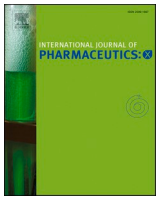
International Journal of Pharmaceutics: X 9 (2025) 100321

Contents lists available at ScienceDirect

International Journal of Pharmaceutics: X

journal homepage: www.sciencedirect.com/journal/international-journal-of-pharmaceutics-x



Non-destructive quantification of low colchicine concentrations in commercially available tablets using transmission raman spectroscopy with partial least squares

Ningzi Guo [a], Sijing Niu [a], Ying Geng [a], Guangzhi Shan [b], Ningyi Wei [a,*], Hua Chen [a,*]

[a] National Institutes for Food and Drug Control, No. 31, Huatuo Road, Beijing 102629, China
[b] Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 1, Tian Tan Xi Li, Beijing 100050, China

ARTICLE INFO

Keywords:
Colchicine
Transmission Raman spectroscopy
Chemometric analysis
Partial least squares
Commercial tablets
Content uniformity

ABSTRACT

The narrow therapeutic index and significant toxicity of colchicine (COL) underscore the importance of content uniformity of dosage units to ensure drug safety and efficacy. In this study, transmission Raman spectroscopy (TRS) technology combined with partial least squares (PLS) regression was used for the non-destructive determination of low concentration levels of COL in commercial tablets (0.83 % *w*/w). Based on a multifactor orthogonal design of experiment, one hundred calibration tablets ranging in drug content from 70 % to 130 % of the label claim were manufactured to develop an initial model which was further calibrated using the HPLC results. The quantitative model displayed good repeatability and high accuracy with a root-mean-standard error for calibration of 0.038 % and root-mean-standard error for cross-validation of 0.039 %. The limits of detection and quantification were 0.13 % and 0.40 % *w*/w, respectively. The absolute value of relative error of the TRS and HPLC content results for commercial tablets varied between 0 and 3.8 %. Notably, the relative standard deviation (RSD) of the TRS method was 1.2 %, lower than the RSD of 2.9 % observed with HPLC. The results demonstrated a fast and non-destructive method for the quality control of highly toxic and low content active pharmaceutical ingredients in commercial products, without human or environmental exposure to toxic substances during sample preparation.

1. Introduction

Colchicine (COL) is a major alkaloid derived from the autumn crocus and other species of the *Colchicum* genus (Karamanou et al., 2018). COL is a well-established first-line treatment for gouty arthritis due to its involvement in various mechanisms that regulate the inflammatory response, including the inhibition of microtubule formation and cell division (Pascart and Richette, 2018; Robinson et al., 2022). Its unique anti-inflammatory properties have also led to its use in the treatment of other conditions, such as familial Mediterranean fever (FMF), Behçet's disease, cirrhosis, pericarditis, and certain types of carcinoma (Bouabdallaoui and Tardif, 2022; Deftereos et al., 2022). Despite its broad clinical applications, COL is associated with significant toxic side effects. Blood concentrations of approximately 3 ng/mL can lead to adverse effects, including nausea, vomiting, diarrhoea, and other gastrointestinal reactions. Due to its narrow therapeutic window, precise control of COL is critical (Aghabiklooei et al., 2014; Molad, 2002).

COL is marketed globally in various oral dosage forms, including tablets and liquid formulations (Lei et al., 2024). In 2009, COL tablets (0.6 mg) were approved by the US Food and Drug Administration (FDA) for treating acute gout and FMF (Portincasa et al., 2013). In 2023, the FDA approved COL (0.5 mg) tablets, (LoDoCo®) as the first anti-inflammatory drug for cardiovascular disease. This formulation reduces the risk of cardiac events in adult patients with a confirmed diagnosis of atherosclerotic cardiovascular disease by an additional 31 % compared to placebo (Lei et al., 2024). In China, COL tablets (1 mg and 0.5 mg) are the only approved COL preparations.

According to the United States Pharmacopoeia (43rd edition), the assay and uniformity of COL dosage units must be tested using HPLC. In addition to HPLC, Raman spectroscopy, including transmission Raman spectroscopy (TRS), has become an increasingly accessible process analytical technology (PAT) tool for the quantitative assessment of active pharmaceutical ingredients (APIs) in both the continuous manufacturing process and the final dosage form (Nagy et al., 2018a;

* Corresponding authors.
E-mail addresses: weiny@nifdc.org.cn (N. Wei), chenhua@nifdc.org.cn (H. Chen).

https://doi.org/10.1016/j.ijpx.2025.100321
Received 3 December 2024; Received in revised form 10 February 2025; Accepted 20 February 2025
Available online 22 February 2025

Table 1
DoE concentration ranges of components in calibration tablets.

Group No.	API (w/w)%	Magnesium stearate (*w*/w)%	Pregelatinised corn starch (w/w)%	Lactose (w/w)%
1	0.58	0.83	33.33	65.25
2	0.66	1.01	26.33	72.00
3	0.66	1.01	40.33	58.00
4	0.66	0.66	26.33	72.35
5	0.83	0.83	43.33	55.00
6	0.83	1.08	33.33	64.75
7	1.01	1.01	26.33	71.65
8	1.01	0.66	26.33	72.00
9	1.01	0.66	40.33	58.00
10	1.08	0.83	33.33	64.75

Nagy et al., 2018b; Triboandas et al., 2024). A typical PAT tool comprises spectroscopy with chemometric tools, which can substantially reduce the analysis time and costs for routine assays of API in tablets (Farrell et al., 2012; Pieters et al., 2013; Triboandas et al., 2024; Wang et al., 2021).

Compared with HPLC, the TRS method has the advantages of being a non-destructive, high-throughput, and high-speed operation. Sample preparation is not required, negating the need for solvent use. It can be used for in-line monitoring of drug content in production lines (Zhang and McGeorge, 2015). Unlike traditional backscatter Raman spectroscopy, TRS collects Raman scatter signals from one side to another side of the tablet; Therefore, the chemical information gathered is more representative of the whole tablet, with more accurate quantitative results, compared to those gathered using traditional backscatter Raman spectroscopy (Doddridge et al., 2022; Eliasson et al., 2008; Li et al., 2016). Several studies have reported the application of TRS for API quantitative analysis (Zhao et al., 2022a; Zhao et al., 2022b), crystal polymorphism analysis (Griffen et al., 2016; Hennigan and Ryder, 2013) (Griffen et al., 2018), co-crystal analysis (Burley et al., 2012) of tablet, ointment formulations (Ohashi et al., 2022), 3D-printed formulations (Trenfield et al., 2022) and liposome preparation (Sato et al., 2024). TRS technology reported in literature has mostly applied to the quantitative analysis of high-dose formulations (>1 % *w*/w), and there is relatively little research on the application of low-dose formulations (<1 % w/w) (Griffen et al., 2018). COL was chosen as a model drug in this study in order to explore the possibility of applying TRS technology in the quantitative determination of the API content of low-dose drug formulations with a view to providing an alternative method to analyse highly toxic APIs without the need for sample preparation to reduce the environmental and the health impact on technical staff involved in the analysis process.

2. Materials and methods

2.1. Materials

The manufactured tablets contained COL (Beijing Helen Medichem Company, Beijing, China), lactose monohydrate (Aladdin Co. Ltd., Shanghai, China), pre-gelatinised corn starch (Shyuanye Co., Ltd., Shanghai, China), and magnesium stearate (Aladdin Co. Ltd., China). The COL reference standard used in HPLC analysis was obtained from the National Institute for Food and Drug Control (Beijing, China). The commercial COL tablets were supplied by a Chinese pharmaceutical manufacturer (anonymity requested).

