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ARTICLES

A Novel Method of the Simultaneous Determination of Spironolactone and Furosemide in Pharmaceutical Tablets

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Abstract—Multivariate regression models, including classical least square, principal component regression, and partial least square, combined with UV-Vis absorbance spectra, have been developed and validated to simultaneously determine furosemide and spironolactone in tablets. The central composite design method is used to design calibration and validation concentration sets for the models. The method was applied in the concentration ranges of 4.4–15.6 mg/L for spironolactone and 2.4–13.6 mg/L for furosemide. The method exhibited good correlation coefficients (R^2) with low root mean square error of calibration values of 0.22, 0.16, and 0.17 mg/L for spironolactone and 0.20, 0.17, and 0.18 mg/L for furosemide, respectively. The root mean square error of prediction values for spironolactone and furosemide are less than 10% of the average concentration values, corresponding to 1 mg/L for spironolactone and 0.8 mg/L for furosemide. Precision assessment encompassed repeatability and intermediate precision, while comparison with the HPLC method revealed no statistically significant disparity ($\alpha = 0.05$). Notably, classical least square, principal component regression, and partial least square methods exhibited successful simultaneous quantification of spironolactone and furosemide within the Spiromide-40 combination tablet. Additionally, the functions and code for the analytical methods implemented in R are provided, facilitating easy usage for the readers.

Keywords: experiment of design, furosemide, multivariate analysis, R language programming, spironolactone

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The combined administration of spironolactone (**SPR**) and furosemide (**FUR**) demonstrates enhanced efficacy in treating hypertension of unknown etiology, congestive heart failure, and primary aldosteronism. Additionally, it provides valuable support in managing edema that is resistant to conventional diuretics, as well as cirrhosis and secondary aldosteronism [1, 2]. Hence, the combination drug form of SPR-FUR enjoys wide acceptance in the pharmaceutical market, necessitating the development of simultaneous quantitative methods to ascertain the content of both SPR and FUR in the formulation, ensuring drug quality.

The simultaneous determination methods of SPR and FUR have been reported, including ultraviolet (**UV**) absorption methods [3] and stability-indicating high-performance liquid chromatography [4]. According to a literature survey, no validated multivariate analysis method is available for the simultaneous determination of SPR and FUR in pharmaceutical formulations.

Multivariate analysis involves studying the relationship between two or more independent variables that influence a dependent variable simultaneously. In spectral analysis, this method is utilized to establish the link between spectral signals and concentration through multivariate calibration methods. Univariate calibration is only performed with a limited number of absorption spectra values at specific wavelengths, potentially leading to data wastage. In contrast, the removal of interfering substances in the analysis sample necessitates the application of methods such as chromatography, physical techniques, or the use of specific reagents for masking or elimination. These approaches, while common, introduce complexity and increase the likelihood of errors during sample processing [5–7]. On the other hand, in multivariate calibration, the elimination of interfering factors is achieved by using mathematical models for the data of the analysis samples. Moreover, incorporating the complete set of obtained spectral values in multivariate calibration enhances the accuracy, reliability, and precision of the calibration model [8].

A multivariate calibration process typically consists of three stages: constructing a calibration model, validating the model, and applying it to analyze real samples. The classical least squares (**CLS**) method, often referred to as the "matrix K method", is derived from Lambert–Beer's law: $A = Kc$, where K represents the

coefficient, and it plays a pivotal role in determining the calibration process's coefficient K. Subsequently, utilizing the acquired coefficient K, the concentration of the analyte in the sample can be calculated [9]. Conversely, the principal component regression (**PCR**) and partial least square 1 (**PLS1**) methods are dimensionality reduction techniques. They combine algorithms with the initial data to identify latent variables in a new space and then use the least squares method to find the linear relationship between these latent variables and the initial concentration [10–12].

R is a software framework that has been collaboratively built and continuously developed by statisticians and mathematicians worldwide. This versatile platform is designed to cater to various statistical analysis and graphing needs, and it stands out as an open-source software, available for free to users. Consequently, individuals can effortlessly access and download R for seamless utilization [13–16]. In the scientific community, an increasing number of mathematicians, statisticians, and researchers are progressively embracing R as their preferred tool for the analysis of scientific data.

The R software furnishes users with a comprehensive repertoire of functions and a computer language designed for elementary and straightforward analysis. However, when faced with the demand for more intricate analyses, additional packages need to be installed on the computer. These packages, devised by statisticians, serve as specialized software tailored to address specific issues and seamlessly integrate with the R system.

One advantage of the UV-Vis method is its affordability, simple implementation process, and less stringent solvent requirements compared to other modern analytical methods such as HPLC. The use of multivariate analysis allows for the utilization of the entire absorbance spectral data obtained from the UV-Vis instrument, minimizing data wastage. Mathematical and statistical methods are employed to enhance the sensitivity, accuracy, and reliability of the method. The combination of the UV-Vis method with multivariate regression eliminates the disadvantages of UV-Vis compared to HPLC. The simultaneous quantification of SPR and FUR using the UV-Vis method combined with certain multivariate regression algorithms represents a novel approach that has not been previously investigated.

