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Simultaneous Determination of Sulfamethoxazole and Trimethoprim Using UV Spectroscopy in Combination with Multivariate Calibration

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The objective of this study is to evaluate the capability of UV-spectrophotometry in combination of multivariate calibration based on Partial Least Square (PLS) regression for simultaneous quantitative analysis and dissolution evaluation of sulfamethoxazole (SUL) and trimethoprim (TRI) in tablet dosage form. The experimental calibration and validation matrixes were constructed with 20 and 10 samples, respectively. The concentration range considered was 1-16 μg mL^{-1} for both SUL and TRI. The absorbance data of the calibration standards were taken between 200-400 nm. For achieving the best calibration model, the related parameters were evaluated. The optimum number of factors was selected by using the cross-validation method. The evaluation of calibration model is relied on the coefficient of determination (R^2) and Root Mean Square Error of Calibration (RMSEC). The coefficient of determination (R^2) for the relationship between actual values and predicted values of SUL and TRI was higher than 0.99 indicating good accuracy of the developed method. The RMSEC values obtained were relatively low, namely 0.167% (SUL) and 0.279% (TRI), which indicate acceptable precision of analytical method. The accuracy of developed method was comparable to that of High Performance Liquid Chromatography (HPLC) method. The UV spectrophotometry in combination with PLS calibration model was successfully used for quantitative analysis and dissolution studies of SUL and TRI sulfamethoxazole and trimethoprim in tablet dosage form.

Key words: UV spectrophotometry, dissolution studies, partial least square, sulfamethoxazole, trimethoprim

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INTRODUCTION

Sulfonamides are used for the prevention and cure of infectious diseases caused by bacteria. The combined two drugs are more effective because they work synergistically (Shamsa and Amani, 2006). The combination of sulfamethoxazole (SUL) and trimethoprim (TRI), which is better known as co-trimoxazole, works as an anti-bacterial widely used in urinary tract infections, respiratory tract and gastro intestinal tract (Sohrabi *et al*., 2010). The chemical structures of sulfamethoxazole and trimethoprim is shown in Fig. 1.

Currently, there was a wide range of both generic and patents co-trimoxazole dosage. To ensure safe and efficacious co-trimoxazole drug, it would require a valid analytical method. Various instrumental method shave been developed to analyze the levels of SUL and TRI simultaneously in tablet dosage forms, including thin layer chromatography (Agbaba *et al*., 1996), FTIR spectrophotometry combined with multivariate calibration (Kargosha and Ahmadi, 1999), capillary electrophoresis (Li *et al*., 2000), UV-Vis spectrophotometry with diazotized (Shamsa and Amani, 2006) and reversed-phase liquid chromatography (Okine *et al*., 2006). Most of these methods require expensive reagents and time consuming making it less suitable for routine quality control. Thus, it was desirable to develop a simpler, faster and cheaper assay by using UV spectrophotometry combined with multivariate analysis.

Spectrophotometric methods provide practical and significant economic advantages over other instrumental methods (Islam *et al*., 2013; Bano *et al*., 2013). The main problems with UV spectrophotometric analysis are limited selectivity, lack of specific chromogenic reactions, as well as strong spectral band overlapping exhibited by most active ingredients in the ultraviolet region. However, the development of chemometric procedures for processing complex signals have allowed the simultaneous determination of analytes, even those suffering from extensive band overlapping (Andrade *et al*., 2013).