2.2. Preparation of calibration samples

Table 1 lists the proportions of COL, magnesium stearate, pre-gelatinised corn starch, and lactose used for the calibration set of COL tablets during the multifactor orthogonal design of experiment (DoE) stage. Based on the concentrations listed in Table 1, which range from 70 % to 130 % of the label claim, ten sets of calibration tablets were prepared. To prepare the calibration tablets, the API was initially mixed with the pre-mixed placebo in a mortar to create a powder with ten times the intended tablet strength. This ten-fold powder mixture was then combined with additional placebo material, placed in a sealed bag, shaken manually for 30 min to ensure homogeneity, with air introduced to achieve the target concentrations. The resulting mixture was compressed into tablets using a round mould with a diameter of 7 mm and a height of 3 mm on a DP30A single-punch tablet machine, producing 10 tablets per concentration level. Each tablet had an average weight of 120 mg.

2.3. Transmission Raman spectroscopy measurements

Raman spectra of the API and its mixture were collected using a TRS instrument (Agilent TRS100, Cheshire, UK) with Content QC software. The acquisition settings included a laser beam diameter of 4 mm, laser power of 650 mW, and low-area collection optics. Tablets were scanned for 50 accumulations at 0.4 s per accumulation, resulting in a total scan time of approximately 20 s per tablet. The measured wavenumber range was 57 cm^{-1} to 2000 cm^{-1}.

2.4. HPLC analysis

One tablet was added to a 50 mL volumetric flask, approximately 30 mL mobile phase was added and ultrasonicated for 30 min to dissolve COL. The mixture was allowed to cool and then diluted to volume with the mobile phase. The mixture was mixed well and filtered. Subsequently, successive filtrates were used for analysis.

The experiment was carried out on an LC-30 A high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) using Lab Solutions software. The separation was carried out at 30 °C using an Agilent HC-C8 column (4.6 × 250 mm, 5 μm), with a mobile phase containing methanol: water (50:50) at a flow rate of 1 mL min^{-1}. Detection was performed at 254 nm. The measurements were repeated twice for each sample. The concentration of each sample solution was calculated using an external standard method. The concentration of the sample solution was corrected using the sample weight and the label claim (1 mg per tablet) to obtain the assay expressed as percentage of dosage weight (% *w*/w) and of label claim (%) respectively.

2.5. Chemometric analysis

Chemometric analysis and model building were conducted using Solo software (ver. 8.6.2, Eigenvector Research Inc., Manson, WA, USA). To develop the PLS quantitative model for assaying COL, ten levels of calibration tablets were selected, with each level scanned twice. The Raman spectra of these samples were the input variables (X), while the HPLC content was used as the response variable (Y). All spectra were baseline-corrected, subjected to multivariate scatter correction (MSC), and the variables were mean-centred before being imported into the Solo software to construct a calibration model.

The predicted drug content values ($\hat{y}_i$) were compared to the actual HPLC content values (y_i), where n represents the number of samples used for prediction. The Root Mean Square Error of Calibration (RMSEC), Root Mean Square Error of Cross-Validation (RMSECV), and Root Mean Square Error of Prediction (RMSEP) were calculated using Eq.1 (Ohashi et al., 2022). The RMSEC was calculated using the predicted drug content values and the sample size of the calibration set. Similarly, the RMSECV was determined using the predicted drug content values obtained from cross-validation and the sample size of the calibration set, while the RMSEP was derived using the predicted drug content values and the sample size of the validation set. Cross-validation was performed using the Venetian blinds method.

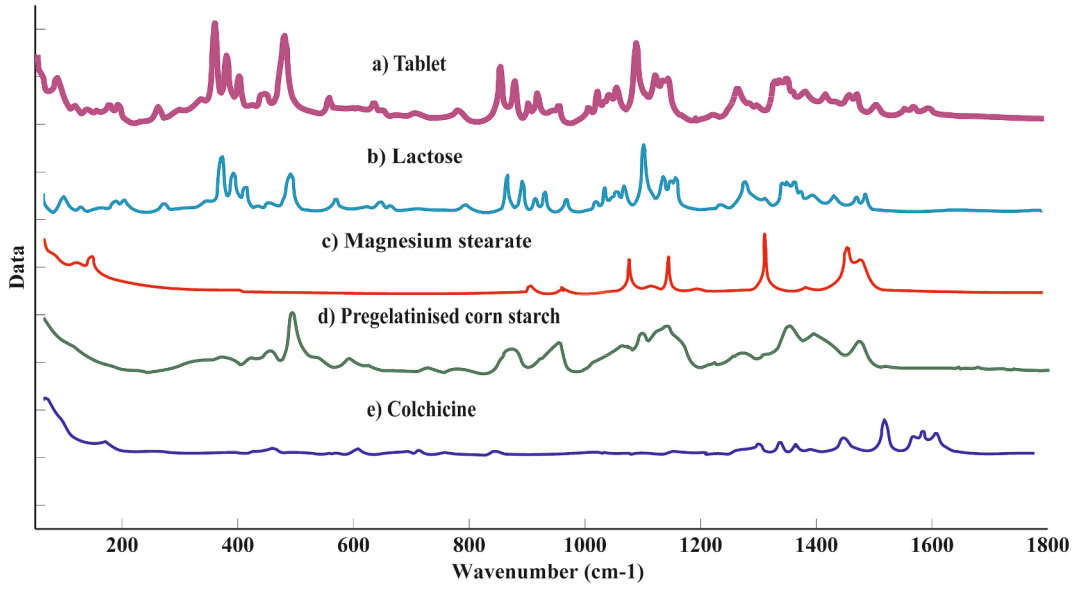


Fig. 1. TRS spectra of COL, excipients, and tablet.

