DEVELOPMENT OF QUANTITATION METHOD FOR ACYCLOVIR IN TABLET OF KNOWN MANUFACTURING FORMULA USING RAMAN SPECTROSCOPY

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***Abstract**: A fast, simple, nondestructive method using Raman spectrometers was developed for quantitation of acyclovir (ACV) in tablet powder with known composition. The method employed spectra of reference mixture containing from 30% to 84% ACV (w/w) recorded in wavenumber region from 150 cm^{-1} to 2800 cm^{-1} to build PLS assay model using 4 factors. Raman spectra of the tabets were pre-processed in the wavebands of 320 cm^{-1} to 520 cm^{-1}, 820 cm^{-1} to 900 cm^{-1} and 1000 cm^{-1} to 1200 cm^{-1} combined with partial least squares (PLS) regression to establish a quantitative model. The method was validated according to current international requirements on performance of analytical method, and was proven to have specificity, linearity, accuracy, and precision.*

***Keywords:** Acyclovir, Raman spectroscopy, PLS*

1. INTRODUCTION

Acyclovir (ACV) was approved by the FDA as an active pharmaceutical ingredient for human use on March 29, 1982 [1]. In Vietnam, there are currently about 293 products containing ACV that have been granted marketing authorization by the Ministry of Health [2]. ACV is listed as an essential medicine in the category of antiviral drugs, according to Circular No. 19/2018/TT-BYT dated August 30, 2018 by the MOH; it is widely used to treat infections caused by *Herpes simplex* virus types 1 and 2 on the skin and mucous membranes, shingles, and chickenpox [3]. ACV has low bioavailability. To achieve therapeutic effectiveness, high doses may be required, reaching over 1 g/day, and overdose may lead to adverse effects. Therefore, quantitative determination of ACV is important to ensure that the actual dose does not deviate significantly from the theoretical dose, thereby reducing the risk of safety issues.

Currently, the most common method for quantifying ACV in pharmaceutical products is high-performance liquid chromatography (HPLC) [4]. The reliability and popularity of this technique have been well established; however, the method still requires a complex sample preparation process and uses organic solvents that are less environmentally friendly and may pose health risks to laboratory personnel. Raman spectroscopy is a green analytical technique with characteristics well-suited to develop quantitative methods. Thanks to its rapid and simple analysis capabilities, as well as the ability to directly analyze samples without requiring transformation, dissolution, or separate extraction of the analyte, it is a promising technique for application in pharmaceutical testing. However, there are very few studies published on the quantification of ACV using Raman spectroscopy in combination with chemometric processing models. In 2003, Skoulika and colleagues developed a rapid method to quantify ACV in tablets through poly (vinyl chloride) blister packaging using FT-Raman spectroscopy [5], but the method was affected by various factors such as tablet thickness, measurement angle, surface flatness,... and the results showed considerable deviation compared to the HPLC method. Therefore, to leverage the advantages

of the technique, we conducted a study to develop a quantitative analysis procedure for ACV in self-prepared tablet samples using Raman spectroscopy.

2. EXPERIMENTS

2.1. Equipments, Instruments, Chemicals, and Reference Standards

2.1.1. Equipments and Instruments

In this study, Raman spectra were measured using a QTRam transmission Raman spectrometer from B&W TEK (USA). Accurate weighing was performed using a Mettler Toledo analytical balance (Switzerland, precision d = 0.01 mg), a Specac tablet press was used to prepare self-prepared tablets. All equipment was managed and calibrated in accordance with ISO/IEC 17025 and GLP regulations.

The uniform mixing of excipients (Table 1) to prepare the placebo blend and the uniform mixing of ACV with the placebo blend for tablet compression were conducted using an agate mortar and pestle designed for laboratory use.

2.1.2. Materials

The ACV raw material (Batch No. 246456810, manufactured by Zhejiang Charioteer Pharmaceutical Co., Ltd. - China) was determined by the research team to contain 93.60% ACV (original state) using the assay method described in the USP 2024 monograph for Acyclovir [4] (results not published in this article). The excipients used to formulate the tablets included: lactose monohydrate, microcrystalline cellulose (101), sodium starch glycolate (type A), povidone K30, and magnesium stearate, all meeting the standards of the Vietnamese Pharmacopoeia, Edition V.

