Application Note

Content Uniformity, Assay and Identification

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Quantitative Analysis of Warfarin Tablets Containing Salt-Form Impurities Using Transmission Raman Spectroscopy

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Abstract

This Application Note demonstrates the use of transmission Raman spectroscopy (TRS) for the quantification of Warfarin in whole intact pharmaceutical tablets at 0.5 % w/w with 20 second measurement time[1]. TRS can differentiate between and quantify two forms of Warfarin without sample preparation. Traditional chromatography techniques cannot do this as the form information is destroyed by solvation. TRS enables fast, automated, nondestructive quantitative bulk analysis of pharmaceutical oral solid dose forms for content uniformity and polymorph analysis.

Introduction

Pure Warfarin is available as an amorphous sodium salt (WS) and a crystalline clathrate (WSC) form. In storage, the clathrate form may dissociate into the amorphous sodium salt, and this change is indistinguishable by HPLC (both forms contribute to the assay result). Quantification of individual forms of an active pharmaceutical ingredient (API) within a dosage can be part of the product critical quality attribute. A method of quantifying both forms quickly, which is nondestructive, uses no solvents, generates no waste, and is less resource intensive in terms of personnel and materials is beneficial. This Application Note looks at the viability of measuring Warfarin at the lowest dose strength (1 mg, 0.5 % w/w) for content uniformity, and differentiating between the two salt forms at this low level.

Experimental

Figure 1 shows the Raman spectra from the pure Warfarin forms. The two forms have very similar spectra. There are subtle spectral differences in peak position and intensity at:

- 680 cm^{-1}
- 818 cm^{-1}
- 1,030 cm^{-1}
- 1,420 cm^{-1}
- 1,460 cm^{-1}
- 1,635 cm^{-1}

Figure 1. Warfarin sodium and Warfarin sodium clathrate transmission Raman spectra, with calculated spectral differences.

Accurate prediction of concentration uses a calibration following a design of experiments (DoE) process covering all variations in the formulation (components and physical factors). The main variants used were dye (strong Raman contribution), Warfarin forms (0.35 to 0.71 % w/w), and lactose, as in Figure 2. Seven tablet compacts were pressed from each of the 19 calibration samples.

Tablets were placed into a beam enhancer measurement tray (see Figure 3), and scanned in an Agilent TRS100 Raman quantitative pharmaceutical analysis system. The acquisition setting for the samples was 650 mW laser power (830 nm) and 20 seconds total accumulations per tablet.

The beam enhancer recycles reflected laser light from the tablet surface back onto the tablet. As a consequence, a greater amount of laser light penetrates through the sample, causing a higher transmission Raman intensity than without the enhancer[2].

Results were obtained in triplicate, resulting in 399 spectra (19 samples × 7 tablets × 3 scans).

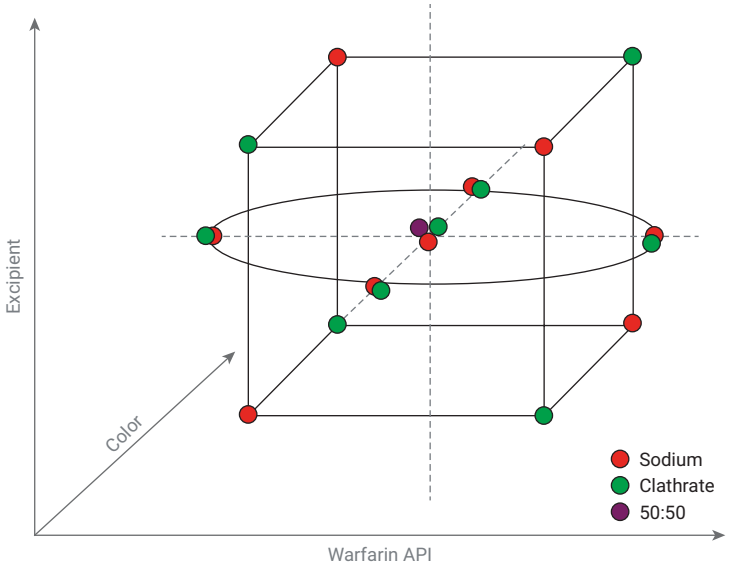

Figure 2. DoE centric cubic design, 19 samples.