In this study, we employed UV-Vis spectral data of SPR and FUR, coupled with multivariate analysis techniques encompassing CLS, PCR, PLS1, and the R software version 4.3.0. Through these methodologies, we aimed to calculate and determine the content of SPR and FUR concurrently in Spiromide-40 tablets.

EXPERIMENTAL

Materials. Standard grade SPR (99.6%) and FUR (99.7%) were purchased from the Drug Testing Institute of Ho Chi Minh City, Vietnam. Methanol was purchased from Fisher. Distilled water is obtained from the Bibby Scientific A4000D water distillation unit (United Kingdom).

Standard solutions. Standard solutions of SPR and FUR, each with a concentration of 1000.0 mg/L, precisely weighing 50.0 mg of each respective standard substance and dissolving it in sufficient MeOH to achieve a final volume of 50.0 mL in a volumetric flask. These accurately prepared standard solutions were then stored in a refrigerator for preservation. For daily usage, working solutions were directly prepared from the standard solutions by employing calculated ratios. Before the preparation of working solutions, the standard solutions were taken out from the refrigerator and allowed to equilibrate to room temperature (25°C) to ensure consistency in the analytical process.

Sample preparation. Twenty tablets were accurately weighed and ground into powder ($\bar{m}$ = 0.2398 g). An amount of powder equivalent to half a tablet (m = 0.1199 g) was dissolved in a 50 mL volumetric flask with sufficient MeOH solvent, sonicated for 5 min, and the solution was filtered through a 0.45 μm filter membrane. Exactly 200 μL of the filtrate was transferred to a 10 mL volumetric flask, MeOH was added up to the mark, and the solution was thoroughly mixed. The final solution was then subjected to UV-Vis absorbance measurement in the range of 200–300 nm ($\Delta\lambda$ = 0.5 nm).

Commercial pharmaceutical preparation. The pharmaceutical formulation of Spiromide-40 in tablet form comprises SPR (50 mg per tablet) and FUR (40 mg per tablet). This medication was procured from Hapharco J.S.C Branch, a reputable pharmaceutical company located in Hanoi, Vietnam, and is readily available in Ho Chi Minh City. The specific batch number of the product is C0033, produced on 06/12/2021, and the expiration date is 05/12/2023. Each box of the medication contains three vials, and within each vial, there are 10 tablets.

Design of experiment. In multivariate calibration, the design of experiments plays a crucial role in accurate calibration [17]. Without an optimized experimental design, there can be either too many experiments, resulting in a waste of time and chemicals without effectively optimizing the calibration, or too few experiments, leading to a calibration that does not cover all possible cases that may occur during the analysis process. Therefore, the calibration set should contain all components present in the sample or at least include all the components that need to be analyzed. The concentrations in the calibration set should cover all concentrations encountered during the working process. Additionally, for the calibration process to be effective, the samples in the calibration set should be in similar conditions as the samples being analyzed and should be independent of each other [9, 18].

Figure 1 presents the calibration set design, accomplished through the central composite design method, with the goal of establishing a robust calibration model for SPR and FUR. The central point of the design mirrors the analyte concentrations observed in real samples, while the neighboring points encompass concentration variations ranging from 50 to 150% of the central concentration [19]. The detailed concentration values of the calibration and validation samples are presented in Table 1, where samples T1–T9 represent the concentrations of the calibration mixture set, and samples V1–V6 represent the concentrations of the validation mixture set. The spectra of the standard and validation solution mixtures in the range of 200–300 nm ($\Delta\lambda$ = 0.5 nm) were recorded, and then the obtained data were stored for late treatment. In R, the experimental design for the calibration concentration range is conducted using the "rsm" package [20].

Software. All algorithms and statistical processing in this report are performed on R software, version 4.3.0. In this regard, to calculate using the CLS method, the algorithms were implemented in R using a custom program, which is introduced in the supplementary information section. The PCR and PLS methods are instructed in the "pls" package [21]. Additionally, statistical methods and graph plotting are available in the "stats", "graphics", "ggplot2", and "datasets" packages.

RESULTS AND DISCUSSION

Stability of the absorption spectra of SPR and FUR. To prepare the solutions of SPR and FUR, concentrations of 10.0, 8.0 mg/L, and a mixture of 8.0–10.0 mg/L for both substances were utilized. Absorbance spectra for all three solutions were recorded in the range of 200–300 nm, with a wavelength increment of 0.5 nm, employing six scans with measurements taken every 30 minutes. The outcomes of this analysis are depicted in Fig. 2.

