattering technique used for structural characterization and quantitation of Raman active materials. In transmission mode, light from a laser diffuses across the sample through multiple scattering processes, as illustrated in Figure 1. The light that is then collected by the detector is representative of the bulk of the material. This bulk analysis property is the reason why Transmission Raman Spectroscopy (TRS) is especially suited for Uniformity Content Analysis (UCA) of pharmaceuticals in solid dose forms.[1–3] For quantitative measurements by TRS, prediction models are built using a series of calibration standards containing the component of interest, for example, the active pharmaceutical ingredient (API). The TRS method can then be used for the direct, non-destructive quantitative measurement of the target component in samples.

The reproducibility of TRS readings, measured in terms of the standard deviation of the prediction model errors, is limited by two factors: sample thickness and the distribution of different components in the sample. The choice of laser beam size, which ranges between 2 and 8 mm in diameter, helps to increase the representation of the sample in heterogeneous samples. Optimizing the beam diameter can bring the reproducibility of TRS close to the levels of HPLC, where aliquots of a diluted sample are analyzed. It is also important to use a robust model for TRS, meaning a model that continues to predict accurately after long periods of time.

Sensitivity is another important aspect of TRS. The sensitivity of the technique depends on the existence of distinctive and unique bands for the components of a sample that are used for quantitation. Sensitivity is typically expressed in terms of limits of detection (LOD) and limits of quantitation (LOQ).

To demonstrate the reproducibility and sensitivity of the Agilent TRS100 for UCA of APIs in a typical case scenario, a pharmaceutical company supplied the sample and calibration standards.

Experimental

Instrumentation

A simple representation of the TRS100 spectrometer is shown in Figure 1. The instrument uses an 830 nm red laser with a 650 mW maximum output power at the sample position. A system of lenses is used to accommodate the spot size of the laser (2, 4, and 8 mm diameter) to the size of the sample. The TRS100 is also equipped with a set of three different collection lenses (small, medium, and large).

In this study, a 4 mm diameter laser beam and a medium collection lens were used. The exposure time per accumulation was set to the top of the dynamic range of the detector (40 K counts) and the number of accumulations was set to achieve a suitable signal-to-noise ratio. Instrument operating parameters are given in Table 1.

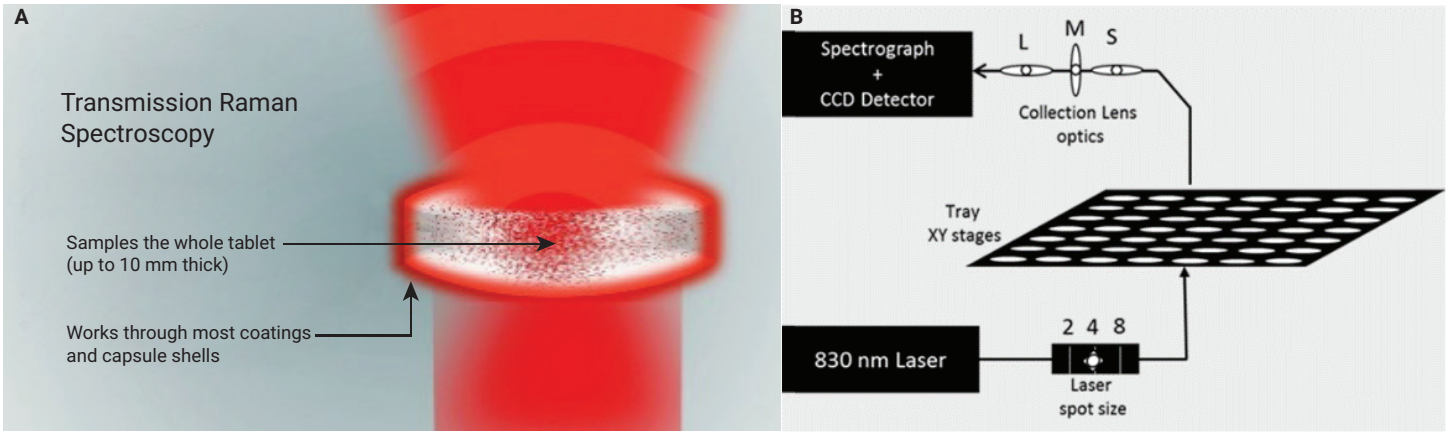

Figure 1. (A) Diagram of how light diffuses across a sample (tablet) in TRS. (B) The adjustable hardware settings of the Agilent TRS100 spectrometer.