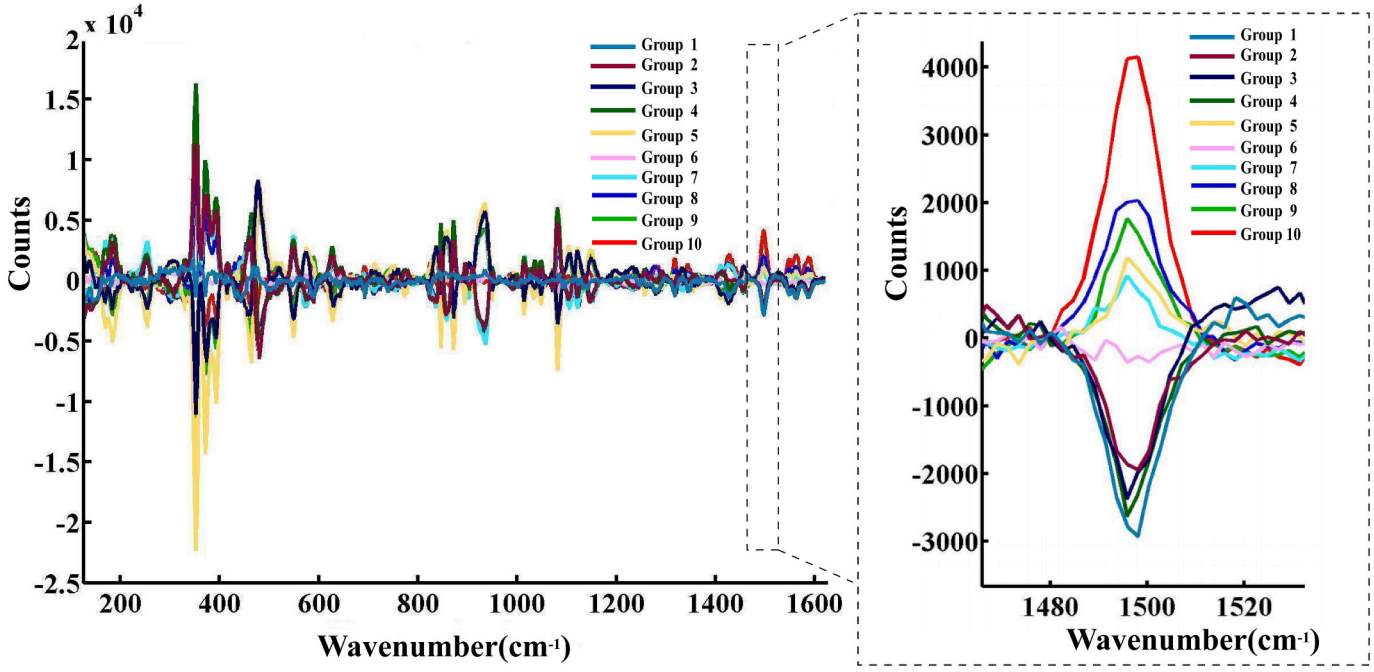


Fig. 2. TRS spectra of the calibration samples used in the model building.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y_i} - y_i)^2}{n}} \quad (1)$$

The prediction of linearity was evaluated by fitting a straight line to the "predicted (TRS) versus measured (HPLC) concentrations" graph, resulting in a determination coefficient (R^2). The limit of detection (LOD) which was the minimum concentration that can be detected with a 95 % confidence level, was calculated using the following equation (Koide et al., 2020).

$$LOD = 3 \times \text{standard deviation } (\delta)/\text{slope of the regression curve } (s) \quad (2)$$

Similarly, the limit of quantification (LOQ) was calculated using Eq.3.

$$LOQ = 10 \times \delta/s \quad (3)$$

3. Results and discussion

3.1. Method feasibility

Fig. 1 shows the Raman spectra of COL tablets and their individual components. The API and COL tablets exhibited identical characteristic Raman signals in the range of 1510 cm^{-1} to 1594 cm^{-1}, derived from the stretching vibrations of benzene. These distinct absorption peaks, absent in the placebo spectra, were identified as key features in the TRS spectra for quantifying the API content in COL tablets.

Fig. 2 illustrates the Raman spectra of calibration samples with

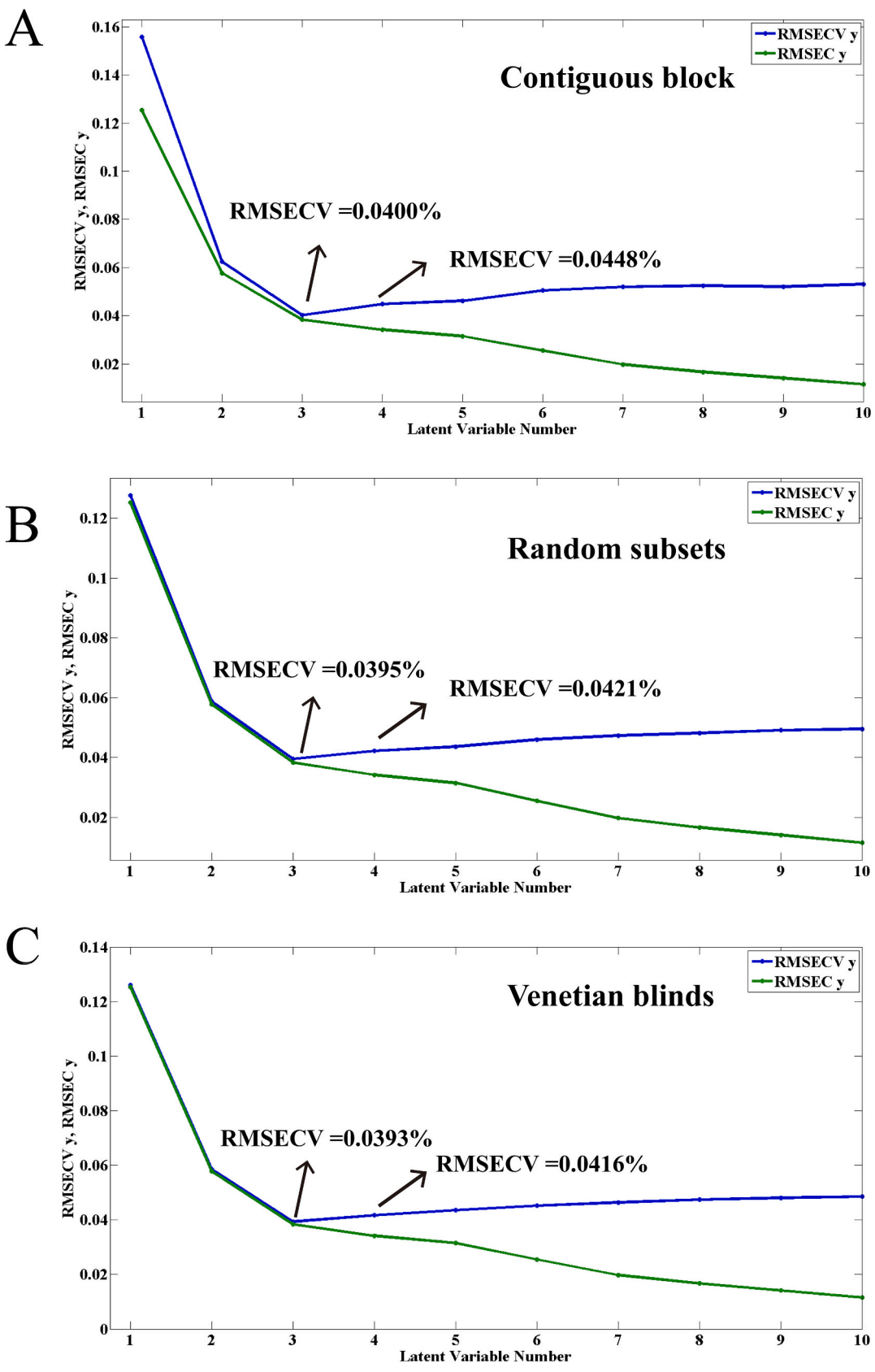


Fig. 3. The RMSECV and RMSEC values (%) for different PLS factors using three cross-validation methods.

Table 2
Performance of PLS regression model using different preprocessing methods.

No	Preprocessing method	Spectral region (cm^{-1})	Number of PLS factor	R^2	RMSEC (%)	RMSECV (%)
1	MSC(median)	170–1700	3	0.940	0.0425	0.0432
2			4	0.950	0.0386	0.0398
3	Mean centre		3	0.944	0.0411	0.0420
4			4	0.950	0.0389	0.0404
5	MSC(median) + Savitzky-Golay 1st derivative (window size: 15points, polynomial order: 2)		3	0.938	0.0433	0.0440
6			4	0.948	0.0395	0.0406
7	MSC(median) + Savitzky–Golay 1st derivative (window size: 17 points, polynomial order: 2)		3	0.938	0.0433	0.0440
8			4	0.949	0.0394	0.0405
9	MSC(median) + Mean centre	170–1700	3	0.951	0.0383	0.0393
*10			4	0.961	0.0342	0.0416
11		300–1600	4	0.952	0.0379	0.0392
12		500–1600	4	0.954	0.0372	0.0397

varying COL concentrations (one spectrum per group). For tablets containing 70–130 % of the labelled COL concentration, a clear correlation was observed between the Raman scattering intensities in the range of 1510 cm^{-1} to 1594 cm^{-1} and the drug concentration. Variations in spectral intensity confirmed measurable differences corresponding to COL concentration levels. The lowest API concentration is shown in blue (Group 1), and the highest concentration is shown in red (Group 10).