According to Fig. 2, SPR exhibits maximum absorption at a wavelength of 238 nm, while FUR has two maximum absorptions at wavelengths of 233.5 and 274 nm. The mean absorption values are as follows: $\bar{A}_{SPR}$ = 0.464420 (λ = 238 nm), $\bar{A}_{FUR}$ = 1.007488 (λ = 233.5 nm), $\bar{A}_{Mix}$ = 1.482973 (λ = 233.5 nm). The corresponding relative standard deviations (**RSD**) are 1.53 (SPR), 2.35 (FUR), and 0.85% (Mix). Furthermore, Fig. 2 demonstrates that the spectra of the standard solutions obtained from the measurements exhibit a high degree of overlap, indicating clear and non-interfered spectra. The low RSD values suggest that the differences between measurements at different times are insignificant, indicating that the absorption spectrum of the mixture remains relatively unchanged and unaffected under working conditions. The absorption spectra of both individual compounds and mixtures also demonstrate good additivity over the investigated range of wavelengths.

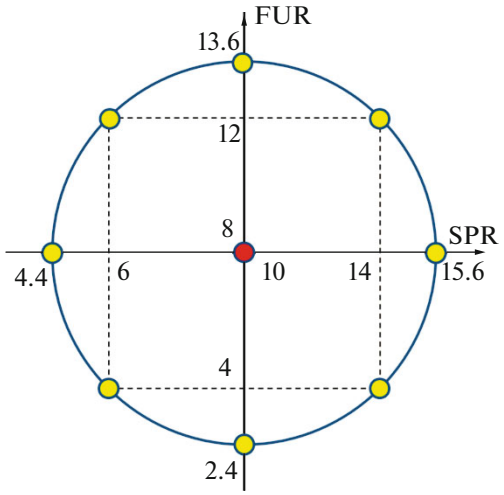

Fig. 1. Experimental design according to the central composite design.

Optimization of the variables for the PCR and PLS1 model. The calibration and prediction performance when using PCR and PLS1 methods relies on the

Table 1. Concentration data of the different mixtures used in the calibration and validation set for the determination of spironolactone and furosemide

Sample	SPR, mg/L	FUR, mg/L	Sample	SPR, mg/L	FUR, mg/L
T1	6.0	4.0	V1	8.0	6.4
T2	14.0	4.0	V2	12.0	6.4
T3	6.0	12.0	V3	8.0	9.6
T4	14.0	12.0	V4	12.0	9.6
T5	10.0	8.0	V5	10.0	8.0
T6	4.4	8.0	V6	7.2	8.0
T7	15.6	8.0	V7	12.8	8.0
T8	10.0	2.4	V8	10.0	5.8
T9	10.0	13.6	V9	10.0	10.2

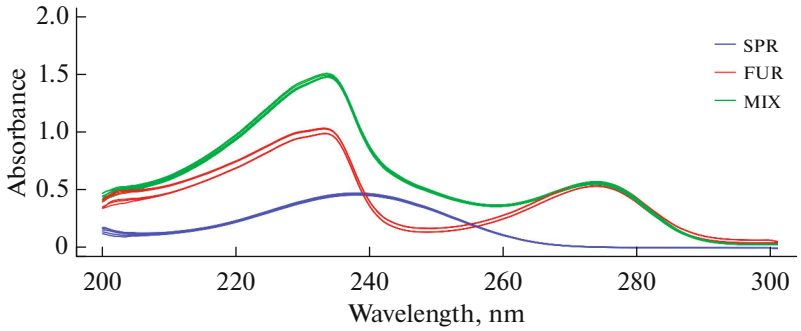

Fig. 2. Absorption spectra of spironolactone 10.0 mg/L, furosemide 8.0 mg/L, and the mixture was repeated six times.

optimal selection of the number of principal components or latent variables from the calibration set. This is a crucial step because if more factors are retained than required, it introduces noise into the model, leading to inaccurate prediction capability. Conversely, if too few factors are retained, important and necessary information for calibration may be overlooked, resulting in poor calibration accuracy. The number of retained factors should not exceed half of the total number of factors in the entire model [6]. The method used to optimize the number of factors is called leave-one-out cross-validation, which involves cross-validation using 9 leave-one-out segments. For the set of 9 calibration spectra corresponding to the samples in the training set in Table 1, PCR and PLS1 calibration were performed on the 8 training spectra, and using these calibrations, the concentration of the left-out sample during calibration was determined. This process was repeated for each training sample, with each sample being left out once.

In Fig. 3, it is evident that as the main components in the model range from ncomp = 1 to ncomp = 3 (PCR model) or ncomp = 1 to ncomp = 2 (PLS1 model), the root mean square error of cross-validation (**RMSECV**) values decrease. This signifies the effectiveness of the data compression process, with the first variables containing crucial information related to absorbance in the calibration set. These variables explain 99.99% of the spectral variance, leading to the selection of 3 (PCR model) or 2 (PLS1 model) factors for constructing a calibration model to predict the concentration of substances in the analyzed samples using the principal component regression or partial least squares regression method.

However, when increasing the number of latent variables from 4 to 7 (PCR) or from 3 to 7 (PLS1), a slight increase in the values of RMSECV is observed. This suggests that the additional latent variables do not provide relevant information and may be considered as noise signals. The use of more latent variables can result in overfitting and lead to inaccurate diagnostic models.

