

## SPECTROPHOTOMETRIC DETERMINATION OF ACETYLSALICYLIC ACID, PARACETAMOL AND ASCORBIC ACID BY CHEMOMETRIC METHODS

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**Abstract.** Acetylsalicylic acid, paracetamol, and ascorbic acid which are used for the pain reliever and fever reducer were analyzed without any prior reservation by means of spectrophotometry-chemometry. The experimental calibration matrix was designed by measuring the absorbance within the range of 200–400 nm for 19 samples of acetylsalicylic acid, paracetamol, and ascorbic acid. Absorbance and concentration values were analyzed using Minitab and other chemometric programs to calculate estimated concentrations by PCR and PLSR. In the first step the synthetic mixtures including acetylsalicylic acid, paracetamol, and ascorbic acid were prepared and absorbance values were obtained according to spectrophotometry. In the second step, amounts of acetylsalicylic acid, paracetamol, and ascorbic acid were calculated in effervescent tablet (Afebryl®). Standard deviations were accomplished. This study encouraged us to apply the developed methods for drug analysis. The methods presented in this paper are rapid and do not need any separation process or preliminary treatments for the analysis.

**Keywords:** paracetamol, acetylsalicylic acid, ascorbic acid, PLSR, PCR.

### 1. Introduction

Paracetamol is a medicine with pain reliever and anti-fever effect. When paracetamol is taken orally, it is rapidly absorbed in the gastrointestinal tract. The drug reaches maximum plasma concentrations after 30–60 min of ingestion [1, 2]. Acetylsalicylic acid is a pain-killer and anti-inflammatory drug commonly used for minor pain and infestation. There is also blood diluent effect and long-term low dosage is used to protect against heart attack [3]. Vitamin C is not stored by the body and is sweated with urine and sweat because it is among the

water-soluble vitamins. For this reason, it needs to be taken back every day. Vitamin C plays an important role in the renewal of strong immune system, collagen production, skin, cartilage, tendon, ligament and blood vessels [4].

Chemometric calibration methods are observed to be the best techniques to determinate the amount of each component in the complex mixture. The most accepted chemometric methods in a drug analysis are principal component regression (PCR) and partial least squares regression (PLSR) [5, 6]. A relationship to be established between matrices of chemical data is determinated at chemometric methods [7].

Chemometrics is the science that accords with extracting valuable information from the raw data. PCR and PLSR calibration methods are intended to be full spectrum computational procedures [8].

Partial least squares regression (PLSR-regression) is the most commonly used chemometric multivariate calibration method [9] and for quantitative analysis of spectroscopic data to determine the amount of drug in multicomponent pharmaceutical products [10].

The Minitab 17 program (Inova, Ankara, Turkey) was used for the analysis of all the concentration and absorbance data to do the statistical calculations. Minitab is a statistical analysis software. In addition to statistical research, statistics can be used to learn [11].

The main aim of this work was the determination of acetylsalicylic acid, paracetamol, and ascorbic acid of tablet sample (Afebryl® effervescent tablet) by chemometric methods. The validation of the proposed UV spectroscopic technique has added precision, accuracy, selectivity and brings successful results with principal component regression (PCR) and partial least squares regression (PLSR) – chemometric methods. In this study, PCR and PLSR were successfully performed to simultaneous determination of acetylsalicylic acid, paracetamol, and ascorbic acid in a commercial tablet formulation without any separation method. Mean recoveries and standard deviation of PCR and PLSR were calculated for the validation of the methods. The acquired results were statistically compared with each other.

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## 2. Experimental

### 2.1. Materials

All materials used were of analytical grade. Stock solutions of 25 mg/250 ml of acetylsalicylic acid (Sigma), paracetamol (Sigma) and ascorbic acid (Sigma) were prepared with 0.1M HCl. A training set and validation set contain the drugs in various proportions, 19 synthetic mixtures (for validation) were made.

Shimadzu UV-1700 PharmaSpec Spectrophotometer connected to an IBM PC with UV Probe Software was used for all measurements and data processing. A pair of 1.0 cm quartz cuvettes was used for absorbance measurements.

### 2.2. Absorbance Measurements

Absorbance spectra of acetylsalicylic acid, paracetamol, and ascorbic acid were recorded between 200 and 400 nm with 0.1 step. The calibration matrix, training and validation sets contain three component mixtures at different rates. To calculate concentrations, the concentration sets and analysis of effervescent tablet have been done using PLSR and PCR.