3.2. Development of a partial least squares (PLS) regression model

The main multivariate analysis methods included partial least squares (PLS), PCA, partial least squares discriminant analysis (PLS-DA), and constrained regularisation (CR) (Shih, 2015). PLS is a multivariate statistical data analysis method based on principal component regression, recognised for its high selectivity and predictive accuracy in mixture analysis. To develop a calibration model with good prediction performance, the number of PLS factors, preprocessing methods, and spectral region were discussed in the study. The R^2, RMSEC, and RMSECV were used as predictive performance indicators of the calibration model.

Internal cross-validation was conducted to evaluate model performance and fine-tune parameters, such as the number of components for PLS. This process occurs during the calibration phase and is typically completed automatically in chemometric software packages. It involves building a model through various iterations by systematically including and excluding specific samples and then assessing the prediction accuracy for the remaining samples. Fig. 3 presents the RMSEC and RMSECV values for PLS factors 1 to 10, calculated using three cross-validation methods: random subset, contiguous block, and venetian blinds, with the number of splits set to 10. Minimal difference in RMSECV values were observed between the three methods. RMSECV values plateaued at three or four PLS factors, as further detailed in Table 2. The venetian blinds cross-validation method yielded the lowest RMSECV value and was, therefore, selected for use in the model, regardless of whether the number of PLS factors was set to three or four (Fig. 3).

In the next step, the number of PLS factors was determined. The primary objective of factor selection is to identify a subset of predictor variables that optimise the performance of the model. Selecting PLS factors in a manner that avoids overfitting (which occurs when the model captures the intended variability in the data and the noise, reducing its predictive accuracy and generalisability) is crucial. As shown in Table 2, the predictive performance of four PLS factors consistently outperformed those with three factors in terms of R^2 and RMSEC, regardless of the wavelength or preprocessing method. In addition to accounting for the number of variables in the DoE table (Table 1), physical factors arising from the tablet production process were considered. Consequently, four latent variables were selected for model building, as this provided the best performance without overfitting.

The study compared three spectral preprocessing methods: MSC; Savitzky–Golay first derivative; and mean centring. MSC, a scattering correction technique, effectively reduced physical variations between tablet samples, such as differences in size, shape, and hardness. The Savitzky–Golay derivative method enhanced differences in drug concentrations between samples by applying baseline correction and smoothing to the Raman spectra. Mean centring removed all common features, thereby emphasising the spectral differences (Ohashi et al., 2022). Among the twelve calibration models evaluated, calibration model No.10, which utilised MSC and mean centring as correction methods, exhibited the best performance, achieving the lowest RMSEC (0.0342 %) and the highest R^2 (0.961).

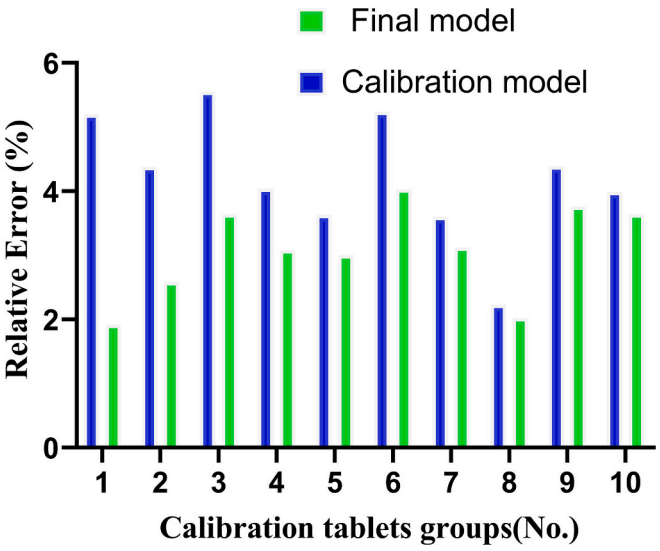


Fig. 4. Average relative error of predicted TRS and HPLC results predicted by the calibrated and final models, determined using ten groups of calibration tablets during internal cross-validation.

The wavenumber region below 200 cm^{-1} was intentionally overexposed during measurements and was, therefore, excluded from model building to improve signal quality at higher wavenumber regions. The wavenumber range of 200–1700 cm^{-1} demonstrated marginally better performance during method development compared to the other two models (300–1600 cm^{-1} and 500–1600 cm^{-1}).

3.3. Optimising the calibration model with commercially available tablets

In an ideal scenario, the preparation process of calibration tablets should closely mimic that of commercially available tablets to establish a robust and reliable model. However, differences between laboratory-simulated small-scale preparation methods and commercial manufacturing processes, such as variations in granulation, are