Development of multivariate analysis methods. The development of a multivariate analysis method was conducted on standard and validation samples. The standard and validation samples are known concentration samples designed according to the section "Design of experiment". The absorbance spectra of samples containing SPR and FUR were measured in the wavelength range of 200–300 nm ($\Delta\lambda = 0.5$ nm). The suitability of the model was evaluated using the algorithms of CLS, PCR, and PLS1.

The regression model was evaluated using the coefficient of determination R^2 and the root mean square error (**RMSE**), a measure of the variation between the predicted values and the reference values for a set of samples. The RMSEs for calibration, validation, and prediction were calculated using Eq. (1).

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(c_{\mathrm{pred}} - c_{\mathrm{known}})^2}{n}}, \qquad (1)$$

where c_{pred} and c_{known} are the predicted and known concentration values of the analyte, respectively, and n is the number of samples used. **RMSEC** is the root mean square error of calibration, indicating the goodness of fit of the model. The prediction ability of the model for a new sample is expressed by the root mean square error of cross-validation, explaining the robustness of the model. When applying the model to a new dataset, the root mean square error of prediction (**RMSEP**) can be calculated if the reference values for the new dataset are known in advance [22].

In a linear regression model, an R^2 value quantifies the relationship between the actual concentration and the predicted concentration of the model. An R^2 value of 0 indicates no linear relationship, while an R^2 value closer to 1 indicates a better predictive model.

The results in Table 2 and Fig. 4 show that the CLS, PCR, and PLS1 models were constructed for SPR and FUR at concentration ranges of 4.4–15.6 and 2.4–13.6 mg/L, respectively, with an R^2 value > 0.9970. This demonstrates that the difference between the predicted concentration and the actual concentration of the substances in the standard sample is insignificant. The RMSECV values for the PCR and PLS1 methods

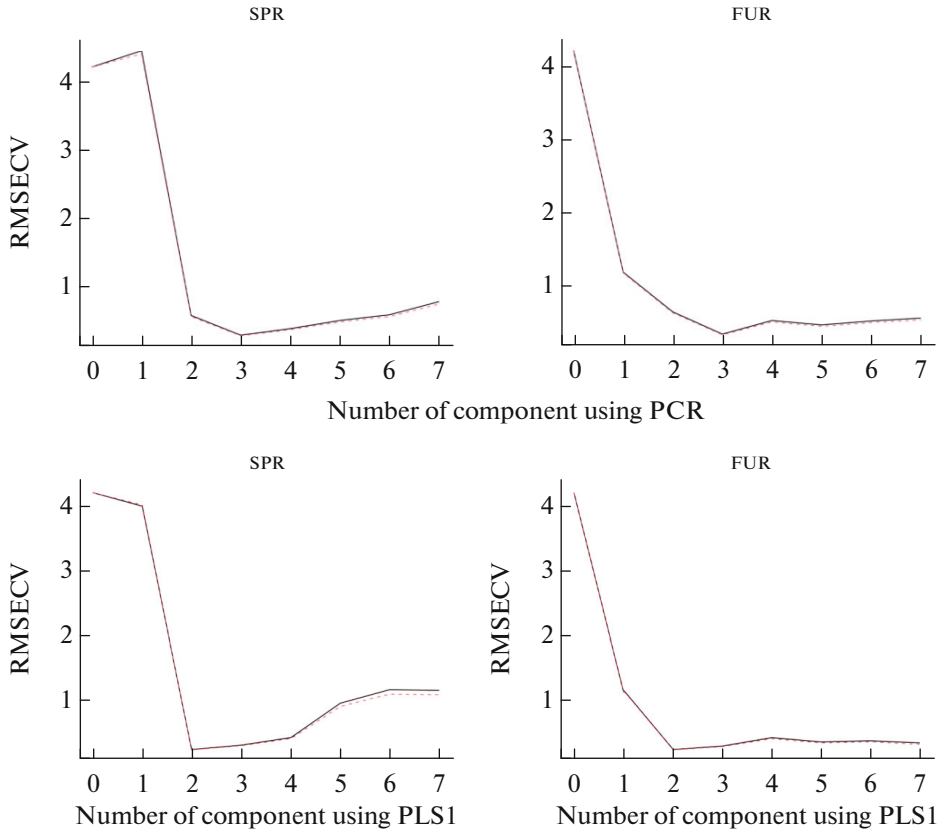

Fig. 3. RMSEP plot of a calibration set prediction using cross validation using PCR and PLS1 models.

are 0.31 and 0.26 mg/L for SPR, and 0.34 and 0.28 mg/L for FUR, respectively. The small RMSECV values indicate the high accuracy of the model's predictions using cross-validation. The RMSEC values for the CLS, PCR, and PLS1 methods are 0.22, 0.16, and 0.17 mg/L for SPR, and 0.20, 0.17, and 0.18 mg/L for FUR, respectively, indicating the good performance of these methods.