Samples of 5.0 and 35.0 ppm of drugs (alone or in combination) were placed in volumetric flasks (25 ml) and dissolved in 0.1M HCl. The mixture was shaken for 20 min and filtered. Dry tight standards were prepared in the same manner as described, except those with the

reagents and drug. A training set and validation set contain the drugs in various proportions, *i.e.* 19 synthetic mixtures (for validation) were prepared and presented in Table 1.

### 2.3. Pharmaceutical Preparations

Afebryl® effervescent tablet produced by Galepharma, containing 300 mg of acetylsalicylic acid, 300 mg of ascorbic acid and 200 mg of paracetamol per tablet were analyzed by the principal component regression (PCR) and partial least squares regression (PLSR) chemometric methods. For this purpose 1 g of the samples were mechanically mixed with 0.1M HCl and transferred into 25 ml bottles. All the techniques were applied to the final solution.

## 3. Results and Discussion

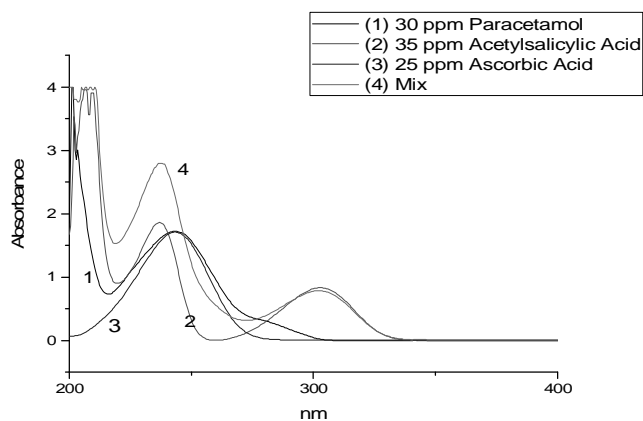
### 3.1. Absorption of Acetylsalicylic Acid, Paracetamol, Ascorbic Acid and Mix Solution

Absorption of acetylsalicylic acid, paracetamol, ascorbic acid and mix solution are all in the visible region of high absorbent substances. Fig. 1 shows the absorbance-wavelength curves. The spectra of acetylsalicylic acid, paracetamol, and ascorbic acid are in the range of 200–400 nm.

Table 1

Concentration set for acetylsalicylic acid, paracetamol and ascorbic acid

No.	Concentration, ppm		
	Acetylsalicylic acid	Paracetamol	Ascorbic acid
1	6	7	5
2	12	7	10
3	18	7	15
4	24	7	20
5	30	7	25
6	6	14	5
7	12	14	10
8	18	14	15
9	24	14	20
10	30	14	25
11	6	21	5
12	12	21	10
13	18	21	15
14	24	21	20
15	6	28	5
16	12	28	10
17	18	28	15
18	6	35	5
19	12	35	10



**Fig. 1.** Original absorption spectra of 30 ppm paracetamol, 35 ppm acetylsalicylic acid and 25 ppm ascorbic acid and their mixture in 0.1M HCl

### 3.2. Principal Component Regression (PCR) and Partial Least Squares Regression (PLSR)

The PLSR method and absorption spectra can be used individually or overlapped with multiple simultaneous detections of various linear components. Some statistical parameters were given for validation of calibrations for synthetic mixtures of drugs.

The statistical parameters were found to produce a satisfactory validity for the PLSR and PCR methods. The PLSR and PCR methods have reliable accuracy and higher precision. Judging from the results obtained, the standard deviation values are sufficiently small and the recovery values are sufficiently close to 100. This shows us that the results obtained are appropriated.

