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Simultaneous Determination of Binary Drug Components in Pharmaceutical Formulations with Chemometric Methods

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ABSTRACT: A synchronous definition of paracetamol and amoxicillin quantities in pharmaceutical formulations was performed using spectrophotometric methods. Principal component regression (PCR) and partial least squares regression (PLS) were used as multivariate calibration techniques. Spectrophotometric-chemometric methods were used for the simultaneous quantification of paracetamol and amoxicillin in the laboratory prepared mixtures and in the drug tablets without any requirements for a prior separation. The linearity of the calibration curves for each active substance in the synthetic mixtures demonstrated that concentration ranges were appropriate (r^2>0.9997). The investigation of the accuracy and repeatability of the two methods resulted in high recovery rates and lower standard deviation values. Achieving high recovery and low standard deviation values, this study encouraged us to proceed further in pharmaceutical assay. The recommended methods are highly sensitive and precise as these methods have been successfully applied to quantify active substances in pharmaceutical samples.

Keywords: Paracetamol, Amoxicillin, PLS, PCR.

Farmasötik Formülasyonlardaki İkili İlaç Bileşenlerinin Eş Zamanlı Olarak Kemometrik Metotlarla Tayini

ÖZET: Farmasötik formülasyonlardaki parasetamol ve amoksisilin miktarlarının spektrofotometrik olarak eş zamanlı tayini yapılmıştır. Çok değişkenli kalibrasyon tekniklerinden temel bileşen regresyonu (TBR) ve kısmi en küçük kareler yöntemi (KEKK) kullanılmıştır. Spektrofotometrik-kemometrik metotlar kullanılarak, hem laboratuvarda hazırlanan sentetik karışımların hem de ilaç tabletlerinin hiçbir ön ayırma yapılmadan analizi sağlanmıştır. Sentetik karışımlardaki her aktif madde için kalibrasyon eğrilerinin doğrusallığı, konsantrasyon aralıklarının uygun olduğu gözlenmiştir (r^2> 0,9997). İki yöntemin doğruluğu ve tekrarlanabilirliği incelendiğinde yüksek geri kazanım ve düşük standart sapma değerleri elde edilmiştir. Yüksek geri kazanım ve düşük standart sapma değerleri, bu çalışmada ilaç tablet analizleri konusunda bizi cesaretlendirmiştir. Önerilen metotlar, farmasötik örneklerdeki aktif bileşenlerin tayininin başarıyla uygulanması yönünden oldukça yüksek kesinlik ve duyarlılığa sahiptir.

Anahtar Kelimeler: Parasetamol, Amoksisilin, PLS, PCR.

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INTRODUCTION

Amoxicillin, a semi-synthetic β-lactam in the penicillin group, is a type of antibiotic is in the treatment for gastrointestinal system infections with *Helicobacter pylori* (Horoz et al., 2004; Rojanarata et al., 2010; Aktaş et al., 2017). Amoxicillin is chemically $C_{16}H_{19}N_3O_5S$ (Reiss et al., 2015). Paracetamol, $C_8H_9NO_2$, is widely used as an analgesic and antipyretic drug worldwide (Mohamed et al., 2017; Shanker et al., 2017). Paracetamol is commercially available in several medications in combination with other pharmaceuticals at different rates (Cunha et al., 2017). Paracetamol is quantified by UV spectroscopy (Dinç, 2003; Hajian et al., 2012; Behera et al., 2012; Hajian and Soltaninezhad, 2013; Aktaş and Kitiş, 2014; Saraan et al., 2015; Zahira et al., 2015; Wedian, 2016; Glavanovic et al., 2016) and HPLC (Attimarad, 2011; Talluri et al., 2012; Sharma et al., 2016). Amoxicillin is determined with HPLC (Aktaş et al., 2017) and UV spectroscopy (Ertokuş and Bağrıaçık, 2017), too.

Spectrophotometric methods are widely used for the simultaneous quantification of distinct molecules in combinations. The results are reported to be accurate, precise and it is stated that the method is inexpensive (Mansour, 2018). Partial least squares regression (PLS) and principal component regression (PCR) are the most frequently used chemometric methods (Dinç and Baleanu, 2002). The analysis of multicomponent pharmaceutical products using these methods are well-accepted (Kenneth, 1997).

The mathematical science (Aktaş and Ünlü, 2016) supported chemometry allows analytical analysis of two or more substances, even in overlapping spectra (Aktaş and Toprak, 2017). The Minitab 17 program (Inova, Ankara, Turkey) (İnova Danışmanlık, 2019) is a statistical analysis software used not only for performing statistical analyses but for training in statistics as well. It allows statistical analysis by using the relationship between Minitab and absorbance-concentration.