2.2. Subjects and Research Methods

2.2.1. Research Subject

The tablet samples consisted of ACV raw material and excipients simulating a tablet formulation with an ACV content of 60.0% (w/w), with components as listed in Table 1.

Table 1. *Formulation for the Research Tablet*

No.	Name of materials	Mass
1	Acyclovir	200 mg
2	Lactose monohydrat	70 mg
3	Microcrystalline cellulose (101)	41 mg
4	Sodium starch glycolate (type A)	14 mg
5	Povidone K30	6 mg
6	Magnesium stearate	2.5 mg
Total		**333.5 mg**

The self-prepared tablet samples were mixed from ACV raw material and the placebo blend (consisting of excipient ratio listed in Table 1 without the active ingredient ACV) at varying ratios.

2.2.2. Research Methodology

2.2.2.1. Development of a Quantitative Model Using Raman Spectroscopy

A quantitative model was developed based on Raman spectra of tablet samples prepared from ACV and the placebo blend with precisely known ACV content levels (%, w/w) at five concentrations surrounding the target ACV content in the formulation (60%, w/w), including 30%, 48%, 60%, 72%, and 84% (w/w). Two samples were prepared at each concentration level, corresponding to ACV content ranging from 50% to 140% of the target concentration. The information and actual ACV content values (α) of the mixtures used to prepare these tablet samples are summarized in Table 2.

Table 2. *ACV and Placebo Mixtures Used for Preparing Samples for Model Development*

No.	Sample name	m_{acv} (mg)	$m_{placebo}$ (mg)	α (%, w/w)
1	30	106.98	226.24	30.05
2		107.85	226.52	30.19
3	48	170.73	163.94	47.75
4		171.46	163.65	47.89
5	60	214.09	119.22	60.12
6		214.25	120.37	59.93
7	72	257.34	77.16	72.01
8		257.63	77.75	71.90
9	84	298.37	35.65	83.61
10		299.18	35.03	83.79

In which: the coefficient α (%, w/w) represents the content of ACV in the tablet, calculated as the actual weight of ACV over the total weight of the tablet.

The spectrum of each sample was randomly recorded 10 times using a transmission Raman spectrometer in the wavenumber range from 150 cm^{-1} to 2800 cm^{-1}, with a scan time of 4 seconds and a resolution of 4 cm^{-1}, the laser source intensity was set at 90%. The spectra obtained at each ACV content level (20 spectra from 2 samples) were randomly divided into two parts: 16 spectra (80%) were used to train the model (calibration), and 4 spectra (20%) were used to validate and optimize the model (validation).

All spectra were preprocessed using the following tools: first derivative of spectral data (Savitzky - Golay 1st Differential), standard normal variate (SNV), and mean centering (center) to eliminate the influence of optical path variations in the Raman measurement technique. The preprocessed spectral data corresponding to Raman shifts in the wavenumber regions from 320 cm^{-1} to 520 cm^{-1}, 820 cm^{-1} to 900 cm^{-1}, and 1000 cm^{-1} to 1200 cm^{-1} were used to develop a quantitative model using the Partial Least Squares (PLS) algorithm with 4 factors.

To quantify ACV-containing tablet samples, each sample was randomly measured 5 times, and the average of these 5 measurements was taken. The relative standard deviation (RSD) of these 5 measurements must not exceed 2.0% to ensure the internal uniformity of the pharmaceutical sample.

The model was validated for specificity, linearity, accuracy, repeatability, and intermediate precision according to ICH [5], AOAC International [6], and EMA [7] guidelines.

2.2.2.2. Data Processing Method

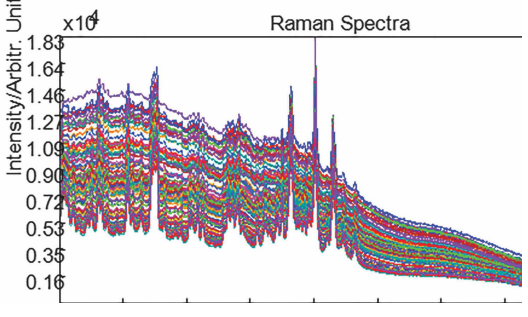
Result calculations and evaluations were performed using BWIQ software version 4.1.4 (including spectral preprocessing algorithms and qualitative/quantitative modeling using appropriate chemometric algorithms) and Microsoft Excel version 2019.