*Table 2*

**Composition of prediction set and recovery results obtained in synthetic mixtures for PLSR method**

Actual concentration, ppm			Prediction concentration, ppm			Recovery, %		
Acetyl-salicylic acid	Para-cetamol	Ascorbic acid	Acetyl-salicylic acid	Para-cetamol	Ascorbic acid	Acetyl-salicylic acid	Para-cetamol	Ascorbic acid
7	6	5	7.01	5.97	4.83	100.15	99.5	96.6
7	12	10	6.82	11.51	9.85	97.43	95.92	98.5
7	18	15	6.95	17.58	14.85	99.29	97.67	99
7	24	20	7.05	24.04	20.01	100.72	100.17	100.05
7	30	25	7.03	30.06	24.95	100.43	100.2	99.8
14	6	5	14.05	5.93	5.01	100.36	98.84	100.2
14	12	10	14.06	11.83	9.69	100.43	98.59	96.9
14	18	15	14	17.82	14.69	100	99	97.94
14	24	20	13.53	23.62	19.68	96.65	98.42	98.4
14	30	25	13.96	29.51	24.86	99.72	98.37	99.44
21	6	5	20.71	5.54	4.89	98.62	92.34	97.8
21	12	10	20.63	11.52	9.65	98.24	96	96.5
21	18	15	21.01	17.82	14.51	100.05	99	96.74
21	24	20	20.84	23.83	19.8	99.24	99.30	99
28	6	5	27.73	5.84	4.89	99.04	97.34	97.8
28	12	10	27.83	11.82	9.87	99.40	98.5	98.7
28	18	15	27.71	17.96	14.96	98.97	99.78	99.74
35	6	5	34.85	5.74	4.85	99.58	95.67	97
35	12	10	35.02	11.94	10.01	100.06	99.5	100.1
						Mean: 99.37 Standard deviation: 1.07	Mean: 98.11 Standard deviation: 1.95	Mean: 98.43 Standard deviation: 1.27

Table 3

**Composition of prediction set and recovery results obtained  
in synthetic mixtures for PCR method**

Actual concentration, ppm			Prediction concentration, ppm			Recovery, %		
Acetyl-salicylic acid	Para-cetamol	Ascorbic acid	Acetyl-salicylic acid	Para-cetamol	Ascorbic acid	Acetyl-salicylic acid	Para-cetamol	Ascorbic acid
7	6	5	6.95	5.89	4.78	99.29	98.17	95.6
7	12	10	6.87	11.82	9.98	98.15	98.5	99.8
7	18	15	6.66	17.89	14.95	95.15	99.39	99.67
7	24	20	6.92	23.95	19.86	98.86	99.80	99.3
7	30	25	7.01	29.85	25.01	100.15	99.5	100.04
14	6	5	13.95	6.01	5.01	99.65	100.17	100.2
14	12	10	14.01	11.85	9.78	100.08	98.75	97.8
14	18	15	13.85	17.56	14.56	98.93	97.56	97.07
14	24	20	13.95	23.86	19.96	99.65	99.42	99.8
14	30	25	13.81	29.92	24.97	98.65	99.74	99.88
21	6	5	19.8	5.85	4.96	94.29	97.5	99.2
21	12	10	21	11.95	9.87	100	99.59	98.7
21	18	15	21.06	17.95	14.62	100.29	99.73	97.47
21	24	20	20.66	23.96	19.98	98.39	99.84	99.9
28	6	5	27.84	5.87	4.9	99.43	97.84	98
28	12	10	27.57	11.89	10.01	98.47	99.09	100.1
28	18	15	27.51	17.92	14.98	98.25	99.56	99.87
35	6	5	34.73	5.86	4.98	99.23	97.67	99.6
35	12	10	35.01	11.92	9.97	100.03	99.34	99.7
						Mean: 98.78 Standard deviation: 1.59	Mean: 99.00 Standard deviation: 1.87	Mean: 99.03 Standard deviation: 1.28

### 3.3. Validation of the Method

For calibration the prediction residual error sum-of-squares (PRESS) was calculated as:

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

where  $C_i^{added}$  – actual concentration, the added concentration of drug;  $C_i^{found}$  – predicted concentration, the calculated concentration of drug.

According to the actual and predicted concentrations of the samples, *PRESS* values of acetylsalicylic acid, paracetamol, and ascorbic acid were calculated and listed in Table 2.

It is important to emphasize that this is not a correct way to normalize the *PRESS* values when not all of the data sets contain the same number of samples. But standard error of prediction (*SEP*) values contain the number of samples. Some statistical parameters

determined the effectiveness of the calibration. The *SEP* was calculated using the following expression:

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

where  $n$  – the total number of synthetic mixtures.

*PRESS* and *SEP* values are close to zero, the degree of accuracy is increasing. The obtained *PRESS* and *SEP* values are close to zero for PLSR and PCR methods.

### 3.4. Analysis of Pharmaceutical Formulation

The experimental results of two methods for pharmaceutical formulation are given in Table 5. One can see that the obtained results are very close to each other.

In order to compare the performances of the investigated chemometric techniques according to UV spectrophotometric method for real samples we applied Snedecor's *F*-test [12].