Samples and calibration standards

All samples and calibration standards were supplied by a pharmaceutical company. The targeted API concentration in the final drug product was 17.00% w/w (commonly referred to as the Label Claim, LC). Round tablets containing the API within a ±15% range relative to the targeted concentration were used as calibration standards (Table 1). The calibration standards had been prepared gravimetrically and the API-concentrations had been determined by HPLC by the pharmaceutical company. The HPLC data (referred to a "measured") was used as a Y-block to create the TRS prediction model. Twenty tablets at each API concentration level were loaded in the sample tray. A tray that best fits the dimensions of the drug was used, as shown in Figure 2.

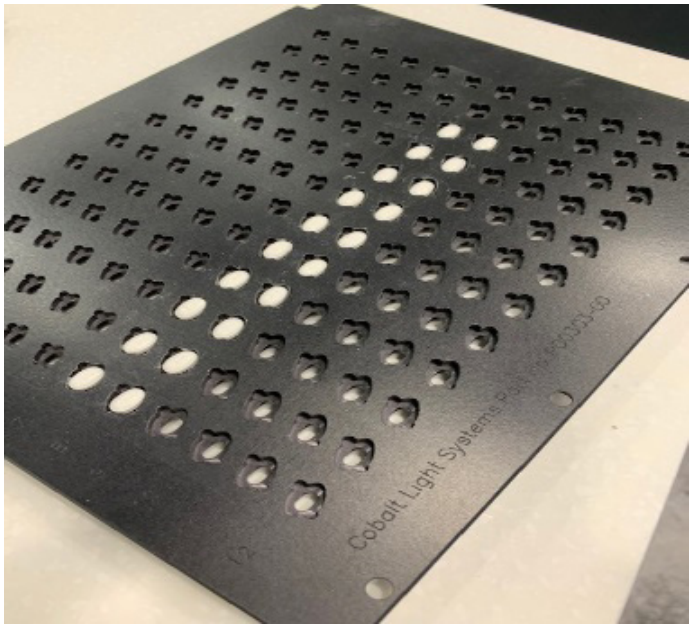

Figure 2. 20 replicate calibration standard tablets containing one level of API concentration in an Agilent TRS100 sample tray.

Table 1. Calibration standard API concentrations, number of replicates, Agilent TRS100 settings, and total measurement times.

API Content of Calibration Standard			TRS100	
Intended Label Claim %	Intended w/w%	Number of Replicates	Operating Conditions	Total Measurement Time per Sample (s)
85	14.45	20	Laser 0.65 W, exposure 1.3 s, accumulations 20	26
90	15.30	20	Laser 0.65 W, exposure 1.2 s, accumulations 20	24
95	16.15	20	Laser 0.65 W, exposure 1.2 s, accumulations 20	24
100	17.00	20	Laser 0.65 W, exposure 1.6 s, accumulations 20	32
105	17.85	20	Laser 0.65 W, exposure 1.2 s, accumulations 20	24
110	18.70	20	Laser 0.65 W, exposure 1.2 s, accumulations 20	24
115	19.55	20	Laser 0.65 W, exposure 1.3 s, accumulations 20	26

Data processing and data analysis

The calibration standards (20 replicate tablets per API concentration level) were measured using the TRS100. The spectra were trimmed between 200 and 1,800 cm^{-1}. To correct for fluorescence, the spectra were baselined by applying a Whittaker filter[4] with an asymmetry value of 0.001 and a smoothing factor of 100. To correct for inconsistencies in tablet thickness, the spectra were normalized by area (1-Norm, Area = 1). Finally, they were mean centered before partial least squares (PLS) modeling in SOLO (eigenvector[5]). The PLS regression model was validated by cross-validation (CV) using Venetian blinds with 10 splits and one sample/blind (10% data out).