Furthermore, residual analysis is an important part of evaluating the suitability of a regression model for predicting concentration variables. It is essential to ensure that the discrepancies between known concentrations and predicted concentrations obtained from the constructed methods are symmetrically distributed around zero, random, and independent. The results of the residual analysis from the models indicated that the residual values are randomly distributed, demonstrating constant variance, independence, and close adherence to a zero-centered distribution—as depicted in Fig. 5. Shapiro–Wilk test conducted using the R software showed that all p-values were greater than 0.05. Therefore, there is not enough evidence to reject the null hypothesis (H_0) or conclude that the residuals follow a normal distribution (Table 2).

Evaluation of the validation set. To assess the level of accurate and reliable prediction achieved by the CLS, PCR, and PLS1 algorithms, 9 mixed samples containing SPR and FUR at different concentrations as shown in Table 3 were prepared. A smaller RMSEP value is considered better, typically requiring it to be less than 10% of the median value of the concentration range of substances in the calibration set [23]. The results in Table 3 show that the RMSEP values for the CLS, PCR, and PLS1 methods are 0.43, 0.34, and 0.39 mg/L for SPR, and 0.37, 0.41, and 0.38 mg/L for FUR, respectively. These values are all less than 10% of the median value, which is 1 mg/L for SPR and 0.8 mg/L for FUR. The recovery and relative standard deviation were calculated. The results indicated that the recovery was within the acceptable range of 80–110% (according to AOAC) [24]. The results obtained demonstrate that the CLS, PCR, and PLS1 methods can be used simultaneously to determine SPR and FUR in pharmaceutical samples.

Evaluation of the analysis process. *Accuracy.* Additional standards were prepared at three different concentration levels, with each level repeated three times. Therefore, the developed methods, including CLS, PCR, and PLS1, were used to determine the presence of SPR and FUR in the tablets. The accuracy of the

Table 2. Statistical parameters for simultaneous determination of spironolactone and furosemide using CLS, PCR, PLS1 methods

Sample	Known concentration, mg/L		Predicted concentration, mg/L					
			CLS		PLS1		PCR	
	SPR	FUR	SPR	FUR	SPR	FUR	SPR	FUR
T1	6.0	4.0	5.66	4.48	5.86	4.32	5.84	4.22
T2	14.0	4.0	13.67	3.81	13.70	3.80	13.72	3.85
T3	6.0	12.0	5.80	12.02	5.89	11.96	5.88	11.96
T4	14.0	12.0	14.28	11.79	14.15	11.89	14.16	11.79
T5	10.0	8.0	10.19	7.95	10.14	7.94	10.07	7.92
T6	4.4	8.0	4.38	7.87	4.57	7.73	4.56	7.77
T7	15.6	8.0	15.79	8.06	15.64	8.16	15.61	8.17
T8	10.0	2.4	10.00	2.55	10.19	2.44	10.25	2.47
T9	10.0	13.6	9.92	13.73	9.86	13.77	9.92	13.85
n comp			—	—	2	2	3	3
RMSECV			—	—	0.26	0.28	0.31	0.34
RMSEC			0.22	0.20	0.17	0.18	0.16	0.17
R^2			0.9975	0.9973	0.9980	0.9977	0.9981	0.9979
Shapiro test (*p*-value)			0.48	0.35	0.15	0.93	0.85	0.39

Table 3. Recovery (%) results obtained in synthetic mixtures for CLS, PCR, PLS1 methods

Mixtures added, mg/L		CLS		PCR		PLS1	
SPR	FUR	SPR	FUR	SPR	FUR	SPR	FUR
8.0	6.4	97.9	98.6	96.9	98.6	99.3	97.0
12.0	6.4	102.5	102.8	101.4	104.2	102.8	102.8
8.0	9.6	100.6	105.1	98.4	105.8	101.5	104.6
12.0	9.6	104.2	105.5	101.7	105.9	103.8	106.0
10.0	8.0	100.8	100.9	99.8	102.0	101.5	100.5
7.2	8.0	91.7	105.5	89.3	103.5	92.8	104.5
12.8	8.0	107.3	107.1	104.3	108.9	106.3	108.0
10.0	5.8	100.3	101.2	100.2	101.6	101.1	100.2
10.0	10.2	102.5	103.8	101.1	104.9	103.0	103.8
Mean recovery, %		100.9	103.4	99.2	103.9	101.3	103.1
SD		4.35	2.75	4.27	2.98	3.75	3.35
RMSEP		0.43	0.37	0.34	0.41	0.39	0.38

methods was assessed based on the recovery rate, which is presented in Table 4. For the CLS method, the average recovery rates were found to be 104.0 and 103.9% for SPR and FUR, respectively. The PCR method exhibited recovery rates of 96.6 and 105.7% for SPR and FUR, respectively. Lastly, the PLS1 method showed recovery rates of 98.8 and 108.3% for SPR and FUR, respectively. All the recovery values, along with their corresponding relative standard deviations, were found to fall within the specified range as per AOAC guidelines, confirming the reliability and accuracy of the developed methods.

Precision. Precision assessment encompasses two important aspects: repeatability and intermediate precision. The samples were meticulously prepared following the procedure described in the "Sample preparation" section. Subsequently, the entire process was repeated six times to ensure robustness and accuracy.