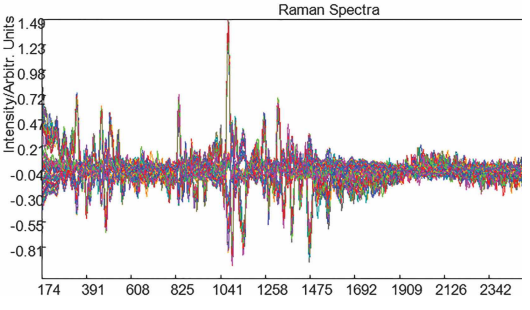
3. RESULTS AND DISCUSSION

3.1. Development of the Quantitative Model

Tablet samples with accurately known ACV content values as shown in Table 2 were measured using Raman spectroscopy, each sample scanned 10 times. The obtained spectra and their corresponding α values were used as data for developing the quantitative model.

Figure 1A shows representative Raman spectra measured from mixtures with different α values before spectral preprocessing. The raw spectra of the tablet samples, corresponding to different α values, exhibit inconsistent variations in shape relative to ACV content. In spectroscopic analysis, the spectral profile of the analyzed sample mainly depends on the nature of the analyte and the optical path of the measurement. To evaluate the variation of spectral signals in function of ACV mass content (%, w/w) in the samples (the intrinsic sample characteristics), appropriate spectral preprocessing methods are needed to eliminate the influence from inherent instability of optical path in Raman spectroscopy as well as to enhance the intensity of signal changes associated with variations in ACV content in the tablet samples..





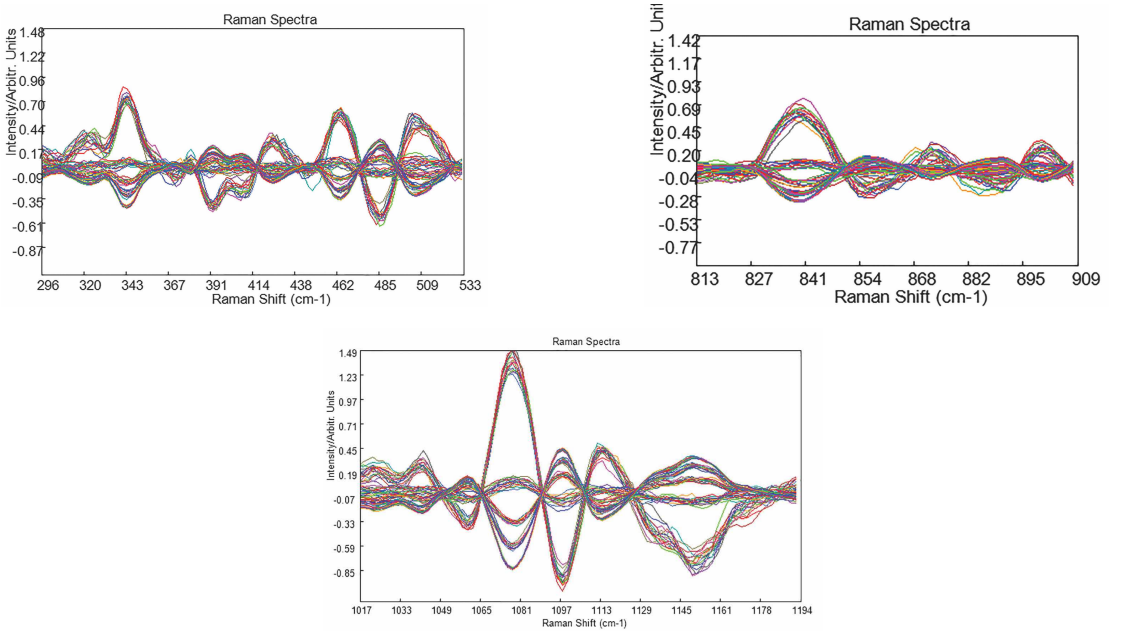


Figure 1. *Raman spectra of self-prepared ACV tablet samples at different concentrations before transformation (A) and after spectral preprocessing and transformation using PLS (B: wavenumber range from 150 cm^{-1} to 2800 cm^{-1}; C: wavenumber regions from 300 cm^{-1} to 530 cm^{-1}; D: wavenumber regions from 800 cm^{-1} to 900 cm^{-1}; E: wavenumber regions from 1000 cm^{-1} to 1200 cm^{-1}).*

