

Quantitative Analysis of Active Compounds in a Three-Component Pharmaceutical Formulation Using Multivariate Calibration Methods

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Quantitative Analysis of Active Compounds in a Three-Component Pharmaceutical Formulation Using Multivariate Calibration Methods

Üç Bileşenli Bir İlaç Formülasyonundaki Aktif Bileşiklerin Çok Değişkenli Kalibrasyon Yöntemleri Kullanılarak Kantitatif Analizi

SUMMARY

A rapid, simple, and cost-effective spectrophotometric method combined with multivariate chemometric calibration was developed for the simultaneous determination of paracetamol (PRC), caffeine (CFN), and propyphenazone (PRPN) in a ternary pharmaceutical formulation without any prior separation step. The direct UV spectrophotometric analysis of these compounds is challenging due to the significant overlap of their absorption spectra within the 220-300 nm wavelength range, which limits the applicability of conventional methods. To overcome this issue, Partial Least Squares (PLS) and Principal Component Regression (PCR) techniques were applied to resolve the overlapping spectral data and enable accurate quantification. Calibration models were constructed using a training set of 35 laboratory-prepared mixtures in the concentration ranges of 3.0-16.0 µg/mL for PRC, 2.5-12.0 µg/mL for CFN, and 3.0-12.0 µg/mL for PRPN. The performance of the models was evaluated using statistical parameters such as SEC, SEP, and RMSEP, demonstrating strong predictive ability with low error values. Validation studies confirmed high accuracy (recoveries close to 100%) and precision (RSD < 3%), while robustness was verified through intra-and inter-day analyses. The method was successfully applied to commercial tablets, providing reliable and consistent results suitable for routine quality control applications.

Keywords: Paracetamol, caffeine, propyphenazone, chemometrics, multivariate calibration.

ÖZ

Üçlü bir farmasötik formülasyonda parasetamol (PRC), kafein (CFN) ve propifenazonun (PRPN) eş zamanlı olarak belirlenmesi için, önceden herhangi bir ayırma işlemine gerek kalmadan, çok değişkenli kemometrik kalibrasyonla birleştirilmiş hızlı, basit ve uygun maliyetli bir spektrofotometrik yöntem geliştirilmiştir. Bu bileşiklerin doğrudan UV spektrofotometrik analizi, 220-300 nm dalga boyu aralığında absorpsiyon spektrumlarının önemli ölçüde örtüşmesi nedeniyle zordur ve bu da geleneksel yöntemlerin uygulanabilirliğini sınırlar. Bu sorunun üstesinden gelmek için, örtüşen spektral verileri çözmek ve doğru nicelleştirmeyi sağlamak amacıyla Kısmi En Küçük Kareler (PLS) ve Temel Bileşen Regresyonu (PCR) teknikleri uygulanmıştır. Kalibrasyon modelleri, PRC için 3,0-16,0 µg/mL, CFN için 2,5-12,0 µg/mL ve PRPN için 3,0-12,0 µg/mL konsantrasyon aralıklarında laboratuvarında hazırlanan 35 karışımdan oluşan bir eğitim seti kullanılarak oluşturulmuştur. Modellerin performansı, SEC, SEP ve RMSEP gibi istatistiksel parametreler kullanılarak değerlendirilmiş ve düşük hata değerleriyle güçlü tahmin yeteneği gösterilmiştir. Doğrulama çalışmaları, yüksek doğruluk (geri kazanımlar %100'e yakın) ve hassasiyet (RSD < %3) teyit ederken, sağlamlık gün içi ve günler arası analizlerle doğrulanmıştır. Yöntem, ticari tabletlere başarıyla uygulanmış ve rutin kalite kontrol uygulamaları için uygun, güvenilir ve tutarlı sonuçlar sağlamıştır.

Anahtar Kelimeler: Parasetamol, kafein, propifenazon, kemometri, çok değişkenli kalibrasyon.

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INTRODUCTION

In recent years, the pharmaceutical and healthcare sectors have increasingly focused on the development and use of multicomponent pharmaceutical formulations rather than single-drug preparations. This tendency is largely attributed to the enhanced therapeutic efficacy and improved clinical outcomes that can be achieved through combination therapy. Numerous over-the-counter analgesic products are formulated as multi-component systems to provide synergistic pharmacological effects. Among these combinations, formulations containing PRC, CFN, and PRPN are widely manufactured and marketed by many pharmaceutical companies.