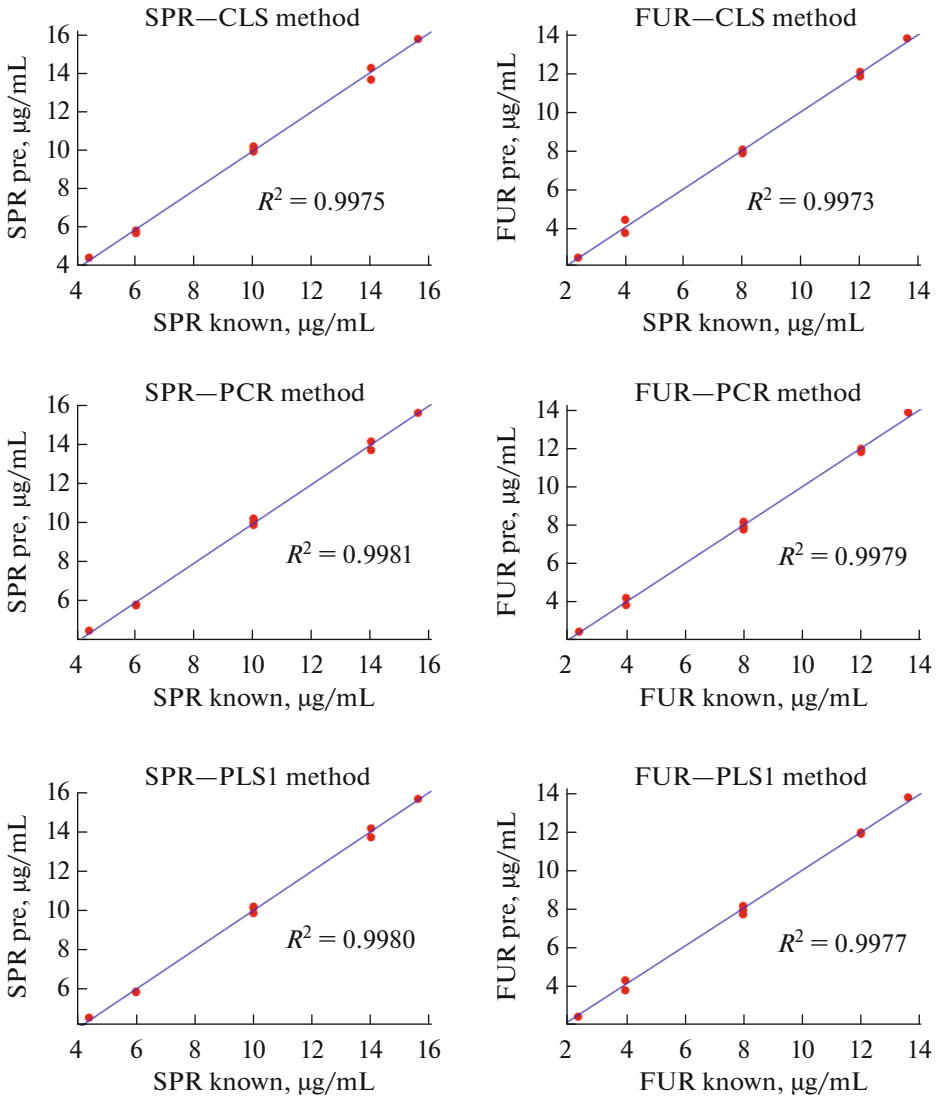

Fig. 4. Predicted concentrations vs. known concentrations for spironolactone and furosemide using CLS, PCR and PLS1 methods.

The detailed results of the repeatability and intermediate precision evaluations are presented in Tables 5 and 6, respectively.

Repeatability. The RSD values of 6 for the analysis of Spiromide-40 drug sample using the CLS, PCR, and PLS methods show that these values are smaller than half of the RSD calculated using the Horwitz function (with $RSD_{Horwitz} = 2^{1-0.5\log c}$). Therefore, both the analysis processes exhibit good repeatability for both the SPR and FUR components.

Intermediate precision. The intermediate precision evaluation results indicate good repeatability for both components, with the value of $RSD < 1/2RSD_{Horwitz}$. Intermediate precision is performed similarly to repeatability but on different days and by different analysts. It demonstrates the influence of random variables on the accuracy of the analytical procedure. An *F*-test is conducted to confirm the similarity between the results of two different experiments. The *F*-test results show no significant difference in repeatability when the experiments are conducted on different days and by different analysts for all three methods: CLS, PCR, and PLS1 in the quantification of SPR and FUR.