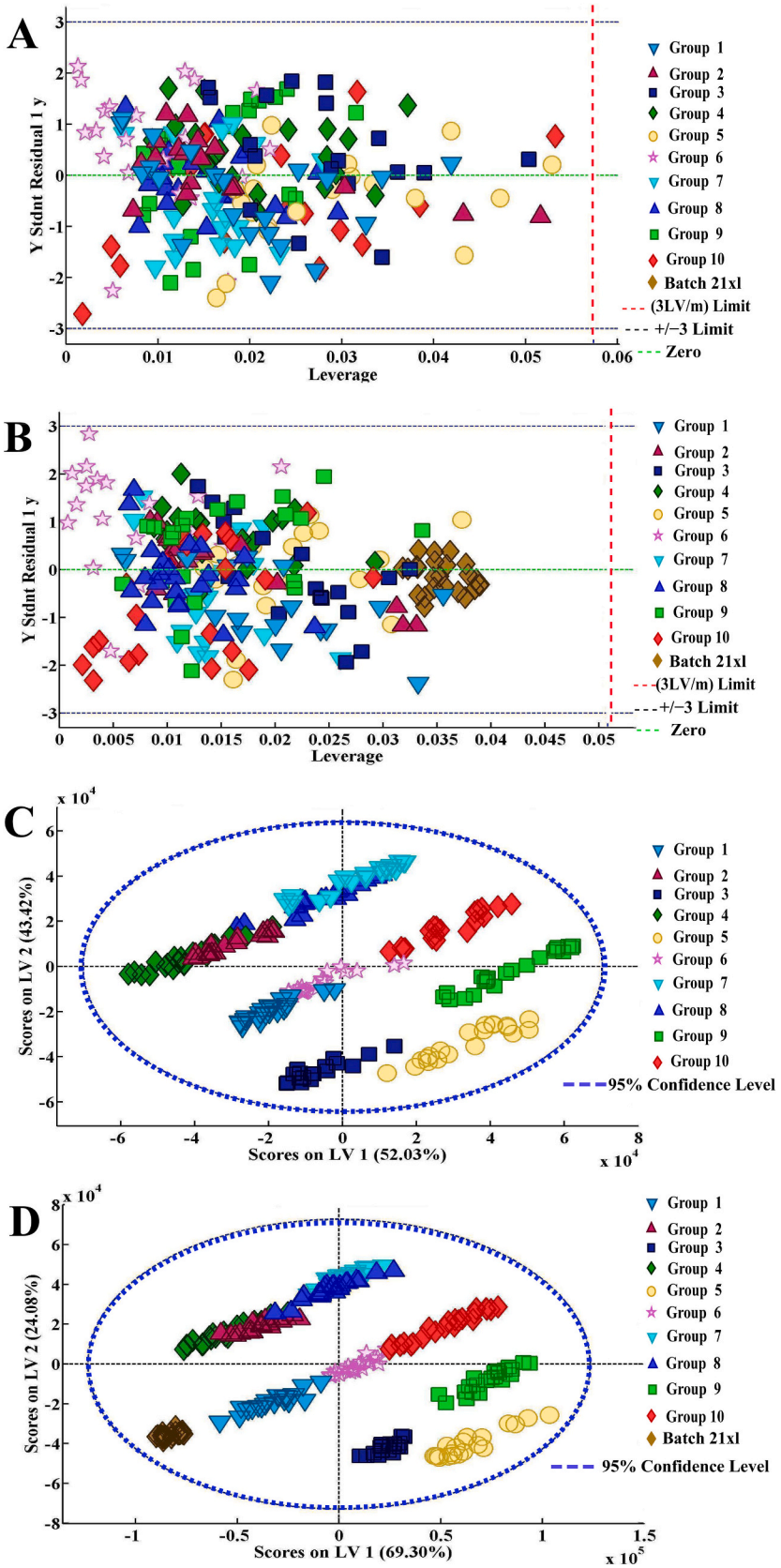


Fig. 5. Residuals vs. Leverage plots of the A) calibrated model and B) final model; Score plots of the C) calibrated model and D) final model. Different colours represent the different groups listed in Table 1.

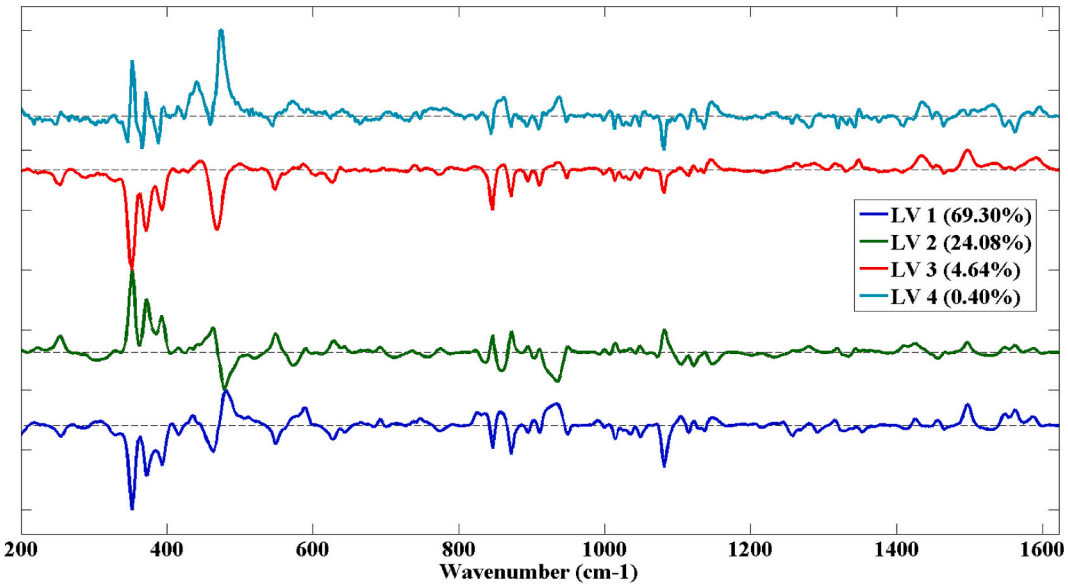


Fig. 6. Loading vectors of the COL assay final model.

unavoidable (Ohashi et al., 2023). Developing TRS models for high-concentration active components is generally more straightforward compared to low-concentration components. High-concentration ingredients tend to form more homogeneous samples, enabling the development of more accurate TRS models. In contrast, low-concentration samples often suffer from poor mixing, resulting in greater variability. This variability can be observed in Fig. 4, where certain calibration sample group with lower concentrations exhibit relatively large errors, likely due to the challenges in sample preparation and inhomogeneities inherent in low-dose formulations.

This study aimed to evaluate the feasibility of establishing a TRS model for a low-dose, highly toxic drug. To improve the applicability of the model to commercially available tablets, a batch of ten commercially available tablets (LOT 21X1) was scanned twice and incorporated into the existing calibration model for optimisation. The spectral region, number of PLS factors, and preprocessing methods of the final model remained consistent with those used in the calibration model.

As shown in Fig. 4, the relative error (Eq. (4)) between HPLC values and TRS-predicted values using the optimised model was smaller than that predicted by the calibrated model.

$$relative\ error = \left|\frac{\hat{y_i} - y_i}{y_i}\right| \times 100\% \quad (4)$$

TRS values were predicted automatically during internal cross-validation, where a portion of the calibration data was used for modelling and the remainder for prediction. This approach improved the applicability and accuracy of the model for determining the COL content in the commercially available tablets.

Fig. 5 depicts the evaluation of the dispersion of the data and the quality of the samples in both the calibrated and final models. The score of Residuals vs. Leverage in Fig. 5 A and B was used to judge whether there were extreme points. All the samples are within the limit range, indicating no outliers. The score plots in Fig. 5 C and D demonstrate that all sample points were within the 95 % confidence interval, clustered consistently by concentration level, and exhibited no significant differences within each concentration level.

The loading vectors of PLS factor 1 (69.30 % contribution) exhibited peaks at wavenumbers similar to those in the pre-processed spectra of COL and pre-gelatinised corn starch. The loading vector of PLS factor 2 (24.08 % contribution) showed peaks at wavenumbers similar to those of the pre-processed spectra of lactose, which also contained characteristic peaks of COL (Fig. 6). This correlation indicated that the developed calibration model accurately reflected changes in COL concentration within the samples. The loading vector of PLS factor 3, contributing 4.64 % to the variance, showed peaks similar to the pre-processed spectra of magnesium stearate. Meanwhile, the loading vector of PLS factor 4, with a contribution of 0.40 %, was inferred to be related to physical factors such as thickness, hardness, porosity (Bawuah et al., 2023), or granule size. To address these variations, MSC and mean centring were employed as correction methods during the development of the PLS regression calibration model (Andrews et al., 2018). Furthermore, a batch of commercial tablets was incorporated into the model. The optimised model demonstrated good repeatability and high accuracy, thereby enhancing the applicability of the model (Zhao et al., 2022b).

The model performance was also reflected in the RMSEC and RMSECV values, with a lower value being preferable, as shown in Fig. 7. For the calibrated model, RMSEC and RMSECV were 0.0342 % and 0.0416 %, respectively. After the model was optimised using commercial samples, these values converged to RMSEC (0.0376 %) and RMSECV (0.0389 %), the RMSEC/RMSECV ratio served as a strong indicator of the model's robustness. Additionally, the RMSEP value decreased significantly from 0.286 % to 0.017 %, indicating an enhancement in predictive accuracy.

As the linearity inferred here is inherently different from HPLC methods, one simply assesses whether the detector response produces a linear response for the reference material at different concentrations. This was a comparison of the two methods being assessed (Villaumié et al., 2019). Both models exhibit linearity with $R^2 = 0.96$ and 0.95 across a range of ±30 % of the nominal API concentration, which has shown good linear correlation between TRS and HPLC results within 95 % confidence limit. The linear regression equation of the final model was $Y = 0.9451 \times X + 0.000454$ ($p < 0.0001$, where X was the content measured using HPLC and Y was the content measured using TRS).

In multivariate analysis, multiple signals (responses) and components in a mixture make the estimation of LOD less straightforward. A simplified ICH recommendation was followed to calculate the LOD and LOQ, where δ was calculated as 0.038 % based on the standard deviation of the residual error, slope obtained from the curve parameters as 0.95 (Fig. 7.B). The final model yielded values of 0.13 % and 0.40 % (*w*/w) for the LOD and LOQ, respectively.