Paracetamol (PRC), also known as acetaminophen (N-(4-hydroxyphenyl) acetamide) (Figure 1a.), is one of the most extensively used analgesic and antipyretic agents worldwide and is commonly prescribed for the treatment of mild to moderate pain

and fever (Warwick, 2008). The World Health Organization recommends PRC as a first-line therapy for several pain conditions (Saragiotto et al., 2019). Caffeine (1,3,7-trimethylxanthine or 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione) (Figure 1b.) is a central nervous system stimulant belonging to the methylxanthine class. In pharmaceutical formulations, it is frequently incorporated as an adjuvant analgesic because it enhances the effectiveness of other pain-relieving agents and contributes to the relief of headaches and similar disorders (Reddy et al., 2024). Propyphenazone (1,5-dimethyl-2-phenyl-4-propan-2-yl-pyrazol-3-one) (Figure 1c.) is a pyrazolone derivative with well-known anti-inflammatory, analgesic, and antipyretic activities (Aronson, 2006; Boerlin et al., 1986). In clinical practice, it is commonly combined with PRC and CFN in formulations intended for the treatment of primary headache and related pain syndromes.

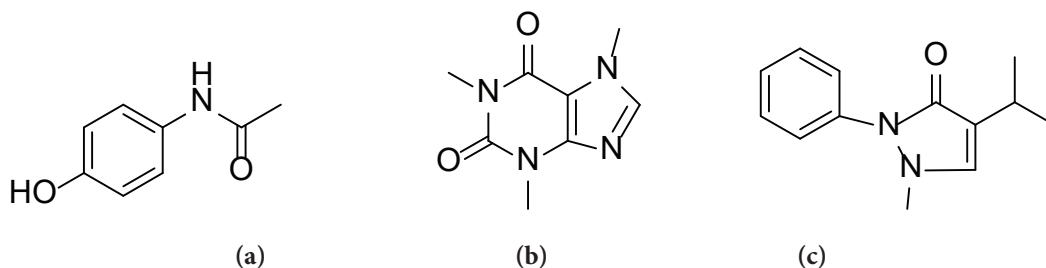


Figure 1. Chemical structure of a) Paracetamol, b) Caffeine, and c) Propyphenazone

The reliable quantitative analysis of such ternary pharmaceutical systems is essential for monitoring manufacturing processes, ensuring product quality, and performing routine quality control analyses. Nevertheless, the simultaneous determination of multiple active ingredients in combined dosage forms remains analytically challenging. Conventional analytical procedures often require complex sample preparation steps, long analysis times, and expensive instrumentation, and may suffer from problems such as co-elution of analytes or overlapping signals under experimental conditions.

Several chromatographic techniques have been reported for the determination of this drug combination, including HPLC-DAD (Soponar et al., 2013), thin-layer chromatography (Bober-Majnusz & Pyka-Pajak, 2024; Ibrahim et al., 2021), micellar electrokinetic capillary chromatography (Emre & Ozaltin, 2007), and gas chromatography (Markovic & Kusec, 1990). Although these methods provide reliable results, they frequently involve extensive solvent consumption, skilled operators, and relatively time-consuming procedures. In contrast, UV spectrophotometric techniques combined with chemometric data processing offer a rapid and economical alternative

for the quantitative analysis of multicomponent pharmaceutical mixtures. Moreover, from the perspective of Green Chemistry, spectrophotometric methods combined with chemometric tools offer important environmental and economic advantages over conventional chromatographic techniques. The proposed approach reduces organic solvent consumption, eliminates complex separation procedures, and shortens analysis time, thereby minimizing chemical waste generation and operational costs. These characteristics make the method an environmentally friendly alternative for routine pharmaceutical quality control laboratories. Such approaches allow the extraction of useful analytical information even in cases where the spectral profiles of the analytes significantly overlap.

Various spectrophotometric strategies have previously been proposed for the analysis of this ternary drug system, including front-column methods (Domínguez Vidal et al., 2003), absorption ratio techniques (Pulungan et al., 2018), net analyte signal-based procedures (Gergov et al., 2018), derivative ratio spectrophotometry (Dinç et al., 2001c), principal component analysis approaches (Rosaldi et al., 2023), and higher-order derivative spectrophotometric methods (Özgür et al., 2002). In addition, chemometric calibration techniques such as partial least squares (PLS) and principal component regression (PCR) have been widely applied to resolve complex multicomponent analytical systems (Dinç et al., 2001a; 2001b). These multivariate approaches establish mathematical relationships between spectral data matrices and concentration profiles, allowing the prediction of analyte concentrations in samples with unknown compositions. PLS and PCR methods provide more precise and accurate results compared to the classical multiple linear regression model. This advantage stems from the noise elimination feature in their algorithms.

