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Research article

Development and validation of a Raman spectroscopy-based chemometric model for content uniformity assessment of amlodipine besylate tablets

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ABSTRACT

This study aimed to develop and evaluate a rapid, non-destructive Raman spectroscopy method combined with chemometric modeling for content uniformity (CU) assessment of Amlodipine Besylate tablets. Feasibility studies were conducted using API, excipients, and placebo to identify spectral regions suitable for quantification. Raman spectra were collected from tablet samples spanning 70% to 130% of the label claim using a TRS 100 analyzer. Baselining, normalizing, and mean centering were examples of spectral preprocessing. Using Solo Eigenvector software, a Partial Least Squares (PLS) regression model was created with Raman spectrum data as predictors (X-axis) and HPLC-derived assay results as the reference (Y-axis). Blind pill samples at 100% concentration were used to validate the model. In the spectral band of 200-1800 cm-1, there were clear API peaks. A principal component analysis indicated that one main component explained ~85% of the variation of the data, suggesting very good sample homogeneity and data quality. The PLS model showed good prediction accuracy with $R^2 > 0.9$. Prediction study of blind samples gave assay results of 99% and 100% verifying the correctness of the model. The presented Raman spectroscopy method coupled with PLS chemometric modeling offers a reliable, quick and non-destructive approach for the assessment of content uniformity of Amlodipine Besylate tablets, having a great potential for routine pharmaceutical quality control applications.

Keywords: Raman spectroscopy, Content uniformity, Amlodipine besylate, Chemometrics, PLS modelling.

INTRODUCTION

Every tablet in a batch is supposed to deliver the same dose. Content uniformity testing is how manufacturers prove it: individual tablets are sampled, and the amount of active pharmaceutical ingredient in each one is measured. For a widely used blood pressure and angina medicine such as amlodipine besylate, where the dose per tablet is small, consistency is essential for both safety and efficacy. Content uniformity is a critical quality attribute for solid oral dosage forms, ensuring that each dosage unit contains an acceptable range of the active pharmaceutical ingredient. High-performance liquid chromatography is the traditional method used for CU testing, which is accurate and validated, but time-consuming, destructive, and involves considerable sample preparation [1].

Raman spectroscopy is a fast, non-destructive vibrational spectroscopic technique based on the inelastic scattering of monochromatic light, providing a molecular fingerprint of a material. Raman spectra are directly taken from pharmaceutical tablets with little or no sample preparation for content uniformity testing of tablets. Generally, spectral data are preprocessed by baseline correction, normalization and mean centring to remove fluorescence, intensity variation and common spectral characteristics. Then a multivariate calibration model, e.g. Partial Least Squares regression, is built utilizing reference assay values (e.g. from HPLC) as the

response variable and Raman spectrum data as predictors. Once validated, the model can predict API content in unknown tablets within seconds [2].

Its key advantages are that it is non-destructive, rapid, and solvent-free, and it can test many more tablets, potentially closer to the production line. However, a limitation is that the blind test was very small, so full validation is still needed. This would require a larger independent test set, error metrics such as RMSEC and RMSEP, and robustness checks across batches and instruments. Raman spectroscopy looks like a promising, greener complement to HPLC for content uniformity assay [3].

In the case of Amlodipine Besylate, the 200-1800 cm-1 range showed separate API peaks. PCA proved the homogeneity of the samples and the PLS model obtained R 2 > 0.9. The accuracy of the predictions of the blinded 100% tablets was 99% and 100%. Thus, Raman spectroscopy provides a robust, solvent-free and high-throughput alternative to conventional HPLC for routine CU testing, enabling real-time quality control and Process Analytical Technology applications [4].

Amlodipine Besylate is a dihydropyridine calcium channel blocker, one of the most recommended drugs for hypertension and angina, and is an excellent choice for Raman investigation because to its unique spectral characteristics. The purpose of this study was to create and evaluate a chemometric model of CU evaluation based on Raman spectroscopy and compare the predictions with the standard HPLC methodology for Amlodipine Besylate tablets [5].

MATERIALS AND METHODS

Amlodipine besylate active pharmaceutical ingredient (API), the excipients and the placebo blend were supplied by the manufacturer. Tablets containing amlodipine besylate were used for calibration and validation. All reagents and solvents used for the reference HPLC assay were of analytical grade.

Raman instrumentation and spectral acquisition

Raman spectra were acquired directly from intact tablets, without sample preparation, using a TRS 100 Raman analyzer controlled by Content QC software. Spectra were recorded over the range 200–1800 cm^{-1}, with 30 accumulations and an exposure time of 0.4 s per accumulation. Medium-setting collection optics was used, giving a laser spot diameter of 4 mm at the tablet surface. The large spot was chosen to sample a greater tablet area and improve the sampling coverage of each measurement.

Feasibility assessment

The active pharmaceutical ingredient, the individual excipients, the placebo and the tablet were measured separately under the conditions in Section 2.2. Their spectra were compared to identify spectral regions containing API-specific bands that were free of significant interference from the placebo and therefore suitable for quantification.

