

Research Article

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Chemometric determination of common cold infection drugs in human urine

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Abstract: In this work, spectrophotometric identification of acetylsalicylic acid (ASA), paracetamol (PCM), and caffeine (CAF) (common cold infection drugs) in human urine samples was investigated. For ASA, PCM, and CAF, chemometric analysis of human urine samples has proved successful. Spectrophotometric analysis of common cold infection drugs was performed using multivariate calibration methods (principal component regression [PCR] and partial least-squares regression). For the simultaneous prediction of common cold infection drugs in prepared mixes and human urine samples without prior separation, two spectrophotometric-chemometric approaches were proposed. The synthetic mixes were made with common cold infection drugs in the first stage, and the absorbance values were obtained using spectrophotometry. The quantities of common cold infection drugs in the human urine sample were calculated in the second stage. The calibration curves for each medication are linear in the concentration range of the synthetic mixes. The two methods were tested for accuracy and repeatability, and high recoveries and low standard deviations were calculated. sum of prediction residual errors, observation limit, and detection limit, and % recovery values, which are the analytical properties of the proposed methods, were 0.00029, 0.096, and 0.290, respectively; 0.0069, 0.086, and 0.260; 0.0077, 0.094, and 0.285; 0.0049, 0.066, and 0.199 for PCM, ASA, and CAF for the principal component regression method, respectively; 0.0059, 0.066, and 0.199; 0.0065, 0.069, and 0.210. The results produced using the employed chemometric methods are quick, easy, and consistent. The proposed methods are extremely sensitive and precise and have thus been effectively employed to detect active chemicals (ASA, PCM, and CAF) in human urine samples.

Keywords: acetylsalicylic acid, paracetamol, caffeine, chemometry, human urine

1 Introduction

Acetylsalicylic acid (ASA) [1], paracetamol (PCM) [2,3], and caffeine (CAF) [4] are used to relieve painful conditions and flu-like infections. ASA, PCM, and CAF in combination provide potent, fast-acting analgesia. The synergistic combination of ASA, PCM, and CAF provides greater pain relief than either component alone. When administered alone, this preparation relieves pain associated with migraine headaches as well as other conditions. The combination is prescribed for temporary relief of headache, mild to moderate pain associated with migraine, pain associated with sinusitis or colds, muscle pain, pain associated with menstrual cramps, toothache and mild arthritis pain, and pain associated with fever [5]. The chemical structures of ASA, PCM, and CAF are shown in Figure 1.

Various chemometric and analytical methods for the analysis of the three active ingredients can be found in the literature. PCM was determined by ultraviolet (UV) spectroscopy [6–10], high-performance liquid chromatography (HPLC) [10], and near-infrared spectroscopy [11]. ASA was investigated by spectroelectrochemical methods [12], UV spectroscopy [13], Raman spectroscopy and capillary electrophoresis [14], thin-layer chromatography [15], and near-IR spectroscopy [16]. CAF has also been analyzed by various methods in the literature, such as UV spectroscopy [17,18], HPLC [19–21], and liquid–liquid microextraction, which can be used together [22]. UV spectroscopic studies involved in chemometric studies were also evaluated in the review of methods [23,24].

Mathematical models support the chemometric method [25]. The simultaneous determination of two or more chemicals in the same sample without prior chemical separation has improved thanks to multivariate calibration methods [26]. Chemometric techniques are recently preferred statistical methods to calculate the amount of

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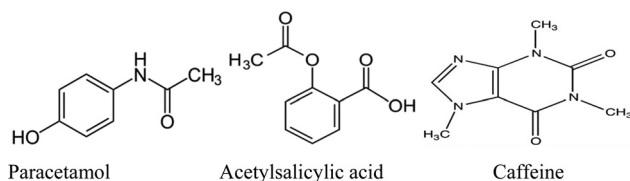


Figure 1: Chemical structures of the drugs.

each component in a complex mixture. One of the most difficult challenges in analytical chemistry is analyzing these compounds in mixtures containing two or more active components without any separation. In rare circumstances where chromatographic and spectrophotometric methods for studying sample mixtures do not yield satisfactory findings, studies utilizing other chromatographic and spectrophotometric methods are available. Furthermore, despite the use of advanced analytical equipment for the analysis of smaller samples, efforts are being made to increase the precision and correctness of the results by exposing the data collected by traditional analytical equipment utilizing chemometrics to various mathematical algorithms. As a result, the procedure is less expensive, much faster, and more precise than other analytical methods. Principal component regression (PCR) and partial least-squares (PLS) are the most extensively used chemometric approaches in the study of pharmacological samples [27].