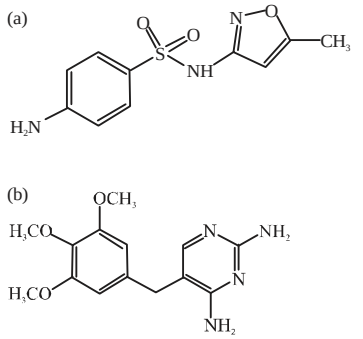

Fig. 1(a-b): Chemical structure of (a) Sulfamethoxazole and (b) Trimethoprim

Currently, the application of chemometric techniques, especially multivariate calibrations are playing a very important role in the multicomponent analysis of pharmaceutical mixtures (De Luca *et al*., 2009). The most adopted multivariate methods in pharmaceutical analysis and frequently used for instrumental methods without separation techniques like ultraviolet and infrared spectroscopies are Principal Component Regression (PCR), Stepwise Multiple Linear Regression (SMLR) and Partial Least-squares (PLS) (Ragno *et al*., 2004; El-Gindy *et al*., 2006; Rohman, 2012). The PLS has a high potential as a calibration-prediction methodology for processing absorbance signals of drugs (Ghasemi and Vosough, 2002; Rohman *et al*., 2013). The PLS has several advantages such as it employs full spectral data that a critical for the resolution of multicomponent mixtures, analytical procedures can be carried out in a short time, usually with no sample clean-up or physical separation and PLS calibration models ignore the concentrations of all other components except a selected analytes of interest in the studied samples (Escandar *et al*., 2006).

The UV spectrophotometry coupled with PLS calibration model has been successfully used for analysis of theophylline in blood serum (Goicoechea *et al*., 1999), direct determination of diclofenac in pharmaceutical formulations containing B vitamins (Sena *et al*., 2004), analysis of preservatives in syrup (Blanco *et al*., 1994) and simultaneous determination of ethinylestradiol and levonorgestrel (Nevado *et al*., 1997). However, using literature review there is no reports available regarding analysis and dissolution studies of SUL and TRI using UV-spectrophotometry-PLS. In the present work, UV spectrophotometry in combination with multivariate calibration of PLS is reported for the simultaneous determination of SUL and TRI without prior separation. Tablet analysis and dissolution test were carried out according to Indonesian Pharmacopeia with satisfactory accuracy and precision.

MATERIALS AND METHODS

The standards of sulfamethoxazole (SUL) and trimethoprim (TRI) were of Reference Standard of Indonesian Pharmacopeia and were obtained from the National Agency of Drug and Food Control, Republic of Indonesia. The chemicals and reagents used were of pro analytical grade. The solvents used for HPLC were of liquid chromatography grade. The tablet dosage form was obtained from pharmacy in Yogyakarta.

Analysis of sulfamethoxazole and trimethoprim in tablet dosage forms using UV spectrophotometry: The standard solutions were obtained by dissolving of 10 mg of sulfamethoxazole and trimethoprim in methanol:aqueous solution 0.1 N NaOH (80:20 v/v) and used for preparing calibration (20 samples) and validation samples (10 samples).

The composition of calibration and validation samples are shown in Table 1 and 2, respectively. For quantitative analysis, 20 tablets of co-trimoxazole were weighted and ground to fine powder. Then a proportion of powder equivalent to one average tablet is taken and added with methanol:aqueous solution 0.1 N NaOH (80:20 v/v) until 100 mL. The solution is shaken vigorously for 30 min. The solution is filtered using Whatman paper. Each solution mixture of calibration samples was scanned using UV-Vis spectrophotometer (Shimadzu) at 200-400 nm. At each 2 nm, their absorbance were recorded and used for the optimization the calibration models.

Analysis of sulfamethoxazole and trimethoprim in tablet dosage forms using high performance liquid chromatography: The mobile phase was prepared by mixing 1400 mL of acetonitril, 400 mL of distilled water and 2.0 mL of triethanolamine (TEA) in a 2000 mL volumetric flask and then pH of the solution was adjusted to 5.9±0.1 using 0.2 N sodium hydroxide or glacial acetic acid solution (1 in100) and then diluted with distilled water to the mark. It was then filtered through a 0.45 μm cellulose membrane filter before use. Standard solution is made by carefully weighing a number of SUL and TRI, dissolved in methanol then diluted with mobile phase to each level of approximately 0.16 and 0.032 mg mL^{-1}. Test solution is prepared by carefully weighing of certain amount of powdered tablets equivalent to approximately160 mg SUL, put in a pint flask of 100 mL and then diluted with methanol up to the mark. A 5.0 mL of the clear filtrate was pipetted into the measuring flask and then diluted with 50 mL of the mobile phase up to the mark. The HPLC separation was performed using a Shimadzu LC 20 AD with a 254 nm UV detector and the column used was C-18 (4.6×250 mm, 5 μ). The detection was set at 254 nm.