Calibration samples and reference analysis

Tablets covering seven concentration levels, from 70% to 130% of the label claim (70, 80, 90, 100, 110, 120 and 130%), were prepared. For each level, seven tablets were measured by Raman spectroscopy and subsequently assayed by the reference HPLC method. The HPLC assay results for individual tablets were paired with their corresponding Raman spectra to form the calibration dataset.

Spectral preprocessing

Raw spectra were preprocessed in the following order: (i) baseline correction, to remove background fluorescence; (ii) normalization, to compensate for overall differences in spectral intensity; and (iii) mean centering, to remove spectral features common to all samples and emphasize variation related to API content.

Chemometric analysis

Multivariate analysis was performed using Solo software (version 8.6; Eigenvector Research, Inc., Manson, WA, USA). Principal component analysis (PCA) was first applied to the preprocessed spectra to assess data structure, homogeneity, outliers and any instrumental drift or batch-related trends, using a 95% confidence limit. A partial least squares (PLS) regression model was then constructed, with the preprocessed Raman spectra as the predictor matrix (X-block) and the HPLC assay values as the response (Y-block). The model used three latent variables [state how this number was selected]. Model performance was evaluated using the coefficient of determination (R^2), the root mean square error of calibration (RMSEC) and the root mean square error of cross-validation (RMSECV).

Model validation

The model was challenged with two blind tablets at the 100% level, which were not included in model building. Their Raman spectra were acquired as described in Section 2.2, and their API content was predicted using the saved model in Content QC software [6].

RESULTS AND DISCUSSION

Feasibility Assessment

Comparative spectral analysis of API, blend, and placebo revealed clear, distinguishable API peaks throughout the 200–1800 cm^{-1} region. Figure 1 demonstrates prominent API peaks across the full spectral range, while Figures 2–5 show zoomed-in regions highlighting specific peak clusters.

The absence of significant spectral overlap between API and placebo confirmed the feasibility of Raman-based quantification for this formulation [7].