The main objective of this work was to perform a statistical analysis of the determination of ASA, PCM, and CAF in a healthy human urine sample by chemometric methods. The proposed UV spectroscopy technique was supported by PLS and PCR and the sensitivity, accuracy, and selectivity of the results obtained by chemometric methods used were determined [28–30]. In this study, PLS and PCR were performed for the simultaneous detection of ASA, PCM, and CAF in human urine without using a sorting method in triumph. The average recoveries (%) and standard deviations of the chemometric methods were calculated to validate the methods. The results were statistically compared with each other. By applying analysis of variance to the results, it was tested whether the differences between the applied methods were significant [31,32]. The obtained F values were cross-checked with the value read from the F table (α : 0.05), and the Student's t -test was applied. The difficulty in working with human urine samples using both the classical UV method and other instrumental methods was overcome by chemometric methods in this study. In this study, the urine sample is investigated by chemometric methods due to its complex structure, and a contribution to the literature is made. Previous studies include UV spectroscopy and

chemometric analysis of active ingredients in drug tablets. In this study, a human urine sample was used. The urine sample has a matrix structure, especially due to the proteins it contains. By eliminating the proteins and adding chemometric calculations to the urine sample, a statistical determination of drug substances was performed by UV spectroscopy.

2 Materials and methods

HCl (0.1 M) was used to dissolve analytical-grade stock solutions of 25 mg/250 mL acetylsalicylic acid (Aldrich, Darmstadt, Germany), paracetamol (Aldrich, Darmstadt, Germany), and caffeine (Aldrich, Darmstadt, Germany). For all measurements and data processing, a Shimadzu UV-1700 PharmaSpec spectrophotometer (Kyoto, Japan) was linked to an IBM PC running UV Probe software.

2.1 Preparation of validation and calibration solutions

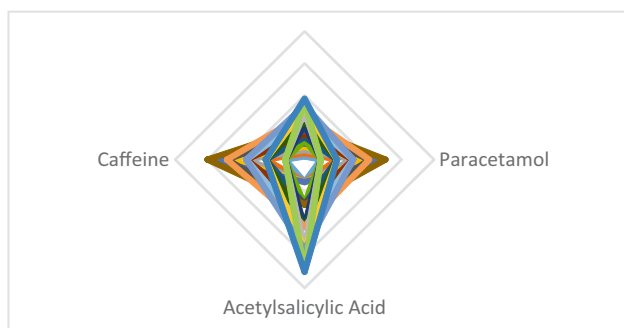
ASA, PCM, and CAF absorption spectra were recorded between 210 and 310 nm. Three-component mixtures with varying concentrations were used in the training and validation sets. PLS and PCR were used to compute concentration levels and analyze human urine samples. Drug samples containing 4.0 and 25.0 mg·L⁻¹ were placed in volumetric flasks (25 mL) and dissolved with 0.1 M HCl. The medications were administered at varying rates in the training set and the validation set, and 19 synthetic mixes (for validation and calibration) were created (Table 1 and Figure 2). The calibration set was constructed using a partial factorial design. Chemometric procedures were based on a well-designed experiment. Nineteen samples were created after the data were evaluated according to the experimental design.