After evaluating several spectral preprocessing methods, the combination of the first derivative of spectral data (Savitzky - Golay 1st Differential), standard normal variate, and mean centering (center) effectively addressed the above-mentioned issues. With this set of preprocessing tools the resulting preprocessed spectra of the ACV-containing tablet samples are shown in Figure 1B. Over the entire preprocessed spectral range from 150 to 2800 cm^{-1}, the spectral regions from 320 cm^{-1} to 520 cm^{-1}, 820 cm^{-1} to 900 cm^{-1}, and 1000 cm^{-1} to 1200 cm^{-1} showed clear and consistent intensity variations among samples with different α values (Figure 1B - 1E). Therefore, preprocessed spectral data from these regions were selected to construct the ACV quantitative model in order to obtain accurate evaluation of ACV content variations while minimizing errors.

Partial Least Squares (PLS) regression has been widely used to build quantitative models with spectroscopic data especially those from near-infrared and Raman spectroscopies. Therefore, in this study, the PLS model was selected to establish the quantitative method for ACV.

The PLS model was optimized by selecting the number of main variables (factors) to meet the performance requirements of routine quantitative methods [6, 7], as well as the reference criteria in the EMA guideline for quantification method using NIR spectroscopy [8]. According to these requirements, the correlation coefficients of the calibration set (Rc) and the internal validation set (Rv) must not be less than 0.998, the root mean square error of calibration (RMSEC) and validation (RMSEV) must be sufficiently low and as close to each other as possible.

According to the model evaluation results at different numbers of factors, starting from 4 factors and above, the correlation coefficients between the actual and predicted values for both the calibration dataset (Rc) and the internal validation dataset (Rv) remained virtually unchanged and consistently above 0.998 (Figure 2A). Furthermore, from 4 factors onward, RMSEC and RMSEV did not show significant changes. At 4 factors, these two values were the closest to each other, whereas using more than 4 factors led to an increasing difference between RMSEV and RMSEC - an indication of overfitting. This suggests the model becomes overly specific and localized to the calibration dataset, reducing its predictive accuracy for external data (e.g., the internal validation dataset or spectral data from new samples requiring ACV quantification) (Figure 2B). Therefore, using 4 factors to build the ACV quantitative model is the most appropriate choice.