Figure 1: API Peaks observed in tablet Blend from 200–1800 cm⁻¹

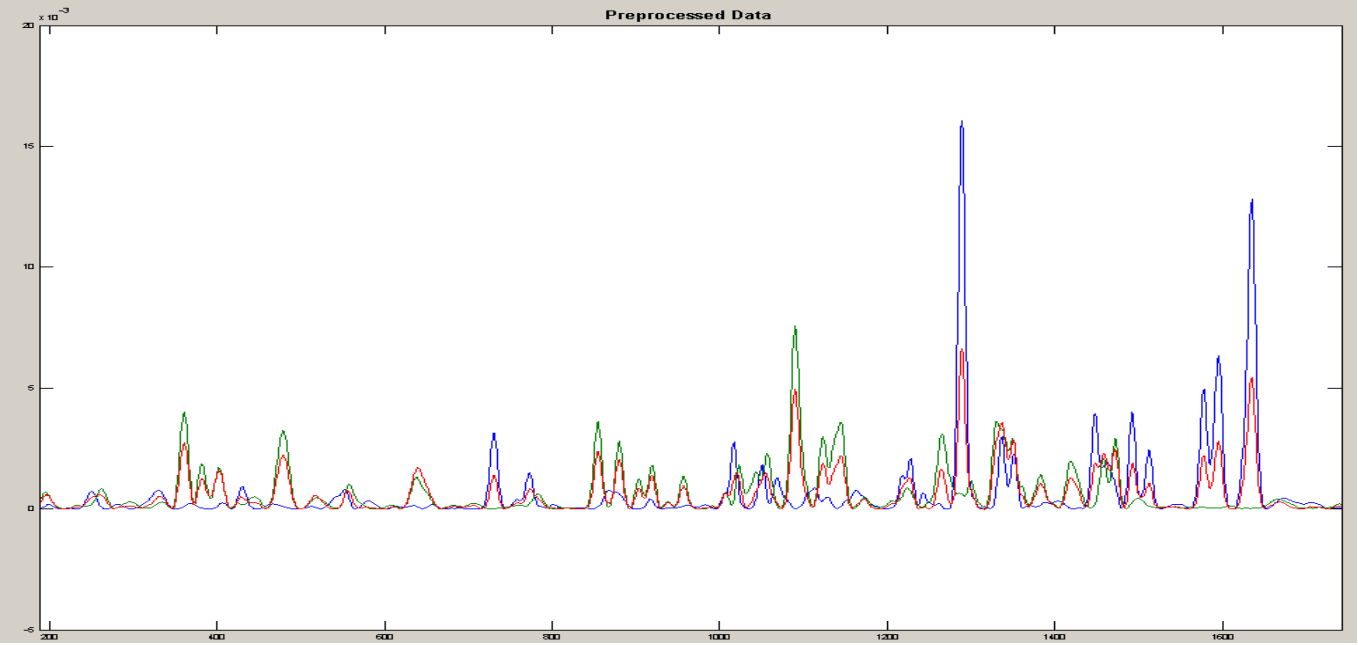


Figure 2: API Peaks observed in tablet Blend from 200–400 cm⁻¹

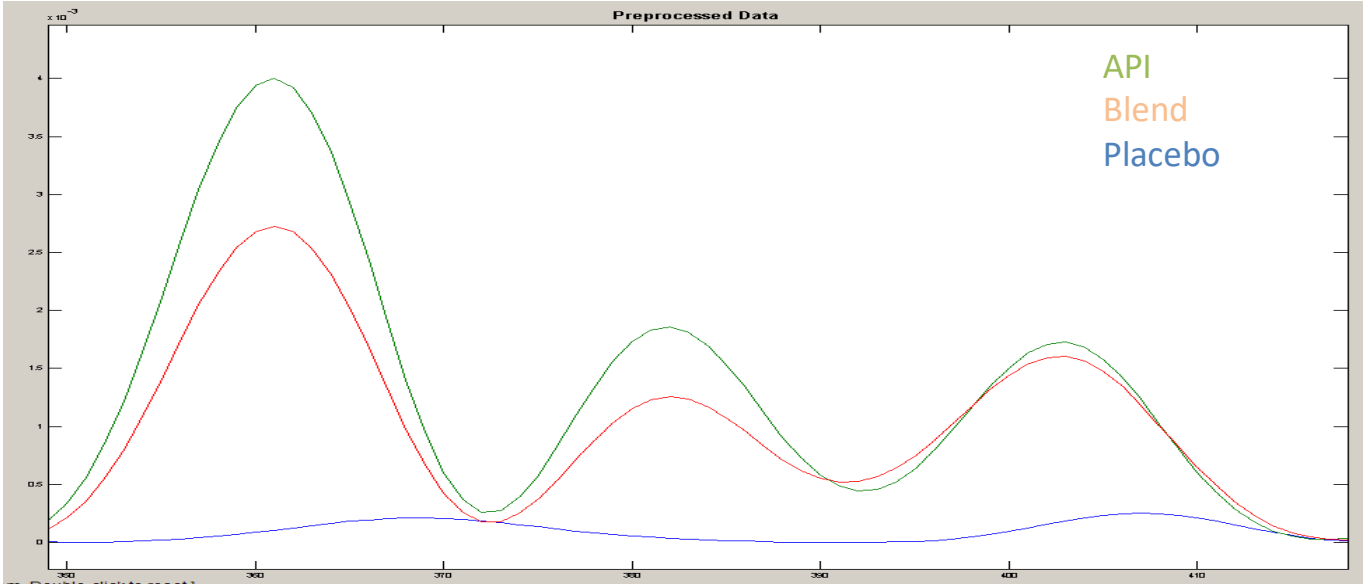


Figure 3: API Peaks observed in tablet Blend at 1050 and 1140 cm⁻¹.