2.2 Preparing for analysis of human urine samples

To prevent the matrix effect, healthy human urine samples were diluted 20 times with deionized water. Two milliliters of diluted urine were put into a tube, along with 3 mL of acetonitrile. To 5 mL of urine sample, 4 mL of 10% acetonitrile and various concentrations of the

Table 1: Concentration set for ASA, PCM, CAF

No.	Concentration (mg·L ⁻¹)		
	PCM	ASA	CAF
1	5	7	6
2	10	7	12
3	15	7	18
4	20	7	24
5	25	7	30
6	5	14	6
7	10	14	12
8	15	14	18
9	20	14	24
10	25	14	30
11	5	21	6
12	10	21	12
13	15	21	18
14	20	21	24
15	5	28	6
16	10	28	12
17	15	28	18
18	5	35	6
19	10	35	12

**Figure 2:** Concentration set design for the preparation of PCR and PLS calibrations.

pharmaceutical ingredients were added and mixed. After the preparations, the individual spectra of the solutions were recorded. Human urine samples studied were obtained from healthy persons who did not consumed any other drugs that they used continuously throughout their lives. A lot of water was drunk even before the sample was taken. Thus, the most suitable urine matrix for drug analysis was prepared.

3 Results

Absorption spectra of ASA, PCM, CAF, and the mix solution were obtained between certain wavelengths. The absorbance-wavelength graphs are shown in Figure 3.

By plotting the absorbance–concentration diagrams for three drug substances, we see that the absorbance values increase proportionally to an increase in concentration. The fact that the regression coefficient [34] approximates the individual values confirms the linear connection [33] between absorbance and concentration (Table 2).

For the basic component analysis [35], a standard series of stock solutions of ASA, PCM and caffeine-containing solutions were prepared using stock solutions with a linear working range of 5–30 ppm. As can be seen from the plot of the eigenvalues obtained from the chemometric data in Figure 4, the eigenvalues increased from the second to the third order.

The first two factors are more reliable than 99% of the total variance. This is the expected behavior in PCA. PCA is a technique whose main purpose is to obtain the data set with the highest variance in high dimensional data while achieving dimensionality reduction. It finds the

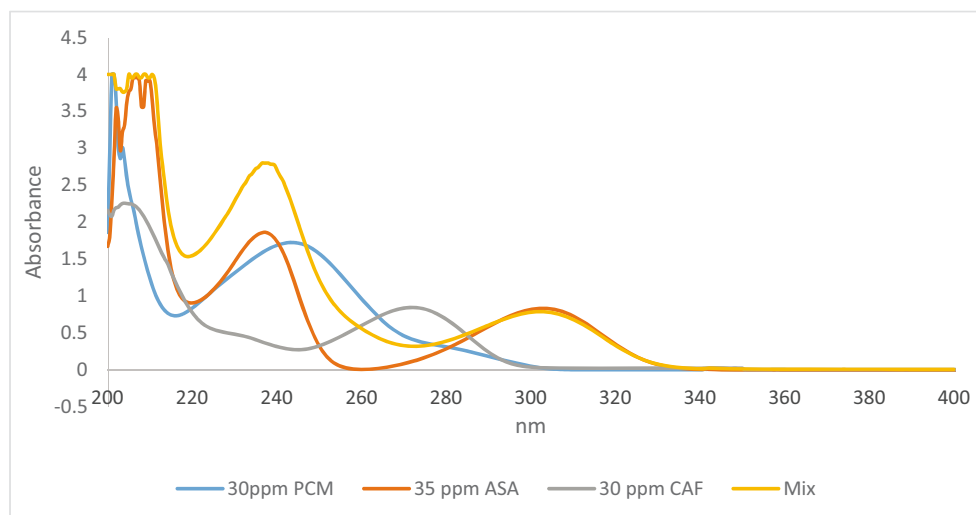
**Figure 3:** The absorption spectrum of ASA, PCM, caffeine mixtures.

Table 2: Spectroscopic properties of drugs

	λ_{max} (nm)	Correlation coefficient
PCM	243.50	0.9997
ASA	302.5	1.0000
CAF	273	0.9989

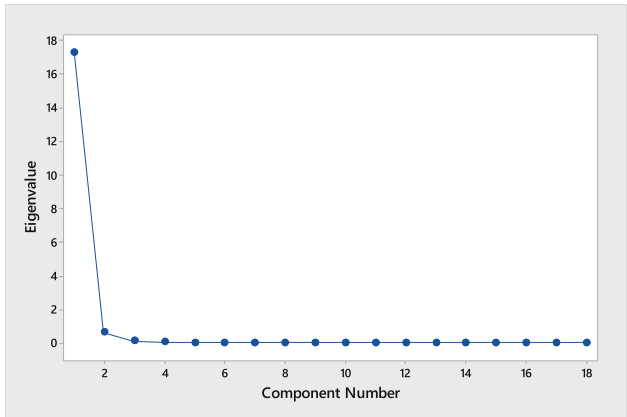


Figure 4: Diagram of eigenvalues obtained from chemometric data.