In the present study, PLS and PCR chemometric calibration models were developed and applied for the simultaneous spectrophotometric determination

of PRC, CFN, and PRPN in commercial tablet formulations without the need for prior separation.

The proposed methods were validated by evaluating recovery values and relative standard deviations, and their analytical performance was assessed using synthetic mixtures and pharmaceutical tablet samples. The obtained results were statistically compared to demonstrate the applicability and reliability of the developed chemometric approaches.

MATERIALS AND METHODS

Chemicals and reagents

Reference standards of paracetamol (PRC, 99.6% purity), caffeine (CFN, 99.7% purity), and propyphenazone (PRPN, $\geq 99\%$ purity) were obtained from Sigma-Aldrich (Steinheim, Germany). Methanol of gradient grade used for the preparation and dilution of the solutions was purchased from Sigma-Aldrich (USA). All solutions were freshly prepared on the day of analysis and protected from light throughout the experimental procedure to maintain their stability.

A commercial tablet formulation, Minoset Plus 30 (Bayer Türk Chemical Industry Ltd., Ümraniye, İstanbul, Turkey), was selected for the analytical application of the proposed methods. Each tablet contains 250 mg of PRC, 50 mg of CFN, and 150 mg of PRPN. The formulation also includes several pharmaceutical excipients such as microcrystalline cellulose, hydroxypropyl methylcellulose, esma spreng (formaldehyde casein), corn starch, talc, magnesium stearate, and colloidal anhydrous silica.

Instruments and software

UV absorption spectra of the studied compounds and their mixtures were recorded using a UV-VIS spectrophotometer (Shimadzu UV-2550, Kyoto, Japan) operated with Shimadzu UVPC software. Spectral measurements were carried out in the wavelength range of 220-300 nm with a spectral interval of $\Delta\lambda = 0.1$ nm. Data acquisition and subsequent statistical analyses were performed using MATLAB 7.0 and Microsoft Excel. The PLS and PCR chemometric cal-

ibration models were implemented in the MATLAB environment using a custom-written M-file.

Preparation of stock, calibration, and validation solutions

Stock solutions of PRC, CFN, and PRPN were prepared by accurately weighing 10.0 mg of each compound and dissolving them separately in methanol in 100 mL volumetric flasks. The obtained stock solutions were subsequently diluted with methanol to prepare the calibration, validation, intra-day, and inter-day working solutions.

For the construction of the PLS and PCR calibration models, a set of 35 calibration mixtures was prepared within the concentration ranges of 3.0-16.0 µg/mL for PRC, 2.5-12.0 µg/mL for CFN, and 3.0-12.0 µg/mL for PRPN. In addition, thirteen synthetic mixtures containing different concentration levels of PRC, CFN, and PRPN within the working range were prepared as an external validation set:

Precision studies were carried out by preparing intra-day and inter-day samples at three concentration levels. The selected concentrations were 5.0, 10.0, and 15.0 µg/mL for PRC; 3.0, 6.0, and 9.0 µg/mL for CFN; and 4.0, 12.0, and 16.0 µg/mL for PRPN. For the standard addition experiments, known amounts of PRC, CFN, and PRPN standard solutions (0, 2.0, 4.0, and 6.0 µg/mL for each analyte) were added to the commercial tablet sample solution, and each level was prepared in triplicate.

Preparation of tablet sample

Ten tablets of Minoset® Plus (Bayer Türk Chemical Industry Ltd., Turkey) were accurately weighed and finely powdered in a mortar to obtain a homogeneous mixture. An amount equivalent to one-twentieth of a tablet was transferred into a 100 mL volumetric flask. Methanol was added to the flask, and the suspension

was stirred mechanically for 1 hour to ensure complete extraction of the active ingredients. The mixture was then filtered through a nylon membrane filter with a pore size of 0.45 µm (ISOLAB, Wertheim, Germany).