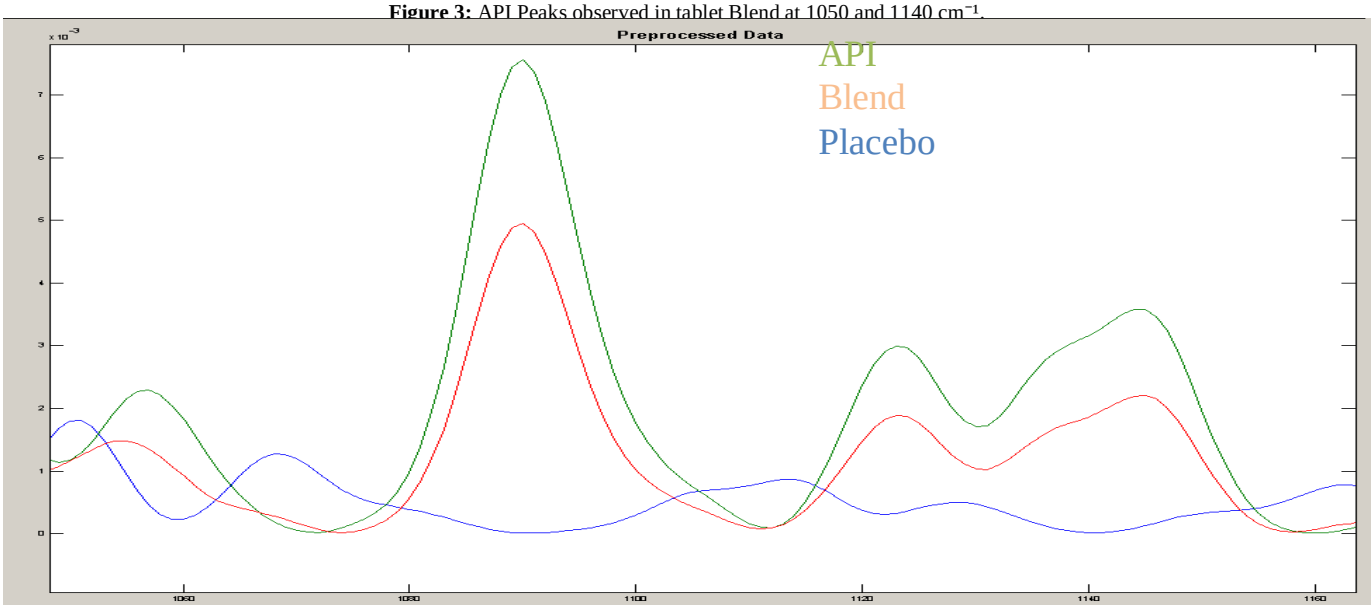


Figure 4: API Peaks observed in tablet Blend at 1360 and 1420 cm⁻¹

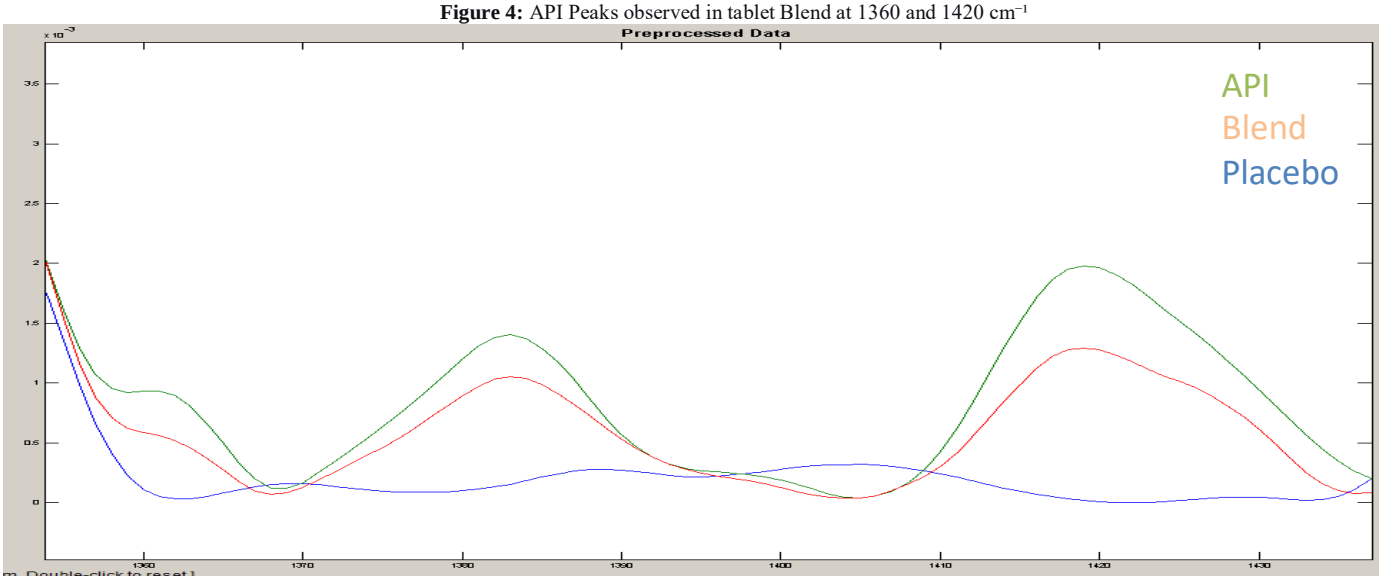


Figure 5: API Peaks observed in tablet Blend at 840–960 cm⁻¹

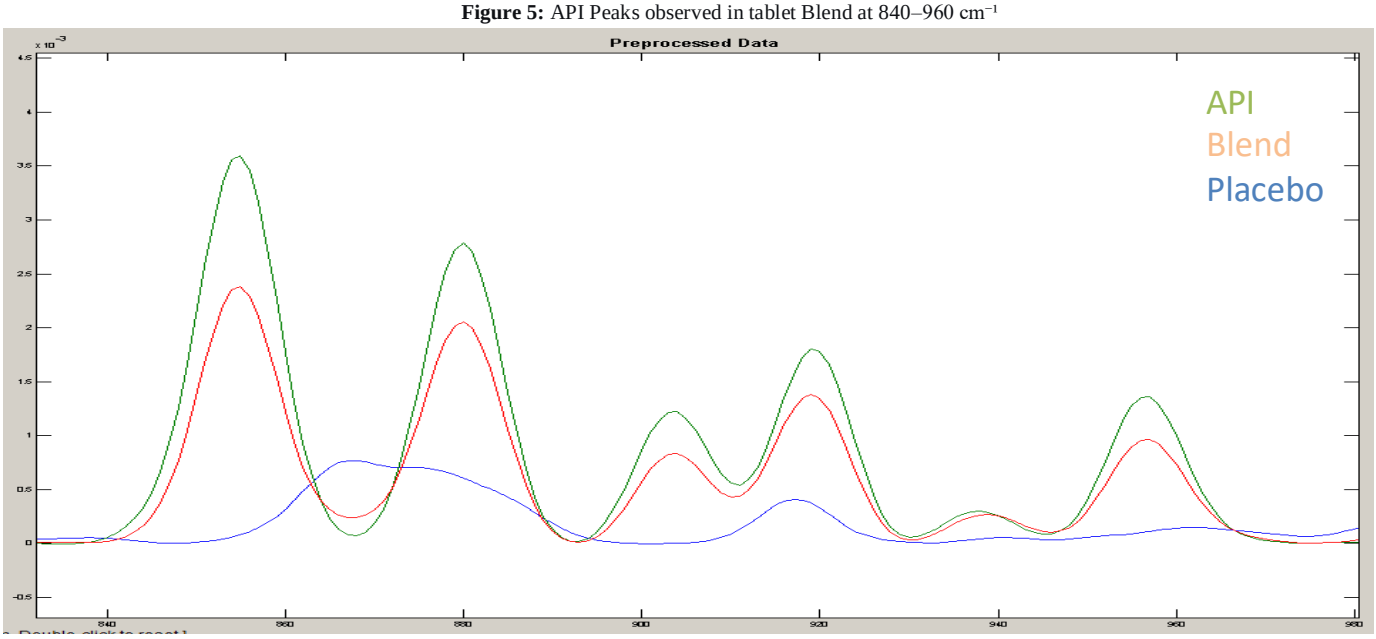