general features in overplanning and provides their dimensional use and data availability. Principal component analysis is a model used to reduce the size or reduce

the size of multivariate values. The method was validated according to ICH (International Conference on Harmonisation) guidelines for validation of analytical procedures for different validation parameters [36]. The concentrations found are compared against known concentrations for cross-validation [37,38] in the calculation. In this way, errors are avoided in the calibration of solutions used in commercial sample processing. A concentration set consisting of 19 artificial mixed solutions containing drugs was prepared. First, the pure substances were examined to determine the spectral range of each component. Measurements were made between 200 and 800 nm. Then, the wavelength range for the interval concentration set and in the direction of the statistical program to be used was narrowed down to 210–310 nm. While the concentration set was prepared, the absorbance of the spectra recorded in the pure form was evaluated.

3.1 PCR and PLS regression

The PLS and PCR techniques were found to be valid by the statistical parameters used to validate the ASA, PCM, and CAF calibrations. The PLS and PCR methods are extremely precise. As indicated in the results (Tables 3 and 4), the standard deviation values

Table 3: For the PLS approach, the composition of the prediction set and the recovery outcomes obtained in synthetic mixtures

Added (mg·L ⁻¹)			Found (mg·L ⁻¹)			% Recovery		
ASA	PCM	CAF	ASA	PCM	CAF	ASA	PCM	CAF
7	5	6	6.96	4.89	5.87	99.43	97.80	97.83
7	10	12	6.87	9.99	11.96	98.14	99.90	99.67
7	15	18	6.89	14.96	17.95	98.43	99.73	99.72
7	20	24	6.99	19.89	23.95	99.86	99.45	99.79
7	25	30	6.97	24.95	29.89	99.58	99.80	99.63
14	5	6	13.96	4.96	5.97	99.71	99.20	99.50
14	10	12	13.97	9.96	11.94	99.79	99.60	99.50
14	15	18	13.90	14.97	17.96	99.29	99.80	99.78
14	20	24	13.92	19.98	23.87	99.43	99.90	99.46
14	25	30	13.87	24.98	29.96	99.07	99.92	99.87
21	5	6	20.95	4.98	5.76	99.76	99.60	96.00
21	10	12	20.97	9.99	11.96	99.86	99.90	99.67
21	15	18	20.96	14.96	17.97	99.81	99.73	99.83
21	20	24	20.89	19.97	23.89	99.48	99.85	99.54
28	5	6	27.89	4.86	5.97	99.61	99.20	99.50
28	10	12	27.85	9.95	11.90	99.46	99.50	99.17
28	15	18	28.01	14.96	17.98	100.03	99.73	99.89
35	5	6	35.01	4.74	5.96	100.03	94.80	99.33
35	10	12	34.89	9.95	11.97	99.69	99.50	99.75
			Mean: 99.50% RSD: 0.49			Mean: 99.21% RSD: 1.29	Mean: 99.34% RSD: 0.93	

Table 4: For the PCR approach, the composition of the prediction set and the recovery outcomes obtained in synthetic mixtures