Table 1: Composition of synthetic mixture consisting of sulfamethoxazole and trimethoprim used in calibration samples

No. of Samples	SUL (μg mL^{-1})	TRI (μg mL^{-1})
1	13.0	16.0
2	9.0	12.0
3	16.0	16.0
4	13.0	2.0
5	3.0	3.0
6	1.0	12.0
7	6.0	6.0
8	1.0	13.0
9	8.0	3.0
10	12.0	8.0
11	11.0	14.0
12	9.0	12.0
13	9.0	10.0
14	16.0	8.0
15	5.0	9.0
16	12.0	5.0
17	1.0	15.0
18	14.0	10.0
19	15.0	3.0
20	6.0	3.0

SUL: Sulfamethoxazole, TRI: Trimethoprim

Table 2: Composition of synthetic mixture consisting of sulfamethoxazole and trimethoprim used in validation samples

No. of samples	SUL (μg mL^{-1})	TRI (μg mL^{-1})
1	10.0	16.0
2	10.0	9.0
3	3.0	4.0
4	12.0	16.0
5	3.0	9.0
6	1.0	8.0
7	6.0	5.0
8	16.0	8.0
9	9.0	3.0
10	7.0	1.0

SUL: Sulfamethoxazole, TRI: Trimethoprim

Dissolution test for tablets: Dissolution test of tablets were carried out in 60 min according to Indonesian Pharmacopeia edition IV method (75 rpm) in 900 mL 0.1 N HCl at 37.0±0.1°C (n = 6). The samples were taken by means of an injector with membrane filter (0.20 μm) and diluted to appropriate concentrations to be analyzed using UV-Vis spectrophotometry and HPLC method, respectively.

Data analysis: The absorbance data and concentration were subjected to PLS regression model as input and output, respectively. The PLS analysis is carried out using Minitab software version 16 (Minitab Corp., USA). The concentration of sulfamethoxazole and trimethoprim in tablet dosage forms is calculated based on the optimized calibration model.

RESULTS AND DISCUSSION

The absorption UV spectra of pure sulfamethoxazole (SUL) and trimethoprim (TRI) in methanol: aqueous solution 0.1 N NaOH (80:20 v/v) at wavelength 200-400 nm showed the strong overlapping of the components. This hinders the resolution of the mixture by conventional spectrophotometry. Some efforts have been made by some researches to solve this problems such as the use of spectral treatment of derivative spectrophotometry (Palabiyik *et al*., 2004; Tomsu *et al*., 2004; Markopoulou *et al*., 2004), the use of H-point standard addition methods to overcome the problems of interference due to the partial spectral overlapping (Sabry and Khamis, 2000). Multivariate calibration using principle component regression and partial least square are the common methods used to overcome the extensive overlapping of spectra which allow to facilitate the calibration between response and concentration of analytes (Marwada *et al*., 2014; Rohman, 2012; Mohamed and Mikre, 2009).

In order to make a good model for quantitative analysis of SUL and TRI, the Uv spectra of the mixture of raw material and the evaluated drugs form have to be similar as shown in Fig. 2. Quantitative analysis of SUL and TRI was performed with the aid of Partial Least Squares (PLS) calibration. For achieving the optimum model, different spectral regions were