The filtrate was appropriately diluted with methanol to obtain a final solution containing 15.0 µg/mL PRC, 3.0 µg/mL CFN, and 9.0 µg/mL PRPN. This prepared tablet solution was subsequently analyzed using UV-VIS spectrophotometry for the application of the PLS and PCR chemometric methods.

RESULTS AND DISCUSSION

Application of PLS and PCR chemometric models

In the present work, PLS and PCR chemometric approaches were applied to spectrophotometric data for the simultaneous determination of PRC, CFN, and PRPN in a ternary pharmaceutical system without the need for a separation step. The UV absorption spectra of the individual compounds and their mixtures were recorded within the wavelength range of 220-300 nm with a spectral interval of $\Delta\lambda = 0.1$ nm. The recorded spectra are presented in Figure 2.

As illustrated in Figure 2, the absorption profiles of PRC, CFN, and PRPN significantly overlap within the selected spectral region. This spectral overlap prevents the direct determination of the individual components using conventional spectrophotometric methods. To overcome this limitation, multivariate chemometric calibration techniques were employed.

For the development of the calibration models, a symmetrical calibration design consisting of 35 mixtures was constructed. The concentration ranges used for the calibration set were 3.0-16.0 µg/mL for PRC, 2.5-12.0 µg/mL for CFN, and 3.0-12.0 µg/mL for PRPN. Detailed information regarding the composition of the calibration mixtures is given in Table 1.

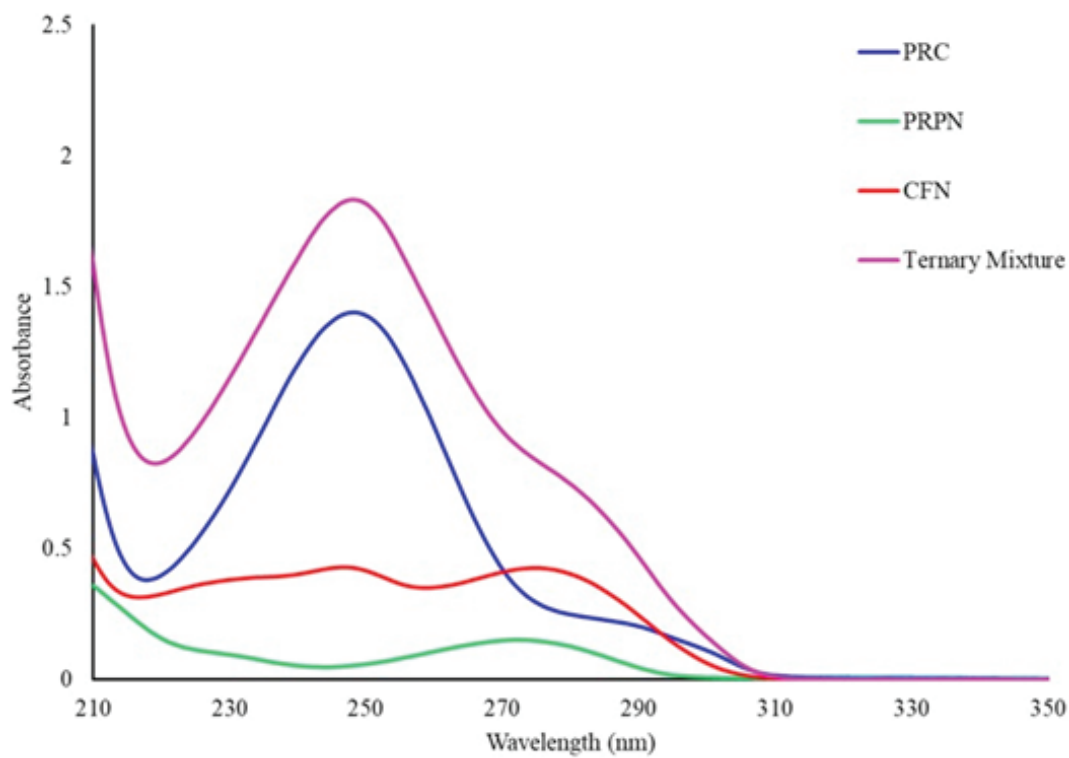


Figure 2. UV absorption spectra of 15.0 $\mu\text{g/mL}$ PRC (—), 3.0 $\mu\text{g/mL}$ CFN (—), 9.0 $\mu\text{g/mL}$ PRPN (—), and their ternary mixture (—)