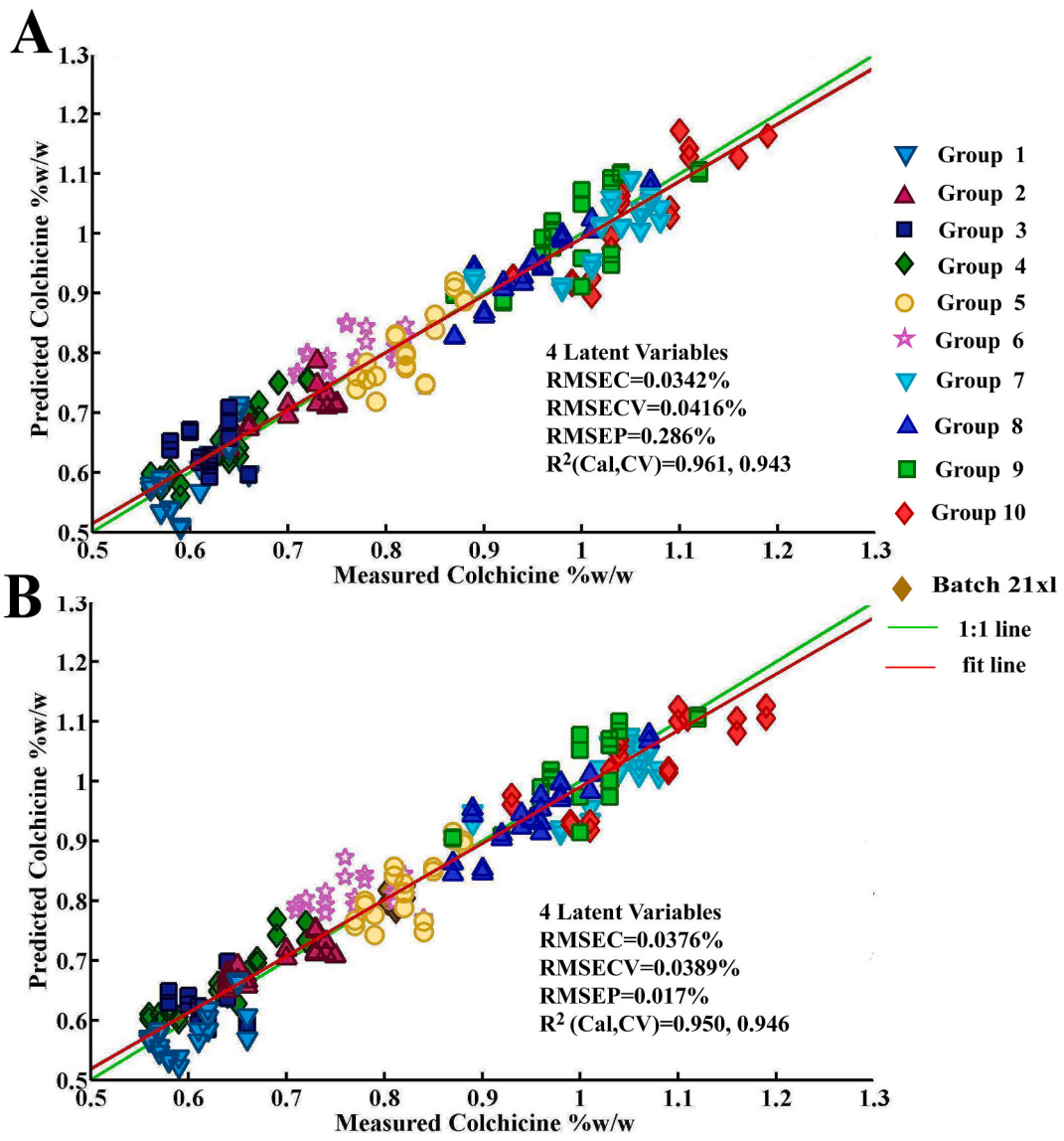


Fig. 7. Plot of measured HPLC assay versus predicted TRS assay (calculated as a percentage of dosage weight *w*/w,%). A) calibrated model; B) final model.

3.4. Content prediction for commercially available tablets

To evaluate the predictive ability of the final model, it was challenged to predict the COL content expressed as percentage of label claim (%) in four batches. The prediction test used a total of 40 commercial tablets from the same manufacturer that were not used in the calibration phase. Compared to HPLC results, the average COL content predicted by TRS differed by 1.2 %, as shown in Table 3. The relative standard deviation (RSD) of the API content predicted by TRS were 1.2 %, which was notably lower than the RSD values of 2.9 % obtained from HPLC. The absolute value of the relative error of the two methods varied between 0 and 3.8 % (Fig. 8), indicating the strong predictive capability of the TRS model. This result demonstrates the stability and repeatability of the final TRS model.

To illustrate the uniformity in the COL amount between batches of samples, the acceptance values of content uniformity (AV) were calculated based on the content (percentage of label claim) according to the standards of the United States Pharmacopoeia (USP 43rd). The AVs of the four batches calculated by both methods did not exceed 15.0, indicating content uniformity of the validation commercial tablets meet the requirements of USP 43rd. For the four batches of samples, the differences in AVs calculated by HPLC and TRS methods (Table 3) were 1.5, 1.8, 0.3, and 2.3. The minor differences indicate that the two methods provide consistent assessments of batch uniformity. In conclusion, TRS has proven suitable for content uniformity measurement and can be used as a quality control tool in the production line as a supplementary choice for HPLC (Griffen et al., 2015).

3.5. Environmental, time and personnel considerations

Despite the clinical use of COL, there are concerns regarding the protection of operation personnel and the environment in the pharmaceutical process for such a toxic drug. While content uniformity determination using the HPLC has been specified in the Chinese and US Pharmacopoeia, the adverse effects of the HPLC method on the environment and operation personnel include many recognised drawbacks in terms of: (i) inevitable exposure to COL solution which poses a high risk to personnel health during sample preparation and (ii) the large amount of mobile phase waste containing COL increases the difficulty of treatment and environmental pollution. (iii) Long sample preparation and measurement times (at least two days batch uniformity analysis). To this end, an alternative option is to utilise TRS spectroscopy, which has become a popular and non-destructive tool for the quantitative determination of toxic compounds such as COL. Overall, the experiment

Table 3
The content prediction and uniformity calculation.

Batch	Sample	Content (percentage of label claim)		Acceptance value of content uniformity (AV)	
		HPLC (%)	TRS (%)	HPLC	TRS
21 F1	1	98.2	95.2	3.4	4.9
	2	101.1	96.8		
	3	98.8	94.6		
	4	100.4	96.4		
	5	96.0	96.3		
	6	98.6	94.8		
	7	99.0	96.5		
	8	98.4	97.4		
	9	97.6	96.5		
	10	97.9	94.9		
21X1	1	98.6	94.1	3.3	5.1
	2	100.6	95.8		
	3	99.9	95.1		
	4	96.5	94.6		
	5	99.7	96.7		
	6	97.8	95.7		
	7	99.4	94.6		
	8	100.3	95.5		
	9	97.4	94.6		
	10	99.8	95.2		
22 L1	1	93.2	92.1	6.6	6.9
	2	92.5	93.0		
	3	93.4	93.5		
	4	93.7	93.4		
	5	94.7	95.1		
	6	92.9	94.2		
	7	93.0	94.7		
	8	93.1	93.5		
	9	93.0	94.8		
	10	93.6	94.1		
22 K1	1	93.4	94.0	7.4	5.1
	2	97.1	96.0		
	3	92.6	95.2		
	4	95.4	95.1		
	5	93.2	94.9		
	6	94.2	95.7		
	7	95.7	94.9		
	8	94.5	95.2		
	9	93.7	96.3		
	10	94.3	97.1		
	Average content (%)	96.3	95.1	/	
	RSD(%)	2.9	1.2		

Note: sd (Sample standard deviation),$\bar{x}$(Mean of individual contents), Acceptance value of content uniformity(AV). AV = $|M-\bar{x}| + 2.4 \times sd$ (If 98.5% $\leq\bar{x} \leq$101.5%, then M = $\bar{x}$ (AV =2.4× sd). If $\bar{x}$ <98.5%, then M = 98.5% (AV = 98.5 –$\bar{x}$ + 2.4 × sd)).