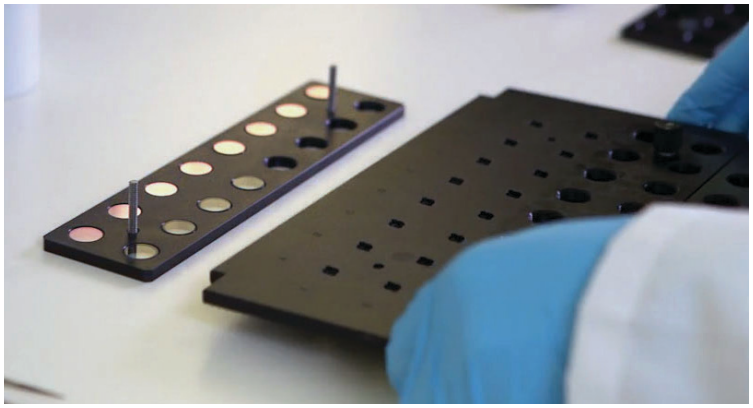

Figure 3. Beam Enhancer tray assembly.

Results and discussion

Figure 4 shows the resulting calibration spectra.

After baseline and normalization corrections were applied to the calibration spectra, we saw clear spectral differences due to Warfarin salt concentration changes. Figure 5 shows a Warfarin peak of interest at ~680 cm^{-1}.

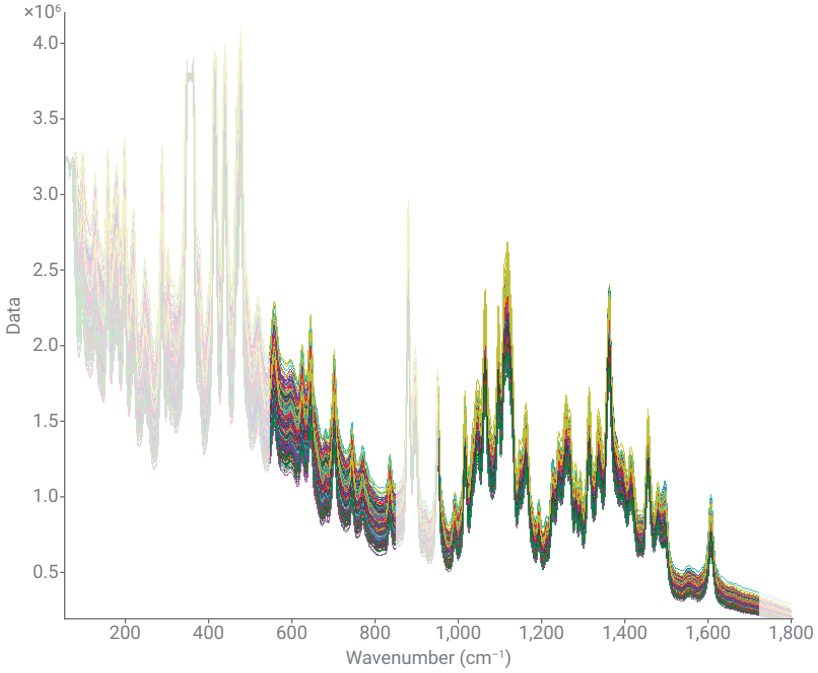

Figure 4. Three hundred ninety-nine TRS spectra of the calibration samples, with the spectral range used in the model building shown.