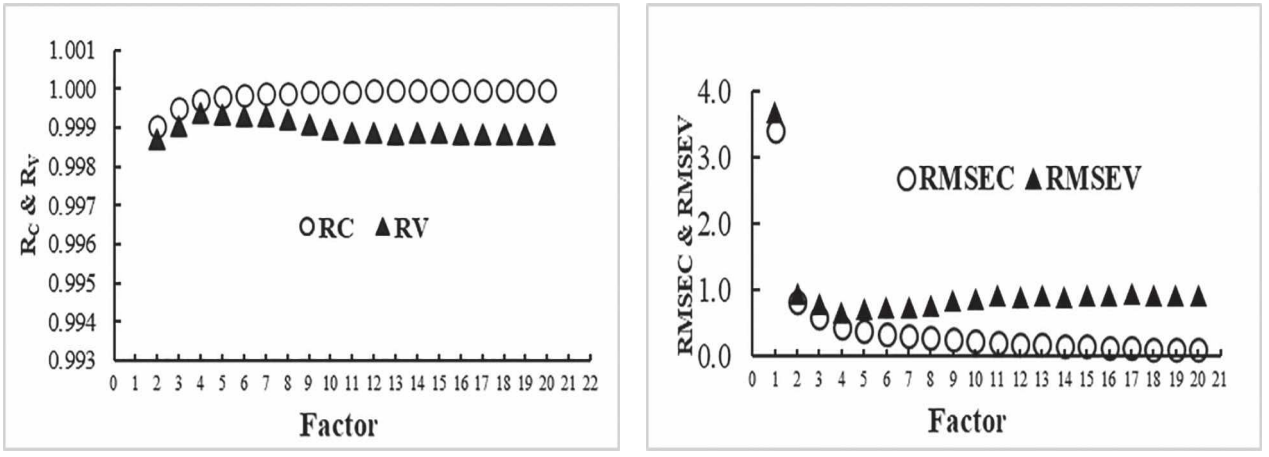


***Figure 2**. Model optimization results based on the number of factors*

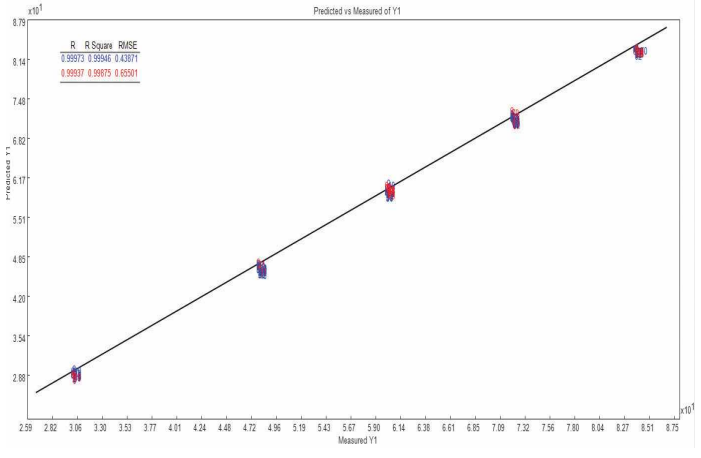


***Figure 3**. Comparison results between actual values and model-predicted values for the self-prepared samples with different α values*

Once the model has been established, for ACV-containing samples with the same matrix composition as in the formula shown in Table 1, it is only necessary to measure the Raman sectra and load the spectral data on model, the model will automatically calculate the ACV content in samples.

3.3. Validation of the ACV Quantification Procedure Using Raman Spectroscopy

To meet the requirements of a routine quantitative model, the ACV quantification model was validated with respect to selectivity, system suitability, linearity, accuracy, repeatability, and intermediate precision.

Regarding to the method's selectivity, Raman spectra are considered fingerprint spectra for sample identification. The Raman spectra of the ACV raw material and the sample matrix were used to select analytical regions and to construct a method that characterizes these components (active ingredient and excipient matrix) in the analytical samples. The appearance of characteristic spectral signals from both tablet samples used to build the model and the ACV raw material confirms the specificity of the analyte (Figure 4). Moreover, the accuracy assessment results showed that the error at various evaluated concentrations did not exceed 0.6% compared to the actual values (see results in the accuracy evaluation section). Thus, the developed method ensures selectivity for the quantification of ACV in tablet formations.

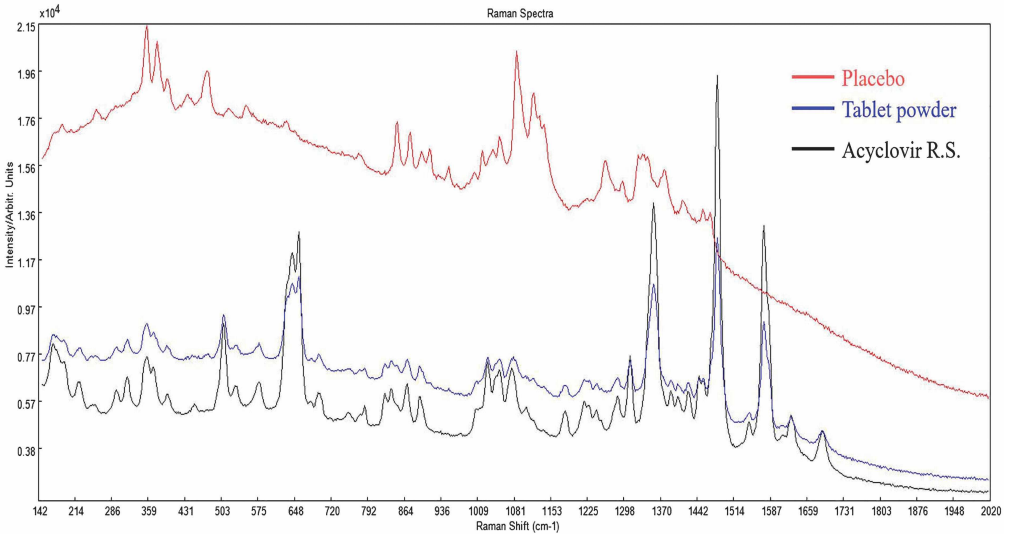


***Figure 4**. Raman spectra of ACV raw material, placebo, and self-prepared tablet samples in the wavenumber range of 150 - 2800 cm⁻¹*