Table 1. A concentration set that includes mixtures of PRC, CFN, and PRPN drug compounds

Sample No.	Actual PRC (µg/mL)	Actual CFN (µg/mL)	Actual PRPN (µg/mL)
1	3.0	2.5	3.0
2	8.0	2.5	3.0
3	12.0	2.5	3.0
4	16.0	2.5	3.0
5	3.0	4.0	6.0
6	8.0	4.0	6.0
7	12.0	4.0	6.0
8	16.0	4.0	6.0
9	3.0	8.0	9.0
10	8.0	8.0	9.0
11	12.0	8.0	9.0
12	16.0	8.0	9.0
13	3.0	12.0	12.0
14	8.0	12.0	12.0
15	12.0	12.0	12.0
16	16.0	12.0	12.0
17	3.0	2.5	6.0
18	8.0	2.5	6.0
19	12.0	2.5	6.0
20	16.0	2.5	6.0
21	3.0	4.0	3.0
22	8.0	4.0	3.0
23	12.0	4.0	3.0
24	16.0	4.0	3.0
25	3.0	8.0	12.0
26	8.0	8.0	12.0
27	12.0	8.0	12.0
28	16.0	8.0	12.0
29	3.0	12.0	9.0
30	8.0	12.0	9.0
31	12.0	12.0	9.0
32	16.0	12.0	9.0
33	15.0	0.0	0.0
34	0.0	0.0	9.0
35	0.0	3.0	0.0

PRC: Paracetamol

CFN: Caffeine

PRPN: Propyphenazone

The absorbance data obtained from the calibration mixtures constituted the X-matrix, while the corresponding concentration values were used as the Y-matrix for the construction of the PLS and PCR calibration models. While the PCR method, based on singular value decomposition of absorbance measurements, relies on the maximum variance between variables, the PLS calibration method is a calibration

methodology based on the covariance of dependent and independent variables.

The predictive ability of the models was evaluated through cross-validation procedures. Prediction error profiles as a function of the number of latent variables for both PLS and PCR models are shown in Figures 3 and 4.

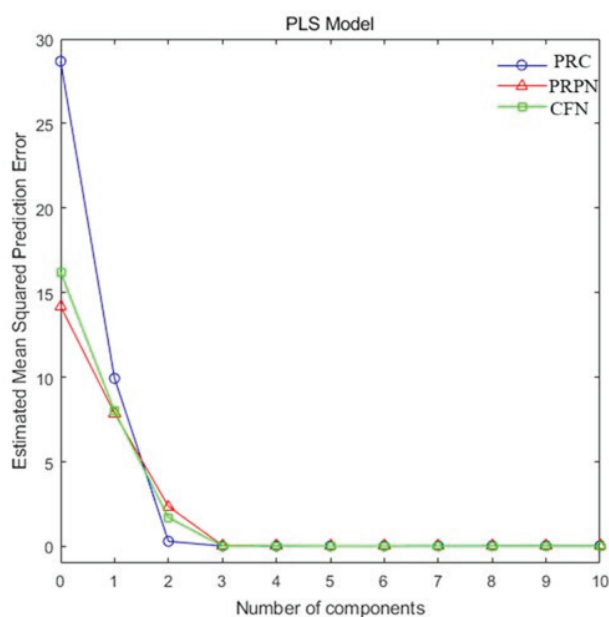


Figure 3. Prediction error against the number of components for the PLS model

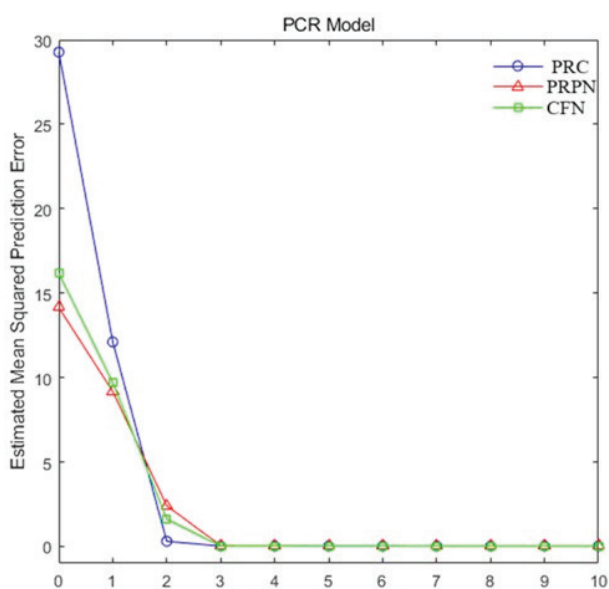


Figure 4. Prediction error against the number of components for the PCR model

Based on the cross-validation results, the optimal number of factors for both calibration models was determined to be three, since the prediction error reached its minimum value at this point and remained essentially constant with the inclusion of additional components.