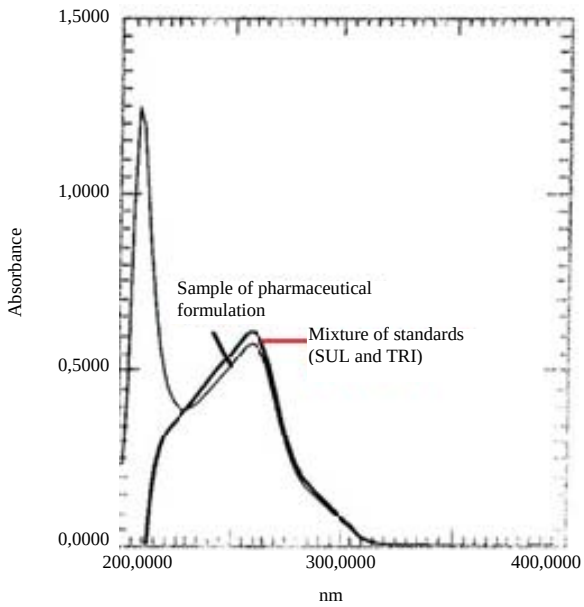

Fig. 2: Overlay of UV spectra of mixture of standard (sulfamethoxazole and trimethoprim) and sample of pharmaceutical drug

evaluated and the wavelength capable of providing the best correlation between actual and predicted value of SUL and TRI were selected. Finally the wavelength of 230-318 and 258-318 nm was preferred for quantification of SULand TRI, respectively due to its capability provide the highest values of coefficient of determination (R^2) and the lowest values of error expressed as Root Mean Square Error of Calibration (RMSEC). The correlation between actual value and predicted values of SUL and TRI as determined using Uv spectrophotometry without any separation processes was shown in Fig. 3. The R^2 values obtained for such correlation is high, namely 0.999 for SUL and 0.997 for TRI. Meanwhile, the RMSEC values obtained are relatively low, i.e., 0.167% (SUL) and 0.279% (TRI). The Relative Standard Deviation (RSD) for precision evaluation for each studied drugs meet the requirements because its value is less than 2%.

One of the potential disadvantages when using multivariate calibrations is over-fitting of the regression model. It means that the model generates an optimistic model on the set of data used for calibration, but the model would not perform well on other datasets with similar material (Miller and Miller, 2005). Cross-validation of calibration samples using "leave-one out" technique can be used to assess this problem. One of the calibration samples is left out from PLS model and the remaining samples are used to make PLS model. Furthermore, the removed sample is calculated using the new developed PLS model. This procedure was repeated; leaving each calibration sample out in turn. Then, the difference between the actual and predicted value for each specimen is calculated. The sum of the squares of these differences is called the Predicted Residual Error Sum of Squares (PRESS). The smaller the PRESS value, the better

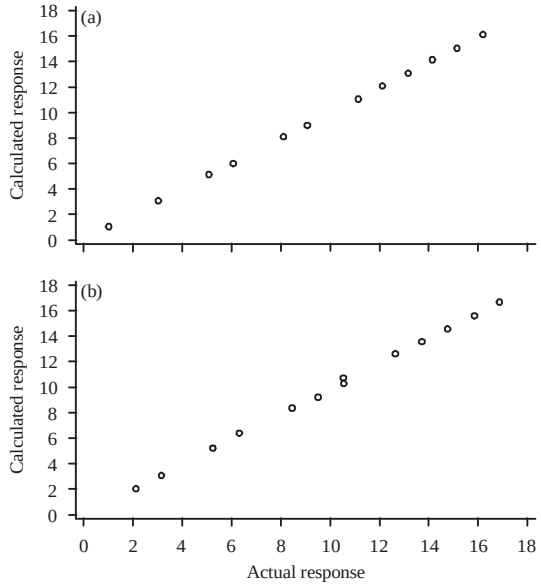

Fig. 3(a-b): Correlation between actual value and predicted values of (a) Sulfamethoxazole and (b) Trimethoprim as determined using UV spectrophotometry at 230-318 and 258-318 nm with the aid of partial least squares

the predictive power of the model (Rohmanand and Man, 2011). The PRESS values obtained are 1.520% (SUL) and 2.561% (TRI). Regarding this result, the over- fitting does not happen in the developed PLS model, because the values of PRESS are relatively similar to those of RMSEC values. Figure 4 revealed the correlation between actual value and predicted values of SUL and TRI during cross validation.