Method's repeatability was assessed using tablet samples containing ACV at 100% of the target formulation concentration (ACV-to-total tablet weight ratio of 60.0% w/w, used as Trial 1 in the repeatability evaluation). Following six consecutive measurements, the mean ACV content was found to be 60.6% with a relative standard deviation (RSD) of 0.9%. These results meet the system suitability requirement (RSD $< 2.0\%$). Thus, the developed method ensures system suitability for the quantification of ACV in tablet formulations.

Method's linearity was evaluated by the correlation coefficient between the actual ACV concentrations used in model construction and the ACV concentrations predicted from the model. As shown in Figure 3, the model's correlation coefficient is 0.9999, meeting the AOAC requirement ($R \geq 0.998$). Thus, the developed method ensures linearity for the quantification of ACV in tablet formulations.

To evaluate the method's accuracy, ACV raw material was spiked into the placebo matrix to obtain powder blends with ACV contents (w/w) of approximately 48%, 60%, and 72%, corresponding to 80%, 100%, and 120% of the nominal working concentration, respectively. At each concentration level, six independent samples were prepared. For each sample, spectra were measured five times and analyzed using the model to determine ACV content. The average of the five measurements was taken as the result for each sample, and each average was only accepted if the RSD of the five measurements was not greater than 2.0%. The results of the accuracy and precision assessments are presented in **Table 3**.

For each sample used in the accuracy evaluation, the RSD of five independent measurements ranged from 0.2% to 1.7%, meeting the requirement for sample homogeneity control ($RSD \leq 2.0\%$). The model showed recovery from 98.6% to 101.7% across the three evaluated α levels. The variability in recovery among the six samples at each α level had RSDs ranging from 0.4% to 0.6%, meeting the AOAC International criteria [7] (recovery between 98% and 102%, $RSD \leq 1.7\%$). Therefore, the developed quantification method meets the accuracy requirements within the working range as specified by ICH [6] for quantifying ACV in self-prepared tablet samples.

The method's precision was evaluated on tablet samples prepared with α values around 60% (equivalent to 100% of the routine working concentration) on two different days, by two different analysts, with six independent tablets analyzed each day (Table 3). For repeatability, the RSDs for the six independently analyzed tablets on the same day by the same analyst were not greater than 0.6%. For intermediate precision, across two days and two analysts, the RSD was 0.7%. For analyte content around 60%, AOAC International recommends an RSD not exceeding 1.9% [7]. Therefore, the developed method provides suitable precision for quantifying ACV in tablets.

Based on the evaluations of linearity and accuracy, the developed method has a working range, in terms of α values (which correspond to ACV content (% w/w) in self-prepared tablet samples), from 48% to 72%. Given that the ACV content in the self-prepared samples is approximately 60% (w/w), this working range corresponds to 80 - 120% of the routine working concentration, which complies with current ICH regulations regarding analytical method performance [6].

Table 3. *Results of accuracy and precision validation*

Sample	Actual α values (%, w/w)	Predicted α values from the model after five replicate measurements (%, w/w, mean ± SD)	RSD (%) of α values across 5 replicate measurements	Recovery based on the mean predicted α value from the model (%)	RSD (%) of recovery at each α value (n = 6)
α = 48 %					
Sample 1	47.7	47.6 ± 0.2	0.5	99.8	0.4
Sample 2	47.6	47.4 ± 0.8	1.7	99.6	
Sample 3	48.1	47.6 ± 0.2	0.4	99.0	
Sample 4	48.0	47.7 ± 0.6	1.3	99.4	
Sample 5	47.8	47.5 ± 0.5	1.0	99.5	
Sample 6	48.1	47.4 ± 0.7	1.4	98.6	
α = 60 %					
Day 1, analyst 1					
Sample 1	60.0	60.6 ± 0.5	0.9	101.0	0.6
Sample 2	60.1	60.4 ± 0.6	0.9	100.5	
Sample 3	60.1	60.3 ± 0.5	0.9	100.3	
Sample 4	59.8	60.8 ± 0.1	0.2	101.7	
Sample 5	60.0	60.1 ± 0.3	0.5	100.2	
Sample 6	60.0	60.1 ± 0.4	0.6	100.2	
Day 2, analyst 2					
Sample 1	60.3	60.7 ± 0.6	1.0	100.7	0.5
Sample 2	59.9	60.6 ± 0.5	0.8	101.1	
Sample 3	59.8	60.6 ± 0.7	1.2	101.4	
Sample 4	59.6	60.8 ± 0.6	1.0	102.0	
Sample 5	59.9	60.9 ± 0.4	0.7	101.7	
Sample 6	59.7	60.8 ± 0.2	0.4	101.7	
Intermediate precision assessment (Day 1 + Day 2, n = 12)					0.7
α = 72 %					