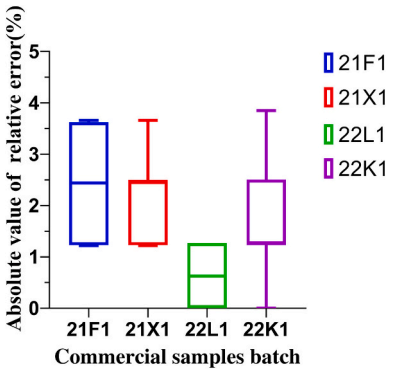


Fig. 8. The boxplot of relative error between HPLC and TRS content(calculated as percentage of the dosage weight *w*/w,%).

required less than 10 min for the analysis of one batch of samples, which lessens the risk of prolonged personnel and environmental exposure to toxic substances, aligning with the concept of green chemistry.

4. Conclusions

In conclusion, low COL concentrations (0.83 % *w*/w) in commercial tablets has been successfully quantified using TRS with PLS, which is applicable either during manufacturing or in QC lab tests for content uniformity. The quantitative model has displayed satisfactory predictive ability, good repeatability, and high accuracy, with an RMSEC of 0.038 % and an RMSECV of 0.039 %. The LOD and LOQ were estimated to be 0.13 % and 0.40 % w/w. The model has been used to predict COL content in a total of 40 commercial tablets from four batches from the same manufacturer. The absolute value of relative error of the TRS and HPLC content results((calculated as percentage of the dosage weight w/w,%)) varied between 0 and 3.8 %. The RSD of the API content of both forms predicted by TRS were 1.2 %, surprisingly lower than that of HPLC results, which demonstrated good stability and repeatability of the TRS final model.

Overall, this study demonstrates that TRS is a suitable tool for the rapid analysis of low-level COL content in final pharmaceutical products. The analysis was completed without sample preparation, which minimised the exposure of operational personnel to toxic chemicals, including solvents and the API.

Author contributions

All authors have given approval to the final version of the manuscript.

Ningzi Guo: Conceptualization, Software, Formal analysis, Writing. **Sijing Niu**: Project Data curation and Administration. **Ying Geng:** Project Data curation and Administration. **Guangzhi Shan**: Investigation; Methodology. **Ningyi Wei**: Conceptualization, editing and Supervision. **Hua Chen**: Writing - review & editing.

Funding

This work was supported by the Youth Development Research Foundation of National Institutes for Food and Drug Control (Grant Number 2024A4),China.

CRediT authorship contribution statement

Ningzi Guo: Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sijing Niu:** Software, Methodology, Investigation. **Ying Geng:** Supervision, Software. **Guangzhi Shan:** Resources. **Ningyi Wei:** Writing – review & editing, Supervision, Investigation. **Hua Chen:** Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We are grateful to Jiwen Cai, Jingjing wang, and Xiaodan Zhang et al. from Agilent Technologies (China) Co., Ltd. for their Support in method development and for the instrument used in this work.

References

Aghabiklooei, A., Zamani, N., Hassanian-Moghaddam, H., Nasouhi, S., Mashayekhian, M., 2014. Acute colchicine overdose: report of three cases. Reumatismo 65, 307–311. https://doi.org/10.4081/reumatismo.2013.720.

Andrews, D., Geentjens, K., Igne, B.T., Mcgeorge, G., Owen, A., Pedge, N., Villaumié, J., Woodward, V., 2018. Analytical Method Development using Transmission Raman Spectroscopy for Pharmaceutical Assays and Compliance with Regulatory Guidelines—Part I: Transmission Raman Spectroscopy and Method Development. J. Pharm. Innov. 13, 121–132. https://doi.org/10.1007/s12247-018-9311-7.

Bawuah, P., Evans, M., Lura, A., Farrell, D.J., Barrie, P.J., Kleinebudde, P., Markl, D., Zeitler, J.A., 2023. At-line porosity sensing for non-destructive disintegration testing in immediate release tablets. Int. J. Pharm. X 5, 100186. https://doi.org/10.1016/j.ijpx.2023.100186.

Bouabdallaoui, N., Tardif, J.C., 2022. Repurposing Colchicine for Heart Disease. Annu. Rev. Pharmacol. Toxicol. 62, 121–129. https://doi.org/10.1146/annurev-pharmtox-052120-020445.

Burley, J.C., Alkhalil, A., Bloomfield, M., Matousek, P., 2012. Transmission Raman spectroscopy for quality control in model cocrystal tablets. Analyst 137, 3052–3057. https://doi.org/10.1039/C2AN35216H.

Deftereos, S.G., Beerkens, F.J., Shah, B., Giannopoulos, G., Vrachatis, D.A., Giotaki, S.G., Siasos, G., Nicolas, J., Arnott, C., Patel, S., Parsons, M., Tardif, J.C., Kovacic, J.C., Dangas, G.D., 2022. Colchicine in Cardiovascular Disease: In-Depth Review. Circulation 145, 61–78. https://doi.org/10.1161/CIRCULATIONAHA.121.056171.

Doddridge, G., Hong, E., Tan, D.C.T., Liu, Y., 2022. A Non-destructive Quantitative Transmission Raman Spectroscopy Method for active Pharmaceutical Ingredient in Drug Product In-Use Samples prepared in Dosing Vehicles. AAPS PharmSciTech 23, 132. https://doi.org/10.1208/s12249-022-02286-w.

Eliasson, C., Macleod, N.A., Jayes, L.C., Clarke, F.C., Hammond, S.V., Smith, M.R., Matousek, P., 2008. Non-invasive quantitative assessment of the content of pharmaceutical capsules using transmission Raman spectroscopy. J. Pharm. Biomed. Anal. 47, 221–229. https://doi.org/10.1016/j.jpba.2008.01.013.

Farrell, J.A., Higgins, K., Kalivas, J.H., 2012. Updating a near-infrared multivariate calibration model formed with lab-prepared pharmaceutical tablet types to new tablet types in full production. J. Pharm. Biomed. Anal. 61, 114–121. https://doi.org/10.1016/j.jpba.2011.11.003.

Griffen, J., Owen, A., Matousek, P., 2015. Comprehensive quantification of tablets with multiple active pharmaceutical ingredients using transmission Raman spectroscopy—a proof of concept study. J. Pharm. Biomed. Anal. 115, 277–282. https://doi.org/10.1016/j.jpba.2015.07.019.

Griffen, J.A., Owen, A.W., Burley, J., Taresco, V., Matousek, P., 2016. Rapid quantification of low level polymorph content in a solid dose form using transmission Raman spectroscopy. J. Pharm. Biomed. Anal. 128, 35–45. https://doi.org/10.1016/j.jpba.2016.05.017.