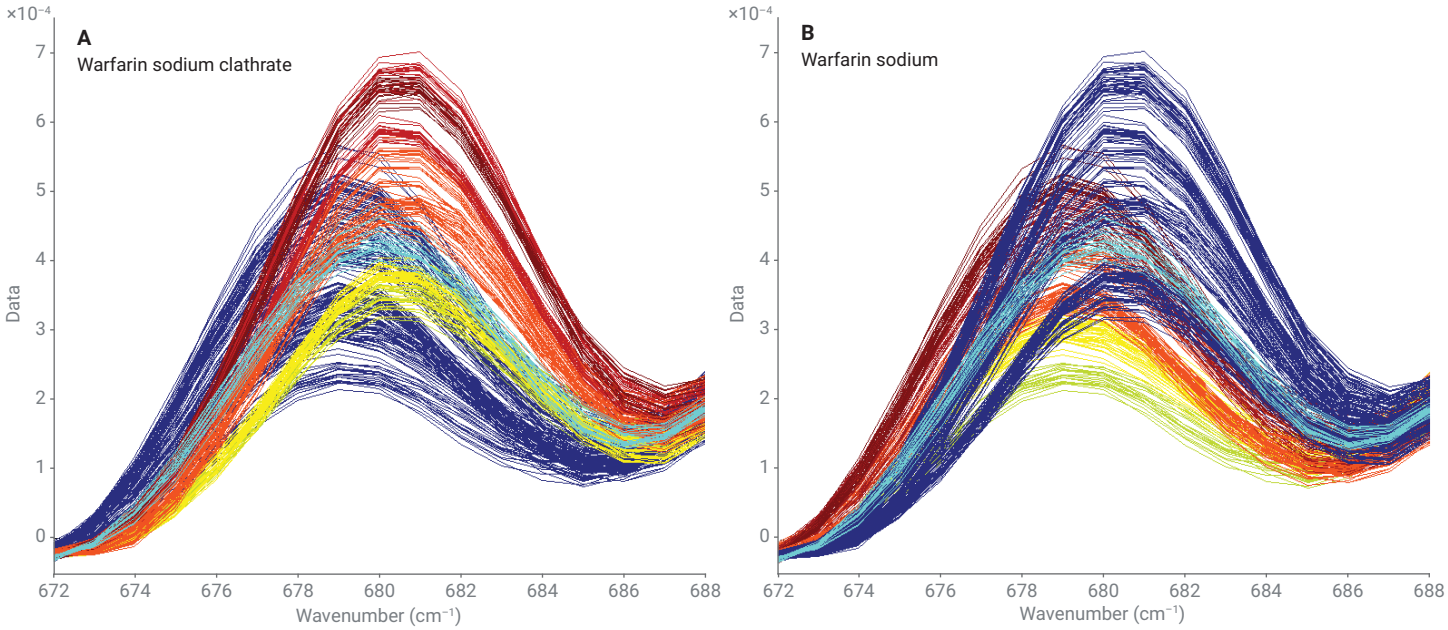

Figure 5. A zoomed in spectral region showing all 399 calibration spectra colored according to Warfarin salt concentration (blue is low to red as high). A) Peak at 681 cm^{-1} indicates spectral changes in concentration of Warfarin sodium clathrate. B) Peak at 679 cm^{-1} indicates spectral changes in concentration of Warfarin sodium.

Figure 6 shows chemometric partial least squares (PLS) models built using the calibration spectra. The optimal model parameters used first derivative, normalize, and mean center preprocessing over the spectral range 500 to 850 and 950 to 1,750 cm^{-1}. Model performance of R^2 = 0.99 and RMSEC and CV both = 0.019 indicate good model characteristics. The RMSEC and CV indicates a calibration error of ±0.019% w/w, which when normalized to the nominal concentration gives a model error of 4%.

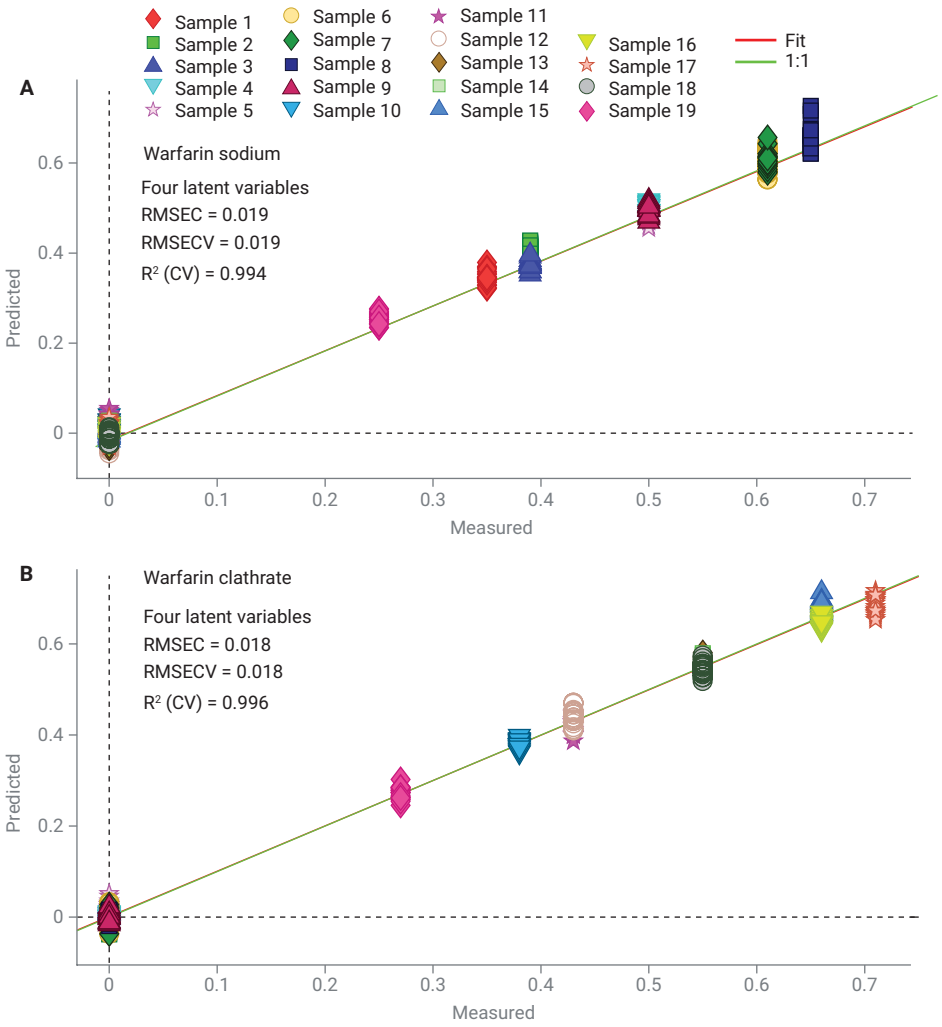

Figure 6. Chemometric PLS calibration models.