Sample	Actual α values (%, w/w)	Predicted α values from the model after five replicate measurements (%, w/w, mean ± SD)	RSD (%) of α values across 5 replicate measurements	Recovery based on the mean predicted α value from the model (%)	RSD (%) of recovery at each α value (n = 6)
Sample 1	72.0	72.4 ± 0.4	0.5	100.5	0.5
Sample 2	72.3	71.8 ± 0.7	0.9	99.3	
Sample 3	71.7	72.0 ± 0.2	0.3	100.5	
Sample 4	72.3	71.9 ± 0.4	0.6	99.5	
Sample 5	72.2	71.8 ± 0.3	0.4	99.5	
Sample 6	72.2	71.9 ± 0.3	0.4	99.7	

3.4. Discussion

For a tablet with a well-defined formulation, the results presented in Sections 3.2 and 3.3 demonstrate the feasibility and reliability of using Raman spectroscopy as a technique for quantifying ACV content in solid dosage forms.

The Raman spectra, after being pre-processed using simple, standard tools available in common spectral data processing software, help minimize variations in absolute intensity between spectral measurements caused by optical system instability. After spectral preprocessing, the Partial Least Squares (PLS) algorithm - integrated in most commercial spectral data processing software - is used to build the quantitative model for ACV. This allows model builders to simply select built-in normalization tools from the software without the need to apply more complex chemometric approaches, which would require users to have a certain level of understanding in chemometrics and programming skills. This is therefore an advantage for routine application in analytical laboratories equipped with Raman spectroscopy, as it does not demand deep expertise in chemometrics or data processing from the operator.

The results also demonstrate that the Raman spectroscopy method, applied directly to tablets with known and stable manufacturing formula, achieves acceptable accuracy and repeatability according to established guidelines, indicating its potential for routine technical application. In cases where the matrix composition of ACV-containing tablets is known in advance and the matrix stability can be controlled, this method enables direct quantification of tablets in a fast and convenient manner. As such, it can be applied for rapid assessment of ACV tablet quality during manufacturing, storage, and distribution once a precise model has been established for the tablet matrix. Since the Raman spectroscopy-based quantification method does not require the use of any additional solvents or chemicals during sample analysis, it is also considered a green, safe, and cost-effective analytical method.

However, a limitation of the quantification method developed in this study is that it is applicable only on samples with the same manufacturing formula, and unsuitable for quantitation of ACV in tablets with different manufacturing formula. Further evaluation is necessary to assess the influence of the matrix or individual matrix components on the quantification results in order to accurately determine the applicability of this method.

4. CONCLUSION

A quantification method for ACV in self-prepared tablets with known compositions using Raman spectroscopy was developed, utilizing pre-processed spectral data in the wavenumber regions of 320 cm^{-1} to 520 cm^{-1}, 820 cm^{-1} to 900 cm^{-1}, and 1000 cm^{-1} to 1200 cm^{-1}, and applying the Partial Least Squares (PLS) algorithm to build the quantitative model. The principles of spectral processing and model development are simple, using readily available chemometric algorithms and tools integrated into commercial spectral software such as Savitzky - Golay 1st Differential, standard normal variate, and mean centering (center). This makes the method suitable for routine use without requiring deep understanding of chemometrics or specialized software. The method was validated in accordance with current regulations on performance

evaluation of analytical methods in pharmaceutical quality control. Validation results demonstrate that the method is reliable for the intended application.

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