Griffen, J.A., Owen, A.W., Matousek, P., 2018. Quantifying low levels (<0.5% w/w) of warfarin sodium salts in oral solid dose forms using Transmission Raman spectroscopy. J. Pharm. Biomed. Anal. 155, 276–283. https://doi.org/10.1016/j.jpba.2018.04.008.

Hennigan, M.C., Ryder, A.G., 2013. Quantitative polymorph contaminant analysis in tablets using Raman and near infra-red spectroscopies. J. Pharm. Biomed. Anal. 72, 163–171. https://doi.org/10.1016/j.jpba.2012.10.002.

Karamanou, M., Tsoucalas, G., Pantos, K., Androutsos, G., 2018. Isolating Colchicine in 19th Century: an Old Drug Revisited. Curr. Pharm. Des. 24, 654–658. https://doi.org/10.2174/1381612824666180115105850.

Koide, T., Takeuchi, Y., Otaki, T., Yamamoto, K., Shimamura, R., Ohashi, R., Inoue, M., Fukami, T., Izutsu, K.I., 2020. Quantification of a cocrystal and its dissociated compounds in solid dosage form using transmission Raman spectroscopy. J. Pharm. Biomed. Anal. 177, 112886. https://doi.org/10.1016/j.jpba.2019.112886.

Lei, Y., Yang, Y., Yang, G., Li, A., Yang, Y., Wang, Y., Gao, C., 2024. Delivery strategies for colchicine as a critical dose drug: reducing toxicity and enhancing efficacy. Pharmaceutics 16, 222. https://doi.org/10.3390/pharmaceutics16020222.

Li, Y., Igne, B., Drennen 3rd, J.K., Anderson, C.A., 2016. Method development and validation for pharmaceutical tablets analysis using transmission Raman spectroscopy. Int. J. Pharm. 498, 318–325. https://doi.org/10.1016/j.ijpharm.2015.11.049.

Molad, Y., 2002. Update on colchicine and its mechanism of action. Curr. Rheumatol. Rep. 4, 252–256. https://doi.org/10.1007/s11926-002-0073-2.

Nagy, B., Farkas, A., Borbás, E., Vass, P., Nagy, Z.K., Marosi, G., 2018a. Raman Spectroscopy for Process Analytical Technologies of Pharmaceutical secondary Manufacturing. AAPS PharmSciTech 20, 1. https://doi.org/10.1208/s12249-018-1201-2.

Nagy, B., Farkas, A., Magyar, K., Démuth, B., Nagy, Z.K., Marosi, G., 2018b. Spectroscopic characterization of tablet properties in a continuous powder blending and tableting process. Eur. J. Pharm. Sci. 123, 10–19. https://doi.org/10.1016/j.ejps.2018.07.025.

Ohashi, R., Fujii, A., Fukui, K., Koide, T., Fukami, T., 2022. Non-destructive quantitative analysis of pharmaceutical ointment by transmission Raman spectroscopy. Eur. J. Pharm. Sci. 169, 106095. https://doi.org/10.1016/j.ejps.2021.106095.

Ohashi, R., Koide, T., Fukami, T., 2023. Effects of wet granulation process variables on the quantitative assay model of transmission Raman spectroscopy for pharmaceutical tablets. Eur. J. Pharm. Biopharm. 191, 276–289. https://doi.org/10.1016/j.ejpb.2023.09.009.

Pascart, T., Richette, P., 2018. Colchicine in Gout: an Update. Curr. Pharm. Des. 24, 684–689. https://doi.org/10.2174/1381612824999180115103951.

Pieters, S., Saeys, W., Van den Kerkhof, T., Goodarzi, M., Hellings, M., De Beer, T., Heyden, Y.V., 2013. Robust calibrations on reduced sample sets for API content prediction in tablets: definition of a cost-effective NIR model development strategy. Anal. Chim. Acta 761, 62–70. https://doi.org/10.1016/j.aca.2012.11.034.

Portincasa, P., Scaccianoce, G., Palasciano, G., 2013. Familial mediterranean fever: a fascinating model of inherited autoinflammatory disorder. Eur. J. Clin. Investig. 43, 1314–1327. https://doi.org/10.1111/eci.12170.

Robinson, P.C., Terkeltaub, R., Pillinger, M.H., Shah, B., Karalis, V., Karatza, E., Liew, D., Imazio, M., Cornel, J.H., Thompson, P.L., Nidorf, M., 2022. Consensus Statement regarding the Efficacy and Safety of Long-Term Low-Dose Colchicine in Gout and Cardiovascular Disease. Am. J. Med. 135, 32–38. https://doi.org/10.1016/j.amjmed.2021.07.025.

Sato, T., Haneishi, K., Hisada, H., Fujii, M.Y., Koide, T., Fukami, T., 2024. Real-Time Quantitative Evaluation of a Drug during Liposome Preparation using a Probe-Type Raman Spectrometer. Langmuir 40 (15), 7962–7973. https://doi.org/10.1021/acs.langmuir.3c03872.

Shih, W.C., 2015. Constrained regularization for noninvasive glucose sensing using Raman spectroscopy. J. Innov. Opt. Health Sci. 8, 4. https://doi.org/10.1142/S1793545815500224.

Trenfield, S.J., Januskaite, P., Goyanes, A., Wilsdon, D., Rowland, M., Gaisford, S., Basit, A.W., 2022. Prediction of Solid-State form of SLS 3D Printed Medicines using NIR and Raman Spectroscopy. Pharmaceutics 14, 589. https://doi.org/10.3390/pharmaceutics14030589.

Triboandas, H., Bezerra, M., Almeida, J., de Castro, M., Santos, B., Schlindwein, W., 2024. Optimizing extrusion processes and understanding conformational changes in itraconazole amorphous solid dispersions using in-line UV-Vis spectroscopy and QbD principles. Int. J. Pharm. X 8, 100308. https://doi.org/10.1016/j.ijpx.2024.100308.

Villaumié, J., Andrews, D., Geentjens, K., Igne, B., Mcgeorge, G., Owen, A., Pedge, N., Woodward, V., 2019. Analytical Method Development using Transmission Raman Spectroscopy for Pharmaceutical Assays and Compliance with Regulatory Guidelines—Part II: Practical Implementation Considerations. J. Pharm. Innov. 14, 245–258. https://doi.org/10.1007/s12247-018-9350-0.

Wang, X., Mao, D.-Z., Yang, Y.-J., 2021. Calibration transfer between modelled and commercial pharmaceutical tablet for API quantification using backscattering NIR, Raman and transmission Raman spectroscopy (TRS). J. Pharm. Biomed. Anal. 194, 113766. https://doi.org/10.1016/j.jpba.2020.113766.

Zhang, Y., McGeorge, G., 2015. Quantitative Analysis of Pharmaceutical Bilayer Tablets using Transmission Raman Spectroscopy. J. Pharm. Innov. 10, 269–280. https://doi.org/10.1007/s12247-015-9223-8.

Zhao, X., Wang, N., Liu, Y., Jiang, Y., Sun, S., Wang, X., Yao, J., Shan, G., 2022a. Fast quantification of diclofenac sodium content in commercially available tablets by transmission Raman spectroscopy with PLS. J. Chemom. e3462. https://doi.org/10.1002/cem.3462.

Zhao, X., Wang, N., Zhu, M., Qiu, X., Sun, S., Liu, Y., Zhao, T., Yao, J., Shan, G., 2022b. Application of Transmission Raman Spectroscopy in Combination with Partial Least-Squares (PLS) for the Fast Quantification of Paracetamol. Molecules 27, 1707. https://doi.org/10.3390/molecules27051707.