Figure 6: Preprocessed spectrum showing API (green) and Placebo (blue) tre

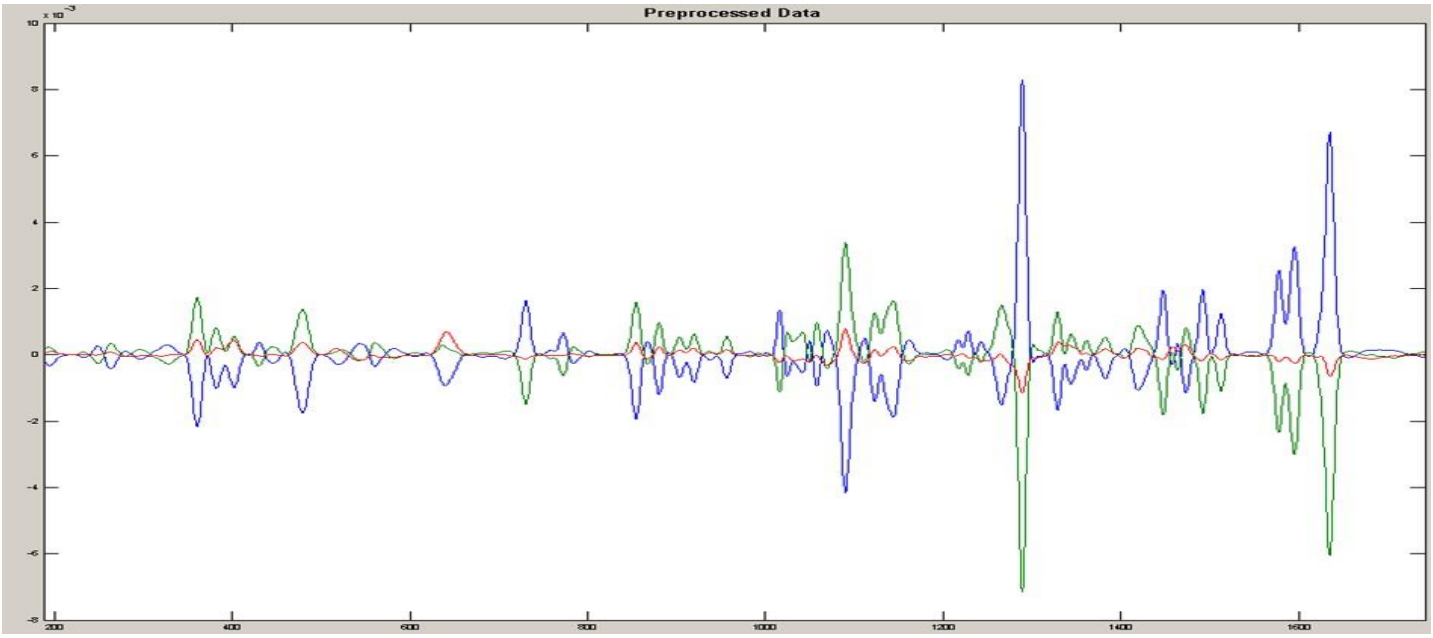


Figure 7: Trend in the collection of spectrum from 70% to 130%

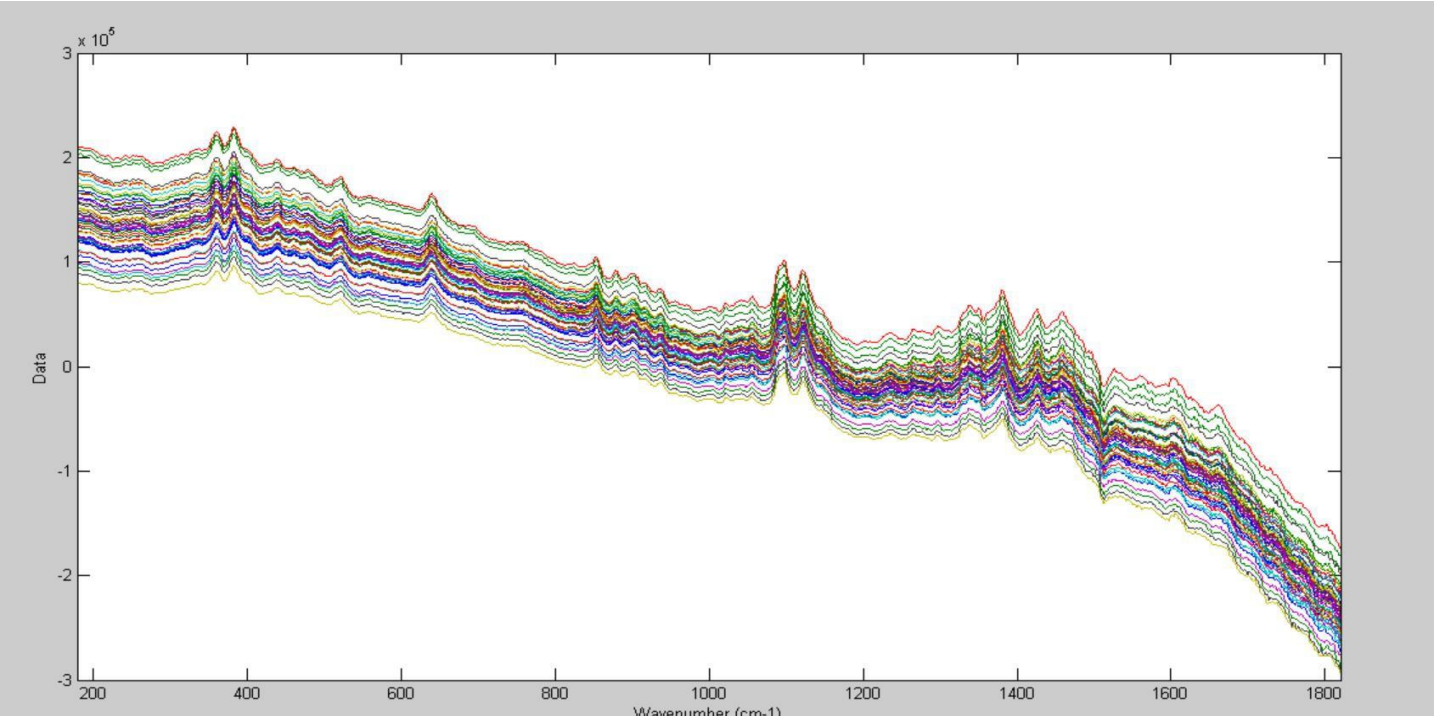