Results and discussion

Figure 3 shows the different steps in the processing of the TRS spectra (raw, trimmed, normalized, and mean centered) and the PLS analysis results in terms of "predicted (TRS) versus measured (HPLC)" concentrations. In Figure 3C, the spectra were color-coded for clearer visualization of the signals that trend with concentration. The lowest API concentration is shown in blue, and the highest concentration is shown in red.

Only the first latent variable (LV) was used for the model. The predictions were evaluated by fitting a straight line to the "predicted versus measured" concentration graph, which resulted in a determination coefficient (R^2) of 0.967 and a Root Mean Square Error (RMSE) of 1.7166.

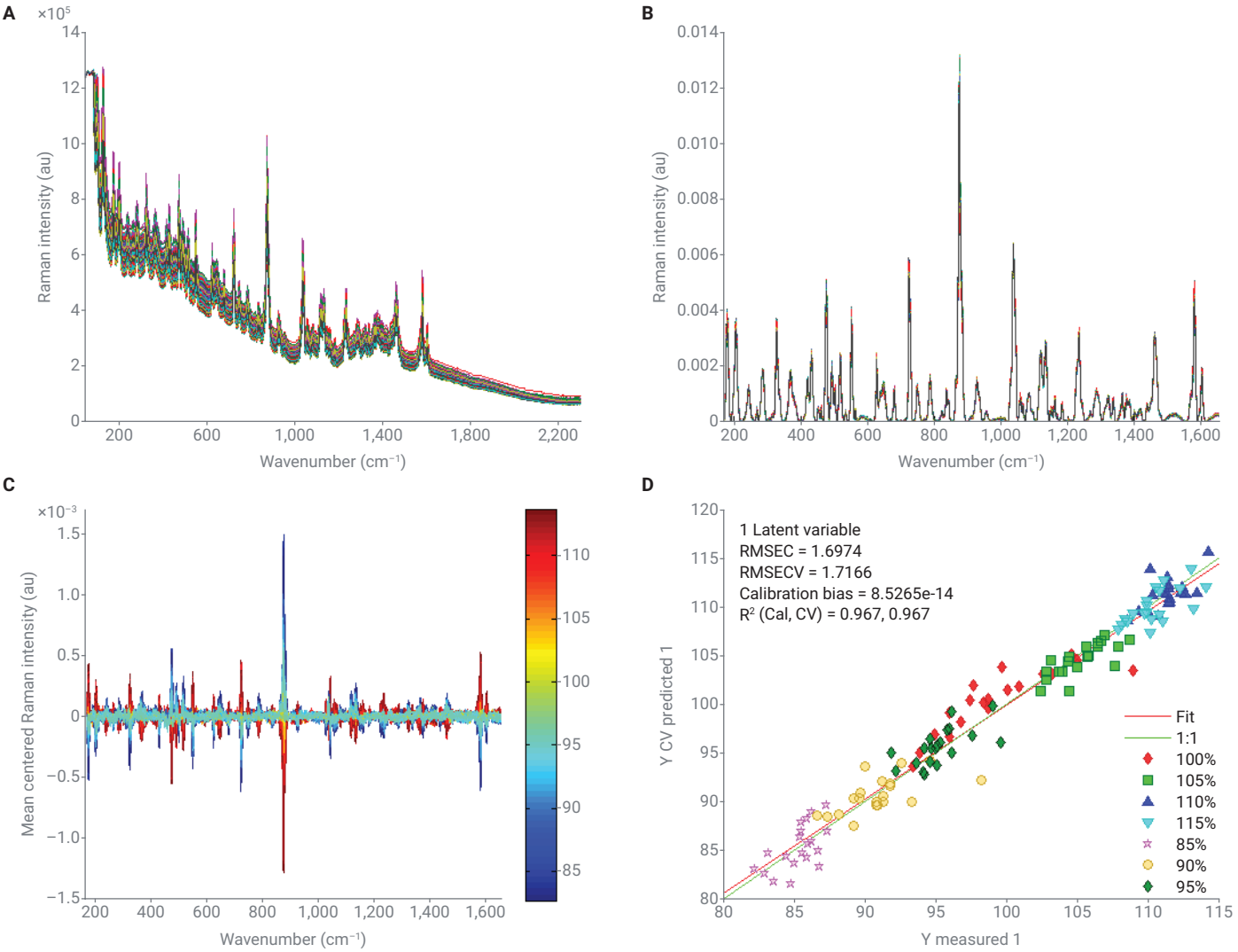

Figure 3. (A) Raw spectra, (B) processed spectra, (C) mean centered spectra, and (D) "predicted vs. measured" concentrations.

Repeatability

Repeatability was evaluated by comparing the prediction and measured values for each calibration standard (20 tablets). Figure 4 shows the relative errors for each individual sample, and the relative errors in the form of a histogram and boxplot (Figures A, B, and C, respectively).

Figure 4D compares the absolute prediction values with values obtained by HPLC. The relative errors seem to be normally distributed with no bias (mean, most frequent value, and median ~0). Also, 96.6% of the predictions were within ±4% relative error, which is below the accepted industry range of ±5%.