Comparison with the HPLC method. To ascertain the credibility of the research method, samples were submitted to the Thua Thien Hue Pharmaceutical and Cosmetic Testing Center in Vietnam for quantitative analysis, utilizing the standard HPLC method. A comprehensive comparison and assessment of the analysis outcomes between the two methods were conducted through statistical means. The findings, pre-

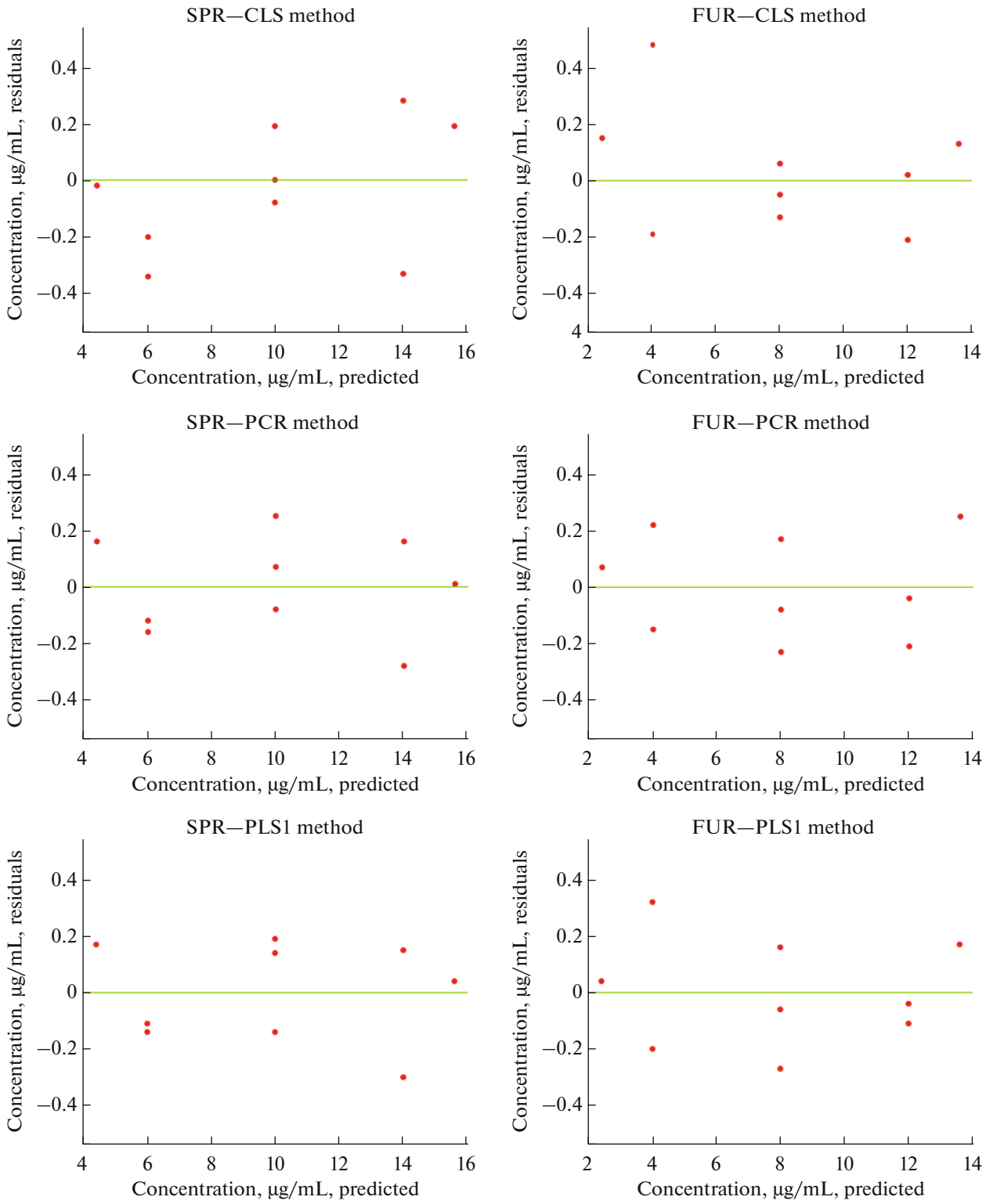

Fig. 5. Residual vs. known concentration plots for spironolactone and furosemide using CLS, PCR and PLS1 methods.

sented in Table 7, demonstrate that both F_{cal} and t_{cal} statistical values are smaller than the corresponding F_{theory} and t_{theory} values when comparing the repeatability and quantitative results of the CLS, PCR, and PLS1 methods with the HPLC method, at a significance level of $\alpha = 0.05$. This observation substantiates

Table 4. Evaluation results of the recovery (%) performance using additional standardization techniques via CLS, PCR, and PLS1 methods

Added to tablet, mg/L		CLS		PCR		PLS1	
SPR	FUR	SPR	FUR	SPR	FUR	SPR	FUR
3.0	2.4	106.7	103.8	95.7	100.4	98.7	108.8
3.0	2.4	107.0	104.2	97.3	112.1	101.3	109.2
3.0	2.4	103.0	104.2	95.0	107.9	98.0	108.3
5.0	4.0	106.2	105.3	100.2	109.3	101.8	109.5
5.0	4.0	107.8	105.0	99.8	104.5	102.8	109.5
5.0	4.0	105.2	104.0	98.2	107.0	100.6	108.0
7.0	5.6	102.3	101.4	97.9	106.8	98.0	105.5
7.0	5.6	99.6	104.1	93.0	101.4	94.7	108.2
7.0	5.6	98.4	103.9	92.6	102.0	93.7	108.0
Mean		104.0	103.9	96.6	105.7	98.8	108.3
RSD, %		3.25	1.05	2.84	3.71	3.17	1.13