Figure 8: PCA scores plot showing clustering and 95% confidence regio

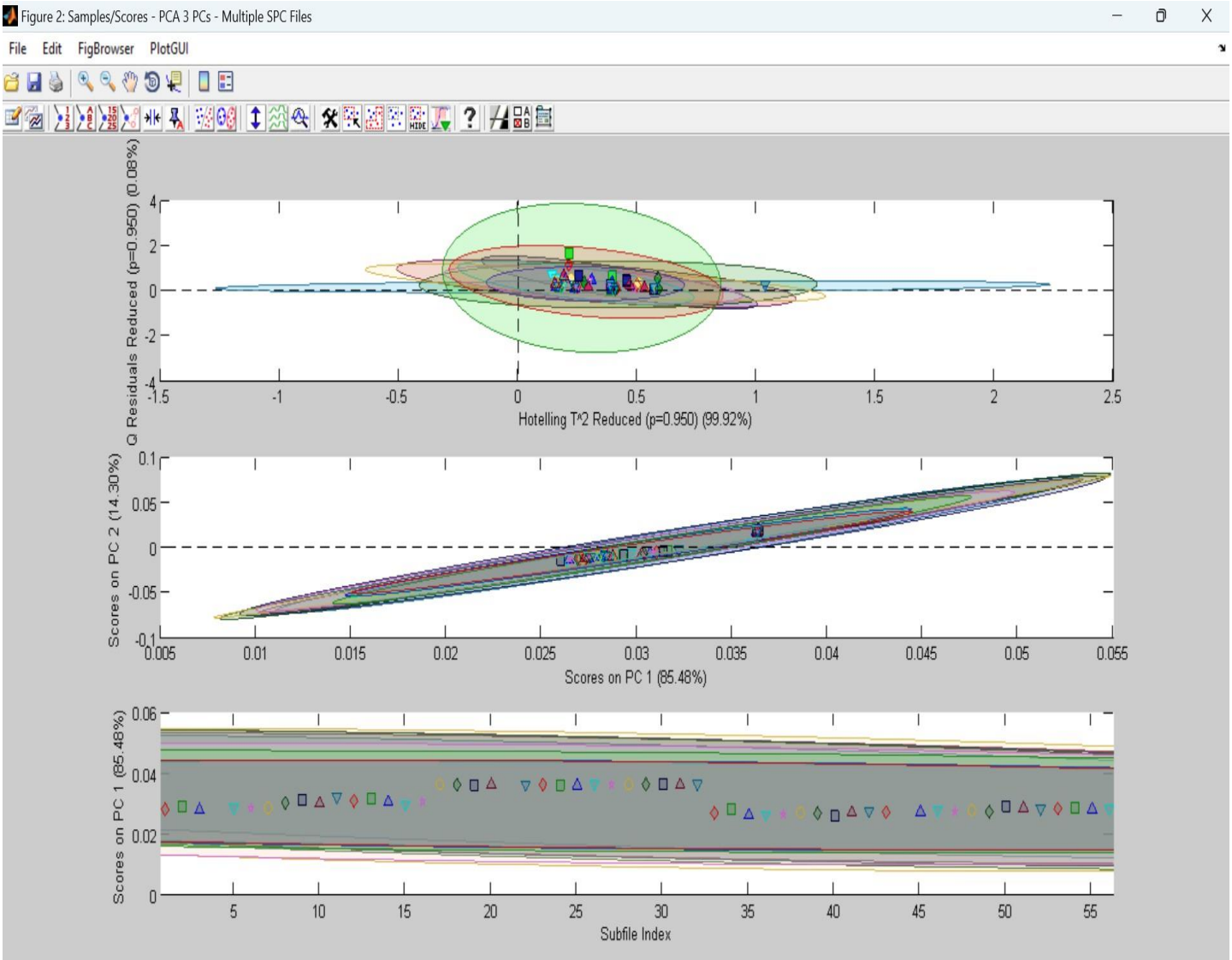


Table 1: HPLC reference values for calibration standards (70%–130%)

Tablet no.	70%	80%	90%	100%	110%	120%	130%
1	68.97	76.9	87.12	97.69	102.6	115.3	134.16
2	65.67	77.2	86.34	97.59	106.69	117.2	132.81
3	65.69	85	87.1	96.01	105.09	115.9	117.93
4	67.72	80	85.98	95.61	105.69	122.2	125.2
5	69.38	81.6	86.15	97.44	109.61	125.1	127.82
6	67.8	79.2	88.2	100.9	108.1	116.1	122.1
7	70.1	80.3	89	99.8	108.2	120.6	128.6