Table 3: Result for Sulfamethoxazole and trimethoprim obtained from commercial formulations

			Sample 1		Sample 2	
			Found (%)		Found (%)	
	Analytes	Labeled amount (mg tablets^{-1})	PLS	HPLC	PLS	HPLC
Assay test	SUL	800	99.37	95.46	99.38	95.01
	TRI	160	96.66	96.34	93.62	93.62
Requirements of Indonesian pharmacopeia : 93-107%						
Dissolution test	SUL	800	93.61	76.78	96.57	79.46
	TRI	160	100.56	80.08	97.3	80.98
Requirements of Indonesian pharmacopeia : ≥75%						

SUL: Sulfamethoxazole, TRI: Trimethoprim, PLS: Partial least square, HPLC: High performance liquid chromatography

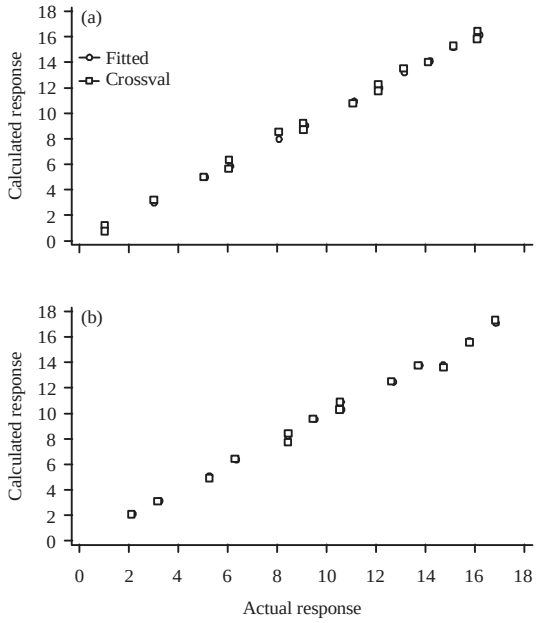

Fig. 4(a-b): Partial least squares model for the correlation between actual value and predicted values of (a) Sulfamethoxazole and (b) Trimethoprim during cross validation

The PLS model was subsequently used to predict the level of independent samples in prediction/validation models. The prediction performance was assessed using R^2 and Root Mean Square Error of Prediction (RMSEP) values obtained; the small RMSEP and the high R^2 values indicated that the prediction model of new sample has less error. Using PLS model, the RMSEP values obtained for SUL and TRI are 0.173 and 0.231 %, respectively.

In order to test the performance of the proposed method, the produced model was used to predict the concentrations of the SUL and TRI in pharmaceutical formulation. The results obtained by UV spectrophotometry are compared with those by official methods (HPLC). The levels of SUL and TRI obtained by UV spectrophotometry in combination with PLS and by HPLC are shown in Table 3. The proposed method was also applied to the dissolution test of the tablets without using any separation procedure. More than 75% of sulfamethoxazole and trimethoprim were dissolved after 60 min in the dissolution medium. From Table 3, it is known that results obtained by UV spectrophotometry are comparable to those determined by HPLC.

CONCLUSION

A study of the use of UV spectrophotometric in combination with PLS for the simultaneous determination of sulfamethoxazole (SUL) and trimethoprim (TRI) in a binary mixture has been accomplished. The results obtained confirmed the suitability of the proposed method for simple, accurate and precise analysis of SUL and TRI in pharmaceutical preparations. The proposed methods do not need prior separation of SUL and TRI before analysis. The Indonesian Pharmacopeia chromatographic standard procedure is rather time consuming and expensive for routine assays. In addition, the proposed methods are suitable for application without interference from the excipients and can be applied directly to the commercial preparations without previous treatment.

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