Added (mg·L ⁻¹)			Found (mg·L ⁻¹)			% Recovery		
ASA	PCM	CAF	ASA	PCM	CAF	ASA	PCM	CAF
7	5	6	6.85	4.96	5.94	97.86	99.20	99.00
7	10	12	6.86	9.87	11.95	98.00	98.70	99.58
7	15	18	6.95	14.88	17.94	99.29	99.20	99.67
7	20	24	6.90	19.89	23.86	98.57	99.45	99.42
7	25	30	6.98	24.96	29.95	99.71	99.84	99.83
14	5	6	13.95	4.96	5.98	99.64	99.20	99.67
14	10	12	14.01	9.92	11.95	100.07	99.20	99.58
14	15	18	13.89	14.99	17.89	99.21	99.93	99.39
14	20	24	13.94	19.97	23.89	99.57	99.85	99.54
14	25	30	13.98	24.95	29.97	99.86	99.80	99.90
21	5	6	20.99	4.99	5.89	99.95	99.80	98.17
21	10	12	20.98	9.97	11.92	99.90	99.70	99.33
21	15	18	21.01	14.95	17.89	100.05	99.67	99.39
21	20	24	20.95	19.98	24.01	99.76	99.90	100.04
28	5	6	27.98	4.95	5.90	99.93	99.00	98.33
28	10	12	28.01	9.89	11.94	100.04	98.90	99.50
28	15	18	28.00	14.95	17.89	100.00	99.67	99.39
35	5	6	34.87	4.89	5.92	99.63	97.80	98.67
35	10	12	34.89	9.96	11.99	99.69	99.60	99.92
Mean: 99.51% RSD: 0.67						Mean: 99.39% RSD: 1.53	Mean: 99.3% RSD: 0.51	

are sufficiently low, and the recovery values are sufficiently close to 100, indicating that the results obtained are reasonable.

3.2 Validation of the method

The chemometric approach was validated in terms of linearity, accuracy, and precision within one day and between two days and in terms of the limit of detection and limit of quantification in accordance with international conference on harmonisation criteria [39–41]. The sum of prediction residual errors (PRESS) is used for calibration (Eq. 1) [42] was calculated as follows:

$$\text{PRESS} = \sum_{i=1}^n (C_i^{\text{added}} - C_i^{\text{found}})^2 \quad (1)$$

where C_i^{added} is actual concentration, the added concentration of drug; and C_i^{found} is predicted concentration, the calculated concentration of drug. According to the actual and predicted concentrations of the samples, PRESS values of ASA, PCM, and CAF were calculated (Table 5).

It is critical to note that this method of normalizing PRESS values is incorrect unless all data sets have the same amount of samples. However, the number of samples is included in the standard error of calibration (SEC)

(Eq. 2). The efficiency of the calibration was determined by a few statistical criteria. The SEC was determined using the following formula:

$$\text{SEC} = \sqrt{\frac{\sum_{i=1}^n (C_i^{\text{added}} - C_i^{\text{found}})^2}{n - 1}} \quad (2)$$

where n is the total number of synthetic mixtures. Another validation parameter is root mean square error of calibration (RMSEC) [43] as shown below:

$$\text{RMSEC} = \sqrt{\text{PRESS}/n} \quad (3)$$

The observation limit (LOD) and detection limit (LOQ) parameters are related but have different definitions (Eqs. 4 and 5) [44]:

$$\text{LOD} = 3\text{Sa}/m \quad (4)$$

$$\text{LOQ} = 10\text{Sa}/m \quad (5)$$

where m – slope, Sa – the corrected standard deviation value $\text{LOQ} > \text{LOD}$ and $\text{LOQ} = \text{LOD}$ were considered in the evaluation of the calculated LOD values [45].

The values of PRESS and SEC are close to zero, indicating that the degree of accuracy is increasing. The calculated PRESS and SEC values are close to zero for the PLS and PCR methods (Table 5).

The performance of the explored chemometric approaches with the UV spectrophotometric method for

Table 5: Statistical parameter values for calibrating step-simultaneous ASA, PCM, and CAF determinations using PLS and PCR techniques

Parameters	Method	PCM	ASA	CAF
SEC	PLS	0.019	0.023	0.023
	PCR	0.020	0.019	0.024
PRESS	PLS	0.00029	0.0069	0.0077
	PCR	0.0049	0.0059	0.0065
RMSEC	PLS	0.00391	0.0602	0.0201
	PCR	0.0160	0.0557	0.0185
LOD ($\mu\text{g}\cdot\text{mL}^{-1}$)	PLS	0.096	0.086	0.094
	PCR	0.066	0.091	0.069
LOQ ($\mu\text{g}\cdot\text{mL}^{-1}$)	PLS	0.290	0.260	0.285
	PCR	0.199	0.276	0.210
Accuracy (% recovery $\pm$ SD)	PLS	99.21 $\pm$ 1.29	99.50 $\pm$ 0.49	99.34 $\pm$ 0.93
	PCR	99.39 $\pm$ 1.53	99.51 $\pm$ 0.67	99.39 $\pm$ 0.51
Precision (reproducibility)				
Intraday (% recovery $\pm$ SD) (n: 6)	PLS	99.89 $\pm$ 0.85	99.88 $\pm$ 0.54	99.84 $\pm$ 0.55
	PCR	99.84 $\pm$ 0.64	99.87 $\pm$ 0.45	99.94 $\pm$ 0.32
Interday (% recovery $\pm$ SD) (n: 6)	PLS	99.66 $\pm$ 0.24	98.87 $\pm$ 0.87	98.88 $\pm$ 0.78
	PCR	99.65 $\pm$ 0.11	99.95 $\pm$ 0.89	99.99 $\pm$ 0.79