In this study; the quantification of paracetamol and amoxicillin in pharmaceutical combinations (Parol and Alfoxil) were performed by PLS and PCR. Amoxicillin and paracetamol are usually given to patients to be taken simultaneously for a combination of their antibiotic and analgesic effects. Starting from this point, using the available medications of these two molecules, we prepared a drug sample containing a combination of amoxicillin and paracetamol. The validation of the chemometric-spectrophotometric method used in this study is included as an appendix to this article presenting the precision, accuracy, and selectivity of the methods. PLS and PCR methods successfully identified and quantified paracetamol and amoxicillin present in the drug combination simultaneously without any prior separation. Mean recoveries (%), the standard deviation of principal component regression (PCR), and partial least squares regression (PLS) methods were used for the validation of the methods used in the study. The data were statistically crosschecked with their respective counterparts.

MATERIAL AND METHODS

Stock solutions were prepared at 25 mg in 250 ml concentration using Paracetamol (Sigma) and amoxicillin (Sigma) HCl in analytical purity. A concentration set was prepared to contain these two drugs in various proportions. Consequently, 25 distinct synthetic mixtures were prepared to be used for validation and calibration and the absorption measurement of the substance was done with s Shimadzu UV-1700 PharmaSpec Spectrophotometer.

Absorption values of drug active compounds were saved at 200 to 350 nm. The training and validation sets containing two component in different proportions were used for calculating the concentrations and the concentration sets. The analysis of the drug mixtures was performed using chemometric methods. Mixtures of 5.0-25.0 mg L^{-1} of active substance were put in volumetric flasks (25 ml) and dissolved in 0.1M HCl. A training set, a validation set, and 25 synthetic mixtures (for

validation and calibration) containing the drugs in various proportions were prepared. They are presented in Table 1.

Table 1. Concentration set for paracetamol and amoxicillin (Ertokuş, 2018).

No.	Concentration, mg L^{-1}				
	Paracetamol	Amoxicillin	No.	Paracetamol	Amoxicillin
1	5	5	**13**	15	15
2	5	10	**14**	15	20
3	5	15	**15**	15	25
4	5	20	**16**	20	5
5	5	25	**17**	20	10
6	10	5	**18**	20	15
7	10	10	**19**	20	20
8	10	15	**20**	20	25
9	10	20	**21**	25	5
10	10	25	**22**	25	10
11	15	5	**23**	25	15
12	15	10	**24**	25	20
			25	25	25

Preparation of Pharmaceutical Preparations

A commercial dosage form Parol tablets produced by Atabay, containing 500 mg of paracetamol per tablet and Alfoxil tablets produced by Actavis, containing 500 mg of amoxicillin per tablet were analyzed by multivarite calibration methods. While preparing the drug sample to be examined, both the parol and all of the alfoxil drugs were mixed. The mixture was crushed and mixed in agate air to provide homogeneity. Paracetamol and amoxicillin were determined from the prepared mixture. For this purpose, weighing one tablet was dissolved in 0.1 M HCl and then the final volume is completed to 25 mL.

RESULTS AND DISCUSSION

Absorption of paracetamol and amoxicillin and of the mixed solution were all in the visible range of highly absorbent substances (Figure 1.).

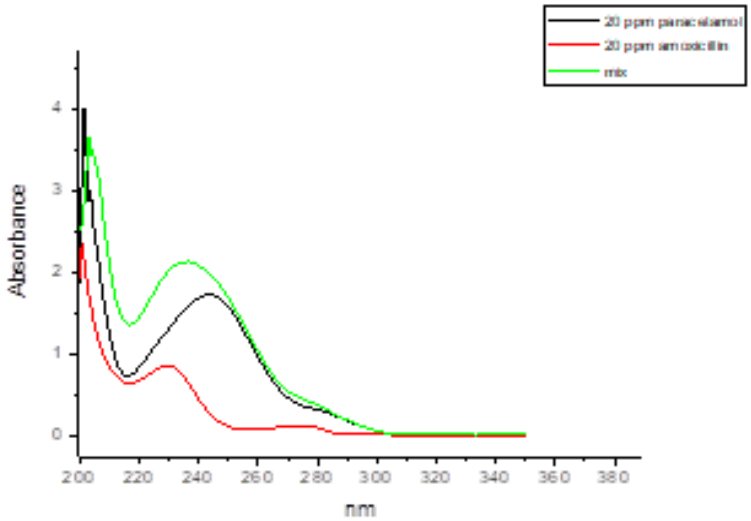

Figure 1. Absorption spectra of 20 mg L^{-1} paracetamol, 20 mg L^{-1} amoxicillin and and their mixture.