The performance of the developed chemometric models was assessed using several statistical param-

eters, including the predicted residual error sum of squares (PRESS), the standard error of prediction (SEP), and the standard error of calibration (SEC). These statistical indicators were calculated in order to evaluate the accuracy and predictive capability of the models. The obtained results are summarized in Table 2, where the agreement between actual and predicted concentrations of PRC, CFN, and PRPN in the calibration and prediction steps can be observed.

Table 2. Statistical parameters in the calibration and prediction steps for PCR and PLS

	Analyte	SEP	SEC	RMSEP
PLS	PRC	0.2258	0.1529	0.8155
	CFN	0.1005	0.1788	0.1615
	PRPN	0.1868	0.234	0.5585
PCR	PRC	0.2555	0.1137	0.4138
	CFN	0.1435	0.0914	0.1994
	PRPN	0.2277	0.089	0.2533

SEC: The standard error of calibration

SEP: The standard error of prediction

RMSEP: The root mean squared error of prediction

PRC: Paracetamol

CFN: Caffeine

PRPN: Propyphenazone

Validation of the chemometric methods

The reliability of the proposed chemometric approaches was evaluated through validation studies. For this purpose, thirteen synthetic mixtures contain-

ing different concentration levels of PRC, CFN, and PRPN were prepared as an independent validation set. The analytical results obtained from these mixtures are summarized in Table 3.

Table 3. Recovery results obtained by the application of PLS and PCR to the validation set consisting of PRC, CFN, and PRPN

Added (µg/mL)						PLS			PCR			PLS			PCR		
Found (µg/mL)						Found (µg/mL)			Found (µg/mL)			Recovery (%)			Recovery (%)		
PRC	CFN	PRPN	PRC	CFN	PRPN	PRC	CFN	PRPN	PRC	CFN	PRPN	PRC	CFN	PRPN	PRC	CFN	PRPN
3.0	3.0	9.0	2.81	2.96	9.13	2.83	3.03	9.06	93.7	98.8	101.5	94.3	100.9	100.7			
8.0	3.0	9.0	8.11	2.98	9.13	8.10	3.04	9.1	101.4	99.2	101.5	101.2	101.2	101.1			
12.0	3.0	9.0	11.95	3.11	9.05	11.92	3.15	9.02	99.6	103.7	100.6	99.4	105.1	100.2			
16.0	3.0	9.0	15.94	3.06	9.22	15.92	3.07	9.29	99.6	102.0	102.5	99.5	102.3	103.3			
15.0	2.5	9.0	14.99	2.50	9.25	15.01	2.57	9.31	100.0	100.2	102.8	100.1	102.7	103.4			
15.0	4.0	9.0	14.98	4.10	9.17	14.99	4.12	9.23	99.9	102.4	101.9	100.0	103.1	102.5			
15.0	8.0	9.0	15.53	7.67	9.15	15.53	7.67	9.2	103.5	95.9	101.7	103.6	95.9	102.2			
15.0	12.0	9.0	15.55	11.92	9.16	15.58	12.07	9.12	103.7	99.4	101.7	103.9	100.6	101.3			
15.0	3.0	3.0	15.34	3.00	2.93	15.31	3.10	2.91	102.3	99.9	97.5	102.1	103.2	97.2			
15.0	3.0	6.0	15.19	3.02	5.81	15.23	3.05	5.88	101.3	100.6	96.8	101.5	101.5	98.0			
15.0	3.0	9.0	15.16	3.10	9.16	15.16	3.11	9.28	101.1	103.3	101.8	101.1	103.7	103.1			
15.0	3.0	12.0	14.98	3.03	12.02	14.98	2.96	12.18	99.8	101.0	100.2	99.9	98.5	101.5			
15.0	3.0	9.0	15.02	3.09	9.01	15.03	3.10	9.16	100.1	102.9	100.1	100.2	103.2	101.8			
						Mean			100.5			100.5			101.7		
						SD			2.47			2.35			2.41		
						RSD			2.46			2.34			2.37		

SD: Standard Deviation

RSD: Relative Standard Deviation

PRC: Paracetamol

CFN: Caffeine

PRPN: Propyphenazone

The recovery values obtained for both PLS and PCR methods were close to 100%, with RSD values generally below 3%, demonstrating satisfactory accuracy and precision.