analyzing the materials was evaluated using Snedecor's *F*-test [46]. To analyze the differences between the disposable tests, the ANOVA method was used on the real samples for each medication. In this investigation, the Snedecor *F*-values were calculated and compared to the experimental *F*-values. An identical mathematical process was applied to each drug. The experimental (calculated) *F*-values did not exceed the *F*-values in the analysis of variance. For both chemometric techniques, the within-group degrees of freedom were 1 and 36, respectively, and the *F*-table value was 4.11 with a 95% confidence interval for both. Using the PLS technique, the *F*-test value for ASA was calculated as 0.00049, with a *p*-value of 0.98; the *F*-test value for PCM was calculated as 0.00071, with a *p*-value of 0.98; and the *F*-test value

for CAF was calculated as 0.00070, with a *p*-value of 0.98. In the PCR method, the *F*-test value for ASA was 0.00030, with a *p*-value of 0.98; for PCM, the *F*-test value was 0.00030, with a *p*-value of 0.98; and the *F*-test value for CAF was 0.00071, with a *p*-value of 0.98 (see Figure 5). As a result, all of these techniques were shown to be considerably different.

3.3 Analysis of human urine samples

Table 6 shows the experimental values of the PCR and PLS techniques for human urine samples. The results can be shown to be extremely near. As indicated in Table 6, chemometric methods were successfully used to determine

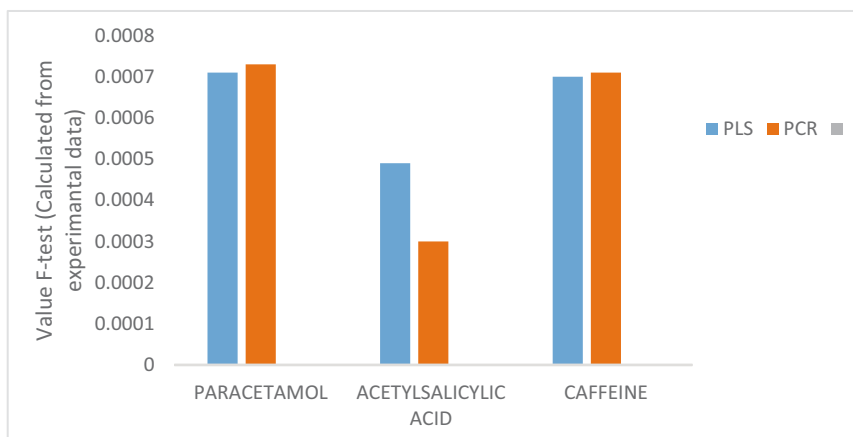
**Figure 5:** The results of the one-way ANOVA test according to Snedecor's *F*-test.

Table 6: Determination of ASA, PCM, CAF in human urine using PLS and PCR methods

Mix no.	Acetylsalicylic acid (PLS)			Paracetamol (PLS)			Caffeine (PLS)		
	Added (mg·L ⁻¹)	Found (mg·L ⁻¹)	Recovery (% mean)	Added (mg·L ⁻¹)	Found (mg·L ⁻¹)	Recovery (% mean)	Added (mg·L ⁻¹)	Found (mg·L ⁻¹)	Recovery (% mean)
1	5	4.89	97.80	5	4.99	99.80	5	5.01	100.2
2	10	9.96	99.60	10	9.97	99.70	10	9.88	98.80
3	15	14.95	99.67	15	14.96	99.73	15	14.87	99.13
4	20	19.96	99.80	20	19.89	99.45	20	19.96	99.80
5	25	24.98	99.92	25	24.97	99.88	25	24.97	99.88
Mean ± SD			99.36 ± 0.88			99.71 ± 0.16			99.56 ± 0.58