Table 4

**Statistical parameter values for calibration step-simultaneous determination of acetylsalicylic acid, paracetamol, and ascorbic acid using PLSR and PCR methods**

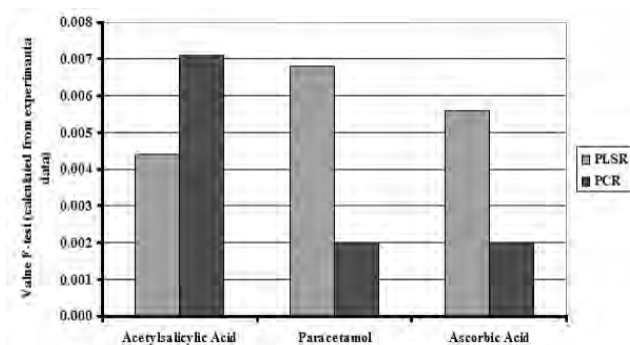
Parameter	Method	Compound, ppm		
		Acetylsalicylic acid	Paracetamol	Ascorbic acid
PRESS	PLSR	0.04	0.07	0.04
	PCR	0.11	0.02	0.02
SEP	PLSR	0.05	0.07	0.05
	PCR	0.06	0.04	0.03

Table 5

**Determination of acetylsalicylic acid, paracetamol, and ascorbic acid in pharmaceutical formulation using PLSR and PCR methods**

No	PLSR			PCR		
	Acetylsalicylic acid	Paracetamol	Ascorbic acid	Acetylsalicylic acid	Paracetamol	Ascorbic acid
1	0.295	0.198	0.278	0.281	0.192	0.297
2	0.296	0.186	0.29	0.284	0.193	0.298
3	0.281	0.182	0.284	0.286	0.187	0.296
4	0.279	0.194	0.281	0.29	0.182	0.285
5	0.293	0.178	0.293	0.295	0.186	0.284
Mean	0.289	0.188	0.285	0.287	0.188	0.292
Standard deviation	0.0081	0.0083	0.0062	0.0055	0.0045	0.0069

The method used to compare the differences between the one-way ANOVA test was applied to the actual samples for each food drug. In this study, Snedecor's  $F$ -values were calculated and compared with the  $F$  value. The same computation process was repeated for each drugs. Fig. 2 shows ANOVA results. The experimental (calculated)  $F$ -values did not exceed the  $F$ -value in the variance analysis. Among all these methods, it was concluded that there was a meaningful difference. All statistical parameters and numeric values are suitable for simultaneous identification in the actual samples.



**Fig. 2.** The results of the one-way ANOVA test according to Snedecor's  $F$ -test

## 4. Conclusions

The partial least squares method and principle component regression successfully applied at the same time were able to identify drugs in synthetic solutions and pharmaceutical formulation. For all values low prediction errors and high correlation coefficients emphasize the high linear relationship between the predicted and actual concentrations. The results obtained with this ternary mixture and some ratios of component concentrations show excellent predictive ability with these methods.

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### СПЕКТРОФОТОМЕТРИЧНЕ ВИЗНАЧЕННЯ АЦЕТИЛСАЛІЦИЛОВОЇ КИСЛОТИ, ПАРАЦЕТАМОЛУ ТА АСКОРБІНОВОЇ КИСЛОТИ ХЕМОМЕТРИЧНИМИ МЕТОДАМИ

*Анотація.* Проведено спектрофотометрично-хеометричний аналіз без будь-яких попередніх застережень ацетилсаліцилової кислоти, парацетамолу та аскорбінової кислоти, які використовують як анаболіки та препарати для зниження температури. Розроблено експериментальну калібрувальну матрицю внаслідок вимірювання поглинальної здатності в діапазоні 200–400 нм для 19 зразків ацетилсаліцилової кислоти, парацетамолу та аскорбінової кислоти. Проаналізовано значення поглинальної здатності та концентрації, використовуючи Minitab та інші хеометричні програми для розрахунку концентрацій за допомогою методів PCR і PLSR. На першому етапі готували синтетичні суміші з ацетилсаліцилової кислоти, парацетамолу та аскорбінової кислоти, і отримали значення поглинальної здатності відповідно до спектрофотометрії. На другому етапі кількість ацетилсаліцилової кислоти, парацетамолу та аскорбінової кислоти розраховувалася в шипучій таблетці (Afebryl®). Розраховані стандартні відхилення, які дали можливість запропонувати розроблені методи для аналізу препаратів. Показано, що методи є швидкими і не потребують будь-якого процесу розділення або попереднього оброблення для проведення аналізу.

**Ключові слова:** парацетамол, ацетилсаліцилова кислота, аскорбінова кислота, PLSR, PCR.