Method precision was also examined through intra-day and inter-day experiments performed at three concentration levels for each analyte. The calculated statistical parameters, including mean recovery, relative RSD, and relative error (RSE) values, are presented in Table 4.

Table 4. The recovery results obtained in the intra-day and inter-day samples by the proposed PLS and PCR methods (n=3 for every concentration level)

		Found (µg/mL)								
		Added (µg/mL)			PLS			PCR		
		PRC	CFN	PRPN	PRC	CFN	PRPN	PRC	CFN	PRPN
Inter-day	5	3	4	4.93	2.96	3.88	4.9	2.89	3.86	
	10	6	12	9.85	6.14	11.77	9.81	5.94	11.9	
	15	9	16	14.92	9.21	15.88	14.88	8.93	16.11	
Intra-day	5	3	4	4.94	2.98	3.88	4.92	2.9	3.95	
	10	6	12	9.81	6.14	11.78	9.76	5.97	11.96	
	15	9	16	14.91	9.2	15.65	14.87	8.99	15.93	
Recovery (%)										
		PLS			PCR					
		PRC	CFN	PRPN	PRC	CFN	PRPN			
Inter-day		98.6	98.6	96.9	98.1	96.4	96.4			
		98.5	102.4	98.1	98.1	99	99.2			
		99.4	102.4	99.2	99.2	99.2	100.7			
Intra-day		98.8	99.2	97.1	98.3	96.6	98.8			
		98.1	102.3	98.2	97.6	99.4	99.7			
		99.4	102.2	97.8	99.1	99.9	99.5			
RSD (%)										
		PLS			PCR					
		PRC	CFN	PRPN	PRC	CFN	PRPN			
Inter-day		1.01	0.97	2.88	0.96	1.26	2.12			
		1.06	0.27	1.33	1.12	0.56	0.8			
		0.91	0.61	1.19	0.94	0.54	0.76			
Intra-day		0.77	1.12	1.35	0.78	1.25	2.36			
		1.06	0.58	1.28	1.23	1.99	1.08			
		0.41	1.22	1.00	0.42	0.97	0.8			
RE (%)										
		PLS			PCR					
		PRC	CFN	PRPN	PRC	CFN	PRPN			
Inter-day		-1.37	-1.39	-3.06	-1.93	-3.56	-3.6			
		-1.45	2.35	-1.88	-1.88	-0.96	-0.83			
		-0.56	2.38	-0.75	-0.83	-0.76	0.69			
Intra-day		-1.16	-0.8	-2.9	-1.66	-3.39	-1.25			
		-1.94	2.34	-1.8	-2.37	-0.56	-0.34			
		-0.61	2.19	-2.2	-0.89	-0.07	-0.46			

RSD: Relative Standard Deviation

RE: Relative Error

PRC: Paracetamol

CFN: Caffeine

PRPN: Propyphenazone

The obtained results indicate that both chemometric methods provide reliable and reproducible analytical performance.

The selectivity of the proposed approaches and the possible influence of tablet excipients were evaluated using the standard addition technique. Known con-

centrations of PRC, CFN, and PRPN stock solutions (0, 2.0, 4.0, and 6.0 µg/mL for each compound) were added to aliquots of the tablet sample solution, and each concentration level was prepared in triplicate. The corresponding recovery values and relative standard deviations are listed in Table 5.