Table 5. Repeatability assessment results

Sample	SPR, mg/L			FUR, mg/L		
	CLS	PCR	PLS1	CLS	PCR	PLS1
S1.1	9.60	9.34	9.57	7.59	7.73	7.57
S1.2	10.19	10.05	10.23	8.05	8.20	8.03
S1.3	9.79	9.60	9.79	7.75	7.93	7.72
S1.4	9.64	9.34	9.59	7.67	7.86	7.65
S1.5	9.65	9.39	9.62	7.73	7.90	7.70
S1.6	9.90	9.47	9.77	7.89	8.00	7.89
Mean	9.80	9.53	9.76	7.78	7.94	7.76
SD	0.22	0.27	0.25	0.16	0.16	0.17
RSD, %	2.29	2.85	2.54	2.12	1.98	2.18
$1/2RSD_{Horwitz}$	5.66			5.85		

the concurrent applicability of the CLS, PCR, and PLS1 methods with HPLC in determining the presence of SPR and FUR in tablets.

Application. CLS, PCR, and PLS1 methods were applied to simultaneously determine SPR and FUR in Spiromide-40 tablets. The quantitative results showed that the content of SPR and FUR fell within the permissible range of 90.0 to 110.0% compared to the labeled content according to the Vietnamese Pharmacopoeia V.

CONCLUSIONS

Previous studies have predominantly relied on proprietary and commercial software, which require licensing and incur fees, to process and compute results from data. In this paper, the *R* programming language was successfully applied to handle data and compute results. The findings demonstrated that multivariate analysis, combined with *R* packages and the flexibility of the *R* programming language, allowing users to write their own programs for data processing and computation. This method development provides analysts with an additional tool for data handling, computation, and analysis.

The CLS, PCR, and PLS methods were successful in simultaneously quantifying spironolactone and furosemide in combined tablet formulations. The statistical results complied with the AOAC regulations and the Vietnamese Pharmacopoeia standards.

FUNDING

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Table 6. Intermediate precision evaluation results

Sample	SPR, mg/L			FUR, mg/L		
	CLS	PCR	PLS1	CLS	PCR	PLS1
S2.1	10.01	9.72	9.98	7.87	8.00	7.85
S2.2	9.77	9.63	9.84	7.72	7.94	7.68
S2.3	10.03	9.79	10.04	7.90	8.05	7.88
S2.4	9.91	9.64	9.90	7.83	7.89	7.80
S2.5	9.71	9.61	9.78	7.70	7.81	7.66
S2.6	9.97	9.93	10.07	7.84	8.04	7.80
Mean	9.90	9.72	9.94	7.81	7.96	7.78
SD	0.13	0.12	0.11	0.081	0.094	0.09
RSD, %	1.33	1.26	1.15	1.04	1.18	1.16
$1/2RSD_{Horwitz}$		5.66			5.85	
F_{cal}	2.88	4.90	4.68	4.11	2.82	3.57
$F_{theory\ (0.05;\ 5;\ 5)}$		5.82			5.82	

Table 7. Comparison of quantitative results (content: *H*, mg/tablet) between CLS, PCR, PLS1 methods with HPLC method

No.	SPR				FUR			
	CLS	PCR	PLS1	HPLC	CLS	PCR	PLS1	HPLC
1	50.94	50.24	51.14	49.75	40.23	40.98	40.13	39.65
2	48.96	48.01	48.96	49.15	38.73	39.63	38.58	39.97
3	49.49	47.35	48.84	50.02	39.45	40.00	39.45	40.25
Mean	49.8	48.53	49.65	49.64	39.47	40.2	39.39	39.96
RSD, %	2.05	3.12	2.60	0.90	1.90	1.74	1.97	0.75
F_{cal}	5.26	11.55	8.40		6.23	5.41	6.67	
$F_{theory\ (0,05;2;2)}$		15.44				15.44		
t_{cal}	0.25	1.22	0.011		1.05	0.56	1.19	
$t_{theory\ (0.05;4)}$		2.776				2.776		

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

ADDITIONAL INFORMATION

In terms of CLS method, the code to build a calibration model for the CLS method is as follows:

CLS.model = function(A.sample) {K = A.train %*% t(*c*.train) %*% solve(*c*.train %*% t(*c*.train))

c = solve(t(K) %*% K) %*% t(K) %*% A.sample

c = round(*c*, 2)}

In which, *c*.train is the concentration matrix in the calibration set with dimensions of *n* components × I samples, and A.train is the absorption matrix with dimensions of J wavelengths × I samples obtained from UV-Vis absorption spectra measurements of the samples in the calibration set.

The concentration in the sample matrices, *c*.sample, is determined as follows:

c.sample = CSL.model(A.sample).

In which, A.sample is the obtained absorption matrix of the samples for which the concentrations need to be determined.

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