Mix no.	Acetylsalicylic acid (PCR)			Paracetamol (PCR)			Caffeine (PCR)		
	Added (mg·L ⁻¹)	Found (mg·L ⁻¹)	Recovery (% mean)	Added (mg·L ⁻¹)	Found (mg·L ⁻¹)	Recovery (% mean)	Added (mg·L ⁻¹)	Found (mg·L ⁻¹)	Recovery (% mean)
1	5	4.88	97.60	5	4.95	99.00	5	4.97	99.40
2	10	9.87	98.70	10	9.94	99.40	10	9.97	99.70
3	15	14.97	99.80	15	14.95	99.67	15	14.97	99.80
4	20	19.99	99.95	20	19.96	99.80	20	19.98	99.90
5	25	24.56	98.24	25	24.69	98.76	25	24.78	99.12
Mean ± SD			98.86 ± 1.01			99.33 ± 0.44			99.58 ± 0.32

Mix no.	Acetylsalicylic acid (classical UV)			Paracetamol (classical UV)			Caffeine (classical UV)		
	Added (mg·L ⁻¹)	Found (mg·L ⁻¹)	Recovery (% mean)	Added (mg·L ⁻¹)	Found (mg·L ⁻¹)	Recovery (% mean)	Added (mg·L ⁻¹)	Found (mg·L ⁻¹)	Recovery (% mean)
1	5	4.96	99.20	5	4.98	99.60	5	4.94	98.80
2	10	9.96	99.60	10	9.98	99.80	10	9.97	99.70
3	15	14.89	99.27	15	14.95	99.67	15	14.95	99.67
4	20	19.97	99.85	20	19.95	99.75	20	19.96	99.80
5	25	24.89	99.56	25	24.96	99.84	25	24.98	99.92
Mean ± SD			99.50 ± 0.26			99.73 ± 0.097			99.58 ± 0.45

drugs in healthy human urine samples, yielding high-accuracy results. The obtained statistical values appear to be appropriate for detecting these substances in human urine samples at the same time.

4 Discussion

Simultaneously, PLS and PCR were successful in determining pharmaceuticals in synthetic solutions with pharmaceutical formulations. Low estimation errors and high correlation coefficients highlight the strong linear relationship between anticipated and actual concentrations for all values. The results obtained with this ternary mixture, as well as the component concentration ratios, reveal that these approaches have high predictive ability. The

UV spectroscopy data were evaluated chemometrically to analyze the drug compounds in the sample containing three different active ingredients. The UV spectra of the active ingredients ASA, PCM, and CAF were recorded to determine the purity at which the study could be conducted, and analytical studies were performed. For ASA, PCM, and CAF the method was statistically supported by improving the UV spectrum. The regression analysis of the standard curves of the method was performed, and the results were statistically calculated. The *F*-test was considered in the analysis of the data obtained by the chemometric program. The results were also compared with the synthetic model created during the experimental design before using the effervescent tablet sample. The synthetic models were compared with the experimental results obtained from the chemometric program. The *F*-test was performed using the within-group and between-

group degrees of freedom. We decided to apply the model we used after the F -test result to the mixture of the drug sample, but we cannot apply it. Now that we have passed the decision, we have decided based on the F test result.

5 Conclusions

After certain processes, proteins were precipitated from urine, and measurements were made in the UV-visible range. The high recoveries also showed that the drugs were not bound to the urine proteins. The mixtures containing ASA, PCM, and CAF errors were predicted in the validation process. The values of the sum of squares (PRESS) and SEC are also calculated. PRESS and SEC values converge to zero. The closer they are, the better the accuracy. The chemometric methods are applied to drug samples and human urine samples. The sensitivity is high for drug analysis, so the results are reproducible. The obtained results suggest that this method may be suitable for the simultaneous determination of ASA, PCM, and CAF in human urine.

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Conflict of interest: The author states no conflict of interest.

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