When the absorbance-concentration relationship of paracetamol and amoxicillin is examined, it is observed that the absorbance value increases with increasing concentration. The linear relationship

(Miao et al., 2018) between absorbance and concentration values is evaluated by the high value of the regression coefficient (Uyanık, 2012) (Table 4.).

The concentration values in the range of 5.0-25.00 ppm used in the study are the area where the linearity for each component was to be determined. According to Lambert-Beer (Uyanık, 2012), when the relationship between absorbance and concentration is examined, it is observed that the linear correlation coefficient (Sharma et al., 2017) between the two variables is close to each other (Table 4.).

Chemometric Methods

Some statistical parameters were introduced for the validation of calibrations for synthetic mixtures of drugs. Recovery and relative standard deviation (rsd) values calculated for each chemometric method 1 are shown in Table 2, Table 3,. While the concentrations against the added concentrations were calculated, the cross-validation procedure was applied to prevent errors in the drug sample (Porfire et al., 2015; Tarhan and Kara, 2017).

Method Validation

The chemometric method was validated in accordance with ICH guidelines (Abbai and Parameswari, 2009; Aravind and Kamarapu, 2013; Despande and Mandawad, 2018;) with respect to linearity, accuracy, intraday and interday precision, limit of detection, and limit of quantitation. For calibration, According to the actual and predicted concentrations of the samples, the prediction of the residual error sum-of-squares (PRESS) and standard error of prediction (SEC) (Table 4.) was calculated with the formula below:

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

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

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

where n – the total number of synthetic mixtures.

Another validation parameter is RMSEC (Bilgili et al., 2014). It is given in the below equation 3.

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

The equations of the observable limit (LOD) and detection limit (LOQ) parameters are shown below. These expressions are interrelated but have different equations (equation 4 and 5) (Shrivastava and Gupta, 2011).

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

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

m: Slope

LOQ> LOD and LOQ = LOD were taken into consideration while evaluating the calculated LOD values (Armbruster and Pty, 2008).

Table 2. Recovery results for PLS method

No	Paracetamol			Amoxicillin		
	Added (ppm)	Found (ppm)	Recovery %	Added (ppm)	Found (ppm)	Recovery %
1	5	4.99	99.80	5	5.00	100.00
2	5	4.87	97.40	10	9.98	99.8
3	5	4.85	97.00	15	14.96	99.73
4	5	4.88	97.60	20	19.97	99.85
5	5	4.92	98.40	25	24.97	99.88
6	10	9.96	99.60	5	4.96	99.20
7	10	9.87	98.70	10	9.92	99.20
8	10	9.84	98.40	15	14.97	99.80
9	10	9.95	99.50	20	18.89	94.45
10	10	9.92	99.20	25	24.95	99.80
11	15	14.96	99.73	5	5.01	100.20
12	15	14.97	99.80	10	9.96	99.60
13	15	14.89	99.27	15	14.92	99.47
14	15	14.94	99.60	20	19.92	99.60
15	15	14.95	99.67	25	24.89	99.56
16	20	19.98	99.90	5	4.97	99.40
17	20	19.95	99.75	10	9.99	99.90
18	20	19.89	99.45	15	14.97	99.80
19	20	19.97	99.85	20	19.96	99.80
20	20	19.97	99.85	25	24.96	99.84
21	25	24.96	99.84	5	4.96	99.20
22	25	24.98	99.92	10	9.97	99.70
23	25	24.97	99.88	15	14.93	99.53
24	25	25.01	100.04	20	19.87	100.04
25	25	25.01	100.04	25	24.97	100.04
			Mean=99.29 % RSD=0.87			Mean=99.50 % RSD =1.08

Table 3. Recovery results for PCR method

No	Paracetamol			Amoxicillin		
	Added (ppm)	Found (ppm)	Recovery %	Added (ppm)	Found (ppm)	Recovery %
1	5	5	100.00	5	4.98	99.60
2	5	4.98	99.60	10	9.89	98.90
3	5	4.97	99.40	15	14.87	99.13
4	5	4.89	97.80	20	19.99	99.95
5	5	4.9	98.00	25	24.92	99.68
6	10	9.95	99.50	5	4.89	97.80
7	10	9.92	99.20	10	9.95	99.50
8	10	9.94	99.40	15	14.99	99.93
9	10	9.97	99.70	20	19.96	99.80
10	10	10.01	100.10	25	24.89	99.56
11	15	14.97	99.80	5	4.92	98.40
12	15	14.96	99.73	10	9.87	98.70
13	15	14.92	99.47	15	14.92	99.47
14	15	15.01	100.07	20	19.87	99.35
15	15	14.89	99.27	25	24.9	99.60
16	20	19.97	99.85	5	4.91	98.20