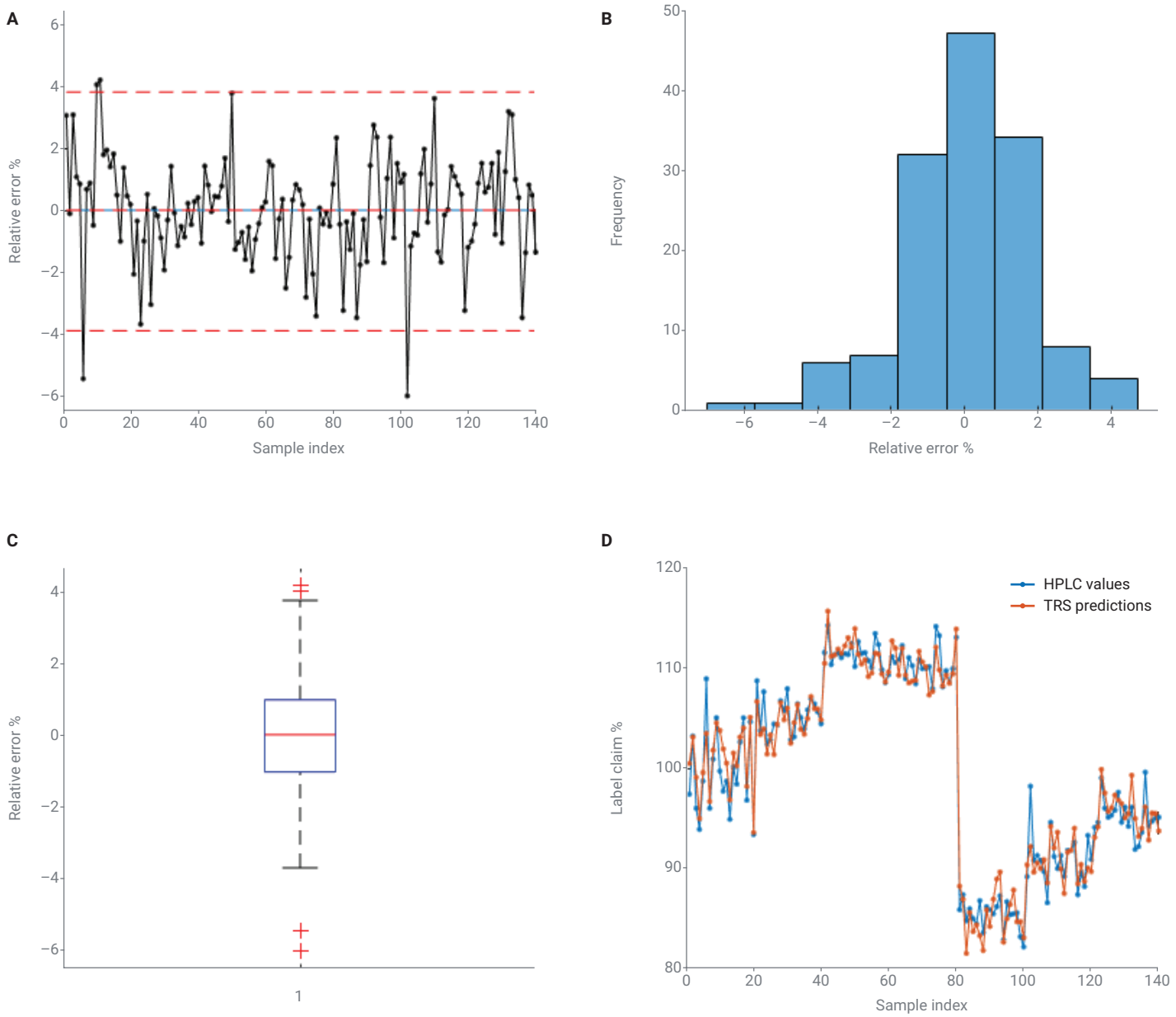

Figure 4. (A) Relative errors for each individual sample (98.3% of significance), (B) relative errors histogram, (C) relative errors boxplot, and (D) comparison of predicted (TRS) with reference (HPLC) values.

Sensitivity

The LOD, which is the minimum concentration that can be detected with a 99.7% confidence level, is calculated as 3 × standard deviation/slope of the calibration curve.[6,7] In multivariate analysis, there are multiple signals (responses). Also, there are multiple components in a mixture, so the estimation of the LOD is not always straightforward.

In this work, we used the predicted concentration as a response. As shown in Figure 3 bottom-right, the predicted (TRS) versus measured (HPLC) were linearly fitted, resulting in an R^2 value of 0.967. The deviations of the predictions from the fitted curve were used to estimate the standard deviation (s) of the blank and the slope (m) was obtained from the curve parameters. The LOQ is defined as 3.3 × LOD.[6] The estimated values for the LOD and LOQ are shown in Table 2, which shows that an analyst can detect 0.850% API and quantify down to 2.805% w/w API for this particular product.

Table 2. Limits of detection and quantitation for API as label claim (LC) and w/w %.

Standard Deviation (s)	Slope (m)	LOD (LC) %	LOQ (LC) %	LOD (w/w) %	LOQ (w/w) %
1.6452	0.97169	5.0793	16.7616	0.850	2.805

Conclusion

In this work, a typical case scenario of UCA of tablets by TRS was presented based on a drug with an API content of 17.00% w/w. A single LV-PLS model was created to evaluate the repeatability and sensitivity (LOD and LOQ) of the Agilent TRS100. Reference data were obtained using HPLC. Repeatability was estimated from the relative errors of the cross-validation predictions across the calibration range. 96.6% of the predictions fell within the ±4% relative error range, which is within the industry-accepted range of ±5%. The sensitivity of TRS in terms of LOD (0.850 w/w%) and LOQ (2.805 w/w%) were estimated from the predicted (TRS) versus reference (HPLC) values of the API.

The study has shown that the TRS100 provides the repeatability and sensitivity needed for direct UCA of pharmaceuticals in solid dose forms. TRS also enables fast, direct, nondestructive measurement of samples, making it highly suitable for quality control testing of tablets.

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