Table 5. The recovery results obtained in the standard addition samples by the proposed PLS and PCR methods

Added (µg/mL)			Found (µg/mL)						
	PLS			PCR					
	PRC	CFN	PRPN	PRC	CFN	PRPN	PRC	CFN	PRPN
Formulation	2.00	2.00	2.00	1.98	2.01	2.01	1.98	1.95	2.02
Formulation	4.00	4.00	4.00	3.95	4.02	4.03	3.94	4.02	4.02
Formulation	6.00	6.00	6.00	5.96	5.93	6.19	5.96	6.06	6.19
Recovery (%)									
	PLS			PCR					
	PRC	CFN	PRPN	PRC	CFN	PRPN	PRC	CFN	PRPN
99		100.6	100.5	98.8	97.3	100.8			
98.8		100.5	100.8	98.6	100.4	100.6			
99.3		98.9	103.1	99.4	101	103.2			
RSD (%)									
	PLS			PCR					
	PRC	CFN	PRPN	PRC	CFN	PRPN	PRC	CFN	PRPN
0.49		1.18	1.75	0.75	12.68	3.44			
0.51		1.83	1.31	0.63	5.29	2.61			
1.67		0.71	0.27	1.76	4.24	0.37			

RSD: Relative Standard Deviation
PRC: Paracetamol
CFN: Caffeine
PRPN: Propyphenazone

These results demonstrate that there is no effect of tablet excipients on the simultaneous analysis of PRC, CFN, and PRPN in the commercial tablet samples.

Analysis of commercial tablet formulation

The applicability of the developed PLS and PCR

chemometric calibration models was further tested by analyzing a commercial tablet formulation containing PRC, CFN, and PRPN. The predicted amounts of the active compounds in the tablet samples are presented in Table 6.

Table 6. Analysis results obtained by applying PLS and PCR to tablets

ExpNo.	mg/tablet					
	PLS			PCR		
	PRC	CFN	PRPN	PRC	CFN	PRPN
1	252.6	50.0	150.4	252.6	50.0	150.4
2	250.3	49.9	148.5	250.3	49.9	148.5
3	243.5	48.4	150.6	243.5	48.4	150.6
4	255.5	48.4	151.3	255.5	48.4	151.3
5	254.9	52.1	146.7	254.9	52.1	146.7
6	247.7	50.8	150.5	247.7	50.8	150.5
7	250.1	50.8	152.7	250.1	50.8	152.7
8	249.9	50.3	152.7	249.9	50.3	152.7
9	251.9	49.4	152	251.9	49.4	152
10	254.3	50.4	152	254.3	50.4	152
Mean	251.07	50.06	150.73	251.07	50.06	150.73
SD	3.64	1.12	1.9	3.64	1.12	1.9
RSD	1.45	2.24	1.26	1.45	2.24	1.26

SD: Standard Deviation

RSD: Relative Standard Deviation

PRC: Paracetamol

CFN: Caffeine

PRPN: Propyphenazone

A good agreement was observed between the results obtained using the two chemometric methods. Furthermore, the calculated statistical parameters, including standard deviation and RSD values, confirmed the reliability of both approaches for the quantitative analysis of the pharmaceutical formulation.

CONCLUSION

In this study, multivariate chemometric calibration models based on PLS and PCR were successfully developed for the simultaneous spectrophotometric determination of PRC, CFN, and PRPN in a ternary pharmaceutical formulation without requiring a prior separation step. The strong spectral overlap observed in the 220-300 nm region was effectively resolved using multivariate data analysis techniques.

The developed models demonstrated satisfactory calibration and prediction performance, as indicated by low SEC, SEP, and RMSEP values. Validation

studies using independent synthetic mixtures yielded mean recovery values close to 100% with relative standard deviations generally below 3%, confirming the accuracy and precision of the proposed methods. In addition, intra-day and inter-day precision experiments verified the robustness and reproducibility of the chemometric models. Standard addition experiments confirmed that tablet excipients did not interfere with the determination of the analytes.

The developed PLS and PCR models were successfully applied to the analysis of a commercial tablet formulation, and the obtained results were consistent with the labeled content of the active ingredients. When compared with conventional chromatographic techniques, the proposed chemometric spectrophotometric approach offers several advantages, including reduced solvent consumption, shorter analysis time, simpler experimental procedures, and lower operational cost.

Consequently, the results demonstrate that multivariate calibration methods combined with UV spectrophotometry provide a powerful and efficient analytical tool for resolving highly overlapping spectra in multicomponent pharmaceutical systems. Therefore, the proposed methods can be considered suitable alternatives for routine quality control analysis of pharmaceutical formulations containing PRC, CFN, and PRPN.

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AUTHOR CONTRIBUTION STATEMENT

Developing hypothesis, statistics, analysis of interpretation of the data, reviewing the text (ED). Experimenting, preparing the study text, literature research (AÜ). Experimenting, uploading the text, editing the article (NS).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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