Table 3. Recovery results for PCR method (contitnued)

No	Paracetamol			Amoxicillin		
	Added (ppm)	Found (ppm)	Recovery %	Added (ppm)	Found (ppm)	Recovery %
17	20	19.99	99.95	10	9.88	98.80
18	20	19.97	99.85	15	14.94	99.60
19	20	19.96	99.80	20	19.95	99.75
20	20	19.94	99.70	25	24.94	99.76
21	25	24.96	99.84	5	4.86	97.20
22	25	24.97	99.88	10	9.88	98.80
23	25	24.93	99.72	15	14.92	99.47
24	25	24.98	100.04	20	19.88	100.04
25	25	24.97	100.04	25	24.85	100.04
			Mean=99.59			Mean =99.24
			% RSD=0.57			%RSD =0.73

Table 4. Calculated Analytical Parameters

Parameters	Method	Paracetamol	Amoxicillin
λ_{max} (nm)		243.50 nm	229.50 nm
Regression Equation*(y)	Y=a+bx	Y=0.064x-0.011	Y=0.022x-0.009
Slope (b)		0.064	0.022
Intercept (a)		0.011	0.009
Correlation Coefficient (R^2)		0.9997	0.9992
SEC	PLS	0.021	0.031
	PCR	0.017	0.029
PRESS	PLS	0.0063	0.055
	PCR	0.004	0.009
RMSEC	PLS	0.016	0.047
	PCR	0.013	0.019
LOD (µg/mL)	PLS	0.060	0.334
	PCR	0.053	0.064
LOQ(µg/mL)	PLS	0.183	1.010
	PCR	0.159	0.195
Accuracy (% Recovery±SD)	PLS	99.29±0.87	99.50±1.08
	PCR	99.59±0.57	99.24±0.73
Precision (Reproducibility)			
Intraday (% Recovery ±SD) (n:6)	PLS	99.57±0.92	99.95±0.54
	PCR	99.89±0.65	99.97±0.55
Interday (% Recovery ±SD) (n:6)	PLS	99.69±0.51	99.55±0.86
	PCR	99.87±0.52	99.64±0.98

* y=a+bx, where y is the absorbance and x is the concentration of paracetamol and amoxicillin.

PRESS and *SEP* values were close to zero with the PLS and PCR methods. The degree of accuracy showed an increasing pattern.

Analysis of the Pharmaceutical Formulations

The calculated values of chemometric methods for the pharmaceutical formulations are shown in Table 5. The results obtained are very close to each other.

Snedecor's *F*-test (Bajpai et al., 2017) and student's t-test were applied to chemometric results. For student's t-test, $p > 0.05$ was found and the variances were homogeneous; i.e., the results obtained with PLS and PCR methods were compatible. For Paracetamol, the p-value was 0.194 and for amoxicillin, the p-value was 0.450. Considering all these methods, it was concluded that there was a meaningful difference. All statistical parameters and numeric values were appropriate for simultaneous identification in pharmaceutical formulations.

Table 5. The amount of paracetamol and amoxicillin in the pharmaceutical formulation harvested using PLS and PCR methods.

	Paracetamol (gram)		
NO	**PLS**	**PCR**	**Classical UV**
1	0.480	0.495	0.492
2	0.495	0.497	0.496
3	0.492	0.498	0.495
4	0.497	0.497	0.495
5	0.499	0.496	0.495
Mean±SD*	0.4926±0.0075	0.4966±0.0011	0.4946±0.0015
	Amoxicillion(gram)		
NO	**PLS**	**PCR**	**Classical UV**
1	0.485	0.498	0.496
2	0.496	0.497	0.494
3	0.495	0.489	0.497
4	0.497	0.488	0.499
5	0.496	0.496	0.498
Mean±SD*	0.4938±0.0050	0.4936±0.0047	0.4968±0.0019

*SD: Standard Deviation

CONCLUSION

The proposed multivariate calibration methods are fast, precise and correct for the simultaneous determination of pharmaceutical formulations containing paracetamol and amoxicillin drugs having overlapping spectra. In this study, PLS and PCR methods were applied to the simultaneous quantitative prediction of paracetamol and amoxicillin in pharmaceutical formulations without requiring any separation step. For all values, low prediction errors and high correlation coefficients emphasized the high linear relationship between the predicted and actual concentrations. The results obtained from the binary mixtures and the results associated with the concentration ratios of some components showed excellent predictive ability with these methods.

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