Table 2: Prediction results for blind samples

Tablet No.	Sample Label	Sample Position	Y1 Prediction
1	t1	A04	99
2	t2	B04	100

Figure 9: Chemometric PLS Model development using Solo Eigenvector Software

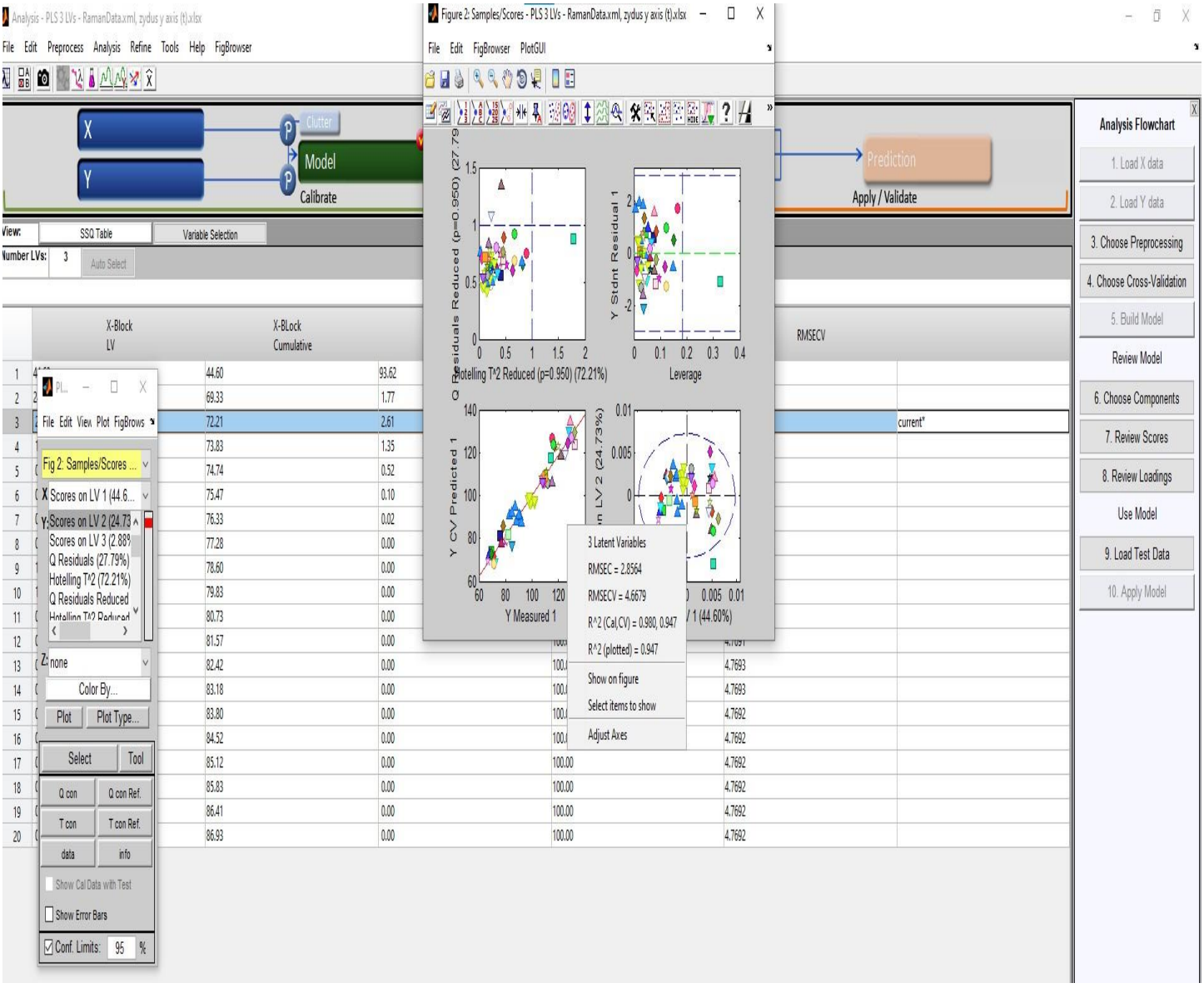


Figure 10: Prediction analysis of unknown/blind

Individual Results

Individual Results : zydus

Assay	Tablet N°	Sample Label	Sample Position	Y1 Prediction		Hotelling's T^2			Q Residual	
1	1	t1	A04	99	OK	✓	15	OK	✓	0	OK
1	2	t2	B04	100	OK	✓	15	OK	✓	0	OK

Calibration development

Raman spectra collected from 70% to 130% concentration levels (Figure 7) demonstrated a clear, progressive trend correlating with API concentration. This concentration-dependent spectral variation confirmed the method's sensitivity for quantitative analysis.

PCA analysis

Principal Component Analysis showed that one single principal component accounts for roughly 85% of the total variance. That tells us a lot. The sample homogeneity is solid, and we are not seeing any wild batch-to-batch swings or weird instrument drift messing with the data. There were no outlier samples throwing things off either.

Almost every single data point landed nicely inside the 95% elliptical confidence region, which you can see in Figure 8. Honestly, it just confirms the data quality is right where it needs to be to build a really robust PLS model.

PLS model development

The PLS regression model incorporating HPLC reference values (Table 1) demonstrated excellent predictive performance:

a. R^2 (Calibration): 0.941
b. R^2 (Validation): >0.9
c. RMSEC: 4.4597
d. RMSECV: 6.0518
e. Bias: 3.93e-07

The model utilized 3 latent variables, balancing predictive accuracy with model simplicity. Regression diagnostics confirmed linearity and homoscedasticity of residuals. Figure 9 shows the Solo Eigenvector software interface during model development.

Blind sample prediction

Prediction analysis of two blind 100% concentration tablets yielded assay values of 99% and 100% (Table 2 and Figure 10), demonstrating excellent agreement with expected values and validating the model's predictive capability.

Method advantages

Compared to standard HPLC, this new Raman-PLS approach has a ton going for it.

First off, it is completely non-destructive, meaning your samples actually stay intact so you can run other tests on them later. And it is fast. You get answers in mere seconds instead of waiting hours for HPLC runs to finish.

Sample prep is basically a non-issue too. There is no tedious extraction or messing with chromatography columns. That naturally cuts down on solvent waste, which is a nice win for the lab's environmental footprint. Put all that together, and you get real-time data right on the factory floor, making it way easier to catch things and make calls on the spot [8].

CONCLUSION

We basically proved that Raman spectroscopy paired with PLS modeling is a totally viable way to check content uniformity for Amlodipine Besylate tablets. When we looked at the spectra between 200 and 1800 cm^{-1}, the active ingredient peaks stood out clearly enough to get reliable numbers. PCA checks also showed the samples were remarkably homogeneous, with zero weird outliers or drifting equipment throwing things off. The PLS model itself turned out super solid. It hit an R^2 greater than 0.9, meaning it predicts accurately right across that 70 to 130% concentration spread. We even ran some blind samples and pulled assay values right at 99 and 100%, which pretty much locks in how accurate the model is. Best of all, this gives the lab a fast, nondestructive way to test content uniformity instead of relying solely on traditional HPLC. It is a genuinely useful step forward for pharmaceutical quality control, especially if manufacturing teams want real-time, at-line testing down the road. Moving forward, the main things to tackle are testing how well the model transfers to other instruments and sites, plus validating it across multiple production batches to make sure it holds up over the long haul.

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