The models were validated using production and placebo samples, which were predicted to contain Warfarin sodium clathrate and no Warfarin sodium (Figure 7). An RMSEP result indicates a prediction error of ±0.026% w/w, which when normalized to the nominal concentration gives a prediction error of 5%.

Conclusion

This Application Note demonstrates the ability to not only distinguish between Warfarin sodium salts in whole intact tablets but to also quantify them at levels in the lowest dose strength (1 mg, 0.5 % w/w) in the presence of one another.

This formulation was particularly challenging due to the presence of a strong Raman-active dye that varied between batches in production material and overlapped with the API in the region 1,400 to 1,700 cm^{-1}. The limitation of this study is the external validation of the model using samples that contain a mix of salt forms. This would require further development.

This work demonstrates the ability to use TRS to quantify Warfarin at 0.5 % w/w, which is applicable for pharmaceutical QC lab tests for content uniformity. It is also applicable to quantifying the two forms to verify the efficacy of the drug form in the tablet. Other applications may include stability testing to examine WSC degradation to WS.

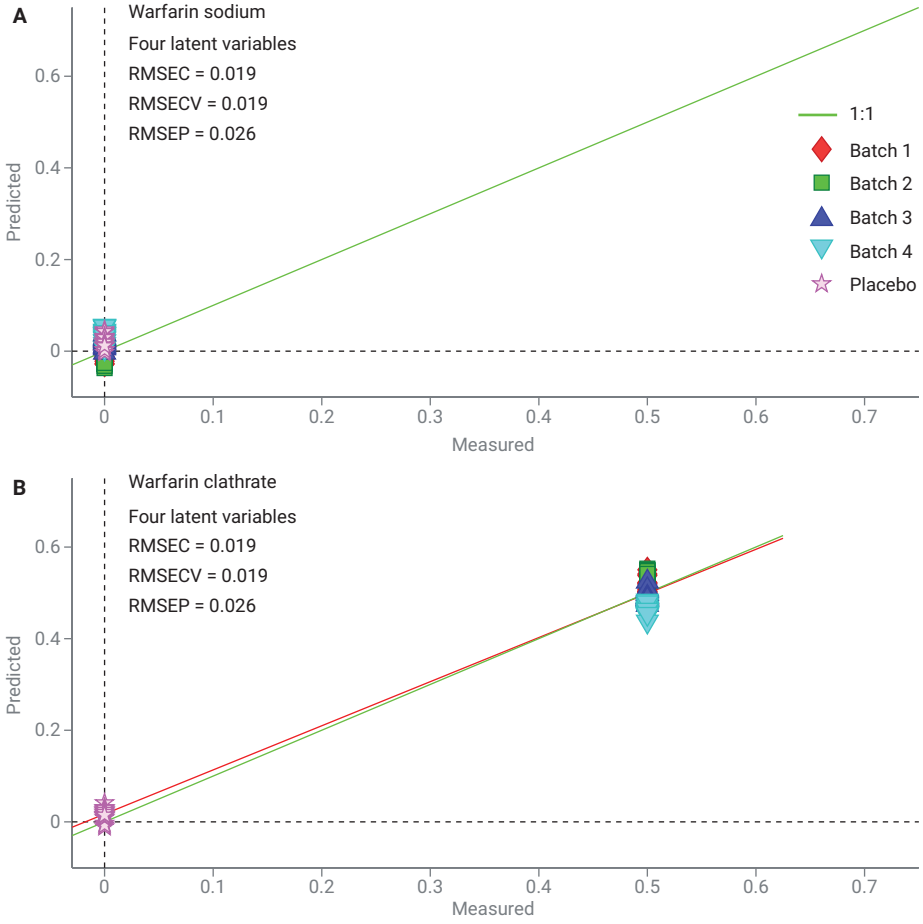

Figure 7. Validation samples, predicting production batches and placebo samples.

References

1. Griffen, J. A.; Owen, A. W.; Matousek, P. Quantifying low levels (<0.5% w/w) of warfarin sodium salts in oral solid dose forms using Transmission Raman spectroscopy, *J. Pharm. Biomed. Anal*. **2018**, *155*, 276–283.
2. Matousek, P. Raman signal enhancement in deep spectroscopy of turbid media. *Appl. Spectrosc*. **2007**, *61*, 845–854.

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